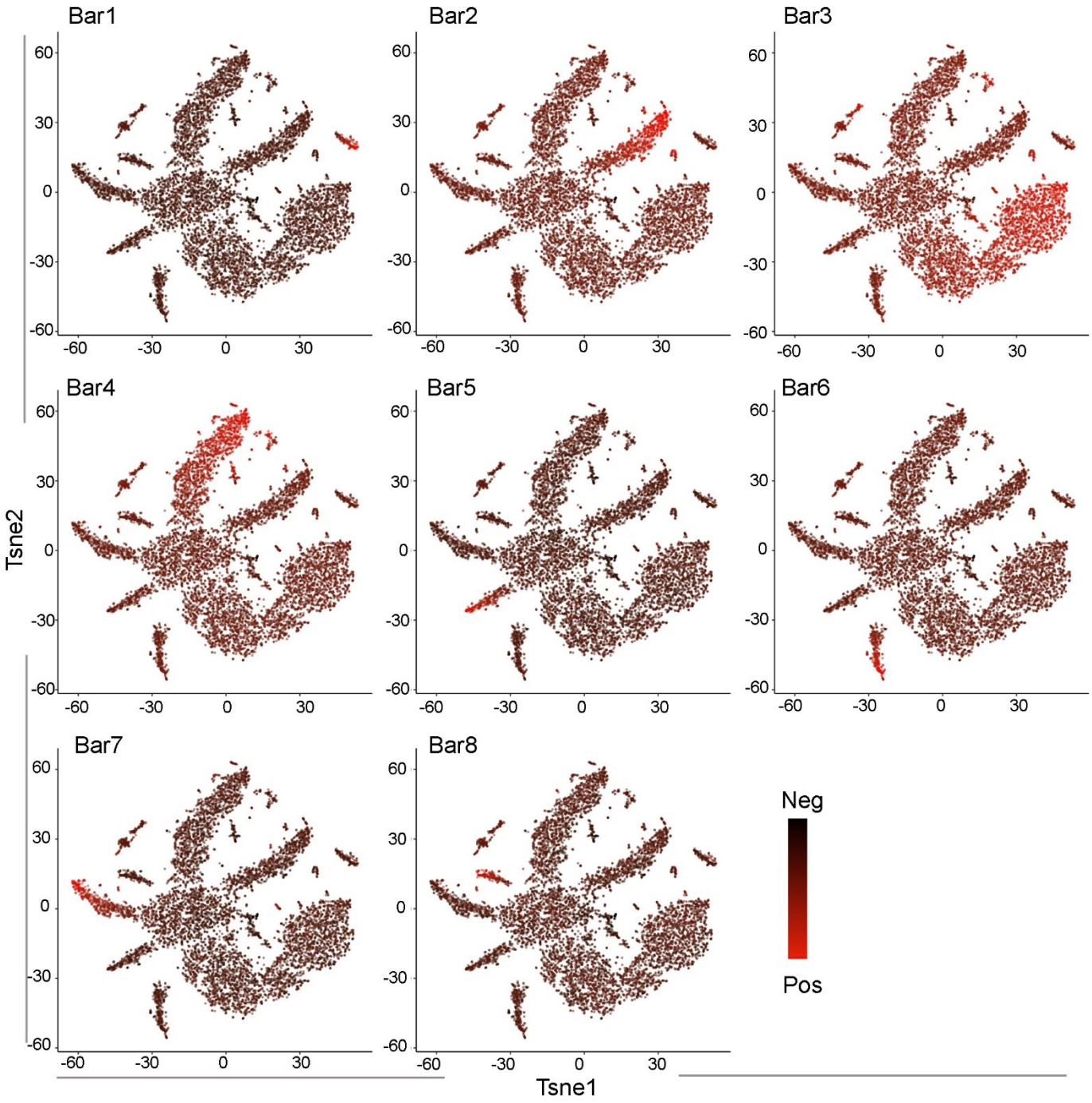



Fig S1. The expression pattern of MULTI-seq barcodes in the E18_P1 dataset. The red signal represents high enrichment, and the black signal represents low or negative signal.

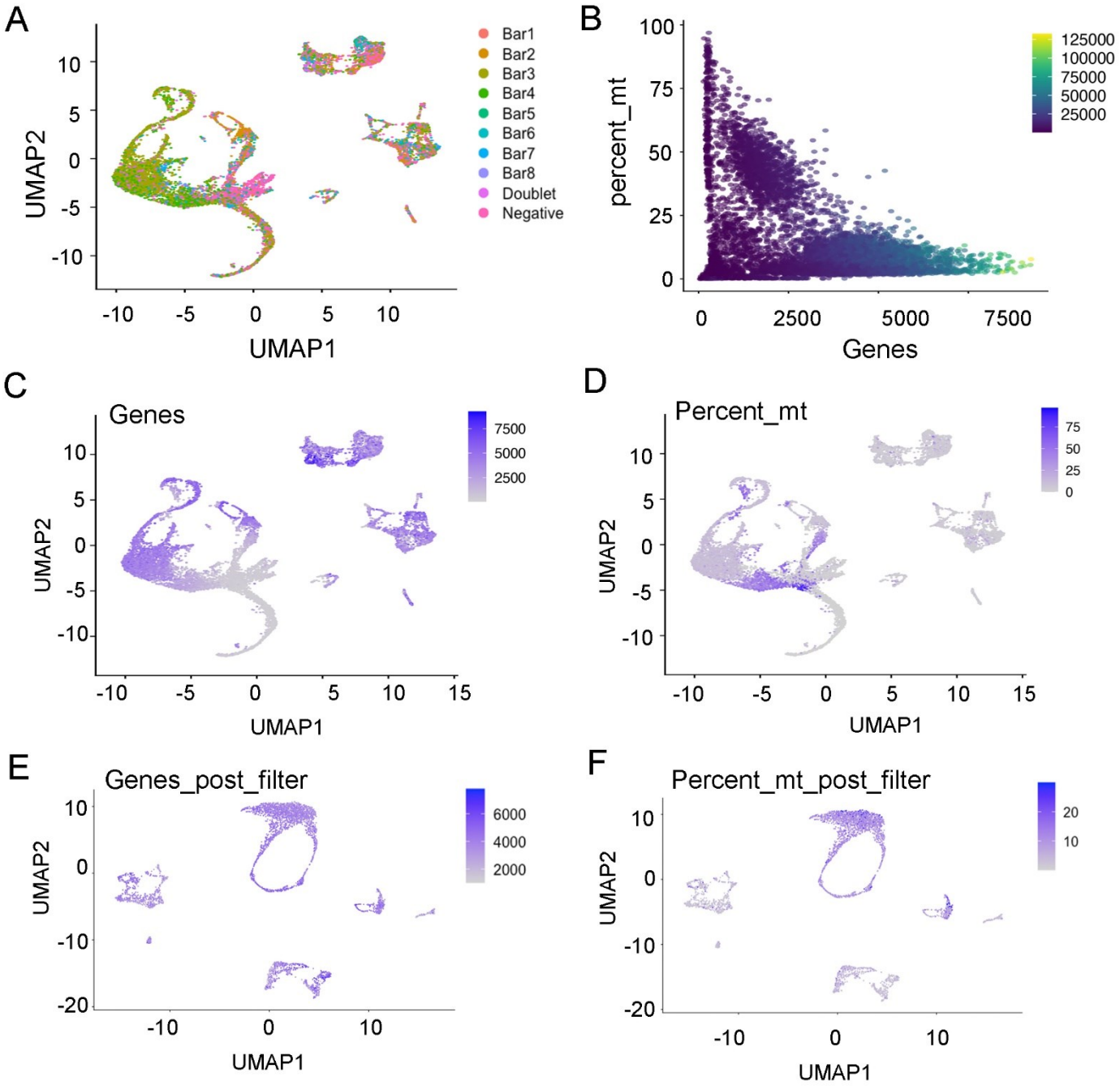



Fig S2. Quality control analysis of the E18_P1 dataset. (A) Identification of singlets, doublets, and negative cells in the E18_P1 dataset. (B) Analysis of the number of expressed genes (x-axis), the percentage of mitochondria genes (y-axis), and the total number of molecules detected in each cell (color). (C, D) UMAP plots of the gene numbers and mitochondria gene percentages in each single cell before filtering. (E, F) UMAP plots of the gene numbers and mitochondria gene percentages in each single cell after filtering.

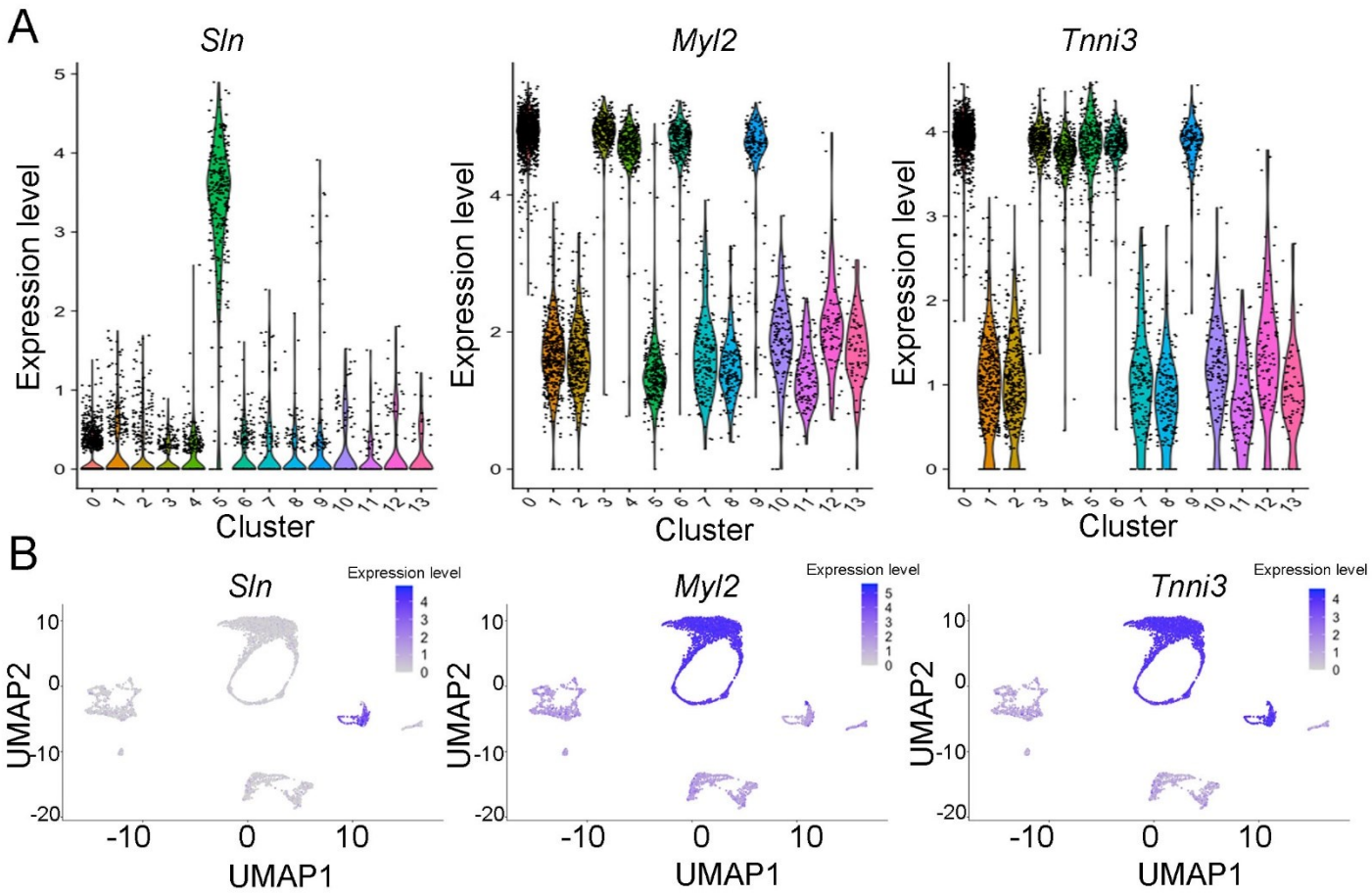



Fig S3. The expression pattern of *Sln* and *Myl2* in E18_P1 single cells. (A, B) Violin plots and Feature plots show that the clusters with atrial and ventricular CMs (*Tnni3* positive) highly express *Sln* and *Myl2*, respectively.

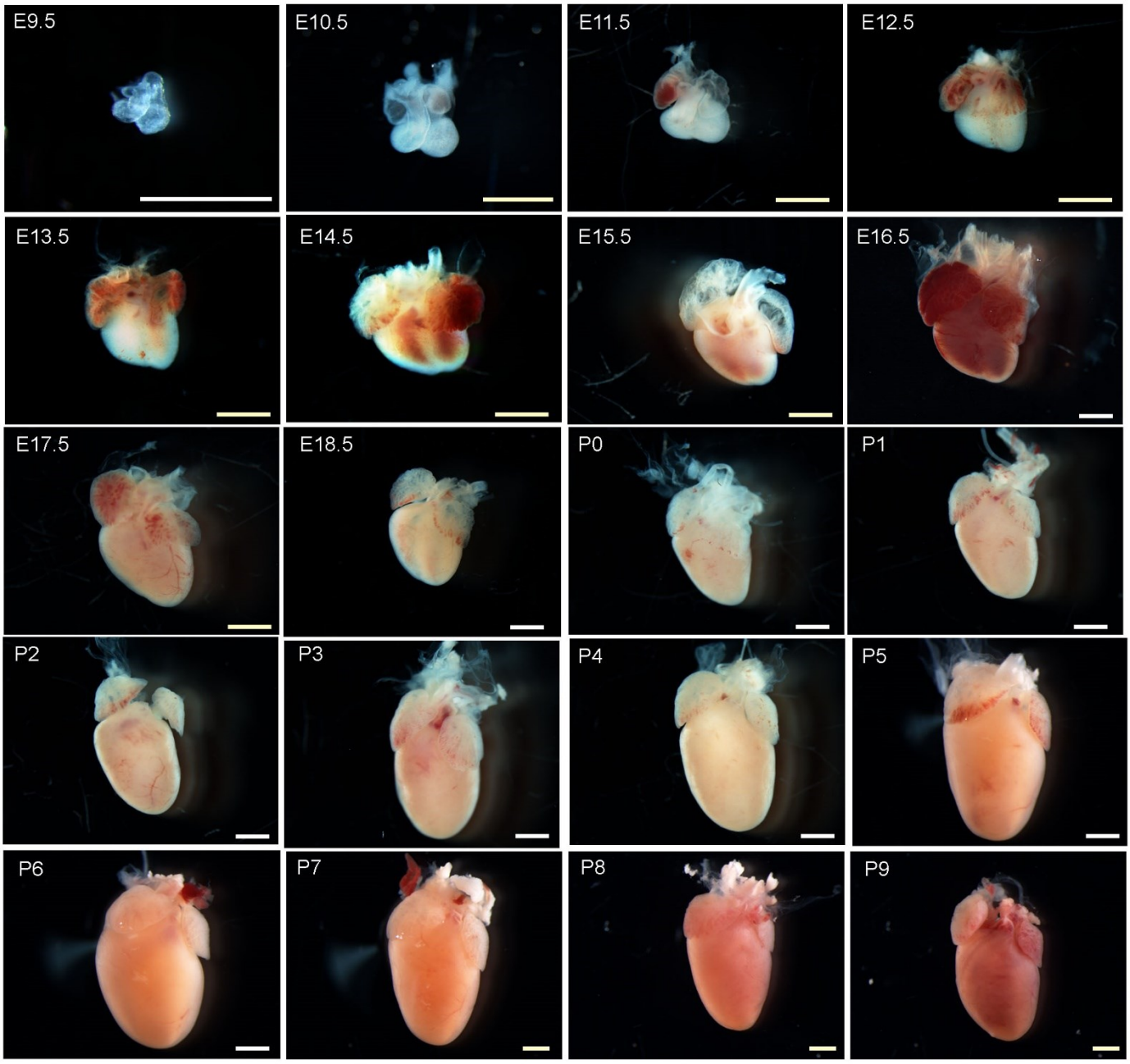



Fig S4. Representative images of staged hearts. Scale bar=1mm.

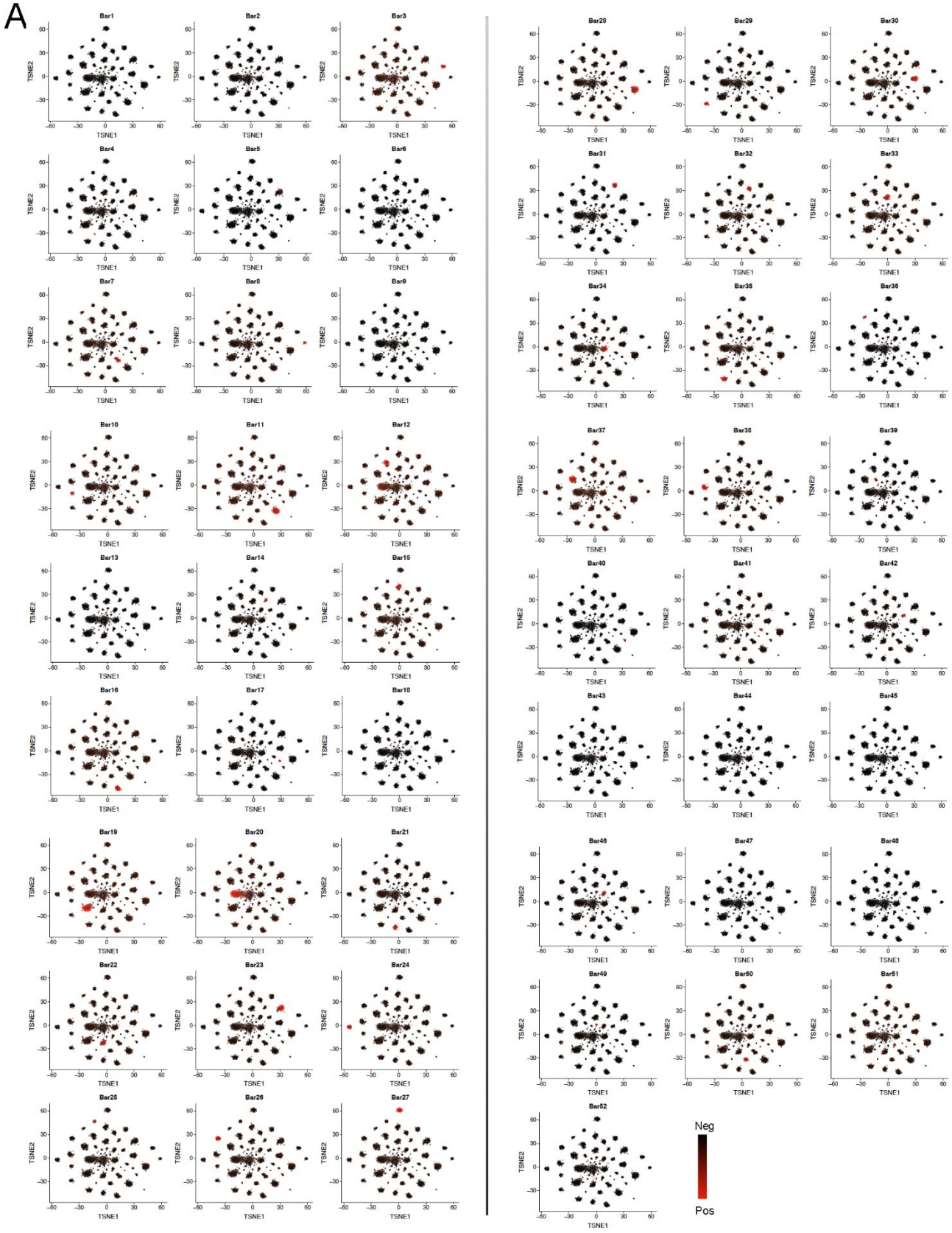



Fig S5. The expression pattern of MULTI-seq barcodes in the 5k_1 dataset. Red color represents a positive signal, and black represents the background. Each barcode was mainly enriched in one cluster of cells.

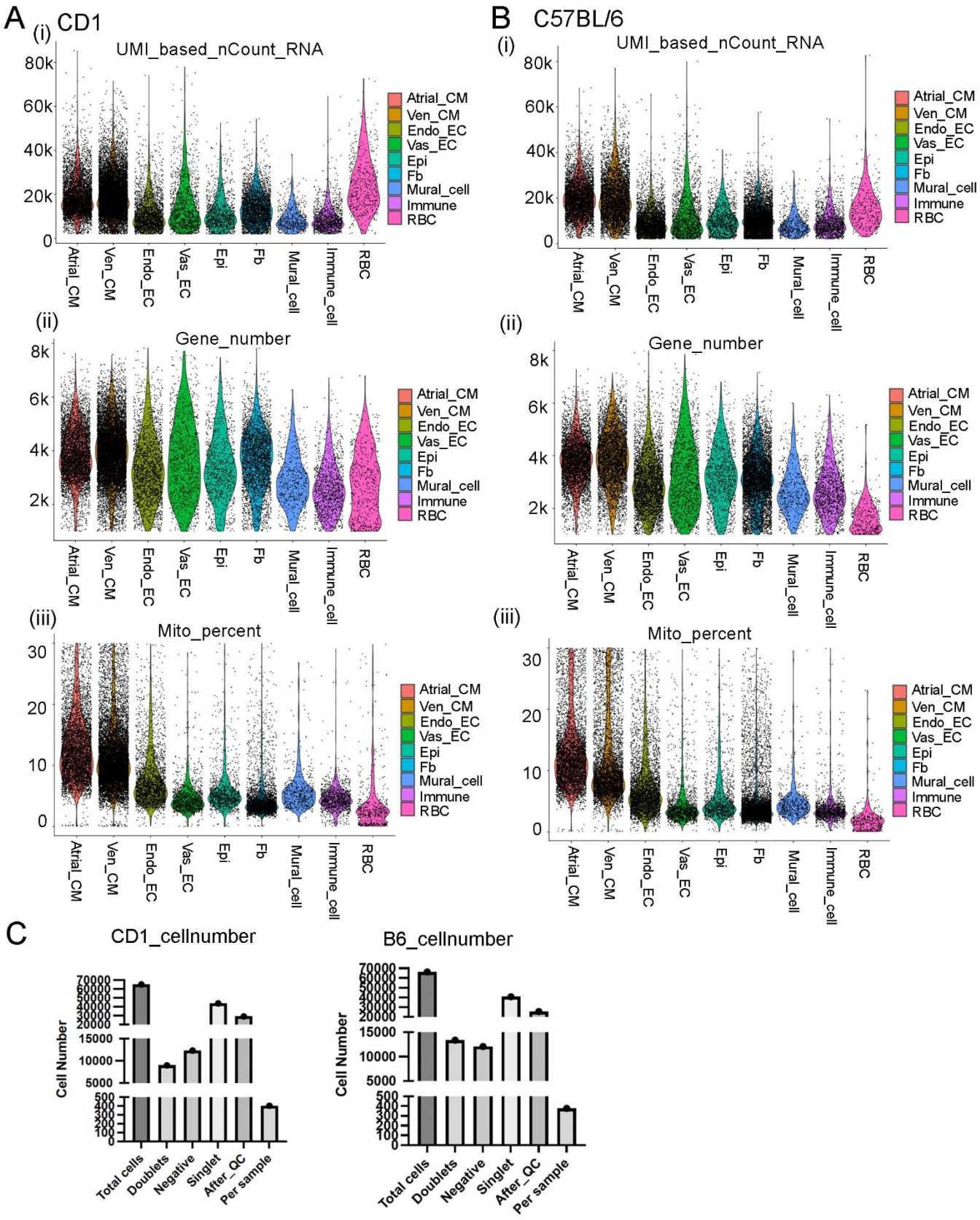



Fig S6. Quantitative analysis of the cells in CD1 and C57BL/6 datasets. (A, B) The number of sequencing reads, gene numbers, and percentage of mitochondria genes at each cell type in CD1 and C57BL/6. (C, D) The number of cells at different QC steps in CD1 and C57BL/6 datasets.

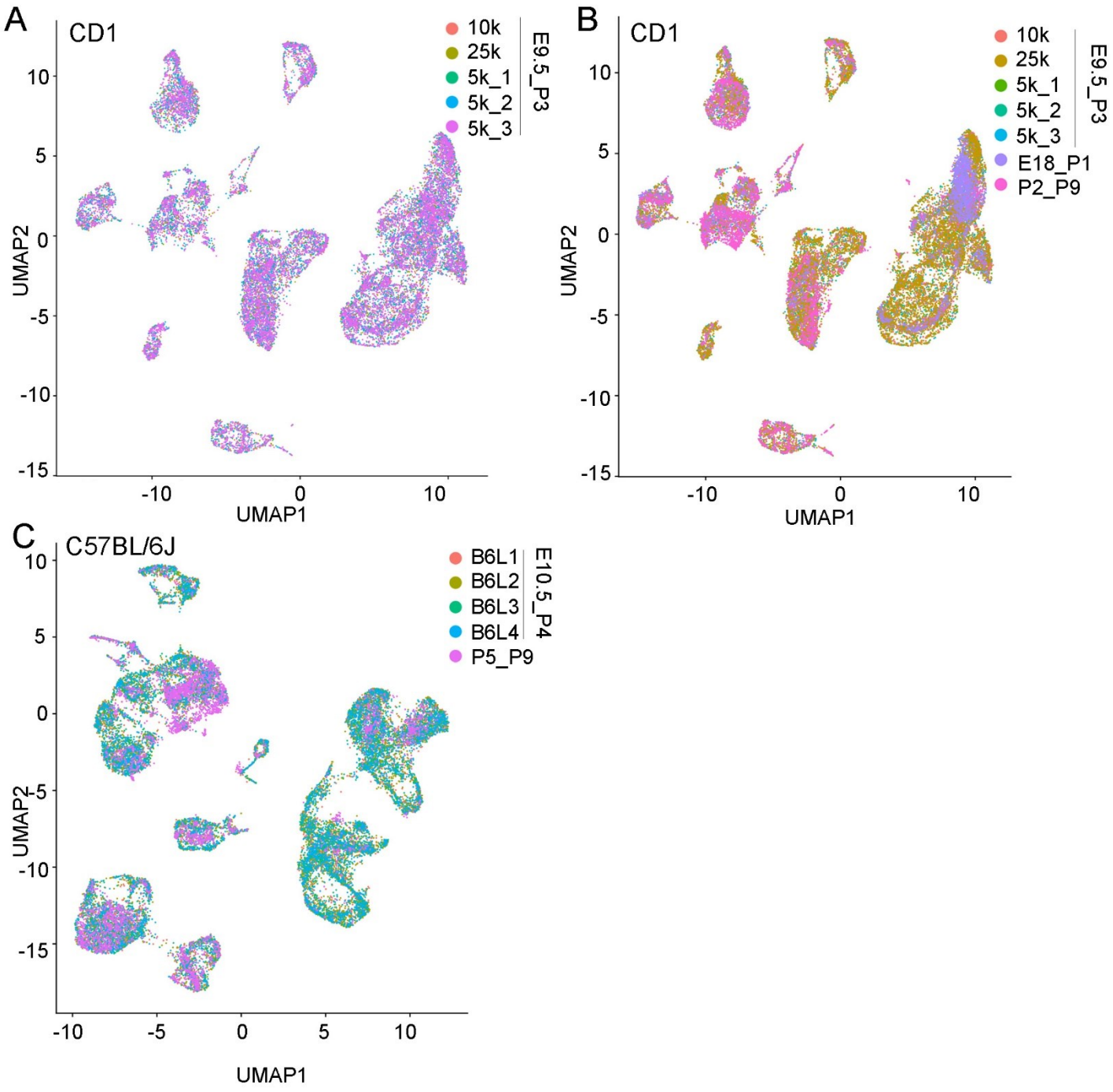



Fig S7. Integrative analysis of the single cells from different experiments. (A) The CD1 E9.5_P3 single cells were from five different loading wells with different targeting cell numbers. (B) UMAP plot of the CD1 data from different experiments (E9.5_P3, E18_P1, P2_P9). (C) The C57BL/6 single cell data profiled at different experiments. The UMAP plots were labeled by loading wells or experiments.

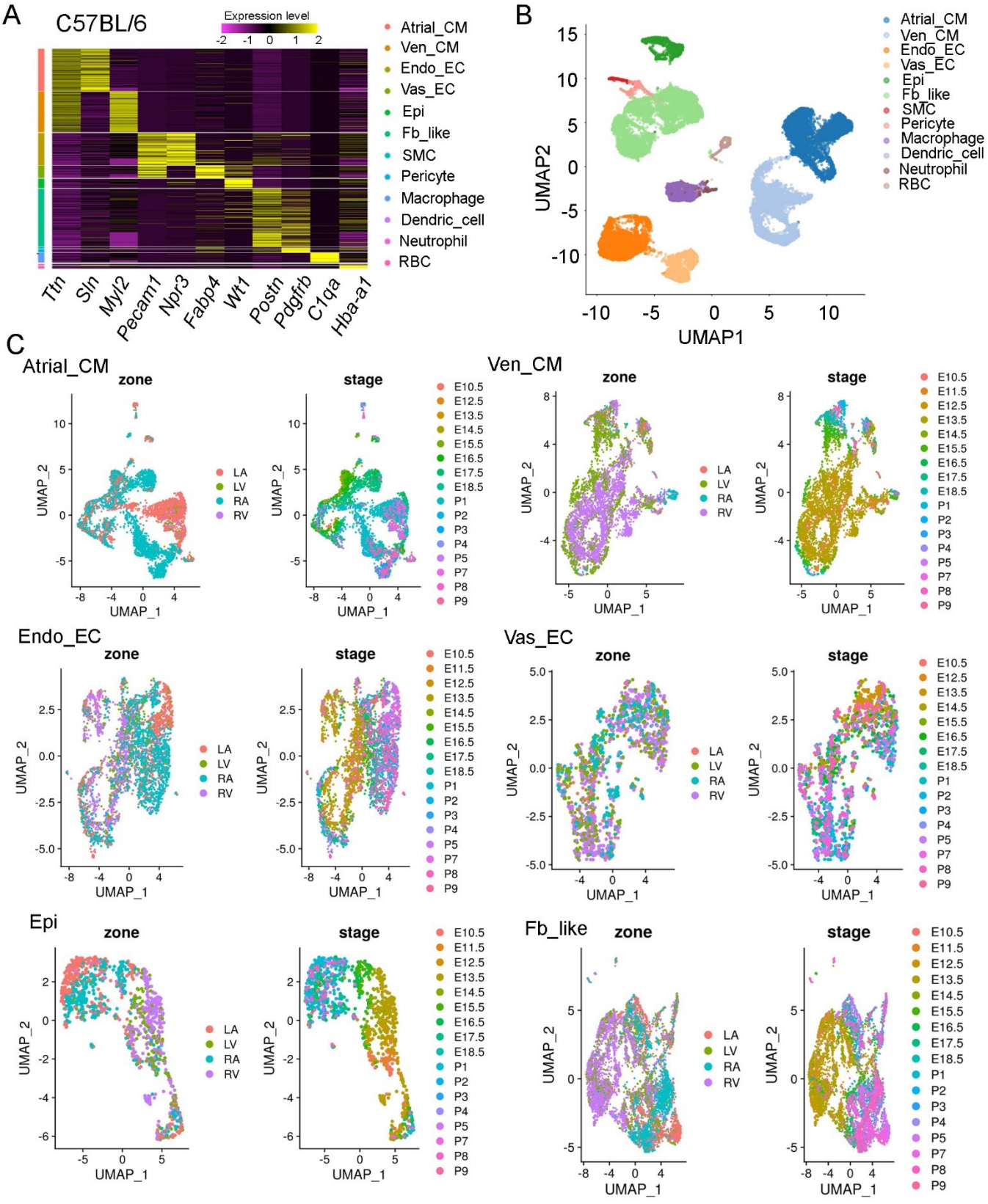



Fig S8. Analysis of the C57BL/6 single cell data. (A) Heatmap showing the expression pattern of cardiac lineage genes. (B) UMAP plot of the single cells labeled by cell types. (C) Zone and stage analysis of the cells in each cell type.

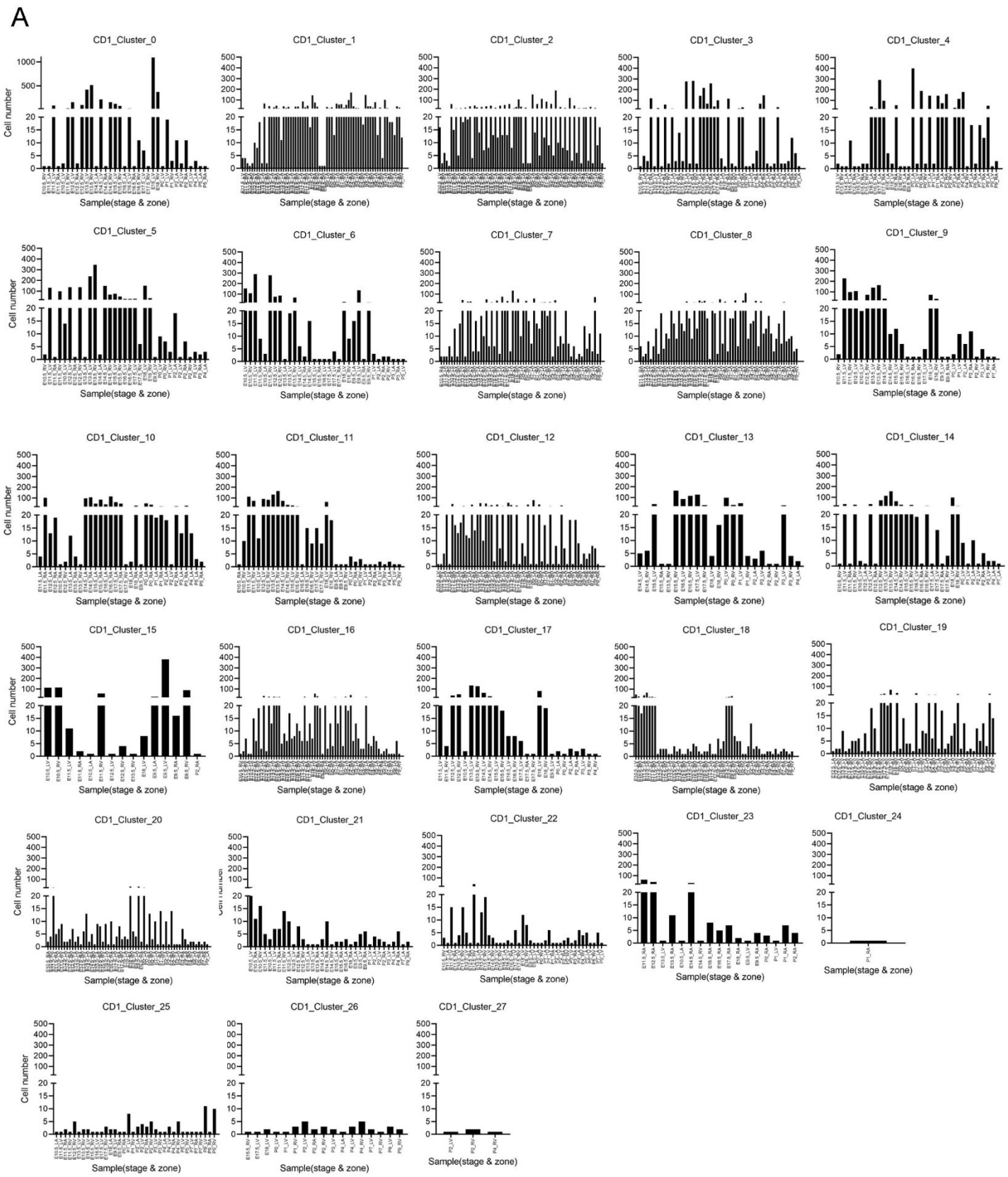



Fig S9. The number of cells in each sample at each CD1 cluster.

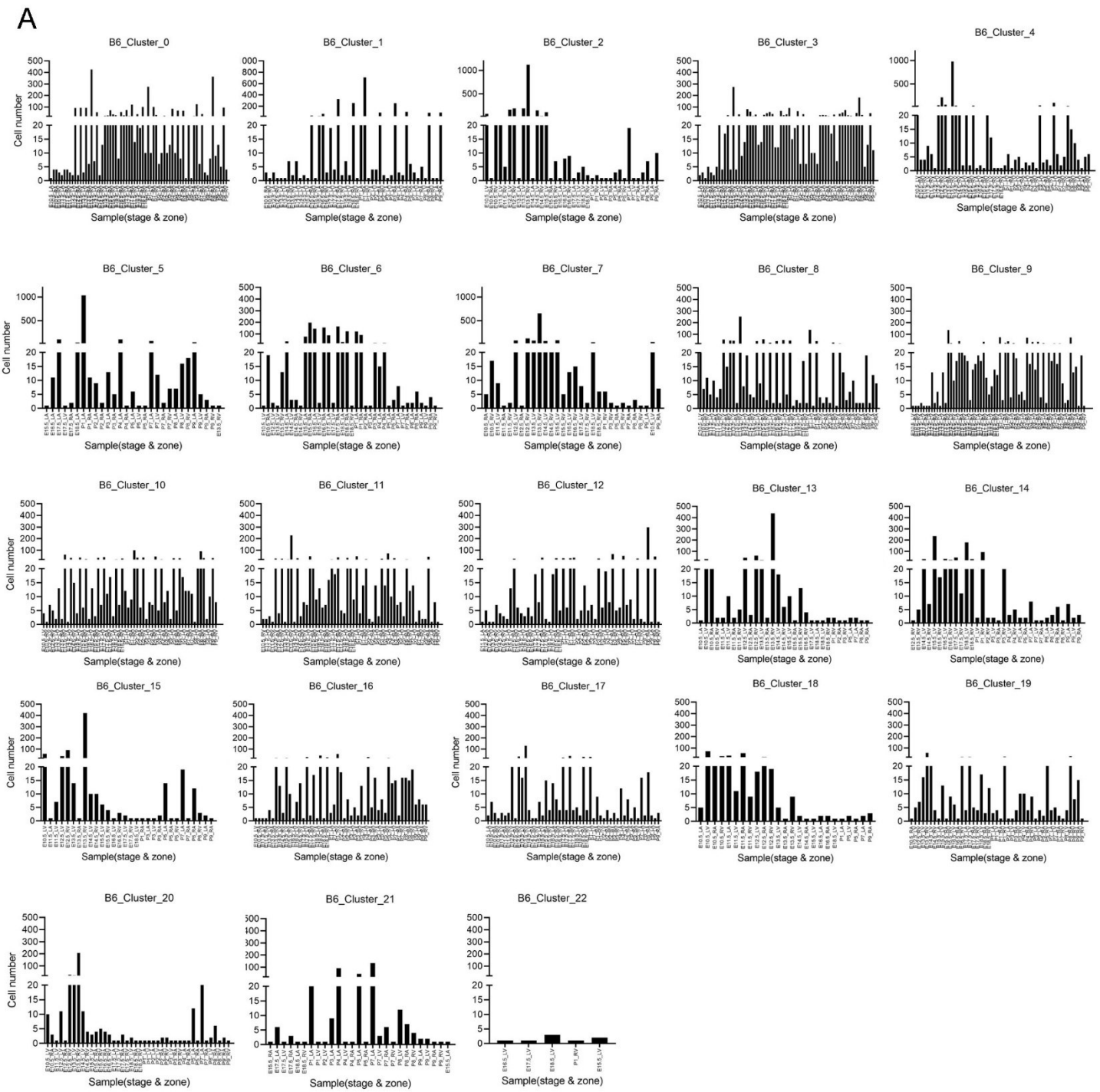



Fig S10. The number of cells in each sample at each C57BL/6 cluster.

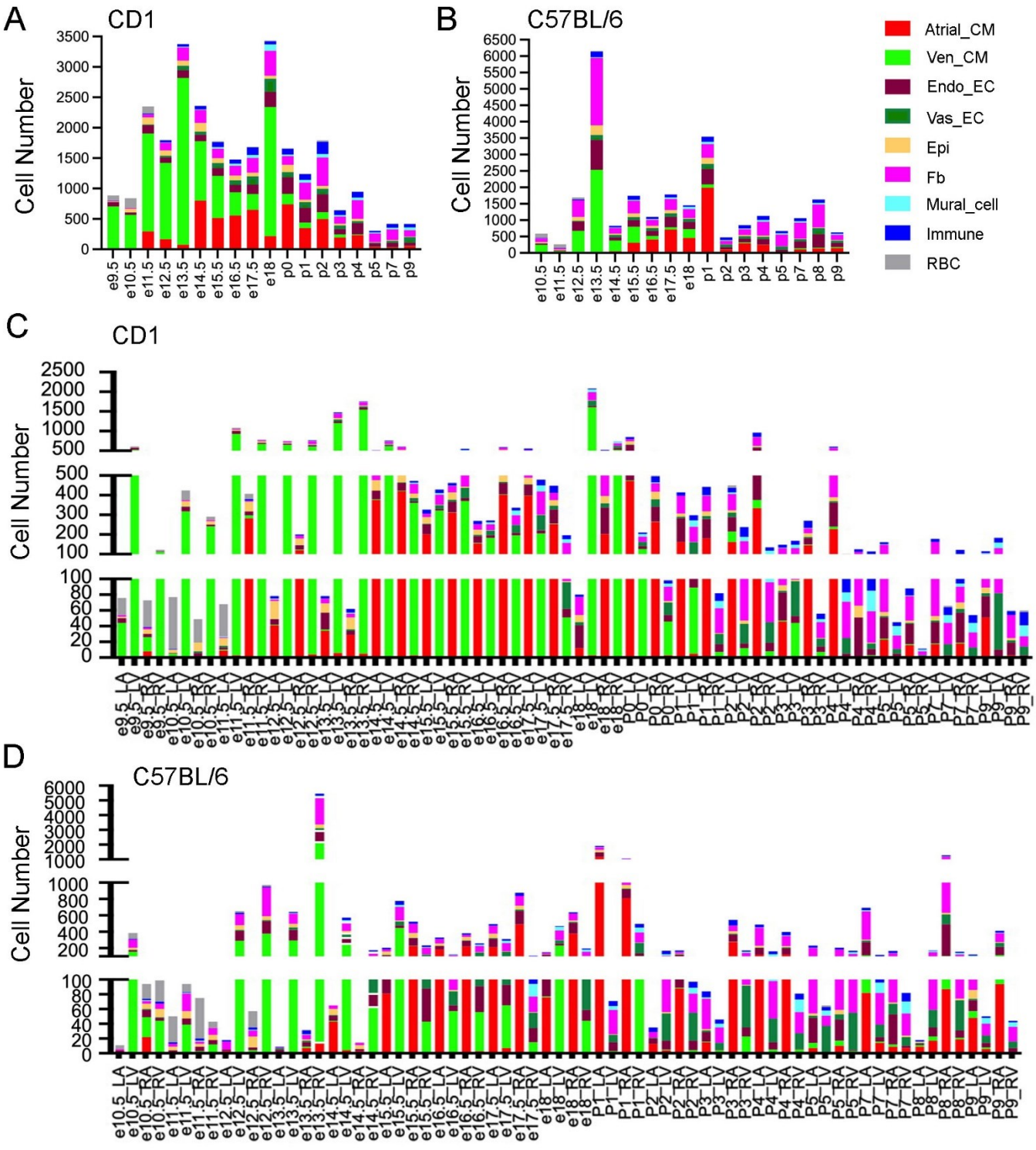



Fig S11. The cell type compositions at each stage and sample. (A, B) The cell type compositions at each stage in CD1 and C57BL/6 datasets. (C, D) The cell type compositions in each sample in CD1 and C57BL/6 datasets.

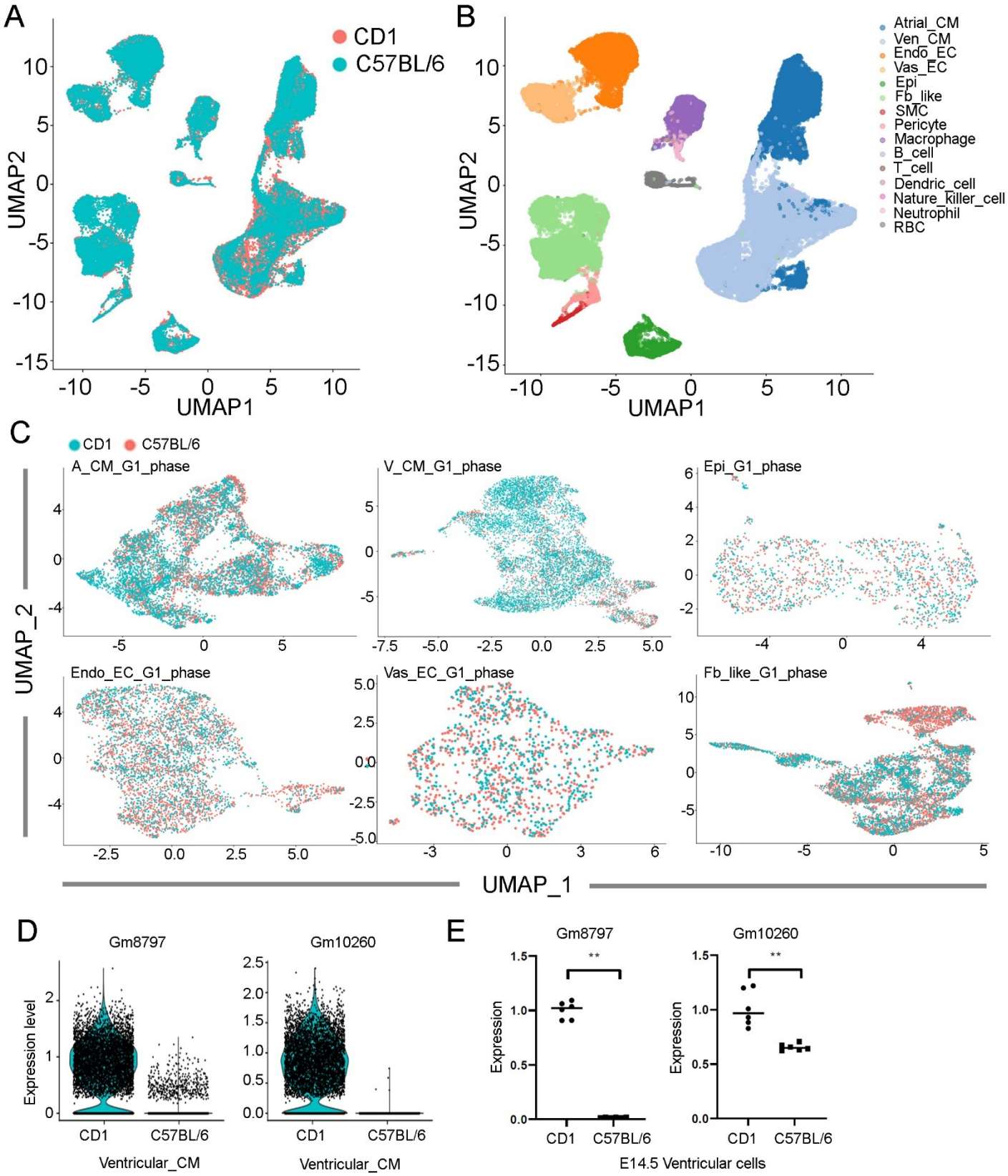



Fig S12. Comparative analysis of the CD1 and C57BL/6 single cell datasets. (A, B) UMAP plot of the integrated CD1 and C57BL/6 datasets labeled by mouse strain or cell type. (C) UMAP plot of the integrated G1 phased cells in each cell type. (D) The two representative genes differentially expressing in CD1 and C57BL/6 ventricular _CMs. (E) qPCR analysis confirmed the differential expression of the two genes between strains. N=2 biologically independent experiments with 3 replicates in each experiment. Student's t-test with two-tailed distribution was used for the statistical analysis. ** indicates the significance with p value <0.01.

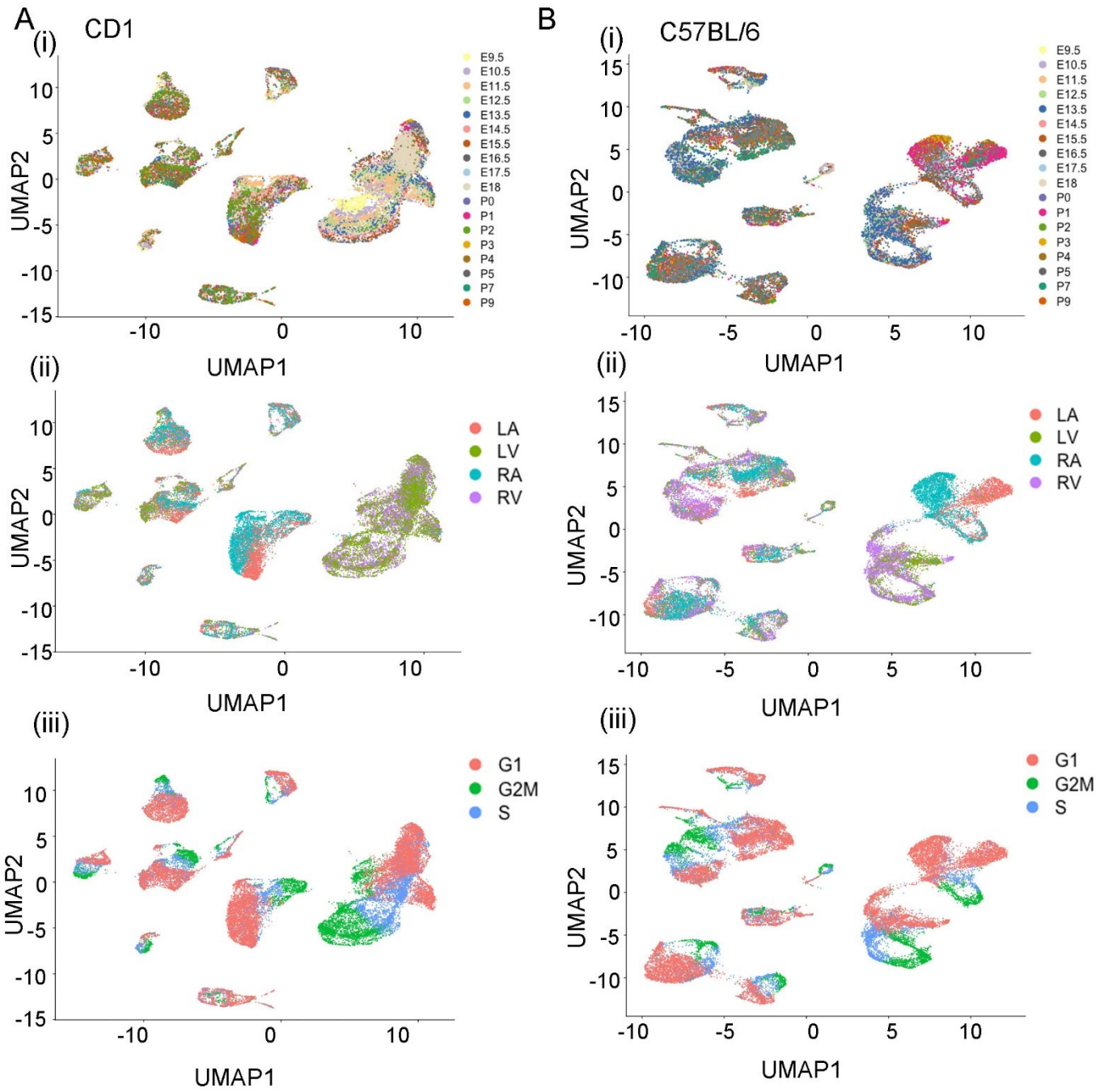



Fig S13. UMAP plots of the CD1 and C57BL/6 single cells with different labels. (A, B) The single cells from CD1 and C57BL/6 strains were labeled by stage, zone, or cell cycle phase.

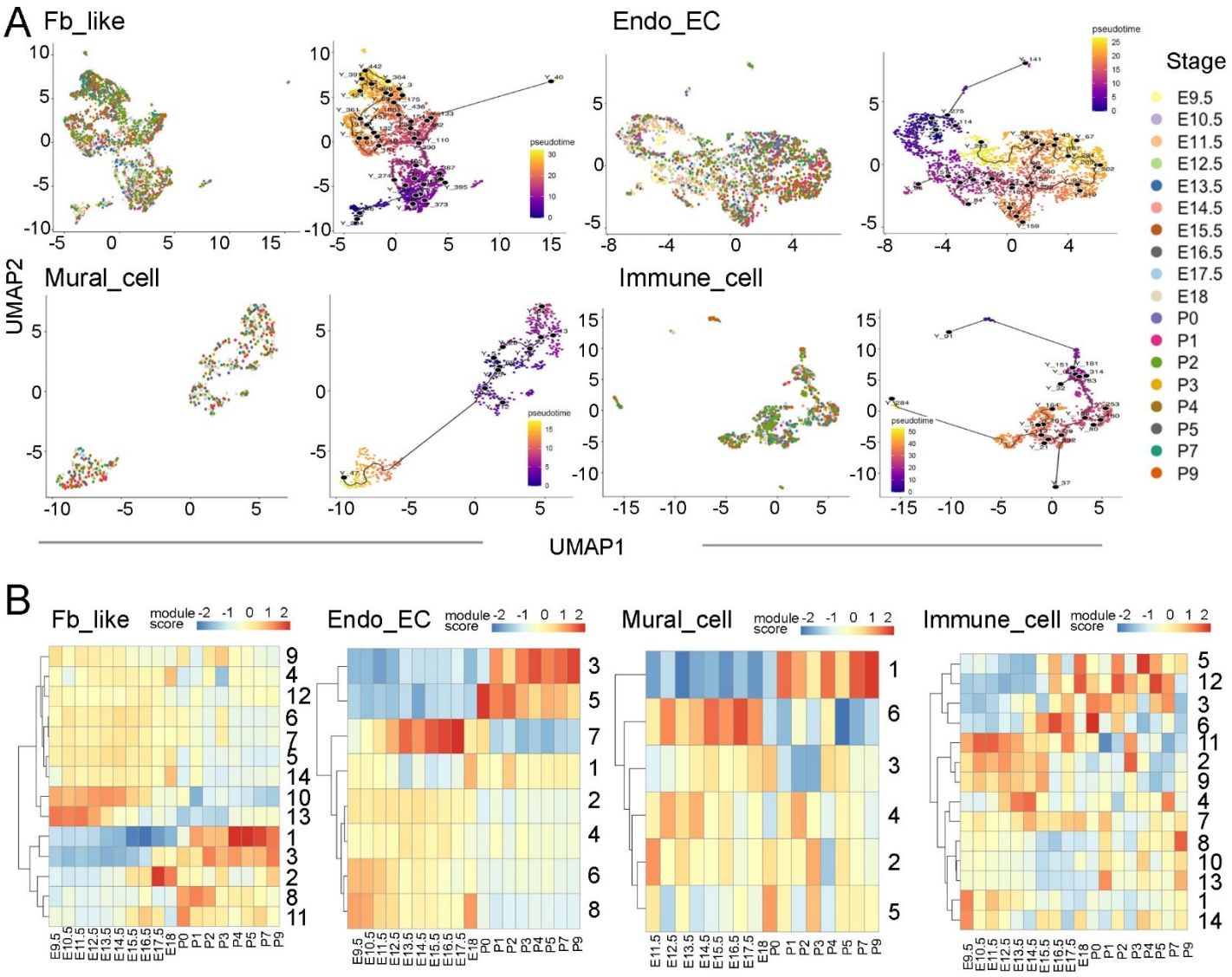



Fig S14. Stage analysis of the single cells in Fb_like, Endo_EC, Mural cell, and Immune cell. (A) UMAP plot of the single cells labeled by stage or pseudotime. (B) Identification of gene modules in each cell type. The color represents module score.

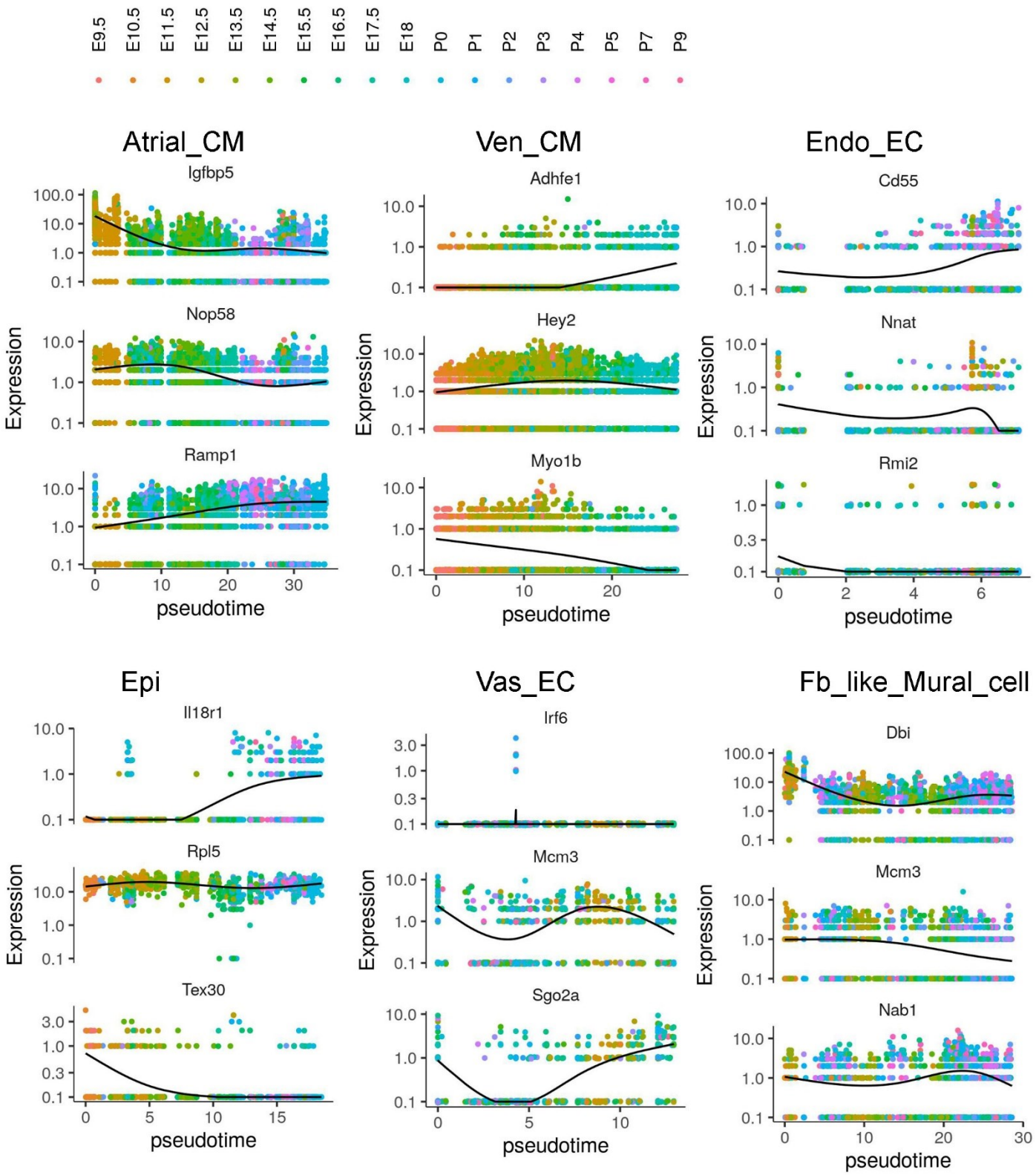



Fig S15. Representative genes that display pseudotime stage-specific expression pattern in each cell type. The color represents real stages. The cells were ordered along the x-axis by pseudotime, and the Y-axis represents gene expression level.

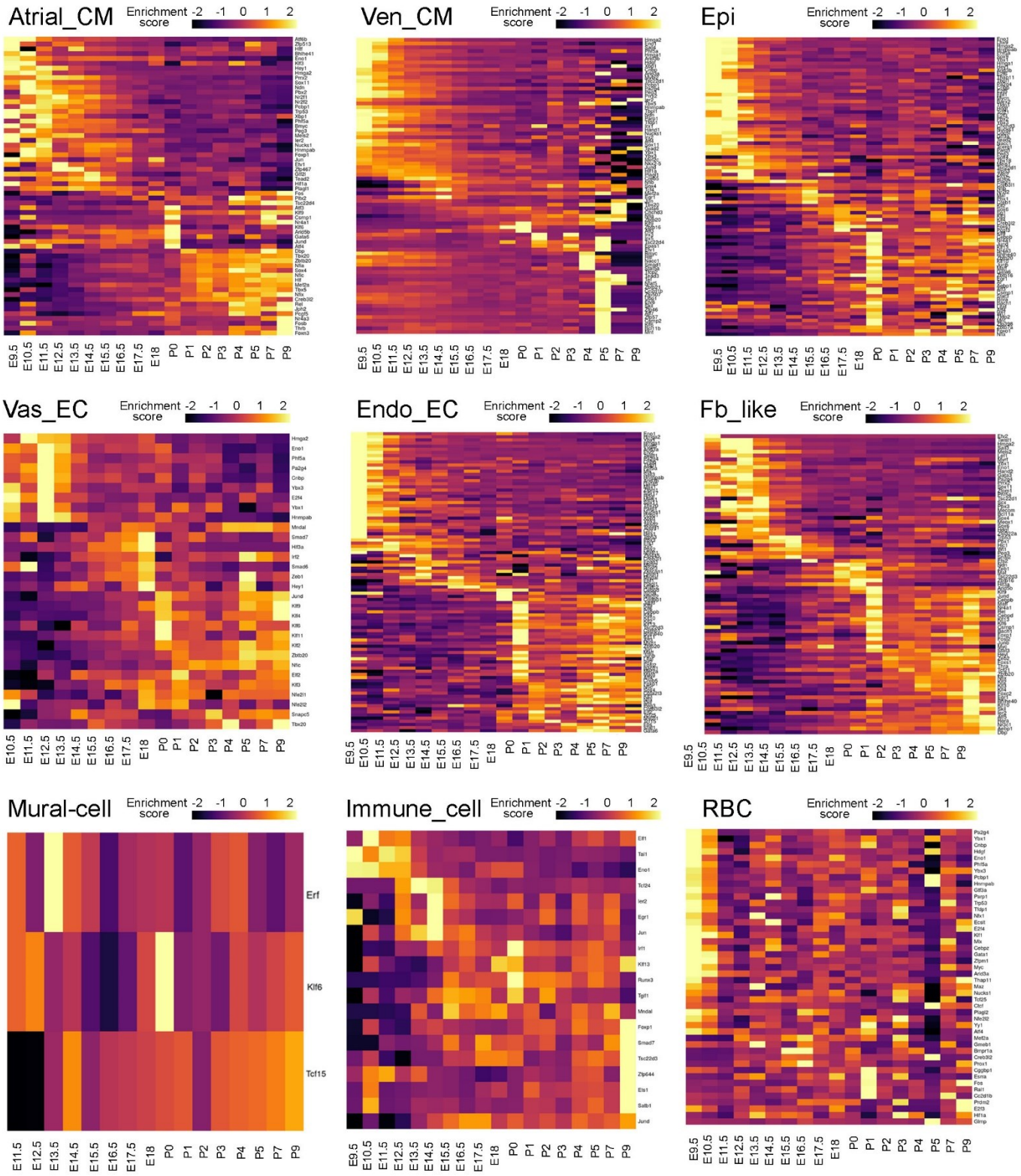



Fig S16. The stage specifically expressed transcription factors in each cell type. The gene expression level reduced from yellow to blue to black.

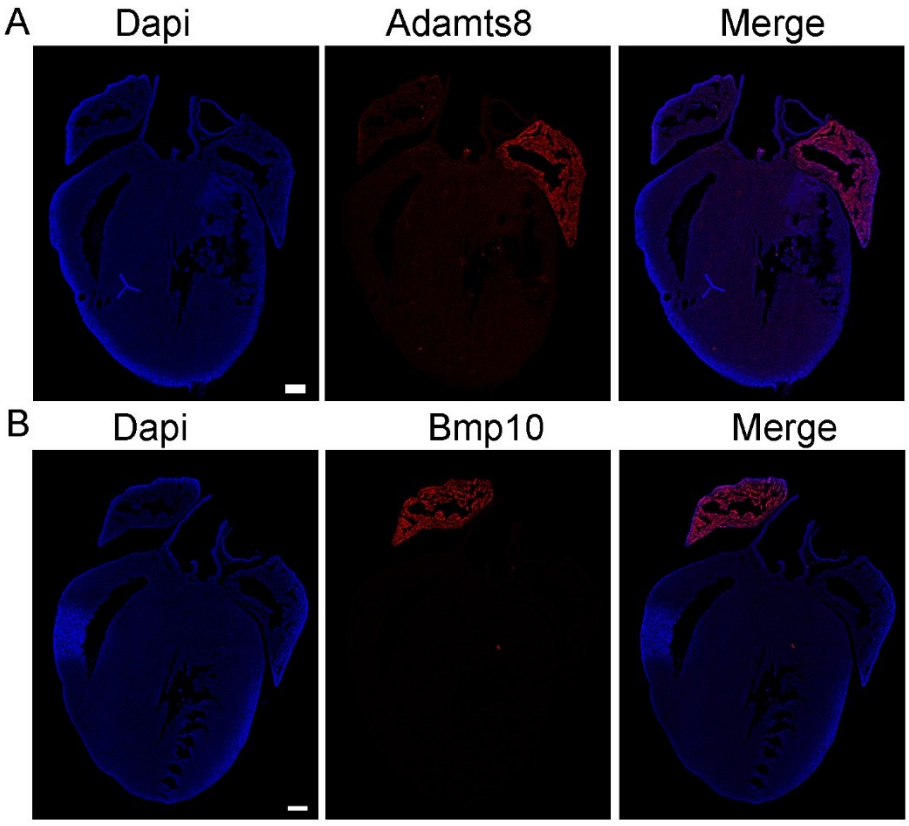



Fig S17. Staining analysis of *Adamts8* and *Bmp10*. (A, B) mRNA staining showed that *Adamts8* and *Bmp10* were specifically expressed in LA and RA at P3 hearts, respectively. The staining experiments were repeated twice with similar results. Scale bar=500 μm.

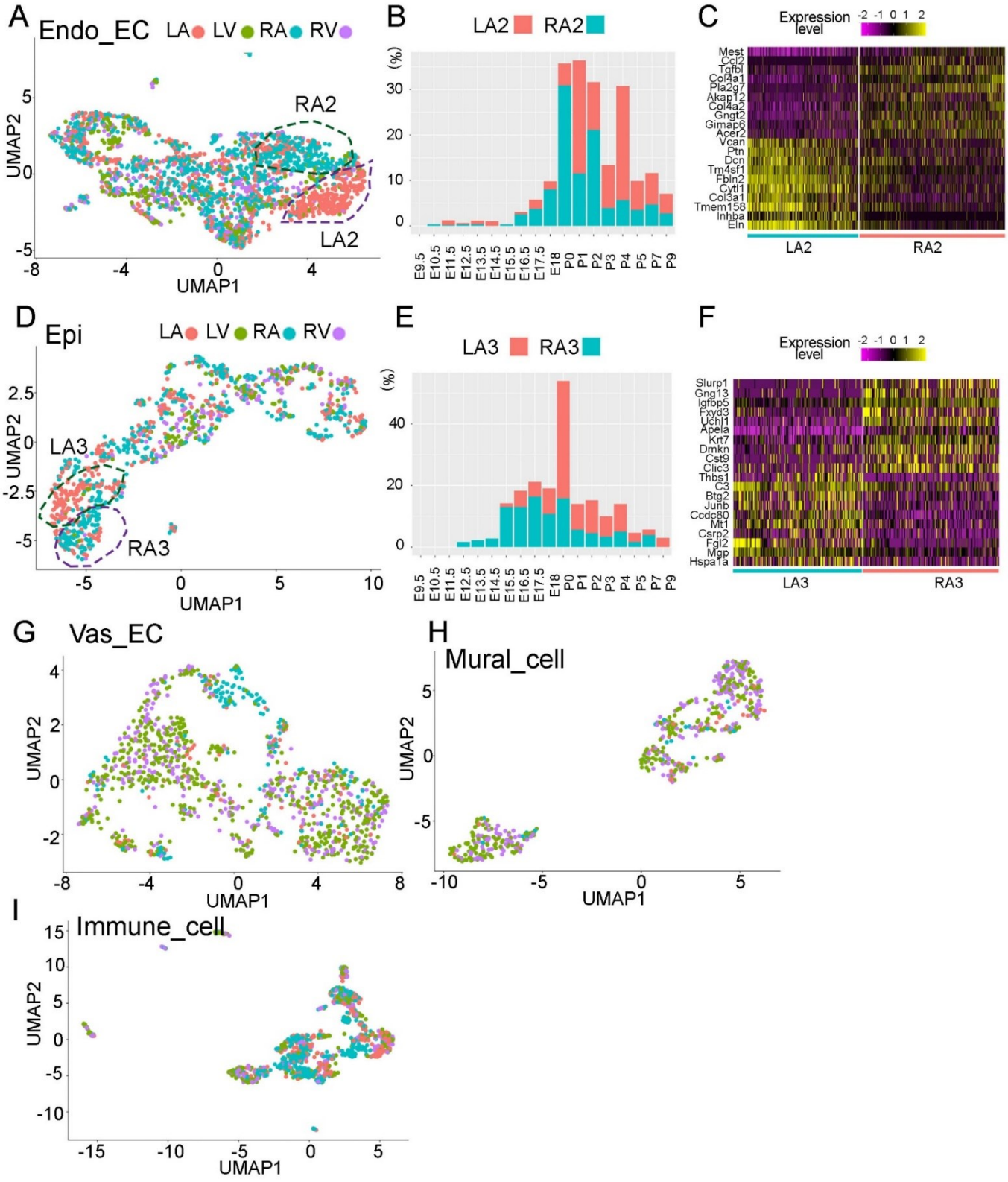



Fig S18. The zone-specific molecular signatures in five cell types. (A) UMAP plot of Endo_ECs labeled by zones. One LA and one RA-specific cell populations (LA2, RA2) were identified. (B) Stage analysis of the cells in LA2 and RA2. (C) Expression heatmap of the top 20 genes that were differentially expressed in LA2 and RA2 cells. (D) UMAP plot of epicardial cells labeled by zones. One LA and one RA-specific cell populations (LA3, RA3) were identified. (E) Stage analysis of the cells in LA3 and RA3. (F) Expression heatmap of the top 20 genes that were differentially expressed in LA3 and RA3 cells. (G-I) UMAP plots of Vas_EC, Mural_cells, and Immune cells labeled by zones.

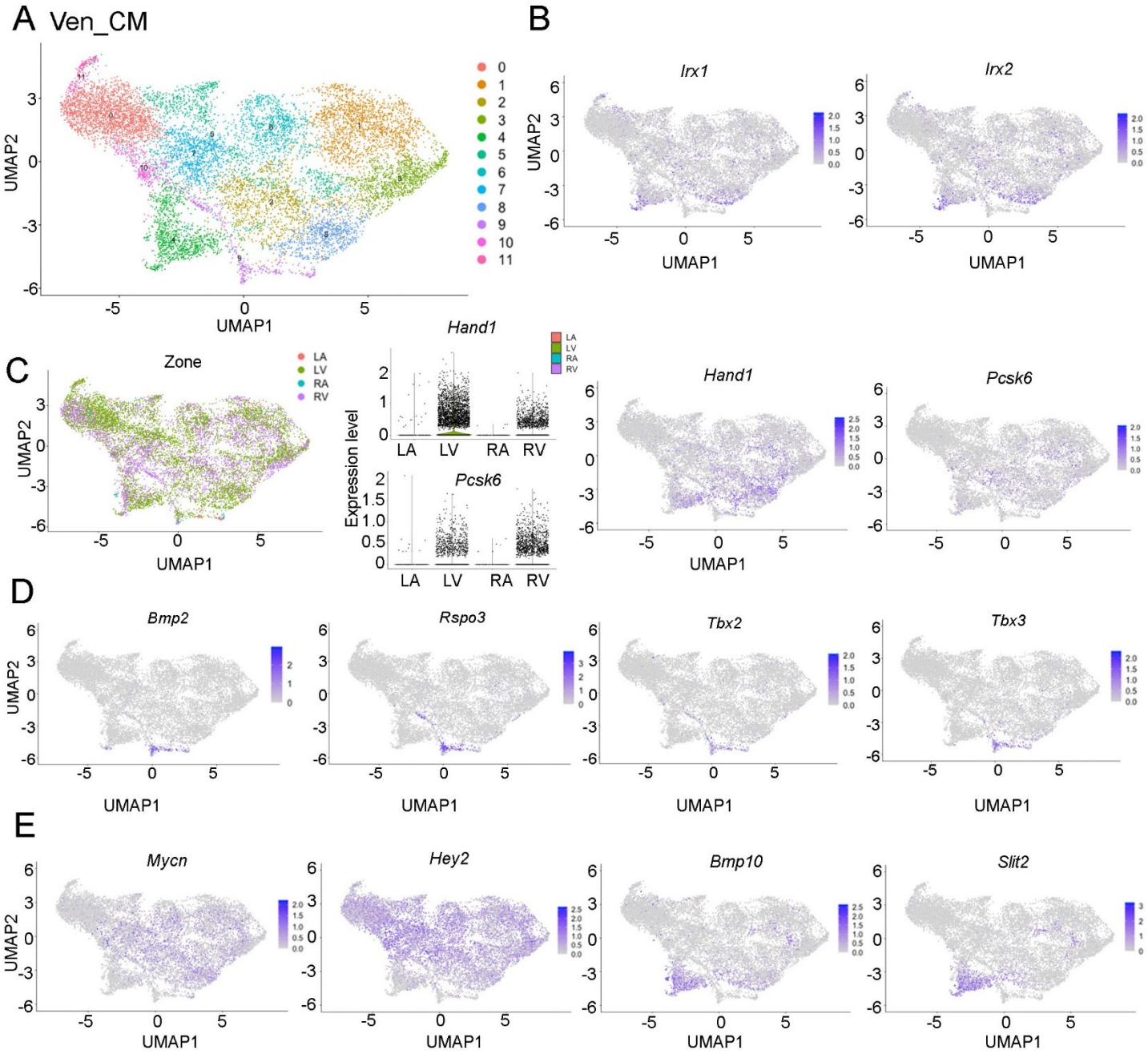



Fig S19. The cellular heterogeneity in Ven_CM. (A) UMAP plot of Ven_CM labeled by clusters. (B) *Irx1* and *Irx2* positive CMs were distributed in multiple cell clusters. (C) UMAP plot of Ven_CMs labeled by zone. No LV and RV-specific clusters were identified. Violin plots showed a preferential expression of *Hand1* and *Pcsk6* in LV and RV CMs, respectively. The feature plots did not identify cluster-specific expressions of *Hand1* and *Pcsk6*. (D) The cluster 9 CMs highly expressed AVC marker genes *Bmp2*, *Rspo3*, *Tbx2*, and *Tbx3*. (E) The cluster 4 CMs highly specifically expressed trabecular myocardium genes *Bmp10* and *Slit2*, and the cells in other clusters expressed compact myocardium genes *Mycn* and *Hey2*.

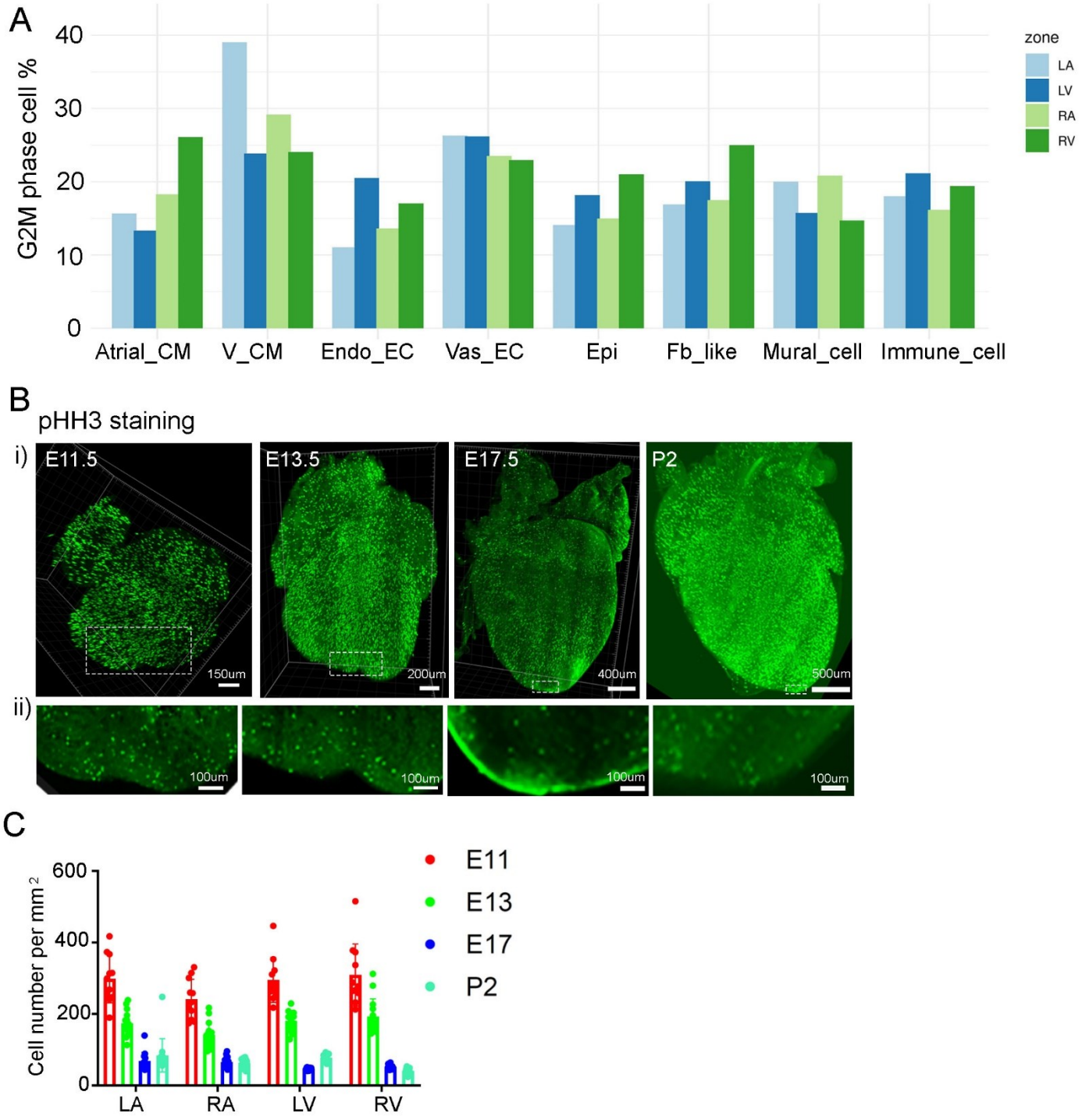



Fig S20. The proportion of cells in proliferation at different stages and zones. (A)The percentages of G2M phased cells in each cell type at each chamber. (B) (i) Overview and (ii) enlarged portion of cleared hearts with pHH3 staining at four stages. (C) The pHH3 positive cells declined along developmental progression in all four chambers. iDISCO images scale bar: E11.5 = 150 μm; E13.5 = 200 μm; E17.5 = 400 μm; P2 = 500 μm. N= around 10 section images from one cleared heart at each stage were analyzed. The error bars represent SD. ANOVA with Tukey's multiple comparisons were used for the statistical analysis. All comparisons except the E17 and P2 pair are statistical significant with p value<0.01.

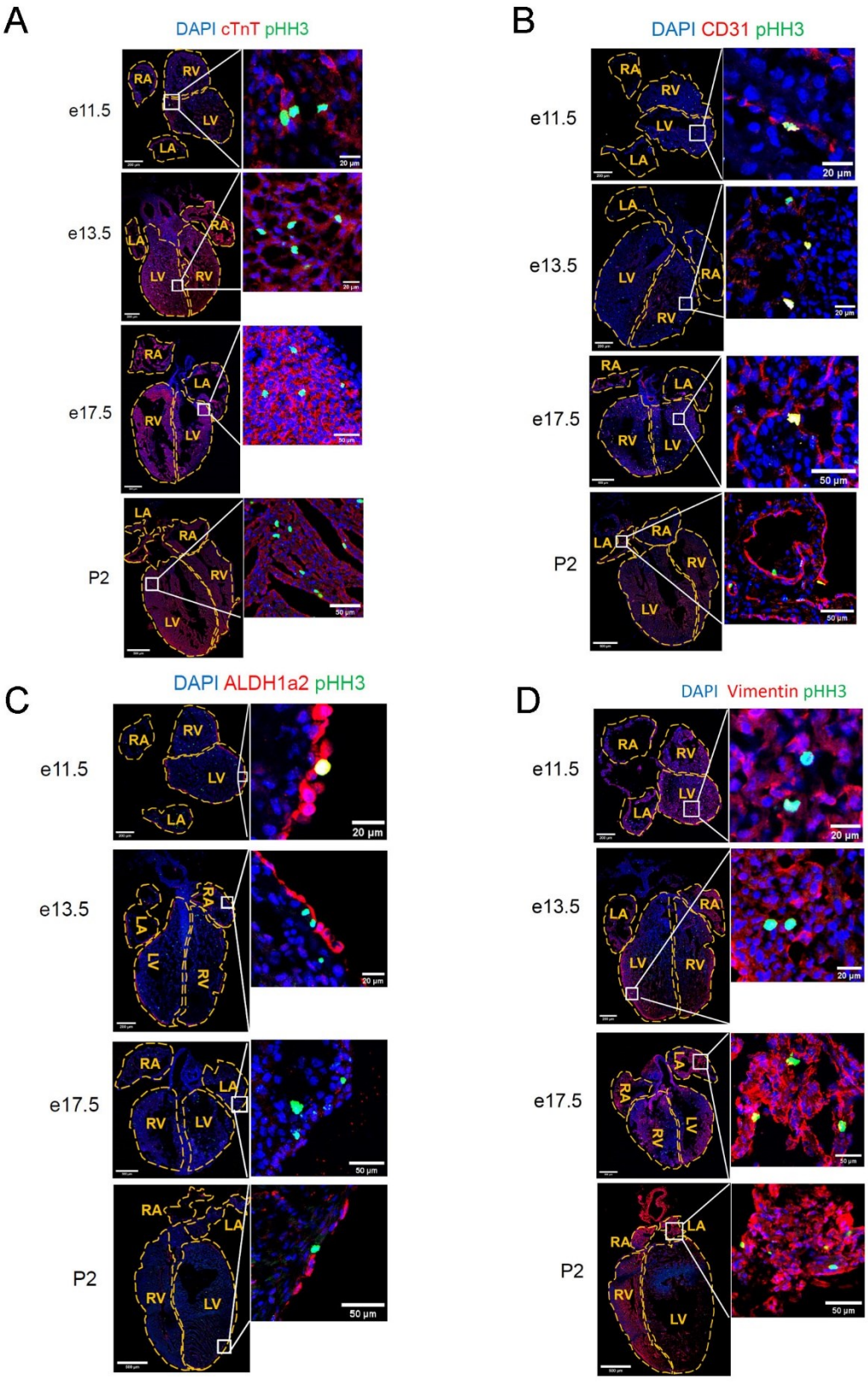



Fig S21. Representative images staining for pHH3 together with (A) CM lineage gene cTNT, (B) endothelial cell gene CD31, (C) epicardial cell gene ALDH1A2, and (D) fibroblast gene VIM. Scale bar=200μm in the images with whole heart sections at E11.5 and E13.5. Scale bar=500μm in the images with whole heart sections at E17.5 and P2. Scale bar=20μm in the enlarged images at E11.5 and E13.5. Scale bar=50μm in the E17.5 and P2 enlarged images. The staining experiments were repeated on three heart sections with similar results.

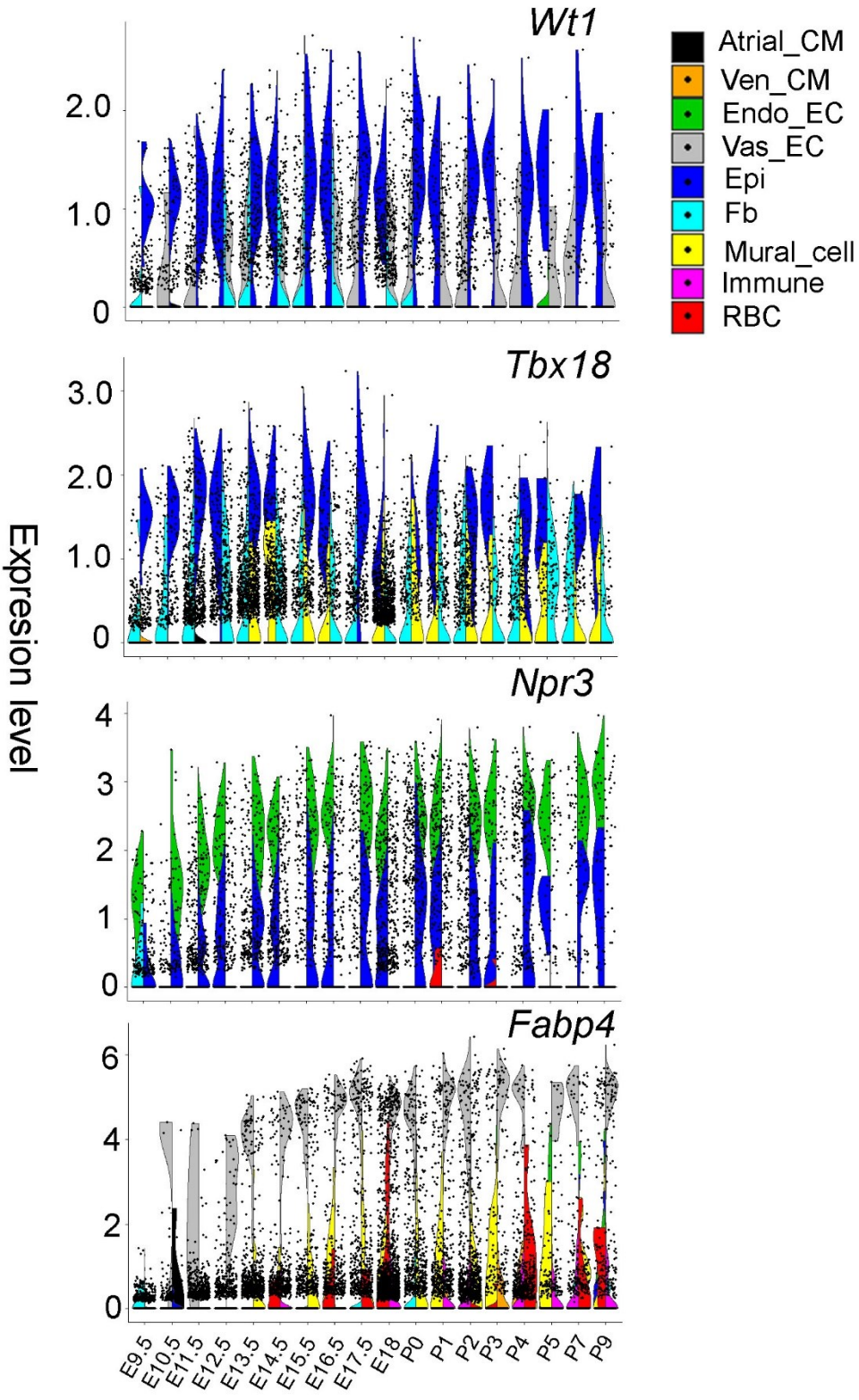



Fig S22. Violin plots showing the expression of genes *Wt1*, *Tbx18*, *Npr3*, and *Fabp4* in each cell type across the stages.

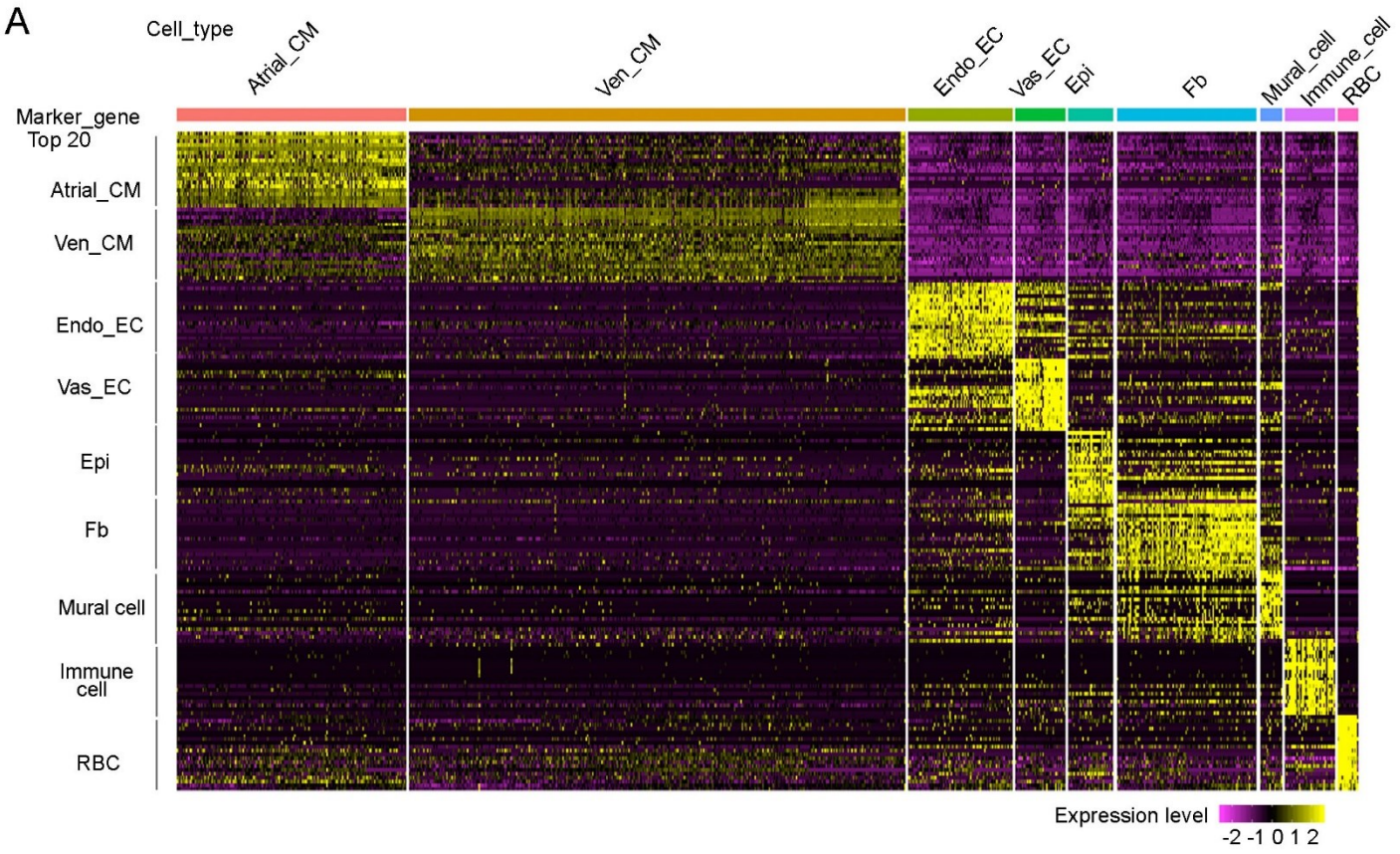



Fig S23. Expression heatmap of the top 20 genes that were uniquely expressed in each cell type.

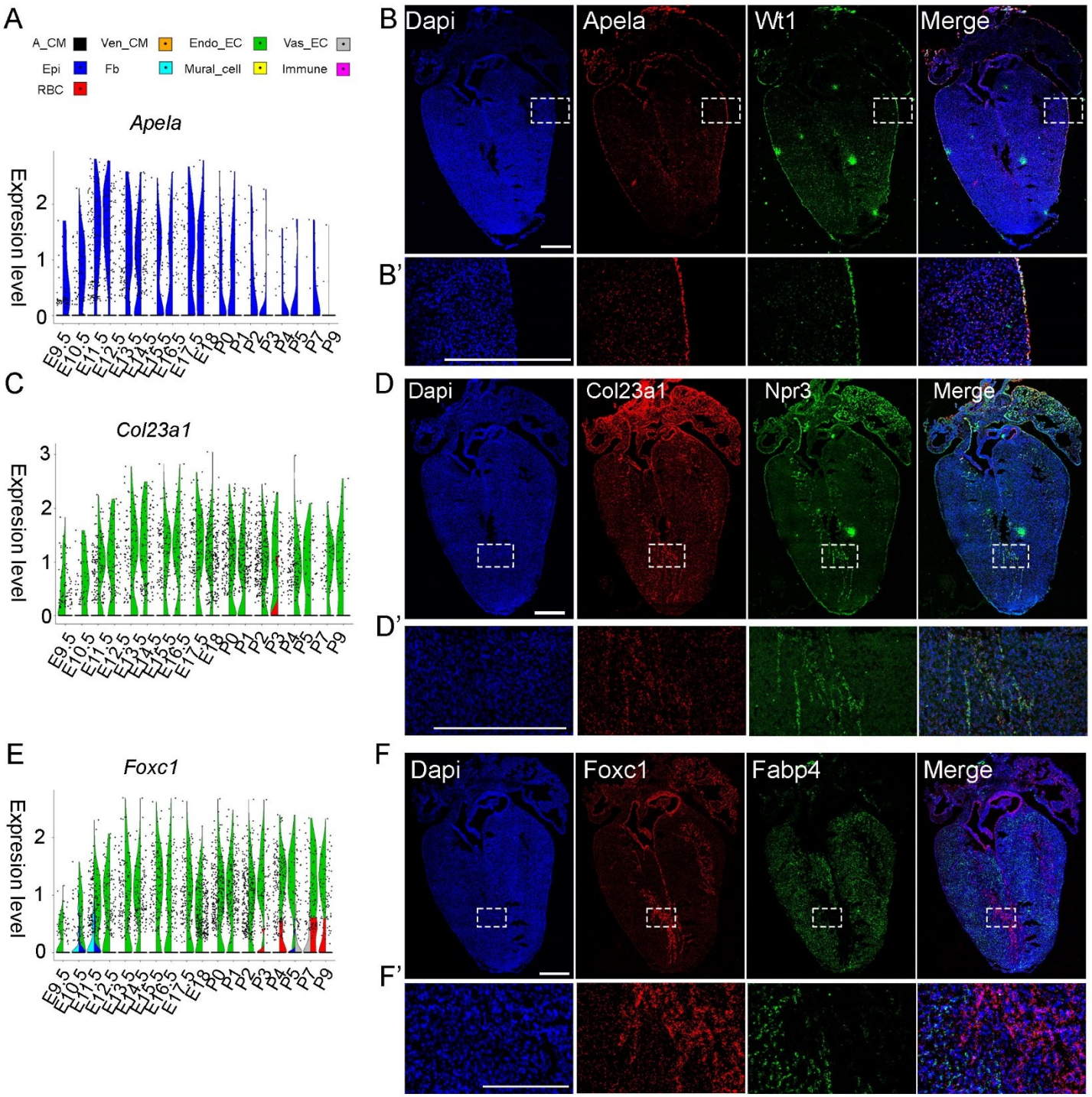



Fig S24. Expression analysis of the newly identified lineage genes. (A) Violin plot showing that *Apela* specifically expressed in epicardial cells at all stages. (B, B') *In situ* RNA staining of *Apela* confirmed its epicardium-specific expression in P2 hearts. (C, E) *Col23a1* and *Foxc1* were found to be specifically expressed in Endo_EC at most stages. (D, D') In situ RNA staining of *Col23a1* with *Npr3* at P2 hearts confirmed its Endo_EC-specific expression. (F, F') *In situ* RNA staining of *Foxc1* and *Fabp4* confirmed their complement expression pattern, indicating *Foxc1* expresses in Endo_EC. The staining experiments were repeated twice with similar results. Scale bar=500 μm.

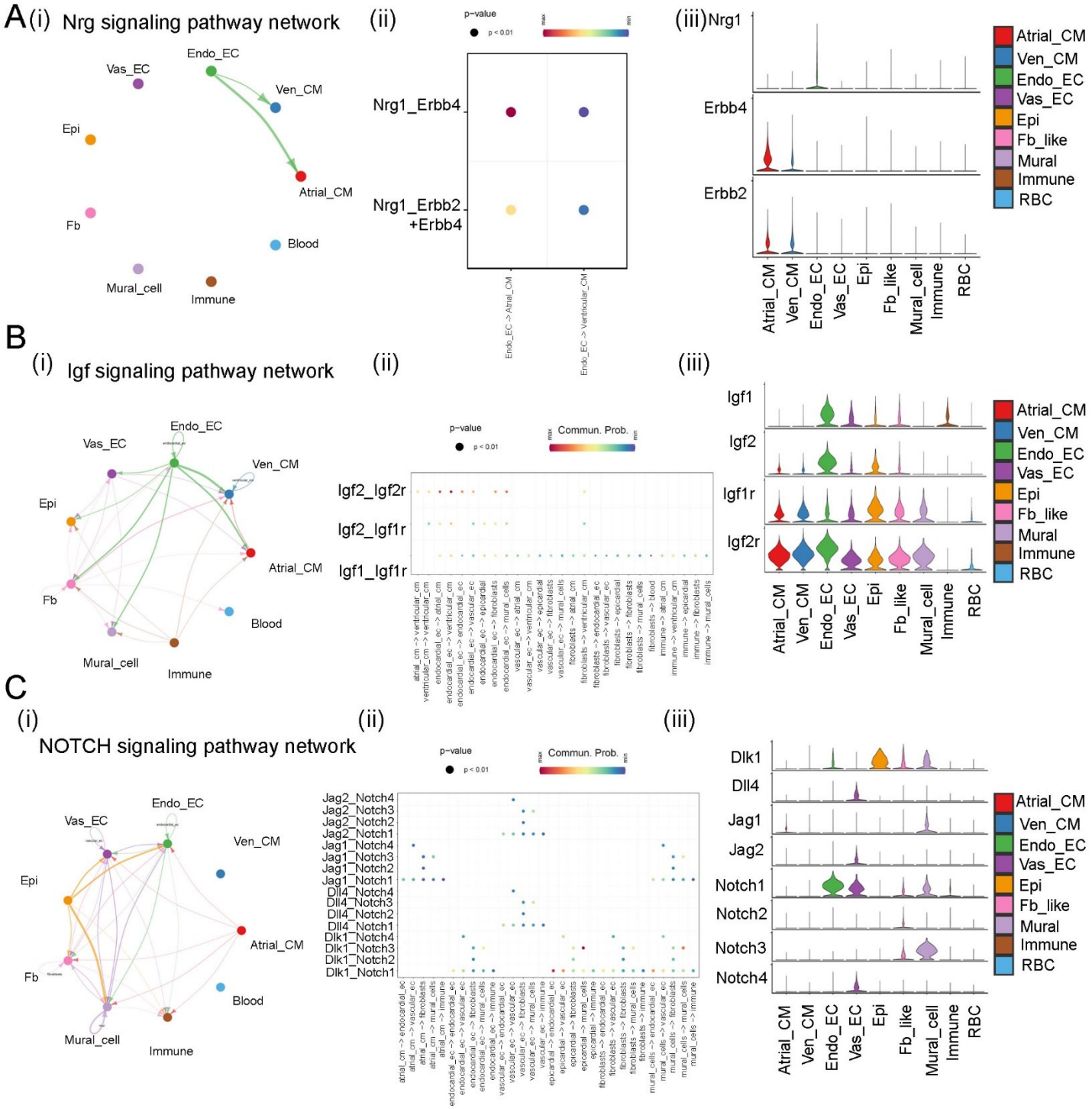



Fig S25. The ligand-receptor interactions in three representative signaling pathways. (A)(i) The network of Nrg signaling pathway. The Loops outside represent autocrine signals for each cell type; the lines connecting cell types represent paracrine signals. The lines with the same color as the cell type dots indicate the cell type secreted ligand in the interactions; the line thickness correlates with the amount of interactions. (ii) The specific ligand-receptor interactions. Each dot represents interactions between the pair of cell types. The color represents interaction probabilities. (iii) Violin plots showing the expression of ligands and receptors at each cell type. (B-C) The signaling pathway networks, ligand-receptor interactions, and ligand and receptor expression patterns in Igf and Notch signaling pathways.

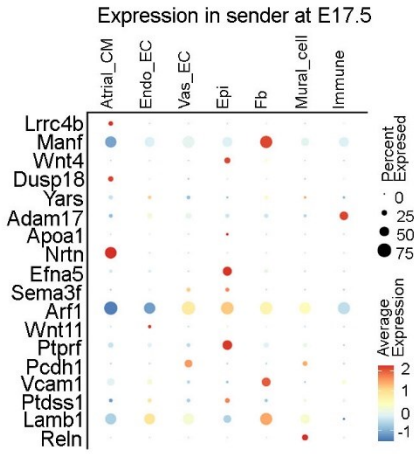



Fig S26. The expression pattern of epicardial cell derived ligands that potentially regulated genes expression in Ven_CMs at E17.5.

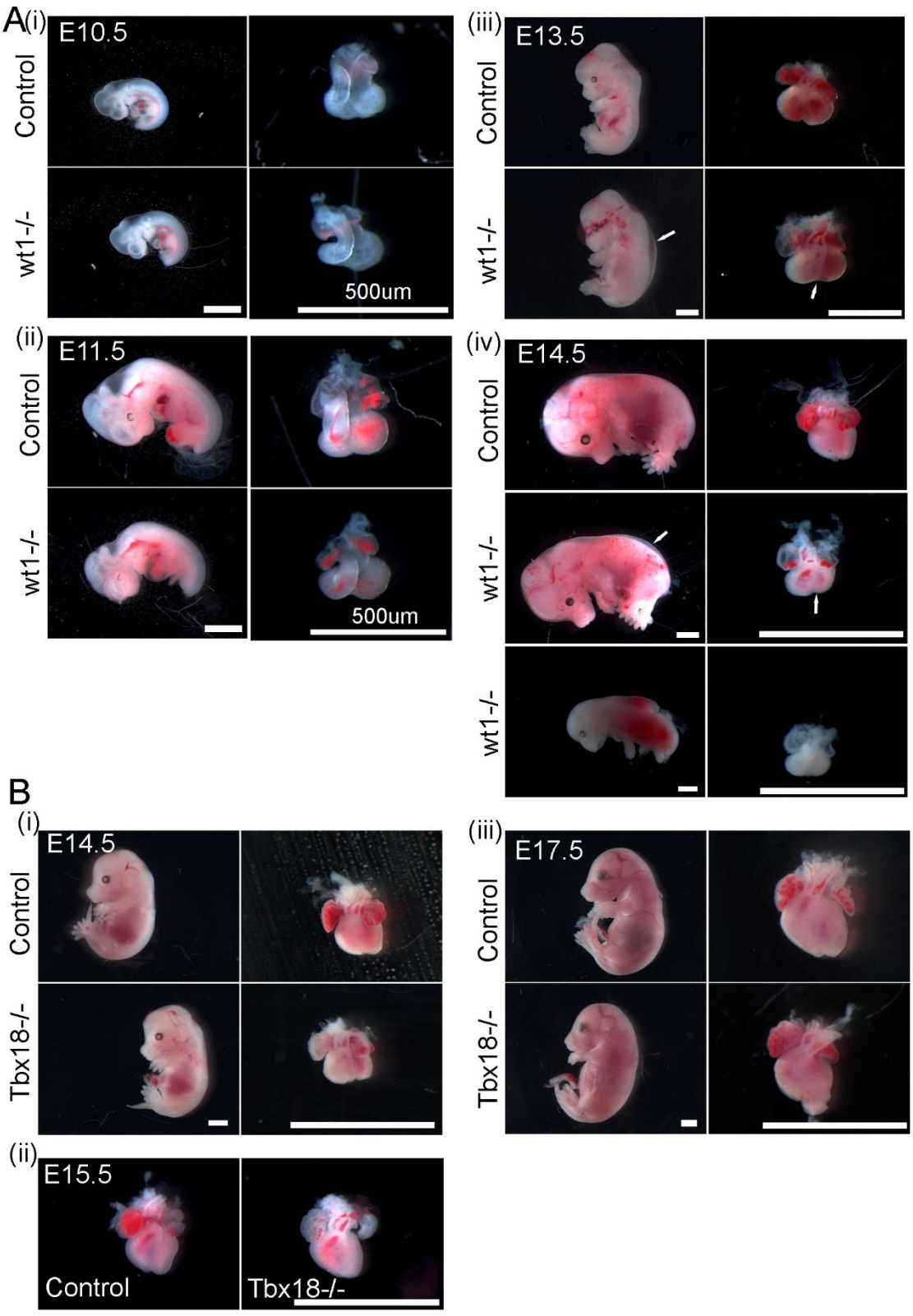



Fig S27. Representative images of control and *Wt1* or *Tbx18* mutant embryos and hearts. (A)(i-iv) Representative embryos and hearts of *Wt1* mutants and wildtype controls from the same litter at different stages. At E13.5 and E14.5, the *Wt1* mutant embryos have obvious body wall edema, and their hearts have more rounded and bifid apices, as pointed by the arrows. (B) (i-iii) Representative *Tbx18* mutant and control embryos and hearts at E14.5, E15.5, and E17.5. Except for the two labeled scale bars that represent 500um, all the other scale bars=1mm.

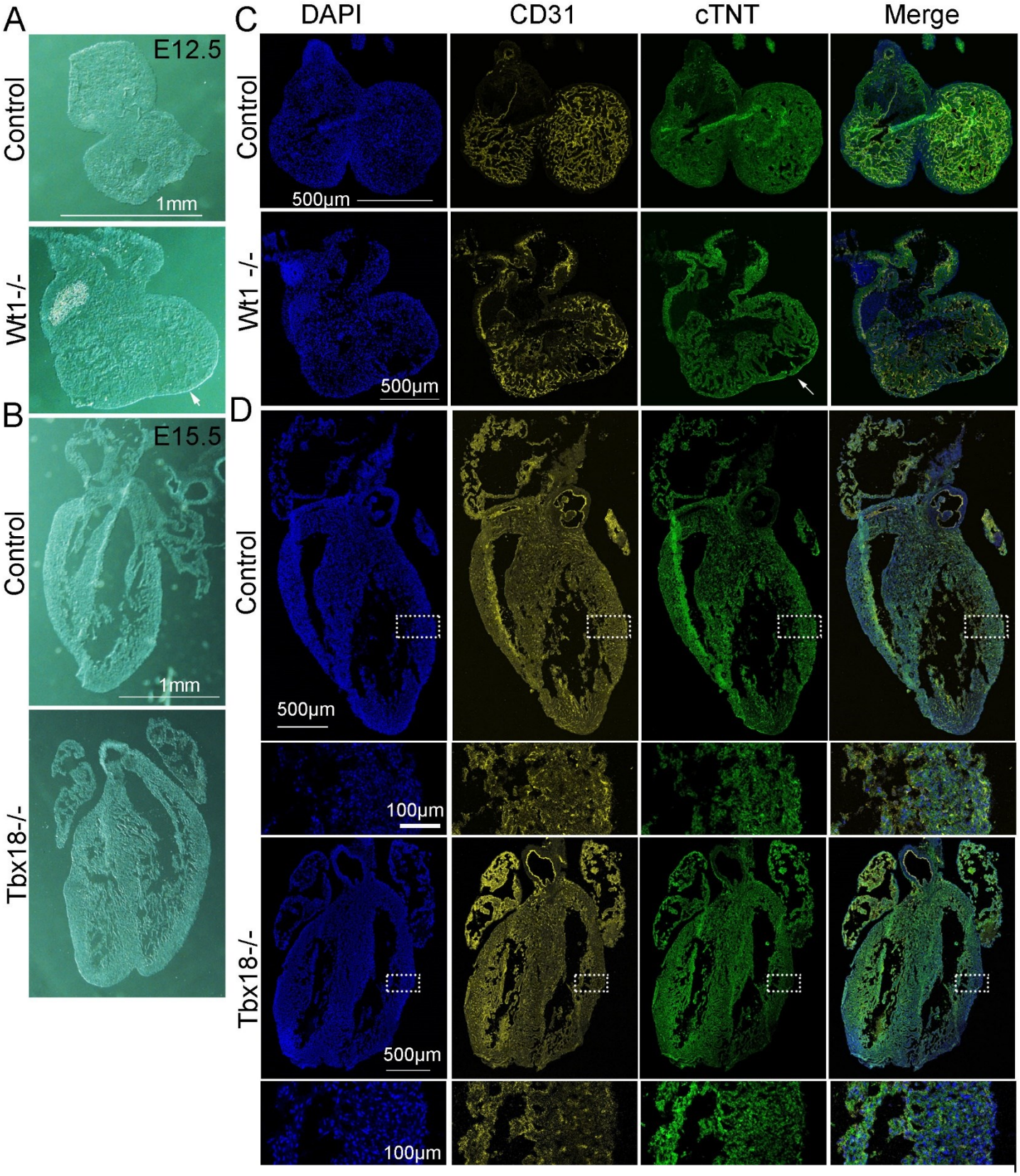



Fig S28. Histological and staining analysis of *Wt1* and *Tbx18* control and mutant hearts. (A, B) Histological images (cryo-sectioning) of *Wt1* control and mutant hearts at E12.5 and *Tbx18* control and mutant hearts at E15.5. (C) Staining analysis of CD31 (endothelial cell marker) and cTNT (cardiomyocyte marker) on control and Wt1 mutant heart sections at E12.5. Note that thinner myocardium was observed in *Wt1* mutant hearts than the control hearts (point by arrow). (D) Staining analysis of CD31 and cTNT on *Tbx18* control and mutant heart sections at E15.5. No obvious changes in vessel density were observed between controls and mutants. The staining experiments were repeated on three heart sections with similar results.

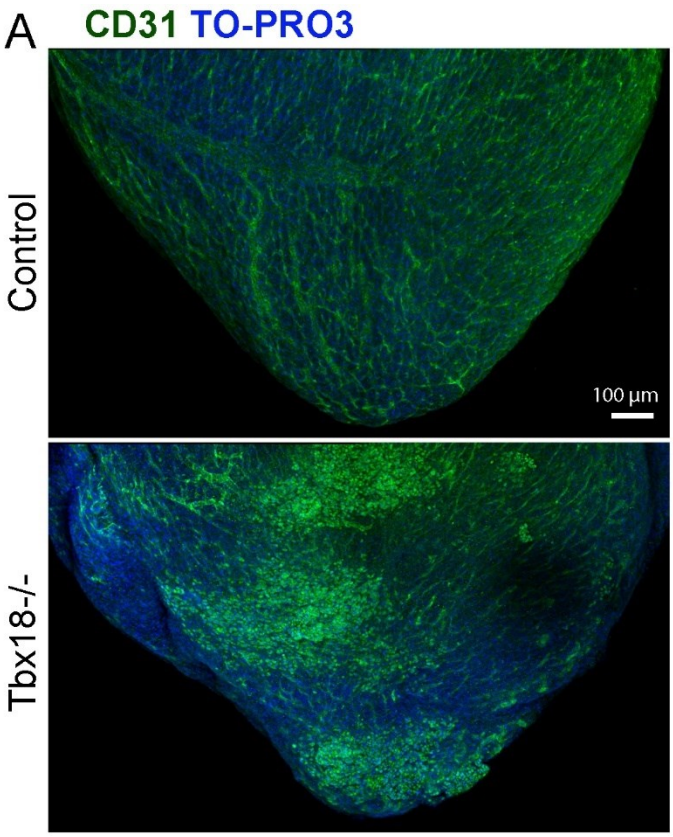



Fig S29. Whole mount staining analysis of CD31 in *Tbx18* control (*Tbx18*+/-) and mutant (*Tbx18*-/-) hearts at E17.5. Ectopic nodules with CD31-positive cells were observed in *Tbx18* mutant hearts. Only one mutant heart at this stage was analyzed, and its defects were observed. Scale bar=100 µm.

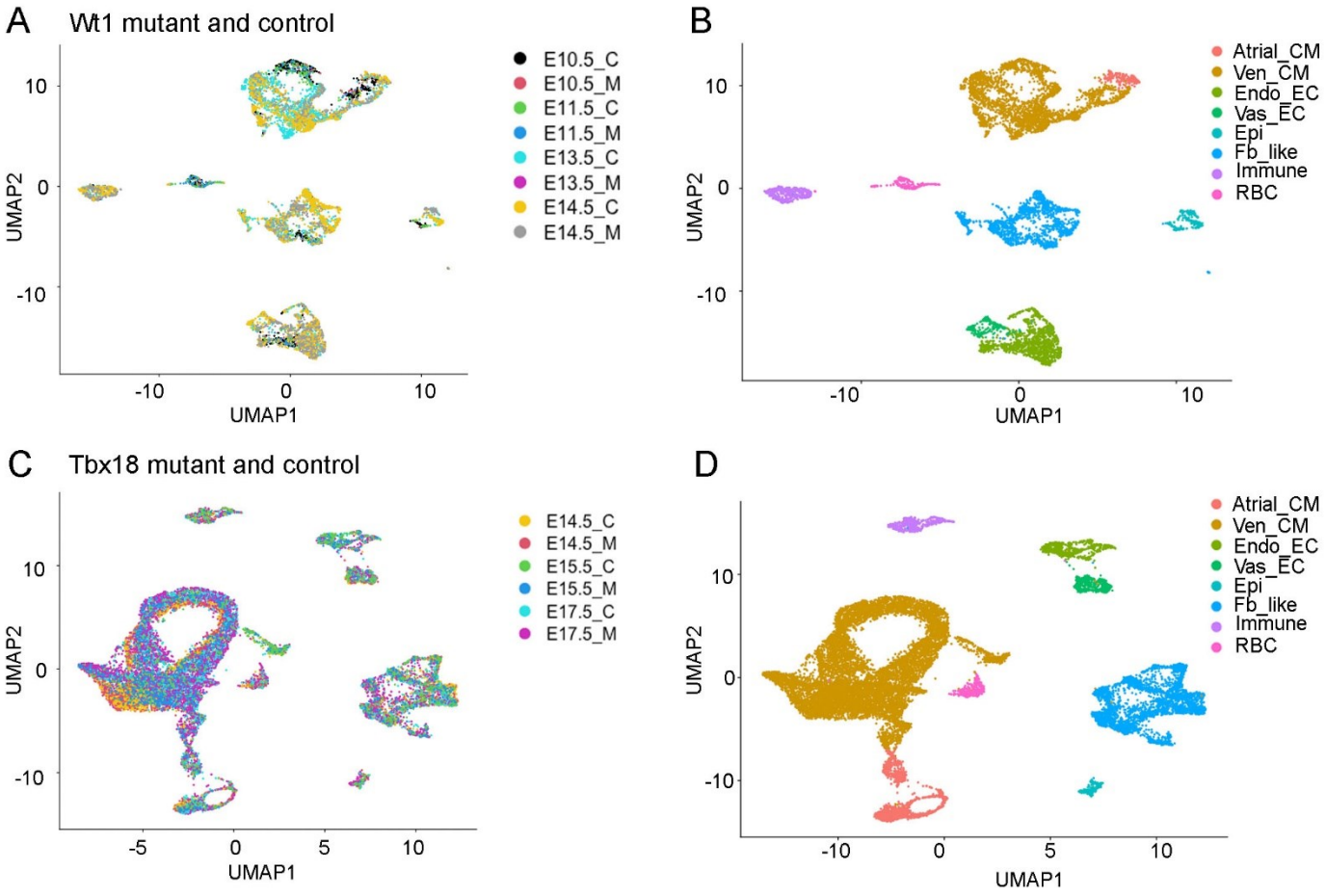
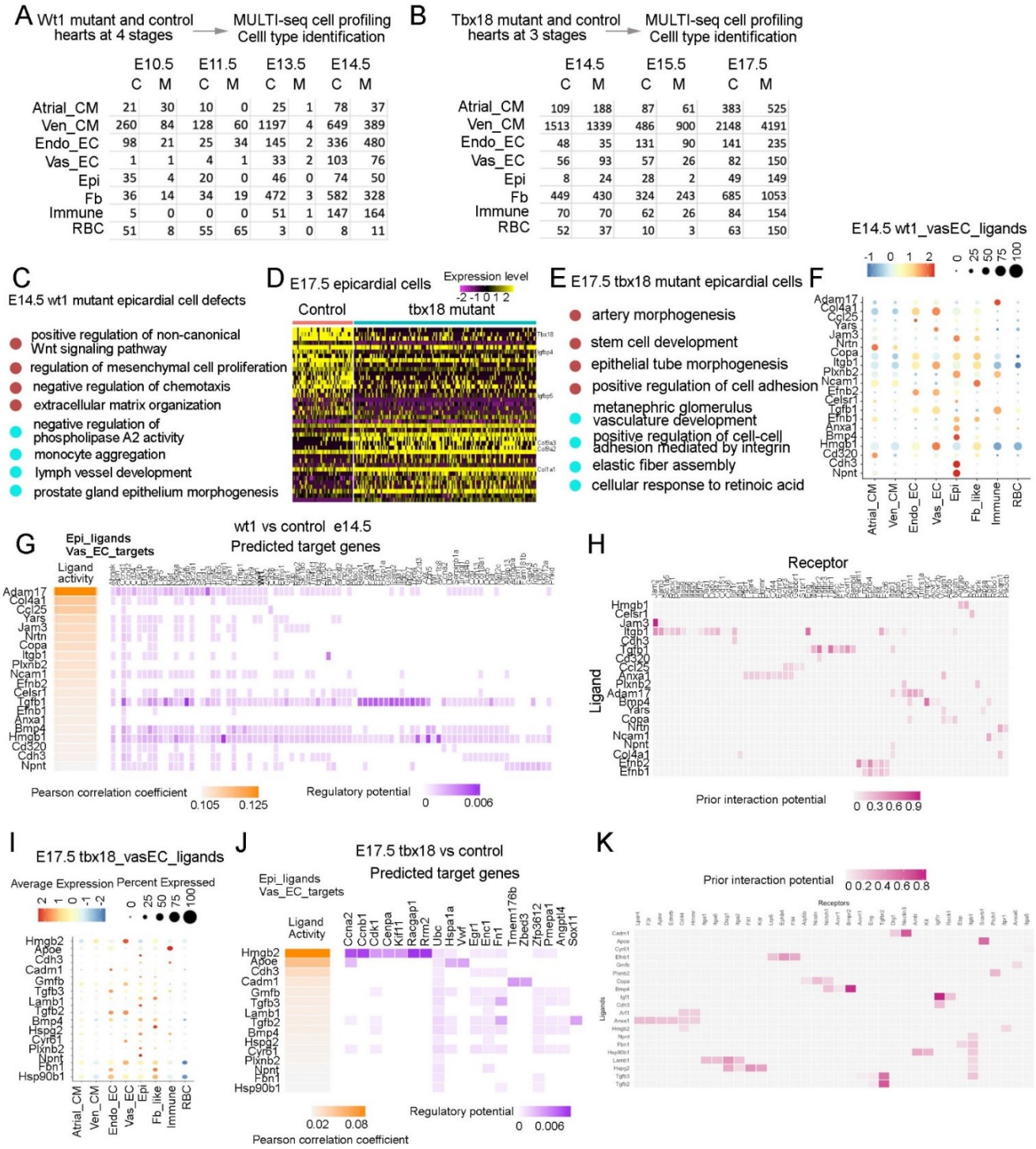



Fig S30. Unsupervised clustering analysis of the scRNA-seq data from *Wt1* and *Tbx18* mutant and control samples. (A, B) UMAP plots of *Wt1* mutant and control cells labeled by sample or cell type. (C, D) UMAP plots of *Tbx18* mutant and control cells labeled by sample or cell type.




| A | E10.5 | | E11.5 | | E13.5 | | E14.5 | |
|---|---|---|---|---|---|---|---|---|
| | C | M | C | M | C | M | C | M |
| Atrial_CM | 21 | 30 | 10 | 0 | 25 | 1 | 78 | 37 |
| Ven_CM | 260 | 84 | 128 | 60 | 1197 | 4 | 649 | 389 |
| Endo_EC | 98 | 21 | 25 | 34 | 145 | 2 | 336 | 480 |
| Vas_EC | 1 | 1 | 4 | 1 | 33 | 2 | 103 | 76 |
| Epi | 35 | 4 | 20 | 0 | 46 | 0 | 74 | 50 |
| Fb | 36 | 14 | 34 | 19 | 472 | 3 | 582 | 328 |
| Immune | 5 | 0 | 0 | 0 | 51 | 1 | 147 | 164 |
| RBC | 51 | 8 | 55 | 65 | 3 | 0 | 8 | 11 |

| B | E14.5 | | E15.5 | | E17.5 | |
|---|---|---|---|---|---|---|
| | C | M | C | M | C | M |
| Atrial_CM | 109 | 188 | 87 | 61 | 383 | 525 |
| Ven_CM | 1513 | 1339 | 486 | 900 | 2148 | 4191 |
| Endo_EC | 48 | 35 | 131 | 90 | 141 | 235 |
| Vas_EC | 56 | 93 | 57 | 26 | 82 | 150 |
| Epi | 8 | 24 | 28 | 2 | 49 | 149 |
| Fb | 449 | 430 | 324 | 243 | 685 | 1053 |
| Immune | 70 | 70 | 62 | 26 | 84 | 154 |
| RBC | 52 | 37 | 10 | 3 | 63 | 150 |

Fig S31. Detailed analysis of the *Wt1* and *Tbx18* mutant and control data. (A, B) The number of cells recovered in each cell type and sample after QC. (C) The gene pathways that are abnormally expressed in *Wt1* mutant epicardial cells at E14.5. (D, E) The expression heatmap and pathways of genes that differentially expressed in control and *Tbx18* mutant epicardial cells at E17.5. (F) The expression pattern of epicardial cell derived ligands that potentially regulated the gene's expression in Vas_ECs at e14.5 (analysis of *Wt1* mutant). (G, H) The activity of epicardial cell derived ligands, and their prior interaction potentials with receptors and regulatory potentials on target genes expression in Vas_ECs at e14.5 (analysis of *Wt1* mutant). (I) The predicted activity of epicardial cell derived ligands and their regulatory potentials on target genes expression in Vas_ECs at e17.5, and (J) the ligands' expression pattern in different cell types and (K) their prior interaction potentials with receptors (analysis of *Tbx18* mutant).

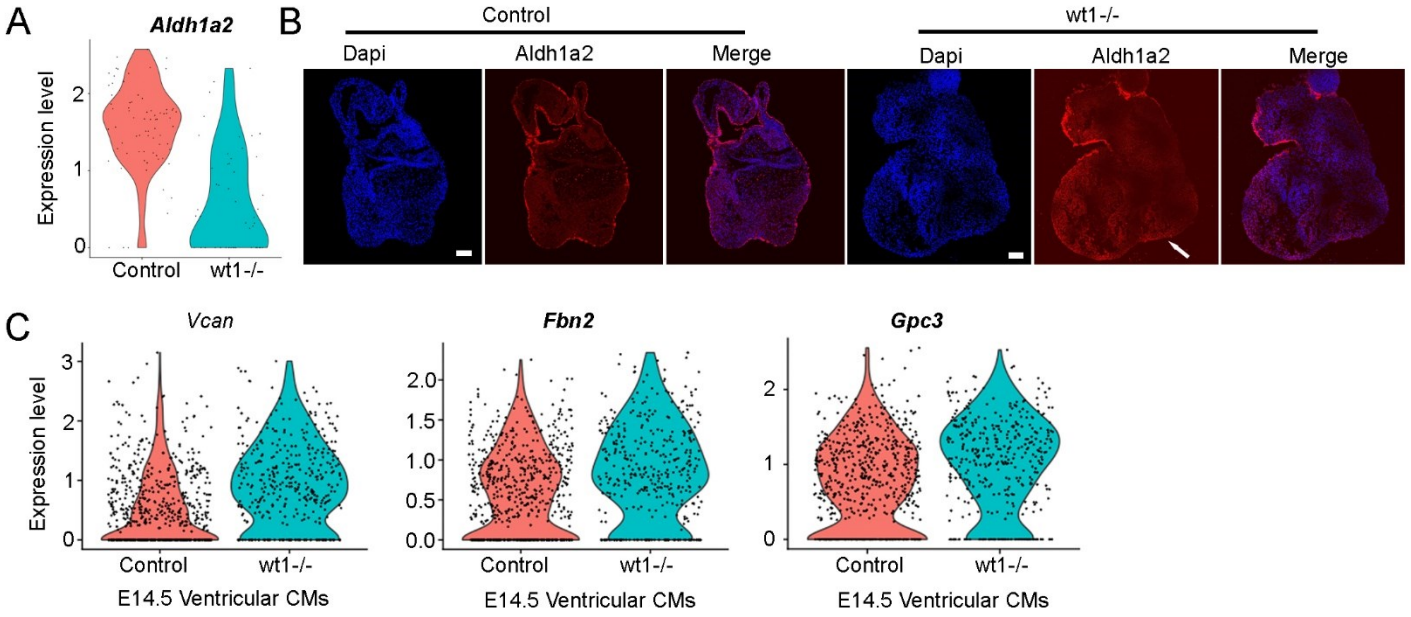



Fig S32. Gene expression analysis of the Wt1 mutant hearts. (A) ScRNA-seq data revealed a reduction of *Aldh1a2* expression in Wt1 mutant epicardial cells. (B) Antibody staining confirmed the reduction of *Aldh1a2* in *Wt1* mutant ventricular cells (arrow). However, the reduction was not observed in atrial epicardial cells. The staining experiments were repeated on three heart sections with similar results. (C) ScRNA-seq data revealed an upregulation of Tgfb3 target genes *Vcan*, *Fbn2*, and *Gpc3* in *Wt1* mutant ventricular CMs compared to control CMs. Scale bar=100 μm.

Table S1. The sequence of barcodes used in the MULTI-seq experiments.

| | |
|---|---|
| Anchor LMO | 5’-TGGAATTCTCGGGTGCCAAGGgtaacgatccagctgtcact-Lipid-3’ |
| Co-Anchor LMO | 5’-Lipid-AGTGACAGCTGGATCGTTAC-3’ |
| MULTI-seq Additive Primer | 5’-CTTGGCACCCGAGAATTCC-3’ |
| TruSeq RPIX Primer | 5’-CAAGCAGAAGACGGCATACGAGATNNNNNNGTGACTGGAGTT CCTTGGCACCCGAGAATTCCA-3’ |
| TruSeq Universal Adapter Primer | 5’-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGA CGCTCTTCCGATCT-3’ |
| BC1 | CCTTGGCACCCGAGAATTCCAGGAGAAGAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC2 | CCTTGGCACCCGAGAATTCCACCACAATGAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC3 | CCTTGGCACCCGAGAATTCCATGAGACCTAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC4 | CCTTGGCACCCGAGAATTCCAGCACACGCAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC5 | CCTTGGCACCCGAGAATTCCAAGAGAGAGAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC6 | CCTTGGCACCCGAGAATTCCATCACAGCAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC7 | CCTTGGCACCCGAGAATTCCAGAAAAGGGAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC8 | CCTTGGCACCCGAGAATTCCACGAGATTCAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC9 | CCTTGGCACCCGAGAATTCCAGTAGCACTAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC10 | CCTTGGCACCCGAGAATTCCACGACCAGCAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC11 | CCTTGGCACCCGAGAATTCCATTAGCCAGAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC12 | CCTTGGCACCCGAGAATTCCAGGACCCCAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC13 | CCTTGGCACCCGAGAATTCCACCAACCGGAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC14 | CCTTGGCACCCGAGAATTCCATGACCGATAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC15 | CCTTGGCACCCGAGAATTCCAGCAACGCCAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC16 | CCTTGGCACCCGAGAATTCCACAATCGGTAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC17 | CCTTGGCACCCGAGAATTCCAATAGCGTCAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC18 | CCTTGGCACCCGAGAATTCCAGAATCTCGAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC19 | CCTTGGCACCCGAGAATTCCACTAGCTGAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC20 | CCTTGGCACCCGAGAATTCCAAGACCTTGAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC21 | CCTTGGCACCCGAGAATTCCAGAAGGAAGAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |

| | |
|---|---|
| BC22 | CCTTGGCACCCGAGAATTCCACTACGACAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC23 | CCTTGGCACCCGAGAATTCCAAGAAGAGGAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC24 | CCTTGGCACCCGAGAATTCCAGTACGCATAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC25 | CCTTGGCACCCGAGAATTCCACGAAGCCCAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC26 | CCTTGGCACCCGAGAATTCCAACATGCGTAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC27 | CCTTGGCACCCGAGAATTCCATAAGGCTCAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC28 | CCTTGGCACCCGAGAATTCCAGGAAGGAAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC29 | CCTTGGCACCCGAGAATTCCACCATGGCGAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC30 | CCTTGGCACCCGAGAATTCCAAAAGGGGAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC31 | CCTTGGCACCCGAGAATTCCATTACGGTGAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC32 | CCTTGGCACCCGAGAATTCCAGCATGTACAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC33 | CCTTGGCACCCGAGAATTCCACAAGGTCTAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC34 | CCTTGGCACCCGAGAATTCCAATACGTGCAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC35 | CCTTGGCACCCGAGAATTCCAGCAGTATCAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC36 | CCTTGGCACCCGAGAATTCCAAGATTCCGAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC37 | CCTTGGCACCCGAGAATTCCATCAGTCGAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC38 | CCTTGGCACCCGAGAATTCCAGAACTCTGAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC39 | CCTTGGCACCCGAGAATTCCACGATTGACAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC40 | CCTTGGCACCCGAGAATTCCAACAGTGCTAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC41 | CCTTGGCACCCGAGAATTCCATAACTGGCAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC42 | CCTTGGCACCCGAGAATTCCACCAGTTAGAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC43 | CCTTGGCACCCGAGAATTCCAGGCTAACTAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC44 | CCTTGGCACCCGAGAATTCCACCCGAAGCAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC45 | CCTTGGCACCCGAGAATTCCATGCTACAGAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC46 | CCTTGGCACCCGAGAATTCCAGCCGACCAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |
| BC47 | CCTTGGCACCCGAGAATTCCACACCACGGAAAAAAAAAAAAAAAA AAAAAAAAAAAAA |

| | |
|---|---|
| BC48 | CCTTGGCACCCGAGAATTCCATCCGAGATAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC49 | CCTTGGCACCCGAGAATTCCAGACCAGCCAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC50 | CCTTGGCACCCGAGAATTCCACTCAAGGTAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC51 | CCTTGGCACCCGAGAATTCCAAGCTAGTCAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC52 | CCTTGGCACCCGAGAATTCCAGTCAATCGAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC53 | CCTTGGCACCCGAGAATTCCACGCTATGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC54 | CCTTGGCACCCGAGAATTCCAACCGATTGAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC55 | CCTTGGCACCCGAGAATTCCAGTCTCAAGAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC56 | CCTTGGCACCCGAGAATTCCACGCGCACAAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC57 | CCTTGGCACCCGAGAATTCCAACCCCAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC58 | CCTTGGCACCCGAGAATTCCAGGCGCCATAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC59 | CCTTGGCACCCGAGAATTCCAAACACCGTAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC60 | CCTTGGCACCCGAGAATTCCATTCTCCTCAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC61 | CCTTGGCACCCGAGAATTCCAGCCCCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC62 | CCTTGGCACCCGAGAATTCCACACACGCGAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC63 | CCTTGGCACCCGAGAATTCCAATCTCGGAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC64 | CCTTGGCACCCGAGAATTCCATGCGCGTGAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC65 | CCTTGGCACCCGAGAATTCCAGACACTACAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC66 | CCTTGGCACCCGAGAATTCCACTCTCTCTAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC67 | CCTTGGCACCCGAGAATTCCAAGCGCTGCAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC68 | CCTTGGCACCCGAGAATTCCATCCCCTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC69 | CCTTGGCACCCGAGAATTCCACTCGGAATAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC70 | CCTTGGCACCCGAGAATTCCAAGCCGACCAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC71 | CCTTGGCACCCGAGAATTCCATCCAGAGTAAAAAAAAAAAAAAAAAAAAAAAAAAAA |
| BC72 | CCTTGGCACCCGAGAATTCCAGACTGATCAAAAAAAAAAAAAAAAAAAAAAAAAAAA |

Table S2. The sequence of H probes that were used for single molecular in situ hybridization (PLISH) analysis.

| Name | Sequence |
|---|---|
| cy5-mApela-Right-1 | atgtgtggaaagacggccatTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mApela-left-1 | TAGCGCTAACAACTTACGTCGTTATGaggacgtgatgtactggtat |
| cy5-mApela-Right-2 | attcagacaaacgcatgtgcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mApela-left-2 | TAGCGCTAACAACTTACGTCGTTATGtttaacctcgtctgttttcc |
| cy5-mApela-Right-3 | tgaaaagccatccacggtacTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mApela-left-3 | TAGCGCTAACAACTTACGTCGTTATGtctgaaacagcctctgttgc |
| cy5-mApela-Right-4 | cgtctgtaaatcgcagtatcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mApela-left-4 | TAGCGCTAACAACTTACGTCGTTATGtgtccggctcaccccacatc |
| cy5-mApela-Right-5 | ccattcaggcacgcagtaacTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mApela-left-5 | TAGCGCTAACAACTTACGTCGTTATGttaagcgggaagtctctgca |
| cy5-mLrrn4-Right-1 | gttaccagaaggccagttttTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mLrrn4-left-1 | TAGCGCTAACAACTTACGTCGTTATGaactgtaactctggaaatac |
| cy5-mLrrn4-Right-2 | tactccgtatatccctcagtTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mLrrn4-left-2 | TAGCGCTAACAACTTACGTCGTTATGaccaagtgagatactacact |
| cy5-mLrrn4-Right-3 | tactgagggatatacggagtTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mLrrn4-left-3 | TAGCGCTAACAACTTACGTCGTTATGactgaccaatgtaaaggatg |
| cy5-mLrrn4-Right-4 | ggagctagtaagacaggcgtTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mLrrn4-left-4 | TAGCGCTAACAACTTACGTCGTTATGagtgagaacaactataggag |
| cy5-mLrrn4-Right-5 | cagctcaaggccaacagaacTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mLrrn4-left-5 | TAGCGCTAACAACTTACGTCGTTATGtcattgtgctaaaatcgggc |
| cy5-mAdamts8-Right-1 | AGGTCAGCACATAAGAGGGCTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mAdamts8-left-1 | TAGCGCTAACAACTTACGTCGTTATGTGATAGTGCAGCTTGCTGAG |
| cy5-mAdamts8-Right-2 | AGTGGGTAATAGGTAGGGACTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mAdamts8-left-2 | TAGCGCTAACAACTTACGTCGTTATGTGTTCCAGGTTAATAGCACC |
| cy5-mAdamts8-Right-3 | CTAGACAATTTCATTGGTGTTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mAdamts8-left-3 | TAGCGCTAACAACTTACGTCGTTATGAAGATCACACTTTAGTTCGG |
| cy5-mAdamts8-Right-4 | ATTTTTCCCCGCTGTGAGGTTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mAdamts8-left-4 | TAGCGCTAACAACTTACGTCGTTATGAGATTGATTATTGGGGAAAC |
| cy5-mAdamts8-Right-5 | CGGACGCAGATGGACAGAGTTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mAdamts8-left-5 | TAGCGCTAACAACTTACGTCGTTATGATCCGGTCCACACAGAGTGC |
| cy5-mDgit4l-Right-1 | ccagagaactgctaacgggtTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mDgit4l-left-1 | TAGCGCTAACAACTTACGTCGTTATGaaaagatctatgtccatgcc |
| cy5-mDgit4l-Right-2 | ggcccagtcttgtgaagactTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mDgit4l-left-2 | TAGCGCTAACAACTTACGTCGTTATGagctccgtggtacctcccca |
| cy5-mDgit4l-Right-3 | ttgctgaatagaacagagttTTATACGTCGAGTTGAACGTCGTAACA |
| cy5mDgit4l-left-3 | TAGCGCTAACAACTTACGTCGTTATGacgtggaaggtaaattgtct |
| cy5-mDgit4l-Right-4 | tttcatatctagtctgtggtTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mDgit4l-left-4 | TAGCGCTAACAACTTACGTCGTTATGagcaacaacatctcagagtc |
| cy5-mDgit4l-Right-5 | acaaagcagcaatgtgacctTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mDgit4l-left-5 | TAGCGCTAACAACTTACGTCGTTATGagtggatccgaaagggacgg |
| cy5-mAdm-Right-1 | tagttccctcttcccacgacTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mAdm-left-1 | TAGCGCTAACAACTTACGTCGTTATGttagcgcccacttattccac |

| | |
|---|---|
| cy5-mAdm-Right-2 | tctgggtaggaactgtcgtcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mAdm-left-2 | TAGCGCTAACAACTTACGTCGTTATGtcatcagcgagtcccgtagg |
| cy5-mAdm-Right-3 | gcttcgctctgattgctggcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mAdm-left-3 | TAGCGCTAACAACTTACGTCGTTATGttgtagggggccagttgtgt |
| cy5-mAdm--Right-4 | tcaatgctgtcacccgcaccTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mAdm--left-4 | TAGCGCTAACAACTTACGTCGTTATGtatatcctaaagagtctgga |
| cy5-mAdm--Right-5 | cgcaggcgccaacgggatacTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mAdm--left-5 | TAGCGCTAACAACTTACGTCGTTATGtcgcccgactgttcaatgct |
| cy5-mBmp10-Right-1 | cttcttccaggggcgactgcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mBmp10-left-1 | TAGCGCTAACAACTTACGTCGTTATGtcaaggcccataatggggct |
| cy5-mBmp10-Right-2 | tgaagtcaataccatcttgcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mBmp10-left-2 | TAGCGCTAACAACTTACGTCGTTATGtccgtgaagatatcatcaaa |
| cy5-mBmp10-Right-3 | cctcgctaccgtctgcactcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mBmp10-left-3 | TAGCGCTAACAACTTACGTCGTTATGtctagtacctcaaaaatggt |
| cy5-mBmp10-Right-4 | ctcctcgctaccgtctgcacTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mBmp10-left-4 | TAGCGCTAACAACTTACGTCGTTATGtctctagtacctcaaaaatg |
| cy5-mBmp10-Right-5 | ctgttggttccgtagatctcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mBmp10-left-5 | TAGCGCTAACAACTTACGTCGTTATGtgttgatactaagaccagca |
| cy5-mBmp10-Right-6 | cactgttggttccgtagatcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mBmp10-left-6 | TAGCGCTAACAACTTACGTCGTTATGtctgttgatactaagaccag |
| cy5-mMest-Right-1 | aagaaatcaagggcgatcacTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mMest-left-1 | TAGCGCTAACAACTTACGTCGTTATGtcgatggaacctcagggtca |
| cy5-mMest-Right-2 | ccgaccacaccgacagaatcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mMest-left-2 | TAGCGCTAACAACTTACGTCGTTATGttggtagaagatgcgtaggc |
| cy5-mMest-Right-3 | gccgaggcgcccgcagcgatTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mMest-left-3 | TAGCGCTAACAACTTACGTCGTTATGacaggatcggaggtggcgtc |
| cy5-mMest-Right-4 | attaatgtactgtaaaagacTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mMest-left-4 | TAGCGCTAACAACTTACGTCGTTATGtgtcgatgaccaggttgccg |
| cy5-mMest-Right-5 | ctgcatttgggctatggaacTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mMest-left-5 | TAGCGCTAACAACTTACGTCGTTATGtgcgcaagcgatctcggcgc |
| cy5-mSfrp2-Right-1 | attcgcctgacatggcaaccTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mSfrp2-left-1 | TAGCGCTAACAACTTACGTCGTTATGtaagtgcacggtataagcca |
| cy5-mSfrp2-Right-2 | ggccggcaggaggtggtcgc TTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mSfrp2-left-2 | TAGCGCTAACAACTTACGTCGTTATGtactagcgagggggatgcag |
| cy5-mSfrp2-Right-3 | cacggctggatggtctcatcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mSfrp2-left-3 | TAGCGCTAACAACTTACGTCGTTATGtaggtcgtcgagacagacag |
| cy5-mSfrp2-Right-4 | acgcatgcaatggtcagagcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mSfrp2-left-4 | TAGCGCTAACAACTTACGTCGTTATGtcatgagggcataaaacgag |
| cy5-mSfrp2-Right-5 | gcagccgcatgttctggtacTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mSfrp2-left-5 | TAGCGCTAACAACTTACGTCGTTATGtcgatgccgtggcacagctg |
| cy5-mCldn5-Right-1 | cctacttcaccgatgaaatcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mCldn5-left-1 | TAGCGCTAACAACTTACGTCGTTATGtgagcgttccacccacgtcg |
| cy5-mCldn5-Right-2 | agcgctcctcccgtgagtgcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mCldn5-left-2 | TAGCGCTAACAACTTACGTCGTTATGtacccgtgccttaactgggc |
| cy5-mCldn5-Right-3 | ctggacattaaggcagcatcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mCldn5-left-3 | TAGCGCTAACAACTTACGTCGTTATGtagtgcccccaggatctcag |

| | |
|---|---|
| cy5-mCldn5-Right-4 | cggtctttcccatcgcgggcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mCldn5-left-4 | TAGCGCTAACAACTTACGTCGTTATGttagctgcgggaagagcccc |
| cy5-mCldn5-Right-5 | gcaccgtcggatcatagaacTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mCldn5-left-5 | TAGCGCTAACAACTTACGTCGTTATGtcgcggacaacgatgttggc |
| cy5-mCol23a1-Right-1 | ctgtgacaactcaaagctttTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mCol23a1-left-1 | TAGCGCTAACAACTTACGTCGTTATGaaggacagggaatctagtaa |
| cy5-mCol23a1-Right-2 | cagctagtaaccatcggaatTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mCol23a1-left-2 | TAGCGCTAACAACTTACGTCGTTATGaaaactcacccagaggtgac |
| cy5-mCol23a1-Right-3 | acagatgagtggtgttatttTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mCol23a1-left-3 | TAGCGCTAACAACTTACGTCGTTATGaagatgccgtaacaagggct |
| cy5-mCol23a1-Right-4 | gccctaacgatgttgttcttTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mCol23a1-left-4 | TAGCGCTAACAACTTACGTCGTTATGaggatgaaggaggagtgctt |
| cy5-mCol23a1-Right-5 | ttcaggttcatggtgtccgtTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mCol23a1-left-5 | TAGCGCTAACAACTTACGTCGTTATGaccatttactaaggaggcct |
| cy5-mFoxc1-Right-1 | agttgttcaagccgatccgcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mFoxc1-left-1 | TAGCGCTAACAACTTACGTCGTTATGtgagactcgaacatttcccg |
| cy5-mFoxc1-Right-2 | ggaggaacgttcggatcaacTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mFoxc1-left-2 | TAGCGCTAACAACTTACGTCGTTATGttccaggcgcagtcggggca |
| cy5-mFoxc1-Right-3 | cagtctcttgcgccggccgcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mFoxc1-left-3 | TAGCGCTAACAACTTACGTCGTTATGttaaggaagcattgggaaaa |
| cy5-mFoxc1-Right-4 | gggctgcggctgcggcgtatTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mFoxc1-left-4 | TAGCGCTAACAACTTACGTCGTTATGaaggcccgtaggcgcgcgcc |
| cy5-mFoxc1-Right-5 | gttccattccatttggctctTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mFoxc1-left-5 | TAGCGCTAACAACTTACGTCGTTATGaagagtgccgggaatagggt |
| cy5-mPlvap-Right-1 | ggaagctggcgttgatgcgcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mPlvap-left-1 | TAGCGCTAACAACTTACGTCGTTATGtccatctcacgtcgcgtagt |
| cy5-mPlvap-Right-2 | ctaagatccacccggaacgcTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mPlvap-left-2 | TAGCGCTAACAACTTACGTCGTTATGtcatctacaattgaggccct |
| cy5-mPlvap-Right-3 | cgcgctctagctccaacttc TTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mPlvap-left-3 | TAGCGCTAACAACTTACGTCGTTATGtggcgccgcagttctgcatt |
| cy5-mPlvap-Right-4 | atctggaaaccctagtggccTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mPlvap-left-4 | TAGCGCTAACAACTTACGTCGTTATGtttaagagggaggctggaaa |
| cy5-mPlvap-Right-5 | cggcatcagctggtagtggtTTATACGTCGAGTTGAACGTCGTAACA |
| cy5-mPlvap-left-5 | TAGCGCTAACAACTTACGTCGTTATGagggcagggtttccaaggta |
| cy3-mWt1-Right-1 | cggaacccatgaggtgcggcTTATACGTCGAGTTGACCGACGTATTG |
| cy3-mWt1-left-1 | TATTCGTTCGAACTTACGTCGTTATGtcggatgcggacggttctcc |
| cy3-mWt1-Right-2 | aaagtgaccgtgctgtatccTTATACGTCGAGTTGACCGACGTATTG |
| cy3-mWt1-left-2 | TATTCGTTCGAACTTACGTCGTTATGttggttgcggatggtaggct |
| cy3-mWt1-Right-3 | gttactcaaaacctatgagcTTATACGTCGAGTTGACCGACGTATTG |
| cy3-mWt1-left-3 | TATTCGTTCGAACTTACGTCGTTATGtttaacaatcgcctgtagaa |
| cy3-mWt1-Right-4 | cacacagtgatgcagctaatTTATACGTCGAGTTGACCGACGTATTG |
| cy3-mWt1-left-4 | TATTCGTTCGAACTTACGTCGTTATGaccatacacctcctaatttt |
| cy3-mNpr3-Right-1 | ttgcaaggagagctgttggtTTATACGTCGAGTTGACCGACGTATTG |
| cy3-mNpr3-left-1 | TATTCGTTCGAACTTACGTCGTTATGatgctccacgattctggtct |
| cy3-mNpr3-Right-2 | taagctgaacagacttctgtTTATACGTCGAGTTGACCGACGTATTG |
| cy3-mNpr3-left-2 | TATTCGTTCGAACTTACGTCGTTATGacatcttcagccagactagc |

| | |
|---|---|
| cy3-mNpr3-Right-3 | cctagctcactgtccaagctTTATACGTCGAGTTGACCGACGTATTG |
| cy3-mNpr3-left-3 | TATTCGTTCGAACTTACGTCGTTATGatgcatcatttgtggtggct |
| cy3-mNpr3-Right-4 | tagaataggaagctatatgtTTATACGTCGAGTTGACCGACGTATTG |
| cy3-mNpr3-left-4 | TATTCGTTCGAACTTACGTCGTTATGagctggtgcagctttgactc |
| cy3-mFabp4-Right-1 | ctgcagcacaggagggtgctTTATACGTCGAGTTGACCGACGTATTG |
| cy3-mFabp4-left-1 | TATTCGTTCGAACTTACGTCGTTATGatgagcctctgaagtccaga |
| cy3-mFabp4-Right-2 | gtggcaaagcccactcccacTTATACGTCGAGTTGACCGACGTATTG |
| cy3-mFabp4-left-2 | TATTCGTTCGAACTTACGTCGTTATGttctttcatgtaatcatcga |
| cy3-mFabp4-Right-3 | tggtgaccaaatccccatttTTATACGTCGAGTTGACCGACGTATTG |
| cy3-mFabp4-left-3 | TATTCGTTCGAACTTACGTCGTTATGacgctgatgatcatgttggg |
| cy3-mFabp4-Right-4 | gtggaagtcacgcctttcatTTATACGTCGAGTTGACCGACGTATTG |
| cy3-mFabp4-left-4 | TATTCGTTCGAACTTACGTCGTTATGaacacattccaccaccagct |

Table S3. The sequence of qPCR primers and mouse genotyping primers.

| | |
|---|---|
| Atf3 Forward Primer | GAGGATTTTGCTAACCTGACACC |
| Atf3 Reverse Primer | TTGACGGTAACTGACTCCAGC |
| Eno3 Forward Primer | CACAGCCAAGGGTCGATTCC |
| Eno3 Reverse Primer | CCCAGGTATCGTGCTTTGTCT |
| Klf9 Forward Primer | TTATTGCACGCTGGTCACTATC |
| Klf9 Reverse Primer | CTCATCGGGACTCTCCAGAC |
| Fhl2 Forward Primer | ATGACTGAACGCTTTGACTGC |
| Fhl2 Reverse Primer | CGATGGGTGTTCCACACTCC |
| Rps2 Forward Primer | GGGGCTCGTGGAGGTAAAG |
| Rps2 Reverse Primer | TCTCAGACTCCTTAATGGGCAG |
| Ranbp1 Forward Primer | CGAGGACCATGATACTTCCACA |
| Ranbp1 Reverse Primer | CCTCCAGCGTTTTAATTTCTTGC |
| Per1 Forward Primer | GAATTGGAGCATATCACATCCGA |
| Per1 Reverse Primer | CCCGAAACACATCCCGTTTG |
| Gapdh Forward Primer | GGATTTGGTCGTATTGGG |
| Gapdh Reverse Primer | GGAAGATGGTGATGGGATT |
| GM8797 Forward Primer | GGCAAGACCATCACCCTAGA |
| GM8797 Reverse Primer | TAATAGCCACCCCTCAGACG |
| GM10260 Forward Primer | GATCCCAGACTGGTTCCTGA |
| GM10260 Reverse Primer | TAAGAGCAAAGGCCCAGAGA |
| Fbn2 Forward Primer | CAACTCCGAAGGAAGCTACG |
| Fbn2 Reverse Primer | AAGAGCCCTTCGTGTTCTCA |
| Gpc3 Forward Primer | CCAACATGCTGCTCAAGAAA |
| Gpc3 Reverse Primer | CTGGAAAGAGGCTGTCGAAC |
| Vcan Forward Primer | GGCCAACAGTGGTTTCAAGT |
| Vcan Reverse Primer | TTCTGTGAAGGCTGTCGATG |

Mouse genotyping primers

| | |
|---|---|
| Tbx18-Cre wt Forward Primer | AACGCCAGAGAAAGAGGAAAC |
| Tbx18-Cre wt Reverse Primer | TCAGTGCCTCCACAGAGAAG |
| Tbx18-Cre mutant Forward Primer | AACGCCAGAGAAAGAGGAAAC |
| Tbx18-Cre mutant Reverse Primer | AGGCAAATTTTGGTGTACGG |
| Wt1-Cre wt Forward Primer | CCTACCATCCGCAACCAAG |

| | |
|---|---|
| Wt1-Cre wt Reverse Primer | CCCTGTCCGCTACTTTCAGA |
| Wt1-Cre mutant Forward Primer | ATCGCAGGAGCGGAGAAC |
| Wt1-Cre mutant Reverse Primer | GAACTTCAGGGTCAGCTTGC |