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Supplemental Information

Enteroendocrine Cells Support Intestinal Stem-Cell-Mediated Homeostasis in *Drosophila*

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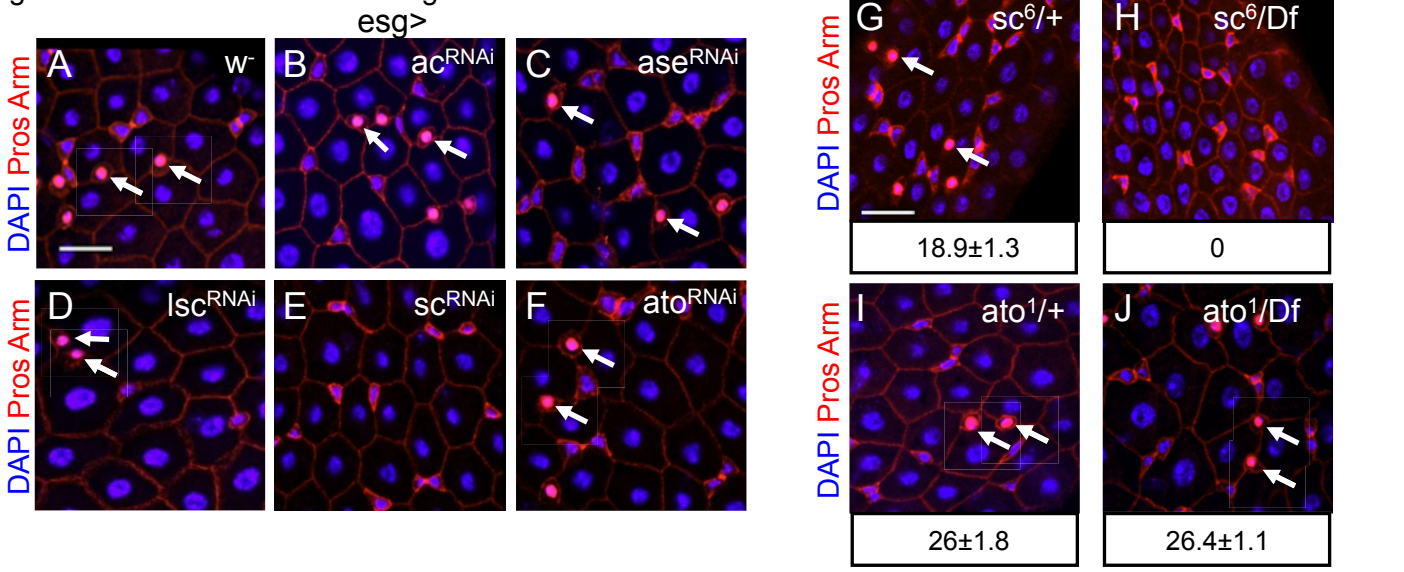
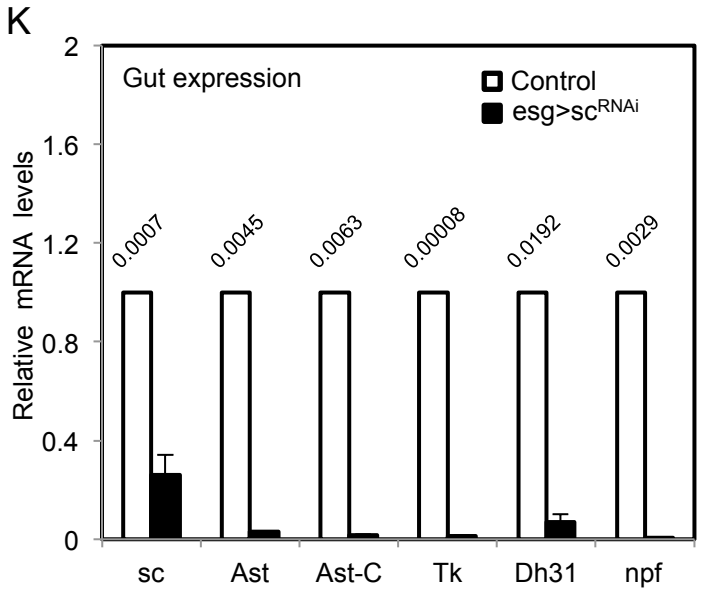
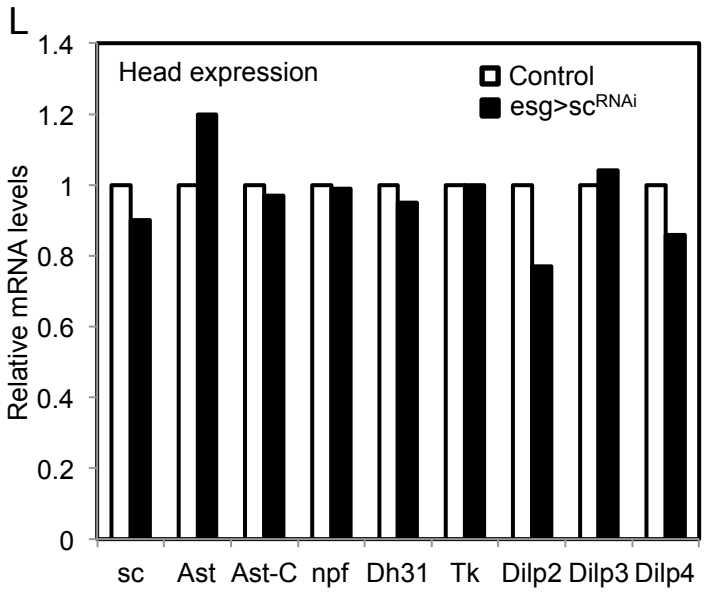
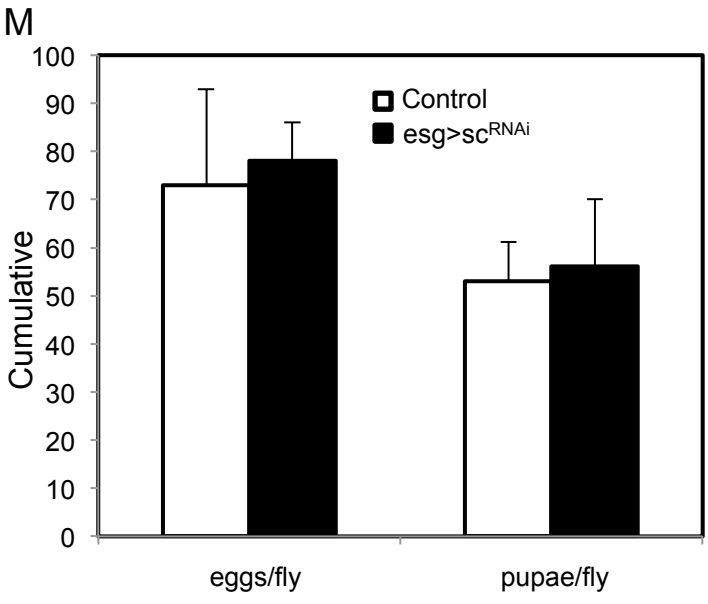
Figure S1. Related to main text Figure 1A-B.









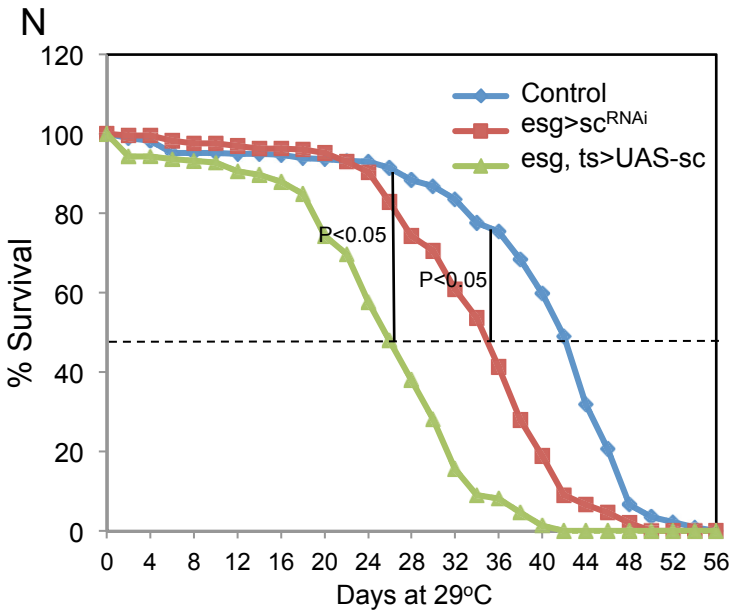


Figure S1. EE number, hormone gene expression and life span after loss of *sc* function. (A-F) Confocal images of adult midgut surface views. esg-Gal4 flies were crossed with *w-* or the indicated UAS-RNAi constructs. 7 days old flies were used for dissection and staining. For all images in this figure, blue is DAPI for DNA, red membrane is Armadillo (β-catenin) and red nuclear is Prospero. The arrows point to examples of EEs. Scale bar is 20 μm. (G-J) Confocal images from the midguts of flies with the indicated genotypes. The deficiency for *sc* was *Df(1)sc*$^{10-1}$ and for *ato* was *Df(3R)p13*. (K, L) Peptide hormone gene expression in midgut and head. 5 days old flies were used for dissection and RNA isolation from heads and midguts. The cycle number of each gene was normalized with that of *rp49* in a parallel reaction of the same RNA sample. The level of expression relative to *rp49* in control sample is shown at the top of each white bar, and this normalized expression was set as 1 for each gene. The expression of that gene in the *sc*^{RNAi} guts was calculated as the ratio to that in control. (M) For fecundity assay, males and females were put together and transferred to new vials every day. Eggs were counted in each vial for 10 days. Four independent experiments were performed and the average cumulative number of eggs laid per female fly was plotted. After flies were transferred out, the vials were kept and the embryos were let grown and the pupae were counted. Four independent experiments were performed and the average pupae number per female fly was plotted. (N) 100 flies of each genotype were kept at 29^{o}C in normal food vials and transferred to fresh vials every other day. The percent of flies survived at each time was recorded. The experiment was performed 3 times, and the average at each time point was plotted. The P value was calculated by comparing the survival of control sample on the same day as 50% survival of the *sc* samples.

Figure S2. Related to main text Figure 1C-H.

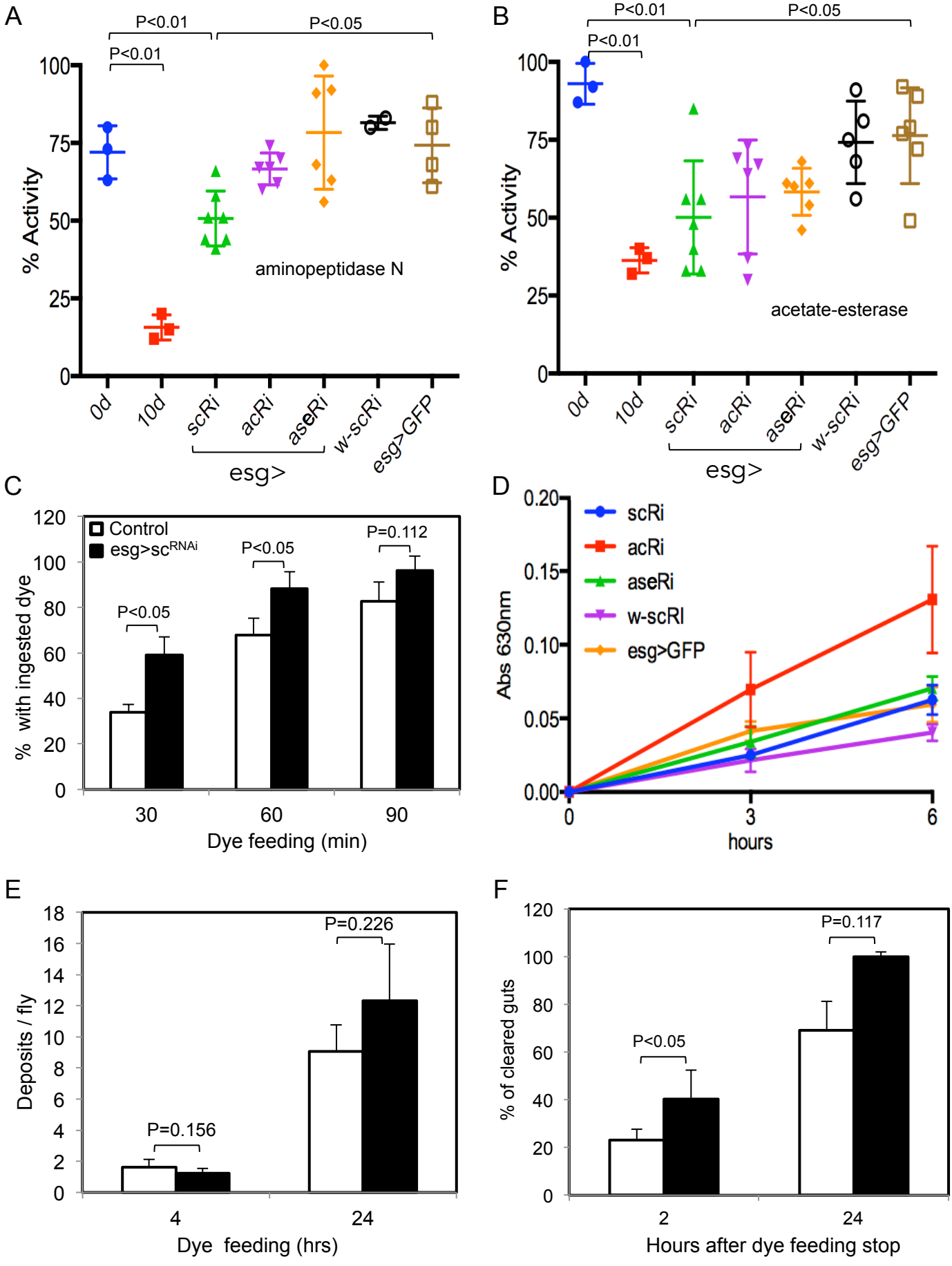


Figure S2. Digestive enzyme activities, food intake and food passage assays in EE-less flies. (A-B) For digestive enzyme assay, midguts from 7 days old females were homogenized and mixed with the corresponding substrate for the indicated enzymatic assay. Each genotype analyzed had 5-6 samples of 10 midguts each. (C) 20 control and esg>sc^{RNAi} flies were transferred to plastic vials containing a filter paper soaked with 5% sucrose in water and 0.5% bromophenol blue (BPB) sodium salt. Every 30 minutes flies that had visible ingested dye in the abdomen were counted. Six independent experiments were performed and the average accumulative at each time point was plotted. (D) 10 midguts from 7 days old females of each genotype were pooled at 3 and 6 h after transferring the flies to a plastic vial containing a solution of sucrose 50 mM (1.71%) and 2.5% (w/v) blue dye (food colorant E131). Food intake was monitored by the increase in absorbance at 630 nm (Mithras LB 940, Berthold Technologies) of the supernatant after midgut homogenization and centrifugation (10.000x g 10 min). Average of 3 experiments was plotted. (E-F) The fly strains were kept in 5% sucrose and 0.5% BPB. At the indicated times, blue deposits on the plastic vials were counted. Similarly, 10 flies were well fed to have visible blue abdomens and then transferred to a new vial containing 5% sucrose solution. Flies that had clear abdomens were counted after the time as indicated. Three independent experiments were performed and the average was plotted.

Figure S3. Related to main text Figure 3.

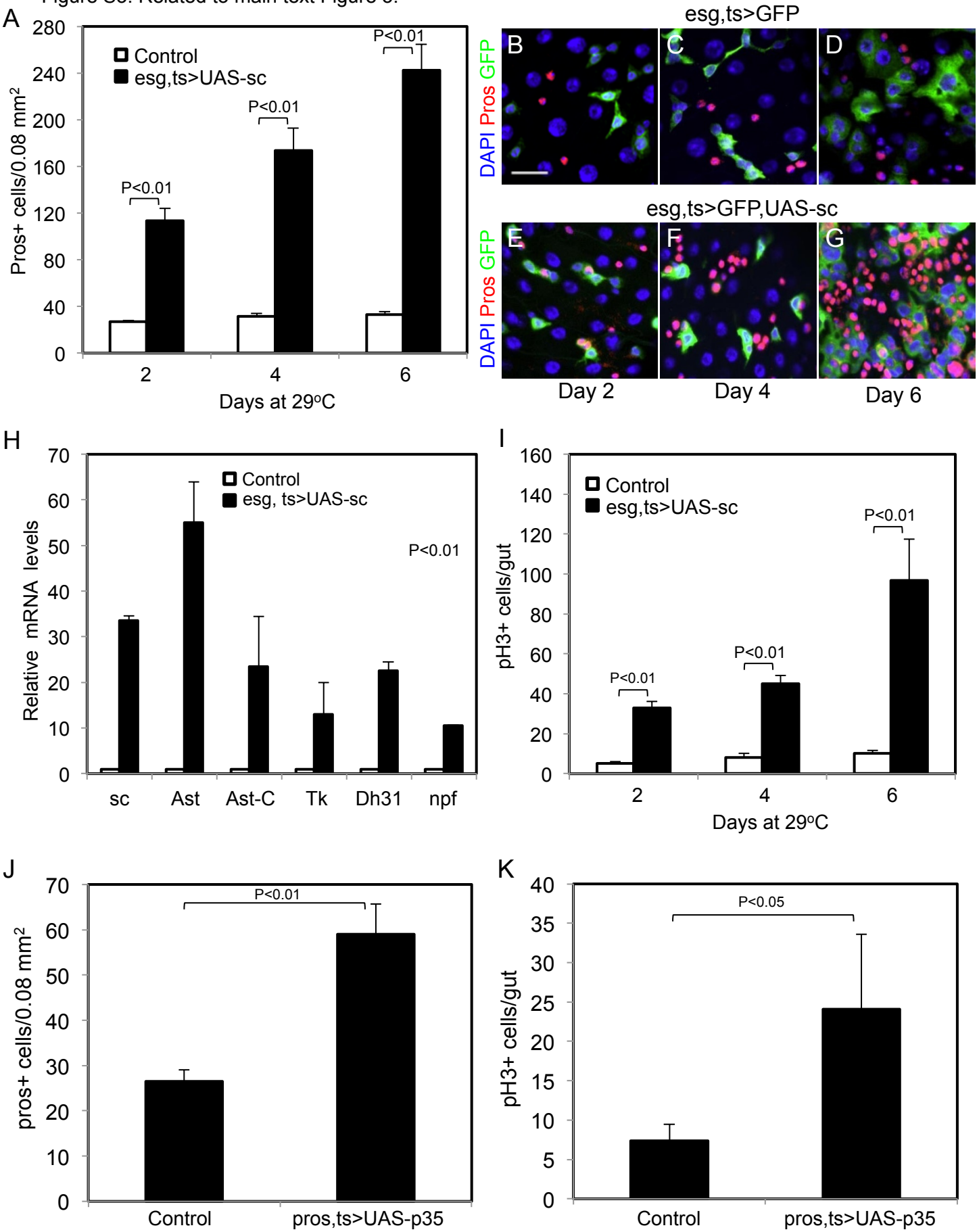
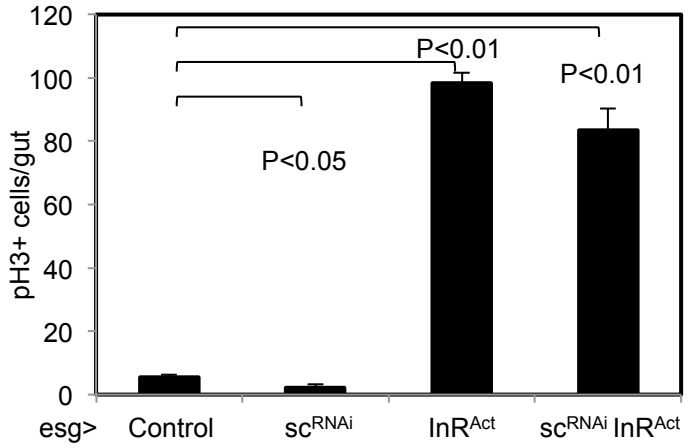


Figure S3. EE number correlates with ISC proliferation. (A) The flies approximately 7 days old were shifted to 29°C for 2 to 6 days to inactivate Gal80^{ts} and allow Gal4 to activate UAS-*sc* expression. Quantification of Prospero+ nuclei were performed from midguts of esg^{ts}>GFP control and esg^{ts}>GFP, UAS-*sc* flies. (B-G) Representative confocal images of midgut surface views from control and *sc* over-expression flies. Blue is DAPI for DNA, red nuclear is Prospero and green is mCD8GFP. The scale bar is 20 μm. (H) The esg^{ts}>GFP control and the esg^{ts}>GFP, UAS-*sc* flies were shifted to 29°C for 4 days and used for gut dissection and RNA isolation. The primer sets for the indicated genes were used for qPCR. The cycle number of each gene was normalized with that of *rp49* in a parallel reaction of the same RNA sample. The normalized expression of each gene in the control sample was set as 1 and the expression of that gene in the UAS-*sc* background was calculated as fold change. (I) Average number of p-H3+ cells in whole midguts of esg^{ts}>GFP control and esg^{ts}>GFP, UAS-sc flies. 7 days old flies were shifted to 29°C to initiate *sc* overexpression. At the days indicated, a portion of the flies was used for midgut dissection and p-H3 staining. (J-K) The *pros* promoter-Gal4 and tubulinGal80^{ts} (pros^{ts}>)were crossed with UAS-GFP control and UAS-p35 transgenic flies. 5 days old flies were shifted to 29°C for 4 days and then used for gut dissection, Pros and p-H3 staining, and quantification.

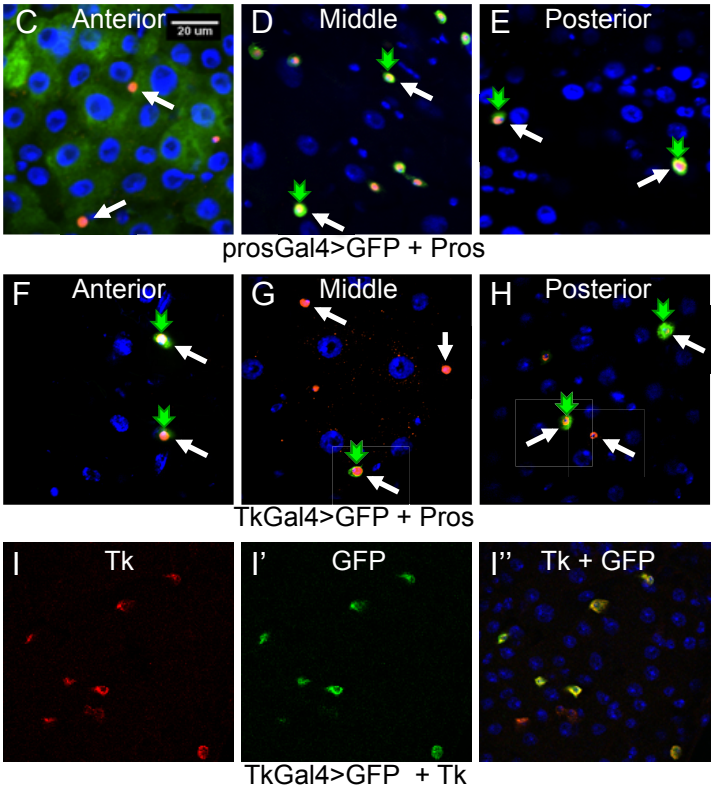
Figure S4. Related to main text Figure 4.

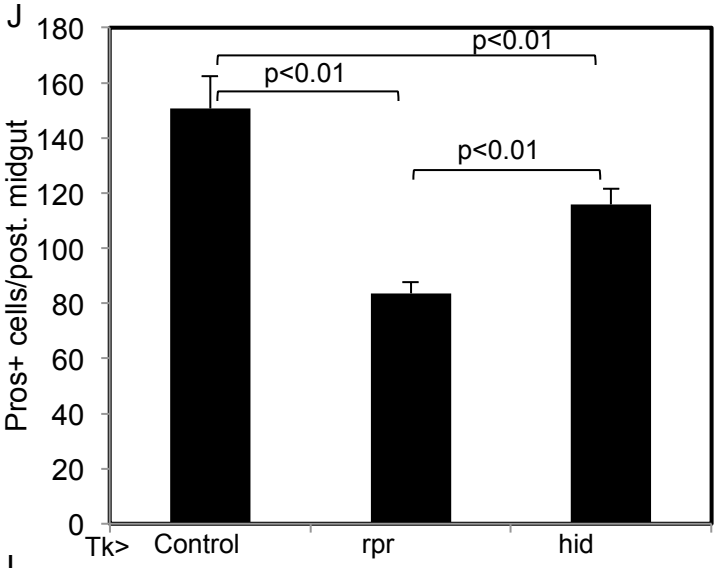
A

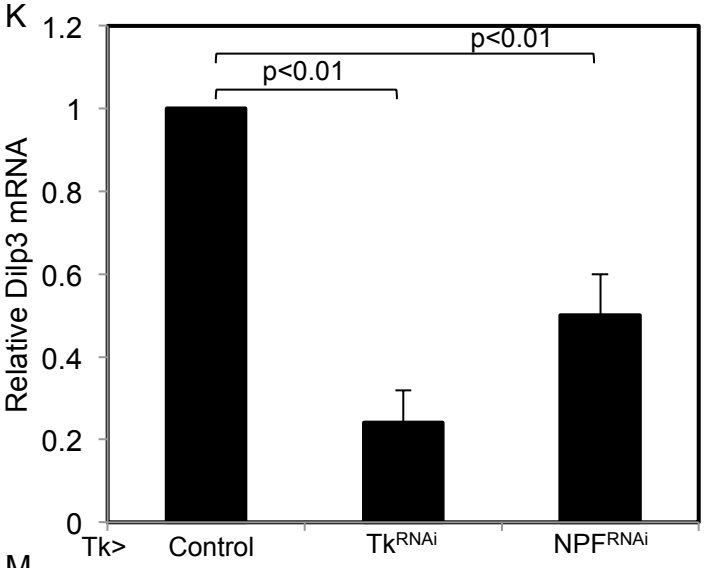


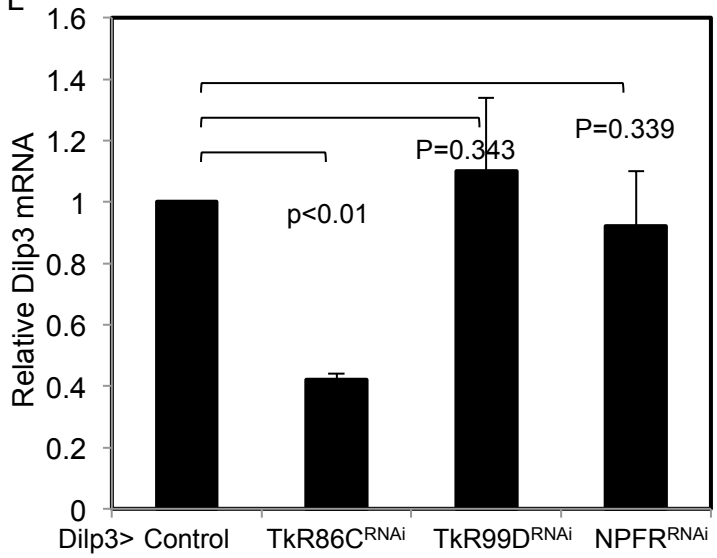
B

	% Gal4+ cells that are EEs	% EEs that are Gal4+
	Pros+/prosGal4>GFP+(%)	ProsGal4>GFP+/Pros+ (%)
Middle Midgut	100 +/- 0	88 +/- 7.7
Posterior Midgut	69 +/- 13	35 +/- 6.9
	Pros+/TkGal4>GFP+ (%)	TkGal4>GFP+/Pros+ (%)
Middle Midgut	100 +/- 0	12 +/- 6.4
Posterior Midgut	100 +/- 0	61 +/- 4.3









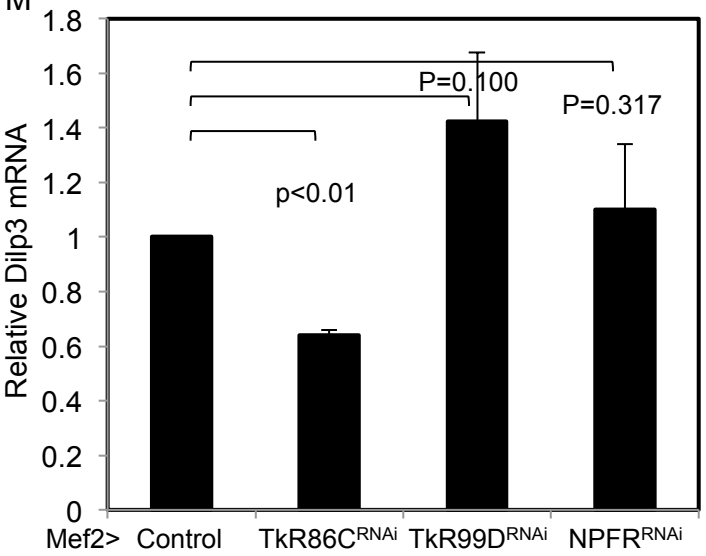


Figure S4. Midgut expression of pros-Gal4 and Tk-Gal4 and functional assays of TK signaling. (A) The esg-Gal4 was used to express an activated insulin receptor (InR^{Act}), together with the scRNAi. Flies 3 days after eclosion were used for gut dissection and p-H3 quantification. (B) Quantification of Pros+ staining overlapping with the Gal4 driven GFP+ cells. The ratios are calculated to show the percentage of GFP+ cells that should be EEs, or to show the percentage of EEs that have the Gal4>GFP expression. (C-I'') UAS-GFP were crossed with the Gal4 drivers as indicated and midguts of 5-7 days old flies were dissected and stained using antibodies for Pros (C-H) or Tk (I-I'') proteins. The arrows indicate examples of Pros+ nuclear staining, and the wide green arrows indicate overlapping GFP+ staining in some EEs. The GFP signal in panel C reveals pros-Gal4 expression in the ECs of a small anterior midgut region. Although we did not observe obvious increase of p-H3+ cells in this anterior region from the pros-Gal4/UAS-p35 experiments, we counted p-H3 staining from middle and posterior midguts only for these experiments. (J) The same experiments as in Fig. 4C, and the guts were stained and quantified for Pros+ EEs. (K) The same experiments as in Fig. 4D, and the guts were used for PCR quantification of *Dilp3* mRNA. (L, M) The same experiments as in Fig. 4E and F, and the guts were used for PCR quantification of *Dilp3* mRNA.

qPCR primer sequences. Related to Real-time qPCR in Experimental Procedures.

scute: 5'-CAATTCGGCAACGAAGAT and 5'-CAGCGGGTTGATTTTGAT

allatostatin: 5'-TCCGCAACCCTTCAACTT and 5'- TGAATAAGTGCGCCATCC

allatostatin C: 5'-AGTTAACGCGACCAAAGG and 5'-AGTTTTTCGGCCTTGATG

neuropeptide F: 5'-ACCATGGCAACGTCACTA and 5'-AACTATTGCCCCGAAAAA

diuretic hormone: 5'-CAAACGAACCGTGGACTT and 5'-CAGACCCATGCGATGTTT

tachykinin: 5'-TACGCGAGCATTTGGACA and 5'-GAAATCGATGCGCTGAAG

phyllopod: 5'-CCTCCTCGGAATACCTGAAAC and 5'-GCCTGGATTAGCTGAACGATA

Dilp2: 5'-ATGGTGTGCGAGTATAATCC and 5'-TCGGCACCGGGCATG

Dilp3: 5'-AGAGAACTTTGGACCCCGTGAA and 5'-TGAACCGAACTATCACTCAACAGTCT

Dilp4: 5'-GCGGAGCAGTCGTCTAAGGA and 5'-TCATCCGGCTGCTGTAGCTT

rp49: 5'-CGGATCGATATGCTAAGCTGT and 5'-GCGCTTGTTCGATCCGTA