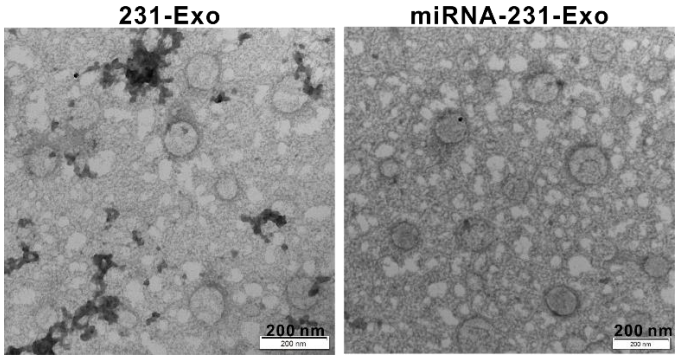
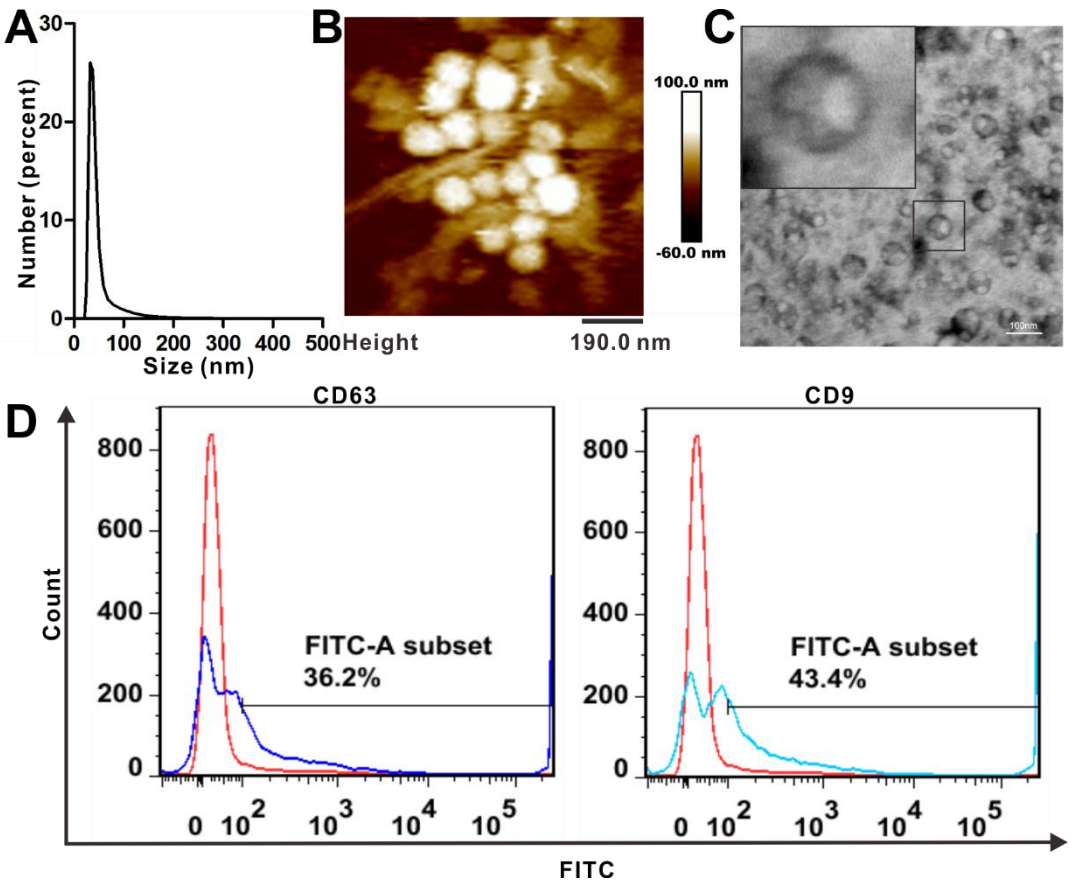
Electronic Supplementary Material (ESI) for Nanoscale.

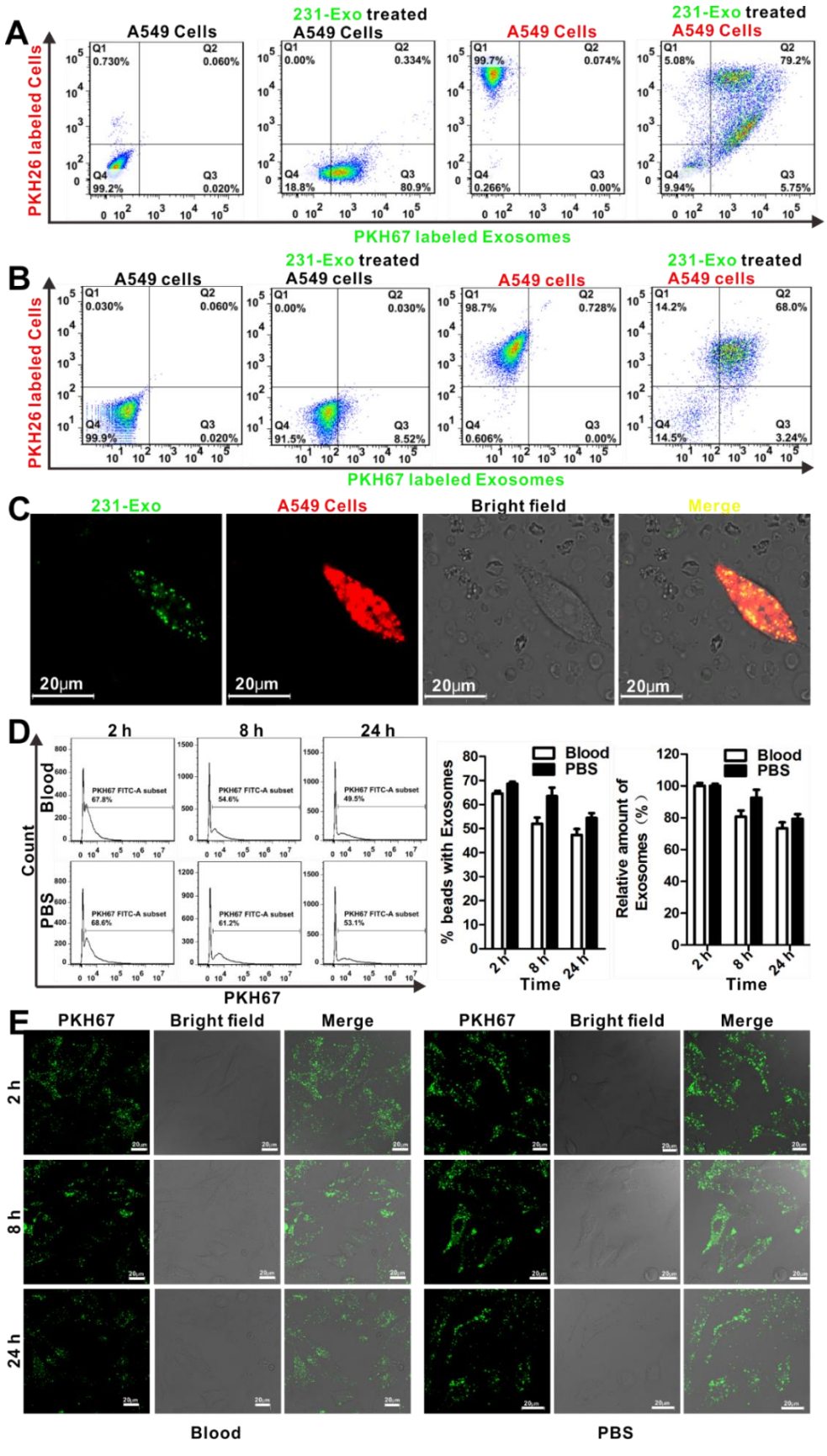
SUPPLEMENTARY INFORMATION



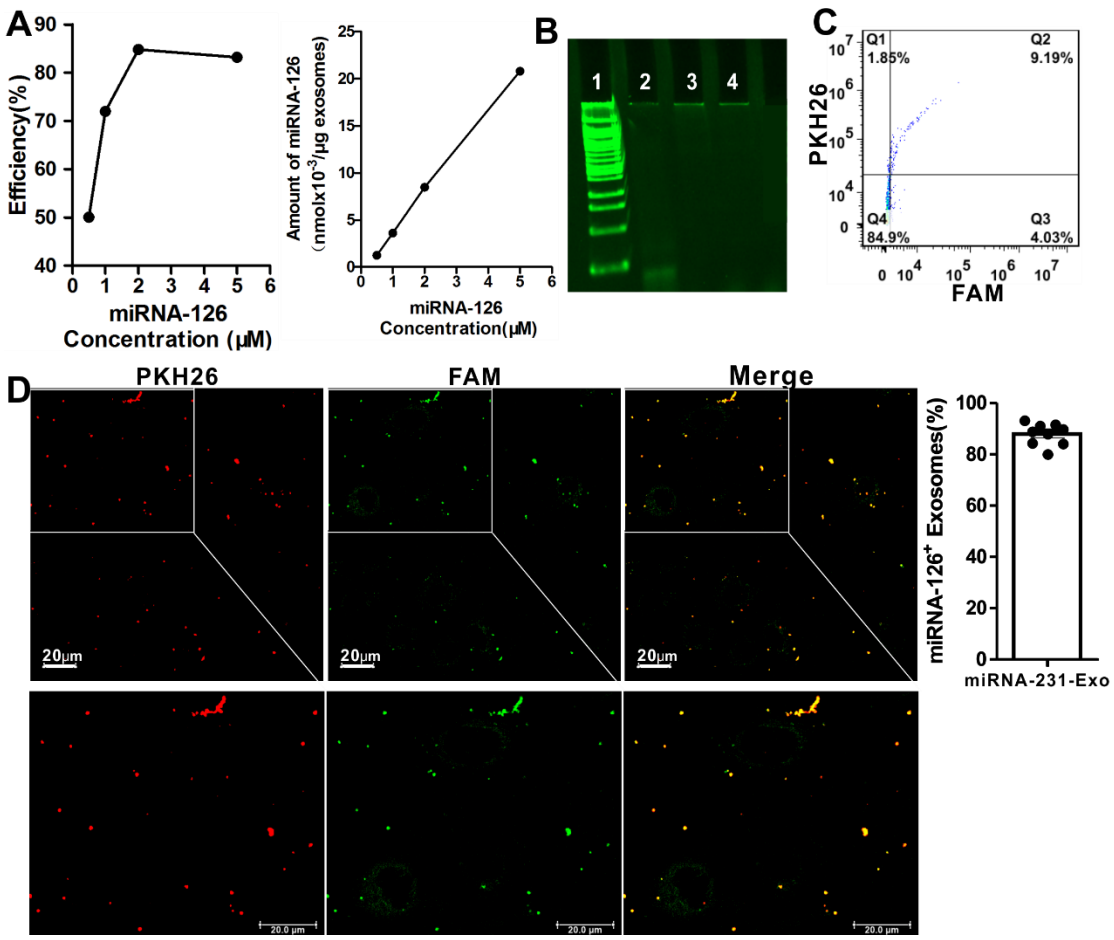
Supplementary Fig. 1. Characterization of exosomes and miRNA-loaded exosomes. MiRNA-126 was transfected into 231-Exo (miRNA-231-Exo) by small RNA transfection reagent. The homogenous morphology of miRNA-231-Exo was detected by transmission electron microscopy. Blank 231-Exo was provided to show exosome morphology before and after miRNA loading.



Supplementary Fig. 2. Characterization of 29-Exo. (A) Malvern Zetasizer Nano ZS analysis showed that the size distribution of 29-Exo was about 30-120 nm. (B) Homogenous morphology of 29-Exo was detected by AFM. (C) TEM image showed that 231-Exo was round-shaped nanovesicles surrounded by membranes. (D) Use of flow cytometry to study CD63 and CD9 abundance in 29-Exo sample.

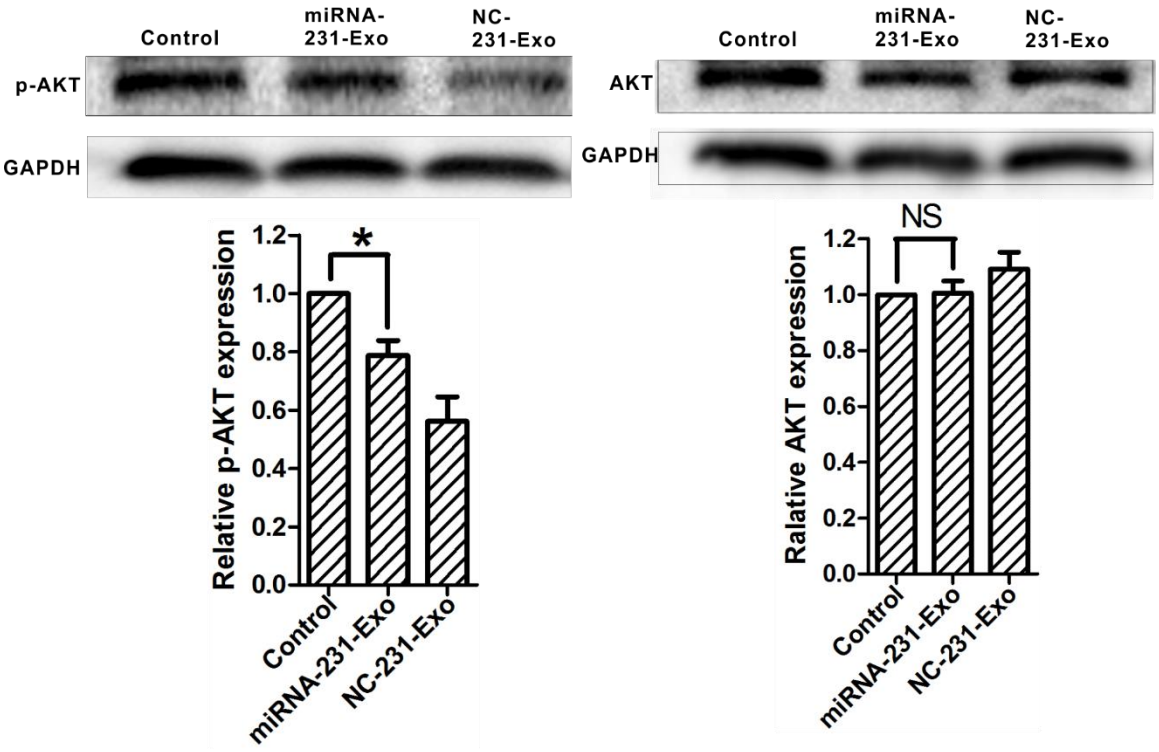

A
A549 Cells
231-Exo treated A549 Cells
A549 Cells
231-Exo treated A549 Cells
PKH26 labeled Cells
PKH67 labeled Exosomes
Q1 0.730%
Q2 0.060%
Q4 99.2%
Q3 0.020%
Q1 0.00%
Q2 0.334%
Q4 18.8%
Q3 80.9%
Q1 99.7%
Q2 0.074%
Q4 0.266%
Q3 0.00%
Q1 5.08%
Q2 79.2%
Q4 9.94%
Q3 5.75%
B
A549 cells
231-Exo treated A549 cells
A549 cells
231-Exo treated A549 cells
PKH26 labeled Cells
PKH67 labeled Exosomes
Q1 0.030%
Q2 0.060%
Q4 99.9%
Q3 0.020%
Q1 0.00%
Q2 0.030%
Q4 91.5%
Q3 8.52%
Q1 98.7%
Q2 0.728%
Q4 0.606%
Q3 0.00%
Q1 14.2%
Q2 68.0%
Q4 14.5%
Q3 3.24%
C
231-Exo
A549 Cells
Bright field
Merge
20μm
D
2 h
8 h
24 h
Blood
PBS
Count
PKH67
PKH67 FITC-A subset 67.8%
PKH67 FITC-A subset 54.6%
PKH67 FITC-A subset 49.5%
PKH67 FITC-A subset 68.6%
PKH67 FITC-A subset 61.2%
PKH67 FITC-A subset 53.1%
% beads with Exosomes
Relative amount of Exosomes (%)
Blood
PBS
Time
2 h
8 h
24 h
E
PKH67
Bright field
Merge
2 h
8 h
24 h
Blood
PBS

Supplementary Fig. 3. Specific recognition of exosomes to A549 cells and the stability of 231-Exo in blood. PKH26-labeled A549 cells and PKH67-labeled exosomes were incubated in C57BL/6 mouse blood for 3 h. After the erythrocytes were removed, the PKH26 and PKH67 fluorophores were analyzed by flow cytometry (A, B) or confocal microscope (C). Please notice that we found the incubation conditions, *i.e.* orbital incubator for (A) *vs* static incubator for (B, the second panel) may significantly impact the cellular readout. We decided to rely on panel (A), which is data generated by orbital incubator, because this condition may closely mimic the *in vivo* scenario. (C) Confocal microscopic images exhibited the effective recognition of 231-Exo by A549 cells as compared to white blood cells in blood. (D, E) Confirmative co-culture experiment using human blood. The experiment is similar to Fig. 4D in the main manuscript. Our data confirm the murine data, *i.e.* ~74% of 231-Exo existed in the human blood after incubation for 24 h.

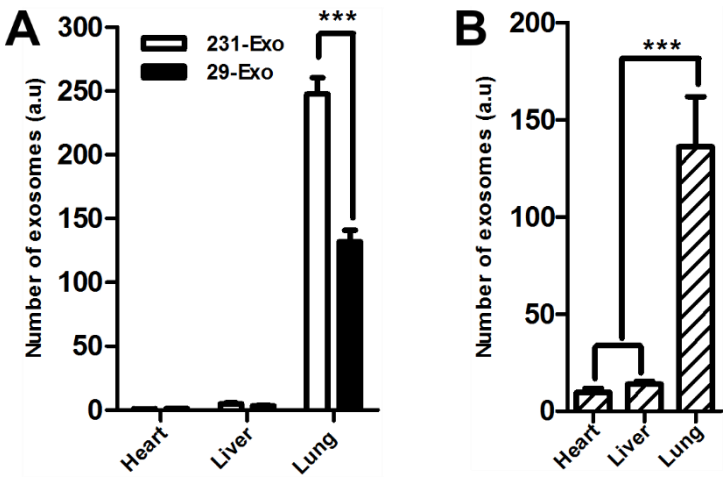


Supplementary Fig. 4. Assessment of miRNA loading efficiency in exosome. (A) Different amount of FAM-labeled miRNA-126 (0.5, 1, 2, 5 μM) was transfected into 1 μg of 231-Exo. The mixture was centrifuged with Millipore of 30 kD at 14,000×g for 30 min. The amount of FAM-labeled miRNA in lower solution was calculated by measuring the fluorescence intensity (left). And the relationship between miRNA adding and miRNA loading efficiency (right). (B) PAGE electrophoresis analysis showed the loading of miRNA-231-Exo. Lane 1, molecular markers; Lane 2, free FAM-labeled miRNA-126; Lane 3, FAM-labeled miRNA-231-Exo (post transfection and centrifugation purification);

4, FAM-labeled miRNA-231-Exo (post transfection and RNase treatment). (C) and (D) After the loading effect, the result products (including FAM-labeled miRNA-231-Exo and blank 231-Exo) were labeled with PKH26 and measured with flow cytometry (C) and confocal microscope (D).



Supplementary Fig. 5. The expression of p-AKT and AKT in A549 cells. A549 cells were seeded in a 6-well plates and treated with miRNA-231-Exo or NC-231-Exo for 48 h. Then A549 cells were homogenized in a RIPA lysis buffer. The expression of p-AKT and AKT in A549 cells was analyzed by western blot. Data are mean ± s.e.m. NS, not significant; *, $P< 0.05$; one-way ANOVA.



Supplementary Fig. 6. Lung enrichment effect in mice receiving IV injected breast cancer derived exosomes. (A) Healthy C57BL/6 mice received IV injected 231-Exo that was labeled using PKH67 dye. A colon cancer derived 29-Exo sample labeled with the same dye was used for comparison. Two hours post IV injection, lung tissues were collected to prepare the thin tissue slides. The number of exosome was quantified under confocal microscopy. We also collected liver (where nanoparticles tend to biodistribute) and heart (in which nanoparticle usually has low chance of access) for comparison. We were able to obtain a statistically significant increased number of breast cancer derived exosome (231-Exo) than a colon cancer exosome (29-Exo). Since the targeting motif (*i.e.* integrin β_4) is conserved among species, this was in the line with the high potential of lung metastasis that was frequently observed in the breast cancer patient. No major uptake was founded in heart and liver in this experiment. (B) The same 231-Exo sample was used to generate the biodistribution data in nude mice, in which a similar trend was observed. Data are mean ± s.e.m. ***, $P< 0.001$; one-way ANOVA.