Supplementary material

Quantitative real-time PCR

The following primer pairs were used for amplification: Ki-67 (forward), CTTTGGGTGCGACTTGACG, (reverse), GTCGACCCCGCTCCTTTT; CCL2 (forward), GTTGGCTCAGCCAGATGCA, (reverse), AGCCTACTCATTGGGATCATCTTG; IL1β (forward), AGTGTGGATCCCAAGCA, (reverse), CACTGTTGTTTCCCAGGA; IL10 (forward), CAAAGGACCAGCTGGACA, (reverse), ATCGATGACAGCGCCTCA; Col1A1 (forward), ACG GGAGGGCGAGTGCTGTG, (reverse), CGGGTCCCCTTGGGCCTTGG; IL-6 (forward), ATG AAGTTCCTCTCTGCAAGAGACT, (reverse), CACTAGGTTTGCCGAGTAGATCTC; GM-CSF (forward), ACCACCTATGCGGATTTCAT, (reverse),TCATTACGCAGGCACAAAAG; TGGTCGTAATCAGCAGCA; GAPDH (forward), ACCCAGAAGACTGTGGATGG, (reverse), CACATTGGGGGTAGGAACAC; Pax7 (forward), GTCCCAGTCTTACTGCCAC, (reverse), TGTGGACAGGCTCACGTTTT; MyoD (forward), TGGGACCCCTCCGATAGATC, (reverse), GGTGGTGCATCTGCCAAAAG; Myogenin (forward), GCATGGAGTTCGGTCCCAA, (reverse), TATCCTCCACCGTGATGCTG.

Table S1. Antibodies used for flow cytometry analysis

Antibody	Clone	Dilution	Provider and Cat.No
CD45	30-F11	1:6000	Biolegend, 103114
CD3	17A2	1:200	Biolegend, 100218
Ki-67	16A8	1:1000	Biolegend, 652405
I-A^b	AF6-120.1	1:200	Biolegend, 116405
Ly6g	1A8 and RB6-8C5	1:3000	eBioscience, 61-9668; 61-5931
Ly6c	HK1.4	1:100	Biolegend,128033

Siglec-F	E50-2440	1:500	BD-Pharmingen, 552126
F4/80	BM8	1:1000	Biolegend, 123116
CD206	C068C2	1:50	Biolegend, 141716
CD11b	M1/70	1:3000	Biolegend, 101226
CCR2	475301	1:20	R&DSystems,FAB5538F-100
CX3CR1	SA011F11	1:1000	Biolegend, 149005
Sca1	Ly-6A/E	1:100	eBioscience, 11-5981-82
CD34	RAM34	1:100	Thermo Fisher, 13-0341-81
CD117 (cKit)	2B8	1:100	BD Biosciences, 553355
TER-119	TER-119	1:100	e-Bioscience,48-5921-82
CD31	MEC 13.3	1:100	Biolegend, 102514
CD31	390	1:100	e-Bioscience,48-0311-82
CD45	30-F11	1:100	e-Bioscience,48-0451-82

Supplementary figures

Figure S1

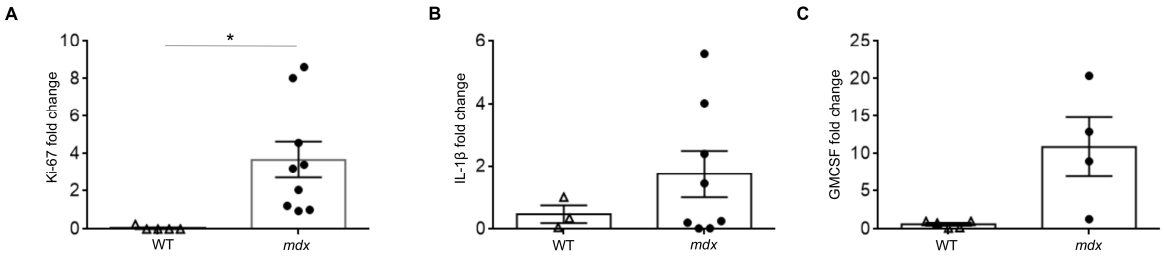

Figure S1. Myeloid activation and differentiation cytokine production in *mdx* spleen. (A) Expression levels of the proliferation marker Ki67and (B) myeloid cell cytokines IL1-β and (C) GM-CSF in *mdx* spleen at 2 weeks compared to WT assessed by quantitative real-time PCR. Data are presented as mean ± s.e.m. *P<0.05 by Student's *t* test.

Figure S2

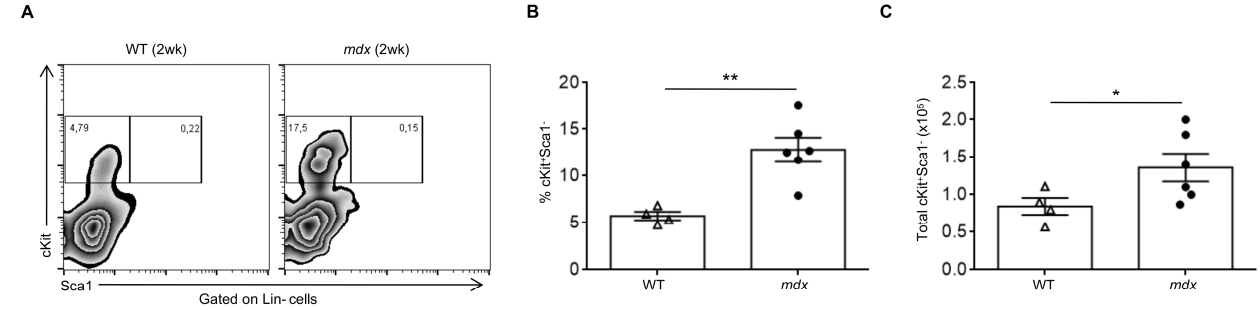

Figure S2. Expansion of myeloid progenitor cells in *mdx* spleen. (A) Representative flow cytometry plots showing the gating strategy for the identification of myeloid progenitor cells in the spleen. Myeloid progenitor cells were identified as $Lin^{-}cKit^{+}CD34^{+}Sca1^{-}$ cells. (B) Frequency and (C) total number of myeloid progenitor cells in *mdx* (n=6) versus WT (n=4) spleen at 2 weeks of age. Data are presented as mean ± s.e.m. *P<0.05, **P<0.01 by Student's *t* test.

Figure S3

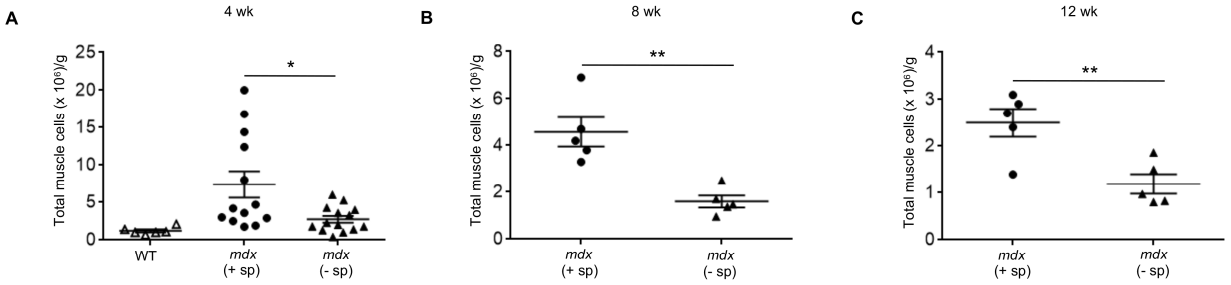

Figure S3. Total mononuclear cells are reduced in *mdx* muscle following splenectomy. (A) Total mononuclear cells in limb muscle of WT (n=6); *mdx* control (n=13); and splenectomised *mdx* (n=14) at 4, (B) 8 (n=5 for both groups) and (C) 12 (n=5 for both groups) weeks off age analysed by flow cytometry. Data are presented as mean ± s.e.m. *P<0.05, **P<0.01 by Student's *t* test.

Figure S4

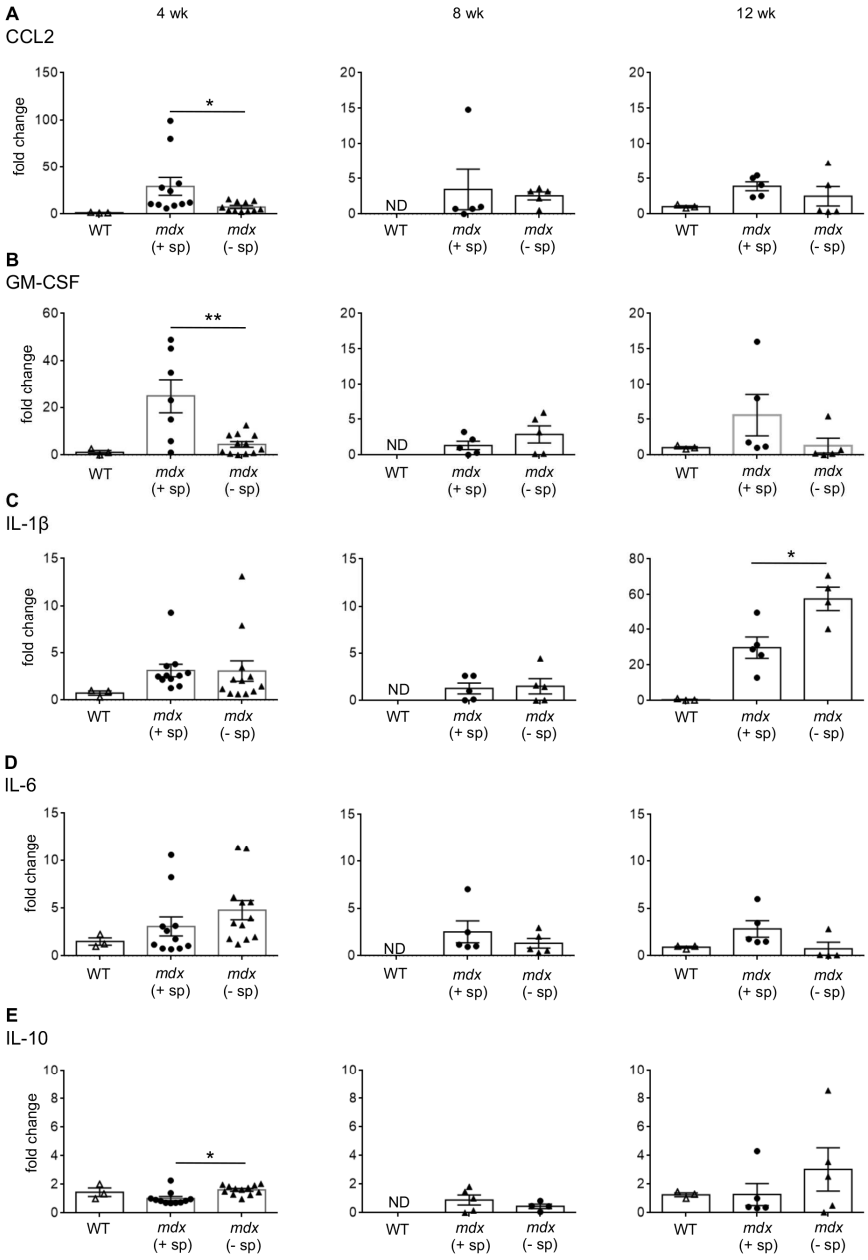

Figure S4. Changes in cytokine mRNA levels in *mdx* muscle following splenectomy. Expression levels of CCL2, GM-CSF, IL-β, IL-10 and IL6 in muscle of *mdx* control and splenectomised mice at 4, 8 and 12 weeks of age (each symbol represents an individual mouse). Data are presented as mean ± s.e.m. *P<0.05, **P<0.01 by Student's *t* test.

Figure S5

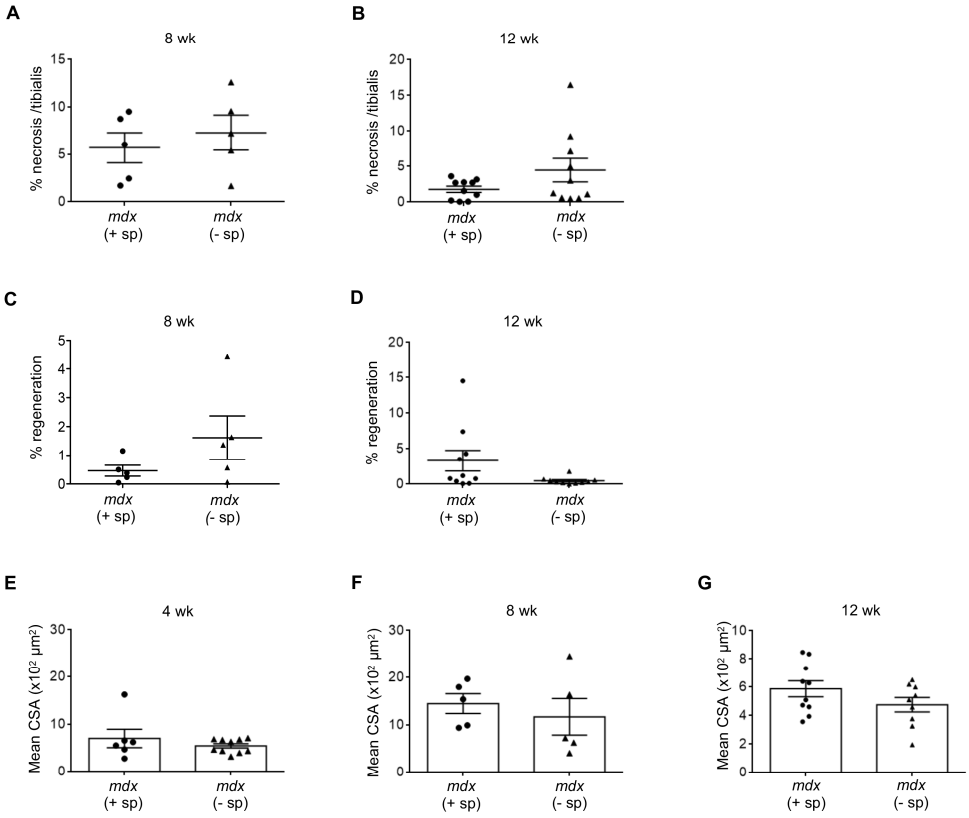

Figure S5. Effect of splenectomy on extent of necrosis and regeneration in *mdx* muscle. Tibialis Anterior muscle sections were stained with IgG to visualise necrotic muscle fibers. (A) Summary graph of histological analysis of necrosis showing percent necrotic area in muscle sections from splenectomised and control *mdx* mice at 8 (n=5 for both groups) and (B) 12 weeks of age (n=10 for both groups). TA muscle sections were stained for embryonic myosin heavy chain (eMHC) to visualise regenerating muscle fibers. (C) Summary graphs of histological analysis of eMHC positive fibers showing percent regenerating area at 8 (n=5 for both groups) and (D) 12 weeks of age (n=10, *mdx* control; n=9, *mdx* splenectomised). (E) Mean cross sectional area (CSA) of regenerating fibers in TA muscle from control and splenectomised mice at 4 weeks of age (n=6, *mdx* control; n=10 *mdx* splenectomised), (F) 8 weeks of age (n=5 for both groups) and (G) 12 weeks of age (n=10, *mdx* control; n=9 *mdx* splenectomised).

Figure S6

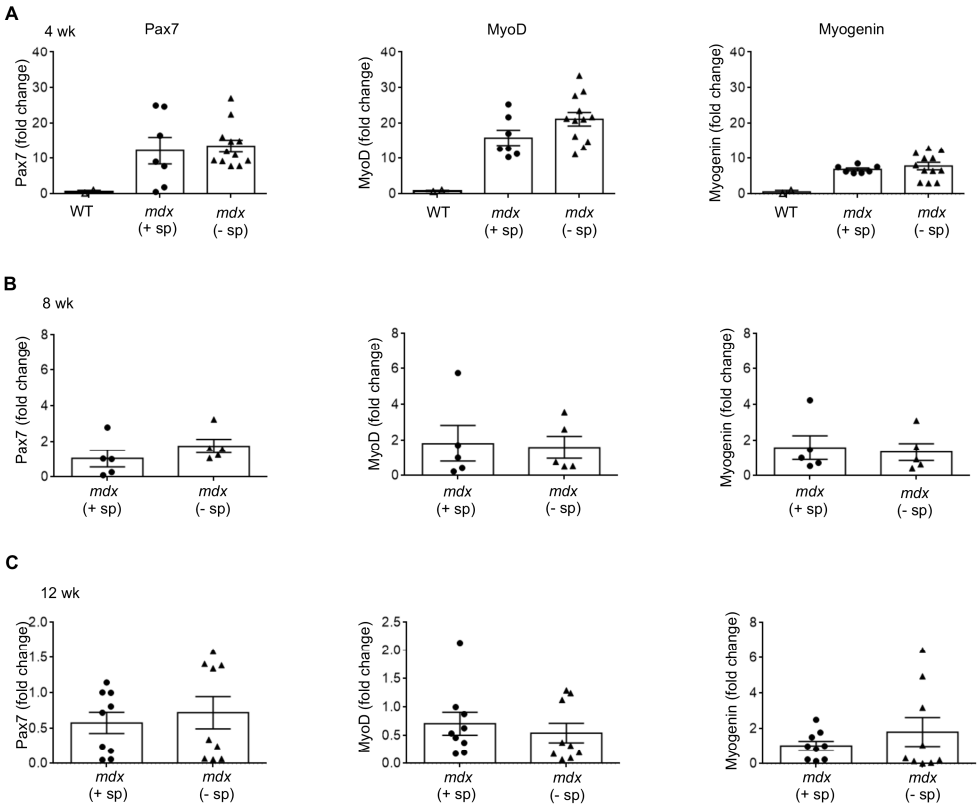

Figure S6. Myogenic marker mRNA expression levels in muscle of splenectomised *mdx* mice. (A) Expression levels of Pax7, MyoD and myogenin mRNA in limb muscle of control and splenectomised *mdx* mice at 4 (n=7, mdx control; n=12 mdx splenectomised), (B) 8 (n=5 for both groups) and (C) 12 weeks of age (n=9 for both groups).

Figure S7

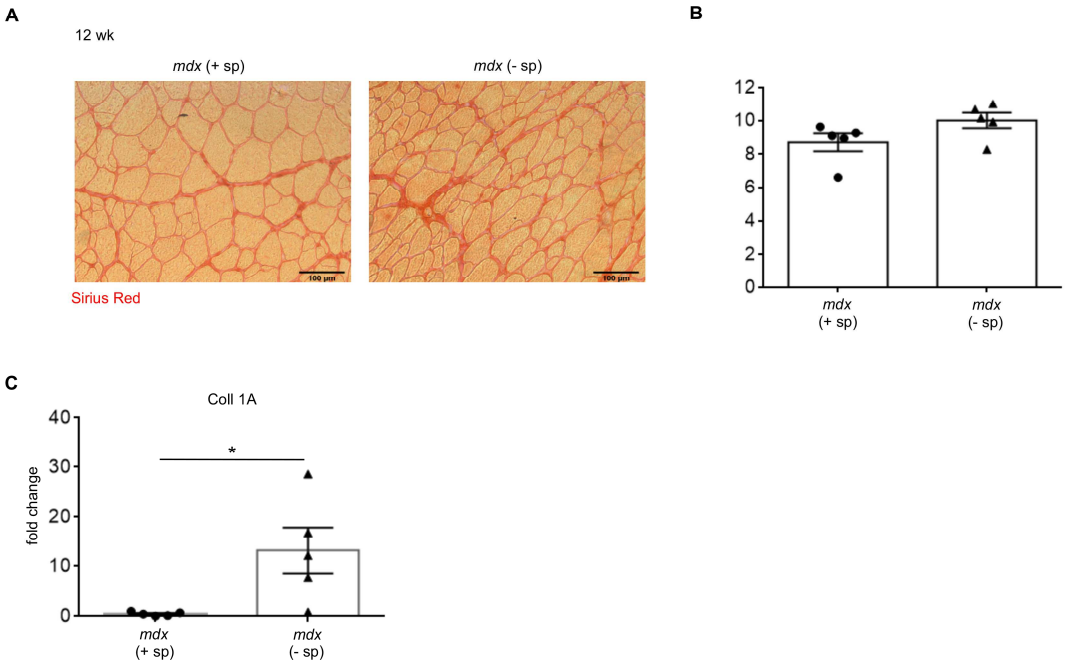

Figure S7. Effect of splenectomy on fibrosis in *mdx* limb muscle. (A) Representative images of TA muscle sections stained with sirius red for the analysis of collagen deposition and (B) summary graph of histological analysis showing percent fibrosis in control and splenectomised *mdx* mice (n=5 for both groups) at 12 weeks of age. (C) Expression levels of Collagen 1A mRNA in muscle of control and splenectomised *mdx* mice at 12 weeks of age assessed by quantitative real-time PCR. Data are presented as mean ± s.e.m. *P<0.05 by Student's *t* test.