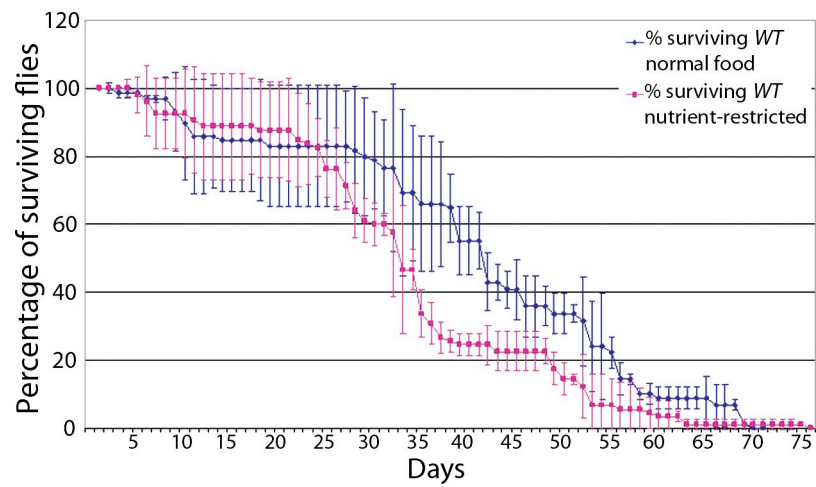Figure S1. **WT flies survive up to 75 d on nutrient-restricted diets.** WT flies fed yeast or nutrient-restricted diets were scored daily for survival. Error bars represent standard deviations.
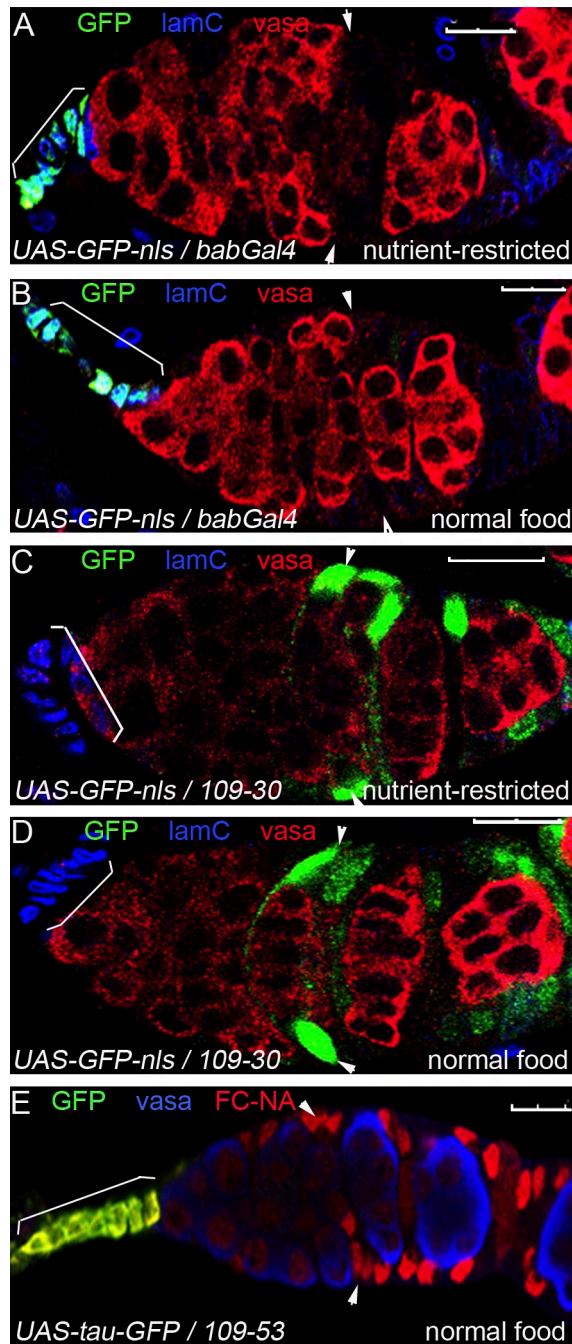
Figure S2. **Expression patterns of Gal4 drivers in germaria.** (A–D) Flies bearing *Gal4* transgenes were crossed to flies bearing a *UAS-GFP-nls* reporter transgene. GFP indicates the expression pattern of the promoter driving Gal4 expression. (A and B) *bab-Gal4* is expressed predominantly in apical cells in both normal food and nutrient-restricted conditions. (C and D) *109-30-Gal4* is expressed in FSCs and their daughter cells in the germarium through stage 3 in both normal food and nutrient-restricted conditions. (E) Flies bearing the 109-53-*Gal4* transgene were crossed to flies bearing a *UAS-tau-GFP* reporter transgene. *109-53-Gal4* is expressed predominantly in apical cells. Arrowheads indicate FSCs. Brackets indicate apical cells. Bars, 10 μM.
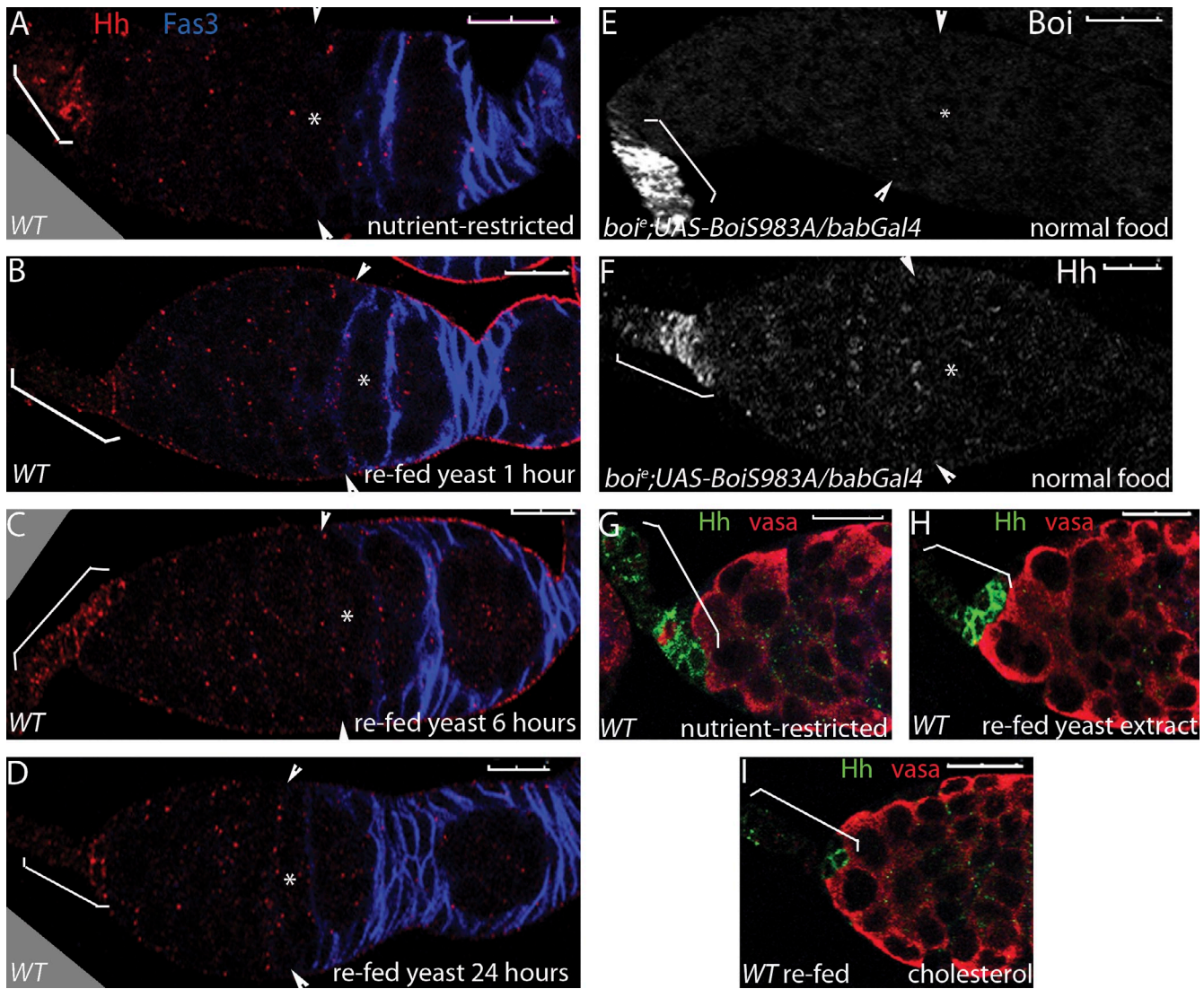
Figure S3.   **Localization of endogenous Hh in nutrient-deprived and refed *WT* flies.** (A–D) Nutrient-restricted *WT* flies were refed yeast for the indicated times, and endogenous Hh localization was analyzed using an Hh antibody. Hh and follicle cells (Fas3) are shown. (E and F) Expression of Boi[S983A] in *boi[e]* mutant flies (*boi[e];UAS-boi983A/bab-Gal4*) rescues Hh sequestration in apical cells. Boi expression is shown in E, and Hh localization is shown in F. (G–I) Nutrient-deprived *WT* flies were refed yeast extract ± 0.2 mg/g cholesterol for 6 h, and endogenous Hh localization was analyzed using an Hh antibody. Hh and germ cells (Vasa) are shown. Asterisks indicate flattened germline cyst at the region 2A/2B border. Arrowheads indicate FSCs. Brackets indicate apical cells. The gray triangles indicate areas that were not viewed in the microscope when the image was acquired. Bars, 10 µM.
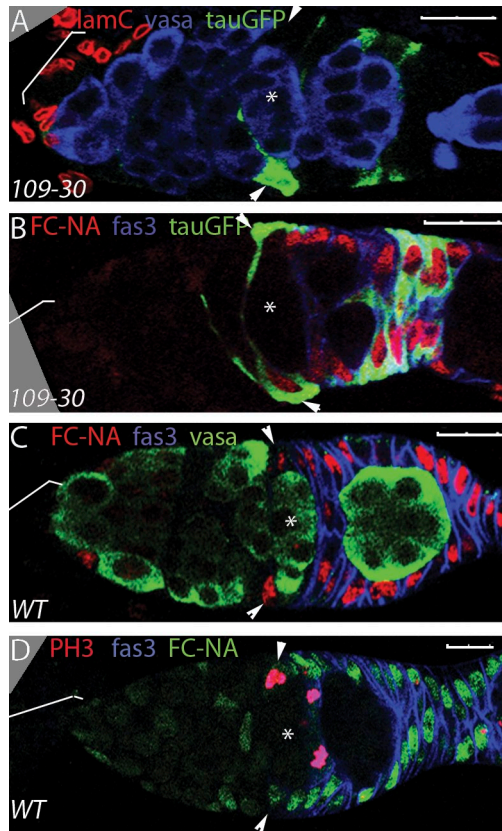
Figure S4. **FSCs can be identified by specific characteristics.** (A–D) FSCs can be identified by their position before the first flattened egg chamber (germ cells [Vasa]) in a MARCM clone (green; A), by triangular nuclear staining of FC-NA, low Fas3, and FSC position as the first marked cell in a MARCM clone (green; B) and by triangular nuclear FC-NA staining, low Fas3, and position before the first flat egg chamber (Vasa; C). (D) Dividing FSCs are identified by PH3-positive staining of the triangular shaped nucleus in a FC-NA–positive cell and low Fas3. Asterisks indicate flattened germline cyst at the region 2A/2B border. Arrowheads indicate FSCs. Brackets indicate apical cells. Bars, 10 µM.
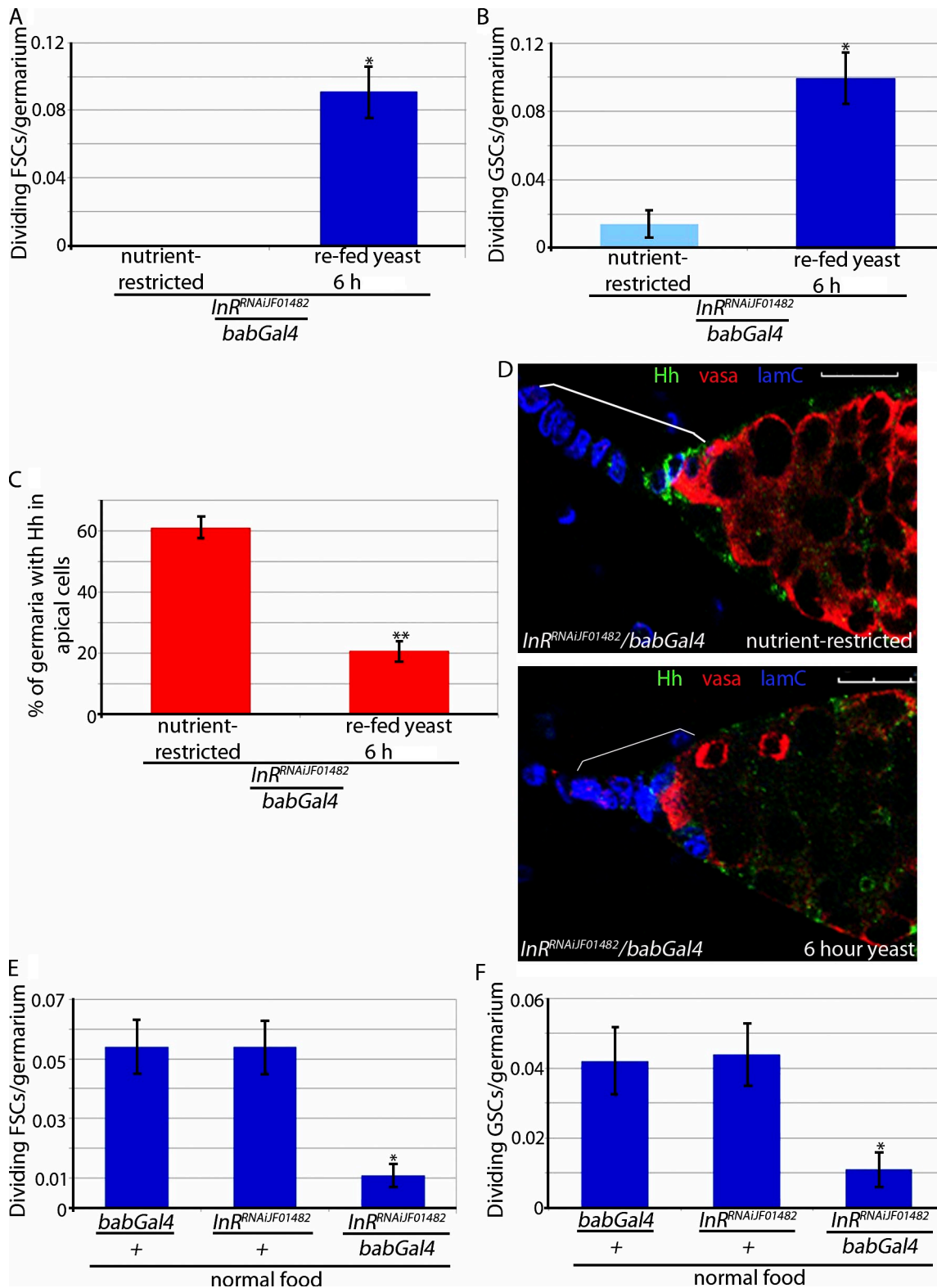
Figure S5. **Loss of InR in apical cells does not block FSC proliferation after refeeding.** (A–D) Nutrient-restricted $InR^{RNAiJF01482}$/bab-Gal4 flies were refed yeast for 6 h. (A) Mean numbers of dividing FSCs (PH3+) per germarium are shown *, P < 0.0002 versus nutrient-restricted $InR^{RNAiJF01482}$/bab-Gal4. Error bars represent SEs (n = 146 and 364; Table 1). (B) Mean numbers of dividing GSCs (PH3+) per germarium are shown. *, P < 0.00001 versus nutrient-restricted $InR^{RNAiJF01482}$/bab-Gal4. Error bars represent SEs (n = 208 and 412). (C and D) Localization of endogenous Hh was determined. (C) The percentage of germaria with endogenous Hh localized to apical cells was scored. **, P < 0.00001 versus nutrient-restricted $InR^{RNAiJF01482}$/bab-Gal4 flies (n = 164 and 155). Error bars represent SEs. (D) Hh was observed in >60% of apical cells of nutrient-restricted $InR^{RNAiJF01482}$/bab-Gal4 flies but was released from refed flies as observed in WT (Fig. 2). Apical cells are labeled in blue (Lamin C [lamC]), and germ cells are labeled red (Vasa). Brackets indicate apical cells. Bars, 10 µM. (E and F) Loss of InR reduces FSC and GSC proliferation in flies on a normal food diet. (E) Mean numbers of dividing FSCs (PH3+) per germarium are shown *, P < 0.00001 versus bab-Gal4/+. Error bars represent SEs (n = 552–611). (F) Mean numbers of dividing GSCs (PH3+) per germarium are shown. *, P < 0.002 versus bab-Gal4/+. Error bars represent SEs (n = 426–527).

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