Supplementary Materials

Genotype	Defective StC morphogenesis (%)	Number of egg chambers counted
c306>GFP	4	25
c306>Inx1 RNAi	0	14
c306>Inx2 RNAi	85.7***	35
c306>Inx3 RNAi	0	14
c306>Inx7 RNAi	0	15

Table S1. Quantitative analysis of egg chambers with defective StC morphogenesis by Fisher's exact test of independence and post-hoc test, *** $p<0.001$.

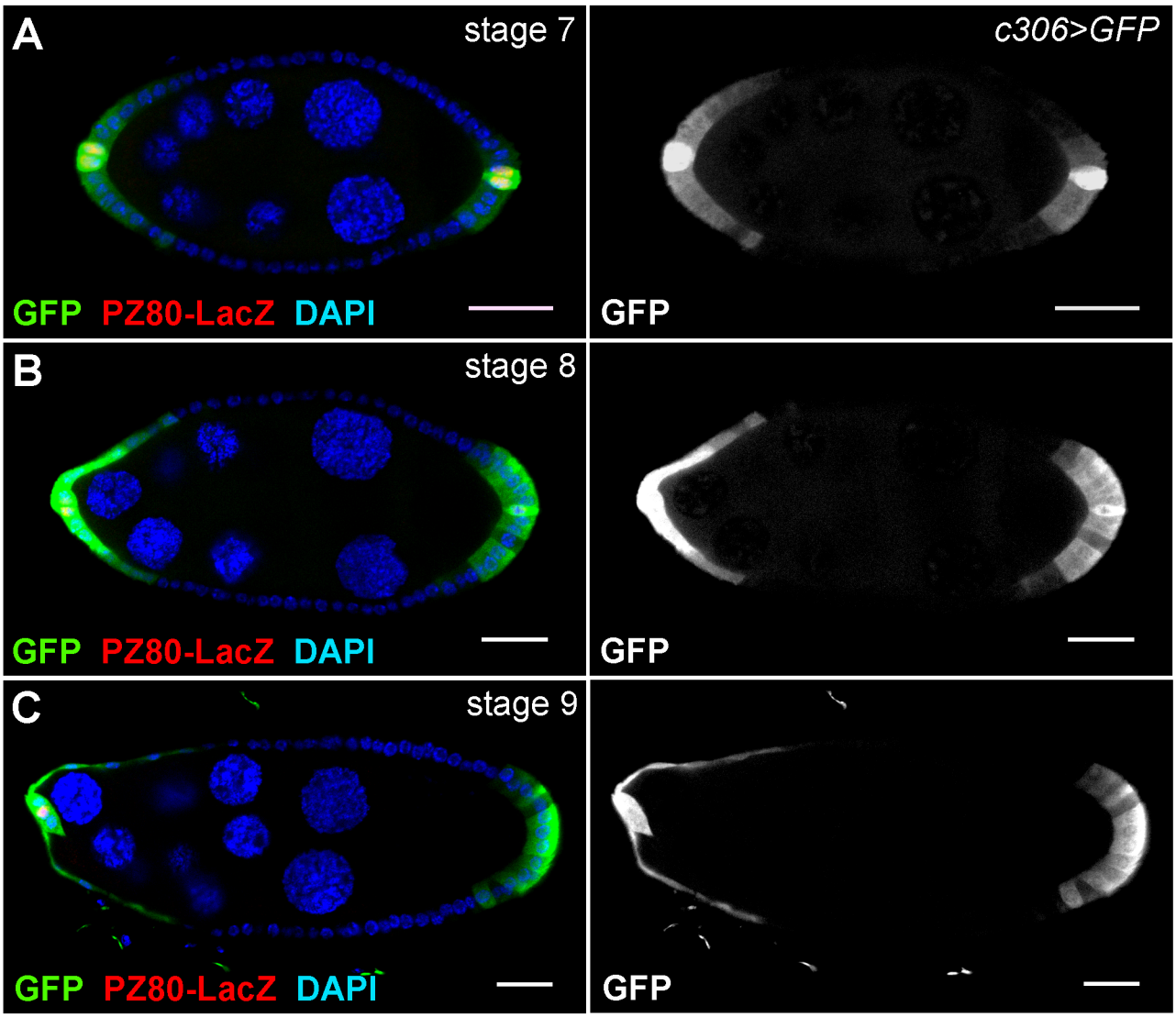

Figure S1. *c306-GAL4* was expressed in polar cells, border cells, StCs and anterior/posterior follicle cells.

Egg chambers from *c306-GAL4; UAS-GFP* at different stages were selected and oriented as anterior to the left. Ovaries were stained with anti-GFP, anti-β-Galactosidase and DAPI for DNA in cell nuclei. *PZ80-LacZ* is expressed in polar cells. *UAS-GFP* driven by *c306-GAL4* was used to label *c306-GAL4* expressing cells. (A, B) GFP was detected in polar cells and anterior/posterior follicle cells throughout stage 7 to stage 8. (C) GFP was detected in polar cells, border cells, StCs and posterior follicle cells at stage 9. Length of scale bar is 20 μm.

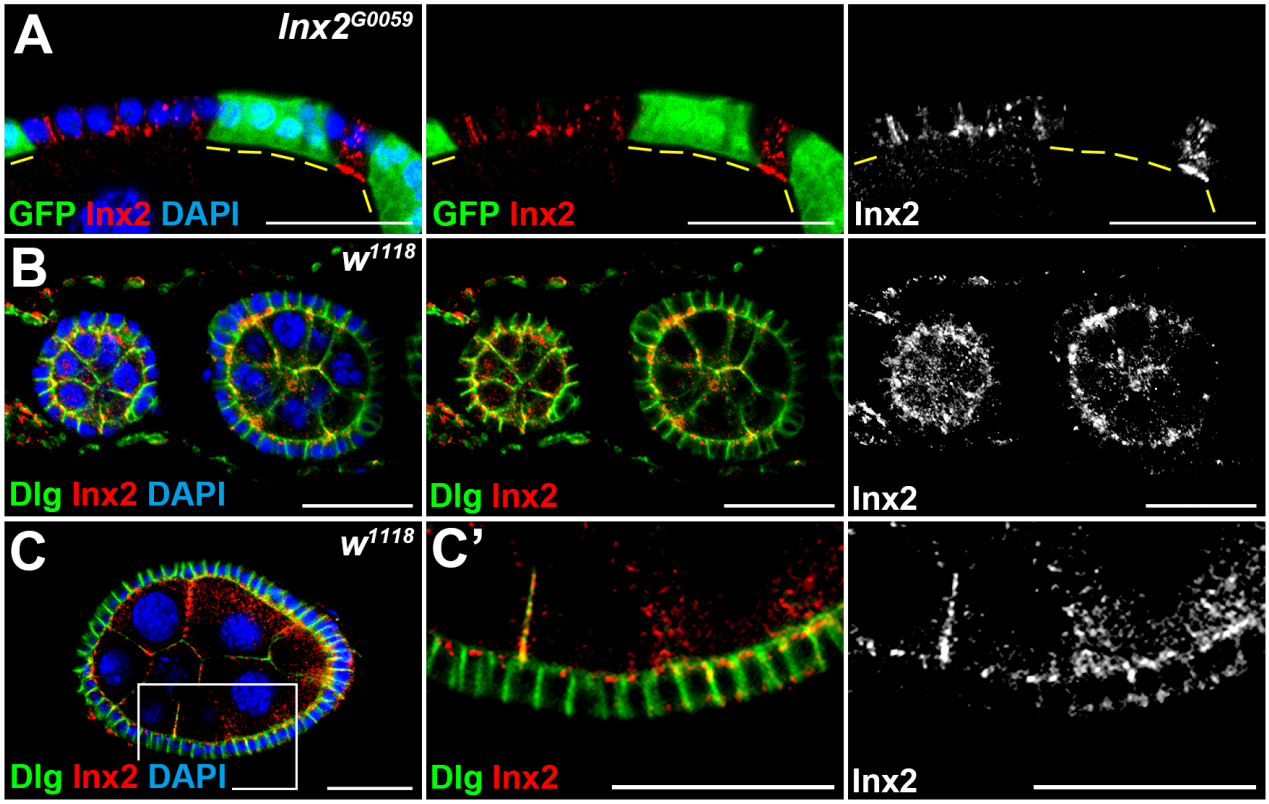

Figure S2. Inx2 was detected at the apical and lateral membrane of follicle cells during oogenesis.

Egg chambers at different stages were selected and oriented as anterior to the left. Ovaries were stained with anti-GFP (A), anti-Inx2 (A-C), anti-Dlg (B-C) for labeling the lateral membrane of follicle cells, and DAPI for DNA in cell nuclei. High magnification views are shown in (C'). (A) GFP-positive *Inx2*^{G0059} mutant clones (yellow dashed lines) were generated by using MARCM and examined six days after clone induction. Inx2 was not detected in GFP-positive *Inx2* mutant cells. (B) At stage 3 and 4, Inx2 was detected on the apical membrane of follicle cells and the membrane between germline cells. (C, C') At stage 6, Inx2 was detected on the apical membrane of follicle cells. Length of scale bar is 20 μm.

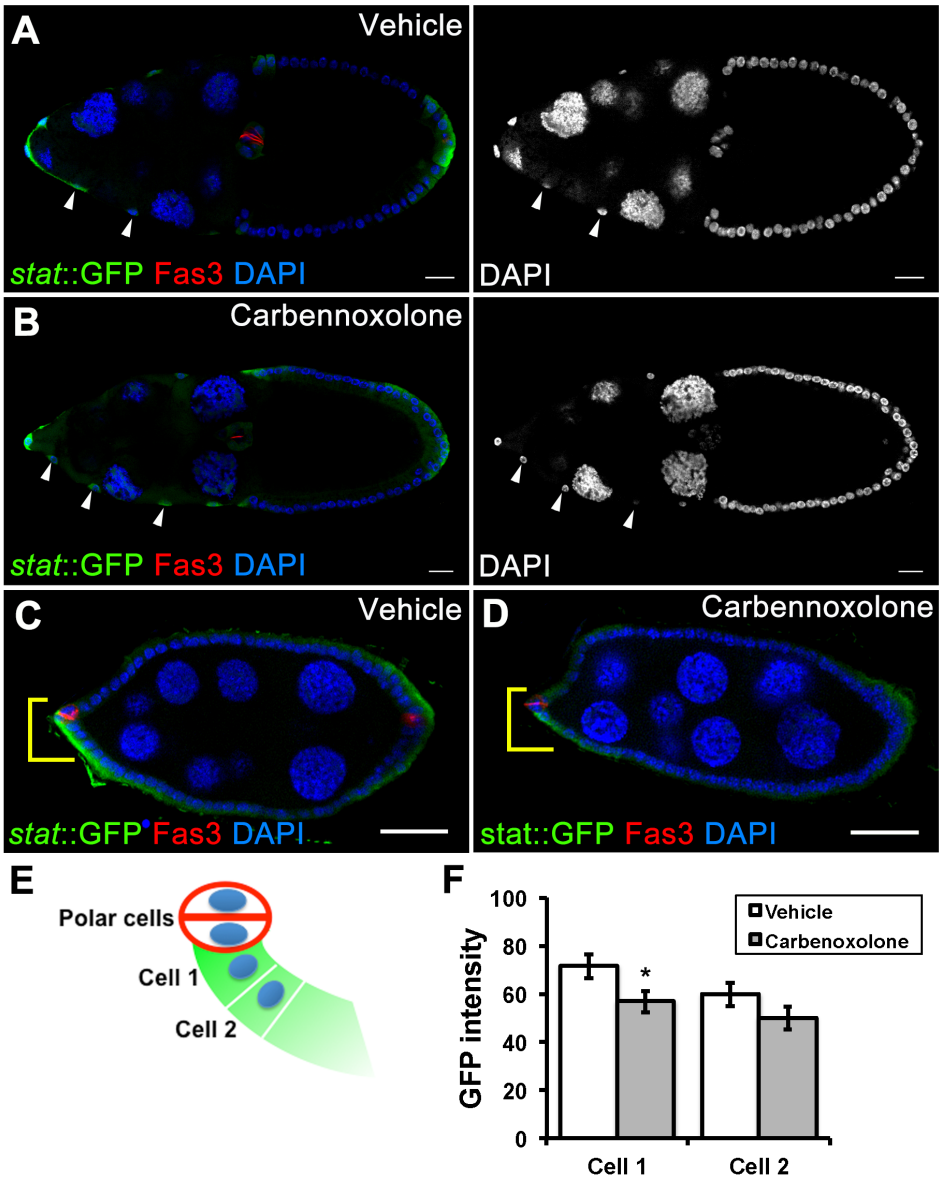

Figure S3. The gap junction functions could be blocked by carbenoxolone treatment in the anterior follicle cells.

Ovaries dissected from *10xstat::GFP* (*stat::GFP*) flies were cultured in Schneider's medium with vehicle control or carbenoxolone (0.2 mM) and immunostained with anti-GFP and anti-Fas3. Nuclear DNA was stained with DAPI. Egg chambers we oriented as the anterior towards left. (A, B) After cultured for six hours, stage 10 egg chambers were selected. StC nuclei were sparsely distributed in both vehicle and carbenoxolone groups (Vehicle: n=32. StC nuclei of 28 egg chambers were sparsely distributed. Carbenoxolone: n=33, StC nuclei of 29 egg chambers were sparsely distributed). White arrowheads indicate the nuclei of StCs. (C, D) After cultured for four hours, stage 8 egg chambers were selected. The GFP immunofluorescent intensity is weaker after incubation in carbenoxolone comparing with that of the vehicle control. Yellow brackets indicate the region of polar cells and *stat*::GFP-positive cells. (E) A diagram demonstrating the cells for quantification of the GFP immunofluorescent intensity. (F) Mean fluorescent intensity of polar cells and those cells adjacent to polar cells were measured by using ImageJ. Mean fluorescent intensity of polar cells was used as background for subtraction. The GFP immunofluorescent intensity of cell 1 after carbenoxolone treatment was weaker than that of the vehicle control. The GFP immunofluorescent intensity of cell 2 after carbenoxolone treatment was weaker but not significantly different from that of the vehicle control. The bar graph is shown as mean ± SEM. Vehicle: n=36; carbenoxolone: n=26. Student's T-test. *p <0.05. Scale bars are 20 μm.

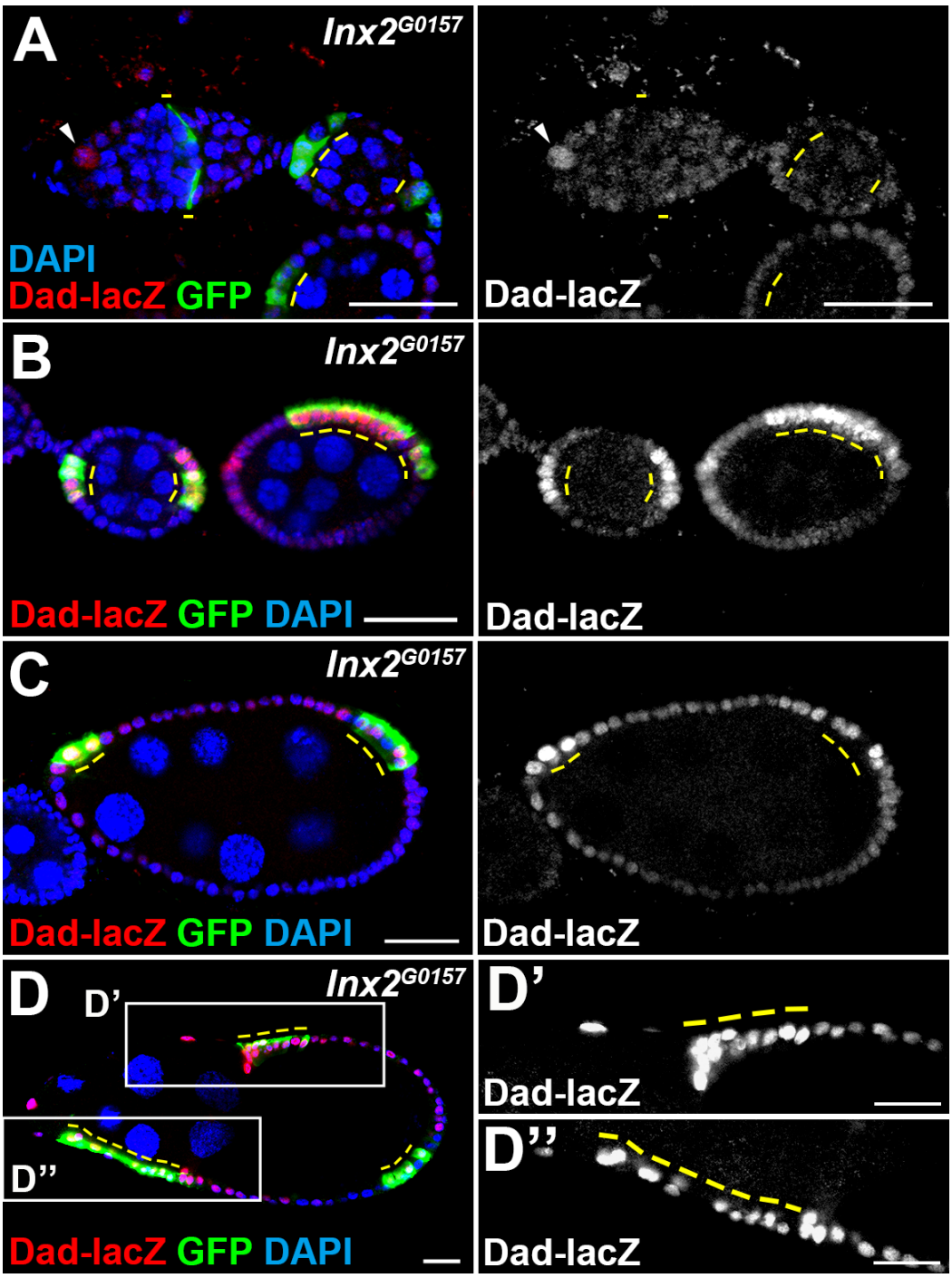

Figure S4. The activity of TGF-β pathway is increased in *Inx2* mutant follicle cells from stage 4 to 9.

Inx2^{G0157} mutant MARCM clones (yellow dashed lines) were generated in a TGF-β pathway activity reporter line *Dad-lacZ* and examined six days after clone induction. Ovaries were stained with anti-GFP, anti-β-Galactosidase (β-Gal) and DAPI. Egg chambers at different stages were selected and oriented as anterior to the left. (A) β-Gal was detected in germline stem cells (white arrowheads). No β-Gal signal was detected in GFP-negative control follicle cells, *Inx2* mutant follicle stem cells and follicle cells (yellow dashed lines) at stages 2 and 3. (B, C) At stages 4, 5 and 7, β-Gal was increased in *Inx2* mutant clones in comparison with that of the neighboring GFP-negative control cells. (D) At stage 10B, the fluorescent intensity of β-Gal in *Inx2* mutant cells was similar to that of the neighboring GFP-negative control cells. High magnification views were shown in the right panels (D', D'') Length of scale bar is 20 μm.

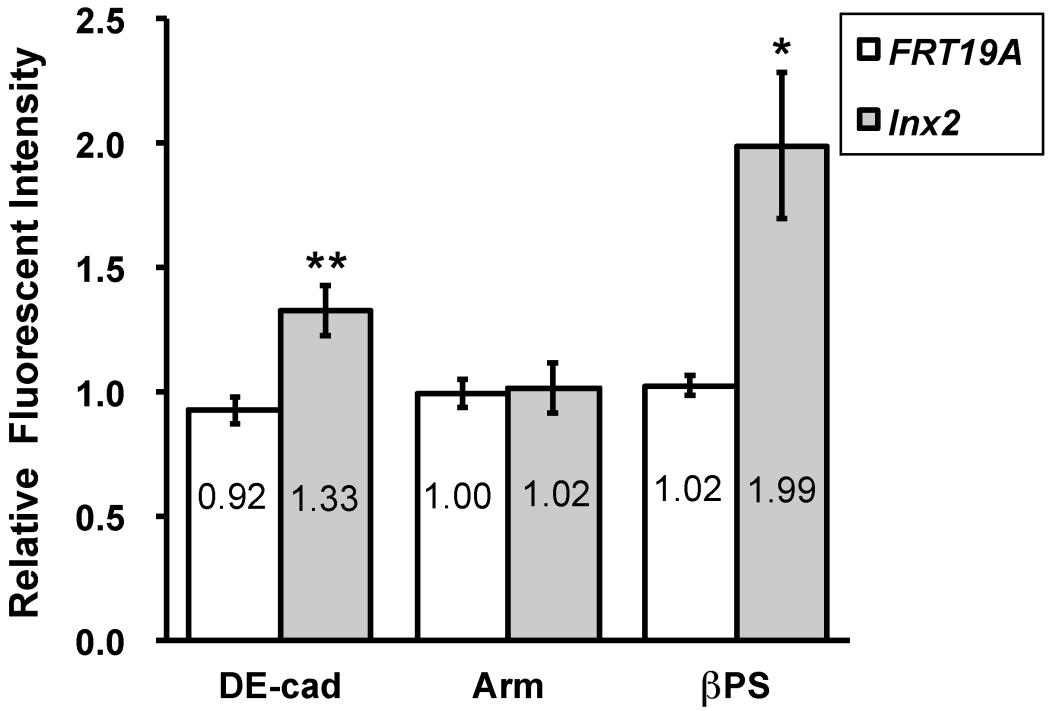

Figure S5. The immunofluorescent intensities of DE-cad and βPS were increased in *Inx2* mutant StCs.

GFP-positive *FRT19A* or $Inx2^{G0157}$ mutant clones were generated by using MARCM and examined six days after clone induction. Ovaries were stained with anti-GFP and anti-DE-cad anti-Arm, or anti-βPS, and DAPI. Egg chambers at stage 9 were selected. The ratio of DE-cad, Arm, and βPS immunofluorescent intensities of GFP-positive *FRT19A* StCs to the adjacent GFP-negative StCs were close to 1.0. The ratio of Arm immunofluorescent intensities of GFP-positive *Inx2* mutant StCs to the adjacent GFP-negative StCs were also close to 1.0. The ratio of DE-cad and βPS immunofluorescent intensities of GFP-positive *Inx2* mutant StCs to the adjacent GFP-negative StCs were significantly higher than 1.0, demonstrating that the levels of DE-cad and βPS are increased in *Inx2* mutant StCs. Bar graph is shown as mean ± SEM. *: $p<0.05$, **: $p<0.01$ determined by using Student *t*-test.

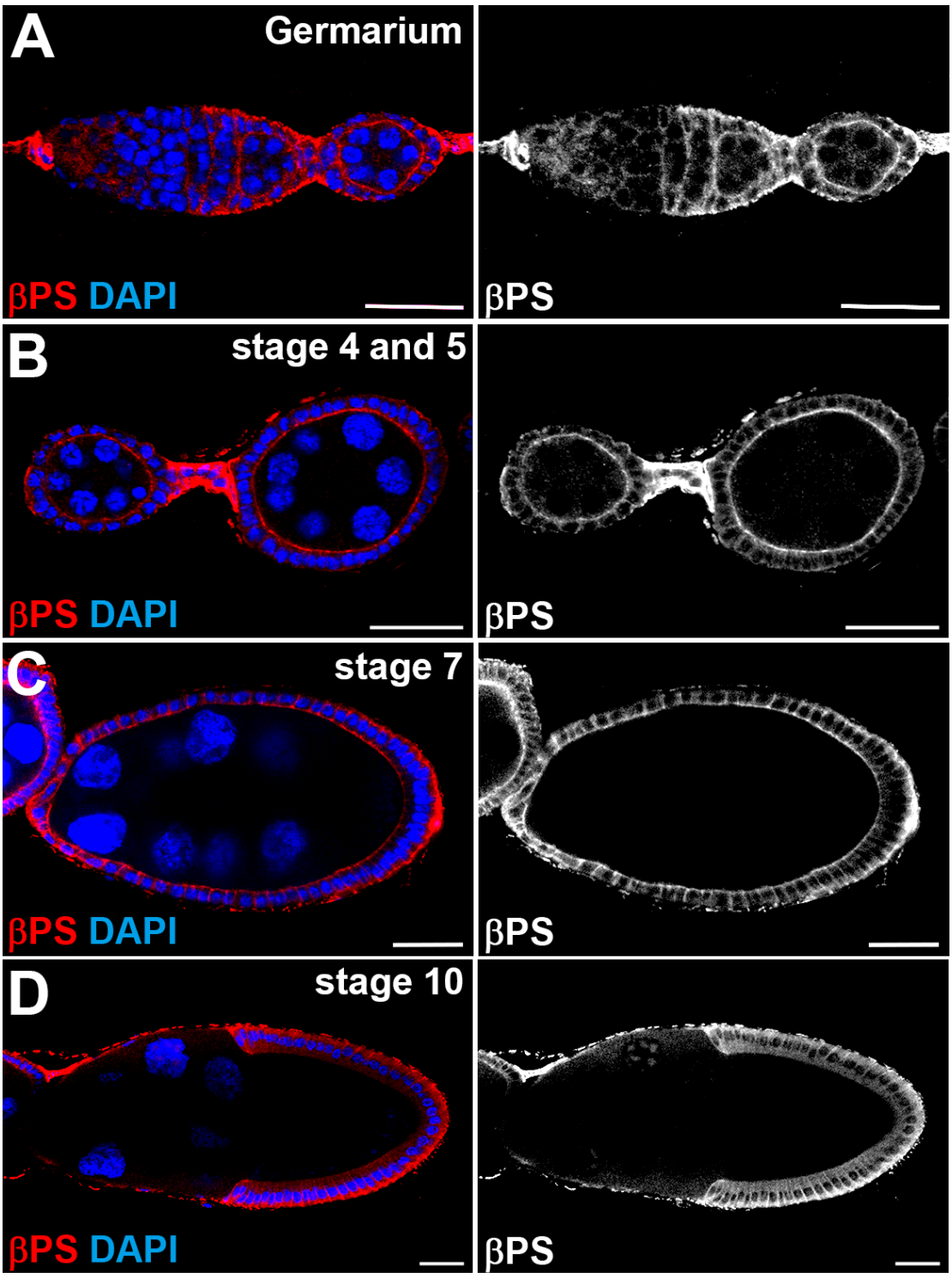

Figure S6. Dynamic distribution pattern of βPS in follicle cells during oogenesis.
Ovaries of *c306-GAL4;UAS-GFP* flies were dissected and stained with anti-βPS and DAPI. Egg chambers at different stages were selected and oriented as anterior to the left. (A) βPS was detected in both apical and basal membrane of follicle cell precursors and follicle cells in the germarium and the stage 1 egg chamber. (B) βPS was detected at a high level surrounding stalk cells and at a moderate level on the apical membrane of follicle cells at stages 4 and 5. (C) At stage 7, βPS was detected at the apical membrane of most follicle cells and at basal membrane of posterior follicle cells. (D) After StC morphogenesis at stage 10, weak βPS was detected in StCs. In posterior follicle cells, βPS was detected on the basal and lateral membranes. Length of scale bar is 20 μm.

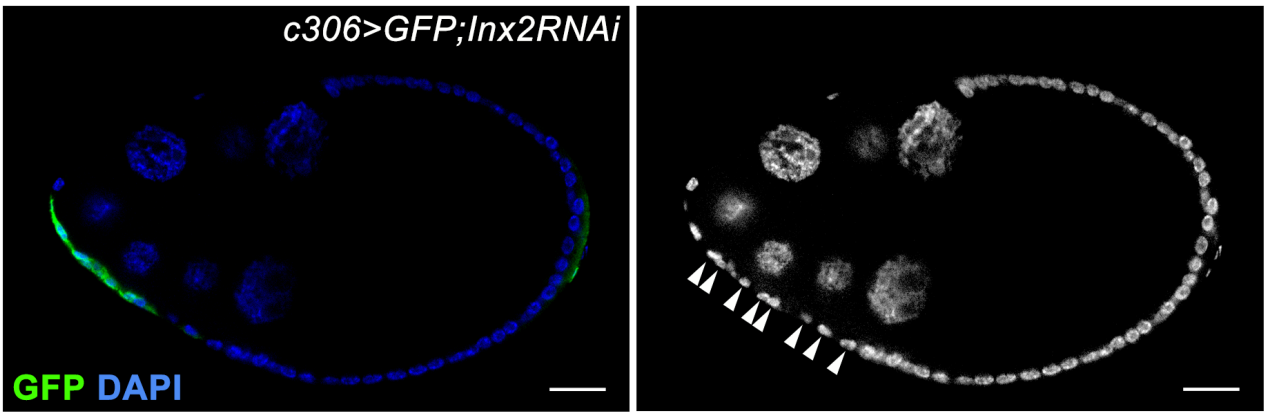

Figure S7. Expression of GFP and shRNAs targeting *Inx2* simultaneously driven by *c306-GAL4* shows similar StC morphogenesis defect as expression of shRNAs targeting *Inx2* alone driven by *c306-GAL4*.

c306-GAL4 was used to drive expression of gene or shRNA in follicle cell precursors and follicle cells in the anterior and posterior of the egg chamber. Flies were collected and grown at 29°C for six days after eclosion. Ovaries were dissected and immunostained with anti-GFP. Nuclear DNA was stained with DAPI. An egg chamber at stage 9 was selected and oriented as the anterior towards left. White arrowheads indicate the nuclei of StCs. When *GFP* was overexpress and *Inx2* was knocked down simultaneously, StCs failed to flatten and the nuclei of StCs were not separated from one another as shown in Fig. 5B. Scale bars are 20 μm.

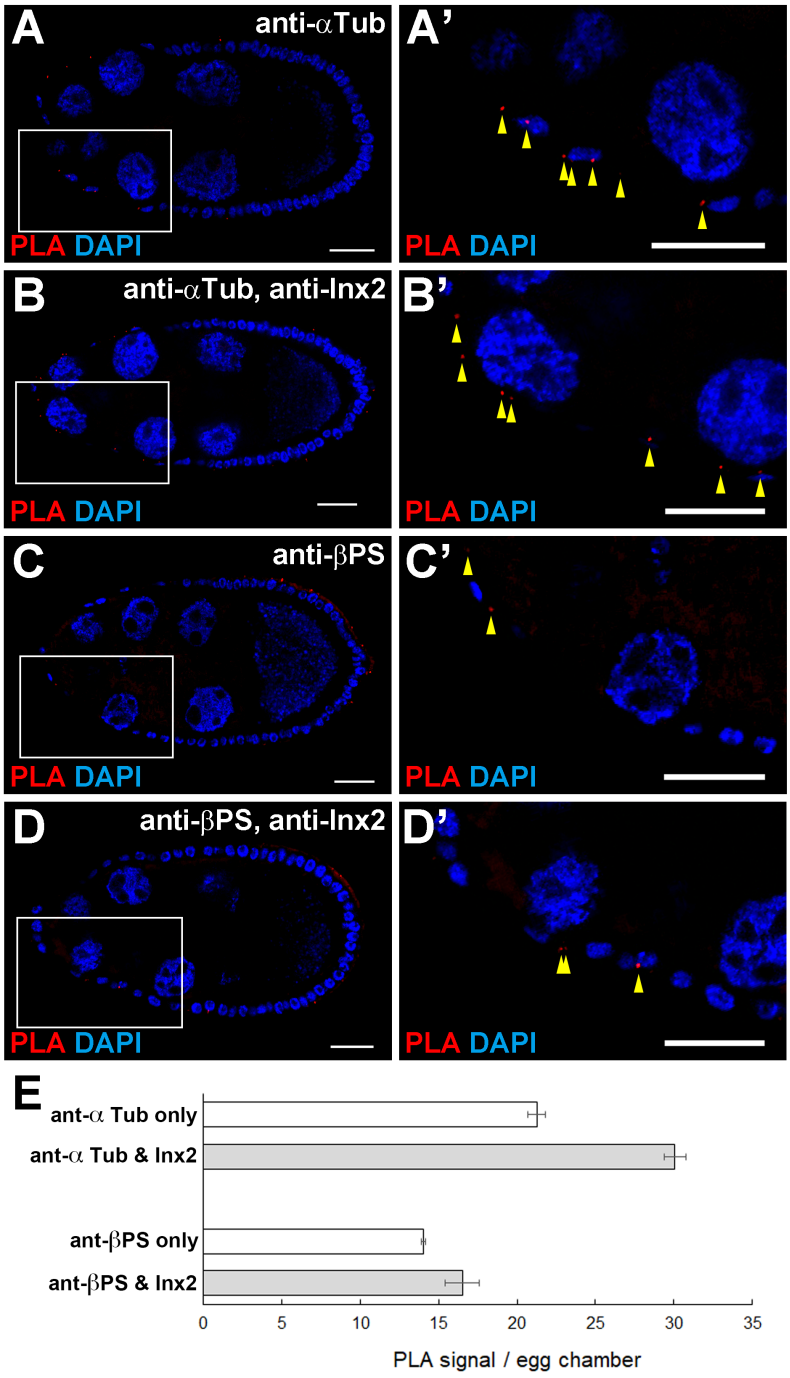

Figure S8. Inx2 does not directly interact with βPS or α Tubulin.
Ovaries of *c306-GAL4;UAS-GFP* flies were dissected for PLA. Egg chambers at stage 9 were selected and oriented as anterior to the left. Signals of PLA were labeled in red indicated by yellow arrowheads and nuclear DNA was stained with DAPI. (A, C) Egg chambers stained with anti-α Tubulin (α Tub) or anti-βPS along for PLA was used as controls. Few PLA signals were observed. (B) Egg chambers stained with anti-α Tubulin and anti-Inx2 for PLA. Few PLA signals were observed. (D) Egg chambers stained with anti-βPS and anti-Inx2. Few PLA signals were observed. Length of scale bar is 20 μm. (E) Quantitative analysis of PLA signals. Bar graph is shown as mean ± SEM. Student *t*-test was used for analysis. No significant difference was observed between controls and anti-α Tubulin and anti-Inx2 or anti-βPS and anti-Inx2.