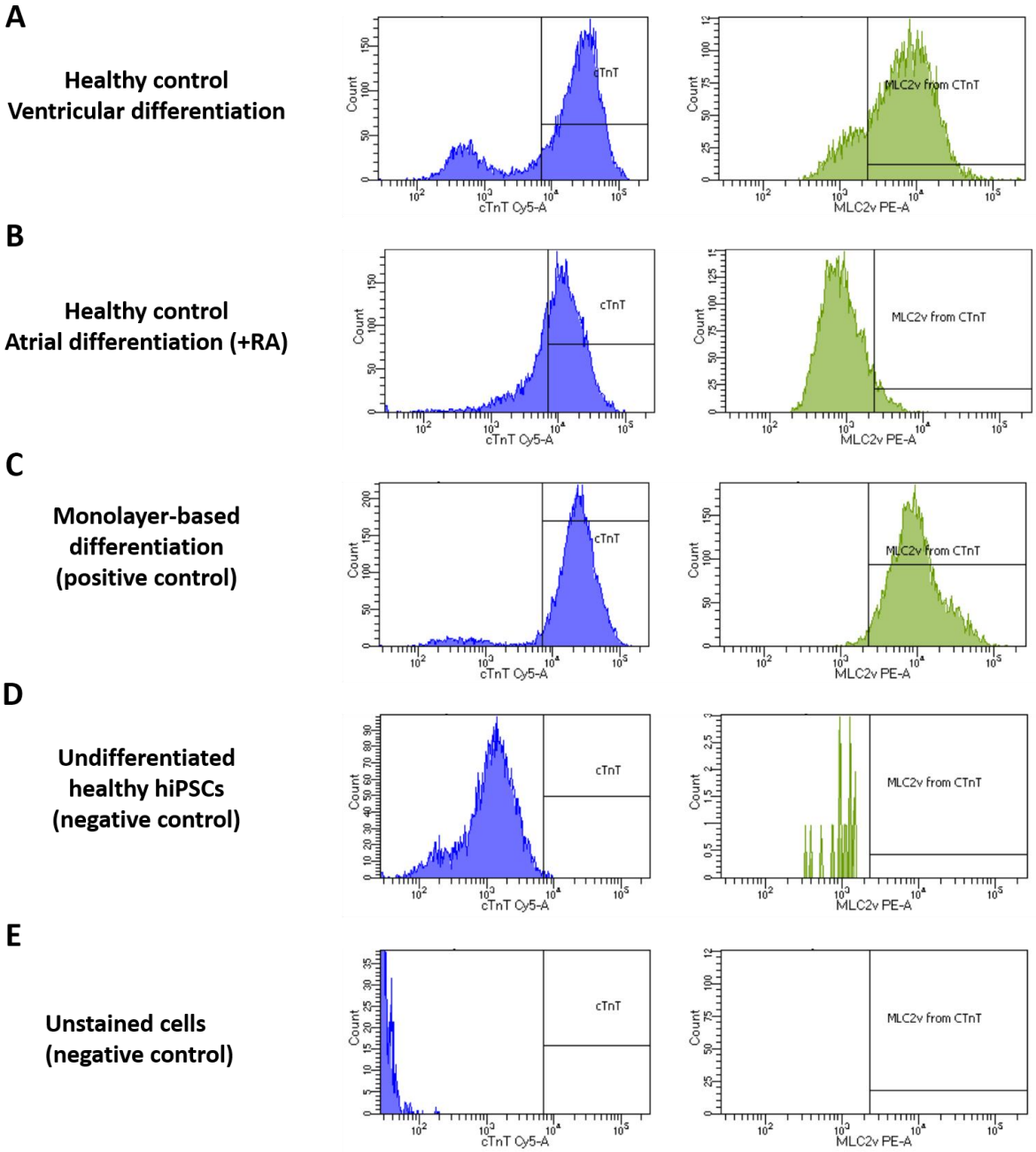
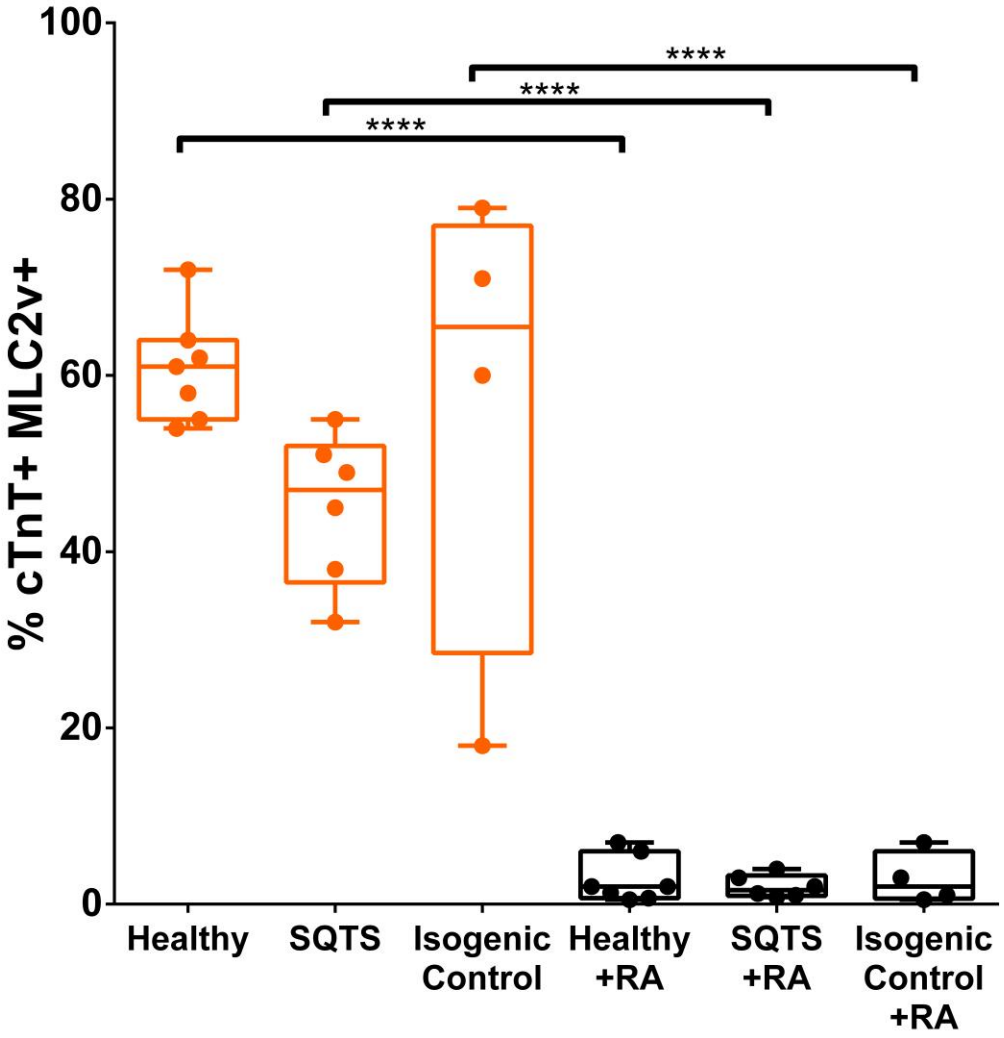
Supplementary Material

Supplementary Figures

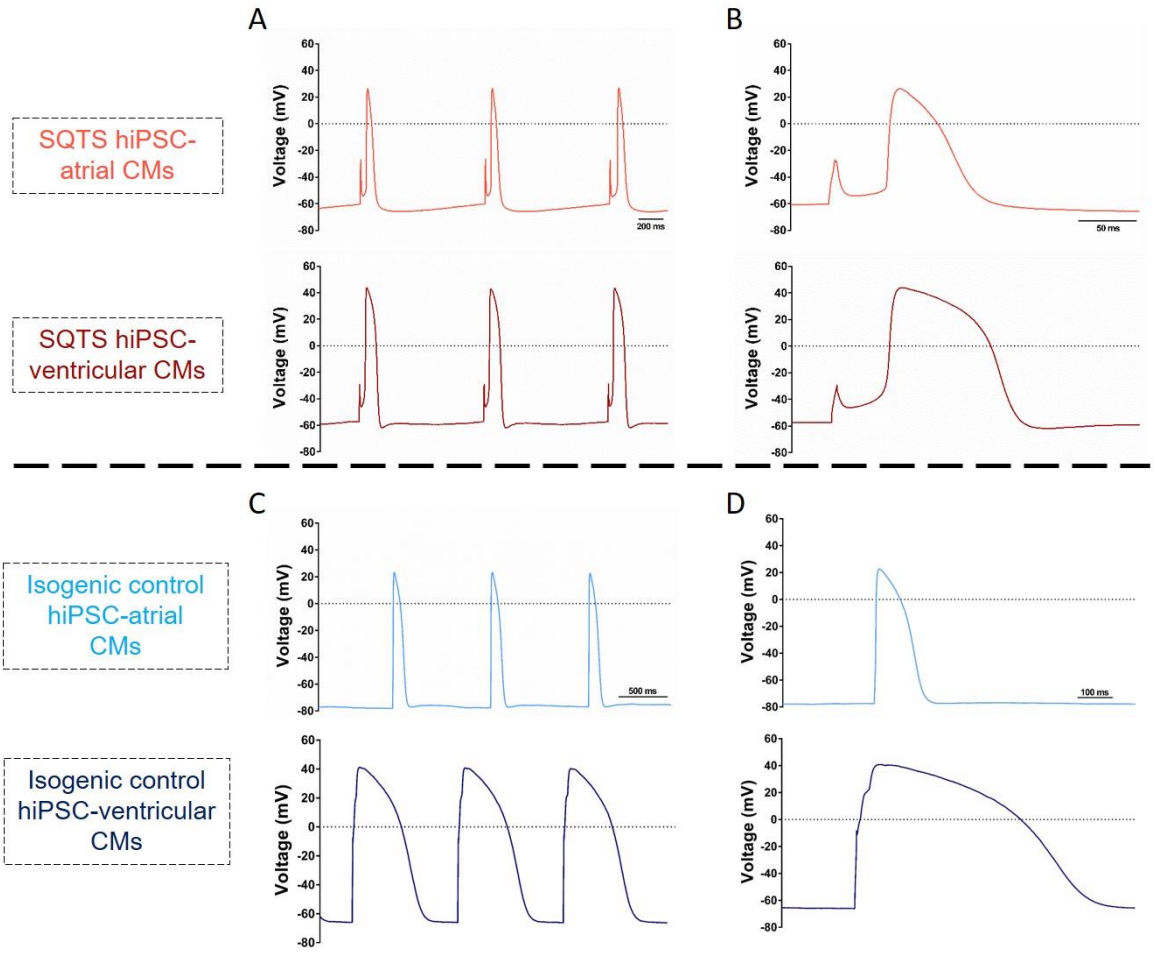


Supplementary Figure 1. Flow-cytometry analysis of subtype-specific differentiations and controls. Flow-cytometry analysis assessing cTnT and MLC2v expression in the following different conditions – [A,B] Ventricular and atrial (RA supplemented) differentiations of healthy hiPSCs, [C] positive control consisting of monolayer based cardiac

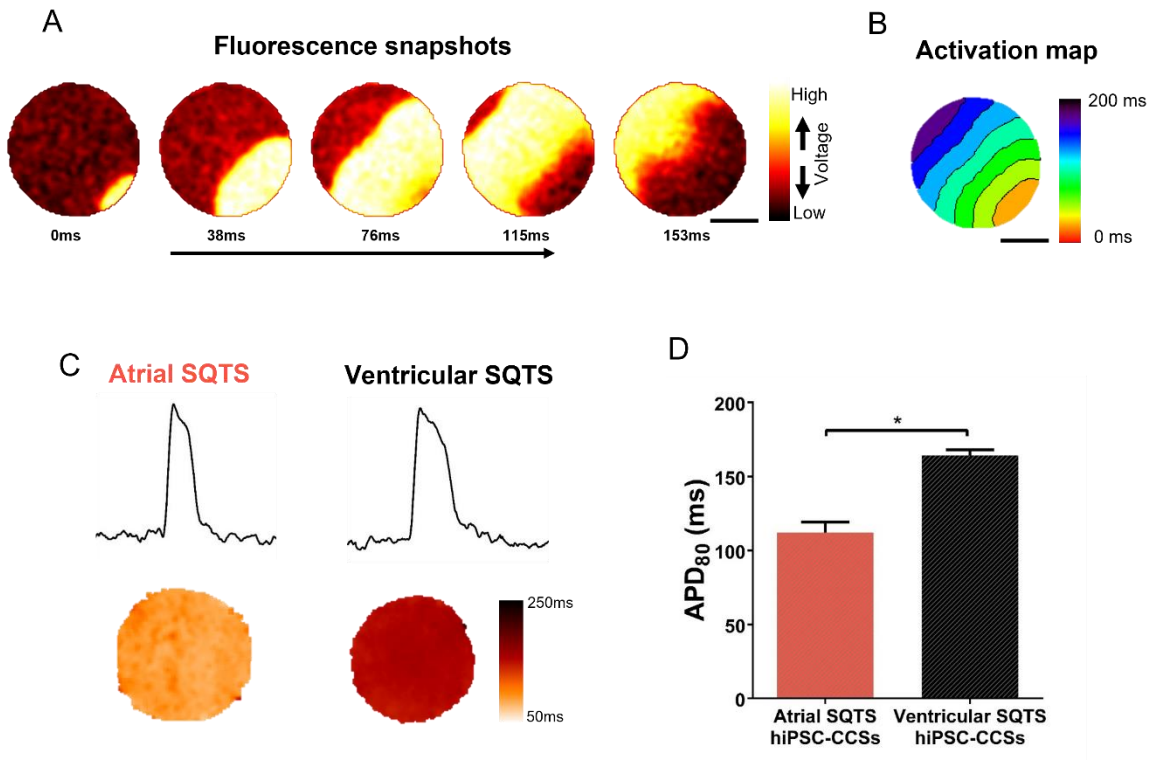
differentiations protocol yielding high proportion of cardiomyocytes with ventricular phenotype (Burridge P., Nat Methods, 2014); [D] negative control of undifferentiated healthy hiPSCs; [E] negative control of unstained cells.



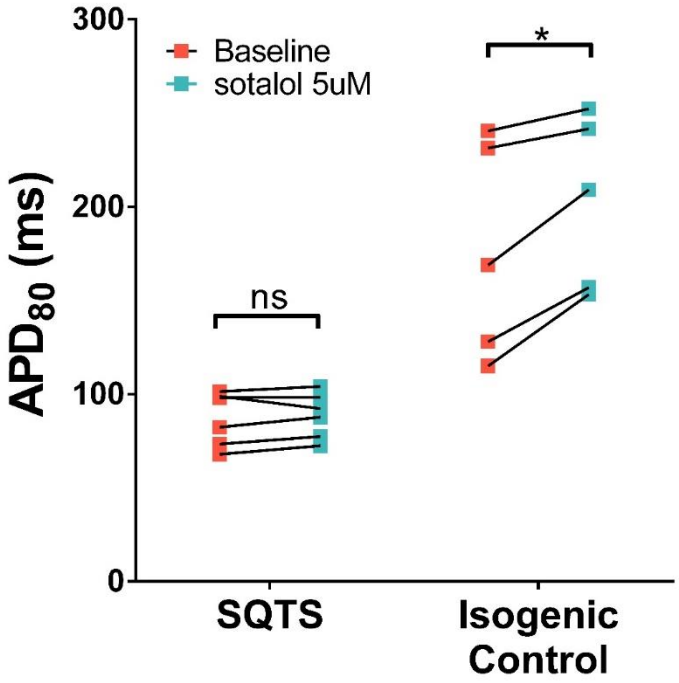
Supplementary Figure 2. Flow cytometry analysis of chamber-specific differentiations. Summary of flow cytometry experiments showing the percentages of cTnT+ MLC2v+ cells in both atrial and ventricular differentiations from the three different hiPSC lines utilized -healthy control, SQTS, and isogenic control. Values are presented in box and whiskers plots indicating minimum, lower quartile, median, upper quartile and maximum as well as the individual data points.



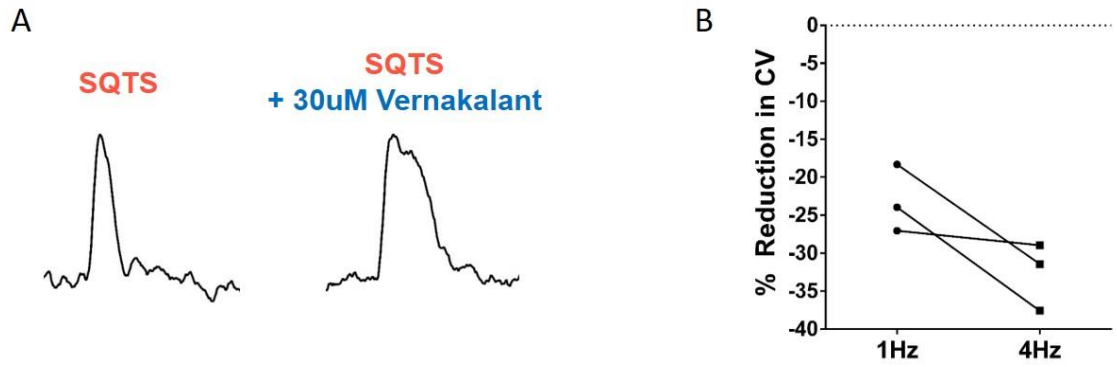
Supplementary Figure 3. Parallel AP recordings of atrial and ventricular CMs. Patch-clamp recordings of atrial and ventricular cardiomyocytes from both SQTS **(A-B)** and isogenic-control **(C-D)** cell lines at similar days of maturation (approximately 30 days old cardiomyocytes). All cells were paced at a similar frequency of 1Hz. Note the shorter AP and steeper repolarization in atrial myocytes, when compared to APs from their ventricular counterpart.



Supplementary Figure 4. Optical mapping of atrial SQTS-hiPSC-CCSs. [A-B] a sequence of fluorescence snapshots and the corresponding activation map as obtained for SQTS-hiPSC-ACS showing normal electrical activity propagating from the pacemaker site throughout the tissue. **[C]** Representative optical APs and the derived APD80 maps obtained from atrial and ventricular SQTS-hiPSC-CCSs. **[D]** Summary of mean APD80 values measured from atrial and ventricular SQTS-hiPSC-CCSs. Values are presented as means ±SEM. *, $p<0.05$



Supplementary Figure 5. APD response to sotalol. Summary of the APD80 values obtained for SQTS and isogenic control hiPSC-ACSs at baseline and upon treatment with 5uM sotalol. *, $p<0.05$



Supplementary Figure 6. Rate-dependent effects of vernakalant. **[A]** Representative optical AP obtained from SQTS-hiPSCs-ACSs at baseline and upon treatment with 30uM vernakalant. **[B]** Summary of the percentage of reduction in CV in SQTS-hiPSC-ACSs upon treatment with 30uM vernakalant at different pacing frequencies of 1Hz and 4Hz.

Supplementary Movie Legends

Supplementary Movie 1. Beating embryoid-body (EB) resulting from the hiPSC chamber-specific ventricular and atrial differentiation protocols.

Supplementary Movie 2. Fluorescence movie of normal AP propagation in SQTS- and isogenic control- hiPSC-ACSs.

Supplementary Movie 3. Fluorescence (top) and phase (bottom) movies of spiral-waves (rotors) induced in SQTS- and isogenic control- hiPSC-ACSs.

Supplementary References

Burridge, Paul W et al. “Chemically defined generation of human cardiomyocytes.” *Nature methods* vol. 11,8 (2014): 855-60. doi:10.1038/nmeth.2999