Expanded View Figures

Figure EV1. EV uptake by fibroblasts and characterization by nanoparticle tracking analysis, Western blots, and RT–qPCR.

A WI-38, NIH3T3, and CAF were incubated with Dil-labeled EVs (red) for 24 h before fluorescent and phase contrast images were captured. Bar = 100 μm.

- B Female NSG mice received mammary fat pad injection of MDA-MB-231 cells mixed with GFP-labeled NIH3T3 cells. After 5 weeks, Dil-labeled EVs or PBS were injected into the mammary tumor, which was collected after 24 h and sectioned for fluorescent microscopy. DAPI (4',6-diamidino-2-phenylindole) indicates nuclear stain. Bar = 100 μm.
- C Western blot analysis of CAF treated with PBS or EVs from indicated cells for 48 h.
- D EVs pelleted at 110,000 \times g were analyzed by nanoparticle tracking analysis. The black line indicates mean, whereas the red shaded area indicates standard error of the mean (SEM) (n = 3 biological replicates for each type of EVs).
- E Western blot analysis of cell lysates (CL) and EVs for known EV markers (TSG101, ALIX, CD63, and CD9), a Golgi marker (GM130), and a marker for non-vesicular particles (Ago2).
- F Levels of miR-105 and miR-204 in EVs treated with Proteinase K (PK, 10 μg/ml) followed by RNase If (RNase, 40 U) or with PBS (as control) in the presence or absence of 1% Triton X-100 (TX-100). RT–qPCR data were normalized to an ath-miR159a spike-in control added after all treatments (*n* = 3 biological replicates for each group). Data are presented as mean ± SD. **P* < 0.001 (Student's *t*-test).

Source data are available online for this figure.



Figure EV1.

Figure EV2. EV characterization by density gradient fractionation.

- A, B Western blot and density measurement of EV fractions collected from indicated cell lines to detect EV markers.
- C, D RT-qPCR of EV fractions collected from indicated cell lines to detect miR-105 and miR-204 levels (n = 3 biological replicates for each fraction). Data are presented as mean \pm SD. ND: not detected.
- E A schematic representation of the density gradient separation procedure to further characterize the 110,000 \times g pellets.

Source data are available online for this figure.



Figure EV2.