

Supplementary Materials

Supplemental Figures

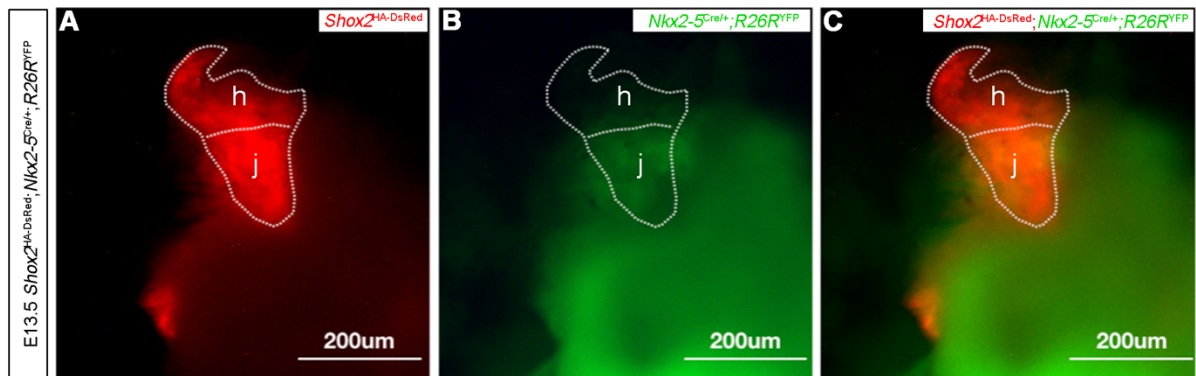


Figure S1

Figure S1. Colocalization of YFP and DsRed in the SAN junction of *Shox2^{HA-DsRed};Nkx2-5^{Cre/+};R26^{RYFP}* mice.

(A-C) Visualization of the heart of an E13.5 *Shox2^{HA-DsRed};Nkx2-5^{Cre/+};R26^{RYFP}* mouse under fluorescent stereomicroscope reveals colocalization of YFP and DsRed in the SAN junction. h, SAN head; j, SAN junction.

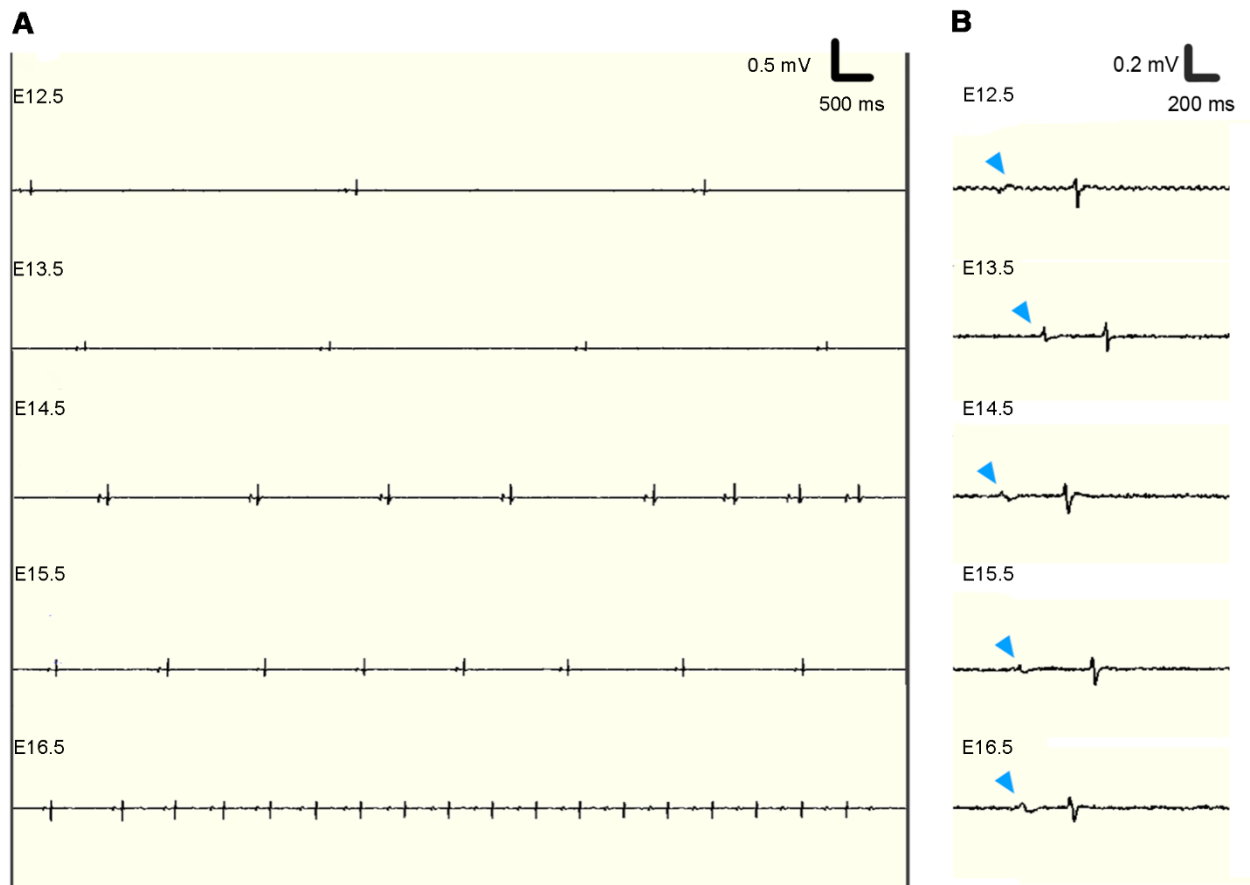


Figure S2

Figure S2. ECG recordings on developing mouse embryos.

(A) Representative ECG recordings of lead II on the embryos at E12.5, E13.5, E14.5, E15.5 and E16.5, respectively. (B) Representative single PQRST compress recordings on the embryos at different stage. Blue arrowheads point to P wave.

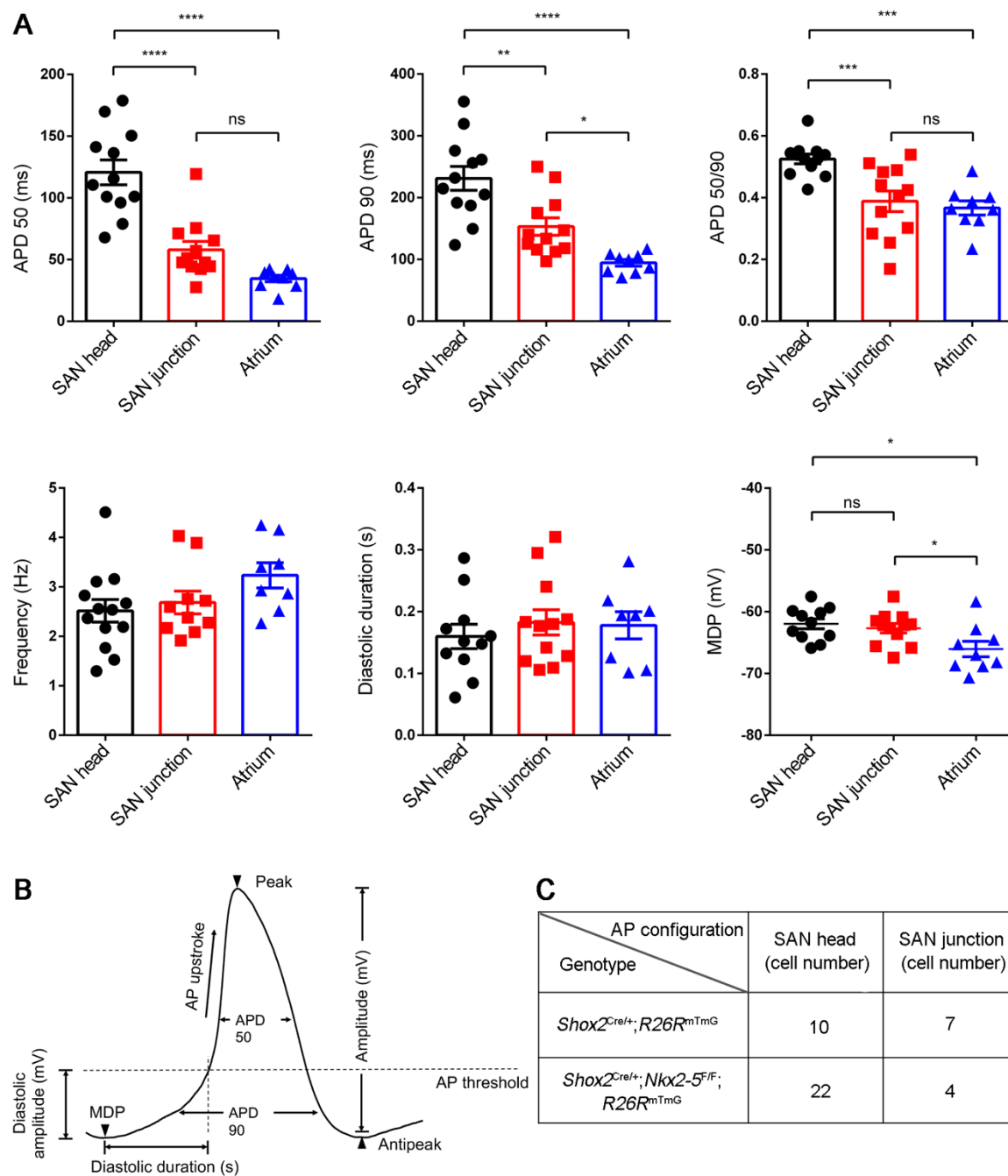


Figure S3

Figure S3. Comparison of the AP parameters between the SAN head, SAN junction, and atrial myocardial cells.

(A, B) Histograms show AP parameters of the SAN head, SAN junction, and atrial myocardial cells at E13.5. (C) A table summarizing cell numbers exhibiting the SAN head and SAN junction AP configurations, respectively, by patch clamp recording on GFP-positive cells

isolated from the SAN of E13.5 *Shox2*^{Cre/+};*R26R*^{mTmG} and *Shox2*^{Cre/+};*Nkx2-5*^{F/F};*R26R*^{mTmG} mice.

ns, non-significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

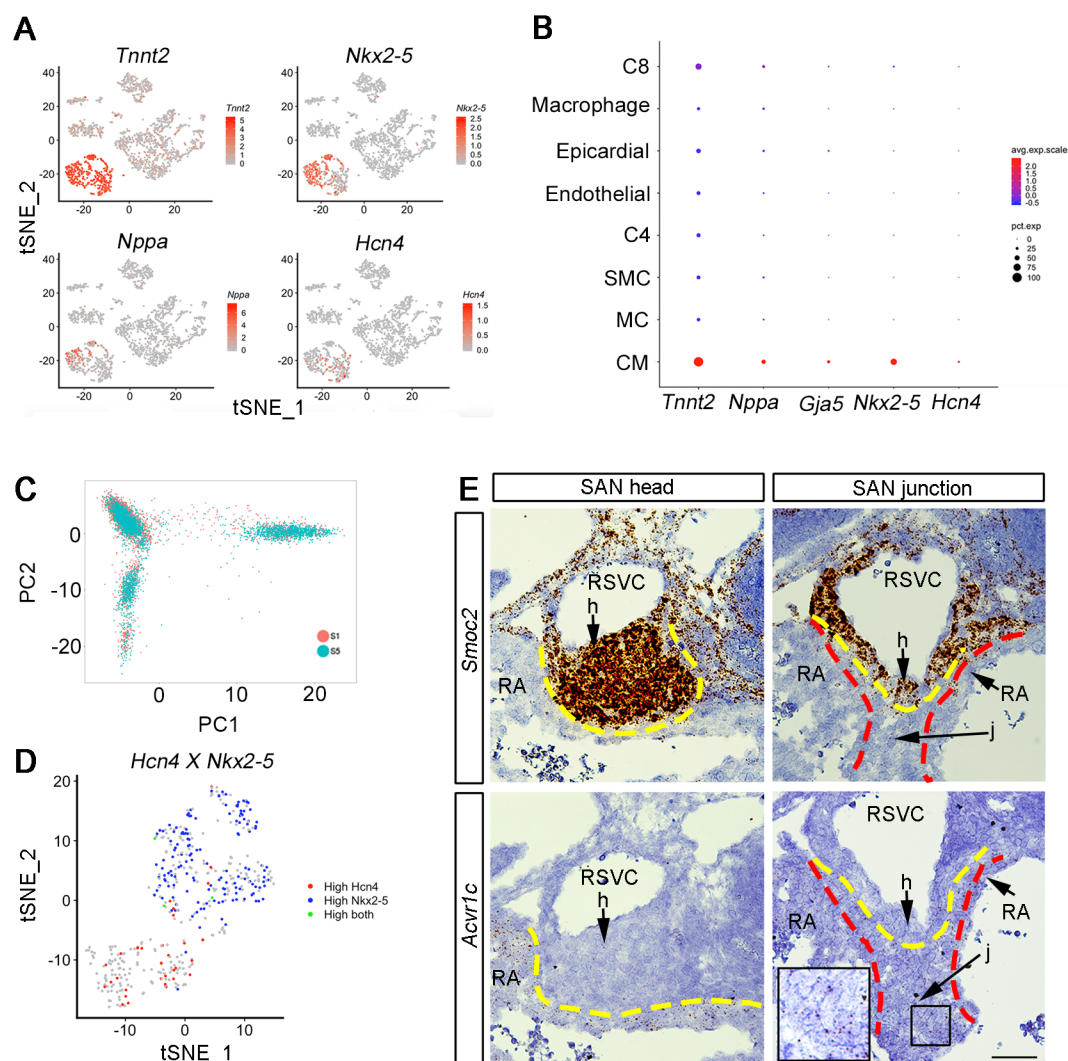


Figure S4

Figure S4. Quality control and cell selection for deeper scRNA-seq data analysis.

(A, B) The expression of *Tnnt2*, *Hcn4*, *Nkx2-5*, *Gja5* and *Nppa* within the CM cluster is visualized by FeaturePlot (A) and DotPlot (B). (C) PCAPlot on S1 and S5 shows the high coincidence between them. (D) FeaturePlot shows the co-expression of *Hcn4* and *Nkx2-5* within different clusters. (E) *In situ* hybridization shows specific *Smoc2* expression in the SAN head and *Acvr1c* expression in the SAN junction and atrial cells at E13.5 wild type embryo, respectively. h, SAN head; j, SAN junction; CM, cardiomyocyte; EC, endothelial cells; MC, mesenchymal cells; RA, right atrium; EPI, epicardial cells; SMC, smooth muscle cells; RSVC, right superior vena cava. C4, C8, cluster number 4, 8. Bar: 50 μ m.

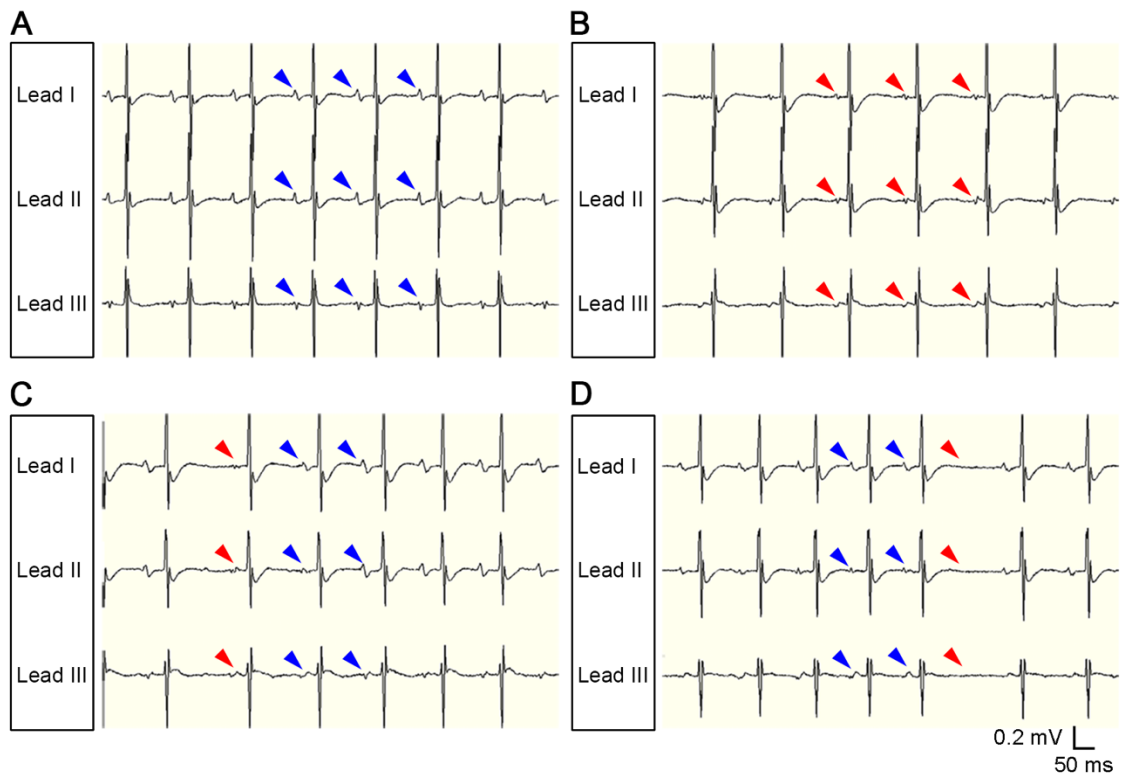


Figure S5

Figure S5. Closer looks at the surface ECG records on *Shox2^{Cre/+};Nkx2-5^{F/F}* mice

(A-D) Typical examples of surface ECG recordings (lead I, lead II and lead III) of the adult *Shox2^{Cre/+};Nkx2-5^{F/F}* mice. Blue arrowheads point to normal P wave. Red arrowheads point to abnormal P wave.