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ii. Cell Physiology

Supplement

Long-QT syndrome related sodium channel mutations probed by dynamic action potential clamp technique

Running title:

SCN5A mutations probed by dAPC technique

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Figure S1.

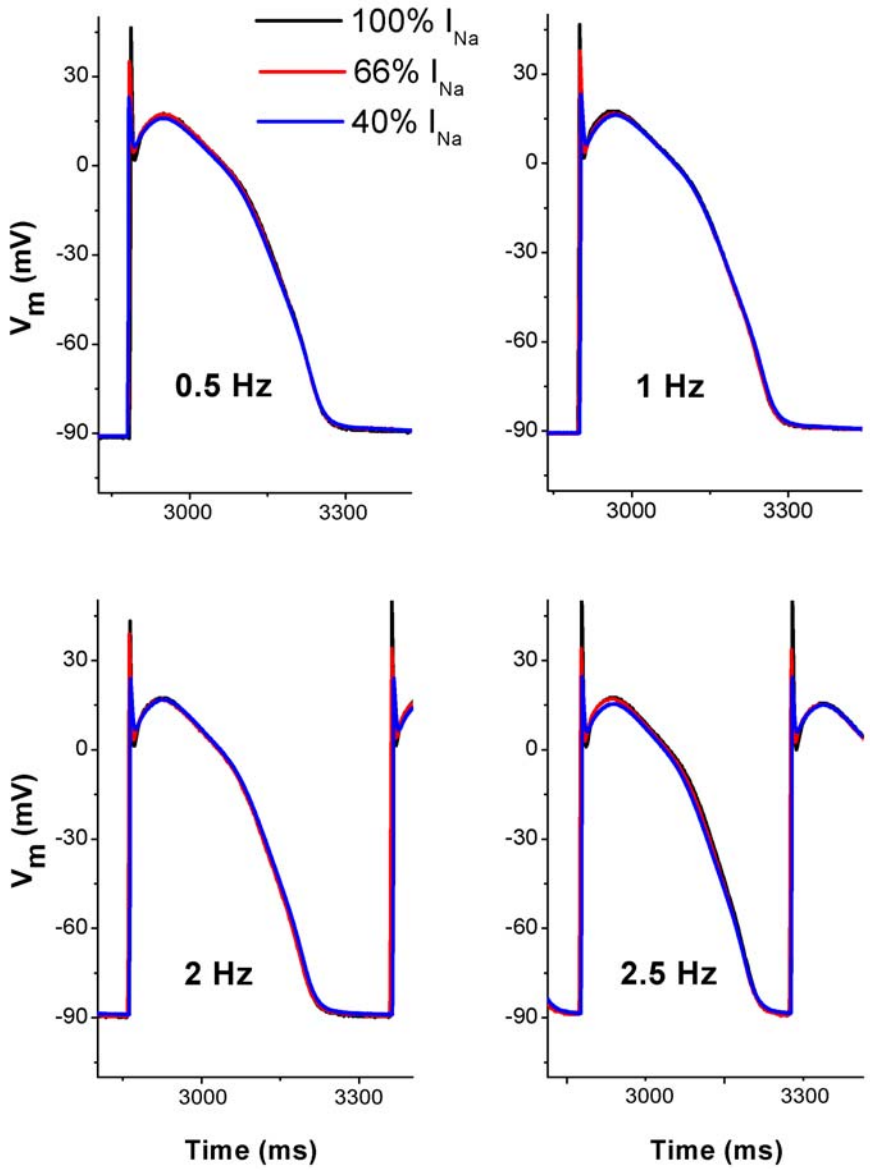

Figure S1. Effects of reducing model cell I_{Na} density on action potential duration (APD). I_{Na} reductions by 34 and 60% do not have APD-shortening effects at physiologically relevant stimulation rates.

Figure S2.

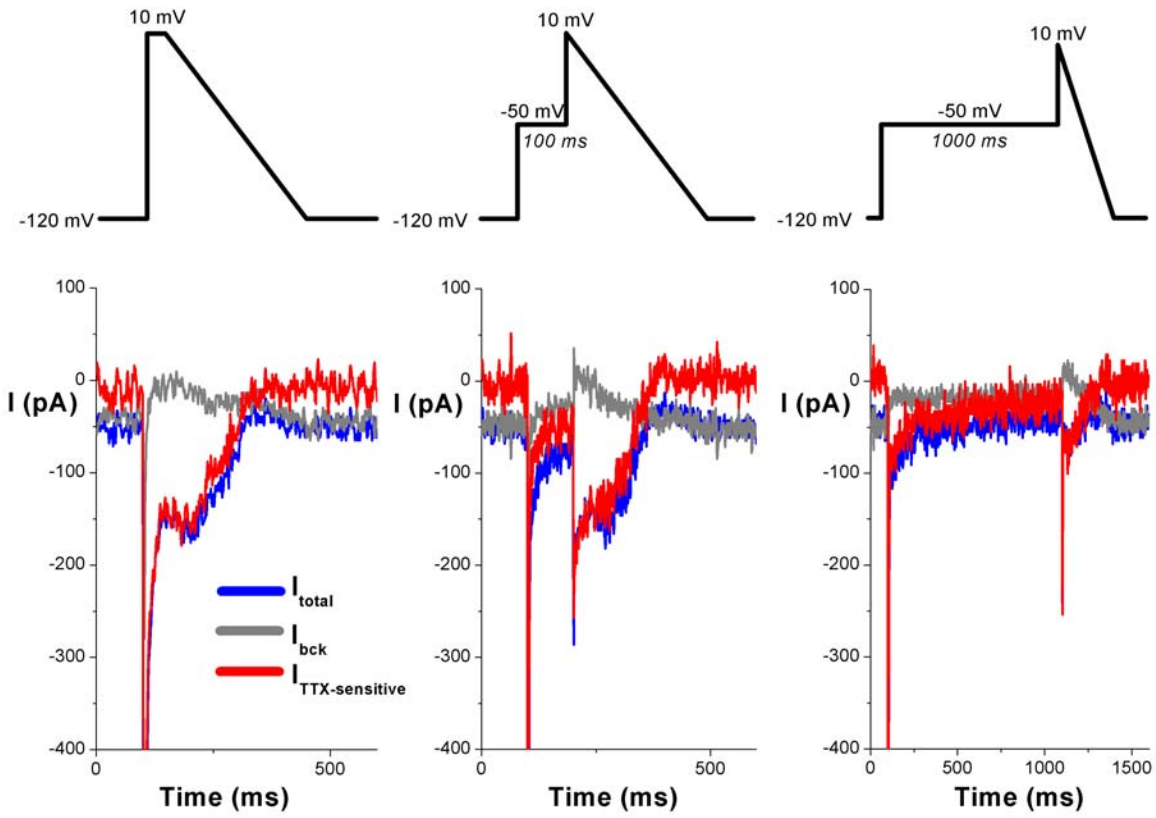

Figure S2. Magnitude of late I_{Na} during repolarising ramps depends on the duration of the depolarising voltage prepulse preceding the ramps. Note that a 1000-ms depolarising prepulse (right) almost fully inactivates I_{Na} (i.e. the TTX-sensitive current, I_{TTX}-sensitive), while the step-ramp (left) allows defining I_{bck}-V relationships in (transfected) individual HEK-293 cells using $I_{bck} = I_{total} - I_{TTX\text{-}sensitive}$ (see main text for details).

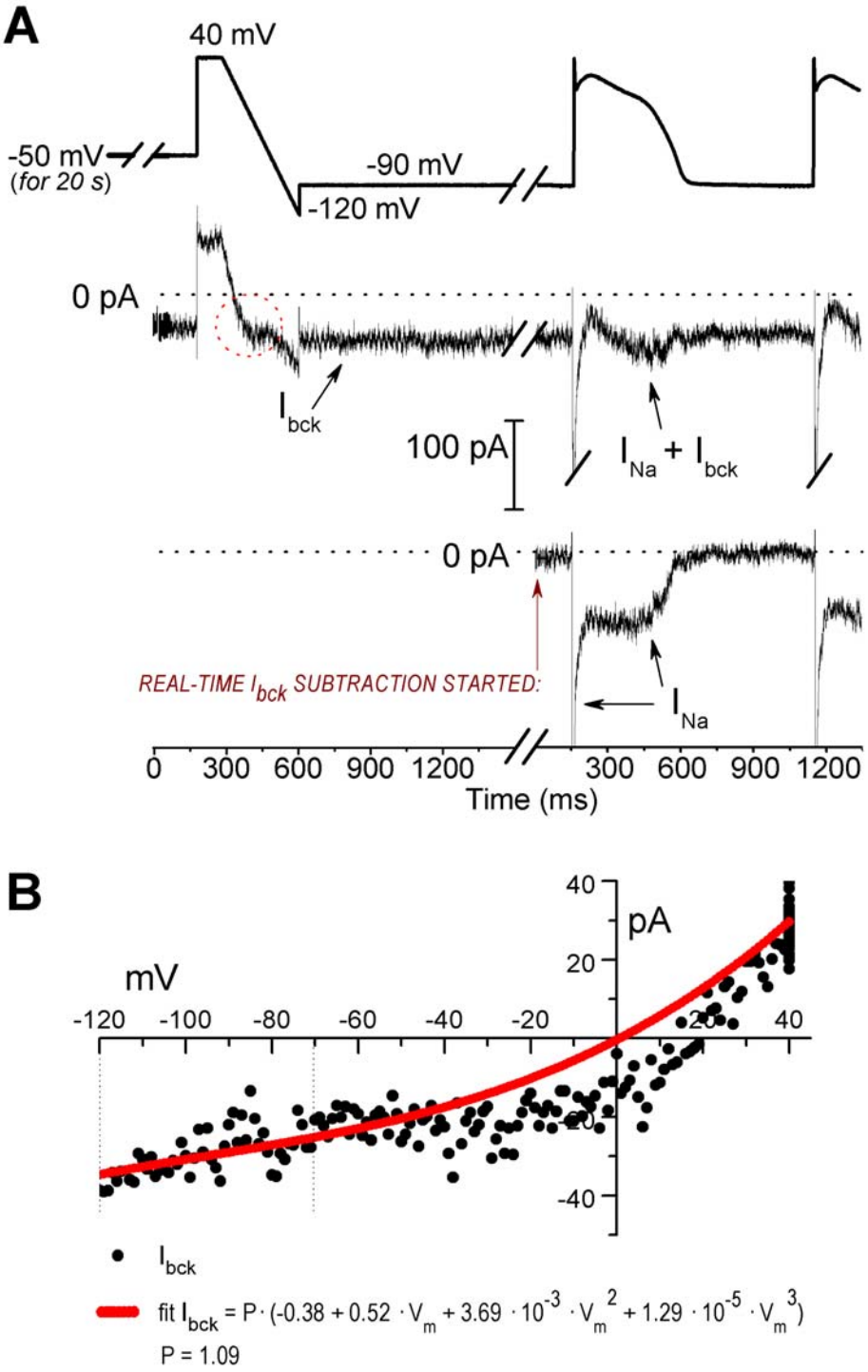

Figure S3. Real-time HEK-293 cell I_{bck} subtraction in a dAPC experiment, using Y1795C SCN5A cDNA-transfected HEK-293 cell. (A) Step-ramp voltage protocol to estimate I_{bck}

followed by APs elicited in a subepicardial model cell (top); Middle: I_{Na} recorded in the presence of I_{bck} (arrow); Bottom: Y1795C I_{Na}, after I_{bck}-removal (the vertical arrow indicates the start of real-time I_{bck} subtraction). Note the massive late (Y1795C) I_{Na} during AP plateau and repolarization. Dotted line shows zero current level. (B) I-V relationship of HEK cell I_{bck} in the experiment shown in A, described by the third order polynomial equation with scaling factor P (fit I_{bck}). Here, the 20-s long depolarizing prepulse-induced (Y1795C) I_{Na} inactivation was incomplete, as indicated by an inward "hump" current during the repolarising voltages (and by the dotted circle in A). To avoid any eventual contribution of late I_{Na} to I_{bck}, equation 1 (bottom) was fitted only to the data points in the -120 to -70 mV range (between the dotted lines) (see main text for details).

Figure S4.

Table S1.
Relative densities of selected ionic currents in the subendocardial, midmyocardial (M), and subepicardial cell models.

Current	*Subendocardial*	*M*	*Subepicardial*
I_{to}	25%	87%	100%
I_{Ks}	92%	46%	100%
I_{K1}	89%	74%	100%

All densities are percentage relative to the standard densities in the PB model that essentially describes a human subepicardial ventricular myocyte (Berecki et al. 2005).

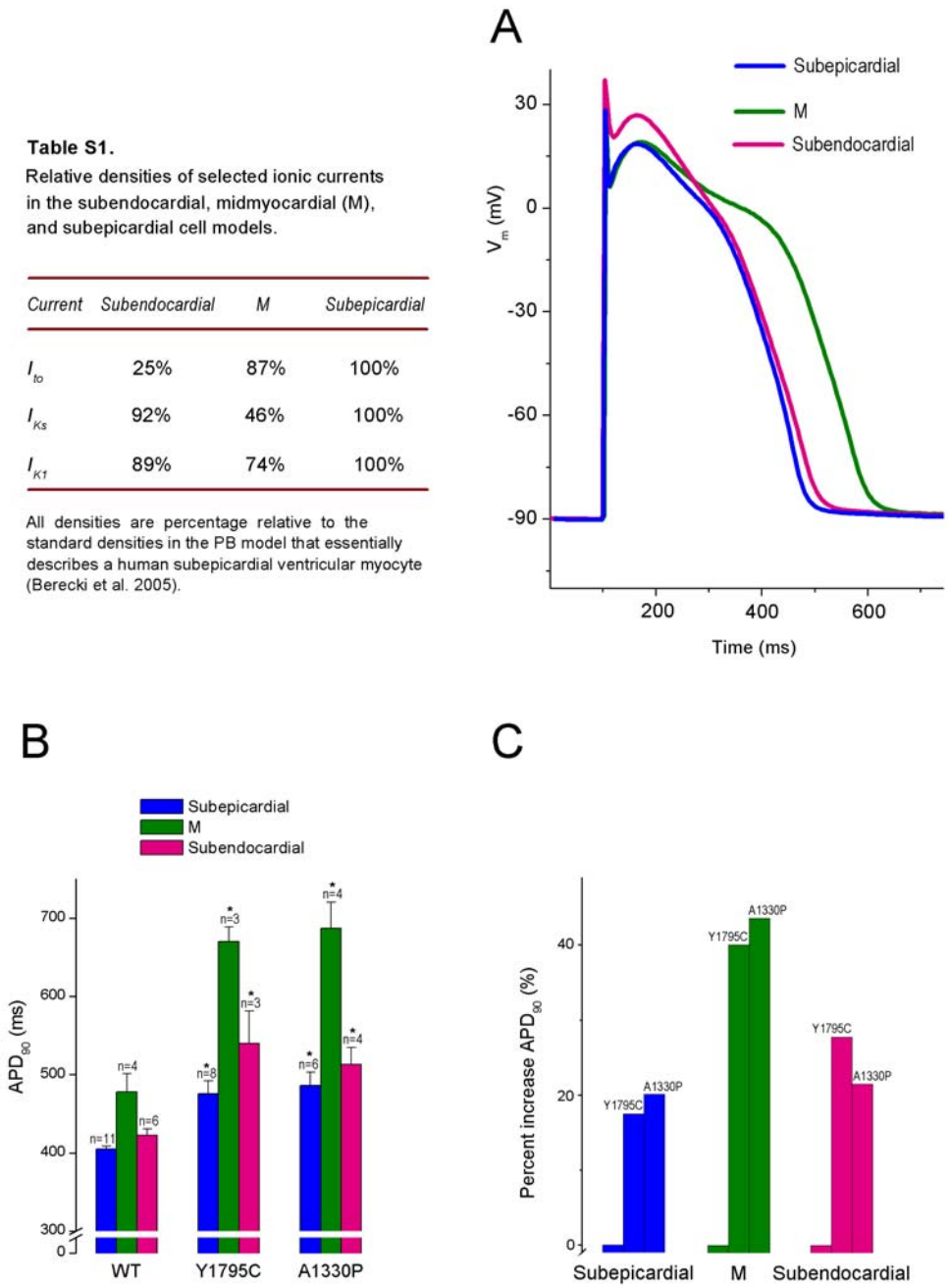

Figure S4. Regional AP heterogeneity and AP prolongation caused by LQT3 syndrome-related SCN5A mutations in dAPC experiments. (A) Subepicardial, midmyocardial (M), and

subendocardial APs were generated by adjusting selected membrane ionic currents in the Priebe & Beuckelmann (PB) model cell (Priebe & Beuckelmann, 1998) according to Table S1, and by implementing wild-type (WT) HEK-293 cell I_{Na} to the PB model cell. Stimulus rate was 1 Hz. (B) AP duration at 90% repolarisation (APD_{90}) values obtained by implementing WT, Y1795C and A1330P HEK cell I_{Na} to the subepicardial, M, or subendocardial PB model cells, at 1 Hz. Asterisks indicate significant difference versus control ($P < 0.05$ for mutant *versus* WT). (C) M cells exhibit a larger increase in APD_{90} with Y1795C and A1330P mutants compared to subepicardial or subendocardial cells. Mean APD_{90} values were normalised for WT APD_{90} within a cell type using data from B. The results are consistent with the decreased repolarising current densities of subendocardial and M cells (Table S1).

References

Berecki G, Zegers JG, Verkerk AO, Bhuiyan ZA, de Jonge B, Veldkamp MW, Wilders R & van Ginneken AC (2005). HERG channel (dys)function revealed by dynamic action potential clamp technique. *Biophys J* **88**, 566-578.

Priebe L & Beuckelmann DJ (1998). Simulation study of cellular electric properties in heart failure. *Circ Res* **82**, 1206-1223.