Investigating the coordination of germline stem cell fate by ecdysone signaling and BMP signaling in the *Drosophila* ovary

By

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ABSTRACT

Germline stem cells (GSCs) receive multiple secreted signals that regulate their fate. How these signals are integrated to balance self-renewal with differentiation are not well understood. Here, we use the *Drosophila* ovary as a model to understand how signal convergence is achieved. Ecdysone signaling and Bone Morphogenetic Protein (BMP) signaling are well-known signals that promote GSC self-renewal. Over-expression of *Ecdysone Receptor* (*EcR)*, knock-down of *bag of marbles* (*bam)*, or over-expression of constitutively active *thickveins* (*tkv)* in all germ cells results in germ cell tumors at similar stages of differentiation. This suggests that ecdysone signaling and BMP signaling converge on a common point of regulation. We perfomed single-cell RNA sequencing (scRNAseq) to assess the potential transcriptomic differences between the three tumor genotypes. UMAP distribution and differential gene expression show that the over-expression of *EcR.B1* and constitutively active *tkv* (Tkv^{ACT}) yield undifferentiated cells that are more similar to each other than they are to the *bam*-depleted cells. Knock-down of BMP signaling in the *EcR.B1* over-expression or knock-down of ecdysone signaling in Tkv^{ACT} over-expression did not rescue differentiation. This data suggests that a direct interaction between ecdysone signaling and BMP signaling is unlikely, and that the two pathways likely function indirectly by converging on common transcriptional targets to regulate GSC

maintenance and differentiation. We identified a subset of putative common targets, including *ecdysone inducible protein 63E (Eip63E or E63E)*, because their expression is upregulated in response to *EcR.B1* or Tkv^{ACT} over-expression. We performed other bioinformatic analyses, such as Single Cell rEgulatory Network Inference and Cluster (SCENIC), pseudotime analysis, and single cell velocity (scVelo) to help identify other potential common targets of ecdysone signaling and BMP signaling. Our results suggest that ecdysone signaling and BMP signaling converge on a common point of regulation to coordinate GSC fate.

Investigating the coordination of germline stem cell fate by ecdysone signaling and BMP signaling in the *Drosophila* ovary

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TABLE OF CONTENTS

TITLE i

COPYRIGHT ii

ACKNOWLEDGMENTS iii

LIST OF FIGURES v

LIST OF ABBREVIATIONS vi

CHAPTER 1 Introduction 1

CHAPTER 2 Results 6

CHAPTER 3 Discussion 29

CHAPTER 4 Materials and Methods 33

REFERENCES 38

LIST OF FIGURES

Figure 1. The *Drosophila* ovary and germarium 2

Figure 2. Schematics of BMP signaling and ecdysone signaling 4

Figure 3. Whole ovary single-cell RNA sequencing.....7

Figure 4. Early germline single-cell RNA sequencing 10

Figure 5. Germ cell tumors have increased Tkv protein expression.....12

Figure 6. BMP RNAi screening identifies effective tools 14

Figure 7. Ecdysone signaling and BMP signaling do not directly interact.....16

Figure 8. Eip63E as a potential common target of ecdysone signaling and BMP signaling 18

Figure 9. SCENIC identifies transcription factors as potential common targets.....21

Figure 10. Pseudotime analysis and single-cell velocity reveal stages of differentiation 24

Figure 11. Potential common targets have varying expression patterns.....26

Figure 12. Proposed model of interactions between ecdysone signaling and BMP signaling in the stem cell niche.....30

LIST OF ABBREVIATIONS

baf	barrier to autointegration factor
bam	bag of marbles
BMP	Bone Morphogenetic Protein
CB	cystoblast
crol	crooked legs
Dad	Daughters against dpp
dae	days after eclosion
Dp	DP transcription factor
dpp	decapentaplegic
E(bx)	Enhancer of bithorax
EcR	Ecdysone Receptor
E63E	Ecdysone inducible protein 63E
E93F	Ecdysone inducible protein 93F
gbb	glass bottom boat
GSC	germline stem cell
HmgD	High mobility group protein D
Hts	Hu li tai shao
Hu	humeral
LamC	Lamin C
Mad	Mothers against decapentaplegic
Mov10	Mov10 RISC complex RNA helicase
nej	nejire
nos	nanos
PBS	phospho-buffered saline
PFA	paraformaldehyde
pMad	phosphorylated Mad

Put	punt
Sax	saxophone
Sb	Stubble
Syb	Synaptobrevin
tkv	thickveins
UAS	Upstream Activating Sequence
usp	ultraspiracle
Wdr33	WD repeat domain 33

INTRODUCTION

Stem cells are undifferentiated cells that give rise to differentiated cells in many tissues. They can asymmetrically divide to form a differentiated daughter cell or a new stem cell to maintain the stem cell population. They receive a variety of different signals to determine their fate, such as local signals from within the stem cell niche or short-range signals from other cells within the tissue (Drummond-Barbosa, 2008). Regulation of these signals is crucial to maintaining the stem cell population, but the mechanisms of how the different signals interact is not well understood.

We use the germline stem cells (GSCs) of the *Drosophila melanogaster* ovary to begin to understand the mechanisms of the interactions between different signals. Each ovary is made of chains of developing egg chambers called ovarioles (Figure 1). The GSCs are located in the germarium, which is found at the anterior tip of each ovariole (Kirilly & Xie, 2007). The GSCs are easily identifiable by an organelle called the fusome that is juxtaposed to the cap cells. Each GSC asymmetrically divides to self-renew or to form a daughter cell called the cystoblast. The cystoblast then undergoes four mitotic divisions to form a sixteen-cell cyst. One of these cells will differentiate into the oocyte and the other fifteen will become nurse cells. At the posterior end of the germarium, the cyst will be surrounded by follicle cells and bud off to form an egg chamber.

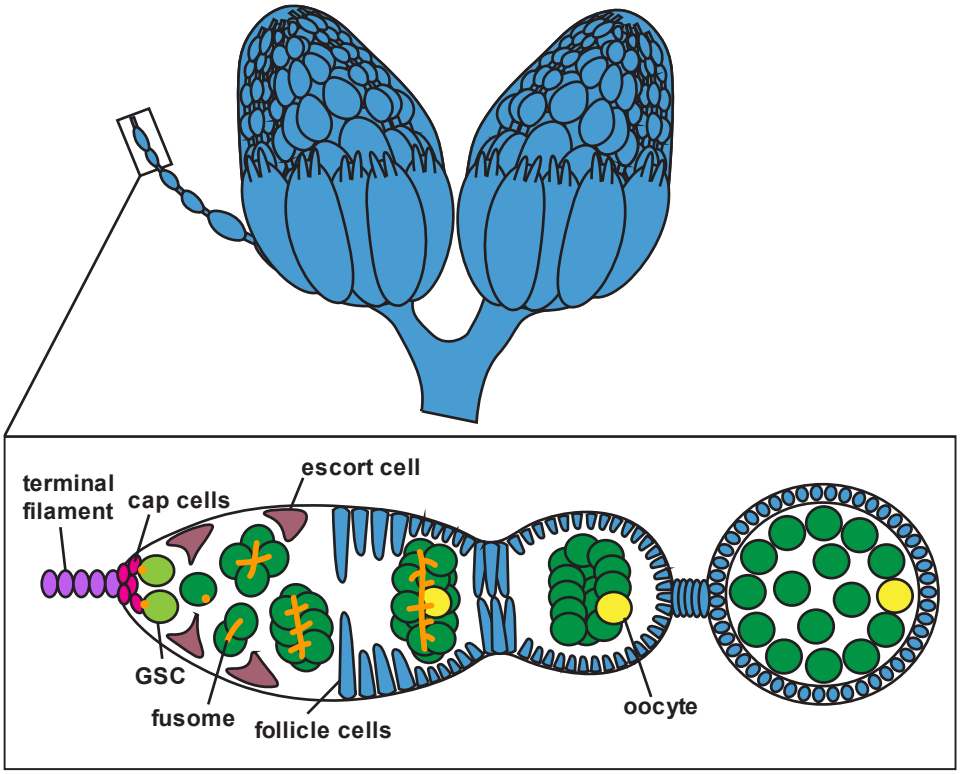


Figure 1: The *Drosophila* ovary and germarium. Each ovary is comprised of ovarioles, which are chains of developing egg chambers with a germarium located at the anterior tip. The germline stem cell (GSC) niche is located at the anterior end of the germarium and is composed of the GSCs (light green) and the cap cells (magenta) The GSCs are easily identifiable by an organelle called the fusome (orange), which is juxtaposed to the cap cells. Each GSC asymmetrically divides to form a cystoblast (dark green) which undergoes four mitotic divisions. The resulting 16-cell cyst consists of one oocyte (yellow) and fifteen nurse cells that are surrounded by follicle cells (blue) to produce an egg chamber.

One local signal that promotes GSC self-renewal is Bone Morphogenetic Protein (BMP) signaling (McKearin & Ohlstein, 1995). The BMP ligands, Decapentaplegic (Dpp) or Glass bottom boat (Gbb) are secreted from the cap cells and received by receptors, Thickveins (Tkv), Saxophone (Sax), and Punt (Put) (Figure 2A). This triggers the phosphorylation of the transcription factor, Mothers against decapentaplegic (Mad). pMad can then repress transcription of the differentiation factor, *bag of marbles* (*bam*), blocking differentiation in the GSCs. When the GSC divides out of the niche to form a cystoblast, it can no longer receive BMP signaling and transcription of bam is activated. BMP signaling also promotes transcription of *Daughters against decapentaplegic (Dad)* which then interacts with the Integrator complex and SMAD specific E3 ubiquitin protein ligase (Smurf) to degrade activated Tkv. (Liu et al., 2023). Over-expression of constitutively active *Tkv* (Tkv^{ACT}) or knock-down of *bam* in all germ cells of the ovary results in undifferentiated germ cell tumors (Xie & Spradling, 1998).

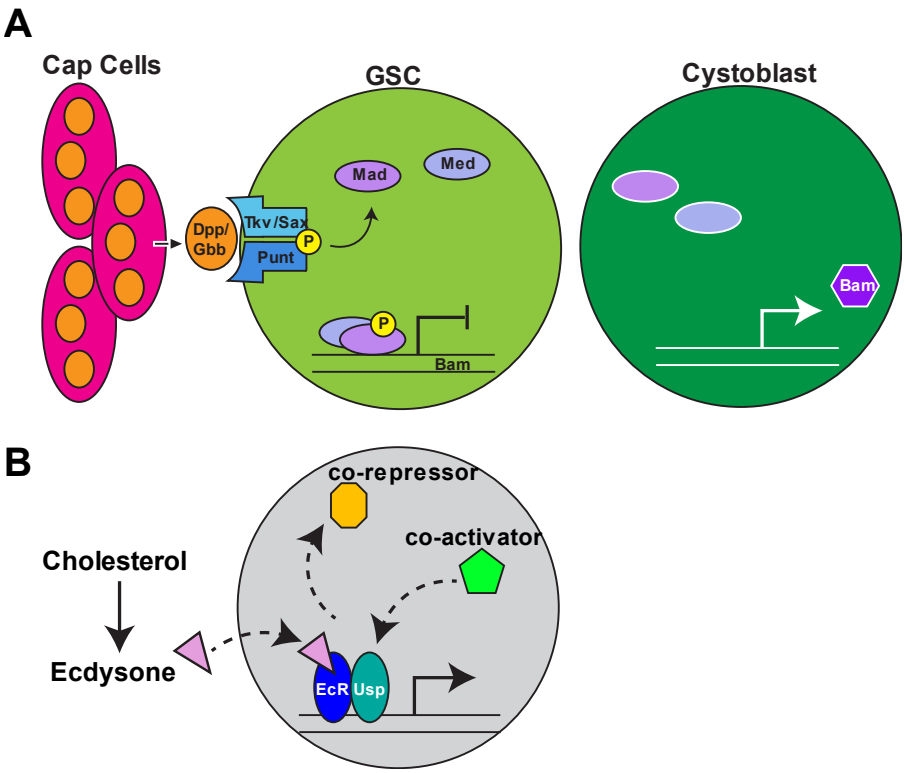


Figure 2: Schematics of BMP signaling and ecdysone signaling. (A) The BMP ligands Dpp and Gbb (orange) are secreted from the cap cells (magenta) and received by the receptors Tkv or Sax (light blue) and Punt (dark blue). This triggers the phosphorylation of Mad (lavender) and Med (light violet) which dimerize and repress transcription of bam. When the GSC asymmetrically divides out of the niche to form cystoblast, bam (dark purple) can be transcribed. (B) Ecdysone (light pink) is synthesized from cholesterol and binds to its receptors EcR (royal blue) and Usp (teal) to regulate transcription of a variety of targets. When the co-repressor (gold) is bound, transcription of these targets is repressed. When the co-activator (lime green) is bound, transcription is activated.

Ecdysone is synthesized from dietary cholesterol and binds to a heterodimer of two nuclear receptors encoded by, *Ecdysone Receptor* (*EcR*) and *ultraspiracle* (*usp*) (Figure 2B). Ecdysone signaling regulates transcription of a variety of targets such as *Eip75B*, *Eip74EF*, *Eip63E*, and *ftz-f1* (King-Jones & Thummel, 2005). Ecdysone from the escort cells provides a short-range signal to the GSCs (Shi et al., 2021). Knock-down of ecdysone signaling causes a loss of GSCs, indicating that it is required for GSC self-renewal (Ables & Drummond-Barbosa, 2010; König et al., 2011). *EcR* is expressed in all germ cells and follicle cells, meaning they are all able to receive ecdysone signaling. Over-expression of *EcR.B1* in all germ cells of the ovary results in undifferentiated germ cell tumors (Jung & Ables, 2024). These look phenotypically similar to the over-expression of Tkv^{ACT} and knock-down of *bam*. They also contain cells at similar stages of differentiation, suggesting that ecdysone signaling and BMP signaling converge on a common point of regulation (Jung & Ables, 2024)

Here, we present evidence that suggests ecdysone signaling and BMP signaling converge on common targets to regulate germline stem cell maintenance and differentiation. To identify potential common targets of ecdysone signaling and BMP signaling, we performed several bioinformatic analyses such as, single cell RNA sequencing (scRNAseq), Single Cell rEgulatory Network Inference and Cluster (SCENIC) analysis, pseudotime analysis, and single cell velocity (scVelo). We demonstrate that ecdysone signaling and BMP signaling converge on a common point of regulation to coordinate GSC fate.

RESULTS

Whole ovary single-cell RNA sequencing confirms in vivo findings

To assess potential transcriptomic differences, we performed single-cell RNA sequencing on over-expression of *EcR.B1* and over-expression of Tkv^{ACT} whole ovaries. We compared this data to wild-type control ovaries from the Fly Cell Atlas and *bam* knock-down mutant, $bam^{\Delta 86}$, ovaries from Sun et al. (Li et al., 2022; Slaidina et al., 2021; Sun et al., personal communication). The *control* ovaries contain few undifferentiated early germline cells and many late germline and somatic cells (Figure 3A). In contrast, the tumorous ovaries of $bam^{\Delta 86}$, *EcR.B1*, and Tkv^{ACT} have an enriched early germline cell population and a depleted late germline and somatic cell population (Figure 3B-D). To determine which cells were early/mitotic and late/meiotic germline we identified cells with the highest expression of the germline markers *nanos* and *vasa*, the early germline marker *HP6*, and the late germline marker *mnd* (Figure 3E-H) (Rust et al., 2020; Slaidina et al., 2021). Previously, it was difficult to study transcriptomic differences between the early germ cell populations because there are so few. The enrichment of the early germ cell populations caused by the over-expression of *EcR.B1*, knock-down of *bam*, and over-expression of Tkv^{ACT} will provide an exciting opportunity to identify novel transcriptomic differences between early germ cell populations.

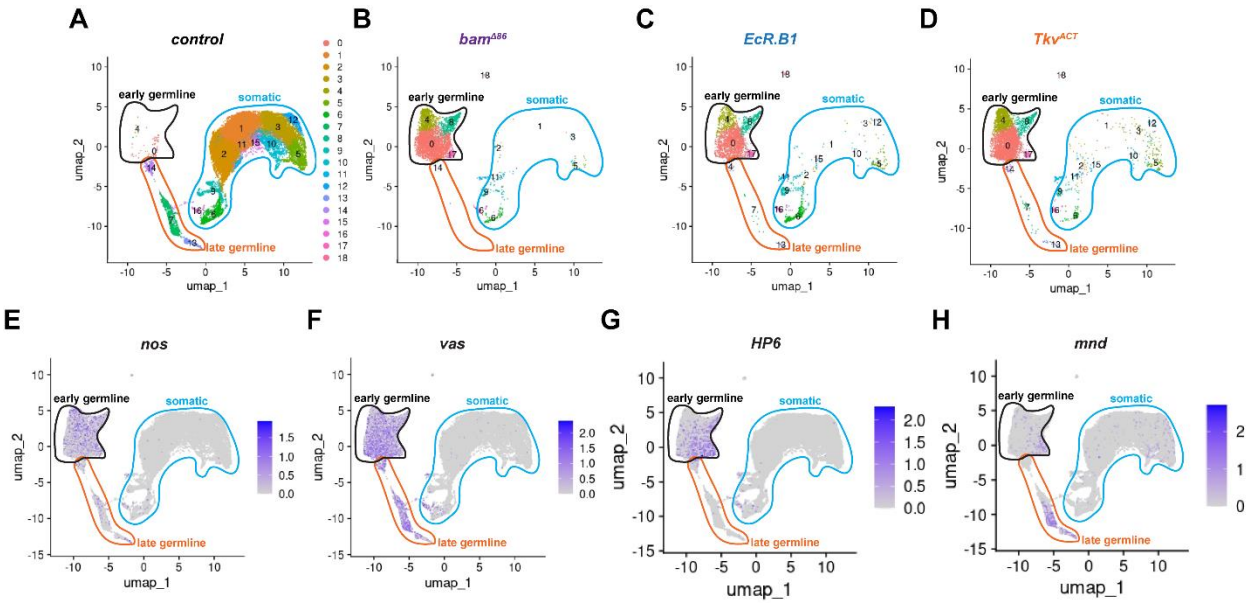


Figure 3: Whole ovary single-cell RNA sequencing. (A) control, (B), bam$^{\Delta 86}$, (C) EcR.B1, and (D) Tkv^{ACT} UMAPs with the early germline (black), late germline (orange), and somatic cells (blue) outlined. Expression of (E) nos, (F) vas, (G) HP6, and (H) mnd on the combined genotype UMAP.

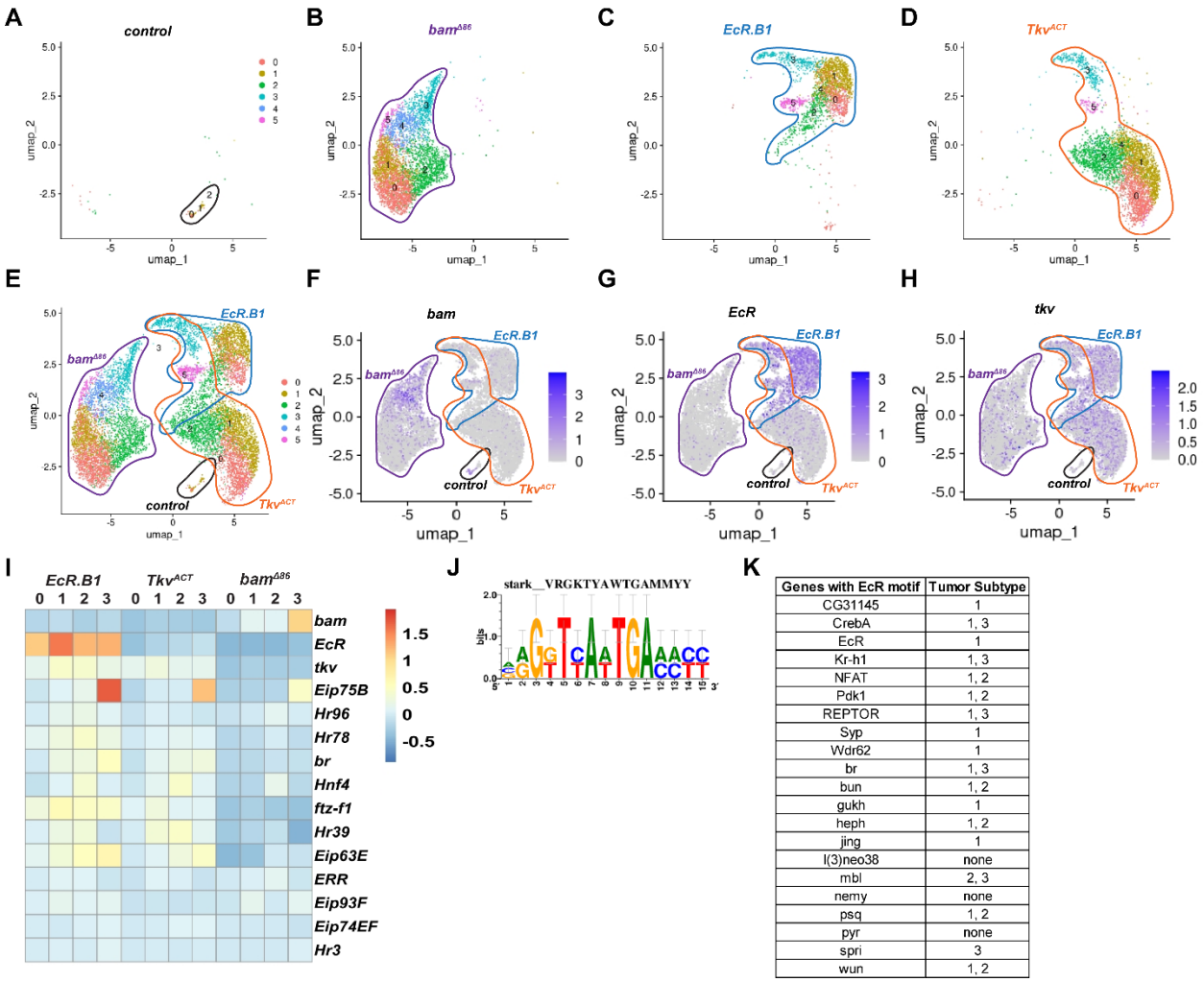
Re-clustering of early germline cells reveals six undifferentiated germ cell populations

To further investigate the differences between the tumor genotypes, the four early germline sub-types were re-clustered. The control shows three undifferentiated germ cell sub-types, 0, 1, and 2, likely representing GSCs, cystoblasts, and 2-cell cysts. The $bam^{\Delta 86}$, *EcR.B1*, and Tkv^{ACT} tumor genotypes show six undifferentiated germ cell sub-types, 0, 1, 2, 3, 4, and 5 (Figure 4A-D). Undifferentiated germ cell sub-type 4 is unique to $bam^{\Delta 86}$ (Figure 4B). Although they all cluster into the same six sub-types, cells from independent genotypes fall in different areas of the UMAP, indicating that they are transcriptomically distinct. When all four genotypes are combined on the same UMAP, *EcR.B1* and Tkv^{ACT} are closer together, suggesting that they are more similar to each other than they are to $bam^{\Delta 86}$ (Figure 4E). Based on their location on the UMAPs, all three tumor genotypes converge on undifferentiated germ cell sub-types 2 and 3.

To validate the differences between genotypes, we examined the expression of the genotype markers *bam*, *EcR*, and *tkv*. (Figure 4F-H). The highest *bam* expression was found in the $bam^{\Delta 86}$ cells, because it does not block transcription of *bam*, only translation (Figure 4F) (McKearin & Ohlstein, 1995). As expected, the highest *EcR* expression was found in the *EcR.B1* cells (Figure 4G). Interestingly, expression of *tkv* was at similar levels in the *EcR.B1*- and Tkv^{ACT}-expressing cells, suggesting that ecdysone signaling also elevates *tkv* expression to similar levels as over-expressing Tkv^{ACT} (Figure 4H). Visualization of marker gene expression in each genotype sub-divided by cluster yielded similar comparisons (Figure 4I).

There are a variety of known ecdysone signaling targets that contain EcR/Usp binding sites, or motifs. One known binding sequence is shown in Figure 4J. Using the Fly Cell Atlas, we could identify genes that have this motif in a wild-type ovary and compare their expression in

our germ cell tumor scRNAseq data (Slaidina et al., 2021). EcR expression is highest in undifferentiated germ cell subtype 1, so we expected ecdysone signaling to be most active in these cells (Figure 4I). Unsurprisingly, genes like *Syp*, *Wdr62*, *gukh*, *jing*, and many others, were upregulated in undifferentiated germ cell subtype 1 of the combined tumor data where ecdysone signaling was most active (Figure 4K).



Genes with EcR motif	Tumor Subtype
CG31145	1
CrebA	1, 3
EcR	1
Kr-h1	1, 3
NFAT	1, 2
Pdk1	1, 2
REPTOR	1, 3
Syp	1
Wdr62	1
br	1, 3
bun	1, 2
gukh	1
heph	1, 2
jing	1
l(3)neo38	none
mbl	2, 3
nemy	none
psq	1, 2
pyr	none
spri	3
wun	1, 2

Figure 4: Early germline single-cell RNA sequencing. UMAPs of re-clustered early germline cells (A) *control*, (B) *bam*$^{\Delta 86}$, (C) *EcR.B1*, and (D) *Tkv*^{ACT}. Each undifferentiated germ cell subtype (or cluster) is represented by a different color, 0 in peach, 1 in yellow, 2 in green, 3 in teal, 4 in blue, and 5 in pink. The different genotypes are outlined in different colors, control in black, *bam*$^{\Delta 86}$ in purple, *EcR.B1* in blue, and *Tkv*^{ACT} in orange. (E) Combined genotype UMAP with each genotype outlined. Expression of genotype markers in combined tumors UMAP (F) *bam*, (G) *EcR*, and (H) *tkv*. (I) Heat map of the mRNA expression of known ecdysone signaling targets within undifferentiated germ cell subtypes 0-3 of *EcR.B1*, *Tkv*^{ACT}, and *bam*$^{\Delta 86}$. (J) Sequence of EcR motif. (K) Table of genes with EcR motif and which combined tumor subtype(s) they are upregulated in.

EcR-expressing cells have increased Tkv protein levels

Tkv mRNA expression was elevated in EcR-over-expressing GSCs (Figure 4H). To test whether this upregulation also reflected increased Tkv protein levels, we used a Tkv2 antibody described in Peterson et al., to visualize the protein expression of Tkv isoforms A, B, and C (2022). It was previously suggested that Tkv-A is predominantly expressed in the germline (Tseng et al., 2018). In contrast, Tkv-D is predominantly expressed in the soma and Tkv-B and -C are minimally expressed in the ovary. As BMP signaling is known to be important for GSC self-renewal, we expected GSCs to have the highest Tkv expression. Surprisingly, wild-type germaria showed the highest expression of Tkv in 8-cell cysts, but low expression in the early germ cells (Figure 5A-A'). Interestingly, Tkv^{ACT} did not show a drastic increase in Tkv protein levels (Figure 5B-B'). In a wild-type ovary, activated Tkv is degraded by a negative feedback loop involving the Integrator complex, Dad, and Smurf (Liu et al., 2023). The scRNAseq data showed an increase in *tkv* mRNA in Tkv^{ACT}, but the constitutively active Tkv protein could have been degraded so that upregulated protein accumulation is not detected in the tissue. When *EcR.B1* is over-expressed, there is a discernible increase of Tkv protein levels in the germ cell tumors (Figure 5C-C'). This could indicate that Tkv is upregulated when EcR.B1 is over-expressed, but it is not active, so it is not degraded and can still be detected in the tissue. The *bam RNAi* did show an unexpected increase in Tkv protein (Figure 5D-D'). The unexpected results from *bam* RNAi could be from the difference in the type of knock-down used. The scRNAseq was done on a global *bam* knock-down mutant, $bam^{\Delta 86}$, that still transcribes mRNA but blocks translation to protein, while the in vivo experiments were done with a *bam* RNAi driven only in the germline (McKearin & Ohlstein, 1995).

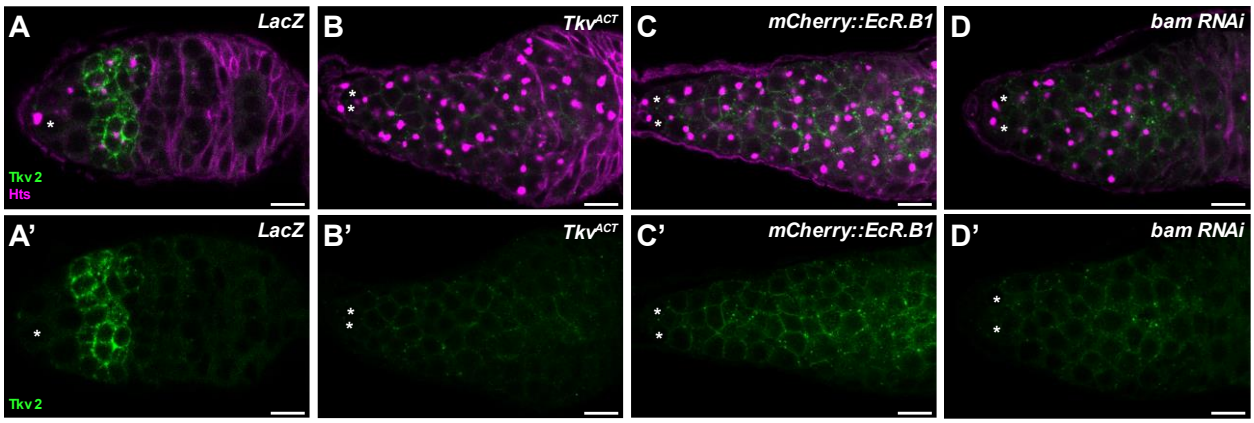


Figure 5: Germ cell tumors have increased Tkv protein expression. (A-A') *UASp-LacZ*, (B-B') *UASp-Tkv^{ACT}*, (C-C') *UASz-mCherry::EcR.B1*, and (D-D') *bam RNAi*. (A-D) are stained with Hts (fusomes) in magenta and Tkv2 in green. (A'-D') show only Tkv2 in green. The GSCs are marked by *. Scale bars represent 10µm. All driven with *nos-Gal4::VP16*.

Taken together, these data suggest that ecdysone signaling and BMP signaling interact on a common point of regulation in GSCs. There are two basic mechanistic models for how they interact, direct or indirect. To test if *EcR* is a direct target of *tkv*, we can knock-down *EcR* and over-express Tkv^{ACT} simultaneously. To test if *EcR* is directly targeting BMP signaling, we can simultaneously over-express *EcR.B1* while individually knocking-down BMP signaling receptors *tkv*, *sax*, and *put*, or the BMP ligand, *dpp*. The first step in these experiments was to identify available tools.

RNAi screening identified a variety of available fly lines that effectively disrupt BMP signaling

We screened available RNAi lines for *tkv*, *sax*, and *put* to find the most effective knock-down in the germ cells (Figure 6). An effective knock-down of the BMP signaling receptors would result in a loss of GSCs because BMP signaling is required for GSC self-renewal (Xie & Spradling, 1998). The RNAi lines were crossed with *nos-Gal4::VP16* to drive expression in all germ cells and the number of GSCs were counted (Figure 6). The most effective *tkv* RNAi line was $tkv^{HMS02185}$ because it resulted in a total loss of GSCs (Figure 6B, P). The Tkv2 antibody was used to determine if the other *tkv* RNAi lines effectively knocked down *tkv*, despite not having a phenotype. We found that $tkv^{GL01338}$, $tkv^{HMS04501}$, and $tkv^{GLV210189}$ effectively knocked down Tkv below detectable limits of the Tkv2 antibody (Figure 6M-N, Q-R). $tkv^{GL00035}$ still has Tkv2 expression, but less than the wild-type, indicating that it has an intermediate knock-down of Tkv (Figure 6O). The most effective *sax* RNAi line was $sax^{HMS04520}$, identified because of its total loss of GSCs (Figure 6I). Another *sax* RNAi line, $sax^{HMJ02118}$, was also determined to be effective because it caused an intermediate GSC loss (Figure 6H). The *put* RNAi lines did not have a significant loss of GSCs, but $put^{GLV21066}$ has been shown to cause GSC loss in the male germline (Figure 6K) (Ridwan et al., 2024).

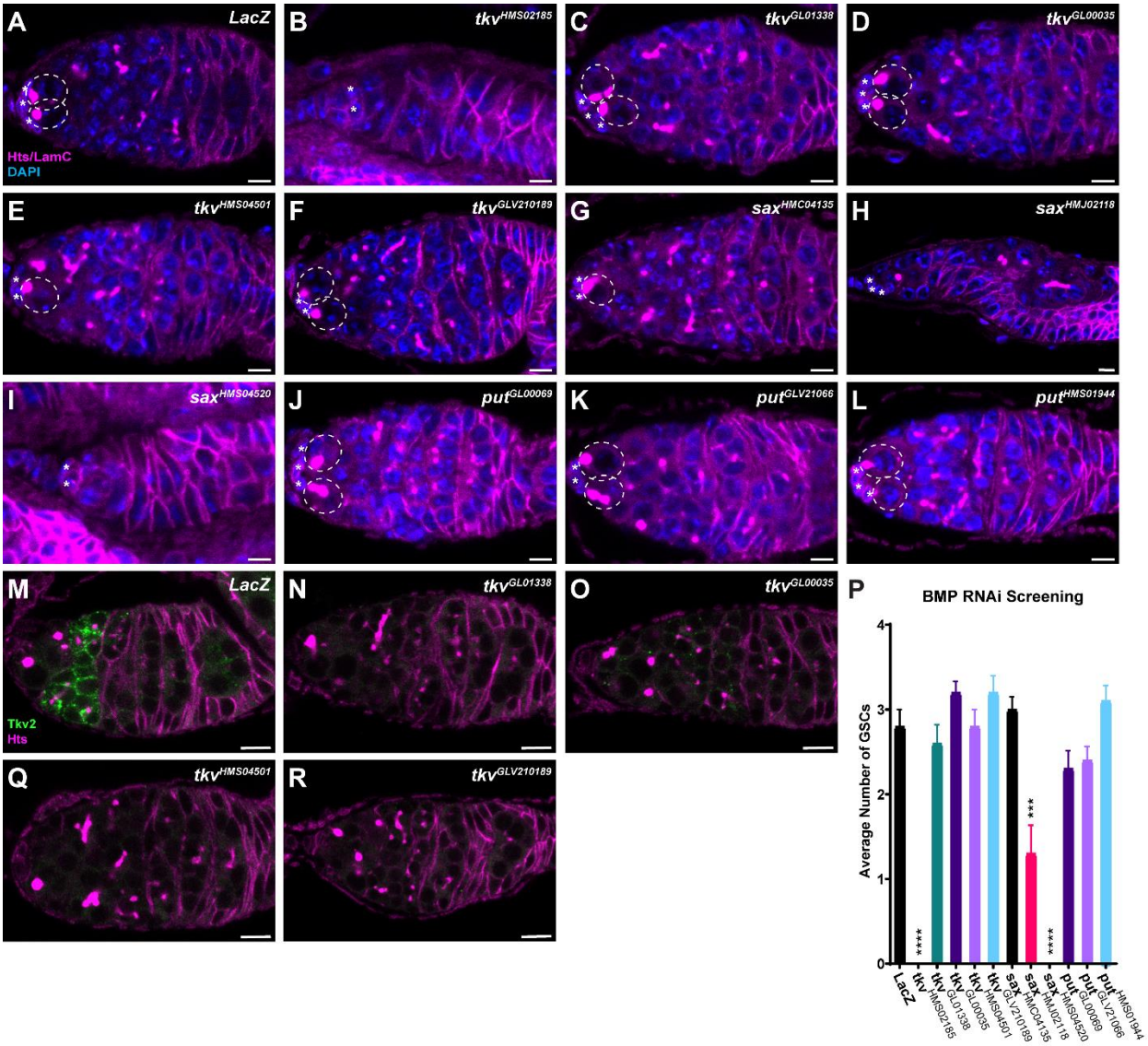


Figure 6: BMP RNAi screening identifies effective tools. (A) *LacZ* control germarium. (B) $tkv^{HMS02185}$, (C) $tkv^{GL01338}$, (D) $tkv^{GL00035}$, (E) $tkv^{HMS04501}$, (F) $tkv^{GLV210189}$, (G) $sax^{HMC04135}$, (H) $sax^{HMJ02118}$, (I) $sax^{HMS04520}$, (J) $put^{GL00069}$, (K) $put^{GLV21066}$, and $put^{HMS01944}$ stained with Hts (fusomes) and LamC (nuclear lamina and cap cells) in magenta and DAPI (DNA) in blue. Cap cells are marked by *. GSCs are outlined dashed circles. Scale bars represent 5µm. (M) *LacZ* control. (N) $tkv^{GL01338}$, (O) $tkv^{GL00035}$, (Q) $tkv^{HMS04501}$, and (R) $tkv^{GLV210189}$ stained with Tkv2 in green and Hts in magenta. Scale bars represent 10µm. All driven with *nos-Gal4::VP16*. (P) Bar graph of the average number of GSC's in each BMP RNAi line. Sample size was n = 10 germaria. Significance was measured using Student's t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$

Depletion of tkv in EcR.B1-expressing germ cells does not rescue differentiation

Using a *tkv* RNAi, we knocked down *tkv* in the over-expression of EcR.B1 germ cells (Figure 7A-C). If *tkv* was a target of ecdysone signaling, then knocking down *tkv* in the *EcR.B1* germ cells would rescue differentiation, resulting in normal ovaries. The resulting ovaries were tumorous, indicating *tkv* is not a direct target of ecdysone signaling. We also knocked down the BMP ligand, *dpp*, in the over-expression of *EcR.B1* germ cells and did not rescue differentiation (Figure 7D-G). These ovaries contain more differentiated cells because an altered protocol was used due to the temperature-sensitive nature of the *dpp* mutants (Materials and Methods). Future experiments will test knock-down of *sax* and *put* using the RNAi lines we identified (Figure 6). We do not expect to see different results when knocking down *sax* or *put*, due to the loss of BMP signaling from the knock-down of *dpp*. The lack of rescue of differentiation from knocking down the ligand indicates that it is unlikely that any component of BMP signaling is a direct target of ecdysone signaling.

***Depletion of EcR in* Tkv^{ACT}-expressing germ cells does not rescue differentiation**

Using an effective *EcR RNAi* previously to be efficient in germ cells, we knocked down *EcR* in *Tkv^{ACT}*-expressing germ cells (Swain & Ables, 2025). If *EcR* is a target of BMP signaling, then knocking it down in the over-expression of *Tkv^{ACT}* germ cells would rescue differentiation, resulting in normal ovaries. The result was tumorous ovaries, indicating that it is unlikely that *EcR* is a direct target of BMP signaling (Figure 7H-J).

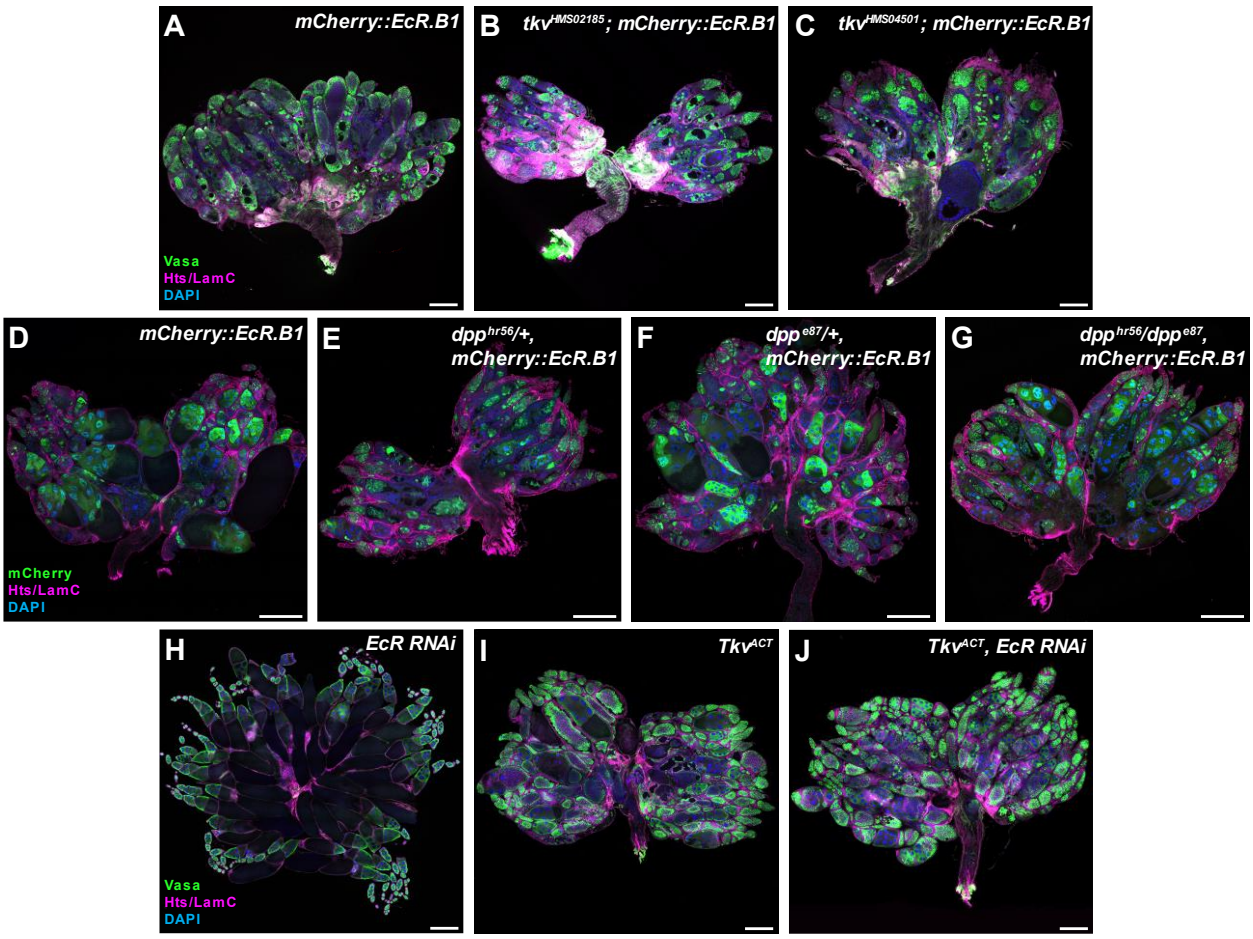


Figure 7: Ecdysone signaling and BMP signaling do not directly interact. (A-C) Knock-down of *tkv* in *EcR* over-expression does not rescue differentiation. (A) *UASz-mCherry::EcR.B1*, (B) *tkv*^{HMS02185} *RNAi; UASz-mCherry::EcR.B1*, (C) *tkv*^{HMS04501} *RNAi; UASz-mCherry::EcR.B1* stained with Vasa (germ cells) in green, Hts (fusomes) and LamC (nuclear lamina and cap cells) in magenta, and DAPI (DNA) in blue. Scale bars represent 50µm. (D-G) Knock-down of dpp in EcR over-expression does not rescue differentiation. (D) *UASz-mCherry::EcR.B1*, (E) *dpp*^{hr56}*, UASz-mCherry::EcR.B1*, (F) *dpp*^{e87}*; UASz-mCherry::EcR.B1*, and (G) *dpp*^{hr56}*/dpp*^{e87}*; UASz-mCherry::EcR.B1* stained with mCherry (EcR) in green, Hts and LamC in magenta, and DAPI in blue. Scale bars represent 200µm. 200µm. (H-J) Knock-down of *EcR* in *Tkv*^{ACT} over-expression does not rescue differentiation. (H) *EcR RNAi*, (I) *UASp-Tkv*^{ACT}, and (J) *UASp-Tkv*^{ACT}*, EcR RNAi* stained with Vasa in green, Hts and LamC in magenta, and DAPI in blue. Scale bars represent 50µm. (A-J) Driven with *nos-Gal4::VP16.*

Eip63E as a potential target of ecdysone signaling and BMP signaling

To identify potential common targets of ecdysone signaling and BMP signaling, we started by comparing the expression of known ecdysone signaling targets in the over-expression of *EcR.B1* and Tkv^{ACT} datasets (Figure 4I). *Eip63E*, or *E63E*, was interesting because it is upregulated in undifferentiated germ cell subtypes 2 and 3 of the over-expression of *EcR.B1* and Tkv^{ACT}, but not in $bam^{\Delta 86}$. *E63E* is a cyclin-dependent kinase (CDK) that is required for Drosophila development (Stowers et al., 2000). It is expressed in mitotically dividing germ cells, but its role in the germline is not characterized (Buszczak et al., 2007). Using an E63E protein trap, we can visualize its presence in the germ cell tumors (Figure 8A-D). To compare expression levels between genotypes, we applied a rainbow heat map filter to the GFP channel of the E63E protein trap. The lighter the color, the higher the expression level. The *bam RNAi* tumors have similar levels of E63E expression when compared to the *LacZ* control (Figure 8A-B). However, the *EcR.B1* and Tkv^{ACT} tumors do show an increase of E63E expression in germ cells found in the posterior of the germarium (Figure 8C-D). The upregulation of E63E protein in the *EcR.B1* and Tkv^{ACT} germ cell tumors validates the upregulation of E63E mRNA shown in the scRNAseq data (Figure 4I).

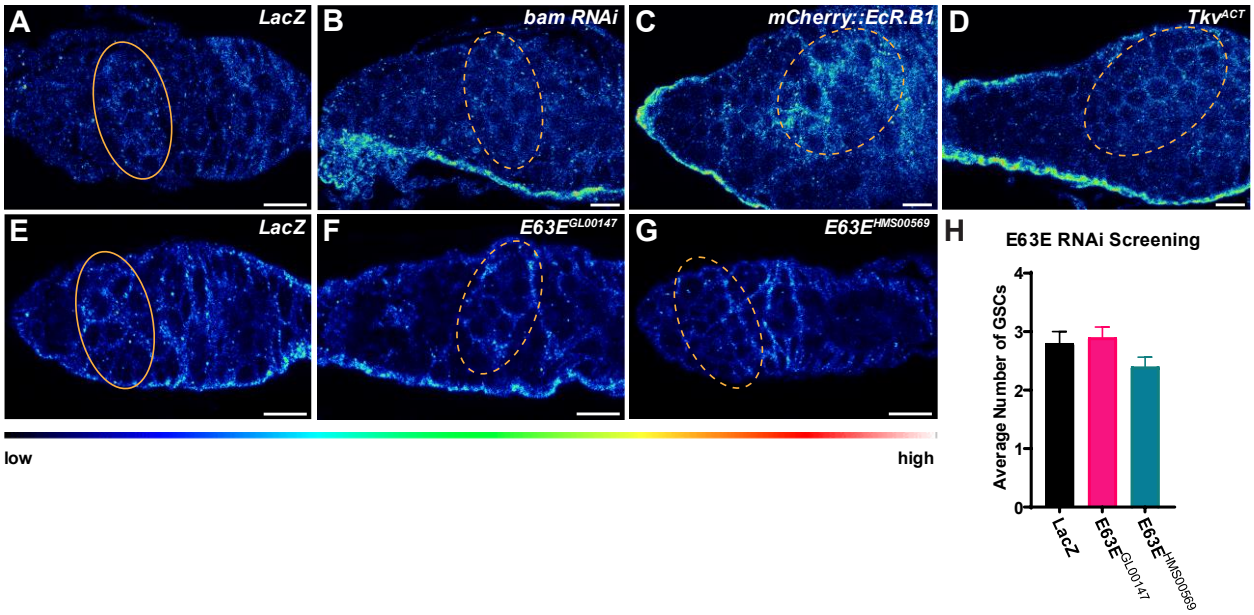


Figure 8: Eip63E as a potential common target of ecdysone signaling and BMP signaling. (A-D) E63E protein trap with (A) *LacZ*, (B) *bam RNAi*, (C), *mCherry::EcR.B1*, and (D) Tkv^{ACT}. (E-H) E63E protein trap with (E) *LacZ*, (F) $E63E^{GL00147}$, and (G) $E63E^{HMS00569}$. (A-G) All driven with *nos-Gal4::VP16* and stained with GFP. The 7-color Rainbow LUT filter on Zen was applied to visualize expression as a heat map. Scale bars = 10μm. (H) Bar graph of the average number of GSCs within the LacZ control, $E63E^{GL00147}$, and $E63E^{HMS00569}$ RNAi lines. Sample size was n = 10 germaria. Significance was measured using Student's t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$

The presence of E63E in the *EcR.B1* and Tkv^{ACT} germ cell tumors indicates that it could be a potential common target of ecdysone signaling and BMP signaling. To test these potential interactions, we first had to identify available tools. We knocked down E63E in the germ cells using two available *E63E RNAi* lines, $E63E^{GL00147}$ and $E63E^{HMS00569}$. There were no visible germ cell phenotypes, and there was no significant change in the average number of GSCs (Figure 8E-H). To test if these lines were effective, we used the E63E protein trap to see if there was a decrease in E63E expression. The expression of *E63E* in the RNAi lines looks unchanged from the *LacZ* control, indicating that both RNAi lines do not effectively knock down *E63E* in the germ cells (Figure 8E-G).

We have identified *E63E* as a potential common target of ecdysone signaling and BMP signaling and have visualized its presence in the germ cell tumors but have been unable to effectively test its interactions with *EcR* or *Tkv*. In the future, we plan to build new tools to over-express or knock-down E63E to test these potential interactions

SCENIC identifies transcription factors as potential targets of Ecdysone signaling and BMP signaling

Single Cell rEgulatory Network Inference and Cluster (SCENIC) predicts the transcriptional state of cells based on their gene regulatory network, which is composed primarily of transcription factors (Aibar et al., 2017). The combined tumor data was used to calculate the regulon specificity score (RSS) to identify cell-type-specific regulons. A regulon is a group of genes regulated by the same regulatory protein or transcription factor (Aibar et al., 2017). The genes with the highest RSS per cluster are shown in Figure 9A. To get a broader understanding of active regulons, we looked at the regulon activity within the combined tumor

data, *EcR.B1*, and Tkv^{ACT} (Figure 9B-D). Using SCENIC, we can identify potential common targets of ecdysone signaling and BMP signaling based on transcription factor activity.

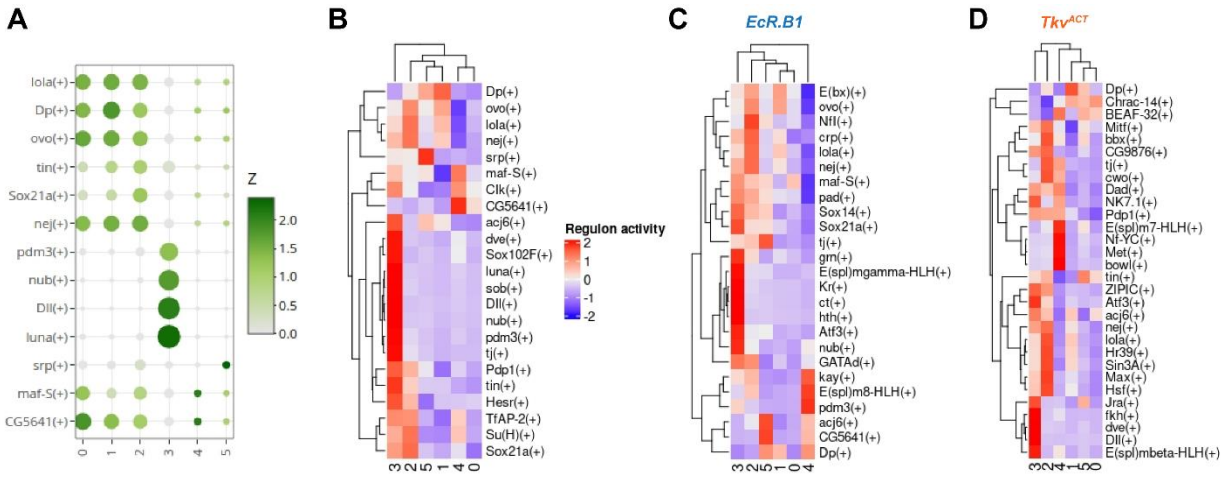


Figure 9: SCENIC identifies transcription factors as potential common targets. (A) Highest regulon specificity score (RSS) per cluster for the combined tumor data. (B) Heat map of the average regulon activity per cluster for the combined tumor data. Scale for regulon activity indicates that red is higher regulon activity and blue is lower regulon activity. (C) Heat map of the average regulon activity per cluster for EcR.B1. (D) Heat map of the average regulon activity per cluster for Tkv^{ACT}.

One gene of particular interest is *longitudinals lacking (lola)*, a transcription factor required for germline stem cell maintenance and differentiation in males, but its role in the female germline is not well understood (Davies et al., 2013; Paige Bass et al., 2007). It was identified by its presence in both the RSS and regulon activity (Figure 9). *lola* has the highest RSS in undifferentiated germ cell subtypes 0, 1, and 2 and the highest regulon activity in subtype 2. Undifferentiated germ cell subtype 2 is also where *lola* mRNA is most highly expressed according to the scRNAseq data (Figure 11J). We can use a Lola antibody to visualize its expression in the germ cell tumors (Zhang et al., 2003). Lola is expressed in the mitotically dividing germ cells, but not in the GSCs of a wild-type germarium (Figure 11A).

The SCENIC data also identified several other potential common targets of ecdysone signaling and BMP signaling. *DP transcription factor* (*Dp*) is a cell cycle regulator required in the nurse cells during oogenesis and is upregulated in undifferentiated germ cell subtype 1 (Royzman et al., 2002). *Sox21a* is a transcription factor that regulates proliferation in the intestinal stem cells (Meng & Biteau, 2015). *Nejire* (*nej*) encodes the transcriptional co-activator CBP that can remodel active chromatin in other tissues and is upregulated in undifferentiated germ cell subtypes 1, 2, and 3 (Ludlam et al., 2002). Their expression patterns and gene ontology are shown in Figure 11J. *Dp*, *Sox21a*, and *nej* were identified based on their high RSS in undifferentiated germ cell subtypes 0, 1, and 2 (Figure 9A).

Enhancer of bithorax (*E(bx)*) is the histone-binding component of the nucleosome remodeling factor (NURF) and is known to interact with ecdysone signaling during development and GSC maintenance as a coactivator of EcR (Badenhorst et al., 2005) (Ables & Drummond-Barbosa, 2010). *E(bx)* has the highest regulon activity in EcR.B1 undifferentiated germ cell subtypes 1, 2, and 3 and its expression is highly upregulated in subtype 1. E(bx) can be

visualized using a GFP-tagged protein trap and, in a wild-type germarium, is expressed in all the follicle cells and germ cells (Figure 11D). We plan to use this GFP line to visualize E(bx) expression in the germ cell tumors. We are currently working to identify available tools to test potential interactions of these genes with ecdysone signaling and BMP signaling.

Pseudotime and scvelo reveal stages of differentiation

Pseudotime analysis and single-cell velocity (scVelo) can predict the stages of differentiation of the germ cell tumors. Pseudotime analysis measures the geodesic, or shortest, distance back to a root node to determine pseudotime (Cao et al., 2019). The longer the geodesic distance, the greater the pseudotime, and the more differentiated the cell is. Single-cell velocity (scVelo) determines the state of differentiation by measuring the rate of gene expression change based on the ratio of its spliced and unspliced mRNA (Bergen et al., 2020). The pseudotime analysis shows that undifferentiated germ cell subtypes 2, 3, and 4 (more yellow) contain the most differentiated cells in all tumor genotypes, while subtypes 0 and 1 (more purple) are the least differentiated (Figure 10A-C). Subtype 5 seems to be less differentiated in the $bam^{\Delta 86}$, but more differentiated in *EcR.B1*, and Tkv^{ACT}. The scVelo shows that the direction of differentiation also flows towards undifferentiated germ cell subtypes 2, 3, and 4, meaning they are more differentiated (Figure 10D-F). Subtypes 0 and 1 are the least differentiated, but there is no clear starting point, indicating that they are at similar stages of differentiation. All differentiated cells start as germline stem cells, so we would expect to see an obvious starting point for differentiation, but we do not. This indicates that there might be two different types of GSCs in the tumor models.

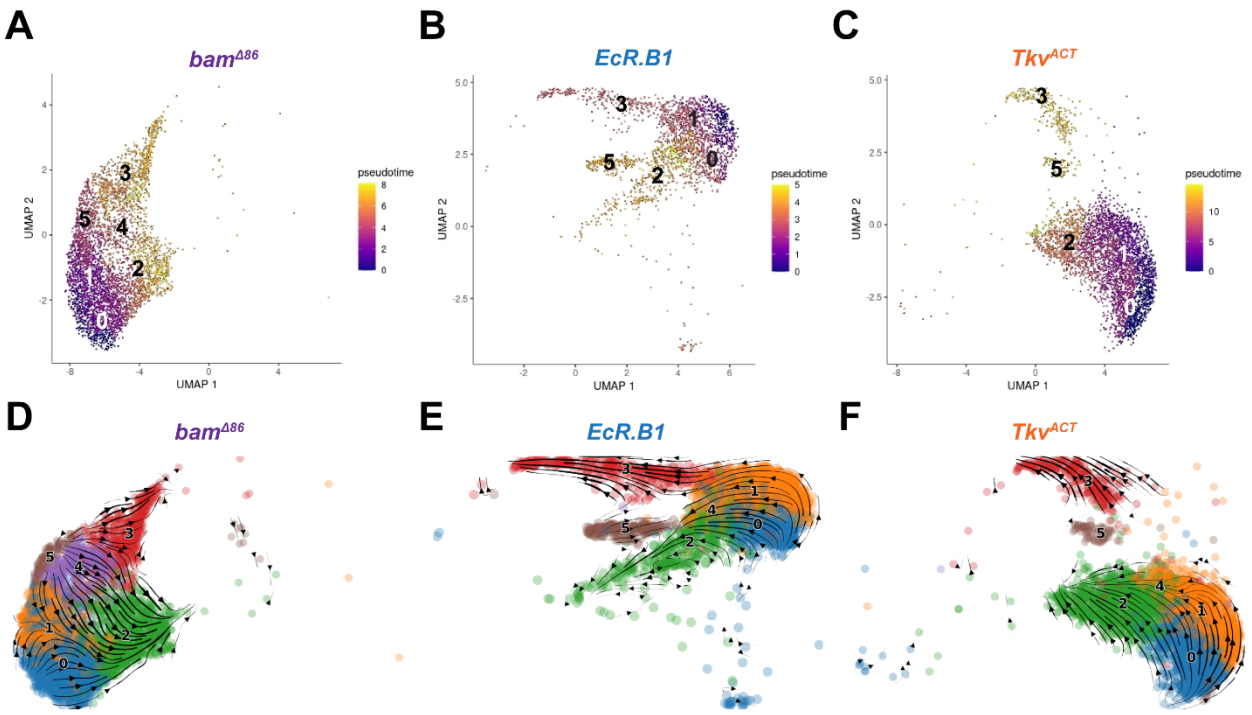


Figure 10: Pseudotime analysis and single-cell velocity reveal stages of differentiation. (A-C) Pseudotime analysis of (A) *bam*$^{\Delta 86}$, (B) *EcR.B1*, and (C) *Tkv*^{ACT}. The lighter the color of the cells, the more differentiated they are. (D-F) Single cell velocity analysis of (D) *bam*$^{\Delta 86}$, (E) *EcR.B1*, and (F) *Tkv*^{ACT}. Undifferentiated germ cell subtype 0 is in blue, 1 in orange, 2 in green, 3 in red, 4 in purple, and 5 in brown.

Potential common targets of ecdysone signaling and BMP signaling

To attempt to visualize these subtypes in vivo, we have been working to identify unique markers for each subtype. We first started by comparing undifferentiated germ cell subtypes 0 and 1, as they are more similar to each other. *Mov10 RISC complex RNA helicase* (*Mov10*) was discovered to be highly upregulated in undifferentiated germ cell subtype 1, but not in subtype 0, making it an ideal marker for subtype 1 (Figure 11J). *Mov10* is a gene that encodes a component of the microRNA gene silencing complex (miRISC) and has been shown to play a role in inhibiting the proliferation of intestinal stem cells (Takemura et al., 2021). Interestingly, the BMP inhibitor, *Dad,* is a direct target of Mov10, indicating a potential interaction with BMP signaling. *Mov10* is upregulated in undifferentiated germ cell subtype 1 of the *EcR.B1* and Tkv^{ACT} germ cell tumors, but not in subtype 0, making it an ideal marker for subtype 1. Using an HA-tagged *Mov10* knock-in line described in Takemura et al., 2021, we can visualize the expression of *Mov10* in the undifferentiated germ cells of a wild-type germarium (Figure 11B). In the future, we plan to use this line to visualize Mov10 expression in the *bam RNAi*, *EcR.B1*, and Tkv^{ACT} germ cell tumors.

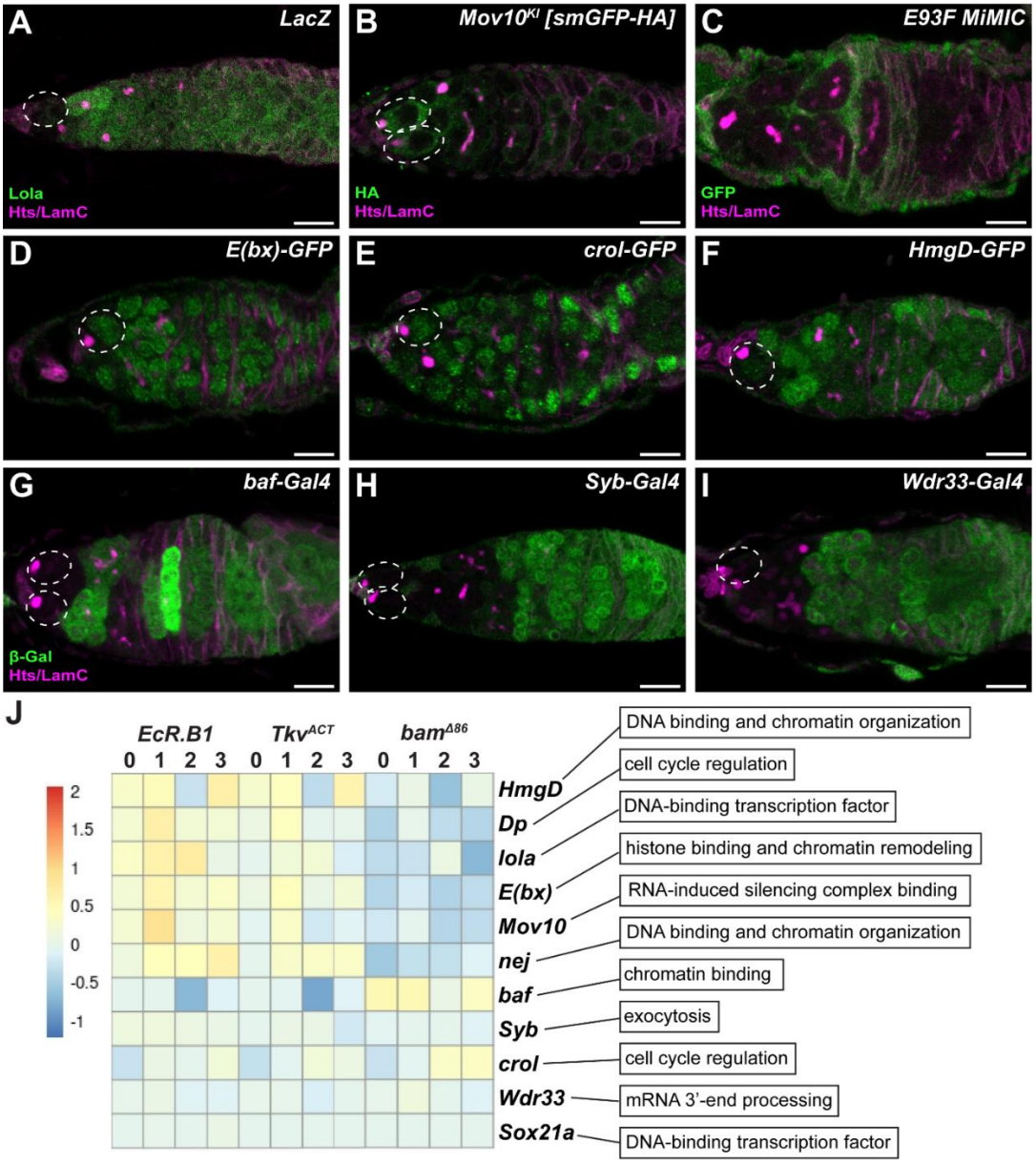


Figure 11: Potential common targets have varying expression patterns. (A) Expression of Lola in a *UASp-LacZ* control. Stained with anti-Lola in green and Hts (fusomes) and LamC (nuclear lamina and cap cells) in magenta. (B) Expression of Mov10 in *Mov10^{KI} [smGFP-HA]*. Stained with HA in green and Hts and LamC in magenta. (C-F) Expression of (C*) E93F MiMIC*, (D) *E(bx)-GFP*, (E) *crol-GFP*, and (F) *HmgD-GFP* stained with GFP in green and Hts/LamC in magenta. (G-I) Expression of (G) *baf-Gal4*, (H) *Syb-Gal4*, and (I) *wdr33-Gal4* crossed with *UASp-LacZ* and stained with β-Galactosidase in green and Hts/LamC in magenta. (A-I) Scale bars = 10µm. (J) Heat map of the expression of potential common targets in undifferentiated germ cell subtypes 0-3 of *EcR.B1*, *Tkv^{ACT}*, and *bam^{Δ86}*. The gene ontology is shown next to the heat map.

Ecdysone inducible protein 93F (*E93F*) is a transcription factor that regulates chromatin accessibility and controls enhancer activity in the wing (Uyehara et al., 2017). It is upregulated in undifferentiated germ cell subtype 1 of the combined tumor data (Figure 4I). Using a GFP-tagged protein trap, we can visualize the presence of *E93F* in the escort cells, but not in the germ cells. (Figure 11C). As it is not normally expressed in germ cells, we plan to use this protein trap as a negative control in the germ cell tumors.

Crooked legs (*crol*) is a known target of ecdysone signaling and is required for cell cycle progression in the wing (Mitchell et al., 2008). It is upregulated in undifferentiated germ cell subtypes 2 and 3 of the combined tumor data (Figure 11J). We have identified a GFP-tagged protein trap that shows *crol* expression in the germ cells and follicle cells (Figure 11E).

High mobility group protein D (*HmgD*) regulates chromatin remodeling by promoting DNA accessibility (Ragab & Travers, 2003). *HmgD* is upregulated in undifferentiated germ cell subtypes 1 and 3 of the combined tumor data (Figure 11J). Using a GFP-tagged protein trap we can visualize that it is more highly expressed in the mitotically dividing cells and at low levels in the GSCs (Figure 11F).

Barrier to autointegration factor (*baf*) encodes a chromatin binding protein that is required for GSC maintenance in the ovary (Duan et al., 2020). It is upregulated in undifferentiated germ cell subtypes 0 and 1 of the combined tumor data (Figure 11J). Using a *baf-Gal4* driver, we can visualize *baf* expression starting in the 8-cell cysts (Figure 11G).

Synaptobrevin (*Syb*) is a vesicle associated membrane protein that is involved in exocytosis (Bhattacharya et al., 2002). It is upregulated in undifferentiated germ cell subtype 1 in the combined tumor data (Figure 11J). Using a *Syb-Gal4* driver we can visualize its expression

starting in the escort cells, follicle cells, and differentiated germ cells, starting at the 16-cell cysts (Figure 11H).

WD repeat domain 33 (*Wdr33*) plays a role in 3' mRNA processing, specifically polyadenylation. It has been shown to play a role in the mitotic to meiotic transition in the male germline and was found to cause germ cell tumors when overexpressed in the mitotically active spermatogonia (Shan et al., 2017). *Wdr33* is upregulated in undifferentiated germ cell subtype 1 in the combined tumor data (Figure 11J). We can visualize its expression in the follicle cells and post-mitotic germ cells, starting in the 16-cell cysts, using a *Wdr33-Gal4* driver (Figure 11I).

Mov10, *E93F*, *crol*, *HmgD*, *baf*, *Syb*, and *Wdr33* are potential common targets of ecdysone signaling and BMP signaling. They have interesting expression patterns that will help us validate our scRNAseq data and visualize the undifferentiated germ cell subtypes in vivo.

DISCUSSION

Based on the scRNAseq data (Figures 3 and 4) and genetic experiments (Figure 7), our data suggests that ecdysone signaling and BMP signaling in the early germ cells converge on common targets that block differentiation resulting in germ cell tumors (Figure 12). However, there is another potential model in which ecdysone signaling activates an unknown gene that triggers a transcriptional cascade to target *tkv*. This could also explain the upregulation of *tkv* in the *EcR*-expressing cells (Figure 4H, 5C).

One potential common target is *E63E*, its upregulation in the over-expression of *EcR.B1* and Tkv^{ACT} tumors is shown in Figure 8. We have determined that it is expressed in the germ cell tumors, but future experiments are needed to investigate potential interactions of ecdysone signaling and BMP signaling with *E63E*. Other potential targets such as *lola, Dp, Sox21a, nej,* and *E(bx)* were identified from the SCENIC data (Figure 9). We are currently working to visualize their expression in the germ cell tumors and identify available tools to test potential interactions with ecdysone signaling and BMP signaling.

Pseudotime analysis and scVelo reveal that undifferentiated germ cell subtypes 0 and 1 are the least differentiated, while subtypes 2, 3, and 4 are the most differentiated (Figure 10). To validate each undifferentiated germ cell subtype, we are working to identify unique markers to visualize each subtype in vivo. One marker we are pursuing for subtype 1 is *Mov10*, which is expressed in the GSCs and cystoblasts of a wild-type germarium (Figure 11B). Other markers and potential targets we are investigating include *E93F*, *crol*, *HmgD*, *baf*, *Syb*, and *wdr33*, of which we have identified available tools to visualize in vivo (Figure 11). We also plan to identify tools to investigate these genes as potential common targets of ecdysone signaling and BMP signaling.

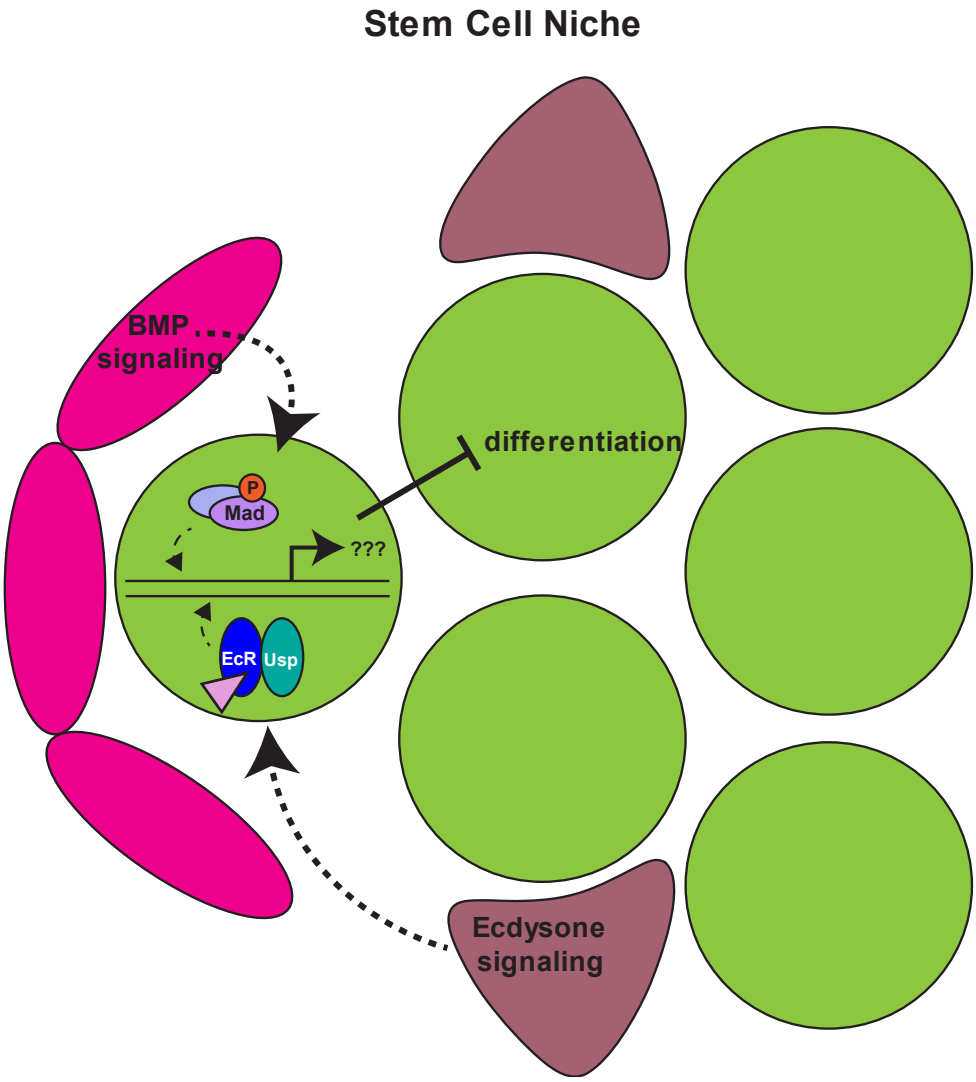


Figure 12: Proposed model of interactions between ecdysone signaling and BMP signaling in the stem cell niche. BMP signaling (purple) from the cap cells (magenta) and Ecdysone signaling (blue) from the escort cells (mauve) converge on common targets within the GSCs (green) to block differentiation, causing stem-like germ cell tumors (green).

Lola was identified as a potential common target of ecdysone signaling and BMP signaling from the SCENIC data (Figure 9). The Lola regulon has increased activity in undifferentiated germ cell subtype 2 in all tumor genotypes. *lola* is unique in that it encodes twenty different protein isoforms with a variable C-terminal region and a common N-terminal Broad-complex, Tramtrack, and Bric à brac (BTB) domain (Zhang et al., 2003). Seventeen of these isoforms contain at least one zinc finger domain in the C-terminal region. These variable zinc finger domains could allow each isoform to have variable functions in different tissues (Goeke et al., 2003). Lola-B, K, L, N and P isoforms have been shown to have cell-type specific roles during spermatogenesis in the testis (Davies et al., 2013). Lola-K and L have been shown to be required for axon guidance in the central nervous system (Goeke et al., 2003). It has also been suggested that Lola-K has role in nuclear organization and chromatin condensation during programmed cell death in the ovary (Paige Bass et al., 2007). Certain isoforms of Lola could have a role in regulating GSC maintenance and differentiation as a common target of ecdysone signaling and BMP signaling. We are currently working to identify isoform-specific expression in the germ cells using GFP-tagged Lola- B, C, D, E, G, I, J, and R.

The highest protein levels of Tkv, E63E, Lola, Baf, Syb, and Wdr33 are found posteriorly in the germarium, where the germ cells undergo the mitotic to meiotic transition. Over-expressing of *EcR.B1* and Tkv^{ACT} may enrich for a population of cells that normally expresses these genes. The location of these cells within the germarium could indicate that these genes are receiving an activating spatial signal, despite remaining undifferentiated. This could provide evidence to the hypothesis that the levels of BMP signaling and ecdysone signaling are important for regulating spatial and temporal cues.

We discovered an upregulation of many genes that regulate chromatin remodeling, including *E93F*, *nej*, *HmgD*, and *baf*, indicating that chromatin remodeling is actively occurring in certain subtypes. Using scVelo and pseudotime analysis, we identified subtypes 0 and 1 as potential GSC populations. Pang et al. previously showed that GSCs undergo two different chromatin states in quick succession (2023). The GSCs start in an open chromatin state and then quickly switch to heterochromatin to begin differentiation. This could suggest that both subtypes 0 and 1 are true GSCs, but at different chromatin states. This could indicate that the over-expression of *EcR.B1* and Tkv^{ACT} has not completely blocked differentiation of the GSCs but severely slowed it down to the point that we can observe the transcriptomic differences between them.

In conclusion, our data suggests that ecdysone signaling and BMP signaling converge on a common point of regulation via common transcriptional targets to coordinate GSC fate. Identification of these target genes is pertinent to understanding the mechanism of interaction. *Mov10* and *lola*, along with many others, were identified from the scRNAseq data as potential common targets of ecdysone signaling and BMP signaling that we plan to investigate in the future.

MATERIALS AND METHODS

Drosophila strains and husbandry

Flies were kept at 22-25°C on a diet of cornmeal/molasses/yeast/agar. Progeny were collected within 24 hours after eclosion, fed with additional wet yeast paste, and dissected 5-6 days after eclosion.

For temperature sensitive mutants: Dpp mutant experiments were set at room temperature (22°C), the progeny were collected within 24 hours of eclosion, and transferred to the 25°C incubator to be fed with wet yeast paste for 5 days before dissection.

Ovary dissection and immunostaining

For Standard Protocol: Ovaries were dissected in Grace's Insect Medium and fixed in cold 5.3% paraformaldehyde for 13 minutes on a nutator at room temperature. They were rinsed with 0.1% phosphate-buffered saline with Triton X-100 (PBST), washed for 10 minutes three times, and permeabilized in 0.5% PBST for 20 minutes. Samples were blocked with a 5% BSA, 5% normal goat serum, and 0.1% PBST solution for 1-3 hours at room temperature. Samples were incubated with primary antibodies in block at 4ºC overnight. After four, 30-minute washes with 0.1% PBST, the samples were incubated with secondary antibodies in block at 4ºC overnight in the dark. After four, 30-minute washes with 0.1% PBST, the samples were incubated with DAPI (1:1000 in 0.1% PBS-Triton) in the dark at room temperature for fifteen minutes. After three 10-minute washes, the samples were stored in mounting media (90% glycerol and 20% n-propyl gallate) at 4ºC.

For Grieder Protocol: Ovaries were fixed with room temperature 5.8% paraformaldehyde in Grace's Insect Medium for 10 minutes at room temperature. The rinse and washes were

performed per the standard protocol and the samples were blocked with room temperature 5% normal goat serum in 0.1% PBST for 30 minutes at room temperature. (Grieder et al., 2000). This protocol was used for Tkv2 and Vasa antibodies.

Microscopy

Zeiss LSM 700 confocal microscope 63X plan apo objective with Zen Black was used to image mounted ovaries in 1-micron optical sections.

Phenotypic Analysis

GSCs were identified by the location of their fusome (marked with Hts) adjacent to the cap cells (marked with LamC). The presence of germ cell tumors was determined by the presence of many germ cells with round fusomes and absence of germ cell cysts with branched fusomes.

Statistical Analysis

Student's t-test (two-tailed, equal variance) was performed in Excel.

Library preparation and sequencing:

Single cells prepared were appraised using the Countess 3 FL (ThermoFisher) for quantity and quality check, and subject to library construction using the Chromium Single Cell 3' Gene Expression kit (10x Genomics), according to the manufacturer's protocol. Sequencing of the pooled libraries were performed on the NextSeq 2000 platform (Illumina).

Bioinformatics analysis:

Raw sequencing reads in FASTQ files were obtained using cellranger (v7.2.0) mkfastq. The alignment to *Drosophila melanogaster* reference genome BDGF6.46 (Ensembl) and the followed quantification was conducted using cellranger count. Control scRNAseq raw sequencing data

were downloaded from NCBI GEO database (GSE162192). Subsequently, Seurat (v5.1.0) package in RStudio (Build 369) with R (v4.4.1) were used for data analysis. Briefly, single cells were firstly filtered with nFeature_RNA (more than 1000 but less than 6000). Doublets or multiplets were identified by DoubletFinder (v2.0.4), mainly in the GC of control samples (4cc, 8cc, and 16cc) and were thus not eliminated. Batch effects were corrected using Harmony (v1.2.0). Gene expression data were then aggregated, normalized and variance stabilized with sctransform regularized negative binominal regression (SCTransform in Seurat), regressing out Dimensionality reduction was performed by principal component analysis (PCA) and uniform manifold approximation and projection (UMAP) embedding (dims = 1:30) with RunPCA and RunUMAP, respectively. Cell clusters were subsequently identified with FindNeighbors (dims = 1:30) and FindClusters (resolution = 0.2 for initial clustering and 0.5 for refined clustering); and annotated based on expression of cell type-specific gene biomarkers. Differentially expressed biomarkers among cell types were assessed with FindAllMarkers or FindMarkers for genes detected in at least 10% of cells using a log(foldchange) threshold at 0.25. Bonferroni-adjusted p-values were used to determine significance at a false discovery rate (FDR) < 0.05.

***Drosophila* Stocks**	**Source**	**Identifier**
UASp-LacZ	D. Drummond-Barbosa	
nos-Gal4::VP16	D. Drummond-Barbosa (Rørth, 1998b; Van Doren et al., 1998)	BDSC #4937 or 7253
UASz-EcR.B1$^{\Delta C655}$/CyO	Jung & Ables, 2024	
UASz-mCherry::EcR.B1$^{\Delta C655}$/CyO	Jung & Ables, 2024	
UASz-mCherry::EcR.B1$^{\Delta C655}$/TM6	Jung & Ables, 2024	
UASp-tkv^{Q199D}/TM6	(Bolivar et al., 2006)	
UASp-EcR RNAi 6-4	Jung & Ables, 2024	
y^{1} v^{1}; P{TRiP.HMJ22155}attP40 (bam RNAi)	Bloomington Drosophila Stock Center (BDSC)	BDSC #58178
ry^{506} e^{1} bam$^{\Delta 86}$/TM3, ry^{RK} Sb^{1} Ser^{1}	BDSC	BDSC #5427
y^{1} v^{1}; P{TRiP.HMS02185}attP40	BDSC	BDSC #40937
y^{1} sc* v^{1} sev^{21}; P{TRiP.GL01338}attP2	BDSC	BDSC #41904

y^1 sc^* v^1 sev^{21}; P{TRiP.GL00035}attP2	BDSC	BDSC #35166
y^1 sc^* v^1 sev^{21}; P{TRiP.HMS04501}attP40	BDSC	BDSC #57303
y^1 sc^* v^1 sev^{21}; P{TRiP.GLV21018}attP2	BDSC	BDSC #35653
y^1 v^1; P{TRiP.HMC04135}attP2	BDSC	BDSC #55865
y^1 v^1; P{TRiP.HMJ02118}attP40/CyO	BDSC	BDSC #42546
y^1 sc^* v^1 sev^{21}; P{TRiP.HMS04520}attP40	BDSC	BDSC #57319
y^1 sc^* v^1 sev^{21}; P{TRiP.GL00069}attP2	BDSC	BDSC #35195
y^1 sc^* v^1 sev^{21}; P{TRiP.GLV21066}attP2	BDSC	BDSC #35701
y^1 sc^* v^1 sev^{21}; P{TRiP.HMS01944}attP40	BDSC	BDSC #39025
y^1 w^*; Mi{PT-GFSTF.1}Eip63EMI00413-GFSTF.1/TM6C, Sb^1 Tb^1	BDSC	BDSC #59763
y^1 sc^* v^1 sev^{21}; P{TRiP.GL00147}attP2	BDSC	BDSC #35576
y^1 sc^* v^1 sev^{21}; P{TRiP.HMS00569}attP2	BDSC	BDSC #34075
w; dMov10[KI, smGFP-HA]/SM; Pr.e/TM3 Ser	H. Nakato (Takemura et al., 2021)	
y^1 w^*; Mi{MIC}Eip93F^{MI05200}/TM3, Sb^1 Ser^1	BDSC	BDSC #43675
y^1 w^*; P{E(bx)-GFP.FPTB}attP40	BDSC	BDSC #68180
y^1 w^*; PBac{crol-GFP.FPTB}VK00031	BDSC	BDSC #93087
w^{1118}; PBac{HmgD-GFP.FPTB}VK00033	BDSC	BDSC #55827
y^1 w^*; Df(2L)CRIMIC-CR70536 TI{KozakGAL4}baf$^{CR70536\text{-}KO\text{-}kG4}$/SM6a	BDSC	BDSC #97351
y^1 w^*; TI{CRIMIC.TG4.2}Syb$^{CR01649\text{-}TG4.2}$ Csgalnact$^{CR01649\text{-}TG4.2\text{-}X}$/SM6a	BDSC	BDSC #86480
y^1 w^*; TI{CRIMIC.TG4.2}Wdr33$^{CR02726\text{-}TG4.2}$/TM3, Sb^1 Ser^1	BDSC	BDSC #97582
Chemicals and Reagents	**Source**	**Identifier**
Grace's insect medium	Caisson Labs	
Phosphate buffered saline (pH 7.4)	Fisher Scientific	
Triton X-100	Fisher Scientific	BP151-500
5% bovine serum albumin	Sigma Aldrich	
5% normal goat serum	MP Biomedicals	
16% Paraformaldehyde	Fisher Scientific	18505
Antibodies and Stains		
Mouse anti-Hts (1:10)	Developmental Studies Hybridoma Bank (DSHB)	1B1; RRID: AB_528070
Mouse anti-LamC (1:100)	DSHB	LC28.26; RRID: AB_528339
Chicken anti-GFP (1:2000)	Abcam	#AB13970
Rabbit anti-Vasa (1:1000)	P. Lasko (Lasko & Ashburner et a., 1990)	

Rabbit anti-Vasa (1:1000)	Boster Biological	DZ41154
Chicken anti-β gal	Abcam	AB9361
Rabbit anti-tkv2 (1:250)	M. O'Connor (Peterson et al., 2022)	
Rabbit anti-dsRed (1:500)	Takara	632496
Mouse anti-lola (1:20)	DSHB	7F1-1D5 RRID: AB_2721954
Goat anti-mouse AlexaFluor 488, 568, 633 (1:200)	Life Technologies	
Goat anti-chicken AlexaFluor 488 (1:200)	Life Technologies	
Goat anti-rabbit AlexaFluor 488, 568 (1:200)	Life Technologies	
Goat anti-rat Alexa Fluor 488 (1:200)	Life Technologies	
4'-6-diamidino-2-phenylindole (DAPI) (0.5 μg/mL)	Sigma	
Software		
Excel	Microsoft	
Zen Blue	Zeiss	
Zen Black	Zeiss	
Prism 10	Graph Pad	
Photoshop	Adobe	
Illustrator	Adobe	
Seurat	RStudio	
SCENIC	RStudio	
Monocle3	RStudio	
scVelo	RStudio	
Primers	**Sequence (5'-3')**	**Identifier**
UASp-Tkv^{ACT} Forward	GTCATCAAGCTTAGGCCTCCAA	ETA 544
UASp-Tkv^{ACT} Reverse	TCCCGGTCGTCTCATCGTAA	ETA 545
pVALIUM 22 Forward	GGT GAT AGA GCC TGA ACC AG	ETA 019
pVALIUM 22 Reverse	TAA TCG TGT GTG ATG CCT ACC	ETA 020
UASz-EcR.B1 C Term Forward	GCTGTACAAGATGAAGCGGCG CTGGTCG	ETA 442
UASz-EcR.B1 C Term Reverse	AGTGGTACCCTCGAGGGATCCT AGATGGCATGAACGTCCCAG	ETA 443

REFERENCES

Bender, M., Imam, F. B., Talbot, W. S., Ganetzky, B., & Hogness, D. S. (1997). Drosophila Ecdysone Receptor Mutations Reveal Functional Differences among Receptor Isoforms. *Cell*, *91*(6), 777–788. https://doi.org/10.1016/S0092-8674(00)80466-3

Bergen, V., Lange, M., Peidli, S., Wolf, F. A., & Theis, F. J. (2020). Generalizing RNA velocity to transient cell states through dynamical modeling. *Nature Biotechnology*, *38*(12), 1408–1414. https://doi.org/10.1038/s41587-020-0591-3

Bhattacharya, S., Stewart, B. A., Niemeyer, B. A., Burgess, R. W., McCabe, B. D., Lin, P., Boulianne, G., O'Kane, C. J., & Schwarz, T. L. (2002). Members of the synaptobrevin/vesicle-associated membrane protein (VAMP) family in Drosophila are functionally interchangeable in vivo for neurotransmitter release and cell viability. *Proceedings of the National Academy of Sciences*, *99*(21), 13867–13872. https://doi.org/10.1073/pnas.202335999

Bolívar, J., Pearson, J., López-Onieva, L., & González-Reyes, A. (2006). Genetic dissection of a stem cell niche: The case of the Drosophila ovary. *Developmental Dynamics*, *235*(11), 2969–2979. https://doi.org/10.1002/dvdy.20967

Buszczak, M., Freeman, M. R., Carlson, J. R., Bender, M., Cooley, L., & Segraves, W. A. (1999). Ecdysone response genes govern egg chamber development during mid-oogenesis in *Drosophila*. *Development*, *126*(20), 4581–4589. https://doi.org/10.1242/dev.126.20.4581

Buszczak, M., Paterno, S., Lighthouse, D., Bachman, J., Planck, J., Owen, S., Skora, A. D., Nystul, T. G., Ohlstein, B., Allen, A., Wilhelm, J. E., Murphy, T. D., Levis, R. W., Matunis, E., Srivali, N., Hoskins, R. A., & Spradling, A. C. (2007). The Carnegie Protein Trap Library: A Versatile Tool for Drosophila Developmental Studies. *Genetics*, *175*(3), 1505–1531. https://doi.org/10.1534/genetics.106.065961

Cao, J., Spielmann, M., Qiu, X., Huang, X., Ibrahim, D. M., Hill, A. J., Zhang, F., Mundlos, S., Christiansen, L., Steemers, F. J., Trapnell, C., & Shendure, J. (2019). The single-cell transcriptional landscape of mammalian organogenesis. *Nature*, *566*(7745), 496–502. https://doi.org/10.1038/s41586-019-0969-x

Carney, G. E., & Bender, M. (2000). The Drosophila ecdysone receptor (EcR) gene is required maternally for normal oogenesis. *Genetics*, *154*(3), 1203–1211.

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

Cherbas, L., Hu, X., Zhimulev, I., Belyaeva, E., & Cherbas, P. (2003). EcR isoforms in Drosophila: Testing tissue-specific requirements by targeted blockade and rescue. *Development (Cambridge, England)*, *130*, 271–284. https://doi.org/10.1242/dev.00205

Davies, E. L., Lim, J. G. Y., Joo, W. J., Tam, C. H., & Fuller, M. T. (2013). The transcriptional regulator lola is required for stem cell maintenance and germ cell differentiation in the Drosophila testis. *Developmental Biology*, *373*(2), 310–321. https://doi.org/10.1016/j.ydbio.2012.11.004

Davis, M. B., Carney, G. E., Robertson, A. E., & Bender, M. (2005). Phenotypic analysis of EcR-A mutants suggests that EcR isoforms have unique functions during Drosophila development. *Developmental Biology*, *282*(2), 385–396. https://doi.org/10.1016/j.ydbio.2005.03.019

DeLuca, S. Z., & Spradling, A. C. (2018). Efficient Expression of Genes in the Drosophila Germline Using a UAS Promoter Free of Interference by Hsp70 piRNAs. *Genetics*, *209*(2), 381–387. https://doi.org/10.1534/genetics.118.300874

Denton, D., Xu, T., Dayan, S., Nicolson, S., & Kumar, S. (2019). Dpp regulates autophagy-dependent midgut removal and signals to block ecdysone production. *Cell Death & Differentiation*, *26*(4), Article 4. https://doi.org/10.1038/s41418-018-0154-z

Drummond-Barbosa, D. (2008). Stem Cells, Their Niches and the Systemic Environment: An Aging Network. *Genetics*, *180*(4), 1787–1797. https://doi.org/10.1534/genetics.108.098244

Duan, T., Kitzman, S. C., & Geyer, P. K. (2020). Survival of Drosophila germline stem cells requires the chromatin-binding protein Barrier-to-autointegration factor. *Development*, *147*(9), dev186171. https://doi.org/10.1242/dev.186171

Duffy, J. B. (2002). GAL4 system in drosophila: A fly geneticist's swiss army knife. *Genesis*, *34*(1–2), 1–15. https://doi.org/10.1002/gene.10150

Finger, D. S., Holt, V. V., & Ables, E. T. (2019). *Ecdysone signaling promotes expression of multifunctional RNA binding proteins essential for ovarian germline stem cell self-renewal in Drosophila* (p. 321109). bioRxiv. https://doi.org/10.1101/321109

Finger, D. S., Whitehead, K. M., Phipps, D. N., & Ables, E. T. (2021). Chapter Eleven—Nuclear receptors linking physiology and germline stem cells in Drosophila. In G. Litwack (Ed.), *Vitamins and Hormones* (Vol. 116, pp. 327–362). Academic Press. https://doi.org/10.1016/bs.vh.2020.12.008

Gauhar, Z., Sun, L. V., Hua, S., Mason, C. E., Fuchs, F., Li, T.-R., Boutros, M., & White, K. P. (2009). Genomic mapping of binding regions for the Ecdysone receptor protein complex. *Genome Research*, *19*(6), 1006–1013. https://doi.org/10.1101/gr.081349.108

Goeke, S., Greene, E. A., Grant, P. K., Gates, M. A., Crowner, D., Aigaki, T., & Giniger, E. (2003). Alternative splicing of lola generates 19 transcription factors controlling axon guidance in Drosophila. *Nature Neuroscience*, *6*(9), 917–924. https://doi.org/10.1038/nn1105

Gramates, L. S., Agapite, J., Attrill, H., Calvi, B. R., Crosby, M. A., dos Santos, G., Goodman, J. L., Goutte-Gattat, D., Jenkins, V. K., Kaufman, T., Larkin, A., Matthews, B. B., Millburn, G., Strelets, V. B., & the FlyBase Consortium. (2022). FlyBase: A guided tour of highlighted features. *Genetics*, *220*(4), iyac035. https://doi.org/10.1093/genetics/iyac035

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

Haerry, T. E., Khalsa, O., O'Connor, M. B., & Wharton, K. A. (1998). Synergistic signaling by two BMP ligands through the SAX and TKV receptors controls wing growth and patterning in Drosophila. *Development*, *125*(20), 3977–3987. https://doi.org/10.1242/dev.125.20.3977

Hales, K. G., Korey, C. A., Larracuente, A. M., & Roberts, D. M. (2015). Genetics on the Fly: A Primer on the Drosophila Model System. *Genetics*, *201*(3), 815–842. https://doi.org/10.1534/genetics.115.183392

Horiuchi, T., Giniger, E., & Aigaki, T. (2003). Alternative trans-splicing of constant and variable exons of a Drosophila axon guidance gene, lola. *Genes & Development*, *17*(20), 2496–2501. https://doi.org/10.1101/gad.1137303

Housden, B. E., & Perrimon, N. (2014). Spatial and temporal organization of signaling pathways. *Trends in Biochemical Sciences*, *39*(10), 457–464. https://doi.org/10.1016/j.tibs.2014.07.008

Hu, Y., Rodiger, J., Liu, Y., Gao, C., Liu, Y., Qadiri, M., Veal, A., Bulyk, M. L., & Perrimon, N. (2025). *TF2TG: An online resource mining the potential gene targets of transcription factors in Drosophila* (p. 2025.02.13.638157). bioRxiv. https://doi.org/10.1101/2025.02.13.638157

Hudson, A. M., & Cooley, L. (2014). Methods for studying oogenesis. *Methods*, *68*(1), 207–217. https://doi.org/10.1016/j.ymeth.2014.01.005

Jung, L., & Ables, E. T. (2024). *Identification of a novel autonomous role for Ecdysone Receptor during Drosophila ovarian germ cell differentiation* [Thesis]. East Carolina University.

King-Jones, K., & Thummel, C. S. (2005). Nuclear receptors—A perspective from Drosophila. *Nature Reviews Genetics*, *6*(4), Article 4. https://doi.org/10.1038/nrg1581

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

Koelle, M. R., Talbot, W. S., Segraves, W. A., Bender, M. T., Cherbas, P., & Hogness, D. S. (1991). The drosophila EcR gene encodes an ecdysone receptor, a new member of the steroid receptor superfamily. *Cell*, *67*(1), 59–77. https://doi.org/10.1016/0092-8674(91)90572-G

König, A., Yatsenko, A. S., Weiss, M., & Shcherbata, H. R. (2011). Ecdysteroids affect Drosophila ovarian stem cell niche formation and early germline differentiation. *The EMBO Journal*, *30*(8), 1549–1562. https://doi.org/10.1038/emboj.2011.73

Li, H., Janssens, J., De Waegeneer, M., Kolluru, S. S., Davie, K., Gardeux, V., Saelens, W., David, F., Brbić, M., Spanier, K., Leskovec, J., McLaughlin, C. N., Xie, Q., Jones, R. C., Brueckner, K., Shim, J., Tattikota, S. G., Schnorrer, F., Rust, K., … Aerts, S. (2022). Fly Cell Atlas: A single-nucleus transcriptomic atlas of the adult fruit fly. *Science (New York, N.Y.)*, *375*(6584), eabk2432. https://doi.org/10.1126/science.abk2432

Li, T.-R., & White, K. P. (2003). Tissue-Specific Gene Expression and Ecdysone-Regulated Genomic Networks in Drosophila. *Developmental Cell*, *5*(1), 59–72. https://doi.org/10.1016/S1534-5807(03)00192-8

Liu, S., Baeg, G. H., Yang, Y., Goh, F. G., Bao, H., Wagner, E. J., Yang, X., & Cai, Y. (2023). The Integrator complex desensitizes cellular response to TGF-β/BMP signaling. *Cell Reports*, *42*(1), 112007. https://doi.org/10.1016/j.celrep.2023.112007

Ludlam, W. H., Taylor ,Matthew H., Tanner ,Kirk G., Denu ,John M., Goodman ,Richard H., & and Smolik, S. M. (2002). The Acetyltransferase Activity of CBP Is Required for wingless Activation and H4 Acetylation in Drosophila melanogaster. *Molecular and*

Cellular Biology, *22*(11), 3832–3841. https://doi.org/10.1128/MCB.22.11.3832-3841.2002

Maines, J. Z., Park, J. K., Williams, M., & McKearin, D. M. (2007). Stonewalling Drosophila stem cell differentiation by epigenetic controls. *Development*, *134*(8), 1471–1479. https://doi.org/10.1242/dev.02810

McDonald, S. I., Beachum, A. N., Hinnant, T. D., Blake, A. J., Bynum, T., Hickman, E. P., Barnes, J., Churchill, K. L., Roberts, T. S., Zangwill, D. E., & Ables, E. T. (2019). Novel cis-regulatory regions in ecdysone responsive genes are sufficient to promote gene expression in Drosophila ovarian cells. *Gene Expression Patterns*, *34*, 119074. https://doi.org/10.1016/j.gep.2019.119074

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

Meng, F. W., & Biteau, B. (2015). A Sox Transcription Factor Is a Critical Regulator of Adult Stem Cell Proliferation in the *Drosophila* Intestine. *Cell Reports*, *13*(5), 906–914. https://doi.org/10.1016/j.celrep.2015.09.061

Mitchell, N., Cranna, N., Richardson, H., & Quinn, L. (2008). The Ecdysone-inducible zinc-finger transcription factor Crol regulates Wg transcription and cell cycle progression in Drosophila. *Development*, *135*(16), 2707–2716. https://doi.org/10.1242/dev.021766

Morris, L. X., & Spradling, A. C. (2012). Steroid Signaling within Drosophila Ovarian Epithelial Cells Sex-Specifically Modulates Early Germ Cell Development and Meiotic Entry. *PLOS ONE*, *7*(10), e46109. https://doi.org/10.1371/journal.pone.0046109

Mouillet, J.-F., Henrich, V. C., Lezzi, M., & Vögtli, M. (2001). Differential control of gene activity by isoforms A, B1 and B2 of the Drosophila ecdysone receptor. *European Journal of Biochemistry*, *268*(6), 1811–1819. https://doi.org/10.1046/j.1432-1327.2001.02051.x

Ohsako, T., Horiuchi, T., Matsuo, T., Komaya, S., & Aigaki, T. (2003). *Drosophila lola* encodes a family of BTB-transcription regulators with highly variable C-terminal domains containing zinc finger motifs. *Gene*, *311*, 59–69. https://doi.org/10.1016/S0378-1119(03)00554-7

Paige Bass, B., Cullen, K., & McCall, K. (2007). The axon guidance gene *lola* is required for programmed cell death in the *Drosophila* ovary. *Developmental Biology*, *304*(2), 771–785. https://doi.org/10.1016/j.ydbio.2007.01.029

Pang, L.-Y., DeLuca, S., Zhu, H., Urban, J. M., & Spradling, A. C. (n.d.). Chromatin and gene expression changes during female Drosophila germline stem cell development illuminate the biology of highly potent stem cells. *eLife*, *12*, RP90509. https://doi.org/10.7554/eLife.90509

Peterson, A. J., Murphy, S. J., Mundt, M. G., Shimell, M., Leof, E. B., & O'Connor, M. B. (2022). A juxtamembrane basolateral targeting motif regulates signaling through a TGF-β pathway receptor in Drosophila. *PLOS Biology*, *20*(5), e3001660. https://doi.org/10.1371/journal.pbio.3001660

Phipps, D. N., Powell, A. M., & Ables, E. T. (2023). Utilizing the FLP-Out System for Clonal RNAi Analysis in the Adult Drosophila Ovary. In M. S. Giedt & T. L. Tootle (Eds.),

Drosophila Oogenesis: Methods and Protocols (pp. 69–87). Springer US. https://doi.org/10.1007/978-1-0716-2970-3_4

Ragab, A., & Travers, A. (2003). HMG-D and histone H1 alter the local accessibility of nucleosomal DNA. *Nucleic Acids Research*, *31*(24), 7083–7089. https://doi.org/10.1093/nar/gkg923

Riddiford, L. M., Cherbas, P., & Truman, J. W. (2000). Ecdysone receptors and their biological actions. In *Vitamins & Hormones* (Vol. 60, pp. 1–73). Academic Press. https://doi.org/10.1016/S0083-6729(00)60016-X

Ridwan, S. M., Twillie, A., Poursaeid, S., Beard, E. K., Bener, M. B., Antel, M., Cowan, A. E., Matsuda, S., & Inaba, M. (2024). Diffusible fraction of niche BMP ligand safeguards stem-cell differentiation. *Nature Communications*, *15*(1), 1166. https://doi.org/10.1038/s41467-024-45408-7

Rørth, P. (1998). Gal4 in the Drosophila female germline. *Mechanisms of Development*, *78*(1), 113–118. https://doi.org/10.1016/S0925-4773(98)00157-9

Royzman, I., Hayashi-Hagihara, A., Dej, K. J., Bosco, G., Lee, J. Y., & Orr-Weaver, T. L. (2002). The E2F cell cycle regulator is required for *Drosophila* nurse cell DNA replication and apoptosis. *Mechanisms of Development*, *119*(2), 225–237. https://doi.org/10.1016/S0925-4773(02)00388-X

Rust, K., Byrnes, L. E., Yu, K. S., Park, J. S., Sneddon, J. B., Tward, A. D., & Nystul, T. G. (2020). A single-cell atlas and lineage analysis of the adult Drosophila ovary. *Nature Communications*, *11*(1), 5628. https://doi.org/10.1038/s41467-020-19361-0

Salz, H. K., Dawson, E. P., & Heaney, J. D. (2017). Germ cell tumors: Insights from the Drosophila ovary and the mouse testis. *Molecular Reproduction and Development*, *84*(3), 200–211. https://doi.org/10.1002/mrd.22779

Schauer, S., Callender, J., Henrich, V. C., & Spindler-Barth, M. (2011). The N-terminus of ecdysteroid receptor isoforms and ultraspiracle interacts with different ecdysteroid response elements in a sequence specific manner to modulate transcriptional activity. *The Journal of Steroid Biochemistry and Molecular Biology*, *124*(3), 84–92. https://doi.org/10.1016/j.jsbmb.2011.01.013

Shan, L., Wu, C., Chen, D., Hou, L., Li, X., Wang, L., Chu, X., Hou, Y., & Wang, Z. (2017). Regulators of alternative polyadenylation operate at the transition from mitosis to meiosis. *Journal of Genetics and Genomics*, *44*(2), 95–106. https://doi.org/10.1016/j.jgg.2016.12.007

Shi, J., Jin, Z., Yu, Y., Zhang, Y., Yang, F., Huang, H., Cai, T., & Xi, R. (2021). A Progressive Somatic Cell Niche Regulates Germline Cyst Differentiation in the Drosophila Ovary. *Current Biology*, *31*(4), 840-852.e5. https://doi.org/10.1016/j.cub.2020.11.053

Slaidina, M., Gupta, S., Banisch, T. U., & Lehmann, R. (2021). A single-cell atlas reveals unanticipated cell type complexity in Drosophila ovaries. *Genome Research*, *31*(10), 1938–1951. https://doi.org/10.1101/gr.274340.120

Stowers, R. S., Garza, D., Rascle, A., & Hogness, D. S. (2000). The *L63* Gene Is Necessary for the Ecdysone-Induced 63E Late Puff and Encodes CDK Proteins Required for *Drosophila* Development. *Developmental Biology*, *221*(1), 23–40. https://doi.org/10.1006/dbio.2000.9685

Swain, L., & Ables, E. T. (2025). *Ecdysone Receptor modulates EGFR signaling for timely cyst packaging in Drosophila germ cells*.

Szakmary, A., Cox, D. N., Wang, Z., & Lin, H. (2005). Regulatory Relationship among piwi, pumilio, and bag-of-marbles in Drosophila Germline Stem Cell Self-Renewal and Differentiation. *Current Biology*, *15*(2), 171–178. https://doi.org/10.1016/j.cub.2005.01.005

Takemura, M., Bowden, N., Lu, Y.-S., Nakato, E., O'Connor, M. B., & Nakato, H. (2021). *Drosophila* MOV10 regulates the termination of midgut regeneration. *Genetics*, *218*(1), iyab031. https://doi.org/10.1093/genetics/iyab031

Tseng, C.-Y., Su, Y.-H., Yang, S.-M., Lin, K.-Y., Lai, C.-M., Rastegari, E., Amartuvshin, O., Cho, Y., Cai, Y., & Hsu, H.-J. (2018). Smad-Independent BMP Signaling in Somatic Cells Limits the Size of the Germline Stem Cell Pool. *Stem Cell Reports*, *11*(3), 811–827. https://doi.org/10.1016/j.stemcr.2018.07.008

Uyehara, C. M., Leatham-Jensen, M., & McKay, D. J. (2022). Opportunistic binding of EcR to open chromatin drives tissue-specific developmental responses. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(40), e2208935119. https://doi.org/10.1073/pnas.2208935119

Uyehara, C. M., Nystrom, S. L., Niederhuber, M. J., Leatham-Jensen, M., Ma, Y., Buttitta, L. A., & McKay, D. J. (2017). Hormone-dependent control of developmental timing through regulation of chromatin accessibility. *Genes & Development*, *31*(9), 862–875. https://doi.org/10.1101/gad.298182.117

Wang, Z., & Lin, H. (2004). Nanos Maintains Germline Stem Cell Self-Renewal by Preventing Differentiation. *Science*, *303*(5666), 2016–2019. https://doi.org/10.1126/science.1093983

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

Zhang, W., Wang, Y., Long, J., Girton, J., Johansen, J., & Johansen, K. M. (2003). A Developmentally Regulated Splice Variant from the Complex*lola* Locus Encoding Multiple Different Zinc Finger Domain Proteins Interacts with the Chromosomal Kinase JIL-1*. *Journal of Biological Chemistry*, *278*(13), 11696–11704. https://doi.org/10.1074/jbc.M213269200

Zhao, T., Xiao, Y., Huang, B., Ran, M.-J., Duan, X., Wang, Y.-F., Lu, Y., & Yu, X.-Q. (2022). A dual role of lola in Drosophila ovary development: Regulating stem cell niche establishment and repressing apoptosis. *Cell Death & Disease*, *13*(9), 756. https://doi.org/10.1038/s41419-022-05195-9