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Supplemental Information

iPSC Modeling of RBM20-Deficient DCM Identifies Upregulation of RBM20 as a Therapeutic Strategy

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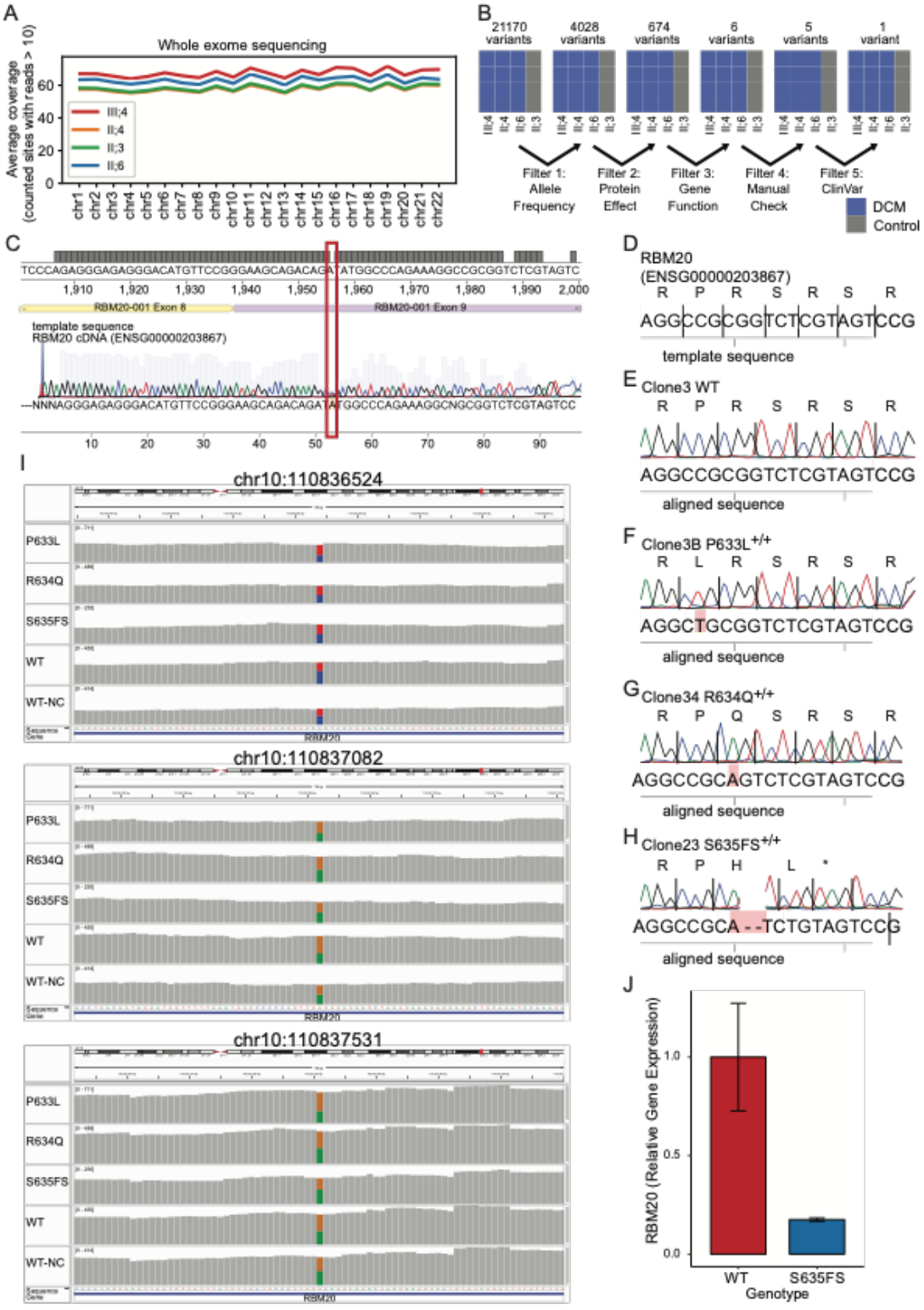
Supplemental Material

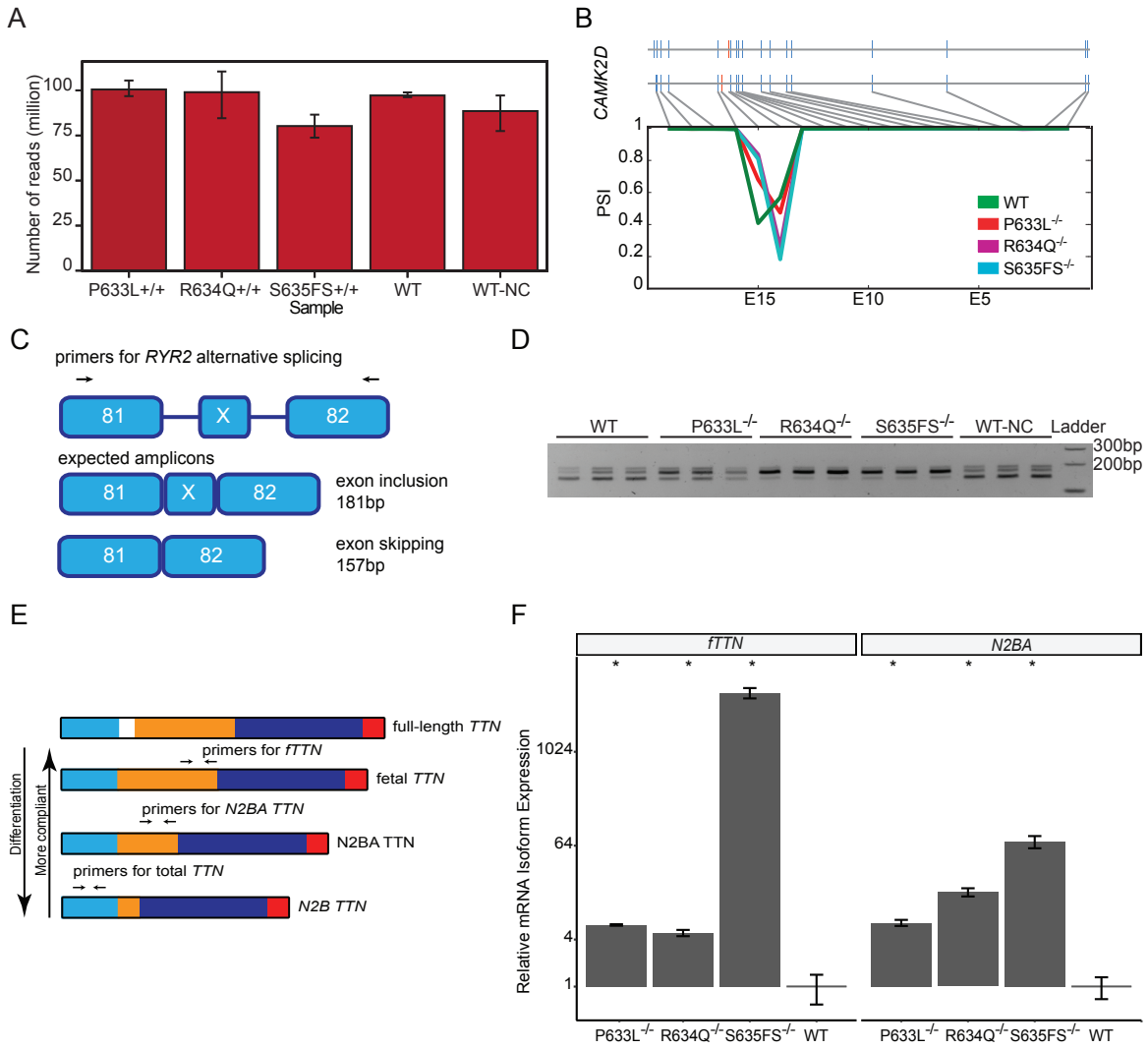
Supplemental Figure 1



Identification of putative DCM causing variants. Related to Figure 1.

(A) Average exome sequencing depth for the three family members across chromosomes. (B) Schematic workflow for the identification of disease-causing variants in the proband's family. Filters from 1 to 5 sequentially were applied to 21,170 variants identified using our variant calling pipeline as being in common between III;4, II;4, II;6 and absent in II;3. Filter 1 excludes variants with frequency higher than 5% in 1000 genome project. Filter 2 excludes variants locating in intergenic, intronic, UTR regions, or having synonymous changes on protein sequences. Filter 3 retains only variants in genes related with heart development or heart diseases. Filter 4 checked all remaining variant calls manually in the raw sequence trace files. Filter 5 removed any variants annotated as benign, likely benign, or variant of uncertain significance in ClinVar, to arrive at RBM20 as containing the most likely disease-causing variant. (C) Electropherogram confirming the RBM20 point mutation in DCM1 iPSC-CMs. Sequence electropherogram showing the *RBM20* mutation hotspot in the edited lines. (D) Reference *RBM20* cDNA sequence (ENSG00000203867) with translation shown on top. (E) Sequence electropherogram for Clone 3, referred to as "WT" in the manuscript. Translation is shown on top. (F) Sequence electropherogram for Clone 3B, referred to as "P633L" in the manuscript. Translation is shown on top. Affected nucleotides are highlighted in pink. (G) Sequence electropherogram for Clone 34, referred to as "R634Q" in the manuscript. Translation is shown on top. Affected nucleotides are highlighted in pink. (H) Sequence electropherogram for Clone 23, referred to as "S635FS" in the manuscript. Translation is shown on top (* = STOP codon). Affected nucleotides are highlighted in pink. (+) = mutant allele; (−) = wild-type allele. (I) Three heterozygous polymorphic variants in the 3' UTR region of RBM20 are present in the parental line and the genome edited lines. Highlighted positions are Chr10:110836524, Chr10:110837082, Chr10:110837531. (J) qRT-PCR analysis of *RBM20* expression in WT and S635FS iPSC-CMs. TBP is used as endogenous control. The WT sample is set to 1. n=6.

Supplemental Figure 2

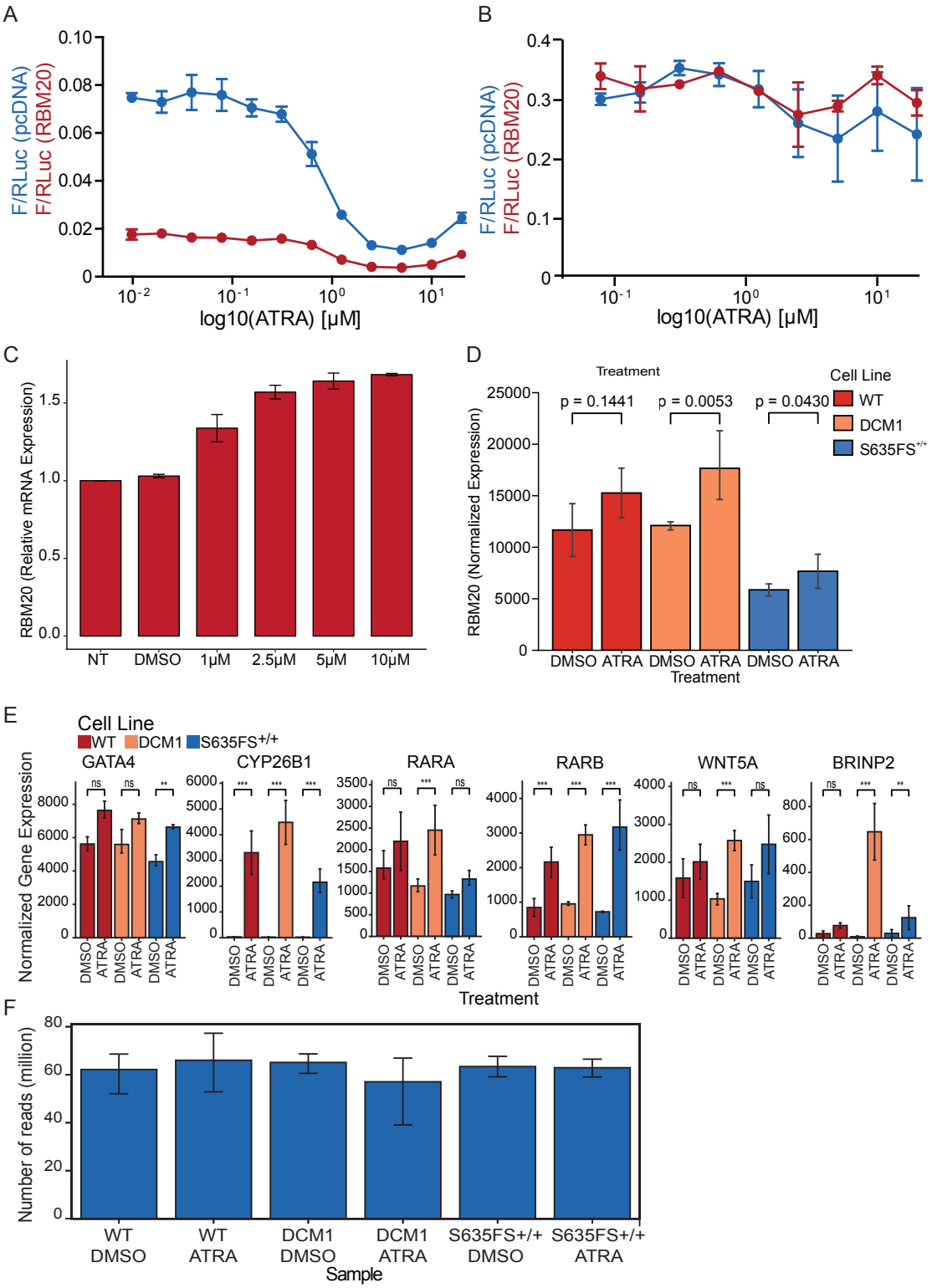


Validation of RBM20-dependent splicing. Related to Figure 2.

A) RNA-Seq library size for splicing effect experiments on the five genotypes each with three biological replicates. Error bars are 95% confidence interval computed with 1000 bootstrap iterations. (+) = mutant allele; (−) = wild-type allele (B) PSI plot of *CAMK2D*. Each line represents the median PSI value of three biological replicates from each of the mutant samples (P633L, R634Q, and S635FS) or six biological replicates from control samples (3 WT + 3 WT-NC). Two exons (*CAMK2D-E14* and *CAMK2D-E15*) are mutually spliced. (C) Schematic of *RYR2* isoforms, positions of primers and expected amplicons. (D) RT-PCR of *RYR2* across the alternatively spliced exon. Bands are resolved on a 3% agarose gel. Ladder is NEB 100 bp. WT is the unedited iPSC line; WT-NC is the parental line. (E) Schematic of *TTN* isoforms, their expression during differentiation, physical property of the protein, and primer positions. (F) qRT-PCR

quantification of relative expression in WT and *RBM20* mutant iPSC-CMs of fetal *TTN* (*fTTN*) on the left and N2BA on the right, normalized to total *TTN*. The WT is set to 1. Y-axis is on a logarithmic scale. Error bars represent standard deviation. WT is the unedited iPSC line. N = 3. (* p-value < 0.05, Wilcox test). (+) = mutant allele; (−) = wild-type allele.

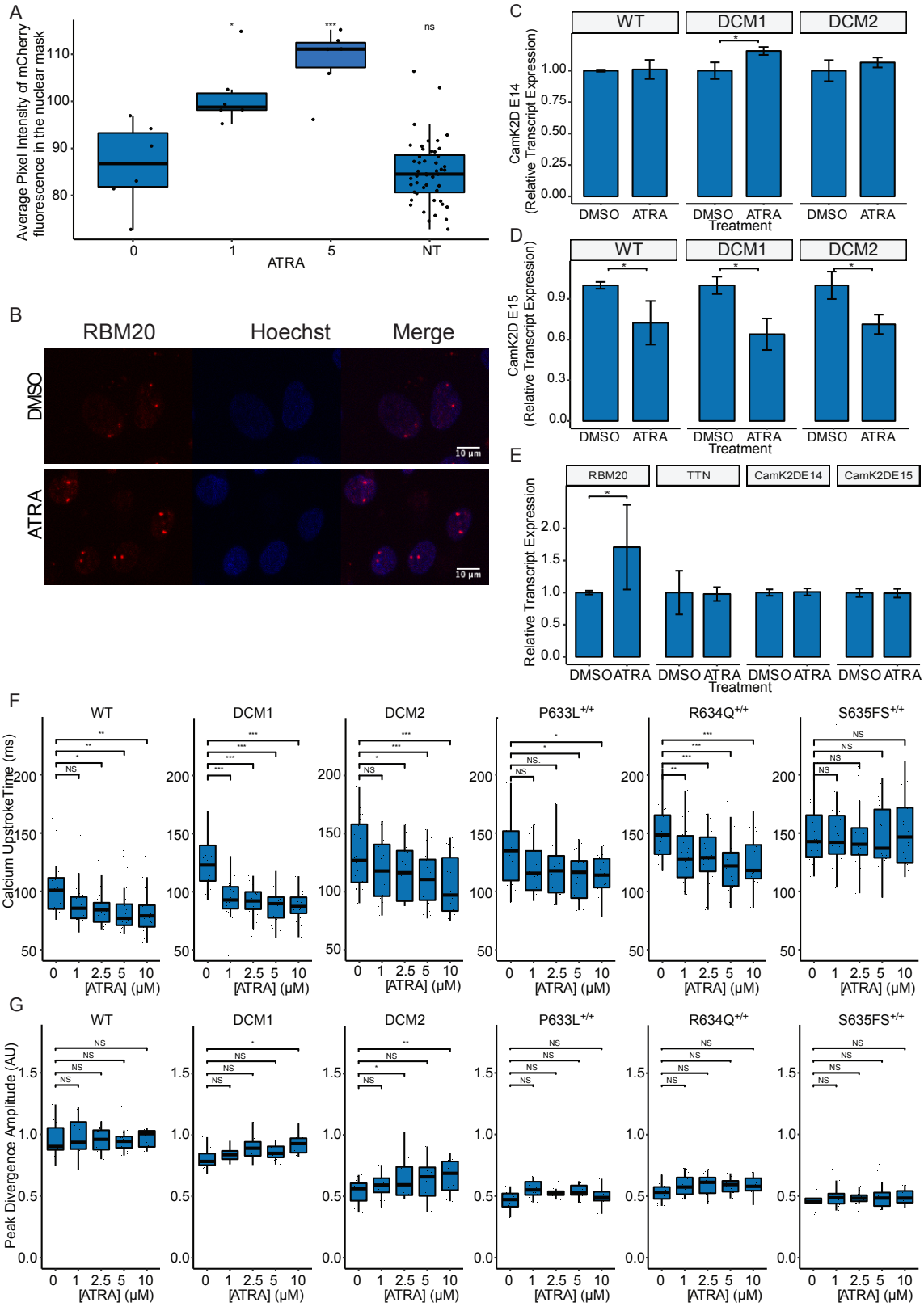
Supplemental Figure 3



ATRA induces RBM20 mRNA expression and enhances titin exon exclusion. Related to Figure 3

(A) Dose response curves of RBM20 activity measured by the Luciferase-based splicing reporter in HEK293T cells cotransfected with either pcDNA-RBM20 (positive control, red line) or pcDNA (blue line). Firefly activity was normalized to Renilla activity (ratios are shown). (B) Dose response curves of Firefly over Renilla luciferase (F/RLuc) activity upon ATRA treatment using the Luciferase-based RBM20-independent splicing reporter (titin exon MEx5) in HEK293T cells cotransfected with either pcDNA-RBM20 (positive control, red line) or pcDNA (blue line). N=3 for expression analysis, N=4 for splice reporter assays. * P<0.05, ** P<0.01, *** P<0.001 versus CTRL (Dunnett's post-test). Data are presented as mean ± SD. (C) qRT-PCR analysis of *RBM20* expression in iPSC-CMs upon treatment with different doses of ATRA. Error bars represent standard deviation. TBP is used as endogenous control. The non-treated (NT) sample is set to 1. NT are non-treated iPSC-CMs; DMSO are iPSC-CMs treated with 0.01% DMSO; 1μM, 2.5 μM, 5 μM, and 10 μM refer to ATRA concentration. (D) RNA-seq based RBM20 expression in WT, patient-derived (DCM1), and knock-out (S635SF) iPSC-CMs upon 5 μM ATRA treatment (n=4). ATRA is always diluted in DMSO such that DMSO concentration in the cell culture media is 0.01%. Error bars represent 95% confidence interval computed with 1000 bootstrap iterations. p is p-values as calculated by DESeq2 with differentiation effect regressed out (Expression ~ Treatment + Differentiation). (+) = mutant allele; (−) = wild-type allele. (E) Expression levels of known ATRA target genes as determined by RNA-Seq of WT, patient-derived (DCM1), and homozygous knock-out (S635FS) iPSC-CMs upon 5 μM ATRA treatment (n=4). ATRA is always diluted in DMSO such that DMSO concentration in the cell culture media is 0.01%. Error bars represent 95% confidence interval computed with 1000 bootstrap iterations. P values were calculated using DESeq2 and adjusted with Benjamini & Hochberg method (* padj <0.05, ** padj <0.01, *** padj < 0.001, padj is adjusted p-value). (+) = mutant allele; (−) = wild-type allele. (F) RNA-Seq library size for the ATRA treatment experiments on the three genotypes (WT, DCM1, and S635FS), each with four replicates from three differentiations. Error bars are 95% confidence interval computed with 1000 bootstrap iterations. (+) = mutant allele; (−) = wild-type allele.

Supplemental Figure 4



Dose-dependent expression of *RBM20* upon ATRA treatment. Related to Figure 4.

(A) mCherry fluorescence in iPSC-CMs upon treatment with different doses of ATRA. NT are non-treated iPSC-CMs; DMSO are iPSC-CMs treated with 0.01% DMSO; 1μM and 5 μM refer to ATRA concentrations. (* padj <0.05, ** padj <0.01, *** padj < 0.001, padj is adjusted p-value). (B) Sample images of the effect of 5μM ATRA on mCherry fluorescence in these iPSC-CMs. Hoechst was used for nuclear staining. (C) qPCR results for *CAMK2D* Exon 14 splicing in WT, DCM1, and DCM2 upon ATRA treatment. (D) qPCR results for *CAMK2D* Exon 15 splicing in WT, DCM1, and DCM2 upon ATRA treatment. Error bars represent standard deviation. * padj <0.05, ** padj <0.01, *** padj < 0.001, padj is adjusted p-value; n=6. (E) qPCR results for *RBM20* mRNA expression and *TTN* and *CAMK2D* splicing in S635FS iPSC-CM upon ATRA treatment. Error bars represent standard deviation. * padj <0.05, ** padj <0.01, *** padj < 0.001, padj is adjusted p-value; n=6. (F) Time-series-based analysis of calcium transients upon treatment with different doses of ATRA. Upstroke time is the calcium influx time (n=18-24). The cell line is indicated on top of each panel. DMSO is iPSC-CMs treated with 0.01% DMSO; 1, 2.5, 5, and 10 refer to ATRA concentration (μM). (G) Time-series-based analysis of contractile profiles of RBM20 WT and mutant iPSC-CMs upon treatment with different doses of ATRA (n=12). AU is arbitrary unit. The cell line is indicated on the top of each panel. DMSO is iPSC-CMs treated with 0.01% DMSO; 1, 2.5, 5, and 10 refer to ATRA concentration (μM). (* padj <0.05, ** padj <0.01, *** padj < 0.001, padj is adjusted p-value). (+) = mutant allele; (−) = wild-type allele.

Table S4. Oligonucleotides List. Related to STAR Methods

oligoName	Sequence
hsaRyR2_Ex81_rv	CTTTGCTGGCACTGATTGTCTG
hsaRyR2_Ex80_fw	GTTGTCATGATGAGGAAGATGACG
hsaTTN_rv	GTTTTGGAGGTGGTGGTTCTGG
hsaTTN_fw	GGAAGAAGTTGTTCTGAAAAGCG
has_eTTN1_rv	GGCGGAAGGCAACTGATACT
has_eTTN1_fw	GAAAAAAGAGGCACCCCCAGCC
has_eTTN2_rv	GGGAGCAGCCATGGGTGTTTC
has_eTTN2_fw	CCAGAGGGAGAAACTCCTATTG
hsaHPRT_fw	TATGCTGAGGATTTGGAAAGGGTG
hsaHPRT_rv	CAGAGGGCTACAATGTGATGGC
hsaRBM20_E8f	CAGAGGGAGAGGGACATGTTCC
hsaRBM20_E9r	GGAGGGCTGTGGGAAGAGCTGC
Fw_Guide_MutationHotspot	CACCGCTCACCGGACTACGAGACCG
Rv_Guide_MutationHotspot	aaacCGGTCTCGTAGTCCGGTGAGC
Fw_Guide_3UTR	CACCGAATCGTGCCACGCTTCCAA
Rv_Guide_3UTR	aaacTTgGAAGCGTGGCACGATTC
R634Q_KI_donor	TGTGGGACCTCGGGGAGAGTGACCGGCTCACCGGACTACGAGAC tGCGGCCTTTCTGGGCCATATCTGTGAGGGAGCCAAGGAGCAGG ATTTAGAATCTTCACACCTCCCATCCCACCCCACCCACA
P633L_KI_donor	TGTGGGACCTCGGGGAGAGTGACCGGCTCACCGGACTACGAGAC CGCaGCCTTTCTGGGCCATATCTGTGAGGGAGCCAAGGAGCAGG ATTTAGAATCTTCACACCTCCCATCCCACCCCACCCACA
RBM20_Fw	CTGGACTAGGGCAATCTTGCCC
RBM20_Rev	CTCATTCTGCTTGGCCTTGGCG
RBM20_LA_FW	gtattaccgcctttgagtgaCACAATTGCCATAGGCCAGC
RBM20_LA_RV	tcctcctcgcccttgctcacgagctTTTTCCTTTCGAAGCG
mCherry_FW	GCTTCGAAAGGAAAAagctcgtgagcaagggcgaggagga
mCherry_RV	cagcagaagcagaagcatcattacttgtacagctcgtccatgcc
RBM20_RA_FW	tggacgagctgtacaagtaatgatgcttctgcttctgctg
RBM20_RA_RV	cgggtgtcggggctggcttaCTACTGTGAGGTGGTTGTACCC
pUC_FW	GTACAACCACCTCACAGTAGtaagccagccccgacacccg