Systematic Investigation on the Intracellular Trafficking Network of Polymeric Nanoparticles

Jinxie Zhang^{1, 2, *}, Danfeng Chang^{1,2,*}, Yao Yang^{3*}, Xudong Zhang^{1, 2, 4, #}, Wei Tao^{1,2}, Lijuan Jiang^{1,2}, Xin Liang^{3,5}, Hsiang-I Tsai^{1, 2}, Laigiang Huang^{1, 2, #} and Lin Mei^{1, 2,5 #}

- 1. School of Life Sciences, Tsinghua University, Beijing 100084, P.R. China
- The Shenzhen Key Lab of Gene and Antibody Therapy, Division of Life and Health Sciences, Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, P.R. China
- College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, People's Republic of China
- Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill and North Carolina State University, Raleigh, North Carolina 27695, United States

5. School of Pharmaceutical Sciences, Sun Yat-sen University, University Town, Guangzhou, 510006, People's Republic of China

* 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> Laiqiang Huang: Tel/Fax: +86 75526036012, E-mail: laiqianghuangthu@163.com Xudong Zhang: Tel/Fax: +1(314) 882-9360, E-mail: x.d.zhang@foxmail.com



Fig. S1. Confocal images of HeLa cells, which were treated with 100 μ g/mL C-6-loaded PLGA nanoparticles for 20 hours. Clathrin, Arf-6, RhoA, Cdc42 and Flotillin were detected with primary antibodies against Clathrin, Arf-6, RhoA, Cdc42 and Flotillin, respectively. (A-E) Scale bars: 10 μ m.



Fig. S2. (A) EEA1 was detected in DsRed-Rab34 transfected HeLa cells with a primary antibody against EEA1; (B) For lysosome detection, the HeLa cells were transfected with DsRed-Rab34, treated with 100 μ g/mL C-6-loaded PLGA nanoparticles for 20 h, and then co-treated with Lyso-Tracker Red probes for 30 min. (C) DsRed-Rab21 transfected HeLa cells were treated with 100 μ g/mL C-6-loaded PLGA nanoparticles for 20 h. Scale bars: 10 μ m.



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



Fig. S4. (A) Confocal images of HeLa cells, DsRed-Rab23 transfected HeLa cells were treated with 100 μ g/mL Coumarin-6-loaded PLGA nanoparticles for 20 h; (B-E) DsRed-Rab23 cells were queried by primary antibodies against RhoA, Cdc42, Flotillin and Arf-6, respectively. Scale bars: 10 μ m.



Fig. S5. (A) Confocal images of HeLa cells. DsRed-Rab4, DsRed-Rab1, DsRed-Rab2 and DsRed-Rab6 transfected HeLa cells, respectively. Then treated with 100 μ g/mL Coumarin-6-loaded PLGA nanoparticles for 20 h; Scale bars: 10 μ m



Fig. S6. (A-D) Confocal images of DsRed-Rab3, DsRed-Rab26, DsRed-Rab32 and DsRed-Rab38 HeLa cells co-transfected with 100 μ g/mL EGFP-LC3, respectively. Scale bars: 10 μ m.