Supplemental Materials

Table S1. The primers of potential off-target sites (POTS) used in this study

	POTS (Mismatches in red)	Position	PCR Primer
sgRNA1	CTGGGGCACGACTTACCGACAGG	chrUN0:-254147	F: CTTAGTGTCACAGAGGCATAG R: GGGTTTCTTACAGTCTCACTT
	ATGGGACAGAATTTACCGAGAGG	chr9:-20541149	F: GTCGAATGAGGAGTGGATTAG R: CCGTGGACATAACCGTAATAG
	CTTTCACAGATCTTACTGACAAG	chrUN0:-3108683	F: GAGTTGGATGGTTGCTTGT R: GCCTACTCAGGTAGCATAGA
	ACTGCACAGAACTTACCCACAGG	chr8:+12350968	F: AACAATGAGCGGACAGAAG R: CTTGGACATAGAGCGAGTTAG
	AATGGACAGAATTTACTGACTAG	chrX:+66615430	F: GCCTTAACAAGATGAGGGTAG R: CAGTGAACTAGATGGCTGAAG
sgRNA2	TTGGGATTTCTAGTTCTGAGCAG	chr17:+57644956	F: ATCTGCTTCACTATGTTCTGG R: AAATTGTCCCTCCATCTCTTC
	TATGCATTTCTAGTTCTGAGGAG	chr7:+83169861	F: GTCTAAAGTCGAAGGAGAACC R: CTGTCACTCTCAAGCAGAAA
	TTTGCTTTTCTAGTTCGGAAAGG	chr14:+155053475	F: GACCGTATCTGGACTCCTTTA R: CCAGGAAATGACAGAGATTGG
	ATTGAAATTCTAGTTCTGAGGAG	chr1:+20744552	F: TCAGAGGTGAGTTCAGATACA R: GCTGTGTAGCTCACGAAAT
	CTGCCTTTTCTATTTCGGAGCAG	chr14:-39465758	F: CATTGTGGGTAAAGGGACAC R: CCAATCCTACATTGCGATTCA

Movie 1. A WT rabbit was capable of climbing up the stair-step.

Movie 2. A *DMD*-KO rabbit failed to climb up the stair-step.

Figure S1. Mutation detection of 33 F0 rabbits by T-cloning and Sanger sequencing. The sgRNA sequences are shown in red; PAM sites are underlined and highlighted in green; insertions are shown in blue; deletions (-); WT, wild-type.

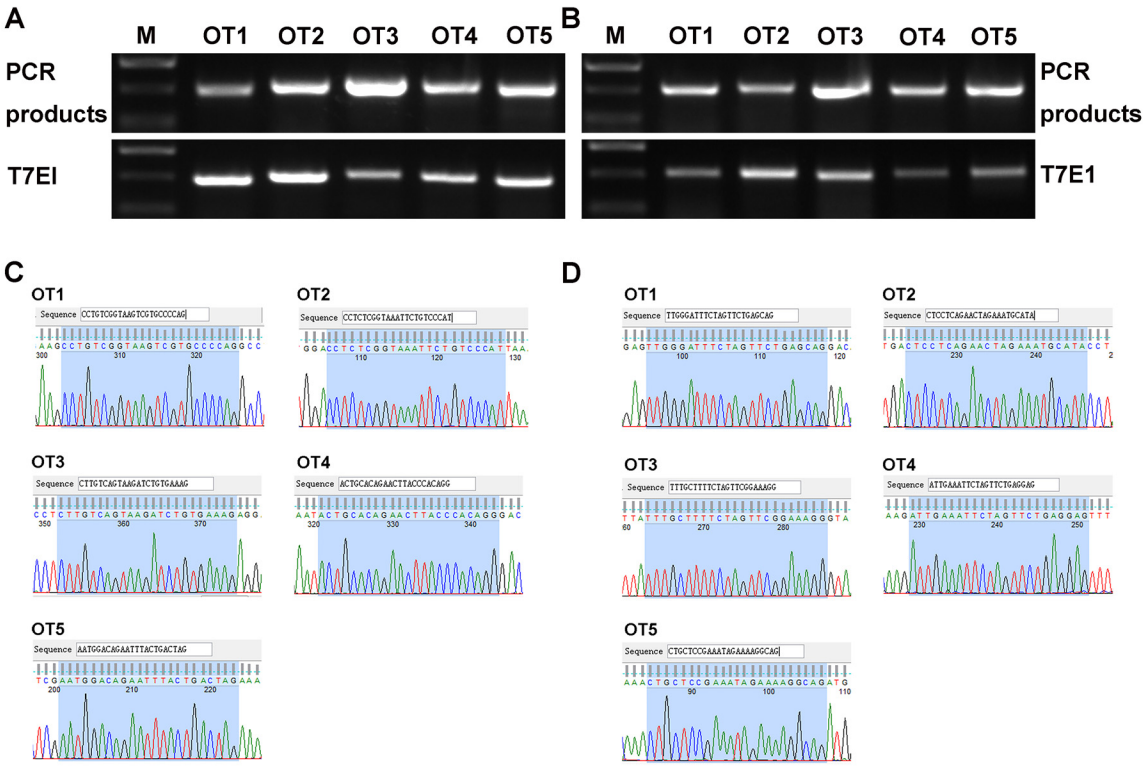

Figure S2. Off-target analysis of *DMD*-KO rabbit. (A, B) The T7E1 cleavage analysis of five potential off-target sites (OT1-5) for sgRNA1 (A) and sgRNA2 (B). M, DNA ladder (DL2000). (C, D) T-cloning and Sanger sequencing of five potential off-target sites for sgRNA1 (C) and sgRNA2 (D).

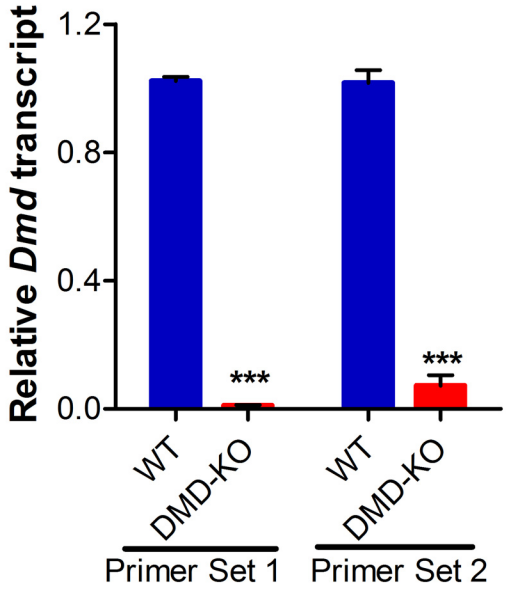

Figure S3. Disrupted dystrophin transcript expression in *DMD*-KO rabbit skeletal muscle. Quantitative reverse transcription-polymerase chain reaction (RT-PCR) showed dystrophin transcript was significantly diminished in the KO skeletal muscle tissues. *** $p < 0.001$.

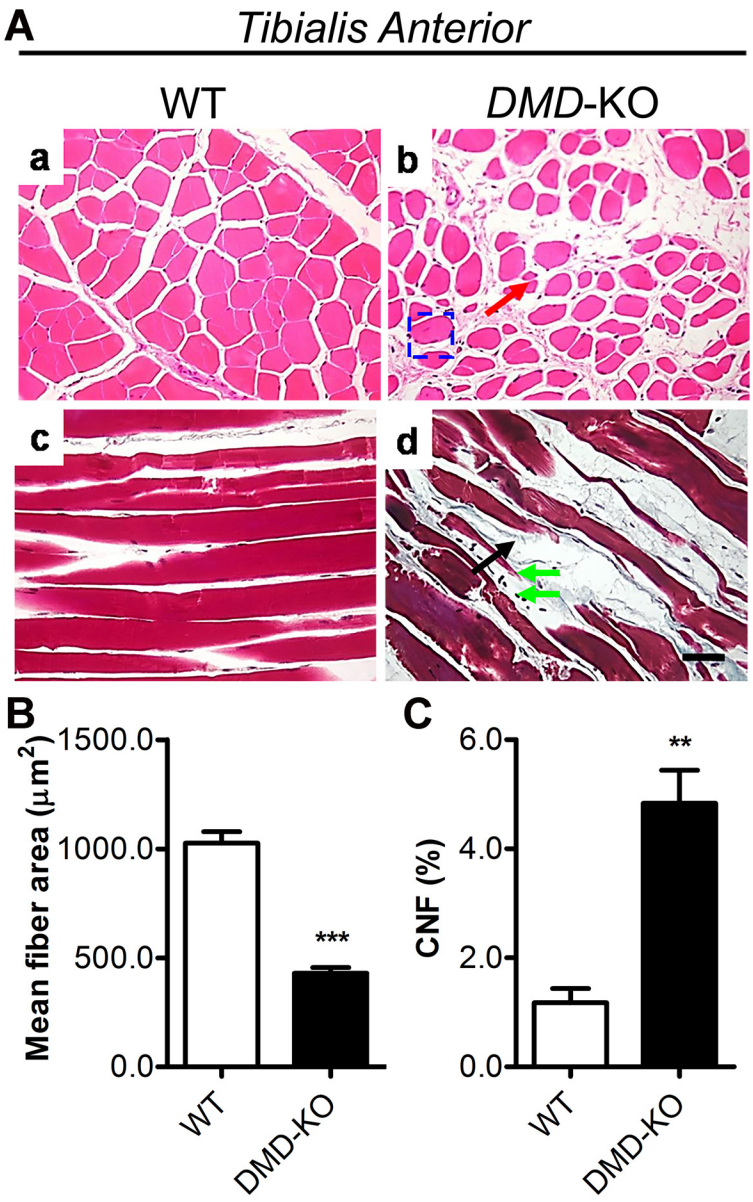

Figure S4. The histological analysis of *tibialis anterior* muscle. (A) Analysis of H&E- and Masson's trichrome-stained sections of *tibialis anterior* muscle from 5-month-old WT and *DMD*-KO rabbits. *DMD*-KO rabbits showed myopathy with excessive fiber size variation (red arrows), fiber necrosis (green double arrow), fibrosis (black arrows) and centrally nucleated fibers (blue rectangle). (B, C) Quantification of mean fiber area (B) and CNF percentage (C) in the *tibialis anterior* muscles of WT and *DMD*-KO rabbits at 5 months of age.

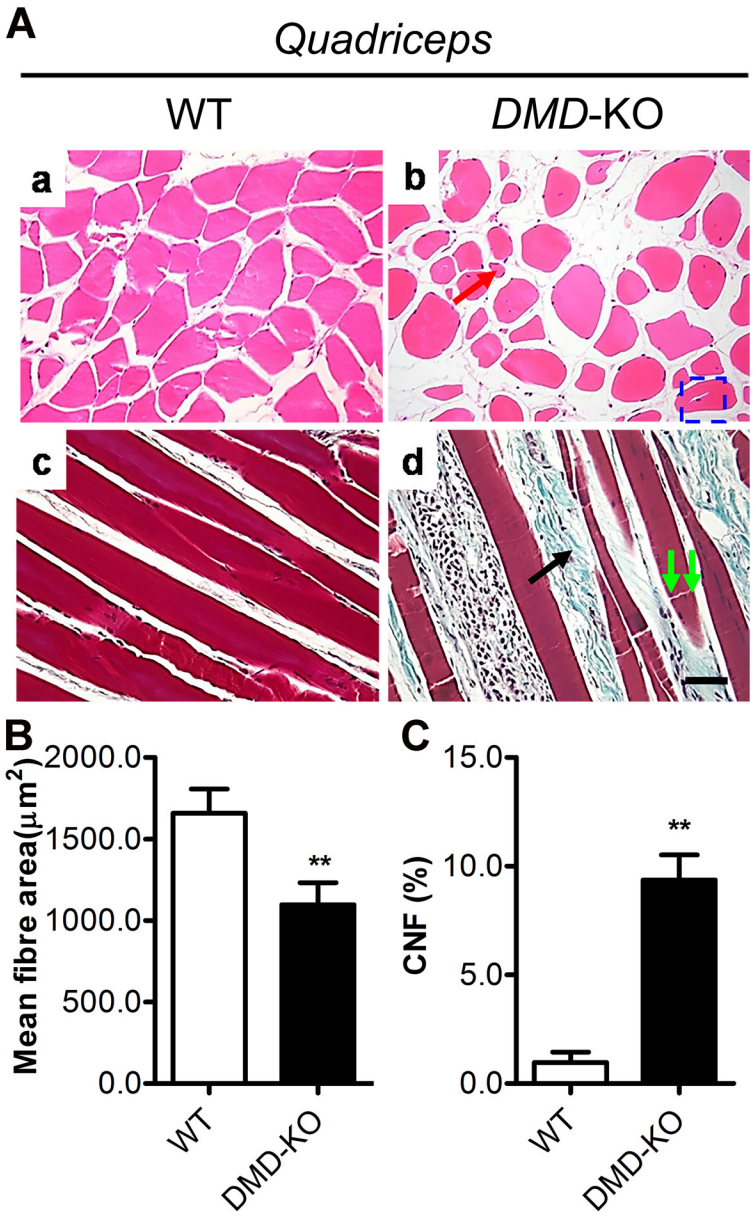

Figure S5. The histological analysis of *quadriceps* muscle. (A) Analysis of H&E- and Masson's trichrome-stained sections of *quadriceps* muscle from 5-month-old WT and *DMD*-KO rabbits. *DMD*-KO rabbits showed myopathy with excessive fiber size variation (red arrows), fiber necrosis (green double arrow), fibrosis (black arrows) and centrally nucleated fibers (blue rectangle). (B, C) Quantification of mean fiber area (B) and CNF percentage (C) in the *quadriceps* muscles of WT and *DMD*-KO rabbits at 5 months of age.

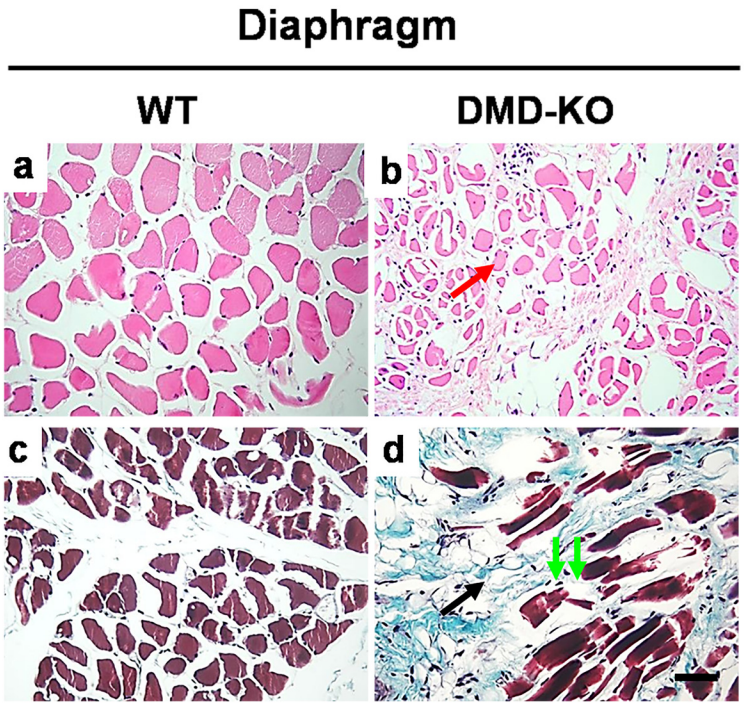

Figure S6. The histological analysis of *diaphragm* muscle. Analysis of H&E- and Masson's trichrome-stained sections of *diaphragm* muscle from 5-month-old WT and *DMD*-KO rabbits. *DMD*-KO rabbits showed myopathy with excessive fiber size variation (red arrows) and fibrosis (black arrows).