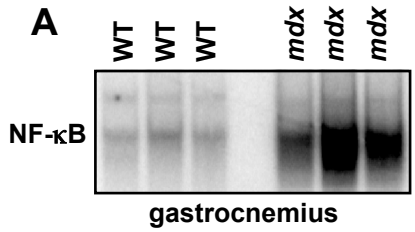
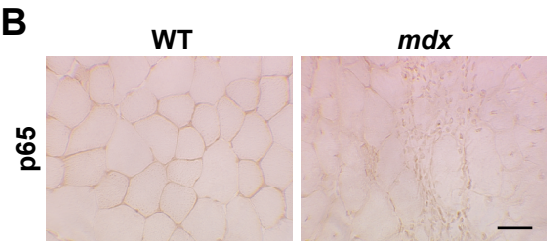
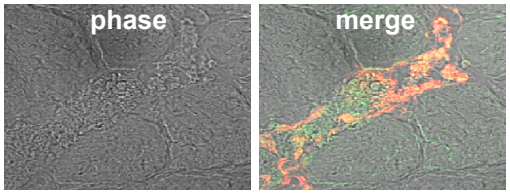
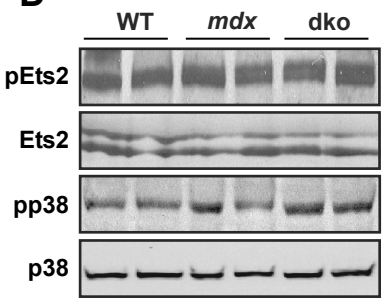
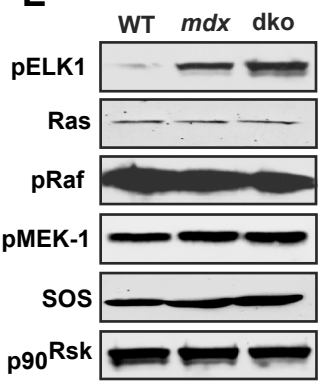
Suppl. Figure 1



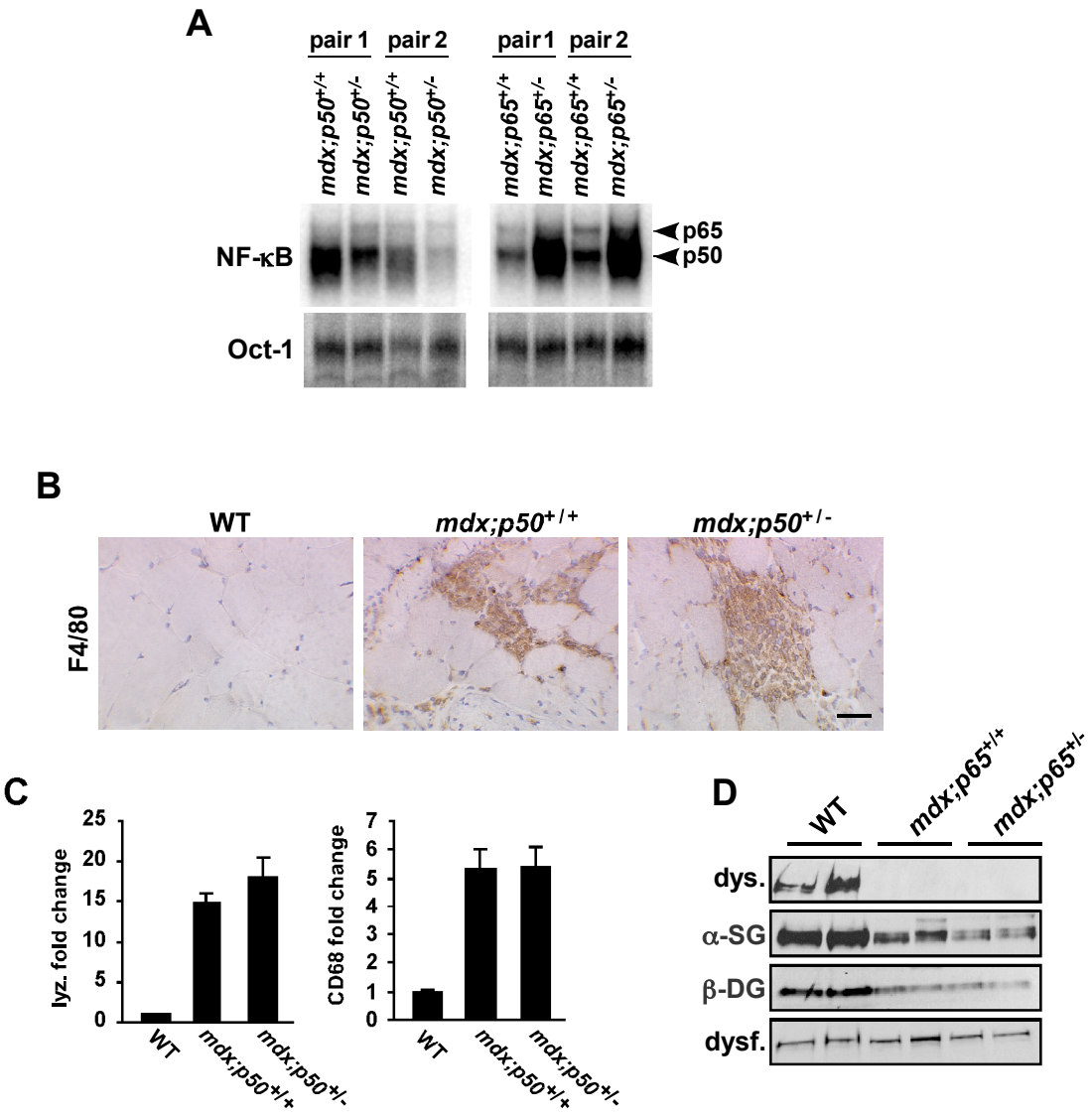




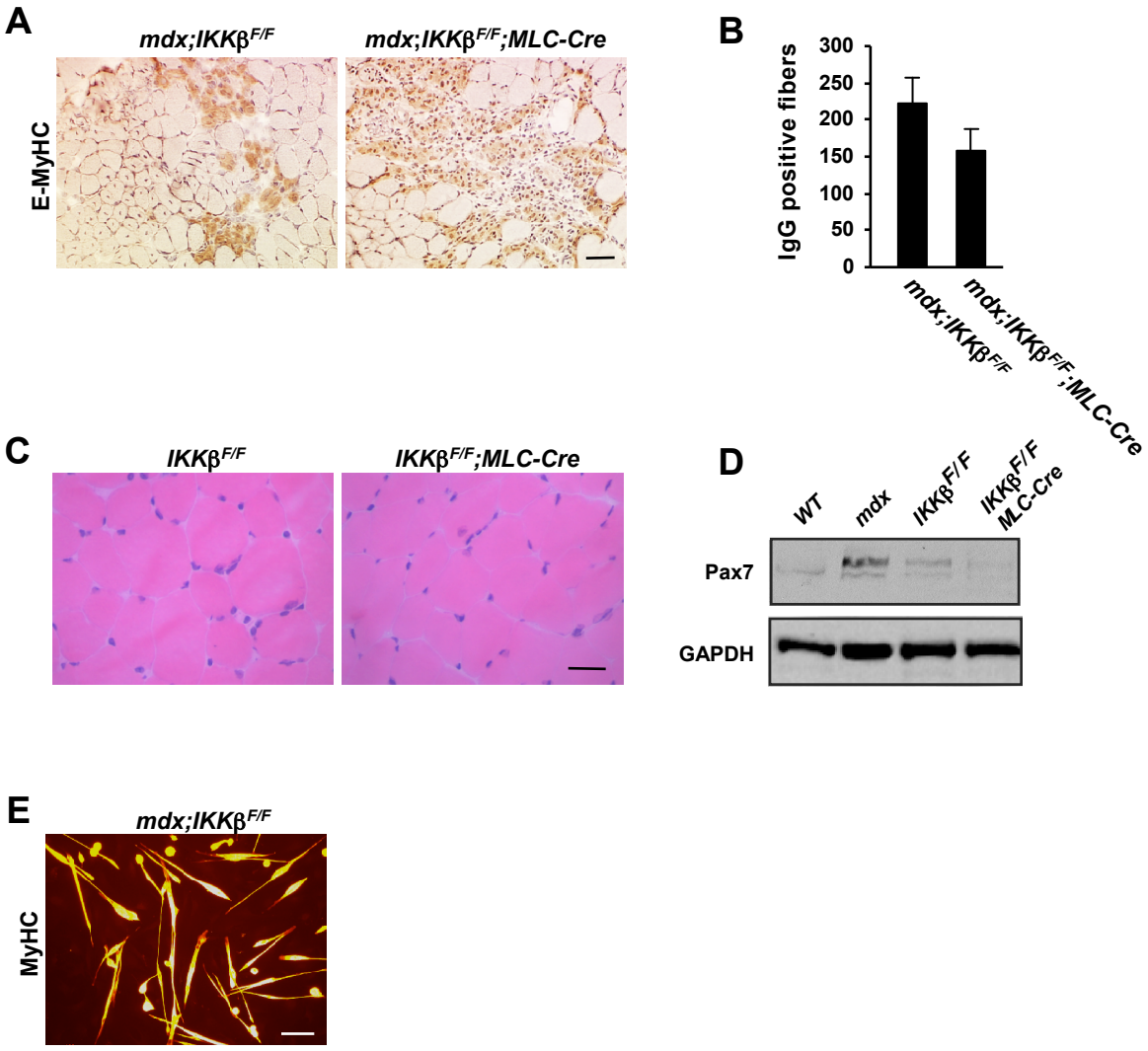




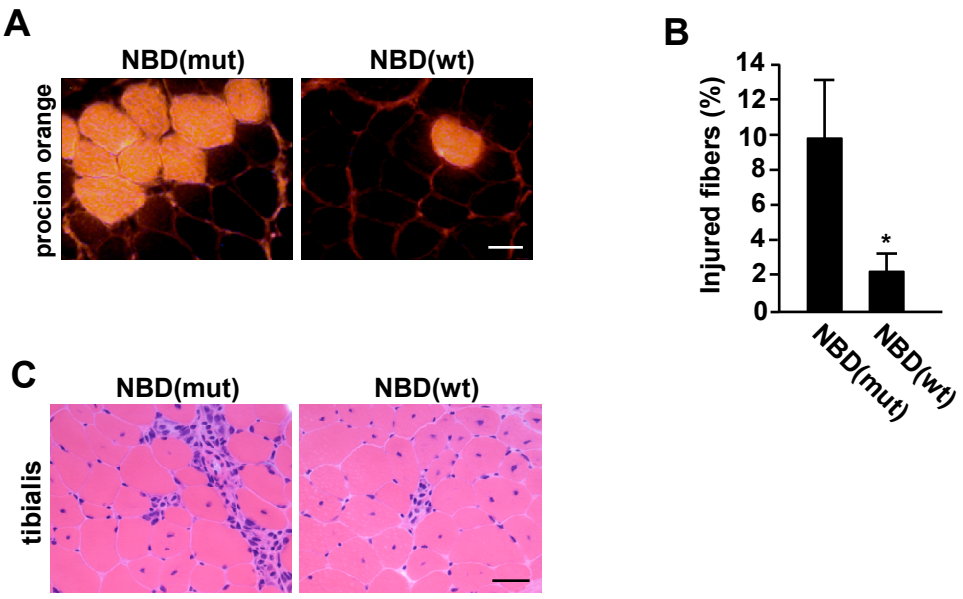
Suppl. Figure 2



Suppl. Figure 3



Suppl. Figure 4



Peptide synthesis

NBD peptide was generated using an ABI 430A solid-phase peptide synthesizer (Applied Biosystems, Foster City, Ca.) with standard tBOC (tert-butyloxycarbonyl)-chemistry. The peptides were cleaved from the resin and deprotected using hydrofluoric acid. The resulting crude materials were purified by HPLC on a Vydac (Vydac Separations, Hesperia, Ca) C18 preparative column using gradients of acetonitrile in 0.10% trifluoroacetic acid. Following lyophilization of the purified fractions, the expected molecular weight of the peptides were confirmed using matrix-assisted laser desorption ionization mass spectrometry.

Primers for Real-Time PCR

CD68, 5'-ACAGGCAGCACAGTGGACATTC-3' forward, 5'-ATGAGAGGCAGCA AGAG-3' reverse; lysozyme, 5'-GTGGGATCAATTGCAGTGCT-3' forward, 5'-TCTCGGTTTTGACAGTGTGCT-3' reverse; TNFα, 5'-CCCAAAGGGATGAGAAG TTCCC-3' forward, 5'-CCTGGTATGAGATAGCA AATCGG-3' reverse; IL1β, 5'-CCAAAAGATGAAGGGCTGCTTCC-3' forward, 5'-GGATGGGCTCTTCTTCAAA GATG-3' reverse; MCP-1; 5'-GGCTCAGCCAGAT GCAGTTAACG-3' forward; 5'-GCTGAAGACCTTAGGGCAGATGC-3' reverse; RANTES, 5'-CTCACCATAGGC TCGGACA-3' forward; 5'-CCTCTGAGTGACAA ACACGA-3' reverse; and MIP-1α, 5'-CAAGTCTTCTCAGCGCCATA-3' forward; 5'-GCAAAGGCTGCTGGTTTC AA-3' reverse. GAPDH primers 5'-GCAAATTCAA CGGCACAGTCAAG-3'

forward, 5'-GGTACAAACACTACCCACACTTG-3' reverse were used as internal controls.

Supp. Fig. 1. Characterization of signaling pathways in *mdx* muscles A. EMSAs were performed from nuclear extracts prepared from gastrocnemius muscles harvested from 5-wk-old wt and *mdx* mice B. Immunostaining was performed for total p65 comparing gastrocnemius sections from 7-wk-old WT or *mdx* mice. Scale bar denotes 50 μm. C. Differential interphase contrast of a muscle section from 4-wk-old mdx mice (left panel) or a merged image with pp65 (green) and CD68 (red). D and E. Western blot analysis was performed on tibialis muscle lysates prepared from the same mice as in (A) probing for Ets-2 and phospho-Ets2 (antibodies kindly provided by M. Ostrowski), p38 (1:500, Santa Cruz), phospho-p38 (1:500, Cell Signaling), phospho-Elk-1, phospho-Raf, MEK-1, and $p90^{Rsk}$ (1:1000, Cell Signaling), Ras (1:500, Calbiochem), and SOS (1:500, Upstate).

Supp. Fig. 2. Effects on pathology in p65 and p50 heterozygous *mdx* mice. A. EMSAs were performed from nuclear extracts prepared from tibialis muscles harvested from 2 pairs of 5-wk-old *mdx;p50*$^{+/+}$ and *mdx;p50*$^{+/-}$ mice and *mdx;p65*$^{+/+}$ and *mdx;p65*$^{+/-}$ mice. B. Gastrocnemius muscle sections from *mdx;p50*$^{+/+}$ and *mdx;p50*$^{+/-}$ mice were immunostained with F4/80. Scale bar denotes 50 μm. B. Real time PCR analysis for lysozyme and CD68 expression was performed on gastrocnemius muscles. C. Western blot analysis of DGC

associated proteins were performed comparing wt, *mdx;p65*$^{+/+}$, and *mdx;p65*$^{+/-}$ mice.

Supp. Fig. 3. Effects of muscle specific deletion of IKKβ in mdx and wild type mice. A. E-MyHC staining in *mdx;IKKβ*$^{F/F}$ and *mdx;IKKβ*$^{F/F}$*;MLC-Cre* gastrocnemius muscles. B. Quantitation analysis of IgG positive fibers from muscles used in A. C. H&E stained gastrocnemius cryosections from 4-wk-old *IKKβ*$^{F/F}$ and *IKKβ*$^{F/F}$*;MLC-Cre* mice (n=3). Scale bar denotes 50 μm. D. Western blot analysis of tibialis anterior muscle lysates from mice used in (C) to probe for Pax7 and α-tubulin. E. CD34^{+}, Sca-1^{-} flow sorted cells from 4 wk-old *mdx;IKKβ*$^{F/F}$ mice were differentiated and stained for MyHC. Scale bar denotes 100 μm.

Supp. Fig. 4. NBD treatment in *mdx* mice ameliorates dystrophic phenotype. A and B. Soleus muscles from 4-wk-old mice were treated with either wt or mut NBD. Dissected muscles were incubated with 0.2% procion orange (Sigma) in Kreb's Ringer solution for 1 hr. Muscles were washed, frozen, sectioned and then viewed by fluorescence microscopy to quantitatively measure myofiber injury (B, asterisk denotes $p<0.05$). C. H&E analysis of tibialis anterior muscles harvested from 50-day-old *mdx* mice injected with either mut or wt NBD peptide. Scale bar in A and C denote 50 μm.