Cohen et al., http://www.jcb.org/cgi/content/full/jcb.200901052/DC1

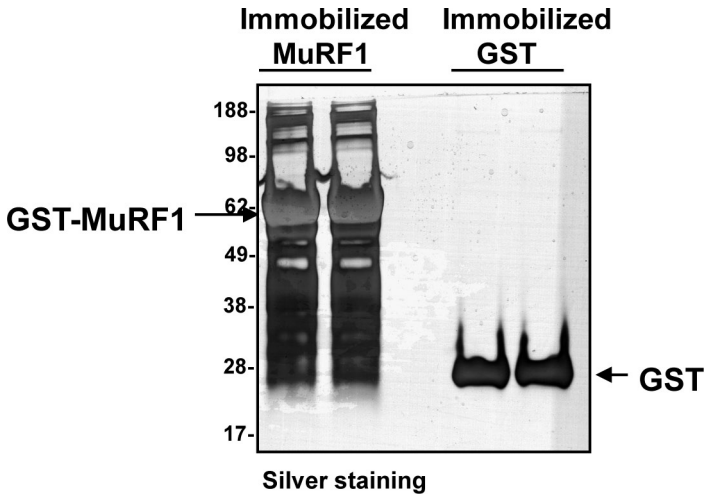

Figure S1. **Analysis of MuRF1's bound proteins by SDS-PAGE.** Immobilized GST-MuRF1 or GST alone was incubated with gastrocnemius extracts to isolate muscle proteins having high affinity for MuRF1. The bound proteins were resolved by SDS-PAGE and silver staining before mass spectrometry.

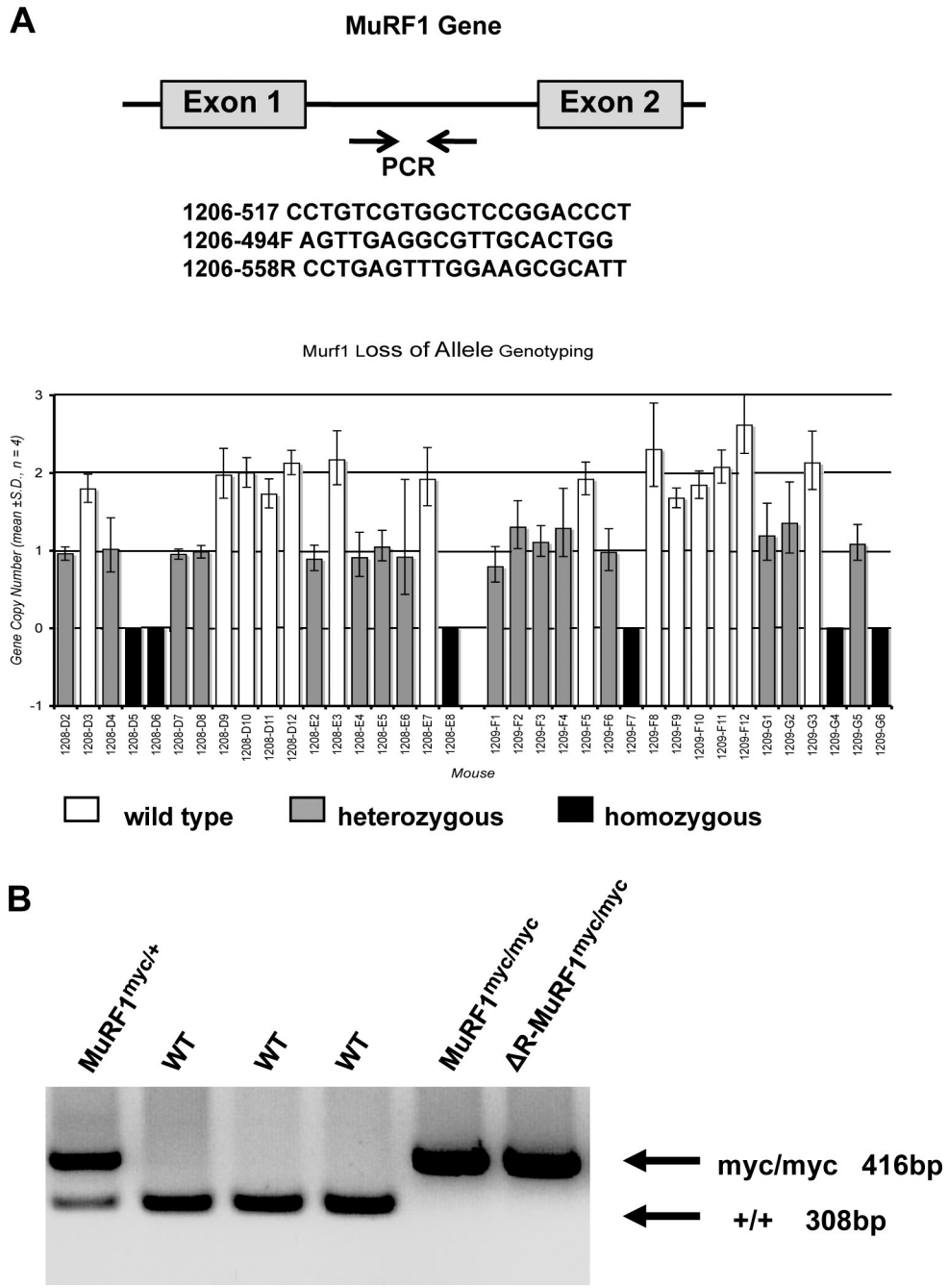

Figure S2. ***MuRF1* loss of allele genotyping.** (A) Representative genotyping data using the LONA assay. Screen of tail DNAs with quantification of MuRF1 alleles versus a reference gene. The first intron of the MuRF1 gene is reduced from two copies in wild-type mice to one copy in heterozygous mice, and to none in homozygous mice. (B) PCR analysis of tail genomic DNA showing the products from the wild-type and targeted alleles.

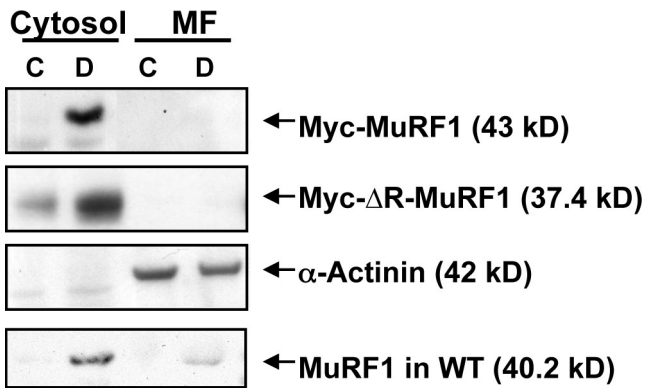

Figure S3. **MuRF1 is primarily a cytosolic protein.** After muscle homogenization, endogenous MuRF1, Myc-MuRF1, and Myc-ΔR-MuRF1 proteins are found mainly in the cytosolic fraction and not in myofibrils (MF); Western blot analysis of isolated myofibrils (0.5 µg) and cytosolic fraction (20 µg) from gastrocnemius muscles of wild-type, MuRF1$^{myc/myc}$, and ΔR-MuRF1$^{myc/myc}$ animals using anti myc-tag. Anti–α-actinin was used to verify isolated myofibrils. C, control; D, denervation for 10 d.

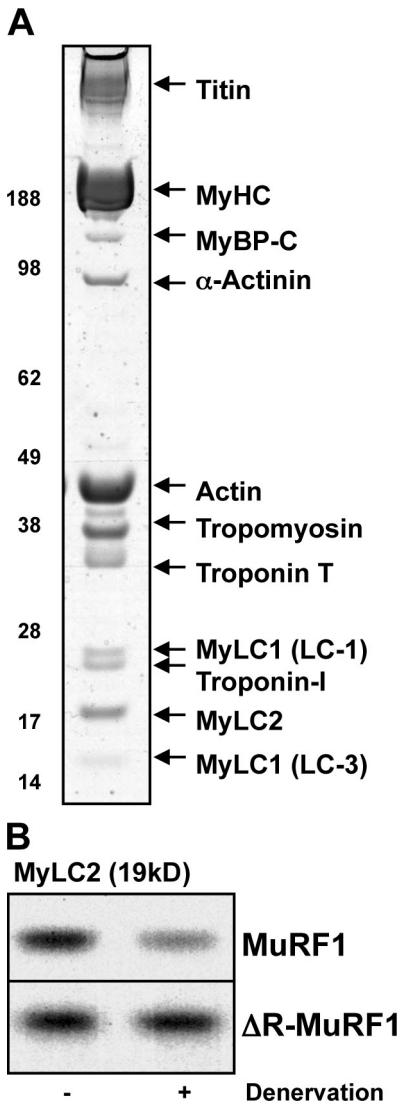

Figure S4. **Identification and measurement of protein bands in isolated myofibrils.** (A) Identification of protein bands in isolated myofibrils by mass spectrometry. Isolated myofibrils (2.5 µg) from gastrocnemius muscles of wild-type mice were separated on SDS-PAGE and stained with Coomassie blue. Specific bands were analyzed by mass spectrometry. (B) Densitometric measurement of specific bands. Equal amounts of the myofibrillar fraction (2.5 µg) of innervated and denervated (10 d) muscles from MuRF1$^{myc/myc}$ and ΔR-MuRF1$^{myc/myc}$ mice were analyzed by SDS-PAGE and Coomassie blue staining. MyLC2 bands are presented.

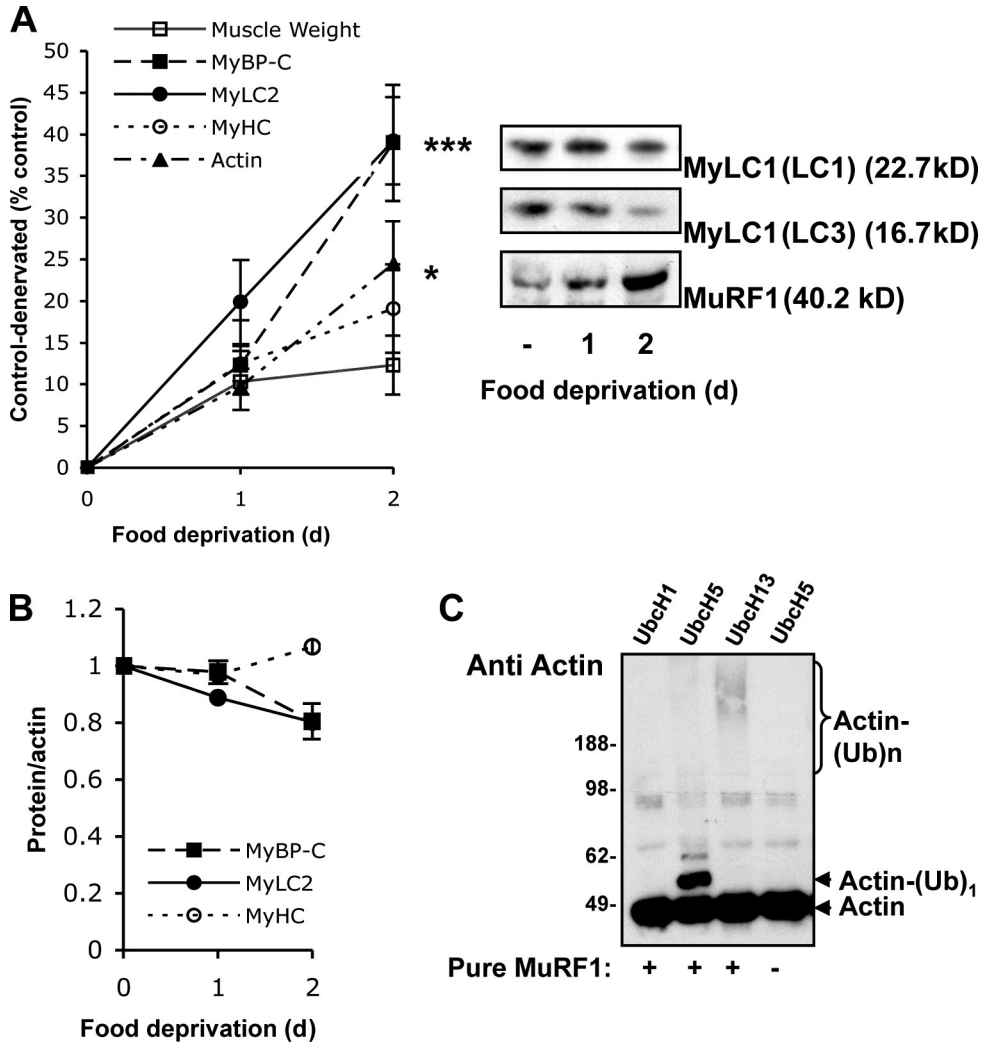

Figure S5. **MyBP-C and myosin light chains are lost selectively in muscle wasting induced by fasting.** Isolated myofibrils (2.5 μg) from gastrocnemius muscles of six mice fasted for 1 or 2 d were analyzed by SDS-PAGE and Coomassie blue staining (left). Shown on the right is Western blot analysis of myofibrillar fraction (0.5 μg) and cytosolic fractions (20 μg) from innervated and denervated muscles from wild-type mice. The intensities of Coomassie blue–stained bands were measured. The mean intensities of the bands are shown as percent loss of each protein per muscle (A) or protein content relative to actin (B). Error bars represent SEM (n = 6, $P < 0.001$). (C) Actin is a substrate of MuRF1. In vitro ubiquitylation of actin by MuRF1 and three different E2s (UbcH13/Uev1).