SUPPLEMENTARY INFORMATION

Supplementary Figures

Figure S1. Generation of genome-edited hiPSC lines. **A**, Using CRISPR/Cas9 technology, the c.242C>T p.S81L *GNB5* mutation was inserted into a control hiPSC line in heterozygous and homozygous state. **B**, Location of the c.242C>T mutation in the *GNB5* transcript and the surrounding sequence. The gRNA sequence that was used is indicated in blue, the PAM sequence in bold. In the single-stranded oligonucleotide (ssODN) that was applied for homologous recombination the c.242C>T mutation is indicated in red and bold, whereas red and italic indicates the synonymous mutation generating a restriction site used for colony screening. **C**, Sanger sequences of wildtype (upper panel), S81L heterozygous (middle panel) and S81L homozygous (lower panel) clones. **D and E**, Immunocytochemistry of the pluripotency markers OCT4, TRA1-81, NANOG, and SSEA4 in the S81L heterozygous (D) and homozygous (E) lines. Scale bars are 100 µm. **F and G**, Normal karyotypes exhibited by hiPSC lines S81L^{het} (F) (verified by COBRA-FISH) and S81L^{homo} (G) (verified by G banding).

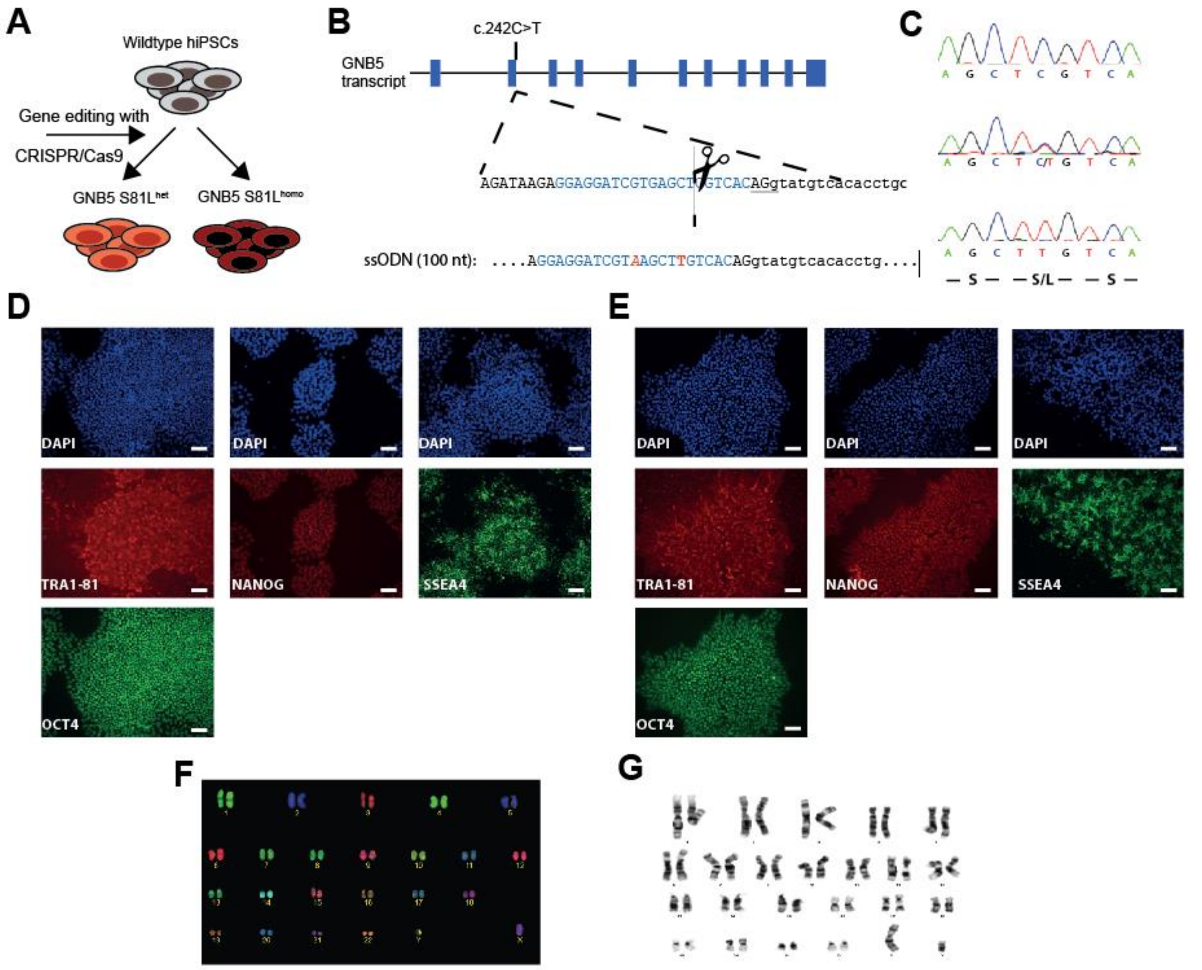


Figure S2. Action potential (AP) characteristics of retinoic acid (RA) and DMSO-treated hiPSC-CMs. A. Typical example of an action potential when paced at 1 Hz. **B.** Action potential duration at 20%, 50% and 80% of repolarization (APD_{20}, APD_{50} and APD_{80} respectively). In RA-treated hiPSC-CMs, APD is significantly shorter as compared to DMSO-treated hiPSC-CMs (*$P<0.05$, two-sided t-test). **C.** Individual plateau amplitudes, demonstrating lower values in RA-treated hiPSC-CMs in comparison with DMSO-treated hiPSC-CMs (*$P<0.05$, , two-sided t-test) . **D.** Upstroke velocity. **E.** Effects of carbachol (CCh) on maximal diastolic potential (MDP) in DMSO- and RA-treated hiPSC-CMs. While no effect on MDP is found in DMSO-treated hiPSC-CMs, RA-treated hiSPC-CMs display a significant hyperpolarization upon CCh application (*$P<0.05$, paired t-test). B-E. DMSO, n=7; RA, n=17 hiPSC-CMs.

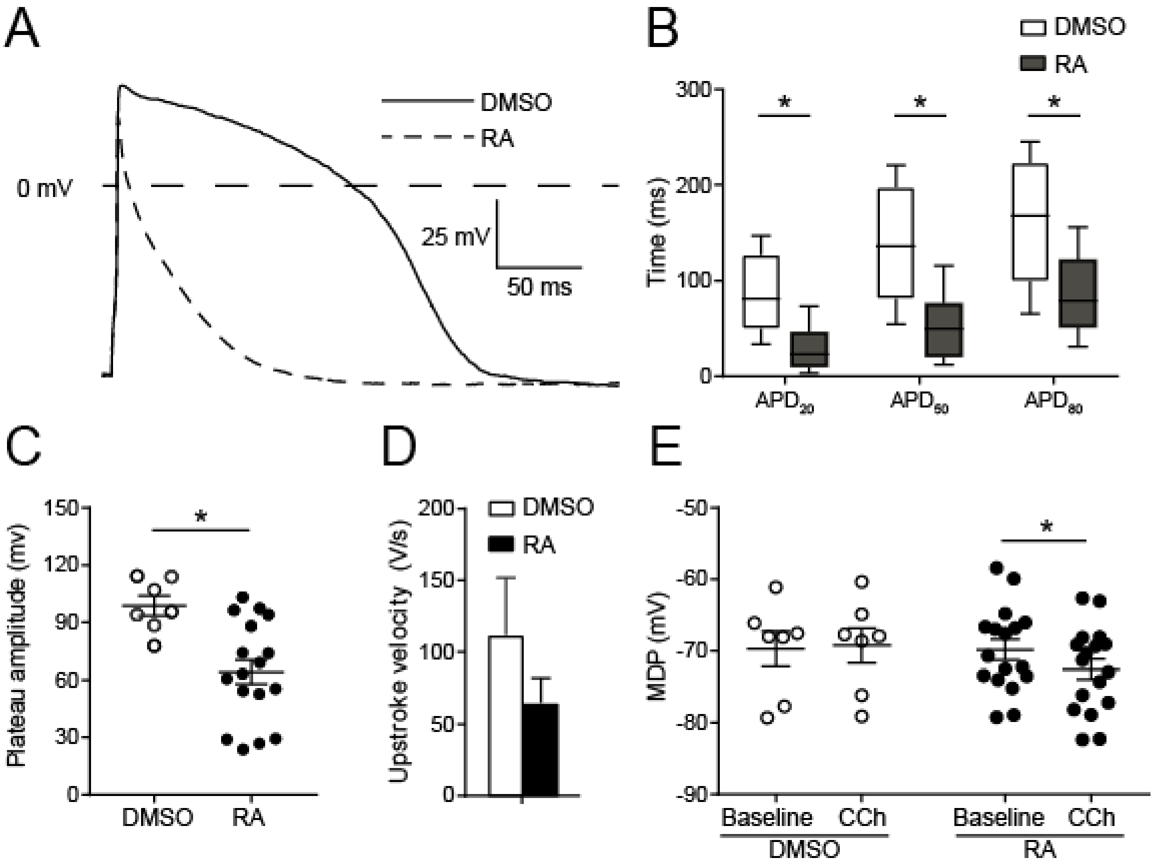
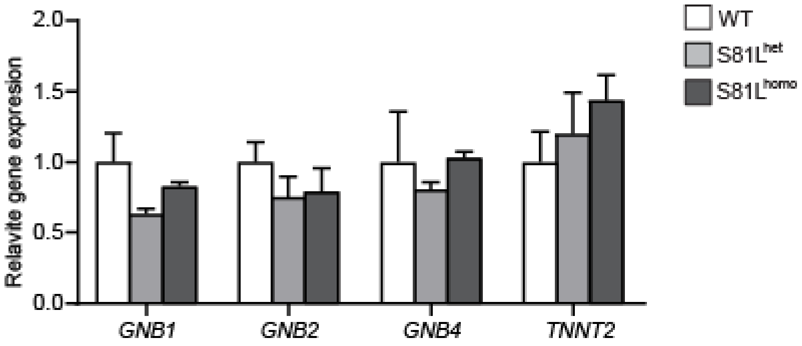


Figure S3. Expression level of genes *GNB1,2,4* and *TNNT2* as assessed by RT-qPCR in WT, S81L^{het} and S81L^{homo} hiPSC-CMs. Data for each gene are presented as mean+/-s.e.m. of three biological replicas and are normalized to the expression of *TBP*. Quantification is expressed as relative to the expression in the wild type (WT) clone.



Supplementary Tables

Table S1. Primer sequences used for PCRs.

Gene	**Forward 5’ to 3’**	**Reverse 5’ to 3’**
GNB5-screen	AGCAAGGGGACTGACTATGG	AAACCCGCTCACCTGTTATG
TBP	GCTCACCCACCAACAATTTAG	TCTGCTCTGACTTTAGCACCTG
NR2F2	GCCATAGTCCTGTTCACCTCA	AATCTCGTCGGCTGGTTG
NPPA	GCTCCTTCTCCACCACCAC	GGGCACGACCTCATCTTCTA
KCNJ3	ACCTATCCCAGCGAAGCAT	AAAACGATGACCCCAAAGAA
KCNJ5	GAGCCCTTTCTGGGAGATGT	TTTCCAAGGTGAGGACTGGTG
GNB5	CGTGTCGCCTCTATGACCTG	CTTTGAGAACATCCCAGACG
RGS6	AAATCTGGCAAGAGTTTCTGG	GGGCATAGCTGTCACTCTTCA
GNB5-OT1	GGTCCCAGGAGAGTTTCTGT	GCTTTGAGAACGAGGGTGTC
GNB5-OT2	TGTGGCAAAAACACATAAACG	CAGCTCCTGGCCTTTGTTA
GNB5-OT3	CTCACCCCCAGCCTTAGA	AGAGGCTGGGCTTTTGCTAT
GNB1	GACCGGGCAGGTGTCTTG	GTTCCAGATCTTGAGGAAGCTA
GNB2[*]	AGATCACAGCTGGGCTG	GATGAGCTTCCCATCCTGG
GNB3	GATCTGCTTCTCCCACGAGAG	GATGCCCACACGCTCAGAC
GNB4	AAAAAGTGGGCGTCTCTTGTTG	CAGCTCACACGGTTGTCATGA
TNNT2	GACCAGGGCAGAAGAAGATG	ACTCTCTCTCCATCGGGGAT

[*] **Stallmeyer B, Kuß J, Kotthoff S, Zumhagen S, Vowinkel K, Rinné S, Matschke LA, Friedrich C, Schulze-Bahr E, Rust S, Seebohm G, Decher N, Schulze-Bahr E**.(2017). A Mutation in the G-Protein Gene *GNB2* Causes Familial Sinus Node and Atrioventricular Conduction Dysfunction. Circ. Res. 120(10):e33-e44.

Table S2. Antibodies used for immunocitochemistry.

Primary Antibodies					
Antigen	**Isotype**	**Supplier**	**Cat. Number**	**Dilution**	**Citation (PMID)**
OCT3/4	Mouse IgG2b	Santa Cruz Biotechnology	sc-5279	1:100	30778227
TRA 1-81	Mouse IgM	Abcam	ab 16289	1:67	28344003
SSEA-4	Mouse Ig3	Santa Cruz Biotechnology	sc-59368	1:100	29030611
NANOG	Goat IgG	R&D Systems	AF 1997	1:33	23910086
Secondary Antibodies					
Host species	**Antigen**	**Supplier**	**Cat. Number**	**Dilution**	**Fluorophore**
Goat	Anti-mouse IgG2b	ThermoFisher Scientific	A-21141	1:500	AF488
Goat	Anti-mouse IgM	ThermoFisher Scientific	A-21426	1:500	AF555
Goat	Anti-mouse IgG$_{(H+L)}$	ThermoFisher Scientific	A-11001	1:500	AF488
Donkey	Anti-goat IgG$_{(H+L)}$	ThermoFisher Scientific	A-11057	1:500	AF568

Table S3. Putative genomic off-target sites for the gRNA used to introduce mutation p.S81L in *GNB5*.

Location	**Genomic sequence**	**Mismatches**	**Strand**	**Type**
15:52153939-52153961	GAGGATCGTGAGCTCGTCAC *AGG*			
22:19979742-19979764	GAGG**TC**CGTGAGCT**T**GTCAC *TGG*	3	–	Exonic
16:77913475-77913497	GAGGATCG**A**GAGCT**GA**TCAC *TGG*	3	–	Intronic
1:160408628-160408650	GAGGAT**G**GTGAGCTC**A**TC**T**C *TGG*	3	+	Intronic

Original gRNA: first row. Sequence in italics depicts the PAM site (NGG), in bold and red the mismatches with the original gRNA.

Table S4. Action potential (AP) parameters in WT, S81L^{het} and S81L^{homo} at baseline in spontaneously fast beating (>1 Hz) hiPSC-CMs.

	WT (n=12)	S81L^{het} (n=10)	S81L^{homo} (n=13)
MDP (mV)	−68.5±0.6	−69.3±1.3	−70.2±1.8
APA (mV)	94.7±4.6	86.5±4.9	85.6±4.9
V_{max} (V/s)	40.9±9.9	28.3±8.2	32.9±8.9
APD_{20} (ms)	45.3±5.7	49.4±12.7	39.0±4.4
APD_{50} (ms)	81.5±9.7	82.4±19.2	66.6±6.5
APD_{80} (ms)	109.0±12.6	103.8±19.5	88.8±8.4
$APA_{plateau}$ (mV)	86.1±4.8	75.0±7.7	77.9±5.5

Data are mean±s.e.m., MDP, maximal diastolic potential; APA, AP amplitude; V_{max}, maximal upstroke velocity; APD_{20}, APD_{50}, APD_{80}, AP duration at 20, 50, and 80% of repolarization, respectively; $APA_{plateau}$, AP plateau amplitude.