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Supplemental information

Positional information modulates transient regeneration-activated cell states during vertebrate appendage regeneration

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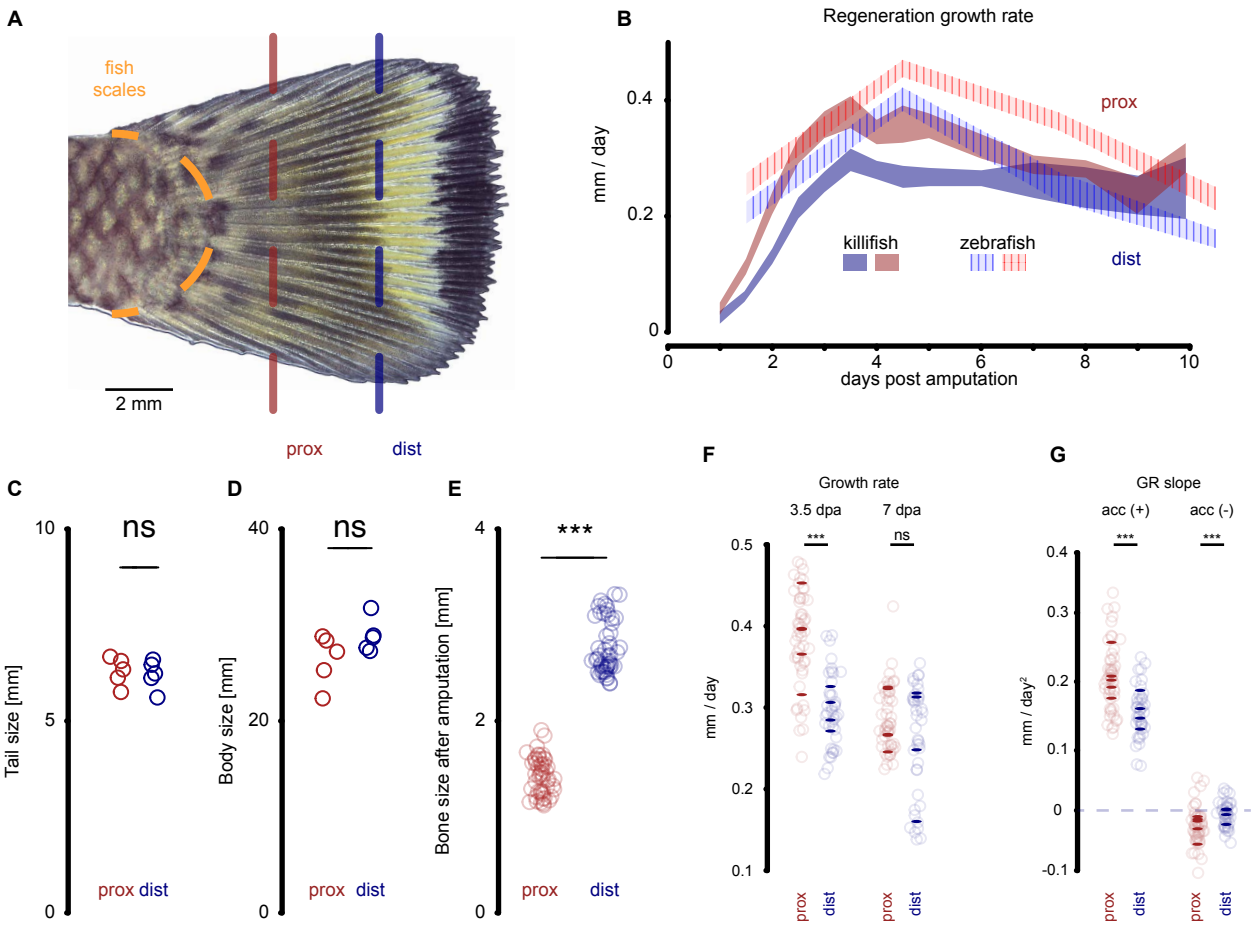
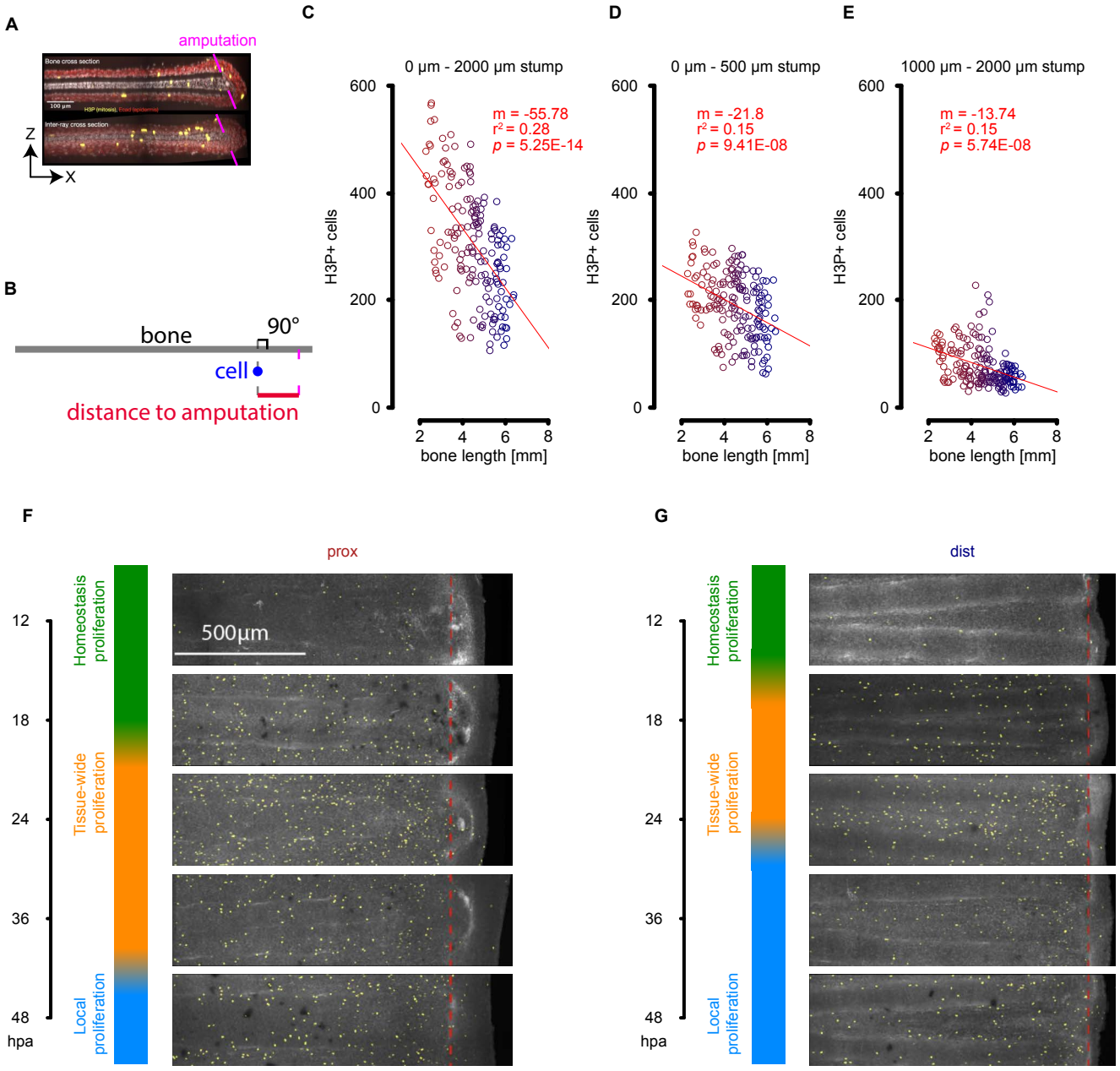


Supplementary Figure S1. Fish size and growth rate comparison, Related to Figure 1.
(A) Representative image of the male caudal fin in killifish, the boundary between the scales and the caudal fin is delineated by the orange dotted line. The distal amputation is performed between the spotted pigmentation region and the yellow pigmentation line perpendicular to the anterior and posterior axis. The proximal amputation is performed at the coloring transition from bright to dark within the spotted pigmentation region perpendicular to the anterior and posterior axis. Both cuts correspond to the second and first bifurcation of the fin rays respectively. Scale bar: 2 mm.
(B) Back calculation of growth rate measurements in zebrafish published by Uemoto et.al. 2020 superimposed with killifish regeneration growth rate of equivalent size fish. Polygon area represents mean ± SEM.
(C) Tail fin length of individual fish used in Figure 1 measured from the base of the fin to the edge of the tissue prior to amputation.
(D) Anterior-Posterior body length of individual fish used in Figure 1 measured from the mouth to the base of the fin.
(E) Bone length measured from the base of the fin where the scales meet the tail to the amputation plane of all bones that support the model in Figure 1.
(F) Statistical analysis of growth rate quantified at 3.5 and 7 dpa in Figure 1F. Open circles reflect individual bones, filled ovals correspond to individual fish mean values.
(G) Statistical analysis of growth acceleration and slowing down in Figure 1F. Open circles and filled ovals represent the same as in (F).
ns not significant, *** p<0.001, Wilcoxon rank sum test.



Supplementary Figure S2. Mitotic cells mainly correspond to dividing epidermis, Related to Figure 2.

(A) 24 hpa bone and inter-ray 10 µm MAX projections of orthogonal views of high-magnification confocal stacks. Scale bar: 100 µm.

(B) Number of H3P⁺ nuclei inside 2 mm window from the amputation plane along the bone axis.

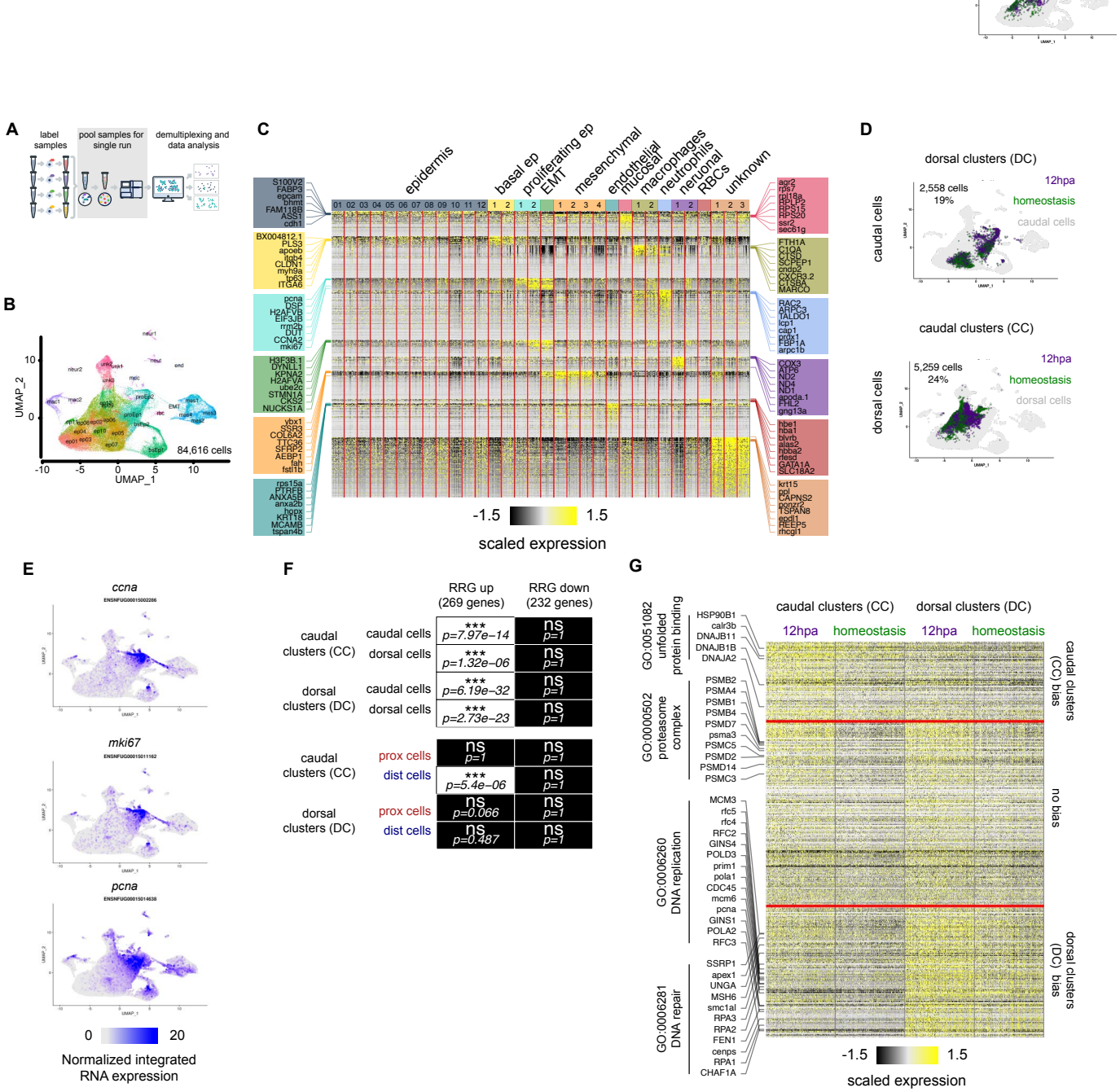
(C) Number of H3P⁺ nuclei inside 0.5 mm window from the amputation plane along the bone axis.

(D) Number of H3P⁺ nuclei inside 1 mm window counting from 1 mm from the amputation plane to 2 mm from the amputation plane along the bone axis.

(E) Definition of distance to amputation used to calculate proliferation profiles and peak proliferation.

(F) Representative images of proliferation time course in proximal injuries where homeostasis, tissue-wide and local proliferation are indicated. Scale bar: 500 µm.

(G) Representative images of proliferation time course in distal injuries where homeostasis, tissue-wide and local proliferation are indicated.



Supplementary Figure S3. CellPlex workflow and single cell atlas cell type definition, Related to Figure 3.

(A) Multiplexed scRNAseq workflow using 10X CellPlex reagents.

(B) Dimensional reduction UMAP plot of the integrated dataset with cell type definitions clustered at resolution 1.0, colors are randomly selected to each cluster.

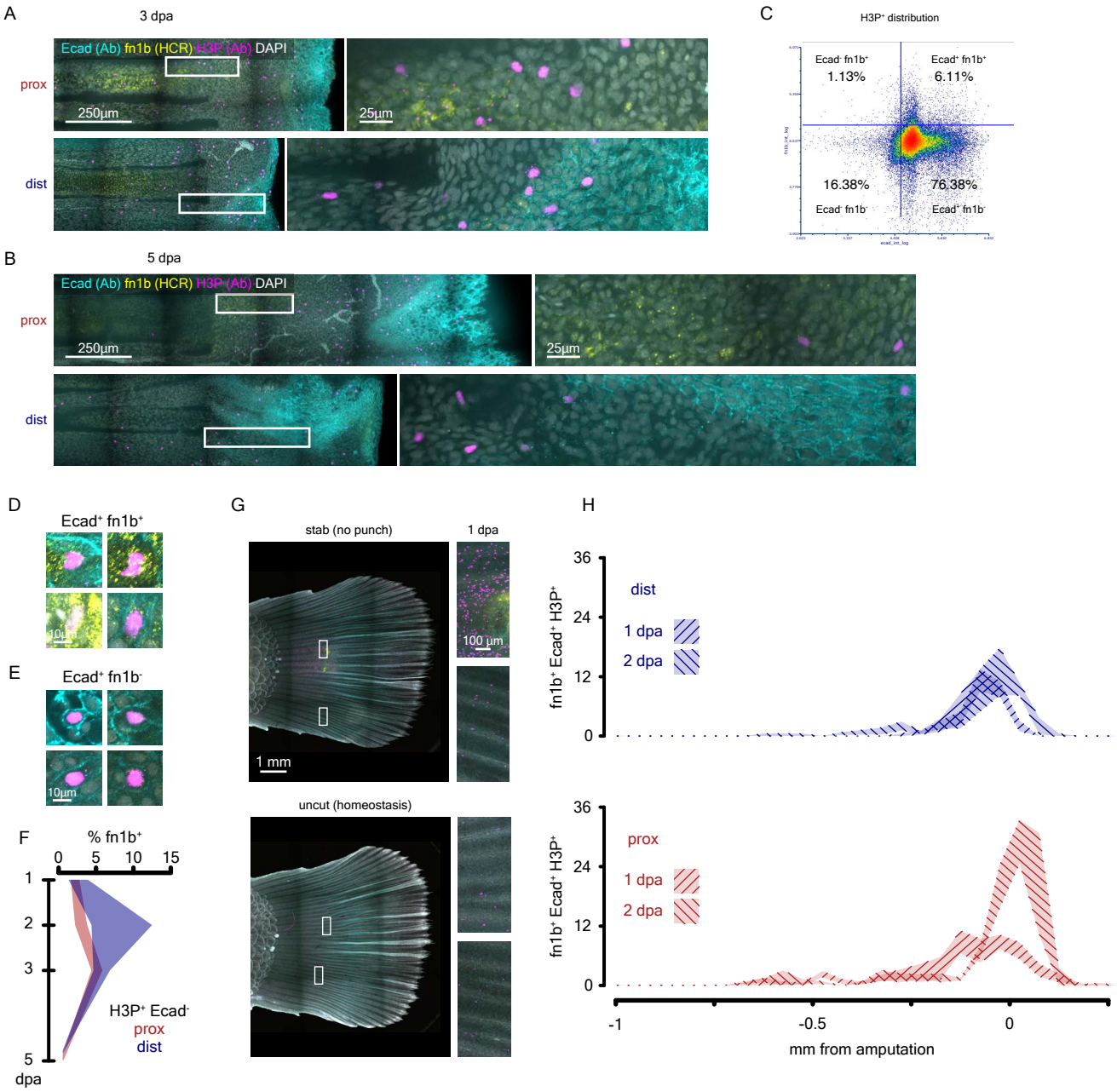
(C) Heatmap of cell type markers.

(D) Dimensional reduction UMAP distribution of regeneration and homeostasis cells captured within the caudal clusters (CC) and dorsal clusters (DC) in both caudal and dorsal datasets.

(E) *ccna*, *mki67* and *pcna* expression within the integrated dataset.

(F) Heatmap of enrichment analysis between RRG and caudal, dorsal, proximal, and distal cells within caudal clusters (CC) and dorsal clusters (DC). Fisher's exact test.
ns, not significant; ***, *FDR < 0.001*.

(G) Heatmap of regeneration upregulated genes within caudal clusters (CC) and dorsal clusters (DC) split by any bias towards one or the other cluster group. and top GO terms associated with the gene subset (*FDR < 0.05*). Cells were randomly sampled to balance all four sample groups.



Supplementary Figure S4. *fn1b*+ expression shuts down at later time points, *fn1b* and Ecad cytometry analysis, and spatial distribution of *fn1b*⁺ Ecad⁺ H3P⁺ cells, Related to Figure 4.

(A) 3 dpa *fn1b* (HCR), Ecad (Ab), H3P (Ab) whole mount staining. Scale bar: 250 µm on the left and 25 µm on the right.

(B) 5 dpa *fn1b* (HCR), Ecad (Ab), H3P (Ab) whole mount staining. Scale bar: 250 µm on the left and 25 µm on the right.

(C) Ecad and *fn1b* cytometry analysis on H3P⁺ cells.

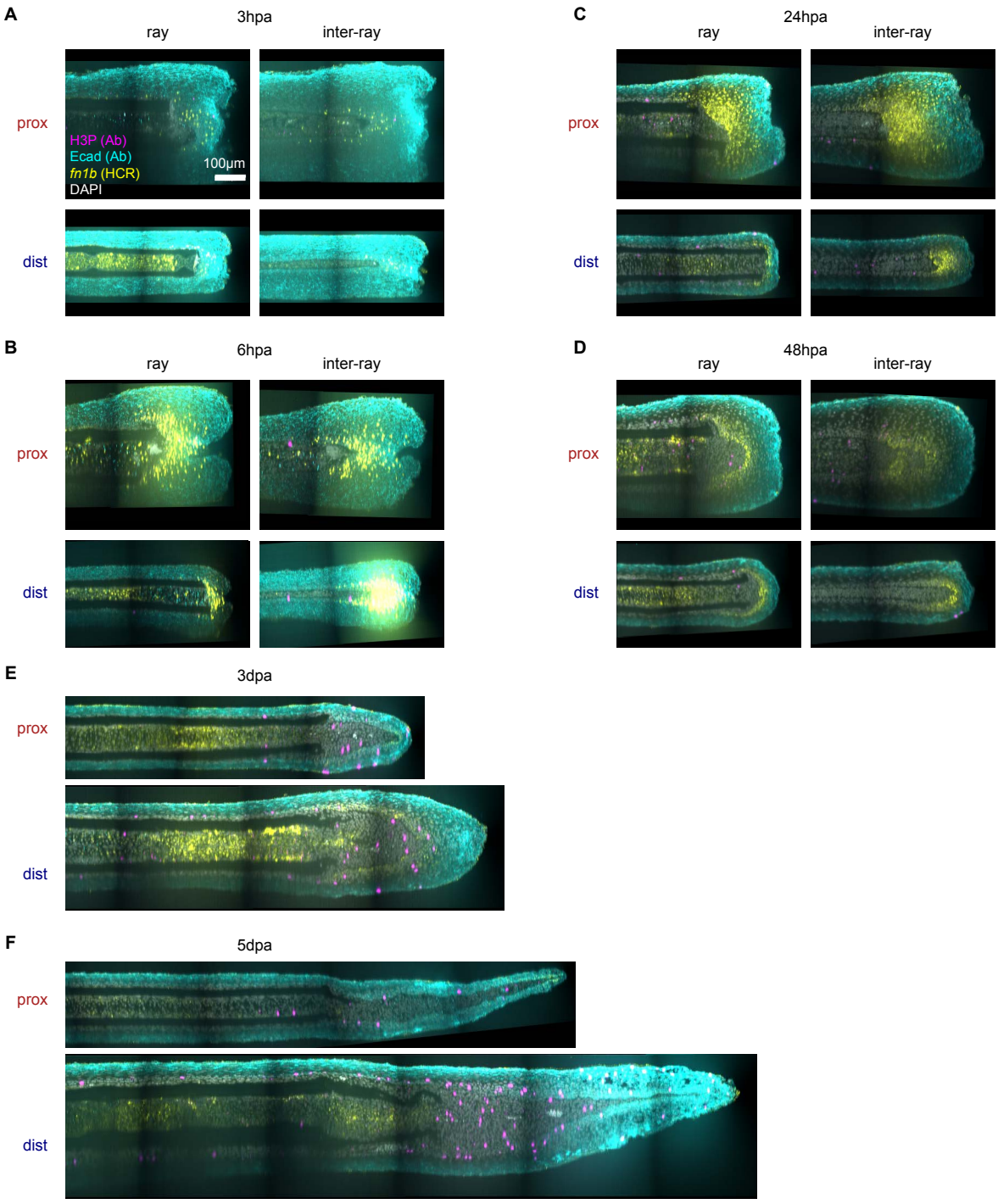
(D) Representative H3P⁺ Ecad⁺ *fn1b*⁺ cells for cytometry analysis. Scale bar: 10 µm.

(E) Representative H3P⁺ Ecad⁺ *fn1b*⁻ cells for cytometry analysis. Scale bar: 10 µm.

(F) % of *fn1b*⁺ cells within the Ecad⁻ H3P⁺ population over time. Polygon area represents mean ± SEM.

(G) Stab injury on the left and uncut control on the right shows *fn1b* (HCR), Ecad (Ab), and H3P (Ab) stains at 1 days post injury. Scale bar: 1 mm on the left and 100 µm on the right.

(H) Spatial distribution of *fn1b*⁺ Ecad⁺ H3P⁺ cells in distal (top) and proximal (bottom) injuries at 1 and 2 dpa. Polygon area represents mean ± SEM.



Supplementary Figure S5. Orthogonal analysis of regeneration time course, Related to Figure 5.

(A) Ray and inter-ray orthogonal views of regenerating proximal and distal samples at 3 hpa *fn1b* (HCR), Ecad (Ab), H3P (Ab) whole mount staining. Scale bar: 100 µm.
(B) Same as in (A) at 6 hpa *fn1b* (HCR), Ecad (Ab), H3P (Ab) whole mount staining.
(C) Same as in (A) at 1 dpa *fn1b* (HCR), Ecad (Ab), H3P (Ab) whole mount staining.
(D) Same as in (A) at 2 dpa *fn1b* (HCR), Ecad (Ab), H3P (Ab) whole mount staining.
(E) Ray orthogonal views of regenerating proximal and distal samples at 3 dpa *fn1b* (HCR), Ecad (Ab), H3P (Ab) whole mount staining.
(F) Same as in (D) at 5 dpa fn1b (HCR), Ecad (Ab), H3P (Ab) whole mount staining.