Supporting information

Designed Gramicidin Inspired Stabilized Peptide-based Therapeutics to Potentiate Immunotherapy Against Aggressive Kidney Cancer.

Argha Mario Mallick, Ananya Chatterjee, Arun Bahadur Gurung, Prithviraj Uttarasili, Archana Tripathi, Monjuri Hembram, Anand Srivastava, Rituparna Sinha Roy**

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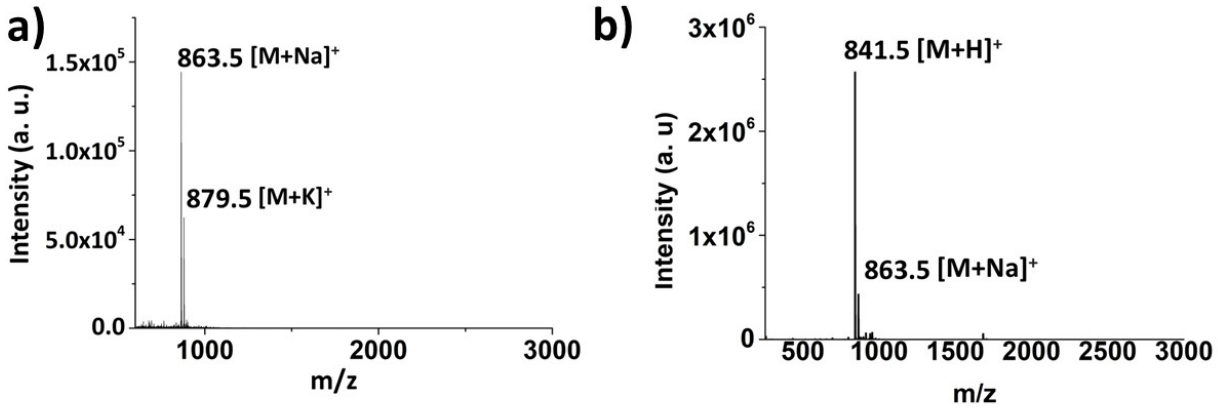

Figure S1. (a) MALDI-TOF mass spectrum of LD8Δ. Calculated mass: 840 Da; Observed mass: 863.5 Da $[M+Na]^+$ and 879.5 Da $[M+K]^+$. (b) ESI-MS spectrum of LD8Δ. Calculated mass: 840 Da; Observed mass: 841.5 Da $[M+H]^+$ and 863.5 Da $[M+Na]^+$

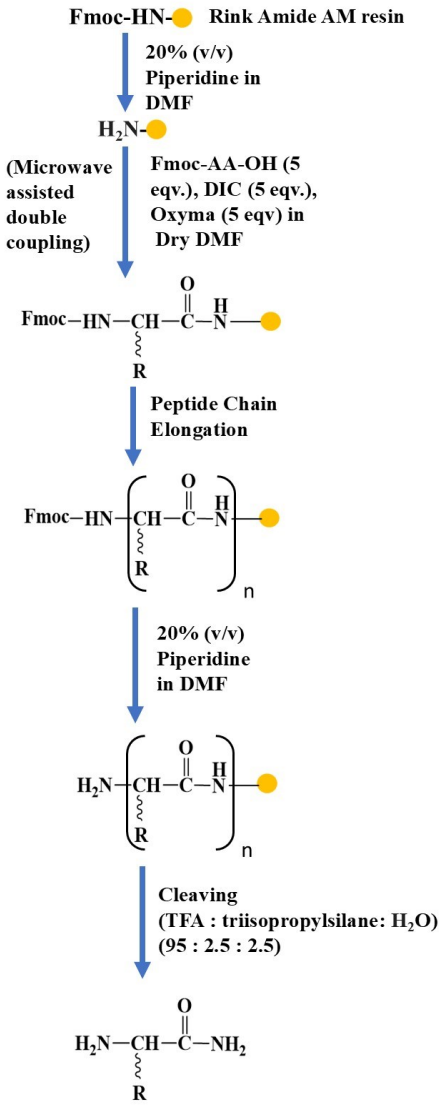

Figure S2. General synthesis scheme of peptide LD8Δ.

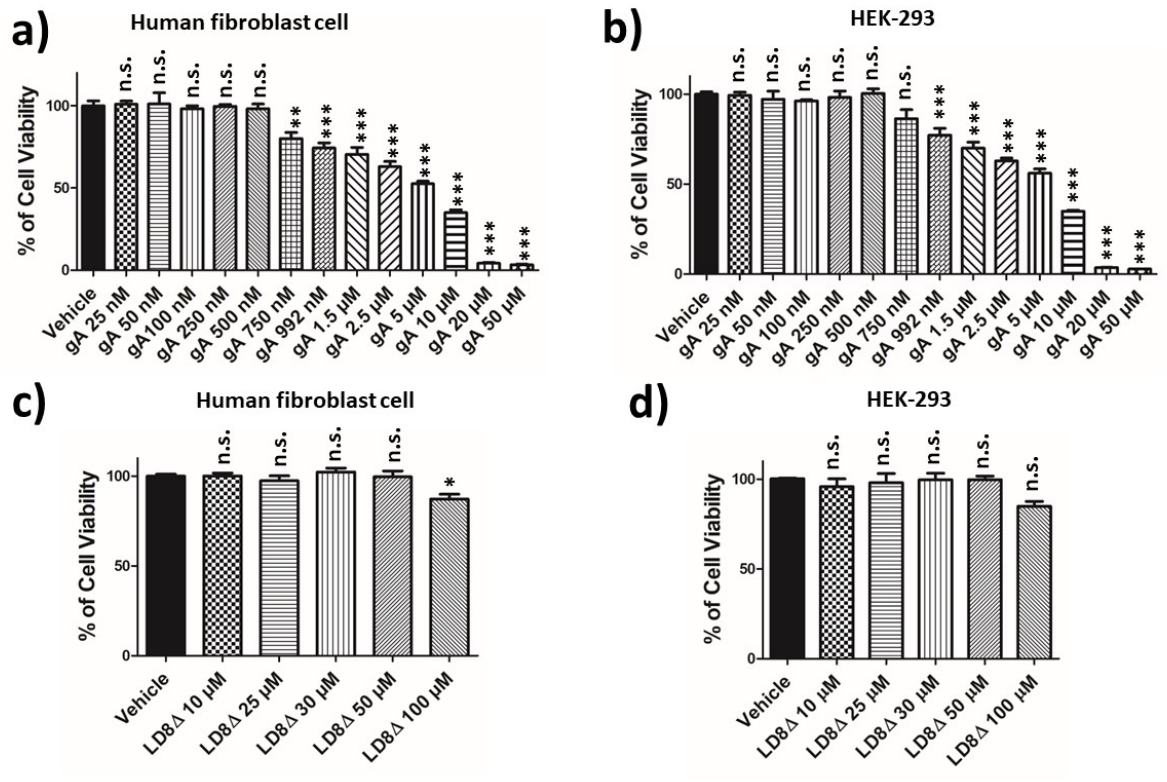

Figure S3. (a) Cytotoxicity analysis of gA at 72 h time point in human fibroblast cell line. (b) Cytotoxicity analysis of gA at 72 h time point in human HEK-293 cell line. (c) Cytotoxicity analysis of peptide LD8Δ at 72 h time point in human fibroblast cell line. (d) Cytotoxicity analysis of peptide LD8Δ at 72 h time point in human HEK-293 cell line. Cytotoxicity data reveals that LD8Δ is biocompatible to both human fibroblast cells and HEK-293 cells, whereas gA induces toxicity to both the cell lines at 992 nM whereas, LD8Δ was non-toxic to both the cell lines till 50 µM. Please note that, the maximum concentration of LD8Δ used for all the experiments in this study is 30 µM.

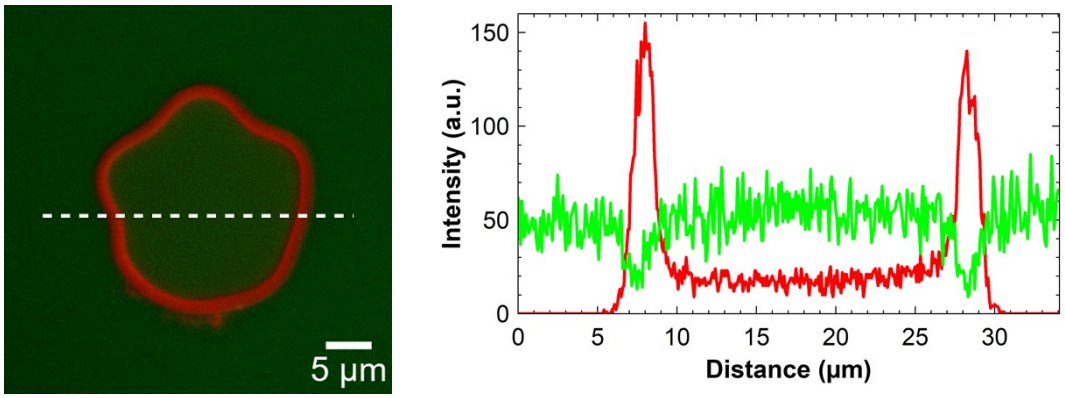

Figure S4. Representative examples of some population of plasma membrane mimicking GUVs exhibiting leakage (dye influx) in addition to structural deformation in the presence of LD8Δ 27.95 µM after 1 h incubation. Right panel shows the respective intensity profiles of FAM dye (green) and CM-Dil dye (red) along the radial axis of GUVs (white dotted line). Among all the conditions performed in this study (gA, LD8Δ and melittin) as represented in Figure 3d only LD8Δ exhibited structural deformations of GUV.

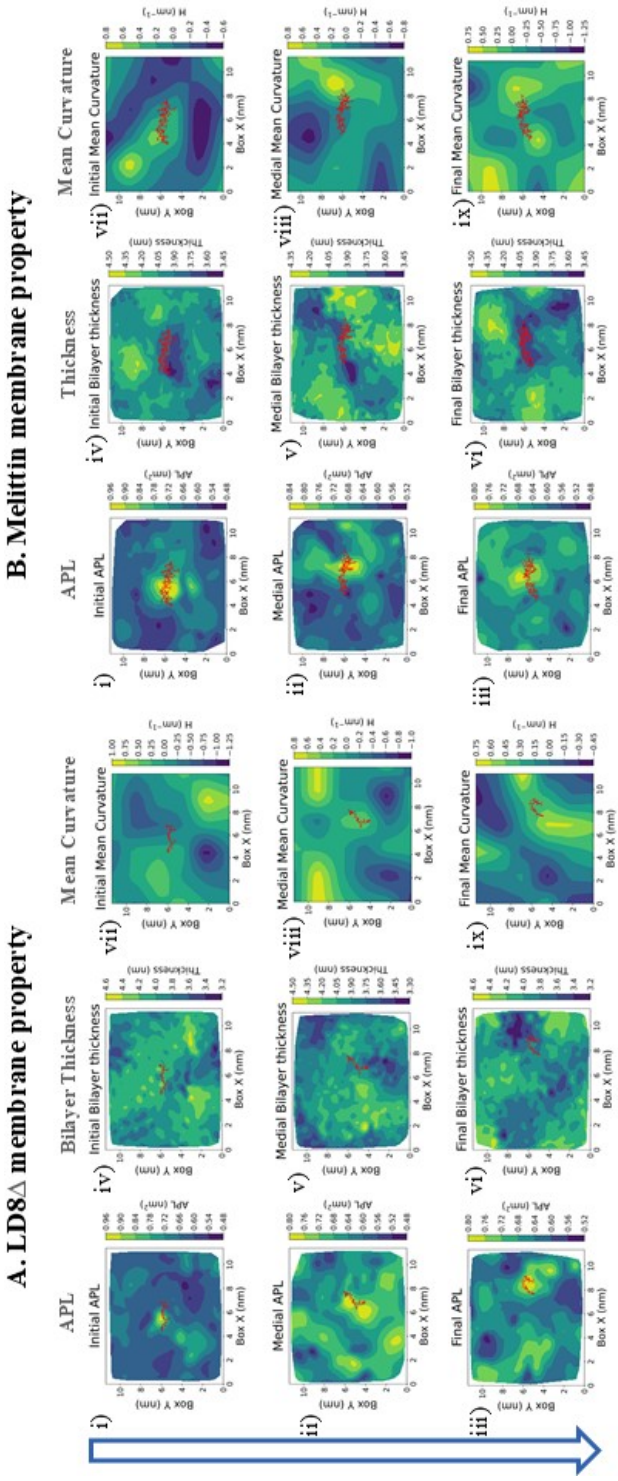

Fig S5. (Computational data) Temporal progress of membrane properties for (A) LD8Δ system and (B) Melittin system. Contour plots for (i-iii) APL, (iv-vi) membrane thickness, and (vii-ix) mean curvature shown at initial, medial and final stages (shown as the downward arrow from initial to final stage) of trajectory averaged over 500ps. The peptide position along X-Y plane was depicted by the red dots in the plot.

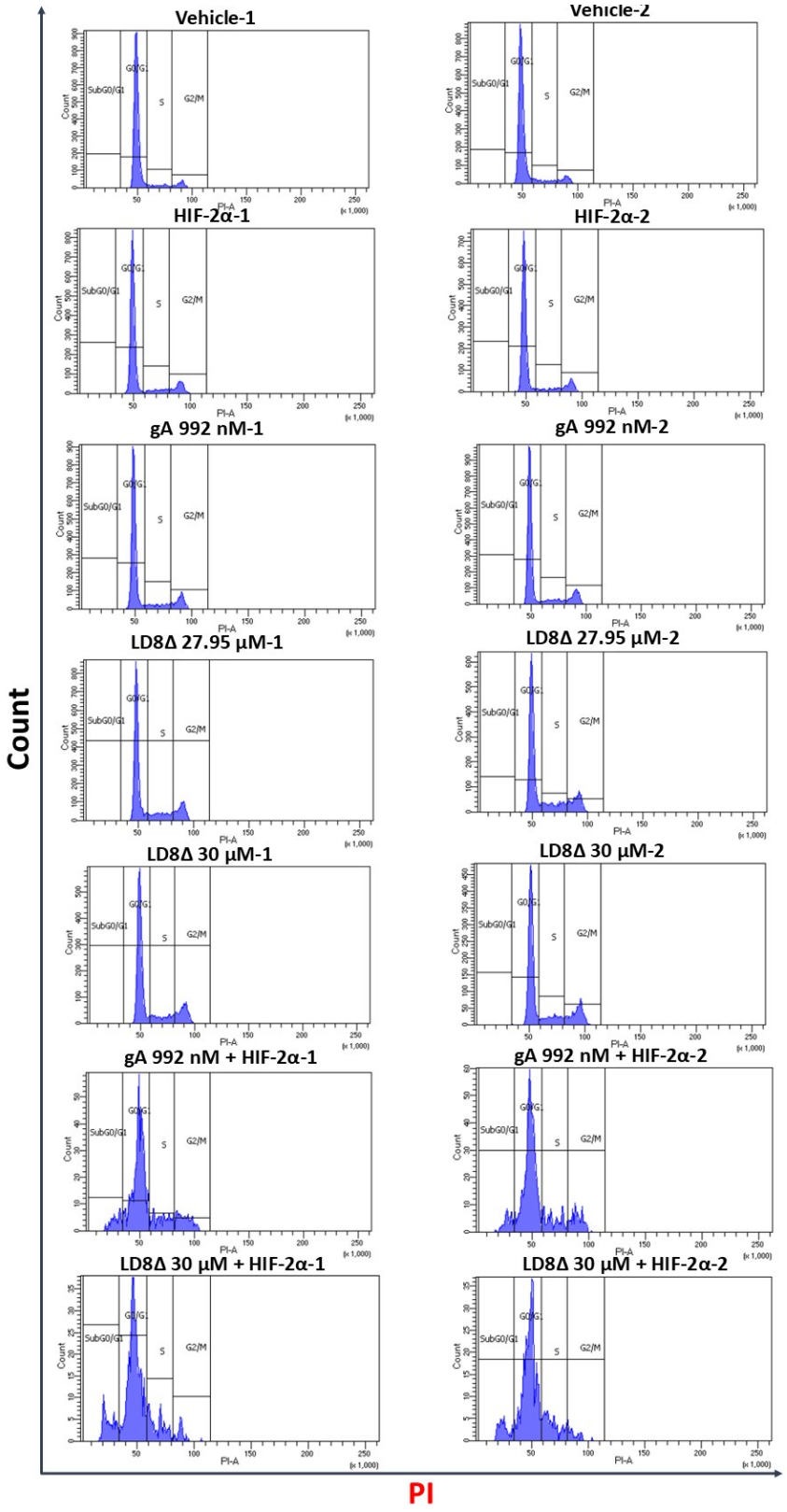

Fig S6. Flow cytometry analysis for assessing cell cycle arrest of SK-RC-45 cells treated with individual conditions taken in the study for 72 h by PI staining.

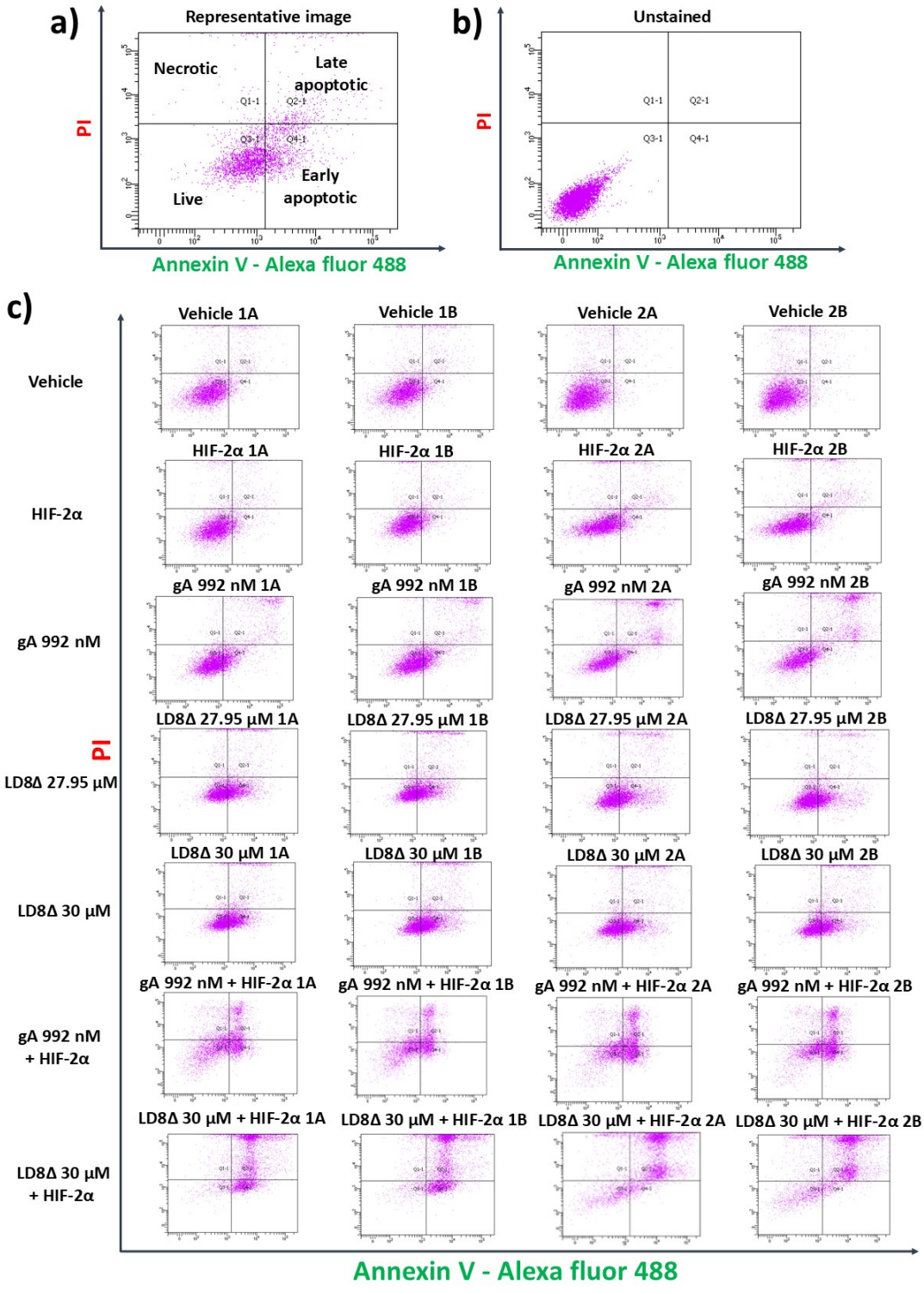

Fig S7. (a) Representative image describing the cell populations in each quadrant in apoptosis and necrosis assay by Annexin V and PI staining flow cytometry method. Flow cytometry analysis for assessing apoptosis and necrosis of (b) unstained SK-RC-45 cells and (c) SK-RC-45 cells treated with individual conditions taken in the study after 72 h incubation.

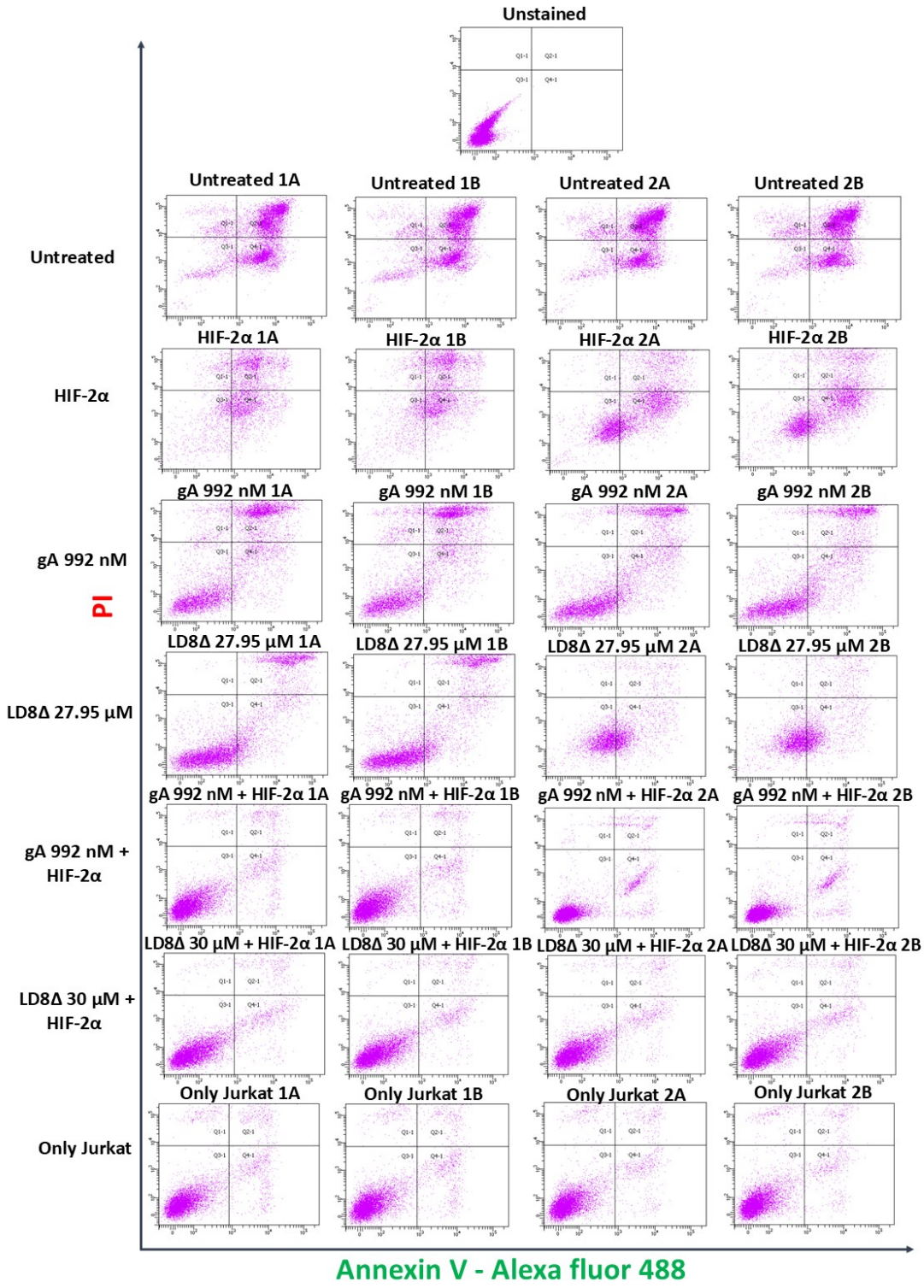

Fig S8. Flow cytometry analysis for assessing apoptosis of Jurkat cells mediated by SK-RC-45 cells treated with individual conditions used in the study for 72 h in a co-culture of SK-RC-45 cells and Jurkat cells (3:1) for 72 h.

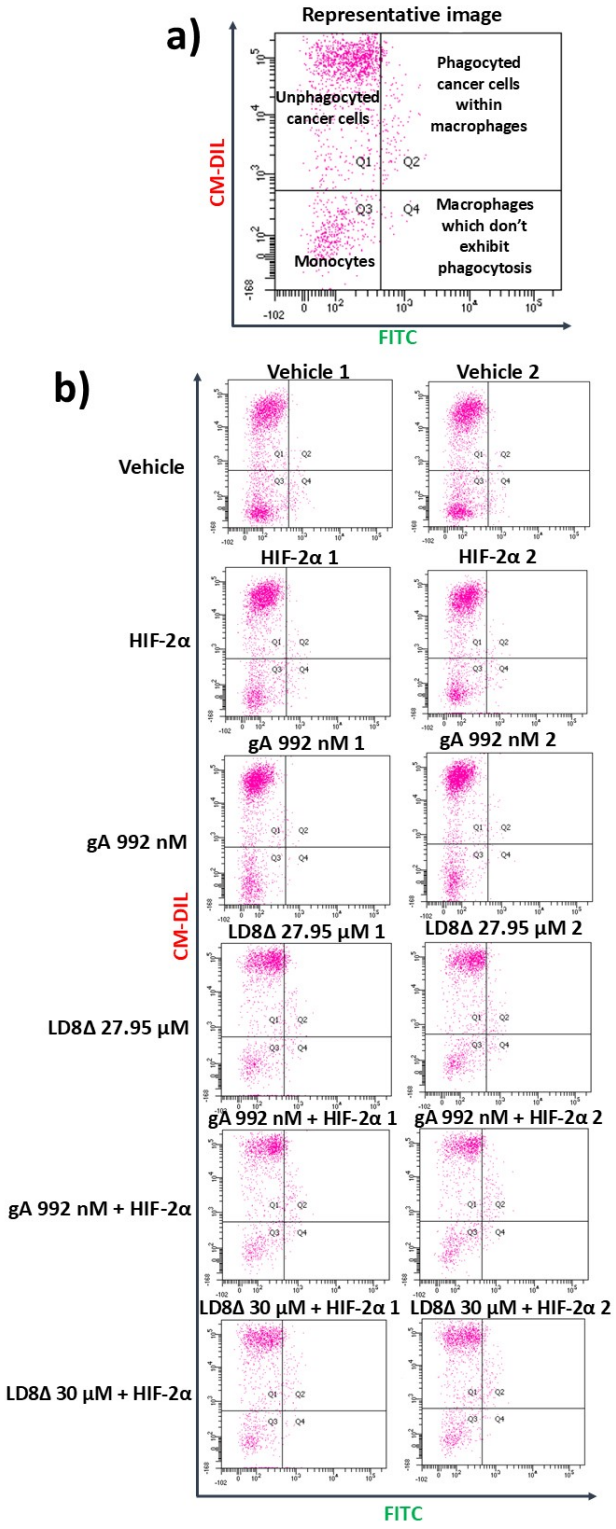

Fig S9. (a) Representative image describing the cell populations in each quadrant for assessing phagocytic ability of THP-1 cells by flow cytometry. **(b)** Flow cytometry analysis for assessing phagocytic ability of THP-1 cells for SK-RC-45 cells treated with individual conditions taken in the study for 72 h in a co-culture of SK-RC-45 cells and THP-1 cells (4:1) for 4 h.

Table S1. Primer sequences used for real time PCR.

Gene name	Forward primer	Reverse primer
GAPDH	5’-GTCTCCTCTGACTTCAACAGCG-3’	5’-ACCACCCTGTTGCTGTAGCCAA-3’
VEGF	5’-TGCAGATTATGCGGATCAAACC-3’	5’-TGCATTCACATTTGTTGTGCTGTAG-3’
HIF-1α	5′-CCACAGGACAGTACAGGATG-3′	5′-TCAAGTCGTGCTGAATAATACC-3′
HIF-2α	5′-GTCTCTCCACCCCATGTCTC-3′	5′-GGTTCTTCATCCGTTTCCAC-3′
VHL	5′-ATGGCTCAACTTCGACGGC-3′	5′-CAAGAAGCCCATCGTGTGTC-3′
m-TOR	5’-AGCATCGGATGCTTAGGAGTGG-3’	5’-CAGCCAGTCATCTTTGGAGACC-3’
CD-47	5’-TATCCTCGCTGTGGTTGGACTG-3’	5’-TAGTCCAAGTAATTGTGCTAGAGC-3’
PDL-1	5’-TGCCGACTACAAGCGAATTACTG-3’	5’-CTGCTTGTCCAGATGACTTCGG-3’
DAB2IP	5′-TCATCGCCAAGGTCACCCAGAA-3′	5′-CGCTGCATGTTGGTCCACTCAT-3′
GM2-synthase	5’-AAGCTACCAGACCAACACAGCAGA-3’	5’-GGCAGCTTCAGTTTGGATGCATGA-3’