1 **RESEARCH ARTICLE**

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3 **SMC5/6 complex-mediated SUMOylation stimulates DNA-protein crosslink**

4 **repair in Arabidopsis**

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6 Eva Dvořák Tomaštíková[a,3], Klara Prochazkova[a], Fen Yang[a,b], Jitka Jemelkova[a,c], Andreas

7 Finke[d,2], Annika Dorn[e], Mahmoud Said[a,f], Holger Puchta[e], Ales Pecinka[a,b,c,3]

8

9 [a]Centre of Plant Structural and Functional Genomics, Institute of Experimental Botany (IEB),

10 Czech Acad Sci, Šlechtitelů 31, 77900 Olomouc, Czech Republic.

11 [b]Department of Cell Biology and Genetics, Faculty of Science, Palacký University, Olomouc,

12 Czech Republic.

13 [c]Functional Genomics and Proteomics, National Centre for Biomolecular Research (NCBR),

14 Faculty of Science, Masaryk University, Kamenice 5, 62500 Brno, Czech Republic.

15 [d]Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, 50829 Cologne,

16 Germany.

17 [e]Botanical Institute, Molecular Biology and Biochemistry, Karlsruhe Institute of Technology,

18 Fritz-Haber-Weg 4, Karlsruhe, 76131, Germany.

19 [f]Field Crops Research Institute, Agricultural Research Centre, 9 Gamma Street, Giza, 12619,

20 Cairo, Egypt.

21

22 [2]Present address: Hamilton Bonaduz AG, Application Laboratory, CH-4123 Allschwil,

23 Switzerland.

24

25 **Short title:** DPC repair by SMC5/6 complex

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29 The author(s) responsible for distribution of materials integral to the findings presented in this

30 article in accordance with the policy described in the Instructions for Authors

31 (https://academic.oup.com/plcell/) is: Ales Pecinka (pecinka@ueb.cas.cz).

32

33 [3]Corresponding authors: Ales Pecinka (pecinka@ueb.cas.cz), Eva Dvořák Tomaštíková

34 (tomastikova@ueb.cas.cz)

35

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ABSTRACT

DNA-protein crosslinks (DPCs) are highly toxic DNA lesions consisting of proteins covalently attached to chromosomal DNA. Unrepaired DPCs physically block DNA replication and transcription. Three DPC repair pathways have been identified in Arabidopsis (*Arabidopsis thaliana*) to date: the endonucleolytic cleavage of DNA by the structure-specific endonuclease MUS81; proteolytic degradation of the crosslinked protein by the metalloprotease WSS1A; and cleavage of the crosslink phosphodiester bonds by the tyrosyl phosphodiesterases TDP1 and TDP2. Here we describe the evolutionary conserved STRUCTURAL MAINTENANCE OF CHROMOSOMEs SMC5/6 complex as a crucial component involved in DPC repair. We identified multiple alleles of the SMC5/6 complex core subunit gene *SMC6B* via a forward-directed genetic screen designed to identify the factors involved in the repair of DPCs induced by the cytidine analog zebularine. We monitored plant growth and cell death in response to DPC-inducing chemicals, which revealed that the SMC5/6 complex is essential for the repair of several types of DPCs. Genetic interaction and sensitivity assays showed that the SMC5/6 complex works in parallel to the endonucleolytic and proteolytic pathways. The repair of zebularine-induced DPCs was associated with SMC5/6-dependent SUMOylation of the damage sites. Thus, we present the SMC5/6 complex as an important factor in plant DPC repair.

IN A NUTSHELL

Background: Cellular DNA is constantly damaged by various internal and external factors that eventually lead to mutations, reduced growth or even death. To ensure genome stability, organisms have evolved sophisticated and intricate DNA repair systems. We understand how cells remove some types of DNA damage, but the mechanisms of detoxification from other types of damage remain poorly characterized. For example, DNA-protein crosslinks, i.e. proteins covalently attached to DNA molecule, hinder the essential processes of replication and transcription.

Question: Our aim is to identify molecular factors protecting plants from toxic DNA-protein crosslinks. We set up a forward-directed genetic screen to identify mutants hypersensitive to the cytidine analog zebularine, which crosslinks DNA METHYLTRANSFERASE1 (MET1) protein to the 45S rDNA repeats, and characterized the first candidate.

Findings: We mapped *HYPERSENSITIVE TO ZEBULARINE 1* (*HZE1*) candidate as *SMC6B*, a core component of the Structural maintenance of chromosomes 5/6 (SMC5/6) complex. HZE1 plays a key role in DNA protein crosslink repair as it is needed for the repair of different classes of crosslinks. We also showed that the SMC5/6 complex acts in parallel with the known proteolytic and nucleolytic DNA-protein crosslink repair pathways. To shed

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light on the possible mechanism of SMC5/6 action, we focused on the Small ubiquitin modifier (SUMO) ligation activity of this complex. We showed the SMC5/6 complex–dependent accumulation of SUMO at the crosslinked foci induced by zebularine.

Next steps: We will focus further on the role of SUMO in plant DNA damage repair and will characterize other HZE candidates coming from the forward-directed genetic screen. This will help us understand the mechanisms of DNA-protein crosslink repair in plants.

INTRODUCTION

Cellular DNA is constantly exposed to various genotoxic factors that may alter its structure and result in DNA lesions. A common type of DNA damage is DNA-protein crosslinks (DPCs), which form when proteins covalently bind to DNA. DPCs are among the most toxic yet least studied lesions that impede DNA-related processes. Indeed, if not repaired, DPCs may lead to mutations, genomic instability, and eventually cell death (Barker et al., 2005). Based on their nature and origin, DPCs can be classified into three main categories: enzymatic, non-enzymatic, and DPC-like traps (Zhang et al., 2020). Enzymatic DPCs occur with proteins that form short-term covalent reaction intermediates as part of their enzymatic cycle (e.g. topoisomerases, DNA methyltransferases). Such DPCs are formed by stabilizing the covalent bond with a specific poison. Non-enzymatic DPCs are caused by the covalent crosslinking of proteins located in the vicinity of DNA. Last, DPC-like trapping occurs when a protein becomes firmly bound to DNA and behaves as a DPC (Stingele et al., 2016, 2017; Klages-Mundt and Li, 2017; Weickert and Stingele, 2022).

Both endogenous and exogenous DPC inducers have been described. Endogenous crosslinkers occur naturally in cells as products of metabolism and include reactive aldehydes such as acetaldehyde and formaldehyde (Nakamura and Nakamura, 2020). Exogenous crosslinkers are induced environmentally, e.g., after exposure to ultraviolet (UV) or ionizing radiation (Kojima and Machida, 2020). Therapeutic crosslinkers represent particular types of exogenous crosslinkers that were identified as potent chemotherapeutic agents. Well-known examples of enzymatic poisons that intercalate at the DNA-protein interface and cause covalent trapping of the target protein to DNA are camptothecin (CPT), etoposide and 5-azacytidine or zebularine, which crosslink TOPOISOMERASE 1 (TOP1; type-3 DPC) (Pommier and Marchand, 2012), TOPOISOMERASE 2 (TOP2; type-4 DPC) (Nitiss et al., 2009), or DNA METHYLTRANSFERASE 1 (DNMT1/MET1; type-1 DPC) (Maslov et al., 2012; Prochazkova et al., 2022), respectively.

Owing to the structural and chemical diversity of the proteins that can be crosslinked and the DNA contexts in which they occur, DPCs can be challenging lesions for repair. Several DPC repair pathways have been reported (Pouliot et al., 1999; Regairaz et al., 2011;

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Stingele et al., 2014; Sun et al., 2020a, 2020b). First, proteolytic cleavage of the protein component of DPCs includes the recently-identified metalloproteases Weak suppressor of Smt3 (Wss1) in yeasts and SPARTAN (SPRTN) in animals (Stingele et al., 2016; Vaz et al., 2016). Wss1/SPRTN proteolytic activity has no defined protein specificity but depends on DNA binding. Second, direct enzymatic hydrolysis of the 3' phosphate from DNA and the active tyrosyl residue of class I Topoisomerases was described, catalyzed by Tyrosyl-DNA phosphodiesterase 1 (TDP1) in yeast *Saccharomyces cerevisiae* (Pouliot et al., 1999). Wss1 and TDP1 define parallel genetic pathways for the repair of CPT-induced DPCs in yeast (Stingele and Jentsch, 2015). The Arabidopsis (*Arabidopsis thaliana*) genome contains two *Wss1* homologs, *WSS1A* and *WSS1B* (Enderle et al., 2019). However, only *wss1a* mutant plants were hypersensitive to the DPC-inducing agents camptothecin (CPT) and cisplatin, and no additive phenotype was observed in the *wss1a wss1b* double mutant. Therefore, WSS1A is currently thought to be the only protease involved in the DPC repair in Arabidopsis. Moreover, *wss1a* plants showed severe growth defects and reduced fertility, probably due to the accumulation of natural DPCs. In contrast to animals, TDP1 only plays a minor role in the repair of TOP1 crosslinks in Arabidopsis and may function as a backup pathway to MUS81 and WSS1A-mediated repair (Enderle et al., 2019). Additionally, TDP2 contributes to the repair of TOP2 crosslinks in Arabidopsis (Hacker et al., 2022). Last, DPCs can be directly processed by DNA endonucleases. The heterodimeric MMS AND UV SENSITIVE 81 (MUS81) and ESSENTIAL MEIOTIC ENDONUCLEASE 1A (EME1) endonuclease complex acts preferentially on DNA substrates that mimick stalled replication forks, nicked Holliday junctions (HJs), and D-loops (Chen et al., 2001; Doe et al., 2002). In Arabidopsis, MUS81 processes HJs, aberrant replication intermediates, and acts in homologous recombination (HR) (Hartung et al., 2006; Mannuss et al., 2010). Plants lacking MUS81 activity are hypersensitive to CPT and cisplatin, indicating their possible function in processing DPCs next to single-strand breaks or stalled replication forks (Enderle et al., 2019).

The STRUCTURAL MAINTENANCE OF CHROMOSOMES 5/6 (SMC5/6) complex is an evolutionary conserved DNA-stimulated ATP-dependent molecular machine involved in organizing DNA and preserving genome stability. The core SMC5/6 complex is composed of the ring structure of SMC5 and SMC6 heterodimers and several NON-SMC ELEMENT (NSE) subunits (Diaz and Pecinka, 2018; Palecek, 2019). The SUMO-ligase subunit NSE2 is positioned at the SMC5 arm, and SUMOylates HR factors to stimulate DNA damage repair (Varejão et al., 2018; Whalen et al., 2020). SUMO modification of DPCs also facilitates their repair (Schellenberg et al., 2017; Borgermann et al., 2019) but has not been connected to the SMC5/6 complex so far.

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147   The Arabidopsis genome encodes two SMC6 (SMC6A, SMC6B), one SMC5 and six

148   NSE subunits (NSE1–3, NSE4A and NSE4B, ARABIDOPSIS SNI1 ASSOCIATED PROTEIN

149   1 [ASAP1], and SUPPRESSOR OF NPR1-1, INDUCIBLE 1 [SNI1]). However, only SMC6B,

150   NSE2, and NSE4A have been firmly associated with DNA damage repair (Watanabe et al.,

151   2009; Liu et al., 2015; Díaz et al., 2019). Our understanding of biological events controlled by

152   the plant SMC5/6 complex and its individual subunits is rather limited. Mutants defective in

153   each subunit are hypersensitive to DNA-damaging treatments, show delayed repair of DNA

154   strand breaks, and accumulate toxic replication intermediates originating during somatic and

155   meiotic HR (Liu et al., 2015; Diaz et al., 2019; Nowicka et al., 2020; Yang et al., 2021), but

156   the exact repair mechanism is unknown.

157   Recently, we showed that zebularine caused enzymatic DPCs in Arabidopsis by

158   covalently trapping the DNMT1 ortholog MET1 to DNA (Prochazkova et al., 2022). The

159   presence of zebularine-induced DPC is signaled by both ATM and ATR kinases (Liu et al.,

160   2015) and triggered genome instability (Nowicka et al., 2020). Here, we introduce a forward

161   genetic screen aimed at the identification of genes involved in the repair of zebularine-

162   induced DNA damage and present the first mapped complementation group

163   *HYPERSENSITIVE TO ZEBULARINE 1* (*HZE1*, pronounced as "haze", to refer to the long

164   unclear DNA damaging effects of zebularine). We mapped the high-effect candidate gene

165   *HZE1* to the *SMC6B* locus. Using several DNA-protein crosslinking agents and constructing

166   higher-order mutants, we show that SMC5/6 repairs DPCs in parallel to known DPC repair

167   pathways. Furthermore, our data suggest that the SUMOylation of MET1-DPCs by the

168   SMC5/6 complex is involved in DPC repair.

169   **RESULTS**

170   **A forward genetic screening identifies *HZE1* as *SMC6B***

171   For the genetic screen, we mutagenized seeds of the Arabidopsis W35 line (Willing et

172   al., 2016) (hereby referred to as wild type, WT) with ethyl methanesulfonate (EMS) and

173   screened M2 seedlings for a decrease in root length when grown on half-strength Murashige

174   and Skoog (MS) medium containing 7.5 µM zebularine. We validated the candidate mutant

175   using M_3 seedling grown in the presence of 20 µM zebularine; we considered the candidates

176   as positive when showing at least a 60% reduction in root length compared to their mock-

177   treated control (for details, see Materials and Methods and Supplemental Figure S1). For

178   reference, the reduction in root length of treated WT seedlings relative to untreated WT was

179   40%. The first candidate identified in the screen showed an over 90% reduction in root length

180   (9.4% ± 2.7% of mock-treated seedlings), indicating a strong sensitivity to zebularine (Figure

181   1A-C, Supplemental Table S1). We named this candidate *hze1-1* for *hypersensitive to*

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182 *zebularine 1*. We performed mapping-by-sequencing (MBS) to identify the causal gene using
183 a pool of ~100 F_2 zebularine-sensitive seedlings derived from a backcross to WT
184 (Supplemental Figure S2A). We located the *hze1-1* mutation to the telomere-proximal region
185 on the bottom arm of chromosome 5 (Supplemental Figure S2B). We analyzed this region for
186 moderate to high-effect mutations within protein-coding regions and identified a G-to-A
187 transition in *SMC6B* at 3,627 bp downstream from the ATG (Figure 1D, Supplemental Figure
188 S2C), resulting in a D513N substitution. Notably, the putative causal mutation in *hze1-1* was
189 located within the SMC6B hinge domain (Figure 1E, 1F) in the highly conserved α-helix of
190 subdomain I (Alt et al., 2017) that is responsible for proper folding (Figure 1F). Homology
191 modeling using the budding yeast SMC6B crystal structure (Hallett et al., 2022) as template
192 revealed that the D513N substitution likely causes a subtle change in charge of the SMC6B
193 hinge domain, which may affect proper folding of the hinge domain (Figure 1F). We validated
194 *SMC6B* as the causal gene by analyzing sensitivity to zebularine in F_1 seedlings from a cross
195 between *hze1-1* and *smc6b-1,* which confirmed that *HZE1* is allelic to *SMC6B* (Supplemental
196 Figure S3).

197 To assess whether other mutant alleles in our collection affect *SMC6B*, we analyzed
198 the phenotypes of the remaining selected *hze* mutants on zebularine and found four
199 additional zebularine hypersensitive but otherwise phenotypically WT-like candidates. We
200 performed complementation crosses between these candidates and *smc6b-1,* followed by
201 zebularine sensitivity assays, which suggested that they are all allelic (Supplemental Figure
202 S3). Consequently, these candidates were named *hze1-2* to *hze1-5* (Figure 1A-D,
203 Supplemental Figure S3, Supplemental Table S1). We sequenced their *SMC6B* cDNA by
204 Sanger sequencing and modeled the effect of the identified substitutions using the *in silico*
205 predicted SMC6B structure (Figure 1D, 1E). In *hze1-2*, we detected a G-to-A transition 7,233
206 bp downstream of the *SMC6B* ATG, which overlapped with a splicing donor/acceptor site.
207 The *hze1-2* mutation resulted in alternative splicing of exon 28 that generated a 10-bp
208 deletion, introducing a premature stop codon in the sequence encoding the Walker B motif of
209 the ATPase head domain of SMC6B (Figure 1D, 1E). In *hze1-3*, we initially did not find any
210 mutations, but we failed to amplify one genomic region using primer pairs validated on WT
211 genomic DNA. We hypothesized that this region might be rearranged and therefore used
212 inverse PCR for isolation. Indeed, the sequencing of inverse PCR products suggested a
213 reciprocal translocation between chromosomes 5 and 4 with a breakpoint 5,472 bp
214 downstream of the *SMC6B* ATG and its fusion with a fragment of *NEXT TO BRCA1 GENE 1*
215 (*NBR1*) (Figure 1D, 1E, Supplemental Figure S4). We confirmed the translocation by a
216 standard PCR assay with individual primers positioned in *SMC6B* and *NBR1,* respectively
217 (Supplemental Figure S4). The *hze1-4* mutant carried a G-to-A transition 1,332 bp
218 downstream of the *SMC6B* ATG (Figure 1D, 1E, Supplemental Figure S3), which overlapped

with a splicing donor/acceptor site and resulted in the deletion of four amino acids (181–184, ΔFFFK) in the DNA-binding motif of the ATPase head domain SMC6B (Yu et al., 2022). The *hze1-5* mutant had a G-to-A transition 264 bp downstream of the *SMC6B* ATG (Figure 1D, 1E, Supplemental Figure S3), which overlapped with a splicing donor/acceptor site and caused the retention of the 3rd intron in the final transcript. This retained intron added nine amino acids and a premature stop codon in the ATPase head domain (after amino acid 97). To exclude the possibility that sensitivity to zebularine was due to an SMC6B function independent from the SMC5/6 complex, we also tested *nse4a-2,* which carries a mutation in the kleisin subunit of the complex (Díaz et al., 2019) and confirmed its strong sensitivity to 20 µM zebularine (Figures 1A-C). Analyses of root cell viability using propidium iodide (PI) staining revealed an increased number of dead cells in the root meristematic zone of *hze1* and control *smc6b-1* and *nse4a-2* seedlings (Figure 1C). The *nse4a-2* seedlings showed fewer dead cells, most likely because this is not a null mutant allele (Díaz et al., 2019).

In conclusion, we identified five new EMS-induced *SMC6B* mutant alleles, *hze1-1* to *hze1-5* (corresponding to *smc6b-5* to *smc6b-9* alleles), and showed that the SMC5/6 complex participates in the repair of zebularine-induced type-1 DPCs.

The SMC5/6 complex is also involved in the repair of TOP1 and TOP2 DPCs

The severity of *hze1* hypersensitivity to zebularine raised the question as to whether the SMC5/6 complex might also be involved in the repair of other types of DPCs. Accordingly, we analyzed root length in response to 20 nM CPT treatment. CPT crosslinks TOP1 and induces type-3 DPCs (Hacker et al., 2020). Root length in CPT-treated WT seedlings was 41.9% ± 0.7% that of untreated control seedlings, while *smc6b-1* mutant seedlings displayed a significantly stronger reduction in root length, reaching only 23.4% ± 2.6 of the original root length (one-way ANOVA and Tukey's HSD post hoc test, $P < 0.05$; Figure 2A, C; Supplemental Table S2). In addition, CPT treatment shortened the meristematic zone and increased cell death in the roots of *smc6b-1* seedlings (Figure 2D). We observed a similar sensitivity in all tested *hze1* alleles (Figure 2A, 2B, 2D). Although *nse4a-2* seedlings showed a relatively strong reduction in root length (50.3% ± 8.3%), this effect was not significantly different from that of WT seedlings ($P = 0.169$), consistent with the classification of the *nse4a-2* mutant as a hypomorphic allele (Diaz et al., 2019). In agreement with published data, we observed sensitivity to CPT for *mus81-1* and *wss1a-1* seedlings, but not for *tdp1-3* or *tdp2-5* (Supplemental Figure S5B, S5D, S5E) (Enderle et al., 2019; Hacker et al., 2022).

Second, we analyzed the role of the SMC5/6 complex in the repair of type-4 DPCs, typically associated with the crosslinking of TOP2 to its cleavage sites. This type of DPC is also caused by the TOP2 poison etoposide, which inhibits the religation of cleaved DNA

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segments, resulting in TOP2 binding to the cleaved DNA ends (reviewed in Nitiss et al., 2009). Because etoposide shortens root length only at very high concentrations (Hacker et al., 2022), we screened several other TOP2 poisons and inhibitors used in mammalian research and observed a strong effect after treatment with bisdioxopiperazine dexrazoxane (ICRF-187) (Figure 2B-D, Supplemental Table S2). Seedlings from *smc6b-1*, *nse4a-2,* and all tested *hze1* alleles (*-1* to *-3*) showed a massive root length reduction to less than 25% of mock-treated controls, while the root length in WT seedlings was only weakly affected in response to 10 µM ICRF-187, with a root length of 81.0% ± 1.7% relative to the mock treatment. In all cases with significantly reduced root length, we also detected more dead cells in the root meristems of *smc6b-1*, multiple alleles of *hze1* and *nse4a-2* seedlings (Figure 2D). We also observed hypersensitivity to ICRF-187 in *wss1a-1,* which was in agreement with the known function of WSS1A in the repair of TOP2 crosslinks (Hacker et al., 2022) (Supplemental Figure S5C-E, Supplemental Table S3). ICRF-187 is a highly-specific TOP2 inhibitor that links the interface between two ATPase protomers of TOP2 (Classen et al., 2003; Nitiss et al., 2009; Lee et al., 2017, 2022). To test the role of the SMC5/6 complex in the repair of type-2 crosslinks, we tested several chemicals known to induce Poly (ADP-ribose) polymerase 1 (PARP1) DPCs in mammalian cells (Waldman and Waldman, 1990; Bernges and Zeller, 1996; Menear et al., 2008). However, there were no visible differences between WT and *smc6b-1* (Supplemental Figure S6), preventing us from evaluating the repair of type-2 crosslinks. Collectively, these findings provide strong evidence that the SMC5/6 complex is a critical component in the repair of different types of DPCs and establish ICRF-187 as a new drug for plant DPC repair research.

SMC6B, MUS81, and WSS1A function non-redundantly during the repair of endogenous DNA damage

The endonuclease MUS81 and the protease WSS1A are required for DPC repair in Arabidopsis (Enderle et al., 2019). To uncover a possible genetic interaction between the SMC5/6 complex and these factors, we generated *mus81-1 smc6b-1* and *wss1a-1 smc6b-1* double mutant plants and analyzed them under mock conditions with spontaneously occurring DNA damage (Figure 3A, B, Supplemental Table S4). The root length of *smc6b-1* (1.18 cm ± 0.11) and *mus81-1* (1.41 cm ± 0.11) seedlings was comparable to that of WT seedlings (1.34 cm ± 0.03), while the roots of *wss1a-1* (0.44 cm ± 0.05 cm) were significantly shorter (one-way ANOVA and Tukey's HSD post hoc test, P < 0.05, note: the same test was used throughout this section). The *smc6b-1 mus81-1* double mutant grown under mock conditions showed a significant 75% reduction in root length (0.31 cm ± 0.06 cm) relative to mock-treated WT (Figure 3A, B). Furthermore, we observed more dead cells in the root meristems of *smc6b-1 mus81-1* seedlings compared to WT and the respective single

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291   mutants (Figure 3C). This increased number of dead cells was accompanied with modest

292   changes in root morphology (Figure 3C). The roots of *smc6b-1 wss1a-1* seedlings (0.14 cm ±

293   0.01 cm) showed a drastic 90% length reduction relative to WT (Figure 3A, B), and their

294   anatomy was compromised with irregularly positioned and sized cells, a minimal

295   meristematic zone, and root hairs close to the root tip (Figure 3C). Although the total number

296   of dead cells in this double mutant appeared similar to that of *wss1a-1* seedlings (Figure 3C),

297   we speculate that this may reflect a bias caused by generally fewer cells in the root meristem

298   and transition zones. Adult *smc6b-1* and *mus81-1* single mutant plants were

299   indistinguishable from WT, but the *smc6b-1 mus81-1* double mutant showed severe growth

300   defects, including tiny rosettes and a shorter stem height by about 40% (Figure 3D, 3F).

301   Similarly, *smc6b-1 wss1a-1* double mutant plants also had a smaller rosette size and were

302   generally shorter compared to WT and single mutant controls (Figure 3D, 3F). Altogether,

303   these results indicate that the SMC5/6 complex functions in pathways parallel to MUS81

304   and/or WSS1A in the repair of spontaneously occurring DNA damage.

305   To explore whether the SMC5/6 complex contributes to both the MUS81 and WSS1A

306   pathways or whether it represents an independent yet unidentified pathway, we generated

307   the *smc6b-1 mus81-1 wss1a-1* triple mutant by crossing the above described homozygous

308   double mutant plants. We grew three independent *smc6b-1 mus81-1/MUS81 wss1a-*

309   *1/WSS1a* F1 plants and expected 25% triple homozygous offspring upon selfing. However,

310   we obtained only 1% to 4% seedlings with the triple mutant genotype (2 out of 200, 4 out of

311   200, and 8 out of 200, in three independent replicates), indicating an additive effect on plant

312   lethality. Several triple homozygous mutant plants were at least partially fertile, allowing us to

313   analyze the phenotype of the progeny closer (Figure 3D, 3F). We selected the ten best-

314   looking plants for each double and triple mutant combinations from equally sized populations

315   (Figure 3D). The *smc6b-1 mus81-1 wss1a-1* plants showed stunted growth compared to the

316   respective double mutant plants, never developed proper roots or shoots, and were smaller

317   compared to the double mutants. Moreover, they were often dark-colored after prolonged

318   cultivation on MS medium (Figure 3F).

319   Based on these findings, we conclude that the SMC5/6 complex, MUS81, and

320   WSS1A function in at least partially unique pathways during the repair of spontaneously

321   occurring DNA damage.

322   **SMC5/6, MUS81, and WSS1A act additively during the repair of zebularine-induced**

323   **DPCs**

324   To uncover whether the SMC5/6 works together with MUS81 and WSS1A in the

325   repair of type-1 DPCs, we tested the zebularine sensitivity of *mus81-1 smc6b-1* and *wss1a-1*

326   *smc6b-1* double mutant plants, which revealed a dose-dependent phenotype (Figure 4A,

327 Supplemental Table S5). The double mutants showed a significant additive hypersensitivity,
328 compared to both single mutants, in response to a low zebularine concentration of 5 µM
329 (one-way ANOVA and Tukey's HSD post hoc test, $P < 0.05$). On the contrary, a higher
330 zebularine concentration of 20 µM fully inhibited *smc6b-1* growth, and we observed no
331 further enhancement of sensitivity in the double mutants. The PI staining of 20 µM
332 zebularine-treated *smc6b-1 mus81-1* seedlings showed moderately altered root anatomy
333 with uneven cell files and a similar number of dead cells as in the root meristematic zone of
334 *smc6b-1* seedlings (Figure 4B). That differences are best observed at the lower zebularine
335 concentration for the fresh weight assay, and at the higher concentration in the cell death
336 assays reflects the different durations of these experiments.

337   WSS1/SPRTN proteins have a unique role in DPC repair (Stingele et al., 2016;
338 Reinking et al., 2020). Therefore, testing the sensitivity of *smc6b-1 wss1a-1* to zebularine
339 allowed us to unambiguously test the role of the SMC5/6 complex in DPC repair (Figure 4A,
340 Supplemental Table S5). Again, a high-dose zebularine treatment caused the greatest
341 sensitivity in *smc6b-1*, and we did not observe a further increase in the sensitivity of the
342 double mutants. For the 5-µM zebularine dose, we measured a statistically significant
343 additive effect (one-way ANOVA and Tukey's HSD post hoc test, $P < 0.05$) in the *smc6b-1*
344 *wss1a-1* seedlings compared to the WT and respective single mutants. Due to the severely
345 affected root anatomy and a high number of dead cells under mock conditions, we could not
346 precisely estimate the effect of zebularine on the number of dead cells within the root
347 meristematic zone of *smc6b-1 wss1a-1* seedlings (Figure 4B). In general, the roots were
348 short and appeared very thick with minute meristematic zones. Because of the severe
349 developmental phenotype of the *smc6b-1 mus81-1 wss1a-1* triple mutants, we were not able
350 to analyze their response to zebularine.

351   In conclusion, our findings indicate that the SMC5/6 complex acts in a parallel
352 pathway to MUS81 for the repair of zebularine-induced DPCs. Moreover, we provide solid
353 genetic evidence that the SMC5/6 complex is specifically involved in DPC repair and
354 functions in the pathway(s) parallel to WSS1A protease.

SUMOylation targets MET1 crosslinks in an SMC5/6-dependent manner

356   In animals, proteins covalently trapped to DNA are targeted by SUMOylation
357 (Borgermann et al., 2019; Liu et al., 2021; Ruggiano et al., 2021). The SMC5/6 complex
358 contains the evolutionary conserved E3 SUMO ligase subunit NSE2 (Varejão et al., 2018).
359 Therefore, we wondered whether SMC6B might link the repair of zebularine-induced DPCs
360 with the SUMOylation activity of the SMC5/6 complex. We previously showed that zebularine
361 induces cytologically detectable DPC arrays at *45S* rDNA in Arabidopsis by crosslinking a
362 large number of fluorescently-tagged MET1 (Prochazkova et al., 2022). To establish whether

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363 zebularine-induced MET1-DPCs are targeted for SUMOylation, we analyzed SUMO
364 enrichment at MET1-RFP (red fluorescent protein) foci after zebularine treatment (Figure
365 5A). To this end, we performed immunolabeling with antibodies specific to SUMO1 or
366 SUMO3 on MET1-RFP positive nuclei isolated by flow sorting from mock- and zebularine-
367 treated wild-type plants as described (Prochazkova et al., 2022). We observed no
368 immunostaining of foci with antibodies against SUMO3 (Supplemental Figure S7). By
369 contrast, SUMO1 showed dispersed signals under mock conditions but largely colocalized
370 with MET1-RFP foci after a 40-µM zebularine treatment (Figure 5A, 5B). To test whether
371 SMC5/6 is responsible for the zebularine-induced deposition of SUMO1 on MET1-DPCs, we
372 repeated the immunolabeling with the anti-SUMO1 antibody on MET1-RFP positive nuclei
373 from *smc6b-1* seedlings. Indeed, most zebularine-stimulated SUMO1 localization of MET1-
374 RFP foci was effectively abolished in the *smc6b-1* background (Figure 5A, B, D). Wild-type
375 plants showed 81% ± 9% colocalization between MET1-RFP and SUMO1 signals upon
376 zebularine treatment, while *smc6b-1* reached only 22% ± 4% (Figure 5D). To unequivocally
377 support a role for the SMC5/6 complex in SUMOylation at MET1-RFP foci, we repeated the
378 experiment with the *nse2-2 MET1-RFP* line. As with *smc6b-1*, about 24% ± 3% of *nse2-2*
379 nuclei showed SUMO1 colocalization with MET1-RFP (Figure 5A, B, D).

380 This finding shows that the SMC5/6 complex adds SUMO1 to crosslinked MET1-RFP
381 upon zebularine treatment, thus highlighting the importance of the SMC5/6 complex in
382 SUMOylation of DPCs. The persistence of SUMO1 at around 20% of MET1-RFP foci also
383 suggests a role for another E3 SUMO ligase in labeling a subset of DPCs.

384 **DISCUSSION**

385 Here, we describe the SMC5/6 complex as an important component involved in the
386 repair of DNA-protein crosslinks, possibly through its E3 SUMO ligase activity. DPCs are
387 highly toxic DNA adducts that represent a major threat to the maintenance of genome
388 integrity (reviewed in Weickert and Stingele, 2022). DPCs, such as TOP1 crosslinks, are
389 formed during normal plant metabolism but are rapidly removed through a number of repair
390 pathways involved in their elimination. Unlike the repair of other types of DNA damage,
391 detoxification of DPCs has been studied in depth only recently (Stingele and Jentsch, 2015;
392 Vaz et al., 2016; Enderle et al., 2019; Larsen et al., 2019; Reinking et al., 2020; Liu et al.,
393 2021). In plants, three major DPC repair pathways have been described to date: the
394 nucleolytic pathway, which is hallmarked by the structure-specific endonuclease MUS81
395 (Enderle et al., 2019); the DPC-specific proteolytic pathway that depends on the
396 WSS1/Spartan metalloproteases (Stingele et al., 2016; Enderle et al., 2019); and the direct
397 hydrolytic pathway represented by TDP1 and TDP2 (Enderle et al., 2019; Tsuda et al.,

398 2020). We show that the SMC5/6 complex represents an independent or overarching DPC
399 repair pathway.

400   The function of the SMC5/6 complex is traditionally associated with DNA damage
401 repair and maintenance of genome stability. Using a forward genetic screen, we identified
402 five loss-of-function mutant alleles in *HYPERSENSITIVE TO ZEBULARINE 1* (*HZE1*), a
403 putative key player involved in the repair of zebularine-induced DPCs based on the strong
404 sensitivity of its mutants. The *HZE1* complementation group was allelic to *SMC6B*, a gene
405 encoding the core subunit of the SMC5/6 complex. The *hze1-2* mutant harbors a mutation in
406 the ATPase domain and most likely leads to a catalytically dead SMC6B. The *hze1-3* allele
407 carries a large translocation in the 3' end of the gene, effectively breaking the gene into two
408 fragments. The *hze1-4* allele lacks four amino acids in the ATPase head domain necessary
409 for interaction with DNA (Yu et al., 2022). Finally, the *hze1-1* allele represents a unique
410 mutation within subdomain I of the hinge region (Alt et al., 2017). Alignment of this region
411 from SMC6 homologs from different organisms revealed that it is highly conserved among
412 plant, fungal and animal species (Supplemental Figure S8). It is likely that the Asp-to-Asn
413 mutation in *hze1-1* results in aberrant chemical properties of the hinge domain, thus affecting
414 its structure and/or flexibility.

415   We selected the HZE mutants based on their sensitivity to zebularine, which is a
416 cytidine analog incorporated into DNA during replication and enables irreversible trapping of
417 the DNA methyltransferase MET1 in plants (Prochazkova et al., 2022). The exact repair
418 pathway of zebularine-MET1 DPCs is not known but sensitivity studies indicate that the
419 homologous recombination pathway is involved (Liu et al., 2015; Nowicka et al., 2020). The
420 isolation of *SMC6B* in our mutant screen is also in agreement with the role of the SMC5/6
421 complex, as SMC6B is required for efficient DNA damage repair by homologous
422 recombination (Mengiste et al., 1999; Potts et al., 2006; Watanabe et al., 2009). The
423 sensitivity to zebularine of a partial loss-of-function mutant in the kleisin subunit NSE4A
424 indicates that the SMC5/6 complex is involved in the DPC repair as a whole (Díaz et al.,
425 2019).

426   Here we show that the SMC5/6 complex is a universal player in DPC repair, as it was
427 not only involved in the repair of MET1 crosslinked to DNA by zebularine (representing type-
428 1 DPCs) but also in the repair of crosslinked TOP1 (type-3) and TOP2 (type-4).
429 Topoisomerases are enzymes that introduce transient DNA breaks to relax supercoiled or
430 intertwined DNA, thus allowing replication- and transcription-associated complexes to
431 proceed and sister chromatids to separate. In search of effective TOP2 inhibitors in plants,
432 we tested several compounds used for animal research and identified ICRF-187 as a new
433 highly potent crosslinker. The most substantial effects were when the drug was applied at
434 early stages of seedling development, possibly concomitant with the large number of

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435 replicating nuclei. ICRF-187 crosslinks TOP2 in the ATP-associated state around double-
436 stranded DNA (dsDNA), hence creating a crosslink on DNA that is not associated with DNA
437 strand break (Classen et al., 2003; Lee et al., 2022). By contrast, cytidine analogs have a
438 very distinctive mode of action. Drugs like 5-azacytidine are incorporated into DNA and act
439 as a pseudosubstrate for DNA methyltransferases (DNMTs), resulting in the covalent
440 trapping of the enzyme without the primary presence of single-stranded (SSBs) or double-
441 stranded breaks (DSBs). Repairing such crosslinks may lead to DSBs (Maslov et al., 2012).
442 We did not observe increased sensitivity of *tdp1-3* or *tdp2-5* seedlings to zebularine-induced
443 DPCs. This result is in agreement with the fact that zebularine induces type-1 DPCs (Hacker
444 et al., 2020) that lack the phosphodiester bond, a common substrate for TDP1 and TDP2.
445 Therefore, the role of the SMC5/6 complex appears more general than that of other DPC
446 repair factors and likely DPC type-independent.

447   It is tempting to speculate how the SMC5/6 complex is involved in DPC repair (Figure
448 6). It has been shown that each of the two SMC6 homologs in Arabidopsis is required for the
449 efficient repair of DNA breakage via intermolecular homologous recombination in somatic
450 cells (Watanabe et al., 2009). Alignment of sister chromatids is enhanced transiently after X-
451 ray irradiation (and mitomycin C treatment) in WT nuclei. In the SMC5/6 complex mutants,
452 the X-ray–mediated increase in sister chromatid alignment is much lower and delayed than in
453 WT. Therefore, we hypothesize that the function of the SMC5/6 complex might be required
454 for the use of the sister chromatid as a template for repair. This mode of action might not only
455 be restricted to the repair of replicative DSBs by HR but also by post-replicative DPC repair
456 (Liu et al., 2021) in which a template-switching mechanism using the sister chromatid might
457 be involved (Torres-Ramos et al., 2002; Chen et al., 2008).

458   Interestingly, protein degradation by the proteasome in the replication-independent
459 pathway depends on the prior SUMOylation of the respective proteins (Liu et al., 2021).
460 Conjugation of SUMO has previously been described for several naturally occurring and
461 chemically-induced DPCs, including TOP1, TOP2, and DNMT1 in animals and yeast
462 (Schellenberg et al., 2017; Borgermann et al., 2019; Liu et al., 2021; Serbyn et al., 2021).
463 The E3 SUMO ligase activity of the SMC5/6 complex might mark crosslinked proteins for
464 degradation (Figure 6) and/or for conjugation with other factors promoting the repair. Hence,
465 SUMOylation via the SMC5/6 complex might be a mechanism integrating and orchestrating
466 various DPC repair pathways in plants. Interestingly, the SUMOylation activity of the SMC5/6
467 complex is unique among all SMC complexes and canonical DNA damage repair factors.
468 Arabidopsis genome encodes eight SUMO proteins, and four of the encoding genes are
469 expressed (*SUMO1, 2, 3, 5*) (Hammoudi et al., 2016). We discovered here that SUMO1, but
470 not SUMO3, is involved in DPC modification and that this is largely SMC5/6 complex-
471 dependent.

13

472   In summary, we identified SMC6B from our forward genetic screen for factors
473 contributing to the repair of zebularine-induced DNA-protein crosslinks. SMC6B is a core
474 subunit of the SMC5/6 complex that functions in several DPC repair pathways. We propose
475 that SUMOylation mediated by this complex plays an important role in DPC repair. Further
476 screening and identification of their candidates is in progress and provides a high potential to
477 identify and characterize additional DPC repair factors in Arabidopsis.

478 **MATERIALS AND METHODS**

479 **Plant materials**

480 Arabidopsis (*Arabidopsis thaliana*) wild type and mutants in the Col-0 background (unless
481 stated otherwise) were used in this study: *smc6b-1* (SALK_123114C), *nse4a-2* (GK-
482 768H08), *nse2-2* (SAIL_77_G06), *mus81-1* (GABI_113F11, (Hartung et al., 2006)), *wss1a-1*
483 (CRISPR/Cas9 line, (Enderle et al., 2019)), *tdp1-3* (CRISPR/Cas9 line with a 1-bp insertion
484 in exon 1 of *TDP1*, (Enderle et al., 2019)), *tdp2-5* (CRISPR/Cas9 line with a 5-bp deletion in
485 exon 1 of *TDP2*, (Hacker et al., 2022)). The double mutants were generated by crossing
486 homozygous single mutants and identification in the F2 generation by PCR-based
487 genotyping. Plants homozygous for the *wss1a-1* mutation were identified by Sanger
488 sequencing of PCR products spanning the mutated site. The primers used for genotyping are
489 listed in Supplemental Table S7. Plants were cultivated in an air-conditioned phytochamber
490 under a long-day photoperiod (16-h light, 150 µmol m^{-2} s^{-1}, 21°C, 8-h dark, 19°C; lights
491 provided by fluorescent tubes MASTER TL-D 18W/840, Philips). For the drug sensitivity
492 assays, seeds were surface-sterilized using 8% (w/v) sodium hypochlorite solution for 6 min,
493 followed by three washes in sterile H_2O, stratification for 2 days at 4°C in the dark. Seeds
494 were evenly distributed on plates containing half-strength Murashige and Skoog medium
495 (MS) with 0.6% (w/v) agar and with or without the addition of DNA-protein crosslinking
496 chemicals, depending on the experimental setup.

497 ***DCPR forward-directed genetic screen setup, candidate mapping-by-sequencing***
498 ***(MBS), and inverse PCR***

499 For the HZE genetic screen (Supplemental Figure S1), an ethyl methanesulfonate (EMS)-
500 mutagenized population was used in the W35 reporter line (Col-0 carrying *ProUVR2:UVR2-*
501 *LUCIFERASE*) background (Willing et al., 2016). The reporter line behaves as wild type, and
502 *UVR2* expression was not monitored in this study. About 10,000 seeds were soaked in 0.1%
503 (w/v) KCl and shaken at 4°C for 8 h; seeds were then washed in distilled water and
504 incubated in 0.2% (v/v) EMS solution at room temperature for 12 h to induce mutations.
505 Afterwards, seeds were washed 2 × 5 min with 100 mM sodium thiosulfate and 3 × 5 min
506 with water. Finally, the seeds were resuspended in 0.1% (w/v) agarose and spread onto soil
507 surface at a density of ~100 seeds per 18 × 14 cm tray. All M1 plants were grown until

maturity; seeds of all plants from one tray were collected together, resulting in 100 M2 seed batches. Approximately 1,500 seeds per M2 batch were surface-sterilized with 8% (w/v) sodium hypochlorite for six min, followed by three washes with sterile water, resuspension in 0.1% (w/v) agarose, and 1,600 seeds were evenly sown onto plates filled with half-strength MS medium containing 20 µM zebularine using a pipette with a sterile cut plastic tip. Each plate included the zebularine-sensitive control *smc6b-1* and resistant wild-type controls. Seedlings were grown in a phytochamber under long-day conditions for 10 days. Afterwards, the plates were visually inspected, and primary candidates with short roots were transferred to soil and grown until maturity, and their M3 seeds were collected. Each primary M2 candidate was further analyzed by phenotyping the M3 generation on half-strength MS medium without or with 20 µM zebularine (approximately 30 seedlings per experimental point). Based on the phenotype, each candidate was classified into one of the following categories: (i) developmental mutants with short roots on both control and zebularine-containing media; (ii) false positive with less than a 60% reduction in root length on control medium compared to zebularine-containing medium; (iii) true candidates with at least a 60% reduction in root length on control medium compared to zebularine-containing medium. Only type (iii) candidates were considered for further work.

The candidates selected for mapping were backcrossed to the non-mutagenized wild type Col-0, and the resulting F2 population was screened on half-strength MS medium containing 20 µM zebularine. Segregation of the zebularine-sensitive phenotype was assessed, which typically matched the expected segregation pattern for a single nuclear recessive locus. About 75 to 150 zebularine-sensitive seedlings were collected, pooled, and their genomic DNA was isolated using a NucleoSpin Plant II kit (Macherey-Nagel). Genomic DNA was sent for sequencing (Novogene LTD, Cambridge, UK), as paired-end 150-bp reads on a Novaseq platform to approximately 50x coverage. Sequencing data were analyzed using bioinformatics tools available at the public platform usegalaxy.org as described (Prochazkova et al., 2022). The clean reads were mapped to the *Arabidopsis thaliana* reference genome (TAIR10) with bowtie2 using default settings (Langmead et al., 2009; Langmead and Salzberg, 2012). Read sorting, SNP calling, and filtering were performed using tools from the MiModD tool set (https://celegans.de/mimodd/) and annotated with the snpEff tool (Cingolani et al., 2012). Sequencing data of the mapping populations were deposited at the NCBI Sequence Read Archive under accession number PRJNA730368. Mapping information of respective candidates was uploaded to the UCSC Genome Browser with the following IDs: http://genome-euro.ucsc.edu/s/KlaProche/candidate%208%2D13%20a.k.a.%20dpcr1.

The *SMC6B* locus was sequenced in the candidates identified as *smc6b* mutants via complementation crosses with *smc6b-1*. Total RNA extraction and first-strand cDNA

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545 synthesis were performed as described previously (Nowicka et al., 2020). The *SMC6B*
546 transcript was divided into six regions, amplified with specific primers (Supplemental Table
547 S1) and PCR products were sequenced by Sanger sequencing. To identify the putative
548 rearranged region in *hze1-3*, inverse PCR was performed. Briefly, 2 µg genomic DNA was
549 digested with 10 units of *Xmn*I for 1 h, 50 ng of linear DNA was religated with 5 units of T4
550 DNA ligase for 1 h at room temperature, and the sample was used as a template for
551 amplification with *SMC6B*-specific primers. The resulting PCR product was cleaned with
552 ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher) and sequenced by Sanger
553 sequencing.

554 **Root length assays and phenotypical analyses of mutant plants**
555 Stratified, surface-sterilized seeds were evenly sown on square culture plates with half-
556 strength MS medium with 0.8% (w/v) agar, and placed horizontally for 7 d. Subsequently, the
557 seedlings were carefully pulled off the agar surface with tweezers and stretched onto fresh
558 agar plates. Seedlings were photographed with a D90 digital camera (Nikon), and the length
559 of the primary root was measured using the ImageJ plugin SmartRoot (Lobet et al., 2011).
560 Detailed photographs were collected using an SZX16 binocular microscope equipped with a
561 Regita 1300 QImaging camera and QCapture x64 software (both Olympus).
562 For sensitivity assays of single mutants, seeds were germinated on half-strength MS medium
563 with 0.8% (w/v) agar containing individual chemicals: 20 µM zebularine (Z4775, Sigma-
564 Aldrich), 20 nM (*S*)-(+)-camptothecin (CPT, C9911, Sigma-Aldrich), 10 µM ICRF-187 (D1446,
565 Sigma-Aldrich), 100 nM, 10 µM and 100 µM AZD2461 (SML1858, Sigma-Aldrich), 100 nM,
566 100 µM and 1 mM 3-methoxybenzamide (M10050, Sigma-Aldrich), 100 µM, 1 mM and 4 mM
567 3-aminobenzamide (A0788, Sigma-Aldrich). Sensitivity to each chemical treatment in
568 individual replicates was determined by calculating mean (treatment) / mean (mock). The
569 experiment was performed as three biological replicates, each with at least 20
570 seedlings/replicate. The means of the three replicates are shown. Statistical significance was
571 tested by one-way analysis ANOVA with post-hoc Tukey HSD in R (Core R Team, 2020).

572 **Drug sensitivity assays**
573 Drug sensitivity assays were performed as described (Dorn and Puchta, 2020). Stratified,
574 surface-sterilized seeds were sown on culture plates with half-strength MS medium with
575 0.6% (w/v) agar, and cultivated for seven days. Subsequently, ten seedlings of each
576 genotype were transferred to a six-well culture plate containing 5 mL of liquid half-strength
577 MS medium (untreated control) or 4 mL of liquid half-strength MS medium (treated samples)
578 per well under sterile conditions. The next day, 1 ml of genotoxin solution diluted in liquid
579 half-strength MS medium was added to obtain the desired final concentration. Seedling fresh
580 weight was measured after 13 days of exposure. Relative fresh weight was determined by

581 comparison of fresh weight between treated and untreated samples for each genotype and

582 concentration. The experiment was performed as three biological replicates, and the means

583 of the three replicates are shown.

584 **Cell death analyses in roots**

585 Seeds were sown on plates containing half-strength MS medium with 0.6% (w/v) agar and

586 grown vertically for five days before transfer to liquid half-strength MS medium without

587 (mock) or with 20 µM zebularine, 20 nM CPT or 10 µM ICRF-187 for 24 h. Afterwards, the

588 seedlings were placed in 10 mg mL^{-1} propidium iodide (PI, Sigma) on slides and immediately

589 analyzed and photographed using a Leica confocal microscope TCS SP8 (Leica, Wetzlar,

590 Germany) and HC PL APO CS2 20x/0.75 DRY objective equipped by Leica LAS-X software

591 with Leica Lightning module laser scanning confocal microscope (Leica). The pattern was

592 checked in at least ten individual seedlings per treatment.

593 **Immunostaining and confocal microscopy**

594 Immunostaining was performed as previously described (Prochazkova et al., 2022). Briefly,

595 5-day-old seedlings were incubated in 0 (mock) or 40 µM zebularine for 24 h. Seedlings were

596 fixed with 4% (w/v) formaldehyde in Tris buffer (10 mM Tris-HCl pH 7.5, 10 mM NaEDTA,

597 100 mM NaCl) at 4°C for 20 min and washed 2 × 10 min with Tris buffer at 4°C. Seedlings

598 were chopped in 500 µL LB01 buffer and filtered through 50-µm and 20-µm cell strainer

599 caps. Flow cytometry analysis and sorting were carried out on a FACSAria II SORP flow

600 cytometer and sorter (Becton Dickinson Immunocytometry Systems, San José, USA). The

601 samples were analyzed at rates of 400–1,400 particles per second. Bivariate flow karyotypes

602 of PI pulse area (PI-A) vs. DAPI pulse area (DAPI-A) fluorescence were acquired, and

603 20,000 events were recorded to create a bivariate flow karyotype for each experiment.

604 Sorted regions were set on the flow karyotypes, and RFP-positive nuclei were sorted onto

605 microscope slides with a 3-µL drop of PRINS buffer supplemented with 2.5% (w/v) sucrose

606 (Kubaláková et al., 1997). Around 3,000 nuclei were sorted per slide. Slides were post-fixed

607 with 4% (w/v) formaldehyde in phosphate-buffered saline (PBS) for 15 min and washed with

608 PBS. For immunolocalization of SUMO1 and SUMO2/3, slides were incubated with a rabbit

609 anti-SUMO1 or anti-SUMO3 primary antibody diluted 1:200 (ab5316 and ab5317, Abcam) at

610 4°C overnight, and a goat anti-rabbit Alexa Fluor 488-conjugated secondary antibody diluted

611 1:250 (A11008, Invitrogen) at room temperature for 2 h. The slides were shortly washed with

612 1× PBS, and nuclei were counterstained with DAPI (300 ng.µL^{-1}) and mounted in

613 Vectashield (H-1000, Vector Laboratories). Imaging was performed with a Leica confocal

614 microscope TCS SP8 (Leica 265 Microsystems) and HC PL PAO CS2 63×/1.4 OIL objective

615 equipped with Leica LAS-X software (Leica). Images were captured separately for each

616 fluorochrome with 546-nm (MET1-RFP), 488-nm (Alexa Fluor 488), and 405-nm (DAPI) laser

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617 lines for excitation and appropriate emission filters. Processing of the final images and

618 quantitative analysis of MET1-RFP colocalization with SUMO1 was performed in ImageJ

619 using a fluorescent intensity profile for both correlated signals. The respective colocalization

620 coefficients were calculated by Pearson's correlation coefficient in Microsoft Excel.

621 **Multiple sequence alignment**

622 Multiple sequence alignment was performed to elucidate the conservation level of mutations

623 in SMC6B. Sequence data of the proteins used in Supplemental Figure S8 were retrieved

624 from UniProt: Q9FII7 (Arabidopsis SMC6B), Q9FLR5 (Arabidopsis SMC6A), D7MV22

625 (*Arabidopsis lyrata* SMC6B), P53692 (*Schizosaccharomyces pombe* SMC6), Q12749

626 (*Saccharomyces cerevisiae* SMC6), Q96SB8 (*Homo sapiens* SMC6), Q8GU52 (*Oryza sativa*

627 SMC6), A0A0Q3L328 (*Brachypodium distachyon* SMC6), M4D8Z6 (*Brassica rapa* SMC),

628 R0G894 (*Capsella rubella* SMC), A0A1S3ZHR2 (*Nicotiana tabacum* SMC6), A0A3Q7GL50

629 (*Solanum lycopersicum* SMC6), A0A2K2B516 (*Populus trichocarpa* SMC6), D7U753 (*Vitis*

630 *vinifera* SMC6). Analyses were performed in the AliView program (Larsson, 2014).

631 **Protein structure analysis**

632 The model of AtSMC6B subunit (UniProt ACC: Q9FIIH) was built using SWISS-MODEL

633 (Bienert et al., 2017; Waterhouse et al., 2018). ScSMC6 (PBDID: 7qcd) (Hallett et al., 2022)

634 was used as a template. More detailed hinge models were generated using AlphaFold2. All

635 models were further processed in PyMOL (Schrödinger, LLC, 2015).

636 **Statistical methods**

637 To determine statistically significant effects, one-way analysis of variance (ANOVA) with

638 post-hoc Tukey HSD ($P ≤ 0.05$) tests were performed in R (Core R Team, 2020). Statistical

639 significance of colocalization between MET1-RFP and SUMO1 in the wild-type, *smc6b-1,*

640 and *nse2-2* backgrounds was calculated with a chi-square test.

641 **Accession numbers**

642 Sequencing data of the mapping populations were deposited at the NCBI Sequence Read

643 Archive under accession number PRJNA730368. Sequence data of the genes used in this

644 article can be found at TAIR under the following accession numbers: *SMC6B* (At5g61460),

645 *NSE4A* (At1g51130), *NSE2* (At3g15150), *MUS81* (At4g30870), *TDP1* (At5g15170), *TDP2*

646 (At1g11800), *WSS1A* (At1g55915).

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655 Czech Republic.
656 The authors declare no conflict of interest.

657 **Authors contributions**

658 AP, EDT, AF, and HP designed the research, EDT, FY, AF, KP, JJ, and MS performed the
659 analysis, AD, and HP developed CRISPR mutant, EDT, FY, KP, JJ, and AP analyzed the
660 data. EDT and AP wrote the article.

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692   Figure legends

693   **Figure 1.** *HYPERSENSITIVE TO ZEBULARINE 1* (*HZE1*) encodes the SMC5/6 complex
694   subunit SMC6B.
695   A, Representative growth phenotypes of seedlings from wild type (WT), *smc6b-1*, *hze1*
696   alleles and *nse42-2* on 0 (Mock) and 20 µM zebularine (ZEB). Scale bar, 1 cm. B, Relative
697   root length of seedlings in (A) under zebularine/mock conditions (% of ZEB/Mock). Data are
698   means ± SD from three biological replicates, each with a minimum of 14 seedlings. Different
699   lowercase letters indicate significant differences (P < 0.05), according to one-way ANOVA
700   followed by Tukey's test. Source data for statistical analyses are available in Supplemental
701   Table S1. The original experiment was split between Figures 1A-C, and Supplemental
702   Figures S5A-D. Therefore, these figures show identical images and data for the controls. C,
703   Representative confocal microscopy images of root tips stained with propidium iodide, which
704   indicates dead cells (dark sectors). Five-day-old seedlings were treated with 20 µM ZEB for
705   24 h prior to analysis. Scale bar, 100 µm. D, Schematic model of the *SMC6B/HZE1* locus
706   (At5g61460) with the positions of individual mutations. E, Detailed position of *hze1-1* to *hze1-
707   5* mutations (magenta) in the AtSMC6B protein structure. The AtSMC6B (UniProt ACC:
708   Q9FIIH) model was built using SWISS-MODEL using *Saccharomyces cerevisiae* SMC6
709   (PBDID: 7qcd) (Hallett et al., 2022) as template. F, Superposed models of the hinge domain
710   from wild-type SMC6B (azure) and SMC6B in *hze1-1* (green) generated using AlphaFold2.
711   Position of the D513N substitution is marked with black circle. The predicted effect on
712   secondary structure is marked with black asterisks.
713
714   **Figure 2.** The SMC5/6 complex is required for the repair of type-3 and type-4 DNA protein
715   crosslinks (DPCs).
716   A, Representative growth phenotype of wild-type (WT) and mutant seedlings on medium
717   without DPC inducers (Mock) or containing 20 nM camptothecin (CPT) or 10 µM ICRF-187
718   (ICRF). Scale bar, 1 cm. B, C, Relative root length of WT and mutant seedlings grown in the
719   presence of 20 nM CPT (B) or 10 µM ICRF (C). Data are means ± SD from three biological
720   replicates, each with at least 20 seedlings. Different letters indicate significant differences (P
721   < 0.05) according to one-way ANOVA followed by Tukey's test. Source data for statistical
722   analyses are available in Supplemental Table S2. The original experiment was split between
723   Figure 2A-C and Supplemental Figures S5A-D. Therefore, these figures show identical
724   images and data for the controls. D, Representative confocal microscopy images of root
725   apices stained with propidium iodide. Five-day-old seedlings were treated for 24 h with 20
726   nM PT or 10 µM ICRF prior to analysis. Dark sectors within the roots indicate dead cells.
727   Scale bar, 100 µm.

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Figure 3. Phenotypic analysis of *smc6b-1*, *mus81-1*, *wss1a-1* and their higher-order mutants under normal conditions. A, Representative growth phenotype of wild type, *smc6b-1*, *mus81-1*, *wss1a-1* and double mutants with *smc6b-1*. Scale bar, 1 cm. B, Quantification of root length from A. At least 20 roots per genotype were analyzed in each of three biological replicates. The lower and upper hinges of the boxplots correspond to the first and third quartiles of the data, the black lines within the boxes indicates the median. Whiskers mark 10% and 90% intervals. Different letters indicate significant differences ($P < 0.05$) according to one-way ANOVA followed by Tukey's test. Source data for statistical analyses in B are available in Supplemental Table S4A. C, Representative confocal microscopy images of root tips stained with propidium iodide. Seedlings were grown for five days on control medium prior to analysis. Dark sectors indicate dead cells. Scale bar, 100 μm. D, Representative phenotypes of two-week-old WT, single mutants, double mutants, and *smc6b-1 mus81-1 wss1a-1* triple mutant seedlings grown on half-strength MS medium. Scale bar, 1 cm. E, Detailed photograph of three-week-old *smc6b-1 mus81-1 wss1a-1* triple mutant seedlings with severe phenotype grown on half-strength MS medium. Scale bar, 1 cm. F, Representative phenotypes of six-week old plants grown on soil. Scale bar, 70 mm.

Figure 4. Sensitivity of *smc6b-1 mus81-1* and *smc6b-1 wss1a-1* plants to zebularine.
A, Fresh weight of plants treated with 5 μM or 20 μM zebularine (ZEB). The sensitivity of double mutant plants was compared to the respective single mutants and WT plants relative to the mock-treated plants of the same genotype. Data are means ± SD of three biological replicates. Different letters indicate significant differences ($P < 0.05$) according to one-way ANOVA followed by Tukey's test. Source data for A are available in Supplemental Table S4. B, Representative confocal microscopy images of root tips stained with propidium iodide. Scale bar, 100 μm.

Figure 5. SMC5/6-dependent SUMOylation of zebularine-induced MET1 crosslinks.
A, Immunolabeling of mock- and zebularine-treated WT, *smc6b-1*, and *nse2-2* root nuclei stained with SUMO1 antibody. MET1-RFP signals were observed directly, and nuclei were counterstained with DAPI. The white lines in Merge indicate intersects for fluorescence intensity measurements shown in (B). Scale bar, 5 μm. B, Fluorescence intensity (FI) plots based on the white lines indicated intersects in (A). The y-axis shows FI intensity in arbitrary units (AU) for MET1-RFP and SUMO1 signals. R indicates Pearson's correlation coefficient assessment of colocalization (1, full colocalization). C, Detailed image of MET1-RFP colocalization with SUMO1 (from A) in WT nuclei after zebularine treatment. Scale bar, 1 μm. D, Percentage of nuclei with MET1-RFP foci colocalizing with SUMO1 in WT, *smc6b-1* and *nse2-2* root nuclei after zebularine treatment. Data are means ± SD from three biological replicates. Statistical significance was tested with chi-square test (*smc6b-1* x^2 (2, N = 544) = 196.6331, p = .000, *nse2-2* (x^2 (2, N = 651) = 231.7348, p = .000). n, total number of nuclei evaluated per genotype. Source data for the analyses are available in Supplemental Table S6.

Figure 6. Working model of zebularine-induced DPC repair. The endonuclease MUS81 cleaves DNA surrounding the crosslink. The SMC5/6 complex deposits SUMO residues on the MET1-DPC or adjacent repair proteins to stimulate repair. Without the SMC5/6 complex, the protease WSS1A proteolytically degrades the protein crosslinked by zebularine.

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References

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Alt, A., Dang, H.Q., Wells, O.S., Polo, L.M., Smith, M.A., McGregor, G.A., Welte, T.,
Lehmann, A.R., Pearl, L.H., Murray, J.M., and Oliver, A.W. (2017). Specialized
interfaces of Smc5/6 control hinge stability and DNA association. Nat Commun **8**,
14011.

Barker, S., Weinfeld, M., and Murray, D. (2005). DNA–protein crosslinks: their induction,
repair, and biological consequences. Mutat Res **589**: 111–135.

Bernges, F. and Zeller, W.J. (1996). Combination effects of poly(ADP-ribose) polymerase
inhibitors and DNA-damaging agents in ovarian tumor cell lines--with special reference
to cisplatin. J Cancer Res Clin Oncol **122**: 665–670.

Bienert, S., Waterhouse, A., De Beer, T.A.P., Tauriello, G., Studer, G., Bordoli, L., and
Schwede, T. (2017). The SWISS-MODEL Repository—new features and functionality.
Nucleic Acids Res **45**: D313–D319.

Borgermann, N., Ackermann, L., Schwertman, P., Hendriks, I.A., Thijssen, K., Liu, J.C.,
Lans, H., Nielsen, M.L., and Mailand, N. (2019). SUMOylation promotes protective
responses to DNA -protein crosslinks . EMBO J **38**: e101496.

Chen, X.B., Melchionna, R., Denis, C.M., Gaillard, P.H.L., Blasina, A., Van de Weyer, I.,
Boddy, M.N., Russell, P., Vialard, J., and McGowan, C.H. (2001). Human Mus81-
associated endonuclease cleaves Holliday junctions in vitro. Mol Cell **8**: 1117–1127.

Chen, Z., Yang, H., and Pavletich, N.P. (2008). Mechanism of homologous recombination
from the RecA–ssDNA/dsDNA structures. Nature **453**: 489–494.

Cingolani, P., Platts, A., Wang, L.L., Coon, M., Nguyen, T., Wang, L., Land, S.J., Lu, X.,
and Ruden, D.M. (2012). A program for annotating and predicting the effects of single
nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster
strain w1118; iso-2; iso-3. Fly (Austin). **6**: 80–92.

Classen, S., Olland, S., and Berger, J.M. (2003). Structure of the topoisomerase II ATPase
region and its mechanism of inhibition by the chemotherapeutic agent ICRF-187. Proc
Natl Acad Sci U S A **100**: 10629–10634.

Core R Team (2020). A language and environment for statistical computing. R Found. Stat.
Comput. **2**: https://www.R--project.org.

Díaz, M. et al. (2019). The SMC5/6 complex subunit NSE4A is involved in DNA damage
repair and seed development. Plant Cell **31**: 1579–1597.

Diaz, M. and Pecinka, A. (2018). Scaffolding for repair: understanding molecular functions
of the SMC5/6 complex. Genes (Basel). **9**: 36.

Diaz, M., Pecinkova, P., Nowicka, A., Baroux, C., Sakamoto, T., Gandha, P.Y.,
Jeřábková, H., Matsunaga, S., Grossniklaus, U., and Pecinka, A. (2019). SMC5/6

22

Complex Subunit NSE4A is Involved in DNA Damage Repair and Seed Development in Arabidopsis. Plant Cell **31**: 1579-1597. **Doe, C.L., Ahn, J.S., Dixon, J., and Whitby, M.C.** (2002). Mus81-Eme1 and Rqh1 involvement in processing stalled and collapsed replication forks. J Biol Chem **277**: 32753–32759.

Dorn, A. and Puchta, H. (2020). Analyzing somatic DNA repair in Arabidopsis meiotic mutants. Methods Mol Biol **2061**: 359-366.

Enderle, J., Dorn, A., Beying, N., Trapp, O., and Puchta, H. (2019). The protease WSS1A, the endonuclease MUS81, and the phosphodiesterase TDP1 are involved in independent pathways of DNA-protein crosslink repair in plants. Plant Cell **31**: 775–790.

Hacker, L. et al. (2020). Repair of DNA-protein crosslinks in plants. DNA Repair (Amst). **87**: 102787.

Hacker, L., Dorn, A., Enderle, J., and Puchta, H. (2022). The repair of topoisomerase 2 cleavage complexes in Arabidopsis. Plant Cell **34**: 287–301.

Hallett, S.T., Harry, I.C., Schellenberger, P., Zhou, L., Cronin, N.B., Baxter, J., Etheridge, T.J., Murray, J.M., and Oliver, A.W. (2022). Cryo-EM structure of the Smc5 / 6 holo-complex. Nucleic Acids Res **50**: 9505–9520.

Hammoudi, V., Vlachakis, G., Schranz, M.E., and van den Burg, H.A. (2016). Whole-genome duplications followed by tandem duplications drive diversification of the protein modifier SUMO in Angiosperms. New Phytol. **211**: 172–185.

Hartung, F., Suer, S., Bergmann, T., and Puchta, H. (2006). The role of AtMUS81 in DNA repair and its genetic interaction with the helicase AtRecQ4A. Nucleic Acids Res. **34**: 4438–4448.

Klages-Mundt, N.L. and Li, L. (2017). Formation and repair of DNA-protein crosslink damage. Sci China Life Sci **60**: 1065–1076.

Kojima, Y. and Machida, Y.J. (2020). DNA-protein crosslinks from environmental exposure: Mechanisms of formation and repair. Environ Mol Mutagen **61**: 716–729.

Kubaláková, M., Macas, J., and Doležel, J. (1997). Mapping of repeated DNA sequences in plant chromosomes by PRINS and C-PRINS. Theor Appl Genet **94**: 758–763.

Langmead, B. and Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. Nat Methods **9**: 357–359.

Langmead, B., Trapnell, C., Pop, M., and Salzberg, S.L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol **10**: R25.

Larsen, N.B., Gao, A.O., Sparks, J.L., Gallina, I., Wu, R.A., Mann, M., Räschle, M., Walter, J.C., and Duxin, J.P. (2019). Replication-Coupled DNA-Protein Crosslink Repair by SPRTN and the Proteasome in Xenopus Egg Extracts. Mol Cell **73**: 574-588.e7.

849 **Larsson, A.** (2014). AliView: A fast and lightweight alignment viewer and editor for large
850   datasets. Bioinformatics **30**: 3276–3278.

851 **Lee, J.H., Mosher, E.P., Lee, Y.S., Bumpus, N.N., and Berger, J.M.** (2022). Control of
852   topoisomerase II activity and chemotherapeutic inhibition by TCA cycle metabolites. Cell
853   Chem Biol **29**: 476-489.e6.

854 **Lee, J.H., Wendorff, T.J., and Berger, J.M.** (2017). Resveratrol: A novel type of
855   topoisomerase II inhibitor. J Biol Chem **292**: 21011–21022.

856 **Liu, C.-H.H., Finke, A., Díaz, M., Rozhon, W., Poppenberger, B., Baubec, T., and**
857   **Pecinka, A.** (2015). Repair of DNA damage induced by the cytidine analog zebularine
858   requires ATR and ATM in arabidopsis. Plant Cell **27**: 1788–1800.

859 **Liu, J.C.Y. et al.** (2021). Mechanism and function of DNA replication-independent DNA-
860   protein crosslink repair via the SUMO-RNF4 pathway. EMBO J **40**: e107413.

861 **Lobet, G., Pagès, L., and Draye, X.** (2011). A novel image-analysis toolbox enabling
862   quantitative analysis of root system architecture. Plant Physiol **157**: 29–39.

863 **Mannuss, A., Dukowic-Schulze, S., Suer, S., Hartung, F., Pacher, M., and Puchta, H.**
864   (2010). RAD5A, RECQ4A, AND MUS81 have specific functions in homologous
865   recombination and define different pathways of dna repair in Arabidopsis thaliana. Plant
866   Cell **22**: 3318–3330.

867 **Maslov, A.Y., Lee, M., Gundry, M., Gravina, S., Strogonova, N., Tazearslan, C.,**
868   **Bendebury, A., Suh, Y., and Vijg, J.** (2012). 5-Aza-2′-deoxycytidine-induced genome
869   rearrangements are mediated by DNMT1. Oncogene **31**: 5172–5179.

870 **Menear, K.A. et al.** (2008). 4-[3-(4-Cyclopropanecarbonylpiperazine-1-carbonyl)-4-
871   fluorobenzyl] -2H-phthalazin-1-one: A novel bioavailable inhibitor of poly(ADP-ribose)
872   polymerase-1. J. Med. Chem. **51**: 6581–6591.

873 **Mengiste, T., Revenkova, E., Bechtold, N., and Paszkowski, J.** (1999). An SMC-like
874   protein is required for efficient homologous recombination in arabidopsis. EMBO J **18**:
875   4505–4512.

876 **Nakamura, J. and Nakamura, M.** (2020). DNA-protein crosslink formation by endogenous
877   aldehydes and AP sites. DNA Repair (Amst). **88**: 102806.

878 **Nitiss, J.L. et al.** (2009). Targeting DNA topoisomerase II in cancer chemotherapy. Nat Rev
879   **9**: 338–350.

880 **Nowicka, A. et al.** (2020). Comparative analysis of epigenetic inhibitors reveals different
881   degrees of interference with transcriptional gene silencing and induction of DNA
882   damage. Plant J **102**: 68–84.

883 **Palecek, J.J.** (2019). SMC5/6: Multifunctional player in replication. Genes (Basel). **10**.

884 **Pommier, Y. and Marchand, C.** (2012). Interfacial inhibitors: Targeting macromolecular
885   complexes. Nat Rev Drug Discov **11**: 25–36.

24

886   **Potts, P.R., Porteus, M.H., and Yu, H.** (2006). Human SMC5/6 complex promotes sister
887   chromatid homologous recombination by recruiting the SMC1/3 cohesin complex to
888   double-strand breaks. EMBO J **25**: 3377–3388.

889   **Pouliot, J.J., Yao, K.C., Robertson, C.A., and Nash, H.A.** (1999). Yeast gene for a Tyr-
890   DNA phosphodiesterase that repairs topoisomerase I complexes. Science **286**: 552–
891   555.

892   **Prochazkova, K., Finke, A., Dvořák Tomaštíková, E., Filo, J., Bente, H., Dvořák, P.,**
893   **Ovečka, M., Šamaj, J., and Pecinka, A.** (2022). Zebularine induces enzymatic DNA–
894   protein crosslinks in 45S rDNA heterochromatin of Arabidopsis nuclei. Nucleic Acids
895   Res **50**: 244–258.

896   **Regairaz, M., Zhang, Y.W., Fu, H., Agama, K.K., Tata, N., Agrawal, S., Aladjem, M.I., and**
897   **Pommier, Y.** (2011). Mus81-mediated DNA cleavage resolves replication forks stalled
898   by topoisomerase I-DNA complexes. J Cell Biol **195**: 739–749.

899   **Reinking, H.K., Kang, H.S., Götz, M.J., Li, H.Y., Kieser, A., Zhao, S., Acampora, A.C.,**
900   **Weickert, P., Fessler, E., Jae, L.T., Sattler, M., and Stingele, J.** (2020). DNA
901   structure-specific cleavage of DNA-protein crosslinks by the SPRTN protease. Mol Cell
902   **80**: 102–113.

903   **Ruggiano, A., Vaz, B., Kilgas, S., Popović, M., Rodriguez-Berriguete, G., Singh, A.N.,**
904   **Higgins, G.S., Kiltie, A.E., and Ramadan, K.** (2021). The protease SPRTN and
905   SUMOylation coordinate DNA-protein crosslink repair to prevent genome instability. Cell
906   Rep **37**: 110080.

907   **Schellenberg, M.J., Lieberman, J.A., Herrero-Ruiz, A., Butler, L.R., Williams, J.G.,**
908   **Muñoz-Cabello, A.M., Mueller, G.A., London, R.E., Cortés-Ledesma, F., and**
909   **Williams, R.S.** (2017). ZATT (ZNF451)–mediated resolution of topoisomerase 2 DNA-
910   protein cross-links. Science **357**: 1412–1416.

911   **Schrödinger, LLC** (2015). The {PyMOL} Molecular Graphics System, Version~1.8.

912   **Serbyn, N., Bagdiul, I., Noireterre, A., Michel, A.H., Suhandynata, R.T., Zhou, H.,**
913   **Kornmann, B., and Stutz, F.** (2021). SUMO orchestrates multiple alternative DNA-
914   protein crosslink repair pathways. Cell Rep **37**: 110034.

915   **Stingele, J. et al.** (2016). Mechanism and regulation of DNA-protein crosslink repair by the
916   DNA-dependent metalloprotease SPRTN. Mol Cell **64**: 688–703.

917   **Stingele, J., Bellelli, R., and Boulton, S.J.** (2017). Mechanisms of DNA-protein crosslink
918   repair. Nat Rev Mol Cell Biol **18**: 563–573.

919   **Stingele, J. and Jentsch, S.** (2015). DNA-protein crosslink repair. Nat Rev Mol Cell Biol **16**:
920   455–460.

921   **Stingele, J., Schwarz, M.S., Bloemeke, N., Wolf, P.G., and Jentsch, S.** (2014). A DNA-
922   dependent protease involved in DNA-protein crosslink repair. Cell **158**: 327–338.

25

923 **Sun, Y., Jenkins, L.M.M., Su, Y.P., Nitiss, K.C., Nitiss, J.L., Pommier, Y., Miller Jenkins,**
924   **L.M., Su, Y.P., Nitiss, K.C., Nitiss, J.L., and Pommier, Y.** (2020a). A conserved
925   SUMO pathway repairs topoisomerase DNA-protein cross-links by engaging ubiquitin-
926   mediated proteasomal degradation. Sci Adv **6**: eaba6290.

927 **Sun, Y., Saha, L.K., Saha, S., Jo, U., and Pommier, Y.** (2020b). Debulking of
928   topoisomerase DNA-protein crosslinks (TOP-DPC) by the proteasome, non-
929   proteasomal and non-proteolytic pathways. DNA Repair (Amst) **94**: 102926.

930 **Torres-Ramos, C.A., Prakash, S., and Prakash, L.** (2002). Requirement of RAD5 and
931   MMS2 for Postreplication Repair of UV-Damaged DNA in Saccharomyces cerevisiae.
932   Mol Cell Biol **22**: 2419–2426.

933 **Tsuda, M., Kitamasu, K., Kumagai, C., Sugiyama, K., Nakano, T., and Ide, H.** (2020).
934   Tyrosyl-DNA phosphodiesterase 2 (TDP2) repairs topoisomerase 1 DNA-protein
935   crosslinks and 3′-blocking lesions in the absence of tyrosyl-DNA phosphodiesterase 1
936   (TDP1). DNA Repair (Amst) **91**–**92**: 102849.

937 **Varejão, N., Ibars, E., Lascorz, J., Colomina, N., Torres-Rosell, J., and Reverter, D.**
938   (2018). DNA activates the Nse2/Mms21 SUMO E3 ligase in the Smc5/6 complex.
939   EMBO J **37**: e98306 .

940 **Vaz, B. et al.** (2016). Metalloprotease SPRTN/DVC1 orchestrates replication-coupled DNA-
941   protein crosslink repair. Mol Cell **64**: 704–719.

942 **Waldman, B.C. and Waldman, A.S.** (1990). Illegitimate and homologous recombination in
943   mammalian cells: differential sensitivity to an inhibitor of poly(ADP-ribosylation). Nucleic
944   Acids Res **18**: 5981–5988.

945 **Watanabe, K., Pacher, M., Dukowic, S., Schubert, V., Puchta, H., and Schubert, I.**
946   (2009). The STRUCTURAL MAINTENANCE of CHROMOSOMES 5/6 complex
947   promotes sister chromatid alignment and homologous recombination after DNA damage
948   in Arabidopsis thaliana. Plant Cell **21**: 2688–2699.

949 **Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer,**
950   **F.T., De Beer, T.A.P., Rempfer, C., Bordoli, L., Lepore, R., and Schwede, T.** (2018).
951   SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic
952   Acids Res. **46**: W296–W303.

953 **Weickert, P. and Stingele, J.** (2022). DNA-Protein Crosslinks and Their Resolution. Annu
954   Rev Biochem **91**: 157–181.

955 **Whalen, J.M., Dhingra, N., Wei, L., Zhao, X., and Freudenreich, C.H.** (2020). Relocation of
956   Collapsed Forks to the Nuclear Pore Complex Depends on Sumoylation of DNA Repair
957   Proteins and Permits Rad51 Association. Cell Rep **31**: 107635

958 **Willing, E.-M.M., Piofczyk, T., Albert, A., Winkler, J.B., Schneeberger, K., and Pecinka,**
959   **A.** (2016). UVR2 ensures transgenerational genome stability under simulated natural

960   UV-B in Arabidopsis thaliana. Nat Commun **7**: 13522.

961   **Yang, F., Fernández Jiménez, N., Majka, J., Pradillo, M., and Pecinka, A.** (2021).

962   Structural Maintenance of Chromosomes 5/6 Complex Is Necessary for Tetraploid

963   Genome Stability in Arabidopsis thaliana. Front Plant Sci **12**: 748252.

964   **Yu, Y., Li, S., Ser, Z., Kuang, H., Than, T., Guan, D., Zhao, X., and Patel, D.J.** (2022).

965   Cryo-EM structure of DNA-bound Smc5/6 reveals DNA clamping enabled by multi-

966   subunit conformational changes. Proc Natl Acad Sci U S A **119**: e2202799119.

967   **Zhang, H., Xiong, Y., and Chen, J.** (2020). DNA–protein cross-link repair: what do we know

968   now? Cell Biosci **10**: 3..

969

A
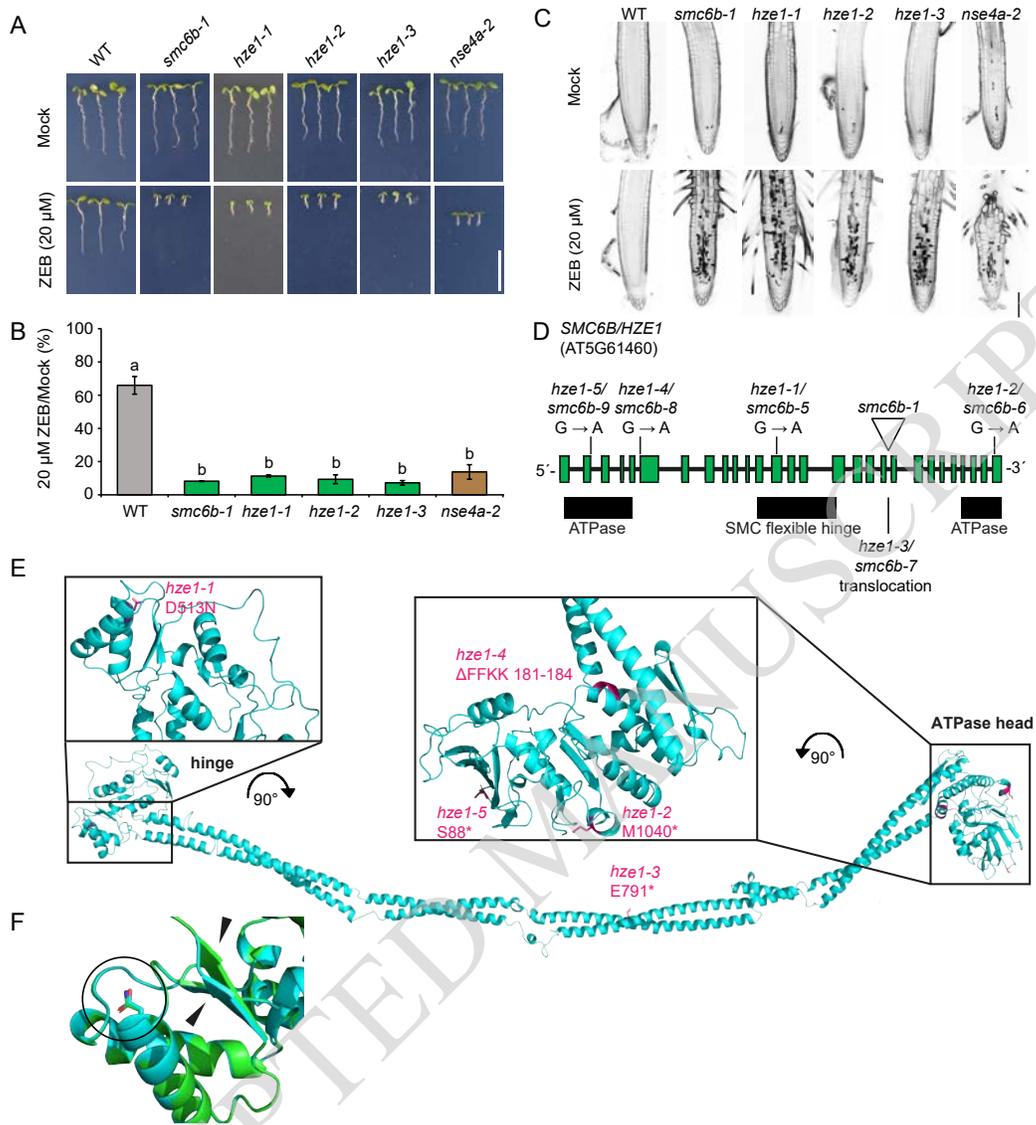
B

C

D   *SMC6B/HZE1*
    (AT5G61460)

E

F

Figure 1. *HYPERSENSITIVE TO ZEBULARINE 1* (*HZE1*) encodes the SMC5/6 complex subunit SMC6B.

A, Representative growth phenotypes of seedlings from wild type (WT), *smc6b-1*, *hze1* alleles and *nse42-2* on 0 (Mock) and 20 µM zebularine (ZEB). Scale bar, 1 cm. B, Relative root length of seedlings in (A) under zebularine/mock conditions (% of ZEB/Mock). Data are means ± SD from three biological replicates, each with a minimum of 14 seedlings. Different lowercase letters indicate significant differences ($P < 0.05$), according to one-way ANOVA followed by Tukey's test. Source data for statistical analyses are available in Supplemental Table S1. The original experiment was split between Figures 1A-C, and Supplemental Figures S5A-D. Therefore, these figures show identical images and data for the controls. C, Representative confocal microscopy images of root tips stained with propidium iodide, which indicates dead cells (dark sectors). Five-day-old seedlings were treated with 20 µM ZEB for 24 h prior to analysis. Scale bar, 100 µm. D, Schematic model of the *SMC6B/HZE1* locus (At5g61460) with the positions of individual mutations. E, Detailed position of *hze1-1* to *hze1-5* mutations (magenta) in the AtSMC6B protein structure. The AtSMC6B (UniProt ACC: Q9FIIH) model was built using SWISS-MODEL using *Saccharomyces cerevisiae* SMC6 (PBDID: 7qcd) (Hallett et al., 2022) as template. F, Superposed models of the hinge domain from wild-type SMC6B (azure) and SMC6B in *hze1-1* (green) generated using AlphaFold2. Position of the D513N substitution is marked with black circle. The predicted effect on secondary structure is marked with black asterisks.

A
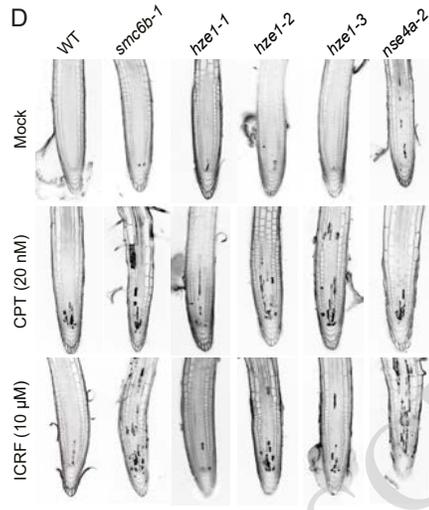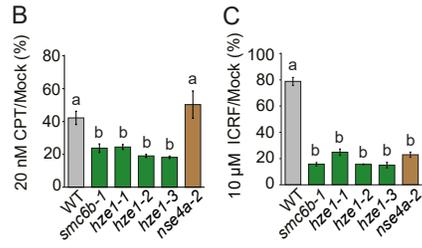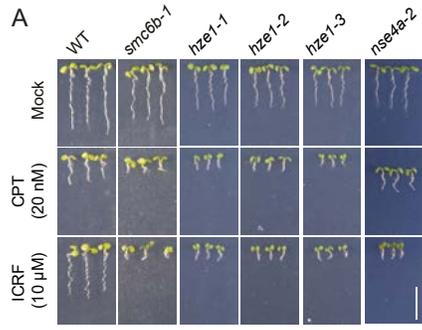
Mock

CPT
(20 nM)

ICRF
(10 µM)

WT smc6b-1 hze1-1 hze1-2 hze1-3 nse4a-2

B

20 nM CPT/Mock (%)

C

10 µM ICRF/Mock (%)

D

Mock

CPT (20 nM)

ICRF (10 µM)

WT smc6b-1 hze1-1 hze1-2 hze1-3 nse4a-2

Figure 2. The SMC5/6 complex is required for the repair of type-3 and type-4 DNA protein crosslinks (DPCs).

A, Representative growth phenotype of wild-type (WT) and mutant seedlings on medium without DPC inducers (Mock) or containing 20 nM camptothecin (CPT) or 10 μM ICRF-187 (ICRF). Scale bar, 1 cm. B, C, Relative root length of WT and mutant seedlings grown in the presence of 20 nM CPT (B) or 10 μM ICRF (C). Data are means ± SD from three biological replicates, each with at least 20 seedlings. Different letters indicate significant differences ($P < 0.05$) according to one-way ANOVA followed by Tukey's test. Source data for statistical analyses are available in Supplemental Table S2. The original experiment was split between Figure 2A-C and Supplemental Figures S5A-D. Therefore, these figures show identical images and data for the controls. D, Representative confocal microscopy images of root apices stained with propidium iodide. Five-day-old seedlings were treated for 24 h with 20 nM PT or 10 μM ICRF prior to analysis. Dark sectors within the roots indicate dead cells. Scale bar, 100 μm.

Figure 3. Phenotypic analysis of *smc6b-1*, *mus81-1*, *wss1a-1* and their higher-order mutants under normal conditions. A, Representative growth phenotype of wild type, *smc6b-1*, *mus81-1*, *wss1a-1* and double mutants with *smc6b-1*. Scale bar, 1 cm. B, Quantification of root length from A. At least 20 roots per genotype were analyzed in each of three biological replicates. The lower and upper hinges of the boxplots correspond to the first and third quartiles of the data, the black lines within the boxes indicates the median. Whiskers mark 10% and 90% intervals. Different letters indicate significant differences ($P < 0.05$) according to one-way ANOVA followed by Tukey's test. Source data for statistical analyses in B are available in Supplemental Table S4A. C, Representative confocal microscopy images of root tips stained with propidium iodide. Seedlings were grown for five days on control medium prior to analysis. Dark sectors indicate dead cells. Scale bar, 100 µm. D, Representative phenotypes of two-week-old WT, single mutants, double mutants, and *smc6b-1 mus81-1 wss1a-1* triple mutant seedlings grown on half-strength MS medium. Scale bar, 1 cm. E, Detailed photograph of three-week-old *smc6b-1 mus81-1 wss1a-1* triple mutant seedlings with severe phenotype grown on half-strength MS medium. Scale bar, 1 cm. F, Representative phenotypes of six-week old plants grown on soil. Scale bar, 70 mm.

Figure 4. Sensitivity of *smc6b-1 mus81-1* and *smc6b-1 wss1a-1* plants to zebularine.
A, Fresh weight of plants treated with 5 µM or 20 µM zebularine (ZEB). The sensitivity of double mutant plants was compared to the respective single mutants and WT plants relative to the mock-treated plants of the same genotype. Data are means ± SD of three biological replicates. Different letters indicate significant differences (P < 0.05) according to one-way ANOVA followed by Tukey's test. Source data for A are available in Supplemental Table S4. B, Representative confocal microscopy images of root tips stained with propidium iodide. Scale bar, 100 µm.

Figure 5. SMC5/6-dependent SUMOylation of zebularine-induced MET1 crosslinks.
A, Immunolabeling of mock- and zebularine-treated WT, *smc6b-1*, and *nse2-2* root nuclei stained with SUMO1 antibody. MET1-RFP signals were observed directly, and nuclei were counterstained with DAPI. The white lines in Merge indicate intersects for fluorescence intensity measurements shown in (B). Scale bar, 5 μm. B, Fluorescence intensity (FI) plots based on the white lines indicated intersects in (A). The y-axis shows FI intensity in arbitrary units (AU) for MET1-RFP and SUMO1 signals. R indicates Pearson's correlation coefficient assessment of colocalization (1, full colocalization). C, Detailed image of MET1-RFP colocalization with SUMO1 (from A) in WT nuclei after zebularine treatment. Scale bar, 1 μm. D, Percentage of nuclei with MET1-RFP foci colocalizing with SUMO1 in WT, *smc6b-1* and *nse2-2* root nuclei after zebularine treatment. Data are means ± SD from three biological replicates. Statistical significance was tested with chi-square test (*smc6b-1* x^2 (2, N = 544) = 196.6331, p = .000, *nse2-2* (x^2 (2, N = 651) = 231.7348, p = .000). n, total number of nuclei evaluated per genotype. Source data for the analyses are available in Supplemental Table S6.

Figure 6. Working model of zebularine-induced DPC repair. The endonuclease MUS81 cleaves DNA surrounding the crosslink. The SMC5/6 complex deposits SUMO residues on the MET1-DPC or adjacent repair proteins to stimulate repair. Without the SMC5/6 complex, the protease WSS1A proteolytically degrades the protein crosslinked by zebularine.

Parsed Citations

Alt, A, Dang, H.Q., Wells, O.S., Polo, L.M., Smith, M.A, McGregor, G.A, Welte, T., Lehmann, A.R., Pearl, L.H., Murray, J.M., and Oliver, A.W. (2017). Specialized interfaces of Smc5/6 control hinge stability and DNA association. Nat Commun 8, 14011.
  Google Scholar: Author Only Title Only Author and Title

Barker, S., Weinfeld, M., and Murray, D. (2005). DNA–protein crosslinks: their induction, repair, and biological consequences. Mutat Res 589: 111–135.
  Google Scholar: Author Only Title Only Author and Title

Bernges, F. and Zeller, W.J. (1996). Combination effects of poly(ADP-ribose) polymerase inhibitors and DNA-damaging agents in ovarian tumor cell lines--with special reference to cisplatin. J Cancer Res Clin Oncol 122: 665–670.
  Google Scholar: Author Only Title Only Author and Title

Bienert, S., Waterhouse, A, De Beer, T.A.P., Tauriello, G., Studer, G., Bordoli, L., and Schwede, T. (2017). The SWISS-MODEL Repository-new features and functionality. Nucleic Acids Res 45: D313–D319.
  Google Scholar: Author Only Title Only Author and Title

Borgermann, N., Ackermann, L., Schwertman, P., Hendriks, I.A, Thijssen, K., Liu, J.C., Lans, H., Nielsen, M.L., and Mailand, N. (2019). SUMOylation promotes protective responses to DNA-protein crosslinks . EMBO J 38: e101496.
  Google Scholar: Author Only Title Only Author and Title

Chen, X.B., Melchionna, R., Denis, C.M., Gaillard, P.H.L., Blasina, A, Van de Weyer, I., Boddy, M.N., Russell, P., Vialard, J., and McGowan, C.H. (2001). Human Mus81-associated endonuclease cleaves Holliday junctions in vitro. Mol Cell 8: 1117–1127.
  Google Scholar: Author Only Title Only Author and Title

Chen, Z, Yang, H., and Pavletich, N.P. (2008). Mechanism of homologous recombination from the RecA–ssDNA/dsDNA structures. Nature 453: 489–494.
  Google Scholar: Author Only Title Only Author and Title

Cingolani, P., Platts, A, Wang, L.L., Coon, M., Nguyen, T., Wang, L., Land, S.J., Lu, X., and Ruden, D.M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin). 6: 80–92.
  Google Scholar: Author Only Title Only Author and Title

Classen, S., Olland, S., and Berger, J.M. (2003). Structure of the topoisomerase II ATPase region and its mechanism of inhibition by the chemotherapeutic agent ICRF-187. Proc Natl Acad Sci U S A 100: 10629–10634.
  Google Scholar: Author Only Title Only Author and Title

Core R Team (2020). A language and environment for statistical computing. R Found. Stat. Comput. 2: https://www.R--project.org.
  Google Scholar: Author Only Title Only Author and Title

Díaz, M. et al. (2019). The SMC5/6 complex subunit NSE4A is involved in DNA damage repair and seed development. Plant Cell 31: 1579–1597.
  Google Scholar: Author Only Title Only Author and Title

Diaz, M. and Pecinka, A (2018). Scaffolding for repair: understanding molecular functions of the SMC5/6 complex. Genes (Basel). 9: 36.
  Google Scholar: Author Only Title Only Author and Title

Diaz, M., Pecinkova, P., Nowicka, A, Baroux, C., Sakamoto, T., Gandha, P.Y., Jeřábková, H., Matsunaga, S., Grossniklaus, U., and Pecinka, A (2019). SMC5/6 Complex Subunit NSE4A is Involved in DNA Damage Repair and Seed Development in Arabidopsis. Plant Cell 31: 1579-1597. Doe, C.L., Ahn, J.S., Dixon, J., and Whitby, M.C. (2002). Mus81-Eme1 and Rqh1 involvement in processing stalled and collapsed replication forks. J Biol Chem 277: 32753–32759.
  Google Scholar: Author Only Title Only Author and Title

Dorn, A and Puchta, H. (2020). Analyzing somatic DNA repair in Arabidopsis meiotic mutants. Methods Mol Biol 2061: 359-366.
  Google Scholar: Author Only Title Only Author and Title

Enderle, J., Dorn, A, Beying, N., Trapp, O., and Puchta, H. (2019). The protease WSS1A, the endonuclease MUS81, and the phosphodiesterase TDP1 are involved in independent pathways of DNA-protein crosslink repair in plants. Plant Cell 31: 775–790.
  Google Scholar: Author Only Title Only Author and Title

Hacker, L. et al. (2020). Repair of DNA-protein crosslinks in plants. DNA Repair (Amst). 87: 102787.
  Google Scholar: Author Only Title Only Author and Title

Hacker, L., Dorn, A, Enderle, J., and Puchta, H. (2022). The repair of topoisomerase 2 cleavage complexes in Arabidopsis. Plant Cell 34: 287–301.
  Google Scholar: Author Only Title Only Author and Title

Hallett, S.T., Harry, I.C., Schellenberger, P., Zhou, L., Cronin, N.B., Baxter, J., Etheridge, T.J., Murray, J.M., and Oliver, A.W. (2022). Cryo-EM structure of the Smc5 / 6 holo-complex. Nucleic Acids Res 50: 9505–9520.
  Google Scholar: Author Only Title Only Author and Title

Hammoudi, V., Vlachakis, G., Schranz, M.E., and van den Burg, H.A. (2016). Whole-genome duplications followed by tandem duplications drive diversification of the protein modifier SUMO in Angiosperms. New Phytol. 211: 172–185.
  Google Scholar: Author Only Title Only Author and Title

Hartung, F., Suer, S., Bergmann, T., and Puchta, H. (2006). The role of AtMUS81 in DNA repair and its genetic interaction with the helicase AtRecQ4A. Nucleic Acids Res. 34: 4438–4448.
  Google Scholar: Author Only Title Only Author and Title

Klages-Mundt, N.L. and Li, L. (2017). Formation and repair of DNA-protein crosslink damage. Sci China Life Sci 60: 1065–1076.
  Google Scholar: Author Only Title Only Author and Title

Kojima, Y. and Machida, Y.J. (2020). DNA-protein crosslinks from environmental exposure: Mechanisms of formation and repair. Environ Mol Mutagen 61: 716–729.
  Google Scholar: Author Only Title Only Author and Title

Kubaláková, M., Macas, J., and Doležel, J. (1997). Mapping of repeated DNA sequences in plant chromosomes by PRINS and C-PRINS. Theor Appl Genet 94: 758–763.
  Google Scholar: Author Only Title Only Author and Title

Langmead, B. and Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. Nat Methods 9: 357–359.
  Google Scholar: Author Only Title Only Author and Title

Langmead, B., Trapnell, C., Pop, M., and Salzberg, S.L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol 10: R25.
  Google Scholar: Author Only Title Only Author and Title

Larsen, N.B., Gao, A.O., Sparks, J.L., Gallina, I., Wu, R.A., Mann, M., Räschle, M., Walter, J.C., and Duxin, J.P. (2019). Replication-Coupled DNA-Protein Crosslink Repair by SPRTN and the Proteasome in Xenopus Egg Extracts. Mol Cell 73: 574-588.e7.
  Google Scholar: Author Only Title Only Author and Title

Larsson, A. (2014). AliView: A fast and lightweight alignment viewer and editor for large datasets. Bioinformatics 30: 3276–3278.
  Google Scholar: Author Only Title Only Author and Title

Lee, J.H., Mosher, E.P., Lee, Y.S., Bumpus, N.N., and Berger, J.M. (2022). Control of topoisomerase II activity and chemotherapeutic inhibition by TCA cycle metabolites. Cell Chem Biol 29: 476-489.e6.
  Google Scholar: Author Only Title Only Author and Title

Lee, J.H., Wendorff, T.J., and Berger, J.M. (2017). Resveratrol: A novel type of topoisomerase II inhibitor. J Biol Chem 292: 21011–21022.
  Google Scholar: Author Only Title Only Author and Title

Liu, C.-H.H., Finke, A., Díaz, M., Rozhon, W., Poppenberger, B., Baubec, T., and Pecinka, A. (2015). Repair of DNA damage induced by the cytidine analog zebularine requires ATR and ATM in arabidopsis. Plant Cell 27: 1788–1800.
  Google Scholar: Author Only Title Only Author and Title

Liu, J.C.Y. et al. (2021). Mechanism and function of DNA replication-independent DNA-protein crosslink repair via the SUMO-RNF4 pathway. EMBO J 40: e107413.
  Google Scholar: Author Only Title Only Author and Title

Lobet, G., Pagès, L., and Draye, X. (2011). A novel image-analysis toolbox enabling quantitative analysis of root system architecture. Plant Physiol 157: 29–39.
  Google Scholar: Author Only Title Only Author and Title

Mannuss, A., Dukowic-Schulze, S., Suer, S., Hartung, F., Pacher, M., and Puchta, H. (2010). RAD5A, RECQ4A, AND MUS81 have specific functions in homologous recombination and define different pathways of dna repair in Arabidopsis thaliana. Plant Cell 22: 3318–3330.
  Google Scholar: Author Only Title Only Author and Title

Maslov, A.Y., Lee, M., Gundry, M., Gravina, S., Strogonova, N., Tazearslan, C., Bendebury, A., Suh, Y., and Vijg, J. (2012). 5-Aza-2′-deoxycytidine-induced genome rearrangements are mediated by DNMT1. Oncogene 31: 5172–5179.
  Google Scholar: Author Only Title Only Author and Title

Menear, K.A. et al. (2008). 4-[3-(4-Cyclopropanecarbonylpiperazine-1-carbonyl)-4-fluorobenzyl] -2H-phthalazin-1-one: A novel bioavailable inhibitor of poly(ADP-ribose) polymerase-1. J. Med. Chem. 51: 6581–6591.
  Google Scholar: Author Only Title Only Author and Title

Mengiste, T., Revenkova, E., Bechtold, N., and Paszkowski, J. (1999). An SMC-like protein is required for efficient homologous

recombination in arabidopsis. EMBO J 18: 4505–4512.

Google Scholar: Author Only Title Only Author and Title

Nakamura, J. and Nakamura, M. (2020). DNA-protein crosslink formation by endogenous aldehydes and AP sites. DNA Repair (Amst). 88: 102806.

Google Scholar: Author Only Title Only Author and Title

Nitiss, J.L. et al. (2009). Targeting DNA topoisomerase II in cancer chemotherapy. Nat Rev 9: 338–350.

Google Scholar: Author Only Title Only Author and Title

Nowicka, A. et al. (2020). Comparative analysis of epigenetic inhibitors reveals different degrees of interference with transcriptional gene silencing and induction of DNA damage. Plant J 102: 68–84.

Google Scholar: Author Only Title Only Author and Title

Palecek, J.J. (2019). SMC5/6: Multifunctional player in replication. Genes (Basel). 10.

Google Scholar: Author Only Title Only Author and Title

Pommier, Y. and Marchand, C. (2012). Interfacial inhibitors: Targeting macromolecular complexes. Nat Rev Drug Discov 11: 25–36.

Google Scholar: Author Only Title Only Author and Title

Potts, P.R., Porteus, M.H., and Yu, H. (2006). Human SMC5/6 complex promotes sister chromatid homologous recombination by recruiting the SMC1/3 cohesin complex to double-strand breaks. EMBO J 25: 3377–3388.

Google Scholar: Author Only Title Only Author and Title

Pouliot, J.J., Yao, K.C., Robertson, C.A., and Nash, H.A. (1999). Yeast gene for a Tyr-DNA phosphodiesterase that repairs topoisomerase I complexes. Science 286: 552–555.

Google Scholar: Author Only Title Only Author and Title

Prochazkova, K., Finke, A, Dvořák Tomaštíková, E., Filo, J., Bente, H., Dvořák, P., Ovečka, M., Šamaj, J., and Pecinka, A. (2022). Zebularine induces enzymatic DNA–protein crosslinks in 45S rDNA heterochromatin of Arabidopsis nuclei. Nucleic Acids Res 50: 244–258.

Google Scholar: Author Only Title Only Author and Title

Regairaz, M., Zhang, Y.W., Fu, H., Agama, K.K., Tata, N., Agrawal, S., Aladjem, M.I., and Pommier, Y. (2011). Mus81-mediated DNA cleavage resolves replication forks stalled by topoisomerase I-DNA complexes. J Cell Biol 195: 739–749.

Google Scholar: Author Only Title Only Author and Title

Reinking, H.K., Kang, H.S., Götz, M.J., Li, H.Y., Kieser, A, Zhao, S., Acampora, A.C., Weickert, P., Fessler, E., Jae, L.T., Sattler, M., and Stingele, J. (2020). DNA structure-specific cleavage of DNA-protein crosslinks by the SPRTN protease. Mol Cell 80: 102–113.

Google Scholar: Author Only Title Only Author and Title

Ruggiano, A, Vaz, B., Kilgas, S., Popović, M., Rodriguez-Berriguete, G., Singh, A.N., Higgins, G.S., Kiltie, A.E., and Ramadan, K. (2021). The protease SPRTN and SUMOylation coordinate DNA-protein crosslink repair to prevent genome instability. Cell Rep 37: 110080.

Google Scholar: Author Only Title Only Author and Title

Schellenberg, M.J., Lieberman, J.A, Herrero-Ruiz, A, Butler, L.R., Williams, J.G., Muñoz-Cabello, A.M., Mueller, G.A, London, R.E., Cortés-Ledesma, F., and Williams, R.S. (2017). ZATT (ZNF451)–mediated resolution of topoisomerase 2 DNA-protein cross-links. Science 357: 1412–1416.

Google Scholar: Author Only Title Only Author and Title

Schrödinger, LLC (2015). The {PyMOL} Molecular Graphics System, Version~1.8.

Serbyn, N., Bagdiul, I., Noireterre, A, Michel, A.H., Suhandynata, R.T., Zhou, H., Kornmann, B., and Stutz, F. (2021). SUMO orchestrates multiple alternative DNA-protein crosslink repair pathways. Cell Rep 37: 110034.

Google Scholar: Author Only Title Only Author and Title

Stingele, J. et al. (2016). Mechanism and regulation of DNA-protein crosslink repair by the DNA-dependent metalloprotease SPRTN. Mol Cell 64: 688–703.

Google Scholar: Author Only Title Only Author and Title

Stingele, J., Bellelli, R., and Boulton, S.J. (2017). Mechanisms of DNA-protein crosslink repair. Nat Rev Mol Cell Biol 18: 563–573.

Google Scholar: Author Only Title Only Author and Title

Stingele, J. and Jentsch, S. (2015). DNA-protein crosslink repair. Nat Rev Mol Cell Biol 16: 455–460.

Google Scholar: Author Only Title Only Author and Title

Stingele, J., Schwarz, M.S., Bloemeke, N., Wolf, P.G., and Jentsch, S. (2014). A DNA-dependent protease involved in DNA-protein crosslink repair. Cell 158: 327–338.

Google Scholar: Author Only Title Only Author and Title

Sun, Y., Jenkins, L.M.M., Su, Y.P., Nitiss, K.C., Nitiss, J.L., Pommier, Y., Miller Jenkins, L.M., Su, Y.P., Nitiss, K.C., Nitiss, J.L., and Pommier, Y. (2020a). A conserved SUMO pathway repairs topoisomerase DNA-protein cross-links by engaging ubiquitin-mediated proteasomal degradation. Sci Adv 6: eaba6290.
Google Scholar: Author Only Title Only Author and Title

Sun, Y., Saha, L.K., Saha, S., Jo, U., and Pommier, Y. (2020b). Debulking of topoisomerase DNA-protein crosslinks (TOP-DPC) by the proteasome, non-proteasomal and non-proteolytic pathways. DNA Repair (Amst) 94: 102926.
Google Scholar: Author Only Title Only Author and Title

Torres-Ramos, C.A, Prakash, S., and Prakash, L. (2002). Requirement of RAD5 and MMS2 for Postreplication Repair of UV-Damaged DNA in Saccharomyces cerevisiae. Mol Cell Biol 22: 2419–2426.
Google Scholar: Author Only Title Only Author and Title

Tsuda, M., Kitamasu, K., Kumagai, C., Sugiyama, K., Nakano, T., and Ide, H. (2020). Tyrosyl-DNA phosphodiesterase 2 (TDP2) repairs topoisomerase 1 DNA-protein crosslinks and 3′-blocking lesions in the absence of tyrosyl-DNA phosphodiesterase 1 (TDP1). DNA Repair (Amst) 91–92: 102849.
Google Scholar: Author Only Title Only Author and Title

Varejão, N., Ibars, E., Lascorz, J., Colomina, N., Torres-Rosell, J., and Reverter, D. (2018). DNA activates the Nse2/Mms21 SUMO E3 ligase in the Smc5/6 complex. EMBO J 37: e98306 .
Google Scholar: Author Only Title Only Author and Title

Vaz, B. et al. (2016). Metalloprotease SPRTN/DVC1 orchestrates replication-coupled DNA-protein crosslink repair. Mol Cell 64: 704–719.
Google Scholar: Author Only Title Only Author and Title

Waldman, B.C. and Waldman, A.S. (1990). Illegitimate and homologous recombination in mammalian cells: differential sensitivity to an inhibitor of poly(ADP-ribosylation). Nucleic Acids Res 18: 5981–5988.
Google Scholar: Author Only Title Only Author and Title

Watanabe, K., Pacher, M., Dukowic, S., Schubert, V., Puchta, H., and Schubert, I. (2009). The STRUCTURAL MAINTENANCE of CHROMOSOMES 5/6 complex promotes sister chromatid alignment and homologous recombination after DNA damage in Arabidopsis thaliana. Plant Cell 21: 2688–2699.
Google Scholar: Author Only Title Only Author and Title

Waterhouse, A, Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F.T., De Beer, T.AP., Rempfer, C., Bordoli, L., Lepore, R., and Schwede, T. (2018). SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res. 46: W296–W303.
Google Scholar: Author Only Title Only Author and Title

Weickert, P. and Stingele, J. (2022). DNA-Protein Crosslinks and Their Resolution. Annu Rev Biochem 91: 157–181.
Google Scholar: Author Only Title Only Author and Title

Whalen, J.M., Dhingra, N., Wei, L., Zhao, X., and Freudenreich, C.H. (2020). Relocation of Collapsed Forks to the Nuclear Pore Complex Depends on Sumoylation of DNA Repair Proteins and Permits Rad51 Association. Cell Rep 31: 107635
Google Scholar: Author Only Title Only Author and Title

Willing, E.-M.M., Piofczyk, T., Albert, A, Winkler, J.B., Schneeberger, K., and Pecinka, A (2016). UVR2 ensures transgenerational genome stability under simulated natural UV-B in Arabidopsis thaliana. Nat Commun 7: 13522.
Google Scholar: Author Only Title Only Author and Title

Yang, F., Fernández Jiménez, N., Majka, J., Pradillo, M., and Pecinka, A (2021). Structural Maintenance of Chromosomes 5/6 Complex Is Necessary for Tetraploid Genome Stability in Arabidopsis thaliana. Front Plant Sci 12: 748252.
Google Scholar: Author Only Title Only Author and Title

Yu, Y., Li, S., Ser, Z, Kuang, H., Than, T., Guan, D., Zhao, X., and Patel, D.J. (2022). Cryo-EM structure of DNA-bound Smc5/6 reveals DNA clamping enabled by multi-subunit conformational changes. Proc Natl Acad Sci U S A 119: e2202799119.
Google Scholar: Author Only Title Only Author and Title

Zhang, H., Xiong, Y., and Chen, J. (2020). DNA–protein cross-link repair: what do we know now? Cell Biosci 10: 3..
Google Scholar: Author Only Title Only Author and Title