## Supplementary Methods

### Plasmids

To construct plasmids pRS426-*SAE2* (2μ) and pRS416-*SAE2* (*CEN*), the 1,038 bp *SAE2* ORF flanked by 370 bp upstream promoter and 36 bp synthetic MYC epitope were amplified by PCR from genomic DNA of LSY0678 and the resulting product was cloned into BamHI-SalI digested pRS426 or pRS416 (primers are available on request). pRS416-*sae2*-5A was made using plasmid pML487 as a template (1). pRS416-*sae2*-S267A and pRS416-*sae2*-T90A, T279A (2A) were made by side-directed mutagenesis of pRS416-*SAE2* (Agilent QuickChange II kit). pRS416-*SAE2*-13MYC was made by PCR amplification of *SAE2* with a 13MYC tag from genomic DNA of LSY3942-8C and the resulting fragment was cloned into NruI-SalI-digested pRS416-*SAE2*. Plasmids pRS416-*sae2*-S267A-13MYC, pRS416-*sae2*-2A-13MYC and pRS416-*sae2*-5A-13MYC were made by sub-cloning NruI-BsiWI fragments containing the phosphorylation site mutant alleles from pRS416-*sae2*-S267A, pRS416-*sae2*-2A, pRS416-*sae2*-5A into NruI-BsiWI-digested pRS416-*SAE2*-13MYC. Sae2 overexpression plasmids, pRS416-*GAL-SAE2* was made by PCR amplifying a 693 bp BglII-NruI fragment containing the *GAL1* promoter and the 5' part of *SAE2* from LSY3942-8C genomic DNA and cloning into BamHI-NruI-digested pRS416-*SAE2*. Then a SpeI-NruI 699 bp fragments containing the *GAL1* promoter and partial 5' *SAE2* from pRS416-*GAL-SAE2* was sub-cloned into SpeI-NruI-digested pRS416-*sae2*-S267A, pRS416-*sae2*-2A, pRS416-*sae2*-5A to obtain pRS416-*GAL-sae2*-S267A, pRS416-*GAL-sae2*-2A, and pRS416-*GAL-sae2*-5A.

**Table S1. *Saccharomyces cerevisiae* strains used in this study**

| Strain | Relevant genotype | Source |
|---|---|---|
| LSY0678 | *MATa ade2-1 can1 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5* | (2) |
| LSY1091 | *MATa sae2::KanMX6* | (3) |
| LSY3366 | *MATa mre11-H125N-NatMX4 (mre11-nd)* | (4) |
| LSY2872-2A | *MATα rad50K81I-URA3 (rad50S)* | (5) |
| LSY2363-28C | *MATa mec1::TRP1 sml1::HIS3* | (5) |
| LSY3769-8C | *MATa mre11-H125N-NatMX4 mec1::TRP1 sml1::HIS3* | This study |
| LSY3770-2A | *MATa rad50S-URA3 mec1::TRP1 sml1::HIS3* | This study |
| LSY3769-12D | *MATa sae2::KanMX6 mec1::TRP1 sml1::HIS3* | This study |
| LSY3299-1D | *MATa sgs1::HphMX4 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3753-6B | *MATa sgs1::HphMX4 mre11-H125N leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3850-2B | *sgs1::HphMX4 rad50S-URA3 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3671-2C | *MATa RAD9-3HA-KanMX6 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY4018-1B | *MATα RAD9-3HA-KanMX6 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3682-3C | *MATα mre11-H125N RAD9-3HA-KanMX6 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3974-9D | *MATα rad50S-URA3 RAD9-3HA-KanMX6 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3681-5C | *MATα sae2::KanMX6 RAD9-3HA-KanMX6 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3684-6C | *MATα 3HA-TEL1-URA3 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | (6) |
| LSY3697-1B | *MATα 3HA-TEL1-URA3 mre11-H125N leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3976-2A | *MATα 3HA-TEL1-URA3 rad50S-URA3* | This study |

| | | |
|---|---|---|
| | *leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | |
| LSY3696-3D | *MATα 3HA-TEL1-URA3 sae2::KanMX6 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3596-1 | *rad9::HIS3* | This study |
| LSY3771-2D | *rad9::HIS3 mre11-H125N-NatMX* | This study |
| LSY3772-1D | *rad9::HIS3 rad50S-URA3* | This study |
| LSY3597-1 | *rad9::HIS3 sae2::KanMX* | This study |
| LSY3849-8A | *rad9::HIS3 sgs1::HphMX leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3849-3C | *mre11-H125N rad9::HIS3 sgs1::HphMX leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3851-9C | *rad50S-URA3 rad9::HIS3 sgs1::HphMX leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3852-1B | *sae2::KanMX rad9::HIS3 sgs1::HphMX leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3590-14D | *MATa tel1-G2611D, D2612A, N2616K, D2631E-LEU2 (tel1-kd)* | This study |
| LSY3690-1B | *tel1-kd-LEU2 mre11-H125N-NatMX* | This study |
| LSY3926-1C | *tel1-kd-LEU2 rad50S-URA3* | This study |
| LSY3590-8D | *MATa tel1-kd-LEU2 sae2::KanMX* | This study |
| LSY3917-1C | *MATα tel1-kd-LEU2 RAD9-3HA-KanMX leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3915-3A | *MATα tel1::HphMX RAD9-3HA-KanMX leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3919-10D | *MATa tel1-kd-LEU2 sae2::KanMX RAD9-3HA-KanMX leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3914-4D | *MATα tel1::HphMX sae2::KanMX RAD9-3HA-KanMX leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY2127 | *MATa rad53-K227A-KanMX* | (Pike *et al.*, 2003) |
| LSY3551 | *MATa rad53-K227A-KanMX sae2::HIS3* | This study |
| LSY3689 | *rad53-K227A-KanMX mre11-H125N-NatMX* | This study |
| LSY3938-11C | *rad53-K227A-KanMX rad50S-URA3* | This study |
| LSY3574 | *rad53-R605A* | (7) |

| | | |
|---|---|---|
| LSY3909-7B | *rad53-R605A mre11-H125N* | This study |
| LSY3920-1A | *rad53-R605A rad50S-URA3* | This study |
| LSY3908-5B | *MATa rad53-R605A sae2::KanMX* | This study |
| LSY3958-2C | *MATa exo1-S372A, S567A, S587A, S692A-13myc-HIS3 (exo1-4S::A)* | (8) |
| LSY3959-2A | *mre11-H125N-NatMX4 exo1-4S::A-13myc-HIS3* | This study |
| LSY3960-2A | *rad50S-URA3 exo1-4S::A-13myc-HIS3* | This study |
| LSY3958-9D | *sae2::KanMX exo1-4S::A-13myc-HIS3* | This study |
| LSY3195-2B | *MATα DNA2-TEV-9MYC-HIS3 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | (6) |
| LSY3699-1C | *MATα sae2 ::KanMX DNA2-TEV-9MYC-HIS3 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3700-4D | *MATα mre11-H125N DNA2-TEV-9MYC-HIS3 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3928-4A | *rad50S-URA3 DNA2-TEV-9MYC-HIS3 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY3965-4B | *MATα sae2::SAE2-13MYC-TRP1 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR RAD9-3HA-KanMX* | This study |
| LSY4003-9A | *MATα mre11-H125N leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR sae2::SAE2-13MYC-TRP1 RAD9-3HA-KanMX* | This study |
| LSY4003-1C | *MATα rad50S-URA3 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR sae2::SAE2-13MYC-TRP1 RAD9-3HA-KanMX* | This study |
| LSY3942-8C | *MATα leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR KanMX-GAL1-SAE2-13MYC-TRP1 RAD9-3HA-KanMX* | This study |
| LSY3942-13A | *MATα mre11-H125N leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR KanMX-pGAL1-SAE2-13MYC-TRP1 RAD9-3HA-KanMX* | This study |
| LSY3943-35A | *MATα rad50S-URA3 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR KanMX-pGAL1-SAE2-13MYC-TRP1 RAD9-3HA-KanMX* | This study |
| LSY4005-8D | *MATα leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |

| | | |
|---|---|---|
| | *KanMX-pGAL1-SAE2-13MYC-TRP1 3HA-TEL1-URA3* | |
| LSY4005-11D | *MATα mre11-H125N leu2::pGAL-HO-LEU2 hml::oripRS hmr::ampR KanMX-pGAL1-SAE2-13MYC-TRP1 3HA-TEL1-URA3* | This study |
| LSY4006-35B | *MATα rad50S-URA3 leu2::pGAL-HO-LEU2 hml::oripRS hmr::ampR KanMX-pGAL1-SAE2-13MYC-TRP1 3HA-TEL1-URA3* | This study |
| LSY3670-3C | *MATa-inc ade3::GAL-HO rad51::LEU2 lys2::NatMX4 AVT2::lys-HOcs::KanMX6-(20kb)-TRP1-ys2 (SSA strain)* | This study |
| LSY3655-2 | *MATa-inc ade3::GAL-HO rad51::LEU2 lys2::NatMX4 AVT2::lys-HOcs::KanMX6-(20kb)-TRP1-ys2 exo1::HIS3* | This study |
| LSY3669-36D | *MATa-inc ade3::GAL-HO rad51::LEU2 lys2::NatMX4 AVT2::lys-HOcs::KanMX6-(20kb)-TRP1-ys2 sae2::KanMX exo1::HIS3* | This study |
| LSY3708-13B | *MATa-inc ade3::GAL-HO rad51::LEU2 lys2::NatMX4 mre11-H125N-URA3 AVT2::lys-HOcs::KanMX6-(20kb)-TRP1-ys2* | This study |
| LSY3925-57A | *MATa-inc ade3::GAL-HO rad51::LEU2 lys2::NatMX4 rad50S-URA3 AVT2::lys-HOcs::KanMX6-(20kb)-TRP1-ys2* | This study |
| LSY3687-1A | *MATa-inc ade3::GAL-HO rad51::LEU2 lys2::NatMX4 sae2::KanMX AVT2::lys-HOcs::KanMX6-(20kb)-TRP1-ys2* | This study |
| LSY3964-26A | *MATa-inc ade3::GAL-HO rad51::LEU2 lys2::NatMX4 exo1-4S::A-13myc-HIS3 AVT2::lys-HOcs::KanMX6-(20kb)-TRP1-ys2* | This study |
| LSY3964-20A | *MATa-inc ade3::GAL-HO rad51::LEU2 lys2::NatMX4 sae2::KanMX exo1-4S::A-13myc-HIS3 AVT2::lys-HOcs::KanMX6-(20kb)-TRP1-ys2* | This study |
| LSY4326-4D | *MATa rad9::NatMX TRP1::rad9-S462A, T474A (rad9-2A)* | (9) |
| LSY4328-4C | *MATa sae2::KanMX rad9::NatMX TRP1::rad9-2A* | This study |
| LSY3539 | *MATa slx4::HIS3* | This study |

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|---|---|---|
| LSY4208-5C | *MATa mre11-H125N-NatMX slx4::HIS3* | This study |
| LSY4208-13A | *MATa rad50S-URA3 slx4::HIS3* | This study |
| LSY3562-2D | *MATa sae2::KanMX slx4::HIS3* | This study |
| LSY4036-6D | *MATα mec1::TRP1 sml1::HIS3 RAD9-3HA-KanMX6 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY4036-8A | *MATα mec1::TRP1 sml1::HIS3 mre11-H125N RAD9-3HA-KanMX6 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY4037-9A | *MATα mec1::TRP1 sml1::HIS3 rad50S-URA3 RAD9-3HA-KanMX6 leu2::GAL-HO-LEU2 hml::oripRS hmr::ampR* | This study |
| LSY4038-11D | *MATα mec1::TRP1 sml1::HIS3 sae2 ::KanMX6 RAD9-3HA-KanMX6 leu2 ::GAL-HO-LEU2 hml ::oripRS hmr ::ampR* | This study |
| LSY4187-8C | *sae2-S73, T90, S249, T279, S289 (sae2-5A)* | This study |
| LSY4309-2B | *mre11-H125N-NatMX sae2-5A* | This study |
| LSY4309-1A | *rad9::HIS3 sae2-5A* | This study |
| LSY4309-1C | *mre11-H125N-NatMX rad9::HIS3 sae2-5A* | This study |
| LSY3832-1C | *dot1 ::HphMX* | This study |
| LSY3832-2A | *sae2 ::KanMX dot1 ::HphMX* | This study |
| LSY3561-1C | *hta1-S129A hta2-S129A* | This study |
| LSY3832-2B | *dot1 ::HphMX hta1-S129A hta2-S129A* | This study |
| LSY3832-3B | *dot1 ::HphMX hta1-S129A hta2-S129A sae2::kanMX* | This study |

All strains are W303 genetic background (*trp1-1 his3-11,15 can1-100 ura3-1 leu2-3,112 ade2-1 RAD5*), only the mating type and differences from this genotype are shown. The MAT genotype was not scored for some of the strains used for spot assays.

**References**


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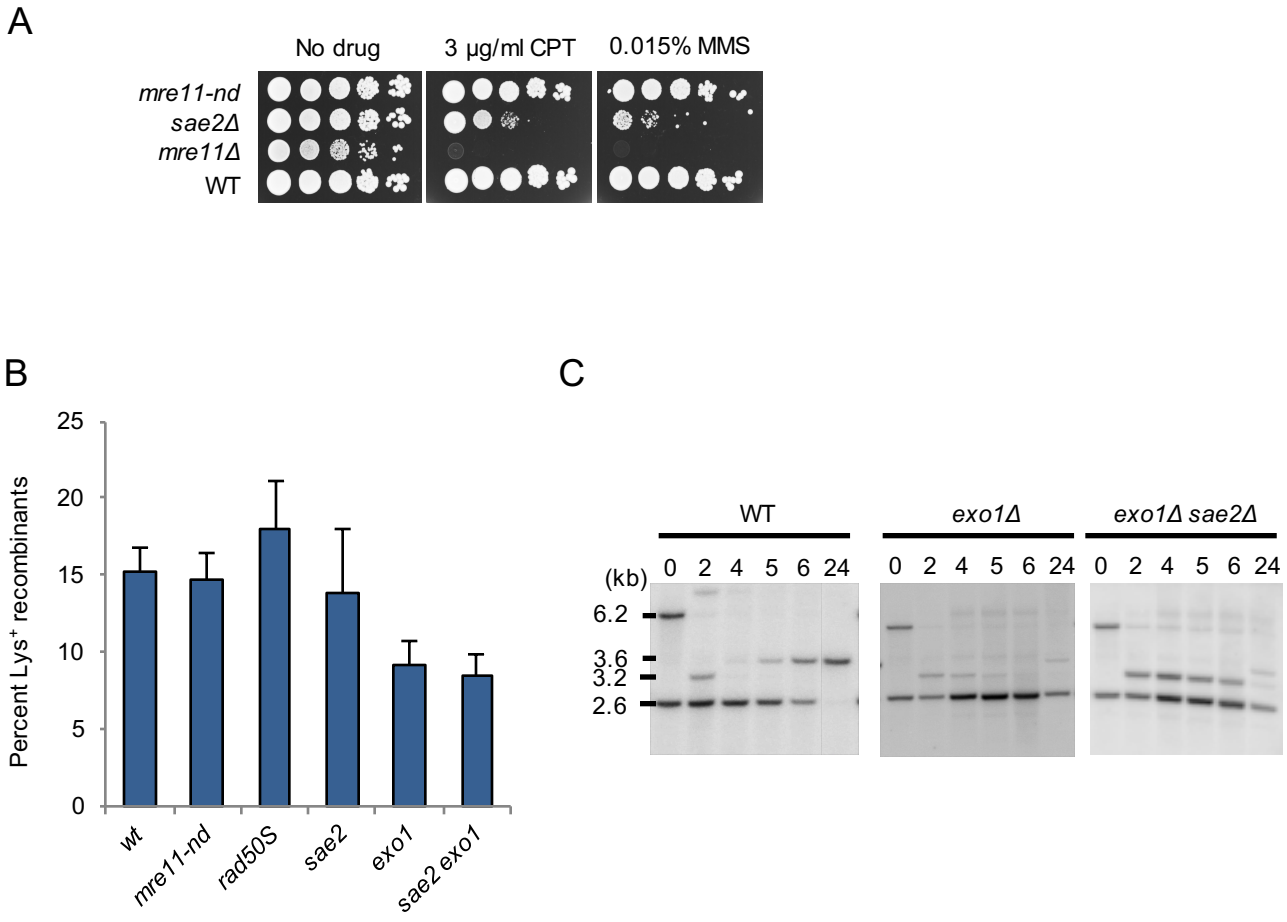


**Figure S1. DNA damage sensitivity and SSA phenotype of resection mutants. A** Ten-fold serial dilutions of the indicated strains spotted on agar plates with no additive, or with CPT or MMS. **B** The percent SSA as determined by the ratio of colony forming units (cfu) on SC-LYS + GAL medium to cfu on SC GLU-containing medium for the indicated strains. **C** Southern blot to detect the SSA product in WT, *exo1Δ* and *exo1Δ sae2Δ* strains.

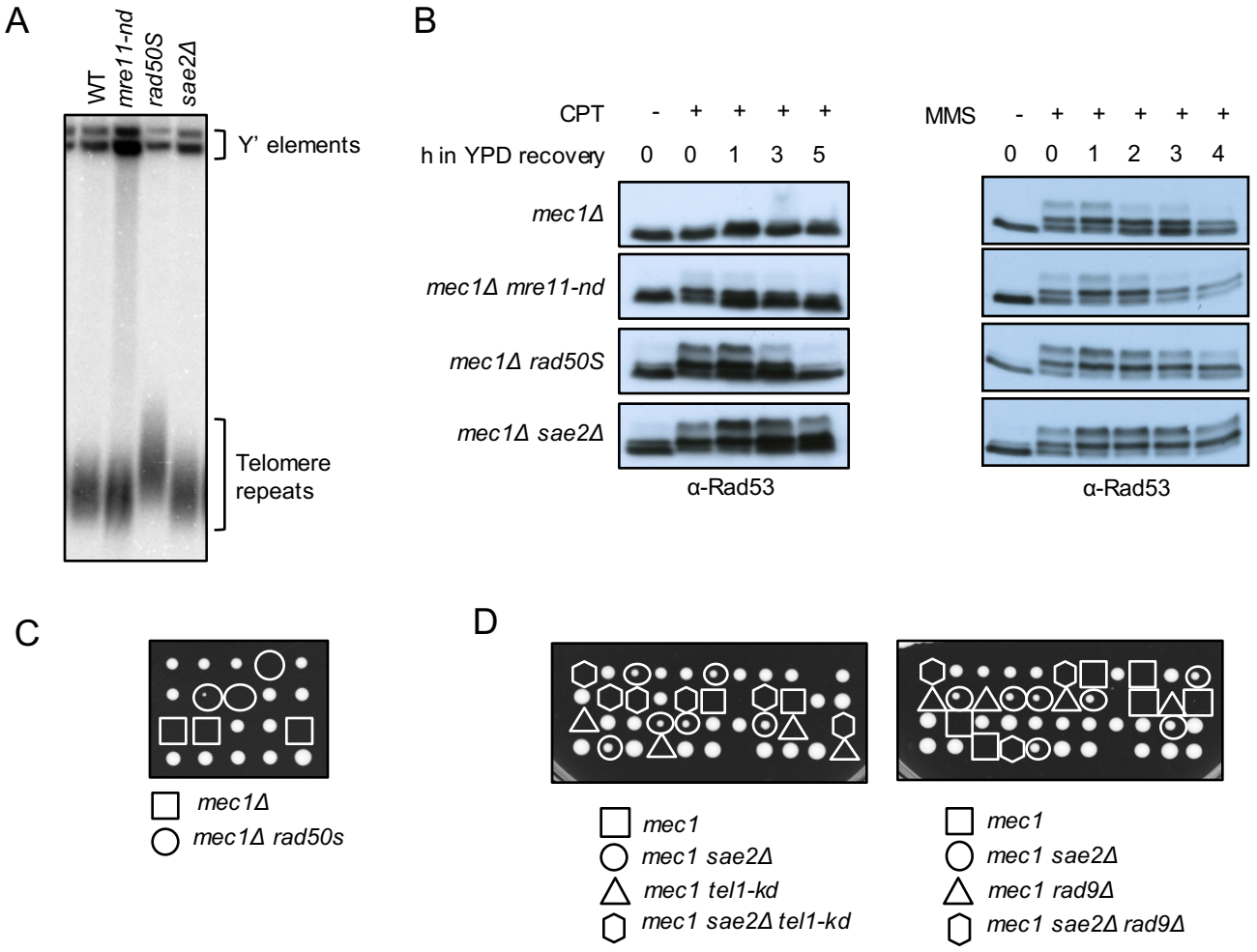


**Figure S2. Tel1 hyper-activation in *rad50S* and *sae2Δ* cells**. **A** Southern blot of XhoI digested genomic DNA probed with a Y'-telomere probe. **B** Western blot analysis showing Rad53 phosphorylation pattern in response to CPT or MMS. Log phase growing cells (t=0) from indicated strains were treated with 10 µg/mL CPT or 0.015% MMS for 1h and released into fresh YPD (t=0-5). Protein samples from different time points before and after drug treatment were analyzed using anti-Rad53 antibodies. **C** Spore colonies from dissection of a *mec1Δ/MEC1 sml1Δ/SML1 RAD50/rad50S* heterozygous diploid. **D** Spore colonies from dissection of *mec1Δ/MEC1 sml1Δ/SML1 SAE2/sae2Δ TEL1/tel1-kd* and *mec1Δ/MEC1 sml1Δ/SML1 SAE2/sae2Δ RAD9/rad9Δ* heterozygous diploids. Note that only *SML1* segregants are indicated.

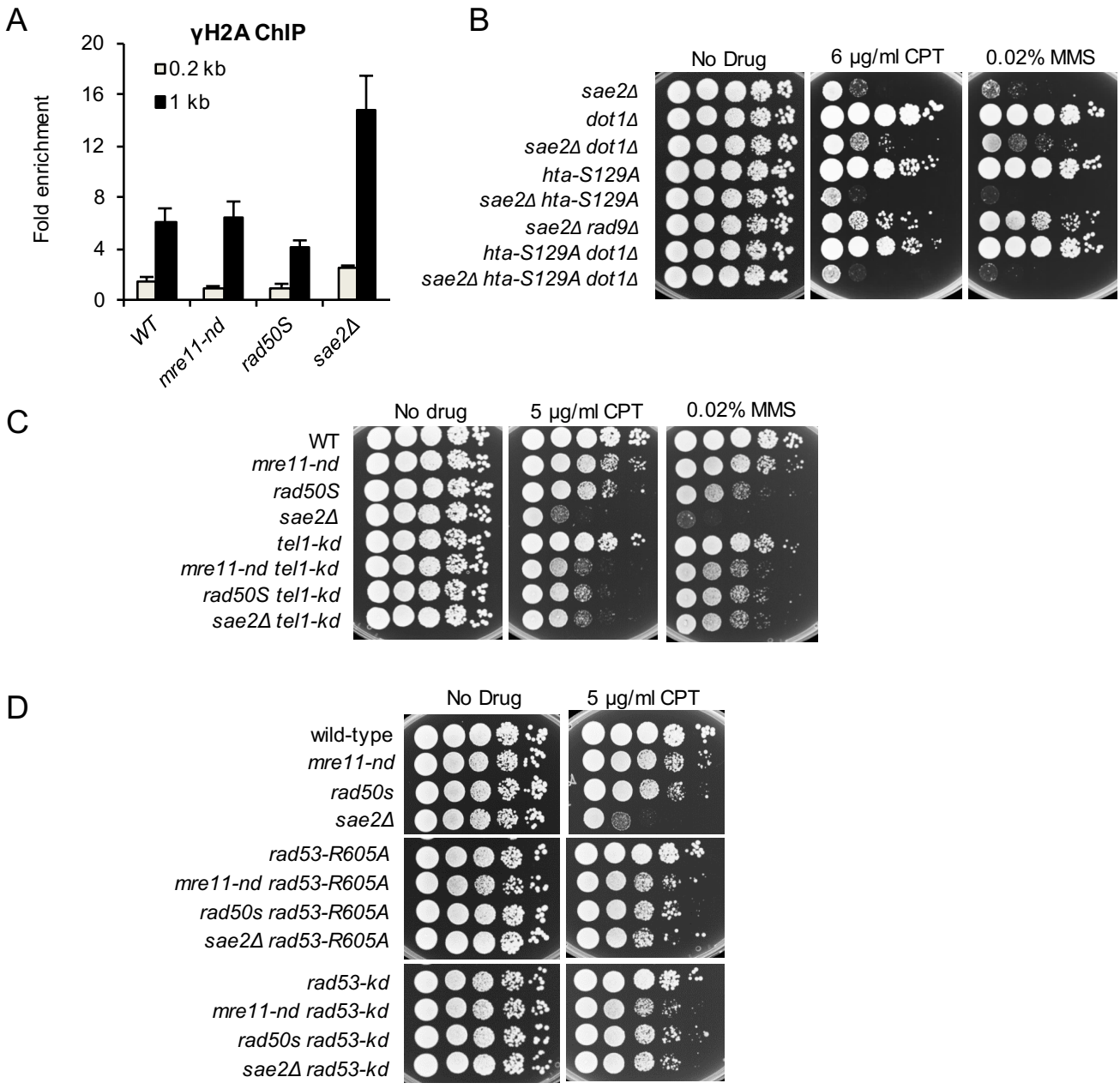


**Figure S3. Rad9 chromatin binding and Rad53 kinase activity contribute to *sae2Δ* CPT sensitivity. A** The relative fold enrichment of γH2A at 0.2 and 1 kb from the HO site was evaluated by ChIP- qPCR 3 hours after HO induction. **B** Ten-fold serial dilutions of *dot1Δ* and *hta-S129A* derivatives spotted on plates without drug, or plates containing CPT or MMS at the indicated concentrations. **C** Ten-fold serial dilutions of *tel-kd* derivatives spotted on plates without drug, or plates containing CPT or MMS at the indicated concentrations. **D** Ten-fold serial dilutions of *rad53-R605A* or *rad53-kd* derivatives spotted on plates without drug, or plates containing CPT at the indicated concentrations.

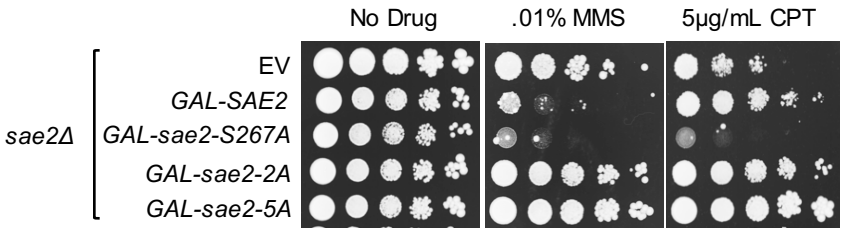


**Figure S4. Sae2 OE is toxic to cells.** Ten-fold serial dilutions of *sae2Δ* cells with EV, or the indicated *SAE2* allele expressed from the *GAL* promoter of a low copy number plasmid spotted on YPGal, YPGal + MMS or YPGal + CPT.