

Supplementary Materials for  
**Modeling long QT syndrome type 2 on a chip via in-depth assessment of  
isogenic gene-edited 3D cardiac tissues**

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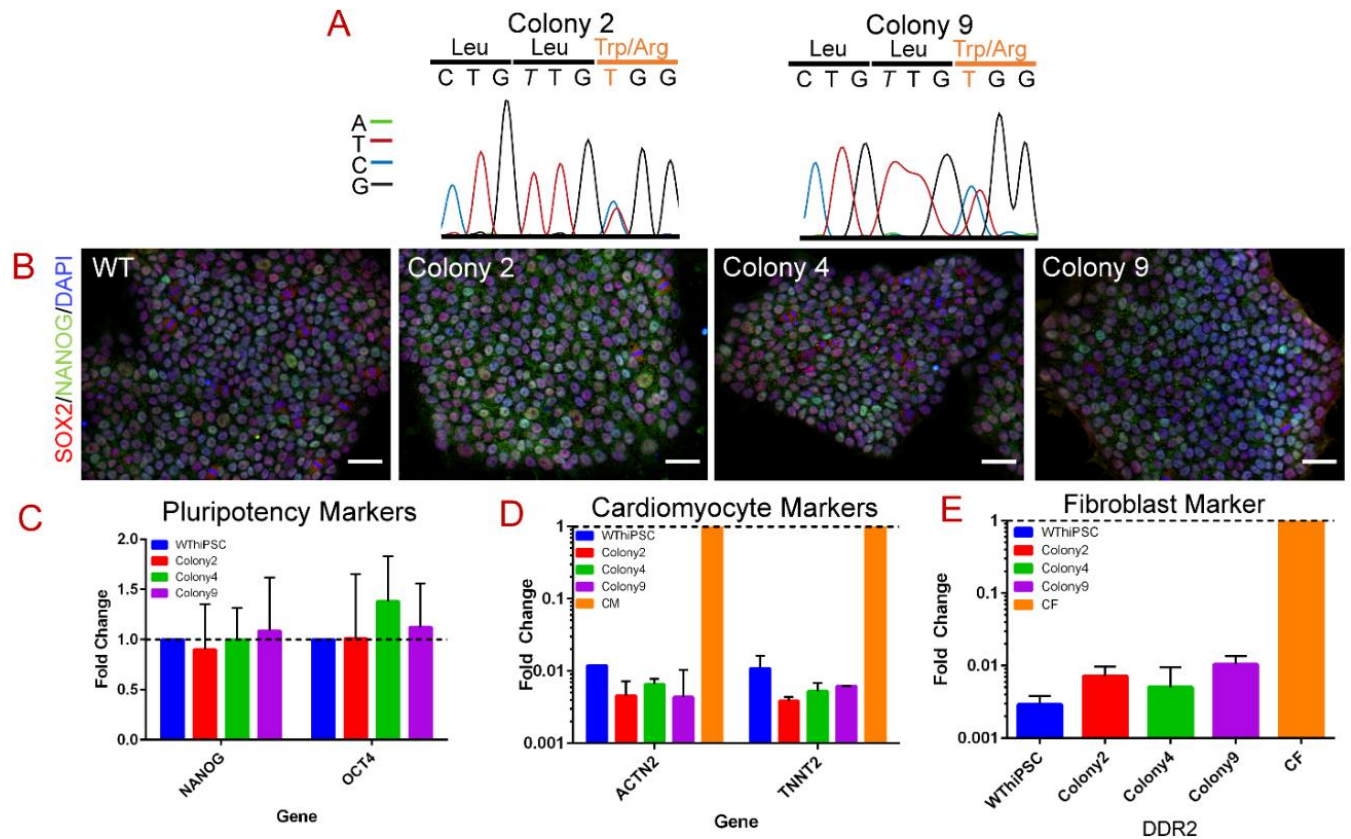
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**The PDF file includes:**

Figs. S1 to S3  
Tables S1 and S2  
Legends for movies S1 to S6  
Legend for supplemental source code

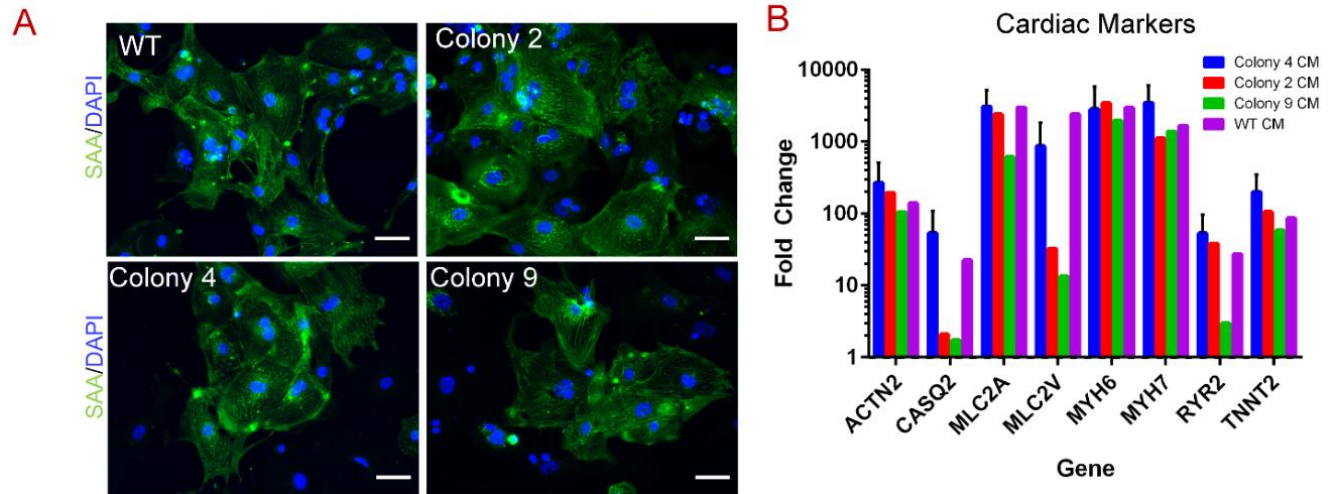
**Other Supplementary Material for this manuscript includes the following:**

Movies S1 to S6  
Supplemental source code



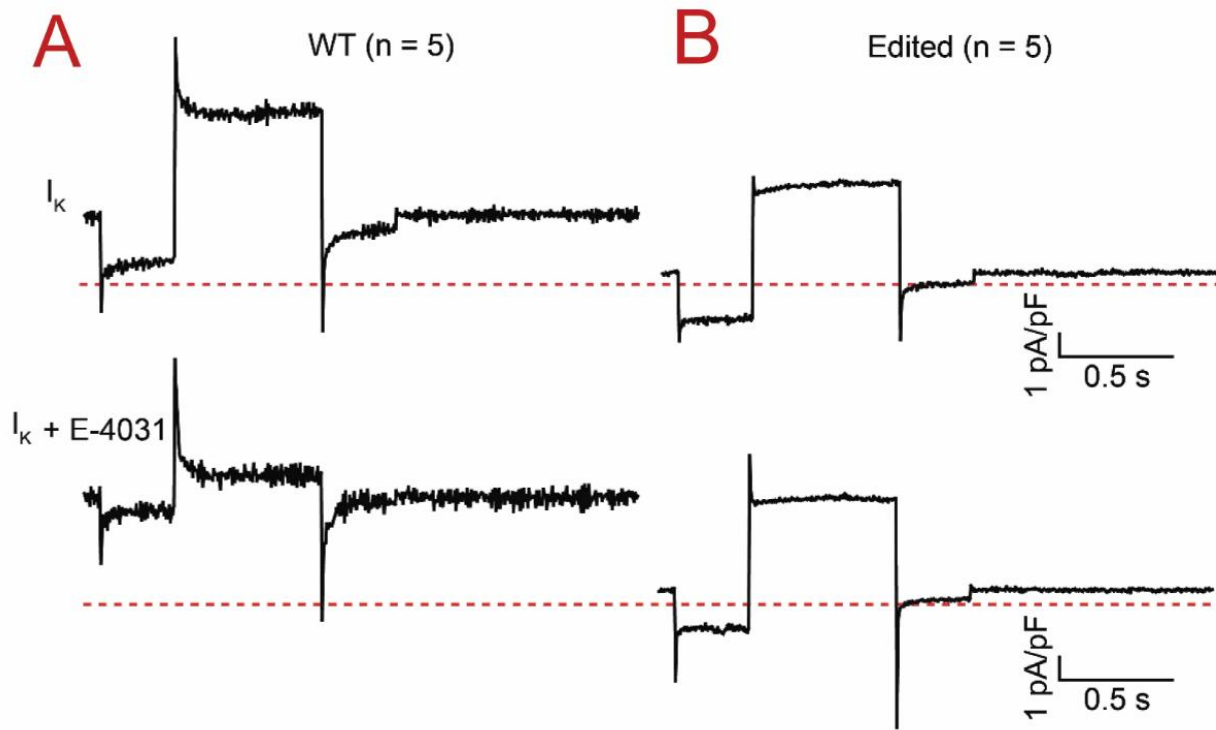
**Fig. S1.**

Characterization of hiPSC clones with R531W edit in *KCNH2*. (A) Trace files of the target site in Colony 2 and Colony 9, showing a heterozygous edit in c.C1591T. (B) Immunostaining of pluripotency markers, where SOX2=red and NANOG=green, for all edited colonies and WT hiPSCs. Gene expression analysis of (C) pluripotency, (D) cardiomyocyte, and (E) fibroblast markers of edited colonies, normalized to the respective positive control.



**Fig. S2.**

Cardiomyocyte differentiation potential of hiPSC clones with R531W edit in *KCNH2*. (A) Immunostaining of sarcomeric  $\alpha$ -actinin and (B) gene expression analysis of an array of cardiac markers of cardiomyocytes differentiated from edited colonies and WT hiPSCs.



**Fig. S3.**

Whole-cell patch-clamp experiments on WT and edited hiPSC-CMs  $I_K$  currents of (A, top) WT and (B, top) edited CMs were collected using extracellular solution supplemented with JNJ-303 (1  $\mu$ M) and Nifedipine (5  $\mu$ M).  $I_K$  + E-4031 currents of (A, bottom) WT and (B, bottom) edited CMs were collected after perfusion of E-4031 (1  $\mu$ M). The data are average traces from n=5 cells.

HEK293 Transfection		
	Bystander C	Target C
US	32.5+/-0.71	0.5+/-0.71
DN	18.5+/-2.12	2.5+/-0.71
BFP	41 +/-7.07	5.5+/-2.12
BFP/GFP	72.5+/-2.38	6.15+/-2.06

hiPSC Transfection		
	Bystander C	Target C
US	11.5+/-6.24	1+/-0.82
DN	14.25+/-4.86	2+/-0
BFP	50.25+/-14.17	2.5+/-0.58
BFP/GFP	66.25+/-4.86	4.5+/-2.08

**Table S1.**  
Editing efficiencies of both HEK and hiPSC transfections

<b>Gene</b>	<b>Forward primer</b>	<b>Reverse primer</b>	<b>Product size (bp)</b>
<i>DSCR3</i>	CACTAACGGGAGAGCTGGTG	AACCATGGAACGTGAGGGTG	153
<i>KIAA1324</i>	AAAATAACCCCTGGCCCGAA	TAGCGTTGTCCTGAGTGAGC	457
<i>TFR2</i>	TGACGTCTACTGCCCTACA	ACTTACCTTCTGGGCGAAGC	274
<i>POU3F3</i>	AGAGTCTGCTCTACTCGCA	CAGCTCTGGCGTGTCCC	149
<i>ACTN2</i>	GGCACCCAGATTGAGAACAT	CCTGAATAGCAAAGCGAAGG	268
<i>CASQ2</i>	GTTGCCCGGGACAATACTGA	CTGTGACATTACACCACCCA	142
<i>MLC2A</i>	CAGCGGCAAAGGGGTGGTGAA	GGTCCATGGGTGTCAGGGCGA	113
<i>MLC2V</i>	GGCGCCAACCTCCAACGTGTT	ACGTTCACTCGCCCAAGGGC	149
<i>MYH6 - 4</i>	TCCTGCGGCCAGATTCTTC	TCTTCCTTGTCATCGGGCAC	193
<i>MYH</i>	CACAGCCATGGGAGATTCCGG	CAGGCACGAAGACATCCTTCT	128
<i>RYR2</i>	AGCCAGTGTCATCCACCAAC	ACTGATCACAGGTGGCTGAA	89
<i>TNNT2</i>	GACAGAGCGGAAAAGTGGGA	CTCCTTGGCCTTCTCCCTCA	127
<i>18S</i>	GTAACCCGTTGAACCCATT	CCATCCAATCGGTAGTAGCG	151
<i>COL1A1</i>	AGTGGTTTGGATGGTGCCAA	GCACCATCATTTCCACGAGC	170
<i>COL3A1</i>	CCAGGAGCTAACGGTCTCAG	CAGGGTTTCCATCTCTTCCA	103
<i>TIMP1</i>	AATTCCGACCTCGTCATCAG	TGCAGTTTTCCAGCAATGAG	230
<i>KCNH2</i>	GAATGTGCCCTTCCCTGTC	ATGCAGGCTAGCCAGTGC	146
<i>DDR2</i>	TTTTTGGGTGGGGAAACGC	CTGGGAGGCATATCAACGGG	249
<i>POSTN</i>	CCCCGTGACTGTCTATAAGC	CCTTGGTGACCTCTTCTTGTA	197
<i>OCT4</i>	CAAAGCAGAAACCCTCGTGC	CTCGGACCACATCCTTCTCG	164
<i>NANOG</i>	CAATGGTGTGACGCAGGGAT	GGACTGTTCCAGGCCTGATT	183

**Table S2.**

Primer sequences utilized in either PCR or qPCR

**Movie S1.**

Phase contrast video of WT tissue on-a-chip after 14 days of culture within microfluidic device.

**Movie S2.**

Phase contrast video of LQTS2 tissue on-a-chip after 14 days of culture within microfluidic device.

**Movie S3.**

Calcium transients of control WT tissues on-a-chip before epinephrine.

**Movie S4.**

Calcium transients of control LQTS2 tissues on-a-chip before epinephrine.

**Movie S5.**

Calcium transients of control WT tissues on-a-chip after epinephrine.

**Movie S6.**

Calcium transients of control LQTS2 tissues on-a-chip after epinephrine.

**Supplemental source code**

Generated code to quantify action potential metrics from calcium transients.