The intragenic microRNA *miR199A1* in the *dynamin 2* gene contributes to the pathology of X-linked centronuclear myopathy

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Running title: **MiR-199a-1 regulates X-linked centronuclear myopathy**

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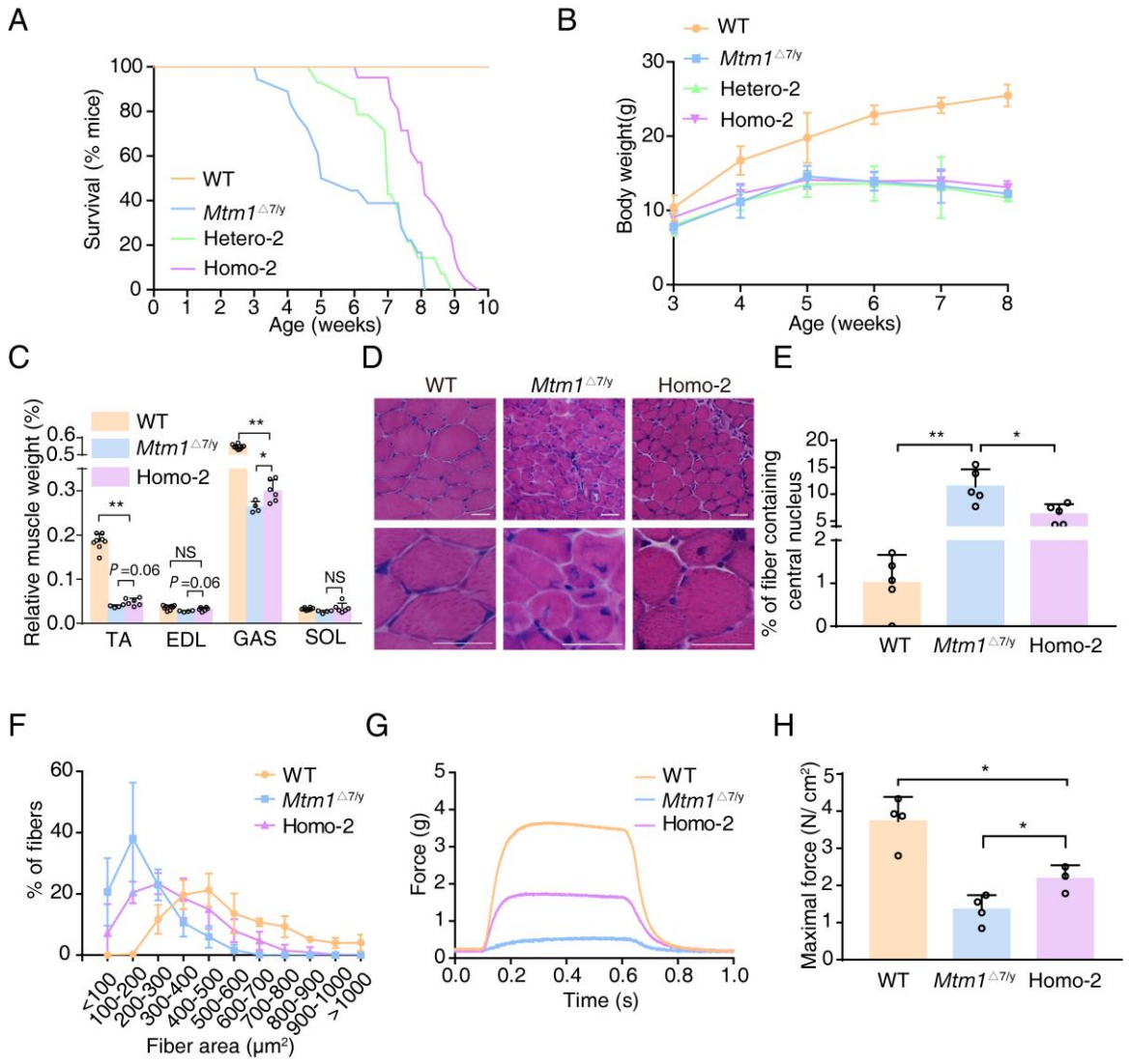

Figure S1. Deletion of miR-199a-1 partially improved symptoms in *Mtm1*$^{\Delta 7/y}$ mice. (*A*) Lifespan of indicated mice (n = 14-30). (*B*) The whole-body weight of indicated mice were recorded from 3 to 8-week-old (n = 12-25). (*C*) Relative muscle weight of labeled muscle from different mice (n = 4-8). TA, tibialis anterior; GAS, gastrocnemius; EDL, extensor digitorum longus; SOL, soleus. (*D*) Characteristic muscle biopsy from different mice. Scale bar = 20 nm. (*E*) The ratio of centronuclear myofiber number to total fiber number (n = 5). (*F*) Distribution of myofibers was grouped according to cross-sectional area (n = 5). (*G*) The typical EDL contraction curve induced by electric stimulus. (*H*) Quantification of maximal forces in *G* (n = 3-4). The data of WT mice were also present in Fig. 2 and 3. All muscles from 6-week-old mice. Graphs represent mean ± SD. NS, no significant difference; **P* < 0.05; ***P* < 0.01 (2-tailed Student's t test).

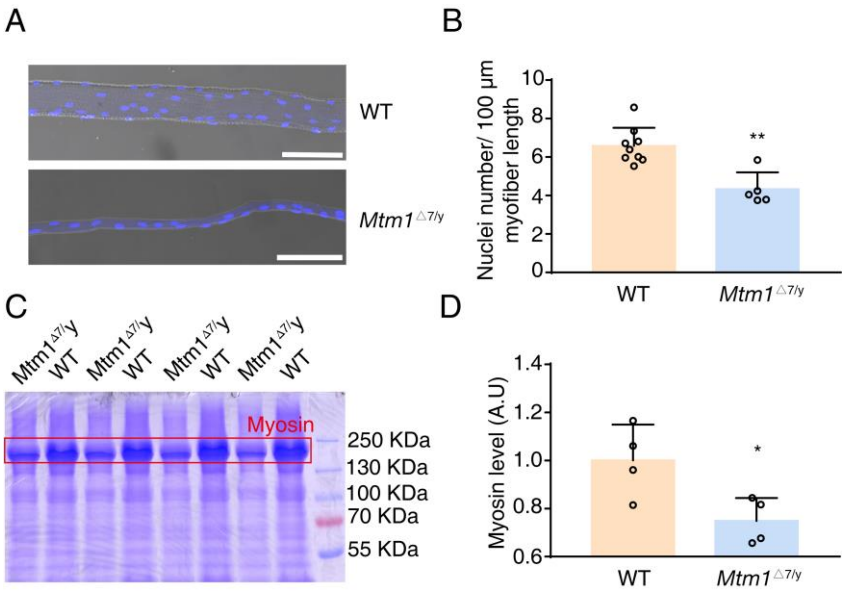

Figure S2. *Mtm1*$^{\Delta 7/y}$ mice exhibits defect in myoblast fusion. (*A*) Immunoflurescence with DAPI staining for single myofiber from 3-weeks-old WT and *Mtm1*$^{\Delta 7/y}$ mice. Scale bar =100 µm. The data of WT mice were also present in Fig. 4*A*. (*B*) Quantification of nuclei number per 100 µm myofiber (n = 5-9). The data of WT mice were also present in Fig. 4*B*. (*C*) Protein lysates from the TA muscle of 3-week-old mice were separated by SDS-PAGE gel and stained by Coomassie brilliant blue. The red box represents the myosin heavy chain band at approximately 230 kDa. (*D*) The expression of myosin was measured by gray level (n = 4). A.U, arbitrary units. Graphs represent mean ± SD. *P < 0.05; **P < 0.01 (2-tailed Student's t test).

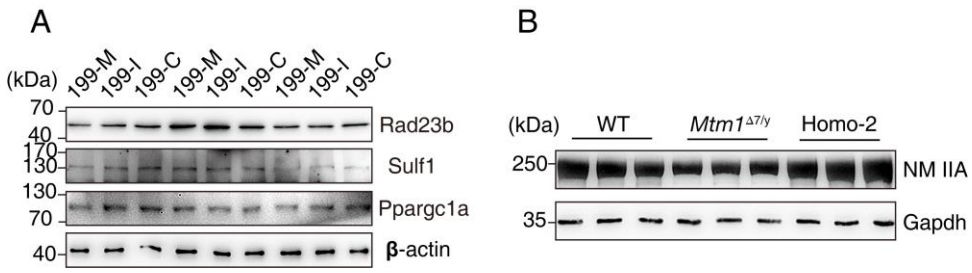

Figure S3. MiR-199a-1 inhibits NM IIA expression. (*A*) C2C12 cells treated with miR-199a-5p oligonucleotides were subjected to Western blot of predicted miR-199a-1 target proteins. 199-M, miR-199a-5p mimic; 199-I, miR-199a-5p inhibitor; 199-C, control oligonucleotides. (*B*) Western blot with NM IIA antibody for lysates of 3-week-old TA muscle. Gapdh was as an internal control.

Table S1. Primer sequence in PCR.

Primer name	Sequence (5'-3')
miR-199a-1 vector forward	AGATCTCCTAGTCTGCTGCAAATGTGC
miR-199a-1 vector reverse	GTCGACTCACAGACCAGGTACGCAAT
Myh9-3'-UTR-WT forward	ACTAGTCCTGCCTTGAGACTGCTCTGACCA
Myh9-3'-UTR-WT reverse	ACTAGTTGTGACGCTCAGTGGAAACAT
Myh9-3'-UTR-mut forward	CCATGGGTCGCTGAGTTCCCT
Myh9-3'-UTR-mut reverse	AGACATTGTAGGGCAAGCTACTCTCTTC
Dnm2-B1-Mut forward	ACTTCCACCCTAAGGAATTCCATGTCCTCTTCTCA
Dnm2-B1-Mut reverse	GGAATTCCTTAGGGTGGAAGTTAAGAGCAGTTGTCA
Dnm2-B2-Mut forward	GGTGTCAGACCTCTGGAACTGGAATCACTGATGGT
Dnm2-B2-Mut reverse	CAGTTCCAGAGGTCTGACACCCTCGTCTGGCCT
Dnm2-WT forward	CTCGAGCAAAAACTAAGAGAAGTGACAAGGC
Dnm2-WT reverse	AAGCTTATCCGGTTCTCAGGCGACAC
miRNA-RT primer	GCTGTCAACGATACGCTACGTAACGGCATGACAGTGT TTTTTTTTTTTTTTTTTTTTA
miR-199a-5p qPCR forward	CCCAGTGTTCAGACTACCTGTTC
miR-199a-5p qPCR reverse	GCTGTCAACGATACGCTACGTAACG
5S rRNA qPCR forward	GTCTACGGCCATACCACCCTGAAC
5S rRNA qPCR reverse	GCTGTCAACGATACGCTACGTAACG
Actb qPCR forward	AGGCCAACCGTGAAAAGATG
Actb qPCR reverse	AGAGCATAGCCCTCGTAGATGG
Gapdh qPCR forward	TGAACGGGAAGCTCACTGG
Gapdh qPCR reverse	TCCACCACCCTGTTGCTGTA
Dnm2 qPCR forward	AAGAGCCGAGTTTGAAGTGTG
Dnm2 qPCR reverse	ACGACTGCTCAATGTCAATCAG
miR-199a-1 qPCR forward	GCCATCCCAGTGTTCAGACTA
miR-199a-1 qPCR reverse	GCCTAACCAATGTGCAGACT