UNIVERSITY OF CALIFORNIA SAN DIEGO

The Effect of Topoisomerase II Beta Mutations on the Transcription/Double-Strand Break Complex

A Thesis submitted in partial satisfaction of the requirements for the degree of Master of Science

in

Biology

by

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The Thesis of Michelle Nichole Peru is approved and it is acceptable in quality and form for publication on microfilm and electronically:

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DEDICATION

I would like to thank my mentor Dr. Lori Broderick for giving me a chance to work and learn from you. This experience has been unlike any other and has expanded my knowledge in so many ways, which is something that can never be taken away from me.

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LIST OF ABBREVIATIONS

ATM	mammalian ataxia telangiectasia mutated
DNA-PK	DNA-dependent protein kinase
DNA-PKcs	DNA-dependent protein kinase catalytic subunit
DNL4	yeast DNA ligase 4
dNTP	deoxyribonucleotide triphosphate
DSB	double-strand break
DTT	dithiothreitol
EDTA	ethylenediamine tetraacetic acid
HA	hemagglutinin
HR	homologous recombination
HRP	horseradish peroxidase
KAN	kanamycin
KU70	mammalian ku 70
KU80	mammalian ku 80
LiAc	lithium acetate
LIG4	mammalian DNA ligase 4
MAT	mating type
MEC1	mitosis entry check point 1
MRE11	meiotic recombination 11
NBS1	mammalian nijmegen breakage syndrome 1
NEJ1	yeast non-homologous end joining defective 1
NHEJ	non-homologous end joining
PAP	peroxidase anti-peroxidase
PCR	polymerase chain reaction
PMSF	phenylmethylsulfonyl fluoride
POL4	DNA polymerase IV
PVDF	polyvinylidene difluoride
RAD27	radiation sensitive 27
RAD50	radiation sensitive 50
RT–qPCR	real-time quantitative polymerase chain reaction
SEM	standard error of mean
TAP	tandem affinity purification
TBST	tris buffered saline with tween
TOP2	yeast topoisomerase 2
TOP2B	mammalian topoisomerase 2 beta
URA	uracil
WCE	whole cell extract
WT	wild-type
XRCC4	mammalian x-ray cross complementing 4
XRS2	yeast x-ray sensitive 2
YKU70	yeast ku 70
YKU80	yeast ku 80

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ABSTRACT OF THE THESIS

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Topoisomerase II beta-mediated immunodeficiency is a novel syndromic immunodeficiency characterized by the lack of B lymphocytes, a reduced number of antibodies in the blood, in addition to facial, limb and genital abnormalities. Four patients with this syndrome have been shown to have heterozygous mutations in the gene DNA Topoisomerase II

Beta (TOP2B). TOP2B has besen shown to be necessary for long gene transcription by creating transient DNA double-stranded breaks (DSBs) that recruit DSB repair proteins in order for transcription to continue. These DSB repair proteins participate in non-homologous end joining (NHEJ) and homologous recombination (HR) pathways. To investigate the molecular mechanism of patient-derived mutations in *TOP2B* and the DSB repair pathways, we used the model organism *Saccharomyces cerevisiae* that contains conserved DSB repair pathways in addition to the highly conserved yeast homolog TOP2. Co-immunoprecipitation analyses demonstrated that TOP2 interacts directly with YKU70, YKU80 and RAD50 and their interaction was unchanged in the presence of top2 mutants. Long gene expression in the presence of top2 mutants showed that long gene expression was significantly decreased. Our results suggest that mutations in *TOP2B* negatively affect the transcription of long genes, possibly due to the interaction with key components of the NHEJ and HR pathways.

INTRODUCTION

Overview of the Immune System

The immune system has evolved distinct lines of defense in order to protect oneself from various types of pathogens. Beyond the layer of skin and mucosal membranes which act as the mechanical and chemical barriers to pathogens, the innate and adaptive immune systems coordinate to recognize and destroy pathogens while preventing reinfection. The innate immune system works quickly by sending macrophages, neutrophils and other monocytes to the area of infection. While the adaptive immune system utilizes antigen-specific receptors on B cells and T cells to carry out an effector response (Parkin & Cohen, 2001). Any defect in these lines of defense may contribute to disease and increased susceptibility to infection and immune dysregulation.

Primary Immunodeficiencies

Primary immunodeficiencies are syndromes in which patients have frequent, often severe infections, and immune dysregulation (such as atopy, autoimmunity and increased risk of malignancy) due to a genetic defect in the immune system. Defects have been described in genes affecting various aspects of the immune system including B cells, T cells, NK cells, neutrophils and complement genes, and now encompass more than 200 different disorders (Bonilla *et al*, 2015). Such defined genetic diseases have been instrumental in our understanding of normal immune system development and function.

Syndromic immunodeficiencies are a unique subset of primary immunodeficiencies in which the physical malformations may be more pronounced than the underlying immunodeficiency, leading to delays in immunologic evaluation (Ming *et al*., 2003; Kersseboom

et al., 2011). Clearly, the genetic defects culminating in the syndromic immunodeficiency disorders must affect key cellular processes in order to impact multiple organ systems and include single gene defects, chromosomal abnormalities and metabolic disruption.

Topoisomerase II Beta-Mediated Immunodeficiency

Topoisomerase II beta-mediated immunodeficiency, formerly known as Hoffman syndrome, is a novel syndromic immunodeficiency characterized by the lack of B lymphocytes, a reduced number of antibodies in the blood, in addition to facial, limb and genital abnormalities (Hoffman *et al.*, 2001). The physical abnormalities include: microcephaly, micrognathia, sickle-shaped eyebrows, hypoplastic alae nasi, thenar hypoplasia, partial syndactyly of toes, recessed great toes, anterior anus, and hypoplastic labia minora (Hoffman *et al*., 2000; Hügle *et al*., 2010). While hypogammaglobulinemia is a common feature of many primary immunodeficiencies, the combination of this condition with conserved dysmorphic features are far less frequent (Hoffman *et al*., 2001). We have recently identified several patients with this syndrome, with heterozygous mutations in DNA topoisomerase II beta (Broderick *et al*., in revision). The patient mutations in *TOP2B* are G633S, S483L and EE587E located in the TOPRIM domain which is important for the catalytic activity of TOP2B.

Role of Topoisomerases

DNA Topoisomerases are essential enzymes that resolve topological problems in DNA during transcription, replication and chromosome segregation by creating single or double-stranded breaks (Pommier *et al*., 2016). Humans have six different types of topoisomerases: TOP1, TOP1mt, TOP2A, TOP2B, TOP3A, TOP3B which differ in their expression pattern or

mechanism of creating DSBs. For the type I topoisomerases, TOP1 and TOP1mt are exclusive to the nucleus and mitochondria respectively, while both function to relieve DNA tension in their respective cellular compartments. The type II topoisomerases TOP2A and TOP2B are similar in sequence with the exception of their C-terminal domains, which accounts for their difference in expression patterns (Gilroy & Austin, 2011). Both isoforms function to resolve DNA constraints but TOP2B is active in dividing and non-dividing cells during transcription, while TOP2A is mostly active in proliferating cells during chromosomal segregation (Haffer *et al*, 2011). In type III topoisomerases, TOP3A functions in both the nucleus and mitochondria while TOP3B resolves R loop formations during transcription elongation. The family of topoisomerases have distinct and shared roles in the essential process of DNA relaxation making five out of the six topoisomerases necessary for viability (Pommier *et al*., 2016).

TOP2B Resolves DNA Topology During Transcription

TOP2B is a homodimeric enzyme that is highly conserved at the amino acid level from *Saccharomyces cerevisiae* to humans and functions to relax supercoiled and catenated (intertwined) strands of DNA. The mechanism of TOP2B decatenation begins by creating a double-stranded break (DSB) in one strand of DNA referred to as the G (gate) segment to pass a second uncut strand of DNA called the T (transfer) segment through the break followed by re-ligation (Nitiss *et al*., 2009). The formation of DSBs followed by strand passage mechanism utilized by TOP2B makes this enzyme more efficient at relaxing positive supercoils than TOP2A which creates single-stranded DNA breaks followed by strand rotation around the uncut strand (Joshi *et al*., 2012). Although type II topoisomerases perform similar functions in different

contexts, the mechanism of DNA relaxation utilized by TOP2B gives this enzyme an important role in the transcriptional regulation of many genes.

DNA topoisomerase II beta-mediated DSBs are required to initiate transcription of long and transcriptionally active genes (Puc *et al*.,2017). It has been shown in mouse neurons that *top2b* knockdown reduces the expression of genes longer than 200 kilobases, and a TOP2B inhibitor also reduced expression of long genes in a dose-dependent fashion (King *et al*., 2013). TOP2B is also known to bind preferentially to promoter regions enabling transcription at sites of active chromatin (Tiwari *et al*., 2012). The structure of chromatin is constantly subject to changes in flexibility due to transcription and DNA replication. During transcription elongation, the structural changes of DNA generate many supercoils in front of the transcriptional machinery as well as behind. Supercoils are the over- or under-winding of DNA named positive and negative supercoiling respectively (Vos *et al*., 2011). These supercoils ultimately affect DNA topology (structure) and changes in nucleosome placement causing increased tension in segments of DNA. For instance, a positive and negative supercoil is made for every ten base pairs transcribed, which makes the transcription of relatively long genes increasingly strenuous (Puc *et al*., 2017). If these supercoils are left unresolved it could prevent the transcription of many genes and negatively impact genomic stability.

While TOP2B is necessary for genome stability, its DNA cutting activity can also be the cause of genomic instability if the DSBs go unrepaired or are repaired by the error prone non-homologous end joining (NHEJ) pathway. Therefore, the catalytic activity of TOP2B itself can also be the cause of the genomic instability that it functions to prevent since it causes DNA damage in order resolve previous DNA damage. As a precautionary measure, DNA damage

response proteins are recruited during TOP2B-mediated DSBs to ensure that the breaks are not deleterious to the stability of the genome.

Transcription/DSB Complex is Required for Gene Transcription

Although TOP2B is capable of strand passage *in vitro* on its own, TOP2B contributes to the processes of transcription and DNA repair. TOP2B has been shown to be a part of a multi-protein transcription/DSB complex where DSB repair proteins are recruited as a result of a TOP2B-mediated DSB. This protein complex includes: RNA polymerase II (Pol II), ataxia-telangiectasia mutated (ATM), Ku70 and Ku80, TOP2B and possibly other DSB repair proteins (Haffner *et al*., 2011). Many of the proteins in the transcription/DSB complex are components of NHEJ, homologous recombination (HR) and variable-diversity-joining [V(D)J] recombination DSB repair machineries. This complex facilitates transcriptional initiation of genes that require DSBs in order to relax the DNA supercoils preparing the DNA to be more accessible by transcriptional machinery (Joshi, *et al*., 2012; Zheng, 2013). The formation of incorrectly repaired DSBs may lead to deletions of gene segments or chromosomal translocations as seen in the development of many types of cancer and genetic disorders. While the formation of persistent DSBs may lead to cell senescence and apoptosis (Haffner *et al*, 2001). Therefore, illegitimate function of TOP2B or the type of DSB repair mechanism used for repair suggest a disturbance in the expression of genes that rely on the DNA relaxing activity of TOP2B. TOP2B and other factors involved in the formation and repair of DSBs in this transcription/DSB complex is not well understood.

DNA Double-Stranded Breaks are Repaired by NHEJ or HR

Non-homologous end joining (NHEJ) and homologous recombination (HR) are two mechanisms conserved throughout evolution from *S. cerevisiae* to humans in order to maintain genome stability in response to DSBs (Pastink *et al.*, 2001). NHEJ and HR pathways have been shown to compete for the repair of DSBs, and there are also many proteins that can influence either pathway (Shirvastav *et al*., 2008). The pathway used for repair varies greatly among cell type, species and phases of the cell cycle (Dudášová *et al*., 2004; Shirvastav *et al*., 2008). In particular, during variable, diversity and joining recombination lymphocytes utilize NHEJ to repair the double-stranded breaks in order to generate unique antigen receptors (Clatworthy *et al*., 2003). Subsequently, *S. cerevisiae* has been the primary model organism for studying DNA damage and repair mechanisms including NHEJ (Dudášová *et al*., 2004).

The proteins required for NHEJ in *S.cerevisiae* are similar to those in humans. The factors necessary for NHEJ in mammals include DNA-dependent protein kinase (DNA-PK), which consists of DNK-PKcs (catalytic subunit) and the KU70-KU80 heterodimer; ARTEMIS and the DNA ligase complex composed of DNA ligase IV (LIG4) and X-ray repair cross complementing 4 (XRCC4) (Dudášová *et al*., 2004). On the other hand, *S.cerevisiae* requires homologues of KU70-KU80, DNL4, in addition to the MRE11-RAD50-XRS2 (MRX) complex, non-homologous end joining defective 1 (NEJ1), DNA polymerase IV (POL4) and radiation sensitive 27 (RAD27) (Dudášová *et al*., 2004). Humans also contain structural and functional homologues of MRE11 and RAD50 of the MRX complex, but instead have functional homologue of XRS2 referred to NBS1 (Dudášová *et al*., 2004). The process of NHEJ begins with two KU70-K80 heterodimers binding to both strands of DNA in order to keep the strands in close proximity allowing DNA-PK to align the strands to make them more accessible. KU70 and

KU80 are highly abundant and are capable of binding to DNA ends within five seconds from the formation of a double-stranded break (Fell *et al*., 2015). The MRE11-RAD50-XRS2 (MRX) complex (MRN complex in humans) also plays an important a role in the recognition and processing of DSBs (Dudášová *et al*., 2004). ARTEMIS directs DNA end processing, leaving the gaps to be filled by DNA polymerase μ or λ which both function to resynthesize damaged or missing nucleotides. The final step in repairing the broken DNA is ligation by the DNA ligase IV/XRCC4 complex.

DNA Repair Mechanisms and Disease

Mutations in the transcription factors involved in DNA repair mechanisms such as NHEJ and HR have been shown to result in various diseases, including primary immunodeficiencies. For example, patients who have a mutation in *NBS1* of the MRN complex are known to have Nijmegen breakage syndrome with symptoms such as: microcephaly, increased susceptibility to tumor growth, and chromosomal instability (Lieber *et al*., 2003). In addition, one cause of severe combined immunodeficiency is caused by a mutation in the gene encoding for ARTEMIS, leaving patients extremely susceptible to infections due to their weakened immune systems (Lieber *et al*., 2003). In order to learn more about the mechanisms behind topoisomerase II beta-mediated immunodeficiency, we examined the effects of patient-identified *TOP2B* mutations on the transcription/DSB complex using the yeast model organism *S. cerevisiae*.

Aims of Research

The TOP2B mutations discovered in patients with topoisomerase II beta-mediated immunodeficiency have been shown to be non-functional in *S. cerevisiae* including slower

growth, an inability to sporulate and failure to decatenate (Broderick *et al*., in revision). Further work in *TOP2B* knockin- and knockout murine models, has shown that the patient-based *TOP2B* mutations have a dominant negative phenotype resulting in reduced B cell development, with the appearance of increased double- and single-stranded DNA breaks (Broderick *et al*., in revision). The molecular mechanism underlying these observations remains unclear.

We hypothesize that mutations in DNA topoisomerase II beta affect protein interactions in the transcription/DSB complex and/or their expression levels contributing to the immune phenotype of Hoffman syndrome. We will address this hypothesis through investigating the expression levels of DSB complex genes, the direct interactions of TOP2 with the DSB complex proteins and the effect on long gene transcription in the presence of top2 mutants.

MATERIALS AND METHODS

Yeast strains and Plasmids

S. cerevisiae strains used in this study are listed in Table 1. C-terminal HA and TAP tags were generated using pRS 314 3xHA KAN and pRS 314 TAP URA plasmids (listed in Table 2) as templates to generate Longtine cassettes (Longtine *et al*., 1998). PCR generation of Longtine cassettes for each gene was done in 100 µl reactions containing 1X PCR Buffer (Applied Biosystems), 1.5 mM $MgCl_2$, 50 µM dNTP's, 1 µM of each primer (listed in Table 3), 100 ng template, and 1 µl of Taq DNA polymerase. Reactions were run for 1 cycle of 5 min at 94˚C, 2 min at 55˚C, 5 min at 30˚C, 2:30 min at 72˚C; followed by 32 cycles of 1 min at 94˚C, 1 min at 55˚C (or appropriate annealing temperature), 3 min at 72˚C; followed by a 7 min extension at 72˚C. Longtine cassettes were then transformed into haploid strains of yeast using a modified LiAc method to be integrated into the genome by homologous recombination (Ito *et al*., 1983). PCR screening of transformants was done in 10 µl reactions containing 1X PCR Buffer, 1.5 mM $MgCl_2$, 50 µM dNTP's, 1 µM of each primer (listed in Table 3), 100 ng template, and 0.1 µl of Taq DNA polymerase. Reactions were run for 1 cycle of 5 min at 94˚C; 30 cycles of 1 min at 94˚C, 1 min at 55˚C, 3 min at 55˚C (or appropriate annealing temperature), 2:30 min at 72˚C; followed by a 7 min extension at 72˚C. PCR reactions were run on 1% agarose gels and imaged using an AlphaImager® (ProteinSimple).

RNA Isolation

S. cerevisiae strains that have a G418-based deletion of genomic TOP2 while carrying wild-type and mutant TOP2 plasmids cells were grown to 15 OD, pelleted and used in the

RNeasy Mini Kit (Qiagen) to isolate total RNA per the manufacturer's instructions. The RNA concentration was determined by NanoDrop™ (Thermo Scientific ND-1000).

RT-qPCR

A 25ng/µl stock of RNA was used with TaqMan Reverse Transcription Reagents per the manufactures instructions to generate cDNA (Life Technologies). The PrimerQuest tool from https://idtdna.com was used to design primers for a SYBR Green system. The samples were run in triplicate for at least a total of three biologic replicates on a BioRad CFX96 Real Time System. Microsoft Excel and GraphPad PRISM were used to graph and statistically analyze data. Relative gene expression was determined by the $2^{-\Delta\Delta Ct}$ method.

Co-Immunoprecipitation

S. cerevisiae strains with genomic TOP2 tagged with three consecutive HA tags and the one double strand break protein tagged with TAP carrying a wild-type, empty vector or mutant TOP2 were grown to 30 O.D. in selection media, and cell pellets were resuspended in 450µl of lysis buffer (50mM Tris pH 8, 50mM NaCl, 0.1% nonidet P-40 (NP-40), 5mM EDTA, 10% glycerol, 0.4mM phenylmethylsulfonyl fluoride (PMSF)). An equivalent volume of 0.5mm diameter of glass beads (BioSpec 11079105) were added to the samples and shaken at 4˚C 3 times for 2 minutes each with a 1-minute rest in between to cool down samples between incubations. Samples were then spun at 13,000 rpm for 10 mins at 4˚C, and the supernatant was used for subsequent steps. 30 µl samples of the supernatant, referred to as whole cell extract, were reserved for a Bradford Assay (Pierce) to asses protein concentration and for SDS-PAGE. For the remaining whole cell extract, lysis buffer was added to bring the whole cell extract to a

final volume of 1 ml and incubated with 30-40µl of anti-HA magnetic beads (Pierce TE263229) for 2-24 hours on a rotator at 4˚C. After incubation, samples were rinsed three times with 1ml of lysis buffer and spun at 2000 rpm for 2 mins at 4˚C. After the last wash, the beads were resuspended in 20µl of 2X Laemmli sample buffer (Bio-RAD) with 50 mM of DTT added fresh.

Western Blot

Whole cell extracts (5 µg) and boiled bead samples were run on a 4-15% gradient gel (BioRad Mini-PROTEAN TGX Precast Gel) and wet transferred onto a polyvinylidene difluoride (PVDF) membrane (BioRad) then blocked in 10% milk with TBST for 1 hour. Following milk block, membranes were incubated with anti-HA primary antibody (Roche, used at 1:2000 dilution in 5% milk with TBST) and goat anti-mouse IgG HRP conjugated secondary antibody (Thermo Scientific OE185739, used at 1:5000 dilution in 5% milk with TBST) for detection of TOP2-3xHA. Anti-Peroxidase Anti-Peroxidase (PAP) HRP conjugated primary antibody (Sigma Aldrich 103M4822, used at 1:2000 dilution in 5% milk with TBST) was used for detection of MRE11-TAP, DNL4-TAP, RAD50-TAP, XRS2-TAP, YKU70-TAP, YKU80-TAP, and MEC1-TAP. Membranes were exposed using 1:10 or 1:20 dilution of SuperSignal™ West Femto (Thermo Scientific TE265689) in 1X TBS. Membranes were stained for total protein with amido black (10% acetic acid, 45% ethanol).

Statistical Analyses

Data are represented as means and standard error of mean (SEM) and comparisons between values were made using a student's *t* test. A p value less than 0.05 was considered

statistically significant. Statistics and graphing were performed using Microsoft Excel (Version 16.17) and GraphPad Prism (Version 5.03; Graph Pad Software Inc., CA).

RESULTS

DSB complex gene and protein expression are unchanged in the presence of top2 mutants

Programmed DNA double-stranded breaks by TOP2B followed by DSB repair have been shown to occur as a means of regulating transcription (Puc *et al*., 2017). To determine the effects of top2 mutants on the expression of the DSB complex genes of interest, we measured the expression levels of each of the complex genes by RTq-PCR (Figure 1). We observed that the strains heterozygous for mutations in top2 showed no significant differences in gene expression of *MRE11*, *RAD50*, *XRS2*, *MEC1*, *DNL4*, *YKU70* and *YKU80*. These results suggest that mutations in TOP2 do not affect the transcription of these DSB complex genes.

Since there were no changes in gene expression, we tagged each of the DSB complex genes to analyze their protein expression in the presence of top2 mutants (Figure 2). We performed an anti-HA and anti-TAP Western blot on haploid *S. cerevisiae* strains that contained genomic TOP2-HA and one DSB complex gene tagged with TAP that was transformed with a WT or mutant top2 plasmid. XRS2 and MEC1 were not successfully tagged indicating that the tags may be affecting the stability of the protein or the formation of the DSB complex.

WT TOP2 interacts directly with YKU70, YKU80, RAD50 and not with DNL4 or MRE11

In multi-protein complexes, determining which proteins are interacting directly can elucidate which proteins in a complex are likely to affect another. To first determine which proteins are most likely to bind to TOP2, we used WT *S. cerevisiae* strains to perform an anti-HA co-immunoprecipitation followed by an anti-HA and anti-TAP Western blot (Figure 3). The WT strains contained genomic WT TOP2 tagged with three consecutive HA tags, one of the DSB complex genes tagged with TAP that was transformed with an untagged WT TOP2

plasmid. We observed that YKU70, YKU80 and RAD50 were present in the anti-HA bead samples that captured TOP2-HA suggesting that TOP2 binds directly to YKU70, YKU80 and RAD50. In contrast, the DNL4-TAP strain showed a faint band was seen that was at a higher molecular weight than the expected size of 128 kDa that was seen in the whole cell extract (Supplemental Figure 1). Therefore, DNL4 was not present in the anti-HA beads suggesting that DNL4 and TOP2-HA do not interact directly (Figure 3). In probing for MRE11, MRE11-TAP was not present in the anti-HA beads suggesting that MRE11 and TOP2-HA do not interact directly (data not shown). We observed that both MRE11 and DNL4 are not present in the anti-HA beads suggesting that TOP2 does not interact directly with MRE11 or DNL4.

Saccharomyces Genome and STRING databases have shown that TOP2 and the DSB complex proteins have been predicted or experimentally determined to associate together in humans and *S. cerevisiae* (Supplemental Figure 2). Experimentally determined associations do not correspond to direct protein-protein interactions between TOP2 and the DSB complex proteins. In addition, the level of confidence in the experimental determinations between TOP2 and the DSB complex proteins are low in humans and *S. cerevisiae* (Supplemental Figure 3).

Given the importance of this complex formation in response to DNA damage, and the essentiality of many of the complex components, we verified that the strains were not negatively affected by the presence of tags through the evaluation of growth and assessment of degradation products. While we did not observe growth differences between the tagged strains (data not shown), we observed that RAD50 (Supplemental Figure 4) and DNL4 (Supplemental Figure 5) tagged strains were shown to have reduced levels of full size TOP2-HA at 168 kDa compared to similar strains. The degradation product of TOP2-HA at 50 kDa suggests that though the

combination of these tags may affect TOP2 stability, the ability of the strains to grow normally and form the DSB complex were unaffected.

WT TOP2 is still able to bind directly to YKU70, YKU80, RAD50 in the presence of top2 mutants

After identifying the direct interactions between WT TOP2 and each of the DSB complex proteins, we examined their interaction in the presence of top2 mutants. We performed an anti-HA co-immunoprecipitation followed by anti-HA and anti-TAP western blot on haploid *S. cerevisiae* strains that contained genomic TOP2-HA and one DSB complex gene tagged with TAP that was transformed with a WT or mutant top2 plasmid. In YKU70 tagged strains, WT TOP2-HA is captured by the anti-HA beads and present at its expected size of 168 kDa while YKU70-TAP is also present in the anti-HA bead sample at its expected size of 90 kDa (Figure 4). Both WT TOP2-HA and YK70-TAP are present in the whole cell extract at their expected sizes (Supplemental Figure 6). In YKU80 tagged strains, both WT TOP2-HA and YKU80-TAP are present in the anti-HA bead samples at 168 kDa and 91 kDa respectively (Figure 5). Both WT TOP2-HA and YK80-TAP are present in the whole cell extract at their expected sizes (Supplemental Figure 7). RAD50-TAP tagged strains show that both WT TOP2-HA and RAD50-TAP are present in the anti-HA bead samples at 168 kDa and 173 kDa (Figure 6). The presence of YKU70, YKU80 RAD50 in the anti-HA bead samples that captured WT TOP2-HA suggests that the top2 mutations do not affect the ability of WT TOP2 from recruiting these proteins.

While MRE11 and DNL4 were not shown to bind directly to TOP2-HA in WT strains, we also examined their interaction in the presence of top2 mutants. In MRE11-TAP tagged

strains, WT TOP2-HA was captured by the anti-HA beads and was present at the expected size of 168 kDa while MRE11-TAP was not detected in the presence of any top2 mutants (Figure 7) and only seen in the whole cell extract (Supplemental Figure 8). In DNL4-TAP tagged strains, only WT TOP2-HA was detected in the anti-HA bead samples at 168 kDa while there were no bands for DNL4-TAP at the expected size of 128 kDa (Figure 8). The lack of direct interaction between TOP2-HA and MRE11 or DNL4-TAP in WT strains was also observed in the presence of top2 mutants suggesting that top2 mutations do not cause these two proteins to bind directly to one another.

Expression of long genes of SEC7 and GCN1 are significantly decreased in the presence of top2 mutants while the expression of short gene CUP1-2 is unchanged

Transcription of long genes greater than 3 kb has been shown to require topoisomerase II relaxation activity to prevent a stall in transcription as the DNA strands accumulate tension during their unwinding (Joshi *et al.*, 2012; King *et al*., 2013). Long B cell transcription factors are also significantly reduced in myeloid cell knockout of TOP2B and heterozygous Top2b+/EE587E mice (Broderick *et al*., in revision). To investigate whether the expression of long genes is affected in TOP2 mutant yeast strains, we measured the gene expression levels of long genes *SEC7* (6029 bp) and *GCN1* (8018 bp) in comparison to a short gene *CUP1-2* (185 bp) (Figure 9). The S483L and EE587E mutant strains showed significantly decreased gene expression of both long genes *SEC7* and *GCN1*, while showing no significant differences in gene expression of the short gene *CUP1-2*. These results suggest that mutations in *TOP2* negatively affect the transcription of long genes

DISCUSSION

The identification of genes that contribute to the onset of disease such as primary immunodeficiencies has led to an increased understanding of the immune system and the onset of disease if processes do not function properly. Since the genes found to be mutated in most primary immunodeficiencies are immune cells, it is unprecedented that *TOP2B* has been shown to be a common gene found in the patients with Hoffman syndrome. DNA double-stranded breaks are necessary for various types of cellular processes including transcription, replication, and in the generation of unique B-cell and T cell receptors. Therefore, a disruption in the process of DSB formation and repair can lead to disease. Hoffman syndrome patients characterized by absent B cell counts, reduced immunoglobulin levels with dysmorphic features has been attributed to differing mutations in the TOPRIM domain of the gene DNA topoisomerase II beta (Broderick *et al*., in revision). The function of topoisomerases is widely studied, but little is known about the molecular mechanism of Hoffman syndrome and how the *TOP2B* mutations contribute to the disease phenotype of this immunodeficiency.

TOP2B is important in maintaining genomic stability by relieving tension in DNA during transcription and does so by creating double-stranded breaks. TOP2B has also been implicated in creating double-stranded breaks to induce transcription of certain genes, which are first repaired by the recruited of DSB repair machinery (Haffner *et al*., 2011). The mRNA expression levels of *MRE11*, *RAD50*, *XRS2*, *MEC1*, *DNL4*, *YKU70* and *YKU80* were measured to determine if this process of DNA repair was being altered in the presence of top2 mutations. There were no significant changes, neither increased nor decreased, in gene expression in all DSB complex genes measured. Since these DSB repair genes are not exceptionally long genes ranging from 1.8 to 2 kb, and are constant through the cell cycle, we expected that the expression of these genes

would not be affected (Cherry *et al*., 2012). The protein expression of the DSB repair machinery is also not affected by the presence of top2 mutants suggesting that top2 mutations do not affect DSB repair transcription or their recruitment to fix DSBs mediated by TOP2.

By co-immunoprecipitation analyses, WT TOP2 was found to interact directly with RAD50, YKU70 and YKU80. RAD50 and YKU70 have not been previously shown to bind directly to TOP2, while YKU80 has not been shown to bind directly to TOP2 by co-immunoprecipitation in *S. cerevisiae* (Szklarczyk *et al*., 2017; Cherry *et al*., 2012). Our data confirms similar results found in transfected HeLa cell overexpression systems showing that TOP2B and KU80 interact directly (Matheos *et al*., 2002). Conversely, WT TOP2 did not interact directly with DNL4 and MRE11 which lead us to focus on the interaction of TOP2 with RAD50, YKU70 and YKU80 in *S. cerevisiae* strains in the presence of Hoffman syndrome mutants. These results are consistent with previous literature as MRE11 and DNL4 has not been shown to bind directly to TOP2. Specifically, MRE11 has been suggested to remove TOP2-DNA covalent complexes in humans and *S. cerevisiae* but has not been shown to interact directly, as MRE11 also does not show experimental evidence for their direct interaction (Lee at al., 2012; Szklarczyk *et al*., 2017; Cherry *et al*., 2012). Additionally, DNL4 and TOP2 have not shown to interact directly but the human homolog LIG4 and TOP2B may be associated due to co-expression (Szklarczyk *et al*., 2017). Further co-immunoprecipitation analyses revealed that the WT TOP2 was still able to recruit DSB repair proteins RAD50, YKU70 and YKU80 in the presence of all top2 mutants. This would suggest that DSB repair is still occurring in strains with top2 mutants, however it remains unclear how efficient this process is.

Since TOP2 decatenation activity is required for the expression of long genes, measuring the mRNA expression levels of long genes in *S. cerevisiae* strains can provide insight into the

ability of TOP2 to resolve topological constraints during transcription in the presence of top2 mutants. The average gene length in *S. cerevisiae* is about 1.6 kilobases in length (Lewin 2008). From gene expression analysis, long gene *GCN1* (8018 bp) mRNA expression levels are significantly decreased in the presence of all three top2 mutants when compared to WT TOP2 strains. *SEC7* (6029 bp) mRNA expression levels were also significantly decreased in S483L and EE587E mutant strains compared to WT strains, while the G633S strain showed no significant differences. Conversely, the mRNA expression levels of short gene *CUP1-2* (185 bp) was unchanged. The significant decrease in long gene expression supports our hypothesis that disease -associated mutations alter the efficiency of TOP2 to relax DNA topology during the transcription of long genes.

We recognize several limitations of our study. The yeast strains generated did not contain tagged top2 mutants that could be directly isolated from immunoprecipitation. Utilizing diploid yeast strains that contain a tagged WT TOP2 on one chromosome and a tagged mutant top2 on the other chromosome from each of the three patient-derived mutations in addition to tagged DSB genes would be ideal to study. Examining the direct interactions between mutant top2 and the DSB complex proteins may provide more insight into the effect of Hoffman syndrome mutations on DSB repair and the transcription of long genes. Two of the eight complex proteins, namely XRS2 and MEC1, were not effectively tagged to examine by co-immunoprecipitation suggesting that these tags may be affecting the stability of the protein or the protein complex as a whole, making the yeast strains nonviable.

Our work suggests that mutations in *TOP2B* negatively affect the transcription of long genes, possibly due to the interaction with key components of the NHEJ and HR pathways. Hoffman syndrome patients are described as having absent peripheral B-cell counts, and since

many B-cell transcription factors are also long genes, our work suggests that the transcription of these genes is greatly affected (Supplemental Figure 9). This effect suggests that many genes other than B-cell transcription factors may also be negatively affected which may provide insight into the dysmorphic features seen in the Hoffman syndrome patients. While proteins that function in NHEJ and HR are still seen binding directly to WT TOP2 in the presence of top2 mutants, long gene transcription is still affected indicating that this repair process may not be adequate enough for transcription of these genes to be completed. In the transcription of long B cell transcription factors, top2 mutations may be hindering the ability of Pol II to continue transcription because of unrepaired DSBs caused by WT/mutant top2 heterodimers. Further work in higher organisms may begin to resolve these remaining questions.

Table 1. Yeast strains used in this study.

Strain	Genotype	Source
RDKY 3023	MATa ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2ΔBgl, hom3-10, ade2Δ1, ade8	RD Kolodner
LBY 158/159	MATa top2::kanR YTOP2+intron EE587E (trp-), YTOP2+intron (ura-)	L Broderick
LBY 160/161	MATa top2::kanR YTOP2+intron S483L (trp-), YTOP2+intron (ura-)	L Broderick
LBY 162/163	MATa top2::kanR YTOP2+intron R503S (trp-), YTOP2+intron (ura-)	L Broderick
LBY 164/165	MATa top2::kanR YTOP2+intron R510Q (trp-), YTOP2+intron (ura-)	L Broderick
LBY 176/177	MATa YTOP2+intron (trp-), YTOP2+intron (ura-)	L Broderick
LBY 206/283	MATa top2::kanR YTOP2+intron G633S (trp-), YTOP2+intron (ura-)	This study
LBY 213	MATα TOP2-3xHA KAN	L Broderick
LBY 248/249	MATa pRS314 TOP2-3xHA KAN, MRE11-TAP URA	This study
LBY 250/251	MATa pRS314 YTOP2+intron, TOP2-3xHA KAN, MRE11-TAP URA	This study
LBY 252/253	MATa pRS314 YTOP2+intron S483L, TOP2-3xHA KAN, MRE11-TAP URA	This study
LBY 254/255	MATa pRS314 YTOP2+intron EE587E, TOP2-3xHA KAN, MRE11-TAP URA	This study
LBY 278/279	MATa pRS314 YTOP2+intron G633S, TOP2-3xHA KAN, MRE11-TAP URA	This study
LBY 258/259	MATa pRS314, TOP2-3xHA KAN, RAD50-TAP URA	This study
LBY 260/261	MATa pRS314 YTOP2+intron, TOP2-3xHA KAN, RAD50-TAP URA	This study
LBY 262/263	MATa pRS314 YTOP2+intron S483L, TOP2-3xHA KAN, RAD50-TAP URA	This study

Table 1. (continued)

Strain	Genotype	Source
LBY 264/265	MATa pRS314 YTOP2+intron EE587E, TOP2-3xHA KAN, RAD50-TAP URA	This study
LBY 266/267	MATa pRS314 YTOP2+intron G633S, TOP2-3xHA KAN, RAD50-TAP URA	This study
LBY 268/269	MATa pRS314, TOP2-3xHA KAN, DNL4-TAP URA	This study
LBY 270/271	MATa pRS314 YTOP2+intron, TOP2-3xHA KAN, DNL4-TAP URA	This study
LBY 272/273	MATa pRS314 YTOP2+intron S483L, TOP2-3xHA KAN, DNL4-TAP URA	This study
LBY 274/275	MATa pRS314 YTOP2+intron EE587E, TOP2-3xHA KAN, DNL4-TAP URA	This study
LBY 276/277	MATa pRS314 YTOP2+intron G633S, TOP2-3xHA KAN, DNL4-TAP URA	This study
LBY 293/294	MATα pRS314, YTOP2-3xHA KAN, YKU80-TAP URA	This study
LBY 295/296	MATα pRS314 YTOP2+intron, YTOP2-3xHA KAN, YKU80-TAP URA	This study
LBY 297/298	MATα pRS314 YTOP2+intron S483L, YTOP2-3xHA KAN, YKU80-TAP URA	This study
LBY 299/300	MATα pRS314 YTOP2+intron EE587E, YTOP2-3xHA KAN, YKU80-TAP URA	This study
LBY 301/302	MATα pRS314 YTOP2+intron G633S, YTOP2-3xHA KAN, YKU80-TAP URA	This study
LBY 303/304	MATα pRS314, YTOP2-3xHA KAN, YKU70-TAP URA	This study
LBY 305/306	MATα pRS314 YTOP2+intron, YTOP2-3xHA KAN, YKU70-TAP URA	This study
LBY 307/308	MATα pRS314 YTOP2+intron S483L, YTOP2-3xHA KAN, YKU70-TAP URA	This study
LBY 309/310	MATα pRS314 YTOP2+intron EE587E, YTOP2-3xHA KAN, YKU70-TAP URA	This study
LBY 311/312	MATα pRS314 YTOP2+intron G633S, YTOP2-3xHA KAN, YKU70-TAP URA	This study

Table 2. Plasmids used in this study.

Strain	Genotype	Source/Reference
LBB 72	pFA6a 3HA KAN MX6	Longtine *et al.*, 1998
LBB 83	pFA6a TAP URA3	Longtine *et al.*, 1998
LBB 7	pRS 314	Sikorski & Heiter, 1989
LBB 124	pRS 315	Sikorski & Heiter, 1989
LBB 55	pRS 316 YTOP2+intron	L Broderick
LBB 67	pRS 314 YTOP2+intron	L Broderick
LBB 113	pRS314 YTOP2+intron S483L	L Broderick
LBB 36	pRS314 YTOP2+intron EE587E	L Broderick
LBB 117	pRS314 YTOP2+intron G633S	L Broderick

Table 3. Primers used in this study.

Primer	Purpose	Primer Sequence (5' – 3')	Source/Reference
LBP 183/184	qPCR MEC1	GCCCTTGTCATACAGGAATCA CCCATAGTTTGGCTTCATTTGG	IDT
LBP 185/186	qPCR XRS2	CGAGTGAGGATTCGTTGGATAAG GTCAGCGGAGTTCGAAGATATG	IDT
LBP 187/188	qPCR DNL4	ACAGGAAACAACTGCCAATTTC TCAAGTTCTGCCCGTGTAATC	IDT
LBP 189/189	qPCR YKU80	GAAACAGACGGGCATCAAATC CTTCGCCTATGGGTTGATAGTAG	IDT
LBP 191/192	qPCR YKU70	GGCTATGCCGATAAACCATTTG TAGGCTTTGTACTTGGACCATC	IDT
LBP 193/194	qPCR MRE11	GACGAAGAGGACGCTAGTTATG CGTCCTTGATGCTCTTCCTT	IDT
LBP 195/196	qPCR RAD50	TGGCAAGCCTCTGACTTTAATA AATACTCCTCCCTTGCTGTTG	IDT
LBP 222/223	qPCR CUP1-2	GCCAATGTGGTAGCTGCAAA TCTTCAGACTTGTTACCGCAGG	Joshi *et al*., 2012
LBP 220/221	qPCR SEC7	CACTGATGACCGTGTGGAAC ATGGGTTCAACGAAAAGACG	Joshi *et al*., 2012
LBP 216/217	qPCR GCN1	GTCGCTGCATTTAAGCTTCC CTTCGAAACAACGTCAGCAA	Joshi *et al*., 2012
LBP 117/118	qPCR ACT1	TCGAACAAGAAATGCAAACCG GGCAGATTCCAAACCCAAAAC	IDT
LBP 202/203	Tag MRE11	GACGGATATTCTTGGAAGTCTCCTTGCTAAGAAAAGAAAA* TTATAAATAGGATATAATATAATATAGGGATCAAGTACAA**	IDT

Table 3. (continued)

Primer	Purpose	Primer Sequence (5' – 3')	Source/Reference
LBP 204/205	Tag XRS2	CGACGGTCCGAAGTTTACGTTCAAAAGAAGAAAAGGATAA* AAATATAATTTAATGAAATTGGAAATACTCGGAAAATTTA**	IDT
LBP 206/207	Tag RAD50	ATCACAAATTGAGTGGGTCGATATTAACAGAGTCACTTAT* CAATCAAAGTCTATCCCTTCGTAGATATTATGGGGTCTTT**	IDT
LBP 208/209	Tag DNL4	CTGTCAAGTGCCTGAAGAAGACTTCCCCGTAGTCAACTAC* AAATAAACTTCAAAAAATTAAGCCTCCGCAAAACGCACCA**	IDT
LBP 175/176	Tag YKU70	AGAAAAGAAGCCCTTTGATAAAAAGCCGAAATTCAATATA* TTGTATGTAACGTTATAGATATGAAGGATTTCAATCGTCT**	IDT
LBP 177/178	Tag YKU80	TGAACAACACAGTAGGGGAAGTCCAAACAATAGCAATAAT* GTGGTGACGAAAACATAACTCAAAGGATGTTAGACCTTTT**	IDT
LBP 226	TAP rev	GCTTTGGCTTGGGTCATCT	IDT
LBP 242	TAP fwd	GAAGCCGTGGACAACAAATTC	IDT
LBP 198	YKU70 fwd confirm	GAGGGATGGATATAACCCATCC	IDT
LBP 199	YKU80 fwd confirm	TGCTGTTGCAAGATATGTGAGC	IDT
LBP 241	XRS2 rev confirm	GAAGAAGTTGGCAGGGCTATTA	IDT
LBP 228	DNL4 fwd confirm	GTAGACCACTCAAGCCAAGAAA	IDT
LBP 230	RAD50 fwd confirm	GGATGAAGCGCAAAGTGTTC	IDT

Table 3. (continued)

Primer	Purpose	Primer Sequence (5' – 3')	Source/Reference
LBP 231	MRE11 fwd confirm	GCGACGCTAATGAGGAAACTA	IDT

*forward gene-specific sequence followed by plasmid-specific sequence CGG ATC CCC GGG TTA ATT AA
**reverse gene-specific sequence followed by plasmid-specific sequence GAA TTC GAG CTC GTT TAA AC

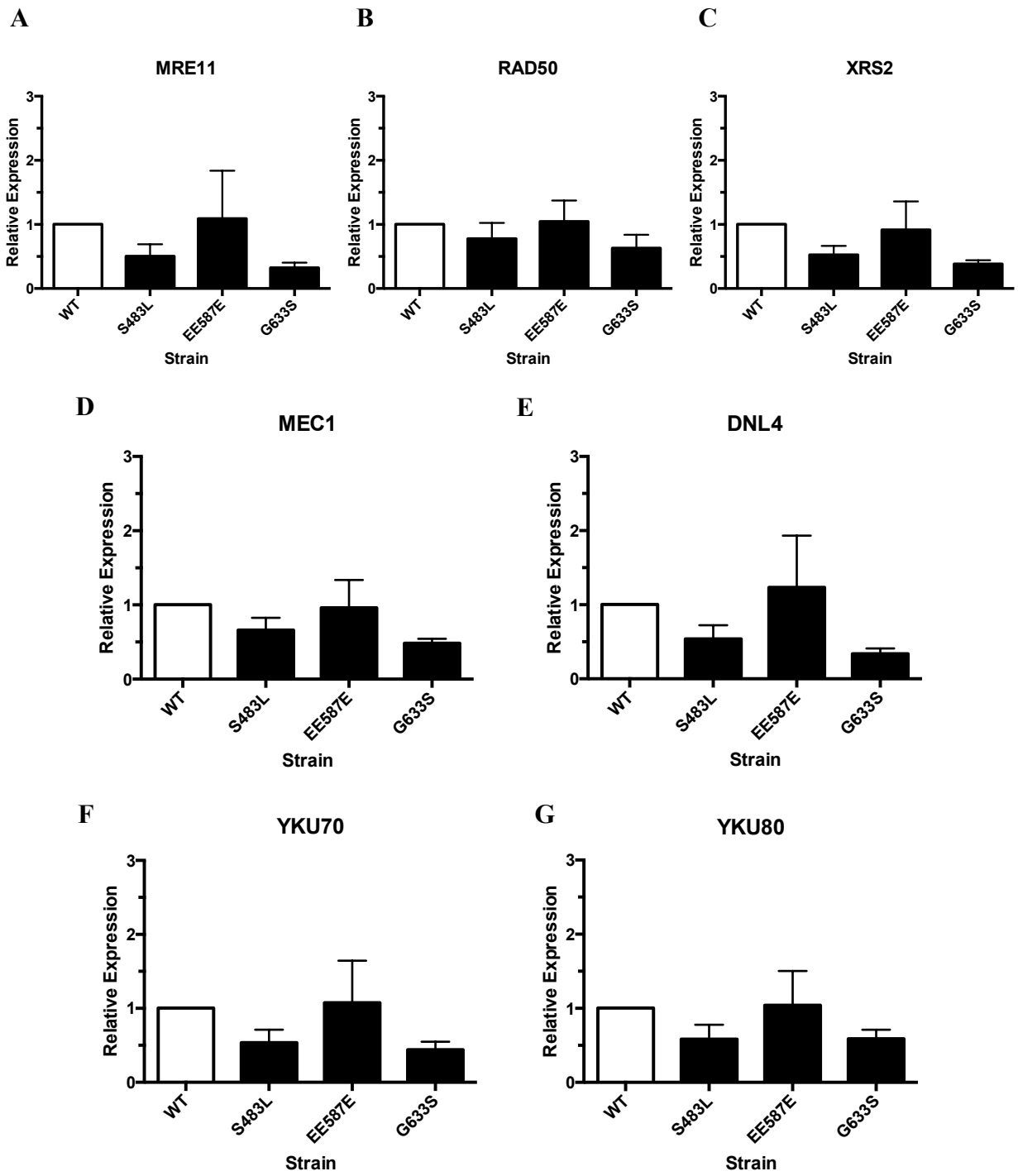


Figure 1. Double-strand break complex gene expression is unchanged in the presence of top2 mutants. RT-qPCR of DSB complex genes in haploid yeast strains with genomic TOP2 deleted in addition to WT and mutant TOP2 plasmids. The data was normalized to WT TOP2 strains. Error bars and mean are the standard error of mean (SEM) for n=3.

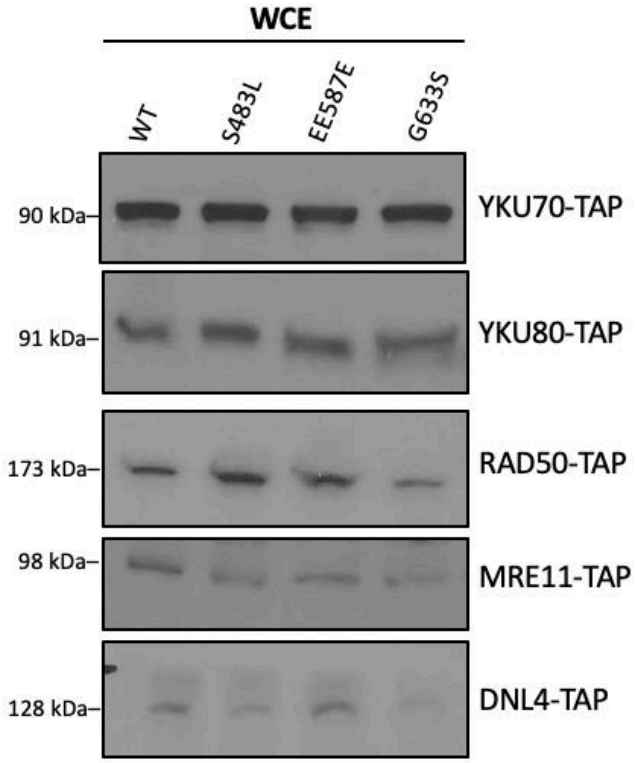


Figure 2. DSB complex protein expression is unchanged in the presence of top2 mutants. Whole cell extracts (WCE) of *S. cerevisiae* strains with genomic WT TOP2-HA, one DSB complex gene tagged with TAP, transformed with a WT or mutant TOP2 plasmid. The molecular weight of each tagged protein is listed to the right of each membrane. n=3.

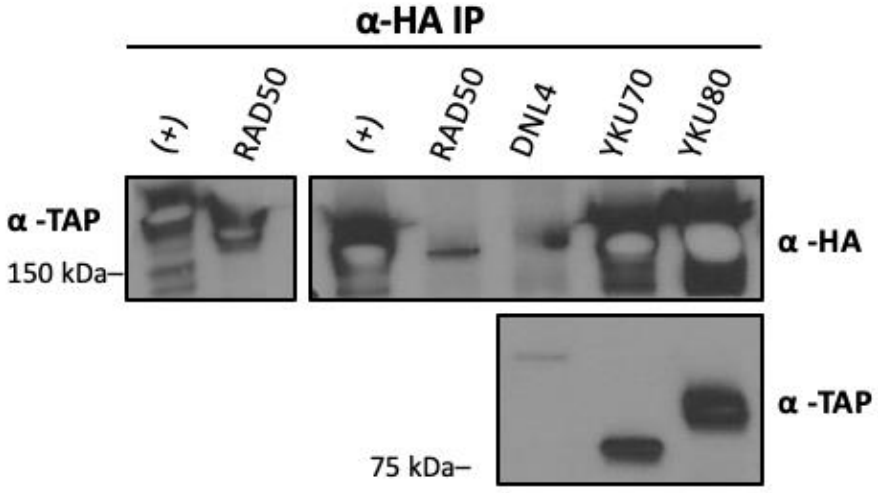


Figure 3. WT TOP2 interacts directly with RAD50, YKU70, YKU80 and not to DNL4. Anti-HA co-immunoprecipitation of *S. cerevisiae* strains with genomic WT TOP2-HA, one DSB complex gene tagged with TAP, transformed with a WT TOP2 plasmid. The positive control is diploid *S. cerevisiae* strain with genomic WT TOP2-HA and WT TOP2-TAP. WT TOP2 in RAD50 and DNL4 strains are visible are at longer exposure. n=3.

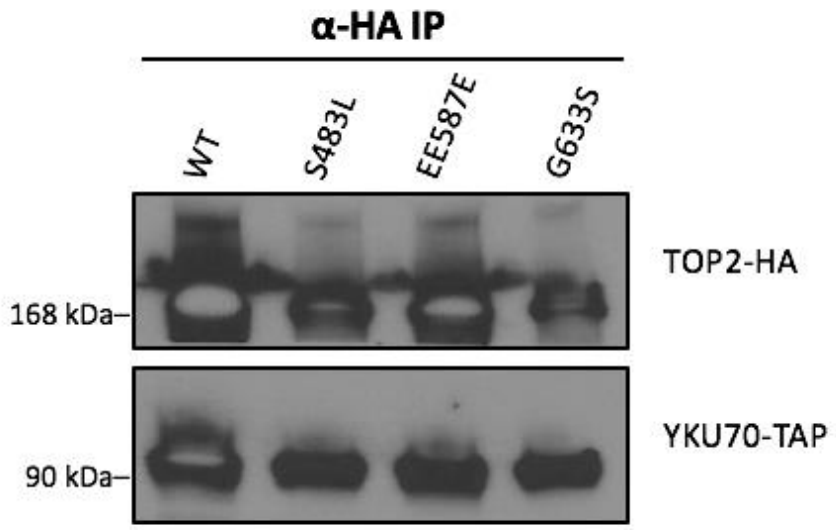


Figure 4. WT TOP2 interacts directly to YKU70-TAP in the presence of top2 mutants. Anti-HA co-immunoprecipitation followed by anti-HA and anti-TAP Western blot of *S. cerevisiae* strains with genomic WT TOP2-HA and YKU70-TAP transformed with a WT or mutant TOP2 plasmid. n=3.

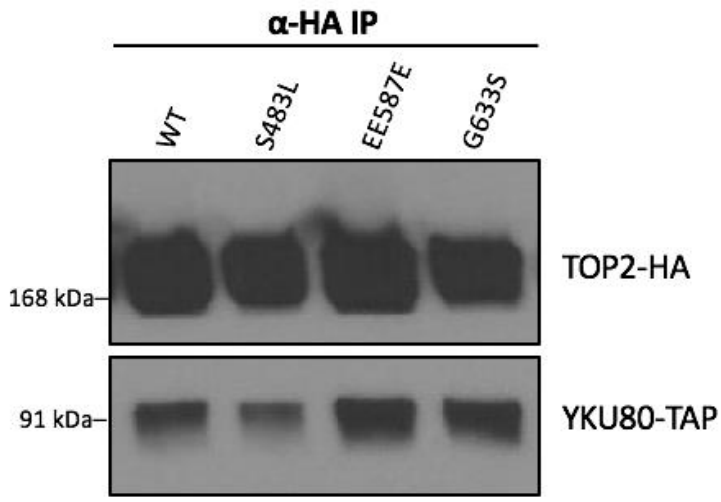


Figure 5. WT TOP2 interacts directly with YKU80 in the presence of top2 mutants. Anti-HA co-immunoprecipitation followed by anti-HA and anti-TAP Western blot of *S. cerevisiae* strains with genomic WT TOP2-HA and YKU80-TAP transformed with a WT or mutant TOP2 plasmid. n=3.

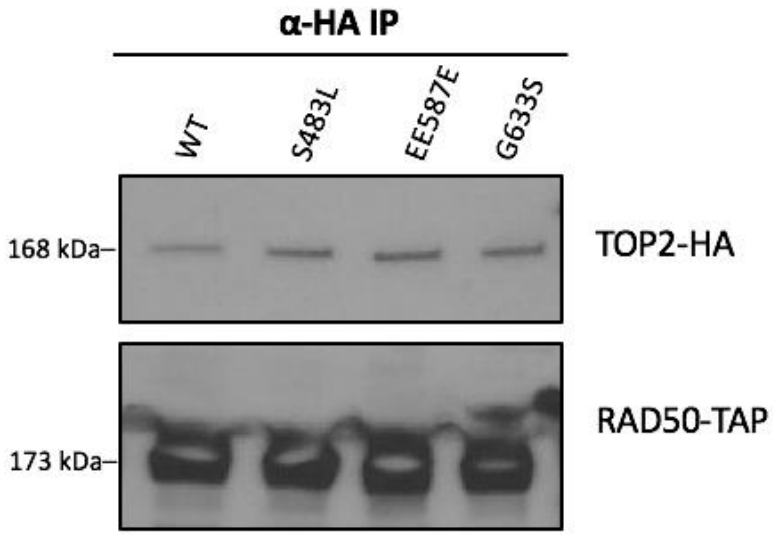


Figure 6. WT TOP2 interacts directly with RAD50 in the presence of top2 mutants. Anti-HA co-immunoprecipitation followed by anti-HA and anti-TAP Western blot of *S. cerevisiae* strains with genomic WT TOP2-HA and RAD50-TAP transformed with a WT or mutant TOP2 plasmid. n=3.

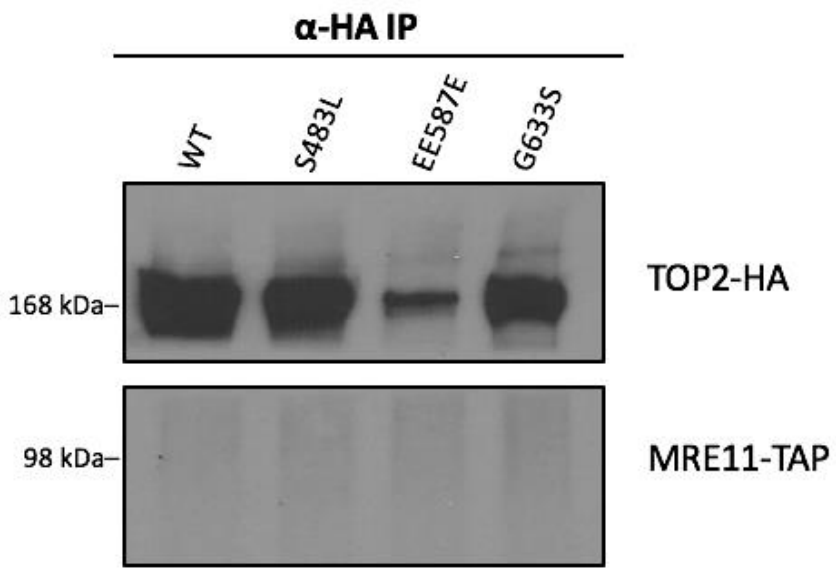


Figure 7. WT TOP2 does not interact directly with MRE11 in the presence of WT or mutant top2. Anti-HA co-immunoprecipitation followed by anti-HA and anti-TAP Western blot of *S. cerevisiae* strains with genomic WT TOP2-HA and MRE11-TAP transformed with a WT or mutant TOP2 plasmid. n=3.

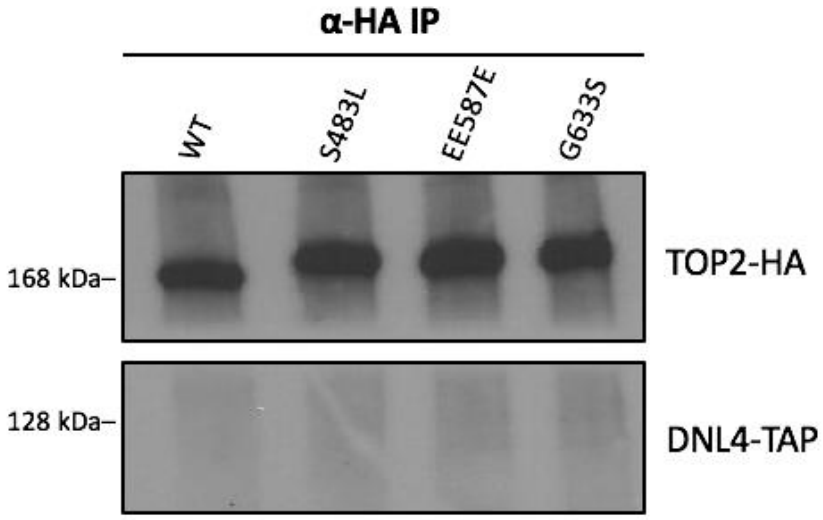


Figure 8. WT TOP2 does not interact directly with DNL4 in the presence of WT or mutant top2. Anti-HA co-immunoprecipitation followed by anti-HA and anti-TAP Western blot of *S. cerevisiae* strains with genomic WT TOP2-HA and DNL4-TAP transformed with a WT or mutant TOP2 plasmid. n=3.

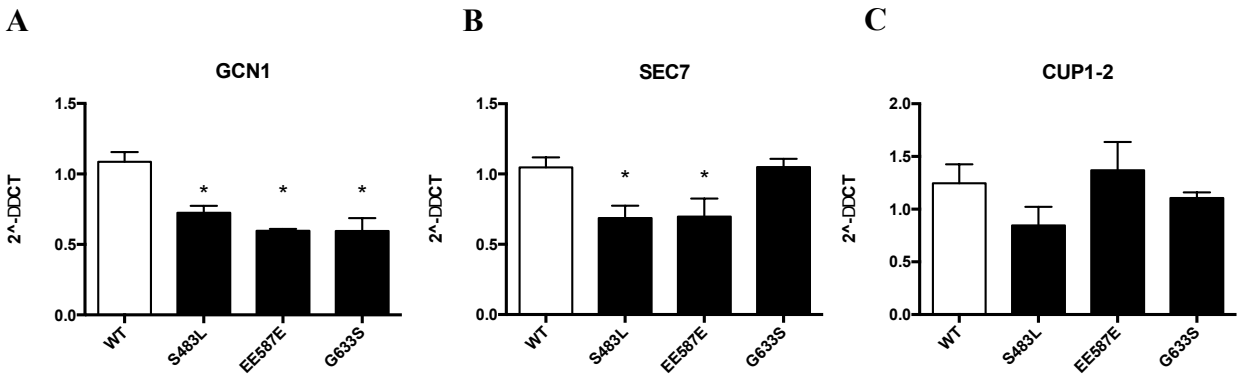
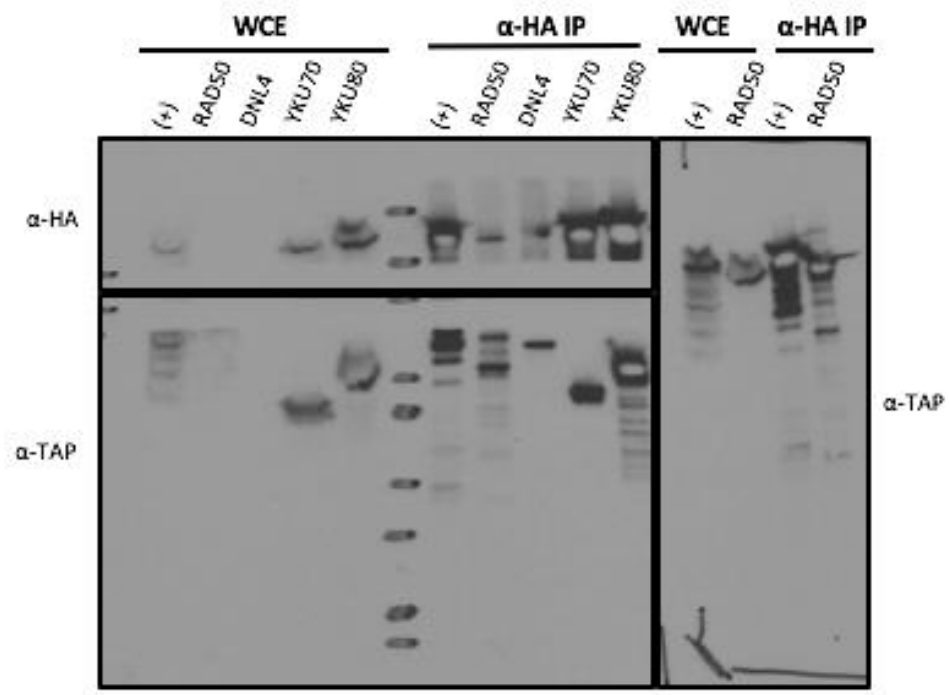
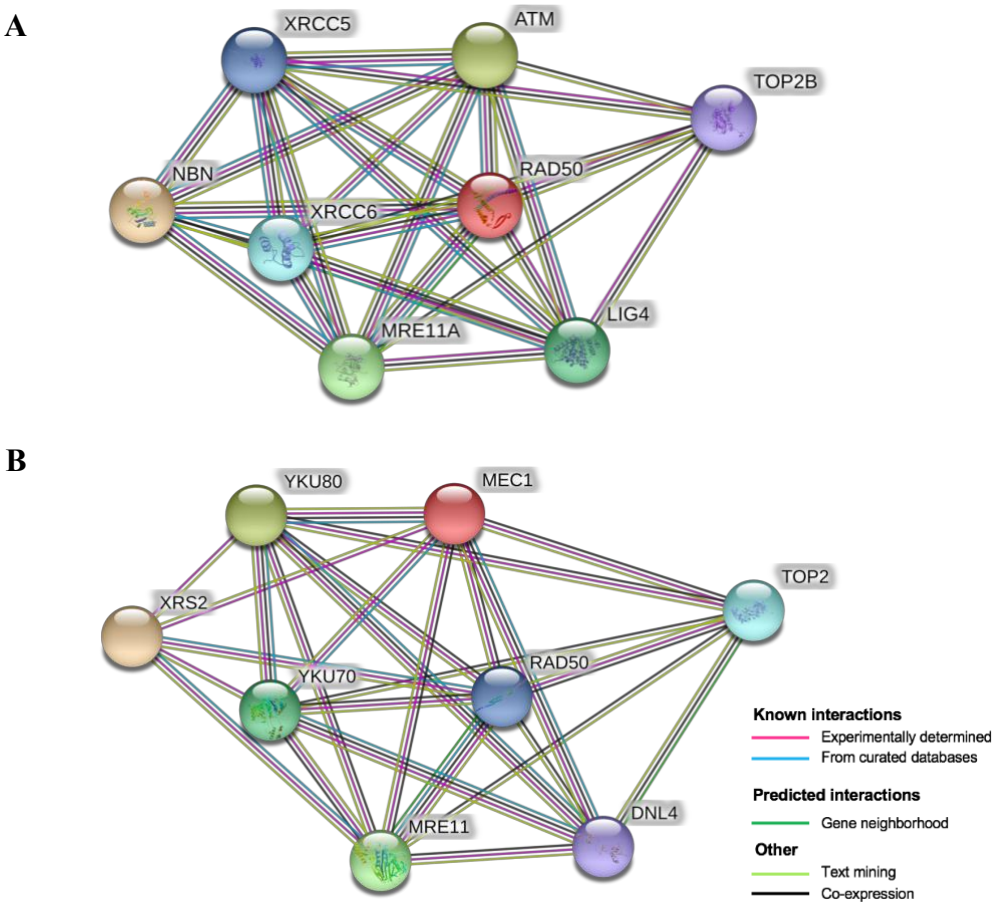


Figure 9. Expression of long genes *GCN1* and *SEC7* are decreased in the presence of top2 mutants while the short gene *CUP1-2* is unchanged. RT-qPCR was performed on *S. cerevisiae* strains with a genomic deletion of TOP2 in addition to WT and mutant TOP2 plasmids for gene expression analyses of *GCN1* (A), *SEC7* (B) and *CUP1-2* (C). The data was normalized to WT TOP2 strains. Error bars and mean are the standard error of mean (SEM) for n=3. An asterisk denotes a significant difference (*$p < 0.05$; by student's two-tailed t-test).

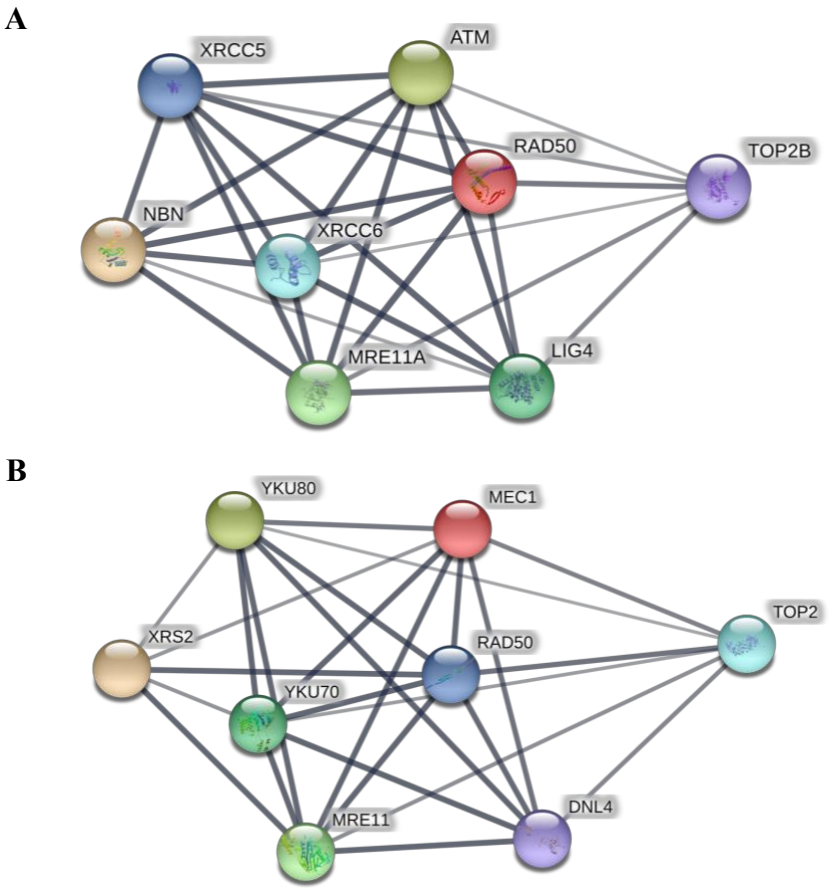
SUPPLEMENTAL FIGURES



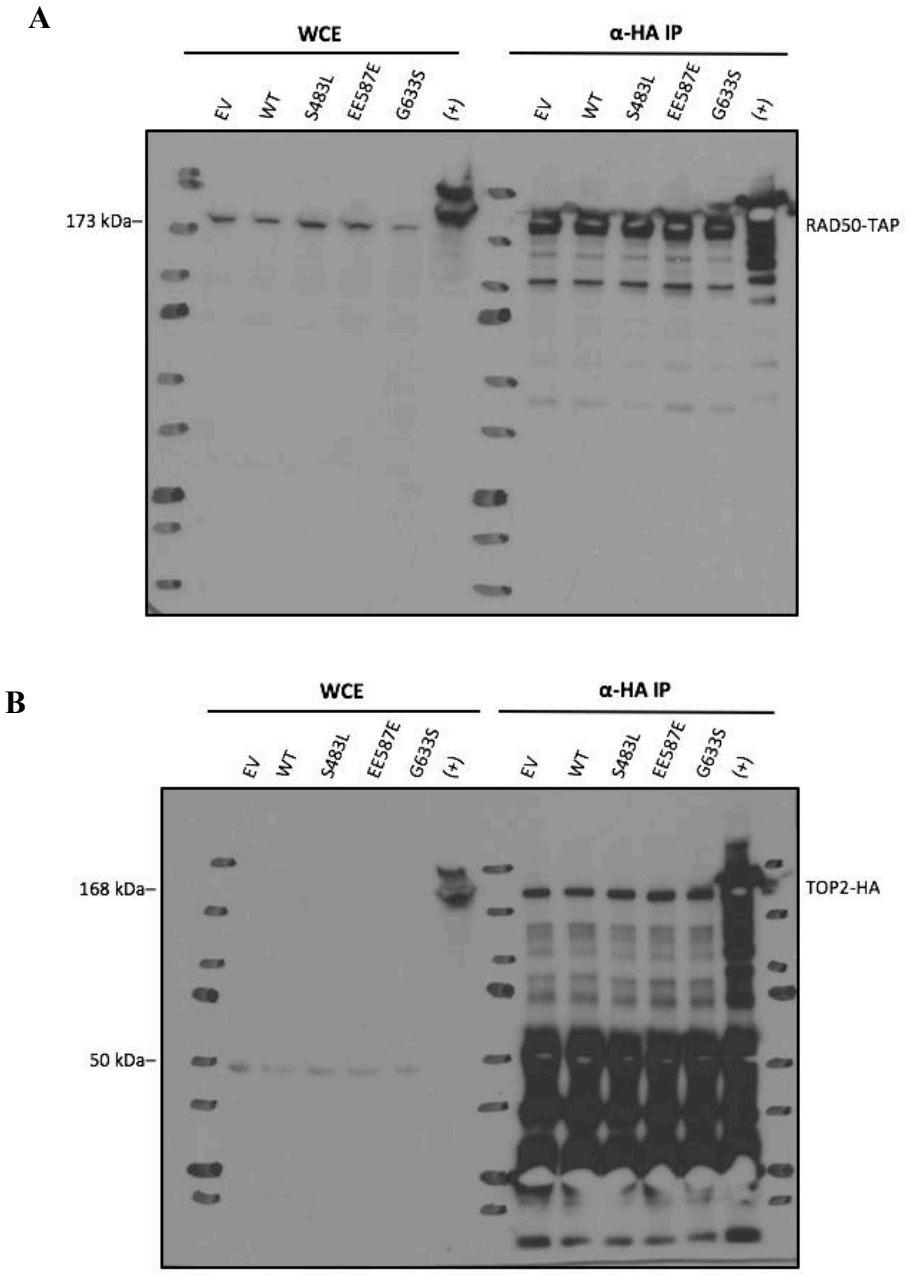
Supplemental Figure 1. Full membrane of WT anti-HA co-immunoprecipitation. Representative full membrane of the anti-HA co-immunoprecipitation of WT tagged strains followed by anti-HA and anti-TAP Western blot. Cuts in membrane to probe for different tags are indicated by the black lines. n=3.



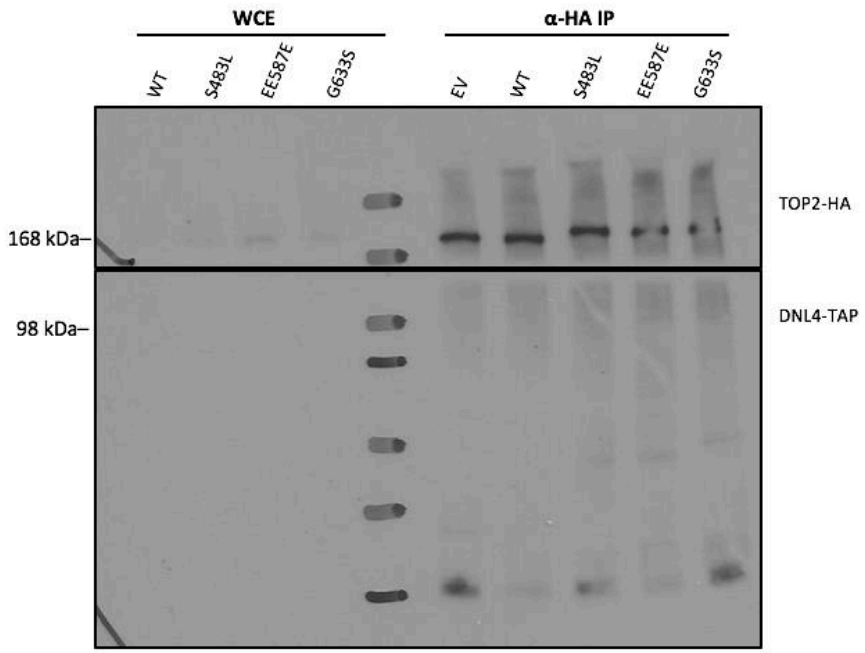
Supplemental Figure 2. STRING database depicts associations between TOP2B and the DSB complex proteins. Protein association between TOP2B and the DSB complex proteins in humans (A) and *S. cerevisiae* (B). The color of the lines connecting each protein represents their association with one another, which does not imply direct protein-protein interaction.



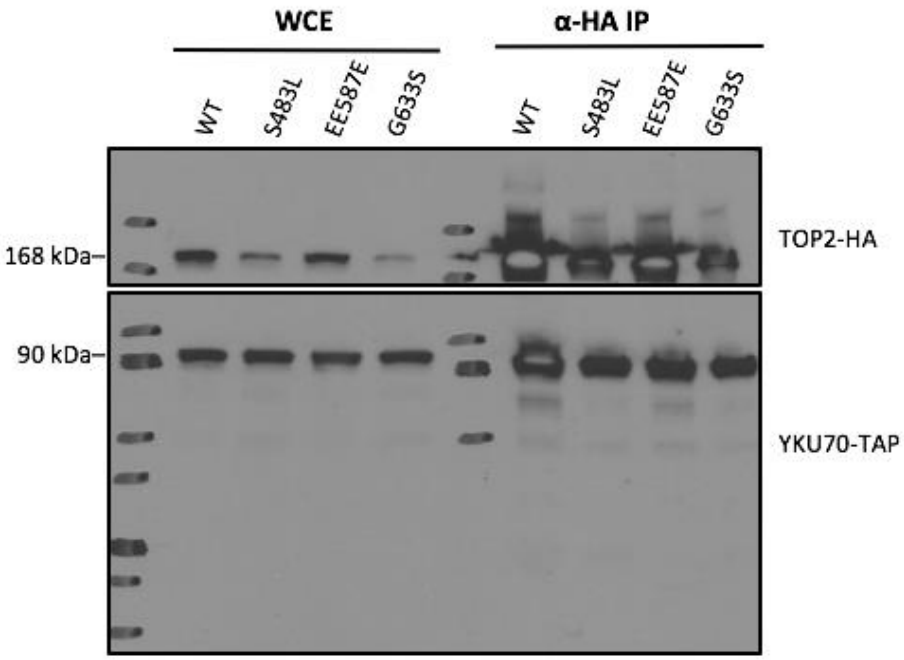
Supplemental Figure 3. STRING database depicts low confidence in associations between TOP2B and the DSB complex proteins. Protein associations between TOP2 and the DSB complex proteins in humans (A) and *S. cerevisiae* (B). The thickness of the lines connecting each protein represents the strength of data support for their protein association, with an increase in thickness correlating to an increase in confidence.



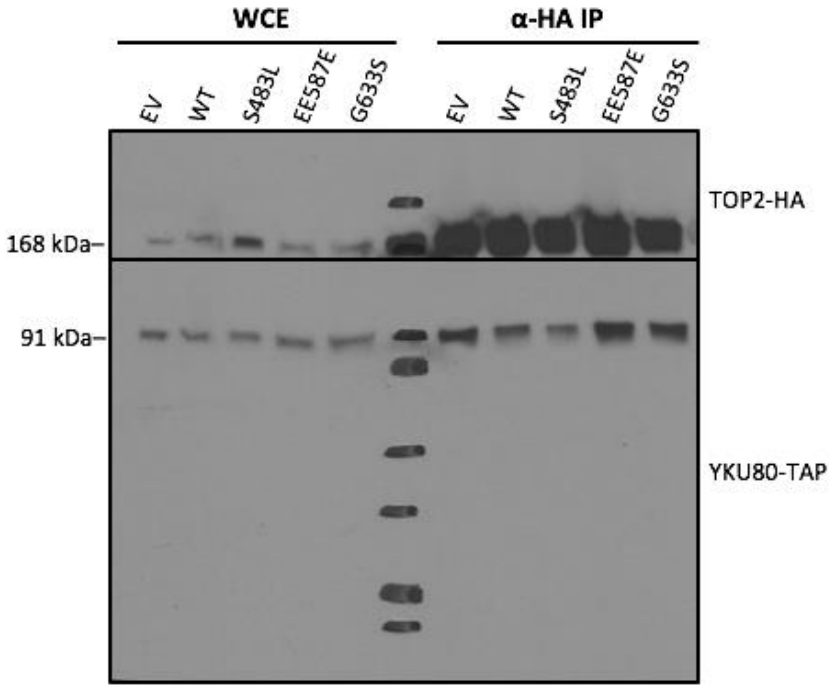
Supplemental Figure 4. Full membrane of anti-HA co-immunoprecipitation of RAD50 tagged strains. Full membranes for anti-TAP (A) and anti-HA (B) Western blots of RAD50-TAP tagged strains with top2 mutations. n=3.



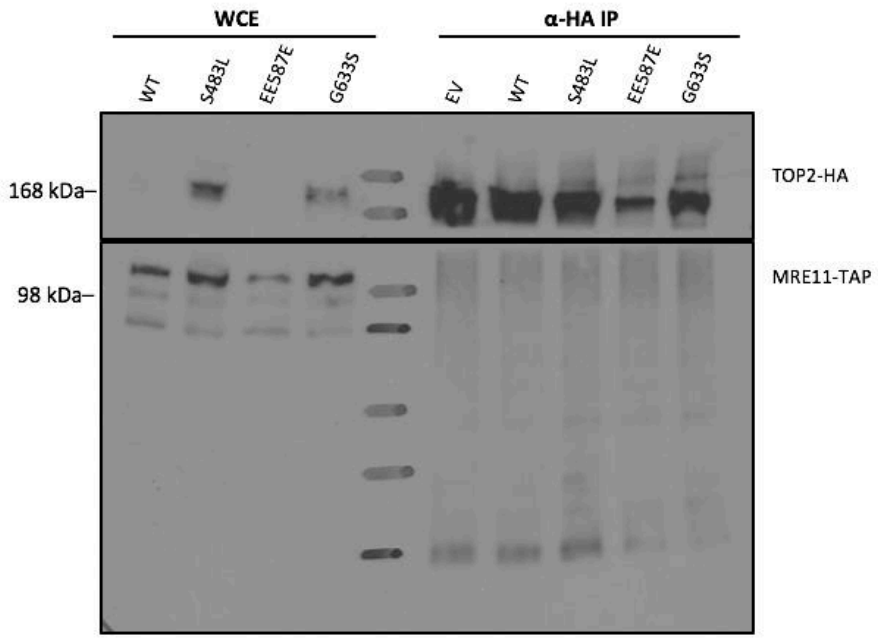
Supplemental Figure 5. Full membrane of anti-HA co-immunoprecipitation of DNL4 tagged strains. Full membrane for anti-HA and anti-TAP Western blots of DNL4-TAP tagged strains with top2 mutations. Cuts in membrane to probe for different tags are indicated by the black lines. n=3.



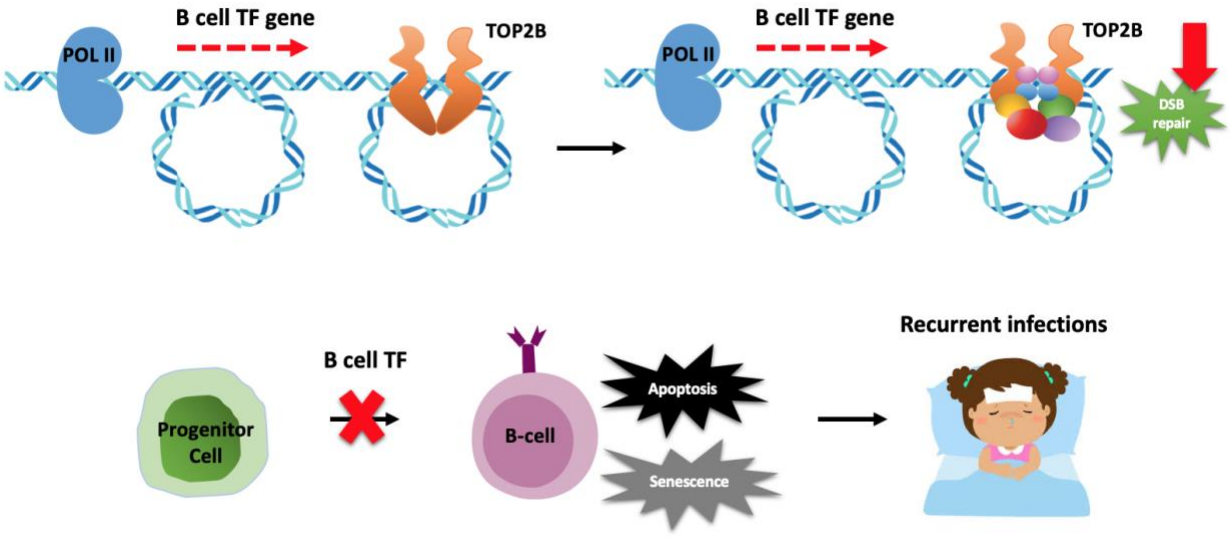
Supplemental Figure 6. Full membrane of anti-HA co-immunoprecipitation of YKU70 tagged strains. Full membrane for the anti-HA co-immunoprecipitation of YKU70 tagged strains in the presence top2 mutations followed by anti-HA anti-TAP Western blot. Cuts in membrane to probe for different tags are indicated by the black lines. n=3.



Supplemental Figure 7. Full membrane of anti-HA co-immunoprecipitation of YKU80 tagged strains. Anti-HA co-immunoprecipitation followed by anti-HA and anti-TAP Western blots of YKU80-TAP tagged strains with top2 mutations. Cuts in membrane to probe for different tags are indicated by the black line. n=3.



Supplemental Figure 8. Full membrane of anti-HA co-immunoprecipitation of MRE11 tagged strains. Anti-HA co-immunoprecipitation followed by anti-HA and anti-TAP Western blots of MRE11-TAP tagged strains with top2 mutations. Cuts in membrane to probe for different tags are indicated by the black lines. n=3.



Supplemental Figure 9. Formation of supercoils during transcription of long B-cell transcription factors affect the development of B-cells. Transcription of long B-cell transcription factors generate increased tension in DNA leading to the formation of supercoils. In order for transcription to continue the supercoils must be relaxed by TOP2B. The DNA double-stranded break formed by TOP2B recruits the double-strand break repair machinery. If this process of double-strand break repair is affected by mutations in *top2*, long B-cell transcription factors are not being transcribed and progenitor cells do not have the necessary transcription factors to continue through B-cell development, leading to recurrent infections.

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