

Fig. S1. Upd distribution is graded prior to specification of follicular epithelium. (A,A') Fluorescence signal intensity of Upd protein (red) as detected by anti-Upd antisera processed for extracellular protein was quantified along the apical surface of the follicle cells at the anterior and posterior poles. DNA is blue. (B,B') Signal intensity is plotted relative to position along the epithelium. Colored arrows on the image and graphs mark corresponding positions. Scale bar: 20 μ m.

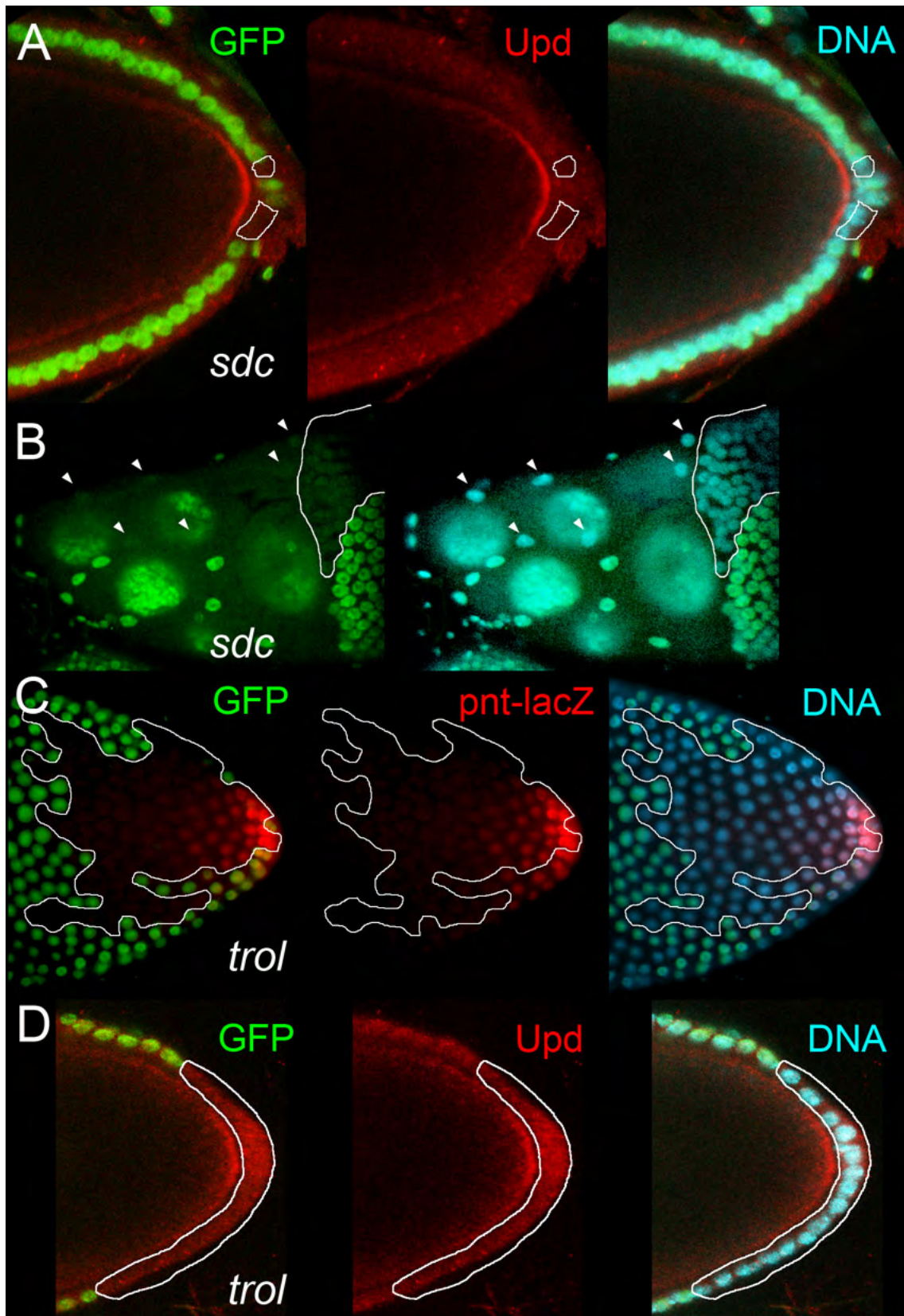


Fig. S2. *sdc* and *trol* do not influence the distribution of Upd during oogenesis. (A-D) Follicular clones of *sdc* (A,B) and *trol* (C,D) mutant cells are outlined in white as marked by lack of GFP (green). Upd distribution (A,D, red) and *pnt-lacZ* expression which serves as a reporter of JAK/STAT activity at the posterior (C, red) are shown. Upd distribution appears to be normal in chambers with mosaic follicle cells of either mutation. Anterior follicle cells mutant for *sdc* (B, loss of GFP) undergo normal morphological transitions for their region, including extension of stretched cells (arrowheads) and migration of the adjacent cells (outline) and cannot be differentiated from the wild-type anterior follicle cells (B, GFP). In large *trol* clones (C, loss of GFP), graded expression of *pnt-lacZ* is unaffected.

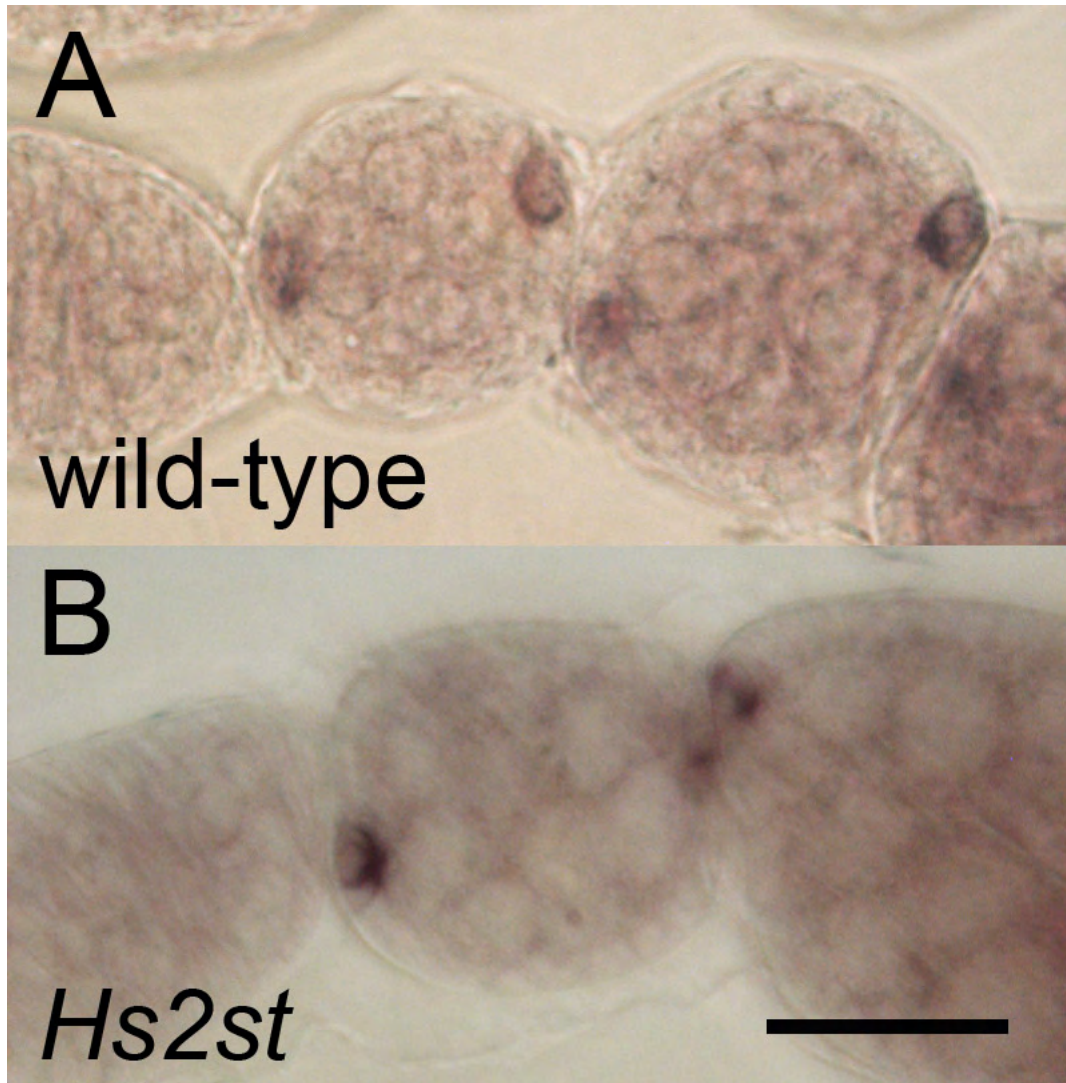


Fig. S3. Loss of *Hs2st* does not alter *upd* expression. (A,B) RNA in situ hybridization to *upd* shows comparable expression between wild-type (A) and *Hs2st* mutant (B) ovaries. Scale bar: 20 μ m.

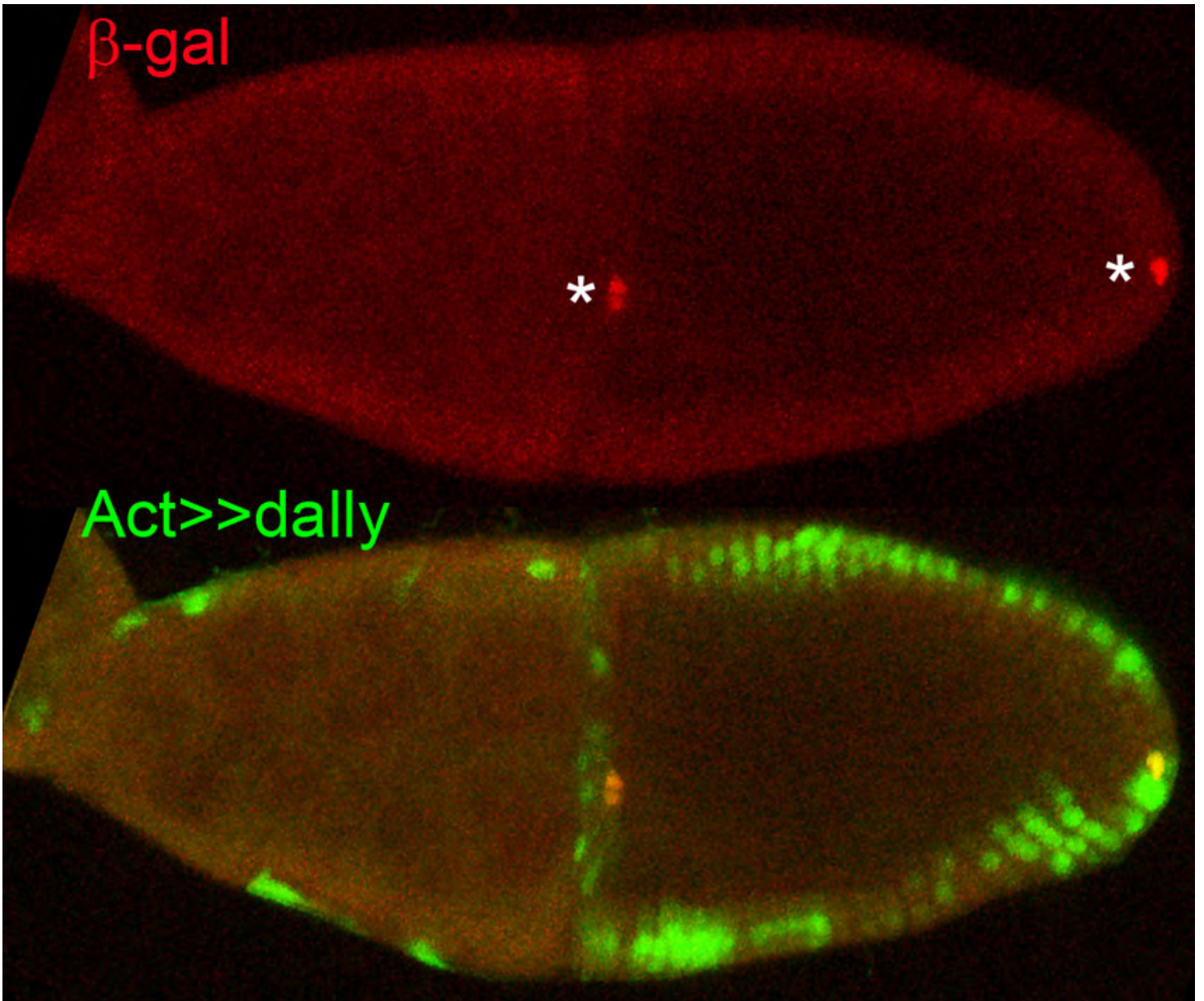


Fig. S4. *dally* misexpression does not alter *upd* expression. Flip-out misexpression clones of *dally* (marked with GFP, green) in the background of an enhancer trap in the *upd* gene (PD) show expression of the β -galactosidase reporter (red) only in the polar cells (asterisks), but not in *dally*-misexpressing cells.

Table S1. Primers used to amplify templates from cDNA for in situ hybridization

Target gene	Primer pairs
<i>dally</i>	5'- <u>GTAATACGACTCACTATAGGGCCAGCTTTTGCTATTTACCCTGC</u> -3' 5'- <u>AATTAACCCTCACTAAAGGGAGTCCGGCATATTCCGCCG</u> -3'
<i>dlp</i>	5'- <u>GTAATACGACTCACTATAGGGCGTGGAGGCCGATACTCTGG</u> -3' 5'- <u>AATTAACCCTCACTAAAGGGCGCCATATAAAGGGGCAGC</u> -3'
<i>upd</i>	5'- <u>GTAATACGACTCACTATAGGGCACCTCCAGTACGGCTTCAGC</u> -3' 5'- <u>AATTAACCCTCACTAAAGGGTCTGCAACTCCAGCAGCACC</u> -3'

Primer sets used to synthesize template for RNA labeling are indicated for each gene of interest.

Underlines indicate T7 and T3 RNA polymerase binding sites, respectively.

Table S2. Stalk size is reduced in HSPG mutant ovarioles

	Number of cells in stalk		
	1-2	3-4	≥5
Control	0	0	39
<i>dally^{gem}/dally⁵²⁷</i>	9	16	15*
<i>dlp^{A187}</i> clone in stalk cells	0	0	3
<i>dlp^{A187}</i> clone in polar cells	0	0	20
<i>dally⁸⁰, dlp^{A187}</i> clone in stalk cells	9	1	0*
<i>dally⁸⁰, dlp^{A187}</i> clone in polar cells	0	0	14

*P<0.05 by Fisher's exact probability test.

The number of stalk cells was counted for stage 2-7 egg chambers of the indicated *dally* mutant combination or for chambers with the indicated locations of mutant clones. For polar cell clones, cells were counted for the adjacent stalk. For stalk cell clones, only clones in which the entire stalk was mutant were scored. Stalk cell numbers that differ statistically from the wild-type control are marked with an asterisk.

Table S3. pSTAT92E in *Hs2st* mutant ovaries

Genotype	pSTAT92E positive germaria	pSTAT92E positive stage 7-9 egg chambers	
		Anterior follicle cells	Posterior follicle cells
Control	84% (n=25)	90.9% (n=33)	78.8% (n=33)
<i>Hs2st</i>	2.9% (n=34)*	12.5% (n=32)*	21.9% (n=32)*

*P < 0.05 χ^2 test

Anti-pSTAT92E fluorescence signal was observed in ovaries of wild-type and *Hs2st* mutants.

Using the non-specific ring canal staining as an internal control, signal was scored as background (negative) or above background (positive). In the posterior germarium, pSTAT92E was scored in the follicle cell precursors. In stage 7-9 egg chambers of the vitellarium, anterior and posterior follicle cells were scored separately for pSTAT92E. Statistically significant differences between wild-type and *Hs2st* ovaries are marked with an asterisk.