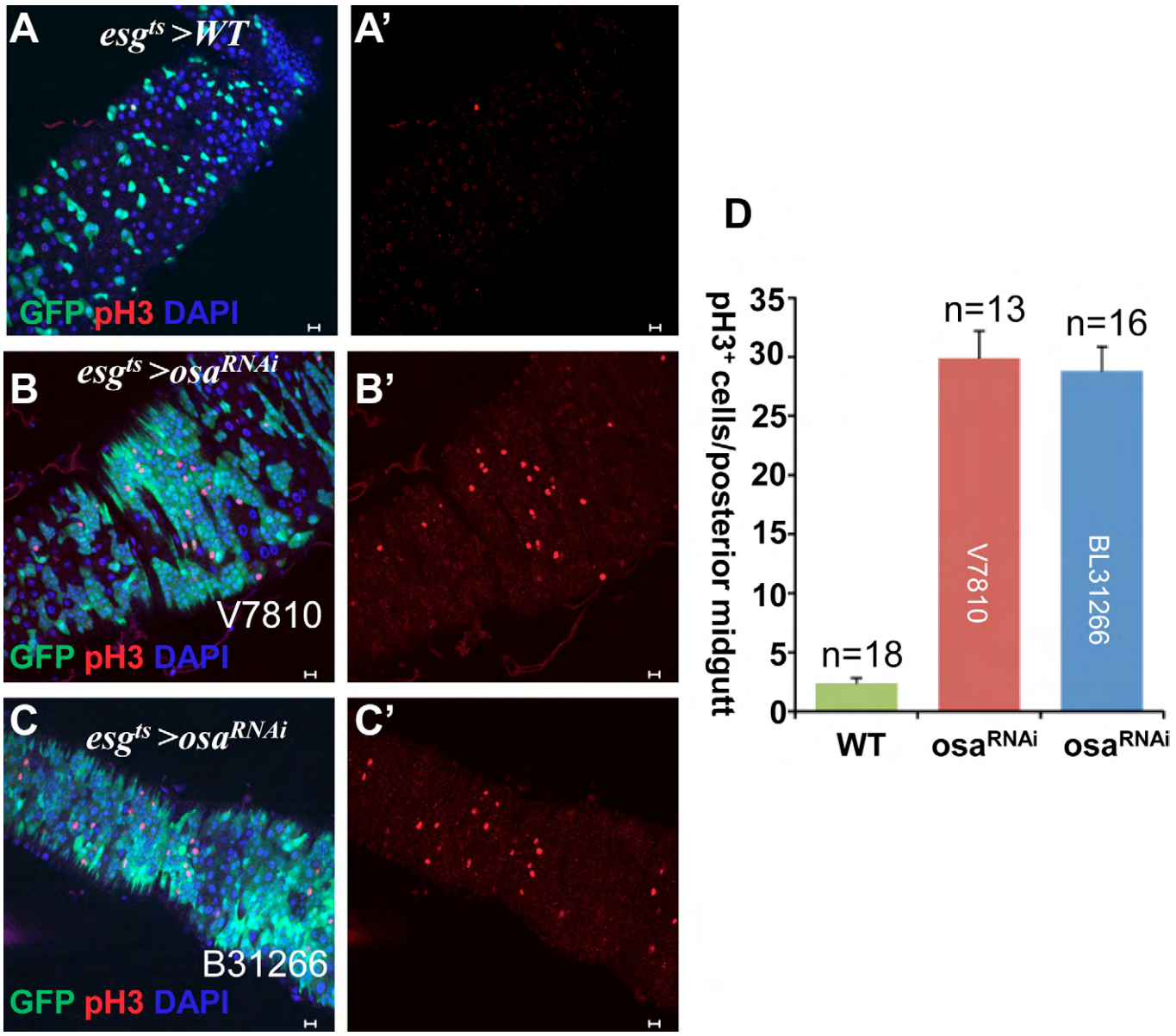

Fig. S1. Knockdown of *osa* increases cell division in posterior midgut. (**A-C′**) The wild-type control and osa^{RNAi} were expressed in the adult midgut using esg^{ts}. As compared with the wild-type control (A,A′), there were more pH3-positive dividing cells in osa^{RNAi} posterior midguts (V7810 in B,B′ and BL31266 in C,C′). (**D**) Quantification of pH3-positive cells in wild-type and osa^{RNAi} posterior midguts. Data are mean ± s.e.m.

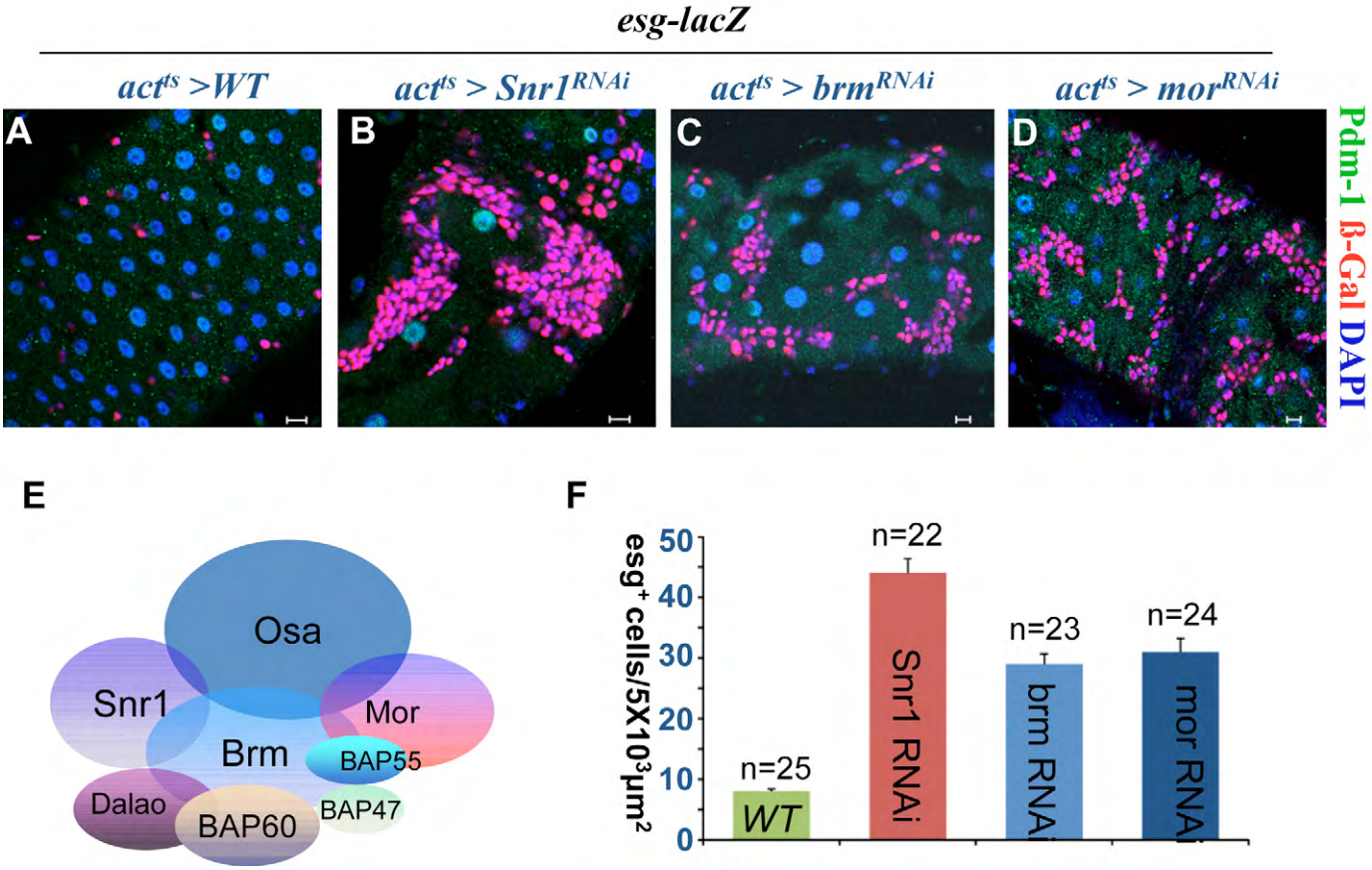

Fig. S2. Knockdown of other components in the SWI/SNF complex results in the expansion of *esg*-expressing cells. (**A-D**) The indicated genes were depleted in the adult *Drosophila* intestine using the *act*^{ts} (*act-Gal4, esg-lacZ /+; tub-Gal80*^{ts}/+) driver. (A) Wild-type control (*WT*). (B) *UAS-Snr1*^{RNAi} *V12645*. (C) *UAS-brm*^{RNAi} *V37721*. (D) *UAS-mor*^{RNAi} *V6969*. Scale bars: 10 μm. (**E**) Illustration of the Osa-containing SWI/SNF (Brm) complex. (**F**) Quantification of *esg*$^{+}$ cells in wild-type, *Snr1*^{RNAi}, *brm*^{RNAi} and *mor*^{RNAi} midguts. *esg-lacZ*-positive cells in 5×10^3 μm^2 gut tissue number eight for wild type (*n*=25); 44 for *Snr1* (*n*=22); 29 for *brm* (*n*=23); and 31 for *mor* (*n*=24). Data are mean ± s.e.m.

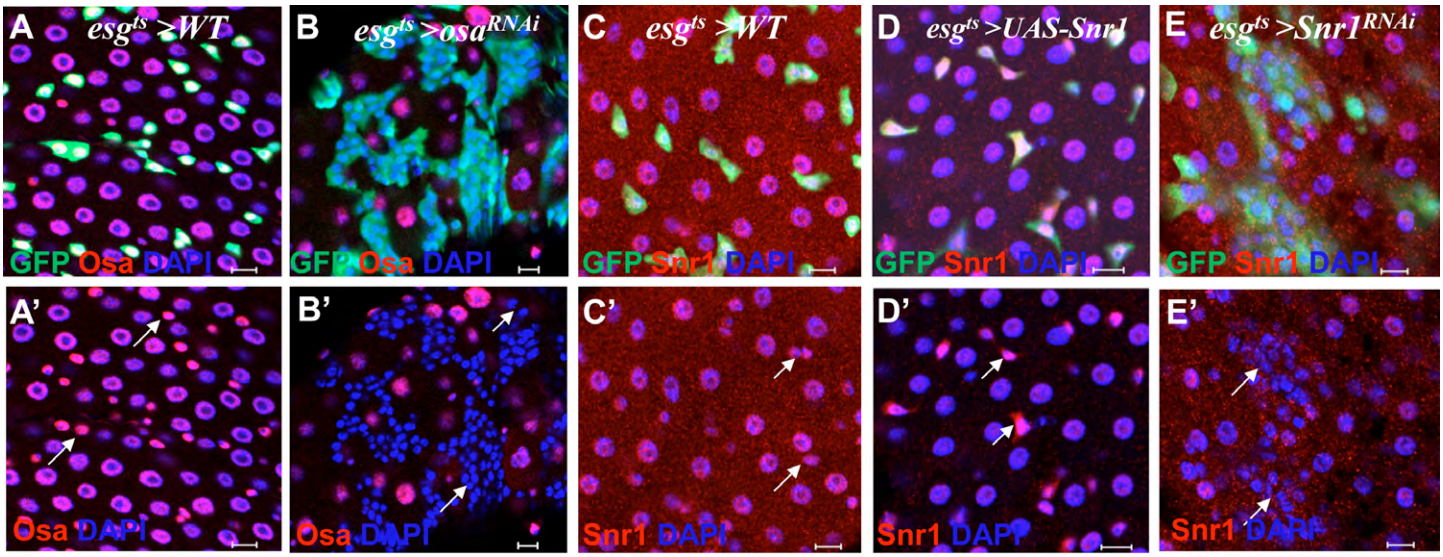

Fig. S3. Osa and Snr1 are expressed in all cell types in the posterior midgut. (**A-B′**) The wild-type control and *osa*^{RNAi} were expressed in the adult midgut using *esg*^{ts}. Osa is expressed in all cell types, including ISCs and EBs (arrows), in the wild-type midgut (A,A′). Osa is undetectable in GFP-labeled *osa*^{RNAi} cells (B,B′, arrows). (**C-E′**) The wild-type control, *UAS-Snr1* and *Snr1*^{RNAi} were expressed in the adult midgut. Snr1 is expressed in all cell types, including ISCs and EBs (arrows), in the wild-type midgut (C,C′). The higher expression of Snr1 was detected in GFP-labeled ISCs and EBs in the *UAS-Snr1*-overexpressing midgut (D,D′, arrows), but the expression of *Snr1* in GFP-labeled *Snr1*^{RNAi} cells (E,E′, arrows) was undetectable. Scale bars: 10 μm.

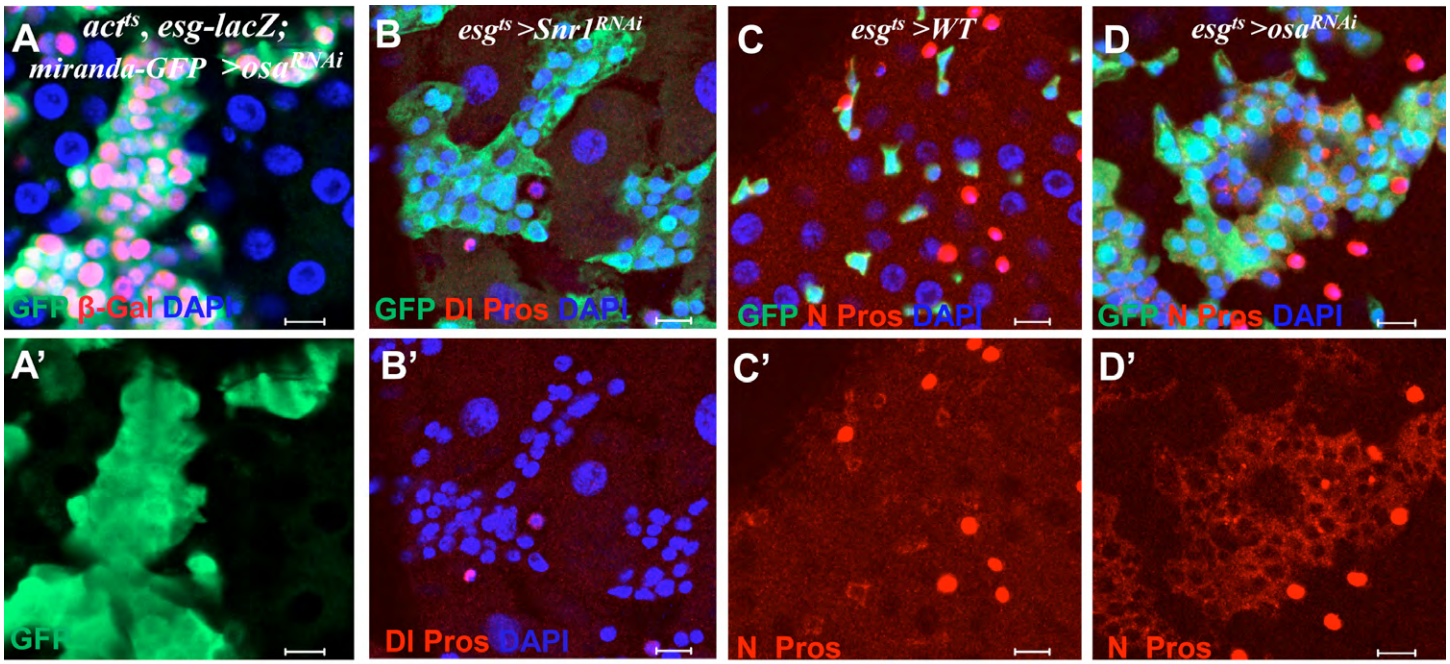

Fig. S4. The expression of Notch is unchanged in *osa*^{RNAi} cells. The wild-type control and *osa*^{RNAi} or *Snr1*^{RNAi} are expressed in the adult midgut using *esg*^{ts}. (**A**,**A′**) The ISC marker Mira-GFP is expressed in all expanded *esg*$^{+}$ cells in the *osa*^{RNAi} midgut, suggesting that these cells are ISC-like cells. (**B**,**B′**) No Dl expression was detected in the expanded ISC-like cells in the *Snr1*^{RNAi} midgut. (**C-D′**) In comparison to the wild-type control (C,C′), the expression of N is unchanged in the *osa*^{RNAi} midgut (D,D′). Scale bars: 10 μm.

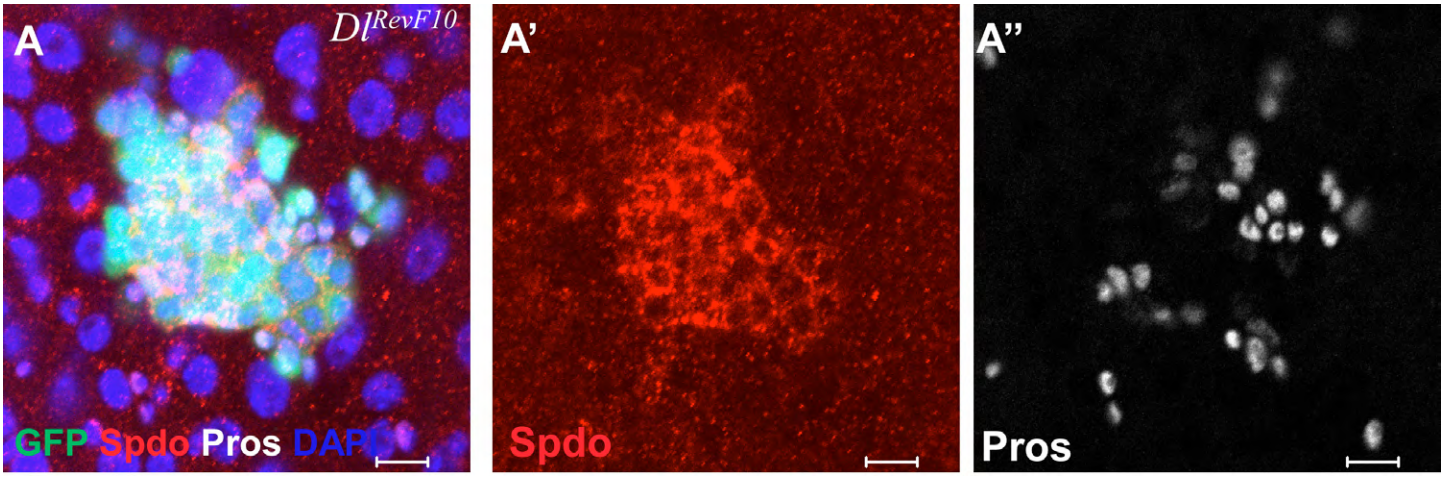

Fig. S5. *Dl* mutant MARCM clone. (**A-A″**) GFP^{+} *FRT*^{82B}-*Dl*^{RevF10} clones were generated in the posterior midguts of flies of the indicated genotypes using the MARCM technique, and the clones were stained at 8 days ACI with the indicated antibodies. In the *Dl* mutant clone, there were many Pros^{+} EEs in addition to Spdo^{+} ISC-like cells. Scale bars: 10 μm.

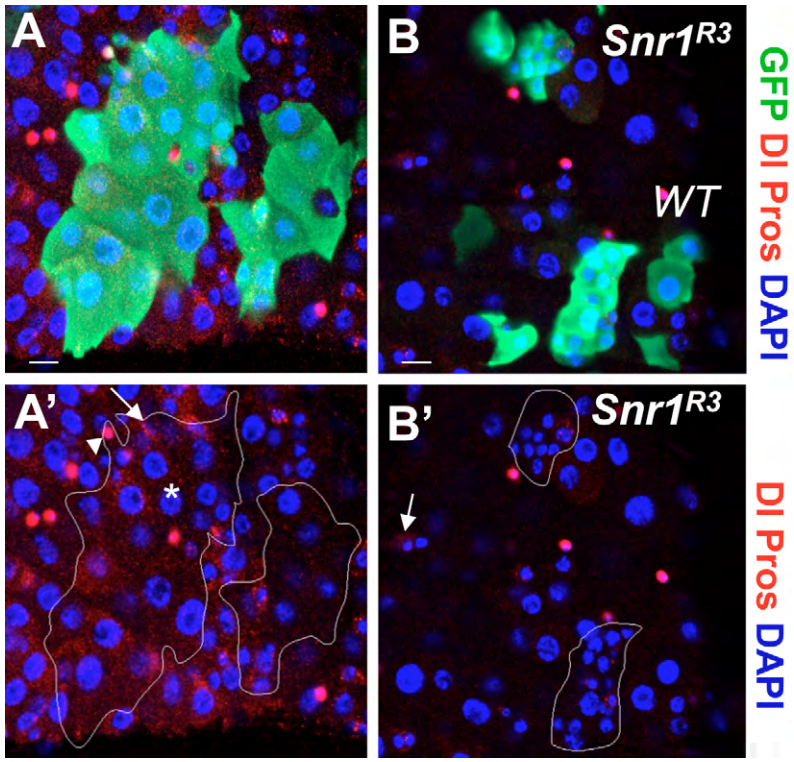

Fig. S6. *Snr1* autonomously regulates ISC fate. GFP$^+$ clones were generated in the posterior midguts of flies of the indicated genotypes using the MARCM technique and stained at 6 days ACI with the indicated antibodies. (**A**,**A′**) FRT^{82B} wild-type control clones. In wild-type clones there are Dl-labeled ISCs (arrow), EBs, differentiated ECs (large nuclei, asterisk) and Pros-positive EE cells (arrowhead). (**B**,**B′**) MARCM clones of $Snr1^{R3}$ lead to ISC-like cell expansion.

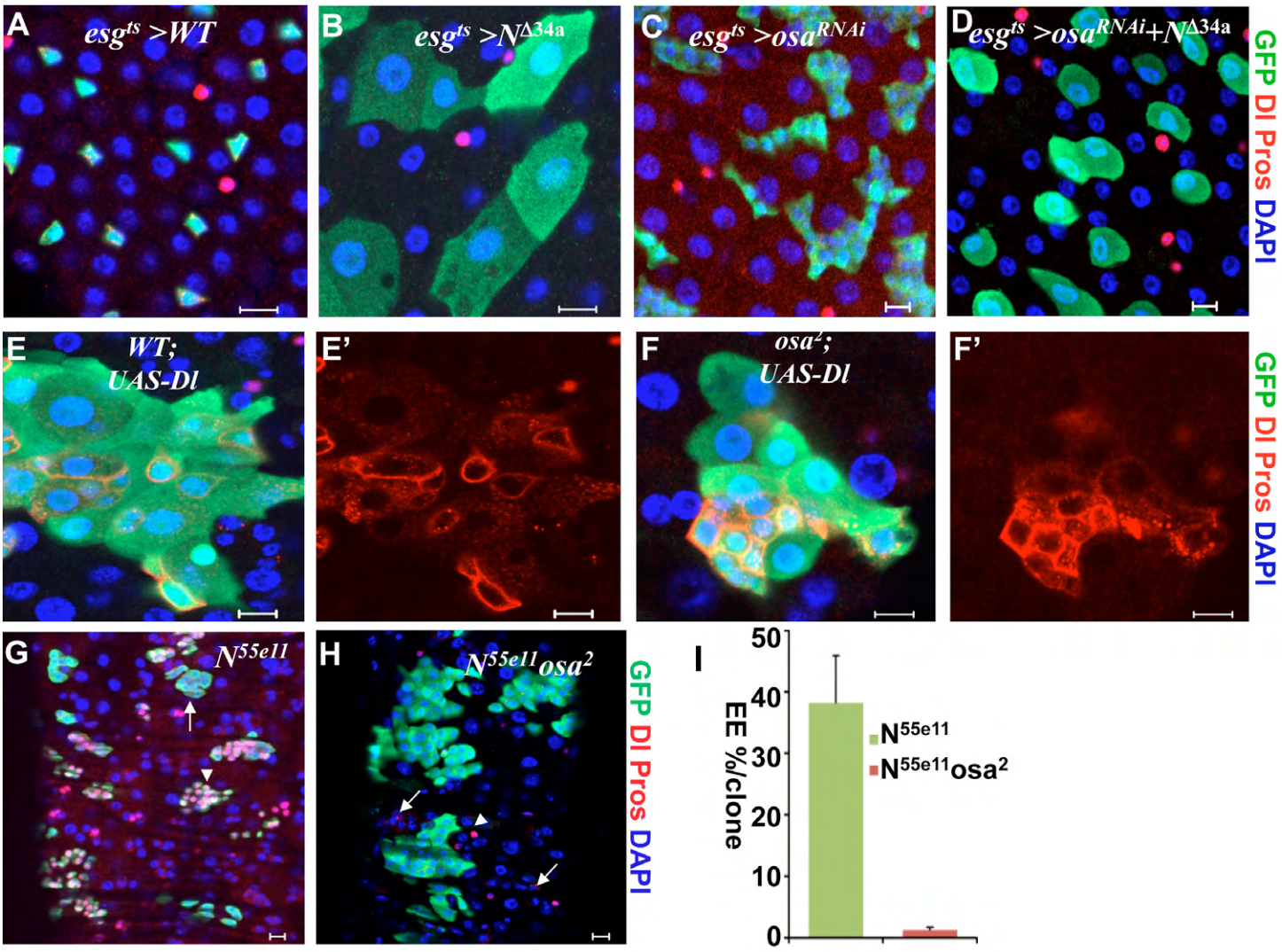

Fig. S7. Osa functions upstream of N in regulating ISC differentiation into ECs but downstream of N in regulating EE cell formation. (**A-D**) Osa functions upstream of N in regulating ISC fate specification. (A) Wild-type control. (B) Overactivation of N by expressing *UAS-N$^{\Delta 34a}$* leads to ISC differentiation into ECs. (C) The expression of *osa^{RNAi}* results in ISC-like cell expansion. (D) The expression of *osa^{RNAi}* plus *UAS-N$^{\Delta 34a}$* results in ISC differentiation into ECs. (**E-F′**) The expression of *UAS-Dl* in *osa^{2}* mutant MARCM clones rescues the ISC-like stem cell tumor phenotype (F,F′). The wild-type MARCM clones expressing *UAS-Dl* were used as a control (E,E′). (**G-I**) Osa functions downstream of N in regulating EE cell formation. GFP^{+} clones were generated in the posterior midguts of flies of the indicated genotypes using the MARCM technique, and the clones were stained at 6 days ACI with the indicated antibodies. The *osa^{2}* mutation suppresses the phenotype of excess EE cells associated with the *N^{55e11}* mutation. Arrows, Dl-positive ISCs; arrowheads, Pros-positive EE cells. (I) Analysis of the percentage of Pros^{+} EE cells in *N* or *N; osa* double-mutant clones at 6 days ACI. *osa^{2}* suppresses the excess Pros^{+} EE cells in the *N^{55e11}* mutant clones. Data are mean ± s.e.m. Scale bars: 10 μm.

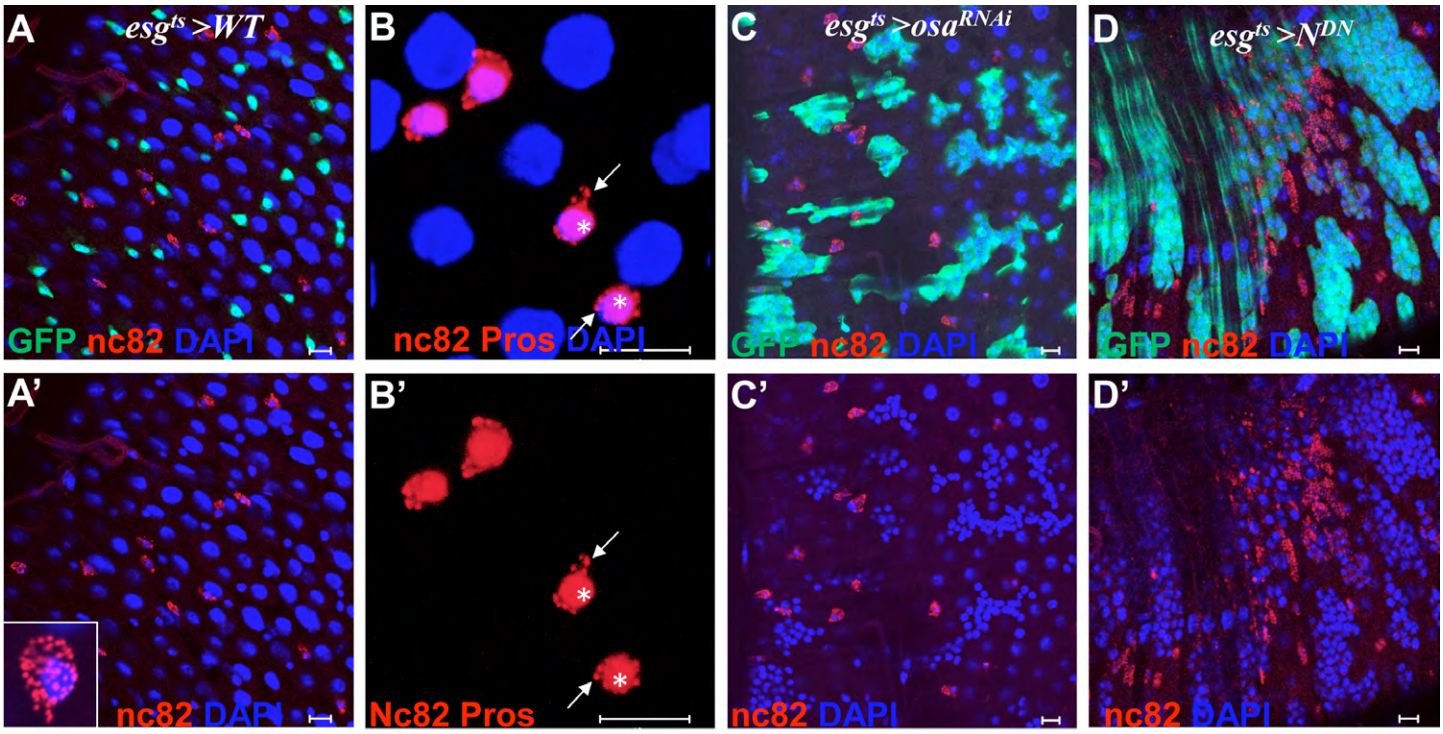

Fig. S8. nc82, a synaptic marker, specifically labels EE cells in the midgut. (**A**,**A′**) nc82 specifically labels *esg*⁻ diploid cells in wild-type flies. Membrane-associated punctate staining by nc82 (inset in A′). (**B**,**B′**) nc82 (arrow, punctate staining) labels Pros-positive (asterisk, nuclear staining) EE cells. (**C**,**C′**) No nc82-positive EE cells were found in *esg*⁺ cell clusters in the osa^{RNAi} midgut. (**D**,**D′**) nc82-positive EE cell clusters in the N^{DN} midgut. All flies were driven by esg^{ts} and stained with the indicated antibodies. Scale bars: 10 μm.

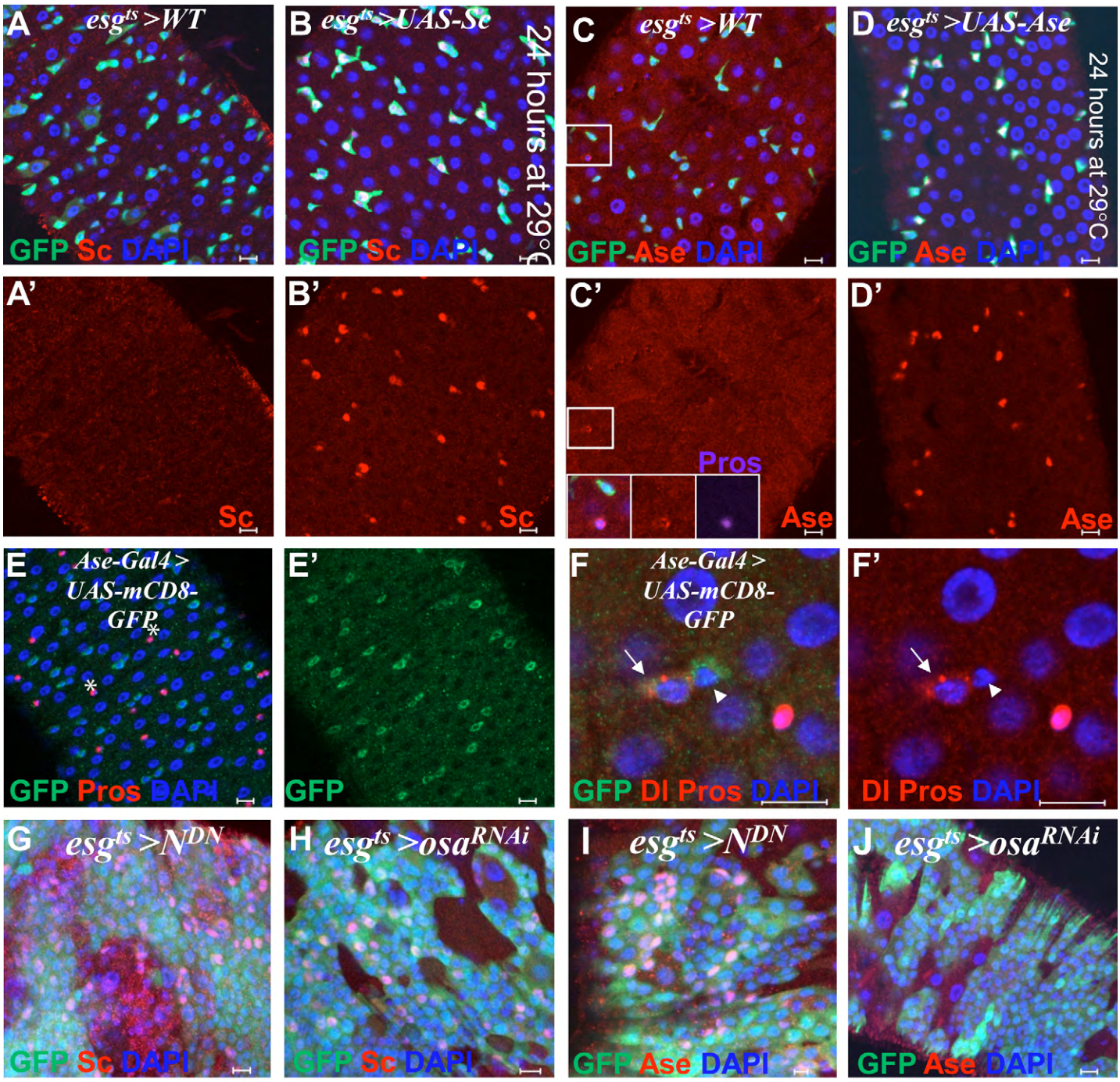

Fig. S9. Ase and Sc are expressed in the midgut at low levels. (**A-B′**) Endogenous Sc is not detected above background levels by immunofluorescence (A,A′). Nevertheless, Sc is readily detected in the gut with ectopic *UAS-sc* expression driven by esg^{ts} for 24 hours at 29°C (B,B′). (**C-D′**) Endogenous Ase is also not detected above background levels by immunofluorescence (C,C′). Nevertheless, Ase is readily detected in the gut with ectopic *UAS-ase* expression driven by esg^{ts} for 24 hours at 29°C (D,D′). There is non-specific staining in some portion of the EE cells by the anti-Ase serum, labeling the membrane and cytoplasm, which is not consistent with the nuclear localization of Ase (inset in C,C′). (**E-F′**) Ase-Gal4 is weakly expressed in ISCs and EBs (E,E′) but not in $Pros^{+}$ EEs (asterisk). GFP is detected in doublet cells, one of which is a Dl-positive ISC (arrow in F,F′) and the other an EB (arrowhead in F,F′). (**G**,**H**) The expression of *sc* is upregulated in both N^{DN} (G) and osa^{RNAi} (H) midguts (compare with A,A′). (**I**,**J**) The expression of *ase* is upregulated in the N^{DN} midgut (I) but not in the osa^{RNAi} midgut (J) (compare with C,C′). The wild-type and osa^{RNAi} were driven by esg^{ts} and stained by the indicated antibodies. Scale bars: 10 μm.

Table S1. Primers

Gene	Forward (5′-3′)	Reverse (5′-3′)
qPCR		
Rp49	TACAGGCCCAAGATCGTGAA	CACGTTGTGCACCAGGAACTTC
Dl	CACTCGACTTGCTCGGAGAC	CAGGGTCTGTGGTTGGTGCAG
ase	GTCAACGGAAGAGGCCCCTG	CTACGGGCAACGGCTTGTG
sc	GCTTCAGGATCTGGTGGATG	ATCCTGCATCTCCACCTGGTAC
ChIP		
Dl	CGAACTGCGGAGTCTTCTCCT	TCGGTGTGTGACGCGAACTGC
spdo	GATCGCGTTGAGACGTCTCAG	ACTGCGACTCGACTGATCTAG
ase	GAGAGAGAGCGTGGCGAAATC	CAGAAGGCATTTGAGGTCCTGCG