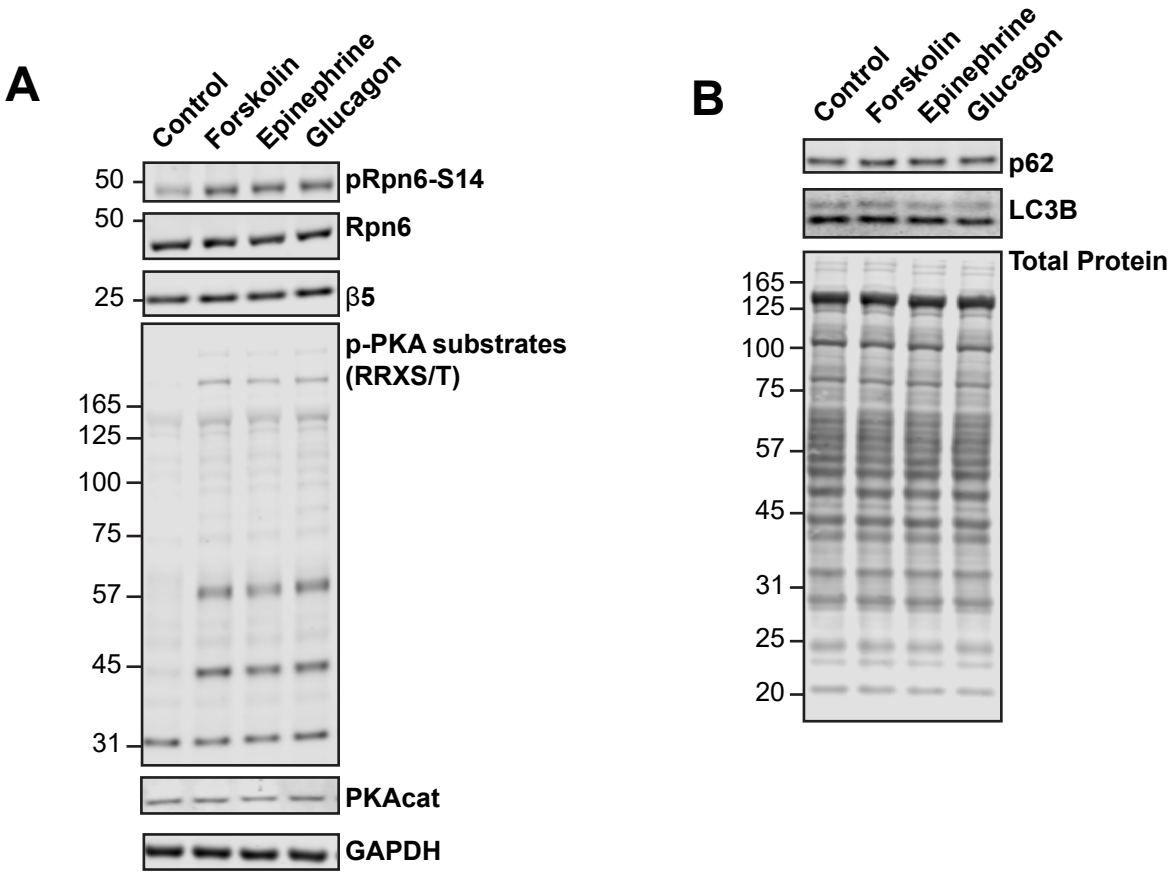
Supplemental Figure 1



Supplemental Figure 1: Raising cAMP in mouse primary hepatocytes with forskolin, epinephrine, or glucagon increases phosphorylation of Rpn6-S14 but does not change levels of proteasome subunits or markers of autophagy.

(A) Forskolin, epinephrine, or glucagon treatment of mouse primary hepatocytes for one hour increased levels of pRpn6-S14.

(B) p62 or LC3B, two markers of autophagy, were not changed by treatment with forskolin, epinephrine, or glucagon.

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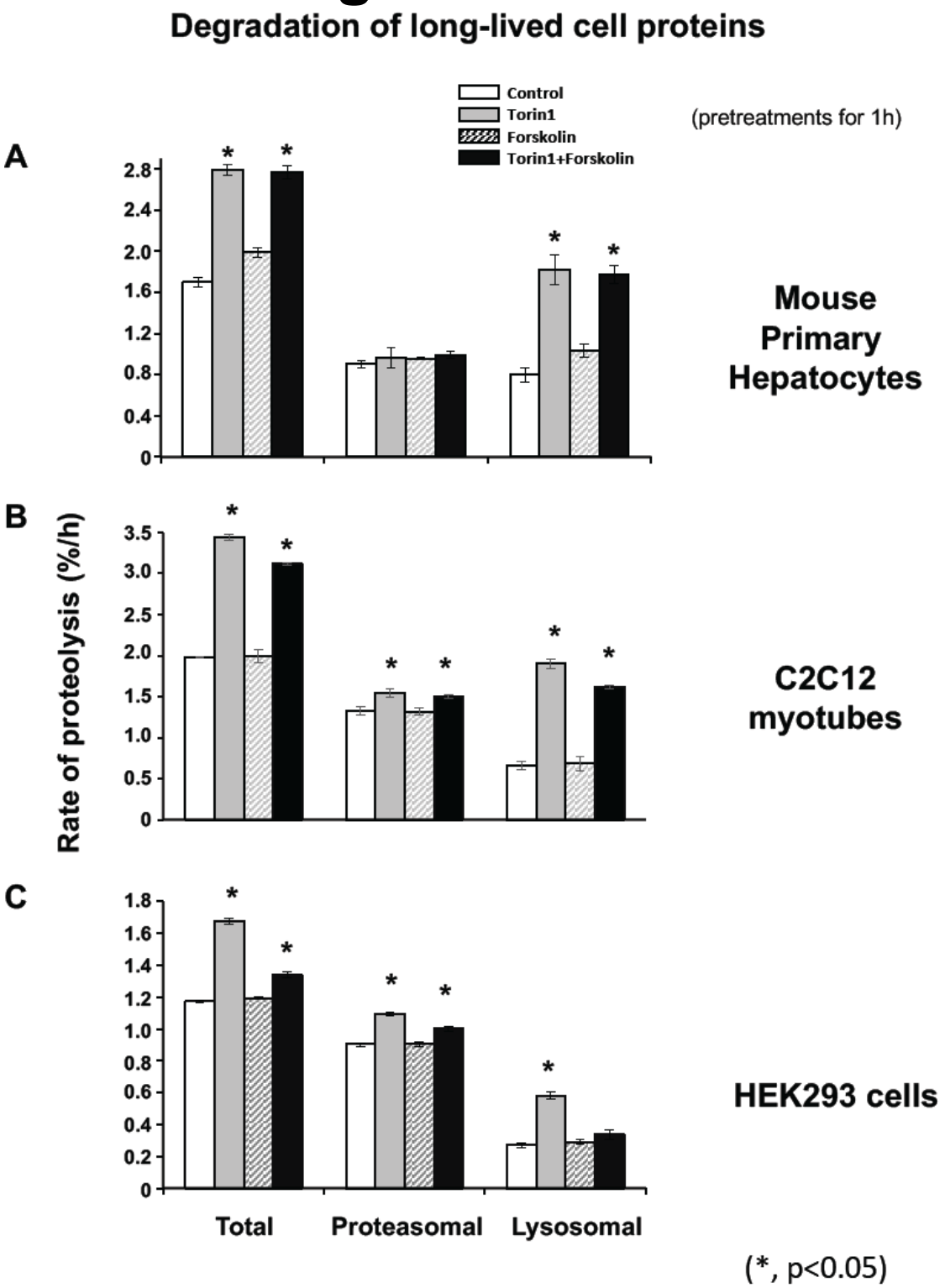
Supplemental Figure 2



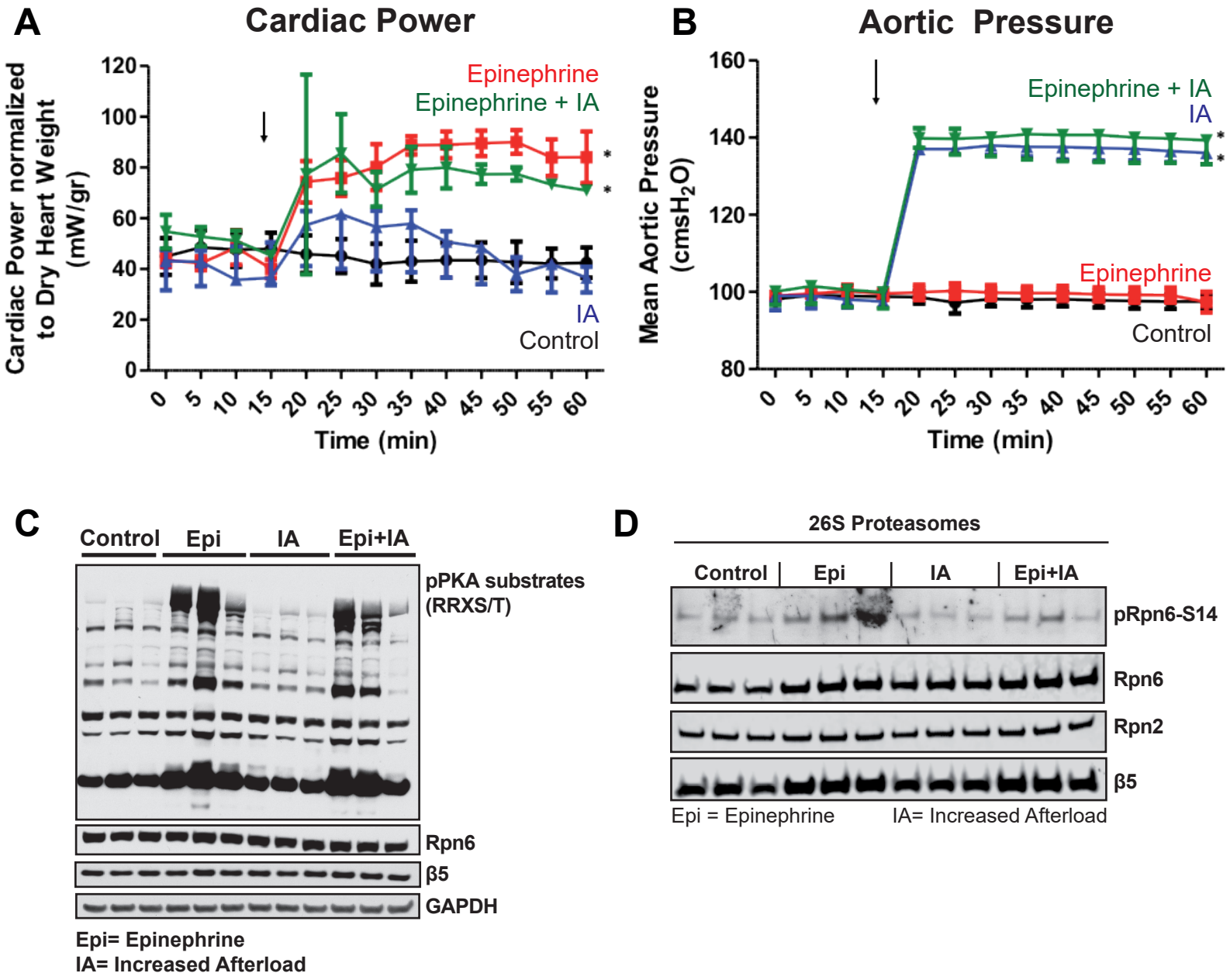
Supplemental Figure 2: Forskolin does not stimulate the lysosomal or proteasomal degradation of long-lived proteins in hepatocytes, myotubes, or HEK29s cells, but can suppress the Torin1-mediated stimulation of lysosomal degradation in myotubes and HEK293 cells.

(A) Mouse primary hepatocytes were incubated with [3H]phenylalanine (1 μCi/ml) for 20 hours and then switched to chase medium containing 2 mM non-radioactive phenylalanine for 2 hours to allow the degradation of short-lived proteins. During this chase period, cells were treated with concanamycin A (200nM) for 2 hours to inhibit lysosomal acidification and with Torin1(250nM), Forskolin (50uM), or both for 1 hour. Three replicates were used for each condition.

(B) Same as in (A), but in mouse C2C12 myotubes.

(C) Same as in (A), but in HEK293 cells.

Supplemental Figure 3



Supplemental Figure 3: Changes in cardiac power and aortic pressure on perfused rat hearts induced by epinephrine, increased afterload (IA), or both.

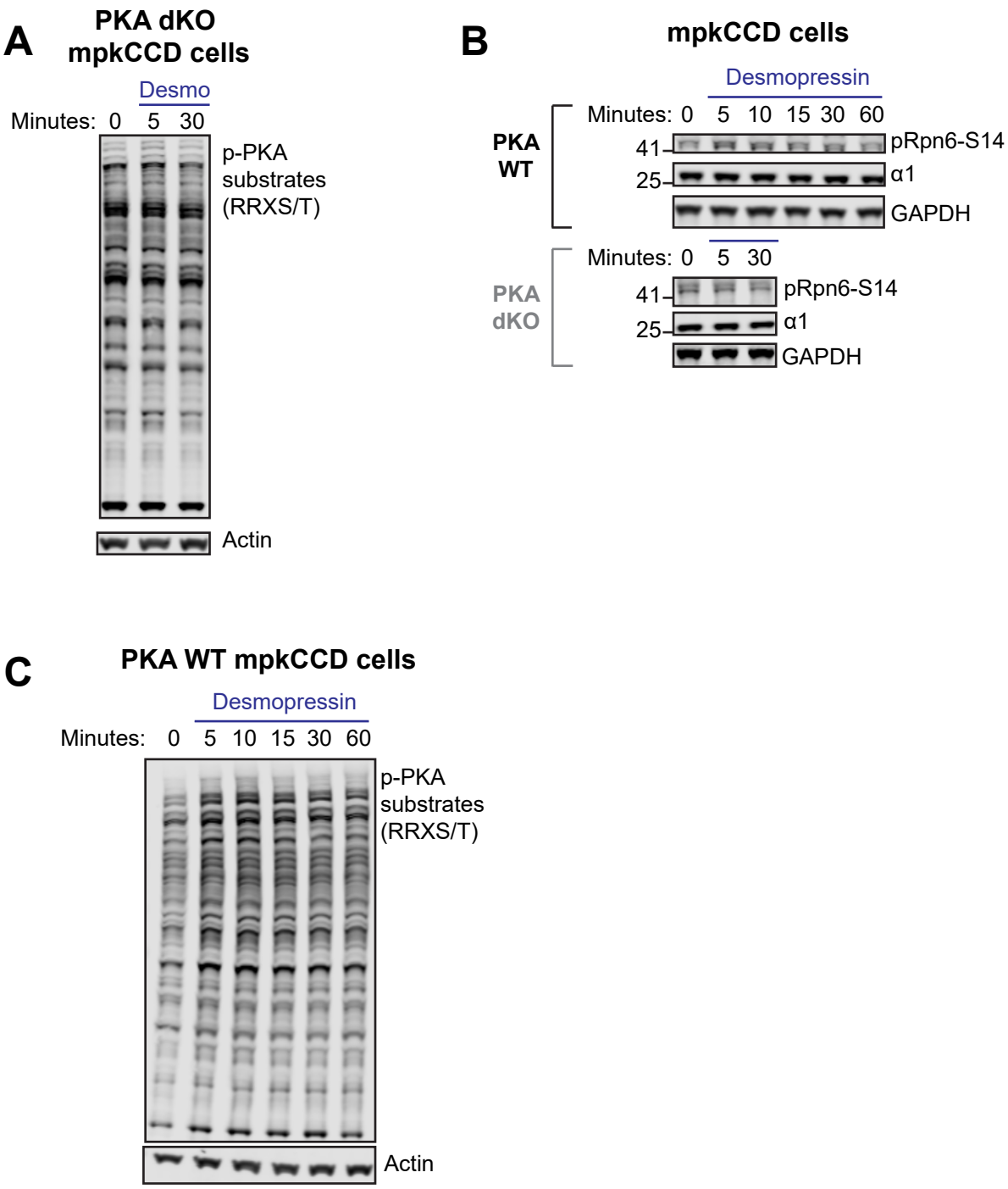
(A) Epinephrine increased cardiac power, normalized to dry heart weight (mW/g). Epinephrine was added to the perfusion at time indicated with arrow.*p<.001.

(B) Mean aortic pressure was increased when the afterload was raised to 140 cm H2O (increased afterload, IA). *p<.001.

(C) Epinephrine treatment stimulated the phosphorylation of PKA substrates. The levels of proteasome subunits in the hearts were not changed by epinephrine, increased afterload, or both.

(D) Epinephrine treatment, but not increased afterload, of working rat hearts stimulated the phosphorylation of Rpn6-S14. 26S proteasomes were affinity-purified by the Ubl-method from control or epinephrine-treated hearts and analyzed by immunoblot for pRpn6-S14, Rpn1, and α1.

Supplemental Figure 4



Supplemental Figure 4: Desmopressin stimulates the phosphorylation of Rpn6-S14 in mouse kidney epithelial cells, but not in cells lacking PKA.

(A) In PKA dKO mpkCCD cells, desmopressin did not stimulate the phosphorylation of PKA substrates.

(B) Desmopressin stimulated the rapid but transient phosphorylation of Rpn6-S14 in PKA WT mpkCCD cells, but not in PKA dKO mpkCCD cells. The levels of proteasome subunits, as determined by western blot for the 20S subunit α1, were not changed by the desmopressin treatments.

(C) PKA substrate proteins remained phosphorylated upon desmopressin treatment of PKA WT mpkCCD cells for 5-60 minutes, unlike Rpn6, whose phosphorylation was maximal at 5 minutes and then decreased (Fig 3D).

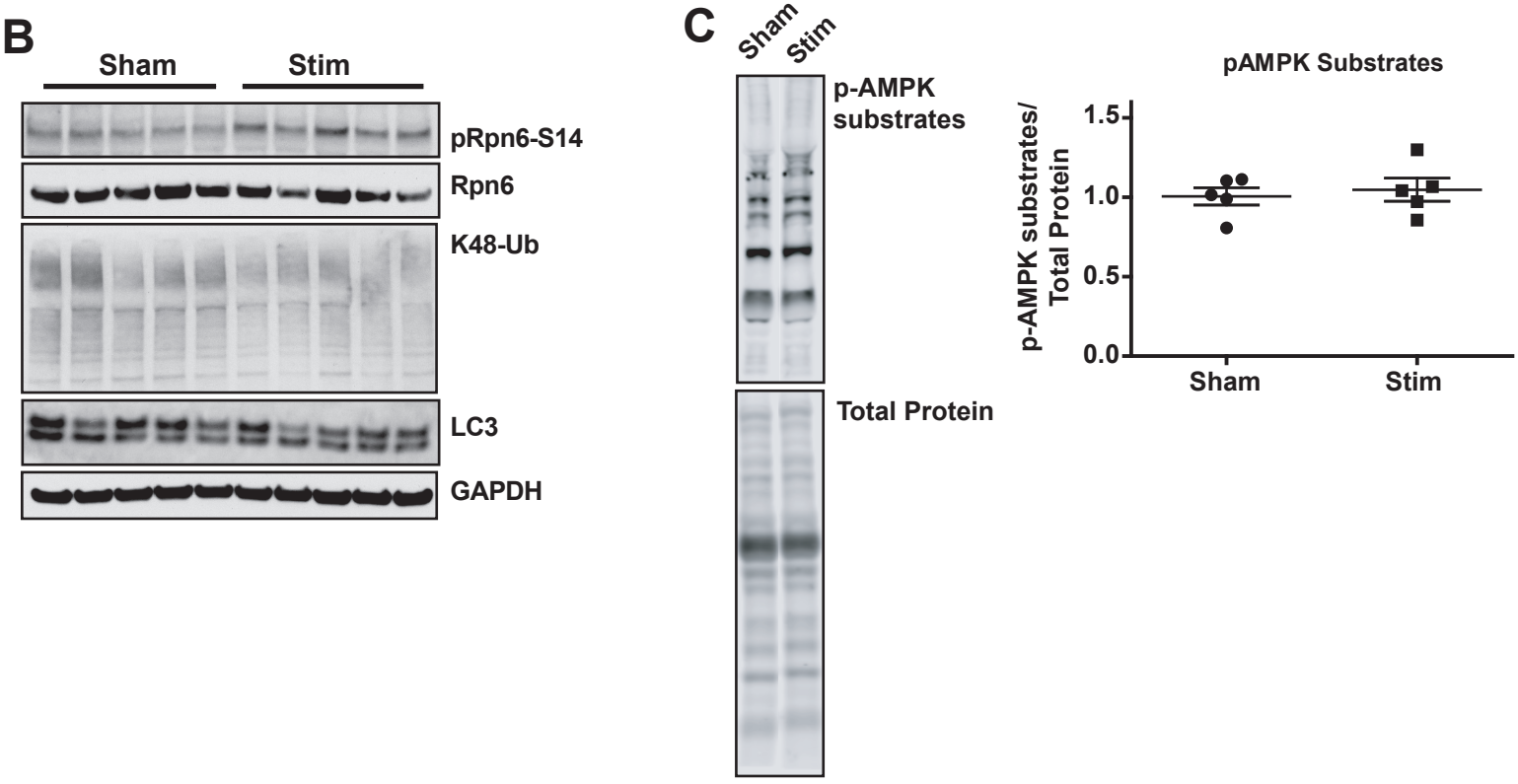
Supplemental Figure 5

A

TMT MS/MS analysis
Phospho-Rpn6 (Exercise/Basal)

Subject #1	Subject #2	Subject #3	Subject #4	Log Fold Change	P-Value
2.548	2.234	3.849	2.339	1.445	0.006

Hoffman et al., 2015



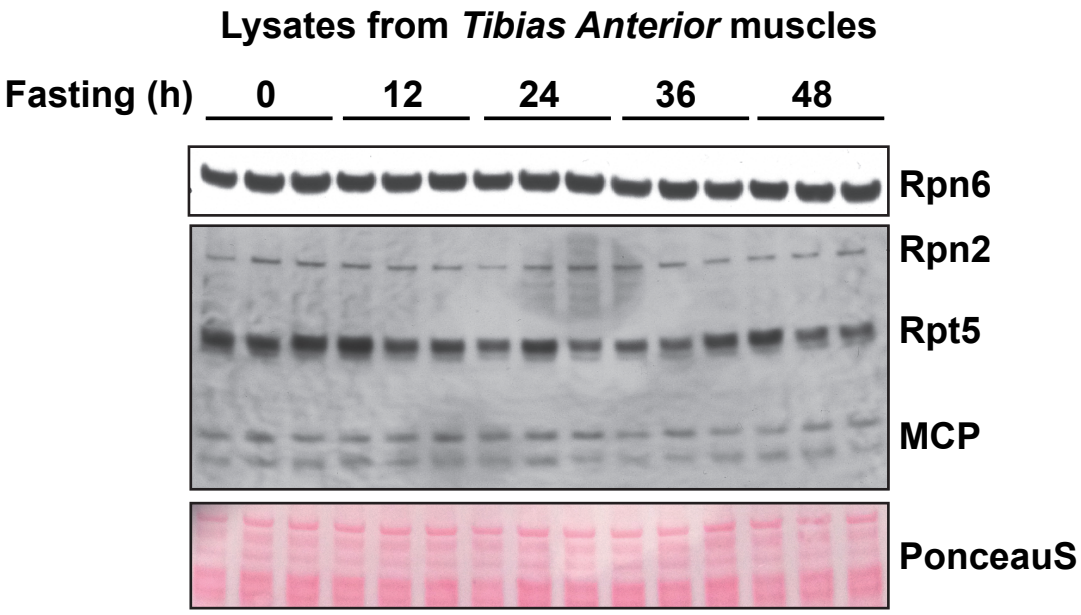
Supplemental Figure 5: Intense exercise in humans and high-intensity repetitive contractions of anterior tibialis muscles in rats enhances phosphorylation of Rpn6-S14.

(A) Phosphorylation of Rpn6-S14 was identified by TMT phosphoproteomic analysis of four human subjects after a single high intensity cycling session. These data are the ratios of the phosphorylation of Rpn6-S14 after intense exercise to basal phosphorylation of Rpn6-S14 for each volunteer and are from the supplemental material (21).

(B) High-intensity repetitive contractions of anterior tibialis muscles in rats increased the levels of pRpn6-S14 over sham controls without changing levels of proteasome subunits or the autophagy marker LC3.

(C) AMPK was not activated by high-intensity repetitive contractions of anterior tibialis muscles. Muscle extracts were analyzed by western blot for phosphorylated AMPK substrates, and the signal was normalized to total protein, as detected by the REVERT stain.

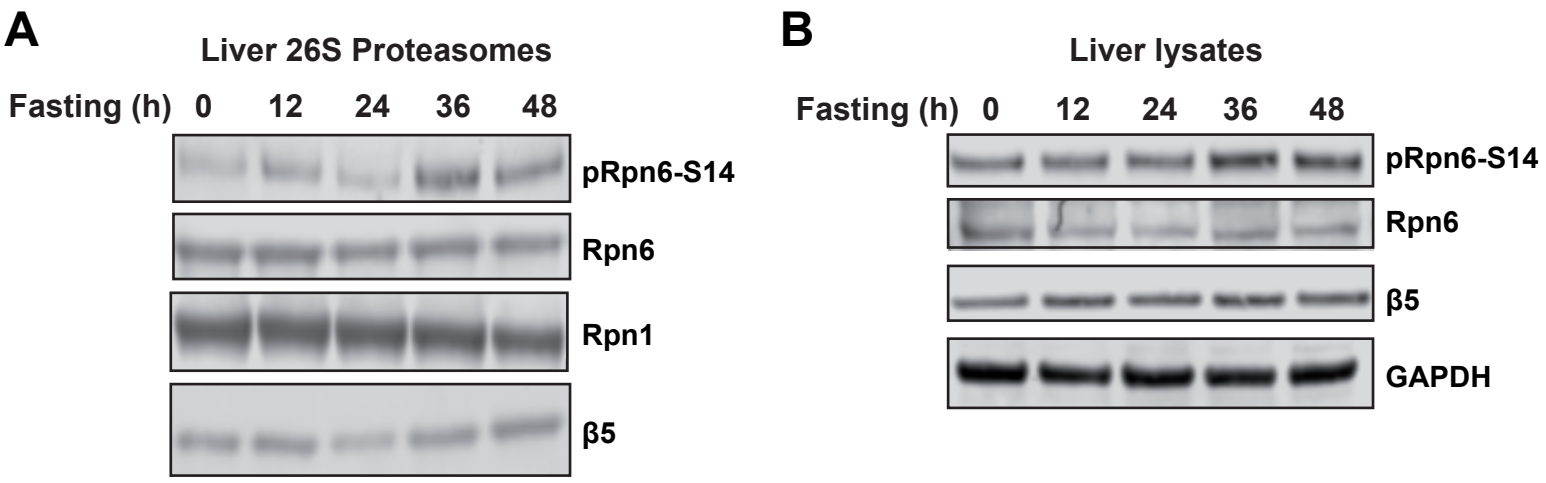
Supplemental Figure 6



Supplemental Figure 6: Food deprivation of mice did not increase the levels of proteasome subunits in tibialis anterior muscles.

(A) The tibialis anterior muscles of mice fasted for indicated time points were homogenized and analyzed by SDS PAGE and western blot for the proteasome 19S subunits Rpn6, Rpn2, and Rpt5, and the 20S α subunits with the MCP antibody.

Supplemental Figure 7



Supplemental Figure 7: Food deprivation of mice promotes the phosphorylation of Rpn6-S14 in livers.

(A) 26S proteasomes purified from the livers of fasted mice exhibited greater levels of pRpn6-S14 than from fed control mice.

(B) The levels of pRpn6-S14 were greater in the liver extracts of fasted mice than in fed controls. Fasting did not change the levels of proteasome subunits in the liver extracts, as determined by western blot for Rpn6 and β5.