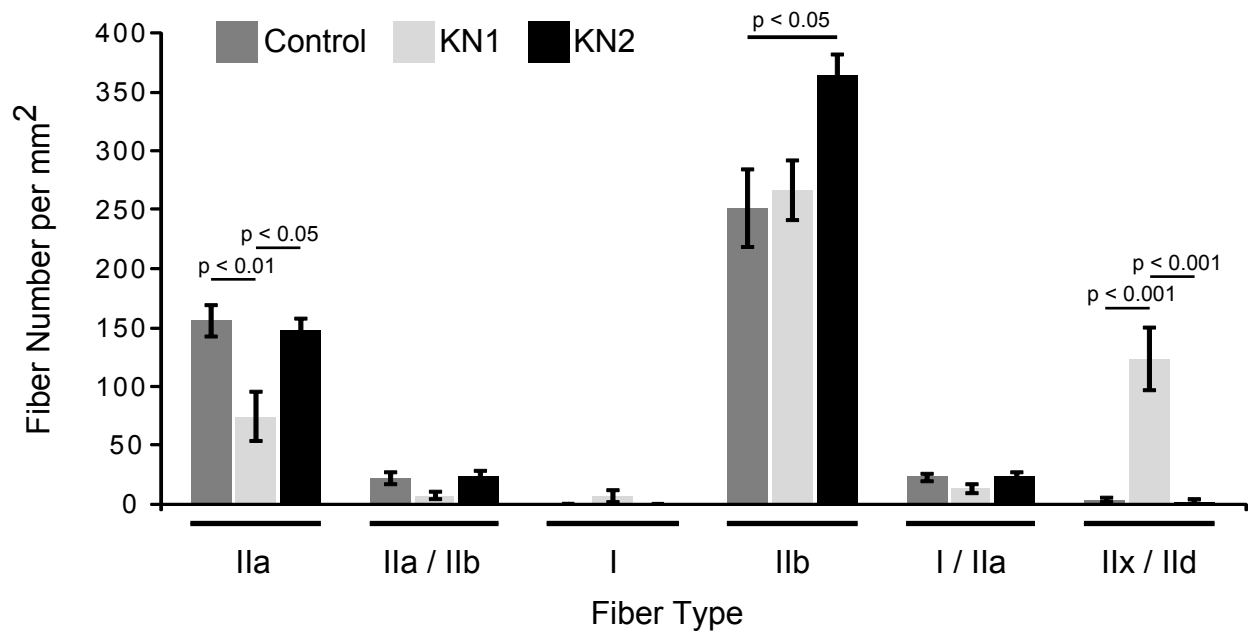
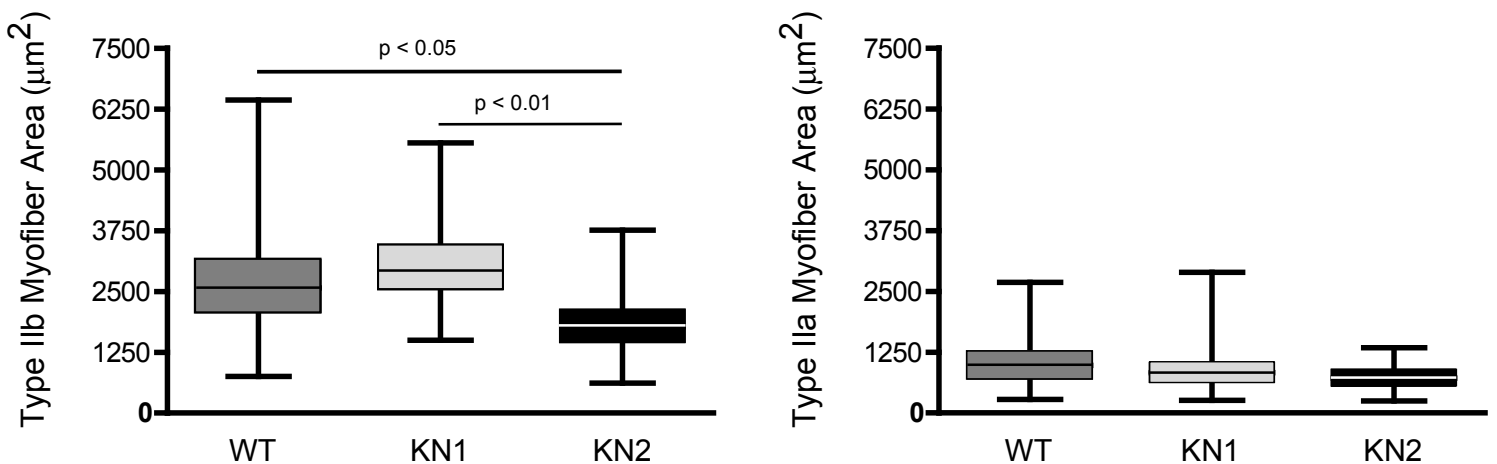
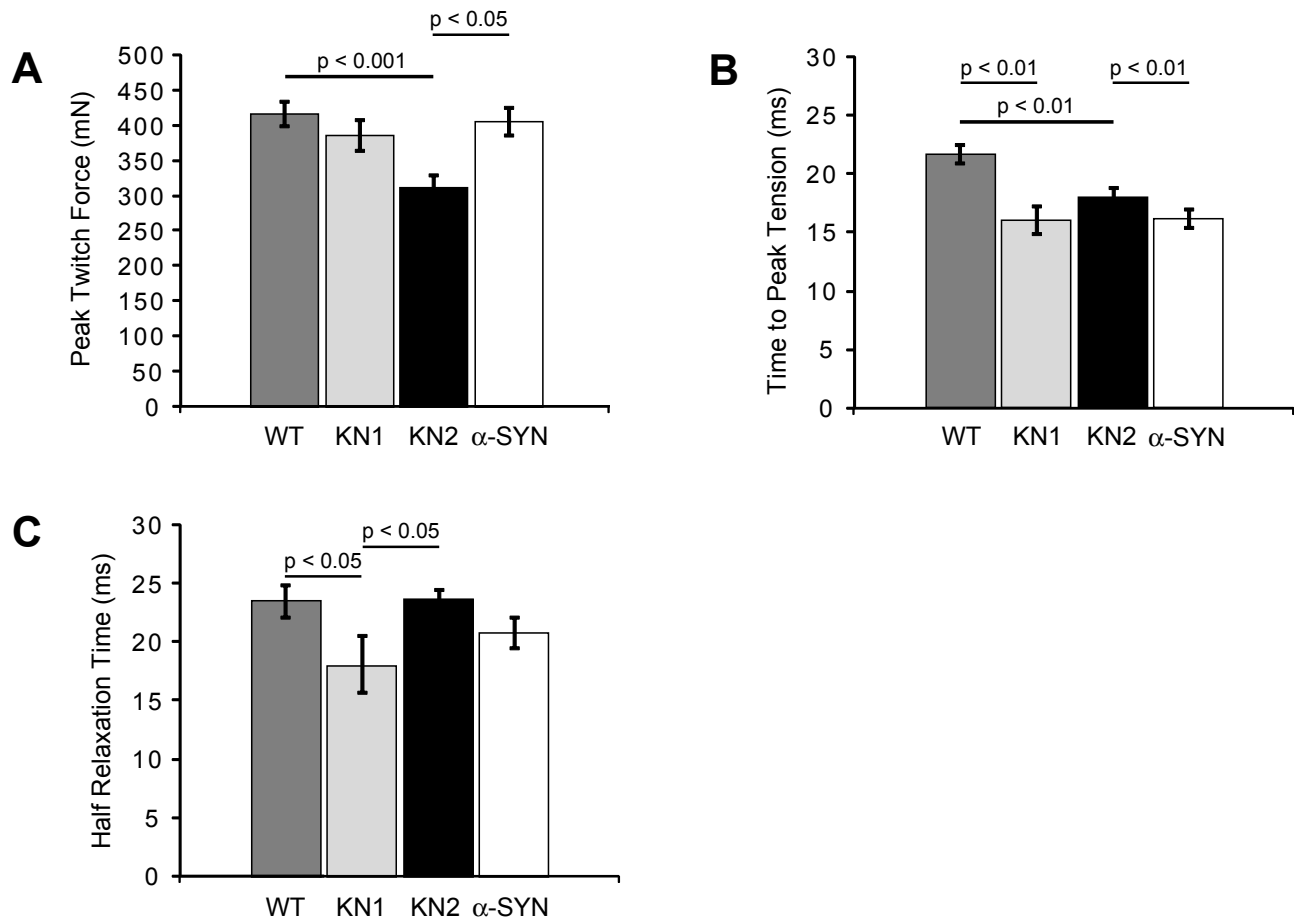


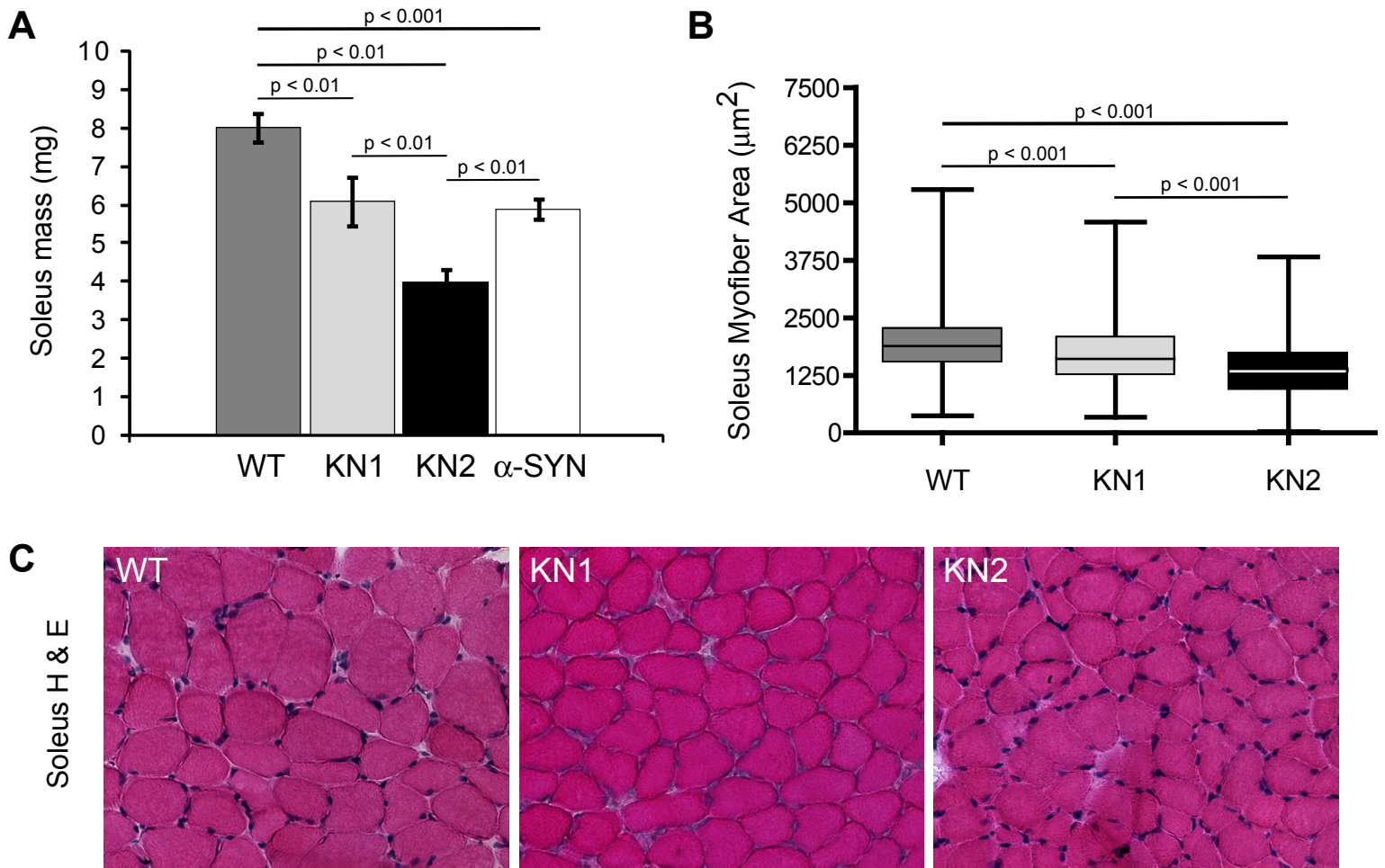
Supplemental Figure 1. The Golgi nNOS splice variant is detected by four distinct pan-specific nNOS antibodies. To confirm that Golgi nNOS labeling was not due to non-specific antibody cross reactivity we tested the ability of four commercially available antibodies raised against unique epitopes to detect the Golgi nNOS. All four antibodies detected the Golgi nNOS. Pan-specific antibodies used were raised against (i) the amino terminus (Invitrogen), (ii) carboxyl terminus residues 1409-1429 (Sigma-Aldrich), (iii) carboxyl terminus residues 1089-1284 (Becton Dickinson Transduction Labs) and (iv) carboxyl terminus residues 1414-1428 (Diasorin). FITC-conjugated anti-GM130 antibody was used to label the Golgi complex.

A**B**

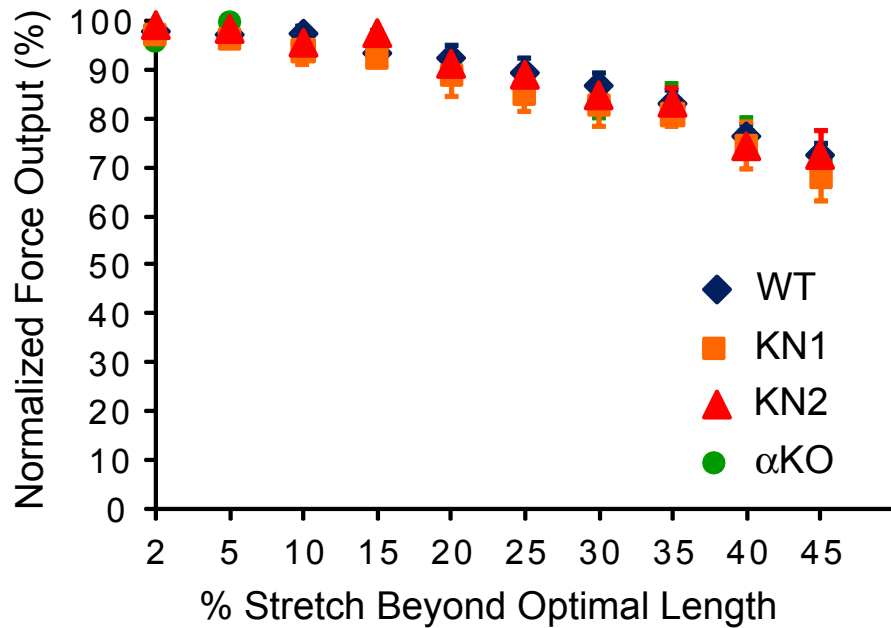
Supplemental Figure 2. (A) nNOS splice variants differentially regulate fiber composition in *tibialis anterior* (TA) muscle. Antibodies specific for myosin heavy chains 1, Ila and Ilb were used to identify slow oxidative type 1 (most fatigue resistant), fast oxidative type Ila (intermediate fatigue resistance) and fast glycolytic type Ilb (least fatigue resistant) fiber types in the TA muscles from WT, KN1 and KN2 mice. Unlabeled fibers were designated fast oxidative Type Ilx / Ild fibers which are also characterized by a high degree of fatigue resistance. The numbers of fast and slow fibers per mm² were determined from entire WT, KN1 and KN2 muscle sections. KN1 muscles exhibited shift to a more fatigue resistant fiber composition characterized by a reduction in Type II a fibers concomitant with an increase in Type Ilx / Ild fibers. In KN2 muscles, there was a 45 % increase in the number of fatigue susceptible fast twitch glycolytic Type Ilb fibers. Mean values \pm S.E.M are shown. $n \geq 4$. **(B)** The cross sectional areas (CSA) of Type Ilb fibers from KN2 TA muscles were significantly decreased by 31 % relative to KN1 and wild type controls. WT and KN1 Type Ilb fiber CSA were not significantly different. Also, the CSA of Type Ila fibers did not differ significantly between wild type, KN1 and KN2 TA muscles. These data suggest that reduced muscle mass of KN2 TA muscles may result from inability of Type Ilb fibers to attain a normal size. The median is represented by the horizontal line in the box. $n = 4$.



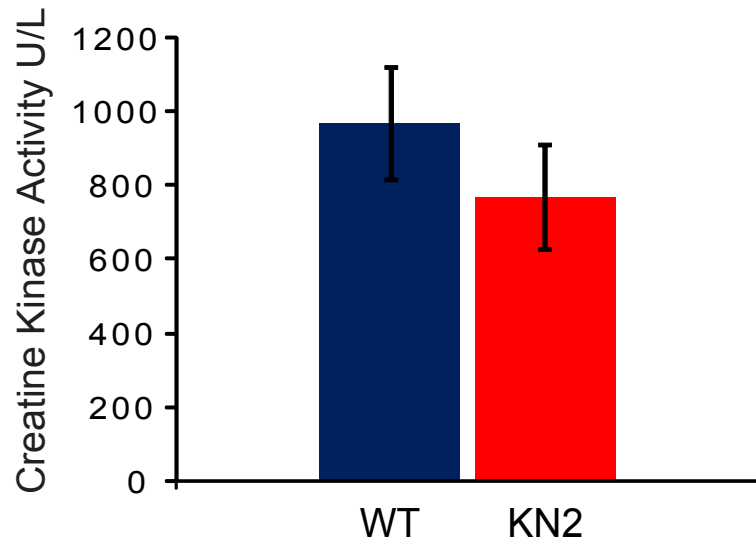
Supplemental Figure 3. nNOS splice variants differentially regulate fiber twitch force properties in *tibialis anterior* (TA) muscle. Because isometric twitch properties depend on muscle fiber composition, we investigated whether the shifts in fiber composition in KN1 and KN2 mice correlated with changes in the contraction and relaxation times of isometric twitch contractions in TA muscle. **(A)** Maximum twitch tension (contractility) was decreased significantly in KN2 muscle only. **(B)** nNOS mutant myofibers contracted at a faster speed, thereby taking a shorter time to generate peak twitch tension than wild type controls. Faster times to peak twitch tension are consistent with an increased number of fast twitch fibers **(C)** nNOS μ -deficient myofibers exhibited a faster relation time than controls. Mean values \pm S.E.M are shown. $n \geq 6$.



Supplemental Figure 4. nNOS splice variant regulation of muscle mass is modulated by muscle type. **(A)** Mistargeting of sarcolemmal nNOS μ in α -SYN mice or loss of sarcolemmal and cytosolic nNOS μ in KN1 mice both lead to a significant reduction in soleus muscle mass (contrast with the TA, see Figure 7). The additional loss of nNOS β lead to a further significant 50 % reduction ($p < 0.01$) in muscle mass. **(B)** KN1 myofibers were significantly smaller ($p < 0.001$) in cross sectional area (CSA) than wild type controls suggesting that nNOS μ could regulate the size of more oxidative muscles. The CSA of KN2 soleus muscles was significantly smaller than KN1 muscle or wild type controls suggesting that decreased muscle mass could be accounted for by reduced muscle cell size. **(C)** Decreased cross sectional area was evident from hematoxylin & eosin labeled TA muscle sections. Taken together, these data argue that nNOS β is a critical regulator of skeletal muscle cell size and nNOS μ regulates the cell size and muscle mass of more oxidative muscles. **(A)** Mean values \pm SEM for muscle mass. **(B)** Median is represented by the horizontal black line in the box. $n \geq 4$.



Supplemental Figure 5. nNOS splice variants do not regulate susceptibility to lengthening contraction-induced injury. To determine if nNOS was required for protection against contraction-induced mechanical injury, TA muscles were subject to a series of lengthening contractions of progressively increasing strain. Strain was induced by stretching TA muscles from 5 to 45 % beyond their optimal length for generating force (L_0). Increased susceptibility would be indicated by decreased force output at increasing strains. The susceptibility to contraction-induced injury in α -SYN (green circles), KN1 (orange squares), and KN2 (red triangles) did not differ from wild type (blue diamonds). $n \geq 6$.



Supplemental Figure 6. nNOS splice variant deficiency does not impact myofiber turnover. Skeletal muscle myofiber instability leads to muscle cell turnover which is correlated with creatine kinase (CK) release into the serum. An unpaired Student's t-test revealed no significant difference between mean WT and KN2 serum creatine kinase activity. $n \geq 15$.