Supplemental material

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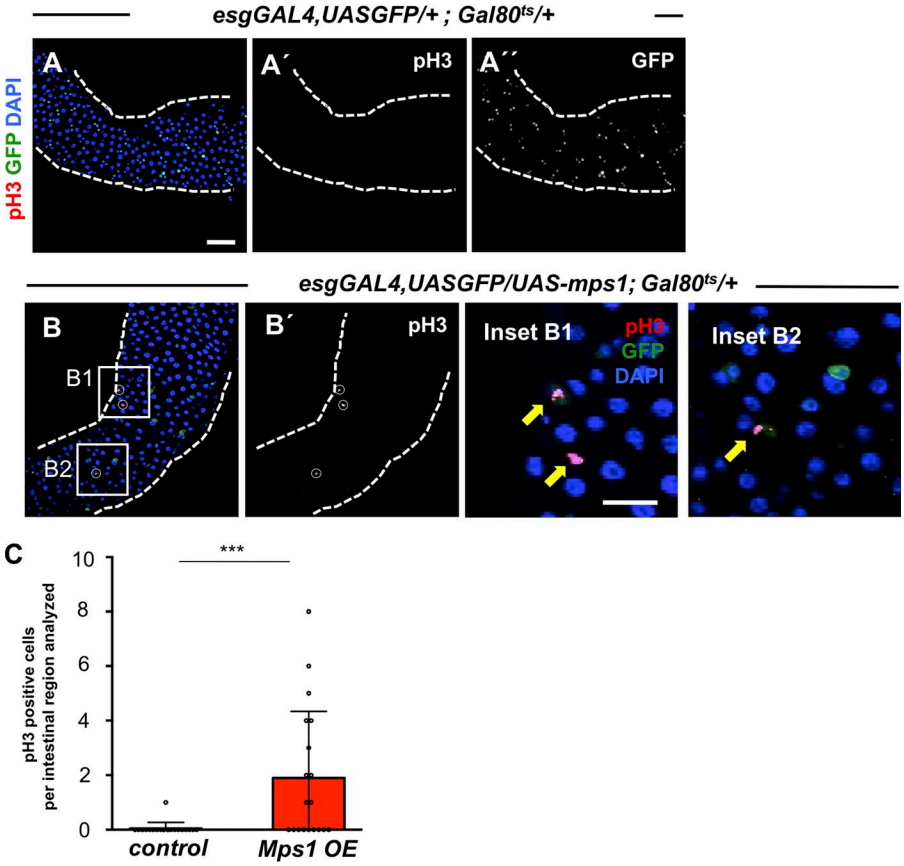

Figure S1. **Mps1 overexpression leads to ISC cell-cycle arrest. (A)** 2-d-old control intestine stained for GFP marking *esg*-positive cells (ISCs/EBs) and pH3-positive cells. In the majority of intestines (20 out of 21 total analyzed), no pH3 was found in the intestinal region analyzed. First two fields of view after the pyloric ring using a 40× objective. **(B)** Intestine where Mps1 was overexpressed under the control of the *esgGal4* promoter during the first 2 d after eclosion showing three pH3-positive cells (white circles; insets B1 and B2; yellow arrows). Bars: 40 µm (main images); 20 µm (insets). **(C)** Number of mitotic cells present in the first two fields of view of the posterior midgut after the pyloric ring (40× objective) from A and B. ***, $P < 0.001$; Mann–Whitney *U* test. *n* (intestines) = 21 for controls; *n* (intestines) = 19 for Mps1 overexpression. In both A and B, GAL4-UAS was suppressed during development using the temperature-sensitive repressor $GAL80^{ts}$ and by raising the flies at 18°C.

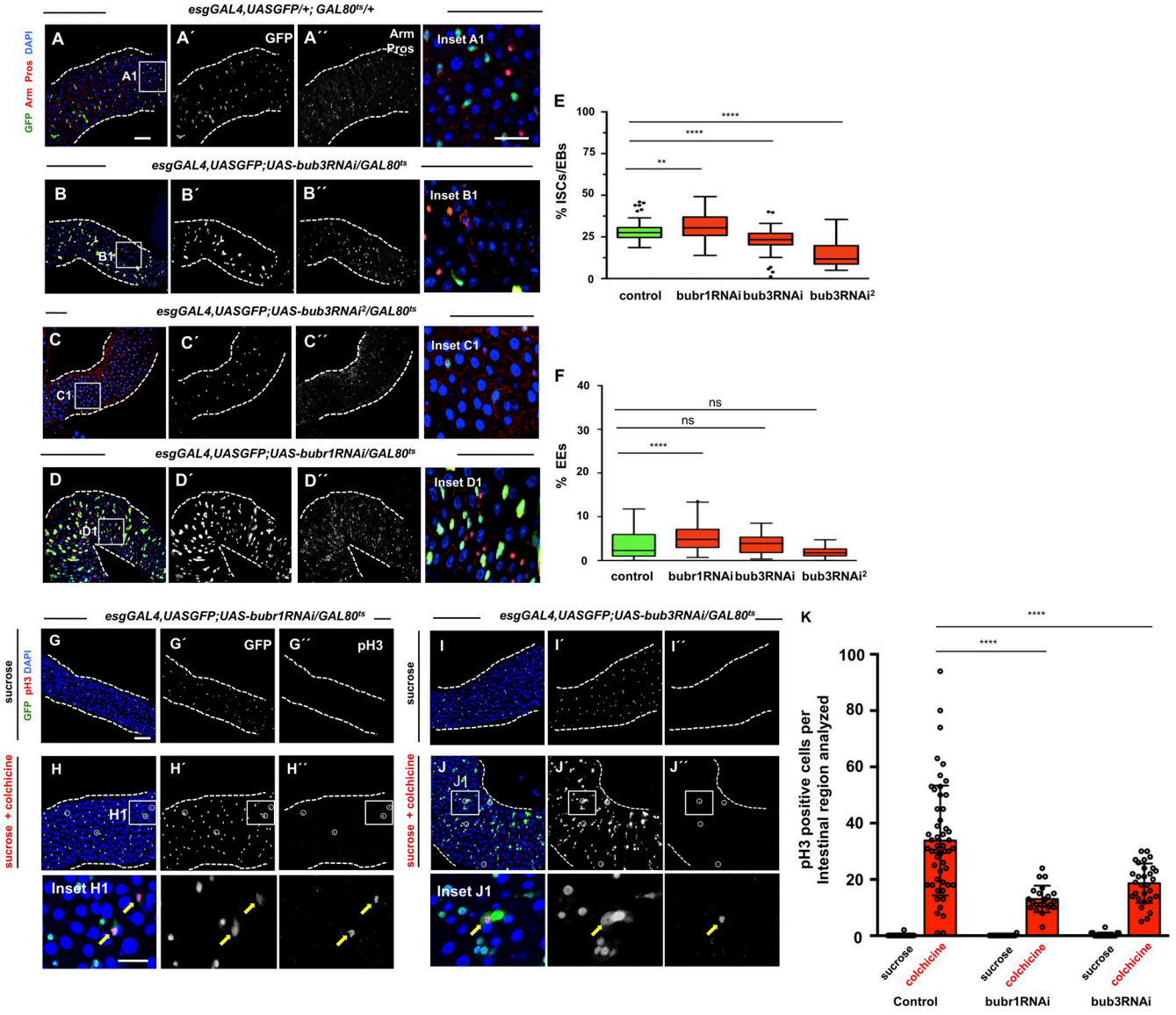

Figure S2. **Unlike Mad2, Mps1, or BubR1 depletion, Bub3 loss of function does not cause a dysplastic phenotype. (A)** 20-d-old control intestine where ISCs and EBs are marked based on *esg* expression (green; A′). EEs can be identified by expression of Pros (nuclear red signal; A″). Arm marks all cell membranes, and DAPI marks cell nuclei. **(B–D)** Intestines where RNAi constructs against SAC genes *bubr1* or *bub3* were expressed under the control of the *esgGal4* promoter during the first 20 d of the *Drosophila* adult. Note accumulation of ISCs/EBs after BubR1 knockdown (compare D′ with A′) and ISC/EB loss after Bub3 knockdown (compare B′ or C′ with A′). In all genotypes, suppression of the GAL4-UAS system was performed during development by using the temperature-sensitive repressor GAL80^{ts} and by raising the flies at 18°C. **(E and F)** Quantification of ISCs/EBs and EEs from A–D. Control data are the same as in Fig. 3. Tukey boxplots; *n* (intestines) = 21 for controls; *n* (intestines) = 24 for bubr1RNAi; *n* (intestines) = 19 for bub3RNAi; *n* (intestines) = 10 for bub3RNAi2. Three to four images per intestine were used for quantifications. **(G–J)** Mitotic cells labelled with PH3 in intestines from *bubR1RNAi* and *bub3RNAi* flies. Flies were kept at 18°C during development to suppress the GAL4-UAS system and then shifted to 29°C at eclosion day. After 48 h at 29°C on regular food, flies were shifted to vials with either sucrose or sucrose + colchicine solutions for 24 h as described in Fig. 1. Compare with controls in Fig. 1 (K and L). **(K)** Number of mitotic cells present in the first two fields of view of the posterior midgut after the pyloric ring (40× objective) from K–P. **, P < 0.01; ****, P < 0.0001; Mann–Whitney *U* test. *n* (intestines) > 22 for all genotypes/conditions. White circles and yellow arrows show pH3-positive cells. Data from controls are the same as in Fig. 1. Bars: 40 µm (main images); 20 µm (insets).

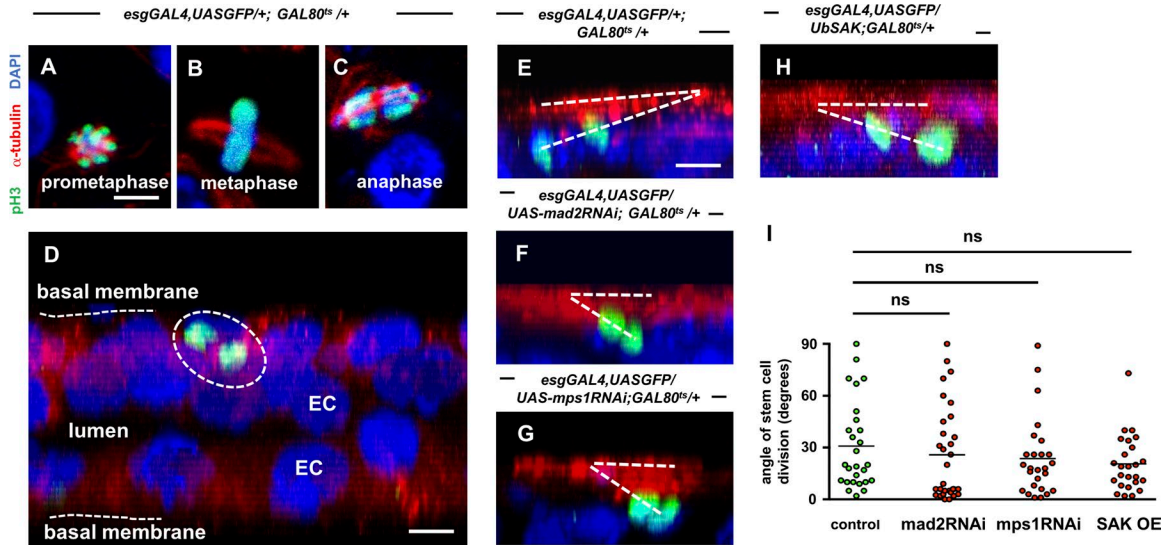

Figure S3. **SAC impairment and centrosome amplification do not affect the angle of stem cell division. (A–C)** Different mitotic phases could be identified in 15–20-d-old intestinal samples using pH3 (green) to label mitotic cells, α-tubulin (red) to stain the spindle, and DAPI to mark the nuclei (blue). **(D)** Example of a broad view of a 3D reconstruction (X axis projection) of multiple z stacks of an intestinal sample. General architecture of the tissue can be appreciated with the ECs facing the lumen found between two epithelial layers and where dividing ISCs (white ellipses) can be found adjacent to the basement membrane (made evident by α-tubulin; red). **(E–H)** Examples of dividing ISCs in control, mad2RNAi, mps1RNAi, and SAK overexpression (OE) 15–20-d-old intestines. Mitotic angle was inferred by the angle formed by two lines drawn: one along the basement membrane and one intersecting the center of the two separating nuclei. Bars, 5 µm. **(I)** Distribution of mitotic angles determined for three conditions described in E–H. *n* = 27 (control); *n* = 31 (mad2RNAi); *n* = 26 (mps1RNAi); *n* = 25 (SAK overexpression). Controls versus mad2RNAi, P = 0.14; controls versus mps1RNAi, P = 0.29; controls versus SAK overexpression, P = 0.24; Mann–Whitney *U* test.

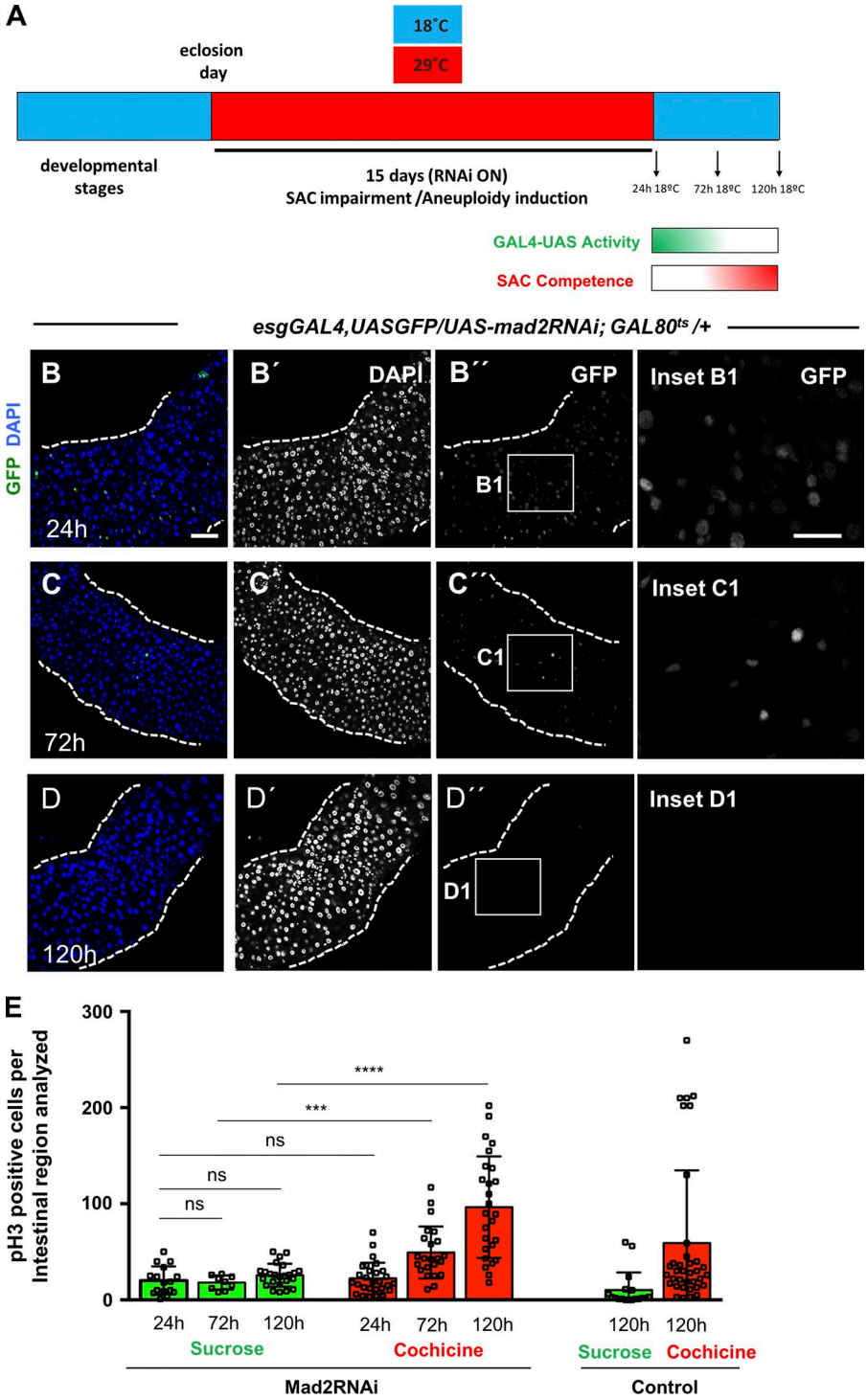

Figure S4. **SAC function is progressively restored in Mad2RNAi intestines upon shift to 18°C. (A)** Scheme illustrating temperature shifts performed in control and mad2RNAi flies. The Gal4-UAS system and mad2RNAi were expressed since eclosion day and maintained during 15 d. Then, flies were shifted to 18°C to turn off the GAL4-UAS system and RNAi expression (GAL80^{ts} ON). After 24, 72, and 120 h at 18C°, we evaluated GFP levels (GAL4-UAS activity) and scored pH3-positive cells in flies fed in sucrose versus fed with colchicine (SAC response). During this period, it was expected that GAL4-UAS activity should go down and, consequently, that SAC function be recovered in mad2RNAi flies. **(B–D)** GAL4-UAS activity drop after shift to 18°C. After 24 h at 18°C, GFP was still present in ISCs/EBs (B′′; inset B1). After 72 h, some intestines already presented no GFP-positive cells, while others presented a smaller number of GFP-positive cells compared with the 24-h time point (C′′; inset C1). After 120 h at 18°C, the majority of flies presented no GFP-positive cells (D′′; inset D1), while the minority of those where GFP-positive cells were found were in a very small number (one to five) and expressing very low levels of GFP. Bars: 40 µm (main images); 20 µm (insets). **(E)** Number of mitotic cells present in the first two fields of view of the posterior midgut after the pyloric ring (40× objective) in mad2RNAi intestines during the same time points described in A. Note that while the number of mitotic cells was not different during this 5-d period in flies fed with sucrose, the number of mitotic cells increased significantly during this period, and after 120 h, SAC response was similar to the one observed for controls (last two graph bars on the right). ***, P < 0.001; ****, P < 0.0001; Mann–Whitney *U* test.

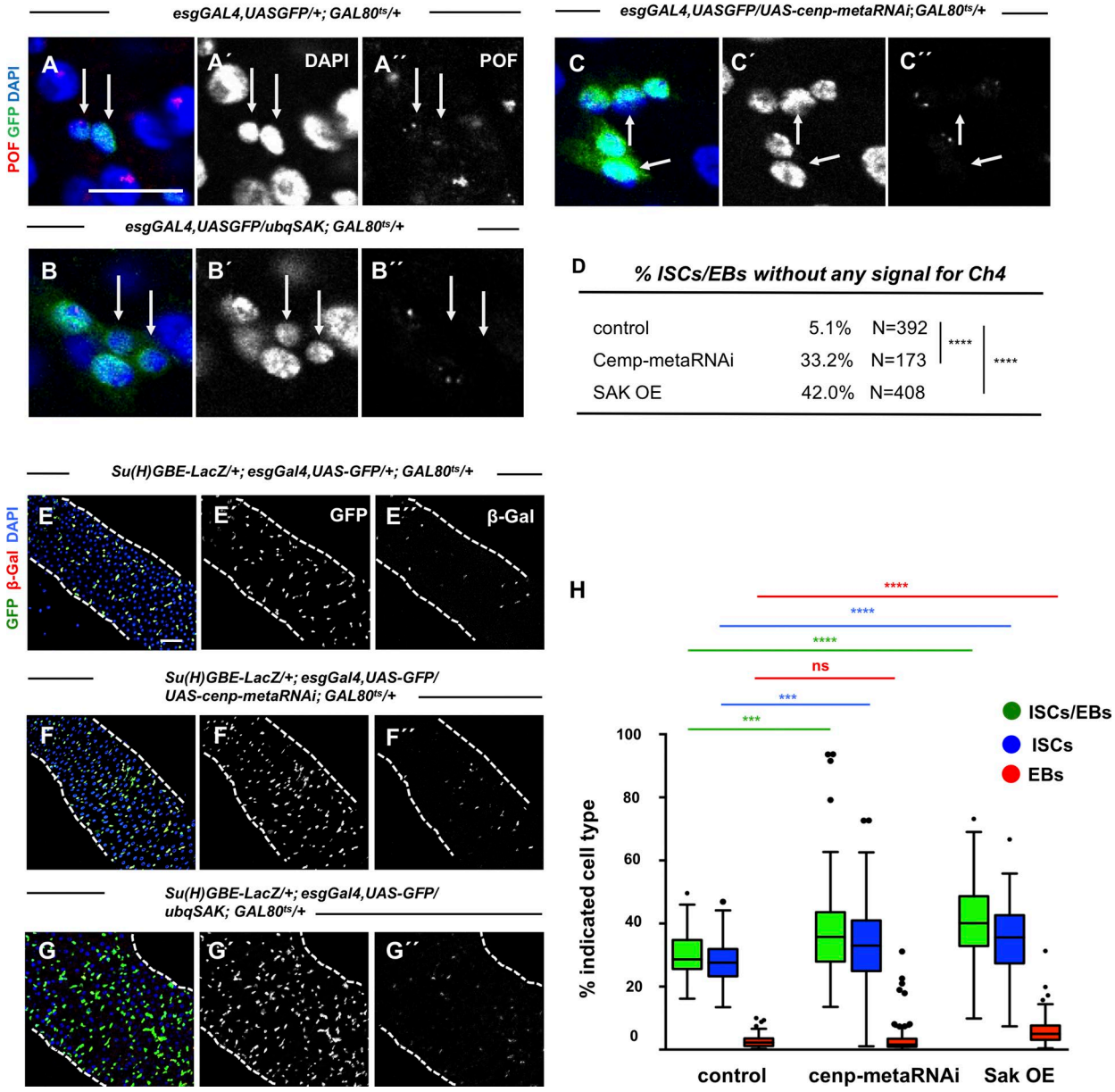

Figure S5. **Kinetochore disruption or centrosome amplification induce aneuploidy and accumulation of ISCs. (A–C)** 15–20 d control, cenp-metaRNAi, and SAK overexpression (OE) intestines stained for anti-POF antibody to label the fourth chromosome. Bar, 5 µm. Arrows in A indicate examples of ISCs/EBs where POF signal was evident; arrows in B and C indicate examples of ISCs/EBs where no signal of POF was detected. **(D)** Percentage of ISCs/EBs where no signal for anti-POF could be observed. N refers to the number of ISCs/EBs analyzed in each genotype. Control data are the same as in Fig. 2. **(E)** 20-d-old control intestine where ISCs and EBs can be distinguished based on expression of the Su(H)GBE-LacZ as described in Fig. 5. **(F)** Example of an intestine where an RNAi construct against *cenp-meta* was expressed under the control of the esgGAL4 driver during the first 20 d of the *Drosophila* adult. **(G)** Example of a 20-d-old intestine where the protein SAK was constitutively overexpressed. Bar, 40 µm. **(H)** Quantification of E–G. Control data are the same as in Fig. 5. Tukey boxplot; ***, P < 0.001; ****, P < 0.0001; Mann–Whitney *U* test. *n* (intestines) > 20 for all genotypes.