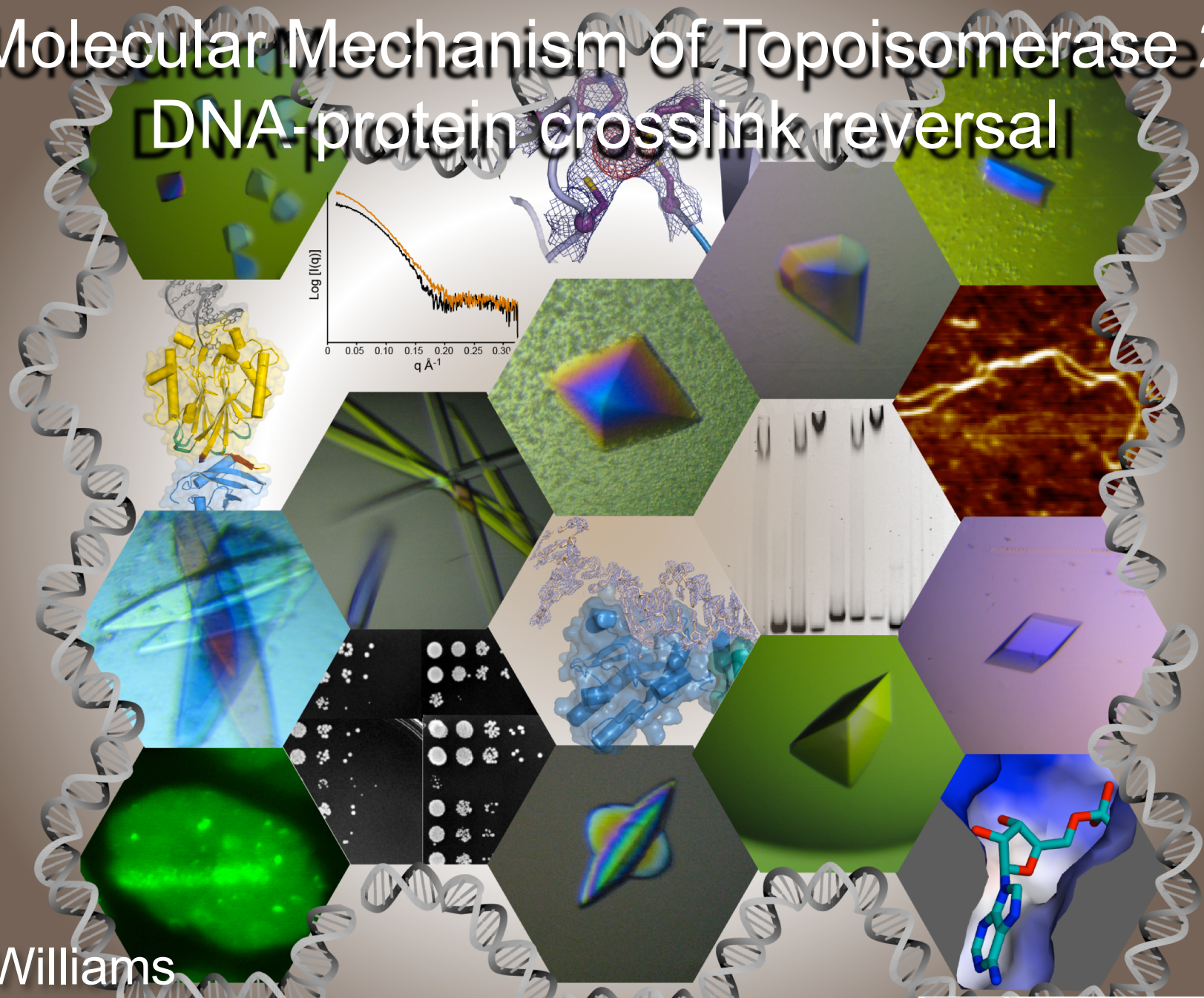
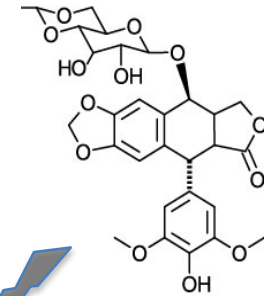
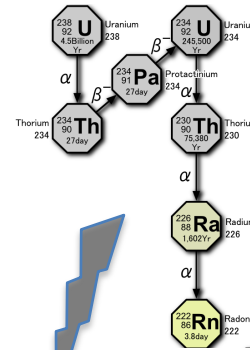
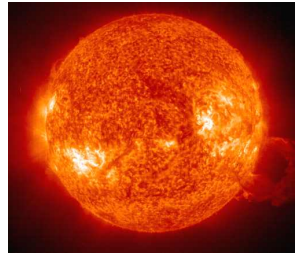
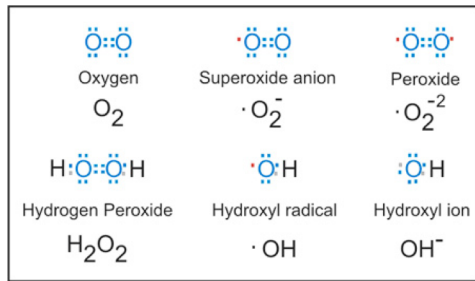


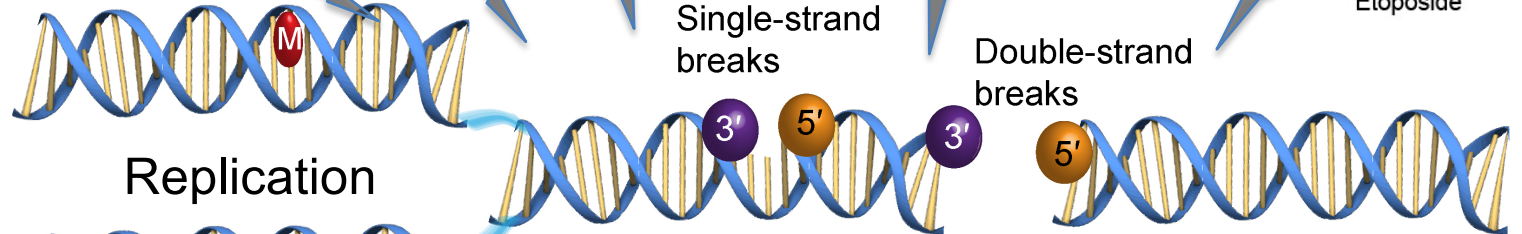
Molecular Mechanism of Topoisomerase 2 DNA-protein crosslink reversal



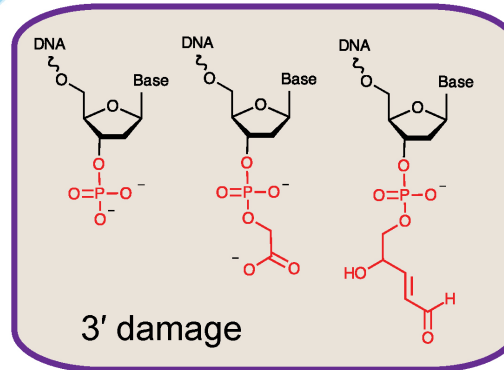
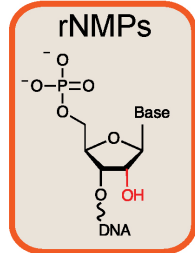
Scott Williams
Genome Integrity and Structural Biology Laboratory



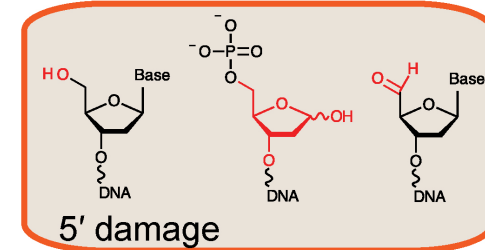
Etoposide



Replication



3' damage



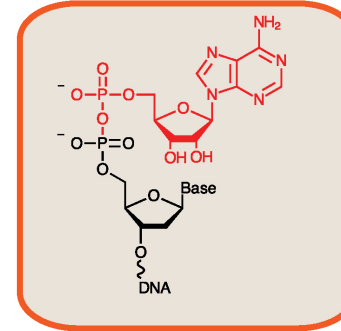
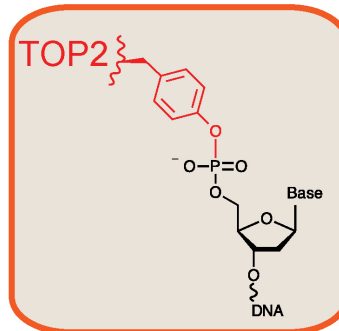
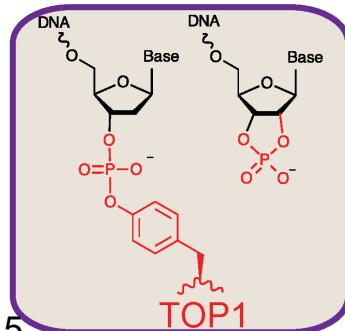
5' damage

Secondary nucleic acid metabolism

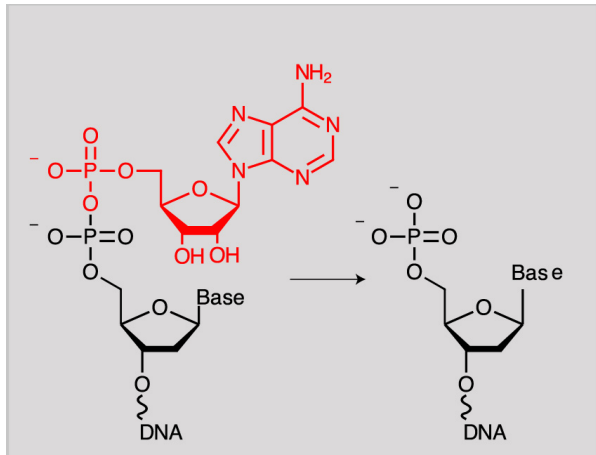
TOP1

TOP2

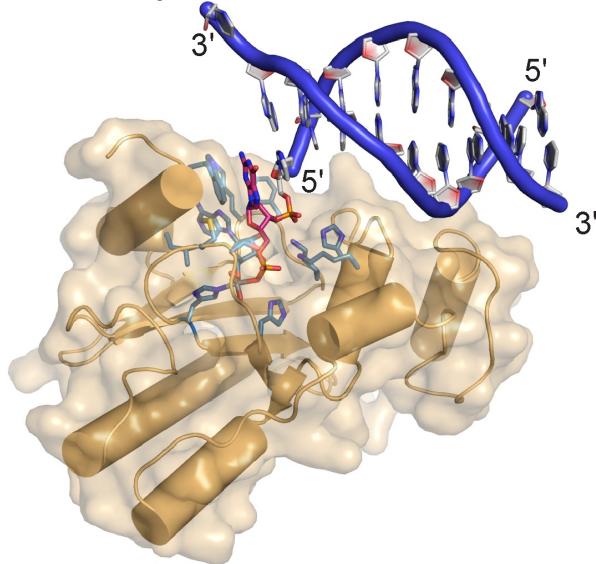
DNA ligase



DNA Adenylation repair

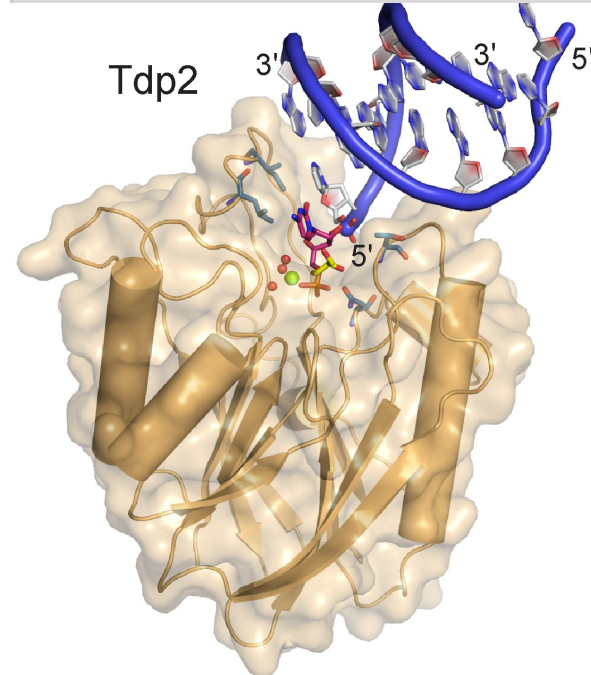
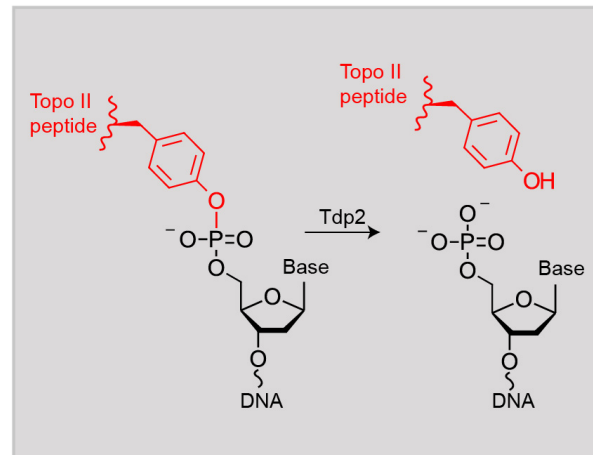


Aprataxin



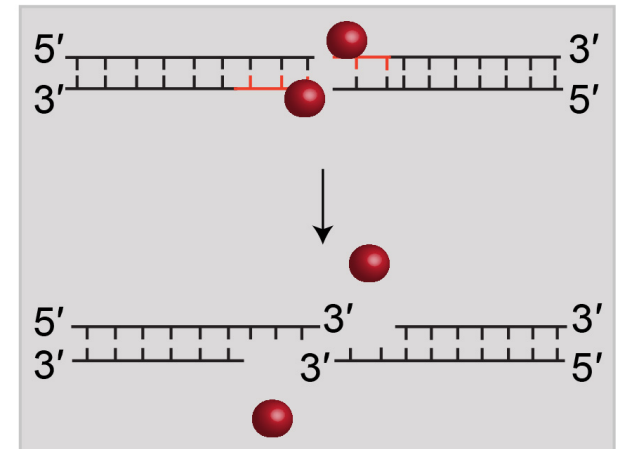
Tumbale, Nature 2014
Schellenberg, Prog Biophys Mol Biol 2015
Wallace, RNA Biol 2014
Tumbale, Nat Struct Mol Biol 2011

Topoisomerase DNA-protein Crosslink repair

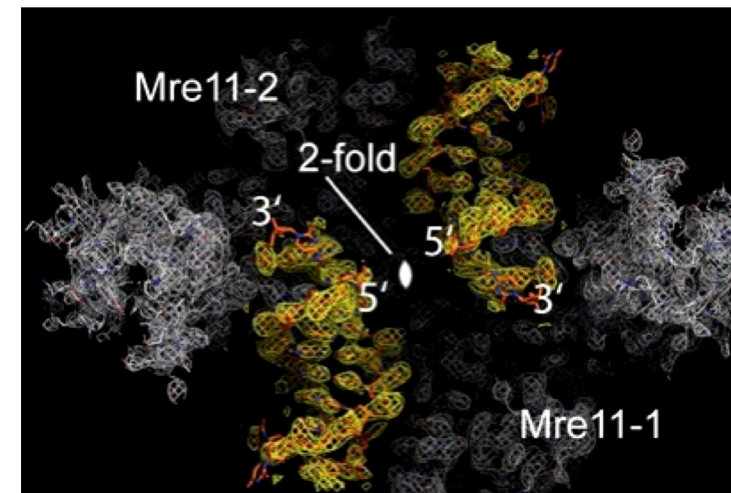


Schellenberg, Nucleic Acids Res 2016
Gao et al, JBC 2014
Schellenberg, Nat Struct Mol Biol 2012
Adhikari, Anal Biochem, 2011

Nucleolytic processing in DSB repair

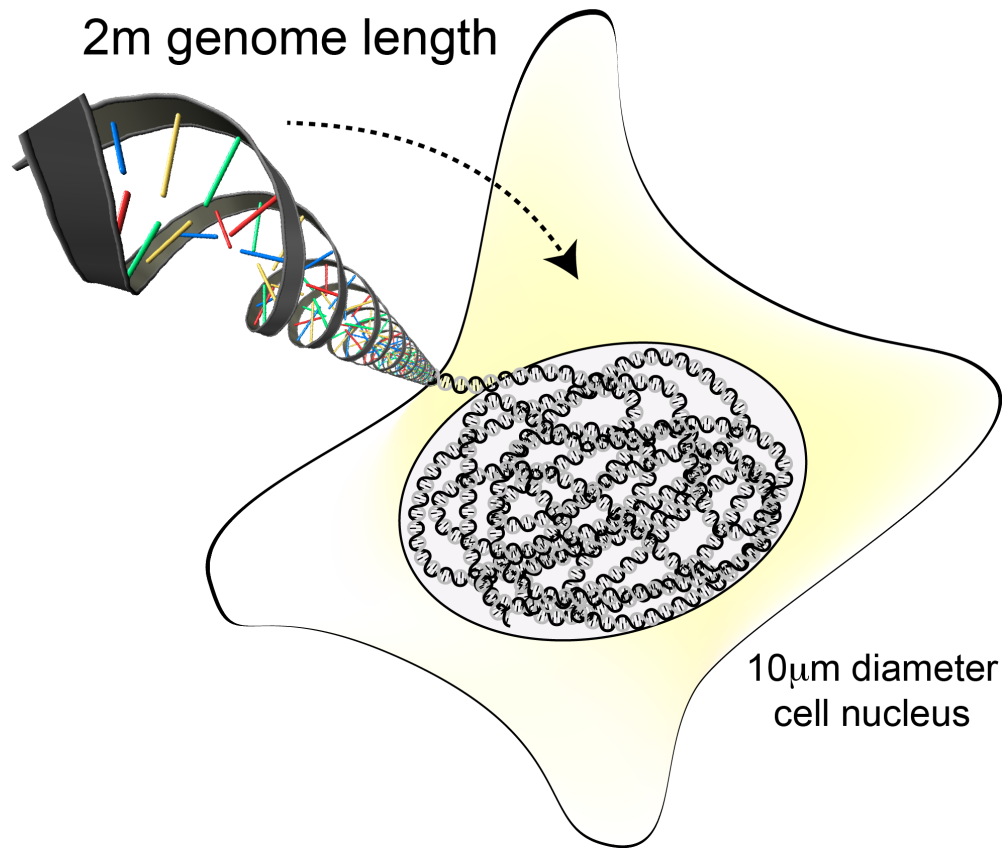


MRN/CtIP, APE2

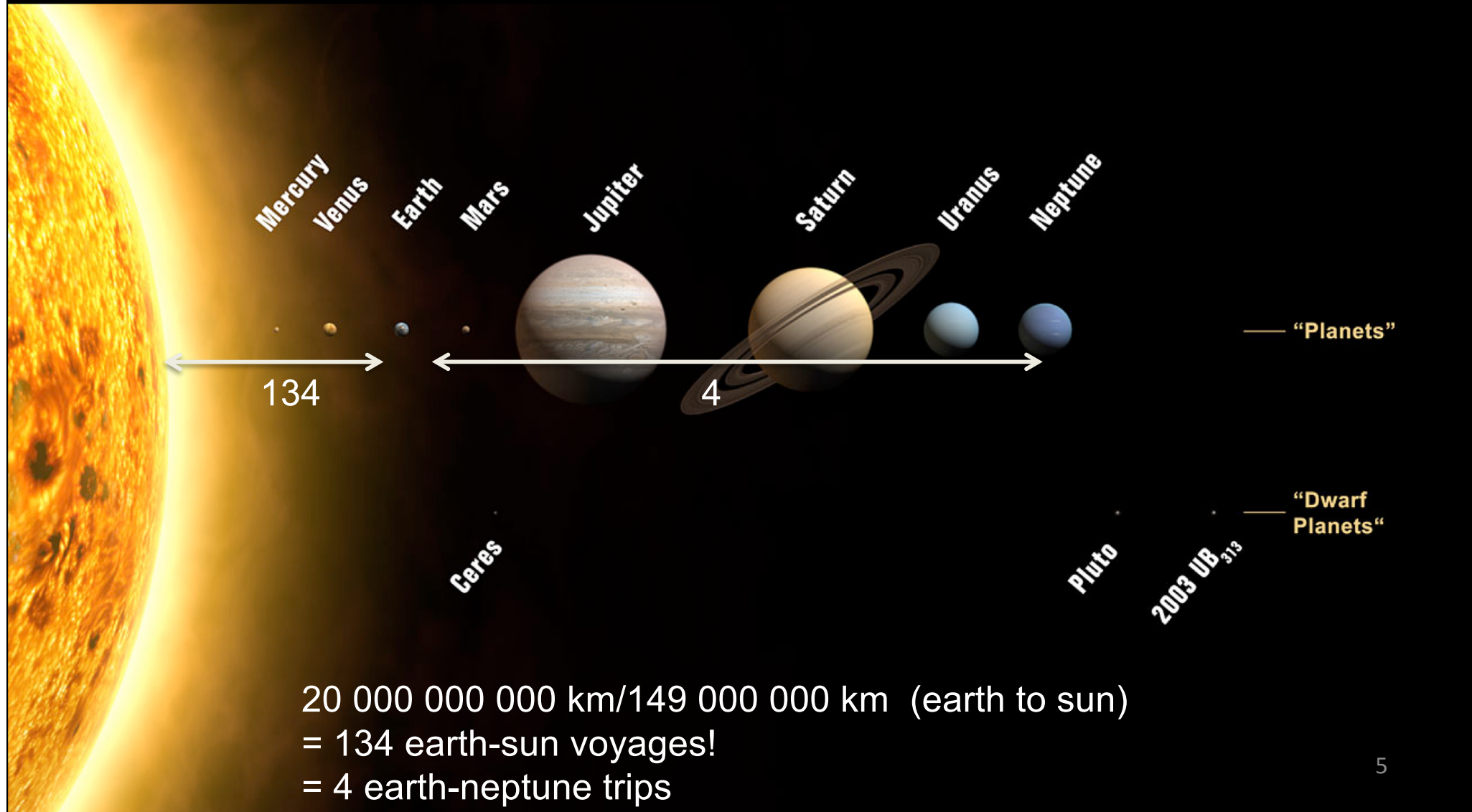
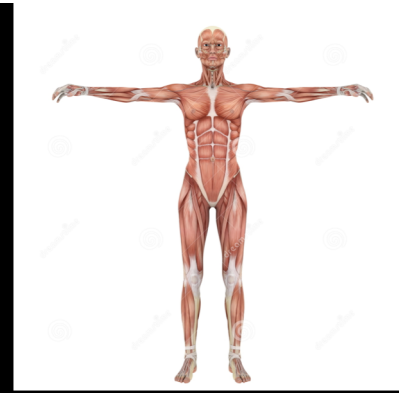


Andres et al DNA Repair 2017
Wallace et al PNAS 2017
Andres et al Nat Struct Mol Biol 2015
Williams et al Nat Struct Mol Biol 2011
Williams et al Cell 2009
Williams et al Cell 2008

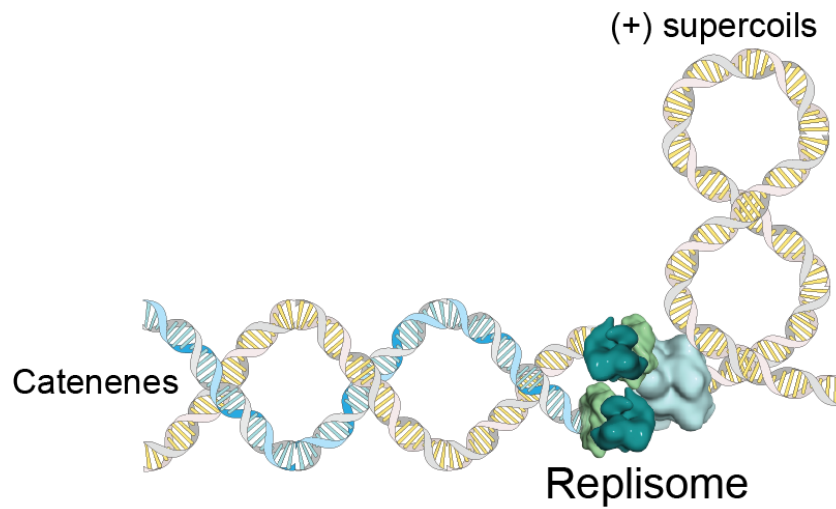
DNA Entanglement



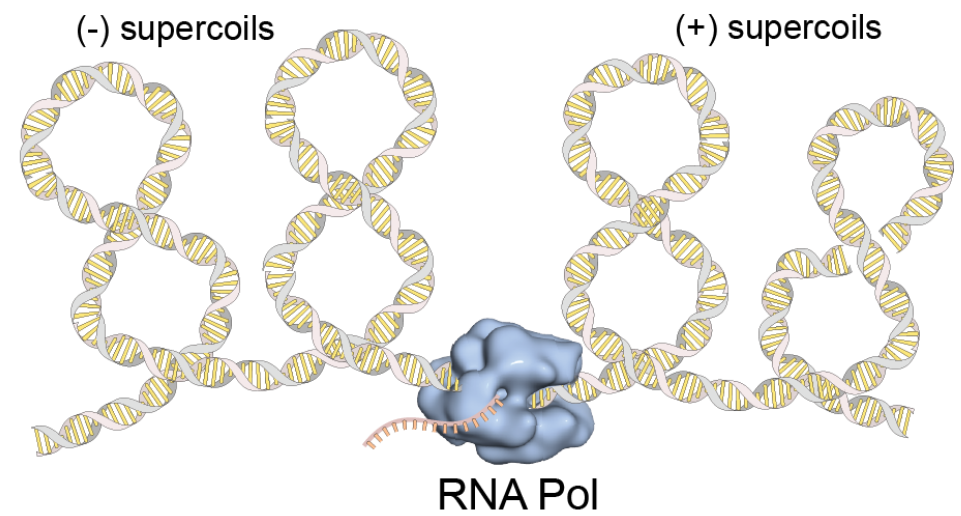
~0.002 km/genome
~10 trillion cells/individual



Topoisomerases resolve DNA entanglement

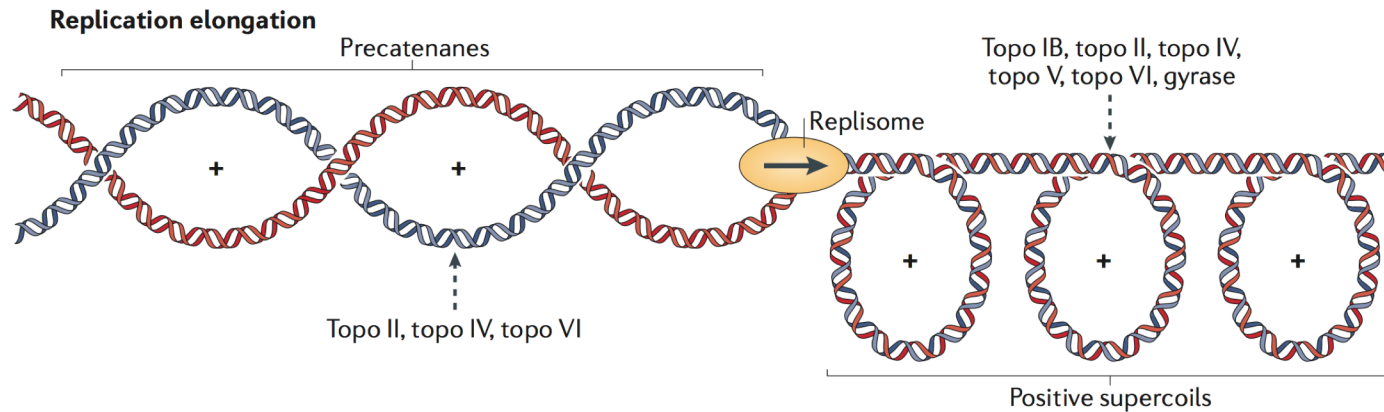


DNA replication



Transcription

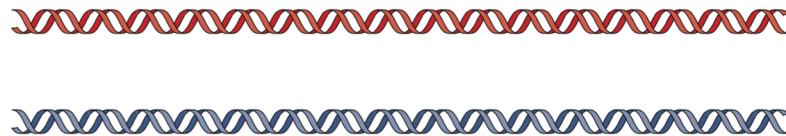
Topoisomerases resolve DNA entanglement



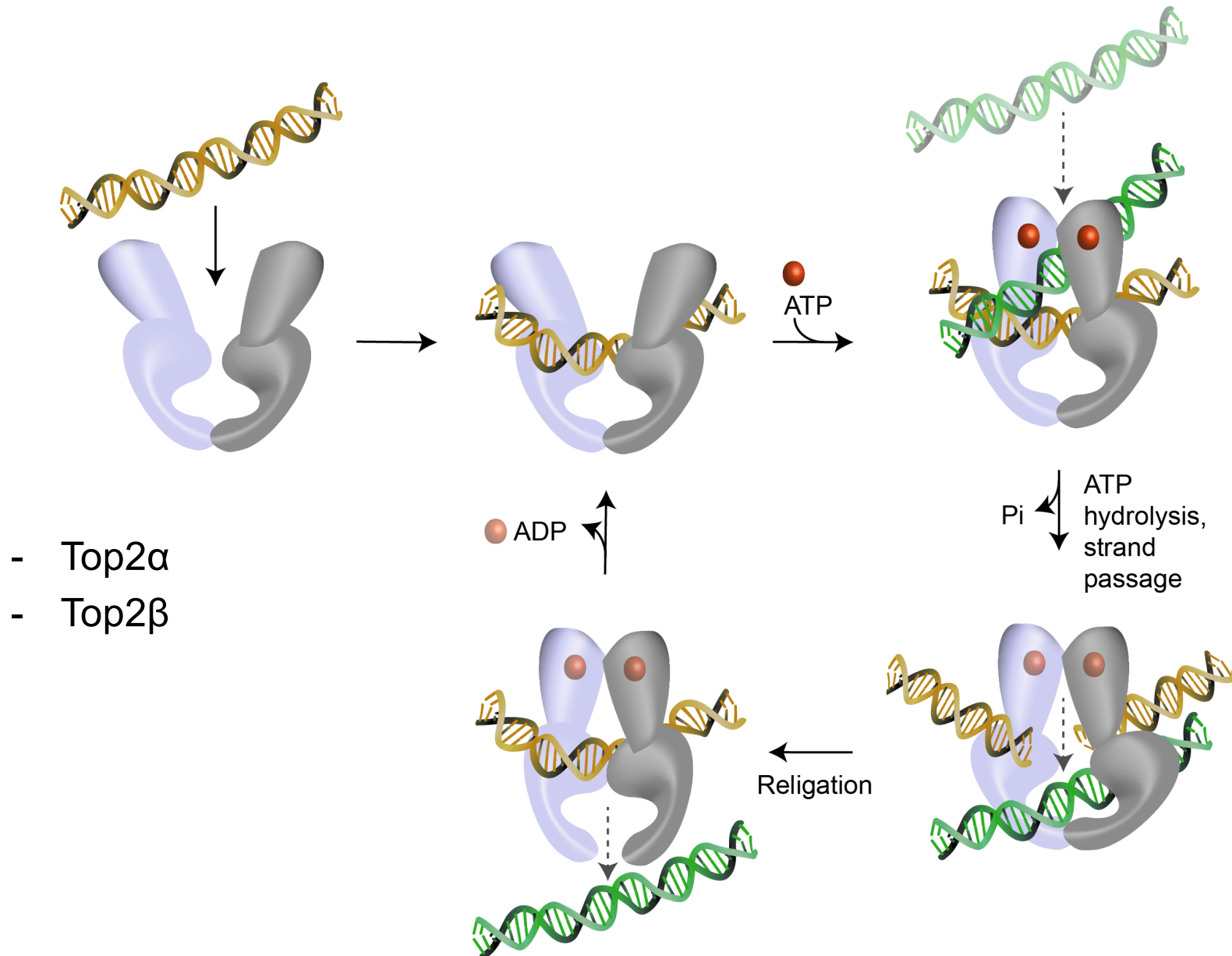
Antibiotics
Chemotherapy

—| X —|

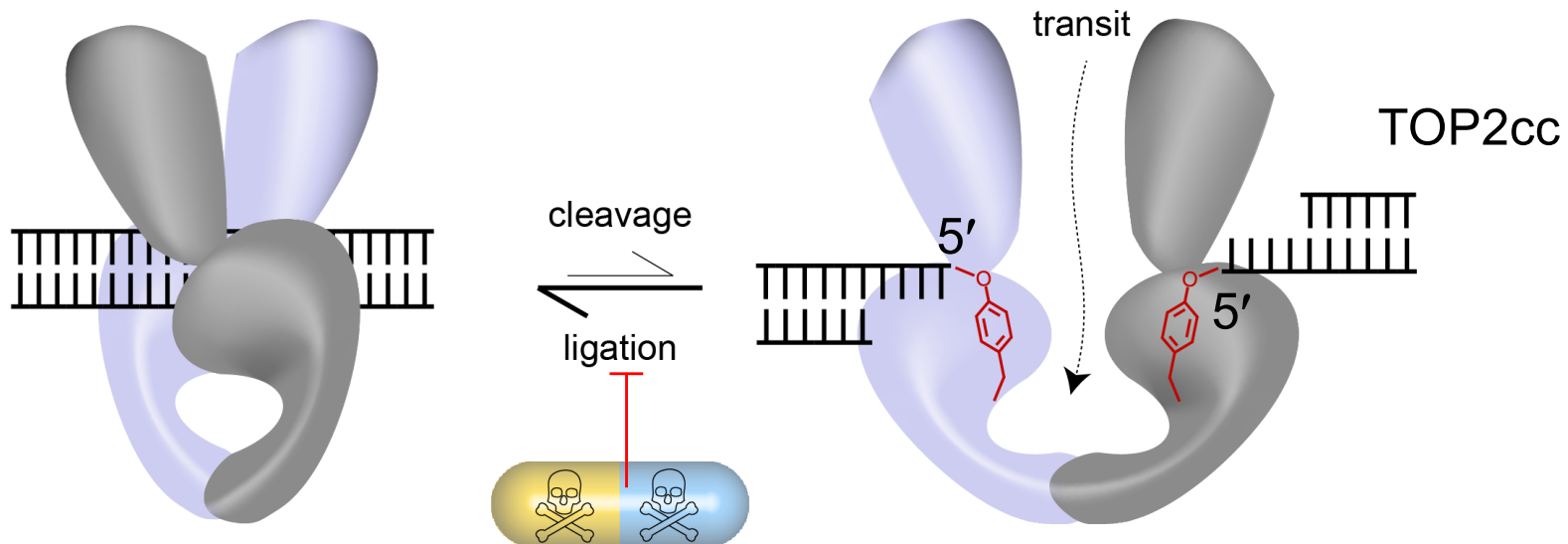
Topo II, topo IV, topo VI



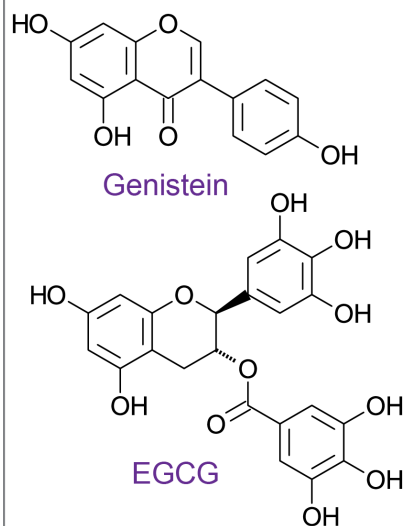
Type IIA Topoisomerase reaction cycle



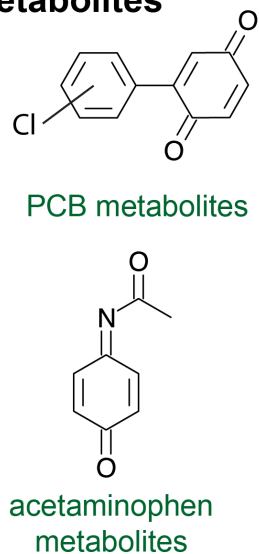
Reversible DNA cleavage by TOP2 can be poisoned



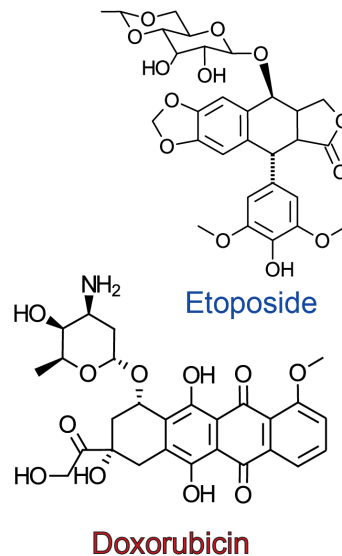
Dietary



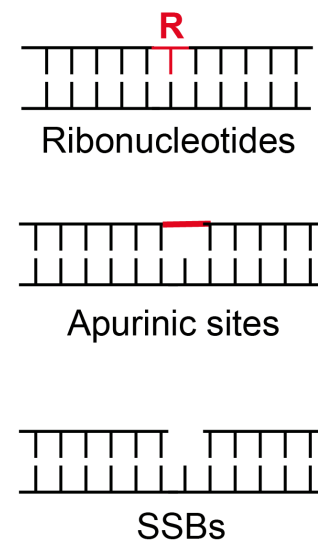
Chemical and drug metabolites

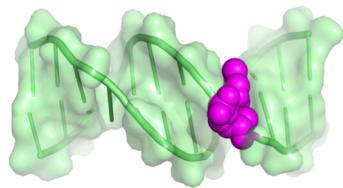


Chemotherapeutic drugs



DNA Damage





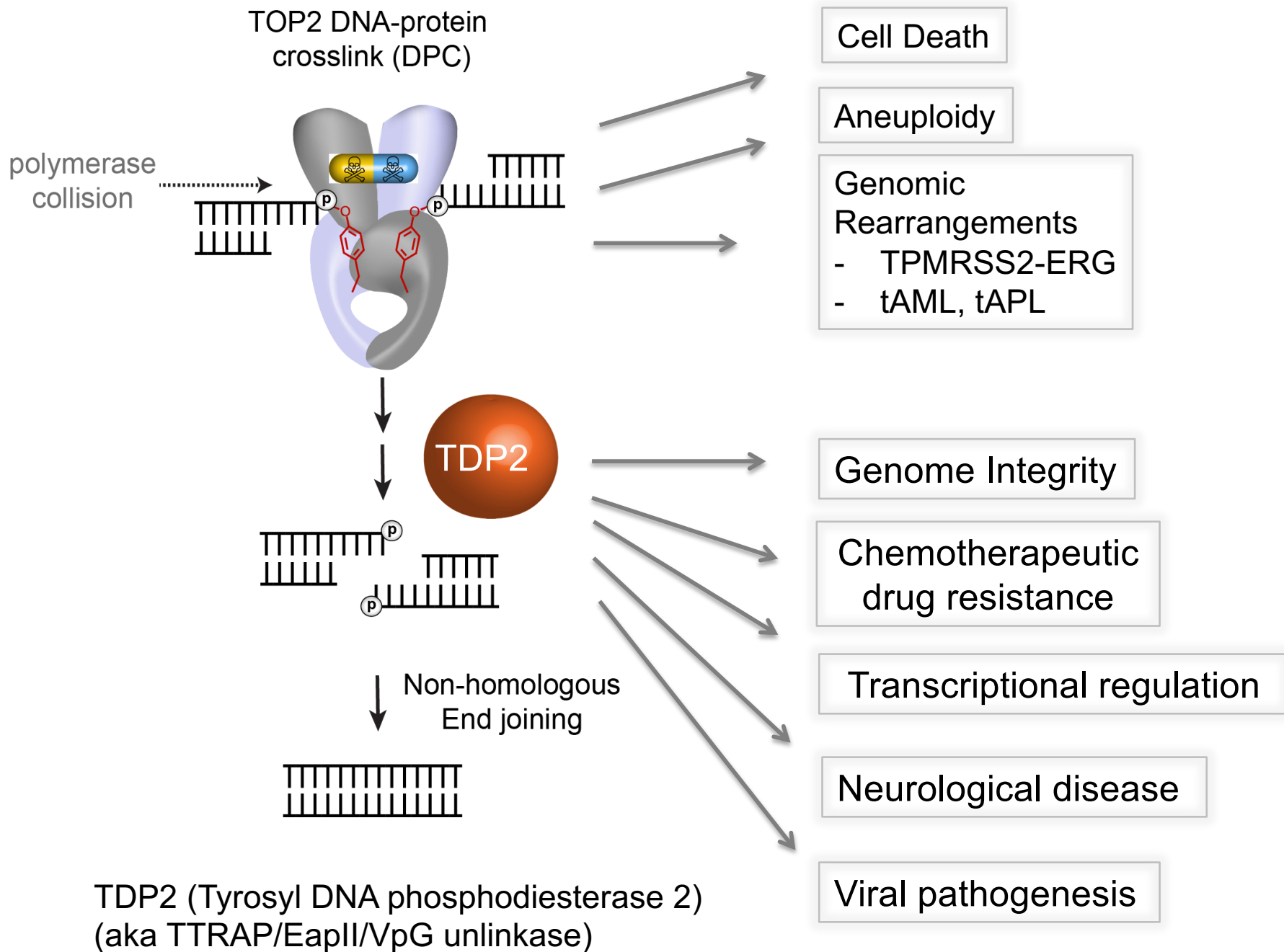
Top2



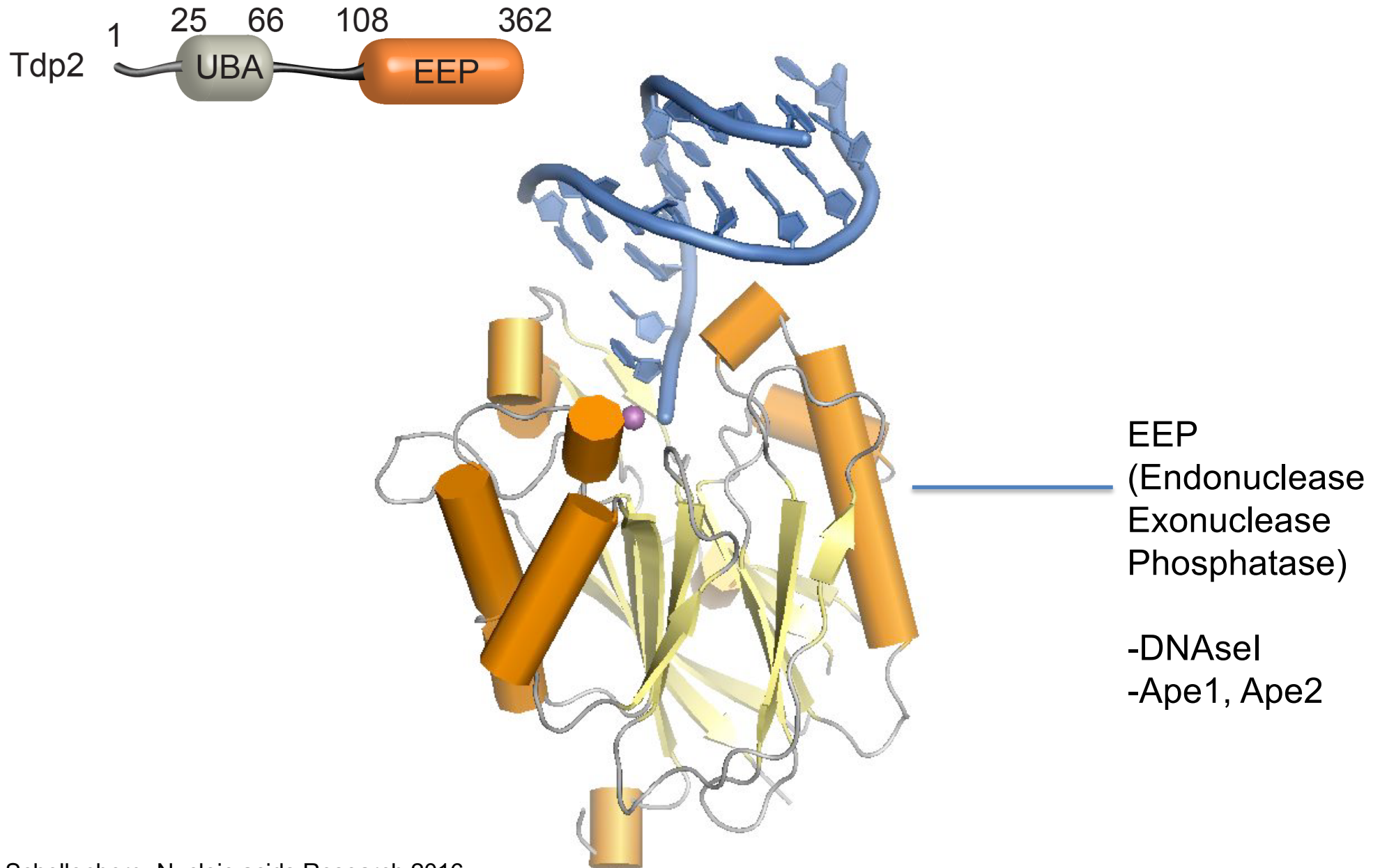
Problem

Bigger
Problem

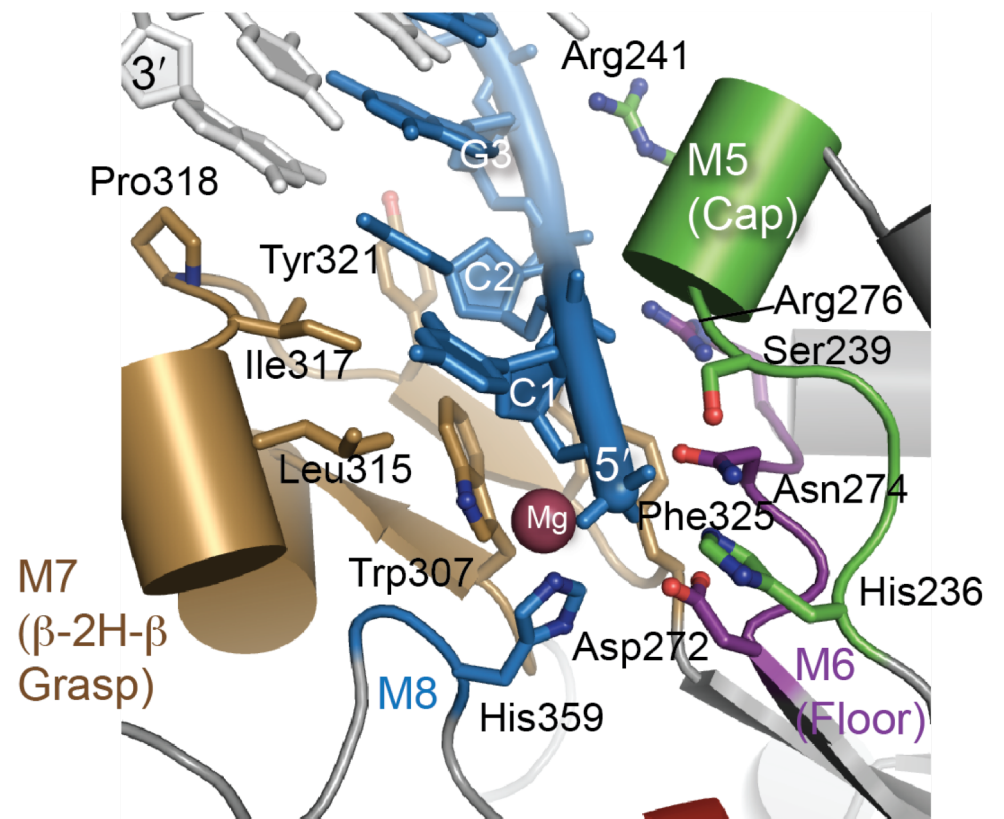
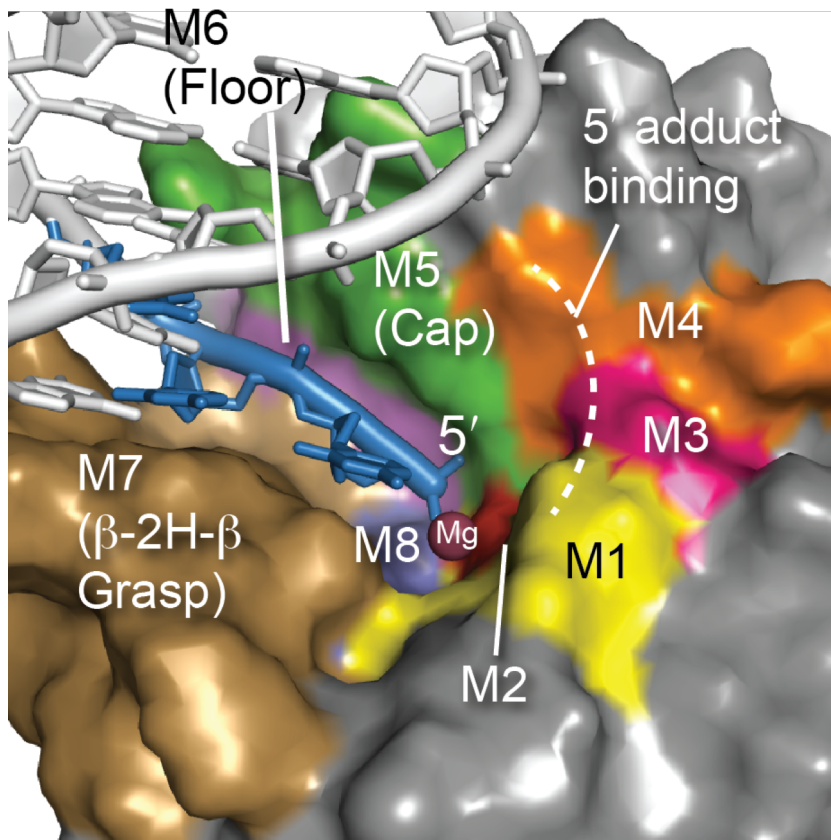
TDP2 resolves Top2-DNA protein crosslinks (DPCs)



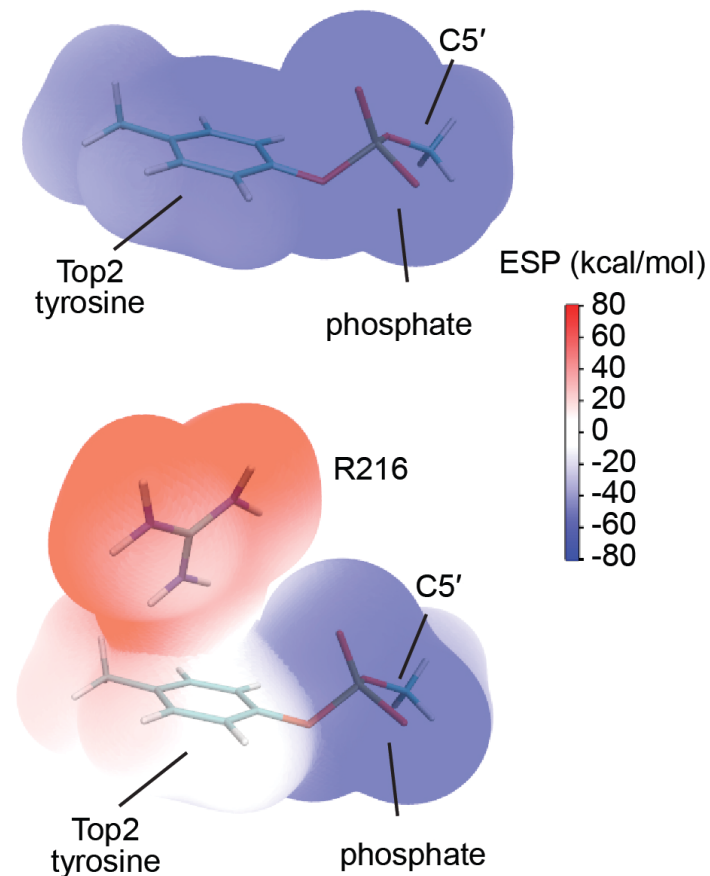
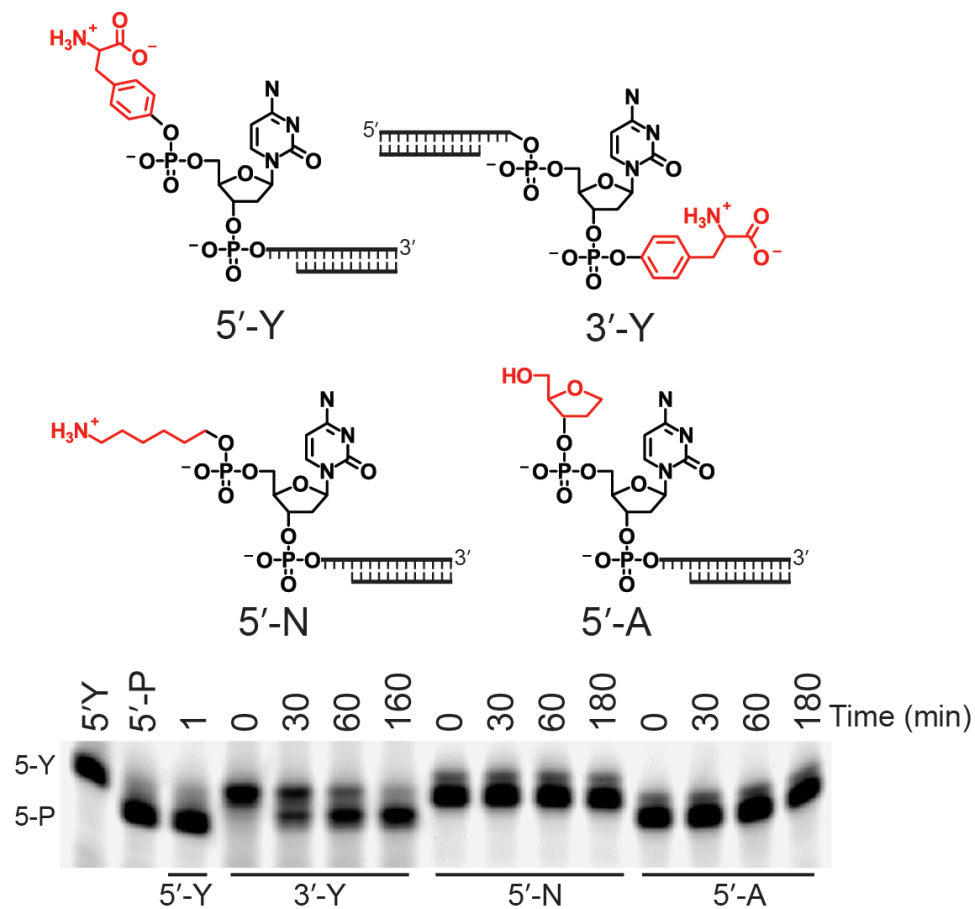
Architecture of the Tdp2-DNA complex



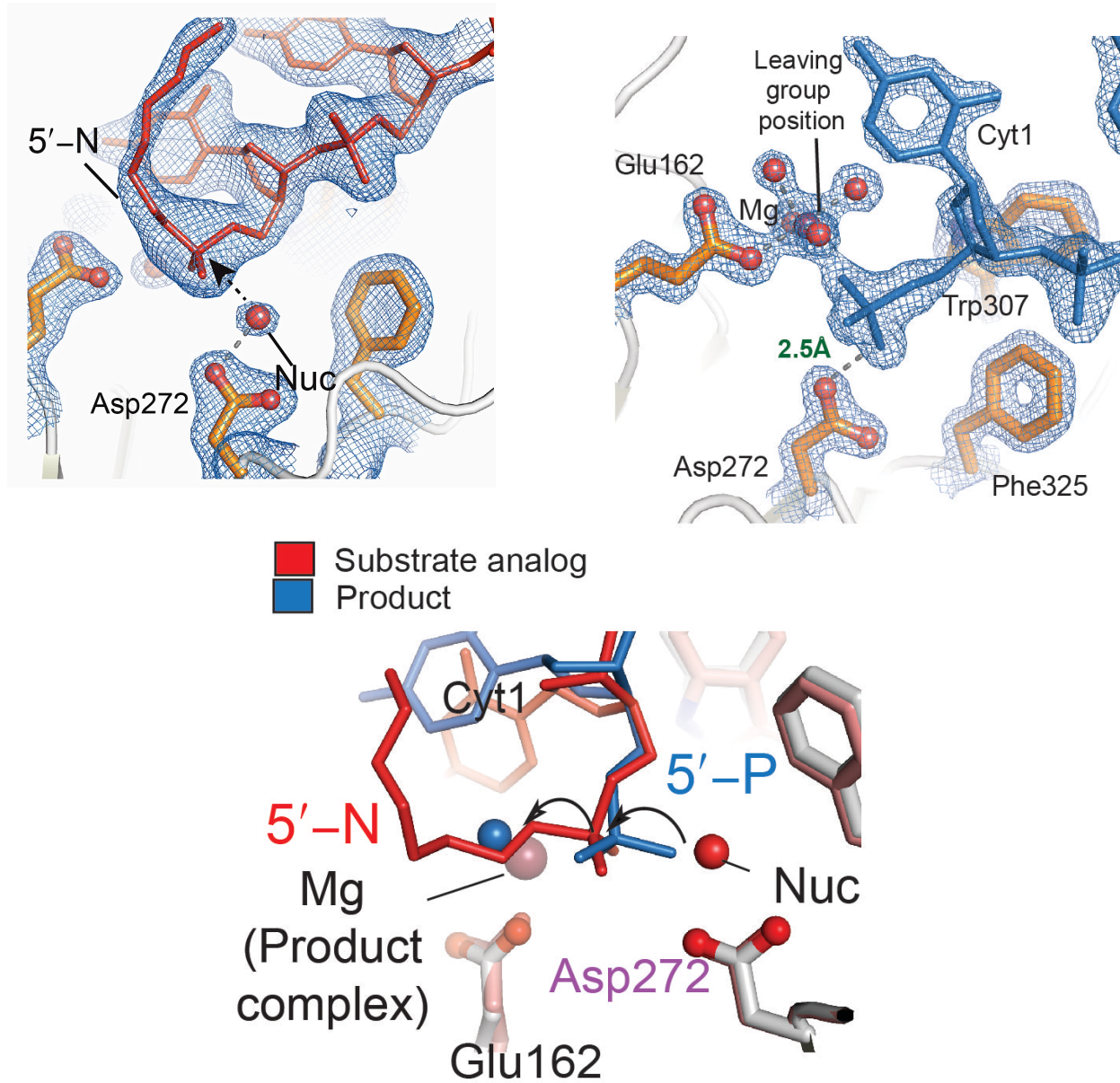
Tdp2 structure-specific DNA Interaction motifs



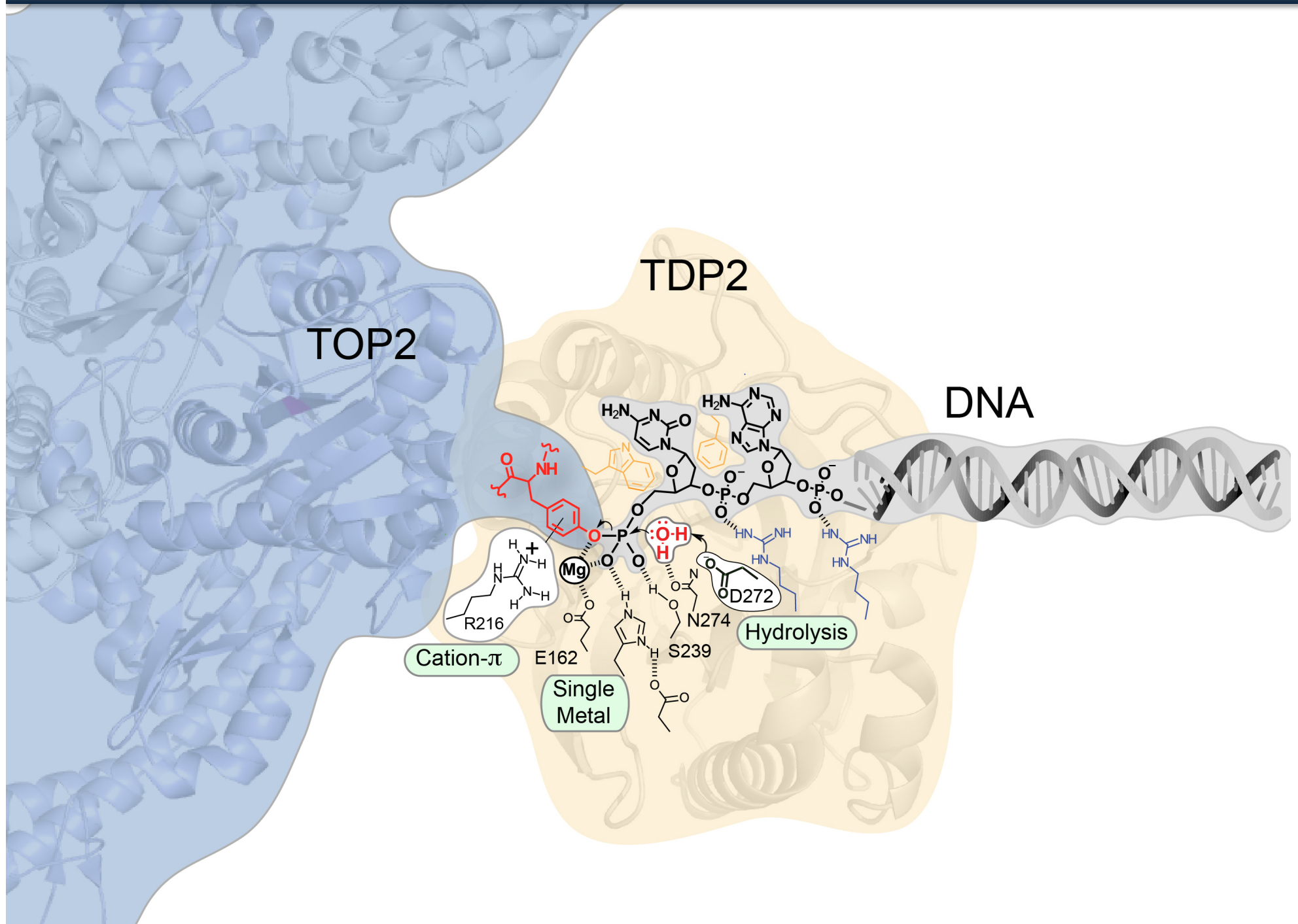
TDP2 is specific for tyrosylated termini



The Tdp2 Active site

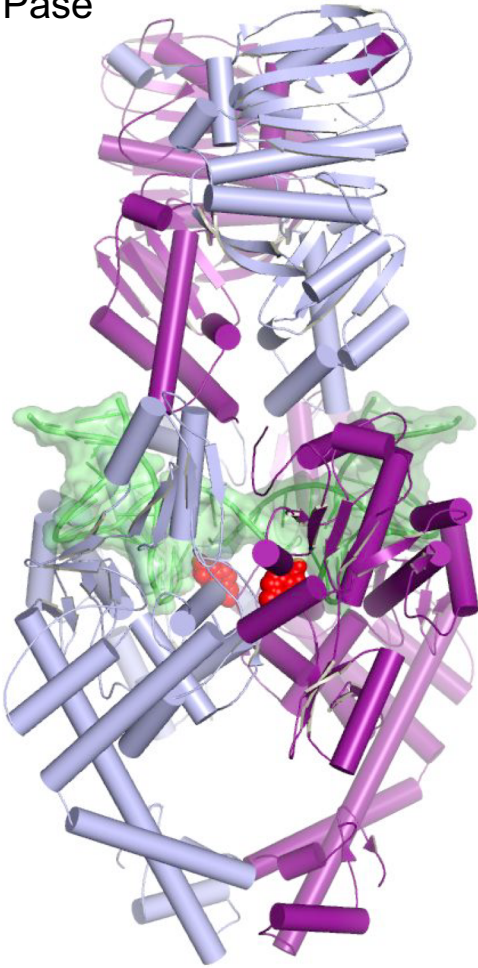


Direct reversal of DNA-protein crosslinks by Tdp2

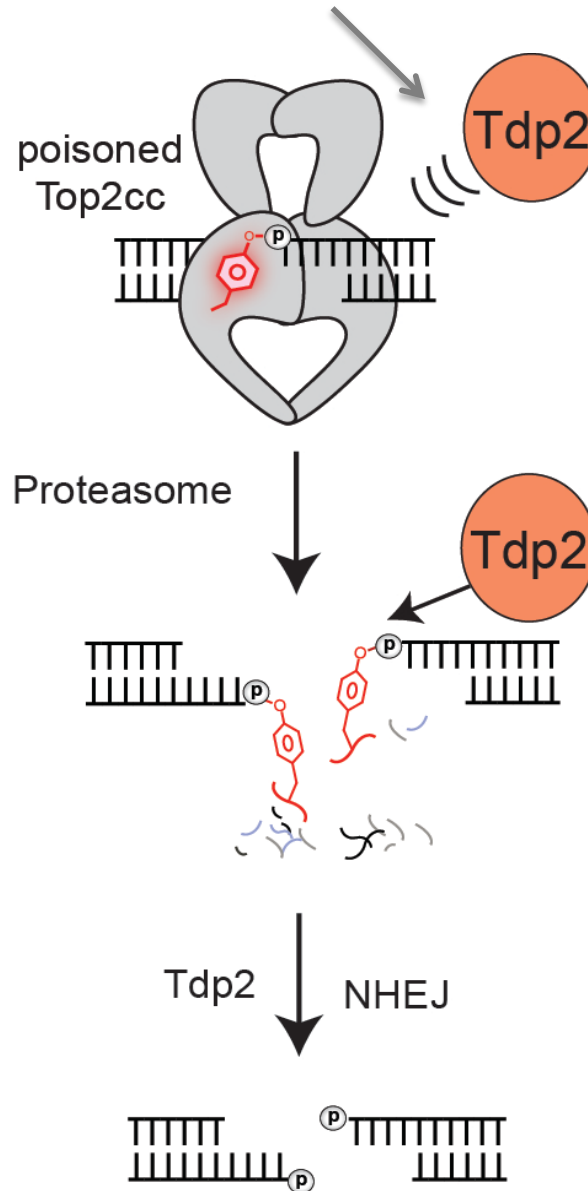


How is Tdp2 activity regulated?

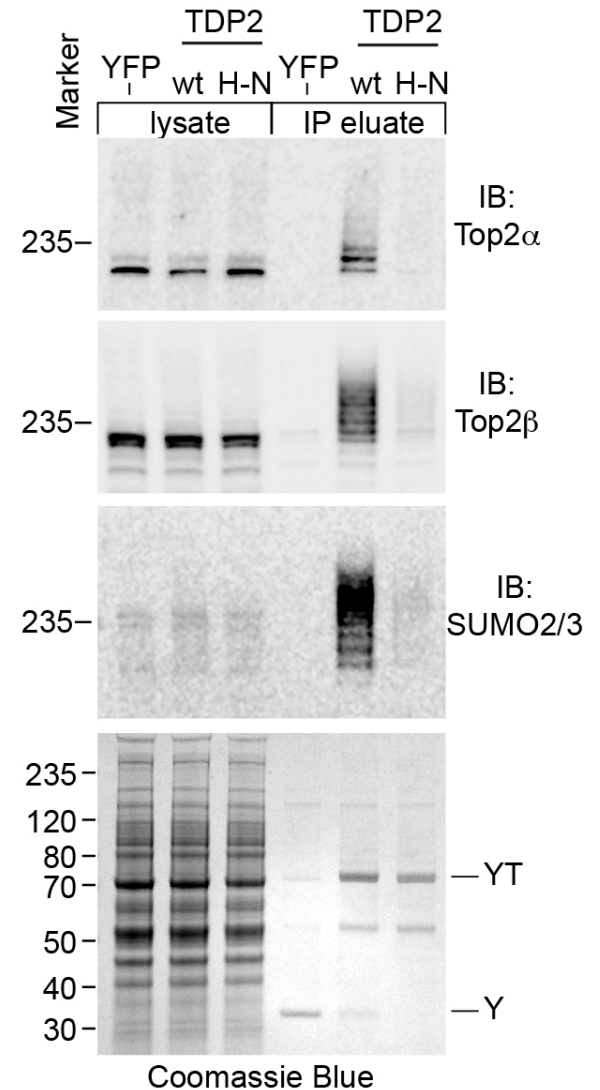
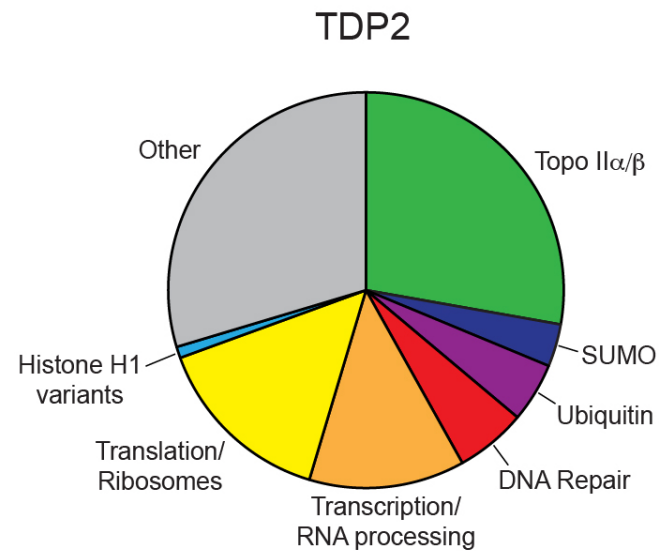
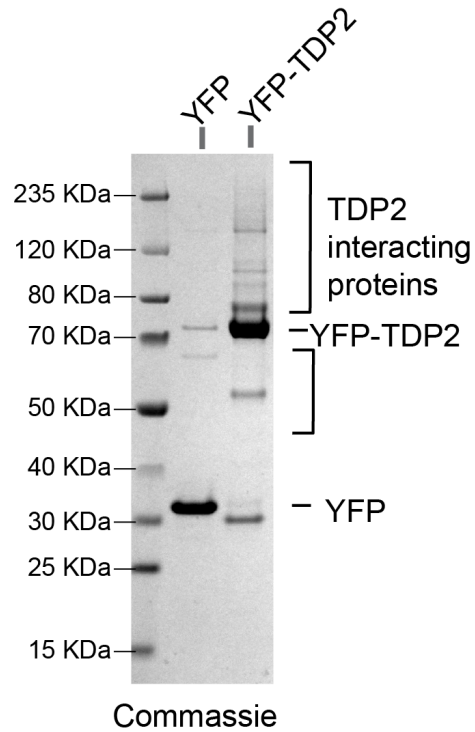
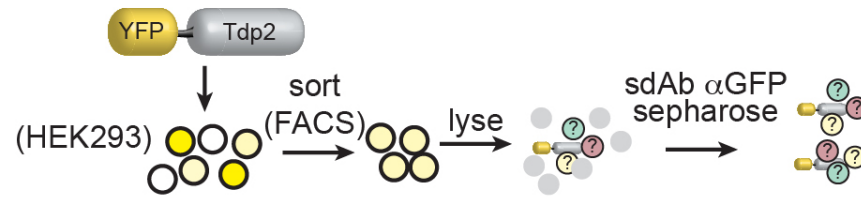
ATPase



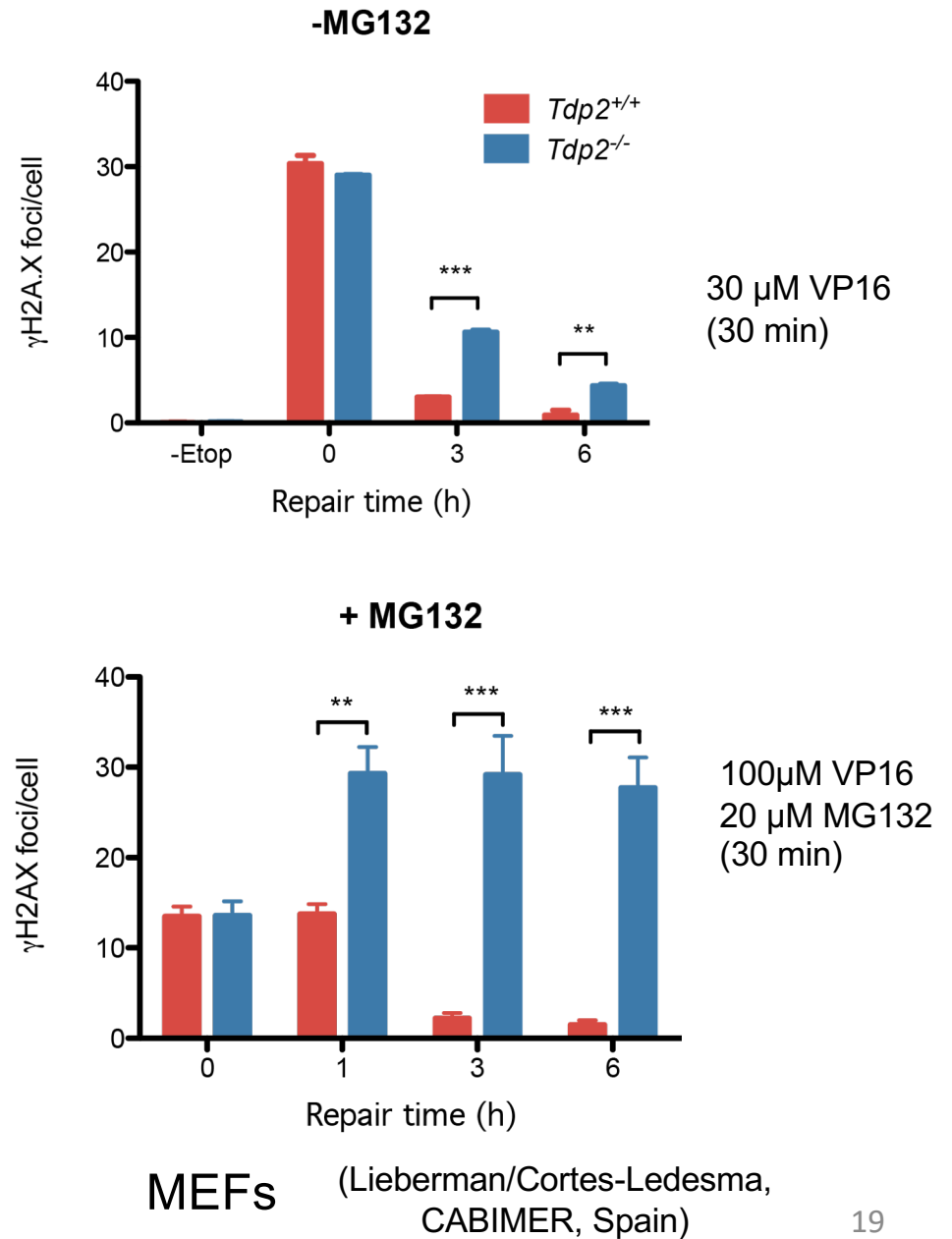
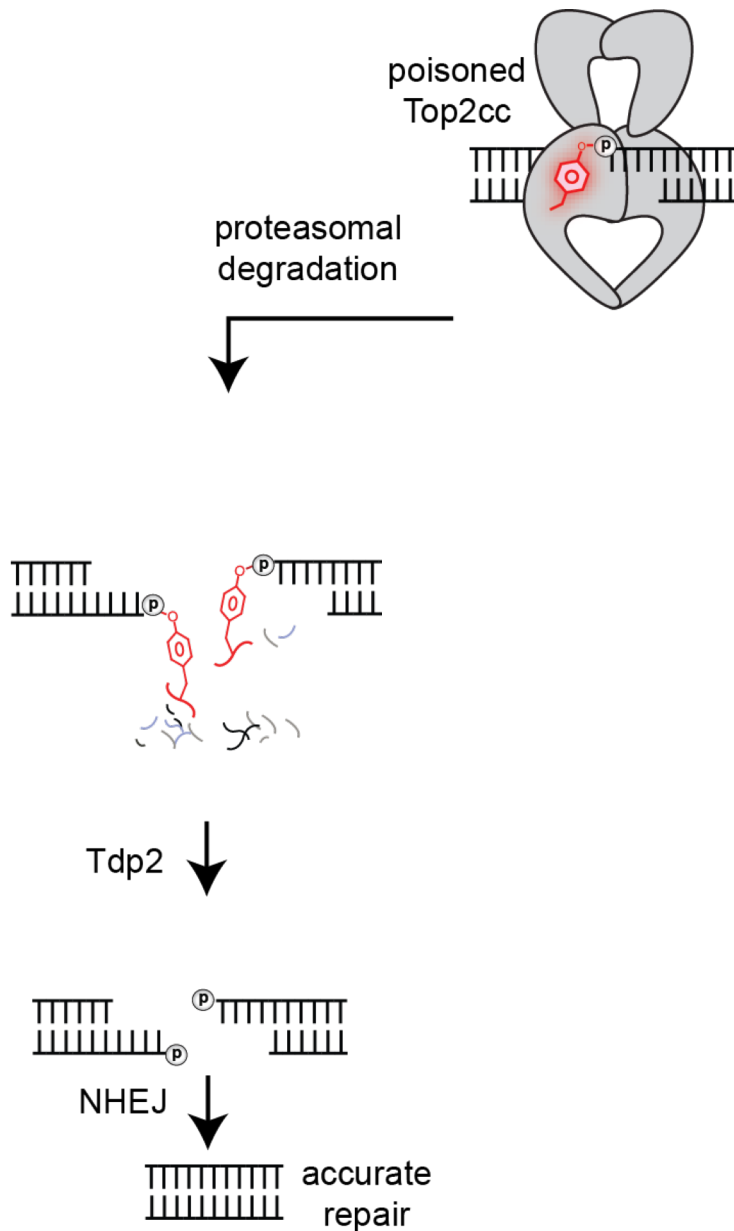
Cleavage/
religation core



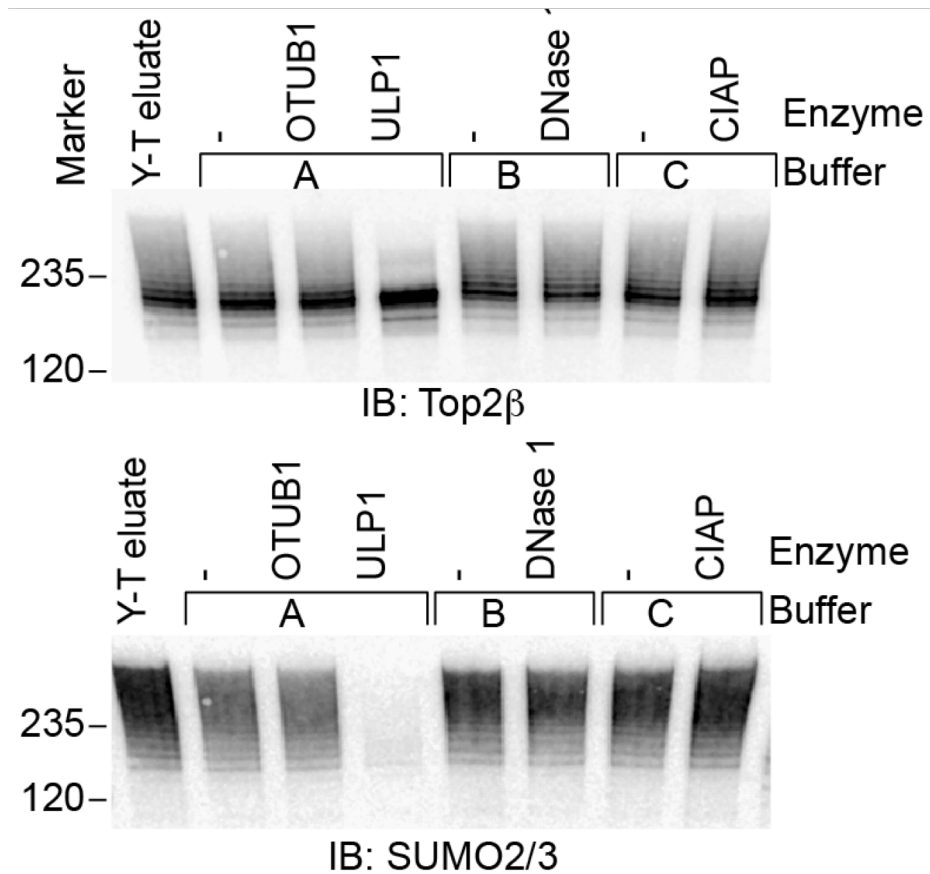
Identification of TDP2 associated proteins



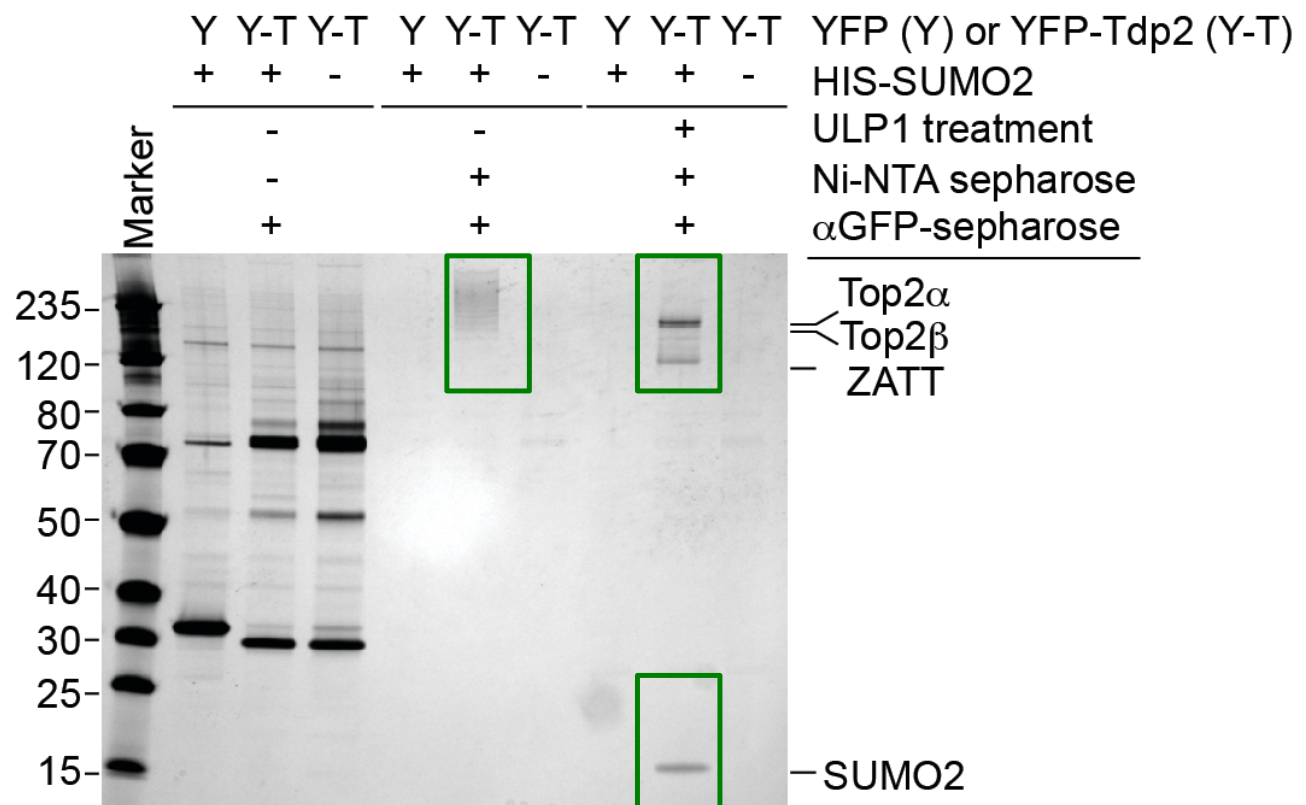
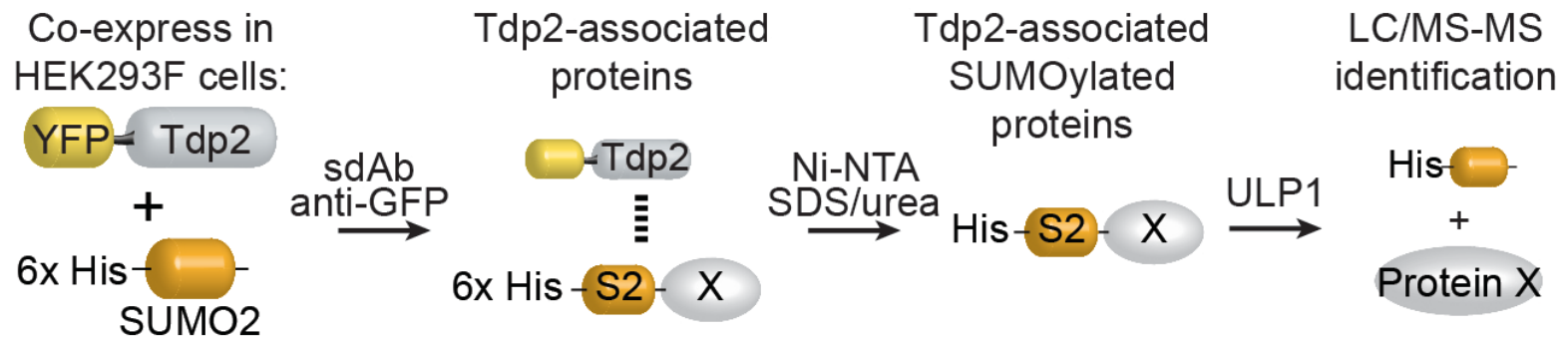
Proteasome independent functions of Tdp2



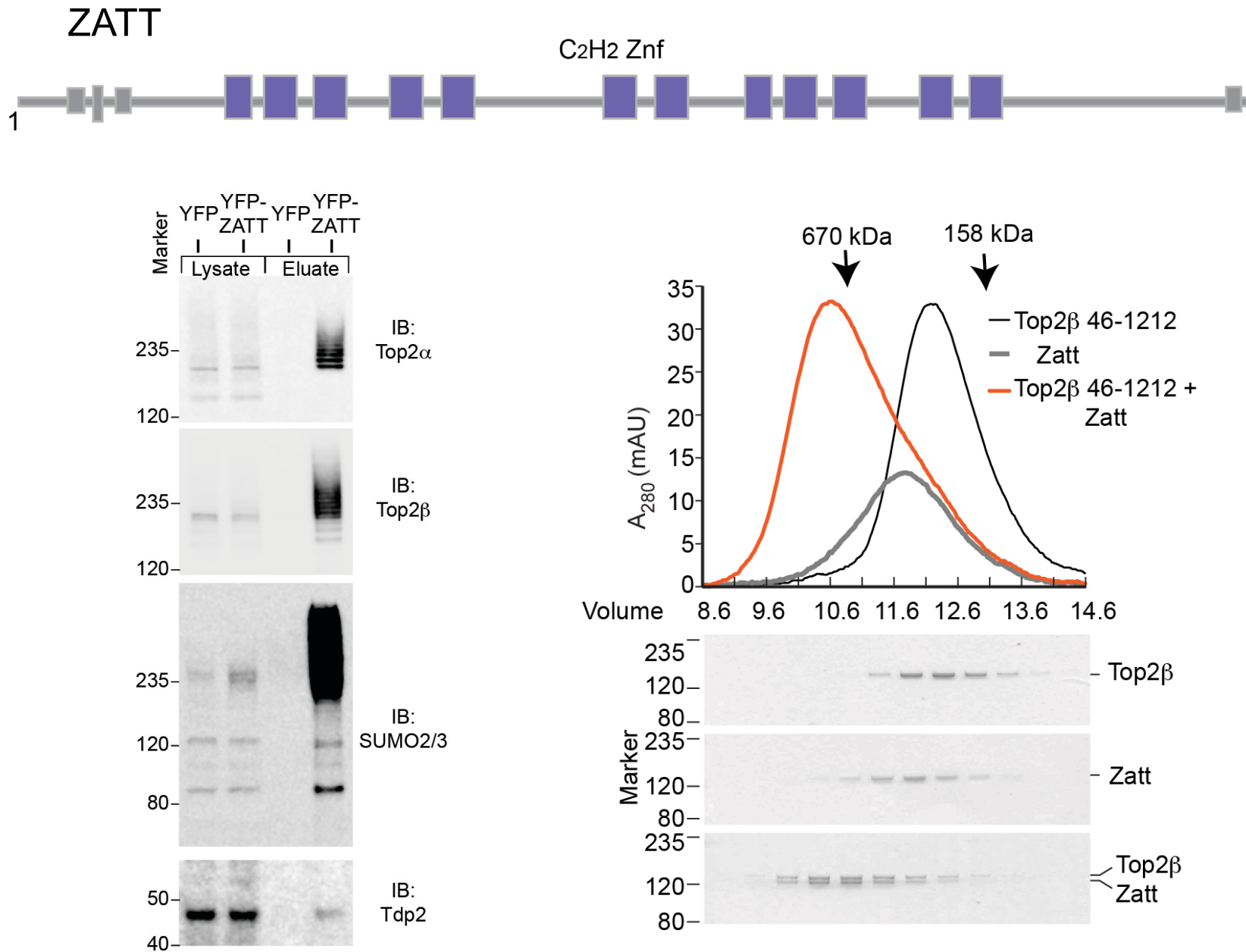
TDP2 co-purifies with SUMOylated Top2 isoforms



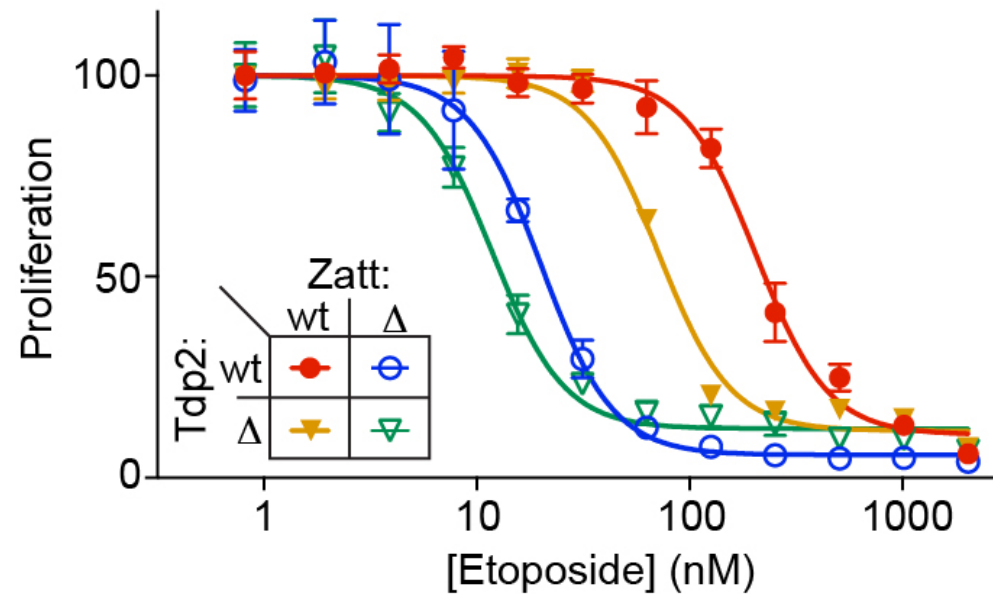
TAP purification of SUMO2ylated Tdp2 interacting proteins



ZATT: poly-Znf protein Associated with Tdp2 and Top2

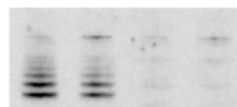


ZATT Modulates cellular sensitivity to Top2 poisons



wt Δ wt Δ TDP2:

wt wt Δ Δ ZATT



IB: ZATT



IB: TDP2

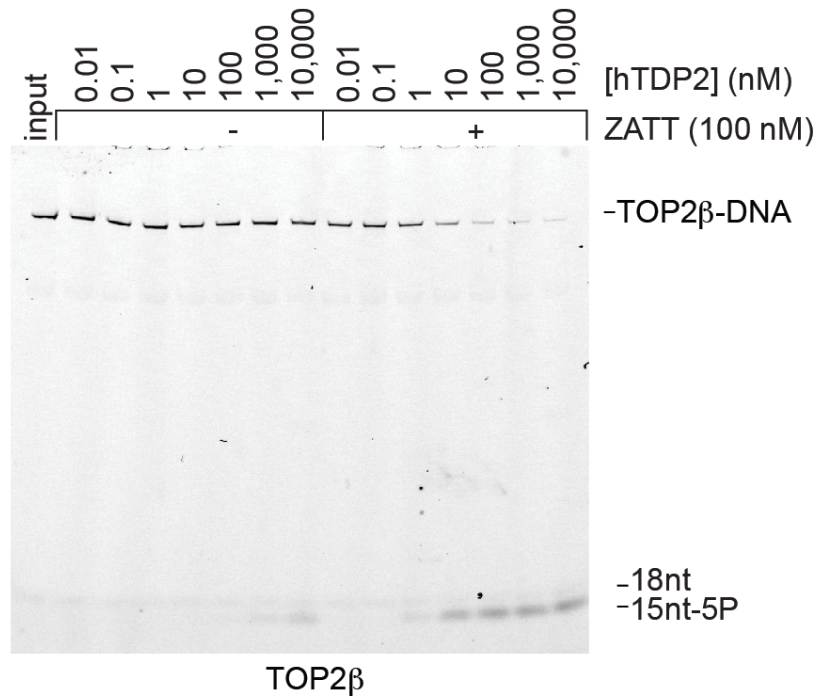
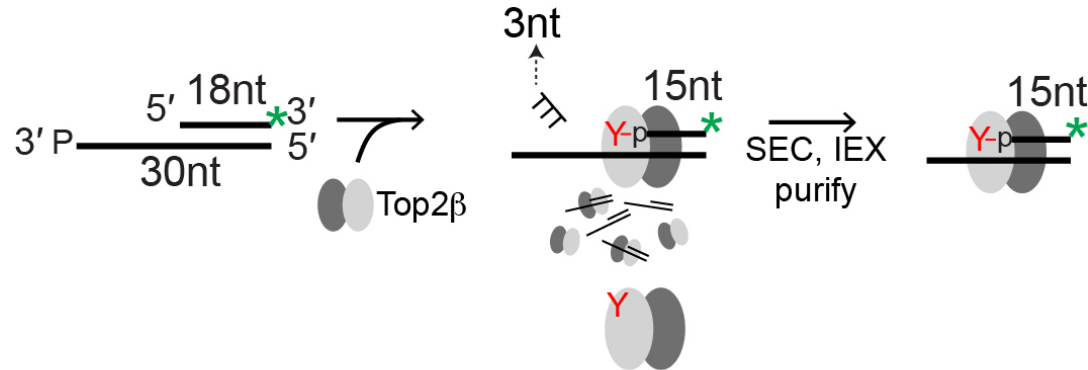


IB: β -Tubulin

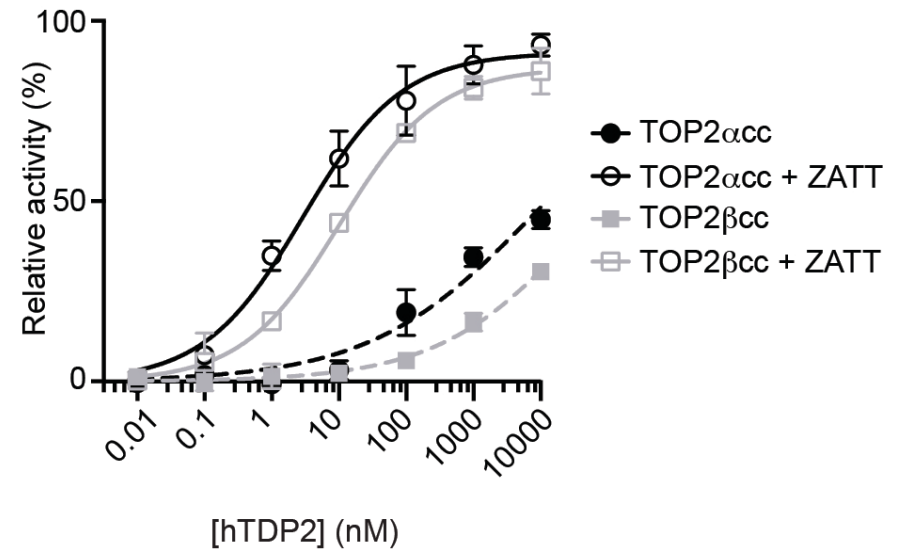
HEK293F

WT	203 ± 9 nM
Δ TDP2	71 ± 3 nM
Δ ZATT	20.1 ± 1
Δ TDP2/ Δ ZATT	11.8 ± 1 nM)

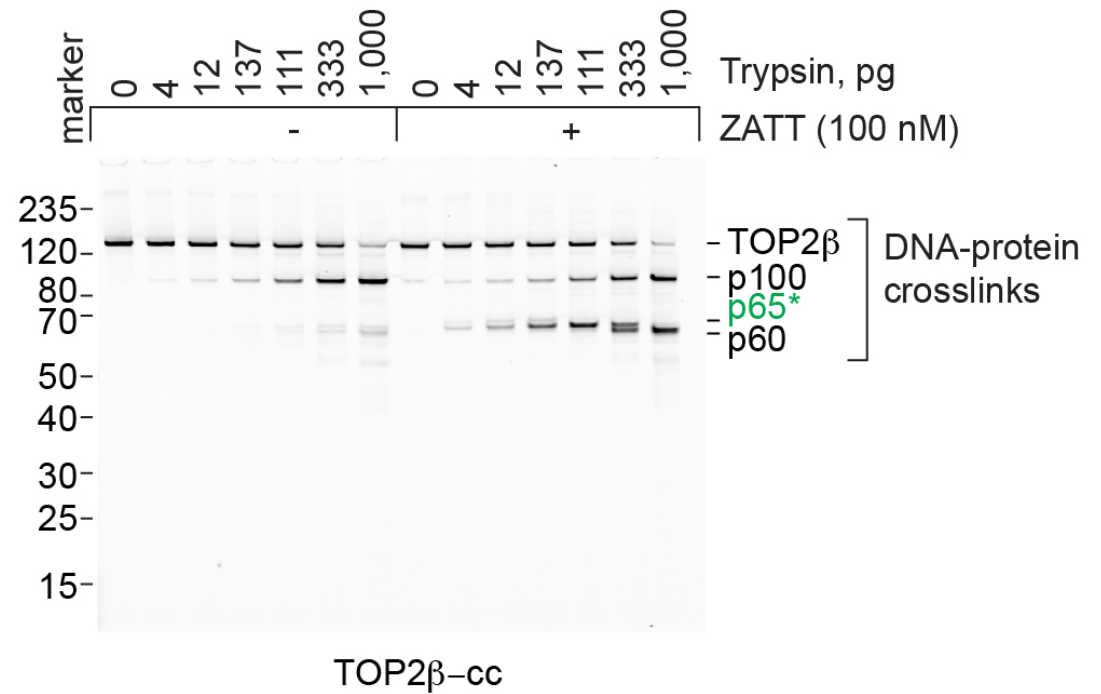
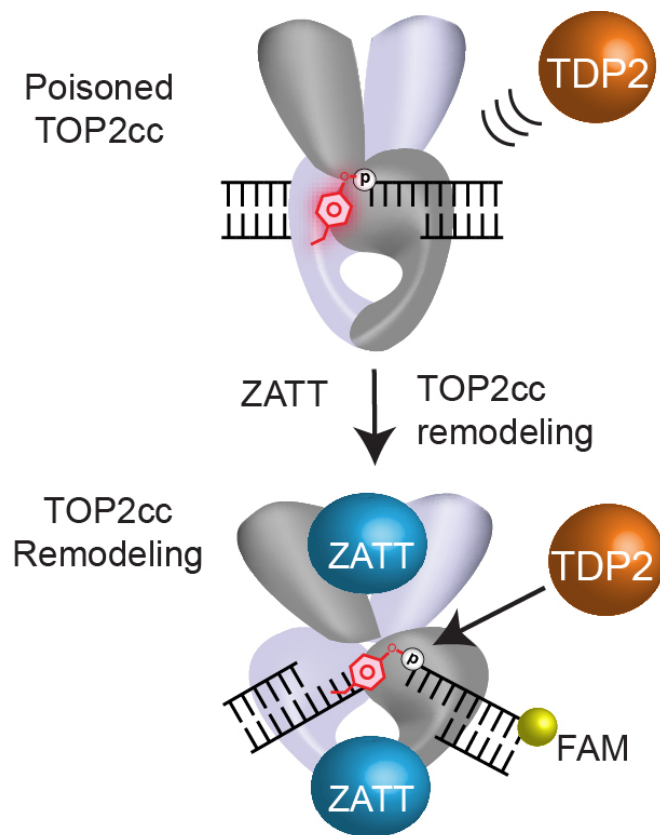
ZATT licenses Direct Reversal of Top2 DNA-protein crosslinks



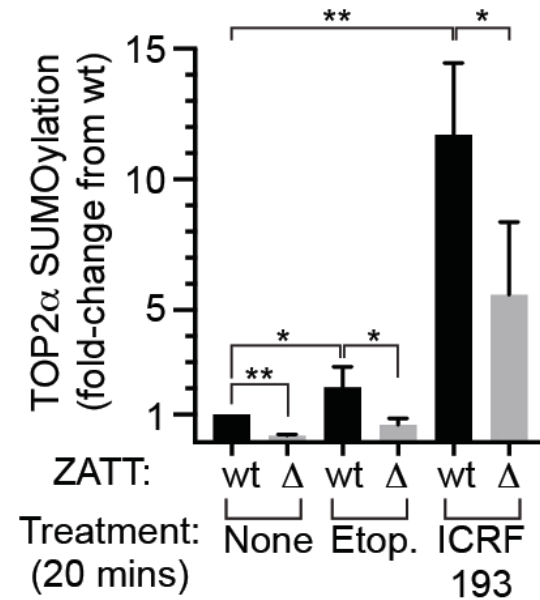
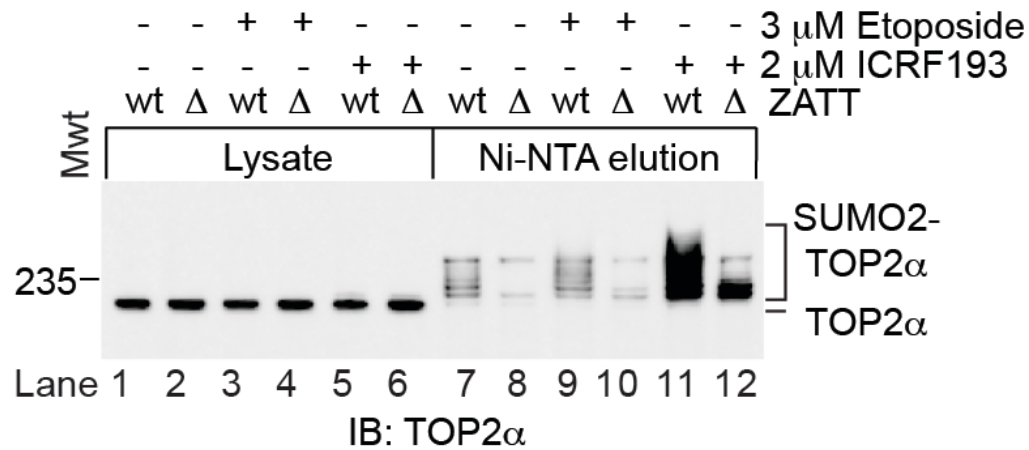
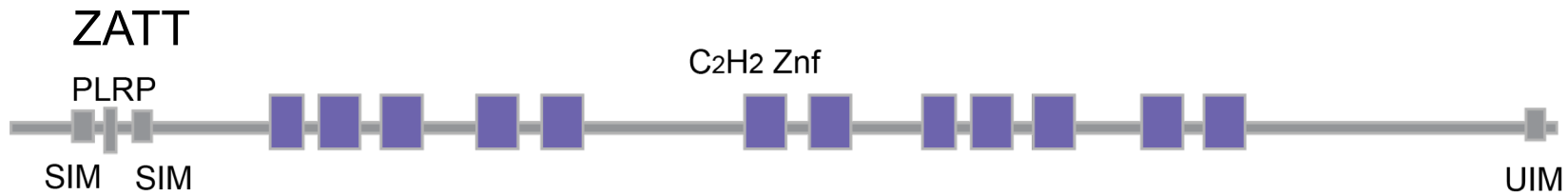
(60 sec.)



ZATT Remodels TOP2cc conformation



ZATT regulates TOP2 sumoylation



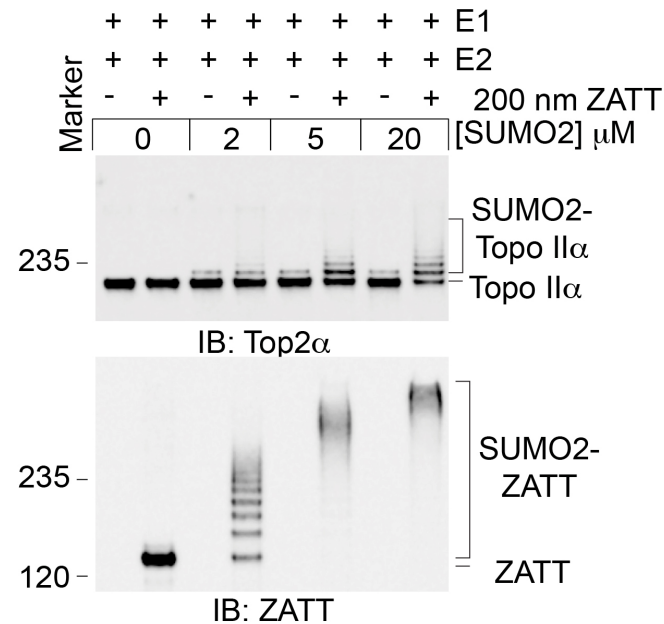
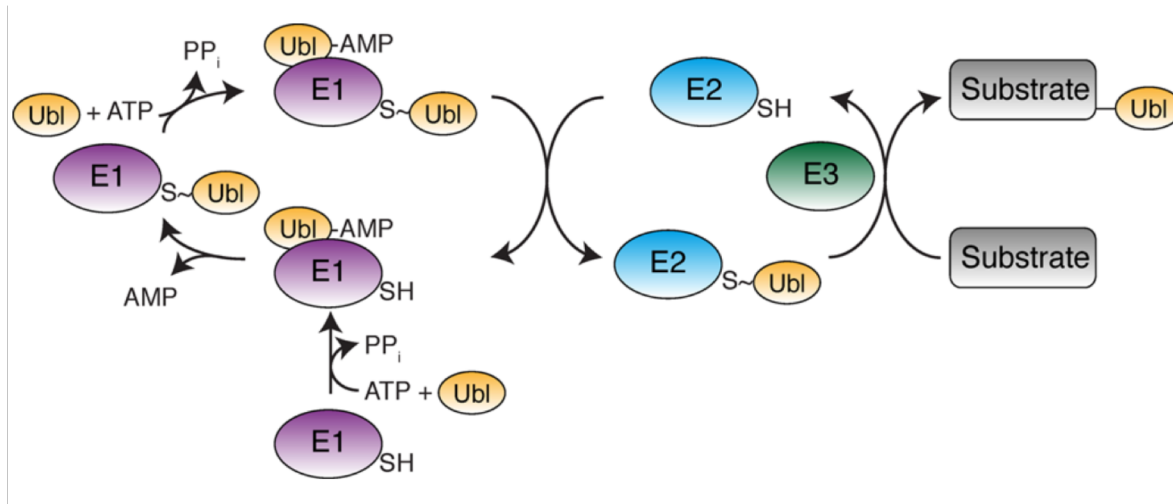
ZATT SUMOylates TOP2

SAE1/
UBA2

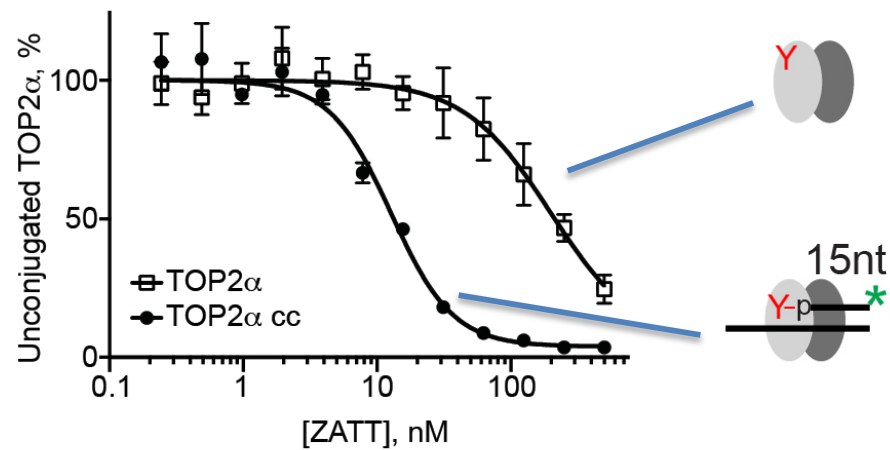
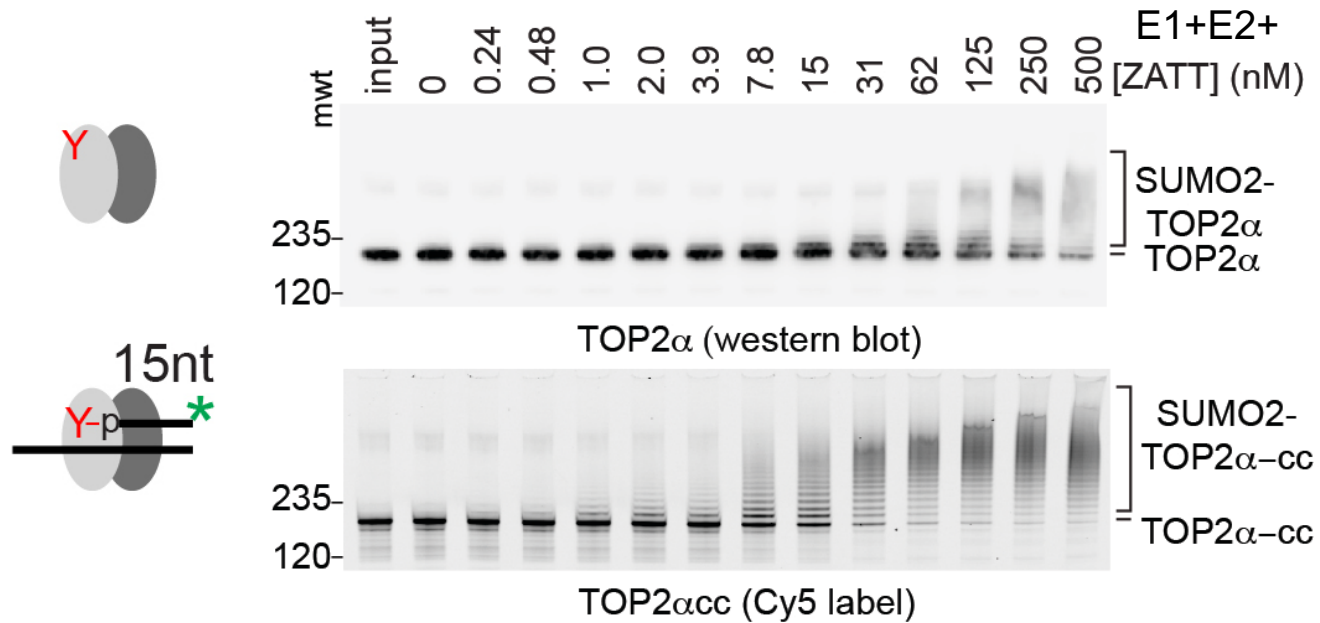
UBC9

ZATT

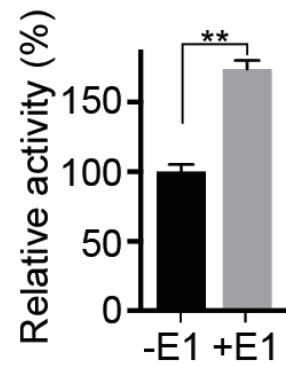
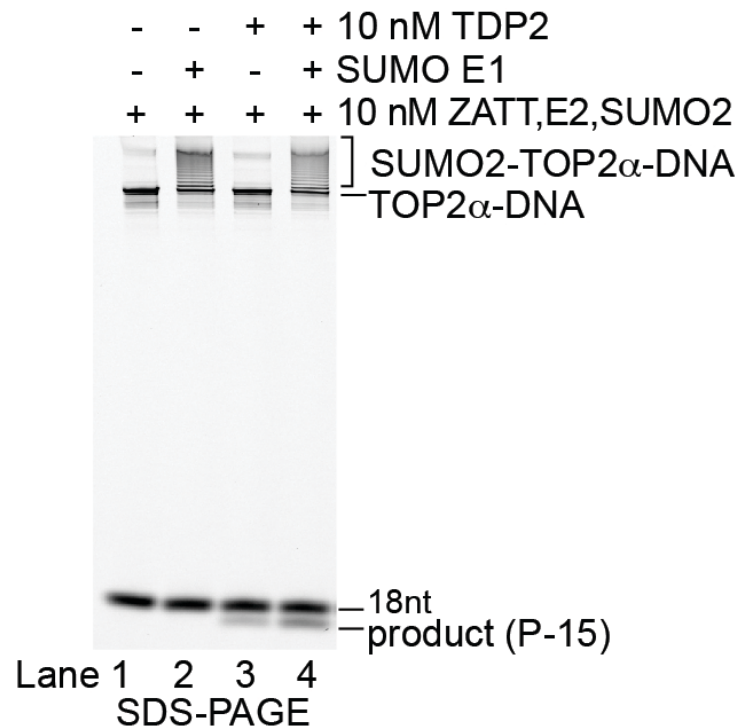
TOP2



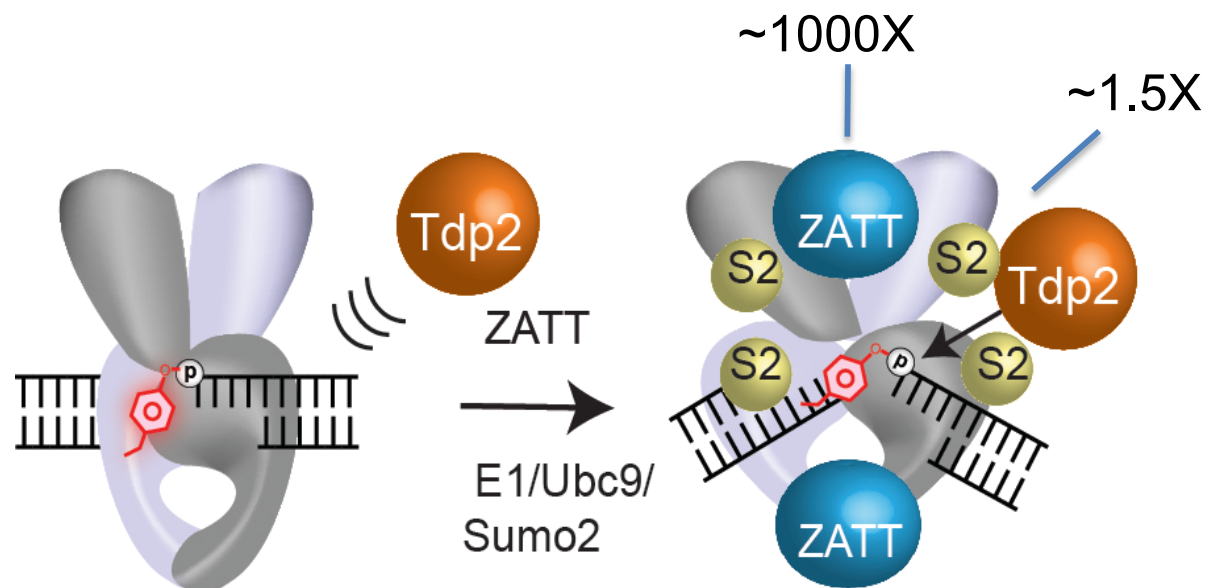
ZATT specifically SUMOylates Top2cc



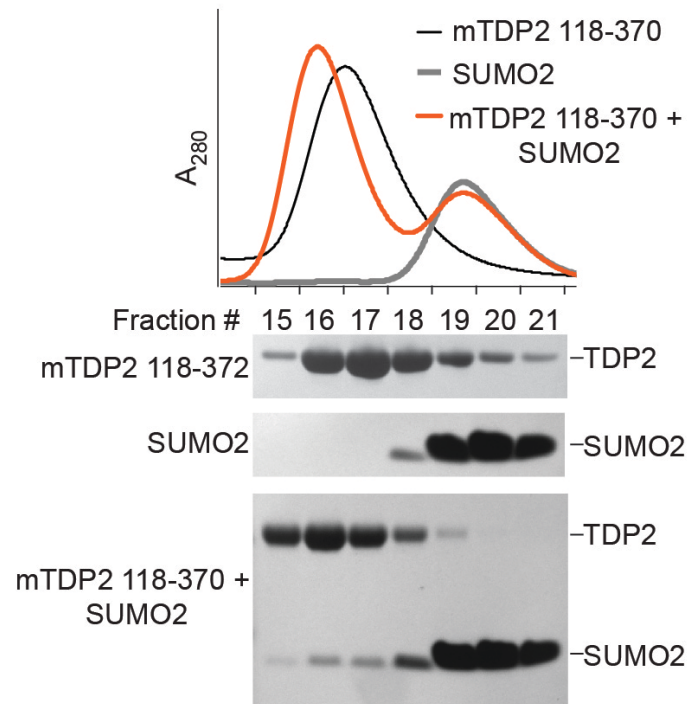
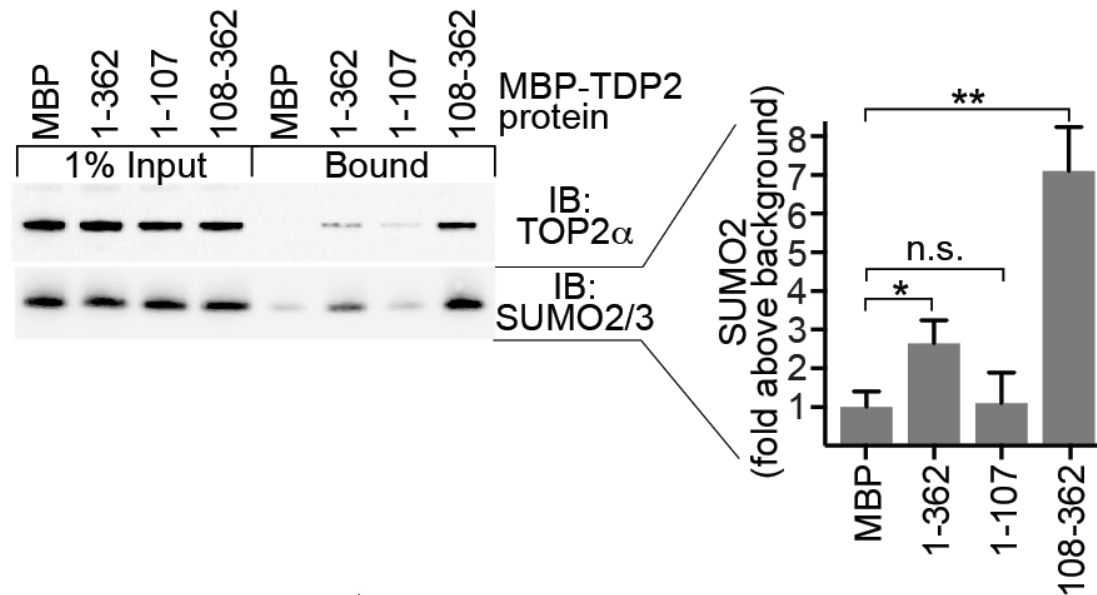
Sumoylation of Top2 and direct reversal



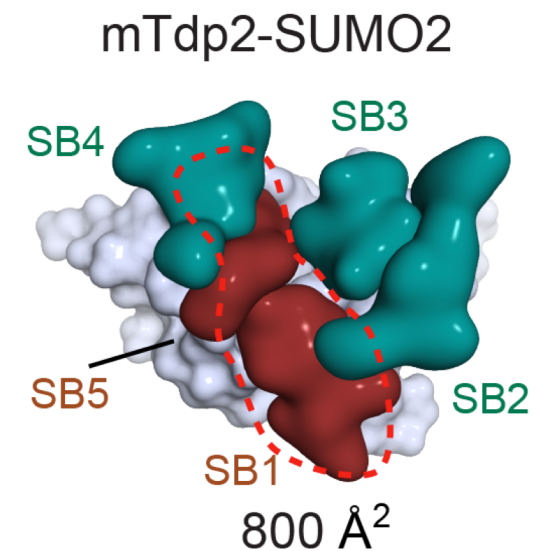
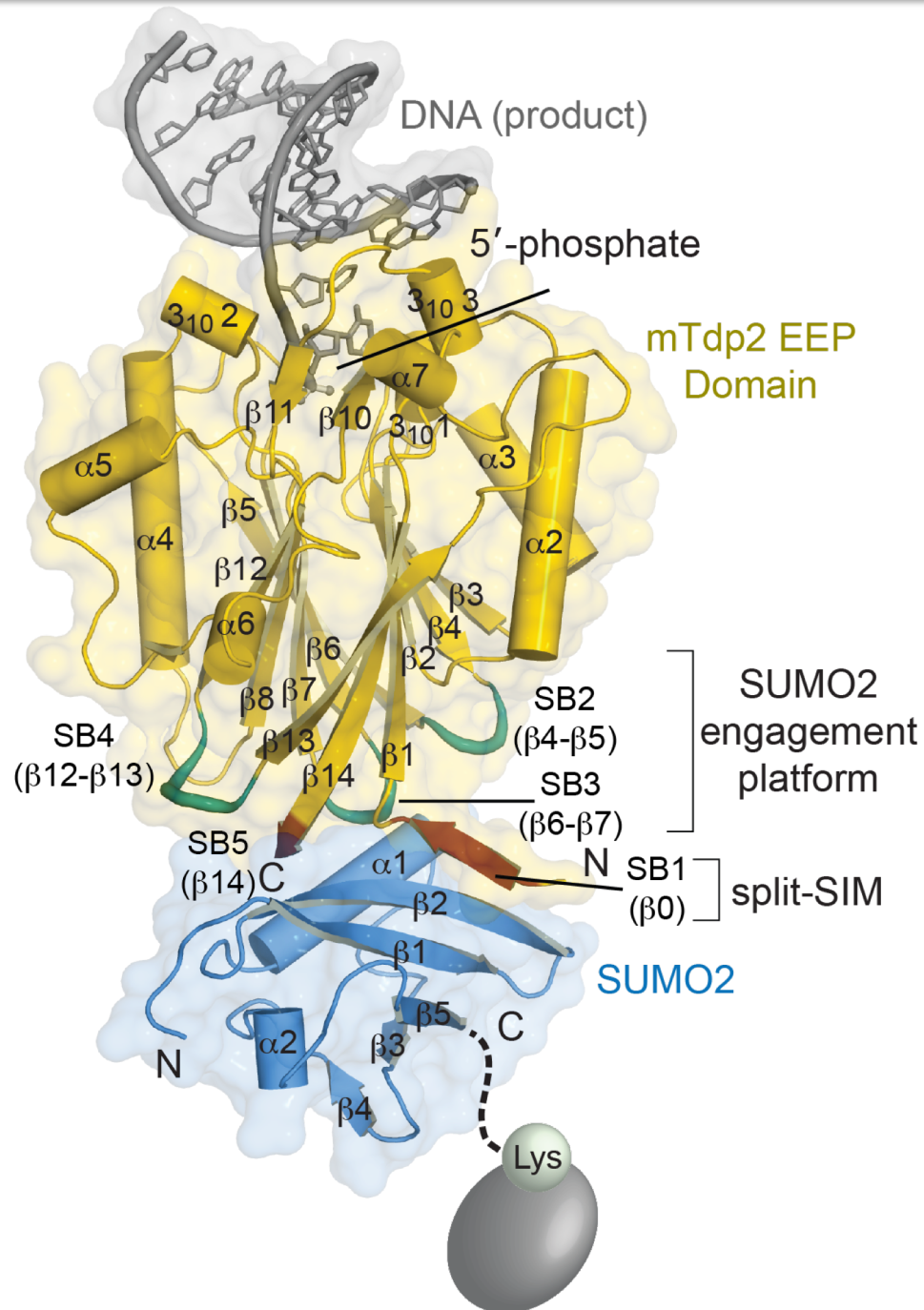
Does SUMO2 modification of TOP2 play a targeting role?



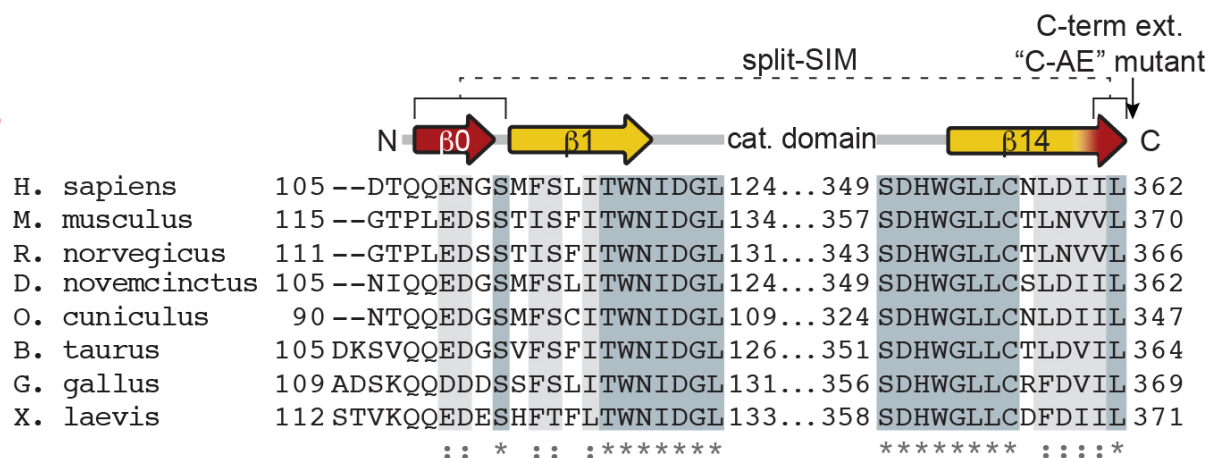
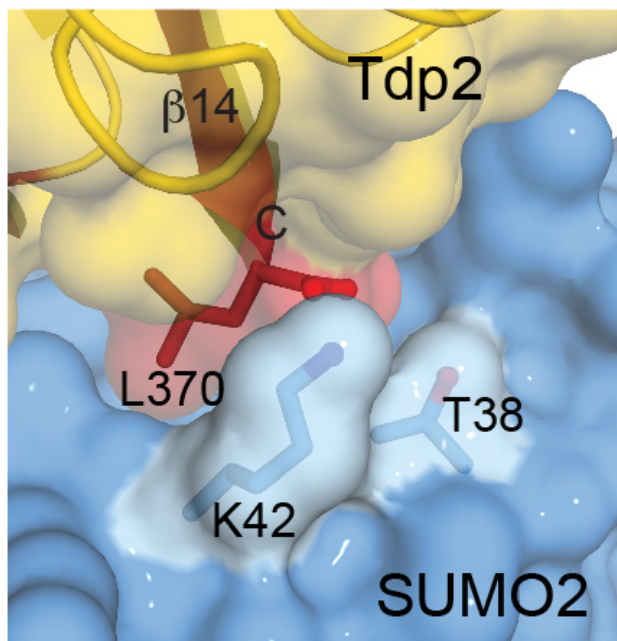
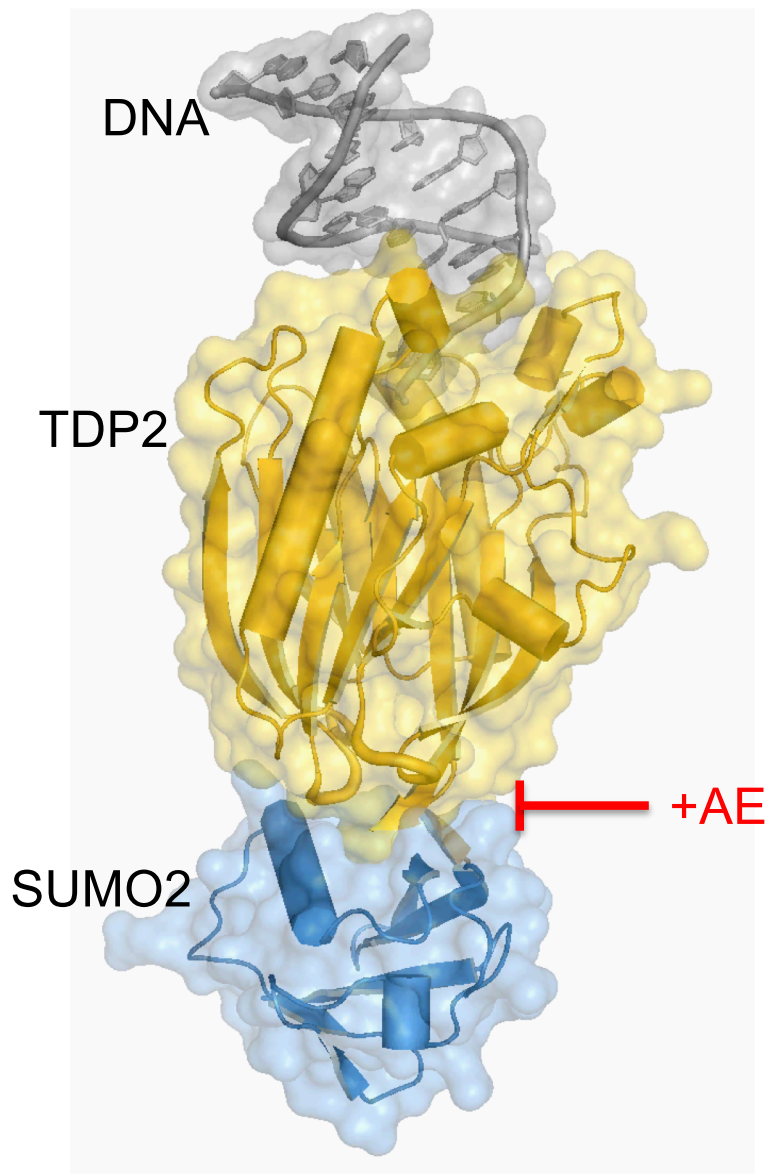
TDP2 binds TOP2 and SUMO2



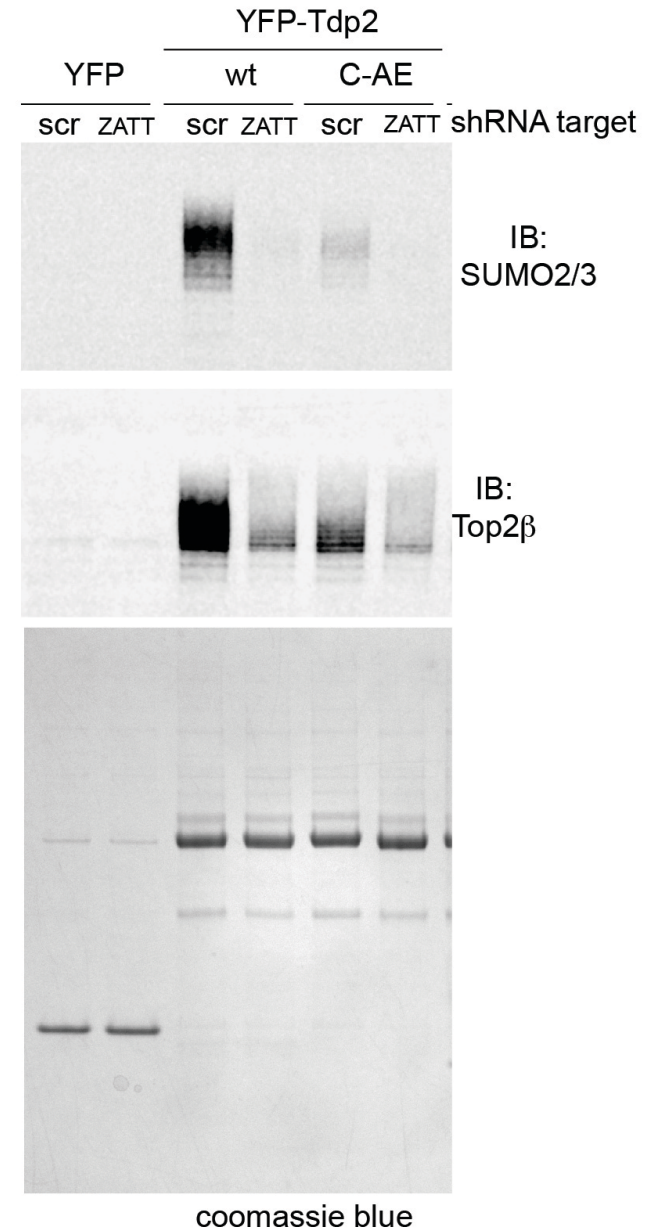
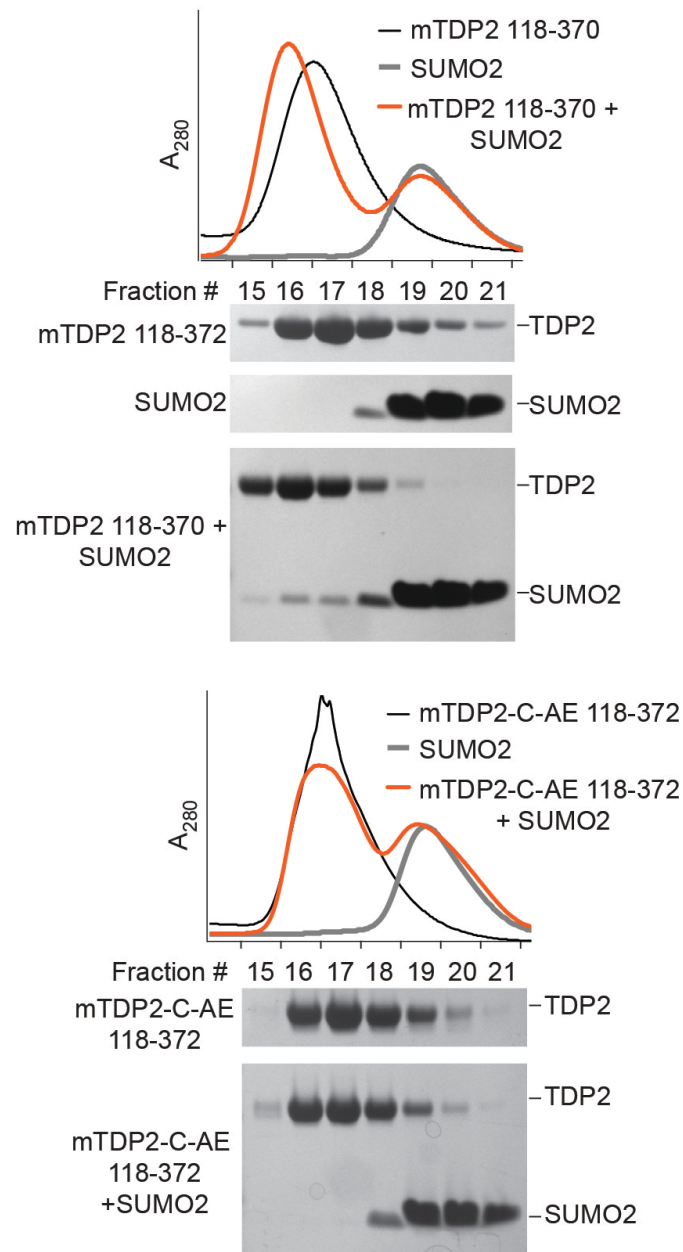
A novel Molecular mechanism for SUMO2 recognition by Tdp2



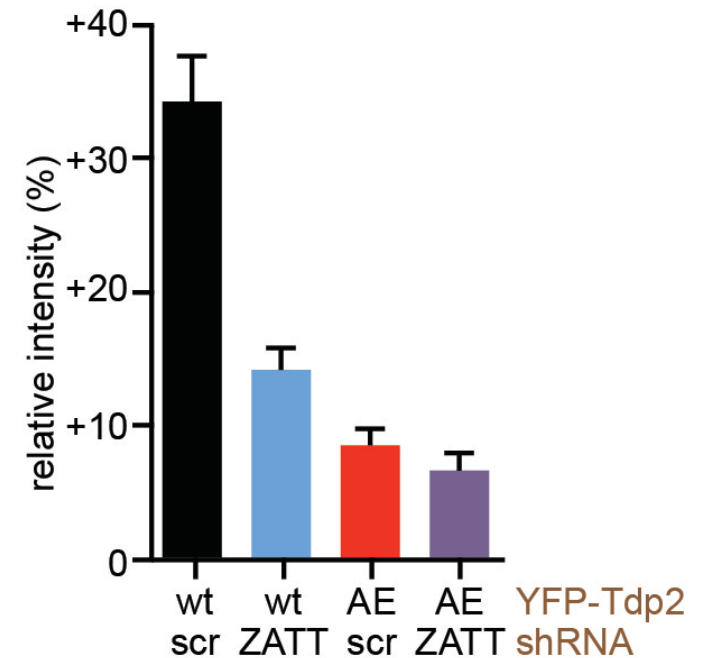
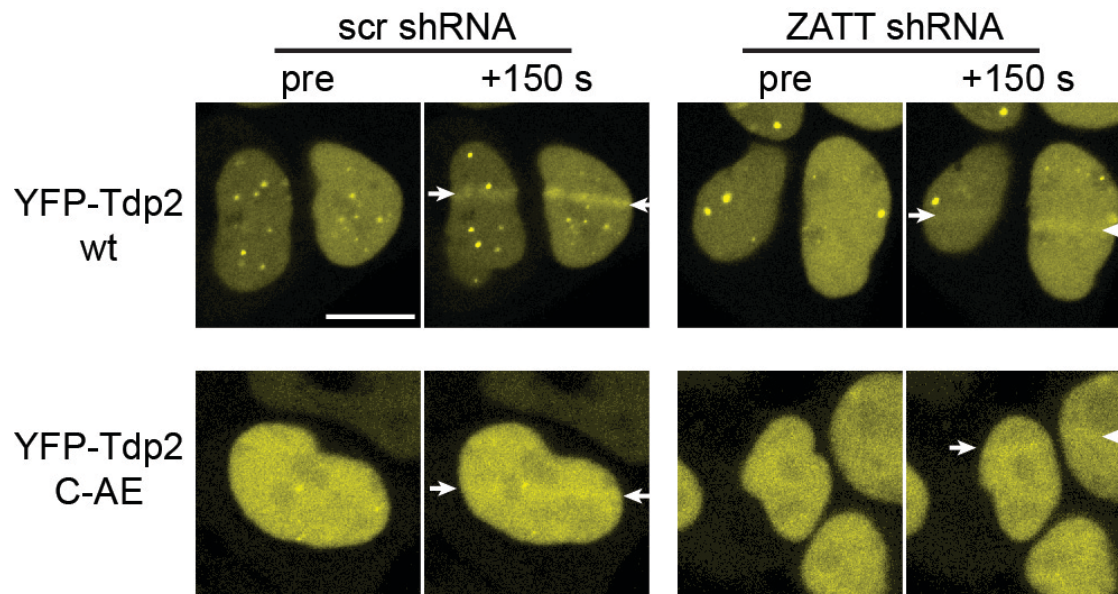
A novel mode for Sumo2 protein-protein interactions



ZATT and the Tdp2-SUMO2 interface facilitate interactions with TOP2

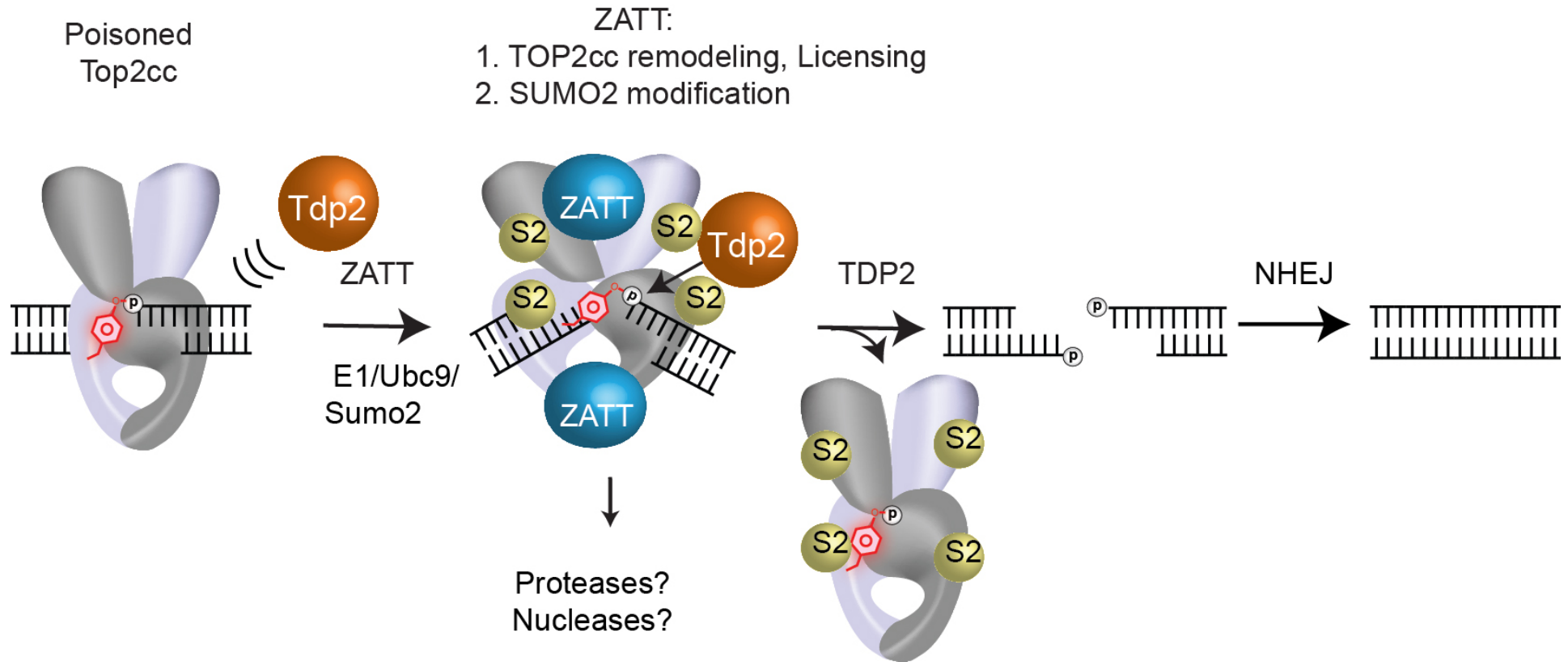


Tdp2 recruitment to DNA damage



ZATT and the Tdp2-SUMO2 binding interface are required for robust Tdp2 recruitment to UV laser induced DNA damage

Conclusions



1. TOP2cc is refractory to direct reversal
2. ZATT binds TOP2cc and remodels TOP2cc conformation
3. ZATT licenses the direct reversal activity of TDP2
4. ZATT is a SUMO2 ligase and SUMOylation of TOP2 by ZATT enforces rapid TDP2 localization to DNA damage

Acknowledgements

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Lars Pedersen

Juno Krahn

Andrea Moon

ALS- SIBYLS

APS- SERCAT

NIEHS PMCF

Jason Williams et al

NIEHS Confocal Microscopy

Jeff Tucker et al

NIEHS Viral vector core

Chip Romeo et al

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Bob Petrovich et al

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