# Supplementary Information

**Ultrasound-enhanced delivery of Morpholino with Bubble liposomes ameliorates the myotonia of myotonic dystrophy model mice**

Michinori Koebis, Tamami Kiyatake, Hiroshi Yamaura, Kanako Nagano, Mana Higashihara, Masahiro Sonoo, Yukiko Hayashi, Yoichi Negishi, Yoko Endo-Takahashi, Yukihiko Aramaki, Dai Yanagihara, Ryoichi Matsuda, Masanori P Takahashi, Ichizo Nishino, Shoichi Ishiura

**a**



**b**
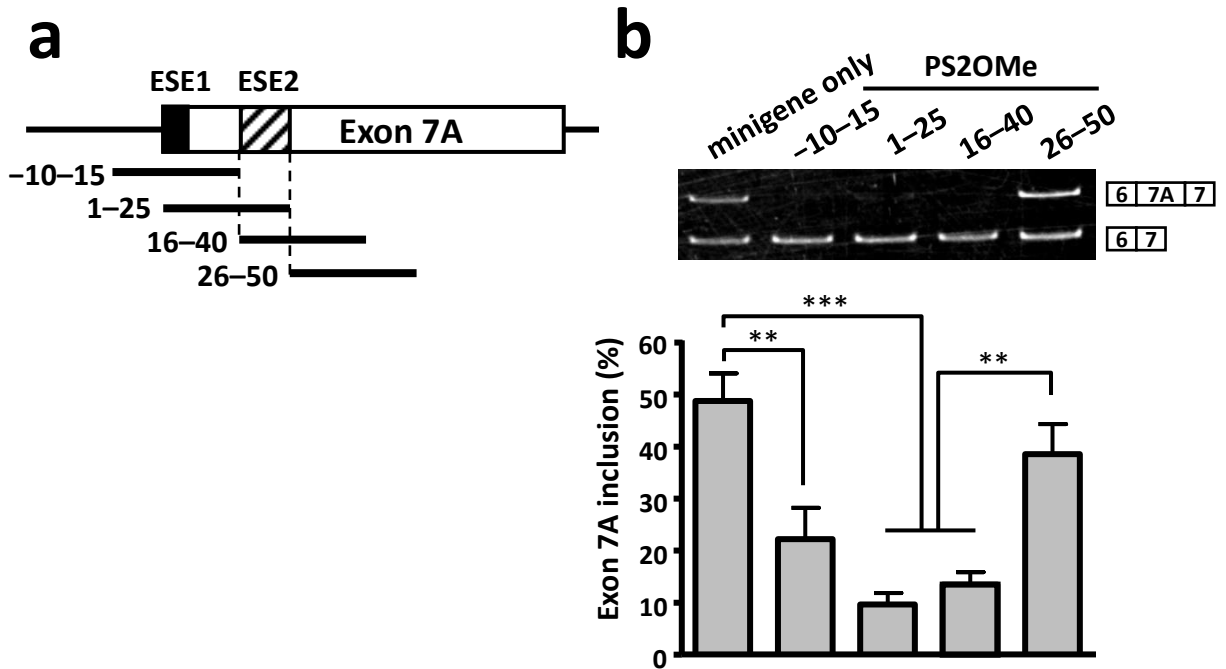


**Supplementary Fig. S1 | Putative ESE in *Clcn1* exon 7A revealed by the cellular splicing assay. a,** Schematic diagram of the genomic region around *Clcn1* exon 7A. We previously showed that an ESE (ESE1) is located at the beginning of exon 7A[27]. **b,** Cellular splicing assay. 16–40, which did not cover ESE1, prevented the inclusion of exon 7A (*n* = 3). Statistical significance was analysed by Tukey's multiple comparison test (** *P* < 0.01, *** *P* < 0.001).

**a**



**b**
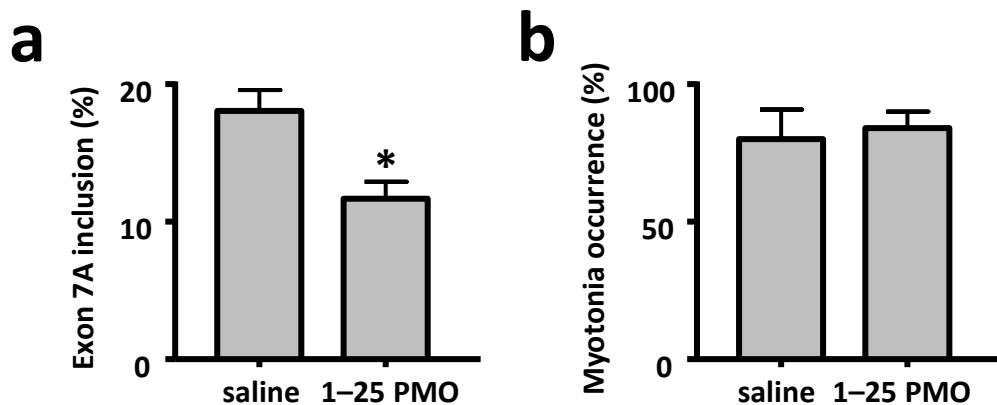


**Supplementary Fig. S2 | Intramuscular administration of 1–25 PMO without Bubble liposomes and ultrasound improved *Clcn1* splicing but not myotonia.** We administrated 60 μg of +1–25 PMO in 50 μl of saline intramuscularly into the TA muscles of *HSA*LR mice four times at weekly intervals. **a,** The alternative splicing of *Clcn1* exon 7A ($n = 5$; Student's *t*-test; *, $P < 0.05$). **b,** The rate of occurrence of myotonia, as determined by needle electrode insertion.

# Supplementary Table ST1 | Sequences of oligonucleotides

| | | sequence (5' to 3') |
|---|---|---|
| 2' O-Methyl RNA | -10-15 | CUGCCCAGGCACGGUCUGCAACAGA |
| | +1-25 | GGAGAUCAAGCUGCCCAGGCACGGU |
| | +16-40 | CACAGGCUGGCACCAGGAGAUCAAG |
| | +26-50 | ACGCCCACUGCACAGGCUGGCACCA |
| | +51-75 | AUCAGGGGCUGCAAAGUAGCAUCCC |
| | +76-90 | UAGGGAGGAAGUGAAACUUGCCUCC |
| | Control | AGACAACGUCUGGCACGGACCCGUC |
| Morpholino | -11-14 | CCAGGCACGGTCTGCAACAGAGAAG |
| | +1-25 | GGAGATCAAGCTGCCCAGGCACGGT |
| | Control | TGGCACGGACCCGTCGAACTAGAGG |

# Supplementary Table ST2 | Sequences of primers

| | target | | sequence (5' to 3') |
|---|---|---|---|
| Primer | Clcn1 minigene | Fw | AAGTCCGGACTCAGATCTCG |
| | | Rv | CATCTCCATCTCCAGCTCCT |
| | Clcn1 | Fw | GTCCTCAGCAAGTTTATGTCC |
| | | Rv | GAATCCTCGCCAGTAATTCC |
| | Cypher | Fw | GGAAGATGAGGCTGATGAGTGG |
| | | Rv | TGCTGACAGTGGTAGTGCTCTTTC |
| | Mbnl1 | Fw | GCTGCCCAATACCAGGTCAAC |
| | | Rv | TGGTGGGAGAAATGCTGTATGC |
| | Ryr1 | Fw | GACAATAAGAGCAAAATGGC |
| | | Rv | CTTGGTGCGTTCCTGATCTG |
| | Serca1 | Fw | GCTCATGGTCCTCAAGATCTCAC |
| | | Rv | GGGTCAGTGCCTCAGCTTTG |