Introducing Commensal Bacteria from *Caenorhabditis elegans* to *Drosophila melanogaster*: Effects on Health, Ageing and Insulin-like Signalling

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Dissertation Declaration

I, Tze Chang (Justin) Ng, declare that the work submitted is my own and is not similar in concept to or based on the work of others, whether published or unpublished, except with full and proper acknowledgment.

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ii. List of Abbreviations

No	Abbreviation	Context	Meaning
1	R	Antibiotic susceptibility test	Resistant
2	S	Antibiotic susceptibility test	Susceptible
3	T	Antibiotic susceptibility test	Trace
4	DST	Bacteria experimentation	Diagnostic sensitivity test
5	OD	Bacteria experimentation	Optical Density
6	Tn7	Bacteria experimentation	A 14-kb bacterial transposon
7	CO_2	Chemicals	Carbon Dioxide
8	EDTA	Chemicals	Ethylenediaminetetraacetic acid
9	EtNa	Chemicals	Ethanol and Sodium solution
10	HCl	Chemicals	Hydrochloric acid
11	IPTG	Chemicals	Isopropyl β-D-1-thiogalactopyranoside
12	KH2PO4	Chemicals	Monopotassium phosphate
13	LB	Chemicals	Lysogeny Broth
14	$MgSO_4$	Chemicals	Magnesium sulfate
15	Na_2HPO_4	Chemicals	Disodium phosphate
16	NaCl	Chemicals	Sodium chloride
17	NaOH	Chemicals	Sodium hydroxide
18	Nipagin	Chemicals	Methyl-p-Hydroxybenzoate
19	PBS	Chemicals	Phosphate-buffered saline
20	PVDF	Chemicals	Polyvinylidene difluoride
21	SY	Chemicals	Sugar and Yeast
22	TBE	Chemicals	Tris-Borate-EDTA
23	TSA	Chemicals	Tryptic Soy Agar
24	JAK-STAT	Cytokine-activated signalling pathway	Janus kinase/signal transducers and activators of transcription
25	MD	Dietary Lifestyle	Mediterranean-style diet
26	DAM	Drosophila experimentation	Drosophila activity monitor
27	dTomato	Fluorophores	Dimer tomato
28	dTomato	Fluorophores	Dimeric Tomato
29	mPlum	Fluorophores	Monomeric Plum
30	mPlum	Fluorophores	Monomeric Plum
31	sfGFP	Fluorophores	Superfolder green fluorescent protein
32	PCR	General Experimentation	Polymerase chain reaction
33	RPM	General Experimentation	Revolutions per minute
34	+	Genetics	Wildtype
35	E. Value	Genetics	Expectation Value
36	InR^{DN}	Genetics	Insulin receptor dominant negative
37	*rpr*	Genetics	Reaper apoptotic-inducing gene
38	TRH	Genetics	Tryptophan hydroxylase
39	UAS	Genetics	Upstream Activation Sequence
40	COSSH	Health and Safety	Control of Substances Hazardous to Health Regulations
41	IL	Immunology	Interleukin
42	MHC	Immunology	Major Histocompatibility Complex

43	AFX	Insulin signalling pathway	Forkhead box O4
44	AKT	Insulin signalling pathway	Protein kinase B Homolog
45	CHICO	Insulin signalling pathway	Insulin receptor substrate 1 homolog (*D. melanogaster*)
46	DAF	Insulin signalling pathway	Dauer abnormal formation protein (*C. elegans*)
47	DILP	Insulin signalling pathway	Drosophila insulin-like peptides (*D. Melanogaster*)
48	FKHR	Insulin signalling pathway	Forkhead in rhabdomyosarcoma
49	FKHRL1	Insulin signalling pathway	Forkhead transcription factor-like 1
50	FOX	Insulin signalling pathway	Forkhead box
51	FOXO	Insulin signalling pathway	Forkhead box (*D. melanogaster*)
52	IGF	Insulin signalling pathway	Insulin-like growth factor (mammals)
53	IIS	Insulin signalling pathway	Insulin/insulin-like growth factor-1 signalling
54	ILPC	Insulin signalling pathway	Insulin-like Producing Cells
55	INR	Insulin signalling pathway	Insulin-like receptor (*D. Melanogaster*)
56	INS	Insulin signalling pathway	Insulin/insulin-like peptide ligands (*C. elegans*)
57	IR	Insulin signalling pathway	Insulin receptor (mammals)
58	IRS	Insulin signalling pathway	Insulin receptor substrates (mammals)
59	IRS	Insulin signalling pathway	Insulin receptor substrate
60	PI3K	Insulin signalling pathway	Phosphoinositide 3-kinases
61	PIP	Insulin signalling pathway	Phosphatidylinositol bisphosphate
62	PK	Insulin signalling pathway	Protein kinase
63	PTEN	Insulin signalling pathway	Phosphatase and tensin homolog
64	SGK	Insulin signalling pathway	serum- and glucocorticoid-inducible kinase (*C. elegans*)
65	TF	Insulin signalling pathway	Transcription factor
66	mTOR	Metabolic pathway	Mechanistic target of Rapamycin
67	SCFA	Metabolic pathway	Short-chained fatty acids
68	NIH	Organisation	The National Institutes of Health (USA)
69	WHO	Organisation	World Health Organisation
70	ARD	Pathology	Age-related disease
71	COVID-19	Pathology	Severe Acute Respiratory Syndrome Coronavirus 2
72	NCD	Pathology	Non-communicable disease
73	GI	Physiology	Gastrointestinal
74	BLAST	Sequencing	Basic Local Alignment Search Tool
75	HSD	Statistics	Honestly significant difference
76	n	Statistics	Sample size
77	SD	Statistics	Standard Deviation
78	SE	Statistics	Standard Error
79	$\tilde{x}$	Statistics	Median
80	Keap1	Stress response pathway	Kelch-like ECH-associated protein 1
81	Nrf2	Stress response pathway	Nuclear factor erythroid 2-related factor 2
82	w/	Writing abbreviation	with

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v. Abstract

Background: With the rise of age-related diseases and challenges associated with ageing, there is a growing demand for innovative approaches to promote healthspan. The gut microbiome, essential for health and homeostasis in vertebrates and invertebrates, emerges as a promising avenue for enhancing overall health and addressing age-related disorders. However, little is known about how gut commensals affect host biological processes, let alone what constitutes a healthy gut microbiome.

Purpose: Given the shared ecological environments and co-evolutionary history between nematode roundworms and fruit flies, this study introduces *Caenorhabditis elegans* commensal bacteria (derived from the CeMbio database) to *Drosophila melanogaster* to probe for bacterial impacts on ageing and interactions with evolutionarily conserved nutrient-sensing pathway—insulin/insulin-like signalling pathway (IIS).

Methods: To ascertain successful bacteria colonisation of the fly gut, fly media was adapted for bacteria growth and candidate bacteria were fluorescently transformed to enable direct observation under fluorescence microscopy. Using the optimal experimental conditions and bacterial combination, the impacts of the introduced bacteria on fly health and ageing were evaluated by assaying fly lifespan, exploratory walking behaviour, gut integrity, sleep, neuromuscular function, and fecundity. To gain insights into potential crosstalk between bacteria and IIS, bacteria were also introduced to flies with impaired IIS achieved through either ablating cells that produce *Drosophila* insulin-like peptides 2-3 (d2-3GAL4/UAS-*rpr*) or downregulating insulin receptors expression in serotonergic neurons (trhGAL4/UAS-InR^{DN}).

Results: From 16 CeMbio bacteria, 11 bacteria grow on fly media in contingent that antifungals were omitted. Thus, all subsequent experiments utilised additive-free fly media. Out of 24 transformations, 6 new fluorescent bacteria were generated and validated for their correct identity. Three fluorescent bacteria—*Ochrobactrum vermis* (MYb71-sfGFP), *Enterobacter ludwigii* (MYb174-dTomato), and *Enterobacter cloacae* (CEent1-mPlum)—successfully colonised the fly gut. When introduced together, these three bacteria reduced fly median lifespan but increased early-age egg laying, resulting in earlier egg exhaustion. Across life, these bacteria attenuated changes to exploratory walking and sleep behaviour induced by IIS reduction. However, fly gut permeability and neuromuscular function remained unaffected.

Conclusions: MYb71-sfGFP, MYb174-dTomato, and CEent1-mPlum may benefit adult flies during early age but be detrimental later as bacterial load increases. Regardless, these bacterial-host interactions crosstalk with IIS to affect complex behaviours like exploratory walking and sleep. These findings provide evidence that the study of ecologically relevant commensal bacteria from C. elegans can be translated onto D. melanogaster to further probe for interactions affecting evolutionarily conserved biological pathways and complex behaviours.

1. Introduction

Ageing represents a complex accumulation of biological changes in an organism over time that can lead to a variety of age-related diseases (ARDs). These changes are influenced by a myriad of genetic and environmental factors and the incidences of ARDs has increased in recent years due to advances in medicine that have extended the human lifespan. In response to this trend, the field of biogerontology and geroscience has emerged to explore ways to reduce the burden of ARDs through biomedical interventions that target the ageing process itself. One area of particular interest in this field is the role of the gut microbiome has on ageing mechanisms. However, our understanding of how bacteria in our gut influence nutrient sensing pathways in model organisms, let alone in humans, remains limited. Our study aims to shed light on how defined gut microbiota interventions affect fitness and ageing in flies with impaired versus control insulin/insulin-like growth factor-1 (IIS). By doing so, this study would represent the first step in our underlying research strategy to identify probiotic bacteria and therapeutic targets for healthspan interventions.

1.1. Longevity, a blessing, or curse?

The “*Corpus Inscriptionum Latinarum*” was a collection of ancient Latin inscriptions that documented life during the Roman Empire, spanning from 509 BCE to 476 CE. Back then, the average life expectancy in Rome was 29.3 years (1). Today, life expectancy has increased significantly, with the World Health Organisation (WHO) reporting a global average of 73.4 years. This increase in longevity was attributed to advancements in modern medicine and public health. Remarkably, antibiotics improved health outcomes for communicable diseases,

vaccination programs have helped eradicated smallpox, and public smoking bans have reduced mortality rates for cardiovascular diseases (2–5). Furthermore, better access to medical care, clean water, and a more diverse diet have contributed to enhanced overall well-being. As healthcare advancements continue, more individuals are able to live longer. While longer life expectancy is typically seen as a positive development, it also brings several challenges affecting society at an economic, social, and institutional level. From these challenges, one concerns the ability of future working-age populations and current healthcare systems to support the increasing number of older individuals. Currently, the aging population above 65 is expected to more than double by 2050 (6). However, the global birth rate has been declining since the 1970s, causing a decrease in working-age populations. As such, the burden of supporting and providing for the growing number of older people falls upon a relatively smaller group of working-age individuals. Making matters worse, healthcare systems with limited staffing pools may be ill-equip to tackle the potential increasing demand for specialised eldercare when needed. This was evident during the COVID-19 pandemic, where the strain on healthcare resources and personnel highlighted the vulnerabilities of healthcare systems in addressing the unique needs of older patients (7–9). To address these challenges, it becomes imperative to consider the well-being and healthspan of longer-living populations and implement appropriate economic and healthcare policies to ensure a high quality of life for all segments of the population.

1.2. Healthspan versus lifespan

Healthspan refers to the number of years an individual spends free of any significant health issues and infirmity, whereas lifespan defines the total number of years a person lives. While lifespan extension may be desirable, healthspan extension is often seen as more important because living a long life while suffering from chronic illness can be burdensome. Ideally, the onset of chronic disease should be delayed as long as possible, and the time spent suffering from disease before death should be minimized (10). However, the current gap between healthspan and lifespan is estimated to be more than 9 years, which means that many people may spend a significant portion of their lives suffering chronic diseases (11). Noticeably, these diseases tend to manifest later in life, suggesting ageing itself a risk factor for developing such conditions.

1.3. Aging, a risk factor and therapeutic target?

As people age, their risk of experiencing a decline in in physical and mental function increases (12,13). These age-related diseases (ARDs), which include non-communicable diseases (NCDs) like cancer, metabolic disease, and Alzheimer's, are more common among older populations (14–16). Alone, these three ARDs account for more than 13 million deaths worldwide and are projected to increase in incidences (17–19). Moreover, many of these older adults suffer from multiple chronic conditions simultaneously, further exacerbating quality of life and healthcare expenditure (20–22). These trends suggest that families and healthcare systems worldwide may struggle to cope with the increasing demands of patient admissions, care, and expenditure in

future. Therefore, research into effective and scalable interventions for preventing or treating such conditions is crucial.

Ageing research first began as an interest in understanding the fundamental processes that drive ageing. Biogerontology, as it was referred to in the 20th century, focused on the biological basis of ageing and ARDs. In 2005, interest grew, and the Buck Institute for Aging Research expanded their research to include how ageing contributes to the development of chronic diseases (23,24). Consequently, the field of geroscience emerged. This term was later adopted by the National Institute of Health (NIH) in 2007, creating initiatives encouraging interdisciplinary approaches to ageing research. (21). Given that ageing is a significant risk factor for all ARDs, ageing has become increasingly recognised as a therapeutic target. In theory, by delaying or reversing the biological aging process, the onset of ARDs can also be delayed, leading to an extension of healthspan. Unlike disease-specific interventions, these approaches aim to treat or prevent multiple diseases simultaneously.

1.4. Current ageing interventions and challenges

Currently, two popular research areas in ARD intervention includes lifestyle and pharmacological interventions. Lifestyle interventions involve changes to individuals' daily habits or behaviours to improve health. Examples include smoking cessation, dietary modifications, and exercise programs. Notably, Mediterranean-style diets (MDs) have been shown to be particularly effective at reducing the risk of non-communicable diseases (NCDs) when paired with regular exercise and reduced cigarette and alcohol consumption (25). However, promoting and adopting dietary interventions like MDs can be

challenging due to geographical, socio-cultural, and personal barriers. For example, individuals in non-Mediterranean countries may have difficulty sourcing or affording ingredients used in MDs (26,27). Also, those in culturally distinct countries with large vegetarian populations, such as India, may be unwilling to adopt a diet that includes meat consumption (28). Furthermore, foods can affect consumers differently based on their ethnicity and genetic background. Notably, East Asian individuals have greater glycaemic responses when consuming rice compared to Europeans (29). Likewise, the cognitive benefits of MDs may be greater among Caucasians than other races (30,31). In addition to genetics, these differences may also be influenced by different gut microbiota composition present across ethnicities (32). Altogether, these challenges may lead to poor adherence, inefficacious practises, and limited coverage effect of lifestyle interventions.

Contrastingly, pharmacological interventions involve the use of drugs to alter biological processes that contribute to aging. One example of this is the use of drugs that inhibit the mechanistic target of rapamycin (mTOR) signalling pathway, which plays a role in regulating cell proliferation, autophagy, and cell death (33–35). Interestingly, mTOR inhibitors such dactolisib and everolimus have been shown to improve immune function in older adults (36). Likewise, another mTOR inhibitor known as rapamycin extends lifespan in worms, flies, and mice (37–39). While drugs may be easier to administer than lifestyle modifications, their efficacies and safety have not been fully evaluated. Thus, more years of research and clinical trials are required to determine their effectiveness as anti-ageing interventions. Furthermore, drug repurposing, let alone *de novo* drug development, takes more than 6 years to complete, costing on average USD 300 million, with a success rate only around 30% (40,41). Being

expensive, difficult, and time-consuming to produce, a safe and effective pharmacological anti-ageing intervention may not be ready in time when the ageing population doubles.

Given the challenges and limitations of lifestyle and pharmacological interventions, it becomes evident that combining these approaches could offer a more comprehensive approach to delay or prevent ARDs effectively. One additional intervention worth considering is the optimisation of the human gut microbiome. Similar to dietary interventions, this strategy focuses on introducing prescribers to identified substances that promote microbiome health, such as probiotics and prebiotics. Probiotics refer to any viable microorganism that benefit host health when ingested (42). On the other hand, prebiotics are ingested food ingredients that improves host health when fermented (43). Unlike drugs, probiotics and prebiotics are generally safe which allows them to be categorised as dietary food supplements unless used to treat specific pathologies (44–46). Therefore, these supplements do not require costly and extensive clinical testing. Furthermore, they are easy to administer, inexpensive, and less labour intensive to produce. Therefore, gut microbiome modulation may serve as a complementary ARD and ageing intervention alongside lifestyle and pharmacological interventions.

1.5. The gut microbiome and health

The gut microbiome is a community of microorganisms that inhabit the digestive tract and can influence host health. These microbes include bacteria, archaea, fungi, and viruses (47–50). Specifically, over 93% of the human gut microbiome consist of bacteria from the phyla *Firmicutes*, *Bacteroidetes*,

Actinobacteria, and *Proteobacteria* (51,52). Dynamic, this composition changes with age, lifestyle, health status and location within the digestive track (53–57)

In addition to aiding in nutrient metabolism and acquisition, the gut microbiome is also involved in immunomodulation, gut integrity regulation, and drug metabolism (58–61). Given the microbiome's many functions, it is not surprising that dysbiosis or altered gut microbiota compositions have been observed in patients with ARDs such as Alzheimer's disease, metabolic syndrome, or cancer (57,62–64). Therefore, optimising the composition of the gut microbiome may help to promote healthy aging and delay the onset of ARDs. However, it is still not clear what constitutes a healthy gut microbiome or how it impacts aging-related mechanisms. While there exist extensive human omics studies characterising the human gut microbiome, further experimental research is necessary to establish causal relationships. To better comprehend the effects of gut microbes on aging, it is crucial to investigate their influence using model organisms. Conducting experiments in these models allows for controlled manipulation, providing valuable insights into the interactions between gut microbes and the aging process, complementing the findings from human studies.

1.6. Model organisms in Biogerontology

Due the copious ethical challenges of conducting human research, the field of biogerontology has extensively relied on model organisms to study the ageing process. Model organisms have shorter lifespans than humans and tractable genetics, which makes them easy to manipulate under laboratory settings. They also share evolutionary conserved biological pathways analogous

with humans which suggest that findings from model organisms can be applied to humans. Some commonly used model organisms include yeasts, worms, mice, and fruit flies.

For over 5000 years, humans have used budding yeast (*Saccharomyces cerevisiae*) for bread making and alcohol brewing. Beyond culinary and industrial applications, these unicellular fungi also expanded our understanding in cell biology and genetics. One of the early publications of biogerontological research using yeast dates as far back as 1959 reporting their finite lifespan and reproductive capacity (65). Since then, yeast has continuously contributed towards our understanding of chronological lifespan, caloric restriction, and longevity genes (66,67). Their smaller genome (around 6000 genes) and lack of organ systems make them the least complex organisms compared to other model organisms mentioned (68). Easily cultured, they possess a short generation time (2 hours) and lifespan (14-24 days) allowing experiments to be conducted in short time frames (69). Likewise, extensive collections can be cryopreserved, allowing convenient distribution while minimizing the need for continuous maintenance (70). Despite these advantages, yeast is less genetically similar to humans compared to other model organisms sharing only 23% of disease genes homologous to humans (71).

Nematode worms (*Caenorhabditis elegans*) emerged as another critical model organism in biogerontology research. Roundworms were first introduced as models for developmental biology dating as far back as 1963 (71). Unlike yeast, roundworms are multicellular organisms that share certain human physiological overlaps such as a digestive and nervous system. Their transparent bodies offer a live visualization of their internal organs and cells without the need for dissections. This allows scientist to track the development of organ

systems and effects of drugs across life. Similar to yeast, *C. elegans* have short lifecycles (14-21 days) and can be cryopreserved (72,73). In addition to large brood sizes, these characteristics allow for high-throughput studies as models for dietary manipulation, diseases, and drug screening (74–76).

Another crucial model organism in biogerontology is the laboratory mice (*Mus musculus*). While the use of mice in research can be traced back to the 17th century, its popularity as a model organism took off after 1909 upon the establishment of inbred mice strains (77). Possessing a respiratory, musculoskeletal, and highly developed immune system, mice share a higher degree of anatomical and physiological similarities with humans compared to the other model organisms. Furthermore, around 90% of mice genes are homologous to humans (78). Altogether, they can recapitulate the phenotypes of human diseases and characterise metabolic changes from interventions in greater detail (79). For example, the benefits of calorie restrictions in mice were detailed by improved insulin sensitivity, decreased cardiovascular disease and inflammatory markers (80). Despite these advantages, mice experiments are more time consuming, laborious, and expensive to carry out. This is in part due to their longer life span (1.5-3 years) and generation time (1-2 months), alongside a greater need for ethical considerations (81).

Finally, the biogerontology field also extensively utilised fruit flies (*Drosophila melanogaster*) in research. During its initial adoption in the early 20th century, flies helped elucidated the roles of chromosomes and effects of radiation-induced mutations (82,83). Since then, use of the organism extended to chronobiology, immunology, and neuroscience (84–86). These experiments can be conducted within short time frames as fruit flies have short generation times (~10 days) and lifespans (2-3 months) (87,88). Due to their high

reproductive output (~120 eggs per day), experiments often include large samples sizes (89). Furthermore, more than 50% of genes, including disease-associated genes from flies are homologous to humans (90,91).

Altogether, each organism offers unique strengths and limitations in research (Table 1). Regardless, each model organism has been well characterised for their similarities to human physiology and genetics. Although they do not perfectly reflect human physiology, they share biological pathways similar to humans. These biological pathways, often essential for life, are crucial regulators of growth, development, and metabolism. Remarkably, one of these conserved pathways include nutrient sensing.

Table 1) A comparison between yeast, worms, mice, and fruit flies as model organisms

Model Organism	**Yeast (*Saccharomyces cerevisiae*)**	**Worms (*Caenorhabditis elegans*)**	**Mice (*Mus musculus*)**	**Fruit Flies (*Drosophila melanogaster*)**
NCBI Taxonomy ID	4932	6239	10090	7227
Generation Time (69,81,87,92)	2 Hours	3 Days	1-2 Months	10 Days
Life Span (72,81,88,93)	14-24 Days	14-21 Days	1.5-3 Years	70-90 Days
Reproductive Output (69,72,81,89)	Double every 1.5 Hours	~140 Eggs per Day	2-12 Pups per Month	~120 Eggs per Day
Genome Size (94)	12.1 Mb	100.3 Mb	2,700 Mb	143.7 Mb
Genes (94)	6,465	46,926	50,562	17,894
Homogeneity to Human Genome (78,91,95,96)	31%	36%	90%	50%
Homogeneity to 2,271 Known Human Disease Genes (90)	23%	52%	99%	55%
Cryopreservability (70,73)	Yes	Yes	No	No
Live-Cell Imaging	Yes	Yes	No	No
Cost	Low	Low	High	Low
Ethical Consideration	No	No	Yes	No
Commonalities	• Genetically well characterised • Can be genetically manipulated • Share evolutionary conserved biological pathways with humans			

1.7. Nutrient sensing: IIS pathway

Nutrient sensing enables cells to detect and respond to the availability of nutrients in their surroundings. This process involves complex signalling pathways regulating nutrient metabolism of glucose, amino acids, and fatty acids. One example of these pathways is the insulin/insulin-like growth factor-1 signalling (IIS) pathway, which regulates nutrient uptake, cell growth, development, and proliferation through intercellular signalling cascades mediated by ligands, receptors, transduction proteins, and transcription factors (97). The IIS pathway is evolutionarily conserved across humans and other organisms, although the number of isoforms of its activators and effectors can vary between species (98–100).

In the IIS pathway (Figure 1), ligands bind to their respective receptors, which are receptor tyrosine kinases (100). Upon dimerization, these receptors provide binding sites for the phosphorylation of insulin receptor substrates (IRSs). The dimerized receptors and IRSs then activate downstream signalling pathways through the activation of signal transducer enzymes, secondary messenger proteins, and protein kinases, which ultimately phosphorylate Forkhead box (FOX) transcription factors resulting in their localisation into the cytoplasm and subsequent ubiquitination (101,102). Consequently, FOX transcription factor dependent transcription of genes responsible for gluconeogenesis, cell cycle inhibition, and oxidative stress responses is downregulated resulting in increased proliferation and glucose metabolism (101–104). Evidently, IIS plays an essential modulator of glucose metabolism, growth, and survival.

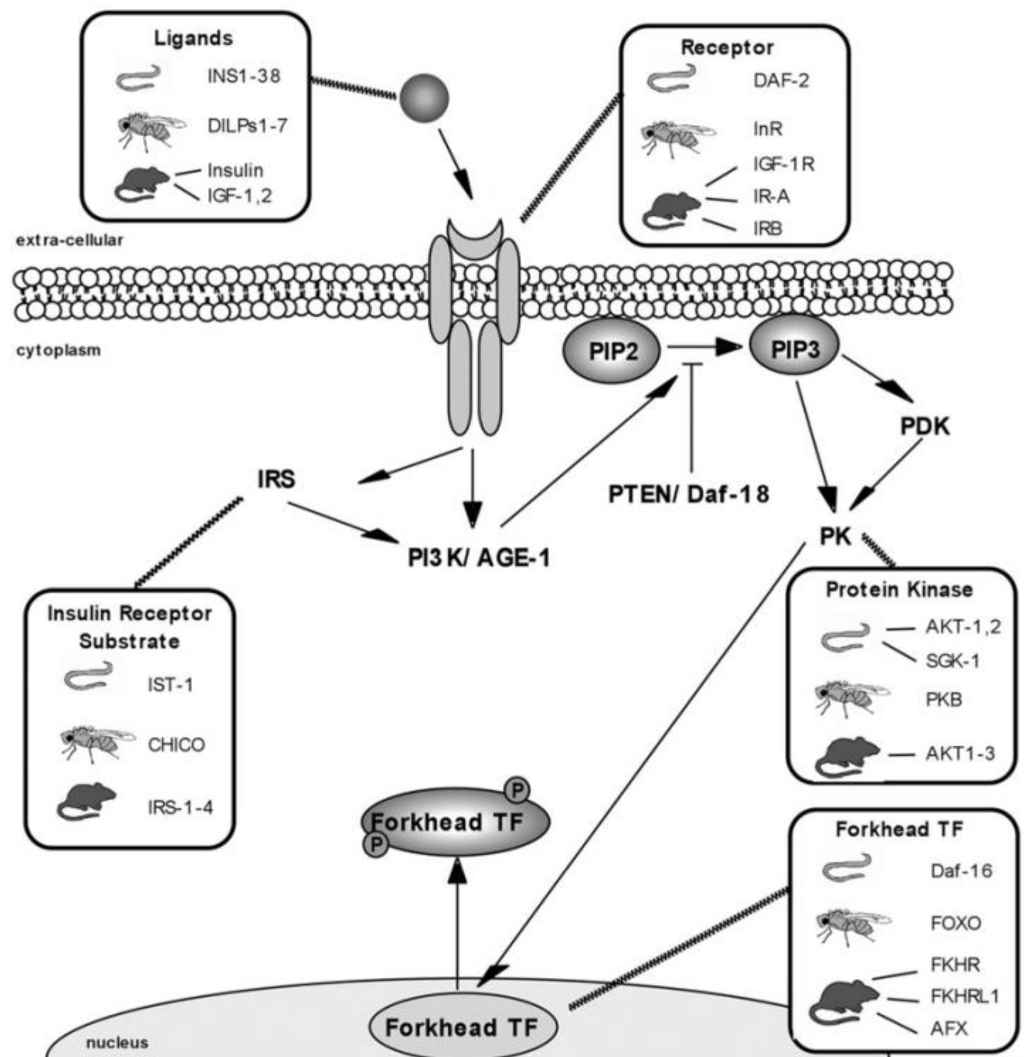


Figure 1) A simplified overview of the IIS pathway across model organisms (100).

Current research has shown that changes to this pathway can impact lifespan and survival. In humans, lower levels of bioavailable IGF-1 in the blood have been associated with a higher survival rate among people in their 90s (105). Similarly, inhibiting the IIS pathway through methods such as dietary restriction or knocking down insulin/IGF-1 receptors has been shown to increase lifespan in yeast, worms, flies, and mice (97,106). Given the known link between the IIS pathway and longevity, it would be interesting to explore the potential role of commensal bacteria in the gut in influencing IIS signalling.

1.8. Gut microbiome and IIS

The presence of gut microbiota in the body has been linked to physical and cognitive development in model organism. In germ-free mice, the absence of gut microbiota has been shown to decrease levels of IGF-1 and impair development (107–110). However, reintroducing gut microbes can restore these levels and improve development (109,110). Here, it is thought that certain commensal bacteria produce metabolites such as short-chain fatty acids (SCFAs) that regulate IGF-1 signalling and impact host development (109). Similarly, these modulatory effects also extend to flies. Particularly, axenic flies exhibit higher degrees of developmental defects under food scarcity compared to normal flies (111). It was found that flies required acetic acid, produced by the commensal bacteria *Acetobacter pomorum*, for proper growth and development. Interestingly, absence of acetic acid increases circulating sugars and stored in lipids in flies, alluding possible interactions with IIS. Collectively, these findings demonstrated the essential role of commensal bacteria in maintaining host health and development emphasising on bacterial-host IIS interactions. While some bacteria-host interactions have been characterised, much remains unknown of many other classified and unclassified bacteria and their effects on IIS. Thus, further research on identifying these bacteria and examining their impact on IIS can be a valuable avenue for investigation.

2. Project Background

The goal of this project is to: 1) to investigate whether natural gut microbes from the microbiota of *C. elegans* could colonise the gut of *D. melanogaster* and 2) investigate potential probiotic effects that may be IIS-dependent in flies. To achieve these objectives, experimental bacteria was chosen based on their biological and ecological relevance, as well as their potential to induce probiotic effects or affect the IIS pathway. Furthermore, it is essential to transform the experimental bacteria to express fluorescent markers to enable *in situ* visualisation of colonisation patterns. By generating different bacterial strains expressing distinctive fluorophores, it becomes possible to assess the co-colonisation of multiple bacteria in a single host. Additionally, genetic tools were employed to generate flies with impaired IIS, allowing for the investigation of potential interactions between the bacteria and the IIS pathway.

2.1. Selecting experimental bacteria

The *C. elegans* gut bacteria transformed and introduced to flies in this study were selected from the *C.* elegans microbiome resource CeMbio (112). This resource provides a database of naturally occurring bacteria in the nematode microbiome, detailing their complete genome sequence, diagnostic PCR primer designs, and known host interactions. While the study initially planned to transform and utilise bacteria with reported positive impacts on worm health, these bacteria were not ready during the time of experiment. As such, selection of bacteria was limited to those previously transformed and confirmed via sequencing. Ultimately, the bacteria selected were *Ochrobactrum vermis* (MYb71), *Enterobacter ludwigii* (MYb174), and *Enterobacter cloacae*

(CEent1). These bacteria are quick to colonise the *C. elegans* gut and are not harmful to its host (unpublished data from the Benedetto Lab).

2.2. About selected bacteria

While the bacteria strains used in this experiment — *Ochrobactrum vermis* (MYb71), *Enterobacter ludwigii* (MYb174), and *Enterobacter cloacae* (CEent1) — have not been fully characterised, some probiotic effects and IIS interactions have been discovered from these species affecting various animals. *Enterobacter sp.* have been well studied as probiotics in worms, fishes, and mice. Notably, *E. cloacae* increases the *C. elegans* resistance against *Enterococcus faecalis* infections (113). Simultaneously, lifelong exposure of the bacteria accelerates worm development and shortens lifespan. However, these effects did not translate in worms where the bacteria are not naturally occurring. Notably, unnatural host worms such as *C. briggsae* exposed to *E. cloacae* are not protected against *E. faecalis* infections but experience lifespan extension. Therefore, the effects of *E. cloacae* depend on if the bacterium is naturally occurring in host. In other animals, *E. cloacae* also protects Kenyi cichlids and rainbow trout from *Plesiomonas shigelloides* and *Yersinia ruckeri* infections, respectively (114,115). Both fishes reported improved growth and higher white blood counts during infections suggesting enhanced immune responses. These findings were also consistent in murine models where *E. cloacae* improves nutrient bioavailability and immune response to *Escherichia coli* infections (116). Particularly, serum triglycerides and interleukin (IL)-1α levels were improved during infection periods. It is also suggested that *E. cloacae* protects against

Helicobacter pylori infections as the *Enterobacter* inhibits *H. pylori* growth *in vitro* (117).

Similarly, *E. ludwigii* have largely been characterised for their immunomodulatory effect in fishes and mice. When paired with *Bacillus cereus* and *Paraburkholderia fungorum*, the combination improves immunity against nervous necrosis virus infections in giant groupers through upregulating innate and adaptive immune response genes (118). Here, there were higher expressions of inflammatory factors (IL-10 and IL-1β) and major histocompatibility complex (MHC) 1 in the infected fish's enterocytes. In mice, *E. ludwigii* improves resistance and recovery to colitis through choline supplementation produced by the bacterium (119). As a result, the metabolite improves mouse immune tolerance allowing colitis remission. Similar to *E. cloacae*, *E. ludwigii* sourced from Asian seabass possess antimicrobial abilities to inhibit the *in vitro* growth of two host pathogens, *Vibrio parahaemolyticus* and *Aeromonas hydrophilla* (120).

As for *O. vermis*, the bacterium's effect on animals has not been fully understood. However, transcriptomic analysis in *C. elegans* revealed its impact across 86 genes involved in immunity, metabolism, and development (121). Relevant, the bacterium's establishment in the gut of *C. elegans* is dependent on IIS (122). Downregulation of the worm's insulin receptor gene (*daf*-2) reduces *O. vermis* colonisation. Vice versa, *O. vermis* population increases when its Forkhead transcription factor (*daf-16*) expression increased. Moreover, uptake of this bacteria differs between *C. elegans* strains as wildtype MY2079 but not laboratory N2 worms actively avoid *O. vermis* in favour of other bacteria in media (123).

All in all, *Ochrobactrum vermis* (MYb71), *Enterobacter ludwigii* (MYb174), and *Enterobacter cloacae* (CEent1) can be found naturally occurring in the gut of diverse animals and even colonise unnatural host guts. Significantly, these bacteria interact and affect a multitude of biological processes such as immunity and IIS making them suitable candidates to study in the fly gut. However, these bacteria need to be fluorescently transformed to allow experiment tractability.

2.3. Generating fluorescent bacteria

Fluorescent transformation of bacteria enables the visualisation of bacteria *in vivo*. By utilising distinct fluorophores expressed by each bacterium, it becomes feasible to track the co-colonisation of all three bacteria in a single fly. Bacteria transformation was carried out using methodologies adapted from a Tn7 kill switch counterselection system (124). Briefly, this protocol (Methods 4.2) involves the selection of transformed bacteria through a triparental conjugation of a gentamicin-susceptible target bacteria (Bacteria^{Recipient}), a gentamicin-resistant bacteria donor containing a tn7-tagging vector with a kill switch insert (Bacteria^{Donor}), and a gentamicin-susceptible transposase helper vector (Bacteria^{Helper}). The goal of this protocol is to transform the target bacteria to express fluorescent protein and gentamicin resistance while eliminating vector bacteria or untransformed bacteria through gentamicin treatment and activation of the kill switch via IPTG (isopropyl-β-d-thiogalactopyranoside) exposure. Once transformed, the identity of the fluorescent bacteria was reconfirmed through 16S sequencing.

Like other transformation systems involving conjugation, this method relies on negative screening through selective media to eliminate the donor and

untransformed bacteria. However, conventional approaches relied on prior knowledge on the target bacteria's physiology such as antibiotic susceptibility and auxotrophies to design suitable counterselection media and incubation conditions for each bacteria transformation. In contrast, the adapted tool circumvents this arduous step by incorporating an inducible kill switch counterselector to eliminate donor bacteria. This is advantageous as alternative counterselector systems such as the *sacB*-based sucrose sensitivity system may not be universally compatible with all bacteria, namely gram-positive bacteria, due to its nontoxic nature in gram-positive microenvironments (124,125). Also, the introduction of fluorescence and gentamicin resistance to recipient bacteria allows convenient visual screening for adequately transformed clones. Hence, bacteria transformation using the Tn7 kill switch counterselection system streamlines transformation across diverse bacteria lineages, including novel and uncharacterized bacteria, using a standardised protocol. Importantly, bacteria transformations can be completed in shorter time frames to allow *Drosophila* experimentation.

2.4. Using the *Drosophila* model

As mentioned, the *Drosophila* model was selected for this study to investigate if commensal bacterial-host effects observed in *C. elegans* could be recapitulated or contrasted in flies. From an experimental perspective, flies offer the same advantages of *C. elegans* where their high reproductive output and short lifespan alongside generation time allows experiments with large sample size to be set up and executed in short timeframes, fitting of a master's project. Furthermore, flies allow the convenient and cost-effective study of complex

behaviours relevant to ageing such as exploratory walking, sleep, and negative geotaxis (126–128).

In the fly microbiome, *Acetobacter sp. and Lactobacillus sp.* are the most common and abundant bacteria found in the gut, with *Lactobacillus sp.* being the prevalent genus during the larval stage, and *Acetobacter sp.* emerging later as the dominant genus in adult flies (129,130). Nevertheless, this composition is not consistent with all flies, as evident in wild-caught *Drosophila* from Massachusetts (USA) where *Enterobacter sp.* and *Klebsiella sp.* were the dominant bacteria species instead (131). Therefore, much of the fly gut microbiota is influenced by their geography and food source (129,131). As *Drosophila* share the same natural habitat with *C. elegans*, particularly as larvae growing in rotting fruits or plants, it is possible that the natural commensal bacteria found in the *C. elegans* microbiome also constitutes the microbiomes of the fly (132). As such, studying the transfer of microbiota from one organism to another offers an opportunity to explore ecological dynamics of microbial communities occurring in shared habitats. These interactions can be further explored through the lens of insulin/insulin-like signalling (IIS) using flies with altered IIS.

2.5. IIS pathway manipulation

To assess the effects of bacteria on insulin/insulin-like signalling (IIS), it is necessary to introduce the bacteria to flies with both normal and impaired IIS to allow comparative analysis. To achieve this, two fly models with reduced IIS were utilised — 1) flies with systemic IIS reduction achieved by ablating drosophila insulin-like peptide (dilp)-producing neurosecretory cells and 2) flies

with serotonergic specific IIS reduction obtained through knockdown of IIS receptor in serotonergic neurons.

IIS manipulation in flies was achieved using the GAL4/UAS binary expression system, which allows for the specific and controlled expression of target genes in transgenic organisms (133). The GAL4/UAS system consists of two components: 1) the *gal4* gene, which encodes the GAL4 transcription factor and 2) the UAS (upstream activator sequence) enhancer, which is a specific DNA sequence that serves as a binding site for the GAL4 transcription factor. In transgenic organisms, the GAL4 transcription factor is expressed in a specific tissue or cell type, and the UAS enhancer is inserted into the promoter region of the target gene. When the GAL4 transcription factor is expressed, it activates the transcription of the target gene, resulting in its expression in the tissue or cell type of interest.

To generate flies with systemic knockdown of IIS, d2-3GAL4/UAS-*rpr* flies were produced. Consequently, d2-3GAL4 drives expression of the proapoptotic gene *reaper* in the insulin-like producing cells (IPC) that produce DILP2 and DILP3, resulting in their ablation. Similarly, serotonergic decline of IIS in flies were generated through trhGAL4/UAS-InR^{DN} expression. As tryptophan hydroxylase (TRH) is an enzyme involved in serotonin production and is expressed in mainly neurons, this selective knockdown of IIS was achieved through upregulating dominant negative insulin receptors (UAS-InR^{DN}) in cells that express *trh*.

2.6. *Drosophila* experimentation

As the transfer of *C. elegans* commensal bacteria to *D. melanogaster* was not previously explored, three concerns arose — 1) Are the CeMbio bacteria

viable on fly media? 2) Could the transformed bacteria colonise the fly gut? and 3) What is the optimal bacteria inoculum load for experiments? Therefore, three pilot studies were carried out to address these issues before the main experiment commence.

Methylparaben (Nipagin) and propionic acid are commonly used to inhibit fungal growth in fly media (134,135). Beyond drosophila experimentation, Nipagin is widely used as an antimicrobial preservative for cosmetics and pantry foods while propionic acid prevents fungal growth in farm grains during long-term storage (136–138). Unclear if these additives would be lethal to bacteria inoculum, a pilot study was designed to assessed bacteria viability of different fly media with or without these additives. The media that best supports bacteria growth was chosen for the experiments.

While MYb71-sfGFP, MYb174-dTomato, and CEent1-mPlum can colonise the gut of *C. elegans* (unpublished findings from the Benedetto lab), it was unknown whether they could colonise fly guts. Here, gut colonisation refers to the establishment and persistent presence of microorganisms within the host gut. In a follow-up pilot experiment, 3-day-old flies were exposed once to all possible combinations of selected fluorescent bacteria on fly media. After one week, fly guts were dissected and assessed for bacteria colonisation under fluorescence microscopy.

Finally, it was unknown what the optimal combination of bacteria and inoculum load is for the experiments. Hence, the remaining flies from the gut colonisation cohort were used in a pilot lifespan assay to select the best combination of bacteria that were likely to be probiotic. The bacteria inoculum load was also adjusted based on the lifespan results.

In the main experiment, several assays were carried out to assess fly lifespan, behaviour, gut health, neuromuscular function, and fecundity. Respectively, these experiments include a lifespan, exploratory walking, gut permeability, negative geotaxis, and offspring/egg quantification assay. Due to resource constraints, experiments were conducted in sets of three assays. Each set includes a survival assay to ensure replicability and validate the results of parallel assays. All results from the pilot and main studies were statistically analysed for significance.

2.7. Project timeline

To summarise, the project was divided between five sections: A) bacteria preparation, B) fly generation, C) pilot studies, D) main experiments, and E) statistical analysis (Figure 2). Methodologies for each section were described in the Methods section. The study began with the bacteria preparation phase (Figure 2A) where available bacteria from the CeMbio database was cultured, transformed, sequenced, and prepared for fly inoculation. Once transformed, flies with and without reduced IIS were generated (Figure 2B) for pilot studies and subsequent experiments. Both the transformed bacteria and flies generated were initially utilised in pilot studies (Figure 2C) to assess experiment feasibility. Once the experiment design was optimised, the main experiments (Figure 2D) were carried out accordingly. Each experimental set utilised a new set of flies and were not reused unless stated otherwise. Finally, statistical analysis (Figure 2E) of results were carried out upon completion of all experiments.

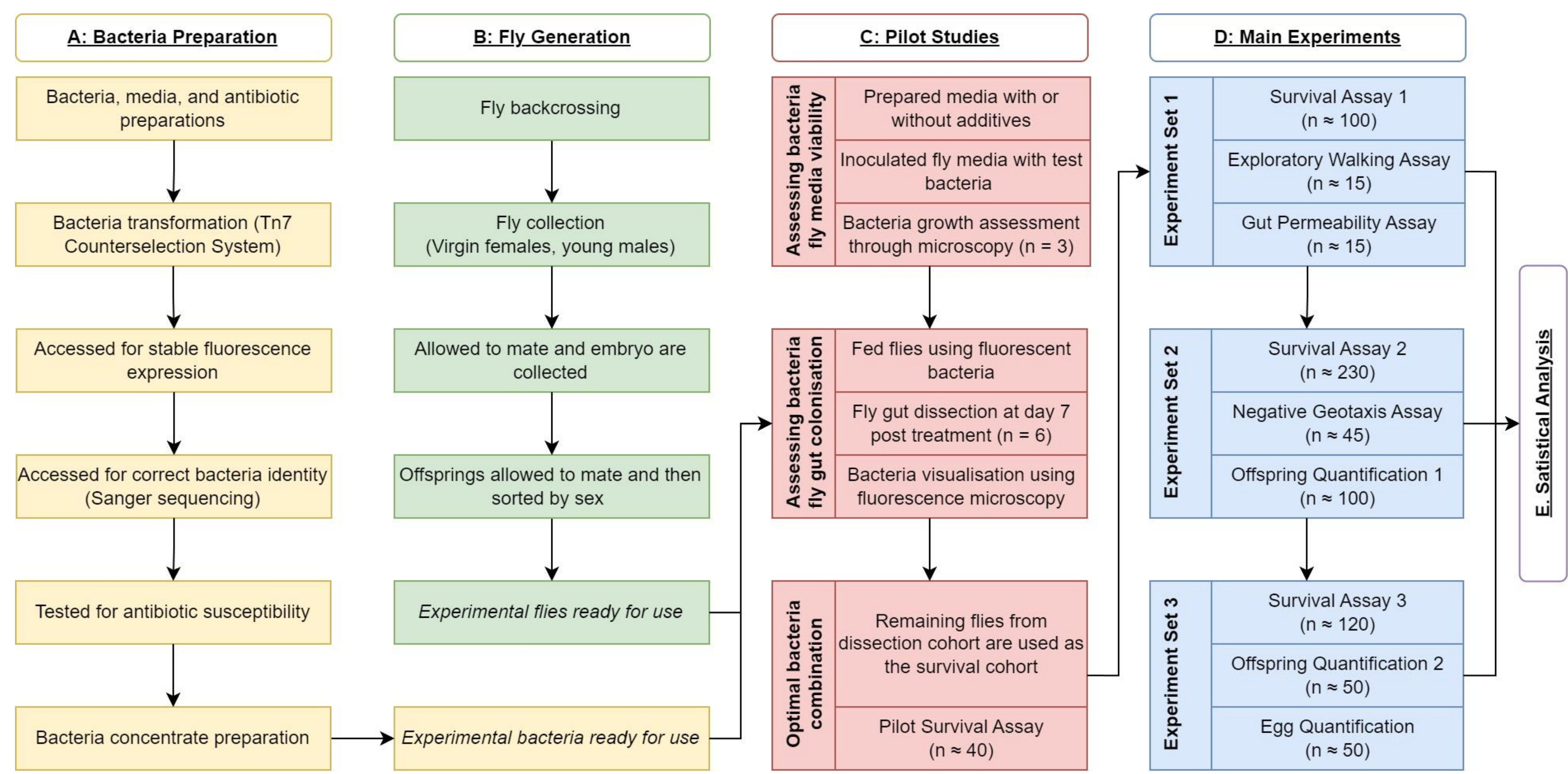


Figure 2) Project timeline includes bacteria preparation, fly generation, pilot study, and main experiment phases.

3. Materials

The following tables outline the materials, resources, and recipes utilised in the experimental design. Table 2 lists the key materials and resources, while Table 3 and Table 4 detailed the recipes used in the experiments. These tables serve as a comprehensive guide for replicating the experiments presented.

3.1. Key resource table

Table 2 presents a complete list of all materials and resources used in the master's project. The formatting and layout of the key resource table was adapted from the STAR Methods guidelines (Cell Press).

Table 2) A key resource table sectioned by A) Bacteria Strains, B) Drosophila melanogaster Strains, C) Chemicals and Reagents, D) Commercial Assays, E) Specialised Equipment, and F) Software and Algorithms.

No	Reagent or Resource	Source	Identifier
	A) Bacteria Strains		
1	*Acinetobacter guillouiae*	Schulenburg Lab, University of Kiel	MYb10
2	*Chryseobacterium scophthalmum*	Schulenburg Lab, University of Kiel	JUb44
3	*Comamonas piscis*	Schulenburg Lab, University of Kiel	BIGb0172
4	*Comamonas sp.*	Schulenburg Lab, University of Kiel	MYb396
5	*Comamonas sp. B-9*	Schulenburg Lab, University of Kiel	MYb21
6	*Comamonas sp. TK41*	Schulenburg Lab, University of Kiel	MYb69
7	*Enterobacter cloacae*	Schulenburg Lab, University of Kiel	CEent1
8	*Enterobacter ludwigii*	Schulenburg Lab, University of Kiel	MYb174
9	*Enterobacter sp. 638*	Schulenburg Lab, University of Kiel	MYb186
10	*Escherichia coli* SM10/pTn7xKS-dTomato	Guillemin Lab, University of Oregon	SM10/pTn7xKS-dTomato
11	*Escherichia coli* SM10/pTn7xKS-mPlum	Guillemin Lab, University of Oregon	SM10/pTn7xKS-mPlum
12	*Escherichia coli* SM10/pTn7xKS-sfGFP	Guillemin Lab, University of Oregon	SM10/pTn7xKS-sfGFP
13	*Escherichia coli* SM10/pTNS2	Guillemin Lab, University of Oregon	SM10/pTNS2
14	*Ochrobactrum anthropi*	Schulenburg Lab, University of Kiel	MYb49
15	*Ochrobactrum vermis*	Schulenburg Lab, University of Kiel	MYb71
16	*Pantoea nemavictus*	Schulenburg Lab, University of Kiel	BIGb0393
17	*Pseudomonas fluorescens*	Schulenburg Lab, University of Kiel	MYb115
18	*Pseudomonas lurida*	Schulenburg Lab, University of Kiel	MYb11
19	*Pseudomonas sp.*	Schulenburg Lab, University of Kiel	MYb16
20	*Pseudomonas sp.*	Schulenburg Lab, University of Kiel	MYb330
21	*Sphingobacterium multivorum*	Schulenburg Lab, University of Kiel	BIGb0170
22	*Stenotrophomonas indicatrix*	Schulenburg Lab, University of Kiel	JUb19
23	*Stenotrophomonas sp.*	Schulenburg Lab, University of Kiel	MYb57
	B) Drosophila melanogaster Strains		
24	dilp2-3GAL4 (d2-3GAL4)	Broughton lab, Lancaster University	(139)
25	dInRA1409K (UAS-InR^{DN})	Bloomington Drosophila Stock Centre	FBal015635
26	trhGAL4	Bloomington Drosophila Stock Centre	#38389
27	UAS-*reaper* (*rpr*)	Bloomington Drosophila Stock Centre	#5823
28	White Dahomey (W^{Dah})	Partridge labs, University College London	(140)
	C) Chemicals and Reagents		
29	16S-1495r Reverse Primer	Merck Group	SY201035515-012
30	16S-27f Forward Primer	Merck Group	SY201035515-011
31	Agar powder	BTP Drewitt	N/A
32	Agarose (genetic analysis grade)	Fisher BioReagents	CAT#9012-36-6
33	Agarose powder (genetic analysis grade)	Fisher BioReagents	9012-36-6
34	Ampicillin Sodium Salt	Sigma-Aldrich	CAS#69-52-3
35	Apple Juice	Spar	SKU#5010358173719
36	Boric Acid	Fisher Scientific	CAS#10043-35-3
37	Brewer's yeast	MP Biomedicals	CAT#903312

38	Brilliant Blue water-soluble powder	Instant Sunshine	E133
39	Diagnostic susceptibility test agar	Mast Group	DM215D
40	Dry active yeast	Saf Levure	SKU#BB-1606
41	Ethanol (99.8%)	Fisher Scientific	E/0650DF/17
42	Ethylenediaminetetraacetic acid (EDTA)	Sigma-Aldrich	CAS#60-00-4
43	Gel Loading Dye, Purple (6X)	New England Biolabs	10043349
44	Gentamicin sulfate	Alfa Aesar	J62834
45	Glycerol	TAAB Laboratories Equipment	G041
46	Gotaq Green PCR Master Mix	Promega	M7122
47	Granulated sugar	Tate and Lyle Sugars	N/A
48	HCl	Fisher Scientific	CAS#7647-01-0
49	Isopropyl-ß-D-thiogalactopyranoside (IPTG)	Acros Organics	CAS#367-93-1
50	KH_2PO_4	BDH Group	CAS#7778-77-0
51	Lysogeny broth (Miller's)	Sigma-Aldrich	L3522
52	Lysogeny broth Agar (Lennox)	Difco, Becton Dickinson	240110
53	Methyl-p-Hydroxybenzoate (Nipagin)	MP Biomedicals	CAT#102341
54	$MgSO_4$	Fisher Scientific	CAS#22189-08-8
55	Na_2HPO_4 Anhydrous	Thermo Scientific	CAS#7558-79-4
56	NaCl	Sigma-Aldrich	CAS#7647-14-5
57	NaOH pallets	Sigma-Aldrich	CAS#1310-73-2
58	n-propyl gallate	Sigma-Aldrich	CAS#121-79-9
59	Paraformaldehyde	Sigma-Aldrich	#30525-89-4
60	Phosphate-buffered saline (PBS) tablets	Sigma-Aldrich	MFCD00131855
61	Propionic acid	Sigma-Aldrich	P5561; CAS: 79-09-4
62	SYBR Safe DNA gel Stain	ThermoFisher Scientific	S33102
63	Tris Base	Fisher Scientific	CAS#77-86-1
64	Triton X-100	Sigma-Aldrich	9036-19-5
65	Tryptic soy agar	NutriSelect Plus, Sigma-Aldrich	22091
66	Tween-20	Sigma-Aldrich	CAS#9005-64-5
	D) Commercial Assays		
67	Antibiotic susceptibility discs	Mastring-s, Mast Group	M13/NCE; M14/NCE; M43/NCE
68	GeneJET PCR Purification Kit	Thermo Scientific	K0702
69	Purple 1 kb Plus DNA Ladder	Quick-Load, New England Biolabs	N0550G
	E) Specialised Equipment		
70	12-well plate (3.8 cm²; 6.9ml)	Falcon, Corning	353043
71	Autoclave	Astell Scientific	AMB430
72	Autoclavable bottle (for media preparation and bacteria culture)	Duran	GL45
73	Automated rocker	Stuart	SSL3
74	Bacteriological petri dish (100 x 15 mm)	Falcon, Corning	351029
75	Camcorder (for exploratory walking assay)	Sony	DCR-SR58
76	Centrifugation (>1000 ml)	Beckman Coulter	Avanti J-26S
77	Centrifugation (>50 ml)	Beckman Coulter	Allegra X-15R
78	Centrifuge tube	Costar, Corning	CAT#05-539-12

79	Class 2 Microbiological safety cabinet	Envair	N/A
80	Cotton Bungs	Genesee Scientific	CAT#51-101
81	Cotton Swabs	Technical Service Consultants	TS/8-A
82	Cryogenic vials	Fisher Scientific	377234
83	Disposable Bacteria Loops and Needles	Nunc, Thermo Scientific	CAT#251586
84	Dissection Tweezers (0.05 x 0.01mm tip)	Dumont	0209-55
85	Drosophila activity monitor (DAM) 2 and tubes	TriKinetics	trikinetics.com/
86	Drosophila bottles	BTP Drewitt	N/A
87	Drosophila Embryo Collection Cage	Flystuff	Flystuff 59-101
88	Drosophila vials	Regina Industries	reginaindustries.co.uk/
89	Fluorescence microscopy – Camera	Zeiss	Axiocam 512 Mono-Camera
90	Fluorescence microscopy – Fluorescence Source	Zeiss	CoolLED PE-4000
91	Fluorescence microscopy – Lens	Zeiss	1x, 2.3x Neufluar
92	Fluorescence microscopy – Stereo Microscope	Zeiss	Axiozoom V16 with
93	Light Microscopy - Camera	AmScope	MU1803
94	Light Microscopy - Len	Leica	0.5x apochromatic
95	Light Microscopy – Light Base	Leica	TL3000
96	Light Microscopy – Stereo Microscope	Leica	M80
97	Microcentrifugation (>1ml)	Eppendorf	5415R
98	Microscope glass cover slips	Scientific Laboratory Supplies	MIC3224
99	Microscope glass slides	Deltalab	D100004
100	Microvolume centrifuge tubes	Sarstedt	72.690.001
101	Microvolume spectrophotometer	Thermo Scientific	CAT#ND-2000
102	PCR Laminar Flow Cabinets	Air Science	PCR-24-A
103	Polymethyl methacrylate block (for exploratory walking assay)	Perspex	N/A
104	Purified water source	Milli-Q	IQ 7000
105	Serological pipette	Thermo Scientific	170358N
106	Serological pipette (for negative geotaxis assay)	Corning	4489
107	Shaker Incubator	Infors HT	Unitron AJ254
108	Stereo Microscope (Fluorescent) – 1x, 2.3x	Zeiss	Axiozoom V16
109	Stereo Microscope (Light) – 0.5x	Leica	M80 with TL3000 Light Base (1x)
110	Syringe (20ml)	Fisher Scientific	12044717
111	Syringe with hydrophilic PVDF filter membrane	Star Lab	CAT#780332
112	Thermal cycler	Techne	Prime
113	Track-etched membranes	Whatman, Cytiva Life Sciences	131135012F
114	Tube revolver rotator	Thermo Scientific	CAT#88881001
115	Visible range cuvettes	Kartell Labware	01938-00
116	Vortexer	Scientific Industries	SI-0266
F) Software and Algorithms			
117	AmScope	AmScope	Version 3.7
118	ClipChamp	Microsoft Corporation	N/A
119	DAMSystem3	TriKinetics	trikinetics.com/
120	Drososleep	Broughton Lab, Lancaster University	Version 2.35
121	EthoVision XT	Noldus	Version 8.5.614

122	Excel	Microsoft Corporation	Version 2108
123	Fiji - ImageJ	(141)	Version 2.5.0
124	Image Lab 4.0	Bio-Rad	Version 4.0
125	JMP	SAS Institute	Version 17
126	NanoDrop 2000	Thermo Scientific	Version 1.6
127	Nucleotide BLAST	National Institute of Health	blast.ncbi.nlm.nih.gov
128	ZEN Pro	ZEISS	Version 2.6

3.2. Recipe table

The recipes for the media and reagents used in this project are presented in Table 3 (for fly experimentation) and Table 4 (for bacteriology work). Detailed preparation protocols for these items can be found in the corresponding Methods sections.

Table 3) Recipes for reagents used in Drosophila media preparation and experimentation.

Protocol Type Fly Media Fly Cages Exploratory Walking Fly Dissection	Standard sugar yeast medium (SY)	Additive-free SY medium	Bacteria-treated SY medium	Dyed SY medium (2.5% wt/vol)	Apple juice plates (1.5% wt/vol)	Exploratory walking agar area (2% wt/vol)	Phosphate-buffered saline (PBS)	Nipagin solution (10% wt/vol)	Paraformaldehyde (4%)	Tris-NaCl-Triton buffer (TNT)	n-propyl gallate (2%)
Water (mL)	700	700	700	700	-	1000	-	-	-	-	-
Agar powder (g)	15	15	15	15	15	20	-	-	-	-	-
Granulated Sugar (g)	50	50	50	50	-	-	-	-	-	-	-
Brewer's Yeast (g)	100	100	100	100	-	-	-	-	-	-	-
Water to add on (mL)*	170	170	170	170	-	-	-	-	-	-	-
Nipagin Solution* (mL)	30	-	-	-	-	-	-	-	-	-	-
Propionic acid (mL)*	3	-	-	-	-	-	-	-	-	-	-
Bacteria inoculant (µL)	-	-	16.5	-	-	-	-	-	-	-	-
Brilliant Blue (g)*	-	-	-	25	-	-	-	-	-	-	-
Apple Juice (mL)	-	-	-	-	1000	-	-	-	-	-	-
Purified Water (mL)	-	-	-	-	-	-	1000	-	-	835	-
PBS tablets (n)	-	-	-	-	-	-	4	-	-	-	-
99% Ethanol (mL)	-	-	-	-	-	-	-	1000	-	-	-
Nipagin powder (g)	-	-	-	-	-	-	-	100	-	-	-
Paraformaldehyde (g)	-	-	-	-	-	-	-	-	40	-	-
PBS solution (mL)	-	-	-	-	-	-	-	-	1000	-	200
5M NaCl (mL)	-	-	-	-	-	-	-	-	-	60	-
Triton X-100 (mL)	-	-	-	-	-	-	-	-	-	5	-
1M Tris (pH 8.0) (mL)	-	-	-	-	-	-	-	-	-	100	-
Glycerol (mL)	-	-	-	-	-	-	-	-	-	-	800
n-propyl gallate (g)	-	-	-	-	-	-	-	-	-	-	20
Final Volume (mL)	~1000										
*Reagents added after sterilisation/heating and cooled to below 60°C											

Table 4) Recipe for reagents used in bacteria media preparation and experimentation.

Protocol Type: Bacteria Media (Lysogeny broth (LB) medium, LB agar plate, Tryptic soy agar plate, Diagnostic susceptibility agar plate); Bacterial Transformation (M9 buffer, Tris-Borate-EDTA buffer x10 pH 8.1, EtNa Extraction Fluid, Suspension fluid, LB Glycerol (15%), Agarose gel, IPTG aliquot); Antibiotic Preparation (Gentamicin Sulphate aliquot, Ampicillin aliquot); PCR (PCR reaction mixture (1 well))

	Lysogeny broth (LB) medium	LB agar plate	Tryptic soy agar plate	Diagnostic susceptibility agar plate	M9 buffer	Tris-Borate-EDTA buffer x10 pH 8.1	EtNa Extraction Fluid	Suspension fluid	LB Glycerol (15%)	Agarose gel	IPTG aliquot	Gentamicin Sulphate aliquot	Ampicillin aliquot	PCR reaction mixture (1 well)
Purified Water (ml)	1000	1000	1000	1000	1000	800	325	935	850	-	1	1	1	0.006
Respective media powder (g)	25	35	40	37.5	-	-	-	-	-	-	-	-	-	-
NaCl (g)	-	-	-	-	5	-	-	-	-	-	-	-	-	-
KH_2PO_4 (g)	-	-	-	-	3	-	-	-	-	-	-	-	-	-
Na_2HPO_4 Anhydrous (g)	-	-	-	-	2.6	-	-	-	-	-	-	-	-	-
1M $MgSO_4$* (mL)	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Tris Base (g)	-	-	-	-	-	108	-	-	-	-	-	-	-	-
Boric Acid (g)	-	-	-	-	-	55	-	-	-	-	-	-	-	-
EDTA powder (g)	-	-	-	-	-	7.4	-	-	-	-	-	-	-	-
0.5M EDTA solution (mL)	-	-	-	-	-	-	5.4	-	-	-	-	-	-	-
4M NaOH (mL)	-	-	-	-	-	-	60	-	-	-	-	-	-	-
99% Ethanol (mL)	-	-	-	-	-	-	610	0.04	-	-	-	-	-	-
50 mM Tris-HCl pH8 (mL)	-	-	-	-	-	-	-	50	-	-	-	-	-	-
Triton X-100 (mL)	-	-	-	-	-	-	-	10	-	-	-	-	-	-
Tween-20 (mL)	-	-	-	-	-	-	-	5	-	-	-	-	-	-
Glycerol (mL)	-	-	-	-	-	-	-	-	150	-	-	-	-	-
Agarose (g)	-	-	-	-	-	-	-	-	-	0.5	-	-	-	-
0.5X TBE (mL)	-	-	-	-	-	-	-	-	-	50	-	-	-	-
DNA gel stain (µL)*	-	-	-	-	-	-	-	-	-	5	-	-	-	-
IPTG (mg)	-	-	-	-	-	-	-	-	-	-	238	-	-	-
Gentamicin sulphate (mg)	-	-	-	-	-	-	-	-	-	-	-	60	-	-
Ampicillin sodium salt (mg)	-	-	-	-	-	-	-	-	-	-	-	-	100	-
Bacteria inoculant (µL)	-	-	-	-	-	-	-	-	-	-	-	-	-	2
16S-27F Primer (µL)	-	-	-	-	-	-	-	-	-	-	-	-	-	1
16S-1495R Primer (µL)	-	-	-	-	-	-	-	-	-	-	-	-	-	1
GoTaq PCR Mastermix (µL)	-	-	-	-	-	-	-	-	-	-	-	-	-	10
Total Volume (mL)	~1000									0.05	1			0.02

*Reagents added after sterilisation/heating and cooled to below 60°C

4. Methods

Before performing any of the laboratory techniques and protocols described below, all necessary training, risk assessments, and COSSH forms were completed.

4.1. Bacterium maintenance

Unless otherwise noted, all bacteriological work was carried out on laboratory benchtops, sanitised with 70% ethanol solution in proximity to an open flame.

4.1.1. Antibiotic preparation

All antibiotics were weighed and mixed with purified water (Milli-Q), vortexed, and then filtered through a polyvinylidene difluoride (PVDF) membrane syringe filter to sterilize the solution. The filtered solution was either used immediately or aliquoted into 1 mL portions and stored at -20°C for later use.

4.1.2. Bacterium media

All ingredients for the bacterial media were measured and combined in autoclave glass bottles (Duran) which were loosely capped and autoclaved at 121°C for at least 15 minutes. Autoclave tape was applied to the bottles to confirm sterilization. Upon removal from the autoclave, the caps were tightened, and the media was allowed to cool to 60°C. At this temperature, any appropriate

antibiotic aliquots (1 mL/L) were added to the media and mixed thoroughly. For agar media, the molten mix was poured from the bottles into petri dishes in volumes of 10 to 15 mL. All media were cooled to room temperature before use or stored at 4°C.

4.1.3. Bacterium culture

Bacterial cultures were streaked and maintained on agar media under conditions specified in Appendix 10.1 and stored lid down. For weekly maintenance, a single colony was transferred to fresh media using an inoculation loop to create an initial streak. The streak was then diluted four times by dragging it out with a new or re-sterilized inoculation loop between streaks.

4.1.4. Bacterium cryopreservation

To preserve bacterial cultures, bacteria were first grown on agar media for up to a week at room temperature to ensure that they were devoid of contamination and that there was sufficient material. Then, a cotton swab was used to collect the bacteria and resuspend it in 1 mL of LB glycerol (20%) in a cryogenic vial, snap-frozen in liquid nitrogen and stored at -80°C for future use.

4.2. Bacterium transformation

This section describes the processes of bacterial transformation and genotype confirmation through 16S DNA sequencing. Methods 4.2.1 and 4.2.8

were performed in a class 2 microbiology safety cabinet close to an open flame, while the remaining methods were conducted on laboratory benchtops that were sanitized with 70% ethanol solution next to an open flame. Unless specified, all bacterial incubation steps were carried out at 25°C.

4.2.1. Tn7-counterselection system

A detailed list of bacteria used in this study can be found in Appendix 10.2. Prior to performing triparental conjugation, $Bacteria^{Recipient}$, $Bacteria^{Donor}$, and $Bacteria^{Helper}$ were cultured overnight in 5mL of LB media, LB media with gentamicin, and LB media with ampicillin, respectively. $Bacteria^{Recipient}$ were incubated at 25°C, while $Bacteria^{Helper}$ and $Bacteria^{Donor}$ were incubated at 37°C. All bacteria cultures were aerated using a tube revolver rotator to prevent sedimentation. The following day, all bacteria were subcultured at a ratio of 1:50 ($Bacteria^{Helper}$ and $Bacteria^{Donor}$) or 1:100 ($Bacteria^{Recipient}$) into 5ml fresh media containing their respective antibiotics and incubated under the same conditions to promote outgrowth.

Once grown to an OD_{600} between 0.4 to 0.6 (and confirmed through spectrophotometry), all subcultured bacteria are mixed 1:1:1 in a tube with at least 500 µL of each bacterium. To remove any antibiotics from the mixture, the mixture is centrifuged at 7000 g and washed with 1 mL of M9 buffer solution. This step is repeated once more, but the mixture is resuspended with 25 µL of M9 buffer solution the second time. The mixture is then thoroughly mixed and pipetted onto a sterile 0.45 µm filter disc placed on a TSA plate. Once dried, the plate is incubated at 25°C for at least 5 hours to allow bacteria mating.

To initiate kill-switch counterselection, the filter disc is washed with 1 mL of M9 buffer solution. The resuspended bacteria are then spread onto TSA plates containing both gentamicin and IPTG. Two plates are prepared with the first plate contains 100 µL of the original bacteria resuspension, while the second plate is inoculated at a 10-fold higher concentration. After the inoculant has dried, the new plates are incubated under the same conditions as before.

Once bacterial growth is apparent, the plates are screened for fluorescence under a fluorescence microscope (AxioZoomV16, Zeiss). Colonies that show uniform fluorescence expressions are then streaked onto TSA plates (without antibiotic treatment) with the same incubation conditions to confirm proper integration of the fluorescence genome. Transformed bacteria with stable fluorescence expression confirmed through fluorescence microscopy proceed for sequencing and antibiotic susceptibility testing.

4.2.2. DNA extraction

In an Eppendorf tube, 100 µL of bacteria were mixed with 450 µL of EtNa extraction fluid and heated at 80°C for 10 minutes. The tube is then centrifuged at 16,060 g for 10 minutes at 4°C. Once a pellet forms, the supernatant was discarded, and the pellet is resuspended in DNA suspension fluid.

4.2.3. DNA amplification

DNA amplification was prepared using the 16S-27F and 16s-1495R primers (Appendix 10.3) and GoTaq Green Mastermix (Promega) at appropriate volumes and run on a thermal cycler under specific parameters (Appendix 10.4).

A negative control was included in each polymerase chain reaction (PCR), in which extracted DNA was replaced with purified water.

4.2.4. Gel electrophoresis

To determine the presence of correct amplicons in amplified DNA, an agarose gel was prepared by mixing agarose powder into an Erlenmeyer flask and heating the mixture in a microwave. The flask was removed and swirled every 30 seconds to ensure proper mixing. Once the powder was fully dissolved, a DNA gel stain was added to the mixture and swirled. The mixture was then poured into a gel cast and a comb was attached to form wells. The solid gel was placed in an electrophoresis chamber and filled with Tris-Borate-EDTA (TBE) buffer until it covered the top of the gel. Each well contained 5 µL of either amplified DNA, negative control reagent, or a 1 kb Plus DNA ladder for molecular weight referencing. All gels ran for 40 minutes at 120 volts before being analysed using Image Lab (Bio-Rad).

4.2.5. DNA purification

Amplified DNA was purified using the GeneJET PCR Purification Kit (Thermo Scientific) according to the manufacturer's protocol (Ref: K0702). The purified DNA sample was then assessed using gel electrophoresis, prepared as described above. However, each well contained 1.8 µL of purified DNA (or negative control reagent) mixed with 4.2 µL of loading dye. DNA purity and concentration were confirmed using microvolume spectrophotometry.

4.2.6. 16S bacterial DNA Sanger Sequencing

As Sanger sequencing is outsourced to an external service provider (Source BioScience, Cambridge), DNA and primers used in DNA amplification were prepared and shipped according to the provider's instructions.

4.2.7. Analysis of bacterium sequences

Both forward and reverse bacterial sequences were analysed using Nucleotide BLAST (National Institute of Health) to identify bacterial classification (142). Successful bacterial transformation was concluded when the taxonomical classification of forward and reverse sequences matched the top 3 BLAST results with more than 90% identical sequences and a close to zero expectation value.

4.2.8. Antibiotic susceptibility test

Bacteria grown on solid media were resuspended in 2 mL of purified water and collected into a tube. The mixture was vortexed, and 1 mL was spread onto diagnostic sensitivity test (DST) agar. Antibiotic rings (Appendix 10.5) were applied to the bacteria-spread plates using a tweezer that was sanitized with ethanol and flamed between uses. The rings were gently pressed down to ensure contact with the bacteria. The plates were allowed to incubate for at least one day before being inspected. Antibiotic susceptibility was determined by examining the antibiotic's zone of inhibition. This test was also repeated using untransformed bacteria to confirm the acquisition of gentamicin resistance and compare antibiotic resistance profiles.

4.3. Bacteria concentrate preparation

Before concentrating bacteria, they were grown to saturation (OD_{600} ~1) in gentamicin (60 mg/L) LB media in a shaker incubator at 25°C. Once the optical density was measured by spectrophotometry, the bacteria and media were poured into 1 L centrifuge bottles and balanced by weight (±1 g) using purified water (MilliQ). The bacteria were then centrifuged at 3000 g for 15 minutes at 20°C. The supernatant was decanted, and the bacteria were washed with plain LB and concentrated to an OD_{600} of 100. The concentrated bacteria were stored at 4°C and used within two weeks.

4.4. Fly maintenance

A list of all flies used in this study was listed on (Appendix 10.6). All stocks had been backcrossed to the white^{Dahomey} (w^{Dah}) outbred background flies at least 6 generations before experimentation. All flies were maintained at 25°C on a 12:12 hour light/dark cycle at constant humidity. Stock flies were fed standard sugar and yeast (SY) medium and reared at standard larval density (140,143). Flies were transferred onto fresh bottles every month.

4.4.1. Fly media

All fly media, except for additive-free media, were prepared on an electric hob. Water was brought to a boil in a cooking pot and then reduced to medium heat. Agar powder was added while stirring continuously, and the mixture was allowed to boil again. Brewer's yeast and granulated sugar were added to the mixture and stirred until it reached an agar-like consistency. The mixture was

then cooled to 60°C and additives such as nipagin and propionic acid were stirred in. Once the media was thoroughly mixed, it was added to vials (~3 mL) or bottles (~70 mL) and allowed to set at room temperature for at least 4 hours before being stored at 17°C.

Additive-free SY food was prepared by omitting nipagin and propionic acid from the standard recipe and autoclaving the mixture under the same conditions as bacteria media preparation. The mixture was dispensed into vials (~3 mL) under sterile conditions (either by an open flame in a fumehood) and stored at 17°C for use within 2-3 days.

4.4.2. Bacterium inoculation of fly media

Prior to bacteria inoculation, bacteria cultures were vortexed to mix the bacteria evenly. In a sterile environment, either in a fume hood or PCR laminar flow cabinet, 50 μL of the bacterial culture were dispensed onto the surface of the fly media, apart from one another. The vials were allowed to dry for at least 2 hours in a class 2 microbiology safety cabinet or 4 hours in a PCR laminar flow cabinet before being stored at 17°C.

4.4.3. Experimental fly generation

Virgin female (collected upon eclosion) and male flies of the appropriate genotypes were collected by CO_2 anaesthesia and kept in standard SY media vials for 3-4 days. Crosses (Appendix 10.7) were set up in Drosophila embryo collection cages equipped with apple juice agar plates containing a paste made of dry active yeast and water. These cages were incubated at 25°C in the dark to

promote egg laying. Every 3 days, the flies were transferred to fresh apple juice agar plates prepared similarly as mentioned. Using Phosphate Buffered Saline (PBS), eggs were washed into tubes and allowed to settle to the bottom. The eggs were transferred using a widened 200 μL micropipette tip, and approximately 500 eggs were placed in each standard SY bottle. The bottles were incubated at 25°C until eclosion (~10 days). Within one day after eclosion, the flies were transferred to fresh bottles to synchronize their age. Unsorted flies were allowed to mate at ages 1-2 as mating status can affect lifespan and fitness (144). At ages 3-4, the flies were anaesthetized and sorted into vials containing their respective fly media with or without bacteria inoculum in groups of 10, separated by sex and genotype.

4.5. Assessing suitability and optimal conditions for bacterium treatment on flies

Pilot studies were conducted to assess the viability of bacteria on fly media and identify the most suitable conditions for inoculating flies with bacteria.

4.5.1. Viability of bacteria on fly media

Different types of SY standard media were prepared using an autoclave, varying on the addition nipagin or propionic acid. The media was then dispensed (2 mL) into 12-well plates near an open flame and allowed to cool to 60°C. Each condition included three technical replicates. Once the agar had set, each plate was inoculated with single colonies of stock bacteria (Methodologies 4.3) and

incubated the plates at 25°C. The plates were monitored and imaged for bacteria growth every 24 hours using the Zeiss AxioZoom V16 for a period of 4 days.

4.5.2. Assessing bacterium colonisation of fly gut

The d2-3GAL4/+ W^{dah} fly strain was selected and tested with three pre-selected bacteria (MYb71-sfGFP, MYb174-dTomato, and CEent1-mPlum) that were prepared as concentrates (Methodology 4.3). At age 3, the flies were transferred into vials treated with selected bacteria, which were prepared similarly to Methodology 4.4.2. Here, the vials contained either a single bacterial species, paired combinations, all three bacterial species, or no bacteria. The inoculation load was 100 µL divided equally between each bacteria combination. The flies were only exposed to bacteria once at age 3 and were subsequently maintained on regular media without bacteria from age 5 days onwards as described in Methodology 4.6. At age 10 days, fly guts were dissected and examined using fluorescence microscopy, as described in Methodology 4.6.7 and 4.7.

4.5.3. Identifying optimal bacterium combination and inoculation volume

To determine which bacteria combination had the greatest impact on lifespan, a survival assay (Methodology 4.6.1) was conducted using the same cohort of flies from Methodology 4.5.2 upon completing the gut dissection assay.

The experimental condition that resulted in the highest survivability was selected for further experimentation.

4.6. Fly experimentation

Unless stated otherwise, all assays were conducted on flies around age 10 and repeated every 10 days at 25°C. The experimental flies were maintained on additive-free standard SY vials (10 flies per vial) and transferred to fresh media every two days. The bacteria treatment of the flies began at age 3. The vials were checked daily for mould and replaced immediately and again a day later to reduce mould concentration. Each experiment (except for the survival assay) included at least 15 flies per experimental condition, genotype, and sex. Apart from the exploratory walking and gut permeability assay, the flies used in the other experimental groups were collected and considered for reuse in future experimental repeats.

4.6.1. Survival assay

Lifespan was measured by counting the number of dead flies every two days. Flies that escaped or died of unnatural causes were censored from the data. The total number of dead flies was recorded in a spreadsheet (Excel).

4.6.2. Exploratory walking assay

This assay utilized a Polymethyl methacrylate block (Perspex) containing four circular arenas (3.5 cm diameter and 1 cm high) with an agar base. Flies

were placed into the arenas and vertically recorded using a camcorder (Handycam DCR-SR58, Sony) for 15 minutes. Each arena in the same block contained a fly with a different genotype, and the agar base was replaced before the block was reused. The videos were analysed using Ethovision XT video tracking software (Noldus). From the recordings, the arenas were virtually divided into two circular zones: a central zone with a 2 cm diameter ring in the centre, and an outer zone covering the entire arena. A video collage showing the flies' walking behaviour was created using ClipChamp (Microsoft Corporation).

4.6.3. Negative geotaxis assay

Flies were divided into groups of 15 of various sexes and treatment types. After being anesthetised with CO_2, the flies were placed on a climbing apparatus and allowed at least 30 minutes to recover as high CO_2 exposure temporarily impairs *Drosophila* flight and climbing abilities (145). The climbing apparatus was constructed using a 25 ml serological pipette (34.4 cm x 1.3 cm) (Costar, Corning) with the tip replaced with cotton. The flies were sharply tapped to the bottom of the column, and the number of flies that climbed above 9.5 cm within 45 seconds was counted. The experiment included three technical replicates. A fly performance index was calculated using the following formula:

$$Performance\ Index = \frac{Flies^{Total} + Flies^{Top} - Flies^{Bottom}}{2 \times Flies^{Total}}$$

4.6.4. Sleep assay

Anesthetised flies were individually placed into 6.5 cm x 0.5 cm glass tubes (TriKinetics) containing standard SY media. The tubes were placed in a DAM System monitor (TriKinetics) to monitor their locomotor activity. The assay was conducted on a 12:12 light/dark cycle at 25°C. Flies were considered asleep if they had been inactive for 5 minutes (146). At least three days of data were collected during each assay, and the raw data was processed using DrosoSleep (Broughton Lab). Results from flies that died during the sleep assay were omitted from the study.

4.6.5. Gut permeability assay

Also known as the 'Smurf' Assay, 15 flies were transferred onto dyed SY media and maintained for at least 9 hours. Disruption of fly GI track was indicated by presence of dye coloration outside the GI tract.

4.6.6. Egg and offspring quantification assay

During each vial transfer, 10 vials were sampled and imaged using a camera-equipped microscope (M80 stereo microscope, Leica). The number of eggs in each vial was counted using Fiji (ImageJ). To quantify the offspring, the imaged vials were stored at 25°C to allow larval development, and the offspring were counted upon fly eclosion.

4.6.7. Fly gut dissection, fixing and mounting

Anesthetised flies were first submerged in 99% ethanol for 30s and then dissected using dissection tweezers (Dumont) on a dissection dish filled with 1x PBS solution. Dissected samples consist of an intact intestinal tract stretching from foregut to hindgut with the crop attached. Fresh samples were temporarily stored in a microcentrifuge tube containing 1ml 1x PBS, chilled with ice. At least 6 guts were collected per experimental condition and sex. Once all necessarily guts were collected, samples were fixed in 4% paraformaldehyde for 20 minutes then washed with 1x PBS. Then samples were washed with TNT solution followed by another PBS wash. All fixes and washes were facilitated with gentle shaking and each washing stage was repeat at least three times. All washed samples were mounted using 2% n-prop gallate and stored at 4°C under dark.

4.7. Fluorescence microscopy

All fluorescent bacteria and fly gut samples were visualised using the Zeiss AxioZoom.V16 under their appropriate excitation range (Appendix 10.8). Images were analysed and processed using Fiji (ImageJ).

4.8. Statistical analysis

Analysed data was presented with means ± standard error (SE). Before statistical analysis, the studentized residuals of all results (except for survival assay data) were calculated and tested for normality using a Shapiro–Wilk test (147). Any skewed data was log transformed to create a normal distribution. Survival data was analysed using the Log Rank test (148). All other data was fitted into a Generalized Linear Model and tested with an effect test. If there

were significant interactions between all test variables, a Tukey-Kramer Honestly Significant Difference (HSD) test was followed up (149,150). Transformed data that remained skewed were analysed using the Median Test instead (151). Any $P<0.05$ was considered statistically significant (JMP, SAS Institute).

5. Results

This section presents the findings from the pilot studies and main experiments as outlined in Project Background 2.7 above. The first half of the results (from Results 5.1 to 5.4) details the viability of bacteria on fly media, generation of new fluorescent bacteria, and colonisability of bacteria in the fly gut. Together, these results validated the project's feasibility and helped optimise the conditions of the main experiments. Subsequently, the latter half of this section (from Results 5.5 to 5.7) reports the impact of fluorescently transformed bacteria (namely MYb71-sfGFP, MYb174-dTomato, and CEent1-mPlum) in flies with and without impaired IIS across life. Several fly ageing phenotypes were assessed which include lifespan, exploratory walking behaviour, gut permeability, sleep, neuromuscular function, and fecundity (represented by egg and offspring counts).

5.1. Nipagin and propionic acid inhibited bacterium growth

While nipagin and propionic acid are well-known preservatives of fly media, it was unknown if these additives could affect the viability of the experimental bacteria, and potentially restrict the bacterial strains we could use. Thus, 16 isolates (Table 5, Appendix 10.2) from the extended CeMbio bacterium collection were evaluated for growth and viability on various fly media. Upon microscopic imaging and screening for bacterial growth, 11 out of the 16 tested bacteria grew on non-additive fly media. The bacteria that showed no growth were for MYb57, MYb69, MYb174, MYb186, and MYb396. Strikingly, none of the bacteria showed growth in media containing nipagin and propionic acid. As such, it was postulated that these additives could negatively impact bacteria viability

and impact gut colonisation in flies. Therefore, nipagin and propionic acid were excluded from fly media from all subsequent experiments. This omission may potentially impact fly ageing and lifespan (Discussion 6.5) due to differences from standard media conditions used in the Broughton lab.

Table 5) Viability of bacteria on fly media with or without additives.

No	Bacteria	No Additive				Nipagin				Propionic Acid				Nipagin + Propionic Acid			
		24h	48h	72h	96h	24h	48h	72h	96h	24h	48h	72h	96h	24h	48h	72h	96h
1	BIGb0170	-	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-
2	BIGb0172	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-
3	BIGb0393	-	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-
4	JUb19	-	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-
5	JUb44	-	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-
6	MYb16	-	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-
7	MYb21	-	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-
8	MYb49	-	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-
9	MYb57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	MYb69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	MYb71	-	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-
12	MYb115	✓	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-
13	MYb174	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	MYb186	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	MYb330	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-
16	MYb396	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ticks (✓) indicate bacterial growth was observed while dashes (-) imply no bacterial growth was found																	

5.2. Generation of 6 new fluorescent bacteria

To visualise and track the growth of bacteria *in vivo*, bacteria would need to be transformed to express fluorescence. At least 24 transformations were conducted and 6 new fluorescent bacteria (MYb71-sfGFP, MYb186-sfGFP, MYb11-sfGFP, MYb11-dTomato, MYb11-mPlum, and BIGb0393-mPlum) were successfully generated (Table 6, Appendix 10.9). Using Nucleotide BLAST (NCBI),

these 6 transformed bacteria resembled more than 90% the sequences of similarly taxonomised bacteria found from BLAST results or the recipient bacteria's pre-transformed genome. As important, the fluorescent protein encoding genes were successfully integrated into the 6 transformed bacteria as demonstrated by stable fluorescent expression observed under fluorescent microscopy (Table 7).

As expected, all transformed bacteria acquired gentamicin resistance, but also exhibited changes in their susceptibility or resistance to other antibiotics (Table 8). The most acquired resistance among the transformed bacteria was Erythromycin resistance, present in all three MYB11 bacteria and MYb71-sfGFP. Additionally, most bacteria developed resistance against multiple antibiotics notably with the addition of Streptomycin resistance found in MYb11-dTomato and MYb11-mPlum. Other observed antibiotic resistances included Chloramphenicol (MYb71-sfGFP), Trimethoprim (MYb186-sfGFP), Tetracycline (MYb11-mPlum), Sulphatriad (BIGb0393-mPlum), and Cotrimoxazole (BIGb0393-mPlum). While all transformed bacteria developed some form of antibiotic resistance, some also lost their resistance towards antibiotics previously unsusceptible. Here, Myb71-sfGFP and BIGb0393-mPlum lost resistant against Oxacillin and Streptomycin respectively, alluding to a collateral sensitivity effect occurring during transformation. Another unexpected observation was that MYb11-mPlum and MYb11-dTomato developed tetracycline and streptomycin resistance under M14/NCE antibiotic strip, but not under M13/NCE antibiotic strip (Appendix 10.10). This discrepancy could be due to improper administration of antibiotic strips.

Among the 18 unsuccessful transformations, 6 fluorescent bacteria were produced but were either identified as *Escherichia sp.* or had improper

fluorescent protein encoding gene integration exhibited by unstable fluorescence expression observed under fluorescence microscopy (not reported here). As for the remaining 12 bacteria, no fluorescent clones were generated. Ultimately, all properly transformed bacteria were cryopreserved at -80°C for future use while unsuccessfully transformed bacteria were discarded. Regrettably, gel electrophoresis images of bacteria DNA were not recorded during DNA extraction.

Table 6) List of all bacteria transformation carried out

No	Identifier	Strain Taxonomy	Fluorophore	Fluorescent Clone Generation	Stable Fluorescence Expression	Sequencing Match	Forward Sequence			Reverse Sequence		
							Sequence Aligned	E Value	% Identical	Sequence Aligned	E Value	% Identical
1	MYb71	*Ochrobactrum vermis*	sfGFP	Yes	Yes	Yes	*Ochrobactrum sp.*	0	95.74	*Ochrobactrum sp.*	0	99.91
2	MYb186	*Enterobacter sp.*	sfGFP	Yes	Yes	Yes	*Enterobacter sp.*	0	90.63	*Enterobacter sp.*	0	99.33
3	MYb11	*Pseudomonas lurida*	sfGFP	Yes	Yes	Yes	*Pseudomonas sp.*	0	91.65	*Pseudomonas sp.* WS01	0	99.773
4	MYb11	*Pseudomonas lurida*	dTomato	Yes	Yes	Yes	*Pseudomonas lurida*	0	99.53	*Pseudomonas lurida*	0	99.54
5	MYb11	*Pseudomonas lurida*	mPlum	Yes	Yes	Yes	*Pseudomonas sp.*	0	100	*Pseudomonas sp.*	0	99.6
6	BIGb0393	*Pantoea nemavictus*	mPlum	Yes	Yes	Yes*	*Enterobacter hormaechei*	0	98.33	*Enterobacter hormaechei*	0	96.78
7	MYb21	*Comamonas sp. B-9*	dTomato	Yes	Yes	No	*Escherichia coli*	0	95.37	*Escherichia coli*	0	98.55
8	MYb69	*Comamonas sp. TK41*	mPlum	Yes	Yes	No	*Escherichia fergusonii*	0	97.15	*Escherichia coli*	0	97.49
9	MYb21	*Comamonas sp. B-9*	mPlum	Yes	Yes	No	*Escherichia coli*	0	99.24	*Escherichia coli*	0	95.37
10	BIGb0172	*Comamonas piscis*	mPlum	Yes	Yes	No	*Escherichia fergusonii*	0	98.3	*Escherichia coli*	0	94.48
11	MYb69	*Comamonas sp. TK41*	sfGFP	Yes	No							
12	BIGb0172	*Comamonas piscis*	dTomato	Yes	No							
13	MYb21	*Comamonas sp. B-9*	sfGFP	No								
14	MYb10	*Acinetobacter guillouiae*	sfGFP	No								
15	BIGb0393	*Pantoea nemavictus*	sfGFP	No								
16	BIGb0172	*Comamonas piscis*	sfGFP	No								
17	MYb71	*Ochrobactrum vermis*	dTomato	No								
18	MYb69	*Comamonas sp. TK41*	dTomato	No								
19	MYb186	*Enterobacter sp.*	dTomato	No								
20	MYb10	*Acinetobacter guillouiae*	dTomato	No								
21	BIGb0393	*Pantoea nemavictus*	dTomato	No								
22	MYb71	*Ochrobactrum vermis*	mPlum	No								
23	MYb186	*Enterobacter sp.*	mPlum	No								
24	MYb10	*Acinetobacter guillouiae*	mPlum	No								

E Value: Expectation Value (A measure of how likely two sequences match by chance alone)
*BIGb03930-mPlum's sequence was confirmed upon further comparison with its pre-transformed genome (Appendix 10.2) reporting highly identical forward (95.75%) and reverse (98.45%) sequences

Table 7) Fluorescent imaging of bacteria generated in this study and previously transformed

	Bacteria generated in this study						Previously transformed bacteria	
	MYb71-sfGFP	**MYb186-sfGFP***	**MYb11-sfGFP**	**Myb11-dTomato**	**Myb11-mPlum**	**BIGb0393-mPlum**	**Myb174-dTomato**	**Ceent1-mPlum**
Brightfield								
Fluorescent								
Merged								

*Further isolation is required for Myb186-sfGFP due to contaminants present

Table 8) A comparison of antibiotic susceptibility profiles between transformed bacteria and stock bacteria

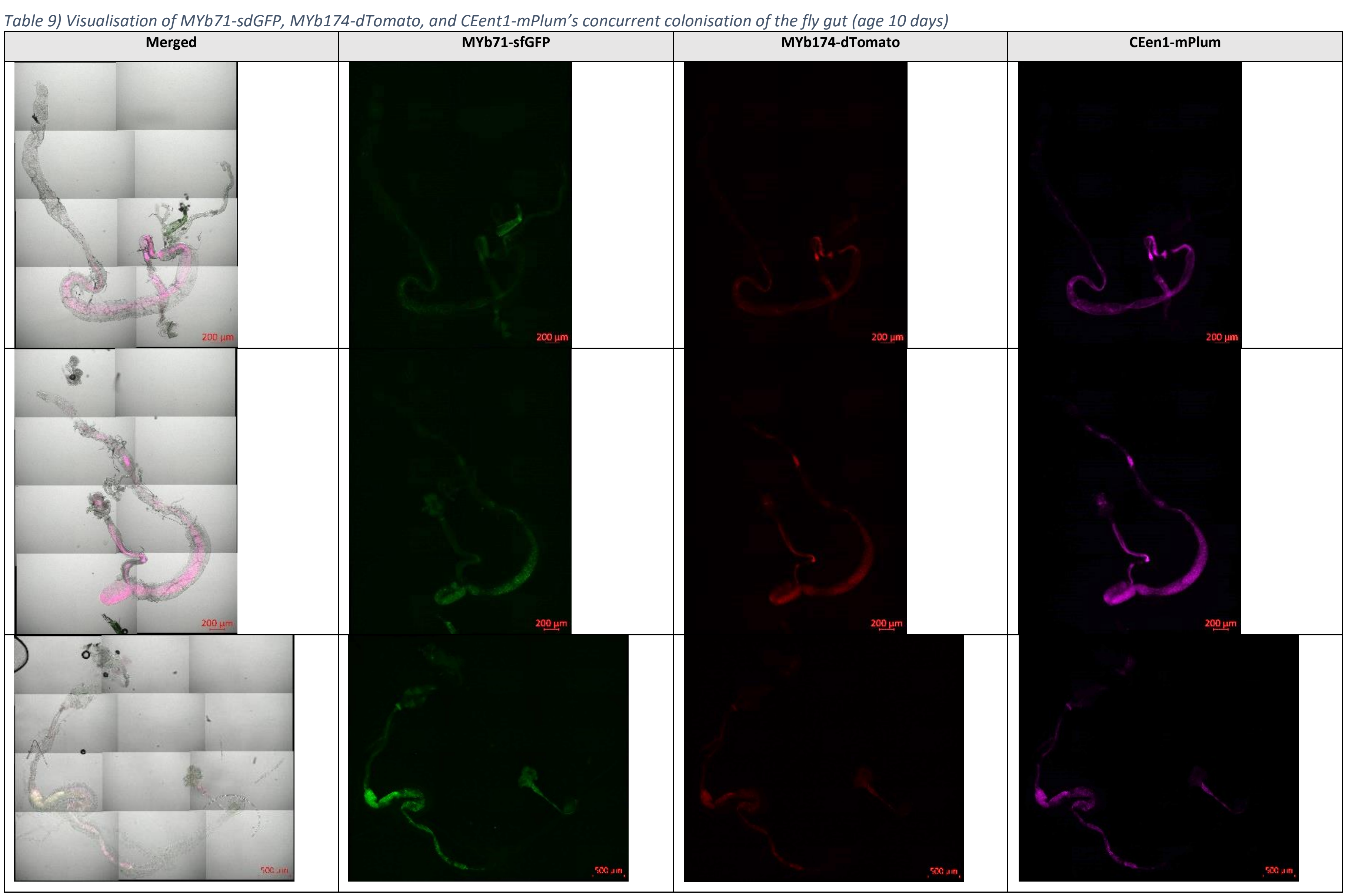
No	Bacteria Strain	Number of Matching Antibiotic Susceptibility Profiles			Gentamicin Resistance Acquisition	Other Resistance Gained	Other Resistance Lost
		M13/NCE	**M14/NCE (excluding gentamicin)**	**M43/NCE (excluding gentamicin)**			
1	MYb71-sfGFP	5/8	7/7	7/7	Yes	• Chloramphenicol • Erythromycin	• Oxacillin
2	MYb186-sfGFP	8/8	7/7	6/7	Yes	• Trimethoprim	-
3	Myb11-sfGFP	7/8	7/7	7/7	Yes	• Erythromycin,	-
4	MYb11-dTomato	7/8	6/7	7/7	Yes	• Erythromycin • Streptomycin	-
5	MYb11-mPlum	6/8	5/7	7/7	Yes	• Erythromycin • Streptomycin • Tetracycline	-
6	BIGb0393-mPlum	8/8	5/7	7/7	Yes	• Sulphatriad • Cotrimoxazole	• Streptomycin
Each bacteria strip contains 8 different antibiotics (Appendix 10.5, Appendix 10.10)							

5.3. Bacteria successfully colonised the fly gut

It was unknown if any of the bacteria from the CeMbio consortium could colonise the fly gut. Therefore, a follow up pilot experiment was carried out introducing these bacteria to flies in various combinations. To verify bacteria colonisation, the bacteria must persist within the host for at least 5 days post-inoculation. Here, the test bacteria included the newly generated MYb71-sfGFP and two previously transformed bacteria which are MYb174-dTomato, and CEent1-mPlum. While Results 5.1 showed that MYb174 could not grow on fly media (Appendix 10.11), MYb174-dTomato was used in this pilot to verify the findings.

Following a single exposure to bacteria, fluorescence microscopy revealed that all three selected bacteria persisted the fly gut at age 10 days, regardless of the combination of bacteria administered (Table 9). However, bacteria quantification was not carried out and therefore could not determine the colonisation patterns of tested bacteria. Regardless, this pilot validated the feasibility of introducing bacteria from the CeMbio collection to flies. Additionally, it was suspected that the inoculum load may be excessive, hence the inoculation volume was reduced from 100 µL to 50 µL for the rest of the project. Overall, these findings contradict Result 5.1 as MYb174-dTomato was able to colonise the fly gut despite not showing growth on fly media. Therefore, it was speculated that the bacteria were induced into a dormant state at some point during concentrate preparation or fly media inoculation. As the project did not further investigate the inoculation of bacteria using additive media, nipagin and propionic acid remained omitted from fly media throughout the study.

Table 9) Visualisation of MYb71-sdGFP, MYb174-dTomato, and CEent1-mPlum's concurrent colonisation of the fly gut (age 10 days)



5.4. MYb71-sfGFP, MYb174-dTomato, and CEent1-mPlum chosen as experiment bacteria

While selecting experimental bacteria, options were limited to MYb71-sfGFP and previously transformed bacteria (not reported here) available from the Benedetto lab. Unexpectedly, MYb186-sfGFP was found contaminated, requiring re-isolation, and thus was not considered for experimentation. Likewise, all MYb11 bacteria was excluded as it may negatively affect host lifespan and increase host susceptibility to toxins (152). Moreover, BIGb0393-mPlum was not selected due to delays acquiring its sequence at the time. Therefore, MYb71-sfGFP, MYb174-dTomato, and CEent1-mPlum were selected as the experimental bacteria in this study due to time constraints and unforeseen delays.

Using flies from the gut colonisation assay, a pilot survival assay was conducted concurrently to scope for any early effects on fly health and identify the best bacteria inoculation combination. The optimal combination was decided when the pilot flies were 25 days old. At the time, the fly group exposed to all three bacteria (MYb71-sfGFP, MYb174-dTomato, and CEent1-mPlum) reported the least number of deaths compared to the rest of the experimental groups (Figure 3). However, the pilot lifespan study later found no significant differences in fly lifespan between any of the bacteria groups and the control. Notably, this may be a favourable outcome as *C. elegans* exposed to the same bacteria yielded shorter lifespans as previously discovered in the Benedetto lab (not reported here). Under the perceived impression that MYb71-sfGFP, MYb174-dTomato, and CEent1-mPlum together may provide the best fly health

outcome, the combination of all three bacteria was chosen as the experimental condition for the main study.

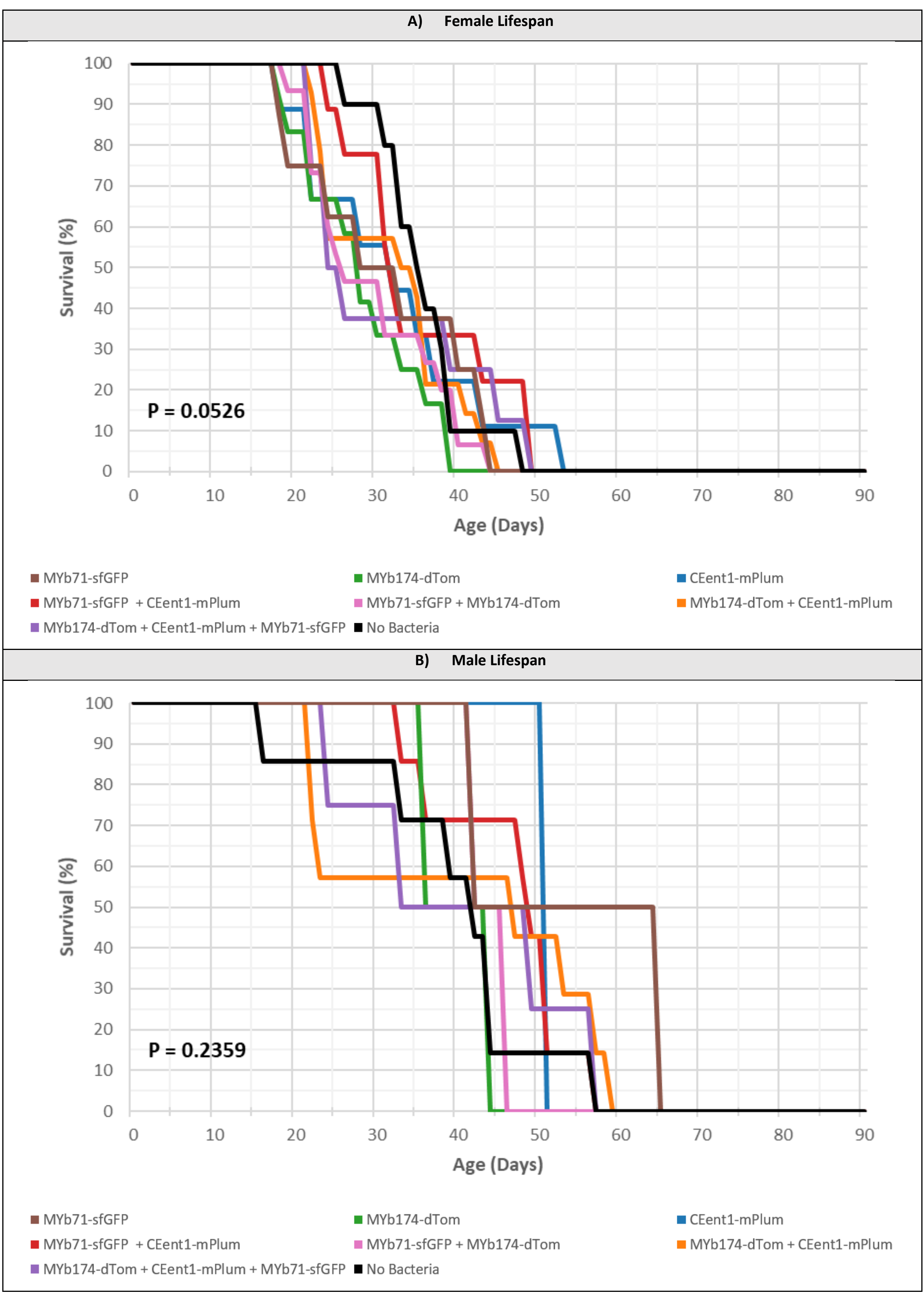


Figure 3) Pilot lifespan of female and male d2-3GAL4/+ flies (n = 2-15) treated with various combinations of bacteria (data prior to age 15 days unavailable).

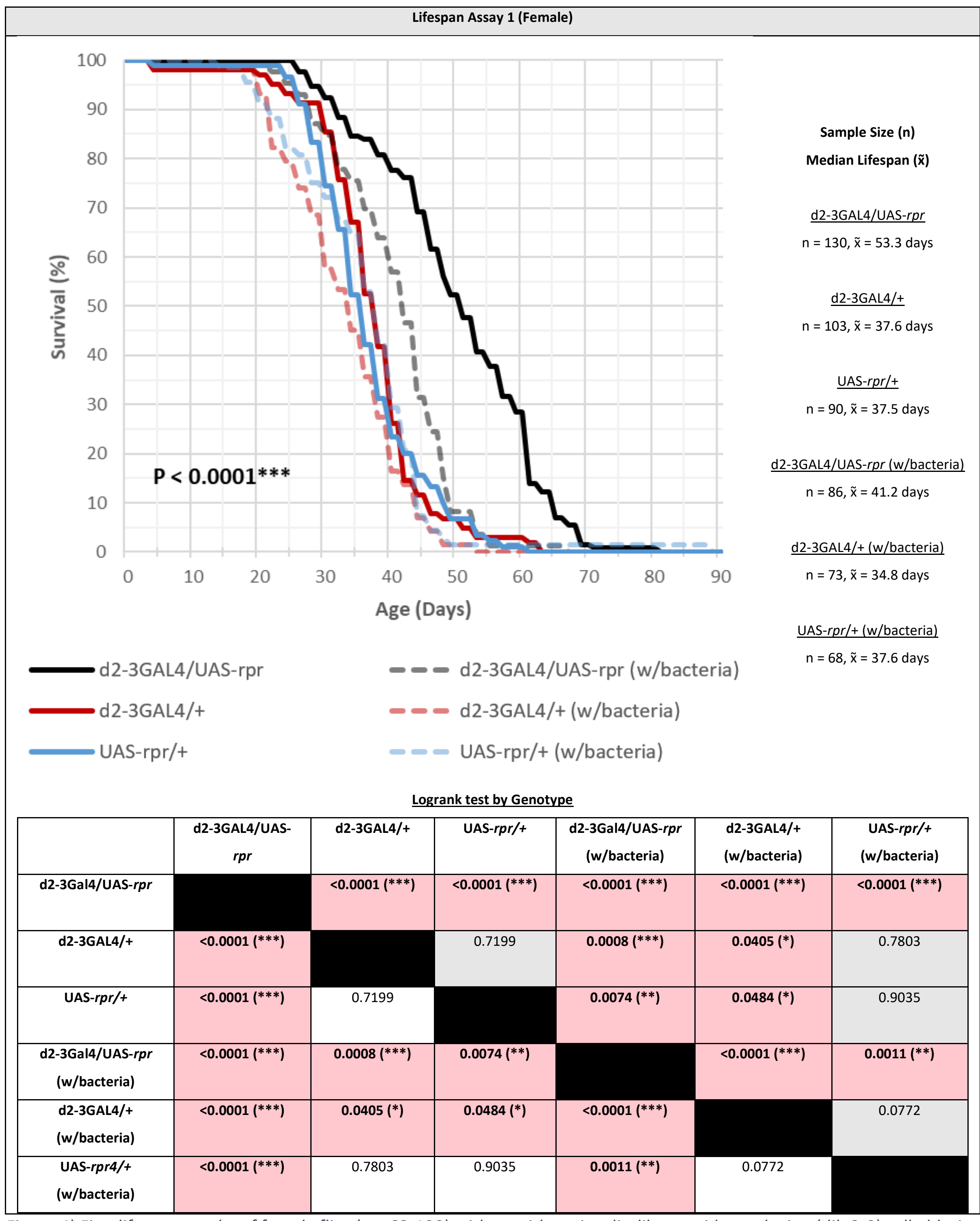
5.5. Bacteria attenuated lifespan extension and exploratory walking behaviour changes in IPC ablated flies

After the optimal fly food media and bacteria inoculant combination was determined, the combined effects of MYb71-sfGFP, MYb174-dTomato, and CEent1-mPlum against fly lifespan, exploratory walking behaviour, and gut permeability were first investigated. Flies with normal and systemic IIS reduction (d2-3GAL4/UAS-*rpr*) were used as experimental groups to assess for bacterial interactions with IIS.

5.5.1. Bacteria attenuated the lifespan-extending effects of IPC ablation

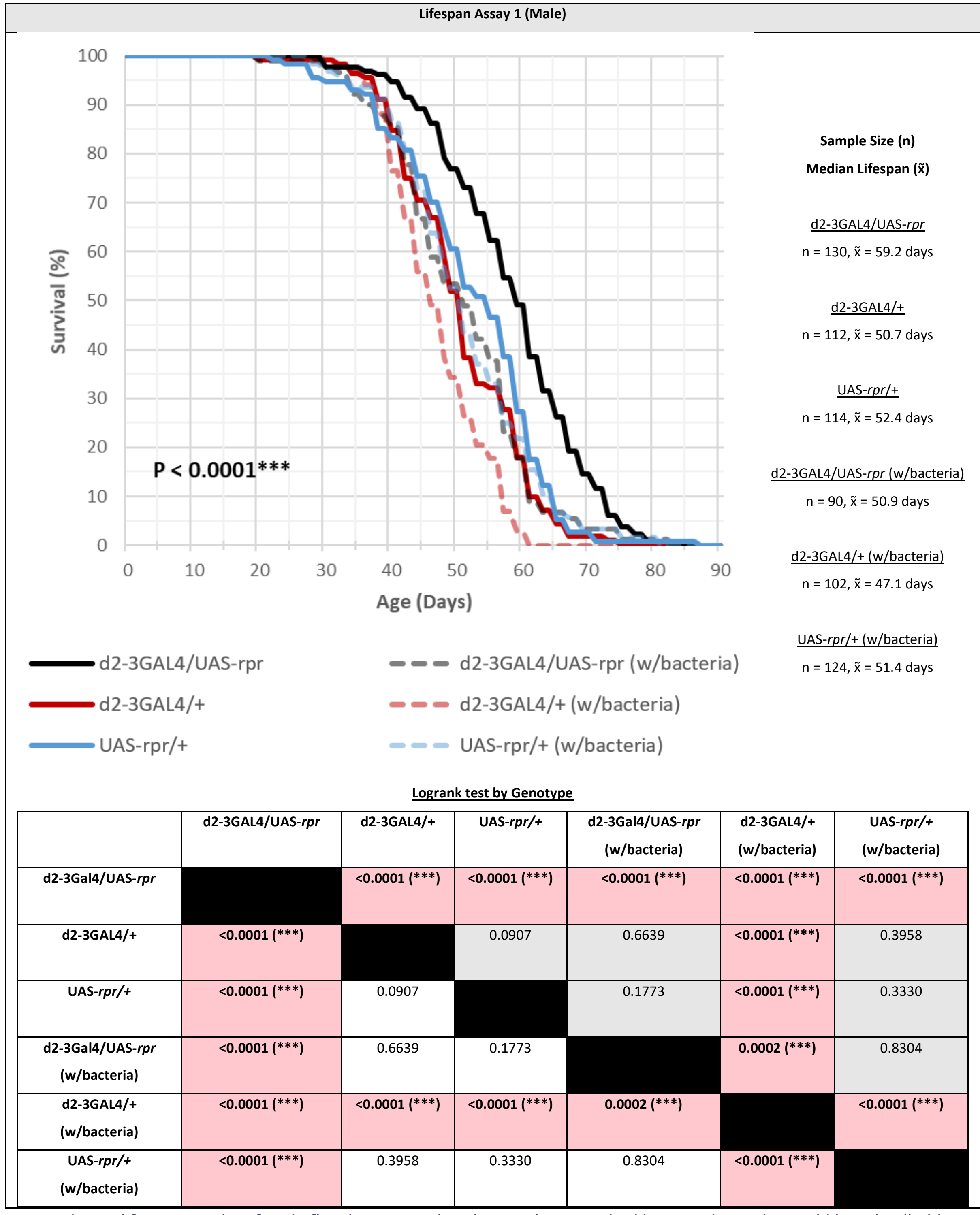
As mentioned, the survival assay monitors the average lifespan of the flies over time allowing comparisons of median lifespan across different experimental groups. From previous studies, systemic IIS reduction increases the median lifespan of flies regardless of sex (140). As anticipated, flies with systemic IIS reduction achieved the longest median lifespan compared to controls among both females (≥29% longer) (Figure 4) and males (≥12% longer) (Figure 5). When treated with bacteria, median lifespan extension was 22% shorter among female bacteria treated d2-3GAL4/UAS-*rpr* flies compared to their non-bacteria treated counterparts. Conversely, no median lifespan extension was observed in systemic IIS reduced males. Between bacteria treated controls, no significant differences in median lifespan were found among flies except for male d2-3GAL4/+ flies, which has a 7% shorter median lifespan than

UAS-*rpr*/+ flies. Altogether, bacteria attenuated the lifespan-extending effects of systemic IIS reduction while further reducing median lifespan on specific fly genotypes.



	d2-3GAL4/UAS-*rpr*	**d2-3GAL4/+**	**UAS-*rpr*/+**	**d2-3Gal4/UAS-*rpr* (w/bacteria)**	**d2-3GAL4/+ (w/bacteria)**	**UAS-*rpr*/+ (w/bacteria)**
d2-3Gal4/UAS-*rpr*		**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**
d2-3GAL4/+	**<0.0001 (***)**		0.7199	**0.0008 (***)**	**0.0405 (*)**	0.7803
UAS-*rpr*/+	**<0.0001 (***)**	0.7199		**0.0074 (**)**	**0.0484 (*)**	0.9035
d2-3Gal4/UAS-*rpr* (w/bacteria)	**<0.0001 (***)**	**0.0008 (***)**	**0.0074 (**)**		**<0.0001 (***)**	**0.0011 (**)**
d2-3GAL4/+ (w/bacteria)	**<0.0001 (***)**	**0.0405 (*)**	**0.0484 (*)**	**<0.0001 (***)**		0.0772
UAS-*rpr4*/+ (w/bacteria)	**<0.0001 (***)**	0.7803	0.9035	**0.0011 (**)**	0.0772	

Figure 4) First lifespan results of female flies (n = 68-130) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure.



Logrank test by Genotype

	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr/+*	d2-3Gal4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr/+* (w/bacteria)
d2-3Gal4/UAS-*rpr*		**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**
d2-3GAL4/+	**<0.0001 (***)**		0.0907	0.6639	**<0.0001 (***)**	0.3958
UAS-*rpr/+*	**<0.0001 (***)**	0.0907		0.1773	**<0.0001 (***)**	0.3330
d2-3Gal4/UAS-*rpr* (w/bacteria)	**<0.0001 (***)**	0.6639	0.1773		**0.0002 (***)**	0.8304
d2-3GAL4/+ (w/bacteria)	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	**0.0002 (***)**		**<0.0001 (***)**
UAS-*rpr/+* (w/bacteria)	**<0.0001 (***)**	0.3958	0.3330	0.8304	**<0.0001 (***)**	

Figure 5) First lifespan results of male flies (n = 90-130) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure.

5.5.2. Bacteria attenuated exploratory walking changes caused by IPC ablation

An exploratory walking assay observes the movement and behaviour of the flies within a novel environment providing insight to how they interact, orient, and explore new surroundings. Typically, flies exhibit high tendencies of wall-following and central avoidance as part of their prey behaviour (128,153). In this assay, both activity levels and prey behaviour were assessed. Activity levels are measured by mean distance moved, mean movement duration, mean activity bouts, and mean velocity. Likewise, the mean time spent in central zone, mean rotations, and latency to first rotation quantifies prey behaviour tendencies. As flies age, these parameters decreased with time and systemic IIS reduction had little effect on exploratory walking senescence with slowed decline found only in female mean movement duration (154). However, systemic IIS reduction here slowed the decline of three additional exploratory walking parameters while bacteria attenuated these affects.

Along age, the mean walking distance of flies declined among females (Figure 6). Compared to controls, female flies with systemic IIS experienced a slower decline in this parameter and a slight, but insignificant, recovery at age 30 days onwards. However, this recovery was not observed among flies of the same genotype treated with bacteria, suggesting that bacteria attenuated the beneficial effects of reduced IIS towards mean distance moved. As for males, the mean distance moved remained unaffected (Figure 7) throughout life regardless of genotype.

Conversely, changes in mean time spent in the central zone were not found among female (Figure 8) but instead among males (Figure 9). While all

male flies appear to spend more time in the central zone as they age, only the UAS-*rpr*/+ controls and both bacteria treated systemic IIS reduced and d2-3GAL4/+ flies showed a significant increase at age 50 days. Paradoxically, bacteria treatment did not affect changes to mean time spent in the central zone except for UAS-*rpr*/+ flies where mean time spent in the central zone remained unchanged across life. Therefore, it is possible that changes to this parameter manifest much later in life (after age 50 days) and any bacterial effects are only present when age-related changes are pronounced.

Consistently, both female (Figure 10) and male (Figure 11) flies exhibited a decline in mean movement duration with age. Despite so, systemic IIS reduction slowed the decline of this parameter among females as seen by greater mean movement duration at age 50 days compared to controls. Again, this finding was not replicated among bacteria treated systemic IIS reduced flies, reiterating bacteria's attenuating effects towards the changes of IIS reduction. Among males, a decline was found across all genotypes except for d2-3GAL4/+ flies which remained relatively constant across age. When treated with bacteria, similar declines were observed across all genotypes, indifferent to controls. Overall, IIS reduction slowed the decline of mean movement duration in female but not male flies. Additionally, bacteria attenuated these changes among females but exerted no effects to males. Therefore, bacteria may modulate the effects of IIS reduction but do not influence fly health in the absence of IIS-induced effects.

While there were no significant changes to mean activity bouts among female flies (Figure 12), an increasing trend was observed in male flies (Figure 13). However, the activity differences between male genotypes and age were

largely insignificant. Therefore, neither systemic IIS reduction nor bacteria treatment appears to affect the number of activity bouts in flies.

Regarding mean velocity, all female flies (Figure 14) excluding the systemic IIS reduced genotype exhibited a decline in this parameter with age. Instead, systemic IIS reduced flies showed a late age recovery at age 50 days outperforming controls. However, bacteria treatment again attenuated this recovery affect in the same genotype. As for males (Figure 15), systemic IIS reduction did not affect mean velocity but bacteria treatment worsened fly performance at age 50 days compared to its control. As mean velocity did not differ between genotypes, it is possible that this late age male decline was driven by factors external to bacteria treatment or age. Similar to mean distance moved, the decline in this parameter was more pronounce among females than males and differences in mean velocity between genotypes were only found at late age.

Interestingly, the number of mean rotations across life declined across all female (Figure 16) flies except for the systemic IIS reduction group where mean rotations were constant throughout life. Despite reporting higher mean rotations than every other, these differences were only significant at age 50 days. Consistent with findings above, bacteria treatment attenuated this positive effect, causing declines akin to controls. Contrastingly, mean rotations trended downwards across age among all male (Figure 17) flies but declines were not significantly consistent across age nor genotypes. Again, the effect strength of both IIS reduction and bacteria attenuation is likely influenced by the magnitude of age-related decline in a given parameter.

Lastly, the latency to first rotation showed an upwards trend across age among female flies (Figure 18). Despite so, this increase was not significant among systemic IIS reduced nor control flies. While bacteria significantly increased the latency to first rotation across all genotypes with age, no significant differences were found between genotypes. Similarly, the increase of latency to first rotation across age trended upwards among males (Figure 19) but no significant increases was found across age nor genotype apart from a late age increase at age 50 days among bacteria treated flies with IIS reduction. As latency to first rotation was not consistently different across genotypes with age, it is unlikely that IIS modulation nor bacteria influenced this parameter.

Overall, fly walking behaviour declined among female but not male flies. This was expected as Results 5.5.1 reported lower female survivability than males at age 50 days, indicating an earlier decline among females. In contrary to previous findings, systemic IIS reduction also slowed the decline mean distance moved, mean velocity and mean rotations across age in addition to mean movement duration. Again, these findings were only present in female flies (Table 10). As male (Table 11) walking behaviour was indifferent across genotypes throughout the assay, it remains inconclusive if systemic IIS reduction affects exploratory walking behaviour. Surprisingly, any changes induced by systemic IIS reduction were attenuated when flies were exposed to bacteria as these flies experienced a decline similar to controls. Interestingly, no bacteria impact was found in parameters unaffected by systemic IIS reduction. Therefore, it is possible that bacteria interact with host IIS and attenuates the changes induced by low IIS expression. Supplementary to this sub-section, a video compilation of female fly walking behaviour across age was prepared in Appendix 10.12.

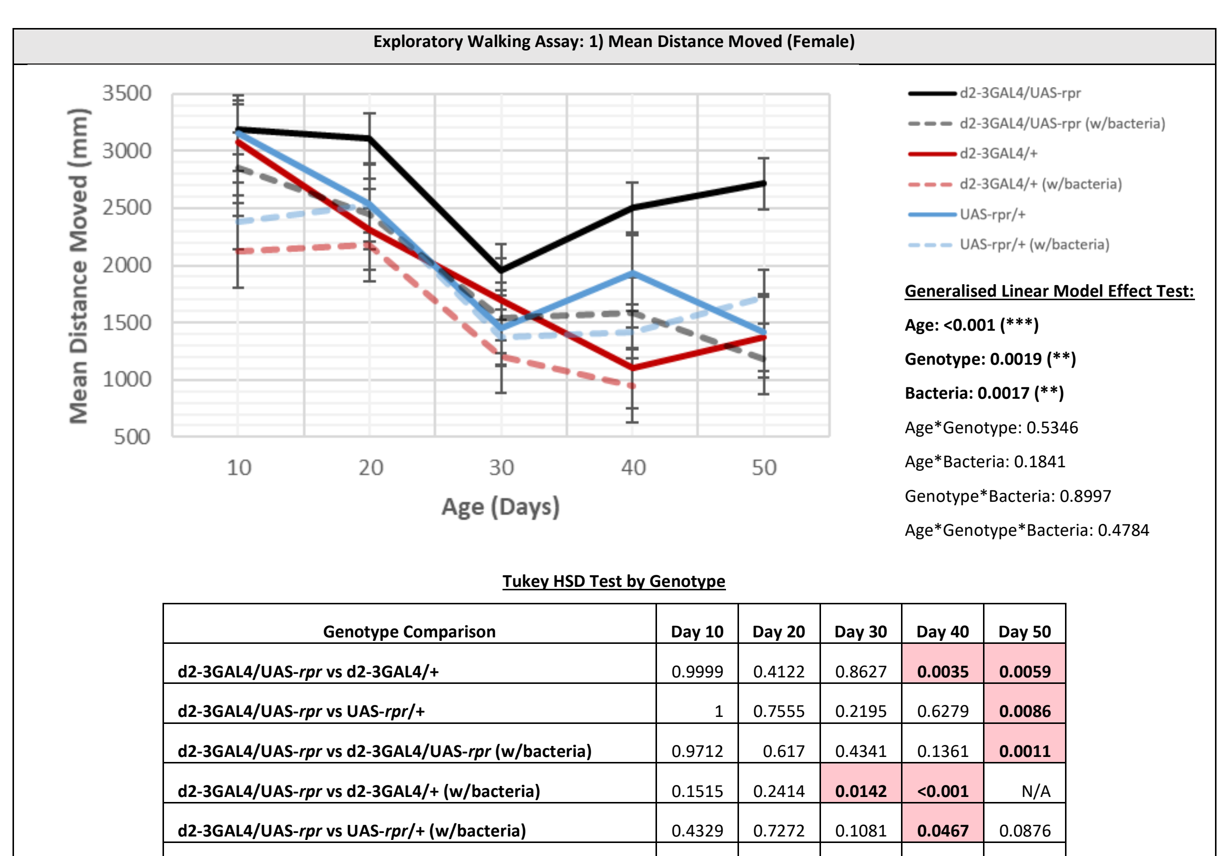
Table 10) Summarised changes to exploratory walking behaviour across age among female and male flies with systemic IIS reduction and/or bacteria exposure

Cohort: Female	Baseline Effect	Changes to Baseline Effect			
Condition / Parameter	**Controls**	**Systemic IIS Reduction**	**Bacteria Treatment**	**Combined Treatment**	**Reference**
1) Mean Distance Moved	Declined	Slowed Declined	No Effect	Attenuated Changes	Figure 6
2) Mean Time Spent in Central Zone	No Change	No Effect	No Effect	No Effect	Figure 8
3) Mean Movement Duration	Declined	Slowed Declined	No Effect	Attenuated Changes	Figure 10
4) Mean Activity Bouts	No Change	No Effect	No Effect	No Effect	Figure 12
5) Mean Velocity	Declined	Late Age Recovery	No Effect	Attenuated Changes	Figure 14
6) Mean Rotations	Declined	Slowed Declined	No Effect	Attenuated Changes	Figure 16
7) Latency to First Rotation	No Change	No Effect	No Effect	No Effect	Figure 18

Table 11) Summarised changes to exploratory walking behaviour across age among male flies with systemic IIS reduction and/or bacteria exposure

Cohort: Male	Baseline Effect	Changes to Baseline Effect			
Condition / Parameter	**Controls**	**Systemic IIS Reduction**	**Bacteria Treatment**	**Combined Treatment**	**Reference**
1) Mean Distance Moved	No Change	No Effect	No Effect	No Effect	Figure 7
2) Mean Time Spent in Central Zone	No Change	No Effect	No Effect	No Effect	Figure 9
3) Mean Movement Duration	Declined	No Effect	No Effect	No Effect	Figure 11
4) Mean Activity Bouts	No Change	No Effect	No Effect	No Effect	Figure 13
5) Mean Velocity	No Change	No Effect	No Effect	No Effect	Figure 15
6) Mean Rotations	No Change	No Effect	No Effect	No Effect	Figure 17
7) Latency to First Rotation	No Change	No Effect	No Effect	No Effect	Figure 19

Exploratory Walking Assay: 1) Mean Distance Moved (Female)



Generalised Linear Model Effect Test:

Age: <0.001 (***)

Genotype: 0.0019 (**)

Bacteria: 0.0017 (**)

Age*Genotype: 0.5346

Age*Bacteria: 0.1841

Genotype*Bacteria: 0.8997

Age*Genotype*Bacteria: 0.4784

Tukey HSD Test by Genotype

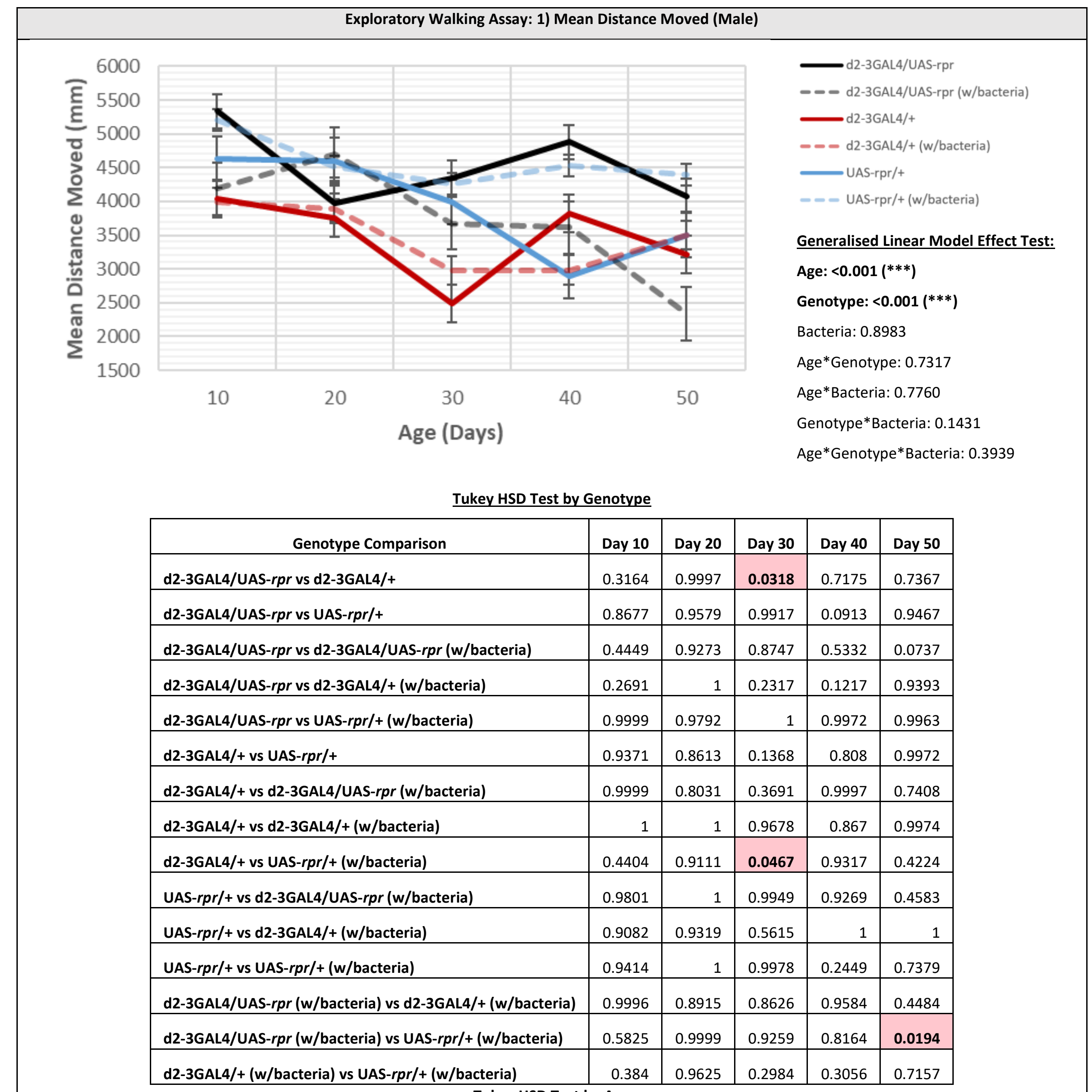
Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.9999	0.4122	0.8627	**0.0035**	**0.0059**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	1	0.7555	0.2195	0.6279	**0.0086**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9712	0.617	0.4341	0.1361	**0.0011**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.1515	0.2414	**0.0142**	**<0.001**	N/A
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.4329	0.7272	0.1081	**0.0467**	0.0876
d2-3GAL4/+ vs UAS-*rpr*/+	1	0.9942	0.8704	0.2287	1
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9949	0.9995	0.9794	0.7817	0.9956
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.2478	0.9995	0.24	0.9981	N/A
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.5914	0.9962	0.6918	0.9541	0.9269
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9825	0.9999	0.9985	0.9354	0.9882
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.1801	0.9542	0.8824	0.0911	N/A
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.485	1	0.9994	0.7389	0.9579
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.5531	0.9877	0.665	0.5135	N/A
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.8855	1	0.9763	0.9978	0.6792
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.9917	0.964	0.9734	0.7884	N/A

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.9997	0.2975	0.4848	0.7969	0.9998	0.9922
10 vs 30 Days	**0.0278**	**0.0065**	**<0.001**	**0.0044**	0.0805	**0.0296**
10 vs 40 Days	0.4537	**<0.001**	**0.0141**	**0.0065**	**0.0117**	**0.0432**
10 vs 50 Days	0.7706	**<0.001**	**<0.001**	**<0.001**	N/A	0.3001
20 vs 30 Days	**0.0464**	0.5264	**0.0366**	0.0961	0.0532	**0.0084**
20 vs 40 Days	0.5757	**0.0229**	0.4821	0.1261	**0.0071**	**0.0128**
20 vs 50 Days	0.8667	0.123	**0.0282**	**0.0063**	N/A	0.1295
30 vs 40 Days	0.6673	0.5486	0.702	0.9999	0.9475	0.9999
30 vs 50 Days	0.3521	0.9133	1	0.8541	N/A	0.8338
40 vs 50 Days	0.9859	0.9612	0.6418	0.7938	N/A	0.8954

Figure 6) Mean distance moved by female flies (n = 15-16) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age

Exploratory Walking Assay: 1) Mean Distance Moved (Male)



Generalised Linear Model Effect Test:

Age: <0.001 (***)

Genotype: <0.001 (***)

Bacteria: 0.8983

Age*Genotype: 0.7317

Age*Bacteria: 0.7760

Genotype*Bacteria: 0.1431

Age*Genotype*Bacteria: 0.3939

Tukey HSD Test by Genotype

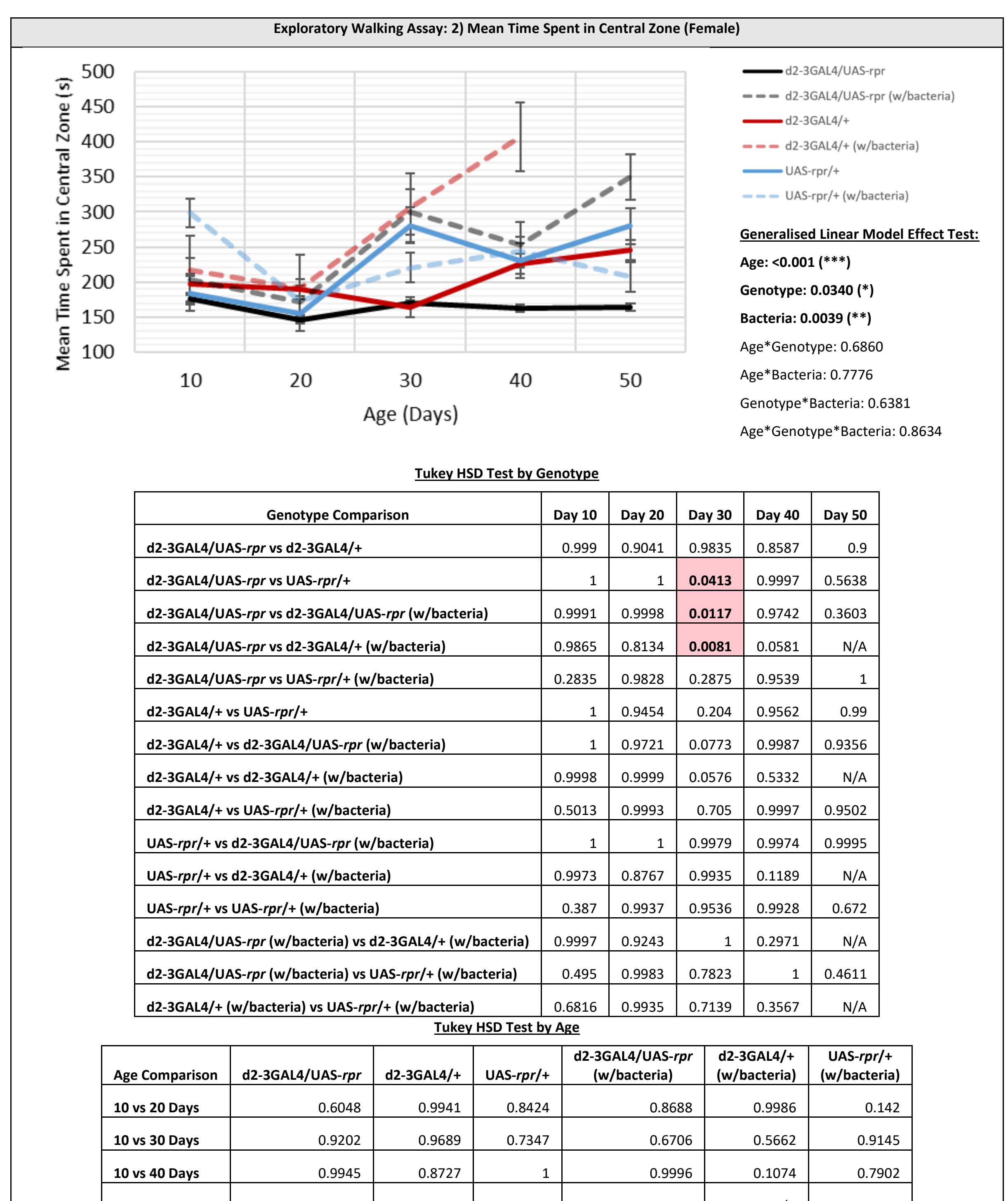
Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.3164	0.9997	**0.0318**	0.7175	0.7367
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.8677	0.9579	0.9917	0.0913	0.9467
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.4449	0.9273	0.8747	0.5332	0.0737
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.2691	1	0.2317	0.1217	0.9393
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.9999	0.9792	1	0.9972	0.9963
d2-3GAL4/+ vs UAS-*rpr*/+	0.9371	0.8613	0.1368	0.808	0.9972
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9999	0.8031	0.3691	0.9997	0.7408
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	1	1	0.9678	0.867	0.9974
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.4404	0.9111	**0.0467**	0.9317	0.4224
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9801	1	0.9949	0.9269	0.4583
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.9082	0.9319	0.5615	1	1
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.9414	1	0.9978	0.2449	0.7379
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9996	0.8915	0.8626	0.9584	0.4484
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.5825	0.9999	0.9259	0.8164	**0.0194**
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.384	0.9625	0.2984	0.3056	0.7157

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.3271	0.991	1	0.9484	0.9998	0.8684
10 vs 30 Days	0.6443	0.1347	0.8696	0.9494	0.4405	0.6808
10 vs 40 Days	0.9683	0.9972	0.0737	0.9237	0.4316	0.881
10 vs 50 Days	0.4141	0.7061	0.4552	0.0732	0.9186	0.7904
20 vs 30 Days	0.9849	0.3177	0.8841	0.5916	0.5404	0.9967
20 vs 40 Days	0.7159	1	0.0801	0.5327	0.5326	1
20 vs 50 Days	0.9999	0.923	0.4766	**0.0101**	0.9615	0.9998
30 vs 40 Days	0.9467	0.2601	0.4569	1	1	0.9955
30 vs 50 Days	0.9961	0.8088	0.9499	0.3195	0.9044	0.9997
40 vs 50 Days	0.8031	0.8805	0.8883	0.3695	0.9039	0.9997

Figure 7) Mean distance moved by male flies (n = 15-16) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age

Exploratory Walking Assay: 2) Mean Time Spent in Central Zone (Female)



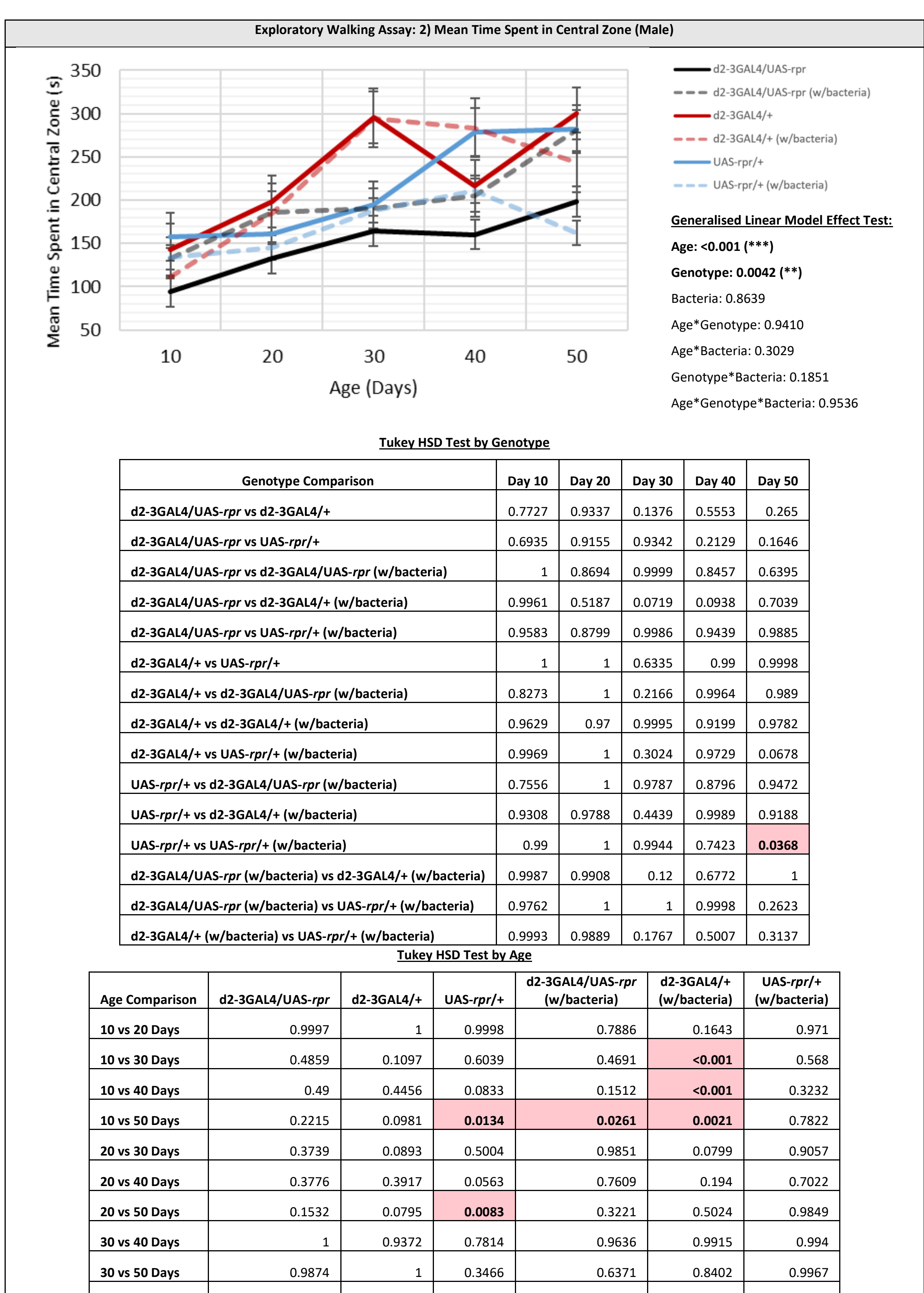
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.999	0.9041	0.9835	0.8587	0.9
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	1	1	**0.0413**	0.9997	0.5638
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9991	0.9998	**0.0117**	0.9742	0.3603
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.9865	0.8134	**0.0081**	0.0581	N/A
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.2835	0.9828	0.2875	0.9539	1
d2-3GAL4/+ vs UAS-*rpr*/+	1	0.9454	0.204	0.9562	0.99
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	1	0.9721	0.0773	0.9987	0.9356
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.9998	0.9999	0.0576	0.5332	N/A
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.5013	0.9993	0.705	0.9997	0.9502
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	1	1	0.9979	0.9974	0.9995
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.9973	0.8767	0.9935	0.1189	N/A
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.387	0.9937	0.9536	0.9928	0.672
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9997	0.9243	1	0.2971	N/A
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.495	0.9983	0.7823	1	0.4611
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.6816	0.9935	0.7139	0.3567	N/A

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.6048	0.9941	0.8424	0.8688	0.9986	0.142
10 vs 30 Days	0.9202	0.9689	0.7347	0.6706	0.5662	0.9145
10 vs 40 Days	0.9945	0.8727	1	0.9996	0.1074	0.7902
10 vs 50 Days	1	0.7885	0.6498	0.5891	N/A	0.499
20 vs 30 Days	0.9734	0.9994	0.1713	0.1556	0.3933	0.5671
20 vs 40 Days	0.8365	0.6516	0.8934	0.7641	0.0557	0.742
20 vs 50 Days	0.5436	0.5403	0.1282	0.1183	N/A	0.946
30 vs 40 Days	0.9927	0.5063	0.6621	0.793	0.8613	0.9986
30 vs 50 Days	0.8858	0.3998	0.9999	0.9999	N/A	0.9421
40 vs 50 Days	0.9875	0.9998	0.5737	0.7201	N/A	0.9894

Figure 8) Mean time spent in central zone by female flies (n = 15-16) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



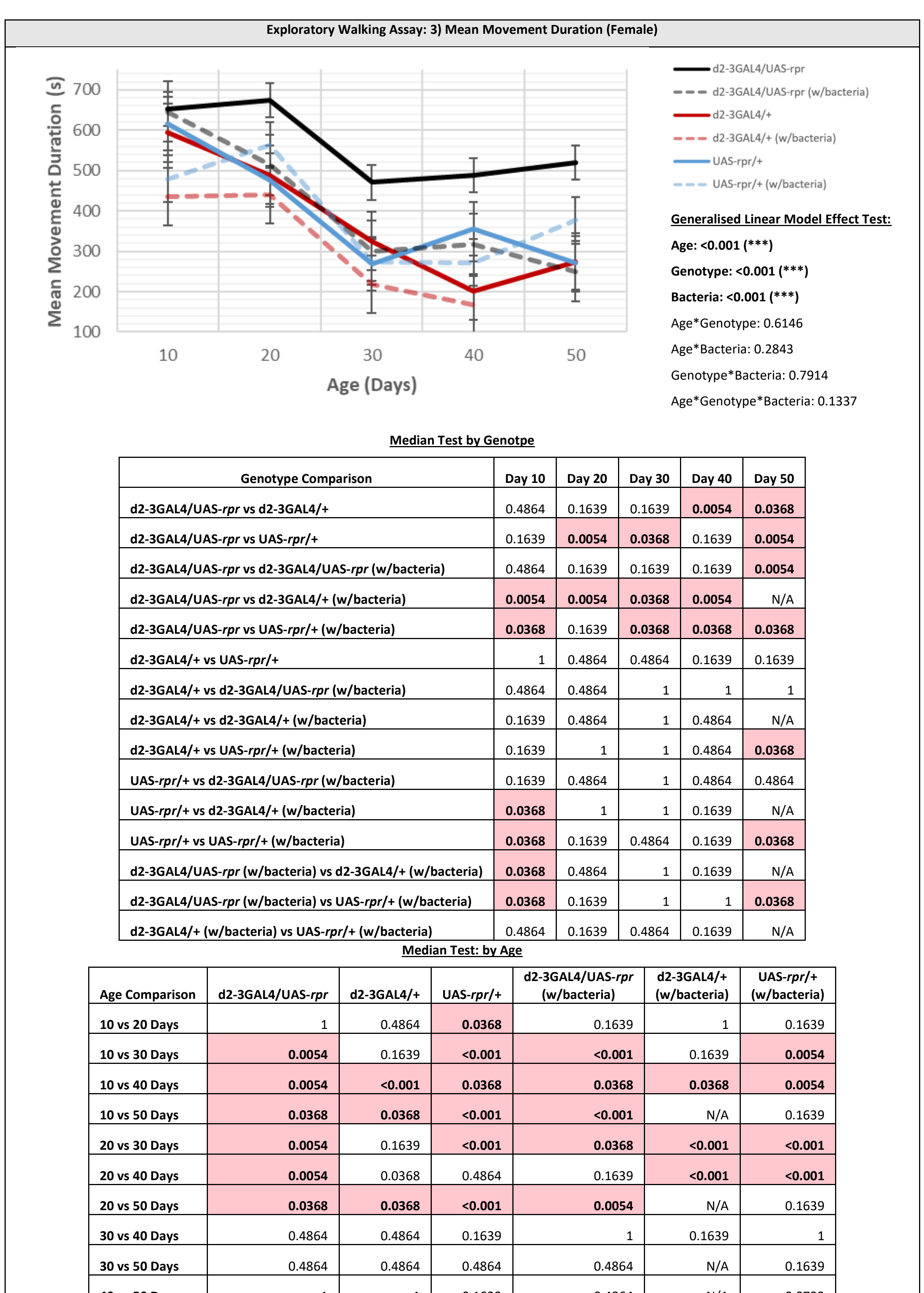
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.7727	0.9337	0.1376	0.5553	0.265
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.6935	0.9155	0.9342	0.2129	0.1646
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	1	0.8694	0.9999	0.8457	0.6395
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.9961	0.5187	0.0719	0.0938	0.7039
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.9583	0.8799	0.9986	0.9439	0.9885
d2-3GAL4/+ vs UAS-*rpr*/+	1	1	0.6335	0.99	0.9998
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.8273	1	0.2166	0.9964	0.989
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.9629	0.97	0.9995	0.9199	0.9782
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.9969	1	0.3024	0.9729	0.0678
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.7556	1	0.9787	0.8796	0.9472
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.9308	0.9788	0.4439	0.9989	0.9188
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.99	1	0.9944	0.7423	**0.0368**
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9987	0.9908	0.12	0.6772	1
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.9762	1	1	0.9998	0.2623
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.9993	0.9889	0.1767	0.5007	0.3137

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.9997	1	0.9998	0.7886	0.1643	0.971
10 vs 30 Days	0.4859	0.1097	0.6039	0.4691	**<0.001**	0.568
10 vs 40 Days	0.49	0.4456	0.0833	0.1512	**<0.001**	0.3232
10 vs 50 Days	0.2215	0.0981	**0.0134**	**0.0261**	**0.0021**	0.7822
20 vs 30 Days	0.3739	0.0893	0.5004	0.9851	0.0799	0.9057
20 vs 40 Days	0.3776	0.3917	0.0563	0.7609	0.194	0.7022
20 vs 50 Days	0.1532	0.0795	**0.0083**	0.3221	0.5024	0.9849
30 vs 40 Days	1	0.9372	0.7814	0.9636	0.9915	0.994
30 vs 50 Days	0.9874	1	0.3466	0.6371	0.8402	0.9967
40 vs 50 Days	0.9868	0.9225	0.9477	0.9501	0.9758	0.9408

Figure 9) Mean time spent in central zone by male flies (n = 15-16) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



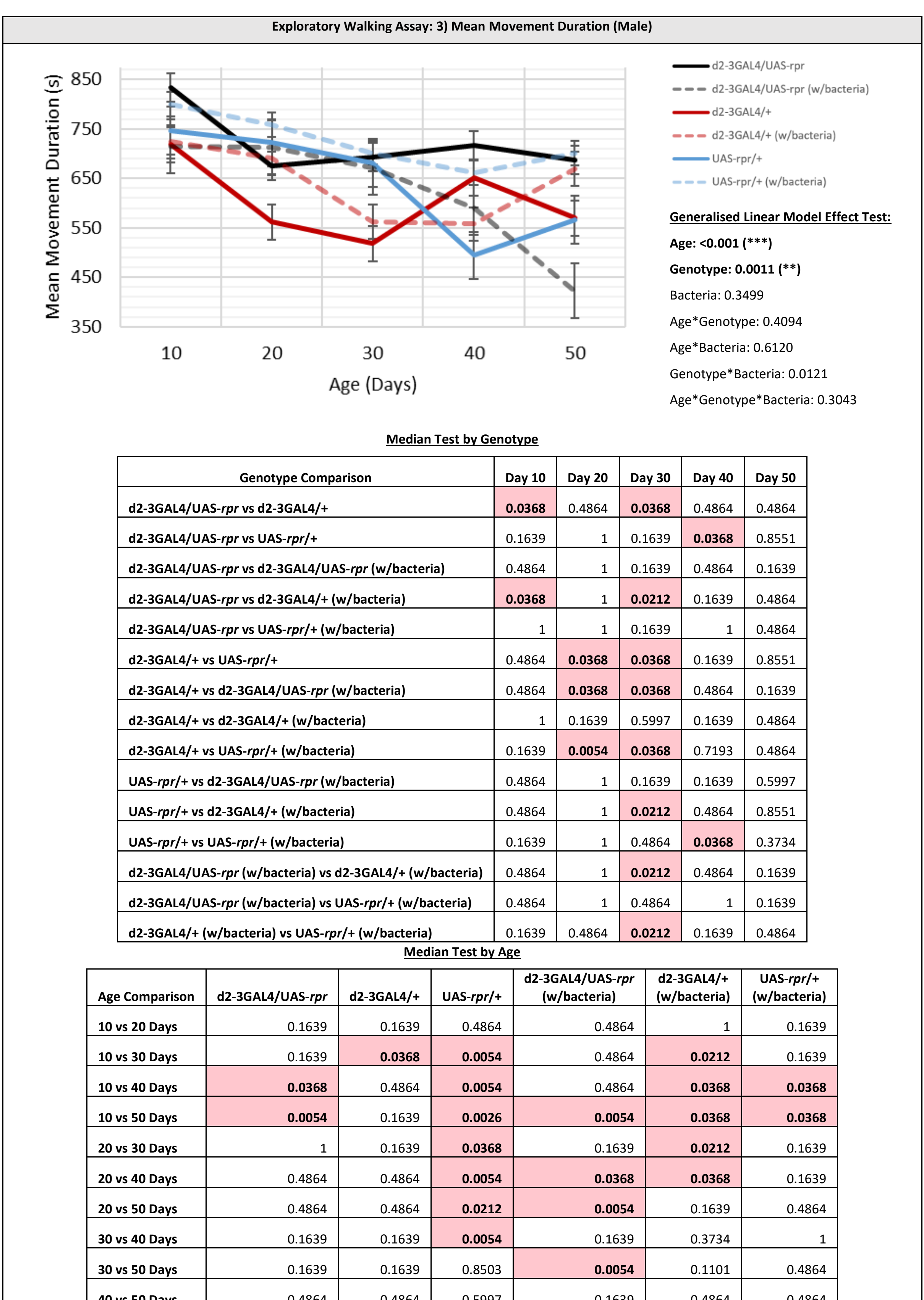
Median Test by Genotpe

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.4864	0.1639	0.1639	**0.0054**	**0.0368**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.1639	**0.0054**	**0.0368**	0.1639	**0.0054**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.4864	0.1639	0.1639	0.1639	**0.0054**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	**0.0054**	**0.0054**	**0.0368**	**0.0054**	N/A
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	**0.0368**	0.1639	**0.0368**	**0.0368**	**0.0368**
d2-3GAL4/+ vs UAS-*rpr*/+	1	0.4864	0.4864	0.1639	0.1639
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.4864	0.4864	1	1	1
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.1639	0.4864	1	0.4864	N/A
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.1639	1	1	0.4864	**0.0368**
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.1639	0.4864	1	0.4864	0.4864
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	**0.0368**	1	1	0.1639	N/A
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	**0.0368**	0.1639	0.4864	0.1639	**0.0368**
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	**0.0368**	0.4864	1	0.1639	N/A
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	**0.0368**	0.1639	1	1	**0.0368**
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.4864	0.1639	0.4864	0.1639	N/A

Median Test: by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	1	0.4864	**0.0368**	0.1639	1	0.1639
10 vs 30 Days	**0.0054**	0.1639	**<0.001**	**<0.001**	0.1639	**0.0054**
10 vs 40 Days	**0.0054**	**<0.001**	**0.0368**	**0.0368**	**0.0368**	**0.0054**
10 vs 50 Days	**0.0368**	**0.0368**	**<0.001**	**<0.001**	N/A	0.1639
20 vs 30 Days	**0.0054**	0.1639	**<0.001**	**0.0368**	**<0.001**	**<0.001**
20 vs 40 Days	**0.0054**	0.0368	0.4864	0.1639	**<0.001**	**<0.001**
20 vs 50 Days	**0.0368**	**0.0368**	**<0.001**	**0.0054**	N/A	0.1639
30 vs 40 Days	0.4864	0.4864	0.1639	1	0.1639	1
30 vs 50 Days	0.4864	0.4864	0.4864	0.4864	N/A	0.1639
40 vs 50 Days	1	1	0.1639	0.4864	N/A	0.0723

Figure 10) Mean movement duration of female flies (n = 15-16) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



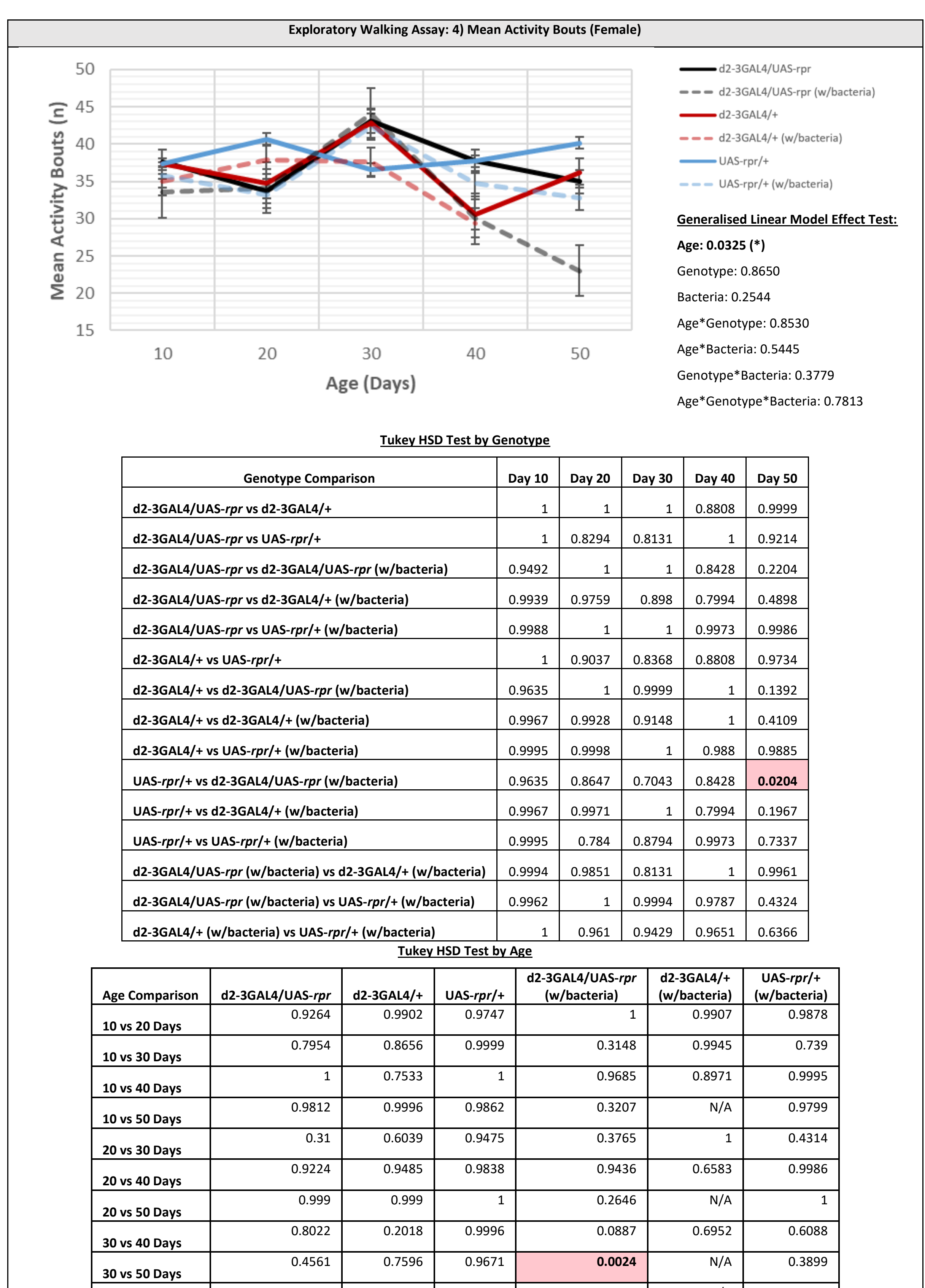
Median Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	**0.0368**	0.4864	**0.0368**	0.4864	0.4864
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.1639	1	0.1639	**0.0368**	0.8551
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.4864	1	0.1639	0.4864	0.1639
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	**0.0368**	1	**0.0212**	0.1639	0.4864
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	1	1	0.1639	1	0.4864
d2-3GAL4/+ vs UAS-*rpr*/+	0.4864	**0.0368**	**0.0368**	0.1639	0.8551
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.4864	**0.0368**	**0.0368**	0.4864	0.1639
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	1	0.1639	0.5997	0.1639	0.4864
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.1639	**0.0054**	**0.0368**	0.7193	0.4864
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.4864	1	0.1639	0.1639	0.5997
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.4864	1	**0.0212**	0.4864	0.8551
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.1639	1	0.4864	**0.0368**	0.3734
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.4864	1	**0.0212**	0.4864	0.1639
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.4864	1	0.4864	1	0.1639
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.1639	0.4864	**0.0212**	0.1639	0.4864

Median Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.1639	0.1639	0.4864	0.4864	1	0.1639
10 vs 30 Days	0.1639	**0.0368**	**0.0054**	0.4864	**0.0212**	0.1639
10 vs 40 Days	**0.0368**	0.4864	**0.0054**	0.4864	**0.0368**	**0.0368**
10 vs 50 Days	**0.0054**	0.1639	**0.0026**	**0.0054**	**0.0368**	**0.0368**
20 vs 30 Days	1	0.1639	**0.0368**	0.1639	**0.0212**	0.1639
20 vs 40 Days	0.4864	0.4864	**0.0054**	**0.0368**	**0.0368**	0.1639
20 vs 50 Days	0.4864	0.4864	**0.0212**	**0.0054**	0.1639	0.4864
30 vs 40 Days	0.1639	0.1639	**0.0054**	0.1639	0.3734	1
30 vs 50 Days	0.1639	0.1639	0.8503	**0.0054**	0.1101	0.4864
40 vs 50 Days	0.4864	0.4864	0.5997	0.1639	0.4864	0.4864

Figure 11) Mean movement duration by male flies (n = 15-16) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



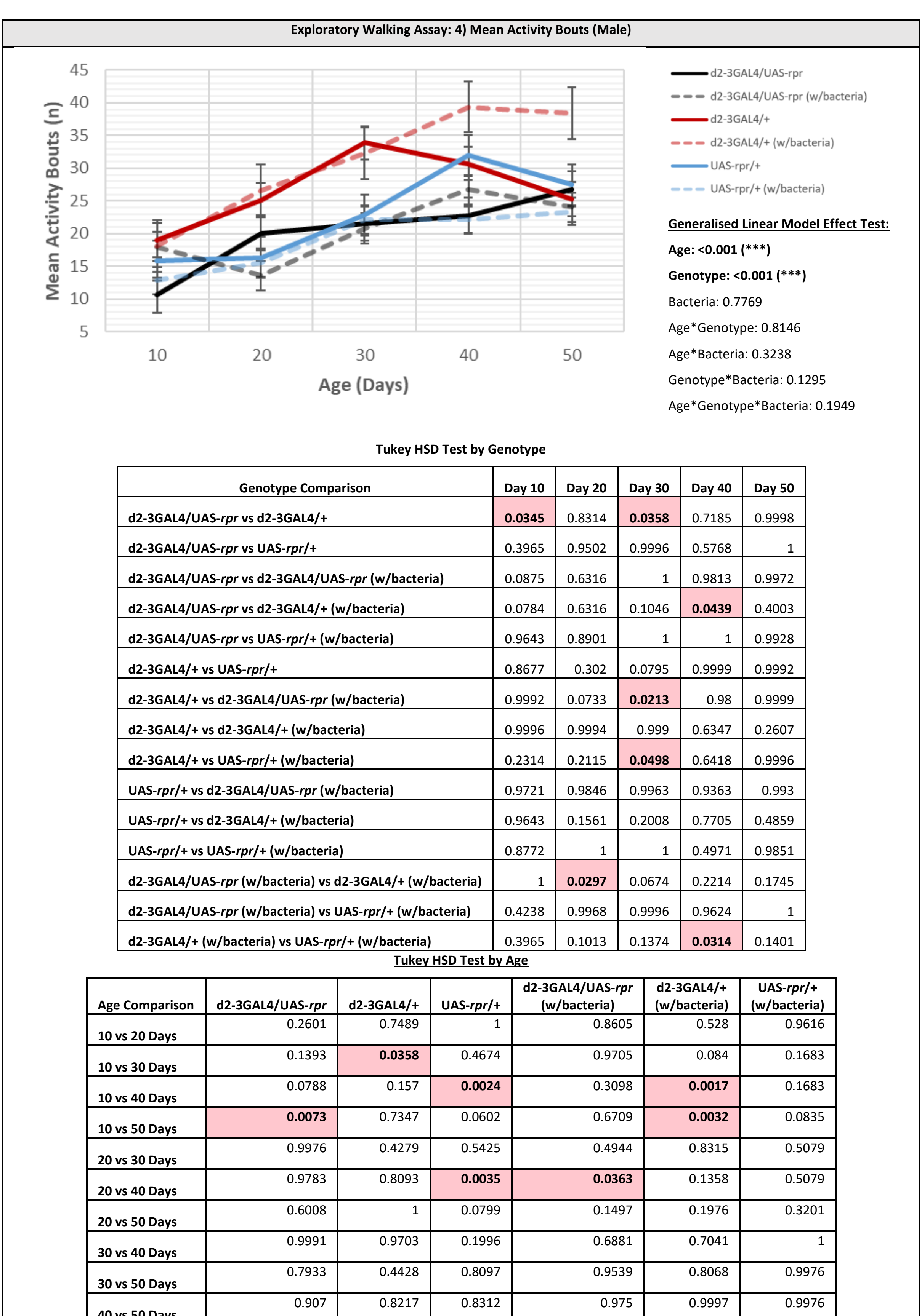
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	1	1	1	0.8808	0.9999
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	1	0.8294	0.8131	1	0.9214
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9492	1	1	0.8428	0.2204
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.9939	0.9759	0.898	0.7994	0.4898
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.9988	1	1	0.9973	0.9986
d2-3GAL4/+ vs UAS-*rpr*/+	1	0.9037	0.8368	0.8808	0.9734
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9635	1	0.9999	1	0.1392
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.9967	0.9928	0.9148	1	0.4109
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.9995	0.9998	1	0.988	0.9885
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9635	0.8647	0.7043	0.8428	**0.0204**
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.9967	0.9971	1	0.7994	0.1967
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.9995	0.784	0.8794	0.9973	0.7337
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9994	0.9851	0.8131	1	0.9961
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.9962	1	0.9994	0.9787	0.4324
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	1	0.961	0.9429	0.9651	0.6366

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.9264	0.9902	0.9747	1	0.9907	0.9878
10 vs 30 Days	0.7954	0.8656	0.9999	0.3148	0.9945	0.739
10 vs 40 Days	1	0.7533	1	0.9685	0.8971	0.9995
10 vs 50 Days	0.9812	0.9996	0.9862	0.3207	N/A	0.9799
20 vs 30 Days	0.31	0.6039	0.9475	0.3765	1	0.4314
20 vs 40 Days	0.9224	0.9485	0.9838	0.9436	0.6583	0.9986
20 vs 50 Days	0.999	0.999	1	0.2646	N/A	1
30 vs 40 Days	0.8022	0.2018	0.9996	0.0887	0.6952	0.6088
30 vs 50 Days	0.4561	0.7596	0.9671	**0.0024**	N/A	0.3899
40 vs 50 Days	0.9795	0.8608	0.992	0.7079	N/A	0.9966

Figure 12) Mean activity bouts of female flies (n = 15-16) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



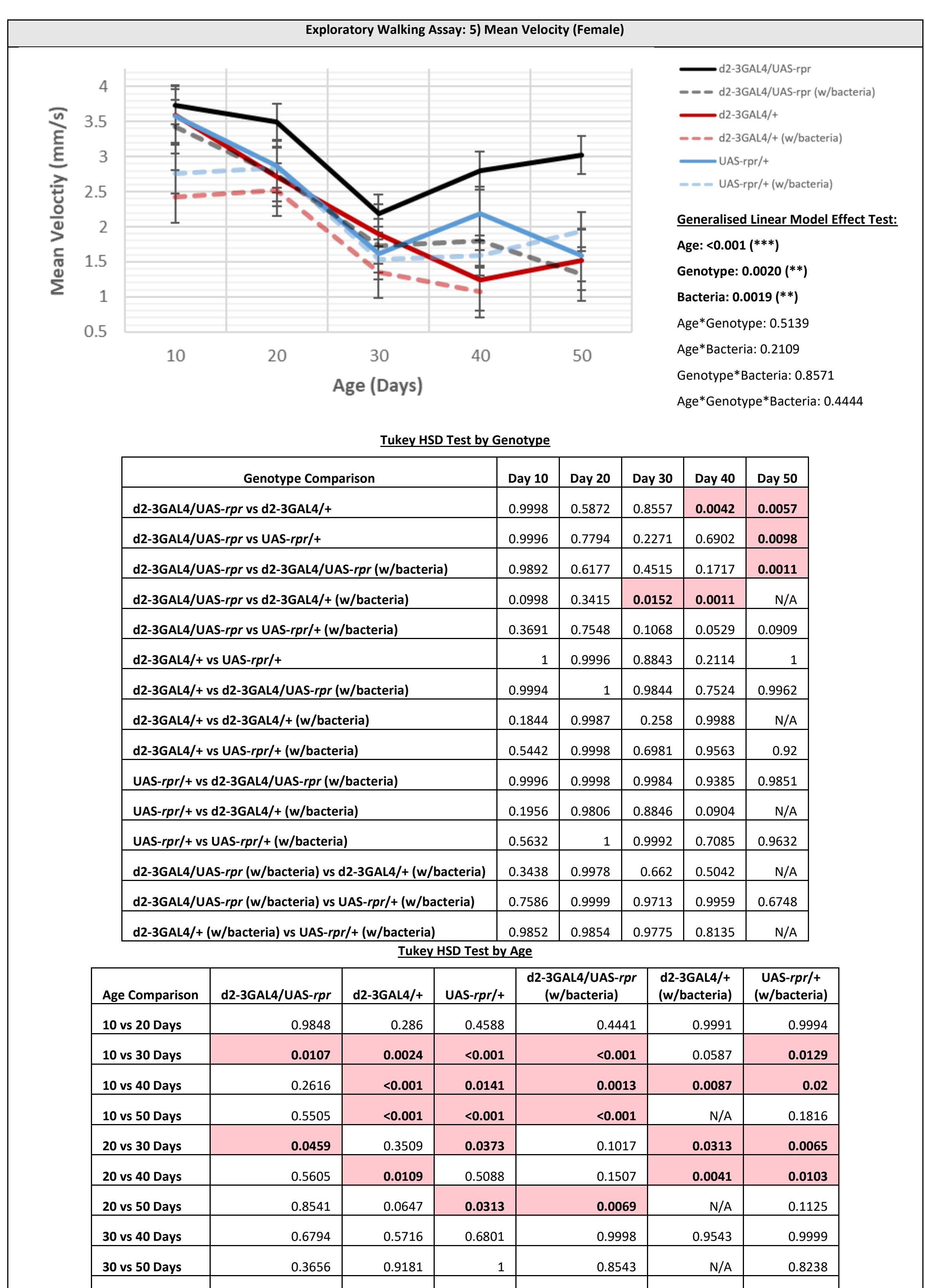
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	**0.0345**	0.8314	**0.0358**	0.7185	0.9998
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.3965	0.9502	0.9996	0.5768	1
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.0875	0.6316	1	0.9813	0.9972
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.0784	0.6316	0.1046	**0.0439**	0.4003
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.9643	0.8901	1	1	0.9928
d2-3GAL4/+ vs UAS-*rpr*/+	0.8677	0.302	0.0795	0.9999	0.9992
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9992	0.0733	**0.0213**	0.98	0.9999
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.9996	0.9994	0.999	0.6347	0.2607
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.2314	0.2115	**0.0498**	0.6418	0.9996
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9721	0.9846	0.9963	0.9363	0.993
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.9643	0.1561	0.2008	0.7705	0.4859
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.8772	1	1	0.4971	0.9851
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	1	**0.0297**	0.0674	0.2214	0.1745
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.4238	0.9968	0.9996	0.9624	1
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.3965	0.1013	0.1374	**0.0314**	0.1401

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.2601	0.7489	1	0.8605	0.528	0.9616
10 vs 30 Days	0.1393	**0.0358**	0.4674	0.9705	0.084	0.1683
10 vs 40 Days	0.0788	0.157	**0.0024**	0.3098	**0.0017**	0.1683
10 vs 50 Days	**0.0073**	0.7347	0.0602	0.6709	**0.0032**	0.0835
20 vs 30 Days	0.9976	0.4279	0.5425	0.4944	0.8315	0.5079
20 vs 40 Days	0.9783	0.8093	**0.0035**	**0.0363**	0.1358	0.5079
20 vs 50 Days	0.6008	1	0.0799	0.1497	0.1976	0.3201
30 vs 40 Days	0.9991	0.9703	0.1996	0.6881	0.7041	1
30 vs 50 Days	0.7933	0.4428	0.8097	0.9539	0.8068	0.9976
40 vs 50 Days	0.907	0.8217	0.8312	0.975	0.9997	0.9976

Figure 13) Mean activity bouts by male flies (n = 15-16) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



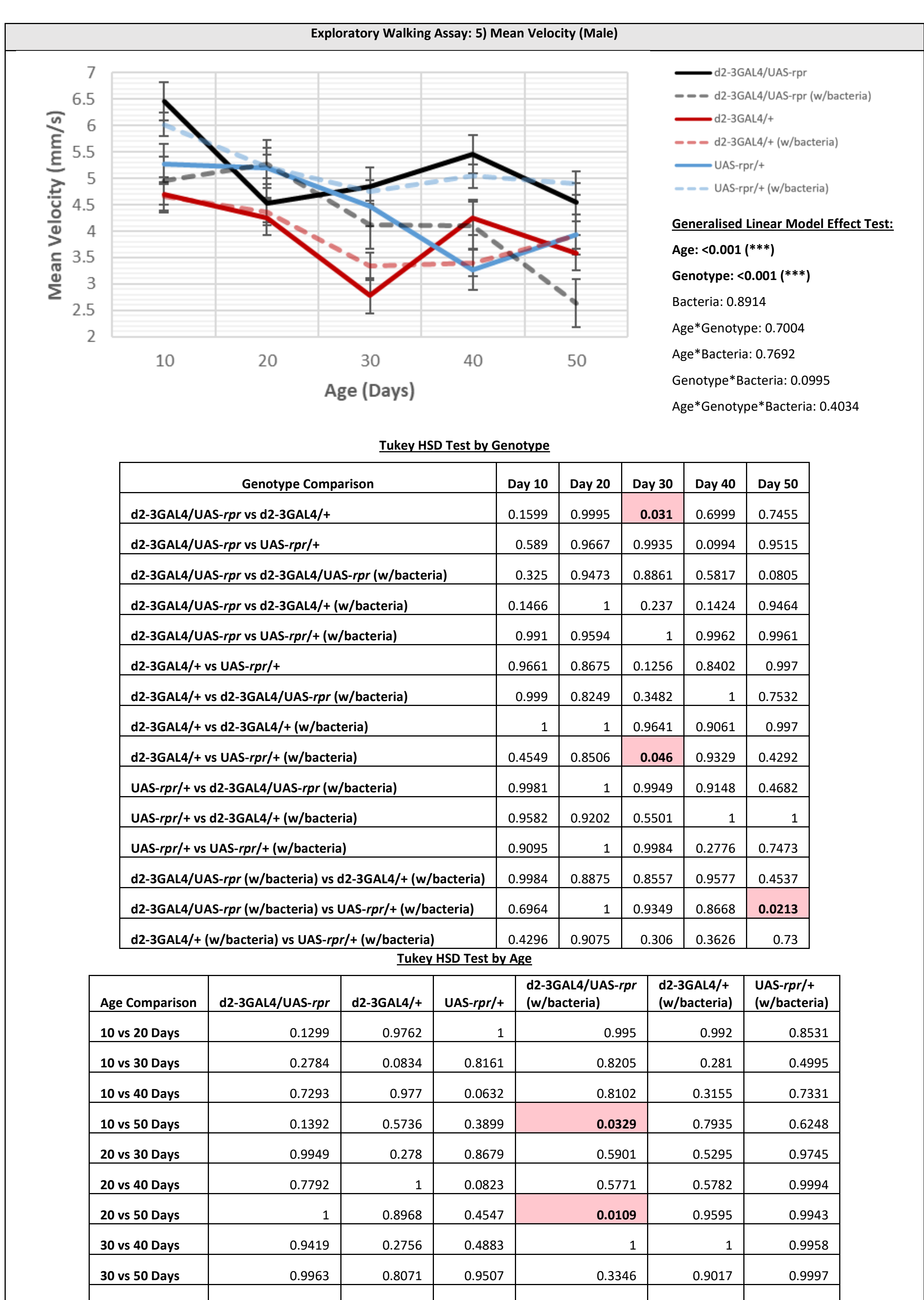
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.9998	0.5872	0.8557	**0.0042**	**0.0057**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.9996	0.7794	0.2271	0.6902	**0.0098**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9892	0.6177	0.4515	0.1717	**0.0011**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.0998	0.3415	**0.0152**	**0.0011**	N/A
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.3691	0.7548	0.1068	0.0529	0.0909
d2-3GAL4/+ vs UAS-*rpr*/+	1	0.9996	0.8843	0.2114	1
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9994	1	0.9844	0.7524	0.9962
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.1844	0.9987	0.258	0.9988	N/A
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.5442	0.9998	0.6981	0.9563	0.92
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9996	0.9998	0.9984	0.9385	0.9851
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.1956	0.9806	0.8846	0.0904	N/A
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.5632	1	0.9992	0.7085	0.9632
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.3438	0.9978	0.662	0.5042	N/A
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.7586	0.9999	0.9713	0.9959	0.6748
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.9852	0.9854	0.9775	0.8135	N/A

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.9848	0.286	0.4588	0.4441	0.9991	0.9994
10 vs 30 Days	**0.0107**	**0.0024**	**<0.001**	**<0.001**	0.0587	**0.0129**
10 vs 40 Days	0.2616	**<0.001**	**0.0141**	**0.0013**	**0.0087**	**0.02**
10 vs 50 Days	0.5505	**<0.001**	**<0.001**	**<0.001**	N/A	0.1816
20 vs 30 Days	**0.0459**	0.3509	**0.0373**	0.1017	**0.0313**	**0.0065**
20 vs 40 Days	0.5605	**0.0109**	0.5088	0.1507	**0.0041**	**0.0103**
20 vs 50 Days	0.8541	0.0647	**0.0313**	**0.0069**	N/A	0.1125
30 vs 40 Days	0.6794	0.5716	0.6801	0.9998	0.9543	0.9999
30 vs 50 Days	0.3656	0.9181	1	0.8543	N/A	0.8238
40 vs 50 Days	0.9864	0.9654	0.639	0.7617	N/A	0.892

Figure 14) Mean velocity of female flies (n = 15-16) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



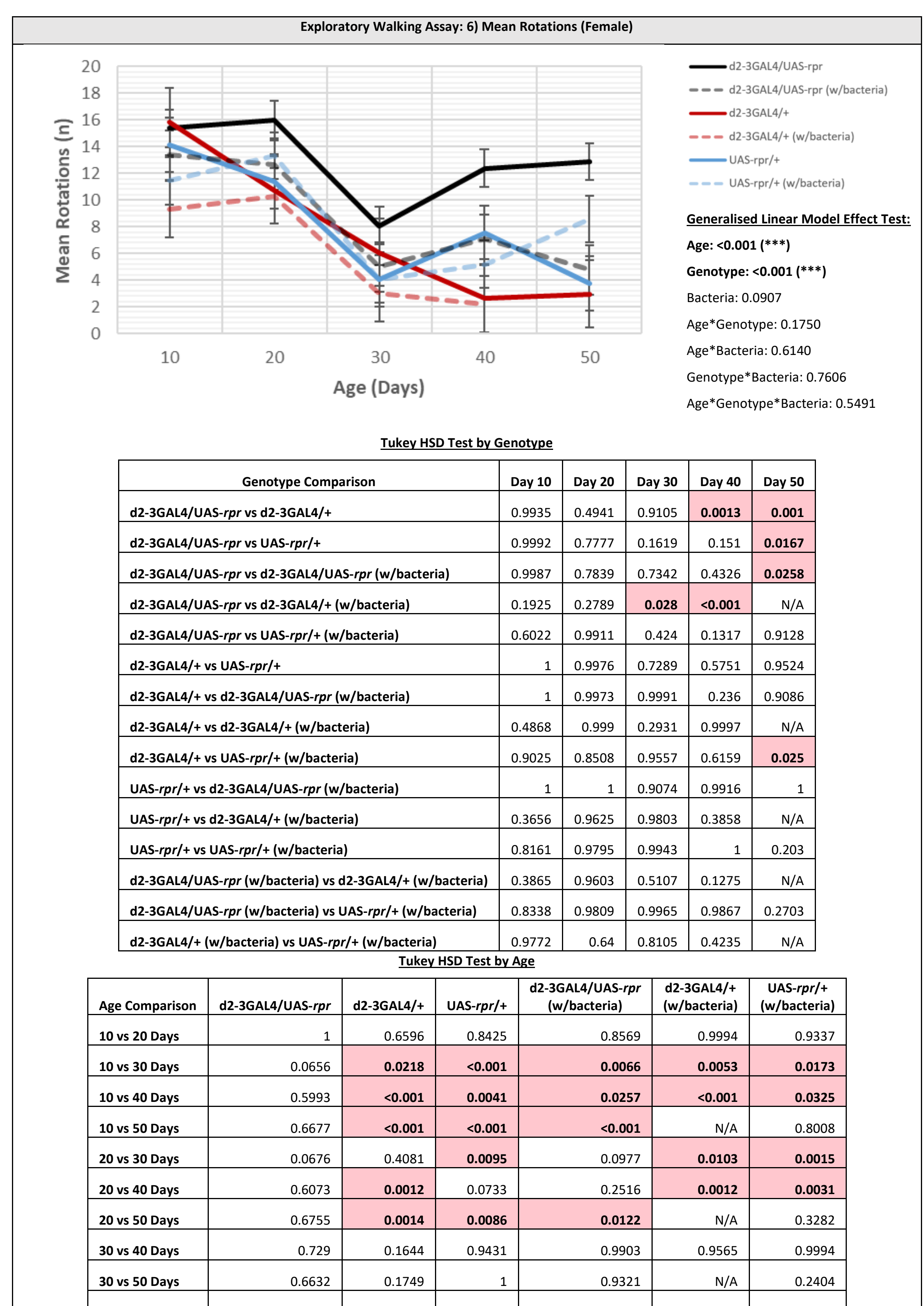
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.1599	0.9995	**0.031**	0.6999	0.7455
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.589	0.9667	0.9935	0.0994	0.9515
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.325	0.9473	0.8861	0.5817	0.0805
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.1466	1	0.237	0.1424	0.9464
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.991	0.9594	1	0.9962	0.9961
d2-3GAL4/+ vs UAS-*rpr*/+	0.9661	0.8675	0.1256	0.8402	0.997
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.999	0.8249	0.3482	1	0.7532
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	1	1	0.9641	0.9061	0.997
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.4549	0.8506	**0.046**	0.9329	0.4292
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9981	1	0.9949	0.9148	0.4682
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.9582	0.9202	0.5501	1	1
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.9095	1	0.9984	0.2776	0.7473
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9984	0.8875	0.8557	0.9577	0.4537
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.6964	1	0.9349	0.8668	**0.0213**
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.4296	0.9075	0.306	0.3626	0.73

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.1299	0.9762	1	0.995	0.992	0.8531
10 vs 30 Days	0.2784	0.0834	0.8161	0.8205	0.281	0.4995
10 vs 40 Days	0.7293	0.977	0.0632	0.8102	0.3155	0.7331
10 vs 50 Days	0.1392	0.5736	0.3899	**0.0329**	0.7935	0.6248
20 vs 30 Days	0.9949	0.278	0.8679	0.5901	0.5295	0.9745
20 vs 40 Days	0.7792	1	0.0823	0.5771	0.5782	0.9994
20 vs 50 Days	1	0.8968	0.4547	**0.0109**	0.9595	0.9943
30 vs 40 Days	0.9419	0.2756	0.4883	1	1	0.9958
30 vs 50 Days	0.9963	0.8071	0.9507	0.3346	0.9017	0.9997
40 vs 50 Days	0.7966	0.8949	0.9058	0.3456	0.9306	0.9998

Figure 15) Mean velocity of male flies (n = 15-16) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



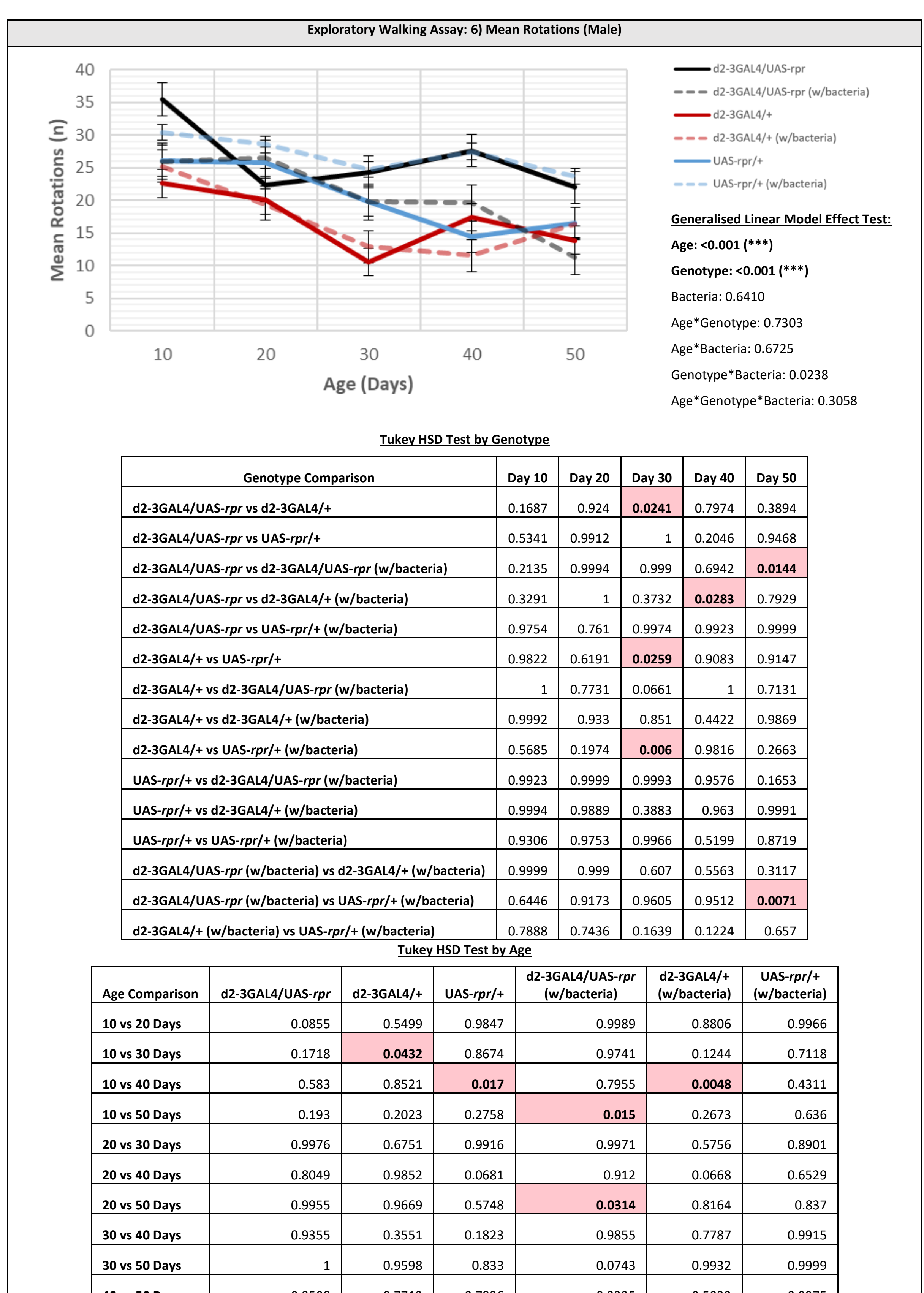
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.9935	0.4941	0.9105	**0.0013**	**0.001**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.9992	0.7777	0.1619	0.151	**0.0167**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9987	0.7839	0.7342	0.4326	**0.0258**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.1925	0.2789	**0.028**	**<0.001**	N/A
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.6022	0.9911	0.424	0.1317	0.9128
d2-3GAL4/+ vs UAS-*rpr*/+	1	0.9976	0.7289	0.5751	0.9524
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	1	0.9973	0.9991	0.236	0.9086
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.4868	0.999	0.2931	0.9997	N/A
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.9025	0.8508	0.9557	0.6159	**0.025**
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	1	1	0.9074	0.9916	1
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.3656	0.9625	0.9803	0.3858	N/A
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.8161	0.9795	0.9943	1	0.203
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.3865	0.9603	0.5107	0.1275	N/A
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.8338	0.9809	0.9965	0.9867	0.2703
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.9772	0.64	0.8105	0.4235	N/A

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	1	0.6596	0.8425	0.8569	0.9994	0.9337
10 vs 30 Days	0.0656	**0.0218**	**<0.001**	**0.0066**	**0.0053**	**0.0173**
10 vs 40 Days	0.5993	**<0.001**	**0.0041**	**0.0257**	**<0.001**	**0.0325**
10 vs 50 Days	0.6677	**<0.001**	**<0.001**	**<0.001**	N/A	0.8008
20 vs 30 Days	0.0676	0.4081	**0.0095**	0.0977	**0.0103**	**0.0015**
20 vs 40 Days	0.6073	**0.0012**	0.0733	0.2516	**0.0012**	**0.0031**
20 vs 50 Days	0.6755	**0.0014**	**0.0086**	**0.0122**	N/A	0.3282
30 vs 40 Days	0.729	0.1644	0.9431	0.9903	0.9565	0.9994
30 vs 50 Days	0.6632	0.1749	1	0.9321	N/A	0.2404
40 vs 50 Days	1	1	0.9339	0.7185	N/A	0.353

Figure 16) Mean rotations of female flies (n = 15-16) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



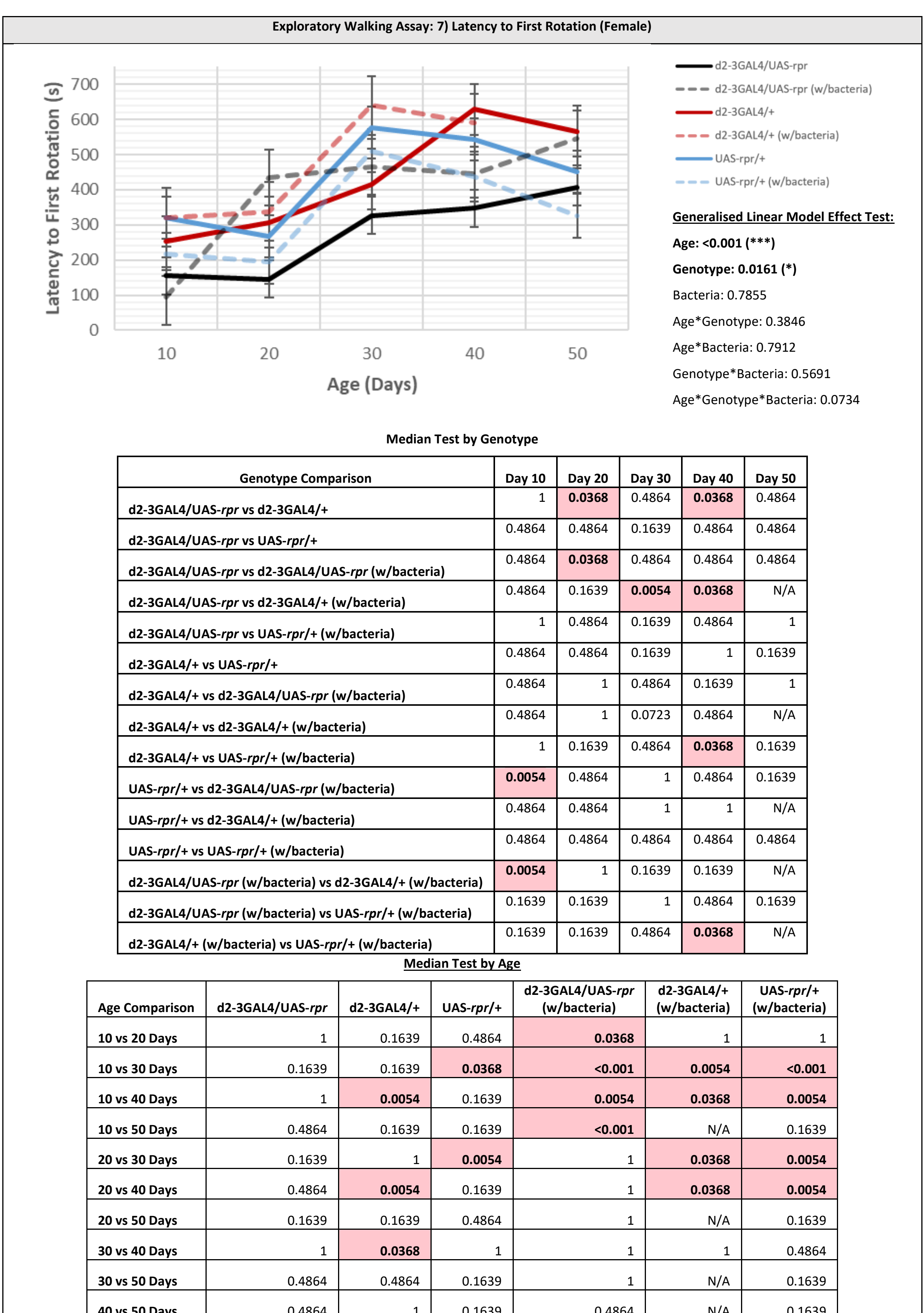
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.1687	0.924	**0.0241**	0.7974	0.3894
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.5341	0.9912	1	0.2046	0.9468
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.2135	0.9994	0.999	0.6942	**0.0144**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.3291	1	0.3732	**0.0283**	0.7929
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.9754	0.761	0.9974	0.9923	0.9999
d2-3GAL4/+ vs UAS-*rpr*/+	0.9822	0.6191	**0.0259**	0.9083	0.9147
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	1	0.7731	0.0661	1	0.7131
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.9992	0.933	0.851	0.4422	0.9869
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.5685	0.1974	**0.006**	0.9816	0.2663
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9923	0.9999	0.9993	0.9576	0.1653
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.9994	0.9889	0.3883	0.963	0.9991
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.9306	0.9753	0.9966	0.5199	0.8719
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9999	0.999	0.607	0.5563	0.3117
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.6446	0.9173	0.9605	0.9512	**0.0071**
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.7888	0.7436	0.1639	0.1224	0.657

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.0855	0.5499	0.9847	0.9989	0.8806	0.9966
10 vs 30 Days	0.1718	**0.0432**	0.8674	0.9741	0.1244	0.7118
10 vs 40 Days	0.583	0.8521	**0.017**	0.7955	**0.0048**	0.4311
10 vs 50 Days	0.193	0.2023	0.2758	**0.015**	0.2673	0.636
20 vs 30 Days	0.9976	0.6751	0.9916	0.9971	0.5756	0.8901
20 vs 40 Days	0.8049	0.9852	0.0681	0.912	0.0668	0.6529
20 vs 50 Days	0.9955	0.9669	0.5748	**0.0314**	0.8164	0.837
30 vs 40 Days	0.9355	0.3551	0.1823	0.9855	0.7787	0.9915
30 vs 50 Days	1	0.9598	0.833	0.0743	0.9932	0.9999
40 vs 50 Days	0.9508	0.7712	0.7836	0.2235	0.5023	0.9975

Figure 17) Mean rotations of male flies (n = 15-16) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



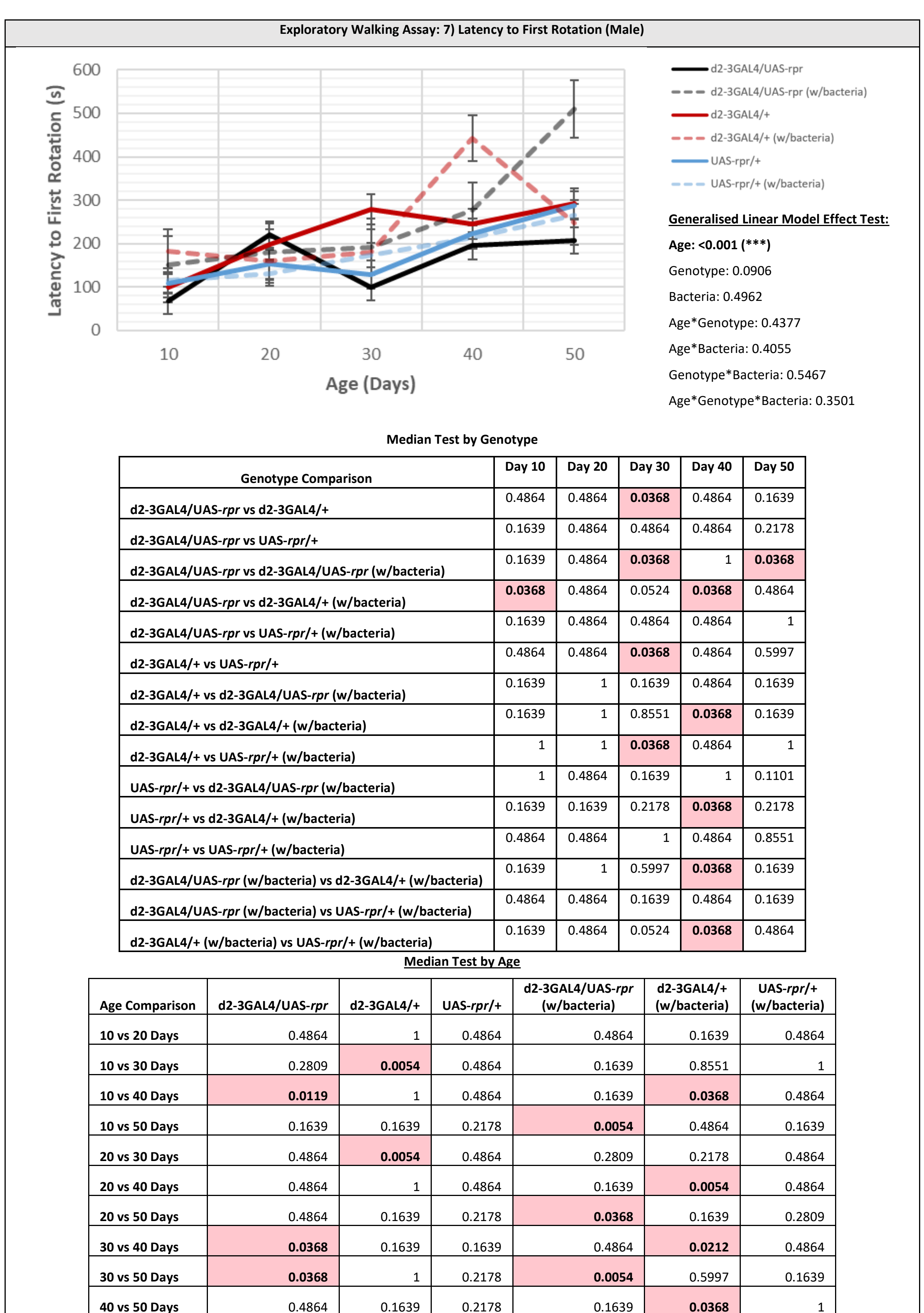
Median Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	1	**0.0368**	0.4864	**0.0368**	0.4864
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.4864	0.4864	0.1639	0.4864	0.4864
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.4864	**0.0368**	0.4864	0.4864	0.4864
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.4864	0.1639	**0.0054**	**0.0368**	N/A
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	1	0.4864	0.1639	0.4864	1
d2-3GAL4/+ vs UAS-*rpr*/+	0.4864	0.4864	0.1639	1	0.1639
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.4864	1	0.4864	0.1639	1
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.4864	1	0.0723	0.4864	N/A
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	1	0.1639	0.4864	**0.0368**	0.1639
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	**0.0054**	0.4864	1	0.4864	0.1639
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.4864	0.4864	1	1	N/A
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.4864	0.4864	0.4864	0.4864	0.4864
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	**0.0054**	1	0.1639	0.1639	N/A
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.1639	0.1639	1	0.4864	0.1639
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.1639	0.1639	0.4864	**0.0368**	N/A

Median Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	1	0.1639	0.4864	**0.0368**	1	1
10 vs 30 Days	0.1639	0.1639	**0.0368**	**<0.001**	**0.0054**	**<0.001**
10 vs 40 Days	1	**0.0054**	0.1639	**0.0054**	**0.0368**	**0.0054**
10 vs 50 Days	0.4864	0.1639	0.1639	**<0.001**	N/A	0.1639
20 vs 30 Days	0.1639	1	**0.0054**	1	**0.0368**	**0.0054**
20 vs 40 Days	0.4864	**0.0054**	0.1639	1	**0.0368**	**0.0054**
20 vs 50 Days	0.1639	0.1639	0.4864	1	N/A	0.1639
30 vs 40 Days	1	**0.0368**	1	1	1	0.4864
30 vs 50 Days	0.4864	0.4864	0.1639	1	N/A	0.1639
40 vs 50 Days	0.4864	1	0.1639	0.4864	N/A	0.1639

Figure 18) Latency to first rotation for female flies (n = 15-16) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



Median Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.4864	0.4864	**0.0368**	0.4864	0.1639
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.1639	0.4864	0.4864	0.4864	0.2178
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.1639	0.4864	**0.0368**	1	**0.0368**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	**0.0368**	0.4864	0.0524	**0.0368**	0.4864
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.1639	0.4864	0.4864	0.4864	1
d2-3GAL4/+ vs UAS-*rpr*/+	0.4864	0.4864	**0.0368**	0.4864	0.5997
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.1639	1	0.1639	0.4864	0.1639
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.1639	1	0.8551	**0.0368**	0.1639
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	1	1	**0.0368**	0.4864	1
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	1	0.4864	0.1639	1	0.1101
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.1639	0.1639	0.2178	**0.0368**	0.2178
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.4864	0.4864	1	0.4864	0.8551
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.1639	1	0.5997	**0.0368**	0.1639
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.4864	0.4864	0.1639	0.4864	0.1639
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.1639	0.4864	0.0524	**0.0368**	0.4864

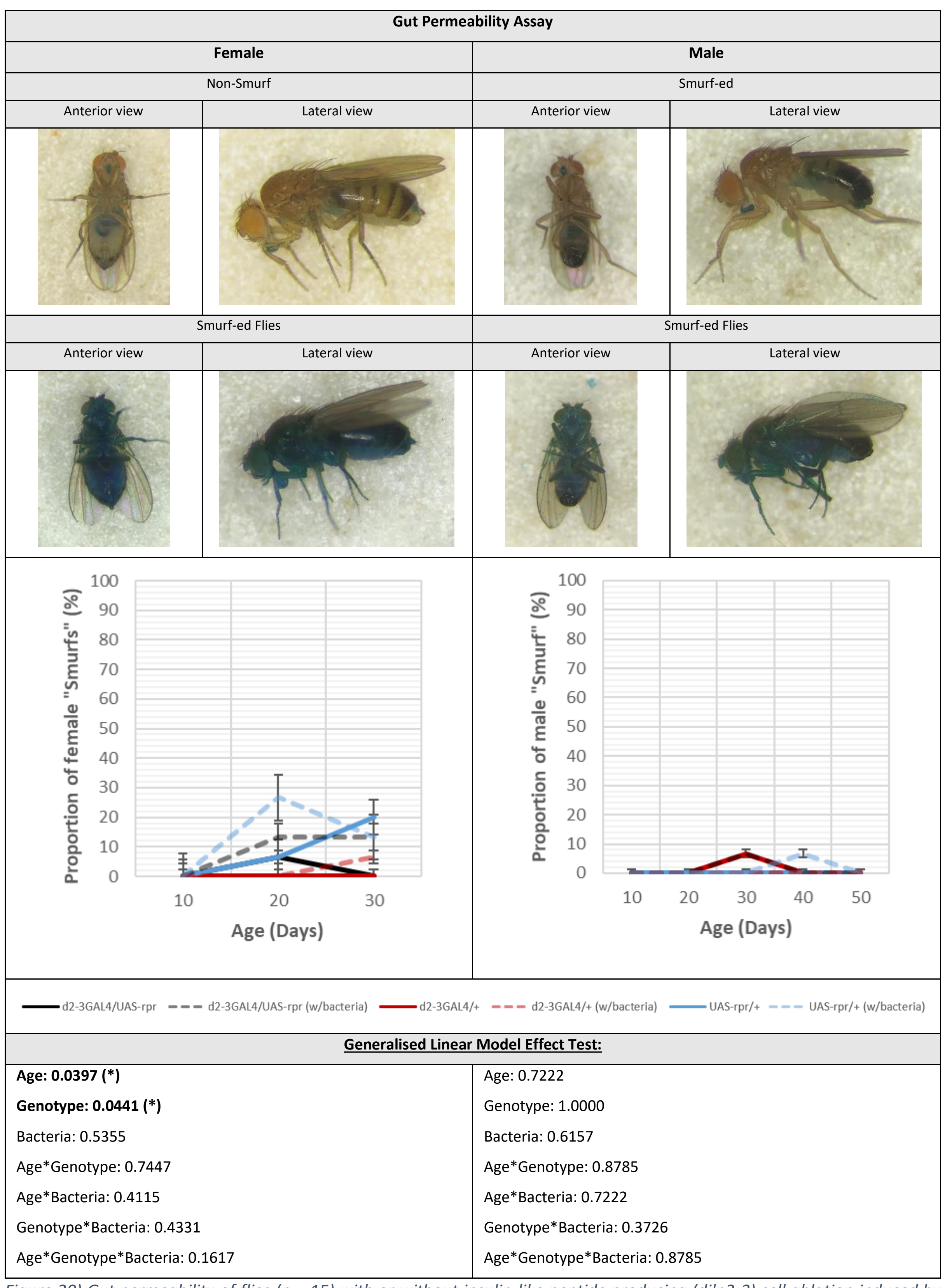
Median Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.4864	1	0.4864	0.4864	0.1639	0.4864
10 vs 30 Days	0.2809	**0.0054**	0.4864	0.1639	0.8551	1
10 vs 40 Days	**0.0119**	1	0.4864	0.1639	**0.0368**	0.4864
10 vs 50 Days	0.1639	0.1639	0.2178	**0.0054**	0.4864	0.1639
20 vs 30 Days	0.4864	**0.0054**	0.4864	0.2809	0.2178	0.4864
20 vs 40 Days	0.4864	1	0.4864	0.1639	**0.0054**	0.4864
20 vs 50 Days	0.4864	0.1639	0.2178	**0.0368**	0.1639	0.2809
30 vs 40 Days	**0.0368**	0.1639	0.1639	0.4864	**0.0212**	0.4864
30 vs 50 Days	**0.0368**	1	0.2178	**0.0054**	0.5997	0.1639
40 vs 50 Days	0.4864	0.1639	0.2178	0.1639	**0.0368**	1

Figure 19) Latency to first rotation for male flies (n = 15-16) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age

5.5.3. Bacteria did not affect gut permeability

The gut permeability or “Smurf” assay evaluates the fly’s gut permeability across age. In aging flies, the gut loses its integrity and are prone to extraintestinal leakage. Therefore, feeding the flies with dyed media allows the visualisation of the compromised intestinal barrier as the dye would have spread beyond the gut. In our experiment, the first instance of flies with altered gut permeability, referred to as "smurfed" flies, served as the benchmark for subsequent assessments (Figure 20). While it is expected that the fly gut permeability increases with age, systemic IIS reduction improves gut integrity in fly by delaying aging-associated dysplasia (155). However, our results reported that systemic IIS reduction and bacteria treatment did not impact the gut permeability of flies across age nor sex. It should be noted that data on gut permeability in female flies was limited to age 30 days, due to a shortage of female d2-3GAL/UAS-*rpr* flies (at age 50 days) and d2-3GAL4/+ flies (at age 40 days onwards).



Generalised Linear Model Effect Test:	
Age: 0.0397 (*)	Age: 0.7222
Genotype: 0.0441 (*)	Genotype: 1.0000
Bacteria: 0.5355	Bacteria: 0.6157
Age*Genotype: 0.7447	Age*Genotype: 0.8785
Age*Bacteria: 0.4115	Age*Bacteria: 0.7222
Genotype*Bacteria: 0.4331	Genotype*Bacteria: 0.3726
Age*Genotype*Bacteria: 0.1617	Age*Genotype*Bacteria: 0.8785

Figure 20) Gut permeability of flies (n = 15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure.

5.5.4. Summary of Results 5.5

Across age, systemic IIS reduction through IIS-producing cell ablation extended the median lifespan of both male and female flies. Similarly, this reduction also slows the decline exploratory walking behaviours in ageing flies. However, these changes are attenuated by the introduction of bacteria as observed from reduced median lifespans and the null effect in exploratory walking parameters. Interestingly, flies with normal IIS function were unaffected by bacteria. Furthermore, neither systemic IIS reduction nor bacteria treatment impacted fly gut integrity in ageing flies. Altogether, these findings suggest that bacteria are not pathogenic to host but affects host IIS.

5.6. Bacteria increased offspring generation but reduced median lifespan and attenuated IIS-reduced changes to sleep behaviour

Building upon Results 5.5, another lifespan assay using a larger sample size was carried out to validate the replicability and Result 5.5.1. Simultaneously, the impacts of bacteria on sleep, neuromuscular function, and fecundity were assessed. Again, systemic IIS reduced flies (d2-3GAL4/UAS-*rpr*) were utilised to probe for any potential bacteria-IIS interactions.

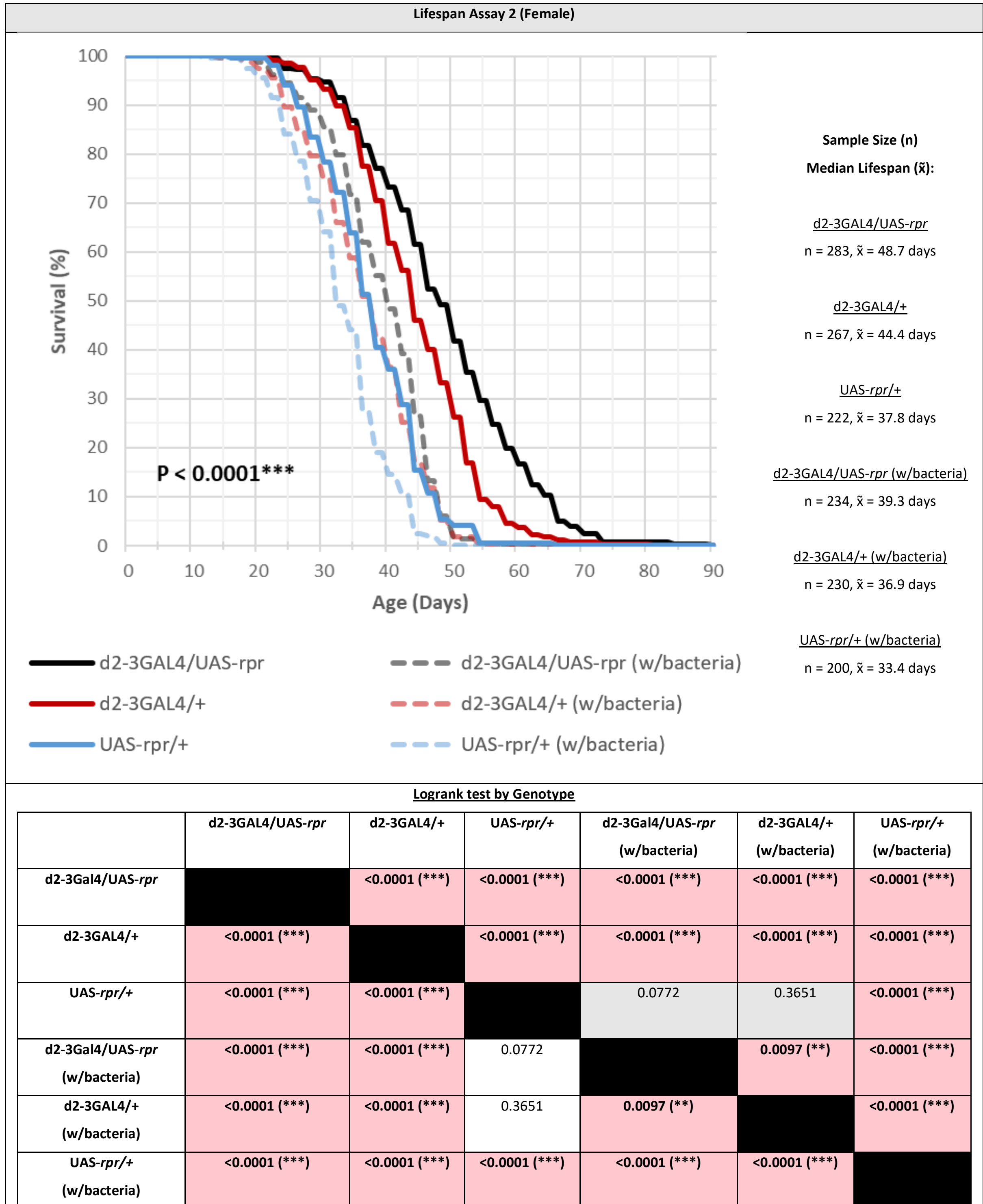
5.6.1. Bacteria reduced the median lifespan of all flies regardless of genotype

This second survival assay aimed to validate Results 5.5.1 using a sample size doubled the previous survival assay. Contrasting above results, systemic IIS reduction only extended median lifespan among female flies (by more than 9% longer) (Figure 21) but not males (Figure 22). When introduced to bacteria, similar results were observed where median lifespan extension, to a lesser extent, reporting a 20% shorter median lifespan than the same flies (with systemic IIS reduction) without bacteria treatment. As both bacteria and non-bacteria treated d2-3GAL4/UAS-*rpr* males do not exhibit lifespan extension, it is possible that the genotype was not properly expressed in male flies in this lifespan assay or external confounders may have disrupted its expression.

Interestingly, all bacteria treated flies regardless of genotype had shorter (at least 10%) median lifespans than their non-bacteria treated counterparts. While this finding was inconsistent in Result 5.5.1, this discrepancy may be due to the difference in sample size. Speculatively, bacteria could be contributing to

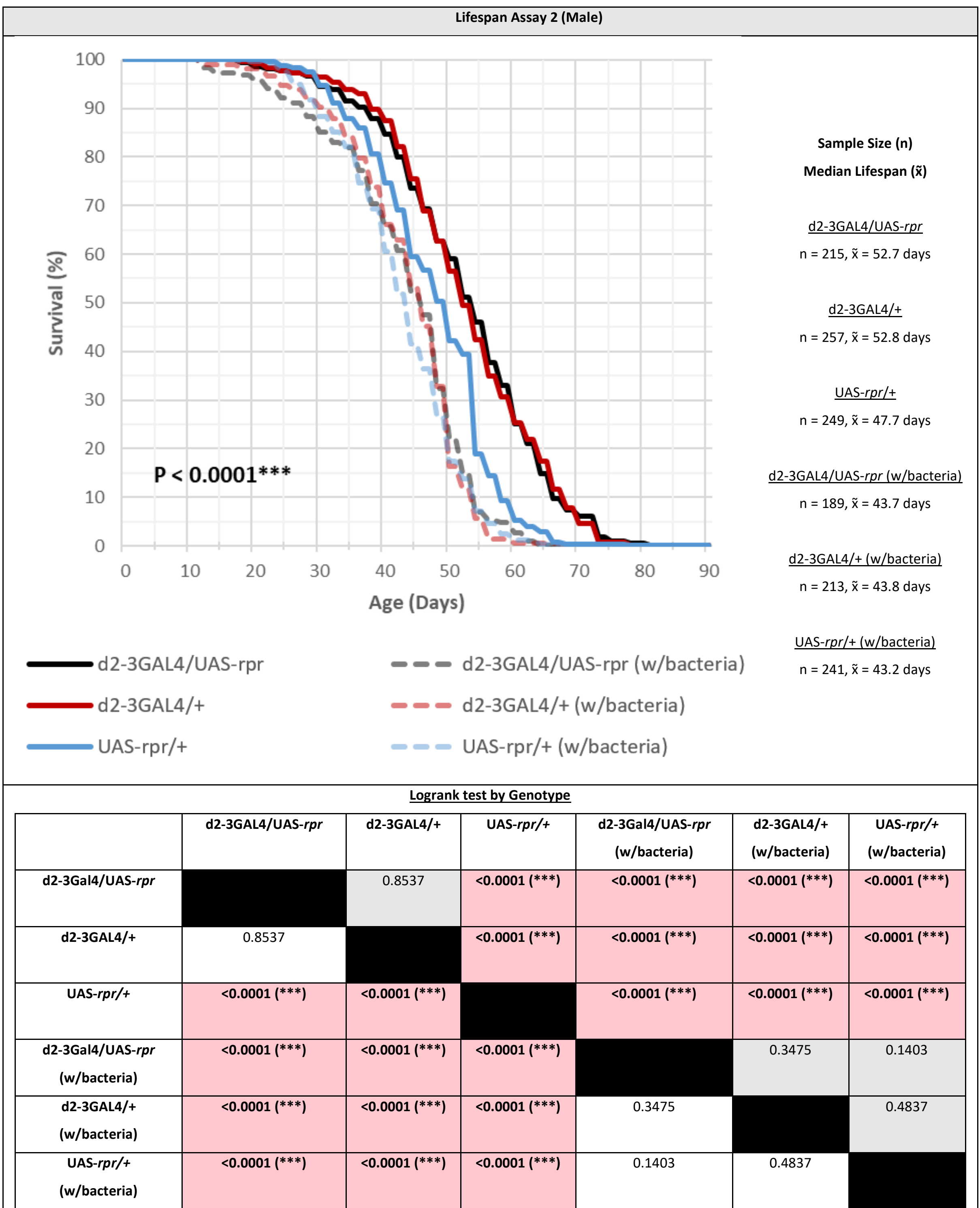
this reduction of median lifespan irrespective of IIS expression. Between controls, the median lifespan of d2-3GAL4/+ flies from both sexes were more than 10 % longer than UAS-*rpr*/+ flies. Similarly observed among bacteria treated groups, these differences suggest that d2-3GAL4 and UAS-*rpr* alone may influence fly lifespan. However, a wildtype control was not included in this assay to validate this result.

Taken together, these results recapitulate the attenuating effects of bacteria against lifespan extension mediated by systemic IIS reduction. However, median lifespan extension was only observed among systemic IIS reduced female flies but not male flies. Regardless, any median lifespan extending effects were attenuated by bacteria treatment. Moreover, bacteria may also reduce fly median lifespan as all flies treated with bacteria reported shorter median lifespans than their respective controls.



	d2-3GAL4/UAS-*rpr*	**d2-3GAL4/+**	**UAS-*rpr*/+**	**d2-3Gal4/UAS-*rpr* (w/bacteria)**	**d2-3GAL4/+ (w/bacteria)**	**UAS-*rpr*/+ (w/bacteria)**
d2-3Gal4/UAS-*rpr*		**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**
d2-3GAL4/+	**<0.0001 (***)**		**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**
UAS-*rpr*/+	**<0.0001 (***)**	**<0.0001 (***)**		0.0772	0.3651	**<0.0001 (***)**
d2-3Gal4/UAS-*rpr* (w/bacteria)	**<0.0001 (***)**	**<0.0001 (***)**	0.0772		**0.0097 (**)**	**<0.0001 (***)**
d2-3GAL4/+ (w/bacteria)	**<0.0001 (***)**	**<0.0001 (***)**	0.3651	**0.0097 (**)**		**<0.0001 (***)**
UAS-*rpr*/+ (w/bacteria)	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	

Figure 21) Second lifespan results of female flies (n = 200-283) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure



Logrank test by Genotype

	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr/+*	d2-3Gal4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr/+* (w/bacteria)
d2-3Gal4/UAS-*rpr*		0.8537	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**
d2-3GAL4/+	0.8537		**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**
UAS-*rpr/+*	**<0.0001 (***)**	**<0.0001 (***)**		**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**
d2-3Gal4/UAS-*rpr* (w/bacteria)	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**		0.3475	0.1403
d2-3GAL4/+ (w/bacteria)	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	0.3475		0.4837
UAS-*rpr/+* (w/bacteria)	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	0.1403	0.4837	

Figure 22) Second lifespan results of male flies (n = 189-257) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure

5.6.2. Bacteria attenuated changes to sleep behaviours driven by IPC ablation

Using Drosophila activity monitors (DAMs), sleep assays allow the analysis of sleep patterns and behaviours of flies across life. As flies age, they sleep longer but experience more sleep fragmentations resulting from the increasing number of sleep bouts (156). Vice versa, systemic IIS reduced flies sleep less but have fewer sleep fragmentations (157,158) In this experiment, sleep length was measured through the parameters of total sleep and total activity. Simultaneously, total sleep bouts and mean sleep bouts represents the number of sleep fragmentation in flies. If a fly exhibits an increased number of sleep bouts, it indicates a higher degree of sleep fragmentation. To further probe these interactions against environmental cues, readings were stratified between dark and light cycles. It should be noted that sample size reported in this assay varies as flies that died during sleep data collection were omitted from the study (Appendix 10.13). Here, changes to sleep behaviour were more prominent among females than males, where bacteria affected sleep changes caused by systemic IIS reduction.

Across age, female (Figure 23) flies with systemic IIS reduction showed a decline in total sleep. Oddly, control flies did not show a consistent pattern as d2-3GAL4/+ flies but not UAS-*rpr*/+ flies reported a similar decline. While declining total sleep among systemic IIS reduced flies may be attributed to impaired IIS, differences between controls may be attributed to unforeseen confounding factors affecting sleep. As d2-3GAL4/+ flies without bacteria treatment were the only control group reporting contrasting sleep results, it was conjectured that d2-3GAL4/+ flies were perturbed by external variables. When

treated with bacteria, all flies had a constant total sleep throughout life regardless of genotype. This meant that bacteria may have compensated for the declining total sleep induced by systemic IIS reduction or confounding factor found among d2-3GAL4/+ flies. As for males (Figure 24), total sleep declined across all genotypes. In the presence of bacteria, there was an increased to total sleep at late age, but the increase was not found significant compared to non-bacteria treated flies apart from d2-3GAL4/+ flies. This demonstrates that bacteria may help recover declining total sleep at beyond age 50 days but there were insufficient results to confirm this.

During dark cycles, female (Figure 25) flies with systemic IIS reduction did not experience changes in total sleep across life. Similarly, d2-3GAL4/+ flies did not experience increases to total dark sleep but UAS-*rpr*/+ flies did. When treated with bacteria, total dark sleep across all genotypes matched that of UAS-*rpr*/+ non bacteria treated controls. On the other hand, total dark sleep declined across all male (Figure *26*) fly genotypes. While bacteria treated flies also showed similar declines, total dark sleep trended upwards at age 50 days. However, these changes were not statistically significant between all genotype and bacteria treatment groups.

As for light cycles, systemic IIS reduced female (Figure 27) flies showed a decline in total sleep. Again, controls do not report consistent findings as d2-3GAL4/+ flies experienced similar declines while UAS-*rpr*/+ flies maintained constant total sleep across age. Among bacteria groups, systemic IIS reduced flies experienced the same decline to total light sleep as their non-bacteria counterparts. Comparatively, control flies reported changes in total light sleep akin to UAS-*rpr*/+ flies without bacteria treatment. Hence, it may be speculated that bacteria treatment does not rescue total light sleep in systemic IIS reduced

flies and confounding factors may have decreased total sleep among d2-3GAL4/+ controls. As for males (Figure 28), total light sleep fluctuated across age but remained constant across all genotypes. Collectively, systemic IIS reduction decreased total sleep in female (but not male) flies, by reducing sleep during light cycles and preventing the increase of sleep during dark cycles throughout life. When treated with bacteria, total sleep among IPC ablated flies was restored to levels similar to controls at later age via increasing sleep during dark cycles. However, total sleep during light cycles remains unaffected. Therefore, the effects of bacteria against IPC ablated fly host sleep behaviour may be photoperiod sensitive.

As total activity is inversely correlated to total sleep, female (Figure 29) flies with systemic IIS reduction showed increased total activity with age. Likewise, findings among controls were inconsistent as d2-3GAL4/+ flies reported increased activity while UAS-*rpr*/+ flies remained unaffected throughout life. Furthermore, bacteria treatment decreased the total activity down of all flies regardless of genotype to baseline levels across age. Therefore, systemic IIS reduction increased total activity in female flies, but this effect was attenuated by bacteria. Similar to total sleep results, there may have been external confounders that increased the total activity of d2-3GAL4/+ flies with age. Among males (Figure 30), total activity trended upwards among all flies regardless of genotype. While an upward trend was also found among bacteria treated flies at late age, there no significant differences when compared to their non-bacteria treated counterparts except for d2-3GAL4/+ flies.

Regarding total sleep bouts, all female (Figure 31) flies regardless of genotype and bacteria treatment experienced an increase in total sleep bouts. This increase began after age 10 days and plateaued after age 20 days.

Conversely, total sleep bouts among male (Figure 32) flies remained relatively constant. During dark cycles, no changes to total sleep bouts were found among female (Figure 33) and male (Figure 34) flies regardless of genotype and bacterial condition. During light cycles, systemic IIS reduction did not affect total sleep bouts across female (Figure 35) flies. Similar to total sleep results above, UAS-*rpr*/+ but not d2-3GAL4/+ control flies experienced an increase in total sleep bouts across age. When treated with bacteria, systemic IIS reduced and UAS-*rpr*/+ flies reported no difference to their respective controls while d2-3GAL4/+ flies saw an increase in total sleep bouts matching UAS-*rpr*/+ flies. Regardless, changes to total sleep bouts during light cycles across age remained relatively similar across genotypes with differences only found at age 30 days. Compared to males (Figure 36), there were no differences of total sleep bouts found across genotype and bacteria treatment across age. Altogether, total sleep bouts naturally increased with age among female flies but not males, while systemic IIS reduction prevents further increase of this parameter during light cycle. Unlike the results of total sleep, bacteria did not affect changes to total sleep bouts regardless of sex nor genotype.

As for mean sleep bout length, all female (Figure 37) flies regardless of genotype or bacteria exposure experienced an early age decline at age 10 days that plateaued after age 20 days. However, no significant differences were found between genotypes across age. Similarly, fluctuations in mean sleep bout length were found across males (Figure 38) but there were no significant differences found between all experimental conditions. During dark cycles, mean sleep bout length remain relatively consistent throughout age among females (Figure 39) and males (Figure 40), with only minor fluctuations found across genotype and bacterial conditions. As for light cycles, all female (Figure

41) shared a declined in total sleep bout length at age 10 days to plateau at later age. However, no differences were observed across all experimental conditions. Likewise, some fluctuations in total sleep bout length were found in males (Figure 42) but no differences were found across any of the experimental conditions. Hence, systemic IIS reduction and bacteria do not influence mean sleep bout length of flies with age.

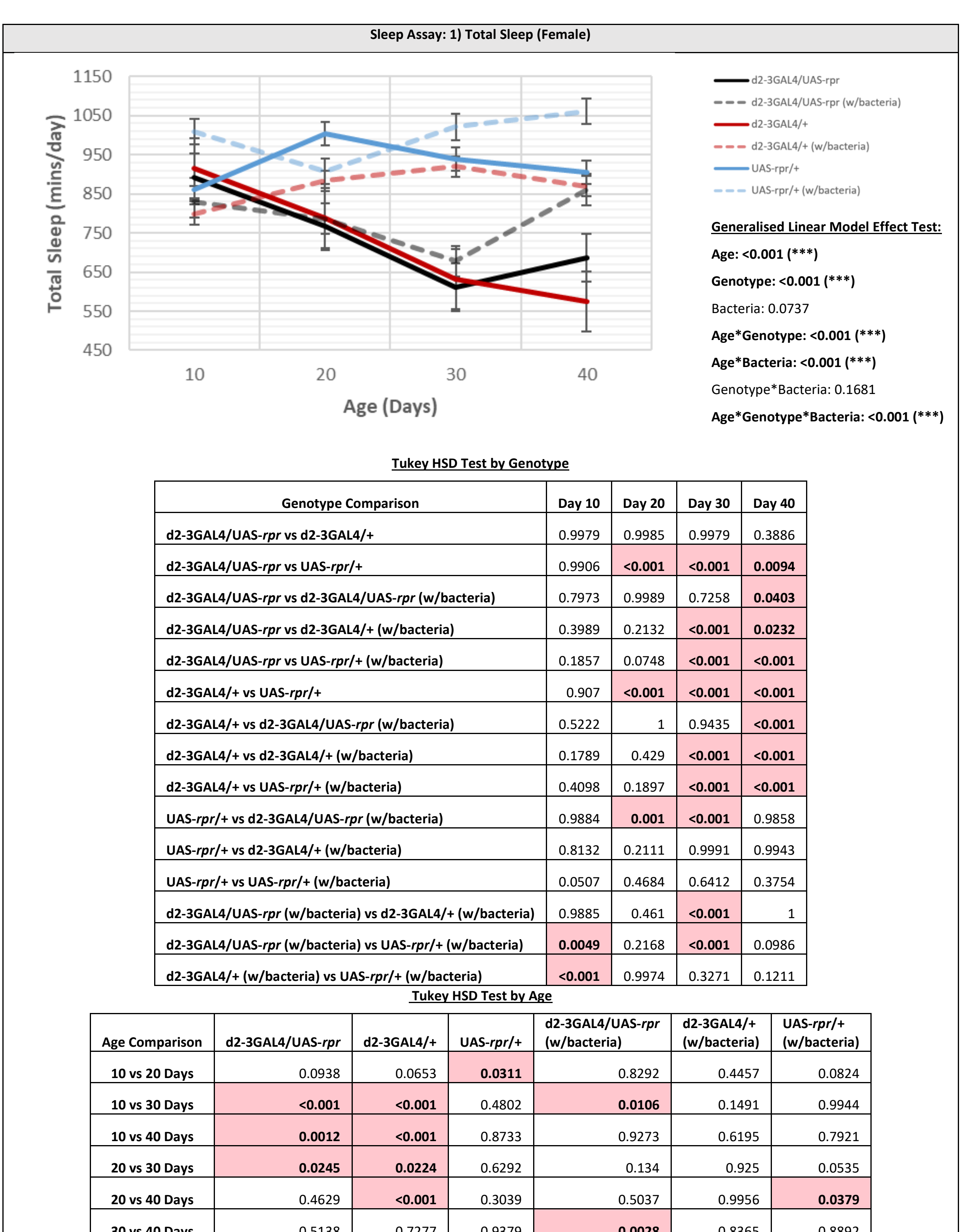
Overall, female (Table 12) flies experience more sleep fragmentations with age due to their increased number of sleep bouts while male (Table 13) flies are sleeping less with age. Oddly, inconsistent findings were observed in normal females between control genotypes, indicating the possibility of confounding factors or genotype-specific interactions affecting sleep results. Nonetheless, systemic IIS reduction decreased total sleep in female flies, and bacteria treatment attenuated these effects, restoring total sleep to levels similar to those found among controls. Despite affecting total sleep, neither bacteria treatment and systemic IIS reduction significantly affect the number nor length of sleep bouts in females. Comparatively, male flies experience declines in total sleep across age but are not affected by systemic IIS reduction nor bacteria treatment. Conflicting with previous studies, it may be possible that d2-3GAL4/UAS-*rpr* was improperly expressed in males (Results 5.6.1) (157,158). All in all, similar to Results 5.5.2, bacteria may only affect host sleep behaviour under the circumstance where host IIS is reduced or when an IPC ablation-induced effect is present.

Table 12) Summarised changes to sleep behaviour across age among female flies with systemic IIS reduction and/or bacteria exposure

Cohort: Female	Baseline Effect	Changes to Baseline Effect			
Condition / Parameter	Controls	Systemic IIS Reduction	Bacteria Treatment	Combined Treatment	Reference
1) Total Sleep	Mixed Results	Declined	Compensatory Effect	Attenuating Effects	Figure 23
2) Total Sleep during Dark Cycles	Mixed Results	Null Effect	Compensatory Effect	Attenuating Effects	Figure 25
3) Total Sleep during Light Cycles	Mixed Results	Declined	Compensatory Effect	Attenuating Effects	Figure 27
4) Total Activity	Mixed Results	Increased	Compensatory Effect	Attenuating Effects	Figure 29
5) Total Sleep Bouts	Increased	No Effect	No Effect	No Effect	Figure 31
6) Total Sleep Bouts during Dark Cycles	No Changes	No Effect	No Effect	No Effect	Figure 33
7) Total Sleep Bouts during Light Cycles	Mixed Results	Null Effect	Compensatory Effect	No Effect	Figure 35
8) Mean Sleep Bout Length	Declined	No Effect	No Effect	No Effect	Figure 37
9) Mean Sleep Bout Length during Dark Cycles	No Changes	No Effect	No Effect	No Effect	Figure 39
10) Mean Sleep Bout Length during Light Cycles	Declined	No Effect	No Effect	No Effect	Figure 41

Table 13) Summarised changes to sleep behaviour across age among male flies with systemic IIS reduction and/or bacteria exposure

Cohort: Male	Baseline Effect	Changes to Baseline Effect			
Condition / Parameter	Controls	Systemic IIS Reduction	Bacteria Treatment	Combined Treatment	Reference
1) Total Sleep	Declined	No Effect	No Effect	No Effect	Figure 24
2) Total Sleep during Dark Cycles	Declined	No Effect	No Effect	No Effect	Figure 26
3) Total Sleep during Light Cycles	No Changes	No Effect	No Effect	No Effect	Figure 28
4) Total Activity	Increased	No Effect	No Effect	No Effect	Figure 30
5) Total Sleep Bouts	No Changes	No Effect	No Effect	No Effect	Figure 32
6) Total Sleep Bouts during Dark Cycles	No Changes	No Effect	No Effect	No Effect	Figure 34
7) Total Sleep Bouts during Light Cycles	No Changes	No Effect	No Effect	No Effect	Figure 36
8) Mean Sleep Bout Length	No Changes	No Effect	No Effect	No Effect	Figure 38
9) Mean Sleep Bout Length during Dark Cycles	No Changes	No Effect	No Effect	No Effect	Figure 40
10) Mean Sleep Bout Length during Light Cycles	No Changes	No Effect	No Effect	No Effect	Figure 42



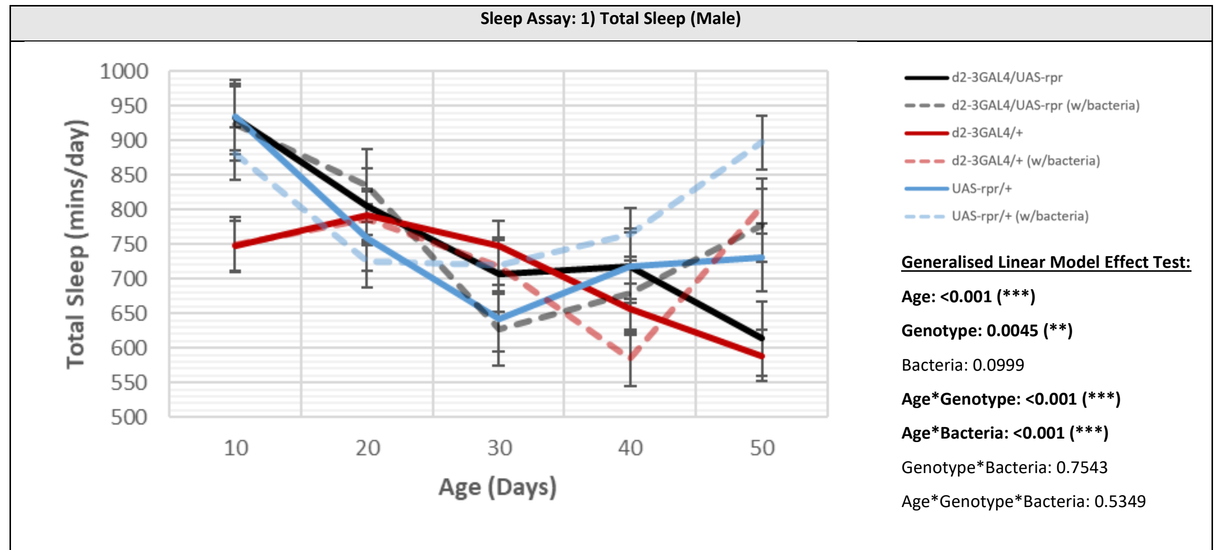
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.9979	0.9985	0.9979	0.3886
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.9906	**<0.001**	**<0.001**	**0.0094**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.7973	0.9989	0.7258	**0.0403**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.3989	0.2132	**<0.001**	**0.0232**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.1857	0.0748	**<0.001**	**<0.001**
d2-3GAL4/+ vs UAS-*rpr*/+	0.907	**<0.001**	**<0.001**	**<0.001**
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.5222	1	0.9435	**<0.001**
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.1789	0.429	**<0.001**	**<0.001**
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.4098	0.1897	**<0.001**	**<0.001**
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9884	**0.001**	**<0.001**	0.9858
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.8132	0.2111	0.9991	0.9943
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.0507	0.4684	0.6412	0.3754
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9885	0.461	**<0.001**	1
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	**0.0049**	0.2168	**<0.001**	0.0986
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	**<0.001**	0.9974	0.3271	0.1211

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.0938	0.0653	**0.0311**	0.8292	0.4457	0.0824
10 vs 30 Days	**<0.001**	**<0.001**	0.4802	**0.0106**	0.1491	0.9944
10 vs 40 Days	**0.0012**	**<0.001**	0.8733	0.9273	0.6195	0.7921
20 vs 30 Days	**0.0245**	**0.0224**	0.6292	0.134	0.925	0.0535
20 vs 40 Days	0.4629	**<0.001**	0.3039	0.5037	0.9956	**0.0379**
30 vs 40 Days	0.5138	0.7277	0.9379	**0.0028**	0.8365	0.8892

Figure 23) Total sleep of female flies (n = 5-15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



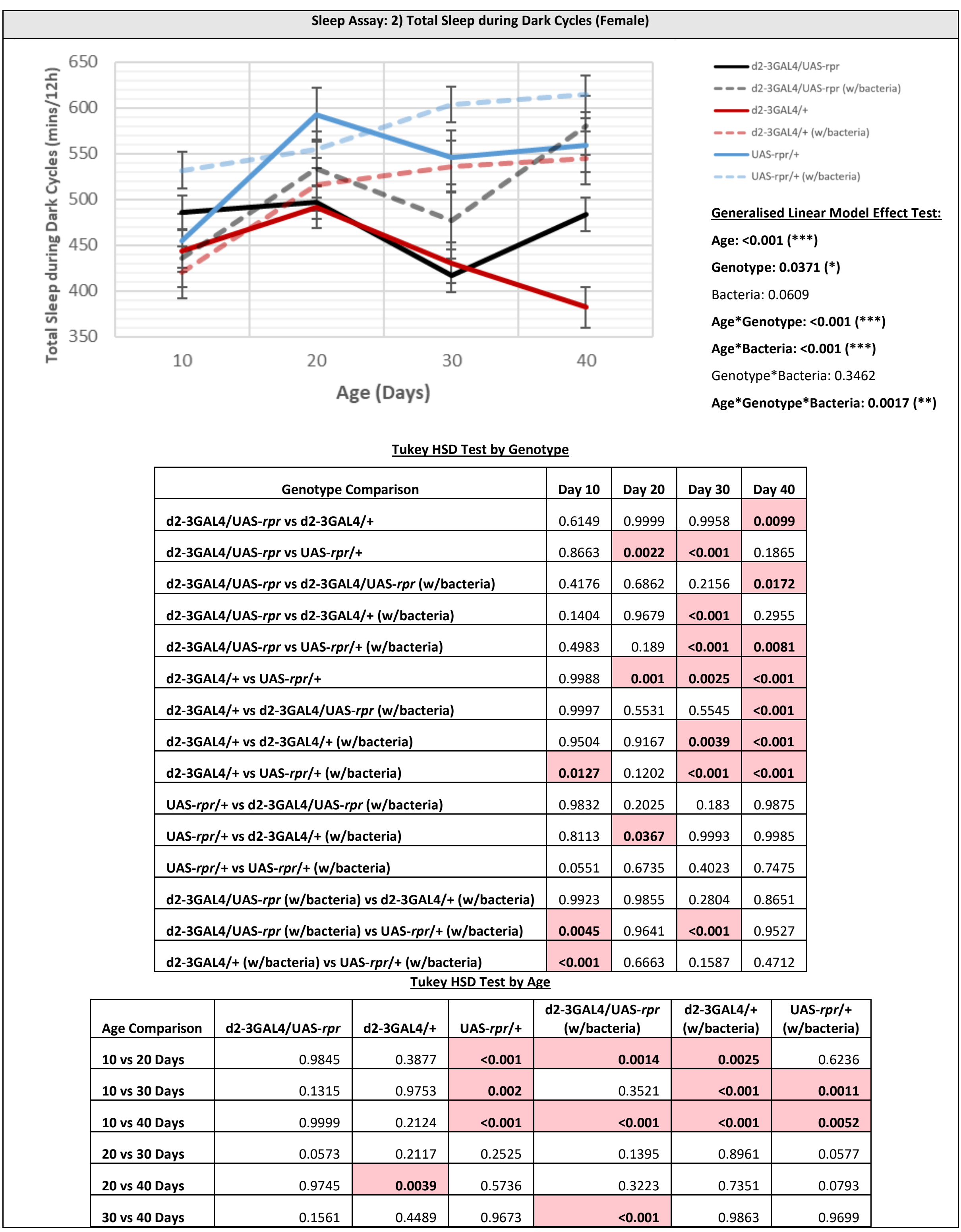
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	**0.0114**	0.9998	0.9828	0.9296	0.9989
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	1	0.9231	0.8941	1	0.3692
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	1	0.9894	0.7712	0.9908	0.0712
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	**0.013**	0.9988	1	0.2899	**0.0382**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.9472	0.5378	0.9999	0.981	**0.0023**
d2-3GAL4/+ vs UAS-*rpr*/+	**0.0096**	0.9813	0.4952	0.9323	0.2161
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	**0.0132**	0.9467	0.3365	0.9994	**0.035**
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	1	1	0.9964	0.8663	**0.0186**
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.1642	0.7165	0.9981	0.571	**0.0011**
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	1	0.6005	0.9998	0.9911	0.968
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	**0.011**	0.9924	0.8245	0.3023	0.8452
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.9429	0.9792	0.8059	0.9823	0.2039
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	**0.015**	0.9161	0.6797	0.6932	0.9976
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.9733	0.1991	0.6586	0.7949	0.5595
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.1784	0.7931	1	0.0682	0.8358

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.1903	0.9316	**0.0133**	0.4753	0.9711	0.0529
10 vs 30 Days	**0.0013**	1	**<0.001**	**<0.001**	0.983	**0.0476**
10 vs 40 Days	**0.0036**	0.5096	**0.0015**	**<0.001**	**0.0461**	0.2789
10 vs 50 Days	**<0.001**	0.0891	**0.0031**	0.0649	0.9149	0.9995
20 vs 30 Days	0.4031	0.9309	0.1805	**0.0019**	0.7844	1
20 vs 40 Days	0.5621	0.1331	0.9453	0.0551	**0.0085**	0.958
20 vs 50 Days	**0.014**	**0.0126**	0.9849	0.8444	0.9987	0.1072
30 vs 40 Days	0.9995	0.5111	0.6342	0.8915	0.1882	0.9396
30 vs 50 Days	0.5339	0.0895	0.4807	0.0577	0.6803	0.097
40 vs 50 Days	0.4275	0.8294	0.9995	0.4406	**0.0089**	0.3499

Figure 24) Total sleep of male flies (n = 5-15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



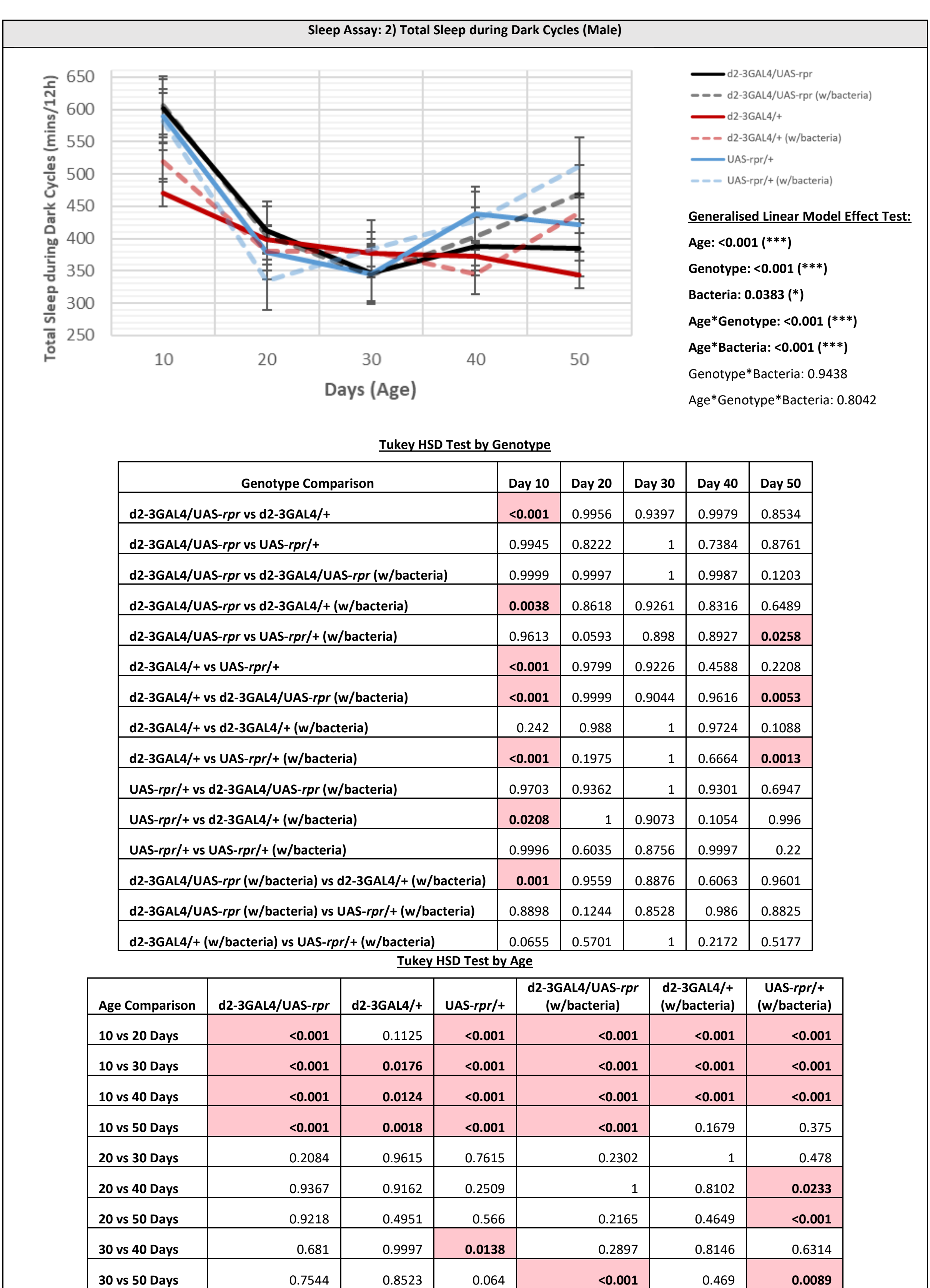
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.6149	0.9999	0.9958	**0.0099**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.8663	**0.0022**	**<0.001**	0.1865
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.4176	0.6862	0.2156	**0.0172**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.1404	0.9679	**<0.001**	0.2955
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.4983	0.189	**<0.001**	**0.0081**
d2-3GAL4/+ vs UAS-*rpr*/+	0.9988	**0.001**	**0.0025**	**<0.001**
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9997	0.5531	0.5545	**<0.001**
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.9504	0.9167	**0.0039**	**<0.001**
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	**0.0127**	0.1202	**<0.001**	**<0.001**
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9832	0.2025	0.183	0.9875
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.8113	**0.0367**	0.9993	0.9985
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.0551	0.6735	0.4023	0.7475
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9923	0.9855	0.2804	0.8651
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	**0.0045**	0.9641	**<0.001**	0.9527
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	**<0.001**	0.6663	0.1587	0.4712

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.9845	0.3877	**<0.001**	**0.0014**	**0.0025**	0.6236
10 vs 30 Days	0.1315	0.9753	**0.002**	0.3521	**<0.001**	**0.0011**
10 vs 40 Days	0.9999	0.2124	**<0.001**	**<0.001**	**<0.001**	**0.0052**
20 vs 30 Days	0.0573	0.2117	0.2525	0.1395	0.8961	0.0577
20 vs 40 Days	0.9745	**0.0039**	0.5736	0.3223	0.7351	0.0793
30 vs 40 Days	0.1561	0.4489	0.9673	**<0.001**	0.9863	0.9699

Figure 25) Total sleep during dark cycles for female flies (n = 5-15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



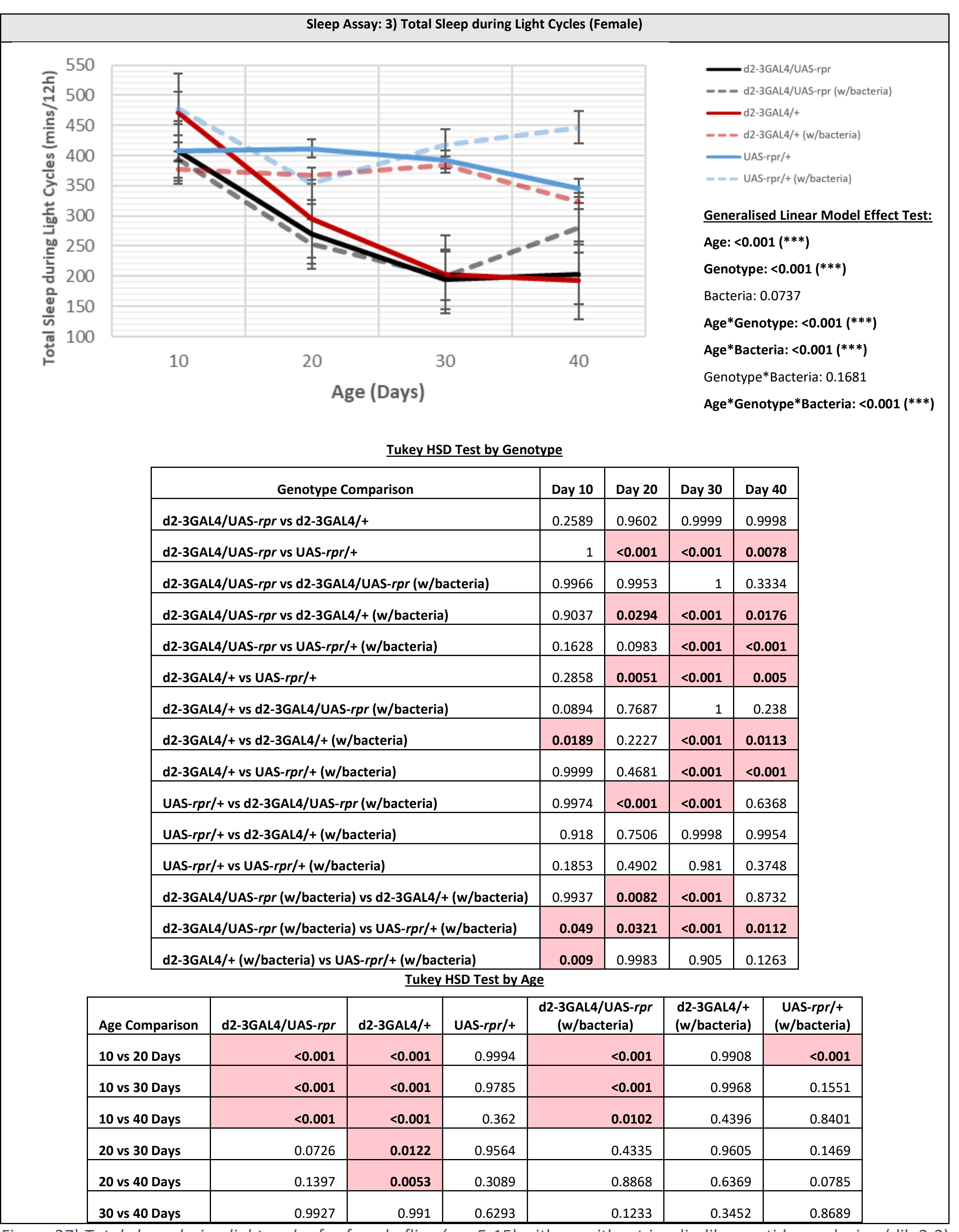
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	**<0.001**	0.9956	0.9397	0.9979	0.8534
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.9945	0.8222	1	0.7384	0.8761
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9999	0.9997	1	0.9987	0.1203
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	**0.0038**	0.8618	0.9261	0.8316	0.6489
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.9613	0.0593	0.898	0.8927	**0.0258**
d2-3GAL4/+ vs UAS-*rpr*/+	**<0.001**	0.9799	0.9226	0.4588	0.2208
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	**<0.001**	0.9999	0.9044	0.9616	**0.0053**
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.242	0.988	1	0.9724	0.1088
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	**<0.001**	0.1975	1	0.6664	**0.0013**
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9703	0.9362	1	0.9301	0.6947
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	**0.0208**	1	0.9073	0.1054	0.996
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.9996	0.6035	0.8756	0.9997	0.22
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	**0.001**	0.9559	0.8876	0.6063	0.9601
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.8898	0.1244	0.8528	0.986	0.8825
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.0655	0.5701	1	0.2172	0.5177

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	**<0.001**	0.1125	**<0.001**	**<0.001**	**<0.001**	**<0.001**
10 vs 30 Days	**<0.001**	**0.0176**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
10 vs 40 Days	**<0.001**	**0.0124**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
10 vs 50 Days	**<0.001**	**0.0018**	**<0.001**	**<0.001**	0.1679	0.375
20 vs 30 Days	0.2084	0.9615	0.7615	0.2302	1	0.478
20 vs 40 Days	0.9367	0.9162	0.2509	1	0.8102	**0.0233**
20 vs 50 Days	0.9218	0.4951	0.566	0.2165	0.4649	**<0.001**
30 vs 40 Days	0.681	0.9997	**0.0138**	0.2897	0.8146	0.6314
30 vs 50 Days	0.7544	0.8523	0.064	**<0.001**	0.469	**0.0089**
40 vs 50 Days	1	0.9243	0.983	0.2194	0.0652	0.1926

Figure 26) Total sleep during dark cycles for male flies *(n = 5-15)* with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (*rpr*) gene activation and bacteria *exposure with age*



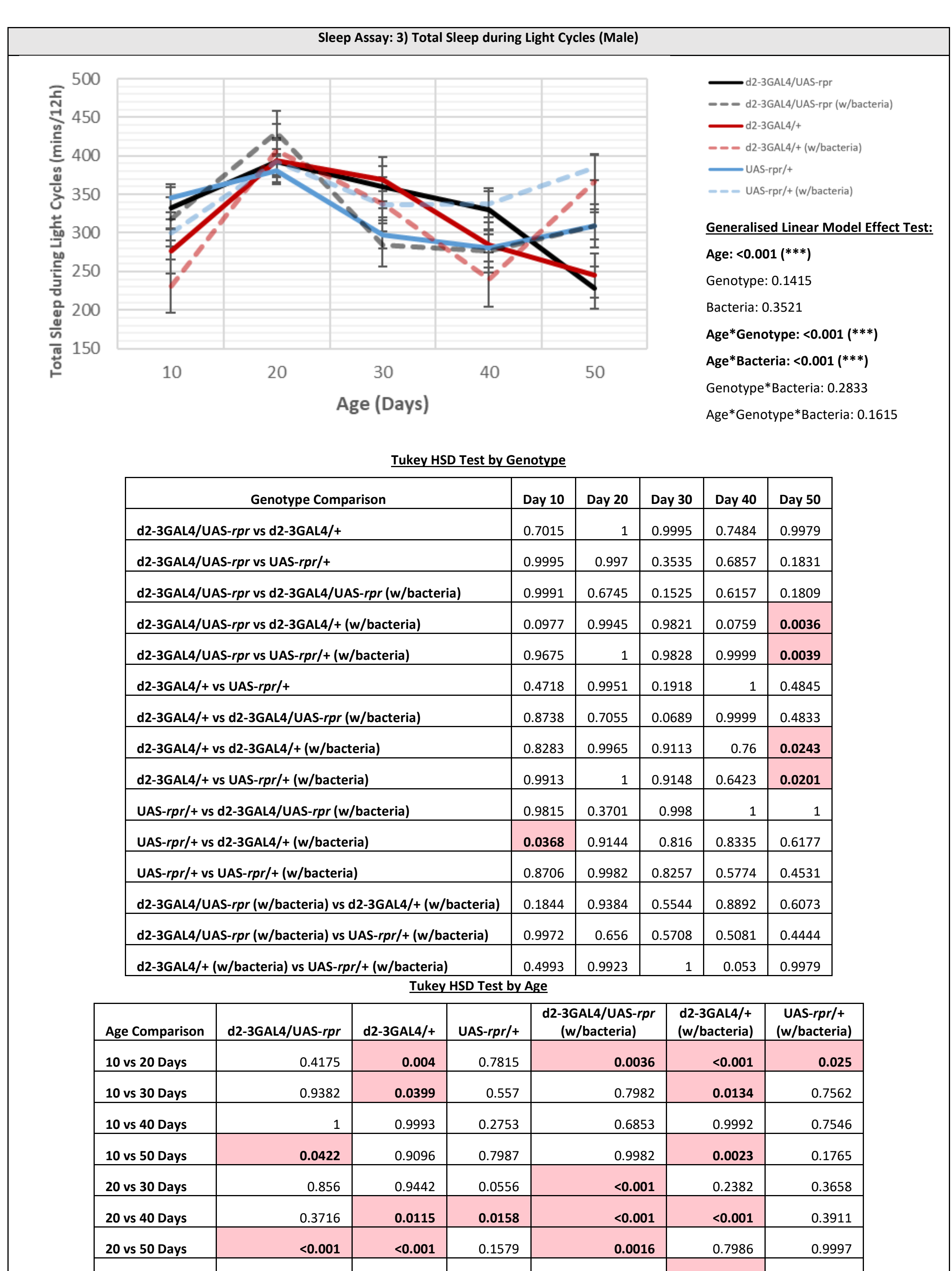
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.2589	0.9602	0.9999	0.9998
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	1	<0.001	<0.001	0.0078
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9966	0.9953	1	0.3334
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.9037	0.0294	<0.001	0.0176
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.1628	0.0983	<0.001	<0.001
d2-3GAL4/+ vs UAS-*rpr*/+	0.2858	0.0051	<0.001	0.005
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.0894	0.7687	1	0.238
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.0189	0.2227	<0.001	0.0113
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.9999	0.4681	<0.001	<0.001
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9974	<0.001	<0.001	0.6368
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.918	0.7506	0.9998	0.9954
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.1853	0.4902	0.981	0.3748
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9937	0.0082	<0.001	0.8732
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.049	0.0321	<0.001	0.0112
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.009	0.9983	0.905	0.1263

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	<0.001	<0.001	0.9994	<0.001	0.9908	<0.001
10 vs 30 Days	<0.001	<0.001	0.9785	<0.001	0.9968	0.1551
10 vs 40 Days	<0.001	<0.001	0.362	0.0102	0.4396	0.8401
20 vs 30 Days	0.0726	0.0122	0.9564	0.4335	0.9605	0.1469
20 vs 40 Days	0.1397	0.0053	0.3089	0.8868	0.6369	0.0785
30 vs 40 Days	0.9927	0.991	0.6293	0.1233	0.3452	0.8689

Figure 27) Total sleep during light cycles for female flies *(n = 5-15)* with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (*rpr*) gene activation and bacteria exposure with age



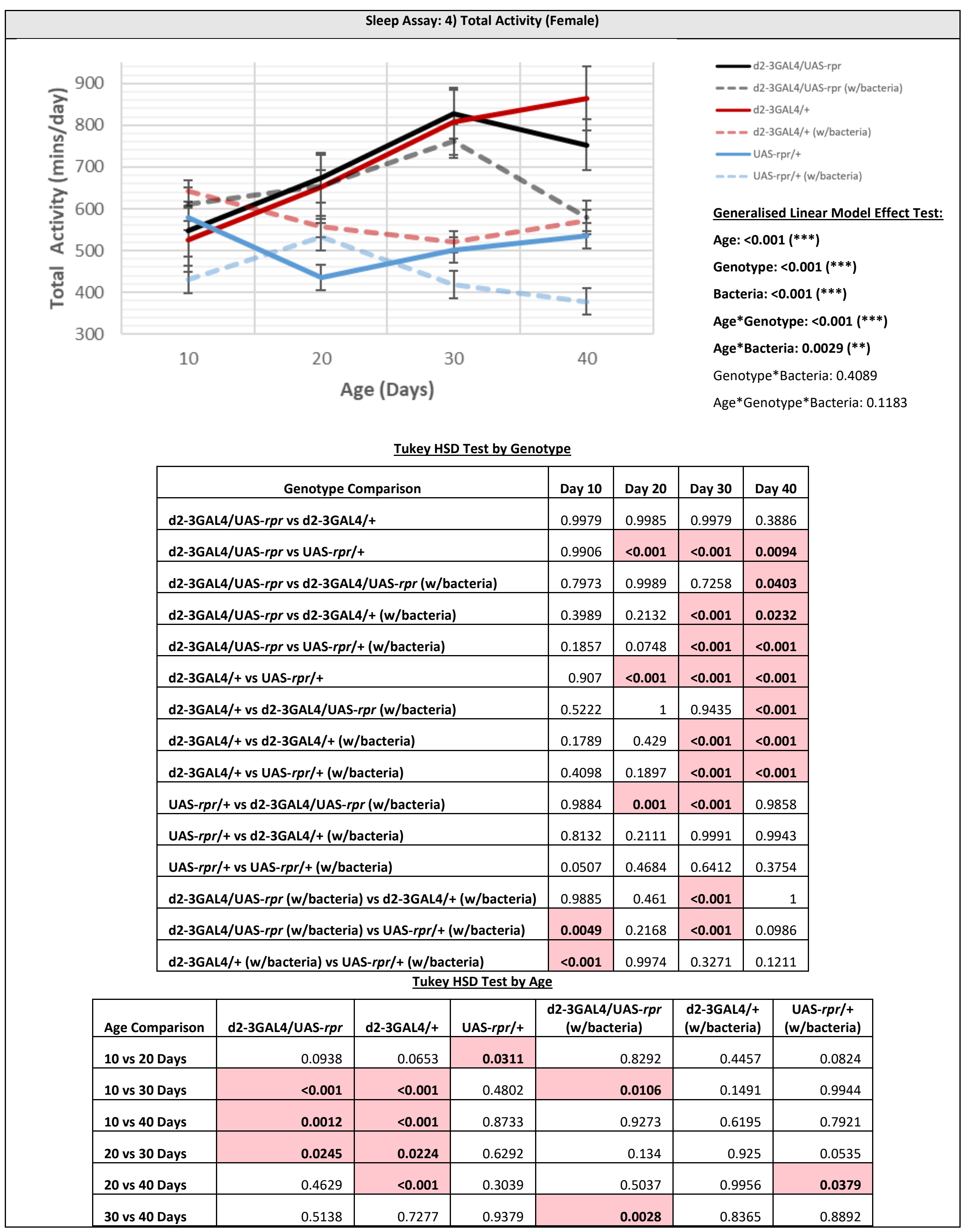
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.7015	1	0.9995	0.7484	0.9979
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.9995	0.997	0.3535	0.6857	0.1831
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9991	0.6745	0.1525	0.6157	0.1809
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.0977	0.9945	0.9821	0.0759	**0.0036**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.9675	1	0.9828	0.9999	**0.0039**
d2-3GAL4/+ vs UAS-*rpr*/+	0.4718	0.9951	0.1918	1	0.4845
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.8738	0.7055	0.0689	0.9999	0.4833
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.8283	0.9965	0.9113	0.76	**0.0243**
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.9913	1	0.9148	0.6423	**0.0201**
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9815	0.3701	0.998	1	1
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	**0.0368**	0.9144	0.816	0.8335	0.6177
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.8706	0.9982	0.8257	0.5774	0.4531
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.1844	0.9384	0.5544	0.8892	0.6073
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.9972	0.656	0.5708	0.5081	0.4444
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.4993	0.9923	1	0.053	0.9979

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.4175	**0.004**	0.7815	**0.0036**	**<0.001**	**0.025**
10 vs 30 Days	0.9382	**0.0399**	0.557	0.7982	**0.0134**	0.7562
10 vs 40 Days	1	0.9993	0.2753	0.6853	0.9992	0.7546
10 vs 50 Days	**0.0422**	0.9096	0.7987	0.9982	**0.0023**	0.1765
20 vs 30 Days	0.856	0.9442	0.0556	**<0.001**	0.2382	0.3658
20 vs 40 Days	0.3716	**0.0115**	**0.0158**	**<0.001**	**<0.001**	0.3911
20 vs 50 Days	**<0.001**	**<0.001**	0.1579	**0.0016**	0.7986	0.9997
30 vs 40 Days	0.9184	0.087	0.9827	0.9992	**0.0325**	1
30 vs 50 Days	**0.0029**	**0.0072**	0.9964	0.9342	0.936	0.7132
40 vs 50 Days	**0.0444**	0.8276	0.9056	0.8589	**0.006**	0.7288

Figure 28) Total sleep during light cycles for male flies *(n = 5-15)* with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (*rpr*) gene activation and bacteria *exposure with age*



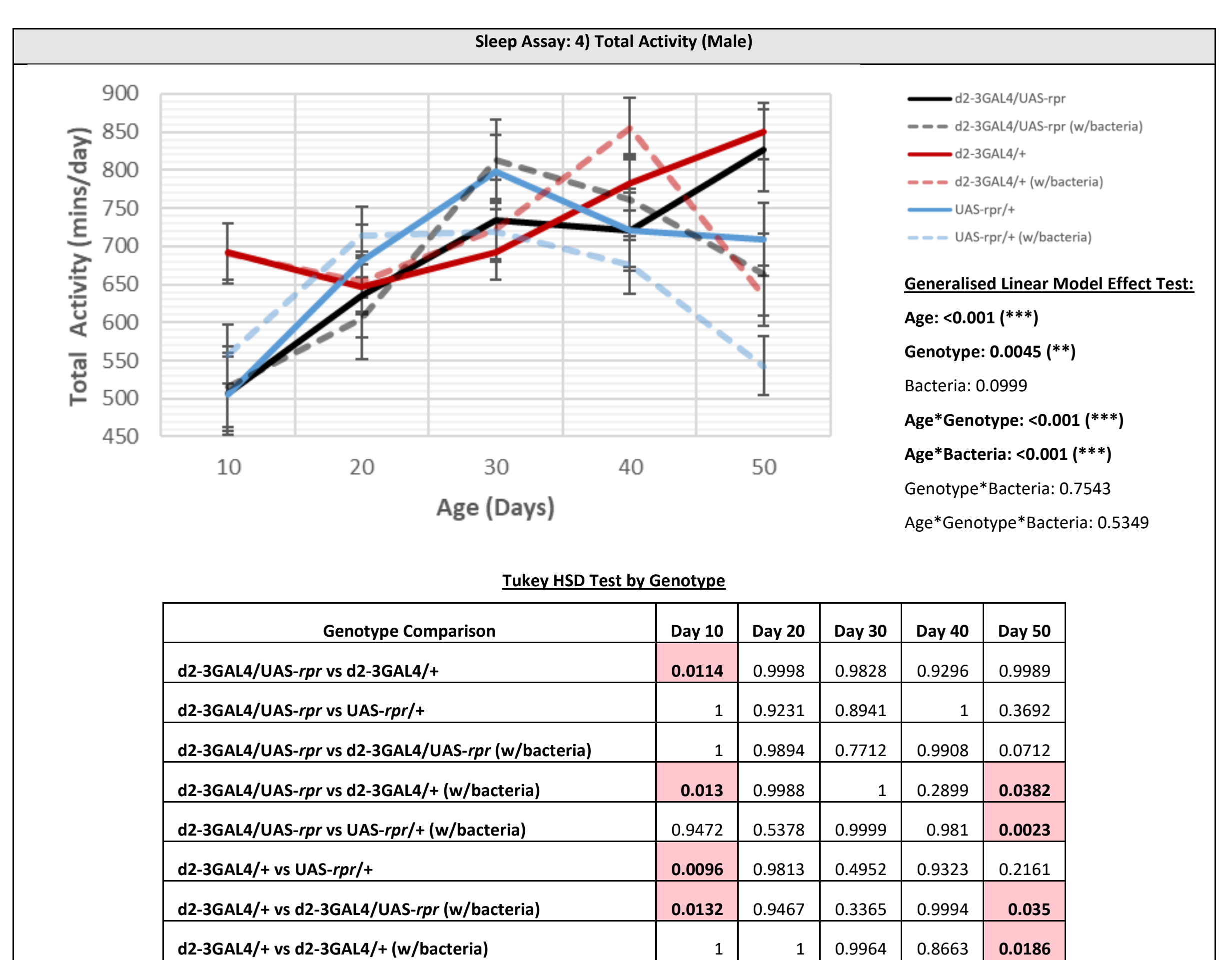
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.9979	0.9985	0.9979	0.3886
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.9906	<0.001	<0.001	0.0094
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.7973	0.9989	0.7258	0.0403
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.3989	0.2132	<0.001	0.0232
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.1857	0.0748	<0.001	<0.001
d2-3GAL4/+ vs UAS-*rpr*/+	0.907	<0.001	<0.001	<0.001
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.5222	1	0.9435	<0.001
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.1789	0.429	<0.001	<0.001
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.4098	0.1897	<0.001	<0.001
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9884	0.001	<0.001	0.9858
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.8132	0.2111	0.9991	0.9943
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.0507	0.4684	0.6412	0.3754
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9885	0.461	<0.001	1
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.0049	0.2168	<0.001	0.0986
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	<0.001	0.9974	0.3271	0.1211

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.0938	0.0653	0.0311	0.8292	0.4457	0.0824
10 vs 30 Days	<0.001	<0.001	0.4802	0.0106	0.1491	0.9944
10 vs 40 Days	0.0012	<0.001	0.8733	0.9273	0.6195	0.7921
20 vs 30 Days	0.0245	0.0224	0.6292	0.134	0.925	0.0535
20 vs 40 Days	0.4629	<0.001	0.3039	0.5037	0.9956	0.0379
30 vs 40 Days	0.5138	0.7277	0.9379	0.0028	0.8365	0.8892

Figure 29) Total activity of female flies (n = 5-15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



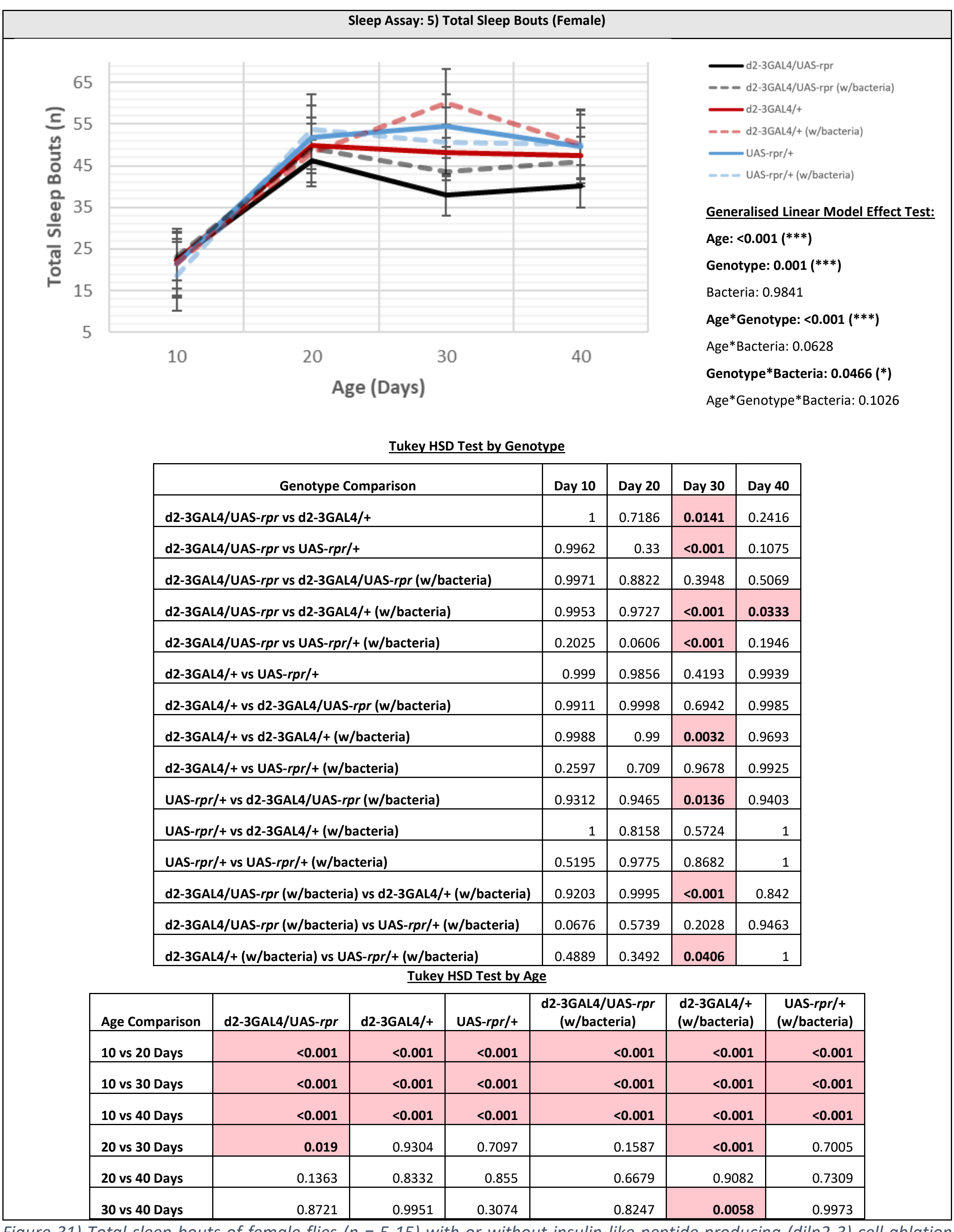
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	**0.0114**	0.9998	0.9828	0.9296	0.9989
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	1	0.9231	0.8941	1	0.3692
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	1	0.9894	0.7712	0.9908	0.0712
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	**0.013**	0.9988	1	0.2899	**0.0382**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.9472	0.5378	0.9999	0.981	**0.0023**
d2-3GAL4/+ vs UAS-*rpr*/+	**0.0096**	0.9813	0.4952	0.9323	0.2161
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	**0.0132**	0.9467	0.3365	0.9994	**0.035**
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	1	1	0.9964	0.8663	**0.0186**
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.1642	0.7165	0.9981	0.571	**0.0011**
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	1	0.6005	0.9998	0.9911	0.968
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	**0.011**	0.9924	0.8245	0.3023	0.8452
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.9429	0.9792	0.8059	0.9823	0.2039
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	**0.015**	0.9161	0.6797	0.6932	0.9976
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.9733	0.1991	0.6586	0.7949	0.5595
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.1784	0.7931	1	0.0682	0.8358

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.1903	0.9316	**0.0133**	0.4753	0.9711	0.0529
10 vs 30 Days	**0.0013**	1	**<0.001**	**<0.001**	0.983	**0.0476**
10 vs 40 Days	**0.0036**	0.5096	**0.0015**	**<0.001**	**0.0461**	0.2789
10 vs 50 Days	**<0.001**	0.0891	**0.0031**	0.0649	0.9149	0.9995
20 vs 30 Days	0.4031	0.9309	0.1805	**0.0019**	0.7844	1
20 vs 40 Days	0.5621	0.1331	0.9453	0.0551	**0.0085**	0.958
20 vs 50 Days	**0.014**	**0.0126**	0.9849	0.8444	0.9987	0.1072
30 vs 40 Days	0.9995	0.5111	0.6342	0.8915	0.1882	0.9396
30 vs 50 Days	0.5339	0.0895	0.4807	0.0577	0.6803	0.097
40 vs 50 Days	0.4275	0.8294	0.9995	0.4406	**0.0089**	0.3499

Figure 30) Total activity of male flies *(n = 5-15)* with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (*rpr*) gene activation and bacteria *exposure with age*



Tukey HSD Test by Genotype

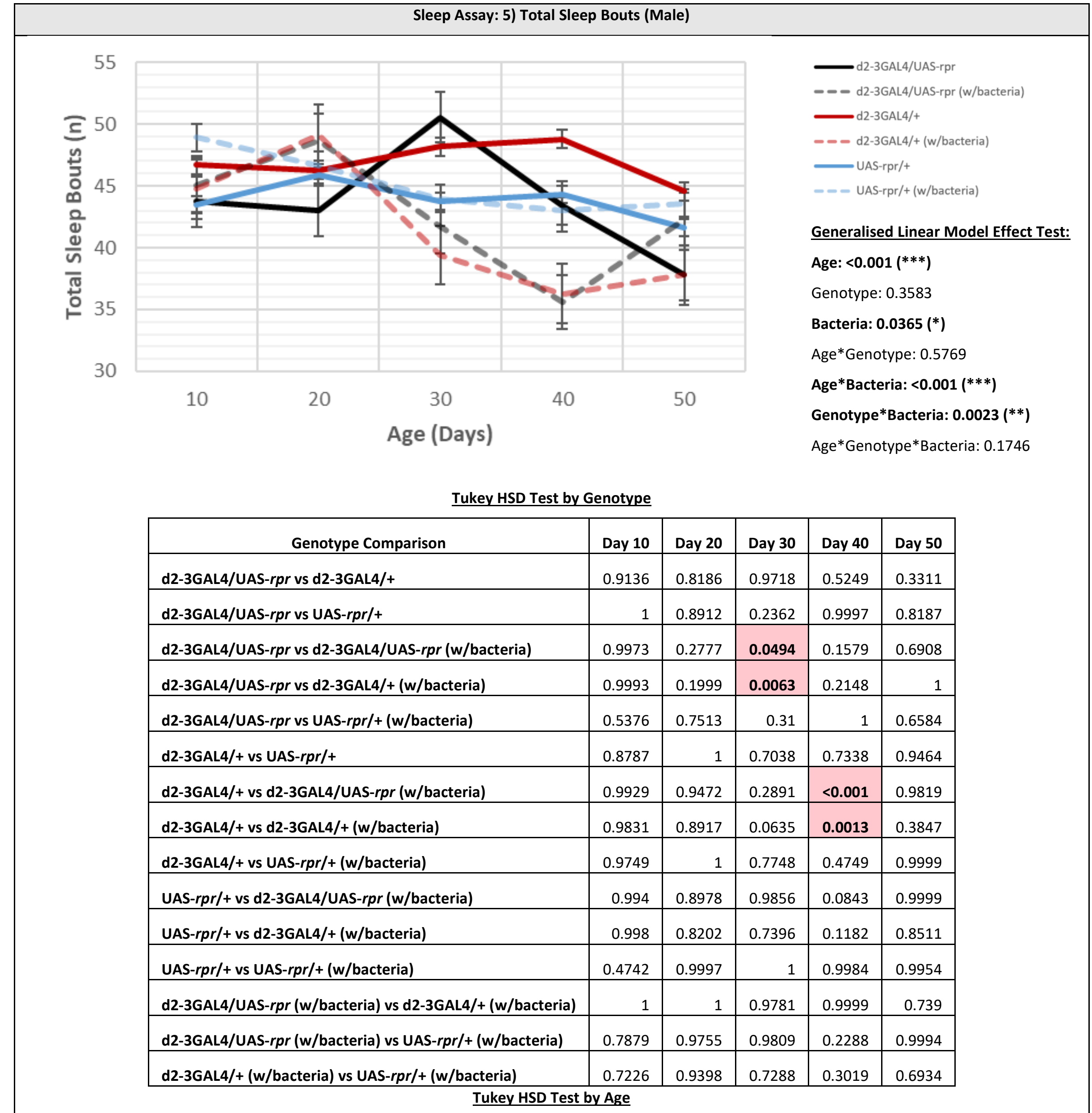
Genotype Comparison	Day 10	Day 20	Day 30	Day 40
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	1	0.7186	**0.0141**	0.2416
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.9962	0.33	**<0.001**	0.1075
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9971	0.8822	0.3948	0.5069
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.9953	0.9727	**<0.001**	**0.0333**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.2025	0.0606	**<0.001**	0.1946
d2-3GAL4/+ vs UAS-*rpr*/+	0.999	0.9856	0.4193	0.9939
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9911	0.9998	0.6942	0.9985
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.9988	0.99	**0.0032**	0.9693
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.2597	0.709	0.9678	0.9925
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9312	0.9465	**0.0136**	0.9403
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	1	0.8158	0.5724	1
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.5195	0.9775	0.8682	1
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9203	0.9995	**<0.001**	0.842
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.0676	0.5739	0.2028	0.9463
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.4889	0.3492	**0.0406**	1

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
10 vs 30 Days	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
10 vs 40 Days	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
20 vs 30 Days	**0.019**	0.9304	0.7097	0.1587	**<0.001**	0.7005
20 vs 40 Days	0.1363	0.8332	0.855	0.6679	0.9082	0.7309
30 vs 40 Days	0.8721	0.9951	0.3074	0.8247	**0.0058**	0.9973

Figure 31) Total sleep bouts of female flies (n = 5-15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age

Sleep Assay: 5) Total Sleep Bouts (Male)



Generalised Linear Model Effect Test:

Age: <0.001 (***)

Genotype: 0.3583

Bacteria: 0.0365 (*)

Age*Genotype: 0.5769

Age*Bacteria: <0.001 (***)

Genotype*Bacteria: 0.0023 (**)

Age*Genotype*Bacteria: 0.1746

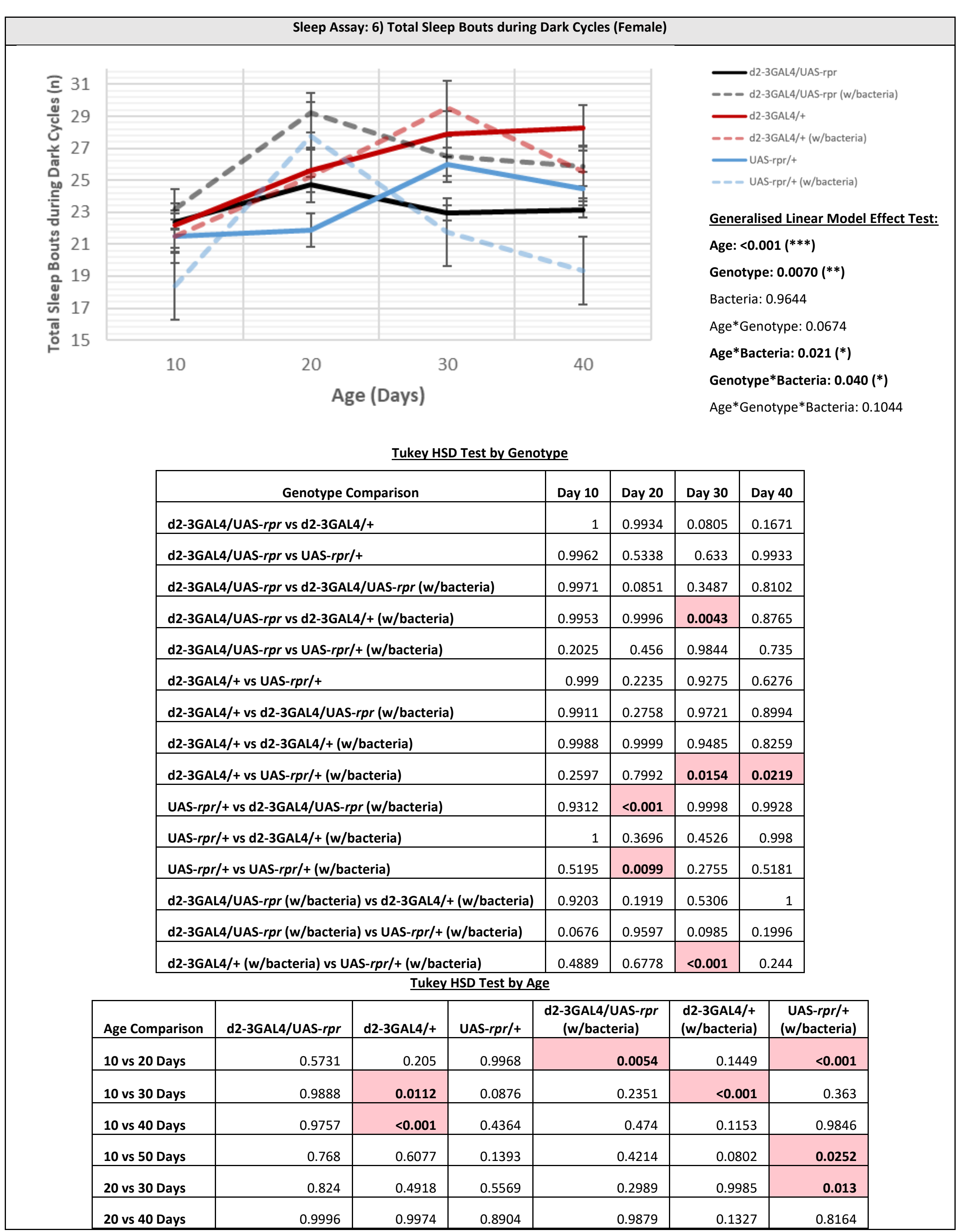
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.9136	0.8186	0.9718	0.5249	0.3311
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	1	0.8912	0.2362	0.9997	0.8187
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9973	0.2777	**0.0494**	0.1579	0.6908
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.9993	0.1999	**0.0063**	0.2148	1
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.5376	0.7513	0.31	1	0.6584
d2-3GAL4/+ vs UAS-*rpr*/+	0.8787	1	0.7038	0.7338	0.9464
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9929	0.9472	0.2891	**<0.001**	0.9819
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.9831	0.8917	0.0635	**0.0013**	0.3847
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.9749	1	0.7748	0.4749	0.9999
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.994	0.8978	0.9856	0.0843	0.9999
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.998	0.8202	0.7396	0.1182	0.8511
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.4742	0.9997	1	0.9984	0.9954
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	1	1	0.9781	0.9999	0.739
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.7879	0.9755	0.9809	0.2288	0.9994
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.7226	0.9398	0.7288	0.3019	0.6934

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.9989	0.9999	0.9227	0.7157	0.6085	0.9476
10 vs 30 Days	0.1123	0.9887	1	0.755	0.4279	0.5053
10 vs 40 Days	0.9999	0.9623	0.9985	**0.0136**	0.0526	0.3319
10 vs 50 Days	0.2686	0.9732	0.9687	0.8738	0.2523	0.6347
20 vs 30 Days	**0.0469**	0.9736	0.9426	0.1088	**0.0196**	0.8985
20 vs 40 Days	0.9999	0.9326	0.9851	**<0.001**	**<0.001**	0.7501
20 vs 50 Days	0.3749	0.9878	0.5841	0.1921	**0.0102**	0.9246
30 vs 40 Days	0.0767	0.9997	0.9996	0.2428	0.861	0.9979
30 vs 50 Days	**<0.001**	0.8345	0.9473	0.9996	0.9909	1
40 vs 50 Days	0.3174	0.7498	0.8906	0.1791	0.9918	0.9999

Figure 32) Total sleep bouts of male flies (n = 5-15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



Tukey HSD Test by Genotype

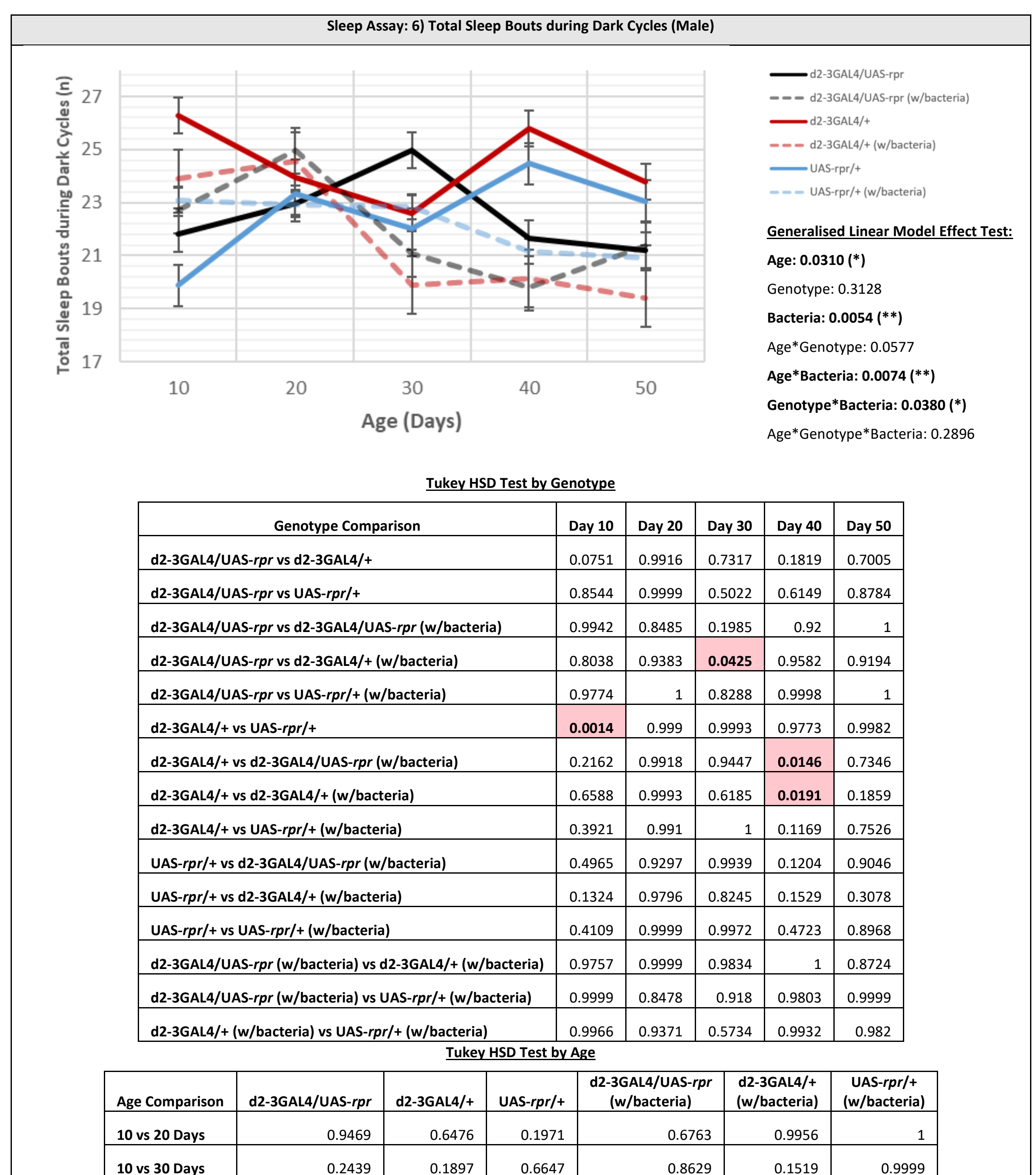
Genotype Comparison	Day 10	Day 20	Day 30	Day 40
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	1	0.9934	0.0805	0.1671
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.9962	0.5338	0.633	0.9933
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9971	0.0851	0.3487	0.8102
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.9953	0.9996	**0.0043**	0.8765
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.2025	0.456	0.9844	0.735
d2-3GAL4/+ vs UAS-*rpr*/+	0.999	0.2235	0.9275	0.6276
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9911	0.2758	0.9721	0.8994
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.9988	0.9999	0.9485	0.8259
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.2597	0.7992	**0.0154**	**0.0219**
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9312	**<0.001**	0.9998	0.9928
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	1	0.3696	0.4526	0.998
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.5195	**0.0099**	0.2755	0.5181
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9203	0.1919	0.5306	1
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.0676	0.9597	0.0985	0.1996
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.4889	0.6778	**<0.001**	0.244

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.5731	0.205	0.9968	**0.0054**	0.1449	**<0.001**
10 vs 30 Days	0.9888	**0.0112**	0.0876	0.2351	**<0.001**	0.363
10 vs 40 Days	0.9757	**<0.001**	0.4364	0.474	0.1153	0.9846
10 vs 50 Days	0.768	0.6077	0.1393	0.4214	0.0802	**0.0252**
20 vs 30 Days	0.824	0.4918	0.5569	0.2989	0.9985	**0.013**
20 vs 40 Days	0.9996	0.9974	0.8904	0.9879	0.1327	0.8164

Figure 33) Total sleep bouts during dark cycles for female flies (n = 5-15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age

Sleep Assay: 6) Total Sleep Bouts during Dark Cycles (Male)



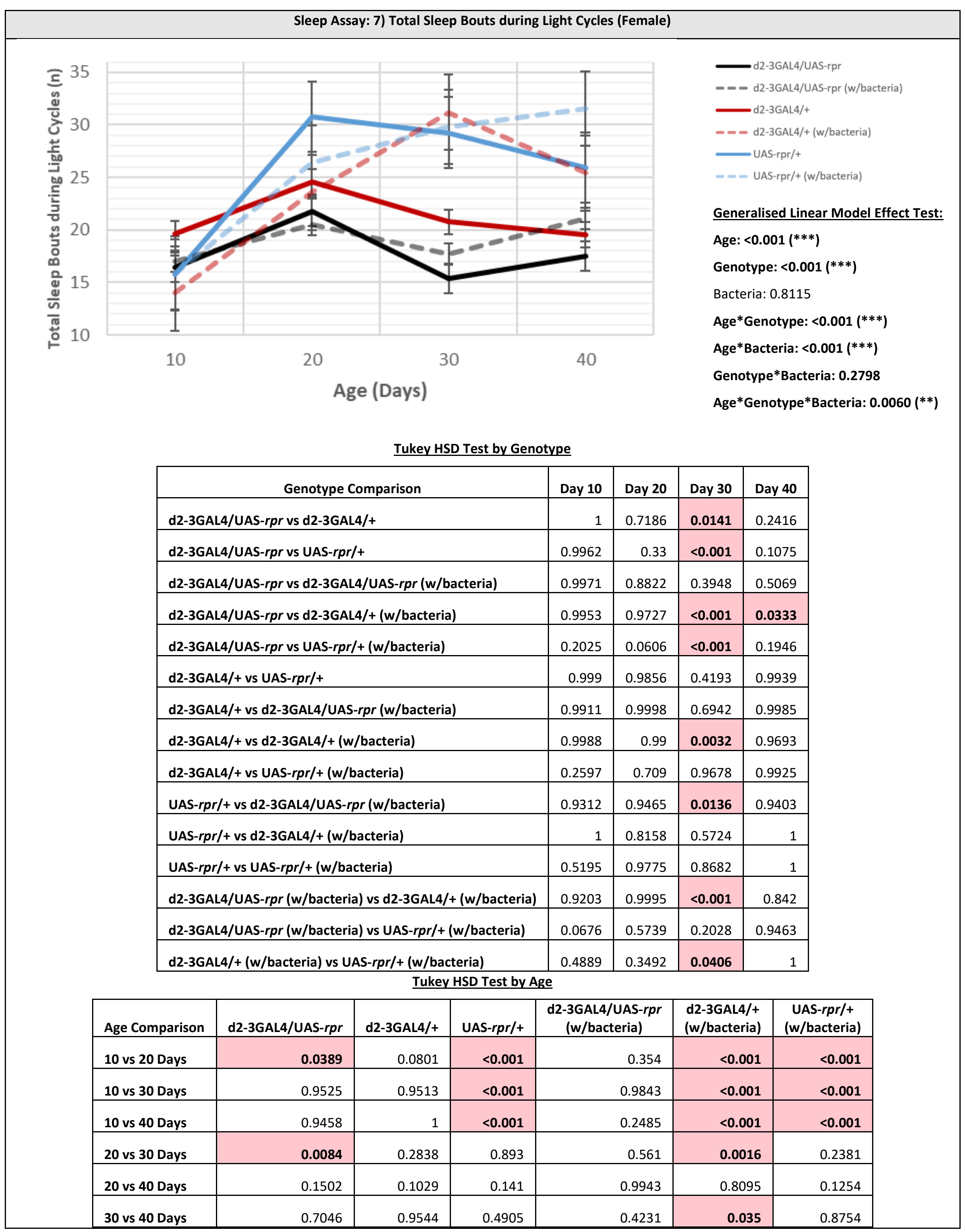
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.0751	0.9916	0.7317	0.1819	0.7005
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.8544	0.9999	0.5022	0.6149	0.8784
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9942	0.8485	0.1985	0.92	1
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.8038	0.9383	**0.0425**	0.9582	0.9194
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.9774	1	0.8288	0.9998	1
d2-3GAL4/+ vs UAS-*rpr*/+	**0.0014**	0.999	0.9993	0.9773	0.9982
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.2162	0.9918	0.9447	**0.0146**	0.7346
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.6588	0.9993	0.6185	**0.0191**	0.1859
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.3921	0.991	1	0.1169	0.7526
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.4965	0.9297	0.9939	0.1204	0.9046
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.1324	0.9796	0.8245	0.1529	0.3078
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.4109	0.9999	0.9972	0.4723	0.8968
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9757	0.9999	0.9834	1	0.8724
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.9999	0.8478	0.918	0.9803	0.9999
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.9966	0.9371	0.5734	0.9932	0.982

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.9469	0.6476	0.1971	0.6763	0.9956	1
10 vs 30 Days	0.2439	0.1897	0.6647	0.8629	0.1519	0.9999
10 vs 40 Days	1	0.9986	**0.044**	0.4734	0.2018	0.8624
10 vs 50 Days	0.9951	0.6825	0.2944	0.9348	0.134	0.8876
20 vs 30 Days	0.6579	0.9296	0.9146	0.1504	0.0699	1
20 vs 40 Days	0.9067	0.8283	0.954	**0.0341**	0.097	0.8834
20 vs 50 Days	0.7941	1	0.9998	0.237	0.0644	0.9051
30 vs 40 Days	0.1815	0.3478	0.5394	0.9563	0.9999	0.9106
30 vs 50 Days	0.1162	0.9684	0.9645	0.9998	0.9992	0.9241
40 vs 50 Days	0.9987	0.8386	0.9121	0.9137	0.9954	1

Figure 34) Total sleep bouts during dark cycles for male flies (n = 5-15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



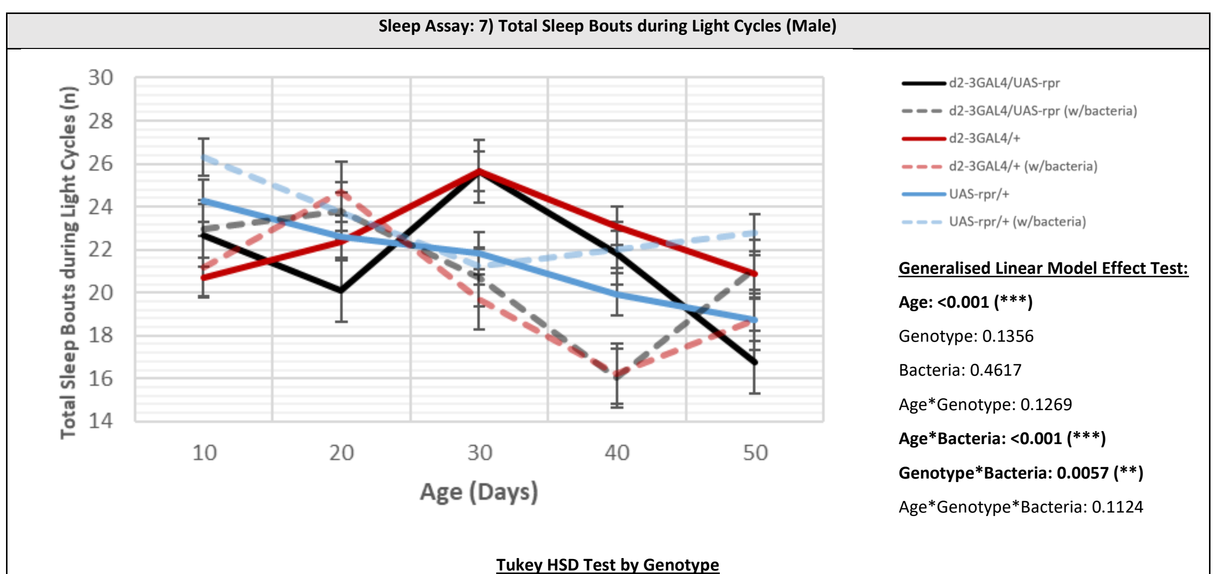
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	1	0.7186	**0.0141**	0.2416
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.9962	0.33	**<0.001**	0.1075
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9971	0.8822	0.3948	0.5069
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.9953	0.9727	**<0.001**	**0.0333**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.2025	0.0606	**<0.001**	0.1946
d2-3GAL4/+ vs UAS-*rpr*/+	0.999	0.9856	0.4193	0.9939
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9911	0.9998	0.6942	0.9985
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.9988	0.99	**0.0032**	0.9693
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.2597	0.709	0.9678	0.9925
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9312	0.9465	**0.0136**	0.9403
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	1	0.8158	0.5724	1
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.5195	0.9775	0.8682	1
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9203	0.9995	**<0.001**	0.842
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.0676	0.5739	0.2028	0.9463
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.4889	0.3492	**0.0406**	1

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	**0.0389**	0.0801	**<0.001**	0.354	**<0.001**	**<0.001**
10 vs 30 Days	0.9525	0.9513	**<0.001**	0.9843	**<0.001**	**<0.001**
10 vs 40 Days	0.9458	1	**<0.001**	0.2485	**<0.001**	**<0.001**
20 vs 30 Days	**0.0084**	0.2838	0.893	0.561	**0.0016**	0.2381
20 vs 40 Days	0.1502	0.1029	0.141	0.9943	0.8095	0.1254
30 vs 40 Days	0.7046	0.9544	0.4905	0.4231	**0.035**	0.8754

Figure 35) Total sleep bouts during light cycles for female flies (n = 5-15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



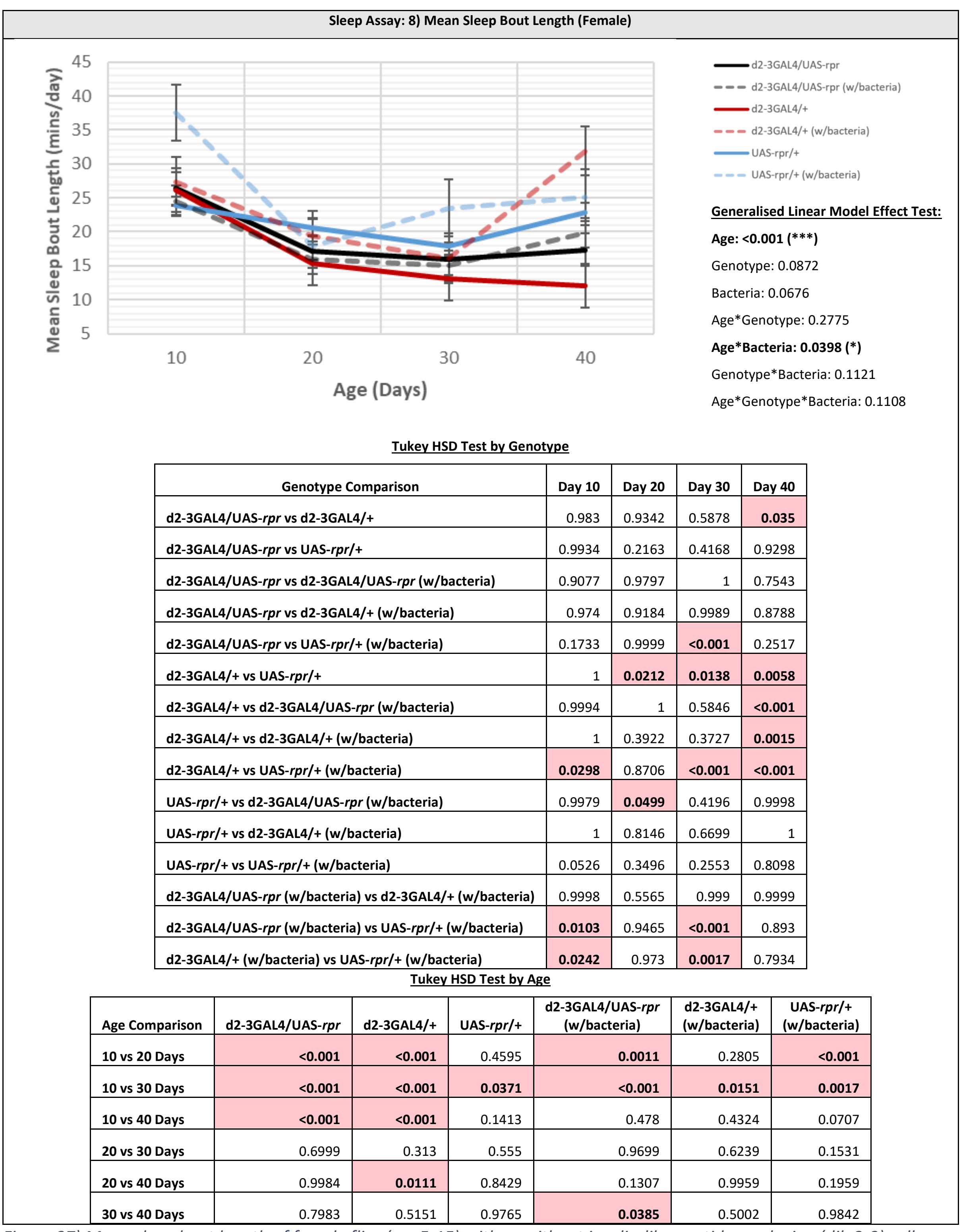
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.9136	0.8186	0.9718	0.5249	0.3311
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	1	0.8912	0.2362	0.9997	0.8187
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9973	0.2777	**0.0494**	0.1579	0.6908
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.9993	0.1999	**0.0063**	0.2148	1
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.5376	0.7513	0.31	1	0.6584
d2-3GAL4/+ vs UAS-*rpr*/+	0.8787	1	0.7038	0.7338	0.9464
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9929	0.9472	0.2891	**<0.001**	0.9819
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.9831	0.8917	0.0635	**0.0013**	0.3847
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.9749	1	0.7748	0.4749	0.9999
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.994	0.8978	0.9856	0.0843	0.9999
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.998	0.8202	0.7396	0.1182	0.8511
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.4742	0.9997	1	0.9984	0.9954
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	1	1	0.9781	0.9999	0.739
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.7879	0.9755	0.9809	0.2288	0.9994
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.7226	0.9398	0.7288	0.3019	0.6934

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.7465	0.927	0.9059	0.9902	0.3579	0.618
10 vs 30 Days	0.6322	0.1078	0.6988	0.71	0.938	**0.0488**
10 vs 40 Days	0.9953	0.7764	0.178	**0.0021**	0.0743	0.1457
10 vs 50 Days	0.0734	1	**0.0371**	0.8429	0.768	0.5216
20 vs 30 Days	0.0587	0.4862	0.9937	0.4224	0.0837	0.6162
20 vs 40 Days	0.9222	0.9965	0.6346	**<0.001**	**<0.001**	0.8687
20 vs 50 Days	0.5665	0.9612	0.2497	0.582	**0.0442**	0.992
30 vs 40 Days	0.3691	0.7327	0.8577	0.0866	0.3882	0.9933
30 vs 50 Days	**<0.001**	0.2085	0.4692	0.9995	0.9912	0.9584
40 vs 50 Days	0.1635	0.8597	0.9711	0.0594	0.7634	0.9971

Figure 36) Total sleep bouts during light cycles for male flies (n = 5-15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



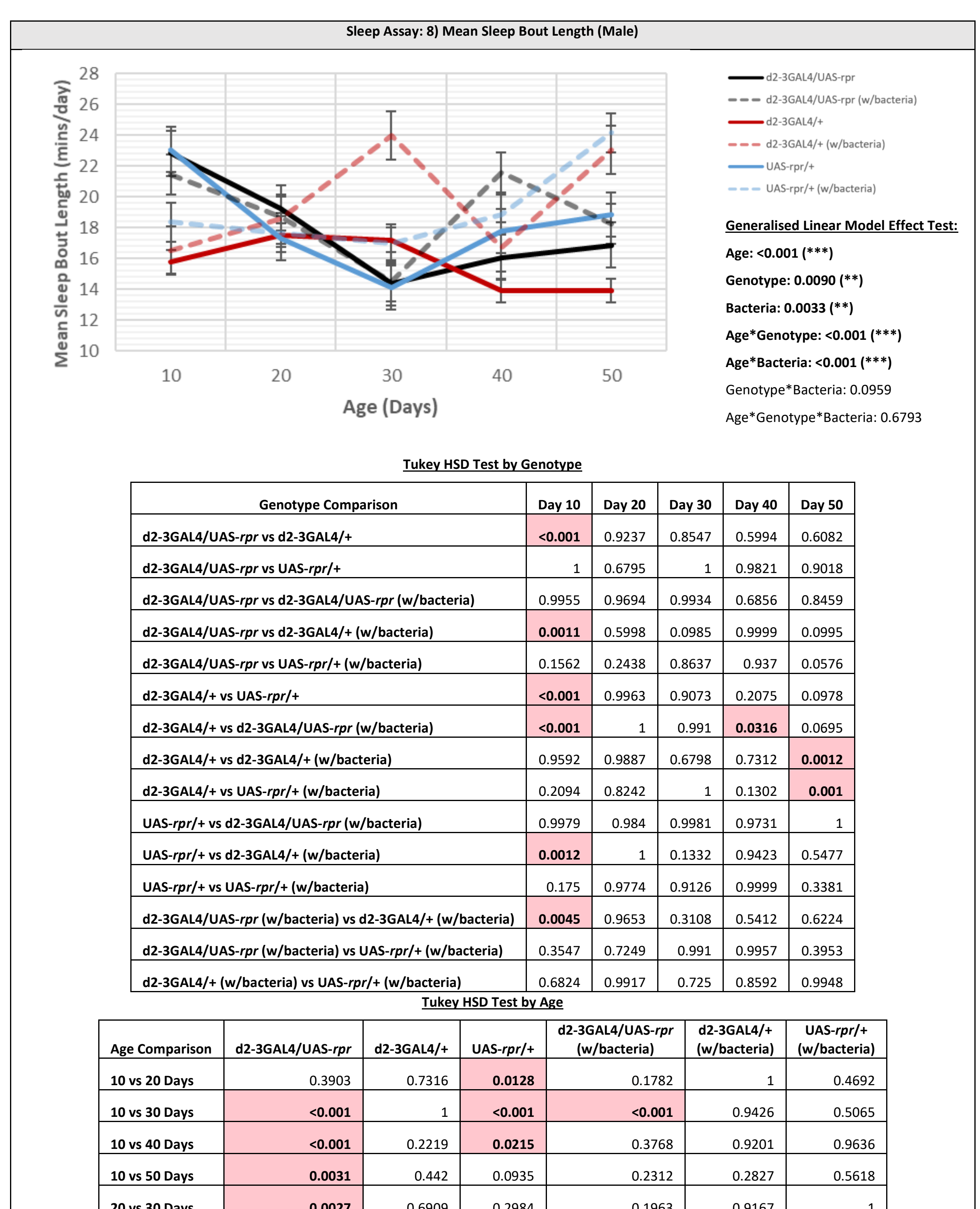
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.983	0.9342	0.5878	**0.035**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.9934	0.2163	0.4168	0.9298
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9077	0.9797	1	0.7543
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.974	0.9184	0.9989	0.8788
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.1733	0.9999	**<0.001**	0.2517
d2-3GAL4/+ vs UAS-*rpr*/+	1	**0.0212**	**0.0138**	**0.0058**
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9994	1	0.5846	**<0.001**
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	1	0.3922	0.3727	**0.0015**
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	**0.0298**	0.8706	**<0.001**	**<0.001**
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9979	**0.0499**	0.4196	0.9998
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	1	0.8146	0.6699	1
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.0526	0.3496	0.2553	0.8098
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9998	0.5565	0.999	0.9999
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	**0.0103**	0.9465	**<0.001**	0.893
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	**0.0242**	0.973	**0.0017**	0.7934

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	**<0.001**	**<0.001**	0.4595	**0.0011**	0.2805	**<0.001**
10 vs 30 Days	**<0.001**	**<0.001**	**0.0371**	**<0.001**	**0.0151**	**0.0017**
10 vs 40 Days	**<0.001**	**<0.001**	0.1413	0.478	0.4324	0.0707
20 vs 30 Days	0.6999	0.313	0.555	0.9699	0.6239	0.1531
20 vs 40 Days	0.9984	**0.0111**	0.8429	0.1307	0.9959	0.1959
30 vs 40 Days	0.7983	0.5151	0.9765	**0.0385**	0.5002	0.9842

Figure 37) Mean sleep bout length of female flies (n = 5-15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



Tukey HSD Test by Genotype

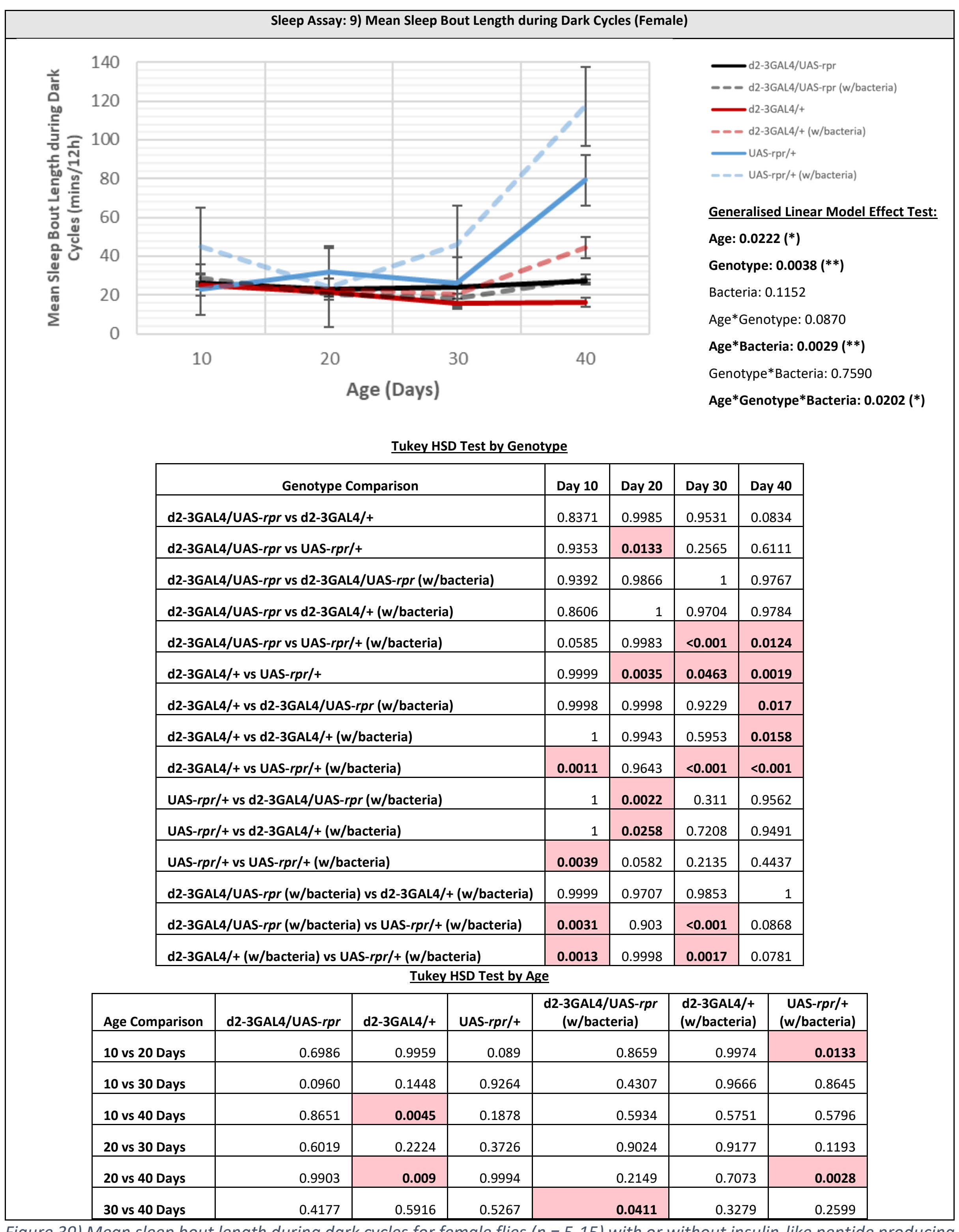
Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	**<0.001**	0.9237	0.8547	0.5994	0.6082
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	1	0.6795	1	0.9821	0.9018
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9955	0.9694	0.9934	0.6856	0.8459
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	**0.0011**	0.5998	0.0985	0.9999	0.0995
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.1562	0.2438	0.8637	0.937	0.0576
d2-3GAL4/+ vs UAS-*rpr*/+	**<0.001**	0.9963	0.9073	0.2075	0.0978
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	**<0.001**	1	0.991	**0.0316**	0.0695
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.9592	0.9887	0.6798	0.7312	**0.0012**
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.2094	0.8242	1	0.1302	**0.001**
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9979	0.984	0.9981	0.9731	1
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	**0.0012**	1	0.1332	0.9423	0.5477
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.175	0.9774	0.9126	0.9999	0.3381
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	**0.0045**	0.9653	0.3108	0.5412	0.6224
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.3547	0.7249	0.991	0.9957	0.3953
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.6824	0.9917	0.725	0.8592	0.9948

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.3903	0.7316	**0.0128**	0.1782	1	0.4692
10 vs 30 Days	**<0.001**	1	**<0.001**	**<0.001**	0.9426	0.5065
10 vs 40 Days	**<0.001**	0.2219	**0.0215**	0.3768	0.9201	0.9636
10 vs 50 Days	**0.0031**	0.442	0.0935	0.2312	0.2827	0.5618
20 vs 30 Days	**0.0027**	0.6909	0.2984	0.1963	0.9167	1
20 vs 40 Days	0.1398	**0.01**	1	0.9971	0.952	0.8716
20 vs 50 Days	0.2659	**0.0455**	0.9659	1	0.2521	**0.036**
30 vs 40 Days	0.7084	0.2512	0.2896	0.1081	0.5263	0.8906
30 vs 50 Days	0.5818	0.4798	0.0835	0.1673	0.718	**0.0423**
40 vs 50 Days	0.9993	0.9993	0.9807	0.9993	0.0585	0.2466

Figure 38) Mean sleep bout length of male flies (n = 5-15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age

Sleep Assay: 9) Mean Sleep Bout Length during Dark Cycles (Female)



Tukey HSD Test by Genotype

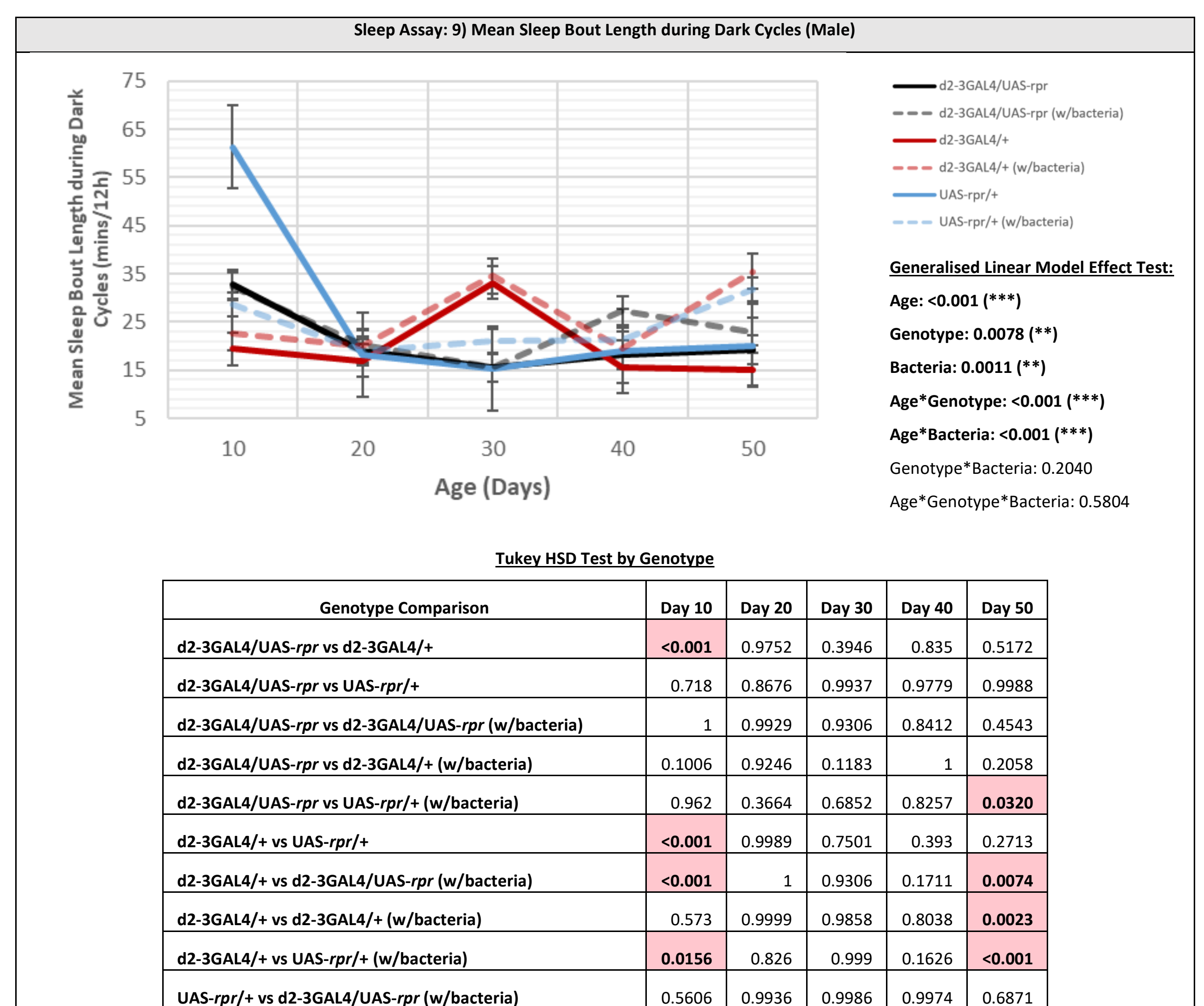
Genotype Comparison	Day 10	Day 20	Day 30	Day 40
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.8371	0.9985	0.9531	0.0834
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.9353	**0.0133**	0.2565	0.6111
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9392	0.9866	1	0.9767
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.8606	1	0.9704	0.9784
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.0585	0.9983	**<0.001**	**0.0124**
d2-3GAL4/+ vs UAS-*rpr*/+	0.9999	**0.0035**	**0.0463**	**0.0019**
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9998	0.9998	0.9229	**0.017**
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	1	0.9943	0.5953	**0.0158**
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	**0.0011**	0.9643	**<0.001**	**<0.001**
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	1	**0.0022**	0.311	0.9562
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	1	**0.0258**	0.7208	0.9491
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	**0.0039**	0.0582	0.2135	0.4437
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9999	0.9707	0.9853	1
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	**0.0031**	0.903	**<0.001**	0.0868
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	**0.0013**	0.9998	**0.0017**	0.0781

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.6986	0.9959	0.089	0.8659	0.9974	**0.0133**
10 vs 30 Days	0.0960	0.1448	0.9264	0.4307	0.9666	0.8645
10 vs 40 Days	0.8651	**0.0045**	0.1878	0.5934	0.5751	0.5796
20 vs 30 Days	0.6019	0.2224	0.3726	0.9024	0.9177	0.1193
20 vs 40 Days	0.9903	**0.009**	0.9994	0.2149	0.7073	**0.0028**
30 vs 40 Days	0.4177	0.5916	0.5267	**0.0411**	0.3279	0.2599

Figure 39) Mean sleep bout length during dark cycles for female flies (n = 5-15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age

Sleep Assay: 9) Mean Sleep Bout Length during Dark Cycles (Male)



Generalised Linear Model Effect Test:

Age: <0.001 (***)

Genotype: 0.0078 (**)

Bacteria: 0.0011 (**)

Age*Genotype: <0.001 (***)

Age*Bacteria: <0.001 (***)

Genotype*Bacteria: 0.2040

Age*Genotype*Bacteria: 0.5804

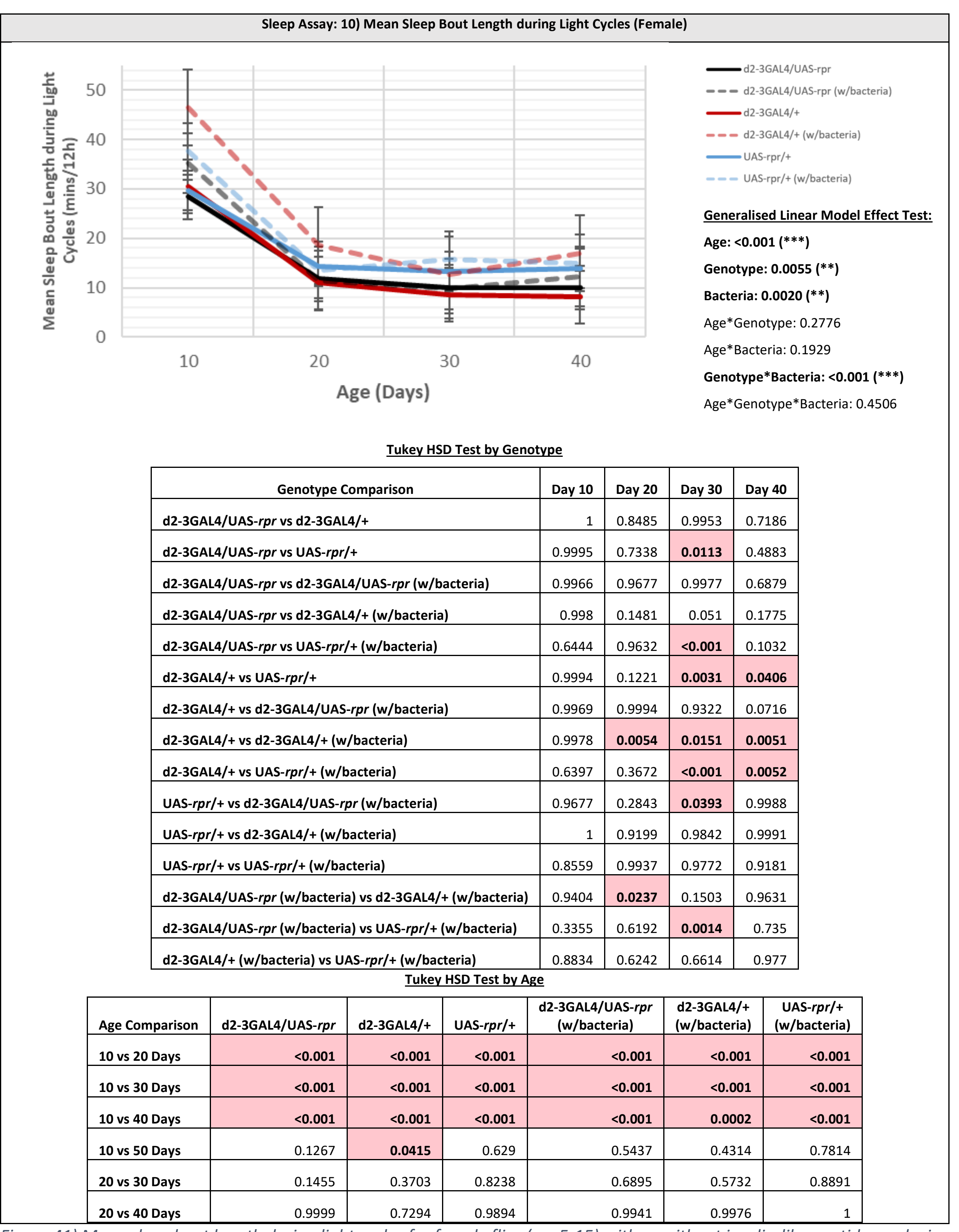
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	**<0.001**	0.9752	0.3946	0.835	0.5172
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.718	0.8676	0.9937	0.9779	0.9988
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	1	0.9929	0.9306	0.8412	0.4543
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.1006	0.9246	0.1183	1	0.2058
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.962	0.3664	0.6852	0.8257	**0.0320**
d2-3GAL4/+ vs UAS-*rpr*/+	**<0.001**	0.9989	0.7501	0.393	0.2713
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	**<0.001**	1	0.9306	0.1711	**0.0074**
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.573	0.9999	0.9858	0.8038	**0.0023**
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	**0.0156**	0.826	0.999	0.1626	**<0.001**
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.5606	0.9936	0.9986	0.9974	0.6871
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	**<0.001**	1	0.3499	0.9838	0.3678
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.2259	0.9588	0.9377	0.9963	0.0673
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.1272	0.9985	0.6005	0.8625	0.9896
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.9851	0.7333	0.9942	1	0.5961
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.5276	0.9257	0.9143	0.8479	0.9132

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	**<0.001**	0.9331	**<0.001**	**<0.001**	0.2158	**<0.001**
10 vs 30 Days	**<0.001**	0.9957	**<0.001**	**<0.001**	0.8737	**0.0033**
10 vs 40 Days	**<0.001**	0.1359	**<0.001**	**0.0078**	0.2527	0.1008
10 vs 50 Days	**<0.001**	0.1897	**<0.001**	0.1384	0.986	0.9991
20 vs 30 Days	0.0569	0.9937	0.9443	0.9197	0.7904	0.8319
20 vs 40 Days	0.87	0.5262	0.874	0.8731	1	0.1891
20 vs 50 Days	0.9972	0.585	0.7985	0.2356	0.1174	**<0.001**
30 vs 40 Days	0.4463	0.2824	0.4434	0.39	0.8324	0.7887
30 vs 50 Days	0.175	0.3495	0.3466	**0.0314**	0.6516	**0.0124**
40 vs 50 Days	0.977	1	0.9999	0.8258	0.1389	0.1529

Figure 40) Mean sleep bout length during dark cycles for male flies (n = 5-15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



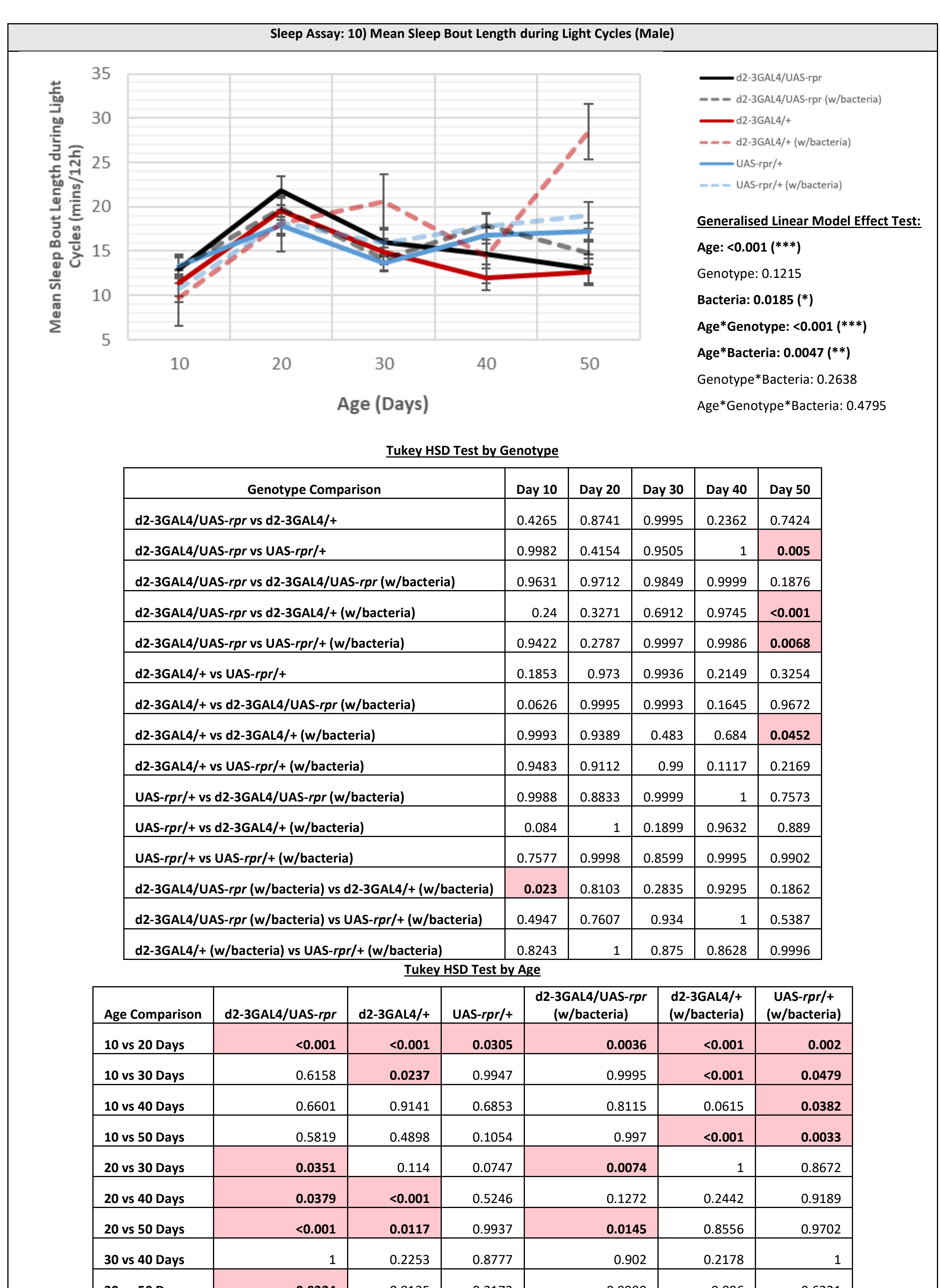
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	1	0.8485	0.9953	0.7186
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.9995	0.7338	**0.0113**	0.4883
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9966	0.9677	0.9977	0.6879
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.998	0.1481	0.051	0.1775
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.6444	0.9632	**<0.001**	0.1032
d2-3GAL4/+ vs UAS-*rpr*/+	0.9994	0.1221	**0.0031**	**0.0406**
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9969	0.9994	0.9322	0.0716
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.9978	**0.0054**	**0.0151**	**0.0051**
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.6397	0.3672	**<0.001**	**0.0052**
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9677	0.2843	**0.0393**	0.9988
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	1	0.9199	0.9842	0.9991
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.8559	0.9937	0.9772	0.9181
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9404	**0.0237**	0.1503	0.9631
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.3355	0.6192	**0.0014**	0.735
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.8834	0.6242	0.6614	0.977

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
10 vs 30 Days	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
10 vs 40 Days	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.0002**	**<0.001**
10 vs 50 Days	0.1267	**0.0415**	0.629	0.5437	0.4314	0.7814
20 vs 30 Days	0.1455	0.3703	0.8238	0.6895	0.5732	0.8891
20 vs 40 Days	0.9999	0.7294	0.9894	0.9941	0.9976	1

Figure 41) Mean sleep bout length during light cycles for female flies (n = 5-15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.4265	0.8741	0.9995	0.2362	0.7424
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.9982	0.4154	0.9505	1	**0.005**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9631	0.9712	0.9849	0.9999	0.1876
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.24	0.3271	0.6912	0.9745	**<0.001**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.9422	0.2787	0.9997	0.9986	**0.0068**
d2-3GAL4/+ vs UAS-*rpr*/+	0.1853	0.973	0.9936	0.2149	0.3254
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.0626	0.9995	0.9993	0.1645	0.9672
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.9993	0.9389	0.483	0.684	**0.0452**
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.9483	0.9112	0.99	0.1117	0.2169
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9988	0.8833	0.9999	1	0.7573
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.084	1	0.1899	0.9632	0.889
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.7577	0.9998	0.8599	0.9995	0.9902
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	**0.023**	0.8103	0.2835	0.9295	0.1862
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.4947	0.7607	0.934	1	0.5387
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.8243	1	0.875	0.8628	0.9996

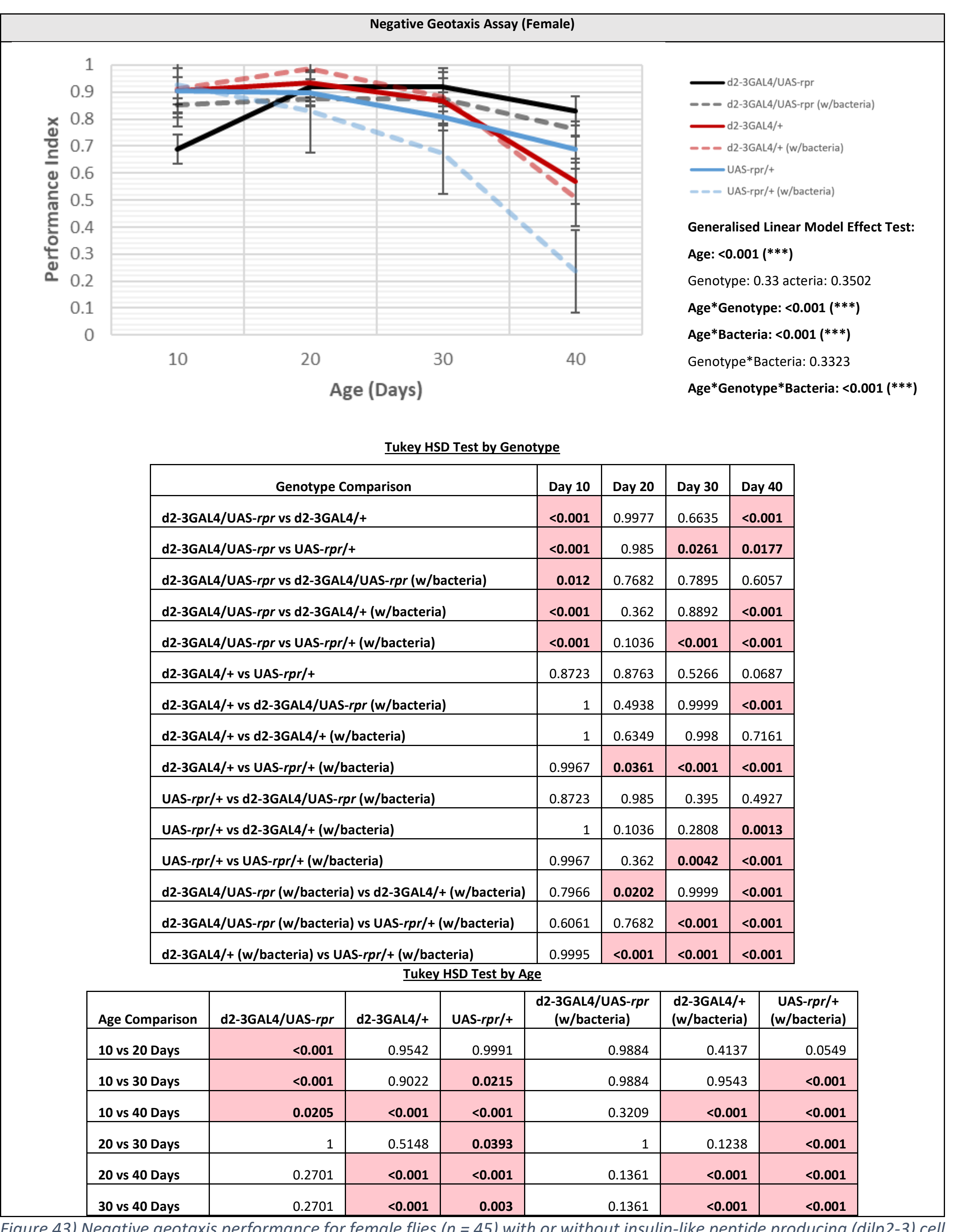
Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	**<0.001**	**<0.001**	**0.0305**	**0.0036**	**<0.001**	**0.002**
10 vs 30 Days	0.6158	**0.0237**	0.9947	0.9995	**<0.001**	**0.0479**
10 vs 40 Days	0.6601	0.9141	0.6853	0.8115	0.0615	**0.0382**
10 vs 50 Days	0.5819	0.4898	0.1054	0.997	**<0.001**	**0.0033**
20 vs 30 Days	**0.0351**	0.114	0.0747	**0.0074**	1	0.8672
20 vs 40 Days	**0.0379**	**<0.001**	0.5246	0.1272	0.2442	0.9189
20 vs 50 Days	**<0.001**	**0.0117**	0.9937	**0.0145**	0.8556	0.9702
30 vs 40 Days	1	0.2253	0.8777	0.902	0.2178	1
30 vs 50 Days	**0.0324**	0.8135	0.2173	0.9999	0.886	0.6321
40 vs 50 Days	**0.0427**	0.9245	0.7949	0.9477	**0.0359**	0.7013

Figure 42) Mean sleep bout length during light cycles for male flies (n = 5-15) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age

5.6.3. Bacteria did not affect fly neuromuscular function

The negative geotaxis assay assesses the negative geotactic and climbing abilities of flies against gravity. This assay allows for the temporal measurement of age-related physical decline, as fly motor function naturally deteriorates with age. From our results, negative geotaxis performance worsened in all control flies from age 40 days onwards. Aligning with previous findings, systemic IIS reduction ameliorated this age-related declined among females (Figure 43), scoring a higher performance index than controls (154). However, systemic IIS reduced males (Figure 44) with systemic IIS reduction only scored a higher performance index than d2-3GAL4/+ flies, but not UAS-*rpr*/+ flies. This difference may be caused by the improper expression of d2-3GAL4/UAS-*rpr* as mentioned earlier (Result 5.6.1). When treated with bacteria, negative geotaxis performance of most flies remained unaffected except for bacteria treated UAS-*rpr*/+ females and untreated d2-3GAL4/+ males. It is possible that bacteria treated UAS-rpr/+ females were already in a state of poor health (as indicated by its shorter median lifespan in Result 5.6.1) which exacerbated its negative geotaxis performance compared to its control. As for non-bacteria treated d2-3GAL4/+ males, its poorer negative geotaxis performance could be influenced by external variables. It should be noted that data for female flies at age 50 days were omitted due to the insufficient sample size. Overall, the negative geotaxis performance of all flies declined with age but are ameliorated through systemic IIS reduction. However, bacteria treatment does not affect negative geotaxis performance of flies regardless of genotype.



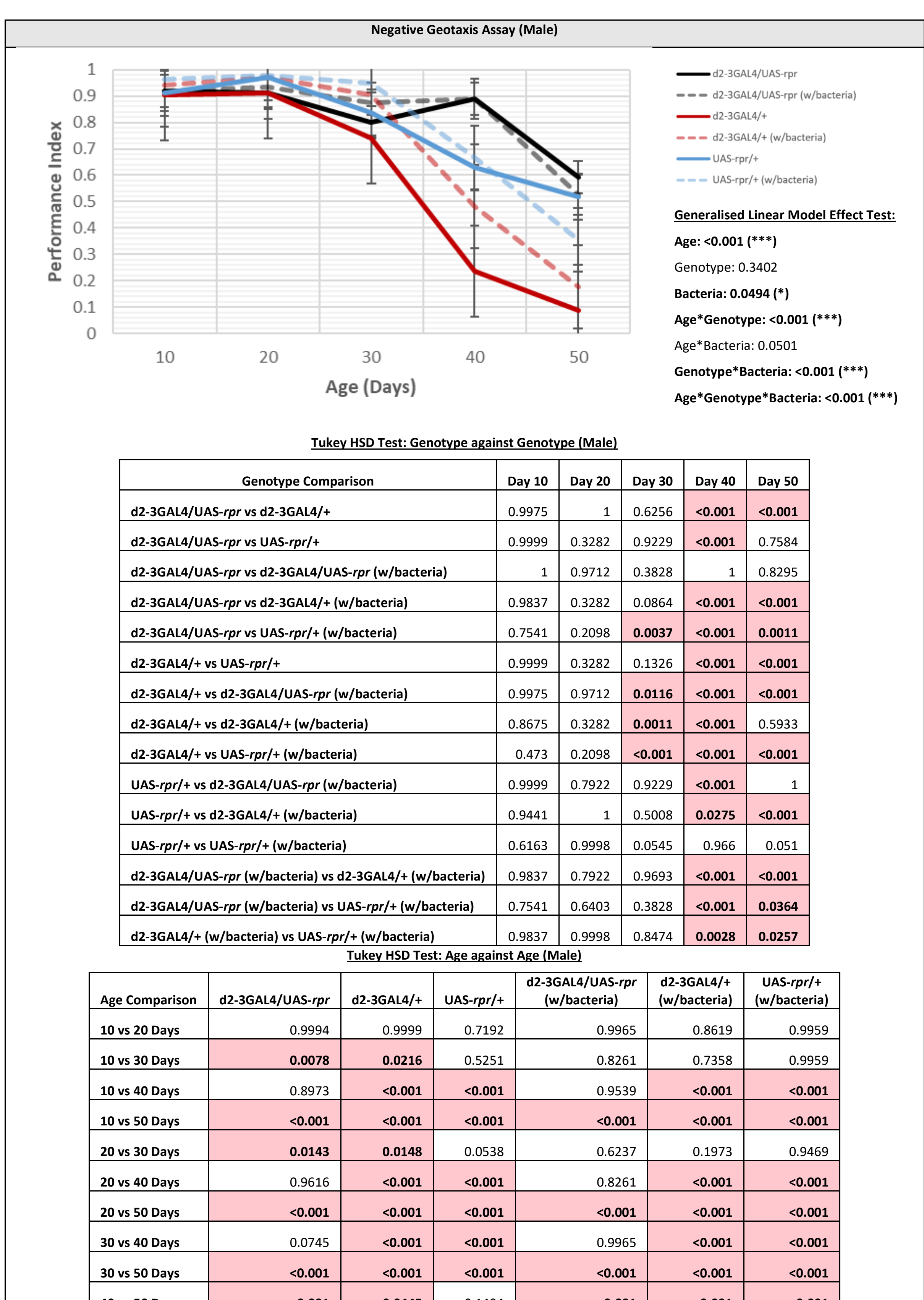
Tukey HSD Test by Genotype

Genotype Comparison	Day 10	Day 20	Day 30	Day 40
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	**<0.001**	0.9977	0.6635	**<0.001**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	**<0.001**	0.985	**0.0261**	**0.0177**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	**0.012**	0.7682	0.7895	0.6057
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	**<0.001**	0.362	0.8892	**<0.001**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	**<0.001**	0.1036	**<0.001**	**<0.001**
d2-3GAL4/+ vs UAS-*rpr*/+	0.8723	0.8763	0.5266	0.0687
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	1	0.4938	0.9999	**<0.001**
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	1	0.6349	0.998	0.7161
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.9967	**0.0361**	**<0.001**	**<0.001**
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.8723	0.985	0.395	0.4927
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	1	0.1036	0.2808	**0.0013**
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.9967	0.362	**0.0042**	**<0.001**
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.7966	**0.0202**	0.9999	**<0.001**
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.6061	0.7682	**<0.001**	**<0.001**
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.9995	**<0.001**	**<0.001**	**<0.001**

Tukey HSD Test by Age

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	**<0.001**	0.9542	0.9991	0.9884	0.4137	0.0549
10 vs 30 Days	**<0.001**	0.9022	**0.0215**	0.9884	0.9543	**<0.001**
10 vs 40 Days	**0.0205**	**<0.001**	**<0.001**	0.3209	**<0.001**	**<0.001**
20 vs 30 Days	1	0.5148	**0.0393**	1	0.1238	**<0.001**
20 vs 40 Days	0.2701	**<0.001**	**<0.001**	0.1361	**<0.001**	**<0.001**
30 vs 40 Days	0.2701	**<0.001**	**0.003**	0.1361	**<0.001**	**<0.001**

Figure 43) Negative geotaxis performance for female flies (n = 45) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



Tukey HSD Test: Genotype against Genotype (Male)

Genotype Comparison	Day 10	Day 20	Day 30	Day 40	Day 50
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.9975	1	0.6256	<0.001	<0.001
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.9999	0.3282	0.9229	<0.001	0.7584
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	1	0.9712	0.3828	1	0.8295
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.9837	0.3282	0.0864	<0.001	<0.001
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.7541	0.2098	0.0037	<0.001	0.0011
d2-3GAL4/+ vs UAS-*rpr*/+	0.9999	0.3282	0.1326	<0.001	<0.001
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9975	0.9712	0.0116	<0.001	<0.001
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.8675	0.3282	0.0011	<0.001	0.5933
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.473	0.2098	<0.001	<0.001	<0.001
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9999	0.7922	0.9229	<0.001	1
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.9441	1	0.5008	0.0275	<0.001
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	0.6163	0.9998	0.0545	0.966	0.051
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.9837	0.7922	0.9693	<0.001	<0.001
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.7541	0.6403	0.3828	<0.001	0.0364
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.9837	0.9998	0.8474	0.0028	0.0257

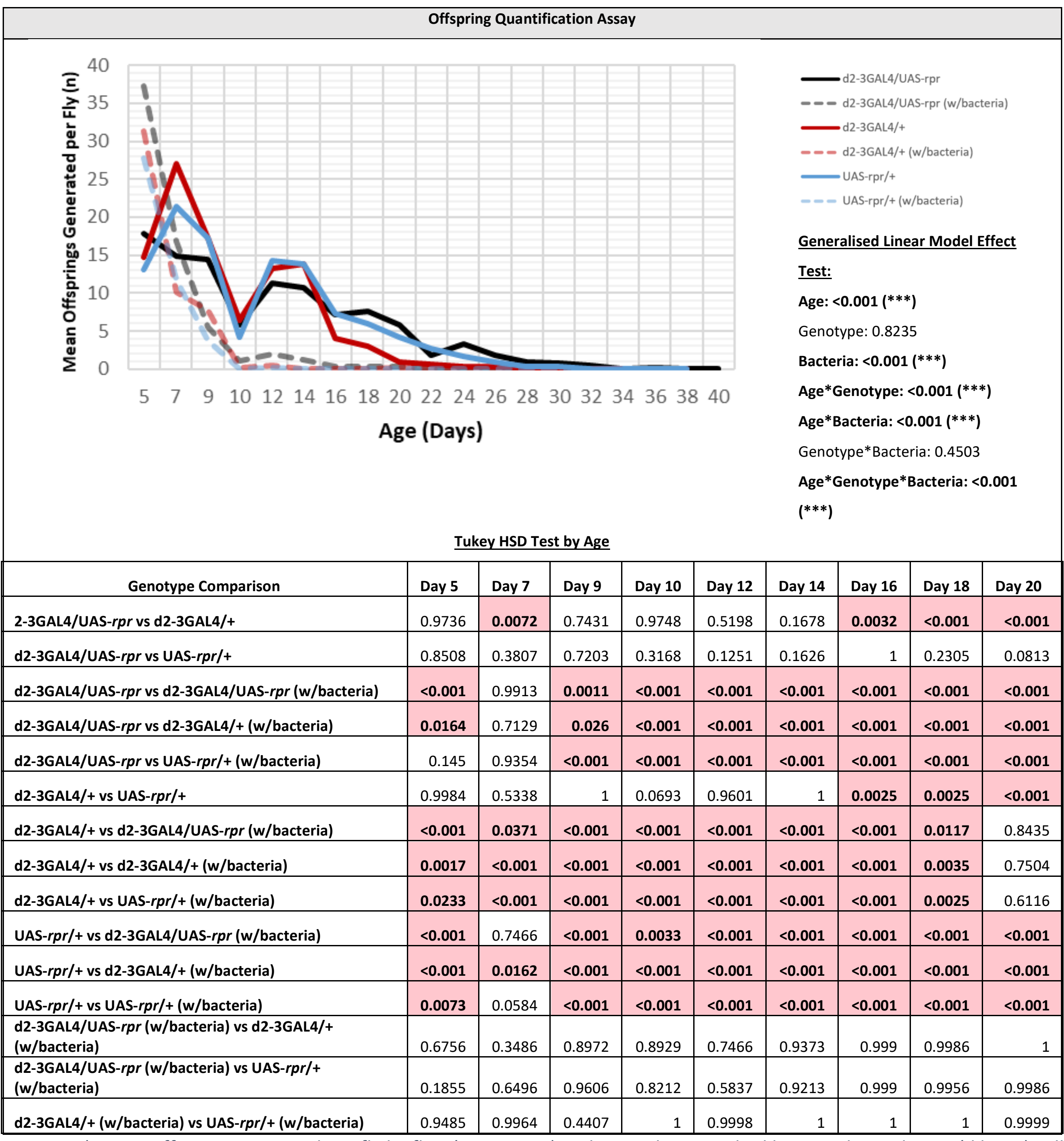
Tukey HSD Test: Age against Age (Male)

Age Comparison	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
10 vs 20 Days	0.9994	0.9999	0.7192	0.9965	0.8619	0.9959
10 vs 30 Days	0.0078	0.0216	0.5251	0.8261	0.7358	0.9959
10 vs 40 Days	0.8973	<0.001	<0.001	0.9539	<0.001	<0.001
10 vs 50 Days	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
20 vs 30 Days	0.0143	0.0148	0.0538	0.6237	0.1973	0.9469
20 vs 40 Days	0.9616	<0.001	<0.001	0.8261	<0.001	<0.001
20 vs 50 Days	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
30 vs 40 Days	0.0745	<0.001	<0.001	0.9965	<0.001	<0.001
30 vs 50 Days	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
40 vs 50 Days	<0.001	0.0445	0.1494	<0.001	<0.001	<0.001

Figure 44) Negative geotaxis performance for male flies (n = 45) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age

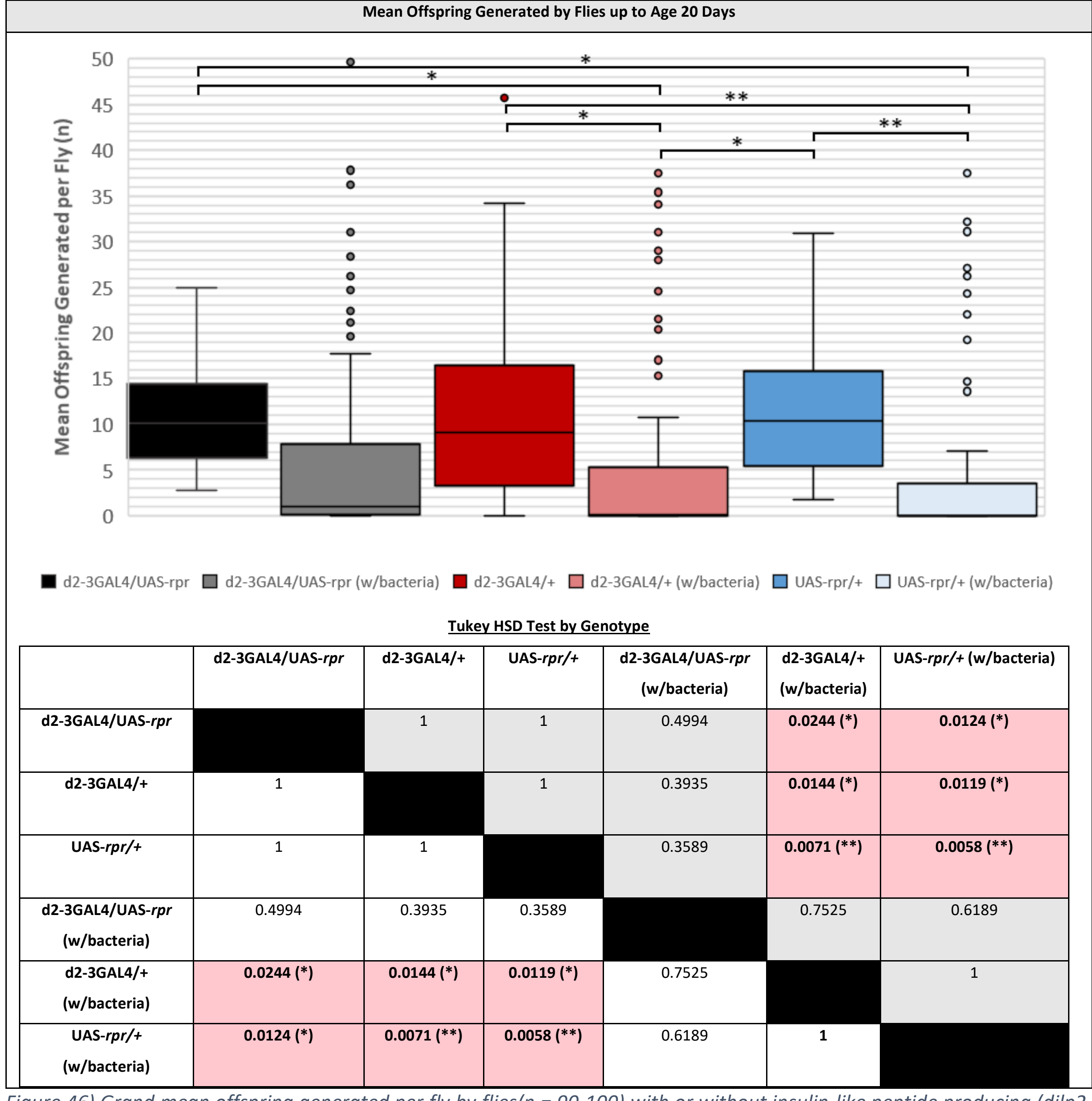
5.6.4. Bacteria increased offspring produced across all flies in early age

The offspring quantification assay measures fly fecundity, providing a snapshot of reproductive health across age. While previous studies demonstrated systemic IIS reduced flies laid fewer eggs than controls, it is unclear if the number of offspring produced was affected (159). As all flies utilised in this project were at least once mated, the number of offspring produced were limited to how much sperm was stored in flies. Here, flies with reduced systemic IIS produced the same number of offspring as its controls despite some discrepancies in daily offspring generation reported at certain ages (Figure 45, Figure 46). When treated with bacteria, all flies produced more than twice the number of offspring than their respective controls at age 5 days. However, generation rates quickly plummeted below that of controls in the subsequent days regardless of genotype. Cumulatively, bacteria treated control flies produced significantly fewer offspring compared to controls without bacteria treatment. However, the mean offspring generated by bacteria treated flies with systemic IIS reduction remain statistically indifferent to all other fly groups. Conflictingly, images of fly vials revealed that more eggs were laid by flies treated with bacteria than in controls between age 4 days and 14 days (Table 14). As such, the number of viable offspring produced did not reflect the high number of eggs laid. It was speculated that while bacteria treatment increases fly egg laying capacity, offspring viability may be compromised consequently.



Genotype Comparison	Day 5	Day 7	Day 9	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20
2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.9736	**0.0072**	0.7431	0.9748	0.5198	0.1678	**0.0032**	**<0.001**	**<0.001**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.8508	0.3807	0.7203	0.3168	0.1251	0.1626	1	0.2305	0.0813
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	**<0.001**	0.9913	**0.0011**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	**0.0164**	0.7129	**0.026**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	0.145	0.9354	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
d2-3GAL4/+ vs UAS-*rpr*/+	0.9984	0.5338	1	0.0693	0.9601	1	**0.0025**	**0.0025**	**<0.001**
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	**<0.001**	**0.0371**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.0117**	0.8435
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	**0.0017**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.0035**	0.7504
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	**0.0233**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.0025**	0.6116
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	**<0.001**	0.7466	**<0.001**	**0.0033**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	**<0.001**	**0.0162**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	**0.0073**	0.0584	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	0.6756	0.3486	0.8972	0.8929	0.7466	0.9373	0.999	0.9986	1
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.1855	0.6496	0.9606	0.8212	0.5837	0.9213	0.999	0.9956	0.9986
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.9485	0.9964	0.4407	1	0.9998	1	1	1	0.9999

Figure 45) Mean offspring generated per fly by flies (n = 90-100) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age.



Tukey HSD Test by Genotype

	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
d2-3GAL4/UAS-*rpr*		1	1	0.4994	**0.0244 (*)**	**0.0124 (*)**
d2-3GAL4/+	1		1	0.3935	**0.0144 (*)**	**0.0119 (*)**
UAS-*rpr*/+	1	1		0.3589	**0.0071 (**)**	**0.0058 (**)**
d2-3GAL4/UAS-*rpr* (w/bacteria)	0.4994	0.3935	0.3589		0.7525	0.6189
d2-3GAL4/+ (w/bacteria)	**0.0244 (*)**	**0.0144 (*)**	**0.0119 (*)**	0.7525		1
UAS-*rpr*/+ (w/bacteria)	**0.0124 (*)**	**0.0071 (**)**	**0.0058 (**)**	0.6189	**1**	

Figure 46) Grand mean offspring generated per fly by flies(n = 90-100) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure

Table 14) Images of eggs laid per vial by flies (n = 10) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age

	dilp2-GAL4/UAS-*rpr*		**Dilp2-GAL4/+**		**UAS-*rpr*/+**	
	No Bacteria	**With Bacteria**	**No Bacteria**	**With Bacteria**	**No Bacteria**	**With Bacteria**
Day 4						
Day 14						
Day 28						

5.6.5. Summary of Results 5.6

Contrasting Results 5.5.1, systemic IIS reduction extended the median lifespan of female flies but not males. When treated with bacteria, all flies regardless of genotype reported shorter median lifespans, implying that bacteria also impacted the lifespan of flies with normal IIS. In terms of sleep, changes to total sleep across age were found in females but not males. Oddly, varying results between female controls obfuscated baseline results and it was suspected that d2-3GAL4/+ female flies may have been affected by confounders. When comparing between d2-3GAL4/UAS-*rpr* to UAS-*rpr*/+ flies, systemic IIS reduction reduced total sleep and increased total activity of female flies across age. Again, these effects can be attenuated by bacteria treatment. In terms of negative geotaxis performance, systemic IIS reduction alleviated neuromuscular function decline across age in only female flies but not males. Interestingly, negative geotaxis performance was not impacted by bacteria irrespective of genotypes. While Result 5.5.1 demonstrates that systemic IIS reduction affects male flies, experiments here did not recapitulate those results nor report differences across sleep nor neuromuscular function. External variables or improper genotype expression may have affected these flies. Lastly, systemic IIS reduction did not affect fly offspring generation, but bacteria treatment increased the number of offspring and eggs found in vials across all genotypes during early life but yielded fewer offspring overall. In summary, these experiments recapitulated findings where systemic IIS reduction extends host median lifespan but reduces total sleep in flies. Moreover, bacteria treatment attenuated these changes induced by IPC ablation without affecting neuromuscular function. Speculatively, these changes in ageing parameters may

be related to the early age increased of offspring generation and quick subsequent depletion of stored sperm by female flies exposed to bacteria.

5.7. Bacteria increased egg laying capacity but reduced median lifespan of flies regardless of IIS expression

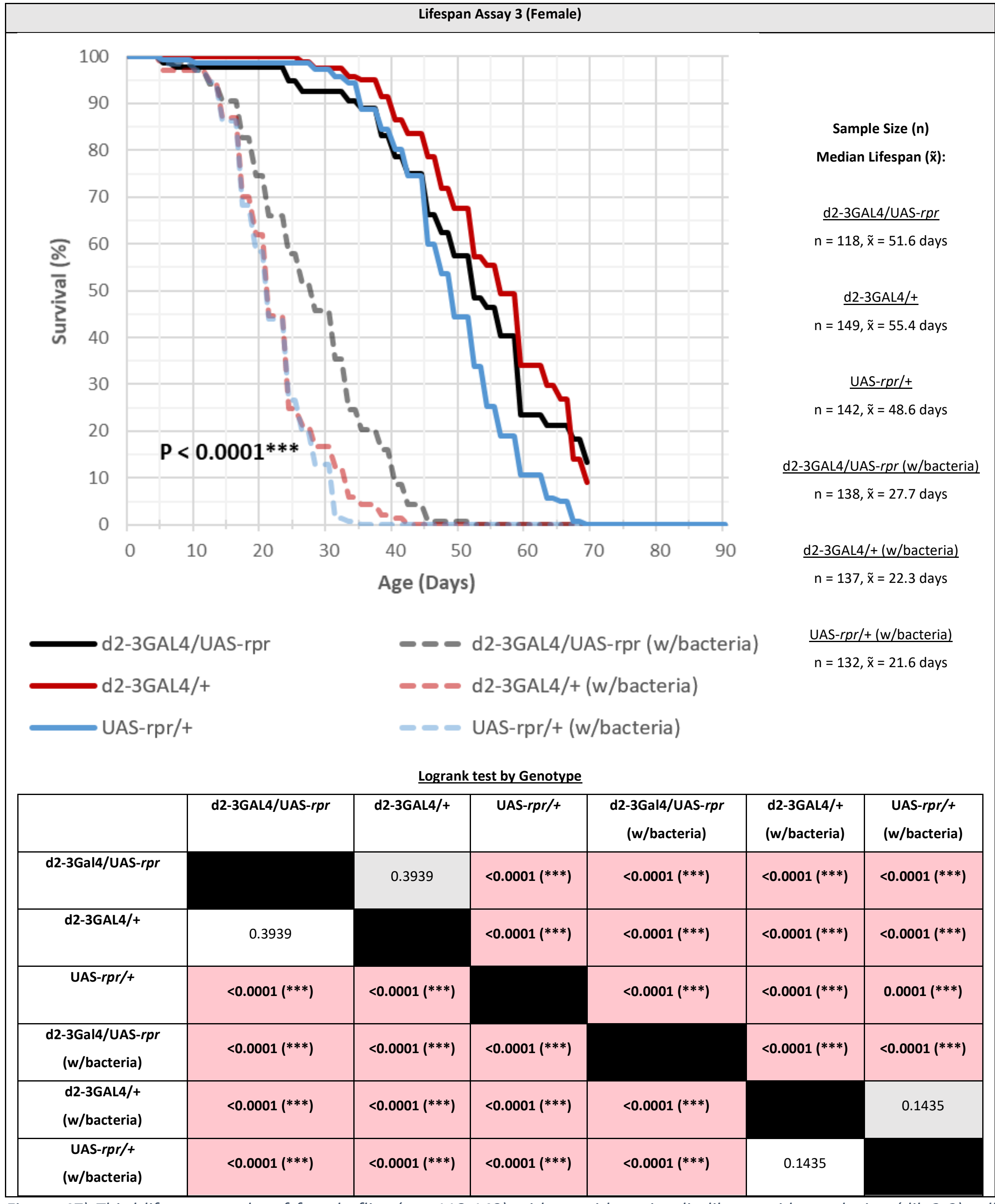
This final section aimed to reconfirm prior observations that feeding flies with bacteria increases their egg laying capacity by repeating the offspring quantification assay while counting egg lays alongside. Additionally, a new experimental group (trhGAL4/UAS-InR^{DN}) was introduced to further probe for bacteria interactions against IIS reduction in serotonergic neuron.

5.7.1. Bacteria further reduced median lifespan of flies regardless of genotype under high concentration

In addition to validating preceding survival assay results, this final survival assay compares the effects of bacteria against flies with systemic IIS reduction and flies with serotonergic neuron IIS reduction. From the former, systemic IIS reduction did not extend median lifespan of non-bacteria treated flies in this assay. Instead, female flies reported ≥6% shorter median lifespans than d2-3GAL4/+ flies (Figure 47) while males had ≥7% shorter median lifespans than both non-bacteria treated controls (Figure 48) flies. Despite the absence of effect among non-bacteria treated flies, systemic IIS reduced female flies treated with bacteria achieved ≥24% longer median lifespans than their respective controls. However, this result was not reciprocated among males as they lived ≥11% shorter than bacteria treated controls. As bacteria treated d2-3GAL4/UAS-*rpr* female flies were the only group reporting increased median lifespan over their respective controls, it could be surmised that external variables attenuated d2-3GAL4/UAS-*rpr* expression in null effect groups.

As for flies with serotonergic neuron IIS reduction, it was expected that this model only extends media lifespan in females, but not males (160). However, non-bacteria treated female (Figure 49) flies here were not long-lived as trhGAL4/UAS-InR^{DN} and trhGAL4/+ flies reported similar median lifespans. Surprisingly, male flies (Figure 50) lived ≥8% longer than both non-bacteria treated controls, contradicting previous findings. When introduced to bacteria, serotonergic reduced IIS flies had longer median lifespans than bacteria treated controls, as seen among female (≥24% longer) and male flies (≥15% longer). Taken together, it is possible that the absence of effect among trhGAL4/UAS-InR^{DN} females may be attributed to confounders and that serotonergic neuron IIS reduction also affects males.

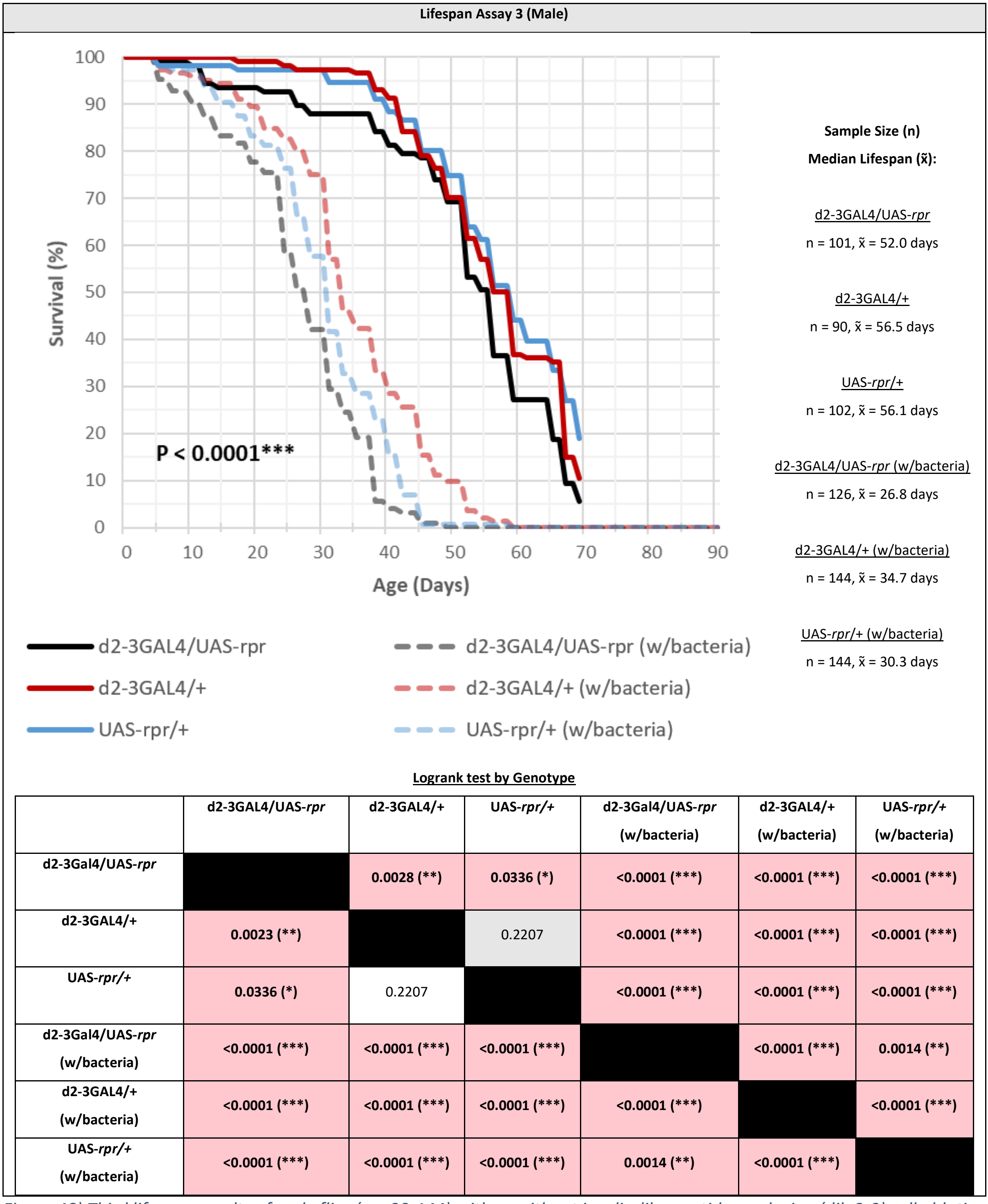
Interestingly, all bacteria treated flies regardless of genotype nor sex reported lower survivability across age compared to non-bacteria treated controls. However, differences of median lifespan between bacteria and non-bacteria groups were far greater here than Result 5.5.1 (≥29%) and Result 5.6.1 (≥23%) where discrepancies were at least 56%. This lower survivability rate among bacteria treated flies may be attributed towards an inadvertent increased in bacteria inoculation load caused by alterations to bacteria inoculation protocols necessitated by logistical complications (as further discussed in Discussion 6.3). Also, it's important to note that the lifespan results for non-bacterial flies are incomplete due to time constraints.



Logrank test by Genotype

	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3Gal4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
d2-3Gal4/UAS-*rpr*		0.3939	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)
d2-3GAL4/+	0.3939		<0.0001 (***)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)
UAS-*rpr*/+	<0.0001 (***)	<0.0001 (***)		<0.0001 (***)	<0.0001 (***)	0.0001 (***)
d2-3Gal4/UAS-*rpr* (w/bacteria)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)		<0.0001 (***)	<0.0001 (***)
d2-3GAL4/+ (w/bacteria)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)		0.1435
UAS-*rpr*/+ (w/bacteria)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)	0.1435	

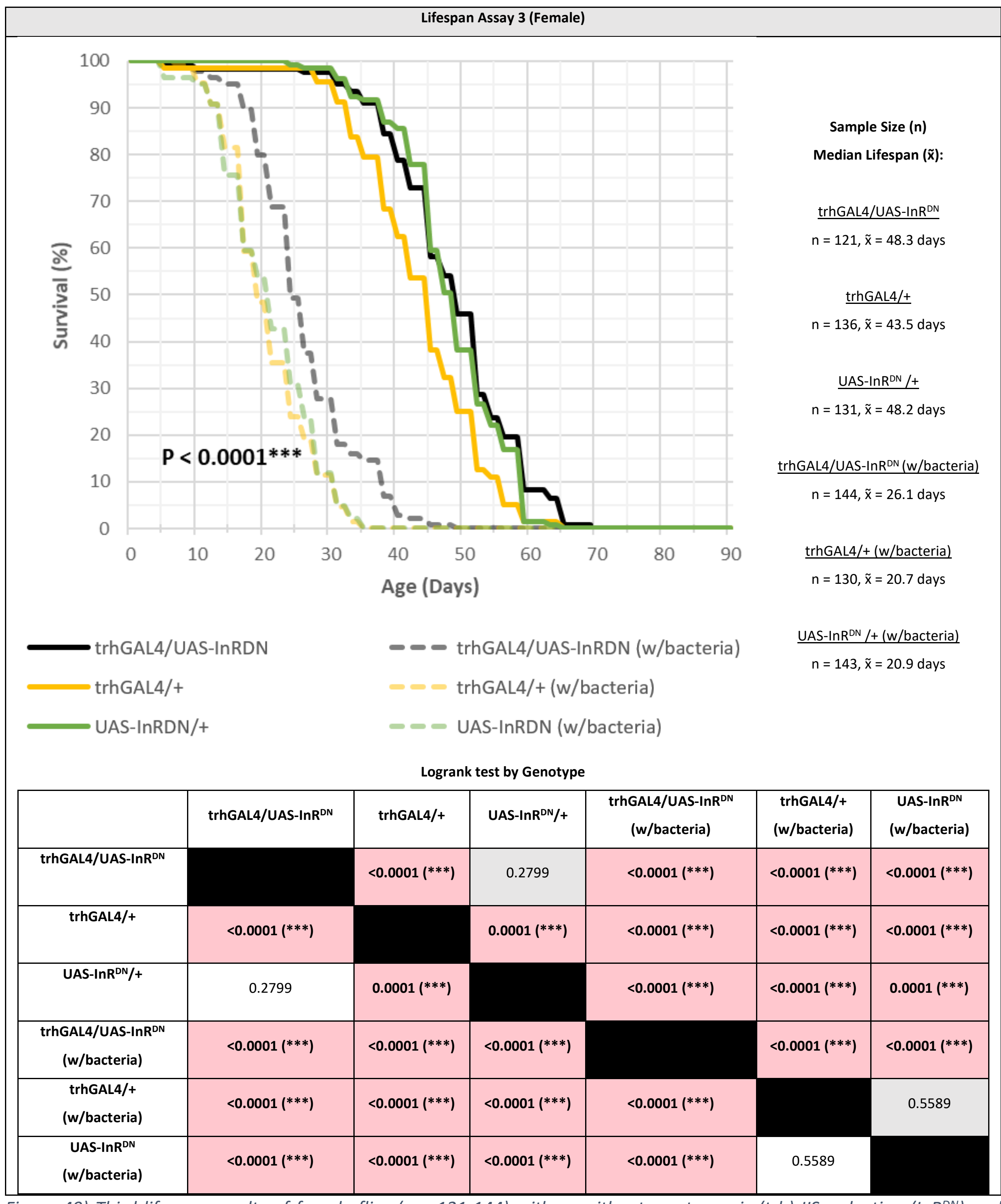
Figure 47) Third lifespan results of female flies (n = 118-149) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure



Logrank test by Genotype

	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3Gal4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
d2-3Gal4/UAS-*rpr*		**0.0028 (**)**	**0.0336 (*)**	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**
d2-3GAL4/+	**0.0023 (**)**		0.2207	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**
UAS-*rpr*/+	**0.0336 (*)**	0.2207		**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**
d2-3Gal4/UAS-*rpr* (w/bacteria)	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**		**<0.0001 (***)**	**0.0014 (**)**
d2-3GAL4/+ (w/bacteria)	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**		**<0.0001 (***)**
UAS-*rpr*/+ (w/bacteria)	**<0.0001 (***)**	**<0.0001 (***)**	**<0.0001 (***)**	**0.0014 (**)**	**<0.0001 (***)**	

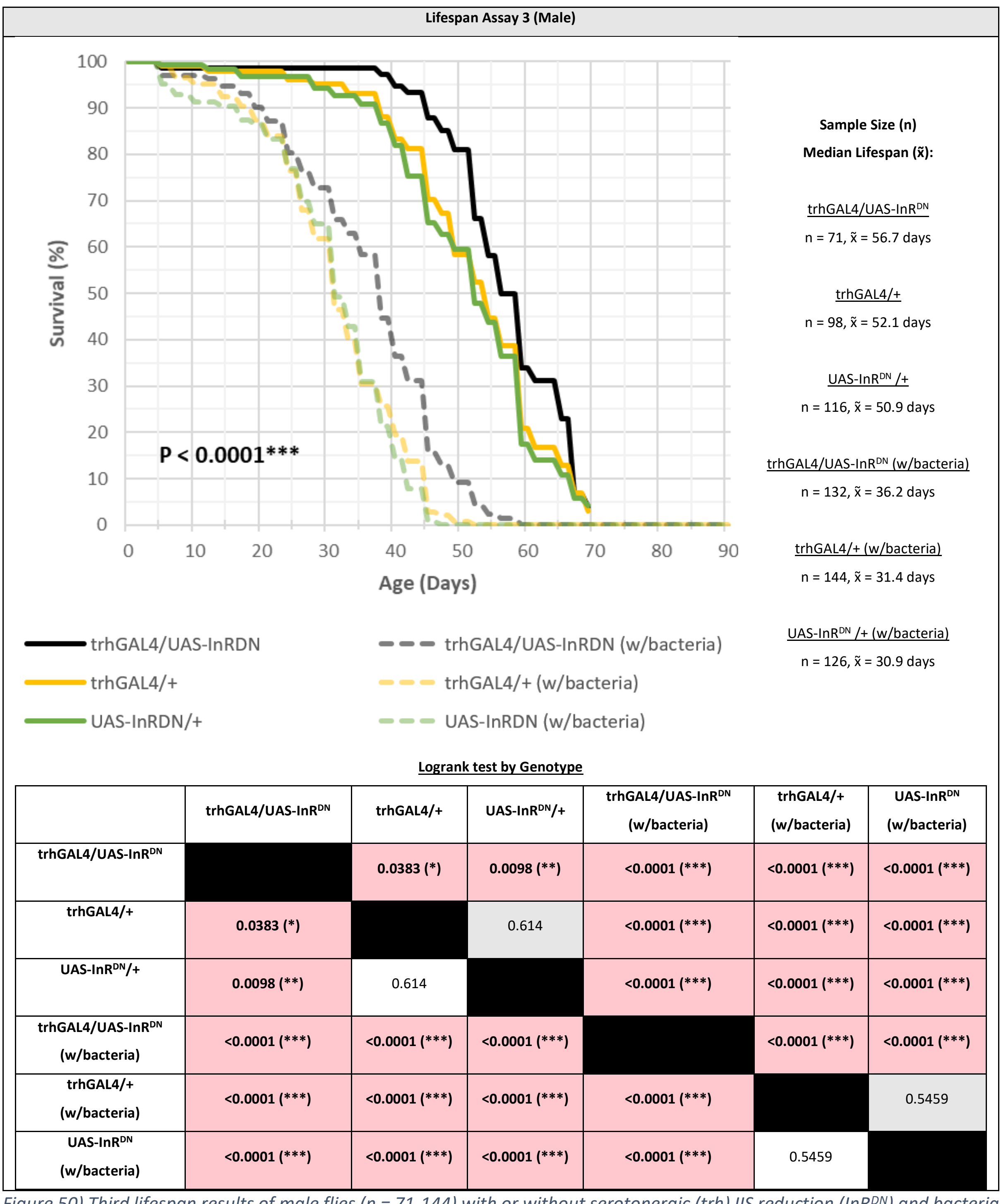
Figure 48) Third lifespan results of male flies (n = 90-144) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure



Logrank test by Genotype

	trhGAL4/UAS-InR^{DN}	trhGAL4/+	UAS-InR^{DN}/+	trhGAL4/UAS-InR^{DN} (w/bacteria)	trhGAL4/+ (w/bacteria)	UAS-InR^{DN} (w/bacteria)
trhGAL4/UAS-InR^{DN}		<0.0001 (***)	0.2799	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)
trhGAL4/+	<0.0001 (***)		0.0001 (***)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)
UAS-InR^{DN}/+	0.2799	0.0001 (***)		<0.0001 (***)	<0.0001 (***)	0.0001 (***)
trhGAL4/UAS-InR^{DN} (w/bacteria)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)		<0.0001 (***)	<0.0001 (***)
trhGAL4/+ (w/bacteria)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)		0.5589
UAS-InR^{DN} (w/bacteria)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)	0.5589	

Figure 49) Third lifespan results of female flies (n = 121-144) with or without serotonergic (trh) IIS reduction (InR^{DN}) and bacteria exposure



	trhGAL4/UAS-InR^{DN}	trhGAL4/+	UAS-InR^{DN}/+	trhGAL4/UAS-InR^{DN} (w/bacteria)	trhGAL4/+ (w/bacteria)	UAS-InR^{DN} (w/bacteria)
trhGAL4/UAS-InR^{DN}		0.0383 (*)	0.0098 (**)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)
trhGAL4/+	0.0383 (*)		0.614	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)
UAS-InR^{DN}/+	0.0098 (**)	0.614		<0.0001 (***)	<0.0001 (***)	<0.0001 (***)
trhGAL4/UAS-InR^{DN} (w/bacteria)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)		<0.0001 (***)	<0.0001 (***)
trhGAL4/+ (w/bacteria)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)		0.5459
UAS-InR^{DN} (w/bacteria)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)	<0.0001 (***)	0.5459	

Figure 50) Third lifespan results of male flies (n = 71-144) with or without serotonergic (trh) IIS reduction (InR^{DN}) and bacteria exposure

5.7.2. Bacteria increased egg laying capacity in all flies regardless of genotype

Results 5.6.4 reported that bacteria increased the number of viable offspring produced at early age but diminished the total number of offspring produced throughout life. Although the assay initially planned to quantify the number of eggs laid by flies, the sample size (n = 10 per vial) used previously yielded far too many eggs to quantify by eye. Therefore, the experiment was repeated using a smaller sample size (n = 5 per vial) to allow feasible egg quantification.

Recapitulating previous findings, systemic IIS reduction flies (Figure 51) laid fewer eggs compared to control groups (159). However, this difference was only significant from age 12 days onwards. When introduced to bacteria, the number of eggs laid was increased at least 60% across all genotypes at age 5 days. This increase was also observed among d2-3GAL4/UAS-*rpr* flies, albeit significantly fewer than bacteria treated controls. Beyond age 5 days, the mean eggs laid among all bacteria treated flies sharply declined along age, falling below the mean eggs laid by their respective non-bacteria controls. When accounting for the mean eggs laid up to age 21 days, bacteria only affected d2-3GAL4/+ flies (Figure 52) where significantly fewer eggs were found compared to its non-bacteria treated control. Altogether, it is possible that bacteria induced earlier oviposition in flies without affecting egg production across life.

Interestingly, systemic IIS reduction largely do not affect offspring production. Despite some fluctuations of offspring numbers from age 7 to 19 days (Figure 53), the total number of offspring generated up to age 21 days remain indifferent between flies with normal or reduced IIS (Figure 54). When

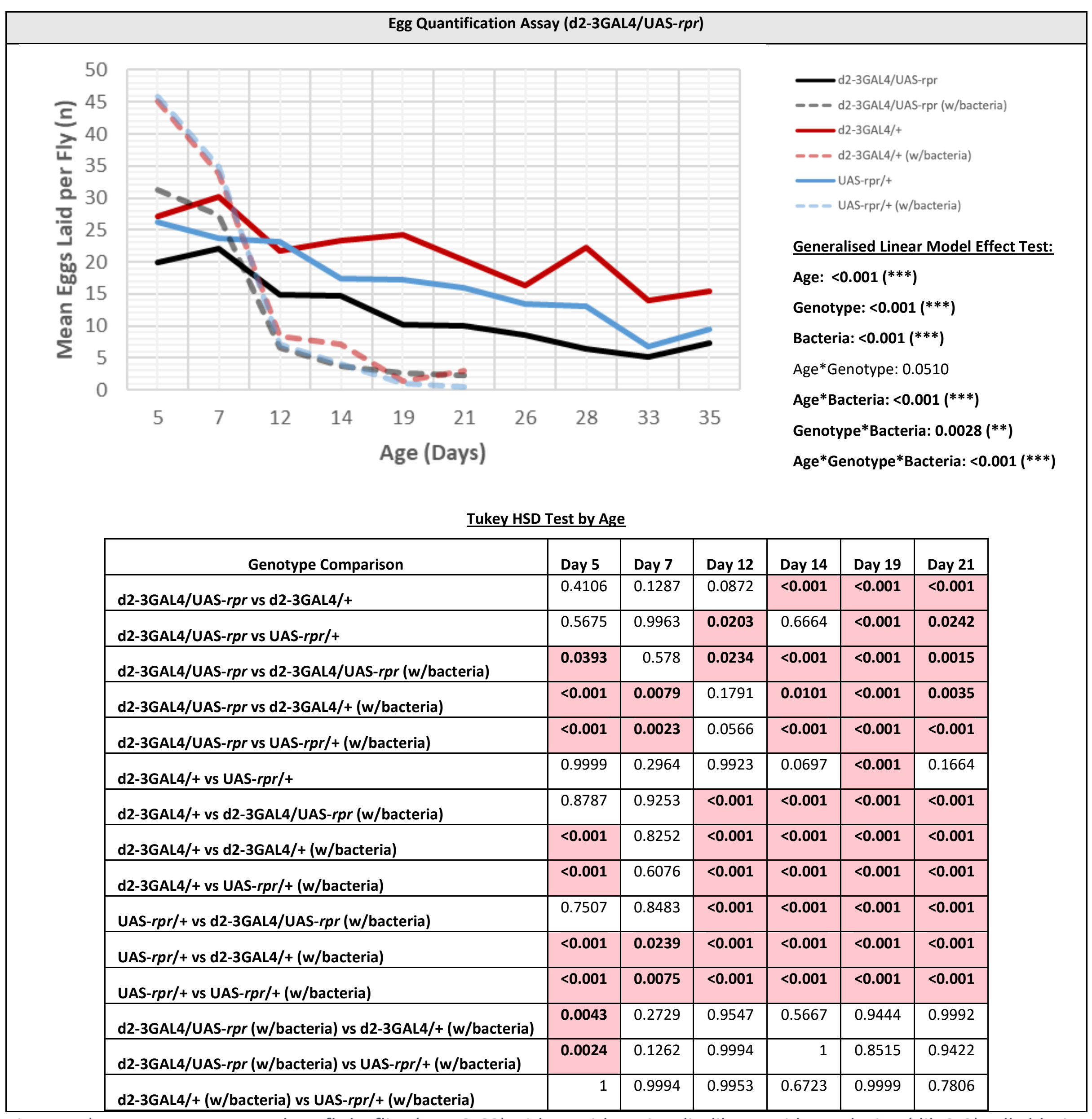
introduced to bacteria, only UAS-*rpr*/+ flies reported a significant increase in offspring generated at early age. Similar to Results 5.6.4, the number of offspring produced by bacteria treated flies greatly dwindled in subsequent days. Although bacteria treatment did not affect the mean offspring generation up to age 21 days among systemic IIS reduced flies, fewer progenies were found from bacteria treated controls than their non-bacteria-treated counterparts. Collectively, the number of offspring produced by bacteria treated flies do not reflect the high egg counts found in early life. Therefore, it is possible that bacteria-treated flies were laying eggs faster that the stored sperm could fertilise resulting lower egg fertilisation rates and earlier egg exhaustion. This in turn could explain the lower offspring generation observed.

As for flies with serotonergic IIS reduction (trhGAL4/UAS-InR^{DN}), these flies did not experience a decrease in egg counts unlike systemic IIS reduced flies. Instead, egg counts of trhGAL4/UAS-InR^{DN} flies remain higher than trhGAL4/+ controls from age 12 days onwards (Figure 55). When treated with bacteria, flies laid more than double the eggs of their respective controls at age 5 days. However, egg counts of bacteria treated flies fell below of controls in the days that follow, akin to effects seen among systemic IIS reduced flies. Contrastingly, there were no difference in the mean eggs laid by flies up to age 21 days regardless of genotype or bacteria exposure. Again, these results demonstrate that bacteria increased oviposition of flies without affecting total egg quantity.

Regarding offspring generation, serotonergic IIS reduction did not affect offspring numbers across life (Figure 57). However, bacteria treatment greatly affected the number of offspring generated across age. Although all fly group produced similar numbers of offspring at age 5 days, the number of offspring produced fell more than 68% in subsequent days across all bacteria treated flies.

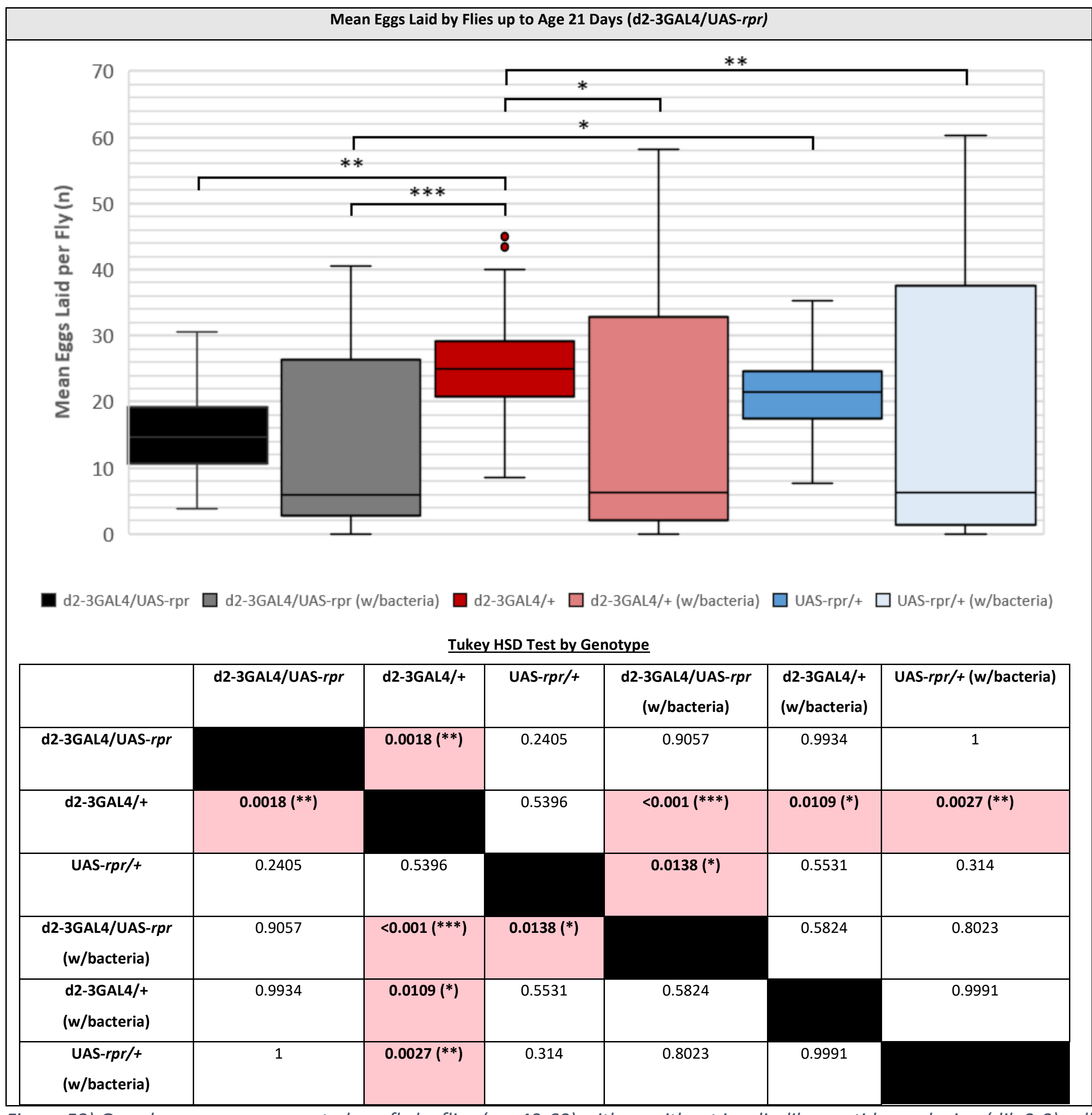
Ultimately, the mean offspring generated per fly was below 5 at age 10 onwards. Similar to the systemic IIS reduced fly cohort, the mean offspring generated by flies up to age 21 days were fewer among bacteria treated controls compared to their respective genotypes while indifferent between serotonergic IIS reduced flies with or without bacteria treatment. Again, bacteria increased the number of eggs laid by flies but not the number of offspring produced, supporting the hypothesis that flies were laying eggs faster than being fertilise.

Taken as a whole, systemic IIS reduction, but not serotonergic IIS reduction, reduced the number eggs laid by flies. Regardless, offspring generation remained unaffected. When treated with bacteria, the number of eggs laid per flies across all genotypes increased during early age, but sharply diminished later in life. Interestingly, increased early age egg laying did not boost offspring generation which conflicts with Results 5.6.4. This difference may be caused by the smaller sample size (n = 40-60 as opposed to n = 90-100) used in this assay. Regardless, bacteria still reduced the mean offspring generated during the first 21 days of life. While it is possible that bacteria-induced premature oviposition can affect offspring generation, it is also possible that bacteria increases both egg lays and offspring generation, but such results may only apply to large sample sizes.



Genotype Comparison	Day 5	Day 7	Day 12	Day 14	Day 19	Day 21
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.4106	0.1287	0.0872	**<0.001**	**<0.001**	**<0.001**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.5675	0.9963	**0.0203**	0.6664	**<0.001**	**0.0242**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	**0.0393**	0.578	**0.0234**	**<0.001**	**<0.001**	**0.0015**
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	**<0.001**	**0.0079**	0.1791	**0.0101**	**<0.001**	**0.0035**
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	**<0.001**	**0.0023**	0.0566	**<0.001**	**<0.001**	**<0.001**
d2-3GAL4/+ vs UAS-*rpr*/+	0.9999	0.2964	0.9923	0.0697	**<0.001**	0.1664
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.8787	0.9253	**<0.001**	**<0.001**	**<0.001**	**<0.001**
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	**<0.001**	0.8252	**<0.001**	**<0.001**	**<0.001**	**<0.001**
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	**<0.001**	0.6076	**<0.001**	**<0.001**	**<0.001**	**<0.001**
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.7507	0.8483	**<0.001**	**<0.001**	**<0.001**	**<0.001**
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	**<0.001**	**0.0239**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	**<0.001**	**0.0075**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	**0.0043**	0.2729	0.9547	0.5667	0.9444	0.9992
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	**0.0024**	0.1262	0.9994	1	0.8515	0.9422
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	1	0.9994	0.9953	0.6723	0.9999	0.7806

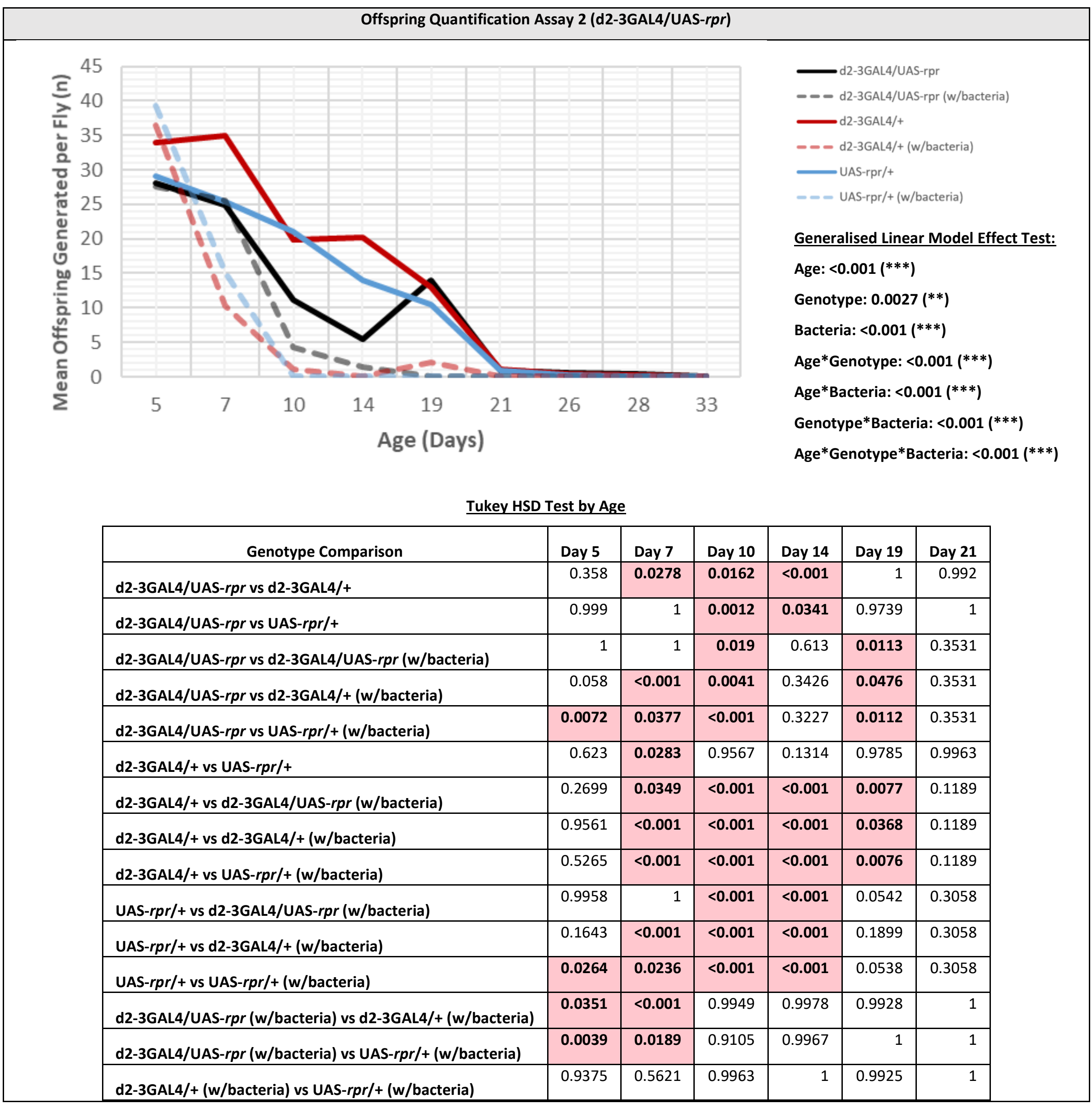
Figure 51) Mean eggs generated per fly by flies (n = 40-60) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



Tukey HSD Test by Genotype

	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
d2-3GAL4/UAS-*rpr*		**0.0018 (**)**	0.2405	0.9057	0.9934	1
d2-3GAL4/+	**0.0018 (**)**		0.5396	**<0.001 (***)**	**0.0109 (*)**	**0.0027 (**)**
UAS-*rpr*/+	0.2405	0.5396		**0.0138 (*)**	0.5531	0.314
d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9057	**<0.001 (***)**	**0.0138 (*)**		0.5824	0.8023
d2-3GAL4/+ (w/bacteria)	0.9934	**0.0109 (*)**	0.5531	0.5824		0.9991
UAS-*rpr*/+ (w/bacteria)	1	**0.0027 (**)**	0.314	0.8023	0.9991	

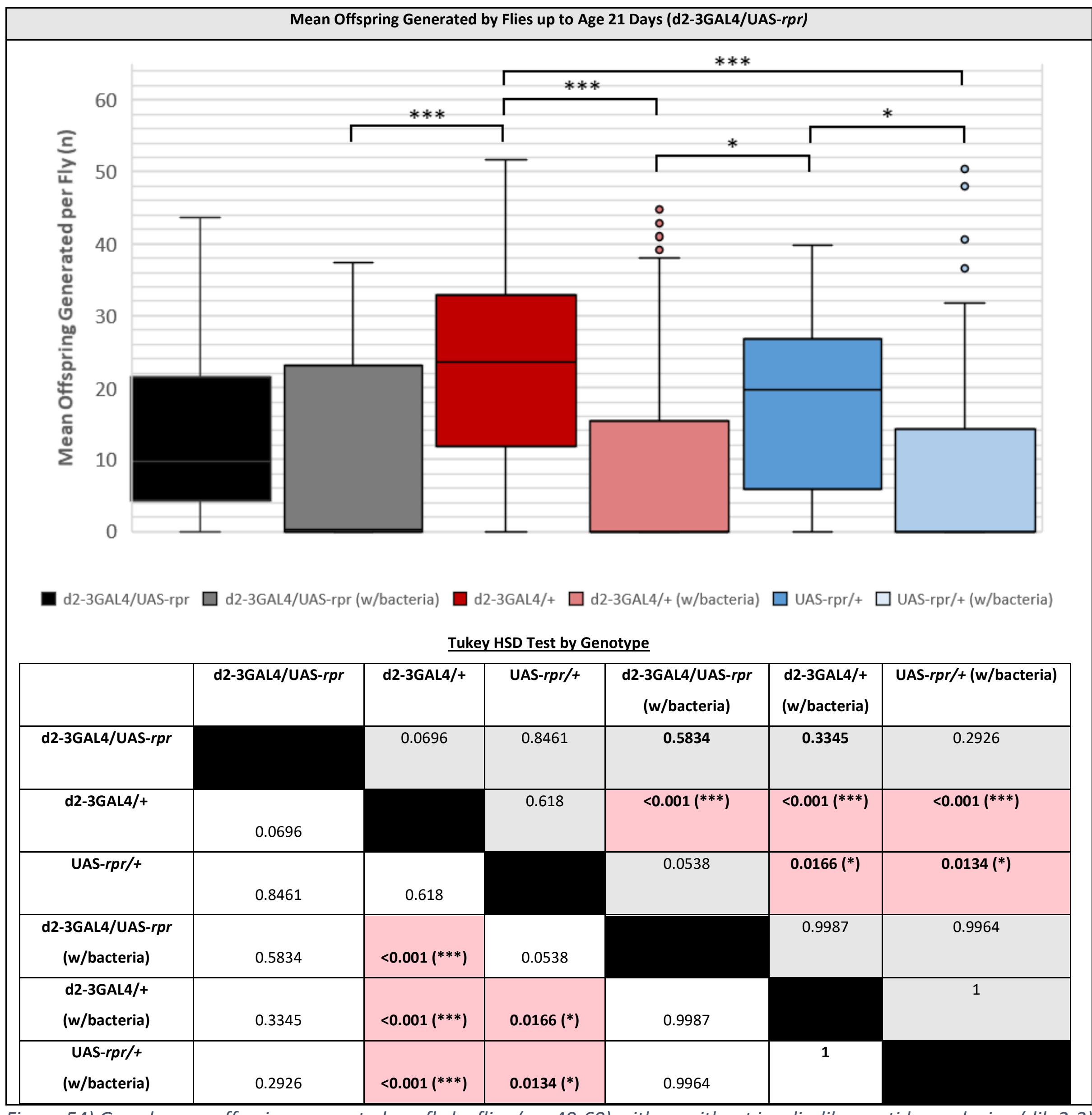
Figure 52) Grand mean eggs generated per fly by flies (n = 40-60) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure



Tukey HSD Test by Age

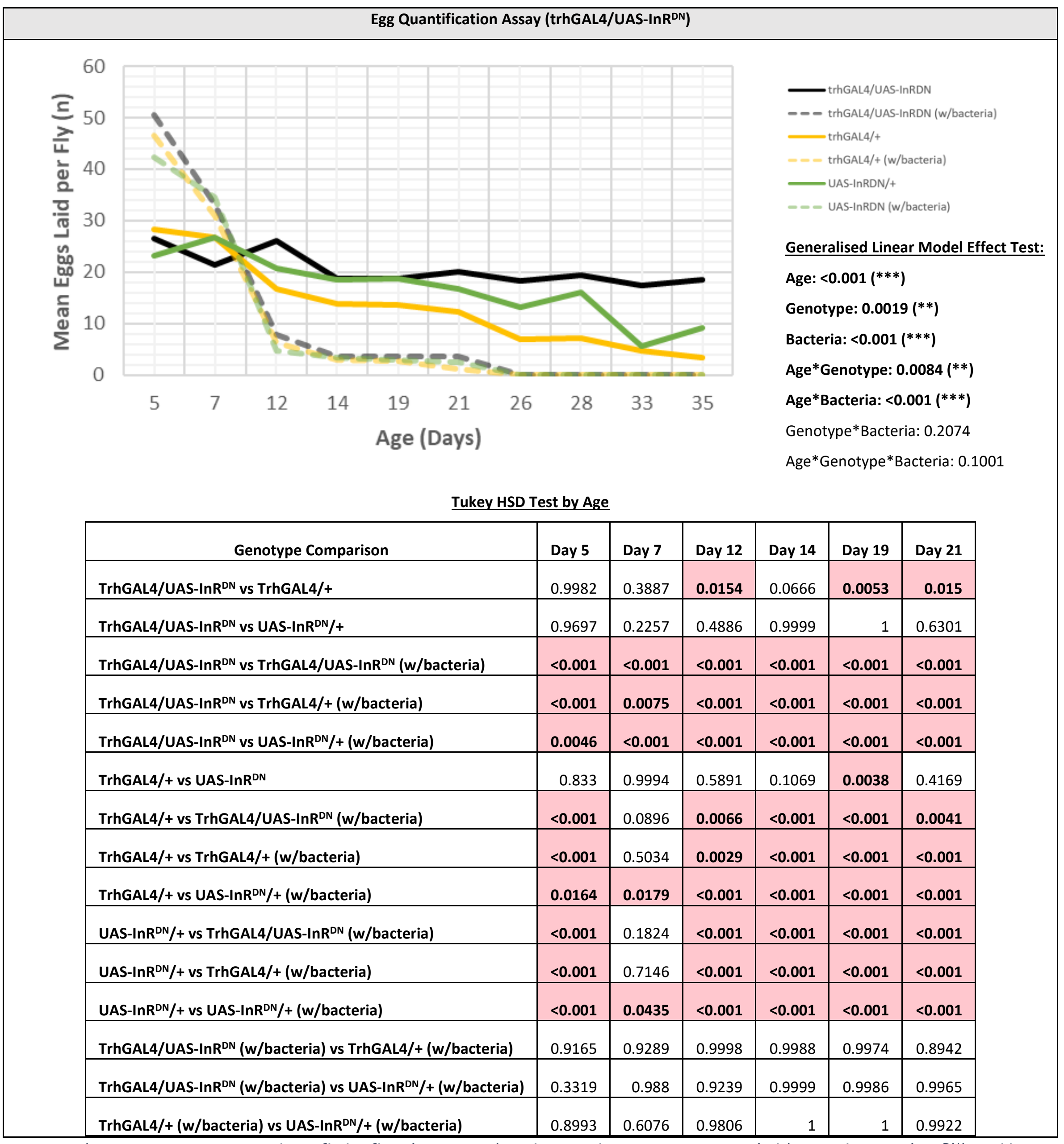
Genotype Comparison	Day 5	Day 7	Day 10	Day 14	Day 19	Day 21
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+	0.358	**0.0278**	**0.0162**	**<0.001**	1	0.992
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+	0.999	1	**0.0012**	**0.0341**	0.9739	1
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/UAS-*rpr* (w/bacteria)	1	1	**0.019**	0.613	**0.0113**	0.3531
d2-3GAL4/UAS-*rpr* vs d2-3GAL4/+ (w/bacteria)	0.058	**<0.001**	**0.0041**	0.3426	**0.0476**	0.3531
d2-3GAL4/UAS-*rpr* vs UAS-*rpr*/+ (w/bacteria)	**0.0072**	**0.0377**	**<0.001**	0.3227	**0.0112**	0.3531
d2-3GAL4/+ vs UAS-*rpr*/+	0.623	**0.0283**	0.9567	0.1314	0.9785	0.9963
d2-3GAL4/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.2699	**0.0349**	**<0.001**	**<0.001**	**0.0077**	0.1189
d2-3GAL4/+ vs d2-3GAL4/+ (w/bacteria)	0.9561	**<0.001**	**<0.001**	**<0.001**	**0.0368**	0.1189
d2-3GAL4/+ vs UAS-*rpr*/+ (w/bacteria)	0.5265	**<0.001**	**<0.001**	**<0.001**	**0.0076**	0.1189
UAS-*rpr*/+ vs d2-3GAL4/UAS-*rpr* (w/bacteria)	0.9958	1	**<0.001**	**<0.001**	0.0542	0.3058
UAS-*rpr*/+ vs d2-3GAL4/+ (w/bacteria)	0.1643	**<0.001**	**<0.001**	**<0.001**	0.1899	0.3058
UAS-*rpr*/+ vs UAS-*rpr*/+ (w/bacteria)	**0.0264**	**0.0236**	**<0.001**	**<0.001**	0.0538	0.3058
d2-3GAL4/UAS-*rpr* (w/bacteria) vs d2-3GAL4/+ (w/bacteria)	**0.0351**	**<0.001**	0.9949	0.9978	0.9928	1
d2-3GAL4/UAS-*rpr* (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	**0.0039**	**0.0189**	0.9105	0.9967	1	1
d2-3GAL4/+ (w/bacteria) vs UAS-*rpr*/+ (w/bacteria)	0.9375	0.5621	0.9963	1	0.9925	1

Figure 53) Mean offspring generated per fly by flies (n = 40-60) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure with age



	d2-3GAL4/UAS-*rpr*	d2-3GAL4/+	UAS-*rpr*/+	d2-3GAL4/UAS-*rpr* (w/bacteria)	d2-3GAL4/+ (w/bacteria)	UAS-*rpr*/+ (w/bacteria)
d2-3GAL4/UAS-*rpr*		0.0696	0.8461	**0.5834**	**0.3345**	0.2926
d2-3GAL4/+	0.0696		0.618	**<0.001 (***)**	**<0.001 (***)**	**<0.001 (***)**
UAS-*rpr*/+	0.8461	0.618		0.0538	**0.0166 (*)**	**0.0134 (*)**
d2-3GAL4/UAS-*rpr* (w/bacteria)	0.5834	**<0.001 (***)**	0.0538		0.9987	0.9964
d2-3GAL4/+ (w/bacteria)	0.3345	**<0.001 (***)**	**0.0166 (*)**	0.9987		1
UAS-*rpr*/+ (w/bacteria)	0.2926	**<0.001 (***)**	**0.0134 (*)**	0.9964	**1**	

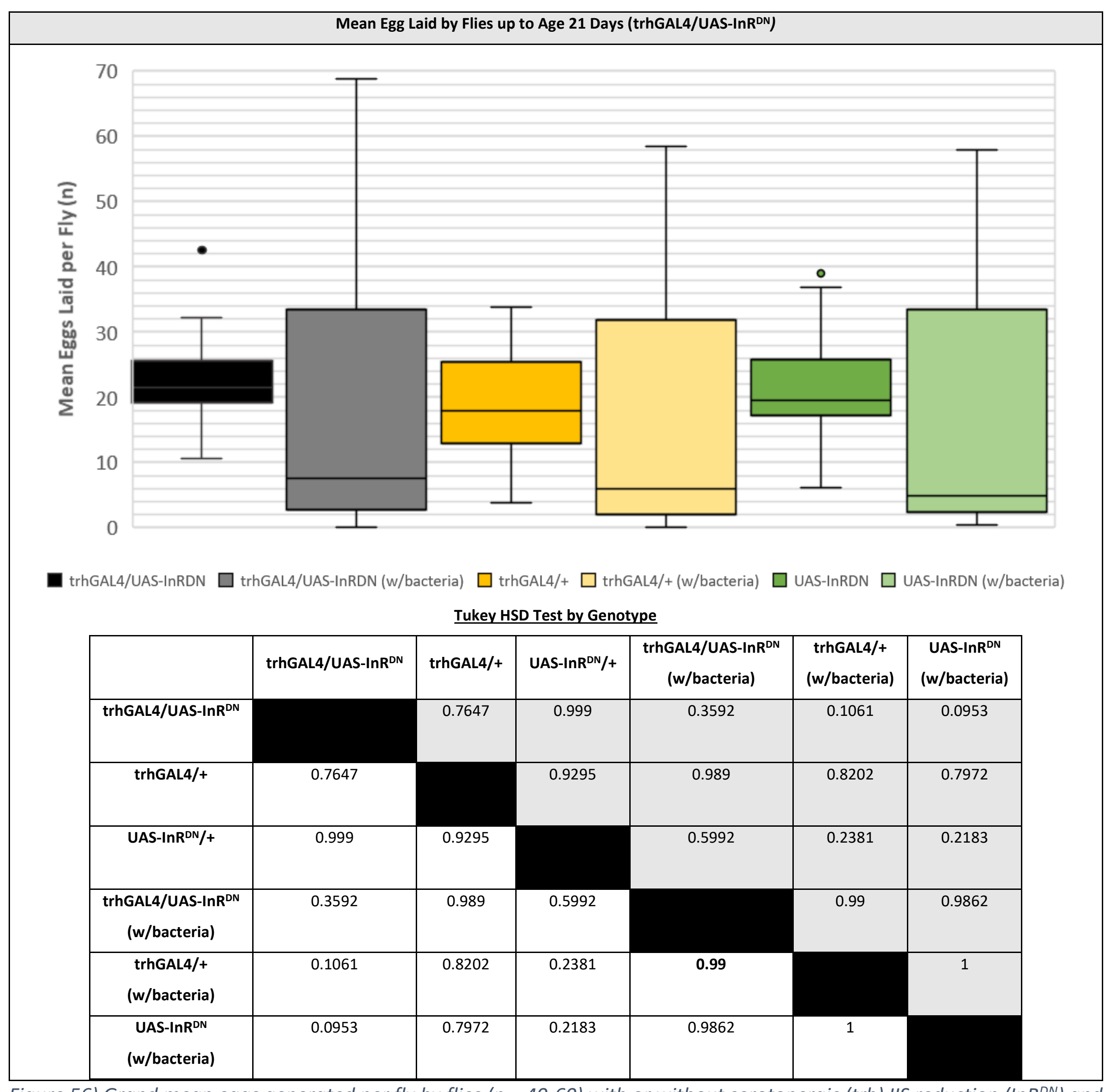
Figure 54) Grand mean offspring generated per fly by flies (n = 40-60) with or without insulin-like peptide producing (dilp2-3) cell ablation induced by reaper (rpr) gene activation and bacteria exposure



Tukey HSD Test by Age

Genotype Comparison	Day 5	Day 7	Day 12	Day 14	Day 19	Day 21
TrhGAL4/UAS-InR^{DN} vs TrhGAL4/+	0.9982	0.3887	**0.0154**	0.0666	**0.0053**	**0.015**
TrhGAL4/UAS-InR^{DN} vs UAS-InR^{DN}/+	0.9697	0.2257	0.4886	0.9999	1	0.6301
TrhGAL4/UAS-InR^{DN} vs TrhGAL4/UAS-InR^{DN} (w/bacteria)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
TrhGAL4/UAS-InR^{DN} vs TrhGAL4/+ (w/bacteria)	**<0.001**	**0.0075**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
TrhGAL4/UAS-InR^{DN} vs UAS-InR^{DN}/+ (w/bacteria)	**0.0046**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
TrhGAL4/+ vs UAS-InR^{DN}	0.833	0.9994	0.5891	0.1069	**0.0038**	0.4169
TrhGAL4/+ vs TrhGAL4/UAS-InR^{DN} (w/bacteria)	**<0.001**	0.0896	**0.0066**	**<0.001**	**<0.001**	**0.0041**
TrhGAL4/+ vs TrhGAL4/+ (w/bacteria)	**<0.001**	0.5034	**0.0029**	**<0.001**	**<0.001**	**<0.001**
TrhGAL4/+ vs UAS-InR^{DN}/+ (w/bacteria)	**0.0164**	**0.0179**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
UAS-InR^{DN}/+ vs TrhGAL4/UAS-InR^{DN} (w/bacteria)	**<0.001**	0.1824	**<0.001**	**<0.001**	**<0.001**	**<0.001**
UAS-InR^{DN}/+ vs TrhGAL4/+ (w/bacteria)	**<0.001**	0.7146	**<0.001**	**<0.001**	**<0.001**	**<0.001**
UAS-InR^{DN}/+ vs UAS-InR^{DN}/+ (w/bacteria)	**<0.001**	**0.0435**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
TrhGAL4/UAS-InR^{DN} (w/bacteria) vs TrhGAL4/+ (w/bacteria)	0.9165	0.9289	0.9998	0.9988	0.9974	0.8942
TrhGAL4/UAS-InR^{DN} (w/bacteria) vs UAS-InR^{DN}/+ (w/bacteria)	0.3319	0.988	0.9239	0.9999	0.9986	0.9965
TrhGAL4/+ (w/bacteria) vs UAS-InR^{DN}/+ (w/bacteria)	0.8993	0.6076	0.9806	1	1	0.9922

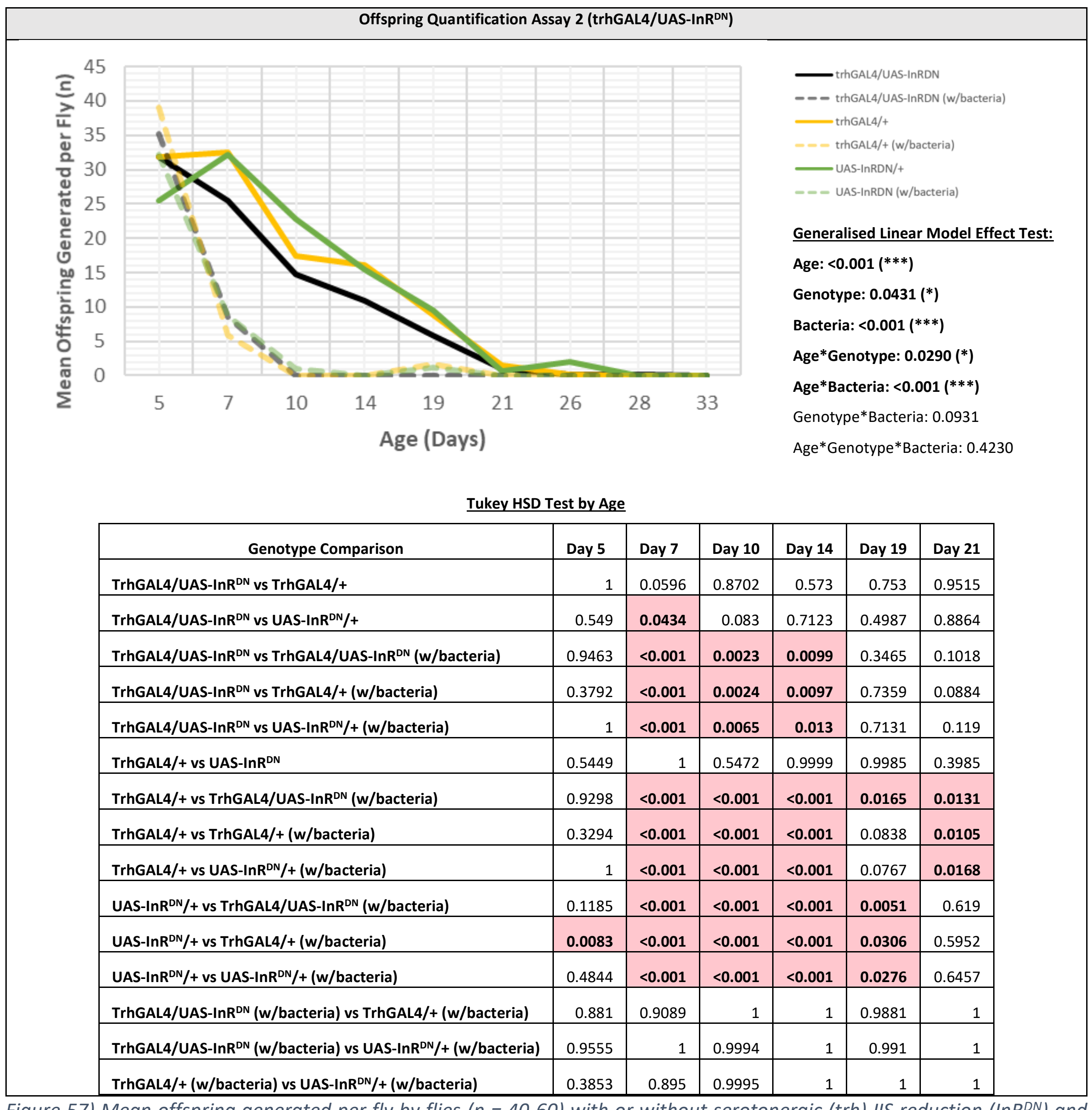
Figure 55) Mean eggs generated per fly by flies (n = 40-60) with or without serotonergic (trh) IIS reduction (InR^{DN}) and bacteria exposure with age



<u>**Tukey HSD Test by Genotype**</u>

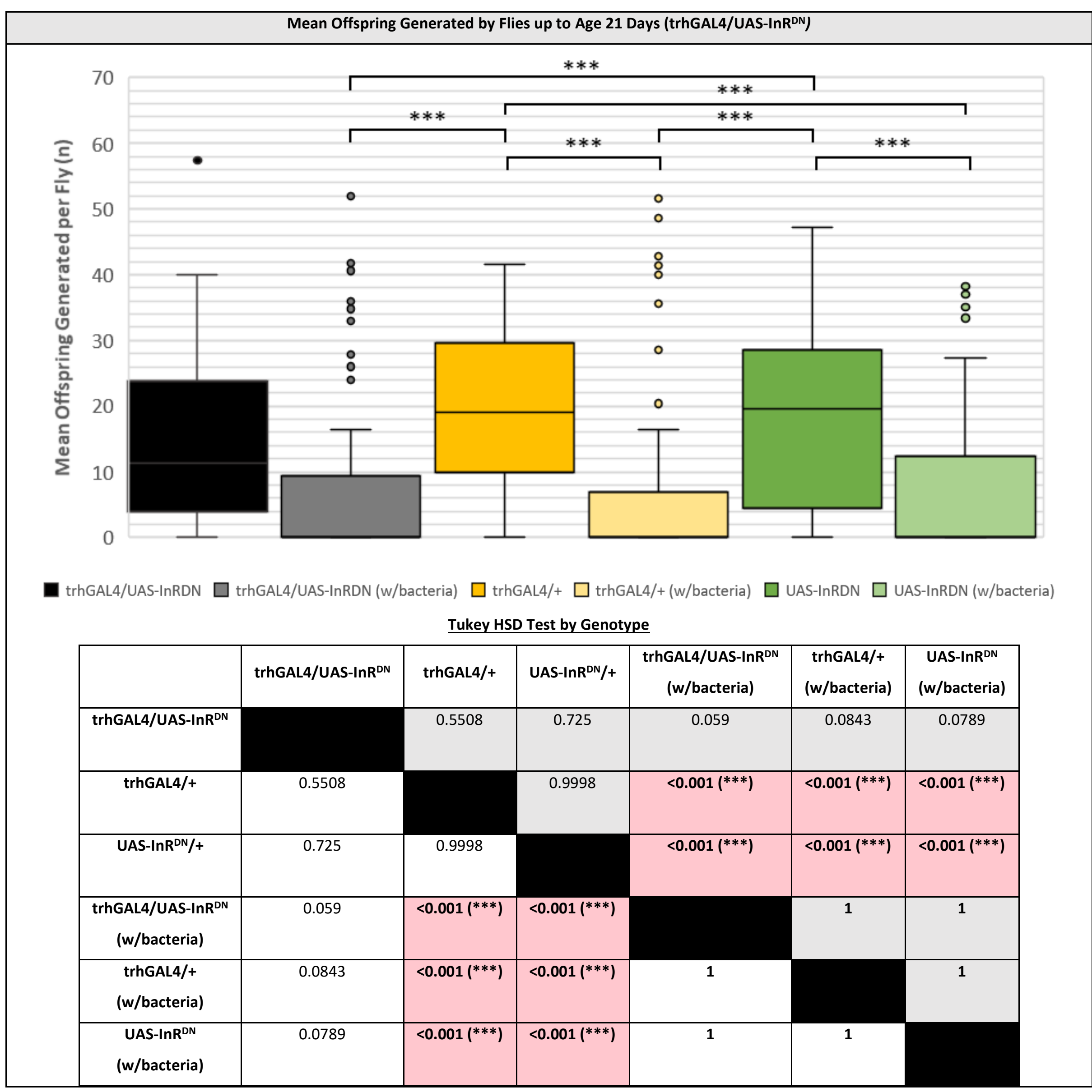
	trhGAL4/UAS-InR^{DN}	**trhGAL4/+**	**UAS-InR^{DN}/+**	**trhGAL4/UAS-InR^{DN} (w/bacteria)**	**trhGAL4/+ (w/bacteria)**	**UAS-InR^{DN} (w/bacteria)**
trhGAL4/UAS-InR^{DN}		0.7647	0.999	0.3592	0.1061	0.0953
trhGAL4/+	0.7647		0.9295	0.989	0.8202	0.7972
UAS-InR^{DN}/+	0.999	0.9295		0.5992	0.2381	0.2183
trhGAL4/UAS-InR^{DN} (w/bacteria)	0.3592	0.989	0.5992		0.99	0.9862
trhGAL4/+ (w/bacteria)	0.1061	0.8202	0.2381	**0.99**		1
UAS-InR^{DN} (w/bacteria)	0.0953	0.7972	0.2183	0.9862	1	

Figure 56) Grand mean eggs generated per fly by flies (n = 40-60) with or without serotonergic (trh) IIS reduction (InR^{DN}) and bacteria exposure



Genotype Comparison	Day 5	Day 7	Day 10	Day 14	Day 19	Day 21
TrhGAL4/UAS-InR^{DN} vs TrhGAL4/+	1	0.0596	0.8702	0.573	0.753	0.9515
TrhGAL4/UAS-InR^{DN} vs UAS-InR^{DN}/+	0.549	**0.0434**	0.083	0.7123	0.4987	0.8864
TrhGAL4/UAS-InR^{DN} vs TrhGAL4/UAS-InR^{DN} (w/bacteria)	0.9463	**<0.001**	**0.0023**	**0.0099**	0.3465	0.1018
TrhGAL4/UAS-InR^{DN} vs TrhGAL4/+ (w/bacteria)	0.3792	**<0.001**	**0.0024**	**0.0097**	0.7359	0.0884
TrhGAL4/UAS-InR^{DN} vs UAS-InR^{DN}/+ (w/bacteria)	1	**<0.001**	**0.0065**	**0.013**	0.7131	0.119
TrhGAL4/+ vs UAS-InR^{DN}	0.5449	1	0.5472	0.9999	0.9985	0.3985
TrhGAL4/+ vs TrhGAL4/UAS-InR^{DN} (w/bacteria)	0.9298	**<0.001**	**<0.001**	**<0.001**	**0.0165**	**0.0131**
TrhGAL4/+ vs TrhGAL4/+ (w/bacteria)	0.3294	**<0.001**	**<0.001**	**<0.001**	0.0838	**0.0105**
TrhGAL4/+ vs UAS-InR^{DN}/+ (w/bacteria)	1	**<0.001**	**<0.001**	**<0.001**	0.0767	**0.0168**
UAS-InR^{DN}/+ vs TrhGAL4/UAS-InR^{DN} (w/bacteria)	0.1185	**<0.001**	**<0.001**	**<0.001**	**0.0051**	0.619
UAS-InR^{DN}/+ vs TrhGAL4/+ (w/bacteria)	**0.0083**	**<0.001**	**<0.001**	**<0.001**	**0.0306**	0.5952
UAS-InR^{DN}/+ vs UAS-InR^{DN}/+ (w/bacteria)	0.4844	**<0.001**	**<0.001**	**<0.001**	**0.0276**	0.6457
TrhGAL4/UAS-InR^{DN} (w/bacteria) vs TrhGAL4/+ (w/bacteria)	0.881	0.9089	1	1	0.9881	1
TrhGAL4/UAS-InR^{DN} (w/bacteria) vs UAS-InR^{DN}/+ (w/bacteria)	0.9555	1	0.9994	1	0.991	1
TrhGAL4/+ (w/bacteria) vs UAS-InR^{DN}/+ (w/bacteria)	0.3853	0.895	0.9995	1	1	1

Figure 57) Mean offspring generated per fly by flies (n = 40-60) with or without serotonergic (trh) IIS reduction (InR^{DN}) and bacteria exposure with age



	trhGAL4/UAS-InR^{DN}	trhGAL4/+	UAS-InR^{DN}/+	trhGAL4/UAS-InR^{DN} (w/bacteria)	trhGAL4/+ (w/bacteria)	UAS-InR^{DN} (w/bacteria)
trhGAL4/UAS-InR^{DN}		0.5508	0.725	0.059	0.0843	0.0789
trhGAL4/+	0.5508		0.9998	**<0.001 (***)**	**<0.001 (***)**	**<0.001 (***)**
UAS-InR^{DN}/+	0.725	0.9998		**<0.001 (***)**	**<0.001 (***)**	**<0.001 (***)**
trhGAL4/UAS-InR^{DN} (w/bacteria)	0.059	**<0.001 (***)**	**<0.001 (***)**		**1**	**1**
trhGAL4/+ (w/bacteria)	0.0843	**<0.001 (***)**	**<0.001 (***)**	**1**		**1**
UAS-InR^{DN} (w/bacteria)	0.0789	**<0.001 (***)**	**<0.001 (***)**	**1**	**1**	

Figure 58) Grand mean offspring generated per fly by flies (n = 40-60) with or without serotonergic (trh) IIS reduction (InR^{DN}) and bacteria exposure

5.7.3. Summary of Results 5.7

Similar to the Result 5.6.1, bacteria treatment is detrimental to host health and increasing its concentration further shortens lifespan. Systemic reductions of IIS did not extend lifespan in flies of both sexes but alleviated the lifespan-shortening effects of bacteria treatment in female flies. Unexpectedly, systemic IIS reduction resulted in a shorter median lifespan of male flies compared to controls. In the case of serotonergic IIS reduction flies, females showed similar results with median lifespan extension observed in bacteria-treated flies but not in non-bacteria treated flies. On the other hand, male flies reported longer median lifespans compared to their respective controls. Contradicting Result 5.5.1 and Result 5.6.1, external factors may have influenced the longevity of the flies, as variations in lifespan were observed among flies of the same generation but different treatments. Regarding fecundity, bacteria treatment increased egg-laying in all flies at early age which led to earlier egg exhaustion. Despite so, the mean number of eggs laid in the first 21 days of life remain indifferent to controls. Unlike Result 5.6.4, the increased egg lays did not increase offspring generation. Instead, bacteria reduced the number of offspring found across life. Hence, early age egg exhaustion may have caused this reduction in offspring found across bacteria treated flies. Alternatively, it could be possible that the smaller sample size used in this experiment did not allow the detection of both increased egg lays and offspring numbers simultaneously.

6. Discussion

The gut microbiome plays an intricate role in host health and its absence can negatively impact fly lifespan, memory, and fecundity (161–163). Despite the importance of the gut microbiome, our understanding of what constitutes a healthy gut and how commensal bacteria benefit the host remains limited. Previously, the Benedetto lab investigated these interactions in *C.* elegans using their naturally occurring gut bacteria referenced from the CeMbio database. Expanding on these studies, this project introduces these same commensal bacteria to *D. Melanogaster* from the Broughton lab, in hopes of discovering bacteria-host interactions that are conserved across species, IIS dependent, or able to affect complex behaviours such as exploratory walking or sleep. In doing so, new experimental protocols and fluorescent bacteria were generated. From results, three fluorescent bacteria (namely MYb71-sfGFP, MYb174-dTomato, and CEent1-mPlum) successfully colonised the fly gut, supporting the notion that these natural *C. elegans* may also naturally occur in the microbiome of *D. melanogaster*. When further assessed for effects against fly health across age, bacteria appeared to directly affect fly lifespan and fecundity while attenuating changes to health induced by IIS reduction. Simultaneously, these experiments recapitulated previous findings and provided additional insight into the effects of IIS reduction on fly health, as observed in exploratory walking experiments. Taken together, these observations suggest that these bacteria affect multiple biological pathways in the fly, where some of these interactions involve IIS. Reflecting on this study, this section discusses these findings while proposing optimisation strategies and ideas for future research.

6.1. MYb174-dTom successfully colonised fly guts despite no growth in fly media

Despite no apparent growth on fly media (Result 5.1), MYb174-dTom was still successful in colonising the fly gut in its subsequent pilot experiment (Result 5.3). These findings suggest that absence of bacteria growth in fly media does not imply bacterial death, but instead bacterial dormancy, likely caused by differences in media composition. While both bacteria and fly media contain similar ingredients, LB agar (Difco, Becton Dickinson) also contains tryptone (10 g/L) and sodium chloride (5 g/L). Importantly, tryptone acts as a source of nitrogen and carbon while sodium chloride provides sodium ions and maintains osmotic balance in media (164,165). Absent in fly media, these conditions may have suppressed bacterial growth but not killed them. As different bacteria react to nutrient deprivation differently, this may explain why some bacteria form colonies earlier than others. Additionally, bacteria inoculated onto fly media containing nipagin and propionic acid may be dormant as well. This is because both compounds were shown to inhibit bacteria growth rather than exerting bactericidal effects (166,167). Therefore, it is worth exploring if all bacteria from the CeMbio database could colonise the fly gut regardless of their growth status in fly media or exposure to additives. Alternatively, tryptone and sodium chloride may be supplemented into fly media to preserve bacteria viability.

6.2. Optimisation strategies for bacteria transformation

Out of 24 attempted transformations, 6 new fluorescent bacteria were successfully generated, resulting in a success rate of only 25%. To improve the efficiency of bacteria transformation in future experiments, several optimization

steps were suggested and referenced (124). In half of the failed transformations, no fluorescent clones were detected after bacterial mating. This suggests that certain target strains may require extended mating time due to strain-specific mating inefficiencies. Additionally, it is possible that transformed strains are slow to integrate or express the acquired gentamicin resistance. Hence, these bacteria may also require a post-mating outgrowth to allow proper expression of gentamicin resistance.

Regarding the occurrence of non-fluorescent clones on selective media, it is possible that these clones were only partially transformed, carrying only the gentamicin resistance without fluorescence. These observations suggest that certain target strains may have a lower success rate of fluorescent plasmid integrations. To increase the chances of generating target bacteria with both fluorescence and antibiotic resistance, it would be worth considering increasing the number of transformation replicates.

Another challenge encountered was the lack of stable fluorescence expression in certain transformed clones when grown on non-selective media. This suggests that the fluorescent gene was not chromosomally integrated into the target strain, resulting in the loss of fluorescence upon cellular division. To address this issue, it is recommended to isolate multiple colonies from the post-mating selection plates to increase chances of obtaining a clone with stable fluorescence. As important, the selection plate should not be discarded until stable fluorescence is confirmed in isolates.

Lastly, some of the transformed bacteria were identified as helper bacteria rather than the intended target strain. Unfortunately, the mechanism by which these donor strains bypassed bacteria selection remains to be

determined. To prevent erroneous clone isolation and waste of lab resources, it is essential to cross-check colony morphologies or antibiotic susceptibilities clones with their respective untransformed strains before performing DNA extraction and sequencing.

6.3. Bacteria reduces fly lifespan

From the main experiments (Result 5.6.1 and 5.7.1), bacteria treatment increased fly fecundity but shortens their lifespan. Although these findings were not reflected in Result 5.4 or inconsistent in Result 5.5.1, it was suspected that low sample size may have reduced the power of these experiments. Regardless, this reduction in lifespan may be attributed to an increased bacteria load. As the experimental flies were not axenic, it is possible that the flies already developed a microbiome prior to bacteria treatment and bacterial administration further increased bacterial load in the fly gut. This was apparent in Results 5.7.1 as flies unintendedly exposed to higher bacteria inoculant concentrations experienced a shorter lifespan compared to other bacteria-treated flies from Results 5.5.1 and 5.6.1. Although the bacteria inoculant load was constant throughout all experiments, the inoculated vials were dried differently. Vials used in Results 5.4, 5.5.1 and 5.6.1 were dried for at least 2 hours in a class 2 microbiology safety cabinet hood, but vials from Results 5.7.1 were dried for 4 hours in a PCR laminar flow cabinet. This change was made due to logistical complications at the time of the experiment. Consequently, the bacteria inoculant from the latter experiments may not have been properly dried, leading to a higher bacteria load that worsened host health.

The effects of different bacteria load on host health were not properly explored here but other studies demonstrated that excessive bacteria load disrupts fly gut integrity leading to the spread of microbes across the body causing chronic inflammation (168). While differences in lifespan results between Result 5.5.1 to Results 5.6.1 and 5.7.1 may be attributed to the disruptions of gut integrity, there were no “smurf” assays performed for the latter experiments to confirm this. Hence, future bacteria experiments may consider incorporating “smurf” assays or bacteria quantification assays to allow comparison of fly gut integrity or bacterial load between multiple experiment sets. Alternatively, measuring the gene/protein expression levels of drosophila antimicrobial peptides (defensin or drosomycin), JAK-STAT and KEAP1-NRF2 signalling may also help gauge the severity of bacterial-induced intestinal inflammation (168–171).

While it could be argued that a lifespan-fecundity trade-off may have caused reduced lifespan in flies, male flies also experienced shorten lifespan when exposed to bacteria. This suggests that though increased early life egg lays may contribute to the reduction in median lifespan, it is not the sole reason for this phenomenon. Another possibility is that bacteria may only be harmful to flies during old age. While bacteria have a beneficial role in early life and development, the build-up of bacteria over time is harmful (168). Evidently, flies introduced to antibiotics at age 31 have an 8% longer median lifespan compared to controls (162). These results may explain why the gut permeability of flies (Result 5.5.3) were unaffected by bacteria during the first half of the fly’s lifespan (Result 5.5.1). To maintain host health, reducing the bacterial load in later life may be necessary. One potential approach could be to introduce

bacteria during the flies' youth to enhance fecundity and administer antibiotics later to reduce bacterial load.

In terms of bacteria's attenuating affects against median lifespan extension of IIS reduction, it is possible that the tested bacteria may have compensated for IIS reduction. Previous research found that *E. ludwigii* (the same species as MYb174) alone boosts dilp2 and dilp5 expression in larvae as they reach adulthood (172). To determine if this compensatory effect was true, future experiments should include quantitative (q)PCR or immunohistochemistry assays to measure the expression of these genes and proteins.

Given that the combination of MYb71-sfGFP, MYb174-dTomato, and CEent1-mPlum negatively affect fly lifespan (Result 5.6.1 and 5.7.1), it would be worth repeating lifespan assays introducing these bacteria individually to find out if these effects were caused by one bacterium or as a collective. Moreover, other bacteria candidates (Appendix 10.2) from the CeMbio database should also be considered.

6.4. Bacteria increases early-life egg laying

Certain commensal bacteria, such as *Acetobacter aceti*, *A. pasteurianus*, *A. pomorum*, and *A. tropicalis,* have been shown to enhance fecundity in flies (173). Another notable bacterium would be *Wolbachia sp*. As an intracellular bacterium, *Wolbachia sp. affects fly* fecundity differently based on host nuclear background and environmental conditions (174,175). Notably, infected flies exhibited decreased fly fecundity in tropical climates but increased fecundity in the same flies under colder climates. Highlighting the important relationship

between commensal bacteria and fecundity, MYb71-sfGFP, MYb174-dTomato, and CEent1-mPlum together significantly boost the egg laying capacity and sometimes offspring numbers of flies during early age. This highlights a potential fitness advantage as early age egg increases the chances of successful reproduction in low-survival rate environments with high predation or scarce food.

Although Result 5.7.2 showed that bacteria treatment increased egg laying in all fly genotypes, lower egg lays was observed across d2-3GAL4/UAS-*rpr* flies regardless of bacteria treatment compared to their controls. This was expected as reduction of dilp2 expression reduces fly fecundity and ovary size, indicating that bacteria only increased the egg-laying capacity but not egg production (139,176). On the other hand, these results were not captured by serotonergic IIS reduced flies indicating that this IIS model does not modulate egg laying. To further probe if the increased egg-laying was caused by bacteria interaction or bacteria-produced metabolites, it would be ideal to repeat the experiment using heat-killed bacteria. Also, future experiments should assay DILP2 and octopamine production as reduction in both pathways are associated to reduced egg laying (159,177). Therefore, bacteria may have increased the production of both compounds resulting in increased egg lays.

In terms of offspring generation, the number of progenies produced by bacteria treated flies does not always reflect the high egg counts observed in early life. Here, bacteria increased the number of offspring generated in flies from Result 5.6.4 but not Result 5.7.1 regardless of genotype. Speculatively, it is possible that a higher bacteria inoculant concentration may have compromised offspring viability or that bacteria-treated flies were laying eggs faster that the stored sperm could fertilise. Alternatively, lower sample size may have reduced

Result 5.7.1's power. To assess these presumptions, an additional comparison group should be included where bacteria treated flies are transferred onto sterile media without bacteria treatment to assay offspring viability. Additionally, it's also unclear if offspring generation was limited by the amount of sperm stored in once-mated females. Therefore, this experiment should be repeated using flies allowed to mate throughout life.

Similar to discussion pertaining to lifespan, the bacteria-induced increase in early life egg lays or offspring generation cannot be attributed towards a single bacterium. Previously, *Enterobacter ludwigii* alone had no effect on fly fecundity or embryo survivability when tested on Oregon-R flies (178). However, it is unclear if the null effect also applies to white Dahomey flies. Hence, it would be worthwhile studying the effects of these bacteria individually on flies.

6.5. Differing results from exploratory walking and sleep analysis to past studies

Previously, the effects of systemic IIS reduction against exploratory walking were largely insignificant except for slower decline in mean walking duration found among female flies (154). However, Result 5.5.2 found that systemic IIS reduction also slowed the decline of mean distance moved, mean velocity, and mean rotations in addition. Comparatively, these changes to exploratory walking behaviour were observed between age 40 to 50 days which was beyond the age range (age 35 days) studied by Ismail et al (2015). Therefore, it is possible that exploratory walking behaviour tend to significantly decline later in life (beyond age 35 days). Regarding sleep behaviour, past studies found that systemic IIS reduction decreases total sleep and sleep bout length in male

flies (158). However, our study showed that systemic IIS reduction decreased total sleep among female flies instead. Comparatively, Cong et al (2015) utilised w^{1118} flies while $W^{Dahomey}$ flies were utilised here. Hence, these contrasting results may be strain specific.

Another consideration when interpretating exploratory walking and sleep assay results is that the survival rate between experimental groups, bacteria conditions, and sex are highly variable. Alone, the survival rate for female flies at age 40 days (16.43%-77.69% in Result 5.5.1 and 14.5-73.14% in Results 5.6.1) were more variable than male flies (76.47%-94.61% and 60.58%-87.54% respectively). Given the lower and variable survivability of female flies beyond age 40 days, the presence dead conspecifics could confound behavioural assay results as death perception reduces lifespan in flies (179). Therefore, it may be necessary to incorporate the number of deaths as a cofactor during data analysis while ensuring dead flies are not transferred into fresh fly media.

Regardless, IIS reduction-mediated changes to exploratory and sleep behaviour were attenuated by bacteria exposure. Speculatively, bacteria may have compensated for the effects of reduced systemic IIS as these parameters were restored to levels similar to controls. To further understand these interactions, future experiments may include a qPCR assay to measure *dilp2* expression of bacteria-treated flies with systemic IIS reduction. Additionally, exploratory walking and sleep assays may be repeated using pan-neuronal reduction of IIS as these flies experience earlier declines in these parameters with age (154,180). In preparation for these experiments, the elavGAL4/+ line has been backcrossed for a minimum of five generations, ready to be used.

6.6. Omitting nipagin and propionic acid may be harmful to flies

Excluding flies with serotonergic IIS reduction, the median lifespan of the experimental flies was 40% shorter (≥ 20 days) compared to similar studies using flies of the same genotypes (159,181). This discrepancy may be due to the omission of nipagin and propionic acid in fly media. As mentioned, the omission of these additives was deemed necessary as Results 5.1 demonstrated their growth inhibitory effects to selected bacteria. Without these additives, mould was found growing in the experimental vials despite frequent transfers. Inevitably, mould may have affected fly health leading to shorter median lifespans overall.

Concerningly, mould may have affected the health of female flies worse than males. Results 5.5.1, 5.6.1, and 5.7.1 revealed that male flies had longer median lifespans than female flies regardless of genotype nor bacteria treatment. These findings conflicted with previous studies reporting females as the longer-lived sex among flies due to their ability to withstand starvation or extreme temperatures better than male flies (182). However, female flies have higher feeding rates than males, which may have increased the chances of ingesting pathogens (168,183). As such, female flies may be more susceptible to mould infections or bacteria overgrowth in the gut which could exacerbate host health (184). Furthermore, nipagin and propionic acid may be beneficial to fly development as larvae exposed with either additive reported increased feeding behaviour and size (185,186). Therefore, it is possible that both additives also contribute to fly health during adulthood. Taken together, all results from this study should be interpreted with consideration of the potential effects resulting from the omission of nipagin and propionic acid in fly media.

6.7. Limitations and future directions

There were several limitations found in this study. Firstly, all flies used in this project were not axenic. As such, all experimental flies may have developed varying background microbiotas that could have influenced interactions with tested bacteria and affect project reproducibility. Therefore, it would be ideal to implement a wild-type control group in future experiments to determine if the observed effects were specific to experimental bacteria. While axenic models may be considered in future experiments, they are more susceptible to diseases and have altered gut physiologies that makes it more difficult to discern bacteria-induced interactions.

Secondly, the protocols used in this experiment may be difficult to replicate. While all experiments were completed using fly media that omits nipagin and propionic acid, the current protocols used in media preparation are laborious, time sensitive, and prone to error. This is in part due to the need to prepare, cool, inoculate, and utilise fly media within a timeframe shorter that the time it takes for mould overgrowth. From the study, the protocol was repeated every 3 days where media were prepared and cooled on day 1, inoculated with bacteria and dried on day 2, and administered to flies on day 3. As the protocol repeats, mould could grow on fly media during any of the 5 days before being replaced. Comparatively, fly media supplemented with nipagin and propionic acid may be prepared a week in advance and utilised whenever needed. While it is possible to shorten the time between certain steps in preparing non-additive fly media, logistical challenges may arise as media preparations requires the use of an autoclave and fumehood which may not be

available at specific times of day. Ultimately, it becomes challenging to efficiently carry out media preparation, fly maintenances and experiments concurrently. Considering these limitations alongside its effects on fly health, it is worth further investigating how nipagin and propionic acid affect bacterial growth and if lowering the concentrations of these additives in media supports bacteria viability while preventing mould growth.

Thirdly, the egg quantification assay used in this study may be further optimised. While initially planned, Result 5.6.4 failed to carry out an egg quantification assay. Although each vial only contained 10 flies, the number of eggs laid by bacteria-treated flies was far too great to be counted by eye. Hence, the sample size was decreased to 5 flies per vial in Result 5.7.2. Despite so, egg numbers remained high and challenging to quantify. Furthermore, Result 5.7.2 intended to report the percentage of viable offspring in relation to the number of eggs laid. However, results reported that the mean offspring generated was greater than the mean eggs laid, implying that the number of eggs laid may be underestimated. To improve the accuracy of future egg quantification assays, automated *Drosophila* egg counting tools such as QuantiFly may be considered (187).

Lastly, the lifespan assay from Results 5.7.1 was not completed due to time constraints, making it uncertain whether IIS reduction was present among non-bacteria treated flies. As lifespan data beyond age 70 days were not available, any lifespan modulatory effects of IIS reduction could only be inferred from the bacteria-treated fly group which lifespans ended before age 70 days. Regardless, these results were still able to demonstrate that bacteria, albeit in high concentrations, negatively impact fly median lifespan regardless of IIS expression.

7. Conclusion

This study demonstrated that bacteria, when fluorescently transformed, provide a useful tool for studying host-microbiome interactions. In addition to generating 6 new fluorescent bacteria, MYb71-sfGFP, MYb174-dTomato, and CEent1-mPlum were found to successfully colonised the fly gut in tandem under fluorescent microscopy. Interested in the effects of these bacteria against fly health, the bacteria were administered to flies with or without IIS reduction and studied for changes across life. While systemic IIS reduction extended fly median lifespan and exploratory walking behaviour, bacteria attenuated these changes. Furthermore, bacteria also attenuated changes to sleep performance mediated by systemic IIS reduction. Notably, changes to exploratory walking and sleep behaviour were only observed among female flies. Surprisingly, bacteria treatment also increased fly egg laying of all flies regardless of genotype during early age with the possibility of increasing offspring generation. Therefore, increased egg laying may have accelerated age-associated decline in female flies resulting in shorter median lifespans than males. Alternatively, the omission of nipagin and propionic acid in fly media may have contributed to earlier female decline as well. Collectively, these results demonstrated that the study of natural gut commensal in *C. elegans* can be translated to *D. melanogaster* to further probe bacteria-host interactions involving IIS and complex behaviours such as exploratory walking and sleep patterns. Ultimately, these experiments allow the comparison of ecologically relevant bacteria-host interactions across different species where findings pertaining to evolutionarily conserved biological pathways may be extrapolated and applied towards human health interventions.

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Completing this Master's degree has been a transformative journey filled with challenges, growth, and self-discovery. Without many of you, this wonderous pursuit would not have been possible. Foremost, I extend my deepest gratitude to **God** for allowing me to pursue my master's degree and granting me opportunities I never thought possible.

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10. Appendices

10.1. Bacteria incubation conditions

No	Bacteria	Media type	Temperature (°C)
1	All *Escherichia coli* SM10/pTn7xKS strains	Gentamicin-treated LB Agar	37
2	Escherichia coli SM10/pTNS2	Ampicillin-treated LB Agar	37
3	All stock bacteria	LB Agar	25
4	All fluorescent bacteria	Gentamicin-treated LB Agar	25

10.2. Bacteria profiles

No	Identifier	Bacteria	Function/Context	Genome Sequence
1	SM10/pTn7x KS-dTomato	*Escherichia coli* SM10/pTn7xKS-dTomato	Donor Bacteria (Transformation)	Not Available
2	SM10/pTn7x KS-mPlum	*Escherichia coli* SM10/pTn7xKS-mPlum	Donor Bacteria (Transformation)	Not Available
3	SM10/pTn7x KS-sfGFP	*Escherichia coli* SM10/pTn7xKS-sfGFP	Donor Bacteria (Transformation)	Not Available
4	SM10/pTNS2	*Escherichia coli* SM10/pTNS2	Helper Bacteria (Transformation)	Not Available
5	BIGb0170	*Sphingobacterium multivorum*	Recipient Bacteria (CeMbio)	GATCCTGGCTCAGGATGAACGCTAGCGGCAGGCCTAATACATGCAAGTCGGACGGGATCCGTCGGAGAGCTTGCTCGAAGACGGTGAGAGTGGCGCACGGGTGCGTAACGCGT GAGCAACCTACCTCTATCAGGGGGATAGCCTCTCGAAAGAGAGATTAACACCGCATAACATATCTGACCGGCATCGGTTRGCTATTAAATATTTATAGGATAGAGATGGGCTCGC GTGACATTAGCTAGTTGGTAGGGTAACGGCTTACCAAGGCGACGATGTCTAGGGGCTCTGAGAGGAGAATCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGG CAGCAGTAAGGAATATTGGTCAATGGGCGGAAGCCTGAACCAGCCATGCCGCGTGCAGGATGACTGCCCTATGGGTTGTAAACTGCTTTTGTCCAGGAATAAACCTTTCTACGTG TAGGAAGCTGAATGTACTGGAAGAATAAGGATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATCCGAGCGTTATCCGGATTTATTGGGTTTAAAGGGTGCGTAGG CGGCCTATTAAGTCAGGGGTGAAATACGGTGGCTCAACCATCGCAGTGCCTTTGATACTGATGGGCTTGAATCCATTTGAAGTGGGCGGAATAAGACAAGTAGCGGTGAAATGC ATAGATATGTCTTAGAACTCCGATTGCGAAGGCAGCTCACTAAGCTGGTATTGACGCTGATGCACGAAAGCGTGGGGATCGAACAGGATTAGATACCCTGGTAGTCCACGCCCTA AACGATGATAACTCGATGTTGGCGATAGACAGCCAGCGTCCAAGCGAAAGCGTTAAGTTATCCACCTGGGGAGTACGCCCGCAAGGGTGAAACTCAAAGGAATTGACGGGGGC CCGCACAAGCGGAGGAGCATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGGCTTGAAAGTTAGTGAAGAATGCAGAGACGCATTCGTCCTTCGGGACACGAAACTA GGTGCTGCATGGCTGTCGTCAGCTCGTGCCGTGAGGTGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTATGTTTAGTTGCCAGCATGTAATGGTGGGGACTCTAAACAGACTG CCTGTGCAAACAGTGAGGAAGGTGGGGACGACGTCAAGTCATCATGGCCCTTACGTCCGGGGCTACACACGTGCTACAATGGATGGTACAGCGGGCAGCTACATAGCAATATGA TGCTAATCTCTAAAAGCCATTCACAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTTGGATTCGCTAGTAATCGCGTATCAGCAATGACGCGGTGAATACGTTCCCGGGCC TTGTACACACCGCCCGTCAAGCCATGAAAGTTGGGGGTACCTAAAGCATGTTACCGCGAGGAGCG
6	BIGb0172	*Comamonas piscis*	Recipient Bacteria (CeMbio)	GCGCCCTCCTTGCGGTTAGGCTACCTACTTCTGGCGAGACCCGCTCCCATGGTGTGACGGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGTGACATTCTGATCCACGATTAC TAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGGACTACGACTGGCTTTATGGGATTAGCTCCCCCTCGCGGGTTGGCAACCCTTTGTACCAGCCATTGTATGAC GTGTGTAGCCCCACCTATAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCCCATTAGAGTGCCCAACTAAATGTAGCAACTAATGGCAAGG GTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTTACGGTTCTCTTTCGAGCACATGTCCATCTCTGGTCACTTCCGTAC ATGTCAAAGGTGGGTAAGGTTTTTCGCGTTGCATCGAATTAAACCACATCATCCACCGCTTGTGCGGGTCCCCGTCAATTCCTTTGAGTTTCAACCTTGCGGCCGTACTCCCCAGGC GGTCAACTTCACGCGTTAGCTTCGTTACTGAGAAAGTTAATTCCCAACAACCAGTTGACATCGTTTAGGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGT GCATGAGCGTCAGTACAGGTCCAGGGGATTGCCTTCGCCATCGGTGTTCCTCCGCATATCTACGCATTTCACTGCTACACGCGGAATTCCATCCCCCTCTACCGTACTCTAGCTATG CAGTCACAAAGGCAGTTCCCAGGTTGAGCCCGGGGATTTCACCTCTGTCTTACATAACCGCCTGCGCACGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTACGTATTACC GCGGCTGCTGGCACGTAGTTAGCCGGTGCTTATTCTTACGGTACCGTCATGGACCCTCTTTATTAGAAAGAGTCTTTTCGTTCCGTACAAAAGTAGTTTACAACCCGAGGGCCTTCA TCCTACACGCGGCATTGCTGGATCAGGCTTTCGCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCGTCCTCTCAG ACCAGCTACAGATCGTCGGCTTGGTAAGCTTTTATCCCACCAACTACCTAATCTGCCATCAGCCGCTCTAGTAGCACAAGGTCTTGCGATCCCCTGCTTTCATCCTTAGATCTCATGC GGTATTAGCTACTCTTTCGAGTAGTTATCCCCCACTACTAGGCACGTTCCGATGTATTACTCACCCGTTCGCCACTCGTCAGCATCCGAAGACCTGTTACCGTTCGACTTGCATGTGT AAAGCATGCCGCCAGCGTTCAATCTGAGC
7	BIGb0393	*Pantoea nemavictus*	Recipient Bacteria (CeMbio)	GCGCCCTCCCGAAGGTTAAGCTACCTACTTCTTTTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTAGCATTCTGATCTACGATTACT AGCGATTCCGACTTCACGGAGTCGAGTTGCAGACTCCGATCCGGACTACGACGCACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGCCATTGTAGCACGT GTGTAGCCCTACTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCAGTTTATCACTGGCAGTCTCCTTTGAGTTCCCGGCCGGACCGCTGGCAACAAAGGATAAGG GTTGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCAGCGTTCCCGAAGGCACCAAAGCATCTCTGCTAAGTTCCCTGGA TGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCG GTCGACTTAACGCGTTAGCTCCGGAAGCCACTCCTCAAGGGAACAACCTCCAAGTCGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGC ACCTGAGCGTCAGTCTTCGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCCCCCTCTACGAGACTCTAGCCTGCC AGTTTCGAATGCAGTTCCCAGGTTAAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCG CGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATCGGTGAGGTTATTA
8	CEent1	*Enterobacter cloacae*	Recipient Bacteria (CeMbio)	CCTAACACATGCAAGTCGAACGGTAACAGGAAGCAGCTTGCTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGG AAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCT

				AGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCC TGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGATAAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAG CACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCC GGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCG AAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCC TTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGG TTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCT CGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTG GGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGT AGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCAT GGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACC
9	JUb19	*Stenotrophomonas indicatrix*	Recipient Bacteria (CeMbio)	CCTAACACATGCAAGTCGAACGGCAGCACAGAGGAGCTTGCTCCTTGGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGAATCTACTTTTTCGTGGGGGATAACGTAGGG AAACTTACGCTAATACCGCATACGACCTACGGGTGAAAGCAGGGGACCTTCGGGCCTTGCGCGATTGAATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCCACCA AGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCC TGATCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCAGCCGGCTAATACCTGGTTGGGATGACGGTACCCAAAGAATAAG CACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTTGTTTAAGTCTGTTGTGAAAGCCCTG GGCTCAACCTGGGAACTGCAGTGGAAACTGGACAACTAGAGTGTGGTAGAGGGTAGCGGAATTCCCGGTGTAGCAGTGAAATGCGTAGAGATCGGGAGGAACATCCATGGCGA AGGCAGCTACCTGGACCAACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAA TTTGGCACGCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGT TTAATTCGATGCAACGCGAAGAACCTTACCTGGCCTTGACATGTCGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTC GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTG GGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTACTACAATGGTAGGGACAGAGGGCTGCAAGCCGGCGACGGTAAGCCAATCCCAGAAACCCTATCTC AGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCA TGGGAGTTTGTTGCACCAGAAGCAGGTAGCTTAACCTTCGGGAGGGCGC
10	JUb44	*Chryseobacterium scophthalmum*	Recipient Bacteria (CeMbio)	CATGCAAGCCGAGCGGTAGAGATCTTTCGGGATCTTGAGAGCGGCGTACGGGTGCGGAACACGTGTGCAACCTGCCTTTATCAGGGGGATAGCCTTTCGAAAGGAAGATTAATA CCCCATAATATATTGAATGGCATCATTTGATATTGAAAACTCCGGTGGATAGAGATGGGCACGCGCAAGATTAGATAGTTGGTAGGGTAACGGCCTACCAAGTCAGTGATCTTTA GGGGGCCTGAGAGGGTGATCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATTGGACAATGGGTGAGAGCCTGATCCAGCCATCCC GCGTGAAGGACGACGGCCCTATGGGTTGTAAACTTCTTTTGTATAGGGATAAACCTTTCCACGTGTGGAAAGCTGAAGGTACTATACGAATAAGCACCGGCTAACTCCGTGCCAG CAGCCGCGGTAATACGGAGGGTGCAAGCGTTATCCGGATTTATTGGGTTTAAAGGGTCCGTAGGCGGATCTGTAAGTCAGTGGTGAAATCTCATAGCTTAACTATGAAACTGCCA TTGATACTGCAGGTCTTGAGTAAAGTAGAAGTGGCTGGAATAAGTAGTGTAGCGGTGAAATGCATAGATATTACTTAGAACACCAATTGCGAAGGCAGGTCACTATGTTTTAACT GACGCTGATGGACGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGCTAACTCGTTTTTGGGTCTTCGGATTCAGAGACTAAGCGAAAGT GATAAGTTAGCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGATTATGTGGTTTAATTCGATGATACGCGAGGAACCTT ACCAAGGCTTAAATGGGAATTGACAGGTTTAGAAATAGACTTTTCTTCGGACAATTTTCAAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTTAGGTTAAGTCCTGCA ACGAGCGCAACCCCTGTCACTAGTTGCCATCATTCAGTTGGGGACTCTAGTGAGACTGCCTACGCAAGTAGAGAGGAAGGTGGGGATGACGTCAAATCATCACGGCCCTTACGCC TTGGGCCACACACGTAATACAATGGCCGGTACAGAGGGCAGCTACCTAGCGATAGGATGCGAATCTCGAAAGCCGGTCTCAGTTCGGATTGGAGTCTGCAACTCGACTCTATGAA GCTGGAATCGCTAGTAATCGCATATCAGCCATGATGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGTTTGGGGTACCTGAAGTCGGTGACCGTA ACAGGAGCTGC
11	MYb10	*Acinetobacter guillouiae*	Recipient Bacteria (CeMbio)	CACATGCAAGTCGAGCGGGGGAGATTGCTTCGGTAATTGACCTAGCGGCGGACGGGTGAGTAATACTTAGGAATCTGCCTATTAATGGGGGACAACATCTCGAAAGGGATGCTA ATACCGCATACGCCCTACGGGGGAAAGCAGGGGATCACTTGTGACCTTGCGTTAATAGATGAGCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGAT CTGTAGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGGAACCCTGATCCAGCC ATGCCGCGTGTGTGAAGAAGGCCTTATGGTTGTAAAGCACTTTAAGCGAGGAGGAGGCTCTCTTGGTTAATACCCAAGATGAGTGGACGTTACTCGCAGAATAAGCACCGGCTAA CTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCGAGCGTTAATCGGATTTACTGGGCGTAAAGCGTGCGTAGGCGGCTTTTTAAGTCGGATGTGAAATCCCCGAGCTTAACTT GGGAATTGCATTCGATACTGGGAAGCTAGAGTATGGGAGAGGATGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGATGGCGAAGGCAGCCAT CTGGCCTAATACTGACGCTGAGGTACGAAAGCATGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGTCTACTAGCCGTTGGGGCCTTTGAGGCTTTAG TGGCGCAGCTAACGCGATAAGTAGACCGCCTGGGGAGTACGGTCGCAAGACTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGC AACGCGAAGAACCTTACCTGGTCTTGACATAGTAAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTTACATACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGAT GTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTTTCCTTATTTGCCAGCACTTCGGGTGGGAACTTTAAGGATACTGCCAGTGACAAACTGGAGGAAGGCGGGGACGACGTCAAG TCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCTACCTAGCGATAGGATGCTAATCTCAAAAAGCCGATCGTAGTCCGGATTGGAGT CTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCGGATCAGAATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTTGTTGCAC CAGAAGTAGGTAGTCTAACCGTAAGGAGGACGCTTACCAC
12	MYb11	*Pseudomonas lurida*	Recipient Bacteria (CeMbio)	CTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAGAGAGAAGCTTGCTTCTCTTGAGAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAG TGGGGGATAACGTTCGGAAACGGACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGT

				GGGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG GACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTTGCCTAATACGTAACTGTTTTGAC GTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAG TTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGACTGACTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGA AGGAACACCAGTGGSGAAGGCGCCCACCTGTRCTAATATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTC AACTAGCCGTTGGAAGCCTTGAGCTTTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACA AGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAACTTTCTAGAGATAGATTGGTGCCTTCGGGAACATTGAGACAGGTGCT GCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCGGTG ACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTACAATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTA ATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTTGTA CACACCGCCCGTCACACCATGGGAGTGGGTTGCACCAGAAGTAGCTAGTCTAACCTTCGGGAGGACGGTTACCACGGTGT
13	MYb16	*Pseudomonas sp.*	Recipient Bacteria (CeMbio)	Not Available
14	MYb21	*Comamonas sp. B-9*	Recipient Bacteria (CeMbio)	GGCTGGCGGCATGCTTTTACACATGCAAGTCGAACGGTAACAGGTCTTCGGATGCTGACGAGTGGCGAACGGGTGAGTAACACATCGGAACGTGCCTAGTAGTGGGGGATAACT ACTCGAAAGAGTAGCTAATACCGCATGAGATCTAAGGATGAAAGCAGGGGACCTTCGGGCCTTGCGCTACTAGAGCGGCTGATGGCAGATTAGGTAGTTGGTGGGATAAAAGCT TACCAAGCCGACGATCTGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGA AAGCCTGATCCAGCAATGCCGCGTGTAGGATGAAGGCCCTCGGGTTGTAAACTACTTTTGTACGGAACGAAAAGACTCTTTCTAATAAAGAGGGTCCATGACGGTACCGTAAGAA TAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTATGTAAGACAGAGGTGAAAT CCCCGGGCTCAACCTGGGAACGGCCTTTGTGACTGCATAGCTAGAGTACGGTAGAGGGGGATGGAATTCCGCGTGTAGCAGTGAAATGCGTAGATATGCGGAGGAACACCGAT GGCGAAGGCAATCCCCTGGACCTGTACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGG AATTAACTTTCTCAGTAACGAAGCTAACGCGTGAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGT GGTTTAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGTACGGAAGTGACCAGAGATGGACATGTGCTCGAAAGAGAACCGTAACACAGGTGCTGCATGGCTGTCGT CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGCCATTAGTTGCTACGAAAGGGCACTCTAATGGGACTGCCGGTGACAAACCGGAGGAAGGTGG GGATGACGTCAAGTCCTCATGGCCCTTATAGGTGGGGCTACACACGTCATACAATGGCTGGTACAAAGGGTTGCCAACCCGCGAGGGGGAGCTAATCCCATAAAGCCAGTCGTA GTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGTCACGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCAT GGGAGCGGGTCTCGCCAGAAGTAGGTAGCCTAACCGTAAGGAGGGCGCTTACCACGGCGG
15	MYb49	*Ochrobactrum anthropi*	Recipient Bacteria (CeMbio)	CACATGCAAGTCGAGCGCCCCGCAAGGGGAGCGGCAGACGGGTGAGTAACGCGTGGGAACGTACCTTTTGCTACGGAATAACTCAGGGAAACTTGTGCTAATACCGTATGTGCC CTTCGGGGGAAAGATTTATCGGCAAAGGATCGGCCCGCGTTGGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCCATAGCTGGTCTGAGAGGATGATCAGCCA CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGT TGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACT GGGCGTAAAGCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCCTTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTC CGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGAA
16	MYb57	*Stenotrophomonas sp.*	Recipient Bacteria (CeMbio)	Not Available
17	MYb69	*Comamonas sp. TK41*	Recipient Bacteria (CeMbio)	GCATGCTTTACACATGCAAGTCGAACGGTAACAGGTCTTCGGATGCTGACGAGTGGCGAACGGGTGAGTAACACATCGGAACGTGCCTAGTAGTGGGGGATAACTACTCGAAAG AGTAGCTAATACCGCATGAGATCTAAGGATGAAAGCAGGGGACCTTCGGGCCTTGCGCTACTAGAGCGGCTGATGGCAGATTAGGTAGTTGGTGGGATAAAAGCTTACCAAGCC GACGATCTGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGAT CCAGCAATGCCGCGTGTAGGATGAAGGCCCTCGGGTTGTAAACTACTTTTGTACGGAACGAAAAGACTCTTTCTAATAAAGAGGGTCCATGACGGTACCGTAAGAATAAGCACCG GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTATGTAAGACAGAGGTGAAATCCCCGGGCT CAACCTGGGAACGGCCTTTGTGACTGCATAGCTAGAGTACGGTAGAGGGGGATGGAATTCCGCGTGTAGCAGTGAAATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGC AATCCCCTGGACCTGTACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGAATTAACTTTC TCAGTAACGAAGCTAACGCGTGAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGTTTAATTC GATGCAACGCGAAAAACCTTACCCACCTTTGACATGTACGGAAGTGACCAGAGATGGACATGTGCTCGAAAGAGAACCGTAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT CGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGCCATTAGTTGCTACGAAAGGGCACTCTAATGGGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTC AAGTCCTCATGGCCCTTATAGGTGGGGCTACACACGTCATACAATGGCTGGTACAAAGGGTTGCCAACCCGCGAGGGGGAGCTAATCCCATAAAGCCAGTCGTAGTCCGGATCGC AGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGTCACGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGCGGGTC TCGCCAGAAGTAGGTAGCCTAACCGCAAGGAGGGCGCTTACCACGGCG
18	MYb71	*Ochrobactrum vermis*	Recipient Bacteria (CeMbio)	TACCGTGGTCCCTGCCTCCTTGCGGTTAGCACAGTGCCTTCGGGTAAAACCAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGA TCCGCGATTACTAGCGATTCCAACTTCATGCACTCGAGTTGCAGAGTGCAATCCGAACTGAGATGGCTTTTGGAGATTAGCTTGCGCTCGCACGCTCGCTGCCCACTGTCACCACCA TTGTAGCACGTGTGTAGCCCAGCCCGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCCAGCTTATCACTGGCAGTCCCCTTAGAGTGCCCAACTAAATGCTGGCAACT AAGGGCGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTATCCGGTCCAGCCGAACTGAAAGACACATCTCTGTG

				TCCGCGACCGGTATGTCAAGGGCTGGTAAGGTTCTGCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACCG TACTCCCCAGGCGGAATGTTTAATGCGTTAGCTGCGCCACCGAAGAGTAAACTCCCCAACGGCTAACATTCATCGTTTACGCGTGGACTACCA
19	MYb115	*Pseudomonas fluorescence*	Recipient Bacteria (CeMbio)	GTGGTAACCGTCCTCCCGAAGGTTAGACTAGCTACTTCTGGTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGACATTCTGATTC GCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGGACTACGATCGGTTTTATGGGATTAGCTCCACCTCGCGGCTTGGCAACCCTTTGTACCGACCATT GTAGCACGTGTGTAGCCCAGGCCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCCTTAGAGTGCCCACCATAACGTGCTGGTAACT AAGGACAAGGGTTGCGCTCGTTACGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTCTCAATGTTCCCGAAGGCACCAATCCATCTCTGGAA AGTTCATTGGATGTCAAGGCCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTA CTCCCCAGGCGGTCAACTTAATGCGTTAGCTGCGCCACTAAAATCTCAAGGATTCCAACGGCTAGTTGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCC CACGCTTTCGCACCTCAGTGTCAGTATCAGTCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCCTATATCTACGCATTTCACCGCTACACAGGAAATTCCACCACCCTCTACCATAC TCTAGCTCGTCAGTTTTGAATGCAGTTCCCAGGTTGAGCCCGGGGCTTTCACATCCAACTTAACGAACCACCTACGCGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCT CTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGGTGCTTATTCTGTCGGTAACGTCAAAATTGCAGAGTATTAATCTACAACCCTTCCTCCCAACTTAAAGTGCTTTACAATCCG AAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATC ATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCTTTCTCCCGT AGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTATCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTCTCAAGAGAAGCAAGCTTCTCTCTACCGCT CGACTTGCATGTG
20	MYb174	*Enterobacter ludwigii*	Recipient Bacteria (CeMbio)	GCCTAACACATGCAAGTCGAGCGGTAACACAGGAGAGCTTGCTCTCTGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCCGATGGAGGGGGATAACTACTG GAAACGGTAGCTAATACCGCATAACGTCTTCGGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAATGGCTCACC TAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGC CTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGCGTTGTGGTTAATAACCGCAGCGATTGACGTTACTCGCAGAAGAA GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCC CGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGC GAAGGCGGCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCC CTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG GTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGC TCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTG GGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGT AGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCAT GGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAAC
21	MYb186	*Enterobacter sp. 638*	Recipient Bacteria (CeMbio)	AGCTGGCGCAGGCCTAAACATGCAAGTCGAGCGGTAGCACGGGGGACCTTGCTCCGTGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGG GATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGT AACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAAT GGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGCATTGTGGTTAATAACCACAGTGATTGACGTTACT CGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGA TGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGA ATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTT GGAGGTTGTTCCCTTGAGGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCG GTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCAT GGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAA CTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTC ATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACC GCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCA
22	MYb330	*Pseudomonas sp.*	Recipient Bacteria (CeMbio)	TGCAAGTCGAGCGGATGAAGAGAGCTTGCTCTCTGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATAC CGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAA CTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCG CGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAATTAATACTTTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTG CCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAAC TGCATTCAAAACTGACGAGCTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGAC TGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCG CAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGC GAAGAACCTTACCAGGCCTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTG GGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTC ATCATGGCCCTTACGGCCTGGGCTACACACGTGCTACAATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCT

				GCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCACCAGAAGTAGCTAGTCTAACCTTCGGGAGGACGGTTACCAC
23	MYb396	*Comamonas sp.*	Recipient Bacteria (CeMbio)	TGCTTTACACATGCAAGTCGAACGGTAACAGGTCTTCGGATGCTGACGAGTGGCGAACGGGTGAGTAACACATCGGAACGTGCCTAGTAGTGGGGGATAACTACTCGAAAGAGTAGCTAATACCGCATGAGATCTAAGGATGAAAGCAGGGGACCTTCGGGCCTTGCGCTACTAGAGCGGCTGATGGCAGATTAGGTAGTTGGTGGGATAAAAGCTTACCAAGCCGACGATCTGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGTAGGATGAAGGCCCTCGGGTTGTAAACTACTTTTGTACGGAACGAAAAGACTCTTTCTAATAAAGAGGGTCCATGACGGTACCGTAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTATGTAAGACAGAGGTGAAATCCCCGGGCTCAACCTGGGAACGGCCTTTGTGACTGCATAGCTAGAGTACGGTAGAGGGGGATGGAATTCCGCGTGTAGCAGTGAAATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGACCTGTACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGAATTAACTTTCTCAGTAACGAAGCTAACGCGTGAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGTTTAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGTACGGAAGTGACCAGAGATGGACATGTGCTCGAAAGAGAACCGTAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGCCATTAGTTGCTACGAAAGGGCACTCTAATGGGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATAGGTGGGGCTACACACGTCATACAATGGCTGGTACAAAGGGTTGCCAACCCGCGAGGGGGAGCTAATCCCATAAAGCCAGTCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGTCACGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGCGGGTCTCGCCAGAAGTAGGTAGCCTAAC

10.3. PCR primer profile

No.	Primer	Primer Code (5'-3')	Gene Target
1	16S-1495r	CTACGGCTACCTTGTTACGA	16s rRNA
2	16S-27f	GAGAGTTTGATCCTGGCTCAG	16s rRNA

10.4. Thermal cycler protocol

No	Stage	Temperature (°C)	Time (s)	Cycles
1	Preincubation	95	180	1
2	3-step Amplification	95	30	35
		54	45	
		72	90	
3	Cooling	72	300	1
4	Final Hold	10	Infinite	Infinite

10.5. Antibiotic ring profile

No	Product Code	Antibiotic	Volume
1	M13/NCE	Chloramphenicol	25 ug
2	M13/NCE	Erythromycin	5 ug
3	M13/NCE	Fusidic acid	10 ug
4	M13/NCE	Oxacillin	5 ug
5	M13/NCE	Novobiocin	5 ug
6	M13/NCE	Penicillin	1 unit
7	M13/NCE	Streptomycin	10 ug
8	M13/NCE	Tetracycline	25 ug
9	M14/NCE	Ampicillin	10 ug
10	M14/NCE	Cephalothin	5 ug
11	M14/NCE	Colistin Sulphate	25 ug
12	M14/NCE	Gentamicin	10 ug
13	M14/NCE	Streptomycin	10 ug
14	M14/NCE	Sulphatriad	200 ug
15	M14/NCE	Tetracycline	25 ug
16	M14/NCE	Cotrimoxazole	25 ug
17	M43/NCE	Penicillin G	1 unit
18	M43/NCE	Clindamycin	2 ug
19	M43/NCE	Gentamicin	10 ug
20	M43/NCE	Fusidic acid	10 ug
21	M43/NCE	Erythromycin	5 ug
22	M43/NCE	Trimethoprim	1.25 ug

23	M43/NCE	Sulphamethoxazole	25 ug
24	M43/NCE	Tetracycline	10 ug

10.6. Fly genotype profile

No	Fly Genotype	Chromosome Location	Purpose
1	d2-3GAL4	3	A transcriptional activator gene that produces GAL4 in DILP2 or DILP 3 expressed cells
2	UAS-*rpr*	X	An enhancer gene whereby GAL4 binds to induce cellular apoptosis
3	TrhGAL4	2	A transcriptional activator gene that produces GAL4 in tryptophan hydroxylase producing cells which mostly includes serotonergic neurons
4	UAS-InR^{DN}	2	An enhancer gene whereby GAL4 binds to induce dominant negative activity of insulin receptor
5	W^{Dahomey}	-	The background used in fly backcrossing to maintain genetic variability

10.7. Fly genotype crosses

<table>
<tr><th rowspan="3">No</th><th colspan="4">Parent</th><th colspan="2">Offspring Genotype</th><th rowspan="3">Purpose</th></tr>
<tr><th colspan="2">Male</th><th colspan="2">Female</th><th rowspan="2">Type</th><th rowspan="2">Collected n (per condition/sex)</th></tr>
<tr><th>Type</th><th>n</th><th>Type</th><th>n</th></tr>
<tr><td rowspan="3">1</td><td>d2-3GAL4</td><td rowspan="6">50</td><td>UAS-rpr</td><td rowspan="6">100</td><td>d2-3GAL4/UAS-rpr</td><td rowspan="6">>150</td><td>Insulin-like peptide producing cells ablation</td></tr>
<tr><td>d2-3GAL4</td><td>W^{Dah}</td><td>d2-3GAL4/W^{Dah}</td><td rowspan="2">No effect (Control group)</td></tr>
<tr><td>W^{Dah}</td><td>UAS-<i>rpr</i></td><td>UAS-<i>rpr</i>/ W^{Dah}</td></tr>
<tr><td rowspan="3">2</td><td>TrhGAL4</td><td>UAS-InR^{DN}</td><td>TrhGAL4/UAS-InR^{DN}</td><td>Serotonergic neurons Insulin-like receptor knockdown</td></tr>
<tr><td>TrhGAL4</td><td>W^{Dah}</td><td>TrhGAL4/W^{Dah}</td><td rowspan="2">No effect (Control group)</td></tr>
<tr><td>UAS-InR^{DN}</td><td>W^{Dah}</td><td>UAS-InR^{DN}/ W^{Dah}</td></tr>
</table>

10.8. Fluorophore profile

No	Fluorophore	FPbase ID	Excitation Maximum (nm)	Emission Maximum (nm)
1	Superfolded (sf) GFP	B4SOW	485	510
2	dTomato	G1DQY	554	581
3	mPlum	XU2WO	590	649

10.9. Transformed bacteria sequence

Successful Transformation

1) MYb71-sfGFP

Forward Sequence:

NNNNNNNNNNNANNCATGCANTCGNCGGTCTCTTCGGAGGNNGTGGCAGACGGGTGAGTAACGCGTGGGAATCTACCTTTTGCTACGGAACAACAGTTGGAAACGACT
GCTAATACCGTATGTGCCCTTCGGGGGAAAGATTTATCGGCAAAGGATGAGCCCGCGTTGGATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCGACGATCCATAGCTG
GTCTGAGAGGATGATCAGCCACACTGGGACTGAGCCACGACCCAANCTCNAACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCC
GCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATAC
GAAGGGGGCTAGCGTTGTTCNNNTTTACTGGGCATAAAGCGCACGTAGGCGGACTTTTAAGTNNGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCCTTTGATACTGG
AAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGC
TGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTAGCCGNNGGGGAGTTTACTCTTCGGTGGCGCAGCTAACG
CATTAAACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGNNTTGACGGNNGCCCGCACAAGCGGTGGAGCATGTGTTTAATTCGAAGCAACGCGCAGA
ACCTTACCAGCCCTTGACATACCGGTCGCGAACACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACAGGTGCTGCATGGCTGTCNNCAGCTCGTGTCGTGAGATGTG
GGTTAAGTCCCGCAACGAGCNNNACCCTCNNCNTTAGTTGCCAGCATTTAGTTNGNCNNTCTAAGGGGACTGCCAGTGATAAGCTGGNGGAAGGNNGGGATGACNNNCA
GTCCTCATNGCCCTTANNGGGTGGNACTACACNNNTGCTACNNTGTTGTGANGNNGGGCANGGAGGGNNNAACCCAAGNNATCTCAAAAGCATCTTAGTCGGATGCCCN
NNNCACNNNGGNNAAGAAGGTGNA

Reverse Sequence:

NNNNNNNNNNNNCCGNNNNCACCTGCCTCCTTGCGGTTAGCACAGTGCCTTCGGGTAAAACCAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT
TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAACTTCATGCACTCGAGTTGCAGAGTGCAATCCGAACTGAGATGGCTTTTGGAGATTAGCTTGCGCTCGCACGCT
CGCTGCCCACTGTCACCACCATTGTAGCACGTGTGTAGCCCAGCCCGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCCAGCTTATCACTGGCAGTCCCCTTAGA
GTGCCCAACTAAATGCTGGCAACTAAGGGCGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTATCCGGTC
CAGCCGAACTGAAAGACACATCTCTGTGTCCGCGACCGGTATGTCAAGGGCTGGTAAGGTTCTGCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCC
CGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGTTTAATGCGTTAGCTGCGCCACCGAAGAGTAAACTCCCCAACGGCTAACATTCATCGTTTACG
GCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTAATGGTCCAGTGAGCCGCCTTCGCCACTGGTGTTCCTCCGAATATCTACGA
ATTTCACCTCTACACTCGGAATTCCACTCACCTCTACCATACTCAAGACTTCCAGTATCAAAGGCAGTTCCGGGGTTGAGCCCCGGGATTTCACCCCTGACTTAAAAGTCCGCC
TACGTGCGCTTTACGCCCAGTAAATCCGAACAACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCCGGGGCTTCTTCTCCGGTTACCGTCATTATCTTCAC
CGGTGAAAGAGCTTTACAACCCTAGGGCCTTCATCACTCACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGC
CGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTATGGATCGTCGCCTTGGTAGGCCTTTACCCTACCAACTAGCTAATCCAACGCGGGCTCATCCTTTGCCG
NTAAAATCTTTCCCCCGAAGGGNNCATTACGGGTATTAGCNNNNCGTTTCCAACTGTTGTTCCGTAGCAAAAAGGTNAATTCCCNNGNNNTTACNNNNCCCGNTTGC

2) MYb186-sfGFP

Forward Sequence:

NNNNNNNNGCTANACATGCAGTCGAGCGGTAGCACNGGNNAGCTTGCTCCCTGGGNGGCGAGCGGCGGACGGGTGAGTAATGTCTGGGANACTGCCTGATGGAGGGG
GATAACTACTGGAAACGGTAGCTAATACCGCATAATGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGG
GGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCACACTCCTACGGGAGGCAGCAGTGGGAAATATT
GCACAATGGGCGCACGCCTGATGCAGCNNNGCCGCGTGTATAAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGTTTTGTGTTTAATAACCACAGTGAT
TGATGTTACTCGCAAAAAAAGCACCGTCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGNNTTAATCNNAATTACTGGGCGTAAAGCGCACGCAGGCGGT
CTGTCAAGTCGGATGTGAAATCCCCGGGCTCNCNNTGANAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGC
ATAGATATCTGGAGGAATACCGGTGGAGAAGGCNNCCCCCTGNACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGATACTCCACG
CCATANACGATGTCGACTTGGAGGTTGTTCCCTTGAGGAGTGTCTTCCNGAGCTAACGCGTTAAGTCGACCGCCTGGAGAGTACGCCCGCAANNNTAAACTCATATGAAT
TGACGGGNNCCCGCACAAGCGGTGGAGCATGTGTTTNNNTTCGATGCANCGNNAANANNCTTACCTACTCTTGACATCCAGAGNNNTTAGCAGAGATGCTTTGGTGCCTT
CGAAAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGAAAAATGTGGGNTTAANNCCCGCANCGAGCNCACNTTTANTTTTTGTGCCGGCGTNNGGCGGG
AACTCAAAGGANNCTNCCAGTAAAAACTGGAGNAGGGGGGGAAGACNNCANNTCATCGNGCCCTTANNAGNNGGGCTACNNNCGTGNNNNANTGGNNNAAAAAAAG
NAAACCACCTCCGGNNAACAAGCNGACCTNAAAAGNNNNGTCGTACTCGGANNNGGANCNNNCACTNNNCCCCCAAAAGCCGAATCCTTAAAA

Reverse Sequence:

NNNNNNNNNNNNGTGANNAGCGCCCTCCCGAAGGTTAAGCTACCTACTTCTTTTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCA
CCGTAGCATTCTGATCTACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCG
CTTCTCTTTGTATGCGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCAGTTTATCACTGGCAGTCTCCTTTGAGTTC
CCGGCCTAACCGCTGGCAACAAAGGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCAGAGTTCCC
GAAGGCACCAAAGCATCTCTGCTAAGTTCTCTGGATGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAAT
TCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGACTTAACGCGTTAGCTCCGGAAGCCACTCCTCAAGGGAACAACCTCCAAGTCGACATCGTTTACGGCGTG

GACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCA
CCGCTACACCTGGAATTCTACCCCCCTCTACAAGACTCTAGCCTGCCAGTTTCGAATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGT
GCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTTCTGCGAGTAACGTCAATCACTGTGGTTAT
TAACCACAATGCCTTCCTCCTCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTG
CCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCA
TCTGGGCACATCTGAATGGCAANAGGCCCGAAGGTCCCCCTCTTTGGNCTTGNNACGTTATGCGGNNTTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCC
C

3) MYb11-sfGFP

Forward Sequence:

NNNNNNNNGCNNNNACNNTGCAGTCGAGCGGTAGAGAGAAGCTTGCTTCTCTTGAGAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAA
CGTTCGGAAACGGACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGNGTCGGATTAGCTAGTTGGTGGGGT
AATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGGAATATTNG
GACAATGGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAAGTCTTCCGATTGTAGAAGCACTTTANNTTTGGGAGGAAAGGCAGTTGCCTAATACGTAACTGT
GTTTGACGTTACCGACAGAATAAAGCACCCNGCTAACTCTGTGCCCAGCAGCCCCCNGTAATACAGAGGGGTGCNAAGCGGTTAATCNNGAANTACTGTGGCGTATAAAC
GCGCCGTACNGTGGGTGTGTTTANGTTTGGATGTGGAGANNCCCCCGGGCTCAACCCTGGGGAACTGCATGTCAAAAACTGAACTGANCTNNCAGTATAGGTAGAANGGT
GGGNGGGANTTTTCCNNTGTTGGCNGTGGAAAATGCNNTANNATAATANGGAAANGAAACACCCNNCCGGCGGAAGGGCGACGCACCCTNNGACTCAATACNNGAACA
CCTGAANNGTGGCGAAAAANCNNGGGGGAGGCGAGACAAGGAATTAAGATAACCCCCGGCNNGTCCGCCGCCNNNAAAANNAATGTTCTAACTAAGCCCGCTTGGGAG
AGCCCTTGNAGCTTTTTTTGTGGNNGNNNGCCTAAACGCCATTAAAATTTGAACCNCCCCGGGGGAGGTACCGGGCCNCCNAGNGTGAAGNAATNCAAAATGNAAATTTN
NNNGGGGGGNCCCGCCNNCAAANCNNNNGGGAAGCAATGNNGGTTTTNNNTTTTTTANNTCCANCGCCNNAAGAAAACCNTTNAACCNNNGGCGCTTNGGACATTCCC
NCCTGGNAANTTTTNNNNNTTAAGAATAAAAAATTNGNGGGNCCCTTCNNNGGAANCCCTTNGAGAAACCAAGGNTGCNCNNGCAANNGGATTGNNNNNTTNNNCCNN
NCCTGGNCCCCNGGANAAANGGNTTGGGGGNTTNAAGGTNNNCGCTTNAACCNNNAAGAGCACAANNCCCCTGGGNNCCCNNTTANNTTTTNCCCNNNCCANACCCTN
NAANNGGGNNGGGGGCGNNNGNNNCNNNAACGGNAAAAAANNNNNCCNNNCGNNNGGCCAA

Reverse Sequence:

NNNNNNNNNNNNGNGGTANNNCTCCGAGGTTAGACTAGCTACTTCTGGTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGC
GACATTCTGATTCGCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGGACTACGATCGGNTTTATGGGANTAGCTCCACCTCGCGGCTTGGCAA
NCCTCTGTACCGACCANTGTAGCACGTGTGTAGCCCANGNCGTAANGGGNCATGANGACTTGACGTCATCCCCCNCCNTCCTCCNGNTTGTCACCNGNAGTCTCCTTANAG
TGNNCACCANTACNTGCTGGGNACTAAAGACAAAGGNTGCGCTCGTTACNGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCANCTGTCTNAAT
GTTNCCGAANGCACCAATCTATCTCTANAAAGNTCATTGGATGTCAANGCCTGGTAAGGTCTTCNCGTTGCTTCNAATTAAAACACATGCTCCACCGCTTGTGCGGGNNCCC
CGCCAATTCATTTGAGTTTTAACCTTGCGGNCGTACTCCCCACGCGGGCAACTTAATGCGTTAGCTGCGCCACTAAAANATCAANGCTTCCNACNGCTAGNTGACATCGTTTT
ACNGCGTGGGANTACCANGGNNNCTAATCCTGTTTNGCTNCCCCACGCCTNTTCNCACCTCACTGTCAGTATTAGTCCAANGTGGGTNGGCCNTTCNCCANCTGGNGNTN
CCTTCCTATATCTACGCNATTTCACCNGCTNNNCAGGAAAATNNNANCCACCCCTCTACCATACTCTANTCAGTCAGTTTTGANGGCANGTTCCCACGNTGANGCGCGGGG
NATTTTNNNTTNCAACTTAANAAAANNAANCTNACNCCNCCNCTNTTACNNCCCAGNAAATTNCNANTAANNNNTTTGNACCCCTCTGTNNTTACCNCCGCCTGCTGGCA
C

4) MYb11-dTomato

Forward Sequence:

NNNNNNNNNNNNNNCNTACNNTGCAGTCGAGCGGTAGAGAGAAGCTTGCTTCTCTTGAGAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGG
ATAACGTTCGGAAACGGACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGG
GGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATT
GGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTTGCCTAATACGTAACTGTTT
TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGT
TTGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGACTGACTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCG
TAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTAATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC
CGTAAACGATGTCAACTAGCCGTTGGAAGCCTTGAGCTTTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATT
GACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAACTTTCTAGAGATAGATTGGTGCCTTCG
GGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGNTTTAAGTCCCGTAACGAGCGCAACCCTTTNTCCTTNGTTACCAGCNCCGTNNGGG
TGGGNCCACT

Reverse Sequence:

NNNNNNNNNNNNNNNGNGGTACCGTCCTNNNAAGGTTAGACTAGCTACTTCTGGTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTC
ACCGCGACATTCTGATTCGCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGGACTACGATCGGTTTTATGGGATTAGCTCCACCTCGCGGCTTG
GCAACCCTCTGTACCGACCATTGTAGCACGTGTGTAGCCCAGGCCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCCTTAGAG
TGCCCACCATTACGTGCTGGTAACTAAGGACAAGGGTTGCGCTCGTTACGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTCTCAATGT
TCCCGAAGGCACCAATCTATCTCTAGAAAGTTCATTGGATGTCAAGGCCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGT

CAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCAACTTAATGCGTTAGCTGCGCCACTAAAAGCTCAAGGCTTCCAACGGCTAGTTGACATCGTTTACGG
CGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGTGTCAGTATTAGTCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCCTATATCTACGCATT
TCACCGCTACACAGGAAATTCCACCACCCTCTACCATACTCTAGTCAGTCAGTTTTGAATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCCAACTTAACAAACCACCTAC
GCGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGGTGCTTATTCTGTCGGTAACGTCAAAACAGTTACGT
ATTAGGCAACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACANACGCGGCATGGCTGGATCAGGCTTTCGCC

5) MYb11- mPlum

Forward Sequence:

NNNNNNNNNNNNTANNNNTGCNNNTCGAGCGGTAGAGAGNAGCTTGCTTCTCTTGAGAGCGGCGGANGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGAT
AACGTTCGGAAACGGACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGG
TAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAAACTCCAACGGGAGGCAGCAGTGGGGAATATTGG
ACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTTGCCTAATACGTAACTGTTTTG
ACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTT
GTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGACTGACTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTA
GATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTAATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCC
GTAAACGATGTCAACTAGCCGTTGGAAGCCTTGAGCTTTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTG
ACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAACTTTCTAGAGATAGATTGGTGCCTTCGG
GAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGG
CACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACCNCGTGCTACAATGGTCGGTANNNAGG
GTTGCCAAGCCGCNAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCNNANNCTG

Reverse Sequence:

NNNNNNNNNNNNNNNNANNGTCCTCCCGAAGNTAGACTAGCTACTTCTGGTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCG
CGACATTCTGATTCGCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGGACTACGATCGGTTTTATGGGATTAGCTCCACCTCGCGGCTTGGCAA
CCCTCTGTACCGACCATTGTAGCACGTGTGTAGCCCAGGCCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCCTTAGAGTGCC
CACCATTACGTGCTGGTAACTAAGGACAAGGGTTGCGCTCGTTACGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTCTCAATGTTCCC
GAAGGCACCAATCTATCTCTAGAAAGTTCATTGGATGTCAAGGCCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAAT
TCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCAACTTAATGCGTTAGCTGCGCCACTAAAAGCTCAAGGCTTCCAACGGCTAGTTGACATCGTTTACGGCGTG
GACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGTGTCAGTATTAGTCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCCTATATCTACGCATTTCAC
CGCTACACAGGAAATTCCACCACCCTCTACCATACTCTAGTCAGTCAGTTTTGAATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCCAACTTAACAAACCACCTACGCGC
GCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGGTGCTTATTCTGTCGGTAACGTCAAAACAGTTACGTATTA
GGCAACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCC
TCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATCCGACC
TAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCTTTTCCCGTAGGACGTANGCGGNNTTAGCGTCCGTTTCCGAACGTTATCCCNACTACCAGGCNNATTCCTAGG
CN

6) BIGb0393-mPlum

Forward Sequence:

NNNNNNNGNNNNCTANNNATGCAGTCGAACGGTAACAGGAAGCAGCTTGCTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGG
GGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGG
TGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT
ATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGATAAGGTTAATAACCTTGTC
GATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGC
GGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA
TGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTC
CACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCATAT
GAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGC
CTTCGCGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTNTGAAATGTTGGGTTAAGTCTCGCAANNGAGGNCACCCNTTATCCTTTGTTNCCANCGGTTAG
GCCGGNAACTCACAGGAGACNGCCCCTGATAAACTGGAGGAAAGGTGTGGATGANNNTCAGATGA

Reverse Sequence:

NNNNNNNCNNAGTGNNNNCGNCTCCCGAAGGTTAAGCTACCTACTTCTTTTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCG
TGGCATTCTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTT
CTCTTTGTATGCGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCAGTTTATCACTGGCAGTCTCCTTTGAGTTCCC
GGCCTAACCGCTGGCAACAAAGGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCAGAGTTCCCG
AAGGCACCAAAGCATCTCTGCTAAGTTCTCTGGATGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATT

CATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGACTTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTTACGGCGTG
GACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCA
CCGCTACACCTGGAATTCTACCCCCCTCTACAAGACTCTAGCCTGCCAGTTTCGAATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGT
GCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATCGACGAGGTTAT
TAACCTTATCGCCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGNCTGNCGCCCATTGTGCAATATTCCCCACTGCT
GCCTCCCGTAGGAANCTGGNACGGTGTCTCACTTCCCGTGTGGNCTGGGTCATCCCCNNNNNAACCAACCTAGGGATCGTCGCCTAAGGTGAGGNNNTTNNCCCCCACCT
ACAAGGCTAATCCCAANNTGGGNCACATCCNAATGGCAANNNGGCCCNNAAGGTCCCCCNTC

Unsuccessful Transformation

7) MYb21-dTomato

Forward Sequence:

NNNNNNNNNNNNNTNNNCATGCACTCGANGGTNCAGGAAGNNCTTGCTTCTTTGCTGACGAGTGGTGGAGGGTGAGTCATGTCTGGGAACTGCCTGATGGAGGGGGA
NACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGNCCTTCNNCCTCTNGNNNTCNGATGTGCCCCNATGGGATTAGCTNGNNGNNGGAA
ANNCGNTTCCNNAGNNANATNCCNAGCTGTNCNGNNGGNNCCCCCCCACACTGNAAGNGCNNNTNANAAAANAANNGGNAGGNNGCAGTTTTTTTAGTGCGGNGGG
NGCGCNNGCNTCCCCNCCCNTNNNTNTAAANNCNNNNNNGTTNGNAATGTATTTTANNGGGGGGGGAGGNAAGGAAATNAANTNNNNNCTTTNCTTTNGACNCNCCA
AAAAAACNGCATCGACTTCCNNCNTGCCCGCNNNNAGAAAAGAGGGAGGGTGCATTNTNAATCATANTACTGCACACAAGCNCCCGNGGGNTTTTTNTATANNANAATG
NNCATCGCNNGNNGCCGGCNNNAANTGTNNNTGNGANTGGGTGCTTNCTCNCGGGNGAGNGGNANNTCCTTCGGGGNGTAGCGATAAAATGNGTAAAGGATGTGGA
AGCCTGCGGGGGGNGACCCCCCCCCCCCNAAAAAAAAAGACTGTCGTNTAAGATAAAGANGGGGTGGGAAAAAAAAAAAANTAATTANATTCCTCTTNNNANCCNNNGA
NNNTAAAANNNGGNNNNTGGGAGGTTGGCCCCNCTGGAGNGGGGCTNCCGGAANTAAANNNNNTAAANNNCGCNNGGGGGNGGAGNNCGCCCGGAAANNANAAA
ATCTTATATTGAGTTGGNCGNGCCCCCNNAAAANGNNNNNGAGTTTTTTNTTTTTNAATTTCGNANGNAANGNCNANAAANNTTTTACTTT

Reverse Sequence:

NNNNNNNNNNNNNGNNNNNNGCNNCTNNGAAGNTAAGCTACCTACTTCTTTTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCA
CCGTGGCATTCTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCG
CTTCTCTTTGTATGCGCCATTGTAGCACGTGTGTAGCCCTGGTCGTAAGGGGCATGATGACTTGACGTCATCCCCACCTTCCTCCAGTTTATCACTGGCAGTCTCCTTTGAGTT
CCCGGCCGGACCGCTGGCAACAAAGGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCACGGTTC
CCGAAGGCACATTCTCATCTCTGAAAACTTCCGTGGATGTCAAGACCAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCA
ATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGACTTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTTACGGCG
TGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTCGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTT
CACCGCTACACCTGGAATTCTACCCCCCTCTACGAGACTCAAGCTTGCCAGTATCAGATGCAGTTCCCAGGTTGAGCCCGAGGATTTCACATCTGACTTAACAAACCGCCTGC
GTGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATGAGCAAAGG
TATTAACTTTACTCCCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGC
TGCCTCCCGTAGGAGTCTGGACCGGGTCTCAGTTCCAGTGTGGCTGGTCATCCCTNTCAAAACCAGCTAGGGAATCGTCGCCCNNGGGNANACCCGTTACC

8) MYb69-mPlum

Forward Sequence:

NNNNNNNGNTANNNNTGCAGTCGAACGGTAACAGGANNNNNCTTGCTNCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGG
ATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGG
GGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATT
GCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCAT
TGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGT
TTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCG
TAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACG
CCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAAT
TGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAATGTGCCTTC
GGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCG
GGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCAGGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAA
ANNAAGNNACCTCCNGNGNGCAAGNGGACCTCAAAANGTGCGTCGTANNCCGAATGGAATCTGCAACTCGANTCCTTGAAGTCGGAATCGCTTGTAATCNGGGNNNAAA
ANGCCACGGTAAAANCTTTCCCGGGCCTTNNNANACCNNCCCGTNNNCCN

Reverse Sequence:

NNNNNNNNNNGCNANNNNTGCAGTCGAACGGTAACAGGANNNNNCTTGCTNCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGG
GGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGG
TGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT
ATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCT
CATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGC

GGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAAT
GCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCC
ACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATG
AATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAATGTGCC
TTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGC
CGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACAACCAGGGCTACNNANGTGCTACAATGGNGNAAAC
AAAGAGAAACGACCTCCCNNGAGCAAGCGGACCTCAAANAGTGCGTCGTAGTCGGGNTGGAGTCTNCAANTCGACTCCNNGAATTCGGAATCNNTAGTAATCCGTGGAN
NAGAAATGCCNGGNNAAANTACTTTCCNGGGNCCTNNTANANCCCGCCCNNNNCCNCAAGGGA

9) MYb21-mPlum

Forward Sequence:

NNNNNNNNNNNTGNTANNNNNNNNCCCGAAGNTAAGCTACCTACTTCTTTTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCG
TGGCATTCTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTT
CTCTTTGTATGCGCCATTGTAGCACGTGTGTAGCCCTGGTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCAGTTTATCACTGGCAGTCTCCTTTGAGTTCCC
GGCCGGACCGCTGGCAACAAAGGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCACGGTTCCCG
AAGGCACATTCTCATCTCTGAAAACTTCCGTGGATGTCAAGACCAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATT
CATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGACTTAACGCGTTAGCTCCGGAAGCCACGCCTCACGGGCACAACCTCCAAGTCGACATCGTTTACGGCGTG
GACTACCAGGGTATCTAATCCTGTTTGCTCACCACGCTTTCGCACCTGAGCGTCAGTCTTCGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCA
CCGCTACACCTGNAATTCTACCCCCCTCTACGAGACTCAAGCTTGCCAGTATCACATGCAGTTTCNCANGTTGAGCGCGGGGATATCACATCTTGACTTAACAACCCGCCTGC
GTGCGCTGTACGCCCAGTAATTTCNAANNAAACGCGNGNNACCCTCNGCTNTTACAGCGNCTGCTNGCAACGGNGNGAGACGAGAGNNNNNNTNTGCGGGCANCGACA
NNGAA

Reverse Sequence:

NNNNNNNNNNNNNNTANNNNTGCAGTCGAACGGTAACAGGAAGNAAGCTTGCTNNTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGA
GGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTA
GGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG
AATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTT
GCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCA
GGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGAGNTAGAATTCCAGGTGTANCGCTGA
AATGCGTAGAGATATGAAGGAATACAGGTGGGGAANTNGGCCCTCTTGTACAACCACTGACGCTCACGTGCGAGAGCGTNNNNAGCTAAAAGGATTAGANTACCCTGCCA
GTCNNCGCCNTAAACGNTGTCGACGTTGCAGCTNGNNCCGTGAAGCNNGCC

10) BIGb0172- mPlum

Forward Sequence:

NNNNNNNNGCTANNNNTGCAGTCGAACGGTAACAGGANNNNNCTTGCTNCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGG
GATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTG
GGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATAT
TGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCA
TTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGG
TTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGC
GTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCAC
GCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAA
TTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAATGTGCCTT
CGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCC
GGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCAGGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCAAACAA
AGAGAAGCGACCTCGCGNNNGCAAGCGGACCTCATAAAGTGCGTCGTAATTCCGGAATGGGAATCTGCAACTCGANTCCCTGAAGTCGGAATCGCTTGTAATCNGGGATC
AGAANGCCACGGTGAAAA

Reverse Sequence:

NNNNNNNNNAGTGNNANCGNNNCCCGAAGGTTAAGCTACCTACTTCTTTTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGT
GGCATTCTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTC
TCTTTGTATGCGCCATTGTAGCACGTGTGTAGCCCTGGTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCAGTTTATCACTGGCAGTCTCCTTTGAGTTCCCG
GCCGGACCGCTGGCAACAAAGGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCACGGTTCCCGA
AGGCACATTCTCATCTCTGAAAACTTCCGTGGATGTCAAGACCAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTC
ATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGACTTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTTACGGCGTGG
ACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTCGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCAC

CGCTACACCTGGAATTCTACCCCCCTCTACGAGACTCAAGCTTGCCAGTATCAGATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCTGACTTAACAAACCGCCTGCGTG
CGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATGAGCAAAGGTATT
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CTCCCGTAGGAAGTCTGGACCGTGTCTCAGTTCCAGTGGNGGCTGGTCATCCTCTCNNACCAGCTAGGGATCGTCGCCTAGGGNGAGCCGTTACCCCCCCNTACTAGCTAA
ATCCNATCTGGGNCACATCCCAATGGCAANNAGGCCCGAANGGTCCCCCCCCT

10.10. Bacteria susceptibility profile

MYb71-sfGFP					
No	Antibiotic Ring	Antibiotic	Fluorescence Bacteria		Match
			Susceptibility	Reference	
1	M13/NCE	Chloramphenicol	R	S	No
2	M13/NCE	Erythromycin	R	S	No
3	M13/NCE	Fusidic acid	R	R	Yes
4	M13/NCE	Oxacillin	T	R	No
5	M13/NCE	Novobiocin	R	R	Yes
6	M13/NCE	Penicillin	R	R	Yes
7	M13/NCE	Streptomycin	R	R	Yes
8	M13/NCE	Tetracycline	S	S	Yes
9	M14/NCE	Ampicillin	R	R	Yes
10	M14/NCE	Cephalothin	R	R	Yes
11	M14/NCE	Colistin Sulphate	R	R	Yes
12	M14/NCE	Gentamicin	R	S	No
13	M14/NCE	Streptomycin	R	R	Yes
14	M14/NCE	Sulphatriad	R	R	Yes
15	M14/NCE	Tetracycline	S	S	Yes
16	M14/NCE	Cotrimoxazole	R	R	Yes
17	M43/NCE	Penicillin G	R	R	Yes
18	M43/NCE	Clindamycin	R	R	Yes
19	M43/NCE	Gentamicin	R	S	No
20	M43/NCE	Fusidic acid	R	R	Yes
21	M43/NCE	Erythromycin	R	R	Yes
22	M43/NCE	Trimethoprim	R	R	Yes
23	M43/NCE	Sulphamethoxazole	R	R	Yes
24	M43/NCE	Tetracycline	S	S	Yes
R: Resistant, S: Susceptible, T: Trace					

MYb186-sfGFP					
No	Antibiotic Ring	Antibiotic	Fluorescence Bacteria		Match
			Susceptibility	Reference	
1	M13/NCE	Chloramphenicol	S	S	Yes
2	M13/NCE	Erythromycin	R	R	Yes
3	M13/NCE	Fusidic acid	R	R	Yes
4	M13/NCE	Oxacillin	R	R	Yes
5	M13/NCE	Novobiocin	R	R	Yes
6	M13/NCE	Penicillin	R	R	Yes
7	M13/NCE	Streptomycin	S	S	Yes
8	M13/NCE	Tetracycline	S	S	Yes
9	M14/NCE	Ampicillin	R	R	Yes
10	M14/NCE	Cephalothin	R	R	Yes
11	M14/NCE	Colistin Sulphate	S	S	Yes
12	M14/NCE	Gentamicin	R	S	No
13	M14/NCE	Streptomycin	S	S	Yes
14	M14/NCE	Sulphatriad	R	R	Yes

15	M14/NCE	Tetracycline	S	S	Yes
16	M14/NCE	Cotrimoxazole	S	S	Yes
17	M43/NCE	Penicillin G	R	R	Yes
18	M43/NCE	Clindamycin	R	R	Yes
19	M43/NCE	Gentamicin	R	S	No
20	M43/NCE	Fusidic acid	R	R	Yes
21	M43/NCE	Erythromycin	R	R	Yes
22	M43/NCE	Trimethoprim	R	S	No
23	M43/NCE	Sulphamethoxazole	R	R	Yes
24	M43/NCE	Tetracycline	S	S	Yes
R: Resistant, S: Susceptible, T: Trace					

MYb11-sfGFP					
No	Antibiotic Ring	Antibiotic	Susceptibility Score		Match
			Susceptibility	Reference	
1	M13/NCE	Chloramphenicol	R	R	Yes
2	M13/NCE	Erythromycin	R	S	No
3	M13/NCE	Fusidic acid	R	R	Yes
4	M13/NCE	Oxacillin	R	R	Yes
5	M13/NCE	Novobiocin	R	R	Yes
6	M13/NCE	Penicillin	R	R	Yes
7	M13/NCE	Streptomycin	S	S	Yes
8	M13/NCE	Tetracycline	S	S	Yes
9	M14/NCE	Ampicillin	R	R	Yes
10	M14/NCE	Cephalothin	R	R	Yes
11	M14/NCE	Colistin Sulphate	S	S	Yes
12	M14/NCE	Gentamicin	R	S	No
13	M14/NCE	Streptomycin	S	S	Yes
14	M14/NCE	Sulphatriad	R	R	Yes
15	M14/NCE	Tetracycline	S	S	Yes
16	M14/NCE	Cotrimoxazole	R	R	Yes
17	M43/NCE	Penicillin G	R	R	Yes
18	M43/NCE	Clindamycin	R	R	Yes
19	M43/NCE	Gentamicin	R	S	No
20	M43/NCE	Fusidic acid	R	R	Yes
21	M43/NCE	Erythromycin	R	R	Yes
22	M43/NCE	Trimethoprim	R	R	Yes
23	M43/NCE	Sulphamethoxazole	R	R	Yes
24	M43/NCE	Tetracycline	R	R	Yes
R: Resistant, S: Susceptible, T: Trace					

MYb11-dTomato					
No	Antibiotic Ring	Antibiotic	Fluorescence Bacteria		Match
			Susceptibility	Reference	
1	M13/NCE	Chloramphenicol	S	S	Yes
2	M13/NCE	Erythromycin	R	S	No
3	M13/NCE	Fusidic acid	R	R	Yes

4	M13/NCE	Oxacillin	R	R	Yes
5	M13/NCE	Novobiocin	R	R	Yes
6	M13/NCE	Penicillin	R	R	Yes
7	M13/NCE	Streptomycin	S	S	Yes
8	M13/NCE	Tetracycline	S	S	Yes
9	M14/NCE	Ampicillin	R	R	Yes
10	M14/NCE	Cephalothin	R	R	Yes
11	M14/NCE	Colistin Sulphate	S	S	Yes
12	M14/NCE	Gentamicin	R	S	No
13	M14/NCE	Streptomycin	R	S	No
14	M14/NCE	Sulphatriad	R	R	Yes
15	M14/NCE	Tetracycline	S	S	Yes
16	M14/NCE	Cotrimoxazole	R	R	Yes
17	M43/NCE	Penicillin G	R	R	Yes
18	M43/NCE	Clindamycin	R	R	Yes
19	M43/NCE	Gentamicin	R	S	No
20	M43/NCE	Fusidic acid	R	R	Yes
21	M43/NCE	Erythromycin	R	R	Yes
22	M43/NCE	Trimethoprim	R	R	Yes
23	M43/NCE	Sulphamethoxazole	R	R	Yes
24	M43/NCE	Tetracycline	S	S	Yes
R: Resistant, S: Susceptible, T: Trace					

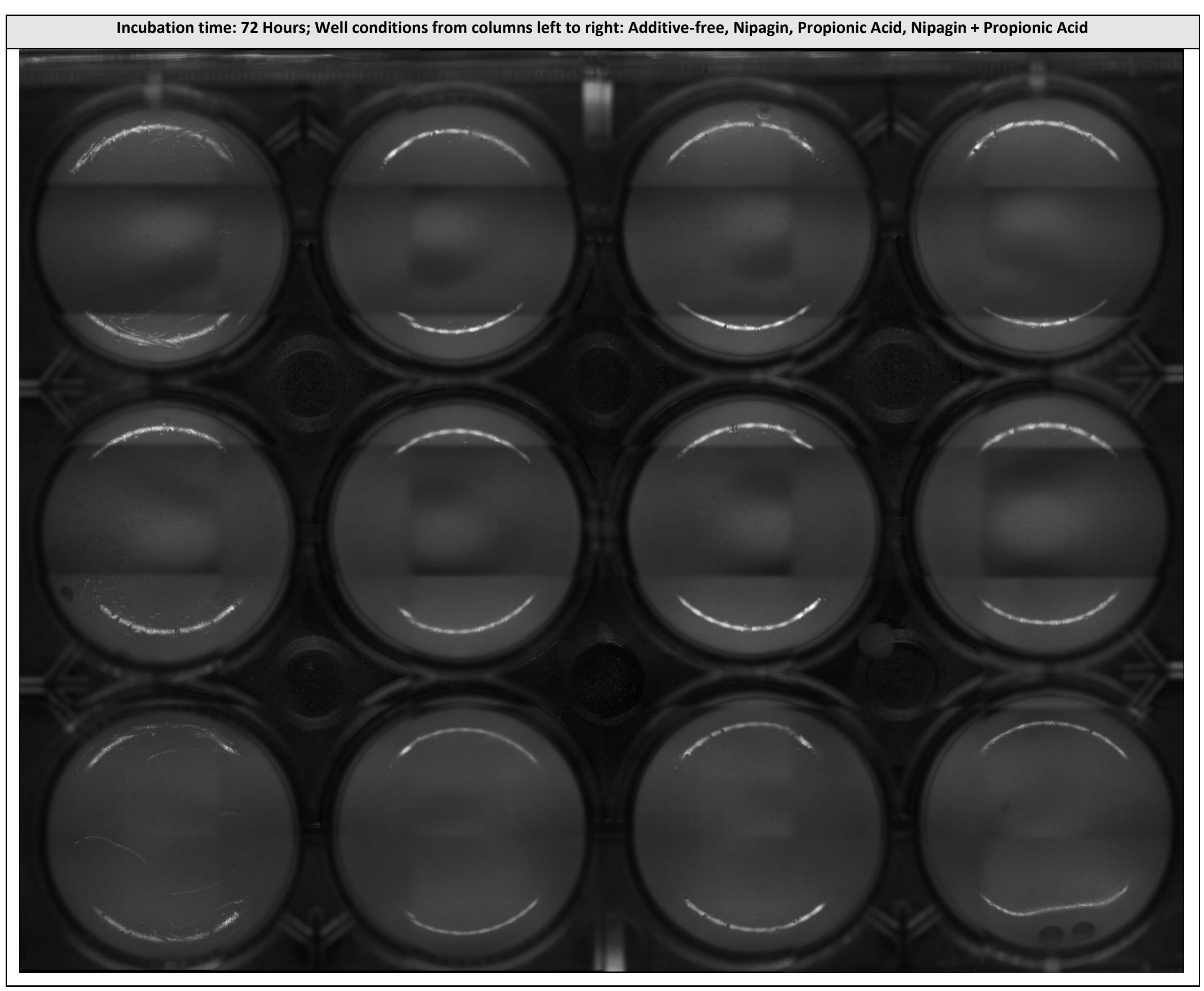
MYb11-mPlum					
No	Antibiotic Ring	Antibiotic	Fluorescence Bacteria		Match
			Susceptibility	Reference	
1	M13/NCE	Chloramphenicol	R	R	Yes
2	M13/NCE	Erythromycin	R	S	No
3	M13/NCE	Fusidic acid	S	S	Yes
4	M13/NCE	Oxacillin	S	S	Yes
5	M13/NCE	Novobiocin	S	S	Yes
6	M13/NCE	Penicillin	S	S	Yes
7	M13/NCE	Streptomycin	R	S	No
8	M13/NCE	Tetracycline	S	S	Yes
9	M14/NCE	Ampicillin	R	R	Yes
10	M14/NCE	Cephalothin	R	R	Yes
11	M14/NCE	Colistin Sulphate	S	S	Yes
12	M14/NCE	Gentamicin	R	S	No
13	M14/NCE	Streptomycin	R	S	No
14	M14/NCE	Sulphatriad	R	R	Yes
15	M14/NCE	Tetracycline	R	S	No
16	M14/NCE	Cotrimoxazole	R	R	Yes
17	M43/NCE	Penicillin G	R	R	Yes
18	M43/NCE	Clindamycin	R	R	Yes
19	M43/NCE	Gentamicin	R	S	No
20	M43/NCE	Fusidic acid	R	R	Yes
21	M43/NCE	Erythromycin	R	R	Yes
22	M43/NCE	Trimethoprim	R	R	Yes
23	M43/NCE	Sulphamethoxazole	R	R	Yes

24	M43/NCE	Tetracycline	S	S	Yes
R: Resistant, S: Susceptible, T: Trace					

BIGb0393-mPlum					
No	**Antibiotic Ring**	**Antibiotic**	**Fluorescence Bacteria**		**Match**
			Susceptibility	**Reference**	
1	M13/NCE	Chloramphenicol	S	S	Yes
2	M13/NCE	Erythromycin	R	R	Yes
3	M13/NCE	Fusidic acid	R	R	Yes
4	M13/NCE	Oxacillin	R	R	Yes
5	M13/NCE	Novobiocin	R	R	Yes
6	M13/NCE	Penicillin	R	R	Yes
7	M13/NCE	Streptomycin	S	S	Yes
8	M13/NCE	Tetracycline	S	S	Yes
9	M14/NCE	Ampicillin	R	R	Yes
10	M14/NCE	Cephalothin	R	R	Yes
11	M14/NCE	Colistin Sulphate	S	S	Yes
12	M14/NCE	Gentamicin	R	S	No
13	M14/NCE	Streptomycin	S	R	No
14	M14/NCE	Sulphatriad	R	S	No
15	M14/NCE	Tetracycline	S	S	Yes
16	M14/NCE	Cotrimoxazole	R	S	No
17	M43/NCE	Penicillin G	R	R	Yes
18	M43/NCE	Clindamycin	R	R	Yes
19	M43/NCE	Gentamicin	R	S	No
20	M43/NCE	Fusidic acid	R	R	Yes
21	M43/NCE	Erythromycin	R	R	Yes
22	M43/NCE	Trimethoprim	R	R	Yes
23	M43/NCE	Sulphamethoxazole	R	R	Yes
24	M43/NCE	Tetracycline	S	S	Yes
R: Resistant, S: Susceptible, T: Trace					

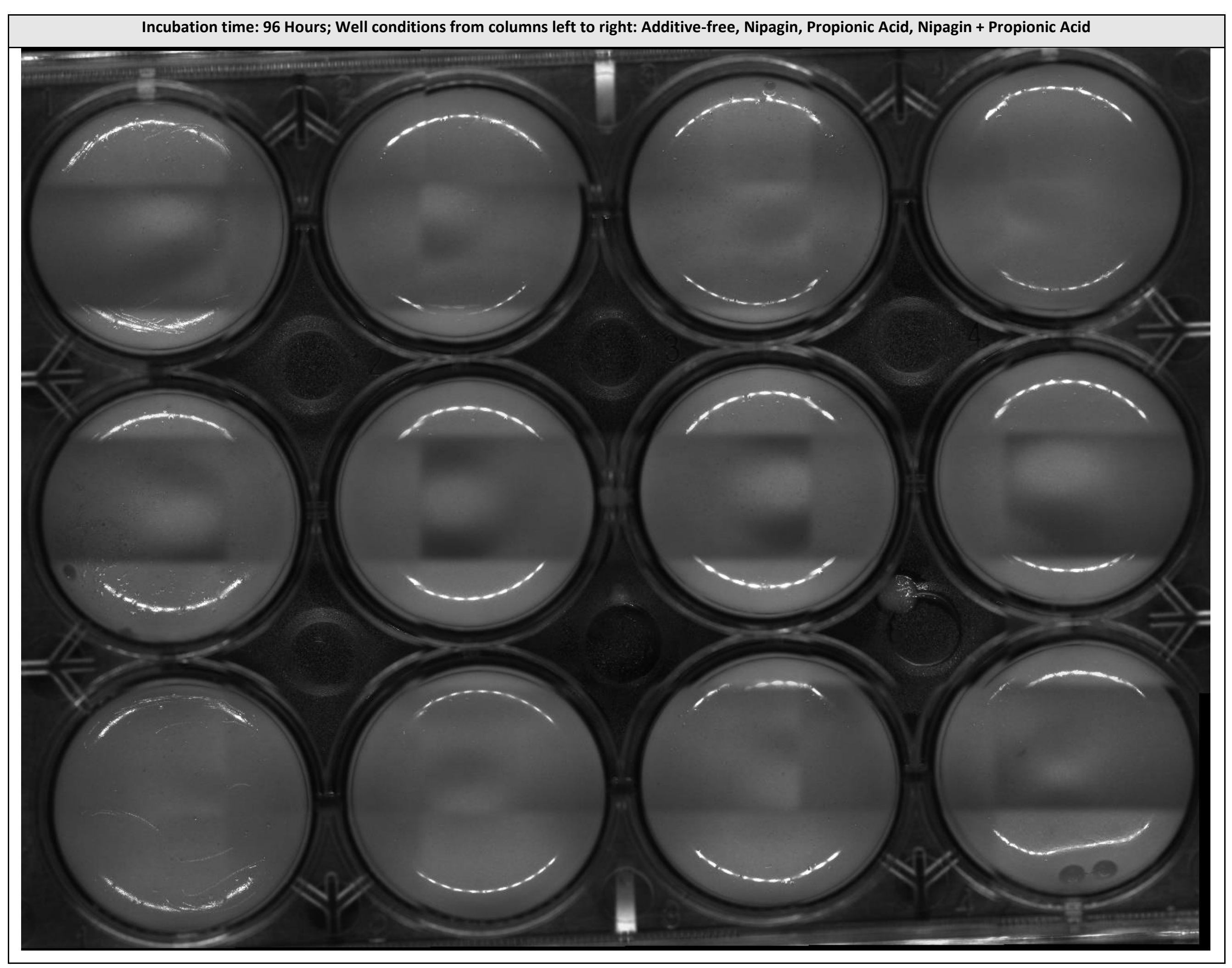
10.11. MYb174 did not grow on fly media

Incubation time: 48 Hours; Well conditions from columns left to right: Additive-free, Nipagin, Propionic Acid, Nipagin + Propionic Acid

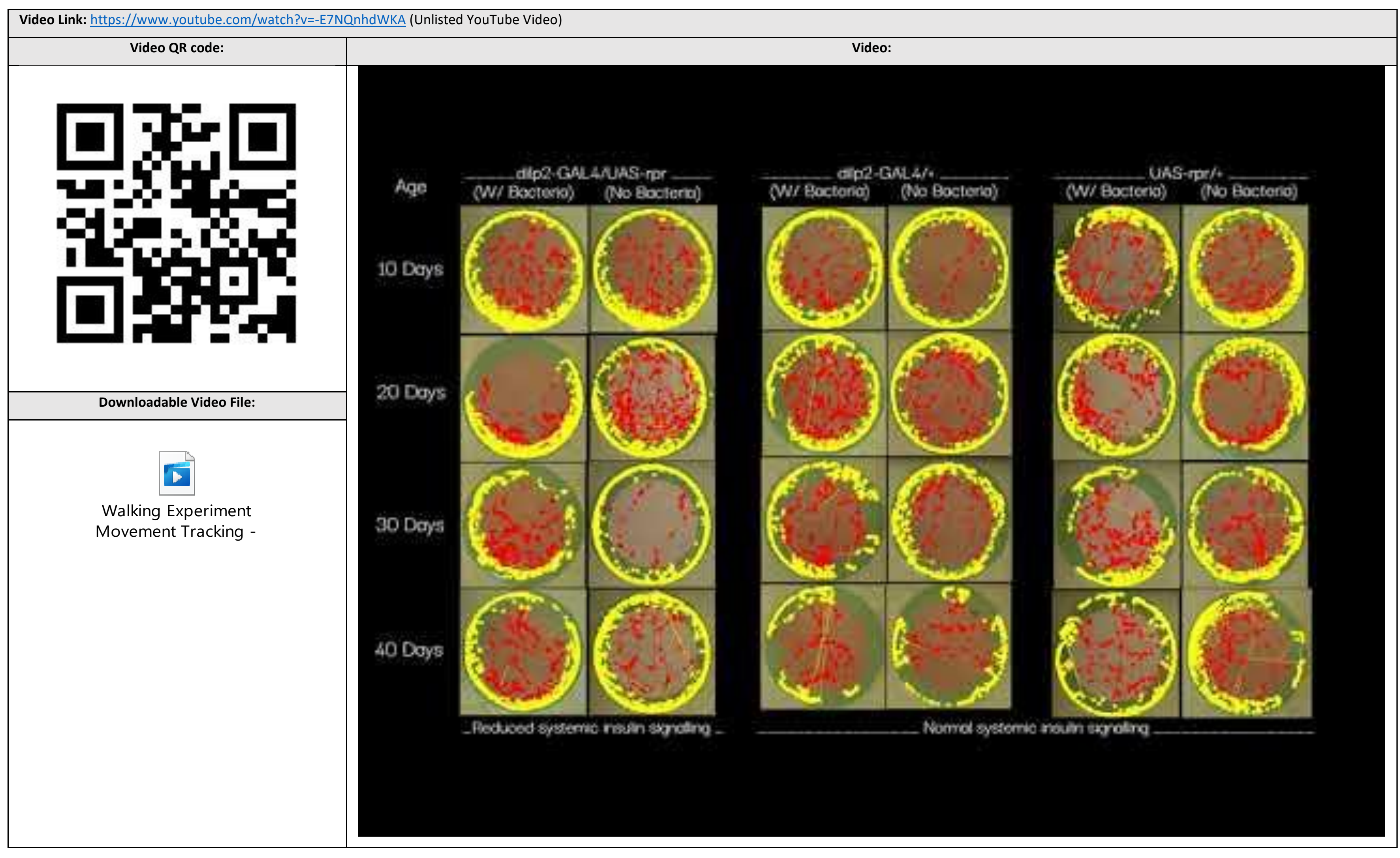


Incubation time: 72 Hours; Well conditions from columns left to right: Additive-free, Nipagin, Propionic Acid, Nipagin + Propionic Acid

Incubation time: 96 Hours; Well conditions from columns left to right: Additive-free, Nipagin, Propionic Acid, Nipagin + Propionic Acid



10.12. Video comparing exploratory walking behaviour of female flies from age 10 to 40 days



Video Link: https://www.youtube.com/watch?v=-E7NQnhdWKA (Unlisted YouTube Video)	
Video QR code:	**Video:**
Downloadable Video File: Walking Experiment Movement Tracking -	

10.13. Sample size of each Experiment

	Female			Male		
Bacteria Condition	**Sample Size**	**Censored**	**Total**	**Sample Size**	**Censored**	**Total**
Pilot Lifespan Assay (Result 5.4)						
MYb71-sfGFP	8	8	16	2	10	12
MYb174-dTom	12	6	18	2	20	22
CEent1-mPlum	9	17	26	2	14	16
MYb71-sfGFP + CEen1-mPlum	9	18	27	7	21	28
MYb71-sfGFP + MYb174-dTom	15	15	30	2	27	29
MYb174-dTom + CEen1-mPlum	14	17	31	7	23	30
MYb174-dTom + CEen1-mPlum + MYb71-sfGFP	8	17	25	4	22	26
No Bacteria	10	18	28	7	17	24

	Female						Male					
Experiment	**d2-3GAL4/ UAS-*rpr***	**d2-3GAL4/ +**	**UAS-*rpr*/ +**	**d2-3GAL4/UAS-*rpr* (w/bacteria)**	**d2-3GAL4/+ (w/bacteria)**	**UAS-*rpr*/+ (w/bacteria)**	**d2-3GAL4/ UAS-*rpr***	**d2-3GAL4/ +**	**UAS-*rpr*/ +**	**d2-3GAL4/UAS-*rpr* (w/bacteria)**	**d2-3GAL4/+ (w/bacteria)**	**UAS-*rpr*/+ (w/bacteria)**
Lifespan Assay 1 (Result 5.5.1)												
Sample Size	130	103	90	86	73	68	130	112	114	90	102	124
Censored	27	19	26	27	50	36	16	11	13	13	14	8
Total	157	122	116	113	123	104	146	123	127	103	116	132
Exploratory Walking Assay (Result 5.5.2)												
Age 10 days	16	16	16	16	16	16	16	16	16	16	16	16
Age 20 days	16	16	16	16	16	16	16	16	16	16	16	16
Age 30 days	16	16	16	16	16	16	16	16	16	16	15	16
Age 40 days	16	16	16	16	16	16	16	16	16	16	16	16
Age 50 days	16	16	16	16	3	16	16	16	15	16	16	16
Gut Permeability Assay (Result 5.5.3)												
Age 10 days	15	15	15	15	15	15	15	15	15	15	15	15
Age 20 days	15	15	15	15	15	15	15	15	15	15	15	15
Age 30 days	15	15	15	15	15	15	15	15	15	15	15	15
Age 40 days	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	15	15	15	15	15	15
Age 50 days	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	15	15	15	15	15	15
Lifespan Assay 2 (Result 5.6.1)												

Sample Size	283	267	222	234	230	200	215	257	249	189	213	214
Censored	2	4	17	15	16	33	10	9	7	26	15	28
Total	285	271	239	249	246	233	225	266	256	215	228	242
Sleep Assay (Result 5.6.2)												
Age 10 days	15	15	13	15	15	15	13	15	14	15	15	12
Age 20 days	15	15	13	13	14	13	15	15	15	14	14	14
Age 30 days	15	13	11	15	14	13	15	15	15	15	14	13
Age 40 days	15	12	9	12	12	6	14	14	13	13	14	12
Age 50 days	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	12	10	13	14	10	6
Negative Geotaxis Assay (Result 5.6.3)												
Age 10 days	45	45	45	45	45	45	45	45	45	45	45	45
Age 20 days	45	45	45	45	45	45	45	45	45	45	45	45
Age 30 days	45	45	45	45	45	45	45	45	45	45	45	45
Age 40 days	45	45	45	45	45	45	45	45	45	45	45	45
Age 50 days	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	45	45	45	45	45	45
Offspring Quantification Assay 1 (Result 5.6.4)												
Age 5 days	100	100	100	100	100	100	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 7 days	100	100	100	100	100	100	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 9 days	100	90	100	99	100	90	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 10 days	98	96	94	100	100	100	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 12 days	100	100	100	100	100	100	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 14 days	100	100	100	100	100	97	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 16 days	100	100	100	97	99	94	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 18 days	99	97	99	97	97	94	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 20 days	100	95	98	91	95	94	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Lifespan Assay 3 (Result 5.7.1)												
Sample Size	118	149	142	138	137	132	101	90	102	126	144	144
Censored	22	20	1	1	1	5	17	26	30	9	6	4
Total	140	169	143	139	138	137	118	116	132	135	150	148
Egg Quantification Assay (Result 5.7.2)												
Age 5 days	45	45	45	50	50	50	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 7 days	40	50	45	50	45	50	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 12 Days	50	50	50	60	55	54	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*

Age 14 Days	50	55	55	50	50	55	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 19 Days	56	50	50	55	40	54	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 21 Days	50	50	50	45	50	50	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Offspring Quantification Assay 2 (Result 5.7.2)												
Age 5 days	50	50	45	55	55	45	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 7 days	44	50	49	50	50	49	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 10 Days	50	50	55	60	55	54	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 14 Days	55	55	54	47	64	64	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 19 Days	61	60	50	61	47	54	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 21 Days	50	50	50	66	44	46	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*

	Female						**Male**					
Experiment	**trhGAL4/ UAS-InR^{DN}**	**trhGAL4/ +**	**UAS-InR^{DN}/ +**	**trhGAL4/UAS-InR^{DN} (w/bacteria)**	**trhGAL4/+ (w/bacteria)**	**UAS-InR^{DN}/+ (w/bacteria)**	**trhGAL4/ UAS-InR^{DN}**	**trhGAL4/ +**	**UAS-InR^{DN}/ +**	**trhGAL4/UAS-InR^{DN} (w/bacteria)**	**trhGAL4/+ (w/bacteria)**	**UAS-InR^{DN}/+ (w/bacteria)**
Lifespan 3 (Result 5.7.1)												
Sample Size	121	136	131	144	130	143	71	98	116	132	144	126
Censored	7	3	6	6	2	0	11	13	12	4	0	7
Total	128	139	137	150	132	143	82	111	128	136	144	133
Egg Quantification Assay (Result 5.7.2)												
Age 5 days	45	45	45	50	50	50	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 7 days	60	50	60	50	50	50	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 12 Days	50	65	55	59	65	70	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 14 Days	50	50	50	50	45	50	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 19 Days	67	50	70	57	41	41	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 21 Days	50	45	50	50	50	50	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Offspring Quantification Assay 2 (Result 5.7.2)												
Age 5 days	45	50	40	50	50	50	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 7 days	60	50	65	50	50	50	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 10 Days	60	70	55	59	65	64	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 14 Days	50	50	50	55	65	62	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 19 Days	62	50	70	57	40	51	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Age 21 Days	50	45	50	60	43	39	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*