Lucía Cócera-Ortega et al. shRNAs Targeting a Common *KCNQ1* Variant Could Alleviate Long-QT1 Disease Severity by Inhibiting a Mutant Allele. *Int. J. Mol. Sci.* 2022

Supplemental tables and figures

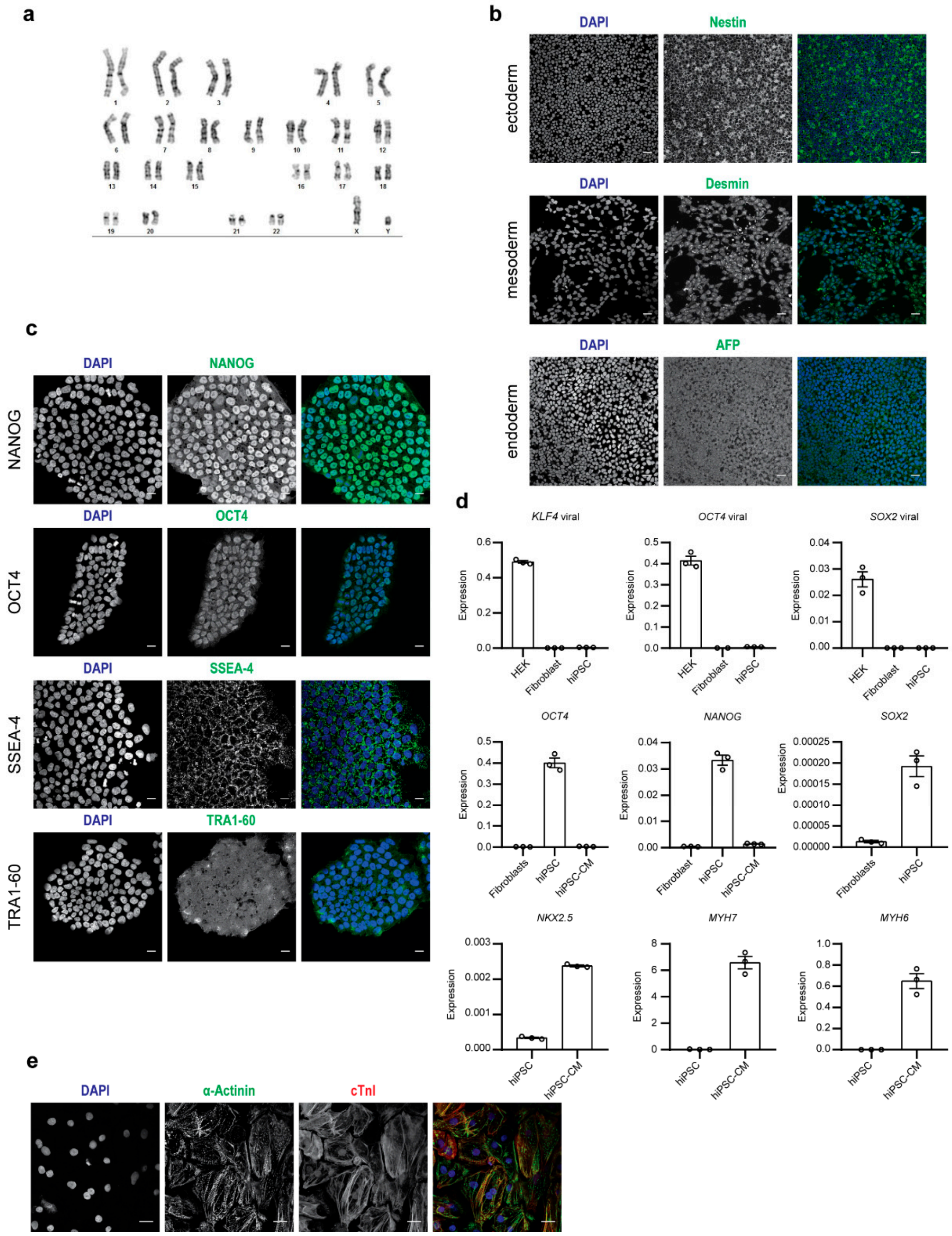


Figure S1. Characterization of Line 2. a) Karyotype analysis of hiPSCs of Line 2. **b)** Immunofluorescence after in vitro 3 lineage differentiation. Scale bars 20 µm. **c)** Immunofluorescence of pluripotency markers in undifferentiated hiPSCs. Scale bars 20 µm. **d)** Downregulation of viral genes in hiPSCs after induction of pluripotency with HEK cells transfected with the viral plasmids as positive control, expression of pluripotency markers OCT4, NANOG and SOX2 in undifferentiated hiPSCs and expression of cardiomyocyte markers NKX2.5, MYH7 and MYH6 in hiPSC-CMs measured by RT-qPCR. Error bars indicate SEM. **e)** Immunofluorescence of cardiomyocyte markers in hiPSC-CMs. Scale bars 20 µm. For all immunofluorescence pictures the separate panels on the left are merged into the rightmost panel with colour coding as indicated above the separate panels. Characterization of line 1 is included elsewhere (Tijsen et al. manuscript in draft).

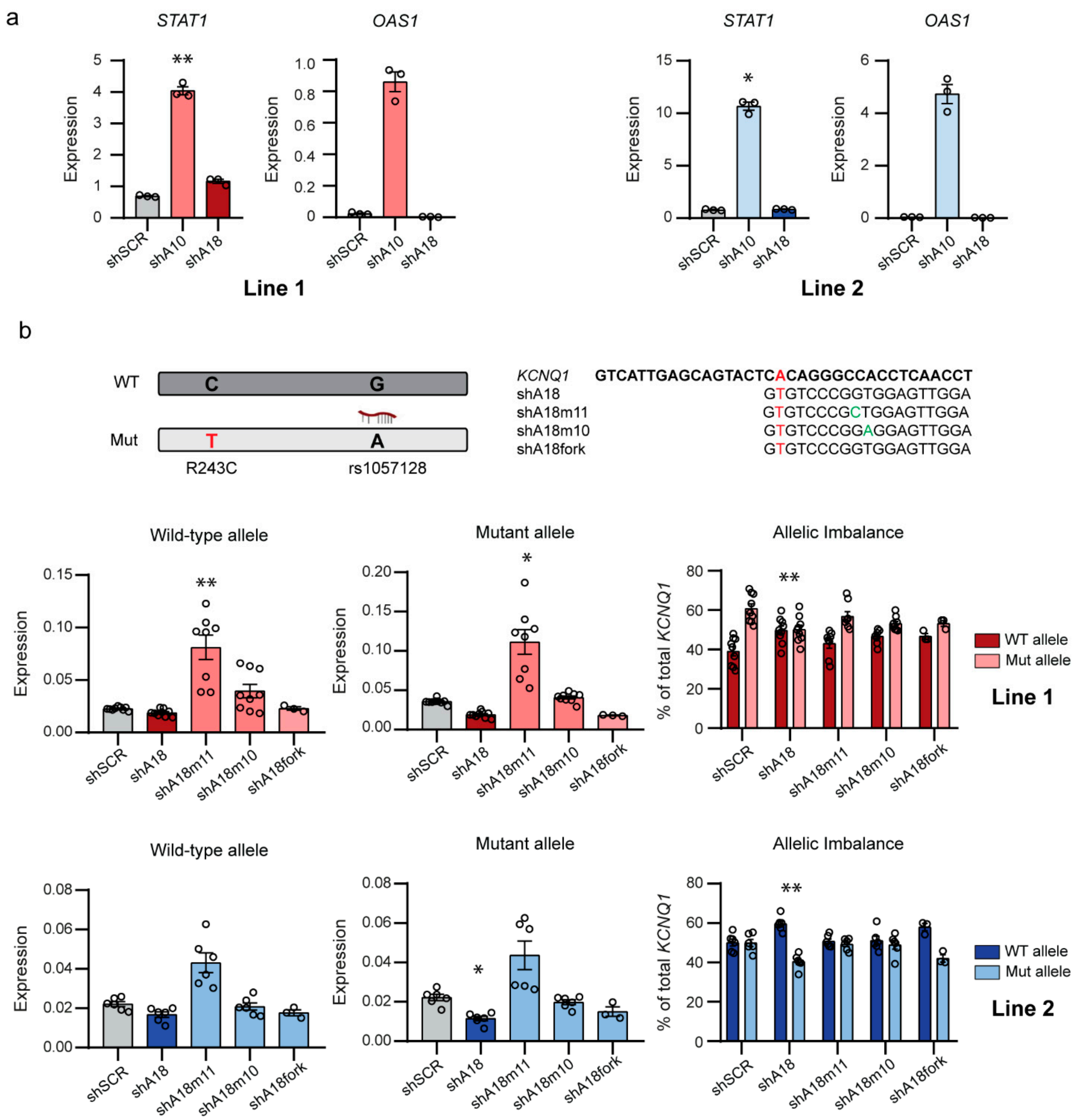


Figure S2. Introduction of additional mismatches does not improve allele-specificity of shRNAs targeting the A allele of SNP rs1057128 on the mutant *KCNQ1* allele. a) Relative mRNA expression of interferon response genes *STAT1* and *OAS1* for the most efficient shRNAs targeting the A allele of rs1057128 in hiPSC-CMs of Line 1 (red; left) and Line 2 (blue; right). **b)** Top, schematic representation of the shRNAs targeting the A allele of rs1057128 on the mutant *KCNQ1* allele with the mismatch positions indicated (original mismatch in red and additional mismatches in green). Middle and bottom, allele-specific relative mRNA expression of the wild-type and mutant *KCNQ1* alleles (left two panels) and the allelic expression of the wild-type and mutant *KCNQ1* alleles presented as % of total *KCNQ1* expression (right panel). In red hiPSC-CMs of Line 1 (middle, n=3-9) and in blue hiPSC-CMs of Line 2 (bottom, n=3-6). *P<0.05; **P<0.025compared to shSCR negative control; error bars indicate SEM.

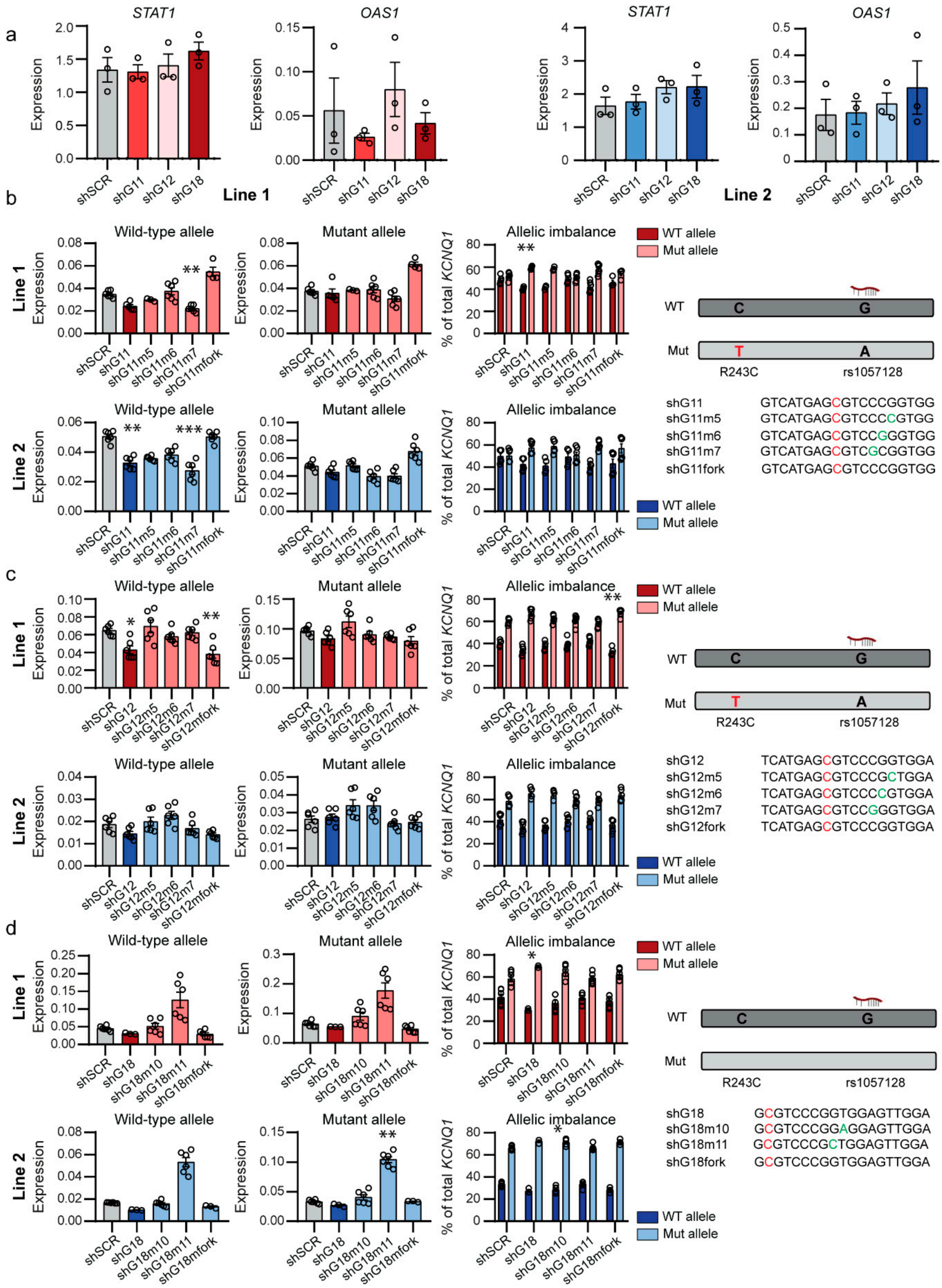

a
STAT1
OAS1
Expression
shSCR
shG11
shG12
shG18
Line 1
Line 2
b
Wild-type allele
Mutant allele
Allelic imbalance
% of total KCNQ1
WT allele
Mut allele
shG11m5
shG11m6
shG11m7
shG11mfork
WT
Mut
C
G
T
A
R243C
rs1057128
shG11 GTCATGAGCGTCCCGGTGG
shG11m5 GTCATGAGCGTCCCCGTGG
shG11m6 GTCATGAGCGTCCGGGTGG
shG11m7 GTCATGAGCGTCGCGGTGG
shG11fork GTCATGAGCGTCCCGGTGG
c
shG12m5
shG12m6
shG12m7
shG12mfork
shG12 TCATGAGCGTCCCGGTGGA
shG12m5 TCATGAGCGTCCCGCTGGA
shG12m6 TCATGAGCGTCCCCGTGGA
shG12m7 TCATGAGCGTCCGGGTGGA
shG12fork TCATGAGCGTCCCGGTGGA
d
shG18m10
shG18m11
shG18mfork
shG18 GCGTCCCGGTGGAGTTGGA
shG18m10 GCGTCCCGGAGGAGTTGGA
shG18m11 GCGTCCCGCTGGAGTTGGA
shG18fork GCGTCCCGGTGGAGTTGGA

Figure S3. Introduction of additional mismatches does not improve allele-specificity of shRNAs targeting the G allele of SNP rs1057128 on the wild-type *KCNQ1* allele. a) Relative mRNA expression of interferon response genes *STAT1* and *OAS1* for the most efficient shRNAs targeting the G allele of rs1057128 in hiPSC-CMs of Line 1 (red; left) and Line 2 (blue; right). **b-d)** Right, schematic representation of the shRNAs targeting the G allele of rs1057128 on the wild-type *KCNQ1* allele with the mismatch positions indicated (original mismatch in red and additional mismatches in green). Left, allele-specific relative mRNA expression of the wild-type and mutant *KCNQ1* alleles and the allelic expression of the wild-type and mutant *KCNQ1* alleles presented as % of total *KCNQ1* expression. In red hiPSC-CMs of Line 1 (top; n=3-9) and in blue hiPSC-CMs of Line 2 (bottom; n=3-6). **P*<0.05; ***P*<0.025; ****P*<0.001 compared to shSCR negative control; error bars indicate SEM.

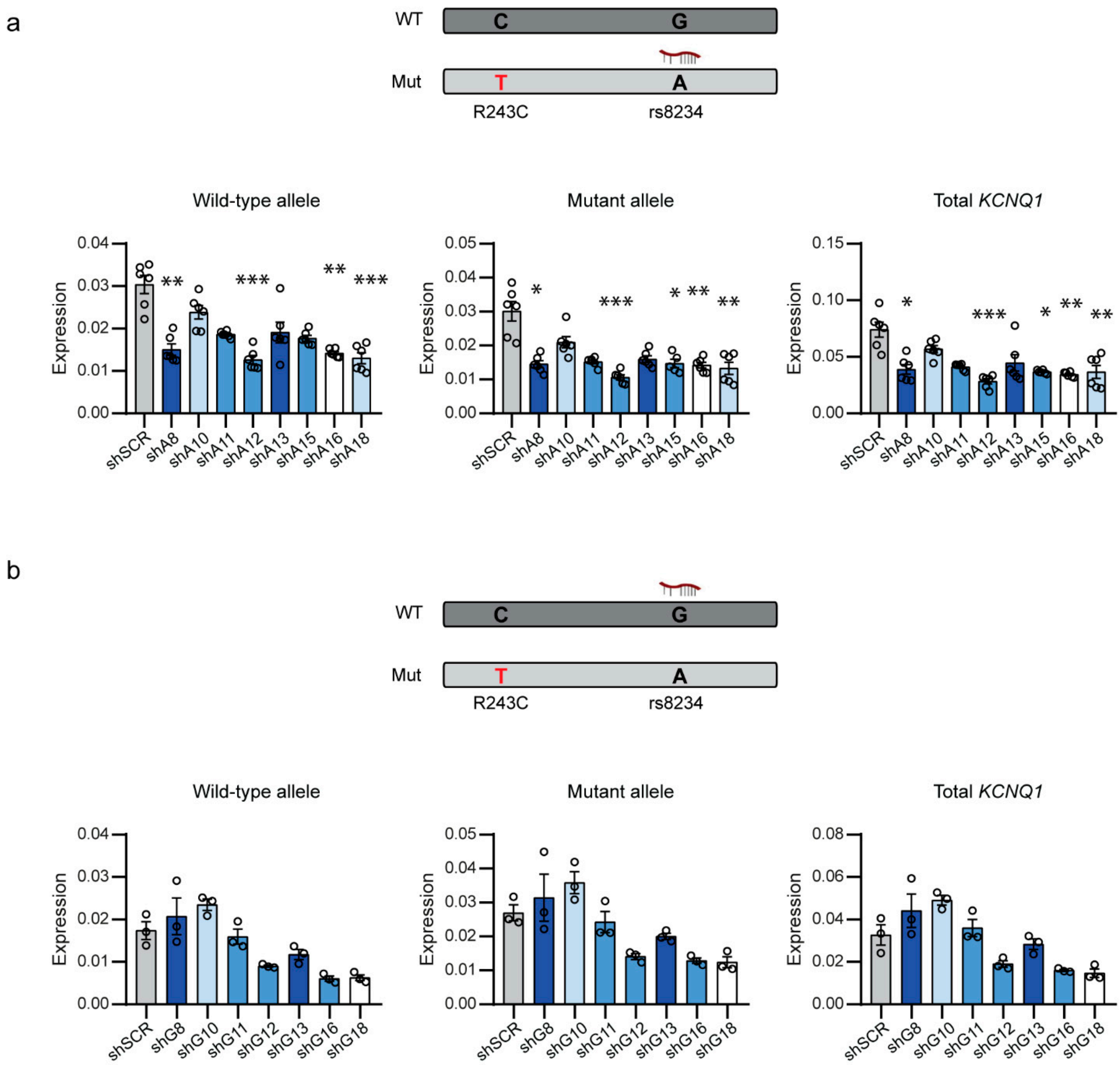


Figure S4. Allele specific shRNAs targeting SNP rs8234 in the 3'UTR of *KCNQ1*. a) Top, representation of the targeting of the A allele of rs8234 on the mutant *KCNQ1* allele in hiPSC-CMs of Line 2. Bottom, relative allele-specific expression of the wild-type and mutant *KCNQ1* allele and total *KCNQ1* (n=6). **b)** Top, representation of the targeting of the G allele of rs8234 on the wild-type *KCNQ1* allele. Bottom, relative allele-specific expression of the wild-type and mutant *KCNQ1* allele and total *KCNQ1* measured by qRT-PCR (n=3). **P*<0.05; ***P*<0.025; ****P*<0.001 compared to shSCR negative control; error bars indicate SEM.

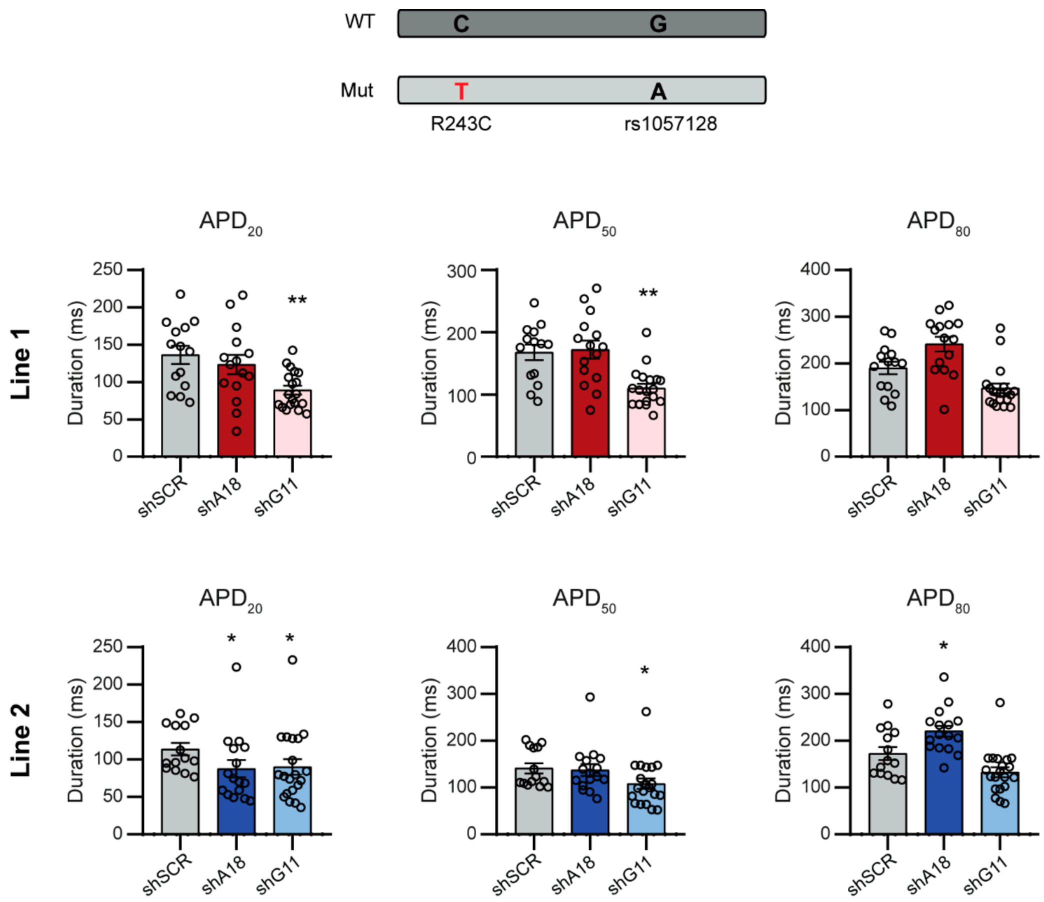
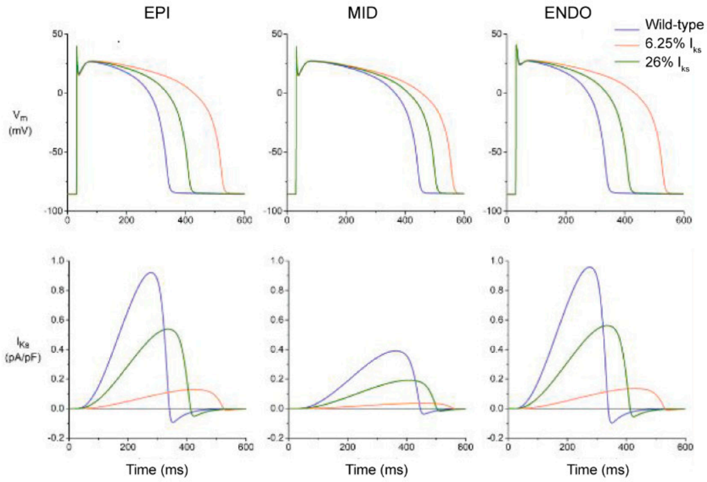


Figure S5. Action potential duration is affected by shifts in allelic balance. Schematic representation of the SNP and mutation in *KCNQ1* (top) and action potential duration at 20, 50 or 80% of repolarization (APD_{20}, APD_{50}, and APD_{80}, respectively) of optical action potentials derived from ArcLight fluorescence changes in hiPSC-CMs from Line 1 (red) or Line 2 (blue) treated with either negative control shSCR, shA18 targeting the mutant *KCNQ1* allele or shG11 targeting the wild-type *KCNQ1* allele stimulated at 2 Hz. *P<0.05; **P<0.025compared to shSCR negative control; error bars indicate SEM.

a



b

	APD_{90} (ms)		
	EPI	**MID**	**ENDO**
Wild-type	311	417	309
6.25% I_{ks}	495	531	501
26% I_{ks}	382	473	383

Figure S6. Effects of changes in the slow delayed rectifier potassium current (I_{Ks}) in a human ventricular cell model. a) Membrane potential (V_m; top) and associated I_{Ks} (bottom) at 1 Hz stimulation under wild-type conditions, upon reduction of functional $K_v7.1$ channels to 1/16th as expected when both alleles are equally expressed (6.25%) or to 26% as expected when the mutant *KCNQ1* allele is downregulated by 60%. **b)** Associated APD_{90} values in the epicardial (EPI), midmyocardial (MID) and endocardial (ENDO) versions of the human cardiomyocyte model.

Table S1

Antibody	Supplier	Species	Dilution	Cat. number
OCT-4	STEMCELL Technologies Vancuver, Canada	Mouse	1:200	60093
SSEA-4	STEMCELL Technologies Vancuver, Canada	Mouse	1:100	60062
Tra1-60	STEMCELL Technologies Vancuver, Canada	Mouse	1:100	60064
NANOG	Peprotech Rocky Hill, NJ,USA	Rabbit	1:200	500-P236
α-Actinin	Abcam Cambridge, UK	Rabbit	1:500	68167
cTnI	HyTest Turku, Finland	Goat	1:400	4T21/2

Nestin	STEMCELL Technologies Vancouver, Canada	Mouse	1:1000	60091
AFP	ThermoFisher Rockford, IL, USA	Rabbit	1:100	PA5-16658
Desmin	ThermoFisher Rockford, IL, USA	Rabbit	1:100	PA5-16705
Alexa fluor 488 Anti-rabbit	Invitrogen Eugene, OR, USA	Goat	1:250 ICC	A32731
Alexa fluor 488 Anti-mouse	Invitrogen Eugene, OR, USA	Goat	1:250 ICC	A32731
Alexa fluor 488 Anti-rabbit	Invitrogen Eugene, OR, USA	Donkey	1:250 ICC	A10037
Alexa fluor 647 Anti-goat	Invitrogen Eugene, OR, USA	Donkey	1:250 ICC	A32814

Table S2

Targeted SNP	Name shRNA	Oligonucleotides for cloning of shRNAs (upper forward and lower reverse oligonucleotide)
rs1057128	shA18	ccggaaCACAGGGCCACCTCAACCTtcaagacAGGTTGAGGTGGCCCTGTGtttttttg
		aattcaaaaaaaCACAGGGCCACCTCAACCTgtcttgaAGGTTGAGGTGGCCCTGTGtt
rs1057128	shA10	ccggaaGCAGTACTCACAGGGCCACtcaagacGTGGCCCTGTGAGTACTGCtttttttg
		aattcaaaaaaaGCAGTACTCACAGGGCCACgtcttgaGTGGCCCTGTGAGTACTGCtt
rs1057128	shA13	ccggaaGTACTCACAGGGCCACCTCtcaagacGAGGTGGCCCTGTGAGTACtttttttg
		aattcaaaaaaaGTACTCACAGGGCCACCTCgtcttgaGAGGTGGCCCTGTGAGTACtt
rs1057128	shA15	ccggaaACTCACAGGGCCACCTCAAtcaagacTTGAGGTGGCCCTGTGAGTtttttttg
		aattcaaaaaaaACTCACAGGGCCACCTCAAgtcttgaTTGAGGTGGCCCTGTGAGTtt
rs1057128	shA16	ccggaaCTCACAGGGCCACCTCAACtcaagacGTTGAGGTGGCCCTGTGAGtttttttg
		aattcaaaaaaaCTCACAGGGCCACCTCAACgtcttgaGTTGAGGTGGCCCTGTGAGtt
rs1057128	shG18	ccggaaCGCAGGGCCACCTCAACCTtcaagacAGGTTGAGGTGGCCCTGCGtttttttg
		aattcaaaaaaaCGCAGGGCCACCTCAACCTgtcttgaAGGTTGAGGTGGCCCTGCGtt
rs1057128	shG10	ccggaaGCAGTACTCGCAGGGCCACtcaagacGTGGCCCTGCGAGTACTGCtttttttg

		aattcaaaaaaaGCAGTACTCGCAGGGCCACgtcttgaGTGGCCCTGCGAGTACTGCtt
rs1057128	shG11	ccggaaCAGTACTCGCAGGGCCACCtcaagacGGTGGCCCTGCGAGTACTGtttttttg
		aattcaaaaaaaCAGTACTCGCAGGGCCACCgtcttgaGGTGGCCCTGCGAGTACTGtt
rs1057128	shG12	ccggaaAGTACTCGCAGGGCCACCTtcaagacAGGTGGCCCTGCGAGTACTtttttttg
		aattcaaaaaaaAGTACTCGCAGGGCCACCTgtcttgaAGGTGGCCCTGCGAGTACTtt
rs1057128	shG16	ccggaaCTCGCAGGGCCACCTCAACtcaagacGTTGAGGTGGCCCTGCGAGtttttttg
		aattcaaaaaaaCTCGCAGGGCCACCTCAACgtcttgaGTTGAGGTGGCCCTGCGAGtt
rs1057128	shG11m5	ccggaaCAGTACTCGCAGGGGCACCtcaagacGGTGCCCCTGCGAGTACTGtttttttg
		aattcaaaaaaaCAGTACTCGCAGGGGCACCgtcttgaGGTGCCCCTGCGAGTACTGtt
rs1057128	shG11m6	ccggaaCAGTACTCGCAGGCCCACCtcaagacGGTGGGCCTGCGAGTACTGtttttttg
		aattcaaaaaaaCAGTACTCGCAGGCCCACCgtcttgaGGTGGGCCTGCGAGTACTGtt
rs1057128	shG11m7	ccggaaCAGTACTCGCAGCGCCACCtcaagacGGTGGCGCTGCGAGTACTGtttttttg
		aattcaaaaaaaCAGTACTCGCAGCGCCACCgtcttgaGGTGGCGCTGCGAGTACTGtt
rs1057128	shG11fork	ccggaaCAGTACTCGCAGGGCCATAtcaagacGGTGGCCCTGCGAGTACTGtttttttg
		aattcaaaaaaaCAGTACTCGCAGGGCCACCgtcttgaTATGGCCCTGCGAGTACTGtt
rs1057128	shG12m5	ccggaaAGTACTCGCAGGGCGACCTtcaagacAGGTCGCCCTGCGAGTACTtttttttg
		aattcaaaaaaaAGTACTCGCAGGGCGACCTgtcttgaAGGTCGCCCTGCGAGTACTtt
rs1057128	shG12m6	ccggaaAGTACTCGCAGGGGCACCTtcaagacAGGTGCCCCTGCGAGTACTtttttttg
		aattcaaaaaaaAGTACTCGCAGGGGCACCTgtcttgaAGGTGCCCCTGCGAGTACTtt
rs1057128	shG12m7	ccggaaAGTACTCGCAGGCCCACCTtcaagacAGGTGGGCCTGCGAGTACTtttttttg
		aattcaaaaaaaAGTACTCGCAGGCCCACCTgtcttgaAGGTGGGCCTGCGAGTACTtt
rs1057128	shG12fork	ccggaaAGTACTCGCAGGGCCACTAtcaagacAGGTGGCCCTGCGAGTACTtttttttg
		aattcaaaaaaaAGTACTCGCAGGGCCACCTgtcttgaTAGTGGCCCTGCGAGTACTtt
rs1057128	shG18m10	ccggaaCGCAGGGCCTCCTCAACCTtcaagacAGGTTGAGGAGGCCCTGCGtttttttg
		aattcaaaaaaaCGCAGGGCCTCCTCAACCTgtcttgaAGGTTGAGGAGGCCCTGCGtt

rs1057128	shG18m11	ccggaaCGCAGGGCGACCTCAACCTtcaagacAGGTTGAGGTCGCCCTGCGtttttttg
		aattcaaaaaaaCGCAGGGCGACCTCAACCTgtcttgaAGGTTGAGGTCGCCCTGCGtt
rs1057128	shG18 fork	ccggaaCGCAGGGCCACCTCAACTAtcaagacAGGTTGAGGTGGCCCTGCGtttttttg
		aattcaaaaaaaCGCAGGGCCACCTCAACCTgtcttgaTAGTTGAGGTGGCCCTGCGtt
rs1057128	shA18m11	ccggaaCACAGGGCGACCTCAACCTtcaagacAGGTTGAGGTCGCCCTGTGtttttttg
		aattcaaaaaaaCACAGGGCGACCTCAACCTgtcttgaAGGTTGAGGTCGCCCTGTGtt
rs1057128	shA18m10	ccggaaCACAGGGCCTCCTCAACCTtcaagacAGGTTGAGGAGGCCCTGTGtttttttg
		aattcaaaaaaaCACAGGGCCTCCTCAACCTgtcttgaAGGTTGAGGAGGCCCTGTGtt
rs1057128	shA18 fork	ccggaaCACAGGGCCACCTCAACTAtcaagacAGGTTGAGGTGGCCCTGTGtttttttg
		aattcaaaaaaaCACAGGGCCACCTCAACCTgtcttgaTAGTTGAGGTGGCCCTGTGtt
rs8234	shA8	ccggaaCTGGGCATTACATCGCATAtcaagacTATGCGATGTAATGCCCAGtttttttg
		aattcaaaaaaaCTGGGCATTACATCGCATAgtcttgaTATGCGATGTAATGCCCAGtt
rs8234	shA10	ccggaaGGGCATTACATCGCATAGAtcaagacTCTATGCGATGTAATGCCCtttttttg
		aattcaaaaaaaGGGCATTACATCGCATAGAgtcttgaTCTATGCGATGTAATGCCCtt
rs8234	shA11	ccggaaGGCATTACATCGCATAGAAtcaagacTTCTATGCGATGTAATGCCtttttttg
		aattcaaaaaaaGGCATTACATCGCATAGAAgtcttgaTTCTATGCGATGTAATGCCtt
rs8234	shA12	ccggaaGCATTACATCGCATAGAAAtcaagacTTTCTATGCGATGTAATGCtttttttg
		aattcaaaaaaaGCATTACATCGCATAGAAAgtcttgaTTTCTATGCGATGTAATGCtt
rs8234	shA13	ccggaaCATTACATCGCATAGAAATtcaagacATTTCTATGCGATGTAATGtttttttg
		aattcaaaaaaaCATTACATCGCATAGAAATgtcttgaATTTCTATGCGATGTAATGtt
rs8234	shA15	ccggaaTTACATCGCATAGAAATCAtcaagacTGATTTCTATGCGATGTAAtttttttg
		aattcaaaaaaaTTACATCGCATAGAAATCAgtcttgaTGATTTCTATGCGATGTAAtt
rs8234	shA16	ccggaaTACATCGCATAGAAATCAAtcaagacTTGATTTCTATGCGATGTAtttttttg
		aattcaaaaaaaTACATCGCATAGAAATCAAgtcttgaTTGATTTCTATGCGATGTAtt
rs8234	shA18	ccggaaCATCGCATAGAAATCAATAtcaagacTATTGATTTCTATGCGATGtttttttg

		aattcaaaaaaaCATCGCATAGAAATCAATAgtcttgaTATTGATTTCTATGCGATGtt
rs8234	shG8	ccggaaCTGGGCATTACGTCGCATAtcaagacTATGCGACGTAATGCCCAGtttttttg
		aattcaaaaaaaCTGGGCATTACGTCGCATAgtcttgaTATGCGACGTAATGCCCAGtt
rs8234	shG10	ccggaaGGGCATTACGTCGCATAGAtcaagacTCTATGCGACGTAATGCCCtttttttg
		aattcaaaaaaaGGGCATTACGTCGCATAGAgtcttgaTCTATGCGACGTAATGCCCtt
rs8234	shG11	ccggaaGGCATTACGTCGCATAGAAtcaagacTTCTATGCGACGTAATGCCtttttttg
		aattcaaaaaaaGGCATTACGTCGCATAGAAgtcttgaTTCTATGCGACGTAATGCCtt
rs8234	shG12	ccggaaGCATTACGTCGCATAGAAAtcaagacTTTCTATGCGACGTAATGCtttttttg
		aattcaaaaaaaGCATTACGTCGCATAGAAAgtcttgaTTTCTATGCGACGTAATGCtt
rs8234	shG13	ccggaaCATTACGTCGCATAGAAATtcaagacATTTCTATGCGACGTAATGtttttttg
		aattcaaaaaaaCATTACGTCGCATAGAAATgtcttgaATTTCTATGCGACGTAATGtt
rs8234	shG16	ccggaaTACGTCGCATAGAAATCAAtcaagacTTGATTTCTATGCGACGTAtttttttg
		aattcaaaaaaaTACGTCGCATAGAAATCAAgtcttgaTTGATTTCTATGCGACGTAtt
rs8234	shG18	ccggaaCGTCGCATAGAAATCAATAtcaagacTATTGATTTCTATGCGACGtttttttg
		aattcaaaaaaaCGTCGCATAGAAATCAATAgtcttgaTATTGATTTCTATGCGACGtt

Table S3

PCR primers			
Gene	**Forward**	**Reverse**	**Annealing temperature**
***KCNQ1*_wt**	TGAGGATGCTACACGTCGACC	AGCCGATGTACAGGGTGGTTATC	66
***KCNQ1*_mut**	TGAGGATGCTACACGTCGACT	AGCCGATGTACAGGGTGGTTATC	66
***KCNQ1*_total**	TCCTGAGGATGCTACACGTC	AGCCGATGTACAGGGTGGTTATC	66
STAT1	ATCACATTCACATGGGTGGAGC	ACAGATACTTCAGGGGATTCTCAGG	60
OAS1	CATCCGCCTAGTCAAGCACT	AAGACCGTCCGAAATCCCTGG	60

GAPDH	ACCCACTCCTCCACCTTTGAC	ACCCTGTTGCTGTAGCCAAATT	60
HPRT	TGACACTGGCAAAACAATGCA	GGTCCTTTTCACCAGCAAGCT	60
TBP	GCTCACCCACCAACAATTTAG	TCTGCTCTGACTTTAGCACCTG	60
NANOG	AGAATAGCAATGGTGTGACGCAG	TGGATGTTCTGGGTCTGGTTGC	60
OCT4	TGGTTGGAGGGAAGGTGAAG	TGTCTATCTACTGTGTCCCAG	60
***OCT4*_viral**	TGTACTCCTCGGTCCCTTTC	CAGGTGGGGTCTTTCATTC	63
SOX2	ACCAATCCCATCCACACTCAC	TCTATACAAGGTCCATTCCCCC	63
***SOX2*_viral**	ATCCCAGTGTGGTGGTACG	AAGGCATTCATGGGCCGCTTG	63
MLC2V	ACAACTGACACCAACACCTGC	AGTCCAAGTTGCCAGTCACGTC	63
NKX2.5	TCTATCCACGTGCCTACAGC	AGAAAGTCAGGCTGGCTCAAG	63
***KLF4*_viral**	CTGCGGCAAAACCTACACAAA	TTATCGTCGACCACTGTGCTGG	63
MYH6	ACCTGTCCAAGTTCCGCAAGG	TTACAGGTTGGCAAGAGTGAGG	60
MYH7	ACCTGTCCAAGTTCCGCAAGG	TTTGCTGGCACCTCCAGGG	60