

Figure S1

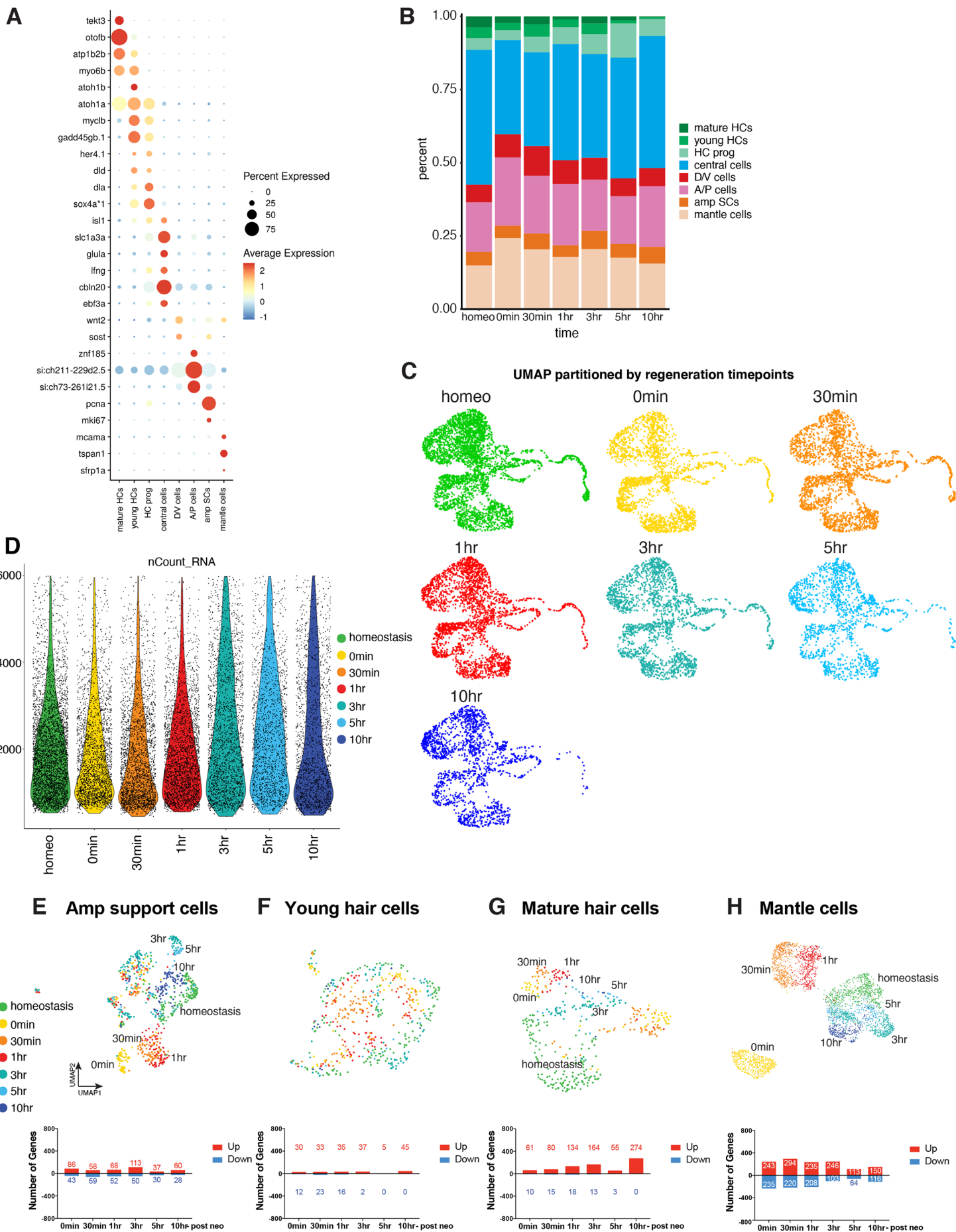


Figure S1. Related to Figure 1.

(A) Dot plot showing marker genes for eight different cell types.

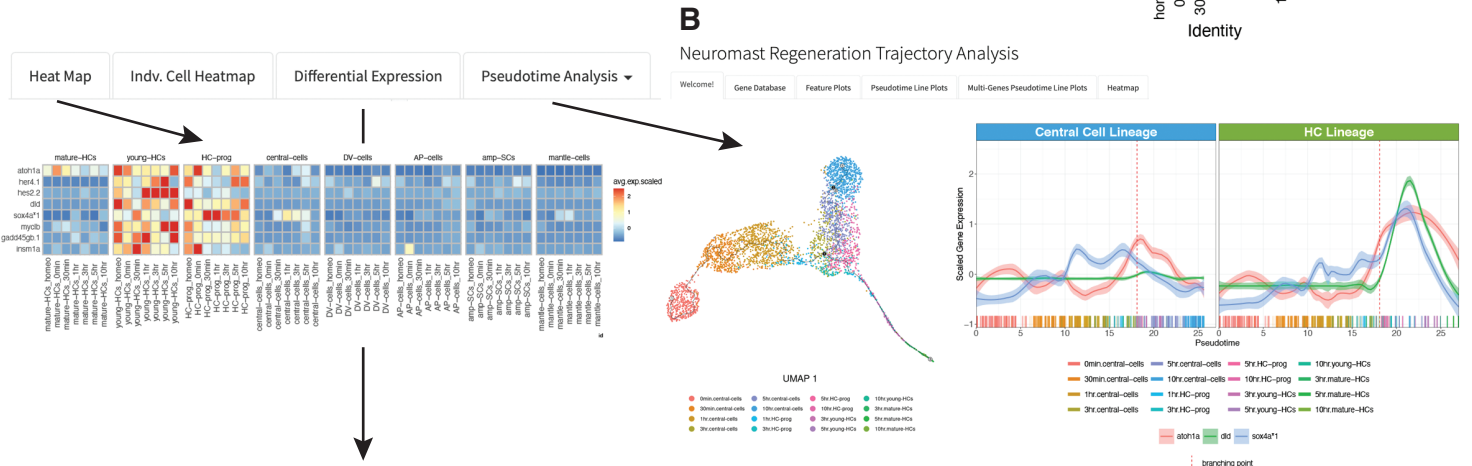
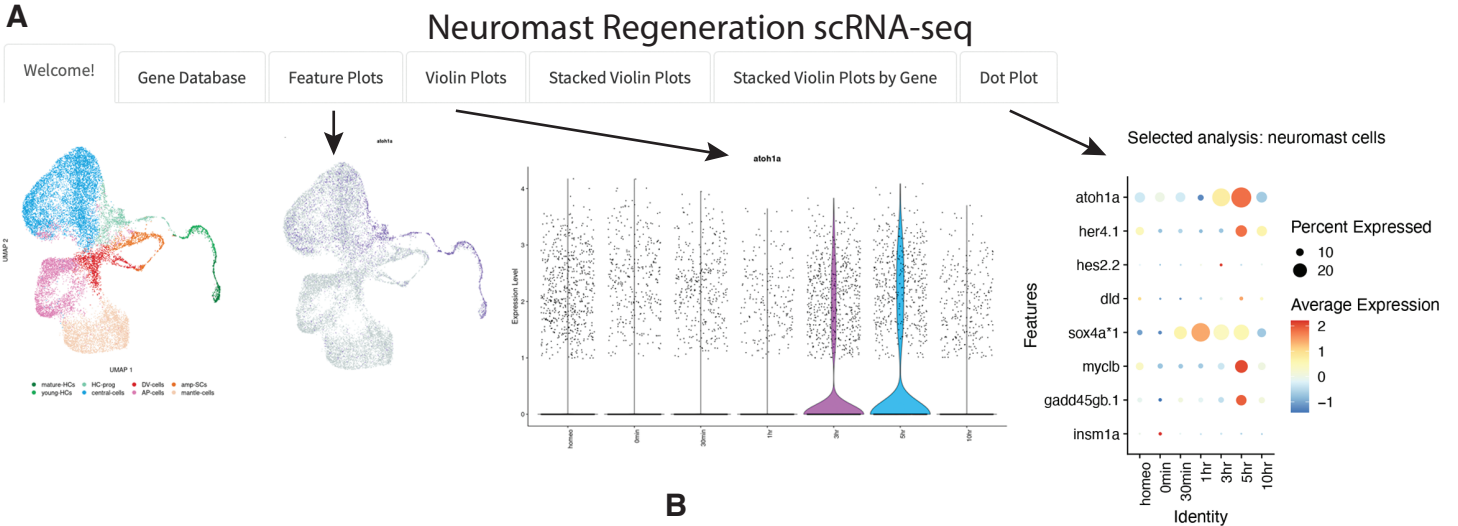
(B) Proportion of cell types in samples of each time point.

(C) UMAPs showing the distribution of cells partitioned by individual time point post-integration.

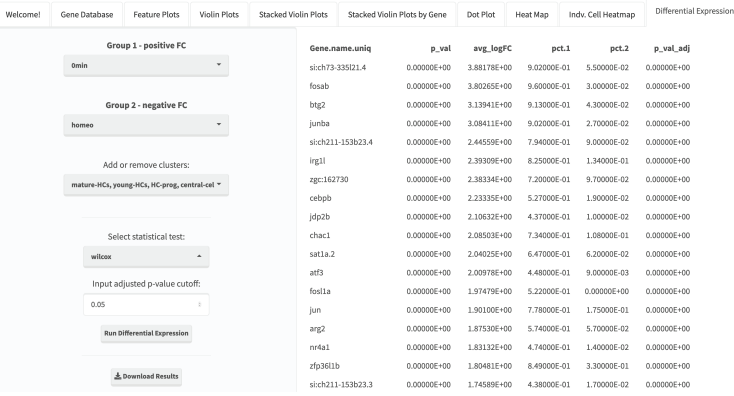
(D) Violin plot visualizing the distribution of raw counts/cell by time point.

(E - H) UMAPs of (E) amplifying cells, (F) young hair cells, (G) mature hair cells, and (H) mantle cells with bar graphs showing the distribution of cells and the number of up and down-regulated genes per time point compared to homeostasis.

Figure S2



Neuromast Regeneration scRNA-seq



C GEAR Profile (atoh1a)

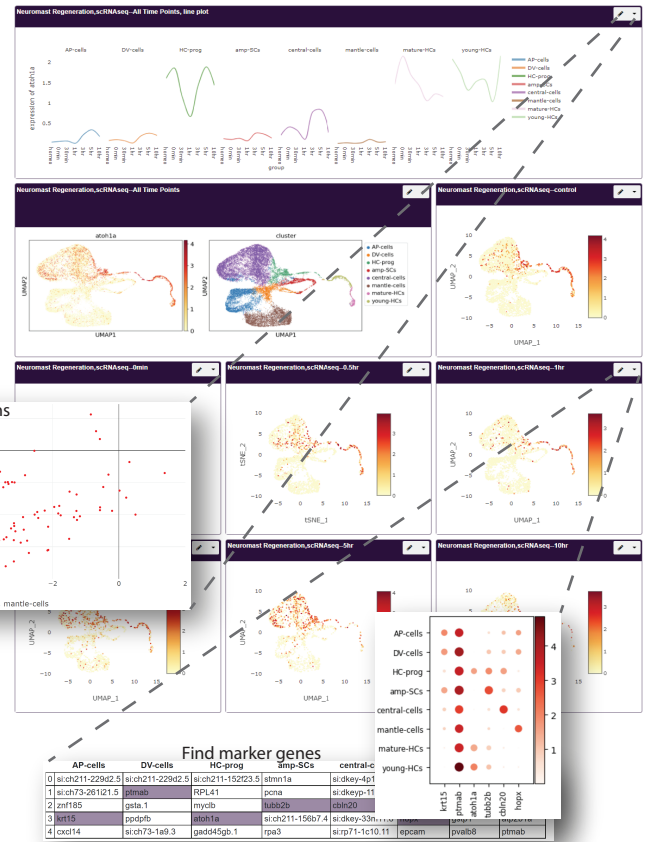


Figure S2. Overview of the interactive shiny application.

(A) Homepage to the interactive shiny application for neuromast regeneration analysis.

Options for different visualization methods are shown in separate tabs.

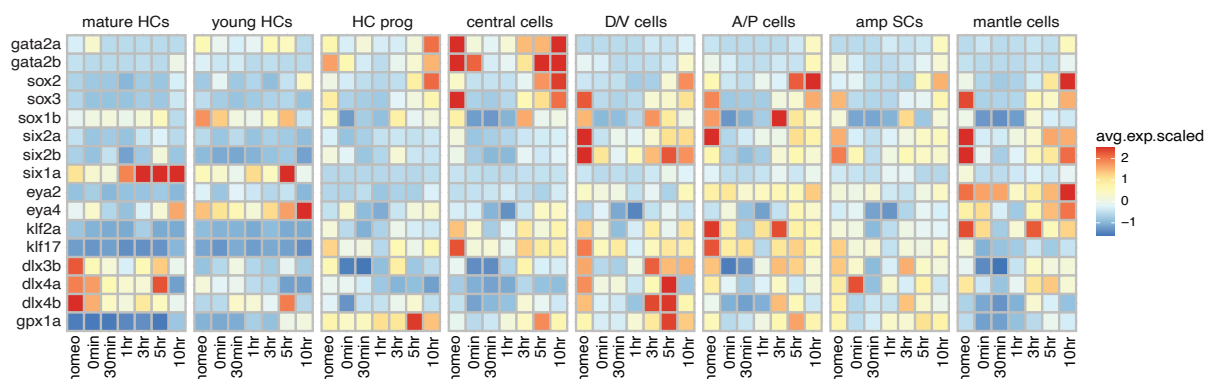
https://piotrowskilab.shinyapps.io/neuromast_regeneration_scRNA-Seq_pub_2021/

(B) The tab 'Pseudotime Analysis' links to interactive shiny app for the neuromast pseudotime analysis with the example of the tab used to graph lineage line plots.

(C) gEAR profile associated with the manuscript (<https://umgear.org/p?l=e0d00950>) (Orvis et al., 2021). *atoh1a* expression is represented. The portal, in addition to browsing gene expression in the datasets associated with the manuscripts, allows users to explore the data using analysis tools. Here shown is a comparison between HCs and Mantle cells in control condition and an example of marker genes for the different clusters.

Figure S3

A



B

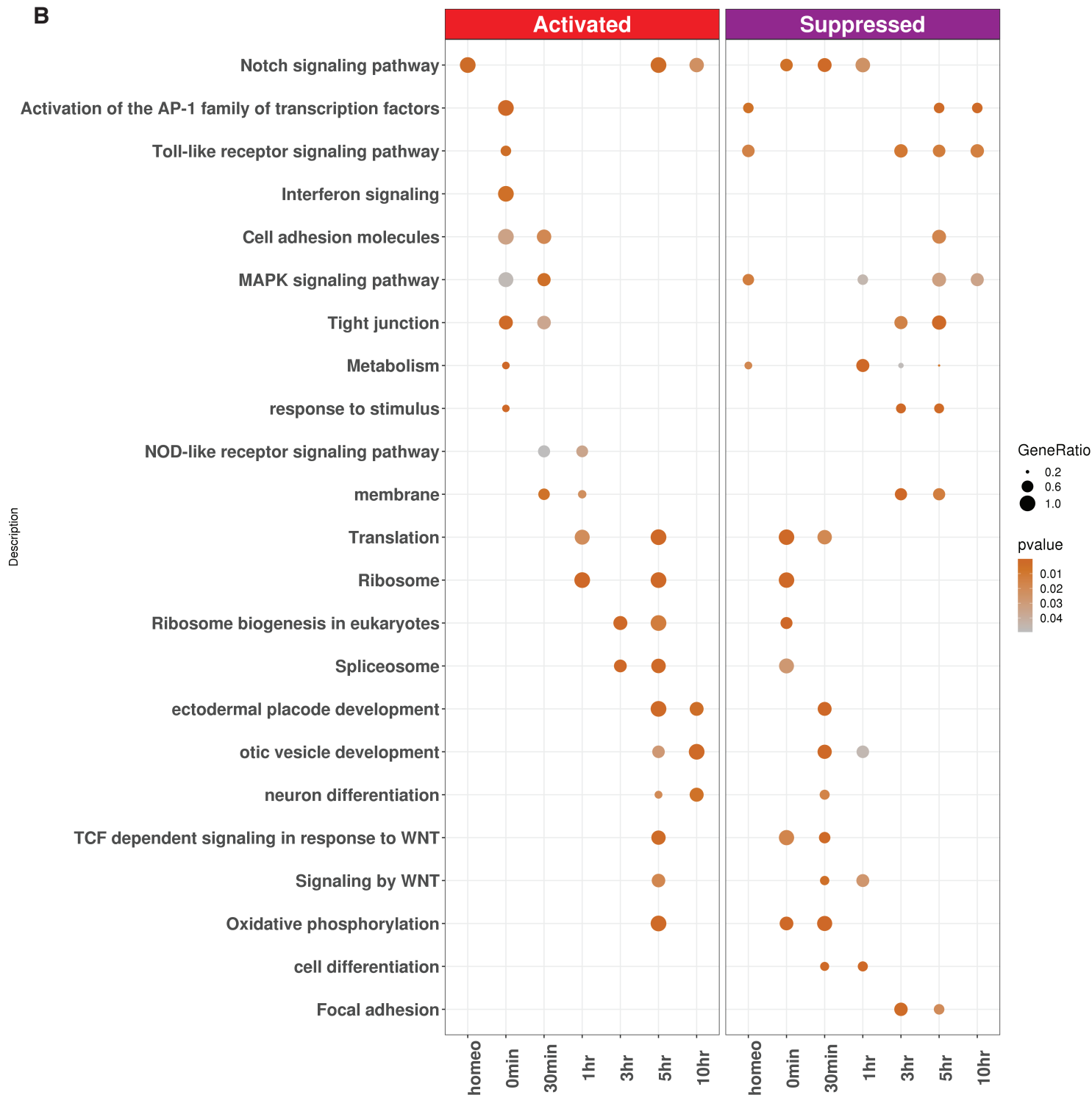


Figure S3. Expression of progenitor/stem cell maintenance/placodal genes and overview of enrichment terms. Related to Figure 2.

(A) Heatmap of the average expression of downregulated of progenitor/stem cell maintenance/placodal genes across eight cell types during hair cell regeneration.

(B) Overview of gene enrichment terms for up- and downregulated per time point compared to all the other time points.

Figure S4 0min upregulated

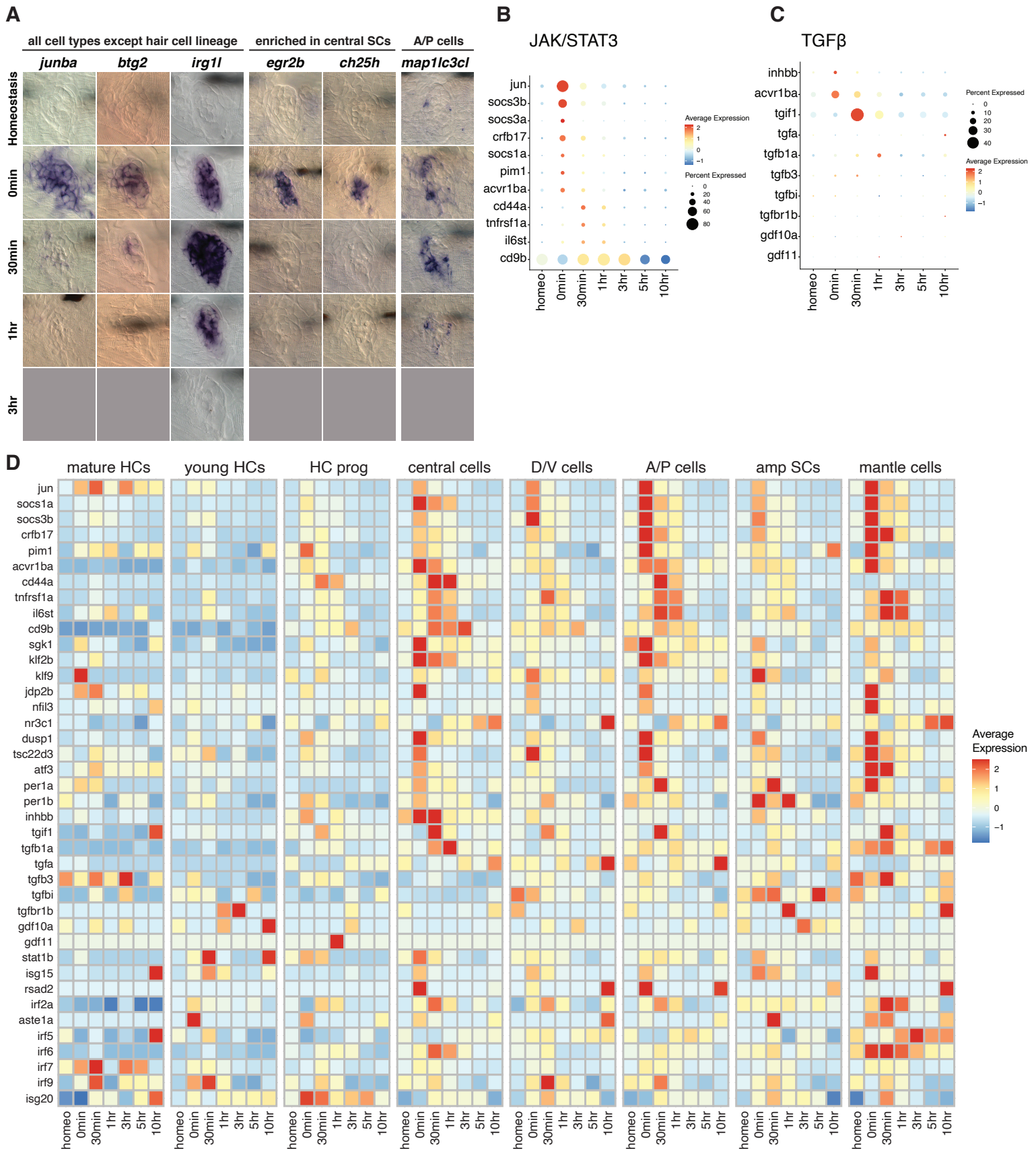


Figure S4. ISH validation, dot plots and heat maps of genes upregulated at 0min. Related to Figure 3.

(A) ISH images of genes upregulated at 0min after hair cell death in either all support cells, mostly central support cells or A/P cells.

(B) Dot plot of the average expression of Jak/Stat3 pathway members during hair cell regeneration.

(C) Dot plot of the average expression of TGF β pathway members during hair cell regeneration.

(D) Heatmap of the average expression of the glucocorticoid, Jak/Stat3 signaling, TGF β signaling and interferon signaling genes shown in Figure 3N-O during hair cell regeneration.

Figure S5 heat map of genes in dot plots

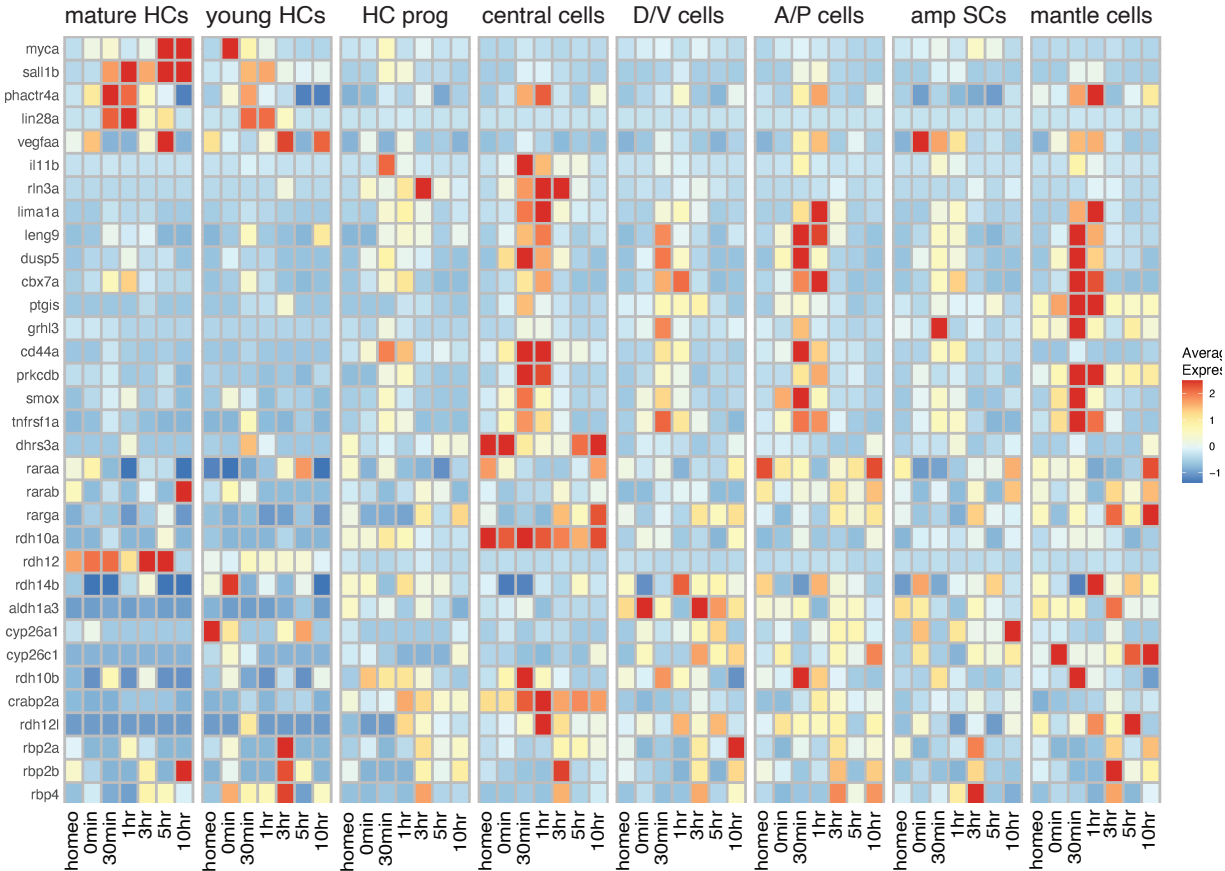


Figure S5. Related to Figure 4.

Heatmap of the average expression of the regeneration enriched genes shown in Figure 4H-I and RA signaling genes in Figure 4J-K during HC regeneration.

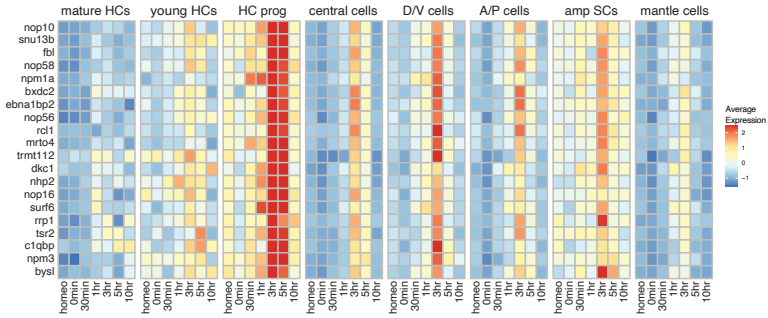
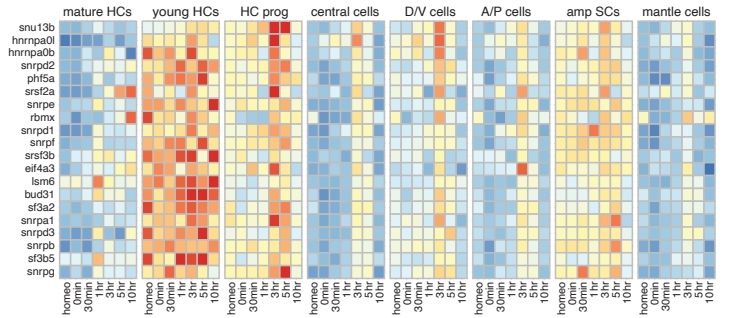
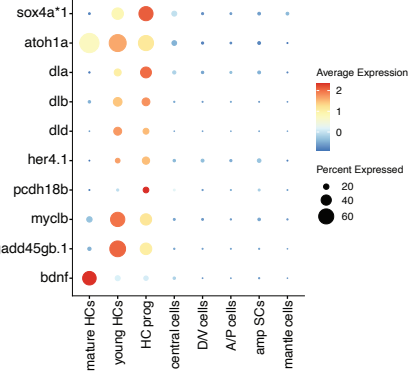
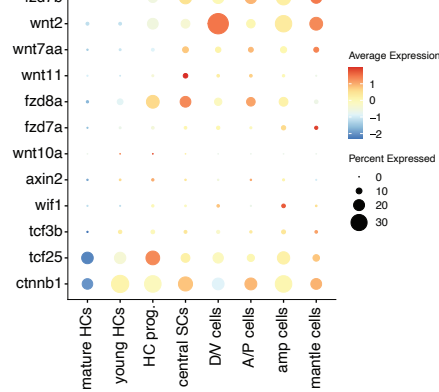
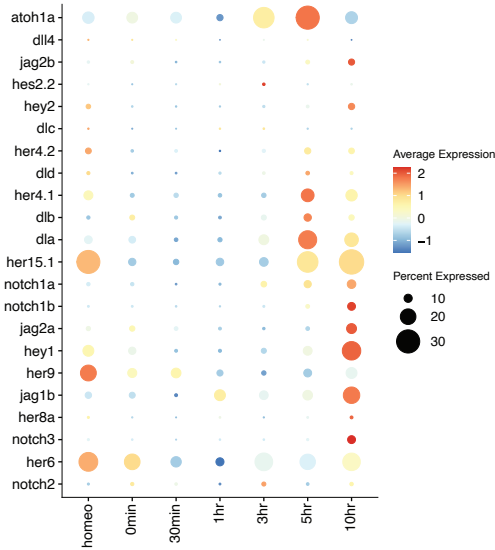
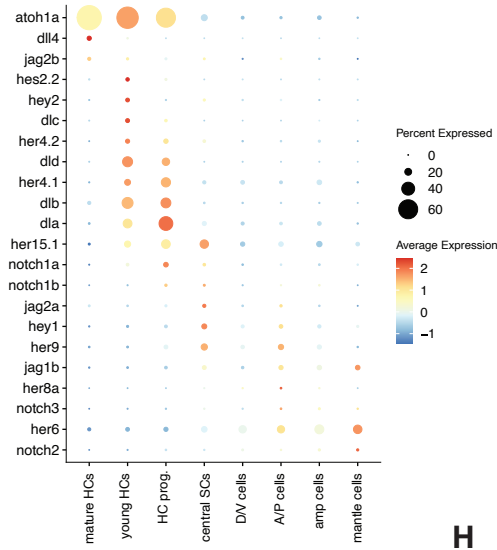
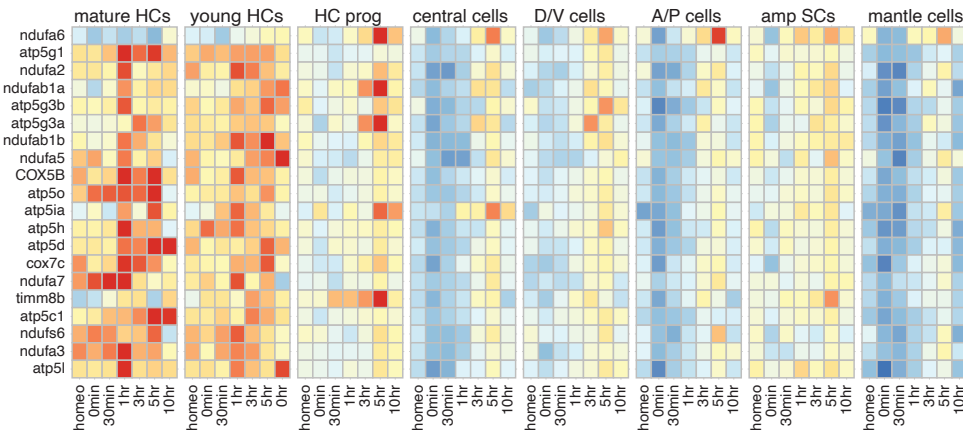
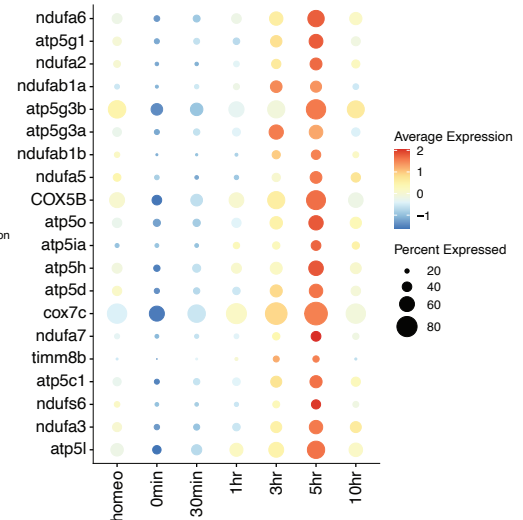
Figure S6**A GO Ribosome biogenesis (3hr vs ALL)****B KEGG Spliceosome (3hr vs ALL)****C****D****E****F****G Hallmark Oxidative phosphorylation (5hr vs ALL)****H**

Figure S6. Expression of ribosome, spliceosome, hair cell lineage, Wnt-, Notch signaling and OXPHOS genes during HC regeneration. Related to Figure 5.

(A) Heatmap showing the average expression pattern of ribosome biogenesis-related genes in eight different cell types during hair cell regeneration.

(B) Heatmap of the average expression of spliceosome-term genes (KEGG) in eight different cell types during hair cell regeneration.

(C) Dot plot visualizing the average expression of hair cell lineage genes in eight different cell types (same genes as in Figure 5I).

(D) Dot plot displaying the average expression pattern of Wnt signaling genes in eight different cell types (same genes as in Figure 5K).

(E and F) Dot plots of the average expression pattern of a more comprehensive list of Notch signaling genes during (E) hair cell regeneration and (F) their enrichment in eight different cell types.

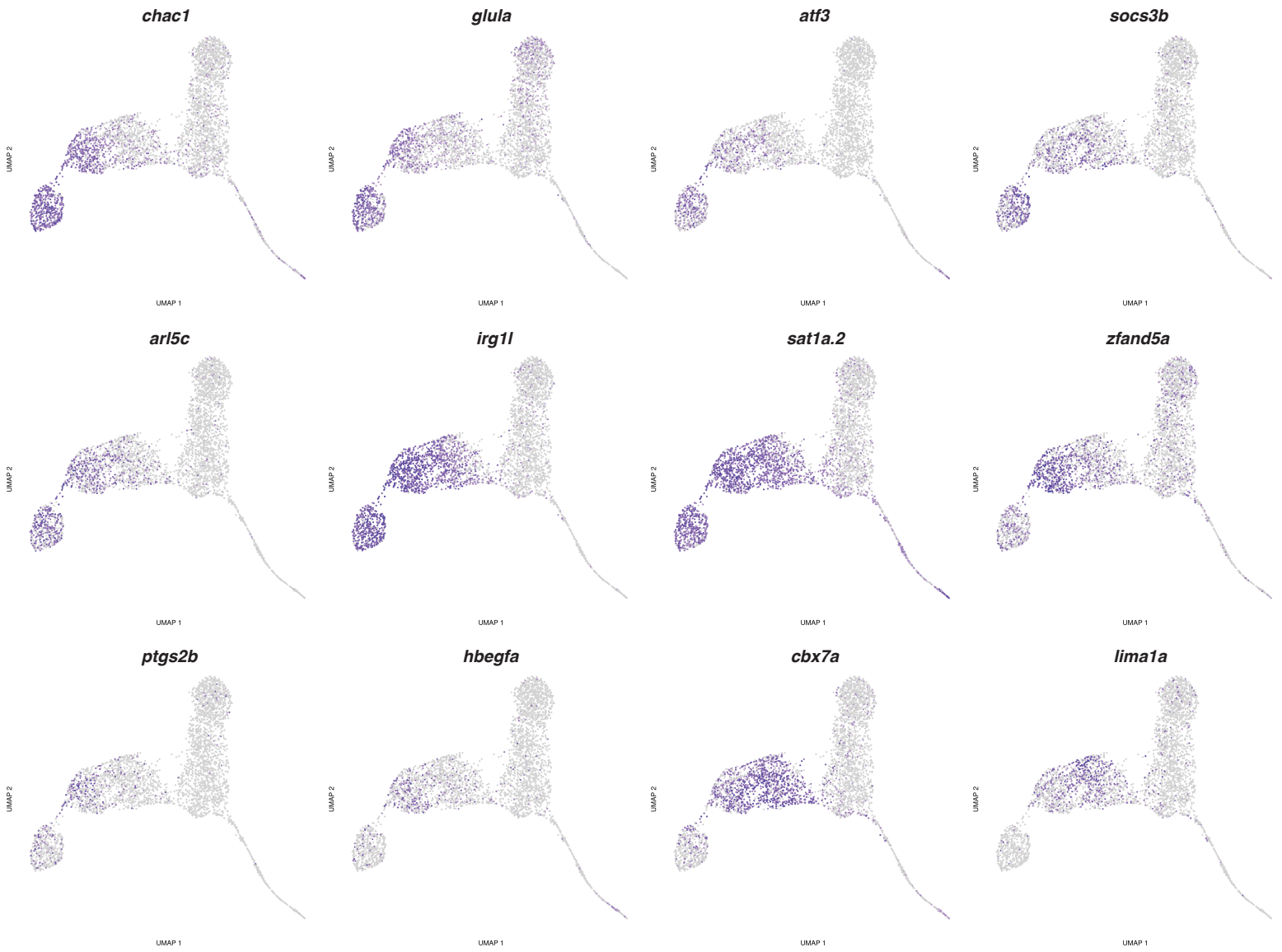
(G and H) (G) heatmap and (H) dot plot showing the cell type enriched expression of oxidative phosphorylation-related genes (G) (Hallmark genes) and their upregulation between 3h- 5h after hair cell death (H).

Figure S7. Related to Figure 5.

Heatmap visualizing the average expression of ribosome biogenesis, spliceosome, HC lineage, cell cycle, Wnt signaling, and Notch signaling genes shown in the dot plots in Figure 5.

Figure S8

A



B

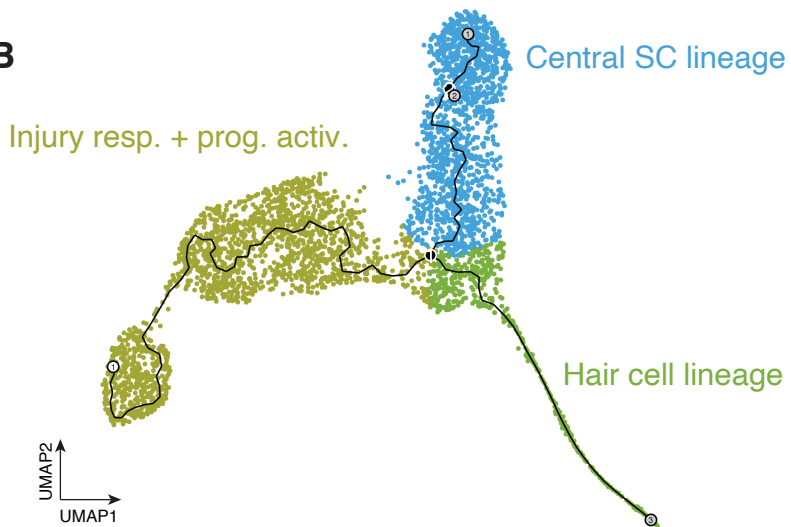


Figure S8. Related to Figure 6.

(A) Gene expression feature plots of representative genes that are enriched in injury response+ progenitor activation clusters (See also in Figure 6H-J).

(B) UMAP showing cell compositions within the Injury response + progenitor activation lineage, central support cell lineage, and hair cell lineage

Figure S9 Central support cell lineage

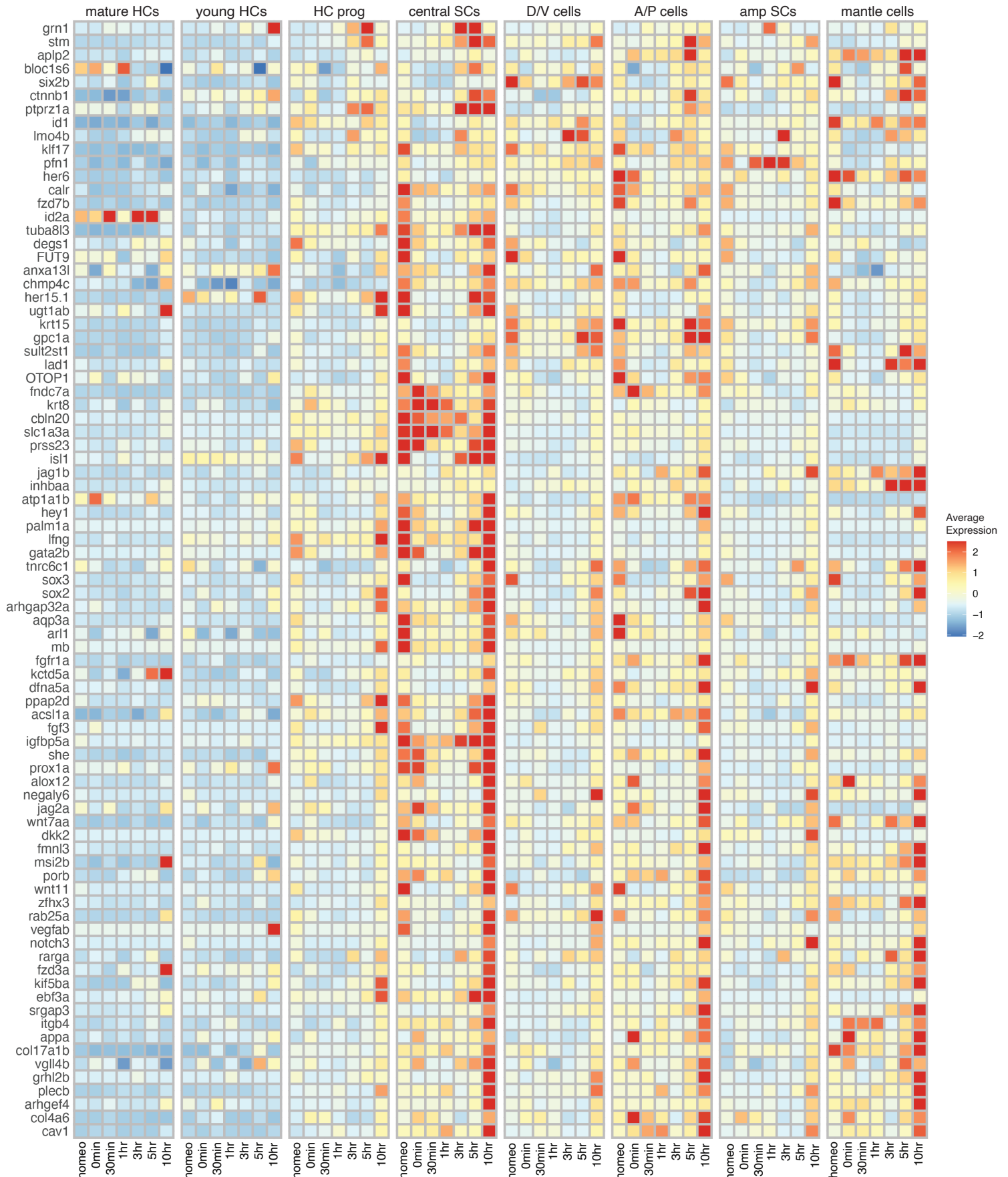


Figure S9. Central support cell lineage genes. Related to Figure 7.

Heatmap displaying the average expression of the central support cell lineage genes in clusters 9, 10, and 4 (see also in Figure 7A) in eight different cell types during hair cell regeneration.

Figure S10 Cell Cycle pseudotime heatmap

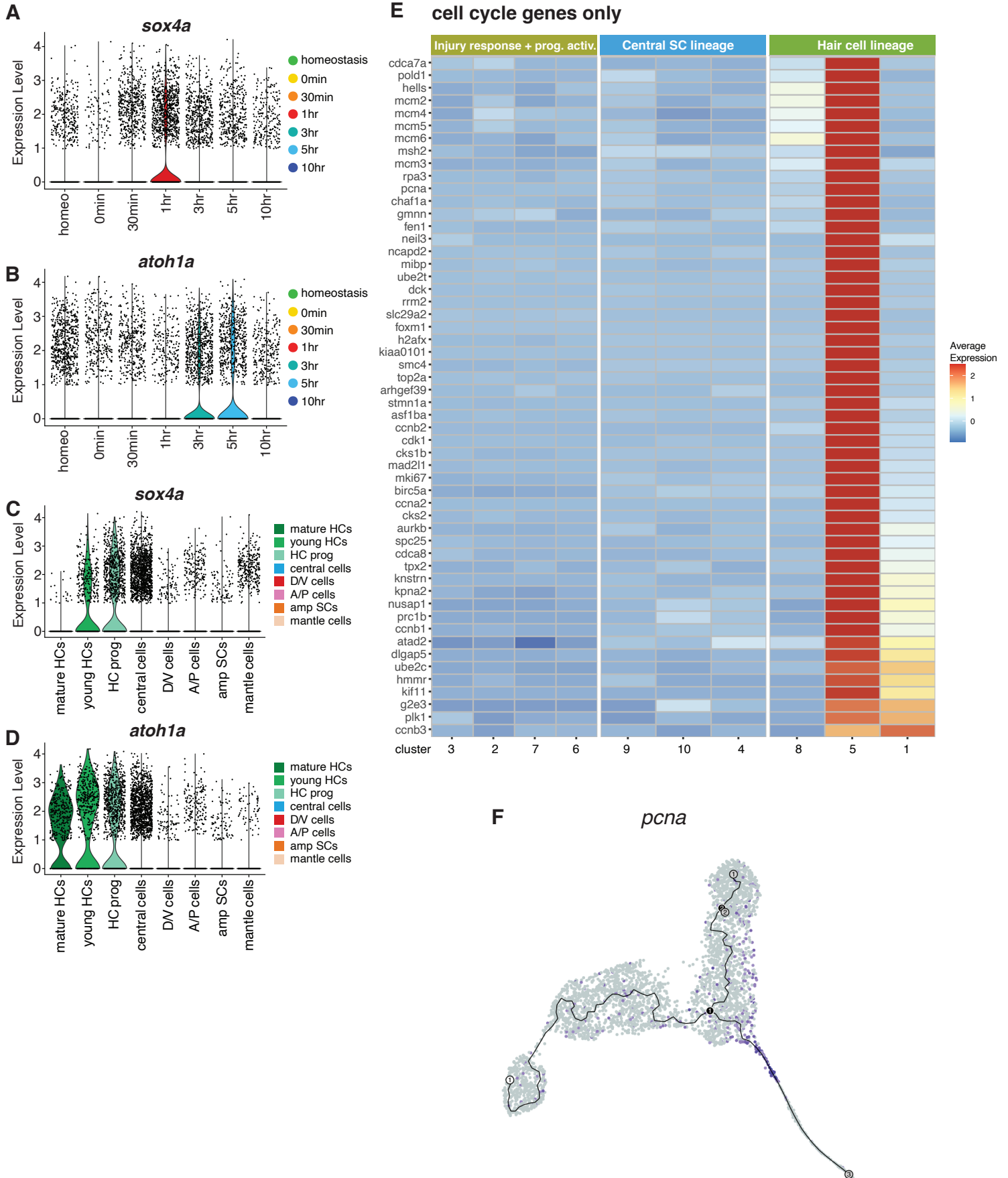


Figure S10. Related to Figure 8.

(A-D) Violin plots of *sox4a* (A and C) and *atoh1a* (B and D) across seven different time points of regeneration and eight different neuromast cell types.

(E) Heatmap showing the expression pattern of cell cycle-related genes in the hair cell lineage.

(F) Feature plot of *pcna*.

Figure S11.

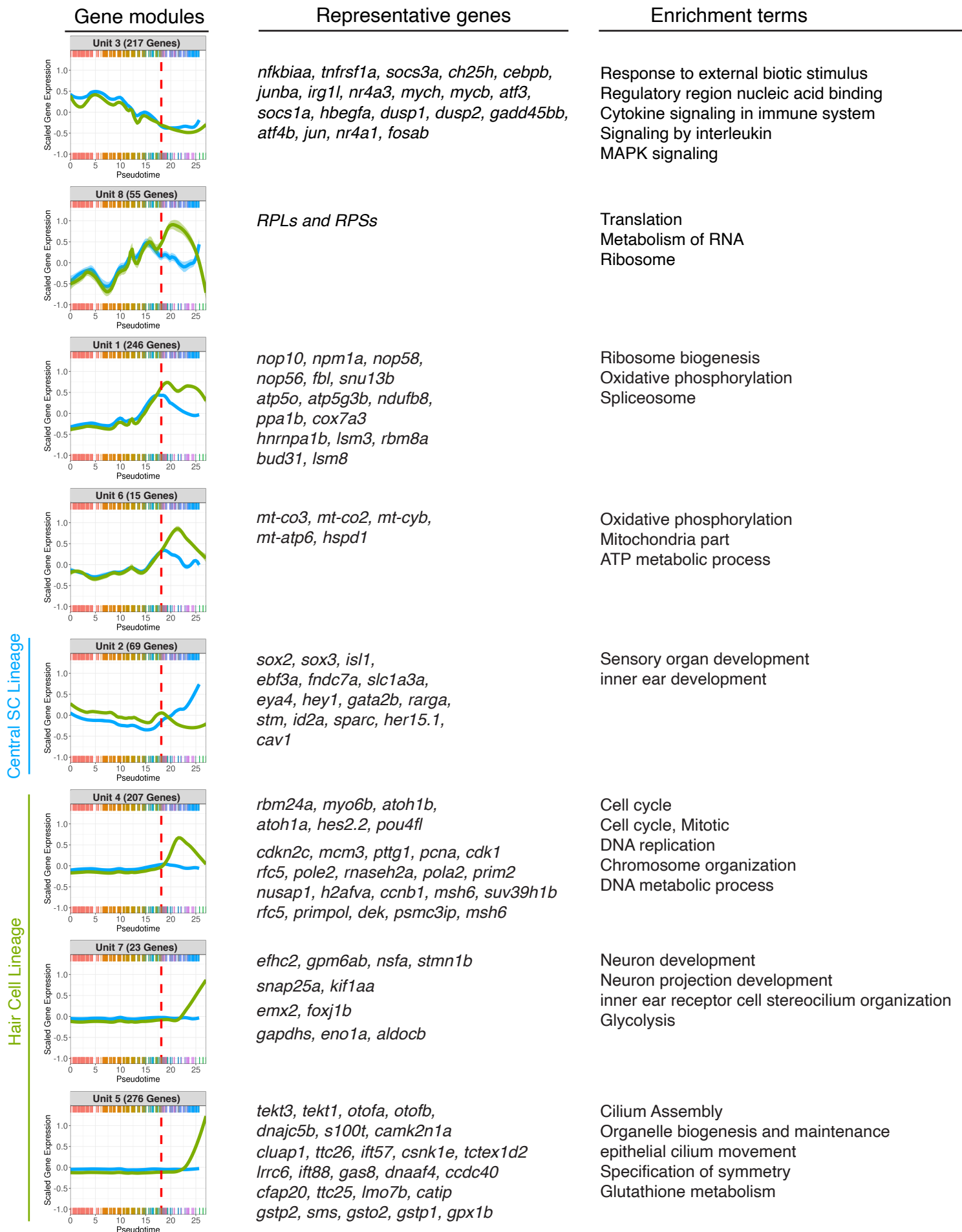


Figure S11. Gene module (unit) analysis based on the pseudotime analysis. Related to Figure 9.

Left column: line plots showing the scaled gene expression profiles of genes in each unit ordered along pseudotime (x-axis). Cells belonging to the central support cell lineage are shown as ticks on the top x-axis of the graph, whereas cells of the hair cell lineages are represented by ticks along the bottom x-axis. Red stitch lines indicate the branch-point between the central support cell and hair cell lineages (Figure 6). Middle column: representative genes in each unit. Right column: representative enrichment terms for each unit.