Supporting Information

Development of a Cell-Permeable Cyclic Peptidyl Inhibitor against the Keap1-Nrf2 Interaction

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Figure S1. Structures, purity (as assessed by reversed-phase analytical HPLC), and HR-MS (MALDI FT-ICR) of peptides used in this work. Note: Some of the NF-labeled peptides eluted as two separate peaks, because the commercially available NF dye is a mixture of 5- and 6-carboxy isomers. The mixtures of two isomers were used in all experiments.

Peptide 1

Crude peptide on semi-preparative reversed-phase HPLC (monitored at 214 nm):

Purity check on analytical reversed-phase HPLC (214 nm):
HRMS:

![HRMS Spectrum]

Peptide 2

![Peptide 2 Structure]

Calcd [M+H]⁺ 1187.5579

Found 1187.5567
Crude peptide on semi-preparative reversed-phase HPLC (monitored at 214 nm):

Purity check (analytical reversed-phase HPLC at 214 nm):

HRMS:

Calcd [M+H]+ 2540.3157
Found 2540.3048
FAM-Peptide 1

Crude peptide on semi-preparative reversed-phase HPLC (monitored at 214 nm):

Purity check on analytical reversed-phase HPLC (214 nm):
HRMS:

Caled [M+H]^+ 1673.6967
Found 1673.6957

FITC-Peptide 2
Crude peptide on semi-preparative reversed-phase HPLC (monitored at 214 nm):

Purity check on analytical reversed-phase HPLC (214 nm):

HRMS:

Calcd [M+H]^+ 3202.5035

Found 3202.5008
NF-Peptide 1

Crude peptide on semi-preparative reversed-phase HPLC (monitored at 214 nm):

Purity check on analytical reversed-phase HPLC (214 nm):
HRMS:

Calcd [M+H]⁺ 1773.7319
Found 1773.7289

NF-labeled Peptide 2
Crude peptide on semi-preparative reversed-phase HPLC (monitored at 214 nm):

Purity check on analytical reversed-phase HPLC (214 nm):

MS (low-resolution):

Calcd [M+H]^+ 3271.5641
Found 3271.976
NF-CPP9

Crude peptide on semi-preparative reversed-phase HPLC (monitored at 214 nm):

Purity check on analytical reversed-phase HPLC (214 nm):
HRMS:

Calcd [M+H]^+ 1828.8712
Found 1828.8686
Figure S2. Serum stability of peptide 2 and a stapled peptide, Ac-GGYPED*ILDK*HLQRVL-(miniPEG)-Dap-CPP9, in which an amide bond is formed between the side chains of D* and K*.

Procedure: Human serum was diluted to 25% in sterile DPBS and incubated at 37 °C for 15 min. Peptide was added to the diluted serum to a final concentration of 100 µM and incubated at 37 °C with gentle mixing. Aliquots of 100 µL were withdrawn at various time points and mixed with 100 µL of 15% trichloroacetic acid in MeOH (w/v) and 100 µL of acetonitrile to quench the reaction. After overnight storage at 4 °C, aliquots were centrifuged at 15000 g for 5 min at 4°C and analyzed by RP-HPLC on a C18 column. The amount of remaining peptide was assessed by integration of the area under the peak in the HPLC chromatogram and comparing it with that at zero-time point.
Figure S3. Enlarged confocal microscopy images from Figure 2 (main text) to show the differential intracellular localization of peptides 1 (a) and 2 (b). Representative round, intensely fluorescent structures (presumably endosomes and lysosomes) are indicated by white arrows, whereas the fibrous structures in (b) are marked with red arrows.
Figure S4. Cytosolic entry efficiencies of peptides 1 and 2. HeLa cells were treated with 5 μM NF-labeled peptide for 2 h in the presence of 10% FBS, washed, and suspended in a pH 6.5 buffer immediately before flow cytometry analysis. All MFI values are relative to that of CPP9-NF (100%).

Figure S5. Effect of peptide 2 on the viability of HeLa and HEK293T cells as monitored by the MTT assay.