

Supporting information

for

Intracellular Mechanistic Understanding of 2D MoS₂ Nanosheets for Anti-Exocytosis Enhanced Synergistic Cancer Therapy

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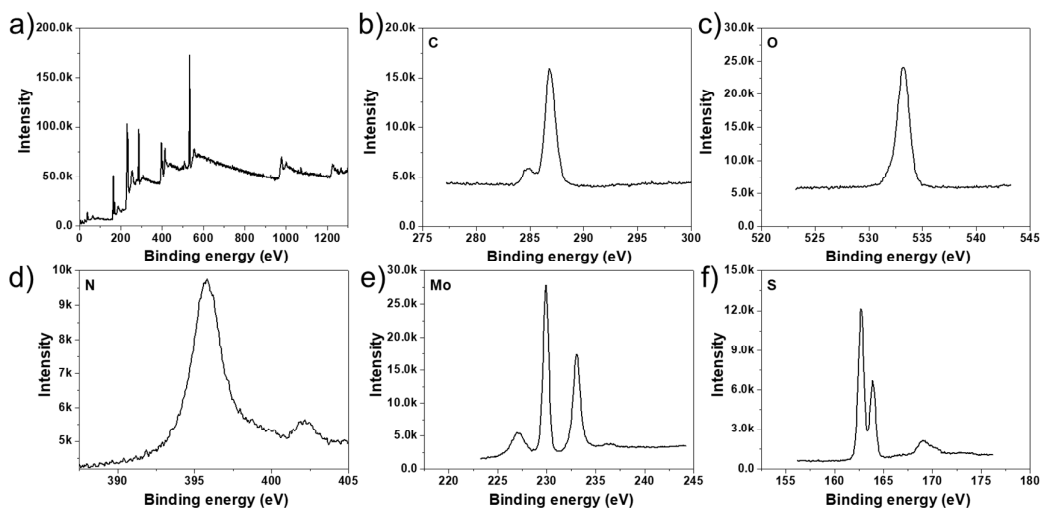


Figure S1. XPS spectra of PEGylated MoS₂/DOX NSs collected from PBS (after 48 h-incubation): (a) full scan, narrow scan for (b) C, (c) O, (d) N, (e) Mo, and (f) S peaks.

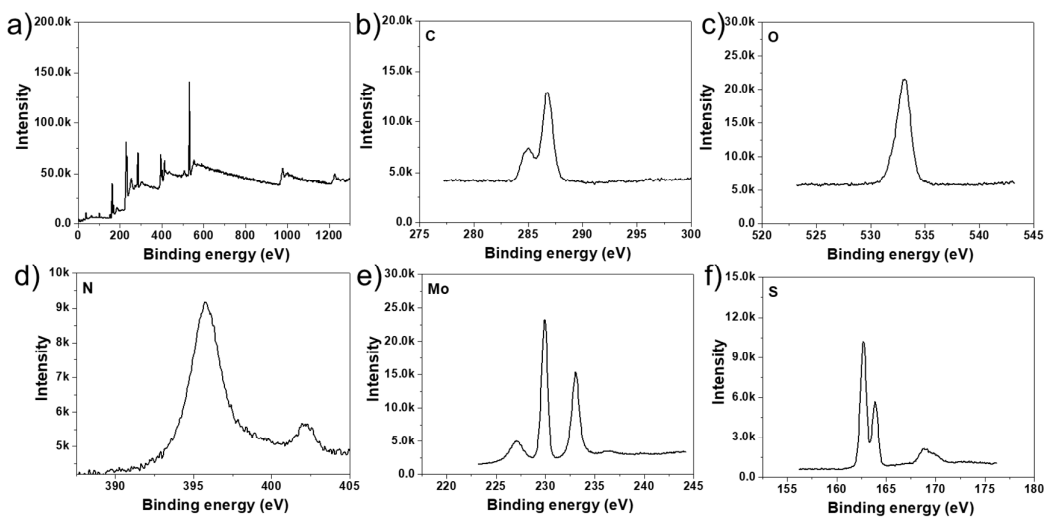


Figure S2. XPS spectra of PEGylated MoS₂/DOX NSs collected from cell culture medium (after 48 h-incubation): (a) full scan, narrow scan for (b) C, (c) O, (d) N, (e) Mo, and (f) S peaks.

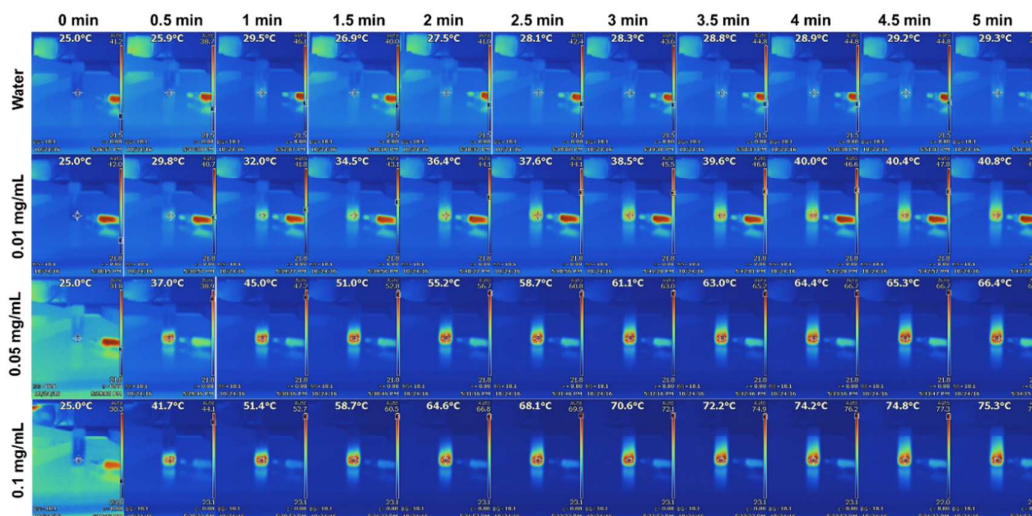


Figure S3. IR thermal images of PEGylated MoS₂ NSs solution with different concentrations (0.01, 0.05, and 0.1 mg/ml) under NIR laser (808 nm, 1 W cm⁻²); irradiation recorded by an IR camera.

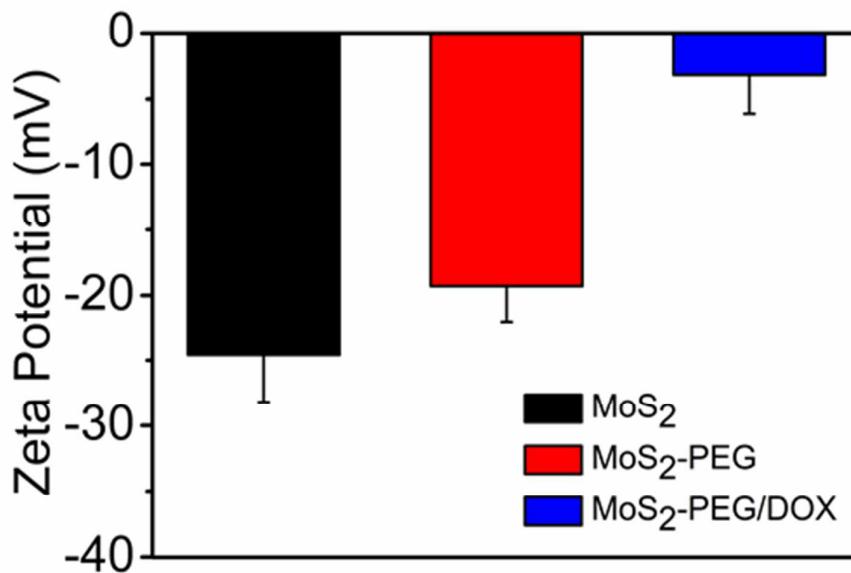


Figure S4. Zeta potential of MoS₂ NSs after PEG coating and DOX loading.

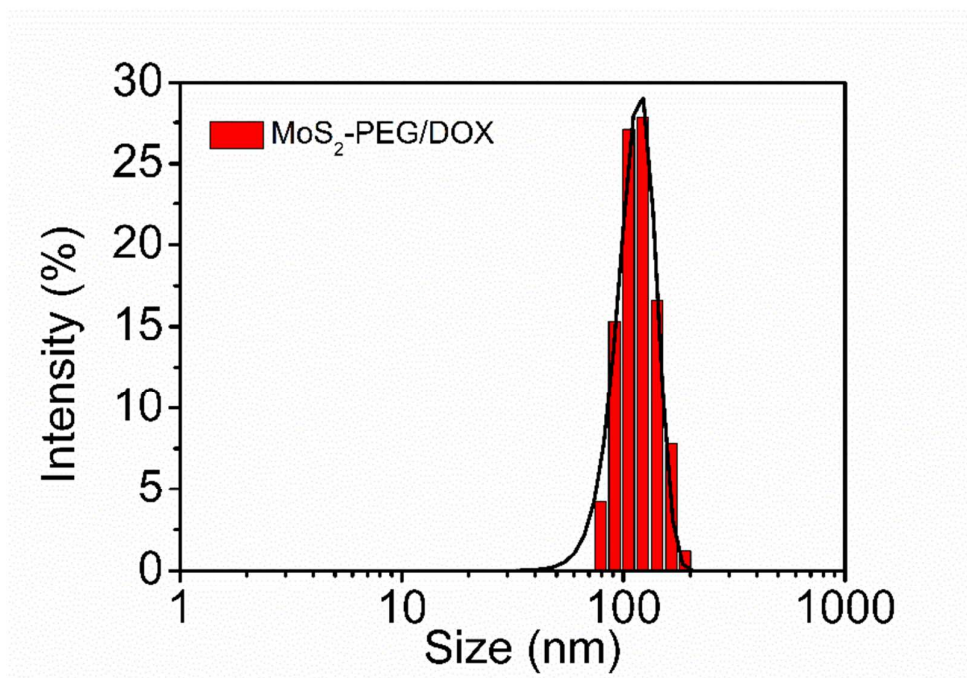


Figure S5. DLS hydrodynamic size distribution of MoS₂-PEG/DOX NSs.

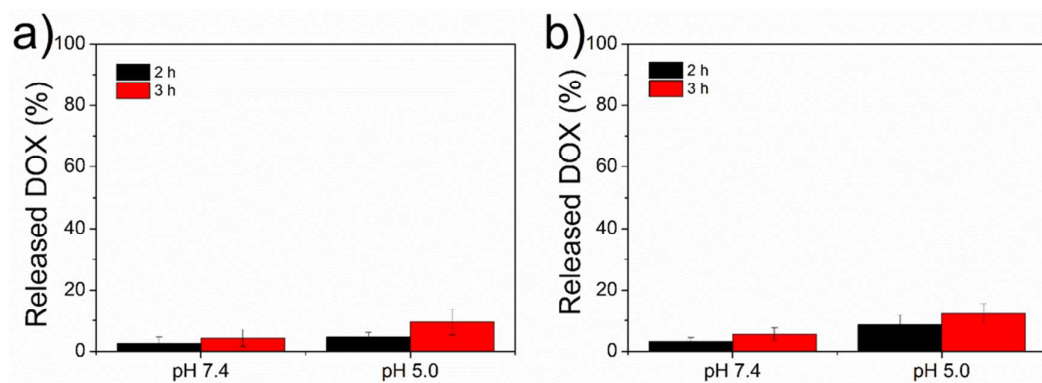


Figure S6. The released DOX percentage from fluorescent NSs at pH 7.4 and 5.0 in (a) cell culture medium and (b) serum after 2 and 3 h incubation.

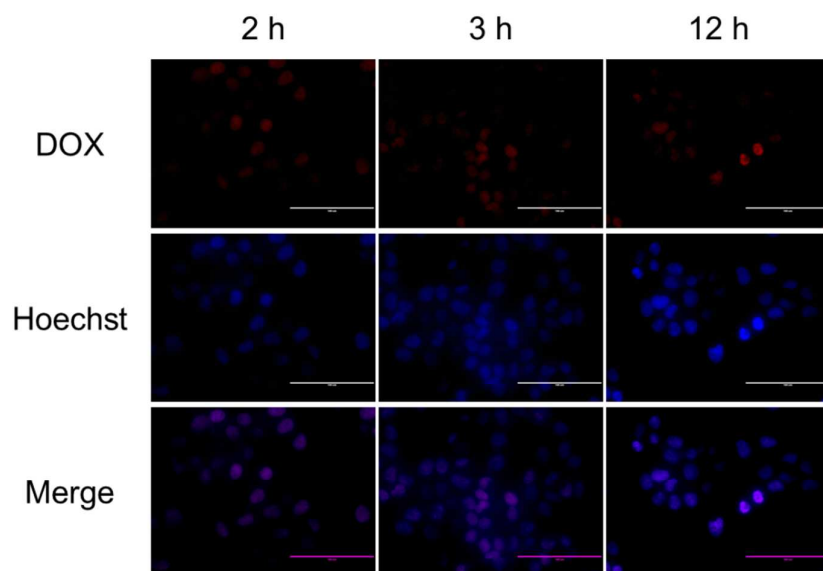


Figure S7. Confocal images of MCF-7 cells after incubation with free DOX molecules taken at different time points. Cell nuclei were stained by Hoechst (blue) and all fluorescence of the free DOX (red) was observed in the nuclei.

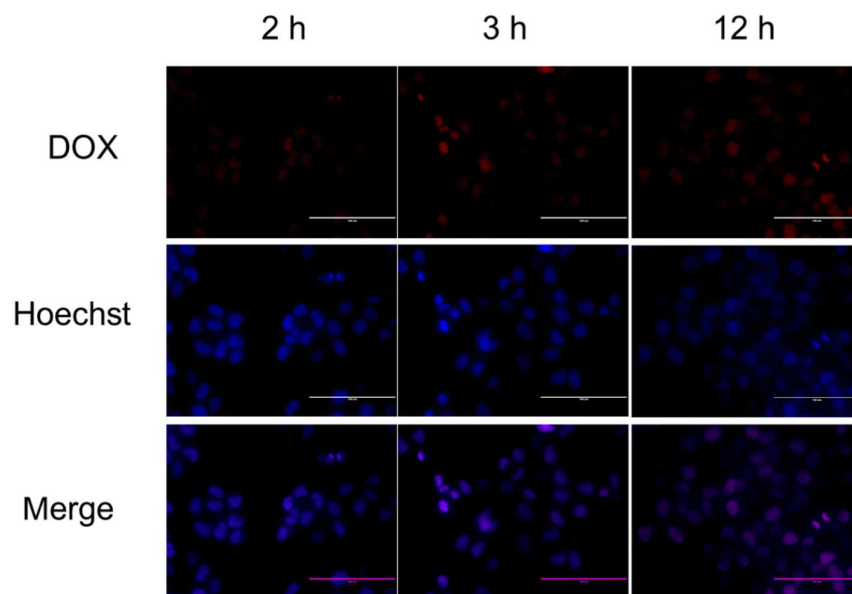


Figure S8. Confocal images of HeLa cells after incubation with free DOX molecules taken at different time points. Cell nuclei were stained by Hoechst (blue) and all fluorescence of the free DOX (red) was observed in the nuclei.

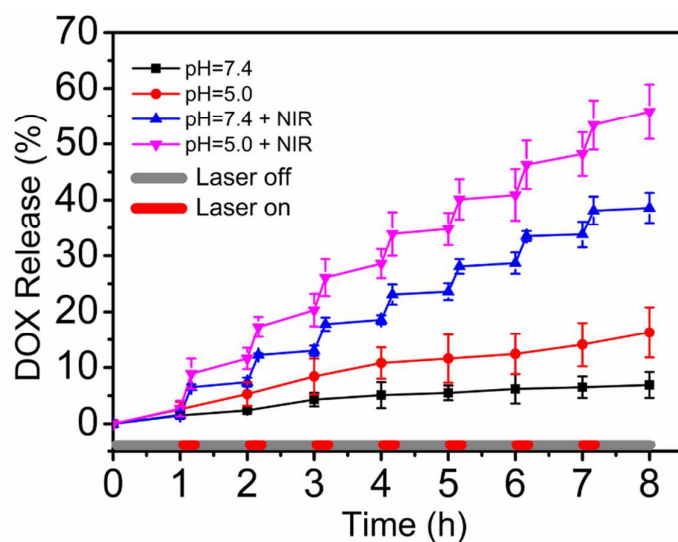


Figure S9. Release profile of DOX in PBS buffer in the absence or presence of 808 nm NIR laser.

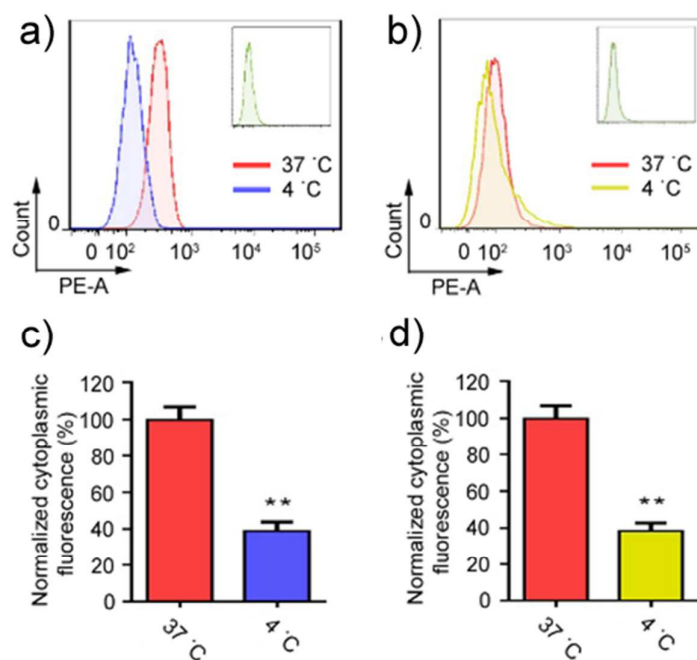


Figure S10. Effect of temperature (37 °C and 4 °C) on MoS₂-based NSs (10 µg/ml) uptake after 2 h-incubation with (a) HeLa cells and (b) MCF-7 cells, measured by flow cytometer. Statistical analysis of cytoplasmic fluorescence in (c) HeLa cells and (d) MCF-7 cells incubated at 37 °C and 4 °C respectively.

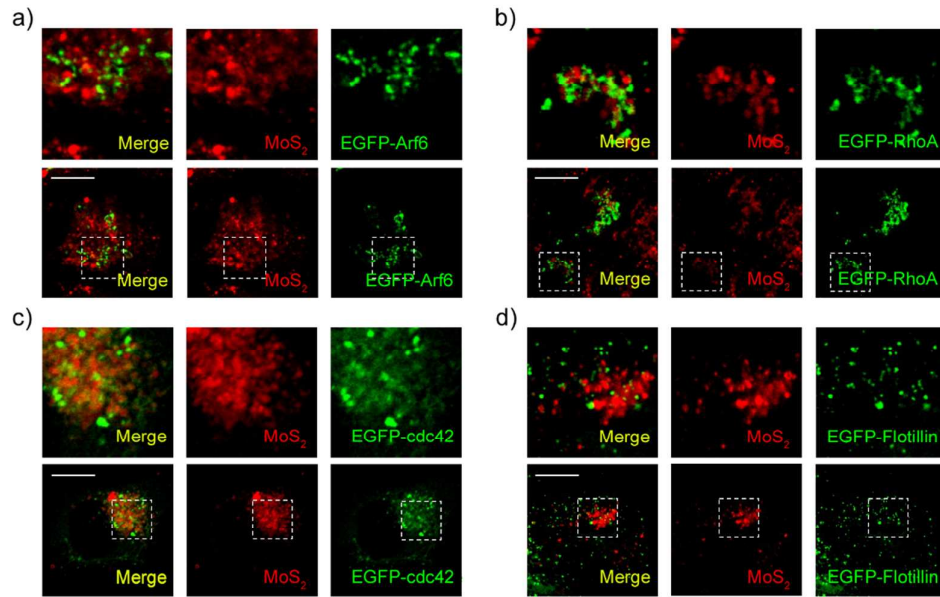


Figure S11. Confocal images of (a) EGFP-Arf6, (b) EGFP-RhoA, (c) EGFP-cdc42, (d) EGFP-Flotillin transfected HeLa cells incubated with fluorescent NSs (10 $\mu\text{g/ml}$) for 2 h. Scale bars: 10 μm .

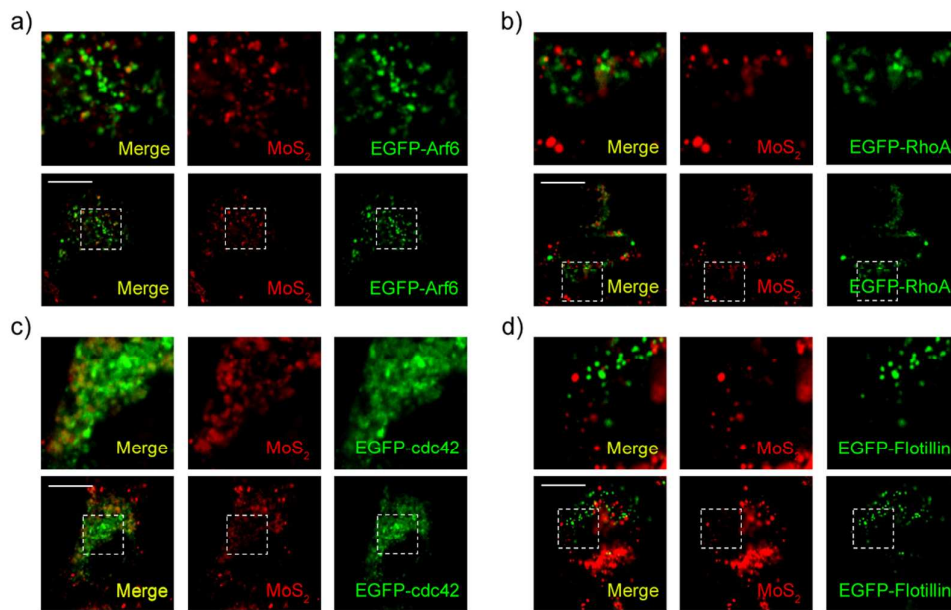


Figure S12. Confocal images of (a) EGFP-Arf6, (b) EGFP-RhoA, (c) EGFP-cdc42, (d) EGFP-Flotillin transfected MCF-7 cells incubated with fluorescent NSs (10 $\mu\text{g/ml}$) for 2 h. Scale bars: 10 μm .

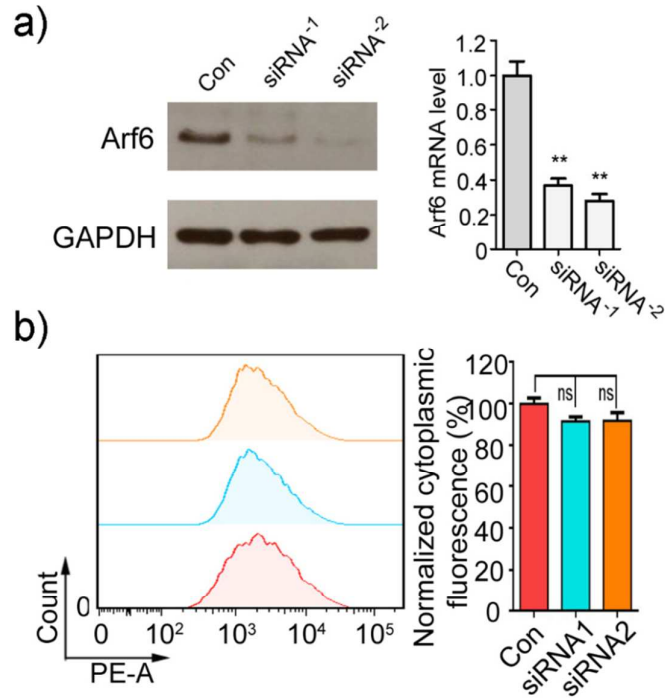


Figure S13. (a) Cells were pretreated with siRNA against Arf6 for 48 h, and then protein and mRNA levels were examined including longer exposure (i.e.). (b) Cells were pretreated with siRNA against Arf6 for 48 h, and then incubated with fluorescent MoS₂-based NSs (10 μ g/ml) for 2 h. Cytoplasmic fluorescence was measured by flow cytometer.

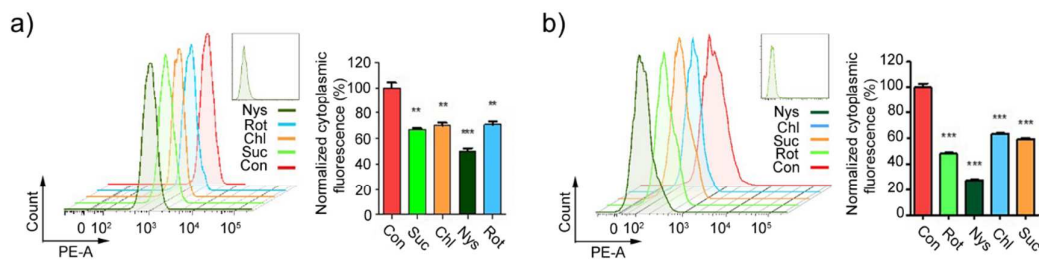


Figure S14. Cells were pretreated with indicated inhibitors (nystatin, 100 μ g/ml; sucrose, 100 mg/ml; chlorpromazine, 8 μ g/ml; rottlerin, 2.6 μ g/ml) for 2 h and then incubated with fluorescent MoS₂-based NSs (10 μ g/ml) for another 3 h. The cytoplasmic fluorescence was measured by flow cytometer in (a) HeLa and (b) MCF-7 cells.

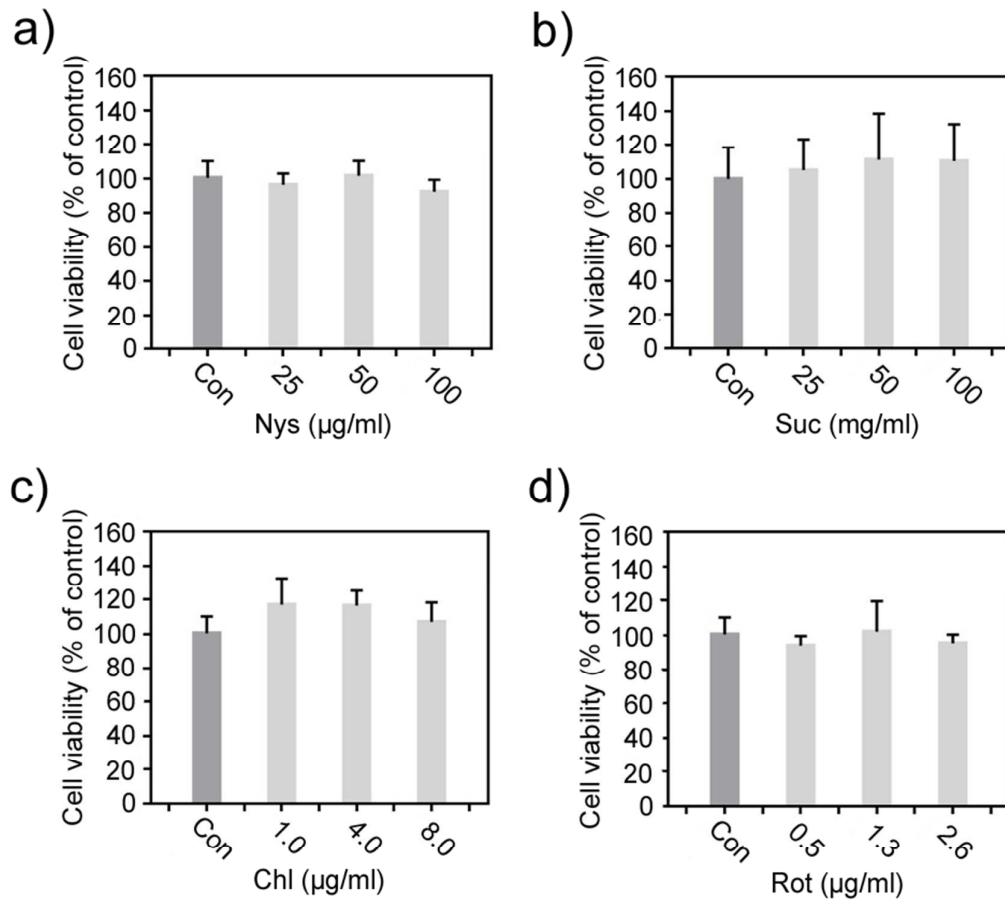


Figure S15. Metabolic activity of HeLa cells after 2 h incubation with different endocytosis inhibitors measured by MTT assay. (a) Nystatin, (b) Sucrose, (c) Chlorpromazine, (d) Rottlerin.

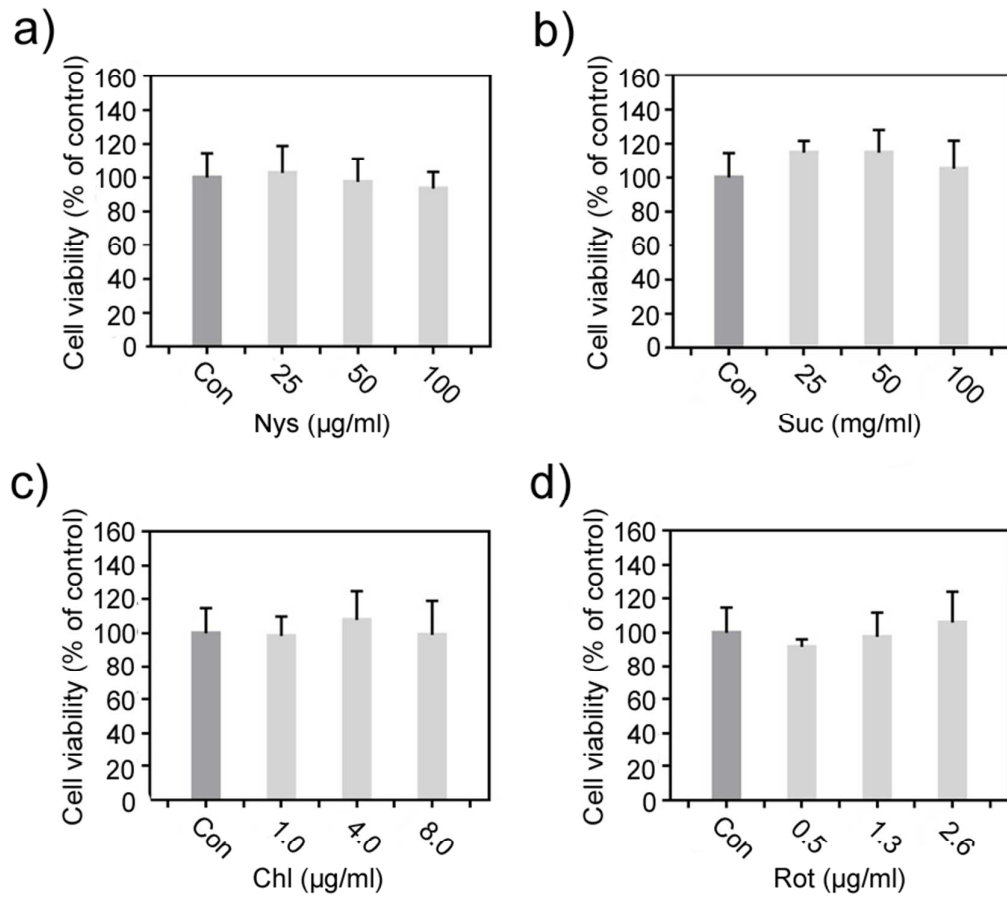


Figure S16. Metabolic activity of MCF-7 cells after 2 h of incubation with different endocytosis inhibitors measured by MTT assay. (a) Nystatin, (b) Sucrose, (c) Chlorpromazine, (d) Rottlerin.

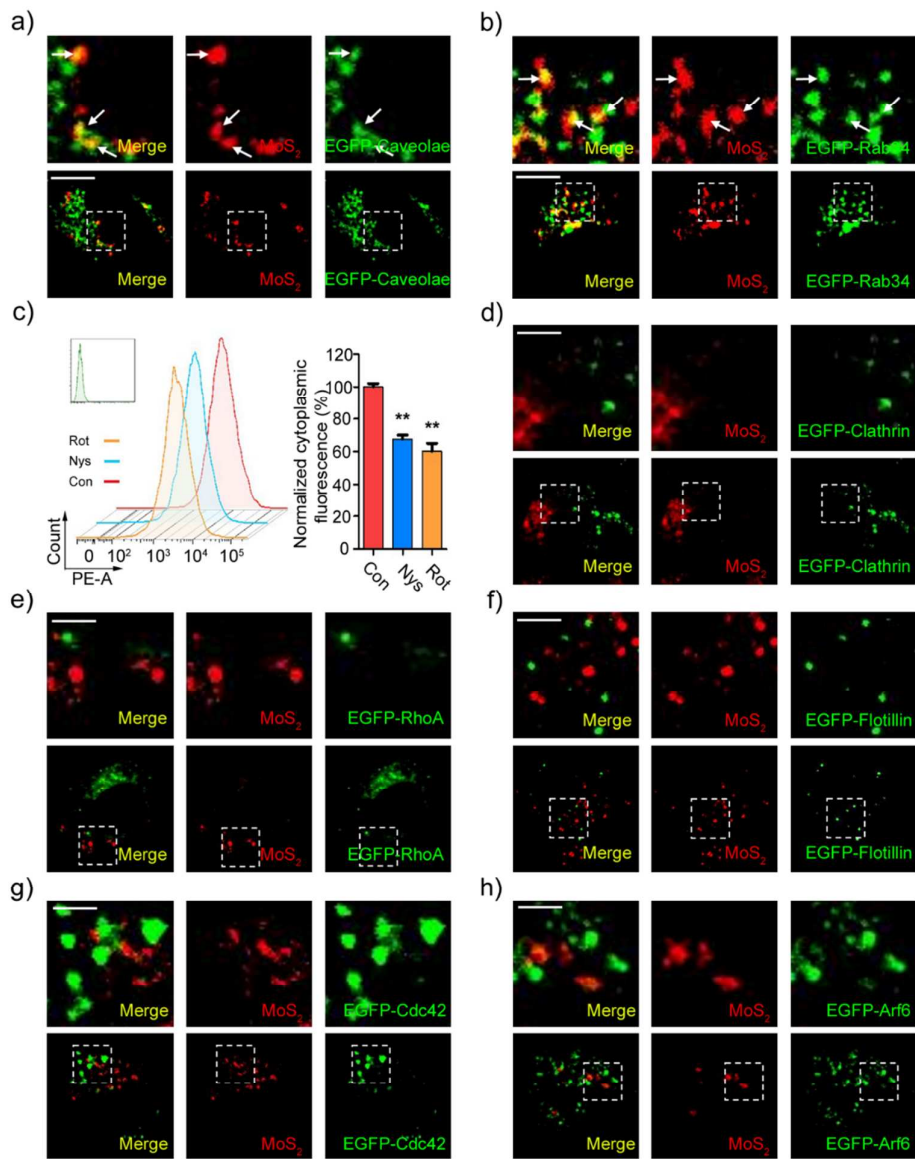


Figure S17. Confocal images of (a) EGFP-Caveolae and (b) EGFP-Rab34 transfected HAOEC incubated with fluorescent MoS₂-based NSs (10 µg/ml) for 2 h. (c) Cells were pretreated with indicated inhibitors (nystatin, 100 µg/ml; rottlerin, 2.6 µg/ml) for 2 h and then incubated with fluorescent MoS₂-based NSs (10 µg/ml) for another 3 h. Cytoplasmic fluorescence was measured by flow cytometer. Confocal images of (d) EGFP-Clathrin, (e) EGFP-RhoA, (f) EGFP-Flotillin, (g) EGFP-Cdc42 and (h) EGFP-Arf6 transfected HAOEC incubated with fluorescent MoS₂-based NSs (10 µg/ml) for 2 h. (Scale bar: 10 µm)

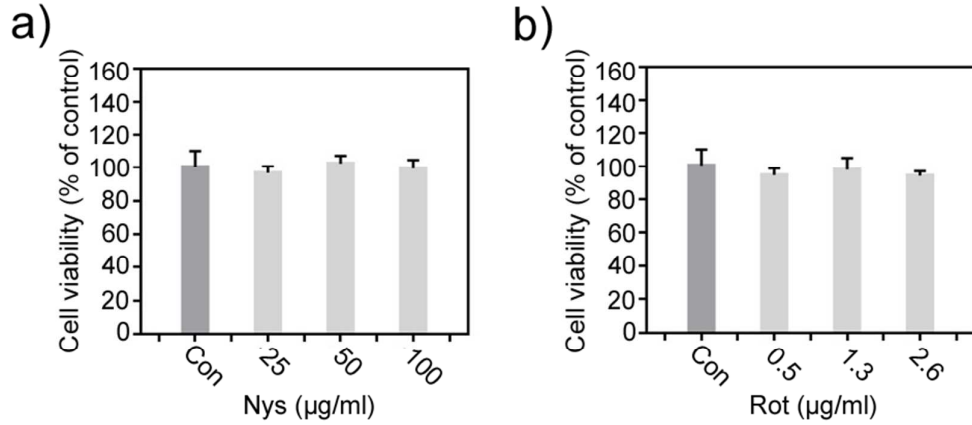


Figure S18. Metabolic activity of HAOEC after 2 h incubation with different pharmacological endocytosis inhibitors measured by MTT assay. (a) Nystatin, (b) Rottlerin.

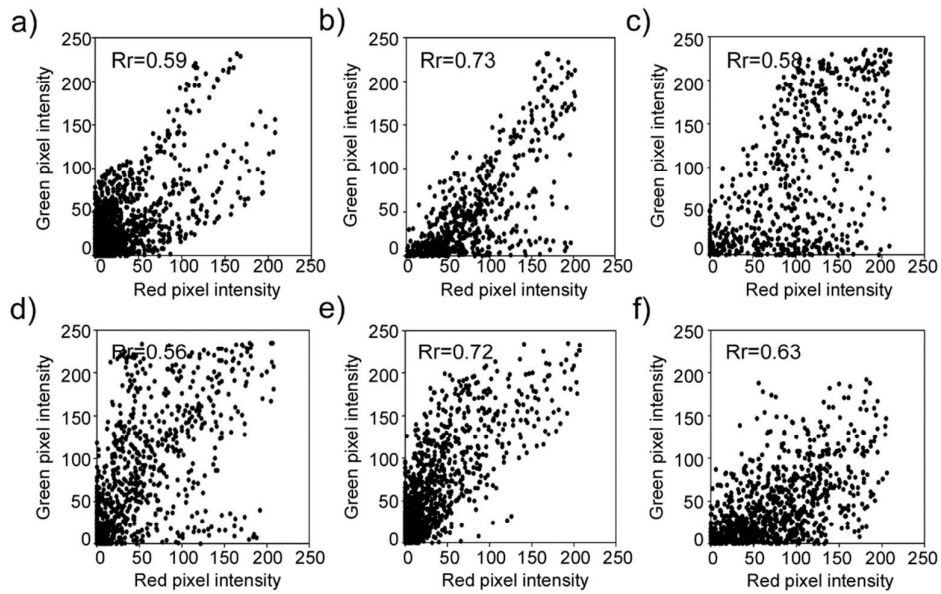


Figure S19. Scatterplot of red and green pixel intensities of the cells shown in (a) Figure 4c, (b) Figure 4d, (c) Figure 4e, (d) Figure 4f, (e) Figure 4g, (f) Figure 4h.

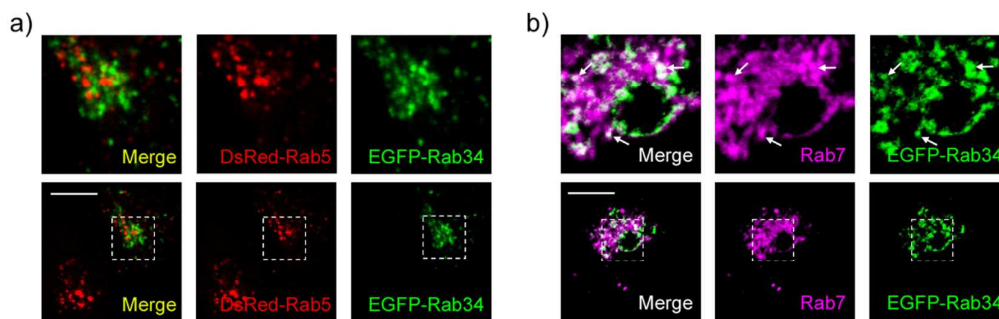


Figure S20. Confocal images of MCF-7 cells. (a) MCF-7 cells were co-transfected with EGFP-Rab34 and DsRed-Rab5. (b) The Rab7 was detected in the EGFP-Rab34 transfected Hela cells with the antibody against Rab7. Scale bars: 10 μm .

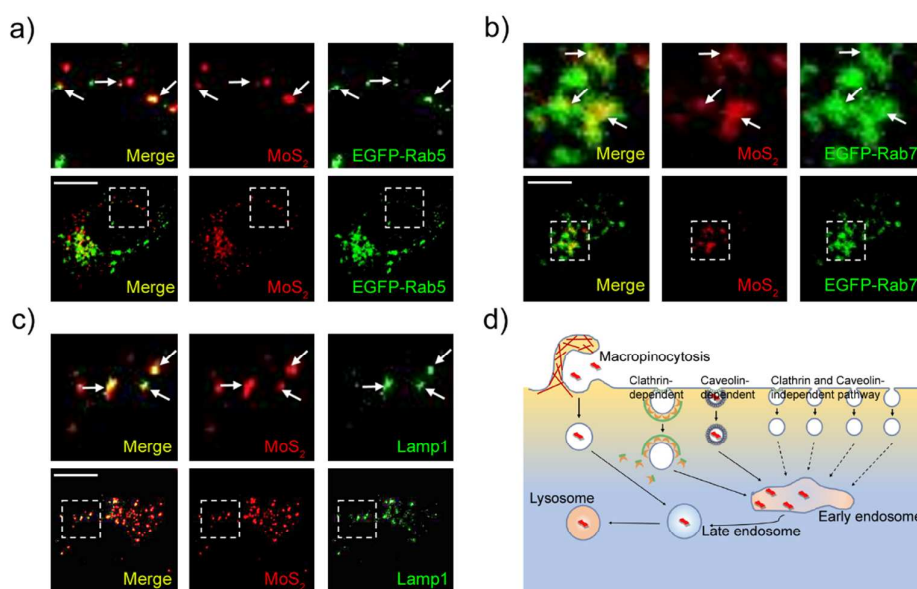


Figure S21. Confocal images of (a) EGFP-Rab5 and (b) EGFP-Rab7 transfected HAOEC incubated with fluorescent MoS₂-based NSs (10 $\mu\text{g}/\text{ml}$) for 2 h. (c) Confocal images of HAOEC, which were incubated with fluorescent MoS₂-based NSs (10 $\mu\text{g}/\text{ml}$) for 2 h, and then an immunofluorescence experiment was performed with the primary antibody against Lamp1. (d) Schematic representation of the routes through which the MoS₂-based NSs enter the cell and accumulate in lysosomes. (Scale bar: 10 μm)

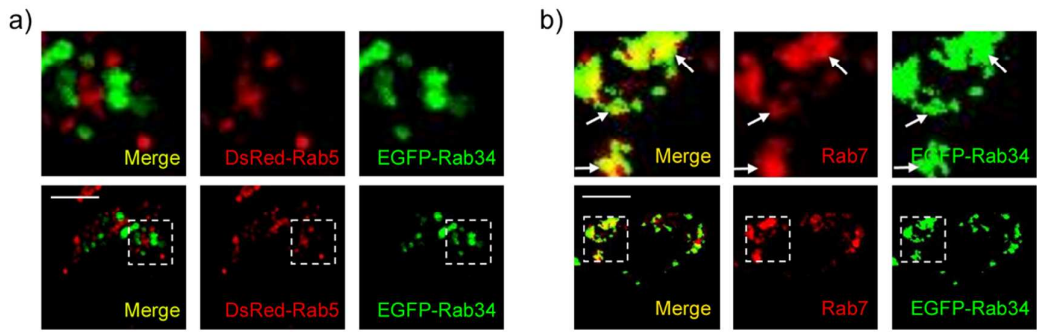


Figure S22. (a) Confocal images of EGFP-Rab34- and DsRed-Rab5-transfected HAOEC cells incubated with MoS₂ (10 μg/ml) for 2 h. (b) Confocal images of EGFP-Rab34- and DsRed-Rab7-transfected HAOEC cells incubated with MoS₂ (10 μg/ml) for 2 h.

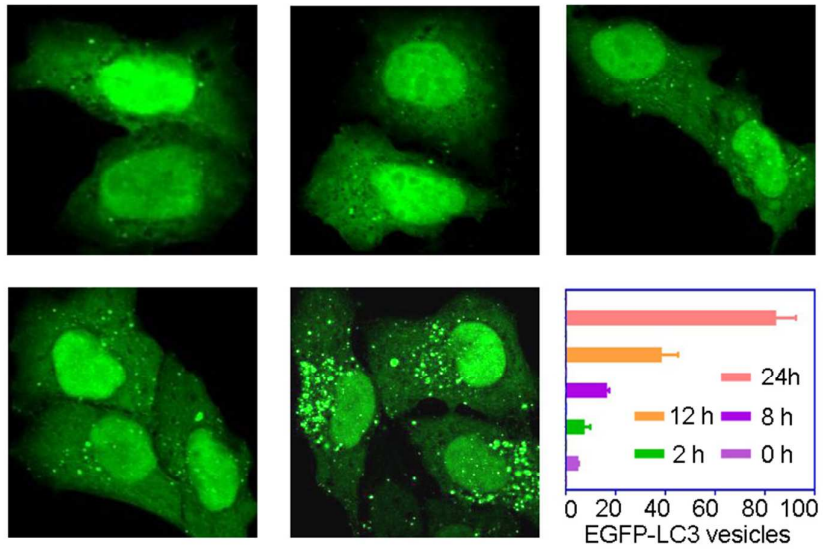


Figure S23. Representative images and quantification of MCF-7 with EGFP-LC3 vesicles (autophagosomes). EGFP-LC3-transfected cells were treated with MoS₂ NSs (10 μg/ml) for the indicated times.

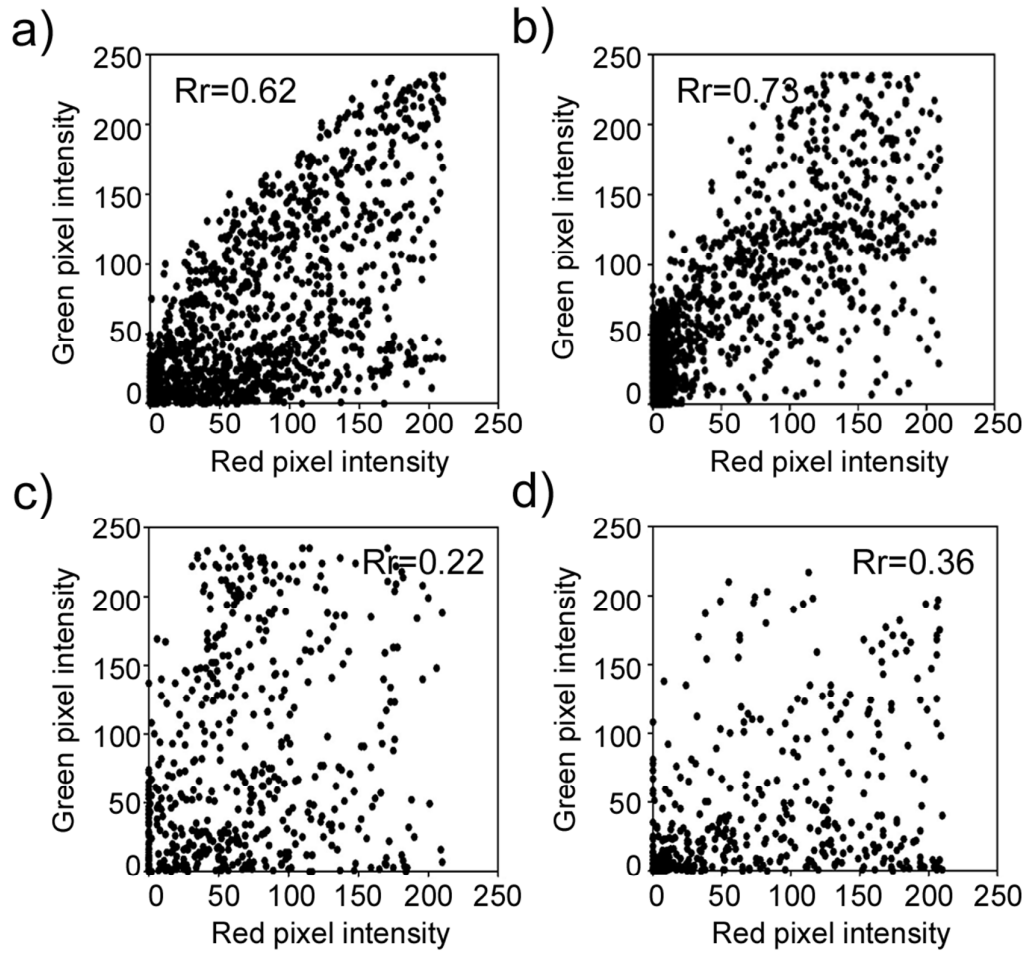


Figure S24. Scatterplot of red and green pixel intensities of the cells shown in (a) Figure 5a, (b) Figure 5b, (c) Figure 5c, and (d) Figure 5d.

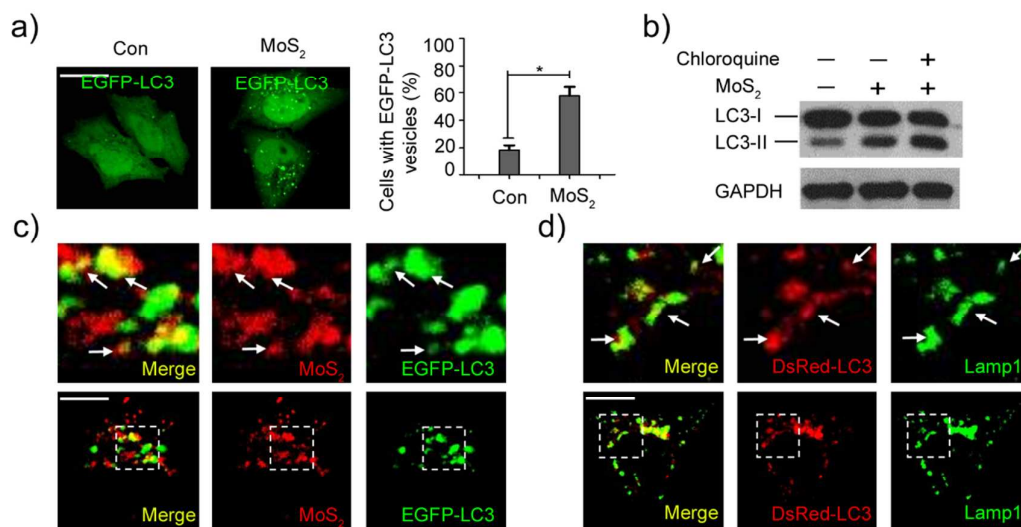


Figure S25. (a) Representative images and quantification of HAOEC with EGFP-LC3 vesicles (autophagosomes). EGFP-LC3-transfected cells were treated with MoS₂ NSs (10 µg/ml) for 24 h. (b) LC3I/II protein levels were analyzed by western blotting in cells treated with MoS₂ NSs (10 µg/ml) for 24 h. (c) Confocal images of DsRed-LC3-transfected HAOEC incubated with fluorescent MoS₂-based NSs (10 µg/ml) for 24 h. (d) Confocal images of EGFP-LC3-transfected HAOEC incubated with fluorescent MoS₂-based NSs (10 µg/ml) for 24 h. Lysosome was detected by Lyso-Tracker. (Scale bar: 10 µm)

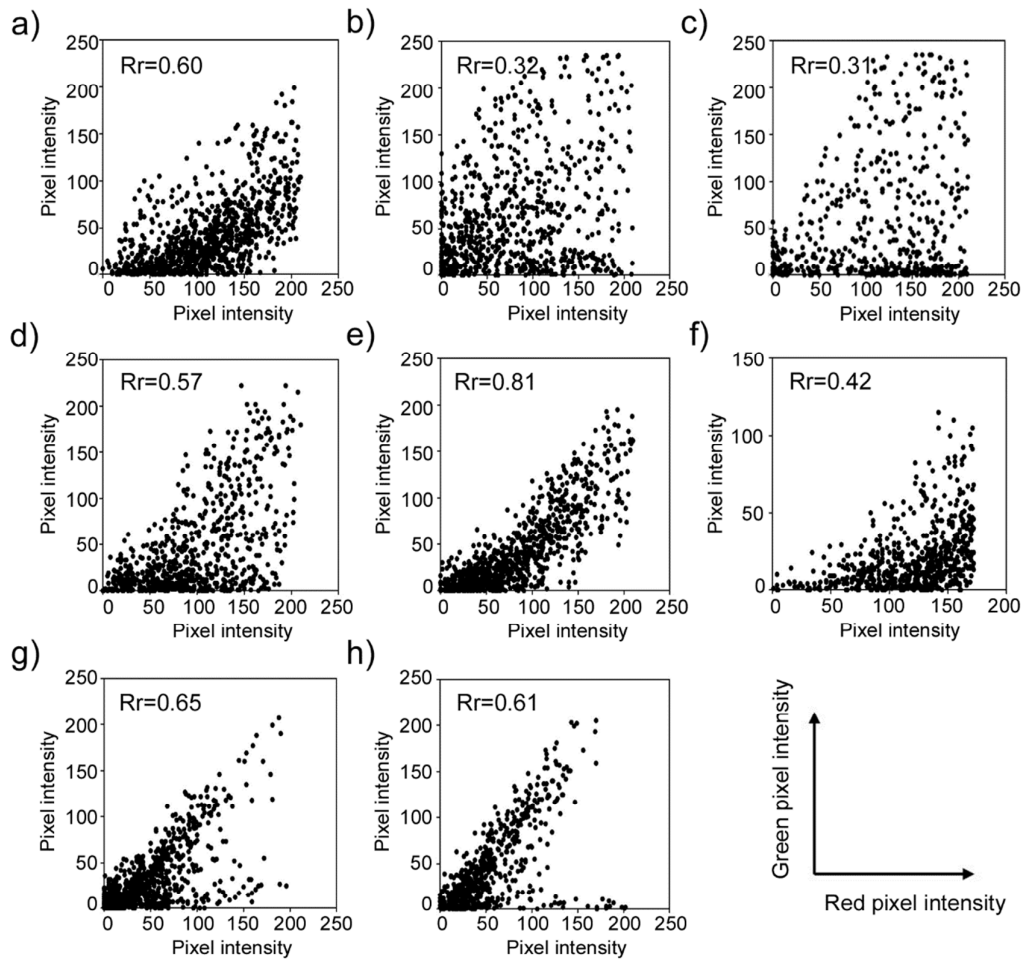


Figure S26. Scatterplot of red and green pixel intensities of the cells shown in (a) Figure 6a, (b) Figure 6b, (c) Figure 6c, (d) Figure 6d, (e) Figure 6e, (f) Figure 6f, (g) Figure 6g, and (h) Figure 6h.

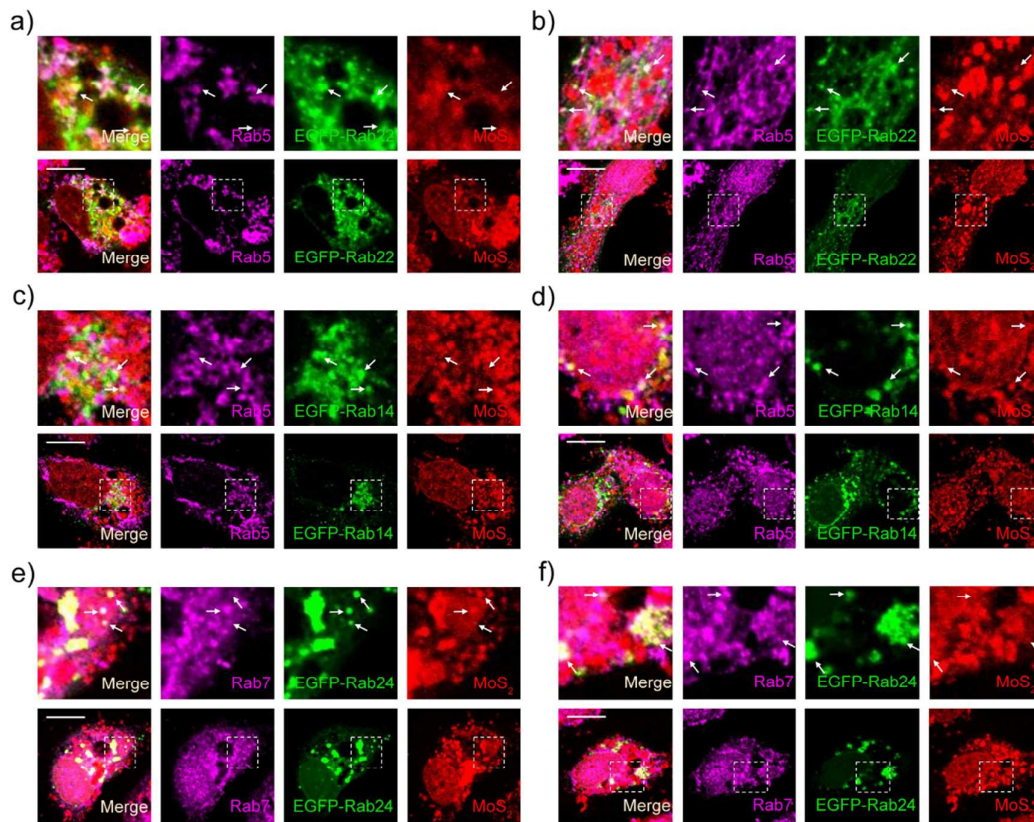


Figure S27. Exocytosis of MoS₂-based NSs. The EGFP-Rab22 transfected (a) HeLa and (b) MCF-7 cells were incubated with fluorescent NSs (10 μg/ml) for 2 h. Rab5 was detected with the antibody against Rab5. The EGFP-Rab14 transfected (c) HeLa and (d) MCF-7 cells were incubated with fluorescent NSs (10 μg/ml) for 2 h. Rab5 was detected with the antibody against Rab5. The EGFP-Rab24-transfected (a) HeLa and (b) MCF-7 cells were incubated with fluorescent NSs (10 μg/ml) for 2 h. Rab7 was detected with the antibody against Rab7. Scale bars: 10 μm.

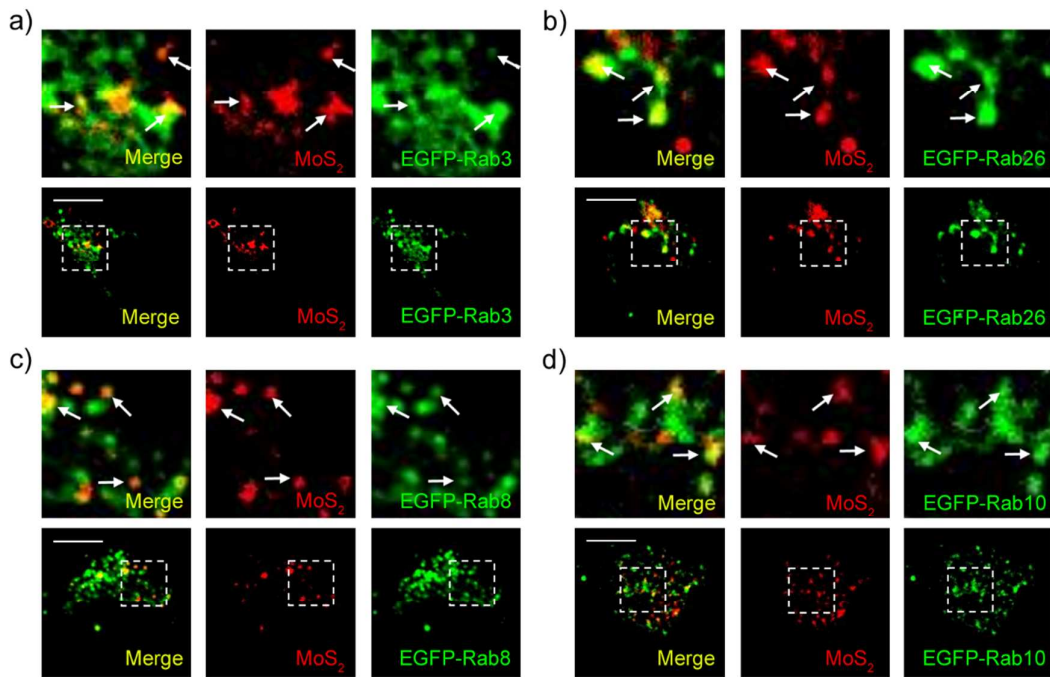


Figure S28. (a) Confocal images of EGFP-Rab3-transfected HAOEC incubated with fluorescent MoS₂-based NSs (10 $\mu\text{g}/\text{ml}$) for 2 h. (b) Confocal images of EGFP-Rab26-transfected HAOEC incubated with fluorescent MoS₂-based NSs (10 $\mu\text{g}/\text{ml}$) for 2 h. (c) Confocal images of EGFP-Rab8-transfected HAOEC incubated with fluorescent MoS₂-based NSs (10 $\mu\text{g}/\text{ml}$) for 2 h. (d) Confocal images of EGFP-Rab10-transfected HAOEC incubated with fluorescent MoS₂-based NSs (10 $\mu\text{g}/\text{ml}$) for 2 h. (Scale bar: 10 μm)

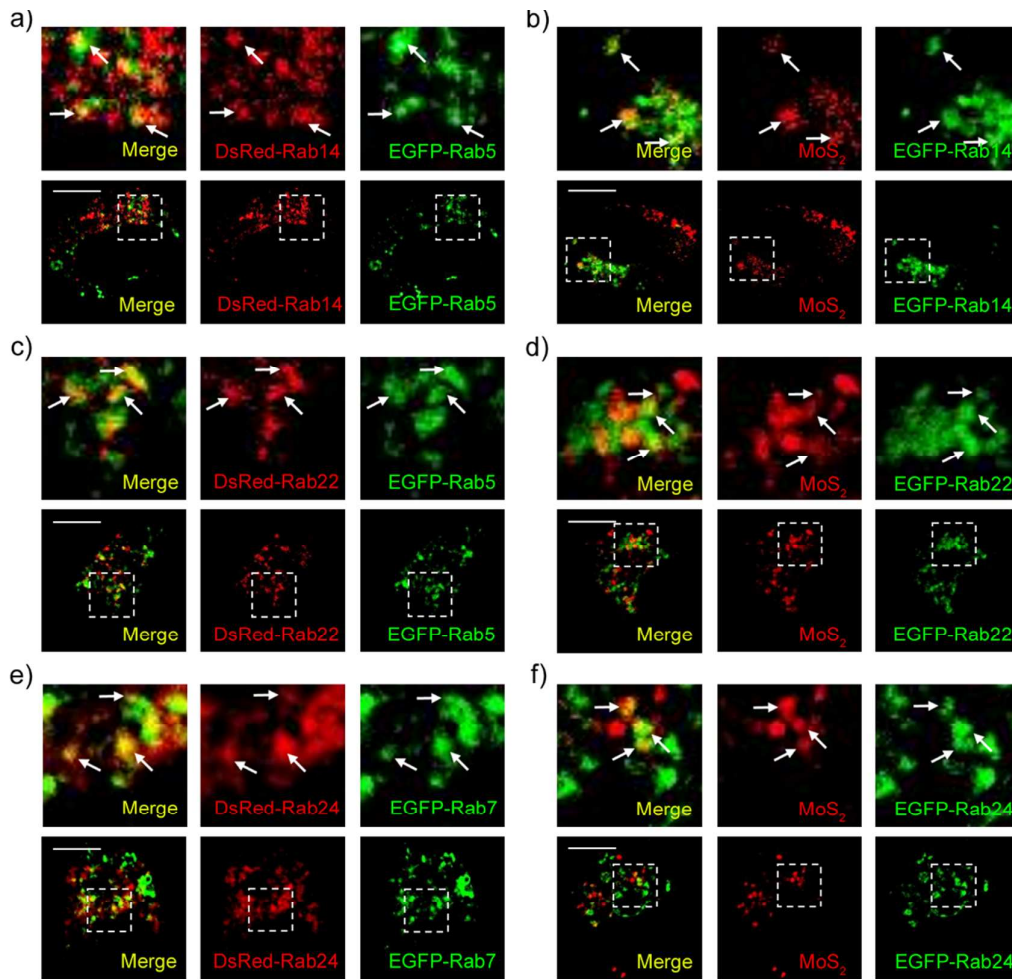


Figure S29. (a) Confocal images of EGFP-Rab5 and DsRed-Rab14 transfected HAOEC incubated with MoS₂ NSs (10 $\mu\text{g/ml}$) for 2 h. (b) Confocal images of EGFP-Rab14-transfected HAOEC incubated with fluorescent MoS₂-based NSs (10 $\mu\text{g/ml}$) for 2 h. (c) Confocal images of EGFP-Rab7- and DsRed-Rab24-transfected HAOEC incubated with MoS₂ (10 $\mu\text{g/ml}$) for 2 h. (d) Confocal images of EGFP-Rab24-transfected HAOEC incubated with fluorescent MoS₂-based NSs (10 $\mu\text{g/ml}$) for 2 h. (Scale bar: 10 μm)

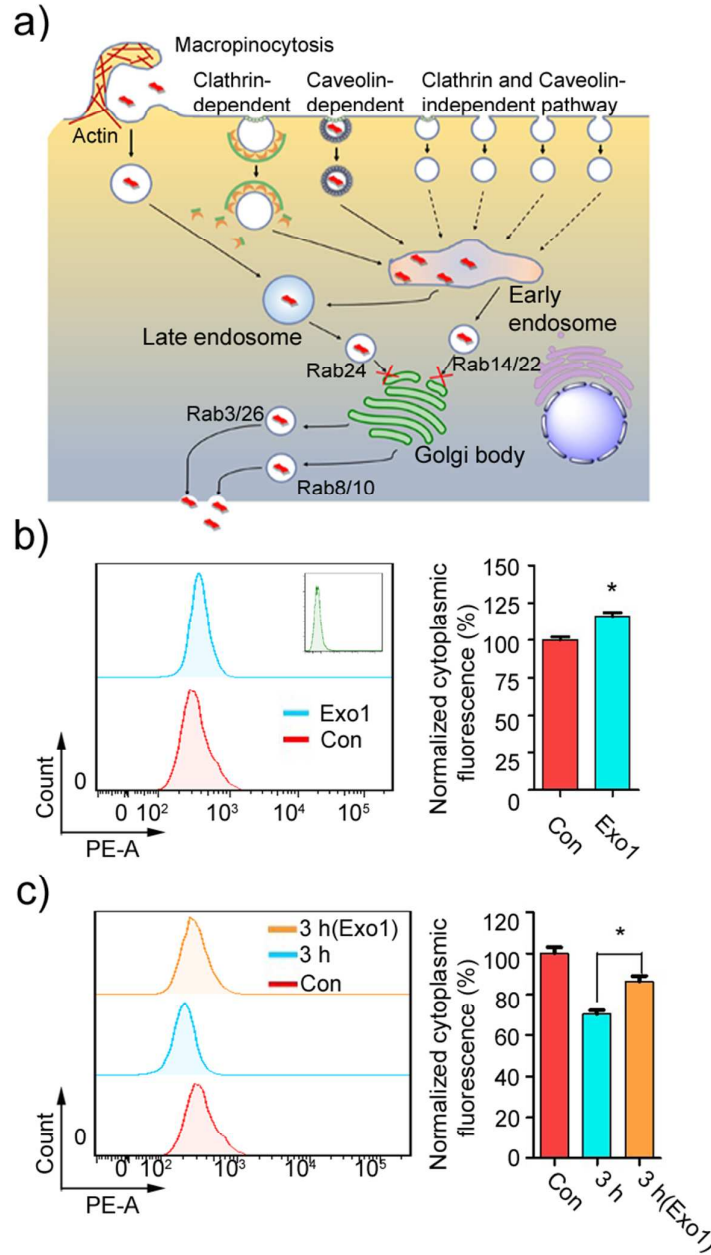


Figure S30. Schematic representation of exocytosis pathways in (a) HAOEC cells. (b) HAOEC cells were pretreated with Exo1 for 2 h. Then cells were incubated with fluorescent MoS₂-based NSs (10 μg/ml) for 3 h. Cytoplasmic fluorescence was measured by flow cytometer. (c) HAOEC cells were incubated in the presence or absence of Exo1 for 2 h after fluorescent MoS₂-based NS (10 μg/ml) treatment. After that, we renewed the culture medium with fresh DMEM and incubated the cells for 3 h. Cytoplasmic fluorescence was measured by flow cytometer.

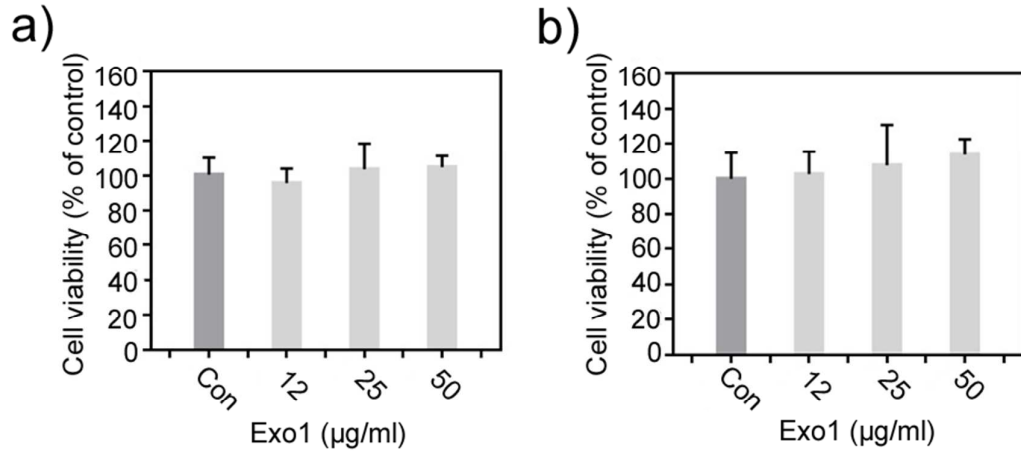


Figure S31. Metabolic activity of (a) HeLa and (b) MCF-7 cells after 2 h of incubation with pharmacological exocytosis inhibitors measured by MTT assay.

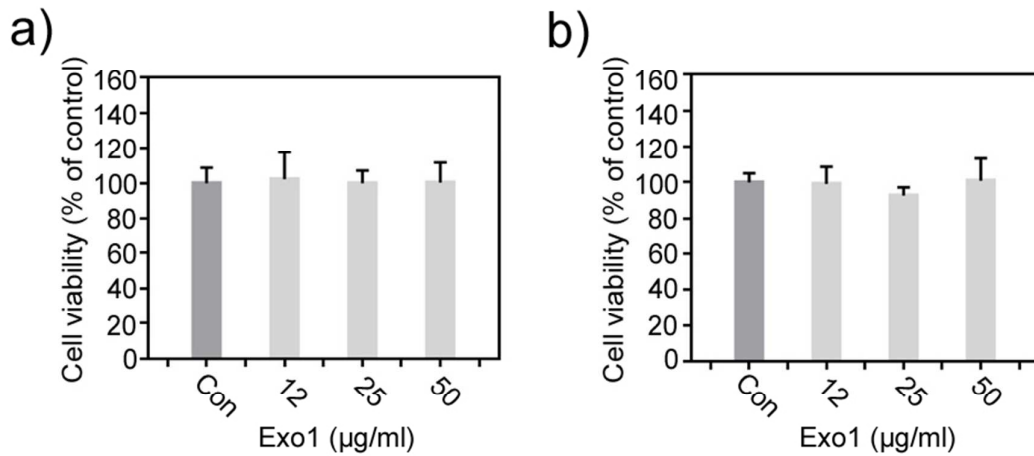


Figure S32. Metabolic activity of MCF-7 cells after (a) 24 h and (b) 48 h of incubation with Exo1 measured by MTT assay.

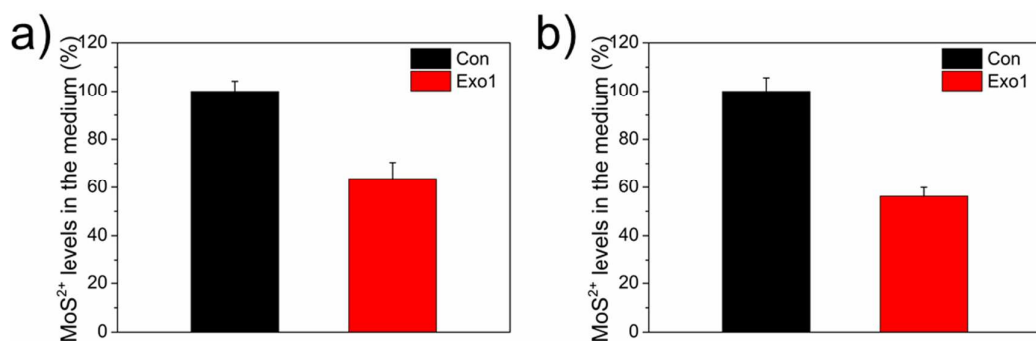


Figure S33. Mo²⁺ levels in medium after treating with (a) HeLa and (b) MCF-7 cells.

Both cell lines were treated with PEGylated MoS₂ NSs in the presence or absence of Exo1 for 2 h. Soon afterwards, the cell culture medium was renewed with fresh medium and the cells were incubated for another 3 h. We then tested the concentration of these NSs by measuring the Mo²⁺ levels in the medium through inductively coupled plasma atomic emission spectroscopy (ICP-AES).

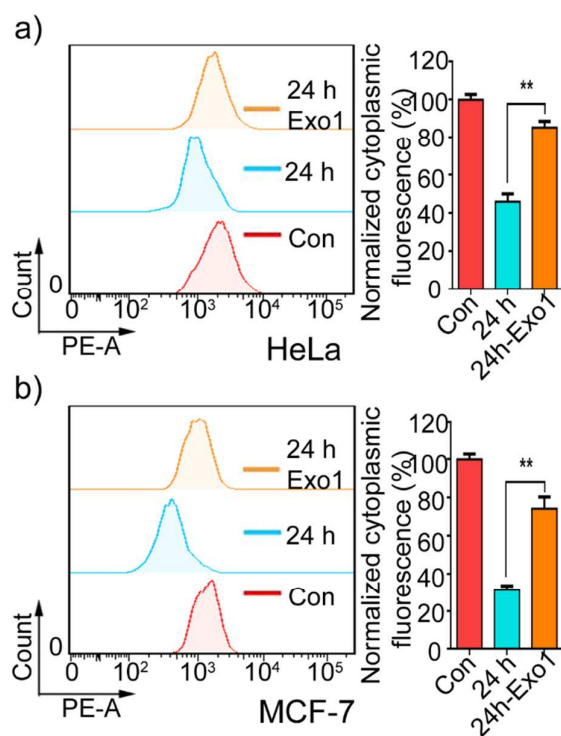


Figure S34. (a) HeLa and (b) MCF-7 cells were incubated in the presence and absence of Exo1 for 2 h after fluorescent MoS₂-based NS (10 μg/ml) treatment. After that, we renewed the culture medium with fresh DMEM every 3 h and incubated the cells for 24 h. Cytoplasmic fluorescence was measured by flow cytometer.

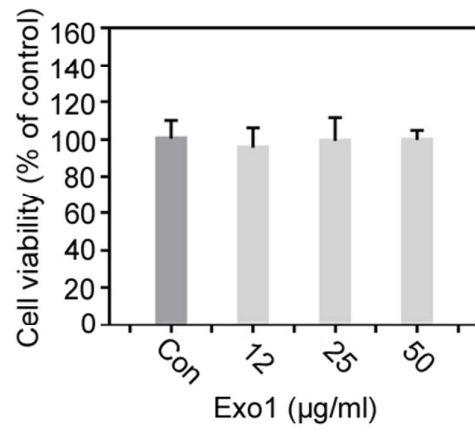


Figure S35 Metabolic activity of HAOEC after 2 h of incubation with Exo1 measured by MTT assay.

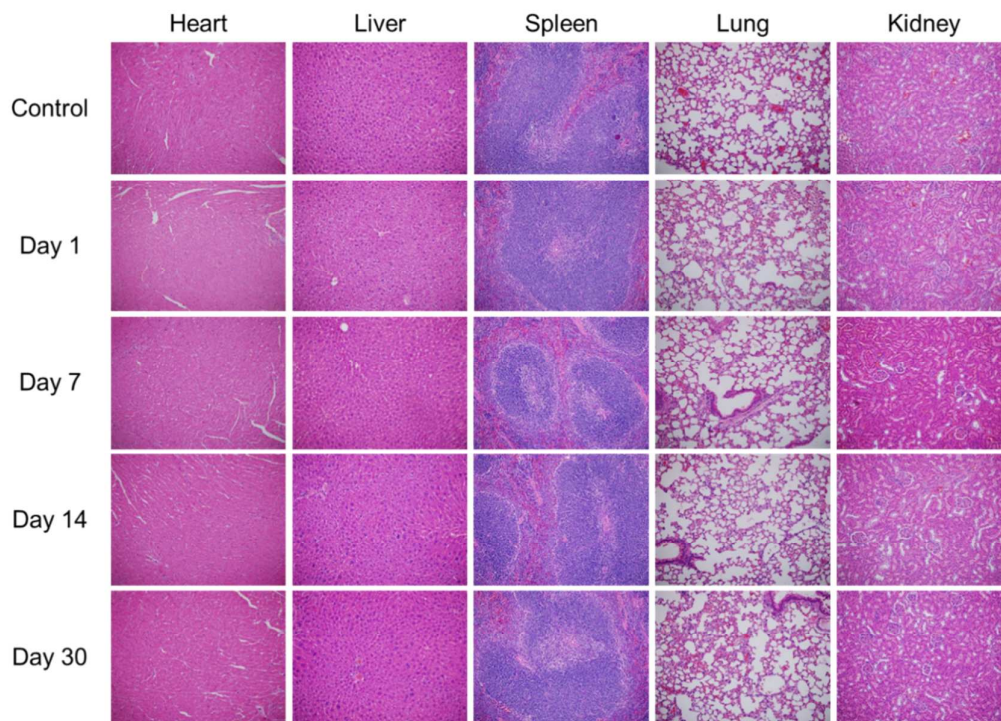


Figure S36. H&E-stained histological images of tissue sections from heart, liver, spleen, lung, and kidney at 1, 7, 14, and 30 days post-intravenous injection with 6 mg/kg of Exo1.

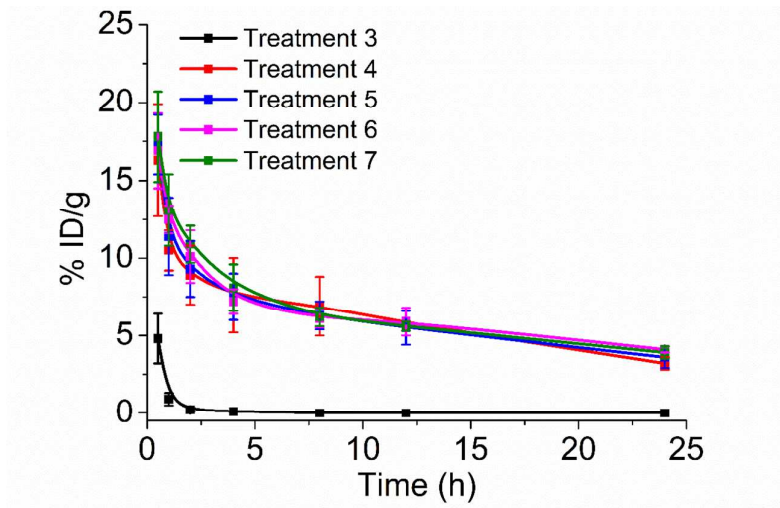


Figure S37. Blood circulation of free DOX (Treatment 3) and PEGylated MoS₂/DOX NSs (Treatments 4-7) after intravenous injection.

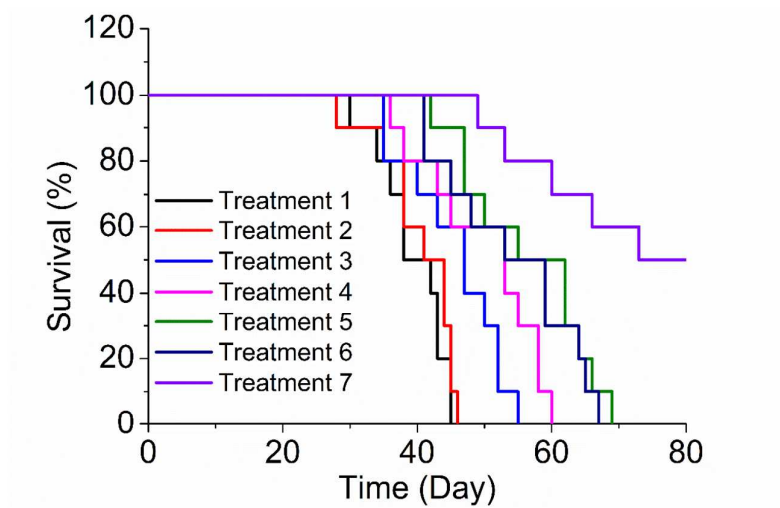


Figure S38. The Kaplan-Meier survival curve of another batch of mice after different treatments (n = 10). Treatment 1: Saline; Treatment 2: Exo1; Treatment 3: DOX; Treatment 4: PEGylated MoS₂/DOX NSs; Treatment 5: PEGylated MoS₂/DOX NSs + Exo1; Treatment 6: PEGylated MoS₂/DOX NSs + NIR; Treatment 7: PEGylated MoS₂/DOX NSs + NIR + Exo1. The doses of PEGylated MoS₂, DOX and Exo1 were 4.01 mg/kg, 4 mg/kg, and 4 mg/kg, respectively. NIR treatment was conducted by irradiating under an 808-nm laser (0.4 W/cm²) for 15 min at the tumor sites.