

Ju et al., <http://www.jcb.org/cgi/content/full/jcb.200908115/DC1>

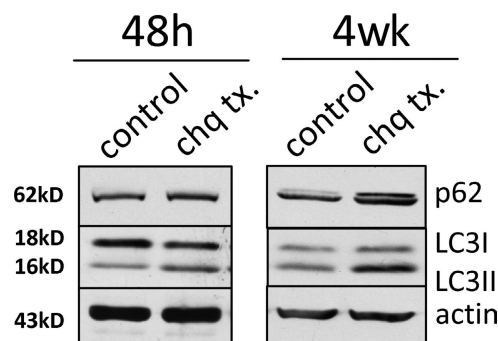


Figure S1. **p62 and LC3 immunoblot analysis of skeletal muscle lysates from chloroquine-treated animals.** Representative immunoblots for p62 and LC3 from quadriceps muscle of 3-mo-old female mice treated for 48 h or 4 wk with daily intraperitoneal saline (control) or 50 μ g/kg chloroquine (chq tx.). Note that the increases in p62 and LC3II upon chemical inhibition of autophagy are consistent with the increases seen in Fig. 1.

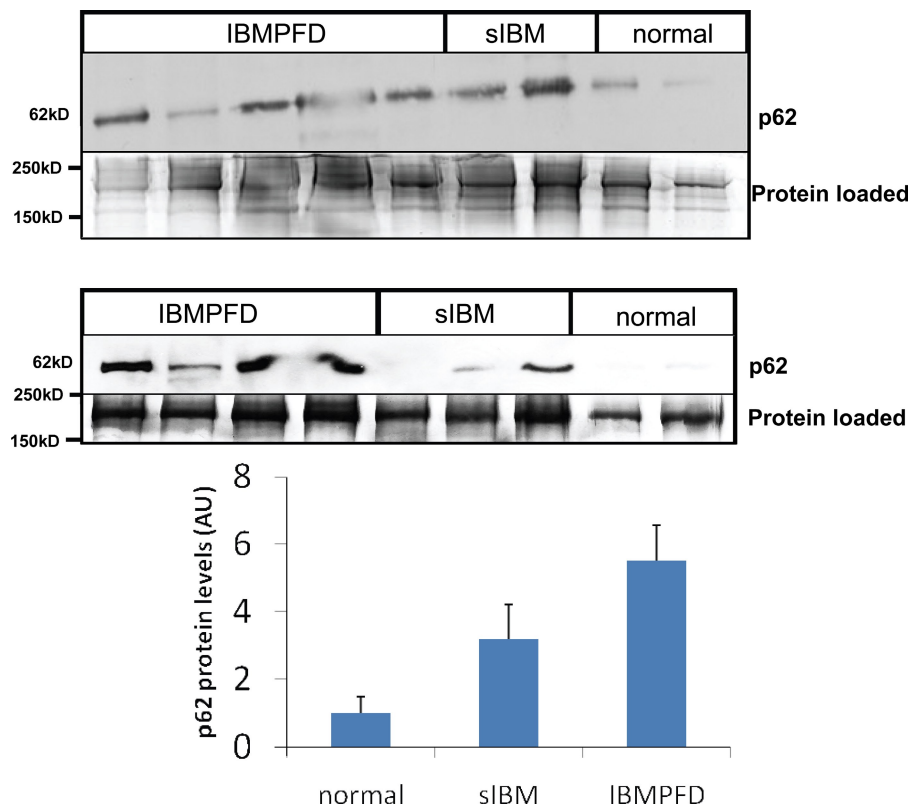


Figure S2. **p62 immunoblots from IBMPFD, sIBM, and control patient muscle.** Nine IBMPFD, five sIBM, and four normal patient skeletal muscle lysates were immunoblotted for p62. Please note that these are archived frozen muscle biopsy samples. Biopsies contain varying degrees of fibrosis, connective tissue, and degradation artifact. Densitometric analysis of p62 protein levels for normal (arbitrarily set as 1), sIBM, and IBMPFD patient muscle is shown graphically below. Total p62 protein levels were plotted for normal (arbitrarily set as 1), sIBM, and IBMPFD patient muscle. Error bars represent the standard error among samples.

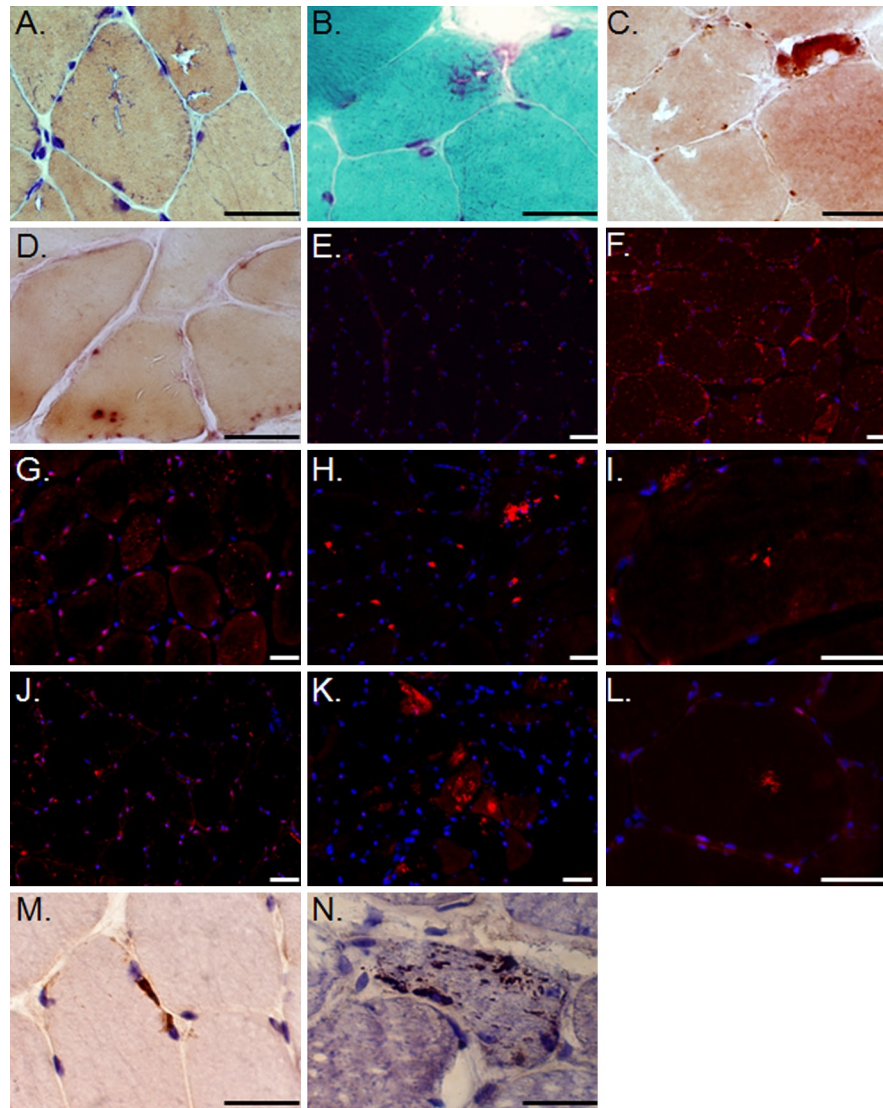


Figure S3. **Histochemical and immunohistochemical analysis of IBMPFD patient skeletal muscle biopsies.** (A–D) Histochemical analysis of IBMPFD patient skeletal muscle. Hematoxylin and eosin (A), Gomori trichrome (B), nonspecific esterase (C), and acid phosphatase (D) are shown. (E and F) Lamp2 immunostaining of normal (E) and IBMPFD (F) patient muscle. (G–I) p62 immunostaining of normal (G) and IBMPFD (H and I) patient muscle. (J–L) LC3 immunostaining of normal (J) and IBMPFD (K and L) patient muscle. (M and N) TDP-43 immunostaining with Congo red counterstaining of IBMPFD patient muscle. Bars, 40 μ m.

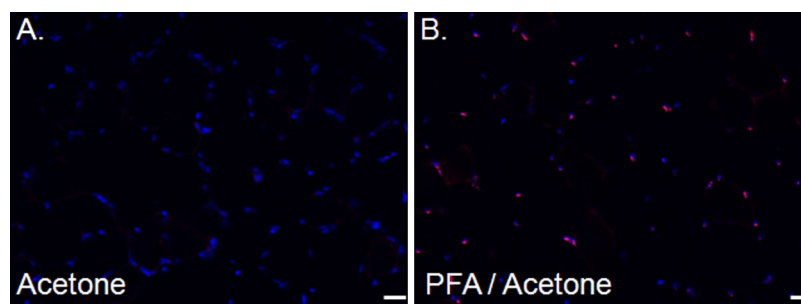


Figure S4. **TDP-43 immunofluorescence in normal mouse muscle.** (A and B) TDP-43 immunofluorescence (red) of 3-mo-old mouse quadriceps using acetone (A) or 4% PFA and then acetone fixation (B). Bars, 30 μ m.

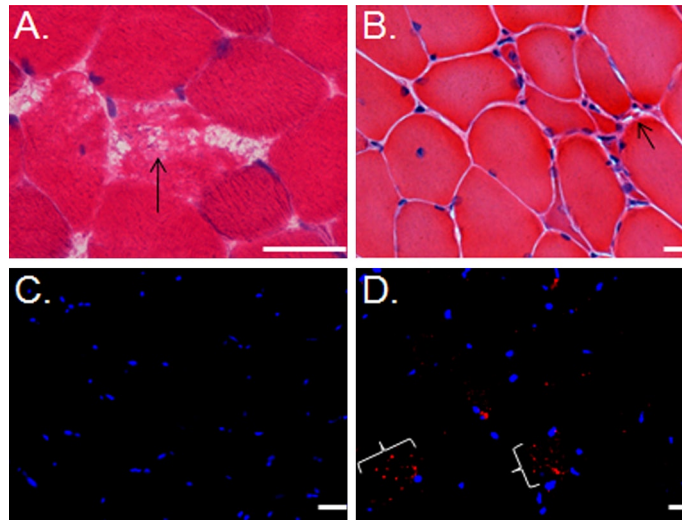


Figure S5. **Skeletal muscle histochemistry and p62 immunohistochemistry of chloroquine-treated mice.** (A and B) Hematoxylin and eosin staining of quadriceps muscle from 3-mo-old mice treated with 50 mg/kg/d chloroquine. Vacuolated fiber (A) and myopathic features, including angular fibers and central nuclei (B), are shown. Arrows denote small atrophic vacuolated fiber. (C and D) p62 (red) immunofluorescence of quadriceps muscle from control (C)- and chloroquine-treated (D) mice. Note that select fibers have prominent p62 puncta. Brackets denote single fibers with prominent p62 puncta. Bars, 30 μ m.