# The ETS-transcription factor Pointed is sufficient to regulate the posterior fate of the follicular epithelium

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## Abstract

The Janus-kinase/Signal Transducers and Activators of Transcription (JAK/STAT) pathway regulates the anterior posterior axis of the *Drosophila* follicle cells. In the anterior, it activates the bone morphogenetic protein (BMP) signaling pathway through expression of the BMP ligand, *decapentaplegic* (*dpp*). In the posterior, JAK/STAT works with the epidermal growth factor receptor (EGFR) pathway to express the T-box transcription factor *midline* (*mid*). While MID is necessary in establishing the posterior fate of the egg chamber, we show that it is not sufficient to determine a posterior fate. The ETS-transcription factor pointed (*pnt*) is expressed in an overlapping domain to *mid* in the follicle cells. This study shows that *pnt* is upstream of *mid*, and it is sufficient to induce a posterior fate in the anterior end, which is characterized by the induction of *mid*, the prevention of the stretched cells formation, and the abrogation of border cells migration. We demonstrate that the anterior BMP signaling is abolished by PNT through *dpp* repression. However, ectopic DPP cannot rescue this repression, suggesting additional targets of PNT participate in the posterior fate determination.

# Introduction

Animal development is an intricate process that is spatiotemporally coordinated by several cell signaling pathways that control cellular proliferation, migration, and differentiation (Davidson and Erwin, 2006; Housden and Perrimon, 2014; Levine, 2010; Levine and Tjian, 2003). During development, body axes formation evolved in animals through different strategies (Genikhovich and Technau, 2017). In *Drosophila melanogaster*, axes formation occurs during oogenesis, prior to egg fertilization (Lynch and Roth, 2011; Moussian and Roth, 2005). Numerous pathways are spatiotemporally coordinated to set the body axes in flies (Deng and Bownes, 1997; Fregoso Lomas et al., 2016; Gonzalez-Reyes and St Johnston, 1998; Moussian and Roth, 2005; Neuman-Silberberg and Schupbach, 1993; Neuman-Silberberg and Schupbach, 1994; Nilson and Schupbach, 1999; Twombly et al., 1996; Xi et al., 2003). However, the targets of these pathways that regulate axes' fates are still not well understood (Fregoso Lomas et al., 2013).

The follicle cells, a layer of follicular epithelium surrounding the developing oocyte, are dynamically patterned along the anterior-posterior axis (Bastock and St Johnston, 2008; Berg, 2005; Hinton, 1969; Horne-Badovinac and Bilder, 2005; Ward and Berg, 2005; Yakoby et al., 2008a). Early activation of the Janus-kinase/Signal Transducers and Activators of Transcription (JAK/STAT) pathway by the secretion of the ligand Unpaired (UPD) from the polar cells sets a mirror symmetry of two ends and main-body fates of the follicle cells (Fig. 1A) (Gonzalez-Reyes and St Johnston, 1998; Xi et al., 2003). The posterior fate is set by the secretion of the TGF-alpha-like ligand Gurken (GRK) from around the oocyte nucleus and activation of the epidermal growth factor receptor (EGFR) pathway in the overlaying follicle cells (Gonzalez-Reyes and St Johnston, 1998; Neuman-Silberberg and Schupbach, 1993; Ray and Schupbach, 1996; Revaitis et al., 2020; Sapir et al., 1998). The anterior end is established by activating the bone morphogenetic protein (BMP) pathway by its ligand Decapentaplegic (DPP), which is induced by the JAK/STAT signaling (Deng and Bownes, 1997; Peri and Roth, 2000; Twombly et al., 1996; Xi et al., 2003; Yakoby et al., 2008c). The anterior and posterior domains are shown in Fig. 1A, B.

The sufficiency of EGFR activation to establish the border between the dorsal-anterior and the main-body follicle cells was initially computationally predicted (Zartman et al., 2011). The Nilson Lab found that the EGFR target *midline* (*mid*), the *Drosophila* homolog of Tbx20, sets this boundary (Fregoso Lomas et al., 2013). MID acts to inhibit *broad* (*br*), a transcription factor that marks the primordia of the future respiratory dorsal appendages on the mature eggshell (Cheung et al., 2013; Deng and Bownes, 1997; Fregoso Lomas et al., 2013; Fuchs et al., 2012; Pyrowolakis et al., 2017; Tzolovsky et al., 1999). Further investigation showed that the JAK/STAT pathway together with EGFR induce *midline* expression by the inhibition of the main body fate determinant *mirror* (MIRR) (Fregoso Lomas et al., 2016; Jordan et al., 2000; Xi et al., 2003). The main body follicle cells are shown in Fig. 1A, B.

The ETS-transcription factor *pointed*-P1 (*pnt*-P1) is expressed dynamically in the follicle cells; in the posterior end during early oogenesis at stage 6 (S6) (the domain is shown in Fig. 1A,B), and later at S10 in the dorsal midline (the domain is shown in Fig. 1C) (Morimoto et al., 1996; Yakoby et al., 2008a). In the dorsal midline, PNT-P1 sets the distance between the two dorsal appendages primordia (Boisclair-Lachance et al., 2009; Deng and Bownes, 1997; Morimoto et al., 1996; Zartman et al., 2009). However, the role of early posterior expression of PNT-P1 is still unknown. Here, we show a new hierarchy in the regulation of the posterior end during early oogenesis. The expression of *midline* is regulated by PNT-P1. Ectopic expression of *pnt*-P1, but not *mid*, is sufficient to repress the early anterior BMP signaling and all associated morphological changes in the anterior domain, which resembles the behavior of cells in the posterior end. Together, we conclude that PNT-P1 is necessary and sufficient to regulate the posterior fate of the follicular epithelium.

# Results

## MID is not sufficient to repress the anterior fate of the follicular epithelium

The follicle cells are extensively patterned prior to specifying different domains (Berg, 2005; Niepielko et al., 2014; Revaitis et al., 2017; Yakoby et al., 2008a). This section clarifies some of the domains discussed in this paper. Up to S7 of oogenesis, the anterior, posterior, and main body domains are set (Fig. 1A). These domains are marked by the posterior expression of MID, the anterior expression of DPP, and the main body expression of MIRR (Fregoso Lomas et al., 2013; Jordan et al., 2000; Twombly et al., 1996; Xi et

al., 2003). At S9, the follicular epithelium progressively engulfs the growing oocyte, and a subsect of anterior cells differentiate to become the stretched follicle cells, which overly the nurse cells (Fig. 1B). In addition, the anterior polar cells recruit ~6 neighboring cells, collectively known as the border cells, that together delaminate and migrate through the nurse cells posteriorly towards the oocyte (Fig. 1B). Later, at S10, the oocyte nucleus is at a dorsal anterior position, which designates the dorsal anterior domain (Fig. 1C). This domain is comprised of the late expression of BR, that marks the primordia of the future dorsal appendages, and PNT that marks the dorsal midline (Morimoto et al., 1996; Tzolovsky et al., 1999). Here, we focus on three main domains: 1) the anterior domain, which includes the border cells and the stretched follicle cells; 2) the posterior domain; and 3) the dorsal-anterior domain.

As previously reported (Fregoso Lomas et al., 2013), MID patterns the posterior domain of the follicular epithelium (Fig. 1D, E). The pattern extends more anteriorly on the dorsal side to generate the posterior border of the dorsal appendage primordia (Fig. 1E"). In agreement with its role (Fregoso Lomas et al., 2013), using the dorsal anterior driver (BR42-GAL4, Fig. 1F-F") to ectopically express *mid* was sufficient to repress BR patterning (Fig. 1G-G"). As was previously reported, in the absence of EGFR signaling both ends of the developing egg chamber maintain an anterior fate (Gonzalez-Reyes and St Johnston, 1998; Neuman-Silberberg and Schupbach, 1994; Peri and Roth, 2000; Twombly et al., 1996). Given that *mid* is a target of EGFR signaling, we aimed to determine if MID is the primary mechanism of EGFR signaling that coverts an anterior to a posterior cell fate.

As mentioned above, the anterior domain of the egg chamber acquires distinct cellular morphologies at S9, including stretched cells formation and the migration of the border cells (Duhart et al., 2017; Kolahi et al., 2009; Montell et al., 1992). We asked whether MID is sufficient to repress these anterior fate characteristics. Ectopic expression of *mid* in the anterior domain (using $GMR^{18E05}$-GAL4, Fig. 1H-H"), and in the polar cells (using Slbo-GAL4, Fig. 1J-L), had no impact on follicle cells' morphogenesis (Fig. 1I-I" and M-M"). We conclude that while MID is necessary to restrict the posterior boundary of the dorsal appendage primordia, it is not sufficient to set a posterior fate.

## PNT regulates the pattern of MID

The ETS-transcription factor *pointed* (PNT-P1) is a regulator of tissue development and is a downstream target of EGFR and JAK/STAT signaling pathways (Gabay et al., 1996; Morimoto et al., 1996; O'Neill et al., 1994; Rebay et al., 2000; Rogers et al., 2017; Wassarman et al., 1995; Xi et al., 2003). Interestingly, the posterior pattern of PNT-P1 fully overlaps the pattern of MID (Fig. Supp. 1A, B). Since PNT-P1 and MID are targets of EGFR and JAK/STAT signaling pathways (Fregoso Lomas et al., 2016; Xi et al., 2003), we aimed to determine whether PNT regulates MID expression in the follicular epithelium. Looking at 84 independent *pnt* null clones, we observed a cell-autonomous complete loss of MID in 55% (Fig. 2A-A'', Fig. Supp. 2A-A"), and a reduced level of MID in 45% of the clones (Fig. Supp. 2B-B").

As expected, ectopic expression of *pnt*-P1 in dorsal anterior domain disrupts the BR pattern and expands the MID domain anteriorly (Fig. 2B-B'' compared to Fig. 1E-E"). Noticeably, MID did not expand to the entire dorsal anterior domain in this background (Fig. 2 B'-B"). The absence of MID can be explained by the expression of MIRR in this domain, which represses *mid* (Fregoso Lomas et al., 2013). Further support for this observation is found in the dorsal midline; while PNT is naturally expressed in this domain at S10 of oogenesis, MID is still absent (Fregoso Lomas et al., 2013; Morimoto et al., 1996) (Fig. 1E', Fig. Supp. 1B). To avoid the inhibition, we used anterior GAL4 drivers that are expressed outside of the endogenous MIRR domain (Fig. 1H, J-L). Indeed, ectopic expression of *pnt*-P1 in the anterior domain, as well as in the polar cells, induced MID expression cell autonomously (Fig. 2 C-D"). Hence, PNT is sufficient to induce *mid* expression.

The *pnt* gene has two isoforms, *pnt*-P1 and *pnt*-P2 (Klambt, 1993; Scholz et al., 1993). As mentioned above, PNT-P1 represses the late *br* to set the dorsal midline (Deng and Bownes, 1997). We tested whether ectopic expression of *pnt*-P2 will impact the BR and MID patterning. Ectopic expression of *pnt*-P2 in the dorsal anterior domain did not change the BR patterning (compare Fig. Supp. 3A, B to Fig. 1D, E). In addition, ectopic expression of *pnt*-P2 in the anterior domain did not induce MID and egg chambers' development continued normally (Fig. Supp. 3 A', B'). This lack of impact on BR patterning is in agreement with the normal expression pattern of *pnt*-P2 in this domain at S10B of oogenesis (Morimoto et al., 1996). We conclude that PNT-P1 is the isoform that activates *mid*.

## Ectopic PNT-P1 represses BMP signaling

It was previously reported that the activation of EGFR in the anterior domain represses BMP signaling (Revaitis et al., 2017) (Fig. 3B, B" compared to A, A"). The expression of a constitutively activated EGFR (ca*Egfr*) in the anterior domain not only abrogated BMP signaling, it also induced MID (Fig. 3A' compared to B', and A" compared to B"). Next, we tested whether PNT-P1, as a target of EGFR, is sufficient to mediate this function. Ectopic expression of *pnt*-P1 in the anterior domain abolished BMP signaling and induced MID (Fig. 3C-C"). To discern between PNT and MID activities, we ectopically expressed MID in the same domain and observed no impact on BMP signaling (Fig. 3D-D"). These results are consistent with the previous observations where the ectopic expression of *mid* in the anterior domain had no observable effect on egg chambers' development (Fig 1I-I", M-M"), whereas ectopic expression of *pnt*-P1 in all anterior cells resulted in abolishing BMP signaling and terminating egg chambers' development at S9. We conclude, PNT-P1 is sufficient to repress BMP signaling in the anterior domain.

The anterior domain of the egg chamber is patterned by BMP signaling as evident by the defects in eggshell morphologies upon perturbations in this pathway (Chen and Schupbach, 2006; Marmion et al., 2013; Marmion and Yakoby, 2018; Peri and Roth, 2000; Twombly et al., 1996). Additionally, BMP signaling is necessary for anterior follicle cell flattening and stretching (Brigaud et al., 2015). Thus, we aimed to understand the role PNT-P1 has on the regulation of BMP signaling. Ectopic expression of *pnt*-P1 in the anterior domain terminated egg chamber development at S9, which is similar to consequences of ectopic expression of ca*Egfr* in this domain (Revaitis et al., 2017). To circumvent lethality, we used the GMR$^{43H01}$-GAL4 driver to limit the expression of PNT-P1 to a portion of the anterior follicle cells, including the border cells and a subset of posterior follicle cells (Revaitis et al., 2017) (Fig. 4 A-A", B-B").

It was previously reported that *dpp* is ectopically expressed in the posterior end of an EGFR signaling mutant (Peri and Roth, 2000). Since ectopic ca*Egfr* represses anterior BMP signaling (Revaitis et al., 2017), we wanted to understand if PNT-P1 represses BMP signaling through *dpp* repression. Using the *dpp*-βGal reporter (DPP-Z) to mark the *dpp* expression domain (Fig. 4C-C"), we ectopically expressed *pnt*-P1 in a small portion of anterior cells (Fig. 4D-D") and monitored changes in the pattern of DPP-Z. As expected, MID was induced in cells ectopically expressing *pnt*-P1 (Fig. 4D'). Interestingly, we found a cell autonomous loss of DPP-Z in these cells (Fig. 4D-D"). Since DPP is a diffusible ligand, we next asked whether surrounding unaffected cells can provide DPP to activate signaling in the cells that do not express *dpp*. In cells expressing *pnt*-P1, marked by ectopic MID (Fig. 4E), we detected BMP activation (Fig. 4E').

## Ectopic PNT represses anterior morphogenesis independently of BMP signaling

As discussed above, while BMP signaling was detected in cells expressing *pnt*-P1 (Fig. 4E'), these cells still remained in the anterior and failed to migrate as border cells (Fig. 4E"). It is possible that the amount of DPP from neighboring cells was not sufficient to activate BMP signaling to a level that induces border cells migration. Therefore, we aimed to determine whether increasing the levels of DPP in these cells would rescue their mobility. Using the Slbo-GAL4 driver, we ectopically expressed both *pnt*-P1 and *dpp*. As expected, MID was detected in the polar cells (Fig. 4F). In addition, BMP signaling was activated in these cells (Fig. 4F'). At the same time, these cells remained in the anterior domain (compare Fig. 4F" to Fig. 1J-L). Interestingly, BMP signaling is a known repressor of *mid* (Fregoso Lomas et al., 2016), however, the anterior BMP signaling could not overcome the induction of *mid* by the ectopic PNT-P1 (Fig. 4F). We conclude that PNT-P1 induction of *mid* abrogates the repression of *mid* by BMP signaling. Furthermore, ectopic expression of *dpp* cannot rescue border cells' migration in the presence of PNT-P1.

As explained above, border cells migration and stretching of cells over the nurse cells are hallmarks of the anterior follicle cells at S9 of oogenesis (Kolahi et al., 2009; Van Buskirk and Schupbach, 1999). The expression of *pnt*-P1 stopped border cells' migration and stretching of the anterior cells, even though BMP signaling was restored. The observed cell clumping in the anterior domain is mirrored in mutant backgrounds of the transcriptional inhibitor *yan*, whereas loss of *yan* resulted in an accumulation of E-cadherin (E-Cad) and revocation of border cells' migration (Schober et al., 2005). Here, we ectopically expressed *pnt*-P1 in the

future border cells, and in agreement with *yan* perturbations, observed a loss of border cell migration and the accumulation of E-cadherin (Compare Fig.4G' to H'). Since PNT-P1 and YAN compete on the same DNA binding motifs (Wei et al., 2010), we proposed that the ectopic PNT-P1 could be outcompeting YAN DNA binding, resulting in the accumulation of E-Cad.

The disruption of EGFR signaling allows for the ectopic expression of *dpp* in the posterior end (Peri and Roth, 2000). We tested whether loss of PNT is sufficient to allow *dpp* expression in the posterior domain. We could not detect DPP-Z expression in large posterior clones null for *pnt* (Fig. Supp. 4A-A'"). In addition, in these clones, we could not detect activation of BMP signaling (Fig. Supp. 4B-B"). We suggest that in this background, EGFR is still activated, therefore factors other than PNT-P1 are likely being induced by EGFR signaling to repress posterior *dpp* expression. We conclude, PNT-P1 is sufficient to repress the anterior *dpp* but it is not necessary to repress posterior *dpp*. Future studies will focus on finding other targets of EGFR that also control posterior *dpp* expression.

## Discussion

The anterior-posterior patterning of the follicular epithelium is an intricate process that sets the initial boundaries of the egg chamber. Here, we investigated the role of PNT-P1 as a regulator of the posterior end, and demonstrated that this ETS-transcription factor is an upstream regulator of MID. Interestingly, in posterior clones null for *pnt* MID is either completely or partially lost. These results are consistent with the reported regulation of MID and PNT. Specifically, in a clonal analysis of cells expressing an amorphic version of either Stat92E (STAT) or Hopscotch (HOP), which are both essential for JAK/STAT signaling, MID was completely lost or only reduced (Fregoso Lomas et al., 2016). In addition, similar perturbations in JAK/STAT signaling led to the complete loss of PNT (Xi et al., 2003). Taken together, the complete loss of PNT and the loss/reduction of MID in JAK/STAT perturbations further support that PNT is an upstream regulator of MID, as shown is our experiments. We reason that *mid* is expressed very early in oogenesis, hence the complete or partial loss of MID in our experiments could reflect a degradation process of MID that was induced earlier to the formation of the clone. Of note, our results are in agreement with the regulation of *mid* by PNT in the developing *Drosophila* cardiac cells (Schwarz et al., 2018).

The BMP signaling pathway has multiple components necessary for signaling, including ligands, receptors, and intracellular components (Chen and Schupbach, 2006; Dobens and Raftery, 1998; Dobens and Raftery, 2000; Marmion et al., 2013; Marmion and Yakoby, 2018; Yakoby et al., 2008b; Yakoby et al., 2008c). It was previously reported that *dpp* is ectopically expressed in the posterior end of an EGFR signaling mutant (Peri and Roth, 2000). Our results demonstrate that ectopic expression of *pnt* in the anterior domain is sufficient to repress *dpp* expression cell autonomously. In addition, restricting the number of anterior cells expressing *pnt* can rescue the activation of BMP signaling by the emanating DPP from surrounding unaffected cells. These results indicated that all other BMP pathway components remain intact in the presence of PNT-P1. Our findings further support our previous prediction that the repression of anterior BMP signaling upon ectopic EGFR activation is due to *dpp* repression (Revaitis et al., 2017).

In the anterior domain, the stretched cells grow to engulf the nurse cells for them to go through apoptosis after releasing their contents into the developing oocyte (Timmons et al., 2016). This process is terminated by the ectopic expression of *pnt*-P1 in the anterior cells. This suggests that PNT-P1 can block the communication between the anterior follicle cells (stretched cells) and the germ-line nurse cells. Since the nurse cells may participate in the anterior fate determination, such as stretch cell formation, the absence of nurse cells in the posterior end may prevent a 'true' anterior from forming in a *pnt* null background (Fig. Supp.4). Further support for the suggested role of PNT-P1 in cell movement is found by the natural expression of *pnt*-P1 in the dorsal midline (Morimoto et al., 1996). These cells do not migrate, while their neighboring cells, the dorsolateral appendage primordia, migrate anteriorly to form the tube-like dorsal appendages (Ward and Berg, 2005). Interestingly in a background of cells expressing MAE, an inhibitor of PNT, the dorsal midline cells move and become part of a single wide dorsal appendage (Yamada et al., 2003). Taken together, we suggest that the expression of PNT anchors groups of cells in tissues.

## Materials and Methods

### Flies and reagents

All flies were raised on standard cornmeal agar and kept at room temperature, unless specified in heat shock trials. The fly strains used in this study were obtained from the following sources: Wild-type *D. melanogaster* (25211), UAS-*pnt*-P1 (869), GMR$^{43H01}$-GAL4 (47931), PNT-GFP (42680), and UAS-*pnt*-P2 (399) were obtained from the Bloomington Drosophila Stock Center. BR-42;*tub*Gal80ts, FRT82B*pnt*Δ88, and *e22cflp*;FRT82B*ubi*GFP were a gift from S. Shvartsman (Princeton University, NJ, USA). UAS-*mid* was a gift from L. Nilson (McGill University, Montreal, Canada). Slbo-Gal4 was a gift from D. Harrison (University of Kentucky, KY, USA). UAS-ca*Egfr* was a gift from T. Schüpbach (Princeton University, NJ, USA). UAS-GFP*nls* was a gift from J. Posakony (University of California San Diego, CA, USA) and UAS-*dpp* a gift from S. Newfeld (Arizona State University, AZ, USA). The p*tub*Gal80ts;GMR$^{18E05}$-GAL4 and the DPP-Z reporter were used here (Revaitis et al., 2017).

### Immunostaining

Immunohistochemistry analysis was done on 2-7 days old flies raised on active yeast for 24 hours at room temperature (23°C) prior to dissection. Ovaries were dissected in 1mL Schneider's Media and fixed in a 4% paraformaldehyde/heptane/0.2% Triton X-100 in PBS (PBST) solution for 20 minutes. Samples were rinsed three times, 5 minutes each time, in 0.2% PBST solution, then permeabilized in 1% PBST solution for 1 hour. Samples were rinsed once in 0.2% PBST then blocked in 0.2% PBST with 1% Bovine Serum Albumin (BSA) solution for 1 hour. Samples were incubated overnight at 4°C in primary antibody cocktail with 0.2% PBST and 1% BSA. After incubation, samples were washed 3 times for 20 minutes each in 0.2% PBST, then secondary antibody cocktail was added with 0.2% PBST and 1% BSA and incubated for 1 hour protected from light at room temperature. Samples were then washed 3 times for 20 minutes each time in 0.2% PBST and mounted in Flouromont-G mounting media. Primary antibody concentrations used were mouse anti-Broad (1:250; DSHB), sheep anti-GFP (1:1000, BioRad), rabbit anti-phosphorylated-Smad (1:3600; gift from E. Laufer, (Yakoby et al., 2008c), rabbit anti-βgalactosidase (1:1000; Invitrogen), guinea pig anti-MID (1:1000; gift from L. Nilson), mouse anti-Fasciclin (1:250; DSHB), rabbit anti-yan (1:250, a gift from S. Shvartsman), and rat anti-DCAD2 (1:50; DSHB). Secondary antibodies used were Alexa Fluor 488 donkey

anti-mouse, Alexa Fluor 488 donkey anti-sheep, Alexa Fluor 568 donkey anti-rabbit, Alexa Fluor 568 donkey anti-mouse, Alexa Fluor 633 goat anti-guinea pig, and Alexa Flour 568 goat anti-rat (1:1250; Invitrogen). DAPI was used for nuclear staining (84ng/mL; Thermo Fisher). Samples were imaged on Leica SP8 Confocal microscope with 20X objective. Images were processed using FIJI (Fiji is Just ImageJ) software (Schindelin et al., 2012).

**Heat Shock Induction of Transgenic Lines**

Fly lines containing a temperature sensitive GAL80 repressive element were raised on active yeast for 3 days at 28°C prior to dissection to alleviate Gal80 and drive ectopic gene expression.

**Generation of Negatively Marked Clones**

The FLP/FRT recombinant technique (Xu and Rubin, 1993) was used to generate loss-of-function null clones marked by the absence of GFP (*ubi*-GFP). The *e22c*flp;FRT82B-*ubi*GFP line was crossed to the FRT82B $pnt^{\Delta 88}$ line (*e22c*>flp; FRT82B $pnt^{\Delta 88}$/FRT82B *ubi*-GFP) to generate mutant clones null for both *pnt*-P1 and *pnt*-P2 isoforms marked with the absence of GFP.

## Quantification and Statistical Analysis

All images were obtained with equal confocal microscopy wavelength settings among images utilizing the same channel. In *pnt* null clonal experiments, boundaries of loss of PNT were drawn according to loss of observable expression of GFP. FIJI software was used for all images for correct orientation and leveling of brightness and contrast. In all images, N-value represents number of egg chambers observed with a similar phenotypic profile.

## Author Contributions

Experimental approach was designed by C.A.S and N.Y, and conducted by C.A.S. GMR fly lines were screened by N.T.R., and R.C. screened MID perturbations. Manuscript was written by C.A.S and N.Y, and edited by N.Y. All authors read and approved of final submission.

## Declaration of Interests

The authors declare no competing interests

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# Figures

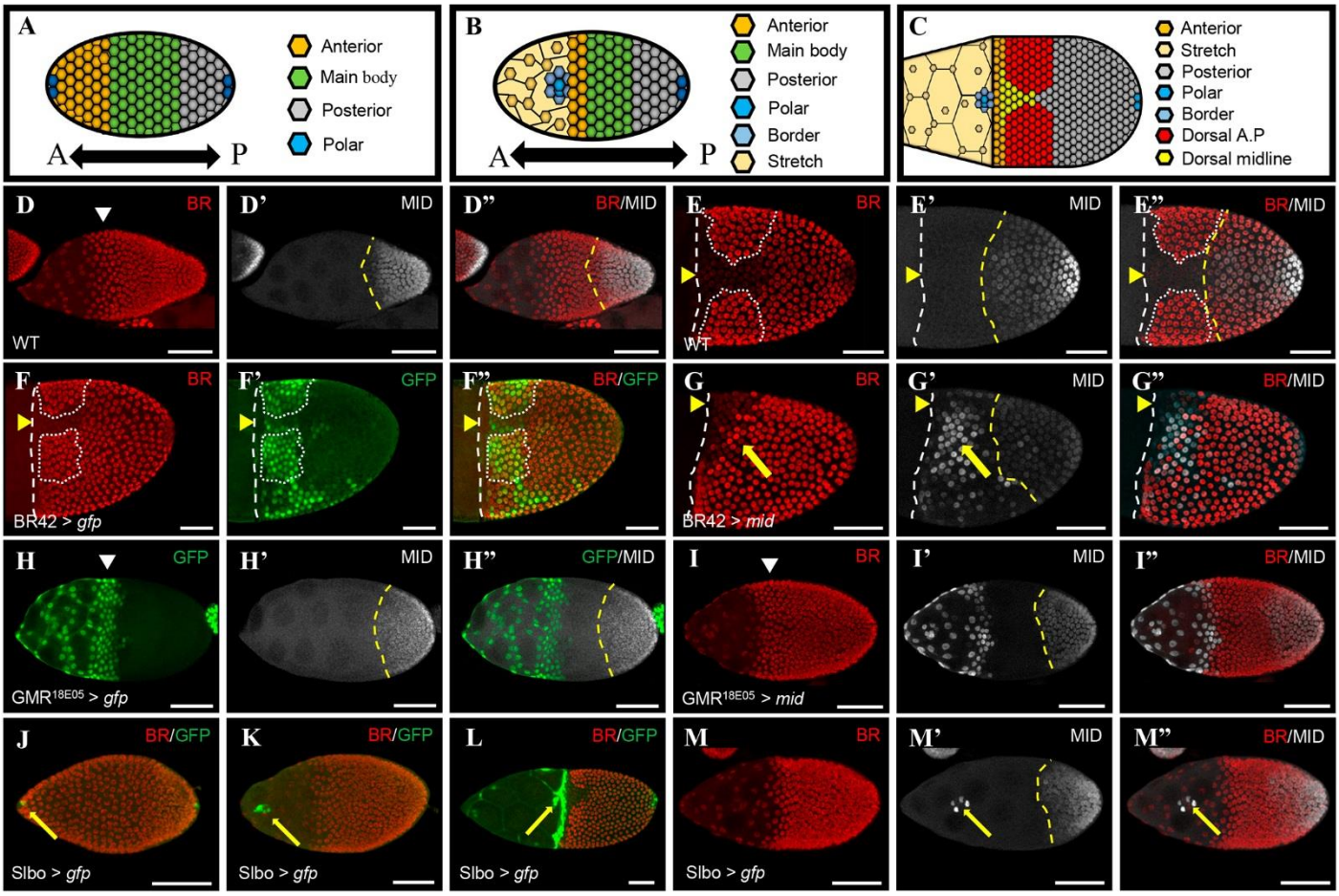



**Figure 1: The *midline* gene is necessary but not sufficient for posterior fate determination.** Cartoon representation of egg chambers at stage 8 (S8) (A), S9 (B), and S10 (C). The different domains are color coded, and described annotation in the legends panels are in bold (**Anterior** domain, **Main body** follicle cells, **Posterior** domain, **Polar** cells, **Border** cells, **Stretch** cells, **Dorsal A.P**- appendage primordia, **Dorsal midline**). (D-E") Wild-type expression of *midline* (MID, white) in early stage 9 (D-D") (n=7), and later at stage 10B oogenesis (E-E") (n=10). The pattern of Broad (BR) is used as a spatial reference for the dorsal midline at S10B. (F-F") The pattern of BR42-GAL4 driver expressing GFP (F', green) (n=4). We focus on the BR domain, which is marked by a dotted white line. (G-G") Ectopic expression of MID by the BR42-GAL4 driver (yellow arrow, G'), disrupts BR patterning (G and G") (n=5). (H-H") The pattern of GMR$^{18E05}$-GAL4 expressing GFP (H, green) in the anterior domain (n=6). (I-I") Ectopic expression of MID by GMR$^{18E05}$-GAL4 (n=8). (J-L) The pattern of Slbo-GAL4 driver expressing GFP (green) during S8 (J, n=6),

S9 (K, n=5) and S10B (L, n=10) of oogenesis. Yellow arrow marks the migrating border cells. (M-M") Ectopic expression of MID by the Slbo-GAL4 (M,' M" yellow arrow) (n=8). Yellow dashed line (D', D'', E', E'', G', H', H", I', M') marks the anterior boundary of MID. Broad (BR, red) expressed early uniformly in the follicular epithelium (D-D"), and later marks the dorsal appendage primordia (E-E" white dotted).White arrowhead (D, H, I) marks posterior migration of follicular epithelium. (E-G") White dash line marks the anterior boundary of follicular epithelium. Yellow arrowhead marks dorsal midline. In all images the anterior is to the left. Reference size-bar in D-M represents 50µm. "n" represents the number of images with similar results.

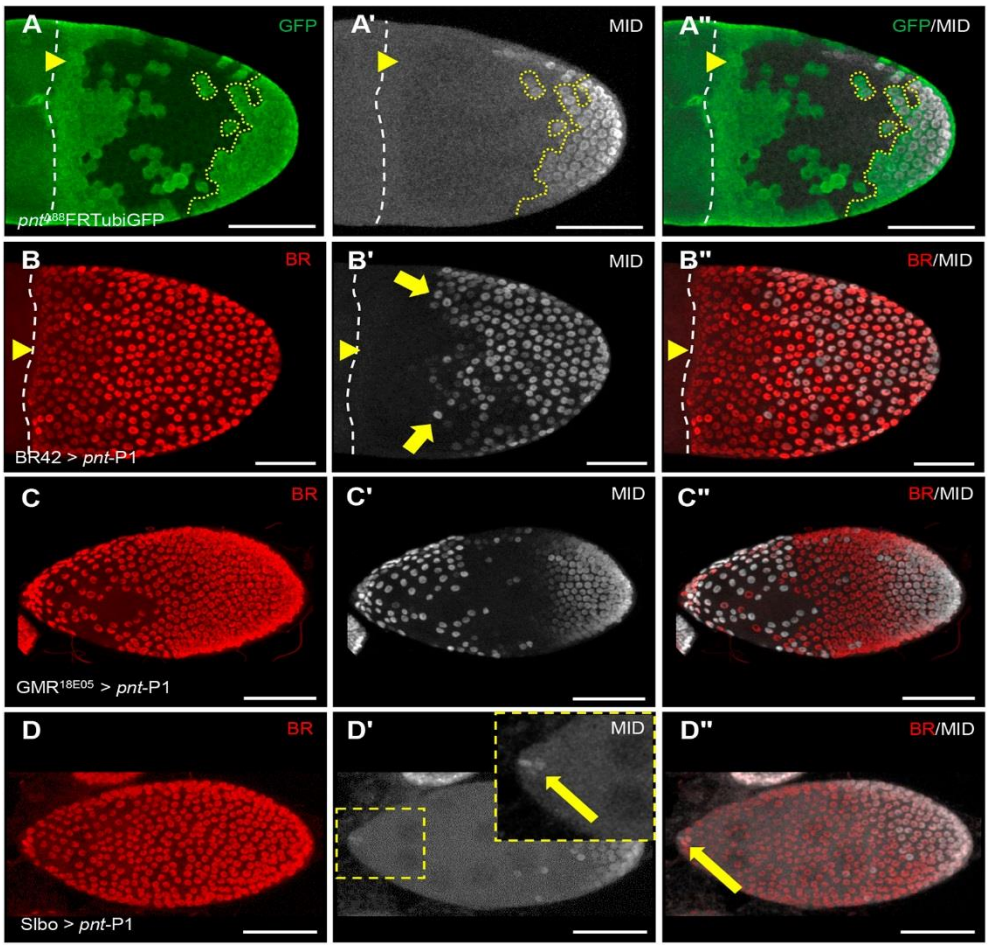



**Figure 2: Pointed is an upstream regulator of *midline* in the follicular epithelium.** (A-A") Null clones of *pointed* (*pnt*$^{\Delta 88}$) negatively marked by the loss of GFP (A). Clonal boundary marked by yellow dotted line exhibiting cell autonomous loss of MID (A', A") (n=46). (B-B") Ectopic expression of *pnt*-P1 by the BR42-GAL4 driver exhibiting ectopic MID (B') and loss of BR (B), and merge (B") (n=4). Yellow arrows in B' mark the ectopic MID. (C-C") Ectopic expression of *pnt*-P1 in the anterior domain using the GMR$^{18E05}$-GAL4 exhibiting ectopic MID (C'). BR is used as a spatial marker (C), and merge (C") (n=11). (D-D") Ectopic expression of *pnt*-P1 in border cells exhibiting ectopic MID (D'). BR is used as a spatial marker (D) and merge (D") (n=12). Yellow arrow (D', inset) marks ectopic MID (D', D"). Yellow arrowhead (A-B") marks dorsal midline. White dashed line marks the anterior boundary of follicular epithelium. In all images anterior is to the left. Reference size-bar in all panels represents 50µm. "n" represents the number of images with similar results.

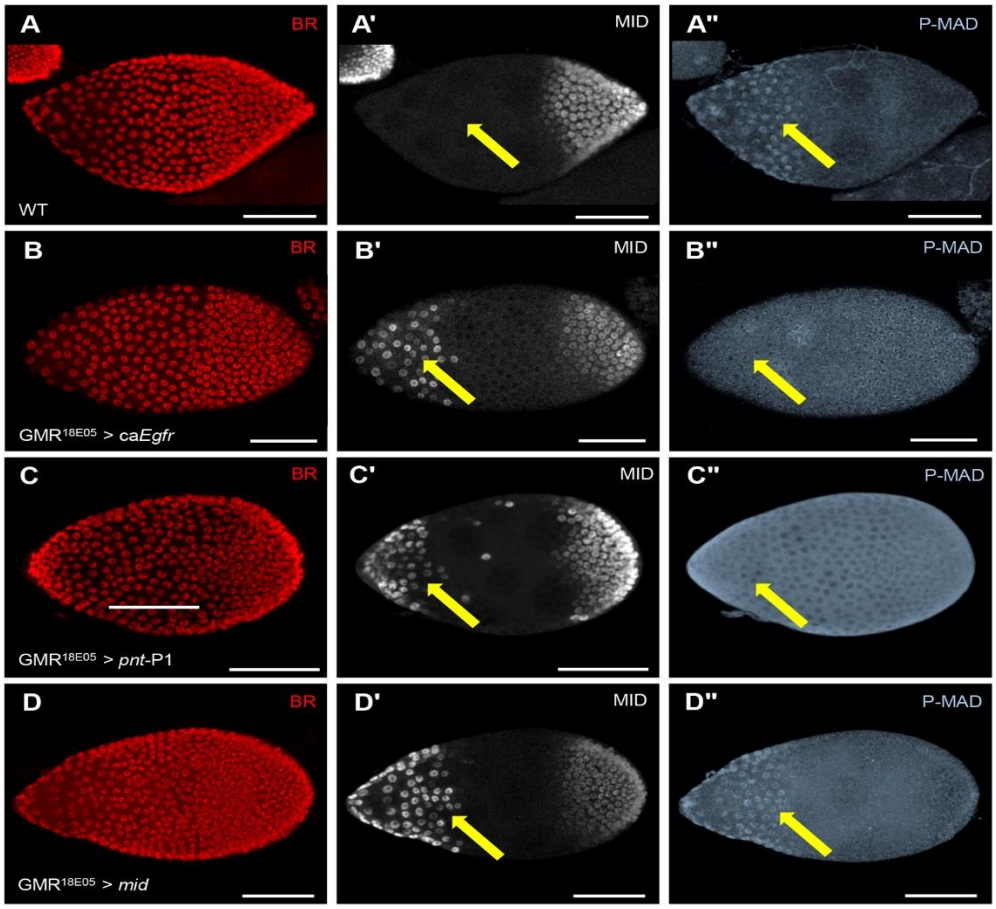



**Figure 3: Ectopic *pointed* represses BMP signaling.** (A-A") Wild-type expression of BR (A), MID (A'), and P-MAD pattern (A") at early stage 9 of oogenesis. Yellow arrow in A' and A" denotes the lack of anterior MID and presence of P-MAD, respectively (n=6). (B-B") Using the GMR$^{180E5}$-GAL4 driver to ectopically express a constitutively active EGFR (ca*Egfr*) in the anterior epithelium at early stage 9 of oogenesis. BR (B), gain of MID (B') and loss of P-MAD (B") are observed, yellow arrow (n=5). (C-C") Using the GMR$^{180E5}$-GAL4 driver to ectopically express *pnt*-P1 in the anterior epithelium. BR (C), gain of MID expression (C'), and loss of P-MAD (C") are observed, yellow arrow (n=11). (D-D") Using the GMR$^{18E05}$-GAL4 driver to ectopically express MID the anterior epithelium. BR (D), gain of MID expression (D'), and intact P-MAD pattern are observed, yellow arrow (n=13). In all images BR marks the follicular epithelium. In all images the anterior of the egg chamber is to the left. Reference size-bar in all panels represents 50µm. "n" represents the number of images with similar results.

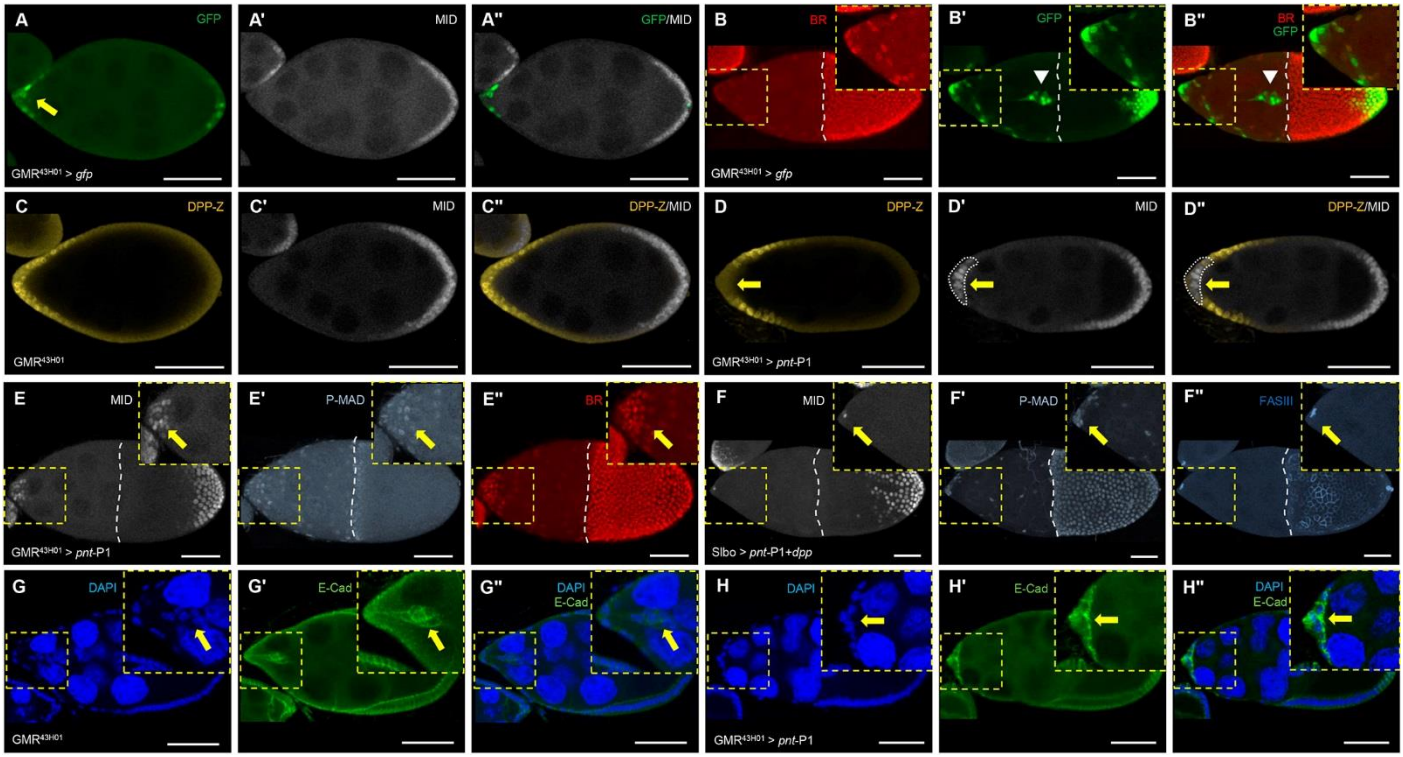



**Figure 4: Pointed represses *dpp* and anterior cell morphogenesis independent of BMP signaling.** (A-A") GMR$^{43H01}$-GAL4 driving expression of GFP at S9 egg chamber, yellow arrow (A') marks anterior GFP (n=6). (A') the pattern of MID. (A") merged image. (B-B") GMR$^{43H01}$-GAL4 driving expression of GFP at S10 (n=10). Expression is observed in some of the anterior stretch follicle cells, migrating border cell cluster, and posterior cells (B', B", white arrowhead). We focus on the anterior and border cells (insets). (C-C") The pattern of a LacZ-DPP reporter (DPP-Z) in the GMR$^{43H01}$-GAL4 driver background cross-section of early stage 9 egg chamber (C), MID pattern (C'), and merged image (C") (n=6). (D-D") GMR$^{43H01}$-GAL4 driver expressing *pnt*-P1 in the anterior resulting in ectopic expression of MID (D', dotted white line, yellow arrow) and loss of DPP-Z (D, D" Merge, yellow arrow) (n=11). (E-E") Ectopic expression of *pnt*-P1 using the GMR$^{43H01}$-GAL4 driver in anterior stretch cells induced MID (E, yellow arrow, inset), and contained P-MAD (E', yellow arrow, inset). These cells did not stretch and remained clustered in the anterior, marked by BR (E", yellow arrow, inset) (n=5). (F-F") Slbo-GAL4 driving expression of both *pnt*-P1 and *dpp*. (F) MID expression observed in anterior polar cells (yellow arrow, inset). (F') P-MAD was present in these cells (yellow arrow, inset). (F") FASIII marks polar cells failing to migrate posteriorly (yellow arrow, inset) (n=6). (G-G") GMR$^{43H01}$-GAL4 exhibiting cell nuclei marked by DAPI (G) and cell boundaries marked by E-cadherin (G'), insets showcase migrating border cells (G-G", yellow arrow) (n=6). (H-H") Ectopic expression

of *pnt*-P1 in anterior stretch cells, (H) DAPI marks cell nuclei, (H') E-Cadherin marks cell boundaries. Insets showcase accumulation of E-Cadherin and loss of border cell migration (H-H", yellow arow) (n=7). Broad (BR) in B, E" marks follicular epithelium. White dashed line (E-F") marks anterior boundary of follicular epithelium. In all images the anterior of the egg chamber is to the left. Reference size-bar in all panels represents 50µm. "n" represents the number of images with similar results.

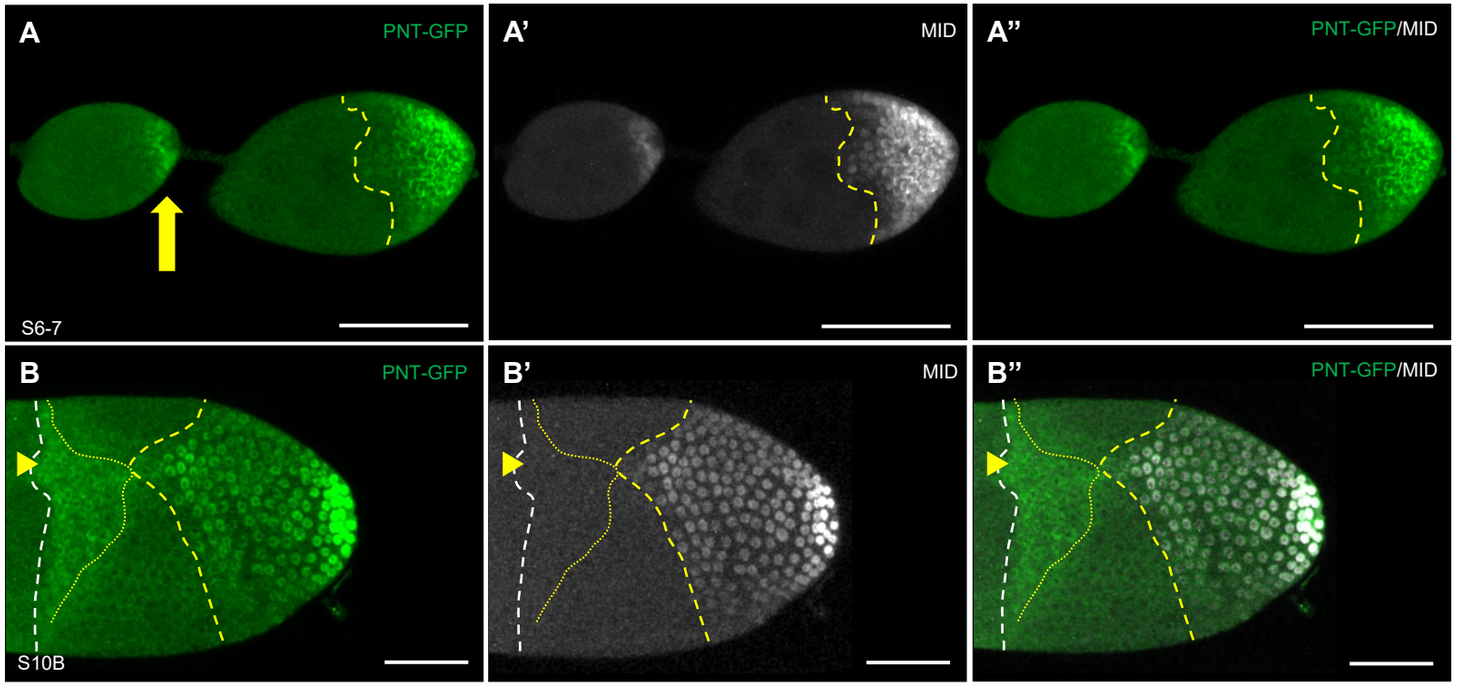



**Figure S1: Pointed and Midline spatiotemporally overlap in the posterior follicular epithelium.** (A-A") Egg chambers in early oogenesis stages 6-7 (S6-7). Chimeric PNT-GFP is detected by anti-GFP (A) starting at S6 (yellow arrow) concurrently with observable MID expression (A') (n=8). Yellow dash line denotes posterior pattern of early PNT. (B-B") PNT-GFP pattern at stage 10B egg chambers. Expression of PNT-GFP is observed overlapping with posterior MID pattern (B") with gain of observable expression in dorsal midline (B) (n=6). Dotted yellow line marks the domain of late PNT in the dorsal midline. White dash line denotes anterior boundary, yellow arrowhead marks dorsal midline. Reference bar in all panels represents 50μm. In all images anterior is to the left.

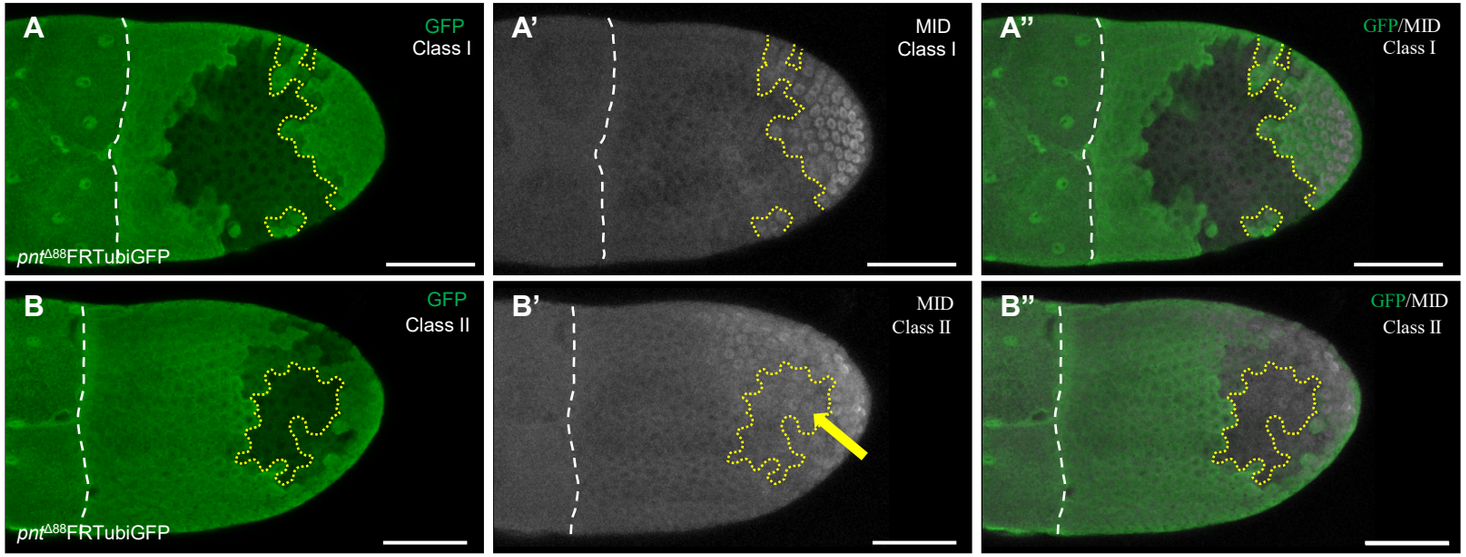



**Figure S2: *pointed* is necessary for wildtype expression pattern of MID.** (A, B) Null clones of *pnt* (*pnt*$^{\Delta 88}$) negatively marked with the loss of GFP. Yellow dotted line marks clonal boundary. (A-A") Class I- null clones of *pnt* (*pnt*$^{\Delta 88}$) exhibiting complete loss of MID (A') (n=46). (B-B") Class II-null clones of *pnt* (*pnt*$^{\Delta 88}$) exhibiting reduced MID expression (B' yellow arrow) (n=38). White dash line (A-B") marks the anterior boundary of the oocyte. Reference bar in all panels represents 50μm. In all images anterior is to the left.

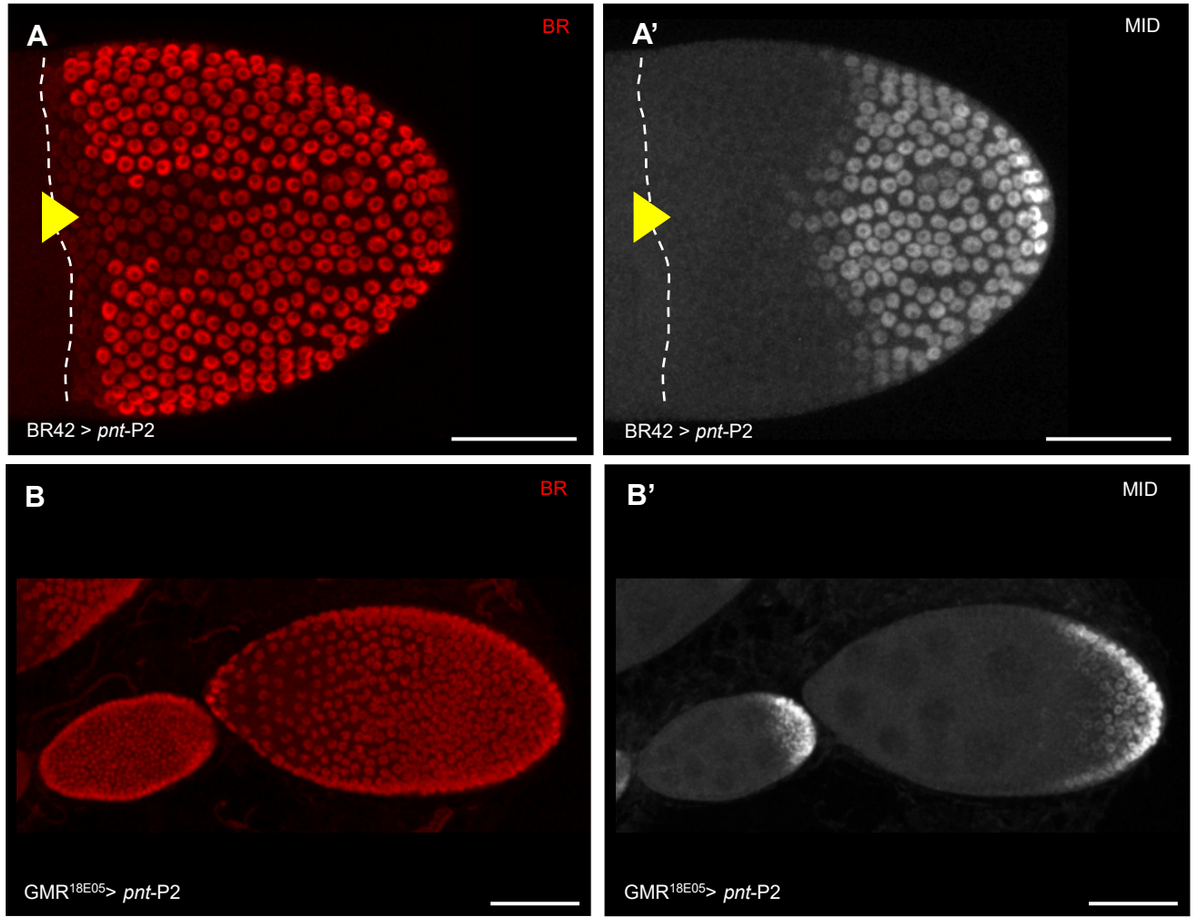



**Figure S3**: ***pointed*-P2 isoform does not impact follicle cells patterning**. (A, A') Ectopic expression of *pnt*-P2 at stage 10B of oogenesis in the dorsal anterior (BR42-GAL4). White dash line marks the anterior boundary between the oocyte and nurse cells. Yellow arrowhead marks the dorsal midline (n=6). (B, B') Early stage egg chambers ectopically expressing of *pnt*-P2 via early anterior driver ($GMR^{18E05}$-GAL4) (n=8). Reference bar in all panels represents 50µm. In all images anterior is to the left.

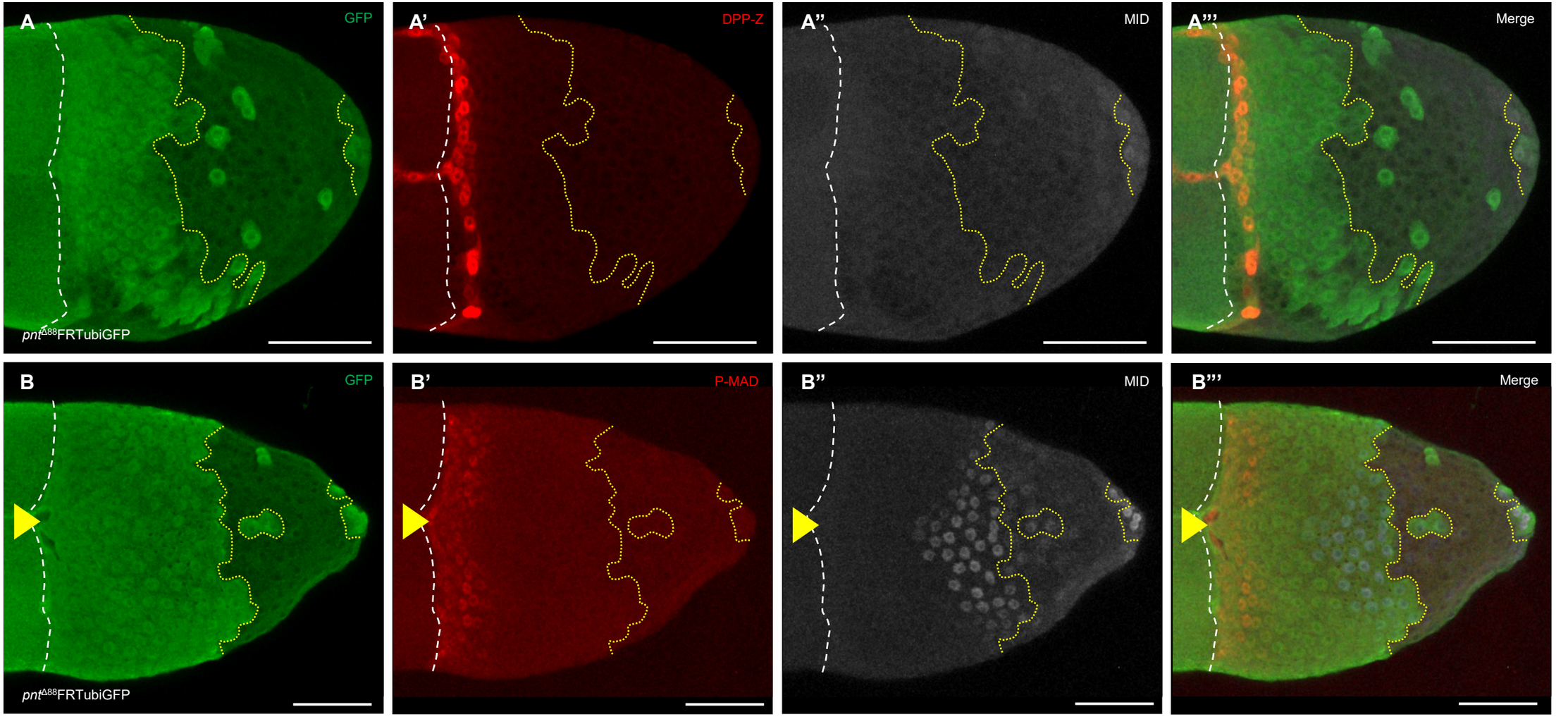
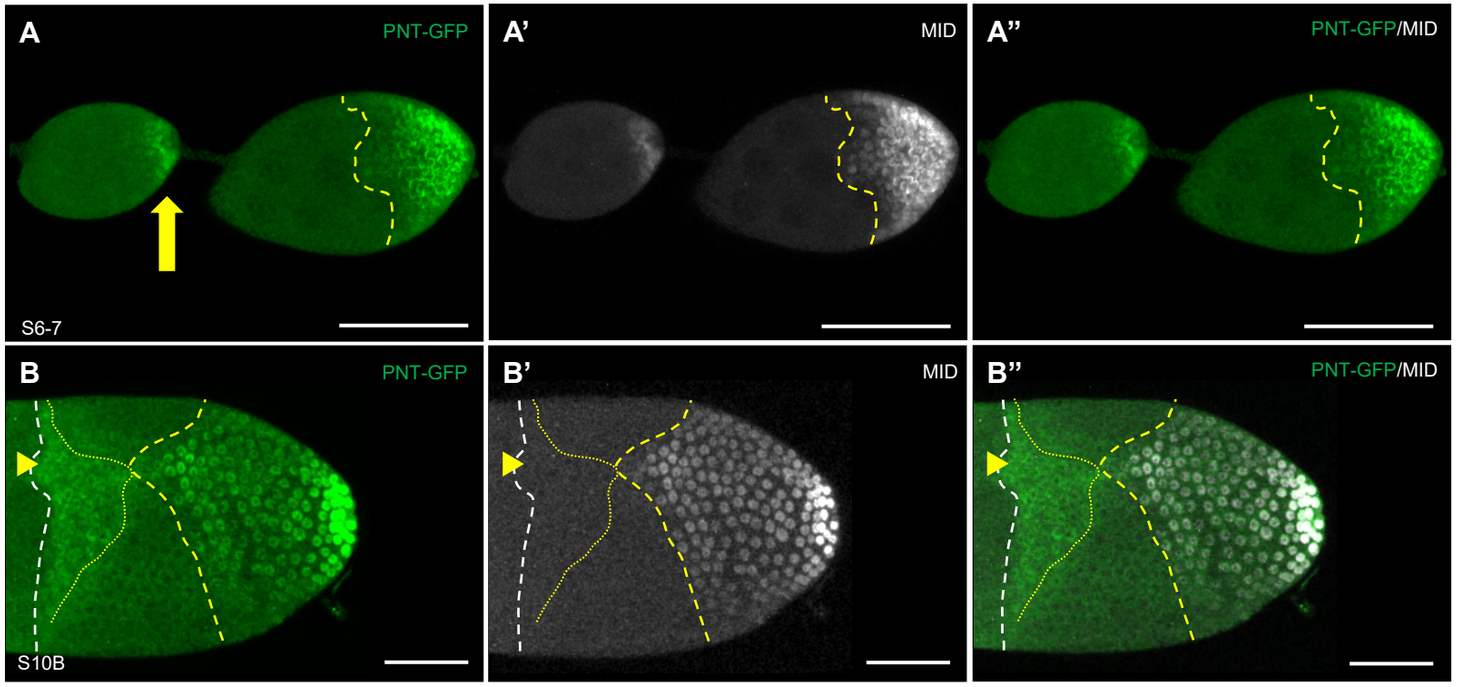



**Figure S4: PNT is not necessary to suppress *dpp* in the posterior end.** (A-A"') Null clones of *pnt* (*pnt*$^{\Delta 88}$) negatively marked with the loss of GFP (A). Clonal boundary is denoted by yellow dotted line. (A') LacZ reporter for DPP (DPP-Z, red). (A") MID expression (white) (n=6). (B-B"') Null clones of *pnt* (*pnt*$^{\Delta 88}$) negatively marked with the loss of GFP (B). Clonal boundary is denoted by yellow dotted line. (B') P-MAD pattern (Red). (B") MID expression (white) (n=6). (A"' and B"') Merged images of A-A" and B-B", respectively. Yellow arrowhead marks dorsal midline. In all images the dashed white line denotes the anterior boundary. Reference bar in all panels represents 50µm. In all images anterior is to the left.

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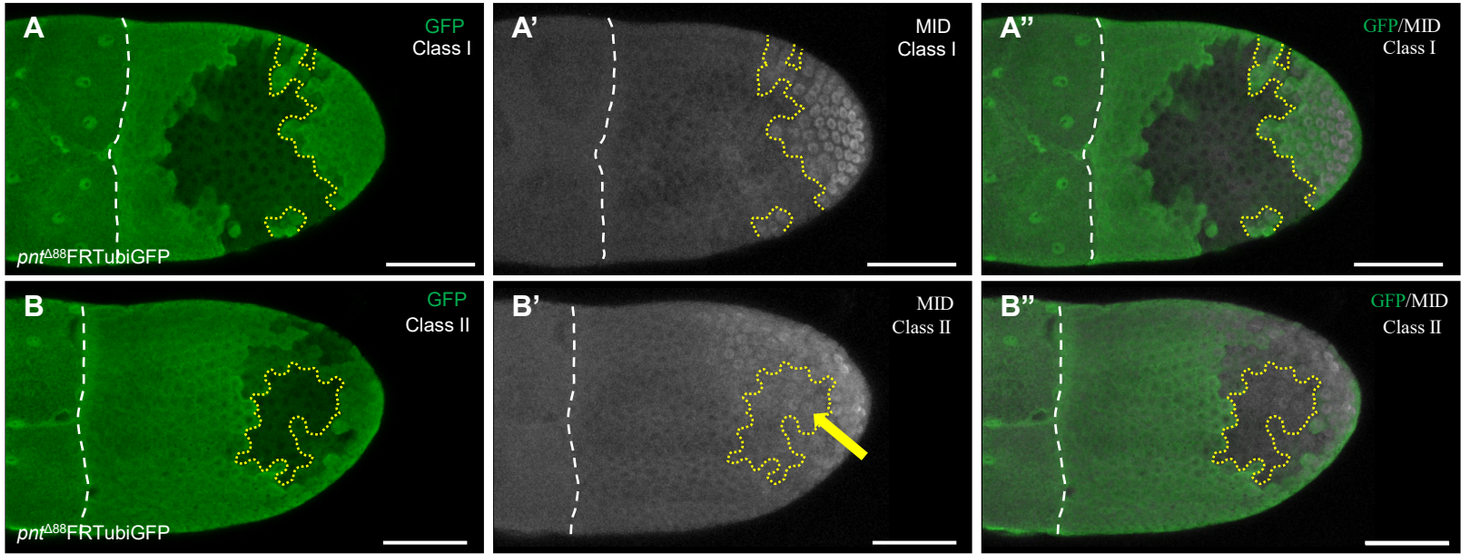


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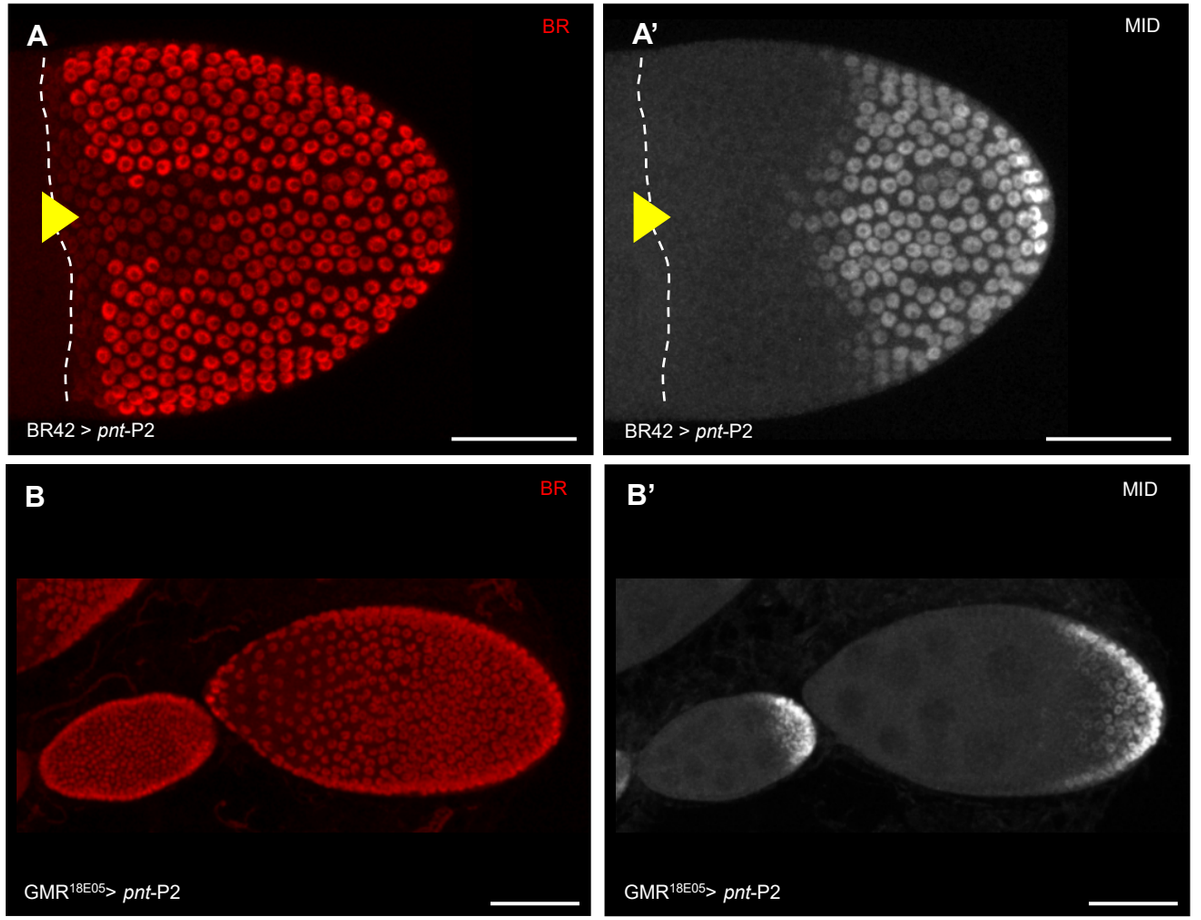


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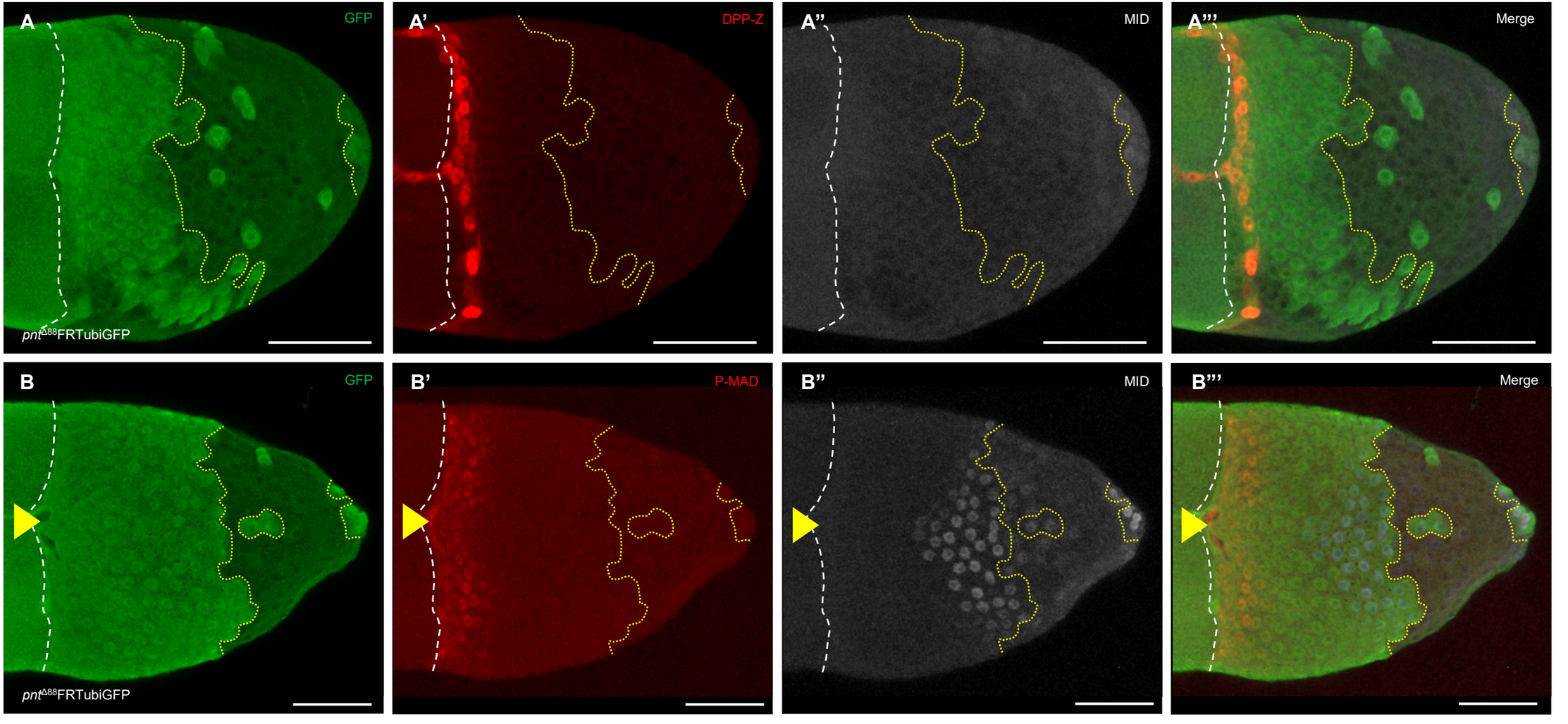


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