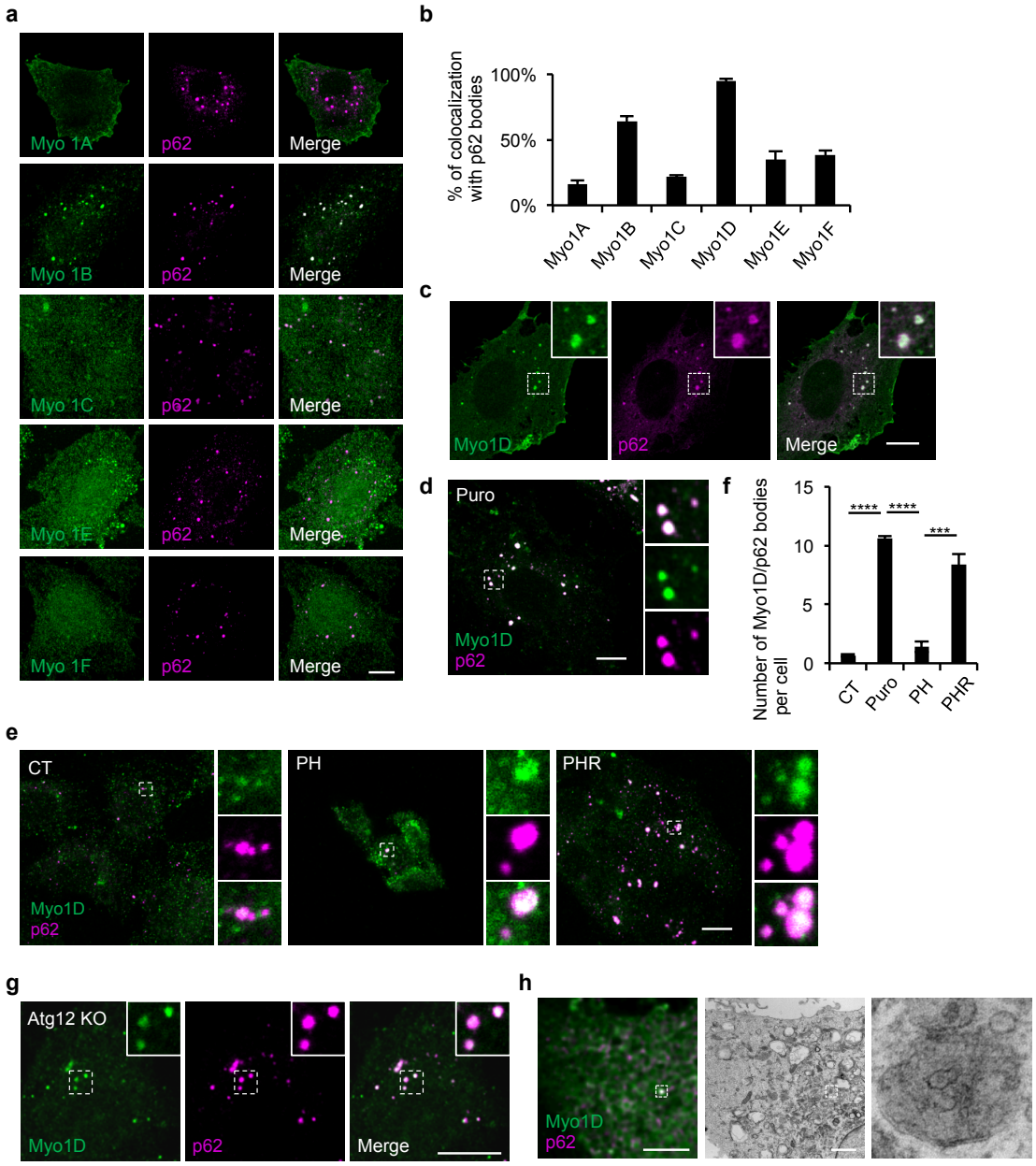

Supplementary information, Figure S3. Myo1D coalesces with p62 bodies during phase separation, related to Figure 3

(a) EGFP-tagged Myo1A was transiently expressed in NRK cells. The cells were treated with puromycin for 8 h, then stained with antibodies against GFP and p62. NRK cells were treated with puromycin for 8 h, then stained with antibodies against Myo1B, Myo1C, Myo1E, Myo1F and p62. Scale bar, 10 μm.

(b) The percentage of Myosin-positive p62 bodies was quantified in cells from (a) (3 independent experiments; 50 cells were assessed per independent experiment).

(c) GFP-Myo1D and tdTomato-p62 were transiently expressed in NRK cells and fluorescence signals were visualized. Scale bar, 10 μm. The boxed region is magnified in the inset.

(d) A549 cells were treated with 1 mg/ml puromycin for 8 h, and stained with antibodies against Myo1D and p62.

(e) Cells were treated without (CT) or with puromycin for 8 h, then puromycin treated cells were next treated with 2% 1,6-hexanediol for 20 min (PH). Cells were then washed and recovered with full medium (PHR) for 2 h. After each treatment, cells were stained with antibodies against Myo1D and p62. Scale bar, 10 μm.

(f) The number of p62 bodies was quantified in images of cells from (e) (3 independent experiments; 50 cells were assessed per independent experiment). P values were calculated using the two-tailed, unpaired t-test. *** $P<0.001$; **** $P<0.0001$.

(g) Atg12 KO cells were treated with 1 mg/ml puromycin for 8 h, and stained with antibodies against Myo1D and p62.

(h) NRK cells were transfected with GFP-Myo1D and tdTomato-p62, then fixed by 2.5% glutaraldehyde. Correlative light and electron microscopy (CLEM) images were acquired. Left: the confocal image, Scale bar, 10 μm; middle: the TEM image. Scale bar, 5 μm; right: the enlarged image of the region outlined in the middle panel.