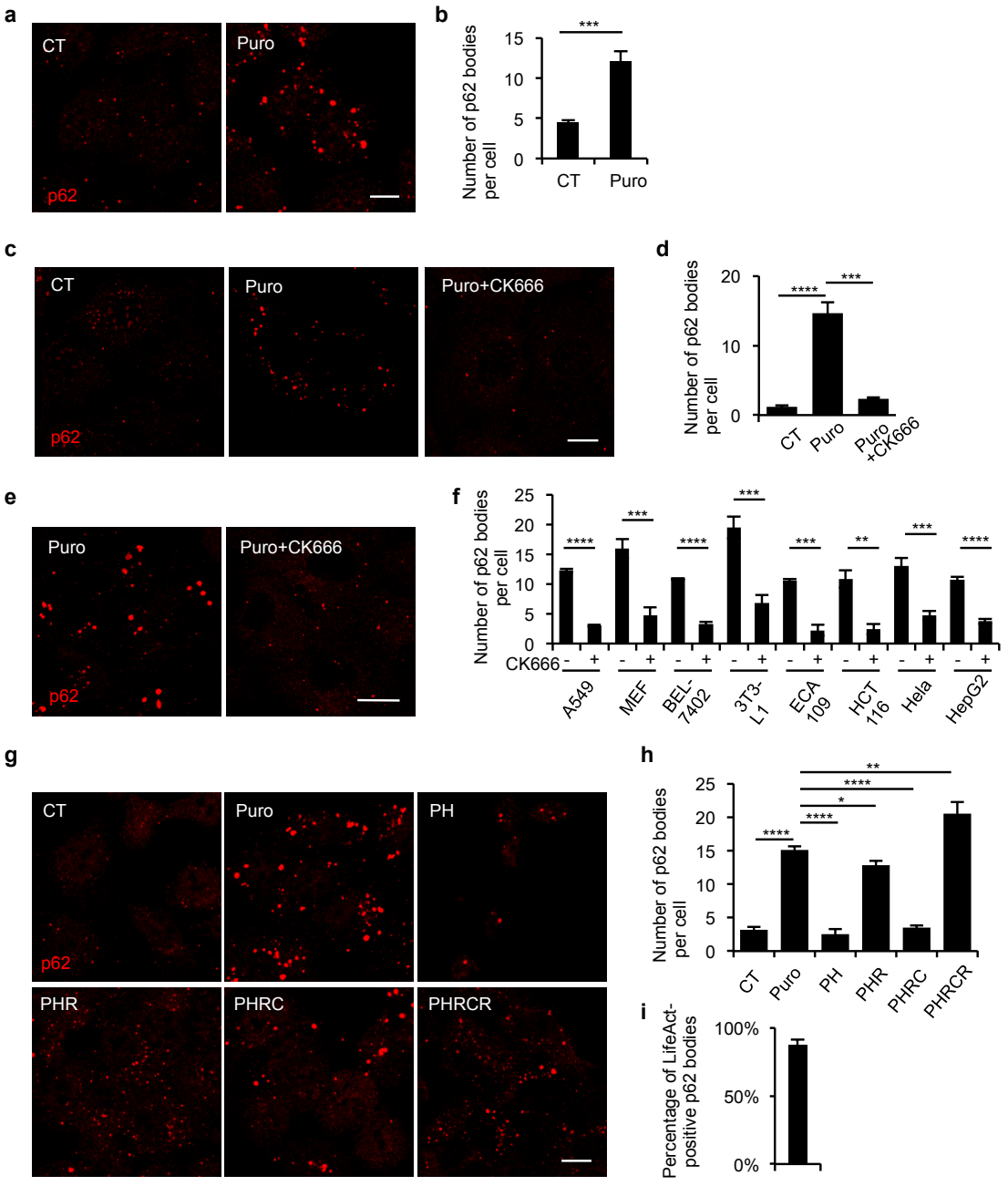

Supplementary information, Figure S1. The branched actin network is required for the phase separation of p62 bodies, related to Figure 1

(a) NRK cells were left untreated or treated with 1 mg/ml puromycin for 8 h, and stained with antibody against p62. Scale bar, 10 μm.

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

(c) NRK cells were left untreated or treated with puromycin with or without CK666 (100 μM), and stained with an antibody against p62.

(d) The number of p62 bodies 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. *** $P<0.001$; **** $P<0.0001$.

(e) A549 cells were treated with puromycin with or without CK666 (100 μM), and stained with an antibody against p62.

(f) The number of p62 bodies was quantified in cells from A549, MEF, BEL-7402, 3T3-L1, ECA 109, HCT116, Hela, and HepG2 cells treated with puromycin with or without CK666 (100 μM) (3 independent experiments; 50 cells were assessed per independent experiment). P values were calculated using the two-tailed, unpaired t-test. ** $P<0.01$; *** $P<0.001$; **** $P<0.0001$.

(g) Reversible effect of CK666 and 1,6-hexanediol on the formation of p62 bodies. 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 minutes (PH). Cells were then washed with PBS three times for one minute each, and recovered with full medium (PHR) or full medium plus CK666 for 2 h (PHRC). Cells recovered with CK666 were then washed again and recovered with full medium for 2 h (PHRCR). After each treatment, cells were stained with an antibody against p62. Scale bar, 10 μm.

(h) The number of p62 bodies was quantified in images of cells from (g) (3 independent experiments; 50 cells were assessed per independent experiment). P values were calculated using the two-tailed, unpaired t-test. * $P<0.05$; ** $P<0.01$; **** $P<0.0001$.

(i) The percentage of LifeAct-positive p62 bodies was quantified in cells transiently expressing GFP-p62 and LifeAct-mRFPruby (3 independent experiments; 50 cells were assessed per independent experiment).