## Supplementary Figures

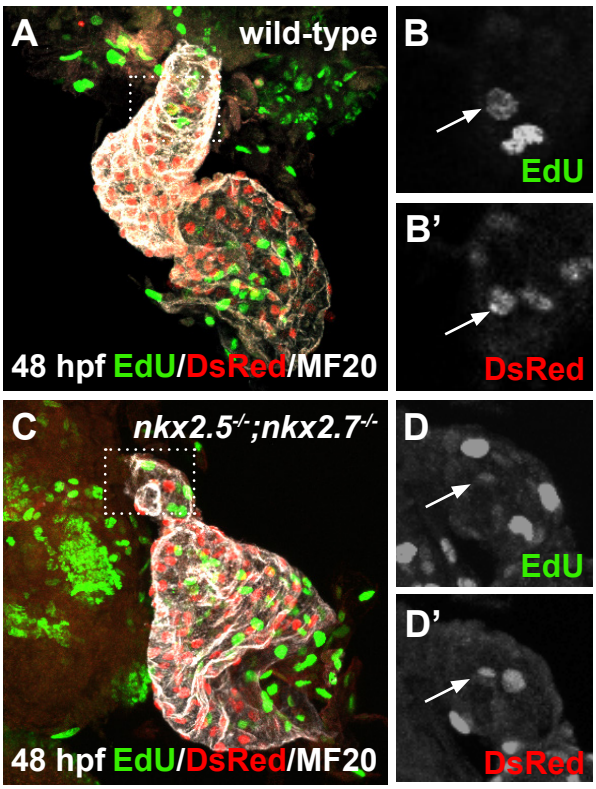


**Figure S1. Proliferation of differentiated arterial pole CMs is not affected**

**(A-D)** Ventral view, anterior to the top, of the arterial pole region at 48 hpf. Confocal projections of immunohistochemistry for EdU (green), DsRed (red), and MF20 (gray) in wild-type (A,B-B') and *nkx2.5*$^{-/-}$*;nkx2.7*$^{-/-}$ (C,D-D') embryos carrying *Tg(-5.1myl7:nDsRed2)* following EdU incubation at 24 hpf. Representative images highlight the detection of proliferating nuclei in the OFT myocardium.

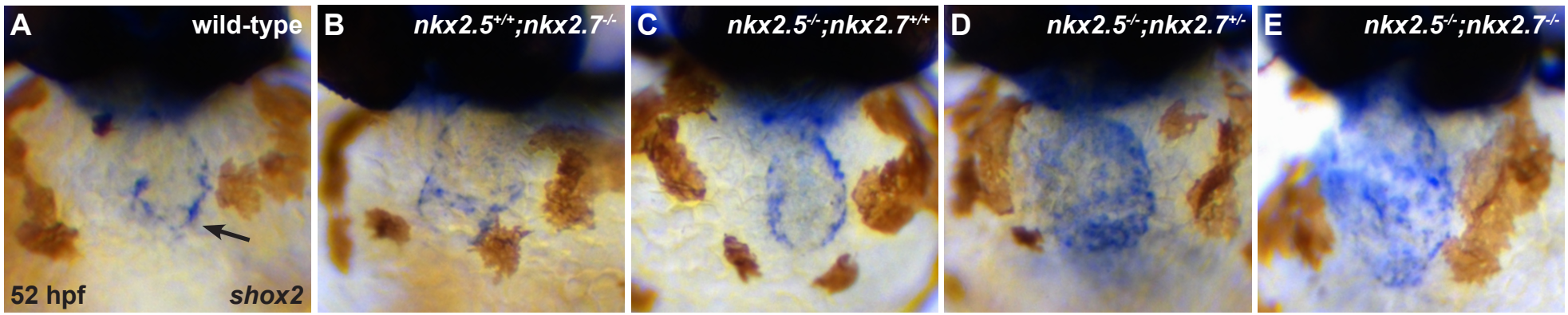


**Figure S2. *nkx* genes regulate *shox2***

**(A-E)** Ventral view, anterior to the top, at 52 hpf illustrates *in situ* hybridization for *shox2*. Expression of *shox2* is restricted to the IFT in wild-type (black arrow) (n = 11/13) (A) and *nkx2.5*$^{+/+}$;*nkx2.7*$^{-/-}$ (n = 5/6) (B) embryos. However, its expression is expanded throughout the cardiac chambers in *nkx2.5*$^{-/-}$;*nkx2.7*$^{+/+}$ (n = 9/10) (C), *nkx2.5*$^{-/-}$;*nkx2.7*$^{+/-}$ (n = 12/13) (D), and *nkx2.5*$^{-/-}$;*nkx2.7*$^{-/-}$ (n = 5/7) (E) embryos.

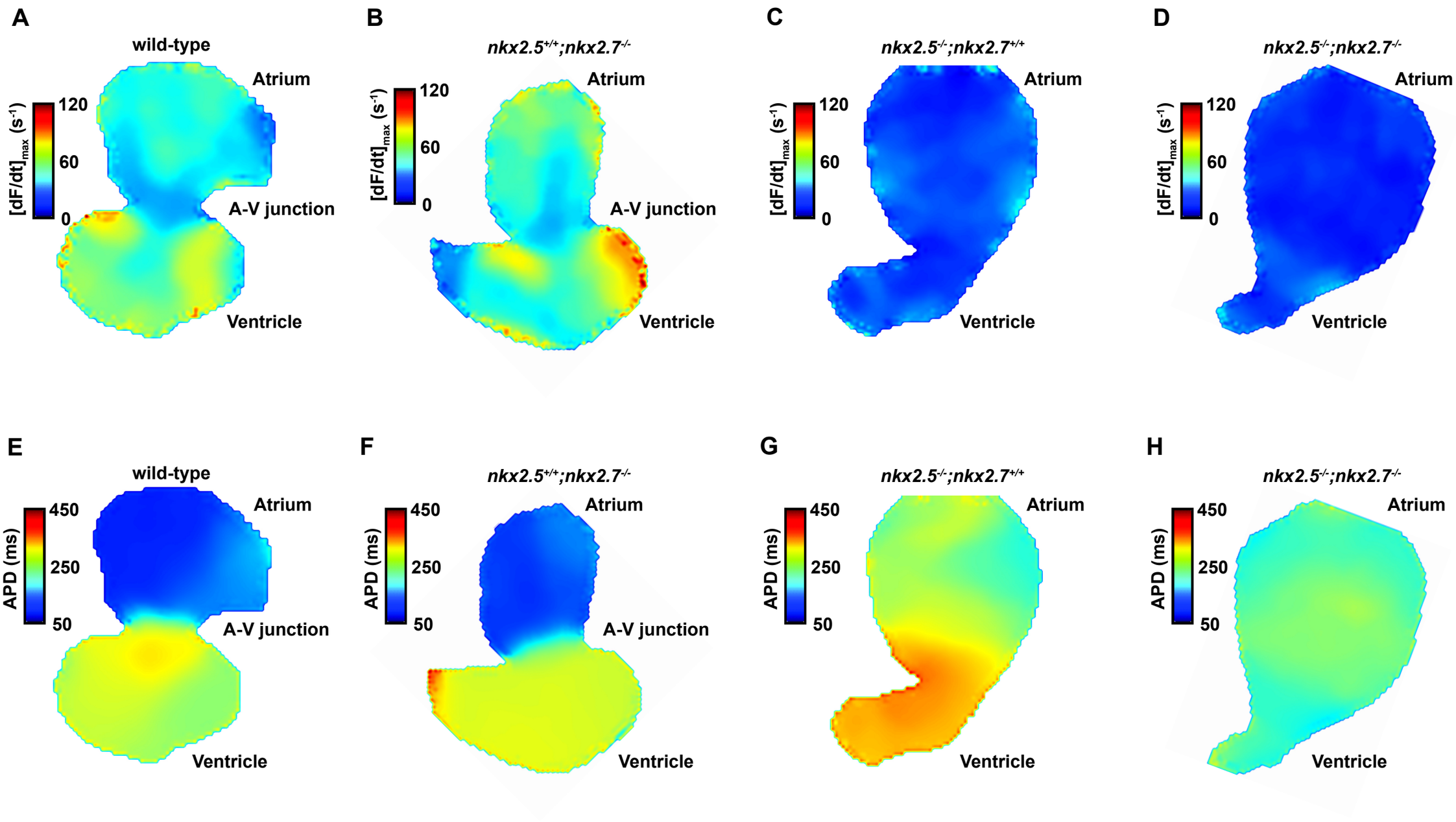


**Figure S3. *nkx* genes are required to establish ventricular and atrial electrophysiological identity**

**(A-D)** Representative heat maps demonstrate the spatial distributions of the maximum slope of the action potentials, $[dF/dt]_{max}$, in wild-type (A), *nkx2.5*$^{+/+}$;*nkx2.7*$^{-/-}$ (B), *nkx2.5*$^{-/-}$;*nkx2.7*$^{+/+}$ (C), and *nkx2.5*$^{-/-}$;*nkx2.7*$^{-/-}$ (D) hearts.

**(E-H)** Representative heat maps show the spatial distributions of the action potential durations (APDs) in wild-type (E), *nkx2.5*$^{+/+}$;*nkx2.7*$^{-/-}$ (F), *nkx2.5*$^{-/-}$;*nkx2.7*$^{+/+}$ (G), and *nkx2.5*$^{-/-}$;*nkx2.7*$^{-/-}$ (H) hearts.