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Supplemental information

Musashi-2 contributes to myotonic dystrophy muscle dysfunction by promoting excessive autophagy through *miR-7* biogenesis repression

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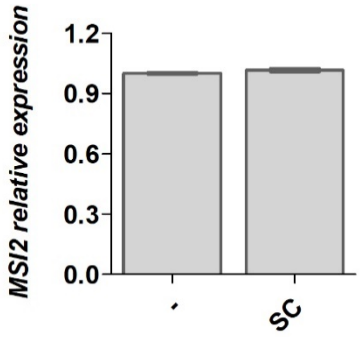

Figure S1. The scrambled (SC) control ASO does not affect *MSI2* levels. Quantification by qRT-PCR of the *MSI2* relative expression levels in healthy TDMs differentiated for 7 days treated with 150 nM of SC. The bar graph shows the mean ± s.e.m. The mean of *GAPDH* and *GPI* expression was used as a reference for normalization (n = 3).

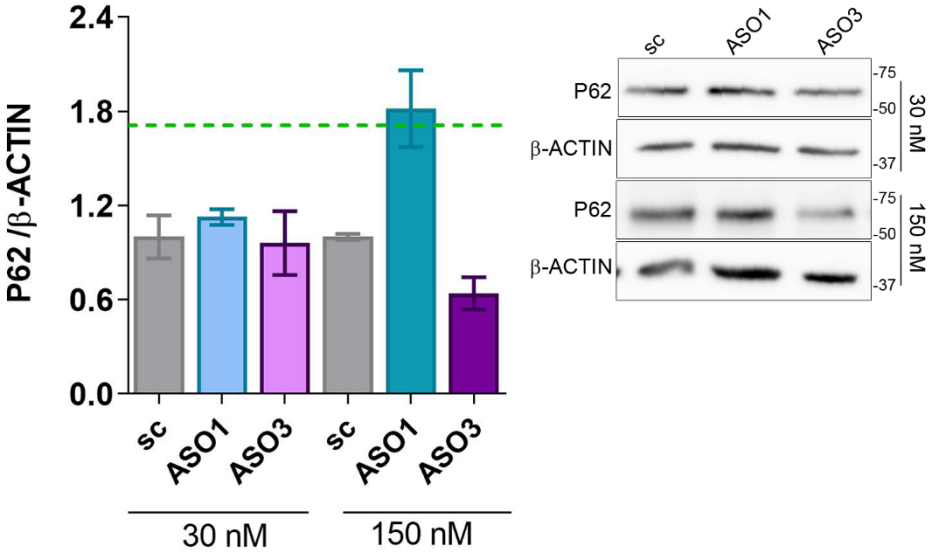

Figure S2. P62 levels of DM1 TDMs remain unchanged upon treatment with MSI2-targeting ASOs. Quantification and representative western blots of the P62 levels in protein extracts from TDMs treated with the indicated ASOs against the *MSI2* transcripts. β-ACTIN expression was used as an endogenous control (n=3). Green dashed line indicates relative levels of P62 detected in control myotubes. Statistical analyses compared experimentally treated-TDMs with the scrambled gapmer at the same concentration and found no significant differences by Student´s t-tests. The bar graphs show the mean ± s.e.m.

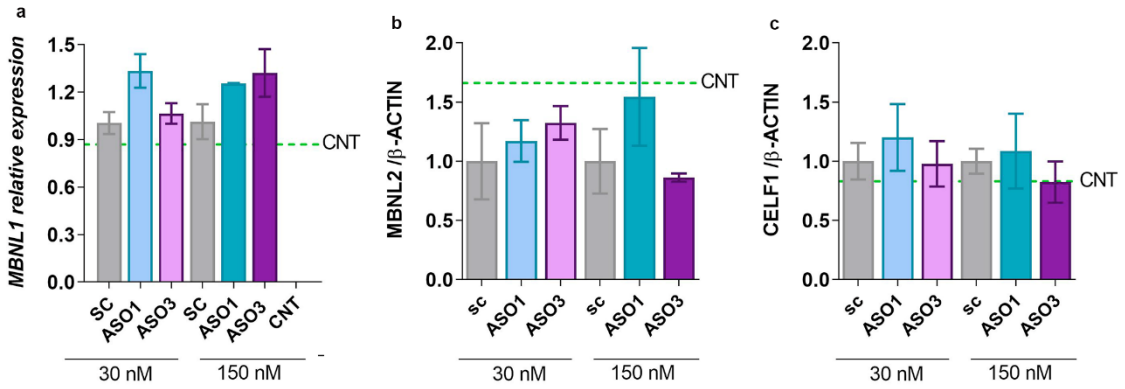

Figure S3. MBNL1 transcripts, MBNL2 and CELF1 protein levels do not significantly change upon treatment with *MSI2* targeting ASOs. (**a**) MBNL1 transcripts quantification by RT-qPCR in DM1 myotubes transdifferentiated for 7 days and treated with the indicated molecules targeting *MSI2* mRNA. *GAPDH* expression levels were used as endogenous controls (n=3). Western blot quantification of MBNL2 (**b**) and CELF1 (**c**) protein expression levels in 7-days transdifferentiated DM1 TDMs after treatment with the indicated ASOs. β-ACTIN expression was used as an endogenous control (n = 3). None of the statistical comparisons of experimental to scrambled-treated TDMs reached the significance threshold. Green dashed lines indicate values detected in control myotubes. The bar graphs show the mean ± s.e.m.

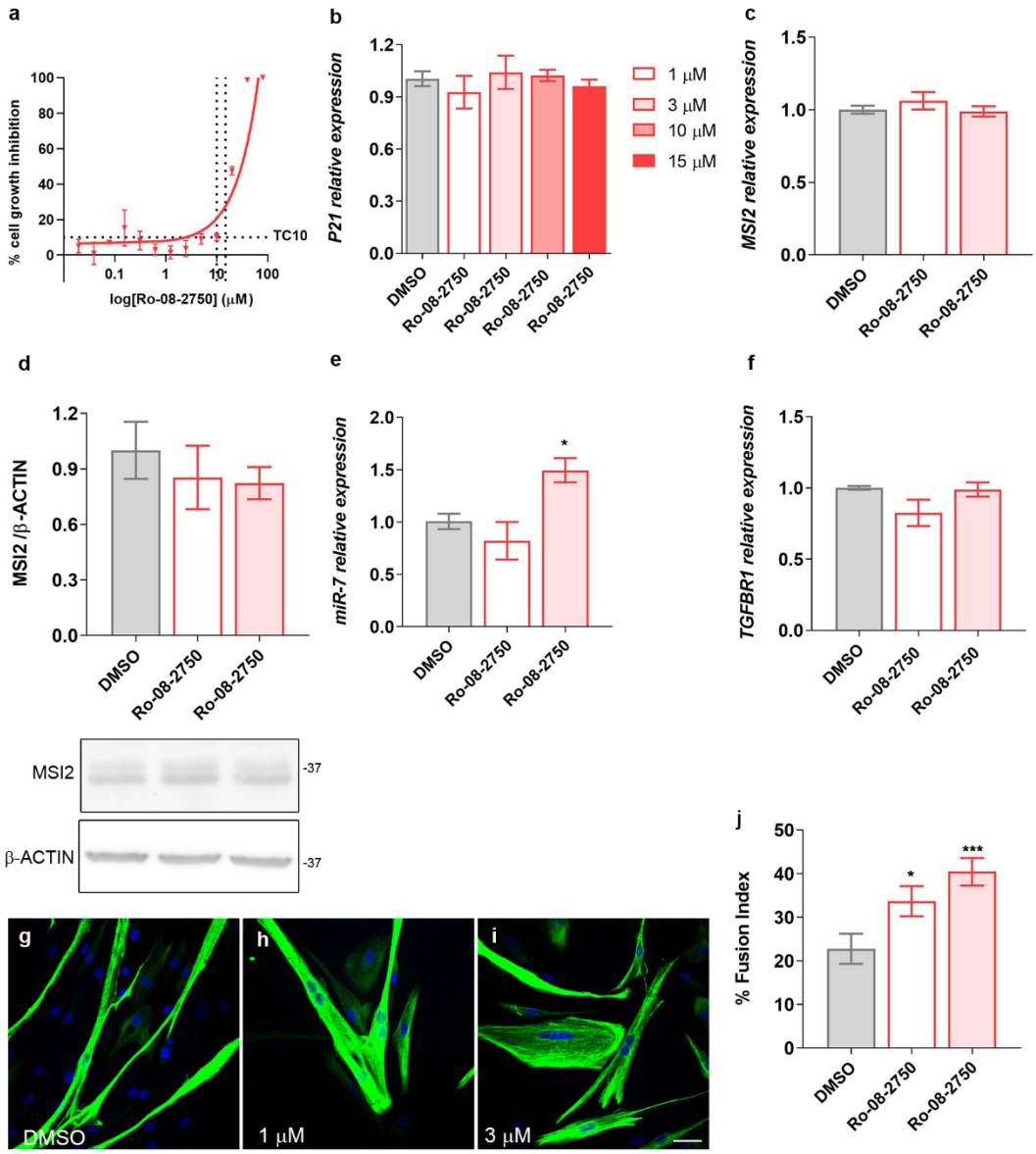

Figure S4. Effect of Ro-08-2750 treatment in DM1 myotubes. (**a**) Cell growth inhibition assay by the MTS method. Human normal TDMs were transfected with a range of Ro 08-2750 concentrations (n=4). TC10 was obtained using the least-squares non-linear regression model. Quantification of mRNA relative expression by qRT-PCR of (**b**) *P21*, (**c**) *MSI2,* (**e**) *miR-7*, and (**f**) *TGFBR1* in DM1 TDMs treated with the vehicle (DMSO), or the indicated concentration of Ro-08-2750. *GAPDH*, *GPI,* and *HPRT1* or *U1* and *U6* expression levels were used as endogenous controls in ***b,c,f*** and ***e,*** respectively (n=3). (**d**) MSI2 relative protein level was quantified by western blot relative to β-ACTIN in myotubes treated with vehicle or with the indicated concentrations of the compound (n=3). Representative blots from each condition are also shown. (**j**) Quantification of the percentage of myogenic fusion index of DM1 TDMs with the indicated concentrations of the

compound (n=10-15 images). Representative confocal images of Desmin-immunostained (green) human DM1 myotubes transdifferentiated for 7 days after treatment with (**g**) DMSO as control or with 1 or 3 μM Ro 08-2750 (**h,i**). Scale bar 40 μm. Nuclei were counterstained with DAPI. The bar graphs show mean ± s.e.m. **P<0.05,* according to Student's t-test.

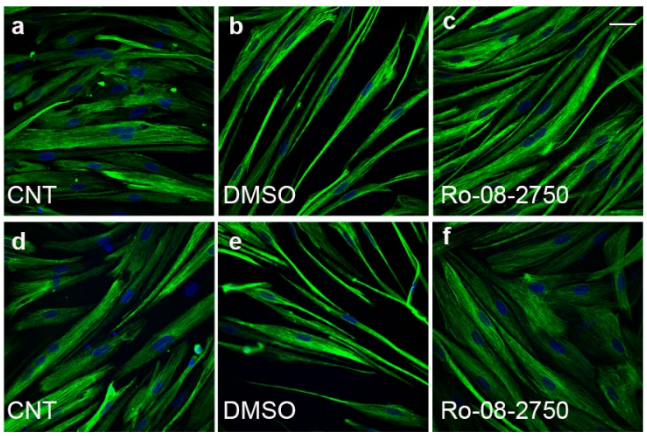

Figure S5 MSI2 inhibition by Ro 08-2750 improves fusion capacity of 10 and 14 days-differentiated DM1 myoblasts. Representative confocal images of Desmin-immunostained (green) immortalized myoblasts from control (**a**,**d**), DM1 treated with 0.8% DMSO (**b,e**) or DM1 treated with 10 μM Ro 08-2750 (**c,f**) differentiated for 10 (**a-c**) or 14 (**d-f**) days. Scale bar 40 μm. Nuclei were counterstained with DAPI.

Table S1 Information of biopsies from skeletal muscle

	Sample	Sex	Age	Muscle	Repeats length
Controls	CNT-1	Female	61	Deltoid	nd
	CNT-2	Male	34	Deltoid	nd
	CNT-3	Female	35	Deltoid	nd
	CNT-4	Male	37	Biceps	nd
	CNT-5	Male	24	Deltoid	nd
	CNT-6	Male	25	Deltoid	nd
	CNT-7	Female	29	Deltoid	nd
	CNT-8	Female	46	Deltoid	nd
	CNT-9	Male	29	Deltoid	nd
	CNT-10(*)	Male	18	Medial gastrocnemius	nd
	CNT-11	Male	50	Deltoid	nd
	CNT-12	Male	63	Deltoid	nd
	CNT-13	Male	59	Deltoid	nd
	CNT-14	Male	47	Deltoid	nd
	CNT-15	Female	34	Deltoid	nd
	CNT-16	Male	55	Deltoid	nd

	CNT-17	Female	37	Deltoid	nd
Patients	DM1-1	Male	30	Deltoid	nd
	DM1-2	Male	61	Deltoid	0.3 kb
	DM1-3	Male	45	Deltoid	3.6 kb
	DM1-4	Female	69	Deltoid	1.1 kb
	DM1-5	Male	28	Deltoid	nd
	DM1-6	Female	65	Deltoid	nd
	DM1-7	Female	33	Deltoid	0.9 kb
	DM1-8	Male	32	Deltoid	0.4 kb
	DM1-9	Female	68	Deltoid	0.2 kb
	DM1-10	Male	27	Deltoid	0.75 kb
	DM1-11	Female	44	Deltoid	nd
	DM1-12	Male	52	Deltoid	nd
	DM1-13	Male	36	Deltoid	3 kb
	DM1-14(*)	Female	50	Deltoid	150
	DM1-15	Female	47	Deltoid	nd
	DM1-16(*)	Male	54	Deltoid	0.8 kb

nd: not determined (*) biopsies used to isolate primary cells

Table S2 Sequences of oligonucleotides used for qRT-PCR and semiquantitative RT-PCR

Primers sequence	**Sequence (5' → 3')**	**qRT-PCR/RT-PCR**	**Species**
GAPDH fwd	CATCTTCCAGGAGCGAGATC	qRT-PCR/ RT-PCR	*Homo sapiens*
GAPDH rev	GTTCACACCCATGACGAACAT	qRT-PCR	*Homo sapiens*
GPI fwd	CAGGGCATCATCTGGGACAT	qRT-PCR	*Homo sapiens*
GPI rev	TCTTAGCCAGCTGCTTTCCC	qRT-PCR	*Homo sapiens*
HPRT1 fwd	TGACACTGGCAAAACAATGCA	qRT-PCR	*Homo sapiens*
HPRT1 rev	GGTCCTTTTCACCAGCAAGCT	qRT-PCR	*Homo sapiens*
IGF1 fwd	CTCTTCAGTTCGTGTGTGGAGAC	qRT-PCR	*Homo sapiens*
IGF1 rev	CAGCCTCCTTAGATCACAGCTC	qRT-PCR	*Homo sapiens*
MSI2 fwd	GCAGACCTCACCAGATAGCCTT	qRT-PCR	*Homo sapiens*
MSI2 rev	AAGCCTCTGGAGCGTTTCGTAG	qRT-PCR	*Homo sapiens*
MSTN fwd	TGAGAATGGTCATGATCTTGCTGT	qRT-PCR	*Homo sapiens*
MSTN rev	TCATCACAGTCAAGACCAAAATCC	qRT-PCR	*Homo sapiens*
mTOR fwd	AGCATCGGATGCTTAGGAGTGG	qRT-PCR	*Homo sapiens*
mTOR rev	CAGCCAGTCATCTTTGGAGACC	qRT-PCR	*Homo sapiens*

NFIX fwd	GAGCCCTGTTGATGACGTGTTCTA	RT-PCR	*Homo sapiens*
NFIX rev	CTGCACAAACTCCTTCAGTGAGTC	RT-PCR	*Homo sapiens*
P21 fwd	AGGTGGACCTGGAGACTCTCAG	qRT-PCR	*Homo sapiens*
P21 rev	TCCTCTTGGAGAAGATCAGCCG	qRT-PCR	*Homo sapiens*
PKM fwd	CTGAAGGCAGTGATGTCGCC	RT-PCR	*Homo sapiens*
PKM rev	ACCCGGAGGTCCACGTCTC	RT-PCR	*Homo sapiens*
SERCA1 fwd	GATGATCTTCAAGCTCCGGGC	RT-PCR	*Homo sapiens*
SERCA1 rev	CAGCTCTGCCTGAAGATGTG	RT-PCR	*Homo sapiens*
TGFBR1 fwd	GACAACGTCAGGTTCTGGCTCA	qRT-PCR	*Homo sapiens*
TGFBR1 rev	CCGCCACTTTCCTCTCCAAACT	qRT-PCR	*Homo sapiens*