Cohen et al., http://www.jcb.org/cgi/content/full/jcb.201111067/DC1

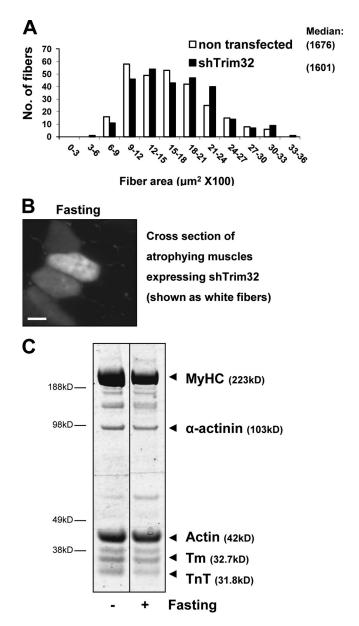


Figure S1. **Effects of shTrim32 on fiber size during fasting.** (A) Down-regulation of Trim32 does not affect fiber size 4 d after electroporation. Cross-sectional areas of 300 fibers transfected with shTrim32 (black bars) versus nontransfected fibers (open bars). Data acquired from five mice. (β) Representative cross sections of muscles from fasted mice expressing shTrim32 (shown as white fibers). Bar, 25 μM. (C) Representative Coomassie blue–stained gels used for densitometric measurements of specific myofibrillar components. To determine the total amounts of each myofibrillar protein in the TA muscles, values were multiplied by the total amount of myofibrils and by muscle weight.

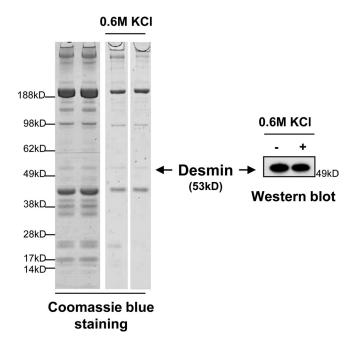


Figure S2. Isolation of desmin filaments from muscle pellet with 0.6 M KCl. Insoluble (myofibrillar) fractions of muscle homogenates were incubated with 0.6 M KCl to extract myofibrillar proteins or left untreated, and were analyzed by SDS-PAGE and Coomassie blue staining or by immunoblotting with an anti-desmin antibody. Under these conditions, myofibrils almost fully disassemble, whereas desmin filaments are stable.

Table S1. Ability of shTrim32 to reduce loss of specific myofibrillar proteins

Myofibrillar component	X = total amount in atrophying muscles expressing shLacz (% fed control)	Y = total amount in atrophying muscles expressing shTrim32 (% fed control)	((Y-X) / (100-X)) * 100 % sparing	Y/X
Actin	56 ± 4.7	88 ± 9.2	73	1.57
Actinin	58 ± 4.7	91 ± 11.4	79	1.56
Tm	$55 \pm 5.3$	88 ±10.3	73	1.60
Tnl	67 ± 5.9	105 ± 11.4	115	1.56
TnT	47 ± 3	89 ± 9.8	79	1.87
MyHC	$50 \pm 4.4$	65 ± 3.1	30	1.29

The total amount of individual myofibrillar proteins was calculated per atrophying muscle expressing either shTrim32 or shLacz, and is shown as percentage of control.

Table S2. qPCR primers and shRNA oligos used in this study

DNA	Gene	Sequence (5' to 3')
shRNA 1	Trim32	GGCTGATTGGTGTCACTGATA
shRNA 2	Trim32	AGCTGCTGGTCTTGGACTGTT
qPCR primer forward	Trim32	GGCAAATCAGCCACTTCTTC
qPCR primer reverse	Trim32	ATGCCCACTGGACAGGTAAG
qPCR primer forward	MuRF1	TGGAAACGCTATGGAGAACC
qPCR primer reverse	MuRF1	TCTTCCTCATCAGCCTCCTC
qPCR primer forward	GAPDH	ATGGTGAAGGTCGGTGTGA
qPCR primer reverse	GAPDH	ATGTAGGCCATGAGGTCCAC