Supplementary Data

Novel TDP2 ubiquitin interactions and their importance for the repair of topoisomerase II-mediated DNA damage

Timsi Rao[1], Rui Gao[2], Saeko Takada[1], Muthana Al Abo[2], Xiang Chen[3], Kylie J. Walters[3], Yves Pommier[2], Hideki Aihara[1,4]

[1]Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, Minnesota, USA

[2]Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institute of Health, Bethesda, Maryland, USA

[3]Structural Biophysics Loboratory, Center for Cancer Research, National Cancer Institute, National Institute of Health, Frederick, Maryland, USA

[4]To whom correspondence should be addressed. Email: aihar001@umn.edu

SUPPLEMENTAL FIGURE 1

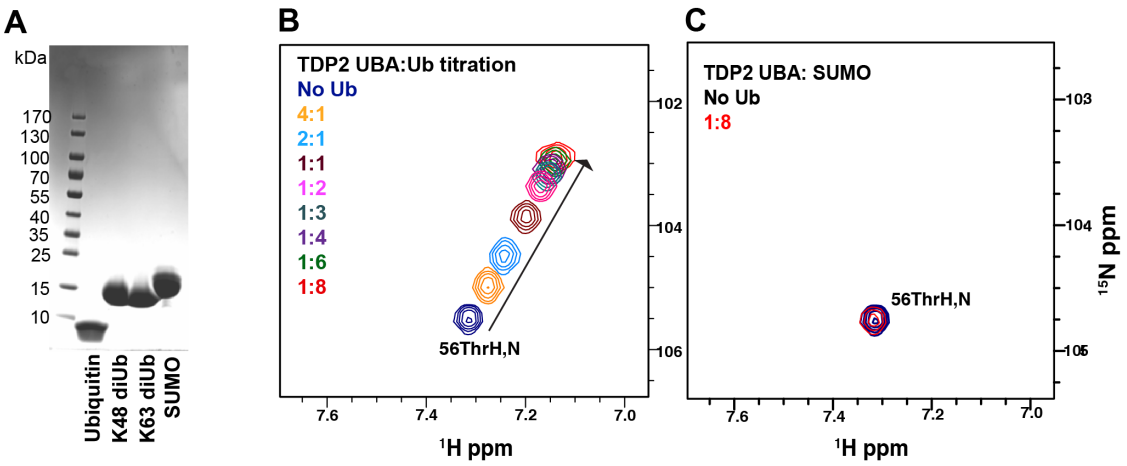

Figure S1. (**A**), Coomassie-stained SDS-PAGE gel (4-20% gradient) for purified Ub, diUbs and SUMO proteins. (**B**), Titration experiment of increasing concentrations of monoUb into ^{15}N labeled *Ce*TDP2 UBA. The region of ^{1}H, ^{15}N HSQC spectra that contains Thr56 is displayed with the peaks at different molar ratios of Tdp2 UBA:Ub color-coded. (**C)**, The same region of the HSQC spectra as in *B*, with and without 8-fold molar excess of SUMO added into ^{15}N labeled *Ce*TDP2 UBA.

SUPPLEMENTAL FIGURE 2

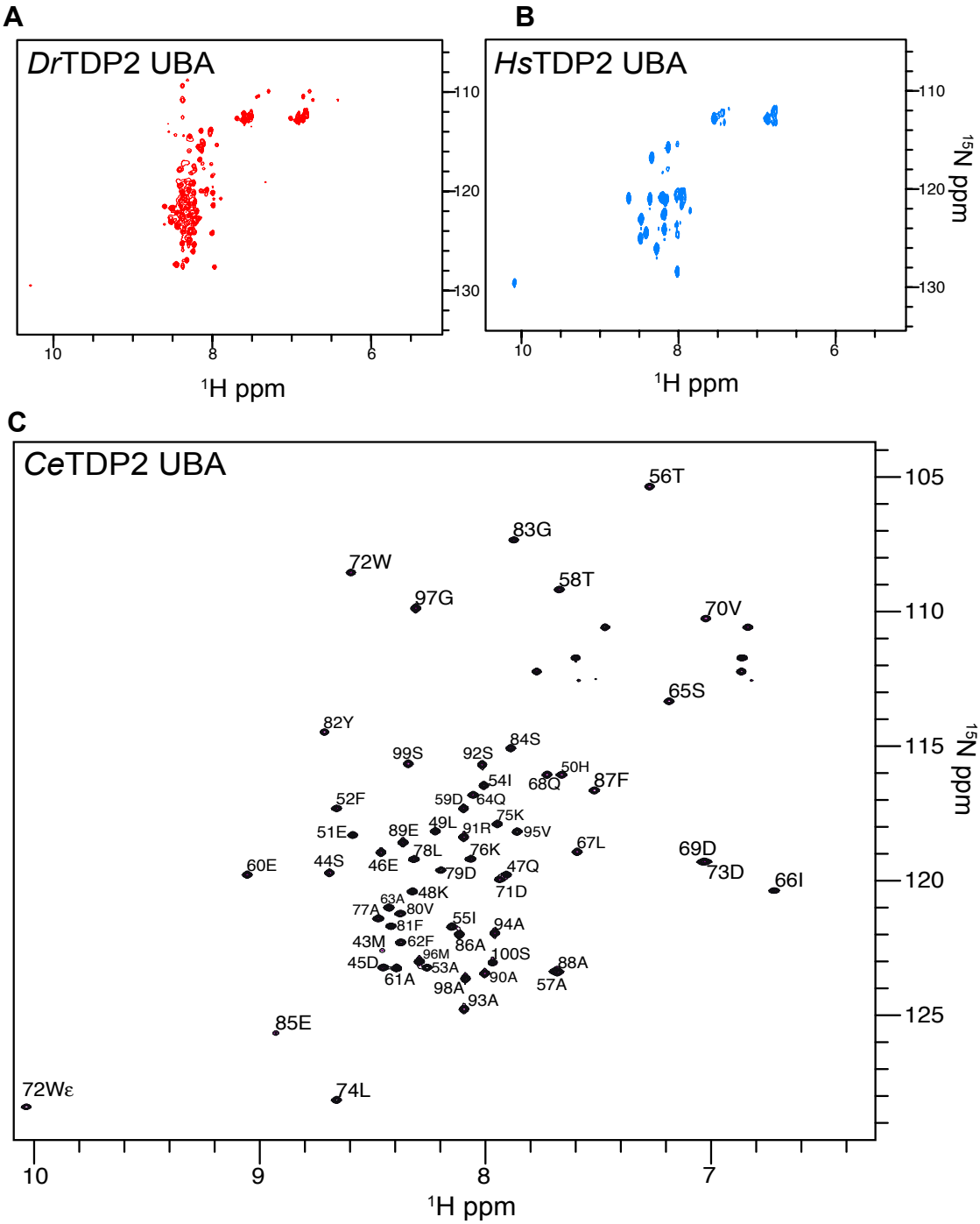

Figure S2. ***Ce*TDP2 UBA gave a well-dispersed 2D ^{15}N HSQC spectrum**. (**A**) and (**B**), 2D ^{15}N HSQC spectra of zebrafish (residues 1-106) and human (residues 24-80) TDP2 UBA. (**C**), 2D ^{15}N HSQC spectrum of *Ce*TDP2 UBA with peak assignments shown.

SUPPLEMENTAL FIGURE 3

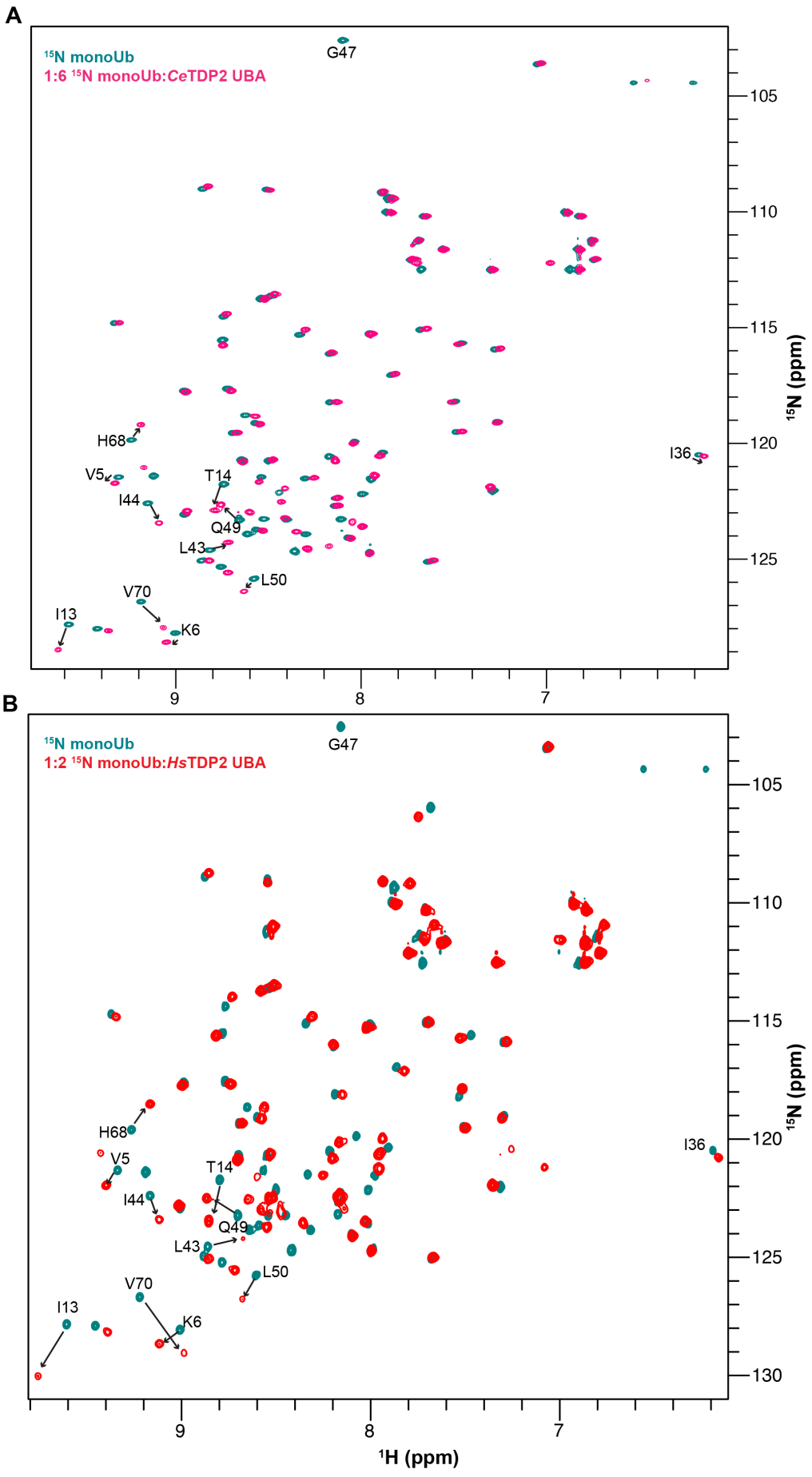

Figure S3. (**A**), $^{1}H,^{15}N$ HSQC spectra of ^{15}N labeled monoUb (teal) and ^{15}N labeled monoUb mixed with excess CeTDP2 UBA (pink) are superimposed. (**B**), $^{1}H,^{15}N$ HSQC spectra of ^{15}N labeled monoUb alone (black) and with 2-fold molar excess *Hs*TDP2 UBA (residues 1-110, red). Some of the significantly shifted peaks are labeled and their bound-state peaks indicated by black arrows on both panels.

SUPPLEMENTAL FIGURE 4

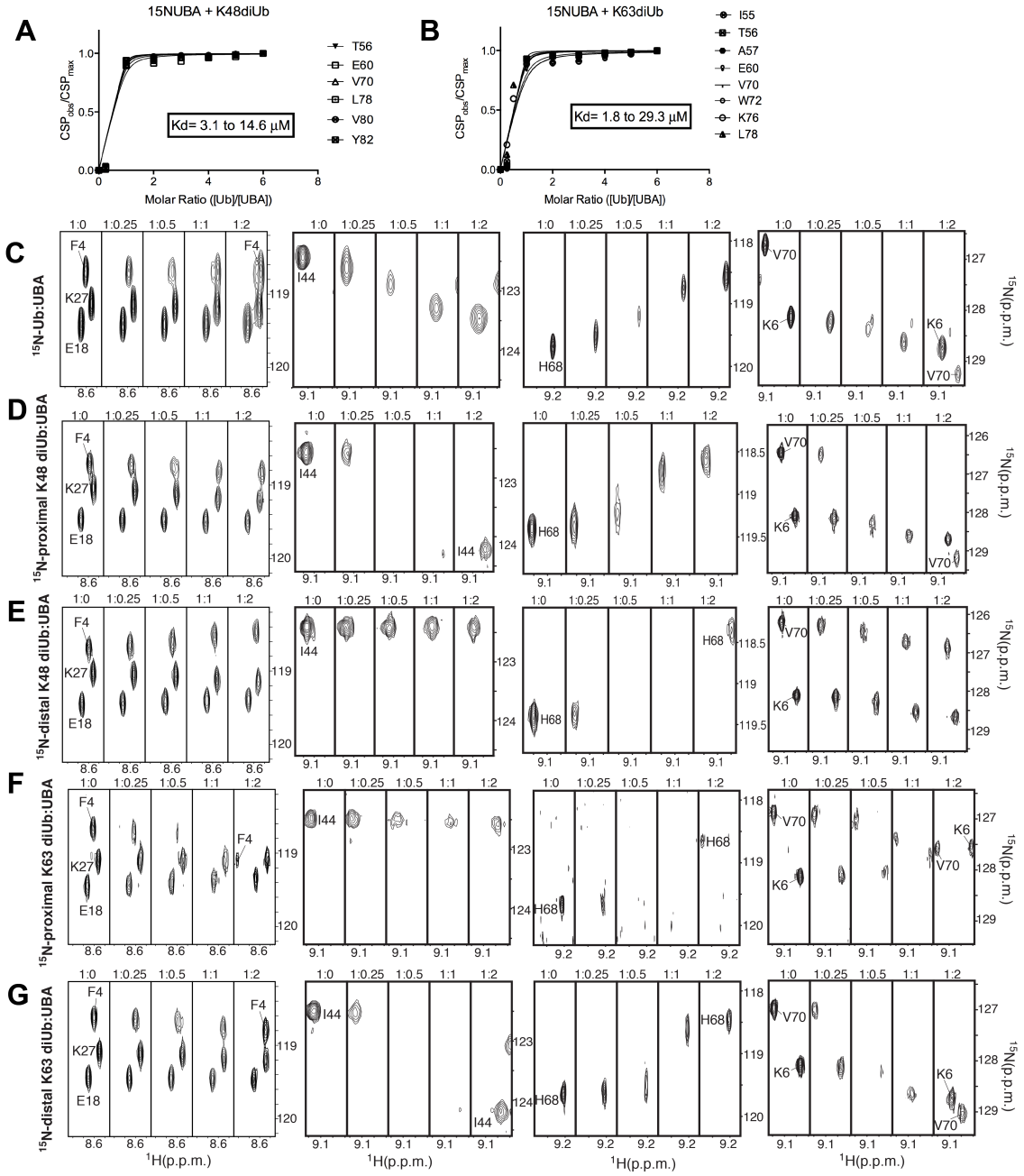

Figure S4. (**A-B**) The combined (^{1}H and ^{15}N) CSPs of significantly shifted peaks of (**A**) ^{15}N labeled *Ce*TDP2 UBA at indicated molar ratios with K48 diUb, and (**B**) ^{15}N labeled *Ce*TDP2 UBA at indicated molar ratios with K63 diUb. The range of K_d values calculated for the chosen residues is shown on each graph. (**C-G**) ^{1}H,^{15}N HSQC spectra strips of significantly shifted peaks upon titration with increasing concentration of unlabeled *Ce*TDP2 UBA are compared for (**C**) ^{15}N labeled monoUb, (**D**) K48 diUb with ^{15}N labeled proximal Ub, (**E**) K48 diUb with ^{15}N labeled distal Ub, (**F**) K63 diUb with ^{15}N labeled proximal Ub, and (G) K63 diUb with ^{15}N labeled distal Ub. The ratio of ^{15}N labeled species to unlabeled UBA is indicated on top of each strip. Residue peaks are labeled.

SUPPLEMENTAL FIGURE 5

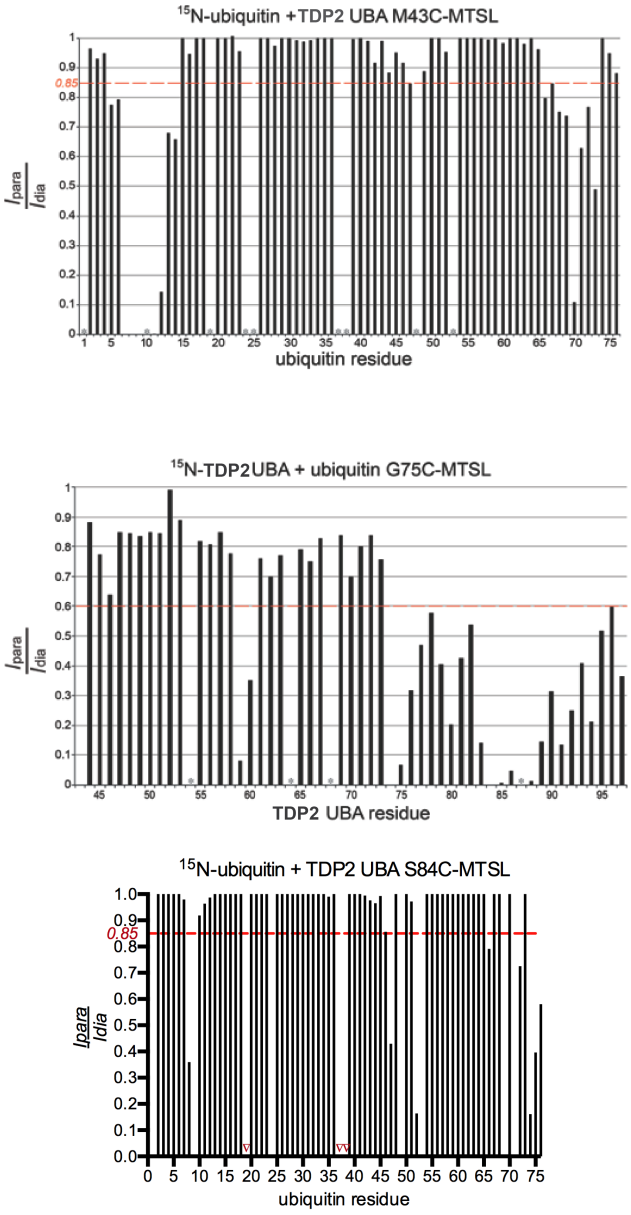

Figure S5. Paramagnetic relaxation enhancement (PRE) data from ^{15}N Ub complexed with TDP2 UBA M43C MTSL (top panel) and ^{15}N TDP2 UBA complexed with Ub G75C MTSL (middle panel). Residues with the intensity ratio below the upper cutoff (shown on each panel with a dashed red line) were categorized in the 1.8 - 23 Å distance range. Paramagnetic relaxation enhancement (PRE) data from ^{15}N Ub complexed with TDP2 UBA S84C MTSL (bottom panel). The upper cutoff for residues categorized in the 1.8 - 23 Å distance range is shown on the panel with a dashed red line. Inverted Red triangles denote Ub residues that were prolines and hence excluded from analyses.

SUPPLEMENTAL FIGURE 6

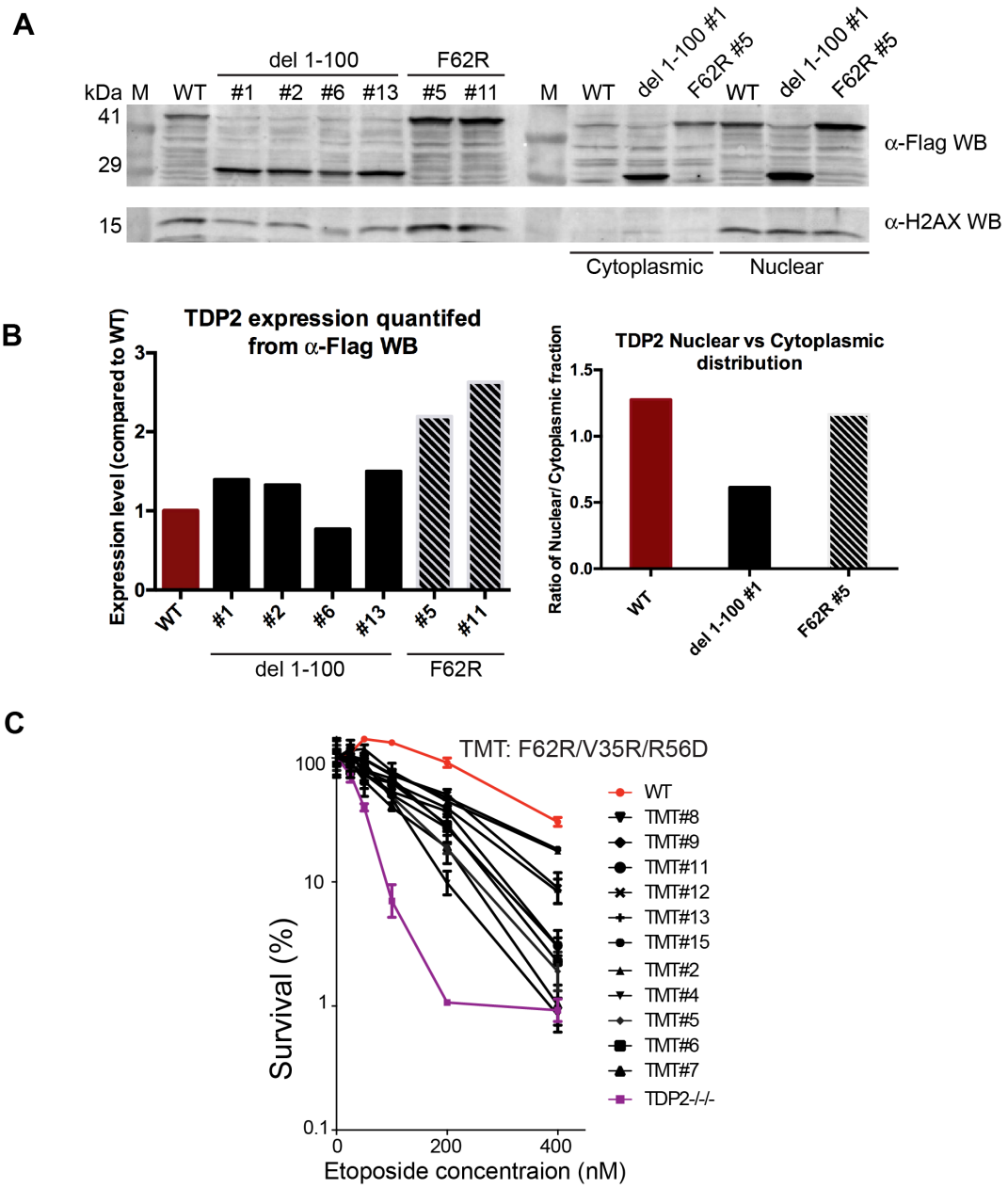

Figure S6. (**A**) Western blot analysis using anti-flag antibody to compare the total expression levels and the nuclear *vs*. cytoplasmic distribution of *Hs*TDP2 wild type (WT) and mutant (del 1-100 or F62R) clones in *TDP2-/-/-* DT40 cells. For the total cell extracts (left), equal number ($2x10^5$) of cells were loaded in each lane. Cytoplasmic load was one third of nuclear load. (**B**) Quantitation of band intensities from panel *A*, showing comparison of the total *Hs*TDP2 expression levels on the left and nuclear/cytoplasmic ratios on the right. (**C**) Cell survival assay (similar to Figure 8B) testing 11 clones transfected with a triple mutant targeting the TDP2 UBA-Ub surface (F62R+V35R+R56D) against increasing concentrations of Top2 poison, etoposide.

SUPPLEMENTAL FIGURE 7

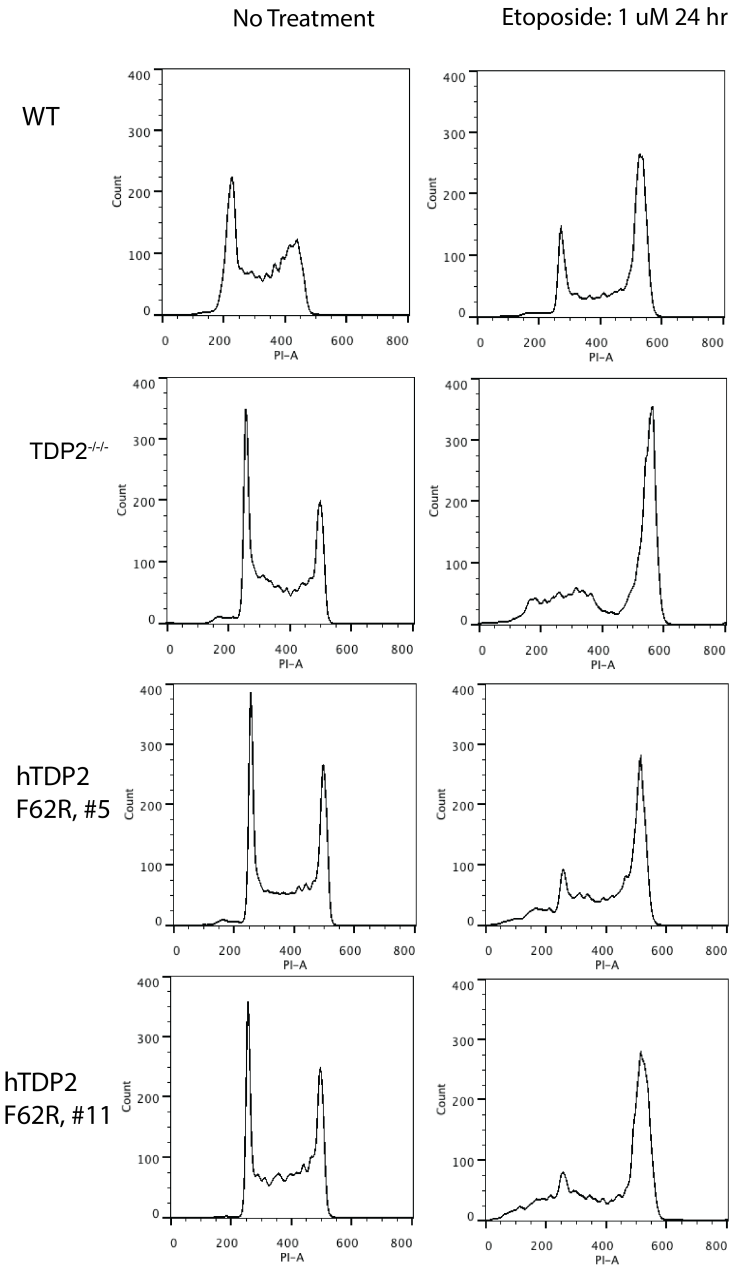

Figure S7. FACS analysis profiles for cells shown in Figure 8B sorted based on intensity of labeling by propidium iodide cell death marker. WT denotes TDP2$^{-/-/-}$ DT40 cells complemented with the full-length wild-type HsTDP2. F62R #5 and 11 are two independent cell lines complemented with HsTDP2 F62R mutant.

FIGURE S8

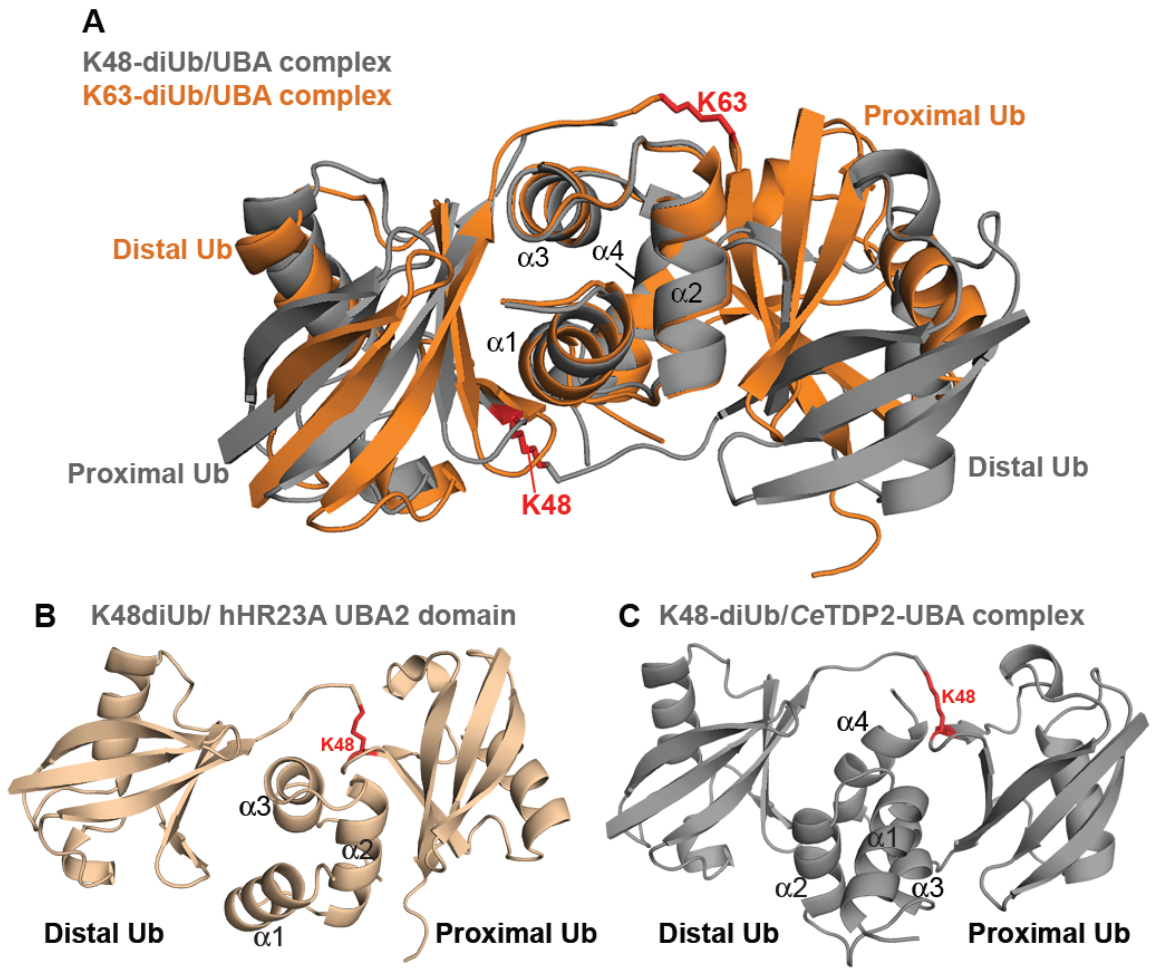

Figure S8. Comparison of TDP2 UBA – diUb models. (**A**) Superposition of hypothetical models of TDP2 UBA with K48-diUb (grey) and K63-diUb (orange). (**B**) TRIDOCK model of human HR23A UBA2 with K48-diUb; pdb id 1ZO6 (1). The K48 side chain is highlighted in red. (**C**) Theoretical model of *Ce*TDP2 UBA with K48-diUb shown in the same conformation as diUb in panel B. The K48 side chain is highlighted in red.

SUPPLEMENTAL FIGURE 9

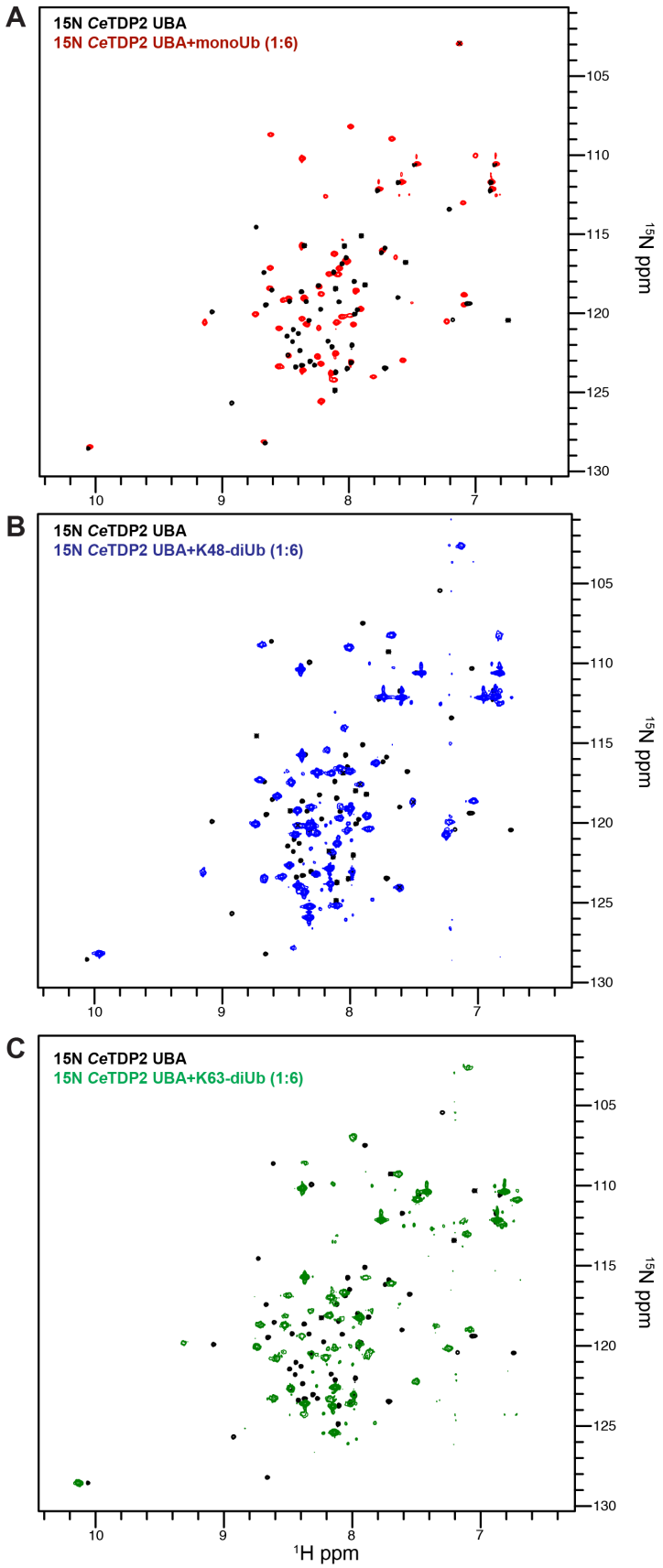

Figure S9. $^{1}H,^{15}N$ HSQC spectra of ^{15}N labeled *Ce*TDP2 UBA alone (black) and in the presence of 6-fold molar excess of (**A**) monoUb (red), (**B**) K48-diUb (blue) or (**C**) K63-diUb (green) superimposed.

Supplemental Table 1: List of active and passive residues involved in TDP2 UBA-Ubiquitin interaction as defined by chemical shift perturbation plots

Protein	Active residues	Passive residues	Flexible segments
TDP2 UBA	55,57,60,61,65,68,75,76,79,80,82, 83,84,86,93,94	51,54,59,64,71,73	49-96
Ubiquitin	6,8,14,42,44,46,47,48,49,68,70,71, 72	9,12,51,66,74	4-16,40-53,64-76

Supplemental Table 2: Comparison of HADDOCK runs with varying numbers of unambiguous restraints

	Ambiguous (Amb) only		Amb+M43C MTSL PREs		Amb+ G75C MTSL PREs		Amb+ half[¶] M43C + half G75C MTSL PREs	
	UBA	Ub	UBA	Ub	UBA	Ub	UBA	Ub
Starting Structure	4GEW	1D3Z	4GEW	1D3Z	4GEW	1D3Z	4GEW	1D3Z
Ambiguous restraints	16	13	16	13	16	13	16	13
Unambiguous restraints (PREs)	0	0	60	0	0	25	30	13
Clusters determined by HADDOCK	9		3		6		2	
Structures in top scored cluster	38		189		16		184	
RMSD from lowest energy structure	10.8±0.2 Å		1.5±0.3 Å		1.6±0.5 Å		1.7±0.4 Å	
Van der Waals energy	-34.7±7.3		-34.5±7.6		-44.5±8.2		-40.2±10.1	
Electrostatic energy	-198.2±66.1		-346.4±50.5		-200.6±58.0		-146.3±64.2	
Desolvation energy	-5.0±7.3		-2.8±6.6		-0.1±4.8		2.0±7.0	
Restraint violation energy	3.6±1.2		3.7±1.3		5.4±1.5		4.7±-1.3	
Buried surface area	1197.1±172.7		1181.0±138.2		1408.2±129.2		1289.1±176.0	
Backbone r.m.s.d. from final best* model	1.9 Å		1.7 Å		1.4 Å		1.5 Å	

* Root mean square deviation (r. m. s. d.) for backbone atoms of the best-scored model from each run was calculated against the best-scored final model (from Amb+85 PRE HADDOCK run). r.m.s.d. was calculated using the “super” script in PyMOL Molecular Graphics System, Version 1.5, Schrodinger, LLC (2). Amb, ambiguous.

[¶] Only half of the restraints for each spin label were included in this modeling run

Supplemental Table 3: validation of the HADDOCK model through comparison of PRE experimental restraints derived from S84C MTSL labeling of TDP2 UBA (Figure S8) to the corresponding distances in the final best scored model

#	UBA Res ID	Ub Res ID	Experimental Restraints PRE range (Å)	On model, distance between HN pairs (Å)
1	S84	L8	2.0 – 23.0	15
2	S84	T9	2.0 – 23.0	17
3	S84	E24	2.0 – 23.0	21
4	S84	A46	2.0 – 23.0	21
5	S84	G47	2.0 – 23.0	19
6	S84	Q49	2.0 – 23.0	15
7	S84	D52	2.0 – 23.0	17
8	S84	G53	2.0 – 23.0	19
9	S84	T66	2.0 – 23.0	24
10	S84	L69	2.0 – 23.0	16
11	S84	L71	2.0 – 23.0	10
12	S84	R72	2.0 – 23.0	9
13	S84	R74	2.0 – 23.0	9
14	S84	G75	2.0 – 23.0	10
15	S84	G76	2.0 – 23.0	10

References

1. Varadan,R., Assfalg,M., Raasi,S., Pickart,C. and Fushman,D. (2005) Structural determinants for selective recognition of a Lys48-linked polyubiquitin chain by a UBA domain. *Mol. Cell*, **18**, 687–698.

2. Schrödinger, LLC. The PyMOL Molecular Graphics System, Version 1.5.0.4.