SUPPLEMENTAL INFORMATION:

Furamidine rescues myotonic dystrophy type I associated mis-splicing through multiple mechanisms

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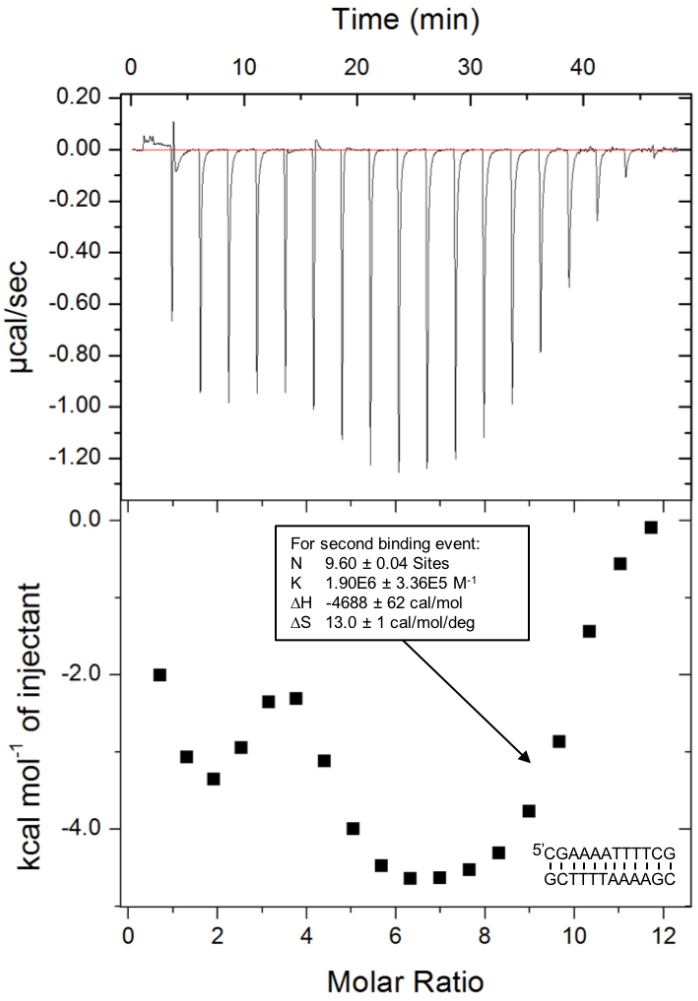

Figure S1. Furamidine binds an AT-rich DNA sequence in the nanomolar range. A representative isothermal titration calorimetry (ITC) binding curve (lower panel) using a d(CGAAAATTTTCG) palindrome sequence (inset lower right) shows that furamidine binds this AT-rich DNA sequence with a two site binding model, containing one higher affinity and one lower affinity site. The lower affinity binding site has a K_D of 543 ± 16 nM. The raw heats of reaction versus time are displayed in the upper panel. Thermodynamic binding properties for the lower affinity site are displayed in the lower panel.

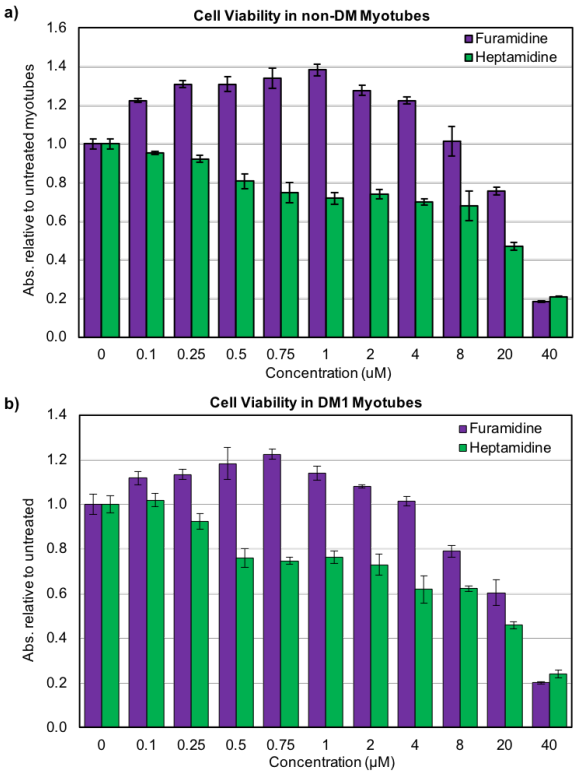

Figure S2. Toxicity study with furamidine and heptamidine across a broad concentration range (0.1 – 40 μM) in non-DM and DM1 patient-derived myotubes. a) In non-DM myotubes, furamidine (purple) did not affect cell viability within the concentration range of 0.1 – 4 μM, however significant toxicity is observed by 20 μM ($p<0.001$). Heptamidine (green) started to display significant cell toxicity after 4 days of treatment at 0.5 μM ($p<0.001$). **b)** In DM1 myotubes, furamidine (purple) did not cause cell toxicity until 4 days of treatment at 8 μM ($p<0.01$), while heptamidine (green) displayed significant cell toxicity after 4 days of treatment at 0.5 μM ($p<0.01$).

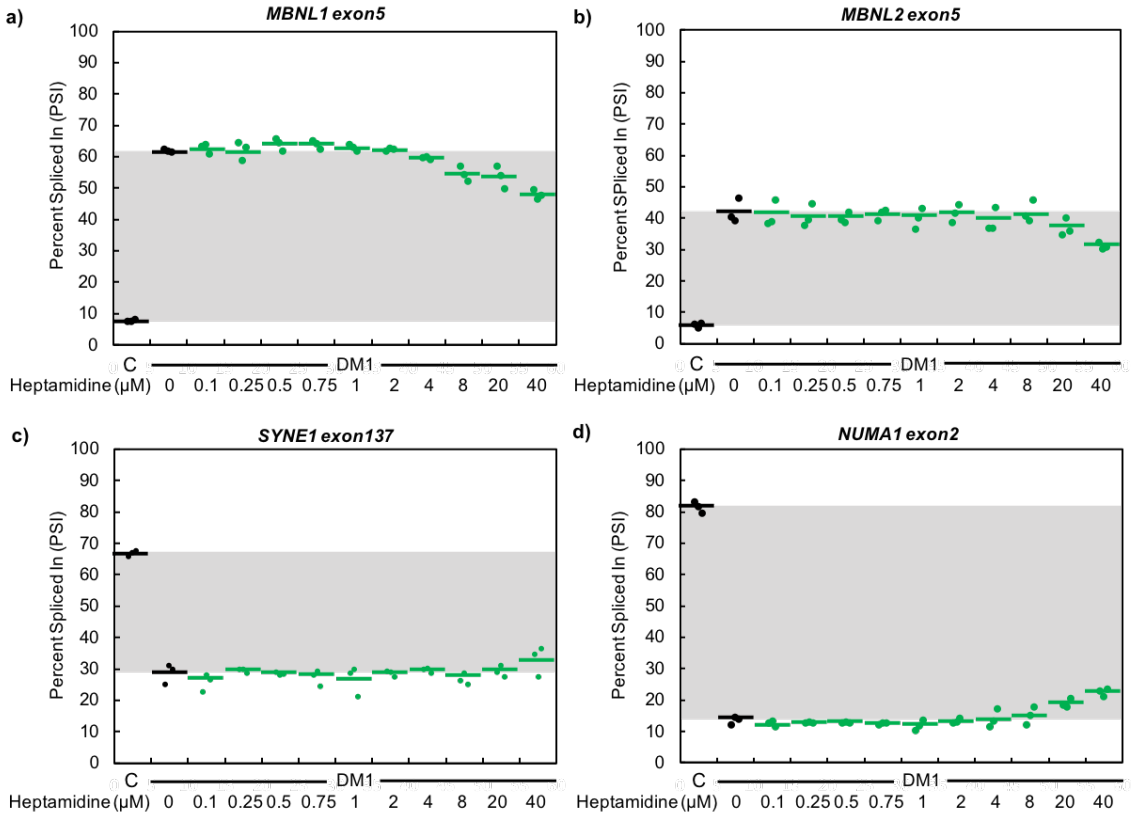

Figure S3. Heptamidine partially rescued mis-splicing in DM1 patient-derived myotubes at high concentrations. a) *MBNL1*, **b)** *MBNL2*, **c)** *SYNE1* and **d)** *NUMA1* events display maximum percent rescue of 26 ± 3%, 30 ± 3%, 13 ± 2%, and 11 ± 12% at 40 μM heptamidine, respectively, after 4 days of treatment (all p<0.01, except NS for *SYNE1*).

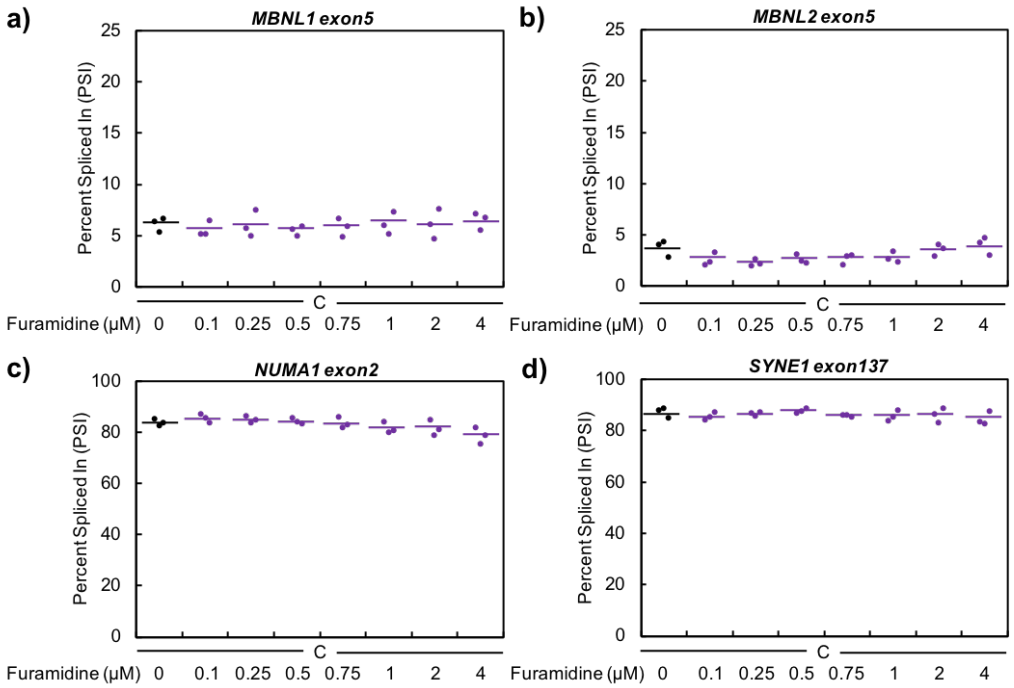

Figure S4. Furamidine does not affect splicing in non-DM patient-derived myotubes. a) *MBNL1*, **b)** *MBNL2*, **c)** *SYNE1* and **d)** *NUMA1* events displayed no significant change in exon inclusion after 4 days of treatment.

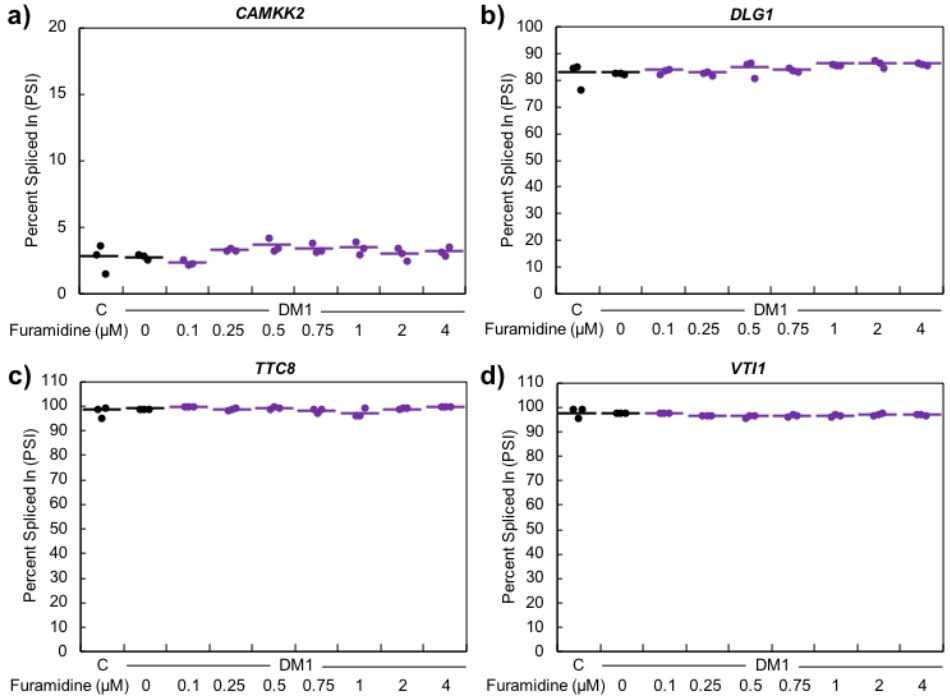

Figure S5. Furamidine did not affect splicing of events not altered in DM1 patient-derived myotubes. a) *CAMKK2 exon15*, **b)** *DLG1 exon14*, **c)** *TTC8 exon3* and **d)** *VTI1 exon5* events show no significant change in exon inclusion after 4 days of furamidine treatment.

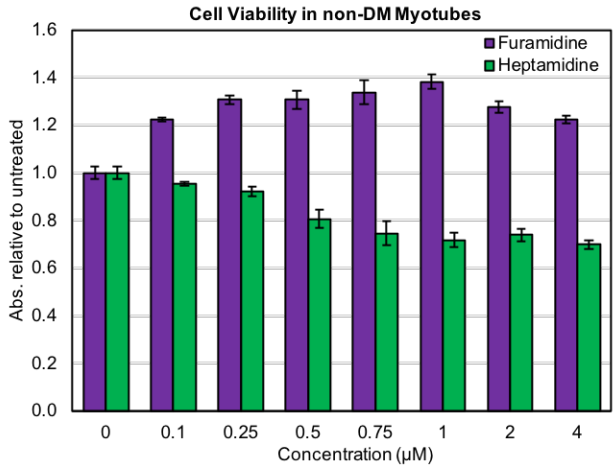

Figure S6. Cell toxicity studies with furamidine and heptamidine in non-DM patient-derived myotubes. Furamidine did not affect cell viability within the concentration range of 0.1 – 4 μM. Heptamidine displayed significant cell toxicity after 4 days of treatment at 0.5 μM ($p<0.001$).

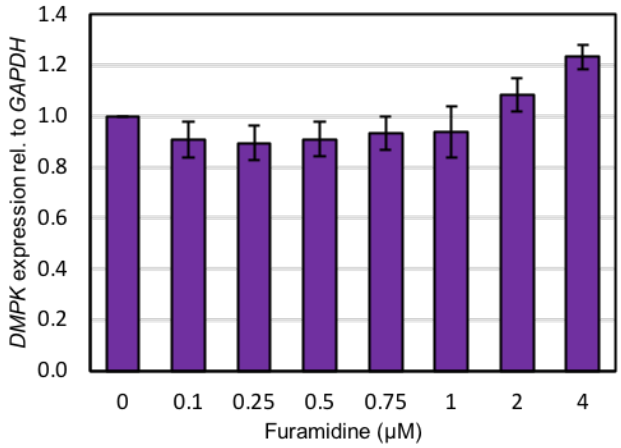

Figure S7. *DMPK* transcript levels were not significantly reduced at furamidine concentrations where mis-splicing rescue is observed in DM1 myotubes. RT-qPCR data showing *DMPK* expression levels in DM1 patient-derived myotubes treated with furamidine for 4 days. Furamidine treatment caused no significant change in DMPK transcript levels between 0.1 and 2 μM. However, *DMPK* levels increased slightly at 4 μM furamidine ($p<0.02$).

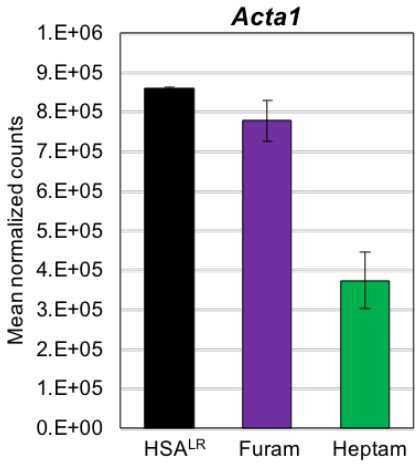

Figure S8. *Acta1* transcript levels did not significantly change with 30 mg kg^{-1} furamidine treatment in HSA^{LR} mice. RNA-seq data showing the mean normalized counts of *Acta1* transcripts in HSA^{LR} mice treated with 30 mg kg^{-1} furamidine (purple) or 30 mg kg^{-1} Heptamidine (green) for 7 days. Furamidine treatment caused no significant change in *Acta1* transcript. However, heptamidine caused *Acta1* levels to decreased significantly to 44 ± 3% of HSA^{LR} control ($p<0.001$).

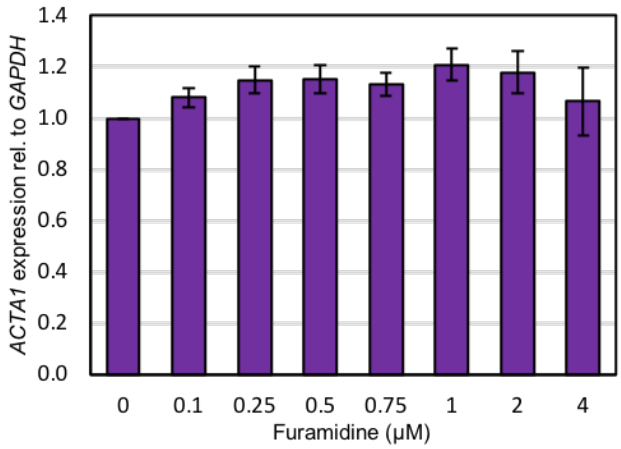

Figure S9. *ACTA1* transcript levels increased modestly with furamidine treatment in DM1 myotubes. RT-qPCR data showing *ACTA1* expression levels in DM1 patient-derived myotubes treated with furamidine for 4 days. Furamidine treatment caused slight increase of *ACTA1* transcript levels between 0.1 and 1 μM ($p<0.05$ or below for all). *ACTA1* expression was not significantly changed at 2 and 4 μM furamidine.

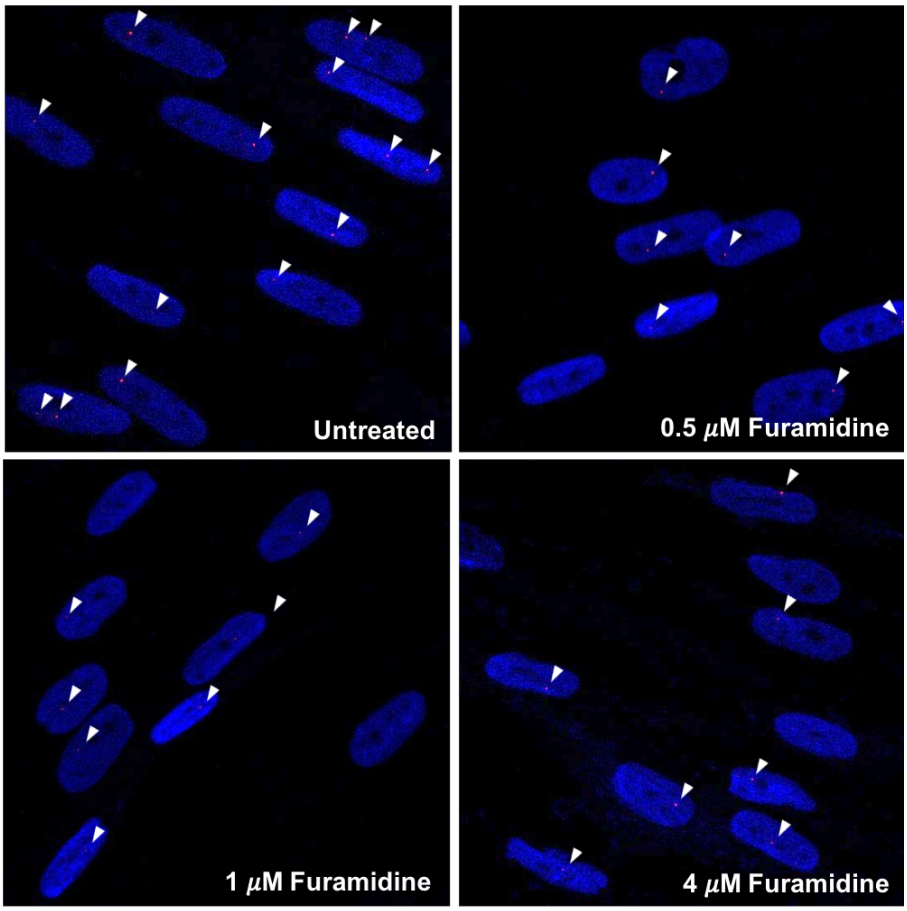

Figure S10. Furamidine reduced ribonuclear foci abundance in DM1 patient-derived myoblasts. Representative fluorescent in situ hybridization (FISH) images using a Cy3-$(CAG)_8$ probe for CUG-repeat RNA foci (red) hybridization and DAPI to stain the nucleus (blue). Foci are highlighted with white arrows. Myoblasts were differentiated for a total of 7 days and treated with the indicated concentration of furamidine for 4 days.

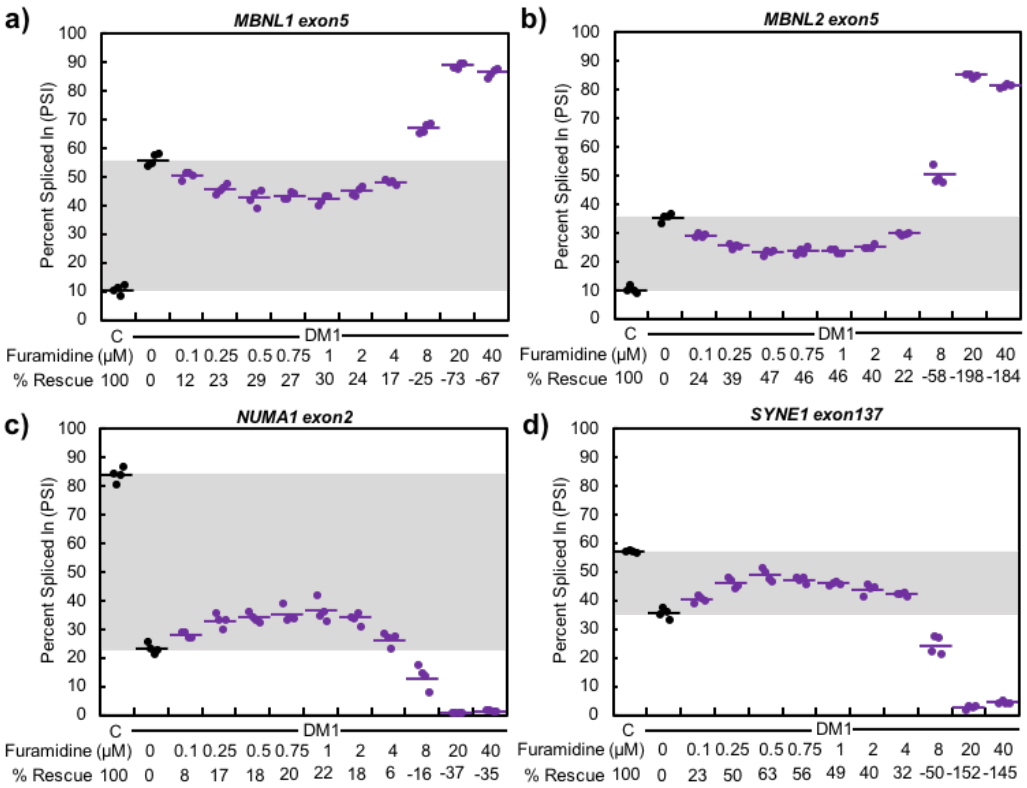

Figure S11. Furamidine partially rescued mis-splicing in DM1 patient-derived myotubes. a) *MBNL1*, **b)** *MBNL2*, **c)** *NUMA1* and **d)** *SYNE1* events displayed maximum percent rescue of 30 ± 3%, 47 ± 4%, 22 ± 6%, and 63 ± 9%, respectively, after 4 days of furamidine treatment ($p<0.01$). Maximum rescue occurred between 0.5 and 1 μM furamidine for all splicing events shown. Exacerbation of mis-splicing was observed for all events starting at 8 μM furamidine for 4 days of treatment. Similar exacerbation of mis-splicing is observed with 24-hour treatments of furamidine at 20 – 80 μM.

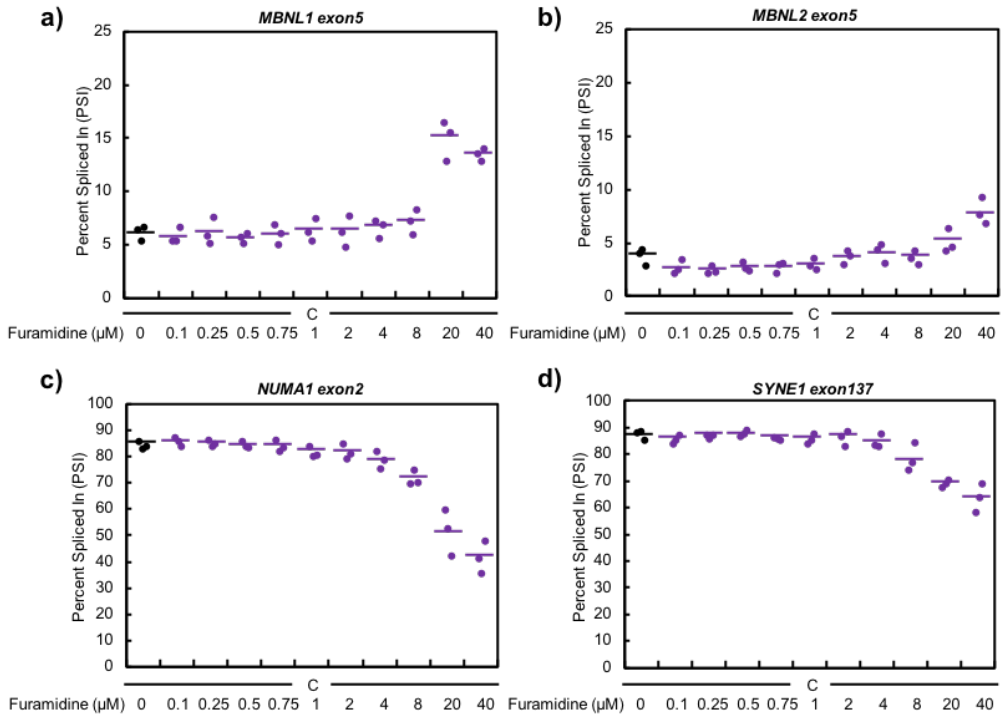

Figure S12. Furamidine causes mis-splicing in non-DM patient-derived myotubes at high concentrations. a) *MBNL1*, **b)** *MBNL2*, **c)** *SYNE1* and **d)** *NUMA1* events display no significant change in exon inclusion until 4 days of treatment at 20 uM. Exacerbation of mis-splicing was observed for all events starting at 20 μM furamidine treatment ($p<0.05$ or better).

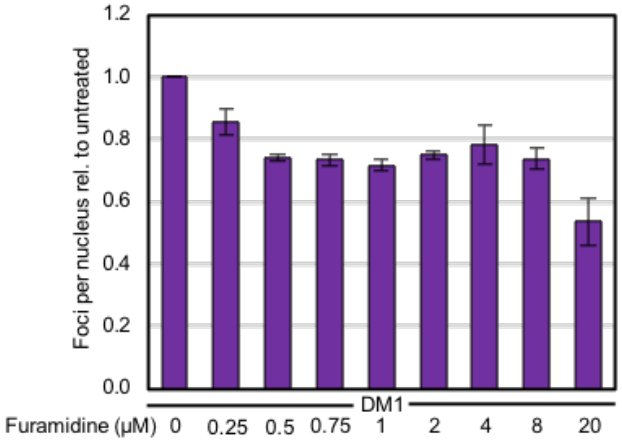

Figure S13. Furamidine treatment reduced ribonuclear foci abundance in DM1 myotubes. Quantified FISH data showing the number of ribonuclear foci per nucleus with foci abundance of untreated DM1 cells set to 1. A reduction in ribonuclear foci per nucleus is observed for all furamidine concentrations tested ($p<0.01$). Foci reduction that occurs at 8 μM furamidine and above are linked to cell toxicity and exacerbation of mis-splicing.

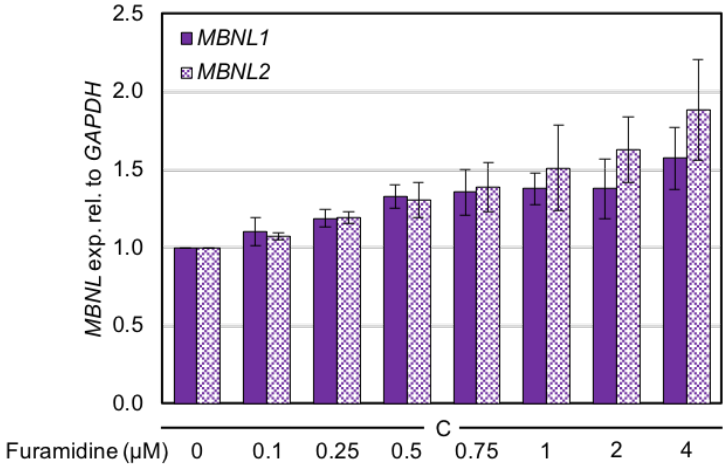

Figure S14. MBNL transcript levels increased with furamidine treatment in non-DM1 myotubes. RT-qPCR data showing *MBNL1* (solid bars) and *MBNL2* (patterned bars) expression levels in non-DM1 patient-derived myotubes treated with furamidine for 4 days. Furamidine treatment causes increased *MBNL1* ($p<0.05$ or better) and *MBNL2* transcripts ($p<0.05$ or better).

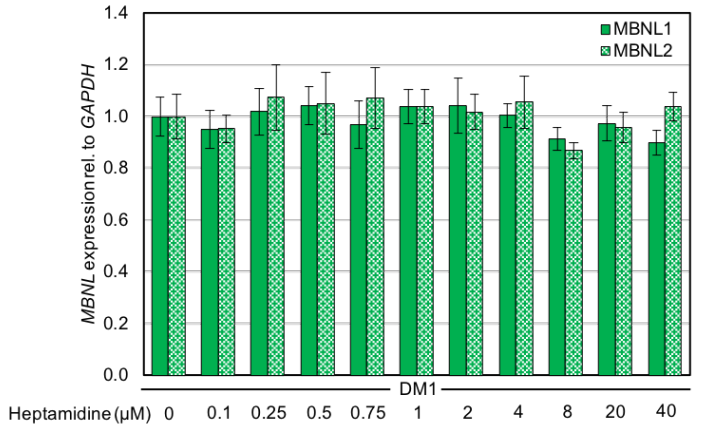

Figure S15. MBNL transcript levels did not increase with heptamidine treatment in DM1 myotubes. RT-qPCR data showing *MBNL1* (solid bars) and *MBNL2* (patterned bars) expression levels in DM1 patient-derived myotubes treated with heptamidine. Heptamidine treatment did not cause statistically significant changes in the levels of *MBNL1* or *MBNL2* transcripts.

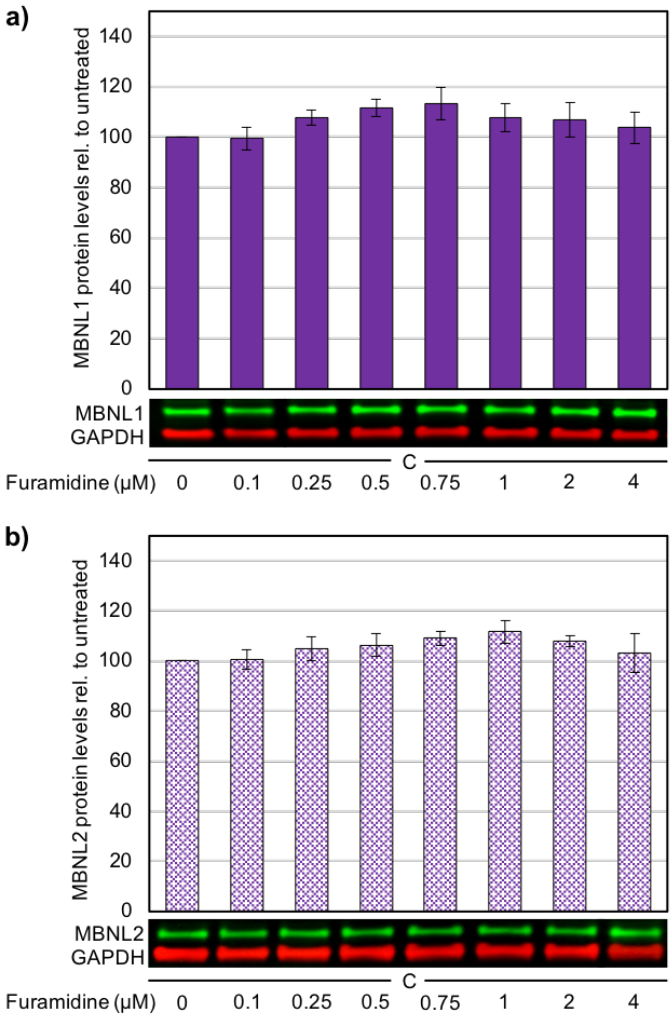

Figure S16. MBNL protein levels increased with furamidine treatment in non-DM1 myotubes. a) MBNL1 and **b)** MBNL2 protein levels in non-DM1 patient-derived myotubes treated with furamidine. Furamidine treatment caused increased levels of MBNL1 at 0.25, 0.75 and 1 μM corresponding to 108 ± 3%, 112 ± 4% and 113 ± 6%, respectively ($p<0.05$ or below for all). Also, MBNL2 protein levels increased at 0.75, 1 and 2 μM furamidine treatment, corresponding to 109 ± 3%, 112 ± 5% and 108 ± 2%, respectively ($p<0.02$ or below for all).

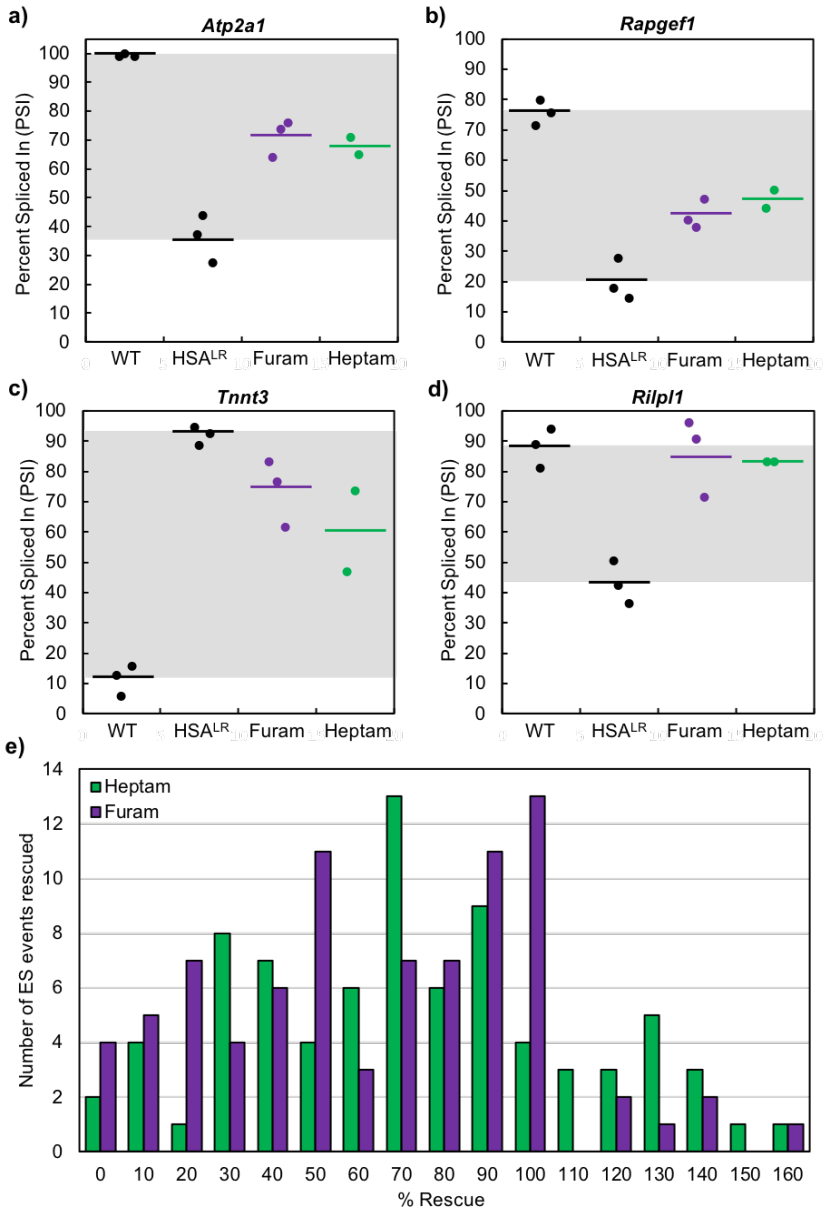

Figure S17: Mis-splicing rescue in HSA^{LR} DM1 mice treated with 30 mg kg^{-1} furamidine or heptamidine. RNA-seq analysis confirming the rescue of **a)** *Atp2a1* mis-splicing event (p<0.01, FDR<0.01). Further, **b)** *Rapgf1,* **c)** *Tnnt3* and **d)** *Rilpl1* mis-splicing events were partially rescued by furamidine (purple) and heptamidine (green) (p<0.01, FDR<0.01). **e)** Distribution of the average percent rescue observed for the mis-splicing events in HSA^{LR} mice that showed evidence of rescue (greater than 10% change in PSI) with treatment of furamidine or heptamidine (p<0.01, FDR<0.01).

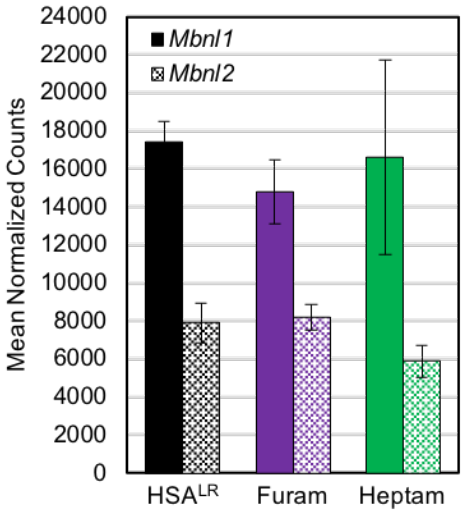

Figure S18. *Mbnl* transcript levels in HSA_{LR} mice did not change with 30 mg kg^{-1} furamidine or heptamidine treatment. Mean normalized *Mbnl1* (solid) and *Mbnl2* (patterned) transcript counts in HSA^{LR} mice treated with 5% glucose in PBS (black), 30 mg kg^{-1} furamidine (purple), or 30 mg kg^{-1} heptamidine (green). There were no statistically significant differences between the expression in HSA^{LR} control versus treatments.

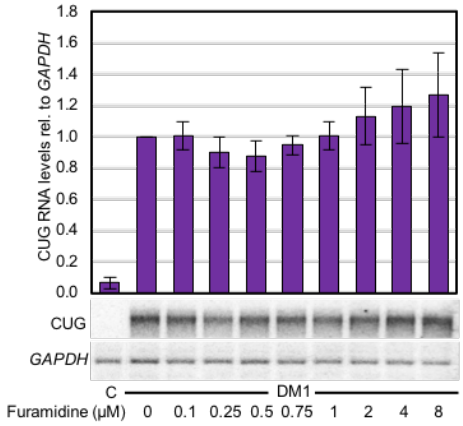

Figure S19. CUG RNA levels are not significantly reduced with furamidine treatment in DM1 myotubes. Northern blot quantification of CUG repeat RNA levels relative to *GAPDH* with the untreated CUG levels set to 1. There were no significant changes in the CUG RNA levels at any concentration of furamidine treatment.

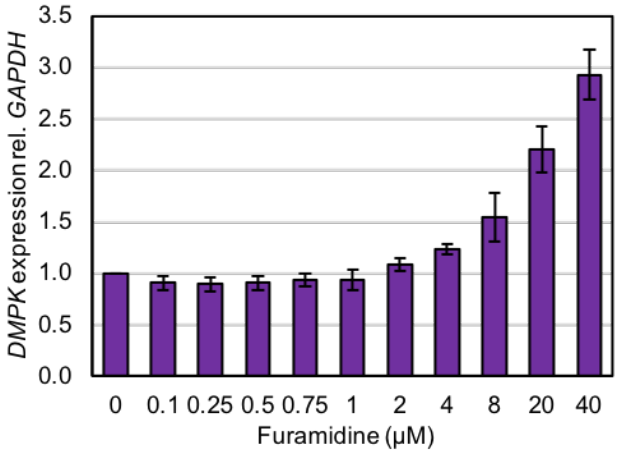

Figure S20. *DMPK* transcript levels increased with furamidine treatment in DM1 myotubes. RT-qPCR data showing *DMPK* expression levels in DM1 patient-derived myotubes treated with furamidine for 4 days. Furamidine treatment caused increasing levels of *DMPK* at high concentrations ($p<0.02$ or below for 4 μM furamidine and above).

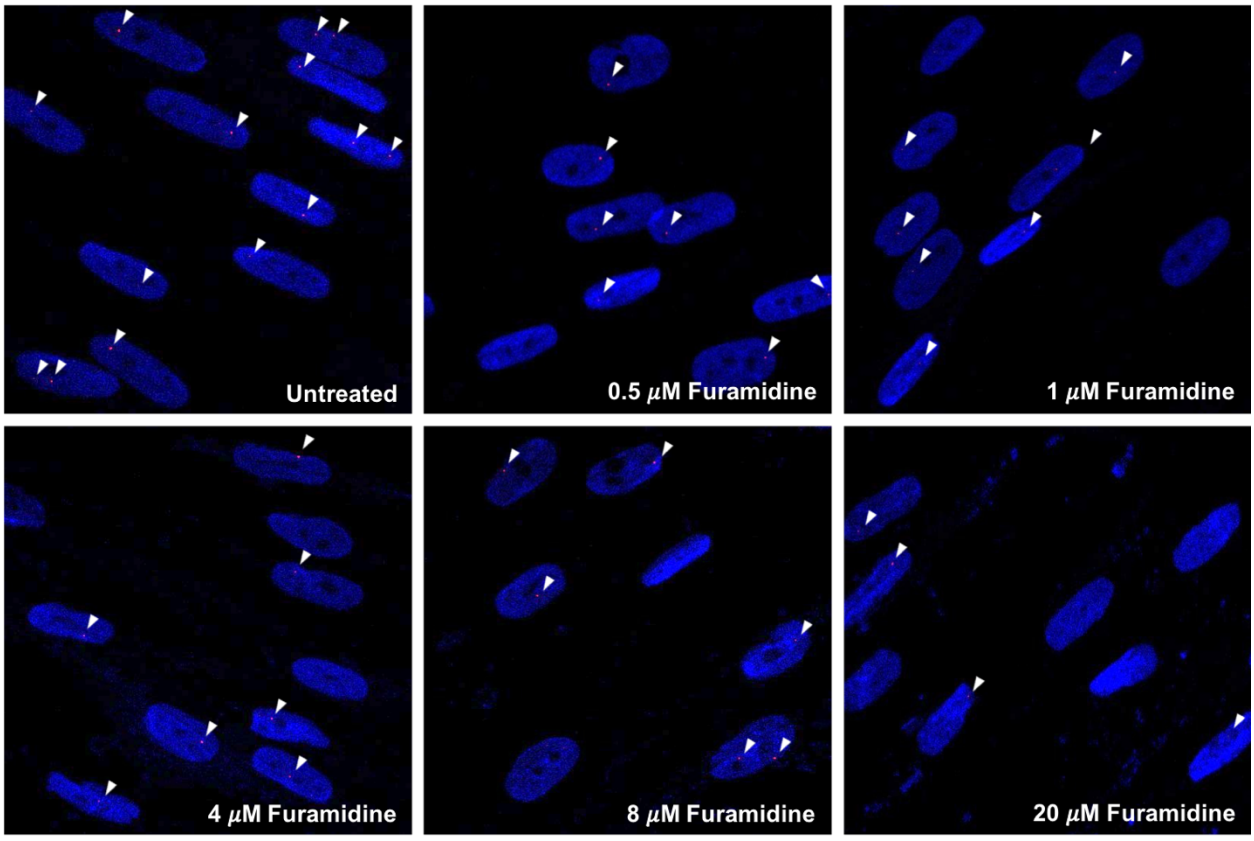

Figure S21. Furamidine reduced ribonuclear foci abundance in DM1 patient-derived myoblasts. Representative fluorescent in situ hybridization (FISH) images using a Cy3-$(CAG)_8$ probe for CUG-repeat RNA foci (red) hybridization and DAPI to stain the nucleus (blue). Foci are highlighted with white arrows. Myoblasts were differentiated for a total of 7 days and treated with the indicated concentration of furamidine for 4 days.

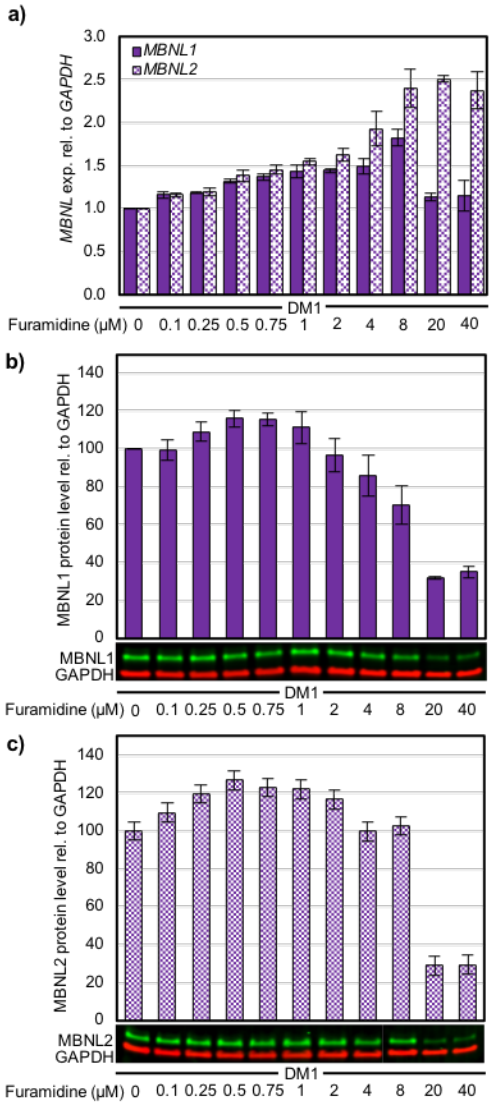

Figure S22. MBNL transcript and protein levels increased with furamidine treatment in DM1 myotubes. a) RT-qPCR data showing *MBNL1* (sold purple bars) and *MBNL2* (patterned purple bars) expression levels and western blot data for **b)** MBNL1 and **c)** MBNL2 protein levels in DM1 patient-derived myotubes treated with furamidine. Furamidine treatment caused increased levels of both *MBNL1* ($p<0.01$ for 0.1 - 8 μM furamidine, NS for 20 and 40 μM furamidine) and *MBNL2* transcripts ($p<0.01$ for all). Also, MBNL1 and MBNL2 protein levels increased between 0.25 and 1 μM furamidine ($p<0.05$ or better). Significant reduction in MBNL1 and MBNL2 protein levels was observed for 20 and 40 μM furamidine ($p<0.01$ for all).

METHODS

RT-PCR splicing analysis. For splicing analysis in cell culture, approximately 1 x 10^5 myoblasts were plated in 12-well plates in SkGM™-2 BulletKit™ growth medium (Lonza). Cells were allowed to reach >90% confluency and then differentiated for 7 days in DMEM/F-12 50/50 medium (Corning) supplemented with 2% (v/v) donor equine serum (Hyclone). Treatments were carried out by adding the indicated concentrations of drug to the differentiation media after three days. Myotubes were harvested at 4 days of drug treatment and 7 days total differentiation. RNA was isolated using an Aurum™ Total RNA mini kit (Bio-Rad) according to package insert with on-column DNase1 treatment. For RT-PCR splicing analysis in mouse model, RNA was TRIZol extracted from quadriceps muscle of HSA^{LR} mice treated with either 5% (w/v) glucose in PBS, 30 mg kg^{-1} heptamidine, or 30 mg kg^{-1} furamidine. For all samples, RNA concentrations were determined using a NanoDrop (Thermo) and reverse transcribed with SuperScript VI using random hexamer primers (IDT). The cDNA was then subjected to polymerase chain reaction for 32 cycles using the primer sets listed in Table S1. Resulting PCR products were run via capillary electrophoresis on Fragment Analyzer using the 1–500 bp DNF-905 kit (Advanced Analytical). Quantification was done using the integration values of the electropherogram peaks corresponding to inclusion and exclusion products from the Prosize 2.0 software (Advanced Analytical). Errors were determined by calculating the standard deviation of replicate data. To determine the % rescue of a given ES event, Eq. **1** was used, where $PSI_{treated}$ = PSI of treated DM1 myotubes, $PSI_{untreated}$ = PSI of treated DM1 myotubes, and $PSI_{untreated}$ = PSI of non-DM myotubes.

$$\% \text{ rescue} = [(PSI_{treated} - PSI_{untreated}) / (PSI_{non\text{-}DM} - PSI_{untreated})] * 100 \quad \textbf{[1]}$$

Toxicity analysis in cell culture. Approximately 1 x 10^4 myoblasts were plated in 96-well plates and treated as described above for RT-PCR splicing analysis. After 4 days drug treatment and 7 days total differentiation, media was replaced and PrestoBlue cell viability reagent (Thermo) was added to the cells according the package insert and incubated at 37°C and 5% CO_2, protected from light for 2 hrs. Absorbance at 570nm and 600nm was read on a BioTek Cytation 3 plate reader. The 570nm/600nm absorbance ratios were calculated for all samples with a background subtraction of the average 570nm/600nm values of no-cell plus furamidine control wells. All samples were

normalized by setting the background subtracted, non-treated cell samples to 1. Errors were determined by calculating the standard deviation of triplicate data.

RT-qPCR for expression analysis. Quantitative real-time PCR was performed using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) according to the package insert. Samples were run on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad) and analyzed using the Quantitative-Comparative (C_T) method. The levels of *MBNL1*, *MBNL2*, *ACTA1* and *DMPK* mRNA in DM1 myotubes were normalized to *GAPDH* mRNA and displayed graphically as relative mRNA levels setting the untreated mRNA levels to 1. Errors were determined by calculating the standard deviation of quadruplicate data. The levels of human skeletal actin (*HSA*) mRNA and *Dmpk* mRNA in HSA^{LR} mice were normalized to *Gtf2b* mRNA and displayed graphically as relative mRNA levels setting the untreated mRNA levels to 1. Two different primer sets were used to assess *HSA* mRNA levels in HSA^{LR} mice and the values were averaged. All primer sets used are shown in Table S2.

Western blot analysis. Approximately 2 x 10^5 myoblasts were plated in 6-well plates and treated as described above for RT-PCR splicing analysis. Protein was harvested by vortexing for 15 min at 4°C in RIPA buffer supplemented with 1 mM PMSF and 1X SigmaFast protease inhibitor (Sigma Aldrich). After centrifugation at 12,000 rpm for 15 min at 4°C, the supernatant was used to determine protein concentration with the Pierce BCA Protein Assay kit (Thermo). A total of 10 μg of protein was denatured for 5 min at 98°C and run on a pre-cast 10% SDS-PAGE mini gel (Bio-Rad) at 200 V for 40 min in 1X running buffer (25 mM Tris base pH 8.3, 192 mM glycine, 0.1% (w/v) SDS). Gel was transferred onto low fluorescence PVDF membrane (Bio-Rad) for 1 hr at 25 V in 1X transfer buffer (25 mM Tris base pH 8.3, 192 mM glycine, 20% (v/v) methanol). Membrane was blocked for 1 hr using SeaBlock (Thermo) and then incubated overnight with primary antibodies [1:2000 MBNL1 (MB1a, Wolfson Centre for Inherited Neuromuscular Disease), 1:500 MBNL2 (3B4, Santa Cruz), 1:1000 GAPDH (14C10, Cell Signaling)]. Blots were incubated at RT for 1 hr with secondary antibodies [1:7500 Goat anti-Rabbit IRDye @680 (Li-Cor), 1:7500 Goat anti-Mouse IRDye @800 (Li-Cor)], washed in TBS-T, and imaged on an Odyssey CLx imager (Li-Cor). Blots were analyzed using ImageStudio

Lite (Li-Cor). The relative levels of MBNL were calculated by first normalizing lanes within the same gel using the GAPDH signal and then by normalizing levels of MBNL in the untreated cells to 1. Errors were determined by calculating the standard deviation of triplicate data.

Fluorescent *in situ* hybridization microscopy. Approximately 5 x 10^4 myoblasts were plated in 8-chamber slides and treated as described for the 4-day furamidine treatments for RT-PCR splicing analysis. Myotubes were fixed with 4% (w/v) PFA, permeabilized using 70% (v/v) ethanol, and pre-hybridized for 30 min at 37ºC. Myotubes were probed for 4 hrs at 50 °C with a Cy3-$(CAG)_8$ probe (IDT). Slides were washed with 42 ºC pre-warmed 40% (v/v) formamide in 2X SSC and mounted using ProLong Diamond Antifade mountant with DAPI (Life Technologies). Myotubes were imaged on a Zeiss LSM 840 confocal scanning microscope with a 40X water objective. Nuclear foci for each cell were quantified using Fiji. At least 100 myotube nuclei were scored blind for all treatments per experiment. The relative foci per nucleus counts were calculated by normalizing the foci per nucleus count of untreated myotubes to 1. Errors were determined by calculating the standard deviation of triplicate data.

Northern blot analysis. DM1 myotubes were drug-treated and harvested as described for RT-PCR splicing analysis. A total of 1 mg of cellular RNA was denatured at 50ºC for 30 min with Glyoxyl Loading Dye (Ambion) and loaded onto a 1% (w/v) agarose gel with 1X Gel prep/Running buffer from the NorthernMax-Gly kit (Ambion). Gel was run in 1X Gel prep/Running buffer at 95 V for 2.5 hrs and transferred overnight to Hybond-XL 0.45 μm nylon membrane (GE) using 20X SSC. The membrane was crosslinked using a UV Stratalinker 1800 (Stratagene) on the optimal crosslink setting and probed at 50°C overnight with radiolabeled oligos in ExpressHyb buffer (ClonTech). Membranes were washed with 0.1% (w/v) SDS in 2X SSC and then with 0.1% (w/v) SDS in 1X SSC and autoradiographed. Probes sequences shown in Table S3. Blots were quantified using ImageQuant (Molecular Dynamics). The relative levels of RNA were calculated by first normalizing lanes within the same gel using the GAPDH signal and by setting levels of CUG repeats in the untreated cells to 1. Errors were determined by calculating the standard deviation of quadruplicate data.

Isothermal calorimetry. ITC experiments were performed with a MicroCal iTC200 instrument (MalVern). In 300 sec intervals, 2 μL of 1.5 mM furamidine in 10 mM cacodylic acid buffer (pH 6.5), 25 mM NaCl and 1 mM EDTA was injected into the instrument sample cell containing degassed 25 μM DNA or RNA duplex (DNA: 5'CTGCTGCAGCAG or 5'CGAAAATTTTCG; RNA: 5'CUGCUGCUGCUG) in the same buffer for a total of 29 injections at 25 °C. Data analysis was performed with the Origin 5.0 package (OriginLab). The observed heat for each injection was measured by integration of the area of power peak with respect to time. Data corresponding to the first injection were discarded. Blank titrations were conducted by injecting the compound into the sample cell containing only buffer under the same conditions. The corrected interaction heat was determined by subtracting the blank heat from that for the compound/DNA titration. Errors were determined by calculating the standard deviation of triplicate data.

Gel shift mobility assay. Before mixing with MBNL1 protein, CUG_8 construct was snap annealed in 20 mM Tris, pH 7.5 at 95°C for 2 min and then put on ice for 5 min. CUG_8 RNA and indicated concentrations of furamidine were then added to MBNL1 protein at indicated concentration. The final reaction conditions were 115 mM NaCl, 20 mM Tris (pH 7.5), 5 mM $MgCl_2$, 10% (v/v) glycerol, 2 mg/mL BSA, 0.1 mg/mL heparin, 1 mM ß-ME, 10 μM EDTA, 0.02% (w/v) xylene cyanol. The binding reaction was incubated for 30 min at room temperature before loaded on a pre-chilled, 6% native acrylamide (37.5:1) with 0.5X Tris-Borate gel that was run at 150 V for 45 min at 4^{o}C. Gels were dried and autoradiographed. Bands were quantified using ImageQuant (Molecular Dynamics). The percent of CUG_8 RNA bound to MBNL1 in complex ($\%CUG_{bound}$) was calculated by quantifying both the free CUG_8 RNA band (UB) and the CUG_8 RNA-MBNL1 complex band (B) within the same lane on the gel and then using the following Eq. **2**:

$$\%CUG_{bound} = [B / (B+UB)] * 100 \quad \textbf{[2]}$$

Errors were determined by calculating the standard deviation of triplicate data. To determine the IC_{50}, Eq. **3** was used, where m_0 = small molecule concentration, $m_1 = IC_{50}$, m_2 = Hill coefficient, and m_3 = fraction MBNL1 bound without furamidine present.

$$Y = m_3 / [1 + (m_0/m_1)^{m2}] \quad \textbf{[3]}$$

Furamidine and heptamidine treatment of mice. Mouse handling and experimental procedures were conducted in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care. Age and sibling matched HSA^{LR} mice were treated with either 5% glucose in PBS, 30 mg kg^{-1} heptamidine, or 30 mg kg^{-1} furamidine as previously described[16, 31].

RNA-seq Library Preparation. RNA was TRIZol extracted from quadriceps muscle of HSA^{LR} mice treated with either 5% (w/v) glucose in PBS, 30 mg kg^{-1} heptamidine, or 30 mg kg^{-1} furamidine. The KAPA Stranded RNA-Seq with RiboErase kit was used to prepare RNA-seq libraries, with a total of 400 ng input RNA from each sample. The manufacturer's protocols were followed, with the following exceptions: RNA was fragmented at 85°C for 3 min, BIOO NEXTflex Rapid Directional qRNA-Seq primers were used to add UMIs, ten cycles of library amplification were performed. The resulting libraries were pooled in equimolar amounts and were sequenced using paired-end, 75 base pair sequencing on the Illumina NextSeq 500 massively parallel sequencer at the University of Oregon Genomics and Cell Characterization Core Facility.

For wild type mouse samples, RNA was TRIZol extracted from quadriceps muscle of FVB mice. RNA quality was checked via capillary electrophoresis on Fragment Analyzer using the RNA Analysis DNF-471 kit (Advanced Analytical). The NEBNext Ultra II Directional RNA Library Prep Kit for Illumina with NEBNext rRNA Depletion Kit was used to prepare RNA-seq libraries, with a total of 500 ng input RNA from each sample. The manufacturer's protocols were followed, with the following exceptions: 40X adaptor dilutions used, all bead incubations done at room temp, used 4X lower concentrations of index primers, and ten cycles of library amplification were performed. The resulting libraries were pooled in equimolar amounts, quantified using the KAPA Library Quant Kit for Illumina, quality checked via capillary electrophoresis on Fragment Analyzer using the NGS Analysis DNF-474 kit (Advanced Analytical), and were sequenced using paired-end, 75 base pair sequencing on the Illumina NextSeq 500 massively parallel sequencer at the University of Florida Center for NeuroGenetics.

Transcriptome analysis from RNA-seq data. Raw reads were checked for quality and

aligned to GRCm38.p5 mouse genome using STAR (version 2.5.1b)[53] and .gtf file generated from Version M16 GENECODE gene models. Uniquely aligning paired sequences were input to Stringtie (version 1.3.4d) and the prepDE.py script (offered with Stringtie package) was used to generate genecounts. Differential expression analysis was performed with DESeq2 (version 1.16.1)[54]. Differential expression was considered significant with $p<0.1$. Of those events, a percent rescue of ≥10% were considered 'Rescues' with furamidine or heptamidine treatment, 'Over-rescues' ≥110%, 'Mis-rescues' ≤ –10%, and 'Off-target' gene expression events were those not in the WT vs HSA^{LR} events. To determine the rescue of a given differentially expressed gene, Eq. **4** was used, where WT_EXP = difference in expression of WT mice versus untreated HSA^{LR} mice, drug_EXP = difference in expression of untreated HSA^{LR} mice versus HSA^{LR} mice treated with furamidine or heptamidine.

$$\% \text{ rescue} = [100 - (WT\_EXP + drug\_EXP) / (WT\_EXP)] * 100 \qquad \textbf{[4]}$$

Splicing analysis from RNA-seq data. UMIs were removed from raw reads using a custom script and adapter sequences were removed using Cutadapt (version 1.10)[55], reads were quality trimmed to 66 bases using Trimmomatic (version 0.36)[56] and reads shorter than 66 bases were removed from the dataset. PCR duplicates were removed using a custom script. After reads were aligned to the mm10 genome, rMATS (version 3.2.5)[57] was used to analyze isoform abundances and compared to three-wild type samples[58]. Wild type (WT) mouse data were obtained from the Sequence Read Archive (SRR5242724, SRR5242725, and SRR5242726). ES events were considered significant with an FDR<0.01 and $p<0.01$. Event were considered mis-spliced in the WT vs HSA^{LR} data sets if the PSI change was ≥10% for a given ES event. Of those events, a percent rescue of ≥10% were considered 'Rescues' with furamidine or heptamidine treatment, 'Over-rescues' ≥110%, 'Mis-rescues' ≤ –10%, and 'Off-target' ES events were those not in the WT vs HSA^{LR} events that had a change in PSI ≥10%. To determine the rescue of a given ES event, Eq. **5** or **6** was used, where HSA_PSI = PSI of untreated HSA^{LR} mice, wt_PSI = PSI of wild type mice, and drug_PSI = PSI of HSA^{LR} mice treated with furamidine or heptamidine.

$$\text{For HSA\_PSI} > \text{wt\_PSI:}$$

$$\text{rescue} = (HSA\_PSI - drug\_PSI) / (HSA\_PSI - wt\_PSI) \qquad \textbf{[5]}$$

For wt_PSI > HSA_PSI:

$$\text{rescue} = (\text{drug\_PSI} - \text{HSA\_PSI}) / (\text{wt\_PSI} - \text{HSA\_PSI}) \quad \textbf{[6]}$$

Table S1. Primers used for RT-PCR splicing analysis.

Target	Forward Primer	Reverse Primer
Human MBNL1 exon5	5'- AGGGAGATGCTCTCGGGAAAAGTG	5'- GTTGGCTAGAGCCTGTTGGTATTGG
Human MBNL2 exon5	5'- ACAAGTGACAACACCGTAACCG	5'- TTTGGTAAAGGATGAAGAGCACC
Human NUMA1 exon2	5'- AAGTATGAGGGTGCCAAGGT	5'- CTTCAGCTTCTGCTGCTGCA
Human SYNE1 exon137	5'- GACAAAGATTTCTACCTCCGGGG	5'- CCCAGTTGTCGGATCTGTGACTC
Mouse Atp2a1 exon22	5'- GCTCATGGTCCTCAAGATCTCAC	5'- GGGTCAGTGCCTCAGCTTTG
Mouse Clcn1 exon7a	5'- TGAAGGAATACCTCACACTCAAGG	5'- CACGGAACACAAAGGCACTG
Human CAMKK2 exon15	5'- CCTGGTGAAGACCATGATACG	5'- GGCCCAGCAACTTTCCAC
Human DLG1 exon14	5'- AGCCCGATTAAAAACAGTGAAA	5'- CGTATTCTTCTTGACCACGGTA
Human TTC8 exon3	5'- AGCTATTTTAGGCGCAGGAAGT	5'- CATTTTCATCCAGCATCATTTCTG
Human VTI1 exon5	5'- GATCGCCTACAGTGACGAAGTA	5'- TCCACTGCTATTTGGTATCCAG

Table S2. Primers used for RT-qPCR expression analysis.

Target	Forward Primer	Reverse Primer
Human ACTA1 set 1	5'- GAGGCTCAGAGCAAGAGAG	5'- TCGTTGTAGAAGGTGTGGTG
Human ACTA1 set 2	5'- GGAGCGCAAATACTCGGTG	5'- CATTTGCGGTGGACGATGG
Mouse Gtf2b	5'- CTTCATGTCCAGGTTCTGCTCC	5'- GGAACCAAGTCCAGCTCCAC
Human DMPK	5'- CACGTTTTGGATGCACTGAGAC	5'- GATGGAGGGCCTTTTATTCGCG
Human MBNL1	5'- CGCAGTTGGAGATAAATGGACG	5'- CACCAGGCATCATGGCATTG
Human MBNL2	5'- CCTGGTGCTCTTCATCCTTTAC	5'- GTGAGAGCCTGCTGGTAGTG
Human GAPDH	5'- AATCCCATCACCATCTTCCA	5'- TGGACTCCACGACGTACTCA

Table S3. Probes used for northern blot analysis.

Target	Probe sequence
CUG repeat RNA	5′- GCAGCAGCAGCAGCAGCAGCAG
GAPDH mRNA	5′- GTCCACCACCCTGTTGCTGTAGCCAAATTC