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Supplementary Information for

The Srs2 helicase dampens DNA damage checkpoint by recycling RPA from chromatin

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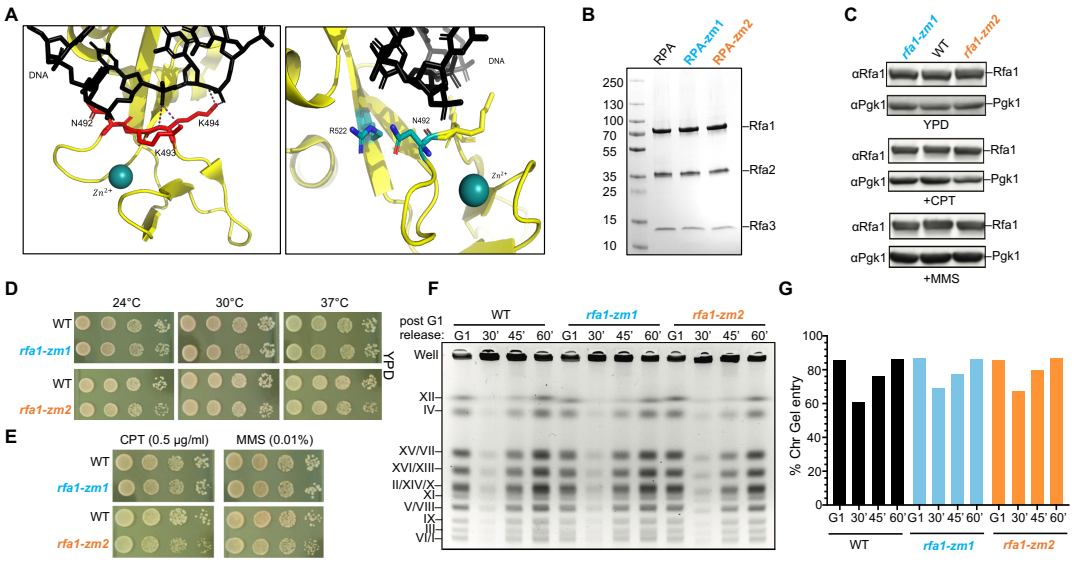

Fig. S1. *rfa1-zm* mutants do not affect cell growth and DNA replication.
(A) Left: molecular contacts around the Zn^{2+}-finger region show direct contacts between the side chains of K493 and K494 in DBD-C and the phosphate backbone of ssDNA. Right: N492 is poised to interact with R522 and likely plays an important role in positioning the loop upon DNA binding.
(B) SDS-PAGE analysis of recombinantly purified wild type RPA, RPA-zm1, and RPA-zm2 proteins.
(C) *rfa1-zm* does not affect Rfa1 protein level. Protein extracts were prepared from asynchronous cultures either untreated or treated with 16μg/ml CPT or 0.01% MMS for 2 hours. Rfa1 was examined using anti-Rfa1 specific antibody. Pgk1 was used as a loading control.
(D) *rfa1-zm* mutants do not compromise cell growth at three different temperatures. 10-fold serial dilution of cells were spotted on YPD plates and growth was assessed after incubation at 24°C, 30°C and 37°C for 2 days.
(E) *rfa1-zm* mutants grow similar to WT at lower concentration of CPT and MMS. Experiments were done as described in Fig. S1D.
(F-G) Chromosomal replication completion in *rfa1-zm* mutant cells is comparable to WT cells. Cultures arrested in G1 were released into YPD for 60 minutes. Samples were taken at the indicated time points and pulse field gel electrophoresis was performed, followed by staining with Ethidium Bromide. (F) shows Ethidium Bromide stained agarose gel, fully replicated chromosomes are able to enter the gel, while chromosomes that have not completed replication are stuck in the well. (G) fraction of replicated chromosomes is quantified relative to unreplicated chromosomes.

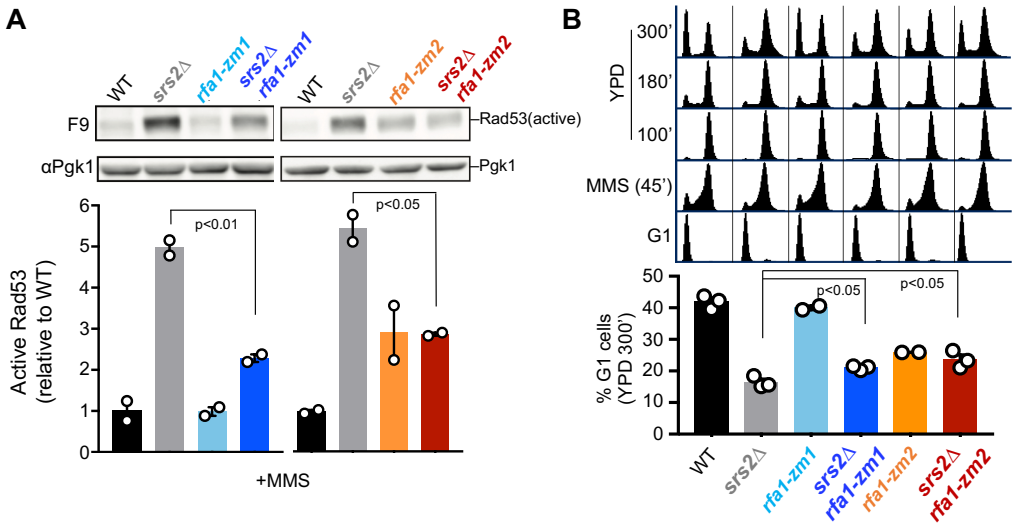

Fig. S2. *rfa1-zm* mutants reduce DDC hyper-activation in *srs2Δ* cells upon MMS treatment.
(A) *rfa1-zm* mutants reduce the level of active Rad53 in *srs2*Δ cells after MMS treatment. Asynchronous cultures were treated with 0.02% MMS (top left) or 0.01% MMS (top right) for one hour and released into fresh YPD for 4 hours (top left) or 2 hours (top right). Activated Rad53 was detected by the F9 antibody by immunoblotting. Active Rad53 signals were compared to the Pgk1 loading control and normalized to wild-type. Mean of two biological isolates per genotype is graphed relative to wild-type, with error bars representing SEM. Statistically significant difference by student's t-test are indicated by p values.
(B) *rfa1-zm* mutants allow better G1 entry of *srs2*Δ cells. G1 arrested cells were released into the cell cycle in the presence of 0.005% MMS for 45 minutes. Following MMS treatment, the cultures were transferred into fresh YPD and FACS samples were collected at the indicated time point. FACS for indicated samples is shown (top) and a graph for percentage of G1 cells after 5 hours following G1 arrest is at the bottom. Mean of three biological isolates per genotype is graphed (two isolates were used for *rfa1-zm1* and *-zm2*), with error bars representing SEM. Statistically significant difference by student's t-test are indicated by p values.

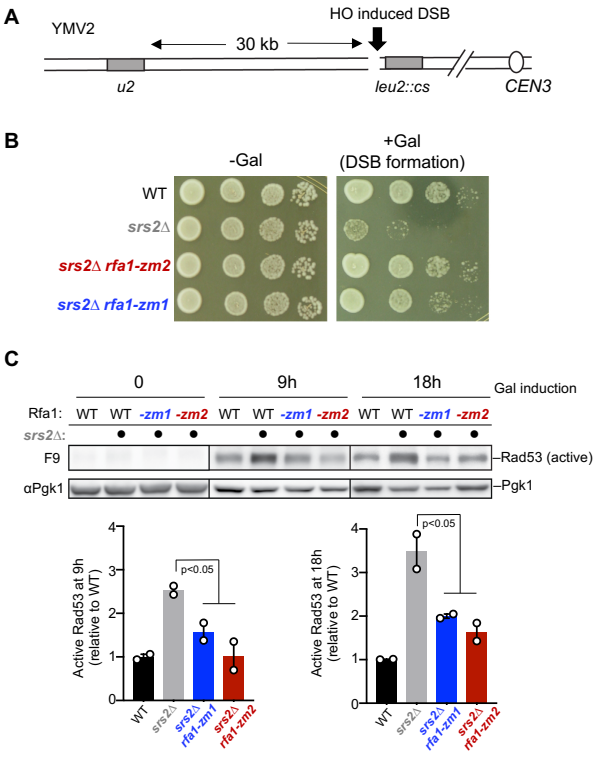

Fig. S3. *rfa1-zm* mutants rescue growth defect and DDC hyper-activation of *srs2*Δ cells upon a single DSB generation.
(A) Schematic of *ChrIII* in the YMV2 strain (1)(adapted from (2)). A HO cleavage site to induce a DSB is inserted into the *LEU2* open reading frame (*leu2-cs*). A partial *LEU2* segment is inserted 30kb away (*u2*) to provide a template for repair.
(B) *rfa1-zm* rescued the growth defect of *srs2*Δ cells upon induction of a single DSB by galactose. Experiments were done as described in Fig. S1D.
(C) *srs2*Δ cells show an increase in the level of active Rad53 upon induction of a single DSB by galactose, while *rfa1-zm* mutants rescue this increase. Galactose was added to asynchronous cultures growing in YP+Raffinose in order to induce DSB formation, and samples were collected at 0, 9 and 18 hours post galactose addition. Protein extracts were prepared and examined by immunoblotting and quantification is presented as in Fig. S2A.

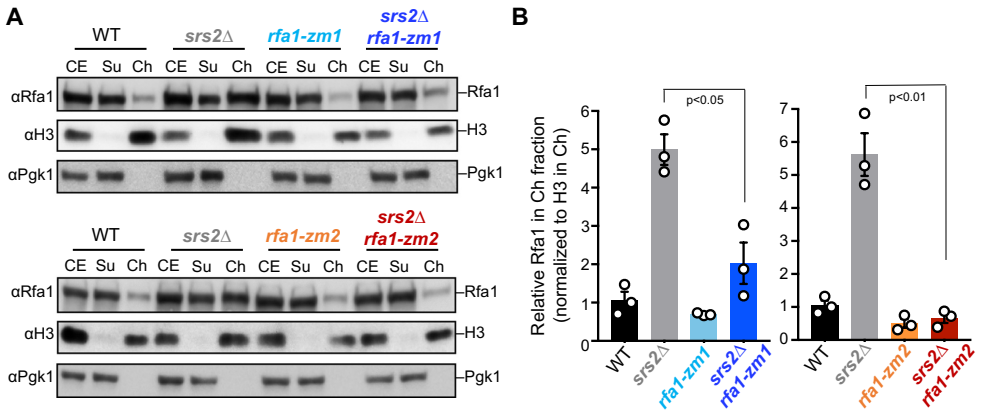

Fig. S4. *rfa1-zm* mutants reduce hyper-chromatin association of RPA in *srs2*Δ cells upon MMS treatment.
(A) *rfa1-zm* mutants reduce chromatin bound Rfa1 in *srs2*Δ cells upon MMS treatment. Cells were treated as in Fig. S2A. Rfa1 in cell extract (CE), chromatin-bound (Ch) and -unbound (Su) fractions was examined. H3 and Pgk1 are markers for Ch and Su fractions, respectively.
(B) Quantification of the levels of chromatin bound Rfa1 after being normalized to H3. Mean of three biological isolates per genotype is graphed with error bars representing SEM. Statistically significant difference by student's t-test are indicated by p values.

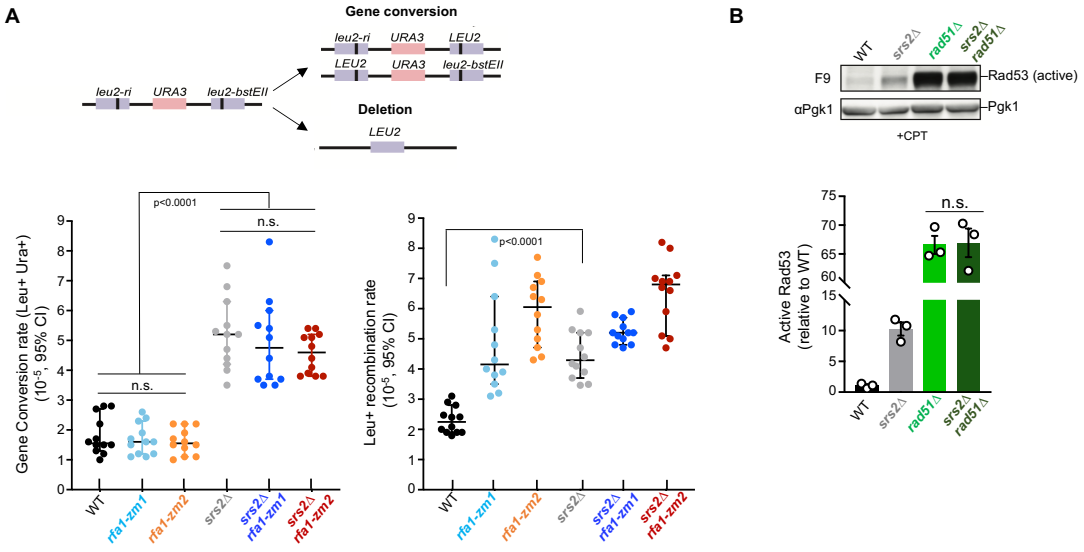

Fig. S5. *rfa1-zm* mutants do not reduce hyper HR in *srs2*Δ cells and *rad51*Δ mutant does not reduce *srs2*Δ DDC hyper-activation.

(A) *rfa1-zm* mutants do not reduce homologous recombination. Top panel: schematic of the homologous recombination assay used. In brief, two different *leu2* defective alleles with mutations in the 5' or 3' end of the gene are separated by a *URA3* gene. Gene conversion or deletion events can produce a *LEU2* gene that supports growth on media lacking leucine and can be scored as Leu+ colonies, while only gene conversion events can retain both *LEU2* and *URA3* marker that can be scored as Leu+Ura+ colonies. Bottom left panel: Leu+ Ura+ gene conversion rates as measured by fluctuation analysis (N=12) are plotted. Bottom right panel: Leu+ recombination rates as measured by fluctuation analysis (N=12) are plotted. For both panels, error bars represent the median with 95% CI. Statistically significant difference by the Mann-Whitney test is indicated by p value.

(B) *rad51*Δ mutant does not reduce active Rad53 level in *srs2*Δ cells. G1 arrested cells were released into the cell cycle in the presence of CPT for two hours. Data are presented and analyzed as in Fig. S2A, except N=3.

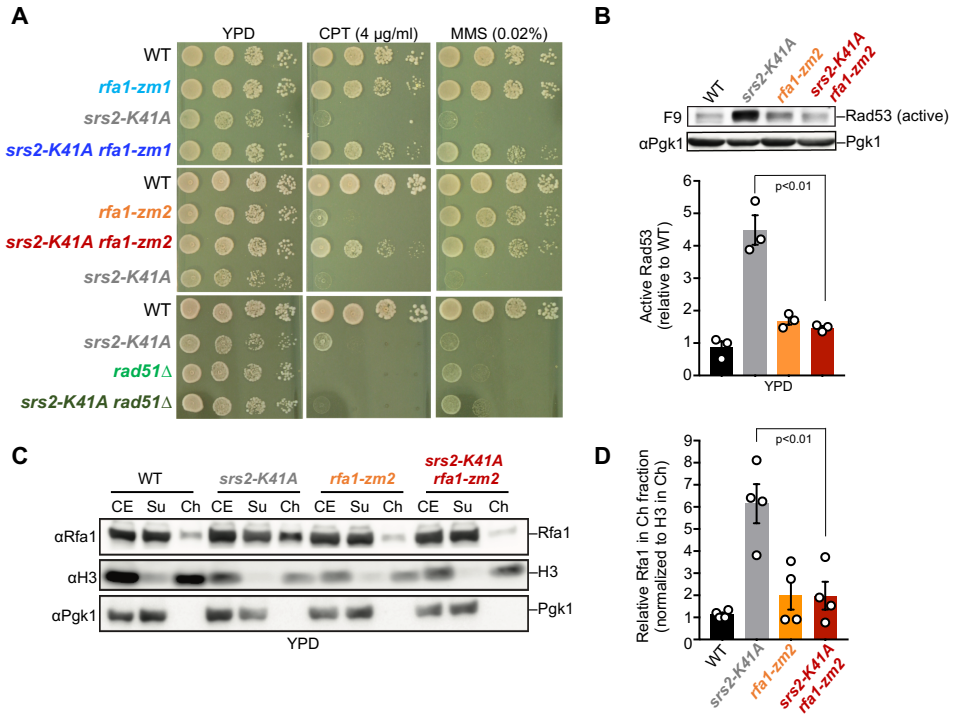

Fig. S6. *rfa1-zm* mutants rescue genotoxic sensitivity of *srs2-K41A* and *rfa1-zm2* rescues multiple defects of *srs2-K41A* cells.
(A) *rfa1-zm* but not *rad51*Δ suppressed *srs2-K41A* sensitivity to CPT and MMS. Experiments were done as described in Fig. S1D.
(B) *rfa1-zm2* reduces the levels of active Rad53 in *srs2-K41A* cells. Protein extracts prepared from cultures growing in YPD were examined by immunoblotting and quantification is presented as in Fig. S2A, except N=3.
(C-D) *rfa1-zm2* reduces chromatin bound Rfa1 in *srs2-K41A* cells. Experiments were done as in Fig. S4A except cells were grown in YPD media. Data are presented and analyzed as in Figs. S4A and S4B, except N=4.

Table S1: Strains used in this study
All strains are derivatives of W1588-4C, a *RAD5* derivative of W303 (*MATa ade2-1 can1-100 ura3-1 his3-11, 15 leu2-3, 112 trp1-1 rad5-535*), unless otherwise noted. 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
X8047-1A	*MATa srs2Δ::HIS3*	This study
X6214-4C	*rfa1-t11*	This study
X6214-9A	*rfa1-t11 srs2Δ::HIS3*	This study
X8211-11B	*MATa rfa1-t48*	This study
X8211-13B	*MATa rfa1-t48 srs2Δ::HIS3*	This study
X6217-5D	*rfa1-D228Y*	This study
X6215-10D	*rfa1-D228Y srs2Δ::HIS3*	This study
X8210-2B	*MATa rfa1-t33*	This study
X8210-3B	*MATa rfa1-t33 srs2Δ::HIS3*	This study
X8049-22A	*MATa rfa1-zm1*	This study
X8049-13A	*MATa rfa1-zm1 srs2Δ::HIS3*	This study
X8047-8A	*MATa rfa1-zm2*	This study
X8047-8B	*MATa rfa1- zm2 srs2Δ::HIS3*	This study
G161 (DMP3295-8B)	*MATa MEC1-HA9::LEU2 DDC2-Myc18:: HIS3*	M.P.Longhese
X8428 I-6C	*MATa MEC1-HA9::LEU2 DDC2-Myc18:: HIS3 srs2Δ::HIS3*	This study
X8428 II-3B	*MATa MEC1-HA9::LEU2 DDC2-Myc18:: HIS3 rfa1-K494A*	This study
X8428 I-20C	*MATa MEC1-HA9::LEU2 DDC2-Myc18:: HIS3 srs2Δ::HIS3 rfa1-zm1*	This study
X8427 I-18D	*MATa MEC1-HA9::LEU2 DDC2-Myc18::HIS3 rfa1-zm2*	This study
X8427 I-2C	*MATa MEC1-HA9::LEU2 DDC2-Myc18:: HIS3 srs2Δ::HIS3 rfa1-zm2*	This study
G564 (W4314-2C)	*MATα rDNA::ADE2-CAN1*	R. Rothstein
X8106-1A	*rDNA::ADE2-CAN1*	This study
X8106-1C	*rDNA::ADE2-CAN1 srs2Δ::HIS3*	This study
X8106-9A	*rDNA::ADE2-CAN1 rfa1-zm2*	This study
X8106-6B	*rDNA::ADE2-CAN1 rfa1-zm2 srs2Δ::HIS3*	This study
X8107-42A	*rDNA::ADE2-CAN1 rfa1-zm1*	This study
X8107-21C	*rDNA::ADE2-CAN1 rfa1-zm1 srs2Δ::HIS3*	This study
X8165-2C	*rDNA::ADE2-CAN1 rad51Δ::LEU2*	This study
X8165-8C	*rDNA::ADE2-CAN1 rad51Δ::LEU2 srs2Δ::HIS3*	This study
X3969-4C	*MATα sgs1Δ::KAN*	Lab stock
X8132-3B	*sgs1Δ::KAN rfa1-zm1*	This study
X8131-1A	*sgs1Δ::KAN rfa1-zm2*	This study
X2426-14A	*MATα mph1Δ::URA3*	Lab stock
X8046-15B	*mph1Δ::URA3 rfa1-zm1*	This study
X8044-18D	*mph1Δ::URA3 rfa1-zm2*	This study
G1086 (hky2070-4a)	*srs2 (1-860)::HphMX*	(3)
G350 (hky660-2A)	*MATα leu2-RI::URA3::leu2-BsteII*	H.Klein
X8102-5C	*leu2-RI::URA3::leu2-BsteII*	This study
X8102-5A	*leu2-RI::URA3::leu2-BsteII srs2Δ::HIS3*	This study
X8102-8C	*leu2-RI::URA3::leu2-BsteII rfa1-zm1*	This study
X8102-8A	*leu2-RI::URA3::leu2-BsteII rfa1-zm1 srs2Δ::HIS3*	This study
X8101-7C	*leu2-RI::URA3::leu2-BsteII rfa1-zm2*	This study
X8101-13B	*leu2-RI::URA3::leu2-BsteII rfa1-zm2 srs2Δ::HIS3*	This study

X8157-9B	*MATa rad51Δ::LEU2*	This study
X8157-2C	*MATa rad51Δ::LEU2 srs2Δ::HIS3*	This study
G852 (E171)	*MATa srs2-K41A*	H.Klein
X8049-7B	*MATα rfa1-zm1*	This study
X8047-1B	*MATα rfa1-zm2*	This study
X3311-3B	*MATα rad51Δ::LEU2*	Lab stock
X8299-5C	*MATα srs2-K41A rfa1-zm1*	This study
X8170-3D	*MATa srs2-K41A rfa1-zm2*	This study
X8235-2A	*MATa srs2-K41A rad51Δ::LEU2*	This study
G1052 (yDD1413)	*ho hml::ADE1 MATa::hisG hmr::ADE1 his4::URA3-leu2(Xho1-to Asp718) -pBR322-his4 leu2::HOcs ade3::GAL:HO ade1 lys5 ura3-52 trp1 (trp1::hisG) DDC2-GFP-NATMX3*	(2)
G1053 (yDD1633)	G1052 *srs2Δ::KAN*	(2)
T2152-4	G1053 *rfa1-zm2*	This study
T2153-10	G1053 *rfa1-zm1*	This study

SI References

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3. S. Colavito *et al.*, Functional significance of the Rad51-Srs2 complex in Rad51 presynaptic filament disruption. *Nucleic Acids Res.* **37**, 6754-6764 (2009).