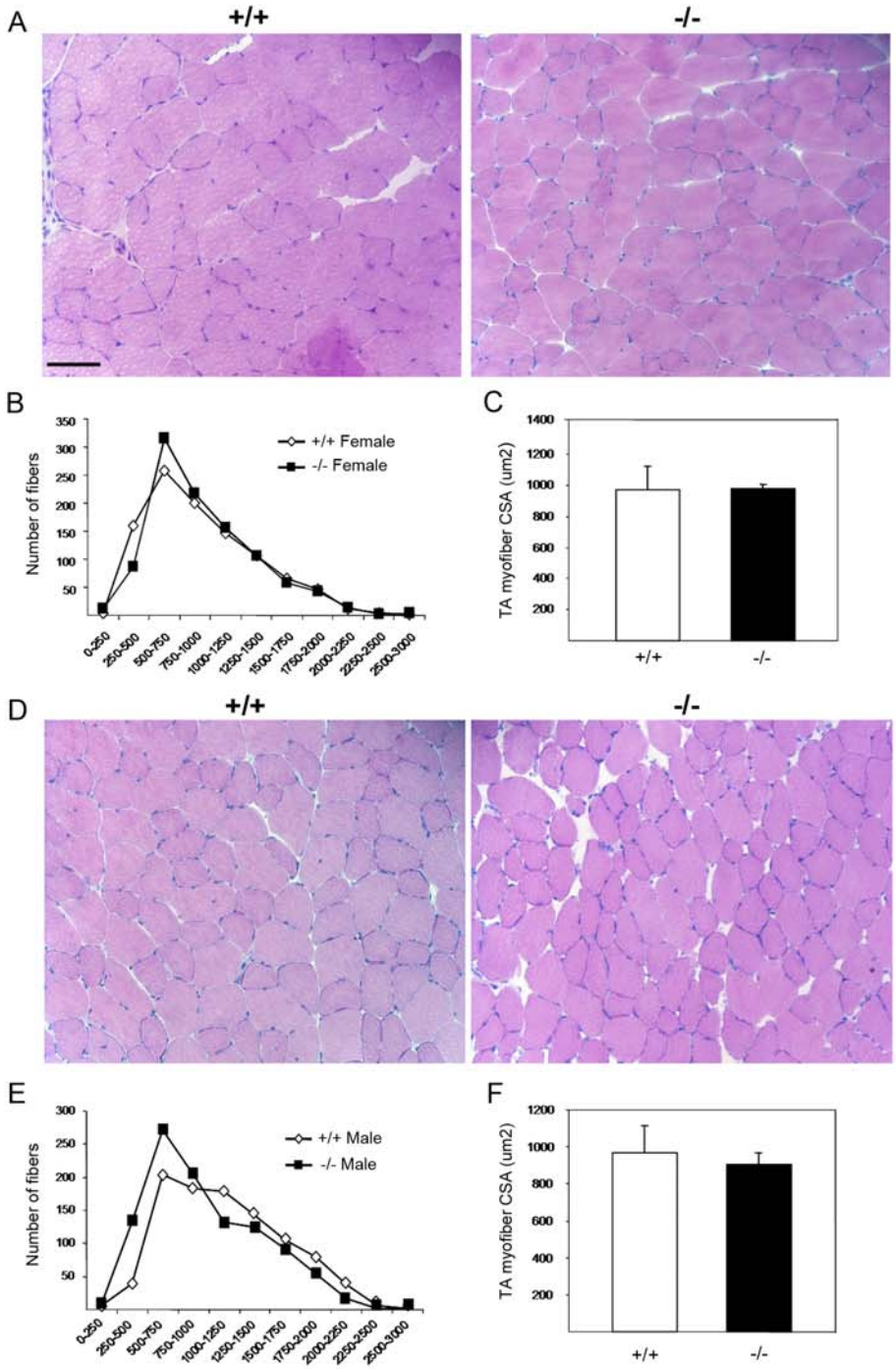
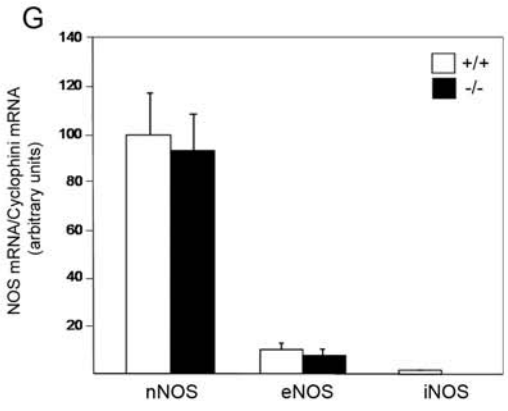
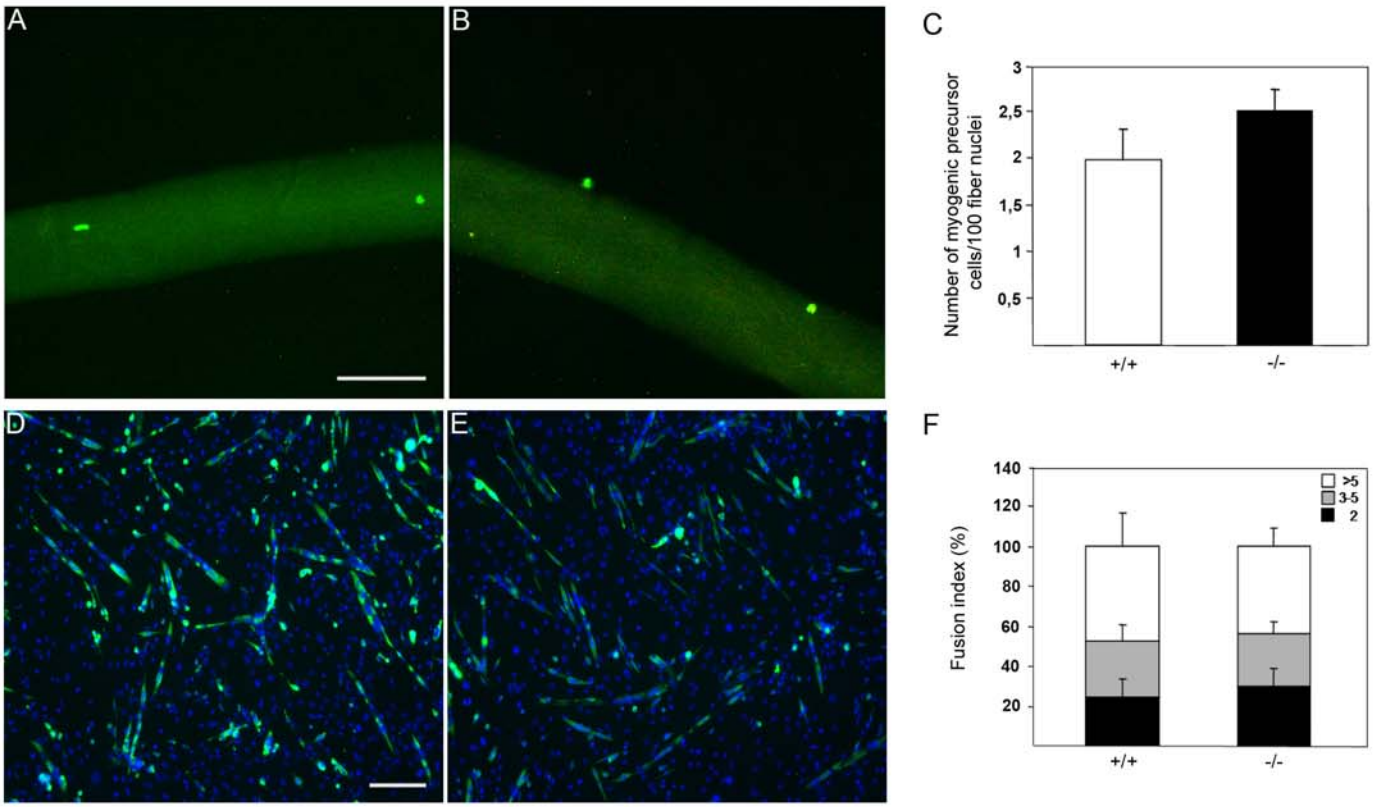




Supplementary figure 1. iNOS does not play a functional role in normal muscle maintenance in vivo

(A-F) iNOS+/+ and -/- muscles show uniform myofiber size and peripherally localized myonuclei. Representative images of TA muscle cross-sections of 2-month-old iNOS+/+ and -/- female (A) and male (D) mice stained with H&E. Magnification 20X. Scale bar: 50 μm. (B,E) Frequency histogram showing the distribution of myofiber cross-sectional area (CSA) in TA muscles of iNOS+/+ and -/- female (B) and male (E) mice. (C,F) Mean myofiber CSA for iNOS+/+ and -/- TA muscles from 2-month-old female (C) and male (F) mice. Data pooled from >1000 fibers from n=5 mice per sex and genotype. (G) iNOS ablation does not affect nNOS and eNOS expression. Quadriceps and TA muscles from 2-month-old iNOS+/+ and -/- mice were collected and processed for RNA extraction. Real time PCR analysis for nNOS, eNOS and iNOS mRNA expression was performed. Results were normalized to cyclophilin mRNA levels and expressed as relative fold change percentage compared to nNOS expression.



Supplementary figure 2. Myogenic precursor cell pool and fusion capacity are not impaired in iNOS-/- mice (A-C) Single muscle fibers were isolated from Gastrocnemius muscles of 2-month-old iNOS+/+ (A) and iNOS -/- (B) mice. Representative images of single fiber-associated myogenic precursor cells, immunostained with a specific antibody for Pax7. (C) Histogram representing the number of quiescent Pax7+ myogenic precursor cells on iNOS+/+ and -/- single myofibers. Results represent the means +/- SEM of 3 independent experiments, normalized for myofiber nuclei number. (D-F) Primary myoblasts from iNOS+/+ and -/- newborn mice were maintained for 24 h in growth medium and then switched to the differentiation medium for 24 and 48 h. Representative images of IF on iNOS+/+ (D) and iNOS-/- (E) primary myoblast culture after 48 h in differentiation medium, using an antibody specific for sarcomeric MyHC. Nuclei are visualized with Hoechst. Magnification 10X. Scale bar: 200 μm. (F) The fusion index indicates the percentage of nuclei within MyHC-positive myotubes with more than two nuclei. Fusion index was calculated after 48 h in differentiation medium by counting the number of myosin positive fibers displaying respectively 2 nuclei (black), 3<nuclei>5 (gray), and >5 nuclei (white). Result represents the means +/- SD of 3 independent experiments performed in triplicate.