SUPPLEMENTARY DATA

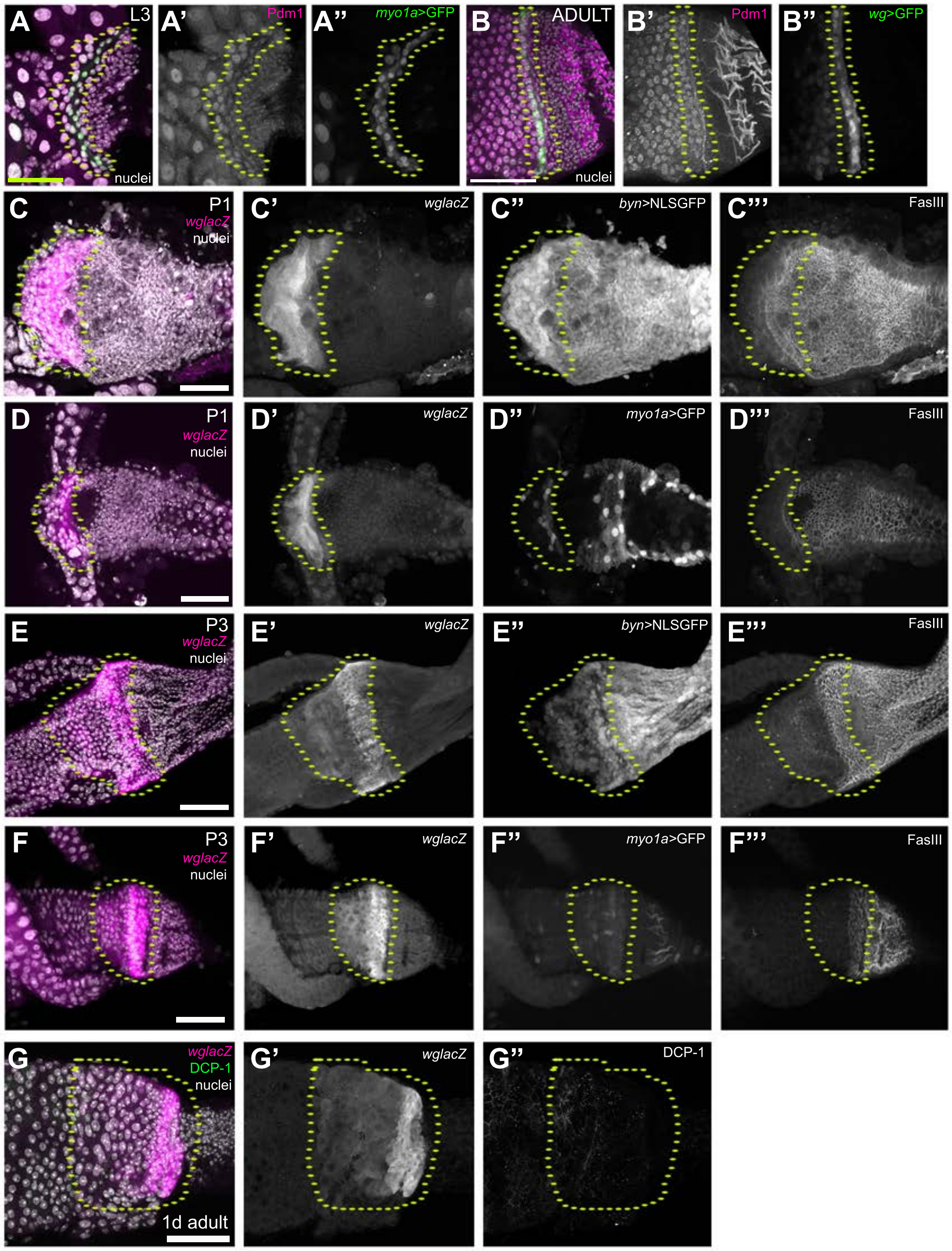

Fig. S1. The HZ is present from late larval development to adulthood. (A) The L3 HZ and (B) adult HZ is also Pdm1+. (C) *wg* and *byn* expression is tightly correlated in the P1 HZ. (D) Many *wg*+ cells are also *Myo1a*+ in the P1 HZ. (E) *wg* and *byn* expression is tightly correlated in the P3 HZ. (F) A few *wg*+ cells are also *Myo1a*+ in the P3 HZ. (G) No significant cell death is observed in 1d old animals, N=5. For all images, genotypes and markers indicated in panels, yellow dotted lines indicate the HZ, scale=50µm.

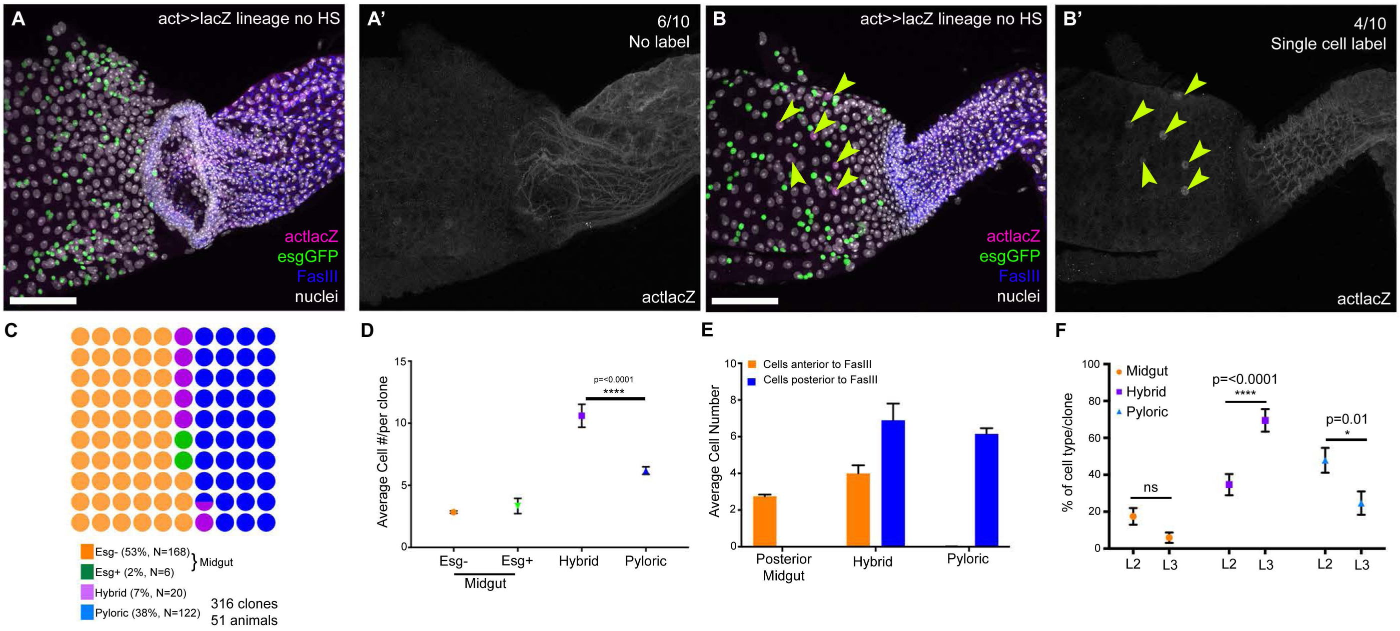

Fig. S2. Lineage tracing of the boundary region in development. (A-B) No HS controls for the lineage tracing show either no label (A) or occasionally single cells are labeled (B). (C) Percentages of the types of clonal patterns observed. (D) Hybrid clones are significantly larger than midgut and pyloric clones. Data represent mean ± SEM. One way ANOVA with Tukey's multiple comparisons test. (E) Hybrid clones cross the FasIII border. Data represent mean ± SEM. (F) Percentage of cell types in clones induced in L2 (2^{nd} instar) and L3 (3^{rd} instar) development. Data represent mean ± SEM. One way ANOVA with Tukey's multiple comparisons test. For all images, genotypes and markers indicated in panels, yellow dotted lines indicate the HZ, white dotted lines indicate clones, scale=50µm.

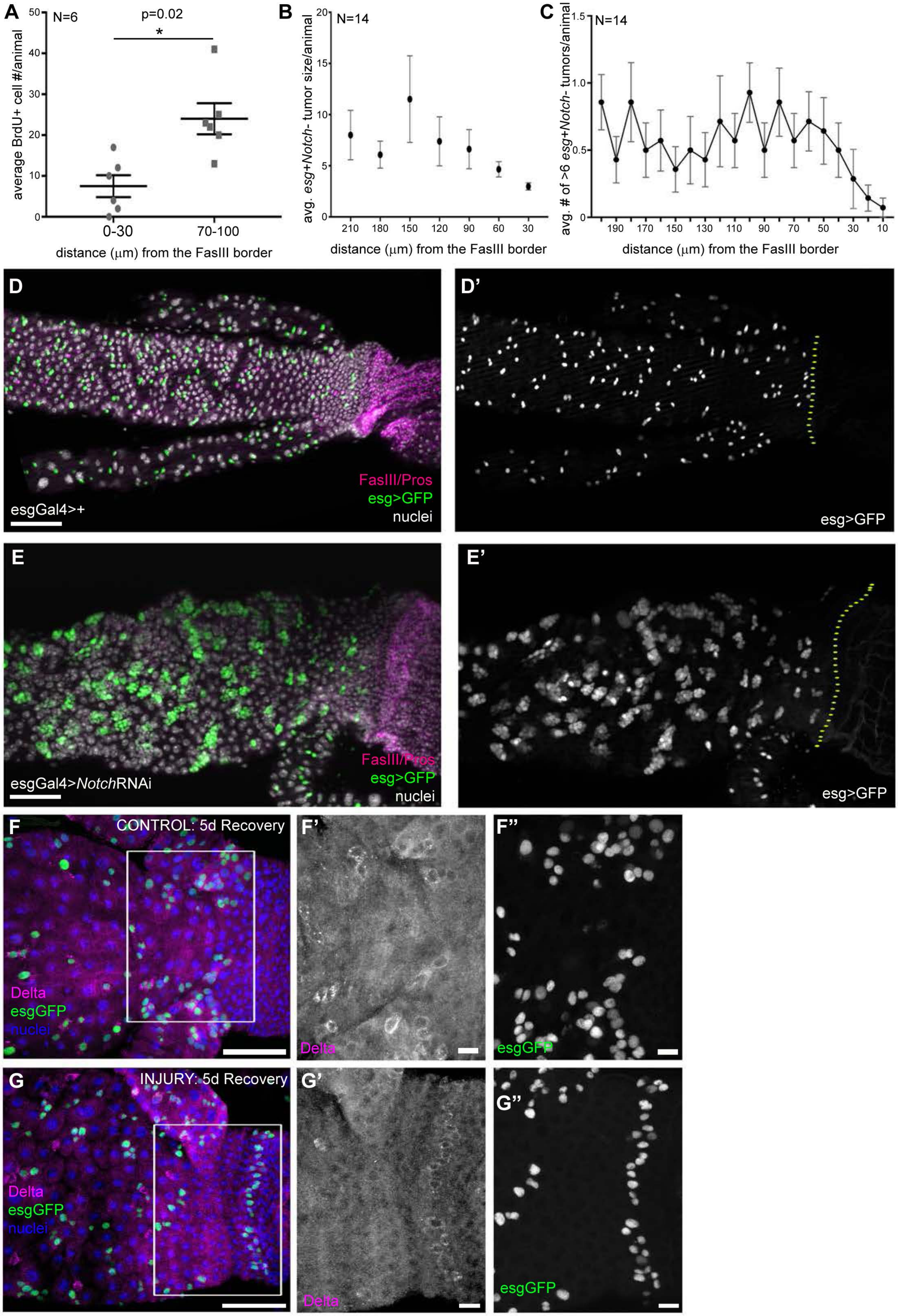
A
N=6
p=0.02
*
average BrdU+ cell #/animal
0
10
20
30
40
50
0-30
70-100
distance (μm) from the FasIII border
B
N=14
avg. esg+Notch- tumor size/animal
0
5
10
15
20
210
180
150
120
90
60
30
distance (μm) from the FasIII border
C
N=14
avg. # of >6 esg+Notch- tumors/animal
0
0.5
1.0
1.5
190
170
150
130
110
90
70
50
30
10
distance (μm) from the FasIII border
D
FasIII/Pros
esg>GFP
nuclei
esgGal4>+
D'
esg>GFP
E
FasIII/Pros
esg>GFP
nuclei
esgGal4>NotchRNAi
E'
esg>GFP
F
CONTROL: 5d Recovery
Delta
esgGFP
nuclei
F'
Delta
F''
esgGFP
G
INJURY: 5d Recovery
Delta
esgGFP
nuclei
G'
Delta
G''
esgGFP

Fig. S3. There is less proliferation near the adult HZ. (A) Fewer BrdU+ cells are observed within 30µm of the HZ. Data represent mean ± SEM. Paired t-test. (B) *Notch* stem cell tumors further from the HZ are larger. Data represent mean ± SEM. (C) *Notch* stem cell tumors with >6 cells are infrequently found near the HZ. Data represent mean ± SEM. (D) In WT animals *esg*+ cells are found in singles or pairs. (E) After depletion of Notch, *esg*+ stem cell tumors form. (F) ISCs near the HZ are Dl+ and (G) after injury, *esg*+Dl+ cells expand , scale=50µm and 10µm. For all images, genotypes and markers indicated in panels, yellow dotted lines indicate the HZ, scale=50µm.

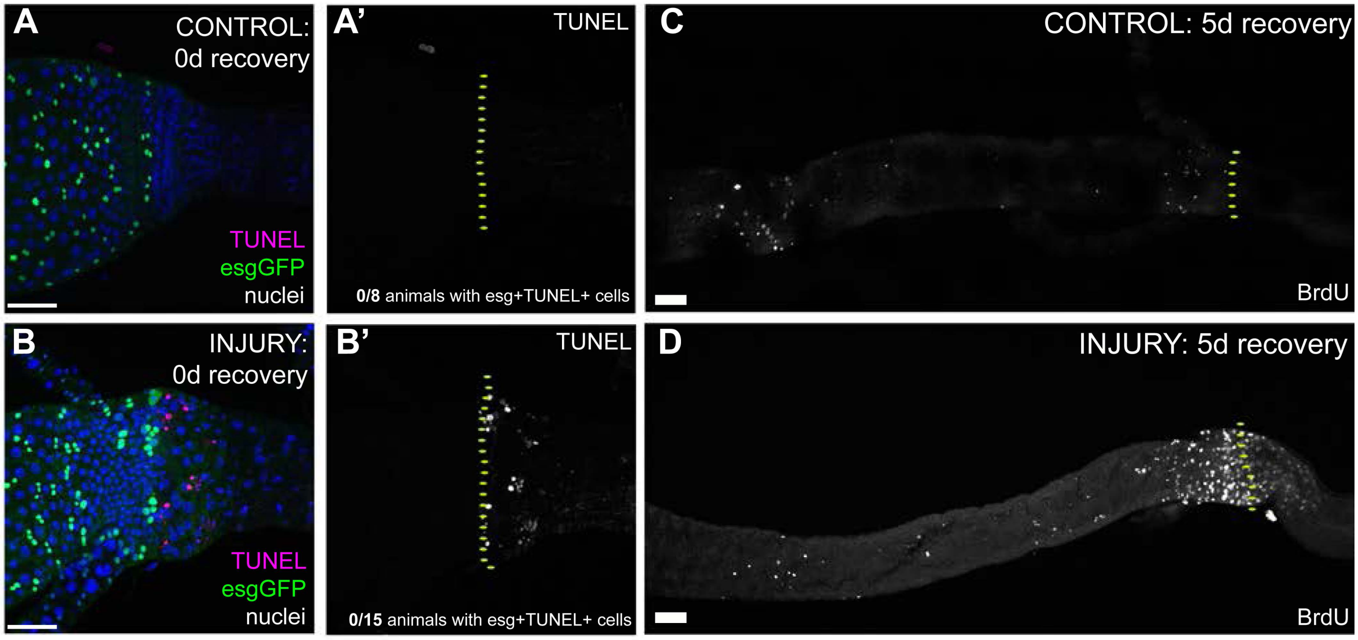

Fig. S4. Injury to the adult HZ/hindgut leads to cell cycle re-entry and OB-ISC expansion.

(A) TUNEL is never observed around the HZ in the absence of injury. (B) After injury, TUNEL is observed in the HZ and hindgut, but never in the midgut or in OB-ISCs, scale for A-B=50µm. (C-D) The cell cycle response is localized to the most posterior part of the midgut, HZ, and hindgut after injury, scale=50µm. For all images, genotypes and markers indicated in panels, yellow dotted lines indicate the HZ.

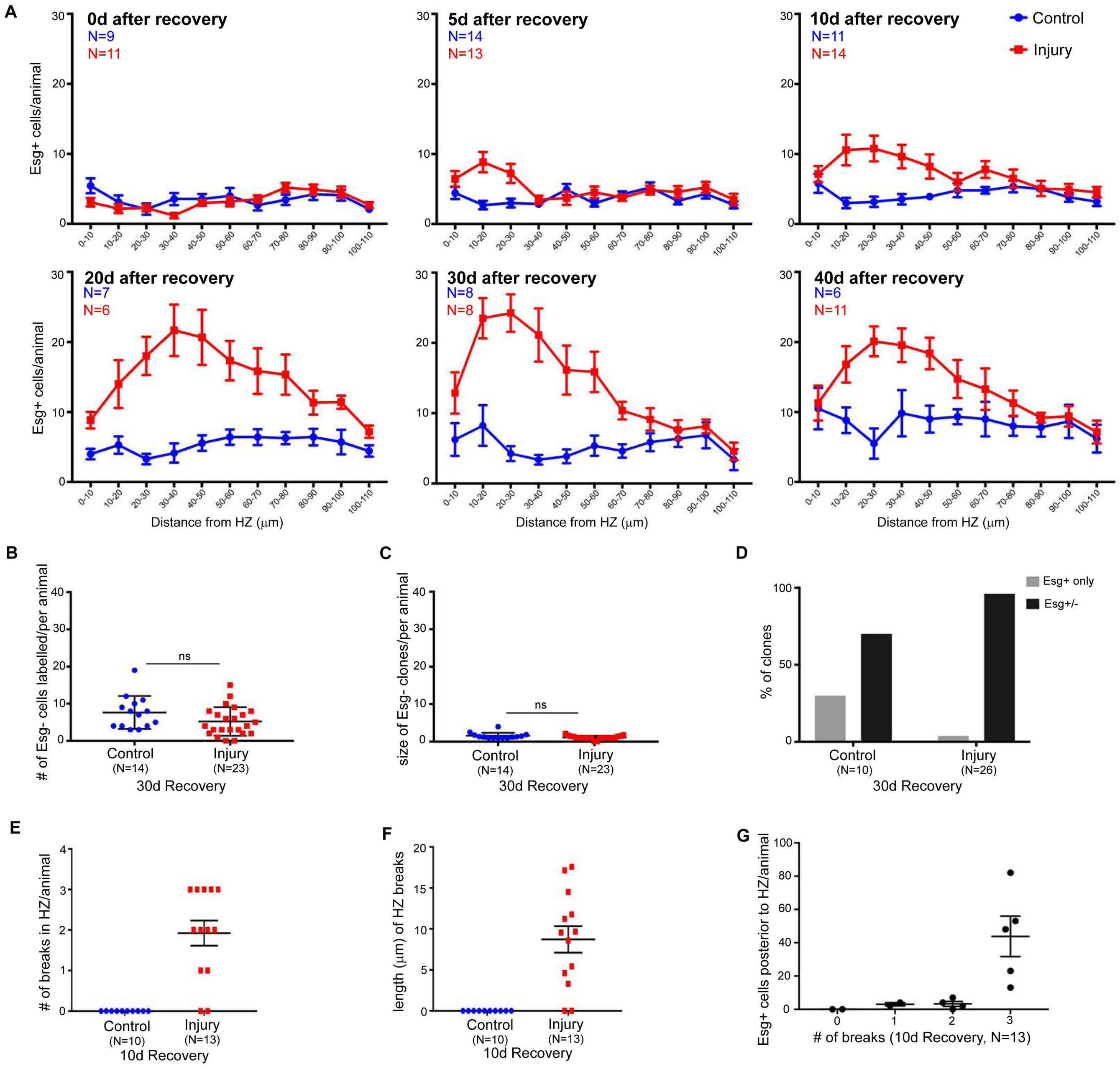
A
0d after recovery
N=9
N=11
5d after recovery
N=14
N=13
10d after recovery
N=11
N=14
Control
Injury
20d after recovery
N=7
N=6
30d after recovery
N=8
N=8
40d after recovery
N=6
N=11
Esg+ cells/animal
0
10
20
30
0-10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
80-90
90-100
100-110
Distance from HZ (μm)
B
# of Esg- cells labelled/per animal
ns
Control
(N=14)
Injury
(N=23)
30d Recovery
C
size of Esg- clones/per animal
ns
Control
(N=14)
Injury
(N=23)
30d Recovery
D
% of clones
Esg+ only
Esg+/-
Control
(N=10)
Injury
(N=26)
30d Recovery
E
# of breaks in HZ/animal
Control
(N=10)
Injury
(N=13)
10d Recovery
F
length (μm) of HZ breaks
Control
(N=10)
Injury
(N=13)
10d Recovery
G
Esg+ cells posterior to HZ/animal
# of breaks (10d Recovery, N=13)

Fig. S5. Injury to the adult HZ/hindgut drives midgut ISC division and HZ breakage. (A) Graphs showing *esg*+ cell expansion by bins of 10µms after different recovery times. The expansion is primarily localized close to the HZ. Data represent mean ± SEM. (B) A similar number of *esg*- cells are labeled in both control and injured animals. Data represent mean ± SEM. Unpaired t-test, two-tails, unequal variance. (C) The size of *esg*- labeled clones are 1-2 cells in both control and injured animals. Data represent mean ± SEM. Unpaired t-test, two-tails, unequal variance. (D) *esg*+ divide both asymmetrically and symmetrically before and after injury. Data represents the % of clones that either *esg*+ only or *esg+/-.* (E) After injury, breaks are observed in the HZ. Data represent mean ± SEM. Unpaired t-test, two-tails, unequal variance, p=<0.0001. (F) Breaks in the HZ vary in length. Data represent mean ± SEM. Unpaired t-test, two-tails, unequal variance, p=0.0002. (G) In injured animals with more breaks, more *esg*+ cells are found posterior to the HZ. Data represent mean ± SEM.

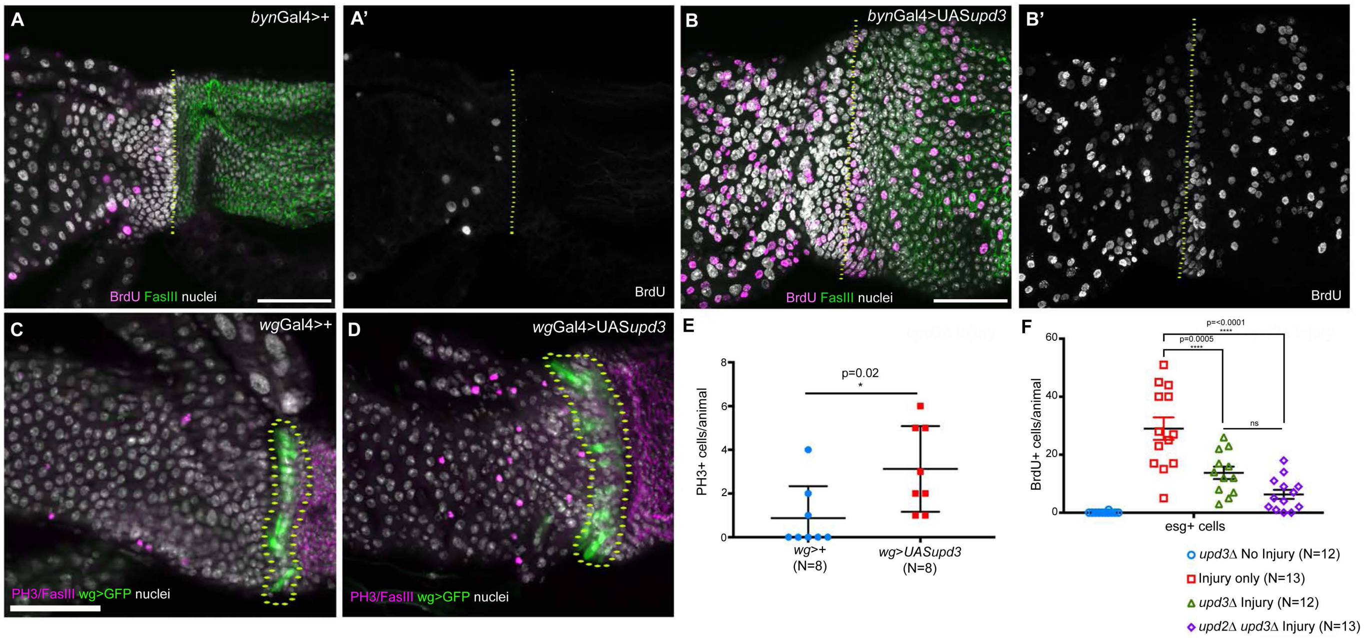

Fig. S6. *upd3* is necessary and sufficient for cell cycling after injury. (A) Few BrdU+ cells are observed in control animals. (B) After ectopic *upd3* expression using *byn*Gal4 (drives in the HZ/hindgut), BrdU+ cells are found in the hindgut, HZ, and midgut. (C-E) Ectopic *upd3* expression using *wgGal4* results in more PH3+ cells in the midgut, scale=50µm. (E) The number of PH3+ cells in the midgut after injury is significantly more than without injury. Data represent mean ± SEM. Unpaired t-test, two tails, unequal variance. (F) Blocking Upd signaling results in a reduction of injury dependent BrdU incorporation *esg*+ cells. Data represent mean ± SEM. One way ANOVA with Tukey's multiple comparisons test. For all images, genotypes and markers indicated in panels, yellow dotted lines indicate the HZ.