

Supplementary Material

Table S1. *Saccharomyces cerevisiae* strains used in this study

Strain	Genotype	Source
HK578-10D	<i>MATa, leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11,15</i>	H. Klein
WXY971	HK578-10D with <i>rad18Δ::TRP1</i>	Lab stock
WXY2561	HK578-10D with <i>rad5Δ::TRP1</i>	This study
HK578-10A	<i>MATa, leu2-3,112 trp1-1, can1-100, ura3-1, ade2-1, his3-11,15</i>	H. Klein
WXY2562	HK578-10A with <i>rad18Δ::TRP1</i>	This study
WXY2563	HK578-10A with <i>rad5Δ::TRP1</i>	This study
WXY939	HK578-10D with <i>pol30Δ::HIS3/pBL211</i>	Lab stock
WXY2972	WXY939 with <i>rad5Δ::TRP1</i>	Lab stock
WXY978	WXY939 with <i>rad18Δ::TRP1</i>	Lab stock
WXY2558	WXY939 with <i>siz1Δ::LEU2</i>	This study
WXY2559	WXY2972 with <i>siz1Δ::LEU2</i>	This study
WXY2560	WXY978 with <i>siz1Δ::LEU2</i>	This study
PJ69-4a	<i>MATa, trp1-901 leu2-3,112 ura3-52, his3-200, gal4Δ, gal80Δ, GAL2-ADE2 LYS2::GAL1-HIS3, MET2::GAL7-lacZ</i>	P. James

Table S2. Oligonucleotides used in this study.

Name	Sequence (5'-3')
pGBT-Pol30-1-258- <i>Bam</i> HI-F	CGCCGCGGATCCATGTTAGAAAGCAAAATTTG
pGBT-Pol30-1-258- <i>Pst</i> I-R	GCAGTTATTCTTCGTCATTAATTTAGGAGCC
pGBT/AD-Rad5-1-1167- <i>Bam</i> HI-F	CGCCGCGGATCCATGAGTCATATTGAACAGG
pGBT/AD-Rad5-1-1167- <i>Pst</i> I-R	GCATGCACTGCAGCTATTCAAACAGCATCTGGATTTC
pGBT-Rad5-HIRAN- <i>Bam</i> HI-F	CGCCGCGAATCCATGATAGGTGCTTTGCAAGTCACTGG
pGBT-Rad5-HIRAN- <i>Pst</i> I-R	TGCACTGCAGTTAGCAATCCAATTGTAGAATAAAGC
pGBT-Rad5-1-100- <i>Bam</i> HI-F	CGCCGCGGATCCATGAGTCATATTGAACAGG
pGBT-Rad5-1-100- <i>Pst</i> I-R	TGCACTGCAGTTATTTTTTCATTAAGTTCCATCAC
pGBT-Rad5-100-200- <i>Bam</i> HI-F	CGCCGCGGATCCATGAAATTCGGCAGTCAGGAGG
pGBT-Rad5-100-200- <i>Pst</i> I-R	GCATGCACTGCAGTTACTTCATCTGAGAGCCGTACTTC
pGBT-Rad5-200-300- <i>Bam</i> HI-F	CGCCGCGGATCCATGAAGCTAAAGAGATCAAGTGAAGAGA
pGBT-Rad5-200-300- <i>Pst</i> I-R	TGCACTGCAGGGATTCTCCATCATTACGTTCCCTC
pGBT-Rad5-300-400- <i>Bam</i> HI-F	CGCCGCGGATCCATGTCCTTGATGAAAAGAAGACG
pGBT-Rad5-300-400- <i>Pst</i> I-R	GCACTGCAGTTATTCTTCTTCGTGCTGAGTATCTC
pGBT-Rad5-400-431- <i>Bam</i> HI-F	CGCCGCGGATCCATGGAAACAATGAACTTGAATCAATTG
pGBT-Rad5-400-431- <i>Pst</i> I-R	TGCACTGCAGATCGCGAGAAGGTTCTGTTTCAGGC
pGBT-Rad5-300-360- <i>Bam</i> HI-F	CGCCGCGGATCCATGTCCTTGATGAAAAGAAGACG
pGBT-Rad5-300-360- <i>Pst</i> I-R	TGCACTGCAGTTATAATGCCTTCTGCTCGTCC
pGBT-Rad5-340-360- <i>Bam</i> HI-F	CGCCGCGGATCCATGGCTCTACTAAAGTTATTTG
pGBT-Rad5-340-360- <i>Pst</i> I-R	TGCACTGCAGTTATAATGCCTTCTGCTCGTCC
pGBT-Rad5-320-380- <i>Bam</i> HI-F	CGCCGCGGATCCATGAATTTTGAAGAACATTGACTG
pGBT-Rad5-320-380- <i>Pst</i> I-R	TGCACTGCAGTTACTCGTCGTTATCTAAATCAATGATTTTC
pGBT-Rad5-100-431- <i>Bam</i> HI-F	CGCCGCGGATCCATGAAATTCGGCAGTCAGGAGG
pGBT-Rad5-100-431- <i>Pst</i> I-R	TGCACTGCAGATCGCGAGAAGGTTCTGTTTCAGGC
pGBT-Rad5-300-340- <i>Bam</i> HI-F	CGCCGCGGATCCATGTCCTTGATGAAAAGAAGACG
pGBT-Rad18-300-340- <i>Pst</i> I-R	TGCACTGCAGTTACAGTCTTTTCGAGCGGC
pGBT-Rad5-320-360- <i>Bam</i> HI-F	CGCCGCGGATCCATGAATTTTGAAGAACATTGACTG
pGBT-Rad5-320-360- <i>Pst</i> I-R	TGCACTGCAGTTATAATGCCTTCTGCTCGTCC
pGBT-Rad5-340-380- <i>Bam</i> HI-F	CGCCGCGGATCCATGGCTCTACTAAAGTTATTTG
pGBT-Rad5-340-380- <i>Pst</i> I-R	CTGCAGTTACTCGTCGTTATCTAAATCAATGATTTTC
pGBT-Rad5-360-400- <i>Bam</i> HI-F	CCGCCGCGGATCCATGTTAGAAAAGCATAAAAATAGAGC
pGBT-Rad5-360-400- <i>Pst</i> I-R	GCACTGCAGTTATTCTTCTTCGTGCTGAGTATCTC
pGBT-Rad5-171-348- <i>Bam</i> HI-F	CGCCGCGGATCCATGACTTGGAAAAGATTTATAGGTGC

pGBT-Rad5-171-348- <i>Pst</i> I-R	GCATGCACTGCAGTTACCTCAATTTATCAAATAAC
pGBT-Rad5-1-321- <i>Bam</i> HI-F	CGCCGCGGATCCATGAGTCATATTGAACAGG
pGBT-Rad5-1-321- <i>Pst</i> I-R	GCAGTTATCCAAAATTTCCATTGTCTTTCTCTC
pGBT/AD-Rad18-1-348- <i>Bam</i> HI-F	CGCCGCGGATCCATGGACCACCAAATAACCACTGCAAGCG
pGBT/AD-Rad18-1-348- <i>Pst</i> I-R	CATGCACTGCAGCAGTGGTAATATATCAGTCATGGATCTTTTGGC
pGBT-Rad18-1-160- <i>Bam</i> HI-F	CGCCGCGGATCCATGGACCACCAAATAACCACTGCAAGCG
pGBT-Rad18-1-160- <i>Pst</i> I-R	GCATGCACTGCAGTTACAGTGGTAATATATCAGTCATGG
pGBT-Rad18-150-258- <i>Bam</i> HI-F	CGCCGCGGATCCGCCAAAAGATCCATGACTGATATATTACCACT
pGBT-Rad18-150-258- <i>Pst</i> I-R	ATGCACTGCAGCACATGTGAGGTCTCACCACATGAGCTTTTGTGC
pGBT-Rad18-240-371- <i>Bam</i> HI-F	CGCCGCGGATCCTTCAAGGTACGAACTCCAGAAGTCGACAAAAG
pGBT-Rad18-240-371- <i>Pst</i> I-R	GCATGCACTGCAGCCTATATGAAGAAGATTTCCC
pGBT-Rad18-300-487- <i>Bam</i> HI-F	CGCCGCGGATCCACTAGGCCAAAACATGATTAAGATACAATCA CTAC
pGBT-Rad18-300-487- <i>Pst</i> I-R	GCATGCACTGCAGTTAATTGTTACCGGGTGGGTC
pGBT-Rad18-1-40- <i>Bam</i> HI-F	CGCCGCGGATCCATGGACCACCAAATAACCACTGCAAGCG
pGBT-Rad18-1-40- <i>Pst</i> I-R	GCATGCACTGCAGTTATAAGACGGGGACTTTTAG
pGBT-Rad18-20-60- <i>Bam</i> HI-F	CGCCGCGGATCCATGTACCAATTGGATACACTTTTGTAG
pGBT-Rad18-20-60- <i>Pst</i> I-R	GCATGCACTGCAGTTATGGTTGGTTATTCAAATGTG
pGBT-Rad18-40-80- <i>Bam</i> HI-F	CGCCGCGGATCCATGTTAACACCTTGTGGCCATAC
pGBT-Rad18-40-80- <i>Pst</i> I-R	GCATGCACTGCAGTTAGACCAGGAACTCACTTCTCAGC
pGBT-Rad18-60-100- <i>Bam</i> HI-F	CGCCGCGGATCCATGCCAAATTGTCCTCTCTGCCTTTTTCGAG
pGBT-Rad18-60-100- <i>Pst</i> I-R	GCATGCACTGCAGTTATATCCTTAGTGCATCTAG
pGBT-Rad18-80-120- <i>Bam</i> HI-F	CGCCGCGGATCCATGGTCAGTGAATAATTCAAAG
pGBT-Rad18-80-120- <i>Pst</i> I-R	GCATGCACTGCAGTTACCATGAAGAATTTTCAGGACCTGG
pGBT-Rad18-100-140- <i>Bam</i> HI-F	CGCCGCGGATCCATGATACCGAAGCCTACCCCTGTCCC
pGBT-Rad18-100-140- <i>Pst</i> I-R	GCATGCACTGCAGTTATTGCAAGTCATCATCAGCGGC
pGBT-Rad18-120-160- <i>Bam</i> HI-F	CGCCGCGGATCCATGTGGATAGAACTCATATCAGAGTCTG
pGBT-Rad18-120-160- <i>Pst</i> I-R	GCATGCACTGCAGTTACAGTGGTAATATATCAGTCATGG
Pol30- <i>Bam</i> HI-F	CGCGGATCCATGTTAGAAGCAAAAATTTGAAGAAGCATCCC
Pol30- <i>Sac</i> I-R	CGAGCTCTTATTCTTCGTCATTAATTTAGGAGCC
Rad5-N431- <i>Bam</i> HI-F	CGCCGCGGATCCATGAGTCATATTGAACAGGAAGAAAGGAAGA
Rad5-N431- <i>Eco</i> RI-R	CCGGAATTCTTAATCGCGAGAAGGTTCTGTTTCAGGC
Rad18-N160- <i>Bam</i> HI-F	CGCCGCGGATCCATGGACCACCAAATAACCACTGCAAGCG
Rad18-N160- <i>Xho</i> I-R	CCGCTCGAGTTACAGTGGTAATATATCAGTCATGG
Rad30- <i>Bam</i> HI-F	CGCCGCGGATCCATGGTTGTAGATATGTTTGGCAATCAGG
Rad30- <i>Eco</i> RI-R	CCGGAATTCTCATTTTTTTCTTGTA AAAAATGATAAGATG

Rad18-<i>SacI</i>-F	CGAGCTCGGAAGCGCGCAACTGAAAAG
Rad18-<i>Bam</i>HI-R	CGCGGATCCATTGTTACCGGGTGGGTCTTTAC
2×Flag-<i>Bam</i>HI-F	CGCGGATCCATGGATTACAAGGATGACGACGATAAGG
2×Flag-<i>Sph</i>I-R	ACATGCATGCTTACTTGTTCATCATCGTCCTTATAG
Rad18-<i>Sph</i>I-F	ACATGCATGCGGTGTGTATATGTAATCAGGCCTG
Rad18-<i>Hind</i>III-R	CCCAAGCTTCACTTCTTTGCCTAATCCAGTGG
Rad5-<i>Bam</i>HI-F	CGCGGATCCCCATCAAAGAAGACAAGACCTGGAG
Rad5-S1-R	CCTGTTCAATATGACTCATCTTGTTCATCATCGTCCTTATAGTCCTT ATCGTCGTCATCCTTGTAATCCATATTGTGATACTGTAAGACTTT AGGTGTG
Rad5-S2-F	CACACCTAAAGTCTTACAGTATCACAATATGGATTACAAGGATG ACGACGATAAGGACTATAAGGACGATGATGACAAGATGAGTCAT ATTGAACAGG
Rad5-S2-R	GCCATCTTCAACGTTTACATGGTCTCTGCTGC
Rad5-S3-F	GCAAGGTCTTACTTGGATGCTAAGGAGGGAGC
Rad5-S3-R	GGTTCCGTTGTGCAGATGGAGCACTCTAAGGACTG
Rad5-S4-F	GGTTACGGAACAAACGGTGGAGCTTGACTC
Rad5-<i>Kpn</i>I-R	CGGGGTACCAAAAAATCTACTAGTTTATTTCCACCTACAC
Pol30-K168-F	GATCACCAAAGAAACAATAGCGTTTGTAGCTGACGGTGATATC GGATC
Pol30- K168-R	CCGTCAGCTACAAACGCTATTGTTTCTTTGGTGATCATGATATTA ATAG
Pol30-K196A-F	CCTGAAACAAGCATCGCACTTGAAATGGATCAACCTGTCTGACTT GACGTTCCG
Pol30-K196A-R	CATTTCAAGTGCGATGCTTGTTTCAGGATGTTCCATATCCACGAA TGTTTTTATTATGAC
Pol30-K164R-F	GATTCTATTAATATCATGATCACCAGAGAAACAATAAAGTTTGT AGCTGACGGTGATATCG
Pol30-K164R-R	CTTTATTGTTTCTCTGGTGATCATGATATTAATAGAATCACTCAA TTGGGACAAG
Pol30-K126,128AA-R	CTGTAATTCTTCCGCCTTCGCGAAATCAGCATCGATATCCATC
Pol30-251-255-AAA-F	CAGTTTTTCTTGGCTGCGGCGGCGGCTGACGAAGAATAA
Pol30-251-255-AAA-R	TTATTCTTCGTCAGCCGCCGCCGAGCCAAGAAAACTGTAGGA ACCCACTC
Rad5-LLF341,343, 344AAA-F (Rad5-3A-F)	GAAAGCCGCTCGAAAAGACTGGCTCTAGCGAAGGCGGCGGATA AATTGAGGCTAAAGCC

Rad5-LLF341,343,344AAA-R (Rad5-3A-R)	CCAAAATAGGCTTTAGCCTCAATTTATCCGCCGCTTCGCTAGAG CCAGTCTTTTCGAGC
Rad5-L341A-F	GAAAGCCGCTCGAAAAGACTGGCTCTAGCGAAGTTATTTGATAA ATTGAGGCTAAAGCC
Rad5-L341A-R	CCAAAATAGGCTTTAGCCTCAATTTATCAAATAACTTCGCTAGAG CCAGTCTTTTCGAGC
Rad5-L343A-F	GAAAGCCGCTCGAAAAGACTGGCTCTACTAAAGGCGTTTGATAA ATTGAGGCTAAAGCC
Rad5-L343A-R	CCAAAATAGGCTTTAGCCTCAATTTATCAAACGCCTTTAGTAGAG CCAGTCTTTTCGAGC
Rad5-F344A-F	GAAAGCCGCTCGAAAAGACTGGCTCTACTAAAGTTAGCGGATAA ATTGAGGCTAAAGCC
Rad5-F344A-R	CCAAAATAGGCTTTAGCCTCAATTTATCCGCTAACTTTAGTAGAG CCAGTCTTTTCGAGC
Rad5-R187E-F	GCTTTGCAAGTCACTGGTATGGCTACCGAACCCACCGTCAGG
Rad5-R187E-R	GCCGTACTTCAAGGGCCTGACGGTGGGTTTCGGTAGCCATAACC
Rad5-RR229,241EE-F (Rad5-2E-F)	GAAAGCGTCCATGGCTAGTTTGGTAGAAATTTTTGATATCCAATA TGATAGAGAAATTGGCGAAGTTTCGGAAGAC
Rad5-RR229,241EE-F (Rad5-2E-R)	GTATTTGAGCAATGTCTTCCGAACTTCGCCAATTTCTCTATCAT ATTGGATATCAAAAATTTCTACCAAAGTAGC
Rad18-LLF64,66,67AAA-F (Rad18-3A-F)	CATTTGAATAACCAACCAAATTTGTCCTGCGTGCGCGGCGGAGTT CAGAGAGTCCTTGCTG
Rad18-LLF64,66,67AAA-R (Rad18-3A-R)	AACTCACTTCTCAGCAAGGACTCTCTGAACTCCGCCGCGCACGC AGGACAATTTGGTTGG
Rad18-L64A-F	CATTTGAATAACCAACCAAATTTGTCCTGCGTGCCTTTTTCGAGTTC AGAGAGTCCTTGCTG
Rad18-L64A-R	AACTCACTTCTCAGCAAGGACTCTCTGAACTCGAAAAGGCACGC AGGACAATTTGGTTGG
Rad18-L66A-F	CATTTGAATAACCAACCAAATTTGTCCTCTCTGCGCGTTTCGAGTTC AGAGAGTCCTTGCTG
Rad18-L66A-R	AACTCACTTCTCAGCAAGGACTCTCTGAACTCGAACGCGCAGAG AGGACAATTTGGTTGG
Rad18-F67A-F	CATTTGAATAACCAACCAAATTTGTCCTCTCTGCCTTGCGGAGTTC AGAGAGTCCTTGCTG
Rad18-F67A-R	AACTCACTTCTCAGCAAGGACTCTCTGAACTCCGCAAGGCAGAG AGGACAATTTGGTTGG

Rad5-K194E-F	GGCTACCAGACCCACCGTCAGGCCCTTGGAGTACGGCTCTCAGA TG
Rad5-K194E-R	CACTTGATCTCTTTAGCTTCATCTGAGAGCCGTACTCCAAGGGCC TGACG
Rad18-C28C-F	GCCTGTACCAATTGGATACACTTTTGAGAAGTCACATTTGTAAAG ATTTTCTAAAAG
Rad18-C28C-R	GACGGGGACTTTTAGAAAATCTTTACAAATGTGACTTCTCAAAA GTGTATCC
Rad18-C65S-F	GAATAACCAACCAAATTGTCCTCTCAGCCTTTTCGAGTTCAGAGA G
Rad18-C65S-R	CAGCAAGGACTCTCTGAACTCGAAAAGGCTGAGAGGACAATTTG GTTGG

Supplementary Figure Legends

Figure S1. Relative sensitivity of *pol30* mutants to DNA-damaging agents by a serial dilution assay. The *pol30* mutants were created by plasmid shuffling. Cells were cultured overnight, density adjusted, serially diluted by tenfold and spotted onto YPD plates containing indicated concentrations of chemicals. For the UV irradiation, the plates were exposed to UV in a UV crosslinker at the given dose. The plates were incubated at 30 °C for two days before photography. At least two sets of independent cultures and multiple doses of DNA damage were employed and only selected representative images are shown.

Figure S2. Assessment of amino acid substitutions in Rad5 and Rad18 by a Y2H assay. (A) Effects of amino acid substitutions in the Rad5 LxLF motif. (B) Effects of amino acid substitutions in the Rad18 LxLF motif. (C) Effects of amino acid substitutions in the Rad18 RING domain. The Quick Exchange method was used to create site-specific mutations that result in single amino acid substitutions. Experimental conditions were as described in Figure 1B.

Figure S3. Assessment of cellular Rad5 and Rad18 protein levels by western blotting. (A) Relative Rad5 protein levels from *rad5Δ* cells transformed with either YCp-Flag-Rad5 or YEp-Flag-Rad5 without (upper panel) or with 0.05% MMS (lower panel) treatment. Experimental conditions were as described in Fig. 5C. (B, C) Quantitative analysis of western blot data as shown in (A). (B) No treatment. (C) 0.05% MMS treatment. (D, E) Quantitative analysis of western blot data as shown in Fig. 5C and D. (B-E) At least three independent western blots from each set of experiment were used for the quantitative analysis. The results are presented as average relative protein levels \pm standard deviations shown as error bars (n=3).

Figure S4. Additional western blot results from the *in vivo* co-IP assays. (A, B) Physical interaction between Rad5 and Pol30. (C, D) Physical interaction between Rad18 and Pol30. Experimental conditions were as described in Figure 5E and G.

Figure S5. Characterization of PCNA post-translation modifications. (A) Western blotting to assess effects of 90-min MMS treatments with different doses on PCNA ubiquitination. All strains are in the *siz1*Δ background. Abbreviations used: Ub₁, Pol30-K164^{Ub}; Ub₂, Pol30-K164^{di-Ub}; SE, short exposure. The asterisk indicates a major uncharacterized band found in all MMS treated samples. Experimental conditions were as described in Figure 6B. (B) Quantitative analysis of mono-ubiquitinated PCNA as shown in Figure 6C. (C) Quantitative analysis of di-ubiquitinated PCNA as shown in Figure 6D. (B, C) At least three independent western blots from each set of experiment were used for the quantitative analysis. The results are presented as average relative protein levels ± standard deviations shown as error bars (n=3). Two-tailed *P* values from the unpaired *t*-test are shown.

Figure S6. Relative sensitivity of *rad18* (A) and *rad5* (B) mutants to DNA-damaging agents by a serial dilution assay. *RAD5* and *RAD18* were cloned in a multicopy plasmid pGAD424 with an *ADHI* promoter that produces Gal4_{AD} fusion proteins. At least two sets of independent cultures and multiple doses of treatments were employed and only selected representative images are shown.

Figure S7. Amino acid sequence alignments of Rad5 (A) and Rad18 (B) in selected yeast species. Residues highlighted by black are identical sequences and those by grey are conserved sequences. Putative LxLF motifs are labelled, and conserved residues are indicated by red asterisks, while conserved Cys residues in the Rad18 RING domain are indicated by blue asterisks.

Figure S8. Predicted Rad5-PCNA and Rad18-PCNA structures. Molscript [1] was used to draw the ribbons representation. The AlphaFold3-predicted PCNA trimeric ring is shown in gray. Each subunit is colored in a different shade of gray. The ring is viewed from its side with its back face on the left. (A) Rad5-PCNA. Residues 1-169 of Rad5 are shown in yellow. The HIRAN domain (residues 170-300) is colored cyan. The intermediate region (residues 301-429) is shown in blue. The helicase domain (residues 430-1169) is colored in green with the embedded RING domain (residues 910-990) in darker green. (B) Rad18-PCNA. Residues 1-99 corresponding to the Rad18

RING Finger domain are shown in blue. Residues 270-280 (shown in cyan) were predicted to make intimate contact with PCNA. The rest of the Rad18 were colored yellow.

Methods and Results:

Modeling of protein complexes using AlphaFold 2 and AlphaFold 3

Both ColabFold [2], which is based on based on AlphaFold 2 [3], and AlphaFold 3 [4] were used to build models for PCNA-Rad5 and PCNA-Rad18 complexes. ColabFold was employed first to predict the two complexes. The five predicted models were consistent for PCNA-Rad5 and for PCNA-Rad18 complexes. We then used ColabFold to build Rad5 and Rad18 complexes with PCNA trimer. In both cases, PCNA trimers were predicted as closed rings, and the resulting interfaces with Rad5 and Rad18 were both consistent with predicted interfaces when monomeric PCNA was modeled. When AlphaFold 3 became available, the same procedure was repeated using AlphaFold 3. The predicted Rad5-PCNA interfaces were consistent with ColabFold predictions when PCNA monomer or trimer were modeled. The predicted Rad18-PCNA monomer interface was also consistent with ColabFold results. However, AlphaFold 3 predicted no interaction between Rad18 and closed trimeric ring of PCNA. This is not surprising since transient protein-protein interfaces remain challenging for the structural prediction.

Molscrip [1] was used to generate molecular graphics. One of the five models of Rad5-PCNA trimer built by AlphaFold3 was visualized. In this AlphaFold 3-predicted model, Rad5 approaches the PCNA trimeric ring from its back face. The HIRAN domain (in cyan) is the only domain of Rad5 that makes contact with PCNA (in gray). The N-terminal side of the HIRAN domain (in yellow) is largely disordered. The LxLF motif (in red) is in the intermediate region (in blue) between the HIRAN domain and the helicase domain (in green). One of the five models of Rad18-PCNA trimer built by ColabFold was also visualized. In this predicted model, Rad18 also approaches the PCNA trimeric ring from its back face. The N-terminal RING Finger domain (in blue), which harbours the LxLF motif (in red), contacts PCNA (in gray). The rest of the predicted Rad18 structure (in yellow) is largely disordered but part of it does appear to fold around the RING Finger domain as the structural core. An extended peptide from residue 270 to 280 (in cyan) makes intimate contact with PCNA.

References:

- 1 Kraulis PJ. A program to produce both detailed and schematic plots of protein structures. *J. Appl. Cryst.* 1991; 24: 946-50.
- 2 Mirdita M, Schutze K, Moriwaki Y, Heo L, Ovchinnikov S, Steinegger M. ColabFold: making protein folding accessible to all. *Nat Methods.* 2022; 19: 679-82.
- 3 Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Zidek A, Potapenko A, Bridgland A, Meyer C, Kohl SAA, Ballard AJ, Cowie A, Romera-Paredes B, Nikolov S, Jain R, Adler J, Back T, Petersen S, Reiman D, Clancy E, Zielinski M, Steinegger M, Pacholska M, Berghammer T, Bodenstein S, Silver D, Vinyals O, Senior AW, Kavukcuoglu K, Kohli P, Hassabis D. Highly accurate protein structure prediction with AlphaFold. *Nature.* 2021; 596: 583-9.
- 4 Abramson J, Adler J, Dunger J, Evans R, Green T, Pritzel A, Ronneberger O, Willmore L, Ballard AJ, Bambrick J, Bodenstein SW, Evans DA, Hung CC, O'Neill M, Reiman D, Tunyasuvunakool K, Wu Z, Zengulyte A, Arvaniti E, Beattie C, Bertolli O, Bridgland A, Cherepanov A, Congreve M, Cowen-Rivers AI, Cowie A, Figurnov M, Fuchs FB, Gladman H, Jain R, Khan YA, Low CMR, Perlin K, Potapenko A, Savy P, Singh S, Stecula A, Thillaisundaram A, Tong C, Yakneen S, Zhong ED, Zielinski M, Zidek A, Bapst V, Kohli P, Jaderberg M, Hassabis D, Jumper JM. Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature.* 2024; 630: 493-500.

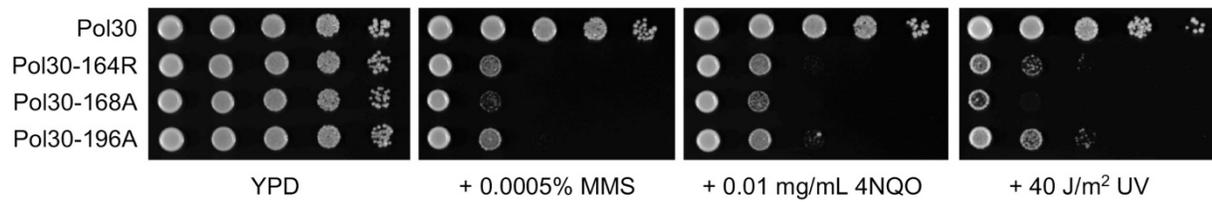


Figure S1

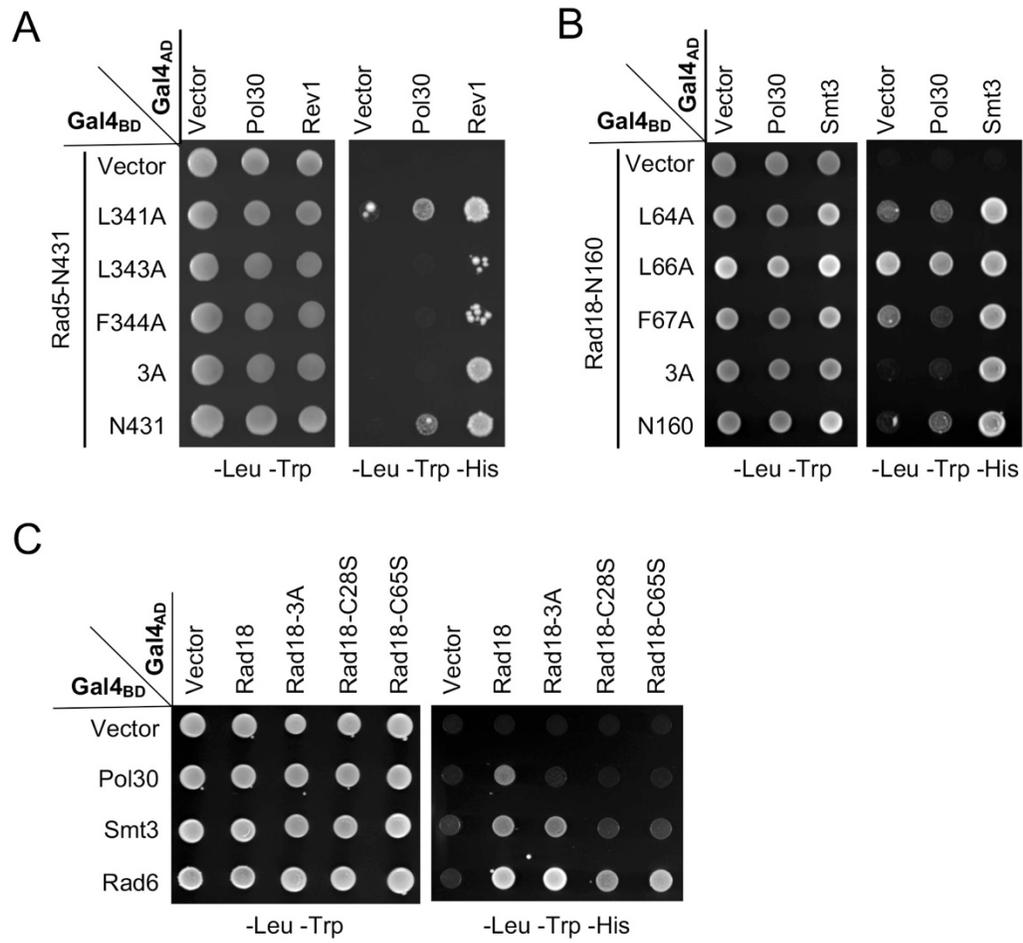


Figure S2

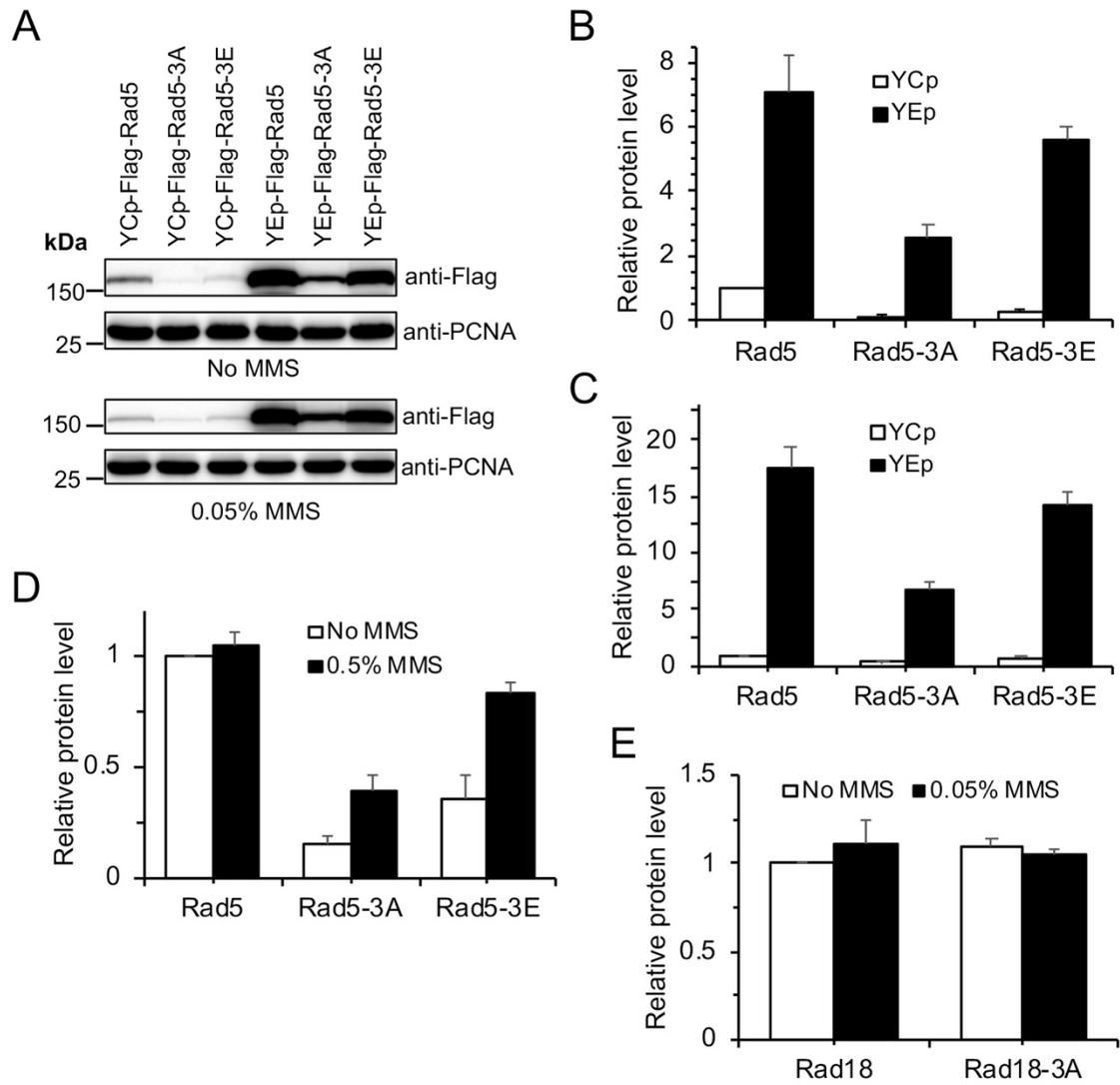


Figure S3

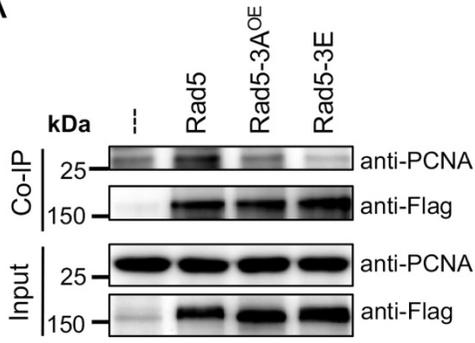
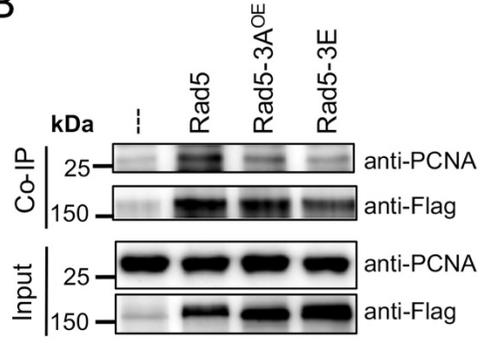
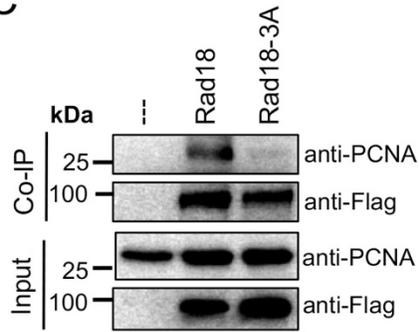
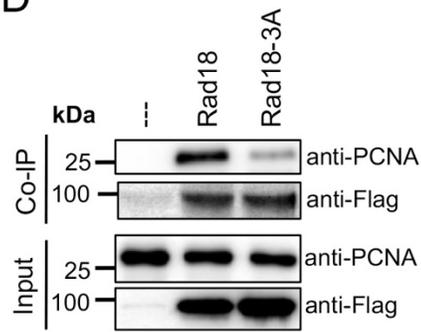
A**B****C****D**

Figure S4

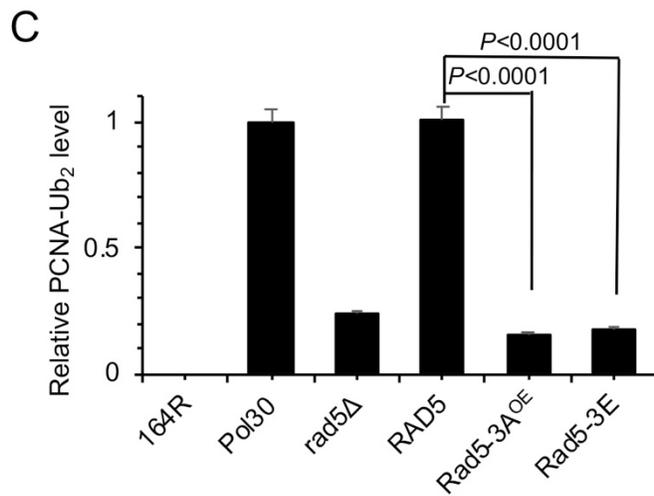
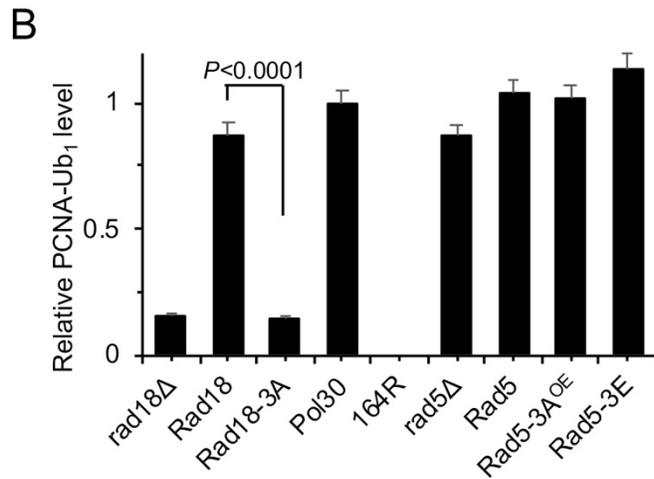
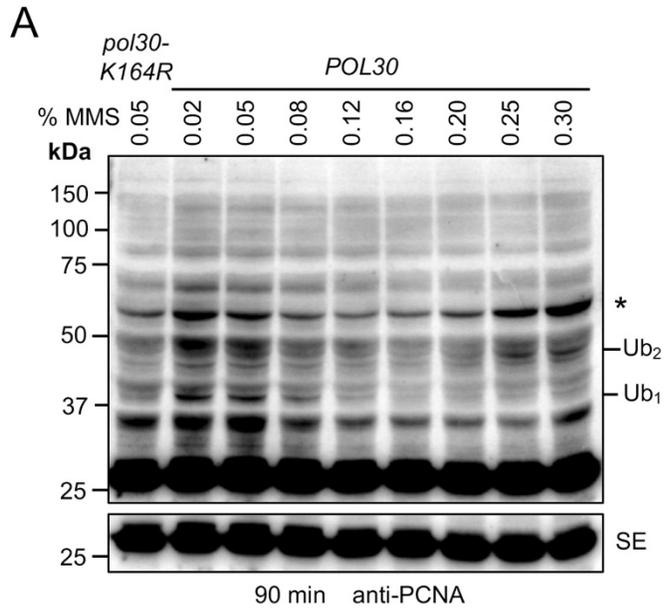


Figure S5

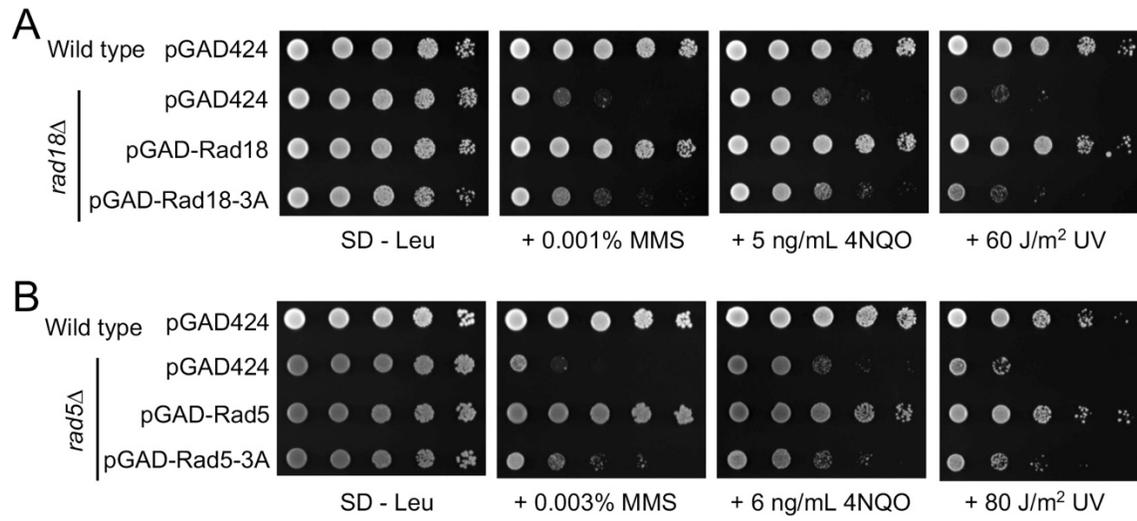
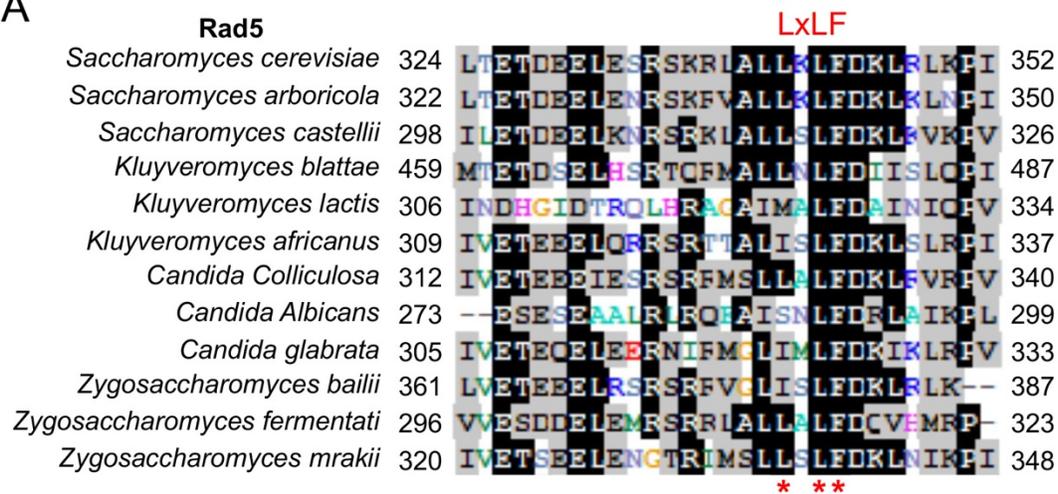


Figure S6

A



B

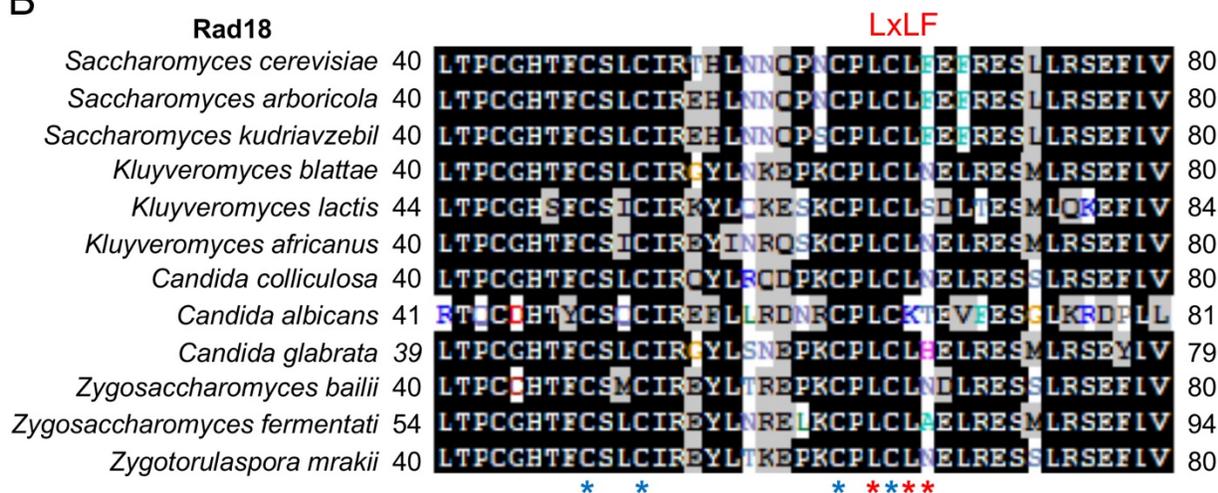
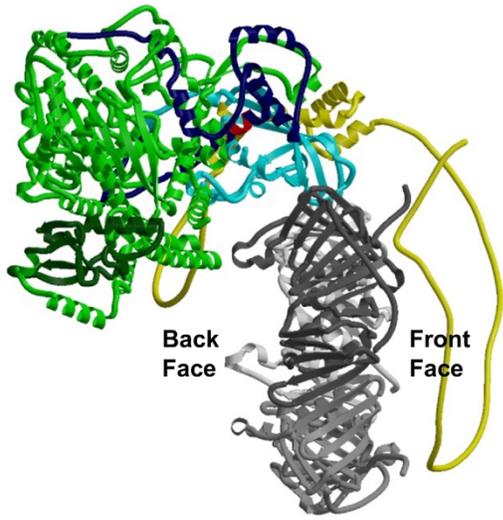


Figure S7

A



B

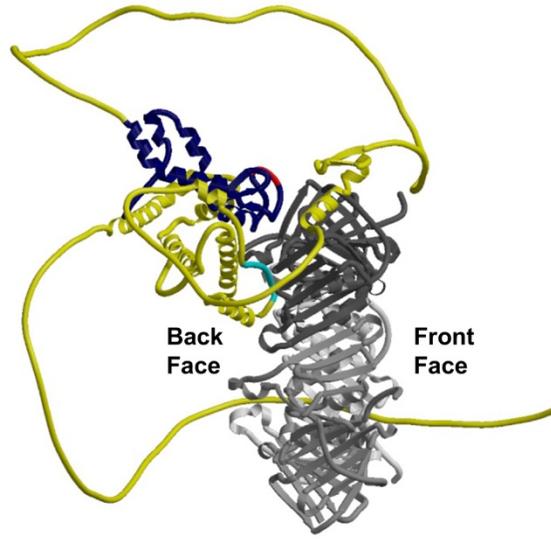


Figure S8