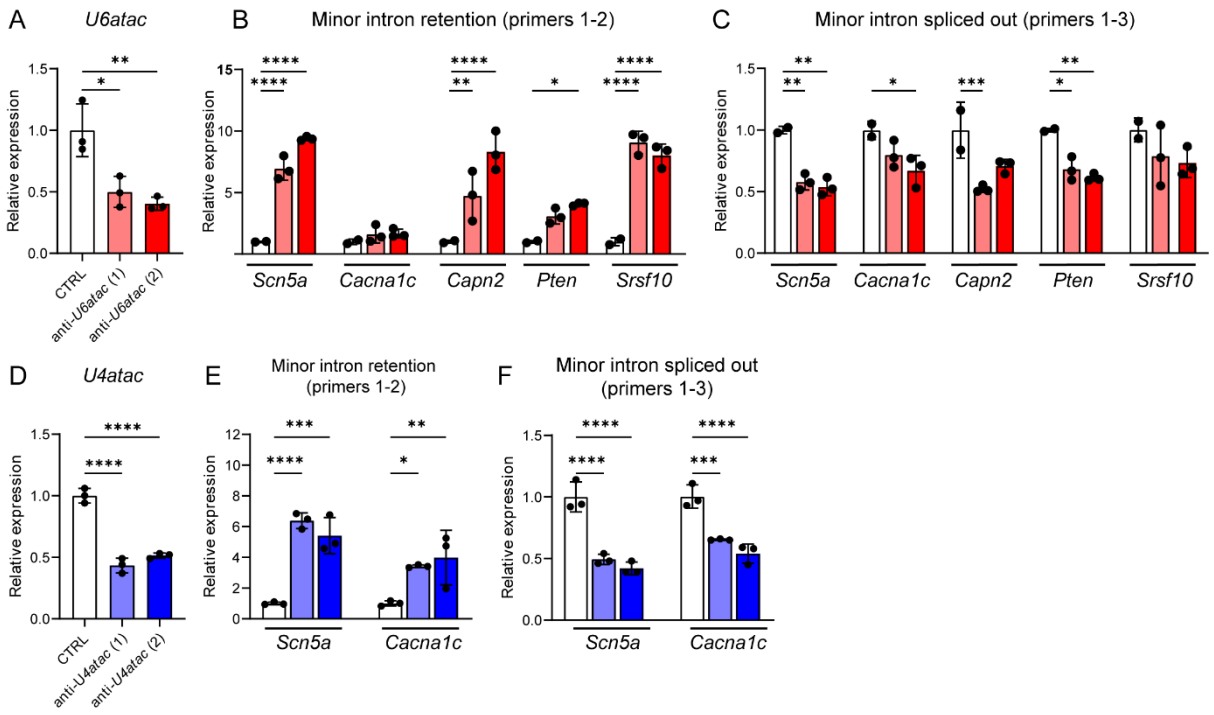

Fig. S1. Specificity of anti-*U6atac* and anti-*U4atac* gapmeRs. (A) Relative expression of the minor splicing component *U6atac*, (B) minor introns and (C) minor intron-flanking exons 24h after transfection with either a non-targeting control or 2 different anti-*U6atac* gapmeRs. (D) Relative expression of the minor splicing component *U4atac*, (E) minor introns and (F) minor intron-flanking exons 24h after transfection NRVMs with either a non-targeting control or 2 different anti-*U4atac* gapmeRs. Reference gene: *Hprt1*. Data are presented as mean ± s.d. Unpaired Student's t-test; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, **** $p < 0.0001$.

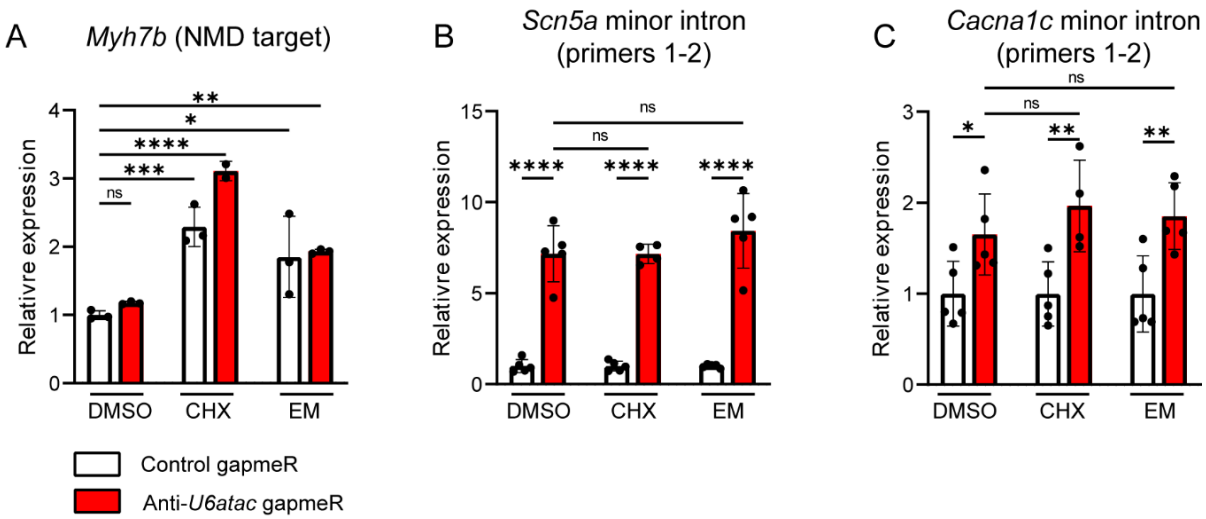

Fig. S2. NMD inhibition do not rescue the effect of minor intron retention. (A) Relative expression of the NMD-target *Myh7b*, (B) minor intron of *Scn5a and* (C) minor intron of *Cacna1c*. Cells were incubated for 3h with DMSO (control), 300 µg/µl cycloheximide (CHX) or 150 µg/µl emetine (EM) 24h after transfection with gapmeRs. Reference gene: *Hprt1*. Data are mean ± s.d. One-way ANOVA, followed by Holm-Sidak test for post hoc analyses; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, **** $p < 0.0001$.

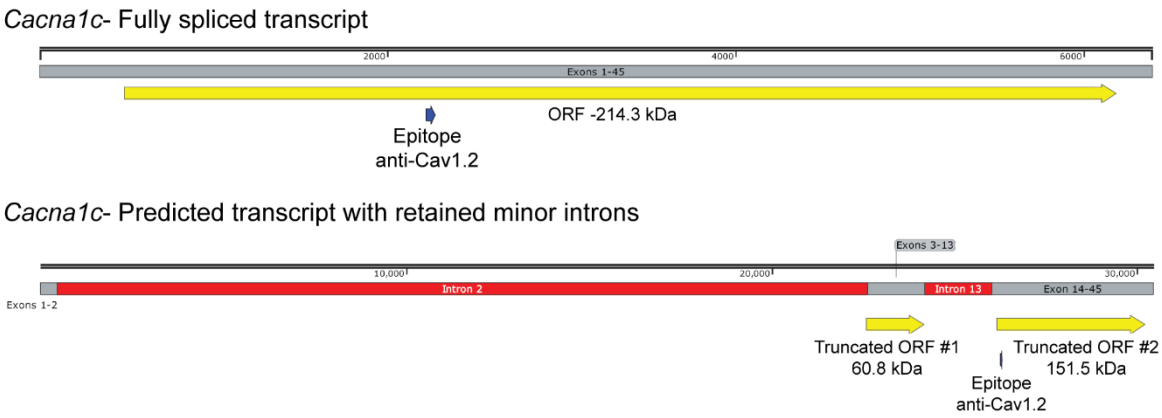

Fig. S3. Predicted size of the transcript and open reading frames of *Cacna1c* after minor intron retention.

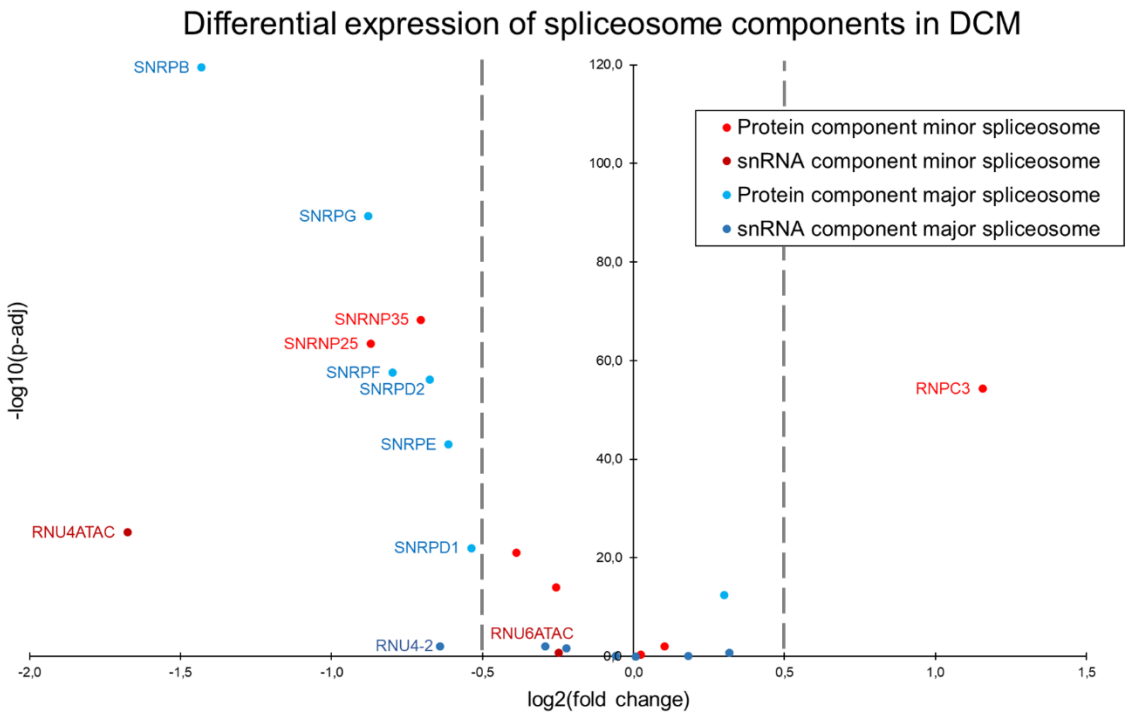

Fig. S4. Volcano plot showing differential expression of the protein and snRNA components of the minor and the major spliceosome in 100 non-diseased (CON) and 128 dilated cardiomyopathy (DCM) hearts. Only genes with TPM ≥ 0.1 were considered. Genes with absolute log2 fold change ≥ 0.58 (dashed lines) and adjusted p-value ≤ 0.05 were considered significantly differentially expressed.

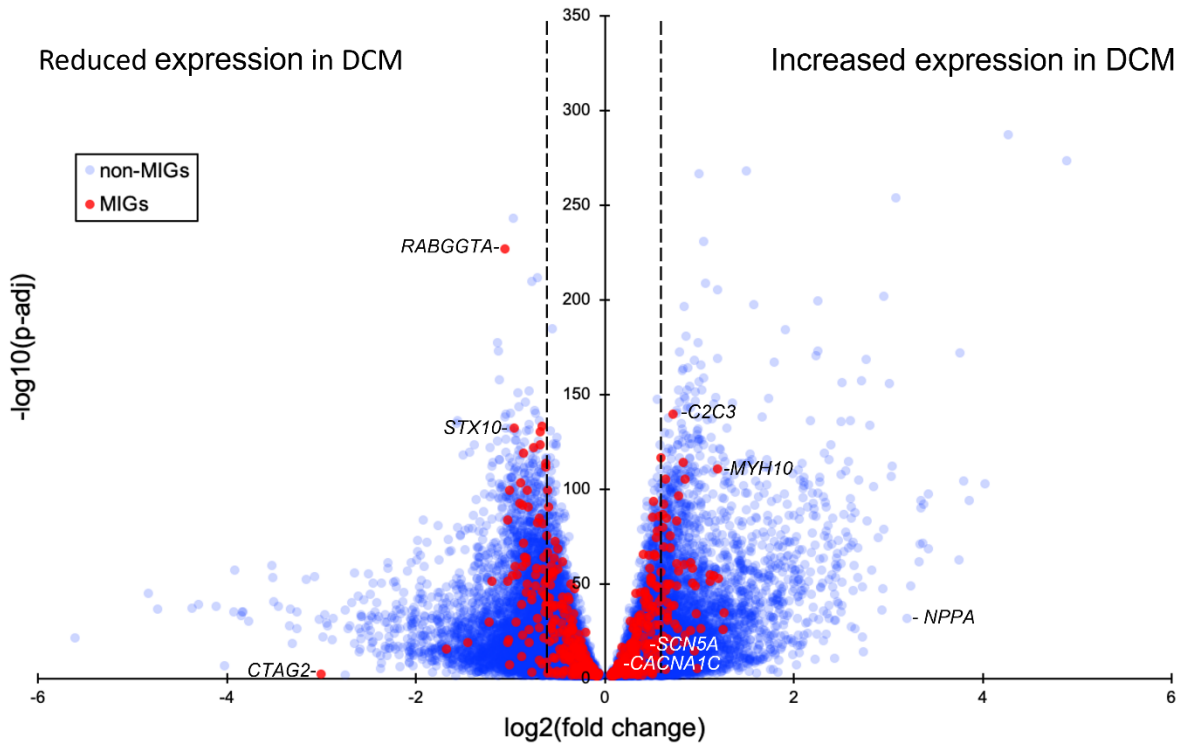

Fig. S5. Volcano plot showing differential expression of minor-intron containing genes (MIGs) and genes non-containing minor introns (non-MIGs) in 100 non-diseased (CON) and 128 dilated cardiomyopathy (DCM) hearts. Only genes with TPM ≥ 0.1 were considered. Genes with absolute log2 fold change ≥ 0.58 (dashed lines) and adjusted p-value ≤ 0.05 were considered significantly differentially expressed.

Table S1. Antisense LNA GapmeR sequences

GapmeR	Sequence 5’ -3’
Non-targeting negative control	AACACGTCTATACGC
Anti-*U6atac* (1)	CAATGCCTTAACCGTA
Anti-*U6atac* (2)	TGCCTTAACCGTATG
Anti-*U4atac* (1)	GTAGCGCAACCCCAAG
Anti-*U4atac* (2)	TAACCGATGCAGGTGT
FAM-labeled control	/56-FAM/AACACGTCTATACGC

Table S2. Primer sequences

Primer	Sequence	Annealing on exon/intron (total number)
EEF1E1 Fw	TCCAGTAAAGAAGACACCCAGA	
EEF1E1 Rv	GACAAAACCAGCGAGACACA	
GAPDH Fw	GGTGGACCTCATGGCCTACA	
GAPDH Rv	CTCTCTTGCTCTCAGTATCCTTGCT	
HPRT1 Fw	TGACTATAATGAGCACTTCAGGGATTT	
HPRT1 Rv	CGCTGTCTTTTAGGCTTTGTACTTG	
MYH7B Fw	CTCAAGCGGGAGAACAAGAATC	
MYH7B Rv	CTGAGGCTGACCTGGTCTGTAA	
U4ATAC Fw	ATCCTTTTCTTGGGGTTGCG	
U4ATAC Rv	CGATGCAGGTGTGTTGTCAG	
U6ATAC Fw	GGTTAGCACTCCCCTTGACA	
U6ATAC Rv	AAGTAGGTGGCAATGCCTTAACC	
U11 Fw	GCGTGCGGAATCGACATCAAG	
U11 Rv	AAGGGCGCCGGGACCAACG	
U12 Fw	ATAACGATTCGGGGTGACGC	
U12 Rv	AGGCATCCCGCAAAGTAGGC	
SCN5A 1 Fw	TCTTCCGGTTCAGTGCCACC	Exon 3 (28)
SCN5A 2 Rv	CAGGACAGATGCGGATTAAGAGC	Intron 3 (27)
SCN5A 3 Rv	GGATGGTGCACATGATGAGCATG	Exon 4 (28)
SCN5A 4 Rv	AGGATGACGATGATGCTGTCG	Exon 15 (28)
SCN5A 5 Fw	ACCGACTCCTTCTCTCTTCTTC	Intron 14 (27)
SCN5A 6 Fw	GAGGAGATGCTGCAGGTCGG	Exon 14 (28)
CACNA1C 1 Fw	TGGGATCATGGCTTATGGCGGC	Exon 13 (45)
CACNA1C 2 Rv	GGCAGCATCAGCACCAAAGG	Intron 13 (44)
CACNA1C 3 Rv	ATCAGCCAGGTTGTCCACCG	Exon 14 (45)
CACNA1C 4 Fw	GAAGTTGTGTCCTCACCGTG	Exon 35 (45)
CACNA1C 5 Rv	TCACAAAGGCAAACAGGTAGC	Intron 35 (44)
CACNA1C 6 Rv	GGCAAACAGTGTAGCATTGAAC	Exon 36 (45)
CAPN2 1 Fw	GGCTTCAGCATCGAGACCTG	Exon 14 (21)
CAPN2 2 Rv	CAGGCTGCCTGTCCACA	Intron 14 (20)
CAPN2 3 Rv	CGTCCAGAGGATGTAGAACTCC	Exon 15 (21)
CAPN2 4 Fw	ATTGGAGATGGATTCAGAAGGC	Exon 16 (21)
CAPN2 5 Rv	GCTAAGGGTCTGAGCGAC	Intron 16 (20)
CAPN2 6 Rv	CTAGAACTCTTCTCAAGATGGTCTG	Exon 17 (21)
PTEN 1 Fw	TCAGCCACAGGCTCCCAGAC	Exon 1 (9)
PTEN 2 Rv	TTCGCATCCGTCTACTCCCACG	Intron 1 (8)
PTEN 3 Rv	ACACCTTCAAGTCTTTCTGCAGG	Exon 2 (9)
PTEN 4 Fw	GTGTGTGGTGACATCAAAGTAG	Exon 7 (9)
PTEN 5 Rv	ACTAATCTCCTAACCAAAGGCAC	Intron 7 (8)
PTEN 6 Rv	TCCTCTGGTCCTGGTATGAAG	Exon 8 (9)
SRSF10 1 Rv	GCGTCTTCAGCATCACGAACATC	Exon 3 (5)

SRSF10 2 Fw	GAGTTGTTTCAGACTTCACAAGCC	Intron 2 (4)
SRSF10 3 Fw	AGATTTACGTCGGGAATTTGGTCG	Exon 2 (5)
SRSF10 4 Rv	TACGCCGTGGTCTTCCAG	Exon 5 (5)
SRSF10 5 Fw	TGTGTATCTTGGATGCTTCATTAAGG	Intron 4 (4)
SRSF10 6 Fw	GGAGGAGATCAAGGAGTCGG	Exon 4 (5)

Table S3. Antibodies and dyes

Antibody	Dilution	Reference
Anti-tubulin	1:5000 (WB)	GeneTex Cat No. GTX628802-01
Anti-$Na_v1.5$(SCN5A)	1:200 (WB)	Sigma S0819
Anti-$Ca_v1.2$ (CACNA1C)	1:200 (WB)	Alomone Cat No. ACC-003
Anti-rabbit-HRP	1:10000 (WB)	Amersham NA9340V
Anti-mouse-HRP	1:10000 (WB)	Amersham NA9310V
Anti-Vimentin	1:1000 (IF)	Abcam AB92537
Anti-alpha-actinin	1:750 (IF)	Sigma A781
Goat anti-rabbit-488	1:250 (IF)	Invitrogen A-11008
Goat anti-mouse-647	1:250 (IF)	Invitrogen A-21235
Wheat germ-agglutinin-488	1:200 (IF)	Invitrogen W11261
DAPI	1:1000 (IF)	Invitrogen D1306

Table S4. Sodium current properties in neonatal rat ventricular myocytes (NRVMs) 48h after transfection with control or anti-*U6atac* gapmeR.

	Control	n	Anti-*U6atac*	n
Current density				
I_{Na} (pA/pF)	-198.8±36.4	12	-82.3±18.5*	12
Activation				
$V_{1/2}$ (mV)	-43.7±1.6	12	-39.3±1.1*	12
***k* (mV)**	6.5±0.3	12	7.5±0.2*	12
Inactivation				
$V_{1/2}$ (mV)	-90±2.7	10	-89.1±1.4	10
***k* (mV)**	-5.9±0.3	10	-6.9±0.5	10

I_{Na}, sodium current density at -25 mV; $V_{1/2}$ of (in)activation, half-voltage of (in)activation; *k*, slope of the (in)activation curve; n, number of cells. * $p < 0.05$ *vs* control; unpaired Student's t-test or Mann-Whitney test when data were not normally distributed.

Table S5. L-type calcium current properties in neonatal rat ventricular myocytes (NRVMs) 48h after transfection with control or anti-*U6atac* gapmeR.

	Control	n	Anti-*U6atac*	n
Current density				
I_{CaL} (pA/pF)	-16.0±1.7	13	-10.3±1.0*	12
Activation				
$V_{1/2}$ (mV)	-13.0±0.8	13	-14.6±1.1	12
***k* (mV)**	7.3±0.2	13	7.3±0.3	12
Inactivation				
$V_{1/2}$ (mV)	-38.3±1.3	10	-37.7±0.7	9
***k* (mV)**	-6.0±0.3	10	-6.1±0.2	9

I_{CaL}, L-type calcium current density at 0 mV; $V_{1/2}$ of (in)activation, half-voltage of (in)activation; *k*, slope of the (in)activation curve; n, number of cells. * p <0.05 *vs* control; unpaired Student's t-test.

Table S6.

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