Supporting Information

Vacuolization of Macrophages Induced by Large Amounts of Inorganic Nanoparticles Uptake to Enhance the Immune Response

1. Synthesis and characterization

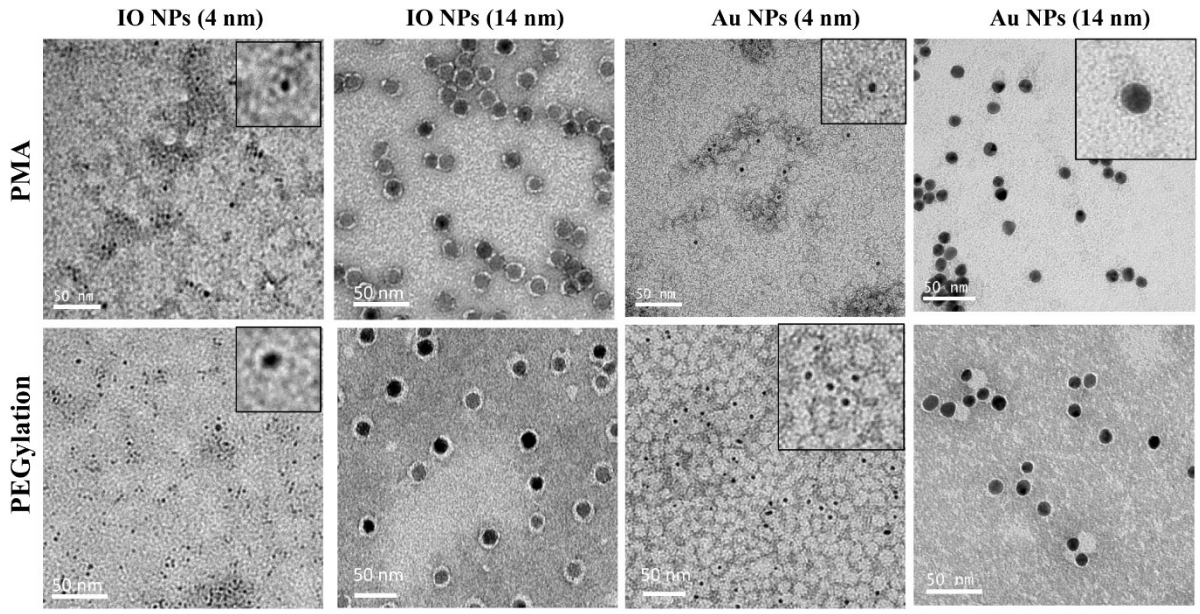


Figure S1. Negatively staining TEM images of all the samples.

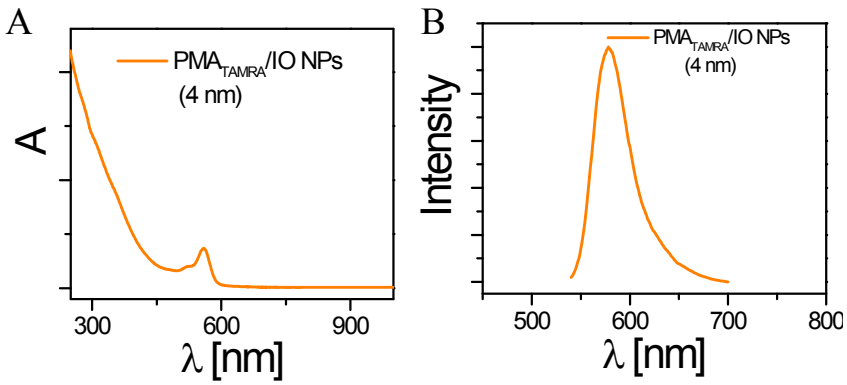


Figure S2. A) UV-Vis spectra and fluorescent spectra of PMA_{TAMRA}/IO NPs with the size diameters of 4 nm. There is an absorption peak at 560 nm, and the emission peak was at 573 nm under excitation peak at 550 nm.

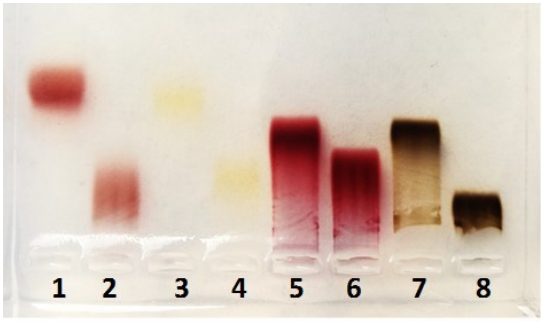


Figure S3. Gel electrophoresis of different NPs with various sizes and surface modifications in a 2 % agarose gel (100 V, 150 mA, 1 h). The samples from 1 to 8 are 4 nm PMA/Au NPs, 4 nm PMA/Au-PEG NPs, 4 nm PMA/IO NPs, 4 nm PMA/IO-PEG NPs, 14 nm PMA/Au NPs, 14 nm PMA/Au-PEG NPs, 14 nm PMA/IO NPs and 14 nm PMA/IO-PEG NPs. The bands of all the inorganic NPs are clearly visible on the image of the agarose gel, the running speed of NPs are highly dependent on the size and surface modification, which bigger size and PEGylation are running much slower than others.

Table S1. The DLS, ζ-potential and layer thickness of inorganic NPs.

Name	DLS [nm]	ζ-potential [mV]	PMA layer [nm]	PEG layer [nm]
PMA/Au (4 nm)	4.6 ± 1.2	-38.3 ± 4.2	3.2 ± 0.54	n.m.
PMA/Au-PEG (4 nm)	7.2 ± 1.5	-28.8 ± 5.5	n.m.	0.8 ± 0.34
PMA/Au (14 nm)	15.9 ± 5.1	-30.4 ± 4.1	1.61 ± 0.23	n.m.
PMA/Au-PEG (14 nm)	17.7 ± 4.2	-26.4 ± 5.5	n.m.	0.45 ± 0.11
PMA/IO (4 nm)	4.8 ± 1.6	-34.5 ± 2.5	1.8 ± 0.34	n.m.
PMA/IO -PEG (4 nm)	8.1 ± 1.7	-29.0 ± 3.4	n.m.	0.75 ± 0.2
PMA/IO (14 nm)	15.8 ± 3.7	-32.4 ± 4.2	2.7 ± 0.6	n.m.
PMA/IO -PEG (14 nm)	19.4 ± 4.9	-20.1 ± 4.4	n.m.	1.6 ± 0.54

Hereby, the PEG layer thickness = the total layer thickness calculated from negatively staining TEM images - the PMA layer thickness. “n.m.”= not mentioned.

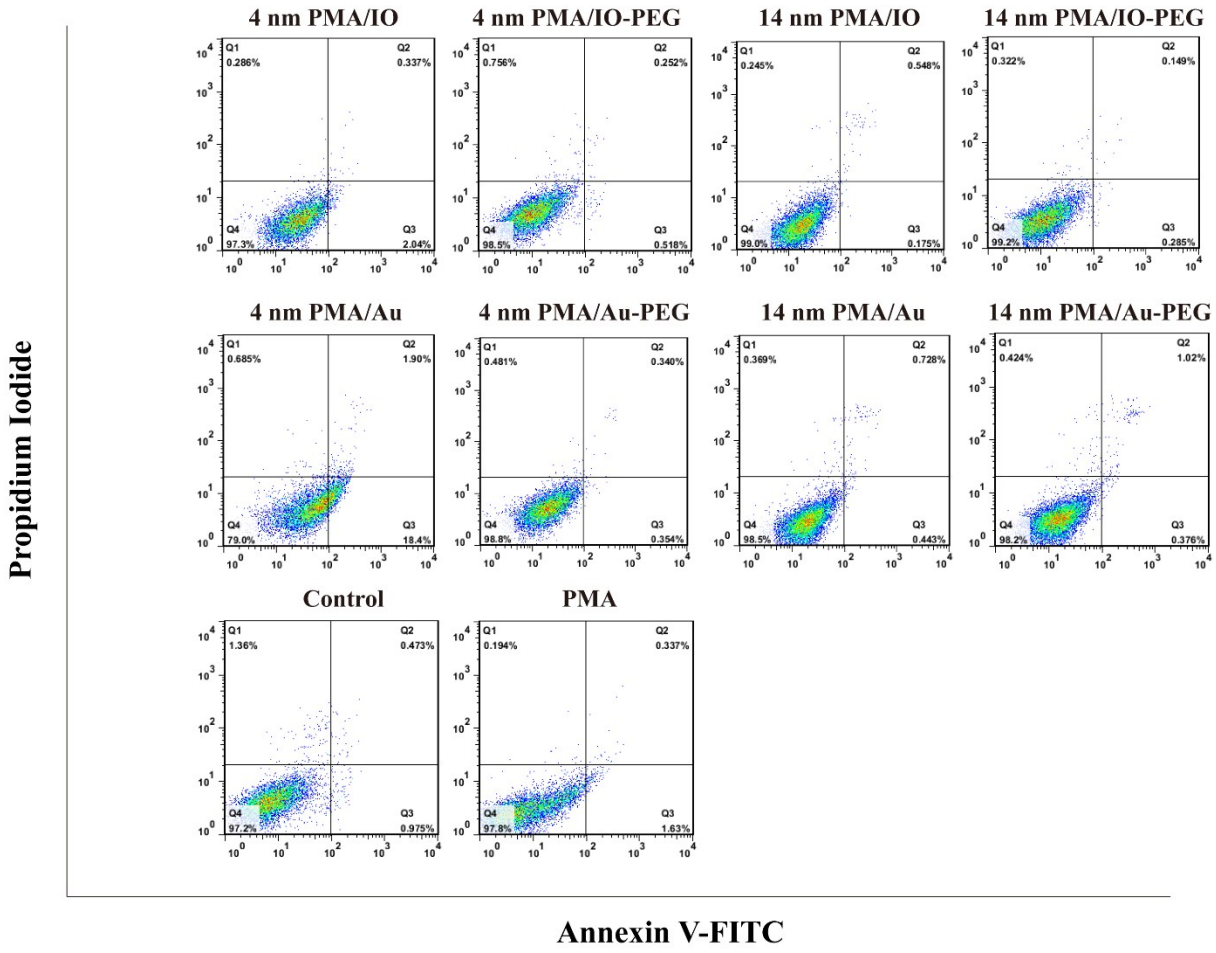


Figure S4. Flow cytometry analysis of RAW264.7 cells incubated with different inorganic NPs (5 nM) for 24 h using Annexin V-FITC and PI staining. The flow cytometry results showed that almost all the particle treated cells maintained alive status without apoptotic phenomenon, except for the smaller size PMA/Au NPs with about 20% of apoptotic cells, which indicated that 4 nm PMA/Au NPs induced higher cytotoxicity than that of other groups.

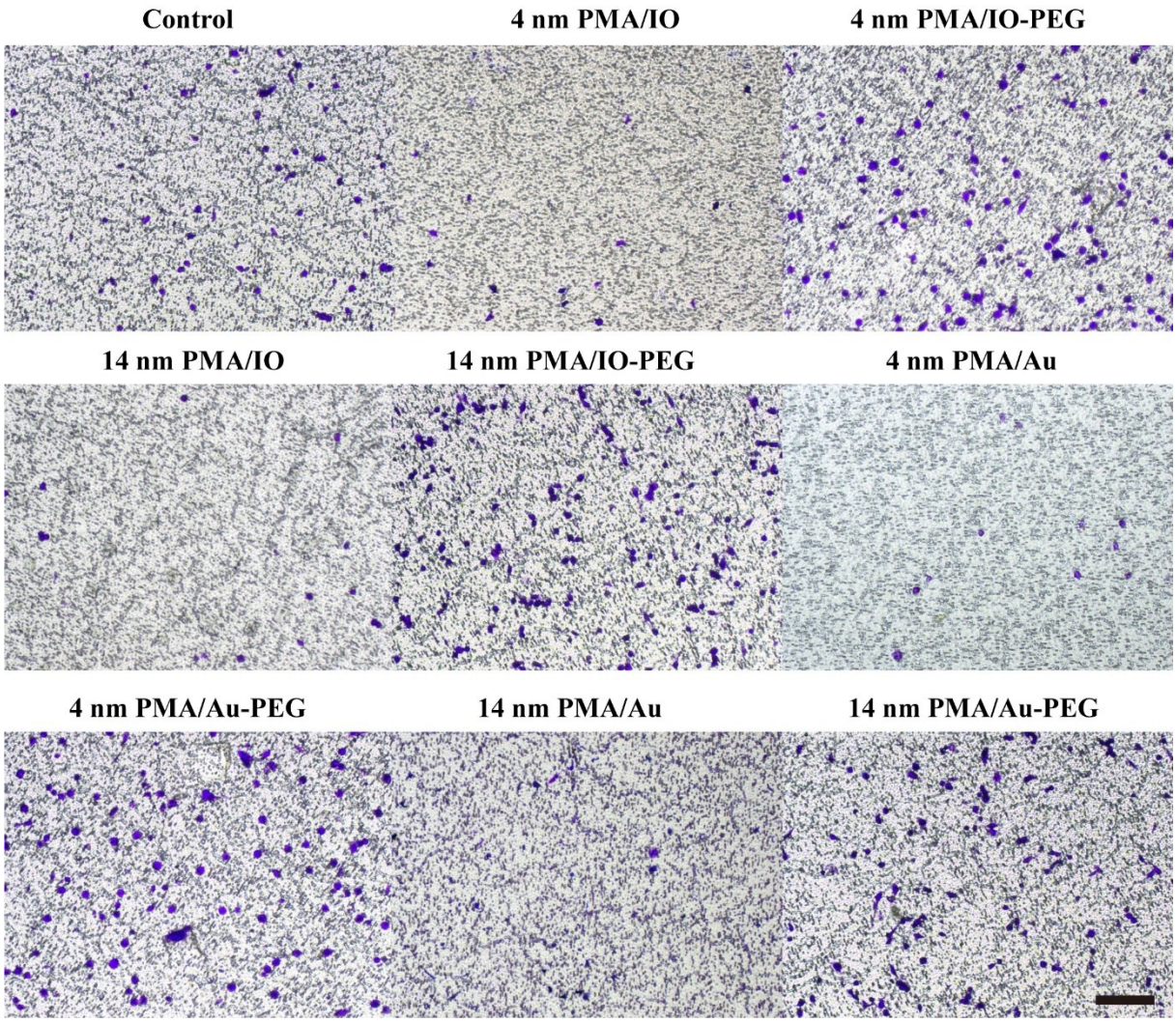


Figure S5. Microscopy images of the migration of RAW264.7 cells incubated with different inorganic NPs for 12 h. (5 nM). Scale bars: 50 μm. The results show that PMA modified inorganic NPs will inhibit the migration of RAW264.7 cells, while the migration of cells exposed to PEGylated NPs was enhanced.

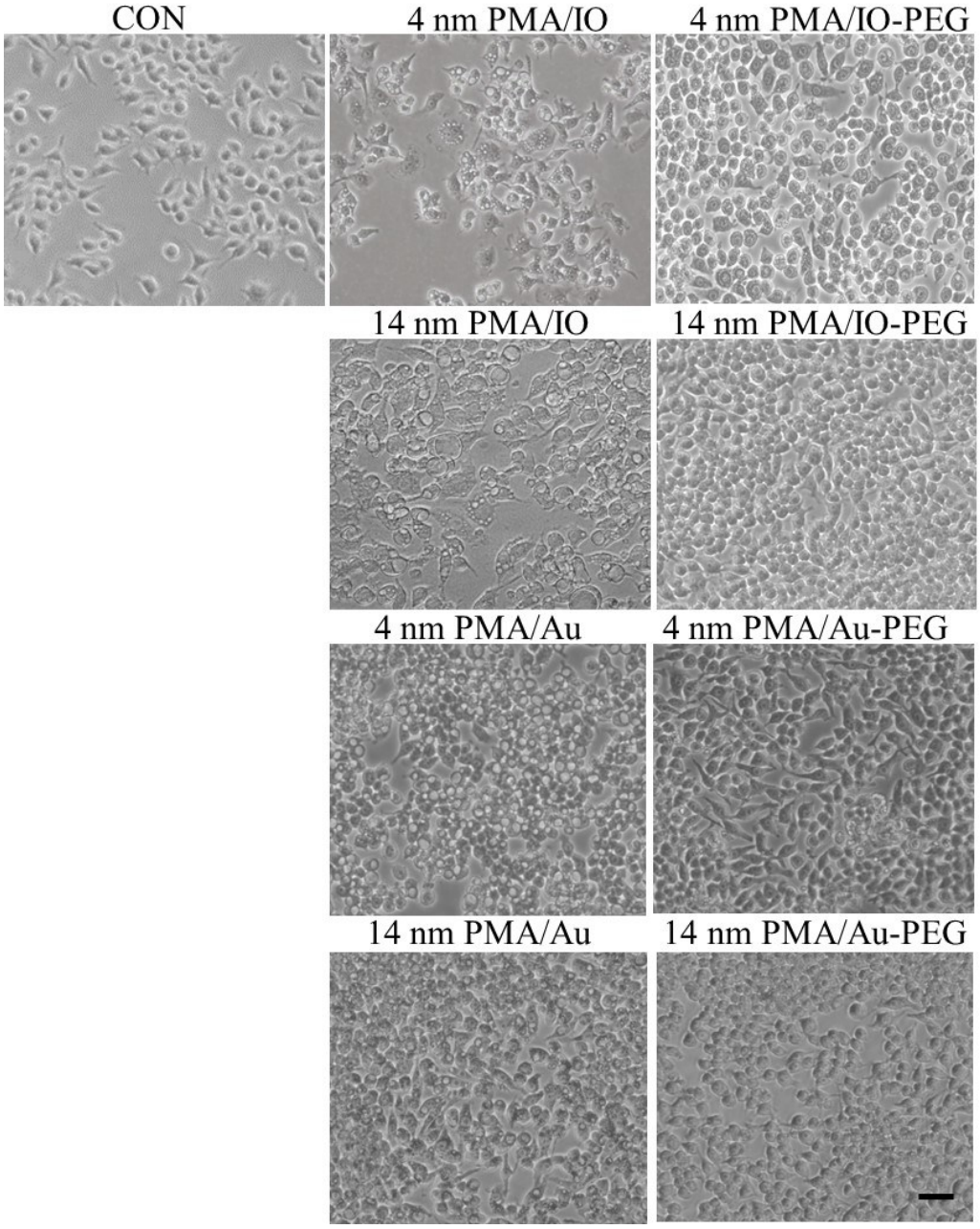


Figure S6. Microscopy images of the RAW264.7 cells incubated with different inorganic NPs for 24 h. (5 nM). Scale bars: 50 μm. The results show that PMA modified inorganic NPs will result in the cytoplasmic vacuolization inside macrophage cells RAW 264.7, while fewer vacuoles were observed after exposed to PEGylated NPs at the same conditions. In addition, the size of IO NPs has effects on the size of vacuoles.

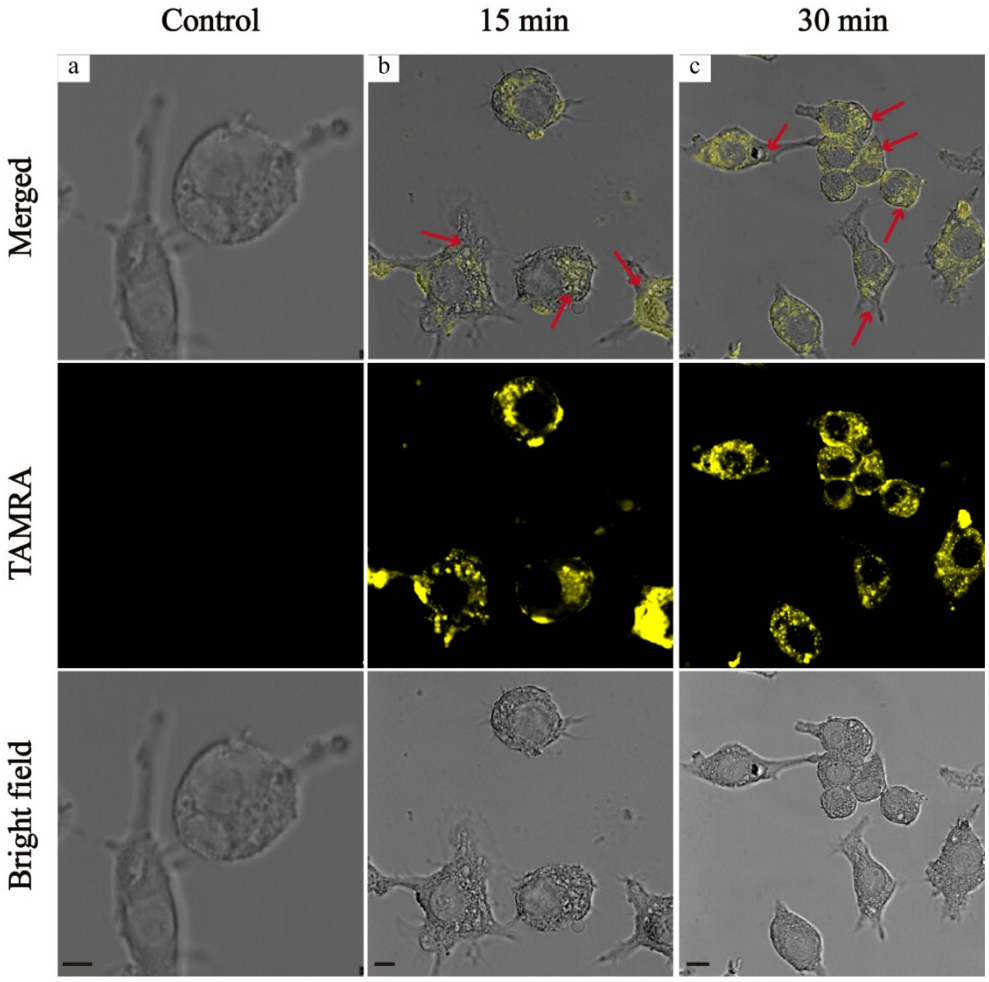


Figure S7. CLSM images of RAW264.7 cells incubated with 4 nm size of PMA_{TAMRA}/IO NPs (50 nM) at 0 min (a), 15 min (b) and 30 min (c), respectively. Scales bar, 10 μm. After exposed to PMA_{TAMRA}/IO NPs, we can see obvious fluorescence around vacuoles inside RAW 264.7 cells. There is no obvious fluorescence inside the vacuoles.

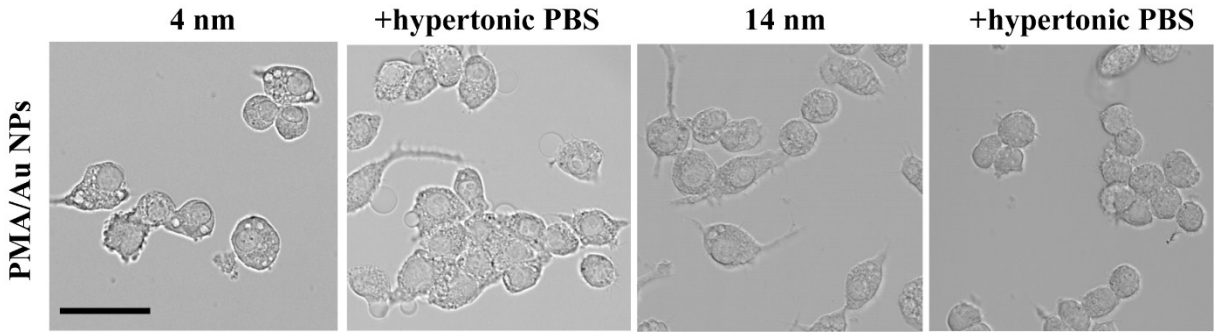
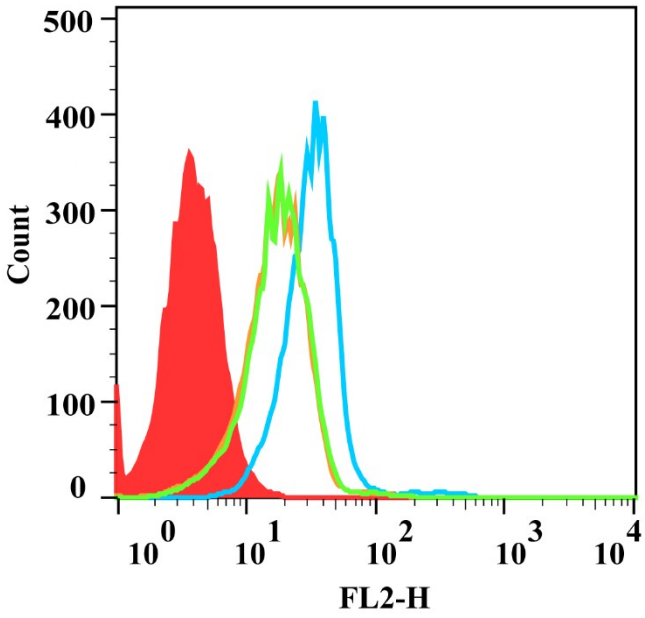


Figure S8. Bright field CLSM images of RAW 264.7 cells after co-incubation with PMA/Au NPs (5 nM) for 24 h with size of 4 and 14 nm, respectively, and further reincubated in hypertonic PBS solution (containing 145 mM NaCl) for 1 h.



Sample Name	Mean, FL2-H
Pre-treated at 4 °C-30 min	21.3
Pre-treated at 4 °C-15 min	20.3
Non-treated cells	40.1
Control	4.26

Figure S9. Flow cytometry analysis of RAW264.7 cells incubated with 4 nm size of PMA_{TAMRA}/IO NPs (50 nM) for 1 h. The cells were pre-treated at 4 °C for 15 and 30 min before incubation with particles. Compared with normal cells without low temperature treatment with the FL2-H intensity of 40.1, the cells pre-treated at 4 °C for 15 min and 30 min have significant uptake decrease of PMA_{TAMRA}/IO NPs, with the FL2-H intensity of 20.3 and 21.3, respectively. The results showed that low temperature could inhibit the uptake of NPs in RAW 264.7 cells, and the inhabitation efficiency could up to 50%. Cells without NPs were considered as control group.

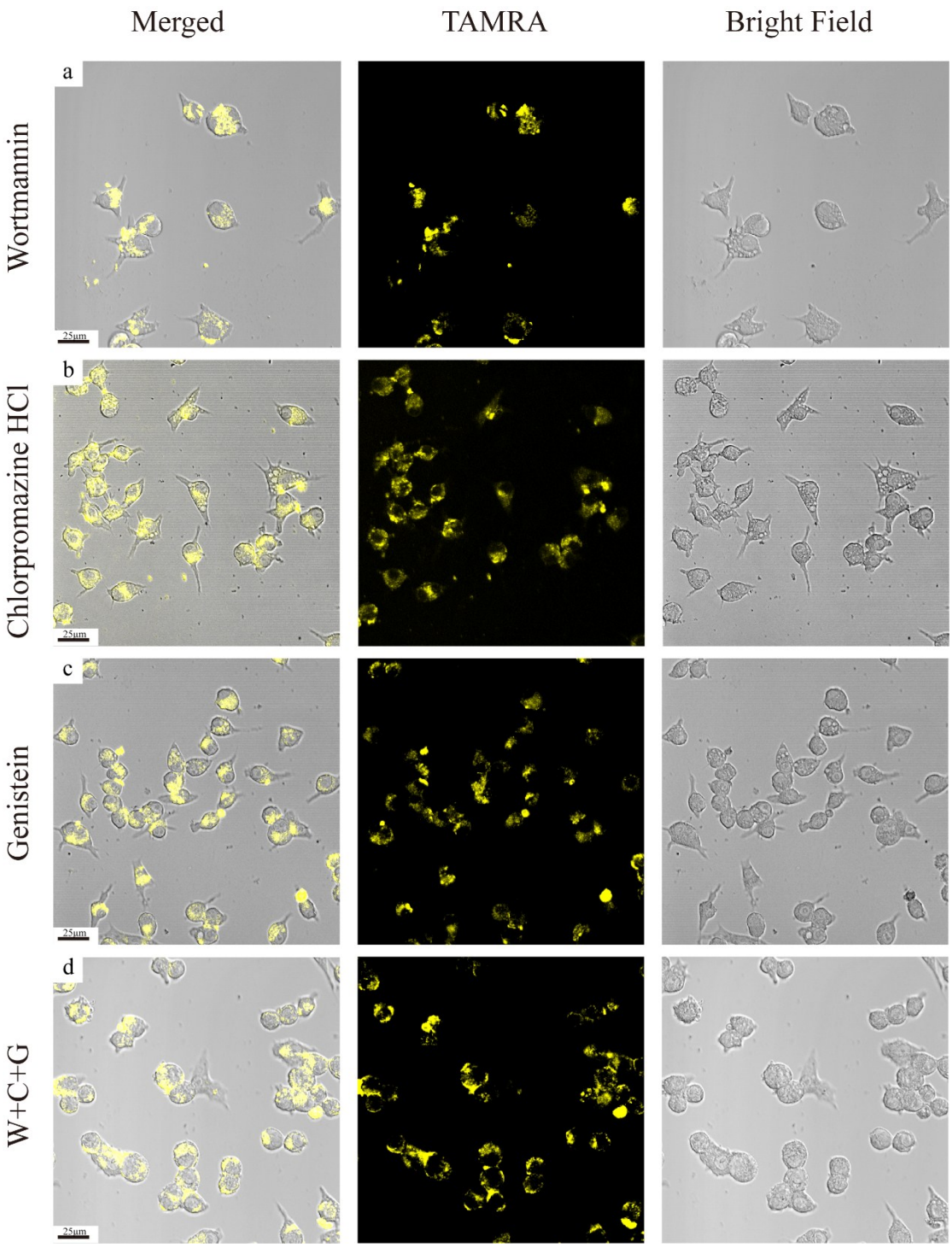


Figure S10. CLSM images of RAW264.7 cells preincubated with wortmannin (0.5 μM) (a), chlorpromazine HCl (15 μM) (b), genistein (50 μM) (c) and all of these three inhibitors (W+C+G, d) for 1 h, and then exposed to 4 nm PMA_{TAMRA}/IO NPs at dosage of 50 nM for 1 h. Results show that the inhibition of pinocytosis has little effects on the uptake of NPs and formation of vacuoles, which implied that the PMA modified NPs can enter the cells in some other ways.

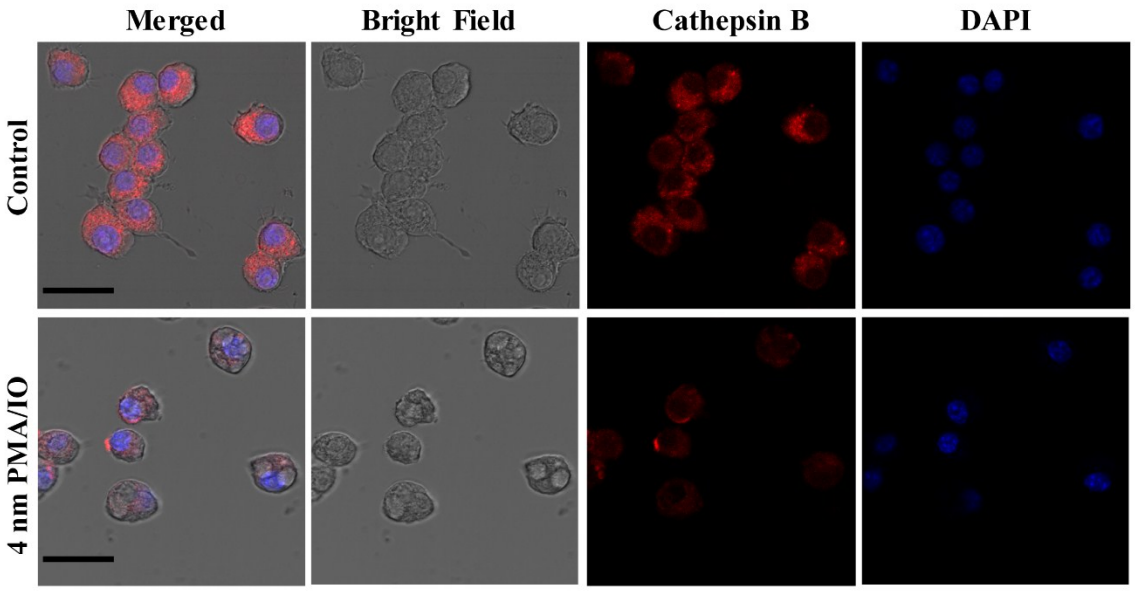


Figure S11. CLSM images to assess lysosome damage. Cells were pre-incubated with 4 nm PMA/IO and then stained with Magic Red-labeled cathepsin B for 30 min. DAPI here was used to reveal nuclear localization. The confocal results show that the intact lysosomes in RAW264.7 cells decreased after exposed to 4 nm PMA/IO NPs. Scales bar, 25 μm.