SUPPLEMENTARY INFORMATION

Supplementary Methods

Protein extraction and detection
For detecting sumoylated proteins, TCA method was used to make cell lysates as described (Foiani et al. 1994). For co-IPs, cells were lysed by bead beating in TMG-125 buffer (10 mM Tris HCl, pH 8, 4 mM MgCl₂, 10% Glycerol, 125 mM NaCl, 0.1 mM EDTA, 0.1 mM DTT, 40 mM NEM). DNA was digested by incubation with Benzonase for 30 min at 4°C, lysates were cleared by centrifugation and incubated in TMG-125 + 0.5% Tween-20 with anti-Rfa1 or anti-myc antibody for 2 h (Rfa1-IP) or o/n (myc-IP) at 4°C. Protein G agarose beads were added and samples were incubated for 1 h at 4°C. Beads were washed with TMG-125 and TMG-125 + 0.5% Tween-20 and proteins were eluted with Laemmli loading dye.

Proteins were separated on 3-8% Tris-acetate gels (Life Technologies) followed by western blotting with antibodies against Rfa1 (a kind gift of S. Brill), TAP- (Peroxidase-Anti-Peroxidase, Sigma), myc-tag (9E10, Bio X Cell) or GFP (Roche).

Chromatin fractionation
Spheroplasting was performed in 0.6 M Sorbitol, 25 mM TrisHCl pH 7.5, YPD, 10 mM DTT with purified Lytic β-1,3-glucanase (the expression plasmid was a kind gift from H. Zhou), and spheroplasts were washed in 0.4 M Sorbitol, 150 mM K acetate, 2 mM Mg acetate, 20 mM PIPES/KOH, pH 6.8, 40 mM NEM, protease inhibitors. Lysis was conducted in 150 mM K acetate, 2 mM Mg acetate, 20 mM PIPES/KOH, pH 6.8, 40 mM NEM, protease inhibitors, 1% Triton-X for 5 min on ice. 100 µl whole cell extract was saved before the lysate was separated by centrifugation (13000 rpm, 20 min) on a sucrose cushion (30% sucrose, 150 mM K acetate, 2 mM Mg acetate, 20 mM PIPES/KOH, pH 6.8, 40 mM NEM, protease inhibitors, 1% Triton-X). 100 µl of supernatant was collected and the chromatin-containing pellet was washed and resuspended in lysis buffer. Equal amounts of whole cell extract, supernatant and pellet fraction were subjected to protein precipitation with TCA and subsequently analyzed by western blotting using either 3-8% Tris-acetate or 4-20% Tris-glycine gels (Life Technologies). Note that the sumoylated forms of proteins could not be consistently maintained in these experiments. Fractionation efficiency was determined by
probing for Pgk1 (Invitrogen) and Orc2 (Abcam) or H3 (Abcam) for soluble and chromatin-associated proteins, respectively.

Cell imaging
Cells were grown in SC media at 25°C, treated with 0.3 mg/ml Zeocin for 2 h, washed with SC, mounted in 1.2% (w/v) low melting agarose and subjected to imaging on a Zeiss Axio Imager.Z1 microscope with a Plan-Apochromat 100x, 1.4 numerical aperture (NA) objective lens. Images were analyzed with ImageJ software.

GST pull-down assay
GST, GST-Siz23-120 and His-Rfa2174-273 were expressed in E. coli BL21 (DE3) and purified by either glutathione (GE Healthcare) or Ni-NTA (Qiagen) metal affinity chromatography using standard procedures. For pull-down assays, proteins were incubated in 20 µl binding buffer (50 mM NaCl, 10 mM Tris HCl pH 7.5, 10% Glycerol, 0.01% NP40, 4 mM MgCl₂, 10 µg/ml BSA) for 2 h at 4°C. 10 µl glutathione sepharose beads were added for 2 h and beads were washed three times with 200 µl binding buffer before proteins were eluted by heating the samples at 95°C for 5 min in 2x Laemmli buffer.

Gel shift assay
His-Siz21-120 wild type and cSAPmut proteins were expressed in E. coli BL21 (DE3) and purified by Ni-NTA (Qiagen) metal affinity chromatography using standard procedures. No protein, or molar ratios of 1:31.3, 1:62.5 and 1:125 were added to 0.4 pmol of a double-stranded 170mer in 50 mM Tris, 20 mM KCl, 10 mM DTT, 7.5% Glycerol and 100 µg/ml BSA. Reactions were incubated for 10 min at 37°C and separated on a 1.2% agarose gel. DNA was detected using SybrGold.

Sensitivity assay
Viability of cells before and after 2 h treatment with 0.1 mg/ml Zeocin was determined by plating cells on YPD plates and counting growth of colonies after two days.
**Supplementary Table S1**

**Strains and plasmids used in this study**

Strains are derivatives of W1588-4C, a RAD5 derivative of W303 (MATα ade2-1 can1-100 ura3-1 his3-11,15 leu2-3,112 trp1-1 rad5-535) (Zhao and Blobel 2005). Only one strain is listed for each genotype, but at least two independent isolates of each genotype were used in the experiments.

| Strain     | Genotype                                                                 | Source          |
|------------|--------------------------------------------------------------------------|-----------------|
| W1588-4A   | MATα his3 ade2-1-100 his3-11,15 ura3-1 TRP1-1 RAD5                       | R. Rothstein    |
| X6020-7C   | RAD52-TAP::HIS3 siz2Δ::KAN                                                | this study      |
| X4540-1B   | RAD52-TAP::HIS3 siz2Δ::KAN                                                | this study      |
| X6021-12D  | RAD52-TAP::HIS3 siz1Δ::KAN                                                | this study      |
| X4511-3B   | RAD52-TAP::HIS3 siz2Δ::KAN                                                | this study      |
| T1361-2    | MATα siz2-cSAPmut-9myc::klTRP1                                            | this study      |
| T1508-2    | MATα KAN::GAL1-SIZ2-9myc::klTRP1                                          | this study      |
| T1500-3    | MATα KAN::GAL1-siz2-SAPmut-9myc::klTRP1                                   | this study      |
| X4691-4D   | RAD52-TAP::HIS3 SIZ2-9myc::klTRP1                                         | this study      |
| X4692-1B   | RAD52-TAP::HIS3 SIZ2-9myc::klTRP1                                         | this study      |
| X5054-2B   | RAD52-TAP::HIS3 KAN::GAL1-siz2-cSAPmut-9myc::klTRP1                       | this study      |
| X5055-1C   | RAD52-TAP::HIS3 KAN::GAL1-siz2-cSAPmut-9myc::klTRP1                       | this study      |
| X5164-2B   | RAD52-TAP::HIS3 KAN::GAL1-SIZ2-9myc::klTRP1                               | this study      |
| X5165-7C   | RAD52-TAP::HIS3 KAN::GAL1-SIZ2-9myc::klTRP1                               | this study      |
| T1559-5    | MATα siz2-SIMmut-9myc::klTRP1                                            | this study      |
| X5493-3D   | RAD52-TAP::HIS3 siz2-SIMmut-9myc::klTRP1                                  | this study      |
| X5494-2C   | RAD52-TAP::HIS3 siz2-SIMmut-9myc::klTRP1                                  | this study      |
| T1354-7    | MATα SIZ2-9myc::klTRP1                                                    | this study      |
| T1549-4    | MATα siz2-CHmut-9myc::klTRP1                                              | this study      |
| X5454-17A  | RAD52-TAP::HIS3 siz2-CHmut-9myc::klTRP1                                   | this study      |
| X5389-2C   | RAD52-TAP::HIS3 siz2-CHmut-9myc::klTRP1                                   | this study      |
| T1565-13   | MATα GPD1-OsTIR::LEU2 RFA1-V5-IAA7::KAN                                   | this study      |
| X5633-1D   | SIZ2-9myc::klTRP1 RFA1-V5-IAA7::KAN                                        | this study      |
| X5633-13A-6| GPD1-OsTIR::LEU2 RFA1-V5-IAA7::KAN SIZ2-9myc::klTRP1                      | this study      |
| X5472-42B  | RAD52-TAP::HIS3 GPD1-OsTIR::LEU2 RFA1-V5-IAA7::KAN SIZ2-9myc::klTRP1       | this study      |
| X5473-14A  | RAD52-TAP::HIS3 GPD1-OsTIR::LEU2 RFA1-3V5-IAA7::KAN SIZ2-9myc::klTRP1       | this study      |
| X6319-7B   | SIZ2-YFP::HIS3 RFA1-CFP                                                   | this study      |
| X6597-2B-1 | SIZ2-YFP::HIS3 GPD1-OsTIR::LEU2 RFA1-V5-IAA7::KAN                         | this study      |
| X6597-1D   | SIZ2-YFP::HIS3 RFA1-V5-IAA7::KAN                                          | this study      |
| X5565-3B   | RAD52-TAP::HIS3 gsa1Δ::HIS3                                               | this study      |
| X5565-19B  | RAD52-TAP::HIS3 exo1Δ::KAN                                                | this study      |
| X5565-5B   | RAD52-TAP::HIS3 gsa1Δ::HIS3 exo1Δ::KAN                                     | this study      |
| Reference | Description |
|-----------|-------------|
| pXZ333    | pOBD-Rad52  |
| pXZ334    | pOBD-Rad59  |
| pXZ327    | pOBD-Rfa1   |
| pXZ328    | pOBD-Rfa2   |
| pXZ329    | pOBD-Rfa3   |
| pXZ292    | pOAD-Siz1   |
| pXZ294    | pOAD-Siz2   |
| pXZ221    | pOBD-SUMO   |
| pXZ767-4  | pOAD-Siz2-1-120 |
| pXZ817-10 | pOAD-Siz2A1-120 |
| pXZ818-3  | pOAD-Siz2-SAP_{Siz1} |
| pXZ797-3  | pOAD-Siz2-6A |
| pXZ819-9  | pOAD-Siz2-4E2K |
| pXZ714-3  | pOAD-Siz2-cSAPmut |
| pXZ799-4  | pOBD-Rfa2-1-167 |
| X5566-2A  | RAD59-TAP::HIS3 sgs1A::HIS3 |
| X5566-4D  | RAD59-TAP::HIS3 exo1A::KAN |
| X5566-14C | RAD59-TAP::HIS3 sgs1A::HIS3 exo1A::KAN |
| T1634-1   | MATalpha EXO1-TAP::HIS3 |
| T1635-2   | MATalpha exo1-ND-TAP::HIS3 |
| X5808-9B  | RAD52-TAP::HIS3 EXO1-TAP::HIS3 |
| X5809-2B  | RAD59-TAP::HIS3 EXO1-TAP::HIS3 |
| X5810-2A  | RAD52-TAP::HIS3 exo1-ND-TAP::HIS3 |
| X5811-4B  | RAD59-TAP::HIS3 exo1-ND-TAP::HIS3 |
| X6454-6A  | RAD52-TAP::HIS3 mre11A::LEU2 |
| X6455-1B  | RAD52-TAP::HIS3 sae2A::KAN |
| X6456-4A  | RAD59-TAP::HIS3 mre11A::LEU2 |
| X6457-7C  | RAD59-TAP::HIS3 sae2A::KAN |
| X6632-5D  | SIZ2-YFP::HIS3 RFA1-CFP exo1A::KAN |
| X4719-9A  | SIZ2-9myc::klTRP1 exo1A::KAN |
| T1807-1A  | MATa siz2-6A-9myc::klTRP1 |
| T1855-11  | MATalpha siz2-4E2K-9myc::klTRP1 |
| T1862-20  | MATalpha KAN::GAL1-siz2-4E2K-9myc::klTRP1 |
| X6644-1C  | RAD52-TAP::HIS3 siz2-6A-9myc::klTRP1 |
| X6645-2B  | RAD59-TAP::HIS3 siz2-6A-9myc::klTRP1 |
| X6770-1A  | RAD52-TAP::HIS3 KAN::GAL1-siz2-4E2K-9myc::klTRP1 |
| X6771-7B  | RAD59-TAP::HIS3 KAN::GAL1-siz2-4E2K-9myc::klTRP1 |
| X6688-3A  | siz2-6A-YFP::HIS3 RFA1-CFP |
| X6801-18A | KAN::GAL1-siz2-4E2K-YFP::HIS3 RFA1-CFP |
| X6615-5C  | KAN::GAL1-siz2-cSAPmut-YFP::HIS3 RFA1-CFP |
| T1854-7   | MATalpha siz2-SAP_{Siz1}-9myc::klTRP1 |
| X6506-1A  | SIZ2-9myc::TRP1 HF-SMT3::LEU2 |
| pJ69-4A   | MATa TRP1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ |
| (James et al. 1996) | |
| pXZ33    | lab collection |
| pXZ334   | lab collection |
| pXZ327   | lab collection |
| pXZ328   | lab collection |
| pXZ329   | lab collection |
| pXZ292   | lab collection |
| pXZ294   | lab collection |
| pXZ221   | lab collection |
| pXZ767-4 | this study |
| pXZ817-10 | this study |
| pXZ818-3 | this study |
| pXZ797-3 | this study |
| pXZ819-9 | this study |
| pXZ714-3 | this study |
| pXZ799-4 | this study |
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Zhao, X. and Blobel, G. 2005. A SUMO ligase is part of a nuclear multiprotein complex that affects DNA repair and chromosomal organization. *Proceedings of the National Academy of Sciences of the United States of America* 102(13): 4777-4782.
**Figure S1**

**A.** Zeocin-induced sumoylation of Rfa1, Rad52, and Rad59 requires Siz2 ligase activity. Sumoylation of the recombination proteins was assessed as in Fig. 1A in the *siz2-CHmut* mutant (C354A, H356A) that has impaired ligase activity.

**B.** *Siz2-cSAPmut* expressed from the endogenous promoter shows a strong reduction in protein level.

**C.** Mutating three conserved residues in the cSAP domain of Siz2 abolishes DNA interaction *in vitro*. His-tagged wild type or cSAPmut Siz21-120 was added in increasing amounts to dsDNA, and DNA was visualized on an agarose gel with SybrGold. Wild-type, but not mutated, form of the Siz2 fragment shifts the DNA bands.

**D.** Siz2-cSAPmut protein levels can be restored by a *GAL1* promoter. *GAL1*-driven expression of the mutant induced by 2% galactose yielded similar protein levels as the wild-type construct. Unmodified form of Siz2 is indicated by a dot, and the sumoylated form by an arrowhead; see panel E for demonstration of the sumoylated form of Siz2.

**E.** Siz2 is sumoylated. Extracts from cells with wild-type SUMO were compared with those from HA-FLAG (HF)-tagged-SUMO expressing cells. Introduction of the tagged SUMO form resulted in an upward shift of the slower migrating Siz2-myc band, indicating that it is the sumoylated form.

**F.** Siz2 is auto-sumoylated. The *siz2-CHmut* mutant that lacks ligase activity abolished its sumoylated form, suggesting that Siz2 sumoylates itself.

**G.** Mutation of the C-terminal SIM of Siz2 protein (SIMmut) does not affect Siz2 protein level or sumoylation.
Figure S2

A. Y2H assay shows that Rfa2 C-terminal fragment (a.a. 174-273), but not its N-terminal fragment (a.a. 1-167), is sufficient for Siz2 interaction. Note that the Rfa2 N-terminal fragment supported the expected interactions with Rfa1 and 3, indicative of correct folding and expression.

B. Rfa1-AID is depleted upon the addition of 1 mM IAA in the presence of the degron co-factor TIR.

C. Depletion of RPA has no effect on Siz2 protein level or sumoylation. Cells carrying AID-tagged Rfa1 and the degron co-factor TIR were treated with 1 mM IAA and 0.3 mg/ml Zeocin, and protein extracts were examined by western blots.

D. Depletion of RPA has no effect on Siz2-YFP protein level or sumoylation. Cells carrying AID-tagged Rfa1 in the absence or presence of the degron co-factor TIR were treated with 1 mM IAA and 0.3 mg/ml Zeocin, and protein extracts were examined by western blots.

E. In undamaged cells, depletion of RPA has no effect on the chromatin-associated portion of Siz2. Chromatin fractionation was performed with cells expressing Rfa1-AID with or without the degron co-factor TIR after incubation with IAA. Note that Rfa1 degradation is induced only in cells expressing TIR.
Figure S3
A. Zeocin-induced sumoylation of Rfa1, Rad52, and Rad59 requires Exo1 but not Sgs1. Experiments were done as in Fig. 1A.
B. Zeocin-induced sumoylation of Rfa1, Rad52, and Rad59 partially depends on Mre11 and Sae2. Experiments were done as in Fig. 1A.
C. Deletion of EXO1 does not affect the protein level or sumoylation of Siz2 tagged with Myc or YFP.
D. Chromatin fractionation shows that in the absence of DNA damage, the pool of chromatin-bound Siz2 is similar in exo1Δ and wild-type cells.
Figure S4
A. Mutating three residues in the DNA-binding cSAP motif of Siz2 does not disrupt its interaction with RPA by Y2H.
B. Co-immunoprecipitation confirms the interaction between Siz2-cSAPmut and RPA. RPA was immunoprecipitated using an anti-Rfa1 antibody and myc-tagged Siz2 wild type or galactose-induced Siz2-cSAPmut was detected using an anti-myc antibody.
Figure S5
A. Control co-immunoprecipitation. Co-IP results performed with or without addition of anti-Rfa1 antibody are shown.
B. The siz2-4E2K mutation at the endogenous locus results in a twofold reduction in protein level compared to the wild-type protein (left). By driving the expression from the same locus under a galactose-inducible promoter and incubating cells with 1.5% galactose/0.5% glucose for 3 h, Siz2 protein levels similar to the wild type could be obtained (right).
C. Auto-sumoylation of Siz2 is not changed in siz2-4E2K cells. The ratio of modified Siz2 to unmodified was measured in five experiments and normalized to the wild type. The mean and SD are shown.
Figure S6

A. Quantifications of western blots as shown in Fig. 5A and 5B from five independent tests with mean and SD. The graph indicates the ratio of sumoylated to unmodified bands of the substrate proteins after exposure to Zeocin in siz2-6A or siz2-4E2K normalized to wild type.

B. The Siz2-YFP protein levels in cells used for Fig. 5C were examined by western blotting to show that the mutations did not change protein levels.

C. Representative image of cells expressing wild-type versions of Rfa1 and Siz2 tagged with CFP and YFP, respectively, after treatment with Zeocin. Experiments were performed at the same time as those shown in Fig. 5C.

D. Chromatin fractionation of cells expressing the Siz2-6A and -4E2K mutant proteins shows that in the absence of DNA damage, these mutants associate with chromatin similarly to wild type.

E. The Siz2-SAP$_{Siz1}$ mutant protein that has the cSAP-eSAP domain (a.a. 1-120) of Siz2 replaced with that of Siz1 (a.a. 1-111) exhibits wild-type levels of chromatin association without DNA damage and reduced levels after Zeocin treatment.

F. siz2-4E2K that is deficient for RPA interaction also displays reduced sumoylation of Rfa1, Rad52, and Rad59 upon treatment with MMS.