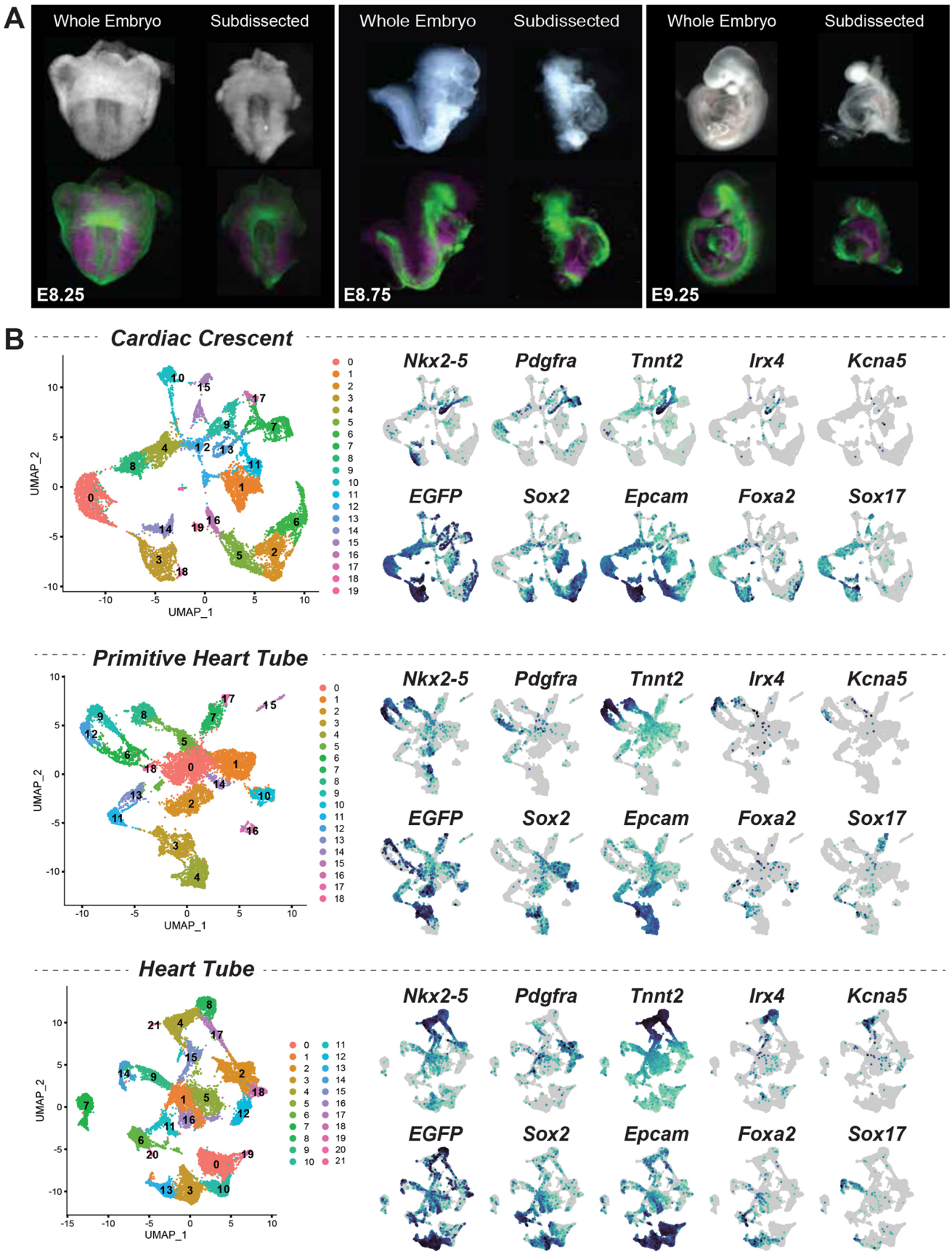


Fig. S1. Representative Dissection Examples and scRNAseq of Individual Stages.

A) Representative images showing brightfield (top) and fluorescence (bottom) in *Foxa2Cre;R26R:mTmG* embryos for each stage sequenced. Left: whole embryo; right: representative example of dissected cardiac regions. **B)** UMAP clustering and feature plots for markers of mesoderm, endoderm, and ectoderm cell types, as well as atrial/ventricular cells at CC, PHT and HT stage.

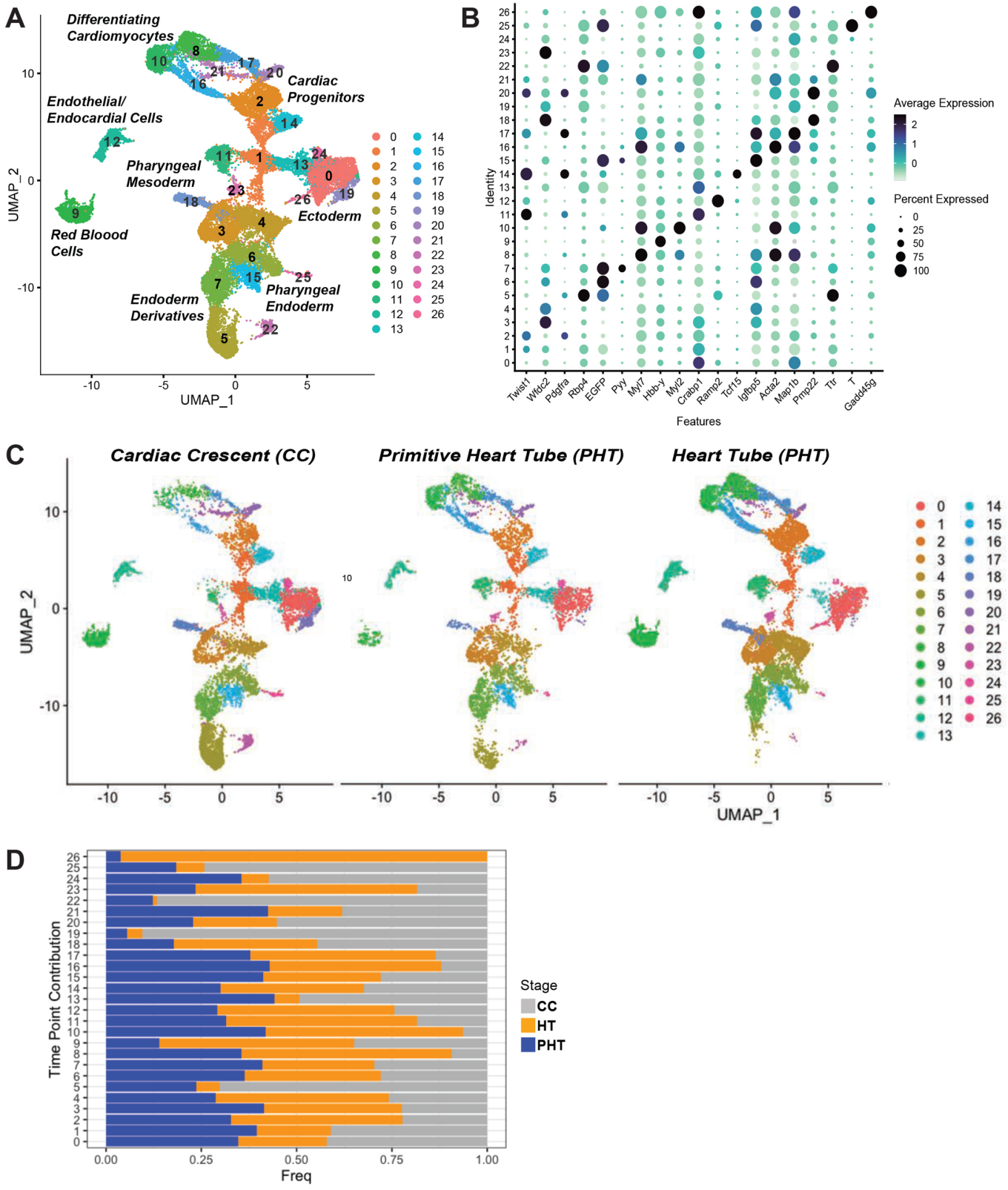


Fig. S2. Merge of Individual Time Point Samples Reveals a Changing Landscape of Cardiac Development and Surrounding Tissues

A) UMAP clustering of all cells sequenced, demonstrating presence of differentiated cardiomyocytes, cardiac progenitors, pharyngeal cell types, endoderm derivatives, and endothelial/endocardial cells. **B)** Bubble plot of top differentially expressed gene for each cluster. Differentially expressed genes for each cluster were calculated using a Wilcoxon rank-sum test with p-value <0.01. **C)** UMAP of all cells sequenced showing contribution from each time point individually. **D)** Quantification of overall contribution of cells from any one particular embryonic time point. Frequency is calculated as the number of cells in a given cluster relative to the total number of cells in the sample.

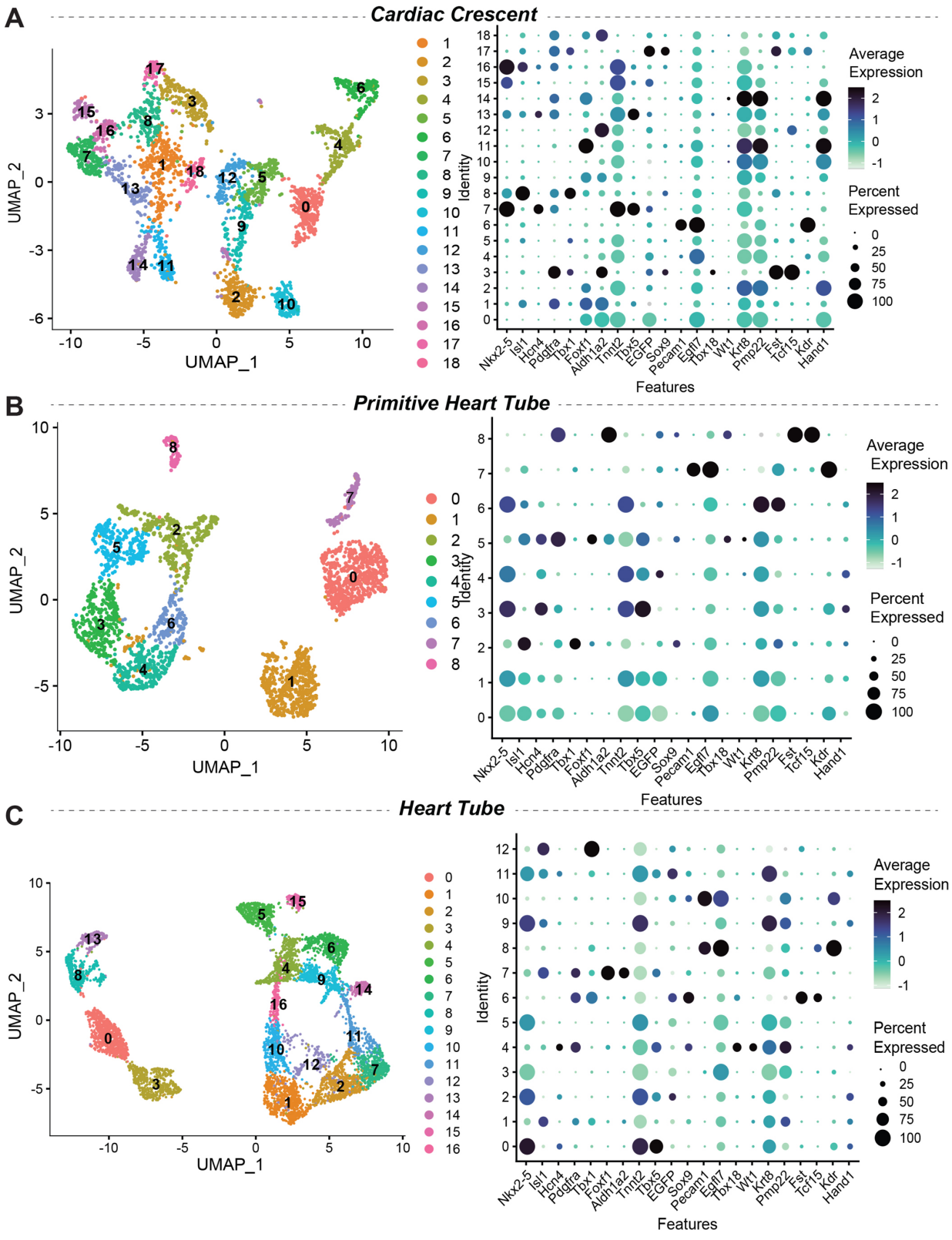


Fig. S3. UMAP Clustering and Selection of Key Markers for Cardiac Subpopulations at Individual Stages

A-C) UMAP clustering (left) and dot plot of selected genes of interest (right) of subclustered cardiac cells at the CC stage (A), the PHT stage (B) and the HT stage (C).

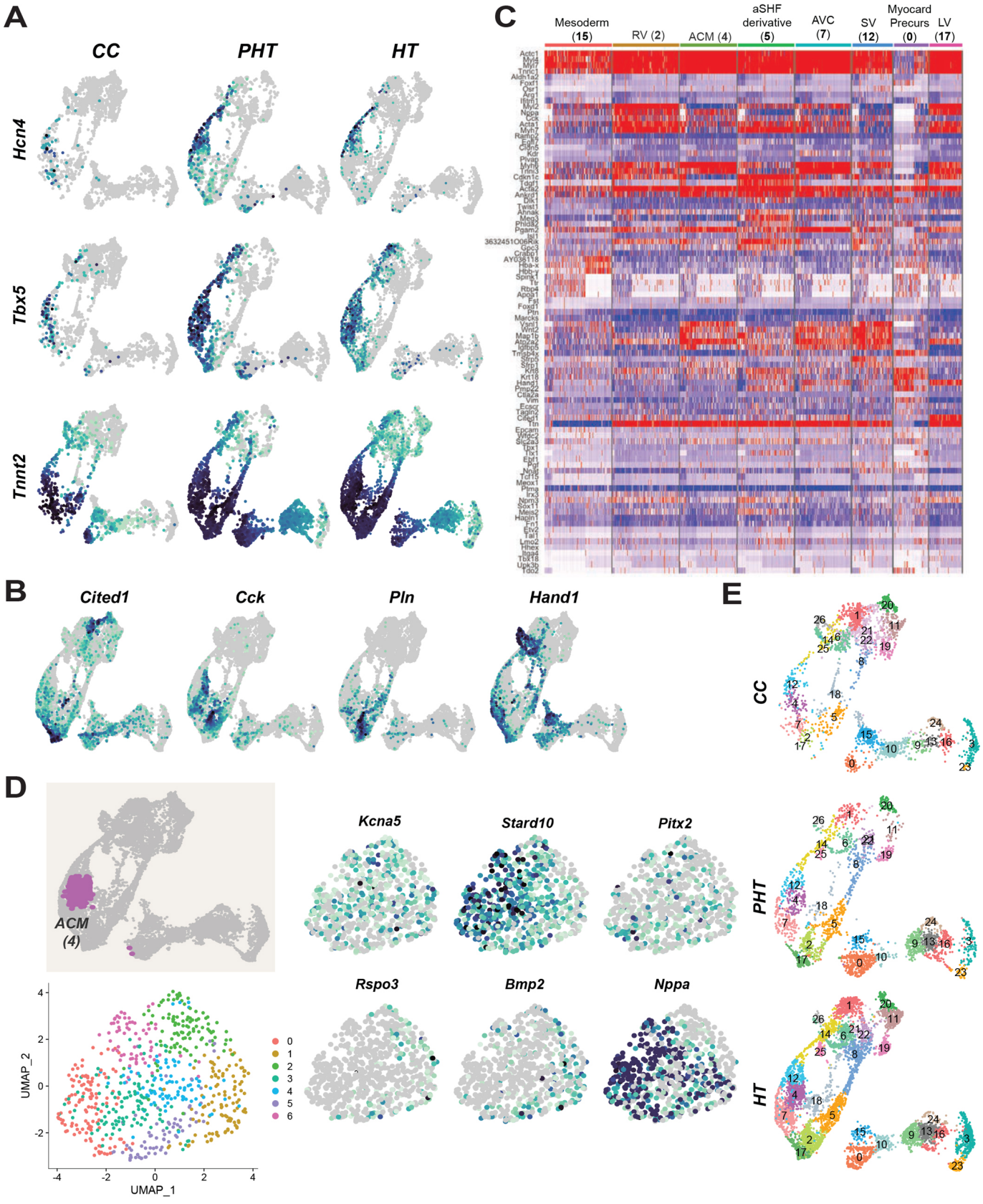
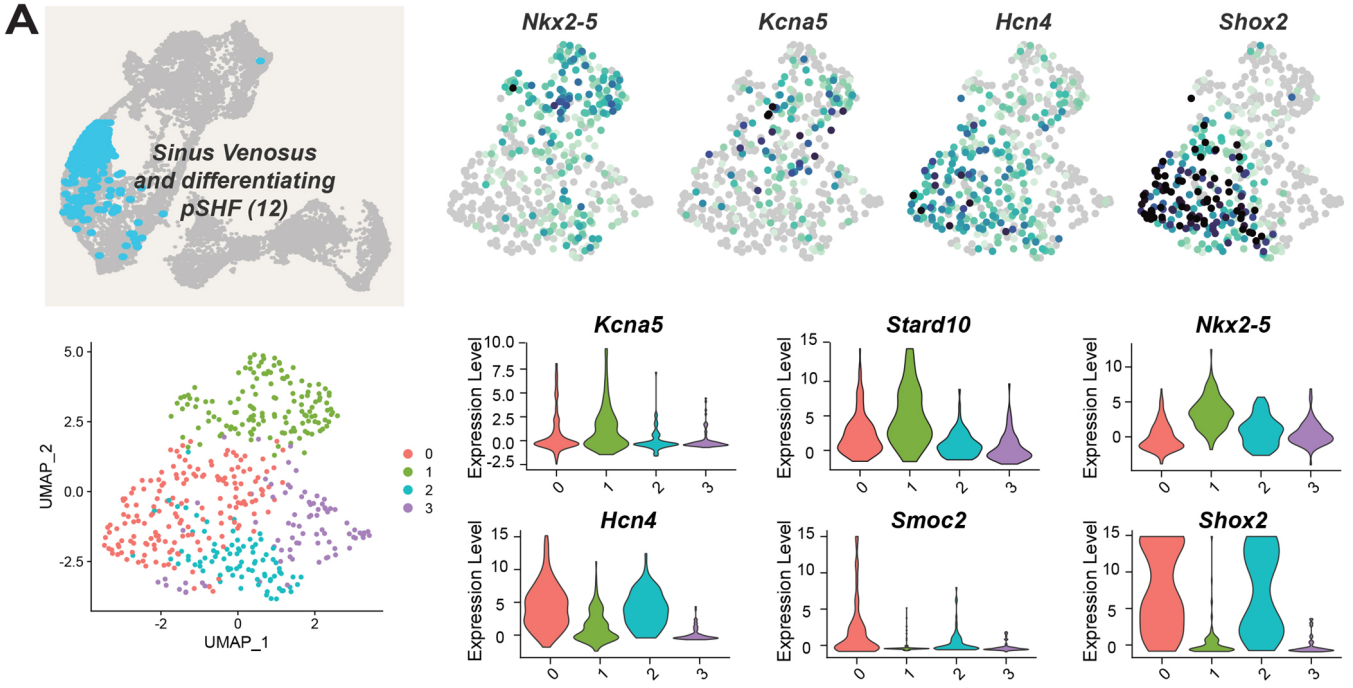


Fig. S4. Characterization of Cardiomyocyte Subpopulations

A) Feature plots for *Hcn4, Tbx5,* and *Tnnt2* split separately across CC, PHT and HT samples. **B)** Feature plots for expression of right (*Cited1/Cck)* and left (*Hand1/Pln)* ventricular identity. **C)** Heatmap demonstrating top 5 most differentially expressed genes for each cluster, identified using FindMarkers function in Seurat. **D)** UMAP clustering of Cluster 4 following re-clustering (bottom left) and Feature plots for key atrial markers (right). **E)** UMAP showing contribution from individual time points to combined cardiac UMAP projection**.**



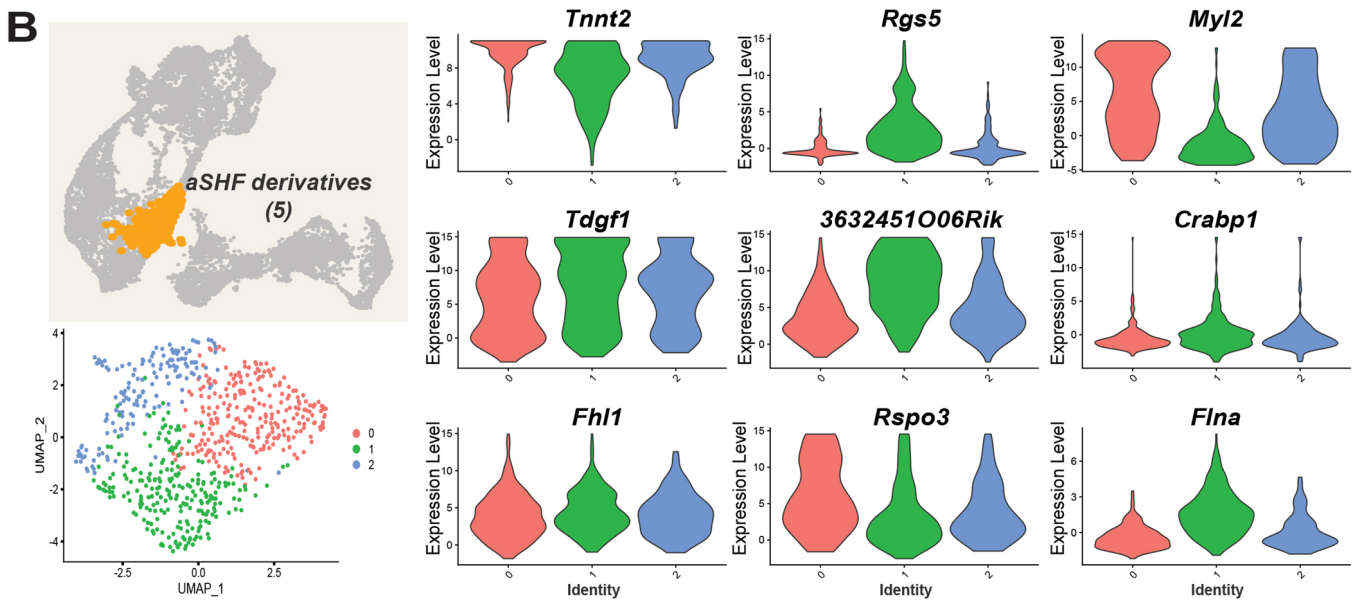


Fig. S5. Mixed Populations Cluster Together Based on Progenitor of Origin

A) *Top left:* Schematic of cluster investigated (Cluster 12)**.** *Bottom left:* UMAP clustering of Cluster 12 alone following re-clustering. *Bottom left:* Feature plots for markers of atrial and sinoatrial nodal identity. *Bottom right:* Violin plots for markers of ventricular and OFT lineages across individual clusters. **B)** *Top left:* Schematic of cluster investigated (Cluster 5)**.** *Bottom left:* UMAP clustering of Cluster 5 alone following re-clustering. *Right:* Violin plots for markers of ventricular and OFT lineages across individual clusters.

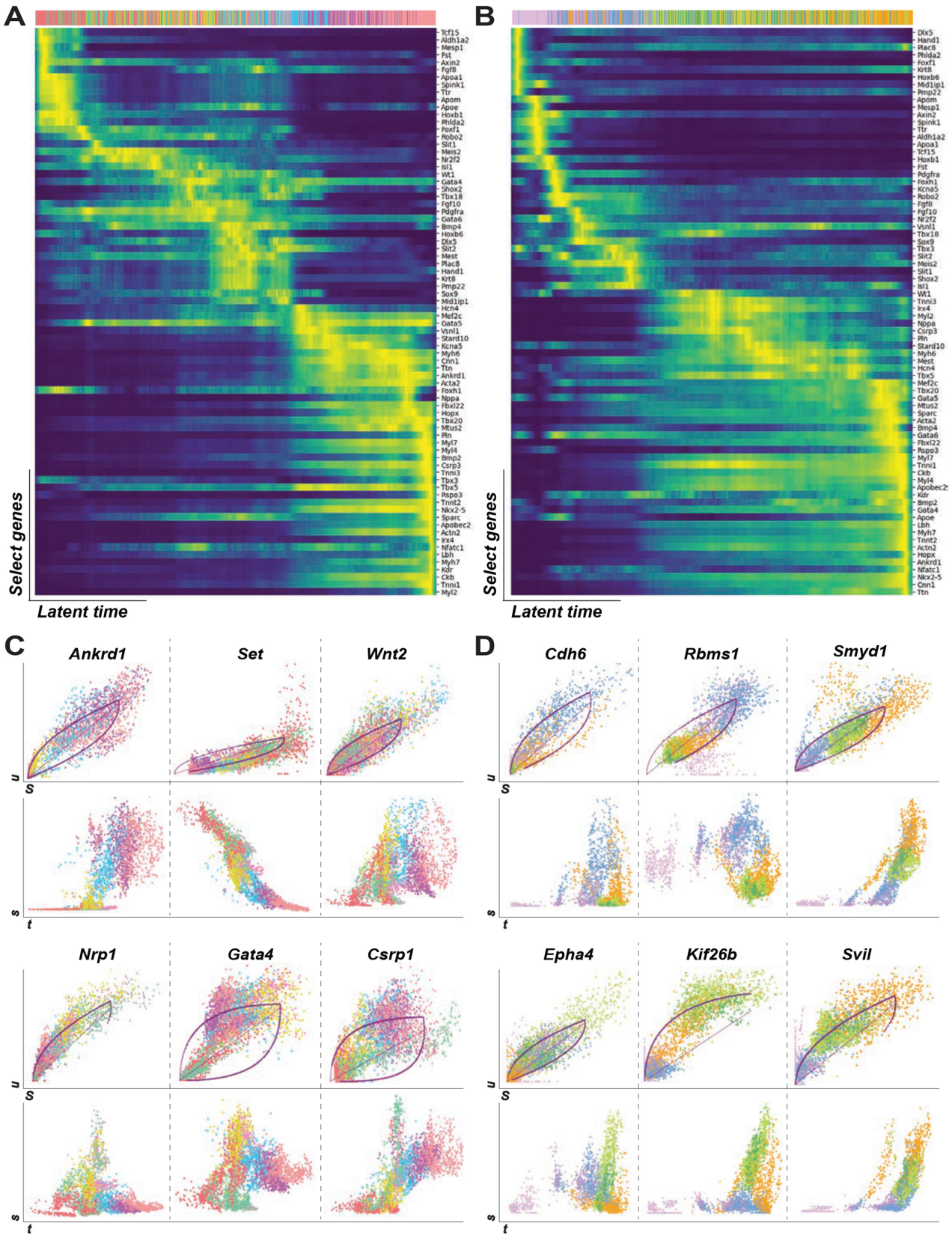


Fig. S6. Visualization of Dynamic Changes in Gene Expression Across LPM/ Heart Field Progenitor Differentiation Streams

A) Heatmap of selected genes of interest plotted against latent time for differentiation trajectory arising from pSHF/LPM progenitors (Figure 3F-H). **B)** Heatmap for selected genes of interest plotted against latent time for differentiation trajectory arising from aSHF progenitors (Figure 3I-K). **C)** Phase portraits (top) and expression dynamics (bottom) for selected genes found to be dynamically regulated across LPM/pSHF differentiation. S = spliced transcript; u = unspliced transcript; t = time. **D)** Phase portraits (top) and expression dynamics (bottom) for selected genes found to be dynamically regulated across aSHF differentiation.

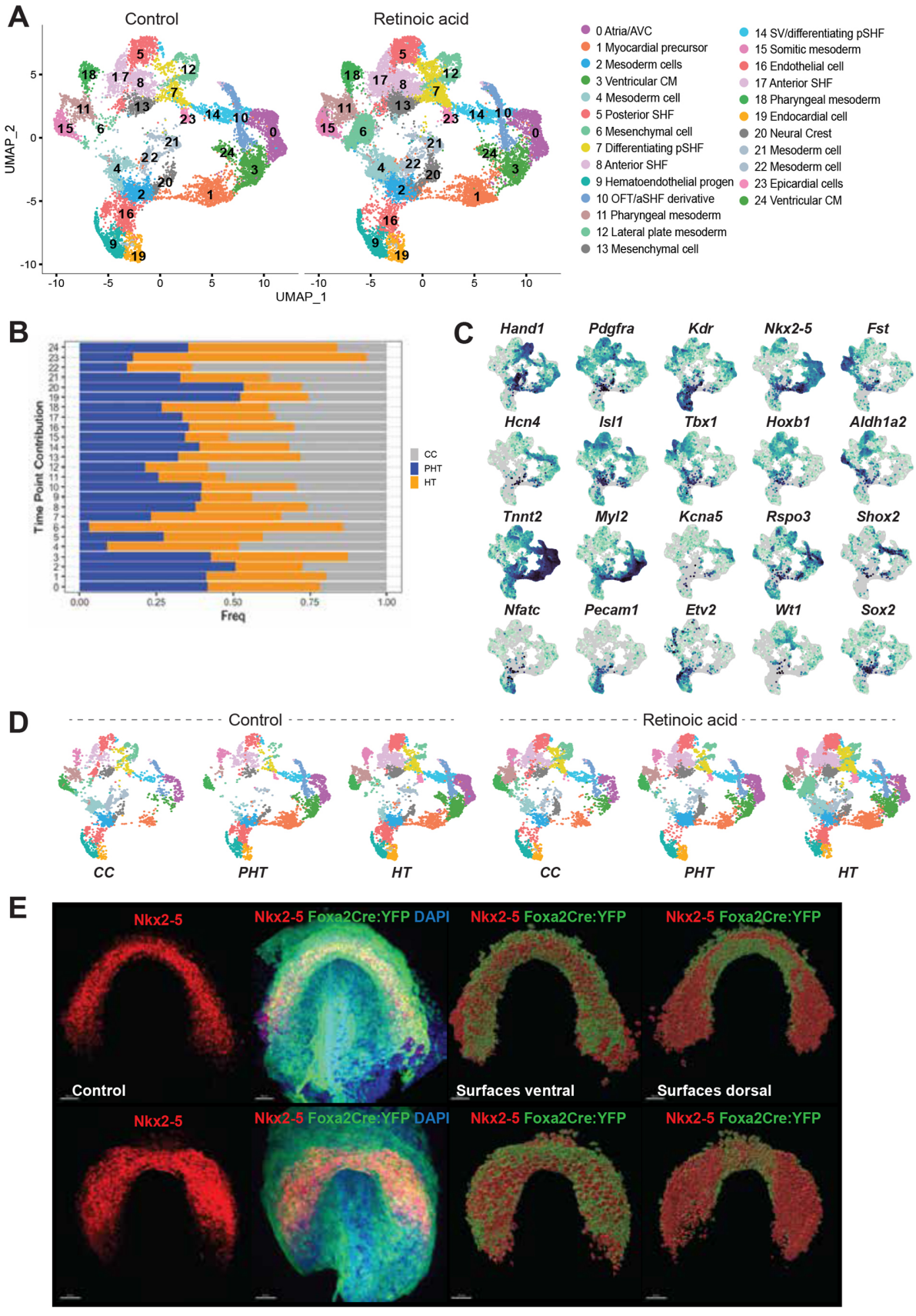


Fig. S7. Contribution of Control and RA-exposed Embryos to Cluster of Merged Data and Whole Mount Immunofluorescence of Cardiac Crescent Samples

A) UMAP projection of Normal/RA merged sample, split by condition (Normal and RA-exposed). **B)** Quantification of overall contribution of cells from any one particular embryonic time point across Normal and RA treated samples. Frequency is calculated as the number of cells in a given cluster relative to the total number of cells in the sample. *Bottom***:** Sample contribution to UMAP projection. **C)** Feature plots for selected candidates of interest on merged Control and RA-treated samples. **D)** UMAP showing contribution from individual time points from normal and RA-exposed samples to combined cardiac UMAP projection. **E)** Representative images of whole mount immunofluorescence used for quantification of *Foxa2*-lineage traced cells within the cardiac crescent. *Left to right:* Single channel Nkx2-5 immunofluorescence; overlay of Nkx2-5 immunofluorescence and YFP (*Foxa2Cre;R26R:YFP* lineage-traced); Ventral view of surface volume construction of Nkx2-5 and *Foxa2* lineage-traced volumes; Dorsal view of surface volume construction.

Table S1. Ranked List of Most Dynamic Genes Per Cluster

Clusters are organized in columns from left to right, cluster ID is indicated in row 1. Genes are organized in descending order, with most dynamical genes at the top of each column.

Click here to download Table S1

Table S2. List of Most Dynamic Genes for pSHF/LPM Derived Differentiation Stream

Genes are listed in second column, organized in descending order for the top 300 genes across differentiation stream.

Click here to download Table S2

Table S3. List of Most Dynamic Genes for aSHF Derived Differentiation Stream

Genes are listed in second column, organized in descending order for the top 300 genes across differentiation stream.

Click here to download Table S3

Table S4. Differential Expression Results of Comparison Between Differentiating aSHF (Cluster 8) and LPM/pSHF (Cluster 14).

Results of differentiation expression testing performed in Seurat (negative binomial) between Cluster 8 and Cluster 14. Results are organized in descending order by adjusted p-value. pct.1 refers to the percentage of cells in test population #1 (Cluster 8) expressing each gene compared to test population #2 (Cluster 14).

Click here to download Table S4

Table S5. Differential Expression Results of Comparison Between Ventricular (Clusters 2&17) and Atrial (Cluster 4) Cells.

Results of differentiation expression testing performed in Seurat (negative binomial) between atrial and ventricular cells. Results are organized in descending order by adjusted p-value. pct.1 refers to the percentage of cells in test population #1 (Cluster 2&17) expressing each gene compared to test population #2 (Cluster 4).

Click here to download Table S5

Table S6. Differential Expression Results of Comparison Between *EGFP*+ and *EGFP*- cells in Cardiac Crescent.

Results of differentiation expression testing performed in Seurat (negative binomial) between EGFP+/- cells within CC sample. Results are organized in descending order by adjusted p-value. pct.1 refers to the percentage of cells in test population #1 (*EGFP* +) expressing each gene compared to test population #2 (*EGFP*-).

Click here to download Table S6