Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Visualization and Intracellular Dynamic Tracking of Exosomes and Exosomal miRNAs using Single Molecule Localization Microscopy

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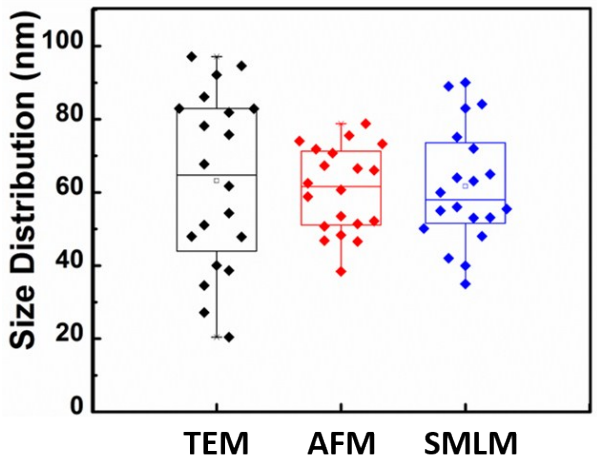

Figure S1 size distribution of exosomes characterized by TEM, AFM and SMLM. Exosomes were stained with CM-DiI while acquiring SMLM images.

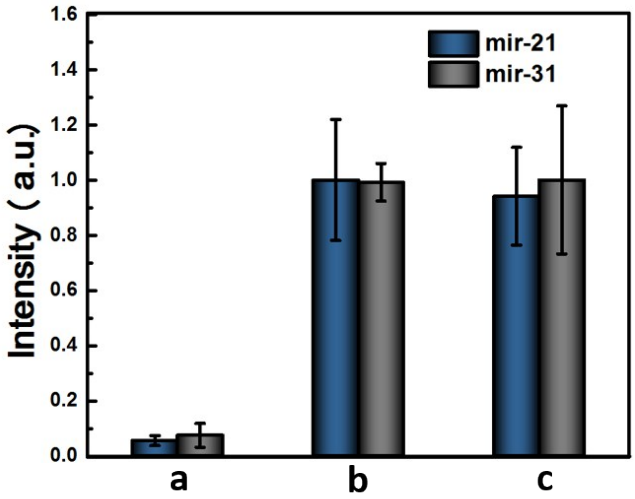

Figure S2. Fluorescence intensity of MBs after RNase A treatment. (a) Only MBs were added in the solution; (b) MBs were incubated with exosomes at 37 °C for 1.5 h. (c) Exosomes solution were incubated with sufficient RNase A (Beyotime, ST578, 10 mg/mL) for 30 min before adding the MBs for hybridization.

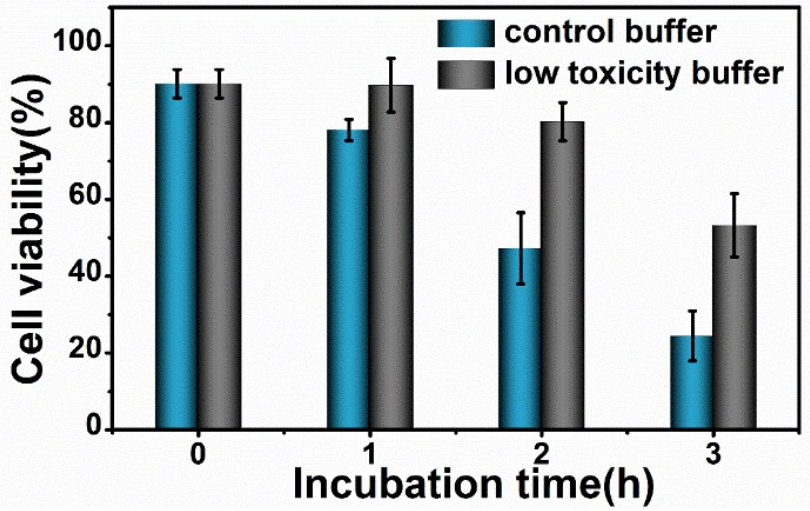

Figure S3. Cell viability of PC12 cells incubated with low toxicity buffer and control buffer. For cell viability assay, PC12 cells were incubated with two kinds of imaging buffers for different times after planted on Lab-Tek Chambered Coverglass for 24 h. Then, 1 mL of culture media containing 4 μL of Propidium Iodide (PI, KG214, KeyGEN BioTECH) and 2 μL of Calcein, AM (C3099, ThermoFisher Scientific) were added to label the cells for 5 minutes before imaging. Cell counting was performed using imageJ software (https://imagej.nih.gov/ij/).

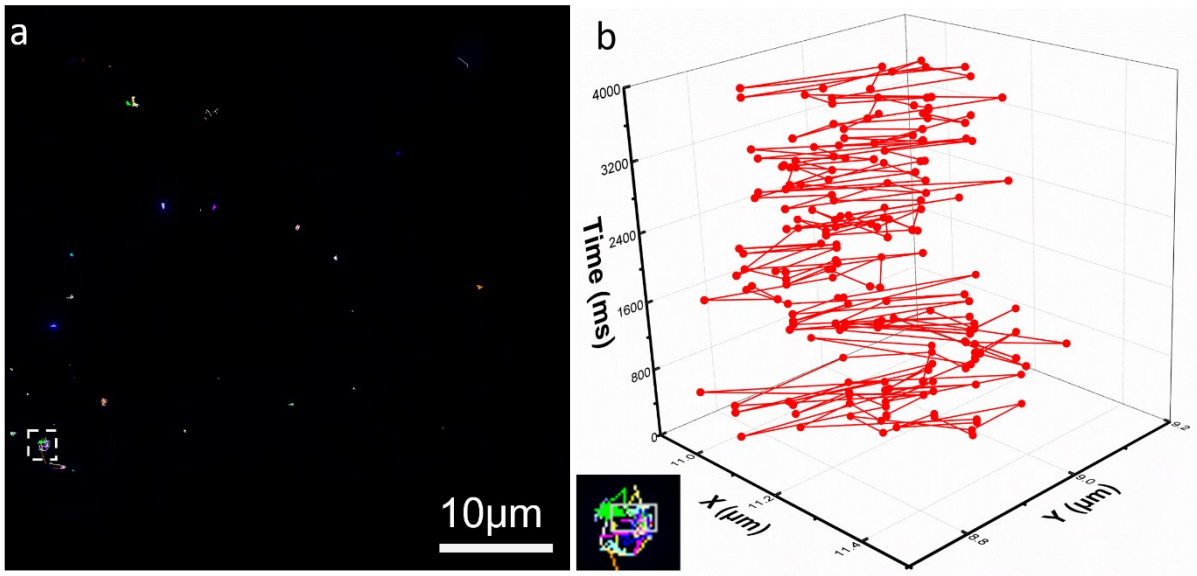

Figure S4 (a) Single particle tracking analysis of exosomes within 4 s. (b) The trajectory of the representive exosome in white rectangular region in (a) with respect to time.

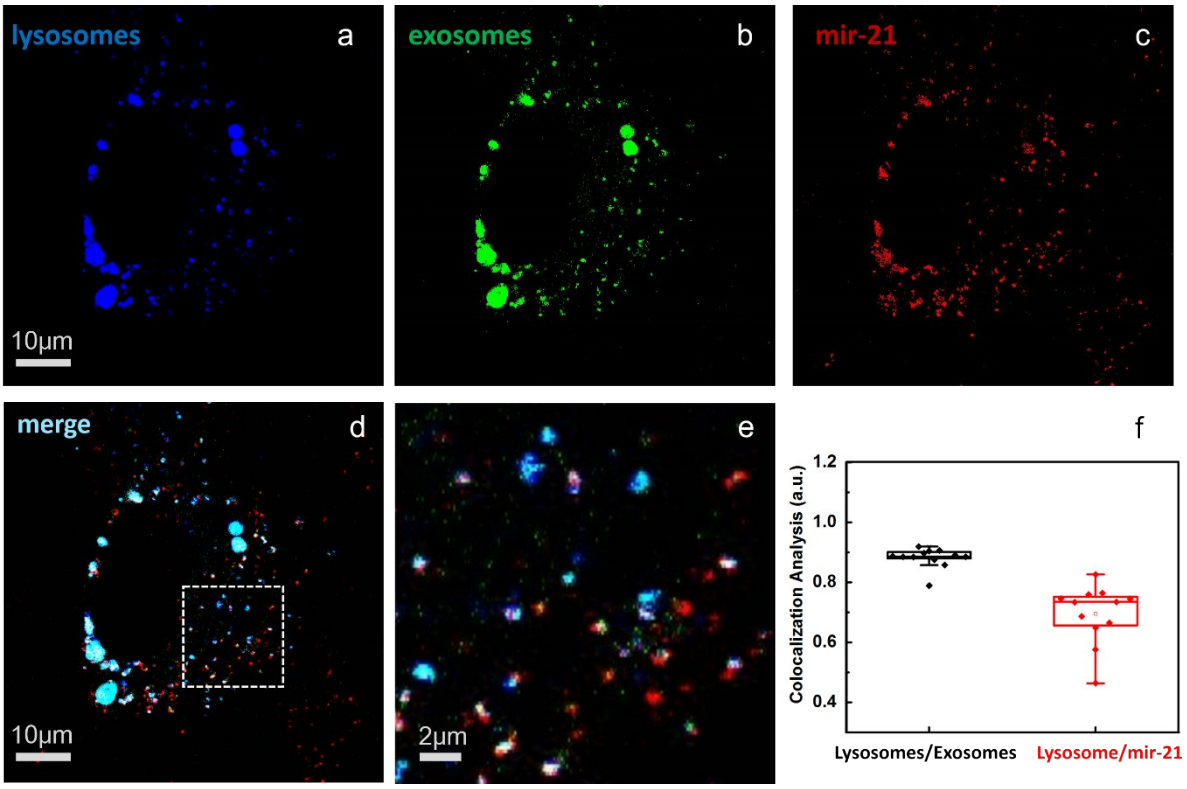

Figure S5 Confocal cololization of exosomes, exosomal mir-21 and lysosomes in PC12 cells. Exosomes were incubated with PC12 cells for 2h at 37°C, then 1mg/mL Lysotracker Green was added to label lysosomes at 37°C for 15 min. The confocal image of lysosomes (a), exosomes (b), exosomal mir-21(c) and merged channel (d), (e) enlarged image of the region indicated by the white box in (d). (f) The colocalization ratio between lysosomes and exosomal mir-21 was much lower compared with lysosomes and exosomes. It means that exosomal mir-21 can be released into cytoplasm.

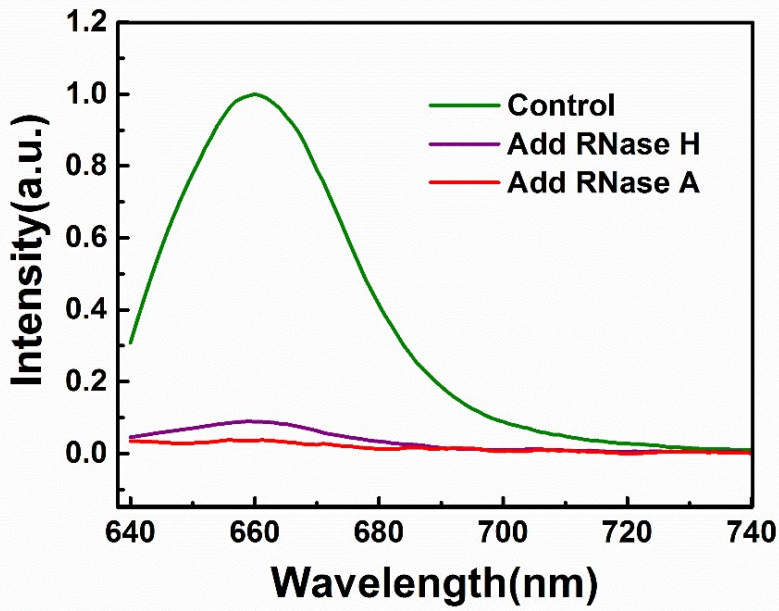

Figure S6. RNase H cleavage assay in solution for evaluation of RNase H activity. MB21 was incubated with synthetic mir-21 in solution for 1 h (PL spectra: green line), then sufficient RNase H (Beyotime, D7089) was added for cleavage (PL spectra: purple line). Meanwhile, RNase A was adopted to evaluate the degradability of RNase H (PL spectra: red line).

Supplementary Movie 1. The TIRF movie presenting the dynamic movements of exosomes in 4s.

Supplementary Movie 2. The super-resolution movie reveals the dynamic motions of exosomes within intercellular filamentous structures.

Supplementary Movie 3. The super-resolution video is merged with a wide field video of the cell.

Supplementary Movie 4. The super-resolution movie showing the dynamic movements of mir-21 in living cells.

Supplementary Movie 5. The super-resolution movie showing the release process of exosomal mir-21 in living cells.