INVESTIGATING THE ROLE OF THE NEUROPEPTIDE DIURETIC HORMONE 31 (Dh31) IN *DROSOPHILA* OOGENESIS

by
Metabel Markwei

A thesis submitted to Johns Hopkins University in conformity with the requirements for the degree of Master of Science

Baltimore, Maryland

August, 2017

ABSTRACT

Background: Adult stem cells are essential for tissue homeostasis. The ability of stem cells to self-renew and generate differentiating progeny allows for the replacement of dying or damaged cells. In the *Drosophila* ovary, germline stem cell (GSC) number and proliferation, as well as later stages in oogenesis, are tightly regulated in response to diet and physiological changes. For example, insulin signaling, which controls cellular nutrient sensing, aging, and many other biological processes, regulates GSC proliferation and maintenance in response to diet. *Drosophila* insulin-like peptides (dILPs) are neuropeptides secreted from the brain, and our lab is currently investigating whether additional neuropeptides may influence GSC proliferation and/or maintenance, or later stages of oogenesis. Neuropeptides are important signaling molecules involved in many processes including metabolism and reproduction. Our lab previously performed a screen of 36 out of 44 *Drosophila* neuropeptides to identify neuropeptides that regulate oogenesis. The Drummond-Barbosa lab measured the rates of egg production within 15 days of brain-specific neuropeptide RNAi knockdown. Interestingly, pan-neuronal knockdown of the neuropeptide diuretic hormone 31 (Dh31) significantly decreased *Drosophila* egg production. These findings sparked our interest in investigating the role Dh31 plays in regulating egg production. Dh31 binds to a G-protein-coupled receptor, Dh31-R, a homolog to the human calcitonin receptor, to positively regulate adenylate cyclase activity. Furthermore, Dh31 is responsible for the regulation of water and NaCl homeostasis in the Malpighian tubules of the fly, in response to changes in diet and environment.

Materials and Methods: RNAi-mediated knockdown experiments were conducted to disrupt Dh31 neuropeptide production, as well as its receptor function, Dh31-R, in a tissue-

specific manner. Using immunohistochemistry together with confocal microscopy, we measured changes in the number of GSCs and their progeny (cysts), as well as the niche size (cap cell number) after Dh31 and Dh31-R RNAi knockdowns.

Results: Dh31-R knockdowns in all somatic cells and Dh31 neuropeptide knockdown in the brain both result in a decrease in the number of eggs laid, no changes in the number of GSCs and CCs, and significant reductions in the number of 4-cell, 8-cell and 16-cell cysts.

Conclusion: Dh31 likely neither affects GSC proliferation and number, nor niche size. Loss of Dh31 neuropeptide from the brain, and Dh31-R somatically, however, leads to a significant decrease in the number of GSC progeny (4-cell cysts, 8-cell cysts and 16 cell cysts). This loss is likely because of cyst death as there is no significant change in GSC proliferation as well. So far, our data suggests that Dh31 signaling is not required in the germline; however, further experiments need to be performed to rule out the possibility.

Keywords: Neuropeptide, Drosophila ovary, *stem cells, germline stem cells, oogenesis, proliferation, cell death, Dh31, calcitonin.*

Thesis Advisor: Daniela Drummond-Barbosa, PhD
Professor
Johns Hopkins Bloomberg School of Public Health
Department of Biochemistry and Molecular Biology

Academic Advisor: Phil Jordan, PhD
Assistant Professor
Johns Hopkins Bloomberg School of Public Health
Department of Biochemistry and Molecular Biology

ACKNOWLEDGEMENTS

I was blessed to have a phenomenal advisor and mentor in Dr. Daniela Drummond-Barbosa. Thank you for your guidance through my seven-month research work and your patience and encouragement through tough times in my own life. You espouse the qualities of a great mentor by seeing things in your mentees that they sometimes may not see in themselves. For that, I say thank you.

To my second reader and academic advisor, Dr. Phil Jordan, thank you for arranging my placement in the Drummond-Barbosa Lab and taking full ownership of my SCM experience. I will never forget your vow to make sure I obtained this degree in a fulfilling manner. For that, I remain indebted.

To the Drummond-Barbosa Lab members, thank you for accepting me as part of the lab family. Your work ethic and ingenuity inspired me every day. A special thank you to Tianlu Ma (Ph.D. candidate) for teaching me all I now know about fly work in only seven months and allowing me to be a part of your exciting project. My sincere thanks to Dr. Lesley Weaver and Dr. Shinya Matsuoka who also taught me techniques with confocal microscopy and fly genetics, you are both stellar scientists and teachers.

My heartfelt thanks also go to Shannon Gaston and Kear Wright, both administrators of the Biochemistry and Molecular Department at the Bloomberg School of Public Health. Thank you for all the time and energy you put into working out the logistics of my research year and your support every step of the way. I owe you both many.

To Ms. Jessica Harrington of the Wellness and Student Assistance Center, thank you for all your guidance, and for creating a safe space for me in your office. You radiate

so much warmth and joy, and I do not take all the life lessons I have learned from our chats and book reads for granted. I will keep our moments in heart forever.

My research would also not be possible without the financial support and aid of the Biochemistry and Molecular (BMB) Department, The Bloomberg School of Public Health at Johns Hopkins (BSPH) and The Ghana Education Trust Fund (GET Fund). Additionally, fly stocks and research materials were provided by the Bloomington *Drosophila* Stock Center, The Vienna *Drosophila* Resource Center, and Dr. Mark Wu at the Johns Hopkins University School of Medicine.

More importantly, a special thank you to my parents, family, close friends and roommate, Joycelyn Cudjoe, for their unwavering support throughout my program and encouraging me every step of the way.

Last but deserving of my last thanks, a thank you to God, the author and finisher of my faith, resilience through my research year and making the lines fall for me in pleasant places. I remain forever grateful.

ACRONYMS:

BDSC	Bloomington *Drosophila* Stock Center
BURS	Bursicon
cAMP	cyclic adenosine monophosphate
CC	Cap cells (the niche)
CRF	Corticotropin releasing factor
Dh31	diuretic hormone-31
dILPs	*Drosophila* Insulin-like Peptides
EdU	Ethynyl deoxyuridine
EH	Eclosion Hormone
GPCR	G-protein Coupled Receptor
GFP	Green Fluorescent Protein
GSC	germline stem cell
LGV	large granular vesicles
MARCM	mosaic analysis with a repressible cell marker
MTD	maternal triple driver
RNAi	RNA-mediated interference
TFCs	thin filament cells
TRP	tachykinin-related peptides
TubP	Tubulin Promoter
UAS	Upstream Activation Sequence
VDRC	Vienna *Drosophila* Resource Center
y w	*yellow white* genotype

FIGURES:

Supplemental Figures:

CONTENTS

INTRODUCTION:

1.1 Background

Neuropeptides are small proteins produced, and secreted by neurons (Merighi, 2011). They are by far the largest and most diverse class of chemical messenger and signaling molecules in the brain. Neuropeptides can act as neurotransmitters directly, as modulators of ongoing neurotransmission by other transmitters, as autocrine and paracrine regulators in a close cellular environment, and as hormones with long range activity (Burbach, 2011). They are usually generated as large precursor polypeptides that are subsequently modified by enzymatic processing and other post-translational modifications (Vanden Broeck, 2001). Many neuropeptides have pleiotropic activity, i.e. they act on a variety of cell types and tissues (Adalberto. Merighi, 2011), thereby forming an integral basis on which intercellular communications occur (Prigge, Kolhekar, Eipper, Mains, & Amzel, 1997).

Cell populations located in the central and peripheral nervous system produce and release these neuropeptides which are stored in large granular vesicles (LGVs) within the neuron (Adalberto. Merighi, 2011). Inside these neurons, the neuropeptides are hosted alongside low-molecular-weight neurotransmitters such as catecholamines and amino acids, typically 50 times smaller than their neighboring neuropeptides (Adalberto Merighi, Salio, Ferrini, & Lossi, 2011). Compared to neurotransmitters, neuropeptides can exist in the brain for much longer and have the additional ability to carry out their biological functions even at low concentrations due to their higher selectivity and higher receptor binding affinity (A Merighi, 2002). As a result, neuropeptides can affect long-term behaviors due to their extended stay within the extracellular fluid of the brain.

Neuropeptides, therefore, can reach targets that are farther away, and these targets reorganize neuronal networks to regulate long-term behaviors (Ludwig & Leng, 2006). Therefore, the controlled secretion of neuropeptides regulates communication between cells, organs and tissues that may be spatially distant and coordinates whole body physiological responses to external stimuli (Burbach, 2011).

The fruitfly is an excellent model organism to study the effects of neuropeptide signaling on several physiological processes due to the development of powerful genetic tools in *Drosophila*, that are simpler and easier to manipulate than in more evolved animals such as mammals (Johnson et al., 2005). With rapid advances in the identification of neuropeptide signaling components, researchers have been able to localize neuropeptide expression, and characterize the physiology of peptidergic and neuroendocrine systems in *Drosophila* (Park, Veenstra, Park, & Taghert, 2008). Using enhancer-trap lines and promoter GAL4 lines combined with reporter systems (Santos et al., 2007), many neuropeptides can be studied. We utilized the temperature-sensitive GAL4/UAS system with $GAL80^{ts}$, in several of our experiments. The GAL4/UAS system is the most widely used binary expression system in *Drosophila melanogaster* (Holtzman, 2010), and, along with $GAL80^{ts}$, allows for temporal and spatial control over the expression of the transgenes used in this study. The GAL4/UAS expression system is imported from yeast (Brand and Perrimon, 1993) and consists of a transcription factor (GAL4) and its target sequence (UAS), which comprises the promoter region for a gene. GAL4 activity can be regulated by the temperature-sensitive $GAL80^{ts}$ inhibitor. At $18^{o}C$, $GAL80^{ts}$ binds the activation domain of GAL4 and prevents it from recruiting other transcriptional activators to the gene of interest. However, when switched to $29^{o}C$, $Gal80^{ts}$ is inactivated, and the GAL4

transcription factor can now promote transcription and expression of the transgene to which the UAS sequence is attached (*Figure 1c*).

With our lab's interest in the role that diet and whole body physiology plays in reproduction, neuropeptides proved interesting to study due to their potential signaling functions between the environment and reproduction in fruit flies. Vast genome-wide information on neuropeptide signaling in *Drosophila* exists due to complete sequencing and annotation of the *Drosophila* genome (Adams et al., 2000). In the fruitfly, neuropeptides are important signaling molecules that regulate several physiological functions such as development, growth, feeding, metabolism, reproduction, muscular control, homeostasis, and longevity, as well as neuromodulation in learning and memory, olfaction and locomotor control (Nässel & Winther, 2010).

There are a total of 44 *Drosophila* neuropeptides identified thus far. Most insect neuropeptides act on G-protein coupled receptors (GPCRs) (Zandawala, Li, Hauser, Grimmelikhuijzen, & Orchard, 2013), whereas some of the insulin-like peptides of *Drosophila* act on tyrosine kinase receptors (Nässel, 2002) and eclosion hormone acts on membrane-bound guanylate cyclase receptors (Zimmer et al., 2009). Most *Drosophila* neuropeptides are likely to have mammalian homologs at the level of primary sequence (Hewes, 2001). In *Drosophila*, several diuretic peptide hormones have been identified, including CRF-related (DH44; CG8348) and calcitonin-related (Dh31; CG13094) diuretic hormones, leucokinin (LK; CG13480) and the capa peptides CAPA-PVK1 (Hewes & Taghert, 2001). Diuretic hormone 31 (CG13094) is a unique neuropeptide in the diuretic hormone class 2 family (Q9VLK4). It derives its name from the fact that it only has 31 residues. Dh31 has a molecular mass of 3149.57 Da, and its gene maps to position 76 –

106 on the 2L chromosome (Adams et al., 2000; Hewes & Taghert, 2001). The displayed sequence illustrated in Figure 1a is further processed into a mature form (Reiher et al., 2011) by enzymatic cleavage. Dh31 is synthesized in several neurons in the brain (mostly neurosecretory cells of the pars intercerebralis) and released into the hemolymph (Vanden Broeck, 2001; Vanderveken & O'Donnell, 2014). Like its human homolog, calcitonin, Dh31 neuropeptide uniquely stimulates fluid secretion from Malpighian tubules by binding its cognate GPCR, Dh31-R, positivity regulating cyclic AMP levels, and ultimately activating the apical membrane V-ATPase in principal cells in the primary secretory segment (Coast, Webster, Schegg, Tobe, & Schooley, 2001). Dh31 is also responsible for the positive regulation of circadian sleep/wake cycles by promoting wakefulness in the fly, as well as stimulating feeding and sexual attraction, together with sex peptide (Kunst et al., 2014). Lastly, Dh31 has been discovered to play a role in coordinating sperm transfer and copulation (Tayler, Pacheco, Hergarden, Murthy, & Anderson, 2012). My ScM research sought to explore the role the neuropeptide, diuretic hormone-31 (Dh31) plays in *Drosophila* oogenesis.

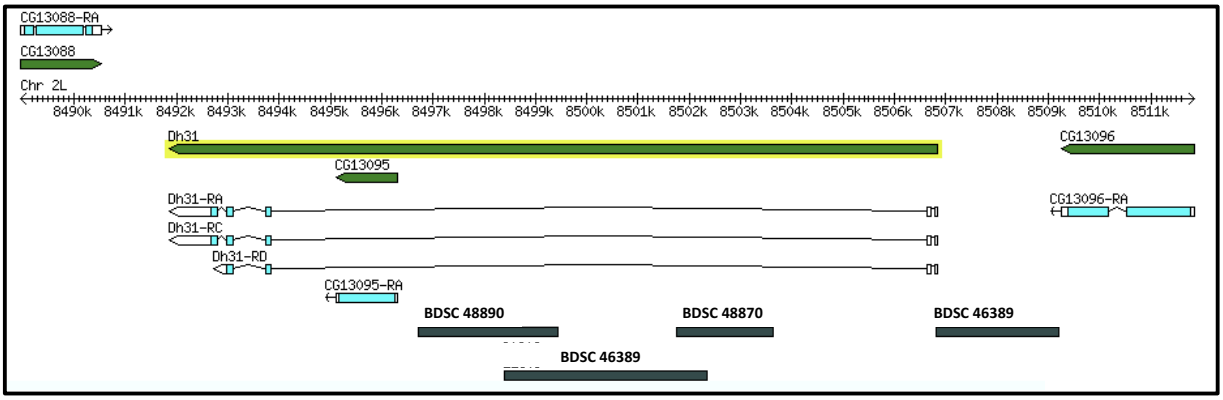
The Dh31 receptor (Dh31-R; CG32843), belongs to the Family B/Group I GPCRs as well as the calcitonin receptor family/calcitonin gene-related peptide (CGRP), and has an extracellular hormone receptor domain, with conserved sites in its gene. In accordance with its function, Dh31-R is expressed in principal cells of *Drosophila* Malpighian tubules, where it is important in controlling H_2O and NaCl homeostasis. Interestingly the DH31 receptor requires a co-expressed receptor component protein (RCP) for its proper activity, like mammalian calcitonin-like receptors (Johnson et al., 2005).

Although researchers know very little about the role of the neuropeptide Dh31 and its receptor Dh31-R in oogenesis (X. Wu, Tanwar, & Raftery, 2008), a previous screen in our lab by a Ph.D. student, Tianlu Ma, showed that ubiquitous somatic RNAi knockdown of Dh31 severely reduces the numbers of eggs laid per female per day compared to controls (Figure 1c).

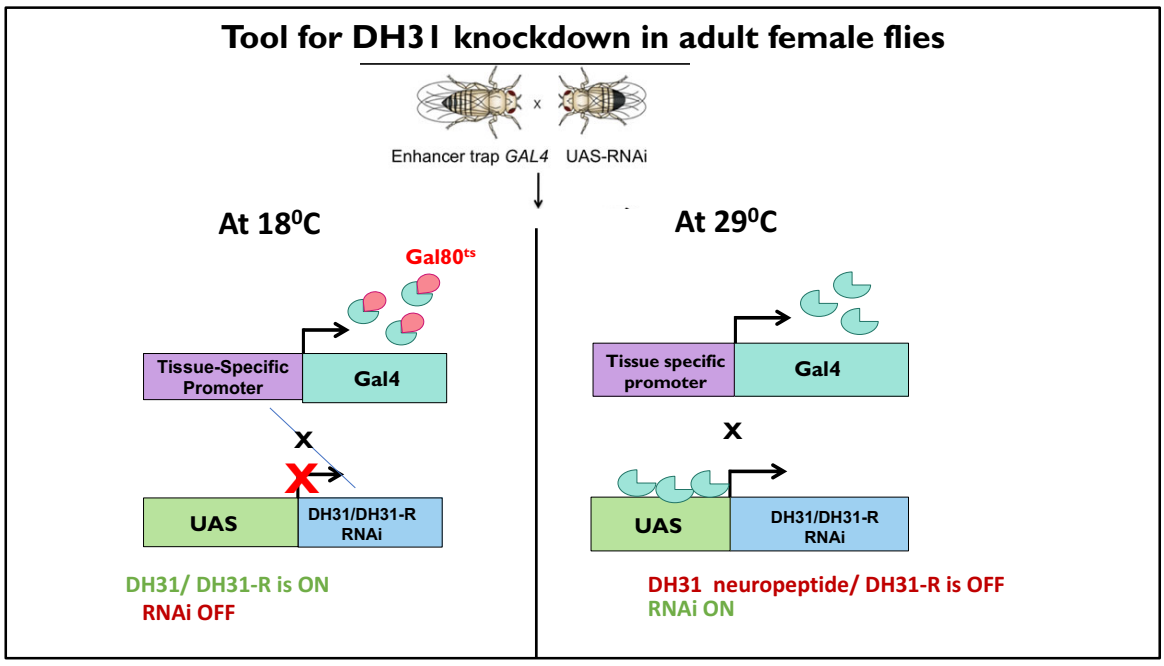
a.

```
         10          20          30          40          50
MTNRCACFAL AFLLFCLLAI SSIEAAPMPS QSNGGYGGAG YNELEEVPDD
         60          70          80          90         100
LLMELMTRFG RTIIRARNDL ENSKRTVDFG LARGYSGTQE AKHRMGLAAA
        110
NFAGGPGRRR RSETDV
```

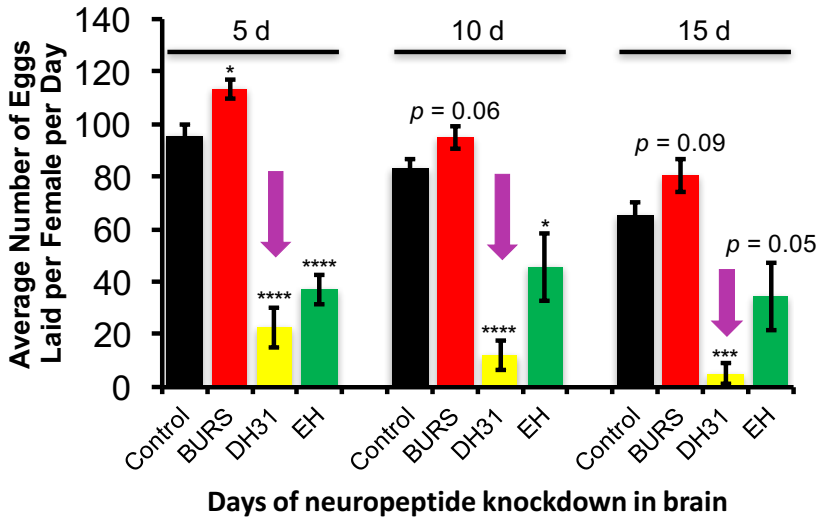
b.



c.



d.



e.

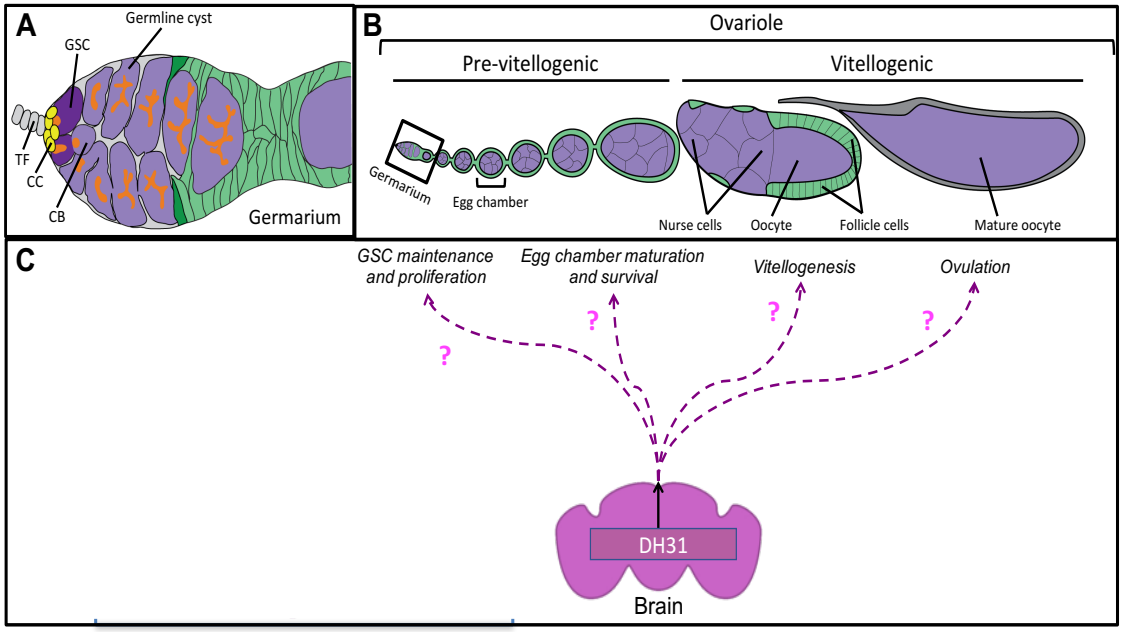


Figure 1| (a) Protein sequence of Dh31(Coast et al., 2001). (b) A gene map showing the different parts of Dh31 regulatory regions that were placed upstream of Gal4 to drive its expression in different subdomains of where Dh31 is endogenously expressed (Flybase). This was used to understand Dh31 expression patterns. (c) Genetic tools used to study the

role of Dh31 in *Drosophila* oogenesis (GAL4-UAS system). At 18^{o}C, GAL80^{ts} binds the activation domain of GAL4 and prevents it from recruiting other transcriptional activators to the gene of interest. However, when switched to 29^{o}C, Gal80^{ts} is inactivated, and the GAL4 transcription factor can now promote transcription and expression of the transgene to which the UAS sequence is attached (Reiher et al., 2011) (d) 36 neuropeptides were screened by Tianlu Ma. Pan-neuronal knockdown of BURS increased egg production relative to controls, while knockdown of EH and Dh31 neuropeptides decreased egg production. *p-value <0.05, **p-value < 0.01, ***p-value<0.001. (e) A schematic diagram showing research questions and hypotheses.

1.2 An Overview of The Drosophila Ovary as a Model System

Drosophila melanogaster (the fruitfly) has developed into the model organism of choice for many scientists over the past hundred years (Holtzman et al., 2010). With the entire *Drosophila* genome sequence published (Adams et al., 2000), its short life cycle, inexpensive rearing media, large repositories of transgenic lines, and online databases, elegant genetic experiments on the fruitfly are readily doable (Holtzman et al., 2010.).

Since the first detailed descriptions of *Drosophila* oogenesis in 1970, nearly half a century of dedicated research and live-culturing innovations have demonstrated the incredible potential of the *Drosophila* ovary as a developmental model (Peters & Berg, 2016). As a result, scientists who work with the *Drosophila melanogaster* ovary enjoy some of the most sophisticated tools developed for easy genetic manipulation (Holtzman et al., 2010). The adult female fruitfly has two artichoke-shaped ovaries, joined by a common oviduct, and each ovary is encased by a muscle sheath (Xie & Spradling, 2000). Each ovary is made up of 15 to 20 ovarioles, progressively bigger and more developed egg chambers linked together by stalks, like beads on a string (Nystul et al., 2007). At the anterior-most end of each ovariole (Figure 2), lies the germarium (Eliazer & Buszczak, 2011). In the female adult germarium, there are two populations of stem cells which give rise to all the other cells within the follicle (a 16-cell germline cyst surrounded by a monolayer of cells).

Stem cells are undifferentiated cells with extraordinary potential to both self-renew and give rise to differentiating daughter cells (de Cuevas and Matunis, 2011). The GSCs sit at the anterior-most region of the germarium, attached to the cap cells which form the niche, a specialized microenvironment in which stem cells reside (Morrison and

Spradling, 2008). The niche is crucial in helping stem cells maintain their "stemness" (i.e. their ability to both self-renew and form differentiated progeny). In the *Drosophila* ovary, two to three GSCs are present in the germarium, tethered to a niche composed of five to eight cap cells, along with terminal filament (TF) cells and a subset of escort cells (Nystul et al., 2007). When one GSC divides, one daughter cell remains as a GSC, and the differentiating daughter, called a cystoblast, undergoes four incomplete rounds of cell division to produce a sixteen-cell cyst connected by ring canals and cytoplasmic bridges called fusomes (Spradling, 1993). Fusomes arise from mitotic spindle 'residues' during the four cystocyte divisions, and form a branching network that extends through each intracellular bridge in the germline cysts as they develop in assembly-line fashion (Yue and Spradling, 1994). These fusomes become handy markers for monitoring the developmental stages (i.e. 2-cell cysts, 4-cell cysts, 8-cell cysts and 16-cell cysts) of these early germline cysts because the fusome becomes progressively branched the further the stage of the cyst. The fusome morphology of germ cells together with the stereotypical position of GSCs near cap cells makes it easy to identify GSCs and determine the developmental stages of GSC progeny in the *Drosophila* germarium (de Cuevas and Spradling, 1998).

A. **B.**

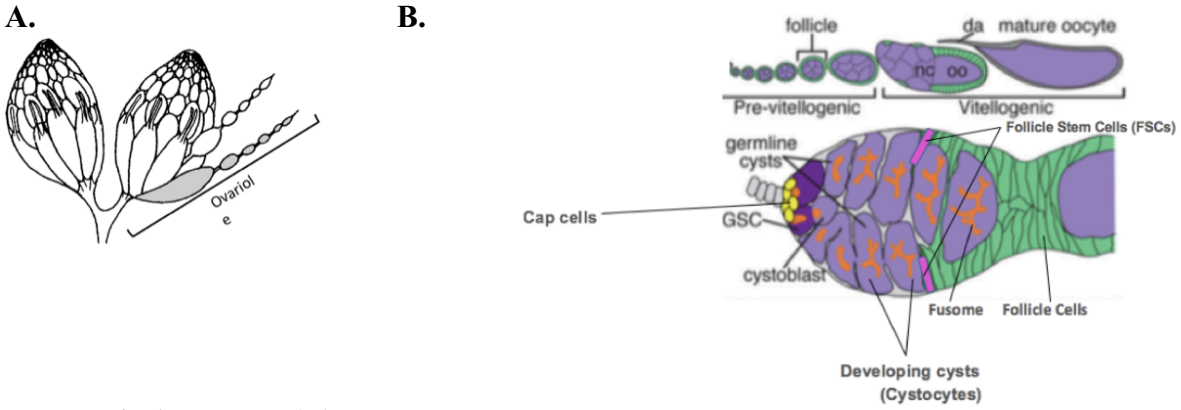


Figure 2| The *Drosophila* ovary,

as illustrated on the left (A), is made up of a 15 to 20 chains of progressively more mature egg chambers called ovarioles. B.) *(Top)* Diagram showing developing follicles in an ovariole. The size of the follicles increases as they mature. *(Bottom)* At the anterior end of the ovariole is the germarium. At apical tip of the germarium (gray) are the terminal filament cells (TFCs), which are anterior to the cap cells (CC; yellow). The GSCs (purple) adhere to the CCs. In each ovariole, there are two or three GSCs that will asymmetrically divide to yield a GSC and a cystoblast that forms a 16-cell cyst (Armstrong, Laws, & Drummond-Barbosa, 2014). Follicle Stem Cells (FSCs, pink) also reside in the germarium and produce follicle cells (in green). The stages of development of the cyst can be identified using a germline-specific structure called the fusome (orange). (*Picture modified from Armstrong et al., 2014*)

1.3 The Drosophila ovary as an excellent model system to study new roles of neuropeptides

Our lab has shown previously that several physiological and nutrient-sensing pathways influence GSC numbers and proliferation. For instance, the number of eggs laid by a female adult fruitfly can change 60-fold when fed a rich diet versus a poor diet (Drummond-Barbosa and Spradling, 2001). More specifically, a poor diet has been shown to cause a fourfold decrease in the rates of division of GSCs and follicle stem cells, a three-fold decrease in the rate of germ cell development in the ovariole, and renders follicle cells unable to enter vitellogenesis (Drummond-Barbosa and Spradling, 2001). Many of these processes are regulated by insulin signaling levels (Drummond-Barbosa and Spradling, 2001).

In the fruitfly brain, two clusters of neural secretory cells produce and release orthologs of insulin called *Drosophila* insulin-like peptides (dILPs) in response to nutrient-rich diets. Neural dILPs, in the form of dILP2 and dILP5, have been shown to directly control the growth and proliferation of *Drosophila* GSCs and their progeny (LaFever and Drummond-Barbosa, 2005). Specifically, ablating dILP2 and dILP5-secreting neurons in *Drosophila* significantly reduces GSC proliferation and also slows down follicle cell divisions, reflecting reduced rates of egg chamber growth on a rich diet. In addition, these processes also require the insulin receptor function in the germline itself (LaFever and Drummond-Barbosa, 2005).

Because of the significant effects of insulin on *Drosophila* oogenesis, our lab predicts that additional neuropeptides produced in the brain of the fruitfly play crucial roles in coordinating reproduction needs, with other environmental inputs. Our curiosity prompted this investigation of the role of neuropeptides such as Dh31 in fruitfly oogenesis.

MATERIALS AND METHODS:

2.1 Drosophila strains, stocks and culture conditions

Fly stocks were maintained on standard fly food (agar, molasses, cornmeal, yeast, water, Tegosept and propionic acid) made in the lab, with dry yeast, at room temperature or 25°C and 60% humidity Specific crosses were first maintained on standard fly food in plastic vials so that males and females could get acquainted for a couple of days, and then transferred to plastic conical bottles and maintained at 18^{o}C. Crosses were tossed every two days, and parents were discarded after one week of tossing. The date and exact genotype of each strain crossed were handwritten on the bottles to ensure clarity during experiments.

The following DH-31 Gal 4 driver lines were used to test the expression patterns in the different Dh31 neurons: **BDSC 51988** *(w[1118]; P{w[+mC]=Dh31-GAL4.TH}2M),* **BDSC 51989** (*w[1118]; P{w[+mC]=Dh31-GAL4.TH}5F),* **BDSC 48870** (*w[1118]; P{y[+t7.7] w[+mC]=GMR20A02-GAL4}attP2),* **BDSC 48890** (*w[1118]; P{y[+t7.7] w[+mC]=GMR20D02-GAL4}attP2),* **BDSC 48936** (*w[1118];P{y[+t7.7]w[+mC]=GME21C09-GAL4}attP2),* **BDSC 48947** (*w[1118]; P{y[t7.7] w[+mC]=GMR21E02-GAL4}attP2),* **BDSC 47885** (*w[1118]; P{y[t7.7] w[+mC]=GMR57F07-GAL4}attP2),* **BDSC 46389** (*w[1118]; P{y[t7.7] w[+mC]=GMR19F09-GAL4}attP2).*

The following stocks were used to test the efficacy of germline-specific drivers: *UAS-nuclearGFP* and *UAS-mCD8GFP* as reporters, crossed with *w[*]; PBac{w[+mW.hs]=GreenEye. nosGAL4}Dmel2,*

PBac{w[+mW.hs]=GreenEye.nosGAL4}Dmel2; tubPGal80ts, and **BDSC 3177** Maternal Triple Driver - GAL4 (MTD-Gal4: *P{w[+mC]=out GAL4::VP16.R}1, w[*]; P{w[+mC]=GAL4-nos.NGT}40; P{w[+mC]=GAL4:: VP16-nos.UTR}CG6325[MVD1])*.

The following fly stocks were used to generate Dh31-receptor *(CG32843)* short-hairpin RNAi knockdowns in the soma: *tubPGal80^{ts}/CyO; tubPGAL4/TM6b* crossed with **VDRC 8777GD** *(w[1118]; P{GD3782} V8777),* **VDRC 101995KK** *(P{KK108756}VIE-260B),* **BDSC 25925** *(y[1] v[1]; P{Y[+t7.7] v[+t1.8]=TRiP.JF01945}attP2/TM3, Sb[1]).*

The following stocks were used for experiments knocking down DH-31 neuropeptide in the brain of the fly, using Dh31 neuropeptide short-hairpin RNAi lines: pan-neuronal nSyb GAL4 with Gal80^{ts} (Mark Wu lab provided the *nSybGAL4* line, *tubpGal80^{ts}; nSybGAL4* was generated by Tianlu Ma (Drummond-Barbosa lab) through standard *Drosophila* crosses*)* crossed to: **VDRC 37763GD** *(w[1118]; P{GD4601} v37763),* **VDRC 50296GD** *(w[1118]; P{GD16889} v50296/TM3),* **BDSC 41957** (*y[1]sc[*]v[1];P{y[+t7.7]v[+t1.8]=TRiP. HMS02354}attP2/TM3, Sb[1]),* **VDRC 50295GD (***w[1118]; P{GD16889} v50295),* **VDRC 37764GD** *(w[1118]; P{GD4601} v37764),* **VDRC 43529GD** (*w[1118]; P{GD4601} v43529/TM3).*

Maternal Triple Driver-GAL4 (*MTD-GAL4*) *(*BDSC 31777) crossed to Dh31-receptor *(CG32843)* RNAi lines: **VDRC8777GD** *(w[1118]; P{GD3782 }V8777),* **VDRC 101995KK** *(P{KK108756}VIE-260B), and* **BDSC 25925** *(y[1] v[1];P{Y[+t7.7]v[+t1.8]=TRiP.JF01945}attP2/TM3, Sb[1])*

These stocks were obtained from the Bloomington Stock Center (BDSC), the Vienna *Drosophila* Resource Center (VDRC), or labs indicated. Information about alleles and transgenes used in this study can be found on FlyBase.

2.2 RNAi knockdown experiments

Because female flies can store sperm from previous mating, virgin females were collected for all experimental crosses. Virgins were identified by their pale pigmentation and a dark spot on their translucent abdomens called the meconium (Greenspan, 2004). At 25^{o}C, female flies will not mate within the first 8 hours of emergence as adults. Bottles were cleaned out the night before virgin collection, to maximize the number of virgins collected and efficiency of recognizing virgins.

For the RNAi knockdown experiments, males were collected from the multiple RNAi lines used in each experiment, while female virgins were collected from specific driver lines (Tubulin, Maternal Triple Driver or pan-neuronal nSyb driver; see Figure 1b). Virgin females were collected from reporter genotypes (e.g.: UASp-lacZ or UAS-nuc-GFP) and crossed with either the nSyb-GAL4 or the MTD-GAL4 male driver, to test expression patterns of reporter genes in the brain and ovary respectively.

For each cross, 20 female virgins were gathered per vial and crossed with 20 males of desired genotypes at room temperature. Crosses were incubated for two days in vials at 18^{o}C for mating to occur and then transferred to bottles of standard fly food and dry yeast. Crosses were raised and maintained at 18^{o}C.

For standard egg-laying assays, five bottles with five females and five males per bottle were set up per RNAi cross and placed at 29^{o}C. Food consisted of a yeasted molasses-agar plate and was changed twice every 24 hours to ensure constant availability of fresh food. The number of eggs deposited within 24 hours

on days 5, 10 and 15 were counted and compared to control RNAi lines using two-tailed Student's *t*-test.

2.3 Dissections and immunostaining.

For dissections, 0 to 3 day-old flies raised at 18°C were transferred to new vials with *y w* male flies and fed a standard cornmeal, molasses and agar diet, supplemented with wet yeast. These vials were switched to the 29°C and changed every day with fresh food and wet yeast. Ovaries were dissected after 0, 7, and 14 days at 29°C. Three biological replicates were done for each time point of dissection (N=1, 2, 3), and samples were processed through immunostaining for confocal and fluorescence microscopy.

Standard ovary dissections and immunostainings were performed following the Drummond-Barbosa Lab protocol (Luo, Chai, & Cai, 2013). Ovaries were dissected in Grace's medium (BioWhittaker). Ovarioles were teased apart starting from the anterior end using a 22.5 Tungsten needle (Becton Dickinson, 115134), but leaving the posterior end intact for easier processing. The ovaries were fixed for 13 minutes at room temperature in 5.3% formaldehyde (Ted Pella) in Grace's medium, and then washed a minimum of 3 times for 15 minutes per wash, in phosphate-buffered saline (PBS, pH 7.0) with 0.1% Triton X-100 (Sigma), and then blocked in 5% bovine serum albumin (BSA; Sigma Aldrich), 5% normal goat serum (NGS; New England BioLabs), and 0.1% Triton X-100 (Sigma) in PBS for 3 hours at room temperature or overnight at 4°C.

Samples were incubated in the following primary antibodies in blocking solution overnight at 4°C: mouse anti-alpha spectrin (3A9) (DSHB; 1:25), mouse anti-lamC (LC28.26) (DSHB; 1:25), rabbit anti-GFP (Torrey Pines, 1:2500), rat anti-Vasa (DSHB 1:20), Following three more washes in PBS with Triton X-100, samples were incubated in species-specific goat secondary antibodies, conjugated with AlexaFluor 488 (green fluorophore) or 568 (red fluorophore), at 1:200 dilution in blocking solution, for 2 hours at room temperature. Samples were washed three times after secondary antibody incubation. Ovaries were mounted onto imaging slides in Vectashield with DAPI (VectaLabs) to visualize nuclei.

For ovary dissections with ethynyl uridine (EdU), ovaries were dissected in Grace's medium at room temperature and incubated in 100 μM EdU (Invitrogen) in Grace's medium for one hour. EdU is a thymidine analog used to label and identify cells undergoing S-phase in vitro (Madhavan, 2007). After fixing the ovaries with formaldehyde and washing and staining as above, EdU is then labeled with AlexaFluor-594 via ClickIt chemistry according to manufacturer's instructions (Invitrogen).

Brain dissections and immunohistochemistry were performed as previously described (J. S. Wu & Luo, 2006), only that, fixation with 5.3% formaldehyde was for 20 minutes, and brain dissections were performed using sharper forceps and samples placed in 0.65mL Eppendorf tubes instead of the 1.5mL Eppendorf tubes.

The gut and Malpighian tubules were also dissected as described above and immunohistochemistry performed using a similar protocol, except that they were fixed with 5.3% formaldehyde for 1 hour. Carcasses were also dissected as

described above in Grace's Medium. They were fixed in 5.3% formaldehyde for 20 minutes, and immunohistochemistry performed using the same protocol.

All organ samples were imaged on a Zeiss microscope after mounting.

2.3 *Imaging and* Confocal *Microscopy*

All images were collected with a Zeiss LSM700 confocal microscope. Pictures of germaria were obtained using the 40x and 63x/1.5 zoom lens, with oil, whereas images for brains were taken at 10x/1.0 zoom and pictures of ovarioles, carcasses, Malpighian tubules and the gut were collected at 20x and 40x (with oil). Images were analyzed using ImageJ software.

2.4 *Statistical Analysis*

Statistical data were analyzed using Microsoft Excel 2016. The total number of cysts was normalized to the number of germline stem cells (0, 1, 2, 3 or 4). Statistically significant differences compared to control samples were determined using two-tailed Student's *t*-test. For egg-laying assays, the number of eggs laid per female per day was counted and compared to control flies. Statistical significance was determined using two-tailed Student's *t*-test. Rate of GSC and CC loss over time was compared to control using two-way ANOVA with interaction to establish statistical significance (Ables & Drummond-Barbosa, 2013; Armstrong et al., 2014; Hsu, LaFever, & Drummond-Barbosa, 2008; LaFever & Drummond-Barbosa, 2005).

2.5 Data Availability

Laboratory notebooks including GSC, CC and egg counts are available to thesis committee or reviewers, upon request. Microsoft Excel Worksheets with

calculations and graphs from raw data can be offered upon request. Stocks and reagents are available in the lab, upon request.

RESULTS

3.1 Characterization of Dh31 expression pattern in 5 to7 day-old adult female CNS.

There are many neurons in the brain that produce the Dh31 neuropeptide (Asahina and Anderson, 2013). We care about the expression pattern of Dh31 because we want to know where the peptide that regulates oogenesis originates from, whether that be from different tissues such as the Malpighian tubules or from specific neurons in the brain.

To date, six Dh31 GAL4 drivers have been developed and their expression patterns characterized in vitro (Asahina and Anderson, 2013). We examined the expression patterns of these six GAL4 drivers in our hands as seen in the brain, the gut, the carcass, and the Malpighian tubules of adult females (Figure 3a, b, c, and d), compared to the standard Janelia Farm Laboratory Research expression patterns available online. Expression patterns below are expressed in the anatomical term.

BDSC 46389 (*w[1118]; P{y[+t7.7] w[+mC] =GMR57F07-GAL4}attP2*) is made from 2,396 amino acid residues, found on coordinates 2L: 8506808-8509204, and expresses GAL4 under the control of DNA sequences in or near Dh31. This construct was inserted into the attP2 site at 68A4 on 3L, by site-specific recombination. It is expressed in the ventral nerve cord inthe brain (Janelia Farms Research Laboratory). In my hands, the BDSC 46389 Gal4 driver was strongly expressed in the ventral nerve cord that runs through the center of the brain as seen in Figure 3a. My findings correspond with standard Janelia Farm maximum projections shown.

BDSC 48947 (*w[1118]; P{y[t7.7] w[+mC]=GMR21E02-GAL4}attP2)* is made from 4,124 amino acid residues, found on coordinates 2L: 8502709-8506833 and expresses

GAL4 under the control of DNA sequences in or near Dh31. It is expressed mainly in the lobula and peripheral medulla (Janelia Farms Research Laboratory). In my hands, the BDSC 48947 Gal4 driver was strongly expressed in the most peripheral section of the medulla, as seen in Figure 3a (Virtual Flybrain). My findings correspond with standard Janelia Farm maximum projections shown.

BDSC 48870 (*w[1118]; P{y[+t7.7] w[+mC]=GMR20A02-GAL4}attP2),* is made from 1,866 amino acid residues and expresses GAL4 under the control of DNA sequences in or near Dh31. It is expressed mainly in the mushroom body, bulb, ellipsoid body, and, fan-shaped body (Janelia Farms Research Laboratory). In my hands, the BDSC 48870 Gal4 driver was strongly expressed in the ellipsoid and fan-shaped body (Virtual Flybrain), as seen in the center of the brain in Figure 3b. My findings correspond with standard Janelia Farm maximum projections shown also in Figure 3b.

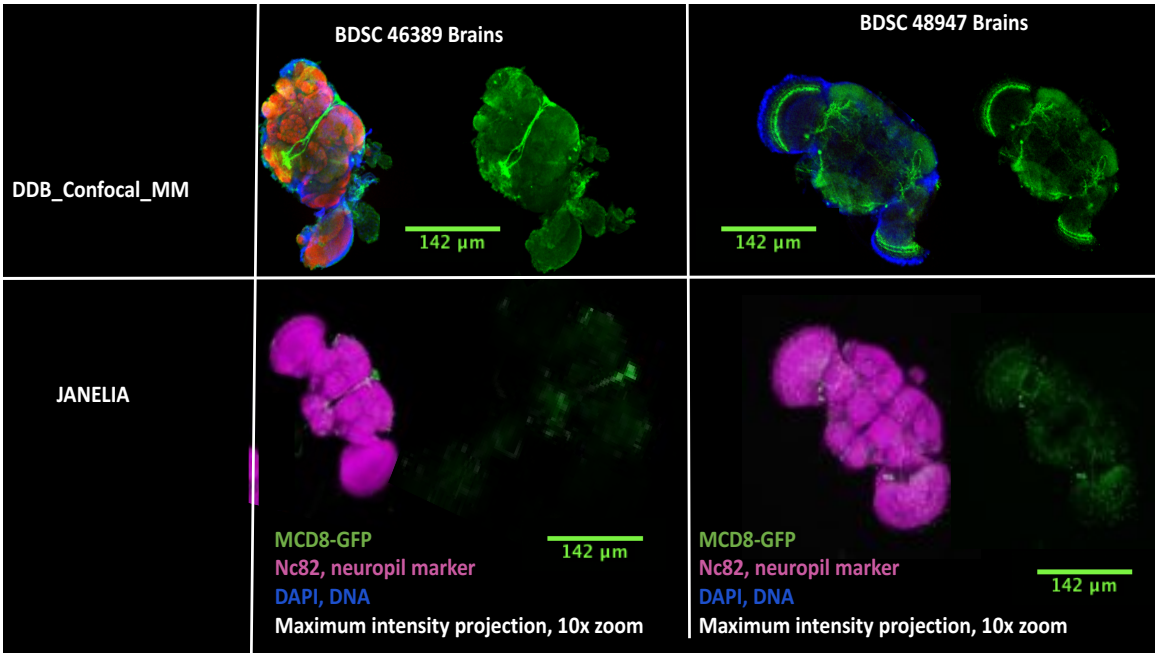
BDSC 48890 (*w[1118]; P{y[+t7.7] w[+mC]=GMR20D02-GAL4}attP2),* is made from 2,704 amino acid residues and expresses GAL4 under the control of DNA sequences in or near Dh31. It is expressed mainly in the protocerebral bridge, and superior posterior slope (Janelia Farms Research Laboratory). In my hands, the BDSC 48890 Gal4 driver was strongly expressed in the protocerebral bridge which is located along the posterior slope, as seen in Figure 3b. My findings correspond with standard Janelia Farm maximum projections shown also in Figure 3b.

BDSC 51988 (w[1118]; P{w[+mC]=Dh31-GAL4.TH}2M), and BDSC 51989 (w[1118]; P{w[+mC]=Dh31-GAL4.TH}5F), express GAL4 under the control of Dh31 regulatory sequences These two larger constructs were created by Kenta Asahina & David Anderson, California Institute of Technology, as a transgenic fusion to different parts of

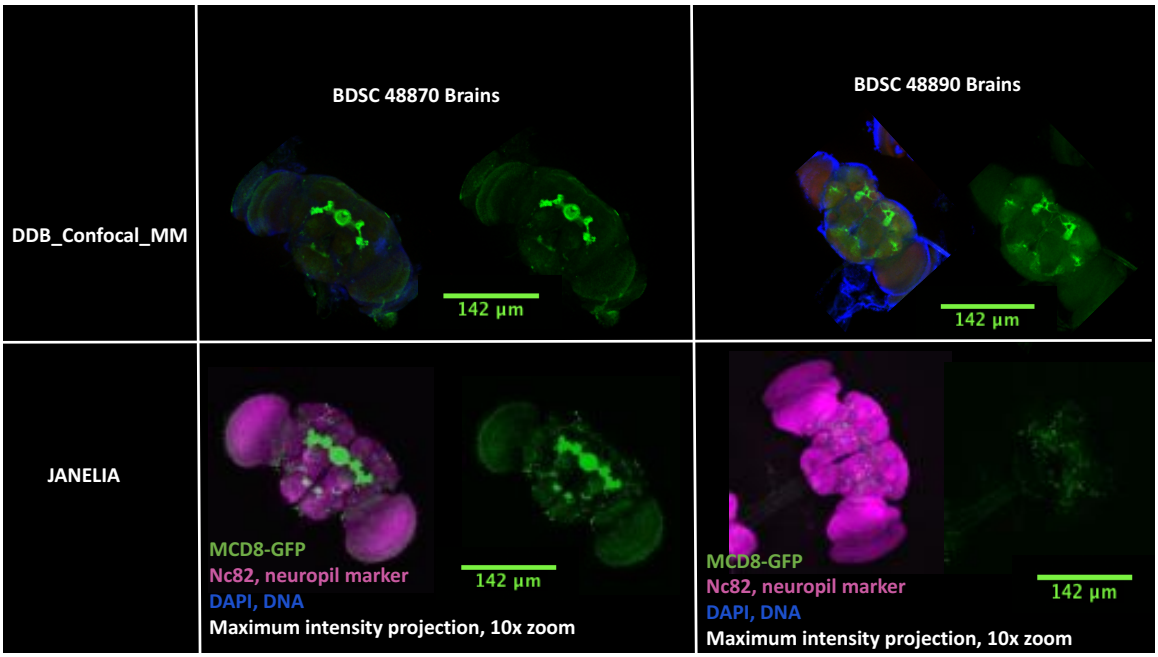
the Dh31 regulatory region. Both insertions were backcrossed for multiple generations into a Canton-S background into which w^{1118} had been introgressed (Asahina and Anderson, 2013).

Each Dh31 Gal4 driver was also tested for expression in the gut, ovary and Malpighian tubules. There were no significant differences in the expression patterns of the different Dh31 Gal4 drivers in these tissues. In general, the ovaries showed no Dh31 expression, the Malpighian tubules and guts showed Dh31 expression that seemed concentrated to one segment of the organ, and the carcass showed no Dh31 expression. In my hands, BDSC 48870 showed the most defined expression pattern multiple times in the ellipsoid body and fan-shaped body of the brain. Therefore, in my hands, I recommend the BDSC 48870 be used first for future experiments to understand how a specific Dh31 neuron affects oogenesis, since it is expressed in a very defined and easily observable region in the center of the brain (the ellipsoid body).

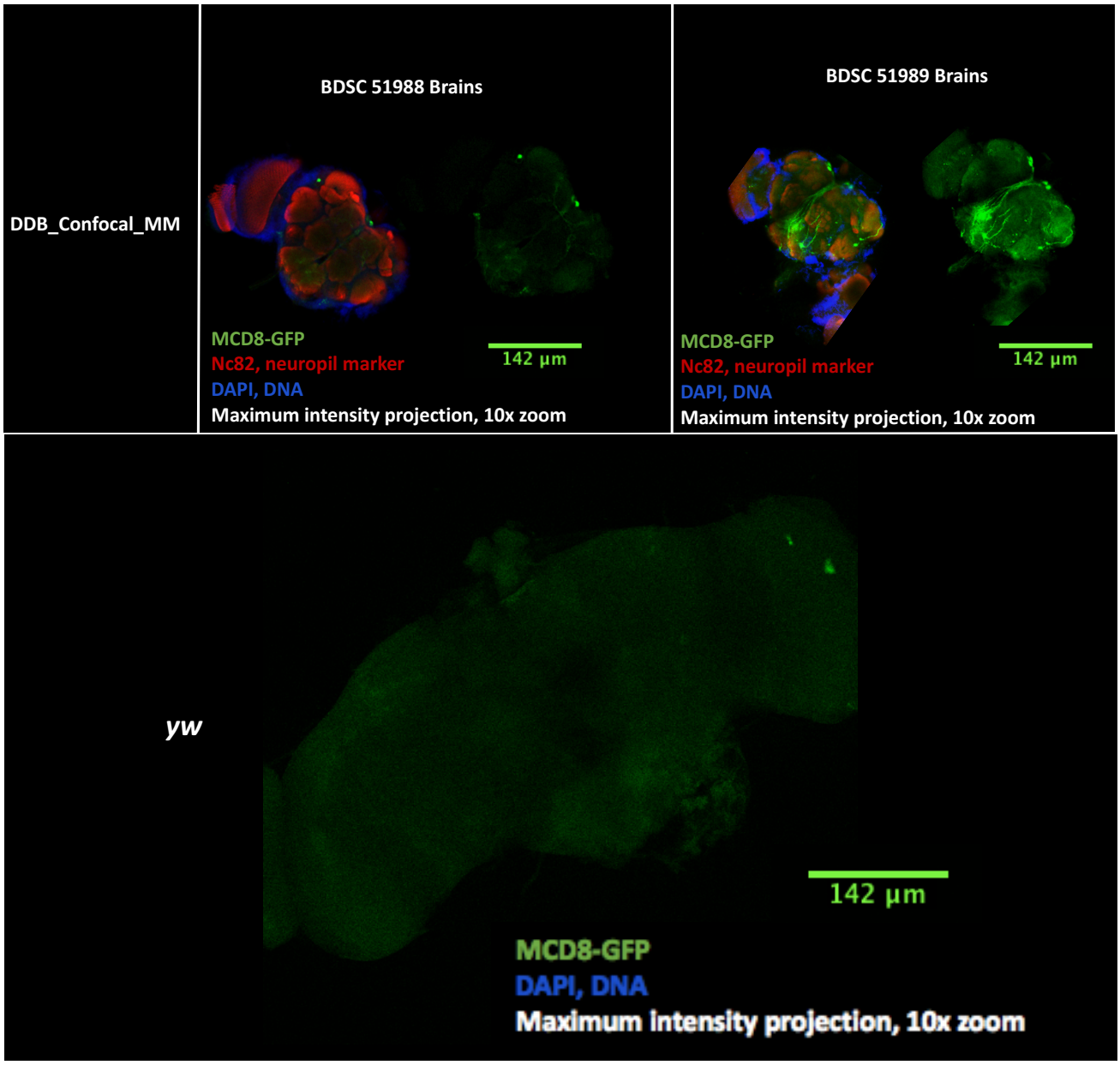
a.


DDB_Confocal_MM
BDSC 46389 Brains
142 µm
BDSC 48947 Brains
142 µm
JANELIA
MCD8-GFP
Nc82, neuropil marker
DAPI, DNA
Maximum intensity projection, 10x zoom
142 µm
MCD8-GFP
Nc82, neuropil marker
DAPI, DNA
Maximum intensity projection, 10x zoom
142 µm

b.


DDB_Confocal_MM
BDSC 48870 Brains
142 µm
BDSC 48890 Brains
142 µm
JANELIA
MCD8-GFP
Nc82, neuropil marker
DAPI, DNA
Maximum intensity projection, 10x zoom
142 µm
MCD8-GFP
Nc82, neuropil marker
DAPI, DNA
Maximum intensity projection, 10x zoom
142 µm

c.


DDB_Confocal_MM
BDSC 51988 Brains
MCD8-GFP
Nc82, neuropil marker
DAPI, DNA
Maximum intensity projection, 10x zoom
142 µm
BDSC 51989 Brains
MCD8-GFP
Nc82, neuropil marker
DAPI, DNA
Maximum intensity projection, 10x zoom
142 µm
yw
142 µm
MCD8-GFP
DAPI, DNA
Maximum intensity projection, 10x zoom

d.

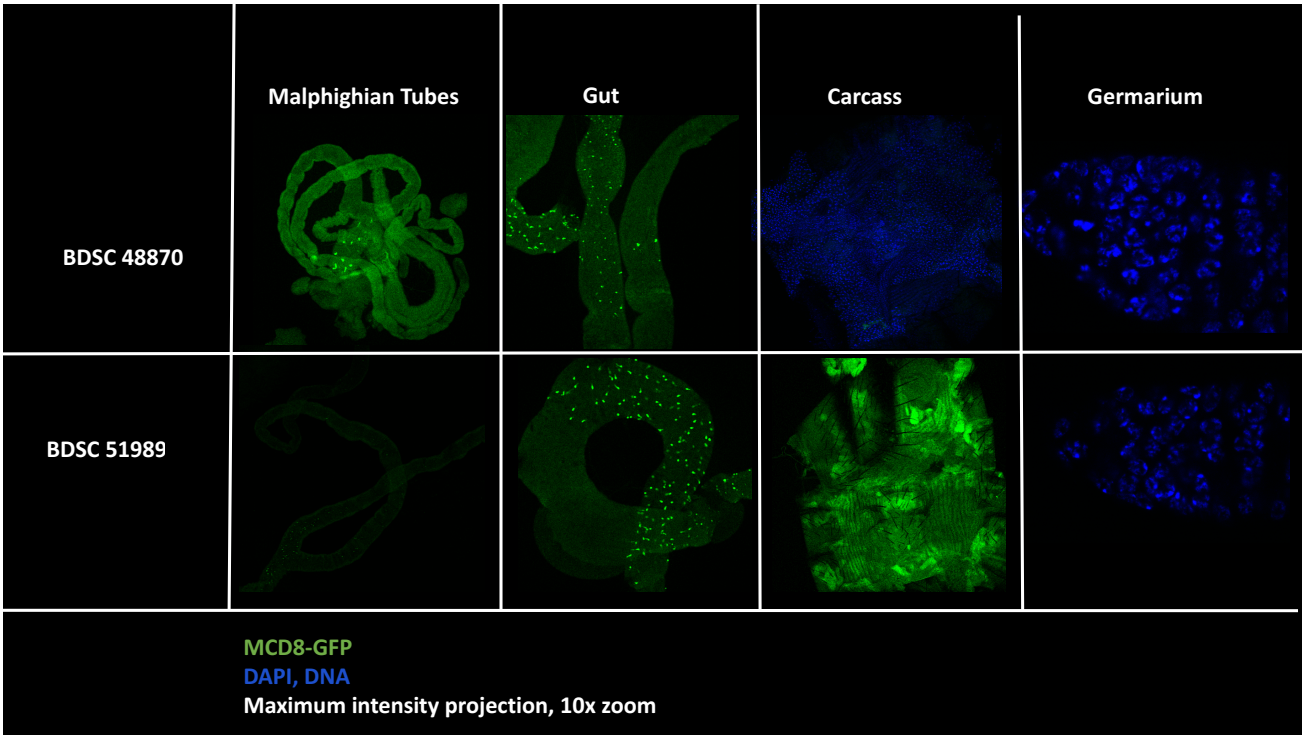


Figure 3| *(a, b, c).* Differential expression of Dh31 GAL4 transgenes in neurons of the adult female brain. Brains of *Dh31-GAL4; UAS-MCD8 GFP* female stained with the anti-GFP antibody (green) and the neuropil marker, nc82. Images are displayed as maximum intensity images. These maximum intensity confocal images were compared to maximum intensity images from the Janelia Farms Research Database for *Drosophila*. BDSC 48870 shows high concentrations in the mushroom body, whereas BDSC 51988 and 51989 as fused segments of the Dh31 gene show less defined localized regions. *y w* flies were used as negative controls and showed no staining. (d) Differential expression of Dh31 GAL4 strains in the Malpighian tubules, gut, carcass, and germarium of adult female flies. Organs of *Dh31-GAL4; UAS-MCD8 GFP* female were stained with anti-GFP antibody (*green*) and DAPI (*blue; DNA*). Due to time constraints, not all driver lines were screened for expression in different tissues.

3.2 Somatic knockdown of Dh31-receptor (Dh31-R) decreases egg-laying

To begin assessing whether Dh31 plays a role in oogenesis, the tubP-GAL4 driver with Gal80^{ts} was used to drive RNAi-mediated knockdown of Dh31 receptors in the soma of adult female flies. Ubiquitous somatic knockdown of Dh31 receptors leads to a significant decrease in the number of eggs laid as seen in Figure 4a, with lines from the Vienna *Drosophila* Resource Center (VDRC 101955KK and VDRC 8777GD) RNAi crosses compared to controls (Luciferase RNAi) showing a greater decrease in the number of eggs laid after 5, 10, and 15 days of RNAi knockdown, compared to the RNAi crosses with Bloomington *Drosophila* Resource Center lines (BDSC 25925), compared to controls. Our observations show a recurring trend: VDRC 8777GD repeatedly has the strongest effect, whereas BDSC 25925 has a weaker effect after 15 days of RNAi knockdown. This likely points to differences in RNAi knockdown efficiency among the RNAi lines used. Primers are currently being designed to test the efficiency of Dh31-R and Dh31 knockdowns through RT-PCR to address this possibility. Additionally, there is an apparent age-dependent decrease in the number of eggs laid over the 15-day period, as observed with shorter bars at Day 15 (Figure 4a). This age-dependent decrease though known to occur naturally in WT flies, is accentuated when DH31-R is knocked down in the soma of the flies.

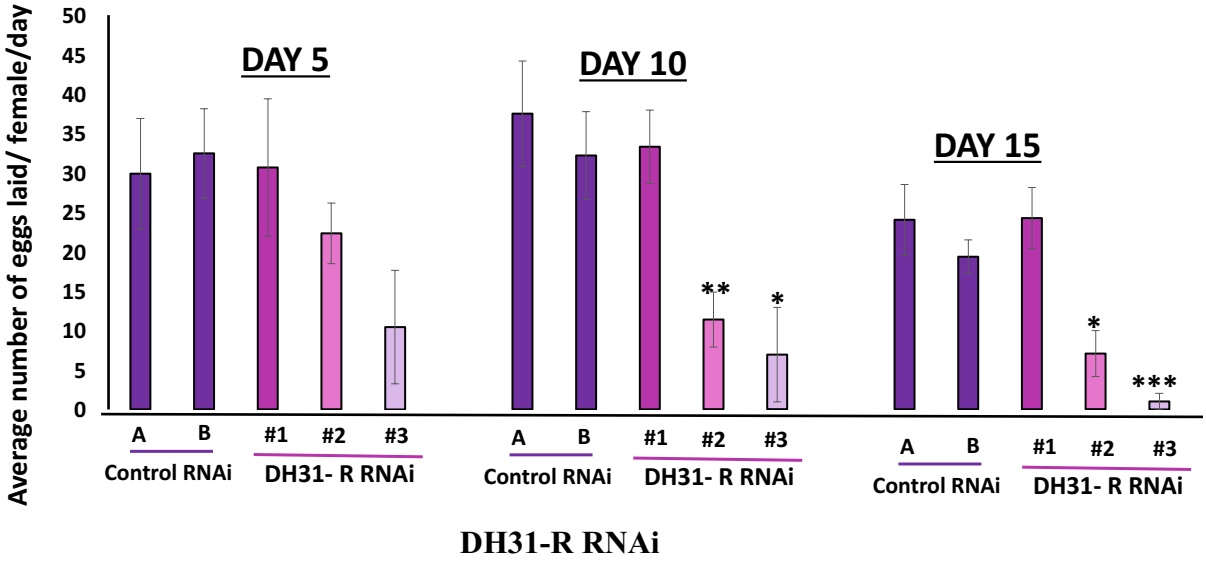


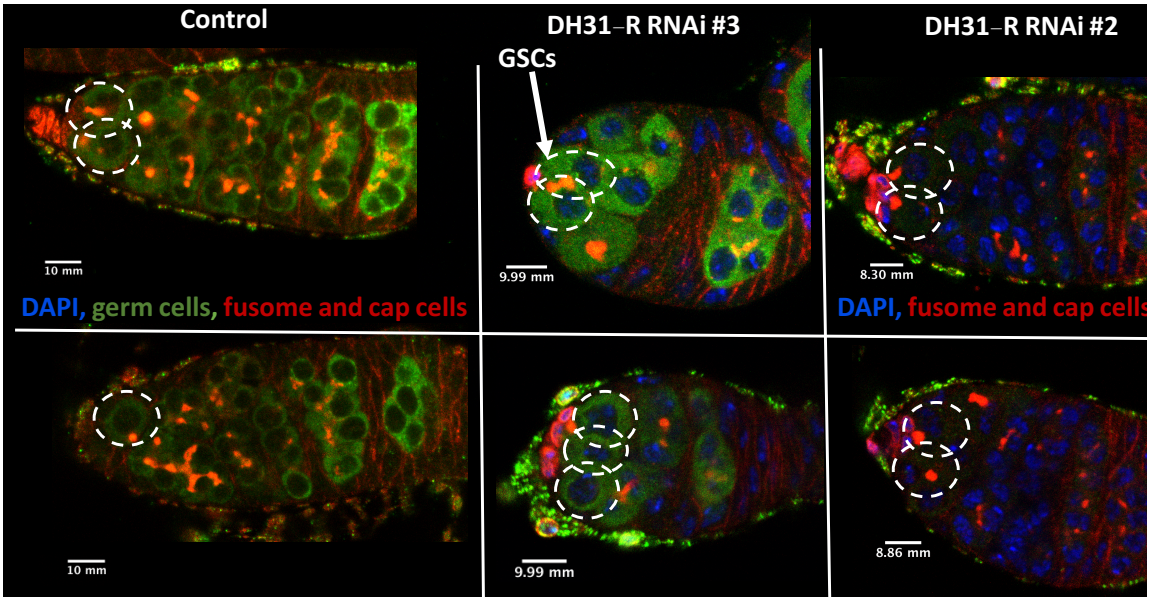
Figure 4| Knockdown of Dh31-R in somatic cells decreased egg production compared to controls. *p<0.01, **p<0.01, ***p<0.001, two-tailed Student's *t*-test.

3.3 Ubiquitous somatic knockdown of Dh31-R does not affect the number of germline stem cells (GSCs) or niche size (number of cap cells, CCs)

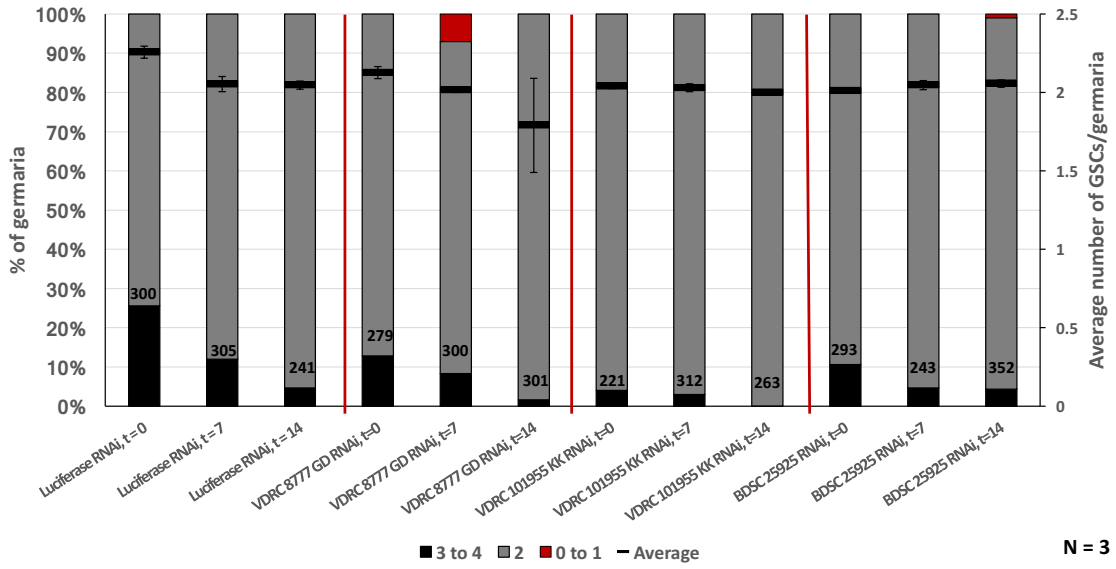
Given that the egg-laying assay was used as a rough read-out of ovarian function, we sought to determine the specific steps of oogenesis affected by Dh31 signaling. To this end, the ovaries of adult female flies with Dh31-R knocked down by the different RNAis, were dissected, immunostained, and mounted on slides for microscopy. Using fusome morphology to identify germline stem cells and the lamin-C antibody to label cap cells (CCs), the GSCs and CCs in each germaria (Figure 5) were counted. Using a two-way ANOVA with interaction test, there was no statistically significant difference in the number of GSCs and CCs between the control germaria (*Luciferase RNAi*) compared to the germaria of Dh31-R RNAi knockdown flies. Our results suggest that the decreased number

of eggs laid after knockdown of Dh31-R using RNAi lines is not because of loss of germline stem cells or a reduction in niche size. Dh31 likely affects oogenesis at later stages.

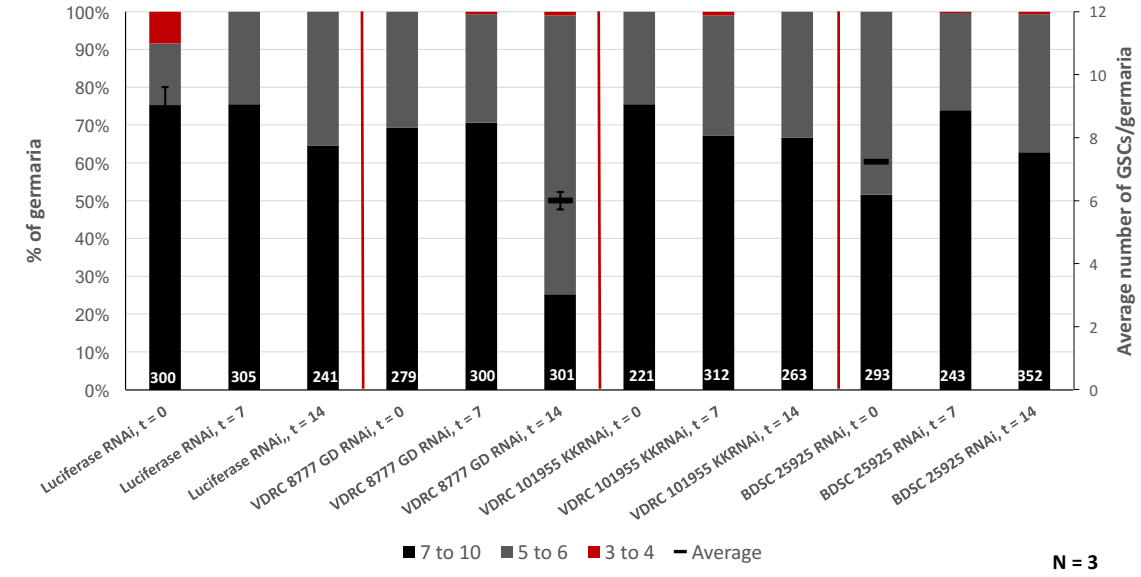
a.


Control
DH31–R RNAi #3
DH31–R RNAi #2
GSCs
10 mm
9.99 mm
8.30 mm
DAPI, germ cells, fusome and cap cells
DAPI, fusome and cap cells
10 mm
9.99 mm
8.86 mm

b.


% of germaria
Average number of GSCs/germaria
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2.5
2
1.5
1
0.5
0
300
305
241
279
300
301
221
312
263
293
243
352
Luciferase RNAi, t=0
Luciferase RNAi, t=7
Luciferase RNAi, t=14
VDRC 8777 GD RNAi, t=0
VDRC 8777 GD RNAi, t=7
VDRC 8777 GD RNAi, t=14
VDRC 101955 KK RNAi, t=0
VDRC 101955 KK RNAi, t=7
VDRC 101955 KK RNAi, t=14
BDSC 25925 RNAi, t=0
BDSC 25925 RNAi, t=7
BDSC 25925 RNAi, t=14
3 to 4
2
0 to 1
Average
N = 3

c.



d.

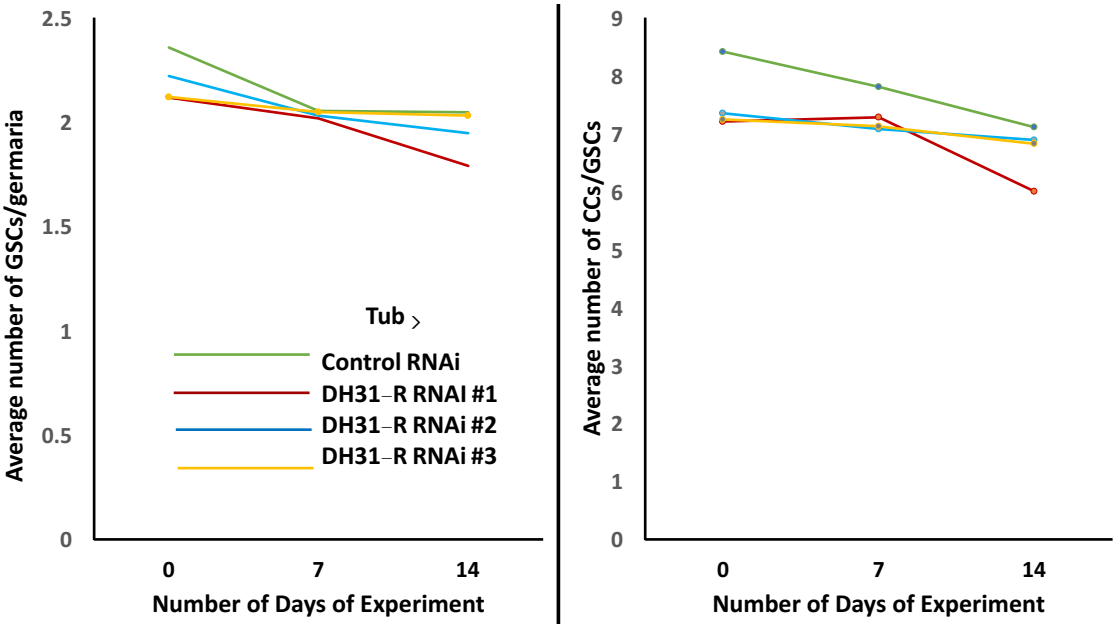


Figure 5| a. Somatic knockdown of Dh31-R did not affect GSC or CC number. Confocal images acquired after 14 days of RNAi knockdown. Images show similar number of GSCs per germaria between controls and RNAi knockdowns (2-3), and an apparent loss of GSC progeny (cysts) (b. - d.) Quantified numbers of GSCs and CCs per germaria, comparing controls and Dh31-R RNAi knockdowns. Somatic knockdown of Dh31-R did not cause a

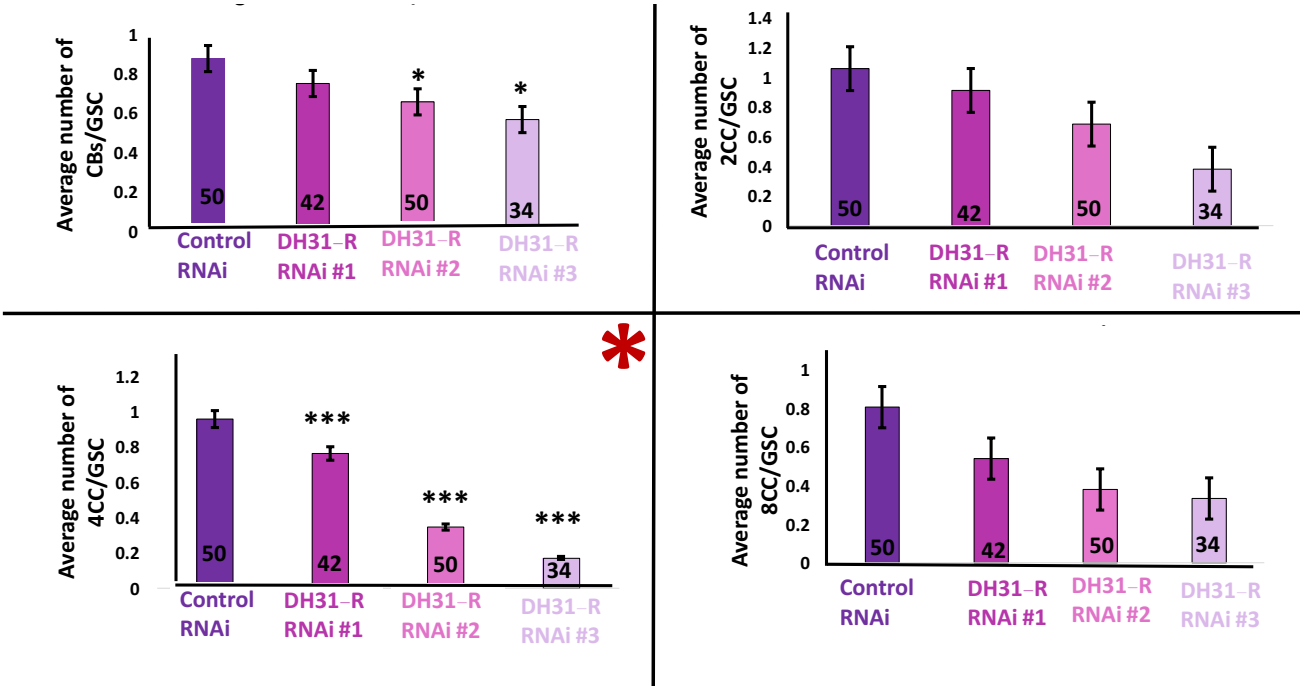
statistically significant change in the rate of GSC or CC loss, as determined through two-way ANOVA with interaction.

3.4 Somatic knockdown of Dh31-R causes a decrease in the number of germline cysts

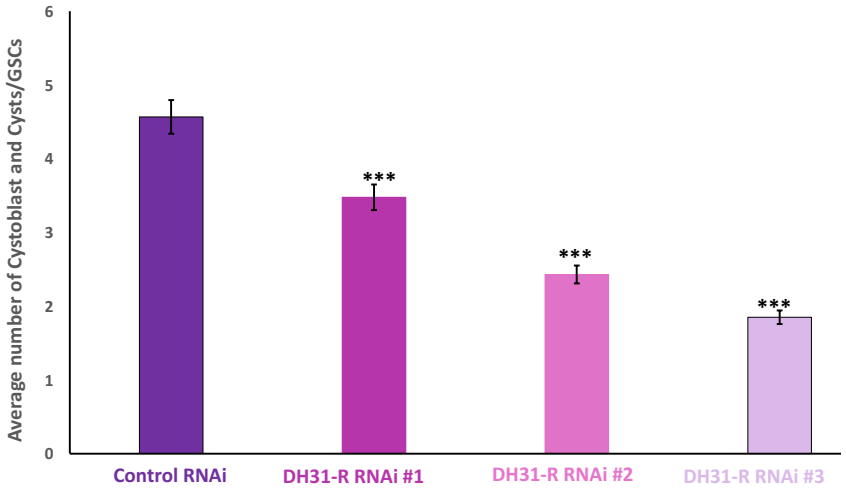
While counting GSCs and CCs in the germarium, I noticed that the Dh31-R knockdown germaria tended to be shorter, with less germ cysts, compared to the control germaria. Therefore, to determine whether systemic Dh31 signaling affects the early differentiating GSC progeny, early germline cysts at various stages of development in the germaria were counted using their specific fusome morphology (stained with *alpha-spectrin*), as described by de Cuevas & Matunis, 2011. The number of cysts per germarium were counted after 14 days of Dh31-R knockdown and compared to control. The different RNAi lines used are as follows: Control (Luciferase RNAi), RNAi#1 (BDSC 25925), RNAi#2 (VDRC 101955KK) and RNAi#3 (VDRC 8777GD). We observed a significant decrease in the numbers of 4-cell cysts and 16-cell cysts in RNAi knockdowns compared to controls (Figure 6a). These results indicate that the decrease in the number of eggs laid in the different Dh31-R RNAi knockdown flies are at least partially due to the loss of GSC progeny that further develop into oocytes and nurse cells. Different reasons could account for the reduced number of early germline cysts. It is possible that cysts are dying at a higher rate because of the lack of Dh31. This possibility can be investigated in future experiments, using cleaved caspase as a marker to determine if more cysts are dying in RNAi knockdowns compared to controls. Alternatively, the reduced number of cysts might be due to reduced proliferation of GSCs. To directly test this hypothesis, ovaries were

incubated *ex vivo* in EdU, a thymidine analog that labels cells in S-phase. By counting the number of EdU-positive GSCs in Dh31-R RNAi knockdown ovaries compared to control ovaries, we found no significant difference in the number of EdU-positive cells in controls compared to knockdowns (Figure 6b). It is therefore likely that cell death accounts for the differences in GSC progeny numbers.

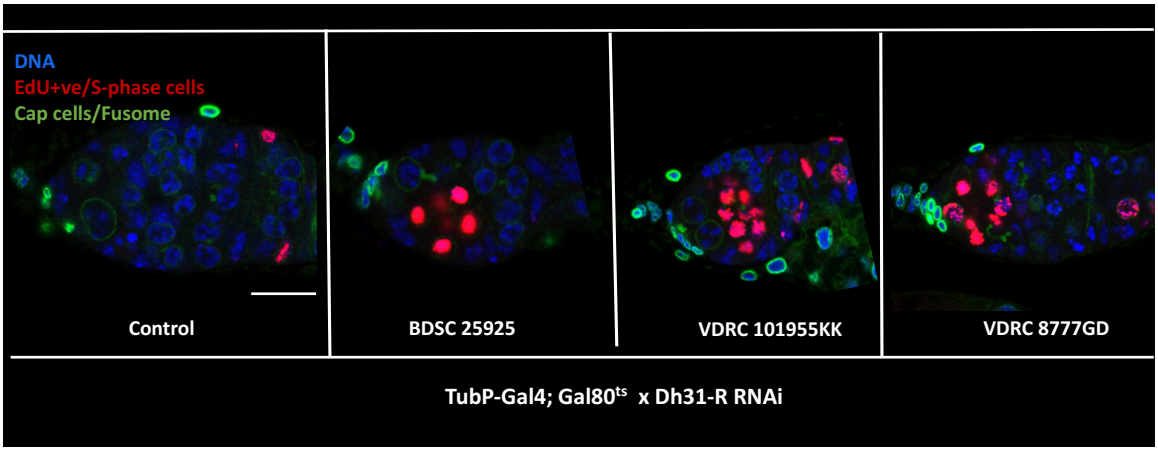
a.



b.


Average number of Cystoblast and Cysts/GSCs
0
1
2
3
4
5
6
***
***
***
Control RNAi
DH31-R RNAi #1
DH31-R RNAi #2
DH31-R RNAi #3

c.


DNA
EdU+ve/S-phase cells
Cap cells/Fusome
Control
BDSC 25925
VDRC 101955KK
VDRC 8777GD
TubP-Gal4; Gal80ts x Dh31-R RNAi

d.

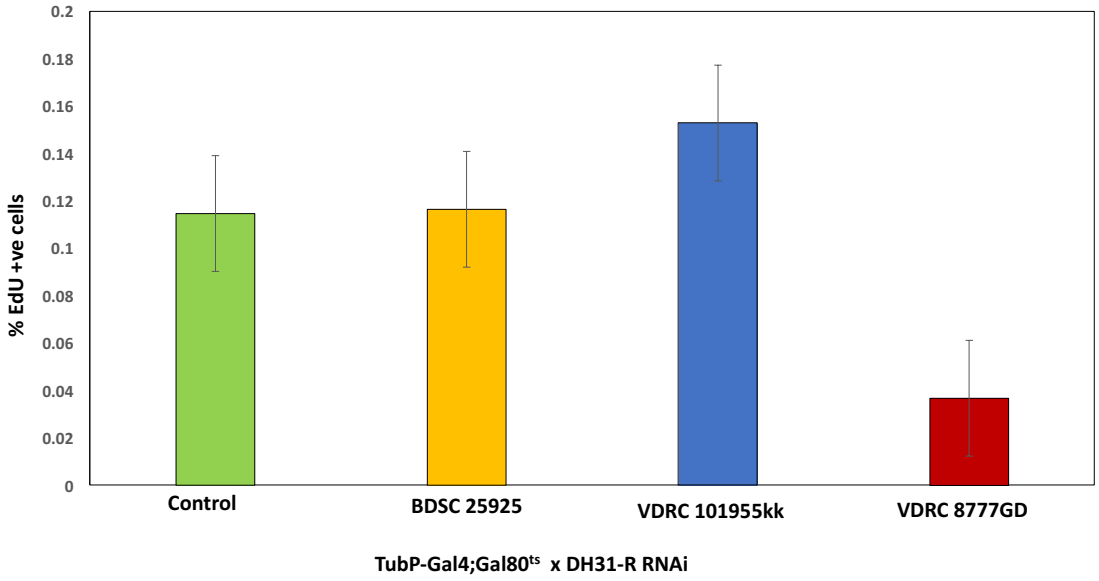


Figure 6| a. There is an overall decrease in the number of early germline cysts after 14 days of Dh31-R RNAi knockdown in the soma. *p<0.05, **p<0.01, ***p<0.001, two-tailed Student's *t*-test. The most significant decreases are observed at the 4-cell cyst stage and b. 16-cell cyst stage. c. Confocal images showing normal EdU proliferation in *Dh31-R* RNAi knockdowns compared to controls. d. Quantification of EdU-positive GSCs in RNAi knockdowns compared to controls. No statistically significant differences were detected using two-tailed Student's *t*-test.

3.5 Adult-specific pan-neuronal knockdown of Dh31 affects egg-laying

To further investigate that Dh31 plays a role in oogenesis, the nSyb-GAL4 driver was used with tubP- Gal80^{ts} to drive RNAi-mediated knockdown of Dh31 receptors pan-neuronally only in adult female flies. This experiment was intended to either validate or reject the phenotype observed when the Dh31-R was knocked down in soma. When Dh31 is knocked down in the brain of adult flies, there are significantly lower numbers of eggs

laid as illustrated in Figure 7a below. The Vienna *Drosophila* Resource Center (VDRC) lines show a greater decrease in the number of eggs laid after 5, 10 and 15 days of RNAi knockdowns, compared to the Bloomington *Drosophila* Stock Center (BDSC) line. These results confirm previous findings from the screen that identified DH31 and are consistent with the DH31-R results.

In addition to the drastic reduction in the number of eggs, ovaries dissected on days 5, 10, and 15 qualitatively, appeared to be more backed up than ovaries that had somatic knockdown of Dh31-R. These results suggest that perhaps neuronal Dh31 signaling and somatic Dh31-R signaling may regulate some distinct processes, and ultimately confirm that Dh31 neuropeptide is playing an important role in oogenesis. Another explanation may be that the knockdown of DH31 in the brain is more effective than the knockdown of DH31-R in all somatic cells. Alternatively, since Dh31-R has genetic and functional similarity to other G-protein coupled receptors of the diuretic hormone peptide family (Coast et al., 2001), perhaps upon ablation of Dh31-R, the neuropeptide partially binds to another diuretic hormone receptor in the class 2 family (Hewes & Taghert, 2001). Taken together, these results confirm that Dh31 neuropeptide plays an important role in oogenesis.

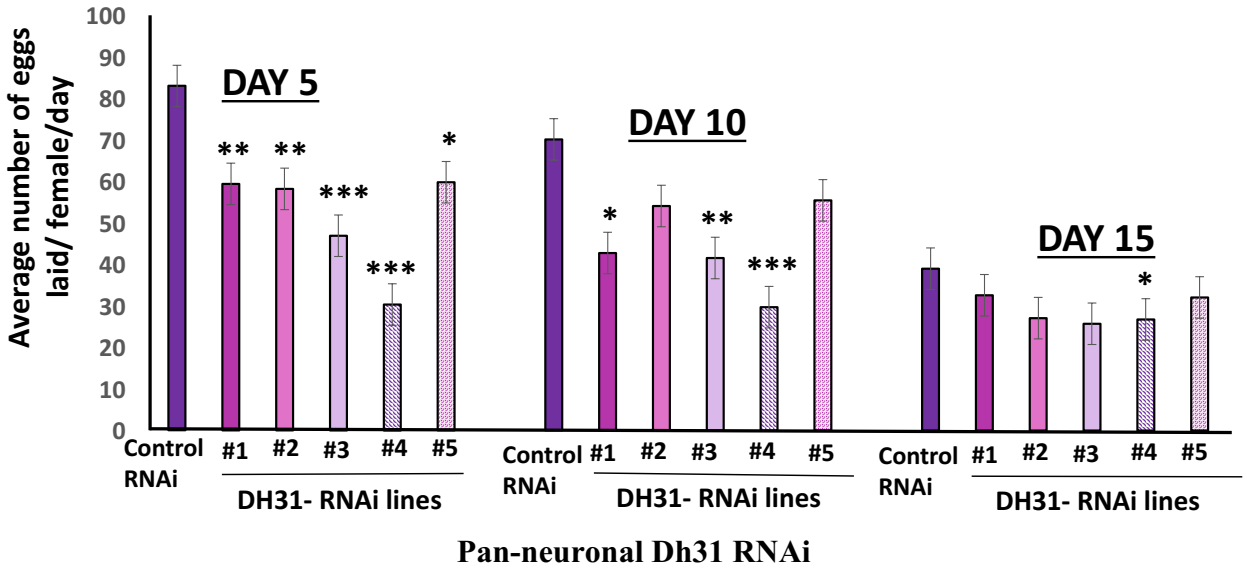
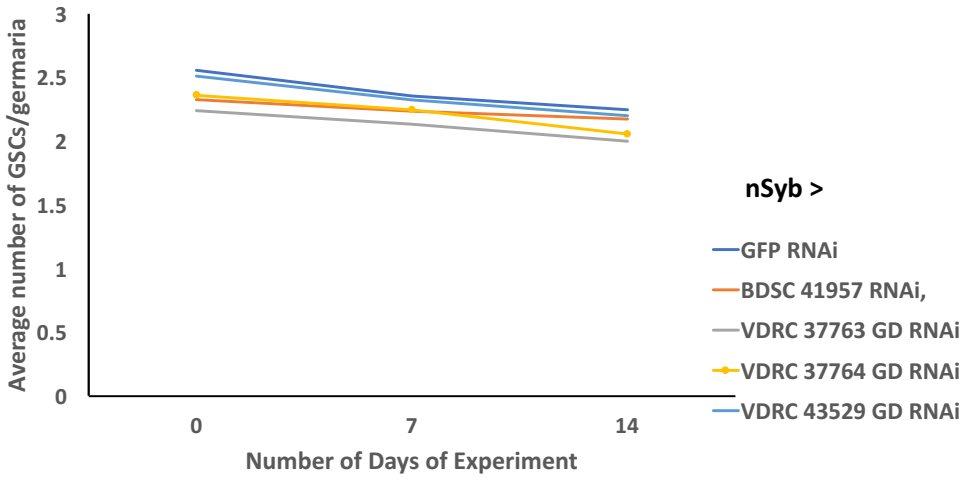


Figure 7| Knockdown of Dh31 only in the brain decreased egg production compared to control. *p<0.05, **p<0.01, ***p<0.001, two-tailed Student's t-test.

3.6 Pan-neuronal knockdown of Dh31 does not affect number of GSCs and the niche size

When Dh31 was knocked down in the brain, the number of GSCs and CCs were not significantly different from the controls, as quantified using immunofluorescence microscopy. These results validate the prior results obtained from the knockdown of Dh31-R in the soma.

a.



b.

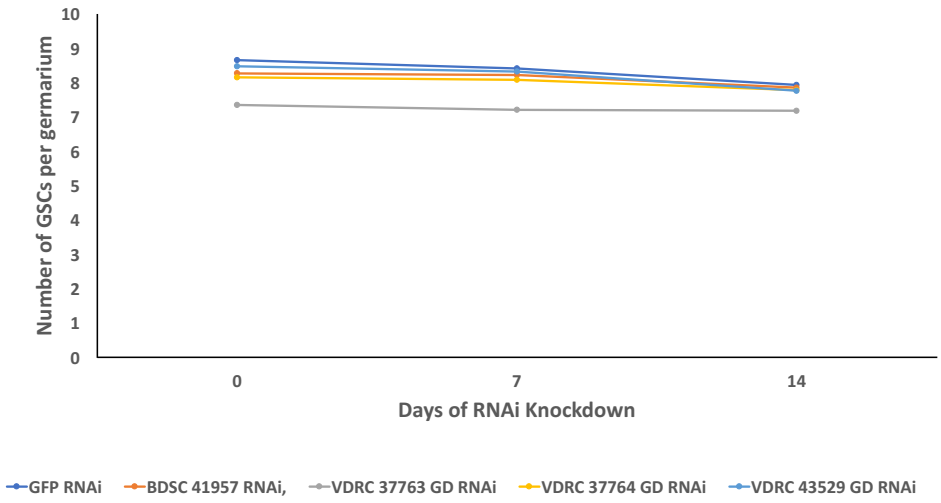


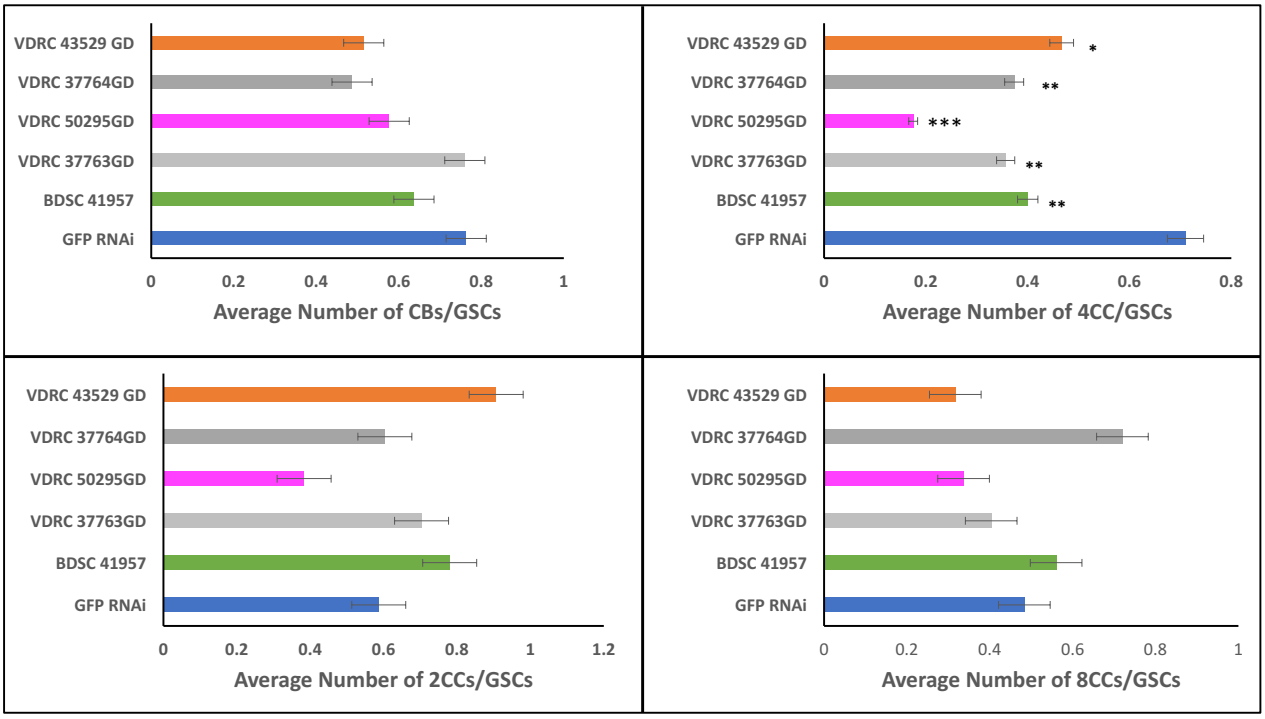
Figure 8| a. Pan-neuronal knockdown of Dh31 did not cause a change in the rate of GSC or b. CC loss. No statistically significant differences were detected using two-way ANOVA with interaction.

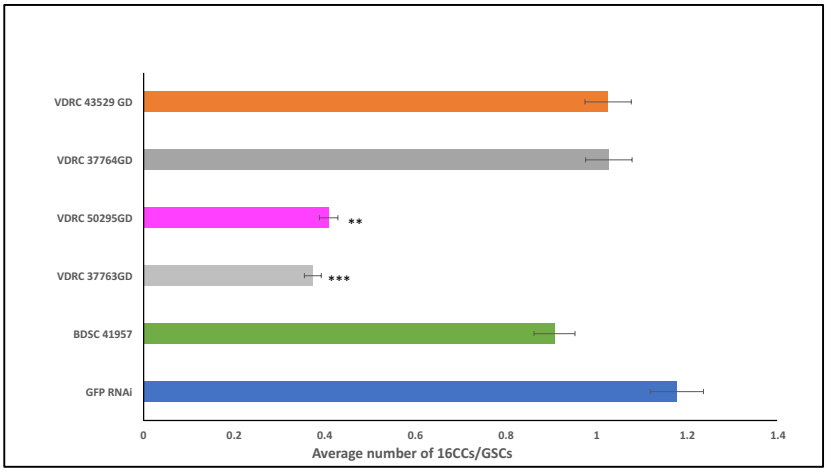
3.7 Pan-neuronal knockdown of Dh31 causes a decrease in the number of germline cysts

Similar to somatic knockdown of Dh31-R, pan-neuronal knockdown of Dh31 also caused a decrease in the number of early germline cysts present in the germaria. The number of cystoblasts, 2-cell cysts, 4-cell cysts, 8-cell cysts and 16-cell cysts were counted using the confocal microscope after 14 days of pan-neuronal Dh31 RNAi (Figure 9a). The most significant decreases are observed at the 4-cell cyst stage, stage when Dh31-R is knocked down only in the soma.

Also similar to the phenotype observed with Dh31-R knockdown, this is not a proliferation defect since the number of EdU-positive GSCs were similar between controls and knockdowns (Figure 9b). Therefore, we predict that the decreased number of cysts is due to an increase in cell death. This hypothesis can be confirmed with a caspase stain, to identify at which stage the cysts are dying.

a.




VDRC 43529 GD
VDRC 37764GD
VDRC 50295GD
**
VDRC 37763GD
***
BDSC 41957
GFP RNAi
0
0.2
0.4
0.6
0.8
1
1.2
1.4
Average number of 16CCs/GSCs

b.

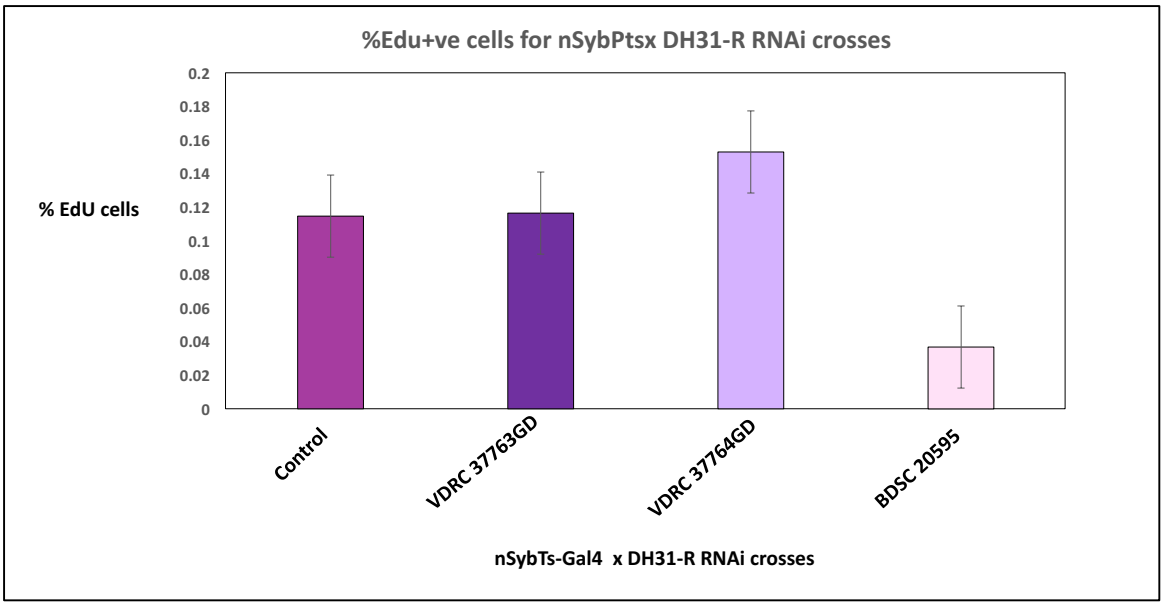


Figure 9| a. There is an overall decrease in the number of early germline cysts after 14 days of pan-neuronal Dh31 RNAi knockdown. *p<0.05, **p<0.01, ***p<0.001, two-tailed Student's *t*-test. The most significant decreases are observed at the 4-cell cyst stage with neuronal knockdown. b. Quantification of percent of EdU-positive GSCs. No statistically significant changes were detected through two-tailed Student's *t*-test.

3.8 *Germline knockdown of Dh31-R does not affect egg laying*

Due to technical limitations, tubP-Gal4 does not express in the female germline. Therefore, to determine if Dh31-R is required in the germline to regulate oogenesis, we used MTD-Gal4 to drive RNAi against Dh31-R specifically in the germline and performed the egg count assay. In Figure 10, the graphs RNAi #1, #2 and #3 represent BDSC 25925, VDRC 101955KK and VDRC 8777GD respectively. We saw no significant changes in the number of eggs laid by Dh31-R knockdown females compared to controls. It is possible that this is because these RNAi lines are inefficient in the female germline. Further analysis

using genetic mosaic analysis, where Dh31-R mutant GSC clones are created in the germline, will be necessary to rule out the requirement for Dh31-R in the germline.

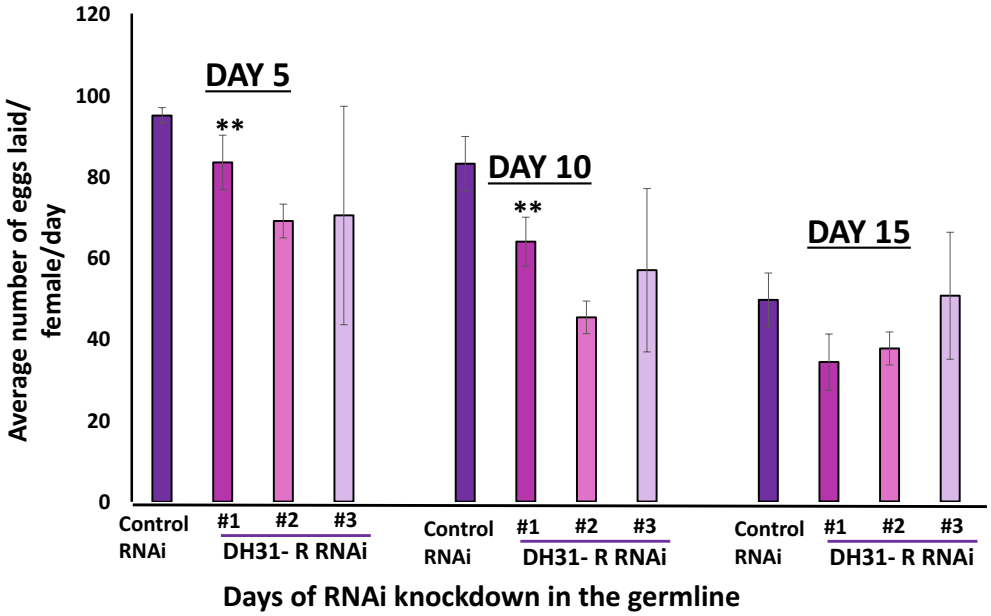


Figure 10| Knockdown of Dh31-R only in the germline may not affect egg production. *p<0.05, **p<0.01, ***p<0.001, two-tailed Student's *t*-test.

DISCUSSION

4.1 Conclusion

In this project, we began to investigate the role of Dh31 signaling in *Drosophila* oogenesis. Based on our results, RNAi knockdown of Dh31 does not cause a statistically significant reduction in the numbers of GSCs nor their niche size (number of CCs). These results were confirmed with somatic knockdown of Dh31-R. However, it is not possible for us to conclude from these experiments alone, whether Dh31 signaling is required in the ovary to regulate oogenesis due to limitations of RNAi experiments. First, it is unclear whether the Dh31-R RNAi lines work well in the ovary, and secondly, RNAi lines are not capable of eliminating Dh31 expression entirely. To investigate the hypothesis that Dh31 is required in the ovary, genetic mosaic analysis needs to be conducted by generating GSC clones of Dh31-R mutants in the ovary.

Our data also shows that disrupting Dh31 signaling mainly affects early progeny of GSCs (the cysts). To investigate whether the lower numbers of cysts were due to a proliferation defect or cell death, dissected ovaries were incorporated in EdU to label proliferating cells in the S-phase of the cell-cycle. Our results show no significant difference in proliferation rates between RNAi knockdowns and controls. Therefore, we suspect that the cysts are dying, and this can be confirmed using a cleaved caspase stain in the future.

4.2 Future Directions

Since Dh31 is expressed in different neurons in the brain (Figure 3a), future experiments can investigate which unique Dh31 neurons in the brain might be responsible for some of the phenotypes observed from our pan-neuronal knockdown experiments of Dh31. This

can be done by over-expressing ion channels in different Dh31-producing neuron to turn an action potential in the neuron on or off. The Dh31-GAL4 drivers would be used to drive expression of two different ion channel: UAS-TrpA, a heat-activated cation channel which turns the neurons on by causing depolarization (sodium ion influx) for the firing of an action potential, or UAS-Kir2.1 with Gal80^{ts}, an inward rectifying potassium channel which turns the neurons off by triggering hyperpolarization (efflux of potassium ion, K+) (Adalberto. Merighi, 2011). *Drosophila* TrpA1 is a cation channel that belongs to the TRP family of cation channels. Members of the TRP family of cations comprise a well-established class of temperature detectors (Ramsey et al., 2006). *Drosophila* TRPA1 functions in the avoidance of noxious tastants (Kang et al., 2010), insect repellents (Pedersen et al., 2005), excessively bright light (Xiang, 2010), and uncomfortably warm and slightly suboptimal temperatures (Viswanath et al., 2003). Kir 2.1 stands for inward rectifier potassium ion channel that is encoded by the KCNJ2 gene (Kang et al., 2012). In neurogenetics, Kir2.1 is used in *Drosophila* research to inhibit neurons, as overexpression of this channel will hyperpolarize cells (Zhong et al., 2009). Additionally, because our experiment knocking down Dh31-R in the germline with the MTD-GAL4 line did not seem to work, we can use genetic mosaic analysis to determine the cell requirement for Dh31-R. We are currently in the process of generating Dh31-R mutants using CRISPR. Lastly, to confirm that Dh31 signaling regulates cyst death, germaria after 0, 7 and 14 days of Dh31 or Dh31-R RNAi knockdown can be stained with caspase for cell death, using immunocytochemistry, and analyzed on the confocal microscope.

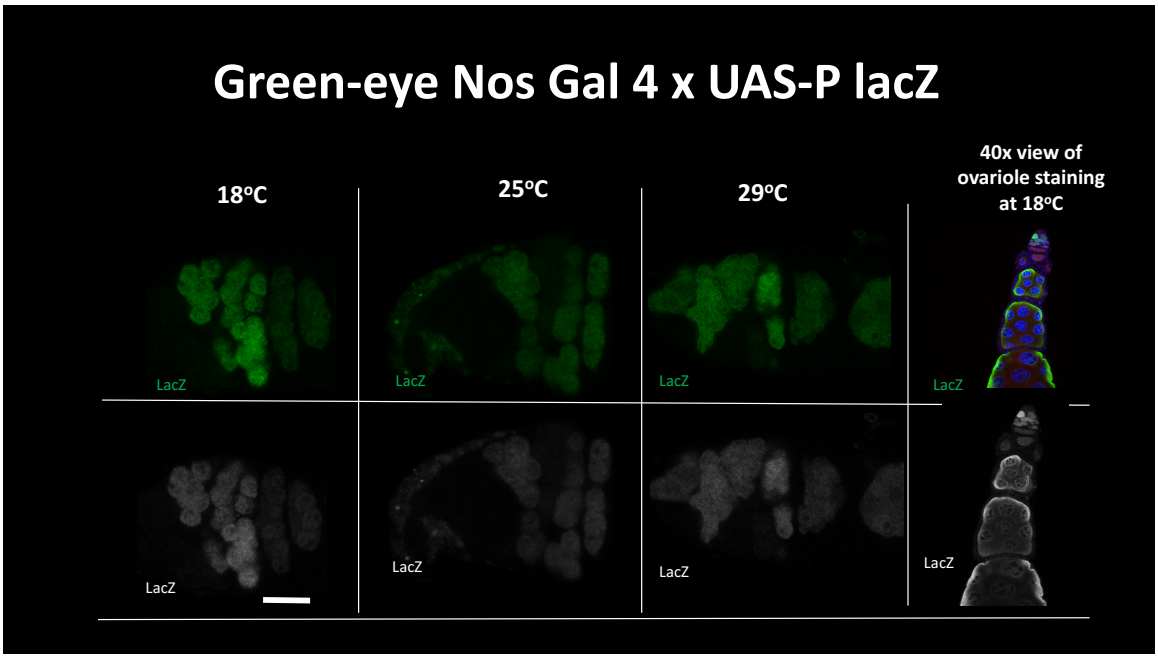
4.3 *Public Health relevance*

Although no germline stem cell populations exist in the adult mammalian ovary, a comparable mitotically proliferating population of germ cells exists in the developing ovary in the mammalian fetus (Nystul et al., 2007). Additionally, many other aspects of germline development are similar. Like cystoblasts in the fruitfly, primordial germ cells in mammals also undergo incomplete mitotic divisions to form germ cell cysts (Nystul et al., 2007). Furthermore, the cysts breakdown and become surrounded by pre-granulosa cells to form a primordial follicle that undergoes further development throughout sexual maturation (Xie et al., 2008), much like the development of egg chambers in the *Drosophila* ovary. Therefore, our understanding of how Dh31 regulates these processes and cell types can give insight on novel therapeutics for any neural or degenerative defects before birth.

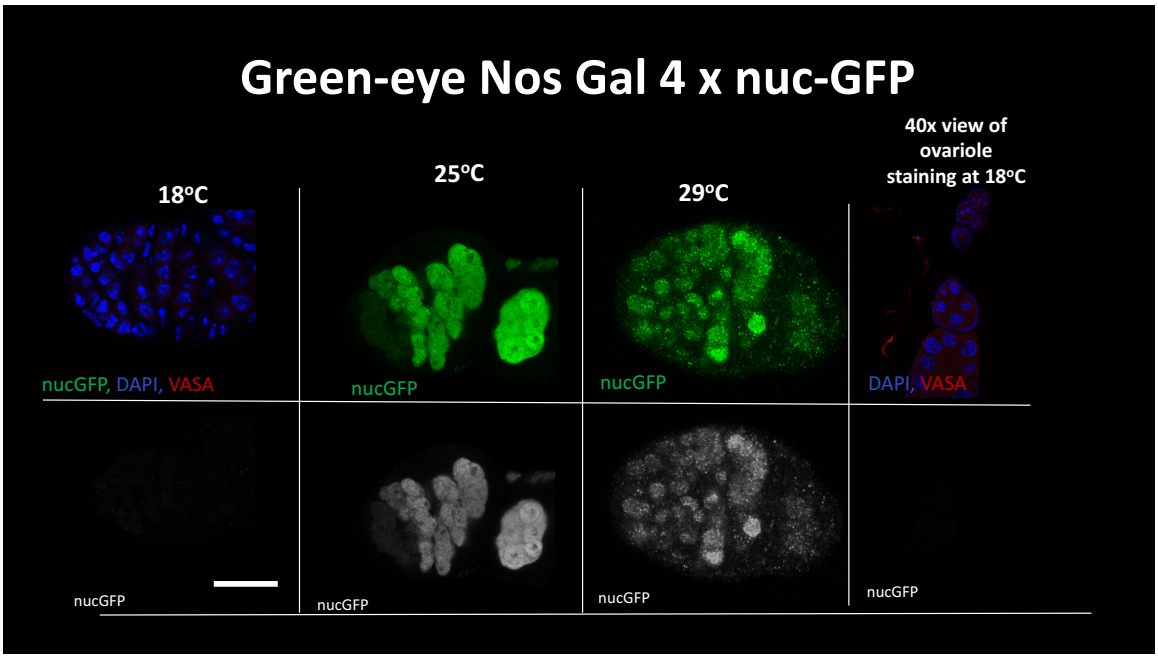
APPENDIX:

I. Characterization of germline drivers

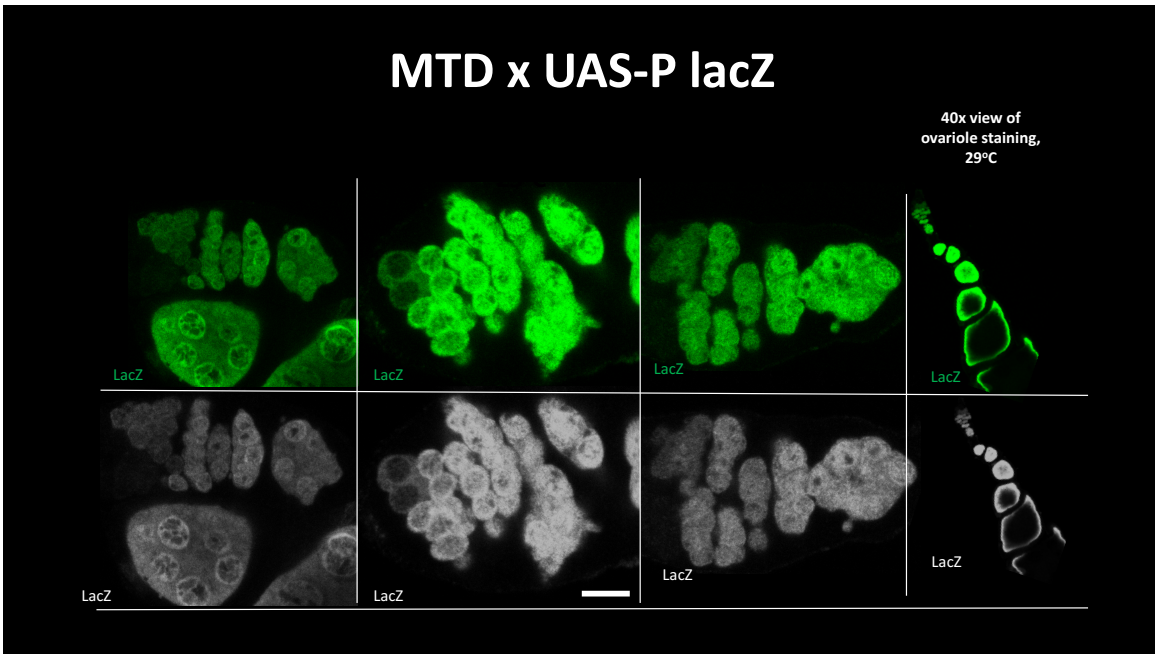
a.



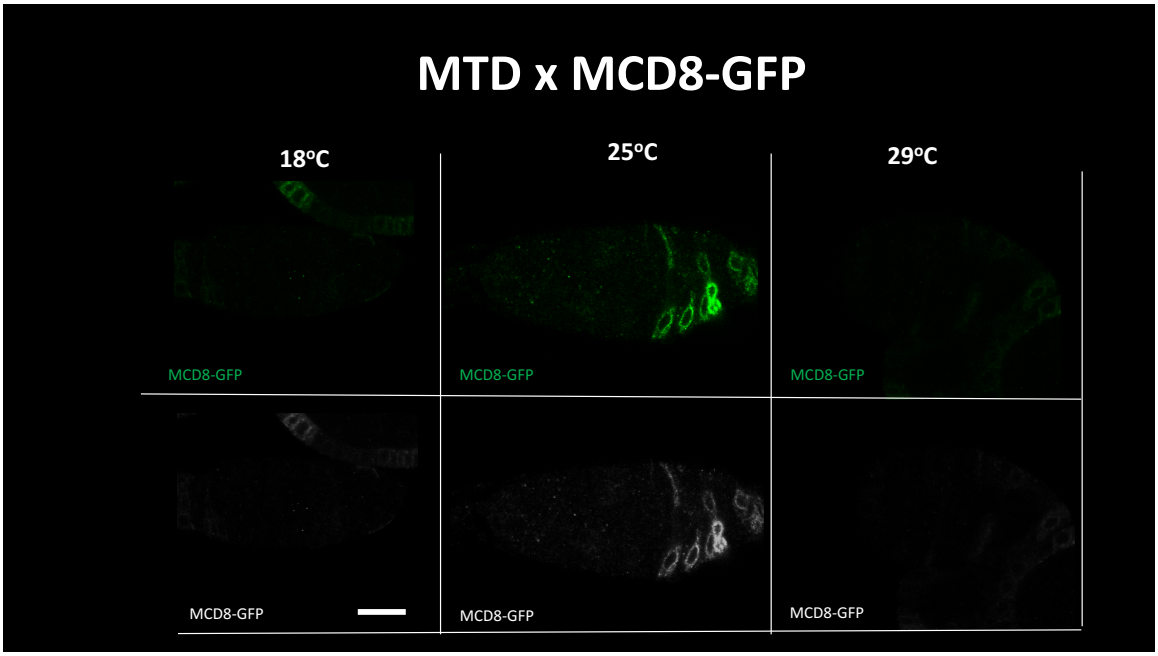
b.



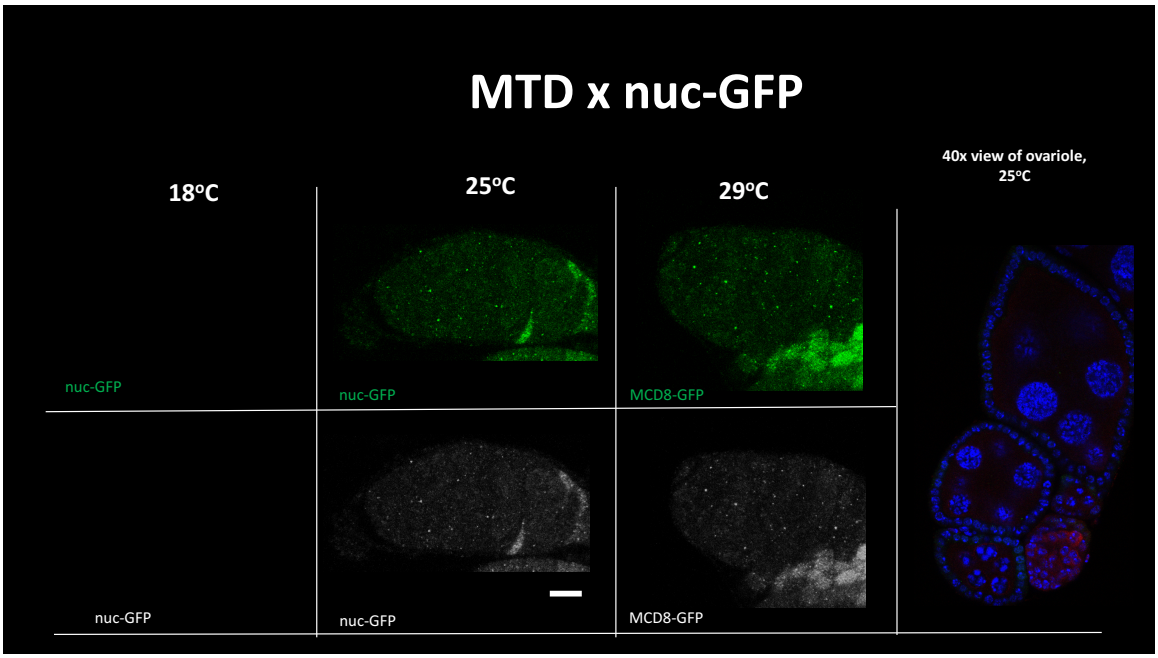
c.


MTD x UAS-P lacZ
40x view of ovariole staining, 29°C
LacZ
LacZ
LacZ
LacZ
LacZ
LacZ
LacZ
LacZ

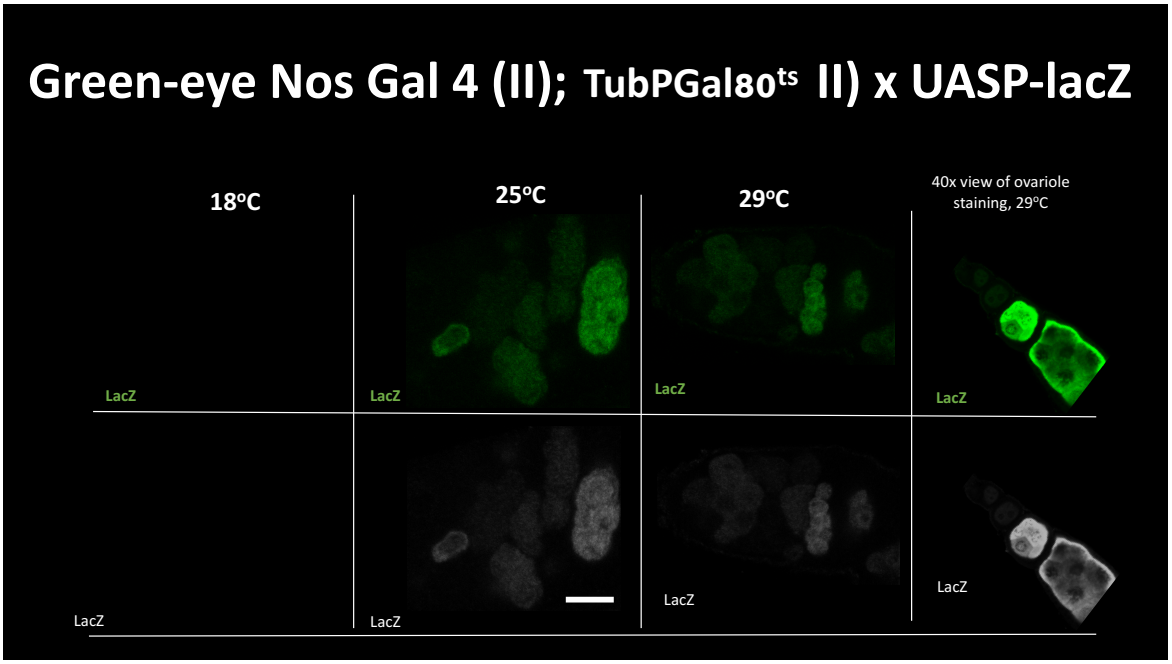
d.


MTD x MCD8-GFP
18°C
25°C
29°C
MCD8-GFP
MCD8-GFP
MCD8-GFP
MCD8-GFP
MCD8-GFP
MCD8-GFP

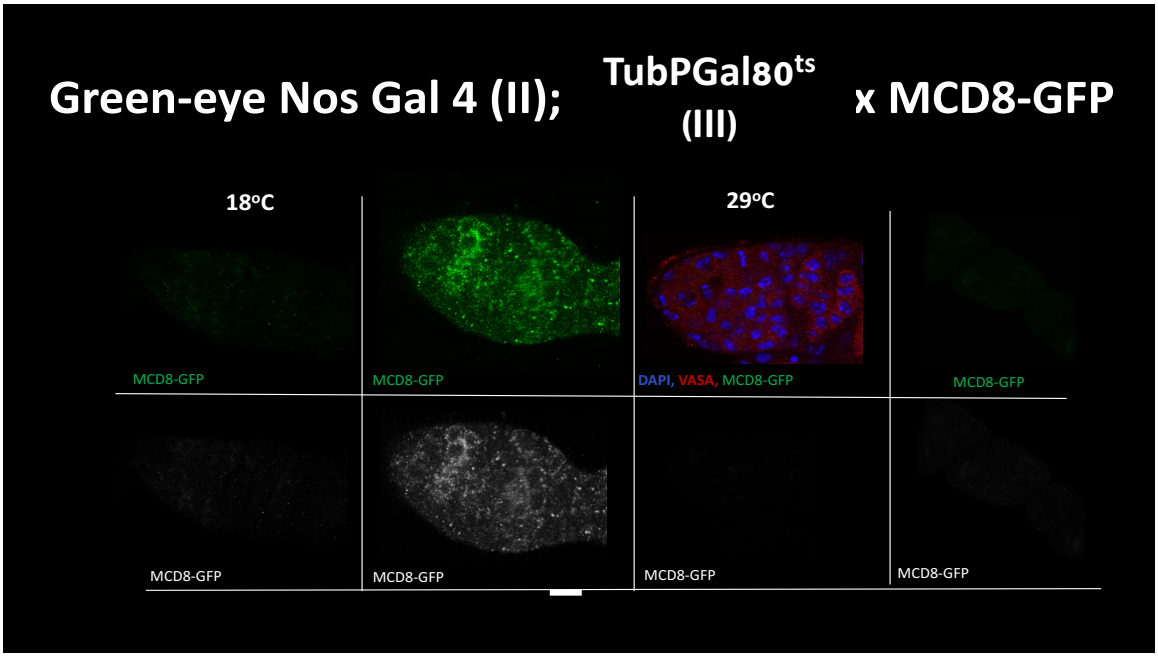
e.


MTD x nuc-GFP
18°C
25°C
29°C
40x view of ovariole, 25°C
nuc-GFP
nuc-GFP
MCD8-GFP
nuc-GFP
nuc-GFP
MCD8-GFP

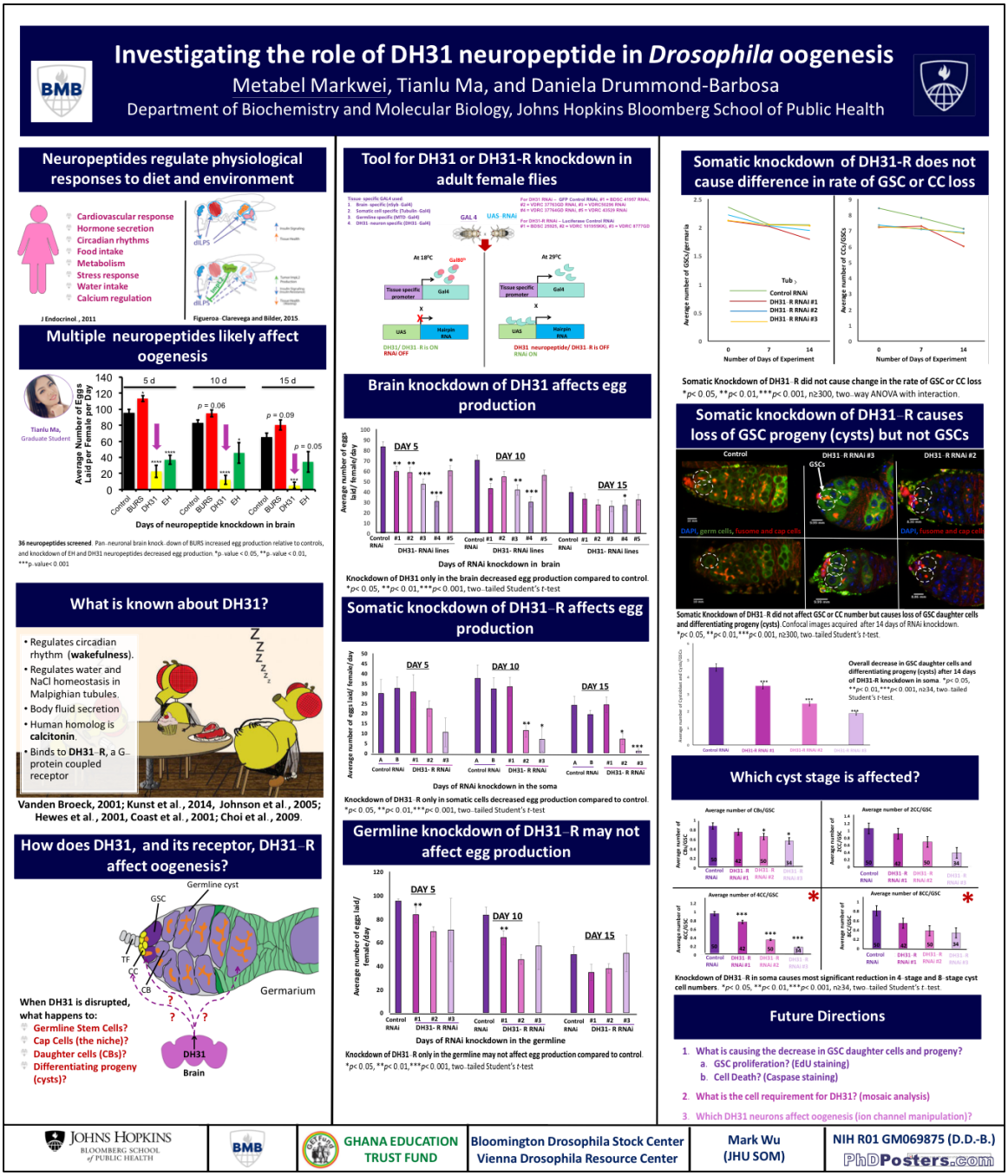
f.


Green-eye Nos Gal 4 (II); TubPGal80ts II) x UASP-lacZ
18°C
25°C
29°C
40x view of ovariole staining, 29°C
LacZ
LacZ
LacZ
LacZ
LacZ
LacZ
LacZ
LacZ

h.



Supplement Figure I| Confocal images show expression patterns of different germline drivers and reporter genes in the germarium. All images were taken at the same laser gain and magnification (2.5 zoom with 40X, 60X) using the MTD-Gal4 driver, Nanos Gal 4 Driver and *PiggyBac* Nanos Gal4 driver. MTD showed the strongest driving effect at all temperatures. Scale bar um



Supplement Figure II| Scientific poster of thesis research work presented at The Johns Hopkins Bloomberg School of Public Health, Department of Biochemistry and Molecular Biology annual retreat. 2nd April 2017.

REFERENCES:

Ables, E. T. (2015). Drosophila oocytes as a model for understanding meiosis: an educational primer to accompany "corolla is a novel protein that contributes to the architecture of the synaptonemal complex of Drosophila". *Genetics*, *199*(1), 17–23. https://doi.org/10.1534/genetics.114.167940

Ables, E. T., & Drummond-Barbosa, D. (2013). Cyclin E controls Drosophila female germline stem cell maintenance independently of its role in proliferation by modulating responsiveness to niche signals. *Development (Cambridge, England)*, *140*(3), 530–40. https://doi.org/10.1242/dev.088583

Adams, M. D., Celniker, S. E., Holt, R. A., Evans, C. A., Gocayne, J. D., Amanatides, P. G., … Venter, J. C. (2000). The genome sequence of Drosophila melanogaster. *Science (New York, N.Y.)*, *287*(5461), 2185–95. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10731132

Agrawal, T., Sadaf, S., & Hasan, G. (2013). A Genetic RNAi Screen for IP3/Ca2+ Coupled GPCRs in Drosophila Identifies the PdfR as a Regulator of Insect Flight. *PLoS Genetics*, *9*(10). https://doi.org/10.1371/journal.pgen.1003849

Amamoto, R., Huerta, V. G. L., Takahashi, E., Dai, G., Grant, A. K., Fu, Z., & Arlotta, P. (2016). Adult axolotls can regenerate original neuronal diversity in response to brain injury. *eLife*, *5*. https://doi.org/10.7554/eLife.13998

Armstrong, A. R., Laws, K. M., & Drummond-Barbosa, D. (2014). Adipocyte amino acid sensing controls adult germline stem cell number via the amino acid response pathway and independently of Target of Rapamycin signaling in Drosophila. *Development*, *141*(23), 4479–4488. https://doi.org/10.1242/dev.116467

Bai, H., Post, S., Kang, P., Tatar, M., Gingras, A., & Crosby, K. (2015). Drosophila Longevity Assurance Conferred by Reduced Insulin Receptor Substrate Chico Partially Requires d4eBP. *PLOS ONE*, *10*(8), e0134415. https://doi.org/10.1371/journal.pone.0134415

Brogiolo, W., Stocker, H., Ikeya, T., Rintelen, F., Fernandez, R., & Hafen, E. (2001). An evolutionarily conserved function of the Drosophila insulin receptor and insulin-like peptides in growth control. *Current Biology : CB*, *11*(4), 213–21. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11250149

Brown, M. R., Crim, J. W., Arata, R. C., Cai, H. N., Chun, C., & Shen, P. (1999). Identification of a Drosophila brain-gut peptide related to the neuropeptide Y family. *Peptides*, *20*(9), 1035–1042. https://doi.org/10.1016/S0196-9781(99)00097-2

Burbach, J. P. H. (2011). What Are Neuropeptides? (pp. 1–36). https://doi.org/10.1007/978-1-61779-310-3_1

Chehrehasa, F., Meedeniya, A. C. B., Dwyer, P., Abrahamsen, G., & Mackay-Sim, A. (2009). EdU, a new thymidine analogue for labelling proliferating cells in the nervous system. *Journal of Neuroscience Methods*, *177*(1), 122–130. https://doi.org/10.1016/j.jneumeth.2008.10.006

Chen, J., Kim, S.-M., & Kwon, J. Y. (2016). A Systematic Analysis of Drosophila Regulatory Peptide Expression in Enteroendocrine Cells. *Molecules and Cells*, *39*(4), 358–366. https://doi.org/10.14348/molcells.2016.0014

Choi, C., Fortin, J.-P., McCarthy, E. v., Oksman, L., Kopin, A. S., & Nitabach, M. N. (2009). Cellular Dissection of Circadian Peptide Signals with Genetically Encoded Membrane-Tethered Ligands. *Current Biology*, *19*(14), 1167–1175. https://doi.org/10.1016/j.cub.2009.06.029

Coast, G. M., Webster, S. G., Schegg, K. M., Tobe, S. S., & Schooley, D. A. (2001). The Drosophila melanogaster homologue of an insect calcitonin-like diuretic peptide stimulates V-ATPase activity in fruit fly Malpighian tubules. *The Journal of Experimental Biology*, *204*(Pt 10), 1795–804. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11316500

Crocker, A., Guan, X.-J., Murphy, C. T., & Murthy, M. (2016). Cell-Type-Specific Transcriptome Analysis in the Drosophila Mushroom Body Reveals Memory-Related Changes in Gene Expression. *Cell Reports*, *15*(7), 1580–1596. https://doi.org/10.1016/j.celrep.2016.04.046

de Cuevas, M., & Matunis, E. L. (2011). The stem cell niche: lessons from the Drosophila testis. *Development*, *138*(14), 2861–2869. https://doi.org/10.1242/dev.056242

de Girolamo, P., & Lucini, C. (2011). Neuropeptide Localization in Nonmammalian Vertebrates (pp. 37–56). https://doi.org/10.1007/978-1-61779-310-3_2

Drummond-Barbosa, D., & Spradling, A. C. (2001). Stem Cells and Their Progeny Respond to Nutritional Changes during Drosophila Oogenesis. *Developmental Biology*, *231*(1), 265–278. https://doi.org/10.1006/dbio.2000.0135

Dzitoyeva, S., Dimitrijevic, N., & Manev, H. (2001). Intra-abdominal injection of double-stranded RNA into anesthetized adult Drosophila triggers RNA interference in the central nervous system. *Molecular Psychiatry*, *6*(6), 665–670. https://doi.org/10.1038/sj.mp.4000955

Eliazer, S., & Buszczak, M. (2011). Finding a niche: studies from the Drosophila ovary. *Stem Cell Research & Therapy*, *2*(6), 45. https://doi.org/10.1186/scrt86

Figueroa-Clarevega, A., & Bilder, D. (2015). Malignant Drosophila Tumors Interrupt Insulin Signaling to Induce Cachexia-like Wasting. *Developmental Cell*, *33*(1), 47–55. https://doi.org/10.1016/j.devcel.2015.03.001

Fontana, J. R., & Crews, S. T. (2012). Transcriptome analysis of Drosophila CNS midline cells reveals diverse peptidergic properties and a role for castor in neuronal differentiation. *Developmental Biology*, *372*(1), 131–142. https://doi.org/10.1016/j.ydbio.2012.09.010

Fuchs, Y., Brown, S., Gorenc, T., Rodriguez, J., Fuchs, E., & Steller, H. (2013). Sept4/ARTS Regulates Stem Cell Apoptosis and Skin Regeneration. *Science*, *341*(6143). Retrieved from http://science.sciencemag.org/content/341/6143/286

Goda, T., Tang, X., Umezaki, Y., Chu, M. L., & Hamada, F. N. (2016). Drosophila DH31 Neuropeptide and PDF Receptor Regulate Night-Onset Temperature Preference. *Journal of Neuroscience*, *36*(46), 11739–11754. https://doi.org/10.1523/JNEUROSCI.0964-16.2016

Harzer, H., Berger, C., Conder, R., Schmauss, G., & Knoblich, J. a. (2013). FACS purification of Drosophila larval neuroblasts for next-generation sequencing. *Nature Protocols*, *8*(6). https://doi.org/10.1038/nprot.2013.062

Hector, C. E., Bretz, C. A., Zhao, Y., & Johnson, E. C. (2009). Functional differences between two CRF-related diuretic hormone receptors in Drosophila. *Journal of Experimental Biology*, *212*(19), 3142–3147. https://doi.org/10.1242/jeb.033175

Hergarden, A. C., Tayler, T. D., & Anderson, D. J. (2012). Allatostatin-A neurons inhibit feeding behavior in adult Drosophila. *Proceedings of the National Academy of Sciences*, *109*(10), 3967–3972. https://doi.org/10.1073/pnas.1200778109

Hewes, R. S., & Taghert, P. H. (2001). No Title, *11*(6). https://doi.org/10.1101/gr.169901

Holtzman, S., Miller, D., Eisman, R., Kuwayama, H., Niimi, T., & Kaufman, T. (n.d.). Transgenic tools for members of the genus Drosophila with sequenced genomes. *Fly*, *4*(4), 349–62. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20890112

Hsu, H.-J., & Drummond-Barbosa, D. (2009). Insulin levels control female germline stem cell maintenance via the niche in Drosophila. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(4), 1117–21. https://doi.org/10.1073/pnas.0809144106

Hsu, H.-J., LaFever, L., & Drummond-Barbosa, D. (2008). Diet controls normal and tumorous germline stem cells via insulin-dependent and -independent mechanisms in Drosophila. *Developmental Biology*, *313*(2), 700–712. https://doi.org/10.1016/j.ydbio.2007.11.006

Jenett, A., Rubin, G. M., Ngo, T.-T. B., Shepherd, D., Murphy, C., Dionne, H., … Zugates, C. T. (2012). A GAL4-Driver Line Resource for Drosophila Neurobiology. *Cell Reports*, *2*(4), 991–1001. https://doi.org/10.1016/j.celrep.2012.09.011

Johnson, E. C., Shafer, O. T., Trigg, J. S., Park, J., Schooley, D. A., Dow, J. A., & Taghert, P. H. (2005). A novel diuretic hormone receptor in Drosophila: evidence for conservation of CGRP signaling. *Journal of Experimental Biology*, *208*(7), 1239–1246. https://doi.org/10.1242/jeb.01529

Jumbo-Lucioni, P., Ayroles, J. F., Chambers, M., Jordan, K. W., Leips, J., Mackay, T. F., & De Luca, M. (2010). Systems genetics analysis of body weight and energy metabolism traits in Drosophila melanogaster. *BMC Genomics*, *11*(1), 297. https://doi.org/10.1186/1471-2164-11-297

Kennerdell, J. R., & Carthew, R. W. (2000). Heritable gene silencing in Drosophila using double-stranded RNA. *Nature Biotechnology*, *18*(8), 896–898. https://doi.org/10.1038/78531

Kirilly, D., & Xie, T. (2007). The Drosophila ovary: an active stem cell community. *Cell Research*, *17*(1), 15–25. https://doi.org/10.1038/sj.cr.7310123

Kiss, B., Szlanka, T., Zvara, Á., Žurovec, M., Sery, M., Kakaš, Š., … Kiss, I. (2013). Selective elimination/RNAi silencing of FMRF-related peptides and their receptors decreases the locomotor activity in Drosophila melanogaster. *General and Comparative Endocrinology*, *191*. https://doi.org/10.1016/j.ygcen.2013.05.023

Klapoetke, N. C., Murata, Y., Kim, S. S., Pulver, S. R., Birdsey-Benson, A., Cho, Y. K., … Boyden, E. S. (2014). Independent optical excitation of distinct neural populations. *Nature Methods*, *11*(3). https://doi.org/10.1038/nmeth.2836

Knapek, S., Kahsai, L., Winther, Å. M. E., Tanimoto, H., & Nässel, D. R. (2013). Short neuropeptide F acts as a functional neuromodulator for olfactory memory in kenyon cells of Drosophila mushroom bodies. *Annals of Internal Medicine*, *158*(6). https://doi.org/10.1523/JNEUROSCI.2287-12.2013

Kula-Eversole, E., Nagoshi, E., Shang, Y., Rodriguez, J., Allada, R., & Rosbash, M. (2010). Surprising gene expression patterns within and between PDF-containing circadian neurons in Drosophila. *Proceedings of the National Academy of Sciences*, *107*(30), 13497–13502. https://doi.org/10.1073/pnas.1002081107

Kunst, M., Hughes, M. E., Raccuglia, D., Felix, M., Li, M., Barnett, G., … Nitabach, M. N. (2014). Calcitonin Gene-Related Peptide Neurons Mediate Sleep-Specific Circadian Output in Drosophila. *Current Biology*, *24*(22), 2652–2664. https://doi.org/10.1016/j.cub.2014.09.077

Kwon, Y., Kim, S. H., Ronderos, D. S., Lee, Y., Akitake, B., Woodward, O. M., … Montell, C. (2010). Drosophila TRPA1 Channel Is Required to Avoid the Naturally Occurring Insect Repellent Citronellal. *Current Biology*, *20*(18), 1672–1678. https://doi.org/10.1016/j.cub.2010.08.016

LaFever, L., & Drummond-Barbosa, D. (2005). Direct Control of Germline Stem Cell Division and Cyst Growth by Neural Insulin in Drosophila. *Science*, *309*(5737), 1071–1073. https://doi.org/10.1126/science.1111410

LaJeunesse, D. R., Johnson, B., Presnell, J. S., Catignas, K., & Zapotoczny, G. (2010). Peristalsis in the junction region of the Drosophila larval midgut is modulated by DH31 expressing enteroendocrine cells. *BMC Physiology*, *10*(1), 14. https://doi.org/10.1186/1472-6793-10-14

Lin, H., Yue, L., & Spradling, A. C. (1994). The Drosophila fusome, a germline-specific organelle, contains membrane skeletal proteins and functions in cyst formation. *Development*, *120*(4). Retrieved from http://dev.biologists.org/content/120/4/947.long

Ludwig, M., & Leng, G. (2006). Dendritic peptide release and peptide-dependent behaviours. *Nature Reviews Neuroscience*, *7*(2), 126–136. https://doi.org/10.1038/nrn1845

Luo, L., Chai, P. C., & Cai, Y. (2013). Immunostaining of Germline Stem Cells and the Niche in Drosophila Ovaries. In *Methods in molecular biology (Clifton, N.J.)* (Vol. 1035, pp. 1–7). https://doi.org/10.1007/978-1-62703-508-8_1

Madhavan, H. (2007). Simple Laboratory methods to measure cell proliferation using DNA synthesis property. *Journal of Stem Cells & Regenerative Medicine*, *3*(1), 12–4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24693014

Marianes, A., & Spradling, A. C. (2013). Physiological and stem cell compartmentalization within the Drosophila midgut. *eLife*, *2*, e00886. https://doi.org/10.7554/eLife.00886

McCall, K. (2004). Eggs over easy: cell death in the Drosophila ovary. *Developmental Biology*, *274*(1), 3–14. https://doi.org/10.1016/j.ydbio.2004.07.017

MCGUIRE, S., Roman, G., & Davis, R. L. (2004). Gene expression systems in Drosophila: a synthesis of time and space. *Trends in Genetics*, *20*(8), 384–391. https://doi.org/10.1016/j.tig.2004.06.012

McLeod, C. J., Wang, L., Wong, C., & Jones, D. L. (2010). Stem Cell Dynamics in Response to Nutrient Availability. *Current Biology*, *20*(23), 2100–2105. https://doi.org/10.1016/j.cub.2010.10.038

Melcher, C., & Pankratz, M. J. (2005). Candidate Gustatory Interneurons Modulating Feeding Behavior in the Drosophila Brain. *PLoS Biology*, *3*(9), e305. https://doi.org/10.1371/journal.pbio.0030305

Merighi, A. (2002). Costorage and coexistence of neuropeptides in the mammalian CNS. *Progress in Neurobiology*, *66*(3), 161–90. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11943450

Merighi, A., Salio, C., Ferrini, F., & Lossi, L. (2011). Neuromodulatory function of neuropeptides in the normal CNS. *Journal of Chemical Neuroanatomy*, *42*(4), 276–287. https://doi.org/10.1016/j.jchemneu.2011.02.001

Merighi, A. (2011). *Neuropeptides : methods and protocols*. Humana Press.

Nässel, D. R. (2002). Neuropeptides in the nervous system of Drosophila and other insects: multiple roles as neuromodulators and neurohormones. *Progress in Neurobiology*, *68*(1), 1–84. https://doi.org/10.1016/S0301-0082(02)00057-6

Nässel, D. R., Kubrak, O. I., Liu, Y., Luo, J., & Lushchak, O. V. (2013). Factors that regulate insulin producing cells and their output in Drosophila. *Frontiers in Physiology*, *4*, 252. https://doi.org/10.3389/fphys.2013.00252

Nässel, D. R., & Winther, Å. M. E. (2010). Drosophila neuropeptides in regulation of physiology and behavior. *Progress in Neurobiology*, *92*(1), 42–104. https://doi.org/10.1016/j.pneurobio.2010.04.010

Nystul, T., Spradling, A., Kirilly, D., Wong, M. D., Xie, T., Morris, R. J., … al., et. (2007). An epithelial niche in the Drosophila ovary undergoes long-range stem cell replacement. *Cell Stem Cell*, *1*(3), 277–85. https://doi.org/10.1016/j.stem.2007.07.009

Ohyama, T., Schneider-Mizell, C. M., Fetter, R. D., Aleman, J. V., Franconville, R., Rivera-Alba, M., … Zlatic, M. (2015). A multilevel multimodal circuit enhances action selection in Drosophila. *Nature*, *520*(7549). https://doi.org/10.1038/nature14297

Park, D., Shafer, O. T., Shepherd, S. P., Suh, H., Trigg, J. S., & Taghert, P. H. (2008). The Drosophila basic helix-loop-helix protein DIMMED directly activates PHM, a gene encoding a neuropeptide-amidating enzyme. *Molecular and Cellular Biology*, *28*(1), 410–21. https://doi.org/10.1128/MCB.01104-07

Park, D., Veenstra, J. A., Park, J. H., & Taghert, P. H. (2008). Mapping Peptidergic Cells in Drosophila: Where DIMM Fits In. *PLoS ONE*, *3*(3), e1896. https://doi.org/10.1371/journal.pone.0001896

Park, J.-H., & Kwon, J. Y. (2011). Heterogeneous Expression of Drosophila Gustatory Receptors in Enteroendocrine Cells. *PLoS ONE*, *6*(12), e29022. https://doi.org/10.1371/journal.pone.0029022

Pearson, J. R., Zurita, F., Tomás-Gallardo, L., Díaz-Torres, A., Díaz de la Loza, M. del C., Franze, K., … González-Reyes, A. (2016). ECM-Regulator timp Is Required for Stem Cell Niche Organization and Cyst Production in the Drosophila Ovary. *PLOS Genetics*, *12*(1), e1005763. https://doi.org/10.1371/journal.pgen.1005763

Pedersen, S. F., Owsianik, G., & Nilius, B. (2005). TRP channels: An overview. *Cell Calcium*, *38*(3–4), 233–252. https://doi.org/10.1016/j.ceca.2005.06.028

Peters, N. C., & Berg, C. A. (2016). In Vitro Culturing and Live Imaging of Drosophila Egg Chambers: A History and Adaptable Method. In *Methods in molecular biology (Clifton, N.J.)* (Vol. 1457, pp. 35–68). https://doi.org/10.1007/978-1-4939-3795-0_4

Prigge, S. T., Kolhekar, A. S., Eipper, B. A., Mains, R. E., & Amzel, L. M. (1997). Amidation of Bioactive Peptides: The Structure of Peptidylglycine α-Hydroxylating Monooxygenase. *Science*, *278*(5341). Retrieved from http://science.sciencemag.org/content/278/5341/1300/tab-article-info

Radford, J. C., Davies, S. A., & Dow, J. A. T. (2002). Systematic G-protein-coupled Receptor Analysis in Drosophila melanogaster Identifies a Leucokinin Receptor with Novel Roles. *Journal of Biological Chemistry*, *277*(41), 38810–38817. https://doi.org/10.1074/jbc.M203694200

Ramsey, I. S., Delling, M., & Clapham, D. E. (2006). AN INTRODUCTION TO TRP CHANNELS. *Annual Review of Physiology*, *68*(1), 619–647. https://doi.org/10.1146/annurev.physiol.68.040204.100431

Reeve, S. (2016). Virtual Flybrain. Retrieved August 22, 2017, from http://www.virtualflybrain.org/site/stacks/index.htm?id=FBbt_00003624

Reiher, W., Shirras, C., Kahnt, J., Baumeister, S., Isaac, R. E., & Wegener, C. (2011). Peptidomics and Peptide Hormone Processing in the *Drosophila* Midgut. *Journal of Proteome Research*, *10*(4), 1881–1892. https://doi.org/10.1021/pr101116g

Santos, J. G., Vömel, M., Struck, R., Homberg, U., Nässel, D. R., Wegener, C., & Zitnan, D. (2007). Neuroarchitecture of Peptidergic Systems in the Larval Ventral Ganglion of Drosophila melanogaster. *PLoS ONE*, *2*(8), e695. https://doi.org/10.1371/journal.pone.0000695

Šimo, L., Koči, J., & Park, Y. (2013). Receptors for the neuropeptides, myoinhibitory peptide and SIFamide, in control of the salivary glands of the blacklegged tick Ixodes scapularis. *Insect Biochemistry and Molecular Biology*, *43*(4). https://doi.org/10.1016/j.ibmb.2013.01.002

Southall, T. D., Gold, K. S., Egger, B., Davidson, C. M., Caygill, E. E., Marshall, O. J., & Brand, A. H. (2013). Cell-type-specific profiling of gene expression and chromatin binding without cell isolation: Assaying RNA pol II occupancy in neural stem cells. *Developmental Cell*, *26*(1). https://doi.org/10.1016/j.devcel.2013.05.020

Tanaka, N. K., Endo, K., & Ito, K. (2012). Organization of antennal lobe-associated neurons in adult *Drosophila melanogaster* brain. *The Journal of Comparative Neurology*, *520*(18), 4067–4130. https://doi.org/10.1002/cne.23142

Tayler, T. D., Pacheco, D. A., Hergarden, A. C., Murthy, M., & Anderson, D. J. (2012). A neuropeptide circuit that coordinates sperm transfer and copulation duration in Drosophila. *Proceedings of the National Academy of Sciences*, *109*(50), 20697–20702. https://doi.org/10.1073/pnas.1218246109

Vanden Broeck, J. (2001). Neuropeptides and their precursors in the fruitfly, Drosophila melanogaster☆. *Peptides*, *22*(2), 241–254. https://doi.org/10.1016/S0196-9781(00)00376-4

Veenstra, J. A. (2014). The contribution of the genomes of a termite and a locust to our understanding of insect neuropeptides and neurohormones. *Frontiers in Physiology*, *5*(Nov). https://doi.org/10.3389/fphys.2014.00454

Viswanath, V., Story, G. M., Peier, A. M., Petrus, M. J., Lee, V. M., Hwang, S. W., … Jegla, T. (2003). Ion channels: Opposite thermosensor in fruitfly and mouse. *Nature*, *423*(6942), 822–823. https://doi.org/10.1038/423822a

Vogel, K. J., Brown, M. R., & Strand, M. R. (2013). Phylogenetic investigation of peptide hormone and growth factor receptors in five dipteran genomes. *Frontiers in Endocrinology*, *4*(DEC). https://doi.org/10.3389/fendo.2013.00193

Winther, ?sa M.E., Siviter, R. J., Isaac, R. E., Predel, R., & N?ssel, D. R. (2003). Neuronal expression of tachykinin-related peptides and gene transcript during postembryonic development of *Drosophila*. *Journal of Comparative Neurology*, *464*(2), 180–196. https://doi.org/10.1002/cne.10790

Wu, J. S., & Luo, L. (2006). A protocol for dissecting Drosophila melanogaster brains for live imaging or immunostaining. https://doi.org/10.1038/nprot.2006.336

Wu, X., Tanwar, P. S., & Raftery, L. A. (2008). Drosophila follicle cells: morphogenesis in an eggshell. *Seminars in Cell & Developmental Biology*, *19*(3), 271–82. https://doi.org/10.1016/j.semcdb.2008.01.004

Xiang, Y., Yuan, Q., Vogt, N., Looger, L. L., Jan, L. Y., & Jan, Y. N. (2010). Light-avoidance-mediating photoreceptors tile the Drosophila larval body wall. *Nature*, *468*(7326), 921–6. https://doi.org/10.1038/nature09576

Xie, T., Song, X., Jin, Z., Pan, L., Weng, C., Chen, S., & Zhang, N. (2008). Interactions between stem cells and their niche in the Drosophila ovary. *Cold Spring Harbor Symposia on Quantitative Biology*, *73*, 39–47. https://doi.org/10.1101/sqb.2008.73.014

Xie, T., & Spradling, A. C. (2000). A niche maintaining germ line stem cells in the Drosophila ovary. *Science (New York, N.Y.)*, *290*(5490), 328–30. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11030649

Xie, T., & Spradling, A. C. (1998). decapentaplegic Is Essential for the Maintenance and Division of Germline Stem Cells in the Drosophila Ovary. *Cell*, *94*(2), 251–260. https://doi.org/10.1016/S0092-8674(00)81424-5

Xu, C., Luo, J., He, L., Montell, C., & Perrimon, N. (2017). Oxidative stress induces stem cell proliferation via TRPA1/RyR-mediated Ca $^{2+}$ signaling in the *Drosophila* midgut. *eLife*, *6*. https://doi.org/10.7554/eLife.22441

Yamamoto, D., & Nakano, Y. (1998). Genes for Sexual Behavior. *Biochemical and Biophysical Research Communications*, *246*(1), 1–6. https://doi.org/10.1006/bbrc.1998.8259

Yamamoto, S., & Seto, E. S. (2014). Dopamine Dynamics and Signaling in Drosophila: An Overview of Genes, Drugs and Behavioral Paradigms. *Experimental Animals*, *63*(2). https://doi.org/10.1538/expanim.63.107

Yu, J., Chen, C., Wang, J., Cheng, Y., Wu, Q., Zhong, Y., & Shen, X. (2012). In vitro effect of adenosine on the mRNA expression of Kir 2.1 and Kir 4.1 channels in rat retinal Müller cells at elevated hydrostatic pressure. *Experimental and Therapeutic Medicine*, *3*(4), 617–620. https://doi.org/10.3892/etm.2012.457

Zhang, D.-Y., Lau, C.-P., & Li, G.-R. (2009). Human Kir2.1 channel carries a transient outward potassium current with inward rectification. *Pfl?gers Archiv - European Journal of Physiology*, *457*(6), 1275–1285. https://doi.org/10.1007/s00424-008-0608-0

Zimmer, M., Gray, J. M., Pokala, N., Chang, A. J., Karow, D. S., Marletta, M. A., … Bargmann, C. I. (2009). Neurons detect increases and decreases in oxygen levels using distinct guanylate cyclases. *Neuron*, *61*(6), 865–79. https://doi.org/10.1016/j.neuron.2009.02.013

METABEL TORI NAA MERLE MARKWEI
metty.markwei@gmail.com | 203-812-0976| 2300 Overlook Rd, Cleveland, OH 44106.

PERSONAL DATA
Born: 27th January 1994
Place of Birth: Accra, Ghana
Citizenship: Ghanaian

EDUCATION

May 2022	*(Expected)* **Doctor of Medicine, with special qualifications in research.** The Cleveland Clinic Lerner College of Medicine, Cleveland, Ohio.
Aug 2017	**Master of Sciences (ScM)**, Department of Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health
May 2015	**Bachelor of Arts (BA),** Medical Anthropology, Yale University *Special Program,* Global Health

FELLOWSHIPS & AWARDS

May 2015	Janifer Lynn Lighten '83 Award
May 2014	Yale Global Health Fellowship Field Research Award
May 2014	CIPE Howard Hilgendorf Memorial Junior Research Fellowship Award
Sept. 2011	Zonta International Young Women in Public Affairs Award

SELECTED PUBLICATIONS

Oct. 2014	Capacity Assessment of selected health care facilities to pilot the implementation of Package for Essential Non-communicable disease intervention in Ghana. Ghana Health Service & WHO. BioMed Central. 2014

RESEARCH EXPERIENCE

Aug '14 – May '15	**Research Assistant**, Yale Reproductive Ecology Lab Assisted biological anthropology research on reproduction among Toba women in Argentina
May – Aug '13	**PEN Research Intern**, Ghana Health Services

- Developed proposal & budget for implementation of WHO Package for Essential NCDs.
- Conducted community health fieldwork in Brong-Ahafo Region.
- Participated in West African Health Organization (WAHO) conference.

Aug '12 – Mar '13 **Research Assistant,** Haven Free Clinic, Research Department
- Collected patient needs and quality of healthcare delivery information for minority patient groups.

ACTIVITIES

Jan – May '16 **Math Tutor,** Wolfe Street Academy, Baltimore City Public Schools.
- Tutored Science & literacy on a weekly basis to Grade 5 children.
- Volunteered as Girls on the Run Coach to empower girls aged 8-13years for 5K race.

May'16 – May '17 **Volunteer**, Johns Hopkins Hospital & Bayview Medical Center
- Assisted orthopedic chief resident at Bayview in the O.R. for surgery
- Patient research in-take for Facial Reconstruction surgery at Ear, Nose and Throat outpatient department, Johns Hopkins Hospital.
-

Jul. – Aug. '12 **History Teacher,** Yale Alumni Service Corps in Yamoransa, Ghana
- Engineered Ghanaian history curriculum for primary school kids.
- Mentored Ghanaian public high school students for a week on college application process.

LANGUAGES

English (Proficient), French (Semi-proficient), West African Languages: Akan (Proficient), Ga (Semi-proficient).