

**Table S1. *S. cerevisiae* strains.**

Strain	Genotype	Source	Strain background*
FF18733	<i>MATa leu2-3, 112 trp1-289 ura3-52 lys1-1 his7-2</i>	F. Fabre	
FF18744	<i>MATa srs2::LEU2</i>	F. Fabre	
FF18742	<i>MATa rad52::URA3</i>	F. Fabre	
ECS2136	<i>MATa rad52::URA3 srs2::NAT</i>	This study	
FF18964	<i>MATa rad50::URA3</i>	F. Fabre	
FF18973	<i>MATa rad54::LEU2</i>	F. Fabre	
FF181495	<i>MATa sgs1::URA3</i>	F. Fabre	
ECS57	<i>MATa rrm3::NATMX</i>	This study	
ECS74	<i>MATa mrc1::NATMX</i>	This study	
ECS75	<i>MATa ctf18::NATMX</i>	This study	
ECS2928	<i>MATa rad51::URA3 YCplac111-RAD52-6H-FFF</i>	This study	
ECS3159	<i>MATa rad52::URA3 srs2::NAT YCplac111-RAD52-V95I-6H-FFF</i>	This study	
ECS3161	<i>MATa rad52::URA3 srs2::NAT YCplac111-RAD52-V129A-6H-FFF</i>	This study	
ECS3163	<i>MATa rad52::URA3 srs2::NAT YCplac111-RAD52-D79N-6H-FFF</i>	This study	
ECS3416	<i>MATa rad52::URA3 srs2::NAT YCplac111-RAD52-V95D-6H-FFF</i>	This study	
ECS3418	<i>MATa rad52::URA3 srs2::NAT YCplac111-RAD52-V95F-6H-FFF</i>	This study	
ECS3420	<i>MATa rad52::URA3 srs2::NAT YCplac111-RAD52-V95T-6H-FFF</i>	This study	
ECS2153	<i>MATa rad52::URA3 srs2::NAT YCplac111</i>	This study	
ECS3031	<i>MATa rad52::URA3 srs2::NAT YCplac111-RAD52-6H-FFF</i>	This study	
ECS3138	<i>MATa rad52::URA3 YCplac111-rad52-V95I-6H-FFF</i>	This study	
ECS3140	<i>MATa rad52::URA3 YCplac111-rad52-V129A-6H-FFF</i>	This study	
ECS3142	<i>MATa rad52::URA3 YCplac111-rad52-D79N-6H-FFF</i>	This study	
ECS2958	<i>MATa rad52::URA3 YCplac111-rad52-R37A-6H-FFF</i>	This study	
ECS2917	<i>MATa rad52::URA3 YCplac111</i>	This study	
ECS2947	<i>MATa rad52::URA3 YCplac111-RAD52-6H-FFF</i>	This study	

ECS3410	<i>MATa rad52::URA3 YCplac111-rad52-V95D-6H-FFF</i>	This study
ECS3412	<i>MATa rad52::URA3 YCplac111-rad52-V95F-6H-FFF</i>	This study
ECS3414	<i>MATa rad52::URA3 YCplac111-rad52-V95T-6H-FFF</i>	This study
ECS3284	<i>MATa rad52::URA3 rad59-9MYC-KAN YCplac111-rad52-6H-FFF</i>	This study
ECS3286	<i>MATa rad52::URA3 rad59-9MYC-KAN YCplac111-rad52-V95I-6H-FFF</i>	This study
ECS3288	<i>MATa rad52::URA3 rad59-9MYC-KAN YCplac111</i>	This study
ECS2142	<i>MATa Rad59::KANMX srs2 ::LEU2</i>	This study
FF181770	<i>MATa Rad59 ::KANMX</i>	F. Fabre
L317.4a	<i>MATa arg4-RV</i>	This study
L318.1d	<i>MATa arg4-Bg</i>	This study
L317.17c	<i>MATa arg4-RV srs2::LEU2</i>	This study
L318.7a	<i>MATa arg4-Bg srs2::LEU2</i>	This study
ECS3230	<i>MATa arg4-RV rad52-V95I</i>	This study
ECS3236	<i>MATa arg4-Bg rad52-V95I</i>	This study
ECS3231	<i>MATa arg4-RV rad52-V95I srs2::LEU2</i>	This study
ECS3235	<i>MATa arg4-Bg rad52-V95I srs2::LEU2</i>	This study
ECS3239	<i>MATa arg4-RV rad52-D79N</i>	This study
ECS3245	<i>MATa arg4-Bg rad52- D79N</i>	This study
ECS3241	<i>MATa arg4-RV rad52- D79N srs2::LEU2</i>	This study
ECS3243	<i>MATa arg4-Bg rad52- D79N srs2::LEU2</i>	This study
EMY110	<i>ade1-100 ura3-52 leu2-3,112 lys5 hml::ADE1 mat::hisG hmr::ADE1 leu2-cs his4::NAT-leu2Δ5' ade3::GAL::HO RAD52-6H-FFF-KANMX</i>	J. Haber YFP17
ECS3220	<i>srs2::HPH</i>	This study
EMY424	<i>rad52-D79N</i>	This study
EMY425	<i>rad52- D79N srs2::HPH</i>	This study
EMY419	<i>rad52-V95I</i>	This study
EMY420	<i>rad52- V95I srs2::HPH</i>	This study
EMY430	<i>rad52-V95I-6H-FFF</i>	This study
EMY441	<i>rad52- V95I-6H-FFF srs2::HPH</i>	This study

EMY443	<i>rad52-R37A-6H-FFF</i>	This study	
EMY444	<i>rad52-R37A-6H-FFF srs2Δ</i>	This study	
tGI354	<i>hml::ADE1 MATalpha hmr::ADE1 arg5,6::MATa-inc::HPH1</i> <i>ade3::GAL::HO</i>	J. Haber	<i>JKM146</i>
ECS2632	<i>srs2::NAT</i>	This study	
EMY421	<i>rad52-V95I</i>	This study	
EMY422	<i>rad52- V95I srs2::NAT</i>	This study	
EMY427	<i>rad52-D79N</i>	This study	
EMY428	<i>rad52- D79N srs2::NAT</i>	This study	
CTY10-5d	<i>MATa ade2 trp1-901 leu2-3,112 his3-200 gal4 gal80 - URA3::lexAop-lacZ</i>	S. Fields	CTY10-5d
TLM285	<i>Rad52::KANMX</i>	This study	

Strains are grouped according to their background, indicated on the first line of each group. Only differences from this genotype are noted subsequently.

\* When different from FF18733.

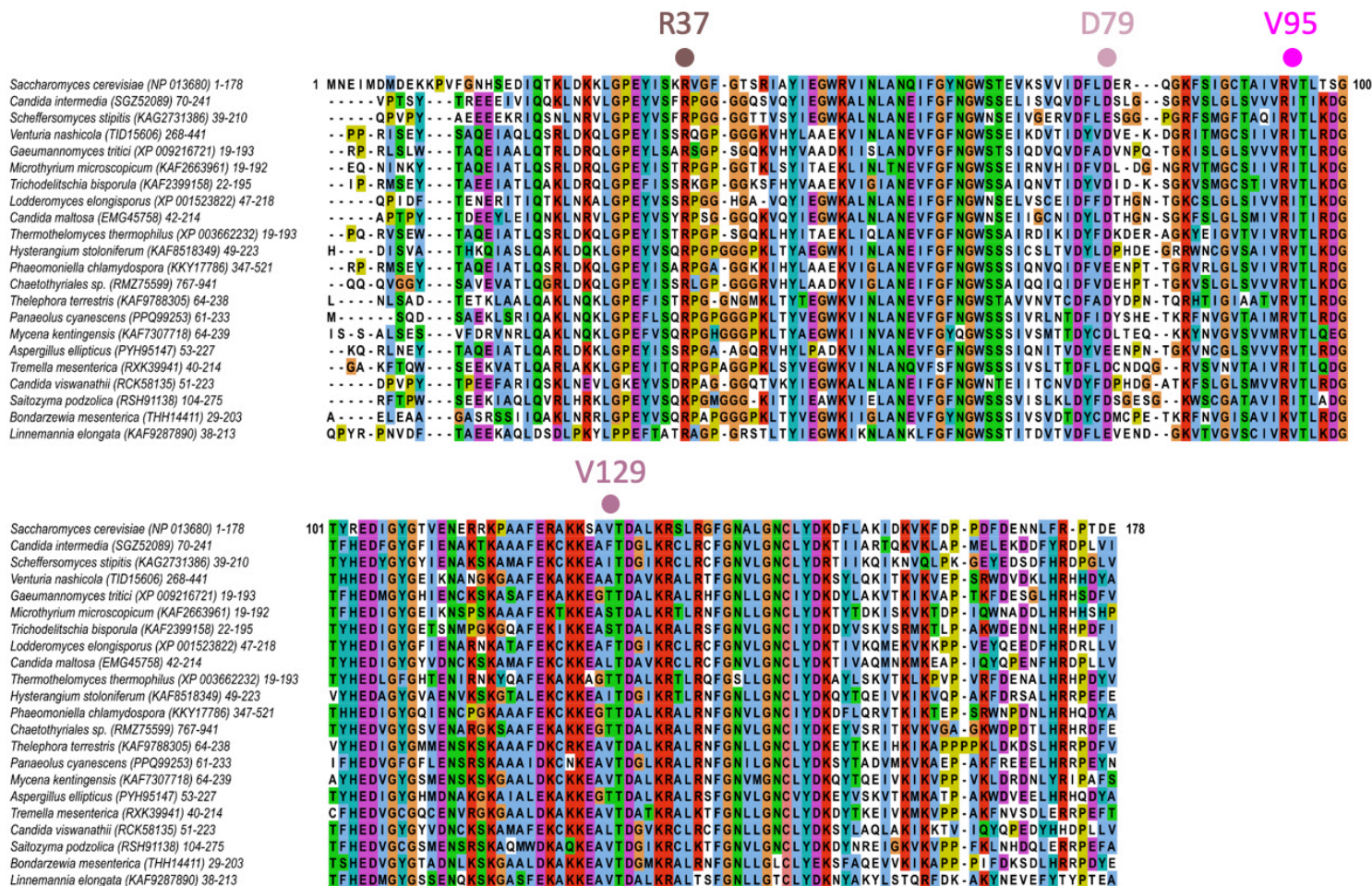
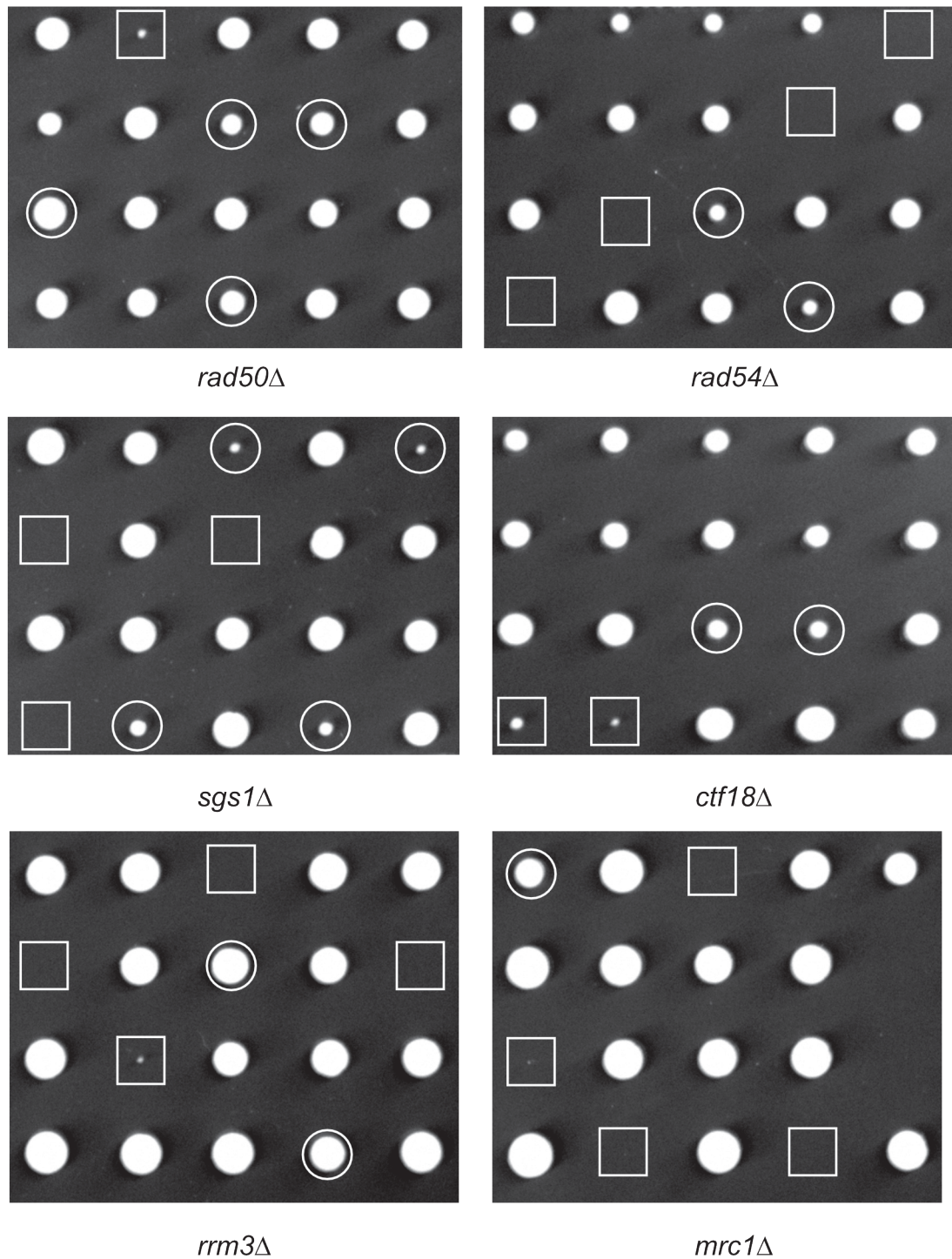


Figure S1. Multiple sequence alignment of a set of 22 representative homologs of *S. cerevisiae* Rad52 (shown on top) restricted to the N-terminal domain region (residues 1-178). The accession number of every homolog is indicated in brackets in the sequence description. The amino acid colors refer to the chemical character of the residues. On top of the alignment the positions of the mutated residues is indicated by a colored circle.

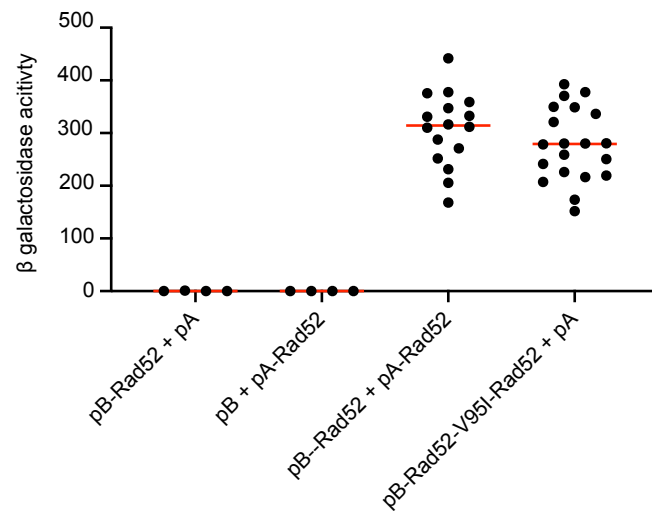
**Table S2. Rad52 mutations that suppress Srs2-deficient cells MMS sensitivity**

Nucleotide change	Triplet Change	Amino acid change
G235A	GAT->AAT	D79N
G283A	GTT->ATT	V95I
T386C	GTT->GCT	V129A





**Figure S2.** The *rad52-V95I* mutation suppresses the effect of mutations that are synthetically lethal with *srs2Δ*. Tetrad analysis of crosses between haploid *rad52-V95I srs2Δ* strains and haploid mutants that are synthetically lethal with *srs2Δ*. Double mutant spores, which do not contain *rad52-V95I*, are indicated by white squares. The white circles mark viable triple mutants.



**Figure S3. Quantitative measurement of the impact of V95I on Rad52-self-interaction by Y2H analyses.** Experiments were done in *rad52Δ* cells to avoid the reduction of the Y2H signal by untagged Rad52. Median  $\beta$ -galactosidase activity is indicated (red line). Controls with empty vectors pBTM116 (pB) or pACT2 (pA) are also shown. The V95I mutation does not affect Rad52-self-interaction.

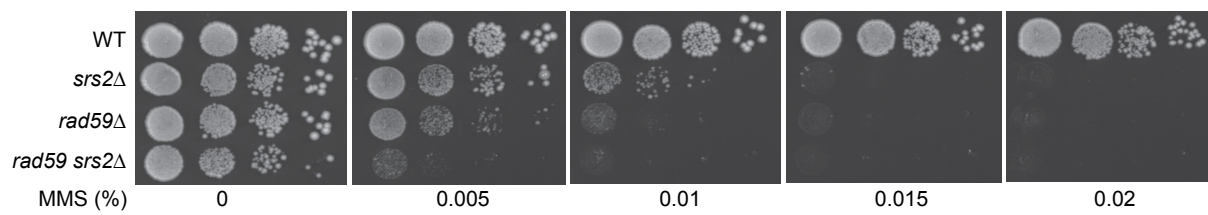
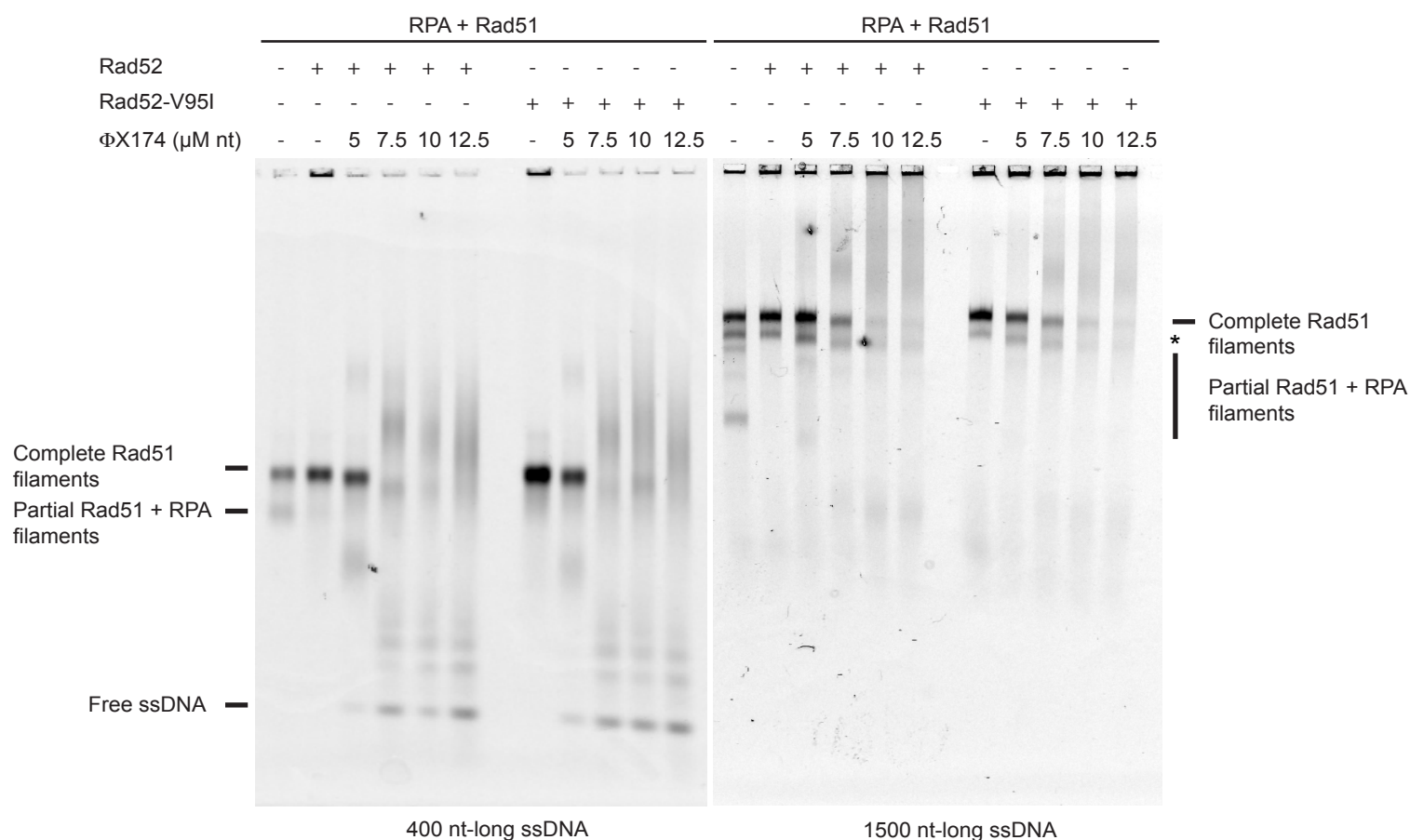


Figure S4. Serial 10-fold dilutions of haploid strains with the indicated genotypes were spotted onto rich media (YPD) containing different MMS concentrations.





**Figure S5.** Rad51 filament stability challenged with excess of ssDNA. All experiments were performed with FLAG-tagged Rad52 proteins. The addition of Rad52 or Rad52-V95I together with Rad51 to RPA-coated 400-nt-long ssDNA or 1500-nt-long ssDNA strongly catalyzes the formation of complete Rad51 filaments. Addition of increasing amounts of competitor  $\Phi$ X174 ssDNA after Rad51 filament formation leads to their destabilization. Note the formation of an uncharacterized band with 1500-nt-long ssDNA (\*).