Supporting Information

A critical assessment of the synthesis and biological activity of p53/Hdm2 stapled peptide inhibitors

Running title: A critical evaluation of stapled peptides

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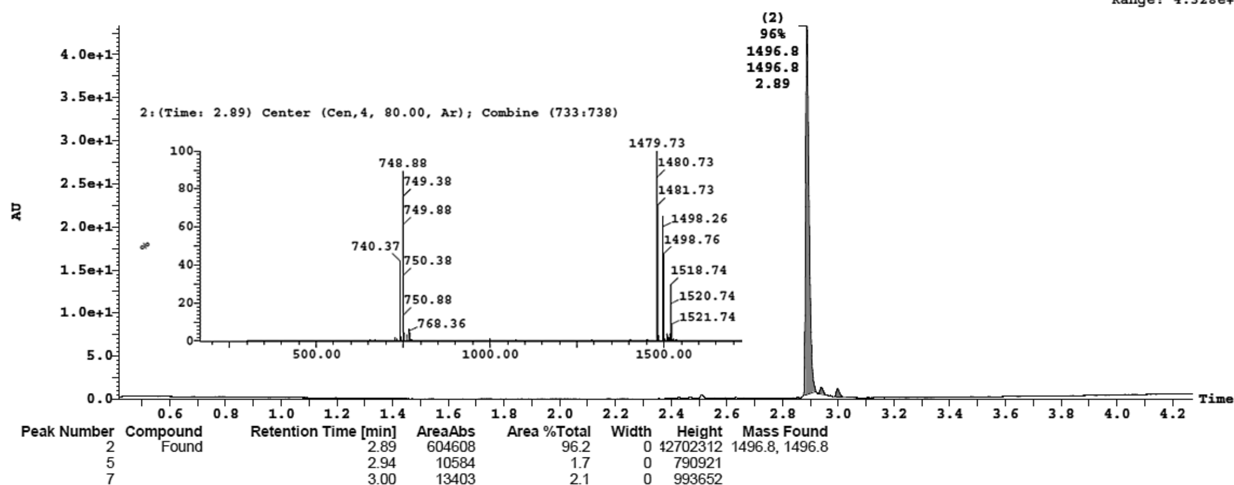


Figure S1: LC-MS of **1a**

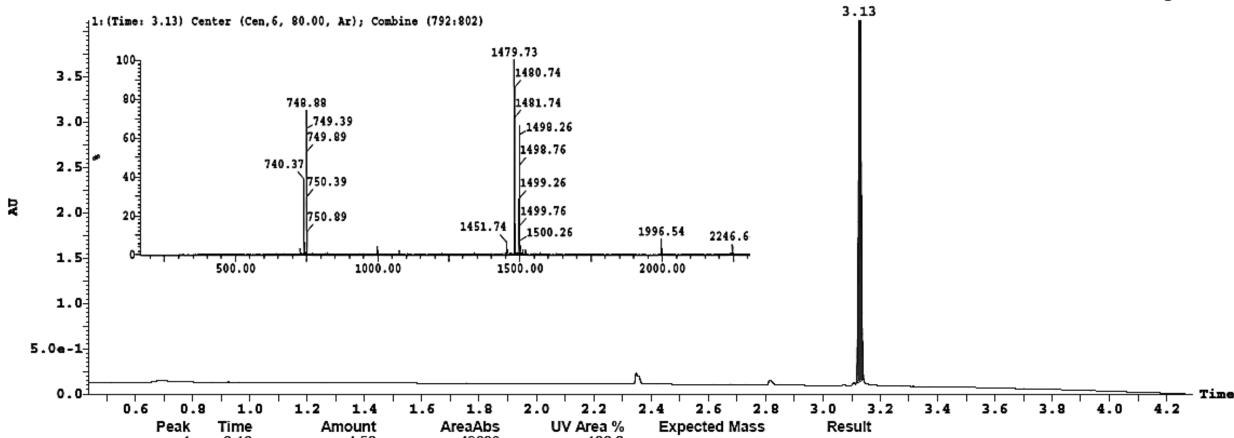


Figure S2: LC-MS of **1b**

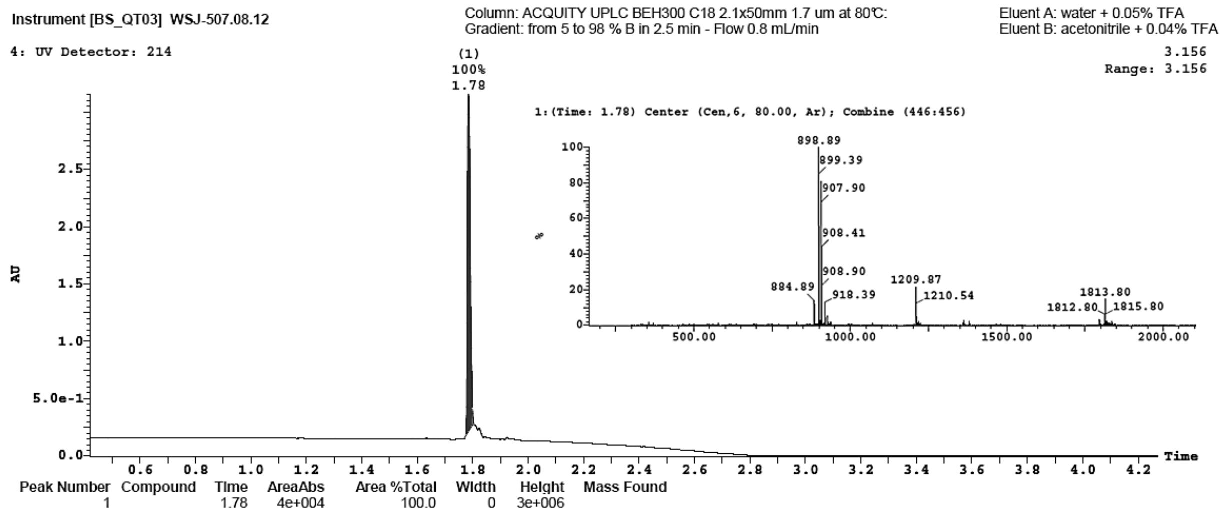


Figure S3: LC-MS of **CF1a**

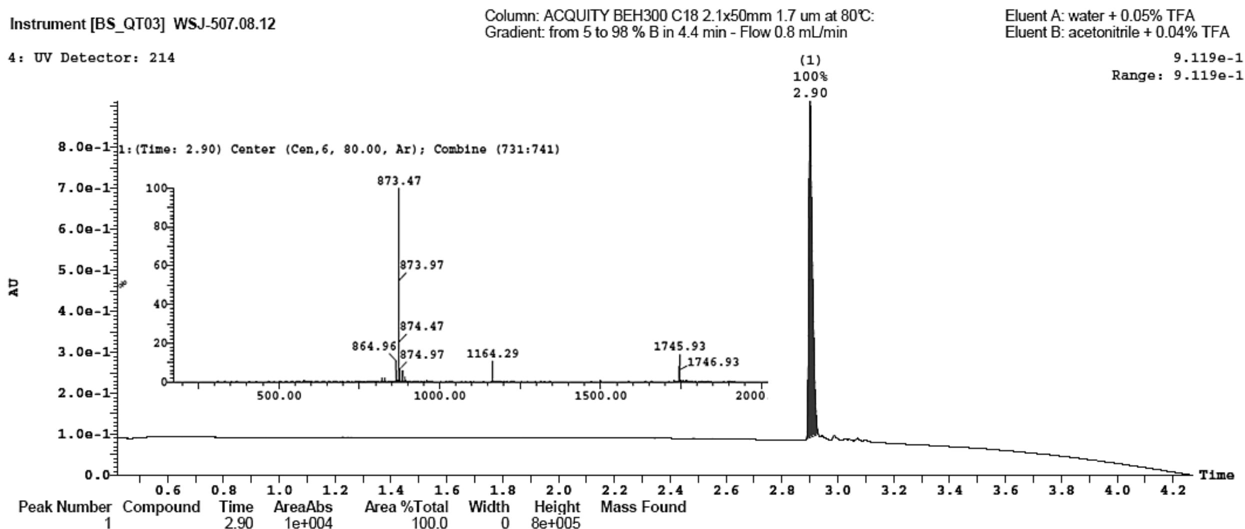


Figure S4: LC-MS of **2a**

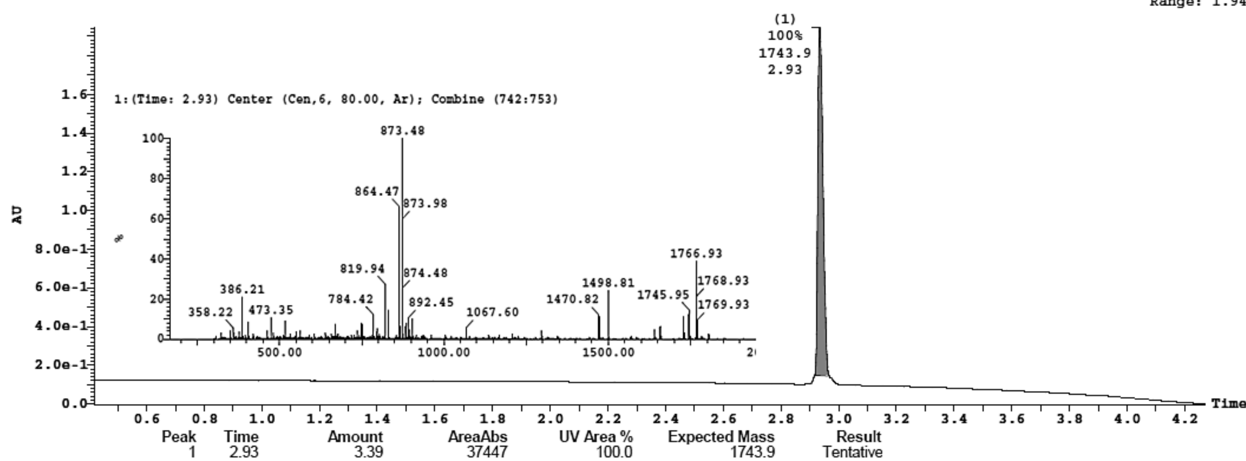


Figure S5: LC-MS of **2b**

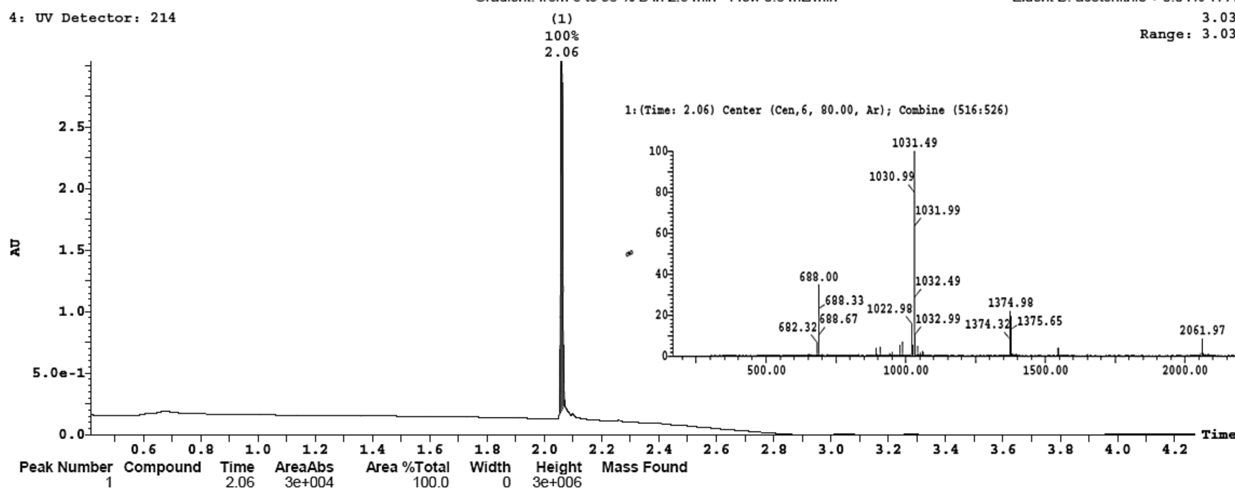


Figure S6: LC-MS of **CF2a**

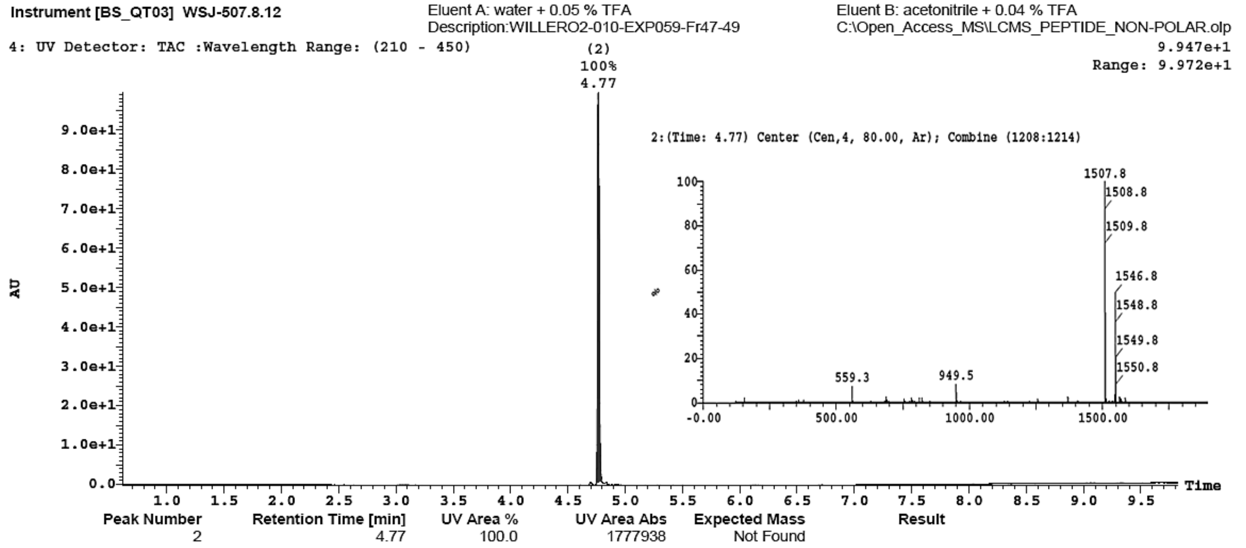


Figure S7: LC-MS of **3**

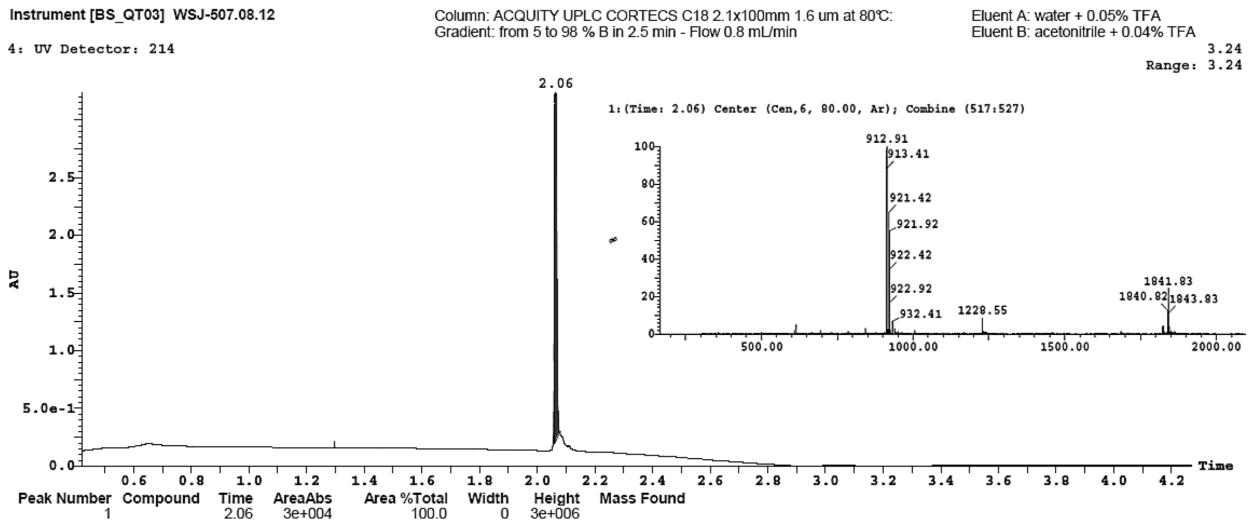


Figure S8: LC-MS of **CF3**

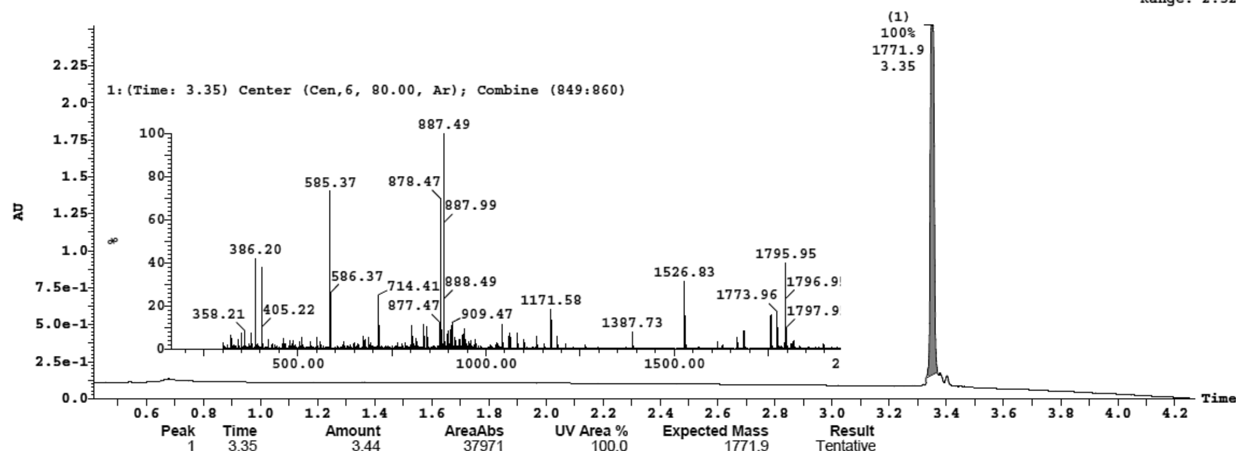


Figure S9: LC-MS of **4**

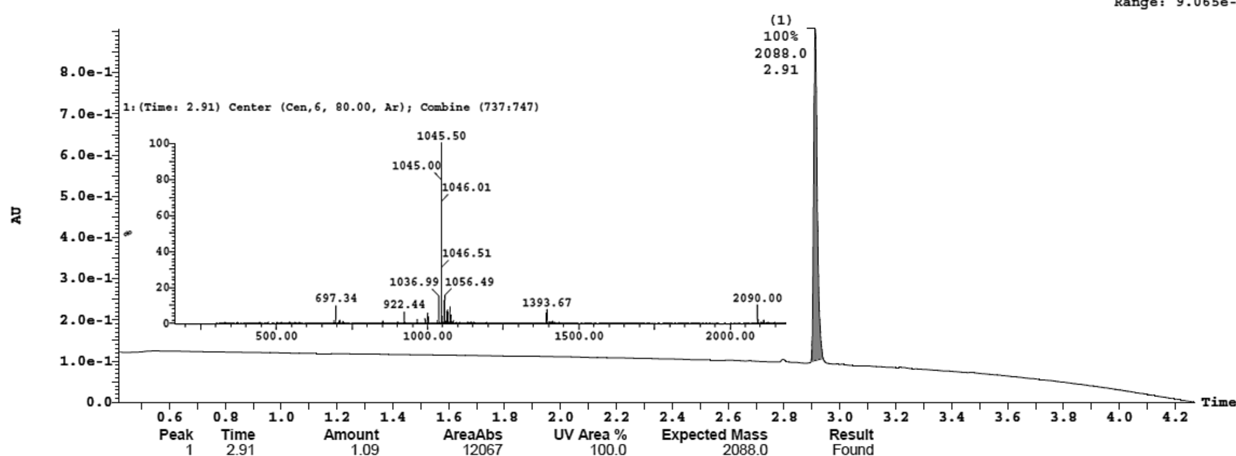


Figure S10: LC-MS of **CF4**

Table S1: Calculated and observed masses for the investigated peptides.

Compound	Formula	Calcd. mass	Found mass
1a	$C_{75}H_{106}ClN_{13}O_{17}$	$[M+H]^{+}$= 1498.2	1498.3
1b	$C_{75}H_{106}ClN_{13}O_{17}$	$[M+H]^{+}$= 1498.2	1498.3
CF1a	$C_{94}H_{114}ClN_{13}O_{22}$	$[M+H]^{+}$= 1814.4	1813.8
2a	$C_{87}H_{125}N_{17}O_{21}$	$[M+H]^{+}$= 1746.0	1745.9
2b	$C_{87}H_{125}N_{17}O_{21}$	$[M+H]^{+}$= 1746.0	1745.9
CF2a	$C_{106}H_{133}N_{17}O_{26}$	$[M+H]^{+}$= 2062.3	2062.0
3	$C_{77}H_{110}ClN_{13}O_{17}$	$[M-H_2O]^{+}$= 1507.2*	1507.8
CF3	$C_{96}H_{118}ClN_{13}O_{22}$	$[M+H]^{+}$= 1842.5	1841.8
4	$C_{89}H_{129}N_{17}O_{21}$	$[M+H]^{+}$= 1774.1	1774.0
CF4	$C_{108}H_{137}N_{17}O_{26}$	$[M+H]^{+}$= 2090.3	2090.0

*Loss of water is attributed to the ionization process during MS analysis.

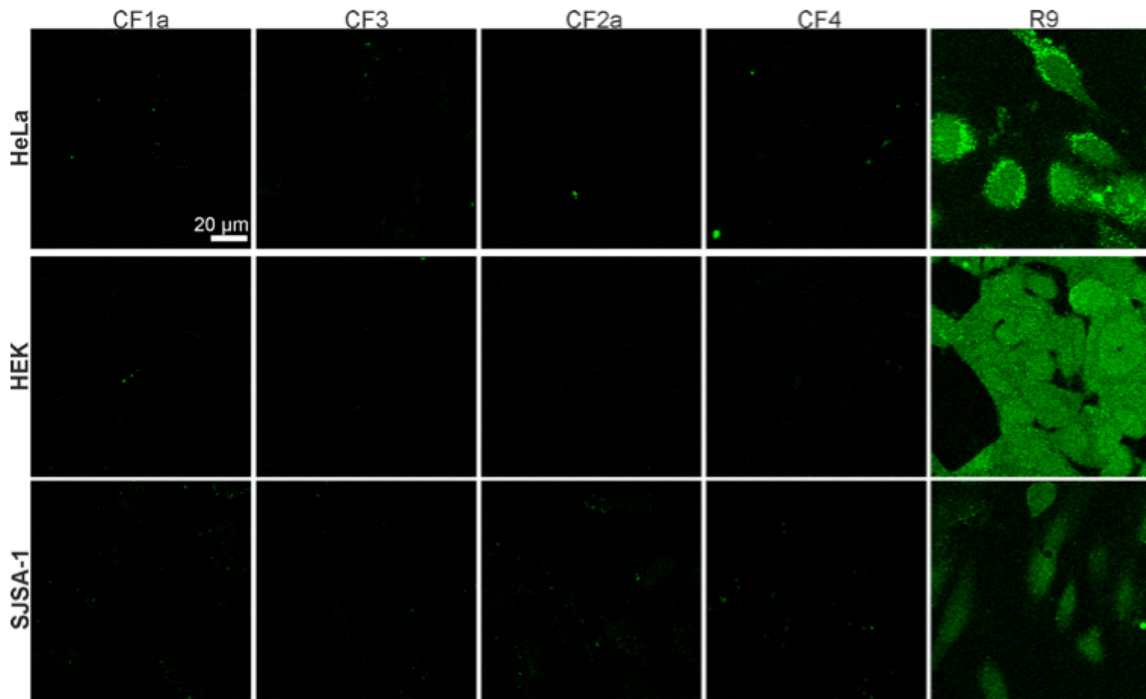


Figure S11: Intracellular distribution of stapled peptides after a 30 min incubation. Cells were incubated with 5 µM of the peptides in RPMI + 10 % FCS, washed and confocal images were recorded. Fluorescein-labeled R9 was used as a reference. Scale bar denotes 20 µm. The figure shows one representative experiment of two independent repetitions.

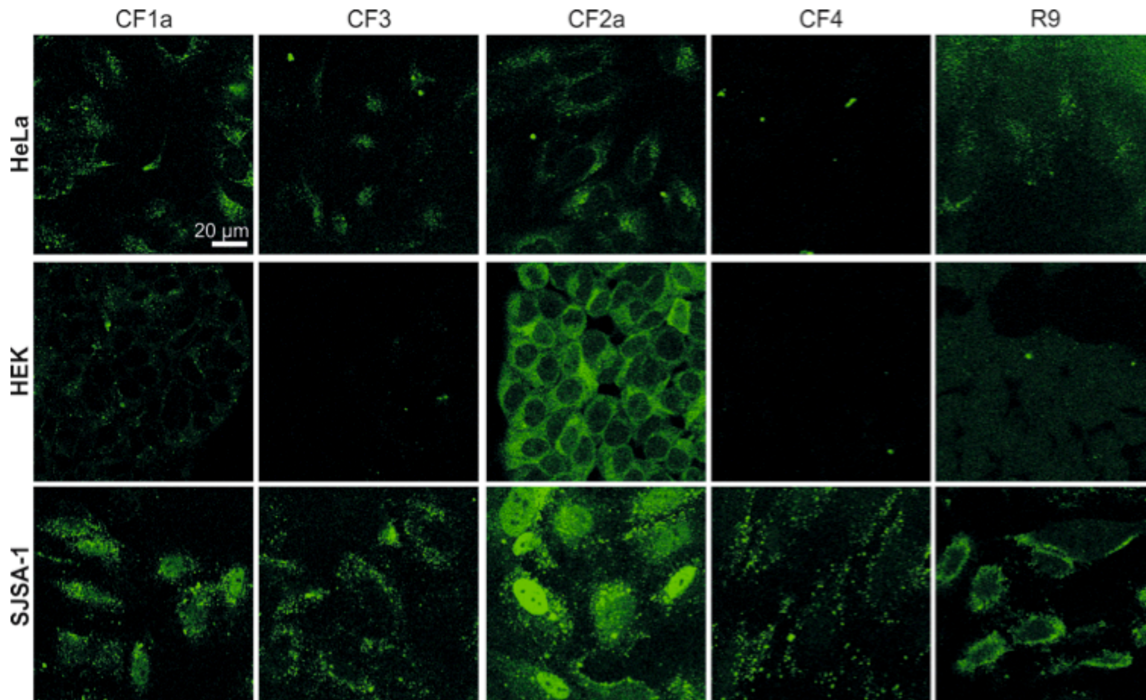
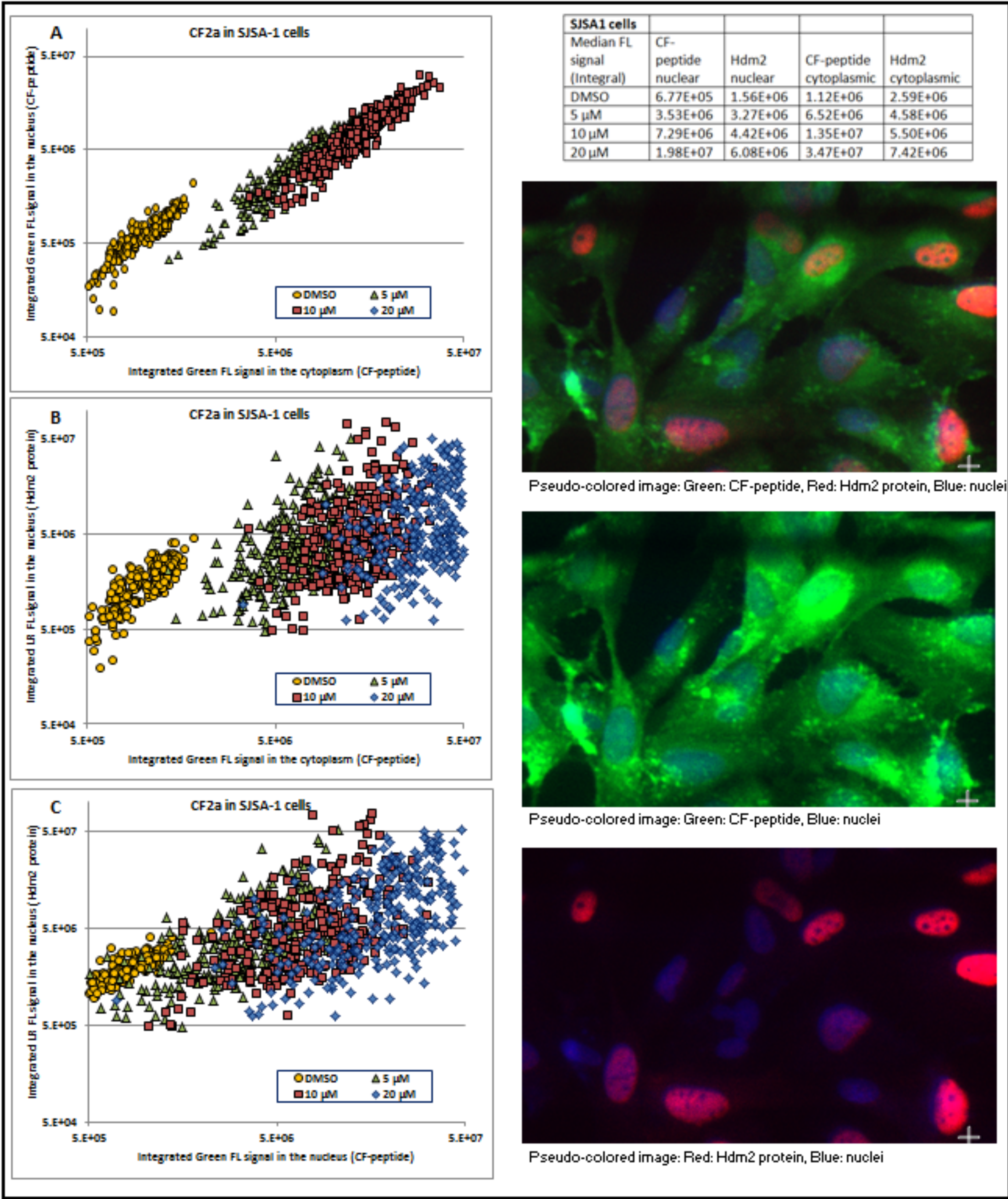


Figure S12: Intracellular distribution of stapled peptides after a 24 h incubation. Cells were incubated with 5 µM of the CF-labeled peptides in RPMI + 10 % FCS, washed and confocal images were recorded. R9 was used as a control CPP. The bars indicate the size. In

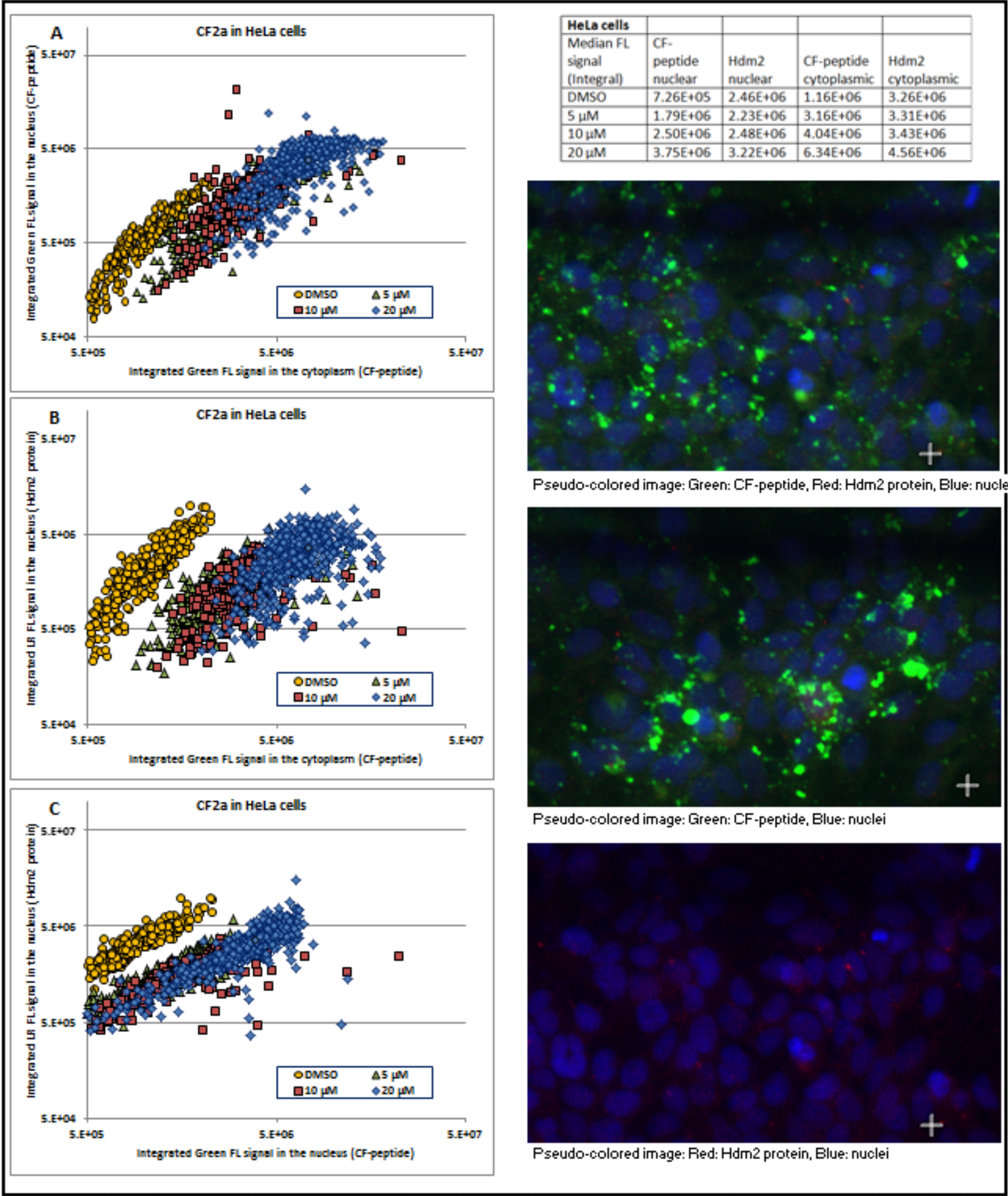
comparison to 20 µM, different contrast settings were used to improve visualization. The figure shows one representative experiments of three independent repetitions.



SJSA1 cells				
Median FL signal (Integral)	CF-peptide nuclear	Hdm2 nuclear	CF-peptide cytoplasmic	Hdm2 cytoplasmic
DMSO	6.77E+05	1.56E+06	1.12E+06	2.59E+06
5 µM	3.53E+06	3.27E+06	6.52E+06	4.58E+06
10 µM	7.29E+06	4.42E+06	1.35E+07	5.50E+06
20 µM	1.98E+07	6.08E+06	3.47E+07	7.42E+06

Figure S13: Colocalization of CF2a and Hdm2 in SJSA-1 cells. After treatment of cells with the carboxyfluorescein-labeled peptide at the indicated concentrations or DMSO control, cells were fixed, stained for detection of nuclei using DAPI and for Hdm2 using immunofluorescence and imaged by multi-channel fluorescence microscopy (right panels). (A) Correlation of integrated fluorescein fluorescence in the nucleus and cytoplasm. (B, C)

Correlation of integrated Hdm2 fluorescence in the nucleus versus carboxyfluorescein fluorescence in the cytoplasm (B) and in the nucleus (C).



HeLa cells Median FL signal (Integral)	CF-peptide nuclear	Hdm2 nuclear	CF-peptide cytoplasmic	Hdm2 cytoplasmic
DMSO	7.26E+05	2.46E+06	1.16E+06	3.26E+06
5 µM	1.79E+06	2.23E+06	3.16E+06	3.31E+06
10 µM	2.50E+06	2.48E+06	4.04E+06	3.43E+06
20 µM	3.75E+06	3.22E+06	6.34E+06	4.56E+06

Figure S14: Colocalization of CF2a and Hdm2 in HeLa cells. After treatment of cells with the carboxyfluorescein-labeled peptide at the indicated concentrations or DMSO control, cells were fixed, stained for detection of nuclei using DAPI and for Hdm2 using immunofluorescence and imaged by multi-channel fluorescence microscopy (right panels). (A) Correlation of integrated fluorescein fluorescence in the nucleus and cytoplasm. (B, C)

Correlation of integrated Hdm2 fluorescence in the nucleus versus carboxyfluorescein fluorescence in the cytoplasm (B) and in the nucleus (C).

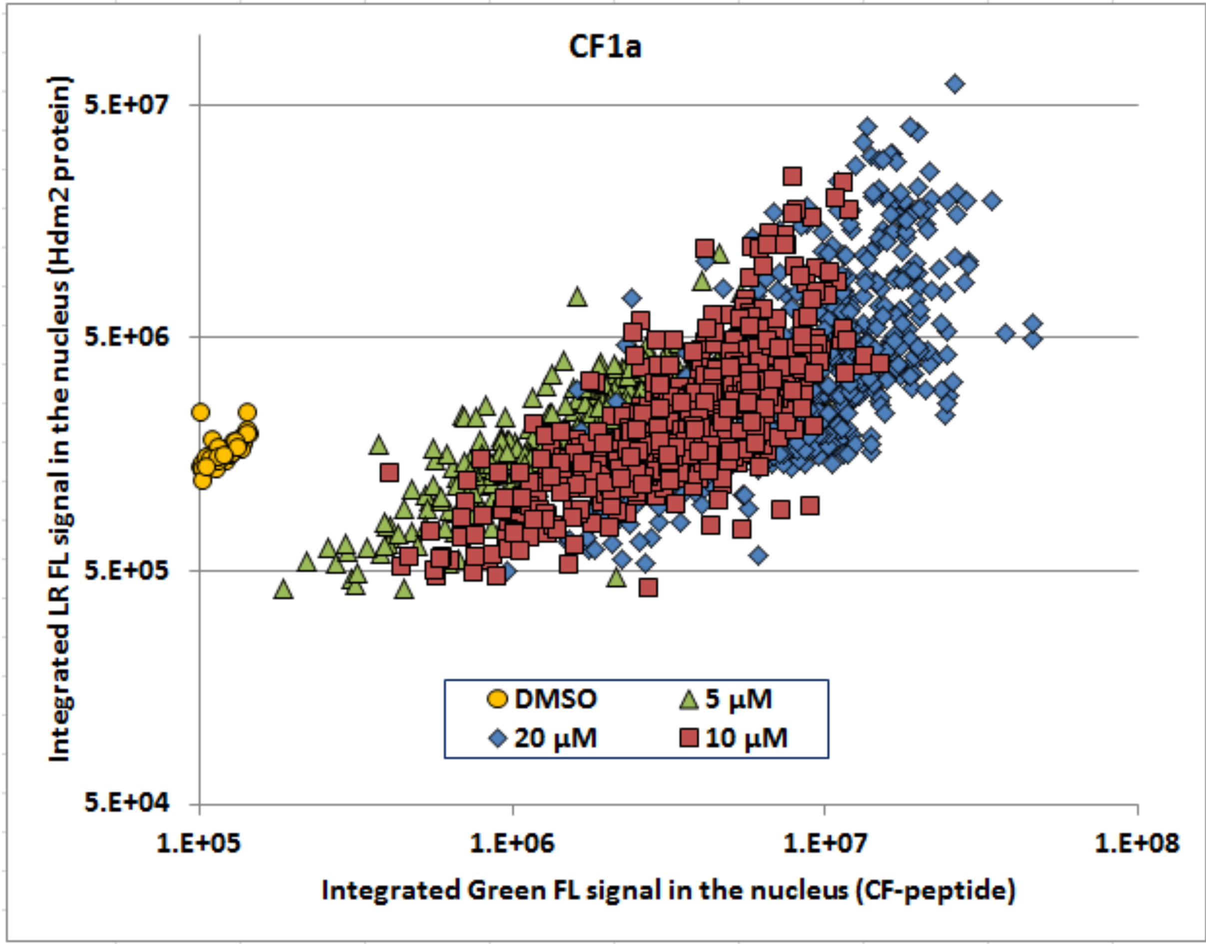


Figure S15: Co-localization of the labeled peptide CF1a with the PD marker Hdm2. After treatment of SJSA1 cells for 24 h with the carboxyfluorescein-labeled peptide at the indicated concentrations, the cells were fixed and stained with a specific antibody against the PD marker Hdm2 linked with a red fluorescent label.