Stem Cell Reports, Volume *10*

Supplemental Information

A Phyllopod-Mediated Feedback Loop Promotes Intestinal Stem Cell Enteroendocrine Commitment in *Drosophila*

Chang Yin and Rongwen Xi

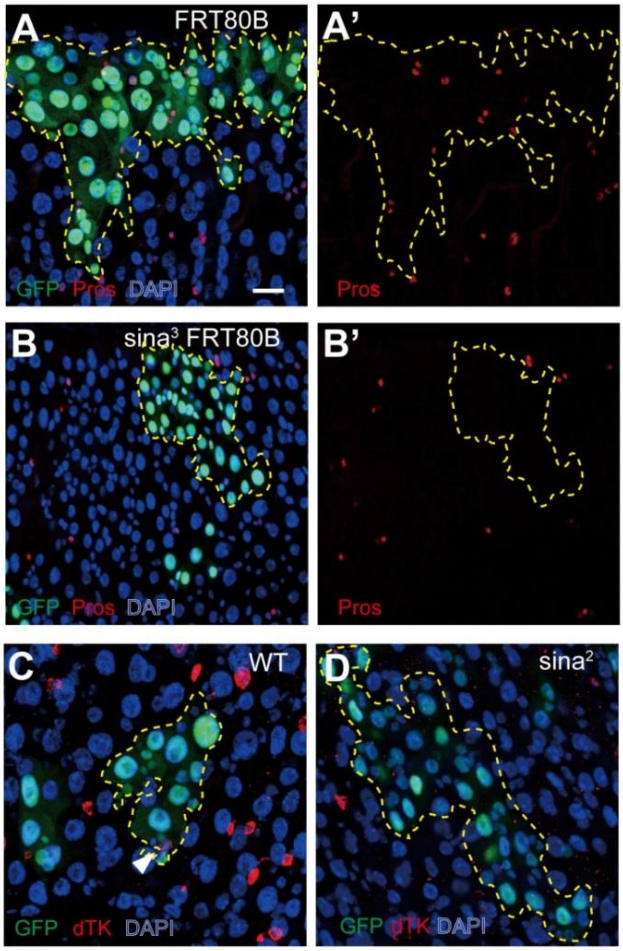

Fig. S1, Related to Figure 1. *sina* is required for EE cell fate specification.

Wild-type or *sina* homozygous mutant clones (GFP, green) were generated by the MARCM system and were examined on 10 day after clone induction (ACI).

(A-B') Clones co-stained with anti-Pros (red). (A,A') A wild-type *FRT80B* clone. (B,B') A *sina*^{3} *FRT80B* clone. Note the absence of Pros^{+} cells in *sina* mutant clones (dashed lines and the separated red channels).

(C-D) Clones co-stained with anti- dTK. (C) A wild-type clone. (D) A *sina*^{2} clone. dTK^{+} cells were not observed in *sina* mutant clones.

Scale bar: 20 μm

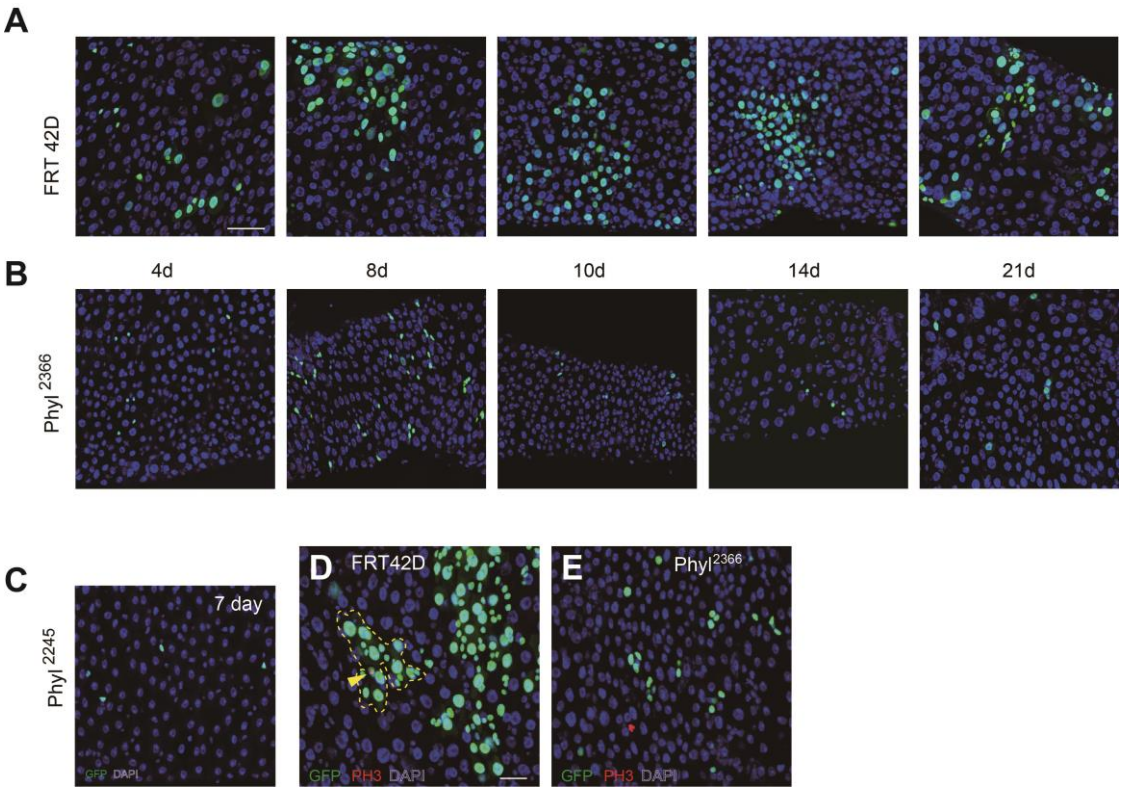

Fig. S2, Related to Figure 1. Clonal analysis of wild-type and *phyl* mutant cells in the midgut epithelia.

(A-B) GFP marked wild-type (upper panels) and *phyl*^{2366} mutant (lower panels) clones (green) were generated by the MARCM system, and examined at the indicated time points (days) ACI. Nuclei were stained with DAPI (blue).

(C) GFP marked *phyl*^{2245} mutant clones at 7 days ACI.

(D-E) Clones co-stained with PH3 to identify mitotic cells. (C) PH3^{+} cells could be detected in some wild-type *FRT42D* clones (yellow arrowhead). (D) PH3^{+} cells could not be detected in *phyl*^{2366} mutant clones.

Scale bar in A: 50 μm, in C: 20 μm.

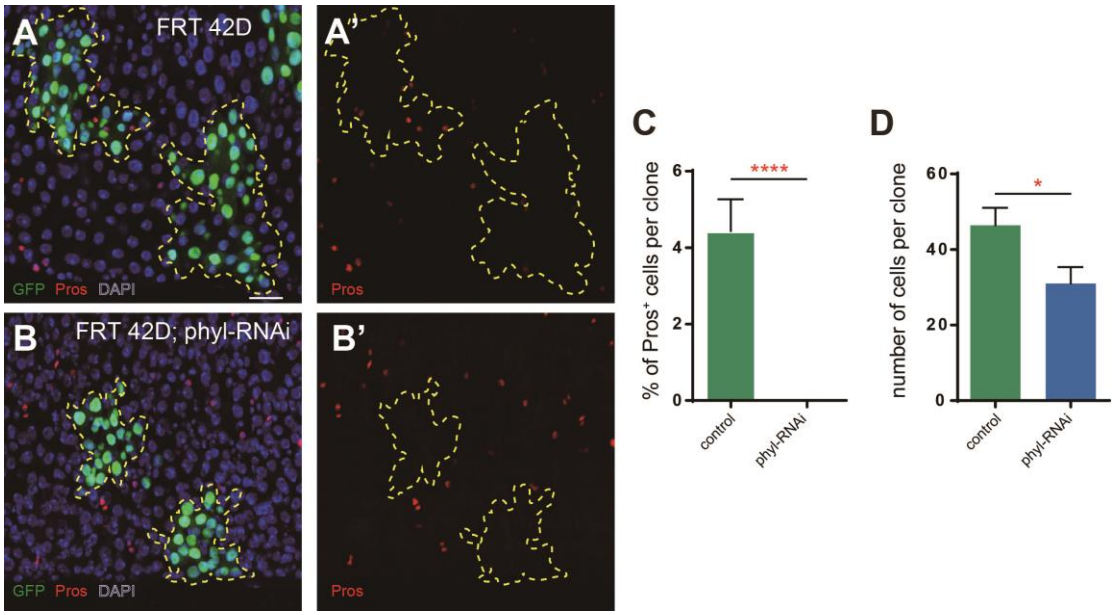

Fig. S3, Related to Figure 1. Effects of *phyl-RNAi* on ISC proliferation and EE differentiation.

Wild-type and *phyl-RNAi* homozygous clones (GFP, green) were generated by the MARCM system and were examined on 10 day after clone induction (ACI).

Clones were stained with anti-Pros (red). Note the absence of $Pros^{+}$ cells in *phyl-RNAi* clones (dashed lines and separated channels).

The proportion of $Pros^{+}$ cells per clone in wild-type and *phyl-RNAi* clones on days 10-14 ACI. Mean ± s.e.m. n=24 for *FRT 42D* clones, n=49 for *phyl-RNAi* clones. ****P<0.0001 (Student's t-test).

Quantification of clone size of wild-type and *phyl-RNAi* clones on days 10-14 ACI. Mean ± s.e.m. n=24 for *FRT 42D* clones, n=49 for *phyl-RNAi* clones. *P<0.05 (Student's t-test).

Nuclei were stained with DAPI (blue). Scale bar: 20 μm.

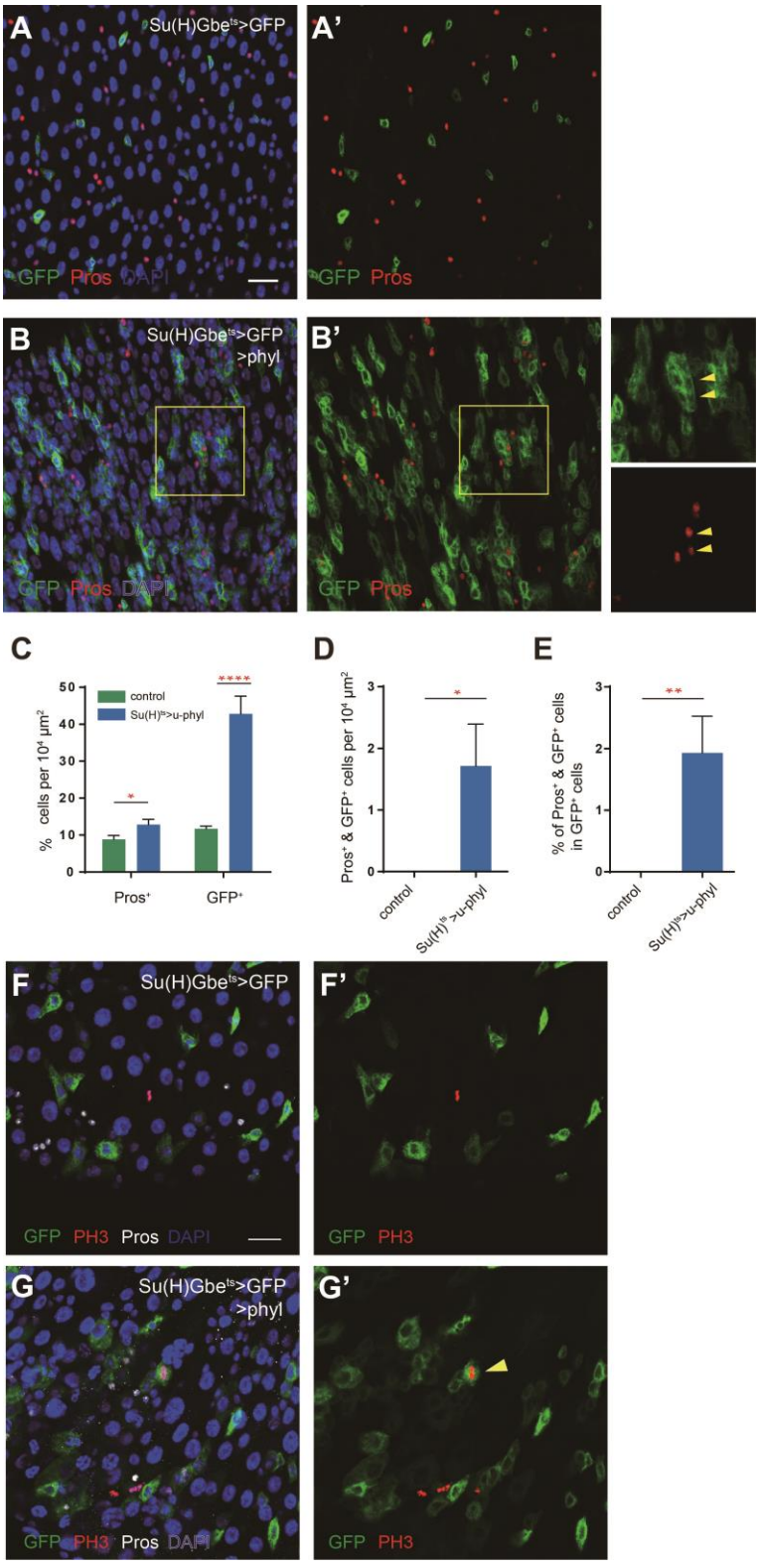

Fig. S4, Related to Figure 2. Overexpression of *phyl* in EBs causes EB accumulation and EE fate switch.

(A-B) Midgut epithelia of indicated genotypes stained with anti-Pros (red). Flies were shifted to the restrictive temperature for 14 days before analysis. Genotypes: *Su(H)Gbe-GAL4^{ts},UAS-GFP* (A); *Su(H)Gbe-GAL4^{ts},UAS-GFP; UAS-phyl* (B). GFP, green. Note that phyl overexpression causes increased number of EBs (GFP$^+$), and some of these EBs adopt EE fate (GFP$^+$ and Pros$^+$).

(C) Quantification of the percentage of Pros$^+$ or GFP$^+$ cells per epithelial area in the intestines of indicated genotypes.

(D) Quantification of the total number of Pros and GFP double-positive cells per epithelial area.

(E) Quantification of the percentage of Pros and GFP double-positive cells in GFP positive cells.

Mean ± s.e.m. n=7 (fields) for *Su(H)Gbe^{ts}>GFP*; n=7 for *Su(H)Gbe^{ts} >UAS-phyl* midguts in C-E. *P<0.05, **P<0.01, ****P<0.0001 (Student's t-test).

(F-G) Mitotic cells, as indicated by PH3 staining (red, yellow arrowhead), were not present in *Su(H)Gbe^{ts}>GFP* cells in wild-type midgut (F). Overexpression of *phyl* driven by *Su(H)Gbe^{ts}* induced some Su(H)Gbe>GFP cells to re-enter mitosis (G).

Scale bars: 20 μm.

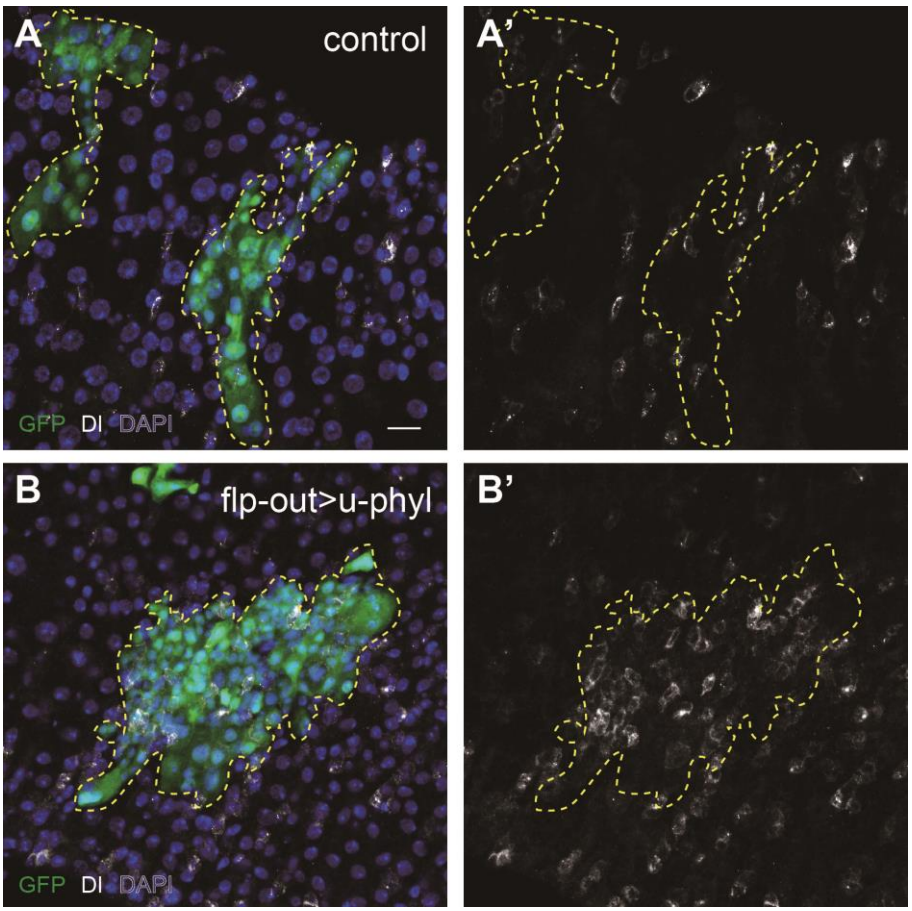

Fig. S5, Related to Figure 2. *phyl*-overexpressed clones contain extra ISC-like cells.

(A-B) Wild-type and *phyl* overexpression clones were generated by the flp-out system and were examined on 7 day ACI. Clones co-stained with anti-Dl (white). Dashed lines depict the clone margin.

Scale bar: 20 μm.

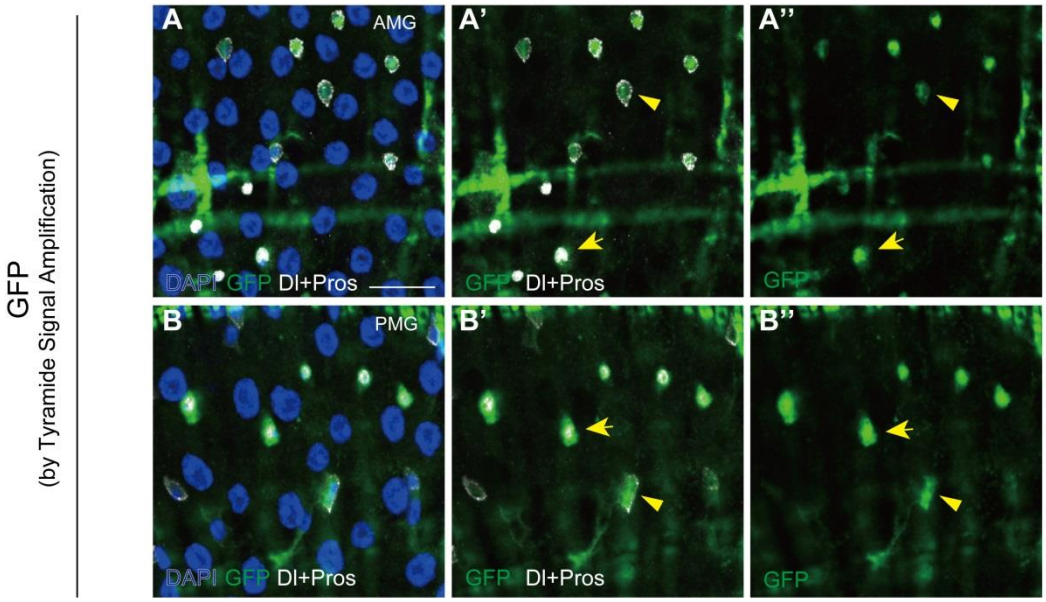

Fig. S6, Related to Figure 5. *phyl3.4-nGFP* expression pattern detected by the TSA method.

(A-B) The Tyramide Signal Amplification (TSA) method was used to amplify the GFP signal in *phyl*$^{3.4}$*-nGFP* intestines. With this method, GFP signal was detected in the majority of Pros^{+} EE s (yellow arrow) and Dl^{+} ISCs (yellow arrowhead) at both the anterior (A) and the posterior (B) midgut regions.

Scale bar: 20 μm.

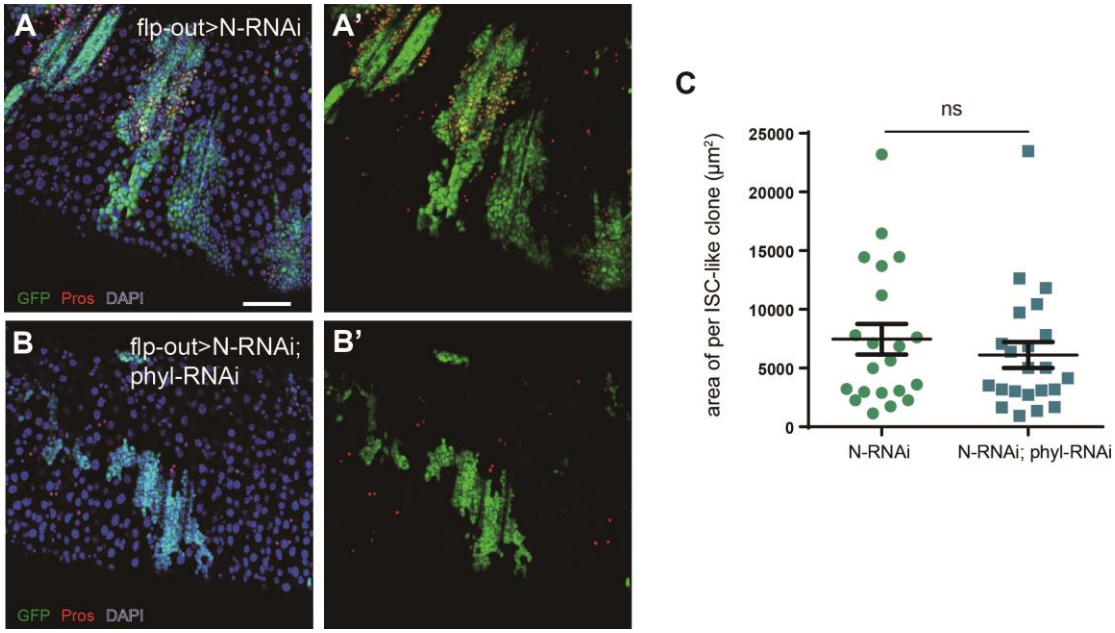

Fig. S7, Related to Figure 6. Depletion of *phyl* blocks *Notch*-loss-induced EE-like tumor fomration.

(A-B) N-RNAi and *phyl*, N double RNAi clones were generated by the flp-out system and were examined on 14 day ACI. Clones co-stained with anti-Pros (red). Dashed lines depict the clone margin. Note the absence of EE-like tumors in *phyl*, *N* double RNAi clones, while ISC-like tumors still could be formed.

(C) Quantification of ISC-like tumor size in *N-RNAi* and *phyl*, *N* double RNAi clones. Mean ± s.e.m. n=21 for *N-RNAi* clones, and n=22 for *phyl*, *N* double RNAi clones. ns, no significant difference.

Scale bar: 20 μm.