



(a) The ratio of p62/ $\beta$ -actin was analyzed in Fig. 5a. P values were calculated using the two-tailed, unpaired t-test, n=3. \*\* P<0.01; ns, not significant.

(b) The ratio of p62/ $\beta$ -actin was analyzed in Fig. 5b. P values were calculated using the two-tailed, unpaired t-test, n=3. \* P<0.05; ns, not significant.

(c) Myo1D KO cells, or Myo1D KO cells transiently transfected with Myc-Myo1D, were starved for 4 h, and then stained with antibodies against LC3 and p62. Scale bar,  $10 \ \mu m$ .

(d) The number of p62 puncta was quantified in cells from (c) (3 independent experiments; 50 cells were assessed per independent experiment). P values were calculated using the two-tailed, unpaired t-test, n=3. \*\*\*\* P<0.0001; \* \* P<0.01.

(e) The expression level of the indicated proteins was confirmed by immunoblotting.

(f) Ubiquitin-modified proteins in WT cells and Myo1D KO cells were analyzed by western blotting with antibodies against ubiquitin and Myo1D before and after puromycin was eluted. P values were calculated using the two-tailed, unpaired t-test, n=3. \* P<0.05.