Bufalino et al., http://www.jcb.org/cgi/content/full/jcb.201207052/DC1

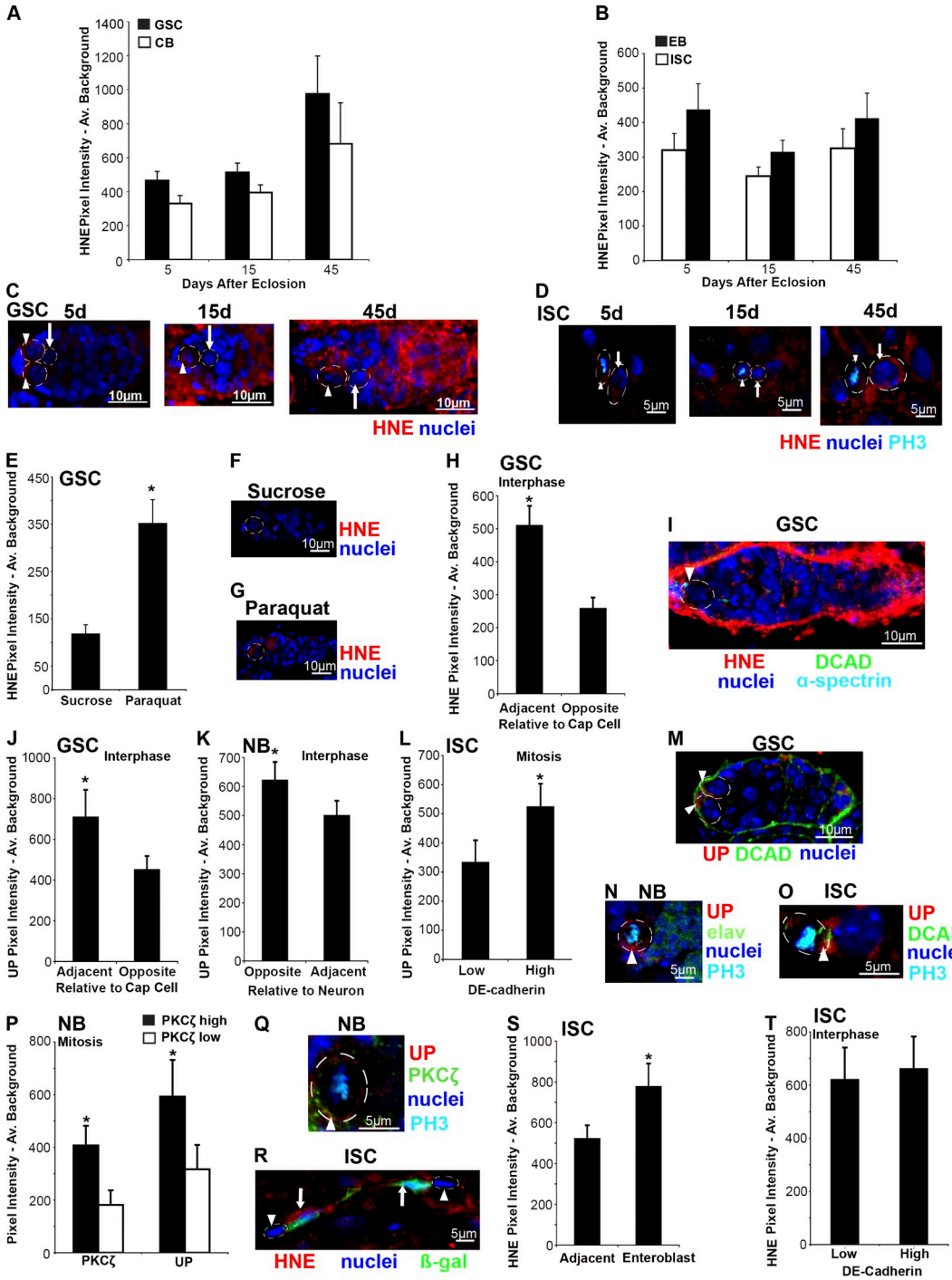

Figure S1. **DPs in the GSC, ISC, and NB.** (A) GSCs contain greater HNE content than adjacent CBs during aging ($n \geq 8$ for each time point). (B) ISCs, identified as the smallest cell in a BrdU-positive lineage, contain less HNE content than adjacent EBs during aging ($n \geq 8$ for each time point). (C) Examples of GSCs (arrowhead) and CBs (arrow) during aging. (D) Examples of ISCs (arrowhead) and EBs (arrow) during aging. (E) Significantly greater HNE content is found in GSCs of flies fed on paraquat for 24 h compared with the sucrose control ($n = 8$). Examples of GSCs (circled) in the sucrose (F) and paraquat (G) conditions. (H) HNE is consistently localized to the cap cell–adjacent side of GSCs in interphase using anti-HNE (Abcam; $n = 11$; 8/11 cells asymmetric). (I) A representative germarium used for the quantification in H is shown. This germarium is the same as one shown in Fig. 1 C. Similar to HNE, UPs are asymmetrically distributed during interphase within GSCs (J; $n = 12$; 8/12 cells asymmetric), during interphase within NBs (K; $n = 11$; 7/11 cells asymmetric), and during mitosis in ISCs (L; $n = 11$; 8/11 cells asymmetric). (M) Example of GSCs (arrowheads) with intense staining for UPs in the anterior tip. (N) Example of a NB (arrowhead) stained for UPs, which are found in small quantities in the L3 larval brain. (O) Example of an ISC (arrowhead) stained for UPs with adjacent progeny to the right. (P) In mitotic NBs, apical PKCs costains with asymmetrically localized UPs ($n = 8$; 7/8 cells asymmetric). (Q) Example of PKCs and UP colocalization in the NB; arrowhead indicates region of colocalization. (R) Example EBs (arrows) and small adjacent cells (arrowheads). (S) EBs identified as LacZ-positive cells from Gbe$^+$Su(H)LacZ flies contain greater levels of HNE (Abcam) than small adjacent cells ($n = 15$). (T) Before mitosis, ISCs identified by delta staining do not display HNE accumulation on the side of the ISC with high DCAD staining ($n = 10$; 3/10 cells asymmetric). The mean (± SEM) represents HNE (or alternative stain) pixel intensity subtracted by average background. *, $P < 0.05$ by paired two-tailed *t* test.

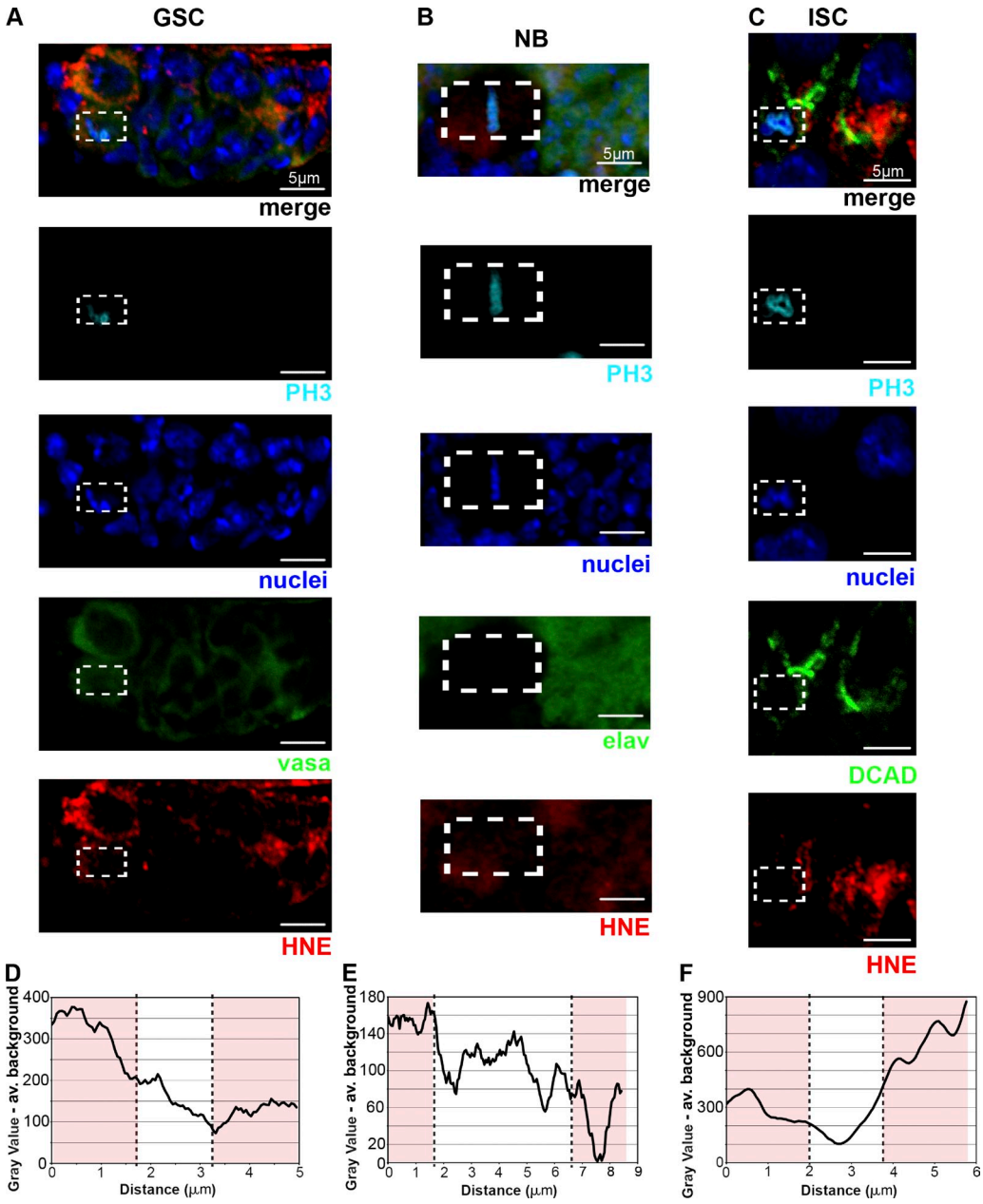

Figure S2. **HNE localization measurements in the mitotic GSC, NB, and ISC.** Color channels from the images shown in Fig. 2 (A–C) are shown individually here for a mitotic GSC (A), NB (B), and ISC (C), respectively. A plot profile is generated in ImageJ by drawing a box within the cell of interest. The program produces the profile of intensity by averaging the pixel intensity across the y-axis and plotting it against the distance (μm) on the x-axis. Across images, the nucleus is considered the center of the cell and is not included in the localization measurements. Consistent between images, the center 30% of the GSC and ISC and the center 60% of NB are identified as the nuclei. The area highlighted in red of the plot profiles of HNE intensity for the GSC (D), NB (E), and ISC (F) display the regions that are included in the localization measurements. The mean of each highlighted region is compared to determine the staining intensity in each region of the cell.

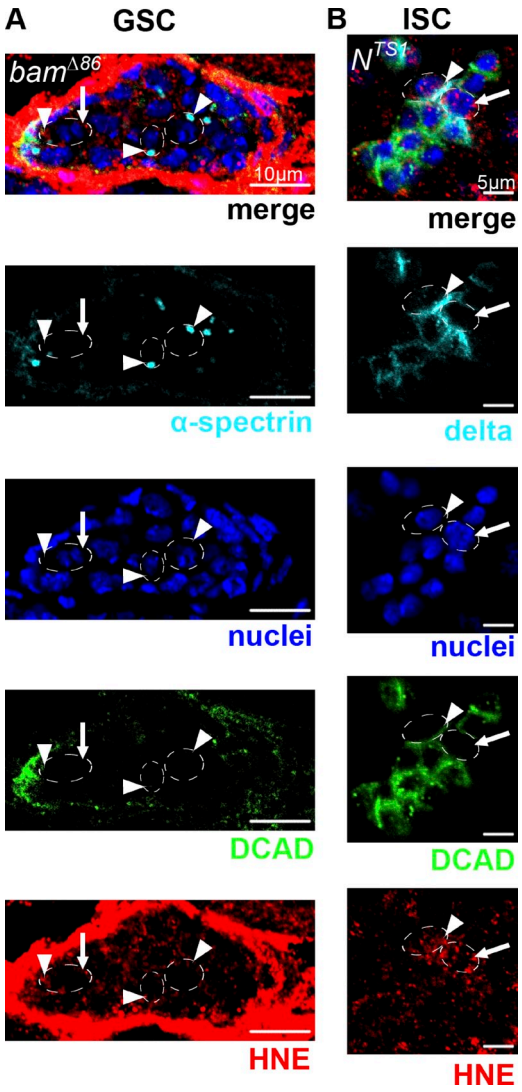

Figure S3. **Localization of HNE in GSCs and ISCs removed from the stem cell niche.** (A) Color channels from the image shown in Fig. 4 A are shown individually here to better visualize greater levels of HNE (Abcam) within the cap cell side (arrowhead) of a GSC (circled) within the niche compared with the opposite end of the cell (arrow) in a $bam^{\Delta 86}/bam^{\Delta 86}$ germarium. In cells separated from the GSC niche, arrowheads indicate regions of cells (circled) containing a spectrosome where enriched levels of HNE are found. (B) Color channels from the image shown in Fig. 4 D are shown individually here to better visualize a region of high (arrowhead) DCAD staining intensity that coincides with high HNE staining intensity within an ISC-like cell (circled) of a N^{ts1}/N^{ts1} midgut. A cell without a strong polarization of DCAD (circled, arrow) does not contain a polarized distribution of HNE.