

APPENDIX

Error-free DNA damage tolerance pathway is facilitated by the Irc5 translocase through cohesin

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Appendix Table S1

Yeast strains used in this work.

Strain	Genotype	Source
W303-1a	<i>MATa ade2-1 can1-100 ura3-1 his3-11,15 leu2-3,112 trp1-1 RAD5</i>	R. Rothstein
IL012	W303-1a, <i>irc5-Δ1::TRP1</i>	Lab collection
W3749-14C	W303-1a, <i>ADE2, bar1::LEU2, RAD52-YFP</i>	R. Rothstein
TB039	W3749-14C, <i>irc5-Δ1::TRP1</i>	This study
BYR52MN	BY4741, <i>MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0</i> <i>RAD52-MN::HIS3MX6</i>	F. Prado
TB040	BYR52MN, <i>irc5-Δ1::kanMX6</i>	This work
W3775-12C	W303-1a, <i>ADE2, bar1::LEU2, RFA1-YFP</i>	R. Rothstein
TB041	W3775-12C, <i>irc5-Δ1::TRP1</i>	This study
TB042	W3775-12C, <i>ura3-1::IRC5-3HA-URA3, irc5-Δ1::TRP1</i>	This study
TB043	W3775-12C, <i>ura3-1::irc5^{DAEA}-3HA-URA3, irc5-Δ1::TRP1</i>	This study
TB044	W3749-14C, <i>ura3-1::IRC5-3HA-URA3, irc5-Δ1::TRP1</i>	This study
TB045	W3749-14C, <i>ura3-1::irc5^{DAEA}-3HA-URA3, irc5-Δ1::TRP1</i>	This study
EMD09	W303-1a, <i>ura3-1::IRC5-3HA-URA3, irc5-Δ1::TRP1</i>	This study
EMD10	W303-1a, <i>ura3-1::irc5^{DAEA}-3HA-URA3, irc5-Δ1::TRP1</i>	This study
MC002	W303-1a, <i>rad51Δ::kanMX6</i>	Lab collection
TB046	W303-1a, <i>irc5-Δ1::TRP1 rad51Δ::kanMX6</i>	This study
MC006	W303-1a, <i>rad52Δ::kanMX6</i>	Lab collection
TB047	W303-1a, <i>irc5-Δ1::TRP1 rad52Δ::kanMX6</i>	This study
MC019	W303-1a, <i>rad18Δ::kanMX6</i>	Lab collection
TB048	W303-1a, <i>irc5-Δ1::TRP1 rad18Δ::kanMX6</i>	This study
MC018	W303-1a, <i>rad5Δ::kanMX6</i>	Lab collection
TB049	W303-1a, <i>irc5-Δ1::TRP1 rad5Δ::kanMX6</i>	This study

MC021	W303-1a, <i>rev3Δ::kanMX6</i>	Lab collection
TB050	W303-1a, <i>irc5-Δ1::TRP1 rev3Δ::kanMX6</i>	This study
MC012	W303-1a, <i>sgs1Δ::kanMX6</i>	Lab collection
TB051	W303-1a, <i>irc5-Δ1::TRP1 sgs1Δ::kanMX6</i>	This study
TB052	W3775-12C, <i>rad18Δ::kanMX6</i>	This study
TB053	W3775-12C, <i>rad18Δ::kanMX6 irc5-Δ1::TRP1</i>	This study
TB054	W3775-12C, <i>rev3Δ::kanMX6</i>	This study
TB055	W3775-12C, <i>rev3Δ::kanMX6 irc5-Δ1::TRP1</i>	This study
TB056	W3775-12C, <i>sgs1Δ::kanMX6</i>	This study
TB057	W3775-12C, <i>sgs1Δ::kanMX6 irc5-Δ1::TRP1</i>	This study
TB058	W3749-14C, <i>rad18Δ::kanMX6</i>	This study
TB059	W3749-14C, <i>rad18Δ::kanMX6 irc5-Δ1::TRP1</i>	This study
TB060	W3749-14C, <i>rev3Δ::kanMX6</i>	This study
TB061	W3749-14C, <i>rev3Δ::kanMX6 irc5-Δ1::TRP1</i>	This study
TB062	W3749-14C, <i>sgs1Δ::kanMX6</i>	This study
TB063	W3749-14C, <i>sgs1Δ::kanMX6 irc5-Δ1::TRP1</i>	This study
JC1315	W303-1a, <i>SCC1-9PK::TRP1 URA3::GPD-TK7</i>	J.Cobb
TB064	JC1315, <i>irc5-Δ1::kanMX6</i>	Lab collection
TB065	W303-1a, <i>POL2-3HA::kanMX6</i>	This study
TB066	TB065, <i>irc5-Δ1::TRP1</i>	This study
Y39	W303-1a, <i>ura3::3xURA3 tetO112, leu2::LEU2 tetR-GFP SCC1-6HA::HIS3</i>	F. Uhlmann
Y4366	W303-1a, <i>scc2-4 ura3::3xURA3 tetO112 leu2::LEU2 tetR-GFP SCC1-6HA::HIS3</i>	F. Uhlmann
TB067	Y39, <i>SCC1, POL2-3HA::kanMX6</i>	This study
TB068	Y4366, <i>scc2-4, SCC1, POL2-3HA::kanMX6</i>	This study
TB069	W303-1a, <i>scc2-4</i>	This study
TB070	W303-1a, <i>scc2-4 irc5-Δ1::TRP1</i>	This study
TB071	W3775-12C, <i>scc2-4</i>	This study

TB072	W3775-12C, <i>scc2-4 irc5-Δ1::TRP1</i>	This study
TB073	W3749-14C, <i>scc2-4</i>	This study
TB074	W3749-14C, <i>scc2-4 irc5-Δ1::TRP1</i>	This study
JC1339	W303-1a, <i>scc1-73; pepΔ::LEU2</i>	J Cobb
TB075	JC1339, <i>irc5-Δ1::TRP1</i>	This study
TB076	W3775-12C, <i>scc1-73</i>	This study
TB077	W3775-12C, <i>scc1-73 irc5-Δ1::TRP1</i>	This study
TB078	W3749-14C, <i>scc1-73</i>	This study
TB079	W3749-14C, <i>scc1-73 irc5-Δ1::TRP1</i>	This study
TB080	W3775-12C, <i>scc2-4 rad18Δ::kanMX6</i>	This study
TB081	W3775-12C, <i>scc2-4 rev3Δ::kanMX6</i>	This study
TB082	W3749-14C, <i>scc2-4 rad18Δ::kanMX6</i>	This study
TB083	W3749-14C, <i>scc2-4 rev3Δ::kanMX6</i>	This study
TB084	W3775-12C, <i>scc1-73 rad18Δ::kanMX6</i>	This study
TB085	W3775-12C, <i>scc1-73 rev3Δ::kanMX6</i>	This study
TB086	W3749-14C, <i>scc1-73 rad18Δ::kanMX6</i>	This study
TB087	W3749-14C, <i>scc1-73 rev3Δ::kanMX6</i>	This study

Appendix Table S2

Oligonucleotide sets used in this work.

Amplified region	Forward primer	Reverse primer
<i>ARS305</i>	TTTGGAGCTCAAGTGGATTGAG	TGAAACTGGACATATTTGAGGAATTT
<i>ARS305 + 7kb</i>	AAGTTCCGCTTTCATATCTTTGTTT	GAGATGAATGGTTGACTAACGTGGTA
<i>ARS607</i>	CGGCTCGTGCATTAAGCTTG	TGCCGCACGCCAAACATTGC
<i>ARS501</i>	GAATGTGTCTACGTAATTGG	CCTTTCTTTTGGAGCTGCTA
<i>POA1</i>	AAACGGCCACATCAAATACC	TCCAAGGGACTCCGAATATG
<i>ACT1</i>	GGCCAAATCGATTCTCAAAA	GCCTTCTACGTTTCCATCCA

Appendix Figure Legends

Appendix Figure S1. Genetic interactions between *irc5-Δ1*, homologous recombination and DNA damage tolerance-related mutants.

Logarithmically growing WT (W303-1a), *irc5-Δ1* (IL012), *rad51Δ* (MC002), *irc5-Δ1 rad51Δ* (TB046) (A), *rad52Δ* (MC006), *irc5-Δ1 rad52Δ* (TB047) (B), *rad18Δ* (MC019), *irc5-Δ1 rad18Δ* (TB048) (C), *rad5Δ* (MC018), *irc5-Δ1 rad5Δ* (TB049) (D), *rev3Δ* (MC021), *irc5-Δ1 rev3Δ* (TB050) (E), *sgs1Δ* (MC012) and *irc5-Δ1 sgs1Δ* (TB051) (F) cultures were incubated for 1 hour with the indicated concentrations of MMS. Next, cells were plated on YPD to determine the survival rate. Error bars represent mean value \pm standard deviations of mean (n=4).

Appendix Figure S2. Levels of Rfa1-YFP and Rad52-YFP DNA repair foci in DTT mutants devoid of *IRC5*.

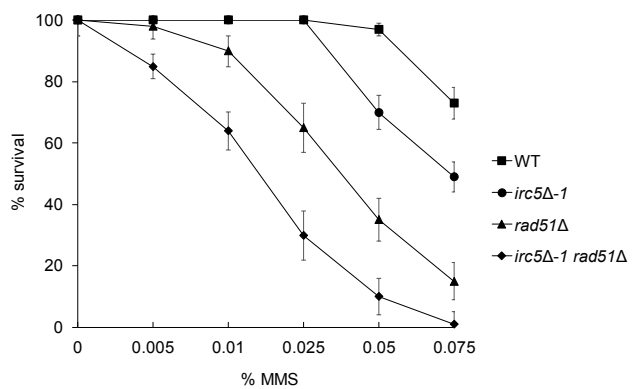
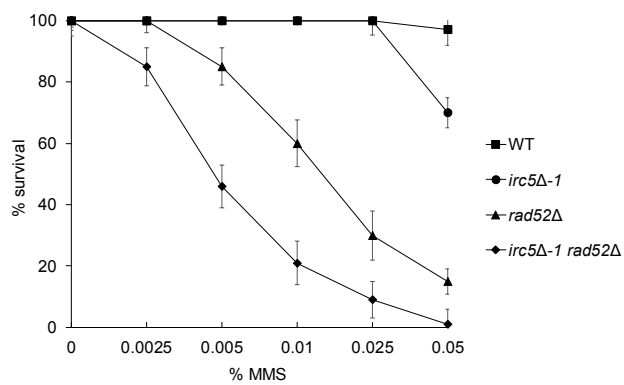
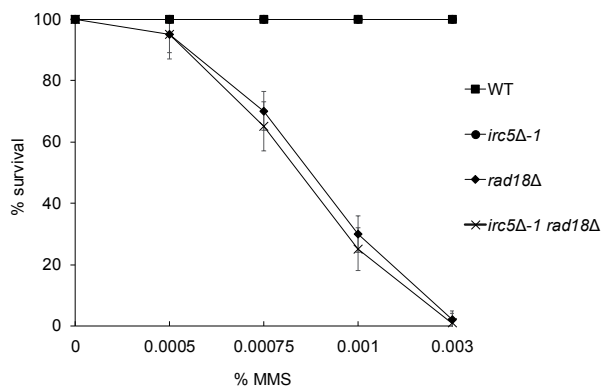
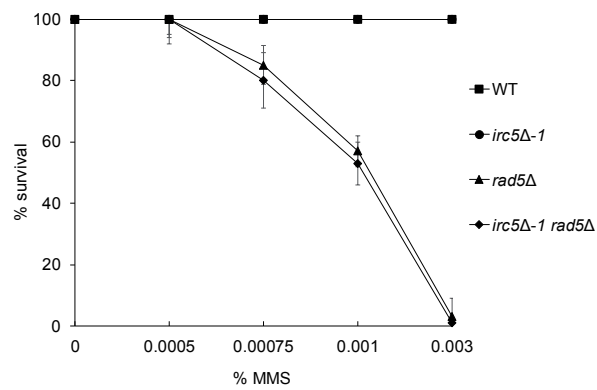
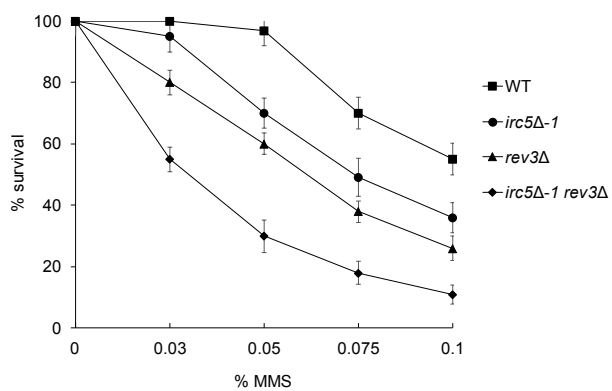
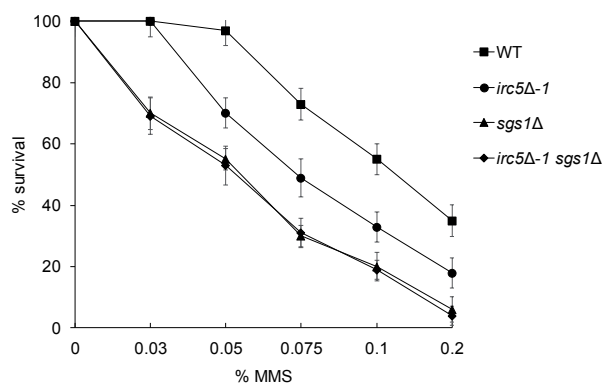
Logarithmically growing WT (W3775-12C or W3749-14C), *irc5-Δ1* (TB039 or TB041), *rad18Δ* (TB052 or TB058), *irc5-Δ1 rad18Δ* (TB053 or TB059) (A and B), *rev3Δ* (TB054 or TB060), *irc5-Δ1 rev3Δ* (TB055 or TB061) (C and D), *sgs1Δ* (TB056 or TB062) and *irc5-Δ1 sgs1Δ* (TB057 or TB063) (E and F) cultures were incubated or not with 0.0025% MMS for 1 hour. Next, cells were washed with water and processed for microscopic analysis. Error bars represent mean value \pm standard deviations of mean (n=4). Unpaired t-test was used to calculate the P-value.

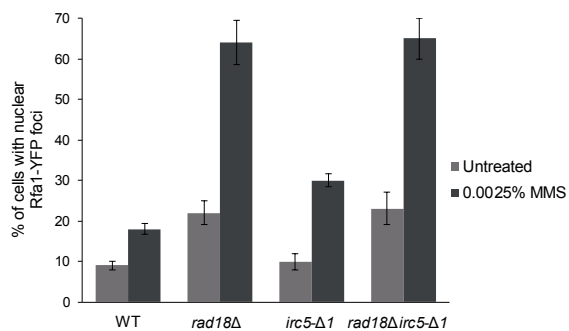
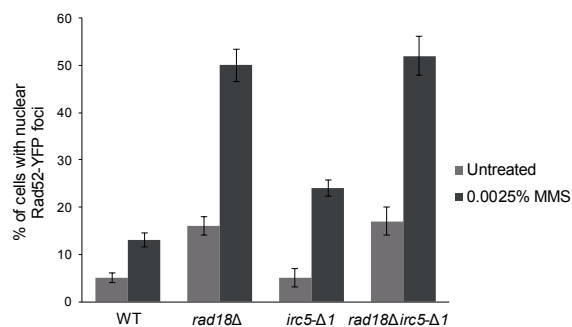
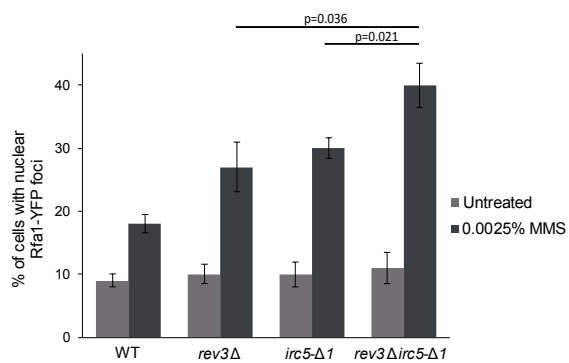
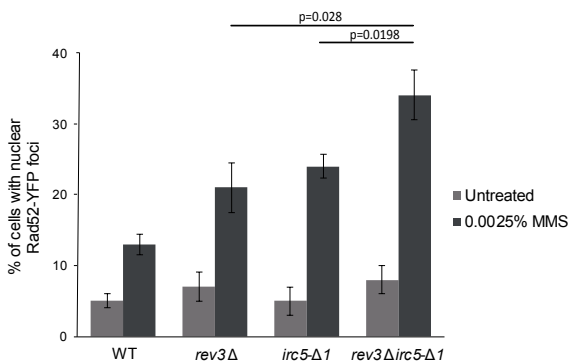
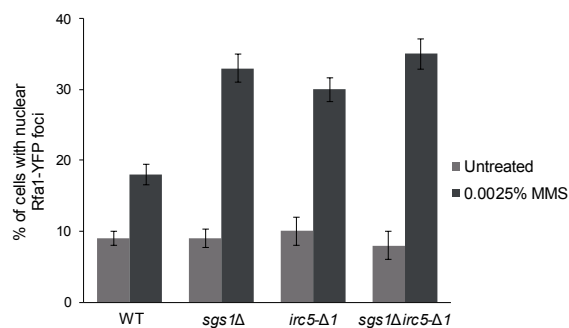
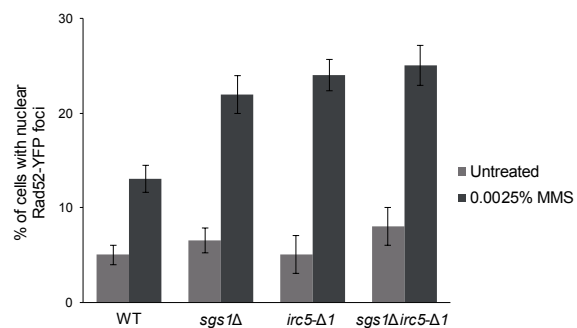
Appendix Figure S3. Disruption of *IRC5* results in decreased formation of recombination intermediates during TS.

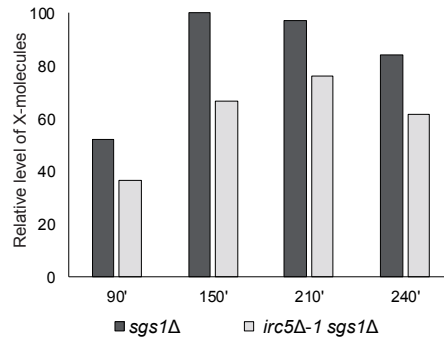
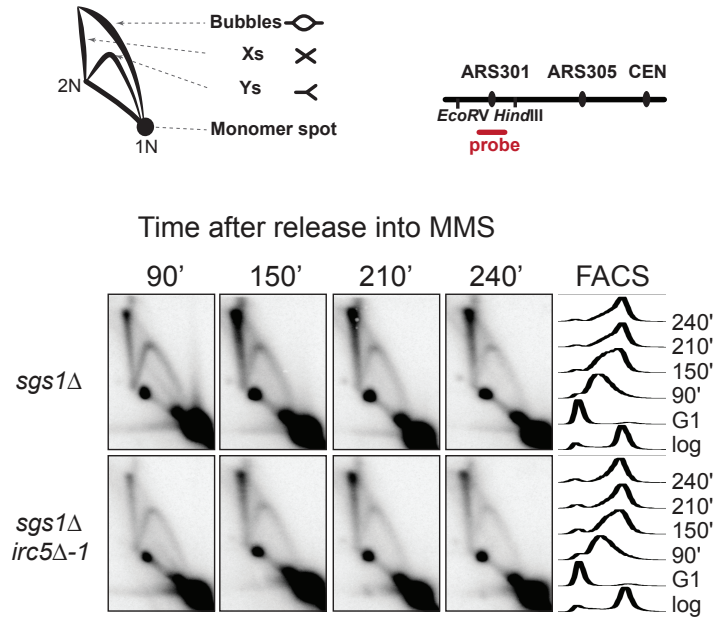
sgs1Δ (MC012) and *irc5-Δ1 sgs1Δ* (TB051) cells were synchronized in G1 with alpha-factor and released in media containing 0.03% MMS. The amount of replication intermediates at *ARS301* was analyzed by 2D gel. Schematic representation of major 2D gel signals and DNA fragment analyzed are presented. Measurement of DNA content by FACS and X-molecule quantification are displayed (n=2).

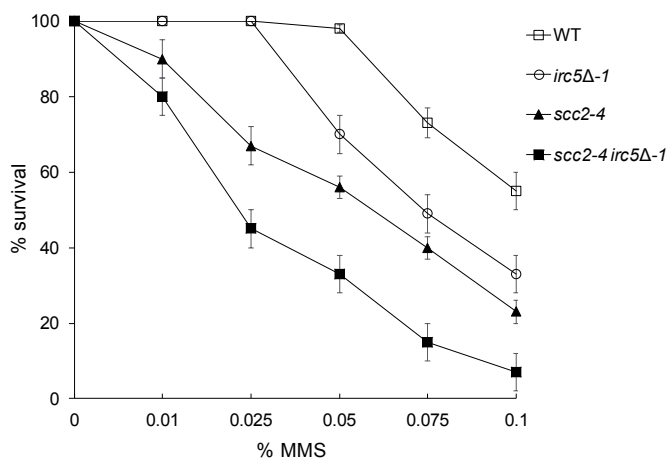
Appendix Figure S4. Genetic interactions between *irc5-Δ1* and cohesin pathway mutants.

Logarithmically growing cultures of wild type (W303-1a), *irc5-Δ1* (IL012), *scc2-4* (TB069) and *irc5-Δ1 scc2-4* (TB070) (A), *scc1-73* (JC1339) and *irc5-Δ1 scc1-73* (TB075) (B) cultures were incubated for 1 hour with the indicated concentrations of MMS at 25°C or 30°C. Next, cells were plated on YPD to determine the survival rate. Error bars represent mean value \pm standard deviations of mean (n=4).

A**B****C****D****E****F**

A**B****C****D****E****F**



A**B**