Electronic Supplementary Information

Challenging metastatic breast cancer with the natural defensin PvD₁

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Figure S1. MDA-MB-231 (A, C) and MCF 10A (B, D) cell membrane roughness (R_{ms}) determined before and after *Pv*D1 (A, B) and HNP-1 (C, D) peptide contact. R_{ms} was evaluated using AFM height images over the nuclear and cytoplasm areas and finally over the entire cell and normalized to control. Number of analysed cells for determination of cell membrane R_{ms} in presence of *Pv*D₁ was 12 for each control and 10 for each *Pv*D₁ concentration tested on MDA-MB-231 cell line while for MFC 10A cell line was: 0.01 µM-10; 0.8 µM- 10 and 50 µM- 7. Cell membrane Rms determination in presence of HNP-1 was based on 12 cells for both cell lines. All experiments were repeated in different days using independently grown cell cultures.



Figure S2. HBMEC cell height (left graph) and cell membrane roughness (R_{ms} , right graph) was determined before and after peptide contact. R_{ms} was evaluated using AFM height images over the nuclear and cytoplasm areas and over the entire cell. Cell height and R_{ms} were normalized to control. Number of analysed cells for determination of HBMEC cell height in presence of PvD_1 was: 0 μ M - 13; 0.1 μ M- 11; 0.8 μ M- 12; 2.5 μ M- 11 and 50 μ M- 12. Cell membrane R_{ms} was based on 10 cells. All experiments were repeated in different days using independently grown cell cultures. A one-way ANOVA followed by a Tukey post-test was employed.



Figure S3. Variations in the cell membrane dipole potential of MCF 10A cells (A, B) and MDA-MB-231 cells (C, D) in the presence of PvD_1 were followed by di-8-ANEPPS fluorescence excitation spectra shifts. Differential spectra (A, C) were obtained by subtracting the normalized excitation spectra (to the integrated spectrum areas) of the suspended labeled cells in the presence of increasing PvD_1 concentration from the spectra obtained in the absence of PvD_1 . Normalized excitation ratios (R_{norm}) were used to quantify the magnitude of dipole potential perturbation (B, D). All experiments were repeated in different days using independently grown cell cultures. Statistical significance was evaluated with an unpaired two-tailed *t*-test. *0.01 < *p*-value < 0.05; **** *p*-value < 0.0001.



Figure S4. Representative Force-distance (F-d) retraction curves obtained during AFM-SCFS experiments in the absence and presence of PvD_1 peptide. (A) MDA-MB-231 – MDA-MB-231; (B) MDA-MB-231 – HBMEC and (C) HBMEC – MDA-MB-231 interactions, respectively.



Figure S5. Adhesion strength (F_D , A) and work (W_D , B) in the absence and presence of PvD_1 are presented for MDA-MB-231 cells –HBMEC-cantilever bound cells. Tethers extraction forces in the absence and presence of peptide are presented for MDA-MB-231 cells –MDA-MB-231-cantilever bound cells (C), MDA-MB-231 cells – HBMEC-cantilever bound cells (D) and HBMEC - MDA-MB-231-cantilever bound cells (E). 16 to 28 cells were analyzed and the number of force-distance curves obtained for each peptide concentration tested was: (A, B, D) 0 μ M - 67; 0.01 μ M- 69; (C) 0 μ M - 65; 0.01 μ M- 63; 0.8 μ M- 47 and 50 μ M- 54; (E) 0 μ M - 97; 0.1 μ M- 123; 0.8 μ M- 63 and 2.5 μ M- 59. Statistical significance was evaluated with a Mann-Whitney test. *0.01 < *p*-value < 0.05; **0.001 < *p*-value < 0.001; ***0.0001 < *p*-value < 0.001; **** *p*-value < 0.0001.