Supplementary Materials for

ZATT (ZNF451) Mediated Resolution of Topoisomerase 2 DNA-Protein Crosslinks

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Supplementary Text

TOP2 cleavage complex composition in MEF cells

In control experiments, we assessed the composition of the chromatin bound Topoisomerase 2 material purified for ICE assays in Mouse Embryonic Fibroblasts (MEF) cells. Under the conditions tested, the majority of the material isolated by the ICE assay corresponds to TOP2β, a fraction of which is modified by SUMO, and preferentially by the 2/3 isoform (figs. S6A–S6C). ICE fractions were digested with micrococcal nuclease to release competent proteins, and western blotting was used to detect TOP2 and SUMO isoforms (fig. S6A). Consistent with the experiment being performed in cell cycle-arrested cells, most of the cleavage complexes detected

corresponded to TOP2β, which mainly migrates with its expected size, and only a minority with a higher molecular weight laddering pattern. SUMO2/3 signal, and more weakly SUMO1 were also observed as slower-migrating ladders. These data show that the majority of the material isolated by the ICE assay in these conditions corresponds to TOP2β, a fraction of which is modified by SUMO, and preferentially by the 2/3 isoform. Further supporting this, we show that TOP2β knock-down in RPE-1 cells greatly diminished the amount of SUMO covalently bound to DNA upon etoposide treatment (fig. S6C), and that denaturing pull-down of His-tagged SUMO from material isolated in the ICE assay recovered TOP2β (fig. S6B). TDP2 deletion also did not affect the amount of TOP2αcc, TOP2βcc and SUMO2 crosslinked to chromatin by etoposide treatment in the presence or absence of the proteasome inhibitor MG132 (fig. S6D).

Materials and Methods

Cloning, plasmid construction, and antibodies.

DNA encoding TDP2 or TDP2 deletion mutants (*7*) was cloned into pENTR-TEV (Invitrogen). Mutant TDP2 plasmids were generated using the Quickchange kit (Stratagene). LR Clonase kit was used to transfer the insert into the appropriate pDEST vector (Table S5). shRNA plasmids targeting ZNF451 (TRCN0000151451) or ZFP451 (TRCN0000241911) were purchased from Sigma. Antibodies used in this work are: Anti-GFP (Roche, 11814460001), anti-TOP2α (Abcam ab52934, Santa Cruz sc-365916), anti-TOP2β (Abcam ab109524, Santa Cruz sc-13059), anti-SUMO1 (DSHB 21C7), anti-SUMO2/3 (Abcam ab81371, Santa Cruz sc-32873), anti- ZNF451 (Sigma SAB2102880, SAB2108741), anti-TDP2 (Bethyl A302-737A, Caprico 100101), anti-GAPDH (Abcam ab128915), anti-SUMO1 (DSHB 21C7), anti-γH2AX (Millipore, 05-636), and anti-H3 (Abcam ab1791), with AlexaFluor 594-conjugated Goat anti-Mouse (ThermoFisher), IRDye 800CW Goat anti-Rabbit IgG (LI-COR), and IRDye 680RD Goat-anti-Mouse (LI-COR) secondary antibodies.

Cell lines and culture conditions

HEK293T and hTERT RPE-1 cells were obtained from the ATCC, and HEK293F cells were obtained from Invitrogen. The Universal Mycoplasma Detection Kit (ATCC) was

used to confirm the absence of mycoplasma. HEK293T and HEK293F cells were cultured in DMEM with 10% (v/v) Fetal Bovine Serum (Gibco), 50 U μL^{-1} penicillin, 50 $\mu g\ mL^{-1}$ streptomycin, 1X Mycozap prophylactic (Lonza), and 0.5 mmol L^{-1} sodium pyruvate at 37°C in 5% CO_2 atmosphere. Lentiviral particles (*33*) were infected at an MOI of 0.1, or plasmid DNA encoding the indicated proteins was transfected using Lipofectamine2000 (Invitrogen). Stable cells were selected with the appropriate antibiotic for 1-3 weeks until stable fluorescence levels were reached. Cells were selected by FACS to obtain cultures with equivalent expression levels. For immunoprecipitations, HEK293F cell lines were grown in Freestyle media (Invitrogen) with 10 U μL^{-1} penicillin and 10 $\mu g\ mL^{-1}$ streptomycin. Primary *Tdp2*$^{-/-}$ MEFs (between P1 and P4) and T121 transformed *Tdp2*$^{-/-}$ MEFs have been previously characterized (*12*). Overexpression of TDP2 was carried out by lentiviral infection at MOI 2.5. For shRNA depletion, cells were infected with equal amounts, as determined by PCR titer, of non-targeting and ZFP451 shRNA lentiviral particles followed by 10 days selection in 2$\mu g\ mL^{-1}$ puromycin to obtain at least 80% knock-down efficiency. For siRNA depletion, cells were transfected with non-targeting siRNA (CGUACGCGGAAUACUUCGA) or TOP2β siRNA (GCTTAACAATCAAGCCCGTTT) (*34*) using Lipofectamine RNAiMAX (Thermo) according to manufacturer's protocol, and experiments were performed 72 h after transfections. To generate CRISPR knockout cell lines, HEK293F cells were transfected with plasmids from the ORIGENE CRISPR knockout kit (KN215720RB for ZNF451, and KN202015LP for TDP2), which encodes CAS9, gRNA, and homology-directed repair template that targets the open-reading frame for replacement with a reporter and antibiotic resistance (RFP-Blasticidin^R for ZNF451, and Luciferase-Puro^R for TDP2), followed by 10 days growth with selection. Parallel transfections with a non-targeting gRNA served as a negative control and yielded far (10-100 fold) fewer stable cells than the on-target gRNA. Clones derived from single cells were obtained by limited dilution plating in a 96-well plate, and the absence of ZNF451 or TDP2 was confirmed by western blotting. To generate TDP2/ZNF451 double knockouts, a clone of ΔZNF451 was transfected with a gRNA targeted to TDP2 as above.

Anti-GFP immunoprecipitation

50 mL cultures of HEK293F cells cultures at a density of 1.5–2.0 x 10^6 cells mL^{-1} were pelleted by centrifugation at 140x g, washed twice in cold PBS, and lysed in 1 packed cell volume of lysis buffer (50mM TRIS pH 8.0, 400 mM NaCl, 0.1% (v/v) NP-40, 1 mM DTT) with 2X Complete Protease Inhibitor cocktail (Roche) for 30 mins at 4 °C. Cells were centrifuged at 16,000 g for 10 mins, and the supernatant was bound to 25 µL anti-GFP nanobody derivatized agarose (*35*) (which also binds YFP–tagged proteins(*36*) for 30 mins at 4 °C on a nutator. Subsequently, the resin was transferred to a mini spin column (BioRad) and centrifuged at 1000 g for 10 s, followed by 8 washes of PBS with 0.1% (v/v) TWEEN 20 and 1mM DTT. Proteins were eluted in 0.1 M glycine-HCl pH 2, and run on a Nupage Bis-Tris 4-12% SDS-PAGE (Invitrogen). For tandem purification of His6-tagged SUMO2, eluates were neutralized with 0.1M Tris base, then diluted to 1 mL in denaturing buffer (20mM HEPES pH 7.5, 10 mM imidazole, 0.5mM TCEP, 8 M urea, 0.1% (w/v) SDS), bound to 25 µL Ni-NTA resin overnight, washed 4 times, then eluted in (20 mM HEPES pH 7.5, 100mM NaCl, 250 mM imidazole, 0.5 mM TCEP, 6 M urea, and 1% (v/v) TWEEN 20). Where indicated, samples were diluted 3-fold in the same buffer without urea or imidazole and cleaved with 10 U of ULP1 (LSBio) for 2 hours at room temperature before running on a Novex 4-12% Bis-Tris SDS PAGE (Novagen). Where etoposide treatment prior to IP is indicated, 50 mL cultures at a density of 1.0–1.3 x 10^6 cells mL^{-1} were treated with etoposide (or DMSO vehicle control) for 16 hours prior to IP. Samples were analyzed by immunoblot (IB) by running on a 4-12% Bis-Tris SDS PAGE (Novagen), transferring to low-fluorescence PVDF (Millipore) in 1X Tris-Glycine transfer buffer (Bio-Rad) supplemented with 0.05 % (w/v) SDS for 60 minutes at 30V. Membranes were then Immunoblotted with the indicated antibodies using the iBind system (Invitrogen) and imaged on an Oddysey Fc imager (Licor). Coomassie stained gels of the HEK293 lysates and IP-MS eluates contained 5X the sample amount used for western blots, and show equivalent loading of samples.

Identification of post-translational modifications on immunoprecipitated TOP2

To identify the covalently linkage to TOP2, IP samples (in 0.1 M glycine pH 2) were neutralized with an equal volume of 0.2M Tris. 5 µL reactions contained 4 µL YFP-TDP2 IP eluate, 0.5 µL 10X buffer, and 0.5 µL of the indicated hydrolase. 10X buffer for

ULP1 and OTUB1 contained 200 mM HEPES pH 7, 500 mM NaCl, 10 mM EDTA, and 20 mM DTT. 10X buffer for DNase I (NEB) and CIAP (Invitrogen) were from the manufacturer. OTUB1 removes Ubiquitin modifications, ULP1 removes SUMO modifications, CIAP removes phosphorylation, and DNAseI can hydrolyze covalently linked DNA. Reactions were incubated for 30 mins at room temperature, then western blotted with the indicated antibodies.

Immunoprecipitations of endogenous ZNF451

100 µL or Protein A/G PLUS Agarose (SCBT) was incubated in 10 mL PBST with 10 uL anti-ZNF451 for 16 hours at 4 °C on a nutator. Resin incubated under the same conditions but without addition of antibody served as negative control for immunoprecipitations. Resin was washed 4 times in PBST to remove free antibody. HEK293F cells (8 $x10^8$) growing at log phase in Freestyle media (Invitrogen) were centrifuged and washed in PBS, then lysed as above for the anti-GFP immunoprecipitations. 2 mL HEK293F cell lysate was incubated with the resin for 30 mins at 4 °C on a nutator, followed by washing and elution as above.

Analysis of protein samples by mass spectrometry

Immunoprecipitated samples were run on a 4-12% Bis-Tris SDS PAGE. Entire gel lanes were manually dissected into 24 equal pieces, minced, and digested with trypsin (Promega) for 8 hours with a Progest robotic digester from Genomic Solutions. Peptides were then analyzed by NanoLC-ESI-MS/MS using an Agilent 1200 nanoLC system on-line with an Agilent 6340 ion trap mass spectrometer with the Chip Cube Interface. 20 µL of the peptide mixture from the in-gel digest were loaded onto an Agilent C18 chip (75 µm x 43 mm), washed, and eluted using a 45-minute linear gradient from 5% (v/v) acetonitrile, 0.1% (v/v) formic acid to 50% (v/v) acetonitrile, 0.1% (v/v) formic acid. The mass spectrometer was used in the positive ion, standard enhanced mode and MS/MS data were acquired using a data dependent acquisition format with the 6 most abundant ions from each MS scan further interrogated by MS/MS. Data were processed and searched against the SwissProt/Uniprot database using the Spectrum Mill software package from Agilent. Proteins present with 10 or more peptides in both of two YFP-

TDP2 replicates (and absent from negative control YFP samples isolated in parallel), and 10 or peptides in the GFP-ZNF451 (and absent from negative control GFP samples isolated in parallel) are included in table S1. Our data validates observations from high-throughput screens that have detected putative ZNF451 interactions with TOP2 and TDP2 (*37-39*).

γH2AX foci analysis

Primary MEFs were grown on coverslips for 7-10 days until confluency arrested. Cells were treated with 30 μM etoposide (-MG132) or 100 μM etoposide and 20 μM MG132 (+MG132) for 30 minutes, then fixed in ice-cold methanol for 10 min at -20°C. Immunofluorescence was carried out as described (*11*). Briefly, cells were permeabilized (2 min in PBS-0.2% (v/v) Triton X-100), blocked (30 min in PBS-5% (w/v) BSA) and incubated with the primary antibody for 1h in PBS-1% (w/v) BSA. Cells were then washed (three times in PBS-0.1% (v/v) TWEEN 20), incubated with the secondary antibody for 30 minutes in 1% (w/v) BSA-PBS, washed again as described above, counterstained with DAPI (Sigma), and mounted in Vectashield (Vector Labs). γH2AX foci were manually counted (double-blind) in 40 cells for each experimental condition.

Chromatin fractionation

Serum-starvation arrested RPE-1 cells were pretreated with 20 μM MG132 or vehicle (DMSO), then 1 h with additional 100 μM etoposide, vehicle (DMSO), or 10 Gy ionizing radiation, and processed as described (*40*). Briefly, cells were washed twice in ice-cold PBS, and pre-extracted twice for 3 minutes on ice in CSK buffer (100 mM NaCl, 300 mM sucrose, 3 mM $MgCl_2$, 0.7% (v/v) Triton X-100, 10 mM PIPES, pH 7.0). After pre-extraction, cells were washed 3 times in ice-cold PBS and harvested in SDS loading buffer. Samples were run in 4-20% precast polyacrylamide gel (Biorad) and transferred/incubated as described above. ZNF451 levels were normalized to the corresponding H3 signal and expressed as fold increase compared to the untreated sample.

Preparation of recombinant proteins

TDP2 proteins were expressed and purified as described (*7*). SUMO2 proteins were expressed in BL21(DE3) Rosetta2 *E coli* (EMD Biosciences) at 25° C for 16 hours. HIS6-tagged SUMO2 was affinity purified from cell lysates with Ni-NTA resin (Qiagen), eluted with 250 mM imidazole, and purified by size exclusion chromatography on a 16/60 S-200 column (GE Life Sciences). After removal of the HIS6 tag with TEV, SUMO was purified on a HiTrap 5 mL Q-sepharose column (GE Life Sciences). Full length SUMO2 (aa 1-93) expressed from pET-DEST42 was purified as above, but elution from Ni-NTA was accomplished by ULP1-mediated tag removal overnight at 4 °C. Recombinant TOP2α protein used was obtained from TOPOGEN or expressed and purified in HEK293F cells. ZNF451 and TOP2β (47-1212) were expressed and purified from HEK293F cells as YFP-fusion proteins linked to the target via a TEV protease linker. Proteins were eluted from GFP/YFP resin (see Anti-GFP immunoprecipitation) with TEV protease, and subsequently purified via a combination of size exclusion chromatography and ion exchange.

Cell viability and AUC growth assays

96-well plates were seeded with 5000 HEK293F cells per well in 100 μL media, grown for 24 hours, then DNA damaging agent was added. For cell viability assays, cells were grown for a further 72 hours, 10 μL CellTitreBlue reagent (Pierce) was added, and viability was measured as per the manufacturer's instructions. Viability measurements were normalized to that of untreated cells, and fit to a 4-parameter log[compound] vs viability model. For AUC growth assays, cells were grown in an Incucyte Zoom live cell imager (ESSEN Biosciences) with pictures taken every 2 hours for 6 days after addition of drug. The confluence percentage for each image was calculated and summed over the course of the experiment for each well, and fit to a 4-parameter log[compound] vs viability model.

Clonogenic survival assays

Survival assays were carried out by seeding T121 transformed MEFs in 100 mm dishes in duplicate for each experimental condition. To compensate for growth defects 4,000 cells were seeded for *Tdp2*$^{+/+}$ cells and 20,000-50,000 cells for *Tdp2*$^{-/-}$. After 6 h, the indicated

concentration of etoposide was added and cells were incubated for 10–12 days. Dishes were fixed and stained for manual colony counting in Crystal Violet solution (0.5% (w/v) Crystal violet in 20% (v/v) ethanol). The surviving fraction at each dose was calculated by dividing the average number of visible colonies in treated versus untreated dishes.

Recombinant TOP2cc preparation and processing by TDP2-ZNF451

TOP2cc (TOP2α and TOP2β) was formed in a 200 μL reaction (50 mM Tris pH 8.0, 100 mM NaCl, 10 mM $MgCl_2$, 5 mM ATP, 0.5mM TCEP) with 10 μM TOP2α-FL or TOP2β (47-1212), and 10 μM DNA substrate, incubated at 37 °C for 1 hour. Reactions were terminated by adding NaCl to 400 mM and EDTA to a final concentration of 20 mM. TOP2cc was purified by sequential chromatography on a Superdex 200 16/60 column (GE) in buffer (20 mM Tris pH 7.5, 500 mM NaCl, 0.5 mM EDTA, and 0.5 mM TCEP), and fractions were run on an SDS-PAGE to identify TOP2cc containing fractions, which were pooled and concentrated. TOP2βcc was further purified on a MonoS 5/5 HR column (GE). Fractions were analyzed by SDS–PAGE, and those containing TOP2β cc largely free of TOP2β were pooled, concentrated, and used for assays.

TDP2-catalyzed hydrolysis of TOP2cc was assayed in 10 μL reactions containing 10mM HEPES pH 7.5, 125 mM NaCl, 2mM ATP, 10mM $MgCl_2$, and 0.5 mM TCEP. ZNF451 and TDP2 proteins were added as indicated. For experiments where the TOP2cc substrate was sumoylated, 1 μM recombinant SUMO2, 50 nM UBC9, and 25 nM SUMO E1 (where indicated) were included and incubated for 30 mins at 37 °C prior to addition of TDP2. After addition of TDP2, reactions were incubated at 37 °C for 60 seconds unless otherwise indicated, then run on an SDS-PAGE and scanned using a Typhoon9500 imager (GE) with a 633nm excitation and 670/30 nm band-pass filter. Gel bands were quantified using ImageJ and data were plotted and analyzed using Prism. Quantification graphs were produced with data from 3 or more replicates as indicated, and were fit to a 4-parameter log[agonist] vs response model using Graphpad Prism 7.

Limited proteolysis of TOP2cc

Proteolysis of TOP2cc was assayed in 10 μL reactions containing 10mM HEPES pH 7.5, 100 mM NaCl, 2mM ATP, 10mM $MgCl_2$, and 0.5 mM TCEP, and the indicated

concentrations of Trypsin (Sigma) at room temperature for 30 minutes. Reactions were terminated by mixing with an equal volume of SDS-PAGE loading dye, and separated on NuPAGE 4-12% Bis-Tris gel (Invitrogen), and imaged with a Typhoon (GE Healthcare) scanner using a 633nm excitation and 670/30 nm band-pass filter to detect only those TOP2β proteolysis fragments that contain the protein-DNA crosslink.

ICE assay

Confluency arrested primary MEFs were pretreated for 1.5 h with 20 μM MG132 or vehicle (DMSO) followed by 100 μM etoposide (Sigma E1383) or vehicle (DMSO) for 1 hour. They were either immediately lysed in 1% (w/v) sarkosyl (Sigma L7414) or washed twice with PBS and incubated at 37 °C in fresh media for the indicated times before lysis. Lysates were processed according to the in vivo complex of enzyme (ICE) assay(*41*). Briefly, sheared samples were centrifuged with a CsCl (Applichem-Panreac, A1098) gradient at 57,000 r.p.m. for 20 h at 25 °C using 3.3 ml 13 x 33 mm polyallomer Optiseal tubes (Beckman Coulter) in a TLN100 rotor (Beckman Coulter).

For slot blotting, ICE samples containing 5 μg of DNA were transferred onto Odyssey Nitrocellulose Membranes (LI-COR Biosciences) using a Bio-Dot SF Microfiltration Apparatus (Biorad). For western blot of ICE, samples were resuspended in 12,000 units of Micrococcal Nuclease (MNase, NEB M0247S), 1 x MNase buffer (NEB, B0247S) and 100 μg mL^{-1} BSA (NEB, B9000S), then incubated at 37 °C for 6 h. Samples were run in 10% SDS-PAGE and transferred to Immobilon-FL Transfer Membranes (Millipore). Membranes were blocked for 1 h in Odyssey Blocking Buffer (LI-COR Biosciences), then incubated for 2 h with primary antibodies in the same buffer with additional 0.1% (v/v) TWEEN 20, washed 3x with TBS-0.1%-TWEEN20, incubated with secondary antibodies for 1 h, and finally washed again. Once the membranes were dry, slots were analyzed and quantified in Odyssey CLx using ImageStudio Odyssey CLx Software.

ICE-His Pull-down

2.5 x 10^6 HEK293T cells were grown in 100 mm dishes for 24h and transiently transfected with PRSV-*Ubc9* alone (Control) or together with PRSV-HIS-*SUMO2*

plasmids using Gene Juice (Novagen) according to manufacturer's protocol. 48h later, cells were treated for 1 h with 200 μM etoposide (Sigma E1383). TOP2-DNA crosslinks were isolated as described above and resuspended in bi-distilled H_2O. 1 mg DNA was degraded with 25U benzonase (Sigma) for 30 mins on ice. Samples were then resuspended in IP buffer (20 mM Tris HCl pH 8, 150 mM NaCl, 8 M Urea, 200 μg ml^{-1} BSA). 2 % input was saved for subsequent analysis. Remaining supernatants were incubated with HIS-Select HF Nickel Affinity Gel (Sigma) for 2 h at RT, beads were washed four times with wash buffer (20mM Tris HCl pH8, 150mM NaCl, 8M Urea, 5mM Imidazole) and finally His-tagged SUMO was eluted using 500mM Imidazole (Sigma). The eluted samples were analyzed by slot blotting as described above.

In vitro TDP2 activity on ICE-isolated TOP2-DNA crosslinks

TOP2-DNA crosslinks were isolated as described above and resuspended in bi-distilled H_2O. samples containing 20 μg of DNA were incubated in reaction buffer (50 mM Tris-HCl pH 7.5, 50 mM KCl, 1 mM DTT, 100 μg ml^{-1} BSA, 1mM $MgCl_2$) with the given concentration of TDP2 in a total volume of 300 μl for 1h at 37°C. Reactions were stopped by the addition of 600 μl STOP buffer (50mM EDTA, 1% (w/v) Sarcosyl) and placed on ice. Samples were centrifuged a second time according to the ICE assay, and 5 μg of DNA was used in a slot blot. Assays of 5′–Tyrosyl DNA phosphodiesterase activity on synthetic 5′–phosphotyrosine DNA oligonucleotides were performed as previously described (*10*).

In vitro pull down assays

To 500 μL reactions in binding buffer (10 mM HEPES pH 7.5, 50 mM NaCl, 0.5 mM TCEP, 2.5% (v/v) glycerol, 0.1 mg mL^{-1} BSA, and 0.1% (v/v) TWEEN 20) containing 1 μM of indicated Maltose Binding Protein (MBP) fusion or MBP protein were added 1 μM recombinant bait protein or 1 μL of TOP2α protein (Topogen), followed by 20 μL of amylose resin (NEB). Reactions were mixed on a nutator at 4° C for 2 hours, transferred to a micro-spin column (Bio-Rad), centrifuged at 1000 g for 1 minute, washed with 100 μL wash buffer, eluted with 100 mM maltose, and run on a Nupage 4-12% Bis-Tris SDS-PAGE, followed by immunoblotting with probes for the bait protein.

SUMOylation assays

In vitro SUMOylation assay reactions (10μL) in buffer (10mM HEPES pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$, 2 mM ATP, and 0.5 mM TCEP) contained 75 nM TOP2α or TOP2β, 1 μM SUMO2, 100 nM UBC9, and 25 nM SUMO E1. TOP2cc SUMOylation assays contained the same buffer with 2 nM TOP2cc and 75 nM TOP2, 50 nM UBC9, and 25 nM SUMO E1 (SAE2/UBA2, Boston Biochem). Reactions were incubated at 37 °C for 30 minutes, then run on a Nupage 4-12% Bis-Tris SDS-PAGE, scanned on a Typhoon imager for the Cy5 label where indicated, then transferred to PVDF (Millipore), and probed with the indicated antibodies. SUMOylation of TOP2 in HEK293F cells was examined in cell lines expressing His6-tagged SUMO2. 2 x 10^6 cells growing in 6-well plates were treated with the indicated concentration of drugs or an equal volume of DMSO for 20 minutes. Media was removed and cells were lysed in 1mL denaturing buffer (20mM Tris pH 8, 8M urea, 0.4% (w/v) SDS, 5 mM Imidazole, and 1 mM TCEP) and bound to 20 μL Ni-NTA resin on a nutator for 16 hours at room temperature. Resin was washed in spin columns four times in the same lysis buffer, followed by elution in 50 μL lysis buffer supplemented with 250 mM Imidazole. Eluates were run on an SDS-PAGE, transferred to PVDF, and probed with the indicated antibodies. Band intensities were measured using ImageJ and data were plotted and analyzed using Prism.

TDP2-SUMO2-DNA crystallization, data collection, and refinement

To prepare samples for crystallization, mouse TDP2 (residues 118-370) and SUMO2 (residues 10-93) were mixed in equimolar concentrations with a 1.2-fold molar excess of oligonucleotide (sequence: [5′-phosphate]CCGAATTCG), at a final protein concentration of 8 mg ml^{-1} in crystallization buffer (10mM HEPES pH 7.5, 200mM NaCl, 4mM $MgCl_2$, and 1 mM TCEP). SUMO2-TDP2–DNA crystals were grown with a precipitant containing 100 mM Citrate-Phosphate buffer pH 4.2, 200mM NaCl, and 12% (w/v) PEG-3350, then cryoprotected in liquid nitrogen in precipitant solution supplemented with PEG-3350 to 25% (w/v) and 12% (v/v) ethylene glycol. Crystals of SUMO2-TDP2 were grown with a precipitant containing 100 mM MES pH 6.0, 18% (w/v) PEG-8000, and

200 mM calcium acetate, then cryoprotected in the precipitant solution supplemented with PEG-8000 to 25% (w/v) and 12% (v/v) glycerol.

X-ray data (Table S4) were collected at 105 K on the SERCAT beamline 22-ID of the Advanced Photon Source at a wavelength of 1.000 Å. X-ray diffraction data was processed and scaled using the HKL2000 suite (*42*). A CC* >0.5 was used to determine the resolution cutoff for data used for scaling and refinement. The mTDP2^{cat}-SUMO2-DNA crystals (5TVP) diffracted anisotropically, and the use of a CC* cutoff included data that would otherwise be discarded using a traditional R_{sym}–based cutoff criteria, resulting in better electron density maps and refined model.

The structures were solved by molecular replacement using the program PHASER(*43*) with PDB entry 4GZ1 chain A (*7*) and entry 1WM2 chain A(*44*) for search models. Initial solutions were improved by iterative rounds of manual fitting and model building in COOT(*45*) with refinement using the program PHENIX(*46*). The mTDP2^{cat}-SUMO2-DNA crystals (5TVP) contained 8 molecules of TDP2, 8 DNA molecules, and 1 molecule of SUMO2. Overall, the mTDP2^{cat}-DNA interface is similar to that of the search model (4GZ1), with the exception that Mg^{2+} is absent from the active site. SUMO2 was assigned the chain identifier "Q", and interacts with Tdp2 chain B via a split-SIM interface. mTdp2^{cat}-SUMO2 crystals (5TVQ) contain 1 TDP2 and 1 SUMO2 molecule, with the TDP2 active site containing a single Ca^{2+} ion derived from Calcium in the precipitant solution. Final refined models display excellent Ramachandran statistics: 5TVP (97% favored, 3% allowed, 0% outliers) and 5TVQ (95% favored, 5% allowed, and 0% outliers).

Small Angle X-ray Scattering

For SAXS studies, mouse TDP2 (residues 118-370) or equimolar mixtures of mouse TDP2 and SUMO2 (1-93) were purified on a S200 HR 10/30 column (GE) in SAXS buffer (20 mM HEPES pH 7.5, 200 mM NaCl, 1mM TCEP, 2mM $MgCl_2$, and 2% (v/v) glycerol), and concentrated in Amicon 3K 0.5mL centrifugal concentrators (Fisher). SAXS data were collected at the ALS beamline 12.3.1 LBNL Berkeley, California (*47*). X-ray wavelength λ 1.03 Å and the sample-to-detector distances were set to 1.5 m resulting in scattering vectors, q, ranging from 0.01 $Å^{-1}$ to 0.33 $Å^{-1}$. The scattering vector

is defined as $q = 4\pi\sin\theta/\lambda$, where 2θ is the scattering angle. All experiments were performed at 20 °C (*48*). SAXS data collection was performed at 4, 2, and 1 mg mL^{-1} protein concentrations with 0.5, 1, 2, and 4 s exposures. Data from the 2 mg mL^{-1} samples 1 s exposures were used for analysis in the program SCATTER (http://bl1231.als.lbl.gov) and compared to the predicted scattering from the crystal structures using the FOXS server (*49, 50*).

Determination of TDP2-SUMO2 k_D by NMR

Mouse TDP2 (amino acids 118-362) and SUMO2 (amino acids 10-93) were buffer exchanged into PBS + 0.1mM TCEP on a Superdex200 10/30 HR column (GE), concentrated with Amicon 10K centrifugal filters (Millipore), and PBS in D_2O was added for a final concentration of 10% Deuterium, and 1 μM DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid) was used as a reference. Protein concentration was determined using a Bradford assay calibrated with lysozyme and BSA standards. SUMO2 protein concentration was held constant at 23 μM and TDP2 protein was titrated from 0 to 40 μM. The assignments of SUMO2 were made by comparison with entry 11267 from the Biological Magnetic Resonance Bank (*51*) (www.bmrb.wisc.edu) and confirmed by analysis of the 3D TOCSY experiments 3D-C(CCO)NH and 3D-H(CCO)NH (*52*). Resonance assignments were in good agreement with the SUMO2:TDP2 interface determined crystallographically. Using the gChmqc NMR experiment on a Varian 800 MHz INOVA spectrometer with a cryogenic probe, the intensity of the Leu43 methyl of SUMO2 in the bound and free states was measured with NMRPIPE(*53*). The k_D was fit to the following equation (*54*):

$$p_B = \frac{k_D + [S_T] + [T_T] - \sqrt{(k_D + [S_T] + [T_T])^2 - 4[S_T][T_T]}}{2[S_T]}$$

where S_T is the total concentration of SUMO2 and T_T is the total concentration of TDP2. Comparison of the uncomplexed and TDP2-complexed SUMO2 resonances shown in the panel utilized a scaling factor of 9 to adjust for relaxation differences between the two species, and the sum of the adjusted free and bound intensities, $p_F + 9p_B$, is also shown.

Microscopy

For localization experiments, HEK293T cells expressing the indicated fluorescent protein-fusions were grown in 35cm glass-bottom petri dishes (MatTek) and imaged using a 40x C-Apochromat (numerical aperture 1.2) water immersion objective in a Zeiss LSM780 confocal microscope (Carl Zeiss MicroImaging). For experiments using UV microirradiation, cells were plated in 35cm glass-bottom petri dishes and grown for 2 days in media with 10 μM bromodeoxyuridine (BrdU; Sigma). DNA damage was induced with a 355 nm UV laser with dose of 0.3 μJ. Under these conditions, recruitment of TOP2(α/β), TDP2, ZNF451, and SUMO2 is dependent on UV sensitivity conferred by BrdU, and is blocked by ICRF193, showing recruitment requires DNA damage and functional TOP2.

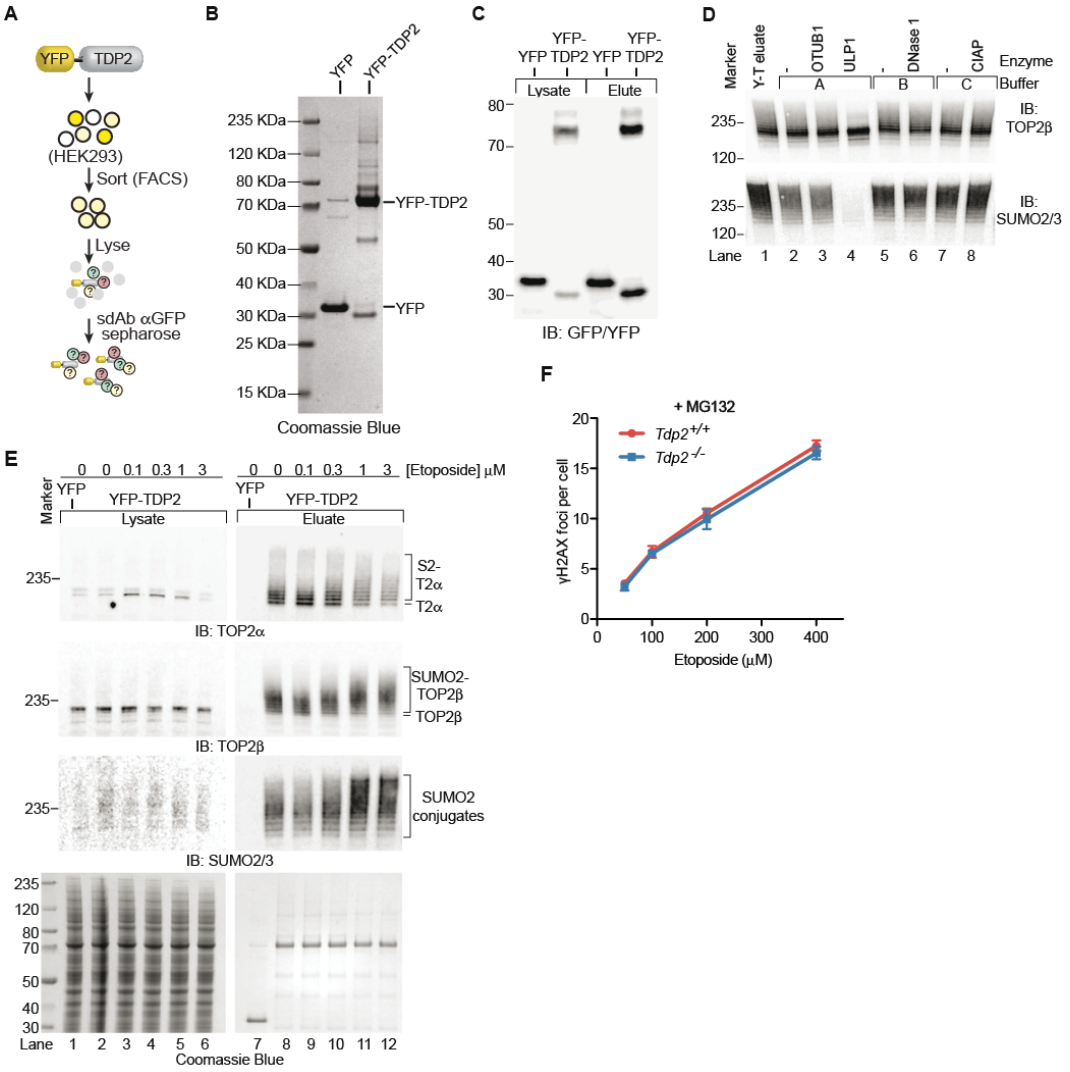

Fig. S1.

Immunoprecipitation of YFP-TDP2 and effect of TDP2 knockout and proteasome inhibition on induction of TOP2-DSBs.

(A) Anti-GFP affinity isolation procedure used for identification of TDP2-associated proteins. HEK293F cells that stably express YFP–TDP2 were generated and sorted by FACS to select consistent YFP expression levels. Camelid sdAb αGFP/YFP resin was used to isolate YFP proteins from soluble cell lysates. (**B)** SDS-PAGE of YFP and YFP-TDP2 IP samples isolated from HEK293F cell lysates stained with coomassie blue. **(C)** Anti-GFP/YFP western blot of YFP or YFP-TDP2 expressing HEK293F cell lysates and immunoprecipitates. **(D)** Immunoblotting of YFP-TDP2 IPs after treatment with the indicated enzymes. Treatment with the SUMO protease ULP1 collapsed the TOP2β ladder down to a single band, showing that TDP2-associated TOP2 is covalently modified with SUMO. **(E)** Immunoblotting of IPs from cells expressing YFP or YFP-TDP2 pretreated with etoposide shows TOP2 poisons modulate the interaction of TDP2 with TOP2-SUMO2 conjugates. **(F)** In controls, γH2AX foci induction was monitored after 30 min treatment with the indicated concentrations of etoposide in the presence of 20 μM proteasome inhibitor MG132 in confluency-arrested primary MEFs.

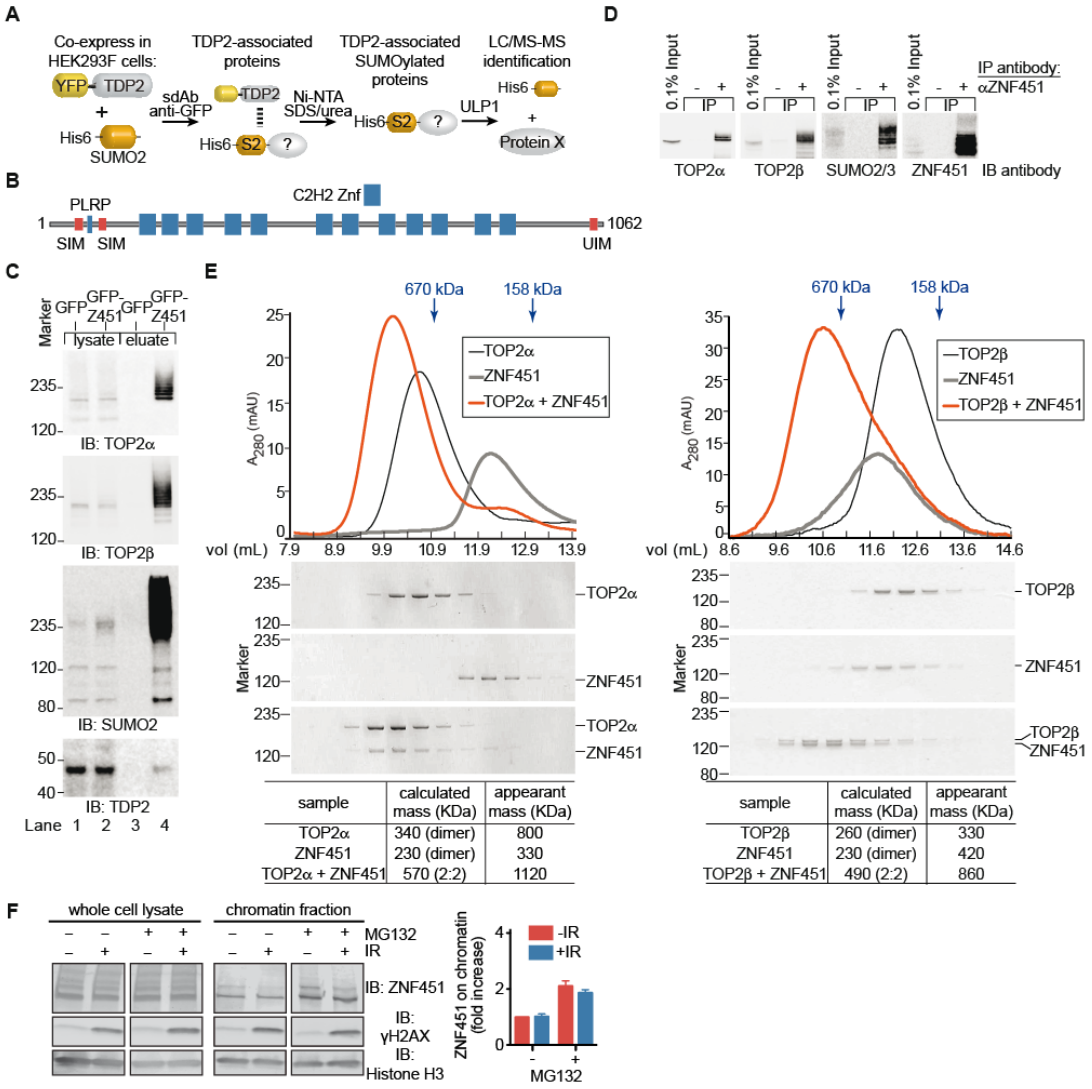

Fig. S2.

ZNF451 Binds TOP2α/β and is recruited to chromatin by poisoned TOP2cc.

(A) Tandem affinity purification procedure used to identify SUMO2–modified proteins that bind to TDP2. **(B)** Domain structure of ZNF451. ZNF451 bears a "SIM-PLRP-SIM" motif and multiple C2H2-type zinc finger motifs **(C)** Immunoblotting of soluble cell lysates and IPs from cells expressing GFP or GFP-ZNF451. Western blotting of GFP-ZNF451 IP shows that ZNF451 is bound to SUMOylated TOP2α and TOP2β, **(D)** HEK293F cell lysate was immunopreciptated with Protein A/G sepharose (-) or protein A/G sepharose pre-bound to anti-ZNF451 antibody (+), and immunoprecipitates were analyzed by western blot with the indicated antibodies. Endogenous ZNF451 binds to sumoylated TOP2α/β. **(E)** Recombinant TOP2α (wt, left) or TOP2β (aa 47-1212, right), ZNF451, or an equimolar mixture of the two proteins were fractionated on a size-exclusion column. Blue arrows denote elution volumes of molecular weight standards. TOP2α migrates anomalously large due to the presence of an extended, disordered C-terminal domain. **A** ZNF451-TOP2 mixture elutes earlier than the individual proteins, indicating they form a stable complex, and ZNF451 binds directly to both TOP2α and TOP2β. **(F)** Chromatin fractionation of RPE-1 cells with or without 10 Gy ionizing

irradiation (IR) in the presence or absence of 20 μM MG132. Representative image (left) of immunoblots against ZNF451, γH2AX and histone H3, and quantification (right) of ZNF451 levels normalized to the corresponding H3 signal and expressed as fold increase compared to the untreated sample. Average ± s.e.m.; N=3.

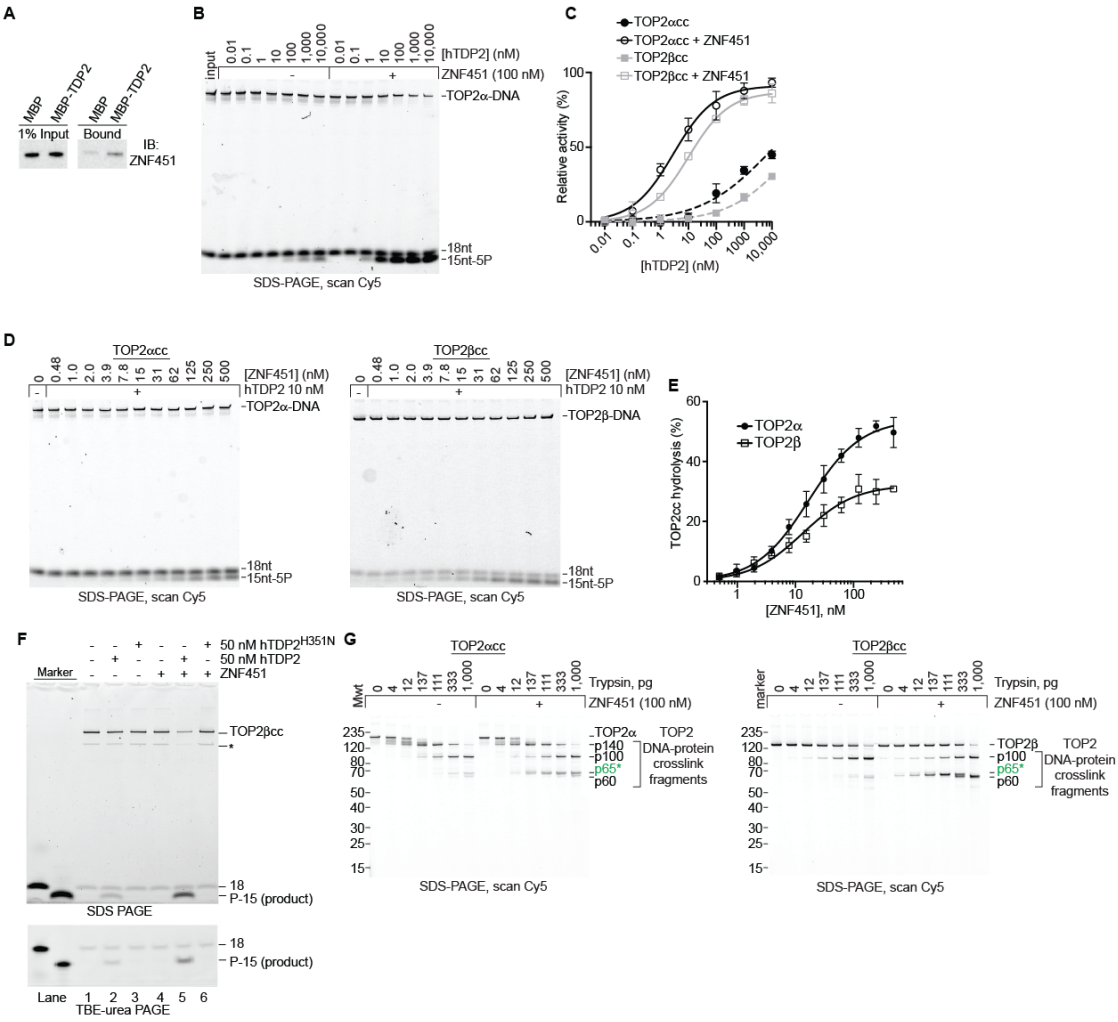

Fig. S3.

ZNF451 stimulates TOP2cc processing by TDP2

(A) MBP pulldown of recombinant ZNF451 by MBP-TDP2 **(B)** Purified TOP2αcc (0.4 nM) was incubated with the indicated proteins for 60 seconds, run on an SDS-PAGE, then scanned with detection of the Cy5 label. Representative gel from 3 replicates is shown. **(C)** Quantification of 15nt 5′-P reaction product release from TOP2αcc and TOP2βcc by TDP2 or TDP2/ZATT from 3 replicates of experiments in panels B and Fig. 2B. Average ± s.d., N=3. **(D)** Purified TOP2αcc (0.4 nM, Left) or TOP2βcc (0.2 nM, Right) were incubated with indicated concentration of ZNF451 and 10 nM hTdp2^{cat} for 60 seconds, run on an SDS-PAGE, then scanned with detection of the Cy5 label. Representative gels from 3 experiments is shown. **(E)** Quantification of 15nt DNA product band from panel D. Data were fit to a 4–parameter agonist vs response model. Average ± s.d., N=3. **(F)** Purified TOP2βcc was incubated with the indicated proteins for 60 minutes, run on an SDS-PAGE and 15% TBE-urea PAGE, then scanned with detection of the fluorescein DNA label. The TDP2 H351N mutation, or ZNF451 alone fail to process TOP2βcc. **(G)** We used limited trypsinolysis to probe the conformation of

TOP2cc, and found that the addition of ZNF451 enhanced protease cleavage at a specific site (p65). This data is consistent with ZNF451 stabilizing or inducing an altered conformation of TOP2αcc. (Left) or TOP2βcc (Right) were incubated +/- ZNF451 and the indicated concentration of trypsin then separated by SDS-PAGE. TOP2cc conformation was assessed by following the covalent DNA-linked fragment of TOP2cc containing the Cy5 label.

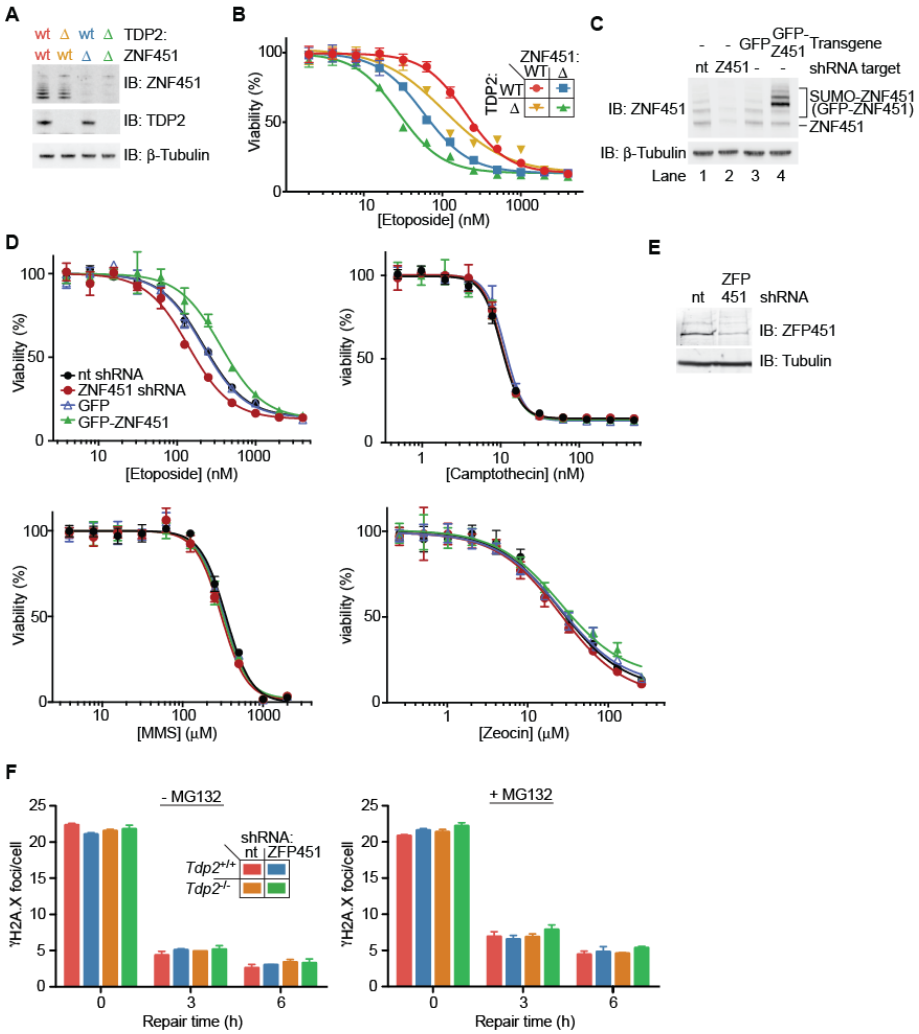

Fig. S4.

ZNF451 is a DNA repair factor for poisoned TOP2.

(A) Western blots of HEK293F ΔTDP2, ΔZNF451, and ΔTDP2ΔZNF451 CRISPR knockouts cell lines used in panel S4B and Fig. 2C. **(B)** Cell viability of indicated HEK293F WT or CRISPR knockout cells was measured 72 hours after treatment with the indicated concentration of etoposide. Average ± s.d., N=4. **(C)** ZNF451 expression levels measured by immunoblotting with anti-ZNF451 (nt, non-targeting shRNA) for ZNF451 knockdown and overexpression cells used in S4D. **(D)** Cell viability of HEK293F cells with ZNF451 knockdown or overexpression after 72 hours treatment with etoposide (TOP2 poison), camptothecin (TOP1 poison), methyl methanesulfonate (DNA alkylation), or Zeocin (radiomimetic DSBs) shows ZNF451 expression levels correlate to resistance to TOP2 poisons, but not any of the other DNA damaging agents. Average ± s.d., N=4. **(E)** ZFP451 levels in wildtype and ZFP451 shRNA knockdown MEF cells used in Fig. 2D. **(F)** Resolution of DSBs marked by γH2AX foci in MEF cells post-exposure to 2 Gy ionizing radiation at the indicated repair time in the presence or absence of MG132. Average ± s.e.m.; N=3. MG132 does not impair the repair of IR induced

DSBs, showing that repair defects observed for etoposide treatment in Fig. 2E are not due to a general role of the proteasome in the DSB repair process.

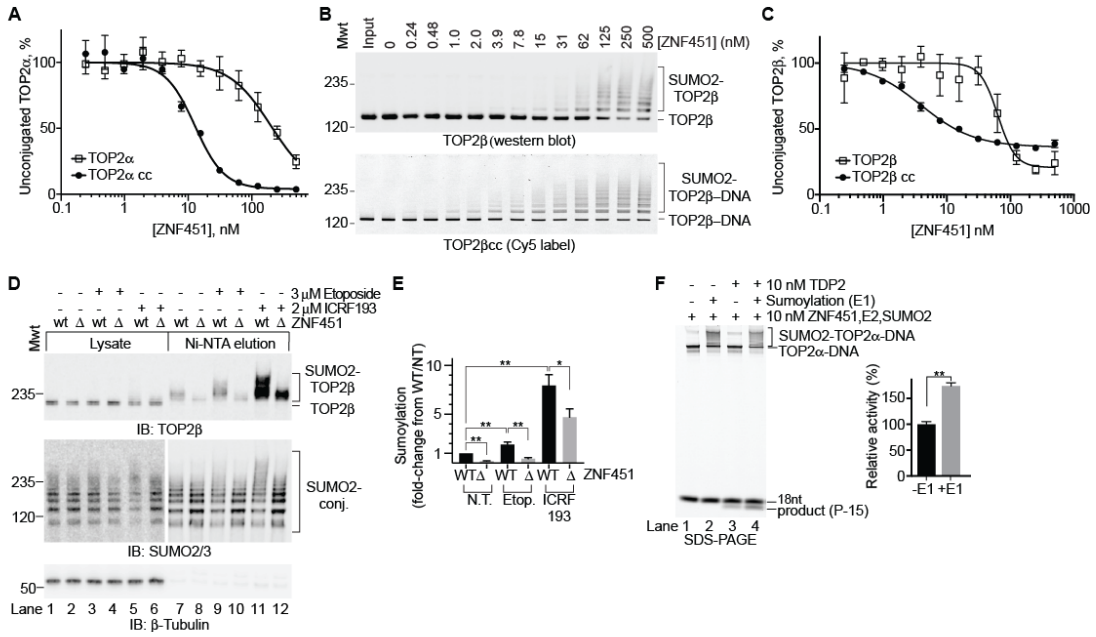

Fig. S5.

ZNF451 Topoisomerase 2 SUMO E3 ligase.

(A) Quantification of un-SUMOylated TOP2α from Fig. 3B. Average ± s.d., N=3. **(B)** ZNF451 catalyzes preferential transfer of SUMO2 to TOP2βcc. Reactions with 2 nM TOP2βcc containing a Cy5 label and 75 nM free TOP2β were incubated with E1, E2, SUMO2, and the indicated concentration of ZNF451, then separated by SDS-PAGE. TOP2cc SUMOylation is monitored by scanning for the Cy5 label, while free TOP2β SUMOylation is monitored by western blotting. **(C)** Quantification of un-SUMOylated TOP2β from panel B. Average ± s.d., N=3. **(D)** Immunoblot of lysates and Ni-NTA purified samples from wildtype and ΔZNF451 HEK293F cells expressing His6-SUMO2 pretreated with the indicated drugs for 20 mins. **(E)** Quantification of SUMO2-TOP2β signal from lanes 7-12 in panel C. Average ± s.d., N=3.; *=p<0.05, **=p<0.001, 2-tailed t-test. **(F)** (Left) Recombinant TOP2αcc was incubated with ZNF451, SUMO E2 (UBC9), and SUMO2. All reactions contain the same concentration of SUMO2, and TOP2cc Sumoylation was triggered by addition of E1 enzyme where indicated prior to addition of TDP2, then reactions were run on SDS-PAGE or 15% TBE-urea PAGE and scanned to detect Cy5-labelled DNA. (Right) Quantification of 15nt 5′-P DNA product from panel F, lanes 3&4. Average ± s.d., N=4.

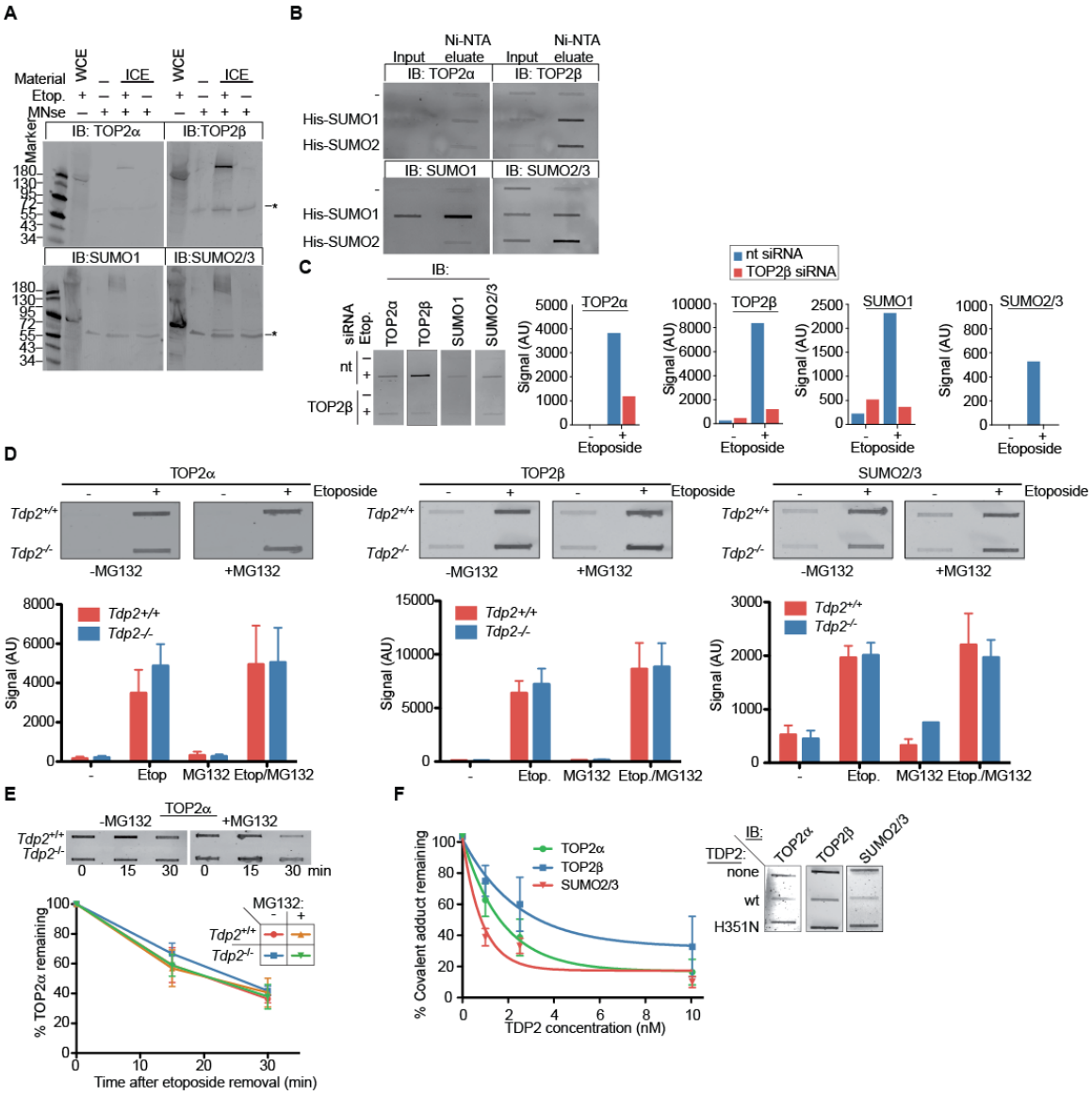

Fig. S6.

ICE samples from etoposide treated cells contain SUMO-modified TOP2.

(A) Western blot of TOP2α, TOP2β, SUMO1 and SUMO2/3 covalently bound to genomic DNA in confluency-arrested primary MEFs following 1 h 100 μM etoposide treatment. Samples were treated by micrococcal nuclease prior to SDS-PAGE to remove covalently linked DNA. * denotes unspecific band derived from BSA in buffer. **(B)** Slot-blot quantification of proteins covalently crosslinked to DNA in material isolated with ICE (input) or after Ni-NTA purification and elution (eluate) from cells expressing His-SUMO1 or His-SUMO2. **(C)** Representative slot-blot image (left) and quantification (right) of proteins covalently crosslinked to DNA in cells pretreated with a non-targeting shRNA (nt) or one targeting TOP2β, with or without etoposide treatment. Average; N=2. **(D)** Representative image (top) and quantification (bottom) of TOP2α, TOP2β, or SUMO2/3 covalently bound to genomic DNA isolated by ICE in $Tdp2^{+/+}$ and $Tdp2^{-/-}$ confluency-arrested primary MEFs with or without prior treatment with 100 μM etoposide for 1 hour in the presence or absence of 20 μM MG132. Average ± s.e.m.;

N≥7. **(E)** Representative image (top) and quantification (bottom) of TOP2α or SUMO2/3 covalently bound to genomic DNA in MEFs following 1 h etoposide treatment and recovery for the indicated time in the presence or absence of 20 μM MG132. Average ± s.e.m.; n≥7; *p<0.05 (two-way ANOVA with Bonferroni post-test). **(F)** Representative image (right) and quantification (left) of covalently–linked TOP2α TOP2β and SUMO2/3 removal from DNA following treatment with the indicated concentrations of recombinant TDP2. Average ± s.e.m.; N≥3; TOP2β *vs.* SUMO2/3 p=0.0027 (F-test, non-linear regression according to an exponential removal model). Right: Catalytic mutant H351N does not remove TOP2cc.

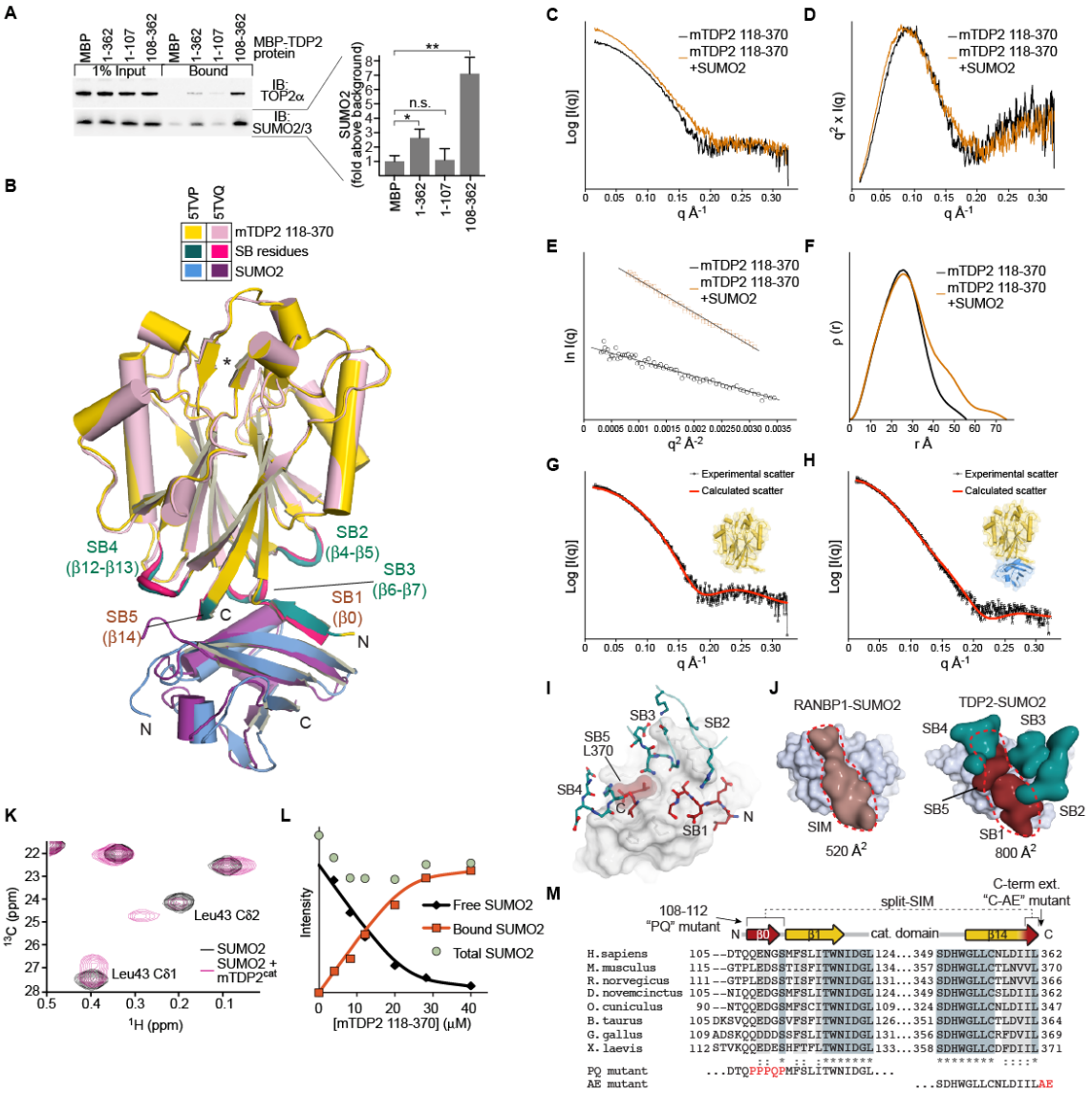

Fig. S7.

Structural characterization of the TDP2-SUMO2 complex

(A) (left) MBP pulldowns mapping SUMO2 and TOP2α binding regions of TDP2 to the catalytic domain. (right) quantification of SUMO2 western blot signal; N=3, Average ± s.d., *=p<0.05, **=p<0.001, 2-tailed t-test. **(B)** Superposition of the mTDP2^{cat}–SUMO2 complexes from crystal form 1 (PDB entry 5TVP; DNA not shown) and crystal form 2 (PDB entry 5TVQ), "*" indicates active-site. **(C)** SAXS curves for the mTDP2^{cat} and mTDP2^{cat}–SUMO2 complexes shows scattering for mTDP2^{cat}-SUMO2 complex that is distinct from that of mTDP2^{cat} alone. **(D)** Kratky plot of scattering data shows a bell-shaped peak, indicating the samples contain globular protein structures. **(E)** Guinier region of scattering curves used to measure Rg values is linear, indicating the sample is monodisperse and not aggregated. **(F)** ρ(r) plot calculated from the SAXS data using the program SCATTER shows the mTDP2^{cat}-SUMO2 is larger than mTDP2^{cat}. **(G)** Comparison of experimentally measured scattering of mTDP2^{cat} and the theoretical scattering calculated from the crystal structure (inset; PDB entry 4GZ1) using the

program FOXS shows agreement between the model and solution scattering data. **(H)** Comparison of experimentally measured scattering of mTDP2^{cat}-SUMO2 complex and the theoretical scattering calculated from the crystal structure (inset; PDB entry 5TVP) using the program FOXS shows agreement between the model and solution scattering data. **(I)** Five SUMO binding elements of TDP2 (SB1-SB5) comprise a SUMO2 (gray) binding interface. **(J)** A typical SIM (RANBP1, PDB entry 3UIN, pink) compared to the mTDP2cat–SUMO2 interface, which is more extensive than a typical SIM (red dashed line). **(K)** Comparison of the methyl region of a gChmqc $^{13}C/^{1}H$ spectra of SUMO2 (black) and SUMO2–mTDP2^{cat} (magenta) shows the Leu43 Cδ2 methyl peak shifts upon binding mTDP2^{cat}. **(L)** The intensities of free and mTDP2^{cat}–bound SUMO2 Leu43 Cδ2 peak were measured, plotted as a function of [mTDP2^{cat}], and fit to a two-state model (solid lines) to calculate a Kd of 0.88 μM. **(M)** Sequence alignment of the split-SIM region of TDP2 homologs with positions of mutations (red) that impair TDP2 interactions with SUMO2.

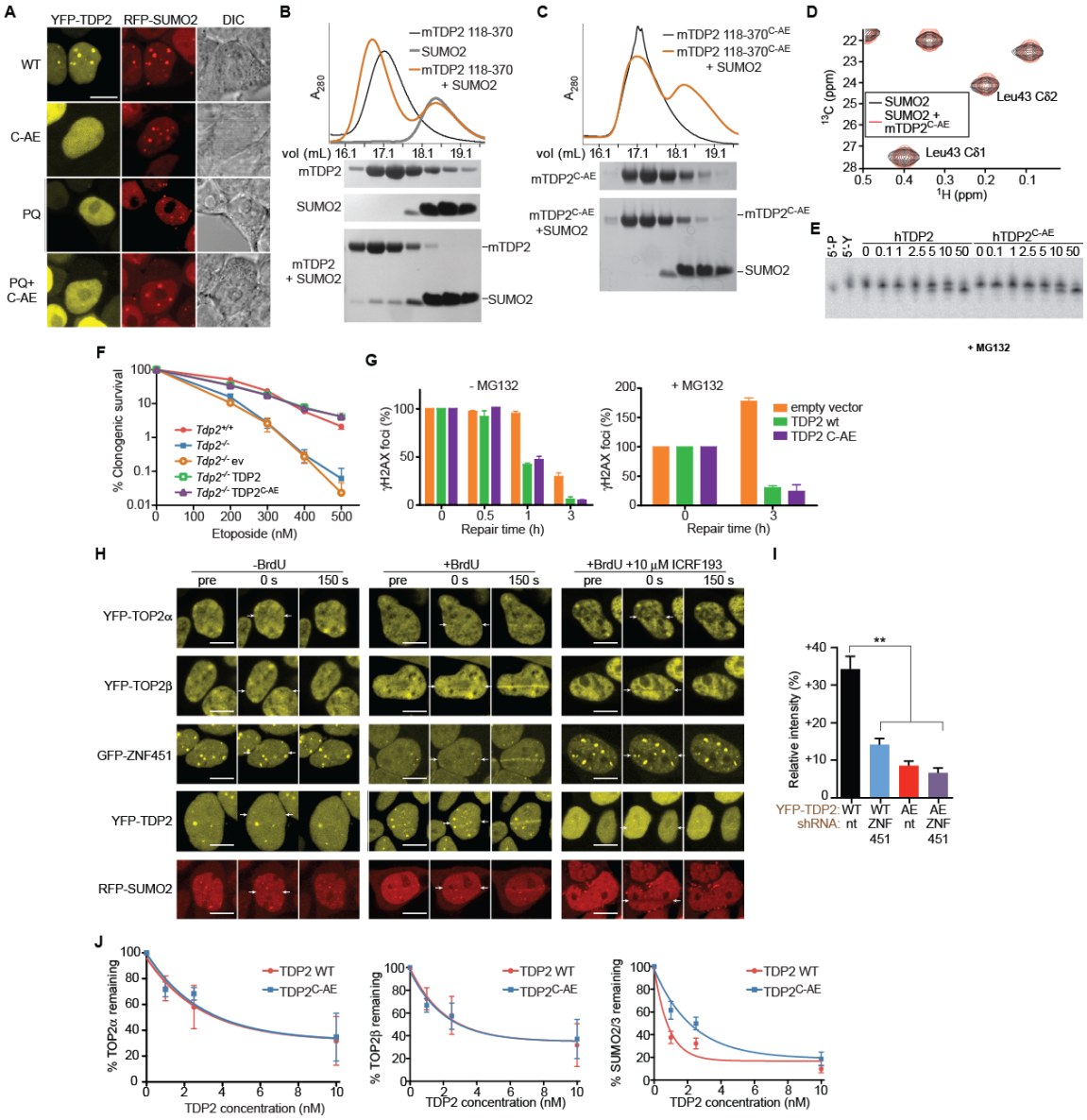

Fig. S8.

Functional Analysis of TDP2$^{C\text{-}AE}$.

(A) Cellular localization of YFP-TDP2 and YFP-TDP2 mutants. Scale bar is 10 μm. **(B)** Gel filtration analysis of TDP2 SUMO2 complexes. 200 μL samples containing 100 μM mTDP2^{cat}, 1 mM SUMO2, or a mixture of the two proteins was fractionated on a size-exclusion column, and A_{280nm} of the column elution was monitored. SDS–PAGE of the fractions is shown in the corresponding lane below. SUMO2 co-elutes with TDP2 **(C)** 200 μL samples containing 100 μM mTDP2^{cat} C–AE or a mixture of 100 μM TDP2$^{cat\ C\text{-}AE}$ and 1 mM SUMO2 was fractionated on a size-exclusion column and analyzed as in panel S8A. The C-AE mutation disrupts TDP2-SUMO2 complex formation. **(D)** Comparison of the methyl region of a gChmqc $^{13}C/^{1}H$ spectra of SUMO2 (black) and SUMO2–mTDP2$^{cat\ C\text{-}AE}$ (red) shows the Leu43 Cδ2 methyl is not shifted in the presence of mTDP2cat C–AE. **(E)** 5′–TDP activity following 1 h incubation of duplex oligonucleotide harboring a terminal 5′–phosphotyrosine moiety with the indicated

TDP2. Reactions were run on a 20% TBE-urea gel. **(F)** Clonogenic survival of transformed *Tdp2*$^{+/+}$ and *Tdp2*$^{-/-}$ MEFs complemented, where indicated, with lentiviral vectors overexpressing wild-type or mutant TDP2 or an empty vector control (ev) following continuous treatment with the indicated concentrations of etoposide. Average ± s.e.m.; N≥4 **(G)** Resolution of γH2AX foci in confluency-arrested *Tdp2*$^{-/-}$ primary MEFs infected with lentiviral vectors overexpressing wild-type or mutant TDP2 or an empty vector control (ev), following 30 min exposure to 30 μM etoposide (-MG132) or 100 μM etoposide (+20 μM MG132) and the indicated repair time. Average ± s.e.m.; N=3. **(H)** HEK293F cells expressing the indicated fluorescently tagged proteins were grown in the absence or presence of 10 μM BrdU, for 2 days, with addition of 10 μM ICRF-193 10 minutes prior to imaging as indicated. Images were taken prior to, immediately after, and 150 s after UV irradiation with a 355nm UV laser between the white arrows. The increased brightness of YFP-TOP2 between the arrows at 150 s in cells grown in BrdU without ICRF-193 treatment indicates TOP2 is recruited and trapped on UV–damaged DNA. In these conditions, TDP2, ZNF451, and SUMO2 are also recruited via a TOP2-dependent mechanism. Scale bar is 10 μm. **(I)** Quantification of the increase in fluorescence at 150s in the UV irradiated region from Fig. 4D. *** $p<0.001$ (2-tailed t-test), Average ± s.e.m. N=4. **(J)** TOP2α, TOP2β, or SUMO2/3 covalently bound to genomic DNA following *in vitro* treatment with the indicated concentrations of recombinant wildtype or mutant TDP2. Average ± s.e.m.; N≥3.

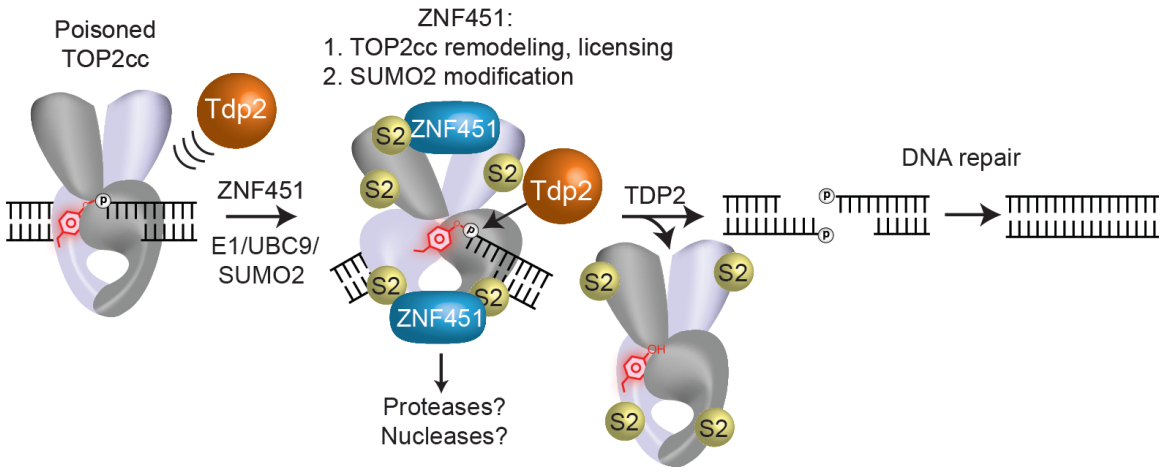

Fig. S9. ZNF451-TDP2 mediates direct resolution of poisoned TOP2cc. Poisoned TOP2cc is refractory to direct resolution by TDP2. ZNF451 participates in the metabolism of TOP2cc in at least two steps. First, ZNF451 binding to TOP2cc and licenses a TDP2 hydrolase activity on TOP2 cleavage complexes, providing a mechanistic basis for the role of the ZNF451-TDP2 axis in dictating TOP2cc metabolism. Second, ZNF451 preferentially SUMOylates TOP2cc and dictates the recruitment kinetics of TDP2 to TOP2 damage via an unconventional TDP2 SUMO-engagement platform (split-SIM).

Table S1.

Proteins identified by LC-MS/MS analysis of YFP-TDP2 and GFP-ZNF451 IP samples

YFP-TDP2 associated proteins				
Uniprot	Protein	Spectral counts (YFP-TDP2)	Unique peptides	Spectral counts (YFP)
P11388	DNA topoisomerase 2-alpha	37	28	0
Q02880	DNA topoisomerase 2-beta	21	16	0
P61956	Small ubiquitin-related modifier 2/3	13	2	0
Q9Y4E5	Zinc finger protein 451	12	6	0

GFP-ZNF451 associated proteins				
Uniprot	Protein	Spectral counts (GFP-ZNF451)	Unique peptides	Spectral counts (GFP)
P11388	DNA topoisomerase 2-alpha	86	28	0
Q02880	DNA topoisomerase 2-beta	58	24	0
Q8NDZ2	SUMO-interacting motif-containing protein 1	17	10	0
P61956	Small ubiquitin-related modifier 2/3	11	2	0

Cutoff criteria: more than 10 spectral counts (Mascot), and absent (zero counts) in YFP/GFP negative control (excludes bait protein).

Table S2.
Peptides identified by LC-MS/MS analysis of YFP-Tdp2 IP samples

Protein	Accession	start amino	Peptide Sequence *	Peptide
DNA Topoisomerase 2 α	P11388	805	YIFTmLSSLAR	22.57
		467	TLAVSGLGVVGR	22.4
		1205	TQmAEVLPSPR	20.81
		1443	RPDALNSGVSQKPDPAK	19.65
		358	NHmWIFVNALIENPTFDSQTK	19.17
		325	HVDYVADQIVTK	19.04
		569	FLEEFITPIVK	18.95
		1444	DPALNSGVSQKPDPAK	18.58
		107	VTIDPENNLISIWNNGK	17.31
		930	TWTQTYKEQVLEPmLNGTEK	16.7
		805	YIFTMLSSLAR	15.87
		307	GFQQISFVNSIATSK	15.23
		1205	TQMAEVLPSPR	13.8
		937	EQVLEPmLNGTEK	13.76
		640	YSGPEDDAAISLAFSK	13.75
		551	GLLINFIHHNWPSLLR	13.62
		937	EQVLEPMLNGTEK	11.01
		243	AYDIAGSTKDVK	9.76
		255	VFLNGNKLPVK	9.71
		135	mYVPALIFGQLLTSSNYDDDEK	8.49
		521	NYEDEDSLKTLR	6.97
		1081	GYDSDPVKAWK	6.63
DNA Topoisomerase 2 β	Q02880	488	SLAVSGLGVIGR	25.3
		328	GFQQISFVNSIATTK	19
		826	YIFTmLSTLAR	18.11
		837	LLFPAVDDNLLK	17.83
		900	mLDGLDPHPmLPNYK	16.17
		1227	KLQLEETmPSPYGR	15.4
		588	HGFLEEFITPIVK	14.33
		940	NTVEITELPVR	13.17
		1093	DLIQmLVQR	12.83
		502	YGVFPLR	7.73
		542	SYDDAESLKTLR	6.85
DNA Topoisomerase 2α/β (ambiguous)	P11388/Q02880	84/105	IFDEILVNAADNK	19.39
		500/521	QIMENAEINNIIK	19.08
		702/723	ELILFSNSDNER	18.21
		500/521	QImENAEINNIIK	16.85
		828/849	FLYDDNQR	11
		481/502	YGVFPLR	7.73
Zinc finger protein 451	Q9Y4E5	972	QIPFTILSGDQGFLELENQFK	20.08
		780	SDEEEQQYVIK	17.96
		972	QIPFTILSGDQGFLELENQFKK	17.06
		931	IYNYLNR	10.27
		1035	NTSIGEEFISTEDVELEEAIRR	8.55
		78	VALTLAR	6.38
Small ubiquitin-related modifier 2 (SUMO2)	P61956	22	VAGQDGSVVQFK	20.84
		8	EGVKTENNDHILNLK	14.13
Small ubiquitin-related modifier 3 (SUMO3)	P55854	21	VAGQDGSVVQFK	20.84
		8	EGVKTENDHILNLK	14.33
Small ubiquitin-related modifier 4 (SUMO4)	Q6EEV6	22	VAGQDGSVVQFK	20.84
		12	TENNNHINLKVAGQDGSVVQFK	19.16

* M=methionine, m=methionine sulfoxide

Table S3.
Peptides identified from GFP-ZNF451 LC-MS/MS analysis

Protein	Accession number	start amino	Peptide Sequence *	Peptide
DNA Topoisomerase 2 α	P11388	805	YIFTmLSSLAR	23.91
		467	TLAVSGLGVVGR	22.83
		325	HVDYVADQIVTK	21.52
		307	GFQQISFVNSIATSK	20.24
		135	mYVPALIFGQLLTSSNYDDDEK	19.72
		1205	TQmAEVLPSPR	19.45
		1205	TQMAEVLPSPR	18.69
		879	LmDGEEPLPmLPSYK	18.3
		569	FLEEFITPIVK	17.47
		1374	SVVSDLEADDVK	17.39
		805	YIFTMLSSLAR	17.21
		107	VTIDPENNLISIWNNGK	17.03
		930	TWTQTYKEQVLEPmLNGTEK	15.81
		640	YSGPEDDAAISLAFSK	15.73
		937	EQVLEPmLNGTEK	15.51
		676	KLLGLPEDYLYGQTTTYLTYNDFINK	14.91
		1443	RPDALNSGVSQKPDPAK	14.91
		135	MYVPALIFGQLLTSSNYDDDEK	14.51
		1012	YDTVLDILRDFFELR	13.99
		567	HRFLEEFITPIVK	13.54
		879	LMDGEEPLPmLPSYK	12.98
		1035	EWLLGmLGAESAK	12.74
		950	TPPLITDYR	11.26
		228	mQSLDKDIVALmVR	10.35
		228	mQSLDKDIVALMVR	8.67
DNA Topoisomerase 2 β	Q02880	488	SLAVSGLGVIGR	23.09
		128	VSIDPESNIISIWNNGK	18.52
		918	GTIQELGQNQYAVSGEIFVVDRNTVEITELPVR	18.42
		940	NTVEITELPVR	18.06
		661	YAGPEDDAAITLAFSK	17.84
		1093	DLIQmLVQR	17.37
		940	NTVEITELPVR	17.15
		837	LLFPAVDDNLLK	17.06
		826	YIFTmLSTLAR	17.05
		1093	DLIQMLVQR	16.82
		588	HGFLEEFITPIVK	16.56
		346	HVDYVVDQVVGK	16.38
		328	GFQQISFVNSIATTK	15.17
		697	RLHGLPEQFLYGTATK	14.98
		713	HLTYNDFINK	14.22
		1228	LQLEETMPSPYGR	13.15
		1228	LQLEETmPSPYGR	13.07
		647	EYFADMER	8.87
		1093	DLIQmLVQR	8.5
		958	EQVELPmLNGTDK	8.29
		105	IFDEILVNAADNKQR	7.61
		290	SYVDLYVK	7.51
		958	EQVELPMLNGTDK	6.49
DNA Topoisomerase 2α/β (ambiguous)	P11388/Q02880	702/723	ELILFSNSDNER	19.9
		500/521	QImENAEINNIIK	18.65
		74/95	EVTFVPGLYKIFDEILVNAADNK	17.58
		84/105	IFDEILVNAADNK	17.33
		500/521	QIMENAEINNIIK	17.33
		536/557	ImImTDQDQDGSHIK	14.95
		828/849	FLYDDNQR	11.69
Small ubiquitin-related modifier 2 (SUMO2)	P61956	22	VAGQDGSVVQFK	21.48
		8	EGVKTENNDHILNLK	10.94
Tyrosyl-DNA phosphodiesterase 2	O95551	193	SQEIIPFPSTK	17.23

* M=methionine, m=methionine sulfoxide

Table S4.

X-ray diffraction data collection and refinement statistics

	mTdp2^{cat}–SUMO2–DNA	mTdp2^{cat}–SUMO2
PDB accession #	5TVP	5TVQ
Data collection		
Space group	P 2_1	P 6_4
Cell dimensions		
a, *b*, *c* (Å)	106.55, 113.77, 125.43	106.84, 106.84, 56.59
α, β, γ (°)	90, 91.52, 90	90, 90, 120
Resolution* (Å)	50–2.40 (2.49–2.40)	50–2.35 (2.43-2.35)
R_{sym}	0.147 (2.58)	0.056 (1.90)
R_{pim}	0.118 (1.65)	0.024 (0.91)
CC1/2	0.98 (0.163)	0.99 (0.177)
CC*	0.99 (0.532)	0.99 (0.548)
$I / \sigma I$	5.9 (0.6)	26.3 (0.9)
Completeness (%)	99.7 (98.9)	99.5 (97.9)
Redundancy	3.7 (3.4)	7.1 (5.0)
Refinement		
Resolution (Å)	48.5–2.40	29.7–2.35
No. reflections	115877	15550
R_{work} / R_{free}	0.193/0.234	0.189/0.225
No. atoms		
Protein	16473	2623
Ligand/ion	1572	5
Water	690	37
B factors (Å^2)		
Protein	54.1	106.5
Ligand/ion	72.0	100.6
Water	41.7	79.6
r.m.s. deviations		
Bond lengths (Å)	0.006	0.013
Bond angles (°)	0.75	1.05

Each dataset was collected from a single crystal.

*Values in parentheses are for highest-resolution shell.

Table S5.

Parameters derived from SAXS experiments

Sample	R_g Guinier	Dmax	R_g Real	Calculated R_g from crystal structure	χ^2 fit to experimental data
mTdp2 118-370	19.2 Å	56 Å	18.9 Å	17.2 Å	0.7
mTdp2 118-370 + SUMO2	22.2 Å	75 Å	22.5 Å	21.5 Å	0.7

Table S6.

DNA Plasmids used in this study.

Plasmid #	Vector	Insert protein	Residues/mutant
1	pCDNA6.2/N-YFP-DEST	Stop codon	none (eYFP only)
2	pCDNA6.2/N-YFP-DEST	hTDP2	1-362/wt
3	pCDNA6.2/N-YFP-DEST	hTDP2	1-362/H351N
4	pDEST566	Stop codon	none (MBP only)
5	pMCSG9	hTDP2	1-362/wt
6	pDEST566	hTDP2	1-107/wt
7	pMCSG9	hTDP2	108-362/wt
8	pMCSG9	hTDP2	1-362/H351N
9	pMCSG9	hTDP2	108-362/H351N
10	pET-DEST42	SUMO2	1-93/wt
11	pMCSG7	SUMO2	10-93/wt
12	pLX302	mRFP1-SUMO2	1-93/wt
13	pCDNA6.2/N-YFP-DEST	hTOP2β	46-1212/wt
14	pLX302	6xHIS-SUMO2	1-93/wt
15	pCDNA6.2/N-YFP-DEST	ZNF451	1-1061/wt
16	pET15d	UBC9	1-158/wt
17	pDEST686	UBCp>eGFP- ZNF451	1-1061/wt
18	pDEST686	UBCp>eGFP-Stop codon	(eGFP only)
19	pCDNA6.2/N-YFP-DEST	hTDP2	1-364/C–AE
20	pCDNA6.2/N-YFP-DEST	hTDP2	1-362/PQ
21	pCDNA6.2/N-YFP-DEST	hTDP2	1-364/C-AE+PQ
22	pMCSG9	mTDP2	118-370/wt
23	pMCSG9	mTDP2	118-372/C–AE
24	pCDNA6.2/N-YFP-DEST	hTOP2α	1-1531/wt
25	pCDNA6.2/N-YFP-DEST	hTOP2β	1-1621/wt

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