

Figure S1 Related to Figure 1

Border cell interactions with the oocyte and cfc during neolamination

Images of *slbo*-Gal4, UAS-LifeAct-GFP egg chambers. (A) Stage 10B egg chamber showing the interaction of border cells and cfc. (A') Magnified view of the region outlined in (A). White arrowhead indicates an inwardly migrating cfc. (B) Anti-E-cad

staining showing the apical side of a border cell cluster at stage 10A as it docks to the oocyte and is surrounded by ring canals that connect nurse cells to the oocyte. (C-E) Projection views of stage 10A (C), 10B (D) and 11 (E) egg chambers. (C'-E') Cross sectional views of the regions outlined by dashed boxes in (C-E). (C''-E'') LifeAct-GFP channel of (C'-E'). (C'''-E''') F-actin channel of (C'-E'). Blue arrowheads indicate the ring canals. White arrows indicate the inward migration of cfcs. Asterisks mark border cells. Scale bars: 20  $\mu$ m.

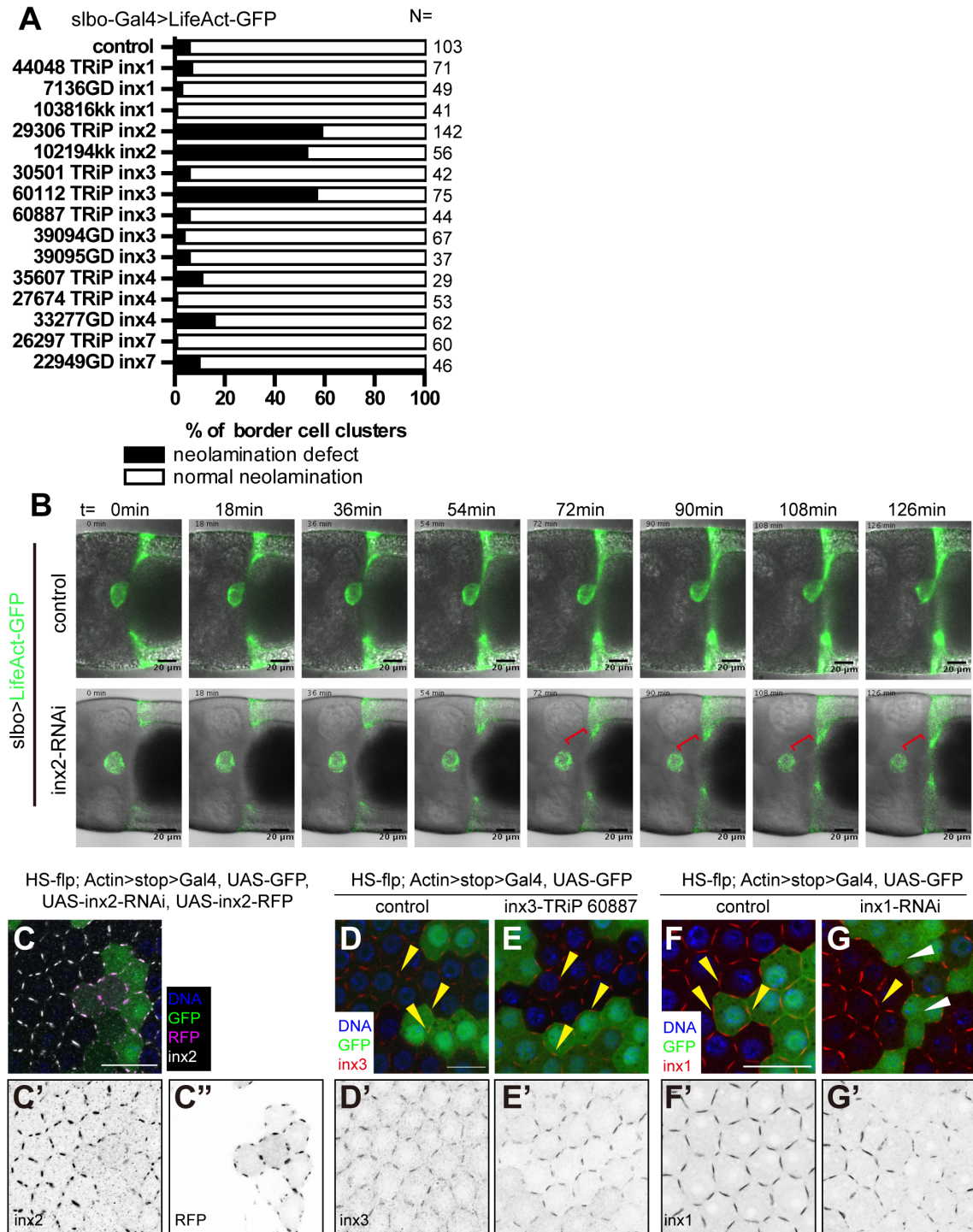


Figure S2 related to Figure 2.

Inx RNAi, rescue, and live imaging

(A) Summary of the frequency of neolamination defects in slbo-Gal4, UAS-LifeAct-GFP driven innexin-RNAi egg chambers. (B) Snapshots of time-lapse movies of a slbo-Gal4,

UAS-LifeAct-GFP/+ egg chambers. The upper row shows a control crossed to  $w^{1118}$ . The bottom row shows *slbo*-Gal4, UAS-LifeAct-GFP/UAS-*inx2*-RNAi egg chamber. Red brackets indicate abnormal spaces between border cells and cfcs. Time (t) is relative to the start of live imaging. (C) Confocal image of overlay of anti-*Inx2* antibody staining (white), RFP (magenta), GFP (green) and DNA (blue) in a patch of follicle cells containing Flp-out clones. Genotype is *HS-Flp; Actin>stop>Gal4, UAS-GFP, UAS-*inx2*-RNAi, UAS-*inx2*-RFP*. (C') Anti-*Inx2* single channel (black). (C'') *Inx2*-RFP single channel (black). *Inx2*-RFP appears resistant to the RNAi. (D-E) Confocal images of overlay of anti-*Inx3* antibody staining (red), GFP (green, cells expressing Gal4) and DNA (blue) in a patch of follicle cells containing Flp-out clones. (E) GFP-positive cells express the ineffective UAS-*inx3*-RNAi line TRiP 60887. (D'-E') Anti-*Inx3* channel only (black). (F-G) Confocal images of overlay of anti-*Inx1* antibody staining (red), GFP (green, cells expressing Gal4) and DNA (blue) in a patch of follicle cells containing Flp-out clones. (F) Control GFP-positive cells show similar staining as GFP-negative cells. (G) GFP-positive cells express the effective UAS-*inx1*-RNAi line and show reduced *Inx1* staining relative to GFP-negative cells. (F'-G') Anti-*Inx1* channel only (black). In (D-G), yellow arrowheads indicate some junctions with *Inx1/3* staining. In (G), white arrowheads indicate some junctions lacking *Inx1* staining. Scale bars: 20  $\mu\text{m}$ .

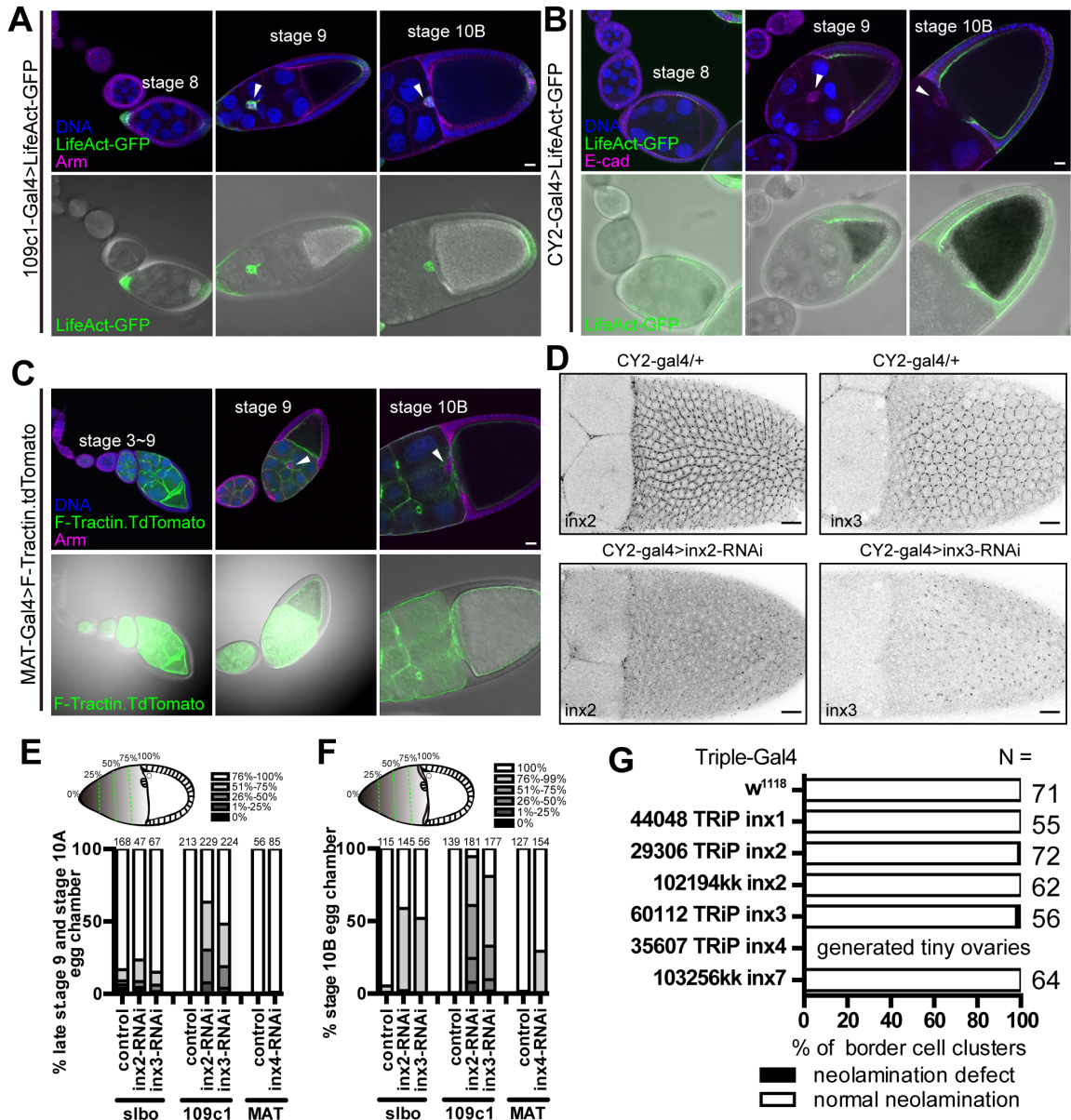


Figure S3 related to Figure 3

Gal4 expression patterns and cell type specific knockdown phenotypes

(A-C) Images of egg chambers showing expression patterns of  $109c1-Gal4$  (A),  $CY2-Gal4$  (B) and  $MAT-\alpha-tub-Gal4$  (C). White arrowheads indicate the border cell clusters.

(D) Images of anti- $inx2/3$  staining showing the knock-down of  $Inx2$  (left panels) and  $Inx3$  (right panels) in follicle cells in  $CY2-gal4 > inx2/3-RNAi$  egg chambers. Scale bars: 20  $\mu m$ .

(E-F) Quantification of migration defects in stage 10A (E) and stage 10B (F) egg

chambers. Histogram showing the spatial distribution of border cell clusters in stage 10A and 10B egg chambers. (G) Summary of the frequency of neolamination defects in triple-Gal4 (MTD-Gal4) driven innexin-RNAi egg chambers.

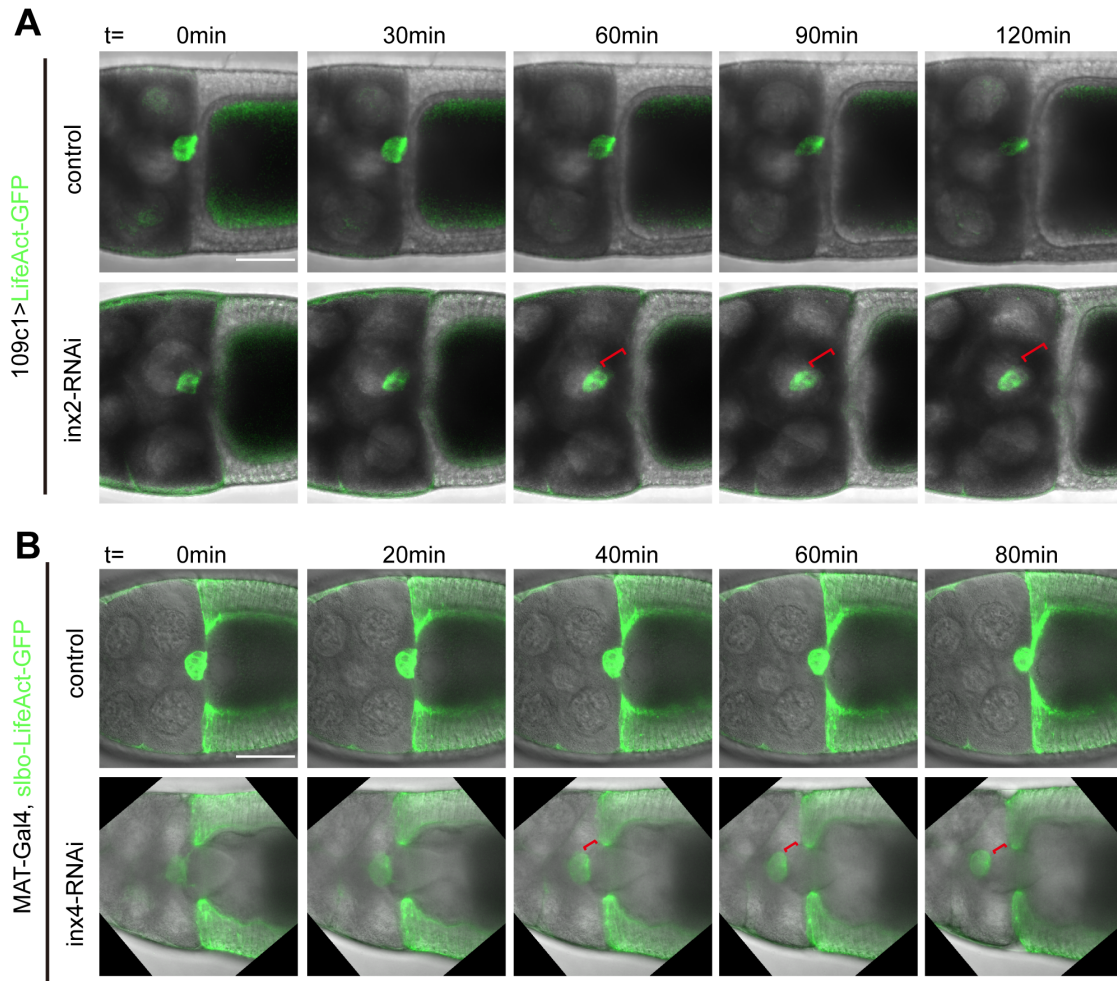


Figure S4 related to Figure 3

Live imaging of egg chambers with border cell *inx2* RNAi and germline *inx4* RNAi

(A) Snapshots of time lapse movies of a *109c1-Gal4, UAS-LifeAct-GFP/+* egg chamber (upper row) and *109c1-Gal4, UAS-LifeAct-GFP/inx2-RNAi* egg chamber (bottom row).

(B) Snapshots of time lapse movies of a *slbo-LifeAct-GFP, MAT- $\alpha$ -tub-Gal4/+* egg chamber (upper row) and *slbo-LifeAct-GFP, MAT- $\alpha$ -tub-Gal4/inx4-RNAi* egg chamber (bottom row). Red brackets indicate the lost interaction of border cells and centripetal cells in *inx2-RNAi* and *inx4-RNAi* egg chambers. Time (t) is relative to the start of live imaging. Scale bars: 20  $\mu$ m.

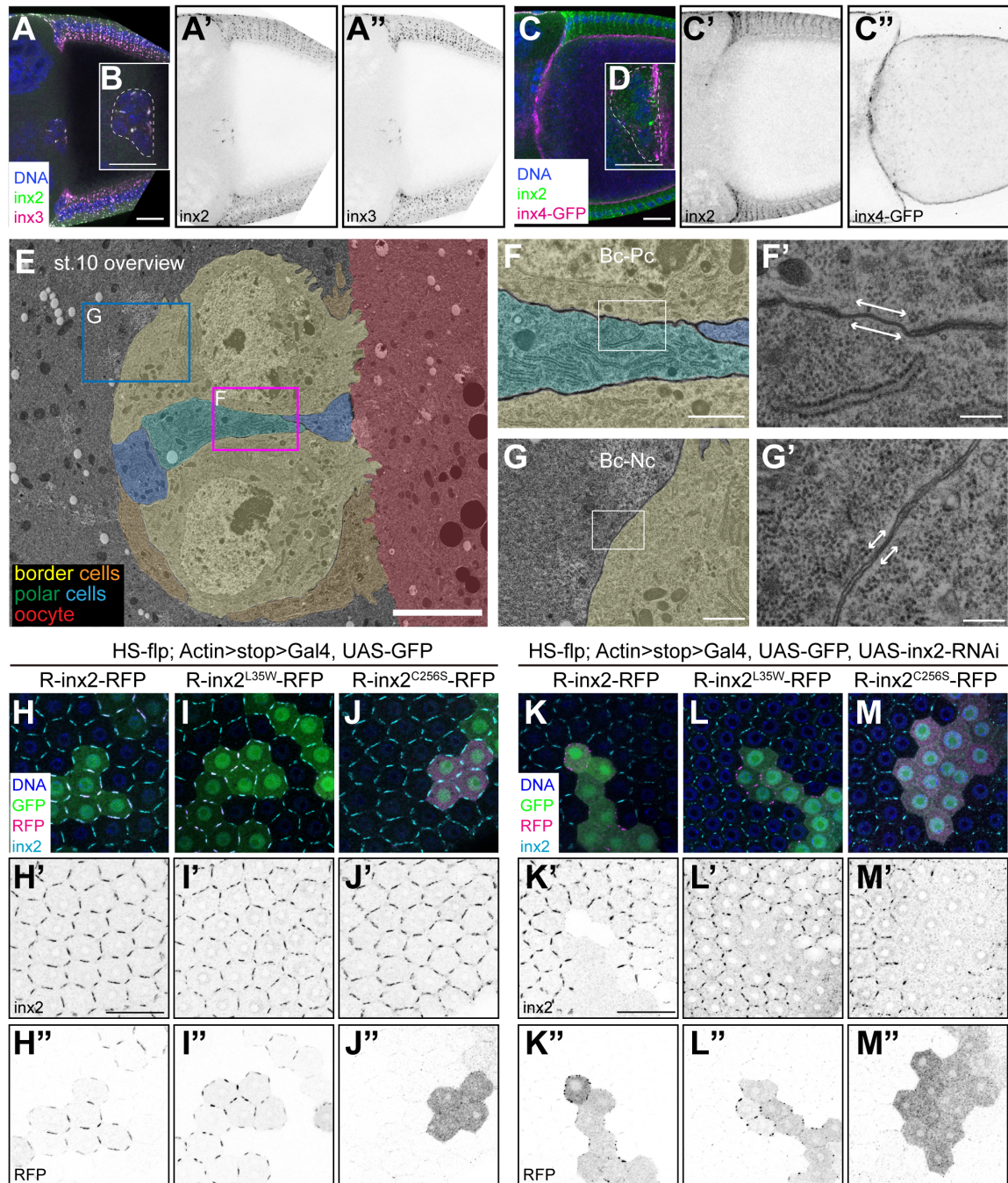


Figure S5 related to Figure 4.

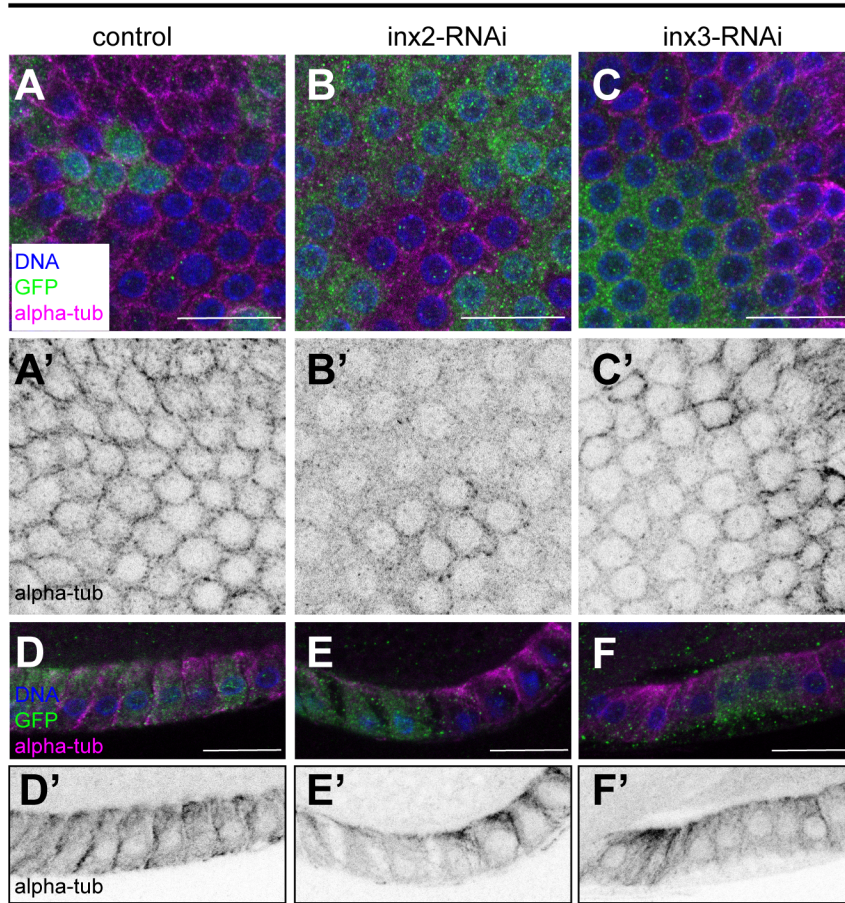
Distributions of Innexins 2,3, and 4 proteins and structural gap junctions in egg chambers

(A) Image of an *w<sup>1118</sup>* egg chamber co-stained with anti-Inx2 and Inx3. (A') Image of Inx2 single channel. (A'') Image of Inx3 single channel. (B) Zoom-in image of border cell



cluster in (A). (C) Image of *inx4*-GFP egg chamber stained with anti-*Inx2*. (C') Image of *Inx2* single channel. (C'') Image of *inx4*-GFP single channel. (D) Zoom-in image of border cell cluster in (C). Scale bars: 20  $\mu\text{m}$ . (E-G) TEM images of a stage 10 egg chamber. (E) Border cells are pseudo-colored yellow and orange color, polar cells green and blue, and the oocyte red. (F-G) Magnified views of the boxes in (E). (F'-G') Magnified view of (F-G). (F) Gap junction between border cell and polar cell. (G) Gap junction between border cell and nurse cell. TEM magnifications: (E) x1.2k, (F) x8k, (G) x6k, (F', G') x30k. Scale bars: (E) 5  $\mu\text{m}$  (F-G) 1  $\mu\text{m}$  (F'-G') 200 nm. (H-M'') HS-flp-out clones showing expression of R-*inx2*-RFP point mutations in follicle cells without (H-J') or with (K-M') *inx2*-RNAi. GFP labels R-*inx2*-RFP-expressing cells. Anti-*Inx2* staining of R-*inx2*-RFP (H-H'', K-K''), R-*inx2*<sup>L35W</sup>-RFP (I-I'', L-L''), R-*inx2*<sup>C256S</sup>-RFP (J-J'', M-M''). Scale bars: 20  $\mu\text{m}$ .

HS-flp; Actin>stop>Gal4, UAS-GFP



slbo-Gal4>UAS-LifeAct-GFP

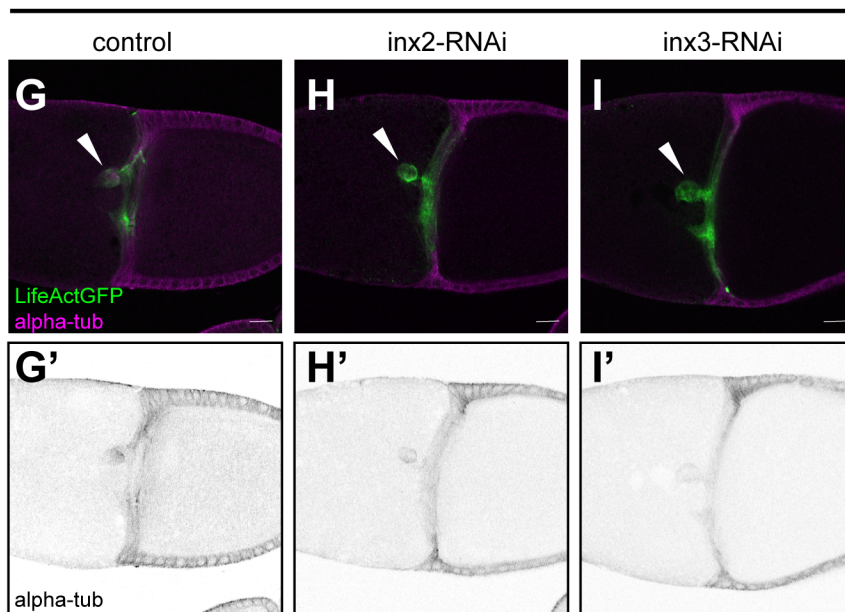


Figure S6 related to Figure 5

Effect of *inx2* and *inx3* RNAi on alpha-tubulin in epithelial follicle cells

(A-F) HSflp-out clone showing the validation of *inx2/3*-RNAi efficiency in follicle cells.

Anti-alpha-tubulin staining of control ( $w^{1118}$ ) (A-A', D-D'), *inx2*-RNAi (B-B', E-E'), *inx3*-RNAi (C-C', F-F'). (G-H) Low magnification view of anti-alpha-tub staining of *slbo-Gal4>UAS-LifeAct-GFP* crossed to  $w^{1118}$  (control) (G-G'), *inx2*-RNAi (H-H') and *inx3*-RNAi (I-I'). White arrowheads indicate the border cell clusters. Scale bars: 20  $\mu\text{m}$ .