Supplementary Information

Cardiac troponin T and autoimmunity in skeletal muscle aging

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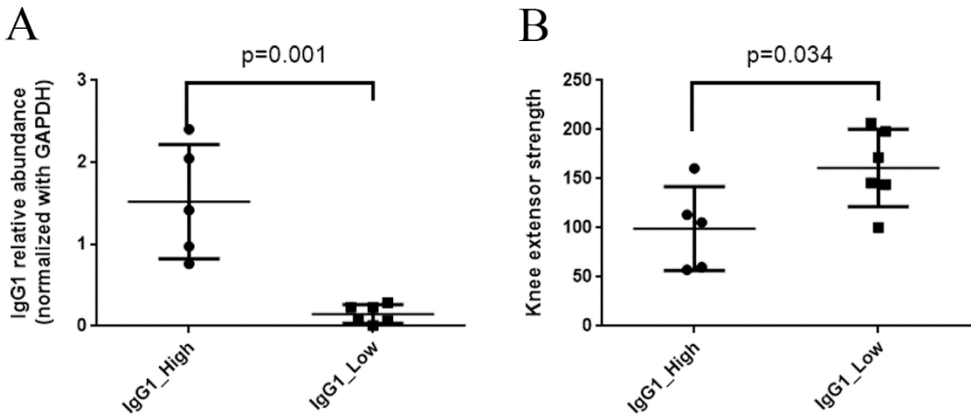
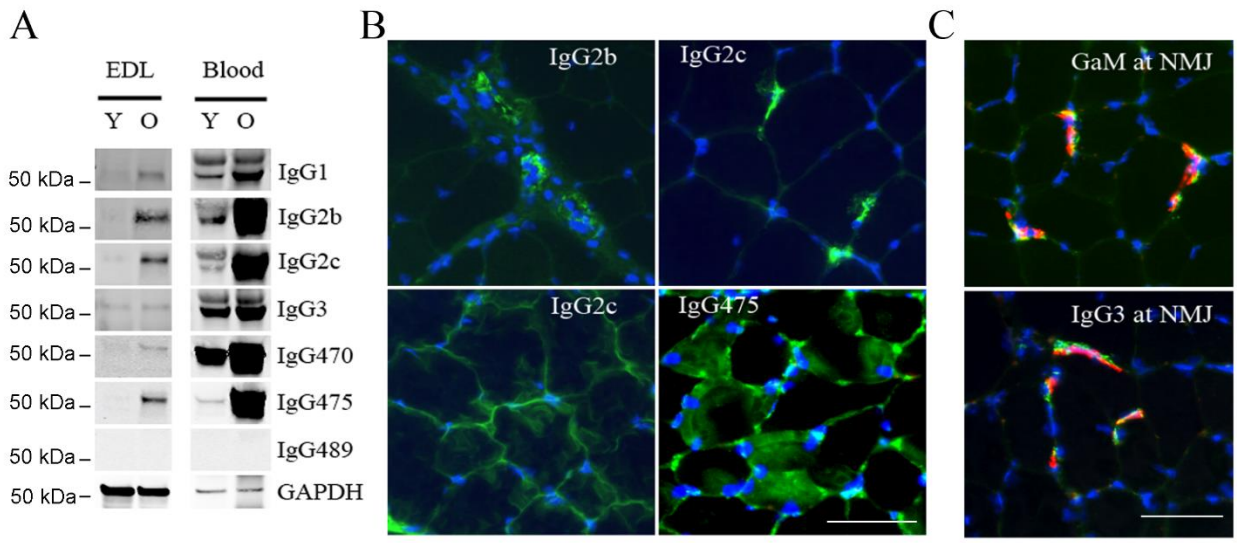
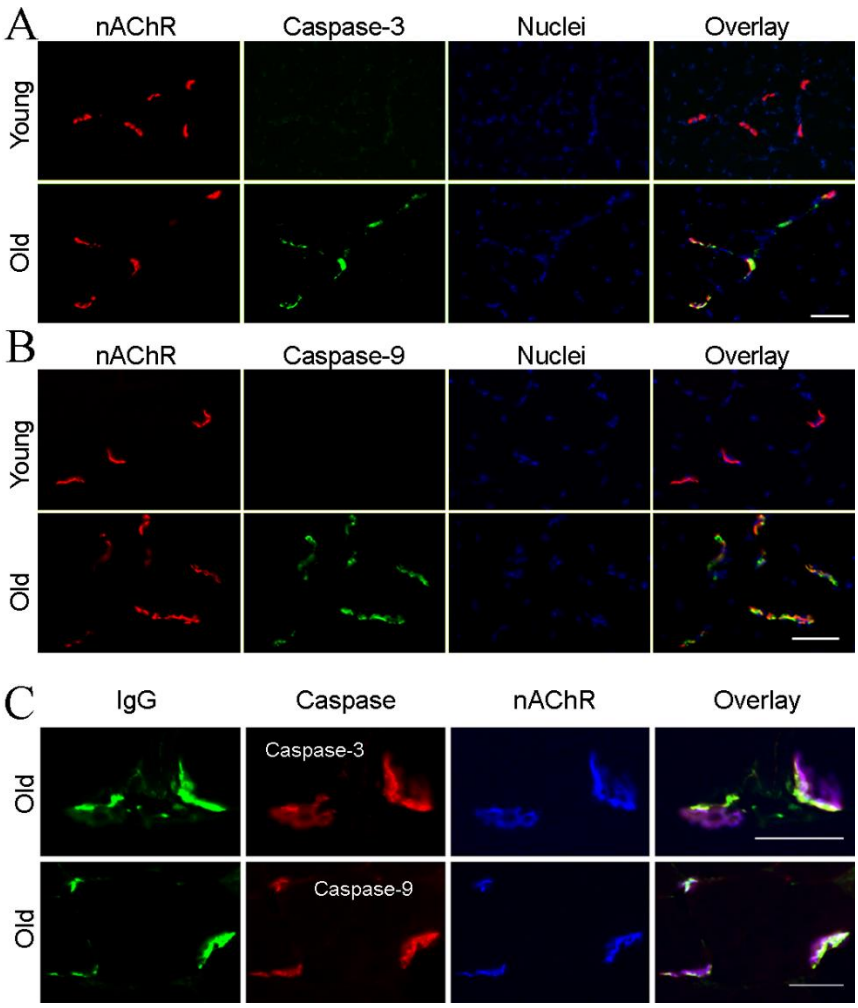


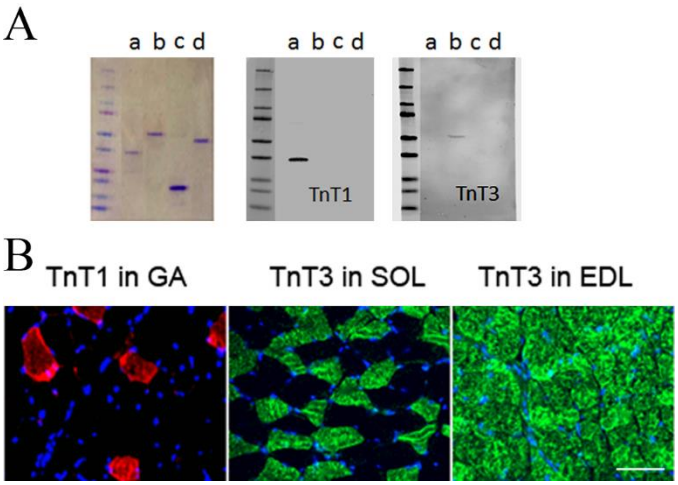
Supplementary Figure 1. Comparison of keen extensor strength between older adults with low or high IgG1 protein levels in their limb muscle. Based on relative abundance of muscle IgG1 determined from immunoblot blot data in Figure. 1C, IMFIT study older adults were further divided into IgG1_low (5 men, 69.6 ± 2.6 years) and IgG1_high (3 women and 3 men, 69.7 ± 4.1 years) groups (A). The knee extensor strength between the two groups was found to be significantly different (B).



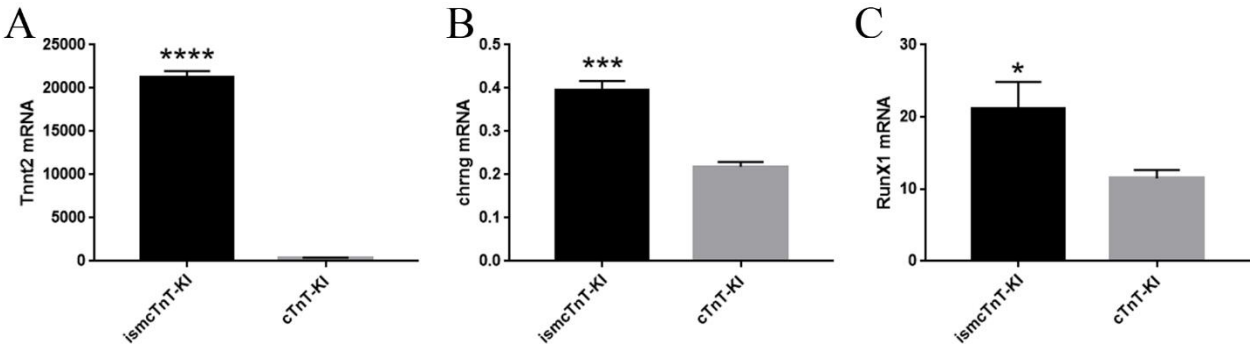
Supplementary Figure 2. IgG levels in blood and in skeletal muscle increases with age in C57BL/6 mice in a subclass specific manner. (A) Representative immunoblot data showing IgG subclass (IgG1, 2b, 2c, and 3) specific abundance in mouse EDL (extensor digitorum longus) muscle and in the blood. IgG antibody IgG470 (ab133470) and IgG475 (ab190475) are mouse IgG specific. Human IgG specific antibody IgG489 (ab109489) did not detect any band at the predicted IgG band region. (B) Representative immunofluorescence staining images of IgG subclasses in TA muscle of age mice are shown. Consistent with findings by immunoblot in A, IgG2b and 2c subclasses are detected clearly in the muscle, either in the interstitial area (IgG2b, 2c) or within myofibers (IgG2c). IgG475 also detected IgG in the myofibers. (C) Goat-anti-mouse secondary antibody alone (GaM) detected IgG deposition (green) at NMJ (red, stained with Alexa Fluor 594-conjugated α-Bungarotoxin) area of some aged mice. IgG3 but not other IgG subclasses was determined to be the main IgG proteins that are deposited at NMJs in aged mice skeletal muscle. Data shown here are representative of all mice examined in Figure 2. Scale bars, 50μm.



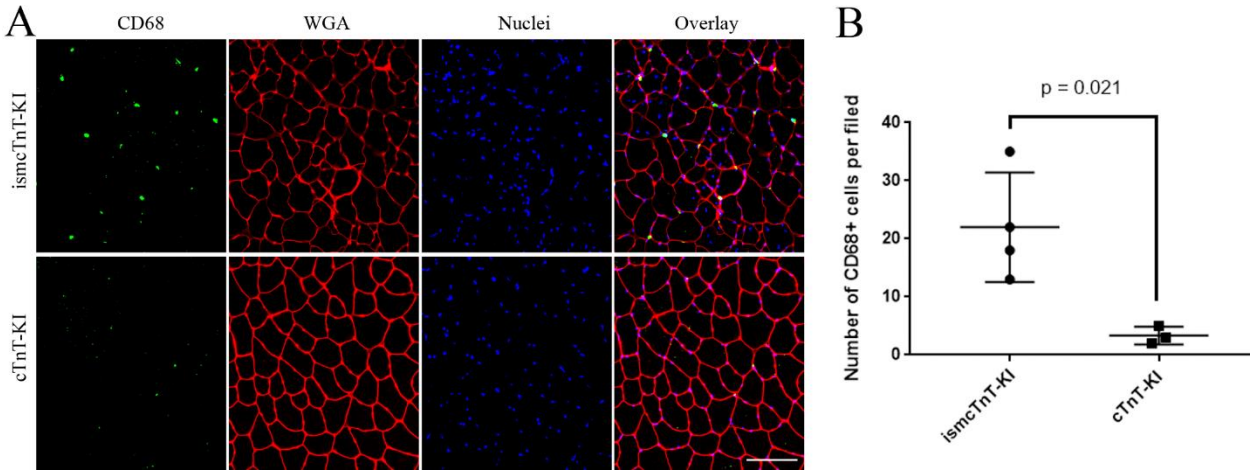
Supplementary Figure 3. Caspase-3 and caspase-9 are co-localized with cTnT and IgG at NMJ of aged mice. Representative immunofluorescence staining image showing enrichment of caspase-3 (A) and caspase-9 (B) at NMJ (nAChR) in tibialis anterior muscle of old but not young mice. (C) All old mice with caspase enrichment at NMJ were also found to have elevated IgG deposition at their NMJs. cTnT was also found to be positively stained at these IgG deposited NMJs (see Figure. 4A). Scale bars, 50μm.



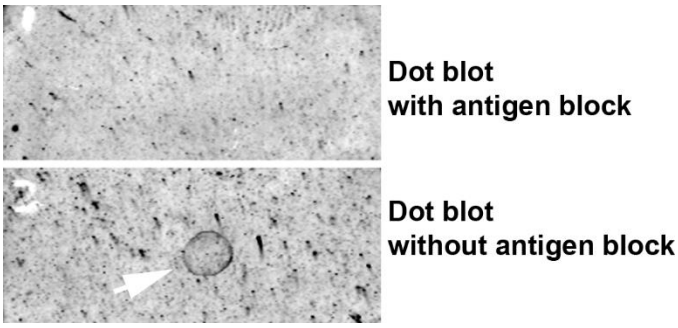
Supplementary Figure 4. TnT1 and TnT3 antibody specificity determined with immunoblot and immunofluorescence staining. (A) Coomassie blue stained SDS-PAGE gel separated recombinant TnT proteins (left column). The same proteins were tested with immunoblot using TnT1 and TnT3 antibodies, which detected TnT1 or TnT3 highly specifically. Recombinant proteins used for immunoblot: a, human TnT1 protein (GTX109585-pro, GeneTex); b, mouse TnT3 isoform 8 protein with a fusion His-SUMO (Small Ubiquitin-like Modifier) tag; c, SUMO tag (GenScript); d, human cTnT isoform 11 protein (ab86685, Abcam). (B) Immunfluorescence staining with TnT1 and TnT3 antibodies labelled slow and fast fibers in GA (gastrocnemius), SOL (soleus), and EDL (extensor digitorum longus) muscles in red or in green, respectively. Nuclei (blue) were stained with DAPI. Scale bar, 50μm.



Supplementary Figure 5. mRNA gene expression level quantitation in the EDL skeletal muscle. ismcTnT-KI mice EDL (extensor digitorum longus) muscle have elevated cTnT encoding gene (*Tnnt2*) mRNA levels and increased levels of NMJ denervation markers (chrng and RunX1) revealed by qRT-PCR. ****, $p < 0.0001$; **, $p < 0.01$.



Supplementary Figure 6. The ismcTnT-KI mice have higher level of macrophage infiltration in their skeletal muscle. (A) Representative immunofluorescence staining image of CD68+ macrophage infiltration (green) in tibialis anterior muscle of ismcTnT-KI and control cTnT-KI mice. Fiber membrane was stained with WGA (Alexa Fluor 594-conjugated Wheat Germ Agglutinin) and irregular myofiber outline shape with smaller size and reduced WGA staining signal was manly seen in ismcTnT mice. Scale bar, 50μm. (B) Quantitation of CD68+ cells in tibialis anterior muscle of ismcTnT mice and control cTnT-KI mice.



Supplementary Figure 7. Specificity validation of anti-cTnT autoantibodies in human plasma with dot blot and antigen blocking. Representative dot blot image shows that dot blotting with antigen block could inhibit the detection of recombinant cTnT protein loaded on the NC membrane. In contrast, without antigen block, the loaded recombinant protein (white arrow) was detected on the NC membrane.