Intracellular Trafficking Network of Protein Nanocapsules: Endocytosis, Exocytosis and Autophagy

Jinxie Zhang^{1,2, *}, Xudong Zhang^{1,2, 3, *, #}, Gan Liu^{1, 2, *}, Danfeng Chang^{1,2}, Xin Liang^{2, 4}, Xianbing Zhu^{1,2}, Wei Tao^{1,2} and Lin Mei^{1, 2, #}

 School of Life Sciences, Tsinghua University, Beijing 100084, P.R. China
Division of Life and Health Sciences, Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, P.R. China
Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill and North Carolina State University, Raleigh, NC 27695, USA
Department of Pharmacological and Physiological Science and Center for Neuroscience, Saint Louis University School of Medicine, St. Louis, Missouri, USA

* These authors contributed equally to this work.

Corresponding authors:

Lin Mei: Tel/Fax: +86 75526036736, E-mail: <u>mei.lin@sz.tsinghua.edu.cn</u>. Xudong Zhang: Tel/Fax: +1(314) 882-9360, E-mail: <u>xzhang60@ncsu.edu</u>



Fig. S1. Confocal images of MCF-7 cells, which were treated with 1 mg/mL FITC-labeled nBSA for 20 hours. Clathrin, Caveolin, RhoA, Cdc42 and Flotillin were detected with primary antibodies against Clathrin, Caveolin, RhoA, Cdc42 and Flotillin, respectively. (A-E) Scale bars: 10 µm.



Fig. S2. (A) EEA1 was detected in DsRed-Rab34 transfected MCF-7 cells with a primary antibody against EEA1; (B) For lysosome detection, the MCF-7 cells were transfected with DsRed-Rab34, treated with 1 mg/mL FITC-labeled nBSA for 20 h, and then co-treated with Lyso-Tracker Red probes for 30 min. (C) DsRed-Rab21 transfected MCF-7 cells were then treated with 1 mg/mL FITC-labeled nBSA for 20 h. Scale bars: 10 μm.



Fig. S3. (A) EGFP-Rab9 cells were co-transfected with DsRed-Rab18; (B) Clathrin was detected in DsRed-Rab18 transfected MCF-7 cells with a primary antibody against Clathrin. Scale bars: 10 μm.



Fig. S4. (A) Confocal images of MCF-7 cells, DsRed-Rab23 transfected MCF-7 cells were treated with 1 mg/mL FITC-labeled BSA-nanocapsules for 20 h; (B-G) DsRed-Rab23 cells were queried by primary antibodies against Clathrin, Caveolin, RhoA, Cdc42, Flotillin and Arf-6, respectively. Scale bars: 10 μm.



Fig. S5. (A) EGFP-Rab11 cells were co-transfected with DsRed-Rab35. (B) Confocal images of MCF-7 cells. DsRed-Rab4 transfected MCF-7 cells were treated with 1 mg/mL FITC-labeled BSA-nanocapsules for 20 h; Scale bars: 10 μm



Fig. S6. (A-D) Confocal images of DsRed-Rab3, DsRed-Rab32, DsRed-Rab26 and

DsRed-Rab38 transfected MCF-7 cells treated with 1 mg/mL FITC-labeled BSA-nanocapsules for 20 h. (E) EGFP-Rab3 cells were co-transfected with DsRed-Rab26; (F) EGFP-Rab32 cells were co-transfected with DsRed-Rab38. Scale bars: 10 µm.



Fig. S7. (A) EGFP-Rab8 cells were co-transfected with DsRed-Rab10. (B-C) Confocal images of MCF-7 cells transfected with DsRed-Rab14 or Rab24 and then treated with 1 mg/mL FITC-labeled BSA-nanocapsules for 20 h. Scale bars: 10 μm.



Fig. S8. (A-C) Confocal images of MCF-7 cells transfected with DsRed-Rab1, DsRed-Rab43, or DsRed-Rab6 and then, treated with 1 mg/mL FITC-labeled BSA-nanocapsules for 20 h. Scale bars: $10 \mu m$.

Fig. S9. (A-G) EGFP-LC3 cells were co-transfected with DsRed-Rab3, DsRed-Rab26, DsRed-Rab32, DsRed-Rab38, DsRed-Rab1 and DsRed-Rab31, respectively. Scale bars: 10 μm.