Supporting Information

Investigation and Intervention of Autophagy to Guide Cancer Treatment with Nanogels

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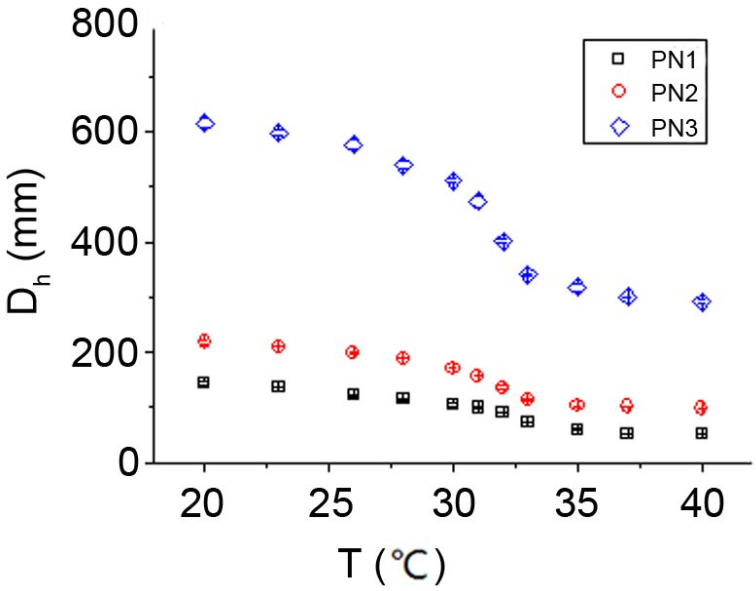

Figure S1. Hydrodynamic diameter (D_h) of the fluorescence-labeled nanogels as a function of temperature. The particles were dispersed in 0.01 M PBS with pH 7.4.

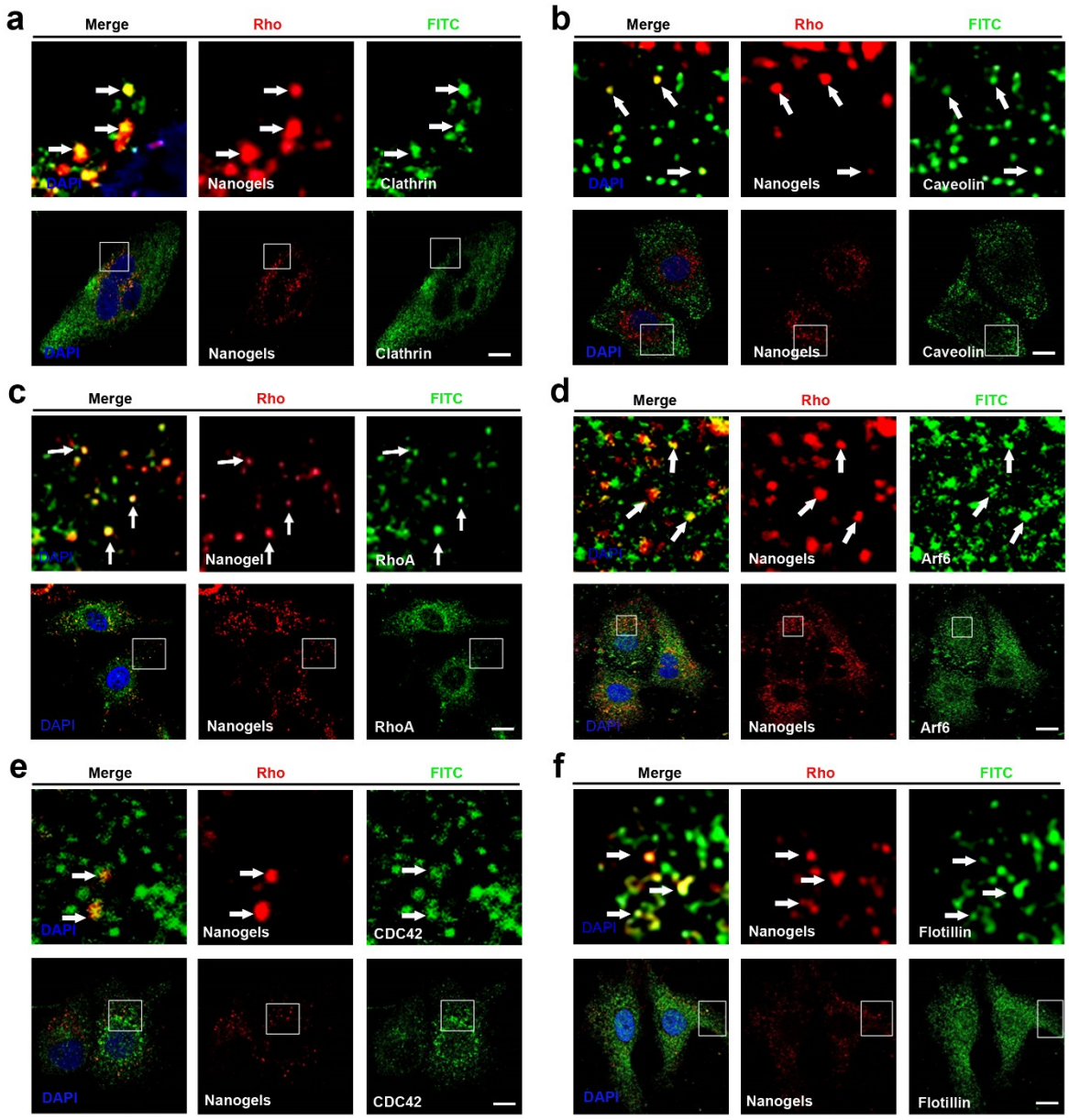

Figure S2. The nanogels incepted by the cells through multiple cell membrane protein dependent invagination of cell membrane. (a-f) Confocal images of MCF-7 cells, which were treated with 1mg/mL Rho-labeled nanogels for 20 hours. Clathrin, caveolin, RhoA, Cdc42 and flotillin were detected with primary antibodies against clathrin, caveolin, RhoA, Arf-6, Cdc42 and flotillin, respectively. Scale bars: 10 μm. The above images are the enlarged ones in the white collar on the underside images. The arrows indicated the localization of Rho-labeled nanogels and the clathrin, caveolin, RhoA, Arf-6, Cdc42 and flotillin positive vesicles.

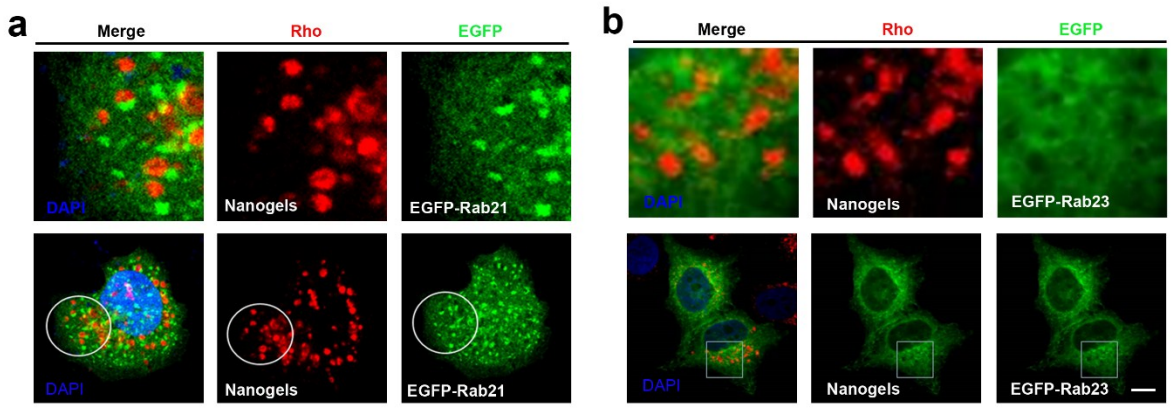

Figure S3. Rab21 and Rab23 positive vesicles did not co-localize with nanogels. (a-b) EGFP-Rab21, 23 transfected MCF-7 cells and then treated with 1mg/mL Rho-labeled nanogels for 20 h. Scale bars: 10 μm. The above images are the enlarged ones in the red collar on the underside images.

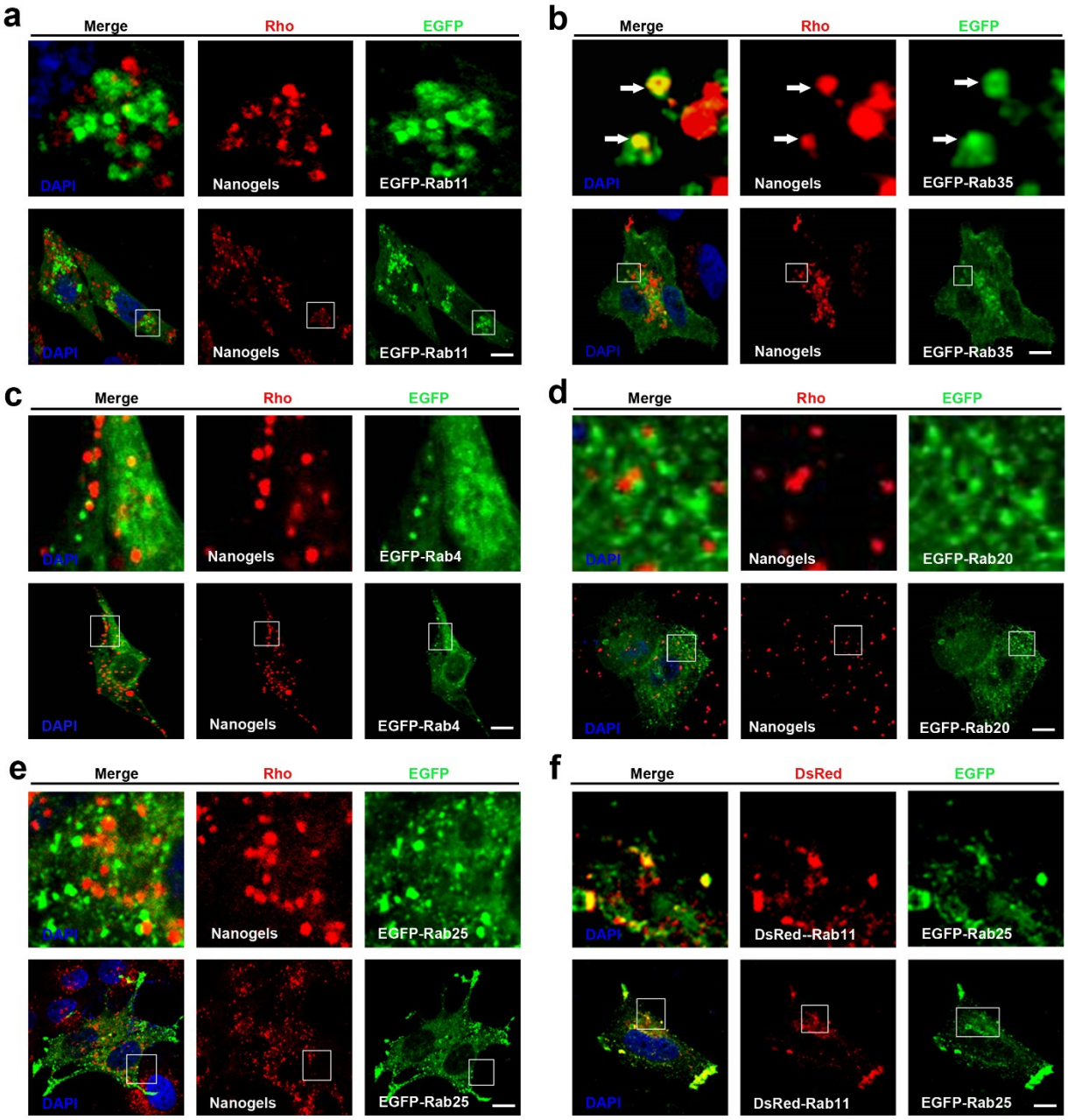

Figure S4. Nanogels transport out of the cells through Rab35 positive slow recycling endosomes but not fast and apical recycling endosomes. (a-e) EGFP-Rab11, 35, 4, 20, 25 transfected MCF-7 cells were treated with 1mg/mL Rho-labeled nanogels for 20 h. The arrows indicated the localization of Rho-labeled nanogels with EGFP-Rab35 positive vesicles. (f) EGFP-Rab25 co-transfected with DsRed-Rab11; Scale bars: 10 μm. The above images are the enlarged ones in the red collar on the underside images.

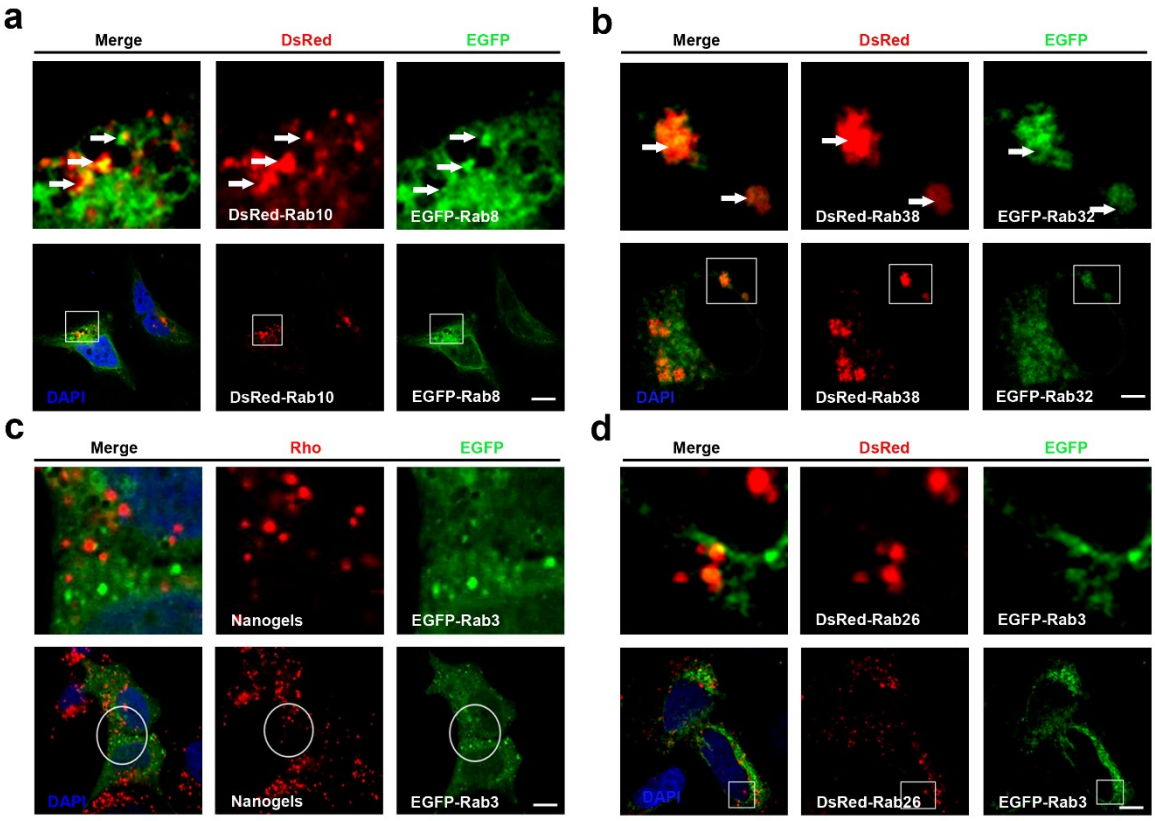

Figure S5. The localization of Rab8 and Rab10 on GLUT4 vesicles; Rab32 and Rab38 on melanosome homologue vesicles. (a) DsRed-Rab10 cells were co-transfected with EGFP-Rab8 in MCF-7 cells for 20 h (b) EGFP-Rab32 cells were co-transfected with DsRed-Rab38 in MCF-7 cells for 20 h. (c) EGFP-Rab3 transfected MCF-7 cells were treated with Rho-nanogels for 20 h. (d) DsRed Rab26 and EGFP-Rab3 were co-transfected in MCF-7 cells for 20 h.

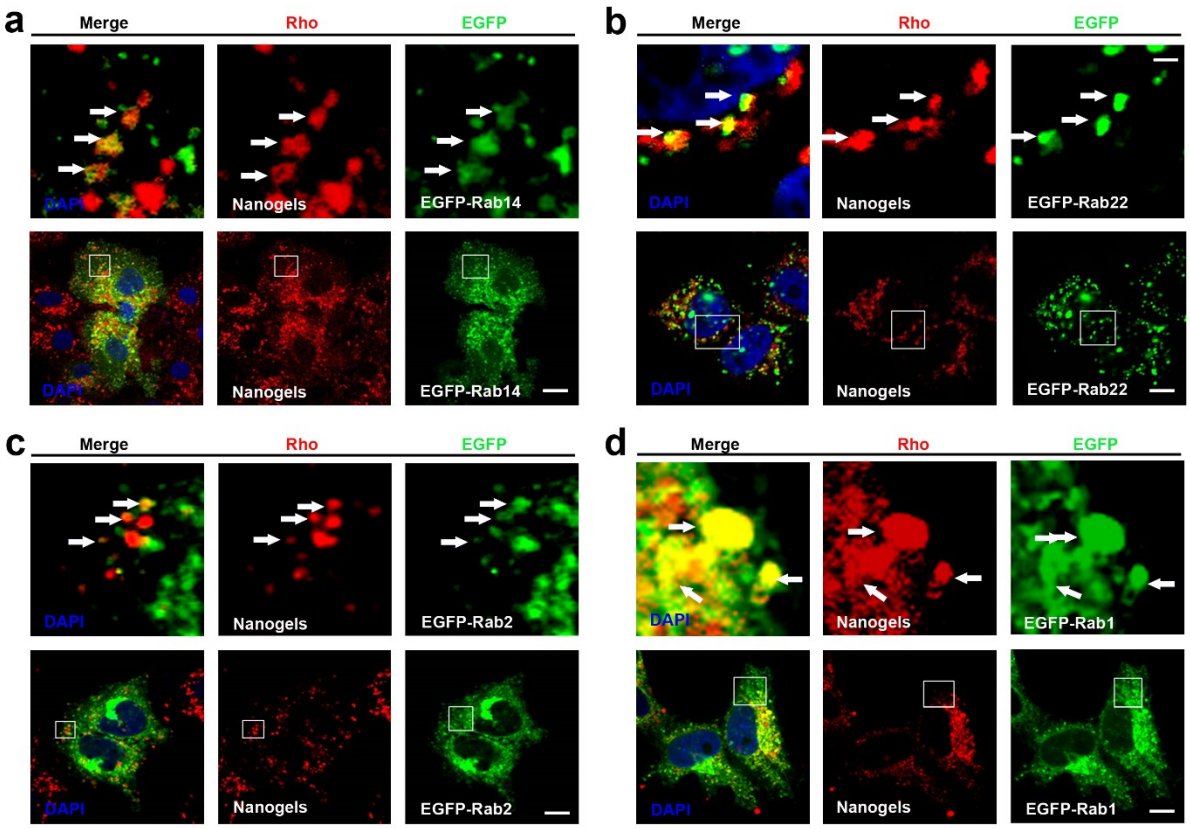

Figure S6. Rab14, 22, 2, 1 positive vesicle co-localized with nanogels. (a-d) EGFP-Rab14, 22, 2, 1 were transfected cells were treated with 1mg/mL Rho-labeled nanogels for 20 h; Scale bars: 10 μm. The above images are the enlarged ones in the red collar on the underside images. The arrows indicated the localization of Rho-labeled nanogels with EGFP-Rab14, 22, 2, 1 positive vesicle.

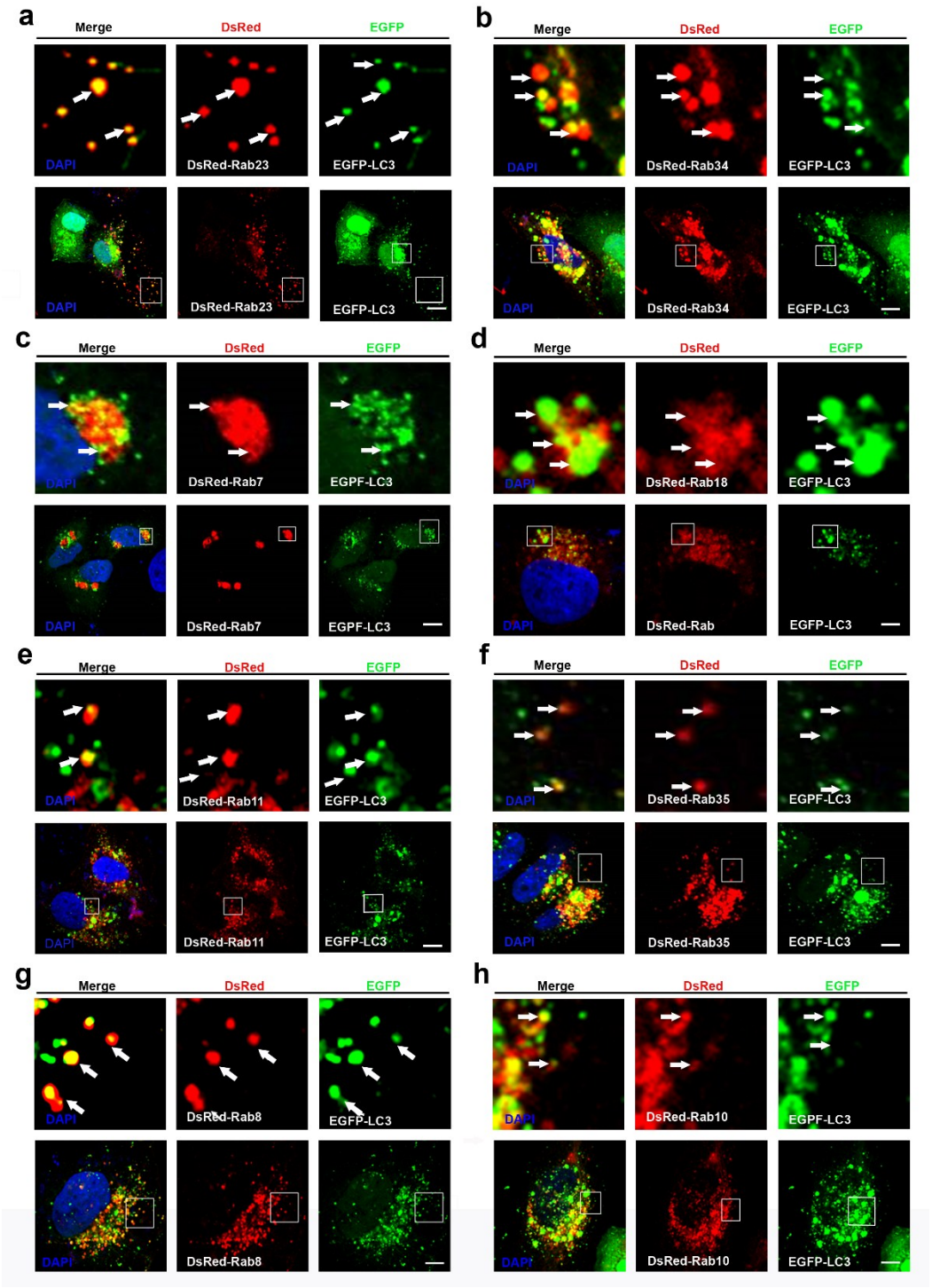

Figure S7. Crosstalk between endocytosis, exocytosis and autophagy. (a-h) EGFP-LC3 cells were co-transfected with DsRed-Rab23, 34, 7, 18, 11, 35, 8 and 10, respectively. Scale bars: 10 μm. The above images are the enlarged ones in the white collar on the underside images. The arrows indicated the localization of EGFP-LC3 positive autophagosomes with DsRed Rab protein positive vesicles.

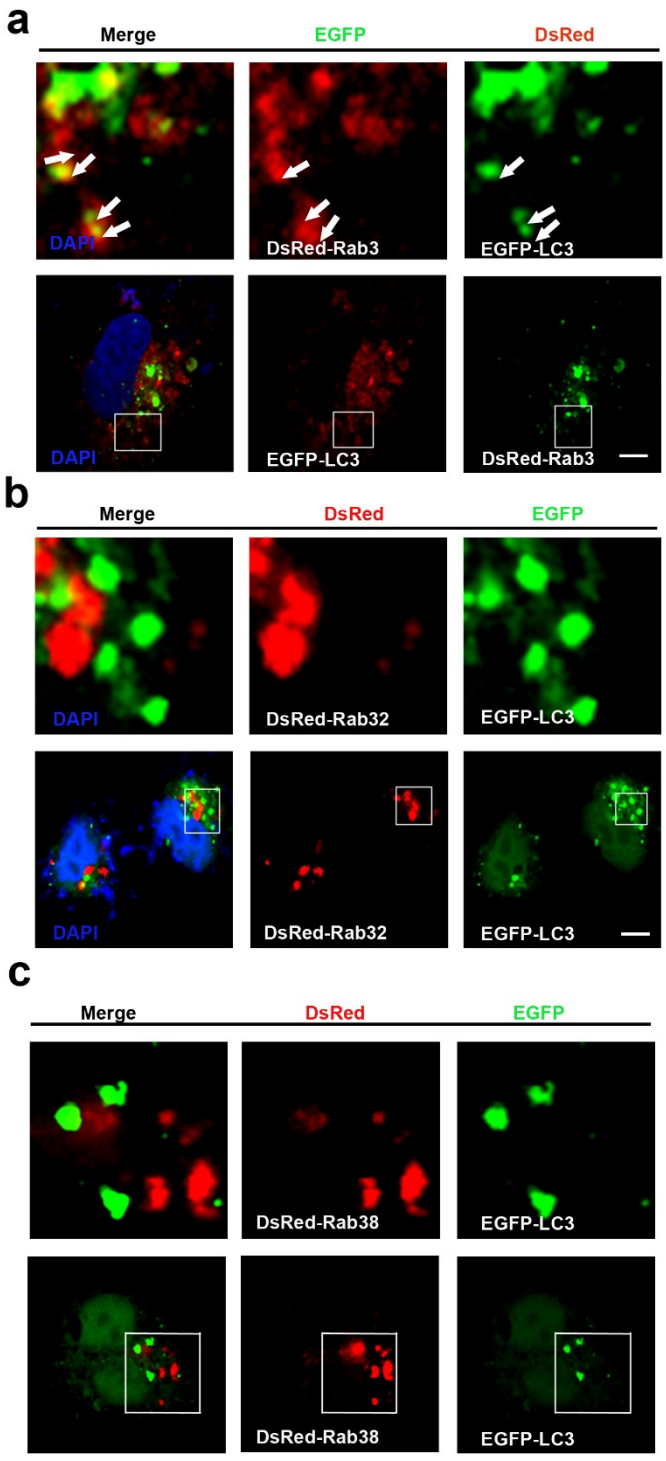

Figure S8. The relationship between Rab3, 32 and 38 positive vesicles with LC3 positive autophagosomes. Fig. S7. (a-c) EGFP-LC3 cells were co-transfected with DsRed-Rab3, DsRed-Rab32, DsRed-Rab38, respectively. Scale bars: 10 μm. The above images are the enlarged ones in the red collar on the underside images. The arrows indicated the localization of EGFP-LC3 positive autophagosomes with DsRed Rab3 positive vesicles.

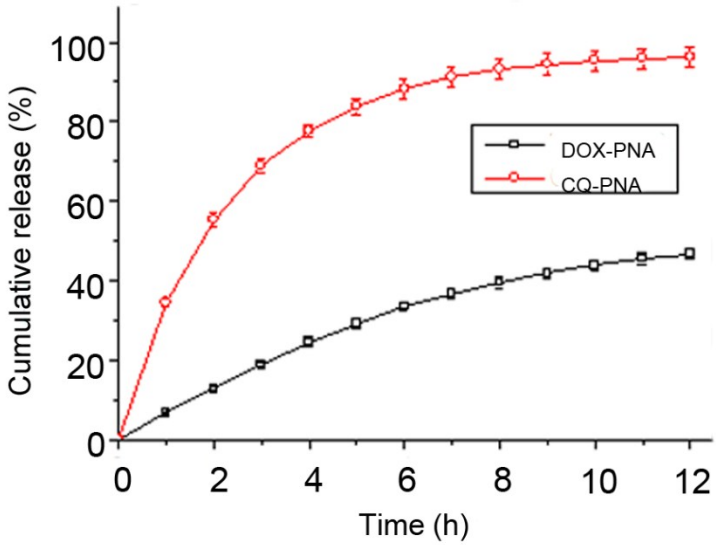

Figure S9. Release profile of DOX and CQ from PNA nanogels particles in pH 7.4 PBS at 37°C.