

Supplementary material

Peptide-conjugated oligonucleotides evoke long-lasting myotonic dystrophy correction in patient-derived cells and mice

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Methods

Synthesis of Peptide-PMO Conjugates. Pip6a Ac-(RXRRBRRXRYQFLIRXRBRXR)-CO OH was synthesized and conjugated to PMO as described previously (1). The PMO sequence targeting CUG expanded repeats (5'-CAGCAGCAGCAGCAGCAGCAG-3') and PMO control reverse (5'-GACGACGACGACGACGACGAC-3') were purchased from Gene Tools LLC.

Animal model and ASO injections. Experiments were carried out in the “Centre d'études fonctionnelles” (Faculté de Médecine Sorbonne University) according to French legislation and Ethics committee approval (#1760-2015091512001083v6). HSA-LR mice are gift from Pr. Thornton. The intravenous injections were performed by single or multiple administrations via the tail vein in mice of 5 to 8 weeks of age. Doses of 12.5 mg/kg of Pip6a-PMO-CAG7 and 12.5 or 200 mg/kg of PMO were diluted in 0.9% saline and given at a volume of 5-6 μ L/g of body weight. Multiple injections were done at 2 weeks apart. Myotonia was evaluated and tissues were harvested 2 weeks after the last injection. For long-term experiments, tissues were harvested 2, 4 weeks or 6 months after the last injection.

In situ myotonia / muscle relaxation measurement. The isometric contractile properties of gastrocnemius muscle were studied in situ as previously described (2). Mice were anesthetized with a solution of ketamine/xylazine (80 mg/kg and 15 mg/kg, respectively). The knee and foot were fixed with clamps and pins. The distal tendon of the gastrocnemius muscle was attached to a lever arm of a servomotor system (305B, Dual-Mode Lever). Data were recorded and analyzed using PowerLab system (4SP, ADInstruments) and software (Chart 4, ADInstruments). The sciatic nerve (proximally crushed) was stimulated by a bipolar silver electrode using a supramaximal (10-V) square wave pulse of 0.1 ms duration. Absolute maximal isometric tetanic force (P0) was measured during isometric contractions in response to electrical stimulation (frequency of 25 to 150 Hz, train of stimulation of 500 ms). Myotonia was measured as the delay of relaxation muscle after the measure of P0.

Cell culture and Pip6a-PMO treatment. Immortalized myoblasts from healthy individual or DM1 patient with 2600 CTG repeats and immortalized MYOD1-inducible DM1 fibroblasts with 1300CTG repeats were previously described (3). Immortalized murine DMSXL MYOD1-inducible fibroblasts with 1500 CTG repeats in the 3'UTR of the human *DMPK* gene are derived from DMSXL mice (4) and were modified as previously described (5). Immortalized myoblasts were cultivated in a growth medium consisting of a mix of M199:DMEM (1:4 ratio; Life technologies) supplemented with 20% FBS (Life technologies), 50 μ g/ml gentamycin (Life technologies), 25 μ g/ml fetuin, 0.5 ng/ml bFGF, 5 ng/ml EGF and 0.2 μ g/ml dexamethasone (Sigma-Aldrich). Fibroblasts are cultivated in a DMEM growth medium supplemented with 15% FBS and 50 μ g/mL gentamycin. Myogenic differentiation was induced by switching confluent cell cultures to DMEM medium supplemented with 5 μ g/ml insulin (Sigma-Aldrich) for myoblasts and a additional 4 μ g/mL of doxycycline (Sigma-Aldrich) for the trans-differentiation of immortalized MYOD1-inducible fibroblasts. For treatment, WT or DM1 cells are differentiated for 4 days. Then, medium was changed with fresh differentiation medium with pip6a-PMO at a 1 μ M concentration. Cells were harvested for analysis 24h after

treatment.

RNA isolation, RT-PCR and qPCR analysis. For mice tissues: prior to RNA extraction, muscles were disrupted in TriReagent (Sigma-Aldrich) using Fastprep system and Lysing Matrix D tubes (MP biomedical). For human cells: prior to RNA extraction, cells were lysed in a proteinase K buffer (500 mM NaCl, 10 mM Tris-HCl, pH 7.2, 1.5 mM MgCl₂, 10 mM EDTA, 2% SDS and 0.5 mg/ml of proteinase K) for 45 min at 55°C. Total RNAs were isolated using TriReagent according to the manufacturer's protocol. One microgram of RNA was reverse transcribed using M-MLV first-strand synthesis system (Life Technologies) according to the manufacturer's instructions in a total of 20 µL. One microliter of cDNA preparation was subsequently used in a semi-quantitative PCR analysis according to standard protocol (ReddyMix, Thermo Scientific). PCR amplification was carried out for 25-35 cycles within the linear range of amplification for each gene. PCR products were resolved on 1.5-2% agarose gels, ethidium bromide-stained and quantified with ImageJ software. The ratios of exon inclusion were quantified as a percentage of inclusion relative to total intensity of isoform signals. To quantify the mRNA expression, real-time PCR was performed using a Lightcycler 480 (Roche). Reactions were performed with SYBR Green kit (Roche) and according to the manufacturer's instructions. PCR cycles were a 15-min denaturation step followed by 50 cycles with a 94°C denaturation for 15 s, 58°C annealing for 20 s and 72°C extension for 20 s. Mouse *Rrlp0* mRNA were used as standard. Data were analyzed with the Lightcycler 480 analysis software. Primers are identified in **Supplementary table 6**.

Fluorescent in situ hybridization / immunofluorescence. Fluorescent in situ hybridization (FISH) experiments were done as previously described (6) using a Cy3-labeled 2'OMe (CAG)7 probe (Eurogentec). For combined FISH-Immunofluorescence experiments, immunofluorescence staining was done after FISH last washing as described previously (7) with a rabbit polyclonal anti-MBNL1 antibody (gift from C. Thornton ; 1:2000) followed by a secondary Alexa Fluor 488-conjugated goat anti-rabbit (1:500, Life technologies) antibody. Single molecule FISH (smFISH) was performed as previously described (8). Briefly cells are incubated with a mix a 24 human *DMPK*-specific DMA probes which are hybridized to a Cy3-labelled universal FLAP oligonucleotide (**Supplementary table 6**). Pictures were captured using an Olympus BX60 microscope and Metamorph software (Molecular Devices). Confocal images were taken with a Nikon Ti2 microscope equipped with a motorized stage and a Yokogawa CSU-W1 spinning disk head coupled with a Prime 95 sCMOS camera (Photometrics). Images were processed with Adobe Photoshop software. Automated counting of nuclei with foci, number of foci per nuclei and/or intensity of FISH signal was done using Fiji software and custom scripts.

RNA sequencing. Total RNA from gastrocnemius and quadriceps muscles of WT, HSA-LR and Pip6a-PMO-CAG7 treated mice were sent to the Wellcome Trust Centre for Human Genetics Sequencing Facility. After complete RNA quality control on each sample (quantification in duplicate and RNA6000 Nano LabChip analysis on Bioanalyzer from Agilent), libraries were generated from each sample using TruSeq Stranded mRNA Library Prep Kit (Illumina), where mRNA are isolated by polyA selection, and pooled together by

multiplexing them using barcoded adapters. The pooled libraries were then sequenced on Illumina's HiSeq4000 sequencing system to obtain 150bp paired end reads. Sequence alignment was provided by the Sequencing Facility where the 150bp paired end reads were trimmed and mapped on *Mus musculus* mm10 reference genome using HISAT software (9). Then, all bioinformatics and statistical analysis were performed using custom scripts. Differentially expressed splicing events (exon_bin) between WT, HSA-LR and Pip6a-PMO-CAG7 samples were identified using DEXSeq software(10) by (i) identifying all possible exon_bins from mm10 Gencode annotation (M12 release) and estimating exon_bin counts for each sample with the provided python scripts (ii) normalization and (iii) differential exon usage tests to quantify log₂ fold changes between the different conditions and their associated p-values. Differential gene expression was computed using DESeq2 software (11) from gene counts generated by FeatureCounts tool (12). Differentially expressed exon_bins or genes were assessed when the log₂ fold change was greater than 2 and the adjusted p-value for multiple comparison above 0.1.

Sample preparation for proteomic analysis. Protein was extracted from WT, HSA-LR untreated and HSA-LR Pip6a-PMO treated (12.5 mg/kg, 3 administrations at 2 weeks intervals) from quadriceps muscle. Tissue was homogenised in lysis buffer (7 M urea, 2 M thiourea, 65 mM CHAPS, 100 mM DTT and supplemented with protease and phosphatase inhibitors) by bead extraction and incubated for 2.5 hours at 4°C. The crude extract was clarified by centrifugation at 16,000xg for 20 min. Protein samples were processed using a ReadyPrep 2-D clean up kit (BioRad) and resuspended in 6 M urea, 2 M thiourea (in 10 mM Tris-HCl, pH 8.0). Protein concentration was determined by BCA. Equalised tissue lysates containing 25 µg protein were prepared for label-free mass spectrometry analysis, as described previously (13). Protein lysates were reduced with 10 mM DTT for 30 min at 37°C and alkylated with 55 mM iodoacetamide for 30 min at room temperature in the dark. Samples were diluted with four volumes 50 mM ammonium bicarbonate and digested with 1 µg trypsin and incubated at 37°C overnight. Digestion was terminated by acidification with 2% trifluoroacetic acid (TFA) in 20% acetonitrile (3:1 (v/v) dilution). Peptides were purified using C18 spin columns (Thermo Fisher), lyophilised and stored at -20°C until mass spectrometry analysis.

Liquid chromatography-mass spectrometry analysis. Peptides were analysed by LC-MS/MS using a Dionex Ultimate 3000 nanoUPLC and a QExactive HF mass spectrometer (Thermo Fisher). Peptides were analysed by LC-MS/MS using a Dionex Ultimate 3000 NanoUPLC system via a nano electrospray source coupled to a QExactive HF mass spectrometer (Thermo Fisher). A 500 mm x 75 µm C18 (2 µm particle size) EASY-Spray column was used to separate peptides over a 60 minute gradient of 2 – 35 % acetonitrile in 5 % DMSO, 0.1 % formic acid with a flow rate of 250 nL/min. The mass spectrometer was operated in data-dependent and positive mode. MS1 scans were acquired at a resolution of 60,000 at 200 m/z. The top 12 most abundant precursor ions with a charge of ≥ 2 were selected for HCD fragmentation.

Mass spectrometry data analysis. MS raw files were processed with MaxQuant software, version 1.5.0.0 (14). The raw files were searched against the mouse, *Mus musculus*, UniProt-

SwissProt FASTA database (16,997 proteins) for MS/MS based peptide identification via Andromeda. Enzyme specificity was set to trypsin, with a minimum number of seven amino acids required for peptide identification, a maximum of two missed cleavages and the FDR was set to <1% at the peptide and protein level. Default settings were used for variable and fixed modifications (variable modification; acetylation (N-terminus) and methionine oxidation; fixed modifications; carbamidomethylation). A target-decoy approach to identify peptides and proteins at an FDR <1%(15). Peptide identification was performed with the precursor mass deviation up to 4.5 ppm after time-dependent mass calibration and an allowed fragment mass deviation of 20 ppm. For label-free protein quantification, the MaxLFQ algorithm was employed for intensity determination and normalisation procedures and is fully compatible with peptide and protein separation prior to MS analysis. The minimum ratio count was set to two (16). Quantitative of high-resolution peptide profiles was based on mass-to-charge (m/z), retention time and intensity values. MaxQuant was used to calculate pairwise protein ratios by taking the median of all pairwise peptide ratios per protein. Only shared identical peptides were considered for each pairwise comparison, with a minimum number of one ratio count required for each pairwise comparison and a least-squares analysis was employed to rebuild the relative abundance profile for individual proteins. This facilitated the conservation of the total summed intensity for a protein across all the samples under analysis. 'Match between runs' was enabled for sample processing to maximise the number of quantification events across samples, permitting the quantification of high-resolution MS1 features that were not identified by MS2 in each single measurement (17). For matching between runs the retention time alignment window was set to 30 min and the match time window was 1 min.

Northern blot. 8-12 μ g of RNA was separated on 1.2% agarose MOPS-EDTA-Sodium Acetate (Sigma-Aldrich) gels containing 0.66 M formaldehyde (Sigma-aldrich) and transferred onto Hybond-N+ membrane (GE Healthcare) by capillary transfer with 10xSSC. Blots were hybridized with random-primed 32P-labeled (AflIII-HindIII fragment of *DMPK* cDNA) probe in a hybridization buffer (2% SDS, 10% dextran sulfate, 1xSSPE, 100 μ g/ml salmon sperm DNA, 2% Denhart's solution) at 65°C overnight. Signals were analyzed on a phosphoimager (Molecular Imager FX, Bio-Rad) and quantified using Quantity One (Bio-Rad). All values were normalized to *18S* rRNA signal after hybridization with 5'-end 32P-labeled *18S* rRNA-oligonucleotide probes.

ELISA based measurements of oligonucleotide concentrations in tissues. Hybridization-Based ELISAs to determine the concentration of PMO oligonucleotides were performed as described (18) using phosphorothioate probes (Sequence (5'->3') [DIG]C*T*G*C*T*G*C*TGCTGCT*G*C*T*G*C*T*G[BIO]) double-labelled with digoxigenin and biotin. This probe was used to detect Pip6a-PMO or naked PMO concentrations in eight different tissues (brain, kidney, liver, lung, heart, diaphragm, gastrocnemius and quadriceps) from treated HSA-LR mice.

Statistical analysis. All group data are expressed as mean +/- SEM. Between groups, the comparison was performed by Mann-Whitney test or one-way ANOVA test followed by a Newman-Keuls or Tukey post-test using Prism 6 software (GraphPad Software, Inc.).

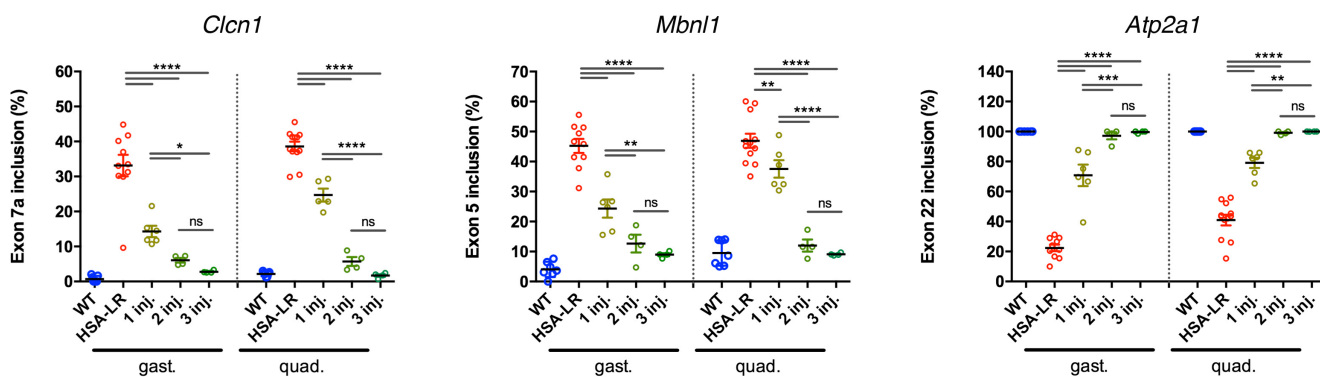
Differences between groups were considered significant when $P < 0.05$ (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, ****, $P < 0.0001$). For proteomics data handling, normalization, statistics and annotation enrichment analysis, open-source bioinformatics platforms Perseus (version 1.5.5.3)(19) or R-studio (version 0.99.903) (R Development Core team, 2011) were employed. For pairwise comparison of proteomes a two-sides t -test statistic was employed.

Data availability: Complete raw data generated from RNA sequencing were deposited in the Gene Expression omnibus database (GEO ; GSE134926). Codes used for analyzing are available from the corresponding author on reasonable request.

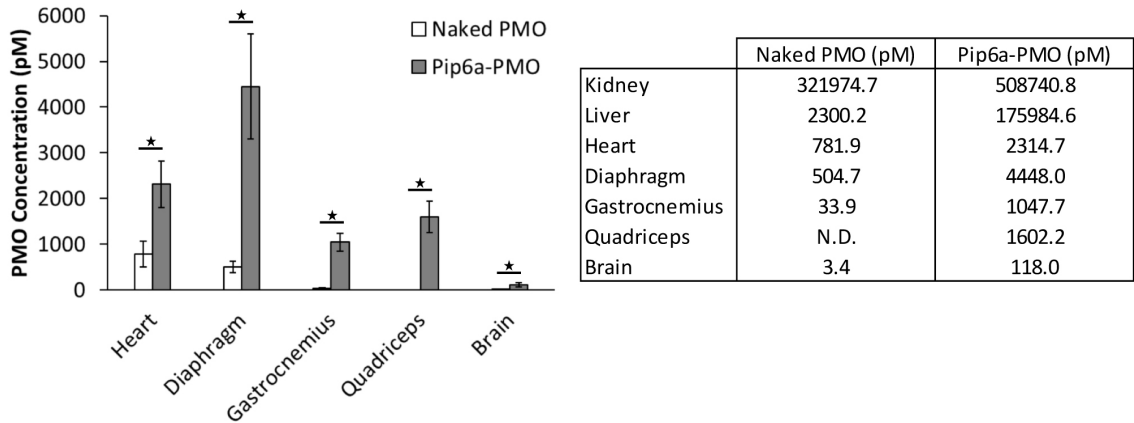
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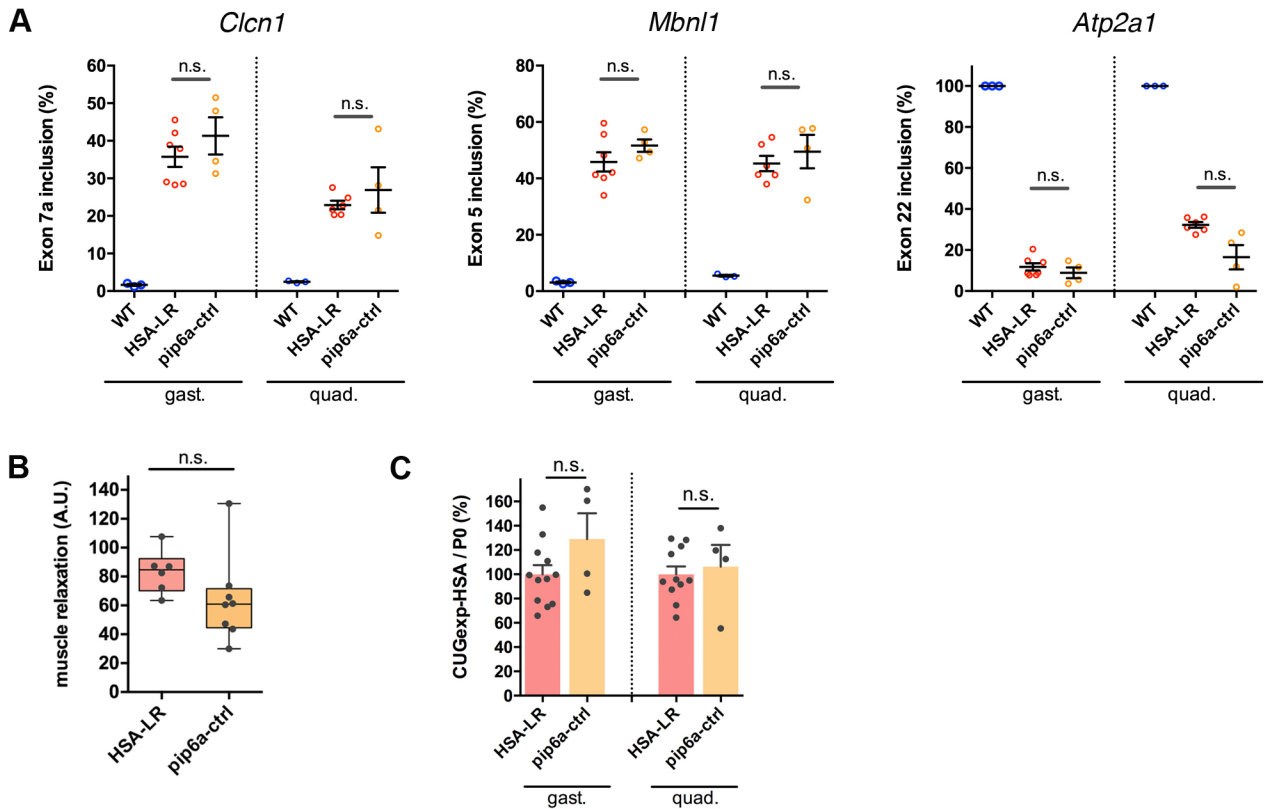
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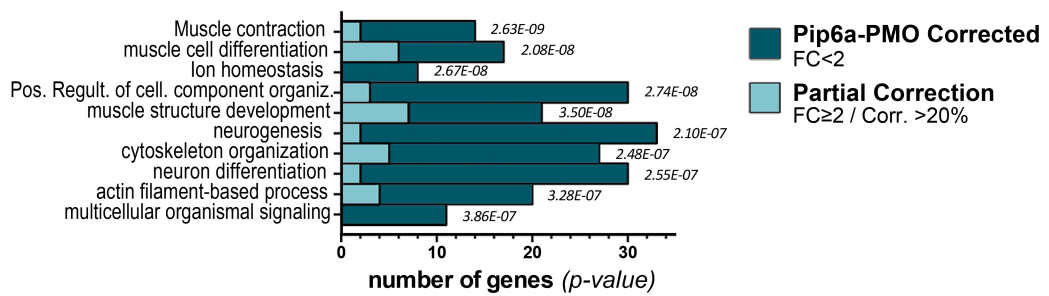
Supplementary figure 1: Dose-dependent effect of Pip6a-PMO on alternative splicing correction in HSA-LR mice. Quantification of the alternative splicing correction in HSA-LR mice treated with multiple intravenous systemic injections of Pip6a-PMO-CAG7 (pip6a-PMO) at the dose of 12.5 mg/kg and sacrificed after 2 weeks (n=7 for WT, n=10 for HSA-LR, n=6 for 1 inj., n=4 for 2 inj. and 3 inj.). Data are expressed as mean \pm SEM. Statistics: One-way ANOVA with Newman-Keuls post-test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.



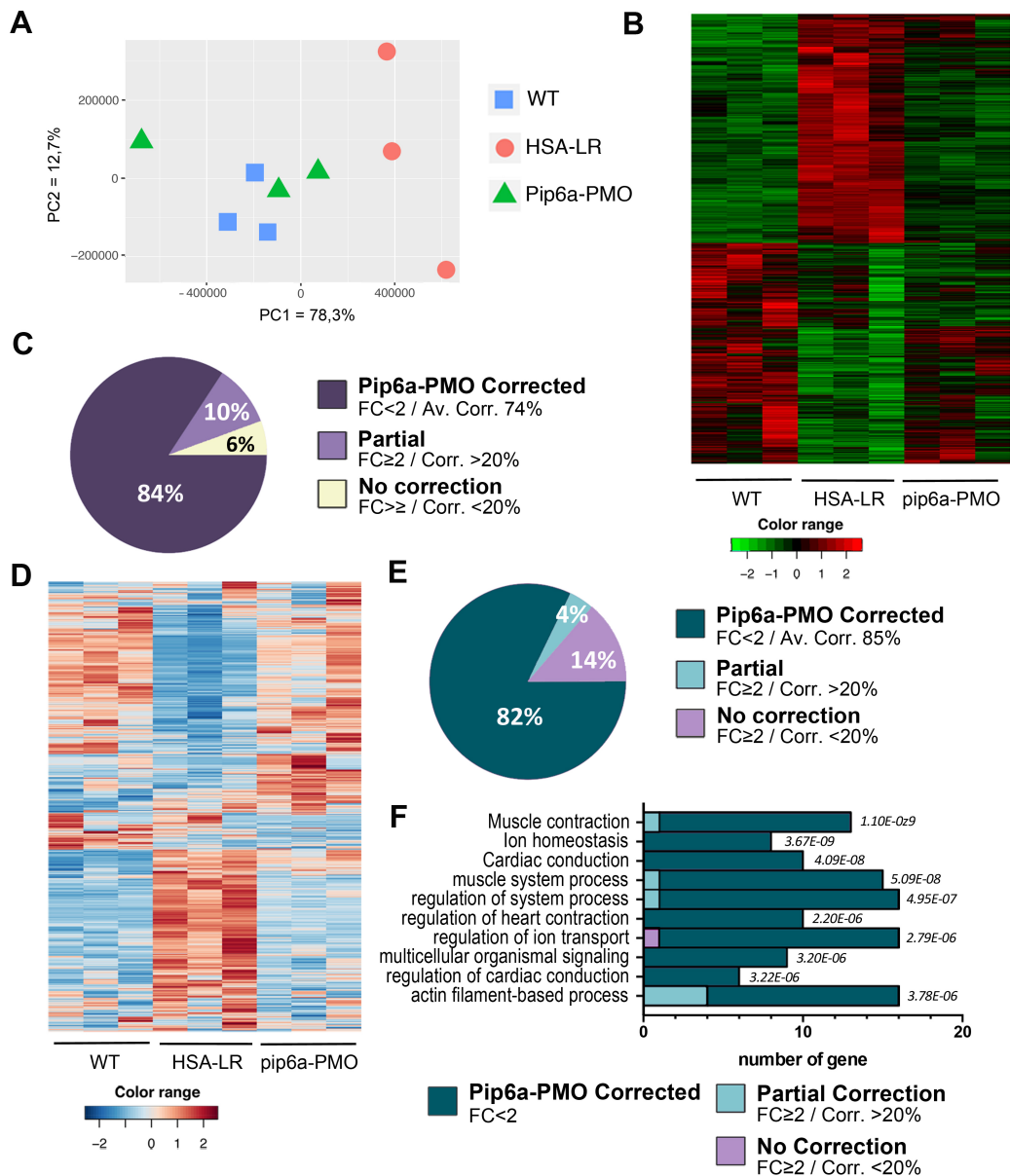
Supplementary figure 2: Evaluation of Pip6a-PMO biodistribution reveals optimal delivery to critically affected tissues in DM1. PMOs were detected by a custom ELISA assay using probes labelled with digoxigenin and biotin after 3 intravenous systemic injections of Pip6a-PMO-CAG7 (Pip6a-PMO) at 12.5 mg/kg or 3x200 mg/kg of naked PMO-CAG7 (Naked PMO) in HSA-LR mice. Two weeks after the Pip6a-PMO injections the concentration of PMO in muscle tissues was still >1nM vs the low pM detected after naked PMO injections (despite of the >20-fold difference in molarity of naked PMO vs Pip6a-PMO treatments) (n=4). *Data are expressed as mean +/- SEM. Statistics: One-way ANOVA with Tukey post-test; *, P<0.05.*



Supplementary figure 3: Treatment with Pip6a-Ctrl (GAC7) has no effect in HSA-LR mice. HSA-LR mice are treated with 3 systemic IV injections of Pip6a-PMO-GAC7 (Pip6a-ctrl) at 12.5 mg/kg and sacrificed 2 weeks after. Treatment with Pip6a-ctrl has no effect on **A**) the alternative splicing profiles of *Clcn1*, *Mbn1* and *Serca1* as analyzed by RT-PCR (n=7 for HSA-LR and n=4 for Pip6a-PMO-ctrl), **B**) in the levels of myotonia of the *gastrocnemius* as measured as the area under the force/time curve during relaxation after maximal muscle contraction (n=6 for HSA-LR and n=8 for Pip6a-PMO-ctrl) and **C**) in levels of *CUGexp* HSA transcripts as determined by real-time PCR (n=12 for HSA-LR and n=4 for Pip6a-PMO-ctrl). Data are expressed as mean \pm SEM. Statistics: A, C: One-way ANOVA with Newman-Keuls post-test; B: Mann-Whitney test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

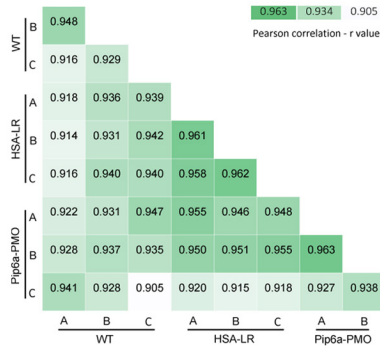


Supplementary figure 4: Treatment with Pip6a-PMO normalizes deregulated pathways in HSA-LR gastrocnemius. Number of genes, p-values and correction level for significantly enriched Biological Process_GO_terms and Pathways in the set of HSA-LR/WT missplicing events using DAVID and Toppgene tools.

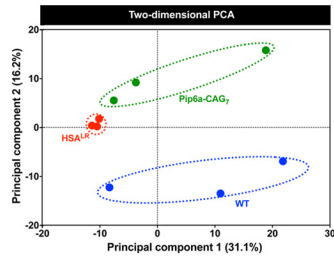


Supplementary figure 5: Treatment with Pip6a-PMO normalizes global transcriptome at both expression and splicing levels. Transcriptomic analysis by RNA sequencing was performed on total RNA of the *quadriceps* muscle of treated mice compared to HSA-LR and wild-type mice (n=3). **A-B)** Principal component analysis (A) and heatmap graphic (B) of all the significantly expressed transcripts (adj. p-value < 0.1) of *quadriceps* muscle from either WT, HSA-LR or Pip6a-PMO-CAG7 (Pip6a-PMO) treated mice reveal a global correction of genes expression profile with Pip6a-PMO treatment. **C)** Treatment with Pip6a-PMO induces the correction of the expression profile in *quadriceps* muscle of treated mice (n=588; FC ≥ 2, adj. p-value < 0.1): 84% of transcripts return to FC < 2 with an average correction index of 76% ; 10% of transcripts remains at FC > 2 but with correction index > 20%; 6% of transcripts are not corrected. **D)** Heatmap graphic of all significant deregulated exon_bin (normalized counts) reveals a global correction of missplicing events with Pip6a-PMO treatment. **E)** Treatment with Pip6a-PMO induces a correction of alternative splicing profiles in *quadriceps* muscle of treated mice (n=242 splicing events; FC ≥ 2, adj. p-value < 0.1): 82% of events return to FC < 2 with an average correction index of 83%; 4% remains at FC > 2 but with correction index > 20%; 14% are not corrected. **F)** Number of genes and p-values for significantly enriched Biological Process_GO_terms and Pathways in the set of HSA-LR/WT missplicing events using DAVID and Toppgene tools.

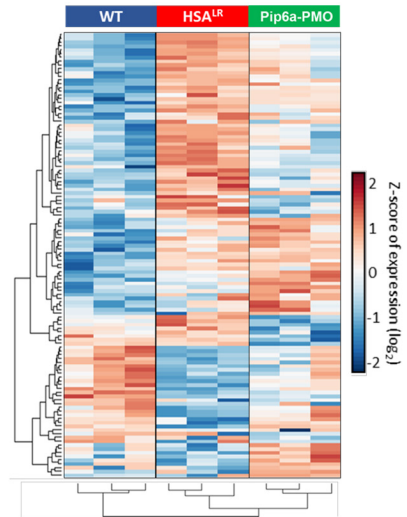
A



B



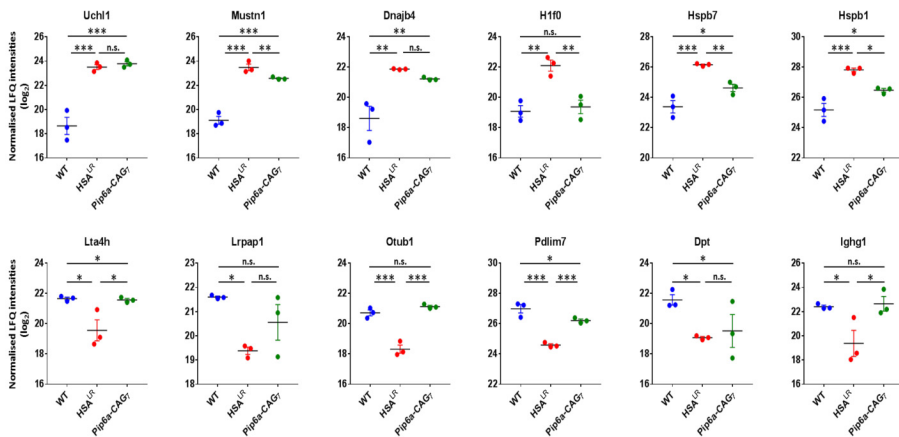
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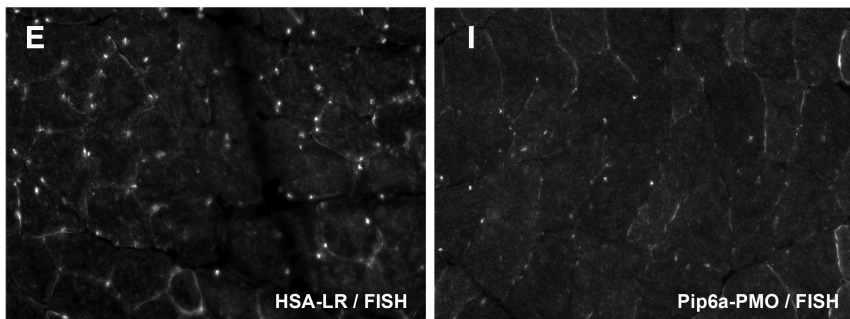
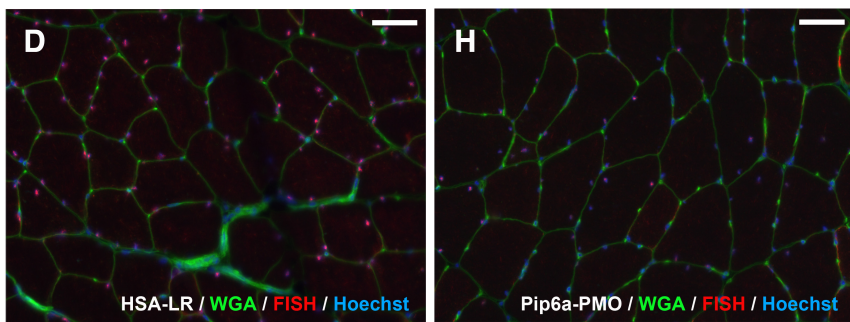
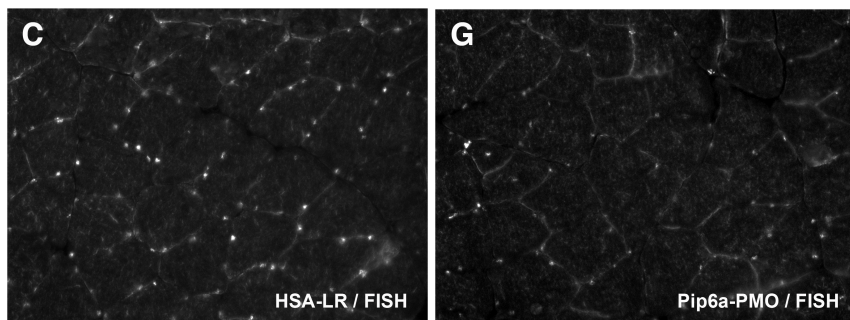
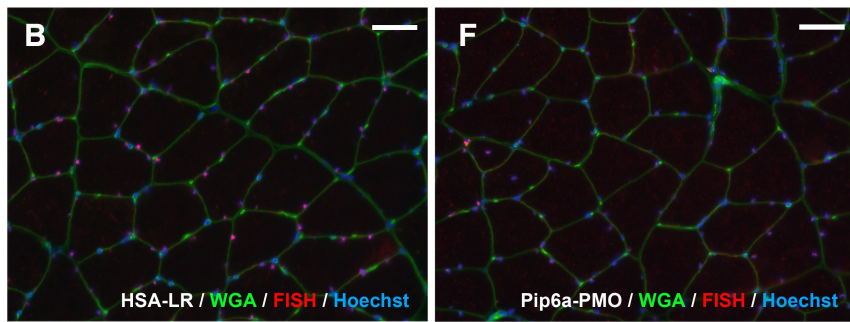
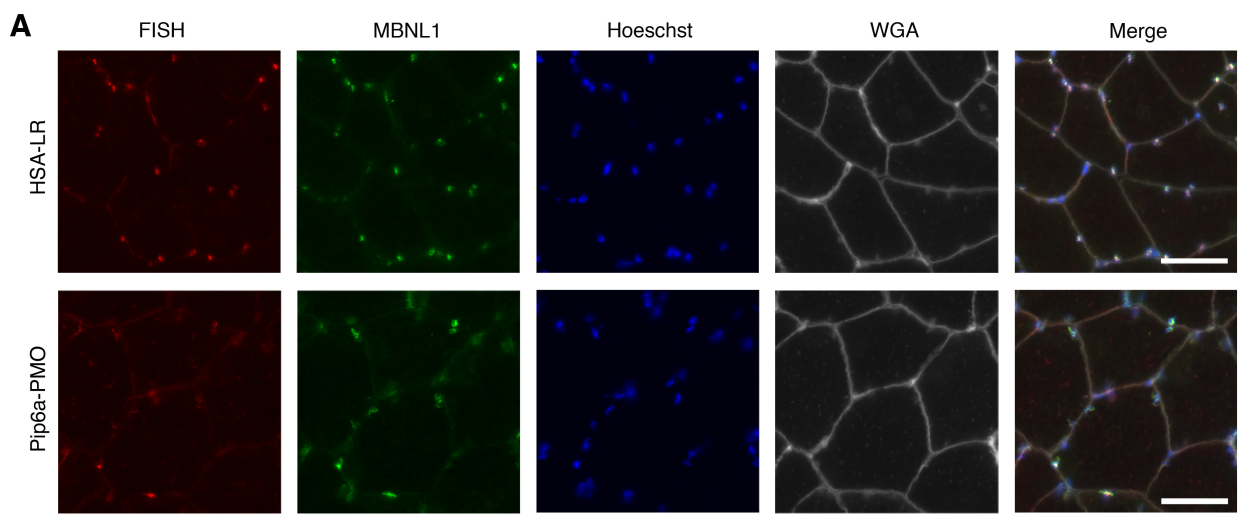
	Protein name	Gene name	Accession number	Fold change	Unique peptides	Unique sequence coverage [%]
Increased	Ubiquitin carboxyl-terminal hydrolase isozyme L1	Uchl1	Q9R0P9	29.02	8	56.5
	Musculoskeletal embryonic nuclear protein 1	Mustn1	Q99J11	20.69	3	48.8
	Dnaj homolog subfamily B member 4	Dnajb4	Q9D832	9.52	7	37.7
	Histone H1.0N-terminally processed	H1f0	P10922	8.12	3	16.5
	Heat shock protein beta-7	Hspb7	P35385	6.85	8	68.0
	Heat shock protein beta-1	Hspb1	P14602	6.25	20	90.9
	Leukotriene A-4 hydrolase	Lta4h	P24527	-4.28	5	11.5
	Alpha-2-macroglobulin receptor-associated protein	Lrpap1	P55302	-4.64	3	8.3
	Ubiquitin thioesterase OTUB1	Otub1	Q7TQ13	-5.26	3	12.2
	PDZ and LIM domain protein 7	Pdlim7	Q3TJD7	-5.28	9	23.0
Decreased	Dermatopontin	Dpt	Q9QZ26	-5.64	2	16.4
	Ig gamma-1 chain C region, membrane-bound form	Ighg1	P01869	-8.16	2	13.6

E

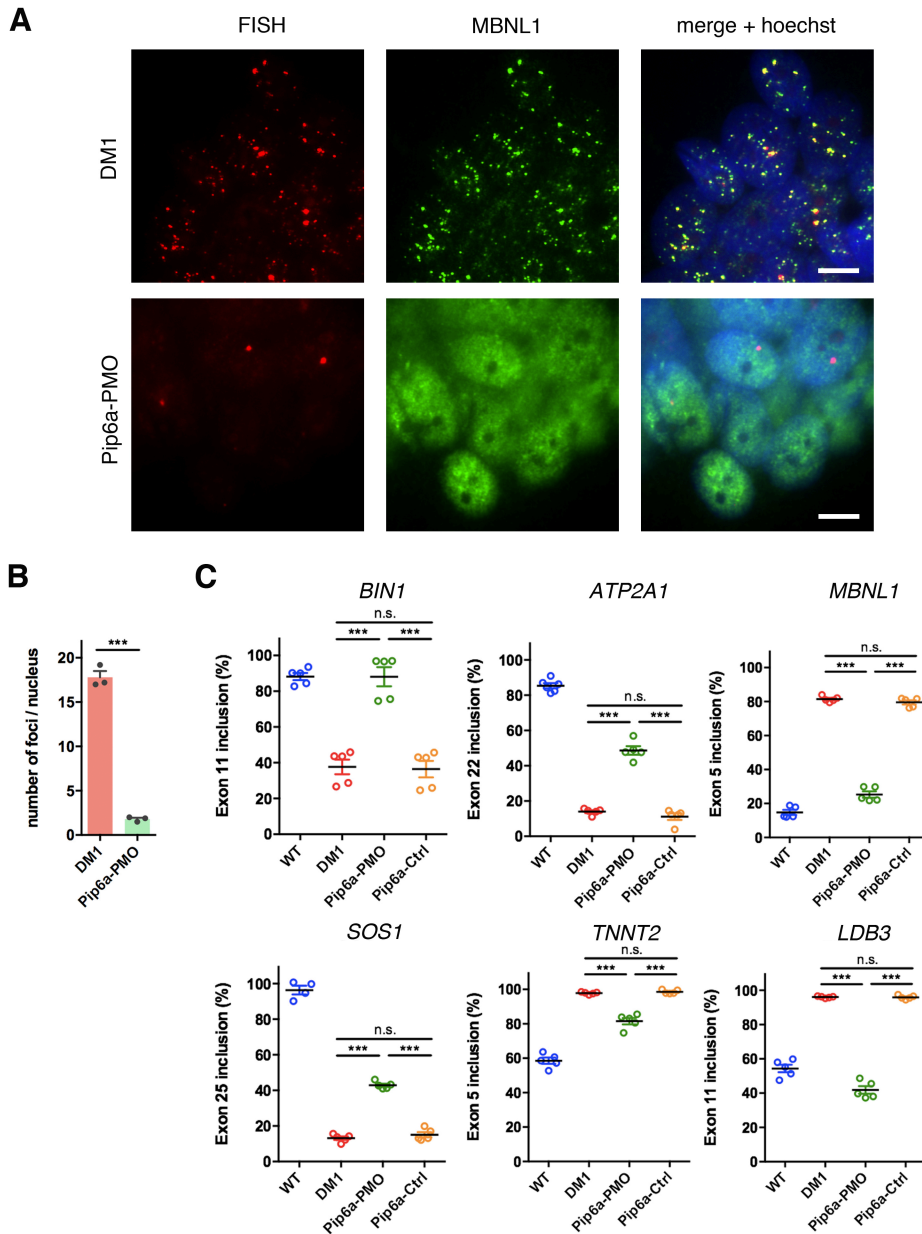


Supplementary figure 6: Treatment with Pip6a-PMO normalizes the DM1 proteomic profile of HSA-LR mice.

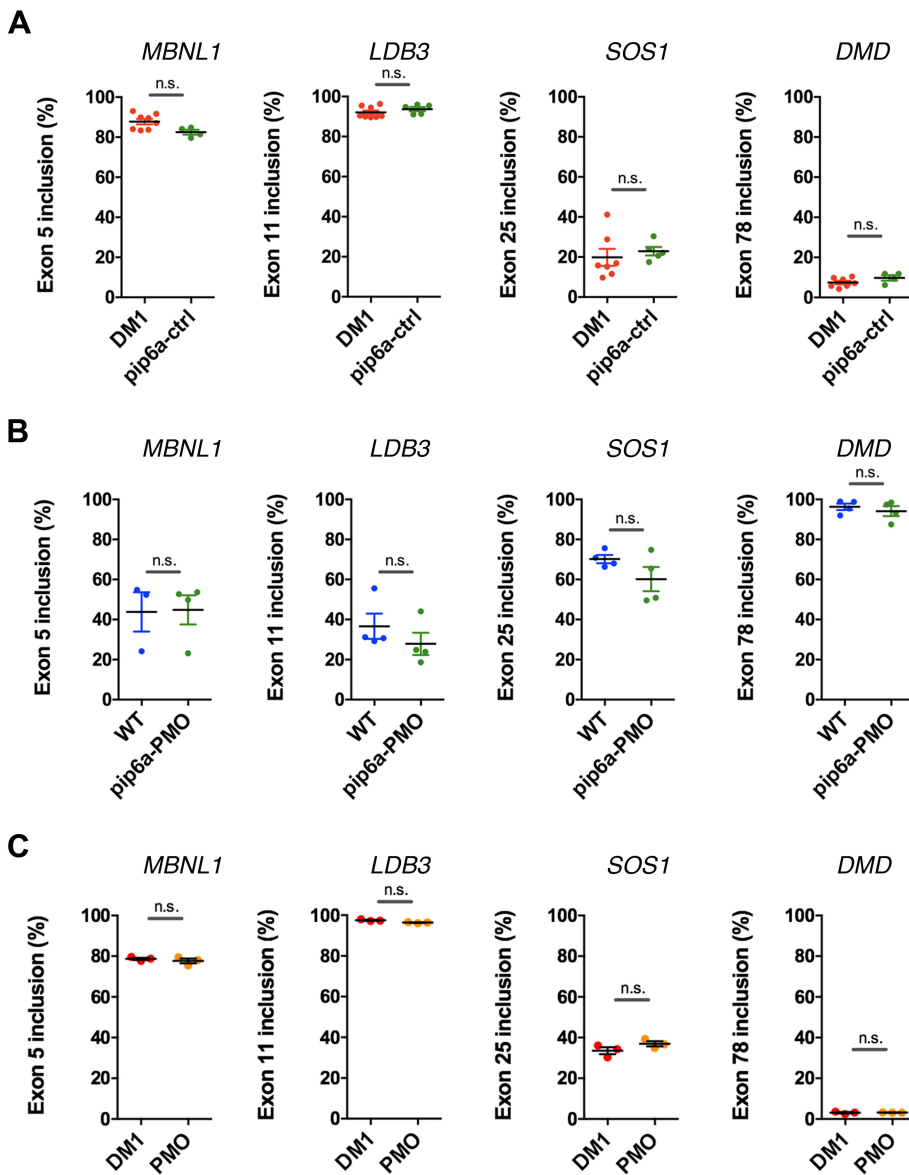
Supplementary figure 6: Treatment with Pip6a-PMO normalizes the DM1 proteomic profile of HSA-LR mice. *Quadriceps* muscle samples from WT, HSA-LR and Pip6a-PMO-CAG7 (Pip6a-PMO) treated HSA-LR mice were analyzed by label-free mass spectrometry. **A)** Correlation plots for quadriceps muscle from WT, HSA-LR and Pip6a-PMO treated HSA-LR mice (n=3 biological replicates per group). Each plot compares the quantitative intensity measurement of LC-MS features between two replicates. The Pearson correlation r-values demonstrate high reproducibility between and across all data sets, with a minimum r-value of 0.905 and a maximum of 0.963. **B)** Principal component analysis (PCA) illustrates a clear separation of the WT (blue), HSA-LR (red) and Pip6a-PMO treated HSA-LR (green) proteome, based on component 1 and component 2, which account for 31.1% and 16.2% of variability, respectively. PCA analysis illustrates Pip6a-PMO treated HSA-LR mice have a protein profile shift from a HSA-LR profile towards a WT profile, indicating Pip6a-PMO treatment can induce protein normalisation. **C)** Heatmap of 118 significant differentially expressed proteins from WT, HSA-LR and Pip6a-PMO treated HSA-LR mice. Differentially expressed proteins were analysed by hierarchical clustering of the z-scored normalised LFQ intensities (log₂) across all experimental groups. Red indicates increased protein abundance and blue indicates decreased protein abundance. **D)** Table with the top 6 increased and decreased proteins from quadriceps muscle from HSA-LR versus WT mice as revealed by label-free mass spectrometry analysis. **E)** Dot plots of specific proteins of interest identified from outlining the dynamic change of normalised LFQ intensities (log₂) from quadriceps muscle samples from WT, HSA-LR and Pip6a-PMO treated HSA-LR mice. *Data are expressed as mean +/- SEM. Statistics: One-way ANOVA and Bonferroni post hoc test; n.s., not significant; *, P≤0.05; **, P≤0.01; ***, P≤0.001.*



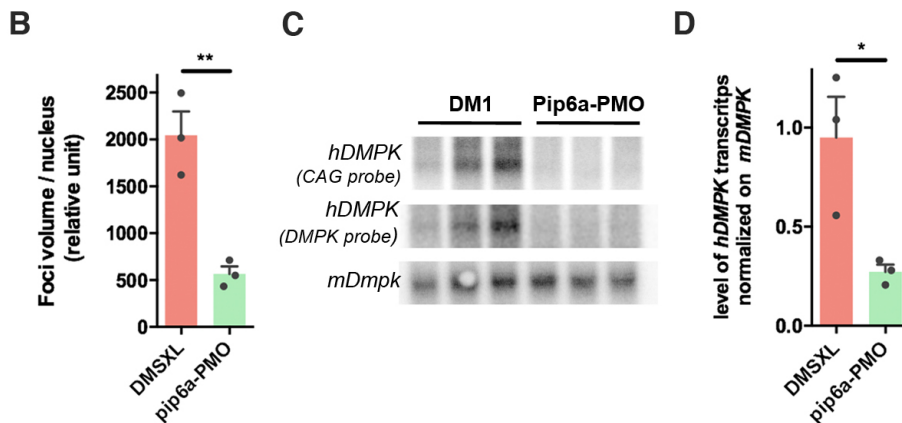
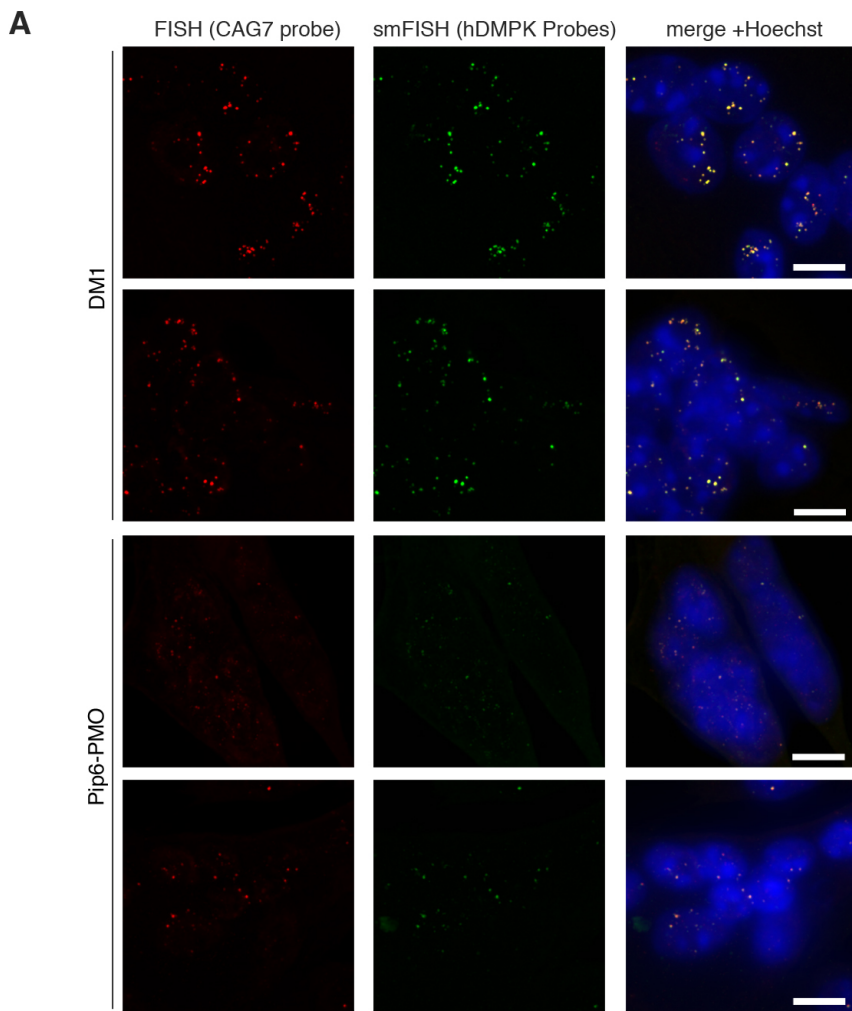
Supplementary figure 7: Treatment with Pip6a-PMO reduces the *foci* level in the muscles of HSA-LR mice. HSA-LR mice were treated with 3 systemic injections of Pip6a-PMO-CAG7 (Pip6a-PMO) at a 12.5 mg/kg dose and sacrificed 2 weeks after treatment. **A)** Representative pictures of gastrocnemius muscle section stained for CUGexp foci (FISH, red), Mbnl1 (green), fibers membrane (WGA, grey) and nucleus (Hoechst, blue) (scale bar : 50 μ m). **B and D)** Representative pictures of non-treated HSA-LR *gastrocnemius* sections stained for CUGexp-RNA foci (CAG probe, red), fibers membrane (WGA, green) and nucleus (Hoechst, blue). **C and E)** Same fields as in B and D where only the FISH signal is shown. **F and H)** Representative pictures of Pip6a-PMO treated HSA-LR *gastrocnemius* stained for CUGexp-RNA foci (CAG probe, red), fibers membrane (WGA, green) and Hoechst (blue). **G and I)** Same fields as in F and H where only the FISH signal is shown. Scale bar: 50 μ m.



Supplementary Figure 8: Pip6a-PMO corrects DM1-specific molecular symptoms in trans-differentiated muscle cells. Four days trans-differentiated immortalized DM1 fibroblasts (1300 CTG repeats) are treated with Pip6a-PMO-CAG7 (Pip6a-PMO) at 1 μ M and analyzed after 24h. **A**) FISH (CAG probe, red) /immuno-fluorescence (MBNL1, green) on DM1 or WT differentiated cells treated with Pip6a-PMO (Scale bar: 10 μ m). **B**) Quantification of mean number of foci per nucleus in treated DM1 differentiated cells (n=3; >300 nucleus per n). **C**) Quantification of splicing corrections, using RT-PCR, induced by Pip6a-PMO treatment in immortalized DM1 trans-differentiated muscle cells (n=5). Data are expressed as mean \pm SEM. Statistics: B, t-test ; C, One-way ANOVA with Newman-Keuls post-test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns: non-significant.



Supplementary figure 9: Effect of Pip6a-PMO on alternative splicing profiles is specific to DM1 cells and CAG antisense sequence. A) Quantification of splicing changes of *LDB3*, *MBNL1*, *SOS1*, *DMD* transcripts in 4 days differentiated immortalized DM1 (2600 CTG repeats) myoblasts treated with Pip6a-PMO-Control (Pip6a-ctrl) at 1 μ M and analyzed after 24 h (n=4). **B)** Quantification of splicing changes of *LDB3*, *MBNL1*, *SOS1*, *DMD* transcripts in 4 days differentiated immortalized WT myoblasts treated with Pip6a-PMO-CAG7 (Pip6a-PMO) at 1 μ M and analyzed after 24 h (n=4). **C)** Quantification of splicing changes of *LDB3*, *MBNL1*, *SOS1*, *DMD* transcripts in 4 days differentiated immortalized DM1 (2600 CTG repeats) myoblasts treated with unconjugated PMO-CAG (PMO) at 1 μ M and analyzed after 24 h (n=3). *Data are expressed as mean \pm SEM. Statistics: One-way ANOVA with Newman-Keuls post-test; ns: non-significant.*



Supplementary figure 10: Pip6a-PMO treatment reduces the quantity of mutated *hDMPK* transcripts in murine DMSXL muscle cells. Three days trans-differentiated immortalized murine DMSXL cells are treated with Pip6a-PMO-CAG7 (Pip6a-PMO) at 1 μ M and analyzed after 24h. **A**) FISH (CAG probe, red) /smFISH (24x *hDMPK* probes hybridized to fluorochrome-labelled FLAP oligonucleotide, green) shows that both CAG and DMPK probes exhibit a complete colocalization in DMSXL cells (both non-treated and treated) and that the number and intensity of foci is reduced in treated cells. **B**) Quantification of the foci volume (mean intensity x area of each foci using smFISH signal) in Pip6a treated cells shows an approx. 70% diminution in the quantity of mutated *hDMPK* transcript (n=3, >300 nucleus per n). **C**) Visualization by Northern blot of *hDMPK* and *mDmpk* transcripts using a CAG or a *DMPK* cDNA probe in DMSXL muscle cells treated with pip6a-PMO. **D**) Quantification by northern blot of *hDMPK* levels (normalized to *mDMPK*) shows an approx. 70% reduction in Pip6a treated cells (n=3). Data are expressed as mean \pm SEM. Statistics: t-test; *, $P < 0.05$; **, $P < 0.01$.

Supplementary table 1: list and annotation of the most deregulated genes in the gastocnemius muscles of HSA-LR and pip6a-PMO treated mice compared to WT mice.

Table columns description:	
Ensembl Gene ID	ENSEMBL gene identifier
Name	HGNC gene name
Description	Gene description and source
Gene type	Gene type
Fold change (log2) WT vs. HSA-LR	Fold change (log2) between WT and HSA-LR mice
Fold Change (Log2) WT vs. Pip6a-PMO	Fold change (log2) between WT and pip6a-PMO treated mice
WT counts (mean)	mean of the WT samples counts
HSA-LR counts (mean)	mean of the HSA-LR samples counts
pip6a-PMO counts (mean)	mean of the pip6a-PMO samples counts
Adj. p-value WT vs. HSA-LR	Adjusted p-values for WT vs. HSA-LR
Adj. p-value WT vs. Pip6a-PMO	Adjusted p-value for WT vs. Pip6a-PMO
Correction	percentage of correction in pip6a-PMO treated compared to HSA-LR

Ensembl_gene_ID	Name	Description	Gene type	Fold change (Log2) WT vs. HSA	Fold change (log2) WT vs. Pip6a	WT counts (mean)	HSA-LR counts (mean)	pip6a-PMO counts (mean)	Adj. p-value WT vs. HSA-LR	Adj. p-value WT vs. Pip6a-PMO	Correction (%)
ENSMUSG0000025401	Myo1a	myosin IA [Source:MGI Symbol;Acc:MGI:107732]	protein_coding	4.149	3.198	48	2378	966	2.19e-71	1.59e-41	59.62
ENSMUSG0000037474	Dtl	denticleless E3 ubiquitin protein ligase [Source:MGI Symbol;Acc:MGI:1924093]	protein_coding	3.275	0.662	14	232	24	5.88e-48	1.19e-01	94.26
ENSMUSG00000051159	Cited1	Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1 [Source:MGI Symbol;Acc:MGI:108023]	protein_coding	3.146	0.306	12	212	15	2.03e-38	7.48e-01	97.78
ENSMUSG00000027827	Kcnab1	potassium voltage-gated channel, shaker-related subfamily, beta member 1 [Source:MGI Symbol;Acc:MGI:109155]	protein_coding	-3.144	-1.597	5611	251	1159	2.43e-37	6.77e-09	18.53
ENSMUSG00000097451	Rian	RNA imprinted and accumulated in nucleus [Source:MGI Symbol;Acc:MGI:1922995]	processed_transcript	3.083	-0.681	866	8496	444	3.94e-77	2.79e-03	104.35
ENSMUSG00000058925	170001103Rik	RIKEN cDNA 170001103 gene [Source:MGI Symbol;Acc:MGI:1922694]	protein_coding	2.976	1.036	50	1059	153	1.99e-30	2.60e-03	89.39
ENSMUSG00000034206	Polq	polymerase (DNA directed), theta [Source:MGI Symbol;Acc:MGI:2155399]	protein_coding	2.65	1.453	66	505	191	7.46e-47	1.22e-12	71.3
ENSMUSG00000091078	Gm17218	predicted gene 17218 [Source:MGI Symbol;Acc:MGI:4938045]	antisense	2.61	0.84	17	238	40	8.87e-24	3.43e-02	88
ENSMUSG00000039145	Camk1d	calcium/calmodulin-dependent protein kinase ID [Source:MGI Symbol;Acc:MGI:2442190]	protein_coding	2.548	0.42	321	2089	390	1.20e-58	1.61e-01	94.63
ENSMUSG00000034457	Eda2r	ectodysplasin A2 receptor [Source:MGI Symbol;Acc:MGI:2442860]	protein_coding	2.531	0.862	1268	8266	2163	4.46e-53	1.88e-05	85.96
ENSMUSG00000032246	Calml4	calmodulin-like 4 [Source:MGI Symbol;Acc:MGI:1922850]	protein_coding	2.502	0.958	66	477	130	9.73e-37	1.50e-04	83.08
ENSMUSG00000097391	Mirg	miRNA containing gene [Source:MGI Symbol;Acc:MGI:3781106]	lincRNA	2.502	-0.215	39	309	26	4.38e-28	8.45e-01	102.97
ENSMUSG00000020798	Spns3	spinster homolog 3 [Source:MGI Symbol;Acc:MGI:1924827]	protein_coding	2.472	0.305	48	576	61	1.16e-20	7.59e-01	96.72
ENSMUSG00000029223	Uchl1	ubiquitin carboxy-terminal hydrolase L1 [Source:MGI Symbol;Acc:MGI:103149]	protein_coding	2.468	0.978	299	1758	538	1.19e-63	6.47e-09	81.65
ENSMUSG00000100410	2310020H05Rik	RIKEN cDNA 2310020H05 gene [Source:MGI Symbol;Acc:MGI:1916853]	lincRNA	2.454	1.083	179	1189	373	2.10e-36	3.09e-06	79.1
ENSMUSG00000019027	Dnah1	dynein, axonemal, heavy chain 1 [Source:MGI Symbol;Acc:MGI:107721]	protein_coding	2.434	0.751	21	167	39	4.59e-27	3.64e-02	87.11
ENSMUSG00000060275	Nrg2	neuregulin 2 [Source:MGI Symbol;Acc:MGI:1098246]	protein_coding	2.412	0.989	19	246	59	1.23e-19	5.94e-03	82.66
ENSMUSG00000060429	Sntb1	syntrophin, basic 1 [Source:MGI Symbol;Acc:MGI:101781]	protein_coding	2.405	0.51	244	1805	345	4.07e-26	2.74e-01	92.77
ENSMUSG00000042045	Sln	sarcolipin [Source:MGI Symbol;Acc:MGI:1913652]	protein_coding	2.371	0.386	339	7670	618	7.65e-17	6.66e-01	95.77
ENSMUSG00000084939	Gm830	predicted gene 830 [Source:MGI Symbol;Acc:MGI:2685676]	processed_transcript	2.367	2.267	29	209	179	7.52e-29	1.68e-25	15.6
ENSMUSG00000055214	Pld5	phospholipase D family, member 5 [Source:MGI Symbol;Acc:MGI:2442056]	protein_coding	2.356	1.787	22	248	140	4.70e-20	9.29e-11	48.35
ENSMUSG00000040828	Catsperd	catsper channel auxiliary subunit delta [Source:MGI Symbol;Acc:MGI:2147030]	protein_coding	2.354	0.239	5	103	8	5.29e-17	8.55e-01	97.08
ENSMUSG00000022865	Cxadr	coxsackie virus and adenovirus receptor [Source:MGI Symbol;Acc:MGI:1201679]	protein_coding	2.338	1.124	40	281	38	1.22e-24	9.32e-01	98.61
ENSMUSG00000028080	Lrba	LPS-responsive beige-like anchor [Source:MGI Symbol;Acc:MGI:1933162]	protein_coding	2.333	2.123	601	3381	2768	3.56e-44	7.14e-36	24.5
ENSMUSG00000075330	A930003A15Rik	RIKEN cDNA A930003A15 gene [Source:MGI Symbol;Acc:MGI:1915412]	protein_coding	2.301	0.77	25	181	43	1.35e-22	7.62e-02	86.97
ENSMUSG00000049928	Glp2r	glucagon-like peptide 2 receptor [Source:MGI Symbol;Acc:MGI:2136733]	protein_coding	2.285	0.477	13	120	21	8.31e-19	4.71e-01	92.28
ENSMUSG00000100550	2310039L15Rik	RIKEN cDNA 2310039L15 gene [Source:MGI Symbol;Acc:MGI:1916879]	lincRNA	2.284	0.306	7	76	9	3.84e-17	7.69e-01	95.3
ENSMUSG00000024524	Gnal	guanine nucleotide binding protein, alpha stimulating, olfactory type [Source:MGI Symbol;Acc:MGI:95774]	protein_coding	2.253	0.736	76	507	132	8.86e-23	3.60e-02	86.16
ENSMUSG00000021913	Ogdhl	oxoglutarate dehydrogenase-like [Source:MGI Symbol;Acc:MGI:3616088]	protein_coding	2.246	0.433	248	1657	331	8.86e-23	4.34e-01	93.17
ENSMUSG00000015745	Plekho1	pleckstrin homology domain containing, family O member 1 [Source:MGI Symbol;Acc:MGI:1914470]	protein_coding	2.245	1.481	908	5001	2510	7.94e-34	8.51e-14	58.27
ENSMUSG00000036882	Arhgap33	Rho GTPase activating protein 33 [Source:MGI Symbol;Acc:MGI:2673998]	protein_coding	2.22	1.162	60	510	187	9.71e-18	2.73e-04	72.78
ENSMUSG00000101227	Gm29506	predicted gene 29506 [Source:MGI Symbol;Acc:MGI:5580212]	sense_overlapping	2.201	1.377	24	164	77	5.08e-22	1.30e-07	60.64
ENSMUSG00000177116	Birc5	baculoviral IAP repeat-containing 5 [Source:MGI Symbol;Acc:MGI:1203517]	protein_coding	2.2	0.052	15	108	14	1.36e-18	9.75e-01	100.01
ENSMUSG00000021268	Meg3	maternally expressed 3 [Source:MGI Symbol;Acc:MGI:1202886]	lincRNA	2.2	-0.611	1335	6378	743	1.43e-52	1.55e-03	109.05
ENSMUSG00000042254	Cilp	cartilage intermediate layer protein, nucleotide pyrophosphohydrolase [Source:MGI Symbol;Acc:MGI:2444507]	protein_coding	2.2	0.075	3540	23270	3292	1.03e-19	9.65e-01	99.4
ENSMUSG00000028185	Dnase2b	deoxyribonuclease II beta [Source:MGI Symbol;Acc:MGI:1913283]	protein_coding	2.185	0.891	3	60	14	7.85e-15	3.21e-02	79.79
ENSMUSG00000026147	Col9a1	collagen, type IX, alpha 1 [Source:MGI Symbol;Acc:MGI:88465]	protein_coding	2.18	1.843	53	407	282	8.31e-19	1.46e-12	34.45
ENSMUSG00000075408	6030408B16Rik	RIKEN cDNA 6030408B16 gene [Source:MGI Symbol;Acc:MGI:1924967]	lincRNA	2.178	0.46	9	77	14	1.03e-16	5.16e-01	90.74
ENSMUSG00000079049	Serpinb1c	serine (or cysteine) peptidase inhibitor, clade B, member 1c [Source:MGI Symbol;Acc:MGI:2445363]	protein_coding	2.137	2.074	83	393	353	2.92e-42	7.07e-39	13.35
ENSMUSG00000068606	Gm4841	predicted gene 4841 [Source:MGI Symbol;Acc:MGI:3643814]	protein_coding	-2.11	-0.271	1679	232	1105	1.14e-19	7.47e-01	71.77
ENSMUSG00000048388	Fam171b	family with sequence similarity 171, member B [Source:MGI Symbol;Acc:MGI:2444579]	protein_coding	2.106	0.083	89	463	83	2.27e-24	9.54e-01	99.25
ENSMUSG00000101257	2310015K22Rik	RIKEN cDNA 2310015K22 gene [Source:MGI Symbol;Acc:MGI:1916815]	lincRNA	2.067	1.028	78	388	160	2.11e-26	1.18e-05	72.13
ENSMUSG00000025789	St8sia2	ST8 alpha-N-acetylneuraminidase alpha-2,8-sialyltransferase 2 [Source:MGI Symbol;Acc:MGI:106020]	protein_coding	2.04	0.099	36	187	34	1.76e-19	9.50e-01	98.9
ENSMUSG00000026764	Kif5c	kinesin family member 5C [Source:MGI Symbol;Acc:MGI:1098269]	protein_coding	2.04	1.572	38	341	193	5.93e-14	1.59e-07	45.6
ENSMUSG00000029026	Trp73	transformation related protein 73 [Source:MGI Symbol;Acc:MGI:1336991]	protein_coding	2.031	2.427	19	152	209	2.92e-15	4.91e-21	-36.53
ENSMUSG00000006642	Tcf23	transcription factor 23 [Source:MGI Symbol;Acc:MGI:1934960]	protein_coding	2.025	0.87	31	222	74	7.33e-15	1.90e-02	78.35
ENSMUSG000000031772	Ctnnap4	contactin associated protein-like 4 [Source:MGI Symbol;Acc:MGI:2183572]	protein_coding	2.017	0.507	6	76	13	8.28e-12	4.60e-01	89.24

ENSMUSG00000097819	Gm26813	predicted gene, 26813 [Source:MGI Symbol;Acc:MGI:5477307]	lincRNA	1.009	-0.271	116	233	80	1.98e-08	6.16e-01	117.84
ENSMUSG00000038453	Srcin1	SRC kinase signaling inhibitor 1 [Source:MGI Symbol;Acc:MGI:1933179]	protein_coding	-1.007	-0.45	333	133	201	3.46e-05	3.50e-01	39.38
ENSMUSG00000040502	March9	membrane-associated ring finger (C3HC4) 9 [Source:MGI Symbol;Acc:MGI:2446144]	protein_coding	1.005	0.732	58	131	96	5.34e-05	2.85e-02	43.29
ENSMUSG00000027215	Cd82	CD82 antigen [Source:MGI Symbol;Acc:MGI:104651]	protein_coding	-1.003	-0.482	790	343	480	1.23e-10	2.90e-02	36.23

	<i>gastrocnemius</i>		<i>quadriceps</i>	
	Genes with CTG	All genes	Genes with CTG	All genes
Genes deregulated in HSA vs WT (FC>2)	17	376	27	588
Genes deregulated in HSA vs WT (FC>2) and corrected in pip6A-treated mice	15	322	20	495
Genes deregulated only in pip6a-treated mice vs WT (FC>2)	1	15	4	20

Supplementary table 2. Number of genes containing CTG tract ($n \geq 7$) significantly deregulated in Pip6a-PMO-CAG7, HSA-LR and WT mice compared to all significantly deregulated genes.

Supplementary table 3: list and annotation of the most deregulated splicing events in the gastrocnemius muscles of HSA-LR and pip6a-PMO treated mice compared to WT mice.

Table columns description:

Ensembl Gene ID	ENSEMBL gene identifier
Name	HGNC gene name
Description	Gene description
Splice event	Splice event
Exon number	Exon number defined using Fosterdb database
Exon_bin	Exon bin identifier
Fold change (log2) WT vs. HSA-LR	Fold change (log2) between WT and HSA-LR mice
Fold Change (Log2) WT vs. Pip6a-PMO	Fold change (log2) between WT and pip6a-PMO treated mice
Gene type	Gene type
chr	Chromosome number
Strand	Strand
Start	Exon bin start
End	Exon bin end
Length	Exon bin length
EUC WT	Exon Usage Coefficient (DEXseq) for WT
EUC HSA-LR	Exon Usage Coefficient (DEXseq) for HSA-LR
EUC pip6a-PMO	Exon Usage Coefficient (DEXseq) for pip6a-PMO treated
Adj. p-value WT vs. HSA-LR	Adjusted p-values for WT vs. HSA-LR
Adj. p-value WT vs. Pip6a-PMO	Adjusted p-value for WT vs. Pip6a-PMO
Correction	percentage of correction in pip6a-PMO treated compared to HSA-LR

Splice events description:

se	Cassette Exon
afe	Alternative First Exon
ale	Alternative Last Exon
ir	Intron Retention
mult	Multiple Exons
mxe	Mutually exclusive Exons
ss	alternative Splice Site

Ensembl Gene ID	Name	Description	Exon number	exon_bin	Fold change (log2) WT vs. HSA-LR	Fold Change (Log2) WT vs. Pip6a-PMO	Splice event	Gene type	Chr.	Strand	Start	End	Length	EUC WT	EUC HSA-LR	EUC pip6a-PMO	Adj. p-value	Correction (%)
ENSMUSG00000061723	Tnnt3	troponin T3, skeletal, fast [Source:MGI Symbol;Acc:MGI:109550]	8	E019	6.854	0.61	se	protein_coding	chr7	+	142 507 770	142 507 788	19	50.598	148.251	58.326	6.44e-61	92.1
ENSMUSG00000038056	Kmt2c	lysine (K)-specific methyltransferase 2C [Source:MGI Symbol;Acc:MGI:2444959]	30	E041	6.603	5.824	se	protein_coding	chr5	-	25 329 241	25 329 273	33	1.157	11.282	8.653	3.65e-14	26
ENSMUSG00000061723	Tnnt3	troponin T3, skeletal, fast [Source:MGI Symbol;Acc:MGI:109550]	8	E018	6.435	0.543	se	protein_coding	chr7	+	142 507 750	142 507 769	20	56.65	149.162	63.801	4.20e-30	92.3
ENSMUSG00000048154	Kmt2d	lysine (K)-specific methyltransferase 2D [Source:MGI Symbol;Acc:MGI:2682319]		E040	4.793	4.32	ss	protein_coding	chr15	-	98 856 069	98 856 077	9	1.514	7.929	6.74	6.04e-08	18.5
ENSMUSG00000021798	Ldb3	LIM domain binding 3 [Source:MGI Symbol;Acc:MGI:1344412]	11	E007	4.241	0.872	se	protein_coding	chr14	-	34 555 343	34 555 528	186	25.112	73.637	32.692	1.45e-43	84.4
ENSMUSG00000026489	Coq8a	coenzyme Q8A [Source:MGI Symbol;Acc:MGI:1914676]		E033	3.993	-0.001	ale	protein_coding	chr1	-	180 179 500	180 179 592	93	5.013	19.382	5.012	1.80e-23	100
ENSMUSG00000028964	Park7	Parkinson disease (autosomal recessive, early onset) 7 [Source:MGI Symbol;Acc:MGI:2135637]		E013	3.965	0.694	ale	protein_coding	chr4	-	150 905 126	150 905 249	124	5.256	20.081	6.675	2.54e-17	90.4
ENSMUSG00000069601	Ank3	ankyrin 3, epithelial [Source:MGI Symbol;Acc:MGI:88026]	32	E067	3.687	0.429	se	protein_coding	chr10	+	69 953 440	69 953 472	33	2.916	10.375	3.383	2.07e-19	93.7
ENSMUSG00000029642	Polr1d	polymerase (RNA) I polypeptide D [Source:MGI Symbol;Acc:MGI:108403]	3	E068	3.6	3.498	se	protein_coding	chr5	+	147 101 300	147 101 303	4	8.6	28.093	27.231	2.14e-02	4.4
ENSMUSG00000057897	Camk2b	calcium/calmodulin-dependent protein kinase II, beta [Source:MGI Symbol;Acc:MGI:88257]	16	E022	3.468	1.05	mxe	protein_coding	chr11	-	5 982 759	5 982 761	3	13.871	40.609	19.616	2.34e-29	78.5
ENSMUSG00000029862	Cttn1	chloride channel, voltage-sensitive 1 [Source:MGI Symbol;Acc:MGI:88417]	7	E010	3.417	0.472	se	protein_coding	chr6	+	42 293 444	42 293 522	79	11.189	33.481	13.121	4.23e-49	91.3
ENSMUSG00000024381	Bin1	bridging integrator 1 [Source:MGI Symbol;Acc:MGI:108092]	7	E007	3.226	0.43	se	protein_coding	chr18	+	32 414 945	32 415 037	93	14.309	38.992	16.509	1.41e-38	91.1
ENSMUSG00000034055	Phka1	phosphorylase kinase alpha 1 [Source:MGI Symbol;Acc:MGI:97576]	19	E017	-3.148	-0.482	se	protein_coding	chrX	-	102 557 096	102 557 272	177	61.227	26.565	54.964	6.17e-88	81.9
ENSMUSG00000062908	Acadm	acyl-Coenzyme A dehydrogenase, medium chain [Source:MGI Symbol;Acc:MGI:87867]		E017	3.128	1.369	afe	protein_coding	chr3	-	153 937 784	153 937 809	26	5.677	16.43	9.082	8.08e-30	68.3
ENSMUSG00000049225	Pdp1	pyruvate dehydrogenase phosphatase catalytic subunit 1 [Source:MGI Symbol;Acc:MGI:2685870]	2	E006	3.045	-0.313	afe	protein_coding	chr4	-	11 965 614	11 965 648	35	7.513	20.862	6.746	2.11e-99	105.7
ENSMUSG00000051747	Ttn	titin [Source:MGI Symbol;Acc:MGI:98864]	11	E379	-3.044	-0.922	se	protein_coding	chr2	-	76 965 777	76 965 914	138	21.573	7.789	15.969	5.40e-26	59.3
ENSMUSG00000028207	Asph	aspartate-beta-hydroxylase [Source:MGI Symbol;Acc:MGI:1914186]	7	E032	-2.961	-0.893	ir	protein_coding	chr4	-	9 630 547	9 630 925	379	35.339	13.903	27.127	4.88e-58	61.7
ENSMUSG00000028207	Asph	aspartate-beta-hydroxylase [Source:MGI Symbol;Acc:MGI:1914186]	7	E033	-2.931	-1.05	ir	protein_coding	chr4	-	9 630 926	9 630 952	27	29.323	11.332	21.136	1.04e-42	54.5
ENSMUSG00000008140	Emc10	ER membrane protein complex subunit 10 [Source:MGI Symbol;Acc:MGI:1916933]	8	E001	-2.805	-0.754	ale	protein_coding	chr7	-	44 489 937	44 490 110	174	23.286	9.178	18.266	2.07e-22	64.4
ENSMUSG00000027763	Mbnl1	muscleblind-like 1 (Drosophila) [Source:MGI Symbol;Acc:MGI:1928482]	7	E038	2.675	0.412	se	protein_coding	chr3	+	60 614 683	60 614 736	54	12.22	29.035	14.035	9.14e-26	89.2
ENSMUSG00000051747	Ttn	titin [Source:MGI Symbol;Acc:MGI:98864]	311	E017	2.649	1.494	mult	protein_coding	chr2	-	76 716 749	76 717 048	300	15.714	35.903	25.447	2.25e-03	51.8
ENSMUSG00000030592	Ryr1	ryanodine receptor 1, skeletal muscle [Source:MGI Symbol;Acc:MGI:99659]		E045	-2.639	-0.146	se	protein_coding	chr7	-	29 056 234	29 056 248	15	83.375	48.086	81.303	1.20e-76	94.1
ENSMUSG00000027601	Mtfr1	mitochondrial fission regulator 1 [Source:MGI Symbol;Acc:MGI:1914722]	5	E014	-2.634	-0.323	se	protein_coding	chr3	+	19 208 491	19 208 527	37	14.116	5.751	12.666	2.07e-11	82.7
ENSMUSG00000058925	3Rik	RIKEN cDNA 170001103 gene [Source:MGI Symbol;Acc:MGI:1922694]	1	E002	2.622	2.515	afe	protein_coding	chr18	+	57 533 829	57 533 881	53	4.127	10.16	9.795	1.99e-03	6
ENSMUSG00000051747	Ttn	titin [Source:MGI Symbol;Acc:MGI:98864]	312	E016	2.614	1.404	mult	protein_coding	chr2	-	76 715 540	76 716 115	576	24.53	51.089	37.252	2.20e-02	52.1
ENSMUSG00000021699	Pde4d	phosphodiesterase 4D, cAMP specific [Source:MGI Symbol;Acc:MGI:99555]		E026	2.571	-0.838	afe	protein_coding	chr13	+	109 757 221	109 757 270	50	6.236	14.948	4.672	8.24e-05	118
ENSMUSG00000076617	Ighm	immunoglobulin heavy constant mu [Source:MGI Symbol;Acc:MGI:96448]		E003	2.569	0.301	ale	IG_C_gene	chr12	-	113 420 741	113 420 928	188	24.955	51.209	27.387	9.56e-33	90.7
ENSMUSG00000061723	Tnnt3	troponin T3, skeletal, fast [Source:MGI Symbol;Acc:MGI:109550]		E010	2.526	0.061	se	protein_coding	chr7	+	142 502 740	142 502 757	18	113.043	150.397	113.935	3.70e-03	97.6
ENSMUSG00000051747	Ttn	titin [Source:MGI Symbol;Acc:MGI:98864]	313	E015	2.505	1.403	mult	protein_coding	chr2	-	76 715 144	76 715 449	306	25.921	51.931	39.109	7.30e-02	49.3
ENSMUSG00000051747	Ttn	titin [Source:MGI Symbol;Acc:MGI:98864]	310	E018	2.48	1.395	mult	protein_coding	chr2	-	76 717 173	76 717 478	306	14.519	31.918	22.898	1.11e-02	51.8
ENSMUSG00000026950	Neb	nebulin [Source:MGI Symbol;Acc:MGI:97292]	86	E091	2.429	1.432	mult	protein_coding	chr2	-	52 221 842	52 221 843	62	11.254	24.976	18.153	7.63e-03	49.7
ENSMUSG00000026950	Neb	nebulin [Source:MGI Symbol;Acc:MGI:97292]	85	E093	2.423	1.339	mult	protein_coding	chr2	-	52 222 901	52 223 048	148	11.456	25.348	17.915	8.43e-03	53.5
ENSMUSG00000026950	Neb	nebulin [Source:MGI Symbol;Acc:MGI:97292]	85	E092	2.411	1.367	mult	protein_coding	chr2	-	52 222 737	52 222 900	164	12.127	26.602	19.1	5.35e-03	51.8
ENSMUSG00000026950	Neb	nebulin [Source:MGI Symbol;Acc:MGI:97292]	83	E096	2.394	1.347	mult	protein_coding	chr2	-	52 225 569	52 225 673	105	10.357	22.875	16.284	1.28e-03	52.7
ENSMUSG00000026950	Neb	nebulin [Source:MGI Symbol;Acc:MGI:97292]		E090	2.372	1.392	mult	protein_coding	chr2	-	52 221 640	52 221 781	142	14.434	30.755	22.742	7.79e-03	49.1
ENSMUSG00000031865	Dctn1	dynaactin 1 [Source:MGI Symbol;Acc:MGI:107745]	7	E013	2.37	-0.268	afe	protein_coding	chr6	+	83 186 428	83 186 445	18	8.605	19.061	7.852	1.76e-09	107.2
ENSMUSG00000049550	Clip1	CAP-GLY domain containing linker protein 1 [Source:MGI Symbol;Acc:MGI:1928401]		E013	2.366	0.019	se	protein_coding	chr5	-	123 581 592	123 581 597	6	15.324	32.376	15.419	2.22e-22	99.4
ENSMUSG00000061462	Obscn	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF [Source:MGI Symbol;Acc:MGI:2681862]	47	E082	2.321	1.328	mult	protein_coding	chr11	-	59 061 424	59 061 687	264	20.686	41.35	31.227	4.36e-07	49

ENSMUSG00000048154	Kmt2d	lysine (K)-specific methyltransferase 2D [Source:MGI Symbol;Acc:MGI:2682319]	21	E043	1.764	1.536	mult	protein_coding	chr15	-	98 856 310	98 856 389	80	9.726	17.582	16.311	1.17e-12	16.2
ENSMUSG00000035247	Hectd1	HECT domain containing 1 [Source:MGI Symbol;Acc:MGI:2384768]	7	E041	1.752	0.578	se	protein_coding	chr12	-	51 800 896	51 800 972	77	8.899	16.068	10.834	5.48e-03	73
ENSMUSG00000047921	Trappc9	trafficking protein particle complex 9 [Source:MGI Symbol;Acc:MGI:1923760]	3	E024	-1.744	-0.018	afe	protein_coding	chr15	-	73 055 748	73 055 812	65	19.783	11.072	19.668	7.68e-18	98.7
ENSMUSG00000061723	Tnnt3	tropoin T3, skeletal, fast [Source:MGI Symbol;Acc:MGI:109550]	1	E020	1.742	0.185	se	protein_coding	chr7	+	142 507 789	142 507 807	19	37.785	58.497	39.762	3.79e-09	90.5
ENSMUSG00000020456	Ogdh	oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide) [Source:MGI Symbol;Acc:MGI:1098267]	8	E017	-1.712	-0.471	se	protein_coding	chr11	+	6 324 878	6 324 922	45	11.43	6.366	9.742	2.91e-02	66.7
ENSMUSG00000061462	Obscn	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF [Source:MGI Symbol;Acc:MGI:2681862]	51	E077	1.709	1.164	mult	protein_coding	chr11	-	59 054 774	59 055 037	264	26.337	43.056	37.135	8.53e-05	35.4
ENSMUSG00000057897	Camk2b	calcium/calmodulin-dependent protein kinase II, beta [Source:MGI Symbol;Acc:MGI:88257]	21	E016	-1.7	0.127	mult	protein_coding	chr11	-	5 977 812	5 977 940	129	37.467	22.487	38.808	1.19e-32	109
ENSMUSG00000048154	Kmt2d	lysine (K)-specific methyltransferase 2D [Source:MGI Symbol;Acc:MGI:2682319]	27	E033	1.683	1.213	mult	protein_coding	chr15	-	98 854 714	98 854 851	138	12.408	21.618	18.573	1.09e-21	33.1
ENSMUSG00000061462	Obscn	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF [Source:MGI Symbol;Acc:MGI:2681862]	114	E001	-1.681	-0.262	mult	protein_coding	chr11	-	58 994 256	58 994 258	3	7.366	4.127	6.731	1.40e-02	80.4
ENSMUSG00000048154	Kmt2d	lysine (K)-specific methyltransferase 2D [Source:MGI Symbol;Acc:MGI:2682319]	33	E027	1.661	1.498	mult	protein_coding	chr15	-	98 851 607	98 851 789	183	15.931	27.136	25.81	4.52e-22	11.8
ENSMUSG00000048154	Kmt2d	lysine (K)-specific methyltransferase 2D [Source:MGI Symbol;Acc:MGI:2682319]	30	E030	1.66	1.39	mult	protein_coding	chr15	-	98 854 033	98 854 106	74	11.107	19.31	17.678	1.84e-19	19.9
ENSMUSG00000049225	Pdp1	pyruvate dehydrogenase phosphatase catalytic subunit 1 [Source:MGI Symbol;Acc:MGI:2685870]	1	E008	1.653	0.108	afe	protein_coding	chr4	-	11 965 884	11 965 927	44	6.747	11.86	7.001	3.13e-04	95
ENSMUSG00000024456	Diaph1	diaphanous related formin 1 [Source:MGI Symbol;Acc:MGI:1194490]	1	E043	-1.647	0.054	afe	protein_coding	chr18	-	37 935 412	37 935 423	12	8.785	4.987	8.948	1.16e-05	104.3
ENSMUSG00000032578	Cish	cytokine inducible SH2-containing protein [Source:MGI Symbol;Acc:MGI:103159]	1	E008	-1.64	1.609	ss	protein_coding	chr9	+	107 299 896	107 299 923	28	7.355	4.18	12.723	5.58e-02	269.1
ENSMUSG00000057897	Camk2b	calcium/calmodulin-dependent protein kinase II, beta [Source:MGI Symbol;Acc:MGI:88257]	22	E015	-1.634	0.102	mult	protein_coding	chr11	-	5 976 765	5 976 893	129	34.667	21.037	35.689	3.10e-24	107.5
ENSMUSG00000057897	Camk2b	calcium/calmodulin-dependent protein kinase II, beta [Source:MGI Symbol;Acc:MGI:88257]	20	E017	-1.633	0.041	mult	protein_coding	chr11	-	5 978 399	5 978 512	114	41.117	25.552	41.566	1.94e-27	102.9
ENSMUSG00000027257	Pacsin3	protein kinase C and casein kinase substrate in neurons 3 [Source:MGI Symbol;Acc:MGI:1891410]	3	E012	1.63	0.605	afe	protein_coding	chr2	+	91 257 329	91 257 334	6	6.938	12.098	8.538	1.02e-08	69
ENSMUSG00000030987	Stim1	stromal interaction molecule 1 [Source:MGI Symbol;Acc:MGI:107476]	1	E021	1.624	0.304	se	protein_coding	chr7	+	102 428 774	102 428 866	93	19.328	32.027	21.322	8.68e-24	84.3
ENSMUSG00000087410	4Rik	2310065F04 RIKEN cDNA 2310065F04 gene [Source:MGI Symbol;Acc:MGI:1921434]	1	E007	1.621	-0.195	afe	lincRNA	chr11	-	67 119 757	67 119 914	158	17.055	28.554	15.988	6.36e-63	109.3
ENSMUSG00000029472	Anapc5	anaphase-promoting complex subunit 5 [Source:MGI Symbol;Acc:MGI:1929722]	1	E043	1.617	-0.46	afe	protein_coding	chr5	-	122 818 543	122 818 583	41	11.85	20.26	10.138	1.22e-22	120.4
ENSMUSG00000026950	Neb	nebulin [Source:MGI Symbol;Acc:MGI:97292]	80	E099	1.615	0.793	mult	protein_coding	chr2	-	52 228 498	52 228 605	108	10.507	18.04	13.733	2.12e-02	57.2
ENSMUSG00000024431	Nr3c1	nuclear receptor subfamily 3, group C, member 1 [Source:MGI Symbol;Acc:MGI:95824]	10	E002	-1.614	-0.786	afe	protein_coding	chr18	-	39 410 547	39 411 986	1440	74.467	52.92	63.696	5.12e-18	50
ENSMUSG00000020190	Mknk2	MAP kinase-interacting serine/threonine kinase 2 [Source:MGI Symbol;Acc:MGI:894279]	1	E020	1.613	-0.449		protein_coding	chr10	-	80 672 252	80 672 291	40	9.364	16.125	8.031	7.80e-11	119.7
ENSMUSG00000009090	Ap1b1	adaptor protein complex AP-1, beta 1 subunit [Source:MGI Symbol;Acc:MGI:1096368]	15	E021	1.606	-0.037	se	protein_coding	chr11	+	5 033 242	5 033 262	21	11.421	19.487	11.279	1.03e-15	101.8
ENSMUSG00000061462	Obscn	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF [Source:MGI Symbol;Acc:MGI:2681862]	50	E078	1.605	0.923	mult	protein_coding	chr11	-	59 055 377	59 055 640	264	30.172	47.05	39.314	2.98e-02	45.8
ENSMUSG00000031352	Hccs	holocytochrome c synthetase [Source:MGI Symbol;Acc:MGI:106911]	1	E014	-1.603	-0.518	afe	protein_coding	chrX	-	169 320 281	169 320 294	14	7.21	4.149	6.033	3.31e-02	61.5
ENSMUSG00000029993	Nfu1	NFU1 iron-sulfur cluster scaffold [Source:MGI Symbol;Acc:MGI:1913290]	1	E013	-1.6	-0.168	se	protein_coding	chr6	+	87 016 176	87 016 394	219	26.039	15.549	24.714	1.28e-06	87.4
ENSMUSG00000064302	Clasp1	CLIP associating protein 1 [Source:MGI Symbol;Acc:MGI:1923957]	28	E060	1.592	0.375	mxe	protein_coding	chr1	+	118 541 675	118 541 698	24	10.275	17.522	11.668	1.51e-23	80.8
ENSMUSG00000039844	Rapgef1	Rap guanine nucleotide exchange factor (GEF) 1 [Source:MGI Symbol;Acc:MGI:104580]	14	E019	-1.588	-0.069	se	protein_coding	chr2	+	29 720 643	29 720 738	96	34.662	21.35	33.979	1.08e-19	94.9
ENSMUSG00000087410	4Rik	2310065F04 RIKEN cDNA 2310065F04 gene [Source:MGI Symbol;Acc:MGI:1921434]	1	E008	1.581	-0.159	afe	lincRNA	chr11	-	67 120 047	67 120 080	34	13.817	23.17	13.101	2.00e-29	107.7
ENSMUSG00000045962	Wnk1	WNK lysine deficient protein kinase 1 [Source:MGI Symbol;Acc:MGI:2442092]	10	E064	1.579	0.813	afe	protein_coding	chr6	-	119 968 476	119 968 663	188	15.322	25.522	20.013	2.58e-02	54
ENSMUSG00000064302	Clasp1	CLIP associating protein 1 [Source:MGI Symbol;Acc:MGI:1923957]	26	E055	1.563	0.146	ir	protein_coding	chr1	+	118 536 135	118 536 197	63	8.217	13.962	8.637	8.57e-07	92.7
ENSMUSG00000048154	Kmt2d	lysine (K)-specific methyltransferase 2D [Source:MGI Symbol;Acc:MGI:2682319]	31	E029	1.557	1.194	mult	protein_coding	chr15	-	98 853 790	98 853 840	51	11.009	18.513	16.431	8.24e-19	27.8
ENSMUSG00000032578	Cish	cytokine inducible SH2-containing protein [Source:MGI Symbol;Acc:MGI:103159]	1	E006	-1.555	1.046	afe	protein_coding	chr9	+	107 299 155	107 299 213	59	7.477	4.376	10.687	7.74e-03	203.5
ENSMUSG00000045962	Wnk1	WNK lysine deficient protein kinase 1 [Source:MGI Symbol;Acc:MGI:2442092]	16	E050	1.554	0.681	se	protein_coding	chr6	-	119 952 869	119 952 969	101	13.038	21.741	16.366	2.38e-02	61.8
ENSMUSG00000033196	Myh2	myosin, heavy polypeptide 2, skeletal muscle, adult [Source:MGI Symbol;Acc:MGI:1339710]	24	E026	1.546	-0.089	ss	protein_coding	chr11	+	67 188 662	67 188 838	177	98.033	120.72	96.741	1.75e-20	105.7
ENSMUSG00000061723	Tnnt3	tropoin T3, skeletal, fast [Source:MGI Symbol;Acc:MGI:109550]	1	E014	1.537	0.369	se	protein_coding	chr7	+	142 504 691	142 504 698	8	45.122	64.557	49.516	8.42e-06	77.4
ENSMUSG00000028619	Tceanc2	transcription elongation factor A (SII) N-terminal and central domain containing 2 [Source:MGI Symbol;Acc:MGI:1913776]	1	E013	1.535	0.71	afe	protein_coding	chr4	-	107 176 616	107 176 624	9	5.847	9.898	7.465	5.53e-03	60.1
ENSMUSG00000028207	Asph	aspartate-beta-hydroxylase [Source:MGI Symbol;Acc:MGI:1914186]	15	E021	1.529	0.039	mult	protein_coding	chr4	-	9 598 733	9 598 780	48	10.957	18.257	11.102	1.12e-11	98
ENSMUSG00000027827	Kcnab1	potassium voltage-gated channel, shaker-related subfamily, beta member 1 [Source:MGI Symbol;Acc:MGI:109155]	6	E006	1.526	0.02	se	protein_coding	chr3	+	65 266 491	65 266 534	44	6.74	11.351	6.786	1.03e-02	99
ENSMUSG00000033196	Myh2	myosin, heavy polypeptide 2, skeletal muscle, adult [Source:MGI Symbol;Acc:MGI:1339710]	23	E025	1.526	-0.076	ss	protein_coding	chr11	+	67 188 308	67 188 550	243	97.395	119.778	96.296	3.05e-19	104.9
ENSMUSG00000028207	Asph	aspartate-beta-hydroxylase [Source:MGI Symbol;Acc:MGI:1914186]	14	E022	1.525	0.135	mult	protein_coding	chr4	-	9 601 307	9 601 363	57	12.18	20.188	12.748	1.41e-06	92.9
ENSMUSG00000058925	3Rik	17000110 gene [Source:MGI Symbol;Acc:MGI:1922694]	2	E004	1.52	1.319	afe	protein_coding	chr18	+	57 538 033	57 538 084	52	8.217	13.762	12.864	9.60e-04	16.2
ENSMUSG00000038485	Socs7	suppressor of cytokine signaling 7 [Source:MGI Symbol;Acc:MGI:2651588]	4	E004	1.513	0.247	mxe	protein_coding	chr11	+	97 376 997	97 377 098	102	15.621	25.453	16.949	6.60e-05	86.5
ENSMUSG00000061462	Obscn	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF [Source:MGI Symbol;Acc:MGI:2681862]	52	E076	1.509	0.965	mult	protein_coding	chr11	-	59 054 162	59 054 425	264	27.042	41.78	35.965	5.84e-05	39.5
ENSMUSG00000030616	Syt12	synaptotagmin-like 2 [Source:MGI Symbol;Acc:MGI:1933366]	1	E026	-1.507	-1.477	afe	protein_coding	chr7	+	90 387 359	90 387 371	13	5.766	3.427	3.462	5.30e-02	1.5
ENSMUSG00000061313	Dhdh2	DDHD domain containing 2 [Source:MGI Symbol;Acc:MGI:1919358]	1	E033	-1.505	-0.567	afe	protein_coding	chr8	-	25 754 267	25 754 280	14	7.975	4.751	6.565	6.48e-02	56.3

ENSMUSG00000048154	Kmt2d	lysine (K)-specific methyltransferase 2D [Source:MGI Symbol;Acc:MGI:2682319]	55	E003	-1.34	-1.174	mult	protein_coding	chr15	-	98 834 301	98 834 592	292	49.982	35.002	36.689	9.45e-28	11.3
ENSMUSG00000024845	Tmem134	transmembrane protein 134 [Source:MGI Symbol;Acc:MGI:1914240]		E017	1.337	0.153	ir	processed_transcript	chr19	+	4 127 698	4 127 739	42	14.439	22.361	15.194	1.07e-12	90.5
ENSMUSG00000023830	Igf2r	insulin-like growth factor 2 receptor [Source:MGI Symbol;Acc:MGI:96435]	5	E050	1.331	0.53	ale	protein_coding	chr17	-	12 748 644	12 748 652	9	7.066	11.133	8.475	6.81e-02	65.4
ENSMUSG00000048154	Kmt2d	lysine (K)-specific methyltransferase 2D [Source:MGI Symbol;Acc:MGI:2682319]	52	E006	-1.327	-1.191	mult	protein_coding	chr15	-	98 835 182	98 835 467	286	48.009	33.525	34.868	1.14e-16	9.3
ENSMUSG00000085779	Atcayos	ataxia, cerebellar, Cayman type, opposite strand [Source:MGI Symbol;Acc:MGI:1916928]		E004	-1.321	-0.091	ale	antisense	chr10	+	81 197 204	81 198 439	1236	58.316	42.212	57.142	5.47e-16	92.7
ENSMUSG00000025162	Csnk1d	casein kinase 1, delta [Source:MGI Symbol;Acc:MGI:1355272]	11	E004	-1.318	-0.106	se	protein_coding	chr11	-	120 964 948	120 965 010	63	26.303	17.251	25.457	1.85e-34	90.7
ENSMUSG00000028207	Asph	aspartate-beta-hydroxylase [Source:MGI Symbol;Acc:MGI:1914186]	29	E004	1.317	-0.407	afe	protein_coding	chr4	-	9 475 390	9 475 458	69	10.491	16.328	9.133	3.45e-04	123.3
ENSMUSG00000008822	Acyp1	acylphosphatase 1, erythrocyte (common) type [Source:MGI Symbol;Acc:MGI:1913454]		E009	-1.316	0.177	afe	protein_coding	chr12	-	85 288 178	85 288 438	261	24.769	16.196	26.167	1.10e-18	116.3
ENSMUSG00000041329	Atp1b2	ATPase, Na ⁺ /K ⁺ transporting, beta 2 polypeptide [Source:MGI Symbol;Acc:MGI:88109]		E019	1.314	0.408	afe	protein_coding	chr11	-	69 603 554	69 603 613	60	14.036	21.603	16.079	4.11e-10	73
ENSMUSG00000036918	Ttc7	tetratricopeptide repeat domain 7 [Source:MGI Symbol;Acc:MGI:1920999]	17	E028	1.313	0.502	ale	protein_coding	chr17	+	87 361 849	87 361 921	73	16.554	25.261	19.509	9.09e-22	66.1
ENSMUSG00000048154	Kmt2d	lysine (K)-specific methyltransferase 2D [Source:MGI Symbol;Acc:MGI:2682319]	17	E047	1.303	0.984	mult	protein_coding	chr15	-	98 857 722	98 857 886	165	11.874	18.33	16.505	3.27e-10	28.3
ENSMUSG00000060913	Trim55	tripartite motif-containing 55 [Source:MGI Symbol;Acc:MGI:3036269]	9	E011	-1.297	-0.019	se	protein_coding	chr3	+	19 674 254	19 674 541	288	43.529	30.22	43.311	7.03e-26	98.4
ENSMUSG00000022744	Cldnd1	claudin domain containing 1 [Source:MGI Symbol;Acc:MGI:2447860]		E006	-1.296	-0.044	se	protein_coding	chr16	+	58 729 158	58 729 172	15	8.588	5.503	8.46	7.82e-03	95.9
ENSMUSG00000073557	Ppp1r12b	protein phosphatase 1, regulatory (inhibitor) subunit 12B [Source:MGI Symbol;Acc:MGI:1916417]	30	E009	-1.294	-0.066	afe	protein_coding	chr1	-	134 765 943	134 766 077	135	38.499	26.337	37.805	2.09e-10	94.3
ENSMUSG00000048154	Kmt2d	lysine (K)-specific methyltransferase 2D [Source:MGI Symbol;Acc:MGI:2682319]	51	E008	-1.293	-1.097	afe	protein_coding	chr15	-	98 837 075	98 837 121	47	37.739	25.763	27.366	1.93e-14	13.4
ENSMUSG00000036918	Ttc7	tetratricopeptide repeat domain 7 [Source:MGI Symbol;Acc:MGI:1920999]	17	E029	1.291	0.613	ale	protein_coding	chr17	+	87 361 922	87 361 943	22	14.744	22.476	18.057	7.22e-15	57.2
ENSMUSG00000052934	Fbxo31	F-box protein 31 [Source:MGI Symbol;Acc:MGI:1354708]		E015	-1.291	-0.009	se	protein_coding	chr8	-	121 564 638	121 564 724	87	41.828	28.936	41.728	1.98e-11	99.2
ENSMUSG00000028207	Asph	aspartate-beta-hydroxylase [Source:MGI Symbol;Acc:MGI:1914186]	19	E016	1.288	-0.023	mult	protein_coding	chr4	-	9 583 812	9 583 856	45	11.921	18.307	11.827	5.32e-04	101.5
ENSMUSG00000053929	Cyhr1	cysteine and histidine rich 1 [Source:MGI Symbol;Acc:MGI:1859320]	2	E009	1.285	0.039	ale	protein_coding	chr15	-	76 659 440	76 659 505	66	10.738	16.526	10.879	7.46e-05	97.5
ENSMUSG00000026773	Pfkfb3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 [Source:MGI Symbol;Acc:MGI:2181202]		E002	-1.283	0.136	ale	protein_coding	chr2	-	11 471 791	11 471 823	33	20.777	13.614	21.694	1.03e-04	112.9
ENSMUSG00000023118	Sympk	sympkin [Source:MGI Symbol;Acc:MGI:1915438]	19	E005	-1.282	-0.539	se	protein_coding	chr7	+	19 027 897	19 028 022	126	19.946	13.055	16.276	1.82e-03	53.3
ENSMUSG00000029446	Psph	phosphoserine phosphatase [Source:MGI Symbol;Acc:MGI:97788]		E011	-1.269	-0.135	se	protein_coding	chr5	-	129 776 830	129 776 865	36	15.051	9.811	14.391	1.60e-09	87.4
ENSMUSG00000038708	Golga4	golgi autoantigen, golgin subfamily a, 4 [Source:MGI Symbol;Acc:MGI:1859646]	25	E048	1.266	0.164	ale	protein_coding	chr9	+	118 579 542	118 579 592	51	9.68	14.842	10.234	3.18e-05	89.3
ENSMUSG00000038708	Golga4	golgi autoantigen, golgin subfamily a, 4 [Source:MGI Symbol;Acc:MGI:1859646]	25	E049	1.264	0.023	ale	protein_coding	chr9	+	118 579 593	118 579 606	14	8.211	12.619	8.275	1.83e-02	98.5
ENSMUSG00000019194	Scn1b	sodium channel, voltage-gated, type I, beta [Source:MGI Symbol;Acc:MGI:98247]		E002	1.259	0.333	ir	protein_coding	chr7	-	31 117 137	31 117 223	87	34.869	48.826	38.293	1.90e-38	75.5
ENSMUSG00000026150	Mff	mitochondrial fission factor [Source:MGI Symbol;Acc:MGI:1922984]		E009	-1.242	-0.129	se	protein_coding	chr1	+	82 728 067	82 728 238	172	28.79	19.483	27.683	1.60e-09	88.1
ENSMUSG00000048154	Kmt2d	lysine (K)-specific methyltransferase 2D [Source:MGI Symbol;Acc:MGI:2682319]	51	E009	-1.237	-1.111	afe	protein_coding	chr15	-	98 837 122	98 837 205	84	38.967	27.171	28.236	2.91e-11	9
ENSMUSG00000036550	Cnot1	CCR4-NOT transcription complex, subunit 1 [Source:MGI Symbol;Acc:MGI:2442402]	1	E084	1.234	0.008	afe	protein_coding	chr8	-	95 807 382	95 807 396	15	6.552	9.996	6.57	9.42e-02	99.5
ENSMUSG00000031812	Map1lc3b	microtubule-associated protein 1 light chain 3 beta [Source:MGI Symbol;Acc:MGI:1914693]		E009	-1.228	-0.398	ir	protein_coding	chr8	+	121 593 546	121 593 650	105	18.933	12.595	16.615	3.01e-05	63.4
ENSMUSG00000061462	Obscn	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF [Source:MGI Symbol;Acc:MGI:2681862]	114	E002	-1.228	0.136	mult	protein_coding	chr11	-	58 994 259	58 994 509	251	70.588	54.273	72.477	4.13e-08	111.6
ENSMUSG00000051910	Sox6	SRY (sex determining region Y)-box 6 [Source:MGI Symbol;Acc:MGI:98368]	22	E002	-1.227	-0.451	ale	protein_coding	chr7	-	115 471 344	115 474 455	3112	85.31	68.093	78.893	3.32e-06	62.7
ENSMUSG00000025586	Cpeb1	cytoplasmic polyadenylation element binding protein 1 [Source:MGI Symbol;Acc:MGI:108442]	7	E013	1.226	0.283	se	protein_coding	chr7	-	81 356 894	81 356 908	15	5.557	8.466	6.125	4.71e-02	80.5
ENSMUSG00000029152	Ociad1	OClA domain containing 1 [Source:MGI Symbol;Acc:MGI:1915345]	7	E022	-1.223	-0.116	afe	protein_coding	chr5	+	73 306 781	73 306 798	18	27.831	18.907	26.862	7.50e-35	89.1
ENSMUSG00000079055	Slc8a3	solute carrier family 8 (sodium/calcium exchanger), member 3 [Source:MGI Symbol;Acc:MGI:107976]	4	E010	1.218	-0.252	mxe	protein_coding	chr12	-	81 214 066	81 214 169	104	18.762	27.59	17.273	5.61e-14	116.9
ENSMUSG00000018661	Cog1	component of oligomeric golgi complex 1 [Source:MGI Symbol;Acc:MGI:1333873]		E025	-1.216	0.096	se	protein_coding	chr11	+	113 661 659	113 661 665	7	21.523	14.446	22.196	4.17e-10	109.5
ENSMUSG00000026150	Mff	mitochondrial fission factor [Source:MGI Symbol;Acc:MGI:1922984]	1	E003	-1.215	0.045	afe	protein_coding	chr1	+	82 724 919	82 724 924	6	7.617	5.015	7.736	7.43e-02	104.6
ENSMUSG00000048154	Kmt2d	lysine (K)-specific methyltransferase 2D [Source:MGI Symbol;Acc:MGI:2682319]	34	E026	1.212	1.089	mult	protein_coding	chr15	-	98 851 395	98 851 531	137	15.175	22.533	21.667	1.96e-10	11.8
ENSMUSG00000023830	Igf2r	insulin-like growth factor 2 receptor [Source:MGI Symbol;Acc:MGI:96435]	21	E035	1.211	0.656	se	protein_coding	chr17	-	12 715 378	12 715 493	116	11.843	17.737	14.762	4.75e-05	50.5
ENSMUSG00000061462	Obscn	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF [Source:MGI Symbol;Acc:MGI:2681862]	107	E009	-1.209	-0.087	mult	protein_coding	chr11	-	58 997 163	58 997 285	123	73.72	57.403	72.512	1.25e-14	92.6
ENSMUSG00000061462	Obscn	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF [Source:MGI Symbol;Acc:MGI:2681862]	106	E010	-1.209	-0.095	mult	protein_coding	chr11	-	58 997 975	58 998 142	168	79.151	62.493	77.811	2.03e-27	92
ENSMUSG00000061462	Obscn	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF [Source:MGI Symbol;Acc:MGI:2681862]	105	E011	-1.209	-0.088	mult	protein_coding	chr11	-	58 998 261	58 998 353	93	75.623	59.174	74.396	2.81e-31	92.5
ENSMUSG00000058056	Palld	palladin, cytoskeletal associated protein [Source:MGI Symbol;Acc:MGI:1919583]		E003	-1.206	-0.026	afe	protein_coding	chr8	-	61 515 021	61 515 034	14	24.384	16.519	24.186	1.76e-22	97.5
ENSMUSG00000025871	9Rik	RIKEN cDNA 4833439L19 gene [Source:MGI Symbol;Acc:MGI:1921162]	3	E014	1.203	0.126	se	protein_coding	chr13	-	54 564 185	54 564 272	88	29.215	41.201	30.34	2.50e-71	90.6
ENSMUSG00000000131	Xpo6	exportin 6 [Source:MGI Symbol;Acc:MGI:2429950]		E008	-1.198	-0.007	se	protein_coding	chr7	-	126 103 623	126 103 715	93	19.41	13.055	19.366	2.92e-10	99.3
ENSMUSG00000026131	Dst	dystonin [Source:MGI Symbol;Acc:MGI:104627]	72	E093	1.198	0.63	se	protein_coding	chr1	+	34 249 804	34 250 130	327	22.108	31.952	26.93	6.80e-02	51
ENSMUSG00000026950	Neb	nebulin [Source:MGI Symbol;Acc:MGI:97292]	152	E010	1.195	0.055	mxe	protein_coding	chr2	-	52 150 537	52 150 629	93	92.817	110.255	89.609	7.72e-03	95.5
ENSMUSG00000048154	Kmt2d	lysine (K)-specific methyltransferase 2D [Source:MGI Symbol;Acc:MGI:2682319]	36	E024	1.195	1.332	mult	protein_coding	chr15	-	98 847 358	98 847 481	124	19.822	28.848	30.056	8.46e-13	-13.4
ENSMUSG00000018661	Cog1	component of oligomeric golgi complex 1 [Source:MGI Symbol;Acc:MGI:1333873]		E026	-1.193	0.062	se	protein_coding	chr11	+	113 661 666	113 661 693	28	21.752	14.718	22.184	1.62e-15	106.1

ENSMUSG00000026207	Speg	SPEG complex locus [Source:MGI Symbol;Acc:MGI:109282]	14	E033	1.002	0.241	ale	protein_coding	chr1	+	75 403 777	75 403 990	214	19.059	26.205	20.605	2.37e-07	78.4
ENSMUSG00000030592	Ryr1	ryanodine receptor 1, skeletal muscle [Source:MGI Symbol;Acc:MGI:99659]	3	E118	1.002	0.428	se	protein_coding	chr7	-	29 117 334	29 117 438	105	44.966	57.312	50.077	9.01e-02	58.6

Supplementary Table 4: Differentially expressed proteins from WT, HSA-LR and Pip6a-PMO treated HSA-LR mice as revealed by label-free mass spectrometry analysis (quadriceps).
(UP: number of unique peptides, US unique sequence coverage %, MW, molecular weight in Kda).

	Protein name	Gene name	Accession #	Fold change	UP	US [%]	MW	p-value
	Ubiquitin carboxyl-terminal hydrolase isozyme L1	Uchl1	Q9R0P9	29,0	8	56,5	24,8	0,0028
	Musculoskeletal embryonic nuclear protein 1	Mustn1	Q99J11	20,7	3	48,8	8,9	0,0005
	Dnaj homolog subfamily B member 4	Dnajb4	Q9D832	9,5	7	37,7	37,8	0,0151
	Histone H1.0; Histone H1.0, N-terminally processed	H1f0	P10922	8,1	3	16,5	20,9	0,0045
	Heat shock protein beta-7	Hspb7	P35385	6,9	8	68,0	18,6	0,0025
	Heat shock protein beta-1	Hspb1	P14602	6,3	20	90,9	23,0	0,004
	Heat shock protein beta-8	Hspb8	Q9JK92	6,2	2	18,9	21,5	0,0351
	Nebulin-related-anchoring protein	Nrap	Q80XB4	6,2	10	6,5	195,8	0,01
	Tubulin polymerization-promoting protein family member 3	Tppp3	Q9CRB6	6,2	2	19,3	19,0	0,0288
	Four and a half LIM domains protein 1	Fhl1	P97447	5,3	21	72,5	31,9	0,0117
	LIM and cysteine-rich domains protein 1	Lmcd1	Q8VEE1	5,2	11	38,4	41,0	0,0167
	COP9 signalosome complex subunit 7a	Cops7a	Q9CZ04	5,1	3	14,5	30,2	0,0203
	Xin actin-binding repeat-containing protein 1	Xirp1	O70373	4,8	15	25,5	123,4	0,0123
	Activator of 90 kDa heat shock protein ATPase homolog 1	Ahsa1	Q8BK64	4,7	2	9,8	38,1	0,0049
	60S ribosomal protein L18	Rpl18	P35980	4,5	4	23,9	21,6	0,0063
	Ras-related protein Rab-2A	Rab2a	P53994	4,5	3	21,7	23,5	0,0002
	Heat shock protein beta-6	Hspb6	Q5EBG6	4,1	8	92,0	17,5	0,0326
	60S ribosomal protein L6	Rpl6	P47911	4,0	2	8,1	33,5	0,0125
	Heat shock 70 kDa protein 1A	Hspa1a	Q61696	4,0	5	10,6	70,1	0,026
	Major vault protein	Mvp	Q9EQK5	3,9	3	9,5	95,9	0,0321
	Myosin-1	Myh1	Q55X40	3,8	69	27,3	223,3	0,0031
	Protein S100-A4	S100a4	P07091	3,7	2	24,8	11,7	0,0009
	Protein diaphanous homolog 1	Diaph1	O08808	3,7	3	2,5	139,3	0,0461
	Adiponectin	Adipoq	Q60994	3,1	2	7,7	26,8	0,0478
	Filamin-C	Flnc	Q8VHX6	2,9	119	62,9	291,1	0,0002
	Biglycan	Bgn	P28653	2,6	5	17,1	41,6	0,0024
	Ankyrin-3	Ank3	G5E8K5	2,4	2	1,2	214,1	0,0221
	Myoglobin	Mb	P04247	2,4	21	87,7	17,1	0,0257
	Endoplasmic reticulum resident protein 44	Erp44	Q9D1Q6	2,3	3	14,3	46,9	0,0173
	Heat shock protein beta-2	Hspb2	Q99PR8	2,3	8	71,4	20,4	0,0002
	PDZ and LIM domain protein 3	Pdlim3	O70209	2,3	11	51,9	34,3	0,0011
	Mitogen-activated protein kinase 1	Mapk1	P63085	2,3	3	15,9	41,3	0,0253
	Alpha-actinin-2	Actn2	Q9JI91	2,2	48	60,1	103,8	0,0059
	ER membrane protein complex subunit 1	Emc1	Q8C7X2	2,2	2	3,4	111,6	0,0072
	Serpin B6	Serpinb6	Q60854	2,2	20	67,2	42,6	0,025
	Calpain-3	Capn3	Q64691	2,1	2	3,9	94,2	0,0034
	Heat shock protein beta-3	Hspb3	Q9QZ57	2,1	2	16,9	17,2	0,0055
	Metaxin-2	Mtx2	O88441	1,9	2	12,5	29,8	0,0153
	Kelch-like protein 40	Klh40	Q9D783	1,9	17	40,3	69,6	9E-05
	BAG family molecular chaperone regulator 3	Bag3	Q9JLV1	1,8	9	21,8	61,9	0,004
	Calpain-2 catalytic subunit	Capn2	O08529	1,8	7	16,1	79,9	0,0004
	Calcium/calmodulin-dependent protein kinase type II subunit delta	Camk2d	Q6PHZ2	1,7	4	8,8	56,4	0,0238
	Annexin A2	Anxa2	P07356	1,7	11	42,8	38,7	0,0002
	PRA1 family protein 3	Arl6ip5	Q8R5J9	1,7	5	20,2	21,6	0,0041
	Asporin	Aspn	Q99MQ4	1,6	9	31,9	42,6	0,0204
	Protein S100-A6	S100a6	P14069	1,6	3	36,0	10,1	0,0007
	Kelch-like protein 41	Klh41	A2AUC9	1,6	31	59,7	68,2	0,001
	Vimentin	Vim	P20152	1,6	24	60,1	53,7	0,0042
	Annexin A1	Anxa1	P10107	1,6	7	26,9	38,7	0,0256
	40S ribosomal protein S24	Rps24	P62849	1,6	2	18,0	15,4	0,0382
	Myotilin	Myot	Q9JIF9	1,6	30	77,6	55,3	0,003
	Small muscular protein	Smpx	Q9DC77	1,6	3	64,7	9,3	0,0047
	Annexin A5	Anxa5	P48036	1,5	9	30,4	35,8	0,0067
	Probable C->U-editing enzyme APOBEC-2	Apobec2	Q9WV35	1,5	11	67,0	25,7	0,0066
	Telethonin	Tcap	O70548	1,5	5	37,1	19,1	0,0003
	Phosphorylase b kinase regulatory subunit beta	Phkb	Q7TSH2	-1,5	27	34,7	123,9	0,0222
	Tropomyosin alpha-1 chain	Tpm1	P58771	-1,5	27	54,6	32,7	0,0125
	Phosphoglucosyltransferase-1	Pgm1	Q9D0F9	-1,6	44	89,7	61,4	0,0018
	Glutathione S-transferase Mu 1	Gstm1	P10649	-1,6	14	59,2	26,0	0,0016
	Leucine-rich repeat-containing protein 20	Lrrc20	Q8CI70	-1,6	4	25,0	20,8	0,049
	Secernin-3	Scrn3	Q3TMH2	-1,6	10	37,3	47,7	0,0009
	AMP deaminase 1	Ampd1	Q3V1D3	-1,6	40	60,5	86,1	0,0102
	Glycerol-3-phosphate dehydrogenase, mitochondrial	Gpd2	Q64521	-1,6	29	48,7	81,0	0,0003
	SH3 and cysteine-rich domain-containing protein 3	Stac3	Q8B271	-1,6	7	21,1	41,0	0,0154
	Alpha-actinin-3	Actn3	O88990	-1,6	63	67,0	103,0	0,0257
	NADP-dependent malic enzyme	Me1	P06801	-1,9	8	28,8	64,0	0,0166
	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	Gpd1	P13707	-1,9	23	61,9	37,6	0,003
	Myosin-binding protein C, fast-type	Mybpc2	Q5XKE0	-2,2	101	84,1	127,4	0,0068
	Trans-1,2-dihydrobenzene-1,2-diol dehydrogenase	Dhdh	Q9DBB8	-2,7	7	43,2	36,3	0,004
	Glutathione S-transferase Mu 2	Gstm2	P15626	-3,0	9	44,0	25,7	0,0025
	Leukotriene A-4 hydrolase	Lta4h	P24527	-4,3	5	11,5	69,1	0,0407
	Alpha-2-macroglobulin receptor-associated protein	Lrpap1	P55302	-4,6	3	8,3	42,2	0,0001
	Ubiquitin thioesterase OTUB1	Otub1	Q7TQI3	-5,3	3	12,2	31,3	0,0019
	PDZ and LIM domain protein 7	Pdlim7	Q3TJD7	-5,3	9	23,0	50,1	0,0013
	Dermatopontin	Dpt	Q9QZ26	-5,6	2	16,4	24,0	0,0021
	Ig gamma-1 chain C region, membrane-bound form	Ighg1	P01869	-8,2	2	13,6	35,7	0,0492
WT versus HSA	Protein name	Gene name	Accession #	Fold change	UP	US [%]	MW	p-value
	Ubiquitin carboxyl-terminal hydrolase isozyme L1	Uchl1	Q9R0P9	34,9	8	56,5	24,8	0,0022
	Musculoskeletal embryonic nuclear protein 1	Mustn1	Q99J11	11,0	3	48,8	8,9	0,0005
	Fatty acid synthase	Fasn	P19096	10,3	3	3,0	272,4	0,0074
	Calnexin	Canx	P35564	6,4	3	12,5	67,3	0,0003
	Dnaj homolog subfamily B member 4	Dnajb4	Q9D832	6,1	7	37,7	37,8	0,0310
	NADH-ubiquinone oxidoreductase chain 1	Mtnd1	P03888	5,5	2	8,8	36,1	0,0198
	Flavin reductase (NADPH)	Blvrb	Q923D2	4,8	5	41,3	22,2	0,0480
	Ras-related protein Rab-2A	Rab2a	P53994	4,8	3	21,7	23,5	0,0003
	Adiponectin	Adipoq	Q60994	4,7	2	7,7	26,8	0,0031
	Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial	Suclg2	Q9Z2I8	3,6	2	3,9	46,8	0,0079
	6-phosphogluconate dehydrogenase, decarboxylating	Pgd	Q9DCD0	2,9	2	7,7	53,2	0,0027
	Cytochrome b5 type B	Cyb5b	Q9CQX2	2,7	2	32,2	16,3	0,0047
	Hemoglobin subunit beta-2	Hbb-b2	P02089	2,7	6	46,3	15,9	0,0500
	Hemoglobin subunit beta-1	Hbb-b1	P02088	2,5	7	58,5	15,8	0,0307
	Plasminogen	Plg	P20918	2,5	3	4,2	90,8	0,0291

Protein name	Gene name	Accession #	Fold change	UP	US [%]	MW	p-value
Heat shock protein beta-1	Hspb1	P14602	2,5	20	90,9	23,0	0,0440
Serpin B6	Serpinb6	Q60854	2,4	20	67,2	42,6	0,0242
Mitogen-activated protein kinase 1	Mapk1	P63085	2,4	3	15,9	41,3	0,0159
Calpain-3	Capn3	Q64691	2,2	2	3,9	94,2	0,0061
Heat shock protein beta-2	Hspb2	Q99PR8	1,9	8	71,4	20,4	0,0029
Protein S100-A4	S100a4	P07091	1,8	2	24,8	11,7	0,0200
Integrin-linked protein kinase	Ilk	O55222	1,8	3	6,9	51,4	0,0017
Annexin A5	Anxa5	P48036	1,7	9	30,4	35,8	0,0025
T-complex protein 1 subunit eta	Cct7	P80313	1,7	6	15,3	59,7	0,0283
Dysferlin	Dysf	Q9ESD7	1,7	32	23,3	237,9	0,0011
Fatty acid-binding protein, adipocyte	Fabp4	P04117	1,7	12	68,9	14,7	0,0481
PDZ and LIM domain protein 3	Pdlim3	O70209	1,7	11	51,9	34,3	0,0150
Alpha-actinin-2	Actn2	Q9J191	1,6	48	60,1	103,8	0,0399
Filamin-C	Finc	Q8VHX6	1,6	119	62,9	291,1	0,0054
Calcium/calmodulin-dependent protein kinase type II subunit delta	Camk2d	Q6PHZ2	1,6	4	8,8	56,4	0,0018
Polyubiquitin-B	Ubb	P0CG49	1,6	2	19,5	14,7	0,0004
Annexin A11	Anxa11	P97384	1,6	10	26,4	54,1	0,0028
40S ribosomal protein S24	Rps24	P62849	1,6	2	18,0	15,4	0,0242
Spectrin beta chain, erythrocytic	Sptb	P15508	1,6	19	13,3	245,3	0,0211
Protein-glutamine gamma-glutamyltransferase 2	Tgm2	P21981	1,5	9	21,1	77,1	0,0009
60S acidic ribosomal protein P0	Rplp0	P14869	1,5	4	16,4	34,2	0,0090
40S ribosomal protein S7	Rps7	P62082	1,5	4	35,1	22,1	0,0238
Protein-arginine deiminase type-2	Padi2	Q08642	-1,6	15	32,7	76,2	0,0340
UTP--glucose-1-phosphate uridylyltransferase	Ugp2	Q91ZJ5	-1,7	32	67,1	57,0	0,0385
Metaxin-2	Mtx2	O88441	-1,8	2	12,5	29,8	0,0257
Myelin protein P0	Mpz	P27573	-1,9	7	25,8	27,6	0,0081
Target of Myb protein 1	Tom1	O88746	-2,0	3	11,4	54,3	0,0131
Phospholipid hydroperoxide glutathione peroxidase, mitochondrial	Gpx4	O70325	-2,2	2	13,2	22,2	0,0487
COP9 signalosome complex subunit 8	Cops8	Q8VBV7	-2,5	2	19,1	23,3	0,0372
Glutathione S-transferase Mu 2	Gstm2	P15626	-2,5	9	44,0	25,7	0,0053
40S ribosomal protein S16	Rps16	P14131	-5,5	4	27,4	16,4	0,0015
Prostaglandin reductase 2	Ptgr2	Q8VDQ1	-5,7	2	14,5	38,0	0,0139
Myosin regulatory light chain 12B	Myl12b	Q3THE2	-7,8	3	27,9	19,8	0,0090
Protein name	Gene name	Accession #	Fold change	UP	US [%]	MW	p-value
Ubiquitin thioesterase OTUB1	Otub1	Q7TQI3	7,0	3	12,2	31,3	0,0006
Fatty acid synthase	Fasn	P19096	4,8	3	3,0	272,4	0,0054
Plasminogen	Plg	P20918	4,7	3	4,2	90,8	0,0108
Leukotriene A-4 hydrolase	Lta4h	P24527	4,0	5	11,5	69,1	0,0464
Glycine--tRNA ligase	Gars	Q9C2D3	3,2	4	10,0	81,9	0,0448
PDZ and LIM domain protein 7	Pdlim7	Q3TJD7	3,1	9	23,0	50,1	0,0003
Cytochrome b5	Cyb5a	P56395	2,5	2	22,4	15,2	0,0063
Myosin-binding protein H	Mybph	P70402	1,9	23	67,1	52,6	0,012
Myosin-binding protein C, fast-type	Mybpc2	Q5XKE0	1,8	101	84,1	127,4	0,0108
Hemopexin	Hpx	Q91X72	1,6	13	27,4	51,3	0,0286
Proline-rich protein 33	Prr33	Q8C494	1,6	4	18,5	28,5	0,0132
Carboxylesterase 1D	Ces1d	Q8VCT4	1,5	7	20,0	61,8	0,0115
Glycogen phosphorylase, muscle form	Pygm	Q9WUB3	1,5	67	58,7	97,3	0,0114
40S ribosomal protein S3a	Rps3a	P97351	-1,5	6	25,4	29,9	0,0039
DnaJ homolog subfamily B member 4	Dnajb4	Q9D832	-1,6	7	37,7	37,8	0,0005
Hydroxyacylglycylglutathione hydrolase, mitochondrial	Hagh	Q99KB8	-1,6	4	17,8	34,1	0,0354
UTP--glucose-1-phosphate uridylyltransferase	Ugp2	Q91ZJ5	-1,7	32	67,1	57,0	0,0175
Filamin-C	Finc	Q8VHX6	-1,8	119	62,9	291,1	0,0007
Heat shock protein beta-3	Hspb3	Q9QZ57	-1,8	2	16,9	17,2	0,0249
Musculoskeletal embryonic nuclear protein 1	Mustn1	Q99J11	-1,9	3	48,8	8,9	0,0304
Myoglobin	Mb	P04247	-1,9	21	87,7	17,1	0,0058
Four and a half LIM domains protein 1	Fhl1	P97447	-2,0	21	72,5	31,9	0,0051
Protein S100-A4	S100a4	P07091	-2,0	2	24,8	11,7	0,004
Alpha-crystallin B chain	Cryab	P23927	-2,0	13	84,6	20,1	0,0328
Protein-arginine deiminase type-2	Padi2	Q08642	-2,1	15	32,7	76,2	0,0127
Coronin-6	Coro6	Q920M5	-2,3	4	11,3	52,6	0,0493
Microfibrillar-associated protein 5	Mfap5	Q9QZ16	-2,4	2	12,8	18,5	0,0003
Heat shock protein beta-6	Hspb6	Q5EBG6	-2,4	8	92,0	17,5	0,0017
Heat shock protein beta-1	Hspb1	P14602	-2,5	20	90,9	23,0	0,0012
Endoplasmic reticulum resident protein 44	Erp44	Q9D1Q6	-2,6	3	14,3	46,9	0,0173
60S ribosomal protein L6	Rpl6	P47911	-2,6	2	8,1	33,5	0,0218
Myosin-1	Myh1	Q55X40	-2,7	69	27,3	223,3	0,0061
Heat shock protein beta-7	Hspb7	P35385	-2,9	8	68,0	18,6	0,0035
Sarcolemmal membrane-associated protein	Slmap	Q3URD3	-3,0	3	4,1	96,9	0,0002
Nebulin-related-anchoring protein	Nrap	Q80XB4	-3,1	10	6,5	195,8	0,0395
Myelin protein P0	Mpz	P27573	-3,1	7	25,8	27,6	0,0069
Metaxin-2	Mtx2	O88441	-3,5	2	12,5	29,8	0,0047
LIM and cysteine-rich domains protein 1	Lmcd1	Q8VEE1	-3,9	11	38,4	41,0	0,0315
40S ribosomal protein S16	Rps16	P14131	-5,6	4	27,4	16,4	0,0019
Histone H1.0;Histone H1.0, N-terminally processed	H1f0	P10922	-6,6	3	16,5	20,9	0,0092
Myosin regulatory light chain 12B	Myl12b	Q3THE2	-8,1	3	27,9	19,8	0,0092

	CTL	2 injections, 2 weeks	3 injections, 2 weeks	3 injections, 4 weeks	3 injections, 6 months
Liver					
infiltration mononuclear cells	- -	- - - -	1 - - -	- - - -	1 - 1 1
hepatocyte vacuolation	- -	- 2 - -	- - - -	2 - - -	- - - -
cytoplasmic rarefaction, hepatocytes	1 1	- - - -	- - 2 -	1 2 - 1	- - - -
hepatocyte hypertrophy	- -	- - - 1	- 1 - 1	- - - -	- - - -
inclusions, hepatocytes	- -	- - - -	- 1 - -	- - - -	- - - -
hepatocyte degeneration and necrosis	- -	1 - - -	- - 1 -	1 - - -	1 - - -
Kidney					
infiltration mononuclear cells	1 -	1 1 - -	1 1 1 1	- 1 1 -	- - - -
tubular basophilia	- -	1 1 - 2	1 2 1 2	1 - - 1	1 - - -
tubular proteinuria	- -	- - 3 2	- 1 - -	1 - - 1	- 1 - 1
tubular hydropic degeneration	- -	- - 3 -	- - - -	- - - -	- - - -
tubular pigment	- -	- - - -	- - - 1	- - - -	- - - -
tubular swelling and dilatation	- -	- - - -	- - - -	- - - -	- 1 1 1

1: Minimal; 2: Mild; 3: Moderate; 4: Marked

Supplementary table 5. Histological observations in HSA-LR mice after Pip6a-PMO treatment.

The toxicology analysis of Pip6a-PMO-CAG7 (Pip6a-PMO) multiple injection treatments in HSA-LR mice indicated the existence of liver changes involving dose dependent hypertrophy and intranuclear inclusions. These changes are present in animals from the 2 injections / 2 weeks and 3 injections / 2 weeks groups but not in the 3 injections / 4 weeks or 3 injections / 6 months groups, suggesting complete recovery. The most significant treatment-related kidney changes included tubular proteinuria and dose dependent tubular basophilia and tubular hydropic degeneration. The reduced incidence and severity of the latter two changes in the 3 injections / 4 weeks and 3 injections / 6 months group suggest partial recovery.

Supplementary table 6. Primers and probes used for PCR, qPCR and FISH/smFISH analysis.

	Transcript	Forward (5' - 3')	Reverse (5' - 3')
Splicing	Mouse		
	<i>Cln1</i>	TTCACATCGCCAGCATCTGTGC	CACGGAACACAAAAGGCACTGAATGT
	<i>Mbn1</i>	GCTGCCCAATACCAGGTCAAC	TGGTGGGAGAAATGCTGTATGC
	<i>Atp2a1</i>	GCTCATGGTCCCTCAAGATCTCAC	GGGTCAGTGCCTCAGCTTTG
	Human		
	<i>LDB3</i>	GCAAGACCCTGATGAAGAAGCTC	GACAGAAGGCCGGATGCTG
	<i>MBNL1</i>	GCTGCCCAATACCAGGTCAAC	TGGTGGGAGAAATGCTGTATGC
	<i>SOS1</i>	CAGTACCACAGATGTTTGCAGTG	TCTGGTCGCTTCGTGGAGGAA
	<i>DMD</i>	TTAGAGGAGGTGATGGAGCA	GATACTAAGGACTCCATCGC
	Expression	<i>HSA</i>	TTCCATCGTCCACCGCAAAT
Internal probe		FAM-AGACACACTCCACCTCCAGCACGCGACTT-TAMRA	
FISH and smFISH	CUG repeats	Cy3-CAGCAGCAGCAGCAGCAGCAG Cy5-CAGCAGCAGCAGCAGCAGCAG	
	<i>hDMPK</i>	TCCGGGGGACACAGCAACCGCTGAATTTACTCTGGACCTCGTCGACATGCATT ACAGGTAGTTCTCATCCTGGAAGGCGAAGTTTACTCTGGACCTCGTCGACATGCATT CAGTCGGACCTCCTTAAGCCTCACCATTACTCTGGACCTCGTCGACATGCATT CAACTGCCGGACGTGTGCCTCTAGGTTTACTCTGGACCTCGTCGACATGCATT TTCTGGTTGTCCGTGCCGATGGCCTCTTACTCTGGACCTCGTCGACATGCATT CGCTGGAAC TGCCACTTCAGCTGTTTTTACTCTGGACCTCGTCGACATGCATT CTGTCCCTGAGGGCCATGCAGGAGTATTACTCTGGACCTCGTCGACATGCATT GTGAGCCCGTCTCCACCAAGTCGAATTACTCTGGACCTCGTCGACATGCATT TGATGTGTCGGTGGCACCTTCGAAATCTTACTCTGGACCTCGTCGACATGCATT GCTCCTTG TAGTGGACGATCTTGCCATTTACTCTGGACCTCGTCGACATGCATT GCGTCTGCCCATAGAACATTTATAGGCTTACTCTGGACCTCGTCGACATGCATT CATCTGCCCGCAGCTTGAGGCAAGAGTTACTCTGGACCTCGTCGACATGCATT GCCACAGCGGTCCAGCAGGATGTTGTTTACTCTGGACCTCGTCGACATGCATT GGTTTGATGTCCCTGTGCACGTAGCCATTACTCTGGACCTCGTCGACATGCATT CCGCCACGTAATACTCCATGACCAGTTACTCTGGACCTCGTCGACATGCATT CGTCTGCTTCATCTTCACTACCGCTATTACTCTGGACCTCGTCGACATGCATT GCCACTGCAAGAAGTCGGCCACGTATTACTCTGGACCTCGTCGACATGCATT CGCACCTTCCCGAATGTCCGACAGTGTACTCTGGACCTCGTCGACATGCATT GTCTCCGCGTGGAAATCCGCGTAGAATTACTCTGGACCTCGTCGACATGCATT CTCCCAAAC TTGCTCAGCAGTGTCATTACTCTGGACCTCGTCGACATGCATT CCCTTTCAGCATGTCCC ACTTGTCTTACTCTGGACCTCGTCGACATGCATT CCCGCTCCGATCACCTTCAGAATCTTACTCTGGACCTCGTCGACATGCATT ACCATCCAGTCGAGGCCAAAGAAGGTTACTCTGGACCTCGTCGACATGCATT AGTAGCCACAAAAGGCAGGTGGACCCTTACTCTGGACCTCGTCGACATGCATT	
	FLAP	Cy3-AATGCATGTCGACGAGGTCCGAGTGTA-Cy3	