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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.

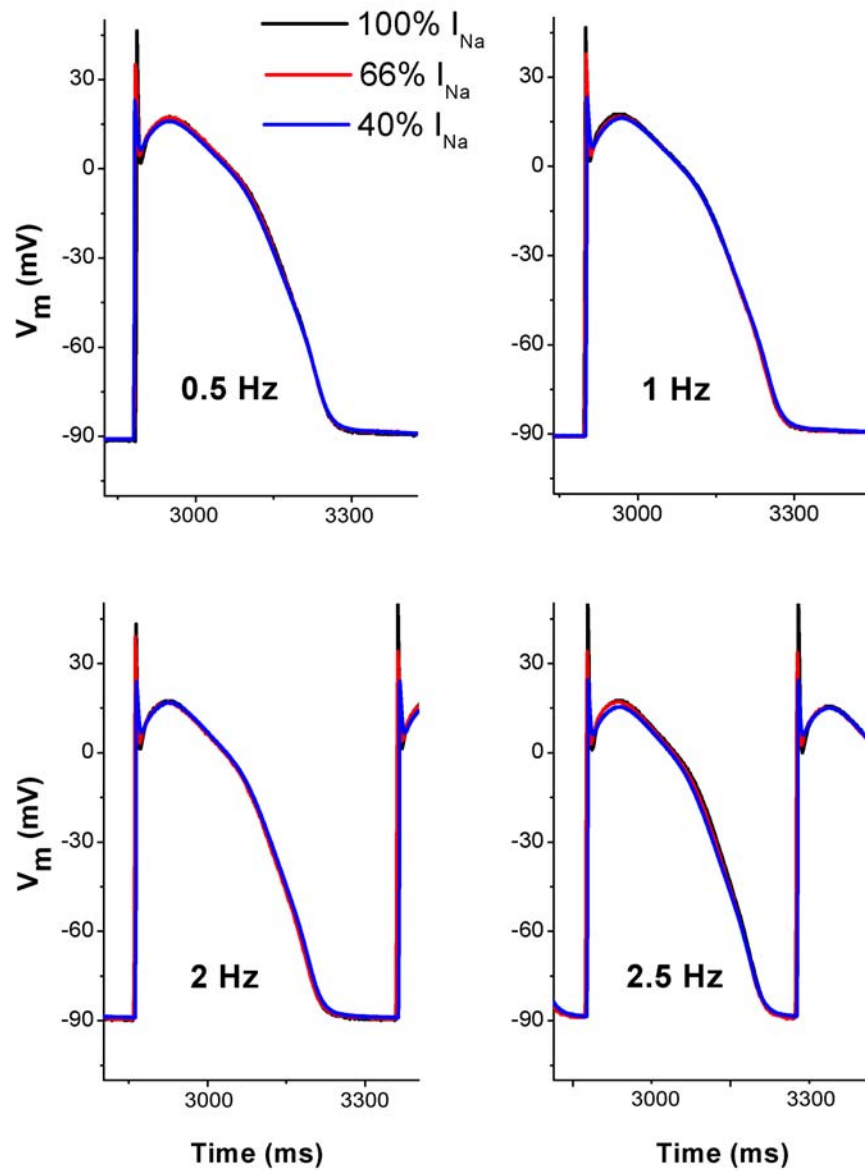


Figure S1. Effects of reducing model cell I_{Na} density on action potential duration (APD). I_{Na} reductions by 34 and 60% do not have APD-shortening effects at physiologically relevant stimulation rates.

Figure S2.

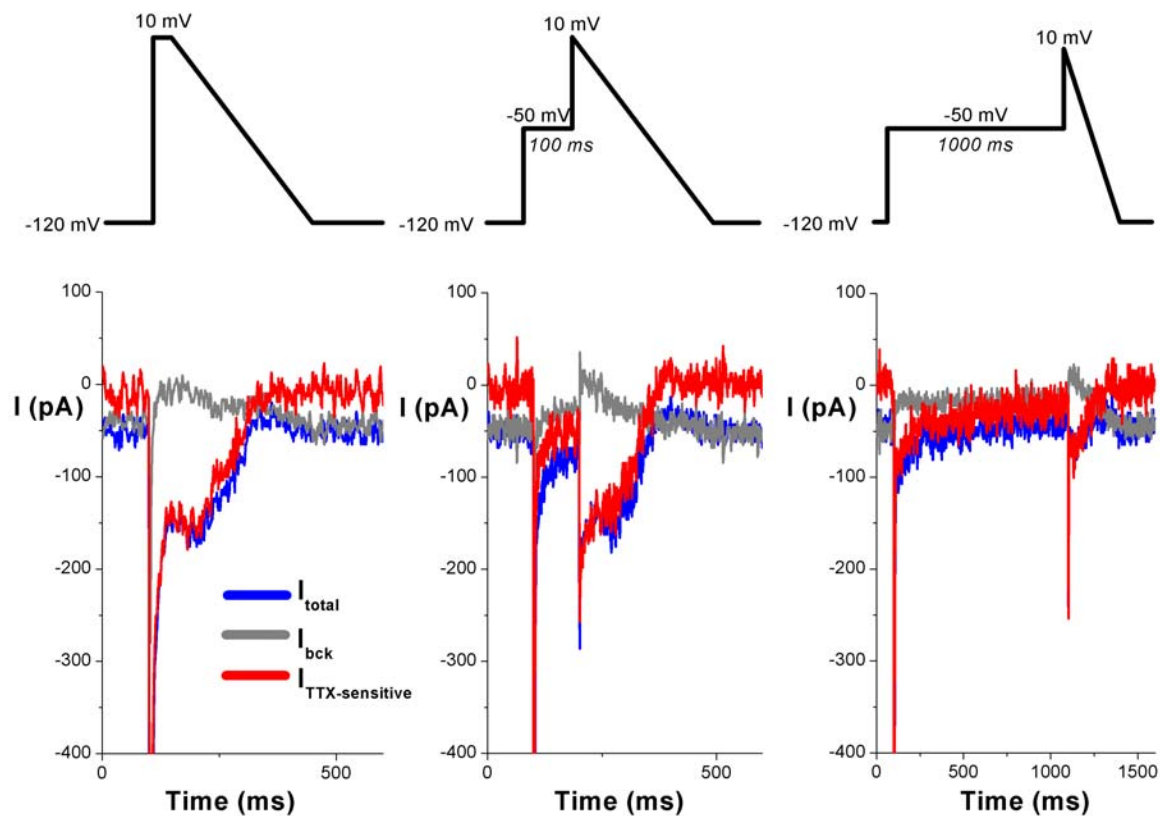


Figure S2. Magnitude of late I_{Na} during repolarising ramps depends on the duration of the depolarising voltage prepulse preceding the ramps. Note that a 1000-ms depolarising prepulse (right) almost fully inactivates I_{Na} (i.e. the TTX-sensitive current, $I_{\text{TTX-sensitive}}$), while the step-ramp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

Figure S3.

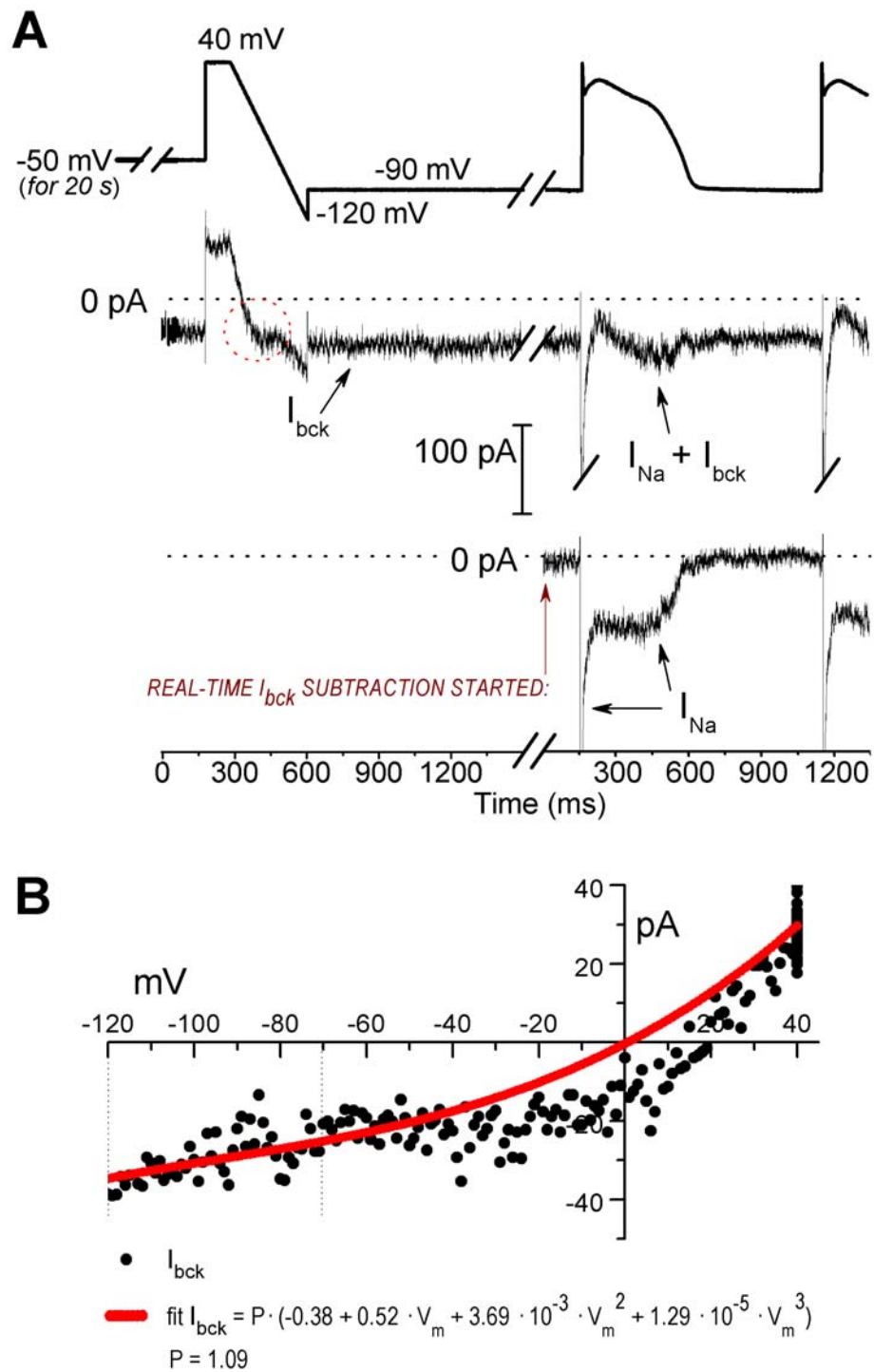


Figure S3. Real-time HEK-293 cell I_{bck} subtraction in a dAPC experiment, using Y1795C SCN5A cDNA-transfected HEK-293 cell. (A) Step-ramp voltage protocol to estimate I_{bck}

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).

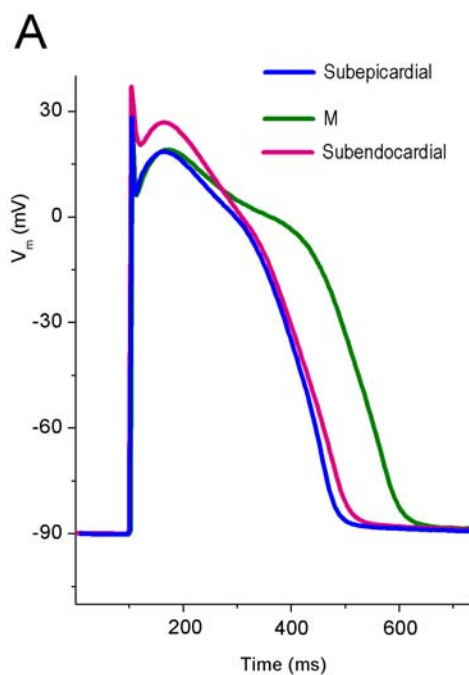
Figure S4.

Table S1.

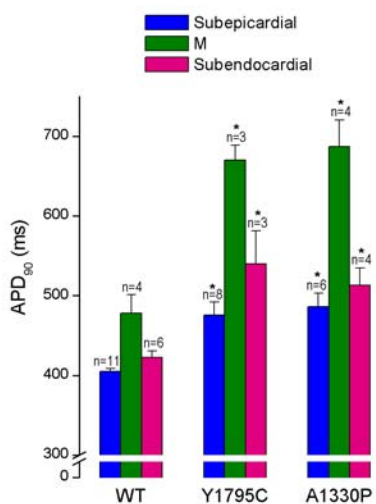
Relative densities of selected ionic currents in the subendocardial, midmyocardial (M), and subepicardial cell models.

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).



B



C

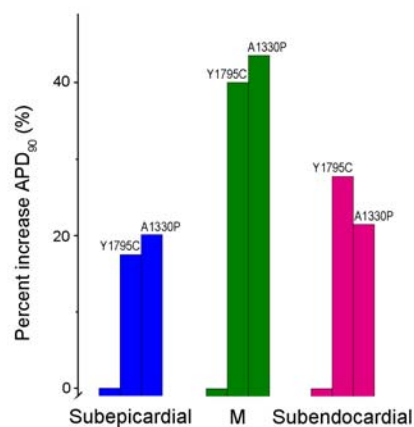


Figure S4. Regional AP heterogeneity and AP prolongation caused by LQT3 syndrome-related SCN5A mutations in dAPC experiments. (A) Subepicardial, midmyocardial (M), and

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.