

## *Supporting Information*

### **Emerging investigator series: Long-term exposure of amorphous silica nanoparticles disrupts the lysosomal and cholesterol homeostasis in macrophages**

Ronglin Ma<sup>1, #</sup>, Xiaoming Cai<sup>2, #</sup>, Ye Zhou<sup>3</sup>, Xi Liu<sup>1</sup>, Di Wu<sup>1</sup>, Huizhen Zheng<sup>1</sup>, Yanxia Pan<sup>1</sup>, Jun Jiang<sup>1</sup>, Shujuan Xu<sup>1</sup>, Qianqian Xie<sup>1</sup>, Jie Jiang<sup>1</sup>, Weili Wang<sup>1</sup>, Nikolai Tarasenko<sup>4</sup>, Fangjun Wang<sup>3\*</sup>,  
Ruibin Li<sup>1\*</sup>

<sup>1</sup> State Key Laboratory of Radiation Medicine and Protection, School for Radiological and Interdisciplinary Sciences (RAD-X), Collaborative Innovation Center of Radiological Medicine of Jiangsu Higher Education Institutions, Soochow University, Suzhou 215123, Jiangsu China

<sup>2</sup> Center for Genetic Epidemiology and Genomics, School of Public Health, Jiangsu Key Laboratory of Preventive and Translational Medicine for Geriatric Diseases, Soochow University, Suzhou 215123, Jiangsu China

<sup>3</sup>CAS Key Laboratory of Separation Sciences for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences (CAS), Dalian 116023, Liaoning P. R. China

<sup>4</sup>B. I. Stepanov Institute of Physics, National Academy of Sciences of Belarus, 68 Nezalezhnasti Ave., 220072 Minsk, Belarus

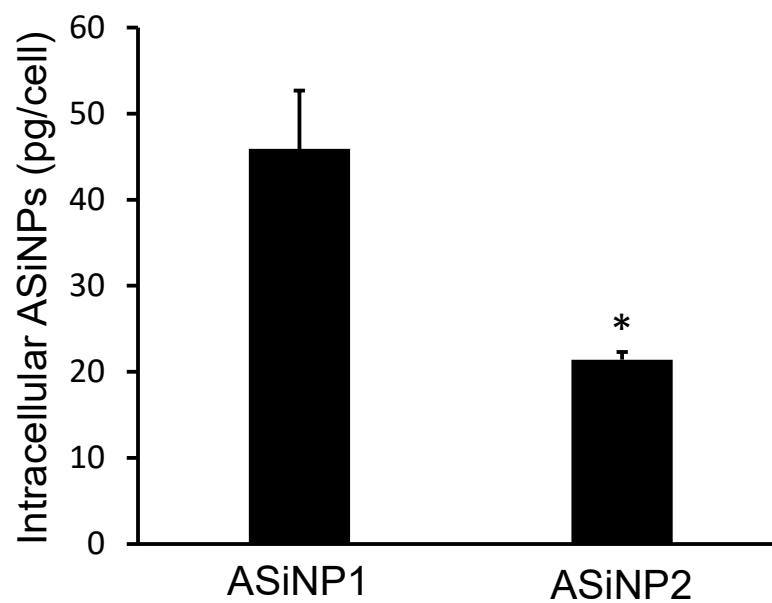
<sup>#</sup>Equal contributions to this paper.

\* Correspondence author.

Dr. Ruibin Li      Email: [liruibin@suda.edu.cn](mailto:liruibin@suda.edu.cn)      Tel: +86-512-65880062

&

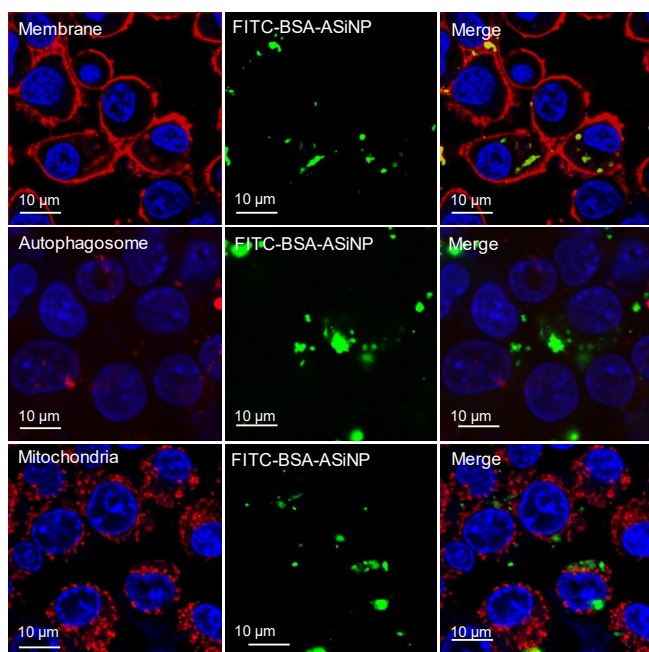
Dr. Fangjun Wang      Email: [wangfj@dicp.ac.cn](mailto:wangfj@dicp.ac.cn)      Tel: +86-411-82464150



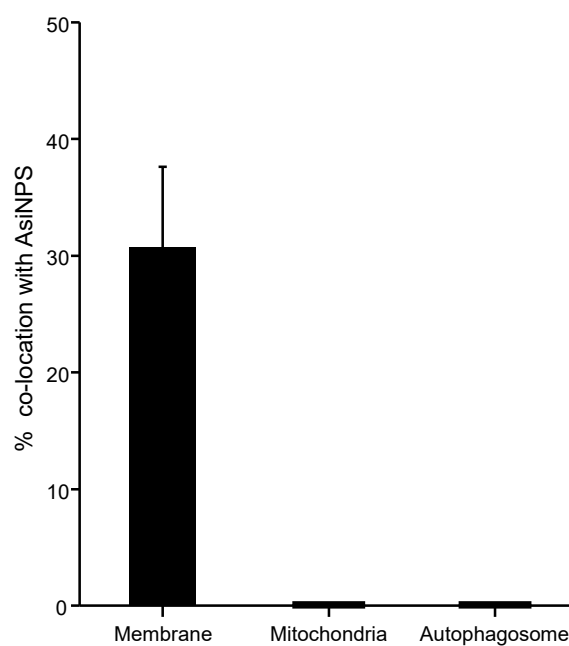
**Figure S1 ICP-OES detection of intracellular silica contents**

RAW 264.7 cells exposed to 100  $\mu\text{g/mL}$  ASiNP-1 or ASiNP-2 solutions for 24 h were collected and pre-digested in nitric acid solution, followed by further digestion in KOH solution. The Si contents in digestions were detected by ICP-OES (n=3). \*,  $p < 0.05$  compared to ASiNP1 by two-tailed Student's T-test.

A



B



**Figure S2. A) Confocal microscopy images of co-localizations of lysosomes with membrane, autophagosome and mitochondria; B) quantification of co-localization coefficients.**

RAW264.7 cells were incubated with 20 μg/mL ASiNPs for 12 h. After three-time washing by PBS, the treated cells were stained by WGA-594, LC-3B immunofluorescence kit and mito-tracker dye for 30 min before visualization by confocal microscopy.

Table S1 Proteins involved mainly in five biological processes

Proteins	MW(Da)	PI	Autophagy	Lipid metabolism	Inflammation	Endocytosis	Apoptosis	Others	Reference
CD63 antigen (CD63) *	25,767	7.00	√	√	√	√	√	/	[1-5], UniProt, KEGG
Two pore segment channel 1 (Tpcn1) *	94,496	8.70	√	/	/	√	√	/	[6], UniProt, KEGG
Rho-related GTP-binding protein RhoB (RhoB)	22,123	4.85	√	√	√	√	√	/	[7-10], UniProt
Vam6/Vps39-like protein (Vps39) *	101,693	6.99	√	/	/	/	/	/	[11] UniProt
Regulatory-associated protein of mTOR (Rptor)	149,471	6.87	√	√	√	/	√	/	[12-14], UniProt, KEGG
Dynamin-2 (DNM-2) *	98,145	7.48	√	/	√	√	√	/	[15], UniProt, KEGG
Vacuolar protein sorting-associated protein 35 (Vps35) *	91,713	5.12	√	/	√	√	/	/	[16], UniProt, KEGG
Ras-related protein Rab-12 (Rab12) *	27,329	8.58	√	√	/	√	/	/	[17], UniProt, KEGG
AP-1 complex subunit beta-1 (Ap1b1) *	103,935	4.79	√	√	/	√	/	/	UniProt, KEGG
Glucocerebrosidase (Gba)	57,622	7.84	√	√	/	/	/	/	[18], UniProt, KEGG
Vesicle-associated membrane protein 7 (Vamp7) *	24,967	8.79	√	√	√	√	/	/	[19], UniProt, KEGG
Interferon-induced transmembrane protein 3 (Ifitm3) *	14,954	7.50	√	/	√	√	/	/	[20], UniProt, KEGG
ADP-ribosylation factor 1 (Arf1)	20,697	6.80	√	√	/	√	/	/	[21], UniProt, KEGG
Lysosomal acid lipase (Lipa) *	45,325	8.21	√	√	√	/	/	/	[22], UniProt, KEGG
Progranulin (Grn)	65,018	6.99	√	√	√	/	/	/	[23], UniProt, KEGG
Vesicle-associated membrane protein 2 (Vamp2)	12,691	8.48	/	√	√	√	/	/	[24-26], UniProt
Beta-glucuronidases (Gusb)	74,195	6.69	/	/	/	/	/	√	UniProt
Ankyrin repeat domain-containing protein 27 (Ankrd27)	116,809	6.64	/	/	/	√	/	/	UniProt, KEGG
N-acetylglucosamine-1-phosphate transferase (Gnptab)	140,984	7.71	√	√	/	/	/	/	[27, 28], KEGG
Serum paraoxonase/arylesterase 2 (Pon2)	39,617	5.69	/	√	/	/	√	/	[29, 30], UniProt, KEGG
Dipeptidyl-peptidase 7 (Dpp7)	56,254	5.02	/	/	/	/	/	√	UniProt
Regulator of MON1-CCZ1 complex (Mic1) *	74,922	7.89	√	√	/	/	/	/	UniProt
Protein Cln8 (Cln8)	33,109	9.02	/	√	/	/	√	/	UniProt
Unconventional myosin-Va (Myo5a)	215,538	8.88	/	√	/	√	/	/	UniProt
Cathepsin C (Ctsc)	52,376	6.88	/	/	/	/	√	/	UniProt, KEGG
Amount			17	16	10	13	8	2	
Percent			25.8%	24.3%	15.1%	19.7%	12.1%	3.0%	

\* represent that proteins are correlated with the fusion of lysosomes and autophagosomes

1. J. J. Pei, M. Q. Zhao, Z. D. Ye, H. C. Gou, J. Y. Wang, L. Yi, X. Y. Dong, W. J. Liu, Y. W. Luo, M. Liao and J. D. Chen, Autophagy enhances the replication of classical swine fever virus in vitro. *Autophagy* 2014, **10**:93-110.
2. F. J. Verweij, M. A. J. van Eijndhoven, E. S. Hopmans, T. Vendrig, T. Wurdinger, E. Cahir-McFarland, E. Kieff, D. Geerts, R. van der Kant, J. Neefjes, J. M. Middeldorp and D. M. Pegtel, LMP1 association with CD63 in endosomes and secretion via exosomes limits constitutive NF-kappa B activation. *Embo J* 2011, **30**:2115-29.
3. U. M. Vischer and D. D. Wagner, Cd63 Is a Component Of Weibel-Palade Bodies Of Human Endothelial-Cells. *Blood* 1993, **82**(4):1184-91.
4. A. R. Mantegazza, M. M. Barrio, S. Moutel, L. Bover, M. Weck, P. Brossart, J. L. Teillaud and J. Mordoh, CD63 tetraspanin slows down cell migration and translocates to the endosomal-lysosomal-MIICs route after extracellular stimuli in human immature dendritic cells. *Blood* 2004, **104**:1183-90.
5. K. K. Jung, X. W. Liu, R. Chirco, R. Fridman and H. R. C. Kim, Identification of CD63 as a tissue inhibitor of metalloproteinase-1 interacting cell surface protein. *Embo J* 2006, **25**:3934-42.
6. D. Hoglinger, P. Haberkant, A. Aguilera-Romero, H. Riezman, F. D. Porter, F. M. Platt, A. Galione and C. Schultz, Intracellular sphingosine releases calcium from lysosomes. *Elife* 2015, **4**:10616.
7. M. D. Liu, T. L. Zeng, X. Zhang, C. Y. Liu, Z. H. Wu, L. M. Yao, C. C. Xie, H. Xia, Q. Lin, L. P. Xie, D. W. Zhou, X. M. Deng, H. L. Chan, T. J. Zhao and H. R. Wang, ATR/Chk1 signaling induces autophagy through sumoylated RhoB-mediated lysosomal translocation of TSC2 after DNA damage. *Nat Commun* 2018, **9**:1-14
8. M. C. A. Pronk, J. Majolee, A. Loregger, J. S. M. van Bezu, N. Zelcer, P. L. Hordijk and I. Kovacevic, FBXW7 regulates endothelial barrier function by suppression of the cholesterol synthesis pathway and prenylation of RhoB. *Mol Biol Cell* 2019, **30**:607-21.
9. S. Y. Liu, L. S. Huang, Z. S. Lin, Y. Q. Hu, R. F. Chen, L. Q. Wang and Y. Shan, RhoB induces the production of proinflammatory cytokines in TLR-triggered macrophages. *Mol Immunol* 2017, **87**:200-06.
10. H. C. Tang, Y. Y. Lai, J. Zheng, H. Y. Jiang and G. Xu, miR-223-3p Inhibits Antigen Endocytosis and Presentation and Promotes the Tolerogenic Potential of Dendritic Cells through Targeting Mannose Receptor Signaling and Rhob. *J Immunol Res* 2020, 2020.
11. D. Iaconis, C. Crina, S. Brillante, A. Indrieri, M. Morleo and B. Franco, The HOPS complex subunit VPS39 controls ciliogenesis through autophagy. *Hum Mol Genet* 2020, **29**:1018-29.
12. A. Y. He, J. M. Dean, D. L. Lu, Y. L. Chen and I. J. Lodhi, Hepatic peroxisomal beta-oxidation suppresses lipophagy via RPTOR acetylation and MTOR activation. *Autophagy* 2020, **16**:1727-28.
13. M. Tsuchiya, S. Kalurupalle, P. Kumar, S. Ghoshal, Y. Q. Zhang, E. Lehrmann, K. G. Becker, M. Gorospe and R. Biswas, RPTOR, a novel target of miR-155, elicits a fibrotic phenotype of cystic fibrosis lung epithelium by upregulating CTGF. *Rna Biol* 2016, **13**:837-47.
14. W. S. Lian, J. Y. Ko, Y. S. Chen, H. C. Ke, S. L. Wu, C. W. Kuo and F. S. Wang, Chaperonin 60 sustains osteoblast autophagy and counteracts glucocorticoid

- aggravation of osteoporosis by chaperoning RPTOR. *Cell Death Dis* 2019, **10**:1-13
15. C. Puri and D. C. Rubinsztein, A location, location, location mutation impairs DNM2-mediated release of nascent autophagosomes from recycling endosomes. *Autophagy* 2020, **16**:1353-54.
16. F. L. Tang, J. R. Erion, Y. Tian, W. Liu, D. M. Yin, J. Ye, B. S. Tang, L. Mei and W. C. Xiong, VPS35 in Dopamine Neurons Is Required for Endosome-to-Golgi Retrieval of Lamp2a, a Receptor of Chaperone-Mediated Autophagy That Is Critical for alpha-Synuclein Degradation and Prevention of Pathogenesis of Parkinson's Disease. *J Neurosci* 2015, **35**:10613-28.
17. T. Matsui and M. Fukuda, Rab12 regulates mTORC1 activity and autophagy through controlling the degradation of amino-acid transporter PAT4. *Embo Rep* 2013, **14**:450-57.
18. M. E. Gegg, D. Burke, S. J. R. Heales, J. M. Cooper, J. Hardy, N. W. Wood and A. H. V. Schapira, Glucocerebrosidase deficiency in substantia nigra of parkinson disease brains. *Ann Neurol* 2012, **72**:455-63.
19. X. Y. Tian, P. L. Zheng, C. Q. Zhou, X. R. Wang, H. Ma, W. Ma, X. K. Zhou, J. L. Teng and J. G. Chen, DIPK2A promotes STX17-and VAMP7-mediated autophagosome-lysosome fusion by binding to VAMP7B. *Autophagy* 2020, **16**:797-10.
20. L. Q. Jiang, T. Xia, Y. H. Hu, M. S. Sun, S. Yan, C. Q. Lei, H. B. Shu, J. H. Guo and Y. Liu, IFITM3 inhibits virus-triggered induction of type I interferon by mediating autophagosome-dependent degradation of IRF3. *Cell Mol Immunol* 2018, **15**:858-67.
21. R. Dechant, S. Saad, A. J. Ibanez and M. Peter, Cytosolic pH Regulates Cell Growth through Distinct GTPases, Arf1 and Gtr1, to Promote Ras/PKA and TORC1 Activity. *Mol Cell* 2014, **55**:409-21.
22. M. Ouimet, V. Franklin, E. Mak, X. H. Liao, I. Tabas and Y. L. Marcel, Autophagy Regulates Cholesterol Efflux from Macrophage Foam Cells via Lysosomal Acid Lipase. *Cell Metab* 2011, **13**:655-67.
23. M. C. Chang, K. Srinivasan, B. A. Friedman, E. Suto, Z. Modrusan, W. P. Lee, J. S. Kaminker, D. V. Hansen and M. Sheng, Progranulin deficiency causes impairment of autophagy and TDP-43 accumulation. *J Exp Med* 2017, **214**:2611-28.
24. C. C. Wang, J. Tu, S. N. Zhang, B. Cai, Z. Y. Liu, S. Q. Hou, Q. L. Zhong, X. Hu, W. B. Liu, G. H. Li, Z. J. Liu, L. He, J. J. Diao, Z. J. Zhu, D. Li and C. Liu, Different regions of synaptic vesicle membrane regulate VAMP2 conformation for the SNARE assembly. *Nat Commun* 2020, **11**:e98311.
25. X. L. Duan, Z. Guo, Y. T. He, Y. X. Li, Y. N. Liu, H. H. Bai, H. L. Li, X. D. Hu and Z. W. Suo, SNAP25/syntaxin4/VAMP2/Munc18-1 Complexes in Spinal Dorsal Horn Contributed to Inflammatory Pain. *Neuroscience* 2020, **429**:203-12.
26. H. F. Renard, M. D. Garcia-Castillo, V. Chambon, C. Lamaze and L. Johannes, Shiga toxin stimulates clathrin-independent endocytosis of the VAMP2, VAMP3 and VAMP8 SNARE proteins. *J Cell Sci* 2015, **128**:2891-02.
27. S. G. Willet, M. A. Lewis, Z. F. Miao, D. Q. Liu, M. D. Radyk, R. L. Cunningham, J. Burclaff, G. Sibbel, H. Y. G. Lo, V. Blanc, N. O. Davidson, Z. N. Wang and J. C. Mills, Regenerative proliferation of differentiated cells by mTORC1-dependent paligenesis. *Embo J* 2018, **37**.

28. H. E. Miller, F. H. Hoyt and R. A. Heinzen, Replication of *Coxiella burnetii* in a Lysosome-Like Vacuole Does Not Require Lysosomal Hydrolases. *Infect Immun* 2019, **87**:e00493
29. O. Rom and M. Aviram, Paraoxonase2 (PON2) and oxidative stress involvement in pomegranate juice protection against cigarette smoke-induced macrophage cholesterol accumulation. *Chem-Biol Interact* 2016, **259**:394-400.
30. M. Kruger, A. M. Pabst, B. Al-Nawas, S. Horke and M. Moergel, Paraoxonase-2 (PON2) protects oral squamous cell cancer cells against irradiation-induced apoptosis. *J Cancer Res Clin* 2015, **141**:1757-66.