CHEMBIOCHEM

Supporting Information

DOI: 10.1002/cbic.200900049

Supporting Information

for

Cell-Permeable β-Peptide Inhibitors of p53•hDM2 Complexation

Elizabeth A. Harker and Alanna Schepartz*

Contents

Table SI-1. Theoretical and MALDI-TOF MS-observed molecular weights for original β^3-peptides used in this study.

β^3-peptide	Formula	Mass calcd ($M+H^+$)	Masses found
β53-8R$_3$	$C_{74}H_{122}N_{22}O_{15}$	1560.9	1563.5 ($M+H^+$), 1585.6 ($M+Na^+$)
β53-12R$_3$	$C_{73}H_{120}F_3N_{21}O_{15}$	1589.9	1592.4 ($M+H^+$), 1614.8 ($M+Na^+$)
β53-12R$_2$	$C_{73}H_{119}F_3N_{18}O_{15}$	1546.8	1549.1 ($M+H^+$), 1571.1 ($M+Na^+$)
β53-8R$_3^{Flu}$	$C_{99}H_{139}N_{23}O_{22}S$	2036.4	2039.5 ($M+H^+$), 2061.4 ($M+Na^+$)
β53-12R3^{Flu}	$C_{98}H_{137}F_3N_{22}O_{22}S$	2065.4	2068.4 ($M+H^+$), 2090.5 ($M+Na^+$)
β53-12R$_2^{Flu}$	$C_{98}H_{136}F_3N_{19}O_{22}S$	2022.3	2025.7 ($M+H^+$), 2067.8 ($M+Na^+$)
β53-12SB$_2$	$C_{75}H_{121}F_3N_{16}O_{15}$	1544.9	1546.0 ($M+H^+$), 1568.0 ($M+Na^+$)
β53-12SB$_3$	$C_{76}H_{126}F_3N_{19}O_{13}$	1571.9	1572.4 ($M+H^+$), 1593.7 ($M+Na^+$)
β53-3SBR$_3$	$C_{77}H_{128}N_{20}O_{13}$	1499.9	1500.8 ($M+H^+$), 1522.8 ($M+Na^+$)
β53-12SB$_2^{Flu}$	$C_{100}H_{138}F_3N_{17}O_{22}S$	2020.4	2021.1 ($M+H^+$), 2044.3($M+Na^+$)
β53-12SB$_3^{Flu}$	$C_{101}H_{143}F_3N_{20}O_{20}S$	2047.4	2048.9 ($M+H^+$), 2071.9 ($M+Na^+$)
β53-3SB$_3^{Flu}$	$C_{102}H_{145}N_{21}O_{20}S$	1976.9	1978.4 ($M+H^+$), 2000.5 ($M+Na^+$)
β53-12R$_6$-1	$C_{73}H_{126}F_3N_{29}O_{10}$	1626.97	1627.0 ($M+H^+$)
β53-12R$_6$-2	$C_{73}H_{126}F_3N_{29}O_{10}$	1626.97	1627.2 ($M+H^+$)
βNEGR$_6$	$C_{63}H_{125}N_{29}O_{10}$	1538.98	1538.9 ($M+H^+$)
β53-12R$_6$-1^{Flu}	$C_{98}H_{143}F_3N_{30}O_{17}S$	2102.47	2103.1 ($M+H^+$)
β53-12R$_6$-2^{Flu}	$C_{98}H_{143}F_3N_{30}O_{17}S$	2102.47	2104.9 ($M+H^+$)
βNEGR$_6^{Flu}$	$C_{88}H_{142}N_{30}O_{17}S$	1924.36	1924.8 ($M+H^+$)
β53-12R$_8$	$C_{121}H_{213}F_3N_{44}O_{23}$	2709.26	2709.8 ($M+H^+$)
β53-3R$_8$	$C_{119}H_{209}N_{45}O_{23}$	2638.22	2638.4 ($M+H^+$)
β53-12R$_8^{Flu}$	$C_{146}H_{230}F_3N_{45}O_{30}S$	3184.76	3185.6 ($M+H^+$)
β533-3R$_8^{Flu}$	$C_{144}H_{226}N_{46}O_{30}S$	3113.72	3113.7 ($M+H^+$)

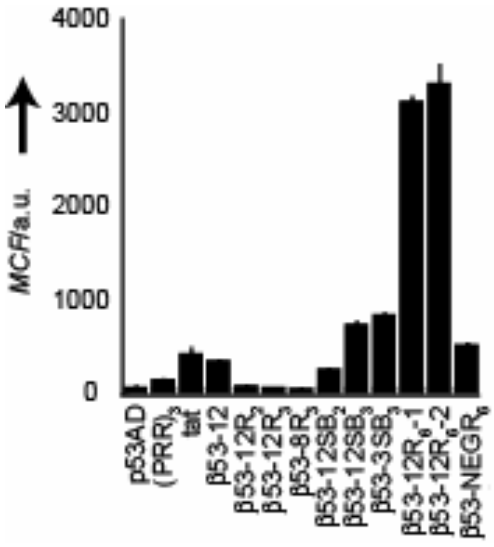

Figure S1:Entry of arginine variants into HCT116 cells quantified by flow cytometry after 4h. Mean cellular fluorescence (*MCF*) was calculated from the histogram of fluorescence intensity and was corrected for background cellular fluorescence by subtracting the geometric mean of cells treated with only PBS. Each value represents the average of three independent trials. Error bars represent the standard error.

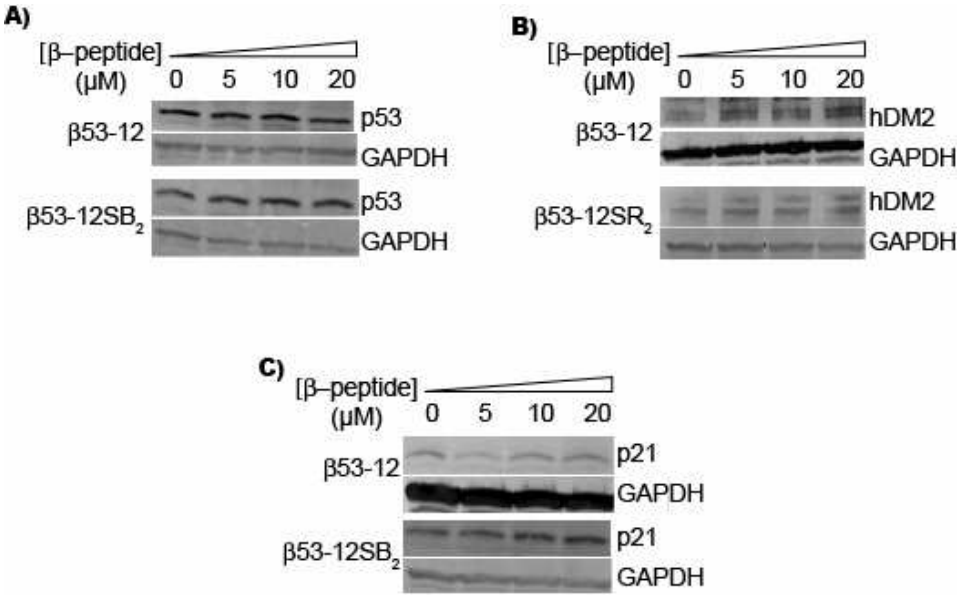

Figure SI- 2. Western blot analysis of the effects of β^3-peptides on (a) p53, (b) hDM2, and (c) p21 levels in HCT116 cells. Cells were incubated with the indicated β^3-peptide for 8 h. Whole-cell lysates were probed with the appropriate primary antibody and corresponding horseradish peroxidase-conjugated secondary antibody and visualized using ECL Plus Western Blotting Detection Reagents (GE Healthcare). Chemiluminescent signal was measured using the Storm 840 imaging system (Molecular Dynamics).

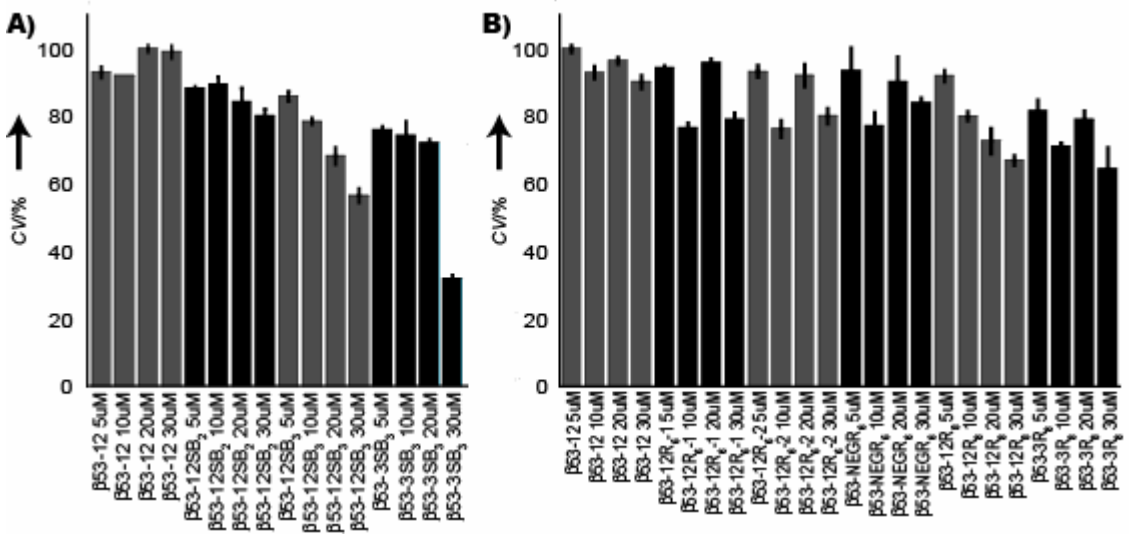

Figure SI- 3. Cellular viability analysis. HCT116 cells were treated with varying concentrations of β^3-peptides ((a) – salt-bridge variants, (b) – 6-arginine and tagged variants) for 8 hours, and cellular viability (CV) was determined by resazurin reduction as described in Experimental Methods. Standard error of three independent experiments, each with three replications, is reported. Alternating colored bars are for clarity to denote a switch to a different peptide.