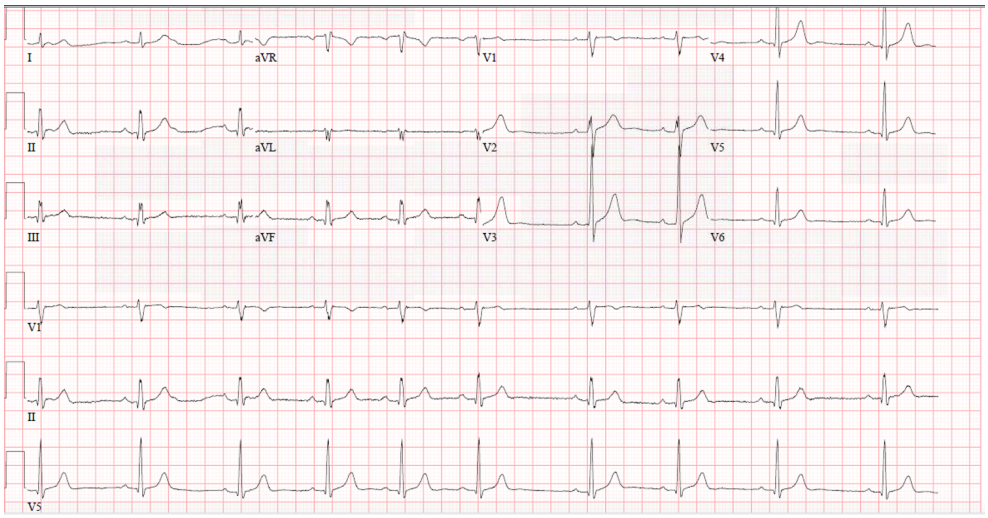
Supplemental Methods

Genetic Modification of iPSC line GM23338

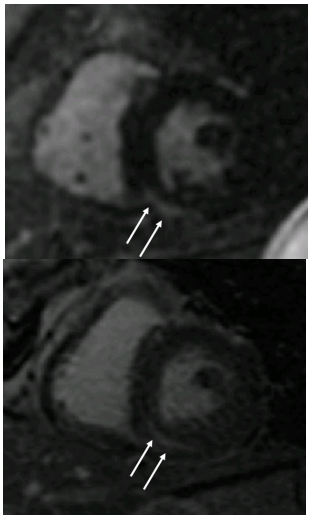
A sgRNA was designed to binding specificially to the DSP gene using http://chopchop.cbu.uib.no/. The guide was designed so the double stranded break would be as close to the mutation base as possible. The guide RNA was cloned into PX459V2 using plasmid digested with bbs1 to allow for ligation of the guide RNA insert. (Addgene plasmid # 62988 ; http://n2t.net/addgene:62988 ; RRID:Addgene_62988). Two 100 bp ultramers from IDT were used as repair template to repair the double stranded break. In order to stop the CRISPR from cutting once targeting was achieved a silent mutation was made in the ssODN. Two ssODNs in total were made for this project. One ssODN contained the mutation of interest (DSP mutation R451G CGT>GGT) along with the silent mutation, the silent mutation created a BSTE11 site. A second ssODN was designed with the silent mutations only keeping the base of interest as the wildtype base pair. GM23338 cells were grown on mitotically inactivated MEFs. 24 hours prior to targeting, cells were treated with ROCKi (Y-27632). The following day the cells were singelized with accutase and pelleted. Cells were resuspended according to the protocol provided in the LONZA 4D nucleofection Primary P3 kit. 2 ug of CRISPR plasmid, and 4 ul of each 100 mM ssODN were added to the nucleofection solution. The cells were nucleofected using program CB-150. Cells were then plated on DR4 MEFs supplemented with ROCKi. The following day .75 ng/ul of puromycin and ROCKi was added to fresh media. This selection was continued for a total of 48 hours. ROCKi was kept on the cells until small colonies were observed. After this point the cell culture was continued with fresh media changes daily. After a total of 15 days colonies were isolated into 24 well plates. 4 days after plating in the 24 well plates, a few colonies from each well was isolated and DNA extracted using the HOT SHOT method to prepare template for PCR. PCR was performed across the region of targeting using Herculase II from Agilent. This PCR product was then digested with BSTEII, NEB R0162S. Clones that showed complete digestion were further analyzed by Sanger sequencing to identify the clones as wildtype, heterozygous, or homozygous. The wildtype will contain the wildtype amino acid as the site of interest as well as the silent mutation to serve as a control line for the experiments.

Supplemental Figure 1

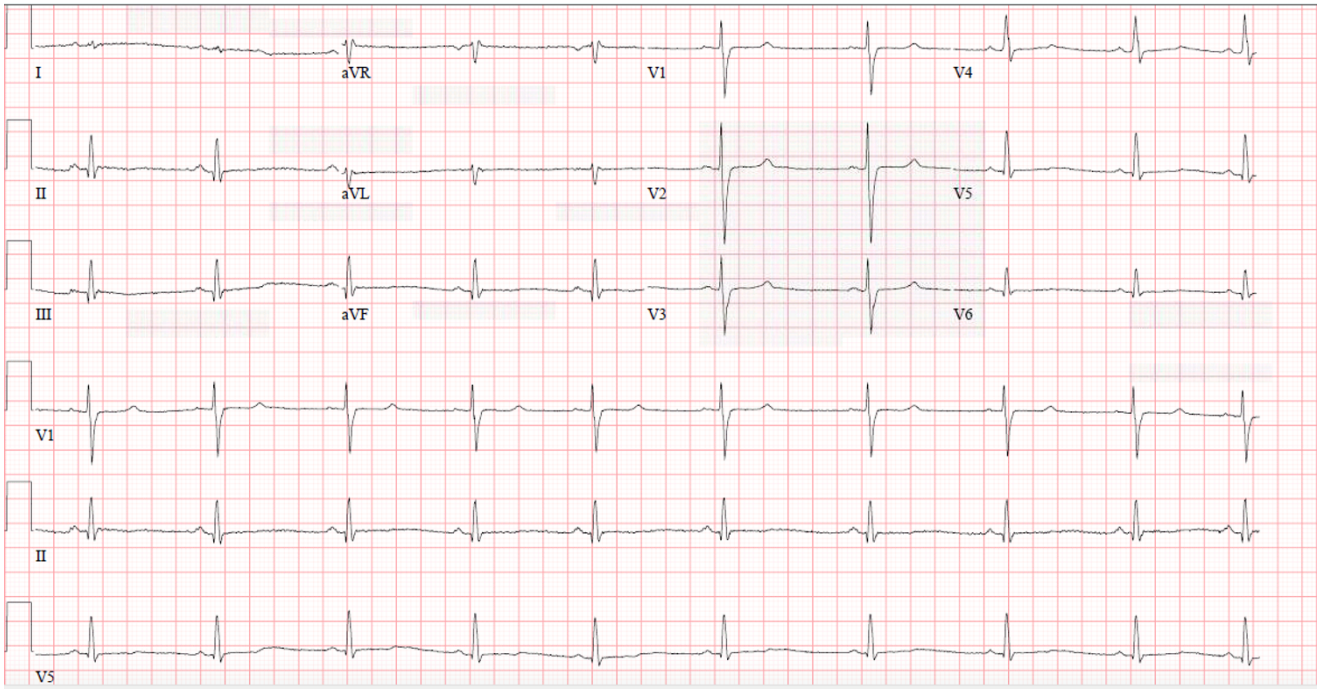
A



B



C



D

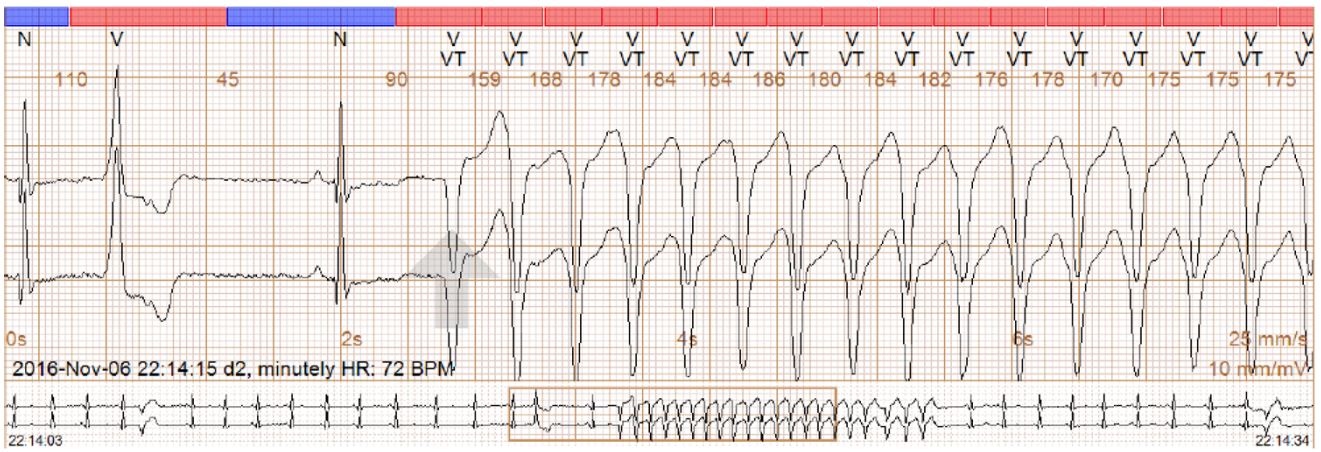
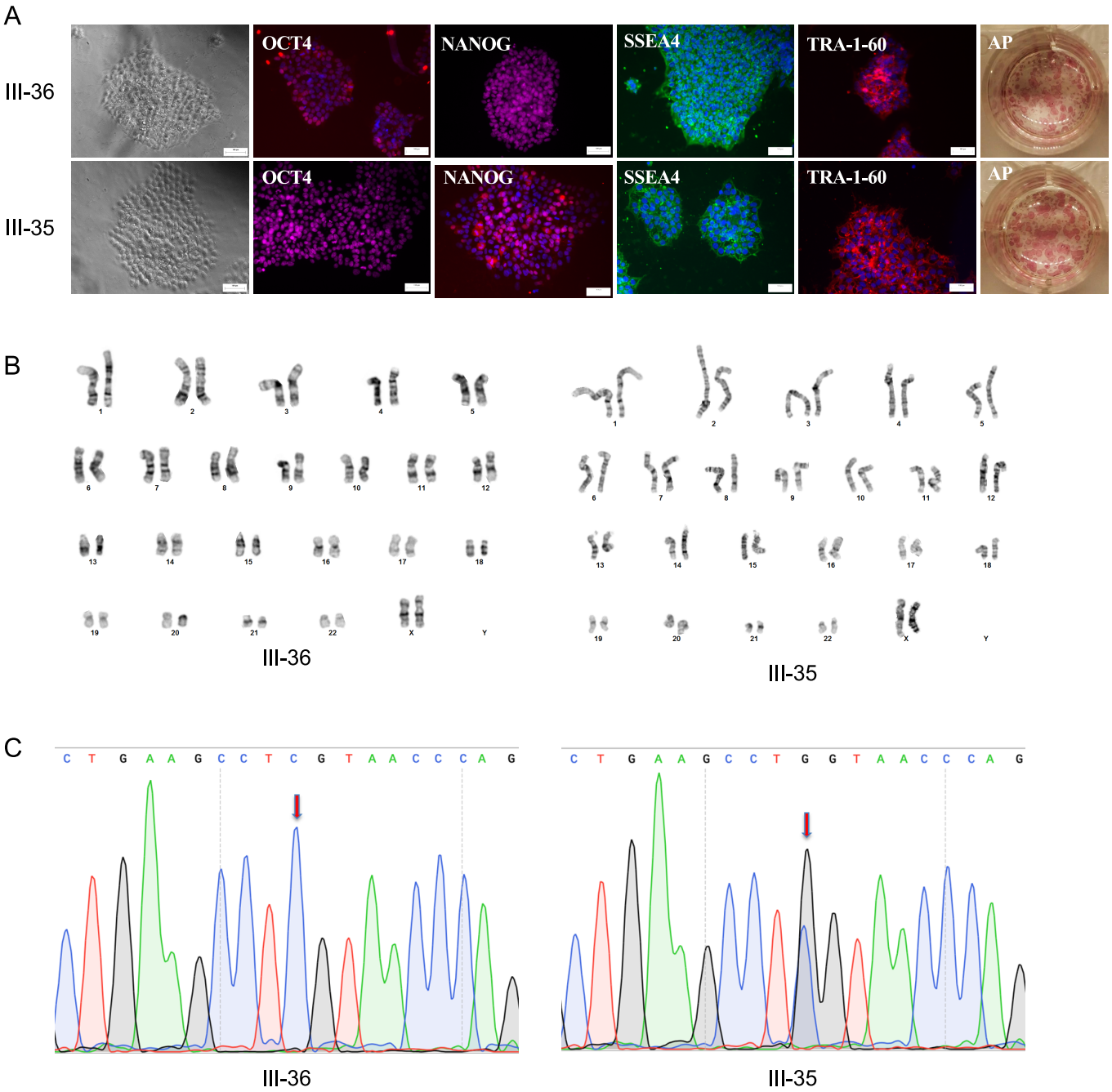


Figure caption on following page

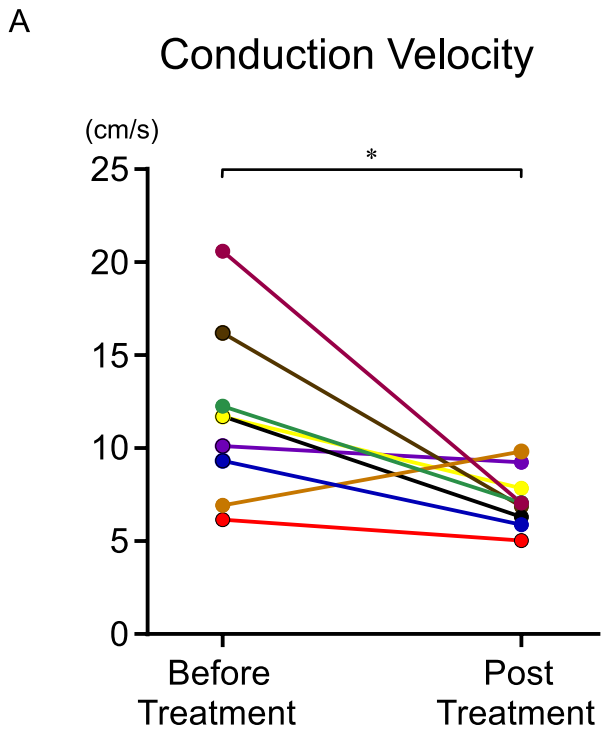
Supplemental Figure 1: R451G is associated with variable ACM presentation **(A, B)** Individual III-23. 60-year-old female with no ventricular arrhythmias, normal LV and RV ejection fraction. Fibrosis can be seen on MRI with late gadolinium enhancement (white arrows). **(C, D)** Individual IV-22. 31-year-old female with normal LV ejection fraction (50%) and normal RV ejection fraction. Non-sustained ventricular tachycardia and frequent ventricular ectopy. Received preventative ICD after meeting implantation criteria.

Supplemental Figure 2



Supplement Figure 2: Characterization of Arrhythmogenic Cardiomyopathy (ACM) induced pluripotent stem cells. **(A)** (From left to right) Typical human iPSC colony, positive immunostaining for pluripotency markers (OCT4, NANOG, SS-EA-4 and TRA-1-60), and positive staining for alkaline phosphatase (AP) were observed in all cell lines. Scale bars 100uM. **(B)** Karyotyping showing chromosomal stability. **(C)** Sequencing chromatograms showing target mutant DNA region in IPSCs from the wild type healthy individual (III-36) and R451G desmoplakin mutant ACM patient (III-35). Red arrow points the exact base, which becomes heterozygous mutant (c.1692CGT>GGT) in ACM patient.

Supplemental Figure 3



Supplement Figure 3: EHT Carbenoxolone treatment **(A)** Treatment of wild type EHTs with gap junction blocker, carbenoxolone (20 minute incubation, 50 μM carbenoxolone), significantly reduced conduction velocity (2 tailed paired t-test. *$p < 0.05$)

Supplemental Table 1

Primer name	Primer sequence
DSP883 5' cloning primer	ATCGAGAATTCATGAGCTGCAACGGAG
DSP883 3' cloning primer	AGTCAGTCGACTTTCTCCAGGTCCCATAATC
DSP883 internal seq. primer 1(nt 720)	CGACCTGCGCGAGAAATCTGCGAT
DSP883 internal seq. primer 2 (nt 1594)	GCCATCTTGGCTCTGTGGAAC
DSP883 S299R mut. primer	CTGTACGACTGGAGGGACAAGAACACCAACATC
DSP883 E422K mut. primer	GGAACAGATCAAGGAGCTGAAGAAAGAACGAGAG
DSP883 S442F mut. primer	CTTGGTAAACAAGTTTAAGAAGATTGTACAGCTGAAGCCTC
DSP883 I445V mut. primer	GGTAAACAAGTCTAAGAAGGTTGTACAGCTGAAGCC
DSP883 R451G mut. primer	GTACAGCTGAAGCCTGGTAACCCAGACTACAG
DSP883 N458Y mut. primer	CCCAGACTACAGAAGCTATAAACCCATTATTCTCAGAG
DSP883 K470E mut. primer	GCTCTCTGTGACTACGAACAAGATCAGAAAATCGTGC
DSP883 S507F mut. primer	GTTGACATGCTTGTTCCCTTTGTGGGGCTGATC
CRISPR Guide RNA	CTTCTGTAGTCTGGGTTACG
DSP CRISPR R451G ssODN 1	AGCGTCAGGTGCAGAACTTGGTAAACAAGTCTAAGAAGATTGTACAGCTGAAGCCTGGTAATCCGGACTACAGAAGCAATAAACCCATTATTCTCAGAGCT
DSP CRISPR R451G ssODN 2	(AGCGTCAGGTGCAGAACTTGGTAAACAAGTCTAAGAAGATTGTACAGCTGAAGCCTCGTAATCCGGACTACAGAAGCAATAAACCCATTATTCTCAGAGCT
DSP CRISPR PCR amplification 5'	TGCAGGTTGAAAATCTCCTCT
DSP CRISPR PCR amplification 3'	GCTTTTGTTCCATAGCTGCTG

Mutagenesis primers indicated are the forward primer. The reverse primer was the reverse complement of this sequence. The sequencing primers all read 5' to 3'.

Sup Movie 1: Horizontal long axis (four chamber) cine view of proband's heart, a few years before his SCD episode. Note left ventricular dysfunction. The proband was completely asymptomatic (NYHA functional class I) and a competitive athlete.