

Supplementary Information

A Bioinspired Pseudopeptide-based Intracellular Delivery Platform Enhances the Cytotoxicity of a Ribosome-inactivating Protein through Multiple Death Pathways

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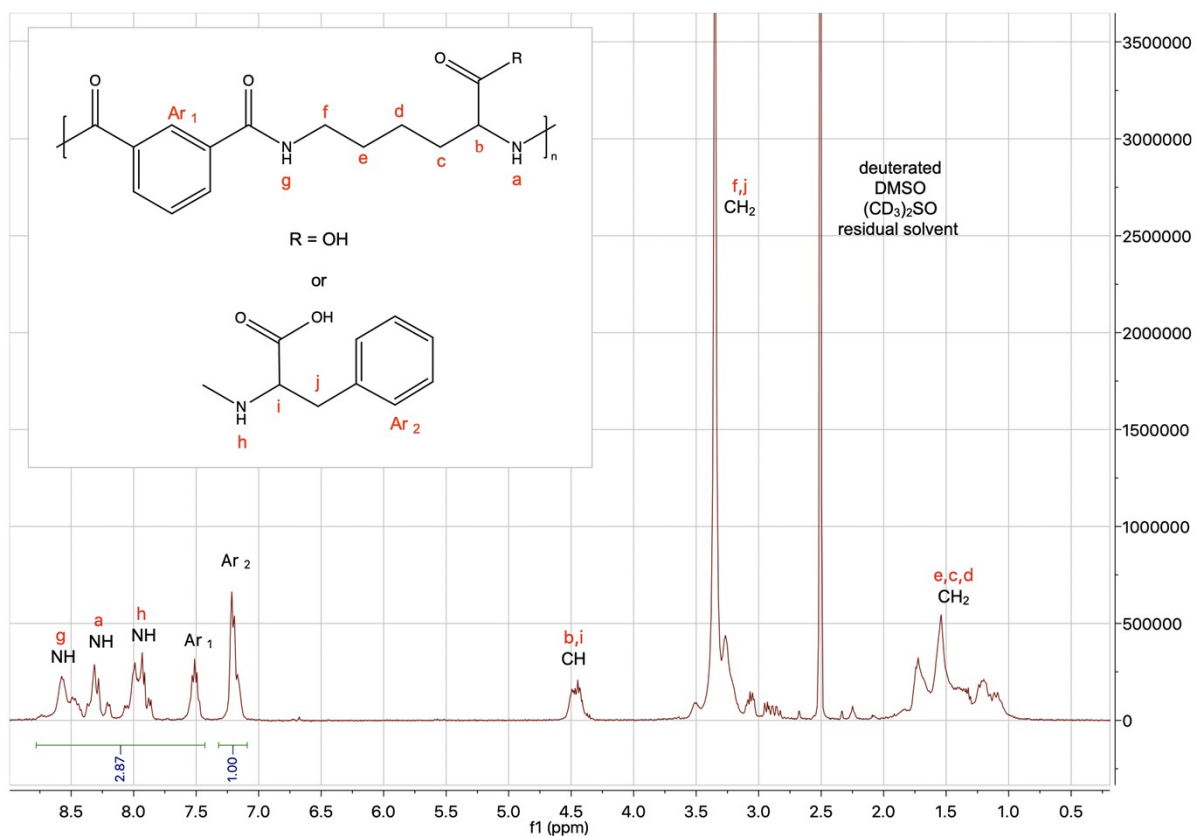
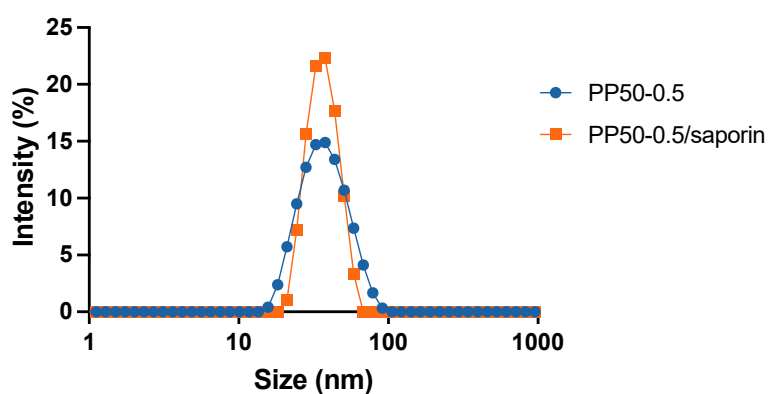


Figure S1. Chemical structure and ^1H -NMR spectrum of PP50 in acidic form in d_6 -DMSO at room temperature.



Sample	Diameter (nm)	PDI
PP50-0.5	39.2 ± 8.1	0.210 ± 0.029
PP50-0.5/saporin	45.5 ± 11.5	0.253 ± 0.018

Figure S2. The hydrodynamic size distributions and the summary table of particle diameters of PP50-0.5 and PP50-0.5/saporin at pH 6.5, respectively, measured by dynamic light scattering ([PP50] = 0.5 mg mL⁻¹; [Saporin] = 1 nM). Mean results ± SD (n=3).

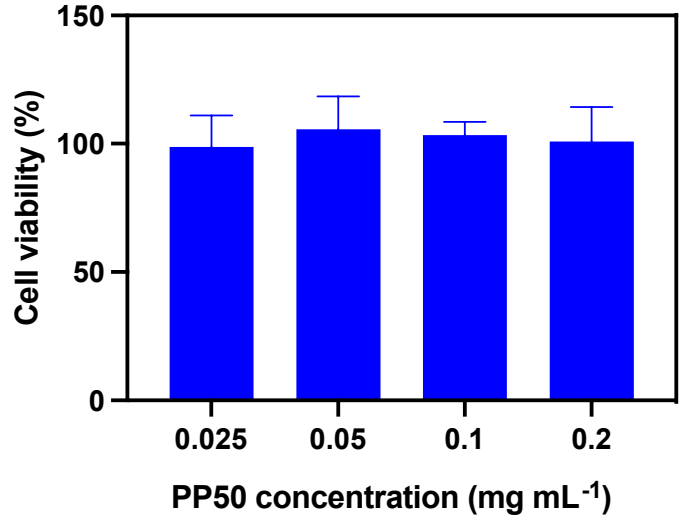


Figure S3. Effects of PP50 at varying concentrations on viability of A549 cells. Cell viability was determined using the CellTiter-Glo® 2.0 Cell Viability Assay after 1 h treatment plus 72 h further incubation in DMEM. Mean results \pm SD (n=3).

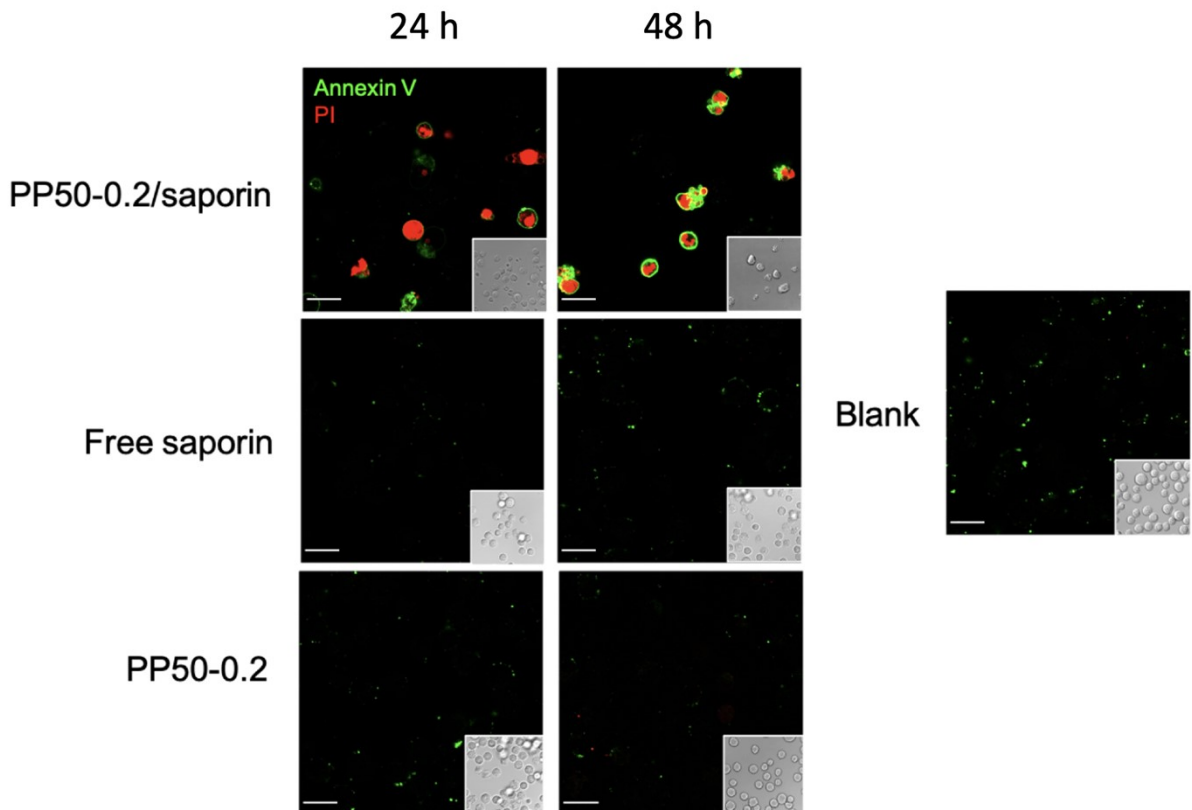


Figure S4. Fluorescence and phase contrast microscopy analysis of Annexin V/PI double staining of A549 cells after exposure to saporin (1 nM) in the presence or absence of PP50-0.2 after 1 h treatment plus 24 or 48 h further incubation in DMEM. Scale bar 20 μ m.

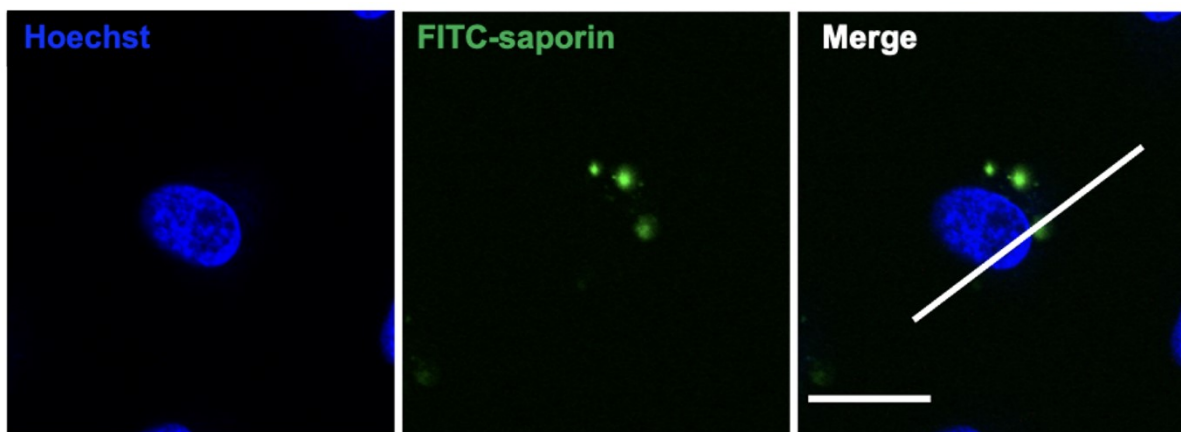


Figure S5. Confocal microscopy images illustrating the intracellular localization in A549 after 1 h treatment with free FITC-saporin ($125 \mu\text{g mL}^{-1}$) plus 24 h further incubation in DMEM. The cell nucleus was counterstained with Hoechst 33342. Scale bar 20 μm .

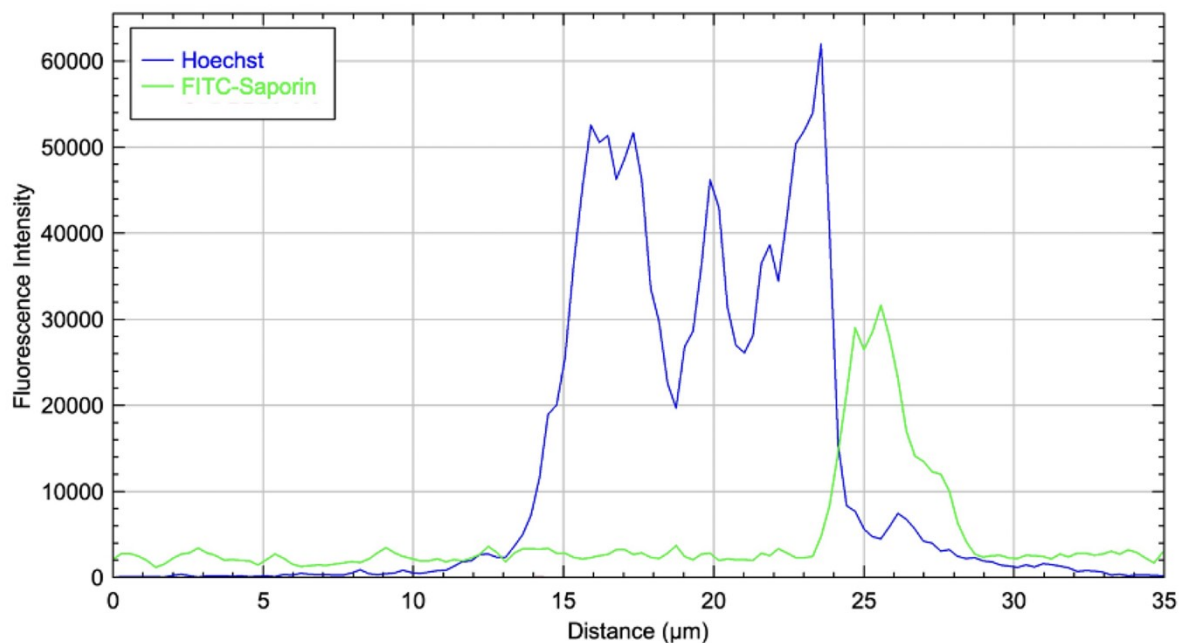


Figure S6. Fluorescence intensity profiles of A549 cells after 1 h treatment with free FITC-saporin ($125 \mu\text{g mL}^{-1}$) plus 24 h further incubation in DMEM, produced from the cross section along the white line in Figure S4.

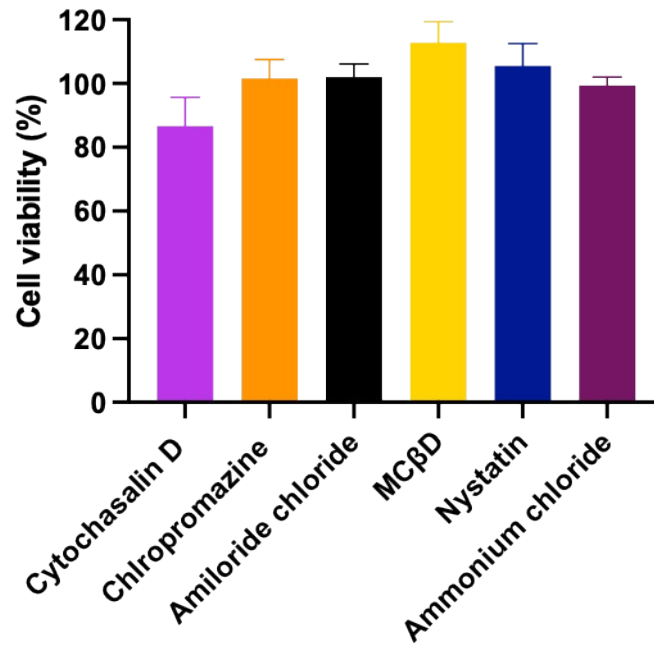


Figure S7. Cell viabilities after inhibitor treatment. A549 cells were pre-incubated with the specific inhibitor for 30 min. Cell viability was determined using the CellTiter-Glo[®] 2.0 Cell Viability Assay after 1 h treatment with corresponding inhibitor plus 72 h further incubation. Mean results \pm SD (n = 4).