

Supplementary information, Figure S4. Droplet formation by LLPS of p62 and Myo1D, related to Figure 3

(a) Droplets formed by LLPS of p62 (10  $\mu$ M) or p62- $\Delta$ PB1 (10  $\mu$ M) in the presence

of Ub8 (6  $\mu M)$  and Myo1D (1  $\mu M)$  in vitro. Scale bar, 5  $\mu m.$ 

(b) Droplets formed by LLPS of p62 (10  $\mu M)$  in the presence of Ub8 (6  $\mu M)$  and of

full-length Myo1D, Myo1D IQ-TH1, Myo1D IQ, Myo1D TH1, Myo1D Motor-IQ or

Myo1D Motor (1  $\mu$ M) in vitro. Scale bar, 5  $\mu$ m.

(c) Representative sedimentation analysis showing Myo1D fragments concentrated in the soluble phase (S) or condensed phase (P). Results were analyzed by western blotting using anti-His tag antibody.

(d) EGFP-fused full-length Myo1D, Myo1D K108R, Myo1D Motor-IQ, Myo1D IQ-TH1, Myo1D IQ, or Myo1D TH1 was transiently expressed with tdTomato-p62 in wild-type NRK cells. Boxed regions are enlarged. Scale bar, 10 μm.