Supporting InformationCathepsin-Mediated Cleavage of Peptides from Peptide Amphiphiles Leads to Enhanced Intracellular Peptide Accumulation

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SUPPORTING FIGURES

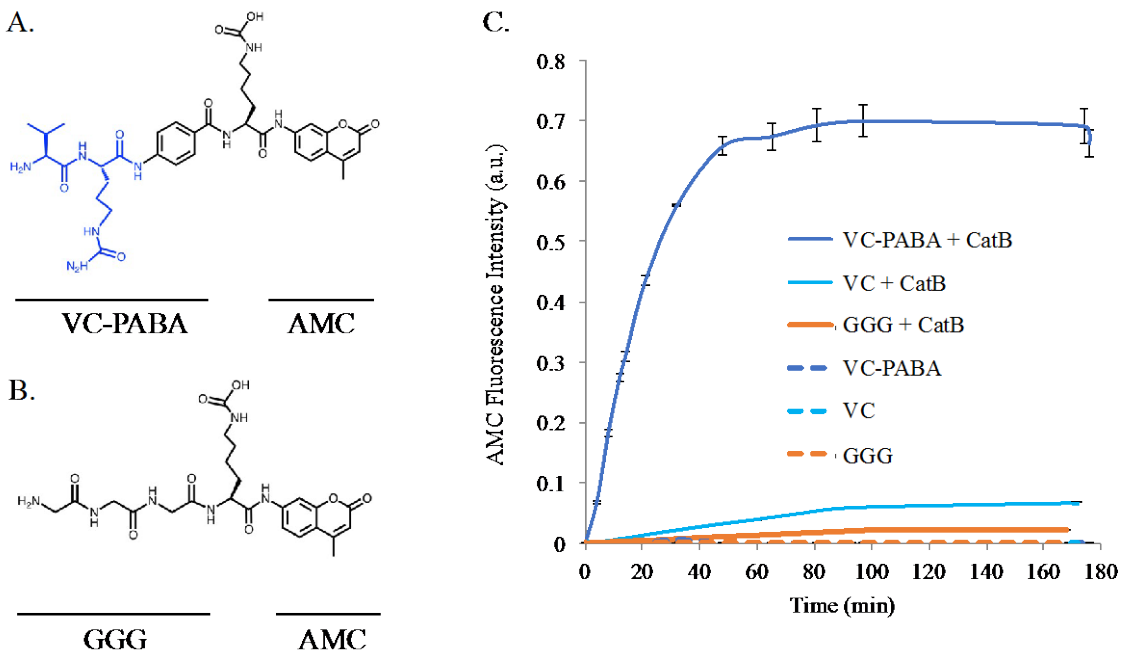


Figure S1. Chemical structure of the enzyme cleavable sequence, (A) Valine-Citrulline-Para(aminobenzoic acid) (VC-PABA)-AMC; (B) Control, non-cleavable sequence, Glycine-Glycine-Glycine (GGG)-AMC; (C) AMC fluorescence intensity change over time following addition of recombinant human cathepsin-B (catB) or PBS control.

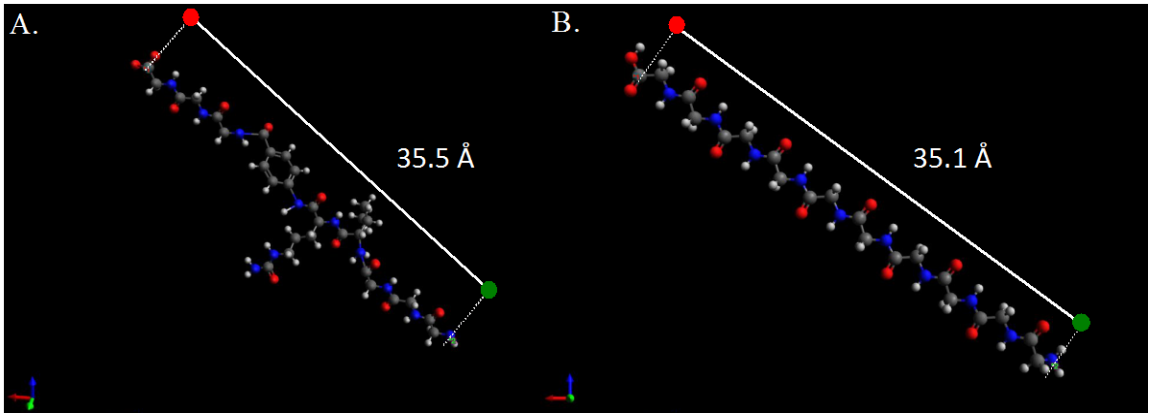


Figure S2. The length between FAM and Tamra FRET fluorophores on the amino acid linkers between (A) VC-PABA-p53$_{(14-29)}$: 35.5 Å, and (B) GGG-p53$_{(14-29)}$: 35.1 Å .

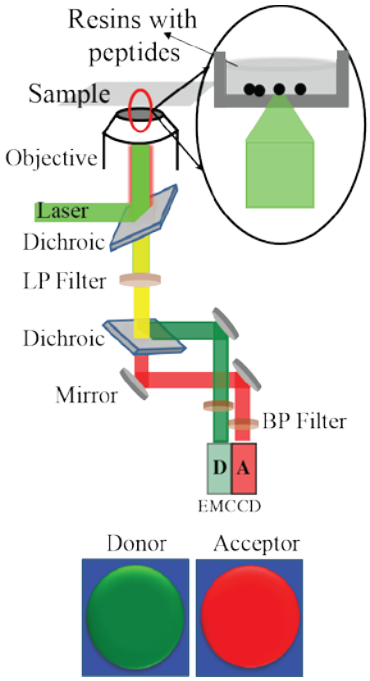


Figure S3. Wide field microscope setup used to measure FRET signal of a small number of peptide targets immobilized on the microscope viewing chamber as depicted in Figure 2.

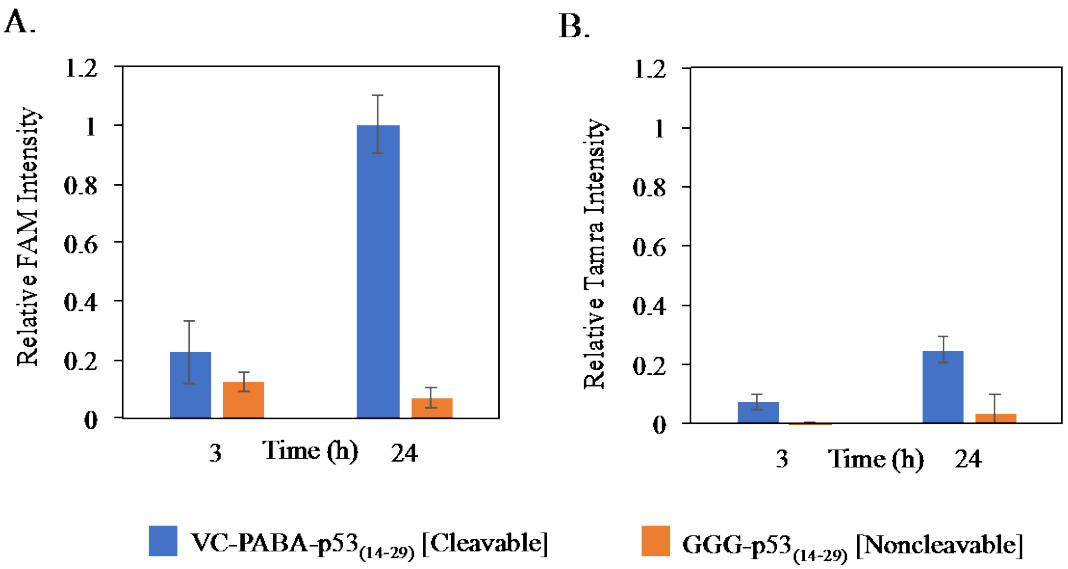
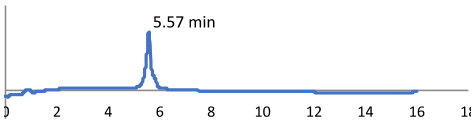


Figure S4. Relative intensity of cleaved (A) FAM and (B) Tamra following the addition of recombinant catB as depicted in Figures 2 and S3. The fluorescence intensity of Tamra remained low even after 24 hours while that of FAM increased over time only in VC-PABA- $p53_{(14-29)}$ samples indicating enzymatic cleavage reliant on VC-PABA and resistance of $p53_{(14-29)}$ to enzymatic degradation.

A.



B.

C.

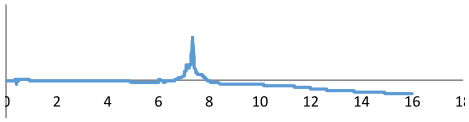
D.

E.

min

Figure S5. LC/MS of diC_{16}-VC-PABA- $p53_{(14-29)}$ shows uniform elution peaks with UV absorbance to detect peptide bonds at 220nm (A), phenylalanine at 280nm (B), FAM at 490nm (C), and Tamra at 565nm (D). Electrospray ionization (ESI) mass spectrum of these peaks confirmed a predicted molecular weight of 4409 g/mol (E).

A.



B.

C.

D.

E.

Figure S6. LC/MS of diC_{16}-GGG- $p53_{(14-29)}$ shows uniform elution peaks with UV absorbance to detect peptide bonds at 220nm (A), phenylalanine at 280nm (B), FAM at 490nm (C), and Tamra at 565nm (D). Electrospray ionization (ESI) mass spectrum of these peaks confirmed a predicted molecular weight of 4249 g/mol (E).

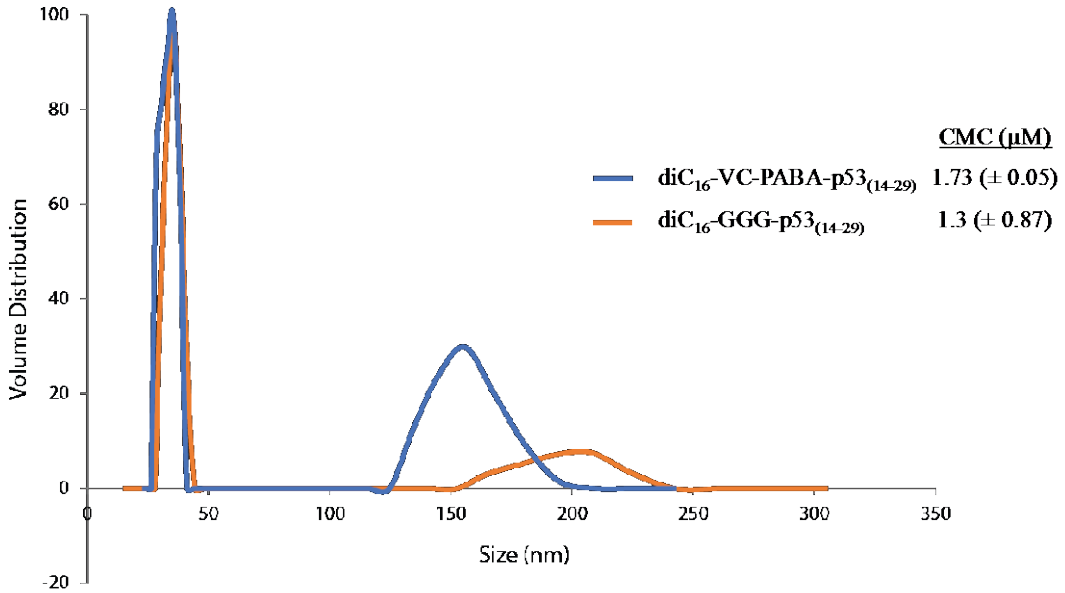


Figure S7. Dynamic light scattering (DLS) of diC_{16}-VC-PABA-$p53_{(14-29)}$ (blue) and diC_{16}-GGG-$p53_{(14-29)}$ (orange) PAs indicate predominant scattering of micelles between 10-40 nm. A second scattering at 150 nm (diC_{16}-VC-PABA-$p53_{(14-29)}$) and 200 nm (diC_{16}-GGG-$p53_{(14-29)}$) is likely secondary to PA aggregation secondary to the FAM and Tamra hydrophobic dyes. Critical micelle concentration (CMC) for PA micelles are similar.

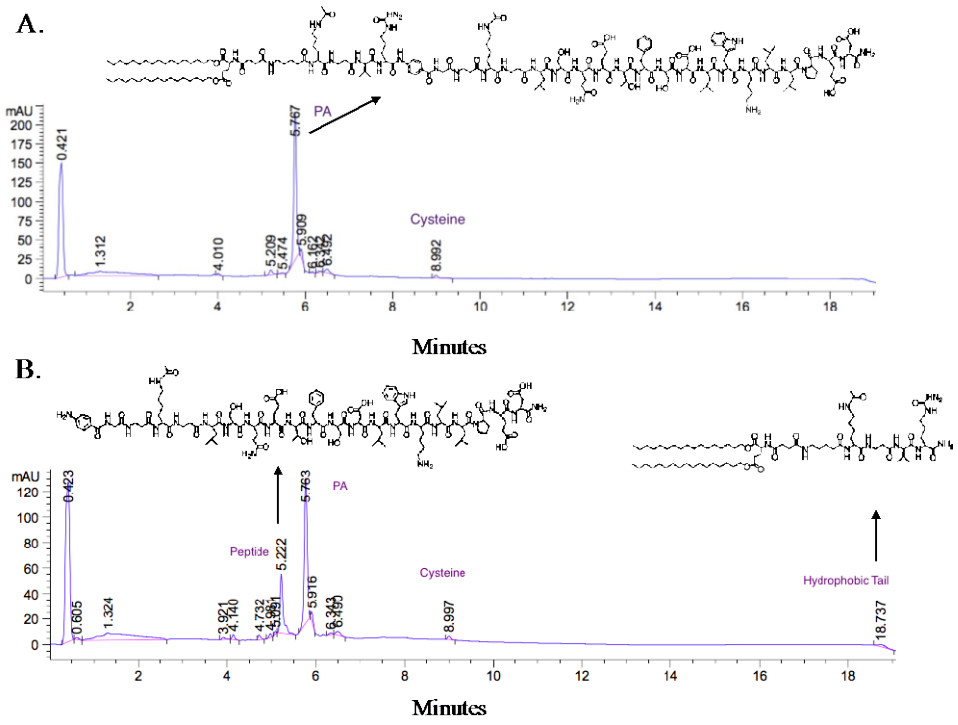


Figure S8. HPLC analysis following recombinant catB enzyme incubation with (A) diC_{16}-GGG-$p53_{(14-29)}$ and (B) diC_{16}-VC-PABA-$p53_{(14-29)}$ indicate cleavage of $p53_{(14-29)}$ from diC_{16} only from diC_{16}-VC-PABA-$p53_{(14-29)}$.