



Title	Transcriptional Repression of reaper by Stand still Ensures Female Germline Development in Drosophila
Author(s)	松井, 将也
Citation	大阪大学, 2025, 博士論文
Version Type	VoR
URL	https://doi.org/10.18910/105271
rights	
Note	

The University of Osaka Institutional Knowledge Archive : OUKA

<https://ir.library.osaka-u.ac.jp/>

The University of Osaka

Doctoral Thesis

**Transcriptional Repression of *reaper*
by Stand Still Ensures Female Germline
Development in *Drosophila***

Masaya Matsui

Graduate School of Frontier Biosciences

The University of Osaka

December 2025

Abstract

Apoptosis is essential for tissue morphogenesis and the maintenance of cellular integrity throughout development. To ensure reproductive success, apoptotic cell death is tightly regulated to balance germline cell number and preserve germline quality. However, the molecular mechanisms governing apoptotic susceptibility in germline cells remain poorly understood.

In this thesis, I characterize *stand still* (*stil*) as a female germline-specific regulator of apoptosis in *Drosophila*. Loss-of-function mutants of *stil* result in an almost complete depletion of germline cells, caused by apoptotic cell death through transcriptional upregulation of the pro-apoptotic gene *reaper* (*rpr*). Genome-wide profiling revealed that Stil associates with upstream regions of *rpr*, and reporter assays in S2 cells confirmed that its N-terminal BED-type zinc finger directly represses *rpr* expression. In contrast, the uncharacterized C-terminal region plays a central role in nuclear co-localization within germline cells. Notably, even in the absence of *stil*, undifferentiated female germline cells exhibit resistance to apoptosis. Analysis of published chromatin datasets indicates that the *rpr* locus adopts a closed, H3K9me3-enriched chromatin configuration in these cells, consistent with a Stil-independent mechanism of transcriptional repression.

My findings uncover two complementary mechanisms that protect the female germline from *rpr*-dependent apoptosis: Stil-mediated transcriptional repression, which functions in both undifferentiated and differentiated germline cells, and a chromatin-based silencing mechanism that operates exclusively in

undifferentiated cells. Collectively, this work highlights how lineage-specific transcriptional and epigenetic programs cooperate to safeguard germline integrity and secure reproductive success in *Drosophila*.

Acknowledgement

I would like to express my deepest gratitude to my supervisor, Prof. Toshie Kai, for her invaluable guidance, encouragement, and continuous support throughout my doctoral studies. Despite financial challenges that required me to work in industry for a period of time, her understanding and patience enabled me to continue my research and bring this project to completion. I am especially thankful for her mentorship, insightful feedback, and for fostering an open and stimulating research environment that greatly contributed to my growth as a researcher. I am also deeply grateful for her generous support with fellowships and financial matters.

I would also like to express my sincere appreciation to the members of my doctoral thesis committee: Prof. Tatsuo Fukagawa, Prof. Makoto Tachibana, and Prof. Hiroshi Sasaki, for their valuable time, thoughtful evaluation, and constructive feedback. Their insightful perspectives and suggestions significantly improved the quality of this work.

I am sincerely grateful to Associate Prof. Taichiro Iki for patiently discussing my research, offering invaluable advice, and constantly encouraging me to pursue new ideas. Our discussions greatly broadened my horizons on both science and life. I also thank Assistant Prof. Shinich Kawaguchi for his generous help with bioinformatics analyses and experiments, as well as for his valuable advice. I am grateful to Assistant Prof. Ritsuko Suyama for her thoughtful guidance and support with experiments and analyses. I would also like to thank the secretaries and technicians, including Kazunori Morikawa, Miwako Okamoto, Minako Moriguchi, Koutaro Taniguchi, Dr. Hilo Yen, and

Chisato Yanagisawa, for their assistance with administrative tasks and experiments. I am indebted to all the members of the Kai lab—Moe Takami, Yuxuan Lin, Lim Lin Xenia, Fengmei Xu, Wakana Isshiki, Xin Xu, Hinano Minami, Ikele Chinyere Marycynthia, Haitian Qin, Miaomiao Zhao, Naoki Murakami, Jinglan Zheng, and Daisuke Kurita—for creating a stimulating and enjoyable research environment.

I deeply appreciate all my friends for their concern and support during difficult times, and I am grateful that our friendship has endured despite time and distance. I also wish to acknowledge FUJIFILM Wako Pure Chemical Corporation, where I worked during my doctoral course, and express my sincere gratitude to my manager Dr. Takashi Torashima, Yoshihiro Kayama, and my senior colleagues Junki Hosaka and Ryota Kasuya for their understanding and support, which allowed me to carry out my doctoral research alongside professional work.

I owe my heartfelt gratitude to my partner, Dr. Hinako Maeda, for standing by me with love, patience, and strength. Her unwavering support sustained me through the most difficult times of my doctoral journey.

Finally, I am profoundly indebted to my family—my father, Tomomi Matsui, my mother, Yukari Matsui, my twin brother, Atsuya Matsui, and my sister, Misaki Matsui—for their constant support, encouragement, and understanding. Their endless love and belief in me have been my greatest source of strength and inspiration throughout this journey.

Table of Contents

Abstract.....	I
Acknowledgement	III
Table of Contents.....	V
List of Figures and Tables.....	VII
1. Introduction	1
1.1 The discovery and evolution of programmed cell death.....	1
1.2 Apoptosis plays central role in developmental regulation	2
1.3 Molecular basis of apoptosis in <i>Drosophila melanogaster</i>	3
1.4 The RHG gene family: central regulators of apoptosis in <i>Drosophila</i>	5
1.5 Development of the germline in <i>Drosophila</i>	7
1.5.1 Embryonic gonad formation	7
1.5.2 Temporal control of larval germline development	9
1.5.3 Oogenesis in the adult ovary	10
1.6 TGF β ligand from the niche maintains female germline stem cell identity.....	12
1.7 Stage-specific regulation of germline cell death during oogenesis	14
1.7.1 Coordinated regulatory mechanisms ensure stringent control of apoptosis in germline stem cells.....	14
1.7.2 Quality control of early cysts at the first checkpoint.....	16
1.7.3 Mid-oogenesis checkpoint and regulation of egg chamber survival.....	17
1.7.4 Programmed cell death of nurse cells in late oogenesis	18
1.8 Identification of <i>stand still</i> (<i>stil</i>) as a key regulator of germline survival	19
1.9 Conserved motif of BED-type zinc finger	21
1.10 DNA damage response and cellular outcome in <i>Drosophila</i>	22
1.11 Meiosis in <i>Drosophila</i> oogenesis	25
1.12 Molecular basis of meiotic recombination in <i>Drosophila</i>	27
1.13 Thesis overview	29
2. Material and Methods	31
2.1 Fly genetics.....	31
2.1.1 Fly husbandry.....	31
2.1.2 Generation of transgenic lines	33
2.2 Immunofluorescence staining	36
2.3 TUNEL-labeling assay	38
2.4 Western blotting	39
2.5 RT-PCR and quantitative RT-PCR.....	39

2.6 mRNA-seq analysis	40
2.7 DamID-seq and data processing	41
2.8 Fertility test.....	42
2.9 S2 cell culture experiments.....	43
2.10 Proximity-dependent biotin identification (BioID/TurboID)	45
2.10.1 Expression of miniTurbo proteins and biotinylation in ovaries	45
2.10.2 Purification of biotinylated proteins	45
2.10.3 LC-MS / MS analysis.....	46
3. Results	47
3.1 Stil Protects Female Germline Cells from Apoptotic Cell Death	47
3.1.1 Loss of <i>stand still</i> (<i>stil</i>) leads to female germline cell loss	47
3.1.2 <i>stil</i> -deficient female germline cells undergo apoptotic cell death	52
3.1.3 Stil regulates proapoptotic genes to suppress apoptosis in female germline cells	59
3.1.4 Stil represses <i>rpr</i> expression by targeting the regulatory regions via its BED-type zinc finger	66
3.2.5 DNA damage response to double-strand breaks promotes germline cell loss in <i>stil</i> mutants	73
3.2.6 Stil regulates meiotic recombination and DNA repair genes to prevent abnormal DSB formation	78
3.2 Epigenetic Regulation Modulates apoptosis in Female Germline Stem Cells	83
3.2.1 Undifferentiated Germline Cells Exhibit Resistance to Apoptosis in <i>stil</i> loss	83
3.2.2 <i>rpr</i> Silencing in Undifferentiated Germline Cells Is Associated with Chromatin States	86
3.3 Identification of Stil's Interacting Partner	89
3.3.1 Identification of C-terminal Binding Protein (CtBP) as a Stil Interacting Protein by TurboID	89
3.3.2 CtBP interacts with Stil, but not necessary for localization and function for Stil ..	94
4. Discussion	99
4.1 Transcriptional repression of <i>rpr</i> by Stil in female germline cells	99
4.2 Stil maintains the apoptotic threshold by balancing Rpr-DIAP1 interactions	100
4.3 Chromatin-based silencing for <i>rpr</i> in germline stem cells	102
4.4 Diptera-specific evolution of apoptosis repression in the female germline	104
5. Reference	108
Publication and Contribution	139

List of Figures and Tables

Figure 1.1 Molecular pathways of <i>Drosophila</i> apoptosis	5
Figure 1.2 Discrete steps in <i>Drosophila</i> germ cell migration	9
Figure 1.3 Development of larval ovary in <i>Drosophila</i>	10
Figure 1.4 Schematic representation of <i>Drosophila</i> oogenesis	12
Figure 1.5 Dpp-signaling pathway maintains the stemness of germline stem cell	14
Figure 1.6 Conservation of the BED-type zinc finger in Stil	22
Figure 1.7 DNA damage response pathway linking damage sensing to cell fate decisions	25
Figure 1.8 Molecular mechanisms underlying DSB formation in <i>Drosophila</i> meiosis ..	29
Table 2.1 <i>Drosophila</i> strains used in the current study	31
Table 2.2 List of primers used for the construction of transgenes in the current study	334
Table 2.3 List of Primary antibodies used for Immunofluorescence staining in the current study	37
Table 2.4 List of primers for RT-PCR and quantitative RT-PCR in the current study ...	40
Table 2.5 List of plasmids used S2 cell experiments in the current study	44
Figure 3.1.1 Disruption of <i>stil</i> induces female germline cell depletion	49
Figure 3.1.2 <i>stil</i> function is indispensable in female germline cells not male germline cells	51
Figure 3.1.3 Germline, but not somatic, knockdown of <i>stil</i> disrupts germline survival and differentiation	53
Figure 3.1.4 <i>stil</i> is irrelevant to sex determination	55
Figure 3.1.5 Loss of <i>stil</i> in the germline increases apoptotic cells	56
Figure 3.1.6 <i>stil</i> loss induces apoptotic cell death in female germline cells	58
Figure 3.1.7 Transcriptome analysis reveals upregulation of pro-apoptotic genes	61
Figure 3.1.8 Loss of <i>stil</i> in the germline elevates <i>rpr</i> expression in ovaries not testes	63
Figure 3.1.9 <i>rpr</i> causes germline cell loss in <i>stil</i> mutants	66
Figure 3.1.10 DamID reveals Stil enrichment in promoter regions, particularly around <i>rpr</i>	67
Figure 3.1.11 BED-type zinc finger in Stil plays a crucial role in germline cell survival	69
Figure 3.1.12 Stil interacts with <i>rpr</i> -transcriptional control region to repress its expression	72
Figure 3.1.13 Accumulation of DNA double-strand breaks in <i>stil</i> mutant oocytes	74
Figure 3.1.14 Germline knockdown of DDR pathway components rescues germline cell	

loss in <i>stil</i> mutants.....	77
Figure 3.1.15 Differential expression of DNA repair and meiotic recombination genes in <i>stil</i> mutants	79
Figure 3.1.16 Germline knockdown of meiotic recombination factors partially recovered germline cell death defects in <i>stil</i> mutants.....	82
Figure 3.2.1 Reduced germline cell number in <i>stil</i> mutant 3rd instar larval ovaries.....	83
Figure 3.2.2 <i>stil</i> mutant germline stem cells resist apoptosis	85
Figure 3.2.3 Chromatin accessibility around <i>rpr</i> changes during female germline development.....	87
Figure 3.2.4 H3K9me3, but not H3K27, is enriched around <i>rpr</i> specifically in germline stem cells	88
Figure 3.3.1 Stil is not co-localized with DNA in somatic cells	889
Figure 3.3.2 Application of TurboID with miniTurbo	91
Figure 3.3.3 C-terminal binding protein (CtBP) as promising protein which binds to Stil	93
Figure 3.3.4 The binding partner CtBP has no effect on Stil localization	97
Figure 4.1 Two-layered defense protecting female germline cells from <i>rpr</i> -dependent apoptosis.....	103
Figure 4.2 Conservation of Rpr and Stil within Diptera.....	107

1. Introduction

1.1 The discovery and evolution of programmed cell death

Cell death is a fundamental biological process by which cells lose their function and are ultimately eliminated from an organism. Initially described in the 19th century, cell death was long regarded as a passive phenomenon and the inevitable endpoint of biological systems (Clarke & Clarke, 1996). The first recognized form of cell death, necrosis, was characterized by German pathologist Rudolf Virchow and is typically associated with injury or pathological conditions (Buja, 2021). In contrast, a distinct, regulated form of cell death—later termed apoptosis—was identified in 1972 by Kerr, Wyllie, and Currie through ultrastructural studies (Jacobson et al., 1997). They observed specific morphological changes in dying cells under physiological conditions, including cell shrinkage, chromatin condensation, preservation of membrane integrity, and rapid clearance by phagocytosis. Unlike necrosis, apoptosis is an active, genetically programmed process critical for development, tissue homeostasis, and immune regulation, and is classified as a form of programmed cell death (PCD) (W. Park et al., 2023).

PCD refers to a controlled sequence of molecular events that culminate in cell death in response to various physiological or developmental cues. Besides apoptosis, PCD encompasses a variety of other forms of regulated cell death, including autophagic cell death, mitoptosis, paraptosis, pyroptosis, NETosis, necroptosis, immunogenic cell death, entosis, methuosis, parthanatos, ferroptosis, autosis, alkaliptosis, oxeiptosis, cuproptosis, and erebosis (W. Park

et al., 2023; Yuan & Kroemer, 2010). Organisms are thought to maintain multiple cell death modalities as backup mechanisms, ensuring that cell elimination can proceed even if one pathway is inhibited.

1.2 Apoptosis plays central role in developmental regulation

Apoptosis, a form of programmed cell death (PCD), is a highly regulated biological process essential for animal development and tissue homeostasis. The term “apoptosis” from Greek ‘apo’ (away) and ‘ptosis’ (falling) — like leaves falling from a tree, which describes the process of cells undergoing controlled self-destruction and detachment from the surrounding tissues (W. Park et al., 2023). Dysregulation of apoptosis —whether excessive or insufficient —can lead to severe consequences and is implicated in various human diseases, including cancer, immune disorders, cardiovascular disorders, and neurodegenerative conditions (Clemens et al., 2000; Hanahan & Weinberg, 2011; Lee & Gustafsson, 2009; Mattson, 2000).

Tissue-specific apoptosis, which selectively activates PCD in particular tissues or cell types, plays a crucial role in developmental processes by preserving the function and integrity of surrounding tissues (Hill et al., 2024). This process is tightly controlled both spatially and temporally through the expression and localization of pro-apoptotic factors. Regulation occurs in response to developmental cues and environmental stimuli, which vary across cellular contexts (Fuchs & Steller, 2011).

In both male and female gonads, apoptosis is meticulously regulated to control cell numbers and maintain germline cell quality, ensuring reproductive

success (Aitken et al., 2011). Defective regulation of apoptosis in germline cells can result in the accumulation of superfluous cells or disruptions in gametogenesis, ultimately leading to infertility or the production of abnormal offspring (Chaudhry et al., 2021; Knudson et al., 1995). Conversely, excessive apoptosis reduces germline cell populations, causing a decline in fertility at an early age (Almeida et al., 2013; Huang et al., 2023). While apoptosis is pivotal for germline maintenance, the precise regulatory mechanisms underlying this process remain an area of critical investigation.

1.3 Molecular basis of apoptosis in *Drosophila melanogaster*

A breakthrough in understanding the mechanism of apoptotic cell death was achieved through genetic studies in the nematode *Caenorhabditis elegans*, followed by research in the fruit fly *Drosophila melanogaster* and the mouse. The identification of mutations that specifically affect programmed cell death and their organization into a genetic pathway demonstrated that cell death is a regulated developmental fate, orchestrated by specific genes that initiate a program of cellular self-destruction (H. M. Ellis & Horvitz, 1986). Subsequent molecular characterization of these genes led to the elucidation of the core apoptotic machinery, notably including a family of evolutionarily conserved cysteine proteases known as caspases (Hengartner, 2000; Thornberry & Lazebnik, 1998). Programmed cell death (PCD), specifically apoptosis, occurs in 131 out of the 1,090 somatic cells during the development of the *C. elegans* hermaphrodite (R. E. Ellis et al., 1991). Mutations in *egl-1*, *ced-3*, or *ced-4* block this cell death, whereas loss-of-function mutations in *ced-9* cause widespread ectopic cell death,

resulting in developmental lethality (Hengartner et al., 1992). The central player in *C. elegans* apoptosis is the caspase protease CED-3, which executes the cell death program (J Li & Yuan, 2008; Yuan et al., 1993). Its activation is mediated by CED-4, the *C. elegans* homolog of the adaptor protein Apaf-1, a key component of the apoptosome (Zou et al., 1999). The pro-apoptotic activity of CED-4 is normally inhibited by the anti-apoptotic protein CED-9 (Hengartner & Horvitz, 1994). The BH3-only protein EGL-1 promotes apoptosis by disrupting the interaction between CED-9 and CED-4, thereby allowing CED-4 to activate CED-3 and trigger cell death (Yarychivska et al., 2024).

Apoptotic cell death has also been well-studied in *Drosophila melanogaster* due to its simple and accessible anatomy, as well as its powerful genetic and molecular biology tools. Furthermore, *Drosophila* consists of more than 1,000-fold the number of cells found in *C. elegans*, providing deeper insights into the roles and regulation of apoptosis in multicellular organisms (Kornbluth & White, 2005). In *Drosophila*, apoptosis is initiated by the transcriptional activation of the pro-apoptotic *reaper* (*rpr*), *hid*, and *grim* (collectively referred to as RHG) genes in response to various apoptotic stimuli (D. Xu et al., 2009a; J. Zhang et al., 2023) (Figure 1.1). The major inhibitor of apoptosis protein, DIAP1, suppresses apoptosis by ubiquitinating the initiator caspase Dronc (a caspase-9-like protein). This suppression is antagonized by RHG proteins, thereby promoting apoptosis. Activated Dronc subsequently cleaves and activates the effector caspases Drice and Dcp-1 (caspase-3-like proteins), which execute the apoptotic program (Nakajima & Kuranaga, 2017). The activity of effector caspases is specifically inhibited by the baculovirus protein P35 (Hay et al., 1994).

Apoptotic cell death is essential for the proper development of all major structures in *Drosophila* and functions as a stringent quality control mechanism to eliminate defective or unnecessary cells during development.

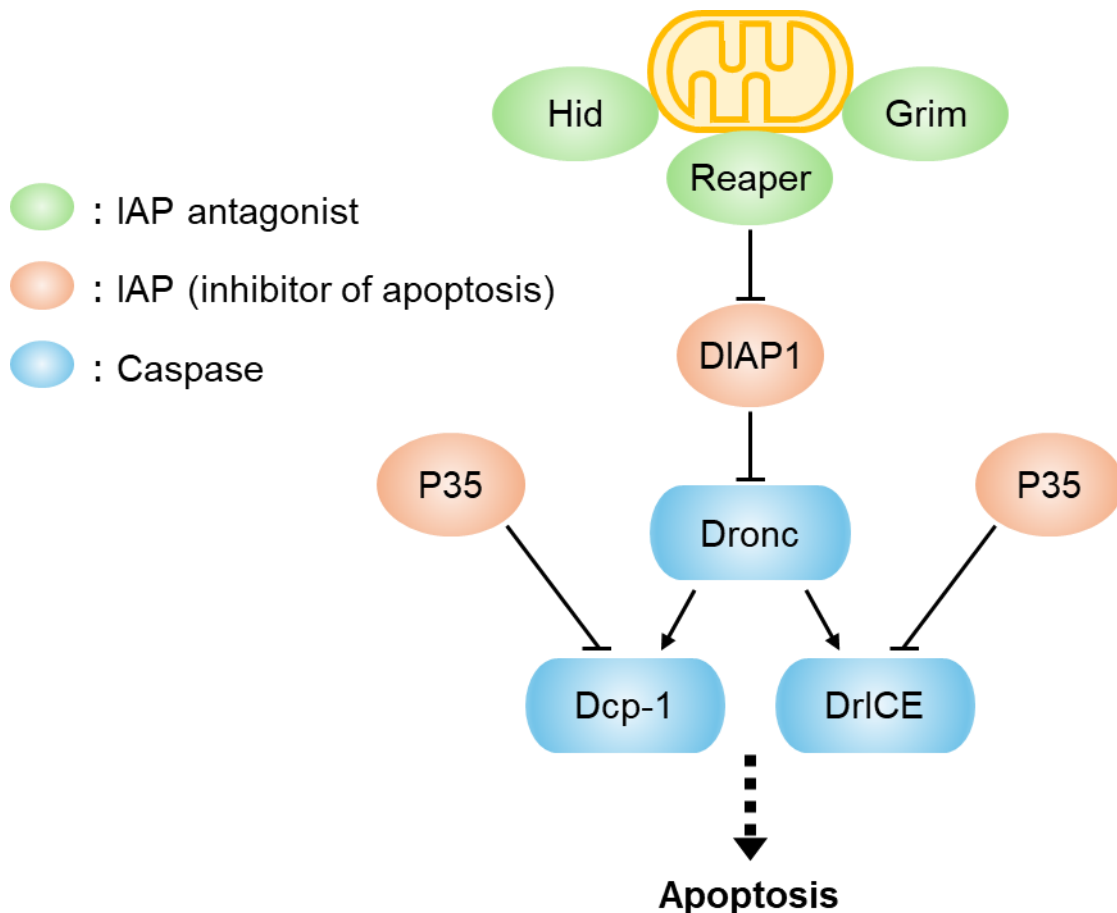


Figure 1.1 Molecular pathways of *Drosophila* apoptosis

The pro-apoptotic RHG genes (*reaper*, *hid*, and *grim*) sense death signals from both intrinsic and extrinsic sources and antagonize the inhibitor of apoptosis (IAP) protein DIAP1. The suppression of DIAP1 leads to the activation of the initiator caspase Dronc, a Caspase-9 homolog, which subsequently activates effector caspases. At the downstream end of the cascade, the effector caspases DrICE and Dcp-1 induce apoptosis by cleaving peptide bonds of target proteins. In addition, the baculoviral protein P35 specifically inhibits the activation of effector caspases.

1.4 The RHG gene family: central regulators of apoptosis in *Drosophila*

Apoptosis is induced to eliminate damaged or unnecessary cells in

response to apoptotic stimuli such as DNA damage, oxidative stress, or nutrient deprivation. These stimuli are sensed and transduced by intracellular signaling pathways. Proper induction of apoptosis requires factors and mechanisms that accurately detect these signals and activate the apoptotic machinery (Steller, 2008). In *Drosophila*, apoptosis is initiated by the transcriptional induction of pro-apoptotic RHG genes, including *reaper* (*rpr*), *head involution defective* (*hid*), *grim*, and *sickle* (*skl*), in response to various signaling cues (Ryoo & Baehrecke, 2010; D. Xu et al., 2009b). Among these, *rpr*, *hid*, and *grim* are essential for proper execution of apoptosis, as deletion of these genes leads to a severe reduction in apoptotic activity (D. Xu et al., 2009a). In contrast, *skl* shares sequence similarity with *rpr*, but is not functionally identical, as it primarily potentiates the activity of other RHG genes rather than inducing apoptosis independently (Ryoo & Baehrecke, 2010; D. Xu et al., 2009b). RHG proteins localize to the mitochondria and share a conserved N-terminal motif known as the IAP-binding motif (IBM), which interacts with the baculovirus IAP repeat (BIR) domains of inhibitor of apoptosis proteins (IAPs) (Clavier et al., 2016; Shi, 2002a). Binding of RHG proteins to IAPs, such as DIAP1, promotes proteasome degradation, thereby releasing initiator and effector caspases like Dronc and DrICE from inhibition, and triggering the apoptotic cascade (Goyal et al., 2000; Holley et al., 2002; Ryoo et al., 2002; Wang et al., 1999; S. J. Yoo et al., 2002; Zachariou et al., 2003). Expression of RHG genes is primarily regulated at the transcriptional level, allowing precise temporal and spatial control of apoptosis during development and stress responses (D. Xu et al., 2009b).

The *rpr* gene encodes a small protein of 65 amino acids containing both

the IBM and the Grim helix 3 (GH3) domain, which is essential for its mitochondrial localization and its ability to induce DIAP1 degradation (Olson et al., 2003; Sandu et al., 2010). Rpr directly binds DIAP1 and promotes its degradation via self-ubiquitination or translational inhibition, resulting in effective activation of downstream caspases (Holley et al., 2002). *rpr* transcription is tightly regulated by multiple signaling pathways and associated transcription factors, which act through cis-regulatory elements located in its extensive upstream control region (Fuchs & Steller, 2011). A well-established regulatory mechanism involves the tumor suppressor p53, which responds to DNA damage by binding to a conserved p53 response element (p53RE) located approximately 4.8 kb upstream of *rpr* (Brodsky et al., 2000). This element, which is evolutionarily conserved from *Drosophila* to humans, functions as a stress-responsive enhancer and retains its activity even when placed in ectopic genomic locations (Link et al., 2013). In addition to p53, other transcriptional regulators such as the homeobox gene *Deformed* (*Dfd*) and the AP-1 complex, a downstream effector of JNK signaling, have been shown to bind to *rpr* enhancers and promote its expression in somatic tissues (Beira et al., 2014; Lohmann et al., 2002). Conversely, the Dpp signaling pathway negatively regulates *rpr* expression through the transcriptional repressor Shn (Shnurri), which binds to its consensus sequences located upstream of *rpr* (Beira et al., 2014).

1.5 Development of the germline in *Drosophila*

1.5.1 Embryonic gonad formation

In sexually reproducing animals, haploid gametes (eggs or sperm) are

produced to maintain the continuity of the species (Gleason et al., 2018). *Drosophila melanogaster* is an excellent model organism for studying genetics and cell biology due to its ease of handling, well-established genetic tools, simple gonadal structure, and the availability of various mutant alleles (Jennings, 2011). The process of egg production, known as oogenesis, is tightly regulated to ensure the generation of progeny across generations.

The embryonic gonad consists of two cell types: germ cells and somatic gonadal cells. These two types of cells are specified in different regions and at distinct developmental stages and subsequently migrate to a common location where the gonad is formed. Germ cells originate from a specialized, maternally supplied cytoplasm called the germ plasm, which is localized at the posterior pole of the early embryo (Mahowald, 1962; Snee & Macdonald, 2004) (Figure 1.2). These cells, referred to as primordial germ cells (PGCs), ingress into the embryo from the dorsal side and are carried to the posterior midgut region. They then actively migrate toward the underlying mesoderm, where they associate with somatic gonadal precursor cells and assemble into the embryonic gonad (Santos & Lehmann, 2004).

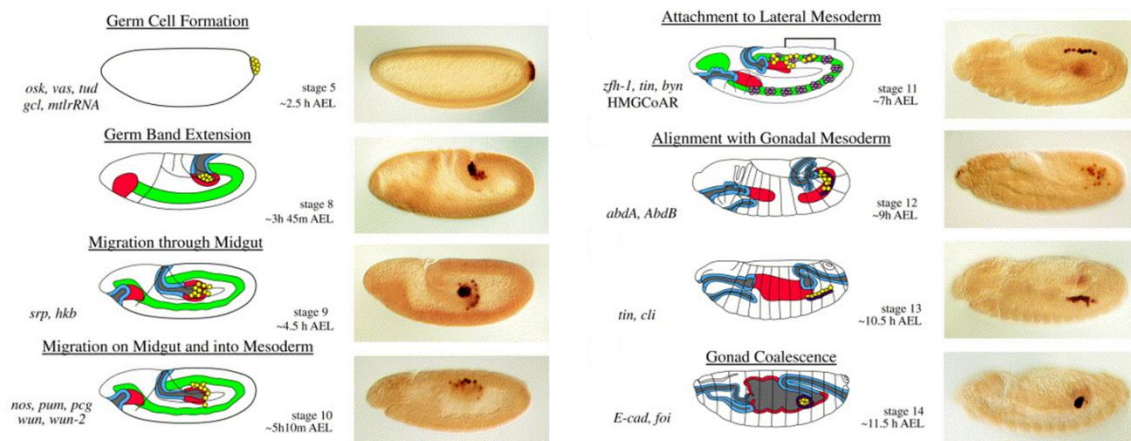


Figure 1.2 Discrete steps in *Drosophila* germ cell migration

The left panels of each stage depict schematic representations of the embryo. Distinct colors indicate different tissues: germ cells (yellow), mesoderm (green), foregut/hindgut (blue), midgut (red), lateral mesoderm (light purple), and somatic gonadal precursors (dark purple). The right panels of each stage show embryos stained with an antibody against the germline cell marker Vasa protein. Genes required for each step of germ cell migration are listed on the left. (Adapted from (Starz-Gaiano & Lehmann, 2001))

1.5.2 Temporal control of larval germline development

After the completion of embryogenesis, the larva hatches and begins larval development, which lasts approximately 120 hours. Larval development proceeds in a temporally defined manner (Gilboa, 2015). During the first four days after egg laying (AEL), PGCs (or early germline stem cells) and somatic gonadal precursor cells proliferate while maintaining an undifferentiated state (Lengil et al., 2015) (Figure 1.3). A key developmental transition from proliferation to differentiation occurs around 96 hours AEL (Lengil et al., 2015; Sahut-Barnola et al., 1996). At this stage, terminal filament (TF) cells, a component of the somatic niche, begin to form and stack (Bartoletti et al., 2012). The formation of TFs induces the differentiation of cap cells from intermingled cells (ICs) via Notch signaling (Song et al., 2007). PGCs are recruited to the cap cells and attach via E-cadherin-mediated adhesion, acquiring the identity of adult germline stem cells

(GSCs) (Song et al., 2002). During the late third instar larval stage (~112h AEL), PGCs that do not associate with the niche begin to differentiate, and the first germline cysts become visible at the prepupal stage (Zhu & Xie, 2003).

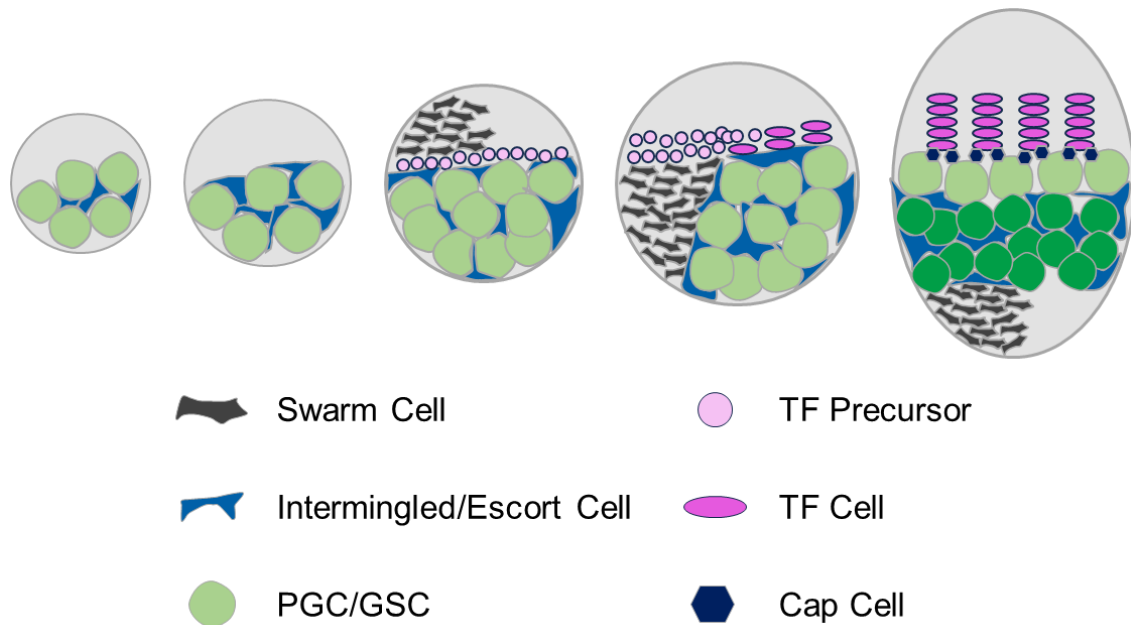


Figure 1.3 Development of larval ovary in *Drosophila*

An illustration of *Drosophila* larval ovaries is shown, covering the period from the onset of larval development (24 h after egg laying) to the end of larval development (120 h after egg laying). Different cell types are indicated in the legend and described in the text. Until 96 h after egg laying, the primary driving force is the growth of both primordial germ cells/germline stem cells (PGCs/GSCs) and ovarian somatic cells. At 96 h, a transition from proliferation to differentiation occurs. During the late third instar stage (120 h after egg laying), the larval ovary contains two types of germline cells: GSCs and differentiating germ cells.

1.5.3 Oogenesis in the adult ovary

The adult female *Drosophila* possesses a pair of ovaries, each consisting of 16–20 tubular units called ovarioles. At the anterior end of each ovariole is the germarium, where early oogenesis events occur, followed by a linear arrangement of developing egg chambers, with the most mature chamber located distally (Kirilly & Xie, 2007) (Figure 1.4). Germline stem cells (GSCs) are

positioned at the anterior tip of the germarium (region 1) and undergo asymmetric division to produce two daughter cells. One daughter cell remains in contact with somatic cap cells, maintaining its stem cell identity, while the other differentiates into a cystoblast (CB) (McLaughlin & Bratu, 2015; A. C. Spradling, 1993). The cystoblast undergoes four rounds of incomplete mitotic divisions to form a 16-cell germline cyst, in which the cells are interconnected by ring canals, cytoplasmic bridges resulting from incomplete cytokinesis (region 2a) (Robinson et al., 1994). Within the cyst, One cell is specified as the oocyte and enters meiosis, while the remaining 15 cells differentiate into nurse cells, which actively synthesize RNAs and proteins that are transferred to the oocyte through ring canals. As the cyst progresses, it becomes surrounded by a monolayer of somatic follicle cells (region 2b). Fully encapsulated cysts bud from the posterior germarium to form stage 1 egg chamber (region 3), which subsequently proceed through 14 morphologically defined developmental stages (He et al., 2011; Lehmann, 2012). Ultimately, mature eggs are ovulated and fertilized to generate embryos (Stetina & Orr-Weaver, 2011).

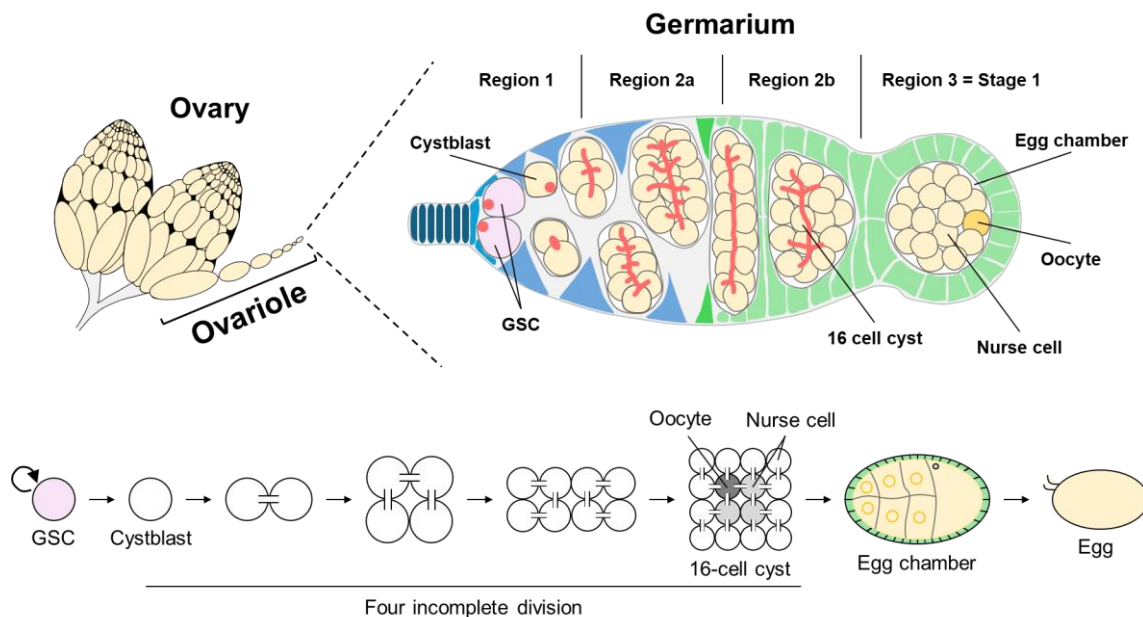


Figure 1.4 Schematic representation of *Drosophila* oogenesis

A female fly possesses a pair of ovaries, each composed of 15–20 single units called ovarioles. Each ovariole contains a germarium at its anterior tip, followed by progressively developing egg chambers. The germarium is subdivided into four functional regions based on cell types and developmental processes. Germline stem cells (GSCs) reside at the anterior end and divide asymmetrically to produce a differentiated cystoblast while self-renewing to maintain the stem cell population. The cystoblast undergoes four rounds of incomplete mitotic division to form a 16-cell cyst, which is encapsulated by somatic cells to form an egg chamber containing 15 nurse cells and a single oocyte.

1.6 TGF β ligand from the niche maintains female germline stem cell identity

Tissue homeostasis relies on a small population of tissue-specific stem cells that are capable of both self-renewal and continuous production of differentiated progeny (L. Liu & Rando, 2011). In the *Drosophila* ovary, germline stem cells (GSCs) reside at a specialized microenvironment called the GSC niche, located at the apical tip of the germarium. This niche is composed of terminal filament (TF) cells and cap cells (Lin, 2002; Ting, 2013). GSCs are physically anchored to the cap cells via adherens junctions and gap junctions, both of which are essential for maintaining GSC identity and self-renewal (Gilboa et al., 2003; Song et al., 2002). Cap cells secrete Decapentaplegic (Dpp), a homolog of

vertebrate bone morphogenetic protein (BMP) 2/4, which serves as the primary niche-derived self-renewal signal. Dpp binds to BMP receptors on the GSC surface, including Thickveins (Tkv), and activates a downstream signaling cascade (Song et al., 2004; Xie & Spradling, 1998). Dpp signaling leads to phosphorylation and nuclear translocation of the transcription factor Mad, which subsequently represses the transcription of *bag-of-marbles* (*bam*), a key differentiation factor (Xie & Spradling, 1998) (Figure 1.5). *bam* encodes a ubiquitin-associated protein and is both necessary and sufficient for germ cell differentiation. Loss-of-function mutations in *bam* cause germ cells to fail to initiate differentiation, leading to the formation of stem cell-like tumors. Conversely, ectopic expression of *bam* in GSCs promotes premature differentiation, resulting in a germarium depleted of germline cells (D. M. McKearin & Spradling, 1990; D. McKearin & Ohlstein, 1995; Ohlstein & McKearin, 1997). Taken together, precise regulation of Dpp/BMP signaling ensures the proper balance between GSC self-renewal and cystoblast (CB) differentiation in a distance-dependent manner, according to the proximity of GSCs to the niche cells (Xie & Spradling, 1998, 2000).

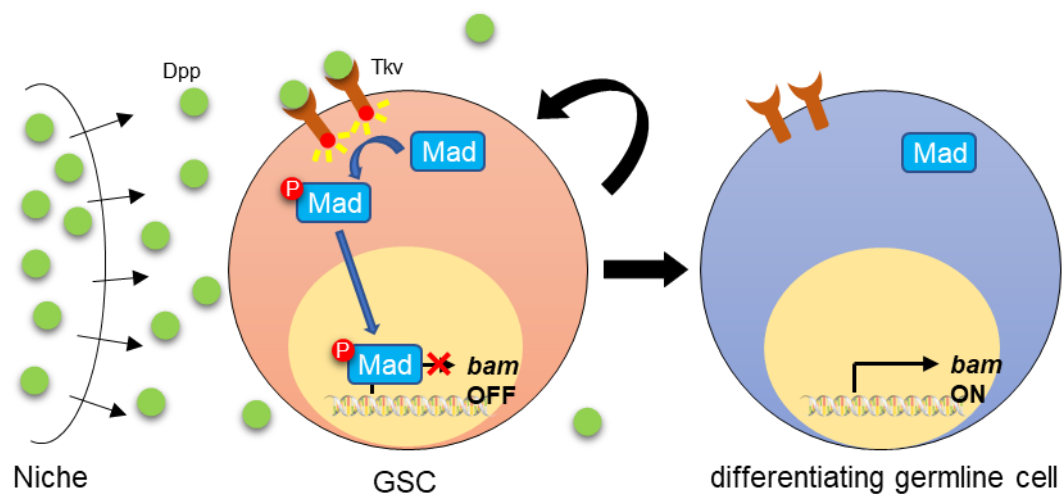


Figure 1.5 Dpp-signaling pathway maintains the stemness of germline stem cell

Niche cells (cap cells) secrete Dpp ligands, which are recognized by the Dpp receptor Thickveins (Tkv) on germline cells. Upon Dpp binding, Tkv activates Mad proteins through phosphorylation. Phosphorylated Mad translocates into the nucleus and represses the expression of *bam*, a key initiator of differentiation. In differentiating germ cells, Dpp signaling is progressively suppressed in a distance-dependent manner from the niche cells.

1.7 Stage-specific regulation of germline cell death during oogenesis

Programmed cell death (PCD) occurs in the germline of many organisms for optimizing reproductive success and allocating resources to the next generation (Baum et al., 2005). In the female germline of *Drosophila*, PCD plays a critical role in regulating both the size and quality of the germ cell population to ensure the production of functional eggs. During oogenesis, stage-specific or stress-responsive PCD events occur, each serving a distinct function (Jenkins et al., 2013; Pritchett et al., 2009a).

1.7.1 Coordinated regulatory mechanisms ensure stringent control of apoptosis in germline stem cells

Oogenesis begins with the asymmetric division of GSCs, enabling both self-renewal and the production of differentiating germline cells (Gleason et al.,

2018). To ensure a continuous supply of germline cells, GSCs are strictly maintained by suppressing cell death (Baonza et al., 2022). Even when exposed to ionizing radiation (IR), which induces DNA damage, GSCs selectively activate the tumor suppressor p53, yet they do not undergo apoptosis (Wylie et al., 2014). This resistance to cell death is mediated by multiple signaling pathways that actively suppress apoptosis within GSCs. One key mechanism involves the microRNA *bantam* (*ban*), which is highly expressed in GSCs and directly targets the 3'-UTR of *hid* mRNA, thereby inhibiting its translation and preventing apoptosis initiation (Brennecke et al., 2003; Xing et al., 2015). Expression of *ban* is upregulated by the receptor tyrosine kinase Tie, a member of the VEGFR/PDGFR family, which is activated by its ligand Pvf1, secreted by neighboring apoptotic cells (Bilak et al., 2014). In addition to *ban*-mediated repression, other mechanisms contribute to the suppression of *hid* expression in GSCs. The *Drosophila* gene *pineapple eye* (*pie*), coding an ortholog of the human G2E3 ubiquitin ligase, promotes the degradation of the transcription factor Forkhead box O (Foxo) (Xing et al., 2012). Foxo, in cooperation with Kay/Fos, can activate *hid* transcription through the JNK signaling pathway. By targeting Foxo for degradation, Pie acts as a negative regulator of *hid* expression (Luo et al., 2007). Although Pie is known to prevent apoptosis in gonadal somatic cells, its deletion does not induce apoptosis in GSCs (Xing et al., 2015). This is likely due to compensatory repression by *ban*, highlighting a layered regulatory system that ensures robust protection of GSCs from apoptotic triggers.

1.7.2 Quality control of early cysts at the first checkpoint

In the germarium, specifically within region 2a/2b, the first checkpoint plays a critical role in ensuring the quality control of early germline cysts during oogenesis. Germline cells exhibiting compromised genomic integrity or an inappropriate ratio of germline to follicle cells are selectively eliminated through apoptosis or autophagy (Drummond-Barbosa & Spradling, 2001; Hinnant et al., 2020). Ionizing radiation (IR) induces DNA double-strand breaks (DSBs), triggering a DNA damage response (DDR). This response activates a cascade of signaling pathways that culminate in the activation of p53, which in turn promotes apoptosis by inducing the expression of *hid* (J.-H. Park et al., 2019). In addition to DNA damage, nutrient deprivation also triggers programmed cell death via both apoptotic and autophagic mechanisms (Pritchett et al., 2009b). Under nutrient-limiting conditions, the insulin-mediated phosphoinositide 3-kinase (PI3K) pathway, which senses nutritional status, acts to suppress apoptosis. However, in the absence of adequate nutrient signaling, this suppression is lifted, allowing apoptosis to proceed (Drummond-Barbosa & Spradling, 2001; Pritchett et al., 2009a; Wu & Brown, 2006). Autophagy is likewise activated under nutrient-deprived conditions and often coincides spatially with apoptotic regions in the germarium (Hou et al., 2008; Nezis et al., 2009; J.-H. Park et al., 2019). Similar to what has been observed in the salivary gland, mutants lacking Dcp-1 exhibit reductions in both apoptosis and autophagy in response to starvation (Hou et al., 2008; Martin & Baehrecke, 2003; Nezis et al., 2009). These findings suggest that the interplay between Dcp-1-mediated apoptosis and autophagy is required for nutrient deprivation-induced programmed cell death at the first checkpoint.

1.7.3 Mid-oogenesis checkpoint and regulation of egg chamber survival

The second checkpoint, occurring specifically at stages 7–8, initiates programmed cell death (PCD) to assess the condition of egg chambers before the onset of energetically costly vitellogenesis (Drummond-Barbosa & Spradling, 2001; Giorgi & Deri, 1976). Unlike most *Drosophila* tissues where PCD is triggered by the IAP antagonists *rpr* and *hid*, these genes are not required for apoptosis during mid-oogenesis in response to nutrient deprivation (Jeanne S. Peterson et al., 2007). Similar to the first checkpoint in the germarium, the effector caspase Dcp-1 is required not only for apoptosis but also for the induction of autophagy under nutrient-limiting conditions (Baum et al., 2007; Laundrie et al., 2003; Mazzalupo & Cooley, 2006; J.S Peterson et al., 2003). The insulin-mediated PI3K signaling pathway also plays a crucial role at this checkpoint. Mutants lacking components of the PI3K pathway exhibit degenerating egg chambers and fail to progress beyond vitellogenic stages, suggesting that insulin signaling negatively regulates cell death during mid-oogenesis (Drummond-Barbosa & Spradling, 2001; LaFever & Drummond-Barbosa, 2005; Yong Zhang et al., 2006). Survival beyond the mid-oogenesis checkpoint is further regulated by ecdysone signaling, which can either promote or suppress apoptosis. The hormones 20-hydroxyecdysone (20E) and juvenile hormone (JH) act in concert to modulate this process (McCall, 2004). Elevated levels of 20E can induce egg chamber degeneration, while reduced 20E signaling also leads to degradation, supporting a model in which a proper balance between 20E and JH is essential for egg chamber survival (Terashima et al., 2005). Alternatively, surpassing a

threshold level of 20E may directly trigger PCD (Gruntenko & Rauschenbach, 2008). In a non-autonomous manner, follicle cells (FCs) promote engulfment and eliminate germline cells via Draper–JNK signaling (Etchegaray et al., 2012). Elevated autophagy in follicle cells under starvation conditions might contribute to engulfment. Draper in FCs recognizes an unknown signal from the NCs, leading to activation of JNK signaling, which promotes engulfment and the progression of germline programmed cell death (J. M. I. Barth et al., 2011; Thomson & Johnson, 2010). Moreover, as observed in other tissues, there is significant crosstalk among the insulin, ecdysone, and c-Jun N-terminal kinase (JNK) signaling pathways, which together coordinate the regulation of 20E and JH levels to ensure appropriate progression of oogenesis (Gruntenko & Rauschenbach, 2008).

1.7.4 Programmed cell death of nurse cells in late oogenesis

During the later stages of oogenesis, nurse cells (NCs) transfer their cytoplasmic contents—nutrients, proteins, mRNAs, and organelles—to the oocyte through ring canals, a process known as “cytoplasmic dumping”. Upon completion of oocyte maturation, NCs are eliminated via programmed cell death (PCD) (Jenkins et al., 2013). The mechanisms underlying NC death in late oogenesis are distinct from those in mid-oogenesis. Although apoptotic markers, such as activated caspases, are detectable in stage 12–13 NCs, canonical pro-apoptotic RHG genes (*reaper*, *hid*, and *grim*) are not required for their death (Foley & Cooley, 1998). Moreover, the expression of caspase inhibitors such as p35 or DIAP1 causes only mild or incomplete suppression of NC clearance,

suggesting that apoptosis plays only a limited role in this context (Baum et al., 2007; Laundrie et al., 2003; Mazzalupo & Cooley, 2006; J.S Peterson et al., 2003). Autophagy has also been implicated in NC death. Autophagy-positive puncta are observed around NC nuclei, and DNA fragmentation in NCs has been shown to require autophagic degradation of dBruce, an IAP family protein (Bass et al., 2009; Nezis et al., 2010; Timmons et al., 2013). These findings support the involvement of autophagic cell death in late oogenesis. However, inhibition of autophagy results in persistence of NC nuclei, indicating that autophagy alone is not sufficient for complete clearance (J. M. Barth et al., 2012; Jeanne S. Peterson & McCall, 2013). Two additional mechanisms have been proposed to contribute to NC death at this stage. First, evidence of reactive oxygen species (ROS) accumulation and propidium iodide (PI) uptake—indicative of compromised membrane integrity—as well as calcium release from the nuclei to the cytoplasm, support the possibility of programmed necrosis (Golstein & Kroemer, 2007; Matova et al., 1999; Timmons et al., 2013). Second, a specific subset of FCs, known as stretch FCs, exhibit phagocytic activity toward the dying NCs. These FCs contain acidified vesicles during corpse processing, similar to phagocytic mechanisms observed in mid-oogenesis (McCall, 2004). Taken together, while features of apoptosis, autophagy, and programmed necrosis are observed in NCs during late oogenesis, the precise machinery and regulatory hierarchy remain incompletely understood.

1.8 Identification of *stand still* (*stil*) as a key regulator of germline survival

Gametogenesis—oogenesis or spermatogenesis—is a fundamental

process for the transmission of genetic information to the next generation, and it is tightly regulated by numerous genes. A chemical mutagenesis screen for recessive female-sterile mutations on the second chromosome—one of the three major chromosomes in *Drosophila*—identified approximately 500 mutant lines affecting various developmental processes in oogenesis. Among these, *stand still* (*stil*) was isolated as a gene whose mutation leads to one of the most severe defects in oogenesis, resulting in the production of very few or abnormal germline cells (Schüpbach & Wieschaus, 1989).

stand still (*stil*) encodes a 35.8-kDa germline-specific protein that localizes to the nucleus of germ cells from embryonic stage 11 through adulthood in both ovaries and testes (Schüpbach & Wieschaus, 1989). Previous studies revealed that EMS (ethyl methanesulfonate)-induced mutant alleles of *stil* cause defects in the specification or maintenance of cytoskeletal components, leading to agametic ovaries devoid of germline cells (Mulligan et al., 1996). In females homozygous or transheterozygous for strong *stil* mutant alleles, the few surviving germ cells exhibit a characteristic morphology resembling primary spermatocytes, distinguished by a prominent round nucleolus with birefringent properties (Pennetta & Pauli, 1997).

The genes *ovo* and *ovarian tumor* (*otu*), which are required specifically in females for germline cell survival, produce phenotypes similar to that of *stil* when mutated. Moreover, *stil* and *ovo* have been shown to positively regulate *otu* transcription through distinct mechanisms (Oliver et al., 1994; Pauli et al., 1993; Sahut-Barnola & Pauli, 1999). Collectively, these studies have established that *stil* plays essential roles in female germline cell survival, differentiation, and sex

determination. However, its precise biological and molecular functions remain largely unknown.

1.9 Conserved motif of BED-type zinc finger

Stand still (Stil) protein contains a characteristic DNA-binding domain known as the BED-type zinc finger, originally named after the *Drosophila* proteins BEAF and DREF (Aravind, 2000). This domain features a canonical zinc finger motif, $Cx_2CxnHx_{3-5}[H/C]$, forming a metal-chelating structure, and a conserved N-terminal motif characterized by two highly conserved aromatic residues (typically tryptophan and phenylalanine) (Figure 1.6). Secondary structure predictions of BEAF and DREF indicate that this N-terminal aromatic motif adopts β -strand structures that encompass the cysteine dyad, while the region surrounding the first of the two histidines at the C-terminal end of the domain forms an α -helix. In some cases, a variable helical region is predicted between the aromatic motif and the zinc-binding site. These features distinguish the BED-type domain from classical C2H2 zinc fingers, despite both adopting metal-chelating folds. The BED domain is broadly associated with DNA binding, and proteins carrying this domain function as transcription factors, chromatin boundary (insulator) element-binding proteins, or transposases (Aravind, 2000; Hayward et al., 2013; Tue et al., 2017a; H. Zhou et al., 2016). Thus, the BED-type zinc finger serves as a versatile module in gene regulation and chromatin architecture.

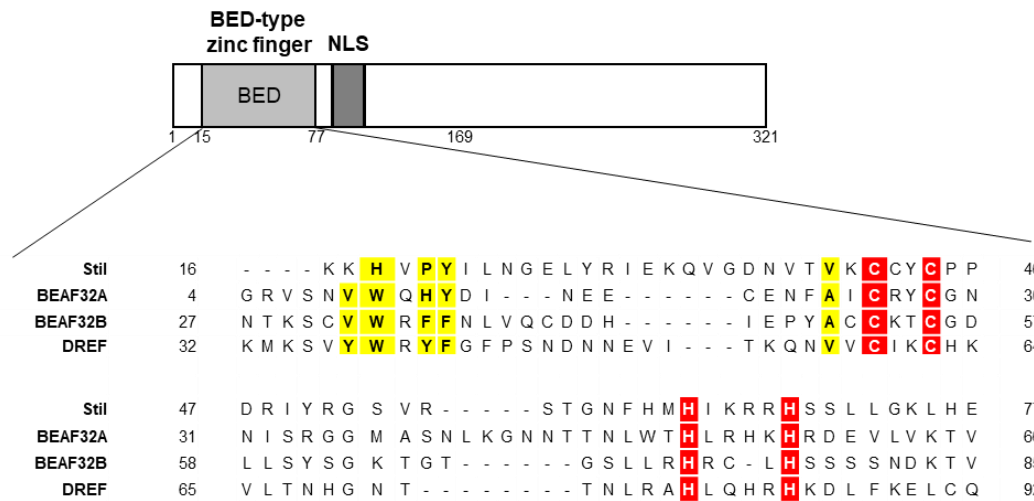


Figure 1.6 Conservation of the BED-type zinc finger in Stil

A schematic presentation of Stil protein, highlighting the BED-type zinc finger (BED, green) and the nuclear localization signal (NLS, blue). A multiple sequence alignment of Stil with three other proteins containing BED-type zinc finger motif is shown. The BED-type zinc finger motif consists of two cysteine and histidine residues (red) and aromatic residues (yellow) in the N-terminal region.

1.10 DNA damage response and cellular outcome in *Drosophila*

Under various physiological and pathological conditions, genotoxic stresses and environmental agents can induce diverse forms of DNA damage, such as base modifications, interstrand crosslinks, and single- or double-strand breaks (DSBs), thereby compromising genomic stability (Sancar et al., 2004). To maintain genomic stability in the face of genomic stress, eukaryotic cells employ a multifaceted set of responses such as cell cycle arrest and DNA repair mechanisms. If the damage exceeds the repair capacity of the cell, programmed cell death pathways, including apoptosis, are activated to prevent the transmission of damaged genetic material to daughter cells (Jackson & Bartek, 2009). Upon encountering DNA damage, cells operate the DNA damage response (DDR), a signaling cascade composed of evolutionary conserved

proteins that function as sensors, transducers, and effectors of the genomic insults (Maréchal & Zou, 2013). Mutations in many genes encoding components of the DNA damage response pathway have been associated with various diseases, including cancer predisposition, as this pathway orchestrates a coordinated cellular response to DNA damage that involves cell cycle checkpoints and apoptosis induction (Baonza et al., 2022; Blackford & Jackson, 2017; Ciccio & Elledge, 2010).

In *Drosophila*, DNA damage is initially detected by the sensor complex MRE11–RAD50–NBS1 (MRN) (Assenmacher & Hopfner, 2004), which activates upstream kinases ATM (ataxia-telangiectasia mutated, known as Tefu in *Drosophila*) and ATR (ATM and Rad3-related, known as Mei-41 in *Drosophila*) (Maréchal & Zou, 2013) (Figure 1.7). Activated Tefu and Mei-41 subsequently phosphorylate and activate the downstream effector kinases Chk2 (Mnk) and Chk1 (Grp), which together mediate cell cycle arrest, DNA repair, and apoptosis (González-Marín et al., 2023). The ATM–Chk2 branch of the DDR primarily induces p53-mediated apoptosis, whereas the ATR–Chk1 axis is essential for DNA repair and cell cycle arrest (Brodsky et al., 2004; Vries et al., 2005). Unlike mammals, which have three p53 family members (p53, p63, p73), *Drosophila* has a single p53 ortholog that primarily functions in apoptosis rather than cell cycle arrest after DNA damage (Brodsky et al., 2000; Ollmann et al., 2000). Chk2 phosphorylates and activates p53, which then induces expression of pro-apoptotic RHG genes (*reaper*, *hid*, and *grim*) by binding to p53-responsive elements (p53REs) (Brodsky et al., 2000, 2004; Peters et al., 2002). In addition

to the canonical ATM–Chk2–p53 pathway, *Drosophila* cells can activate apoptosis independently through JNK signaling and the E2f/Dp transcription factor complex, both of which also regulate RHG gene expression (Baonza et al., 2022). Cell cycle checkpoints, which halt or delay progression to allow time for DNA repair, are predominantly mediated by the ATR/Mei-41 and Chk1/Grapes pathway. This axis inhibits Cdk1 activity and enforces arrest at the G2/M transition (Vries et al., 2005). Furthermore, Chk1/Grapes also regulates the intra-S checkpoint, which delays S-phase entry or slows DNA replication to prevent the duplication of damaged DNA (Ciardo et al., 2019; Jaklevic & Su, 2004).

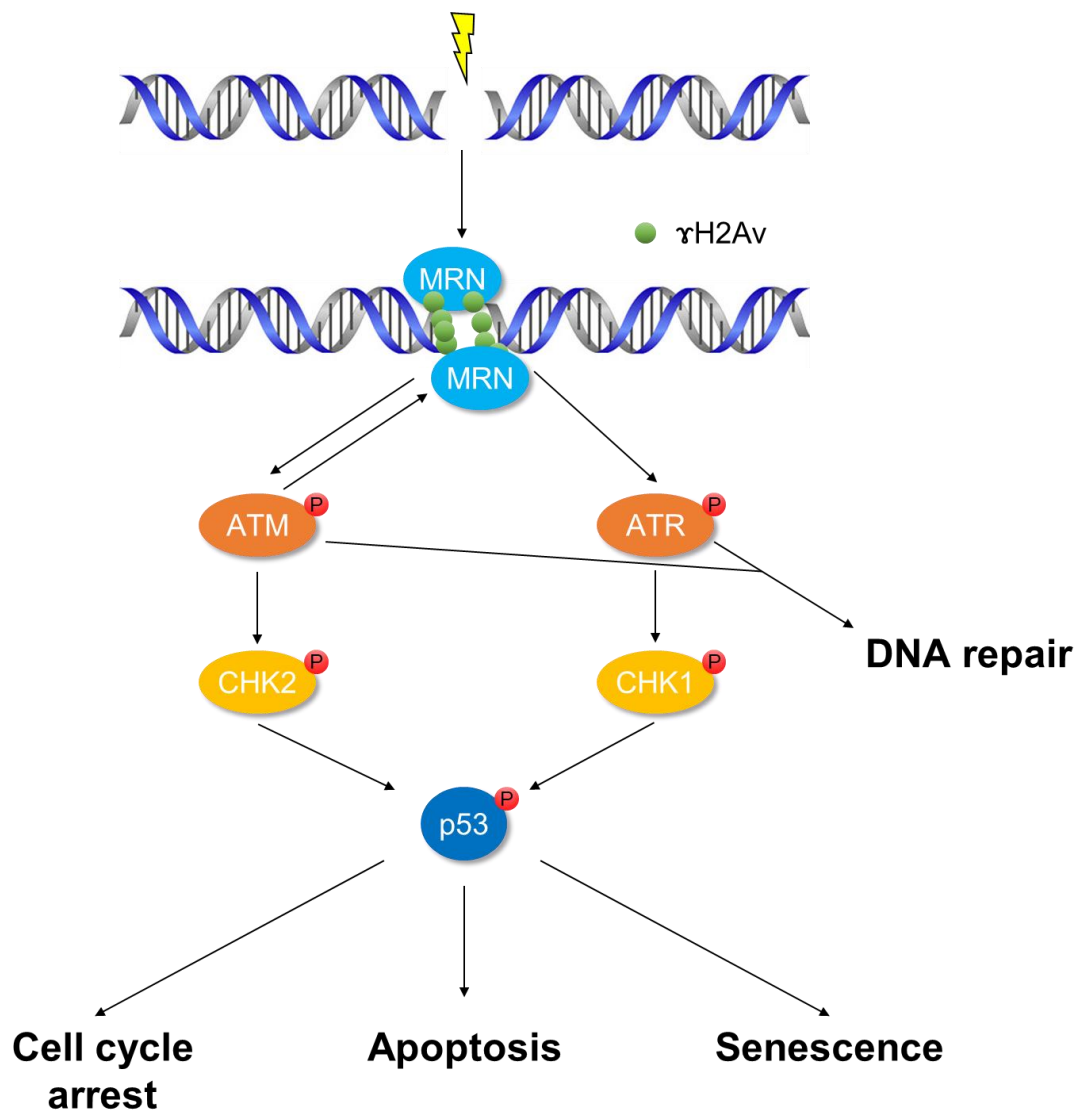


Figure 1.7 DNA damage response pathway linking damage sensing to cell fate decisions
 DNA damage is rapidly detected by the MRN sensor complex, which activates the kinases ATM or ATR through phosphorylation. Activated ATM and ATR subsequently transmit signals to the checkpoint kinases CHK2 and CHK1 or directly initiate DNA repair events. The effector protein p53 is activated by CHK2 and CHK1 and determines cell fate depending on the extent of DNA damage and the efficiency of DNA repair.

1.11 Meiosis in *Drosophila* oogenesis

Sexual reproduction requires the formation of gametes containing half the genetic material of the parent. Meiosis is a specialized cell division process that converts diploid germ cells into haploid gametes through a single round of DNA

replication followed by two successive rounds of chromosome segregation. While meiosis shares several features with mitosis, it is uniquely characterized by homologous recombination with crossover (CO), which reshuffles alleles to promote genetic diversity and enhance adaptability (Carvalho, 2003; Hunter, 2015).

Key meiotic events—such as homolog pairing, synaptonemal complex (SC) formation, recombination, and chromosome segregation—are precisely timed and spatially coordinated by a network of regulatory genes (Hughes et al., 2018). In *Drosophila* females, meiosis initiates within the germarium, located at the anterior tip of each ovariole, where germline stem cells (GSCs) reside and differentiate. The germarium is divided into distinct regions (1, 2A, 2B, and 3) corresponding to progressive meiotic stages. Homologous chromosome pairing begins in region 1, where centromeres of homologs initiate alignment and complete pairing by the 8-cell cyst stage (Christophorou et al., 2013; Joyce et al., 2013). These paired centromeres cluster into two groups, a process dependent on SC components that tether homologous chromosomes. In region 2A, four nuclei within each 16-cell cyst assemble full-length SCs along euchromatic arms. However, only two nuclei retain these SCs in region 2B, and eventually, a single nucleus—designated the pro-oocyte—maintains the SC through region 3/stage 1. This SC is gradually dismantled from stage 5, disappearing entirely by stage 7–8 (Hughes et al., 2018).

Concurrently, meiotic recombination is initiated after SC assembly in region 2A, involving programmed double-strand breaks (DSBs) catalyzed by MeiW68, a *Spo11* homolog, and other associated proteins (Mehrotra & McKim, 2006).

These DSBs are repaired via either the crossover (CO) or non-crossover (NCO) pathway (Hughes et al., 2018). As cysts progress into region 3, DSBs are fully repaired, indicating completion of recombination.

Subsequently, during stages 12–13, germinal vesicle breakdown (GVBD) and meiotic spindle assembly occur in preparation for chromosome segregation (Archambault & Glover, 2009; Głuszek et al., 2015; Radford et al., 2015). At stage 14, homologs align on the spindle and become bioriented toward opposite poles. However, oocytes remain arrested at metaphase I until oocyte activation (Jang et al., 1995). Following activation, anaphase I and the entire meiosis II proceed rapidly to completion (Riparbelli & Callaini, 1996).

1.12 Molecular basis of meiotic recombination in *Drosophila*

Meiotic recombination is a programmed genetic process involving the exchange of genetic material between homologous chromosomes (Lake et al., 2013; Mehrotra & McKim, 2006). It is essential for ensuring genetic diversity, proper chromosome segregation, and genome integrity. This process consists of multiple tightly regulated steps, including the formation of meiotic double-strand breaks (DSBs), resection of DSB ends, strand invasion, Holliday junction formation, and resolution. A variety of highly conserved and specialized proteins coordinate these steps to ensure the successful completion of recombination (Hunter, 2015).

The initiation of meiotic recombination in *Drosophila* begins with the formation of programmed DSBs, which can be visualized in region 2A of the *Drosophila* germarium using the DSB marker γ H2AV, a phosphorylated histone

H2A variant. These DSBs form shortly after the initiation of synaptonemal complex (SC) assembly along the chromosome arms (Lake et al., 2013; Mehrotra & McKim, 2006). In *Drosophila*, six key genes—*mei-W68*, *mei-P22*, *vilya*, *trade embargo* (*trem*), *narya*, and *nenya*—are known to be required for the induction of meiotic DSBs (Hughes et al., 2018; Lake et al., 2019). Mei-W68, the homolog of yeast Spo11, catalyzes the formation of DSBs and is related to the A subunit of archaeal TopoVI DNA topoisomerase (TopoVIA) (Bergerat et al., 1997; McKim & Hayashi-Hagihara, 1998). Mei-P22, which shares structural homology with TopoVIB proteins, is also required for DSB induction (H. Liu et al., 2002; Robert et al., 2016). TopoVI functions as a heterotetramer consisting of two A subunits and two B subunits, suggesting that Mei-W68 and Mei-P22 may act as functional partners in a similar complex to catalyze DSB formation (Mehrotra & McKim, 2006; Robert et al., 2016). The localization of Mei-P22 to discrete chromosomal foci depends on the protein Trem, which contains a zinc finger–associated domain and five C2H2-type zinc fingers (Figure 1.8). Trem is thought to be associated with chromatin and is hypothesized to either facilitate chromatin remodeling to permit Mei-P22 binding or directly recruit Mei-P22 to DSB sites (Lake et al., 2011). Vilya, a protein homologous to the yeast E3 ubiquitin ligase Zip3, also plays a role in DSB formation. *vilya* mutants show increased chromosome nondisjunction and a reduction in DSB foci. Vilya has been shown to interact with Mei-P22, suggesting it may contribute to the regulation or stabilization of DSB formation (Lake et al., 2015). Furthermore, two *Drosophila* paralogs of the Zip3-like family, Narya and Nenia, are also required for meiotic recombination. These proteins interact with Vilya and are essential not only for

proper DSB formation but also for crossover maturation (Lake et al., 2019).

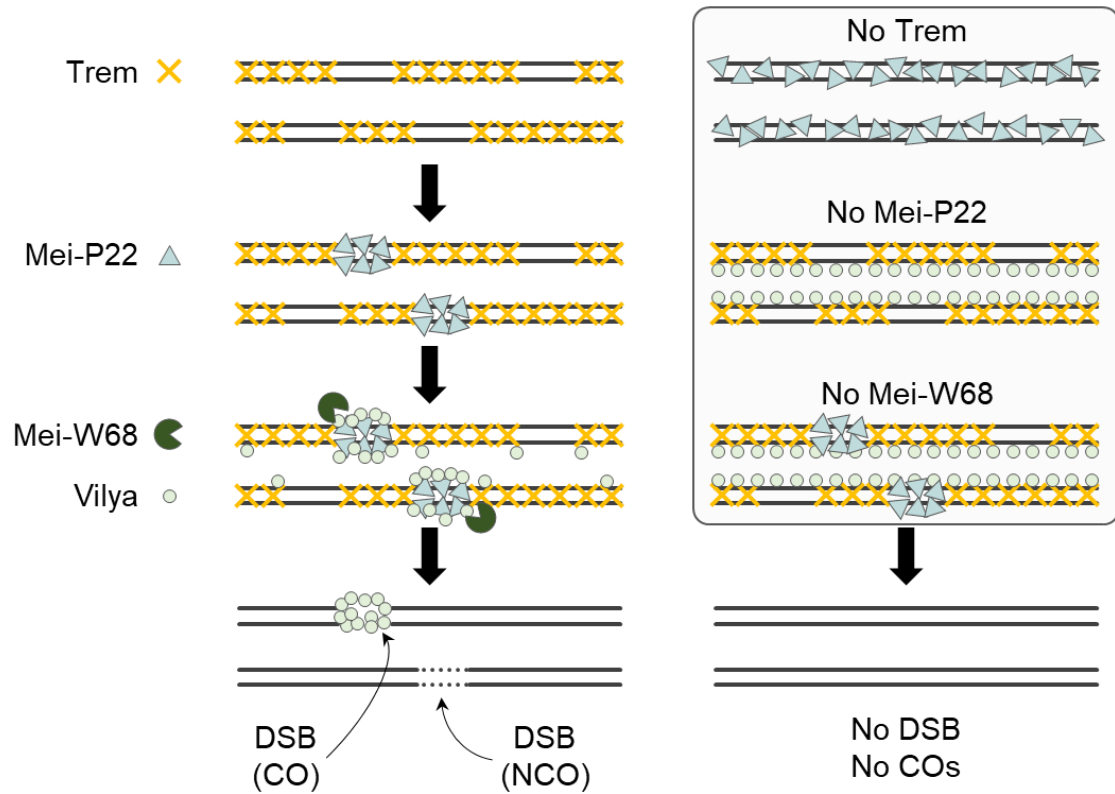


Figure 1.8 Molecular mechanisms underlying DSB formation in *Drosophila* meiosis

Prior to DSB formation, Trem localizes to chromosomes and is required for the recruitment of Mei-P22 to discrete foci. Mei-P22 and Vilya are subsequently recruited to the Mei-P22 sites, leading to DSB formation. In the absence of Trem, Mei-P22 localizes along chromosome arms rather than forming discrete foci. In the absence of either Mei-P22 or Mei-W68, Trem still localizes to chromosome arms, but Vilya fails to form discrete foci. If any of these proteins are absent, DSBs are not generated. CO, crossover; NCO, noncrossover.

1.13 Thesis overview

The aim of my thesis is to elucidate the molecular mechanisms that ensure germline cell survival during *Drosophila* oogenesis, with a particular focus on the role of the *Drosophila*-specific gene *stand still* (*stil*). For reproductive success, programmed cell death is tightly regulated to control germline cell number and quality. However, the molecular mechanisms governing apoptotic

susceptibility in germline cells remain poorly defined.

In this thesis, I demonstrate that loss-of-function mutants of *stil* exhibit massive germline cell loss associated with aberrant apoptosis. Through transcriptomic and chromatin-wide analyses, I reveal that Stil directly represses the pro-apoptotic gene *reaper* (*rpr*) via its BED-type zinc finger domain. In addition to *rpr*, Stil also regulates meiotic recombination and DNA repair genes to prevent aberrant DSB formation. Furthermore, I identified an interacting partner of Stil, the transcriptional cofactor CtBP, which localizes to germline nuclei. In germline stem cells (GSCs), *rpr* expression is strictly repressed by dual mechanisms involving Stil-mediated transcriptional regulation and chromatin-based silencing through H3K9me3. Collectively, this work provides new insights into the unique mechanism by which *Drosophila* preserves female germline cells, highlighting how lineage-specific transcriptional and chromatin-based regulation converge to prevent apoptosis and ensure reproductive success.

2. Material and Methods

2.1 Fly genetics

2.1.1 Fly husbandry

Flies used in this study are *Drosophila melanogaster*. All *Drosophila* stocks and crosses were reared at room temperature (25°C) on standard food [5% (w/v) dry yeast, 5% (w/v) corn flower, 2% (w/v) rice bran, 10% (w/v) glucose, 0.7% (w/v) agar, 0.2% (v/v) propionic acid and 0.05% (w/v) p-hydroxy butyl benzoic acid]. *y w* flies were used as wild type (WT) controls in most of the experiments with exceptions where heterozygotes were sometimes used as controls. *FM6* or *FM7a*, *Sco/CyO*, *TM3 Sb/TM6 Tb* were used as 1st, 2nd and 3rd chromosome balancers. The strains used in this study are listed in Table 2.1

Table 2.1 *Drosophila* strains used in the current study

Fly stock	Reference
<i>y, w</i>	wild type
<i>y, w, FM6</i>	X chromosome balancer
<i>FM7a; Glu/CyO</i>	Multiple chromosome balancer (X & 2nd)
<i>w; Sco/CyO</i>	2nd chromosome balancer
<i>w; TM3, Sb/TM6, Tb Hu</i>	3rd chromosome balancer
<i>w; Pin/CyO; TM3, Sb/TM6, Tb</i>	Multiple chromosome balancer (2nd & 3rd)
<i>P{y[+t7.7]=nos-phiC31/int.NLS}X, y-, sc-, v-, sev-; P{y[+t7.7]=CaryP}attP2</i>	BL#25170
<i>NGT40; NosGal4-VP16</i>	A gift from Dr. Nicole Greider (Grieder et al., 2000)
<i>P{matα4-GAL-VP16}V37</i>	BL#7063

<i>tj-Gal4/CyO</i>	DGRC#104055
<i>P{Sgs3-Gal4.PD}TP1</i>	BL#6870
<i>Bam-Gal4/TM6 Tb</i>	(Chen & McKearin, 2003)
<i>stil</i> ^{EY16156} / <i>CyO</i>	BL#21191 (background mutations were removed by backcrossing)
<i>stil</i> ³ / <i>CyO</i>	BL#533
<i>UASp-P35</i>	A gift from Dr. Andreas Bergmann (Baum et al., 2007)
<i>UASp-Tkv.CA/TM3Sb</i>	A gift from Dr. Dahua Chen
<i>bam</i> ^{Δ86} / <i>TM3 Sb</i>	BL#5427
<i>P{w+mC=UASp-Diap1.P}J8-1</i>	BL#63818
<i>rpr</i> ⁸⁷ / <i>TM6 Tb</i>	BL#83150
<i>P{PZ}hid05014/TM6B, Tb</i>	BL#83349
<i>Df(2R)vg-C/CyO,</i> <i>P{ry[+t7.2]=sevRas1.V12}FK1</i>	BL#754 (Deficiency for <i>stil</i>)
<i>Df(3L)X14/TM6B, Tb</i>	BL#600368 (Deficiency for <i>hid</i>)
<i>P{TRiP.GL01230}attP2/TM3Sb</i>	BL#41648 (<i>stil</i>)
<i>P{TRiP.GL00138}attP2/TM3Sb</i>	BL#44417 (<i>tefu</i>)
<i>P{TRiP.GL00020}attP2/TM3Sb</i>	BL#35152 (<i>loki</i>)
<i>P{TRiP.HMS02286}attP2/TM3Sb</i>	BL#41720 (<i>p53</i>)
<i>P{TRiP.GL00284}attP2</i>	BL#35371 (<i>mei-41</i>)
<i>P{TRiP.HMS01573}attP2</i>	BL#36685 (<i>grp</i>)
<i>UASp-6xMyc-stil-FL/TM3Sb</i>	Previously generated in the lab
<i>UASp-6xMyc-stil-NT/TM3Sb</i>	Previously generated in the lab
<i>UASp-6xMyc-stil-CT/TM3Sb</i>	Previously generated in the lab
<i>UASp-6xMyc-stil-AAYA/TM6Tb</i>	Previously generated in the lab
<i>UASp-3×HA-miniTurbo-Stil-FL</i>	Generated in this study
<i>UASp-3×HA-miniTurbo-Stil-NT</i>	Generated in this study
<i>UASp-3×HA-miniTurbo-CT</i>	Generated in this study
<i>UASp-3×HA-miniTurbo-3×NLS</i>	Generated in this study
<i>mei-W68 TRiP</i>	Generated in this study

<i>UASp-GFP-stop-Dam-alone</i>	Generated in this study
<i>UASp-GFP-stop-Dam-Stil</i>	Generated in this study

2.1.2 Generation of transgenic lines

To generate the 6×Myc-Stil variants (6×Myc-Stil-FL, 6×Myc-Stil-NT, 6×Myc-Stil-CT, and 6×Myc-Stil-AAYA) and 3×HA-miniTurbo-Stil variants (3×HA-miniTurbo-Stil-FL, 3×HA-miniTurbo-Stil-NT, and 3×HA-miniTurbo-Stil-CT), coding sequences of Stil-FL, Stil-NT, Stil-CT, and Stil-AAYA were amplified by PCR using cDNA prepared from *y w* flies. The 3×HA-miniTurbo and 3×HA-miniTurbo-3×NLS fragments were amplified from the plasmid 3×HA-miniTurbo-3×NLS_pCDNA3 (Branon et al., 2018) purchased from Addgene (<https://www.addgene.org/107172/>) as template. For 6×Myc-Stil constructs, Six tandem Myc tags were appended to the N-terminal region of the amplified Stil variant fragments through ligation. For 3×HA-miniTurbo-Stil fusion constructs, Stil-FL, Stil-NT, and Stil-CT fragments were fused to 3' end of 3×HA-miniTurbo fragment using In-Fusion HD Cloning Kit (Takara). All resulting constructs were cloned into XbaI-linearized pUASp-K10-attB vector using In-fusion HD Cloning Kit (Takara).

For DamID constructs, Stil variants were amplified from cDNAs prepared from *y w* ovaries. DamID constructs were designed to contain a primary open reading frame (ORF) encoding GFP, followed by two stop codons, and a secondary ORF encoding Dam or Dam-Stil (Southall et al., 2013). The DNA fragment encoding Dam was amplified from the pUASTattB-LT3-NDam vector (a gift from Dr. Andrea H. Brand). The resulting fragments were cloned into the

pUASp-K10-attB plasmid backbone (Koch et al., 2009).

For *mei-W68* RNAi, Target sites were selected using an algorithm designed to minimize off-target effects (Vert et al., 2006). Sense and antisense oligonucleotides were annealed and cloned into the pWALIUM22 vector (Perkins et al., 2015).

All UASp transgenes and RNAi vectors were integrated into the attP2 landing site by embryo injection using P{CaryP}attP2 strains (BL#25170). Transformants were selected based on mini-white eye color. Primers used for the construction of transgenes are listed in Table 2.2.

Table 2.2 List of primers used for the construction of transgenes in the current study

Fragmen ts or pair	Primer names	Sequences
6×Myc- Stil-FL	Myc-Stil- FL_Fw	CACCATGTCCGTAAACTTGGACGGTT
	Myc-Stil- FL_Rv	TTACAGATTCCGGCTCAAACCTG
6×Myc- Stil-NT	Myc-Stil- FL_Fw	CACCATGTCCGTAAACTTGGACGGTT
	Myc-Stil- NT_Rv	AGCTTTATCTGAAGTGAATCTTC
6×Myc- Stil-CT	Myc-Stil- CT_Fw	CACCATGAATCTACCAAATATCCCTAAGAAC
	Myc-Stil- FL_Rv	TTACAGATTCCGGCTCAAACCTG
6×Myc- Stil- AAYA_5'	Myc-Stil- FL_Fw	CACCATGTCCGTAAACTTGGACGGTT
	Myc-Stil-	CGGAGCGTAGGCGGCTTGACCGTAACATTGTC

fragment	AAYA_5'fr_ Rv	
6×Myc- Stil- AAYA_3' fragment	Myc-Stil- AAYA_3'fr_ Fw	AAGGCCGCCTACGCTCCGCCGGATCGGATTTA
	Myc-Stil- FL_Rv	TTACAGATTTCGGCTCAAACCTG
Stil-FL fragment _Turbo D	TurboID Stil NT Fw	GGTGGAGGCGGTGGCTCCGTAAACTTGGAC
	TurboID Stil CT Rv	CGAGGTCGACTCTAGTTACAGATTTCGGCTC
Stil-NT fragment _Turbo D	TurboID Stil NT Fw	GGTGGAGGCGGTGGCTCCGTAAACTTGGAC
	TurboID Stil NT Rv	CGAGGTCGACTCTAGTTAATTTGGTAGATTAGC
Stil-CT fragment _Turbo D	TurboID Stil CT Fw	GGTGGAGGCGGTGGCAATATCCCTAAGAAC
	TurboID Stil CT Rv	CGAGGTCGACTCTAGTTACAGATTTCGGCTC
3×miniT urbo fragment _Turbo D	TurboID_mi niturbo_Fw -1	ATCTGGATCCTCTAGCAAAATGTACCCGTATGAT
	TurboID_mi niturbo_Rv- 1	GCCACCGCCTCCACCCTTTTCGGCAGACCG
3×miniT urbo- 3×NLS fragment _Turbo D	TurboID_mi niturbo_Fw -1	ATCTGGATCCTCTAGCAAAATGTACCCGTATGAT
	TurboID_mi niturbo_Rv- 2	CGAGGTCGACTCTAGTCACACCTTCCTCTT

GFP fragment	DamID_GF P_Fw	ATCTGGATCCTCTAGcaaaATGGTGAGCAAGGGC
	DamID_GF P_Rv	GCGATTTTTCTTCATGTTATTACTTGACAGCTCGTC
Dam (Dam-Stil) fragment	DamID_Dam_Fw	GACGAGCTGTACAAGTAATAACATGAAGAAAAATCGC
	DamID_Dam (Dam-Stil)_Rv	GCCACCGCCTCCACCCAGATCCTCTTCAGAG
Dam (Dam-alone) fragment	DamID_Dam_Fw	GACGAGCTGTACAAGTAATAACATGAAGAAAAATCGC
	DamID_Dam (Dam-only)_Rv	CGAGGTCGACTCTAGTTACAGATCCTCTTCAGAG
Stil fragment	DamID_Stil_Fw	GGTGGAGGCGGTGGCATGTCCGTAACTTG
	DamID_Stil_Rv	CGAGGTCGACTCTAGTTACAGATTCGGCTC
<i>mei-W68</i> RNAi	<i>mei-W68</i> _TRiP_Fw	CTAGCAGTCCGTGGACTATACTATGACAATAGTTATATTCAAGCATATTGTCATAGTATAGTCCACGGGCG
	<i>mei-W68</i> _TRiP_Rv	AATTCGCCCCGTGGACTATACTATGACAATATGCTTGAATATAACTATTGTCATAGTATAGTCCACGGACTG

2.2 Immunofluorescence staining

Immunofluorescence staining was performed as previously described (F. Xu et al., 2024). Ovaries and testis from adults and the 3rd instar larva were dissected in cold phosphate buffer saline (PBS) and fixed with PBS containing

5.3% (w/v) paraformaldehyde for 10 min. Fixed samples were washed in PBX [PBS with 0.2% (v/v) Triton-X (Nacalai)] for at least 30 min with several changes of solution, then blocked with PBX containing 4% (w/v) bovine serum albumin (BSA) for at least 30 min at room temperature. After rinsing once with PBX, the samples were incubated with the primary antibodies diluted in PBX containing 0.4% (w/v) BSA for more than 4 h at room temperature or overnight at 4°C. After several washes with PBX for at least 1 h, the samples were incubated in secondary antibodies diluted in PBX containing 0.4% (w/v) BSA for more than 2 h at room temperature. The samples were washed several times with PBX for at least 1 h and stained with 1 µg/ml of 4',6-diamidino-2phenylindole (DAPI) (Sigma) diluted in PBX for 10 min at room temperature. Following two rinses with PBX and PBS, tissues were mounted in Fluoro-KEEPER Antifade Reagent (Nacalai). The primary antibodies used in this study are listed in Table 2.3. Secondary antibodies were Alexa Fluor 488-and 555-conjugated goat antibodies against rabbit, mouse and guinea pig IgG (Invitrogen, A11029, A11034, A11073, A21424, A21429 and A21435) at a 1:200 dilution in PBX containing 0.4% (w/v) BSA. Images were captured by Zeiss LSM780 Confocal Microscope with 10x, 20x, and 40x lens and processed with Zen (Zeiss) and Fiji application (Schindelin et al., 2012).

Table 2.3 List of Primary antibodies used for Immunofluorescence staining in the current study

Antibody	Host species	Working dilution	Source
anti-Vasa	guinea pig	1:1000	Previously generated in the

			lab (Patil & Kai, 2010)
anti-Myc	mouse	1:500	Wako (9E10)
anti-Stil	rabbit	1:1000	A gift from Dr. Daniel Pauli (Pennetta & Pauli, 1997)
anti- α -spectrin	mouse	1:200	DSHB (3A9)
anti- γ H2Av	mouse	1:50	DSHB (UNC93- 5.2.1)
anti-dCtBP	rabbit	1:1000	A gift from Dr. David N Arnosti (Struffi & Arnosti, 2005)

2.3 TUNEL-labeling assay

TUNEL assay was conducted using ApopTag® Kits (Millipore), labeling the free 3'-OH DNA termini in situ with fluorescein-nucleotide by terminal deoxynucleotidyl transferase (TdT). Dissected ovaries in cold PBS were fixed with PBS containing 5.3% (w/v) paraformaldehyde and rinsed 3 times with PBX. Ovaries were washed with PBX for 10 min twice and further washed with PBST [PBS with 0.1% (v/v) Tween 20 (Nacalai)] twice. After rinsing with Equilibration Buffer twice, the samples were incubated with TdT reagent [70% (v/v) Reaction Buffer and 30% (v/v) TdT Enzyme] for 1 h at 37°C. After incubation with Stop/Wash Buffer diluted 1:35 for 15 min, samples were rinsed 3 times with PBX and then washed 3 times with PBX for 10 min. The tissue mounting and

microscopic observation were as described in the “Immunofluorescence staining” section.

2.4 Western blotting

5 pairs of ovary lysates mixed with 20% volume of 5× sample buffer [0.25% (w/v) Bromophenol blue, 0.5M DTT, 50% (v/v) Glycerol, 10% (w/v) SDS, and 0.25M (v/v) Tris-HCl (pH 6.8)] were separated by SDS–PAGE and transferred onto 0.45 µm Clear Trans PVDF membranes (Wako). Membranes were blocked with 3% (w/v) skim milk (Nacalai Tesque) in PBST and incubated with primary antibodies diluted in HIKARI signal enhancer (Nacalai Tesque). The primary antibodies used were mouse anti-Myc [Wako, 9E10, 1:1000] and mouse anti-α-Tubulin [Santa Cruz, DM1A, 1:2000]. HRP-conjugated secondary antibodies used were goat anti-mouse IgG [Bio-Rad, 170-6516, 1:3000]. Chemiluminescence detection was performed using the Chemi-Lumi One reagent kit (Nacalai), and immunoreactive bands were visualized with a ChemiDoc Touch imaging system (Bio-Rad) and analyzed using Fiji (Schindelin et al., 2012).

2.5 RT-PCR and quantitative RT-PCR

Total RNA was isolated from ovaries using TRIzol LS Reagent (Invitrogen), following the manufacturer’s protocol. RNA samples (1 µg each) were treated with DNase I (NEB) to digest genomic DNA, followed by inactivation of the enzyme with 5 mM EDTA at 75°C for 10 min. Reverse transcription was performed using the DNase I-treated RNA with oligo(dT)20 primers and

SuperScript III Reverse Transcriptase (Invitrogen), following the manufacturer's protocol. cDNAs were subjected to 30 cycles of RT-PCR with GoTaq DNA polymerase (Promega).

Quantitative PCR (qPCR) was performed with Fast SYBR Green reagents (Invitrogen) using at least 3 biological replicates. $\Delta\Delta C_t$ method was used to obtain the relative transcript levels. α -Tub84B (tubulin) was used as the reference. The sequences of the primers are listed in Table 2.4.

Table 2.4 List of primers for RT-PCR and quantitative RT-PCR in the current study

Primer label	Sequence	Source
stil_RT-PCR_Fw	TGAAGGTGGCTGCACTGGAAG	Current study
stil_RT-PCR_Rv	CTGGCGCGGAGTCTAGTTGG	Current study
stil_qRT-PCR_Fw	TTGGGCAAACCTCCATGAAATGA	Current study
stil_qRT-PCR_Rv	GTGGACATGCAAGCTGCTG	Current study
Tubulin_Fw	GGTAACCGTCGAAATCAGTGTT	(F. Xu et al., 2024)
Tubulin_Rv	TGGCTTTTCTGCTATACGTGTC	(F. Xu et al., 2024)
rp49_Fw	ATGACCATCCGCCAGCATAC	(F. Xu et al., 2024)
rp49_Rv	CTGCATGAGCAGGACCTCCAG	(F. Xu et al., 2024)
sxl_Fw	CTCACCTTCGATCGAGGGTGTA	(Moschall et al., 2019)
sxl_Rv	GATGGCAGAGAATGGGAC	(Moschall et al., 2019)
rpr_Fw	CGATCAGGCGACTCTGTTG	Current study
rpr_Rv	CGGACTTTCTTCCGGTCTTC	Current study
hid_Fw	GAACTGCAGGAGCGAAAGC	Current study
hid_Rv	GTTCTTGTGTCCCGTCAACC	Current study

2.6 mRNA-seq analysis

Total RNAs were extracted from 100 ovaries of control and *stil* mutant

(*stil*^{EY16156}) of Tkv.CA OE (*NGT40; NosGal4-VP16> Tkv.CA*) background and 40 ovaries of control and *stil* mutant of P35 OE (*NGT40; NosGal4-VP16> P35*) background using TRIzol (ThermoFisher) following the manufacturer's instruction. Extracted RNAs were treated with DNase I for 10 min at 37°C and reactions were inhibited by 5 mM EDTA at 70°C for 10 min, following that RNAs were purified using phenol/ chloroform/ isoamyl alcohol (mixed with a ratio of 25: 24: 1, pH 5.2). Purified RNAs were prepared for mRNA-seq libraries with NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB), and deep-sequencing was conducted using NovaSeq6000 (Illumina). Sequencing analysis was conducted using bioinformatic software, CLC Genomics Workbench 12 (QIAGEN). Adaptor sequences of total reads obtained from mRNA-seq were trimmed and reads were mapped to *Drosophila* genomes, r6.32 from Flybase (ftp://ftp.flybase.net/genomes/Drosophila_melanogaster/). After mapping the sequence data, Differential expression analysis was performed between control and *stil* mutant with P35 OE or Tkv.CA OE and calculated the expression level of each gene.

2.7 DamID-seq and data processing

UASp-Dam and UASp-Dam-Stil flies were crossed with *NGT40; NosGal4-VP16* driver and maintained at 25°C. For each replicate, 100 ovaries of 50 flies were dissected in *Drosophila* Schneider's medium. Genomic DNA was extracted using the QIAamp Fast DNA Tissue Kit (QIAGEN cat. no. 51404), and methylated DNA was processed and amplified as described (Díaz-Torres et al., 2021). Briefly, genomic DNA was digested overnight with DpnI (NEB), which cuts

methyated GATC sites, and DamID adaptors were ligated to the resulting fragments. A subsequent digestion with DpnII (NEB), which selectively cuts unmethyated GATC sites, was performed, and fragments containing consecutive methyated GATCs were then amplified by PCR using ligated adaptor-specific primers and PrimeSTAR® HS DNA Polymerase (Takara).

The amplified DNA was sonicated using a Bioruptor (Diagenode) to obtain an average fragment size of 300 bp. DamID adaptors were removed by digestion with AlwI, and the DNA was purified using AMPure XP beads (Beckman Coulter). For next-generation sequencing, the DNA was subjected to end-repair, A-tailing, Illumina adaptor ligation, and PCR amplification. Single-end 100 bp reads were subsequently generated on a NovaSeq 6000 platform (Illumina). Illumina NGS reads were aligned to the *Drosophila melanogaster* reference genome (r6.32), and enrichment profiles were generated using the damidseq_pipeline with default settings (Marshall & Brand, 2015), with replicate libraries subsequently averaged. The Dam-Stil enrichment profile was normalized by the Dam control profile and Dam-Stil peaks were identified using find_peaks and annotated with the ChIPseeker pipeline (Marshall & Brand, 2015).

2.8 Fertility test

For female fertility assays, three virgin females were mated with three y w males for 3 days per replicate. For male fertility assays, three males were mated with three y w virgin females under the same conditions. After the mating period, eggs were collected for 24 hours, followed by incubation for an additional 24 hours. The total number of eggs and the number of hatched eggs were then

counted for each replicate.

2.9 S2 cell culture experiments

Drosophila Schneider S2 cells were cultured at 28°C in Schneider's medium (Thermo) supplemented with 10% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin (Thermo). Plasmids encoding Stil variants were generated using the Gateway cloning system with (Life Technologies) with pAFW, which drives the expression of N-terminal FLAG-tagged fusion proteins under the control of the Actin5C promoter. *rpr*-GFP and *ubiP*-GFP reporter plasmid was constructed by replacing 2.4-kb tubulin promoter of the *tubP*-GFP, a gift from Dr. Steve Cohen (Brennecke et al., 2003), with the PCR-amplified 5-kb promoter region and 5'-UTR of *rpr* and the PCR-amplified 2,137 bp promoter region and 5'-UTR of *ubi-p63E*. The plasmids for transfection to S2 cells used in this study are listed in Table 2.5.

For the reporter assay, a total of 4 µg of plasmid DNA was used for each transfection. Transgenes and reporter plasmids were included at 1 µg each as needed, and the remaining amount was supplemented with the control plasmid pAW without attR sites to reach 4 µg. The plasmid mixture was combined with 20 µL of Hilymax transfection reagent (Dojindo) and transfected into 1 mL of S2 cells seeded one day prior, following the manufacturer's instructions. Two days of post-transfection, cells were collected, sonicated, and followed by centrifugation. The supernatant was mixed with 20% volume of 5× sample buffer. Proteins were then analyzed by Western blotting using the following antibodies and Signal Enhancer Reagent HIKARI (Nacalai): mouse anti-α-Tubulin [Santa Cruz, DM1A, 1:1000]

rabbit anti-GFP [Clonotech, 632569, 1:1000], and rabbit anti-FLAG [MBL, PM020, 1:1000]. Chemiluminescence detection was performed using the Chemi-Lumi One reagent kit (Nacalai), and immunoreactive bands were visualized with a ChemiDoc Touch imaging system (Bio-Rad) and analyzed using Fiji (Schindelin et al., 2012).

For immunostaining, transfected S2 cells were collected at 2-days post-transfection and plated onto poly-L-Lysine-coated coverslips for at least 30 min to promote adhesion. Cells were fixed in 5.3% (w/v) paraformaldehyde for 15 min, permeabilized with PBX for 10 min, and washed three times with PBX. After blocking with PBX containing 4% (w/v) BSA for 30 min, cells were incubated with primary and secondary antibodies for at least 1 hour each, followed by nuclear staining with 1 µg/ml of DAPI for 10 min. After rinsing with PBS, samples were mounted with Fluoro-KEEPER Antifade Reagent (Nacalai). Images were acquired using a Zeiss LSM780 and 900 confocal microscopes with a 63× objective lens and processed using Zen and ImageJ software.

Table 2.5 List of plasmids used S2 cell experiments in the current study

Plasmid	bone
pAW	Previously generated in the lab
FLAG-Stil-FL	pAFW
FLAG-Stil-NT	pAFW
FLAG-Stil-CT	pAFW
FLAG-Stil-AAYA	pAFW
<i>tubP</i> -GFP	A gift from Dr. Steve Cohen
<i>ubiP</i> -GFP	<i>tubP</i> -GFP
<i>rpr</i> -GFP	<i>tubP</i> -GFP

2.10 Proximity-dependent biotin identification (BioID/TurboID)

2.10.1 Expression of miniTurbo proteins and biotinylation in ovaries

Transgenic flies, containing UASp-3×HA-miniTurbo-Stil-FL, UASp-3×HA-miniTurbo-Stil-NT, UASp-3×HA-miniTurbo-Stil-CT, UASp-3×HA-miniTurbo-3×NLS respectively, were crossed with the NGT40-NosGal4-VP16 driver, raised on standard food. After eclosion, adult flies were maintained in fly food containing 100μM biotin for 3 days. Ovaries from 2 females were homogenized with a plastic pestle (Biomasher) and biotinylated protein signals were analyzed as described in “Western blotting” section using streptavidin-HRP (proteintech), anti-HA antibody (Cell Signaling Technology), and anti-rabbit-HRP (BioRad) in 1:1000, 1:3000, 1:3000 respectively.

2.10.2 Purification of biotinylated proteins

50 ovaries were homogenized in BioID lysis buffer [50mM Tris-HCl (pH 7.5), 500mM NaCl, 0.4%(v/v) SDS, 2mM EDTA, 2mM DTT, Complete® EDTA-free protease inhibitor (Roche)] using a plastic pestle (Biomasher). The ovarian lysates were sonicated for 30 sec 3times using Bioruptor UCD-250 (COSOMO BIO CO., LTD). After the 1st sonication, Triton-X-100 was added to the lysates (the final concentration 2%(v/v)). The same sonication program was repeated again, and then the lysates were centrifuged at 15,000g for 10 min at 4°C. The supernatant was diluted with the same volume of 50mM Tris-HCl (pH 7.5), was incubated with 50mM Tris-HCl (pH 7.5)-equilibrated streptavidin-conjugated magnetic beads (TriLink) overnight at 4°C. Beads were washed 5 times with BioID

wash buffer [50mM Tris-HCl (pH 7.5), 250mM NaCl, 0.2%(v/v) SDS, 1mM EDTA, 1mM DTT, 1%(v/v) Triton-X, Complete® EDTA-free protease inhibitor (Roche)]. The beads-bound proteins were extracted by boiling at 95°C for 5min in 2×sample buffer supplemented with 250µM biotin. Biotinylated proteins were separated on 10%(g/v) acrylamide gel. Silver staining was performed using manufacturer's kit (Wako).

2.10.3 LC-MS / MS analysis

The proteins separated with silver staining were digested with trypsin and lysyl endopeptidase. The peptides, labeled with the Tandem Mass Tag™ system (Thermo Fisher Scientific), were separated by UltiMate 3000 Nano LC systems (Thermo Fisher Scientific) using gradient of acetonitrile through ESI-column (0.075×120 mm). Separated peptides were analyzed by Q-Exactive mass spectrometry (Thermo Fisher Scientific). Further, raw data from mass spectrometry was analyzed and calculated by a standard software, Scaffold (Proteome Software, Oregon).

3. Results

3.1 Stil Protects Female Germline Cells from Apoptotic Cell Death

3.1.1 Loss of *stand still* (*stil*) leads to female germline cell loss

Previous studies have demonstrated the necessity of *standstill* (*stil*) function in female germline cells; however, its molecular role remains elusive (Mulligan et al., 1996; Pennetta & Pauli, 1997). To address this, I analyzed a loss of function allele, *stil*^{EY16156}, which harbors a P-element insertion near the 3' end of the second exon of *stil* gene, potentially disrupting the splicing of *stil* transcripts (Figure 3.1.1 A) (Bellen et al., 2004, 2011). RT-PCR revealed that *stil* transcripts were undetectable in homozygous *stil*^{EY16156} ovaries and in *stil*^{EY16156} testes, indicating a complete loss of *stil* expression in both gonads (Figure 3.1.1 B).

Similar to the previously reported loss-of-function allele *stil*³, which exhibited the most severe phenotype (Figure 3.1.2 A, B) (Mulligan et al., 1996), ovaries of both *stil*^{EY16156} homozygous and *stil*^{EY16156}/*Df*(2R)*vg-C* trans-heterozygote, in which the deficiency uncovers the entire *stil* locus, were severely reduced in size and completely lacked germline cells (Figure 3.1.1 C, D). Likewise, *stil*³/*stil*^{EY16156} trans-heterozygote females exhibited more severe germline cell loss than *stil*³/*Df*(2R)*vg-C* (Figure 3.1.2 A, B). However, male germline cells in *stil*^{EY16156} testes progressed to differentiate into mature sperm (Figure 3.1.2 C). Notably, fertility assayed by egg production and hatching rate, was comparable between *stil*^{EY16156} and *stil*^{EY16156}/*CyO* males (Figure 3.1.2 D), indicating that *stil* is specifically required for female germline cells. The loss of germline cells in

stil^{EY16156} ovaries was fully rescued by overexpression of the *UASp-6×Myc-Stil* transgene, driven by the germline-specific *NGT40; NosGal4-VP16* driver (Figure 3.1.1 C, D) (Grieder et al., 2000). Mutant females expressing 6×Myc-Stil, germline cells were differentiated and developed into mature eggs that hatched comparably to those of heterozygous control (Figure 3.1.1 E). Collectively, these results confirm the pivotal role of *stil* in maintaining female germline cells and ensuring proper oogenesis.

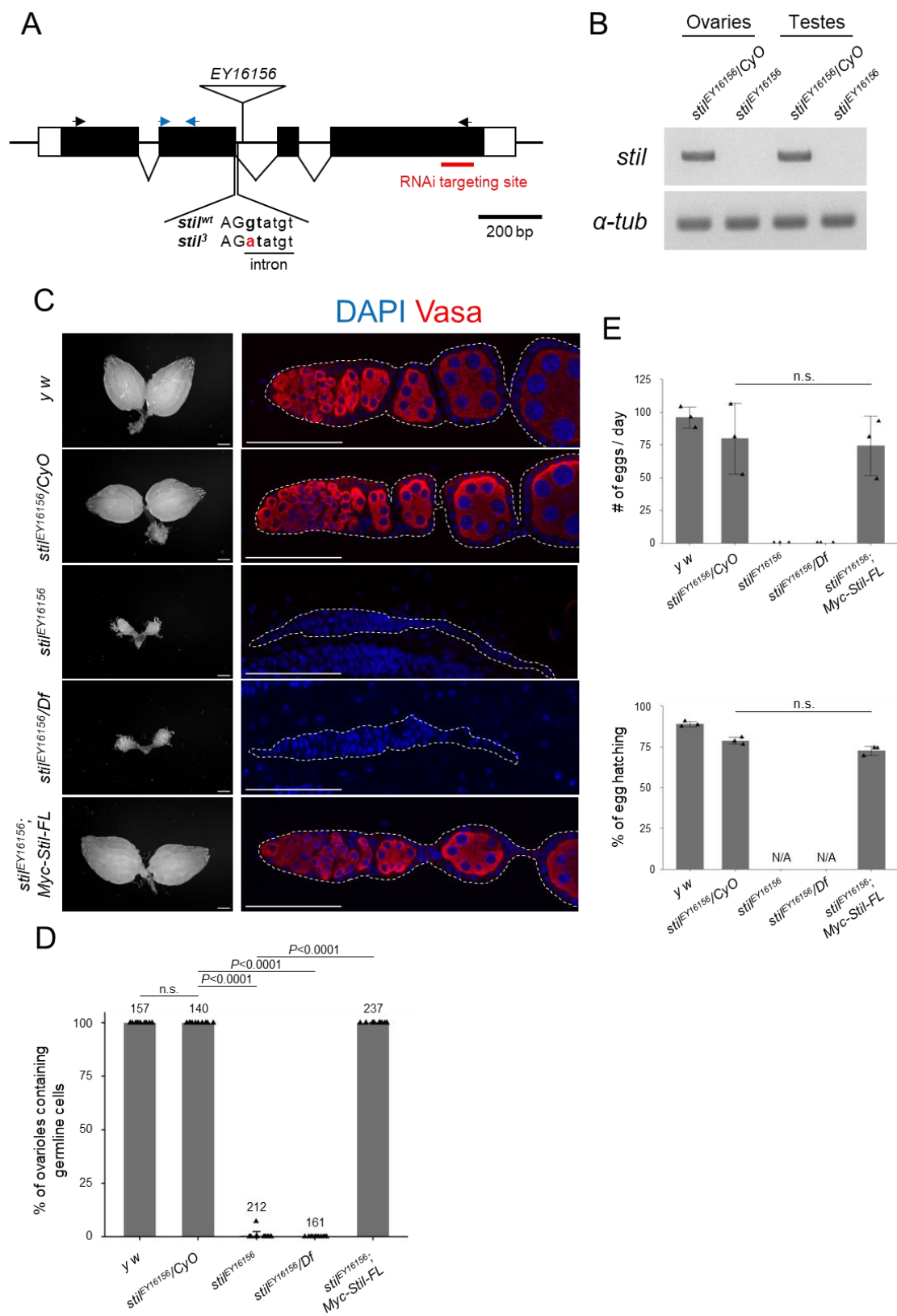


Figure 3.1.1 Disruption of *stil* induces female germline cell depletion

(A) Schematic representation of the *Drosophila stil* gene locus showing the position of P-element insertion, *EY16156*, mutation site of *stil*^β, and the target site of shRNA for knockdown. Black and blue arrows represent primers for RT-PCR and RT-qPCR, respectively. (B) Expression of *stil* and the control α -tub transcripts in *stil*^{*EY16156*} mutant and the heterozygous control ovaries or testes analyzed by RT-PCR. (C) Representative images showing entire ovarian morphology (left panels) and ovarioles immunostained with a germline marker, Vasa (red), and nuclear staining with DAPI (blue) (right panels) from *y w*, *stil*^{*EY16156*}/*CyO*, *stil*^{*EY16156*}, *stil*^{*EY16156*}/*Df*, and *stil*^{*EY16156*} expressing 6×Myc-Stil-FL transgene driven by the germline-specific driver, *NGT40; NosGal4-VP16*. Scale bar: 1 mm for whole ovaries in the left panels, 50 μ m for ovariole in the right panels. (D) Quantification of the percentage of ovarioles containing germline cells per ovary in 2–3-day-old females. Each dot represents an individual ovary. Genotypes are indicated below the graph and the numbers of ovarioles assessed are shown above each bar. Error bars represent standard deviation (s.d.). (E) Analysis of egg laying and hatching rate. The egg numbers and their hatching rate by three females indicated genotypes corresponding to those in (C) mated with three *y w* males were measured daily (*n*=3). Error bars indicate s.d.

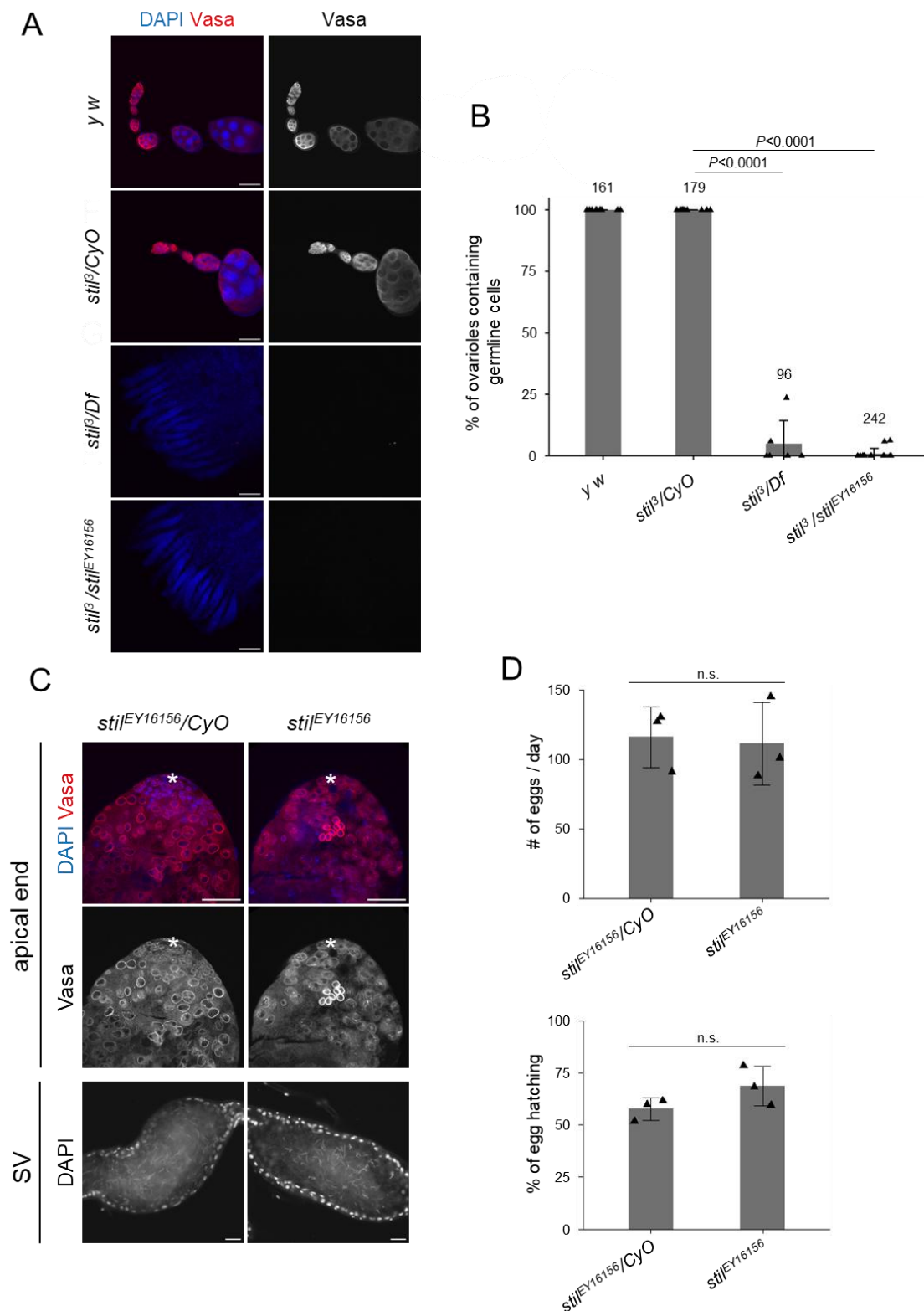


Figure 3.1.2 *stil* function is indispensable in female germline cells not male germline cells

(A) Immunostaining of ovaries from *y w*, *stil³/CyO*, *stil³/Df*, and *stil³/stil^{EY16156}* with antibody against Vasa (red) with DAPI (blue). Scale bar: 50 μ m. (B) Quantification of the percentage of

ovarioles containing germline cells per ovary in 2–3-day-old females. Each dot represents an individual ovary. Genotypes are indicated below the graph and the number of ovarium assessed is noted above each bar. Error bars represent standard deviation (s.d.). (C) Immunostaining of the apical end of testes from *stil^{EY16156}/CyO* and *stil^{EY16156}* with anti-Vasa antibody (red) and DAPI (blue) (top panels). Seminal vesicles (SV) harboring sperm are also stained with DAPI (blue) (bottom panels). Asterisk denotes the apex of testis. Scale bar: 50µm (apical end of testes, top) and 20 µm (SV, bottom). (D) The numbers of egg laying and hatching rate. Daily egg laying by three y w females mated with three males of the indicated genotypes are shown ($n=3$). The number of ovarioles assessed is noted above each bar. Error bar indicates s.d.

3.1.2 *stil*-deficient female germline cells undergo apoptotic cell death

To investigate the cause of female germline cell loss, I knocked down *stil* function by expressing short hairpin RNA targeting the 4th exon of the *stil* transcript (GL01230) using a germline driver (Figure 3.1.1 A). Germline-specific knockdown (*stil*-GLKD) reduced *stil* expression in ovaries by approximately 90% compared to control (Figure 3.1.3 A). *stil*-GLKD females contained significantly smaller ovaries and did not lay any eggs, resembling to *stil* loss-of-function mutant females (Figure 3.1.3 B, E). Unlike the complete germline loss observed in *stil* mutants, however, most ovarioles in *stil*-GLKD retained germline cells, indicating a milder phenotype (Figure 3.1.3 C). Notably, the number of ovarioles containing at least two egg chambers was remarkably fewer (27.4% versus 100% in the control), suggesting that oogenesis was perturbed before mid-oogenesis (Figure 3.1.3 B, D). While *stil*-GLKD exhibited severe defects similar to its loss-of function alleles, its somatic knockdown using a *tj-Gal4* driver (*stil*-STKD) did not affect egg production and the hatching rate at all (Figure 3.1.3 E). These observations suggest that *stil* functions specifically in female germline cells, ensuring their survival and proper egg development, whereas male germline cells remain largely unaffected.

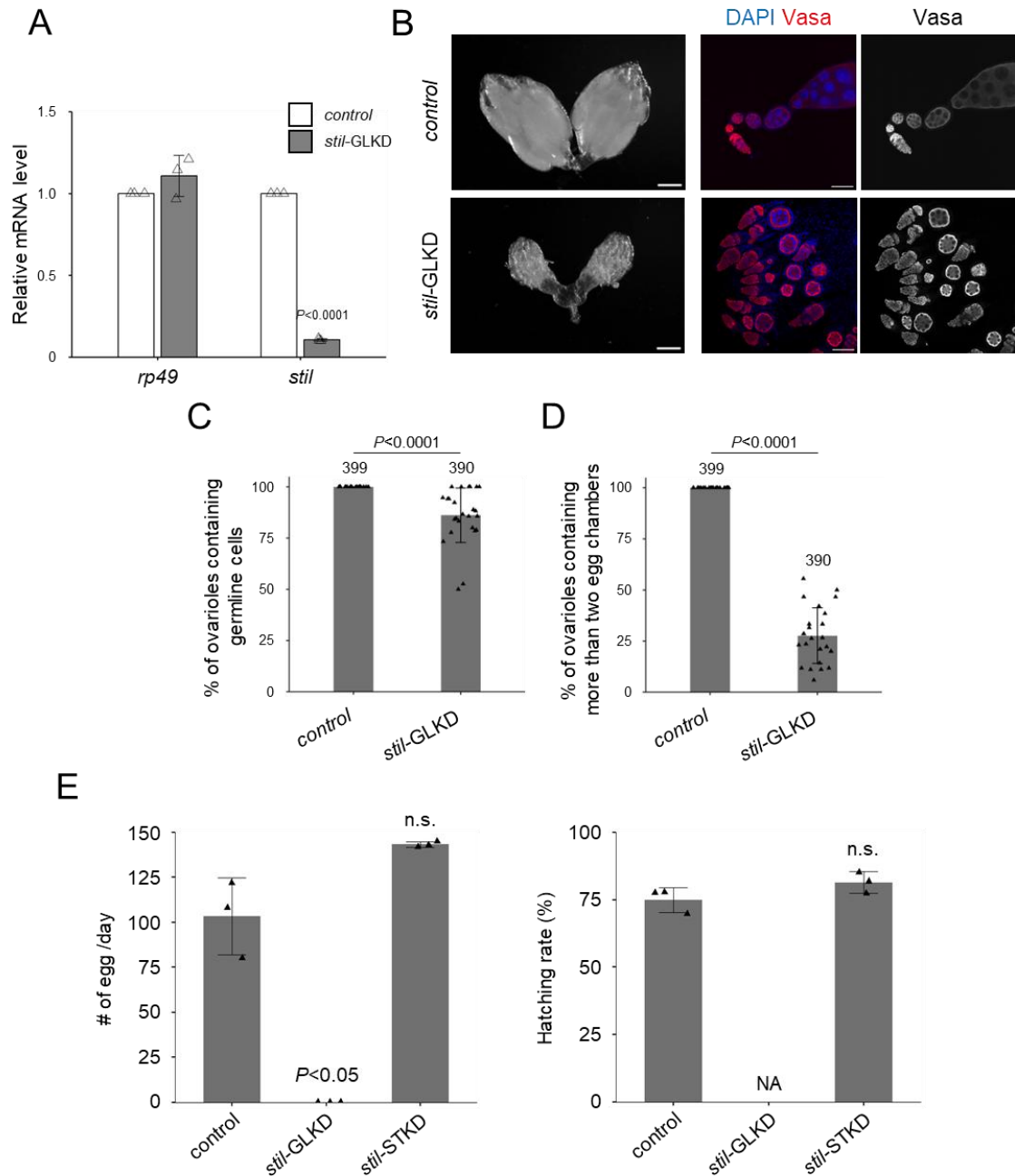


Figure 3.1.3 Germline, but not somatic, knockdown of *stil* disrupts germline survival and differentiation

(A) Quantitative RT-PCR confirming the knockdown efficiency in ovaries of *stil*-germline knockdown (*stil*-GLKD) where shRNA targeting *stil* transcript was driven by *NGT40; NosGal4-VP16*, and the control with shRNA alone. Expression levels of an internal control, *rp49*, and *stil* are normalized to that of α -tub. Error bar indicates s.d. ($n=3$). (B) Representative images showing entire ovarian morphology (left panels) and ovarioles immunostained with a germline marker, Vasa (red), and nuclear staining with DAPI (blue) (right panels) from *stil*-GLKD and the control with shRNA alone. Scale bar: 1 mm for whole ovaries and 50 μ m for ovarioles, respectively. (C) Quantification of the percentage of ovarioles containing germline cells per ovary in 2–3-day-old females. Each dot represents an individual ovary. Genotypes are indicated

below the graph and the number of germarium assessed is noted above each bar. Error bars represent standard deviation (s.d.). (D) Quantification of the percentage of developed ovarioles defined as ovarioles that contain at least two egg chambers beyond the germarium per ovary in 2–3-day-old females. Each dot represents an individual ovary. Genotypes are indicated below the graph and the number of germarium assessed is noted above each bar. Error bars represent standard deviation (s.d.). (E) Analysis of egg laying and hatching rates. The number of laid eggs and their hatching rates are measured daily for three females of the indicated genotypes: control, *stil*-germline knockdown (*stil*-GLKD) driven by *NGT40*; *NosGal4-VP16*, and *stil*-somatic knockdown (*stil*-STKD) driven by *tj-Gal4*, each mated with three *y w* males (*n*=3). Error bars indicate standard deviation (s.d.).

Previous studies have suggested that *stil* may play a role in sex determination, particularly through its genetic interaction with *ovo* and the key sex determination regulator *otu*, which represses the male identity factor Tdrd51 (Pennetta & Pauli, 1997; Primus et al., 2019; Sahut-Barnola & Pauli, 1999). To investigate whether *stil* is involved in sex determination, I examined the transcripts of *sxl*, a master regulator of sex determination of germline cells in *stil* mutant ovaries (Hashiyama et al., 2011). *sxl* pre-mRNA undergoes alternative splicing to generate sex-specific isoforms, producing a functional Sxl protein essential for female development, or a truncated non-functional male-specific isoform (Bell et al., 1991) (Moschall et al., 2019). RT-PCR revealed an enrichment of the female-specific *sxl* isoform in both control and *stil*-GLKD ovaries (Figure 3.1.4). In addition, male-specific *sxl* transcripts, detected in both control and *stil*-GLKD testes, were absent in *stil*-GLKD ovaries, indicating that *sxl* splicing patterns remain unaffected by *stil* depletion. These results suggest that *stil* is irrelevant to sex identity determination in germline cells.

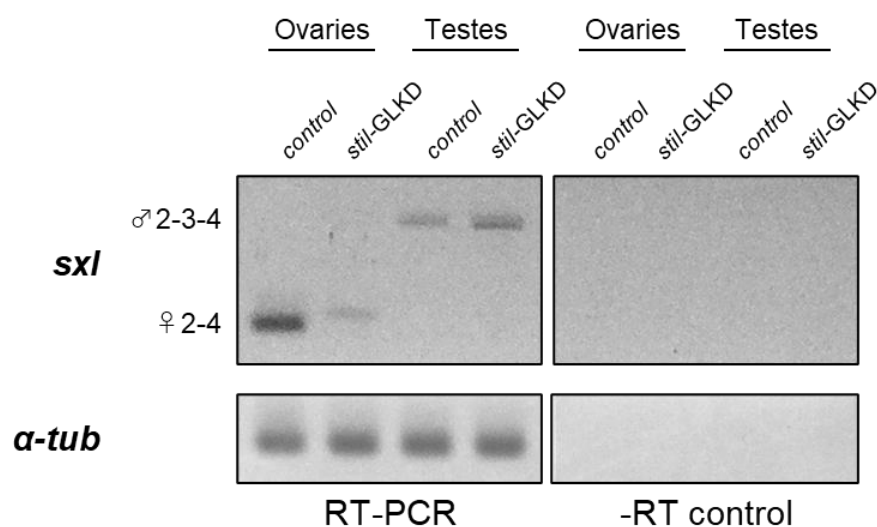


Figure 3.1.4 *stil* is irrelevant to sex determination

RT-PCR analysis of *sxl* and the control, α -tub transcripts in ovaries and testes from control and *stil*-GLKD flies, respectively.

Damaged or abnormal cells are typically removed through regulated cell death mechanisms, including apoptosis, autophagy, and necrosis (Fuchs & Steller, 2011, 2015). In *Drosophila* ovaries, such cell death occurs at two distinct developmental checkpoints during oogenesis: in the germarium and during mid-oogenesis, in response to either developmental cues or environmental stressors (McCall, 2004; Pritchett et al., 2009a). To determine whether germline cell loss in *stil*-deficient ovaries was due to apoptosis, I performed a TUNEL (Terminal deoxynucleotidyl Transferase Biotin dUTP Nick End Labeling) assay, which detects DNA fragmentation indicative of apoptotic cell death. In region 2A and 2B of the germarium, the first developmental checkpoint in oogenesis (Drummond-Barbosa & Spradling, 2001), the number of TUNEL-positive cells significantly increased in *stil*-GLKD ovaries compared to controls (Figure 3.1.5

A, B). These results suggest that *stil*-GLKD germline cells undergoes apoptotic cell death.

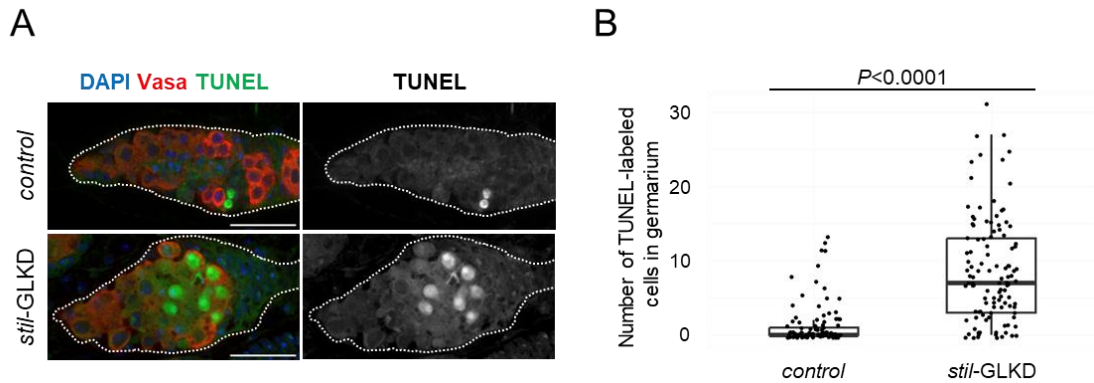


Figure 3.1.5 Loss of *stil* in the germline increases apoptotic cells

(A) Representative images of TUNEL-positive apoptotic cells (green), Vasa (red), and DAPI (blue) in control with shRNA alone and *stil*-GLKD germaria (white dotted). Scale bar: 20 μ m. (B) Quantification of apoptosis in germarium with TUNEL assay in control and *stil*-GLKD.

To further confirm the involvement of *stil* in apoptosis, I overexpressed inhibitor of apoptosis proteins (IAPs) in *stil* mutant germline cells. Both the *Drosophila* protein DIAP1 (Death-associated inhibitor of apoptosis 1) and the baculovirus-derived P35 are well-characterized inhibitors that can effectively suppress apoptosis in *Drosophila*. DIAP1 inhibits the activity of initiator caspase, Dronc (Death regulator Nedd2-like caspase) which activates effector caspase, while P35 directly inhibits effector caspase (Baum et al., 2007; J.S Peterson et al., 2003; Werz et al., 2005). Germline-specific overexpression of P35 fully rescued the germline cell loss observed in *stil*^{EY16156} mutants (Figure 3.1.6 A, B). However, germline development remained arrested, and egg chambers degenerated at the mid-oogenesis (Figure 3.1.6 C). Likewise, DIAP1

overexpression partially restored germline cells (~50% of ovarioles retained germline cells), but did not fully rescue oogenesis defects (Figure 3.1.6 A, B). Altogether, these findings indicate that the loss of *stil* function not only triggers apoptosis that can be suppressed by apoptosis inhibitors but also causes defects in oogenesis progression that are not rescued by blocking cell death.

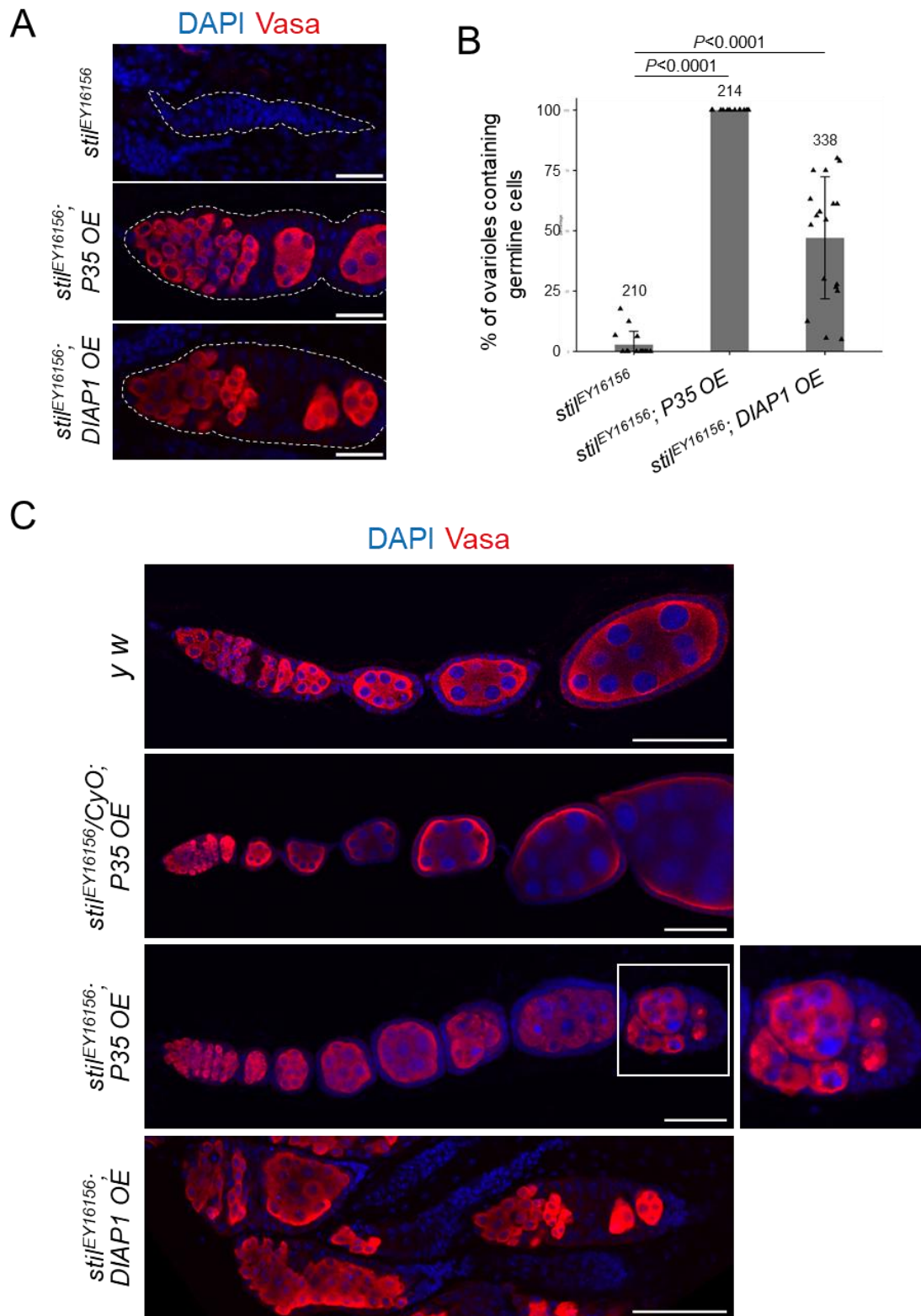


Figure 3.1.6 *stil* loss induces apoptotic cell death in female germline cells

(A) Representative images of germaria (white dotted) from *stil*^{EY16156}, *stil*^{EY16156}; P35 OE (NGT40; NosGal4-VP16> P35), and *stil*^{EY16156}; DIAP1 OE (NGT40; NosGal4-VP16> DIAP1)

immunostained with Vasa (red) with DAPI (blue). Scale bar: 20 μ m. (B) Quantification of the percentage of ovarioles containing germline cells per ovary in 2–3-day-old females. Each dot represents an individual ovary. Genotypes are indicated below the graph and the number of germarium assessed is noted above each bar. Error bars represent standard deviation (s.d.). (C) Immunostaining of ovarioles from *y w, stil^{EY16156}/CyO; P35 OE*, *stil^{EY16156}; P35 OE* and *stil^{EY16156}; DIAP1 OE* flies with antibody against Vasa (red) and DAPI (blue). The enlarged image highlights a degenerated egg chamber at the mid-stage of oogenesis in *stil^{EY16156}; P35 OE*. Scale bar: 50 μ m.

3.1.3 Stil regulates proapoptotic genes to suppress apoptosis in female germline cells

Stil protein harbors a characteristic DNA-binding domain, BED-type zinc finger, containing highly conserved aromatic residues followed by a shared pattern of cysteines and histidines (Figure 1.6) (Aravind, 2000). Given that many of the known BED-type zinc fingers including the founder members, BEAF and DREF, function as transcription factors or chromatin regulators (McKowen et al., 2022; Sokolova et al., 2023; Tue et al., 2017b), Stil may play a role in transcriptional regulation.

To examine this possibility, I analyzed the transcriptional profiles of ovarian RNA of *stil* mutant and control. I took advantage of the ectopic expression of P35 to overcome cell death defect of germline cells due to the loss of in *stil* function. The RNA-seq analysis identified 3,569 (30.7%) differentially expressed genes (DEGs) ($\log_2FC > |1|$ and $p\text{-adj} < 0.01$) out of 11,608 annotated genes (DDBJ: PRJDB20709). Among these, 2,044 genes were up-regulated (17.6 %, red dots) and 1,525 genes were down-regulated (13.1 %, blue dots) in *stil* mutant ovaries (Figure 3.1.7 A). Consistent with the observation of developmental arrest and egg chambers degeneration at the mid-stage of oogenesis (Figure 3.1.7 C), genes known to be highly expressed in late-stage oogenesis, such as proteins composing the chorion, were

significantly downregulated in *stil* mutant ovaries (Figure 3.1.7 B). Of those upregulated, notably, the proapoptotic gene *rpr* (*reaper*) ranked among the top 10 upregulated DEGs. Given the apoptotic phenotype of *stil* mutant germline cells, 67 apoptosis-related genes out of 224 annotated in the dataset (GO:0006915) were identified as DEGs (Figure 3.1.7 C). Among the top 10 genes list of apoptosis-related DEGs, proapoptotic genes, *rpr* and *hid* (*head involution defective*), were significantly upregulated, while the hedgehog signaling genes expressed in later oogenesis such as *ptc*, *duox*, and *rdx* were significantly downregulated (Figure 3.1.7 D). *rpr* and *hid* are the key members of IAP antagonists, known for their central role in initiating apoptosis (Abbott & Lengyel, 1991; White et al., 1994). Their activation through various signaling pathways leads to the ubiquitin-mediated degradation and translational inhibition of DIAP1, which subsequently results in the activation of Caspase proteins (Freel et al., 2008; Holley et al., 2002).

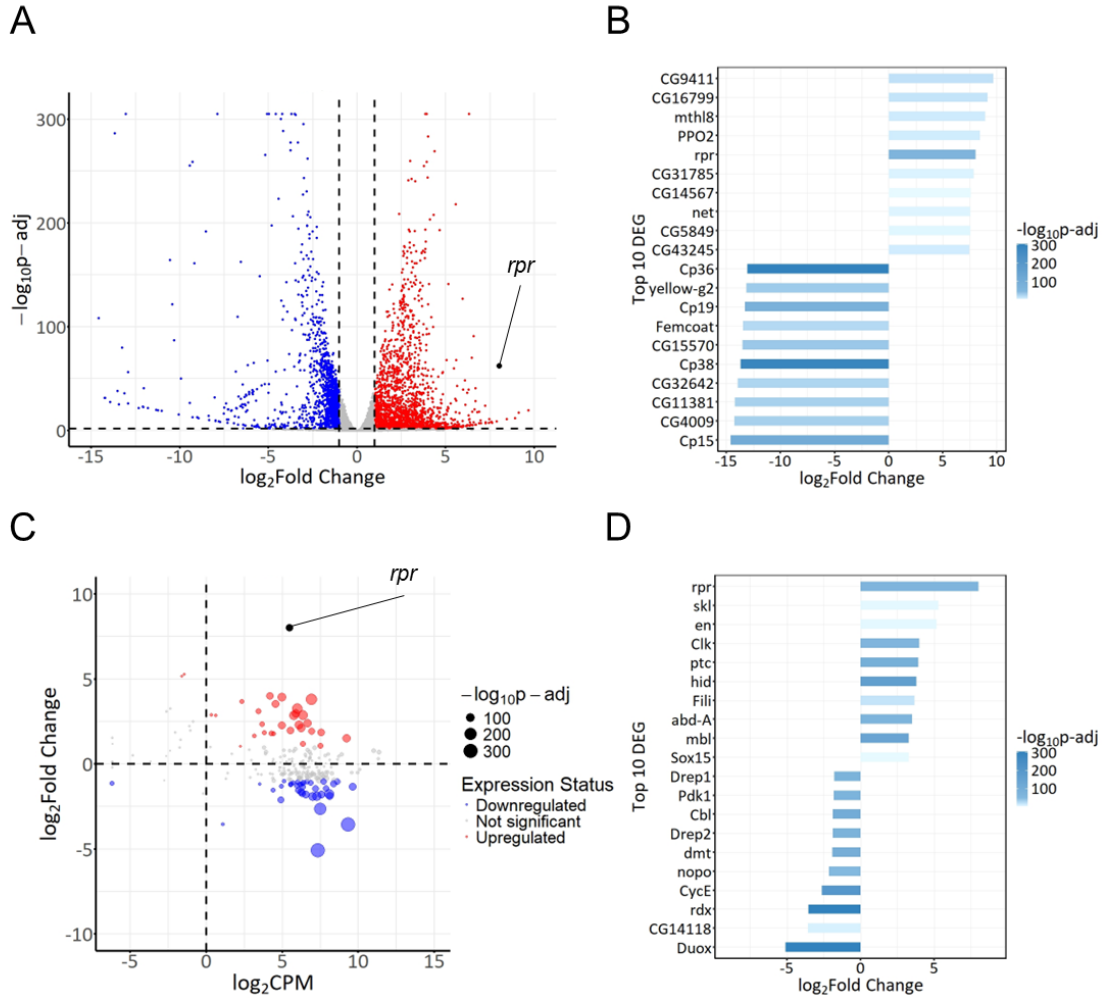


Figure 3.1.7 Transcriptome analysis reveals upregulation of pro-apoptotic genes

(A) Transcriptome analysis of germline-rescued *stil* mutant ovaries expressing P35. Differential expressed genes (DEGs) are obtained with DESeq2. Volcano plot displays the adjusted p-value ($p\text{-adj}$) versus $\log_2$ -fold change ($\log_2FC$) of expression level in *stil^{EY16156}; P35 OE* compared to that in *stil^{EY16156}/CyO; P35 OE*. Significantly upregulated genes ($\log_2FC > 1$, $p\text{-adj} < 0.01$) and downregulated genes ($\log_2FC < -1$, $p\text{-adj} < 0.01$) are highlighted in red and blue, respectively, while non-DEGs are shown in black. (B) Top 10 upregulated and downregulated genes in *stil*-deficient ovaries overexpressing (OE) of the apoptosis inhibitor, P35 in germline cells (*stil^{EY16156}, NGT40; NosGal4-VP16> P35*), compared to control with P35 OE in a heterozygous background (*stil^{EY16156}/CyO, NGT40; NosGal4-VP16> P35*). Bars are according to adjusted p-values ($p\text{-adj}$). (C) MA plot of apoptosis-related genes (GO:0006915) showing their mean gene expression (CPM: counts per million) and fold change in *stil^{EY16156}, P35 OE* comparing *stil^{EY16156}/CyO; P35 OE*. Each dot represents an individual gene, with dot size inversely correlated with $p\text{-adj}$. Larger dots highlight genes with higher statistical significance. The red dots (upregulated genes) and blue dots (downregulated genes) represent the differentially expressed RNAs with fold threshold > 2.0 , and $p\text{-adj} < 0.01$. (D) Top 10 upregulated and downregulated genes related to apoptosis (GO:0006915). Bars represent fold changes and are colored by adjusted p-values ($p\text{-adj}$), as in (B).

Further validation by RT-qPCR demonstrated that *rpr* expression increased approximately 700-fold, while *hid* expression increased 15-fold in the rescued *stil* mutant ovaries by P35 expression (Figure 3.1.8 A). To exclude a possibility that egg chamber degeneration in germline-rescued *stil* mutants affects proapoptotic gene expression, I isolated RNAs from early-stage ovaries (up to stage 5-6 of oogenesis) by manual removal of later stages and re-examined their expression levels. *rpr* expression remained elevated in RNAs prepared from early-stage ovaries, while *hid* expression did not (Figure 3.1.8 B). Furthermore, RT-qPCR analysis using *stil*-GLKD ovaries driven by three different germline drivers, *NGT40*; *NosGal4-VP16*, *Bam-Gal4*, and *mata -Tub-Gal4*, consistently exhibited significant upregulation of *rpr* but not *hid* across all conditions (Figure 3.1.8 C). Conversely, *rpr* was not prominently upregulated in *stil*-GLKD testes with *NGT40*; *NosGal4-VP16* or *Bam-Gal4*, consistent with the phenotypic observations in *stil* mutant testes (Figure 3.1.8 D). These results suggest that *rpr* is robustly upregulated in the absence of *stil* function in female germline cells.

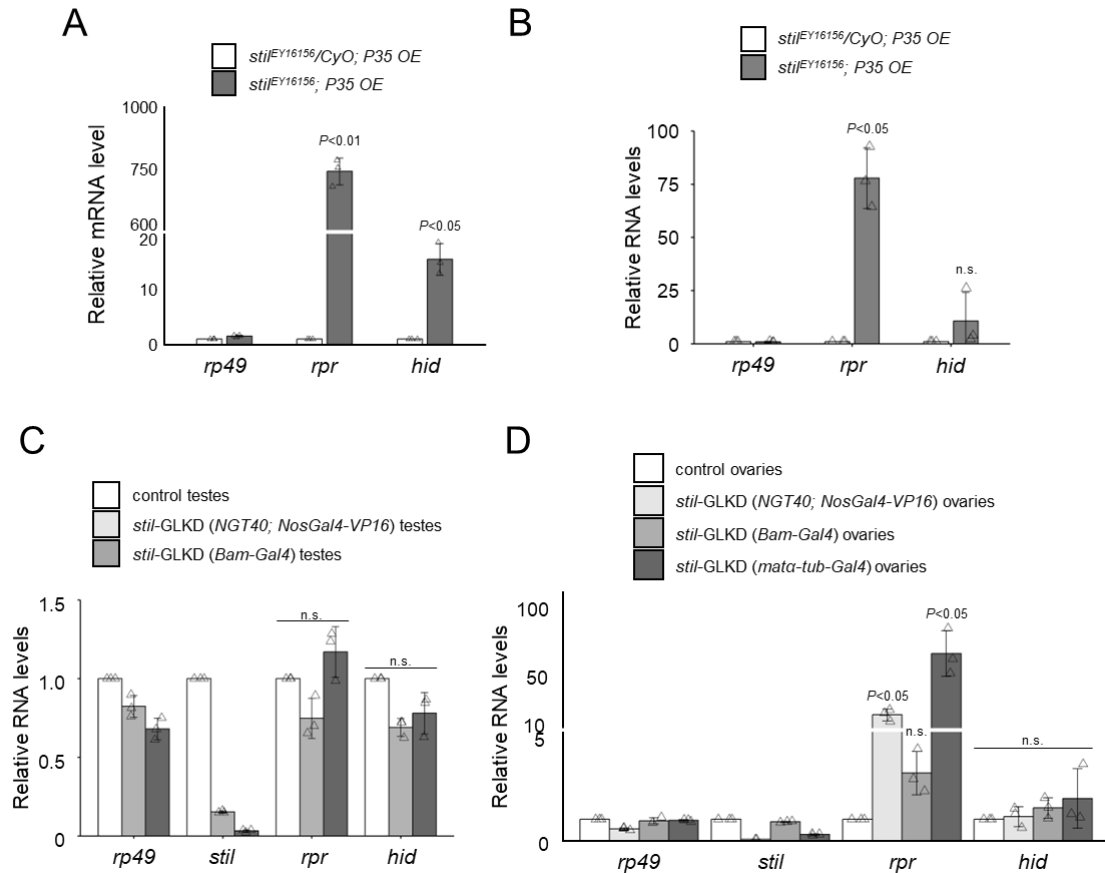


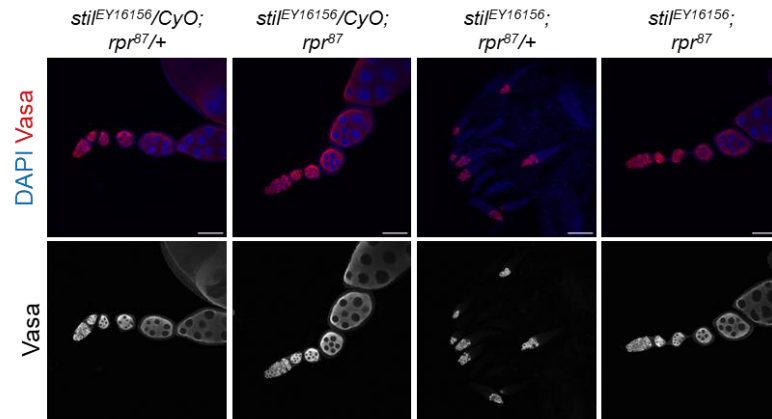
Figure 3.1.8 Loss of *stil* in the germline elevates *rpr* expression in ovaries not testes

(A-D) Quantitative RT-PCR are performed on ovaries from germline-rescued *stil* mutants, as well as ovaries and testes with germline-specific *stil* knockdown using distinct drivers. Expression levels of an internal control, *rp49*, *stil*, *rpr*, and *hid* are normalized to that of α -*tub*. (A) Whole ovaries from *stil^{EY16156}; P35 OE* and *stil^{EY16156}/CyO; P35 OE* ovaries. (B) Early-stage ovaries are isolated by dissecting stage 4–5 oocytes from *stil^{EY16156}/CyO; P35 OE* and *stil^{EY16156}; P35 OE* flies (C) *stil* knockdown in female germline cells is achieved using *NGT40-Gal4*; *NosGal4-VP16*, *Bam-Gal4*, and *mata-tub-Gal4* drivers. (D) *stil* knockdown in male germline cells is achieved using *NGT40-Gal4*; *NosGal4-VP16* and *Bam-Gal4* drivers. Error bars represent standard deviation (s.d.).

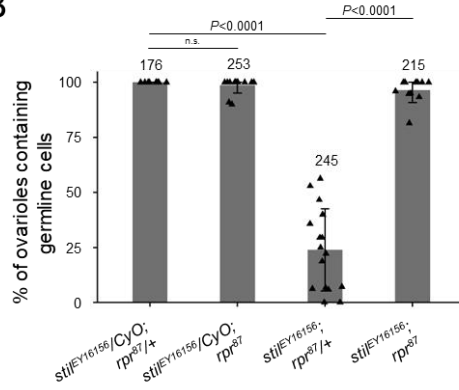
To determine whether proapoptotic genes upregulation directly contributes to apoptosis of *stil* mutant female germline cells, I generated double mutants of *stil* and the proapoptotic gene, *rpr*. Strikingly, the germline cell death and differentiation defects observed in *stil* mutant ovaries were almost completely rescued in the double mutant ovaries (Figure 3.1.9 A, B). Germline development proceeded, resulting in the formation of mature eggs and partial restoration of

fertility; mutant females laid a few eggs and approximately 40 % of those hatched (Figure 3.1.9 C, D). In contrast, *stil-hid* double mutants showed a slight increase in germline cell survival, but no developing egg chambers were observed (Figure 3.1.9 E, F), indicating that *hid* does not play a central role in apoptosis caused by the loss of *stil* function. These findings highlight that *rpr* regulation by *stil* is critical for suppressing apoptosis in female germline cells, establishing *rpr* as a key downstream effector in this pathway.

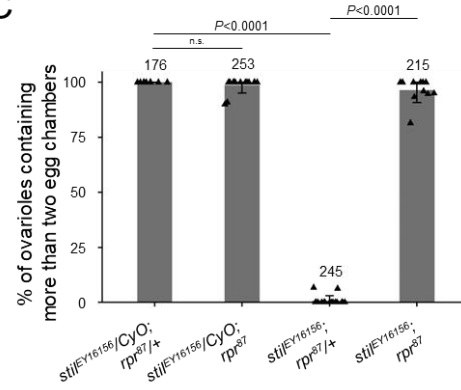
A



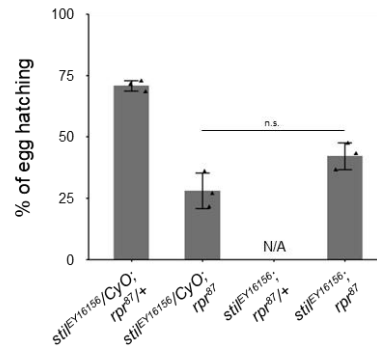
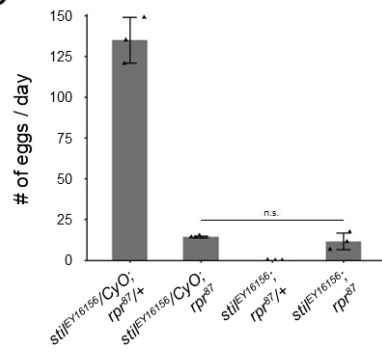
B



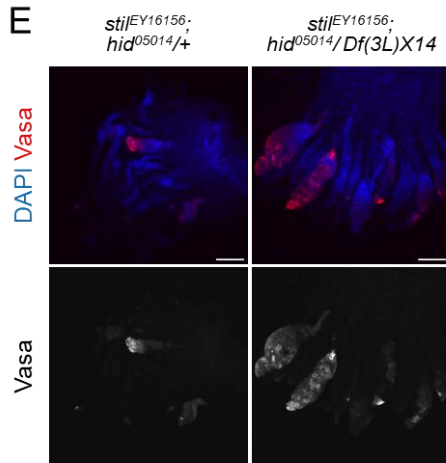
C



D



E



F

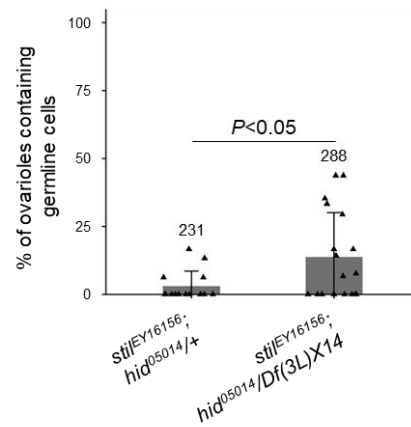


Figure 3.1.9 *rpr* causes germline cell loss in *stil* mutants

(A) Immunofluorescence of Vasa (red) and DAPI (blue) in ovarioles from *stil^{EY16156}/CyO; rpr^{87/+}*, *stil^{EY16156}/CyO; rpr⁸⁷*, *stil^{EY16156}; rpr^{87/+}*, and *stil^{EY16156}; rpr⁸⁷*. Scale bar: 50 μ m. (B) Quantification of the percentage of ovarioles containing germline cells per ovary in 2–3-day-old females. Each dot represents an individual ovary. Genotypes of females and the number of germarium assessed are noted above each bar. Error bars represent standard deviation (s.d.). (C) Quantification of the percentage of developed ovarioles defined as ovarioles that contain at least two egg chambers beyond the germarium per ovary in 2–3-day-old females. Each dot represents an individual ovary. Genotypes females and the number of germarium assessed are noted above each bar. Error bars represent standard deviation (s.d.). (D) Analysis of egg laying and hatching rate. Daily egg laying by three females indicated genotypes mated with three y w male and their hatching rate are shown ($n=3$). Error bar indicates s.d. (E) Immunostaining of *stil^{EY16156}; hid^{05014/+}* and *stil^{EY16156}; hid⁰⁵⁰¹⁴/Df(3L)X14* ovaries with antibody against Vasa (red) with DAPI (blue). Scale bar: 50 μ m. (F) Quantification of the percentage of ovarioles containing germline cells per ovary in 2–3-day-old females. Each dot represents an individual ovary. Genotypes of females and the number of germarium assessed are noted above each bar. Error bars represent standard deviation (s.d.).

3.1.4 *Stil* represses *rpr* expression by targeting the regulatory regions via its BED-type zinc finger

The transcription of *rpr* is governed by a complex network of developmental, environmental, and stress-related signals, each employing distinct regulatory mechanisms. These signals modulate transcription factors such as p53, Dfd, the EcR/USP complex, AP-1, Shn, leading to either the activation or suppression of apoptosis (Beira et al., 2014; Brodsky et al., 2000; Jiang et al., 2000; Lohmann et al., 2002). These transcription factors interact with upstream enhancer and promoter regions of the *rpr* locus (*rpr*-transcriptional control region) to either activate or suppress its transcription, ensuring precise spatial and temporal control of apoptosis (Fuchs & Steller, 2011). To examine whether *Stil* also directly interacts with *rpr*-transcriptional control region, I performed DNA adenine methyltransferase identification (DamID)-seq analysis (Aughey et al., 2019). A fusion of *Stil* with *E. coli* DNA adenine methyltransferase (Dam-*Stil*) or Dam alone as a control were expressed specifically in germline cells (Díaz-Torres et al., 2021; Southall et al.,

2013). Genomic DNA from ovaries was digested with DpnI and subjected to NGS to detect GATC sites methylated by Dam-Stil near Stil-bound regions (DDBJ: PRJDB20722). Genome-wide DamID-seq analysis revealed that ~80% of Stil-enriched peaks were located in promoter regions, supporting a role for Stil in transcriptional regulation (Figure 3.1.10 A). At higher resolution, prominent peaks were observed at the *rpr* locus and its upstream region (5–6 kb), suggesting direct binding of Stil to the *rpr* regulatory element (Figure 3.1.10 B).

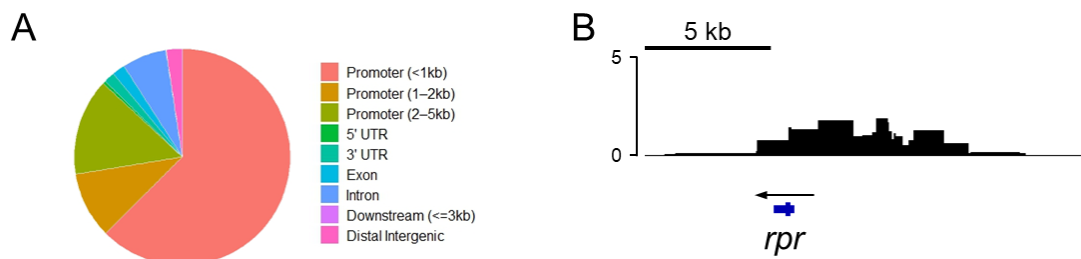


Figure 3.1.10 DamID reveals Stil enrichment in promoter regions, particularly around *rpr*
 (A) Distribution of DamID peaks across entire genome categorized by genomic features including promoter (≤ 1 , 1–2, and 2–3 kb), UTR, exon, intron, distal intergenic, and downstream.
 (B) The plot showing the log₂ ratio of Dam-Stil peaks to Dam-alone control across the *rpr* genomic region. The blue bar on the bottom indicates the *rpr* gene body, and the arrow shows the direction of *rpr* transcription. Three independent biological replicates were performed for both Dam-Stil and Dam-alone and data were merged for visualization.

Next, I examined the role of Stil's BED-type zinc finger in *rpr* repression by generating four Stil variants: Stil-FL (full-length), Stil-NT (N-terminal domain containing the BED-type zinc finger and nuclear localization signal), Stil-CT (C-terminal domain lacking these two motifs), and Stil-AAYA (full-length Stil with a mutated BED-type zinc finger substituting four residues including two critical cysteines) (Figure 3.1.11 A). The expression of all four Stil variant proteins from

the transgenes was confirmed, although Stil-CT showed a slightly reduced expression level (Figure 3.1.11 B). In nurse cells, despite lacking the predicted nuclear localization signal, Stil-CT localized to the nucleus and overlapped with chromosomes, as did Stil-FL and Stil-AAYA, whereas Stil-NT showed diffuse nuclear distribution without being enriched onto chromosomes (Figure 3.1.11 C). Collectively, these data suggest that Stil's nuclear and chromatin localization are mediated by the C-terminal domain, independently of the zinc finger motif. To assess the functional significance of these domains *in vivo*, I expressed the variants in *stil*^{EY16156} germline cells. Stil-FL fully rescued the defects of both germline cell survival and development, leading to the production of mature eggs (Figure 3.1.11 C, D, E). Remarkably, Stil-NT partially rescued germline defects despite its diffuse nuclear localization, while neither Stil-CT nor Stil-AAYA, both lacking functional BED-type zinc fingers, restored germline cell survival or differentiation. These findings suggest that the BED-type zinc finger is essential for Stil function in germline cells possibly as a transcription regulator, while the C-terminal domain mediates nuclear and chromatin localization.

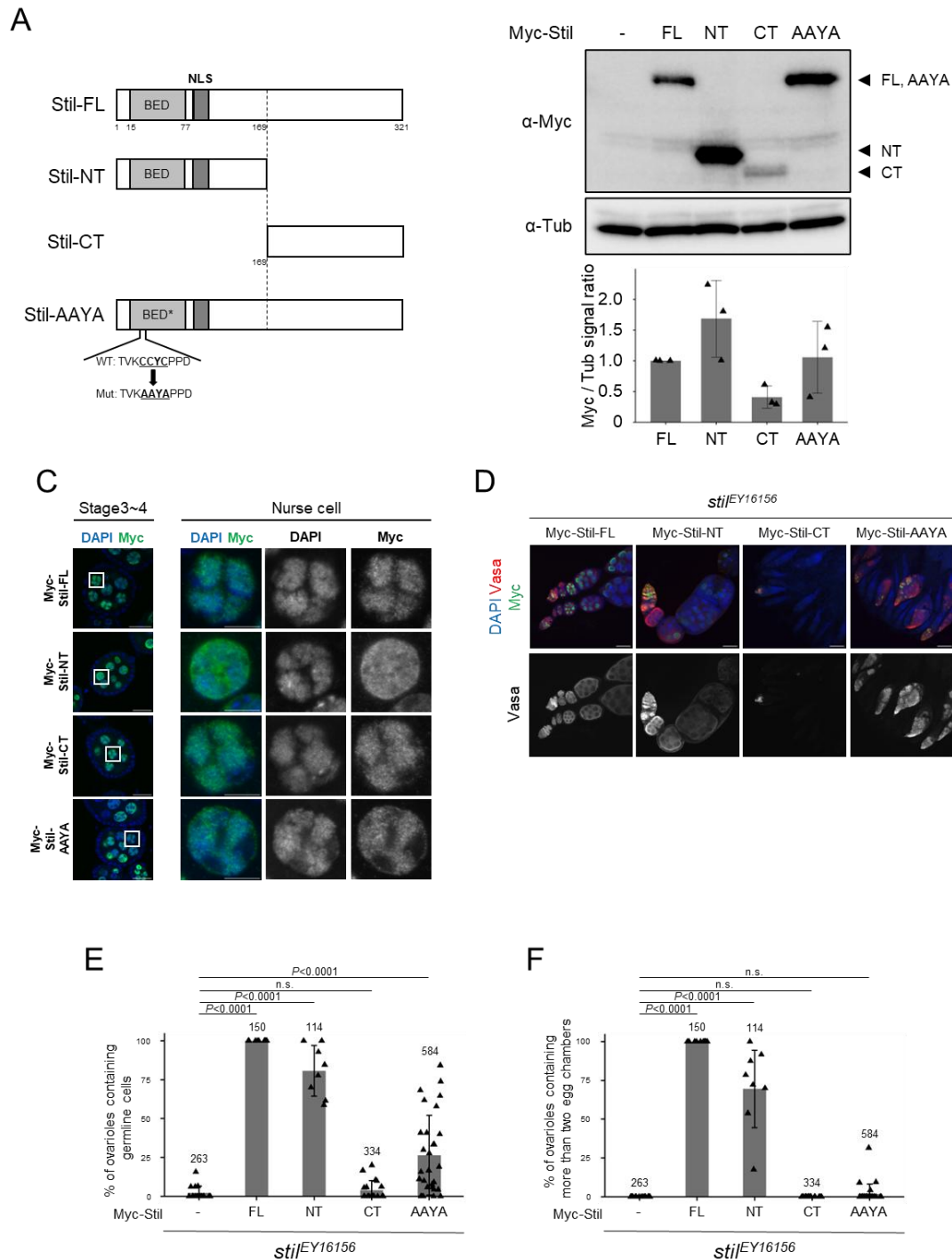


Figure 3.1.11 BED-type zinc finger in Stil plays a crucial role in germline cell survival

(A) Schematic representation of the Stil full-length (Stil-FL), the truncated variants: Stil-NT (1st to 169th aa) and Stil-CT (169th to the end), and amino acid substitutions of the C2H2 zinc finger CCYC: Stil-AA YA. (B) Western blot analysis of 6xMyc-tagged Stil variants (FL, NT, CT, and AAYA) driven by *NGT40-Gal4*; *NosGal4-VP16*, with *yw* as a control. Stil variants were detected with anti-Myc, and α -Tubulin (α Tub) served as a loading control. Arrowheads indicate Stil variant proteins. The lower panel shows quantification of the Myc/ α Tub signal ratio normalized to FL. Error bars indicate standard deviation (s.d.) ($n=3$). (C) Immunofluorescence images showing

Myc-tagged Stil variants (green) and DNA (DAPI, blue) in stage 3~4 egg chambers of the ovaries. Insets show enlarged views of nurse cell nuclei. Scale bar: 50 μm (egg chamber) and 20 μm (nurse cell nuclei). (D) *stil*^{EY16156} ovaries expressing Myc-tagged Stil variants corresponding to C in germline cells. Ovaries are immunostained with a germline marker, Vasa (red), and nuclear staining with DAPI (blue). Scale bar: 50 μm . (E) Quantification of the percentage of ovarioles containing germline cells per ovary in 2–3-day-old females. Each dot represents an individual ovary. Genotypes are indicated below the graph and the number of ovarium assessed is noted above each bar. Error bars represent standard deviation (s.d.). (F) Quantification of the percentage of developed ovarioles defined as ovarioles that contain at least two egg chambers beyond the ovarium per ovary in 2–3-day-old females. Each dot represents an individual ovary. Genotypes are indicated below the graph and the number of ovarium assessed is noted above each bar. Error bars indicate standard deviation (s.d.).

To validate whether the BED-type zinc finger functions in *rpr* repression through interaction with the *rpr* transcriptional control region, I performed a reporter assay using *Drosophila* S2 cells. Stil variants exhibited localization patterns in S2 cells similar to those in nurse cells (Figure 3.1.12 A), supporting their suitability for functional analysis in S2 cells. I constructed a *rpr*-GFP reporter containing the 5-kb upstream region of *rpr*, where DamID-seq signals were detected, fused to GFP (Figure 3.1.12 B) and transfected with Stil variants. Consistent with the *in vivo* rescue experiment (Figure 3.1.11 C, D, E), co-expression of Stil-FL with the *rpr*-GFP reporter significantly repressed GFP expression (Figure 3.1.12 C). Stil-NT containing the BED-type zinc finger also reduced GFP expression, albeit partially. In contrast, Stil-CT and Stil-AAYA failed to exhibit any detectable repressive effects. To confirm the specificity of this Stil-mediated repression, I utilized a *ubiP*-GFP reporter containing the *ubi-p63E* gene promoter fused to GFP (Figure 3.1.12 D). Co-expression of Stil-FL with the *ubiP*-GFP reporter showed no reduction in GFP expression (Figure 3.1.12 E), suggesting that Stil preferentially targets the *rpr* upstream region. These results demonstrate that Stil interacts with the *rpr* transcriptional control region via its BED-type zinc finger, which is critical for transcriptional repression,

while the chromatin association through the C-terminal region is required for full repressive activity in germline cells.

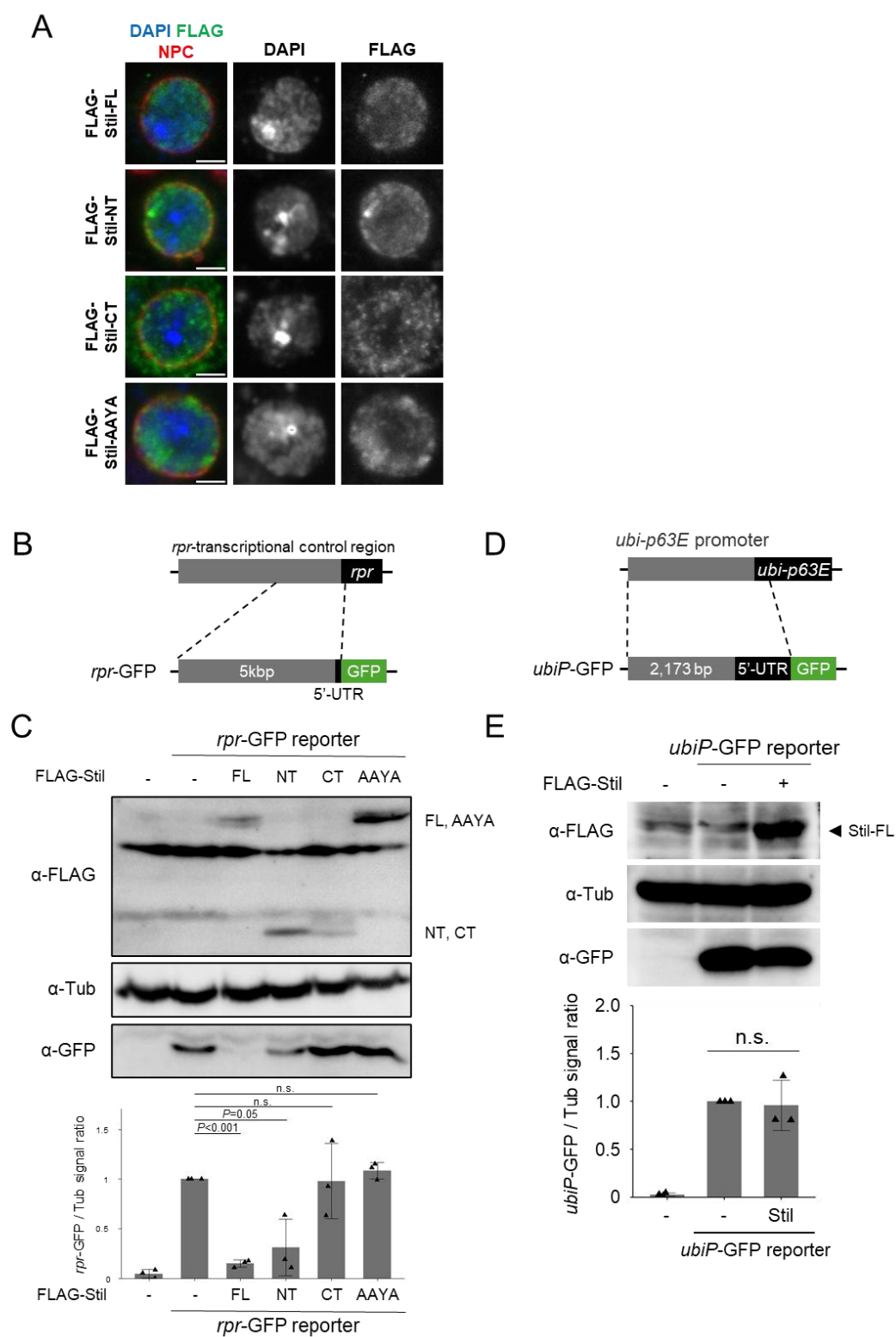


Figure 3.1.12 Stil interacts with *rpr*-transcriptional control region to repress its expression

(A) Immunostaining of S2 cells expressing FLAG-tagged Stil variants with an anti-FLAG (green) antibody and DAPI (blue). Scale bar: 1 μ m (B) Schematic representation of *rpr*-GFP reporter containing the 5 kb upstream region and 5'-UTR of the *rpr* gene fused to GFP CDS. (C) Reporter assay in S2 cells followed by western blot analysis to detect GFP and α -Tubulin (α Tub) as a loading control. Co-transfection of the *rpr*-GFP reporter and FLAG-tagged Stil variants were performed in S2 cells. The lower panel shows the signal ratio of GFP expression to α Tub, normalized to that in cells transfected with the reporter alone. Error bars represent the standard deviation (s.d.) ($n=3$). (D) Schematic representation of the *ubiP*-GFP reporter construct, in which the 2,173 bp upstream region and 5'-UTR of the *ubi-p63E* gene is fused to GFP. (E) Reporter assay in S2 cells followed by western blot analysis to detect GFP expression. Co-transfection of the *ubiP*-GFP reporter and FLAG-tagged Stil-FL were performed in S2 cells. An arrowhead indicates Stil-FL protein. α -Tubulin (α Tub) is used as a loading control. The lower panel quantifies the GFP/ α Tub signal ratio, normalized to that in S2 cells transfected with the reporter alone. Error bars indicate standard deviation (s.d.) ($n=3$).

3.2.5 DNA damage response to double-strand breaks promotes germline cell loss in *stil* mutants

Programmed cell death during oogenesis is tightly regulated by multiple quality-control checkpoints that safeguard both developmental progression and genomic integrity. In *Drosophila*, the first checkpoint operates in the germarium during early oogenesis, eliminating germline cysts that exhibit persistent DNA damage, nutrient deprivation, or defective encapsulation into egg chambers (Benitez et al., 2019; Drummond-Barbosa & Spradling, 2001; J.-H. Park et al., 2019; Smith et al., 2002), whereas the second checkpoint functions during mid-oogenesis to cull defective egg chambers in response to nutrient deprivation, aberrant hormone signaling, or unrepaired DNA damage (Bakhrat et al., 2010; Drummond-Barbosa & Spradling, 2001; Gruntenko & Rauschenbach, 2008; LaFever & Drummond-Barbosa, 2005; J.-H. Park et al., 2019; Pritchett et al., 2009a; Terashima et al., 2005). Expression of the caspase inhibitor P35 suppressed germline cell apoptosis in *stil* mutants; however, their egg chambers still underwent degeneration during mid-oogenesis, even under otherwise normal environmental conditions (Figure 3.1.6 C). This observation indicates

that accumulated intrinsic defects, such as DNA damage, in *stil* mutants are recognized at the second checkpoint, leading to clearance of the affected egg chambers.

To determine whether these defects are associated with DNA damage, I examined phosphorylated histone H2A variant γ H2Av, a well-established marker of DNA double-strand breaks (DSBs) in *Drosophila* (Shim et al., 2014). γ H2Av is normally detected in region 2 of the germarium, where programmed DSBs are generated and subsequently repaired during meiotic recombination (Mehrotra & McKim, 2006). Immunostaining revealed γ H2Av signals in region 2 across all genotypes, which were subsequently lost in region 3 through the DNA repair program specific to meiotic recombination (Figure 3.1.13) (Mehrotra & McKim, 2006). Notably, in *stil* mutants, an additional strong γ H2Av signal was specifically observed in the oocyte, beyond the expected meiotic pattern. These results suggest that persistent DSBs accumulate in *stil* mutant oocytes and may constitute a critical trigger for checkpoint-mediated apoptosis.

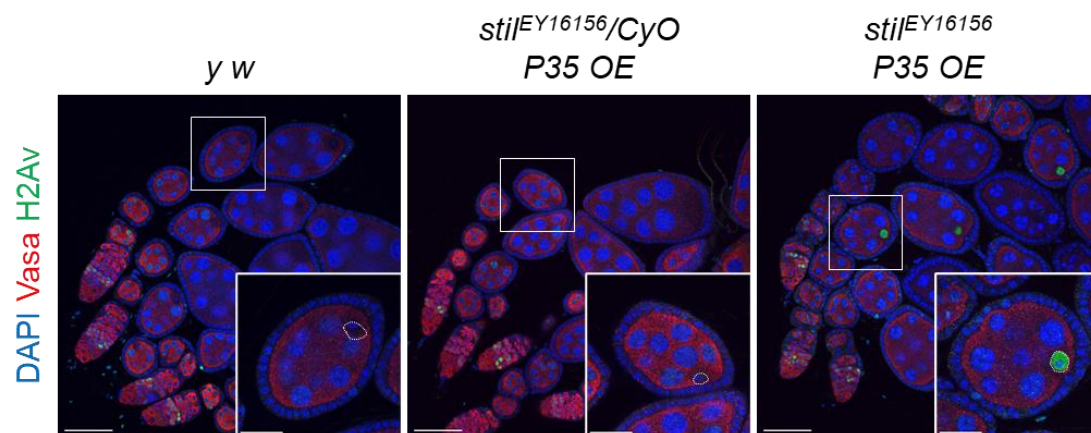


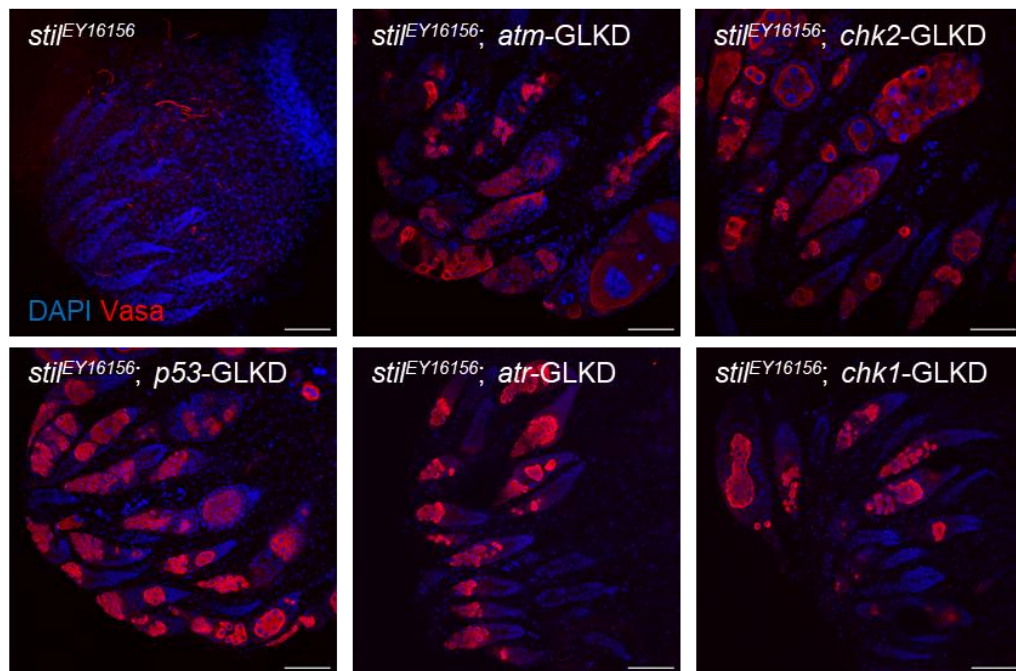
Figure 3.1.13 Accumulation of DNA double-strand breaks in *stil* mutant oocytes

Immunostaining of ovarioles from *y w, stil^{EY16156}/CyO; P35 OE (NGT40; NosGal4-VP16> P35), stil^{EY16156}; P35 OE* flies with antibody against DNA double-strand break marker H2Av (green), Vasa (red), and DAPI (blue). Insets show enlarged views of egg chamber. White dots indicate oocyte nuclei, Scale bar: 50 μ m (ovariole) and 20 μ m (egg chamber).

DNA damage activates the DNA damage response (DDR) pathway, in which the conserved kinases ATM (Ataxia telangiectasia mutated; *tefu* in *Drosophila*) and ATR (ATM- and RAD3-related; *mei-41* in *Drosophila*) are activated and subsequently signal through the downstream effector kinases CHK2 (*loki*) and CHK1 (*grp*), respectively (González-Marín et al., 2023; Maréchal & Zou, 2013). These effector kinases then activate p53, leading to cellular outcomes such as senescence, DNA repair, or apoptosis (Brodsky et al., 2004; Vries et al., 2005). During *Drosophila* oogenesis, DNA damage induced by ionizing radiation (IR) similarly triggers apoptosis through activation of the DDR pathway (J.-H. Park et al., 2019). To confirm whether accumulated DNA damage in *stil* mutant oocytes activates the DDR pathway and thereby induces apoptosis, I performed germline-specific RNAi knockdown of individual DDR pathway components in the *stil* mutant background. Although ovaries from *stil* mutants normally lack germline cells, germline cells were relatively preserved when DDR components were simultaneously knocked down (Figure 3.1.14 A, B). However, most germline cells failed to progress beyond the germarium, suggesting that additional *stil*-dependent functions, independent of the canonical DDR pathway, are required for germline development and survival. Altogether, these results suggest that persistent DNA damage in *stil* mutants contributes to germline apoptosis through activation of the DDR

pathway, but also that other defects intrinsic to *stil* mutants cooperate to ensure elimination of defective germline cells during oogenesis.

A



B

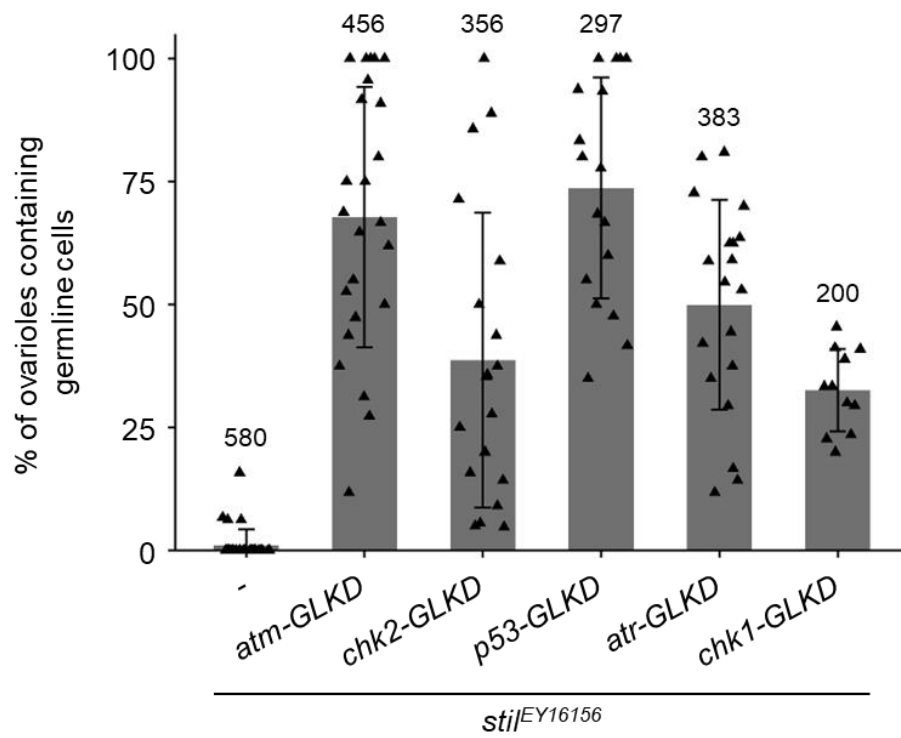


Figure 3.1.14 Germline knockdown of DDR pathway components rescues germline cell

loss in *stil* mutants

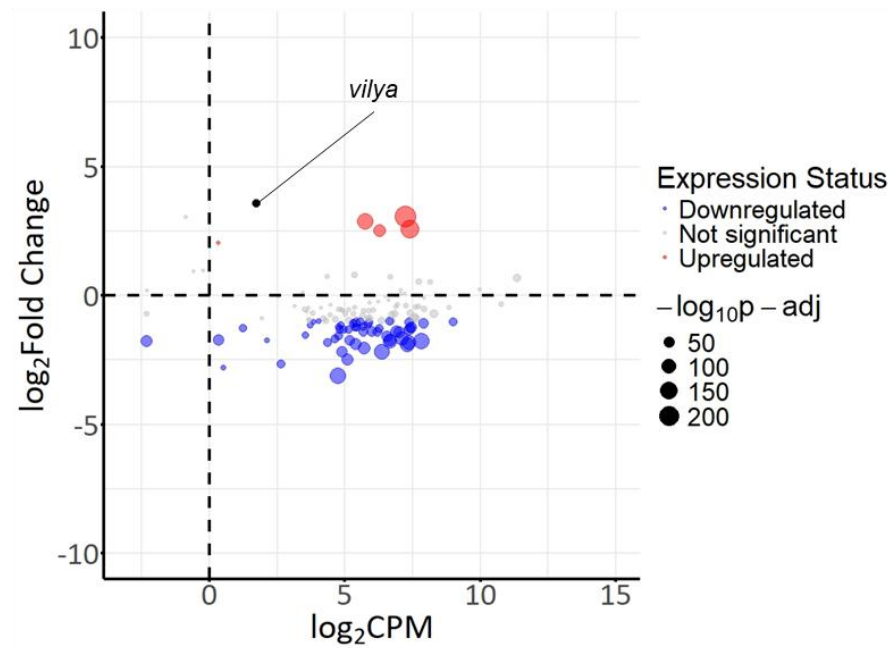
(A) Immunofluorescence of Vasa (red) and DAPI (blue) in ovaries from *stil^{EY16156}*, *stil^{EY16156}; atm-GLKD* (driven by *NGT40; NosGal4-VP16*), *stil^{EY16156}; chk2-GLKD*, *stil^{EY16156}; p53-GLKD*, *stil^{EY16156}; atr-GLKD*, and *stil^{EY16156}; chk1-GLKD*. Scale bar: 50 μ m. (B) Quantification of the percentage of ovarioles containing germline cells per ovary in 2–3-day-old females. Each dot represents an individual ovary. Genotypes of females and the number of germarium assessed are noted above each bar. Error bars represent standard deviation (s.d.).

3.2.6 Stil regulates meiotic recombination and DNA repair genes to prevent abnormal DSB formation

DNA damage can arise from either extrinsic or intrinsic factors, including excessive DNA damage formation or defects in DNA repair during oogenesis.

To investigate why DNA damage accumulates in the germline cells of *stil* mutants, I analyzed the RNA profiles of meiotic recombination (GO:0007131) and DNA repair (GO:0006281) genes by RNA-seq in *stil* mutants expressing P35, given that Stil functions as a transcription factor. Among these genes, 19 out of 34 meiotic recombination genes and 54 out of 116 DNA repair genes were identified as DEGs (Figure 3.1.15 A). Most DEGs in both DNA repair and meiotic recombination categories showed reduced expression in *stil* mutants (Figure 3.1.15 B). In contrast, the meiotic DSB formation–related gene *vilya* was upregulated and exhibited the most pronounced change in expression (Figure 3.1.15 A).

A



B

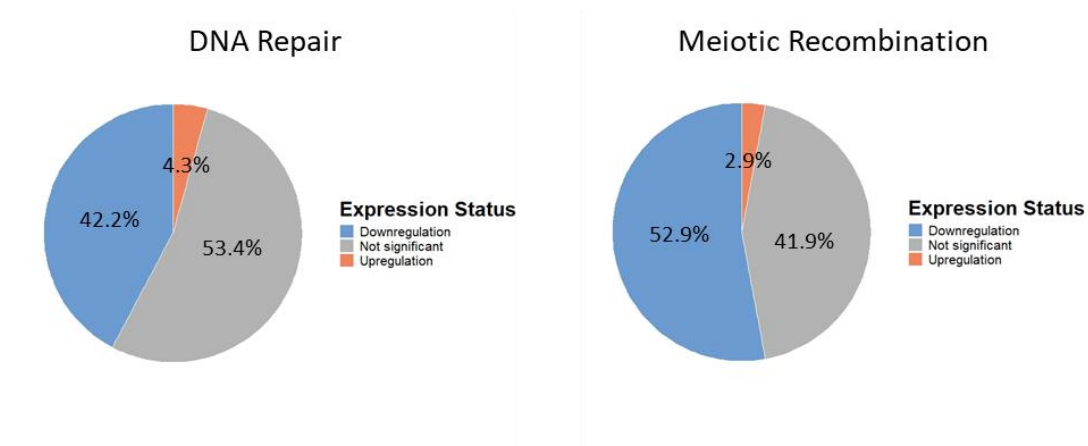


Figure 3.1.15 Differential expression of DNA repair and meiotic recombination genes in *stil* mutants

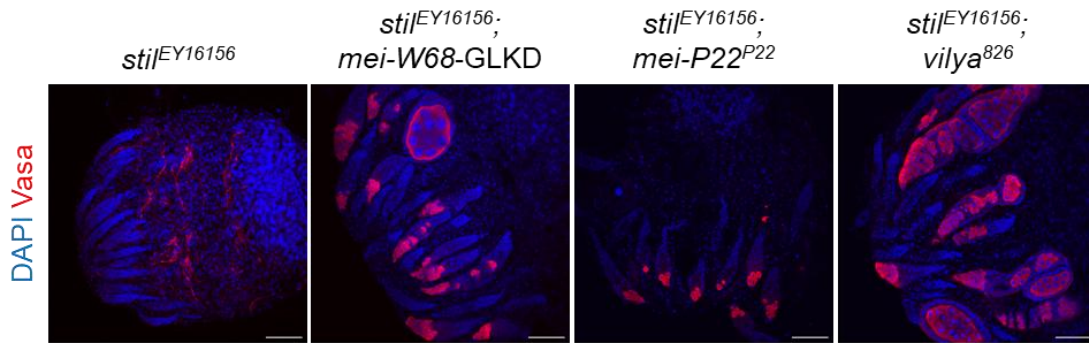
(A) MA plot of DNA repair genes (GO:0006281) and meiotic recombination genes (GO:0007131) showing mean expression levels ($\log_2$ CPM) versus $\log_2$ fold change in *stil*^{EY16156}; *P35* OE compared with *stil*^{EY16156}/CyO; *P35* OE. Each dot represents an individual gene, with dot size proportional to $-\log_{10}$ adjusted p-value (padj), highlighting genes with higher statistical significance. Red dots (upregulated) and blue dots (downregulated) indicate differentially expressed genes defined by $|\log_2$ fold change| > 1 and padj < 0.01. (B) Pie charts summarize the proportions of upregulated, downregulated, and non-significant genes within DNA repair and meiotic recombination categories based on the analysis in (A).

Unexpected DNA damage is rapidly repaired either by homologous recombination (HR) or by non-homologous end joining (NHEJ) (Polo & Jackson, 2011; Reginato & Cejka, 2020), whereas meiotically induced DSBs are repaired exclusively by HR to ensure crossover formation (Miller et al., 2016; S. Yoo & McKee, 2005). During meiotic recombination, DSBs generated in region 2 are rapidly resolved by region 3, where Spn-A and Brca2 primarily function to repair the breaks and promote crossover formation (Mehrotra & McKim, 2006). In *spn-A* mutants, DSB repair is delayed, and the breaks persist in the oocyte through later stages of oogenesis (Staeva - Vieira et al., 2003), a phenotype reminiscent of that observed in *stil* mutants (Figure 3.1.13). These suggest that Stil regulates the expression of DNA repair genes to facilitate the repair of meiotic DSBs. However, in *stil* mutants, meiotically induced DSBs appear to be repaired normally in region 3, even though DSBs accumulate in the oocyte of the egg chamber. Thus, the precise regulation of DNA repair during germline development remains unresolved.

Vilya encodes a Zip3-like RING finger protein essential for meiotic DSB formation (Lake et al., 2015). It interacts with the DSB accessory protein Mei-P22 and functions together with the Spo11 homolog Mei-W68, which catalyzes DSB formation (Hughes et al., 2018; H. Liu et al., 2002; McKim & Hayashi-Hagihara, 1998; Mehrotra & McKim, 2006). To examine whether excess DSB formation driven by *vilya* upregulation contributes to germline cell loss in *stil* mutants, I generated *stil vilya* double mutants. The germline loss defects

observed in *stil* mutants were partially rescued by loss of *vilya* (Figure 3.1.16 A, B). Furthermore, I investigated germline knockdown of *mei-W68* and *mei-P22* mutants in the *stil* mutant background. Similar to *vilya*, germline loss was partially rescued in *mei-W68* germline knockdown and *mei-P22* mutants, although the degree of rescue was weaker than that observed upon germline knockdown of *atm*, *atr*, or *p53*. These results suggest that *vilya* up-regulation contributes, at least in part, to germline cell loss through activation of the DDR pathway. Together, these findings indicate that Stil may regulate the expression of DNA repair and meiotic DSB recombination genes to prevent abnormal DSB accumulation, thereby repressing aberrant activation of the DDR pathway.

A



B

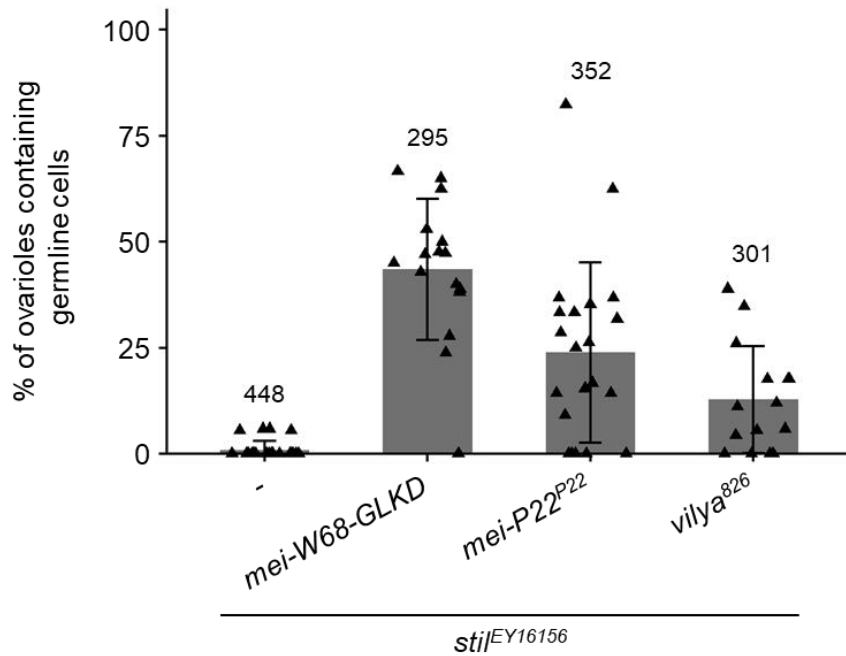


Figure 3.1.16 Germline knockdown of meiotic recombination factors partially recovered germline cell death defects in *stil* mutants

(A) Immunofluorescence of Vasa (red) and DAPI (blue) in ovaries from *stil^{EY16156}*, *stil^{EY16156}; mei-W68-GLKD* (driven by *NGT40; NosGal4-VP16*), *stil^{EY16156}; meiP22^{P22}*, and *stil^{EY16156}; vilya⁸²⁶*. Scale bar: 50 μ m. (B) Quantification of the percentage of ovarioles containing germline cells per ovary in 2–3-day-old females. Each dot represents an individual ovary. Genotypes of females and the number of germarium assessed are noted above each bar. Error bars represent standard deviation (s.d.).

3.2 Epigenetic Regulation Modulates apoptosis in Female Germline Stem Cells

3.2.1 Undifferentiated Germline Cells Exhibit Resistance to Apoptosis in *stil* loss

As *stil* is expressed from embryonic stage 11 (Sahut-Barnola & Pauli, 1999), defects in *stil* mutants are expected to manifest at earlier developmental stages rather than in adults. Loss of *stil* function resulted in a severe depletion of germline cells by the third instar larval stage (120 hours after egg laying, AEL), leaving only a few surviving germline cells in the mutants (Figure 3.2.1A, B). Notably, these surviving germline cells were predominantly found near the terminal filaments and future cap cells, the niche that supports germline stem cell (GSC) (A. Spradling et al., 2001). This observation suggested that GSCs within the niche may be resistant to cell death caused by *stil* loss.

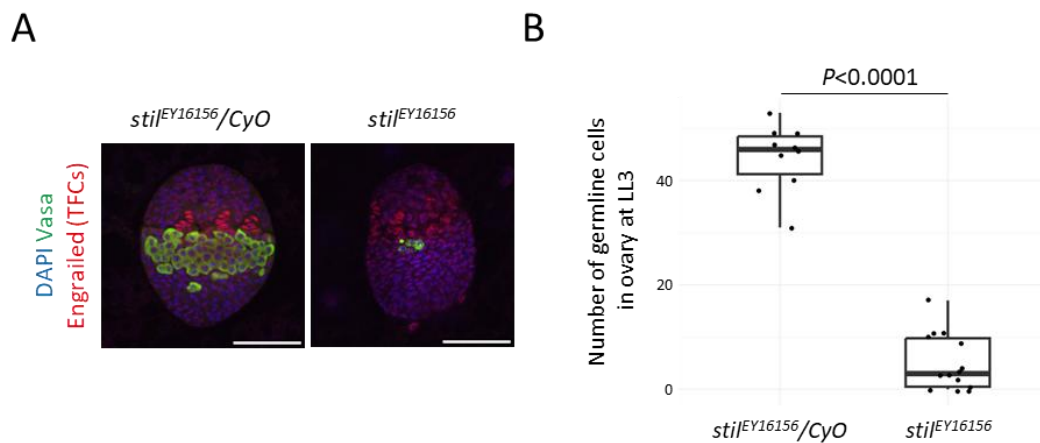


Figure 3.2.1 Reduced germline cell number in *stil* mutant 3rd instar larval ovaries

(A) Fluorescent immunostaining with anti-Engrailed (red), anti-Vasa (green), and DAPI (blue) for terminal filaments of late 3rd instar larval (LL3) ovaries from the heterozygous control, *stil^{EY16156}/CyO* and *stil* mutant, *stil^{EY16156}*. Scale bar: 50 μ m. (B) Quantification of surviving germline cells in 3rd instar larval ovaries from *stil^{EY16156}/CyO* and *stil^{EY16156}*.

To test whether GSCs are inherently resistant to cell death caused by *stil* deficiency, I induced GSC-like properties by overexpressing a constitutively active the Decapentaplegic (Dpp) receptor, Thickveins (Tkv.CA) in germline cells, or the loss of function of *bag of marbles* (*bam*), a key differentiation factor (Casanueva & Ferguson, 2004; Chen & McKearin, 2003). These manipulations resulted in the tumorigenic proliferation of undifferentiated GSC-like cells or precystoblast, respectively (Tiwari et al., 2019). Remarkably, Tkv.CA overexpression and *bam* loss rescued germline cell depletion in *stil* mutant ovaries, leading to the formation of tumorous germaria containing undifferentiated germline cells comparable to the *stil* heterozygous control (Figure 3.2.2 A, B). Indeed, TUNEL assay showed no increase in apoptotic cells in such tumorous germaria (Figure 3.2.2 C, D), indicating that the undifferentiated state of germline cells confers resistance to apoptosis in the absence of *stil*.

Consistent with this observation, RNA-seq and gene Ontology (GO) analysis of those tumorous ovaries revealed that the expression levels of most apoptosis-related genes (GO:0006915), including *rpr*, remain unaffected (DDBJ: PRJDB20709) (Figure 3.2.2 E). Furthermore, RT-qPCR confirmed that *rpr* expression was not significantly upregulated in the GSC-like tumor *stil* mutant ovaries compared to P35-rescued *stil* mutant ones (Figure 3.1.9 A, 3.2.2 E), suggesting that apoptosis-related genes, including *rpr*, are transcriptionally repressed in GSC-like cells even in the absence of *stil* function.

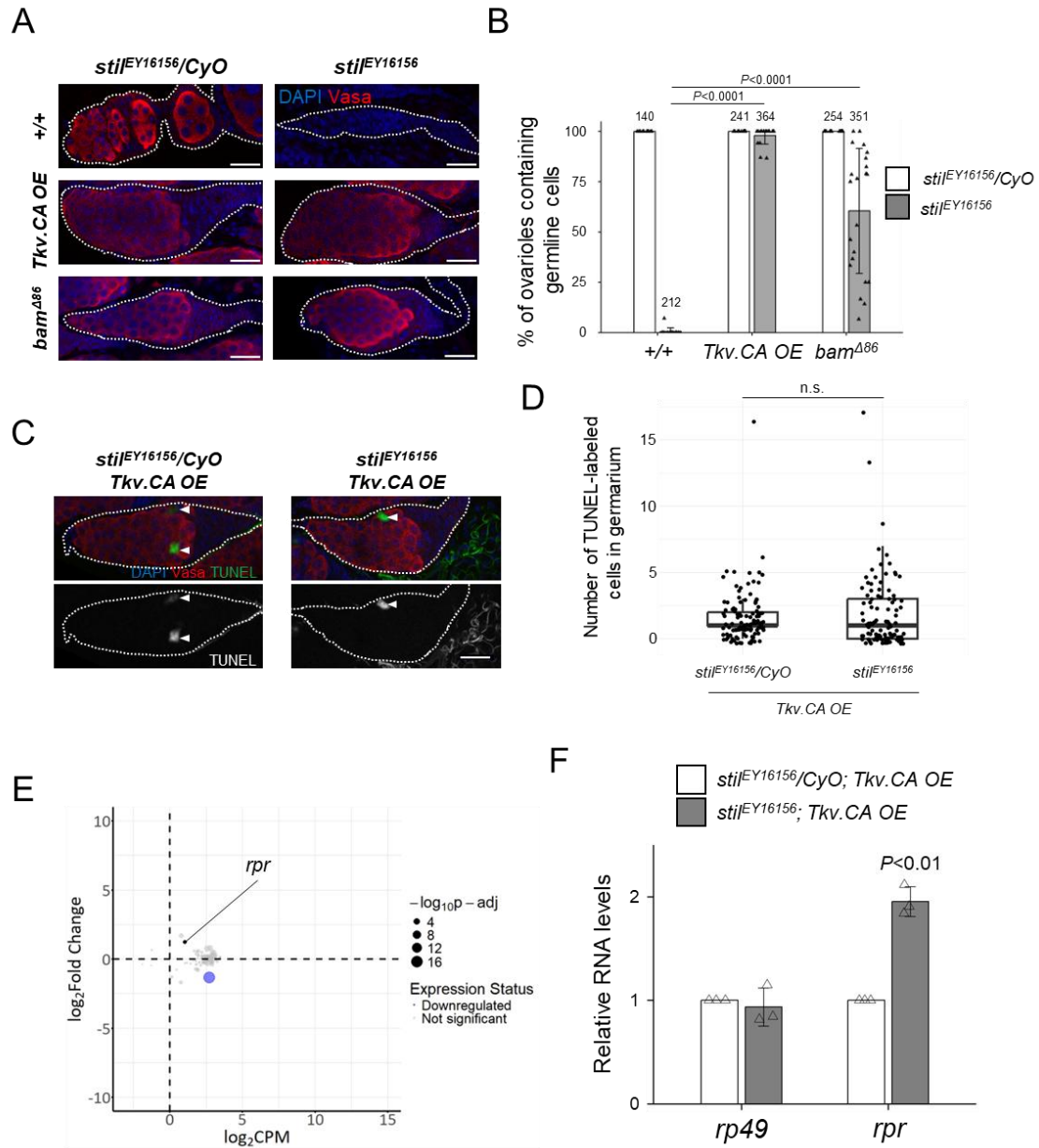


Figure 3.2.2 *stii* mutant germline stem cells resist apoptosis

(A) Immunostaining of Vasa (red) and DAPI (blue) in control, *Tkv.CA* (*constitutive active Thickveins*) OE (*NGT40; NosGal4-VP16>Tkv.CA*), and *bam* mutant (*bam*^{Δ86}) germaria (white dotted) in the control heterozygous (*stiiEY16156/CyO*) or *stii* mutant (*stiiEY16156*) background. Scale bar: 20 μm. (B) Quantification germline cell-containing germaria per ovary in 2-3 days-old females. Each dot represents an individual ovary. Genotypes and the number of germarium assessed are indicated above each bar. Error bars represent standard deviation (s.d.). (C) Representative images of germaria (white dashed lines) showing TUNEL-positive apoptotic cells (green), Vasa (red), and DAPI (blue) in the control tumorous germaria (*stiiEY16156/CyO; Tkiv.CA OE*) and tumorous germaria of *stii* mutant ovaries (*stiiEY16156; Tkiv.CA OE*). White arrowheads indicate TUNEL-positive apoptotic cells. Scale bar: 20 μm. (D) Quantification of apoptosis in germaria from the control tumorous ovaries (*stiiEY16156/CyO; Tkiv.CA OE*) and *stii* mutant ovaries (*stiiEY16156; Tkiv.CA OE*). (E) MA plot of apoptosis-related genes showing the mean gene expression (CPM: counts per million) and fold change in *stii* mutant ovaries

(*stil*^{EY16156}; *Tkv.CA OE*) compared to the control tumorous ovaries (*stil*^{EY16156}/CyO; *Tkv.CA OE*). Each dot represents an individual gene, with dot size inversely correlated with p-adj. The blue dots represent the downregulated genes with fold change < -2.0, and p-adj < 0.01. (F) Quantitative RT-PCR analysis of *rpr* in the control tumorous ovaries (*stil*^{EY16156}/CyO; *Tkv.CA OE*) and *stil* mutant ovaries (*stil*^{EY16156}; *Tkv.CA OE*). Expression levels of an internal control, *rp49*, and *rpr* are normalized to that of α -*tub*. Error bars indicate s.d. (*n*=3).

3.2.2 *rpr* Silencing in Undifferentiated Germline Cells Is Associated with Chromatin States

Previous studies in mammals have established that stem cells are generally resistant to apoptosis. In mouse mammary stem cells and cancer stem cells, this resistance is attributed to several mechanisms, including enhanced DNA repair and overexpression of anti-apoptotic proteins (Chang et al., 2015; Nathansen et al., 2021; Safa, 2016). Similarly, in *Drosophila*, intestinal stem cells were shown to resist cell death through a two-tiered regulation of *rpr*, involving chromatin state and spatial control of its expression (Sulekh et al., 2024). To explore whether chromatin dynamics contribute to GSC apoptosis resistance in *stil* mutants, I analyzed the chromatin status at the *rpr* locus using Assay for Transposase-Accessible Chromatin-seq (ATAC-seq) data of *Drosophila* ovarian cells (Pang et al., 2023). This dataset was generated using fluorescence-activated cell sorting (FACS) of GSCs (2C–4C) and nurse cells (NCs) at various stages of egg chamber maturation, with ploidy levels ranging from 4C to 512C due to endoreplication (Dej & Spradling, 1999). In early mid-stage NCs where *stil* deficient-germline cells undergo apoptosis, ATAC-seq signals were enriched at three upstream regions of *rpr* (-0.5 to 0.5 kb, 1.5 to 2.0 kb, and 3.5 to 5.0 kb relative to the transcription start site [TSS] of *rpr*) (Fig. 6A). Conversely, signals at these three regions were significantly lower in 2C-4C GSCs and 64C-512C NCs. These results suggest that the chromatin state

upstream of *rpr* is closed in GSCs and late-stage NCs (64C-512C) but open in early mid-stage NCs (4C-32C). Notably, despite harboring the same ploidy, 4C GSCs and 4C NCs displayed distinct chromatin accessibility, reflecting regulatory differences likely responsible for their differential responses to apoptosis (Figure 3.2.3).

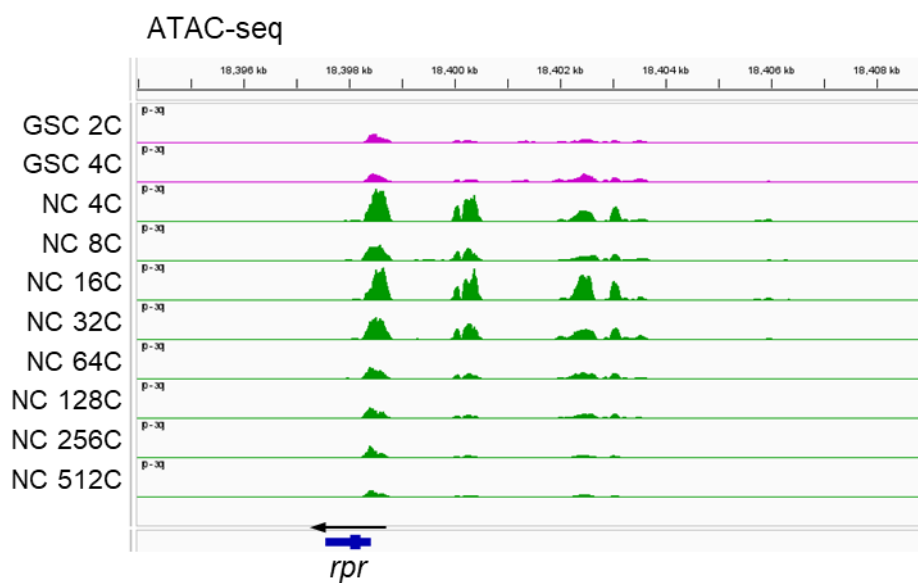


Figure 3.2.3 Chromatin accessibility around *rpr* changes during female germline development

ATAC-seq signal intensity (RPM-normalized) at the *rpr* locus in germline stem cells (GSCs: 2N and 4N) and nurse cells (NCs) at different ploidy levels (4C to 512C). The Y-axis is fixed at 0-30 RPM (reads per million) for all tracks. For 64C, 128C, 256C, and 512C NCs, data from three biological replicates are averaged; all other samples are based on single replicates.

I further analyzed epigenetic marks such as H3K9me3 and H3K27me3 at the *rpr* locus using publicly available data sets (DeLuca et al., 2020; Pang et al., 2023). As internal controls, we confirmed H3K9me3 enrichment at the *light* (*lt*) locus and H3K27me3 enrichment at the *Ultrabithorax* (*Ubx*) locus, consistent with their established chromatin states (Akkouche et al., 2017; Greil et al., 2003;

Torres-Campana et al., 2022); relative to these controls, the *rpr* locus shows H3K9me3 but no clear H3K27me3 enrichment in GSCs. H3K9me3, a histone modification associated with transcriptional repression, was observed around the *rpr* locus and its transcriptional control region in GSCs, whereas only minimal signals were detected in 4C NCs (Figure 3.2.4 A). In contrast, H3K27me3, another repression mark exhibited no difference between GSC and st5 NC (Figure 3.2.4 B) (DeLuca et al., 2020). This suggests that H3K9me3, rather than H3K27me3, may establish a heterochromatic state at the *rpr* loci in GSCs, contributing to transcriptional silencing. These findings imply that chromatin dynamics play a crucial role in regulating *rpr* expression during germline development.



Figure 3.2.4 H3K9me3, but not H3K27, is enriched around *rpr* specifically in germline stem cells

(A) H3K9me3 ChIP-seq signal at the *rpr* locus and the *It* locus (H3K9me3-positive control) in GSCs and 4C NCs. (B) H3K27me3 ChIP-seq signal at the *rpr* locus and the *Ubx* locus (H3K27me3-positive control) in GSCs and 32C NCs.

3.3 Identification of Stil's Interacting Partner

3.3.1 Identification of C-terminal Binding Protein (CtBP) as a Stil Interacting Protein by Turboid

I found that the C-terminal (CT) domain of Stil is required for its localization to DNA, despite lacking any known DNA-binding motifs. Even full-length Stil (Stil-FL) did not colocalize with DNA and was dispersed in follicle cells (Figure 3.3.1), suggesting that Stil may be recruited to DNA or chromatin through interactions with other germline-specific proteins.

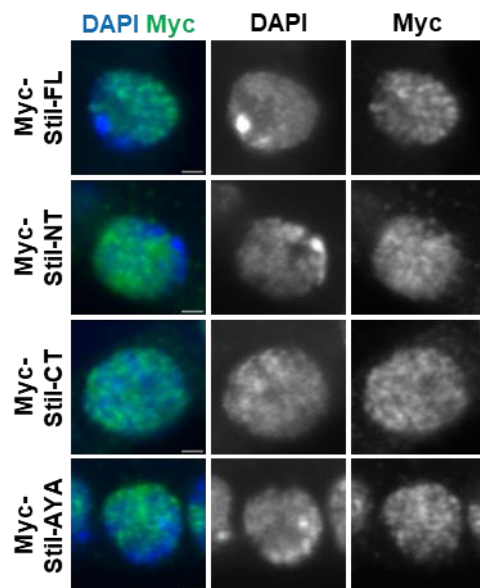


Figure 3.3.1 Stil is not co-localized with DNA in somatic cells

Immunostaining of follicle cells expressing 6×Myc-tagged Stil variants with an anti-Myc (green) antibody and DAPI (blue). Scale bar: 1µm.

To identify such nuclear-localized Stil-interacting proteins, I performed TurboID based on BioID (proximity-dependent biotin identification), using the engineered biotin ligase miniTurbo in place of the conventional *E. coli*-derived BirA (Branon et al., 2018). TurboID enables the identification of protein-protein interactions via proximity labeling, in which a promiscuous biotin ligase biotinylates nearby proteins (Roux et al., 2012). In this approach, the ligase fused to a protein of interest catalyzes the formation of biotin-5'-AMP, which diffuses locally and covalently attaches to lysine residues of proximal proteins (Choi-Rhee et al., 2004; Cronan, 2005). Biotinylated proteins are then purified under stringent conditions using streptavidin beads and analyzed by SDS-PAGE and/or mass spectrometry (Figure 3.3.2). Conventional BioID requires long labeling times (18–24 h) to achieve sufficient biotinylation for proteomic analysis. To overcome this limitation, I used miniTurbo, which enables faster and more efficient labeling—producing ~3–6-fold stronger signals at 30 min and ~15–23-fold stronger signals at 3 h in HEK293T cells (Branon et al., 2018). Previous study reported that a proximity labeling assay using miniTurbo was conducted in the *Drosophila* germline, enabling the identification of potential binding partners of the target protein (Iki et al., 2023).

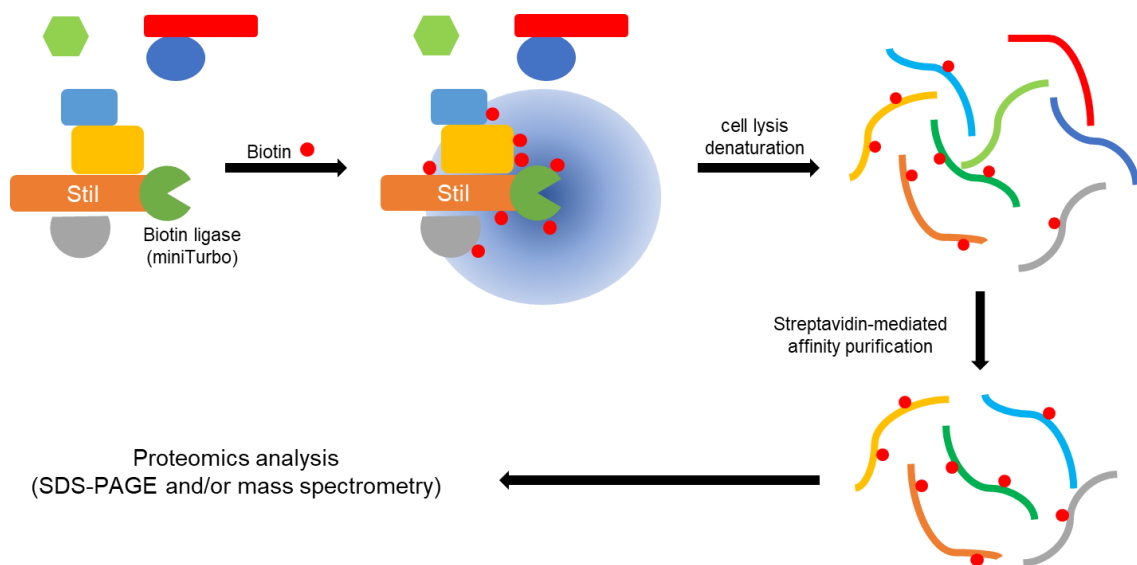


Figure 3.3.2 Application of TurboID with miniTurbo

Procedure of BioID with miniTurbo to identify Stil's interacting partner. Expression of biotin ligase (miniTurbo) fused to Stil variants leads to biotinylation of proximal proteins (radius 10 nm). Extracted proteins, denatured dependent on SDS, are streptavidin-mediated affinity purified to perform proteomics analysis to identify protein-protein interaction.

I generated four transgenes encoding 3×HA–miniTurbo fused to Stil-FL, Stil-NT, Stil-CT, and 3×NLS (negative control) (Figure 3.3.3 A). These constructs were expressed in the germline using the *nanos* (*nos*) driver. The subcellular localization of the miniTurbo–Stil variants matched that of Myc–Stil: all constructs were nuclear, but only Stil-FL and Stil-CT (not Stil-NT) were associated with chromatin (Figure 3.3.3 B). Moreover, miniTurbo–Stil-FL fully rescued the phenotypes of *stil* mutants and restored fertility (data not shown), indicating that the fusion retained endogenous Stil function. Western blotting using streptavidin–HRP confirmed the presence of biotinylated proteins in ovaries of flies fed with biotin for three days (Figure 3.3.3 C), whereas ovaries from non-supplemented flies showed only faint signals, confirming that miniTurbo-dependent biotinylation depends on dietary biotin availability. To identify Stil-binding proteins, I performed pull-down assays using streptavidin-conjugated beads and the miniTurbo–Stil

variants, followed by mass spectrometry. Multiple proteins, including the miniTurbo–Stil variants themselves, were recovered. Notably, a specific ~40 kDa band was detected only in the Stil-FL and Stil-CT fractions (Figure 3.3.3 D). Since the CT domain is likely responsible for interactions with germline-specific proteins, candidates from these fractions were considered potential Stil-binding partners. Mass spectrometry of these unique bands identified C-terminal Binding Protein (CtBP) as the most abundant peptide. Consistent with this, both Stil-FL and Stil-CT yielded high CtBP peptide coverage, whereas the negative controls (miniTurbo–NLS and wild type) showed little to no coverage (Figure 3.3.3 E). These results suggest that CtBP might serve as a potential binding partner of Stil.

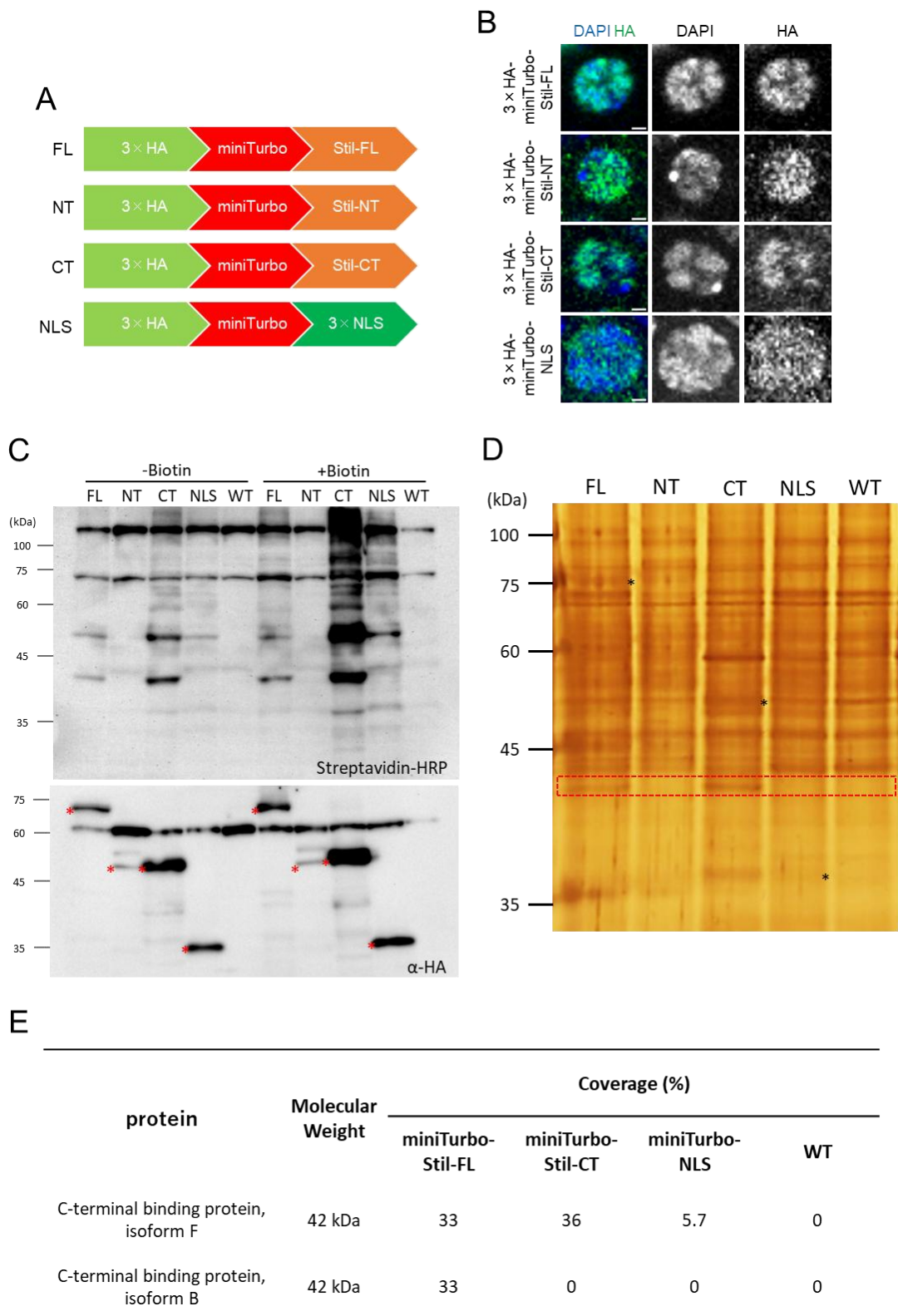


Figure 3.3.3 C-terminal binding protein (CtBP) as promising protein which binds to Stil

(A) Schematic showing Stil variants and 3×NLS fused to 3 ×HA-miniTurbo for BioID. (B) Immunostaining of nurse cells at stage 3~4 egg chamber in HA-miniTurbo-Stil variants and -NLS and WT with anti-HA (green) and DAPI (blue). Scale bar: 2 μm. (C) Western blotting from HA-miniTurbo transgenes expressed flies with or without feeding of biotin for 3 days. Biotinylated proteins were detected by Streptavidin-HRP (upper), and miniTurbo proteins itself were observed by anti-HA (lower). Red asterisks outline miniTurbo proteins. (D) Pull down assay isolated streptavidin binding proteins which were detected by silver staining. Asterisks indicate themselves biotin ligation of miniTurbo. Arrow (white) outlines unique bands only existed in miniTurbo-Stil-FL and miniTurbo-Stil-CT fractions. A red frame indicates the region, where mass spectrometry was conducted, including unique bands. (E) Mass spectrometry showing that CtBP, isoform F was highly enriched in miniTurbo-Stil-FL and miniTurbo-Stil-CT, compared to miniTurbo-NLS and WT. Meanwhile, CtBP, isoform B was only detected in miniTurbo-Stil-FL.

3.3.2 CtBP interacts with Stil, but not necessary for localization and function for Stil

CtBP is a well-studied, evolutionarily conserved transcriptional coregulator, originally identified through its interaction with the human adenovirus E1A protein, and is essential for the regulation of cell survival (Boyd et al., 1993; Chinnadurai, 2002). CtBPs bind to various DNA-binding transcription factors, repress gene expression, and recruit these factors to promoter regions of DNA (Grooteclaes & Frisch, 2000; Vliet et al., 2000). In *Drosophila*, although CtBP has been extensively studied in somatic development—such as pattern formation, imaginal disc development, and nervous system development—several studies have shown that CtBP is also present and functional in germline development (Eusebio et al., 2018; Henarejos-Castillo et al., 2024; Hoang et al., 2010; Nibu et al., 1998; Parhad et al., 2020; Stern et al., 2009; Worley et al., 2012, 2018).

To examine the physical interaction between Stil and CtBP suggested by TurboID analysis, I performed co-immunoprecipitation (Co-IP) using ovaries expressing germline-specific Myc-tagged Stil-FL. Immunoprecipitation with an anti-Myc antibody from *yw* and Myc–Stil-FL ovarian lysates detected both CtBP isoforms (CtBP_L and CtBP_S) in the input and unbound fractions although only

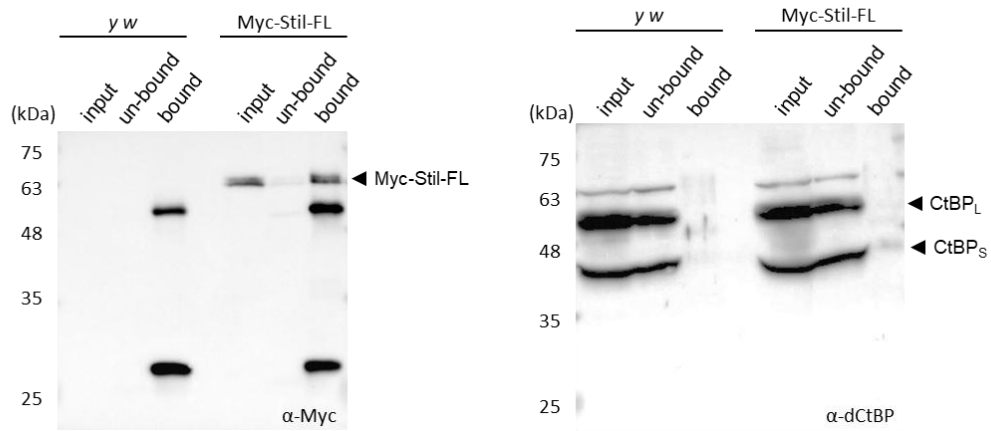
small isoform of CtBP was detected in the bound fraction of Myc–Stil-FL ovarian lysates (Figure 3.3.4A), indicating that Stil physically interacts with CtBP_s in female germline cells.

TurboID and Co-IP experiments suggest that Stil binds to CtBP via its C-terminal region, which is required for DNA co-localization. To test whether the Stil–CtBP interaction contributes to Stil's DNA localization, I examined Stil localization in *ctbp*-GLKD ovaries. CtBP is expressed in both somatic and germline cells in the control *y w* ovaries; however, CtBP signals in nurse cell DNA appeared to be absent in *ctbp*-GLKD (Figure 3.3.4 B), indicating an efficient knocking down. Stil localization in *ctbp*-GLKD nurse cells remained normal, showing no aberrant pattern (Figure 3.3.4 C). These observations suggest that the interaction between Stil's C-terminal domain and CtBP is not required for Stil's localization, implying that Stil may be recruited to DNA by other nuclear factors or through self-association. Collectively, these results indicate that CtBP, despite its physical interaction with Stil's C-terminal region, is dispensable for the DNA localization of Stil.

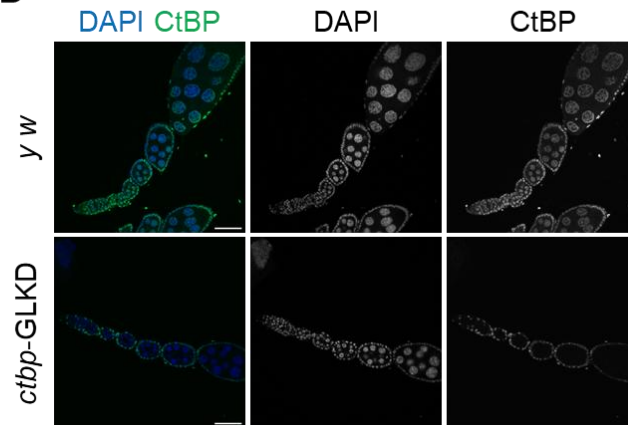
In *ctbp*-GLKD, germline development proceeded normally (Figure 3.3.4 C), in contrast to the survival and developmental defects observed in *stil*-GLKD, despite their physical interaction. This raises the possibility that Stil supports CtBP function in female germline cells. CtBP has been reported to repress canonical transcription of part of transposons during oogenesis and functions as a maternal factor essential for embryonic segmentation (Parhad et al., 2020; Poortinga et al., 1998). Interestingly, although complete loss of CtBP still permits egg production, the resulting eggs fail to hatch due to defective embryonic

segmentation. One possibility is that Stil safeguards genome integrity not only by regulating DNA repair and meiotic recombination pathways but also by cooperating with CtBP to ensure effective transposon silencing. Another possibility is that the Stil–CtBP complex fine-tunes the expression of additional maternal factors required to properly initiate embryogenesis. Together, these studies suggest that the Stil–CtBP interaction may contribute either to transposon suppression or to maternal regulation of early embryonic development.

A



B



C

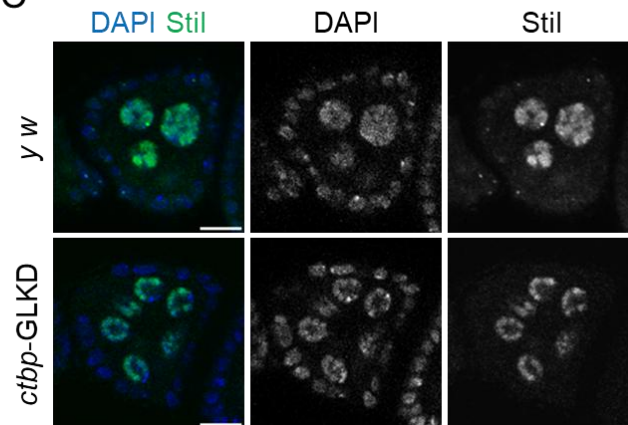


Figure 3.3.4 The binding partner CtBP has no effect on Stil localization

(A) Immunoprecipitation of Myc-Stil-FL in ovarian lysates from *y w* and 6×Myc-Stil-FL transgene driven by the germline-specific driver, *NGT40; NosGal4-VP16* flies. Western blots detect Myc and Drosophila CtBP (dCtBP) showing Myc-Stil-FL and two CtBP isoforms, CtBP_L and CtBP_S. Scale bar: 50 μm. (B) Immunofluorescence of CtBP (green) and DAPI (blue) in ovarioles from *y*

w and *ctbp*-GLKD. Scale bar: 50 μ m. (C) Immunofluorescence images showing Stil (green) and DAPI (blue) in stage 3 egg chambers of the ovaries. Scale bar: 10 μ m.

4. Discussion

During *Drosophila* oogenesis, apoptosis in germline cells eliminates aberrant cells while preserving reproductive capacity. To safeguard the fidelity of germline development, this process is tightly controlled by germline-specific regulatory mechanisms. Stand still (Stil) is a germline-specific factor essential for female germline survival and differentiation. Loss of *stil* results in an almost complete depletion of germline cells in ovaries, but not in testes. However, the molecular mechanisms by which Stil protects female germline cells have remained largely unexplored.

In this thesis, I identified Stil as a novel factor that transcriptionally represses the pro-apoptotic gene *reaper* (*rpr*) through interactions with its upstream regulatory regions. Furthermore, to maintain a continuous supply of gametes, germline stem cells prevent apoptotic cell death by employing both Stil-mediated transcriptional repression and chromatin-based silencing of *rpr*. These findings reveal a unique strategy by which *Drosophila* preserves female germline cells, underscoring how lineage-specific transcriptional and chromatin-based regulation converge to prevent apoptosis and thereby ensure reproductive success.

4.1 Transcriptional repression of *rpr* by Stil in female germline cells

In the absence of *stil*, the expression of the pro-apoptotic gene *rpr* is markedly upregulated in ovaries (Figure 3.1.8 A). The transcription of *rpr* is known to be regulated by multiple transcription factors that bind to cis-regulatory elements located upstream of the gene (Fuchs & Steller, 2011). For instance,

the tumor suppressor protein p53 binds to a well-characterized p53 response element (p53RE) located 4.8 kb upstream of *rpr*, which responds to both intrinsic and extrinsic signals during embryogenesis and in germline cells (Lu et al., 2010; Wylie et al., 2014). However, p53 binding has been observed only in the anterior germarium, and no direct role for p53 in female germline apoptosis has been established (J.-H. Park et al., 2019). Other regulators, including the homeobox transcription factor Deformed (Dfd) and the JNK-responsive AP-1 complex, have also been reported to bind upstream *rpr* enhancers in somatic tissues, but neither is active in the female germline (Beira et al., 2014; Lohmann et al., 2002). DamID profiling demonstrated that Stil binding extended 5–6 kb upstream of the *rpr* locus, encompassing regulatory elements associated with these transcription factors (Figure 3.1.10 B). Consistently, reporter assays showed that Stil repressed *rpr*-GFP reporter containing the 5 kb upstream regulatory region, and this repression required Stil's BED-type zinc finger domain (Figure 3.1.12 C). This domain is known to mediate DNA binding and contribute to transcriptional regulation and chromatin remodeling (Aravind, 2000). Together, these findings support a model in which Stil directly occupies the *rpr* regulatory region, thereby blocking activator access and maintaining transcriptional repression in germline cells. However, the identity of factors that promote *rpr* expression in the absence of *stil* remains unresolved.

4.2 Stil maintains the apoptotic threshold by balancing Rpr-DIAP1 interactions

Although DIAP1 overexpression only partially rescued germline loss in

stil mutants, expression of the viral caspase inhibitor P35 restored germline survival in nearly all cases (Figure 3.1.6 A, B). This highlights a functional distinction between these inhibitors in the context of *rpr*-induced apoptosis. Both suppress apoptosis, but through distinct mechanisms: DIAP1 prevents apoptotic cell death by inhibiting initiator caspases, which in turn blocks the activation of effector caspases, whereas P35, a baculoviral protein, functions as a pseudo-substrate that directly and irreversibly inhibits effector caspases such as DrICE and Dcp-1 (Jingjing Li et al., 2024; Nair & Baker, 2024). Rpr antagonizes DIAP1 via two independent mechanisms: it promotes DIAP1 auto-ubiquitination and subsequent proteasomal degradation, and it also inhibits DIAP1 translation (Freel et al., 2008; Holley et al., 2002). These dual mechanisms reduce cellular DIAP1 levels and thereby lower the apoptotic threshold (Sandu et al., 2010). Therefore, the elevated *rpr* expression in *stil* mutant ovaries likely depletes functional DIAP1, limiting the effectiveness of DIAP1 overexpression in restoring germline survival. By contrast, P35 acts downstream by directly inhibiting effector caspases (Hawkins et al., 2000; Hay et al., 1994; Meier et al., 2000), effectively bypassing DIAP1 loss and producing a more robust rescue. Together, these findings support a model in which Stil maintains the apoptotic threshold by repressing *rpr* expression and thereby balancing the antagonistic interaction between Rpr and DIAP1 (Sandu et al., 2010; Yin & Thummel, 2004). This regulatory balance ensures that apoptosis is properly restrained, preserving germline survival and reproductive capacity.

4.3 Chromatin-based silencing for *rpr* in germline stem cells

Germline cells located near the stem cell niche were relatively spared in *stil* mutants at the larval stage (Figure 3.2.1), and artificially generated GSCs as well as pre-cyst cells (GSC tumor) also survived in this background (Figure 3.2.2 A, B). These observations suggest that the stem cell state confers intrinsic resistance to apoptotic cell death. Consistent with this notion, intestinal stem cells—one of the best-studied tissue stem cell types—also exhibit resistance to apoptosis through chromatin-based repression of *rpr* (Sulekh et al., 2024). Furthermore, irradiation-induced expression of *rpr* has been shown to be epigenetically regulated during embryonic development (Yanping Zhang et al., 2008). Consistent with these observations, my analysis revealed that the chromatin surrounding the *rpr* locus and its upstream regulatory regions is tightly compacted specifically in GSCs (Figure 3.2.3), and these regions are marked by H3K9me3 (Figure 3.2.4 A). Together with previous reports, these results suggest that chromatin-mediated silencing provides an additional layer of protection against apoptosis in female GSCs, independent of *stil*-mediated repression. Nevertheless, despite being protected by dual mechanisms, germline cells, including GSCs, were completely depleted by the adult stage in *stil* mutants, in contrast to the persistence observed in GSC tumors. In GSC tumors, Dpp signaling is extremely upregulated, which form strict stem cell state and may confer stronger resistance to apoptosis than that observed under physiological conditions.

Although Stil is expressed in both male and female germline cells, *stil* mutant testes showed no discernible phenotype (Figure 3.1.2 C, D). Moreover,

germline-specific knockdown of *stil* in testes did not lead to upregulation of *rpr* (Figure 3.1.8 C), suggesting that *rpr*-mediated apoptosis is not enhanced in the male germline. This implies the existence of a sex-specific mechanism of *rpr* repression, potentially involving chromatin-based silencing or a male-specific repressor of *rpr*, although this remains to be investigated.

In summary, my findings uncover two distinct mechanisms that safeguard female germline cells from *rpr*-dependent apoptosis: (i) Stil-mediated transcriptional repression, which functions in both differentiated and undifferentiated germline cells, and (ii) chromatin-based silencing, which operates specifically in undifferentiated germline cells (Figure 4.1).

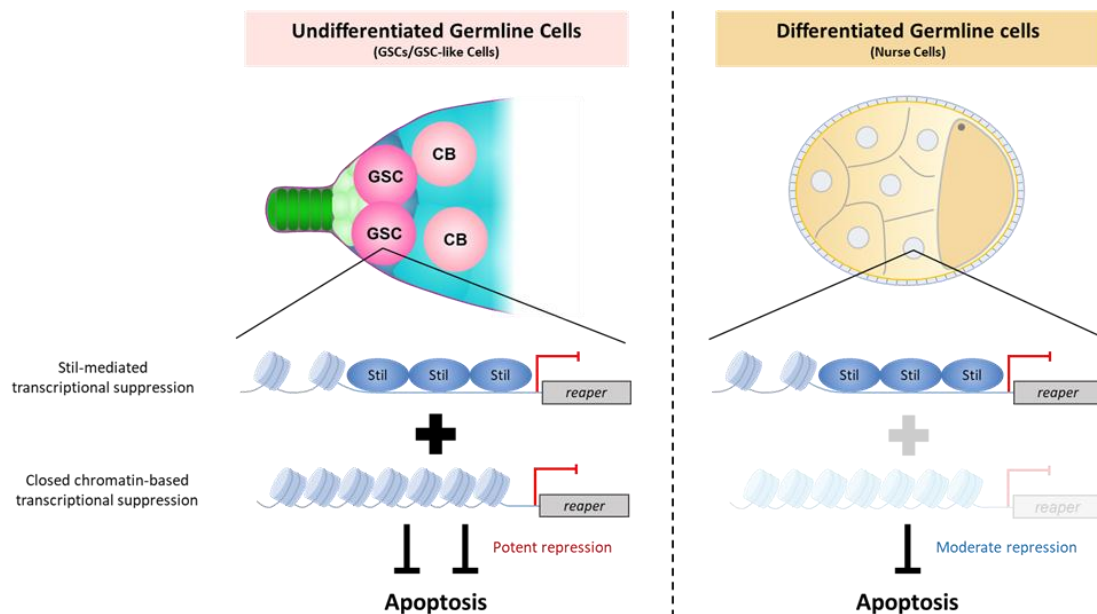


Figure 4.1 Two-layered defense protecting female germline cells from *rpr*-dependent apoptosis

Schematic model illustrating the *rpr* repression to inhibit the apoptotic cell death in undifferentiated germline cells (GSCs or GSC-like cells) and differentiated germline cells (nurse cells).

4.4 Diptera-specific evolution of apoptosis repression in the female germline

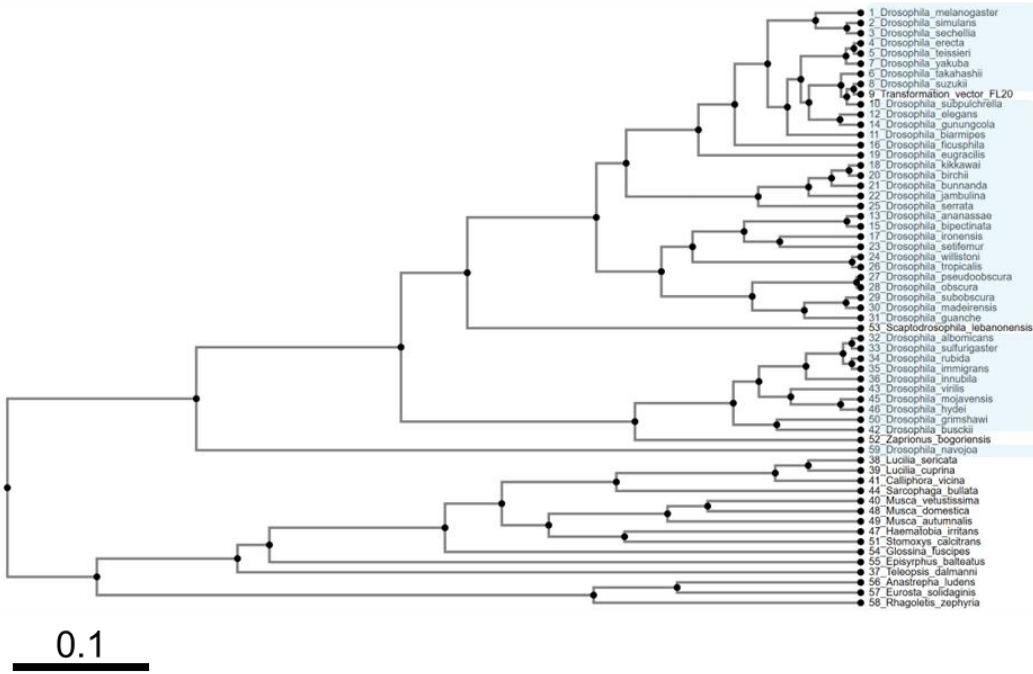
The IAP antagonist *rpr*, which plays a central role in apoptosis initiation in *Drosophila*, is conserved only within Diptera according to my BLAST search (Figure 4.2). *rpr* encodes an IAP-binding motif (IBM) that is essential for apoptosis induction through binding to baculoviral IAP repeat (BIR) domains of IAP proteins (Wing et al., 2001; L. Zhou, 2005). The IBM–BIR interaction represents a conserved mechanism broadly utilized across metazoans, including insects and mammals (Du et al., 2000; Gottfried et al., 2004; Hegde et al., 2002; Shi, 2002b; Verhagen et al., 2000; Vucic et al., 1998). In mammals, IAP antagonists such as Smac/DIABLO interact with IAP proteins including XIAP and cIAP, thereby not only competitively inhibiting caspase binding but also promoting IAP auto-ubiquitination through activation of the RING domain (Arnt et al., 2002; Azoitei et al., 2011; Shiozaki et al., 2003; Srinivasula et al., 2001). Likewise, Rpr binds to DIAP1, competitively blocking caspase–substrate interactions and, unlike Smac/DIABLO, inducing DIAP1 auto-ubiquitination by altering its conformation (Freel et al., 2008; Zachariou et al., 2003). These suggests that *rpr* has uniquely evolved in Diptera as an IBM-containing IAP antagonist.

Similarly, *stil* is also restricted to Diptera, predominantly within *Drosophila* (Figure 4.2). In contrast to the narrow taxonomic distribution of the gene itself, its BED-type zinc finger domain is widely conserved among diverse organisms, including insects, plants, and mammals, where it typically functions as a DNA-binding module in transcriptional regulation or as part of transposases (Aravind, 2000; Hayward et al., 2013; Tue et al., 2017a; H. Zhou et al., 2016). Stil was acquired in Diptera as a germline-specific BED-type zinc finger protein,

distinct from BEAF and DREF. Phylogenetic analyses of *rpr* and *stil* reveal parallel evolutionary patterns, raising the possibility that *stil* was co-opted as a dedicated repressor of *rpr* in germline cells to safeguard their survival. Nevertheless, *rpr* expression can also be suppressed by the Dpp-signaling factor Shn or by epigenetic regulation independent of transcription factors in female germline stem cells (GSCs) and intestinal stem cells (Beira et al., 2014; Sulekh et al., 2024). Why flies specifically evolved *stil* as an additional safeguard remains unresolved, and elucidating this question will be an important direction for future studies.

 Drosophila

Rpr



Stil

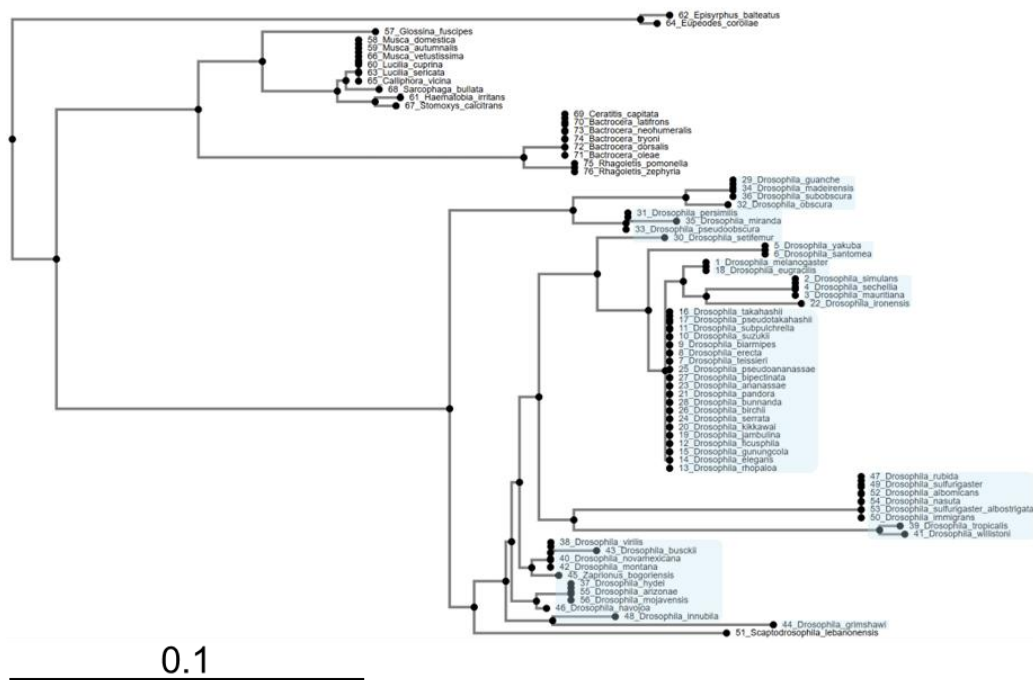


Figure 4.2 Conservation of Rpr and Stil within Diptera

Multiple sequence alignments and phylogenetic analyses were conducted using BLASTp-identified homologs of Rpr and Stil from *Drosophila melanogaster*. Both Rpr and Stil are conserved across Diptera, with the *Drosophila* genus highlighted in blue. Branch lengths represent the expected number of substitutions per site, as indicated by the scale bar.

5. Reference

- Abbott, M. K. & Lengyel, J. A. (1991). Embryonic head involution and rotation of male terminalia require the *Drosophila* locus head involution defective. *Genetics*, 129(3), 783–789. <https://doi.org/10.1093/genetics/129.3.783>
- Aitken, R. J., Findlay, J. K., Hutt, K. J. & Kerr, J. B. (2011). Apoptosis in the germ line. *Reproduction*, 141(2), 139–150. <https://doi.org/10.1530/rep-10-0232>
- Akkouche, A., Mugat, B., Barckmann, B., Varela-Chavez, C., Li, B., Raffel, R., Péliesson, A. & Chambeyron, S. (2017). Piwi Is Required during *Drosophila* Embryogenesis to License Dual-Strand piRNA Clusters for Transposon Repression in Adult Ovaries. *Molecular Cell*, 66(3), 411-419.e4. <https://doi.org/10.1016/j.molcel.2017.03.017>
- Almeida, C., Correia, S., Rocha, E., Alves, Â., Ferraz, L., Silva, J., Sousa, M. & Barros, A. (2013). Caspase signalling pathways in human spermatogenesis. *Journal of Assisted Reproduction and Genetics*, 30(4), 487–495. <https://doi.org/10.1007/s10815-013-9938-8>
- Aravind, L. (2000). The BED finger, a novel DNA-binding domain in chromatin-boundary-element-binding proteins and transposases. *Trends in Biochemical Sciences*, 25(9), 421–423. [https://doi.org/10.1016/s0968-0004\(00\)01620-0](https://doi.org/10.1016/s0968-0004(00)01620-0)
- Archambault, V. & Glover, D. M. (2009). Polo-like kinases: conservation and divergence in their functions and regulation. *Nature Reviews Molecular Cell Biology*, 10(4), 265–275. <https://doi.org/10.1038/nrm2653>
- Arnt, C. R., Chiorean, M. V., Heldebrant, M. P., Gores, G. J. & Kaufmann, S. H. (2002). Synthetic Smac/DIABLO Peptides Enhance the Effects of Chemotherapeutic Agents by Binding XIAP and cIAP1 in Situ *. *Journal of Biological Chemistry*, 277(46), 44236–44243. <https://doi.org/10.1074/jbc.m207578200>

- Assenmacher, N. & Hopfner, K.-P. (2004). MRE11/RAD50/NBS1: complex activities. *Chromosoma*, 113(4), 157–166. <https://doi.org/10.1007/s00412-004-0306-4>
- Aughey, G. N., Cheetham, S. W. & Southall, T. D. (2019). DamID as a versatile tool for understanding gene regulation. *Development*, 146(6), dev173666. <https://doi.org/10.1242/dev.173666>
- Azoitei, M. L., Correia, B. E., Ban, Y.-E. A., Carrico, C., Kalyuzhniy, O., Chen, L., Schroeter, A., Huang, P.-S., McLellan, J. S., Kwong, P. D., Baker, D., Strong, R. K. & Schief, W. R. (2011). Computation-Guided Backbone Grafting of a Discontinuous Motif onto a Protein Scaffold. *Science*, 334(6054), 373–376. <https://doi.org/10.1126/science.1209368>
- Bakhrat, A., Pritchett, T., Peretz, G., McCall, K. & Abdu, U. (2010). Drosophila Chk2 and p53 proteins induce stage-specific cell death independently during oogenesis. *Apoptosis*, 15(12), 1425–1434. <https://doi.org/10.1007/s10495-010-0539-z>
- Baonza, A., Tur-Gracia, S., Pérez-Aguilera, M. & Estella, C. (2022). Regulation and coordination of the different DNA damage responses in Drosophila. *Frontiers in Cell and Developmental Biology*, 10, 993257. <https://doi.org/10.3389/fcell.2022.993257>
- Barth, J. M., Hafen, E. & Köhler, K. (2012). The lack of autophagy triggers precocious activation of Notch signaling during Drosophila oogenesis. *BMC Developmental Biology*, 12(1), 35. <https://doi.org/10.1186/1471-213x-12-35>
- Barth, J. M. I., Szabad, J., Hafen, E. & Köhler, K. (2011). Autophagy in Drosophila ovaries is induced by starvation and is required for oogenesis. *Cell Death & Differentiation*, 18(6), 915–924. <https://doi.org/10.1038/cdd.2010.157>
- Bartoletti, M., Rubin, T., Chalvet, F., Netter, S., Santos, N. D., Poisot, E., Paces-Fessy, M., Cumenal, D., Peronnet, F., Pret, A.-M. & Théodore, L. (2012). Genetic Basis for Developmental Homeostasis of Germline Stem Cell Niche Number: A Network of Tramtrack-Group Nuclear BTB Factors. *PLoS ONE*, 7(11), e49958. <https://doi.org/10.1371/journal.pone.0049958>

- Bass, B. P., Tanner, E. A., Martín, D. M. S., Blute, T., Kinser, R. D., Dolph, P. J. & McCall, K. (2009). Cell-autonomous requirement for DNaseI in nonapoptotic cell death. *Cell Death & Differentiation*, 16(10), 1362–1371. <https://doi.org/10.1038/cdd.2009.79>
- Baum, J. S., Arama, E., Steller, H. & McCall, K. (2007). The Drosophila caspases Strica and Dronc function redundantly in programmed cell death during oogenesis. *Cell Death & Differentiation*, 14(8), 1508–1517. <https://doi.org/10.1038/sj.cdd.4402155>
- Baum, J. S., George, J. P. St. & McCall, K. (2005). Programmed cell death in the germline. *Seminars in Cell & Developmental Biology*, 16(2), 245–259. <https://doi.org/10.1016/j.semcdb.2004.12.008>
- Beira, J. V., Springhorn, A., Gunther, S., Hufnagel, L., Pyrowolakis, G. & Vincent, J.-P. (2014). The Dpp/TGF β -Dependent Corepressor Schnurri Protects Epithelial Cells from JNK-Induced Apoptosis in Drosophila Embryos. *Developmental Cell*, 31(2), 240–247. <https://doi.org/10.1016/j.devcel.2014.08.015>
- Bell, L. R., Horabin, J. I., Schedl, P. & Cline, T. W. (1991). Positive autoregulation of Sex-lethal by alternative splicing maintains the female determined state in Drosophila. *Cell*, 65(2), 229–239. [https://doi.org/10.1016/0092-8674\(91\)90157-t](https://doi.org/10.1016/0092-8674(91)90157-t)
- Bellen, H. J., Levis, R. W., He, Y., Carlson, J. W., Evans-Holm, M., Bae, E., Kim, J., Metaxakis, A., Savakis, C., Schulze, K. L., Hoskins, R. A. & Spradling, A. C. (2011). The Drosophila Gene Disruption Project: Progress Using Transposons With Distinctive Site Specificities. *Genetics*, 188(3), 731–743. <https://doi.org/10.1534/genetics.111.126995>
- Bellen, H. J., Levis, R. W., Liao, G., He, Y., Carlson, J. W., Tsang, G., Evans-Holm, M., Hiesinger, P. R., Schulze, K. L., Rubin, G. M., Hoskins, R. A. & Spradling, A. C. (2004). The BDGP Gene Disruption Project. *Genetics*, 167(2), 761–781. <https://doi.org/10.1534/genetics.104.026427>
- Benitez, M., Tatapudy, S., Liu, Y., Barber, D. L. & Nystul, T. G. (2019). Drosophila anion exchanger 2 is required for proper ovary development and

- oogenesis. *Developmental Biology*, 452(2), 127–133.
<https://doi.org/10.1016/j.ydbio.2019.04.018>
- Bergerat, A., Massy, B. de, Gadelle, D., Varoutas, P.-C., Nicolas, A. & Forterre, P. (1997). An atypical topoisomerase II from archaea with implications for meiotic recombination. *Nature*, 386(6623), 414–417.
<https://doi.org/10.1038/386414a0>
- Bilak, A., Uyetake, L. & Su, T. T. (2014). Dying Cells Protect Survivors from Radiation-Induced Cell Death in *Drosophila*. *PLoS Genetics*, 10(3), e1004220. <https://doi.org/10.1371/journal.pgen.1004220>
- Blackford, A. N. & Jackson, S. P. (2017). ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response. *Molecular Cell*, 66(6), 801–817.
<https://doi.org/10.1016/j.molcel.2017.05.015>
- Boyd, J. M., Subramanian, T., Schaeper, U., Regina, M. L., Bayley, S. & Chinnadurai, G. (1993). A region in the C-terminus of adenovirus 2/5 E1a protein is required for association with a cellular phosphoprotein and important for the negative modulation of T24-ras mediated transformation, tumorigenesis and metastasis. *The EMBO Journal*, 12(2), 469–478.
<https://doi.org/10.1002/j.1460-2075.1993.tb05679.x>
- Branon, T. C., Bosch, J. A., Sanchez, A. D., Udeshi, N. D., Svinkina, T., Carr, S. A., Feldman, J. L., Perrimon, N. & Ting, A. Y. (2018). Efficient proximity labeling in living cells and organisms with TurboID. *Nature Biotechnology*, 36(9), 880–887. <https://doi.org/10.1038/nbt.4201>
- Brennecke, J., Hipfner, D. R., Stark, A., Russell, R. B. & Cohen, S. M. (2003). bantam Encodes a Developmentally Regulated microRNA that Controls Cell Proliferation and Regulates the Proapoptotic Gene hid in *Drosophila*. *Cell*, 113(1), 25–36. [https://doi.org/10.1016/s0092-8674\(03\)00231-9](https://doi.org/10.1016/s0092-8674(03)00231-9)
- Brodsky, M. H., Nordstrom, W., Tsang, G., Kwan, E., Rubin, G. M. & Abrams, J. M. (2000). *Drosophila* p53 Binds a Damage Response Element at the reaper Locus. *Cell*, 101(1), 103–113. [https://doi.org/10.1016/s0092-8674\(00\)80627-3](https://doi.org/10.1016/s0092-8674(00)80627-3)

- Brodsky, M. H., Weinert, B. T., Tsang, G., Rong, Y. S., McGinnis, N. M., Golic, K. G., Rio, D. C. & Rubin, G. M. (2004). *Drosophila melanogaster* MNK/Chk2 and p53 Regulate Multiple DNA Repair and Apoptotic Pathways following DNA Damage. *Molecular and Cellular Biology*, 24(3), 1219–1231.
<https://doi.org/10.1128/mcb.24.3.1219-1231.2004>
- Buja, L. M. (2021). The cell theory and cellular pathology: Discovery, refinements and applications fundamental to advances in biology and medicine. *Experimental and Molecular Pathology*, 121, 104660.
<https://doi.org/10.1016/j.yexmp.2021.104660>
- Carvalho, A. B. (2003). The advantages of recombination. *Nature Genetics*, 34(2), 128–129. <https://doi.org/10.1038/ng0603-128>
- Casanueva, M. O. & Ferguson, E. L. (2004). Germline stem cell number in the *Drosophila* ovary is regulated by redundant mechanisms that control Dpp signaling. *Development*, 131(9), 1881–1890.
<https://doi.org/10.1242/dev.01076>
- Chang, C.-H., Zhang, M., Rajapakshe, K., Coarfa, C., Edwards, D., Huang, S. & Rosen, J. M. (2015). Mammary Stem Cells and Tumor-Initiating Cells Are More Resistant to Apoptosis and Exhibit Increased DNA Repair Activity in Response to DNA Damage. *Stem Cell Reports*, 5(3), 378–391.
<https://doi.org/10.1016/j.stemcr.2015.07.009>
- Chaudhry, S. R., Lopes, J., Levin, N. K., Kalpage, H. & Tainsky, M. A. (2021). Germline mutations in apoptosis pathway genes in ovarian cancer; the functional role of a TP53I3 (PIG3) variant in ROS production and DNA repair. *Cell Death Discovery*, 7(1), 62. <https://doi.org/10.1038/s41420-021-00442-y>
- Chen, D. & McKearin, D. (2003). Dpp Signaling Silences bam Transcription Directly to Establish Asymmetric Divisions of Germline Stem Cells. *Current Biology*, 13(20), 1786–1791. <https://doi.org/10.1016/j.cub.2003.09.033>
- Chinnadurai, G. (2002). CtBP, an Unconventional Transcriptional Corepressor in Development and Oncogenesis. *Molecular Cell*, 9(2), 213–224.
[https://doi.org/10.1016/s1097-2765\(02\)00443-4](https://doi.org/10.1016/s1097-2765(02)00443-4)

- Christophorou, N., Rubin, T. & Huynh, J.-R. (2013). Synaptonemal Complex Components Promote Centromere Pairing in Pre-meiotic Germ Cells. *PLoS Genetics*, 9(12), e1004012. <https://doi.org/10.1371/journal.pgen.1004012>
- Ciardo, D., Goldar, A. & Marheineke, K. (2019). On the Interplay of the DNA Replication Program and the Intra-S Phase Checkpoint Pathway. *Genes*, 10(2), 94. <https://doi.org/10.3390/genes10020094>
- Ciccia, A. & Elledge, S. J. (2010). The DNA Damage Response: Making It Safe to Play with Knives. *Molecular Cell*, 40(2), 179–204. <https://doi.org/10.1016/j.molcel.2010.09.019>
- Clarke, P. G. H. & Clarke, S. (1996). Nineteenth century research on naturally occurring cell death and related phenomena. *Anatomy and Embryology*, 193(2), 81–99. <https://doi.org/10.1007/bf00214700>
- Clavier, A., Rincheval-Arnold, A., Colin, J., Mignotte, B. & Guénal, I. (2016). Apoptosis in Drosophila: which role for mitochondria? *Apoptosis*, 21(3), 239–251. <https://doi.org/10.1007/s10495-015-1209-y>
- Clemens, M. J., Venrooij, W. J. van & Putte, L. B. A. van de. (2000). Apoptosis and Autoimmunity. *Cell Death & Differentiation*, 7(1), 131–133. <https://doi.org/10.1038/sj.cdd.4400633>
- Dej, K. J. & Spradling, A. C. (1999). The endocycle controls nurse cell polytene chromosome structure during Drosophila oogenesis. *Development*, 126(2), 293–303. <https://doi.org/10.1242/dev.126.2.293>
- DeLuca, S. Z., Ghildiyal, M., Pang, L.-Y. & Spradling, A. C. (2020). Differentiating Drosophila female germ cells initiate Polycomb silencing by regulating PRC2-interacting proteins. *eLife*, 9, e56922. <https://doi.org/10.7554/elife.56922>
- Díaz-Torres, A., Rosales-Nieves, A. E., Pearson, J. R., Mateos, C. S.-C., Marín-Menguiano, M., Marshall, O. J., Brand, A. H. & González-Reyes, A. (2021). Stem cell niche organization in the Drosophila ovary requires the ECM component Perlecan. *Current Biology*, 31(8), 1744-1753.e5. <https://doi.org/10.1016/j.cub.2021.01.071>

- 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>
- Du, C., Fang, M., Li, Y., Li, L. & Wang, X. (2000). Smac, a Mitochondrial Protein that Promotes Cytochrome c–Dependent Caspase Activation by Eliminating IAP Inhibition. *Cell*, 102(1), 33–42. [https://doi.org/10.1016/s0092-8674\(00\)00008-8](https://doi.org/10.1016/s0092-8674(00)00008-8)
- Ellis, H. M. & Horvitz, H. R. (1986). Genetic control of programmed cell death in the nematode *C. elegans*. *Cell*, 44(6), 817–829. [https://doi.org/10.1016/0092-8674\(86\)90004-8](https://doi.org/10.1016/0092-8674(86)90004-8)
- Ellis, R. E., Yuan, J. & Horvitz, H. R. (1991). Mechanisms and Functions of Cell Death. *Annual Review of Cell and Developmental Biology*, 7(1), 663–698.
<https://doi.org/10.1146/annurev.cb.07.110191.003311>
- Etchegaray, J. I., Timmons, A. K., Klein, A. P., Pritchett, T. L., Welch, E., Meehan, T. L., Li, C. & McCall, K. (2012). Draper acts through the JNK pathway to control synchronous engulfment of dying germline cells by follicular epithelial cells. *Development*, 139(21), 4029–4039.
<https://doi.org/10.1242/dev.082776>
- Eusebio, N., Tavares, L. & Pereira, P. S. (2018). CtBP represses Dpp-dependent Mad activation during *Drosophila* eye development. *Developmental Biology*, 442(1), 188–198.
<https://doi.org/10.1016/j.ydbio.2018.07.018>
- Foley, K. & Cooley, L. (1998). Apoptosis in late stage *Drosophila* nurse cells does not require genes within the H99 deficiency. *Development*, 125(6), 1075–1082. <https://doi.org/10.1242/dev.125.6.1075>
- Freel, C. D., Richardson, D. A., Thomenius, M. J., Gan, E. C., Horn, S. R., Olson, M. R. & Kornbluth, S. (2008). Mitochondrial Localization of Reaper to Promote Inhibitors of Apoptosis Protein Degradation Conferred by GH3 Domain-Lipid Interactions*. *Journal of Biological Chemistry*, 283(1), 367–379. <https://doi.org/10.1074/jbc.m708931200>

- Fuchs, Y. & Steller, H. (2011). Programmed Cell Death in Animal Development and Disease. *Cell*, 147(4), 742–758.
<https://doi.org/10.1016/j.cell.2011.10.033>
- Fuchs, Y. & Steller, H. (2015). Live to die another way: modes of programmed cell death and the signals emanating from dying cells. *Nature Reviews Molecular Cell Biology*, 16(6), 329–344. <https://doi.org/10.1038/nrm3999>
- Gilboa, L. (2015). Organizing stem cell units in the *Drosophila* ovary. *Current Opinion in Genetics & Development*, 32, 31–36.
<https://doi.org/10.1016/j.gde.2015.01.005>
- Gilboa, L., Forbes, A., Tazuke, S. I., Fuller, M. T. & Lehmann, R. (2003). Germ line stem cell differentiation in *Drosophila* requires gap junctions and proceeds via an intermediate state. *Development*, 130(26), 6625–6634.
<https://doi.org/10.1242/dev.00853>
- Giorgi, F. & Deri, P. (1976). Cell death in ovarian chambers of *Drosophila melanogaster*. *Journal of Embryology and Experimental Morphology*, 35(3), 521–533.
- Gleason, R. J., Anand, A., Kai, T. & Chen, X. (2018). Protecting and Diversifying the Germline. *Genetics*, 208(2), 435–471.
<https://doi.org/10.1534/genetics.117.300208>
- Gluszek, A. A., Cullen, C. F., Li, W., Battaglia, R. A., Radford, S. J., Costa, M. F., McKim, K. S., Goshima, G. & Ohkura, H. (2015). The microtubule catastrophe promoter Sentin delays stable kinetochore–microtubule attachment in oocytes. *Journal of Cell Biology*, 211(6), 1113–1120.
<https://doi.org/10.1083/jcb.201507006>
- Golstein, P. & Kroemer, G. (2007). Cell death by necrosis: towards a molecular definition. *Trends in Biochemical Sciences*, 32(1), 37–43.
<https://doi.org/10.1016/j.tibs.2006.11.001>
- González-Marín, B., Calderón-Segura, M. E. & Sekelsky, J. (2023). ATM/Chk2 and ATR/Chk1 Pathways Respond to DNA Damage Induced by Movento® 240SC and Envidor® 240SC Keto-Enol Insecticides in the Germarium of

- Drosophila melanogaster*. *Toxics*, 11(9), 754.
<https://doi.org/10.3390/toxics11090754>
- Gottfried, Y., Rotem, A., Lotan, R., Steller, H. & Larisch, S. (2004). The mitochondrial ARTS protein promotes apoptosis through targeting XIAP. *The EMBO Journal*, 23(7), 1627–1635. <https://doi.org/10.1038/sj.emboj.7600155>
- Goyal, L., McCall, K., Agapite, J., Hartweg, E. & Steller, H. (2000). Induction of apoptosis by *Drosophila* reaper, hid and grim through inhibition of IAP function. *The EMBO Journal*, 19(4), 589–597.
<https://doi.org/10.1093/emboj/19.4.589>
- Greil, F., Kraan, I. van der, Delrow, J., Smothers, J. F., Wit, E. de, Bussemaker, H. J., Driel, R. van, Henikoff, S. & Steensel, B. van. (2003). Distinct HP1 and Su(var)3-9 complexes bind to sets of developmentally coexpressed genes depending on chromosomal location. *Genes & Development*, 17(22), 2825–2838. <https://doi.org/10.1101/gad.281503>
- Grieder, N. C., Cuevas, M. de & Spradling, A. C. (2000). The fusome organizes the microtubule network during oocyte differentiation in *Drosophila*. *Development*, 127(19), 4253–4264. <https://doi.org/10.1242/dev.127.19.4253>
- Grooteclaes, M. L. & Frisch, S. M. (2000). Evidence for a function of CtBP in epithelial gene regulation and anoikis. *Oncogene*, 19(33), 3823–3828.
<https://doi.org/10.1038/sj.onc.1203721>
- Gruntenko, N. E. & Rauschenbach, I. Y. (2008). Interplay of JH, 20E and biogenic amines under normal and stress conditions and its effect on reproduction. *Journal of Insect Physiology*, 54(6), 902–908.
<https://doi.org/10.1016/j.jinsphys.2008.04.004>
- Hanahan, D. & Weinberg, R. A. (2011). Hallmarks of Cancer: The Next Generation. *Cell*, 144(5), 646–674. <https://doi.org/10.1016/j.cell.2011.02.013>
- Hashiyama, K., Hayashi, Y. & Kobayashi, S. (2011). *Drosophila* Sex lethal Gene Initiates Female Development in Germline Progenitors. *Science*, 333(6044), 885–888. <https://doi.org/10.1126/science.1208146>

- Hawkins, C. J., Yoo, S. J., Peterson, E. P., Wang, S. L., Vernooy, S. Y. & Hay, B. A. (2000). The Drosophila Caspase DRONC Cleaves following Glutamate or Aspartate and Is Regulated by DIAP1, HID, and GRIM*. *Journal of Biological Chemistry*, 275(35), 27084–27093. [https://doi.org/10.1016/s0021-9258\(19\)61483-3](https://doi.org/10.1016/s0021-9258(19)61483-3)
- Hay, B. A., Wolff, T. & Rubin, G. M. (1994). Expression of baculovirus P35 prevents cell death in Drosophila. *Development*, 120(8), 2121–2129. <https://doi.org/10.1242/dev.120.8.2121>
- Hayward, A., Ghazal, A., Andersson, G., Andersson, L. & Jern, P. (2013). ZBED Evolution: Repeated Utilization of DNA Transposons as Regulators of Diverse Host Functions. *PLoS ONE*, 8(3), e59940. <https://doi.org/10.1371/journal.pone.0059940>
- He, L., Wang, X. & Montell, D. J. (2011). Shining light on Drosophila oogenesis: live imaging of egg development. *Current Opinion in Genetics & Development*, 21(5), 612–619. <https://doi.org/10.1016/j.gde.2011.08.011>
- Hegde, R., Srinivasula, S. M., Zhang, Z., Wassell, R., Mukattash, R., Cilenti, L., DuBois, G., Lazebnik, Y., Zervos, A. S., Fernandes-Alnemri, T. & Alnemri, E. S. (2002). Identification of Omi/HtrA2 as a Mitochondrial Apoptotic Serine Protease That Disrupts Inhibitor of Apoptosis Protein-Caspase Interaction*. *Journal of Biological Chemistry*, 277(1), 432–438. <https://doi.org/10.1074/jbc.m109721200>
- Henarejos-Castillo, I., Sanz, F. J., Solana-Manrique, C., Sebastian-Leon, P., Medina, I., Remohi, J., Paricio, N. & Diaz-Gimeno, P. (2024). Whole-exome sequencing and Drosophila modelling reveal mutated genes and pathways contributing to human ovarian failure. *Reproductive Biology and Endocrinology*, 22(1), 153. <https://doi.org/10.1186/s12958-024-01325-4>
- Hengartner, M. O. (2000). The biochemistry of apoptosis. *Nature*, 407(6805), 770–776. <https://doi.org/10.1038/35037710>
- Hengartner, M. O., Ellis, R. & Horvitz, R. (1992). Caenorhabditis elegans gene ced-9 protects cells from programmed cell death. *Nature*, 356(6369), 494–499. <https://doi.org/10.1038/356494a0>

- Hengartner, M. O. & Horvitz, H. R. (1994). Activation of *C. elegans* cell death protein CED-9 by an ammo-acid substitution in a domain conserved in Bcl-2. *Nature*, 369(6478), 318–320. <https://doi.org/10.1038/369318a0>
- Hill, R. J., Bona, N., Smink, J., Webb, H. K., Crisp, A., Garaycoechea, J. I. & Crossan, G. P. (2024). p53 regulates diverse tissue-specific outcomes to endogenous DNA damage in mice. *Nature Communications*, 15(1), 2518. <https://doi.org/10.1038/s41467-024-46844-1>
- Hinnant, T. D., Merkle, J. A. & Ables, E. T. (2020). Coordinating Proliferation, Polarity, and Cell Fate in the *Drosophila* Female Germline. *Frontiers in Cell and Developmental Biology*, 8, 19. <https://doi.org/10.3389/fcell.2020.00019>
- Hoang, C. Q., Burnett, M. E. & Curtiss, J. (2010). *Drosophila* CtBP regulates proliferation and differentiation of eye precursors and complexes with Eyeless, Dachshund, Dan, and Danr during eye and antennal development. *Developmental Dynamics*, 239(9), 2367–2385. <https://doi.org/10.1002/dvdy.22380>
- Holley, C. L., Olson, M. R., Colón-Ramos, D. A. & Kornbluth, S. (2002). Reaper eliminates IAP proteins through stimulated IAP degradation and generalized translational inhibition. *Nature Cell Biology*, 4(6), 439–444. <https://doi.org/10.1038/ncb798>
- Hou, Y.-C. C., Chittaranjan, S., Barbosa, S. G., McCall, K. & Gorski, S. M. (2008). Effector caspase Dcp-1 and IAP protein Bruce regulate starvation-induced autophagy during *Drosophila melanogaster* oogenesis. *The Journal of Cell Biology*, 182(6), 1127–1139. <https://doi.org/10.1083/jcb.200712091>
- Huang, C., Zhao, S., Yang, Y., Guo, T., Ke, H., Mi, X., Qin, Y., Chen, Z.-J. & Zhao, S. (2023). TP63 gain-of-function mutations cause premature ovarian insufficiency by inducing oocyte apoptosis. *Journal of Clinical Investigation*, 133(5), e162315. <https://doi.org/10.1172/jci162315>
- Hughes, S. E., Miller, D. E., Miller, A. L. & Hawley, R. S. (2018). Female Meiosis: Synapsis, Recombination, and Segregation in *Drosophila melanogaster*. *Genetics*, 208(3), 875–908. <https://doi.org/10.1534/genetics.117.300081>

- Hunter, N. (2015). Meiotic Recombination: The Essence of Heredity. *Cold Spring Harbor Perspectives in Biology*, 7(12), a016618.
<https://doi.org/10.1101/cshperspect.a016618>
- Iki, T., Kawaguchi, S. & Kai, T. (2023). miRNA/siRNA-directed pathway to produce noncoding piRNAs from endogenous protein-coding regions ensures Drosophila spermatogenesis. *Science Advances*, 9(29), eadh0397.
<https://doi.org/10.1126/sciadv.adh0397>
- Jackson, S. P. & Bartek, J. (2009). The DNA-damage response in human biology and disease. *Nature*, 461(7267), 1071–1078.
<https://doi.org/10.1038/nature08467>
- Jacobson, M. D., Weil, M. & Raff, M. C. (1997). Programmed Cell Death in Animal Development. *Cell*, 88(3), 347–354. [https://doi.org/10.1016/s0092-8674\(00\)81873-5](https://doi.org/10.1016/s0092-8674(00)81873-5)
- Jaklevic, B. R. & Su, T. T. (2004). Relative Contribution of DNA Repair, Cell Cycle Checkpoints, and Cell Death to Survival after DNA Damage in Drosophila Larvae. *Current Biology*, 14(1), 23–32.
<https://doi.org/10.1016/j.cub.2003.12.032>
- Jang, J. K., Messina, L., Erdman, M. B., Arbel, T. & Hawley, R. S. (1995). Induction of Metaphase Arrest in Drosophila Oocytes by Chiasma-Based Kinetochore Tension. *Science*, 268(5219), 1917–1919.
<https://doi.org/10.1126/science.7604267>
- Jenkins, V. K., Timmons, A. K. & McCall, K. (2013). Diversity of cell death pathways: insight from the fly ovary. *Trends in Cell Biology*, 23(11), 567–574.
<https://doi.org/10.1016/j.tcb.2013.07.005>
- Jennings, B. H. (2011). Drosophila – a versatile model in biology & medicine. *Materials Today*, 14(5), 190–195. [https://doi.org/10.1016/s1369-7021\(11\)70113-4](https://doi.org/10.1016/s1369-7021(11)70113-4)
- Jiang, C., Lamblin, A.-F. J., Steller, H. & Thummel, C. S. (2000). A Steroid-Triggered Transcriptional Hierarchy Controls Salivary Gland Cell Death

- during Drosophila Metamorphosis. *Molecular Cell*, 5(3), 445–455.
[https://doi.org/10.1016/s1097-2765\(00\)80439-6](https://doi.org/10.1016/s1097-2765(00)80439-6)
- Joyce, E. F., Apostolopoulos, N., Beliveau, B. J. & Wu, C. -ting. (2013). Germline Progenitors Escape the Widespread Phenomenon of Homolog Pairing during Drosophila Development. *PLoS Genetics*, 9(12), e1004013.
<https://doi.org/10.1371/journal.pgen.1004013>
- 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>
- Knudson, C. M., Tung, K. S. K., Tourtellotte, W. G., Brown, G. A. J. & Korsmeyer, S. J. (1995). Bax-Deficient Mice with Lymphoid Hyperplasia and Male Germ Cell Death. *Science*, 270(5233), 96–99.
<https://doi.org/10.1126/science.270.5233.96>
- Koch, R., Ledermann, R., Urwyler, O., Heller, M. & Suter, B. (2009). Systematic Functional Analysis of Bicaudal-D Serine Phosphorylation and Intragenic Suppression of a Female Sterile Allele of BicD. *PLoS ONE*, 4(2), e4552.
<https://doi.org/10.1371/journal.pone.0004552>
- Kornbluth, S. & White, K. (2005). Apoptosis in Drosophila: neither fish nor fowl (nor man, nor worm). *Journal of Cell Science*, 118(9), 1779–1787.
<https://doi.org/10.1242/jcs.02377>
- 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>
- Lake, C. M., Holsclaw, J. K., Bellendir, S. P., Sekelsky, J. & Hawley, R. S. (2013). The Development of a Monoclonal Antibody Recognizing the Drosophila melanogaster Phosphorylated Histone H2A Variant (γ-H2AV). *G3: Genes, Genomes, Genetics*, 3(9), 1539–1543.
<https://doi.org/10.1534/g3.113.006833>
- Lake, C. M., Nielsen, R. J., Bonner, A. M., Eche, S., White-Brown, S., McKim, K. S. & Hawley, R. S. (2019). Narya, a RING finger domain-containing

- protein, is required for meiotic DNA double-strand break formation and crossover maturation in *Drosophila melanogaster*. *PLoS Genetics*, 15(1), e1007886. <https://doi.org/10.1371/journal.pgen.1007886>
- Lake, C. M., Nielsen, R. J., Guo, F., Unruh, J. R., Slaughter, B. D. & Hawley, R. S. (2015). Vilya, a component of the recombination nodule, is required for meiotic double-strand break formation in *Drosophila*. *eLife*, 4, e08287. <https://doi.org/10.7554/elife.08287>
- Lake, C. M., Nielsen, R. J. & Hawley, R. S. (2011). The *Drosophila* Zinc Finger Protein Trade Embargo Is Required for Double Strand Break Formation in Meiosis. *PLoS Genetics*, 7(2), e1002005. <https://doi.org/10.1371/journal.pgen.1002005>
- Laundrie, B., Peterson, J. S., Baum, J. S., Chang, J. C., Fileppo, D., Thompson, S. R. & McCall, K. (2003). Germline Cell Death Is Inhibited by P-Element Insertions Disrupting the dcp-1/pita Nested Gene Pair in *Drosophila*. *Genetics*, 165(4), 1881–1888. <https://doi.org/10.1093/genetics/165.4.1881>
- Lee, Y. & Gustafsson, Å. B. (2009). Role of apoptosis in cardiovascular disease. *Apoptosis*, 14(4), 536–548. <https://doi.org/10.1007/s10495-008-0302-x>
- Lehmann, R. (2012). Germline Stem Cells: Origin and Destiny. *Cell Stem Cell*, 10(6), 729–739. <https://doi.org/10.1016/j.stem.2012.05.016>
- Lengil, T., Gancz, D. & Gilboa, L. (2015). Activin signaling balances proliferation and differentiation of ovarian niche precursors and enables adjustment of niche numbers. *Development*, 142(5), 883–892. <https://doi.org/10.1242/dev.113902>
- Li, J & Yuan, J. (2008). Caspases in apoptosis and beyond. *Oncogene*, 27(48), 6194–6206. <https://doi.org/10.1038/onc.2008.297>
- Li, Jingjing, Jordana, L., Mehse, H., Wang, X. & Archambault, V. (2024). Nuclear reassembly defects after mitosis trigger apoptotic and p53-dependent safeguard mechanisms in *Drosophila*. *PLOS Biology*, 22(8), e3002780. <https://doi.org/10.1371/journal.pbio.3002780>

- Lin, H. (2002). The stem-cell niche theory: lessons from flies. *Nature Reviews Genetics*, 3(12), 931–940. <https://doi.org/10.1038/nrg952>
- Link, N., Kurtz, P., O'Neal, M., Garcia-Hughes, G. & Abrams, J. M. (2013). A p53 enhancer region regulates target genes through chromatin conformations in cis and in trans. *Genes & Development*, 27(22), 2433–2438. <https://doi.org/10.1101/gad.225565.113>
- Liu, H., Jang, J. K., Kato, N. & McKim, K. S. (2002). mei-P22 Encodes a Chromosome-Associated Protein Required for the Initiation of Meiotic Recombination in *Drosophila melanogaster*. *Genetics*, 162(1), 245–258. <https://doi.org/10.1093/genetics/162.1.245>
- Liu, L. & Rando, T. A. (2011). Manifestations and mechanisms of stem cell aging. *Journal of Cell Biology*, 193(2), 257–266. <https://doi.org/10.1083/jcb.201010131>
- Lohmann, I., McGinnis, N., Bodmer, M. & McGinnis, W. (2002). The *Drosophila* Hox Gene Deformed Sculpts Head Morphology via Direct Regulation of the Apoptosis Activator reaper. *Cell*, 110(4), 457–466. [https://doi.org/10.1016/s0092-8674\(02\)00871-1](https://doi.org/10.1016/s0092-8674(02)00871-1)
- Lu, W.-J., Chapo, J., Roig, I. & Abrams, J. M. (2010). Meiotic Recombination Provokes Functional Activation of the p53 Regulatory Network. *Science*, 328(5983), 1278–1281. <https://doi.org/10.1126/science.1185640>
- Luo, X., Puig, O., Hyun, J., Bohmann, D. & Jasper, H. (2007). Foxo and Fos regulate the decision between cell death and survival in response to UV irradiation. *The EMBO Journal*, 26(2), 380–390. <https://doi.org/10.1038/sj.emboj.7601484>
- Mahowald, A. P. (1962). Fine structure of pole cells and polar granules in *Drosophila melanogaster*. *Journal of Experimental Zoology*, 151(3), 201–215. <https://doi.org/10.1002/jez.1401510302>
- Maréchal, A. & Zou, L. (2013). DNA Damage Sensing by the ATM and ATR Kinases. *Cold Spring Harbor Perspectives in Biology*, 5(9), a012716. <https://doi.org/10.1101/cshperspect.a012716>

- Marshall, O. J. & Brand, A. H. (2015). damidseq_pipeline: an automated pipeline for processing DamID sequencing datasets. *Bioinformatics*, 31(20), 3371–3373. <https://doi.org/10.1093/bioinformatics/btv386>
- Martin, D. N. & Baehrecke, E. H. (2003). Caspases function in autophagic programmed cell death in *Drosophila*. *Development*, 131(2), 275–284. <https://doi.org/10.1242/dev.00933>
- Matova, N., Mahajan-Miklos, S., Mooseker, M. S. & Cooley, L. (1999). *Drosophila* quail, a villin-related protein, bundles actin filaments in apoptotic nurse cells. *Development*, 126(24), 5645–5657. <https://doi.org/10.1242/dev.126.24.5645>
- Mattson, M. P. (2000). Apoptosis in neurodegenerative disorders. *Nature Reviews Molecular Cell Biology*, 1(2), 120–130. <https://doi.org/10.1038/35040009>
- Mazzalupo, S. & Cooley, L. (2006). Illuminating the role of caspases during *Drosophila* oogenesis. *Cell Death & Differentiation*, 13(11), 1950–1959. <https://doi.org/10.1038/sj.cdd.4401892>
- 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>
- McKearin, D. M. & Spradling, A. C. (1990). bag-of-marbles: a *Drosophila* gene required to initiate both male and female gametogenesis. *Genes & Development*, 4(12b), 2242–2251. <https://doi.org/10.1101/gad.4.12b.2242>
- McKearin, D. & Ohlstein, B. (1995). A role for the *Drosophila* Bag-of-marbles protein in the differentiation of cystoblasts from germline stem cells. *Development*, 121(9), 2937–2947. <https://doi.org/10.1242/dev.121.9.2937>
- McKim, K. S. & Hayashi-Hagihara, A. (1998). mei-W68 in *Drosophila* melanogaster encodes a Spo11 homolog: evidence that the mechanism for initiating meiotic recombination is conserved. *Genes & Development*, 12(18), 2932–2942. <https://doi.org/10.1101/gad.12.18.2932>

- McKowen, J. K., Avva, S. V. S. P., Maharjan, M., Duarte, F. M., Tome, J. M., Judd, J., Wood, J. L., Negedu, S., Dong, Y., Lis, J. T. & Hart, C. M. (2022). The Drosophila BEAF insulator protein interacts with the polybromo subunit of the PBAP chromatin remodeling complex. *G3*, 12(11), jkac223. <https://doi.org/10.1093/g3journal/jkac223>
- McLaughlin, J. M. & Bratu, D. P. (2015). Drosophila Oogenesis, Methods and Protocols. *Methods in Molecular Biology*, 1328, 1–20. https://doi.org/10.1007/978-1-4939-2851-4_1
- Mehrotra, S. & McKim, K. S. (2006). Temporal Analysis of Meiotic DNA Double-Strand Break Formation and Repair in Drosophila Females. *PLoS Genetics*, 2(11), e200. <https://doi.org/10.1371/journal.pgen.0020200>
- Meier, P., Silke, J., Leever, S. J. & Evan, G. I. (2000). The Drosophila caspase DRONC is regulated by DIAP1. *The EMBO Journal*, 19(4), 598–611. <https://doi.org/10.1093/emboj/19.4.598>
- Miller, D. E., Smith, C. B., Kazemi, N. Y., Cockrell, A. J., Arvanitakis, A. V., Blumenstiel, J. P., Jaspersen, S. L. & Hawley, R. S. (2016). Whole-Genome Analysis of Individual Meiotic Events in Drosophila melanogaster Reveals That Noncrossover Gene Conversions Are Insensitive to Interference and the Centromere Effect. *Genetics*, 203(1), 159–171. <https://doi.org/10.1534/genetics.115.186486>
- Moschall, R., Rass, M., Rossbach, O., Lehmann, G., Kullmann, L., Eichner, N., Strauss, D., Meister, G., Schneuwly, S., Krahn, M. P. & Medenbach, J. (2019). Drosophila Sister-of-Sex-lethal reinforces a male-specific gene expression pattern by controlling Sex-lethal alternative splicing. *Nucleic Acids Research*, 47(5), 2276–2288. <https://doi.org/10.1093/nar/gky1284>
- Mulligan, P. K., Campos, A. R. & Jacobs, J. R. (1996). Mutations in the gene stand still disrupt germ cell differentiation in Drosophila ovaries. *Developmental Genetics*, 18(4), 316–324. [https://doi.org/10.1002/\(sici\)1520-6408\(1996\)18:4<316::aid-dvg5>3.0.co;2-c](https://doi.org/10.1002/(sici)1520-6408(1996)18:4<316::aid-dvg5>3.0.co;2-c)

- Nair, S. & Baker, N. E. (2024). Extramacrochaetae regulates Notch signaling in the *Drosophila* eye through non-apoptotic caspase activity. *eLife*, 12, RP91988. <https://doi.org/10.7554/elife.91988>
- Nakajima, Y. & Kuranaga, E. (2017). Caspase-dependent non-apoptotic processes in development. *Cell Death & Differentiation*, 24(8), 1422–1430. <https://doi.org/10.1038/cdd.2017.36>
- Nathansen, J., Meyer, F., Müller, L., Schmitz, M., Borgmann, K. & Dubrovskaya, A. (2021). Beyond the Double-Strand Breaks: The Role of DNA Repair Proteins in Cancer Stem-Cell Regulation. *Cancers*, 13(19), 4818. <https://doi.org/10.3390/cancers13194818>
- Nezis, I. P., Lamark, T., Velentzas, A. D., Rusten, T. E., Bjørkøy, G., Johansen, T., Papassideri, I. S., Stravopodis, D. J., Margaritis, L. H., Stenmark, H. & Brech, A. (2009). Cell death during *Drosophila melanogaster* early oogenesis is mediated through autophagy. *Autophagy*, 5(3), 298–302. <https://doi.org/10.4161/auto.5.3.7454>
- Nezis, I. P., Shrivastava, B. V., Sagana, A. P., Lamark, T., Bjørkøy, G., Johansen, T., Rusten, T. E., Brech, A., Baehrecke, E. H. & Stenmark, H. (2010). Autophagic degradation of dBruce controls DNA fragmentation in nurse cells during late *Drosophila melanogaster* oogenesis. *Journal of Cell Biology*, 190(4), 523–531. <https://doi.org/10.1083/jcb.201002035>
- Nibu, Y., Zhang, H., Bajor, E., Barolo, S., Small, S. & Levine, M. (1998). dCtBP mediates transcriptional repression by Knirps, Krüppel and Snail in the *Drosophila* embryo. *The EMBO Journal*, 17(23), 7009–7020. <https://doi.org/10.1093/emboj/17.23.7009>
- Ohlstein, B. & McKearin, D. (1997). Ectopic expression of the *Drosophila* Bam protein eliminates oogenic germline stem cells. *Development*, 124(18), 3651–3662. <https://doi.org/10.1242/dev.124.18.3651>
- Oliver, B., Singer, J., Laget, V., Pennetta, G. & Pauli, D. (1994). Function of *Drosophila* ovo⁺ in germ-line sex determination depends on X-chromosome number. *Development*, 120(11), 3185–3195. <https://doi.org/10.1242/dev.120.11.3185>

- Ollmann, M., Young, L. M., Como, C. J. D., Karim, F., Belvin, M., Robertson, S., Whittaker, K., Demsky, M., Fisher, W. W., Buchman, A., Duyk, G., Friedman, L., Prives, C. & Kopczynski, C. (2000). Drosophila p53 Is a Structural and Functional Homolog of the Tumor Suppressor p53. *Cell*, 101(1), 91–101. [https://doi.org/10.1016/s0092-8674\(00\)80626-1](https://doi.org/10.1016/s0092-8674(00)80626-1)
- Olson, M. R., Holley, C. L., Gan, E. C., Colón-Ramos, D. A., Kaplan, B. & Kornbluth, S. (2003). A GH3-like Domain in Reaper Is Required for Mitochondrial Localization and Induction of IAP Degradation*. *Journal of Biological Chemistry*, 278(45), 44758–44768. <https://doi.org/10.1074/jbc.m308055200>
- Pang, L.-Y., DeLuca, S., Zhu, H., Urban, J. M. & Spradling, A. C. (2023). Chromatin and gene expression changes during female Drosophila germline stem cell development illuminate the biology of highly potent stem cells. *eLife*, 12(6), RP90509. <https://doi.org/10.7554/elife.90509>
- Parhad, S. S., Yu, T., Zhang, G., Rice, N. P., Weng, Z. & Theurkauf, W. E. (2020). Adaptive Evolution Targets a piRNA Precursor Transcription Network. *Cell Reports*, 30(8), 2672-2685.e5. <https://doi.org/10.1016/j.celrep.2020.01.109>
- Park, J.-H., Nguyen, T. T. N., Lee, E.-M., Castro-Aceituno, V., Wagle, R., Lee, K.-S., Choi, J. & Song, Y.-H. (2019). Role of p53 isoforms in the DNA damage response during Drosophila oogenesis. *Scientific Reports*, 9(1), 11473. <https://doi.org/10.1038/s41598-019-47913-y>
- Park, W., Wei, S., Kim, B.-S., Kim, B., Bae, S.-J., Chae, Y. C., Ryu, D. & Ha, K.-T. (2023). Diversity and complexity of cell death: a historical review. *Experimental & Molecular Medicine*, 55(8), 1573–1594. <https://doi.org/10.1038/s12276-023-01078-x>
- Patil, V. S. & Kai, T. (2010). Repression of Retroelements in Drosophila Germline via piRNA Pathway by the Tudor Domain Protein Tejas. *Current Biology*, 20(8), 724–730. <https://doi.org/10.1016/j.cub.2010.02.046>

- Pauli, D., Oliver, B. & Mahowald, A. P. (1993). The role of the ovarian tumor locus in *Drosophila melanogaster* germ line sex determination. *Development*, 119(1), 123–134. <https://doi.org/10.1242/dev.119.1.123>
- Pennetta, G. & Pauli, D. (1997). stand still, a *Drosophila* gene involved in the female germline for proper survival, sex determination and differentiation. *Genetics*, 145(4), 975–987. <https://doi.org/10.1093/genetics/145.4.975>
- Perkins, L. A., Holderbaum, L., Tao, R., Hu, Y., Sopko, R., McCall, K., Yang-Zhou, D., Flockhart, I., Binari, R., Shim, H.-S., Miller, A., Housden, A., Foos, M., Randkeltv, S., Kelley, C., Namgyal, P., Villalta, C., Liu, L.-P., Jiang, X., ... Perrimon, N. (2015). The Transgenic RNAi Project at Harvard Medical School: Resources and Validation. *Genetics*, 201(3), 843–852. <https://doi.org/10.1534/genetics.115.180208>
- Peters, M., DeLuca, C., Hirao, A., Stambolic, V., Potter, J., Zhou, L., Liepa, J., Snow, B., Arya, S., Wong, J., Bouchard, D., Binari, R., Manoukian, A. S. & Mak, T. W. (2002). Chk2 regulates irradiation-induced, p53-mediated apoptosis in *Drosophila*. *Proceedings of the National Academy of Sciences*, 99(17), 11305–11310. <https://doi.org/10.1073/pnas.172382899>
- Peterson, Jeanne S., Bass, B. P., Jue, D., Rodriguez, A., Abrams, J. M. & McCall, K. (2007). Noncanonical cell death pathways act during *Drosophila* oogenesis. *Genesis*, 45(6), 396–404. <https://doi.org/10.1002/dvg.20306>
- Peterson, Jeanne S. & McCall, K. (2013). Combined Inhibition of Autophagy and Caspases Fails to Prevent Developmental Nurse Cell Death in the *Drosophila melanogaster* Ovary. *PLoS ONE*, 8(9), e76046. <https://doi.org/10.1371/journal.pone.0076046>
- Peterson, J.S, Barkett, M. & McCall, K. (2003). Stage-specific regulation of caspase activity in *drosophila* oogenesis. *Developmental Biology*, 260(1), 113–123. [https://doi.org/10.1016/s0012-1606\(03\)00240-9](https://doi.org/10.1016/s0012-1606(03)00240-9)
- Polo, S. E. & Jackson, S. P. (2011). Dynamics of DNA damage response proteins at DNA breaks: a focus on protein modifications. *Genes & Development*, 25(5), 409–433. <https://doi.org/10.1101/gad.2021311>

- Poortinga, G., Watanabe, M. & Parkhurst, S. M. (1998). Drosophila CtBP: a Hairy-interacting protein required for embryonic segmentation and Hairy-mediated transcriptional repression. *The EMBO Journal*, 17(7), 2067–2078. <https://doi.org/10.1093/emboj/17.7.2067>
- Primus, S., Pozmanter, C., Baxter, K. & Doren, M. V. (2019). Tudor-domain containing protein 5-like promotes male sexual identity in the Drosophila germline and is repressed in females by Sex lethal. *PLoS Genetics*, 15(7), e1007617. <https://doi.org/10.1371/journal.pgen.1007617>
- Pritchett, T. L., Tanner, E. A. & McCall, K. (2009a). Cracking open cell death in the Drosophila ovary. *Apoptosis*, 14(8), 969. <https://doi.org/10.1007/s10495-009-0369-z>
- Pritchett, T. L., Tanner, E. A. & McCall, K. (2009b). Cracking open cell death in the Drosophila ovary. *Apoptosis*, 14(8), 969. <https://doi.org/10.1007/s10495-009-0369-z>
- Radford, S. J., Hoang, T. L., Głuszek, A. A., Ohkura, H. & McKim, K. S. (2015). Lateral and End-On Kinetochore Attachments Are Coordinated to Achieve Bi-orientation in Drosophila Oocytes. *PLoS Genetics*, 11(10), e1005605. <https://doi.org/10.1371/journal.pgen.1005605>
- Reginato, G. & Cejka, P. (2020). The MRE11 complex: A versatile toolkit for the repair of broken DNA. *DNA Repair*, 91, 102869. <https://doi.org/10.1016/j.dnarep.2020.102869>
- Riparbelli, M. G. & Callaini, G. (1996). Meiotic spindle organization in fertilized Drosophila oocyte: Presence of centrosomal components in the meiotic apparatus. *Journal of Cell Science*, 109(5), 911–918. <https://doi.org/10.1242/jcs.109.5.911>
- Robert, T., Nore, A., Brun, C., Maffre, C., Crimi, B., Guichard, V., Bourbon, H.-M. & Massy, B. de. (2016). The TopoVIB-Like protein family is required for meiotic DNA double-strand break formation. *Science*, 351(6276), 943–949. <https://doi.org/10.1126/science.aad5309>

- Robinson, D. N., Cant, K. & Cooley, L. (1994). Morphogenesis of *Drosophila* ovarian ring canals. *Development*, 120(7), 2015–2025.
<https://doi.org/10.1242/dev.120.7.2015>
- Ryoo, H. D. & Baehrecke, E. H. (2010). Distinct death mechanisms in *Drosophila* development. *Current Opinion in Cell Biology*, 22(6), 889–895.
<https://doi.org/10.1016/j.ceb.2010.08.022>
- Ryoo, H. D., Bergmann, A., Gonen, H., Ciechanover, A. & Steller, H. (2002). Regulation of *Drosophila* IAP1 degradation and apoptosis by reaper and ubcD1. *Nature Cell Biology*, 4(6), 432–438. <https://doi.org/10.1038/ncb795>
- Safa, A. R. (2016). Resistance to Cell Death and Its Modulation in Cancer Stem Cells. *Critical Reviews in Oncogenesis*, 21(3–4), 203–219.
<https://doi.org/10.1615/critrevoncog.2016016976>
- Sahut-Barnola, I., Dastugue, B. & Couderc, J.-L. (1996). Terminal filament cell organization in the larval ovary of *Drosophila melanogaster*: ultrastructural observations and pattern of divisions. *Roux's Archives of Developmental Biology*, 205(7), 356–363. <https://doi.org/10.1007/bf00377215>
- Sahut-Barnola, I. & Pauli, D. (1999). The *Drosophila* gene stand still encodes a germline chromatin-associated protein that controls the transcription of the ovarian tumor gene. *Development*, 126(9), 1917–1926.
<https://doi.org/10.1242/dev.126.9.1917>
- Sancar, A., Lindsey-Boltz, L. A., Ünsal-Kaçmaz, K. & Linn, S. (2004). MOLECULAR MECHANISMS OF MAMMALIAN DNA REPAIR AND THE DNA DAMAGE CHECKPOINTS. *Annual Review of Biochemistry*, 73(1), 39–85. <https://doi.org/10.1146/annurev.biochem.73.011303.073723>
- Sandu, C., Ryoo, H. D. & Steller, H. (2010). *Drosophila* IAP antagonists form multimeric complexes to promote cell death. *Journal of Cell Biology*, 190(6), 1039–1052. <https://doi.org/10.1083/jcb.201004086>
- Santos, A. C. & Lehmann, R. (2004). Germ Cell Specification and Migration in *Drosophila* and beyond. *Current Biology*, 14(14), R578–R589.
<https://doi.org/10.1016/j.cub.2004.07.018>

- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P. & Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676–682. <https://doi.org/10.1038/nmeth.2019>
- Schüpbach, T. & Wieschaus, E. (1989). Female sterile mutations on the second chromosome of *Drosophila melanogaster*. I. Maternal effect mutations. *Genetics*, 121(1), 101–117. <https://doi.org/10.1093/genetics/121.1.101>
- Shi, Y. (2002a). A conserved tetrapeptide motif: potentiating apoptosis through IAP-binding. *Cell Death & Differentiation*, 9(2), 93–95. <https://doi.org/10.1038/sj.cdd.4400957>
- Shi, Y. (2002b). Mechanisms of Caspase Activation and Inhibition during Apoptosis. *Molecular Cell*, 9(3), 459–470. [https://doi.org/10.1016/s1097-2765\(02\)00482-3](https://doi.org/10.1016/s1097-2765(02)00482-3)
- Shim, H. J., Lee, E.-M., Nguyen, L. D., Shim, J. & Song, Y.-H. (2014). High-Dose Irradiation Induces Cell Cycle Arrest, Apoptosis, and Developmental Defects during *Drosophila* Oogenesis. *PLoS ONE*, 9(2), e89009. <https://doi.org/10.1371/journal.pone.0089009>
- Shiozaki, E. N., Chai, J., Rigotti, D. J., Riedl, S. J., Li, P., Srinivasula, S. M., Alnemri, E. S., Fairman, R. & Shi, Y. (2003). Mechanism of XIAP-Mediated Inhibition of Caspase-9. *Molecular Cell*, 11(2), 519–527. [https://doi.org/10.1016/s1097-2765\(03\)00054-6](https://doi.org/10.1016/s1097-2765(03)00054-6)
- Smith, J. E., Cummings, C. A. & Cronmiller, C. (2002). daughterless coordinates somatic cell proliferation, differentiation and germline cyst survival during follicle formation in *Drosophila*. *Development*, 129(13), 3255–3267. <https://doi.org/10.1242/dev.129.13.3255>
- Snee, M. J. & Macdonald, P. M. (2004). Live imaging of nuage and polar granules: evidence against a precursor-product relationship and a novel role for Oskar in stabilization of polar granule components. *Journal of Cell Science*, 117(10), 2109–2120. <https://doi.org/10.1242/jcs.01059>

- Sokolova, O., Morgunova, V., Sizova, T. V., Komarov, P. A., Olenkina, O. M., Babaev, D. S., Mikhaleva, E. A., Kwon, D. A., Erokhin, M. & Kalmykova, A. (2023). The insulator BEAF32 controls the spatial-temporal expression profile of the telomeric retrotransposon TART in the *Drosophila* germline. *Development*, 150(12). <https://doi.org/10.1242/dev.201678>
- Song, X., Call, G. B., Kirilly, D. & Xie, T. (2007). Notch signaling controls germline stem cell niche formation in the *Drosophila* ovary. *Development*, 134(6), 1071–1080. <https://doi.org/10.1242/dev.003392>
- Song, X., Wong, M. D., Kawase, E., Xi, R., Ding, B. C., McCarthy, J. J. & Xie, T. (2004). Bmp signals from niche cells directly repress transcription of a differentiation-promoting gene, bag of marbles, in germline stem cells in the *Drosophila* ovary. *Development*, 131(6), 1353–1364. <https://doi.org/10.1242/dev.01026>
- Song, X., Zhu, C.-H., Doan, C. & Xie, T. (2002). Germline Stem Cells Anchored by Adherens Junctions in the *Drosophila* Ovary Niches. *Science*, 296(5574), 1855–1857. <https://doi.org/10.1126/science.1069871>
- 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), 101–112. <https://doi.org/10.1016/j.devcel.2013.05.020>
- Spradling, A. C. (1993). Germline cysts: Communes that work. *Cell*, 72(5), 649–651. [https://doi.org/10.1016/0092-8674\(93\)90393-5](https://doi.org/10.1016/0092-8674(93)90393-5)
- Spradling, A., Drummond-Barbosa, D. & Kai, T. (2001). Stem cells find their niche. *Nature*, 414(6859), 98–104. <https://doi.org/10.1038/35102160>
- Srinivasula, S. M., Hegde, R., Saleh, A., Datta, P., Shiozaki, E., Chai, J., Lee, R.-A., Robbins, P. D., Fernandes-Alnemri, T., Shi, Y. & Alnemri, E. S. (2001). A conserved XIAP-interaction motif in caspase-9 and Smac/DIABLO regulates caspase activity and apoptosis. *Nature*, 410(6824), 112–116. <https://doi.org/10.1038/35065125>

- Staeva-Vieira, E., Yoo, S. & Lehmann, R. (2003). An essential role of DmRad51/SpnA in DNA repair and meiotic checkpoint control. *The EMBO Journal*, 22(21), 5863–5874. <https://doi.org/10.1093/emboj/cdg564>
- Starz-Gaiano, M. & Lehmann, R. (2001). Moving towards the next generation. *Mechanisms of Development*, 105(1–2), 5–18. [https://doi.org/10.1016/s0925-4773\(01\)00392-6](https://doi.org/10.1016/s0925-4773(01)00392-6)
- Steller, H. (2008). Regulation of apoptosis in Drosophila. *Cell Death & Differentiation*, 15(7), 1132–1138. <https://doi.org/10.1038/cdd.2008.50>
- Stern, M. D., Aihara, H., Roccaro, G. A., Cheung, L., Zhang, H., Negeri, D. & Nibu, Y. (2009). CtBP is required for proper development of peripheral nervous system in Drosophila. *Mechanisms of Development*, 126(1–2), 68–79. <https://doi.org/10.1016/j.mod.2008.10.003>
- Stetina, J. R. V. & Orr-Weaver, T. L. (2011). Developmental Control of Oocyte Maturation and Egg Activation in Metazoan Models. *Cold Spring Harbor Perspectives in Biology*, 3(10), a005553. <https://doi.org/10.1101/cshperspect.a005553>
- Struffi, P. & Arnosti, D. N. (2005). Functional Interaction between the Drosophila Knirps Short Range Transcriptional Repressor and RPD3 Histone Deacetylase*. *Journal of Biological Chemistry*, 280(49), 40757–40765. <https://doi.org/10.1074/jbc.m506819200>
- Sulekh, S., Ikegawa, Y., Naito, S., Oji, A., Hiratani, I. & Yoo, S. K. (2024). A feedback loop that drives cell death and proliferation and its defect in intestinal stem cells. *Life Science Alliance*, 7(4), e202302238. <https://doi.org/10.26508/lsa.202302238>
- Terashima, J., Takaki, K., Sakurai, S. & Bownes, M. (2005). Nutritional status affects 20-hydroxyecdysone concentration and progression of oogenesis in Drosophila melanogaster. *Journal of Endocrinology*, 187(1), 69–79. <https://doi.org/10.1677/joe.1.06220>
- Thomson, T. C. & Johnson, J. (2010). Inducible somatic oocyte destruction in response to rapamycin requires wild-type regulation of follicle cell epithelial

- polarity. *Cell Death & Differentiation*, 17(11), 1717–1727.
<https://doi.org/10.1038/cdd.2010.49>
- Thornberry, N. A. & Lazebnik, Y. (1998). Caspases: Enemies Within. *Science*, 281(5381), 1312–1316. <https://doi.org/10.1126/science.281.5381.1312>
- Timmons, A. K., Meehan, T. L., Gartmond, T. D. & McCall, K. (2013). Necrosis, Methods and Protocols. *Methods in Molecular Biology*, 1004, 215–228.
https://doi.org/10.1007/978-1-62703-383-1_16
- Ting, X. (2013). Control of germline stem cell self-renewal and differentiation in the *Drosophila* ovary: concerted actions of niche signals and intrinsic factors. *Wiley Interdisciplinary Reviews: Developmental Biology*, 2(2), 261–273.
<https://doi.org/10.1002/wdev.60>
- Tiwari, M. D., Zeitler, D. M., Meister, G. & Wodarz, A. (2019). Molecular profiling of stem cell-like female germ line cells in *Drosophila* delineates networks important for stemness and differentiation. *Biology Open*, 8(11), bio046789.
<https://doi.org/10.1242/bio.046789>
- Torres-Campana, D., Horard, B., Denaud, S., Benoit, G., Loppin, B. & Orsi, G. A. (2022). Three classes of epigenomic regulators converge to hyperactivate the essential maternal gene deadhead within a heterochromatin mini-domain. *PLoS Genetics*, 18(1), e1009615.
<https://doi.org/10.1371/journal.pgen.1009615>
- Tue, N. T., Yoshioka, Y., Mizoguchi, M., Yoshida, H., Zurita, M. & Yamaguchi, M. (2017a). DREF plays multiple roles during *Drosophila* development. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, 1860(6), 705–712. <https://doi.org/10.1016/j.bbagrm.2017.03.004>
- Tue, N. T., Yoshioka, Y., Mizoguchi, M., Yoshida, H., Zurita, M. & Yamaguchi, M. (2017b). DREF plays multiple roles during *Drosophila* development. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, 1860(6), 705–712. <https://doi.org/10.1016/j.bbagrm.2017.03.004>
- Verhagen, A. M., Ekert, P. G., Pakusch, M., Silke, J., Connolly, L. M., Reid, G. E., Moritz, R. L., Simpson, R. J. & Vaux, D. L. (2000). Identification of

- DIABLO, a Mammalian Protein that Promotes Apoptosis by Binding to and Antagonizing IAP Proteins. *Cell*, 102(1), 43–53.
[https://doi.org/10.1016/s0092-8674\(00\)00009-x](https://doi.org/10.1016/s0092-8674(00)00009-x)
- Vert, J.-P., Foveau, N., Lajaunie, C. & Vandenbrouck, Y. (2006). An accurate and interpretable model for siRNA efficacy prediction. *BMC Bioinformatics*, 7(1), 520. <https://doi.org/10.1186/1471-2105-7-520>
- Vliet, J. van, Turner, J. & Crossley, M. (2000). Human Krüppel-like Factor 8: a CACCC-box binding protein that associates with CtBP and represses transcription. *Nucleic Acids Research*, 28(9), 1955–1962.
<https://doi.org/10.1093/nar/28.9.1955>
- Vries, H. I. de, Uyetake, L., Lemstra, W., Brunsting, J. F., Su, T. T., Kampinga, H. H. & Sibon, O. C. M. (2005). Grp/DChk1 is required for G2-M checkpoint activation in Drosophila S2 cells, whereas Dmnk/DChk2 is dispensable. *Journal of Cell Science*, 118(9), 1833–1842.
<https://doi.org/10.1242/jcs.02309>
- Vucic, D., Kaiser, W. J. & Miller, L. K. (1998). Inhibitor of Apoptosis Proteins Physically Interact with and Block Apoptosis Induced by Drosophila Proteins HID and GRIM. *Molecular and Cellular Biology*, 18(6), 3300–3309.
<https://doi.org/10.1128/mcb.18.6.3300>
- Wang, S. L., Hawkins, C. J., Yoo, S. J., Müller, H.-A. J. & Hay, B. A. (1999). The Drosophila Caspase Inhibitor DIAP1 Is Essential for Cell Survival and Is Negatively Regulated by HID. *Cell*, 98(4), 453–463.
[https://doi.org/10.1016/s0092-8674\(00\)81974-1](https://doi.org/10.1016/s0092-8674(00)81974-1)
- Werz, C., Lee, T. V., Lee, P. L., Lackey, M., Bolduc, C., Stein, D. S. & Bergmann, A. (2005). Mis-specified cells die by an active gene-directed process, and inhibition of this death results in cell fate transformation in Drosophila. *Development*, 132(24), 5343–5352.
<https://doi.org/10.1242/dev.02150>
- White, K., Grether, M. E., Abrams, J. M., Young, L., Farrell, K. & Steller, H. (1994). Genetic Control of Programmed Cell Death in Drosophila. *Science*, 264(5159), 677–683. <https://doi.org/10.1126/science.8171319>

- Wing, J. P., Schwartz, L. M. & Nambu, J. R. (2001). The RHG motifs of *Drosophila* Reaper and Grim are important for their distinct cell death-inducing abilities. *Mechanisms of Development*, 102(1–2), 193–203. [https://doi.org/10.1016/s0925-4773\(01\)00316-1](https://doi.org/10.1016/s0925-4773(01)00316-1)
- Worley, M. I., Alexander, L. A. & Hariharan, I. K. (2018). CtBP impedes JNK- and Upd/STAT-driven cell fate misspecifications in regenerating *Drosophila* imaginal discs. *eLife*, 7, e30391. <https://doi.org/10.7554/elife.30391>
- Worley, M. I., Setiawan, L. & Hariharan, I. K. (2012). Regeneration and Transdetermination in *Drosophila* Imaginal Discs. *Annual Review of Genetics*, 46(1), 289–310. <https://doi.org/10.1146/annurev-genet-110711-155637>
- Wu, Q. & Brown, M. R. (2006). SIGNALING AND FUNCTION OF INSULIN-LIKE PEPTIDES IN INSECTS. *Annual Review of Entomology*, 51(1), 1–24. <https://doi.org/10.1146/annurev.ento.51.110104.151011>
- Wylie, A., Lu, W.-J., D'Brot, A., Buszczak, M. & Abrams, J. M. (2014). p53 activity is selectively licensed in the *Drosophila* stem cell compartment. *eLife*, 3, e01530. <https://doi.org/10.7554/elife.01530>
- 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](https://doi.org/10.1016/s0092-8674(00)81424-5)
- Xie, T. & Spradling, A. C. (2000). A Niche Maintaining Germ Line Stem Cells in the *Drosophila* Ovary. *Science*, 290(5490), 328–330. <https://doi.org/10.1126/science.290.5490.328>
- Xing, Y., Kurtz, I., Thuparani, M., Legard, J. & Ruohola-Baker, H. (2012). Loss-of-Function Screen Reveals Novel Regulators Required for *Drosophila* Germline Stem Cell Self-Renewal. *G3: Genes | Genomes | Genetics*, 2(3), 343–351. <https://doi.org/10.1534/g3.111.001651>
- Xing, Y., Su, T. T. & Ruohola-Baker, H. (2015). Tie-mediated signal from apoptotic cells protects stem cells in *Drosophila melanogaster*. *Nature Communications*, 6(1), 7058. <https://doi.org/10.1038/ncomms8058>

- Xu, D., Woodfield, S. E., Lee, T. V., Fan, Y., Antonio, C. & Bergmann, A. (2009a). Genetic control of programmed cell death (apoptosis) in *Drosophila*. *Fly*, 3(1), 78–90. <https://doi.org/10.4161/fly.3.1.7800>
- Xu, D., Woodfield, S. E., Lee, T. V., Fan, Y., Antonio, C. & Bergmann, A. (2009b). Genetic control of programmed cell death (apoptosis) in *Drosophila*. *Fly*, 3(1), 78–90. <https://doi.org/10.4161/fly.3.1.7800>
- Xu, F., Suyama, R., Inada, T., Kawaguchi, S. & Kai, T. (2024). HemK2 functions for sufficient protein synthesis and RNA stability through eRF1 methylation during *Drosophila* oogenesis. *Development*, 151(14). <https://doi.org/10.1242/dev.202795>
- Yarychkivska, O., Sharmin, R., Elkhailil, A. & Ghose, P. (2024). Apoptosis and beyond: A new era for programmed cell death in *Caenorhabditis elegans*. *Seminars in Cell & Developmental Biology*, 154(Pt A), 14–22. <https://doi.org/10.1016/j.semcdb.2023.02.003>
- Yin, V. P. & Thummel, C. S. (2004). A balance between the diap1 death inhibitor and reaper and hid death inducers controls steroid-triggered cell death in *Drosophila*. *Proceedings of the National Academy of Sciences*, 101(21), 8022–8027. <https://doi.org/10.1073/pnas.0402647101>
- Yoo, S. J., Huh, J. R., Muro, I., Yu, H., Wang, L., Wang, S. L., Feldman, R. M. R., Clem, R. J., Müller, H.-A. J. & Hay, B. A. (2002). Hid, Rpr and Grim negatively regulate DIAP1 levels through distinct mechanisms. *Nature Cell Biology*, 4(6), 416–424. <https://doi.org/10.1038/ncb793>
- Yoo, S. & McKee, B. D. (2005). Functional analysis of the *Drosophila* Rad51 gene (spn-A) in repair of DNA damage and meiotic chromosome segregation. *DNA Repair*, 4(2), 231–242. <https://doi.org/10.1016/j.dnarep.2004.09.009>
- Yuan, J. & Kroemer, G. (2010). Alternative cell death mechanisms in development and beyond. *Genes & Development*, 24(23), 2592–2602. <https://doi.org/10.1101/gad.1984410>

- Yuan, J., Shaham, S., Ledoux, S., Ellis, H. M. & Horvitz, H. R. (1993). The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1 β -converting enzyme. *Cell*, 75(4), 641–652.
[https://doi.org/10.1016/0092-8674\(93\)90485-9](https://doi.org/10.1016/0092-8674(93)90485-9)
- Zachariou, A., Tenev, T., Goyal, L., Agapite, J., Steller, H. & Meier, P. (2003). IAP-antagonists exhibit non-redundant modes of action through differential DIAP1 binding. *The EMBO Journal*, 22(24), 6642–6652.
<https://doi.org/10.1093/emboj/cdg617>
- Zhang, J., Zhang, W., Wei, L., Zhang, L., Liu, J., Huang, S., Li, S., Yang, W. & Li, K. (2023). E93 promotes transcription of RHG genes to initiate apoptosis during *Drosophila* salivary gland metamorphosis. *Insect Science*, 30(3), 588–598. <https://doi.org/10.1111/1744-7917.13135>
- Zhang, Yanping, Lin, N., Carroll, P. M., Chan, G., Guan, B., Xiao, H., Yao, B., Wu, S. S. & Zhou, L. (2008). Epigenetic Blocking of an Enhancer Region Controls Irradiation-Induced Proapoptotic Gene Expression in *Drosophila* Embryos. *Developmental Cell*, 14(4), 481–493.
<https://doi.org/10.1016/j.devcel.2008.01.018>
- Zhang, Yong, Billington, C. J., Pan, D. & Neufeld, T. P. (2006). *Drosophila* Target of Rapamycin Kinase Functions as a Multimer. *Genetics*, 172(1), 355–362. <https://doi.org/10.1534/genetics.105.051979>
- Zhou, H., Hirata, M., Osawa, R., Fujino, K. & Kishima, Y. (2016). Detainment of Tam3 Transposase at Plasma Membrane by Its BED-Zinc Finger Domain. *Plant Physiology*, 173(2), 1492–1501. <https://doi.org/10.1104/pp.16.00996>
- Zhou, L. (2005). The ‘unique key’ feature of the IAP-binding motifs in RHG proteins. *Cell Death & Differentiation*, 12(8), 1148–1151.
<https://doi.org/10.1038/sj.cdd.4401637>
- Zhu, C.-H. & Xie, T. (2003). Clonal expansion of ovarian germline stem cells during niche formation in *Drosophila*. *Development*, 130(12), 2579–2588.
<https://doi.org/10.1242/dev.00499>

Zou, H., Li, Y., Liu, X. & Wang, X. (1999). An APAF-1·Cytochrome c Multimeric Complex Is a Functional Apoptosome That Activates Procaspase-9*. *Journal of Biological Chemistry*, 274(17), 11549–11556.
<https://doi.org/10.1074/jbc.274.17.11549>

Publication and Contribution

Publication

Matsui Masaya, Kawaguchi Shinichi, Kai Toshie. Transcriptional Repression of *reaper* by Stand Still Ensures Female Germline Development in *Drosophila*. Submitting.

Conference presentation

standstill encoding a BED-type zinc finger protein is essential for proper differentiation and maintenance of female germline cells in *Drosophila melanogaster*. (Poster)

The 42nd Annual Meeting of the Molecular Biology Society of Japan. December 3 - 6, 2019.

stand still ensures maintenance and differentiation of germline cells in *Drosophila* female (Poster)

The 43rd Annual Meeting of the Molecular Biology Society of Japan. December 2 - 4, 2020.

Germline cell protection by Stand still (Stil), a female-restricted BED-type zinc finger protein in *Drosophila melanogaster*. (Poster)

The 44th Annual Meeting of the Molecular Biology Society of Japan. December 1 - 3, 2021.

Germline cell protection by Stand still (Stil), a female-restricted BED-type zinc finger protein in *Drosophila melanogaster*. (Oral)

The 14th Japan Drosophila Research Conference. September 13 - 16, 2021.

Drosophila germline factor, Stand still, plays a vital role in preserving female germline cells by inhibiting apoptosis. (Oral)

The 16th Japan Drosophila Research Conference. September 17 - 19, 2024.