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Supplemental Information

Debra-Mediated Ci Degradation Controls

Tissue Homeostasis in *Drosophila* Adult Midgut

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1. Supplemental figures

Li, Fig. S1

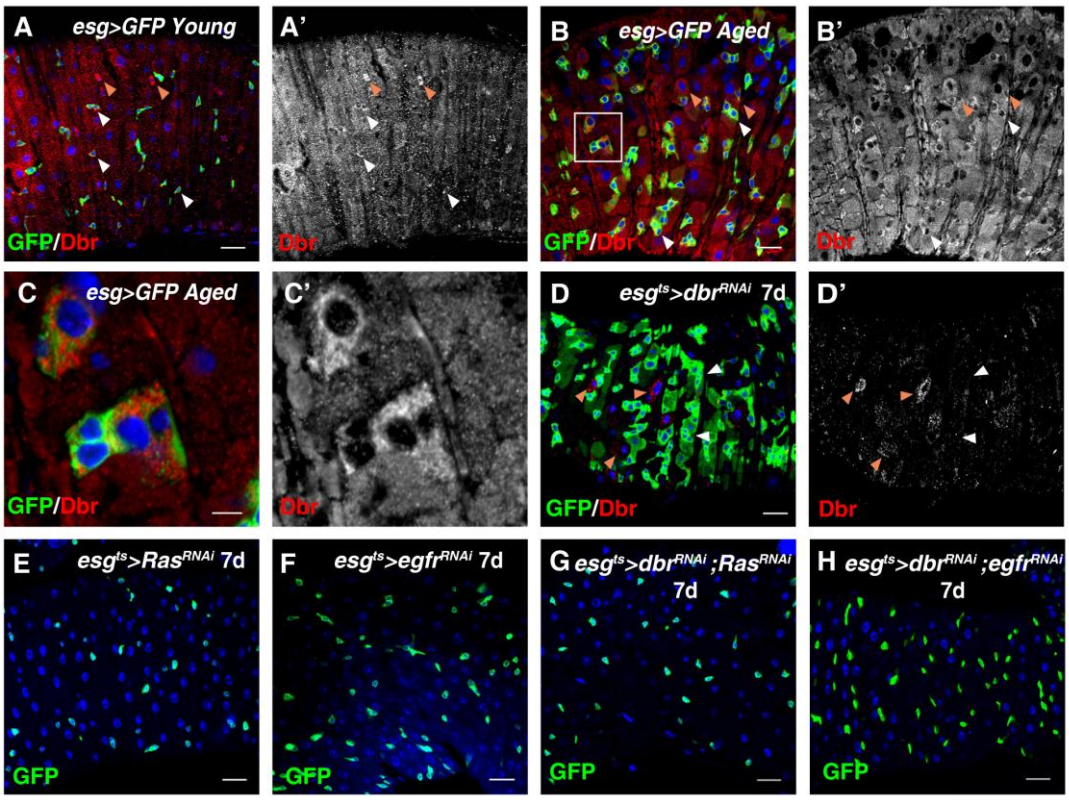

Figure S1 (relates to Figure 1): Tissue homeostasis loss in *esg^{ts}>dbr^{RNAi}* intestines

can be effectively suppressed by inhibition of EGFR signaling

(A-D) Dbr (red) is expressed in different intestinal cell types including the progenitors (green, by *esg>GFP*) (white arrowheads) and other cell types (putatively ECs and ee, orange arrowheads) in adult midgut (A-C). Dbr staining is dramatically reduced in esg^{+} cells (white arrowheads) in $esg^{ts}>dbr^{RNAi}$ intestines (ECs still retain high level of Dbr staining, by orange arrowheads) (D).

(E) esg^{+} cells (green) in $esg^{ts}>Ras^{RNAi}$ flies at 29°C for 7 days. Please refer to Figure 1C for control.

(F) esg^{+} cells (green) in $esg^{ts}>Egfr^{RNAi}$ flies at 29°C for 7 days. Please refer to Figure 1C for control.

(G) The accumulation of esg^{+} cells resulted from *dbr* depletion is effectively suppressed by co-inhibition of Ras ($esg^{ts}>dbr^{RNAi};Ras^{RNAi}$) for 7 days at 29°C. Please refer to Figures 1D, 1K and 1O of $esg^{ts}>dbr^{RNAi}$ for comparison.

(H) The accumulation of esg^{+} cells resulted from *dbr* depletion is effectively suppressed by co-inhibition of EGFR ($esg^{ts}>dbr^{RNAi};Egfr^{RNAi}$) for 7 days at 29°C. Please refer to Figures 1D, 1K and 1O of $esg^{ts}>dbr^{RNAi}$ for comparison.

In all panels, blue indicates DAPI staining for DNA. Scale bars, 20 μm except panel C (5 μm).

Li, Fig. S2

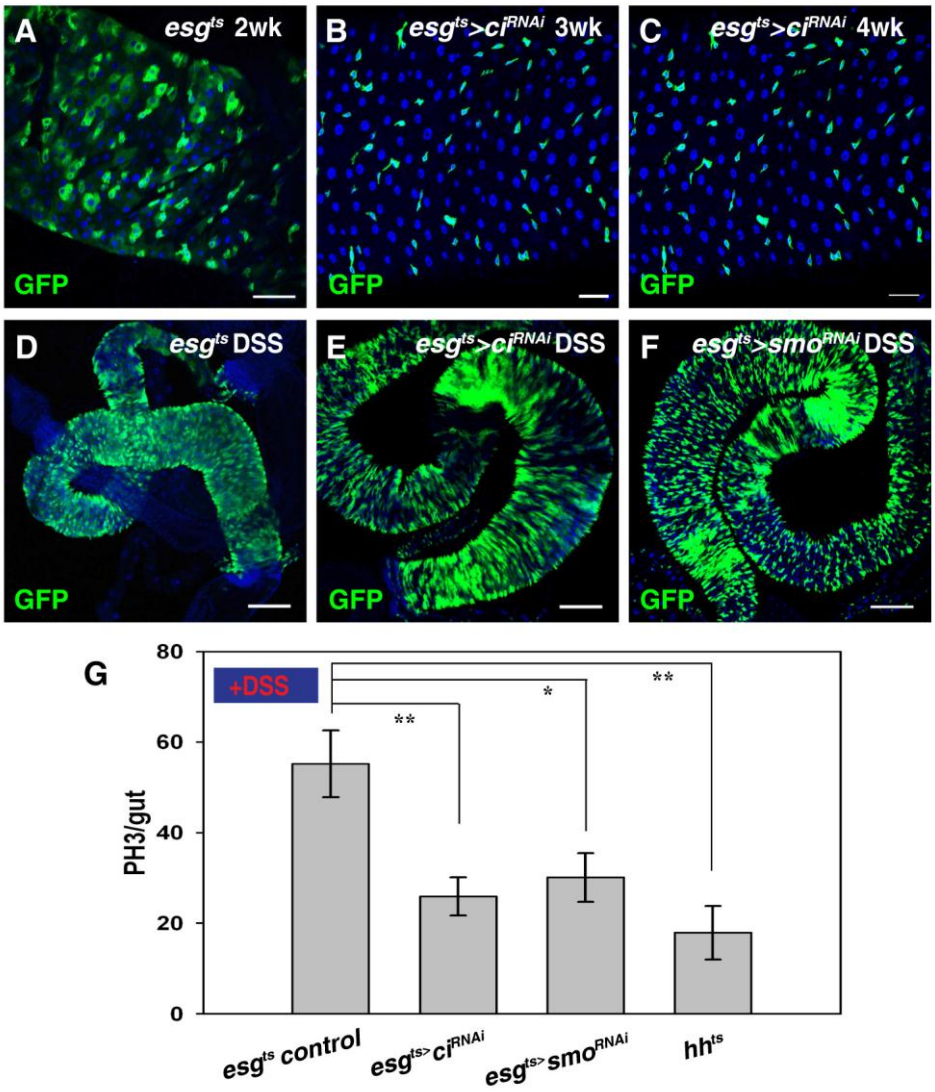

Figure S2 (relates to Figure 2): Depletion of Ci suppresses ISC proliferation while inhibition of Hh signaling leads to compromised midgut regeneration

(A) *esg*$^{+}$ cells (by *esgGal4>GFP*, green) in aged flies (2 weeks at 29°C). Note that the number of *esg*$^{+}$ cells is increased, the morphology of these cells is aberrant, and GFP signal can be observed in many large cells, indicating that midgut homeostasis is lost.

(B) *esg*$^{+}$ cells (green) in *esg*^{ts}*>ci*^{RNAi} flies at 29°C for 3 weeks. Please refer to Figure 1C for control.

(C) *esg*$^{+}$ cells (green) in *esg*^{ts}*>ci*^{RNAi} flies at 29°C for 4 weeks. Please refer to Figure

1C for control.

(D) Massive tissue regeneration can be observed when esg^{ts} flies were fed with DSS. The number of esg^{+} cells (by GFP, green) is dramatically increased.

(E) Accumulated GFP^{+} cells can still be observed when $esg^{ts}>ci^{RNAi}$ flies were fed with DSS.

(F) Accumulated GFP^{+} cells can still be observed when $esg^{ts}>smo^{RNAi}$ flies were fed with DSS.

(G) Quantification of midgut PH3+ cells in different genotypes indicated. Mean±SEM are shown. n=10–15 intestines. *p<0.05, **p<0.01.

In all panels, blue indicates DAPI staining for DNA. Scale bars, panels A-C: 20 μm; panels D-F: 100 μm.

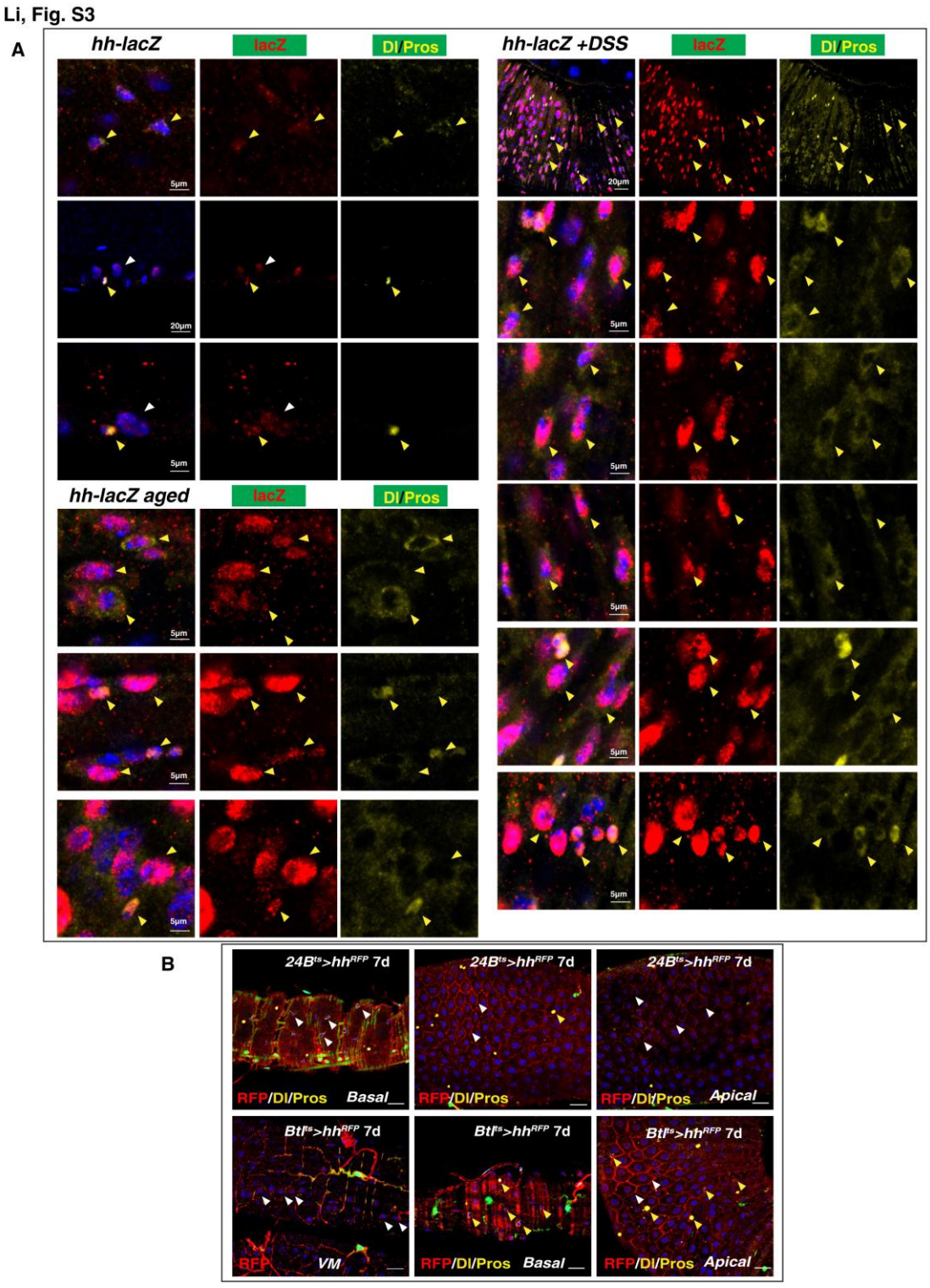

Figure S3 (relates to Figure 3): Hh expression is significantly increased in aged and stress flies and Hh protein can be transported to the midgut epithelium from its producing sources

(A) Beta-gal signal (by beta-gal in red) can be detected at low levels in the nucleus of

Dl^+ and $Pros^+$ cells (by Dl and Pros in yellow, yellow arrowheads) in *hh-lacZ* flies (EC by white arrowhead). The nuclear expression of *hh-lacZ* in Dl^+ and $Pros^+$ cells is more obvious in aged and stressed flies. In all panels, blue indicates DAPI staining for DNA. Scale bars are included in individual panels.

(B) Hh protein can be transported to the midgut epithelium from its producing sources. *24BGal4* is expressed in the visceral muscles (VMs). When HhRFP is ectopically expressed in the VMs (green, by GFP) for 7 days at 29°C, HhRFP protein can be detected in the progenitors (by Dl in yellow, white arrowheads), and also be detected in other intestinal cell types in the midgut epithelium (ee cells by Pros in yellow) (white and yellow arrowheads). *BtlGal4* is expressed in the trachea. When HhRFP is ectopically expressed in the trachea (green, by GFP) for 7 days at 29°C, HhRFP protein can be detected in the VMs (white arrowheads), and also be detected in other intestinal cell types in the midgut epithelium, like the progenitors and ECs (Dl and Pros in yellow, white and yellow arrowheads). Scale bars: 20 μm.

Li, Fig. S4

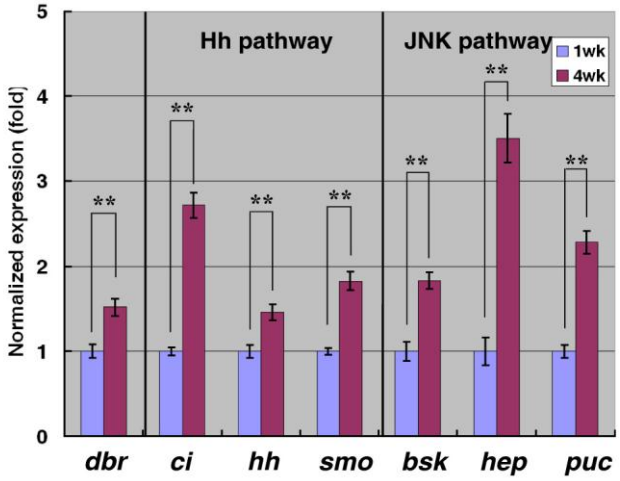

Figure S4 (relates to Figure 4): The expression of Dbr, the Hh pathway and the JNK pathway components is significantly increased in aging intestines

RT-qPCR quantification of mRNA expression of *dbr,* the Hh and JNK pathway components from 1 weak and 4 weeks old whole midguts at 29ºC. Ribosomal gene *RpL11* was used as normalization control. Mean±SEM are shown. **p<0.01.

2. Supplemental Experimental Procedures

Fly Lines and Husbandry

Flies hatched in 18°C incubators were picked and transferred to 29ºC incubator, unless otherwise specified. Flies were transferred to new vials with fresh food every day, and dissected at time points specified. In all experiments, only the female posterior midgut was analyzed. Information for alleles and transgenes used in this study can be found either in FlyBase or as noted: *esgGal4, UAS-GFP, tubGal80^{ts}* (*esg^{ts}*, gift from N. Perrimon), *mefGal4*, *dbr^{RNAi}* (VDRC kk102855), *GBE+Su(H)-lacZ* (gift from S. Bray), *dbr^{EP9}*, *dbr^{EY04764}*, *ci^{RNAi}* (VDRC GD51479), *ptc^{RNAi}* (BL28795), *ptc^{S2}*, *UAS-hh*, *smo^{RNAi}* (BL27037, BL24472, NIG 11561R-1), *smo^{119B6}*, *hh^{p30}* (*hh-lacZ*), *esg-lacZ$^{B7–2-22}$*, *24B^{ts}* (*tubGAl80^{ts}; 24BGal4, UAS-GFP*), *MyoIAGal4; tubGal80^{ts}* (*MyoIA^{ts}*, gift from S. Hou), *tubGal4^{ts}*, *hh^{RNAi} (*BL32489, BL25794*) hh^{AC}*, *hh^{ts}*, *puc^{E69}* (*puc-lacZ*), *UAS-bsk^{DN}* (BL9311), *bsk^{RNAi}* (BL32977), *Egfr^{RNAi}* (BL25781), *Ras^{RNAi}* (VDRC28129), *w(white)^{RNAi}* (BL33613*) & Gal4^{RNAi}* (from TRiP at Harvard Medical School).

Genotypes of Flies Used in Individual Experiments

Figure 1:

w; esgGal4, UAS-GFP

esgGal4, UAS-GFP, tubGal80^{ts}/+;w^{RNAi}/+

esgGal4, UAS-GFP, tubGal80^{ts}/dbr^{RNAi}

Su(H)+GBE-lacZ/+; esgGal4, UAS-GFP, tubGal80^{ts}/+; w^{RNAi}/+

Su(H)+GBE-lacZ/+; esgGal4, UAS-GFP, tubGal80^{ts}/dbr^{RNAi}

hsFLP, UAS-GFP/+;tubGal80, FRT40A/FRT40A;tubGal4/+

hsFLP, UAS-GFP/+;tubGal80, FRT40A-dbr^{EP9}/FRT40A;tubGal4/+

hsFLP, UAS-GFP/+;tubGal80, FRT40A-dbr^{EY04764}/FRT40A;tubGal4/+

Figure 2:

esgGal4, UAS-GFP, tubGal80^{ts}/+;ci^{RNAi}/+

esgGal4, UAS-GFP, tubGal80^{ts}/dbr^{RNAi};ci^{RNAi}/+

hsFLP, UAS-GFP/+;tubGal80, FRT40A-dbr^{EP9}/FRT40A;tubGal4/ci^{RNAi}

hsFLP, UAS-GFP/+;tubGal80, FRT40A-dbr ^{EY04764}/FRT40A;tubGal4/ci^{RNAi}

hsFLP, tubGal4, UAS-GFP/+; tubGal80, FRT42D/FRT42D

hsFLP, tubGal4, UAS-GFP/+; tubGal80, FRT42D/FRT42D-ptc^{S2}

esgGal4, UAS-GFP, tubGal80^{ts}/+;ptc^{RNAi}/+

Su(H)+GBE-lacZ/+; esgGal4, UAS-GFP, tubGal80^{ts}/+; ptc^{RNAi}/+

esgGal4, UAS-GFP, tubGal80^{ts}/+;UAS-Hh/+

esgGal4, UAS-GFP, tubGal80^{ts}/+;smo^{RNAi}/+

hsFLP, UAS-GFP/+;tubGal80, FRT40A-smo^{119B6}/FRT40A;tubGal4/+

Figure 3:

hh-lacZ (P30)/+

y,w

mefGal4, UAS-GFP/+; hh-lacZ (P30)/+

w; esg-lacZ/+;

esg-lacZ, Gal80^{ts}/+ 24BGal4, UAS-GFP/hh^{RNAi}

esg-lacZ, tubGal80ts/+; tubGAl4/hh^{RNAi}

esg-lacZ/+; hh^{AC}/hh^{ts}

Su(H)+GBE-lacZ/+; hh^{AC}/hh^{ts}

Figure 4:

esgGal4, UAS-GFP, tubGal80^{ts}/+;puc-lacZ/+

esgGal4, UAS-GFP, tubGal80^{ts}/+;puc-lacZ/w^{RNAi}

esgGal4, UAS-GFP, tubGal80^{ts}/dbr^{RNAi};puc-lacZ/+

esgGal4, UAS-GFP, tubGal80^{ts}/+;puc-lacZ/ptc^{RNAi}

esgGal4, UAS-GFP, tubGal80^{ts}/+; bsk^{DN}/+

esgGal4, UAS-GFP, tubGal80^{ts}/+; bsk^{RNAi}/+

esgGal4, UAS-GFP, tubGal80^{ts}/dbr^{RNAi}; bsk^{DN}/+

esgGal4, UAS-GFP, tubGal80^{ts}/dbr^{RNAi}; bsk^{RNAi}/+

Transgene Expression

Ectopic expression of transgenes in the adult midgut was achieved using a modified inducible UAS/Gal4 system. Several midgut cell type-specific *Gal4* drivers, including *esgGal4* (midgut progenitor-specific), *24BGal4* (visceral muscle-specific), *MyoIAGal4* (enterocyte-specific) *BtlGal4* and *tubGal4* were combined with *tubGal80^{ts}*. The resulting combinations are called *esg^{ts}*, *24B^{ts}*, *MyoIA^{ts}*, *Btl^{ts}* and *tub^{ts}*. Crosses were raised at 18°C in humidity-controlled incubators, or as otherwise noted. Flies hatched in 18°C incubators were picked and transferred to 29ºC incubator, unless otherwise specified. Flies were transferred to new vials with fresh food every day, and dissected at time points specified.

RT-qPCR

RNA was extracted from 10 midguts using TRIzol (Invitrogen). RNA was cleaned using RNAeasy (QIAGEN), and cDNA was synthesized using the iScript cDNA synthesis kit (Bio-Rad). qPCR was performed using the iScript one step RT-PCR SYBR green kit (Bio-Rad). Data were acquired using an iQ5 System (Bio-Rad). RT-qPCR was performed in duplicate on each of 3 independent biological replicates. All results are presented as Mean±SEM of the biological replicates. Ribosomal gene *RpL11* was used as normalization control.