## Inventory of Supplementary Information

### Supplemental Data

**Figure S1**, showing fluorescence dequenching kinetics in supplemented cell culture medium.

**Figure S2**, showing a comparison between single-tail and double-tail peptide amphiphile internalization by SJSA-1 cells.

**Figure S3**, quantifying peptide amphiphile internalization by SJSA-1 cells in presence of sucrose.

**Figure S4**, confirming **PA** uptake by MDA-MB-435 cells in vitro.

### Supplemental Experimental Procedures.

Detailed description of materials used, synthesis of peptides/peptide amphiphiles, cell culture procedures and cell viability studies.

### Supplemental References

**Supplemental Data**

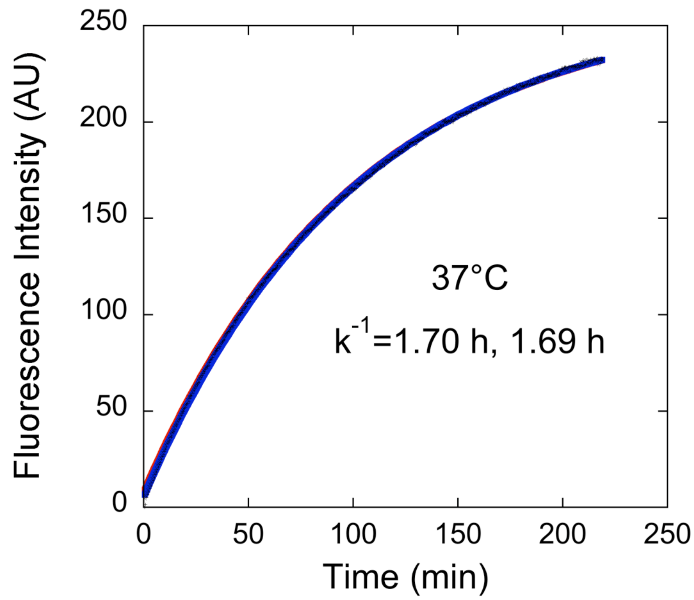


**Figure S1.** Micelle fluorescence de-quenching kinetics monitored in supplemented (10% serum) RPMI 1640 cell culture medium at 37 °C (n=2). Monomer desorption rates were calculated from the best fit using equation $I(t) = [I(0) - I(\infty)]e^{-kt} + I(\infty)$, where k is the monomer desorption constant (see main text for experimental details).

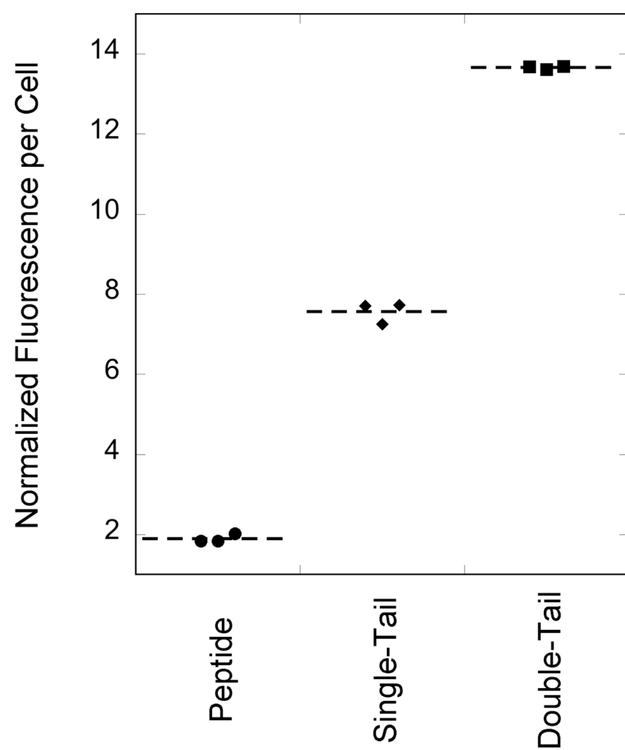


**Figure S2.** Internalization of **peptide,** single-tailed palmitoyl-p53$_{14\text{-}29}$ and double-tailed **PA** by SJSA-1 osteosarcoma cells. Cells were incubated for 4 h with 10 μM of each formulation in supplemented cell culture media. The normalized fluorescence intensity per cell is reported with untreated cells used to normalize fluorescence (intensity=1).

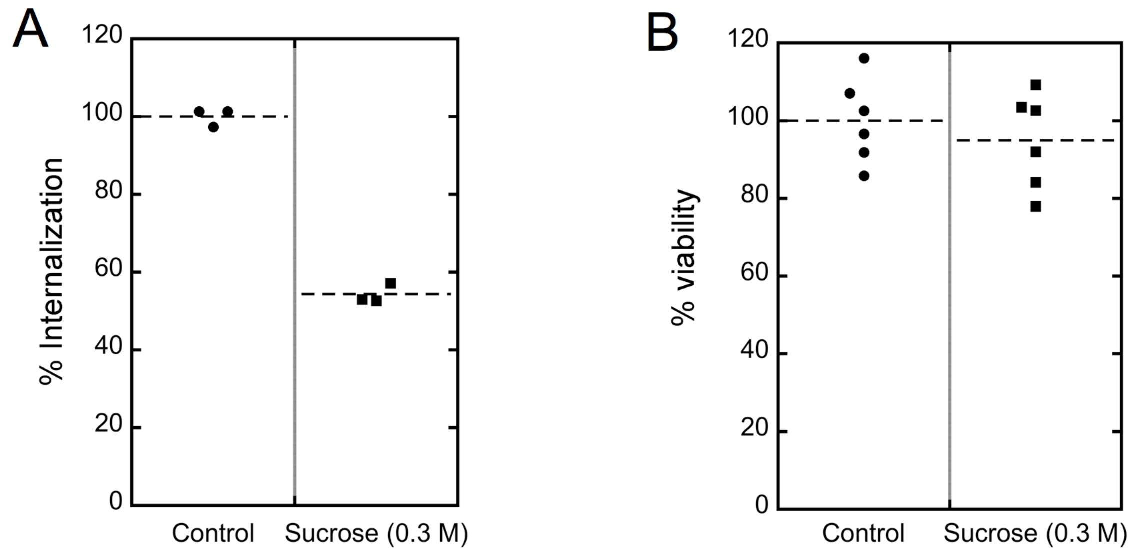


**Figure S3.** Internalization of **PA** (25 μM) by SJSA-1 cells was inhibited 50% in the presence of 0.3 M sucrose following a 4-hour incubation (left). Over the same period of time, no decrease in cell viability was observed in presence of sucrose (right).

**Figure S4. PA** internalize in MDA-MB-435 cells. Cells were treated for 4 h with 10 uM **PA** in supplemented cell culture media, washed and analyzed by flow cytometry. Non-treated cells were used to normalize mean fluorescence per cell (=1 for control).

**Supplemental Experimental Procedures**

*Materials*

Peptide p53$_{14\text{-}29}$ (LSQETFSDLWKLLPEN) was synthesized on Rink amide resin using manual solid-phase peptide synthesis (SPPS) and Fmoc-based chemistry. A lysine was incorporated at the N-terminus of the sequence for fluorescent labeling at its ε-amine with 5,6-carboxytetramethylrhodamine. The di-alkyl lipid acid 4-(1,5-bis(hexadecyloxy)-1,5-dioxopentan-2-ylamino)-4-oxobutanoic (diC$_{16}$COOH) was synthesized as described previously[1]. Coupling of diC$_{16}$COOH at the N-terminus of the peptide was performed on the resin using N-Hydroxybenzotriazole/O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HOBT/HBTU) as coupling reagents. Following cleavage from the resin, the peptide or PAs were purified using high-performance liquid chromatography (HPLC; Shimadzu Corporation) on a reverse-phase C8 column (Luna), with gradients of acetonitrile in water containing 0.1% trifluoracetic acid. Identity of products was verified by electrospray ionization mass spectrometry and purity was determined using analytical HPLC on a reverse-phase C8 column (Luna). Materials of purity greater than 95% were stored dry at -20°C until used.

PAs were dissolved in a 1:1 mixture of chloroform and methanol. The organic solvents were then evaporated under $N_2$ flow to form a film on the glass vial wall, which was then dried in vacuum. Hydration in 10 mM phosphate buffer saline (PBS) at 60°C for 1 hour resulted in dissolution of the film and formation of self-assembled micelles. Following cooling to room temperature the micelle solutions were filtered through a 0.45 µm polycarbonate syringe filter (Millipore) and stored at 4°C until used (within 1 week of preparation).

Small unilamellar vesicles composed of egg phosphotidylcholine (eggPC; Avanti Polar lipids) and with a nominal diameter of 100 nm were prepared as previously described[2].

*Cell Culture*

The human osteosarcoma SJSA-1 cell line was cultured as exponentially growing, sub-confluent monolayers in RPMI-1640 culture medium (ATCC). MDA-MB-435 human cancer cells and NIH 3T3 fibroblasts were cultured as exponentially growing, sub-confluent monolayers in DMEM culture medium (Gibco) and HeLa cells were cultured as exponentially growing, sub-confluent monolayers in MEM-Alpha culture medium (Gibco). All culture media were supplemented with 10% v/v calf bovine serum (ATCC) and 0.1% v/v Penicillin/Streptomycin (Gibco). Cells were grown at 37 °C, humidified atmosphere and 5% $CO_2$. For all *in vitro* studies SJSA-1 cells were seeded at a density of $15\times10^3$ cells/cm$^2$, MDA-MB-435 cells at a density of $10\times10^3$ cells/cm$^2$, NIH 3T3 fibroblasts and HeLa cells at a density of $5\times10^3$ cells/cm$^2$. Cells were allowed to attach on the surfaces overnight (12 h). Internalization of peptides and peptide amphiphiles was assessed using epifluorescence microscopy and flow cytometry analysis.

*Cell viability studies*

Cell viability was assessed using the 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. Briefly, formulations were incubated with cells for 4 hours, the cells washed twice with PBS and further incubated 20 hours with fresh culture medium. Then, 10 μl of MTT solution (5 mg/ml in PBS) were added in each well and cells were incubated for another 3 hours. The medium was removed, cells were

washed twice and 100 μl of a 1:1 mixture of dimethyl sulfoxide (DMSO) and methanol were added in each well. Absorption was measured at 595 nm using a Tecan Safire well plate reader.

Cell viability was determined for amiloride and MβCD treatments by measuring the fractions of cells that showed propidium iodide staining above control levels using flow cytometry. To stain dead cells, a PI solution (1 μg/ml) was and cells were incubated for 15 minutes at 4 °C prior to analysis.

## Supplemental References


1. Berndt, P., Fields, G.B., and Tirrell, M. (1995). Synthetic Lipidation of Peptides and Amino-Acids - Monolayer Structure and Properties. Journal of the American Chemical Society *117*, 9515-9522.
2. Missirlis, D., Khant, H., and Tirrell, M. (2009). Mechanisms of peptide amphiphile internalization by SJSA-1 cells in vitro. Biochemistry *48*, 3304-3314.