

Shh agonist enhances maturation in homotypic Lgr5-positive inner ear organoids

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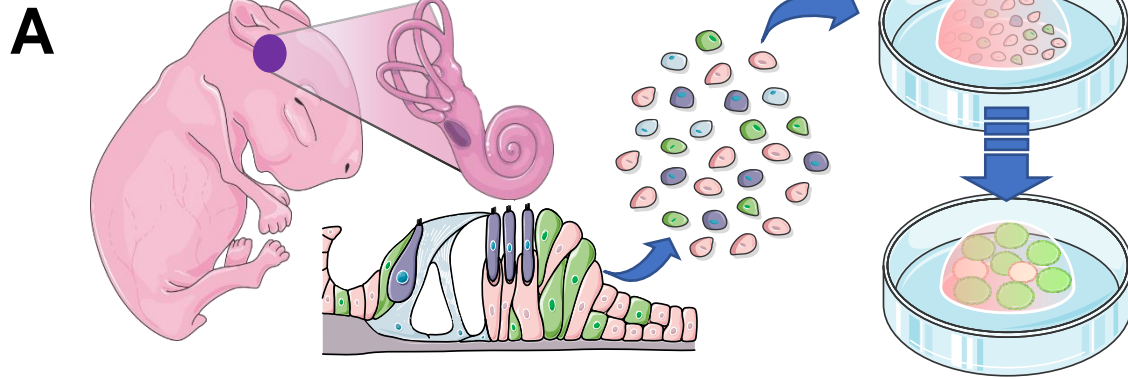
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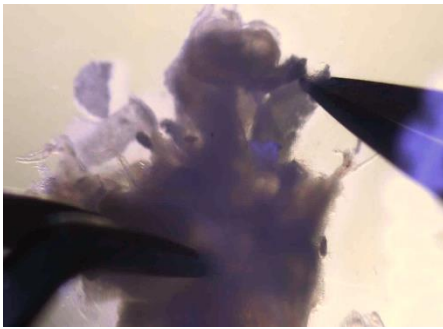
E-mail: eyeglass210@gmail.com

Supplemental Figure 1. Manual isolation technique: (A) Illustration of technique; (B) Experimental images

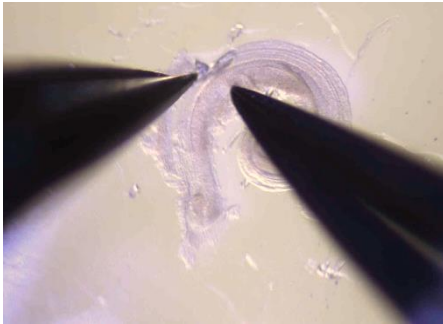


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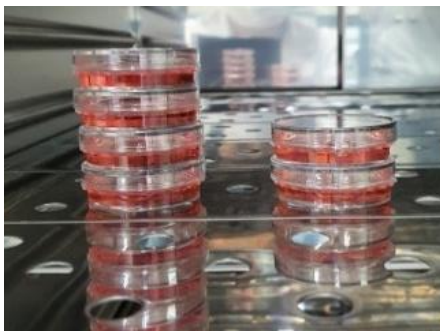
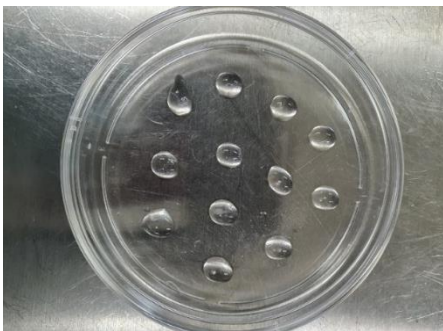
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Stripping

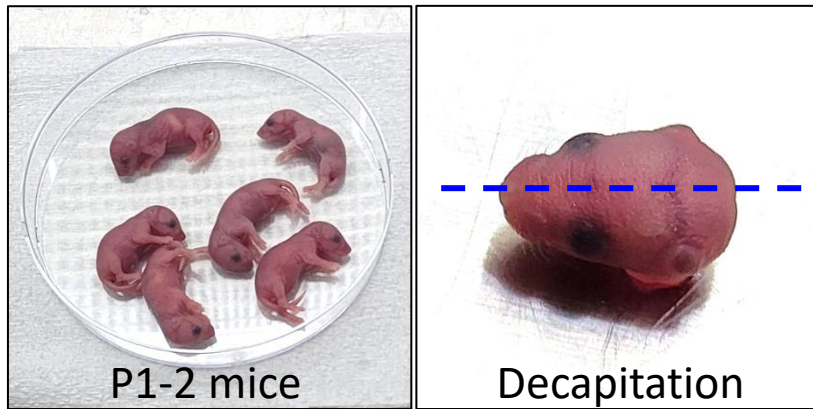


3D culture

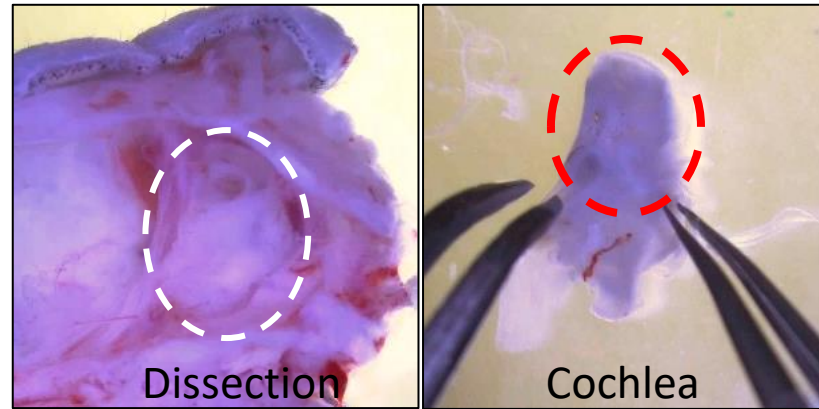


Supplemental Figure 2. MACS isolation technique (A to C: serial images)

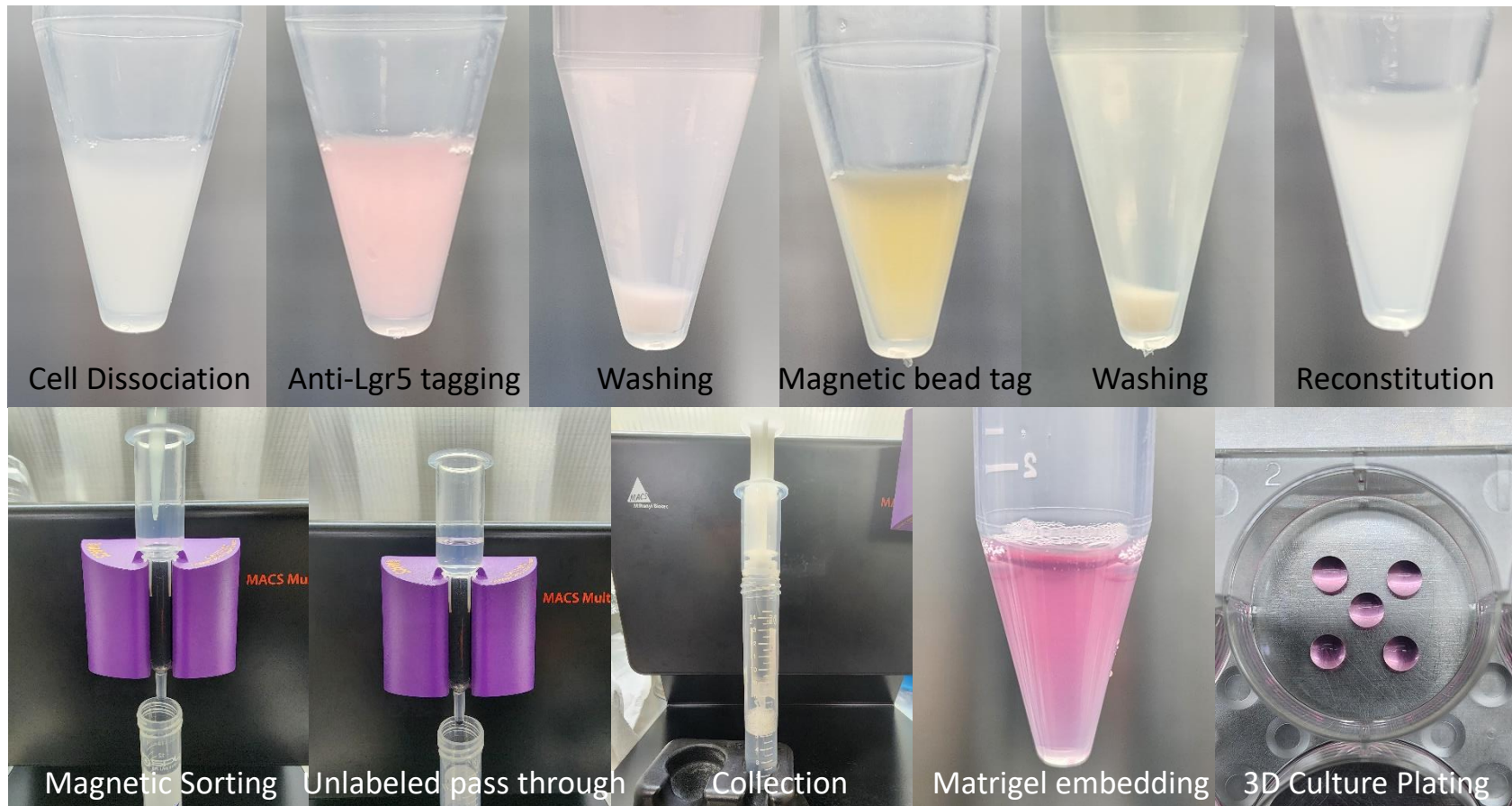
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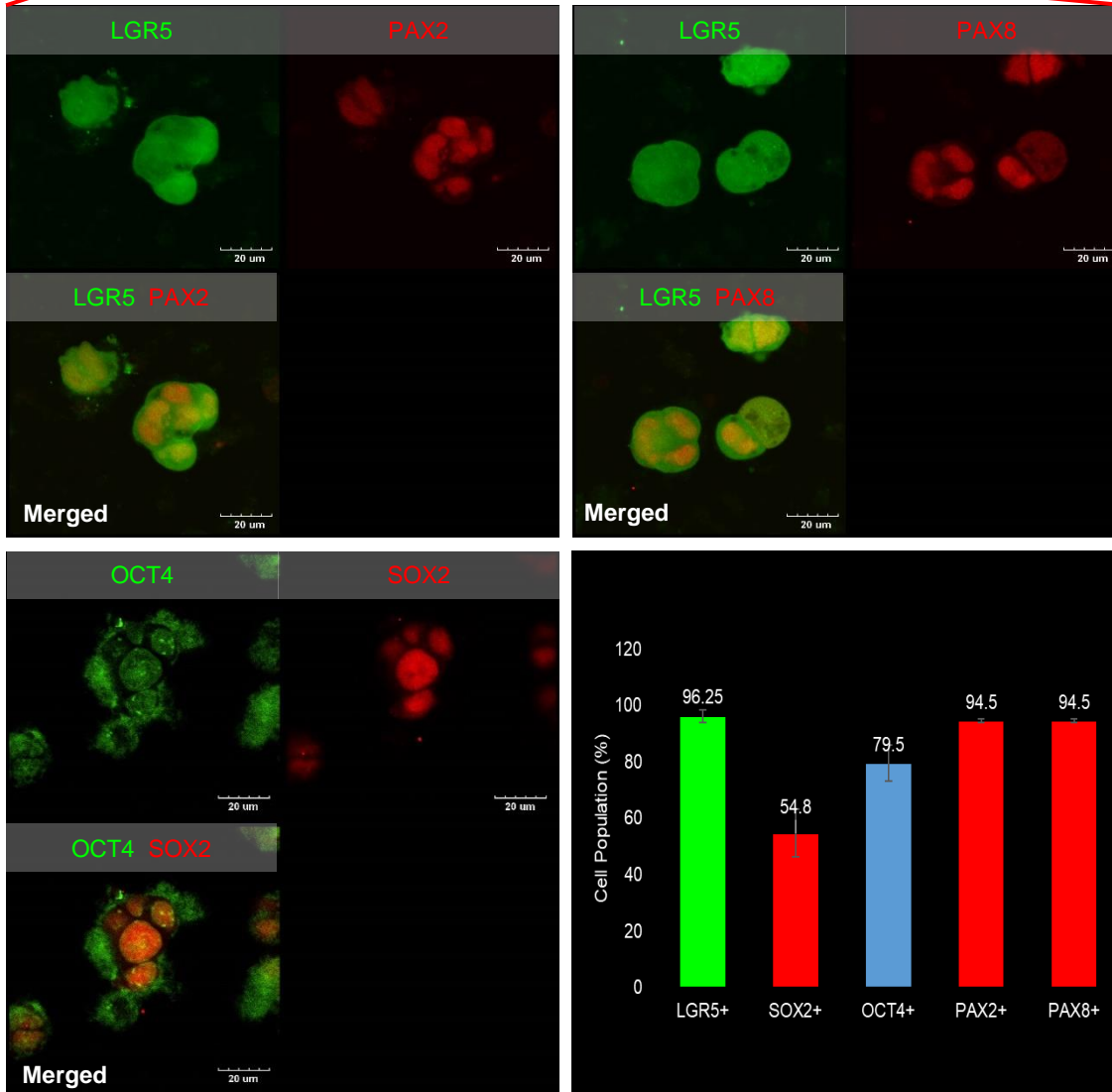
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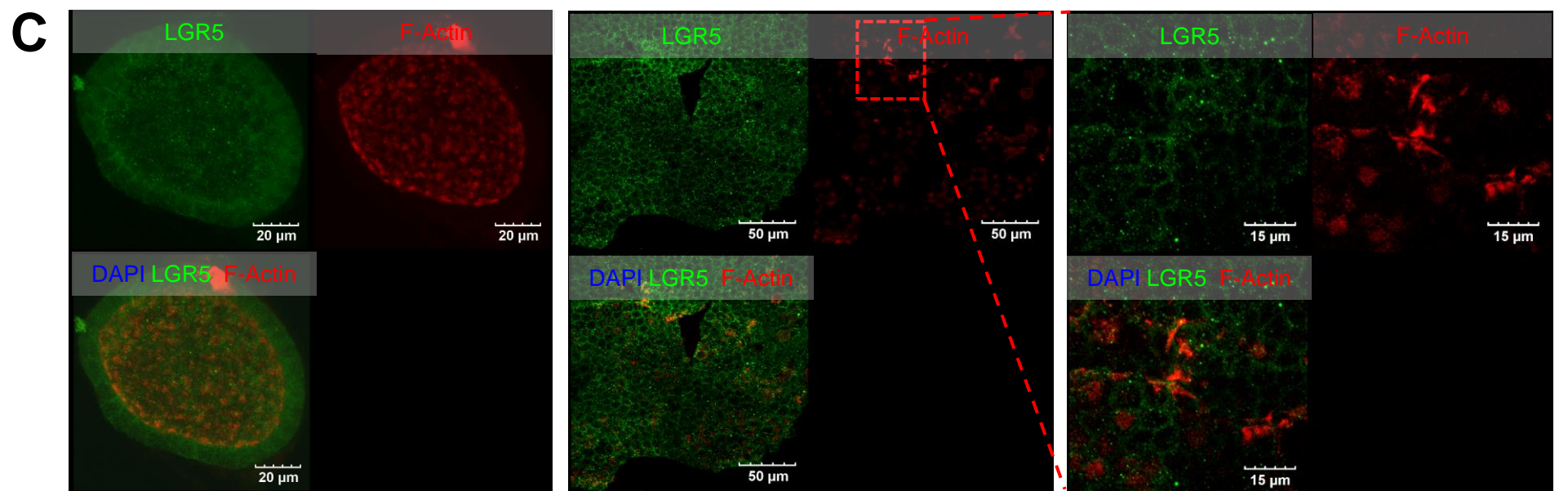
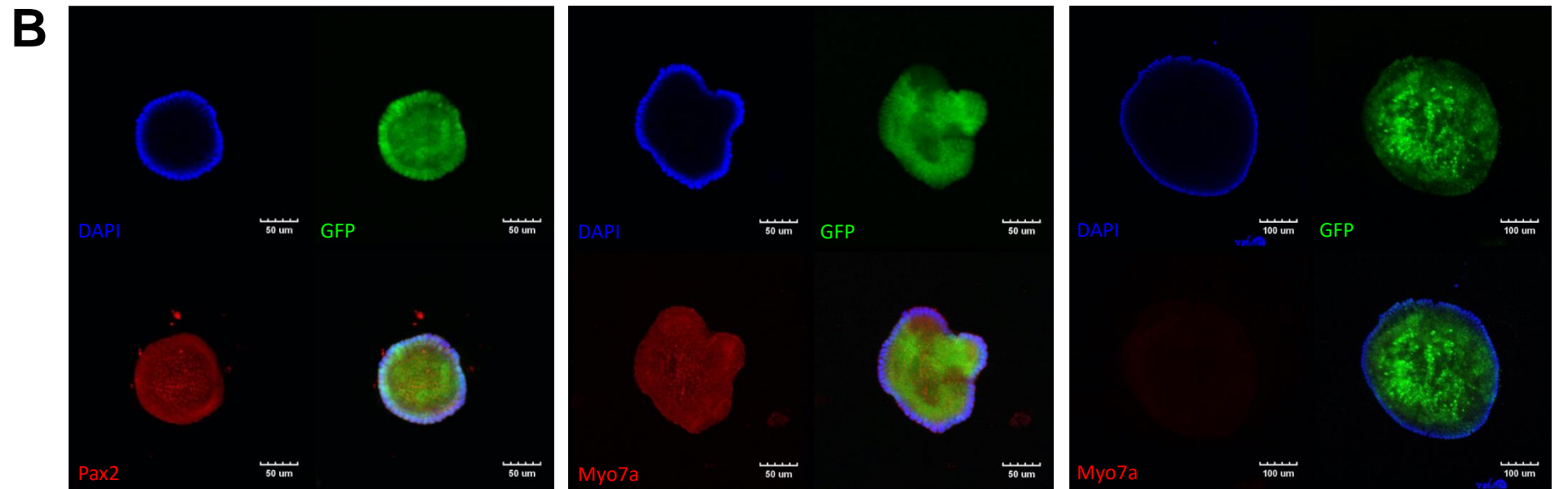
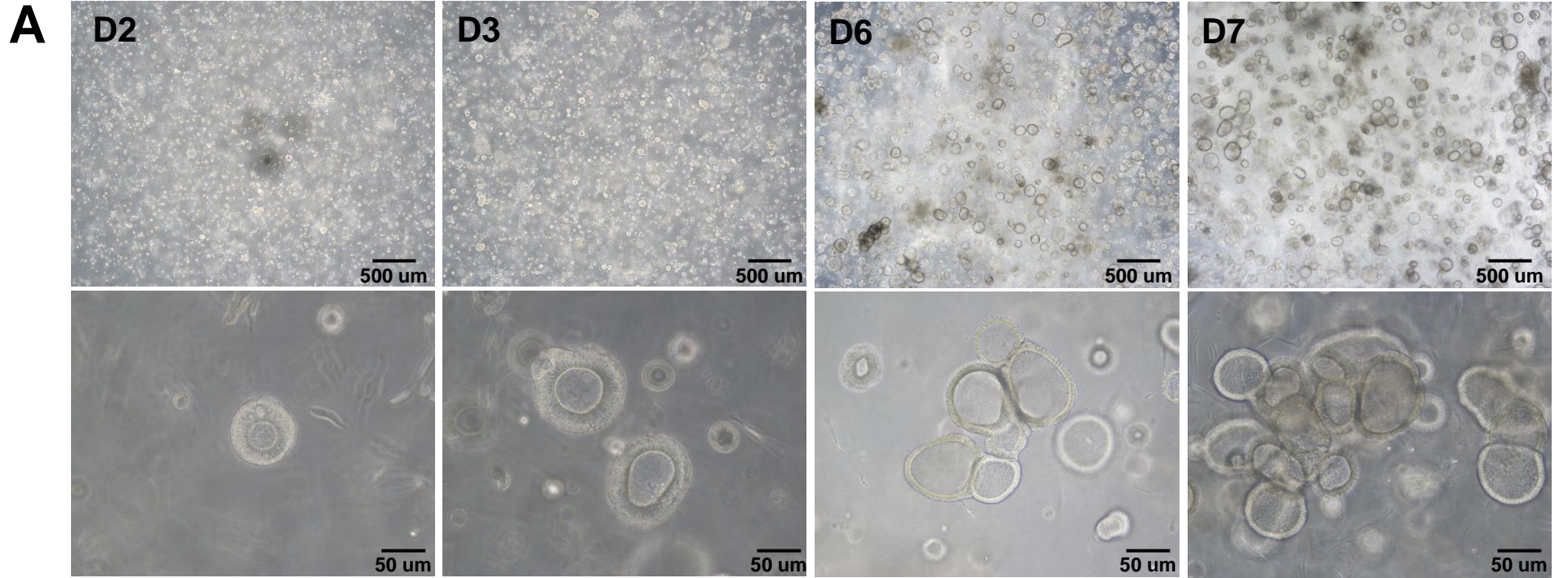
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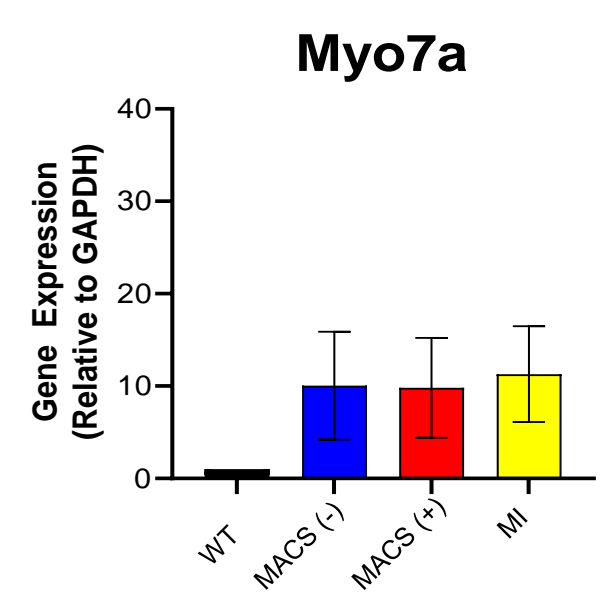
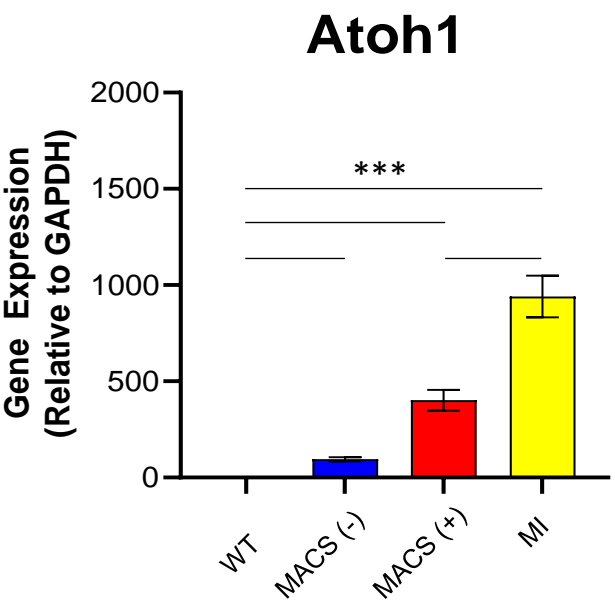
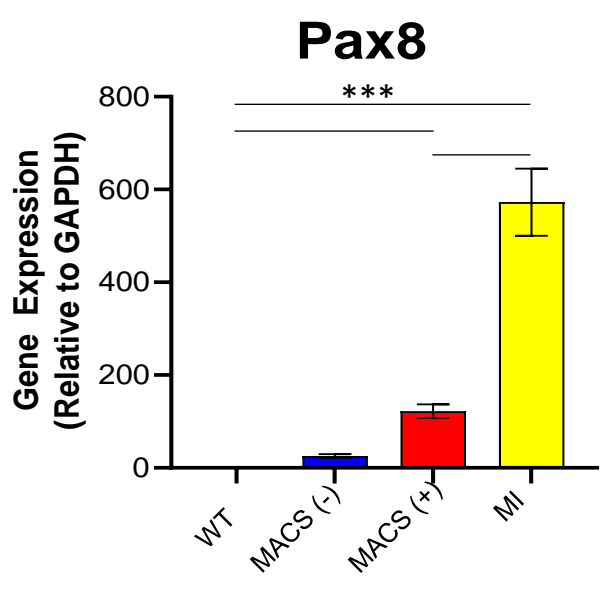
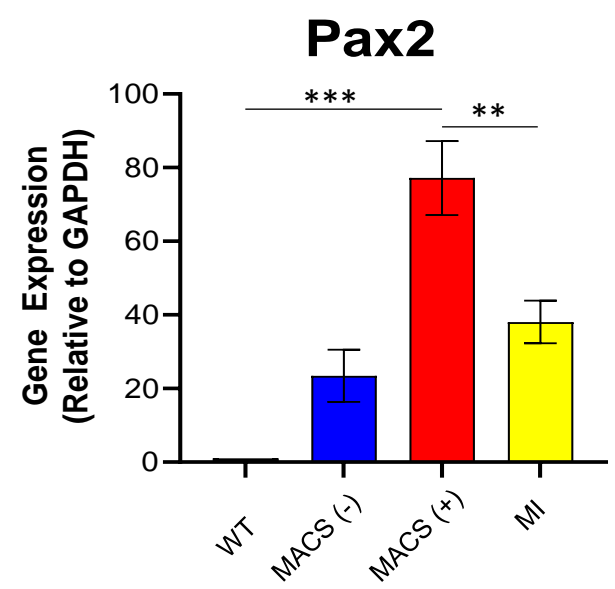
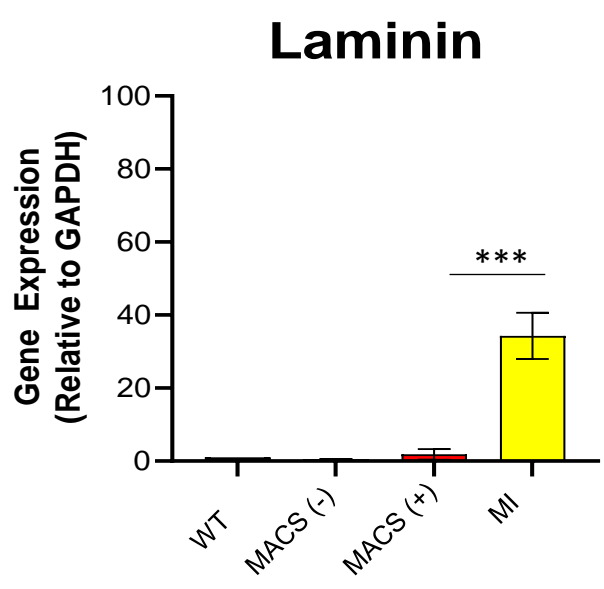
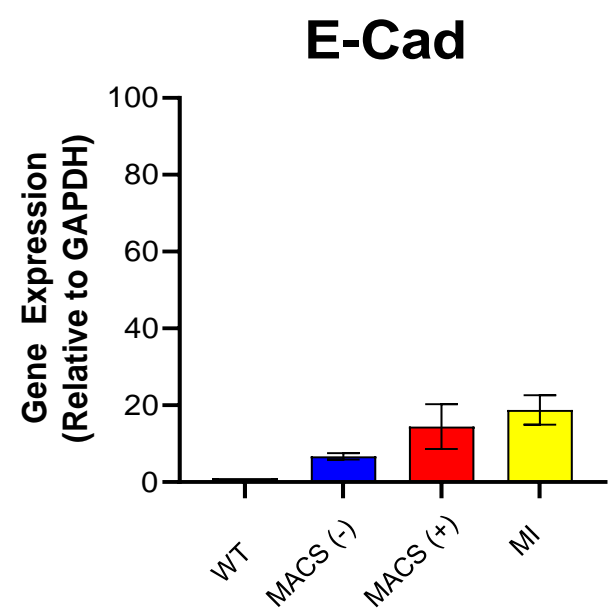
Supplemental Figure 3. Characteristics of MACS sorted LGR5-positive cells at D7



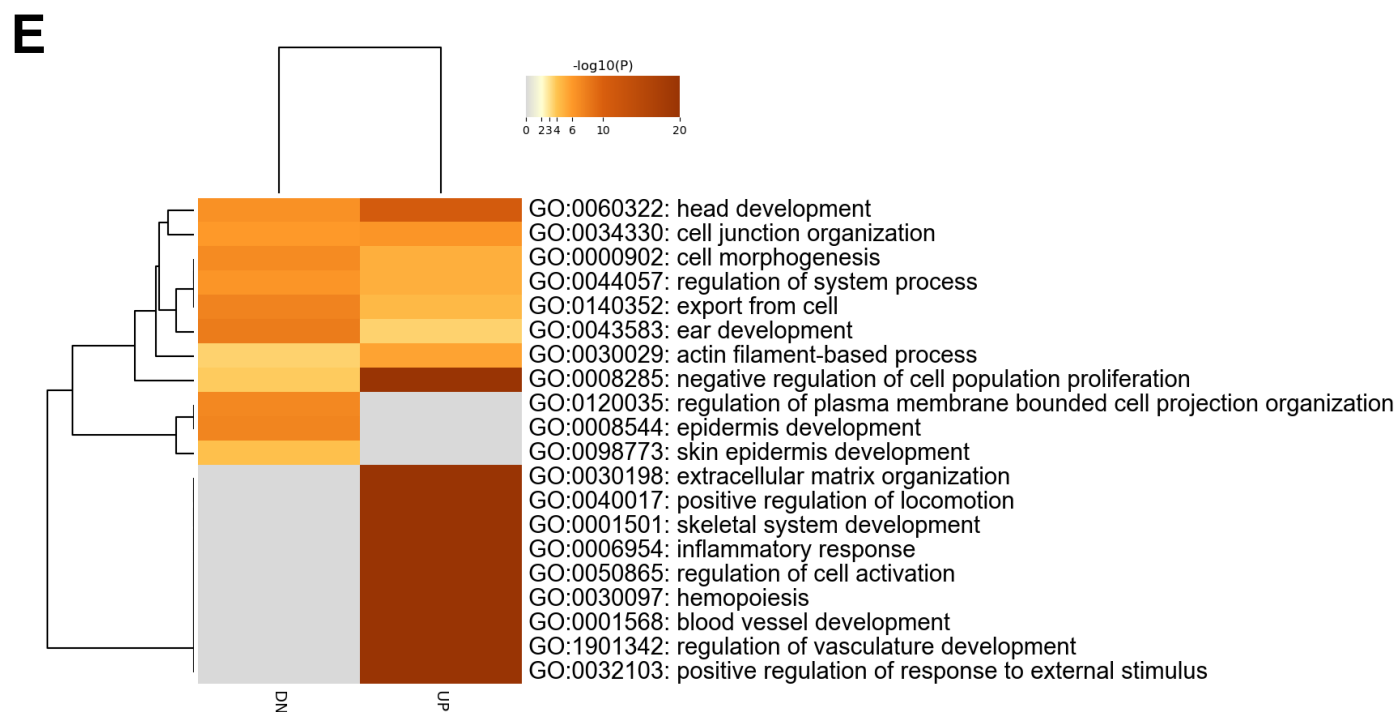
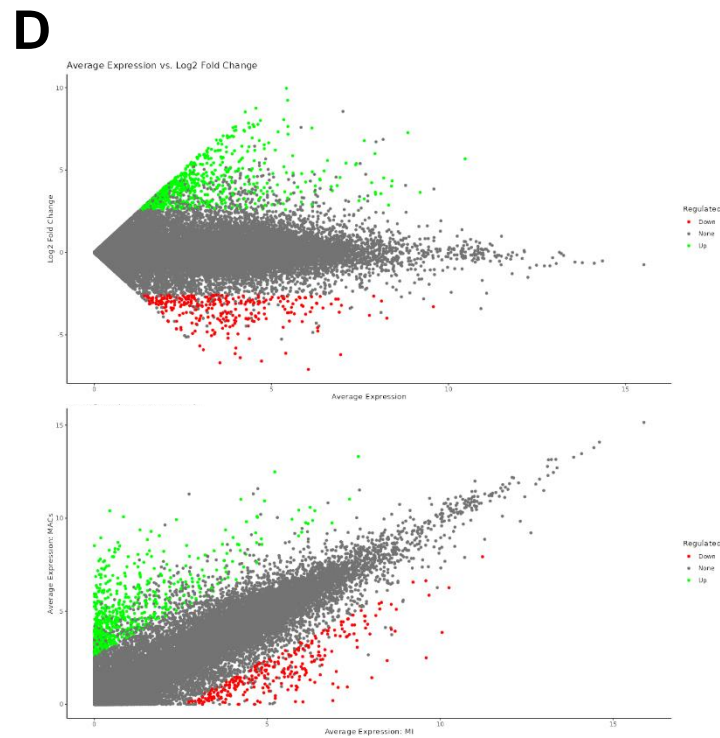
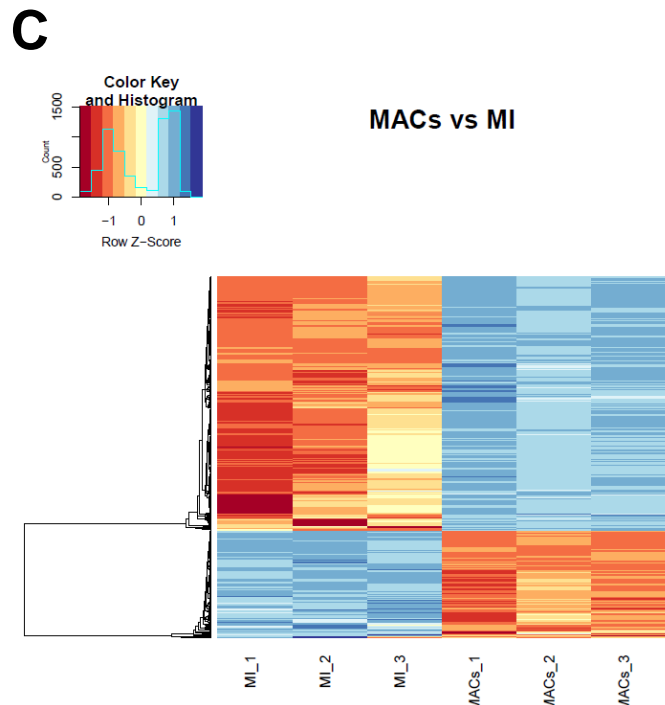
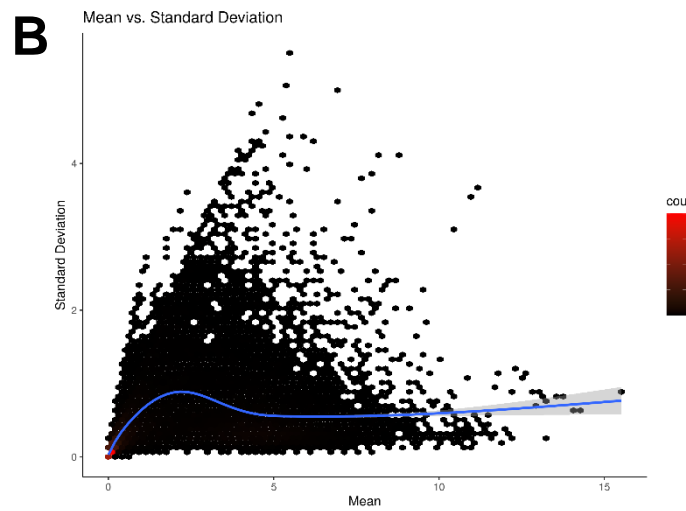
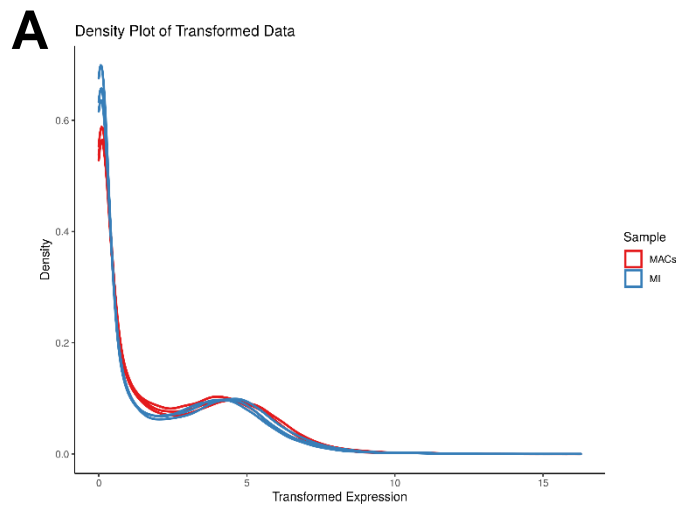
Supplemental Figure 4. Organoid generation from cells by Manual isolation technique (A) light microscopic images; (B) Epifluorescence analysis at D10 ; (C) Epifluorescence analysis at D30



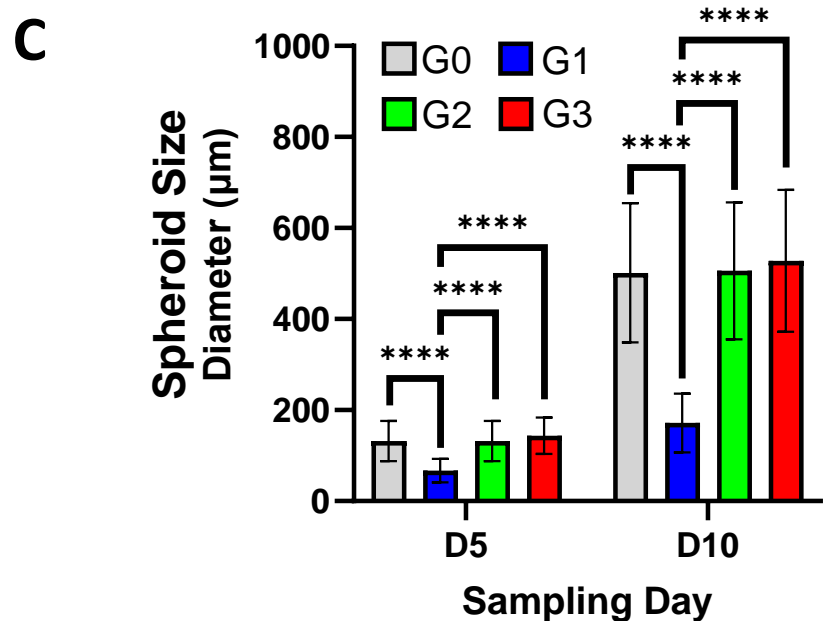
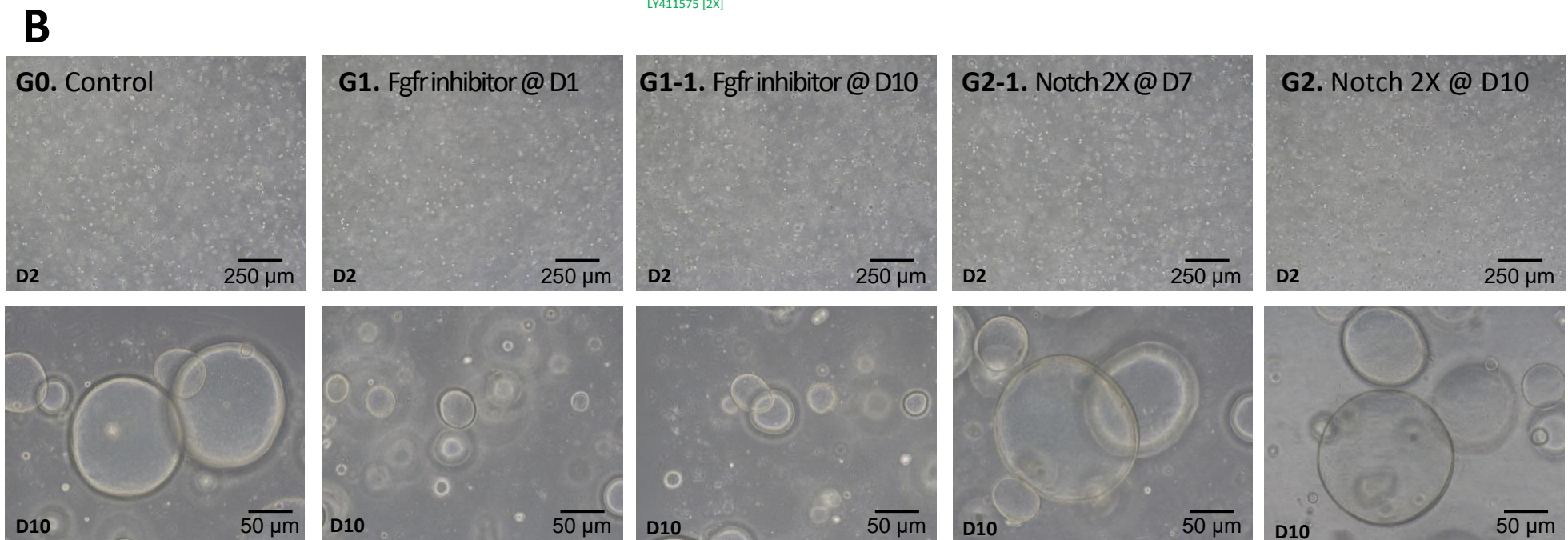
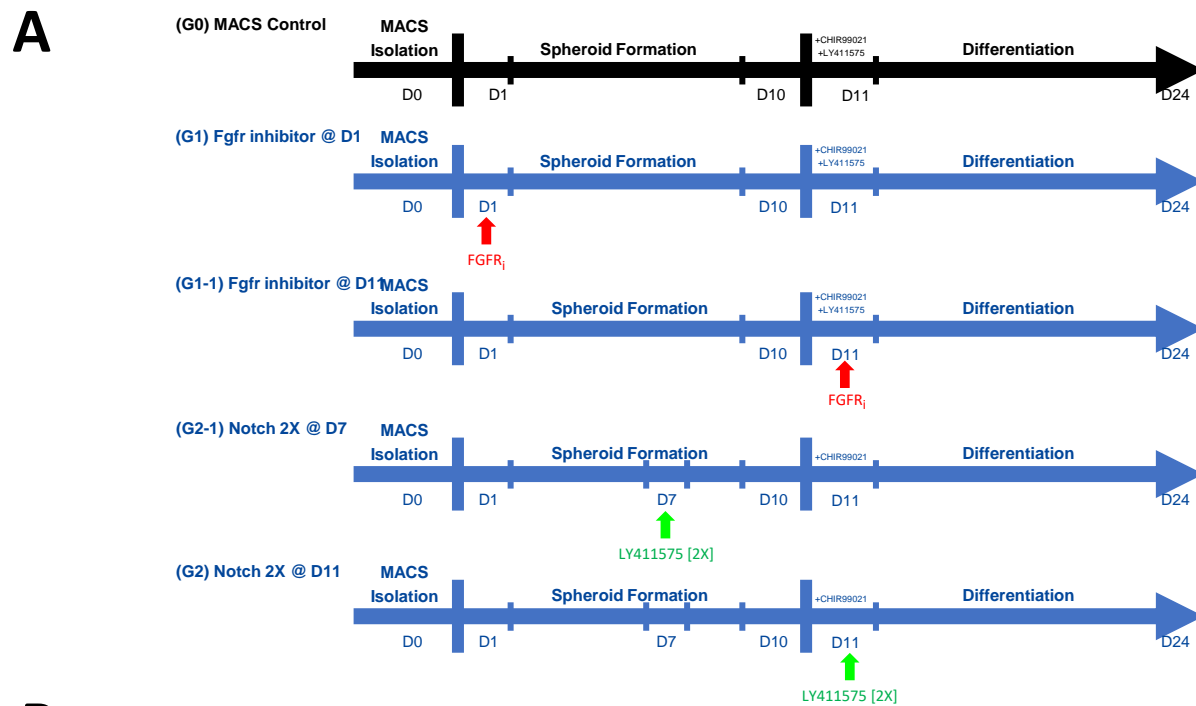
Supplemental Figure 6. Hair cell-associated gene expression between organoids from MACS and Manual isolation at DIV10



Supplemental Figure 7. RNA sequencing comparison of MI and MACS group at D10. (A,B) Statistical validation of RNA sequencing. (C,D) Gene distribution pattern difference of two group. (E) Gene Ontology regarding the differential gene expression between two group (DN: higher expression in MI group).



Supplemental Figure 8. Modifications on the HC differentiation protocol of MACS Lgr5-positive cells (A) Experimental timeline of 5 groups, (B) Representative light microscopic images of 5 groups at D2 and D10, (C) Spheroid sizes at D5 and D10.

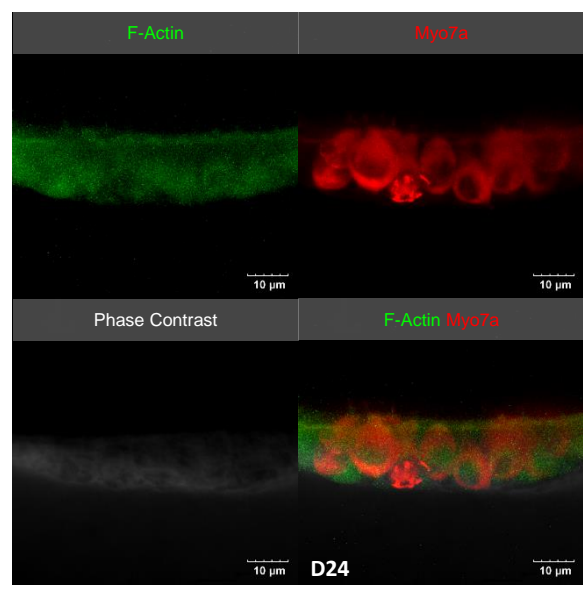
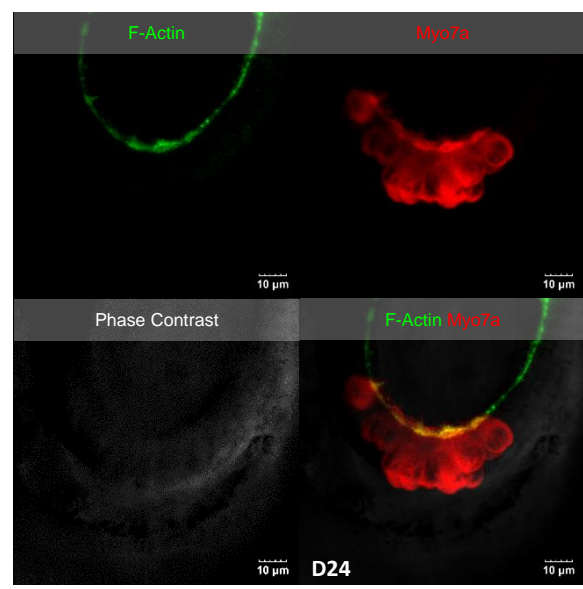
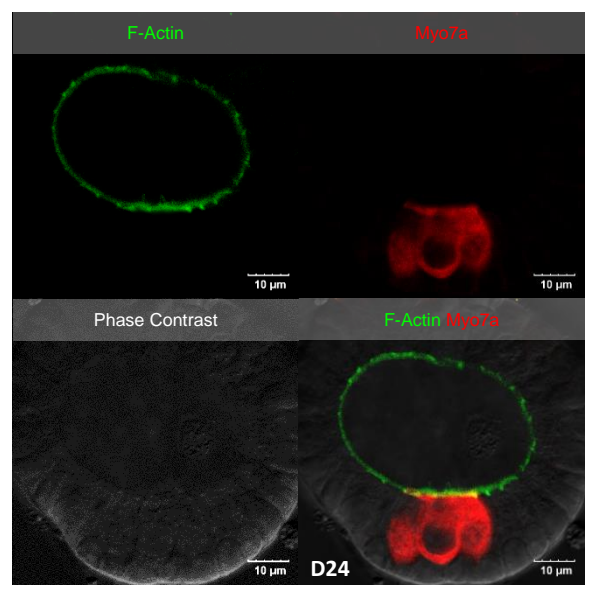


Supplemental Figure 9. HC morphology or organoids from different MACS experimental groups at D24

G1. (-)Fgf2 (+)Fgfr_i @ D1

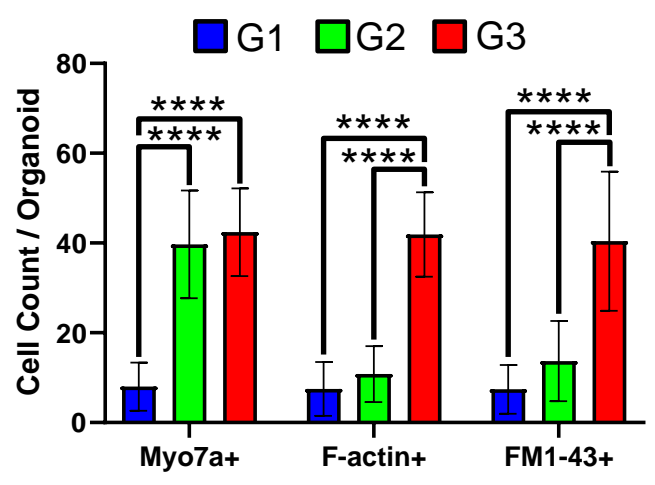
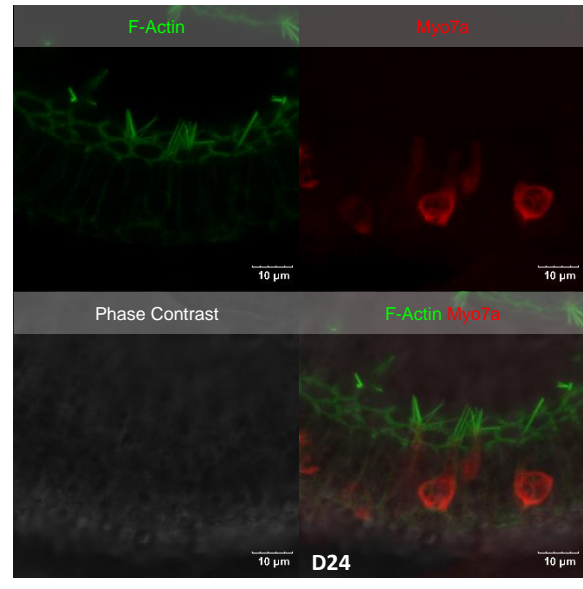
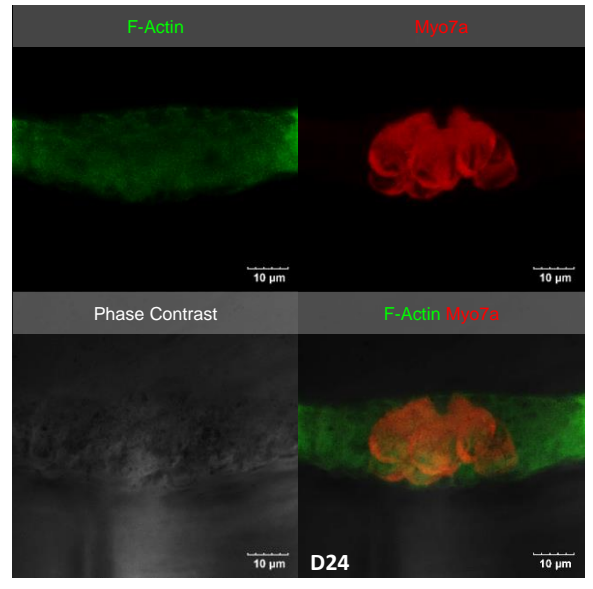
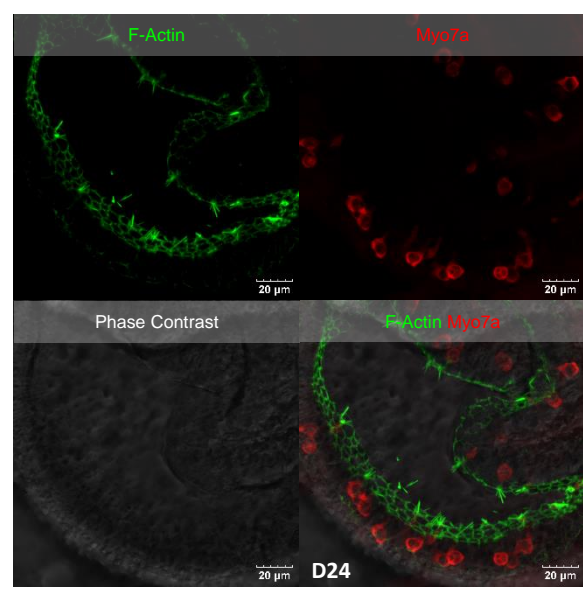
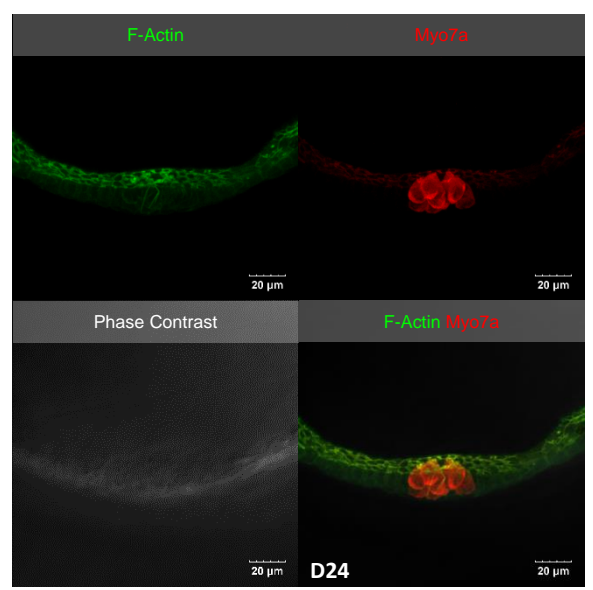
G1-1. (-)Fgf2 @ D1

G2-1. (+) Notch inhibitor 2X @ D7



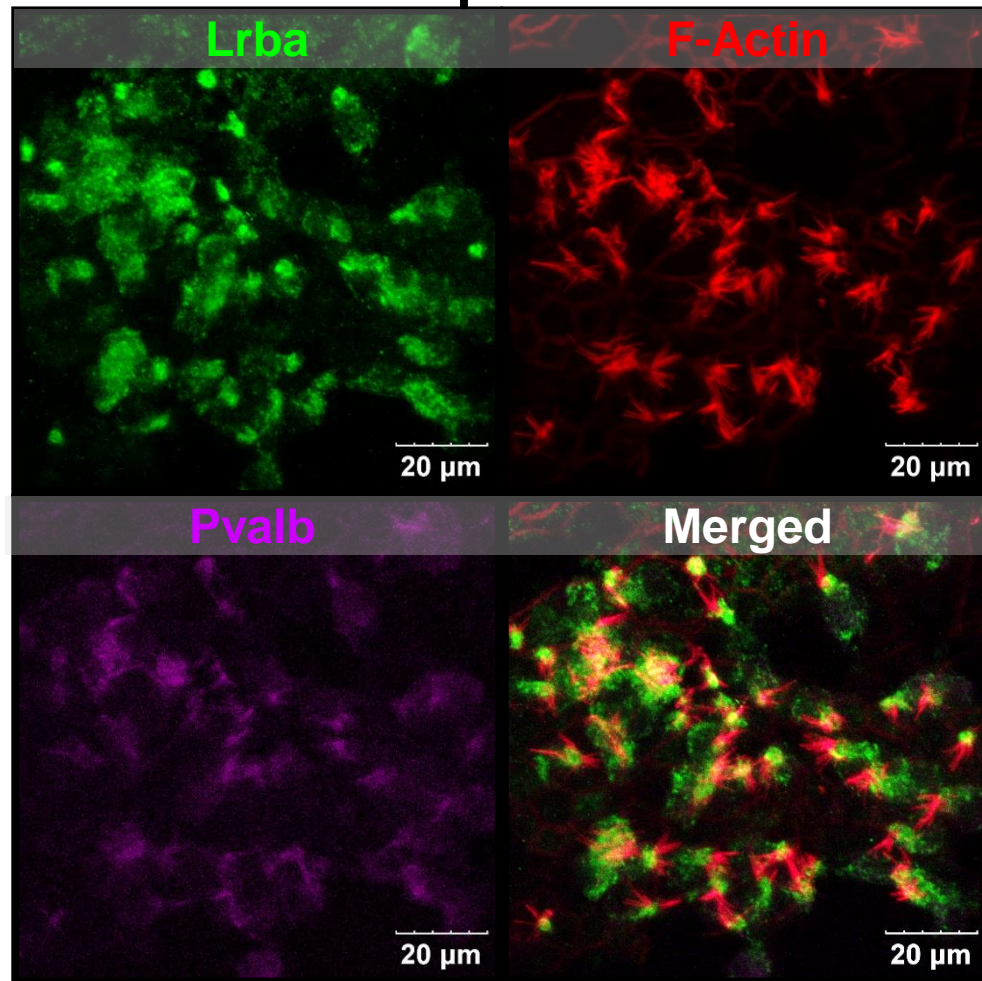
G2. (+) Notch inhibitor 2X @ D11

G3. SHH_a + Wnt_i

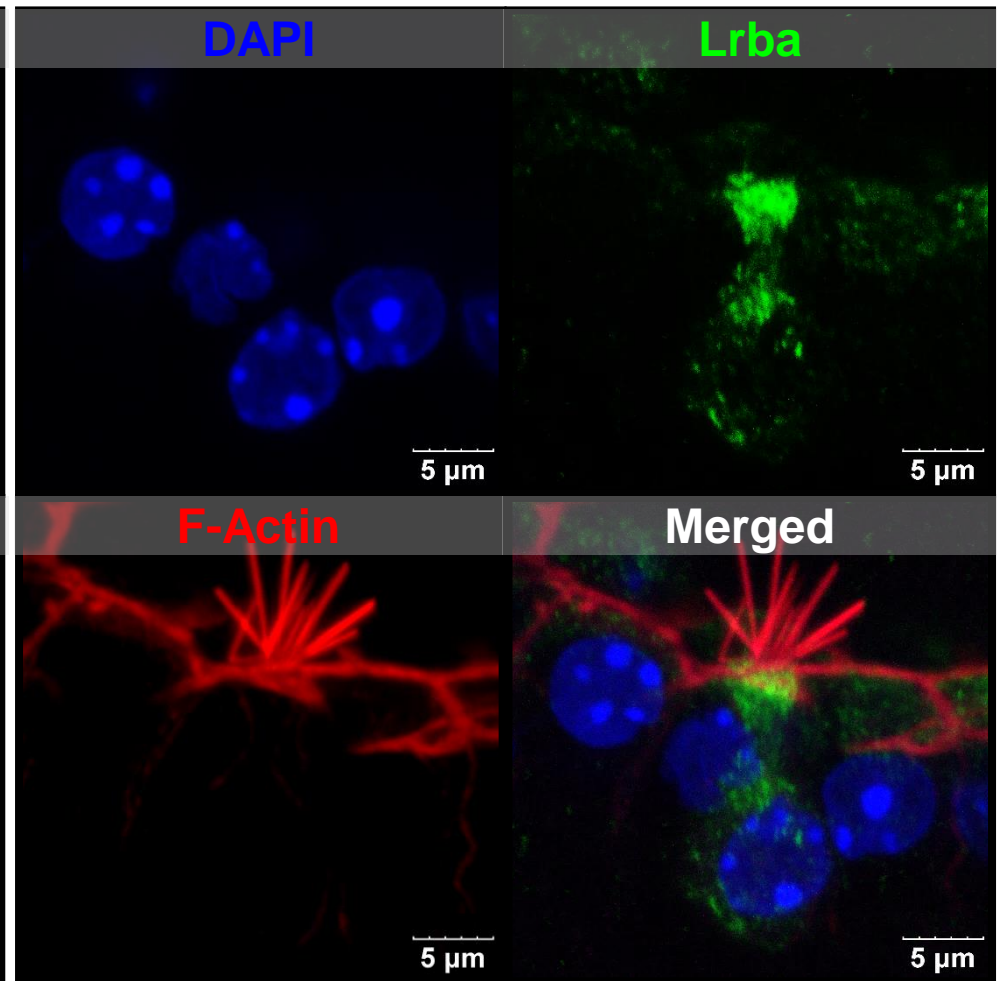


Supplemental Figure 10. Epifluorescence analysis of Lrba expression in the subapical region of hair cells

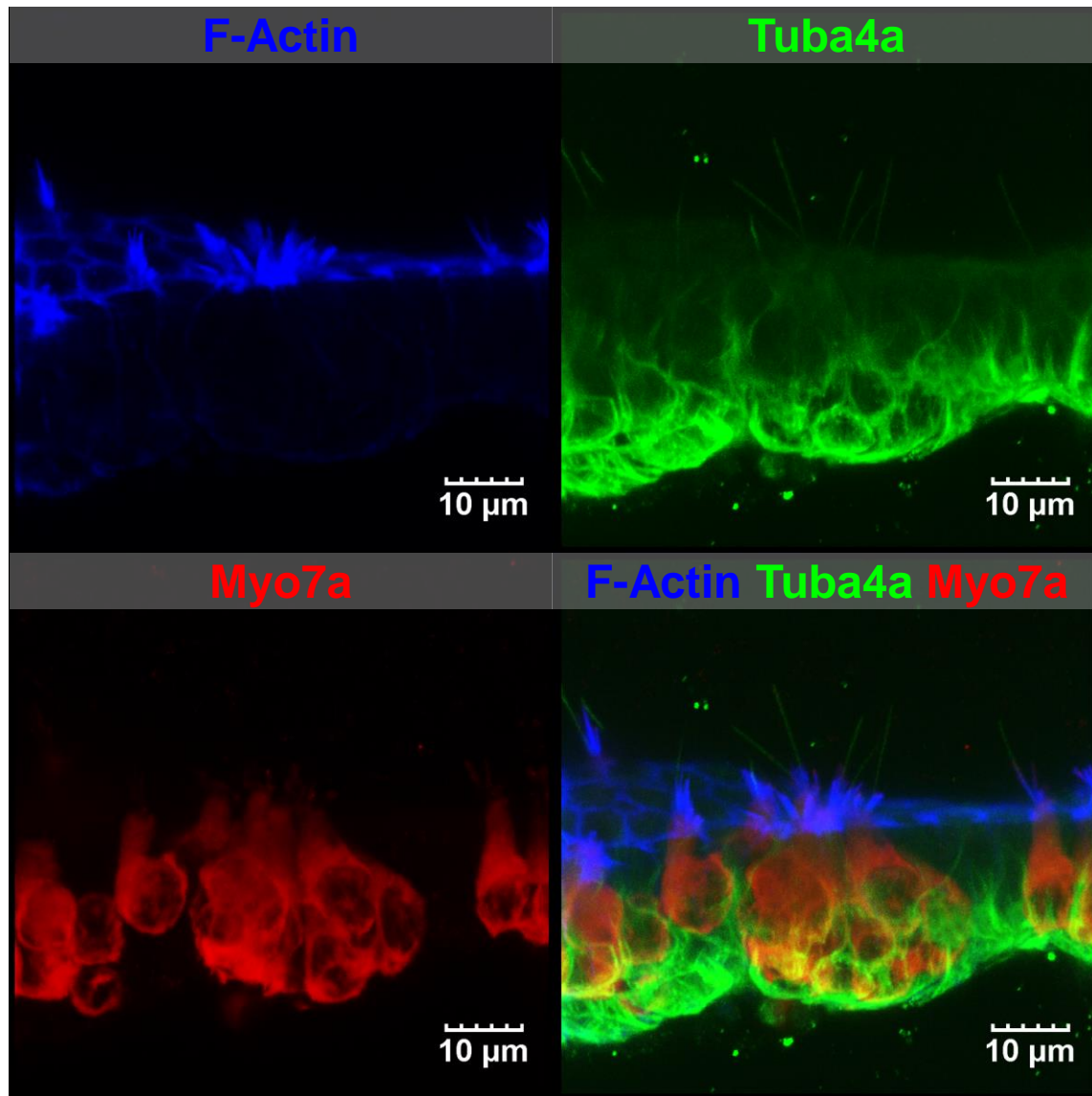
Top View



Side View

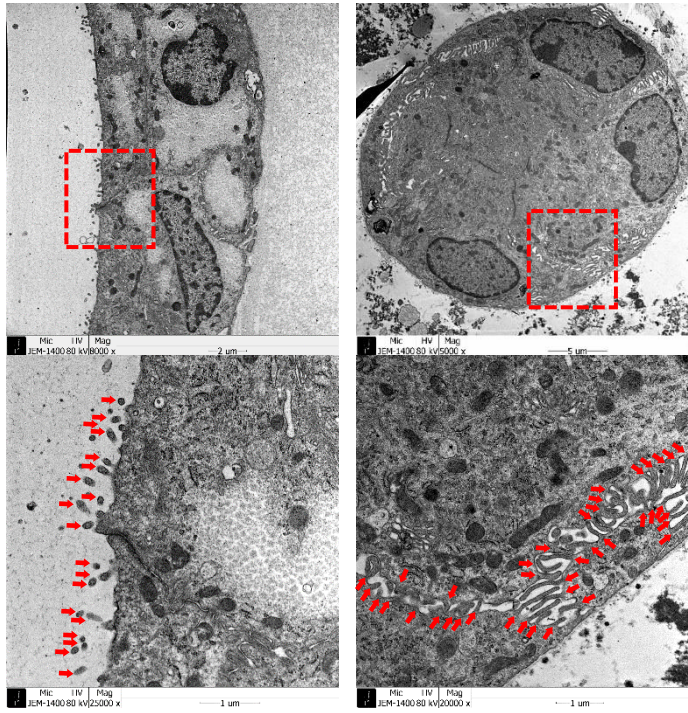


Supplemental Figure 11. Epifluorescence analysis of kinocilium marker, acetylated alpha tubulin (TUBA4A)

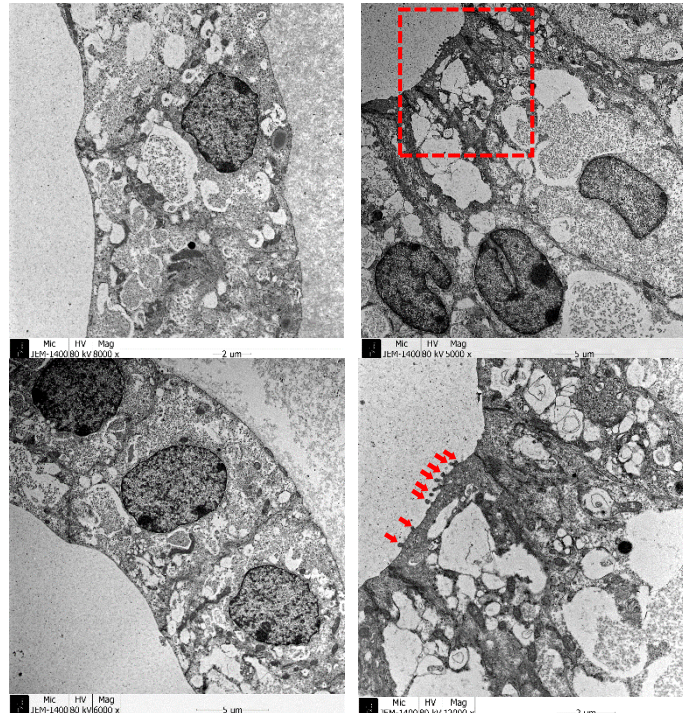


Supplemental Figure 12. TEM imaging of the stereocilia of differentiated HC. Ultrastructural imaging of hair cell stereocilia from LPC-derived IEO that differentiated using modified treatments. A. MACS Control, B. Fgfr inhibited at D1, C. Fgfr inhibited at D10, D. Notch Inhibition 2X at D10

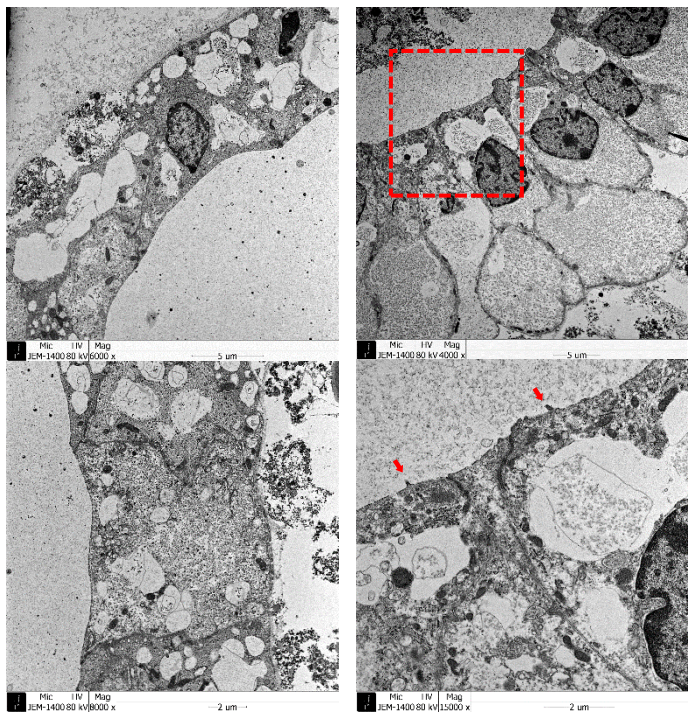
A MACS G0



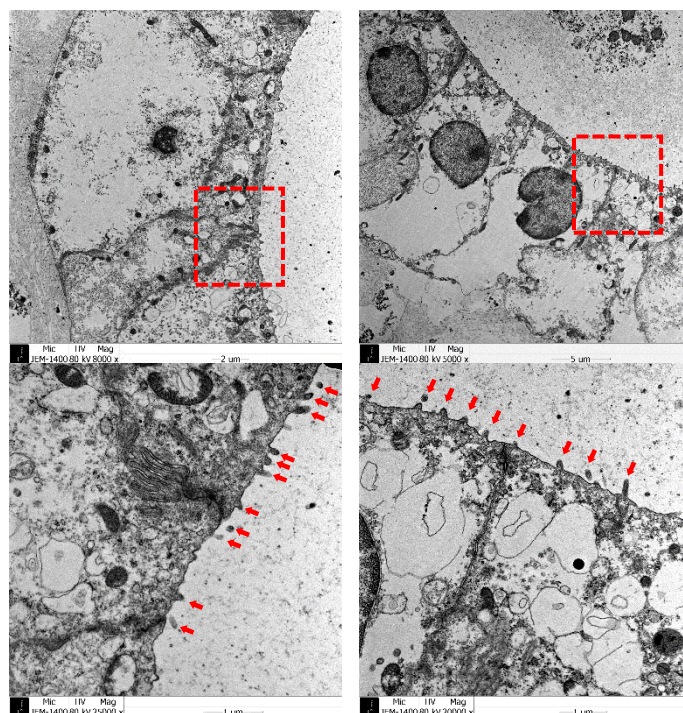
B MACS G1



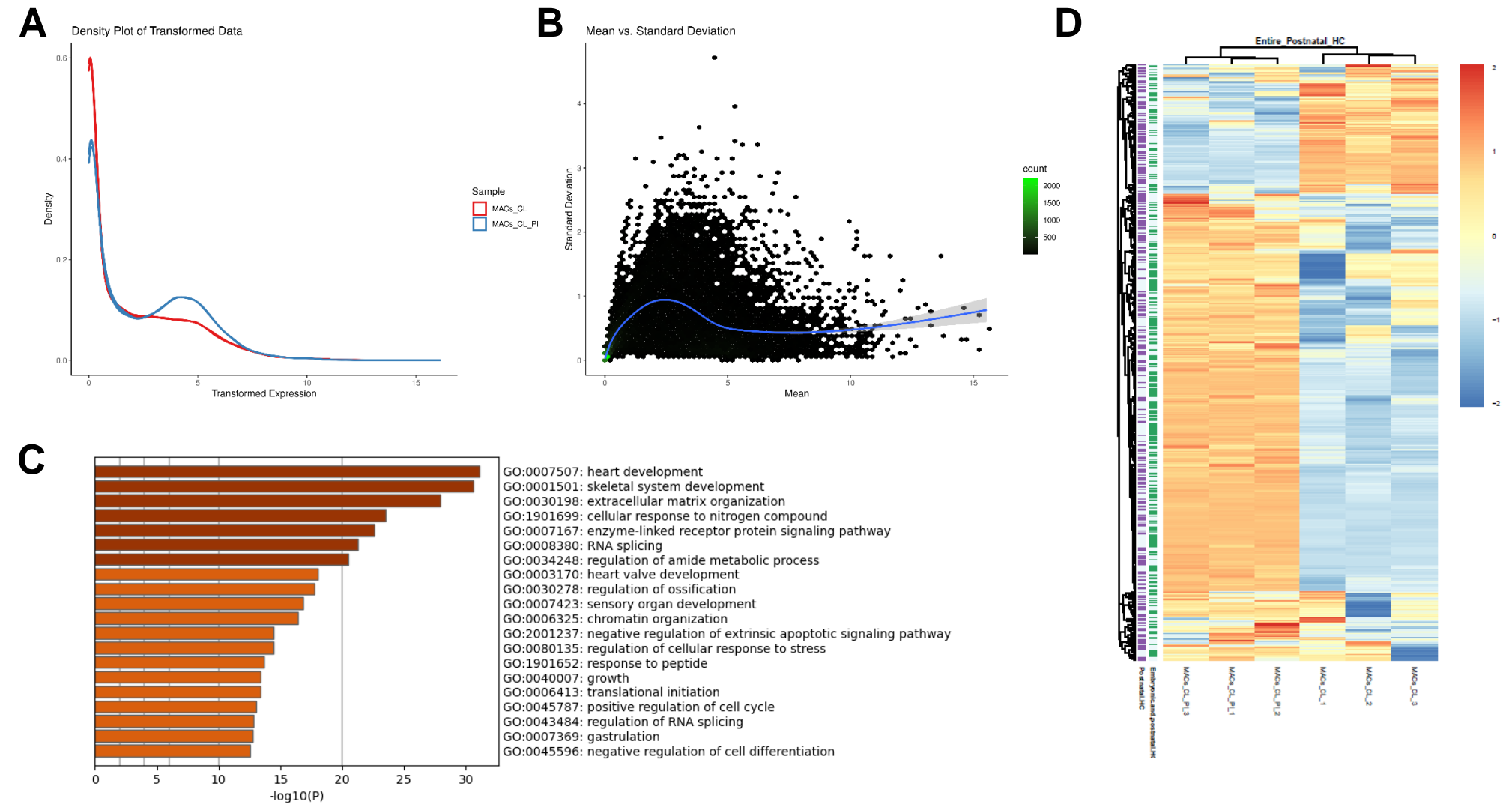
C MACS G1-1



D MACS G2



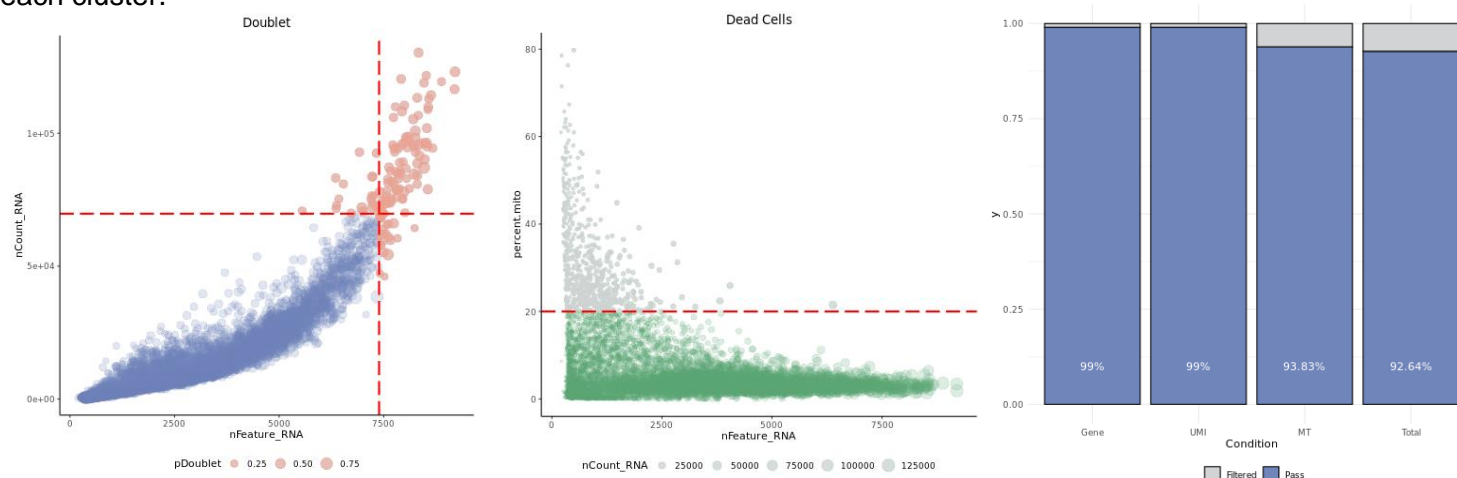
Supplemental Figure 13. RNA sequencing comparison of (G0: MACS_CL) MAC control and (G3: MACS_CL_PI) MACS SHH group at day 24. (A,B) Statistical validation of RNA sequencing. (C) Gene Ontology regarding the differential gene expression between two group. (D) Heatmap for differential gene expressions of postnatal hair cell genes (cite) between two group.



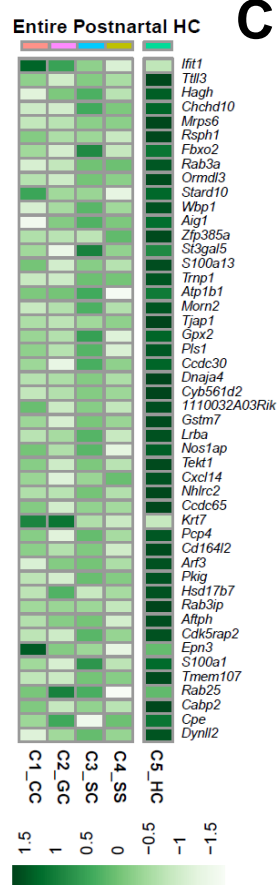
Supplemental Figure 14. Supplementary information for Single Cell (sc) RNA-seq

(A) Statistics summary of Scatter plot and percentage bar plot for filtering criteria and remaining cells. Dead cells and doublets were filtered out. (B) Heat map for postnatal hair cell markers suggested in previous studies. (C) Gene expression comparison heatmap for other cell types associated with the organ of Corti (D) Heatmap comparing expression of IHC and OHC cell markers using both bulk RNA-seq and scRNA-seq data. (B-D) The color intensity indicates higher gene expression levels in the plots. (E) Functional classification of up-regulated genes, identified from five clusters using scRNA-seq. Red boxes were added to emphasize the relevance of cilium development or stereocilia in HC clusters. The scale bars indicate the significant inclusion of genes in each cluster.

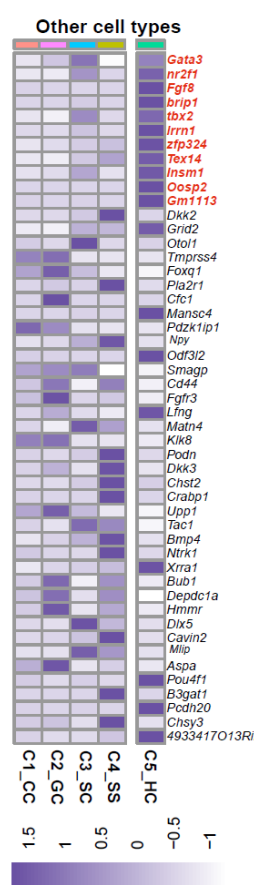
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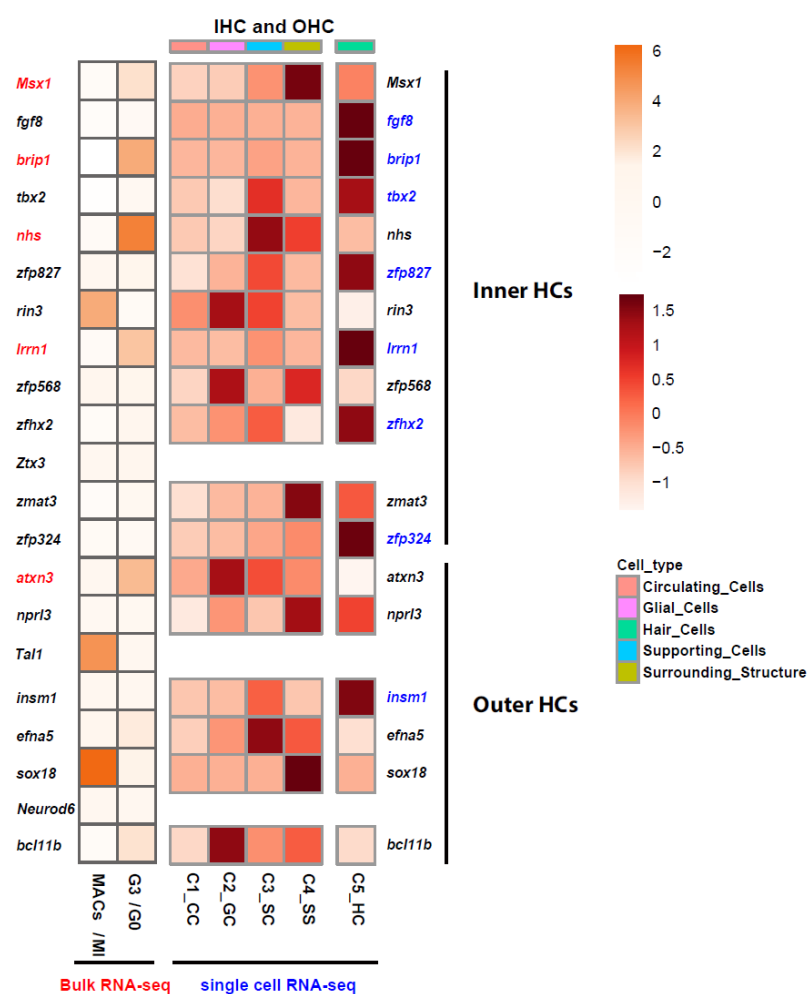
B



C



D



E

