SUPPLEMENTARY DATA

SupplFig1. 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. Genotypes and markers indicated in panels, yellow dotted lines indicate the HZ, scale=50μm.

SupplFig2. 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 do not cross into the midgut. N=4 clones from 3 animals. Genotypes and markers indicated in panels, yellow dotted lines indicate the HZ, white dotted lines indicate clones, scale=50μm.

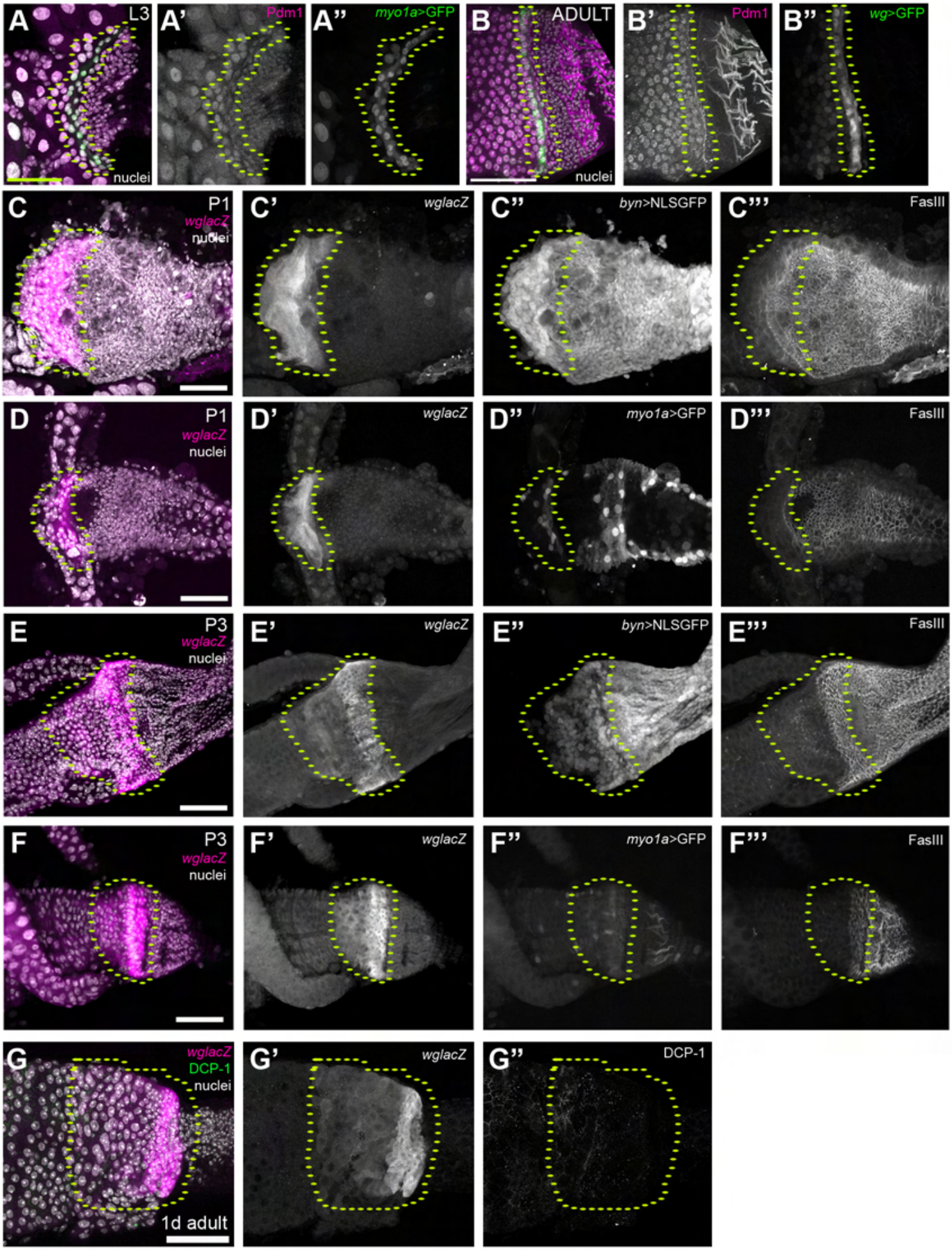
SupplFig3. There is less proliferation near the adult HZ. (A) Fewer BrdU+ cells are observed within 30μm of the HZ. Data represent mean ± SEM. (B) *Notch* stem cell tumors further from the HZ are larger. Data represent mean ± SEM. (C) *Notch* stem cell tumors with >6 cells are infrequently found near 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. Genotypes and markers indicated in panels, yellow dotted lines indicate the HZ, scale=50μm.

SupplFig4. 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. Genotypes and markers indicated in panels, yellow dotted lines indicate the HZ.

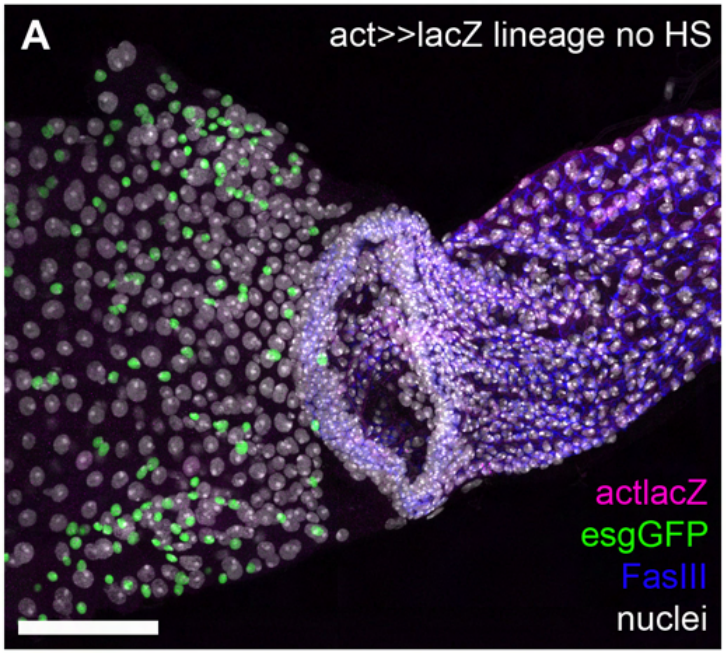
SupplFig5. Injury to the adult HZ/hindgut drives midgut ISC division and HZ breakage. (A) Graphs showing *esg+* cell expansion by bins of 10μms after different recovery times. The expansion is primarily localized close to the HZ. Data represent mean ± SEM. (B) A similar number of *esg-* cells are labeled in both control and injured animals. Data represent mean ± SEM. (C) The size of *esg-* labeled clones are 1-2 cells in both control and injured animals. Data represent mean ± SEM. (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. (F) Breaks in the HZ vary in length. Data represent mean ± SEM. (G) In injured animals with more breaks, more *esg+* cells are found posterior to the HZ. Data represent mean ± SEM.

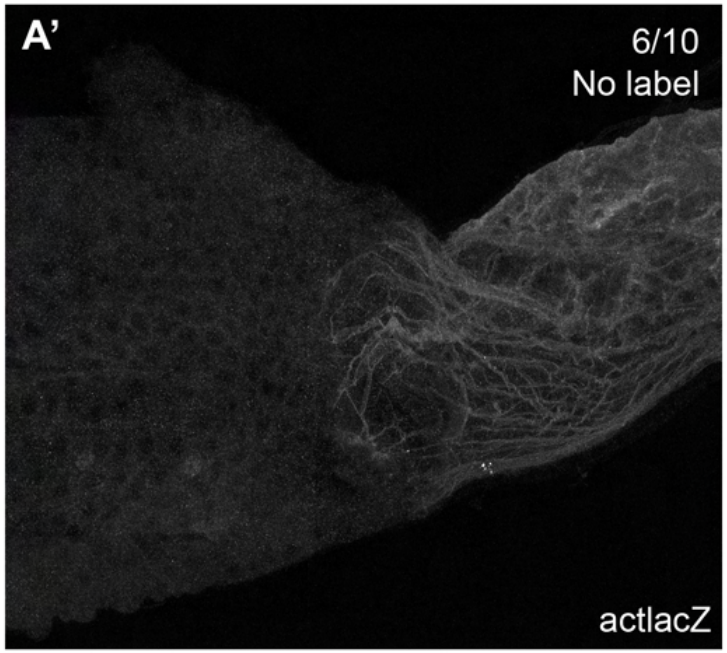
SupplFig6. *upd3* is sufficient to drive cell cycling. (A) Few BrdU+ cells are observed in control animals. (B) After ectopic *upd3* expression using *byn*Gal4 (drives in the HZ/hindgut), BrdU+ cells are found in the hindgut, HZ, and midgut. Genotypes and markers indicated in panels, yellow dotted lines indicate the HZ, scale=50μm.

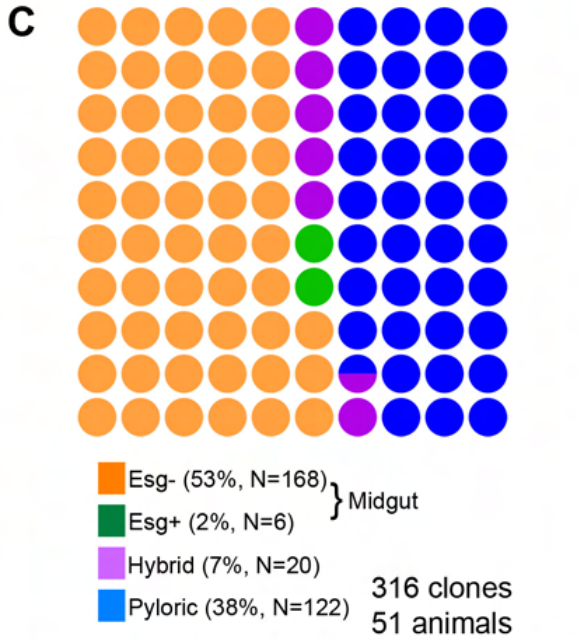
SupplFig1

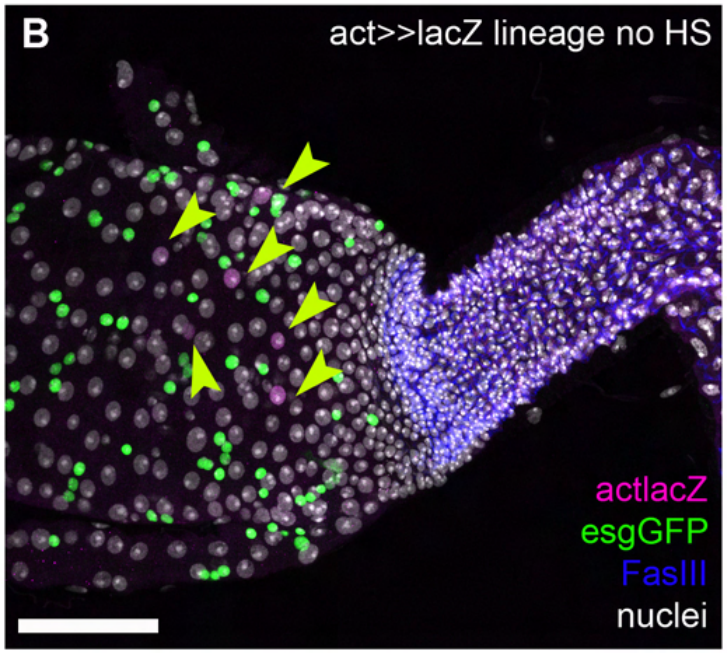

A
L3
nuclei
A'
Pdm1
A''
myo1a>GFP
B
ADULT
nuclei
B'
Pdm1
B''
wg>GFP
C
P1
wglacZ
nuclei
C'
wglacZ
C''
byn>NLSGFP
C'''
FasIII
D
P1
wglacZ
nuclei
D'
wglacZ
D''
myo1a>GFP
D'''
FasIII
E
P3
wglacZ
nuclei
E'
wglacZ
E''
byn>NLSGFP
E'''
FasIII
F
P3
wglacZ
nuclei
F'
wglacZ
F''
myo1a>GFP
F'''
FasIII
G
wglacZ
DCP-1
nuclei
1d adult
G'
wglacZ
G''
DCP-1

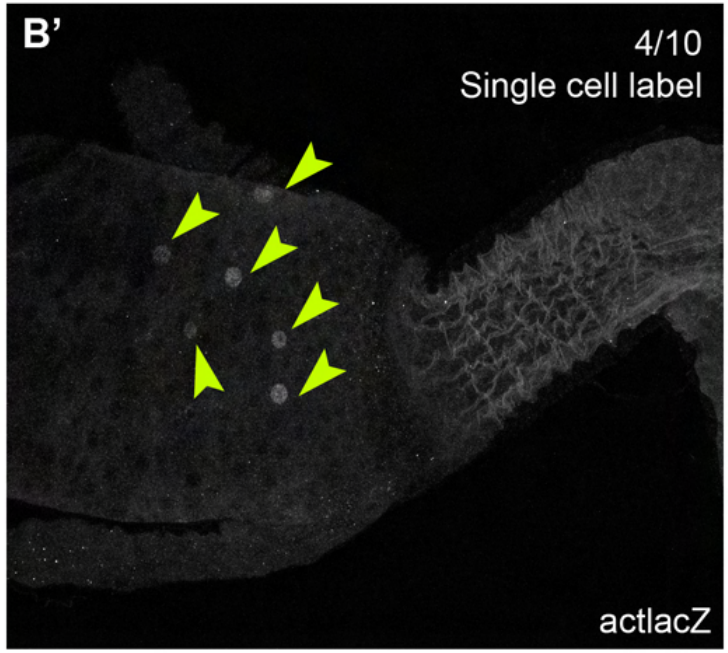
SupplFig2

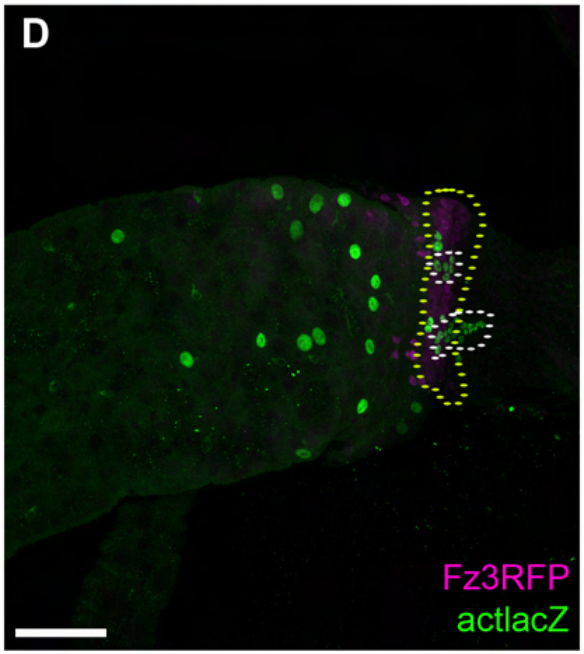

A
act>>lacZ lineage no HS
actlacZ
esgGFP
FasIII
nuclei


A'
6/10
No label
actlacZ

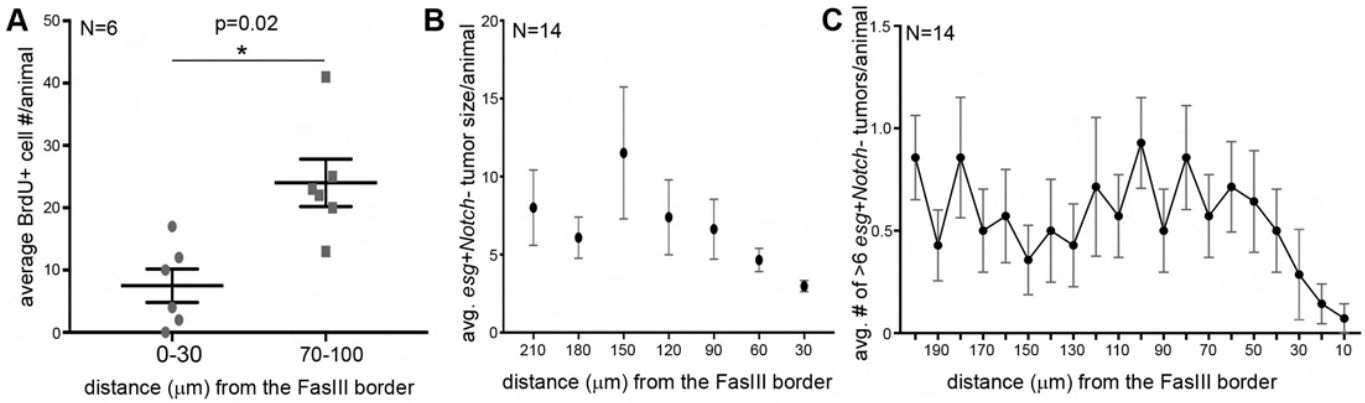

C
Esg- (53%, N=168)
Esg+ (2%, N=6)
Midgut
Hybrid (7%, N=20)
Pyloric (38%, N=122)
316 clones
51 animals

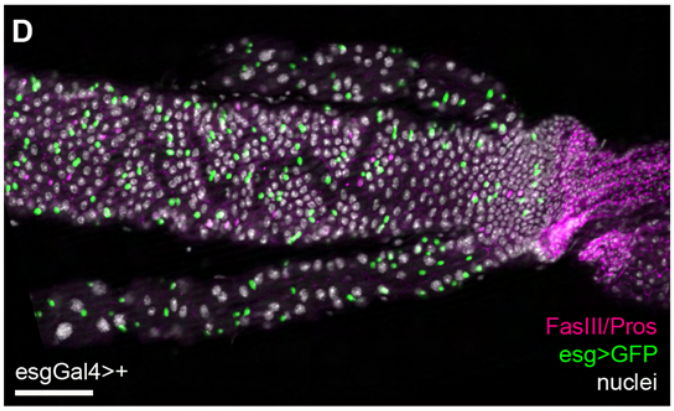

B
act>>lacZ lineage no HS
actlacZ
esgGFP
FasIII
nuclei

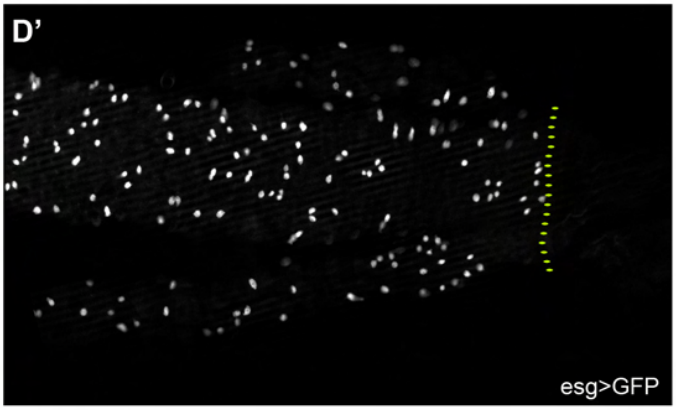

B'
4/10
Single cell label
actlacZ

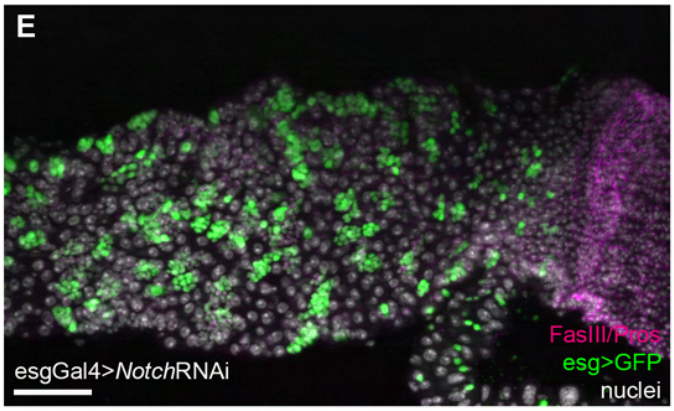

D
Fz3RFP
actlacZ

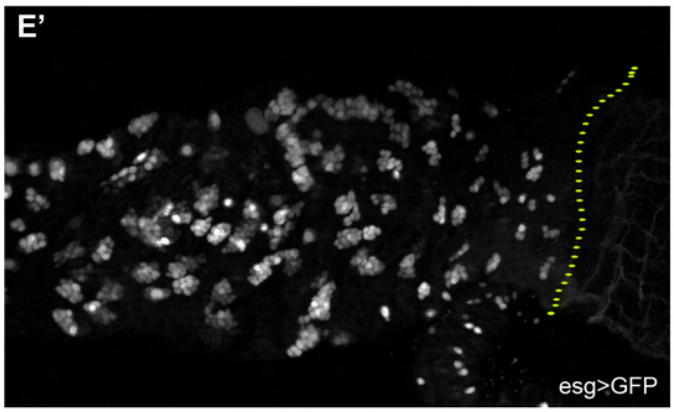
SupplFig3

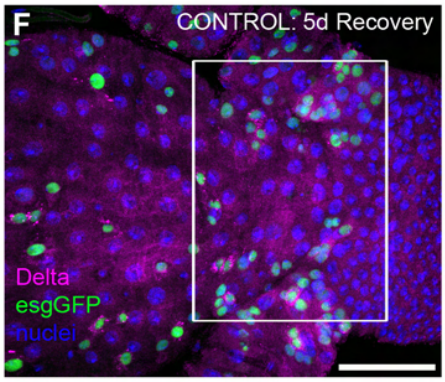

A
N=6
p=0.02
*
average BrdU+ cell #/animal
0-30
70-100
distance (μm) from the FasIII border
B
N=14
avg. esg+Notch- tumor size/animal
distance (μm) from the FasIII border
C
N=14
avg. # of >6 esg+Notch- tumors/animal
distance (μm) from the FasIII border

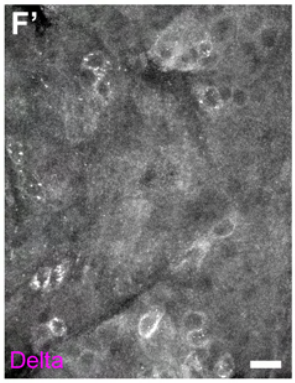

D
esgGal4>+
FasIII/Pros
esg>GFP
nuclei

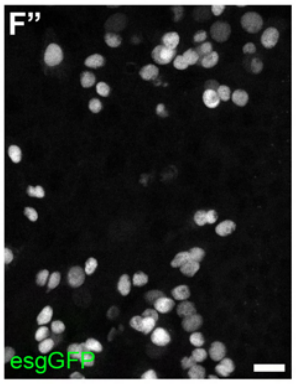

D'
esg>GFP

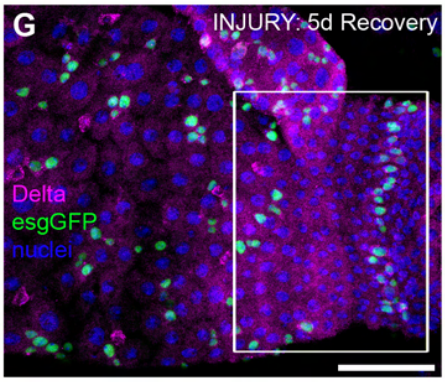

E
esgGal4>NotchRNAi
FasIII/Pros
esg>GFP
nuclei

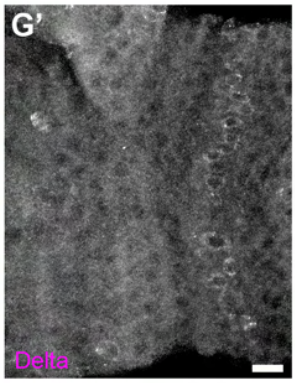

E'
esg>GFP

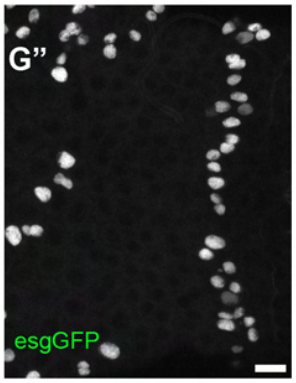

F
CONTROL: 5d Recovery
Delta
esgGFP
nuclei


F'
Delta

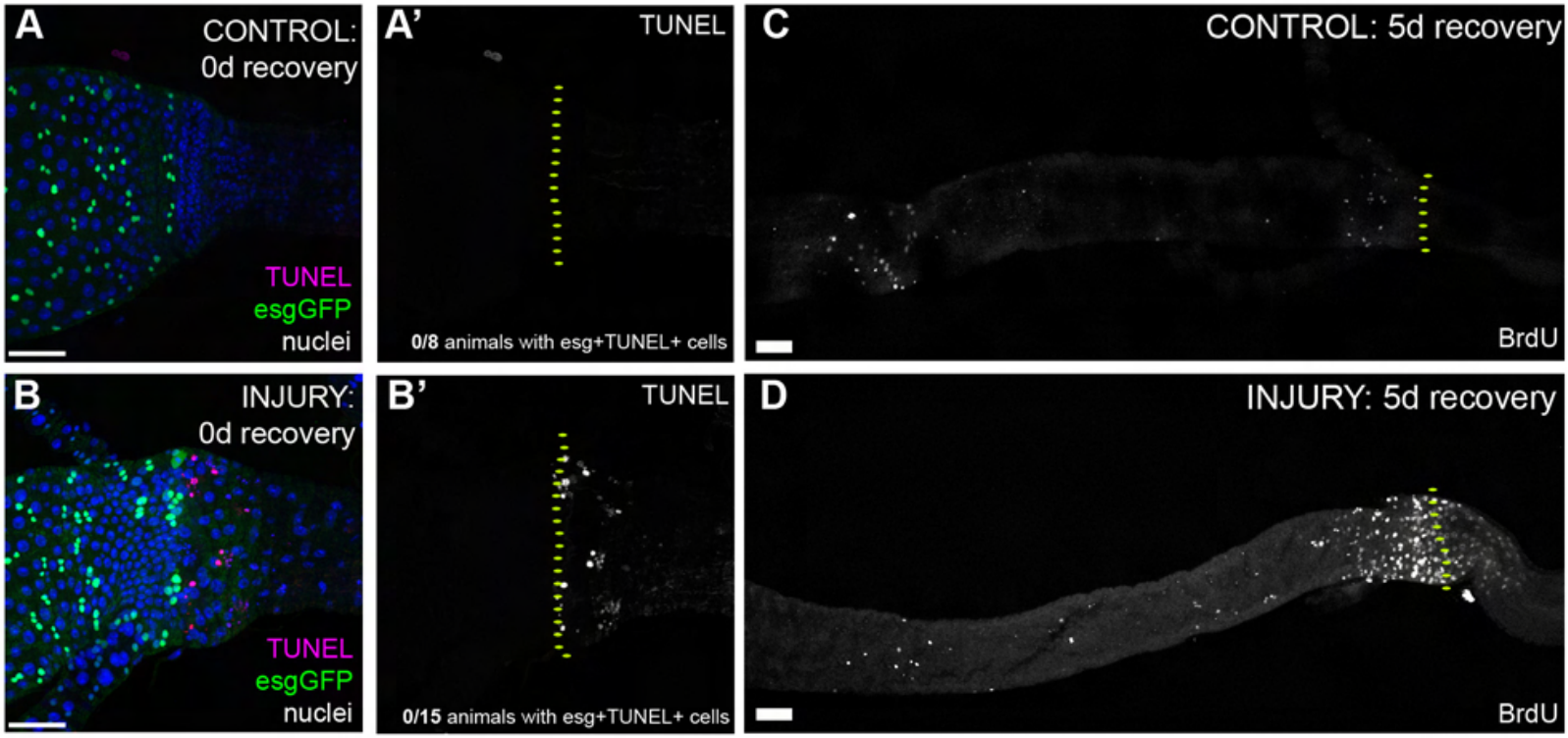

F''
esgGFP


G
INJURY: 5d Recovery
Delta
esgGFP
nuclei

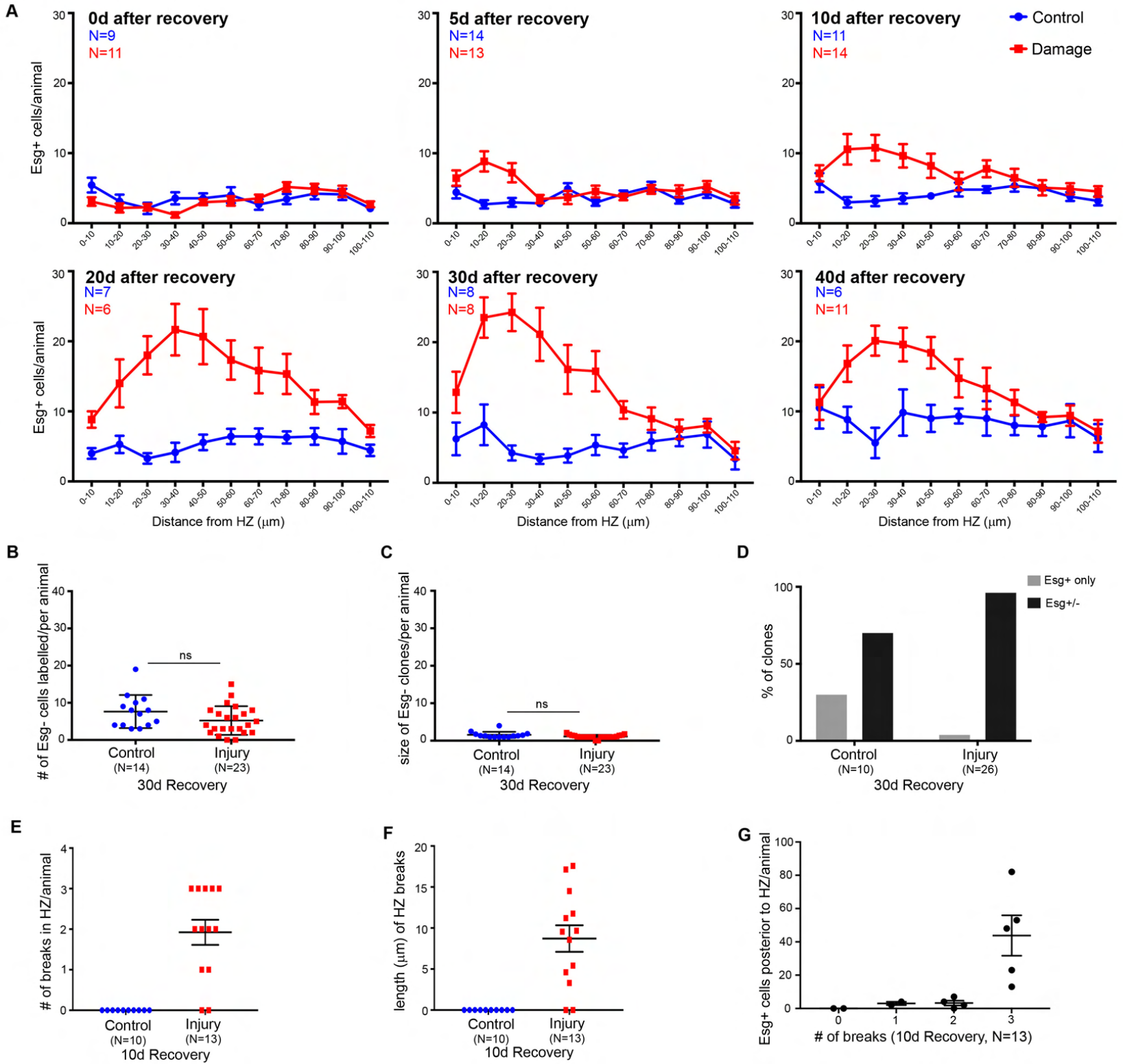

G'
Delta


G''
esgGFP

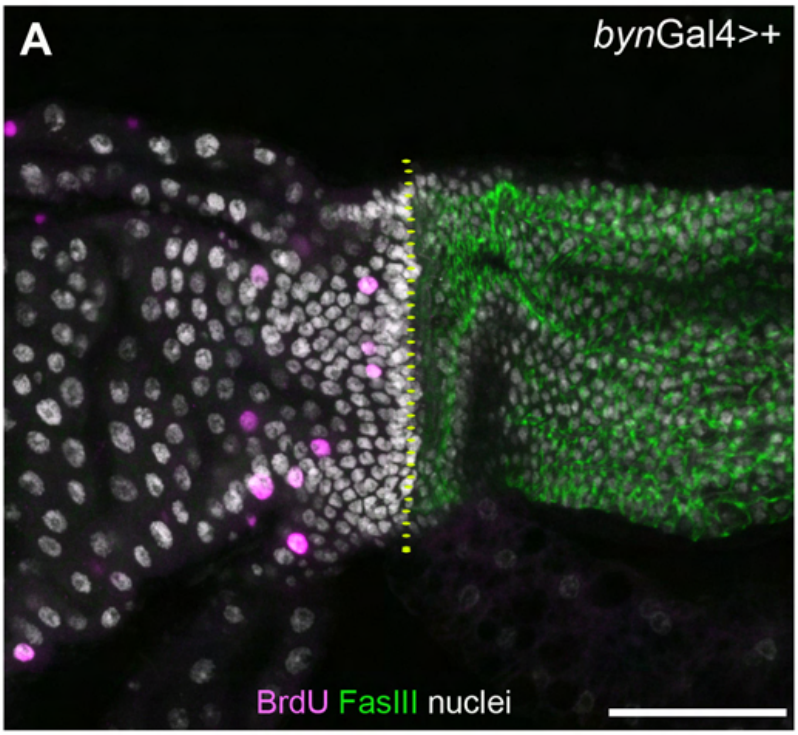
SupplFig4

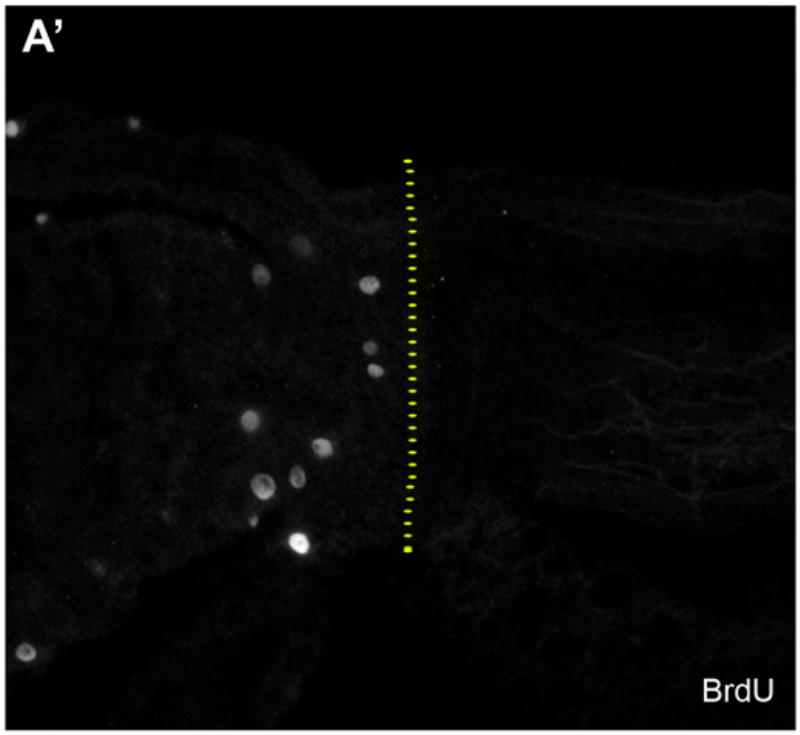

A
CONTROL:
0d recovery
TUNEL
esgGFP
nuclei
A'
TUNEL
0/8 animals with esg+TUNEL+ cells
C
CONTROL: 5d recovery
BrdU
B
INJURY:
0d recovery
TUNEL
esgGFP
nuclei
B'
TUNEL
0/15 animals with esg+TUNEL+ cells
D
INJURY: 5d recovery
BrdU

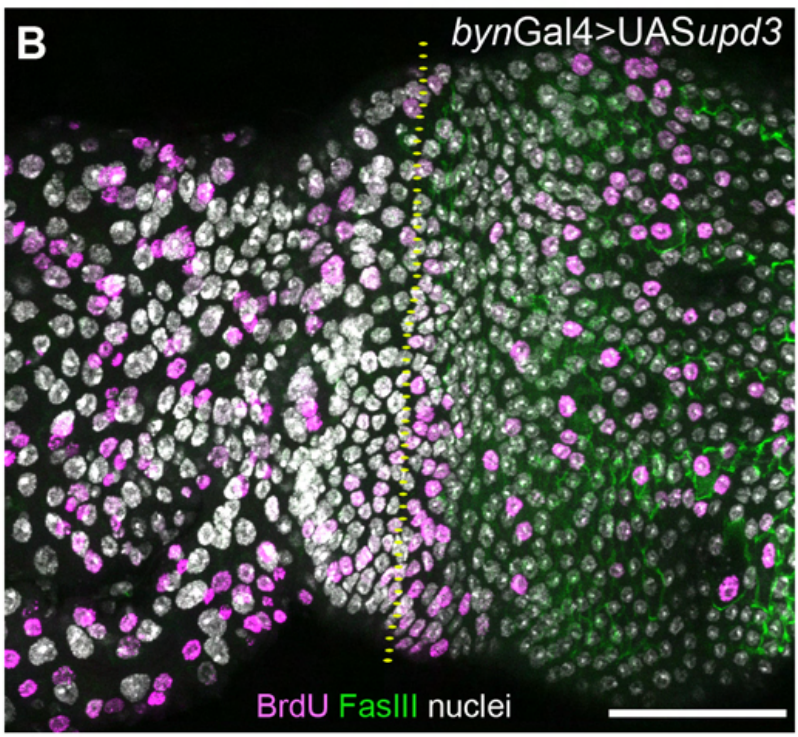
SupplFig5

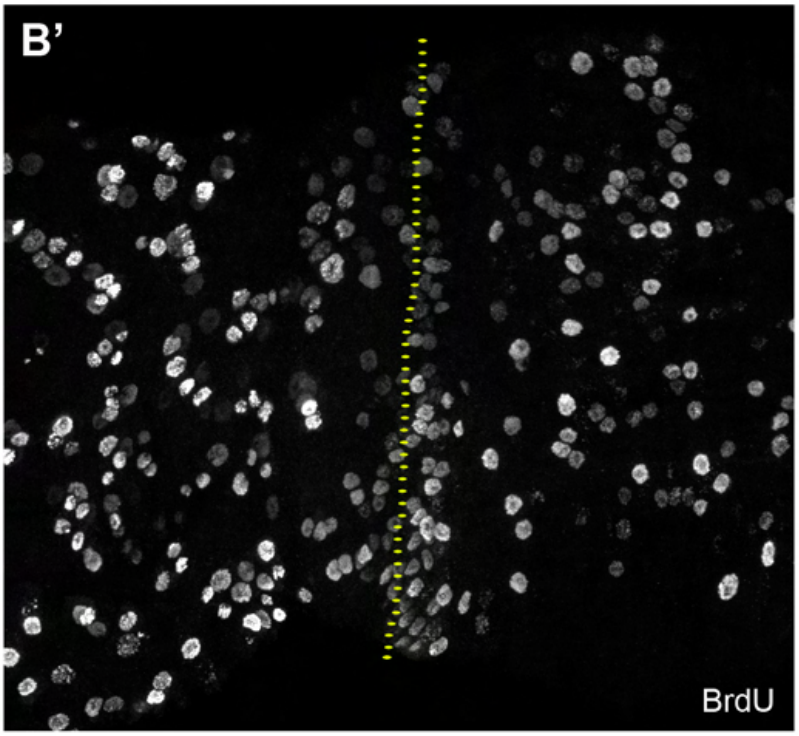

A
0d after recovery
N=9
N=11
5d after recovery
N=14
N=13
10d after recovery
N=11
N=14
Control
Damage
20d after recovery
N=7
N=6
30d after recovery
N=8
N=8
40d after recovery
N=6
N=11
Esg+ cells/animal
Distance from HZ (μm)
0-10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
80-90
90-100
100-110
B
# of Esg- 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)

SupplFig6


A
bynGal4>+
BrdU FasIII nuclei


A'
BrdU


B
bynGal4>UASupd3
BrdU FasIII nuclei


B'
BrdU