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Supplemental Information for

Gap Junction-transported cAMP from the Niche Controls Stem Cell Progeny Differentiation

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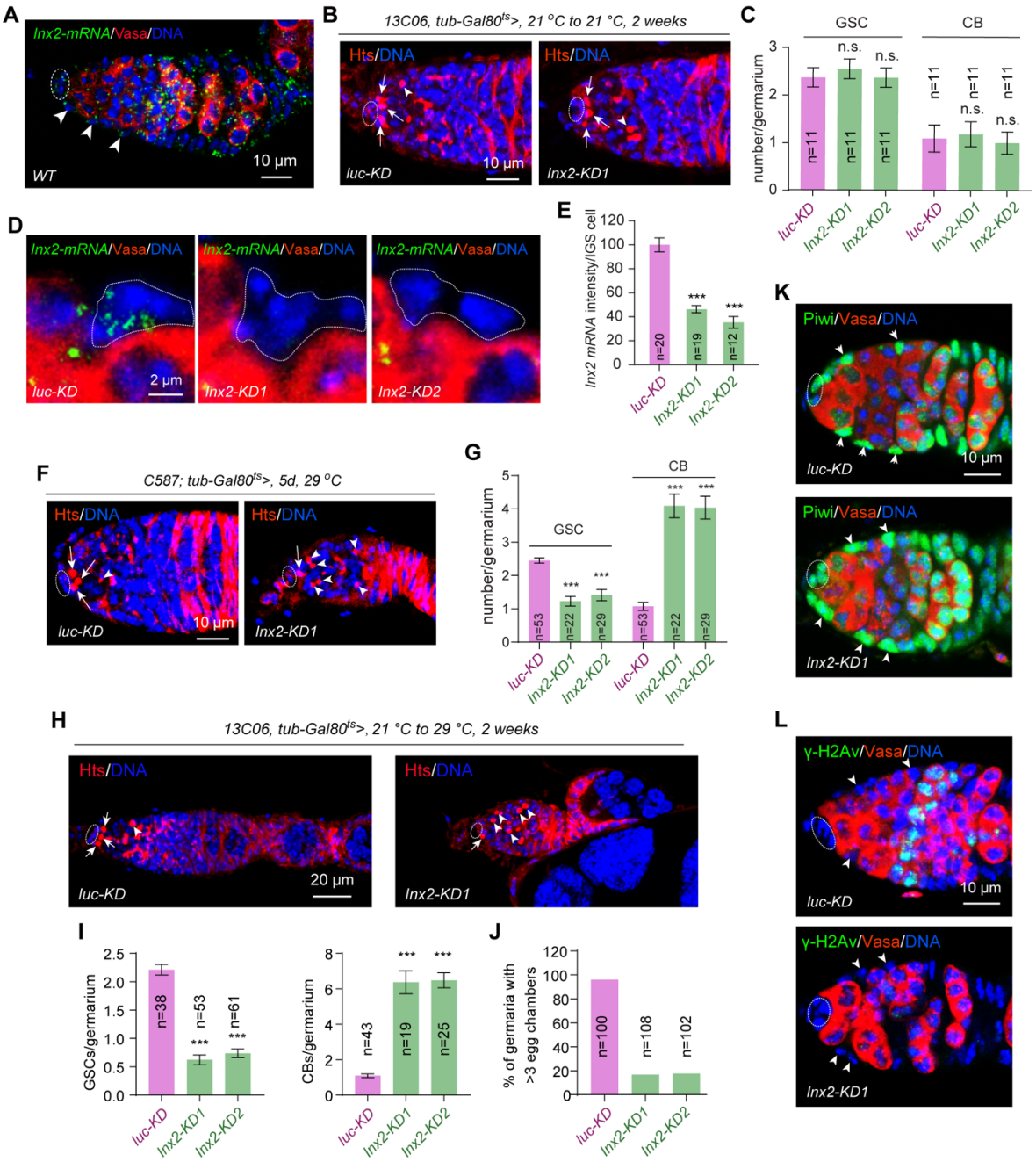

Figure S1. Confirmation of *Inx2* mRNA expression patterns and shRNA knockdown efficiencies in IGS cells.

(**A**) mRNA FISH results show that *Inx2* mRNA is expressed in cap cells (oval), IGS cells (three IGS nuclei by arrowheads), germ cells (Vasa, red) and follicle cells. Scale bars, 10 μm.

(B-C) *13C06, tub-Gal80^{ts}* mediated IGS-specific knockdown at 21 °C of *Inx2* show normal number of GSCs and CBs. **C**: quantification results. n = number of germaria. Scale bars, 10 μm. *Student's t* test: n.s., no significance.

(D-E) Two *Inx2* RNAi lines can efficiently and specifically reduce *Inx2* mRNA expression in IGS cells (highlighted by solid lines). **E:** quantification results of *Inx2* mRNA expression in IGS cells. n = number of IGS cells. Scale bars, 2 μm. Student's t-test: ***p ≤ 0.001.

(**F-G**) *c587-Gal4; tub-Gal80^{ts}* mediated IGS-specific knockdown of *Inx2* decreases GSCs and increases CBs. These phenotypes closely resemble those observed for *13C06^{ts}*-mediated knockdown. **G**: quantification results. n = number of germaria. Scale bars, 10 μm. Student's t-test: ***p ≤ 0.001.

(**H-J**) IGS-specific two-week knockdown of *Inx2* increases undifferentiated CBs, decreases GSCs and disrupt egg chamber development compared to its one-week knockdown. **J**: quantification results. n = number of germaria. Scale bars, 20 μm. Student's t-test: ***p ≤ 0.001.

(K) IGS-specific 3-day knockdown of *Inx2* does not show the obvious loss of IGS cells (arrowheads). The Argonaute/Piwi family protein, P-element induced wimpy testis (Piwi), is highly expressed in Cap cells (highlight by oval), IGS cells and weakly in GSCs and their progeny. Vasa labels GSCs and progeny, IGS cells are identified by Vasa-negative and Piwi-positive staining. Scale bars, 10 μm.

(L) IGS-specific 3-day knockdown of *Inx2* does not cause the accumulation of DNA damage in IGS cells (arrowheads, Vasa-negative). Scale bars, 10 μm.

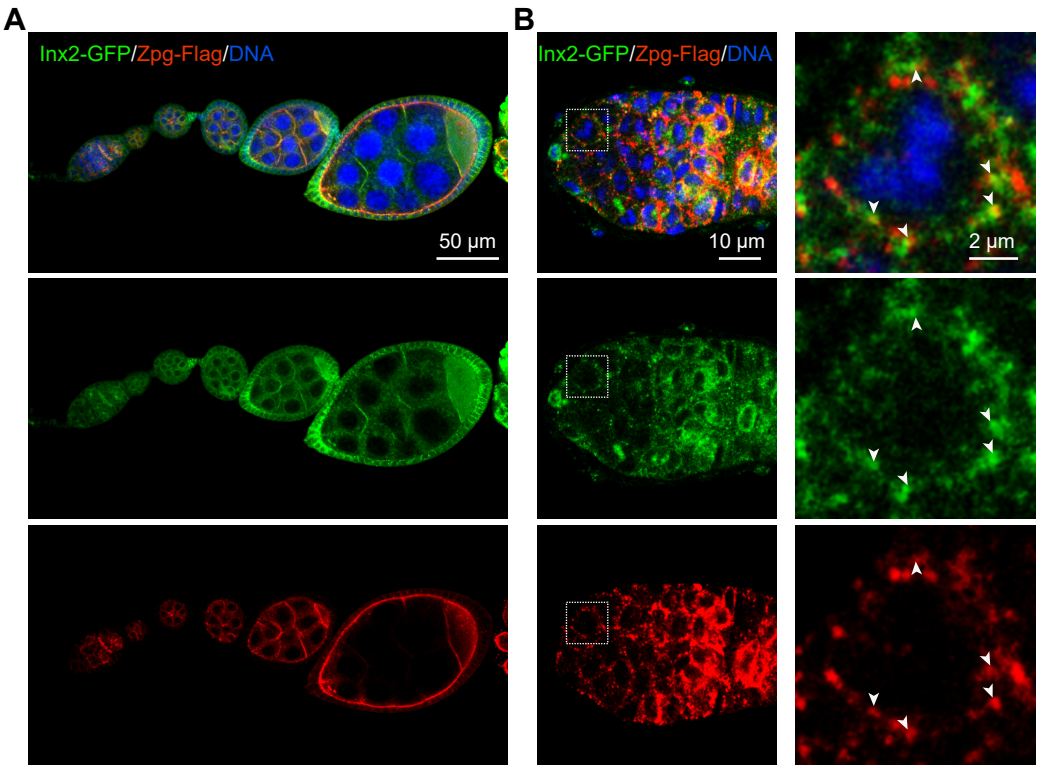

Figure S2. Expression and localization of Inx2-GFP and Zpg-Flag in the germarium and early egg chambers.

(**A**) Inx2-GFP and Zpg-Flag are expressed throughout the ovary. Inx2-GFP is expressed primarily in all somatic cell types of germarium, including IGS cells and follicle cells, while Zpg-Flag is enriched in germ cells. Scale bars, 50 μm.

(**B**) IGS-expressed Inx2-GFP and germline-expressed Zpg-Flag are partly colocalized at the inter-cellular junctions. Scale bars, 10 μm and 2 μm.

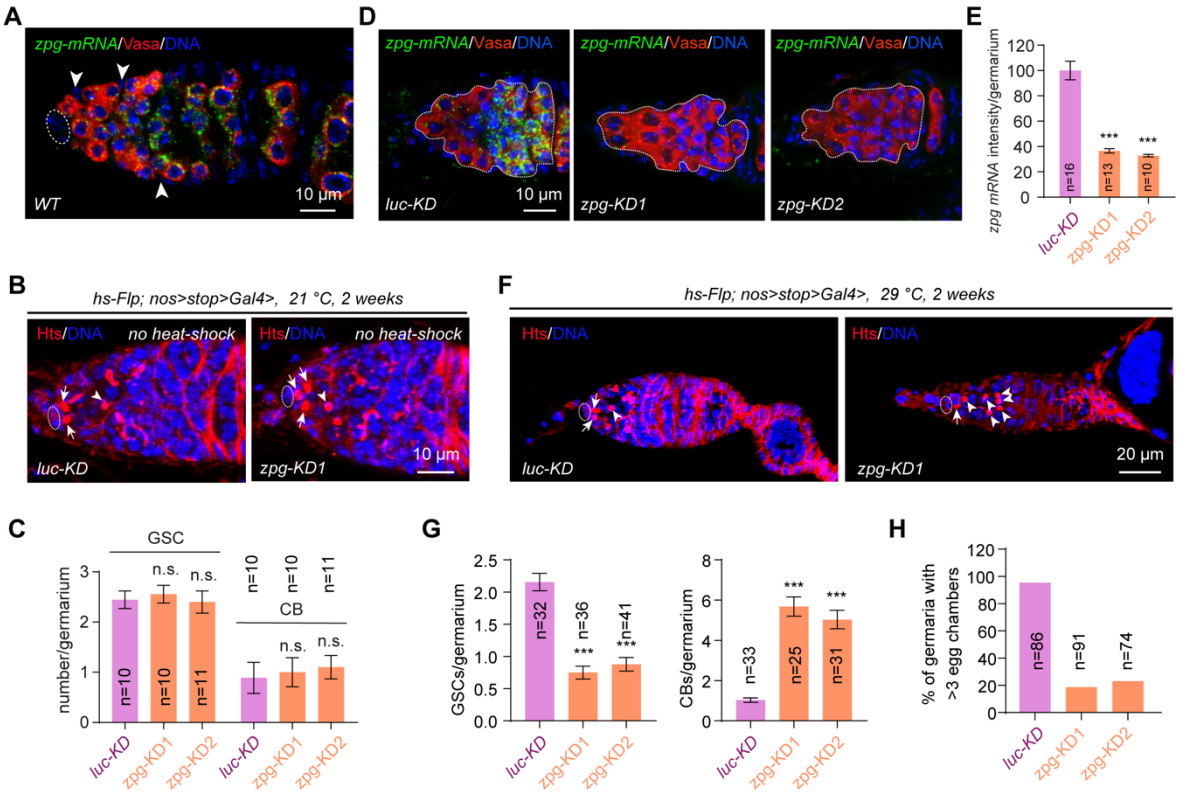

Figure S3. Validation of *zpg* mRNA expression patterns and shRNA knockdown efficiencies in germ cells.

(**A**) *zpg* is expressed in germ cells (Vasa, red) but not in cap cells (oval), IGS cells (three nuclei indicated by arrowheads) and other somatic cells. Scale bars, 10 μm.

(**B-C**) *hsFlp; nos>stop>Gal4* mediated germline-specific knockdown (without 37 °C heat-shock) at 21 °C of *zpg* shows normal number of GSCs and CBs. **C**: quantification results. n = number of germaria. Scale bars, 10 μm. Student's t-test: n.s., no significance.

(**D-E**) Two *zpg* RNAi lines can efficiently and specifically reduce *zpg* mRNA expression in germ cells. **E:** quantification results on *zpg* mRNA FISH in germ cells (highlighted by lines). n = number of germaria. Scale bars, 10 μm. Student's t-test: ***p ≤ 0.001.

(**F-G**) Germline-specific two-week knockdown of *zpg* results in more CBs and less GSCs than its one-week knockdown. **G**: quantification results. n = number of germaria. Scale bars, 10 μm. Student's t-test: ***p ≤ 0.001.

(H) Two-week *zpg-KD* disrupts normal egg chamber development. n = number of germaria.

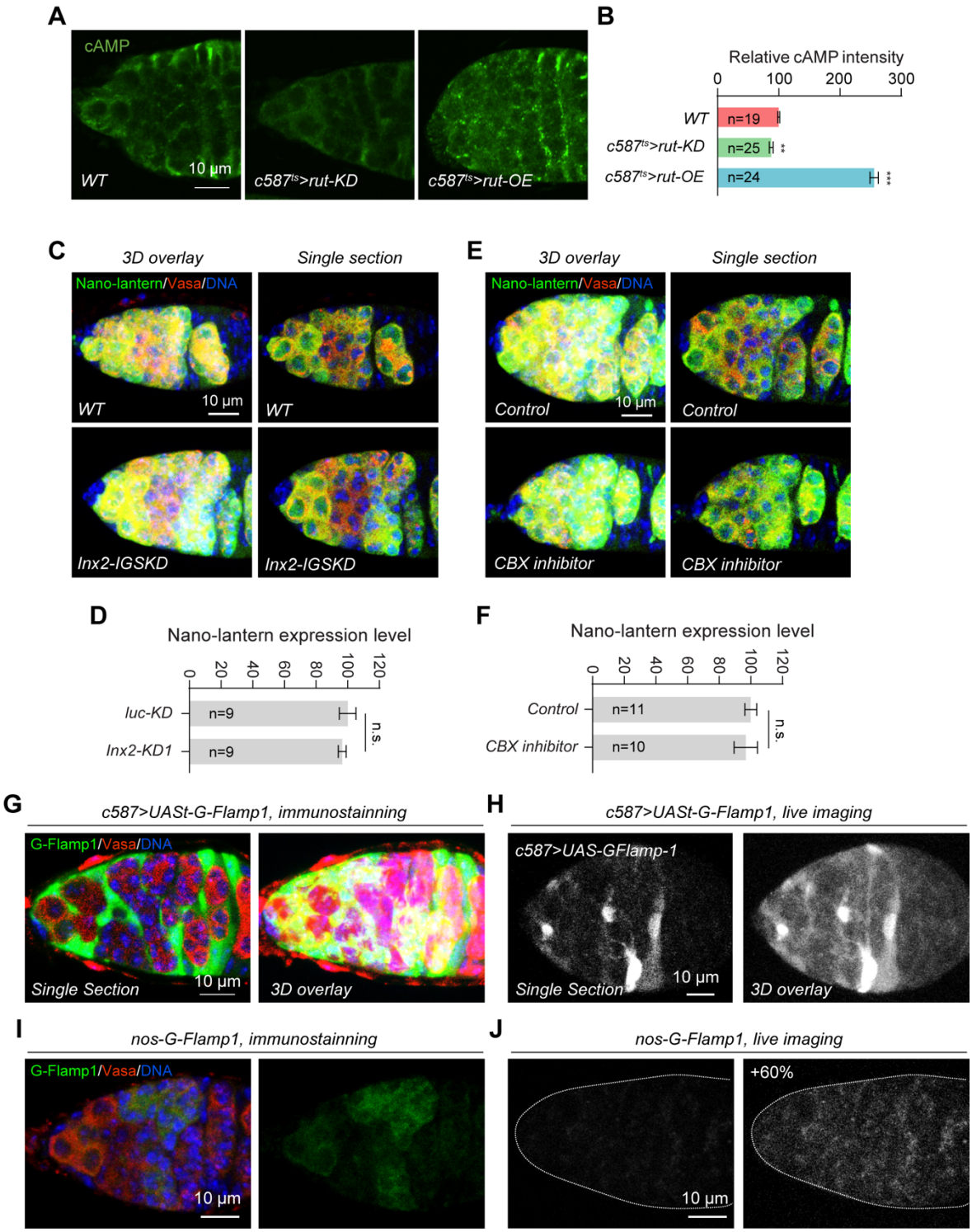

Figure S4. Validation of anti-cAMP antibody and cAMP reporters.

(**A-B**) Rut is required for cAMP production. Single section confocal images showing cAMP levels in IGS cells decrease in the *rut-KD* germarium and increase in the *rut*-overexpressing (*rut-OE*) germarium compared to the control. Scale bar: 10 μm. **B:** Quantification results. n = number of germaria. Student's t-test: ***p ≤ 0.001, **p ≤ 0.01.

(**C-F**) The Nano-lantern is a GFP protein derivative so an anti-GFP antibody can also recognize the Nano-lantern. Single section and 3D overlay confocal images show *nos-Nano-lantern* is specifically expressed in germ cells (labeled by Vasa). The expression level of Nano-lantern is not affected in the *Inx2-KD* or CBX inhibitor-treated germaria. **D** and **F**: quantification results, n.s., no significance, Scale bars, 10 μm.

(**G-H**) Immunostaining (G) and live imaging (H) of germaria using another IGS-specific overexpressed cAMP reporter, G-Flamp1, revealed a remarkably high signal intensity in IGS cells. Scale bars, 10 μm.

(**I-J**) Immunostaining (I) and live imaging (J) of germaria using germline-specific nos-G-Flamp1. However, G-Flamp1 is expressed at relative low levels in germ cells and is almost not visible in live imaging. When increasing by 60%, a weak signal can be observed. Scale bars, 10 μm

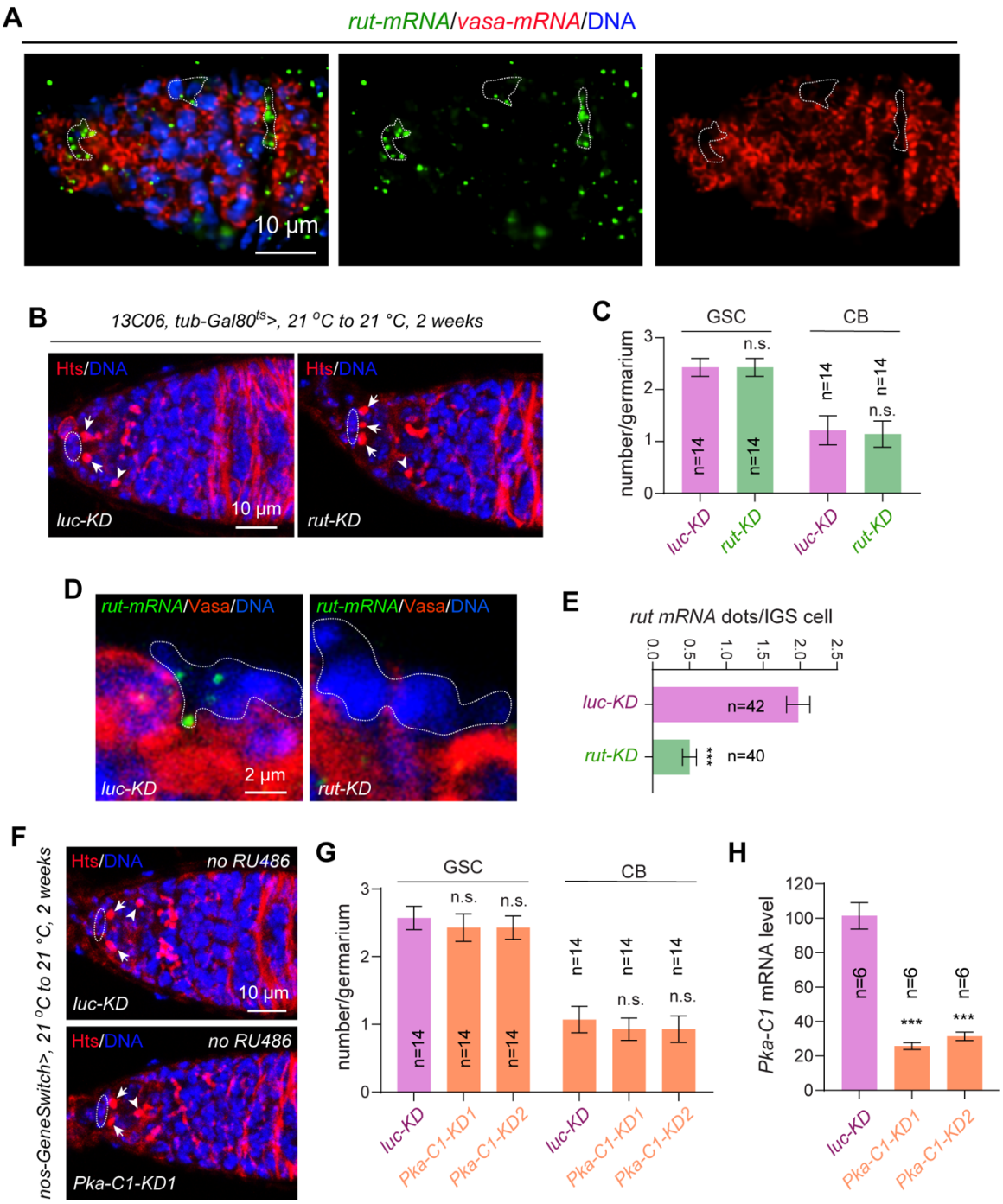

Figure S5. Validation of *rut* expression patterns as well as *rut* and *Pka-C1* knockdown efficiencies in the *Drosophila* germarium.

(**A**) Single section confocal image showing that *rut* mRNA (green) is expressed strongly in IGS cells and weakly in germ cells. *vasa* mRNA (red) labels germ cells. Scale bars, 10 μm.

(**B-C**) *13C06, tub-Gal80*^{ts} mediated IGS-specific *rut* knockdown at 21 ℃ does not have any impact on GSC and CB numbers. **C**: quantification results. n = number of germaria. Scale bars, 10 μm. Student's t-test: n.s., no significance.

(**D**-**E**) *rut* RNAi line can efficiently decrease *rut* mRNA levels in IGS cells (Vasa-negative, highlighted by lines). **E:** quantification results on *rut mRNA* spots per IGS cell. n = number of IGS cells. Scale bars, 2 μm. Student's t-test: ***p ≤ 0.001.

(**F-G**) *nos-GeneSwitch* mediated germline-specific *Pka-C1* knockdown (without RU486 administration) at 21 ℃ does not affect GSC and CB numbers. **F**: quantification results. n = number of germaria. Scale bars, 10 μm. Student's t-test: n.s., no significance.

(**H**) Real-time qPCR results show that two independent *nos-GeneSwitch*-driven *Pka-C1* shRNA-mediated knockdowns can efficiently decrease *Pka-C1* mRNA levels (normalized to internal control, *RpL10*). The primers used in this experiment are as follows: For *Pka-C1*, the forward primer is *attcagttccccttcctcgtct* and the reverse primer is *tactcgaaggccagcacgattt*. For *RpL10*, the forward primer is *atgctaagctgtcgcacaaatg* and the reverse primer is *gttcgatccgtaaccgatgt*. Student's t-test: ***p ≤ 0.001.