## Supplementary materials

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

| Strain | Genotype | Source |
|---|---|---|
| BY4741 | *MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* | SGD Consortium |
| BY4741 *trp1Δ* | BY4741 with *trp1Δ::Kan*$^R$ | SGD Consortium |
| BY4741 *rad5Δ* | BY4741 with *rad5Δ::Kan*$^R$ | SGD Consortium |
| BY4741 *rev1Δ* | BY4741 with *rev1Δ::Kan*$^R$ | SGD Consortium |
| BY4741 *mms2Δ* | BY4741 with *mms2Δ::Kan*$^R$ | SGD Consortium |
| BY4741 *ubc13Δ* | BY4741 with *ubc13Δ::Kan*$^R$ | SGD Consortium |
| WXY1634 | BY4741 with *mms2Δ::Kan*$^R$ *rev1Δ::NAT*$^R$ | Lab stock |
| WXY1810 | BY4741 with *ubc13Δ::Kan*$^R$ *rev1Δ::NAT*$^R$ | Lab stock |
| WXY1912 | BY4741 with *rev1Δ::Kan*$^R$ *rad5Δ::NAT*$^R$ | Lab stock |
| HK578-10D | *MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1* | H. Klein |
| WXY902 | HK578-10D *mms2Δ::HIS3* | Lab stock |
| WXY904 | HK578-10D *ubc13Δ::HIS3* | Lab stock |
| HK578-6B | HK578-10D *rad5Δ::hisG-URA3-hisG* | H. Klein |
| WXY922 | HK578-10D *ubc13Δ::HIS3 rad5Δ::hisG-URA3-hisG* | Lab stock |
| WXY3138 | HK578-10D *mms2Δ::HIS3 rad5Δ::hisG-URA3-hisG* | Lab stock |
| WXY3157 | HK578-10D *rad5Δ::hisG-URA3-hisG rev1Δ::NAT*$^R$ | This study |
| WXY2936 | HK578-10A *rev1Δ::HIS3* | Lab stock |
| DBY747 | *MAT***a** *his3-Δ1 leu2-3,112 trp1-289 ura3-52* | D. Botstein |
| WXY717 | DBY747 *rev1Δ::LEU2* | Lab stock |
| WXY731 | DBY747 *rad5Δ::hisG-URA3-hisG* | Lab stock |
| WXY3163 | DBY747 *rad5-FN13,14AA* | This study |
| WXY2974 | DBY747 *rad5-I916A* | Lab stock |
| WXY3164 | DBY747 *rad5-FN13,14AA I916A* | This study |
| PJ69-4a | *MAT***a** *trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ Met2::GAL7-lacZ LYS2::GAL1-HIS3 GAL2-ADE2* | P. James |

**Table S2.** Oligonucleotides used in this study

| Name | Sequence |
|---|---|
| pM-rad556F | GGATCCATGAGTCATATTGCAGCGGAAGAAAGGAAGAGGTTT |
| pM-rad556R | AAACCTCTTCCTTTCTTCCGCTGCAATATGACTCATGGATCC |
| pM-rad578F | ATGAGTCATATTGAACAGGCAGCAAGGAAGAGGTTTTTAAC |
| pM-rad578R | GTTAAAAACCTCTTCCTTGCTGCCTGTTCAATATGACTCAT |
| pM-rad5910F | CATATTGAACAGGAAGAAGCGGCGAGGTTTTTAACGATGAC |
| pM-rad5910R | GTCATCGTTAAAAACCTCGCCGCTTCTTCCTGTTCAATATG |
| pM-rad51314F | GAAGAAAGGAAGAGGTTTGCTGCCGATGACCTTGACACTTCA |
| pM-rad51314R | TGAAGTGTCAAGGTCATCGGCAGCAAACCTCTTCCTTTCTTC |
| pM-rad51516F | AGGAAGAGGTTTTTAACGCTGCCCTTGACACTTCAGAAACA |
| pM-rad51516R | TGTTTCTGAAGTGTCAAGGGCAGCGTTAAAAACCTCTTCCT |
| pM–rad52426F | GACACTTCAGAAACATCGGCAAACGCCAAATCTGAGAATAAAGAG |
| pM–rad52426R | CTCTTTATTCTCAGATTTGGCGTTTGCCGATGTTTCTGAAGTGTC |
| pM-rad52829F | ACATCGTTAAACTTCAAAGCTGCGAATAAAGAGTCGTTTTTA |
| pM-rad52829R | TAAAAACGACTCTTTATTCGCAGCTTTGAAGTTTAACGATGT |
| pM-rad5R11AF | GAACAGGAAGAAAGGAAGGCGTTTTTAACGATGACCTT |
| pM-rad5R11AR | AAGGTCATCGTTAAAAACGCCTTCCTTTCTTCCTGTTC |
| pM-rad5F12AF | CAGGAAGAAAGGAAGAGGGCTTTTAACGATGACCTTGAC |
| pM-rad5F12AR | GTCAAGGTCATCGTTAAAAGCCCTCTTCCTTTCTTCCTG |
| pM-rad5F13AF | GAAGAAAGGAAGAGGTTTGCTAACGATGACCTTGACACT |
| pM-rad5F13AR | AGTGTCAAGGTCATCGTTAGCAAACCTCTTCCTTTCTTC |
| pM-rad5N14AF | GAAAGGAAGAGGTTTTTTGCCGATGACCTTGACACTTCA |
| pM-rad5N14AR | TGAAGTGTCAAGGTCATCGGCAAAAAACCTCTTCCTTTC |
| pTRad5N21-30F | GATGACCTTGACACTTCAAAAGAGTCGTTTTTATTT |
| pTRad5N21-30R | AAATAAAAACGACTCTTTTGAAGTGTCAAGGTCATC |
| pRad5NT30Stu1F | CCTTAGAAACACACCTAAAGTCTTACAGTATCACAATATGAAAGAGTCGTTTTTATTT |
| pRad5NT60Stu1F | CCTTAGAAACACACCTAAAGTCTTACAGTATCACAATATGCGATCTATTGTGCCAGTC |
| pRad5SalR | GAACTTGTCGACTAATTGGTAGTTTCTTGTACC |

**Table S3** Data collection, phasing and refinement statistics

| | Crystal 1 -native | Crystal 2 -native | Crystal 3 -Hg |
|---|---|---|---|
| **Data collection** | | | |
| Space group | P1 21 1 | P21 21 2 | P21 21 2 |
| Cell dimensions | | | |
| *a*, *b*, *c* (Å) | 58.589, 49.280, 103.074 | 103.125, 49.370, 58.654 | 102.767, 49.114, 57.802 |
| α, β, γ (°) | 90.000, 92.197, 90.000 | 90.000, 90.000, 90.000 | 90.000, 90.000, 90.000 |
| Wavelength (Å) | 0.97911 | 0.97911 | 1.00880 |
| Resolution (Å) | 30-2.0 (2.07-2.00) * | 40-2.04 (2.11-2.04) | 50-2.4 (2.49-2.4) |
| $R_{\mathrm{sym}}$ or $R_{\mathrm{merge}}$ | 0.086 (0.184) | 0.139 (0.626) | 0.125 (0.408) |
| $I / \sigma I$ | 16 (6.2) | 30 (3.0) | 24.7 (4.8) |
| Completeness (%) | 95.7 (89.4) | 99.1 (96.6) | 99.5 (26.3) |
| Redundancy | 3.0 (2.7) | 5.3 (5.2) | 10.8 (9.8) |
| | | | |
| **Refinement** | | | |
| Resolution (Å) | 29-2.0 | | |
| No. reflections | 38367 | | |
| $R_{\mathrm{work}}$ / $R_{\mathrm{free}}$ | 20.1/24.6 | | |
| No. atoms | | | |
| Protein | 4071 | | |
| Ligand/ion | / | | |
| Water | 156 | | |
| *B*-factors (Å$^2$) | | | |
| Protein | 28 | | |
| Ligand/ion | / | | |
| Water | 30 | | |
| R.m.s deviations | | | |
| Bond lengths (Å) | 0.011 | | |
| Bond angles (°) | 1.201 | | |

*Values in parentheses are for highest-resolution shell.

## Supplementary Figures

**Figure S1**. Physical interaction between Rad5 and Rev1 by yeast two-hybrid assays. (**A**) Interaction of full-length Rad5 and Rev1. (**B**) Interaction of truncated Rad5 with full-length Rev1. The Y2H conditions are as described in Figure 1.

**Figure S2.** Complementation of the yeast *rad5* null mutant by N-terminally truncated Rad5. (**A**) Complementation of the yeast *rad5* null mutant by N-terminally truncated Rad5. (**B**) Complementation of the yeast *rad5 ubc13* null mutant by N-terminally truncated Rad5. Experimental conditions are as described in Figure 3.

**Figure S3.** Crystal structures of the Rev1-CTD and Rad5-NTD complex. (**A**) Superimposition of the four copies of the Rev1-Rad5 complex in one AU in the crystals, which show similar Rev1-Rad5 interactions. The four copies of Rev1 are colored blue, grey, orange and magenta. Their contacting Rad5 are colored green, red, cyan and yellow, respectively. Due to crystal packing interactions, the N-terminal end of Rad5 and the C-terminal α5 of Rev1 adopt different conformations among the four copies of the complex in one AU. (**B**) Superimpose of the 2Fo-Fc electron density map around the Rad5 peptide (contour level $\sigma$=1) with the refined model.

**Figure S4**. Rad5-NTD RFF mutations only affect the TLS activity. (**A**) A Y2H assay of Rad5 and its RFF mutations with Ubc13. The Y2H conditions are as described in Figure 1. (**B**) Functional complementation of the *rad5*Δ mutant by *rad5-RFF* mutant alleles by a gradient plate assay.

**Figure S5**. The *rad5-F13A* mutation does not affect expression and its product interaction with

Pol30. (**A**) The *rad5-F13A* mutation does not affect its product interaction with Pol30. The Y2H experimental conditions are as described in Figure 1. (**B**) The *rad5-F13A* mutation does not affect its protein level. Lane 1, molecular weight markers; Lane 2, the pGAD424 vector teransformant; Lane 3, the pGAD-Rad5 transformant; and Lane 4, the pGAD-Rad5-F13A transformant. The host strain is PJ69-4a.

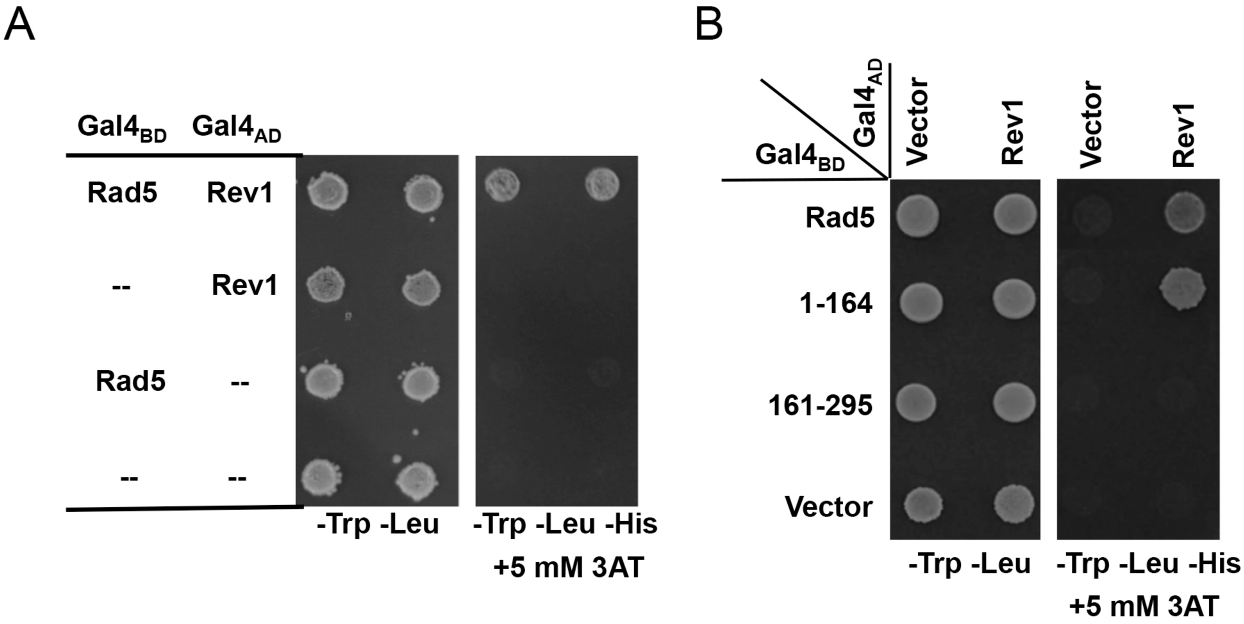
A
Gal4BD
Gal4AD
Rad5
Rev1
--
Rev1
Rad5
--
--
--
-Trp -Leu
-Trp -Leu -His
+5 mM 3AT
B
Gal4AD
Gal4BD
Vector
Rev1
Vector
Rev1
Rad5
1-164
161-295
Vector
-Trp -Leu
-Trp -Leu -His
+5 mM 3AT

Figure S1

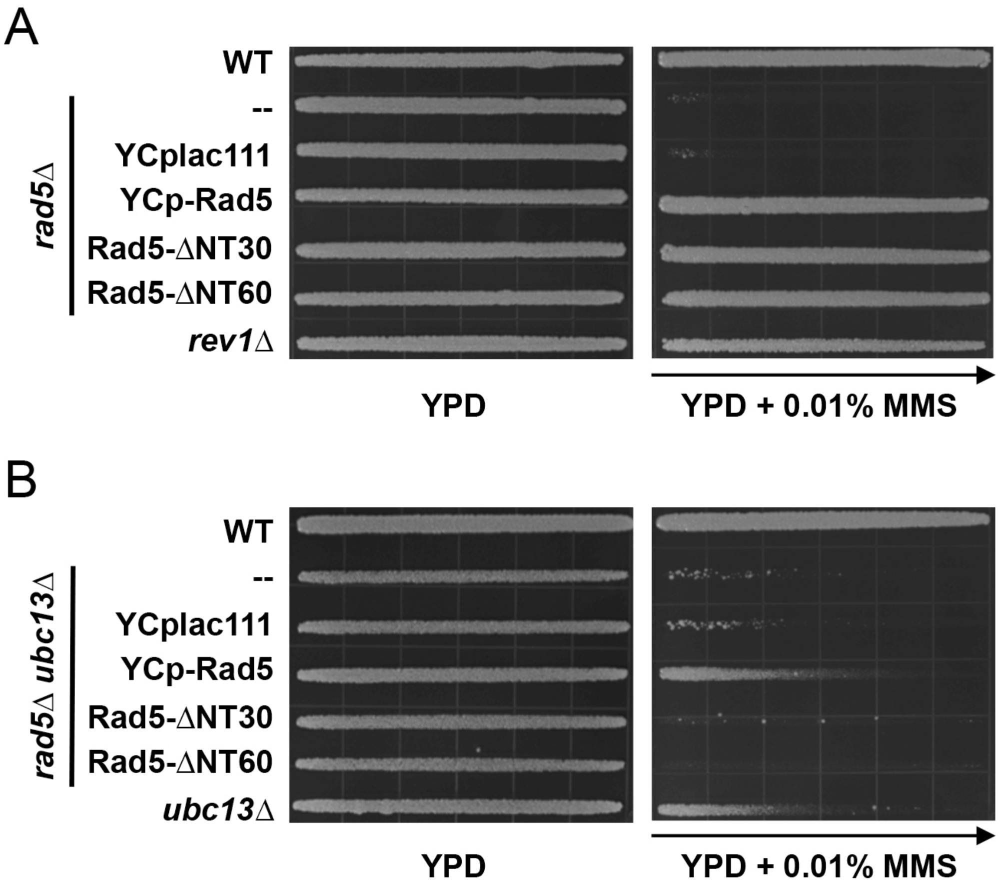
A
WT
--
YCplac111
YCp-Rad5
Rad5-ΔNT30
Rad5-ΔNT60
*rev1*Δ
*rad5*Δ
YPD
YPD + 0.01% MMS
B
WT
--
YCplac111
YCp-Rad5
Rad5-ΔNT30
Rad5-ΔNT60
*ubc13*Δ
*rad5*Δ *ubc13*Δ
YPD
YPD + 0.01% MMS

Figure S2

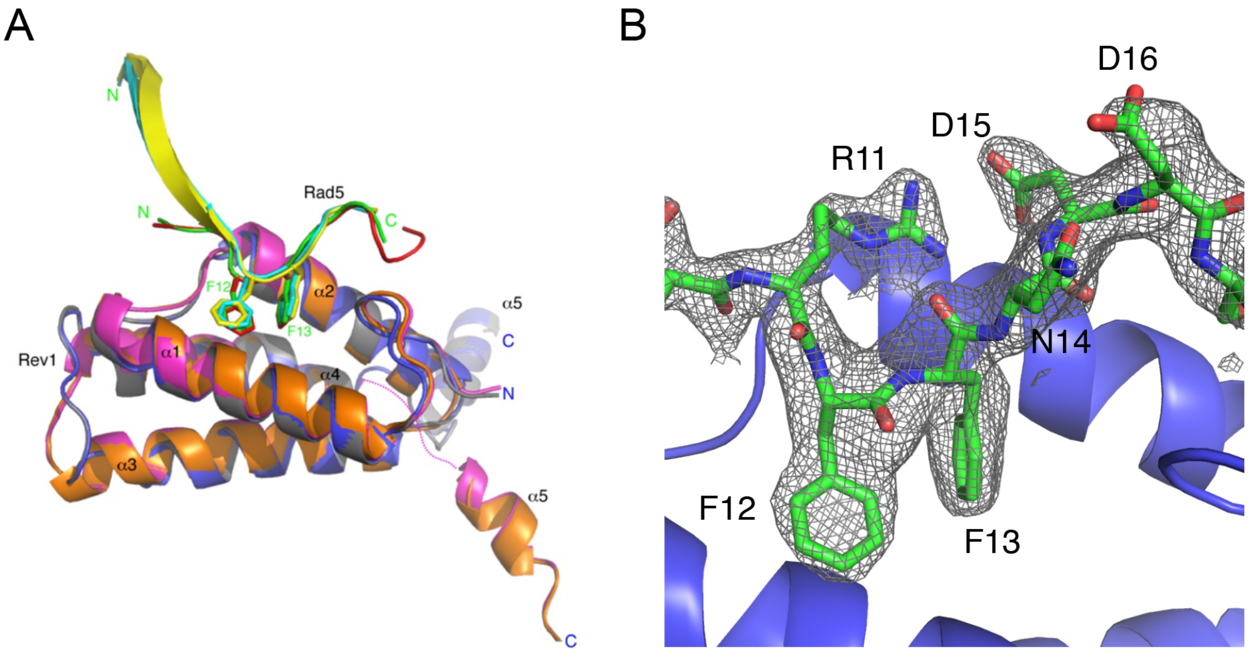
A
N
N
Rad5
C
F12
F13
α2
α5
C
Rev1
α1
α4
N
α3
α5
C
B
D16
D15
R11
N14
F12
F13

Figure S3

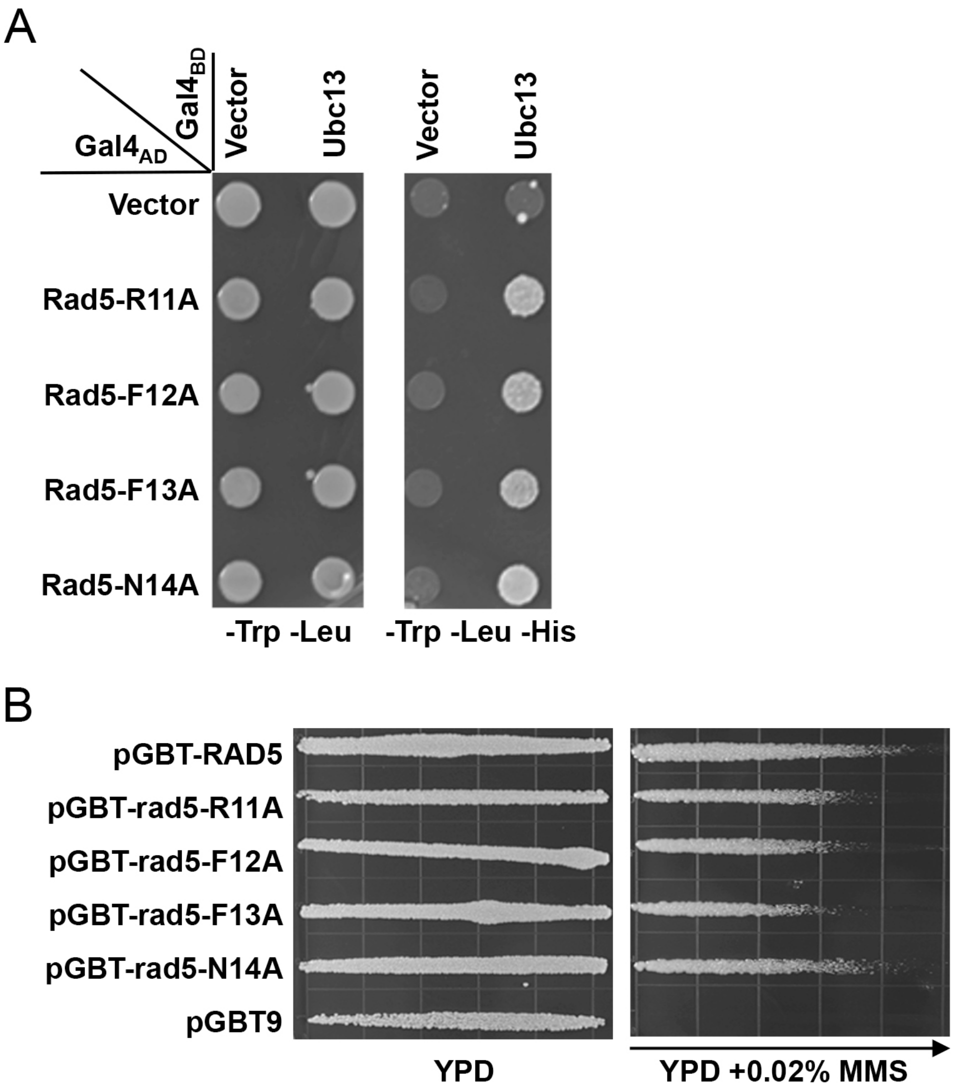
A
Gal4$_{BD}$
Gal4$_{AD}$
Vector
Ubc13
Vector
Ubc13
Vector
Rad5-R11A
Rad5-F12A
Rad5-F13A
Rad5-N14A
-Trp -Leu
-Trp -Leu -His
B
pGBT-RAD5
pGBT-rad5-R11A
pGBT-rad5-F12A
pGBT-rad5-F13A
pGBT-rad5-N14A
pGBT9
YPD
YPD +0.02% MMS

Figure S4

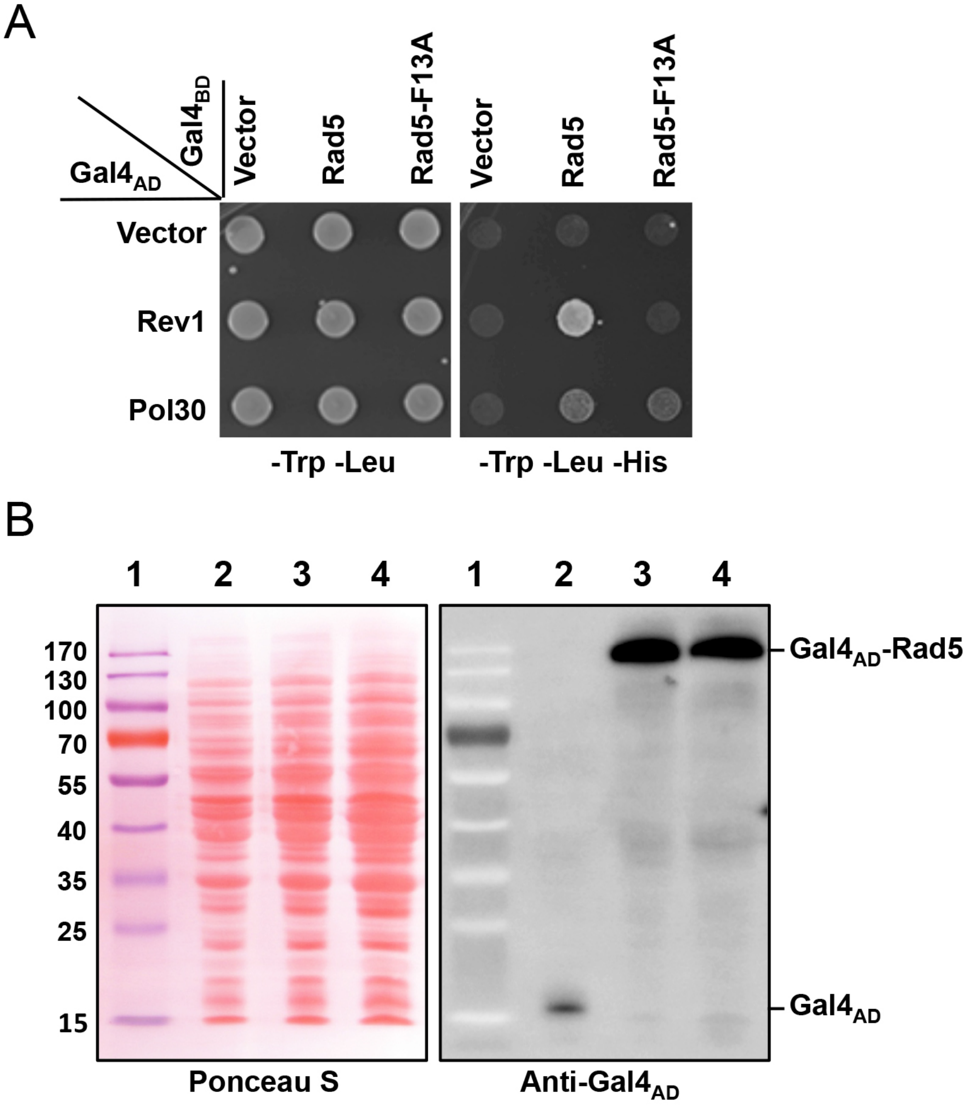
A
Gal4BD
Gal4AD
Vector
Rad5
Rad5-F13A
Vector
Rad5
Rad5-F13A
Vector
Rev1
Pol30
-Trp -Leu
-Trp -Leu -His
B
1
2
3
4
1
2
3
4
170
130
100
70
55
40
35
25
15
Gal4AD-Rad5
Gal4AD
Ponceau S
Anti-Gal4AD

Figure S5