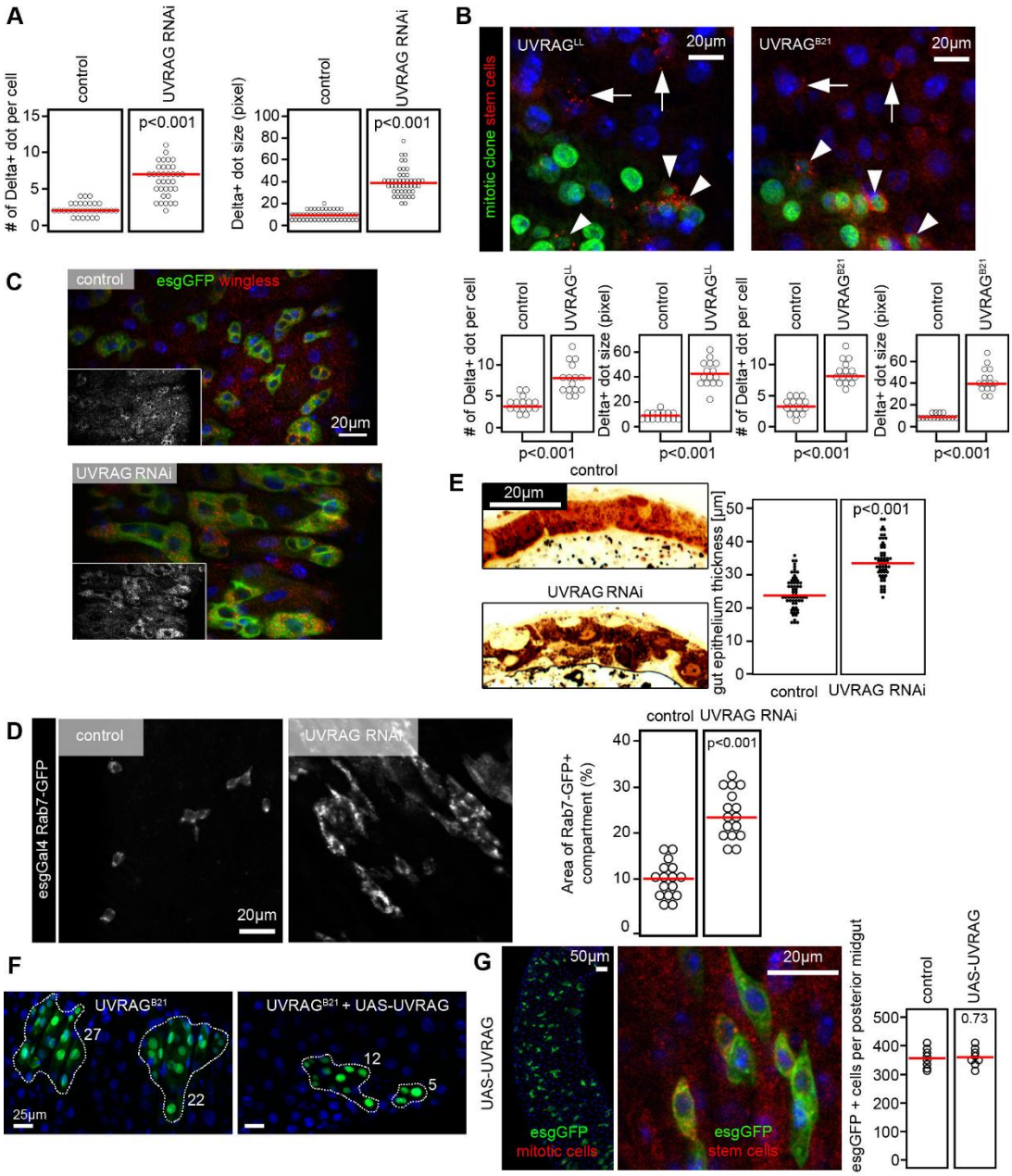

Figure S1. Additional UVRAG mutant data.

A. Quantification of Delta-positive intracellular vesicles in control and UVRAG RNAi stem cells that are shown in Figure 1B. B. Delta-positive intracellular vesicles accumulate in mitotic stem cell clones mutant for either of two different UVRAG null alleles compared to nearby control stem cells. C. Wingless protein accumulates intracellularly in esg-GFP+ cells undergoing UVRAG RNAi. D. Loss of UVRAG leads to expansion of endosomes positive for Rab7-GFP. E. Silver stained semi-thin

sections demonstrate that the wall of the posterior midgut is thicker in animals undergoing UVRAG RNAi in ISCs and EBs than in control animals. F. Positively marked (GFP+) mitotic clones of $UVRAG^{B21}$ null mutant cells contain more cells than control clones two weeks after clone induction, which is suppressed by transgenic expression of wild-type UVRAG. Please see Figure 1E for quantification of data. G. Overexpression of UVRAG does not perturb esg-GFP+ cell numbers compared to controls shown in Figure 1.

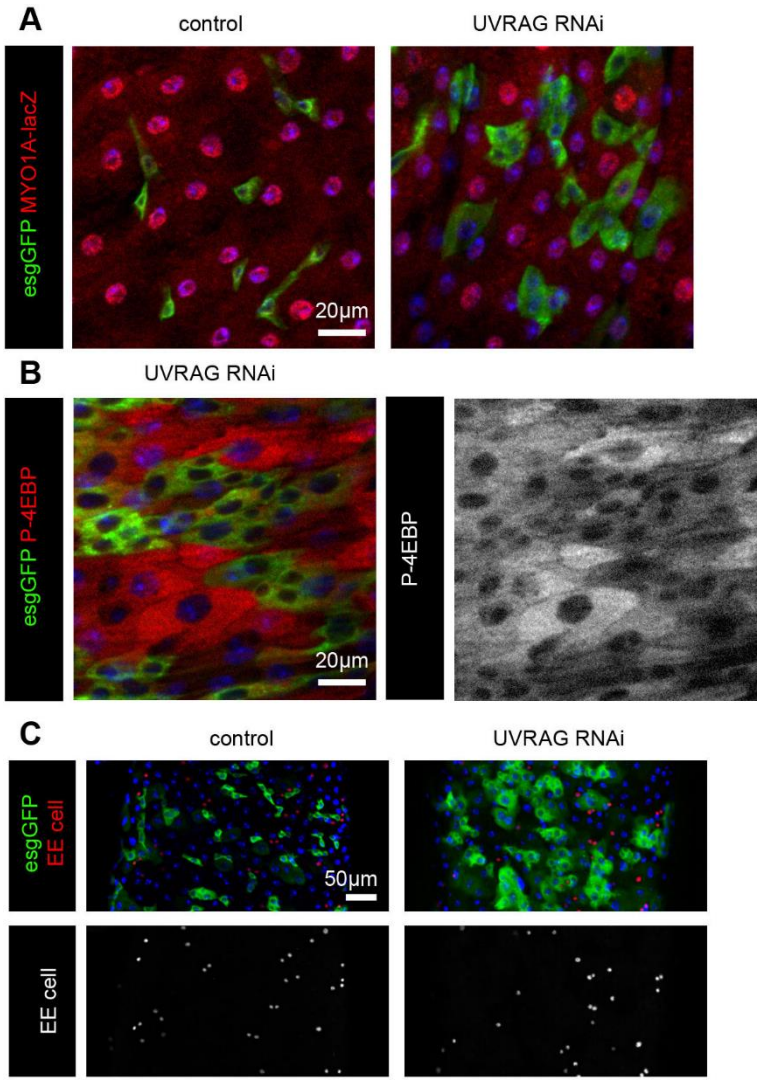

Figure S2. Tor activity and enteroendocrine cell fate in UVRAG knockdown intestines.

A. Differentiated enterocytes express the reporter Myo1A-LacZ in their nuclei in both control and UVRAG RNAi animals. This reporter is repressed in esg-GFP+ cells in both genotypes, indicating that the enlarged polyploid EBs observed in UVRAG RNAi guts fail to differentiate properly. B. Levels of the Tor kinase target phospho-4EBP are not increased upon knockdown of UVRAG in ISCs and EBs. C. Prospero+ enteroendocrine cells differentiate normally in UVRAG depleted posterior midguts.

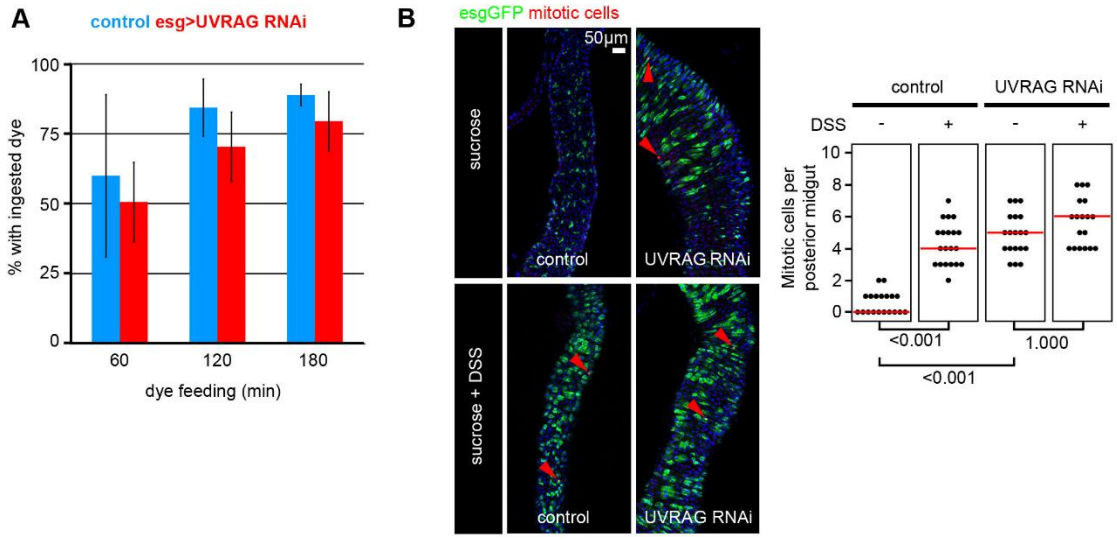

Figure S3. Food intake and DSS-induced regeneration responses in UVRAG RNAi midguts.

A. Animals undergoing UVRAG silencing in esg-GFP+ cells do not display a statistically significant difference from control animals in food intake. B. DSS induces a regeneration response in posterior midguts of control animals, based on the increased number of esg-GFP+ cells and mitoses after treatment. DSS fails to further increase esg-GFP+ cell number and mitoses in UVRAG RNAi animals.

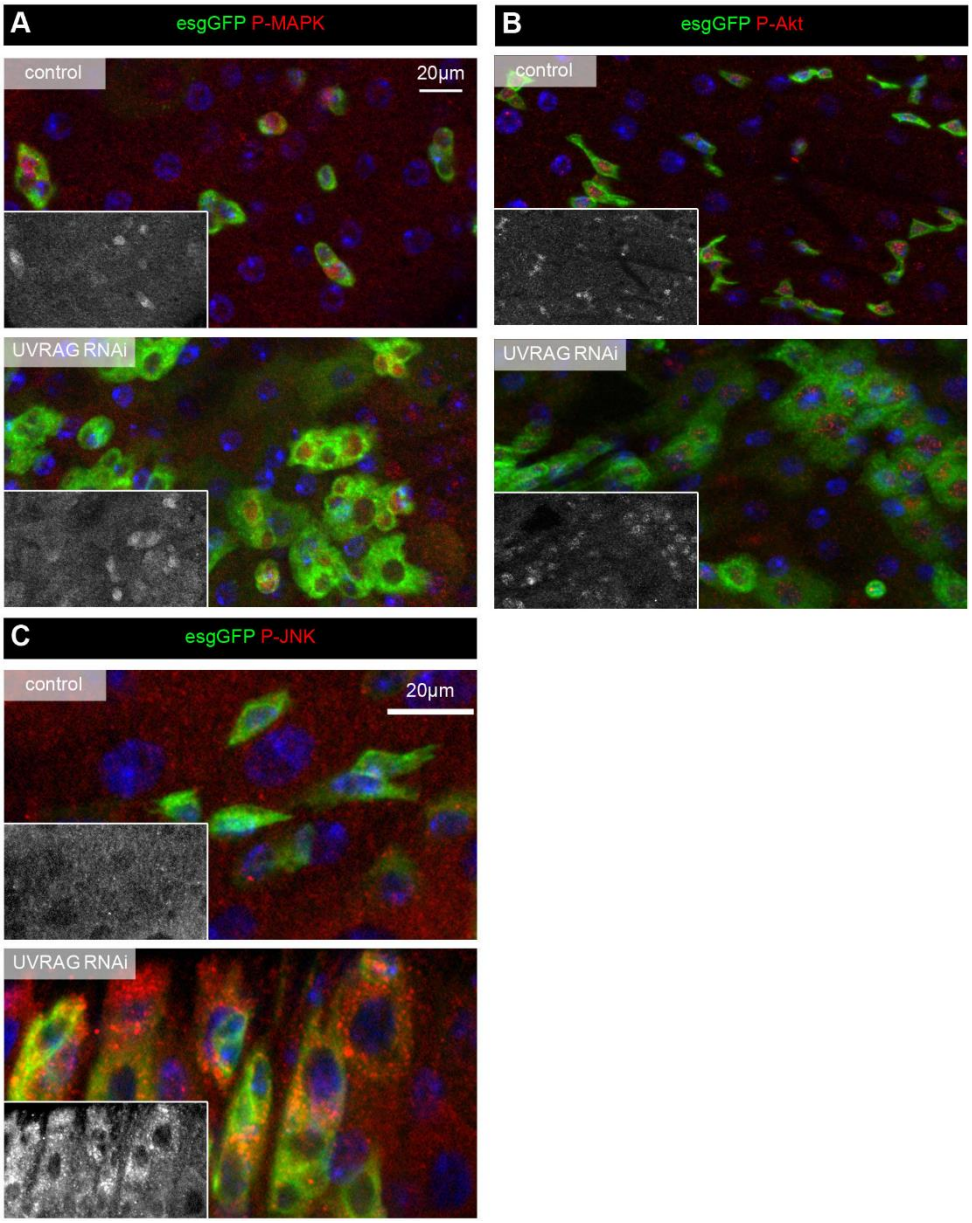

Figure S4. Activity of MAPK, Akt and JNK in UVRAG RNAi cells.

A, B. Loss of UVRAG does not activate MAPK or PI3K/Akt signaling, based on the lack of increased phospho-MAPK (A) or phospho-Akt (B) signal in esg-GFP+ cells. C. UVRAG RNAi upregulates phospho-JNK signal in esg-GFP+ cells.

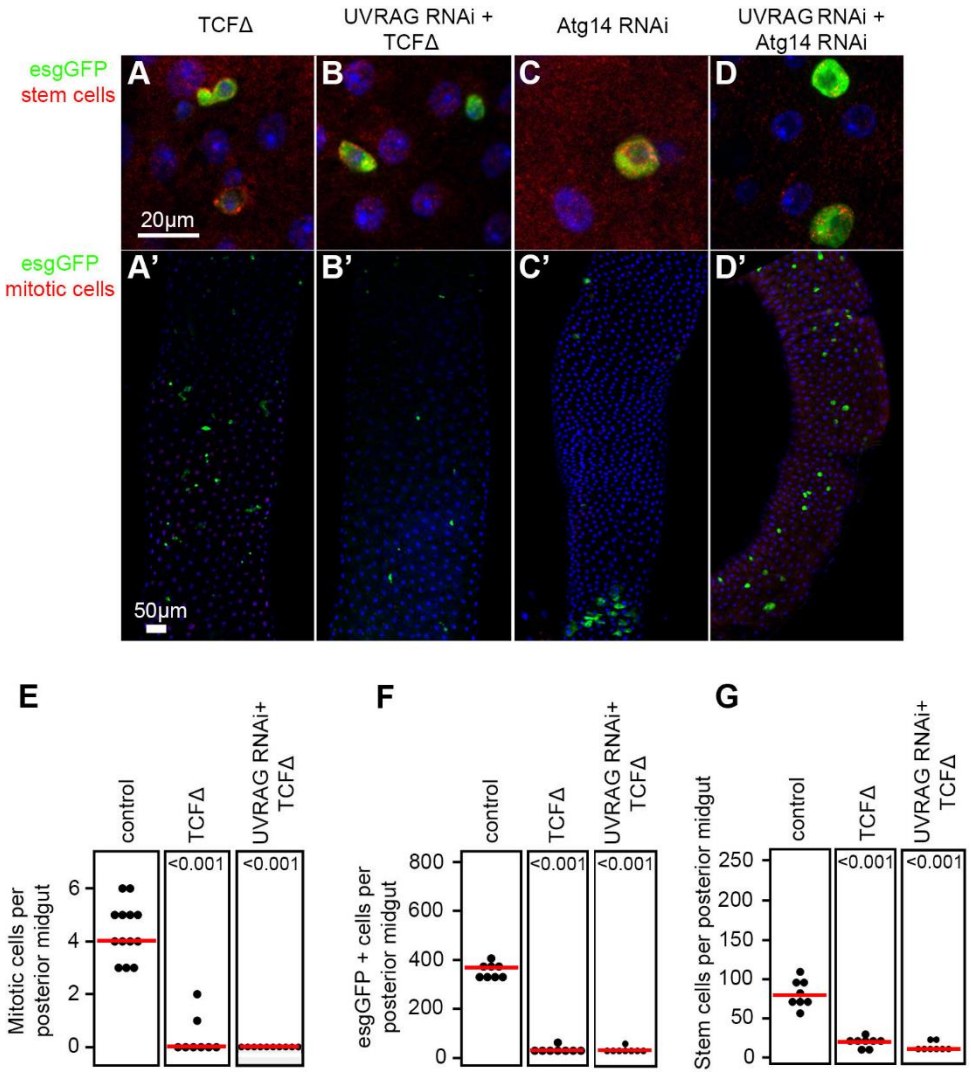

Figure S5. Additional controls and posterior midgut phenotypes.

A. Expression of dominant-negative TCF reduces stem cell numbers and proliferation. B. Dominant-negative TCF eliminates stem cells even when expressed together with UVRAG RNAi. C. Knockdown of Atg14 in esg-GFP+ cells reduces the number of cells and mitoses compared to controls (shown in Figure 1B, D). D. Knockdown of Atg14 prevents the UVRAG RNAi-induced dysplasia and increased mitotic index of esg-GFP+ cells. E-G. Quantification of data from panels A, B and Figure 1B, D.

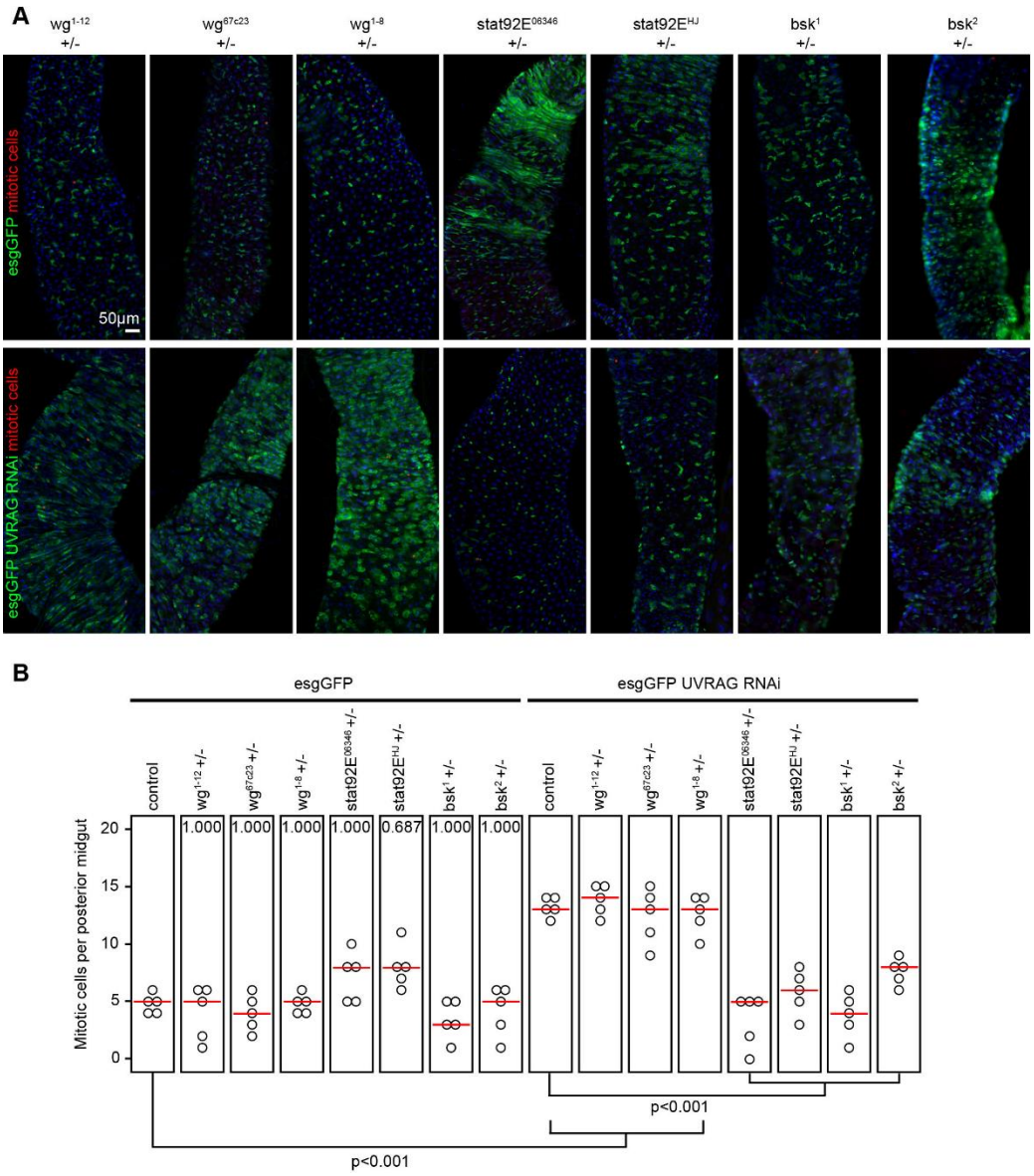

Figure S6. The effect of heterozygosity for wg, stat92E or bsk on wild-type and UVRAG RNAi esg-GFP+ cells.

A. esg-GFP+ cells and mitotic cells in the indicated genotypes. B. Quantification of data. Heterozygosity for wg, stat92E or bsk has no statistically significant effect on the mitotic index of ISCs (left panel). Heterozygosity for stat92E or bsk, but not wg, suppresses the hyperproliferation of UVRAG RNAi ISCs (right panel).

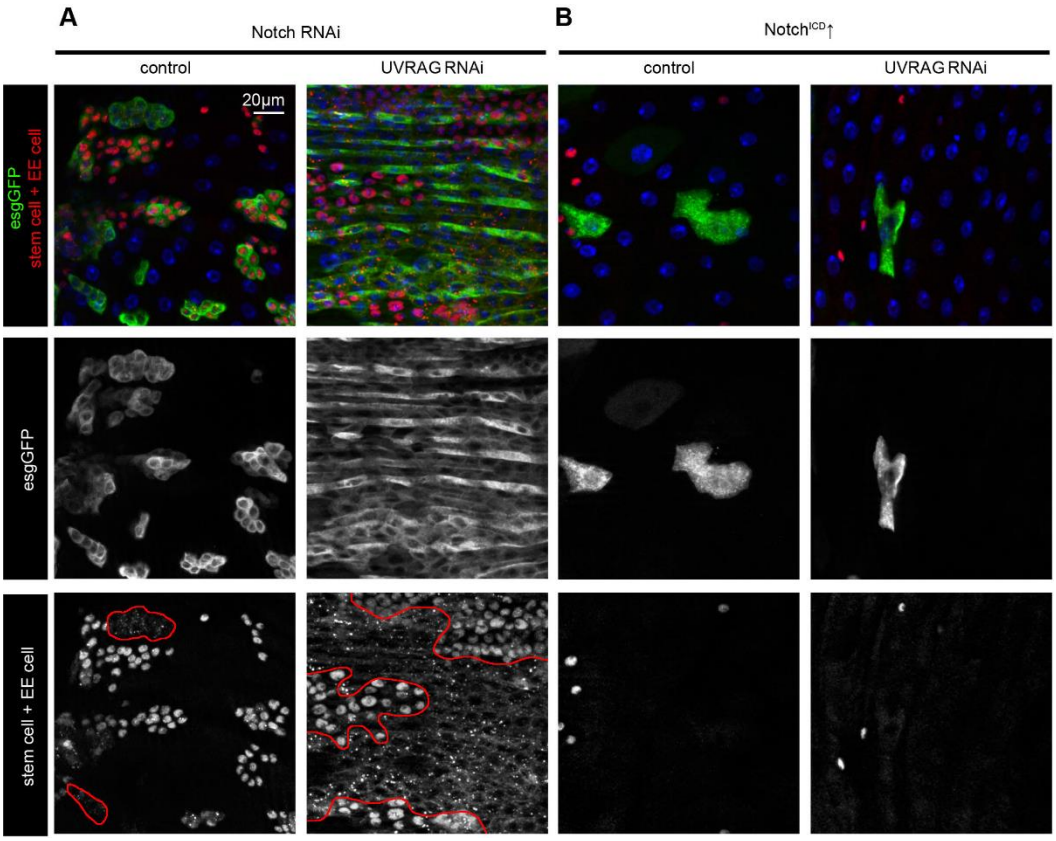

Figure S7. Interactions between UVRAG and Notch.

A. Knockdown of Notch in esg-GFP+ cells leads to accumulation of enteroendocrine (EE) cells that contain Prospero protein in the nucleus, Prospero+ Delta+ stem cells committed to the enteroendocrine fate, and Delta+ positive ISCs (encircled by a red line in the bottom panels). Co-silencing of Notch and UVRAG strongly enhances the accumulation of Delta+ ISCs throughout the posterior midgut. B. Overexpression of active Notch consisting of only the intracellular domain (N^{ICD}) promotes the differentiation of ISCs and EBs into enterocytes both in the presence or absence of UVRAG.