

Supplementary Material

Movie S1. Osmotic shock-induced calcium sparks in non-transfected *mdx* muscle fibers.

Movie S2. Osmotic shock-induced calcium sparks in Cas9/gRNA-transfected *mdx* muscle fibers.

Table S1. List of the plasmids used in this study.

Plasmid Construction			
Plasmid name	Plasmid		Insert
	Vector	cutting site	Template
pLKO-gRNA Temp	pLKO	Nde1 and EcoR1	synthesized minigene
pLKO gRNA	pLKO-gRNA Temp	Sac2 and Kpn1	pEGFP-C1
pLKO-gRNA i20	pLKO gRNA	Bsmb1	
pLKO-gRNA i23	pLKO gRNA	Bsmb1	
pShuttle-gRNA i20	pshuttle	Not1 and Hind3	pLKO-gRNA i20
pShuttle-gRNA i23	pshuttle	Not1 and Hind3	pLKO-gRNA i23
pCDNA3.3-gRNA-GFP-E2A-hCas9	addgene Plasmid #41815	Mlu1 and Sbf1	PCR1 2 3
			PLKO-gRNA
pLOX-hcas9 Temp	pLOX-Tert-Partial	Snab1 and Xma1	addgene Plasmid #41815
pLOX-hcas9	pLOX-hcas9 Temp	BamH1 and Kpn1	addgene Plasmid #41815
			pLOX-CWBmi1
pLOX-EGFP-2A-hCas9	pLOX-hcas9	Snab1 and Sbf1	pCDNA3.3-gRNA-GFP-E2A-hCas9
pshuttle-GFP-E2A-hCas9	pShuttle-CMV	Snab1 and Kpn1	pLOX-EGFP-2A-hCas9
pCDNA3.3-i20 gRNA-GFP-E2A-hCas9	CDNA3.3-gRNA-GFP-E2A-hC	Bsmb1	
pCDNA3.3-i23 gRNA-GFP-E2A-hCas9	CDNA3.3-gRNA-GFP-E2A-hC	Bsmb1	
CDNA3.3-i20 gRNA-mCherry-E2A-hCas9	CDNA3.3-i20 gRNA-mCherry-E2A	Nhe1 and BsrG1	pmCherry-C1
CDNA3.3-i23 gRNA-mCherry-E2A-hCas9	CDNA3.3-i23 gRNA-mCherry-E2A	Nhe1 and BsrG1	pmCherry-C1

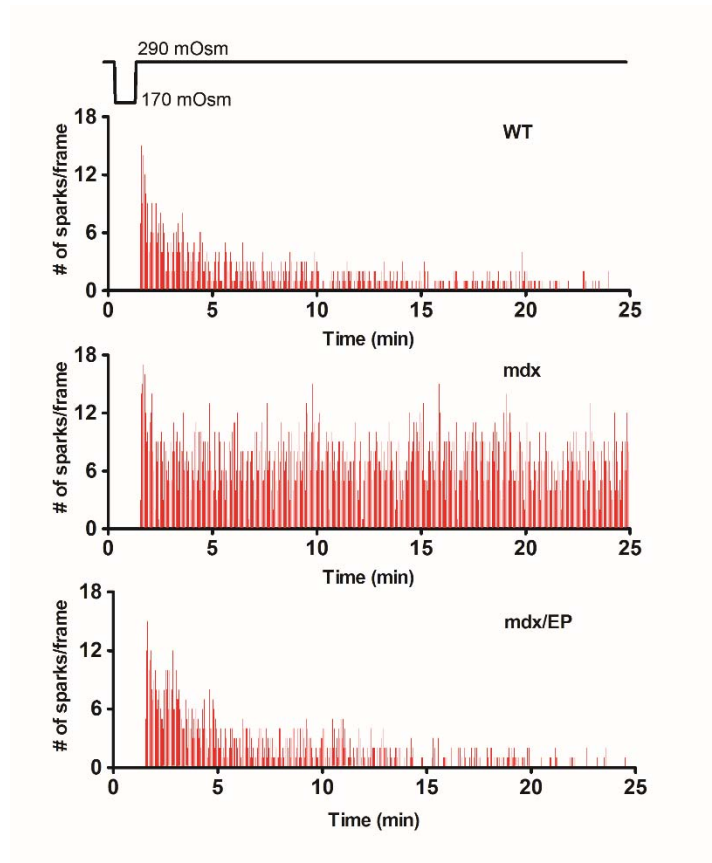


Figure S1. Representative plots of calcium sparks per frame over the 25-minute recording period in isolated FDB muscle fibers of WT, mdx and mdx electroporated with CRISPR plasmids (mdx/EP).

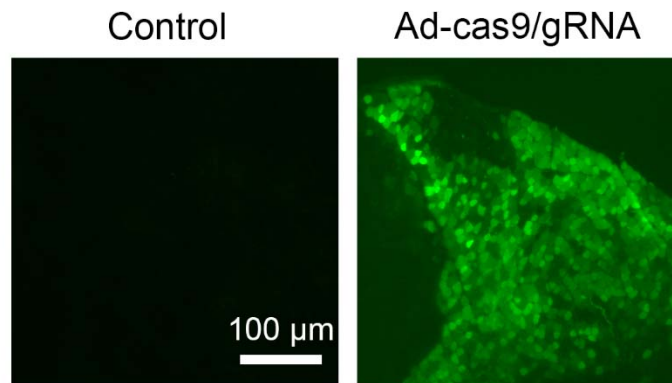


Figure S2. Fluorescence images of GFP fluorescence in the GA muscles of newborn pups two weeks after intramuscular injection with or without cas9/gRNA adenovirus. GFP fluorescence is shown as green. Scale bar: 100 μm. The images are representative of five mice.

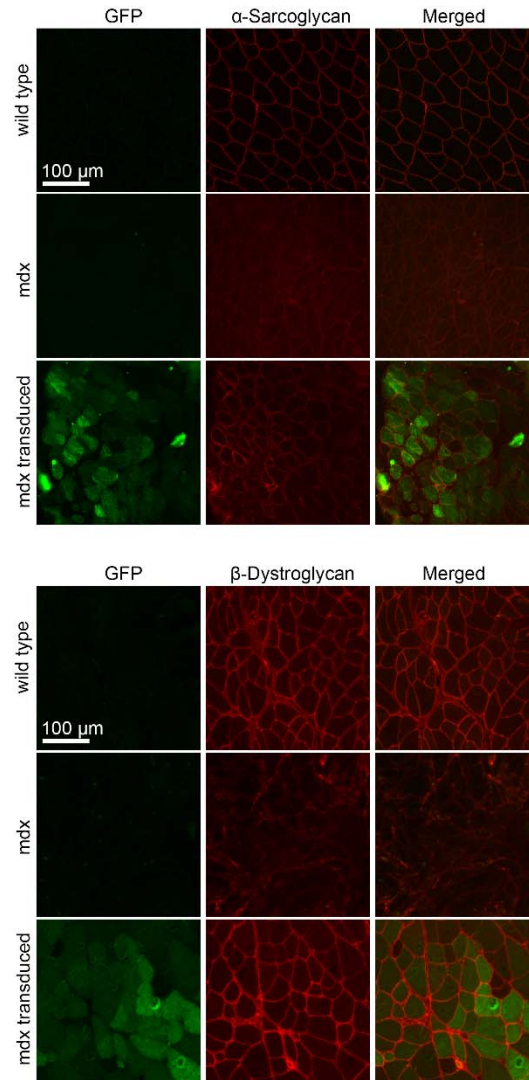


Figure S3. Confocal immunofluorescence images of α -sarcoglycan (a, red) and β -dystroglycan (b, red) in muscle cryosections treated with or without EGFP-2A-cas9/gRNA adenovirus. GFP was shown in green. The images are representative of five experiments.

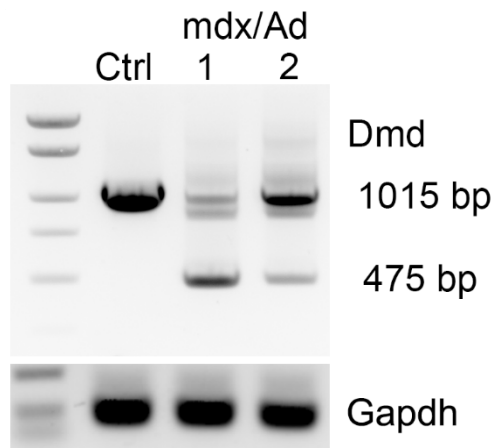


Figure S4. RT-PCR analysis of the total RNA extracted from the gastrocnemius muscles injected with EGFP-2A-Cas9/gRNA adenovirus (*mdx/Ad*). Lane 1 and 2 correspond to two different mice.

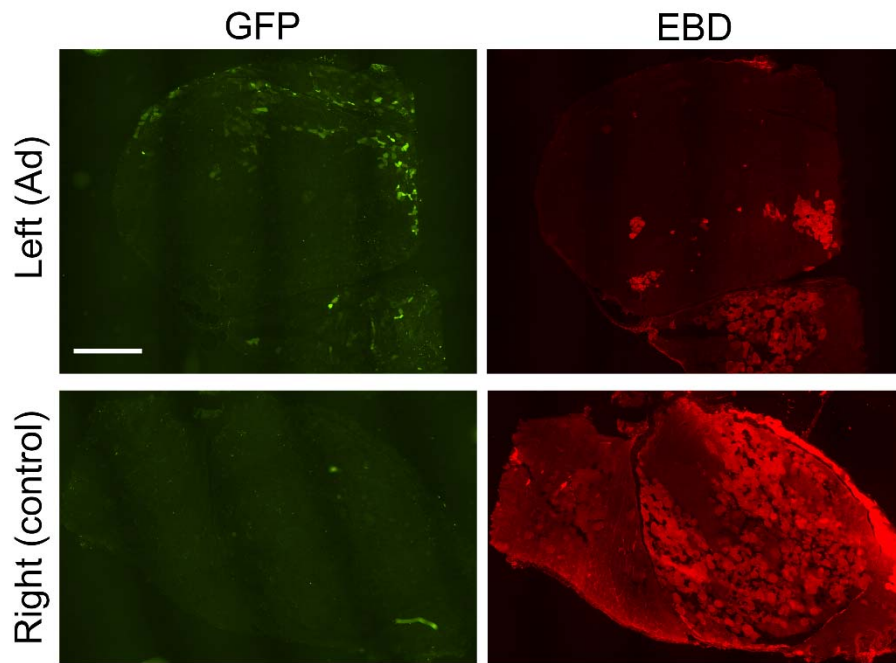


Figure S5. EBD fluorescence micrograph of the entire cross section of both gastrocnemius muscles (the left side was injected with cas9/gRNA adenovirus and the contralateral side was used as control) from the same *mdx* mouse after downhill treadmill running exercise. Scale bar: 1 mm.