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Supplementary Materials for

Cellular diversity of the regenerating caudal fin

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Published 12 August 2020, *Sci. Adv.* **6**, eaba2084 (2020)
DOI: 10.1126/sciadv.aba2084

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Figs. S1 to S6

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/33/eaba2084/DC1)

Tables S1 to S7

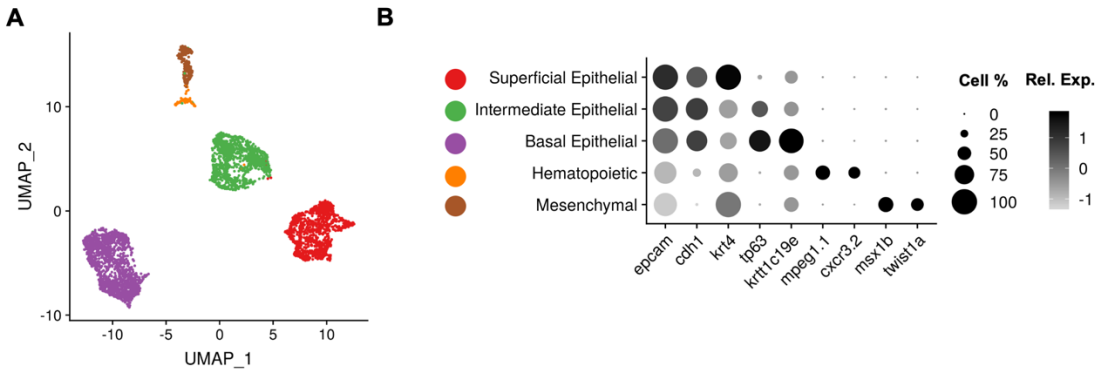

Figure S1. Basic cell type identification in preinjury caudal fins. (A) Clustering assignments for cells collected from preinjury stage only. Cells were plotted on UMAP axes. Color code used is the same for (A) and (B). (B) Relative expression of markers used in cell type identification for preinjury cells. Color gradient: normalized relative expression level. Dot size: percentage of cells in the cluster that express the specified gene.

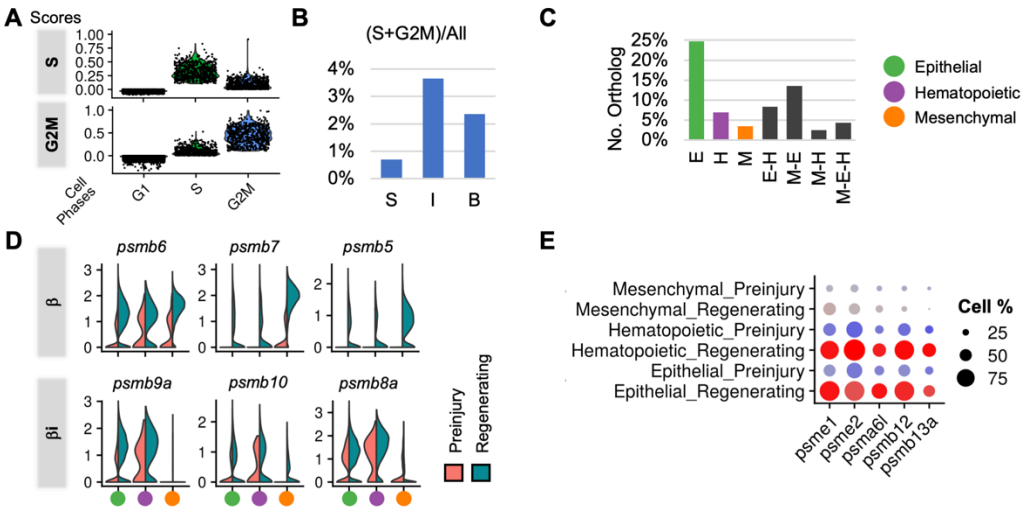

Figure S2. **Common and cell type-dependent cell cycle regulations in S-phase cells.** (A) S phase and G2M phase scores of cells grouped by the assigned cell cycle phases. Phase scores are calculated based on the expression level of cell cycle-related genes (Methods). (B) Percentage of S or G2M phase cells in three epithelial layers. S, Superficial epithelial; Intermediate epithelial; B, Basal epithelial. (C) Percentage of S-phase enriched genes that do not have human orthologs. E: Epithelial, H: Hematopoietic, M: Mesenchymal. (D) Expression level distribution of β and βi proteasome subunits in all cells. Cells were first grouped by major cell types, then separated into preinjury and regenerating stages. Color code is the same as in (C). (E) Differential expression of proteasome subunits in all cells grouped by major cell types and their stage of collection. Color gradient: relative expression level, with blue used for all preinjury groups, and red for all regenerating groups. Dot size: percentage of cells in the cluster that express the specified gene.

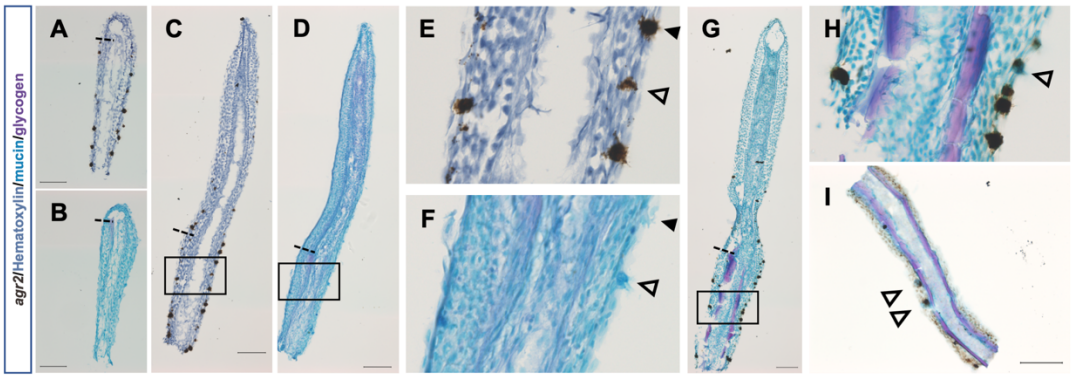

Figure S3. *agr2* expression in uninjured and regenerating fins. (A,B) Consecutive sections from 1dpa fin tissue, targeted by *agr2* probe for *in situ* hybridization (A) or stained by Alcian blue/PAS (B). Brown dots indicate *agr2* mRNA. For Alcian blue/PAS staining, nuclei are in pale blue, glycogen is in magenta, and stromal mucins in blue. Dotted lines indicate the amputation plane. (C,D) Consecutive sections from 4dpa fin tissue, targeted by *agr2* probe for *in situ* hybridization (C) or stained by Alcian blue/PAS (D). (E,F) Zoomed in views for regions highlighted by focus rectangles in (C,D). Arrows point to matching Alcian blue-positive staining and *agr2* mRNA signal. Hollow arrows point to one pair of exact match, while solid arrows point to one pair where Alcian blue staining show a weak positive pattern. (G,H) 4dpa fin section targeted by *agr2* probe for *in situ* hybridization and then stained with Alcian blue/PAS. (H) Zoomed in view for the region highlighted by the focus rectangle in (G). The hollow arrow points to an example cell positive for both *agr2* mRNA signal and Alcian blue. (I) Uninjured fin section targeted by *agr2* probe for *in situ* hybridization and then stained with Alcian blue/PAS. Hollow arrows point to example cells positive for both *agr2* mRNA signal and Alcian blue. All scale bars are 100μm.

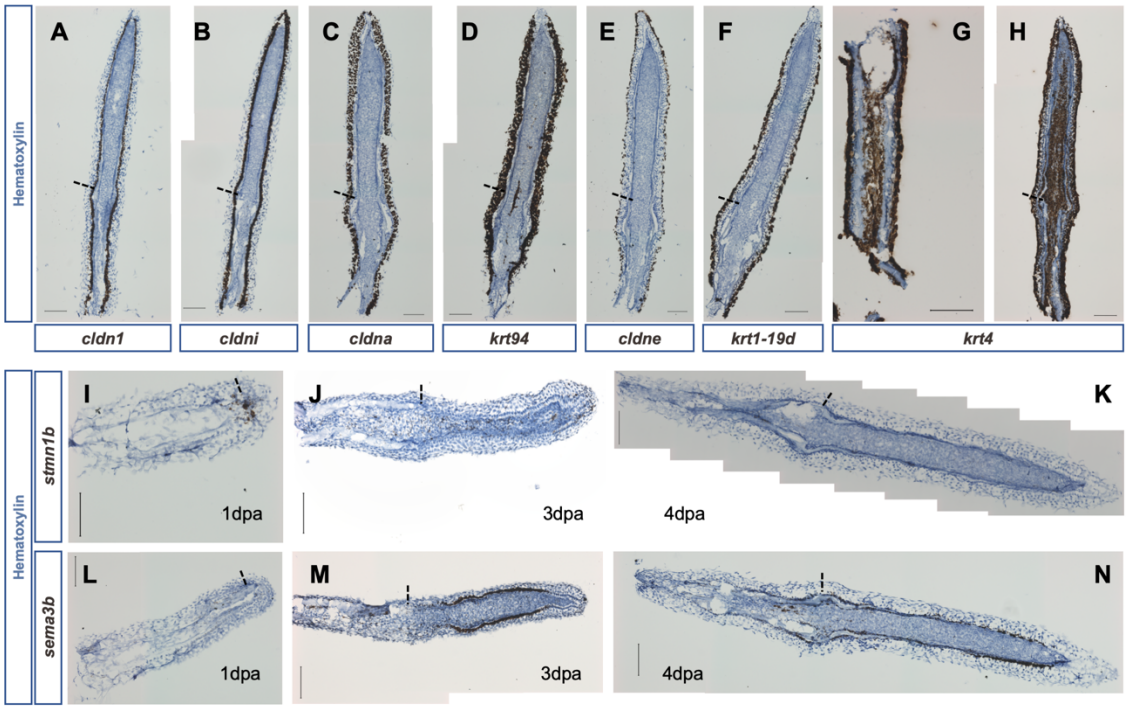

Figure S4. **Expression pattern of candidates separating epithelial subpopulations.** *In situ* hybridization targeting (A) *cldn1*, (B) *cldni*, (C) *cldna*, (D) *krt94*, (E) *cldne*, (F) *krt1-19d*, (G,H) *krt4*, (I-K) *stmn1b*, (L-N) *sema3b*, using (G) preinjury tissue sample, (I,L) 1dpa tissue sample, (J,M) 3dpa tissue sample, or (A-F, H, K,N) 4dpa tissue sample. Brown dots indicate positive RNA signals from target genes, while pale blue blocks represent hematoxylin-stained cell nuclei. Dotted lines indicate the amputation plane. All scale bars are 100μm.

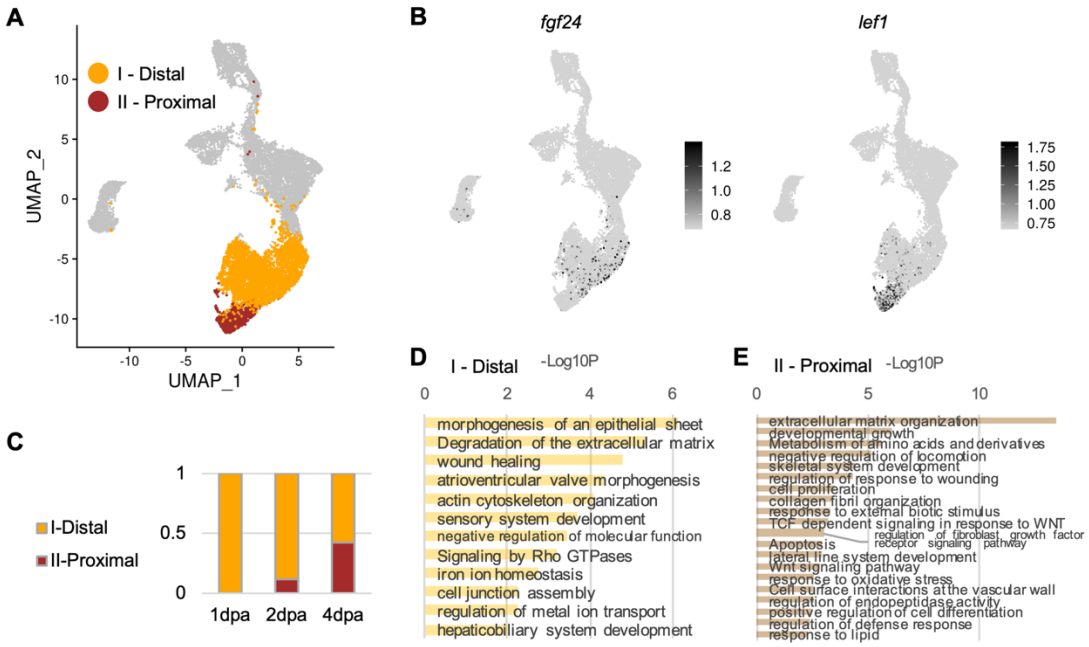

Figure S5. **Features of distal and proximal wound epidermis.** (A) Sub-clustering assignments for regenerating basal epithelial cells shown on the same UMAP axes as in Figure 3D. Clustering results reflected the physical location of the cells. (B) Expression signature of genes marking the distal or proximal region of the wound epidermis in epithelial cells. Cells were plotted on the same UMAP axes as in (A). (C) Proportion of I-Distal and II-Proximal basal epithelial cells in each stage. (D,E) Clustered gene ontology enrichment for genes upregulated in (D) distal or (E) proximal wound epidermis.

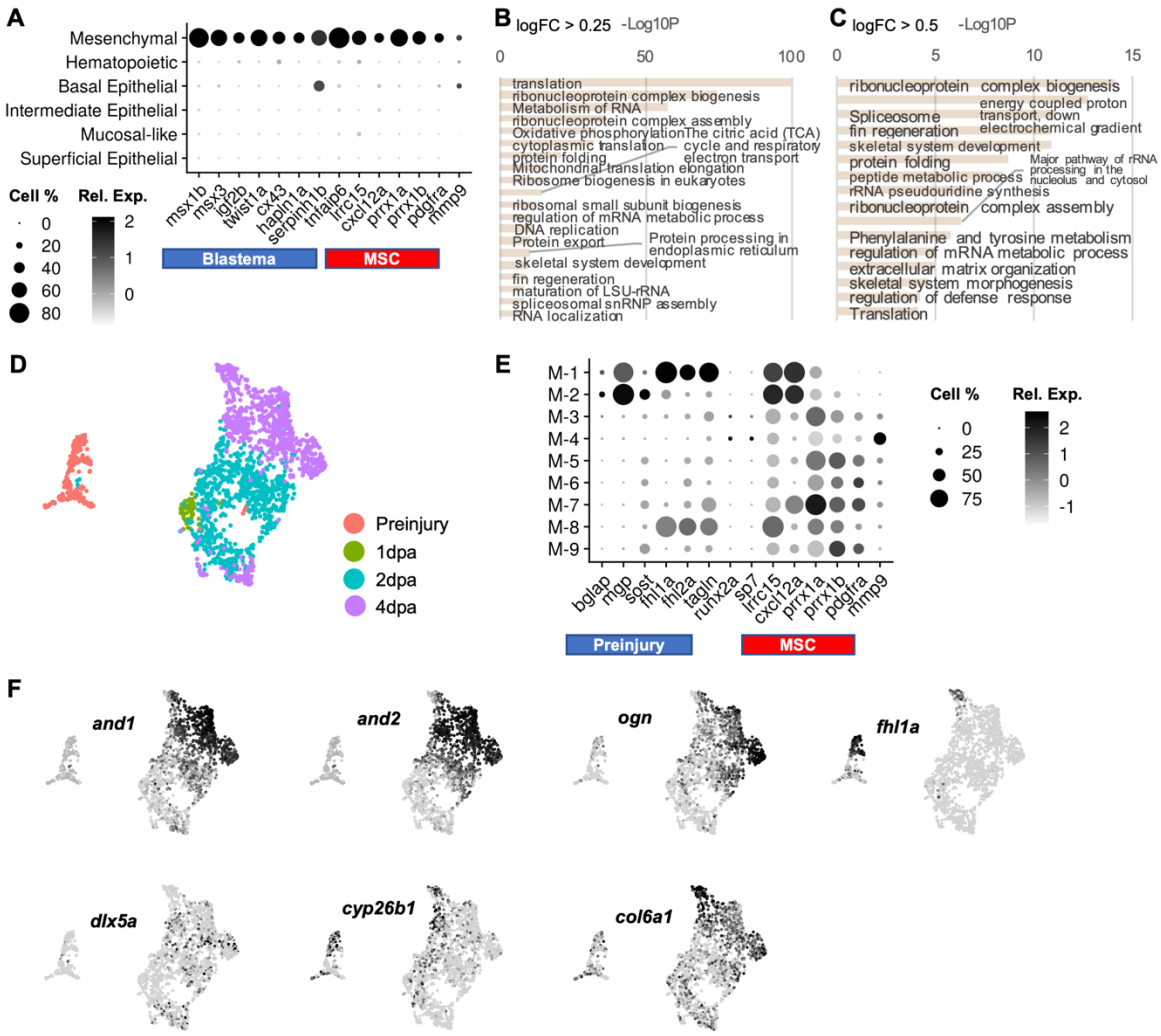

Figure S6. **Expression distribution of blastemal feature genes in the mesenchymal cluster.** (A) Relative expression levels of genes labeling the mesenchymal cluster and orthologs of mammalian MSC markers by major clusters. Color gradient: relative expression level. Dot size: percentage of cells in the cluster that express the specified gene. (B) Stage of collection of mesenchymal cells plotted on the same UMAP axes as in Figure 5A. (C,D) Clustered gene ontology enrichment for genes highly expressed in the mesenchymal cluster with a log fold change over (C) 0.25 or (D) 0.5. (E) Relative expression levels of genes enriched in the two preinjury populations, known bone cell lineage markers (*runx2a*, *sp7*) and orthologs of mammalian MSC markers by mesenchymal sub-clusters. Cluster labels are the same as Figure 5A. Color gradient:

relative expression level. Dot size: percentage of cells in the cluster that express the specified gene. (F) By-gene expression feature plot for genes potentially involved in blastema formation and mesenchymal lineage separations.