Supporting Information

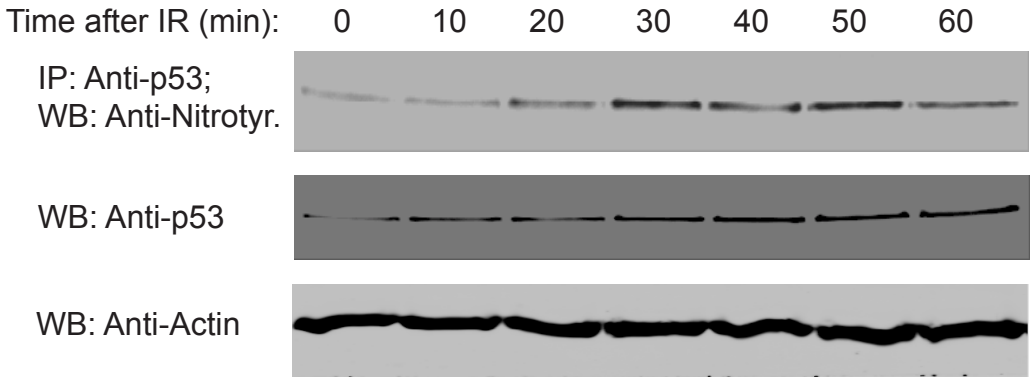

Figure S1. Tyr nitration and phosphorylation of endogenous p53 after radiation. MCF-7 cells were irradiated at 5 Gy and cell lysates were prepared at the indicated time-points for analysis of Tyr nitration and total p53 accumulation. Actin expression was shown as a loading control. NO-donor stimulated nitration was analyzed by IP with anti-p53 IgG and WB with anti-NitroTyr IgG.

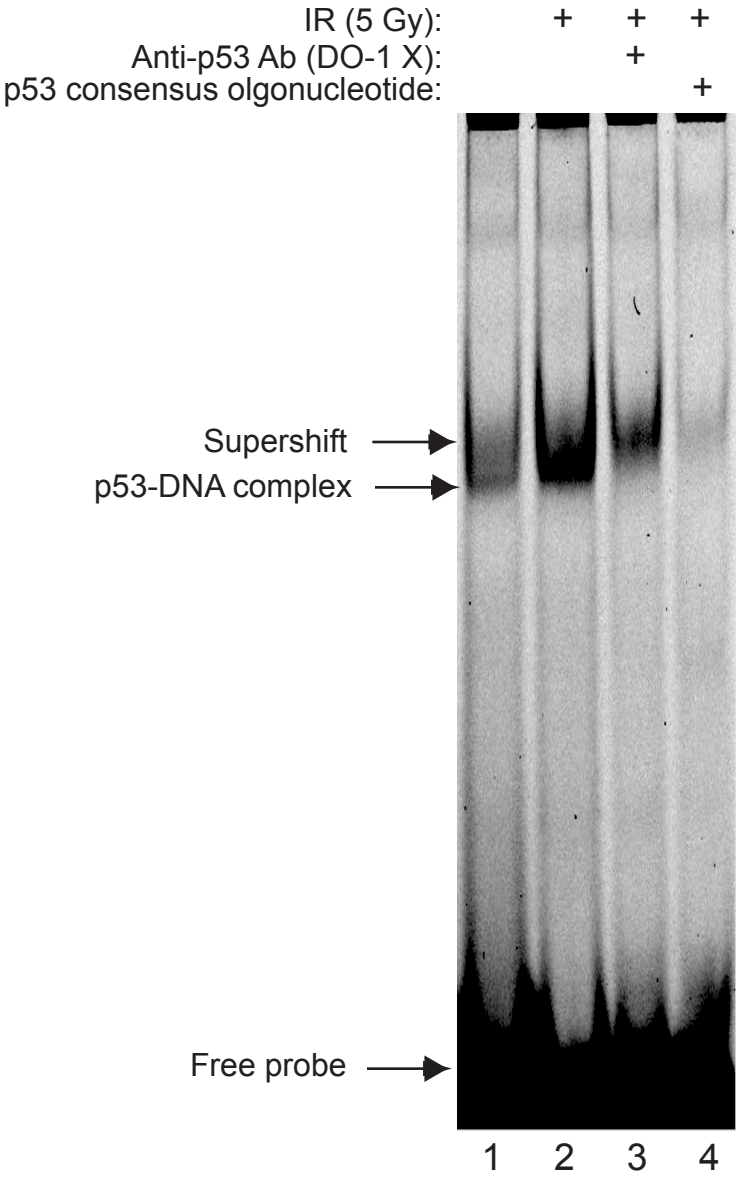

Fig. S2. Gel mobility shift, supershift, and "cold oligonucleotide" control. DNA-binding activity of endogenous p53 in MCF-7 cells was analyzed by EMSA. 5μg of the nuclear extract from non-radited (lane 1) and radiated cells (2 h after IR, lanes 2-4) was used. p53 specific antibody DO-1 was added to supershift p53-DNA complex (lane 3). p53 consensus oligonucleotide was used to indicate specific p53-DNA binding (lane 4). Migration positions of free probe, p53-DNA, and DO-1 Ab-p53-DNA complexes are shown.

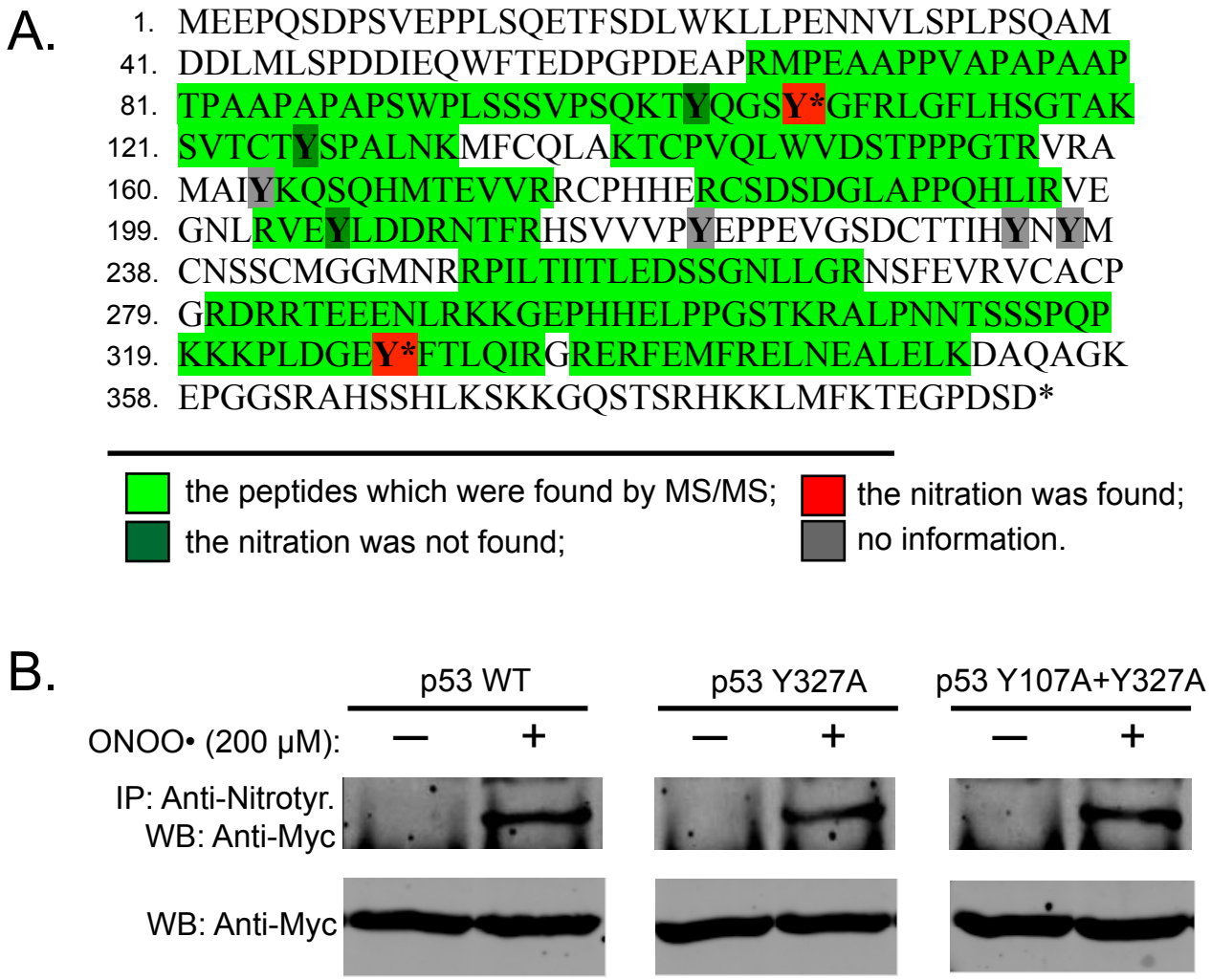

Figure S3. p53 Tyr nitration in vitro. (A) p53 sequence and mass spectrometry results; (B) MCF-7 cells were transfected with equal amounts of Myc-tagged p53 WT and indicated mutants. Cell lysates were nitrated with 200 μM peroxynitrite and anti-nitroTyr immunoprecipitates analyzed by Western Blot with anti-Myc antibody. All top panels are from the same blot. Total cell lysates were also probed with anti-Myc-tag antibody for Myc-tagged p53 expression and loading control (bottom panels).

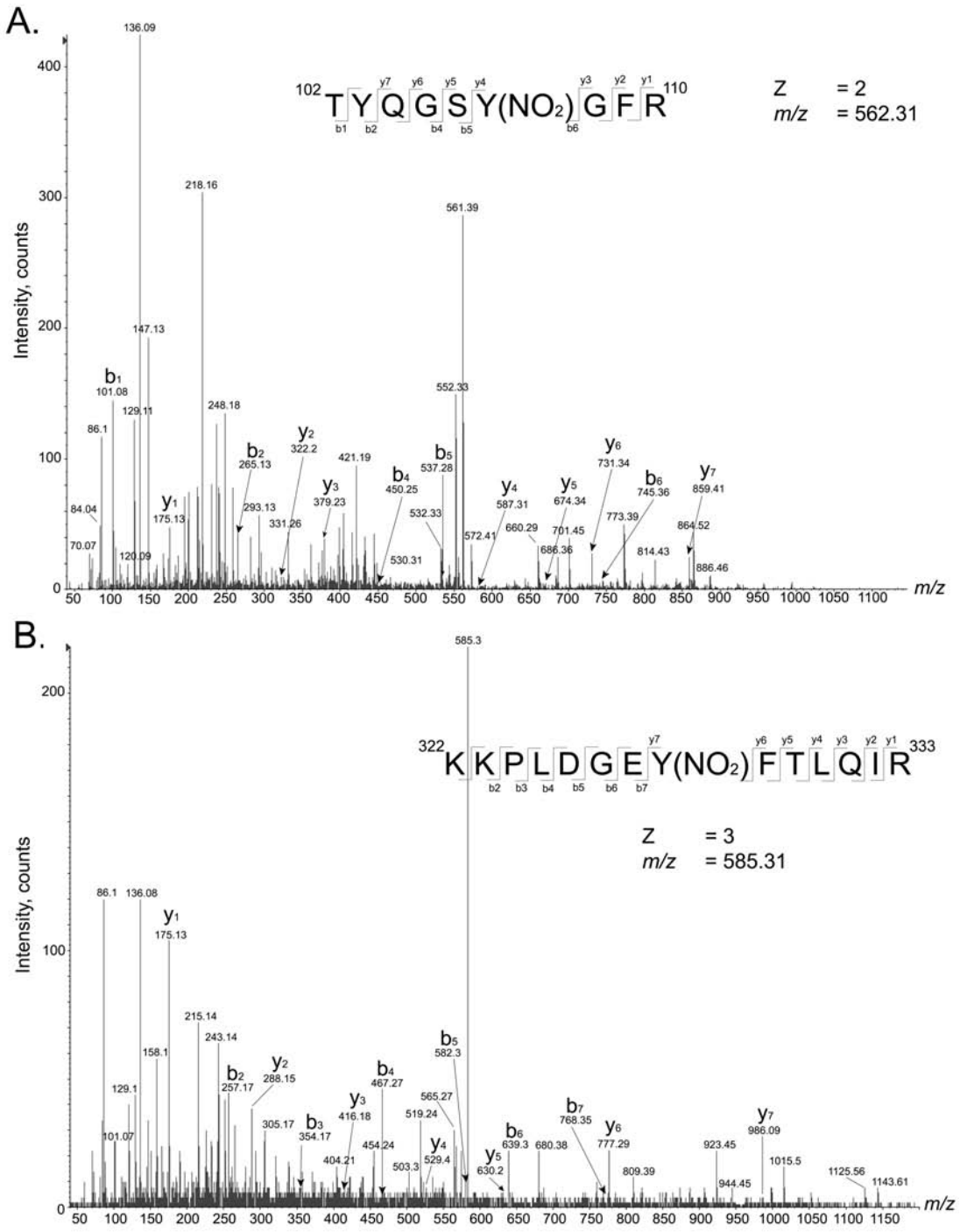

Figure S4. Tandem mass spectrum of the peptides contained Nitro-Tyrosines. (A) The peptide ^{102}TYQGSY(NO$_2$)GFR^{110} with nitrated Tyr107; (B) The peptide ^{320}KKPLDGEY(NO$_2$)FTLQIR^{333} with nitrated Tyr327. The modified peptide was manually verified. The sequence-specific ions are labeled as y and b ions on the spectra.

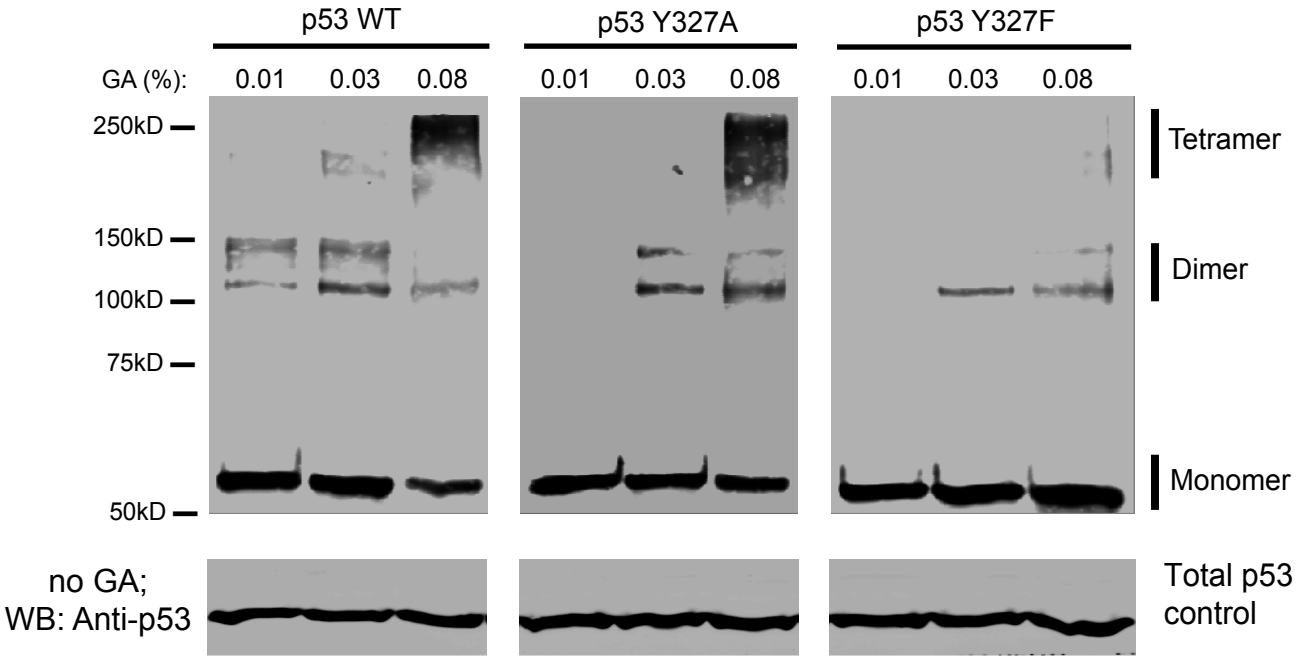

Figure S5. Tetramerization activity of overexpressed p53 WT, Y327A, and Y327F mutants. Saos-2 cells were transfected with the same amount of p53 WT or indicated mutants. 24 h after transfection cells were lysed. Equal amounts of the cell lysates were treated with indicated concentrations of glutaraldehyde. All panels are from the same blot. GA – glutaraldehyde; Tetramerization of p53 was assessed as described in Methods.

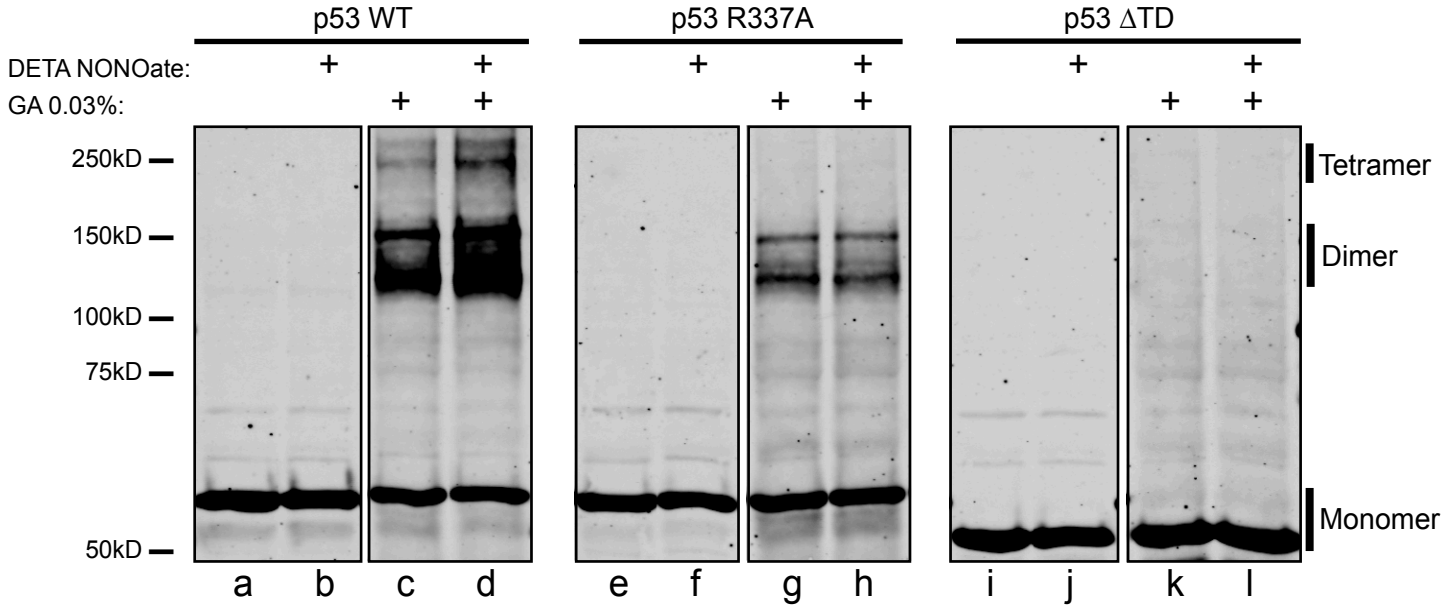

Figure S6. Incubation with DETA NONOate stimulates tetramerization of WT p53, but not Arg337Ala or ΔTD mutants. Saos-2 cells were transfected with identical amounts of p53 WT and mutants. Twenty-four h after transfection, cells were incubated with 200 μM DETA NONOate for 4 h. All panels are from the same blot. GA – glutaraldehyde; Tetramerization of p53 was assessed as described in Methods.

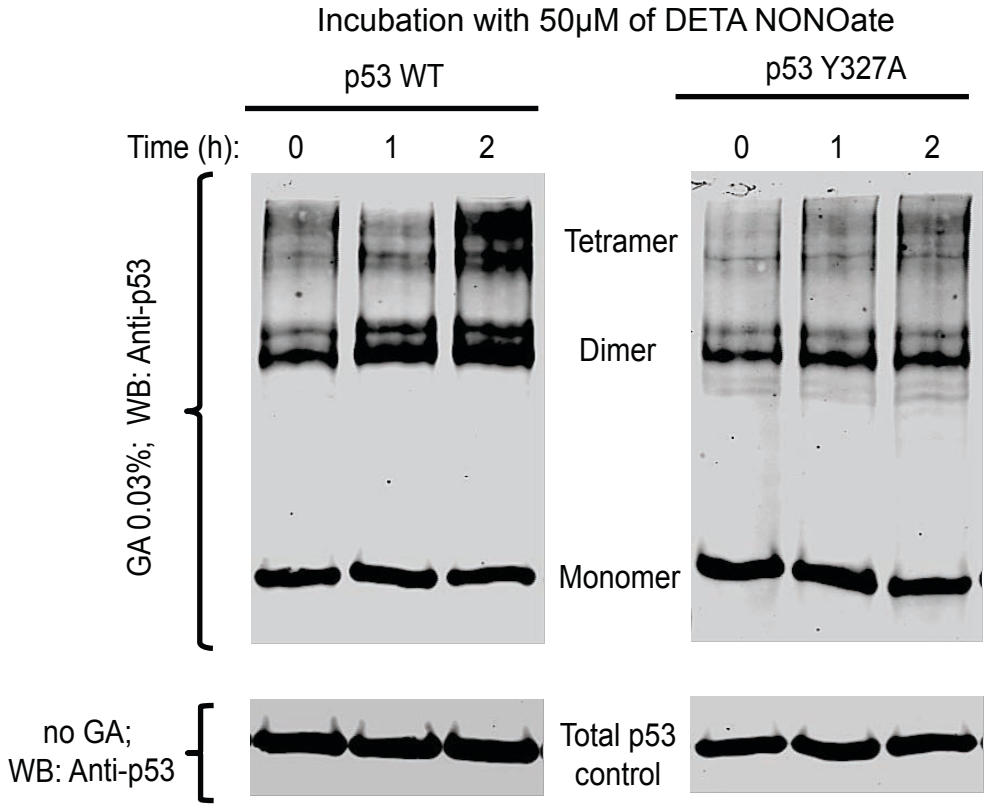

Figure S7. Stimulation of p53 WT tetramerization by 50μM of DETA NONOate. Saos-2 cells were transfected with identical amounts of p53 WT and mutant. Twenty-four h after transfection cells were incubated with 50 μM DETA NONOate and samples were collected at the indicated time-points. p53 Y327A was used as a negative control. All panels are from the same blot. GA – glutaraldehyde; Tetramerization of p53 was assessed as described in Methods.

Table S1. Mass spectroscopy results for p53 protein: observed m/z values and their corresponding A.A. sequence.

Amino Acids	Peptide Sequence	Calculated m/z	Observed m/z	Ion Charge	Modifications
66-101	MPEAAPPVAPAPAAPTPAAPAPAPSWPLSSSVPSQK	861.69	861.98	MH_4^{4+}	None
102-110	TYQGSYGFR	539.75	539.78	MH_2^{2+}	None
102-110	TYQGS**Y***GFR	539.75	562.31#	MH_2^{2+}	NO_2
111-120	LGFLHSGTAK	515.78	515.72	MH_2^{2+}	None
121-132	SVTCTYSPALNK	642.36	642.31	MH_2^{2+}	None
140-156	TCPVQLWVDSTPPPGTR	618.57	618.58	MH_3^{3+}	None
165-174	QSQHMTEVVR	607.79	607.82	MH_2^{2+}	None
182-196	CSDSDGLAPPQHLIR	804.89	805.31	MH_2^{2+}	None
203-213	VEYLDDRNTFR	714.35	714.38	MH_2^{2+}	None
281-290	DRRTEEENLR	659.32	658.80	MH_2^{2+}	None
291-305	KKGEPHHELPPGSTK	547.97	547.95	MH_3^{3+}	None
293-305	GEPHHELPPGSTK	693.34	693.38	MH_2^{2+}	None
307-319	ALPNNTSSSPQPK	670.84	670.87	MH_2^{2+}	None
320-333	KKPLDGEYFTLQIR	569.98	569.92	MH_3^{3+}	None
320-333	KKPLDGE**Y***FTLQIR	569.98	585.31#	MH_3^{3+}	NO_2
336-342	ERFEMFR	507.74	507.77	MH_2^{2+}	None
343-351	ELNEALELK	529.79	529.81	MH_2^{2+}	None

* - Modified amino acid;
\# - Mass is consistent with Tyrosine nitration.

Table S2. HINT score calculations for interactions of Tyr 327 of chain A with chain C before and after nitration. Only interaction scores over 10 are shown. Interactions with NO group are in red.

Before Nitration

Residue	Residue Atom	Tyr Atom	Interaction Score	Interaction Type
GLN331	OE1	CB	-16	Hydroph./Polar
GLN331	OE1	CD2	11	Hydrogen Bond
ILE332	O	O	-13	Base/Base

Total HINT Score 57

After Nitration

Residue	Residue Atom	nitroTyr Atom	Interaction Score	Interaction Type
GLN331	O	ON	-14	Base/Base
GLN331	CG	ON	-13	Hydroph./Polar
GLN331	OE1	NZ	-24	Base/Base
GLN331	OE1	ON	-39	Base/Base
GLN331	OE1	CB	-23	Hydroph./Polar
GLN331	NE2	NZ	87	Hydrogen Bond
GLN331	NE2	ON	43	Acid/Base
GLN331	NE2	ON	184	Hydrogen Bond
GLN331	NE2	OH	22	Acid/Base
ILE332	O	O	-13	Base/Base
ARG333	N	ON	15	Acid/Base
ARG333	CB	NZ	16	Hydrophobic
ARG333	CB	ON	-10	Hydroph./Polar
ARG333	CB	OH	-10	Hydroph./Polar
ARG333	CG	OH	-12	Hydroph./Polar
ARG333	CD	OH	-19	Hydroph./Polar
ARG333	NE	NZ	109	Hydrogen Bond
ARG333	NE	ON	133	Hydrogen Bond
ARG333	NE	ON	52	Acid/Base
ARG333	NE	OH	363	Hydrogen Bond
ARG333	CZ	OH	-16	Base/Base
ARG333	NH1	ON	12	Acid/Base
ARG333	NH1	OH	41	Acid/Base
ARG333	NH2	NZ	64	Acid/Base
ARG333	NH2	ON	192	Hydrogen Bond
ARG333	NH2	ON	26	Acid/Base
ARG333	NH2	OH	457	Hydrogen Bond

Total HINT Score 1688