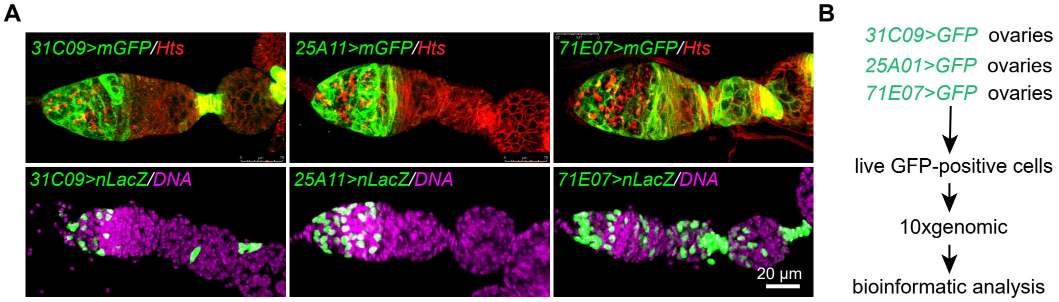

Figure S1. *71E07* and *31C09* are expressed in other somatic cells in addition to IGS cells. Related to Figure 1. (A) *31C09-Gal4-*, *25A11-Gal4-* and *71E07-Gal4-driven UAS-mGFP* or *UAS-nLacZ* expression patterns in the germarium and early egg chambers. In addition to IGS cells, *31C09-Gal4* is also expression in stalk cells, while *71E07-Gal4* is also expressed in stalk cells and follicle cells. Scale bars, 10 µm. (B) Experimental procedures for IGS scRNA-seq.

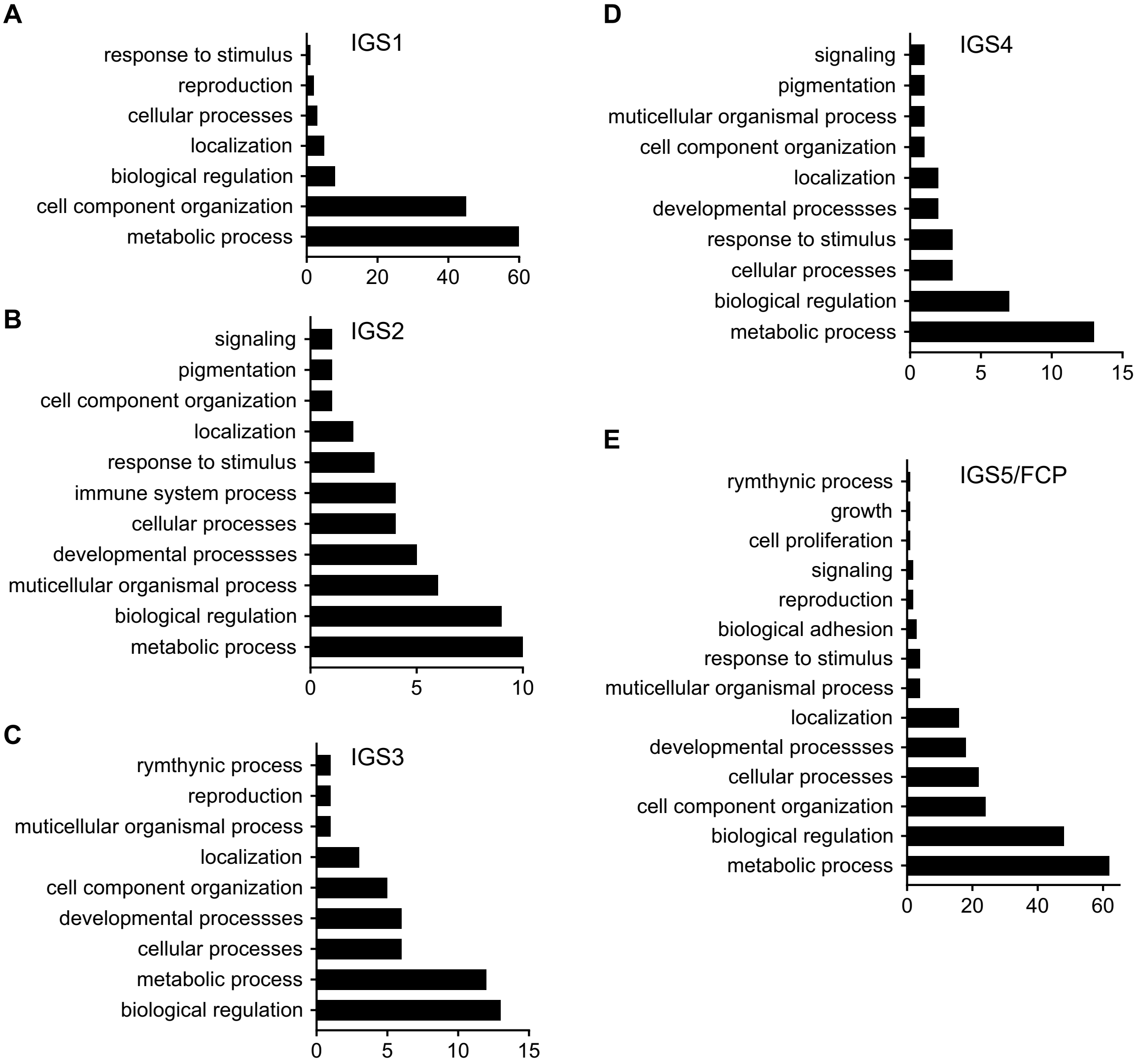

Figure S2. GO term analyses of the five IGS subpopulation-enriched genes. Related to Figure 1. (A-E) Graphs showing the overrepresented GO terms for the genes enriched in IGS1-4 and IGS5 (FCP) subpopulations.

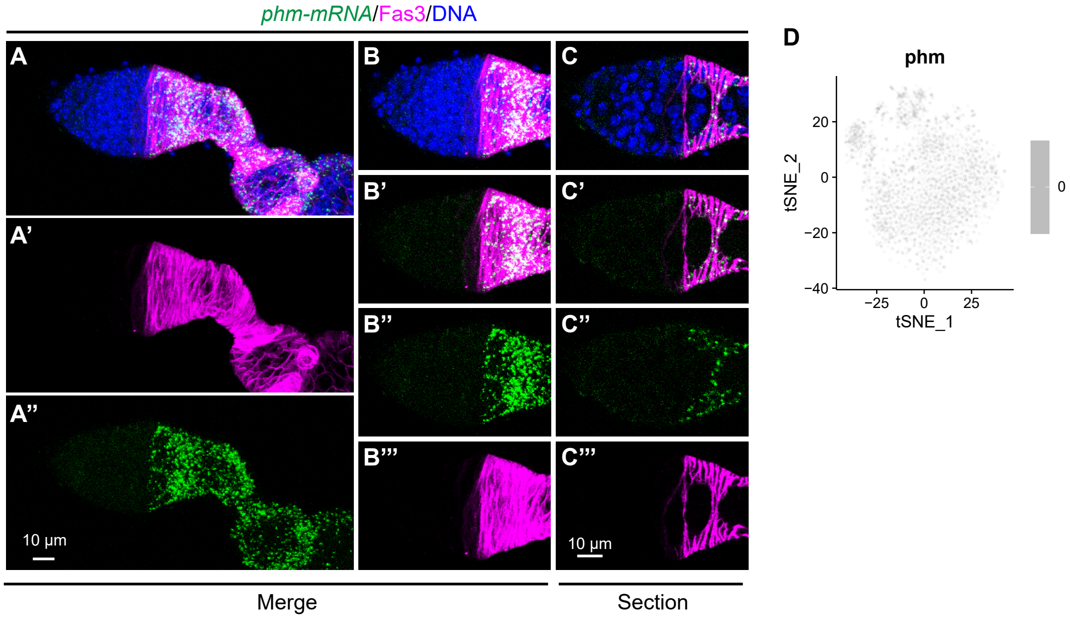

Figure S3. *phm* is expressed in Fas3-expressing differentiated follicle cells. Related to Figure 2. (A-C’’’) Immunostaining and FISH confocal images showing the expression patterns of *phm* and Fas3 in the *Drosophila* germarium. B-B’’’ are enlarged images, C-C’’’ are single section confocal images. Scale bars, 10 µm. (D) t-SNE plot of *phm* showing a lack of its expression in IGS cells.

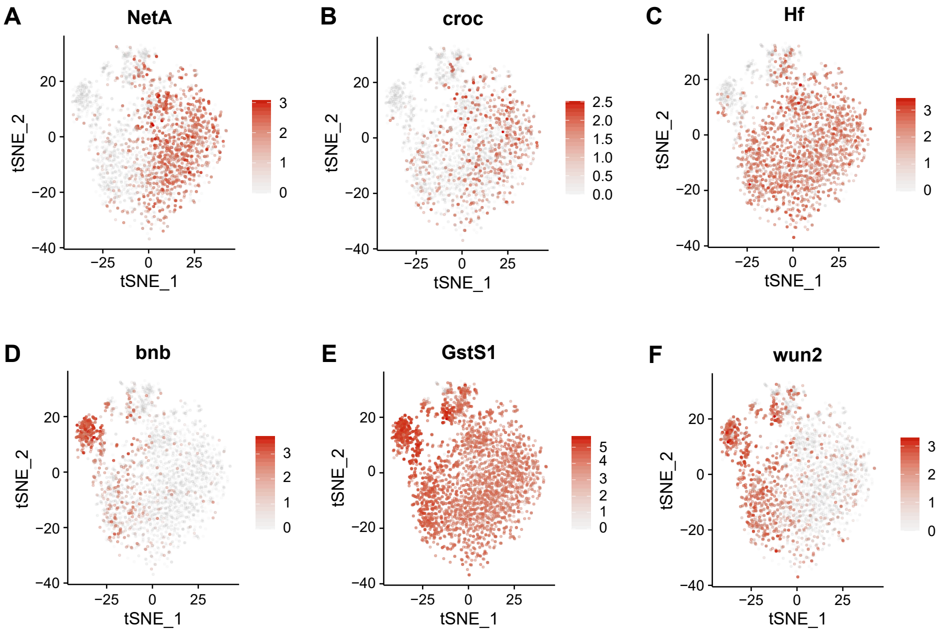

Figure S4. IGS subpopulations-specific genes. Related to Figure 2. (A-F) t-SNE plots of *NetA*, *croc*, *Hf*, *bnb*, *GstS1* and *wun2* in IGS populations.

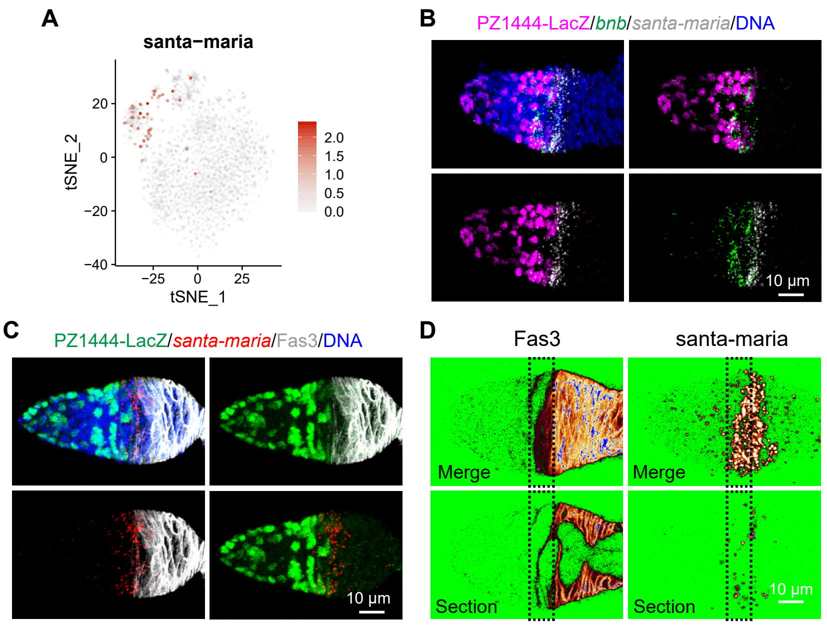

Figure S5. *santa-maria* is mainly expressed in the FCP. Related to Figure 2. (A) t-SNE plot showing *santa-maria* is mainly expressed in the FCP cluster (previously identified as IGS5). (B) Immunostaining and FISH confocal images showing that the *bnb*-expressing region is overlapped with the most posterior PZ1444-positive IGS cells, while the *santa-maria*-expressing region is located posterior to PZ1444-positive IGS cells. Scale bars, 10 µm. (C) Immunostaining and FISH confocal images showing that *santa-maria* is mainly expressed between PZ1444-positive IGS cells and Fas3-positive follicle cells. Scale bars, 10 µm. (D) Section and merged confocal images showing *santa-maria* expression mainly in low Fas3-expressing FCP cells. Scale bars, 10 µm.

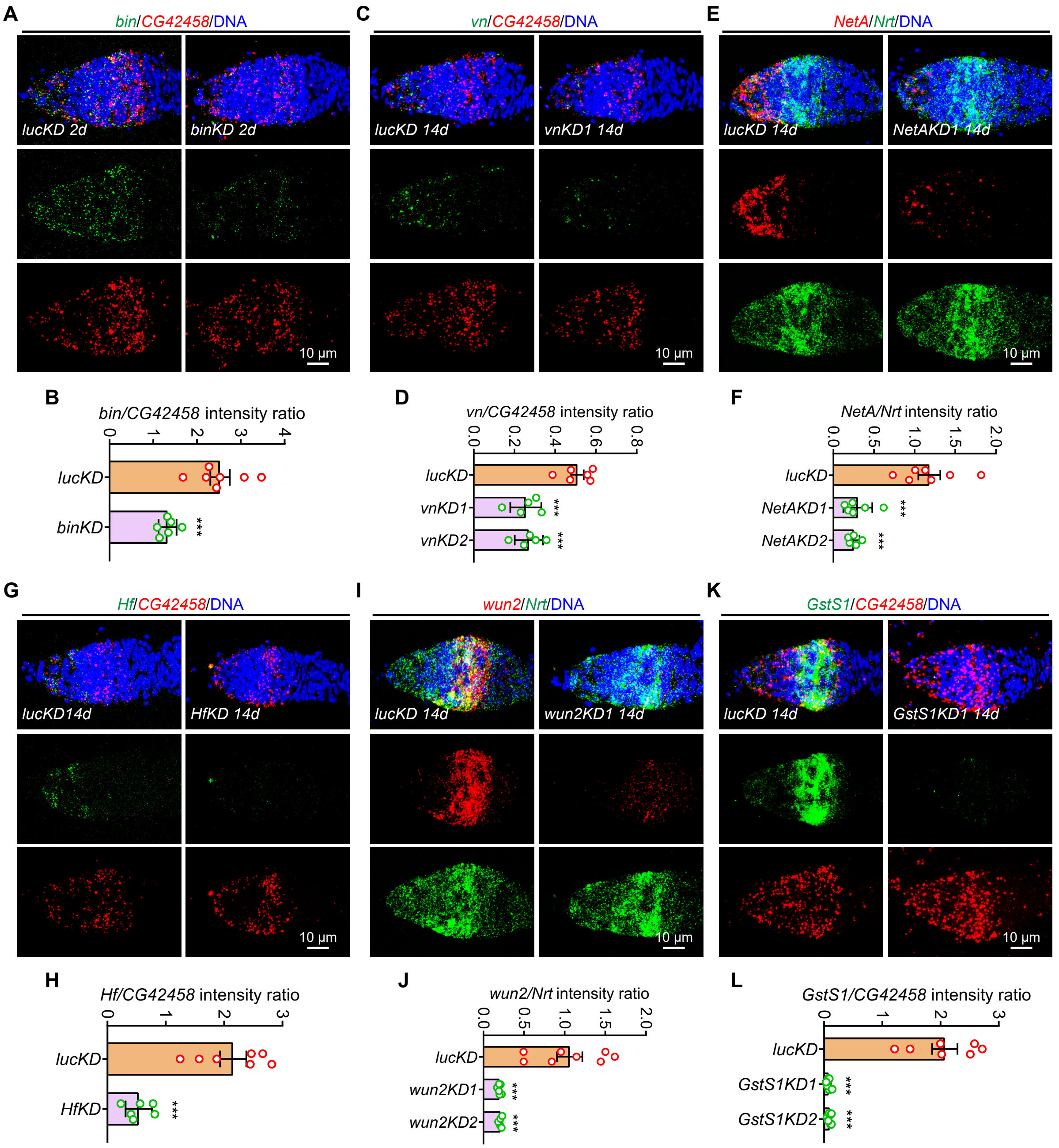

Figure S6. *c587*^{ts}-driven shRNA expression can efficiently knock down *bin*, *vn*, *NetA*, *Hf*, *wun2* and *GstS1* in adult IGS cells. Related to Figures 5, 6 and 7. (A-L) mRNA FISH results show that *c587*^{ts}-driven shRNA expression can significantly descrease the mRNA expression of *bin* (A, B), *vn* (C, D), *NetA* (E, F), *Hf* (G, H), *wun2* (I, J) and *GstS1* (K, L) in adult IGS cells. Internal controls: *CG42458* for A, C, G, K; *Nrt* for E and I. mRNA FISH quantification results: B, D, F, H, J and L. Scale bars, 10 µm. Student's t-test: ***, $P \leq 0.001$.

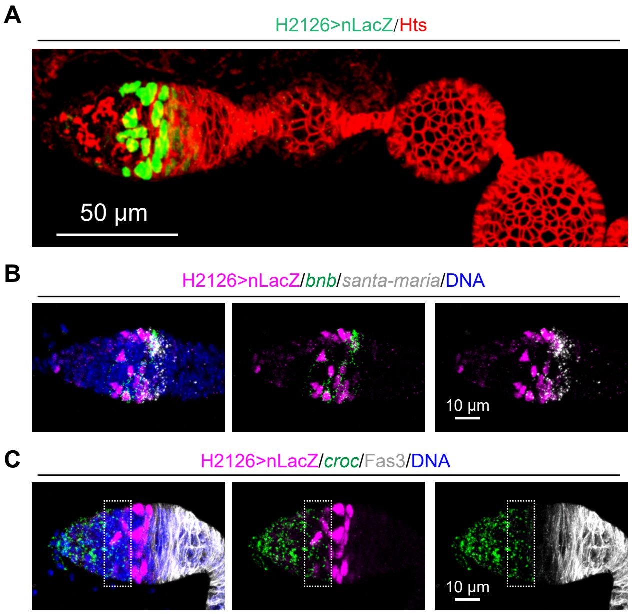

Figure S7. *H2126-SwitchGal4* is mainly expressed in IGS4 and also in some IGS3 cells. Related to Figure 7. (A) Confocal images showing the *H2126*-driven *UAS-nLacZ* expression pattern in the germarium. Scale bars, 50 µm. (B) Immunostaining and FISH confocal images showing that the H2126-expressing region physically contacts the *santa-maria*-expression region and much broader than the *bnb*-expressing region. Scale bars, 10 µm. (C) Immunostaining and FISH confocal images showing that the *H2126*-expressing region is overlapped with the *croc*-expression region. Scale bars, 10 µm.