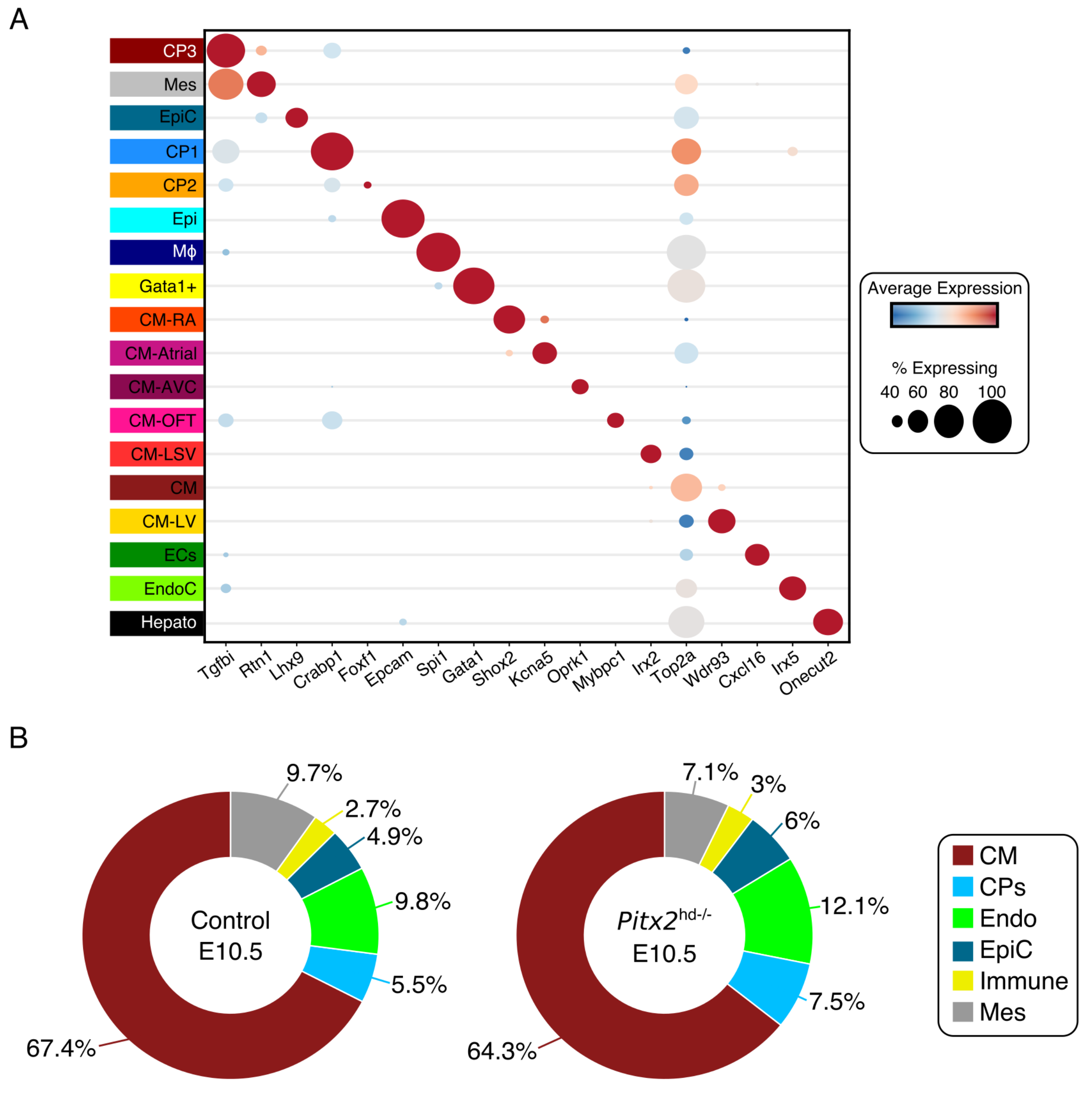
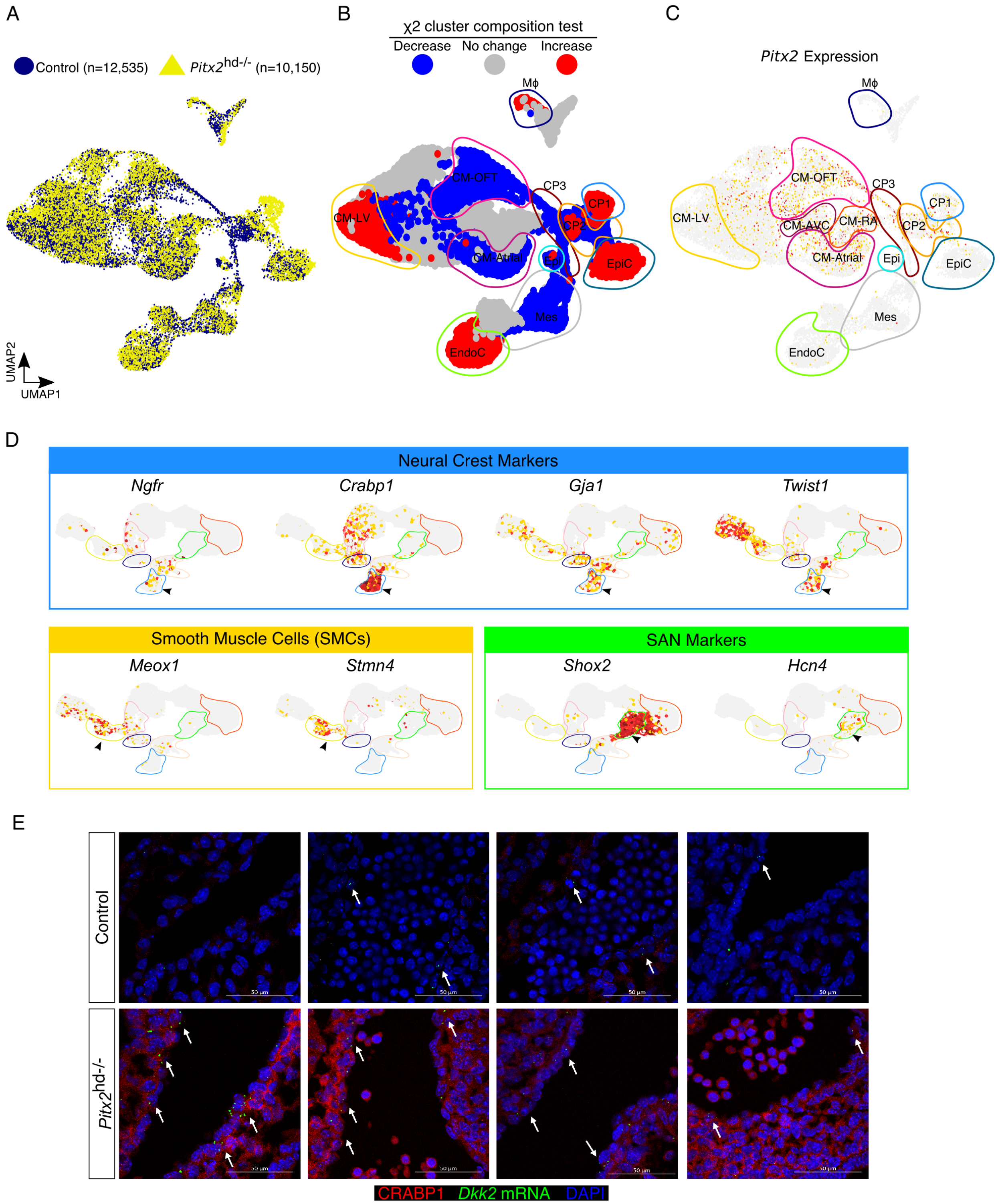


**Figure S1. Related to Fig. 1, E10.5 Cardiac Cell Composition.**

A. Dotplot showing the expression of individual marker genes across the distinct E10.5 clusters.
B. Donut plots displaying the cell composition by major cell type at E10.5.


A
Control (n=12,535)
Pitx2hd-/- (n=10,150)
UMAP2
UMAP1
B
χ2 cluster composition test
Decrease
No change
Increase
Mϕ
CM-OFT
CP3
CP1
CP2
CM-LV
CM-Atrial
Epi
EpiC
Mes
EndoC
C
Pitx2 Expression
Mϕ
CM-OFT
CP3
CP1
CM-LV
CM-AVC
CM-RA
CP2
CM-Atrial
Epi
EpiC
Mes
EndoC
D
Neural Crest Markers
Ngfr
Crabp1
Gja1
Twist1
Smooth Muscle Cells (SMCs)
Meox1
Stmn4
SAN Markers
Shox2
Hcn4
E
Control
Pitx2hd-/-
50 µm
CRABP1 Dkk2 mRNA DAPI

# Figure S2. Related to Fig. 2, Markers for Cardiac Progenitor Cell Populations.

A. UMAP projection of E10.5 scRNA-seq data colored according to genotype. Control cells are colored blue, and *Pitx2*-null cells are colored maize.
B. χ2 cluster composition analysis of E10.5 *Pitx2*-null cardiac tissue compared to control. Blue, cluster that significantly decreases in *Pitx2*-null hearts. Red, cluster that significantly increased in *Pitx2*-null hearts. Gray, no significant change in cluster composition between control and *Pitx2*-null conditions.
C. UMAP feature plot displaying *Pitx2* gene expression at E10.5.
D. UMAP feature plots for indicated genes. Highlighted clusters are colored according to **Fig. 2D**. High expression is denoted in red, and low or zero expression is shown in grey.
E. Additional images from CRABP1 IF (red), and *Dkk2* FISH (green) co-labelling experiments.

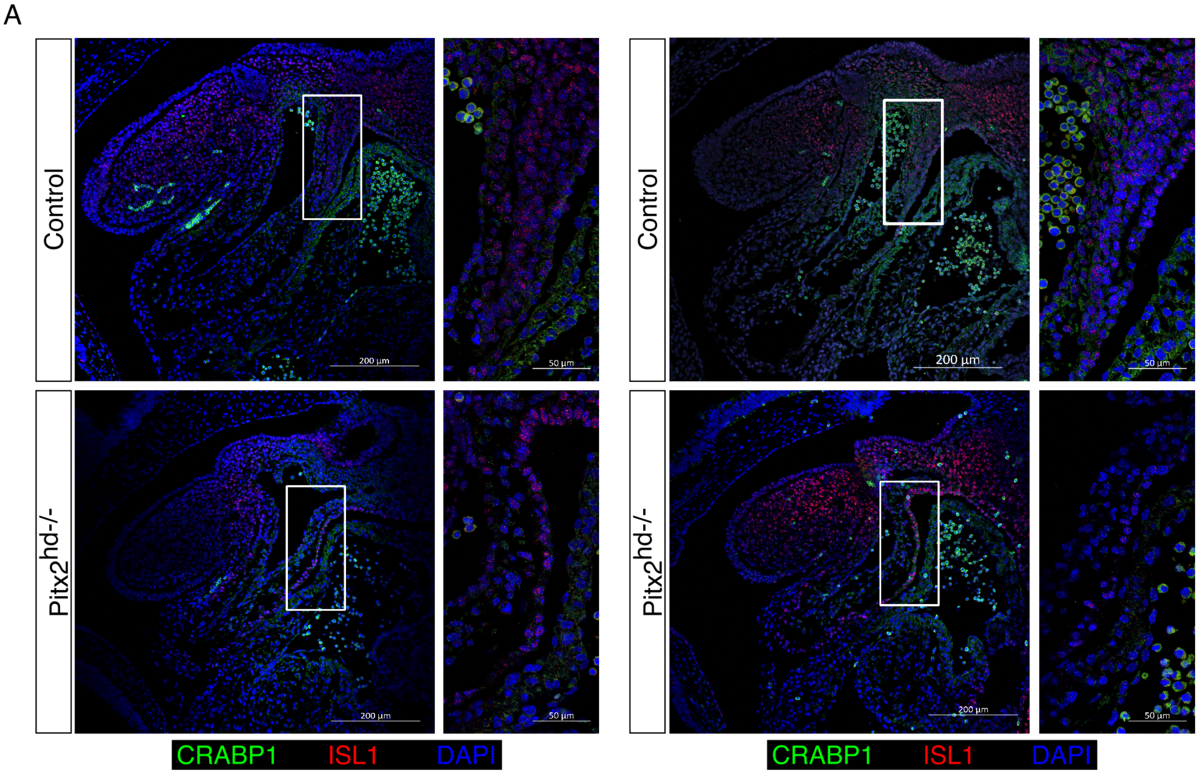


**Figure S3. Related to Figure 2, Decreased ISL1 Positive Progenitors in *Pitx2*-deficient Outflow Tract.**

A. ISL1 and CRABP1 IF co-labelling experiments in the outflow tract.

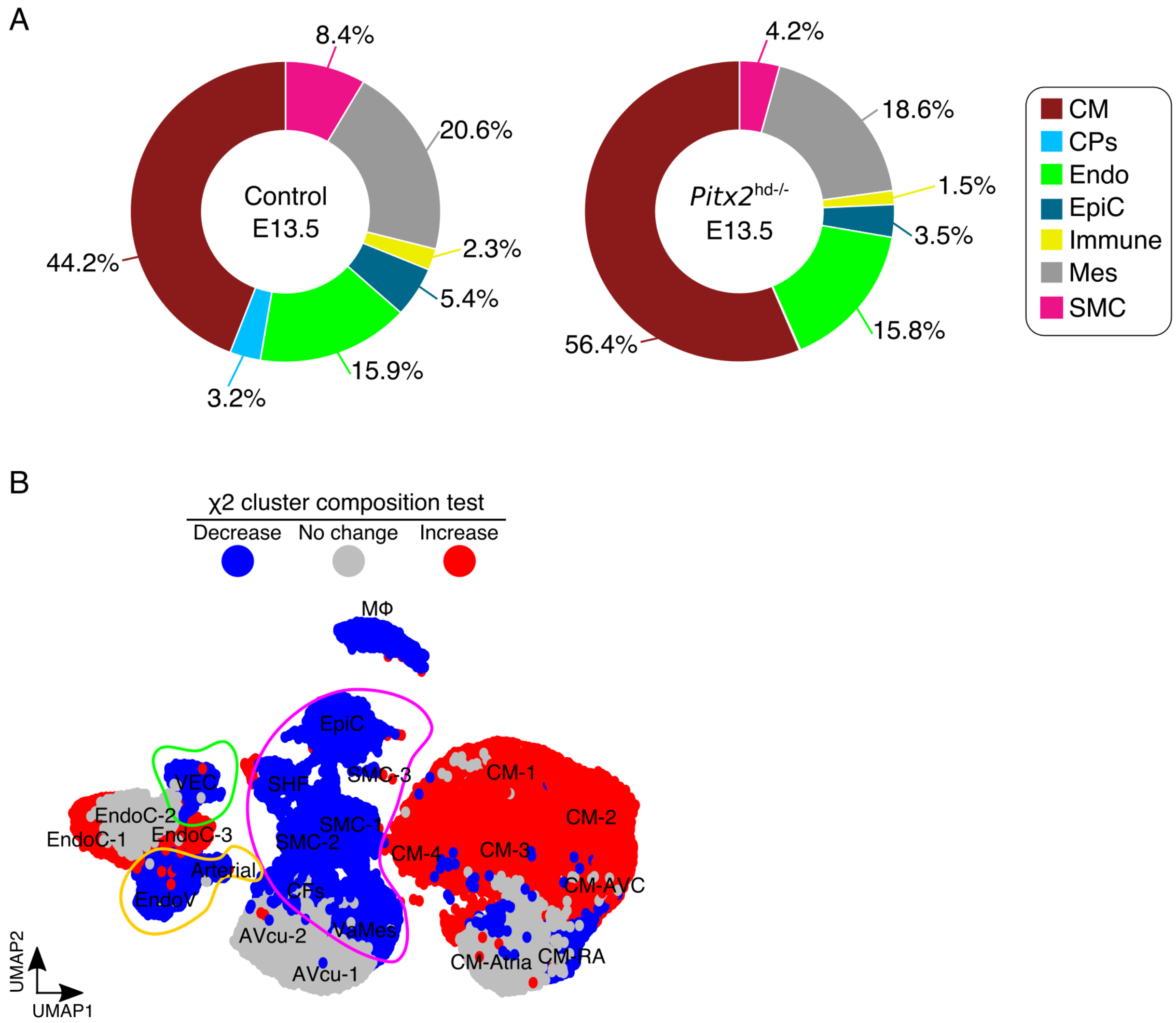


**Figure S4. Related to Figure 4, E13.5 Cardiac Composition.**

A. Donut plots displaying the cell composition by major cell type at E13.5.
B. χ2 cluster composition analysis of subclustered E13.5 *Pitx2*-null cardiac tissue compared to control.

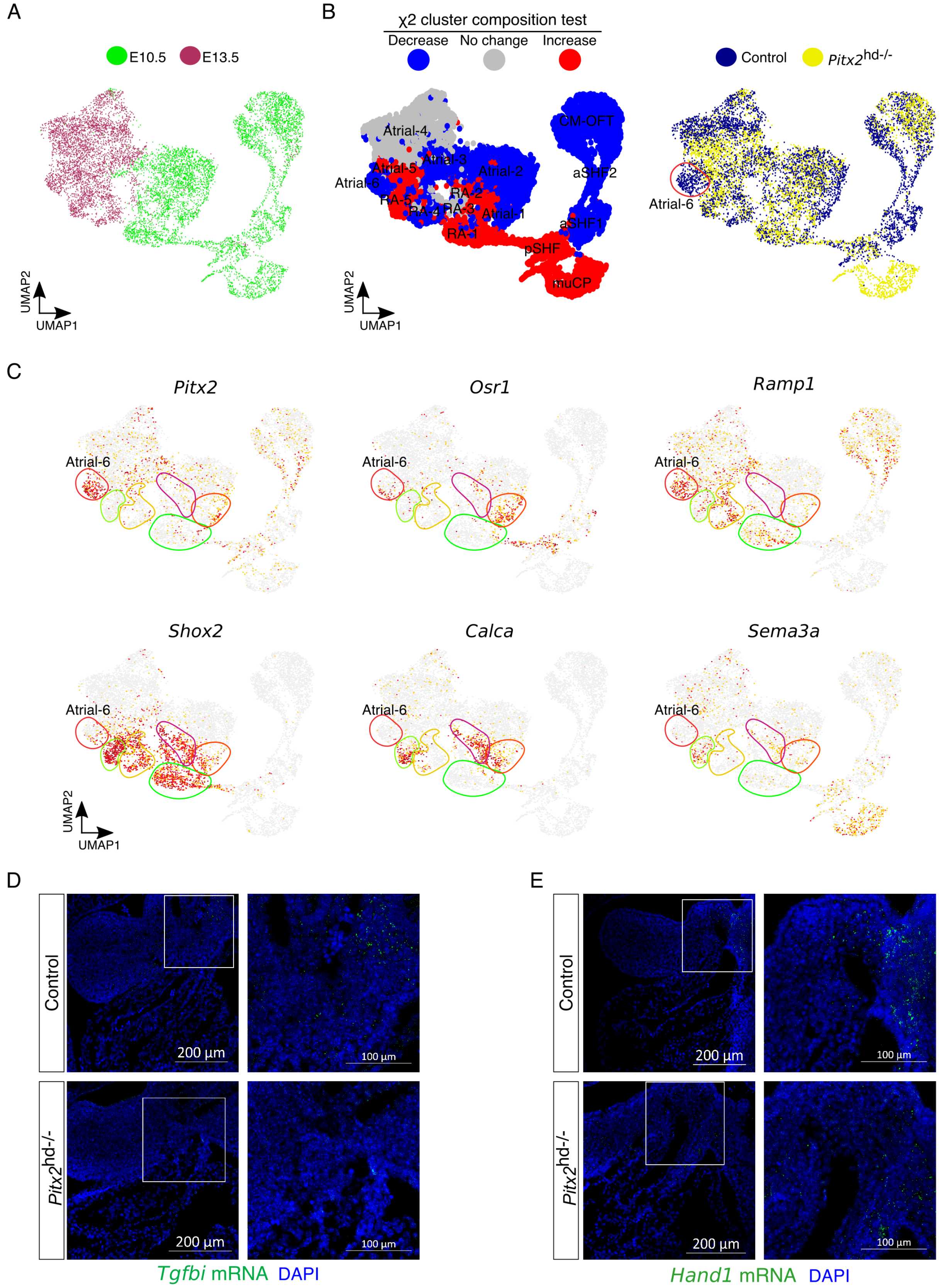

A
E10.5
E13.5
UMAP2
UMAP1
B
χ2 cluster composition test
Decrease
No change
Increase
Atrial-4
Atrial-3
Atrial-5
Atrial-2
Atrial-6
RA-5
RA-2
RA-4
RA-3
Atrial-1
RA-1
CM-OFT
aSHF2
aSHF1
pSHF
muCP
UMAP2
UMAP1
Control
Pitx2hd-/-
Atrial-6
C
Pitx2
Osr1
Ramp1
Atrial-6
Atrial-6
Atrial-6
Shox2
Calca
Sema3a
Atrial-6
Atrial-6
Atrial-6
UMAP2
UMAP1
D
Control
Pitx2hd-/-
200 µm
100 µm
200 µm
100 µm
Tgfbi mRNA DAPI
E
Control
Pitx2hd-/-
200 µm
100 µm
200 µm
100 µm
Hand1 mRNA DAPI

**Figure S5. Related to Figure 4, Atrial and Outflow Tract Cardiomyocyte Differentiation and Maturation.**

A. UMAP plot showing age of each individual cell. E10.5 cells are colored green, and E13.5 cells are highlighted in maroon.
B. (left) $\chi 2$ cluster composition analysis of subclustered *Pitx2*-null cardiac tissue compared to control. (right) UMAP plot colored by genotype.
C. UMAP feature plots of gene expression. Highlighted cell clusters are highlighted and colored according to **Fig. 4A**.
D. Replicates for *Tgfbi* (green) FISH labelling. Nuclei are stained blue (DAPI).
E. Replicates for *Hand1*(green) FISH labelling. Nuclei are stained blue (DAPI).

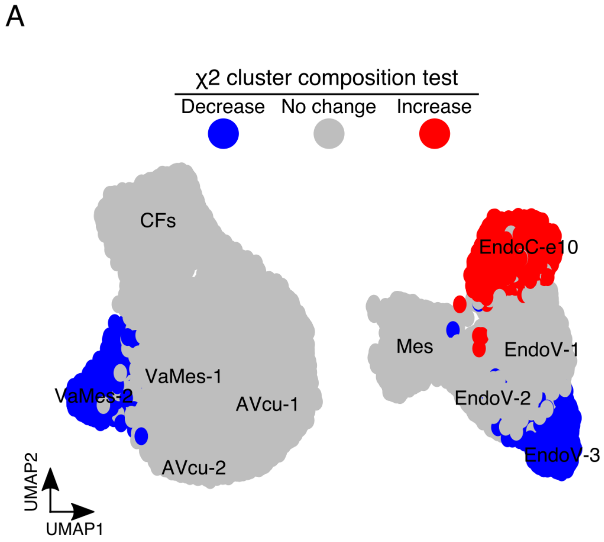


**Figure S6. Related to Figure 6, Cardiac Valve and Cushion Composition.**

A. χ2 cluster composition analysis of subclustered *Pitx2*-null cardiac tissue compared to control.

**Table S1.**

Click here to Download Table S1

**Table S2.**

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**Table S3.**

Click here to Download Table S3