Preparation of Prostate Cancer-Derived EVs.

Two prostate cancer cell lines (PC3 and LNCaP) were used as a model to produce prostate cancer-derived EVs. Cell lines were cultured at 37 °C and 5% CO2 in Dulbecco's modified Eagle medium, RPMI 1640 with L-glutamine (Thermo Fischer Scientific, 11875) supplemented with 10% v/v fetal bovine serum, 10 units/mL penicillin, and 10 µg/mL streptomycin. Medium was refreshed every second day.

When cells reached 80−90% confluence, they were washed three times with PBS and FBS-free RPMI medium supplemented with 1 unit/mL penicillin and 1 µg/mL streptomycin was added to the cells. After 48 h of cell culture, cell supernatant was collected and centrifuged at 1000g for 30 min. The invisible pellet containing dead or apoptotic cells and the biggest in size population of EVs was discarded. The supernatant was pooled, and aliquots of 50 µL were frozen in liquid nitrogen and stored at −80 °C.
Size distribution and presence of the harvested EVs was assessed with nanoparticle tracking analysis (NTA), and transmission electron microscopy (TEM) images were taken to provide some examples of EVs.