Supplemental Figures

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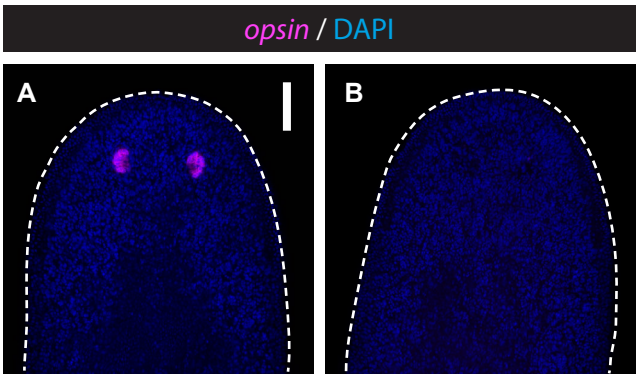
Figure S7. Increased progenitor incorporation into the ventral nerve cords and pharynx does not require specific tissue removal. Related to Figure 7.

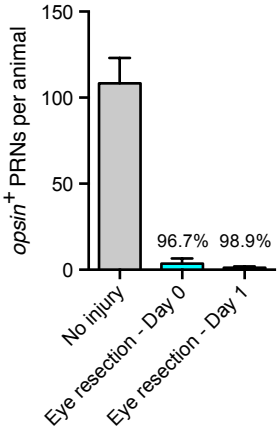
Supplemental Tables

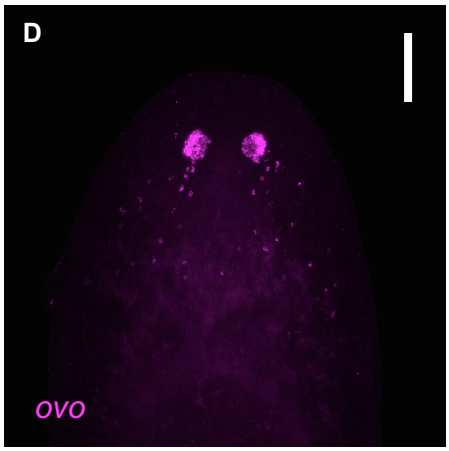
Table S1. Animal numbers for all experiments. Related to all figures.

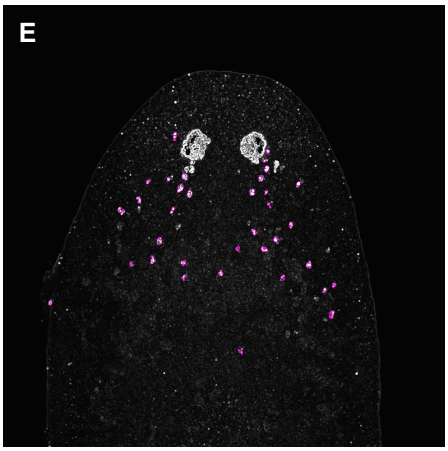
Table S2. Sequences used for FISH probes and dsRNA. Related to all figures.

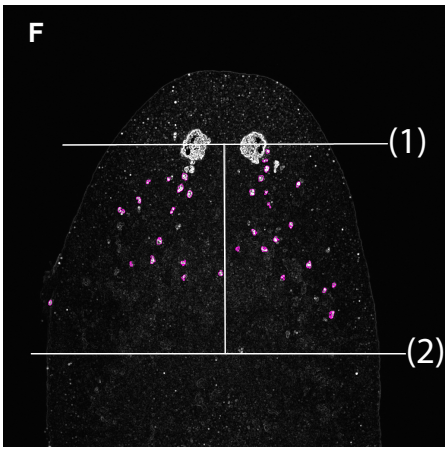
Figure S1

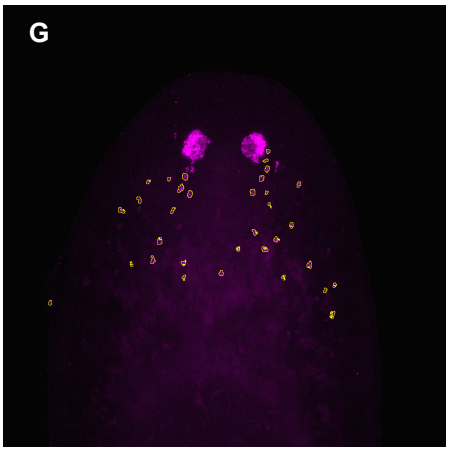

opsin / DAPI
A
B

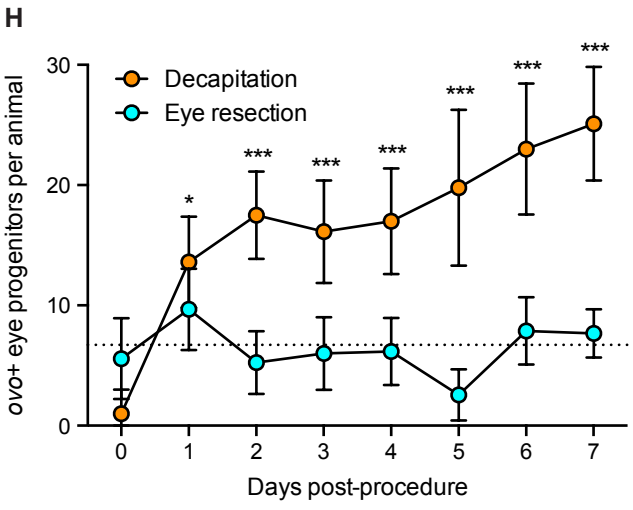

C
opsin+ PRNs per animal
150
100
50
0
96.7%
98.9%
No injury
Eye resection - Day 0
Eye resection - Day 1


D
ovo


E


F
(1)
(2)


G


H
ovo+ eye progenitors per animal
30
20
10
0
Decapitation
Eye resection
*
***
***
***
***
***
***
0
1
2
3
4
5
6
7
Days post-procedure

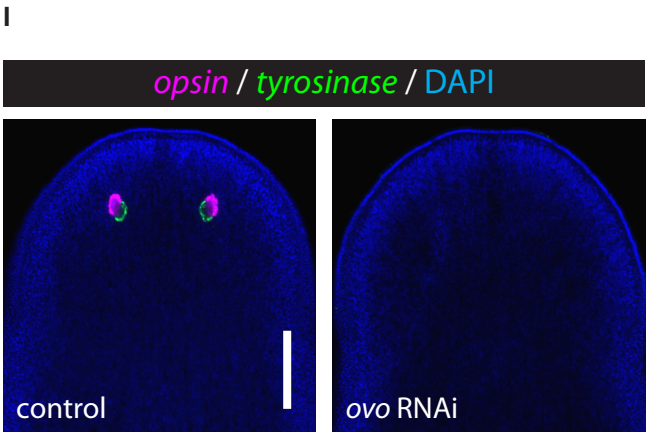

I
opsin / tyrosinase / DAPI
control
ovo RNAi

Figure S1. Eye resection controls and semi-automated eye progenitor counting procedure and results. Related to Figures 1 and 2

(A and B) FISH for *opsin* with DAPI labeling in uninjured animals (A) and eye-resected animals (B) 1 day post-surgery demonstrates PRN removal. Dashed white line indicates animal boundary. Scale bar, 100 μm.

(C) PRNs per animal in uninjured and eye-resected animals, 0 and 1 day post-surgery. $n \geq 6$ animals per condition. Data represented as mean ± SD. Percentages indicate % PRN removal (mean number of PRNs per animal after resection / mean number of PRNs per animal in intact animals x 100). Day 0 and Day 1 eye resection data are also shown in Figure S5 regeneration timecourse.

(D-G) Semi-automated progenitor counting procedure. (D) Representative maximum intensity projection from z-stack of FISH for *ovo* in decapitated animal seven days post-surgery. Scale bar, 100μm. (E) Appearance of image after processing algorithm. Computer-identified eye progenitors are outlined in magenta. (F) Lines indicate area of progenitor counting, defined as the area between a line running through the eyes (1) and a parallel line 300 μm posterior to the first (2). Events touching the line are counted, events completely outside lines or completely inside eye are not counted.

(G) All counted progenitor outlines (yellow) overlayed on original maximum intensity projection.

(H) *ovo*$^{+}$ eye progenitor numbers 0 to 7 days post-surgery, as quantified by semi-automated progenitor counting. Data represented as mean ± SD. Dotted line represents mean of uninjured animals on Day 0. Decapitation but not eye resection results in significantly elevated eye progenitor numbers in comparison to uninjured controls. Statistical significance assessed with respect to uninjured animals by one-way ANOVA (*$p<0.05$, ***$p<0.001$).

(I) FISH for *opsin* and *tyrosinase* with DAPI labeling in eye-resected animals 1 week post-surgery under control RNAi and *ovo* RNAi conditions. Control RNAi animals regenerated normal eyes containing *tyrosinase*$^{+}$ pigmented optic cup cells and *opsin*$^{+}$ PRNs (10 of 10 animals). *ovo* RNAi animals failed to regenerate eyes (10 of 10 animals had ≤3 PRNs, 6 of 10 had 0 PRNs). Scale bar, 200 μm.

Figure S2

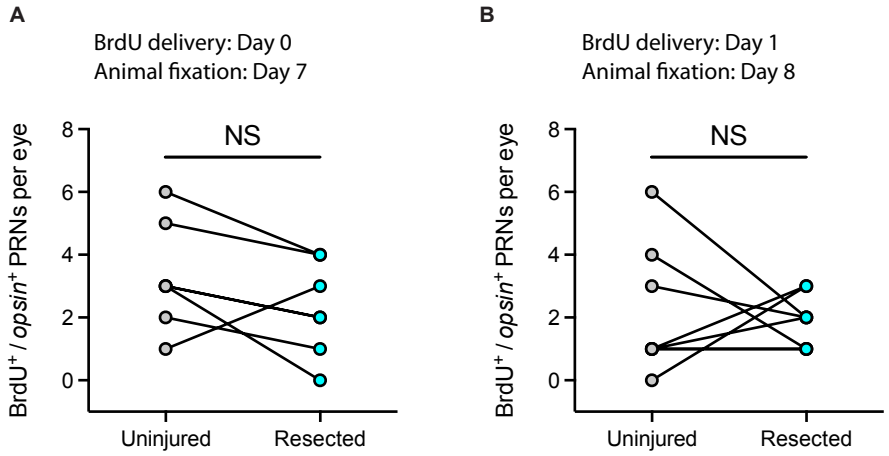

A
BrdU delivery: Day 0
Animal fixation: Day 7
BrdU+ / opsin+ PRNs per eye
NS
0
2
4
6
8
Uninjured
Resected
B
BrdU delivery: Day 1
Animal fixation: Day 8
BrdU+ / opsin+ PRNs per eye
NS
0
2
4
6
8
Uninjured
Resected

Figure S2. Additional single eye resection BrdU incorporation experiments. Related to Figure 3.
(A and B) BrdU/*opsin* double-positive PRNs per eye after single eye resection. (A) BrdU delivery Day 0, animal fixation Day 7. *n*=7 animals. (B) BrdU delivery Day 1, animal fixation Day 8. *n*=8 animals. Statistical significance assessed by paired Student's *t*-tests (NS, not significant).

Figure S3

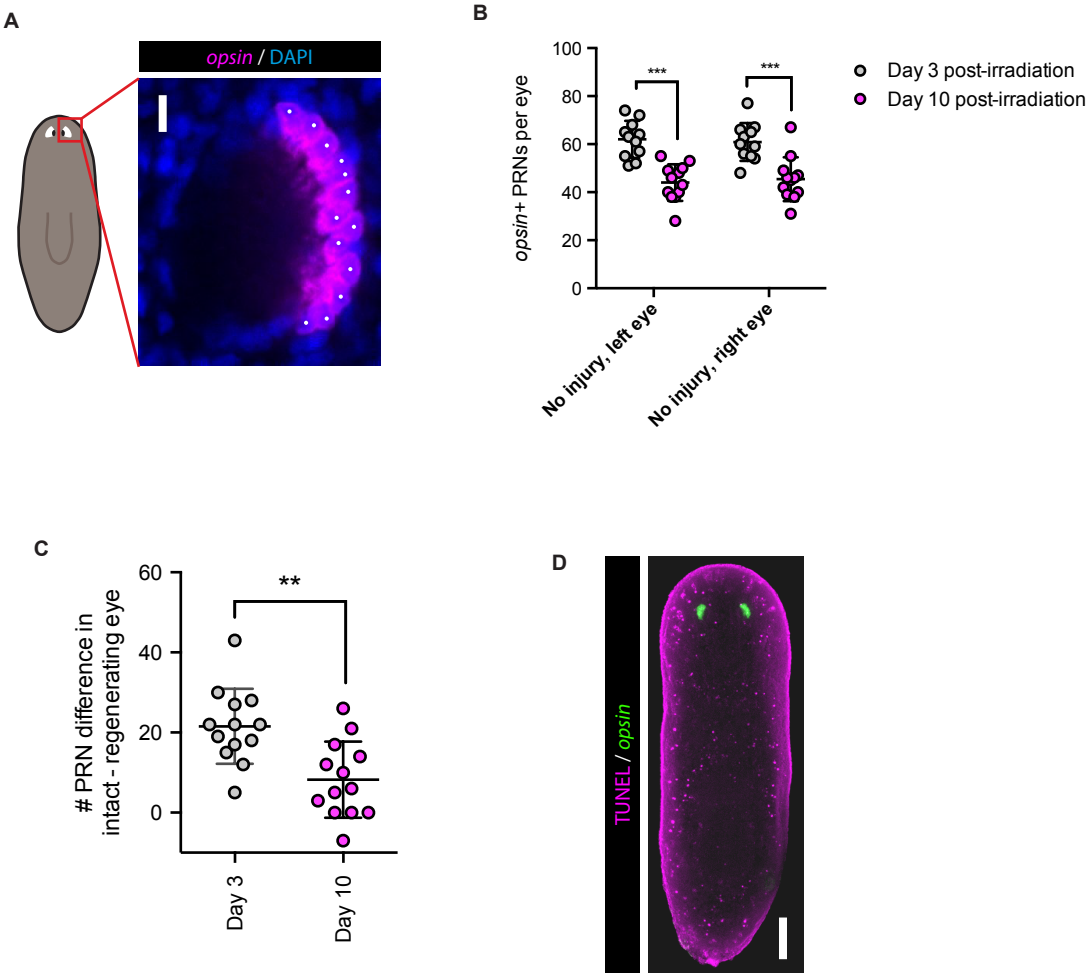

A
opsin / DAPI
B
opsin+ PRNs per eye
100
80
60
40
20
0
***
***
No injury, left eye
No injury, right eye
Day 3 post-irradiation
Day 10 post-irradiation
C
PRN difference in intact - regenerating eye
60
40
20
0
**
Day 3
Day 10
D
TUNEL / opsin

Figure S3. Cell loss controls and combined TUNEL and FISH for *opsin*. Related to Figure 4.
(A) Example of PRN counting in a single eye. FISH for *opsin* with DAPI labeling. Single confocal optical section, showing individual PRNs marked with white spots. All PRN counting was performed blind to condition. Scale bar, 10μm.
(B) PRNs per eye, 3 and 10 days post-irradiation in uninjured animals. Data represented as mean ± SD. Dots represent PRN counts for individual eyes. Statistical significance assessed by Student's *t* test (***$p<0.001$).
(C) Intra-animal difference in PRN number (uninjured – regenerating), decreases from Day 3 to Day 10 post-irradiation. Data represented as mean ± SD. Dots represent differences from individual animals. Statistical significance assessed by paired Student's *t* test (**$p<0.01$).
(D) Combined TUNEL and FISH for *opsin*. Maximum intensity projection. Scale bar, 100 μm.

Figure S4

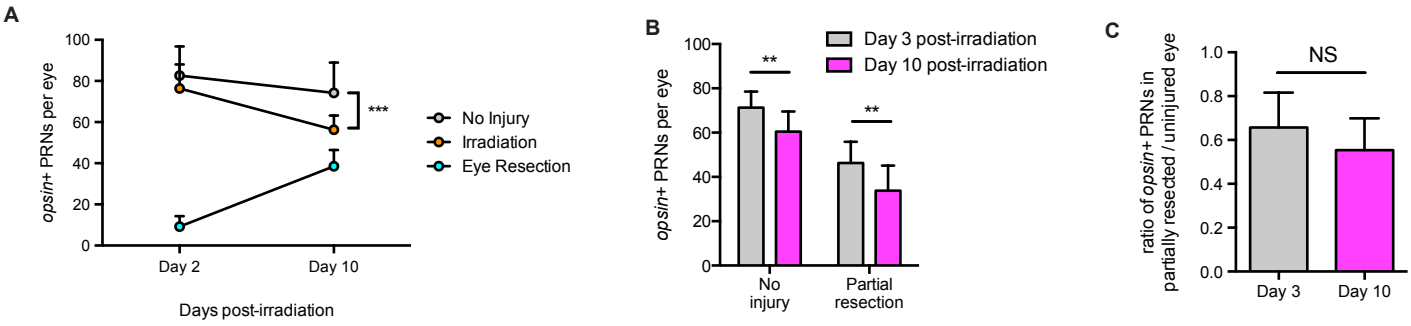

A
100
80
60
40
20
0
opsin+ PRNs per eye
***
Day 2
Day 10
Days post-irradiation
No Injury
Irradiation
Eye Resection
B
Day 3 post-irradiation
Day 10 post-irradiation
**
**
No injury
Partial resection
C
NS
ratio of opsin+ PRNs in partially resected / uninjured eye
1.0
0.8
0.6
0.4
0.2
0.0
Day 3
Day 10

Figure S4. Dynamics of eye growth and de-growth are consistent with a passive model for tissue-specific eye regeneration. Related to Figures 4 and 5.
(A) PRNs per eye Day 2 and Day 10 after indicated manipulation. Data represented as mean ± SD. Significance assessed by Student's *t*-test (***p<0.001). $n \geq 10$ animals per condition.
(B and C) Uninjured and partially resected eyes undergo cell loss at similar rates. (B) PRNs per eye Day 3 and Day 10 post-irradiation for indicated surgeries. (C) Intra-animal ratio of PRNs in regenerating/intact eyes remained constant from Day 3 to Day 10 post-irradiation. Data represented as mean ± SD. Significance assessed by Student's *t*-test (**p<0.01); NS, not significant. $n=12$ animals per timepoint.

Figure S5

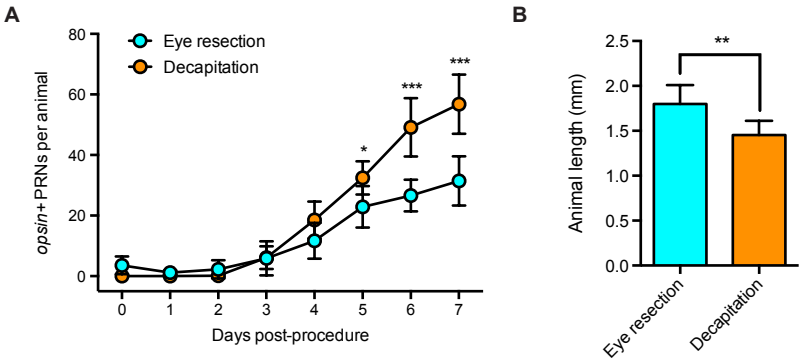

A
Eye resection
Decapitation
opsin+ PRNs per animal
0
20
40
60
80
0
1
2
3
4
5
6
7
Days post-procedure
*
***
***
B
Animal length (mm)
0.0
0.5
1.0
1.5
2.0
2.5
**
Eye resection
Decapitation

Figure S5. Eye-resected animals regenerate eyes more slowly than decapitated animals. Related to Figure 5.

(A) PRN regeneration timecourse following decapitation or eye resection. PRNs per animal Day 0 to Day 7 post-procedure are shown. Decapitated animals have a significantly greater number of PRNs than eye-resected animals at 5, 6 and 7 days post-procedure. Animals are same as used for eye progenitor counting in Figure 2D. Day 0 and Day 1 eye resection data is also shown in Figure S1C. Data represented as mean ± SD. Statistical significance assessed by Student's *t*-test (*$p<0.05$, ***$p<0.001$). $n \geq 4$ animals per condition.

(B) Animal length seven days post-surgery. Decapitated animals are significantly shorter than eye-resected animals. Data represented as mean ± SD. Statistical significance assessed by Student's *t*-test (**$p<0.01$). $n \geq 9$ animals per condition.

Figure S6

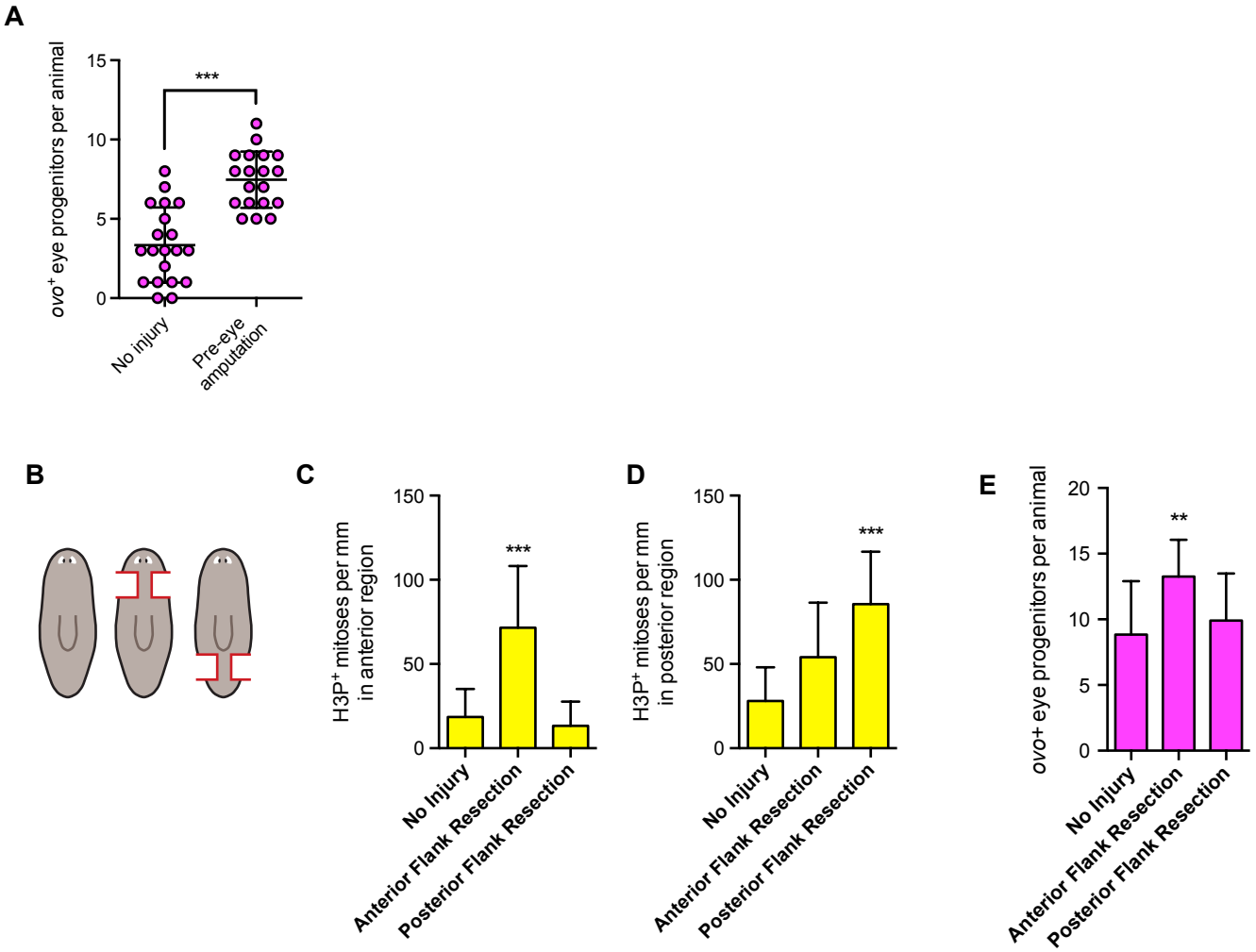

A
ovo+ eye progenitors per animal
15
10
5
0
***
No injury
Pre-eye amputation
B
C
H3P+ mitoses per mm in anterior region
150
100
50
0
***
No Injury
Anterior Flank Resection
Posterior Flank Resection
D
H3P+ mitoses per mm in posterior region
150
100
50
0
***
No Injury
Anterior Flank Resection
Posterior Flank Resection
E
ovo+ eye progenitors per animal
20
15
10
5
0
**
No Injury
Anterior Flank Resection
Posterior Flank Resection

Figure S6. Eye progenitor amplification is associated with wounds that induce proliferation in the location of eye progenitor specification. Related to Figures 6 and 7.
(A) Independent experiment determining the effect of pre-eye amputation on eye progenitor numbers. *ovo*$^{+}$ eye progenitors per animal 4 days after no injury and pre-eye amputation, as depicted in Figure 6G. Pre-eye amputation induces significant increase in eye progenitor numbers. Data represented as mean ± SD. Dots represent values from individual animals. Statistical significance assessed by Student's *t*-test (***p<0.001).
(B) Cartoon depiction of uninjured animal (left), anterior flank resection (center), and posterior flank resection (right).
(C and D) H3P^{+} cells per mm in anterior (C) and posterior (D) regions 48 hours after surgeries indicated in (B). Data from (C) is also displayed in Figure 7D. Data represented as mean ± SD. Statistical significance assessed with respect to uninjured animals by one-way ANOVA (***p<0.001). $n \geq 9$ animals per condition.
(E) *ovo*$^{+}$ eye progenitors per animal 4 days after surgeries depicted in (B). Data represented as mean ± SD. Significance assessed with respect to uninjured animals by one-way ANOVA (**p<0.01). $n \geq 11$ animals per condition.

Figure S7

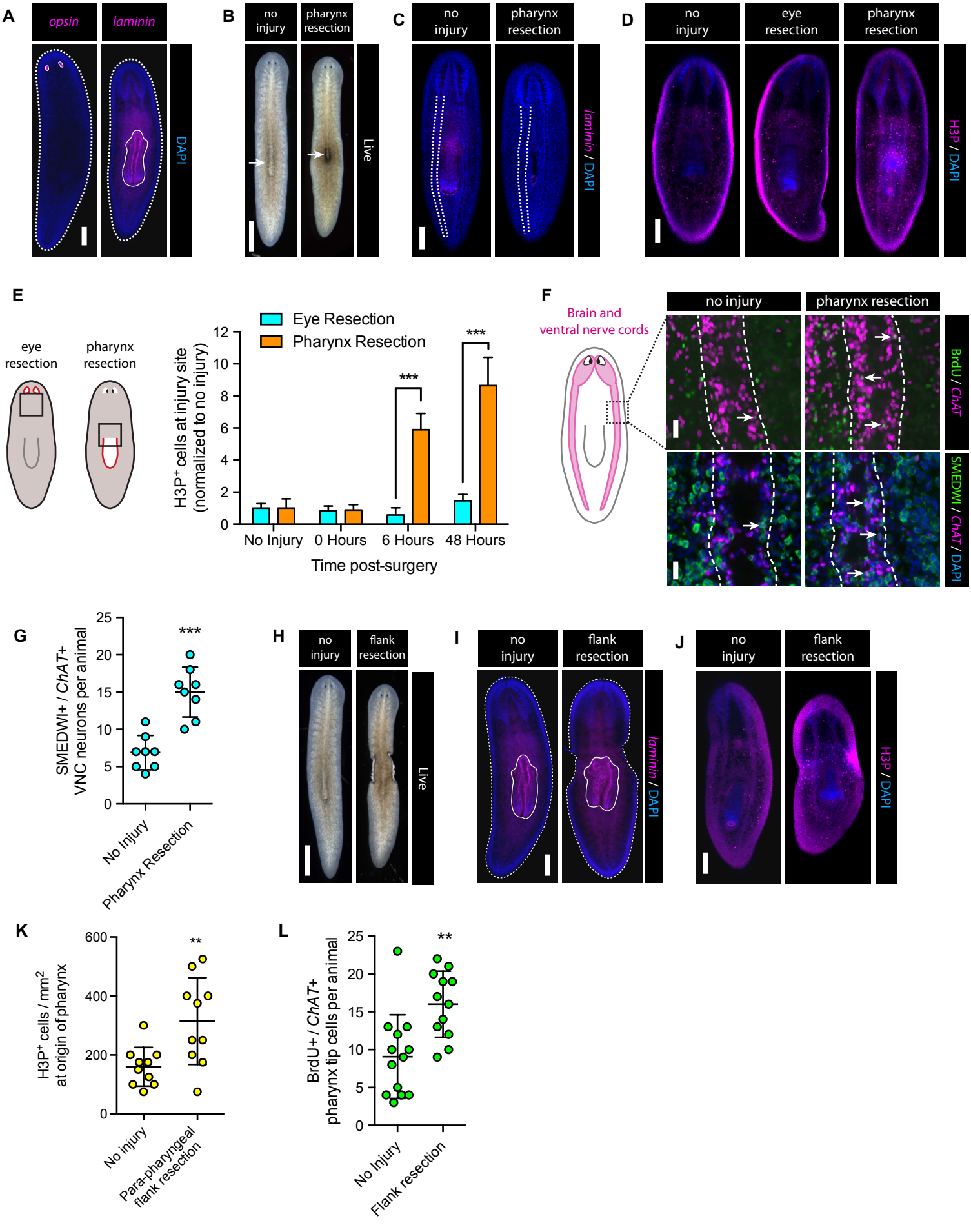

A
opsin
laminin
DAPI
B
no injury
pharynx resection
Live
C
no injury
pharynx resection
laminin / DAPI
D
no injury
eye resection
pharynx resection
H3P / DAPI
E
eye resection
pharynx resection
Eye Resection
Pharynx Resection
H3P+ cells at injury site (normalized to no injury)
***
***
No Injury
0 Hours
6 Hours
48 Hours
Time post-surgery
F
Brain and ventral nerve cords
no injury
pharynx resection
BrdU / ChAT
SMEDWI / ChAT / DAPI
G
SMEDWI+ / ChAT+ VNC neurons per animal
***
No Injury
Pharynx Resection
H
no injury
flank resection
Live
I
no injury
flank resection
laminin / DAPI
J
no injury
flank resection
H3P / DAPI
K
H3P+ cells / mm2 at origin of pharynx
**
No injury
Para-pharyngeal flank resection
L
BrdU+ / ChAT+ pharynx tip cells per animal
**
No Injury
Flank resection

Figure S7. Increased progenitor incorporation into the ventral nerve cords and pharynx does not require specific tissue removal. Related to Figure 7.

(A) FISH for *opsin* or *laminin* with DAPI labeling. *laminin* marks pharynx tissue. Dashed lines indicate animal boundaries, solid lines indicate borders of PRNs or pharynx. Scale bar, 200 μm. The pharynx is much larger than the eyes.

(B) Live images of the same animal immediately before (left) and after (right) dorsal surgical pharynx resection. Arrow indicates location of pharynx (left) or location of dorsal incision through which pharynx was excised (right). Scale bar, 1 mm.

(C) FISH for *laminin* with DAPI labeling after no injury and dorsal surgical pharynx resection. Dashed white lines indicate left ventral nerve cord (VNC). Scale bar, 200 μm. Surgery removes pharynx and leaves VNCs continuous and intact.

(D) IF for H3P with DAPI labeling in uninjured, eye-resected and pharynx-resected animals 48 hours post-surgery. Scale bar, 200 μm. Pharynx-resected but not eye-resected animals display increased mitotic activity near the site of tissue removal.

(E) Cartoons depict surgeries (red lines) and location of quantification (black boxes) for eye- and pharynx-resected animals. Graph indicates mitotic density at indicated times post-surgery, normalized to mean of uninjured animals. Pharynx but not eye resection induces sustained local neoblast proliferation.

(F) IF for BrdU with FISH for *ChAT* (top insets) and IF for SMEDWI with FISH for *ChAT* and DAPI labeling (bottom insets) after no injury or pharynx resection. Dashed white lines indicate VNC boundaries. Arrows indicate $BrdU^+/ChAT^+$ or $SMEDWI^+/ChAT^+$ VNC cells. Scale bars, 20 μm.

(G) $SMEDWI^+/ChAT^+$ VNC neurons per animal 4 days after no injury or pharynx resection. Quantification performed in VNC just anterior to pharynx. Data represented as mean ± SD. Dots represent values from individual animals. Significance assessed by Student's *t*-test (***$p<0.001$).

(H) Live images of the same animal immediately before (left) and after (right) para-pharyngeal flank resection. Scale bar, 1 mm.

(I) FISH for *laminin* with DAPI labeling in animals fixed immediately after no injury or para-pharyngeal flank resection. Dashed white lines indicate animal boundaries, solid white lines outline pharynges. Scale bar, 200 μm. Para-pharyngeal flank resection does not remove pharynx tissue.

(J) IF for H3P with DAPI labeling 48 hours after no injury or para-pharyngeal flank resection. Scale bar, 200 μm. Flank resection increases mitotic activity near the pharynx.

(K) Mitotic density near pharynx 48 hours after no injury or para-pharyngeal flank resection. Data represented as mean ± SD. Dots represent values from individual animals. Significance assessed by Student's *t*-test (**$p<0.01$).

(L) $BrdU^+/ChAT^+$ pharynx tip cells per animal after no injury or para-pharyngeal flank resection. BrdU delivery Day -1, fixation Day 4 with respect to surgery. Data represented as mean ± SD. Dots represent values from individual animals. Significance assessed by Student's *t*-test (**$p<0.01$).

Supplemental Tables

Table S1. Animal numbers for all experiments. Related to all figures.
The table indicates the number of animals used for all experiments involving quantification, with corresponding figure number and condition.

Table S2. Sequences used for FISH probes and dsRNA. Related to all figures.
The table indicates sequences used for FISH probes and dsRNA, with references.