SUPPLEMENTARY MATERIAL

Myotonic dystrophy CTG expansion affects synaptic vesicle proteins, neurotransmission and mouse behavior.

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SUPPLEMENTARY MATERIALS AND METHODS

Mouse genotyping. Animals were kept on a >90% C57BL/6 background. Transgenic status was determined by multiplex PCR. Tail DNA was amplified using DMHR8, DMHR9 and dmm9 oligonucleotide primers (Supplementary Table 1). DMHR8 hybridizes with both human and mouse DMPK gene sequences. DMHR9 hybridizes specifically with the human DMPK gene sequence, generating a 106 bp PCR product. dmm9 was designed to specifically recognize mouse Dmpk gene sequence, generating a 71 bp PCR product. Mouse genotyping is based on the intensity ratio between the bands corresponding to the transgenic DMPK and the endogenous Dmpk gene products: DMPK/Dmpk = 0, wild-type mouse; DMPK/Dmpk = 0.5 hemizygous mouse; DMPK/Dmpk = 1, homozygous mouse. CTG repeat size was determined by PCR of tail DNA with oligonucleotide primers 101 and 102, and after electrophoresis of amplification products through a 0.7% (m/v) agarose gel denaturing gel.

Mouse behavior analysis. *Exploratory behavior; open-field*: Mouse activity was tested in open boxes (open-fields: 42 cm wide, 42 cm large, 40 cm high) made of transparent Plexiglas. Animal activity was recorded with an infrared photobeam detection system (Acti-Track, LSI Letica, Panlab). The animals were subjected to two 30-minute sessions, 24 hours apart. The following data were collected to assess horizontal and vertical animal activity: (a) the distance travelled in the apparatus during each 30-minute session and the total on the two sessions, (b) the number of rearings during each 30-minute session and the total on the two sessions. Inhibition of exploration due to novelty and anxiety-related behavior, was assessed by determining the percentage of rearings during the first minute out of the first five minutes spent in the open-field arena. A percentage of vertical activity lower than 20% indicates that novelty induces behavior inhibition [Mouse age (mean ± SD): DMSXL, 139 ± 9 days;

wild-type, 138 ± 9 days]. *Anxiety, obsessive-compulsive disorder; marble burying test:* Animals were individually placed for 30 minutes in an open-field (42 cm wide, 42 cm large, 40 cm high) made of transparent Plexiglas, covered with sawdust. Twenty-five clean glass marbles were evenly spaced five cm apart on sawdust. At the end of the session the total number of marbles buried by each mouse was carefully counted. A marble was considered to be buried when at least 2/3 of its volume was covered with sawdust. The number of marbles buried is an index of anxiety-related and of obsessive-compulsive behaviors [Mouse age (mean ± SD): DMSXL, 129 ± 11 days; wild-type, 127 ± 13 days]. *Spatial memory; Morris water maze:* Mice were first trained with a cued platform so that they could associate the platform with the escape from the pool. The distance travelled and time spent to reach the platform were measured. The swimming speed was calculated as an indication of motor activity. In memory assessment, animals were trained over five training sessions (one per day), each composed of four trials, to find a hidden platform, always situated at the same place at the centre of a quadrant of the pool (target quadrant). After two rest days, animals were subjected to a 120-second probe trial in which the platform was removed. The number of entries in the target quadrant was used as an indication of spatial memory retention [Mouse age (mean ± SD): DMSXL, 145 ± 11 days; wild-type, 143 ± 13 days]. *Working memory; Morris water maze*: Animals were subjected to four daily working memory sessions. On each session, the platform was situated at a new place. The animal was subjected to two trials, an acquisition trial immediately followed by a retention trial. To begin each trial, the subject is randomly placed in one of five locations in the pool. The animal is allowed 15 seconds of rest on the platform after finding it (or after being put on the platform by the experimenter if it failed to find it within 60 seconds). The distance travelled and time spent to reach the platform on the acquisition and retention trials was measured. Working memory is present if the distance and the time to reach the platform is lower on the retention trial than on the

acquisition trial [Mouse age (mean ± SD): DMSXL, 145 ± 11 days; wild-type, 143 ± 13 days]. *Anhedonia; saccharine intake:* Animals were individually placed in individual cages with food and water available *ad libitum* over a period of four weeks. Two days per week, the bottle containing water was replaced by two pipettes at 5 pm. One pipette contained fresh water; the other contained a saccharin solution (0.2% saccharin in water). The amount of water and saccharine intake was measured on the following day at 9 am. The total amount of saccharine intake and the percentage of saccharine-containing water drunk by each mouse are considered as index of anhedonic behavior [Mouse age (mean ± SD): DMSXL, 153 ± 7 days; wild-type, 153 ± 8 days]. *Non-spatial long-term memory, passive avoidance test.* The test consisted in an acquisition and a retention session, conducted 24 hours apart. On the acquisition session, the animal was placed in the lit chamber. After 60 seconds the guillotine door was opened. As soon as the animal entered the dark chamber, the guillotine door was closed and an electric shock was delivered through the grid-floor. An animal that did not enter the dark chamber within 300 seconds was excluded from the experiment. The retention session was conducted on the same way, without the delivery of an electric shock once the animal entered the dark chamber. The latency to enter the dark chamber in the acquisition and retention sessions was recorded. The difference is an index of long-term memory [Mouse age (mean ± SD): DMSXL, 153 ± 9 days; wild-type, 152 ± 9 days].

Electrophysiological profiling. *PPF protocol:* two pulses with a decreasing inter-stimuli interval (300 ms, 200 ms, 100 ms, 50 ms, 25 ms) were applied at Schaeffer collaterals. Both stimuli were of equal intensity and set at 40% of the maximal amplitude response (Imax). The ratio of the second evoked response compared to the first one ($fEPSP_2/fEPSP_1$) was plotted as a function of the inter-stimulus interval. *LTD protocol:* Following a 10-minute control period to verify the stability of basal synaptic

transmission (stimulus set at 40% Imax and applied every 30 seconds), long-term depression (LTD) was triggered by a 15-minute train of low frequency stimulation (1 Hz) of Schaeffer collateral fibers (stimulus set at 70% Imax). LTD induction and maintain was monitored over a 50-minute period (stimulus set at 40% Imax applied every 30 seconds). *LTP protocol:* Long-term potentiation (LTP) was assessed in five DMSXL homozygotes and three wild type controls, aged four months. fEPCP recordings of synaptic responses were made using microelectrodes (1-3 MΩ) filled with artificial cerebral fluid placed within CA1 area in the stratum radiatum, following electrical stimulation of the Schaffer collaterals using a concentric bipolar stimulating electrode. The signal was amplified with an Axoclamp 2A amplifier and acquired using pClamp software program (Molecular devices, Union City, USA). LTP was induced by three one-second trains of high frequency stimulation (100 Hz) at 50% of Imax. LTP induction and maintain was monitored over a 60-minute period.

Supplementary Table 1. Oligonucleotide primer sequences.

Gene	MGI gene ID[a]	Exon	Alternative exon sequence	Forward primer	Reverse primer	PCR product size (bp)
App	11820	7 8	**Exon 7:** AGGTGTGCTCTGAACAAGCCGAGACCGGG CCATGCCGCGCAATGATCTCCCGCTGGTA CTTTGATGTCACTGAAGGGAAGTGTGTCCC ATTCTTTTACGGCGGATGTGGCGGCAACA GGAACAACTTTGACACGGAAGAGTACTGC ATGGCGGTGTGTGGCAGCGTGT **Exon 8:** CAACCCAAAGTTTACTCAAGACTACCAGTG AACCTCTTCCCCAAGATCCTGATAAAC	ACCGAGAGAACAACCAGCAC	GTCTCTCATTGGCTGCTTCC	556 (+7+8) 499 (+7-8) 331 (-7-8)
Atp2a1/ Serca1	11937	22	GATAACCACCCCCCTCCTCCATGTCTTTGA ACCGTGTCACAG	GCTCATGGTCCTCAAGATCTCAC	GGGTCAGTGCCTCAGCTTTG	218 (+22) 176 (-22)
DMPK	1760		N/A (SybrGreen)	GGAGAGGGACGTGTTG	CTTGCTCAGCAGTGTCA	133
Dmpk	13400		N/A (SybrGreen)	GGAAGAAAGGGATGTATTA	CTCAGCAGCGTTAGCA	132
DMPK	1760	N/A	N/A (genotyping)	DMHR8: TGACGTGGATGGGCAAACTG	DMHR9:	106
Dmpk	13400	N/A	N/A (genotyping)	DMHR8: TGACGTGGATGGGCAAACTG	dmm9: GCTTGTAACTGATGGCTGGG	71
Fxr1	14359	15 16	**Exon15:** ATGATAGTGAAAAAAAACCCCAGCGACGC AATCGTAGCCGCAGGCGTCGTTTCAGGGG TCAGGCAGAAGATAGACAGCCAG **Exon16:** TCACAGTTGCAGATTATATTTCTAGAGCTG AGTCTCAGAGCAGACAAAGAAACCTCCCA AGGGAAACTTTGGCTAAAAACAAGAAAGA AATG	GATAATACAGAATCCGATCAG	CTGAAGGACCATGCTCTTCAATCAC	370 (+15+16) 289 (-15+16) 197 (-15-16)
Grin1/ Nmdar1	14810	5	AGTAAAAAAAGGAACTATGAAAACCTCGAC CAACTGTCCTATGACAACAAGCGCGGACC CAAG	AGCGTCGTCCTCGCTTGCAGAA	GACAAGAGCATCCACCTGAGCT	360 (+5) 297 (-5)
Grin1/ Nmdar1	14810	21	GATAGAAAGAGTGGTAGAGCAGAGCCCGA CCCTAAAAAGAAAGCCACATTTAGGGCTAT CACCTCCACCCTGGCCTCCAGCTTCAAGAG ACGTAGGTCCTCCAAAGACACG	GCAGCTGGCCCTCCTCCCTCTC	ATGCCCCTGCCACCCTCACTTTT	381 (+21) 270 (-21)
Ldb3	24131	11	CACCCCTATTGAGCATGCTCCAGTGTGCAC CAGCCAGGCCACTTCCCCGCTGCTGCCTG CTTCTGCCCAGTCGCCCGCTGCTGCCTCTC CCATTGCGGCCTCGCCAACCCTGGCCACA	GGAAGATGAGGCTGATGAGTGG	TGCTGACAGTGGTAGTGCTCTTTC	761 (+11) 575 (-11)

			GCTGCTGCCACCCATGCTGCTGCCGCCTC TGCTGCAGGCCCTGCCGCAAGTCCCGTGG AGAATCCGAG			
Mapt/ Tau	17762	10	GTGCAGATAATTAATAAGAAGCTGGATCTT AGCAACGTCCAGTCCAAGTGTGGCTCGAA GGATAATATCAAACACGTCCCGGGTGGAG GCAGT	CTGAAGCACCAGCCAGGAGG	TGGTCTGTCTTGGCTTTGGC	367 (+10) 274 (-10)
Mbnl1	56758	7	ACTCAGTCGGCTGTCAAATCACTGAAGCG ACCCCTCGAGGCAACCTTTGACCTG	TGGTGGGAGAAATGCTGTATGC	GCTGCCCAATACCAGGTCAAC	270 (+7) 216 (-7)
Mbnl2	105559	7	ACTCAGTCGACTGCCAAAGCAATGAAGCG ACCTCTCGAAGCAACTGTAGACCTG	CTTTGGTAAGGGATGAAGAGCAC	ACCGTAACCGTTTGTATGGATTAC	255 (+7) 201 (-7)
Rab3A	97843	N/A	N/A (SybrGreen)	GCACCATCACCACAGCCTATTAC	TTGTTTCCCACCAGCAGCAC	154
Rab3A	97843	2	**Exon 2** ATGGCTTCCGCCACAGACTCTCGCTATGG GCAGAAGGAGTCCTCAGACCAGAACTTCG ACTATATGTTCAAGATCCTGATCATTGGGA ACAGCAGCGTGGGCAAAACCTCGTTCCTC TTCCGCTACGCAGATGACTCCTTCACTCCA GCCTTTGTCAGCACCGTTGGCATAGACTTC AAGGTCAAAACCATCTACCGCAACGACAA GAGGATCAAGCTGCAGATCTGG	CGCCAGCGTTGTCTCAGCTTAG	TAGGCTGTGGTGATGGTGCG	299 (+2) 71 (-2)
Rn18s/18S	19339	N/A	N/A (loading control)	CGGGTTGGTTTTGATCTG	CAGTGAAACTGCGAATGG	171
Rn18s/18S	19339		N/A (SybrGreen)	CAGTGAAACTGCGAATGG	CGGGTTGGTTTTGATCTG	165
Syn1	6853	12 and 12A	**Exon 12** GAGGCCCTCCACAGCCAGGCCCAGGACCT CAGCGCCAGGGACCCCCGCTGCAGCAGC GCCCACCCCCACAAGGCCAGCAACATCTTT CTGGCCTTGGACCGCCAGCTGGCAGCCCT CTGCCTCAGCGCCTACCAAGTCCCACCGC AGCACCTCAGCAGTCTGCCTCTCAGGCCA CACCAGTGACCCAGGGTCAAGGCCGCCAG TCGCGGCCAGTGGCAGGAGGCCCTGGAG CACCTCCAGCAGCGCGCCCACCAGCCTCC CCATCTCCACAGCGTCAGGCGGGGGCCCC GCAGGCTACCCGTCAGGCATCTATCTCTG GTCCAGCTCCAACGAAGGCCTCAGGAGCC CCACCCGGAGGGCAGCAGCGCCAGGGCC CTCCCCAAAAACCCCCAGGCCCTGCTGGT CCCACTCGTCAGGCCAGTCAGGCAGGTCC CGGACCTCGCACTGGGCCTCCCACCACAC AGCAGCCCCGGCCCAGCGGCCCAGGTCCT GCTGGACGTCCCGCCAAACCACAGCTGGC CCAGAAACCCAGCCAGGATGTGCCACCAC	CCTCCCCATCTCCACAGCGTC	GCTTTCACCTCGTCCTGGCTAAGG	383 (+12-12A) 299 (-12+12A)

			CCATCACCGCCGCTGCCGGGGGACCCCCG CACCCCCAGCTCAA **Exon 12A** GTCCCACTCGTCAGGCCAGTCAGGCAGGT CCCGGACCTCGCACTGGGCCTCCCACCAC ACAGCAGCCCCGGCCCAGCGGCCCAGGTC CTGCTGGACGTCCCGCCAAACCACAGCTG GCCCAGAAACCCAGCCAGGATGTGCCACC ACCCATCACCGCCGCTGCCGGGGGACCCC CGCACCCCCAGCTCAA			
Tbp	21374	N/A	N/A (loading control)	GGTGTGCACAGGAGCCAAGAGTG	AGCTACTGAACTGCTGGTGGGTC	191

a, http://www.ncbi.nlm.nih.gov/gene; N/A, not applicable;

Supplementary Table 2. Primary antibodies used for protein immunodetection in western blot analysis (WB) and immunofluorescence (IF).

Antigen	Supplier, reference	Application	PAGE (%)	Species origin	Incubation conditions	Ab dilution
Actin	M.Hernández (gift)	WB	10-12	Mouse	5% blotto, 1h, RT	1:3000
CELF1	Upstate, 05-621	WB	10	Mouse	5% blotto, 1h, RT	1:1000
CELF2	Sigma, C9367	WB	10	Mouse	2h, RT	1:1000
GFAP	DakoCytomation, Z0334	IF	N/A	Mouse	1h, RT	1:250
MBNL1	G. Morris, MB1a (gift)	WB	10	Mouse	5% blotto, 1 h, RT	1:1000
MBNL1	M. Swanson (3B10)	IF	N/A	Mouse	1h, RT	1:5000
MBNL2	G. Morris, MB2a (gift)	WB	10	Mouse	5% blotto, 1 h, RT	1:1000
MBNL2	G. Morris, MB2a (gift)	IF	N/A	Mouse	0.1% BSA, 10% NGS, 1h, RT	1:20
NeuN	Chemicon, MAB377	IF	N/A	Mouse	1h, RT	1:400
NSF	Abcam, ab16681	WB	10	Mouse	5% blotto, 1 h, RT	1:10000
Rab1A	Abcam, ab27528	WB	12	Rabbit	5% blotto, 1 h, RT	1:500
Rab3A	Abcam, ab3335	WB	12	Rabbit	10% blotto, 1h, RT	1:1000
RabGDI	Santa Cruz, sc-20447	WB	10	Goat	5% blotto, O/N, 4°C	1:500
Rabphilin-3A	Santa Cruz, sc-14687	WB	10	Goat	5% blotto, 1 h, RT	1:1000
RhoGDI	Abcam, ab52830	WB	12	Mouse	5% blotto, O/N, 4°C	1:7500
ß-Tubulin	Sigma, T4026	WB	10-12	Mouse	5% blotto, 1 h, RT	1:2000
Synapsin I	Abcam, ab8	WB	10	Rabbit	5% blotto, O/N, 4°C	1:10000
Synapsin I Ser553	Epitomics, 1532-1	WB	10	Rabbit	5% blotto, O/N, 4°C	1:5000
Synapsin I Ser9	Epitomics, 2228-1	WB	10	Rabbit	5% blotto, O/N, 4°C	1:5000
Synaptobrevin	Santa Cruz, sc-20039	WB	12	Mouse	5% blotto, 1 h, RT	1:200
Synaptotagmin	Santa Cruz, sc-12466	WB	10	Goat	5% blotto, 1 h, RT	1:1000
Syntaxin	Abcam, ab18010	WB	12	Mouse	5% blotto, 1 h, RT	1:5000

N/A, not applicable; NGS, normal-goat serum; O/N, over-night; RT, room temperature.

Supplementary Table 3. Clinical characteristic of control individuals and DM1 patients.

	Non-DM controls					
	a	**b**	**c**	**d**	**e**	**f**
Sex	M	M	M	M	M	M
Diagnosis	Rheumathoide arthritis	N/A	N/A,	Charcot-Marie-Tooth Disease;	Metastatic brain tumour	Limb-girdle muscular dystrophy
Neuropsychological profile	N/D	N/D	N/D	N/D	N/D	N/D
Neuroimaging	N/D	N/D	N/D	N/D	N/D	N/D
Age; cause of death	76; interstitial pneumonia	53; heart failure	79; *Pneumocystis* pneumonia	71; pneumonia	64; pneumonia	66; cardiac failure

	DM1 individuals								
	g	**h**	**i**	**j**	**k**	**l**	**m**	**n**	**o**
Sex	F	F	M	F	M	F	M	F	F
CTGs in blood (age of analysis)	N/D	>2500 (N/D)	N/D	1730 (73)	N/D	1300-1400 (40)	1600-1800 (40)	1800 (30)	700-1100 (30)
CTGs in brain (age of analysis)	250 (62)	500 (64)	500 (58)	>2500 (73)	>2000 (32)	>2000 (69)	>2000 (62)	>2000 (61)	>2000 (67)
Age of onset	54	N/D	46	40	Birth	40?	40	30	30
Clinical form of DM	Late onset DM1	N/D	Adult DM1	Adult DM1	Congenital DM1	Adult DM1?	Adult DM1	Adult DM1	Adult DM1
DM main symptoms	Cardiac arrhythmia; gait problems	Gait problems.	Limb muscle weakness	Muscle weakness and atrophy in all extremities	Cognitive deficits	Gait problems	Gait problems	Gait problems	Gait problems
Neuropsychological profile	N/D	N/D	N/D	Memory loss	Mental retardation	WAIS-R (VIQ74, PIQ73, IQ73)	N/D	N/D	N/D
Neuroimaging	N/D	General brain atrophy	N/D	Bilateral fronto-temporal atrophy	N/D	Diffuse atrophy	Normal	Temporal lobe atrophy	N/D
Age; cause of death	62; pneumonia	64; ARDS	58; pneumonia	73; pneumonia	32; pneumonia	69; pneumonia	62; heart failure	61; pneumonia	67; pneumonia

ARDS, acute respiratory distress syndrome; N/A not applicable; N/D, not determined.

SUPPLEMENTARY FIGURES

Supplementary Figure 1. Expression of expanded *DMPK* transgene induces regional foci accumulation and is associated with mild CELF1 hyperphosphorylation in the CNS. (**A**) Real-time quantitative PCR of transgene expression in eight dissected CNS regions. The graph shows the average *DMPK* relative expression (±SD) in homozygous DMSXL mice at one month of age and illustrates a region-specific expression profile. (**B**) Representation of regional RNA foci distribution in DMSXL brains. The percentage of foci-containing cells was calculated based on the analysis of three one-month-old DMSXL homozygotes. Arc, arcuate hypothalamic nucleus; CA1, cornu ammonis area 1 (hippocampus); CA3, cornu ammonis area 3 (hippocampus); Cbl, cerebellum; CC, cerebral cortex; CPu, caudate putamen (striatum); MD, mediodorsal thalamic nucleus; PV, paraventricular thalamic nucleus; RN, dorsal raphe nucleus; TC, temporal cortex; VM, ventromedial hypothalamic nucleus; N/D, not determined. RNA foci were also detected in frontal cortex (61±2%) and substantia nigra (45±4% cells) (data not shown). (**C**) FISH and IF techniques revealed nuclear foci of expanded *DMPK* mRNA in GFAP-positive astrocytes in *post-mortem* DM1 brains. (**D**) Western blot analysis of CELF1 and CELF2 in DMSXL (n=3) and wild-type (n=3) frontal cortex and brainstem at one month of age. (**E**) Bi-dimensional electrophoresis of CELF1 and CELF2 at one month of age. Black arrows indicate shifts towards a more acidic isoelectric point of CELF1 in DMSXL mice. The results shown in these representative western blots were reproduced in six of the seven DMSXL transgenic animals studied. (**F**) Western blot analysis of CELF1 and CELF2 in DM1 (n=5; patients g, h, i, j and k) and non-DM (n=3; individuals a, b and c) frontal cortex. Actin was used as loading control. (**G**) Western blot comparison of CELF1, CELF2, MBNL1 and MBNL2 protein levels between

wild-type frontal cortex and brainstem at one month of age. Decreasing amounts of a protein pool of whole cell lysate from three wild-type mice were electrophoresed and immunodetected. Higher CELF1 and CELF2 protein levels are expressed in frontal cortex than in brainstem, whereas brainstem appears to have a higher content of MBNL1 and MBNL2. Actin was used as loading control.

Supplementary Figure 2. Expression of embryonic-like RNA isoforms in adult DMSXL. (**A**) Representative splicing analysis of candidate genes in frontal cortex and brainstem of DMSXL (n=6) and WT (n=6) mice at 1 month of age. Newborn splicing profiles (P1) were determined in a cDNA pool prepared from three wild-type animals. (**B**) Splicing analysis of candidate genes throughout wild-type embryonic development (E12.5, E14.5, E18.5), in newborn (P1), postnatal day 8 (P8), and adult mice aged one (M1), four (M4) and 10 (M10) months. RNA from three individual animals were pooled for each developmental stage. Whole brain RNA was analyzed at E12.5-E18.5. Alternative exons are indicated on the right. *Tbp* and *18S* (data not shown) were used as loading controls.

Supplementary Figure 3. DMSXL mice show anhedonic-like behavior, but no deficits in horizontal activity, short-term or long-term memory. (**A**) Open-field assessment of horizontal (graph on the left) and vertical activity (graph on the right) in DMSXL (n=16) and age-matched controls (n=16). The graphs represent the average of the total distance travelled and total number of rearings (±SEM) during two sessions of 30 minutes each. (**B**) Average swim speed (±SEM) of DMSXL and wild-type in Morris water maze (n=15 per genotype). (**C**) Passive avoidance conditioning test of non-spatial long-term memory. The graphs represent the average latency (±SEM) of DMSXL (n=16) and wild-type (n=16) mice to enter the

dark chamber in the acquisition and retention trials. The statistically significant increase in both genotypes indicated functional long-term memory. ***, $P<0.001$. (**D**) Measurement of the volume of water drank by adult DMSXL homozygotes (n=12) and wild-type mice (n=12) during the habituation period to the graduated drinking pipettes in the anhedonia test of saccharine consumption.

Supplementary Figure 4. RNA missplicing in DMSXL hippocampus**.** Splicing analysis of alternative exons of *Grin1/Nmdar1*, *Mapt/Tau*, *Mbnl1* and *Mbnl2* by RT-PCR in hippocampus of (**A**) one- and (**B**) four-month-old DMSXL homozygous mice (n=4) and wild-type controls (n=4). DMSXL hippocampus exhibited abnormalities in *Grin1/Nmdar1* exon 20 (exclusion), *Mapt/Tau* exon 10 (exclusion), *Mbnl1* exon 7 (inclusion), *Mbnl2* exon 7 (inclusion). Splicing defects showed greater inter-individual variability at four months, when compared to younger one-month-old DMSXL mice. Asterisks (*) denote overt splicing defects in adult DMSXL mice. Newborn splicing profiles (P1) were determined in a cDNA pool prepared from three wild-type animals. Alternative exons are indicated on the right. *Tbp* and *18S* (data not shown) were used as loading controls.

Supplementary Figure 5. Western blot analysis of synaptic proteins in frontal cortex, brainstem and hippocampus of DMSXL mice. (**A**) Representative western blots of RAB3A quantification in frontal cortex, brainstem and hippocampus of DMSXL and wild-type mice aged four months. (**B**) Representative western blot analysis of SYN1 (Ser9, Ser553) phosphorylation in frontal cortex, brainstem and hippocampus of DMSXL homozygous mice and wild-type controls at four months of age. Three of the nine mice studied are represented. (**C**) Western blot analysis of candidate proteins of the exocytotic vesicle (RAB1A, Rabphilin 3A, RabGDI, SYN1,

Synaptobrevin, Synaptotagmin), proteins of the plasma membrane (Syntaxin) and cytoplasmic proteins (NSF). Whole cell protein lysates from frontal cortex, brainstem and hippocampus were prepared from four-month-old homozygous DMSXL mice (n=3) and age-matched wild-type controls (n=3). Actin was used as loading control. (**D**) Quantification of steady-state levels of synaptic proteins in DMSXL frontal cortex, brainstem and hippocampus. The graphs show the average protein levels relative to normalized wild-type littermates (±SEM). None of the additional candidate proteins studied was significantly dysregulated in DMSXL mice. (**E**) Splicing analysis of known alternative exons of *Rab3A* and *Syn1* in frontal cortex of four-month-old DMSXL homozygous mice (n=4) and wild-type controls (n=4).

Supplementary Figure 6. CTG-transfected PC12 cells show RNA foci, missplicing of endogenous transcripts and abnormal protein metabolism. (**A**) FISH detection of RNA foci in PC12 cells transfected with CTG-containing DT960 plasmids. Nuclear RNA foci were observed in ~30% cells, as revealed by FISH, and co-localised with MBNL1 and MBNL2. PC12 cells transfected with no-repeat DMPKS plasmid did not show RNA accumulation (data not shown). DAPI was used for staining of nuclear genomic DNA. (**B**) Analysis of alternative splicing in transfected PC12 cells. The graph represents the average inclusion rate (±SEM) of alternative exons in three independent assays. The expression of expanded DT960 transcripts in PC12 cells resulted in a statistically significant decrease in the inclusion rate of endogenous *Grin1/Nmdar1* exon 21 and *Mapt/Tau* exon 10, relative to mock- and DMPKS-transfected cells. (**C**) Western blot analysis of the expression of RAB3A expression and phosphorylation of SYN1 in PC12 cells. Non-transfected (NT), mock-, GFP- and DMPKS-transfected cells were used as controls. The expression of expanded transcripts in DT960-transfected cells resulted

in RAB3A upregulation and abnormal SYN1 hyperphosphorylation in culture. *, $P<0.05$.