

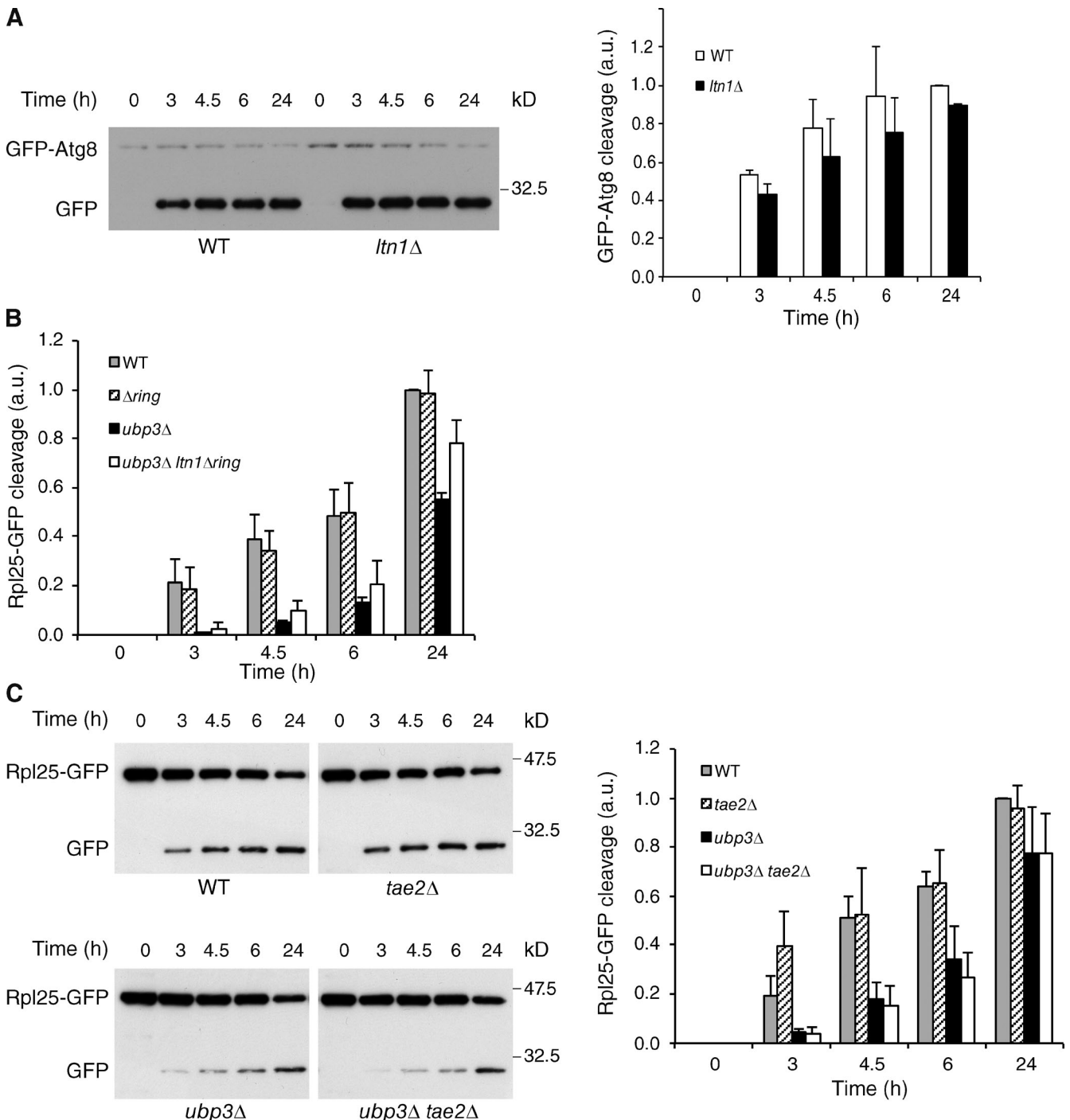
Ossareh-Nazari et al., <http://www.jcb.org/cgi/content/full/jcb.201308139/DC1>

Figure S1. **Ltn1 does not affect general autophagy, and its RING domain, but not its cofactor Tae2, is required for its function in ribophagy.** (A) General autophagy appears normal in *Ltn1*-deficient cells. Cells expressing GFP-Atg8 were starved for the indicated period of time. (left) Degradation of the GFP-tagged Atg8 autophagy marker was analyzed by Western blotting of whole cell extracts using an anti-GFP antibody. (B) Deletion of the RING domain of *LTN1* rescues the ribophagy defect of *UBP3*-null cells. Indicated wild-type (WT) and mutant cells expressing Rpl25-GFP were starved for the indicated periods. (C) The *Ltn1* cofactor *Tae2* is not involved in ribophagy. Indicated wild-type and mutant cells expressing Rpl25-GFP were starved for the indicated periods. (left) Degradation of GFP-tagged proteins was then analyzed by Western blotting in whole cell extracts using an anti-GFP antibody. (right) The ratio between cleaved GFP and full-length protein was quantified for every time point in three independent experiments. The errors bars correspond to standard deviations. a.u., arbitrary units.

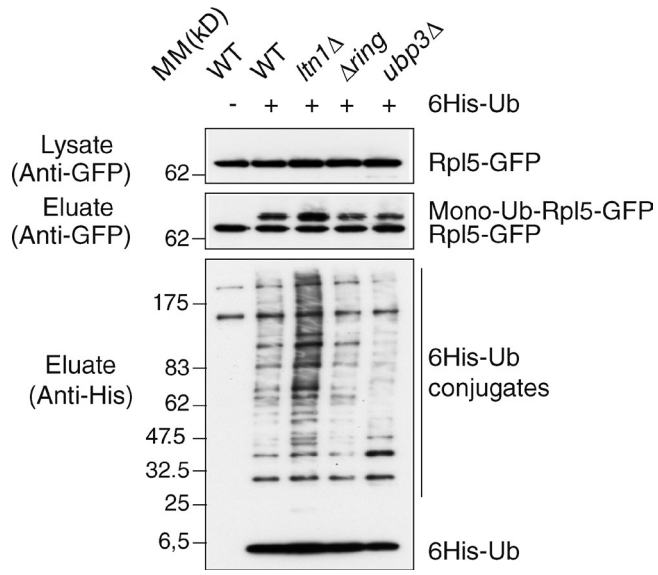


Figure S2. **Ubiquitylation of Rpl5 is not regulated by either Ltn1 or Ubp3.** Analysis of ubiquitin-conjugated forms of Rpl5-GFP in wild-type and the indicated mutant cells as in Fig. 2 A. MM, molecular mass; Ub, ubiquitin.

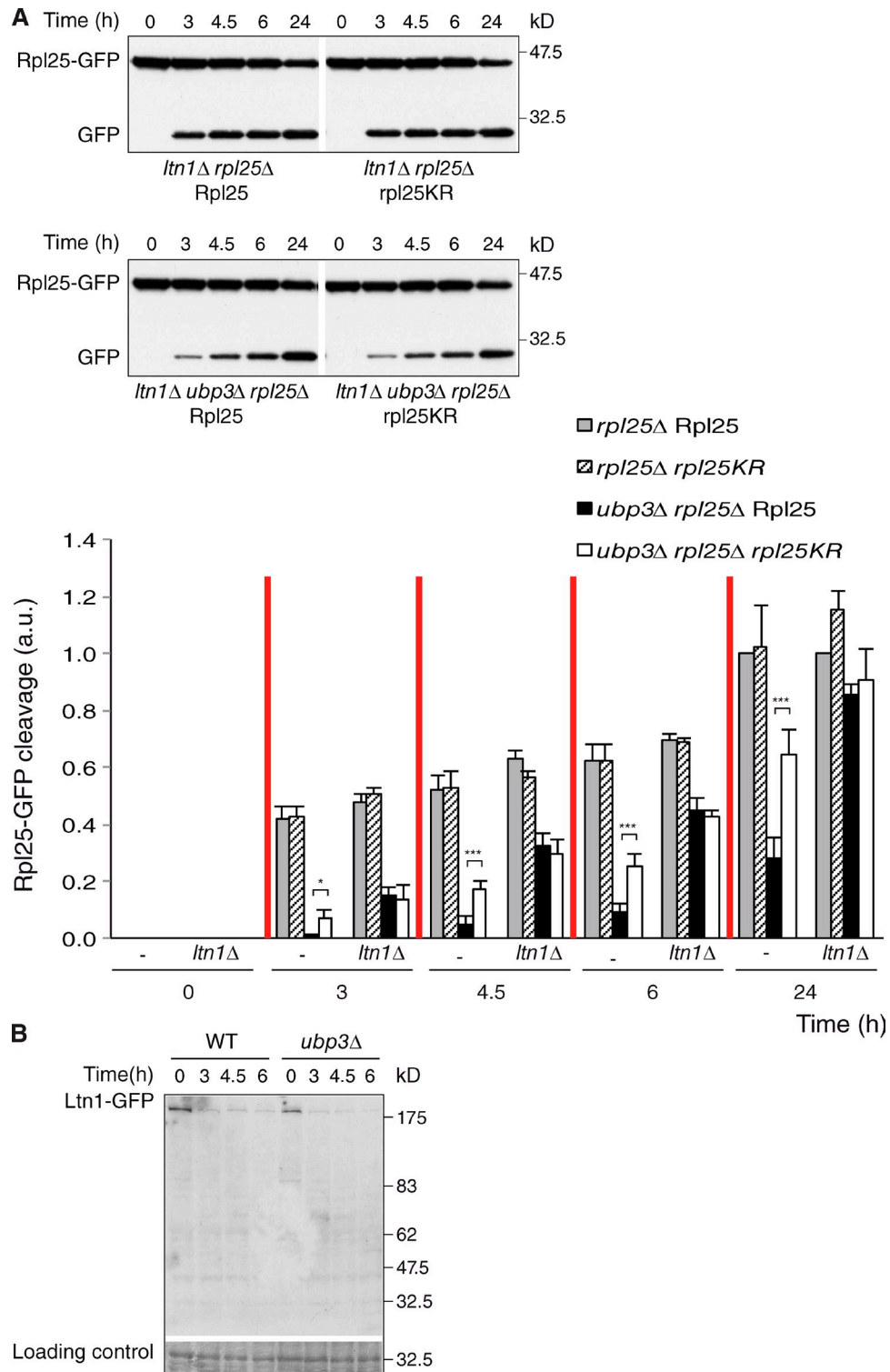


Figure S3. **Combining *LTN1* deletion with K74/75R mutation in Rpl25 has no synergistic or additive effect.** (A) *ltn1Δ* and *ltn1Δubp3Δ* cells expressing Rpl25-GFP (Rpl25) or *rpl25* K74,75R-GFP (*rpl25KR*) were starved for the indicated period of time. (top) Degradation of GFP-tagged proteins was analyzed by Western blotting of whole cell extracts using an anti-GFP antibody. (bottom) The ratio between cleaved GFP and full-length protein was quantified for all time points in three independent experiments and represented together with results from Fig. 3 C. The errors bars correspond to standard deviations. The red bars are used to separate the histograms corresponding to each time point. a.u., arbitrary unit. (B) Wild-type or *ubp3Δ* cells expressing Ltn1-GFP were starved for the indicated period of time. Degradation of tagged proteins was analyzed by Western blotting of whole cell extracts with anti-GFP antibodies. A nonspecific band served as a loading control. WT, wild type. White lines indicate that intervening lanes have been spliced out.