Supporting Information

Cyclic Peptidyl Inhibitors against Human Peptidyl-Prolyl Isomerase Pin1

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Table S1. Structures of Building Blocks Used for the Cyclic Peptide Library

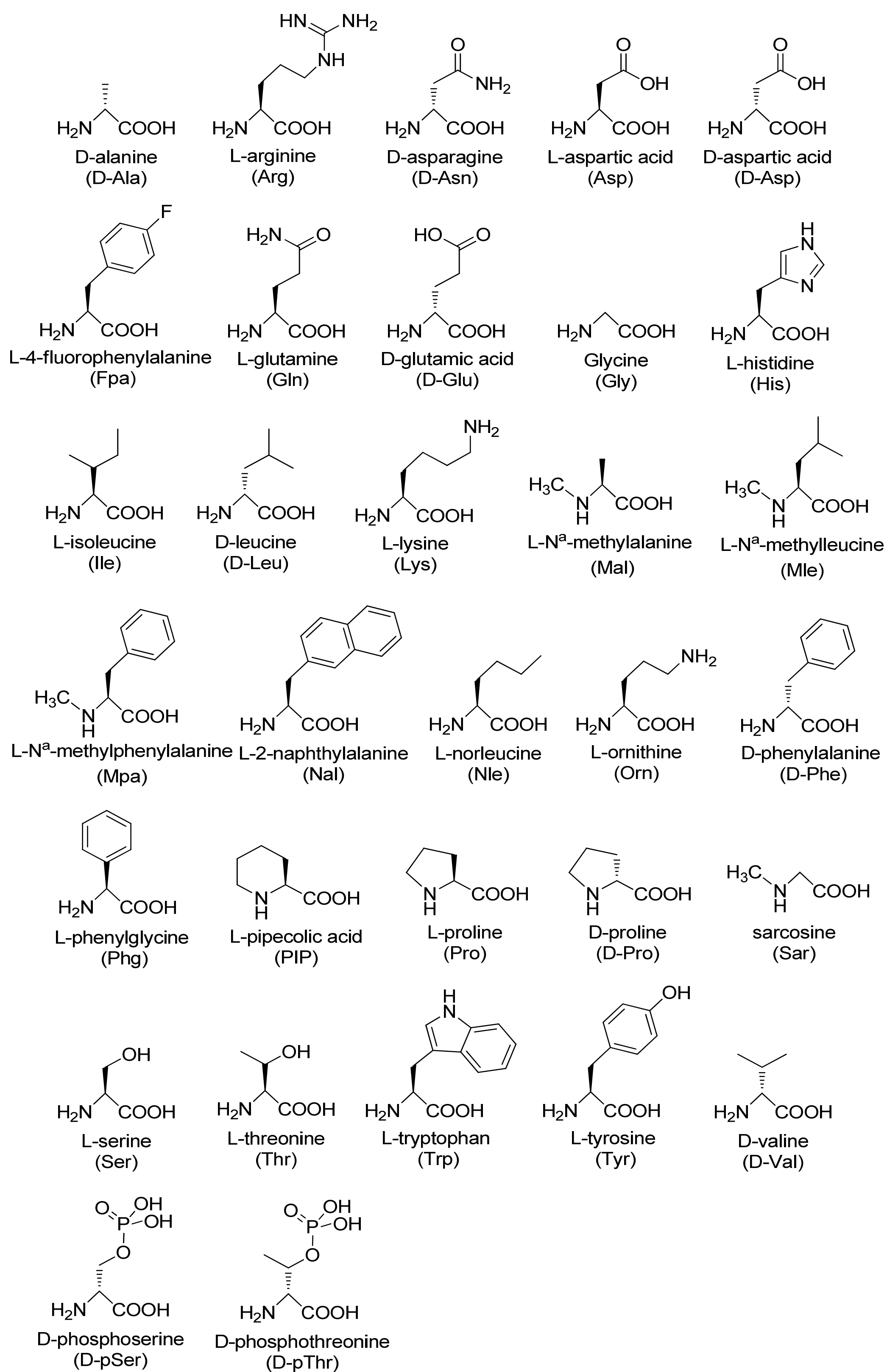
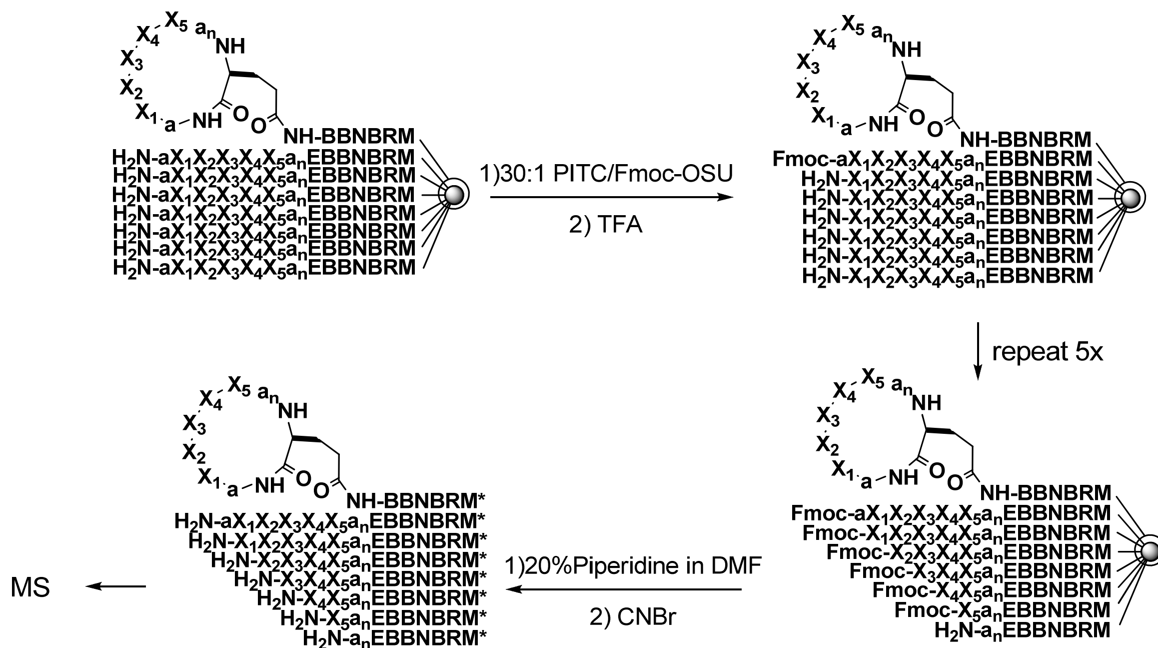


Figure S1. View of a positive bead derived from library screening against Texas Red-labeled Pin1-MBP under Olympus SZX12 fluorescence microscope.

Figure S2. Hit Identification by PED-MS. (a) Partial Edman degradation of resin-bound peptide. PITC, phenylthioisocyanate; Fmoc-OSU, N-(9-Fluorenylmethoxycarbonyloxy) succinimide; M*, homoserine lactone. (b) A representative MALDI-TOF spectrum of PED products derived from a single bead carrying peptide cyclo(D-Ala-Fpa-D-pThr-Pip-Tyr-Arg-D-Ala). *Procedure*: Selected resin beads were pooled and subjected to PED in a single reaction vessel. The beads were suspended in 160 μL of pyridine/water (v/v 2:1) containing 0.1% triethylamine and mixed with an equal volume of pyridine containing 1.13 mg of Fmoc-OSu and 12 μL of PITC. The reaction was allowed to proceed for 6 min with mixing, and the beads were washed with DCM, and TFA. The beads were treated twice with 400μL of TFA for 6 min each. After the resin was washed with DCM, pyridine, and pyridine/water (2:1), the cycle was repeated 5 times. After the final cycle, the N-terminal Fmoc group was removed by 20% piperidine in DMF. For MS analysis, the degraded beads were treated with 500 μL of TFA containing NH_4I (10 mg) and Me_2S (20 μL) on ice for 30 min to reduce any oxidized Met. After washing with water, the beads were transferred into microcentrifuge tubes (1 bead/tube) and each treated with 20 μL of 70% TFA containing CNBr (40 mg/mL) overnight in the dark. The solvent was evaporated under vacuum, and the peptides were dissolved in 5 μL of 0.1% TFA in water. One μL of the peptide solution was mixed with 2 μL of saturated 4-hydroxy-alpha-cyanocinnamic acid in acetonitrile/0.1% TFA (1:1), and 1μL of the mixture was spotted onto a MALDI sample plate. Mass spectrometry was performed at Campus Chemical Instrument Center of The Ohio State University on a Bruker III MALDI-TOF instrument in an automated manner. The data obtained were analyzed by Moverz software (Proteometrics LLC, Winnipeg, Canada).

Figure S2

a



b

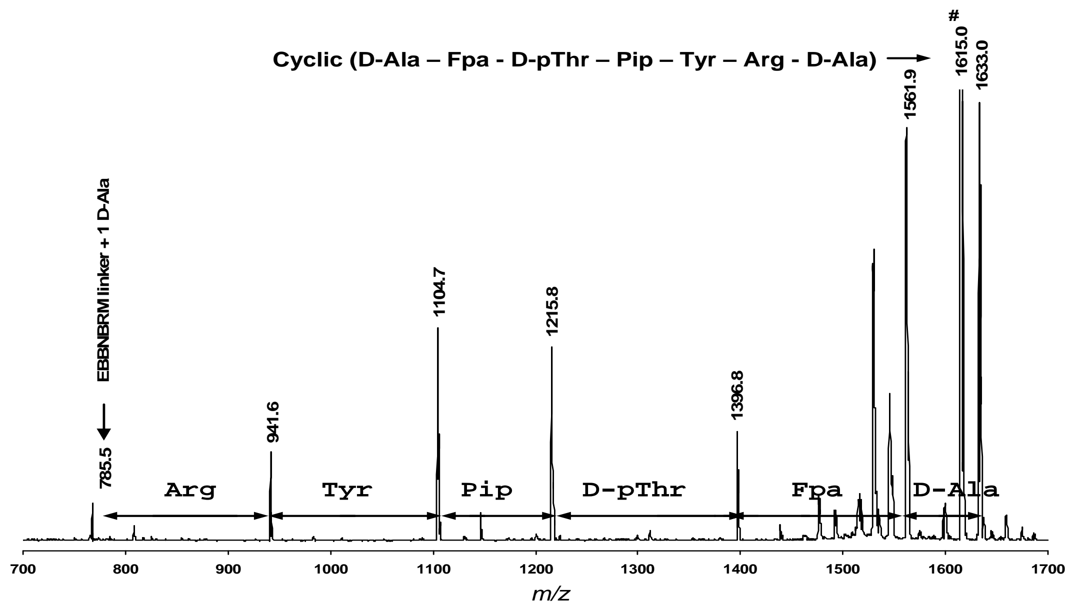
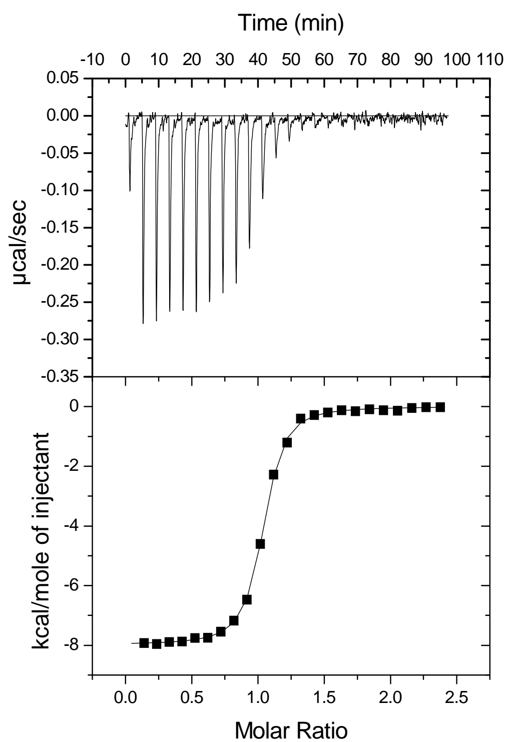
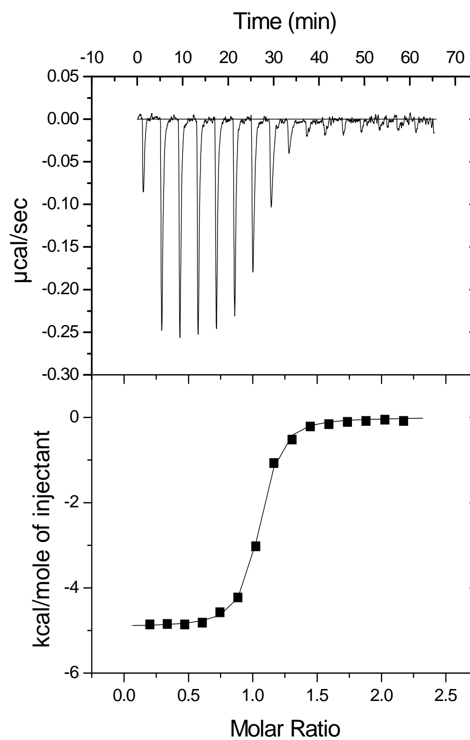


Figure S3. ITC of binding of Pin1 to peptides A, B, C, F, G and H.

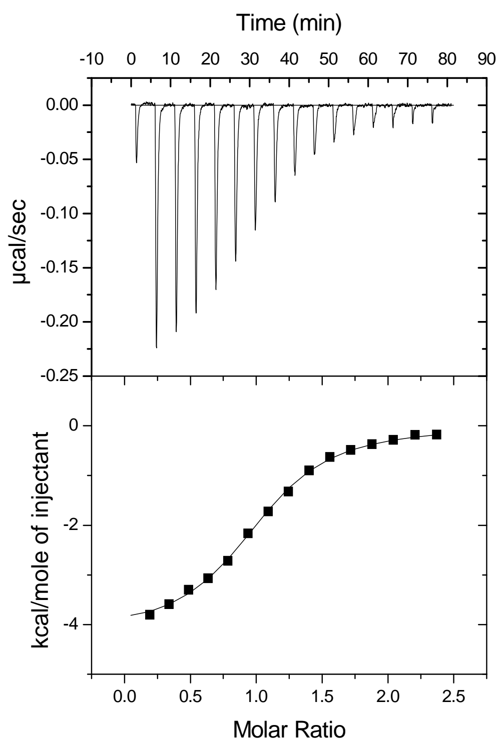
Peptide A, K_D = 47 ± 2 nM



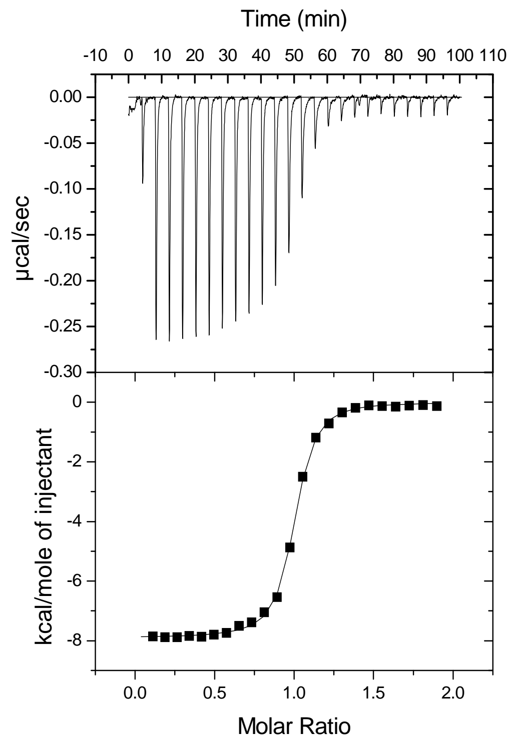
Peptide B, K_D = 48 ± 4 nM



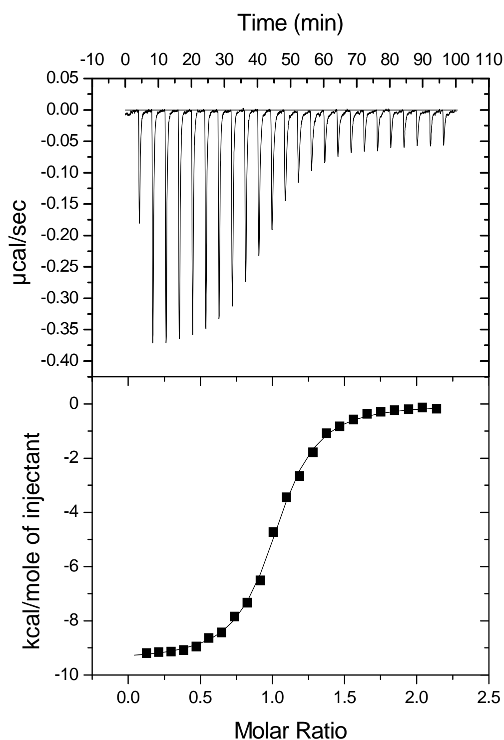
Peptide C, K_D = 920 ± 60 nM



Peptide F, K_D = 33 ± 2 nM



Peptide G, K_D = 170 ± 10 nM



Peptide H, K_D = 980 ± 30 nM

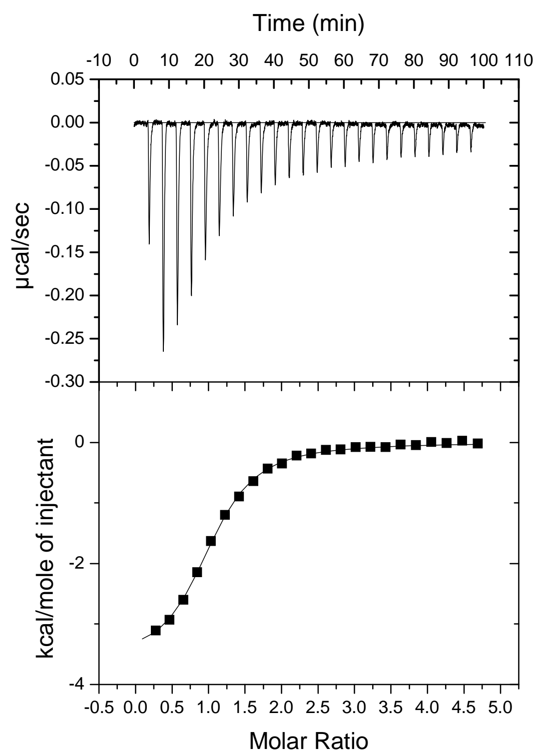
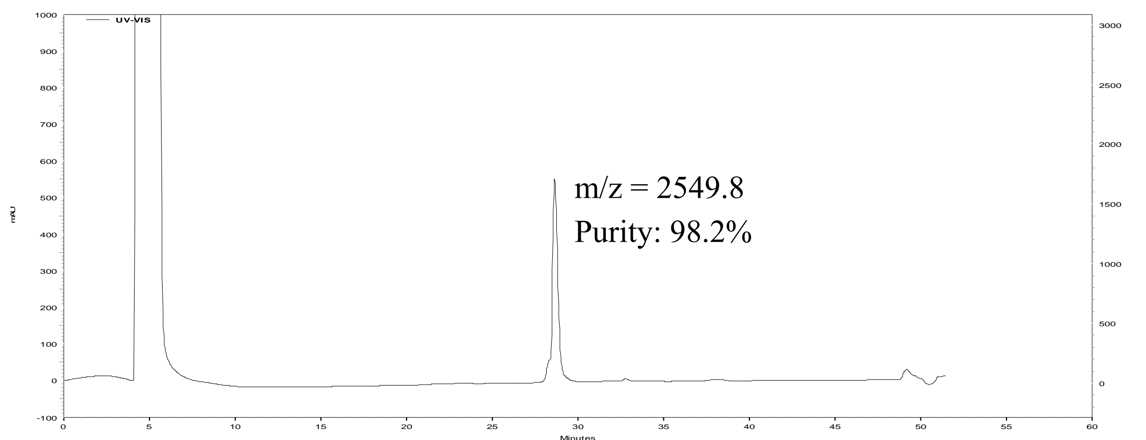
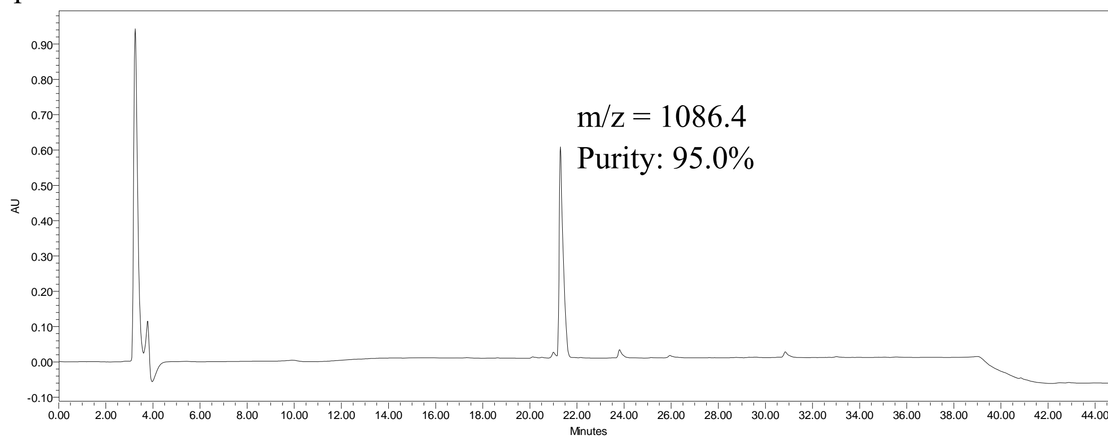


Figure S4. HPLC tracings showing the purity of peptides E−K. HPLC purified peptides were analyzed on a Varian (Palo Alto, CA) C-18 column, which was eluted with a linear gradient of 10-50% acetonitrile in 0.05% TFA in ddH_2O over 30 min (flow rate of 1 mL/min), except for peptide E, which was eluted with a linear gradient of 10-40% acetonitrile in 0.05% TFA in ddH_2O over 40 min.

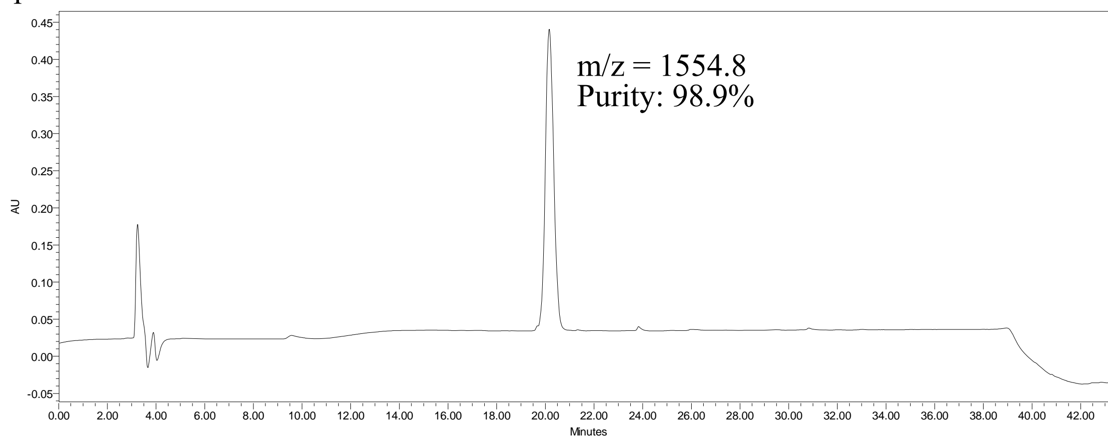
Peptide E



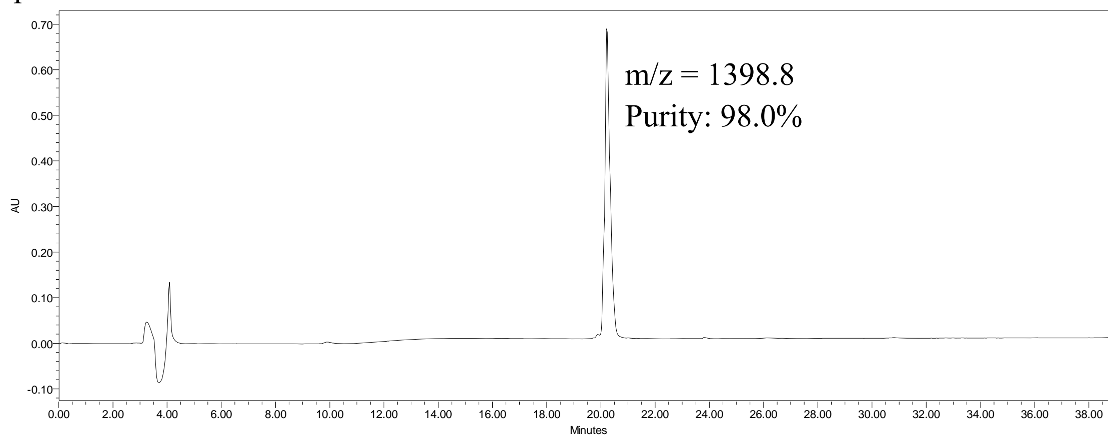
Peptide F



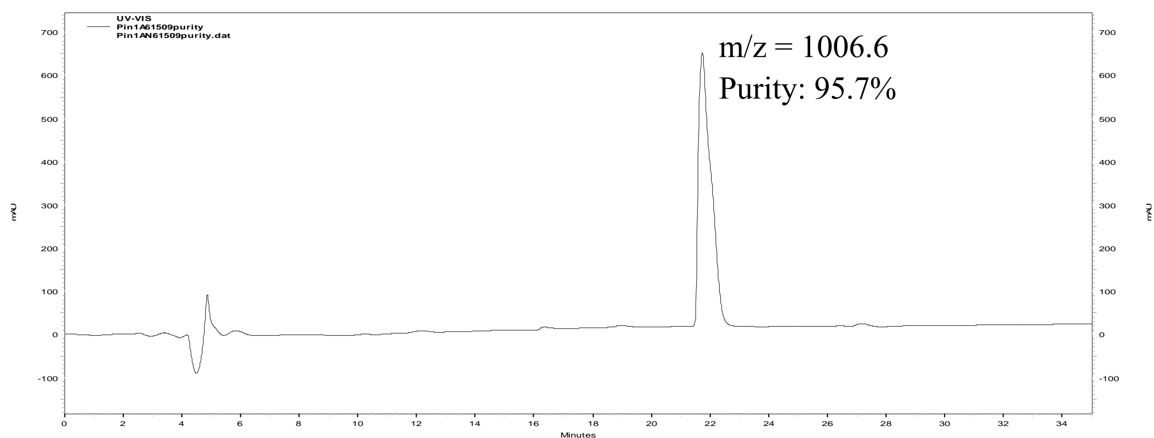
Peptide G



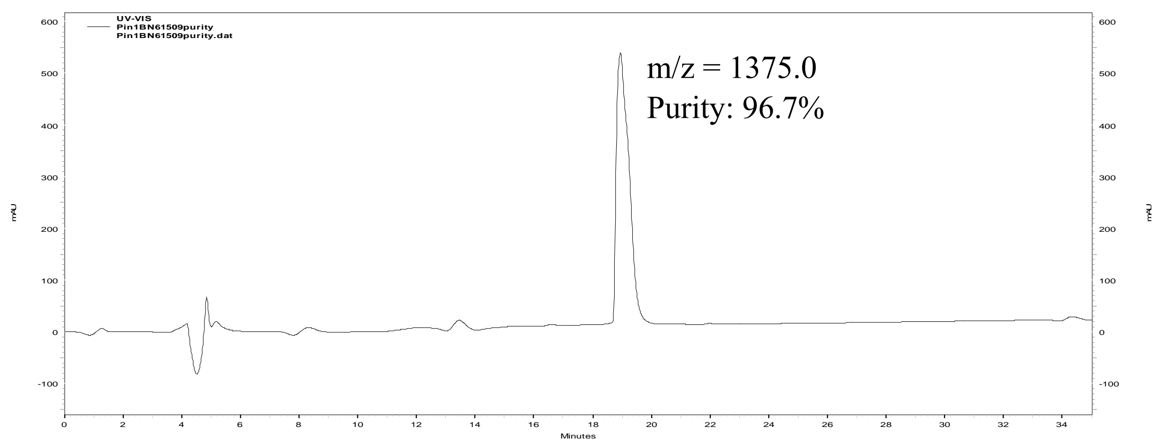
Peptide H



Peptide I



Peptide J



Peptide K

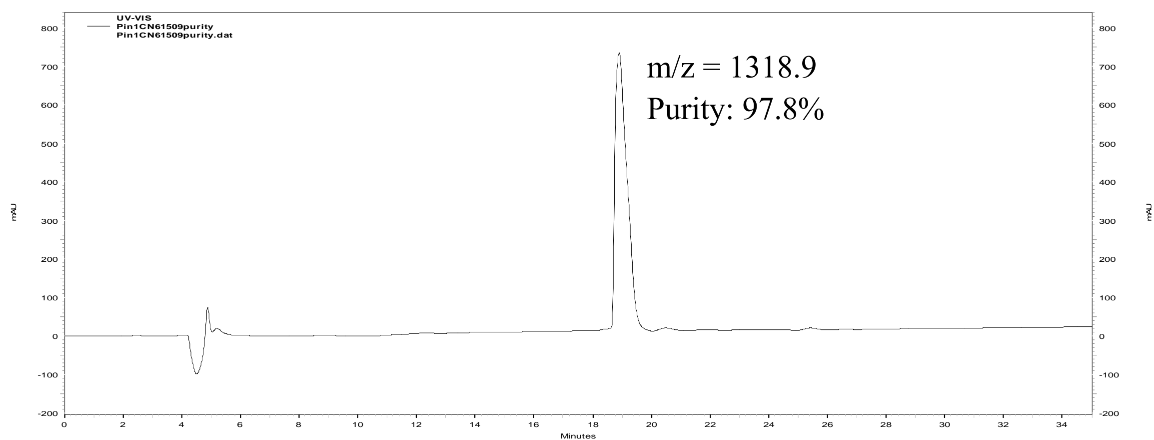
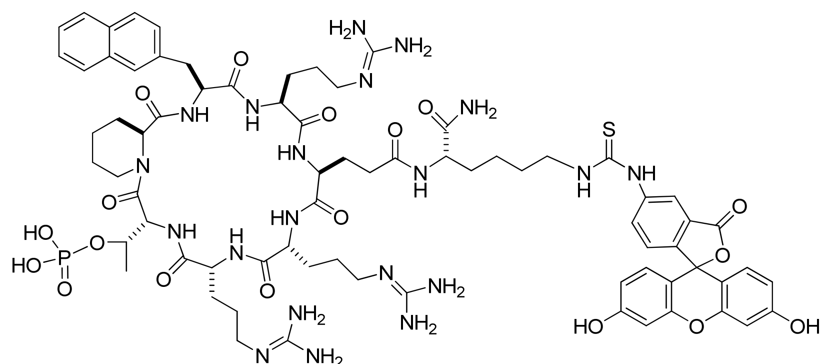


Figure S5. Structure of FITC-labeled peptide F.



Procedure: FITC-labeled peptides F-K were generated by first synthesizing the cyclic peptides with a C-terminal lysine. Each peptide was purified by reversed-phase HPLC on a C18 column, reacted for 1 h with 5 molar equivalents of FITC (Pierce, catalog #46425) in 50 mM sodium bicarbonate (pH 8.5)/DMSO mixture (v/v ration depended on the solubility of each peptide), and purified again by reversed-phase HPLC. The molecular mass of each peptide was confirmed by MALDI-TOF mass spectrometric analyses.

Peptide	*Calcd Mass (M + H^+)*	*Observed m/z*
F	1603.66	1603.76
G	2071.96	2072.21
H	1915.86	1916.02
I	1523.69	1523.76
J	1991.99	1993.15
K	1835.89	1836.07

Figure S6. Fluorescence microscopy showing the internalization and distribution of peptides I–K in Hela cells.