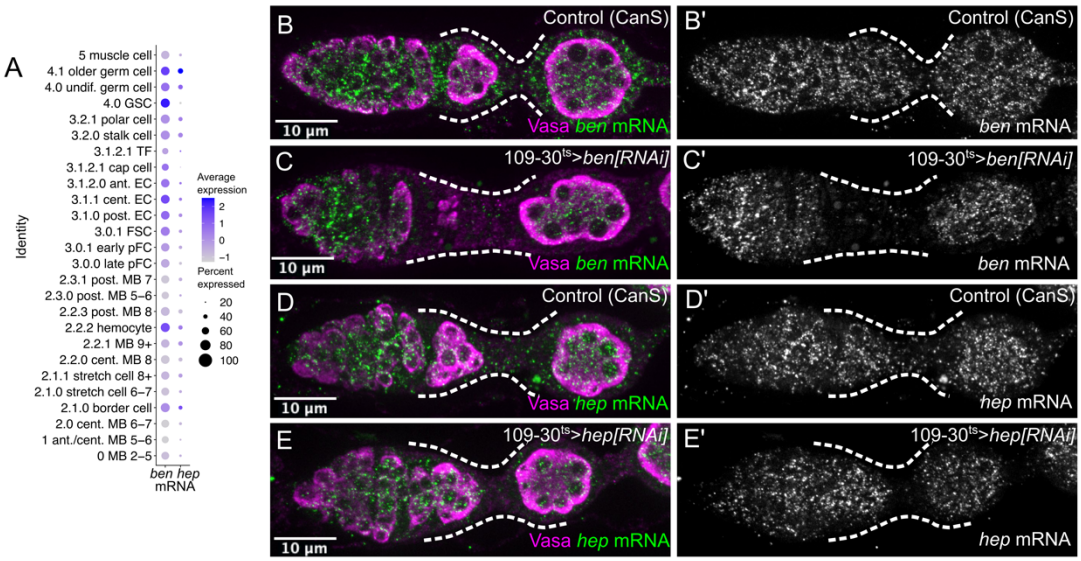

Fig. S1. *ben* and *hep* are broadly expressed in the germarium.

(A) Dotplot showing the expression of *ben* and *hep* mRNA detected in each cell type by single cell RNA sequencing of the ovary (Rust, et al., 2020). (B-E) Ovarioles from Canton-S (CanS) flies (B and D) or with *109-30^{ts}* driving *ben[RNAi]* or *hep[RNAi]* stained for *ben* or *hep* transcripts using HCR (green) and Vasa (magenta). The HCR channel is shown separately in B'-E'. *109-30-Gal4* is expressed in follicle cells within the germarium (white dashed lines) (Hartman et al., 2010; Sahai-Hernandez and Nystul, 2013) absence of signal in these cells in C and E confirms that the HCR probes are specific for their target transcripts and that the RNAi lines are effective in knocking down mRNA expression.

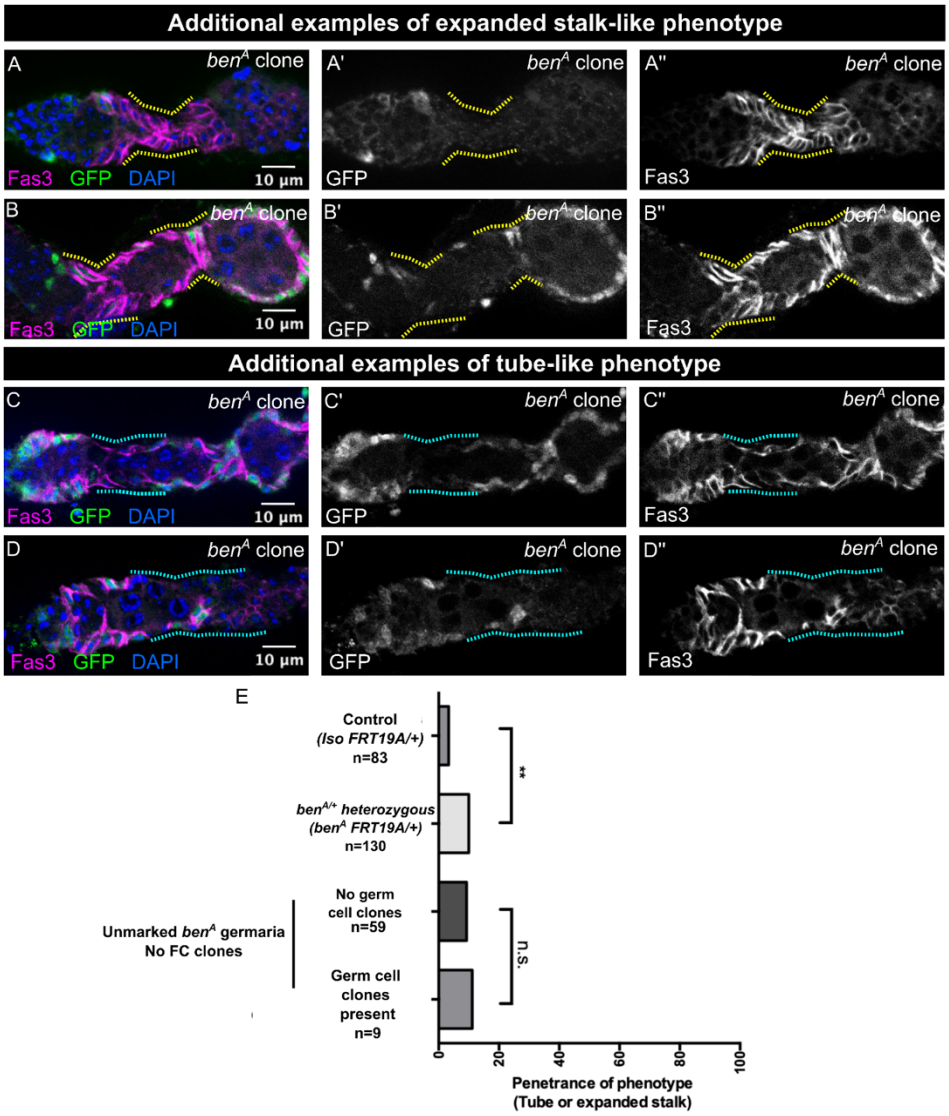

Fig. S2. *ben*^{A} clones exhibit a range of morphological phenotypes.

(A-D) Ovarioles with GFP^{-} *ben*^{A} clones stained for Fas3 (magenta), GFP (green), and DAPI (blue). Expanded stalk (yellow dotted lines) and tube-like phenotypes (cyan dotted lines) are indicated. (E) Quantification of the frequency of ovarioles with either and expanded stalk or tube-like phenotype in wildtype control (*IsoFRT19A*/+), *ben*$^{A/+}$ heterozygous (*ben*^{A}, *FRT19A*/+), and "unmarked" *ben*^{A} germaria (*ben*^{A}, *FRT19A*/*hsFlp*, *Ubi-GFP*, *FRT19A*) that do not have any follicle cell (FC) clones but either do or do not germ cell clones. Chi-squared test, ** = p<0.01, n.s. = not significant, n (number of ovarioles examined) are indicated in (E).

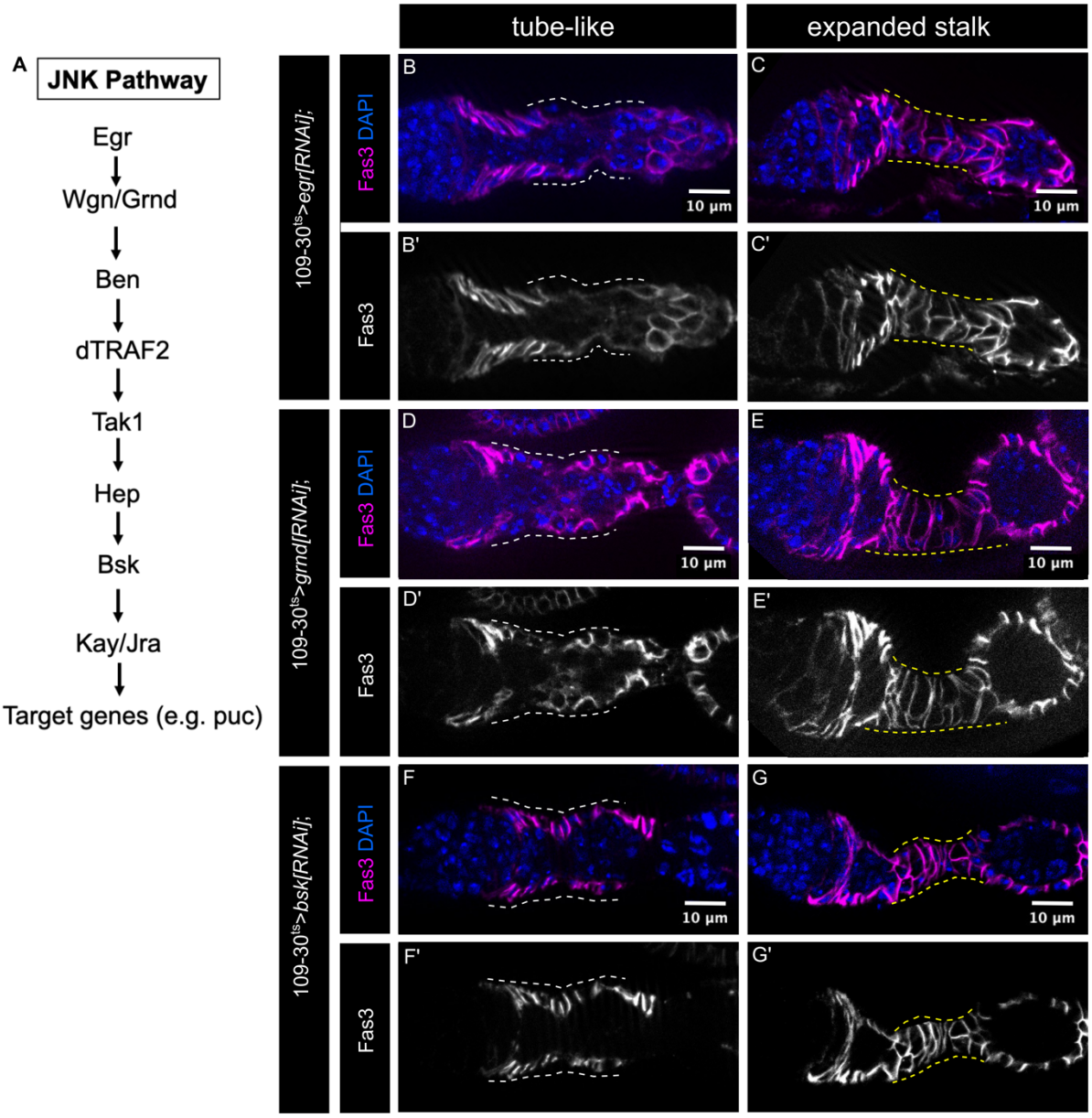

Fig. S3. JNK signaling is required for follicle formation and stalk cell differentiation. (A) Schematic depicting JNK signaling pathway. (B-G) Ovarioles with *109-30^{ts}* driving RNAi against *egr*, *grnd*, or *bsk* stained for Fas3 (magenta), and DAPI (blue). Expanded stalk phenotypes (yellow dashed lines) and tube-like phenotypes (white dashed lines) are indicated.

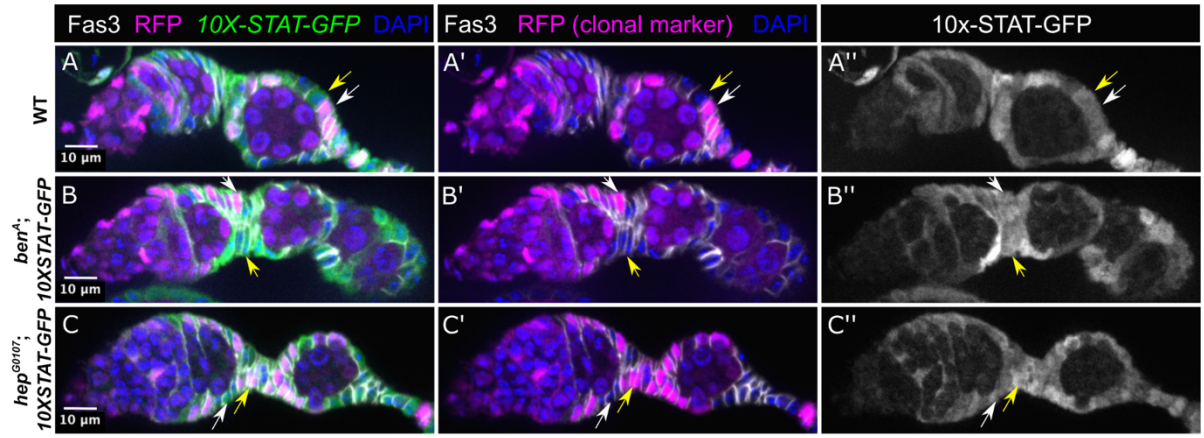

Fig. S4. JNK signaling does not affect the pattern of JAK-STAT signaling in the FSC lineage.

(A-C) Ovarioles with *10x-STAT-GFP* and wildtype, ben^{A}, or hep^{G0107} RFP^{-} clones stained for RFP (magenta) GFP (green) Fas3 (white) and DAPI (blue). The pattern of GFP expression is similar in RFP^{-} (yellow arrows) and RFP^{+} (white arrows) cells.

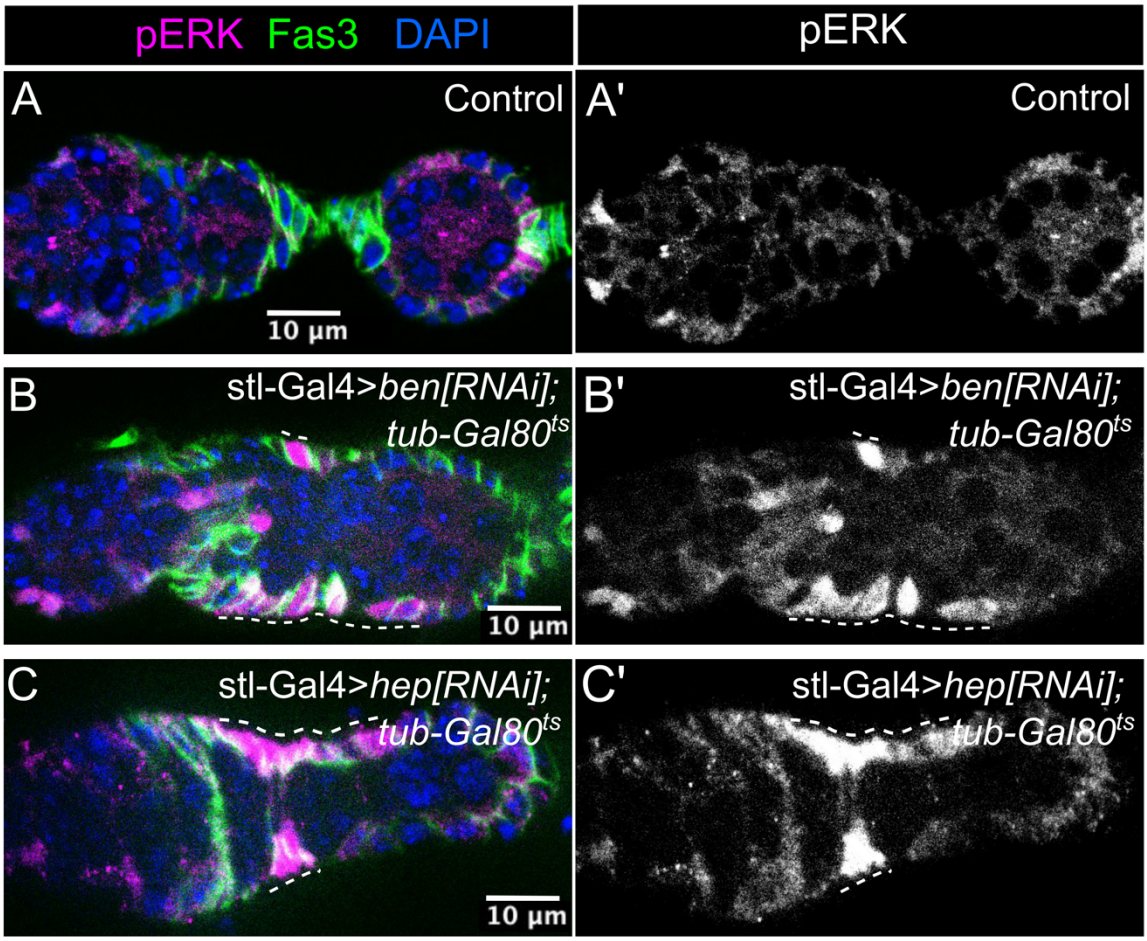

Fig. S5. JNK signaling is required in pFCs to decrease pERK in pFCs.

(A-C) Ovarioles with *stl-Gal4* alone (A) or in combination with *UAS-ben[RNAi]* or *UAS-hep[RNAi]* to knockdown gene expression specifically during adulthood, stained for pERK (magenta), Fas3 (green) and DAPI (blue). The pERK signal is absent in pFCs in the control ovarioles (A) but clearly detectable in the pFCs of the mutant ovarioles (white dashed lines, B-C).

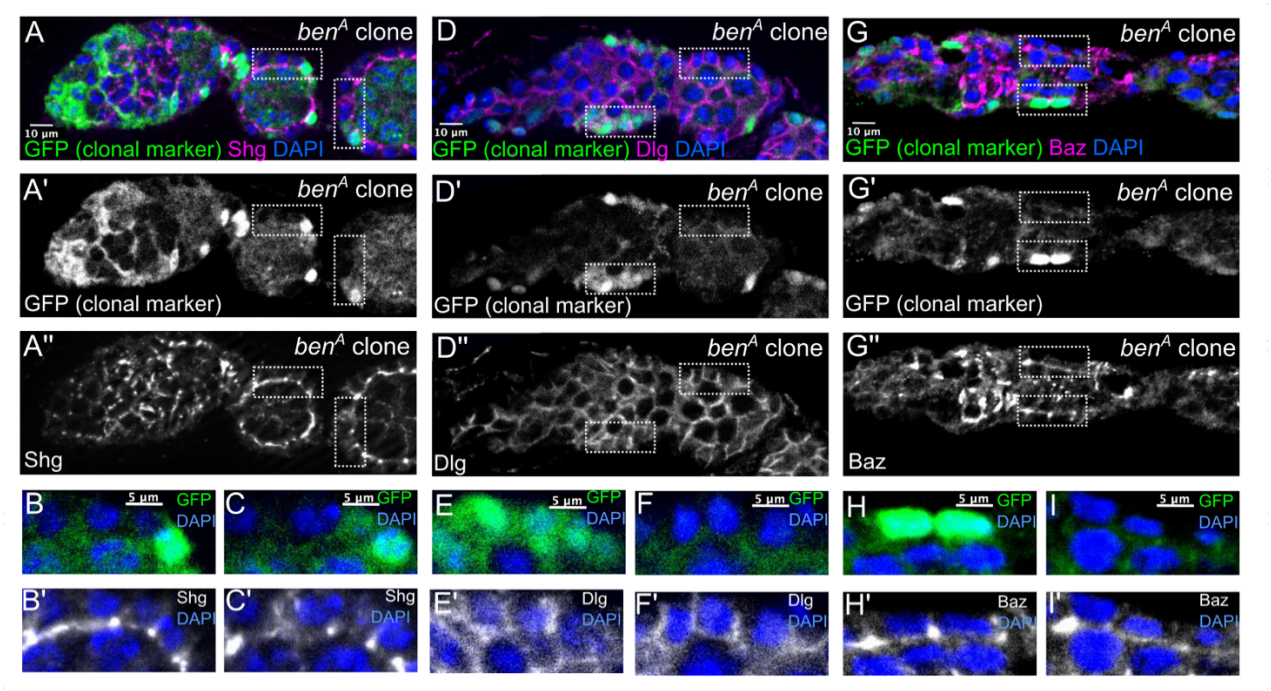

Fig. S6. *ben*^{A} clones do not exhibit cell polarity defects.

(A-I) Ovarioles with *ben*^{A} GFP^{-} clones stained for GFP (green), DAPI (blue) and either Shg (A-C), Dlg (D-F), or Baz (G-I) in magenta (A, D, and G) or white (B, C, E, F, H and I). The GFP and cell polarity marker channels are separated out in A'-A", D'-D", and G'-G". The regions bounded by the rectangular boxes in A, D and G are magnified in B-C, E-F, and H-I (and also rotated in C). There are no detectable differences in the localization of cell polarity markers between GFP^{+} and GFP^{-} cells, as can be seen in these magnified regions.

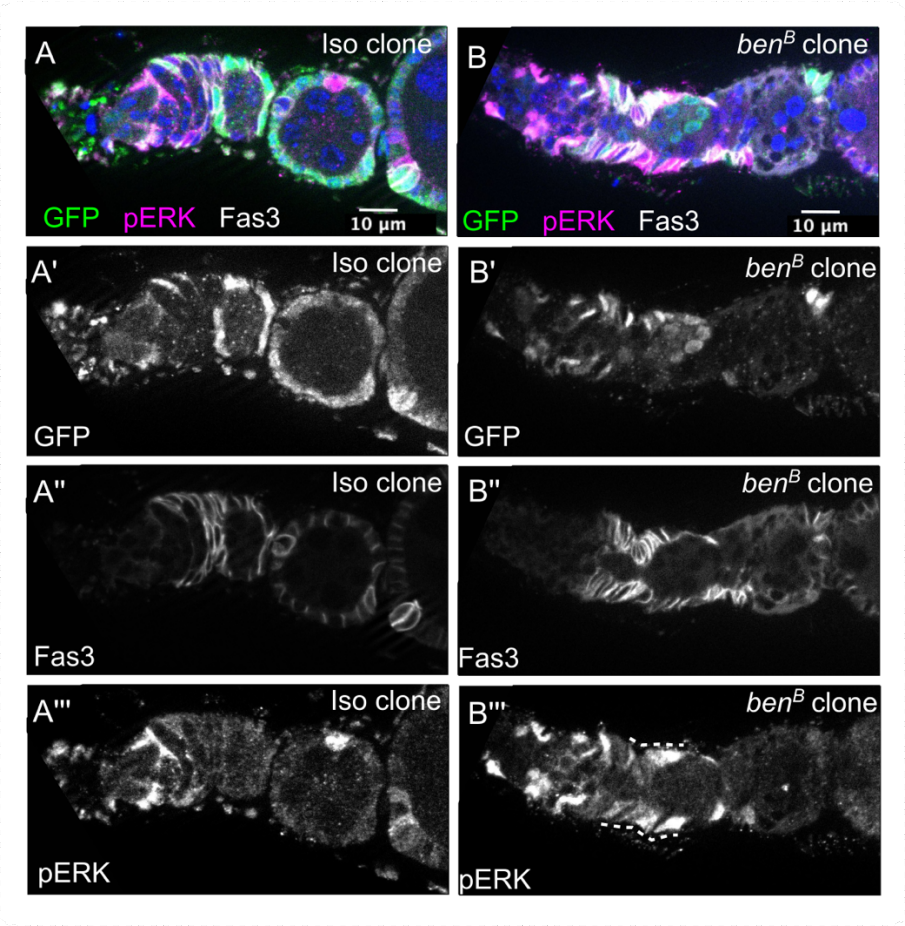

Fig. S7. Ovarioles with *ben^{B}* clones exhibit expanded pERK and tube-like phenotypes.

(A-B) Ovarioles with wildtype or *ben^{B}* GFP^{-} clones stained for GFP (green), pERK (magenta), Fas3 (white), and DAPI (blue). The presence of pERK signal in *ben^{B}* pFCs is indicated in B''' (white dashed line).

Table S1. Raw data

Click here to download TableS1