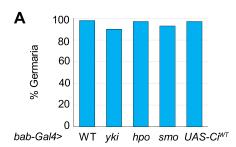
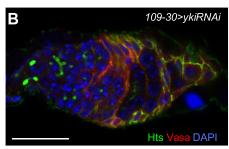


Fig. S1. Activation of Yki in ECs produces no significant defects. (A-G) Germaria from adult females with UAS-CD8-RFP and C587-GAL4 (A) only or together with (B) UAS-yki, (C-E,H) the indicated UAS-RNAi transgenes, or (F) UAS-hpo or (G) UAS-wts. CD8-RFP (red) principally labels the surface of ECs and Hts (green) antibody labels round spectrosomes (arrowheads) and fusomes (arrows), as well as the cortical cytoskeleton of Follicle cells. No major differences were observed in germarial morphology or germline cyst maturation for any of these genotypes. Scale bar (same for all) is 20 μm.





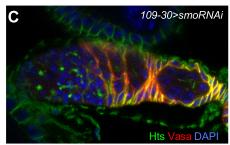


Fig. S2. Germline differentiation defects are not due to Yki and Smo actions in terminal filament, cap cells, or FSCs.

- (A) Percentage of germaria with a normal complement of spectrosomes (less than or equal to six) when the indicated UAS transgenes were expressed in terminal filament and cap cells using bab-GAL4. n= 168, 88, 127, 133 and 129 biologically independent germaria, in the order shown.
- (B,C) Germaria stained with antibody to Hts (green), Vasa (red) as well as DAPI (blue nuclei) and expressing (B) UAS-yki RNAi or (C) UAS-smo RNAi using 109-30-GAL4. No changes in germline differentiation are evident.

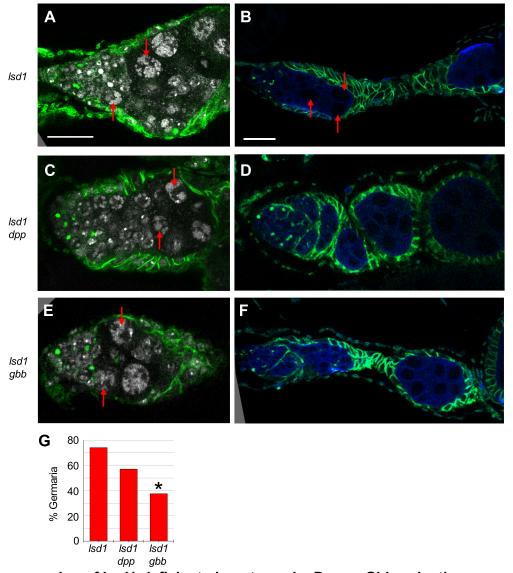


Fig. S3. Suppression of Lsd1-deficient phenotypes by Dpp or Gbb reduction. (A-G) C587-GAL4 was used to express UAS-lsd1 RNAi (A, B) alone or together with (C, D) UAS-dpp RNAi or (E, F) UAS-gbb RNAi. (A,C,E) Germaria labeled with antibody to Hts (green) to visualize spectrosomes (anterior (left) dots) and DAPI (gray) to stain all nuclei exhibited severe morphological defects, including failure of cyst maturation and abnormally large germline nuclei (red arrows). (B,D,F) Germaria stained for Hts (green) and Vasa (blue). (B) Most Isd1 RNAi germaria could bud off egg chambers despite morphological defects. Red arrows indicated abnormally large germline nuclei. (D,F) Germaria were scored as morphologically normal if they did not contain abnormally large germline cells, even if (D) they did contain an abnormal number of spectrosomes. Both the proportion of ovarioles classified as morphologically abnormal due to the presence of large germline nuclei in the germarium (red arrows in A-C,E) and the severity of these defects were reduced by dpp RNAi and by gbb RNAi. Same magnification for (A, C, E) and (B, D, G); scale bars 20 µm. (G) The frequency of morphological defects was slightly reduced by dpp RNAi and more substantially by qbb RNAi. n= 58 (lsd1), 42 (lsd1+dpp) and 40 (lsd1+gbb) biologically independent germaria. Significant differences imposed on Isd1 RNAi phenotypes by dpp RNAi or gbb RNAi were calculated using Fisher's exact two-tailed test (* p<0.01).

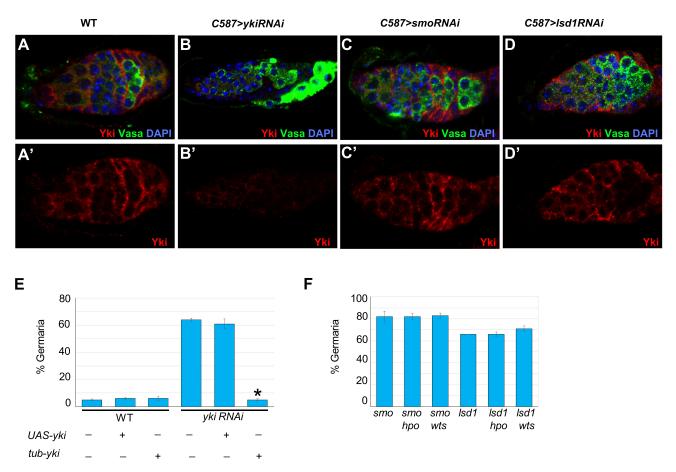


Fig. S4. Yki activity does not mediate Hh pathway actions in ECs that affect germline differentiation.

(A-D) Yki antibody staining (red) together with Vasa antibody staining of germline cells (green) or (A'-D') alone in (A, A') wild-type germaria was (B, B') greatly reduced in ECs by UAS-yki RNAi but unaltered by (C, C') UAS-smo RNAi or (D, D') UAS-lsd1 RNAi expression with C587-GAL4. Scale bar for all is 20 μm. (E) Percentage of germaria with 6 or more spectrosomes for females containing the indicated transgenes in addition to C587-GAL4. The yki RNAi transgene targets regions of yki that are present in both UAS-yki and tub-yki transgenes. Consequently, the rescue activity of those transgenes in the presence of UAS-yki RNAi is expected to be much lower than for genotypes where UAS-yki RNAi is absent (as in Fig. 6). n= 310, 282, 265, 333, 248 and 310 biologically independent germaria, in the order shown. Significant differences imposed on the yki RNAi phenotype by UAS-yki or tub-yki were calculated using Fisher's exact two-tailed test (* p<0.0001; for all others p>0.05). (F) Percentage of germaria with more than 6 spectrosomes for animals containing the indicated transgenes in addition to C587-GAL4. Mean and SEM for three (smo) or two (lsd1) trials are shown using a total of n= 216, 196, 316, 247, 166 and 172 biologically independent germaria, in the order shown. No significant differences (p<0.05) were seen for the effect of hpo RNAi or wts RNAi on smo RNAi or lsd1 RNAi phenotypes by Fisher's exact two-tailed test.