Supplementary Figures

Figure S1. Phenotypes of *rad1* alleles in mating-type switching and nucleotide excision repair. A. High (HC; 2μ) and low (LC; *ARS CEN*) copy plasmids encoding *RAD1* were tested for their ability to complement a *rad1Δ* in the double non-homology mating type switch assay (1). EAY1115 (*rad1Δ)* was transformed with the *RAD1* plasmids and corresponding empty vectors. DSBR activity was compared to that of EAY1042 (*RAD1*). Percent survival (black bars) indicates the number of cells that survived induction of a DSB. Percent switching (white bars) indicates the percentage of survivors that switched their mating-type from *MAT***a** to *MAT*α. Data represents the mean ± SEM of at least three independent experiments with each isolate. **B.** The ten *rad1* alleles were tested for function in DSBR in EAY1115, as described in **A**. **C.** The ten *rad1* alleles were tested for function in NER. Serial dilutions of a saturated overnight were plated and exposed to increasing doses of UV irradiation.

Figure S2. Interactions between rad1 complexes and Saw1 (A, B) or Msh2-Msh3 (C, D) by Far Western analysis. A. 6xHis-Saw1 was spotted on a nitrocellulose membrane (0, 2, 4 pmols shown). The membrane was incubated with His-Rad1-Rad10. α–Rad10 antibody was used to probe the membrane and secondary antibody was used to detect αRad10. As a negative control, the same amounts of BSA were spotted and then incubated with His-Rad1-Rad10, His-rad1R203A K205A-Rad10 or His-radR218A-Rad10, followed by α–Rad10 antibody. **B.** Quantification of His-Rad1-Rad10 (black circles), His-rad1R203A K205A-Rad10 (red squares) and His-rad1R218A-Rad10 (teal triangles) binding to 6xHis-Saw1 was done in Image Lab (Bio Rad). **C.** Msh2-Msh3 was spotted on a nitrocellulose membrane (0-2 pmols). The membrane was incubated with His-Rad1-Rad10, His-rad1R203A K205A-Rad10 or His-rad1R218A-Rad10. The membrane was then probed with α–Rad10, then secondary antibody and exposed by ChemiDoc (Bio Rad). **D.** Quantification of His-Rad1-Rad10 (black circles), His-rad1R203A K205A-Rad10 (red squares) and His-rad1R218A-Rad10 (teal triangles) binding to Msh2-Msh3 was performed in Image Lab (Bio Rad). Standard curves of His-Rad1-

Rad10, His-rad1R203A K205A-Rad10 and His-rad1R218A-Rad10 were also spotted and imaged in parallel with the 6xHis-Saw1 and Msh2-Msh3 membranes. The slopes of the standard curve were used to determine the pmols of His-Rad1-Rad10, His-rad1R203A K205A-Rad10 and His-rad1R218A-Rad10 binding. Data represents the mean ± SEM of at least four independent experiments with at least two independent protein preparations.

Figure S3. Purification of His-Rad1-Rad10/Saw1, His-rad1R203A K205A-Rad10/Saw1 and His-rad1R218A-Rad10/Saw1 from Cobalt column. **A.** The load, flow-through and elution fractions from the Cobalt column of His-Rad1-Rad10/Saw1 (top), His-rad1R203A K205A-Rad10/Saw1 (middle) and His-rad1R218A-Rad10/Saw1 (bottom) were analyzed on an SDS-page (12%) gel. The elution lane is labeled GF load to indicate the gel filtration load. The His-Rad1, His-rad1R203A K205A, His-rad1R218A, Rad10, and Saw1 bands are indicated with arrows. The gels shown are representative of two independent experiments. **B.** The relative amounts of His-Rad1, His-rad1R203A K205A, His-rad1R218A, Rad10, and Saw1 in each GF load were quantified from the gels in **A,** using Image Lab (Bio Rad). Within a lane, the signals for Rad1 (or rad1 mutant), Rad10 and Saw1 were summed. The proportion of that represented by each protein is the number shown in the Table.

Figure S4. *in vitro b*ehavior of His-Rad1-Rad10, His-rad1R203A K205A-Rad10 and His-rad1R218A-Rad10 complexes. A. Analysis of His-Rad1/rad1R203A K205A-Rad10 gel filtration fractions following purification over a Cobalt column, as in the complex purification shown in **Fig. 7**. Fraction numbers start with #1 representing the highest molecular weight fractions following the void. Fractions were analyzed for His-Rad1, His-rad1R203A K205A (Coomassie stain), His-rad1R218A (silver stain) and Rad10 (Western). **B.** Analysis of partially purified His-Rad1-Rad10 and His-rad1R218A-Rad10 following centrifugation. Protein was centrifuged at 16,000*xg* for 10 minutes to detect protein aggregation. An aliquot of each protein was sampled prior to centrifugation (Pre). An equivalent sample was sampled

following centrifugation (Post). The samples were separated by gel electrophoresis and stained with Coomassie. Quantification was performed in ImageLab (Bio-Rad).

Figure S5. UV sensitivity of strains with integrated *rad1R218A*. UV sensitivity of strains with integrated *rad1::HA* alleles, used for short repeat SSA assays in **Fig. 10** was tested. Data represents the mean ± SEM of at least three independent experiments with each of at least two independent isolates.

Figure S6. Saw1 protein levels *in vivo*. SSA strains with short repeats encoding *RAD1-3HA, rad1D825A-3HA, rad1Δ* and *rad1R218A-3HA* were transformed with a 2μ (high copy) plasmid encoding *SAW1.* Cell lysates were prepared from strains transformed with either the *SAW1* plasmid or the corresponding empty vector that had been grown to mid-log phase. Western Blots were performed on whole cell lysates using α–Saw1 as a probe. The Saw1 band is indicated with arrows.

Figure S7. Effect of Rad1 protein partners Rad14 and Saw1 on SSA and UV sensitivity. **A.** UV sensitivity of cells overexpressing *SAW1* from a high copy (2 μ) plasmid under control of the endogenous *SAW1* promoter was determined. **B.** SSA (short repeats) survival assays were performed in the presence and absence of *RAD14*, to determine whether SSA efficiency improved in *rad14Δ* cells.

Figure S8. Summary of *in vivo* and *in vitro* phenotypes of Rad1-Rad10 and mutant rad1 complexes. The rad1 mutants have distinct phenotypes in the different *in vivo* and *in vitro* assays performed in this work. * indicates data from Li et al, 2013.(2) ++ indicates wild-type levels of activity, *in vitro* or *in vivo*; +++ indicates increased activity relative to wild-type, + and +/- indicate decreasing activity levels relative to wild-type; - indicates not detectable activity; NT indicates not tested.

Table S1. Plasmids used in this study

Plasmid	Allele	Copy Number	Reference
pRS424	Empty Vector	2 µ	(3)
pRS425	Empty Vector	2 µ	(3)
pRS414	Empty Vector	*ARS CEN*	(4)
pJAS33	*RAD1*	*ARS CEN*	This study
pRD1	*RAD1*	2 µ	This study
pRD2	*rad1R203A K205A*	2 µ	This study
pRD3	*rad1R218A*	2 µ	This study
pRD4	*rad1E251A*	2 µ	This study
pRD5	*rad1R259A K261A*	2 µ	This study
pRD6	*rad1R301A*	2 µ	This study
pRD7	*rad1K315A*	2 µ	This study
pRD8	*rad1E365A*	2 µ	This study
pRD9	*rad1R399A R402A*	2 µ	This study
pRD10	*rad1D444A*	2 µ	This study
pRD11	*rad1K454A R456A*	2 µ	This study
pCY1	*SAW1*	2 µ	This study
pSP1	*MSH3*	2 µ	This study
pMMR8	*MSH2*	2 µ	(5)
pMMR20	*MSH3*	O/E (Gal)	(5)
pEAE247	*msh3KKAA*	O/E (Gal)	(6)
pJAS104	Empty Vector	O/E (Gal)	This study
pET28A-Saw1	*SAW1*	O/E (IPTG)	(2)
pJAS19	*RAD10*	O/E (IPTG)	This study
pJAS21	*His-RAD1 RAD10*	O/E (IPTG)	(2)
pMF1	*His-rad1R203A K205A RAD10*	O/E (IPTG)	This study
pMF2	*His-rad1R218A RAD10*	O/E (IPTG)	This study
pCY1	*6xHis-SAW1*	2 µ	This study

Table S2: Oligonucleotides used for site directed mutagenesis.

***rad1* allele**	**Oligo name**	**Sequence**
rad1 R203A/K205A	SO87	5'-CGCTGAGCATTGAAAAGGCAAGAGCGCTATATATTTCTGGCGG
	SO88	5'-CCGCCAGAAATATATAGCGCTCTTGCCTTTTCAATGCTCAGCG
rad1R218A	SO89	5'-GAGCATTACTTCTGCAATTCTCATTGTGGATCTC
	SO90	5'-GAGATCCACAATGAGAATTGCAGAAGTAATGCTC
rad1E251A	SO91	5'-GCAGACTCACTTCGACATAATTCGAATGCATCGTTTATATTAGAG
	SO92	5'-CTCTAATATAAACGATGCATTCGAATTATGTCGAAGTGAGTCTGC
rad1R259A/K261A	SO93	5'-GAGATTTACGCGTCTGCAAATACTTGGGGTTTTATTAAAGCC
	SO94	5'-GGCTTTAATAAAACCCCAAGTATTTGCAGACGCGTAAATCTC
rad1R301A	SO95	5'-GCTATGGCCGGCGTTCAGGGTAGAGGTCTC
	SO96	5'-GAGACCTCTACCCTGAACGCCGGCCATAGC
rad1K315A	SO97	5'-CCTGTTTGAATGCCACTAATGCGACGTCACACAATAAAGTC
	SO98	5'-GACTTTATTGTGTGACGTCGCATTAGTGGCATTCAAACAGG
rad1E365A	SO99	5'-CTGGACTGGTGGAATATGGCAAATGTCCTGG
	SO100	5'-CCAGGACATTTGCCATATTCCACCAGTCCAG
rad1R399A/R402A	SO101	5'-CTGGTTAAGGATATAGCATTCCTAGCCCACCTTTTAAAGATGCTC
	SO102	5'-GAGCATCTTTAAAAGGTGGGCTAGGAATGCTATATCCTTAACCAG
rad1D444A	SO103	5'-CCGTGGCTATTGGTCGCTGAGGCACAATTAG
	SO104	5'-CTAATTGTGCCTCAGCGACCAATAGCCACGG
rad1K454A/R456A	SO105	5'-GGCACAATTAGTCATATCGTATGCGGCGAAAGCAATATTTTAC
	SO106	5'-GTAAAATATTGCTTTCGCCGCATACGATATGACTAATTGTGCC

Table S3. Strains used in this study

Strains	Genotype	Plasmid	Reference
EAY1042	*Δho, HMLα MATa::KanMX hmrΔ::ADE1 ade1-100 leu2-3 lys5 trp1::hisG ura3-52 ade3::Gal::HO MSH2-HA4::LEU2*		(1)
JSY2785-87	EAY1042	pEA032	This study
JSY3564-66	EAY1042	pEA032	This study
JSY2866-68	EAY1042	pRD1	This study
JSY3567-69	EAY1042	pRD1	This study
JSY2782-84	EAY1042	pRD2	This study
JSY3570-72	EAY1042	pRD2	This study
JSY2869-71	EAY1042	pRD3	This study
JSY3573-75	EAY1042	pRD3	This study
EAY1115	*Δho, HMLα MATa::KanMX hmrΔ::ADE1 ade1-100 leu2-3 lys5 trp1::hisG ura3-52 ade3::Gal::HO MSH2-HA4::LEU2 rad1Δ*		(1)
JSY251, 367	EAY1115	pEA032	This study
JSY252, 368	EAY1115	pRD1	This study
JSY253, 369	EAY1115	pRD2	This study
JSY254, 370	EAY1115	pRD3	This study
JSY255, 371	EAY1115	pRD4	This study
JSY256, 372	EAY1115	pRD5	This study
JSY257, 373	EAY1115	pRD6	This study
JSY258, 374	EAY1115	pRD7	This study
JSY259, 375	EAY1115	pRD8	This study
JSY260, 376	EAY1115	pRD9	This study
JSY261, 377	EAY1115	pRD10	This study
JSY262, 378	EAY1115	pRD11	This study
JSY4126, 4127	EAY1115	pEAO37	This study
JSY4128, 4129	EAY1115	pJAS33	This study
FY86	*MATα ura3-52 leu2Δ1 his3Δ200*		(7)
FY23	*MATa ura3-52 trp1Δ63 leu2Δ1*		(8)
JSY1540	FY23 + *rad1Δ::KanMX*		This study
JSY1617-19	JSY1540	pEA032	This study
JSY1620-22	JSY1540	pRD1	This study
JSY1913-15	JSY1540	pRD2	This study
JSY1916-18	JSY1540	pRD3	This study
JSY1919-21	JSY1540	pRD4	This study
JSY1922-24	JSY1540	pRD5	This study
JSY1925-27	JSY1540	pRD6	This study
JSY1928-30	JSY1540	pRD7	This study
JSY1931-33	JSY1540	pRD8	This study
JSY1934-36	JSY1540	pRD9	This study
JSY1937-39	JSY1540	pRD10	This study
JSY1940-42	JSY1540	pRD11	This study

EAY1141	*mat::leu2::hisG hmr3 thr4 leu2 trp1 THR4-ura3-A(205bp)-HOcs-URA3-A ade3::GAL10-HO::NAT*		(9)
JSY2876-78	EAY1141 *rad1D825A-3HA::KanMX*		(10)
JSY2885-87	EAY1141 *rad1R218A-3HA::KanMX*		This study
JSY2888-90	EAY1141 *rad1Δ::KanMX*		(10)
JSY2903-05	EAY1141 *rad1-3HA::KanMX*		(10)
JSY3347-49	EAY1141 *rad14Δ::KanMX*		This study
JSY2945-47	EAY1141 *rad1D825A-3HA::KanMX*	pSP1	This study
JSY2948-50	EAY1141 *rad1R218A-3HA::KanMX*	pSP1	This study
JSY2957-59	EAY1141 *rad1-3HA::KanMX*	pSP1	This study
JSY2930-32	EAY1141 *rad1D825A-3HA::KanMX*	pEAO33	This study
JSY2933-35	EAY1141 *rad1R218A-3HA::KanMX*	pEAO33	This study
JSY2942-44	EAY1141 *rad1-3HA::KanMX*	pEAO33	This study
JSY3022-24	EAY1141 *rad1D825A-3HA::KanMX*	pCY1	This study
JSY3025-27	EAY1141 *rad1R218A-3HA::KanMX*	pCY1	This study
JSY3028-30	EAY1141 *rad1Δ::KanMX*	pCY1	This study
JSY3031-33	EAY1141 *RAD1*	pCY1	This study
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JSY3054-56	EAY1141 *rad1D825A-3HA::KanMX*	pEAO32	This study
JSY3057-59	EAY1141 *rad1R218A-3HA::KanMX*	pEAO32	This study
JSY3063-65	EAY1141 *RAD1*	pEAO32	This study
JSY3066-68	EAY1141 *rad1-3HA::KanMX*	pEAO32	This study
JSY 3049-51	EAY1141 *rad1-3HA::KanMX*	pEAO32 pEAE247	This study
JSY2960-62	EAY1141 *rad1D825A-3HA::KanMX*	pEAO32 pMMR20	This study
JSY2963-65	EAY1141 *rad1R218A-3HA::KanMX*	pEAO32 pMMR20	This study
JSY2972-74	EAY1141 *rad1-3HA::KanMX*	pEAO32 pMMR20	This study
JSY3037-39	EAY1141 *rad1D825A-3HA::KanMX*	pEAO32 pEAE247	This study
JSY3040-42	EAY1141 *rad1R218A-3HA::KanMX*	pEAO32 pEAE247	This study
JSY3069-71	EAY1141 *rad1D825A-3HA::KanMX*	pMMR8 pMMR20	This study
JSY3072-74	EAY1141 *rad1R218A-3HA::KanMX*	pMMR8 pMMR20	This study
JSY3081-83	EAY1141 *rad1-3HA::KanMX*	pMMR8 pMMR20	This study
JSY3084-86	EAY1141 *rad1D825A-3HA::KanMX*	pCY1 pMMR20	This study
JSY3087-89	EAY1141 *rad1R218A-3HA::KanMX*	pCY1 pMMR20	This study
JSY3096-98	EAY1141 *rad1-3HA::KanMX*	pCY1 pMMR20	This study
JSY3117-19	EAY1141 *rad1D825A-3HA::KanMX*	pEAO32	This study

JSY3120-22	EAY1141 *rad1R218A-3HA::KanMX*	pJAS104 pEAO32 pJAS104	This study
JSY3129-31	EAY1141 *rad1-3HA::KanMX*	pEAO32 pJAS104	This study
YMV80	*ho*Δ *hml*Δ*::ADE3 MAT*α*Δ::hisG hmr*Δ*::ADE3 leu2-cs ade3::GAL::HO ade1 lys5 ura3-52*		(11)
JSY2894-96	YMV80 *rad1R218A-3HA::KanMX*		This study
JSY3399-01	YMV80 msh2Δ		This study

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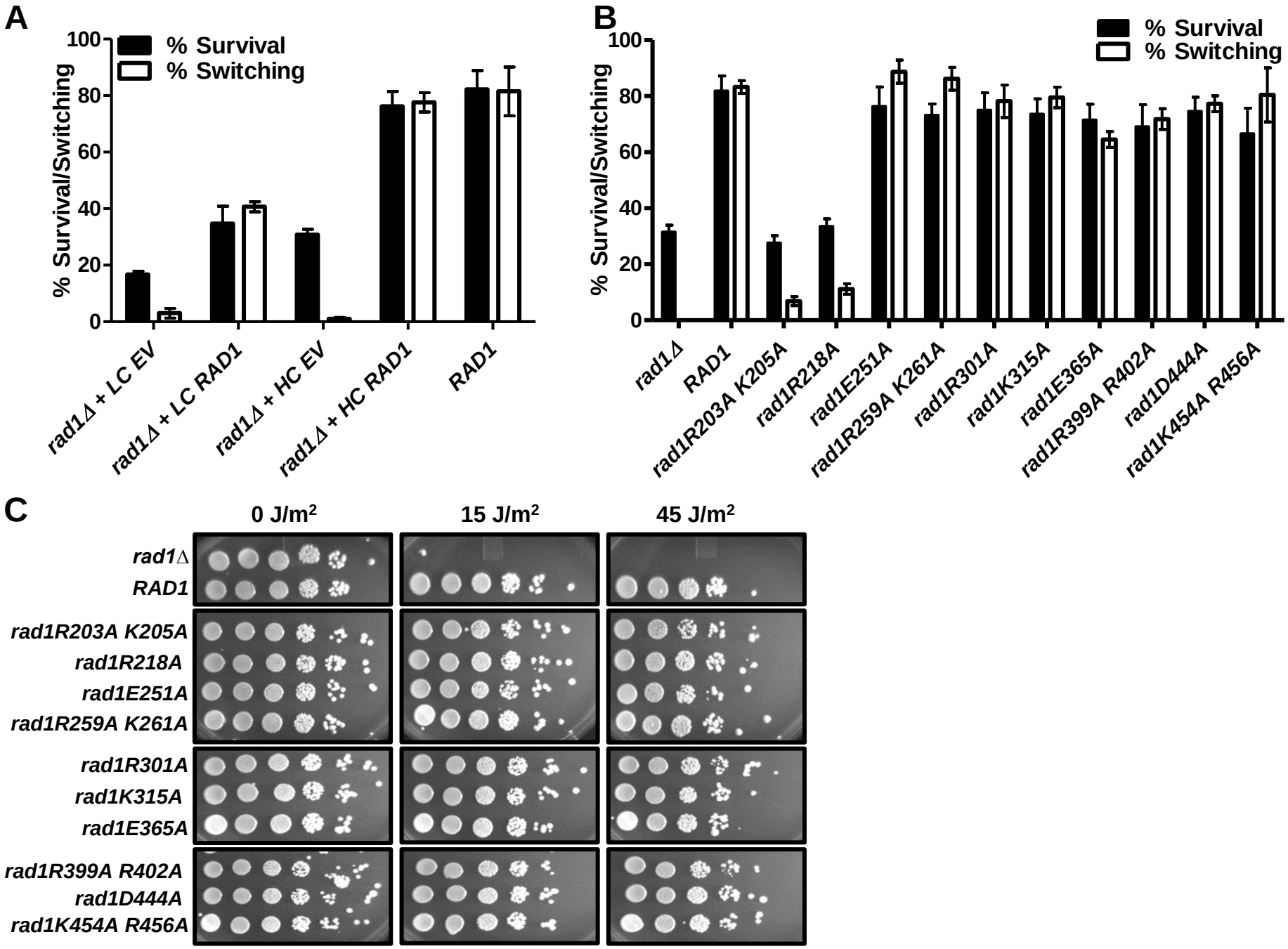
A
% Survival
% Switching
% Survival/Switching
100
80
60
40
20
0
rad1Δ + LC EV
rad1Δ + LC RAD1
rad1Δ + HC EV
rad1Δ + HC RAD1
RAD1
B
% Survival
% Switching
% Survival/Switching
rad1Δ
RAD1
rad1R203A K205A
rad1R218A
rad1E251A
rad1R259A K261A
rad1R301A
rad1K315A
rad1E365A
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C
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15 J/m2
45 J/m2
rad1Δ
RAD1
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rad1R218A
rad1E251A
rad1R259A K261A
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Figure S1.

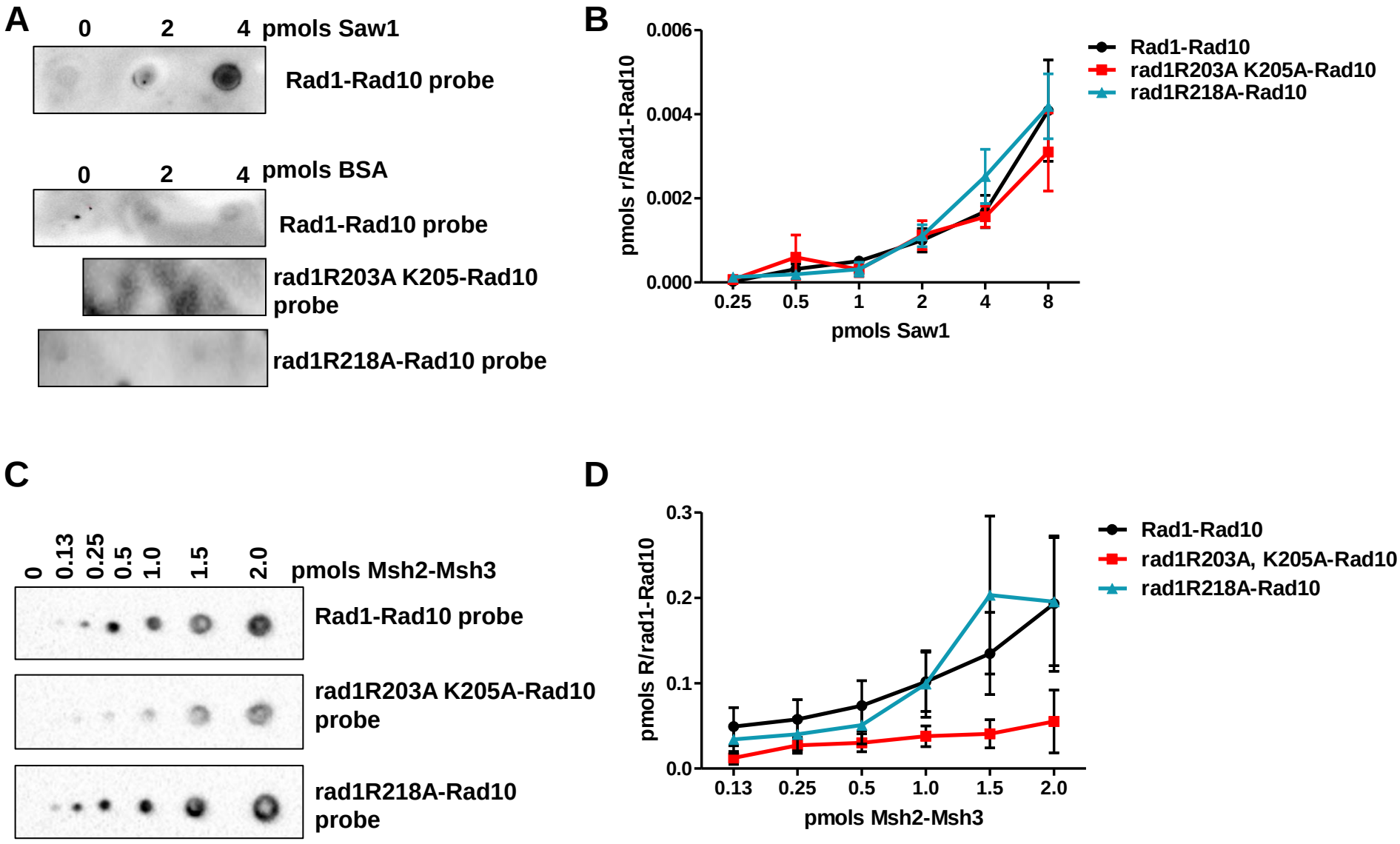
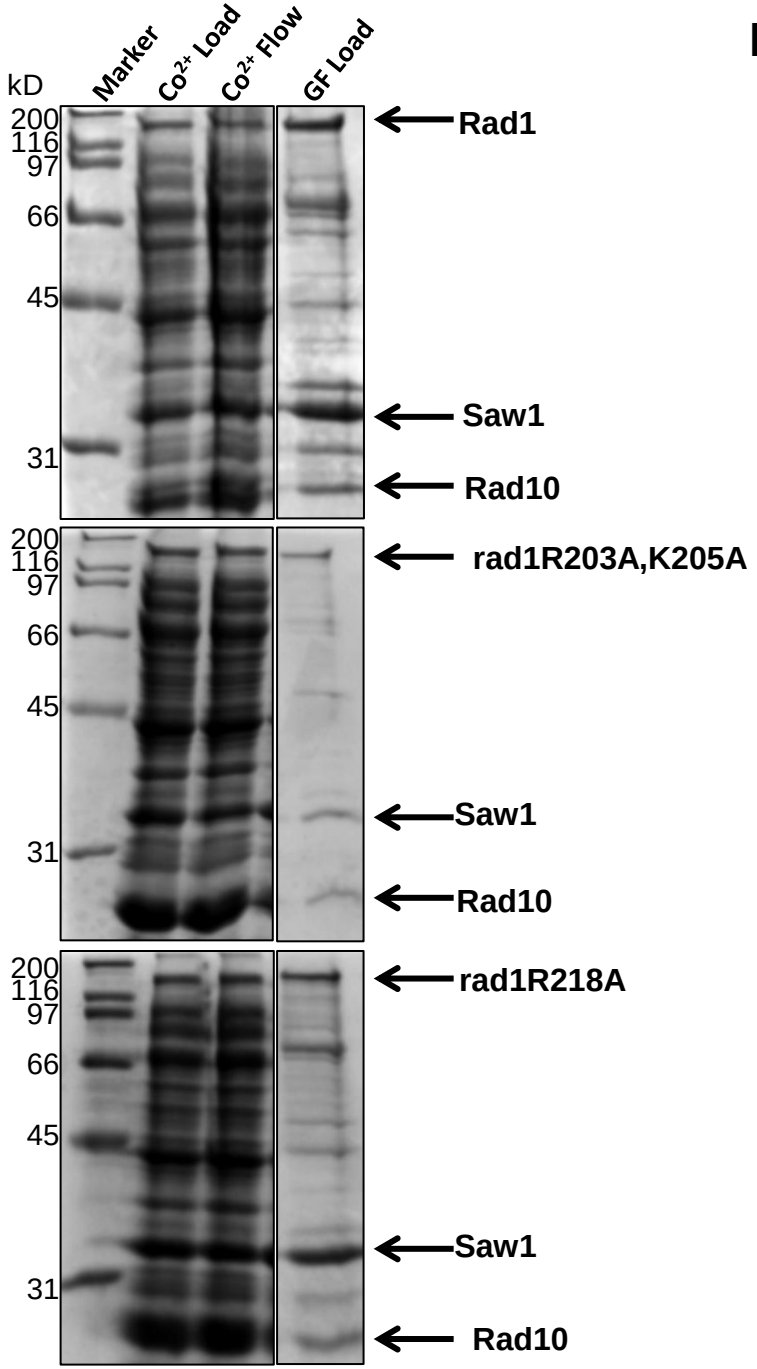

A
0
2
4
pmols Saw1
Rad1-Rad10 probe
0
2
4
pmols BSA
Rad1-Rad10 probe
rad1R203A K205-Rad10 probe
rad1R218A-Rad10 probe
B
pmols r/Rad1-Rad10
0.006
0.004
0.002
0.000
0.25
0.5
1
2
4
8
pmols Saw1
Rad1-Rad10
rad1R203A K205A-Rad10
rad1R218A-Rad10
C
0
0.13
0.25
0.5
1.0
1.5
2.0
pmols Msh2-Msh3
Rad1-Rad10 probe
rad1R203A K205A-Rad10 probe
rad1R218A-Rad10 probe
D
pmols R/rad1-Rad10
0.3
0.2
0.1
0.0
0.13
0.25
0.5
1.0
1.5
2.0
pmols Msh2-Msh3
Rad1-Rad10
rad1R203A, K205A-Rad10
rad1R218A-Rad10

Figure S2.

A

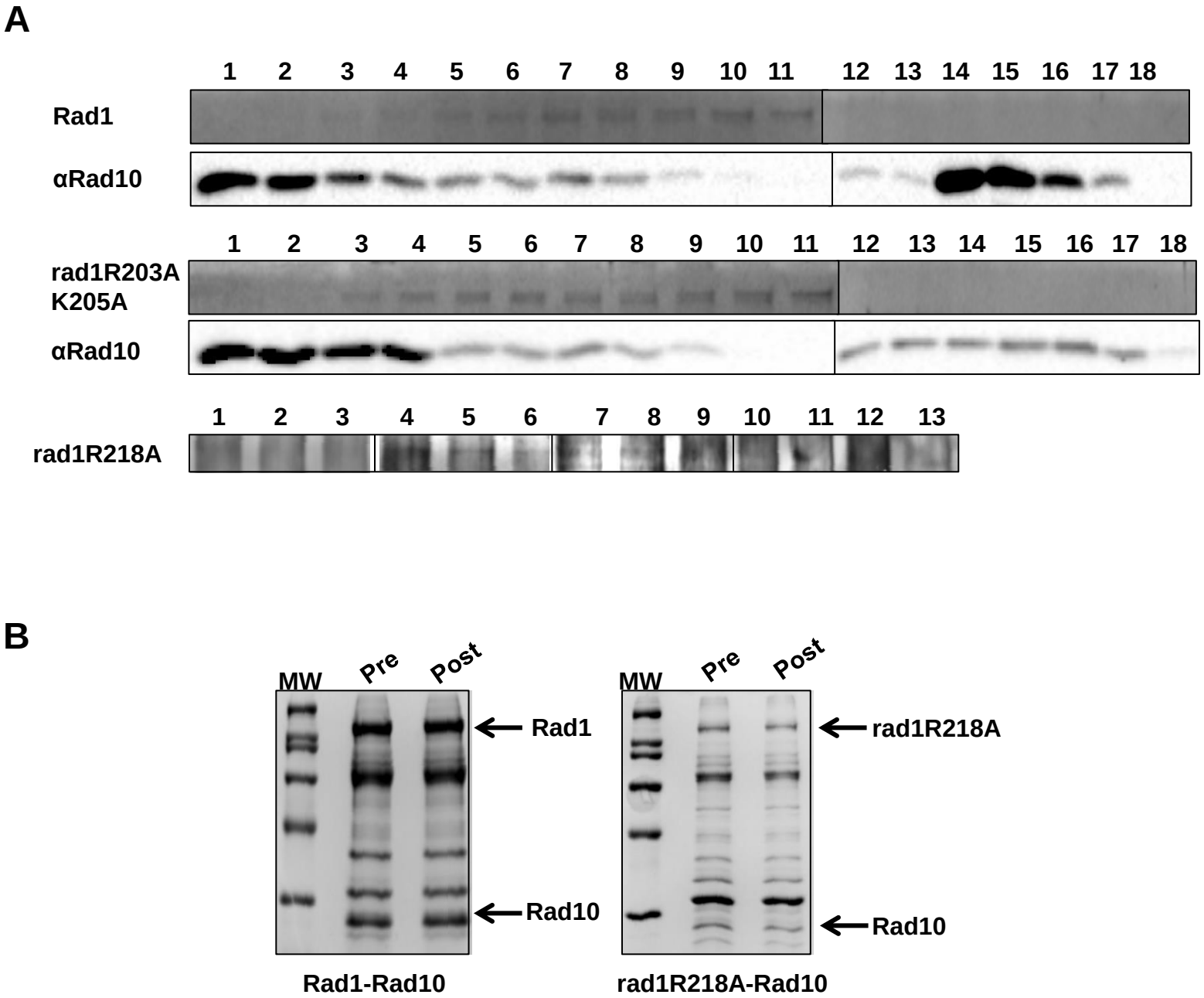


B

Relative amount of each protein in gel filtration load

	r/Rad1	Rad10	Saw1
Rad1	29	18	53
rad1R203A K205A	33	31	36
rad1R218A	22	28	50

Figure S3.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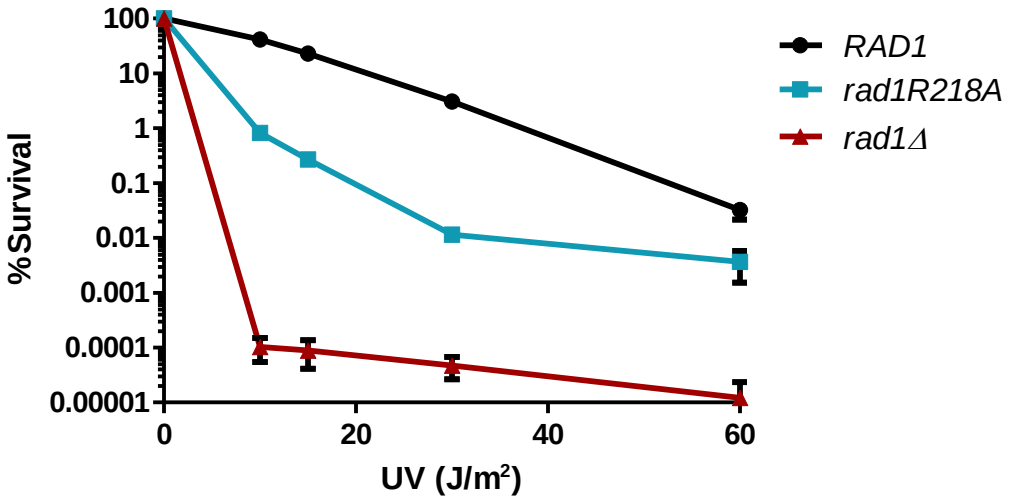
Rad1
αRad10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
rad1R203A
K205A
αRad10
1 2 3 4 5 6 7 8 9 10 11 12 13
rad1R218A
B
MW
Pre
Post
Rad1
Rad10
Rad1-Rad10
MW
Pre
Post
rad1R218A
Rad10
rad1R218A-Rad10

Figure S4.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10
1
0.1
0.01
0.001
0.0001
0.00001
%Survival
0
20
40
60
UV (J/m²)
RAD1
rad1R218A
rad1Δ

Figure S5.

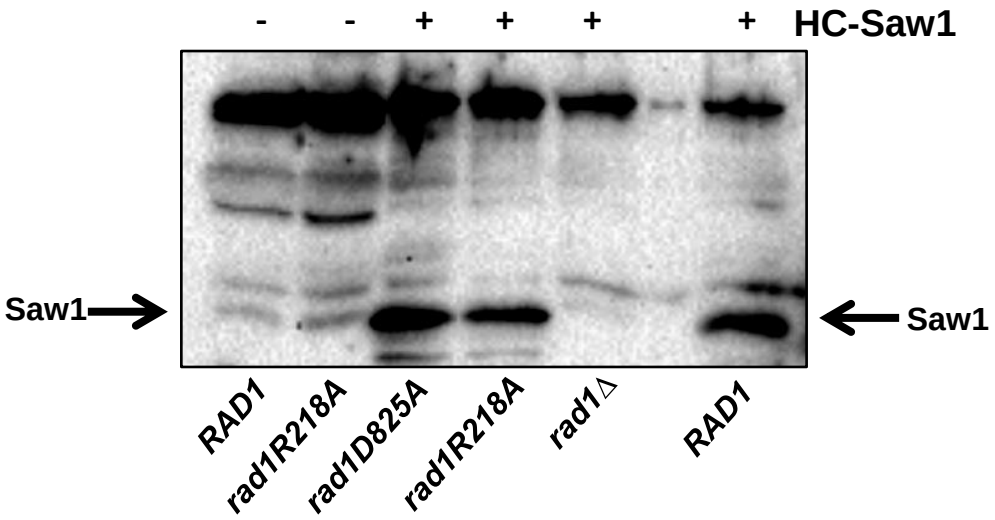
- - + + + + HC-Saw1
Saw1
Saw1
RAD1
rad1R218A
rad1D825A
rad1R218A
rad1Δ
RAD1

Figure S6.

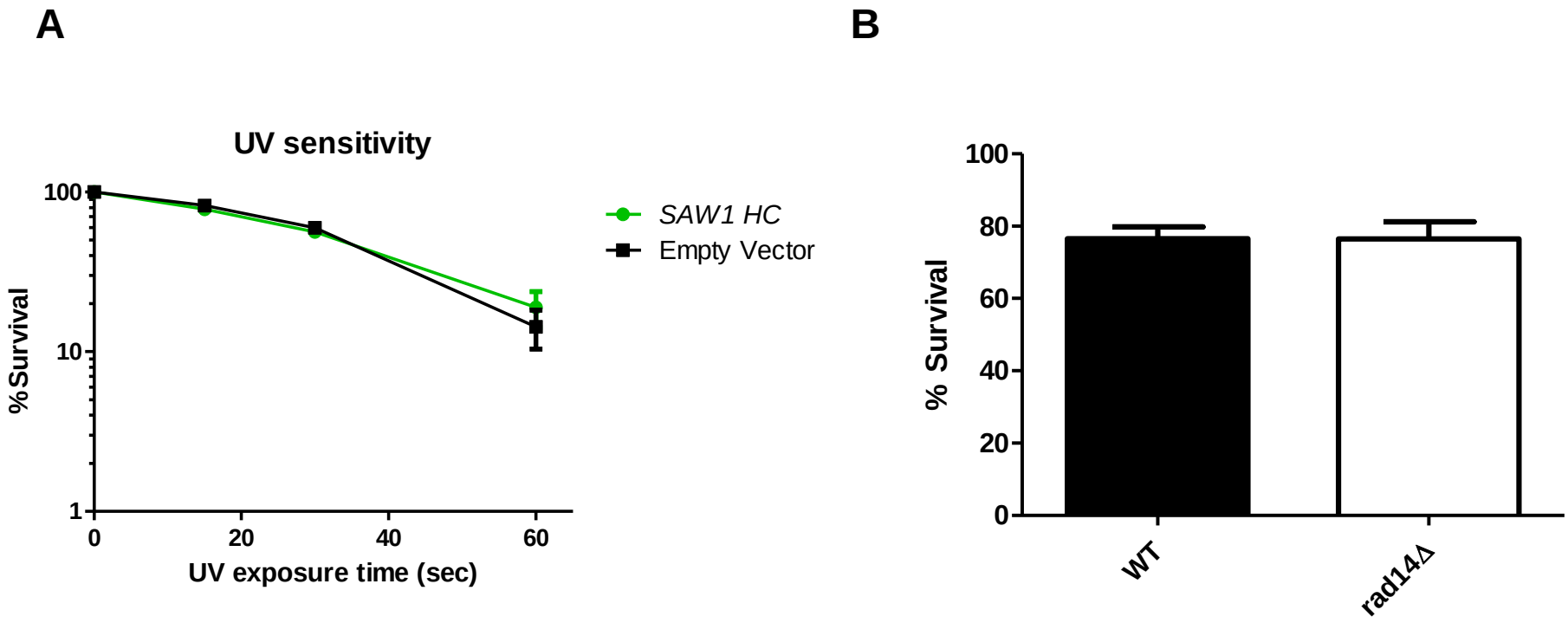
A
UV sensitivity
100
10
1
% Survival
0
20
40
60
UV exposure time (sec)
SAW1 HC
Empty Vector
B
100
80
60
40
20
0
% Survival
WT
rad14Δ

Figure S7.

Pathway Summary

Allele	3' NHTR	NER	ICLR
RAD1	++	++	++
rad1R203A K205A	-	+	++
R218A	-	++	++
D825A	-	-	NT

***In vitro* Summary**

Allele	DNAB	Endo	Saw1 Int.	Stable complex	2-3 Int.	2-3 int. + DNA	Saw1 Int. + 2-3	RPA Stim.
RAD1	++	++	++	++	++	++	++	++
rad1R203A K205A	+	+/-	+	-	+/-	NT	NT	NT
R218A	++	++	++	++	++	+++	+++	-
*D825A**	++	-	NT	NT	NT	NT	NT	NT

***In vivo* Summary**

Allele	SSA - short	SSA - long	ChIP	SSA + Saw1	SSA + 2-3	SSA + KKAA	SSA + Saw1 + Msh3	RPA Int.
RAD1	++	++	-	No change	↓	↓	No change	++
R218A	-	+	+/-	↑	↓	No change	No change	-
D825A	-	NT	++	No change	NT	NT	NT	NT

Figure S8.