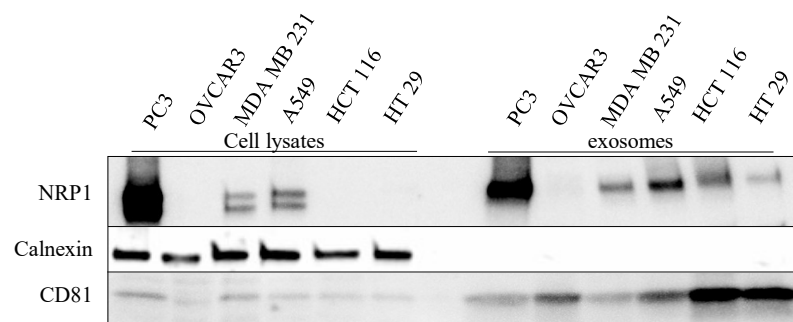
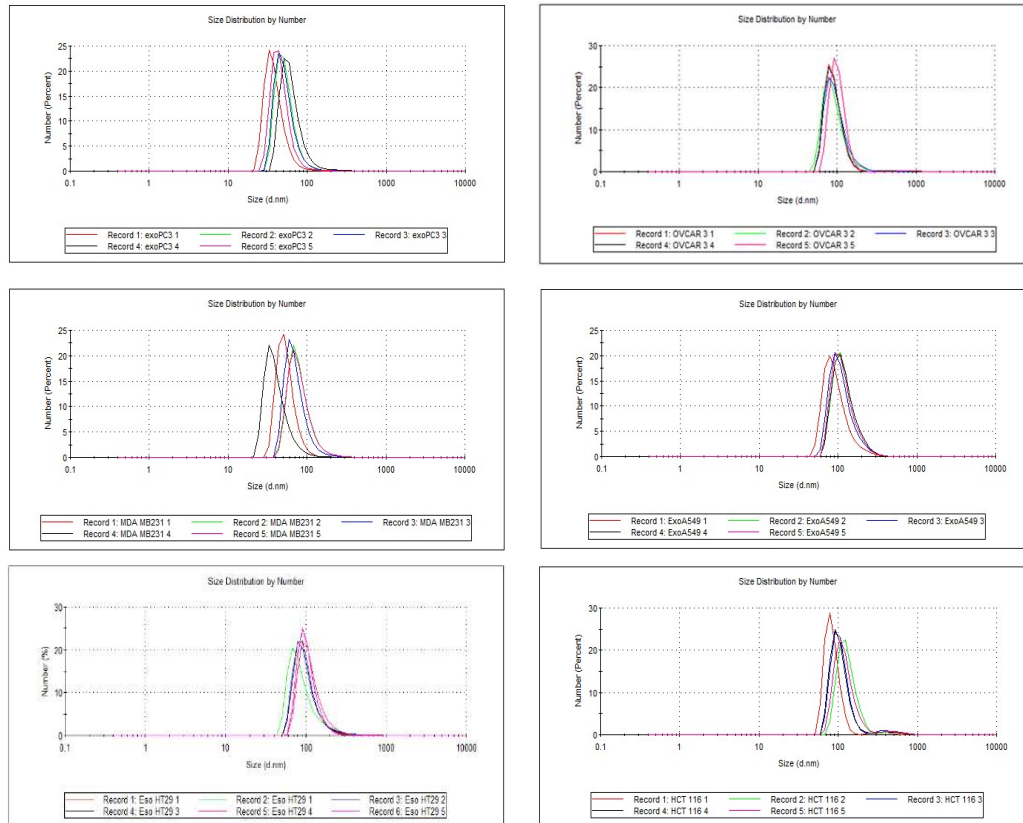


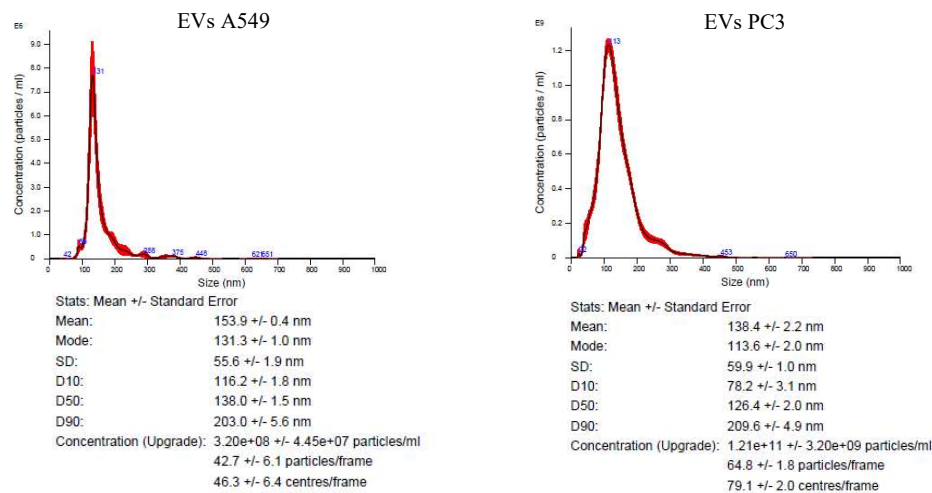
A



B



C

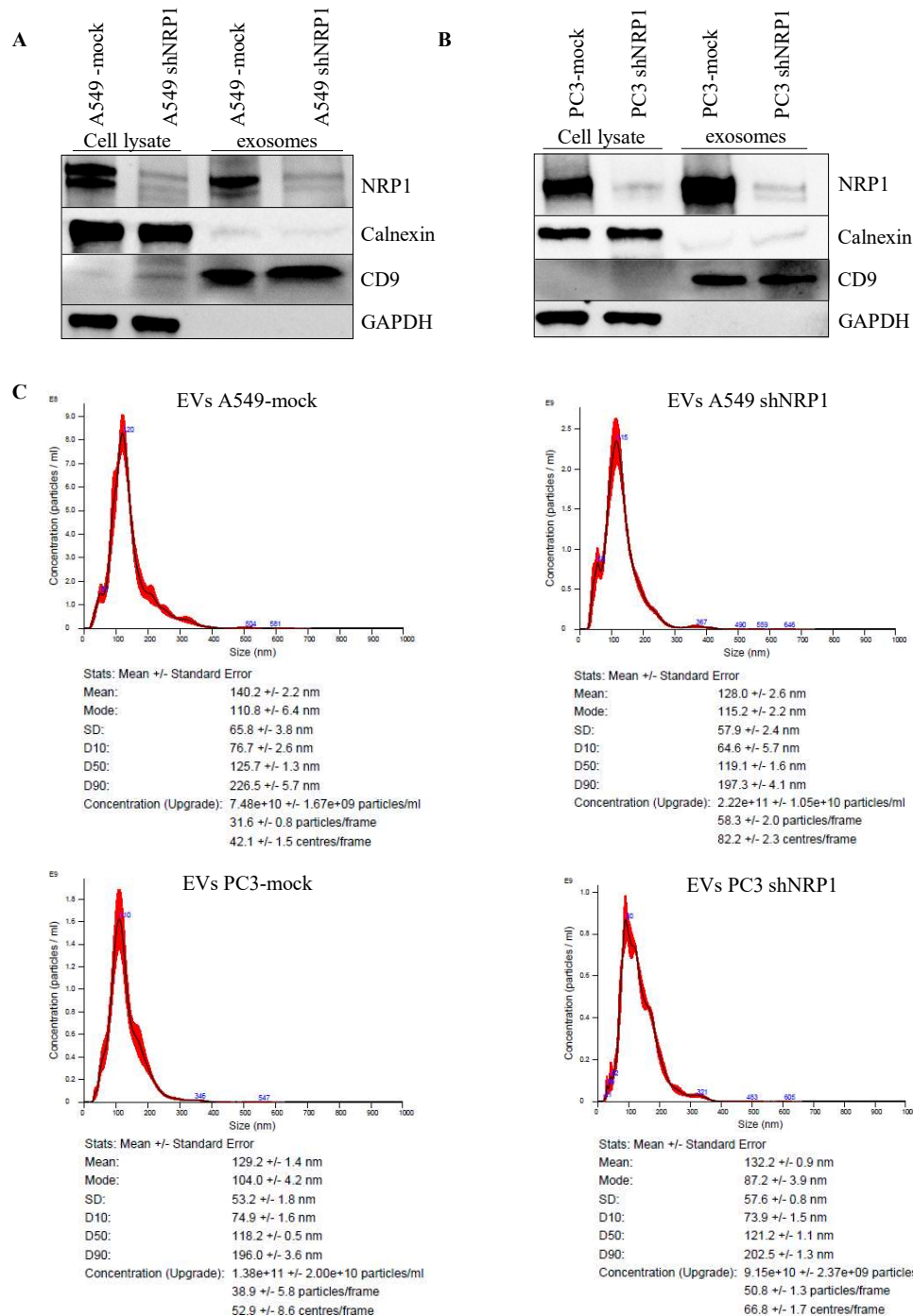
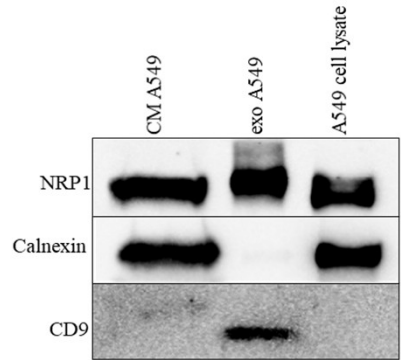


Suppl. Figure 1

(A) Western blot analysis of NRP1 protein levels (130kDa) in exosomes derived from the indicated cancer cell lines. The endoplasmic reticulum marker Calnexin (90kDa) and the specific marker CD81 (26kDa) provided a control for EV purification. (B) Analysis by dynamic light scattering (DLS) of the size distribution profile of EVs purified from the conditioned medium (CM) of prostate cancer cells PC3, ovarian cancer cells OVCAR3, breast cancer cells MDA-MB231, lung cancer cells A549, or colon cancer cells HCT116 and HT29. (C) Assessment of size distribution profile and vesicle concentration by nanoparticle tracking analysis (NTA) of purified EVs derived from A549 and PC3 cancer cells.

Suppl. Figure 2

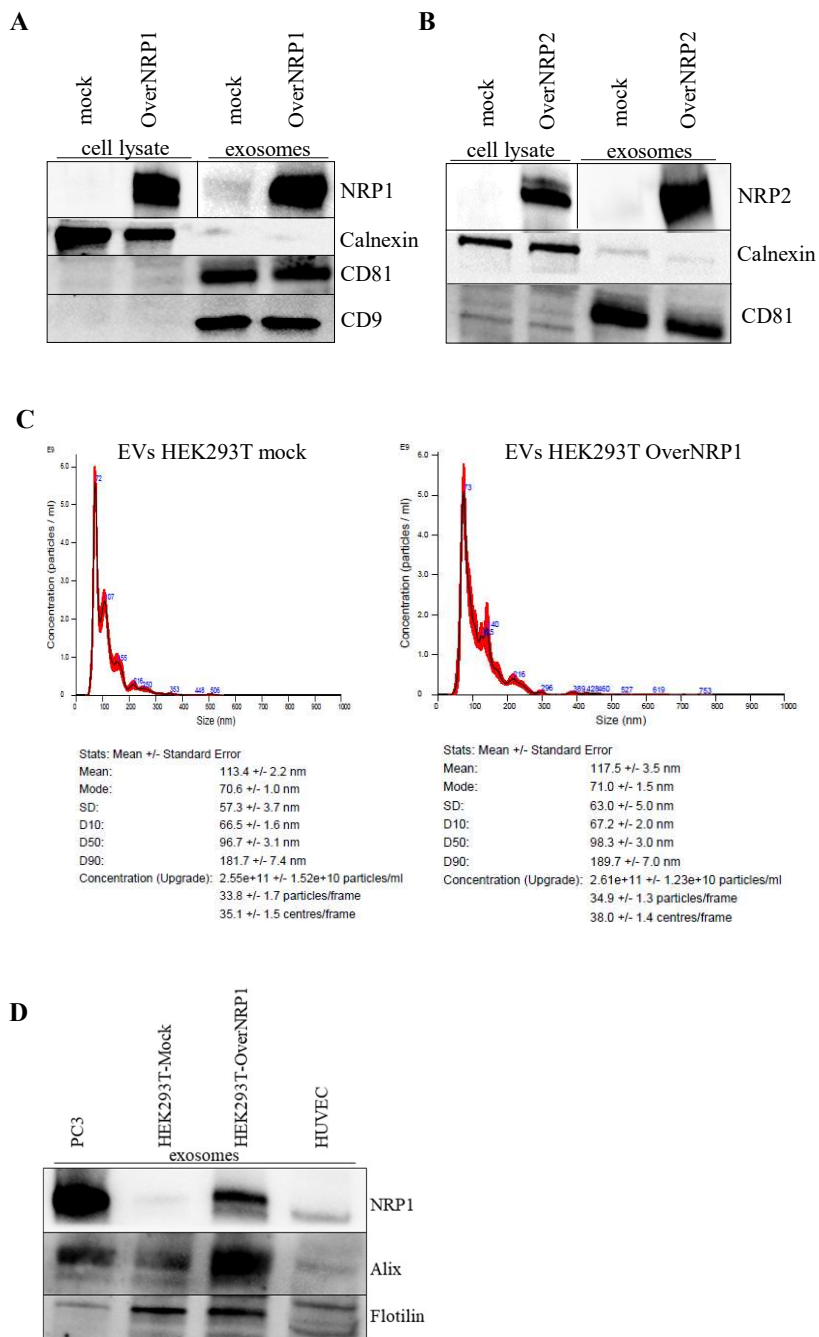
Western blotting analysis of NRP1 (130kDa) content in a given volume (10 microliters) of A549-conditioned medium (CM) and in the exosomal fraction proportionally derived from the same volume (lane 2); in the rightmost lane was loaded a total lysate of the same cells, as a reference. Calnexin (~90kDa) was analyzed in the same samples as a marker of intracellular membranes, and CD9 (24kDa), as exosomal marker.



Suppl. Figure 3

(A-B) Western blotting analysis of NRP1 (130kDa) protein level in cell lysates of A549 and PC3 cell lines and in the derived exosomes, upon lentiviral-mediated transduction with constructs expressing NRP1-targeted shRNA (shNRP1) or mock empty vector pLKO. EV markers calnexin (~90kDa) and CD9 (24kDa), and the cytosolic marker GAPDH (35kDa), were also analysed in the same samples.

(C) Assessment of size distribution profile and concentration of the purified EVs shown in the previous panel, by nanoparticle tracking analysis (NTA).

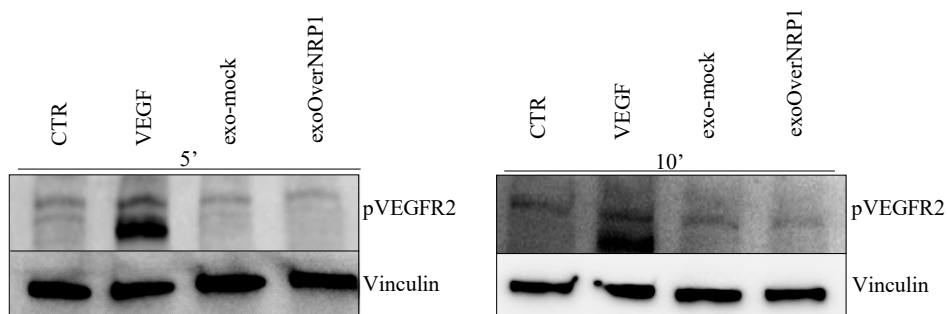


Suppl. Figure 4

(A-B) Western blotting analysis of NRP1 and NRP2 protein level in cell lysates of normal kidney cell line HEK293T and in the derived exosomes, upon transient transfection with either constructs expressing NRP1 or NRP2, or mock-transfected with pLKO empty vector. EV markers calnexin, CD9 and CD81 were also analysed in the same samples.

(C) Assessment of size distribution profile and concentration, by nanoparticle tracking analysis (NTA), of purified EVs shown above.

(D) Western blot analysis of NRP1 protein levels (130kDa) in exosomes derived from the indicated cells, including from transfected HEK293T, as in previous panels. Specific markers Alix (95kDa) and Flotilin (49kDa) provided a control for EV purification.

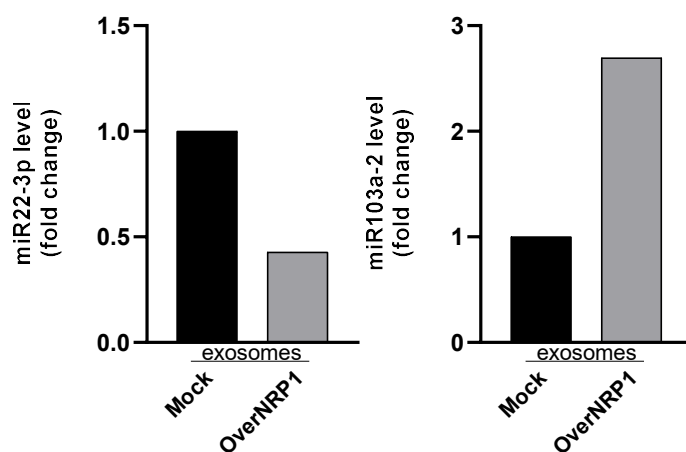
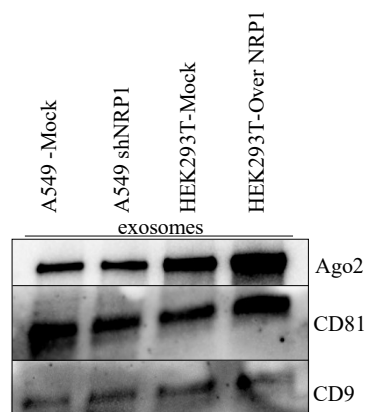


Suppl. Figure 5

Western blot analysis of phospho-tyrosine-VEGFR2 (230kDa) in cell lysates of HUVECs, upon treatment (for 5 or 10 minutes) with 30ng of VEGF (positive control) or 10 μ g of exosomes, either exoOverNRP1 or exo-mock. Vinculin (130kDa) was analyzed to provide a protein loading reference.

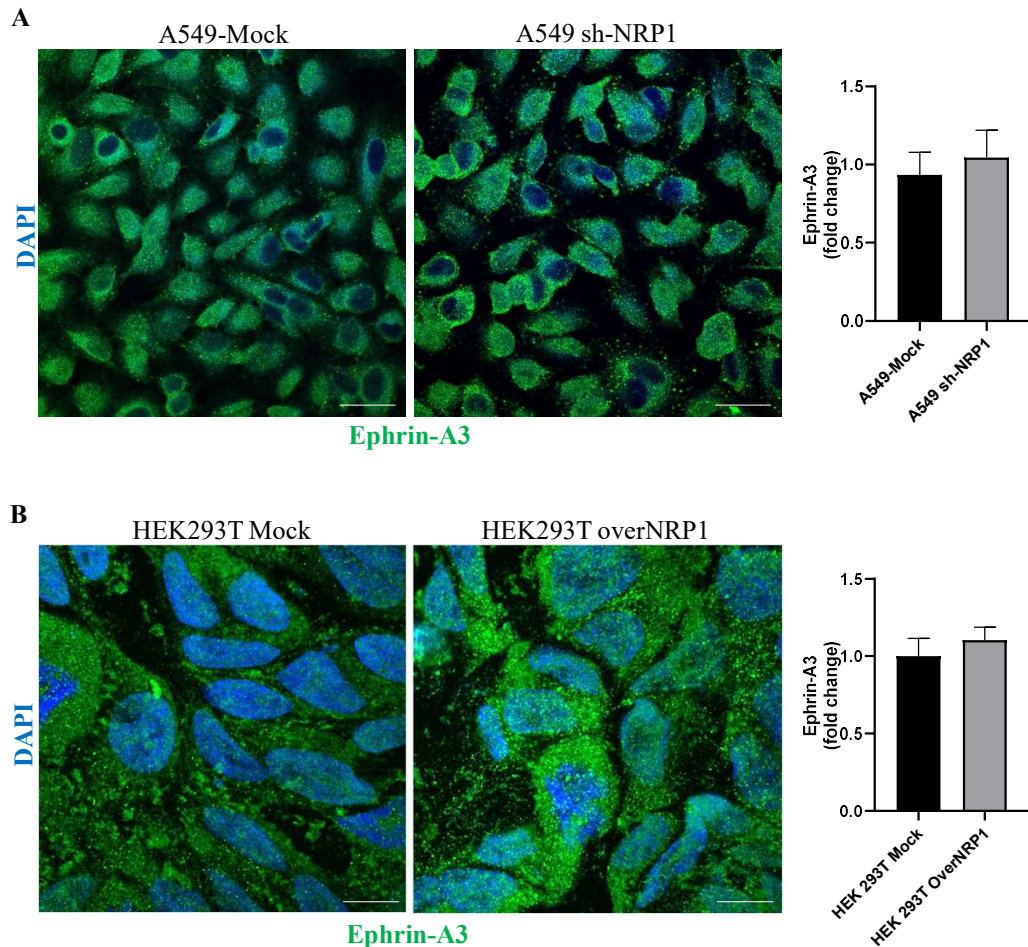
Suppl. Figure 6

Western blotting analysis of Ago2 (97kDa) protein levels in exosomes derived from A549 cells, subjected to NRP1 silencing (shNRP1) or mock-transduced with pLKO (empty vector); as well as from HEK293T cells transfected to overexpress NRP1 (or with an empty vector, as control). EV markers CD81 (26kDa) and CD9 (24kDa) were also analyzed.



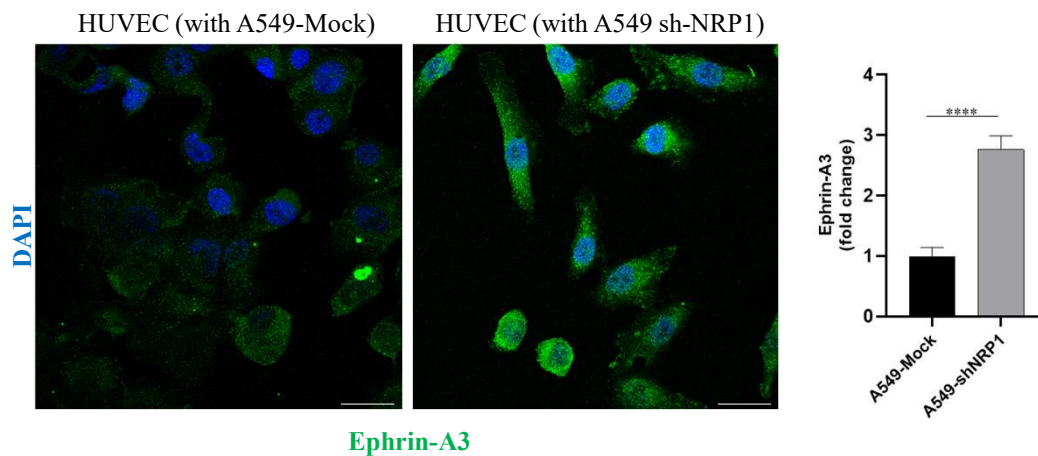
Suppl. Figure 7

miR22-3p and miR103a-2 expression was assessed by qPCR. The analysis shows a downregulation of miR22-3p and an upregulation of miR103a-2 in exoOverNRP1, confirming NGS results.



Suppl. Figure 8

Representative confocal images showing Ephrin-A3 expression (green) in: **(A)** A549 cells depleted for NRP1 (shNRP1) or pLKO-transduced mock controls, scale bar=20 μ m; and **(B)** HEK293T cells overexpressing NRP1 vs. respective empty vector controls; the nuclei were stained by DAPI. Scale bar=10 μ m. The bar graphs on the right show the quantification of ephrin-A3 optical density fold-change upon NRP1 regulation (n = 2).



Suppl. Figure 9

Representative confocal images showing Ephrin-A3 expression (green) in HUVEC incubated in co-culture with A549 cells depleted for NRP1 (shNRP1) or pLKO-transduced mock controls, separated by the semipermeable membrane of a Transwell insert (see Methods for details). The nuclei were stained by DAPI. Scale bar=20 μ m. The bar graph on the right shows the quantification of ephrin-A3 optical density fold-change upon NRP1 regulation (n = 2 experiments, each including triplicate coverslips). The statistical analysis was performed by Student's t-test and asterisks represent p-value: ****p<0.0001.