Supporting information

In situ one-step fluorescence labeling strategy of exosomes *via* bioorthogonal click chemistry for real-time exosome tracking *in vitro* and *in vivo*

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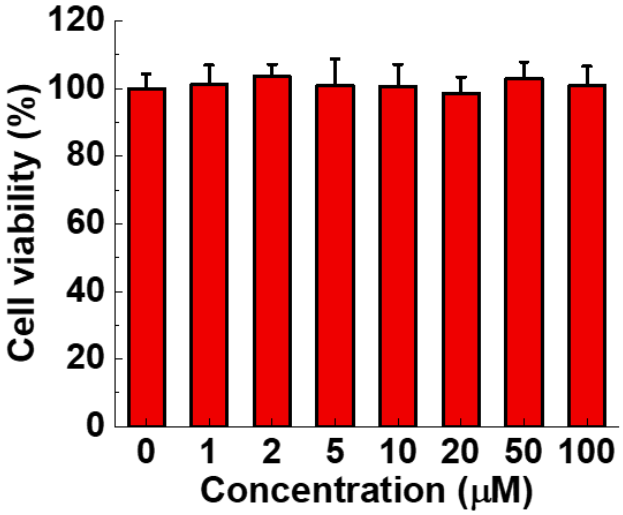
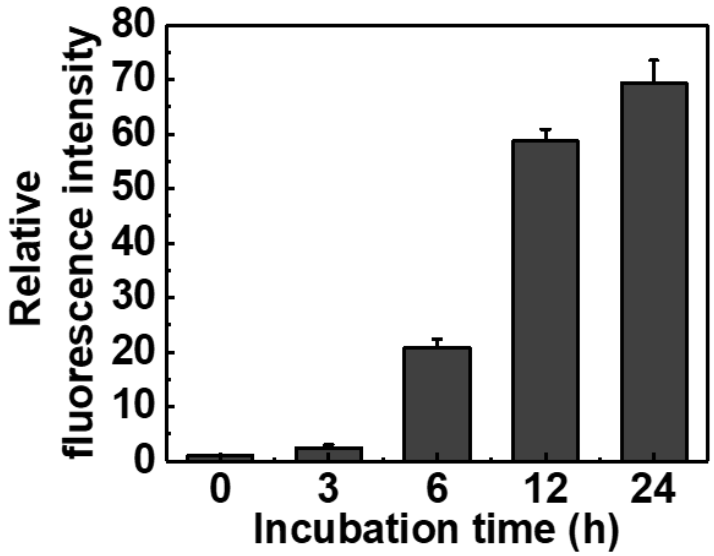
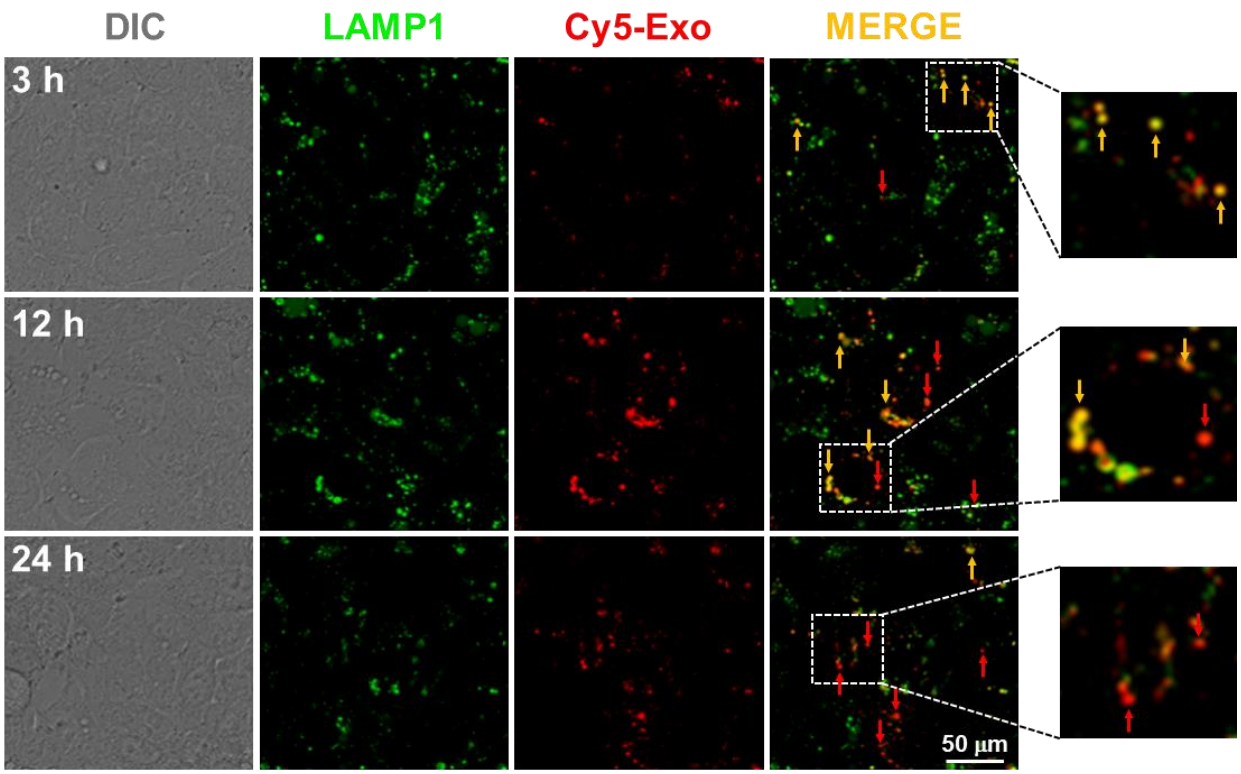


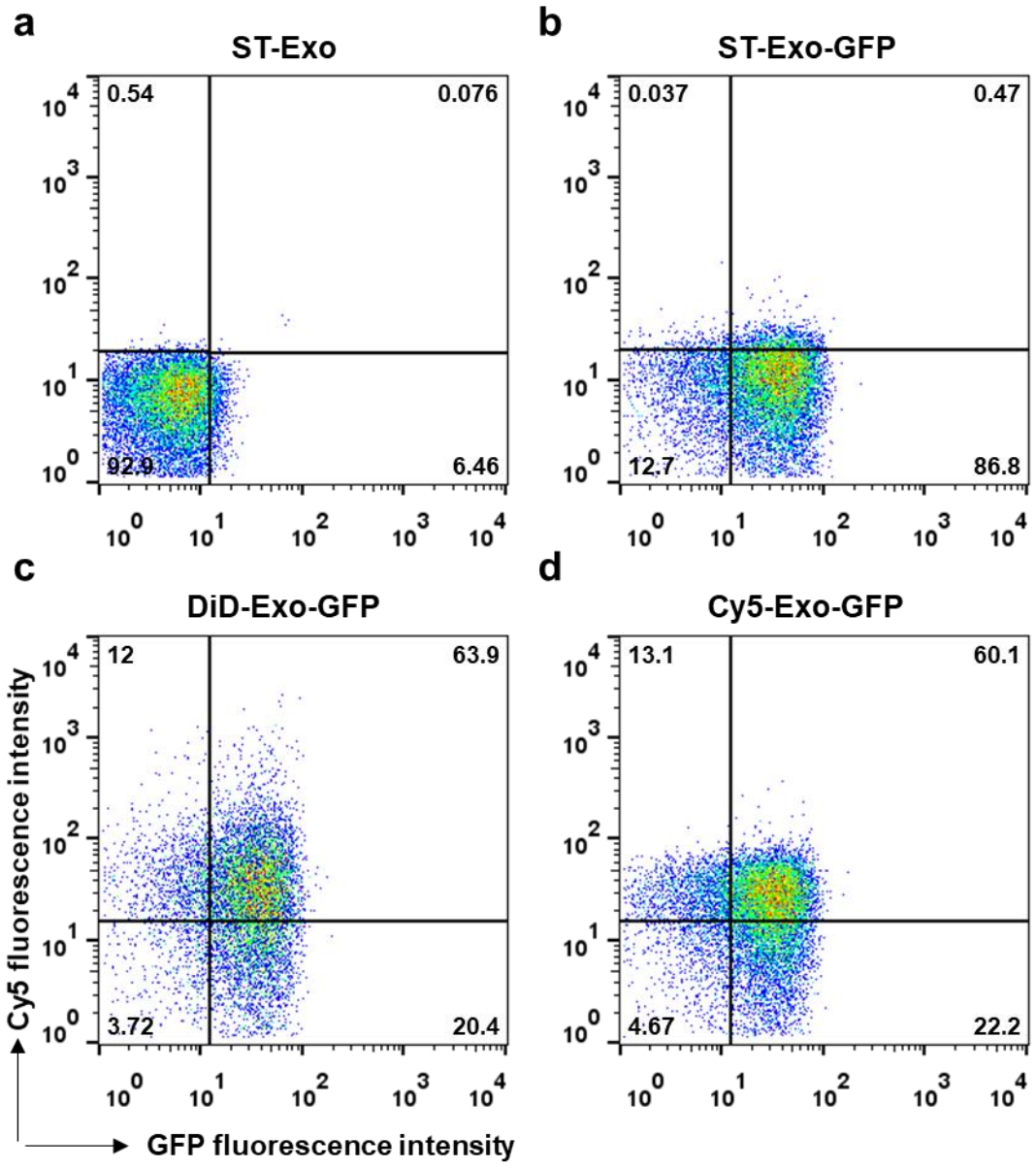
Supplementary Figure 1. To ensure biocompatibility of *in situ* one-step fluorescence labeling strategy of exosomes *via* bioorthogonal click chemistry, toxicity assay of Ac4ManNAz was performed in A549 cells. The A549 cells were incubated with different concentrations of Ac4ManNAz (0 - 100 μM) for 24 h, and cell viability was confirmed *via* cell counting kit-8 (CCK-8).



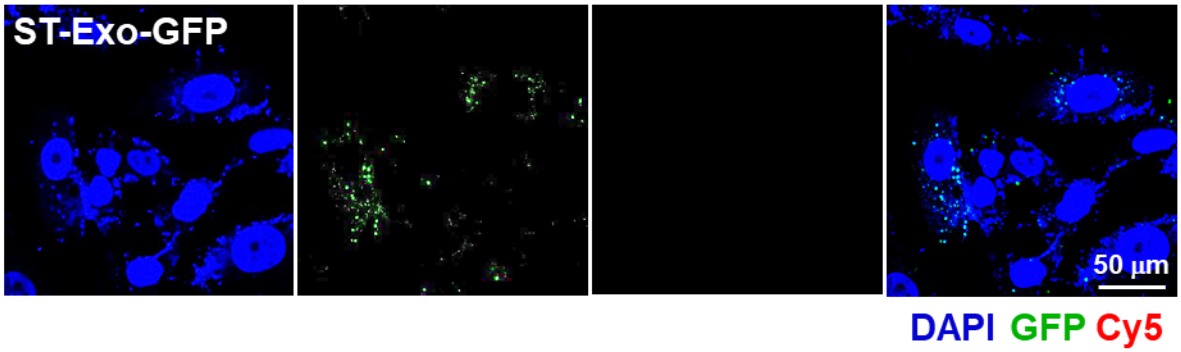
Supplementary Figure 2. Fluorescence intensity of Cy5-Exo in A549 cells after 0, 3, 6, 12 and 24 h of incubation was analyzed using Image-Pro software. For analysis of cellular uptake efficiency, Cy5-Exo (1 μg/ml) were incubated with A549 cells for 0, 3, 6, 12 and 24 h at 37ºC. After the predetermined incubation time, A549 cells were washed with DPBS twice and then fixed with paraformaldehyde fixative. The time-dependent cellular uptake of Cy5-Exo were visulalzied with confocal laser scanning microscopy (CLSM) equipped with 405 diode (405 nm) and HeNe-Red (633 nm) lasers (Leica, Germany).



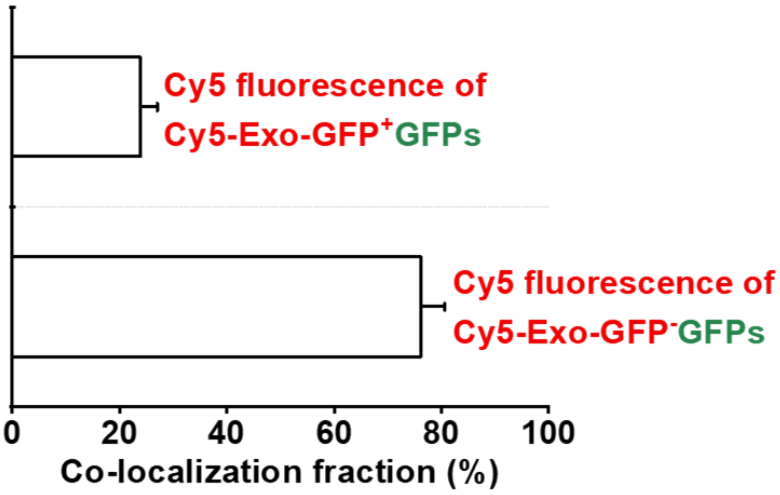
Supplementary Figure 3. Zoom in images of Cy5-Exo treated GFP-LAMP1-expressing A549 cells. The GFP-LAMP1-expressing A549 cells were incubated with Cy5-Exo (1 μg/mL) for 3 h, 12 h and 24 h, and imaged using a confocal laser scanning microscopy (CLSM) equipped with 405 diode (405 nm) and HeNe-Red (633 nm) lasers.



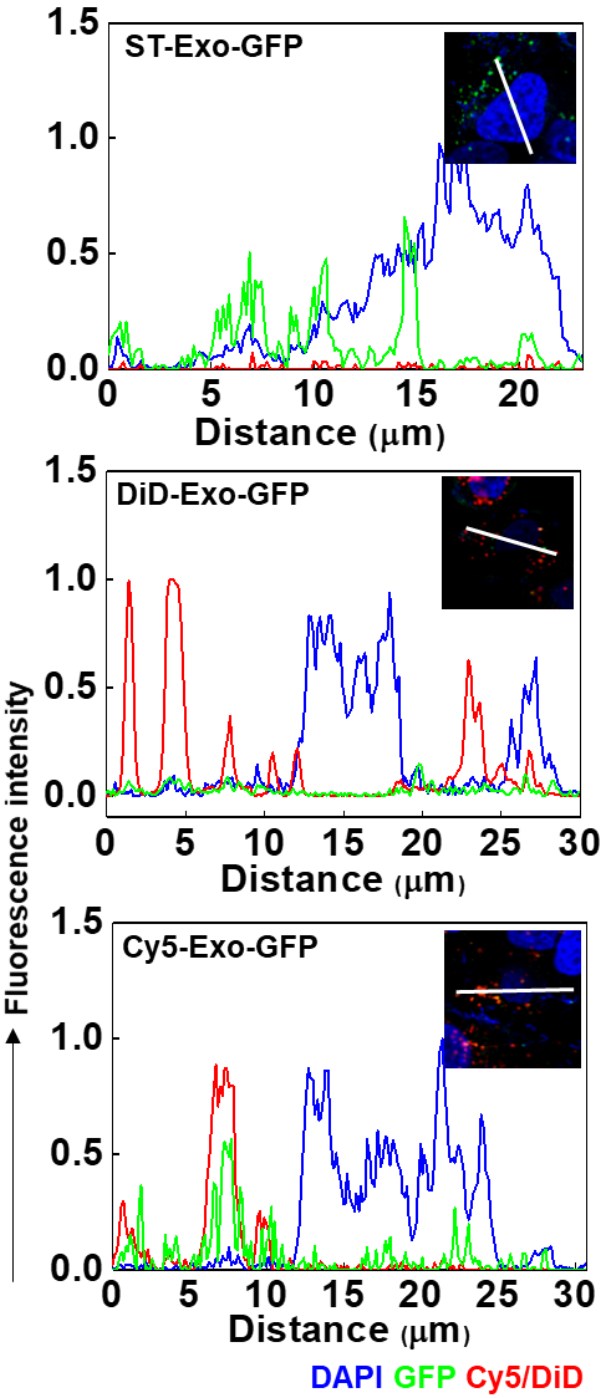
Supplementary Figure 4. FACS analysis of **(a)** ST-Exo, **(b)** ST-Exo-GFP, **(c)** DiD-Exo-GFP and **(d)** Cy5-Exo-GFP. To study drug delivering efficiency of labeled exosomes, ST-Exo-GFP, DiD-Exo-GFP and Cy5-Exo-GFP were isolated from GFP-expressing A549 cells by sequential centrifugation at 200 ×g for 10 min, 2000 ×g for 10 min, 10,000 ×g for 30 min and 150,000 ×g for 3 h. After isolation of exosomes, the fluorescence signals of ST-Exo-GFP, Cy5-Exo-GFP and DiD-Exo-GFP were characterized using flow cytometry and the data was analyzed using the FlowJo software.



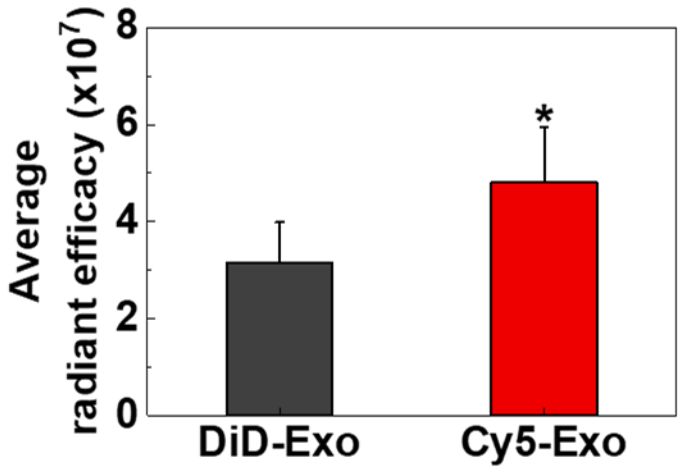
Supplementary Figure 5. The cellular uptake patterns of ST-Exo-GFP in A549 cells were observed using a confocal laser scanning microscopy (CLSM) equipped with 405 diode (405 nm) and HeNe-Red (633 nm) lasers.To assess drug delivering efficiency, ST-Exo-GFP (1 μg/ml) were incubated with A549 cells for 24 h at 37ºC. After incubation, A549 cells were washed with DPBS twice and then fixed with paraformaldehyde fixative.



Supplementary Figure 6. Quantitative analysis to analyze co-localization of Cy5-Exo-GFP and GFPs in Cy5-Exo-GFP-treated A549 cells. To evaluate drug delivering efficiency, Cy5-Exo-GFP were incubated with A549 cells for 24 h. Then, co-localization of GFPs and Cy5-Exo-GFP was analyzed using Image-Pro software.



Supplementary Figure 7. Fluorescence intensity profiles of ST-Exo-GFP-, DiD-Exo-GFP- or Cy5-Exo-GFP-treated A549 cells. To evaluate drug delivering efficiency, A549 cells were incubated with ST-Exo-GFP, DiD-Exo-GFP or Cy5-Exo GFP for 24 h at 37°C. After incubation, the GFPs delivering efficiency of each exosome was observed using a confocal laser scanning microscopy (CLSM) equipped with 405 diode (405 nm) and HeNe-Red (633 nm) lasers. Then, the Fluorescence intensity profiles in A549 cells were analyzed using Leica LAS X software.



Supplementary Figure 8. Quantitative analysis for fluorescence intensities of DiD-Exo and Cy5-Exo in tumor region after 24 h of intravenous injection. To evaluate the tumor targeting efficiency of Cy5-Exo and DiD-Exo, the tumor tissues were collected from mice after 24 h of injection. Then, the Cy5-Exo and DiD-Exo in the tumor tissues were observed using the IVIS Lumina Series III and the NIRF intensity in tumor tissues were calculated from the region of interest (ROI) using the Living Image® software.