PNAS
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# Supplementary Information for

**Increased Muscleblind levels by chloroquine treatment improve myotonic dystrophy type 1 phenotypes in *in vitro* and *in vivo* models.**

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References for SI reference citations



www.pnas.org/cgi/doi/10.1073/pnas.1820297116

**Supplementary Information Text**

## Materials and methods.

### Fly strains and crosses

*w*$^{1118}$ line was obtained from the Bloomington Drosophila Stock Center (Indiana University, Bloomington, IN, USA). *Mhc-Gal4* flies were described in (1). *Mhc-Gal4 UAS-(CTG)480* flies were generated in (2). All crosses were carried out at 25 °C with standard fly food. For oral administration of chloroquine (Chloroquine diphosphate salt solid, ≥98%, C6628 Sigma Aldrich**)**, a maximum of 25 one-day adult flies were collected in tubes containing standard food supplemented with chloroquine (10 or 100 μM). Flies were transferred to tubes containing fresh food every 2-3 days for a total administration time of 7 days.

### Determination of caspase-3 and caspase-7 activity

Ten adult female flies of the indicated genotypes were homogenized in 100 μl of cold PBS buffer using TissueLyser LT (Qiagen, Hilden, Germany). After a 10 min centrifugation, the supernatant was transferred into a white 96-well plate. Caspase-3 and caspase-7 activity was measured using the Caspase-Glo 3/7 Assay Systems (Promega, Fitchburg, WI, USA). Briefly, 100 μl of Caspase-Glo 3/7 reagent was added per well and the plate was incubated at room temperature for 30 min. Then luminescence was measured using an Infinite 200 PRO plate reader (Tecan Life Sciences; Männedorf, Switzerland). Luminescence readings were normalized to total protein using the BCA Protein Assay Reagent Kit (Pierce, Rockford, IL, USA). All graphs show the mean of three biological samples with three technical replicates of each. P-values were obtained using a two-tailed, non-paired Student's *t*-test ($\alpha$=0.05).

### LysoTracker staining

*Drosophila* somatic muscles were processed to stain lysosomes as previously described (3)**.** *Drosophila* muscles were incubated for 30 min at 37ºC with 100 nM of Lysotracker RED-DND99 (Thermo Fisher Scientific, Bremen, Germany) and 5 µg/ml Hoechst 33258 (Sigma-Aldrich; St. Louis, MO USA) in PBS 1X. After three rinses with PBS 1X, *Drosophila* muscles were mounted using fluorescence mounting medium (Dako, Glostrup, Denmark)

**Differential scanning fluorimetry (DSF)**

DSF experiments were performed as described in (4)**.** Six CQ concentrations ranging from 0.01 to 100 µM were used.

**RNA extraction, semiquantitative PCR and real time PCR.**

For RNA extraction from fly and murine samples, tissues were homogenized using TissueLyser LT (Qiagen, Hilden, Germany) in TriReagent (Sigma-Aldrich; St. Louis, MO, USA). Total RNA from human myoblasts was isolated using TriReagent following manufacturer's recommendations. One microgram of RNA was digested with DNaseI (Invitrogen) and reverse-transcribed with SuperScript II Reverse Transcriptase (Invitrogen, Life Technologies, Grand Island, NY, USA) using random hexanucleotides (Roche Life Sciences; Indianapolis, IN, USA). Alternative splicing was analyzed using 20 ng of cDNA in a standard PCR reaction with GoTaq polymerase (Promega, Inc.; Madison, WI, USA) and specific primers (*SI Appendix,* Table. S1). *Rp49, GAPDH,* and *Gapdh* were used as endogenous controls in fly, human and mouse samples, respectively. PCR products were separated in a 2.5% agarose gel and quantified using ImageJ software (NIH). *Serca* exon 13, *LIMCH1* exon 10 and *Limch1* exon 9 expression were analyzed by RT-qPCR as follows. RT-qPCR was performed using 0.2 ng of cDNA as template with 5x HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX) (Solis BioDyne, Tartu, Estonia) using a Step One Plus Real Time PCR System (Applied Biosystems; Foster City CA, USA). As endogenous control we used *Rp49, GAPDH,* and *Gapdh* that were detected using 0.2 ng of cDNA in both, quantitative and semi-quantitative PCR. We used 1 ng of cDNA from human

myoblast or mouse tissue as a template for multiplex RT-qPCR using the QuantiFast Probe PCR Kit reagent. Commercial TaqMan probes (Qiagen) were used to detect human (MBNL1 and MBNL2) or mouse (Mbnl1 and Mbnl2; FAM-labeled probes) and reference (GAPDH; MAXlabeled probe) genes. Results from myoblasts were normalized to *GAPDH* (TAMRA-labeled probe; Integrated DNA Technologies) whereas the mouse results were normalized to Gapdh. HSA transgene expression levels were determined by RT-qPCR as described previously (5). Three biological replicates and three technical replicates per biological sample were performed. Relative expression to endogenous gene and the control group were obtained by the $2^{-\Delta\Delta Ct}$ method. Pairs of samples were compared using two-tailed Student's *t*-test ($\alpha<0.05$), applying Welch's correction when necessary.

**RNAseq and data analysis**

Libraries were prepared using TruSeq Stranded mRNA Library prep kit (Illumina) following manufacturers indications. Libraries were sequenced using paired-end, 75 base pair sequencing with the Illumina NextSeq 550 sequencer in the SCSIE-UCIM from the University of Valencia. For gene expression analysis, raw reads were checked for quality and aligned to GRCh38.p12 genome using STAR software (version 2.6.1a_08-27)(6). Uniquely aligning paired sequences were used as input to the program featureCounts part of the package SubRead (v1.6.3) (7) using GRCh38.95.gtf as annotation file to generate the gene counts. Differential Gene Expression (DGE) were computed using the R Bioconductor package edgeR (version 3.24.3)(8). To consider a gene to be DGE we used a fold change (FC) >= 2 and adjusted p-value<0.05. Percentage of recovery of each gene was used using the following formula:

$$\%\, recovery = \frac{Mean\_treated\_genecount - Mean\_disease\_gene\_count}{Mean\_contol\_gene\_count - Mean\_disease\_gene\_count} X100$$

To analyze transcripts expression raw reads were checked for quality and aligned to GRCh38.p12 using STAR. Uniquely aligning paired sequences were used as input to the program Rsem (v1.3.1)(9) using GRCh38.95.gtf as annotation file to generate the transcripts counts. DGE were computed using the R Bioconductor package edgeR. To

consider a transcript to be DGE we used a fold change (FC) >= 2 and adjusted p-value < 0.05. We used the same formula as before to calculate the percentage of recovery. Gene Ontology analyses were performed using clusterProfiler (10) considering all recovered genes in each treatment with a percentage of correction ranging between 10 and 110. The raw data and the raw count tables are available in GEO repository under the id GSE128844

**Western blotting**

For total protein extraction from *Drosophila*, 20 female thoraces were homogenized in 50 mM Tris-Cl, pH 8 plus protease and phosphatase inhibitor cocktails (Roche Applied Science). For total protein extraction from human myoblasts, cells were sonicated in RIPA buffer while mouse muscles were homogenized in RIPA buffer using TissueLyser LT (Qiagen). Total protein was quantified with BCA protein assay kit (Pierce) using bovine serum albumin as standard. 20 µg of samples were denatured for 5 min at 100°C, resolved in 12% SDS-PAGE gels and transferred onto nitrocellulose 0.45 µm (GE Healthcare) membranes. The membranes were blocked with 5% non-fat dried milk in PBS-T (PBS 1X containing 0.05% Tween 20) and immunodetected following standard procedures. For Mbl protein detection on fly samples, anti-Mbl antibody (11) was pre-absorbed against 0–6 h after egg laying embryos to eliminate non-specific binding of antibody. Membranes were incubated with pre-absorbed primary (overnight, 1:1000). Loading control was mouse anti-α-Tubulin (overnight, 1:5000, Sigma-Aldrich). For human myoblasts and mouse muscle samples, membranes were incubated overnight at 4°C with primary mouse anti-MBNL1 antibody (1:1000, ab77017, Abcam in the case of mouse muscle samples and 1:200, clone MB1a, The Wolfson Centre for Inherited Neuromuscular Disease in the case of cell samples), mouse anti-CUG-BP1 (1:200, clone 3B1, Santa Cruz) antibodies or mouse anti-MBNL2 (1:200, clone MB2a, The Wolfson Centre for Inherited Neuromuscular Disease). Loading controls were the anti-β-ACTIN antibody (1 h, 1:5000, clone AC-15, Sigma-Aldrich) for human myoblast samples and mouse anti-Gapdh (1 h, 1:5000, clone G-9, Santa Cruz) for mouse samples. In all cases,

primary antibodies were detected by horseradish peroxidase (HRP)-conjugated sheep anti-mouse-IgG secondary antibody (1 h, 1:5000, Sigma-Aldrich) except in the case of α-Tubulin, that it was used at 1:3000. Immunoreactive bands were detected using enhanced chemiluminescence. Western Blotting Substrate (Pierce) and images were acquired with an ImageQuant LAS 4000 (GE Healthcare). Quantification was performed using ImageJ software (NIH), and statistical differences were estimated using Student's *t*-test ($p<0.05$) on normalized data.

**Non-fluorescent histological analysis**

Analysis of the indirect flight muscle area in *Drosophila* thoraces was performed as previously described (2). Briefly, six thoraces of three-day-old females were embedded in Epon following standard procedures. After drying the resin, semi-thin sections of 1.5 μm were obtained using an ultramicrotome (Ultracut E, Reichert-Jung and Leica). Images were taken at 100x magnification with a Leica DM2500 microscope (Leica Microsystems, Wetzlar, Germany). P-values were obtained using a two-tailed, non-paired t-test ($\alpha=0.05$), applying Welch's correction when necessary.

For central nuclei quantification of mouse muscles, gastrocnemius and quadriceps were frozen in isopentane and 15 μm-sections were obtained with a Leica CM 1510S cryostat. Sections were stained with hematoxylin-eosin (H&E) and mounted with DPX (Sigma-Aldrich) according to standard procedures. Images were taken at a 100× magnification with a Leica DM2500 microscope. The percentage of central nuclei was quantified in a total of 500 fibers in each mouse.

***Drosophila* functional assays.**

Flight assays were performed at day 5 according to Babcock. et al. 2014 using 100 male flies per group (12). Landing distance was compared between groups using two-tailed t-test ($\alpha=0.05$). To assess climbing velocity groups of ten five-day-old males were transferred into disposable pipettes (1.5 cm in diameter and 25 cm height) after a period of 24 h without anesthesia. The height reached from the bottom of the vial by each fly in a period of 10 s was recorded with a camera. For each genotype, two

groups of 30 flies were tested. Two-tailed Student t-test (α=0.05) was used for comparisons of pairs of samples applying Welch's correction whenever necessary.

### *Drosophila* lifespan analyses

50-80 newly emerged flies were collected in freshly prepared tubes containing standard nutritive medium with or without chloroquine. Males and females were kept in different tubes at 25°C. The number of dead flies was scored daily. Flies were transferred to new tubes twice a week. Survival curves were obtained using the Kaplan–Meier method and were statistically compared according to the Gehan-Breslow-Wilcoxon test (α=0.05).

### Cell culture conditions

A cell model of the disease (kindly provided by Dr. Denis Furling, Institute of Myologie, Paris (13)) consisted of normal (iCDF) and DM1 (iPDF; 1300 CTG repeats) immortalized (hTERT) skin fibroblasts conditionally expressing MyoD. Fibroblast Cells were grown in Dulbecco's modified Eagle Medium (DMEM, Life Technologies, Mulgrave, Victoria, Australia) supplemented with 4.5 g/L of glucose, 1 % of penicillin and streptomycin (P/S) and 10% foetal bovine serum (FBS) (Sigma-Aldrich; St. Louis, MO). Fibroblasts were transdifferentiated into myoblasts by inducing expression of MyoD. Cells were plated in muscle differentiation medium (MDM) made of DMEM 4.5 g/L glucose with 1% P/S, 2% horse serum, 1% apo-transferrin (10 mg/ml), 0.1 % insulin (10 mg/ml) and 0.02 % doxycycline (10 mg/ml). In all cases, the cells were grown at 37 °C in a humidified atmosphere containing 5% CO2. Fibroblasts were in MDM for 96 h. Chloroquine was added to MDM to final concentrations of 0.1 μM, 1 μM and 10 μM for 48 h. Similarly, 30 mM mtf was added for 48 h. It was demonstrated in human cell lines that autophagic flux was not blocked by CQ at acidic pH (14). Then, we measured the pH of the cell culture medium before adding CQ, 24 and 48 h after addition, and observed values ranging from 7.8 to 8, confirming that CQ was indeed inhibiting autophagy.

**Human fibroblasts isolation and culture**

Four different lines from primary human fibroblast were isolated from donors skin and transduced to express MyoD as previously described (15). Fibroblasts from DM1 patients expressing 1000 and 333 CUG repeats were from male donors (33 and 46 years, respectively). Control fibroblasts were obtained from healthy female controls (48 years and unknown). All control and patient materials were obtained with the written informed consent of the donor. Ethical approval for this study was obtained from the ethical review committee at the Hospital Universitario Donostia.

**Double MBNL staining and FISH**

Immortalized fibroblasts were seeded into 24-well plates ($4.0\times10^4$ cells per well). Fibroblasts were differentiated for 96 h. CQ was added to MDM to final concentrations of 0.1 μM and 10 μM for 48 h. Then, cells were fixed in 4% PFA for 15 min at room temperature followed by washes in 1x PBS. Double MBNL and foci detection were performed as previously described (16). Analysis of MBNL and (CAG)7 colocalization in ribonuclear foci was calculated using ZEN 2.6 software analyzing 50-60 nuclei.

**Foci detection**

Immortalized fibroblasts were seeded into 96-well plates ($1.0\times10^4$ cells per well). After chloroquine treatment, cells were fixed in 4% PFA for 15 min at room temperature followed by washes in 1x PBS. Fixed cells were washed with 2x SSC, 30% deionized formamide for 10 min at room temperature and were hybridized with Cy3-(CAG)7-Cy3 labelled probe diluted 1:500 in hybridization buffer (40% formamide, 2x SSC, 0.2% BSA, 10% dextran sulfate, 2 mM ribonucleoside-vanadyl complex, 10% tRNA [10 mg/ml], and 10% herring sperm) for 2 h at 37ºC. After hybridization, cells were washed twice with 2x SSC, 30% deionized formamide for 15 min at 45ºC, rinsed with 1x PBS, incubated with Hoechst 33342 (5 mg/ml) diluted 1:20.000 in 1x PBS for 20 min at room temperature, and mounted with 20% Mowiol. Images were taken and analyzed using an IN Cell Analyzer 2200 Imaging System.

**Immunofluorescent methods**

Immunofluorescence detection of Muscleblind in fly thorax muscles was performed as previously described (2). Briefly, fly thoraces from 5-day-old females were dissected and fixed in 4% paraformaldehyde (PFA) overnight at 4 °C, followed by cryoprotection with 30% sucrose for 48 h at 4°C. Thoraces were then embedded in OCT, and transversal sections (10 μm) were obtained with a Leica CM 1510S cryostat. Cryosections were washed in PBS containing Triton 0.3% (PBS-T), blocked (PBT containing 5% donkey serum and 0.5% BSA) for 30 min at room temperature and incubated with the corresponding antibody (sheep anti-Mbl 1:500) overnight at 4°C. After washes with PBS-T, the tissue was incubated 45 min with biotin-conjugated secondary antibodies (Sigma) at 1:200 dilution. Cryosections were then incubated with Elite ABC kit standard (Vectastain) for 30 min at RT, followed by washes and incubation with streptavidin-FITC (1:1000) (Vector, London, UK) for 45 min. Finally, samples were mounted in Vectashield (Vector, London, UK) with 2 μg/ml DAPI. Anti-Mbl antibody was preincubated with 0 to 6 h-old embryos, which do not express the protein.

MBNL1 and MBNL2 were detected in cells differentiated in MDM for 96 h ($3x10^5$ cells/well in 24 well plates). After 48 h CQ treatment, cells were fixed with 4% PFA in PBS for 15 min at RT. In the case of double staining to detect LysoTracker and MBNL1 or 2, previously to fixation, cells were incubated 30 min at 37°C with 100 nM Lysotracker RED-DND99 (Thermo Fisher Scientific, Bremen, Germany). In all cases, fixed cells were permeabilized with PBS-T and blocked with Blocking Buffer (PBS-T, 0.5% BSA, 1% Goat Serum) 1 h at RT. Cells were incubated overnight at 4ºC with primary mouse anti-MBNL1 (1:200 clone MB1a, The Wolfson Centre for Inherited Neuromuscular Disease), rabbit anti-MBNL2 (1:200, ab105331, Abcam) for single immunofluorescence or mouse anti-MBNL2 (1:200, clone MB2a, The Wolfson Centre for Inherited Neuromuscular Disease) for double staining (LysoTracker plus MBNL1 or -2). Then, cells were incubated for 1 h at RT with a secondary biotin-conjugated anti-mouse-IgG to detect MBNL1 or MBNL2 (1:200, Sigma-Aldrich) in the case of double

immunofluorescence and MBNL1 single staining, or anti-rabbit-IgG (1:200, Sigma-Aldrich) for single MBNL2 staining. Fluorescence signal was amplified using ABC solution for 30 min at RT, followed by a FITC-Streptavidin (1:200, Vector, London, UK) to detect anti-MBNL1 and anti-MBNL2 (double staining with Lysotracker) or streptavidin-Texas Red (1:200, Vector) to detect anti-MBNL2 (single immunofluorescence) for 2 h at RT. Excess antibody was washed off with PBS-T. Finally, cells were washed three times with PBS and mounted in Vectashield (Vector Laboratories, London, UK) with 2 µg/ml DAPI. Images were taken on an LSM800 confocal microscope (Zeiss, Jena, Germany). LysoTracker and MBNL1 or MBNL2 colocalization were calculated using ZEN 2.6 software.

To carry out immunodetection of DESMIN, fibroblasts were seeded in 24-well plates with $2.5x10^4$ cells/well and were transdifferentiated for 7 days. After 48 h of chloroquine treatment, cells were fixed with 4% PFA for 15 min at RT and were processed using anti-DESMIN (1:50, Abcam; Cambridge, MA), biotin-conjugated anti-mouse-IgG (1:200, Sigma-Aldrich) and streptavidin-FITC (1:200, Vector) as primary and secondary antibodies, and fluorophore, respectively.

Images were taken with an LSM800 confocal microscope (Zeiss, Jena, Germany) at 200x magnification. The fusion index was defined as the percentage of nuclei within myotubes (>2 myonuclei) out of the total number of nuclei in each condition. The average number of nuclei per myotube was determined by counting over 250 nuclei from randomly chosen DESMIN-positive cells (5-7 micrographs). Myotube diameters were measured at 5 points along the entire tube. A total of 50 myotubes were examined for each experimental condition. Quantification was performed using ImageJ software (NIH).

For LC3B immunodetection cells were differentiated in MDM for 96 h ($3x10^5$ cells/well in 24 well plates). After 48 h CQ treatment, cells were fixed with 4% PFA in PBS for 15 min at RT. After fixation, cells were permeabilized with methanol 100% for 15 min at -20ºC and blocked with Blocking Buffer (PBS 1X, 0.5% BSA, 1% Goat Serum) for 1 h at RT. Cells were incubated O/N with rabbit anti-LC3B (1:200, ab51520,

Abcam) diluted in blocking buffer. Then, samples were incubated with Goat anti-Rabbit IgG (H+L) Alexa Fluor Plus 594 (1:200, Invitrogen) for 2 h. Between antibodies, cells were washed three times with PBS1X. Finally, cells were washed three times with PBS and mounted in Vectashield (Vector Laboratories, London, UK) with 2 µg/ml DAPI. Images were taken on an LSM800 confocal microscope (Zeiss, Jena, Germany) at 400x magnification.

**Toxicity assay**

Healthy iCDM were aliquoted in 96-well plate with $1.0x10^5$ cells per well. After 24 h, chloroquine or mtf were added to the MDM at concentrations ranging from 0.05 to 200 µM and from 0.05 µM to 200 mM, respectively. Cells were transdifferentiated into myoblasts for 4 days. To measure cell viability, 20 µl MTS/PMS tetrazolium salt was added to each well and cells were incubated for 2 h at 37°C in a humidified chamber with 5% $CO_2$. The conversion of MTS into soluble formazan (accomplished by dehydrogenase enzymes from metabolically active cells) was measured by absorbance at 490 nm (CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay, Promega, Inc.; Madison, WI). Absorbance was measured using an Infinite 200 PRO plate reader (Tecan Life Sciences; Männedorf, Switzerland). Data were transformed to percentage of survival relative to cells not exposed to the drug, which was considered 100% viability.

**Transgenic mice and chloroquine administration.**

Mouse handling and experimental procedures conformed to the European law regarding laboratory animal care and experimentation (2003/65/CE) and were approved by *Conselleria de Agricultura, Generalitat Valenciana* (reference number 2018/VSC/PEA/0182). Homozygous transgenic $HSA^{LR}$ (line 20 b) mice were provided by Prof. C. Thornton (17) (University of Rochester Medical Center, Rochester, New York, USA) and mice with the same genetic background (FVB) were used as controls. Experiments were performed in 4.5-month-old males. Animals received one intraperitoneal shot of 100 µl of compound every 24 h for seven

consecutive days. Experimental groups were treated with PBS (n=8 FVB, n=6 $HSA^{LR}$), 25 mg/kg/dose (n=3 $HSA^{LR}$) and 50 mg/kg/dose (n=5 $HSA^{LR}$). 7 days after the last injection animals were sacrificed and quadriceps and gastrocnemius were harvested. Each muscle was divided into three parts, two were snap frozen in liquid nitrogen for protein and RNA isolation and the third was frozen in isopentane for histological analyses.

**Electromyography studies.**

EMG was performed before starting the treatment and before sacrifice. Analysis was done under general anesthesia, as previously described (18). Briefly, five needle insertions were performed in each quadriceps muscle of both hind limbs, and myotonic discharges were graded on a five-point scale: 0, no myotonia; 1, occasional myotonic discharge in ≤50% of the needle insertions; 2, myotonic discharge in >50% of the insertions; 3, myotonic discharge in nearly all of the insertions; and 4, myotonic discharge in all insertions.

**Forelimb grip strength test.**

The forelimb grip strength was measured with a Grip Strength Meter (BIO-GS3; Bioseb, USA). The peak pull force (measured in grams) was recorded on a digital force transducer when the mouse grasped the bar. The gauge of force transducer was reset to 0 g after each measurement. Tension was recorded by the gauge at the time the mouse released its forepaws from the bar. We performed five consecutive measurements at 30 s intervals. Grip force values were normalized to the body weight of each mouse.

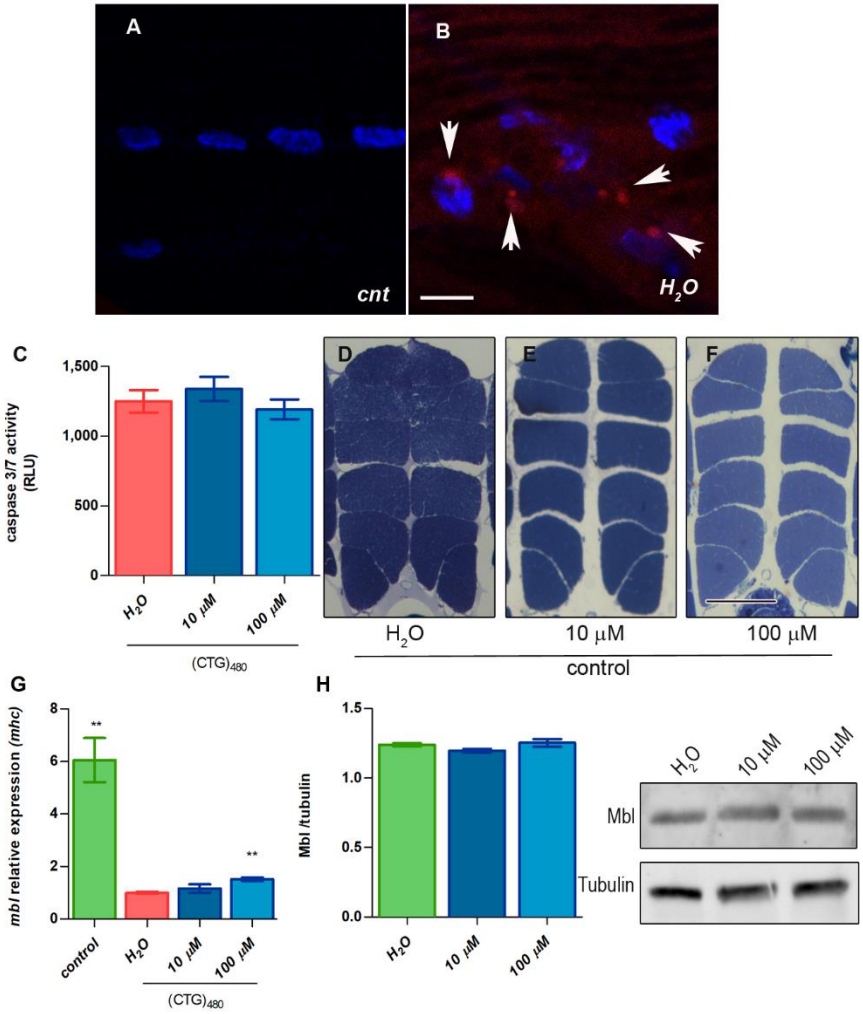


**Fig. S1. Effect of chloroquine treatment on DM1 and control flies.** (*A*,*B*) LysoTracker staining (red) of IFMs from control (cnt, *Mhc-Gal4/+)* (*A*); DM1 ($H_20$; *Mhc-Gal4 UAS-*i*(CTG)480*) (*B*). Arrows indicate LysoTracker signal. Scale bar=10 µm. Nuclei were counterstained with Hoechst 33342 (blue). (*C*) Quantification of caspase 3 and caspase 7 activity in DM1 model flies taking water (red bars) or chloroquine at the indicated concentrations (blue bars). (*D-F*) Representative dorsoventral sections of resin-embedded thoraces of control flies treated with the indicated concentrations of chloroquine. Scale bar=100 µm. (*G*) RT-qPCR amplification of *muscleblind* from control (*Mhc-Gal4/+*; green) and DM1 flies (*Mhc-Gal4 UAS-*i*(CTG)480; red*) treated with 10 or 100 µM (blue bars) of chloroquine after normalization to *mhc*. (*H*) Bar graph and representative images of Muscleblind protein

detection by western blot in healthy controls fed with 10 or 100 μM chloroquine. α-Tubulin protein expression was used as an endogenous control. Graphs show mean ± S.E.M **P<0.01 according to Student's *t*-test. All comparisons are referred to untreated DM1 flies ($H_2O$ in the graphs).

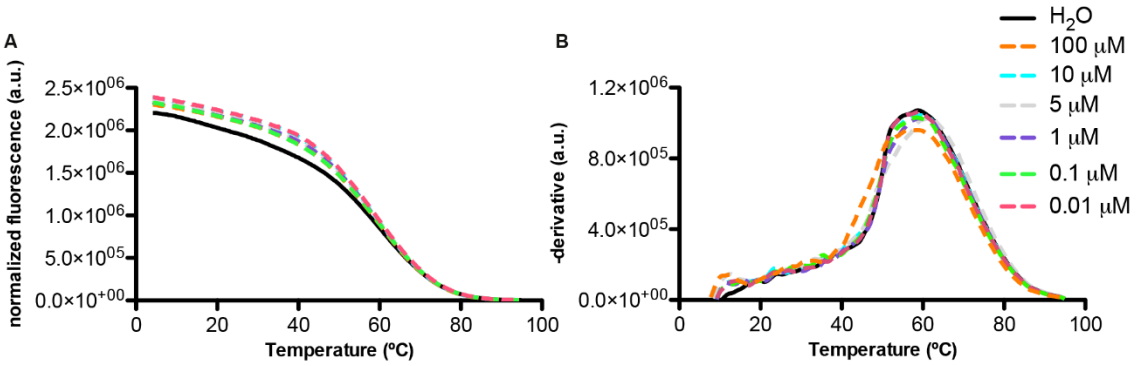


**Fig. S2. Chloroquine does not interact with CUG RNA.** Melting curve representation of Differential Scanning Fluorimetry assay showing fluorescence intensity (*A*) and first derivative (*B*) versus temperature (ºC) of a wide range of chloroquine concentrations (0.01-100 μM). A constant concentration of RNA (600 nM) was used for each condition (n=3) (a.u arbitrary units).

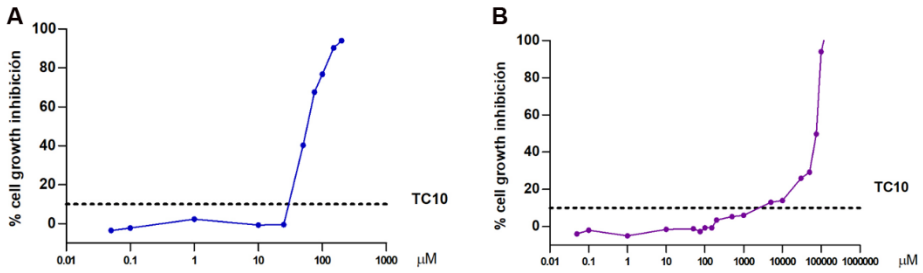


**Fig. S3. Cell growth inhibition assay by MTS method**. Human normal fibroblasts transdifferentiated for 96 h into myoblasts were treated with increasing concentrations of chloroquine (*A*) or mtf (*B*) ranging from 0.05 to 200 µM and 0.05 µM to 200 mM, respectively (n=4). TC10 (27.6 µM for chloroquine and 32 mM for mtf) was obtained using the least squares non-linear regression model.

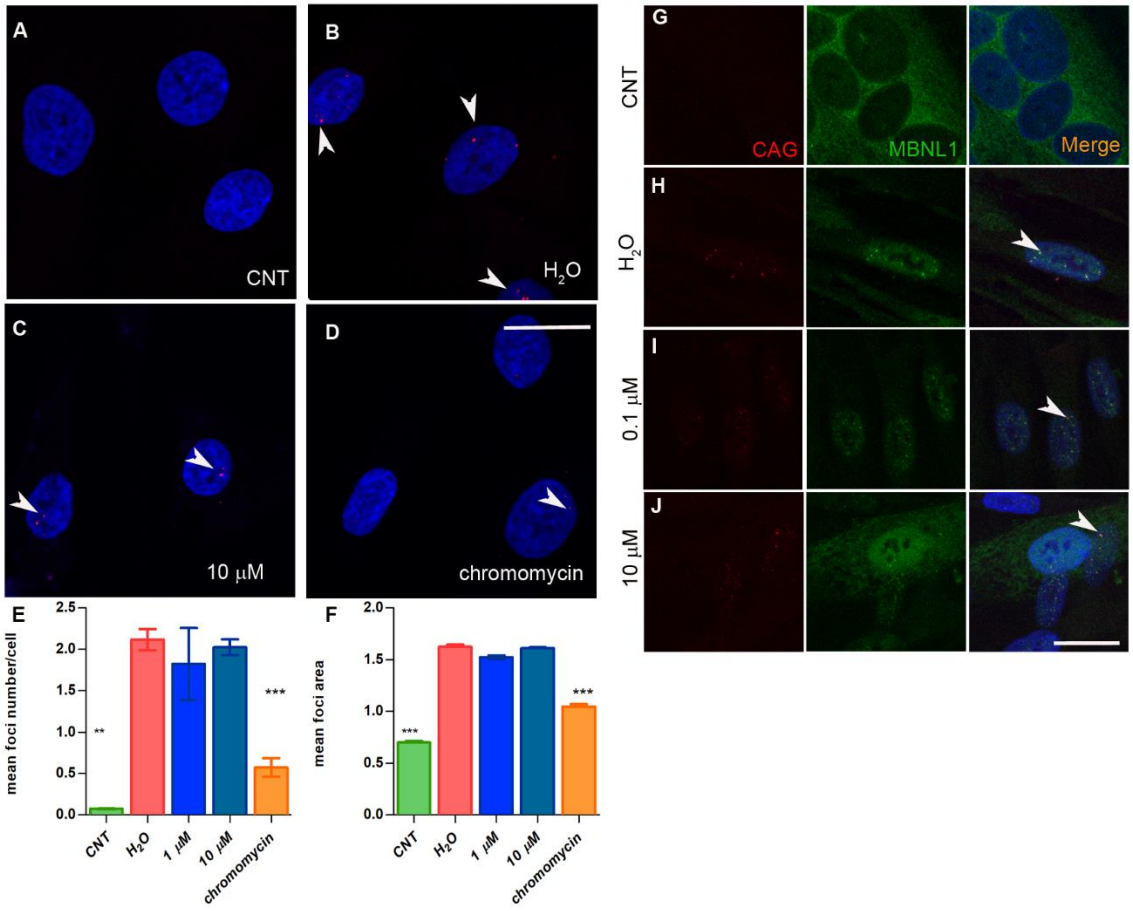


**Fig. S4. Analysis of CQ effect on foci formation.** (*A-D*) Representative images of fluorescence *in situ* hybridization to detect ribonuclear foci in iCDF (*A*), iPDF (*B*) and iPDF treated with CQ (*C*) or chromomycin (*D*) for 48 h. Nuclei were counterstained with DAPI (blue) and rCUG$^{exp}$ RNA foci were detected with a Cy3-labelled probe (red). Quantification of mean foci number per cell (*E*) and the mean foci area (*F*) from the analysis of a minimum of 1000 fibroblast nuclei. Chromomycin was used as a positive control (19). Three biological replicates were used for each condition. (*E* and *F*). (*G-J*) Representative confocal images of MBNL1 immunostaining (green) and *in situ* hybridization to detect ribonuclear foci (red) in iPDM. Nuclei were countestained with DAPI (blue). Arrows indicate MBNL1 and foci co-localization that is makedly green in untreated cells and acquires a redish colour with CQ, indicating lower MBNL1 levels in ribonuclear foci. Scale bars=20 μm.

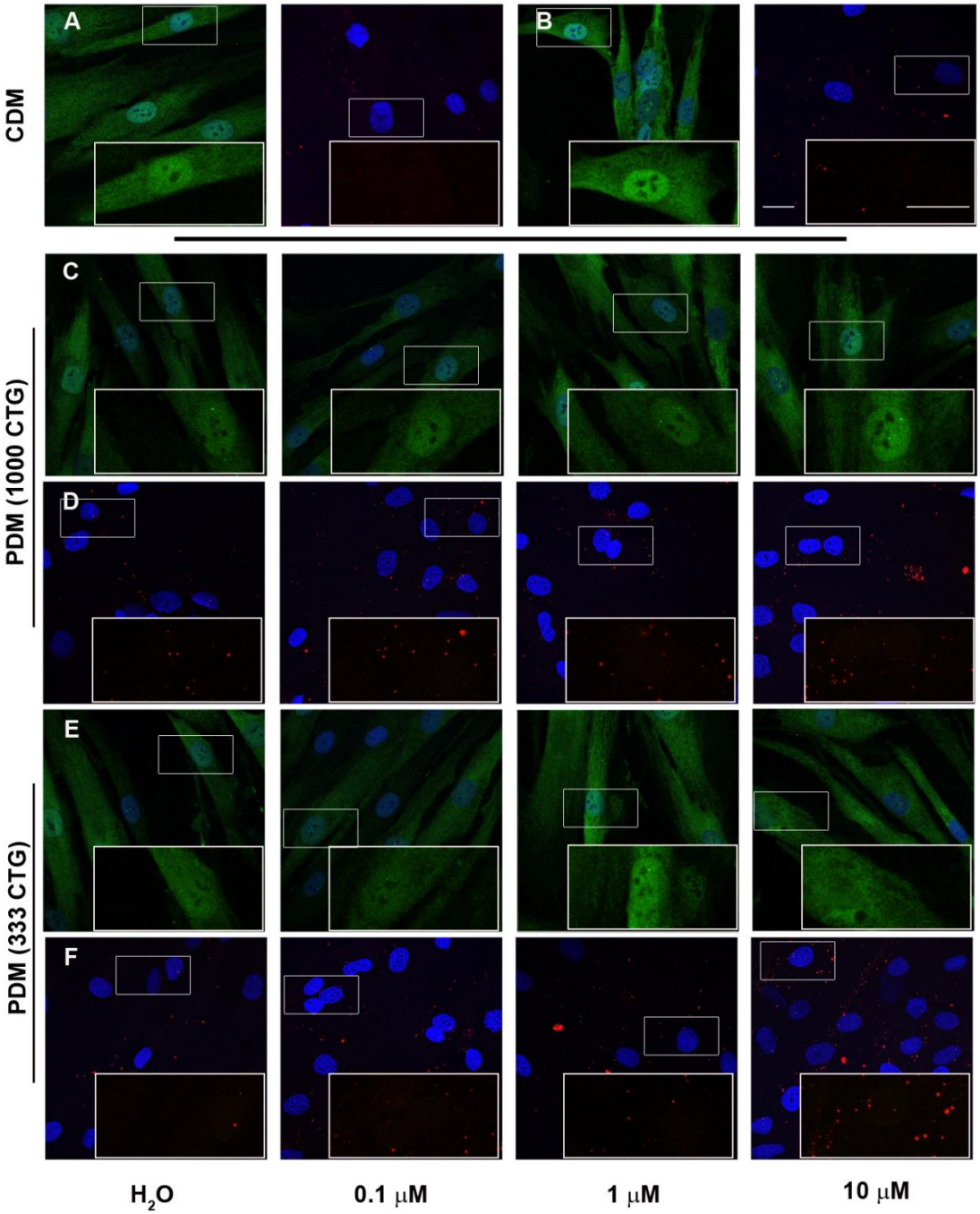


**Fig. S5. MBNL1 and MBNL2 are overexpressed upon chloroquine treatment of PDM.** Representative confocal images of MBNL1 (green) and MBNL2 (red) immunostaining in two different lines of healthy CDM (*A,B*), and two different lines of patient-derived myoblasts expressing 1000 (*C,D*) and 333 (*E,F*) CUG repeats,

respectively, treated with water as control, or with the indicated concentrations of chloroquine as indicated. Cells were differentiated for 96 h. Nuclei were counterstained with DAPI. Scale bars=20 μm.

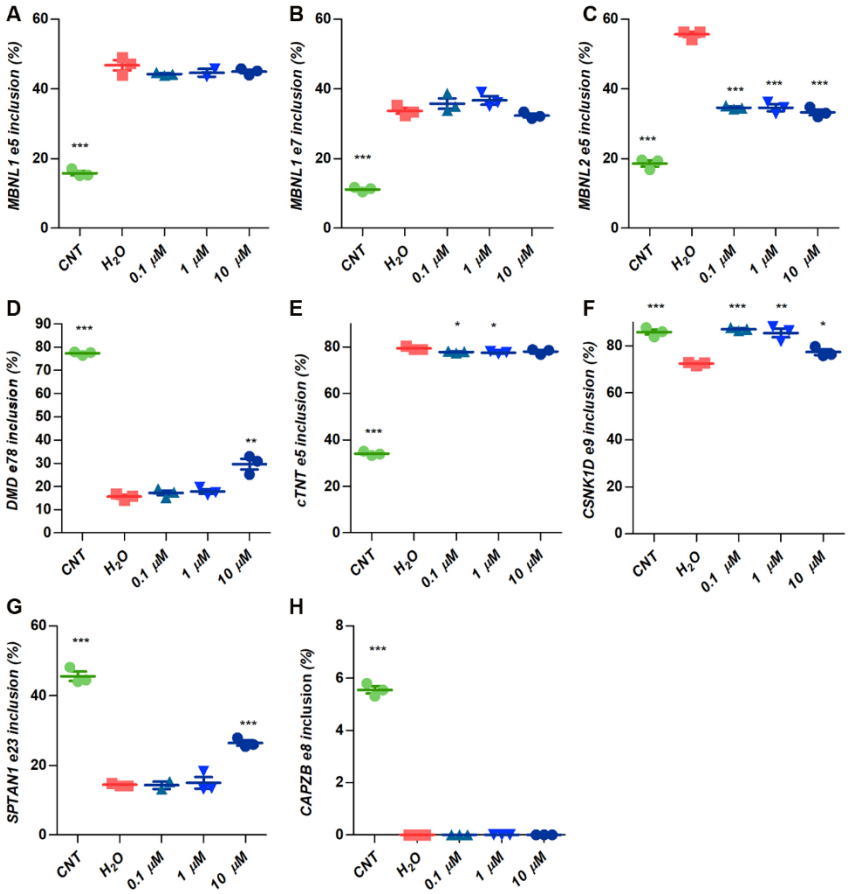


**Fig. S6. Effect of chloroquine on splicing regulation on muscle cells.** (*A-H*) Graphs show the percentage of inclusion of the indicated exons obtained by semiquantitative RT-PCR of *MBNL1* (exons 5 and 7), *MBNL2* (exon 5), *DMD* (exon 79), and *cTNT* (exon 5), which are MBNL1-dependent (*A-F*), and *CSNK* (exon 9) and *SPTAN1* (exon 23), which are MBNL2-dependent (*G* and *H*). Percentage of CELF1-regulated *CAPZB* exon 8 inclusion was determined as a control (*I*). *GAPDH* was used as an internal control. In all analyses sample size was n=3. Data are mean ± S.E.M. *P<0.05, **P<0.01, ***P<0.001 according to Student's *t*-test. Cells were differentiated 96 h and treated with the indicated concentrations of chloroquine. All comparisons are referred to untreated DM1 cells ($H_2O$ in the graphs).

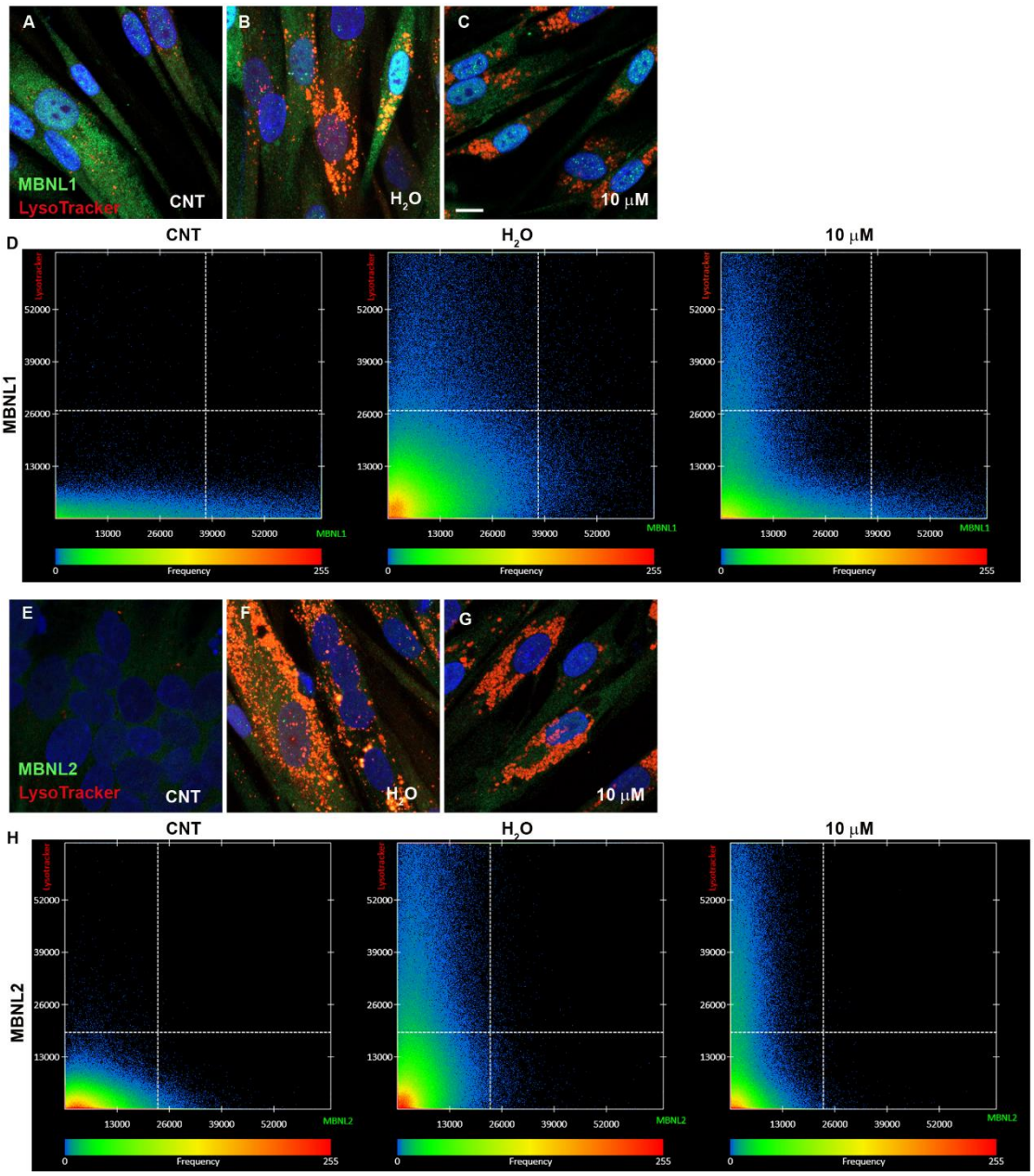


**Fig. S7. The blockade of autophagosome and lysosome fusion by CQ reduces MBNL1 and MBNL2 colocalization with LysoTracker.** Representative confocal images of immunodetection of MBNL1 (green) and LysoTracker (red) (*A*-*C*) or MBNL2 (green) and LysoTracker (red) (*E*-*G*) in iCDM (*A*, *E*, CNT), iPDM treated with vehicle (*B*, *F*, $H_20$) and iPDM treated with 10 μM CQ (*C*, *G*, 10 μM). Scale bar=10 μm. Representative Scatter Plots of fluorescence intensity distribution of pixels for MBNL1/Lysotracker (*D*) and MBNL2/Lysotracker (*H*) from controls (CNT), iPDM

($H_20$) or iPDM cells treated with 10 μM CQ (10 μM). X-axis corresponds to the green channel (MBNL1 (*D*) or MBNL2 (*H*)) and Y-axis is the red channel (LysoTracker). Dashed lines indicate thresholds used to define colocalized signals (upper right quadrant).

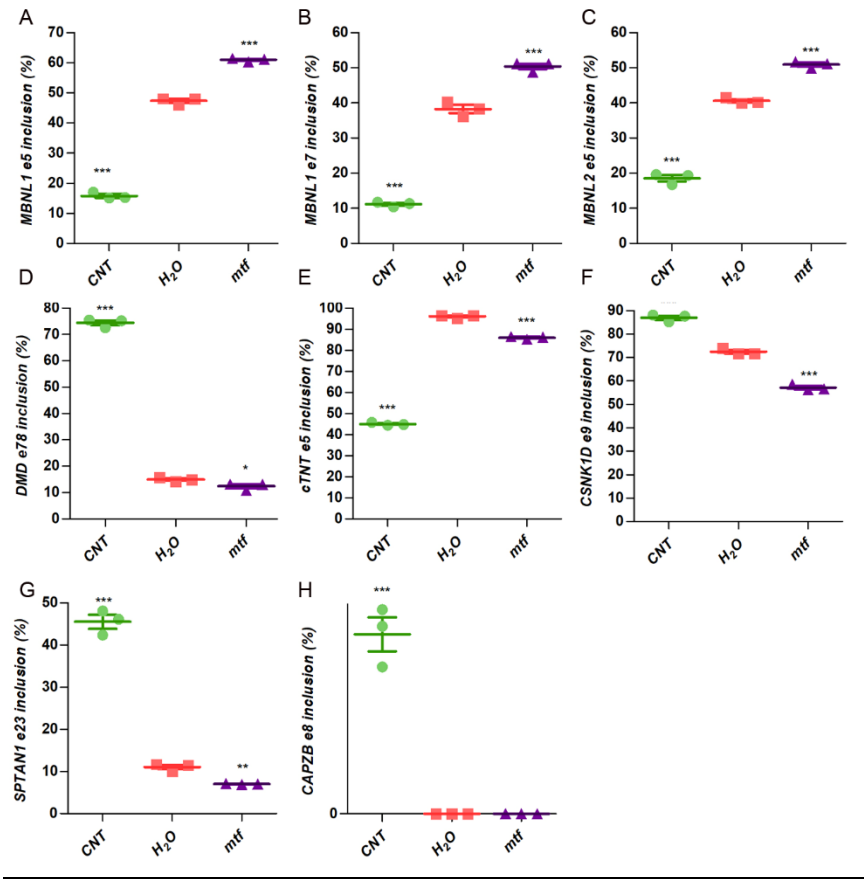


**Fig. S8. Mtf treatment of DM1 muscle cells worsens splicing defects.** (*A-H*) Graphs show the percentage of inclusion of the indicated exons obtained by semiquantitative RT-PCR of *MBNL1* (exons 5 and 7), *MBNL2* (exon 5), *DMD* (exon 78), and *cTNT* (exon 5), which are MBNL1-dependent (*A-F*), and *CSNK* (exon 9) and *SPTAN1* (exon 23), which are MBNL2-dependent (*G* and *H*). Percentage of CELF1-regulated *CAPZB* exon 8 inclusion was determined as a control (*I*). *GAPDH* was used as an internal control. In all analyses sample size was n=3. Data are mean ± S.E.M. *P<0.05, **P<0.01, ***P<0.001 according to Student's *t*-test. Cells were differentiated 96 h and treated with 30 mM mtf. All comparisons are referred to untreated DM1 cells ($H_2O$ in the graphs).

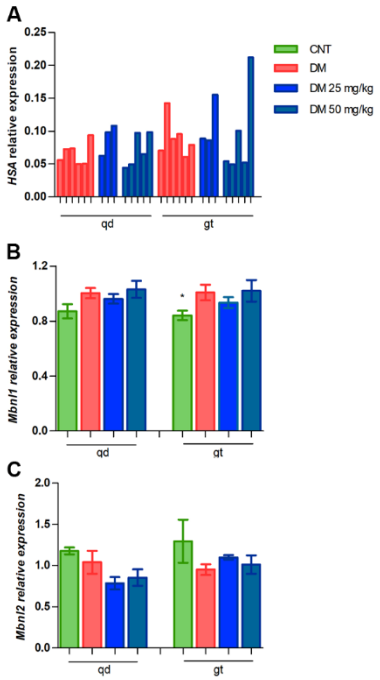


**Fig. S9. *HSA* transgene and *Mbnl* expression quantification.** (*A*) Quantification of the *HSA* transgene by RT-qPCR. Bars show relative expression in each animal. (*B* and *C*) Analyses of the relative expression of *Mbnl1* (*B*) or *Mbnl2* (*C*) by RT-qPCR. Quantifications were performed in control (green), and $HSA^{LR}$ mice treated with PBS (red) or with 25 and 50 mg/kg chloroquine (blue) in quadriceps (qd) and gastrocnemius (gt). *Gapdh* was used as an internal control. Data are mean ± S.E.M. *P<0.05 according to Student's *t*-test. Experimental groups were CNT (FVB; n=8), DM ($HSA^{LR}$; n=6), DM 25 mg/kg ($HSA^{LR}$; n=3), DM 50 mg/kg ($HSA^{LR}$; n=5).

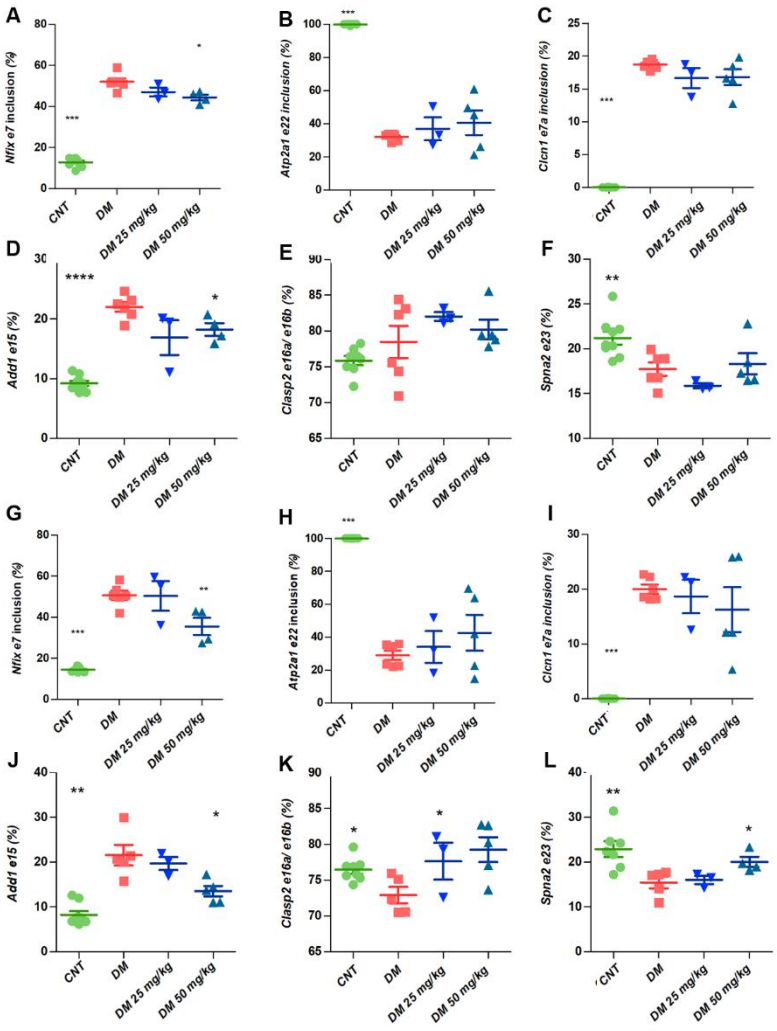


**Fig. S10. Percentage of inclusion of the indicated exons in control and HSA$^{LR}$ mice with or without chloroquine**. *Nfix* (exon 7), *Atp2a* (exon 22), and *Clcn1* (exon 7a) inclusion is regulated by Mbnl1 (*A-C* and *G-I*), while *Add1* (exon 15), *Clasp2* (exon 16), and *Spna2* (exon 23) are regulated by Mbnl2 (*D-F* and *J-L*). Determinations were performed in quadriceps (*A-F*) and gastrocnemius (*G-L*) and *Gadph* values were used as an internal control. Quantifications were performed in control (green), model HSA$^{LR}$ mice treated with PBS (red) or with 25 and 50 mg/kg chloroquine (blue). Data are mean ± S.E.M. *P<0.05, **P<0.01, ***P<0.001 according to Student's *t*-test. Experimental groups were CNT (FVB; n=8), DM (HSA$^{LR}$; n=6), DM 25 mg/kg (HSA$^{LR}$; n=3), DM 50 mg/kg (HSA$^{LR}$; n=5). All comparisons are referred to untreated HSA$^{LR}$ mice (DM in the graphs).

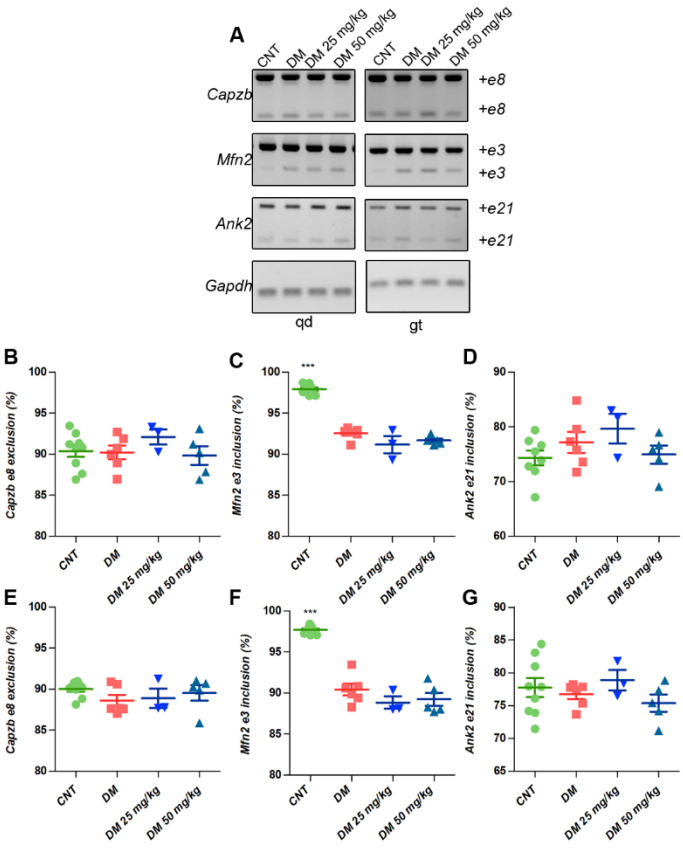


**Fig. S11. CELF1-dependent splicing remains unchanged in chloroquine-treated HSA$^{LR}$ mice.** (*A*) Representative gels used for quantification in *B-G* from quadriceps (qd, left) and gastrocnemius (gt, right) muscles. (*B-G*) RT-PCR analysis of Celf1-dependent splicing impaired in HSA$^{LR}$ mice; *Capzb* (exon 8), *Mfn2* (exon 3), and *Ank2* (exon 21), from quadriceps (*B-D*) and gastrocnemius (*E-G*). *Gadph* values were used for normalization in the quantification of the exon inclusion. Quantifications were performed in control FVB mice (green), model HSA$^{LR}$ mice treated with PBS (red) or with 25 and 50 mg/kg chloroquine (blue). Data are mean ± S.E.M. ***P<0.001 according to Student's *t*-test. Experimental groups were CNT (FVB; n=8), DM (HSA$^{LR}$; n=6), DM 25 mg/kg (HSA$^{LR}$; n=3), DM 50 mg/kg (HSA$^{LR}$; n=5). All comparisons are referred to untreated HSA$^{LR}$ mice (DM in the graphs)

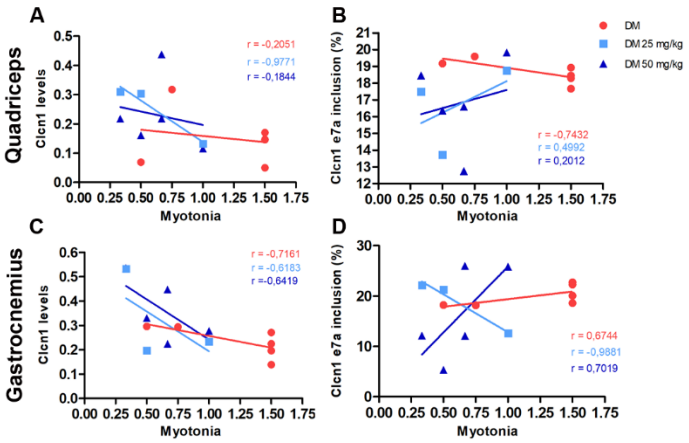


**Fig. S12. Clcn1 protein levels, *Clcn1* splicing, and myotonia are correlated.** Pearson´s correlations between Clcn1 protein levels (A and C) or Clcn1 exon 7a (B and D) and myotonia grade. Experimental groups were DM ($HSA^{LR}$; n=6), DM 25 mg/kg ($HSA^{LR}$; n=3), DM 50 mg/kg ($HSA^{LR}$; n=5). Data from myotonia assay (x-axis) were expressed as the ratio between myotonia measurement after (ai) and before (bi) chloroquine injection (ai/bi). Clcn1 protein levels were normalized to Gapdh.

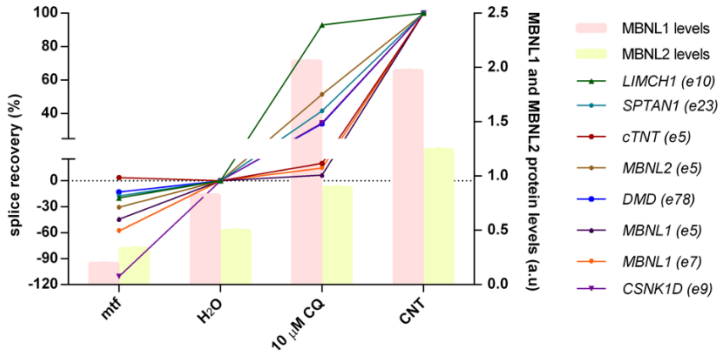


**Fig. S13 Effect of the treatments on DM1-related phenotypes.** MBNL levels (right Y-axis) are downregulated in iPDM ($H_2O$) compared to iCDM (CNT). MBNL1 (red bars) and MBNL2 (yellow bars) levels quantified in iPDM were reduced or increased after mtf or CQ treatment, respectively. Dots represent the percentage of recovery of exon inclusion (left Y-axis) after mtf or CQ treatment considering 0% values in iPDM ($H_2O$) and 100% those obtained for iCDM (CNT)

**Table S1 Sequences of oligonucleotides used for qRT-PCR and semiquantitative RT-PCR**

| Primers sequence | Sequence (5' → 3') | RT-qPCR/RTPCR | Species |
|---|---|---|---|
| *amy-d fwd* | GGACCATCTGATTGATCTCG | RT-qPCR | *Drosophila* |
| *amy-d rev* | GGTGTTCAGGTTCTTGAGG | RT-qPCR | *Drosophila* |
| *cyp6w1 fwd* | TTGCGCACAAAAATCTCTCC | RT-qPCR | *Drosophila* |
| *cyp6w1 rev* | GTCCTGCAAGTTCTTTCCAA | RT-qPCR | *Drosophila* |
| *mbl fwd* | TTGAATCAAAATTATAGCCCAAGCT | RT-qPCR | *Drosophila* |
| *mbl rev* | CGATTTTGCTCGTTAGCGTTT | RT-qPCR | *Drosophila* |
| *mhc fwd* | CCAGGAGCGCAACGCCAAGT | RT-qPCR | *Drosophila* |
| *mhc rev* | GCGGGCATCCTCCTCCTGAGT | RT-qPCR | *Drosophila* |
| *rp49 fwd* | GGATCGATATGCTAAGCTGTCGCACA | RT-qPCR/RTPCR | *Drosophila* |
| *rp49 rev* | GGTGCGCTTGTTCGATCCGTAACC | RT-qPCR/RTPCR | *Drosophila* |
| *serca fwd* | GCAGATGTTCCTGATGTCG | RT-qPCR | *Drosophila* |
| *serca rev* | CGTCCTCCTTCACATTCAC | RT-qPCR | *Drosophila* |
| *CAPZB fwd* | GGAGAAGGATGAAACTGTGAGTG | RT-PCR | *homo sapiens* |
| *CAPZB rev* | CAGAGGTTTAGCATTGCTGCT | RT-PCR | *homo sapiens* |
| *CSNK1D fwd* | GATACCTCTCGCATGTCCACCTCACA | RT-PCR | *homo sapiens* |
| *CSNK1D rev* | GCATTGTCTGCCCTTCACAGCAAT | RT-PCR | *homo sapiens* |
| *DMD fwd* | GTGAGGAAGATCTTCTCAGTCC | RT-PCR | *homo sapiens* |
| *DMD rev* | CTCCATCGCTCTGCCCAAATC | RT-PCR | *homo sapiens* |
| *GAPDH fwd* | CATCTTCCAGGAGCGAGATC | RT-PCR | *homo sapiens* |
| *GAPDH rev* | GTTCACACCCATGACGAACAT | RT-PCR | *homo sapiens* |
| *LIMCH1 fwd* | CGGAAGCTGCCAGATGTGAAGAAG | RT-qPCR | *homo sapiens* |
| *LIMCH1 rev* | CCTCCTCACACCGCATGTCAAA | RT-qPCR | *homo sapiens* |
| *MBNL1 (e5) fwd* | AGGGAGATGCTCTCGGGAAAAGTG | RT-PCR | *homo sapiens* |
| *MBNL1 (e5) rev* | GTTGGCTAGAGCCTGTTGGTATTGGAAAATAC | RT-PCR | *homo sapiens* |
| *MBNL1 (e7) fwd* | GCTGCCCAATACCAGGTCAAC | RT-PCR | *homo sapiens* |
| *MBNL1 (e7) rev* | TGGTGGGAGAAATGCTGTATGC | RT-PCR | *homo sapiens* |
| *MBNL2 (e5) fwd* | ACAAGTGACAACACCGTAACCG | RT-PCR | *homo sapiens* |
| *MBNL2(e5) rev* | TTTGGTAAAGGATGAAGAGCACC | RT-PCR | *homo sapiens* |
| *SPTAN1 fwd* | GATTGGTGGAAAGTGGAAGTGAACGAT | RT-PCR | *homo sapiens* |
| *SPTAN1 rev* | TGATCCATTGCTGTAGTTCATTCGC | RT-PCR | *homo sapiens* |
| *cTNT fwd* | ATAGAAGAGGTGGTGGAAGAGTAC | RT-PCR | *homo sapiens* |
| *cTNT rev* | GTCTCAGCCTCTGCTTCAGCATCC | RT-PCR | *homo sapiens* |

| **Primers sequence** | **Sequence (5' → 3')** | **RT-qPCR/RTPCR** | **Species** |
|---|---|---|---|
| *Add1 fwd* | GGATGAGACAAGAGAGCAGAAAGAGAAGA | RT-PCR | *mus musculus* |
| *Add1 rev* | CTGGGAAGGCAAGTGCTTCTGAA | RT-PCR | *mus musculus* |
| *Ank2 fwd* | GAACGTGGTTCTCCGATTGT | RT-PCR | *mus musculus* |
| *Ank2 rev* | CGTCTCCTGGGGGTATGTCAG | RT-PCR | *mus musculus* |
| *Atp2a1 fwd* | GCTCATGGTCCTCAAGATCTCAC | RT-PCR | *mus musculus* |
| *Atp2a1 rev* | GGGTCAGTGCCTCAGCTTTG | RT-PCR | *mus musculus* |
| *Capzb fwd* | GCACGCTGAATGAGATCTACTTTG | RT-PCR | *mus musculus* |
| *Capzb rev* | CCGGTTAGCGTGAAGCAGAG | RT-PCR | *mus musculus* |
| *Clasp2 fwd* | GTTGCTGTGGGAAATGCCAAGAC | RT-PCR | *mus musculus* |
| *Clasp2 rev* | GCTCCTTGGGATCTTGCTTCTCTTC | RT-PCR | *mus musculus* |
| *Clcn1 fwd* | GTCCTCAGCAAGTTTATGTCC | RT-PCR | *mus musculus* |
| *Clcn1 rev* | GAATCCTCGCCAGTAATTCC | RT-PCR | *mus musculus* |
| *HSA fwd* | ACGGGTGCGTGGTGTCTC | RT-qPCR | *mus musculus* |
| *HSA rev* | GGTCAGGATACCTCTCTTGCT | RT-qPCR | *mus musculus* |
| *Gapdh fwd* | ATCAACGGGAAGCCCATCAC | RT-PCR | *mus musculus* |
| *Gapdh rev* | CTTCCACAATGCCAAAGTTGT | RT-PCR | *mus musculus* |
| *Limch1 fwd* | CGGAAGTTGCCAGATGTGAAGAAA | RT-qPCR | *mus musculus* |
| *Limch1 rev* | CCTCCTCACACCGCATGTCAAA | RT-qPCR | *mus musculus* |
| *Mfn2 fwd* | AGCCATGTCCACGATGCCCA | RT-PCR | *mus musculus* |
| *Mfn2 rev* | ATGTAGGCCCCAGCTGCTCAA | RT-PCR | *mus musculus* |
| *Nfix fwd* | TCGACGACAGTGAGATGGAG | RT-PCR | *mus musculus* |
| *Nfix rev* | CAAACTCCTTCAGCGAGTCC | RT-PCR | *mus musculus* |
| *Spna2 fwd* | GATTGGTGGAAAGTGGAAGTGAATGAC | RT-PCR | *mus musculus* |
| *Spna2 rev* | TGATCCACTGCTGTAACTCGTTTGCT | RT-PCR | *mus musculus* |

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