

Supplementary Information

Plant defensin PvD₁ modulates the membrane composition of breast tumour-derived exosomes

Authors: Julia Skalska^a, Filipa D. Oliveira^a, Tiago N. Figueira^a, Érica O. Mello^b, Valdirene M. Gomes^b, Grant McNaughton-Smith^c, Miguel A. R. B. Castanho^a, Diana Gaspar^a

Affiliations: ^aInstituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal;

^bLaboratório de Fisiologia e Bioquímica de Microrganismos do Centro de Biociências e Biotecnologia da Universidade Estadual do Norte Fluminense Darcy Ribeiro, Rio de Janeiro, Brazil;

^cCEAMED - Centro Atlántico del Medicamento, S.A., San Cristobal de La Laguna, S/C Tenerife, Spain

Correspondence: dianagaspar@medicina.ulisboa.pt,
macastanho@medicina.ulisboa.pt

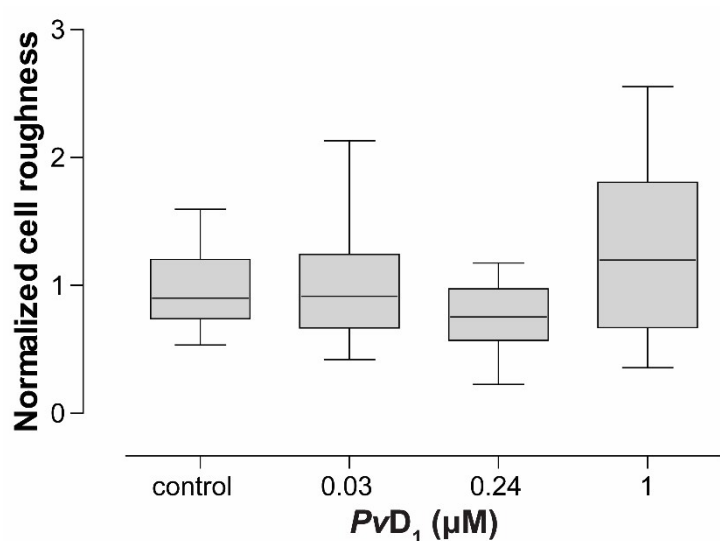


Figure S1. Root mean square roughness (S_q) of MCF-7 cells after treatment with PvD_1 . Cell membrane roughness (S_q) was determined using AFM height images. Squares of $2.5 \mu m \times 2.5 \mu m$ were drawn in different cell areas (nucleus and cytoplasm) using Gwyddion version 2.50 software corresponding the final S_q values to the average of these $2.5 \mu m \times 2.5 \mu m$ areas. The number of cells used for the analysis was 29 for the control and in the presence of PvD_1 at the concentration of $0.03 \mu M$ – 24 cells, $0.24 \mu M$ – 22 cells and for $1 \mu M$ – 19 cells. Independently grown cultures were used and imaged in different days.

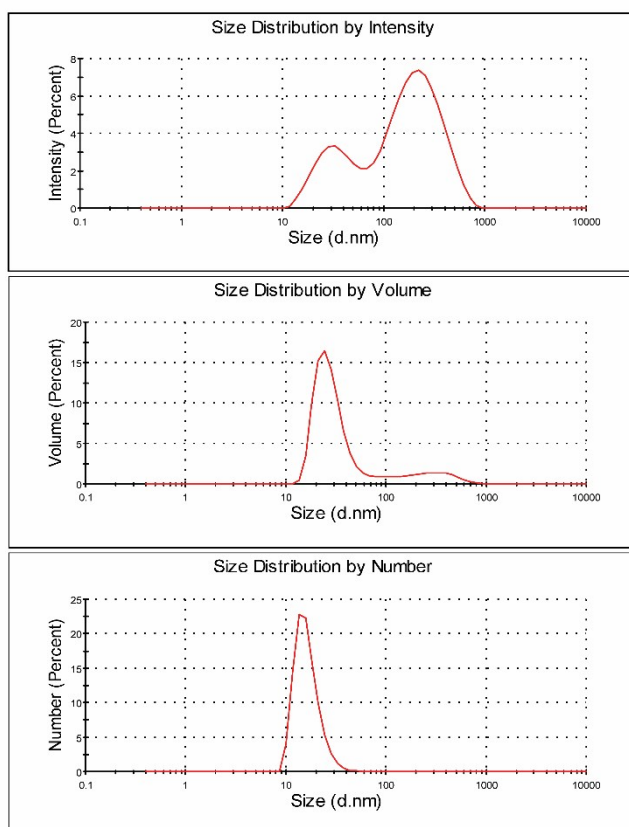
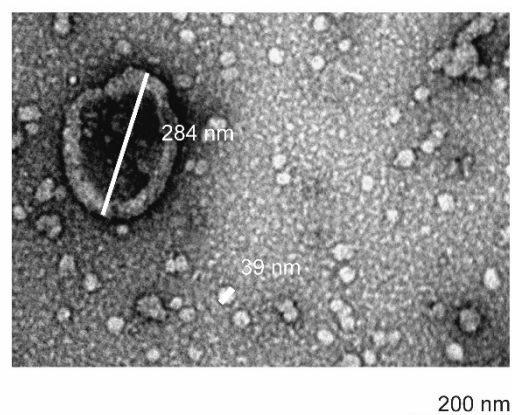
A.**B.**

Table 1. Size distribution of MCF-7 derived exosomes determined by DLS

Exosomes Size Distribution by DLS		
Weighted by:	Peak 1	Peak 2
Intensity	253 (87.9%)	36.4(12.2%)
Volume	272.1 (15.6%)	28.40 (84.4%)
Number	22.54(100%)	N/A
Z-Average (d. nm)	118.9	
PDI	0.579	

Figure S2. Size analysis of MCF-7 breast tumor derived exosomes. The size of isolated exosomes was determined by intensity, volume and number distributions obtained by Dynamic Light Scattering (DLS) (A). Each measurement consisted of 15 individual runs, each run corresponding to an averaged autocorrelation curve obtained from at least 12 repeated sample scans. Representative transmission electron microscopy (TEM) image showing two populations of MCF-7 extracellular vesicles (EVs) (B). The values of MCF-7 exosomes' size distribution obtained from DLS measurements are presented in Table 1.

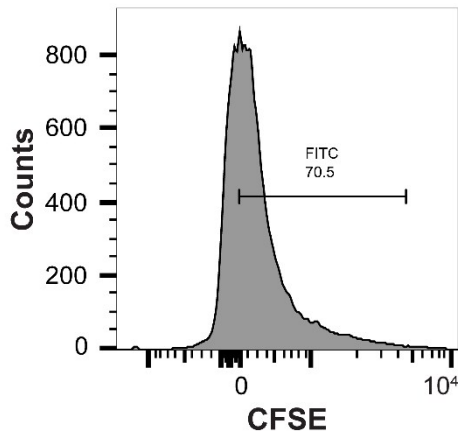
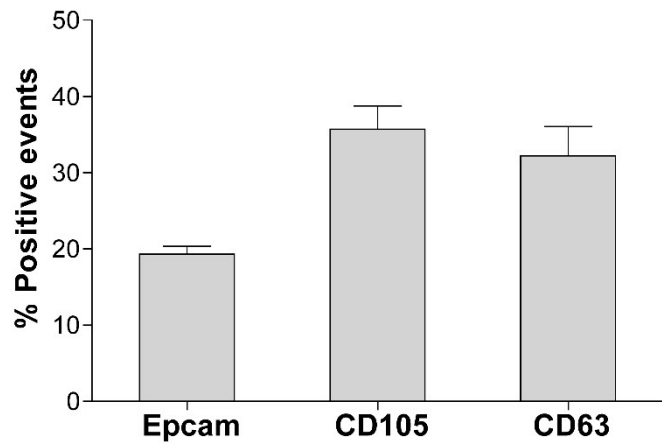
A.**B.**

Figure S3. Flow cytometry analysis of exosomal markers in MCF-7 breast tumor derived exosomes. A representative population of exosomes positive to CFSE detected by FITC channel is shown (A). Expression of exosomal markers Epcam, CD105 and CD63 in MCF-7 derived exosomes represented by the percentage of CFSE-positive exosomes' population (B). Experiments were repeated on different days using independent exosome samples.

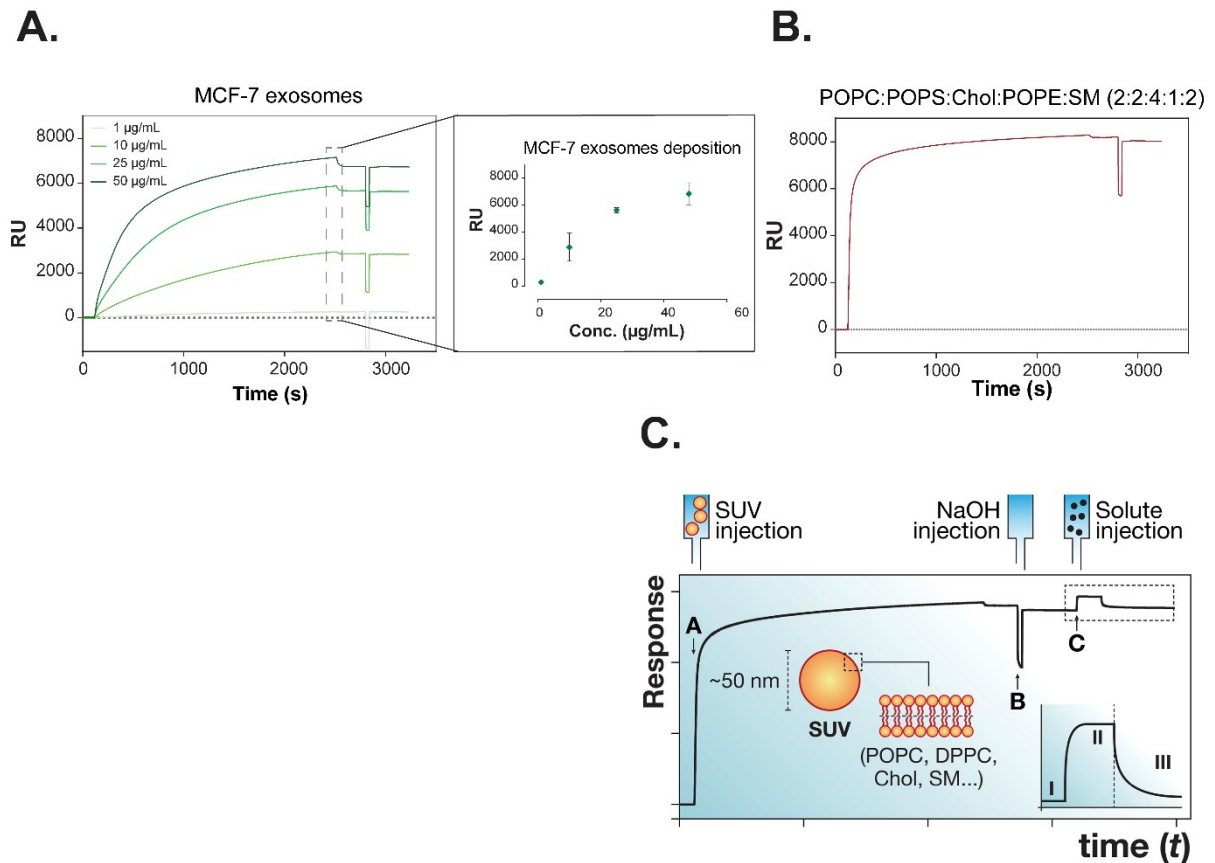


Figure S4. Deposition of MCF-7 derived exosomes and SUVs on the surface of L1 sensor chip. Concentration dependent deposition of MCF-7 derived exosomes (A). Representative spectrum deposition of SUVs mimicking the membrane lipid composition of exosomes (B). Scheme representing the spectrum obtained during an SPR experiment: A) deposition of vesicles on the surface of the L1 sensor chip; B) washing of the surface of L1 sensor chip with NaOH for removing unbound vesicles; C) injection of the peptide over the surface covered by vesicles; I) stable response; II) association phase; III) dissociation phase (C).

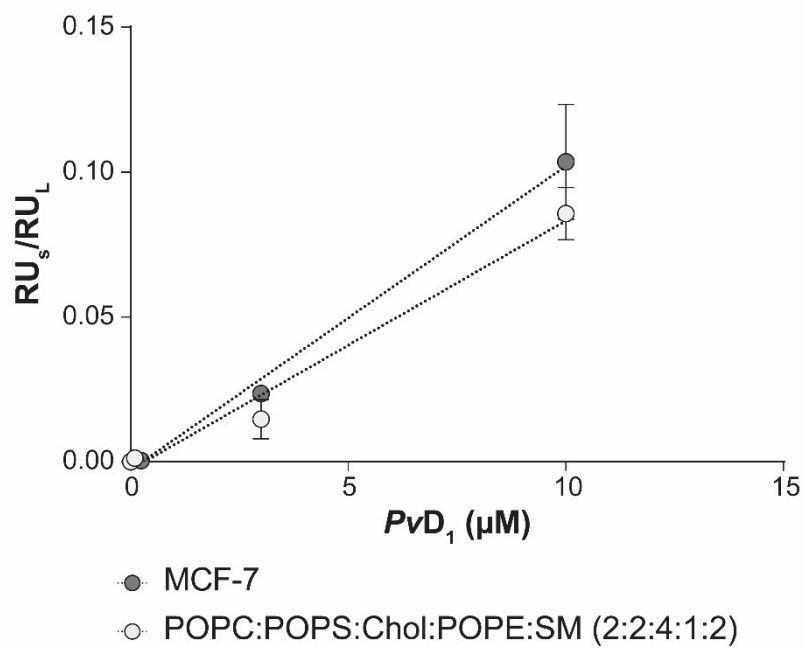
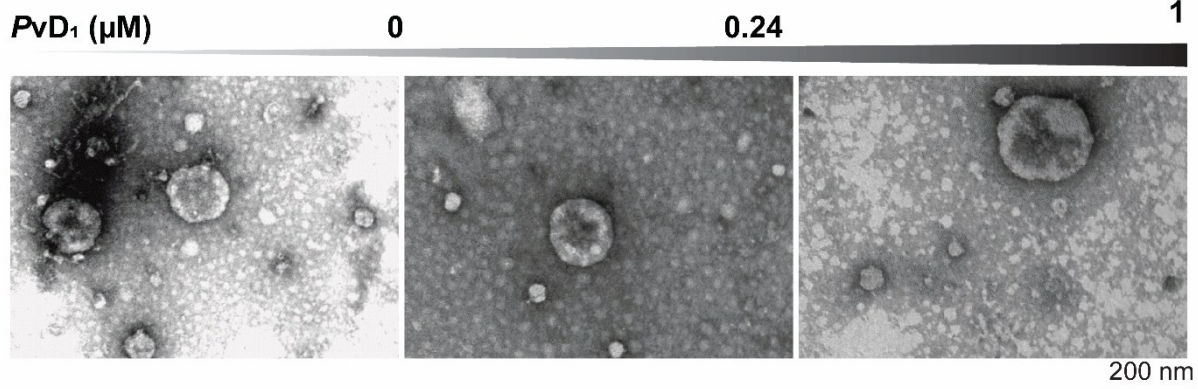


Figure S5. Affinity of PvD_1 to exosomes evaluated by SPR. Comparison between PvD_1 binding to MCF-7 exosomes and exosome-mimicking SUVs (POPC:POPS:Chol:POPE:SM 2:2:4:1:2).

A.

MCF-7 exosomes



B.

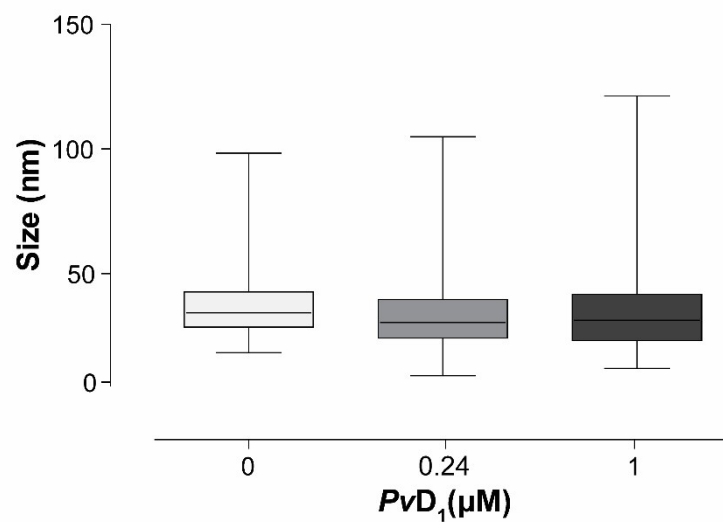


Figure S6. Transmission electron microscopy (TEM) size analysis of MCF-7 derived exosomes after contact with *PvD*₁. Representative TEM images of exosomes isolated from MCF-7 cells after treatment with *PvD*₁ (A). Average size derived from TEM images' analyses of exosomes isolated from MCF-7 cells treated with *PvD*₁ (B). Experiments were performed on different days using independent cell cultures.

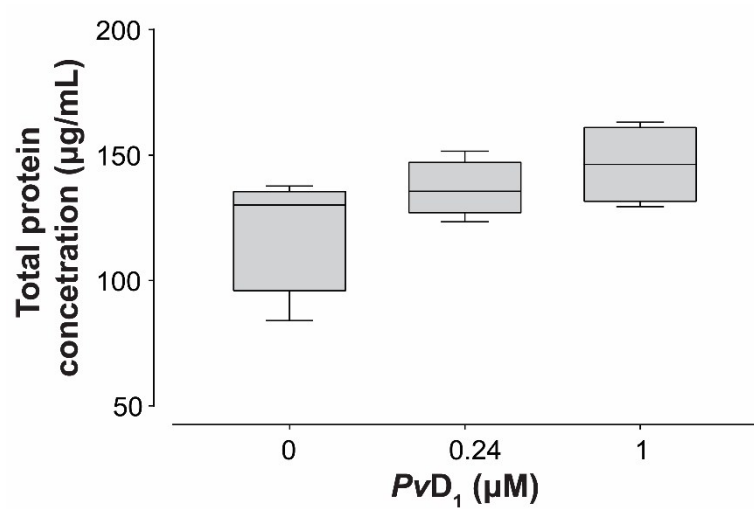


Figure S7. Quantification of total protein content of MCF-7 derived exosomes after cell contact with *PvD*₁. Total protein concentration (µg/mL) of MCF-7 exosomes, determined by MicroBCA assay.