

Figure S1

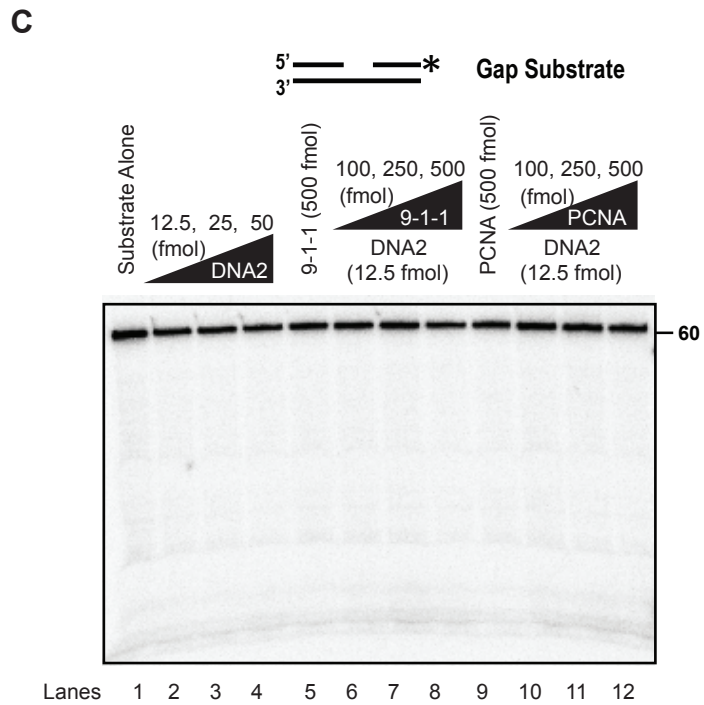
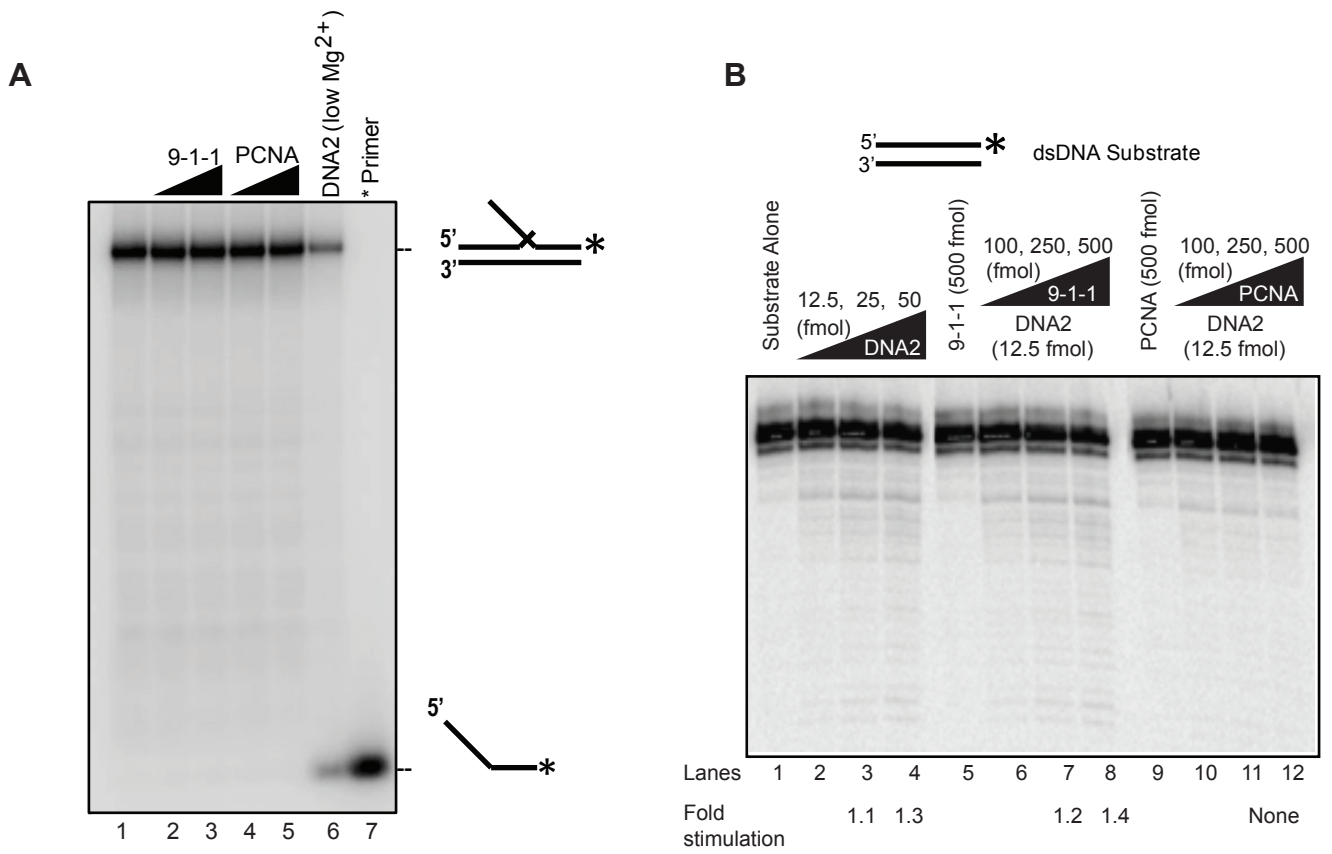
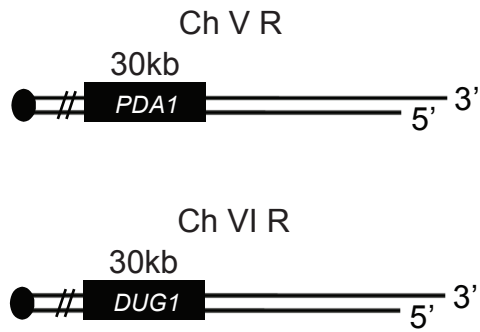
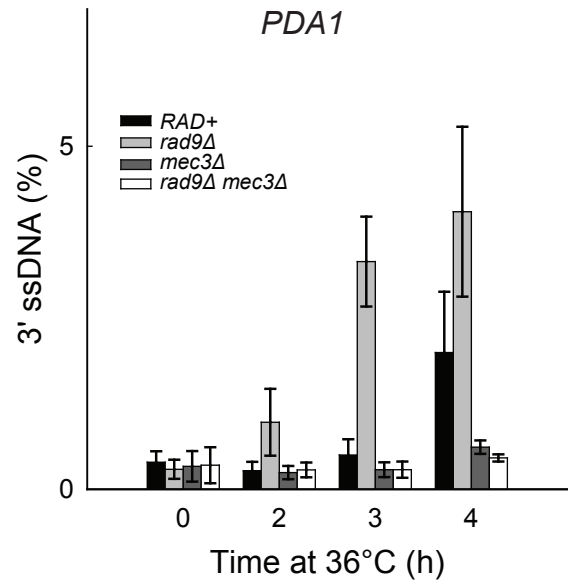


Figure S2

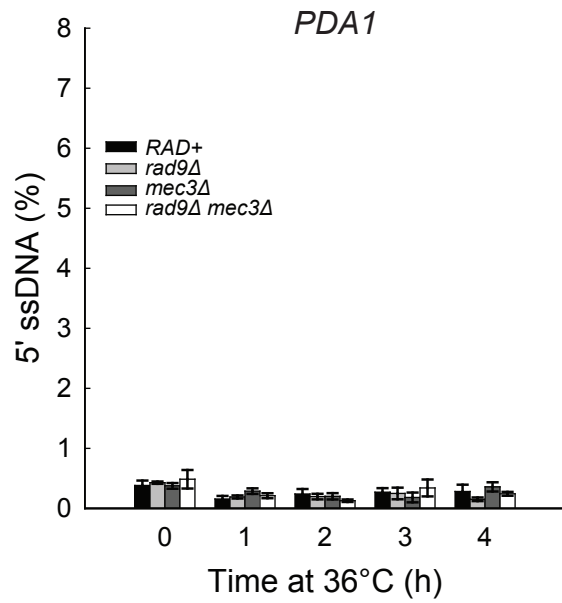
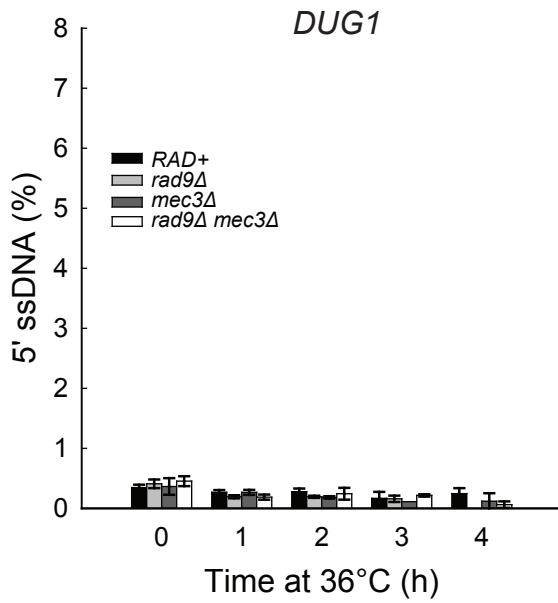
A



B



C



D

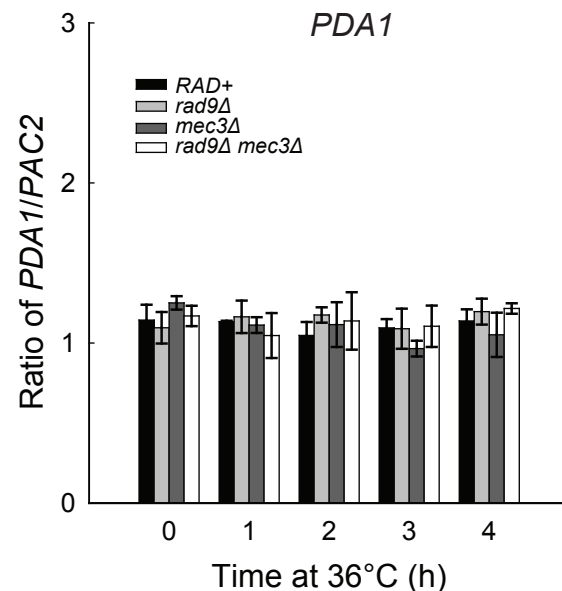
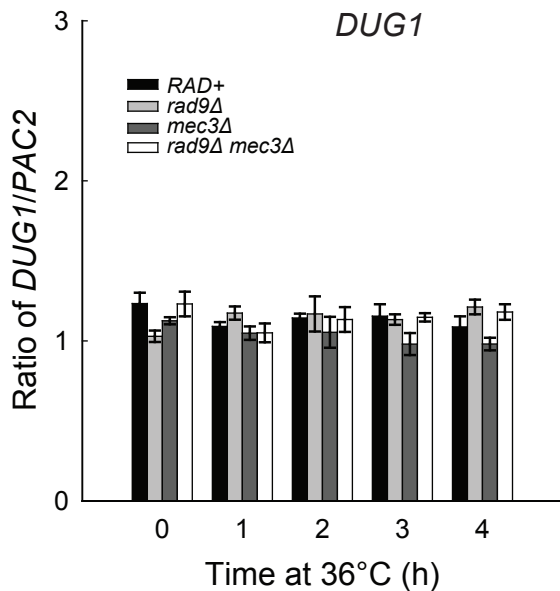
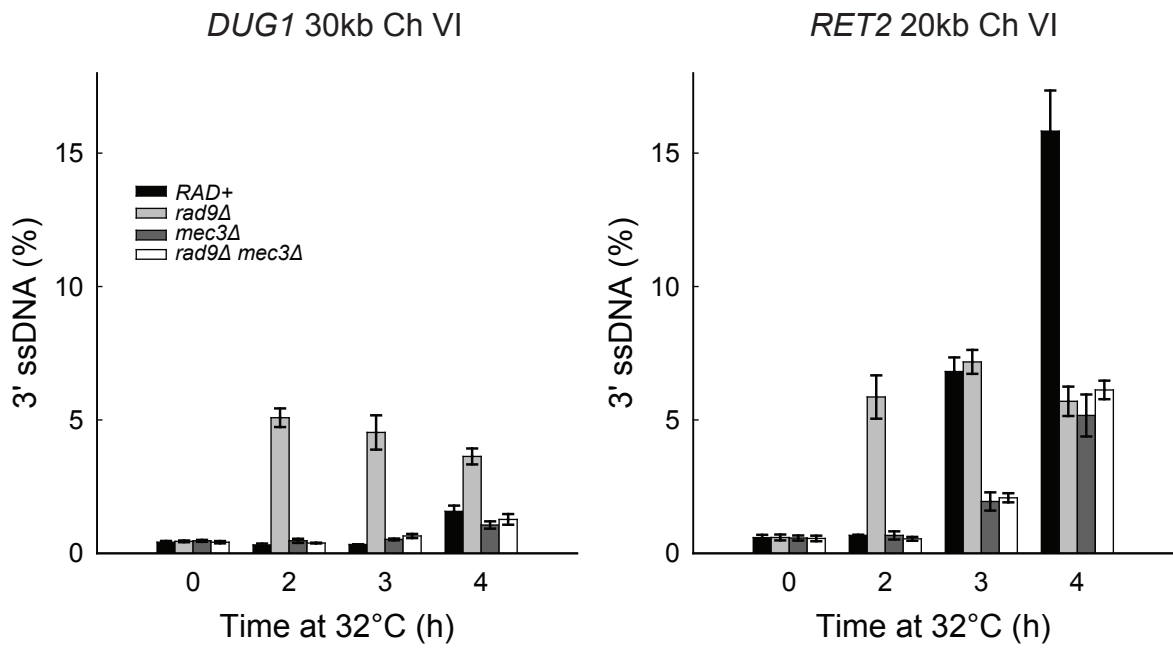


Figure S3

A



B

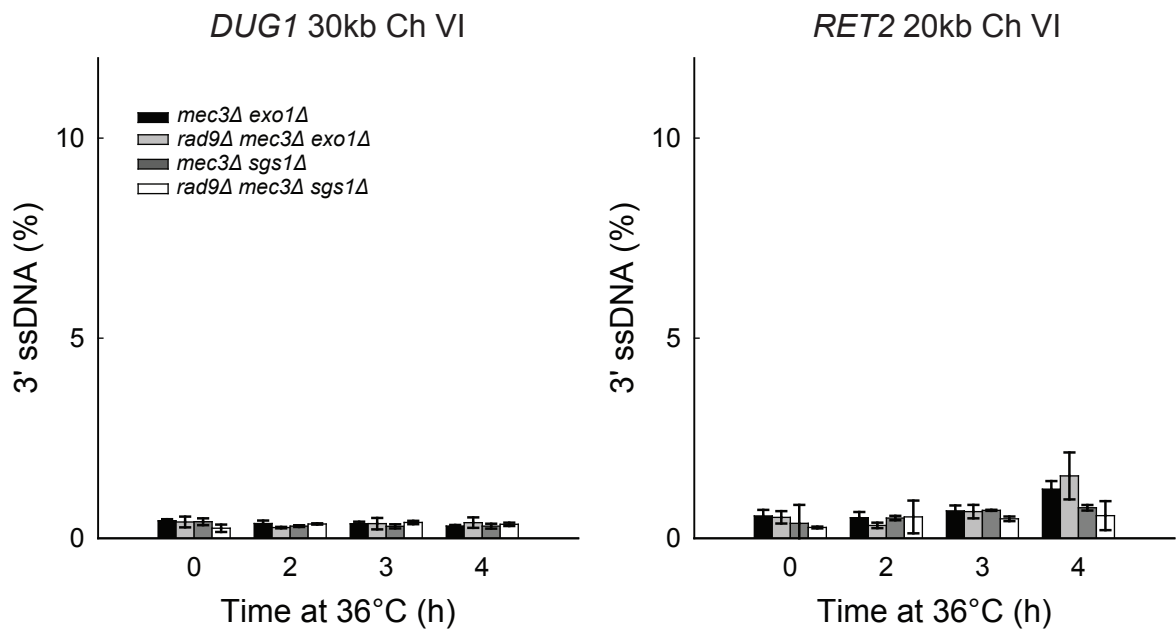


Figure S4

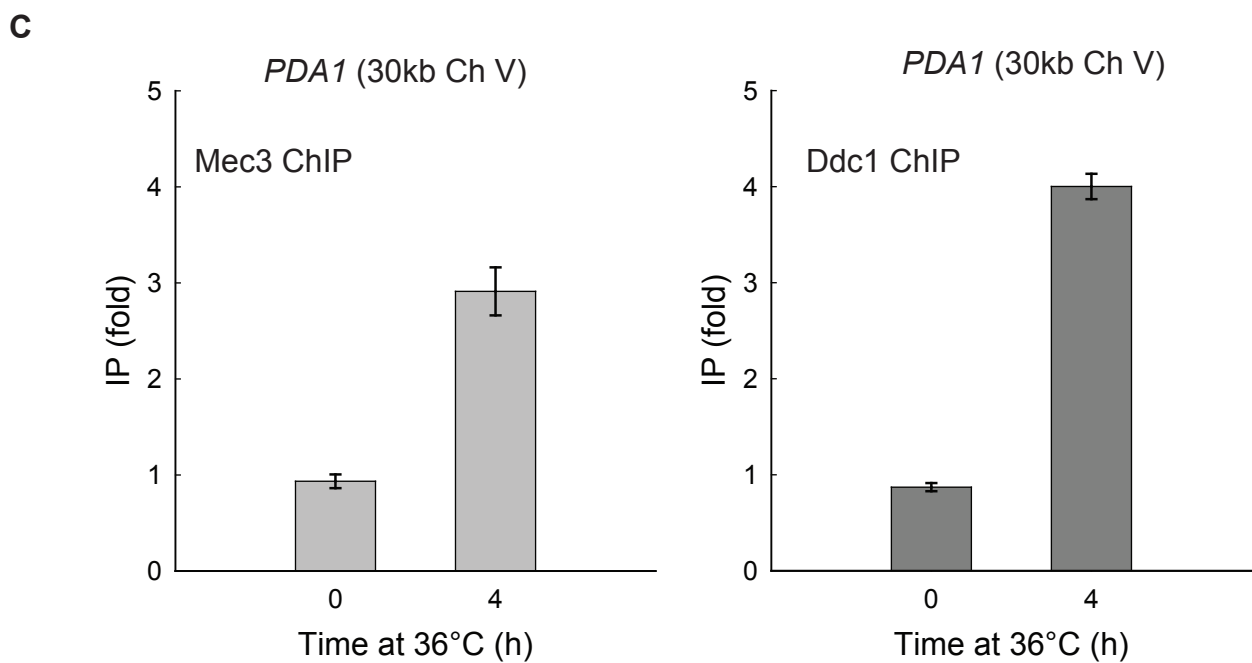
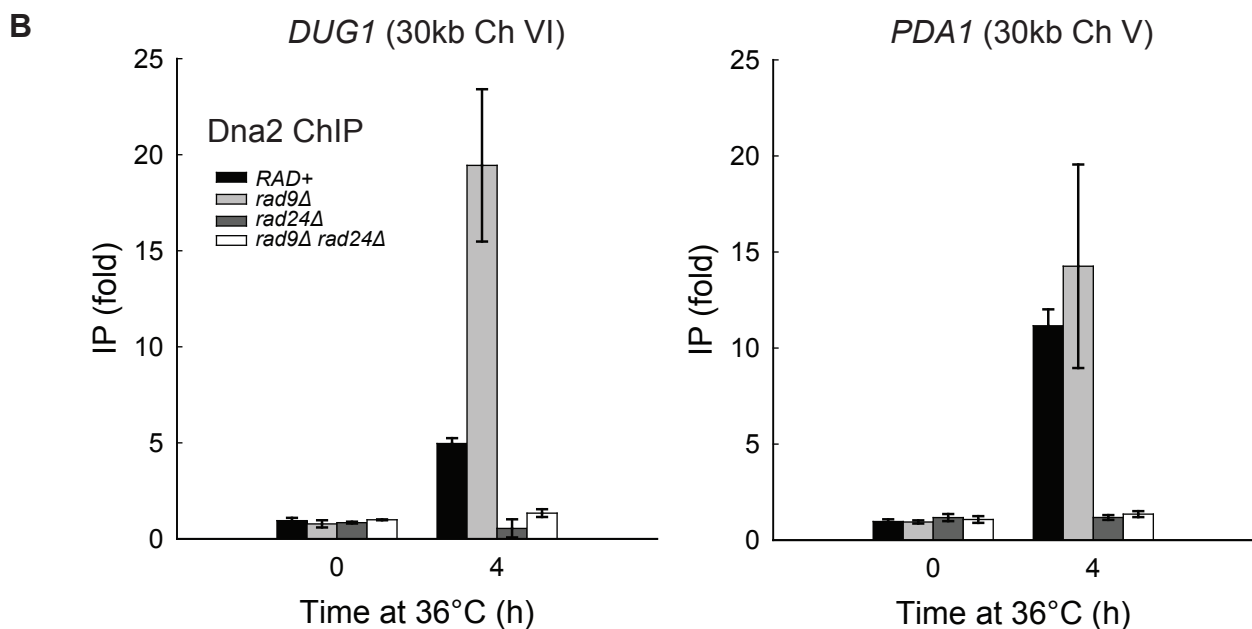
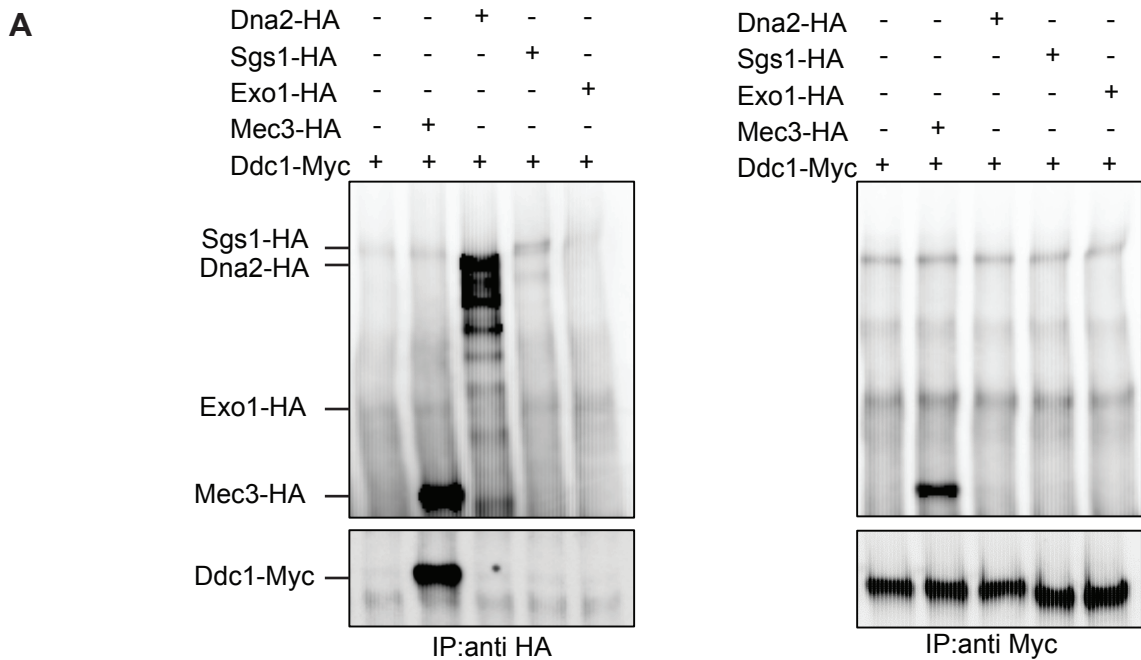


Figure S5

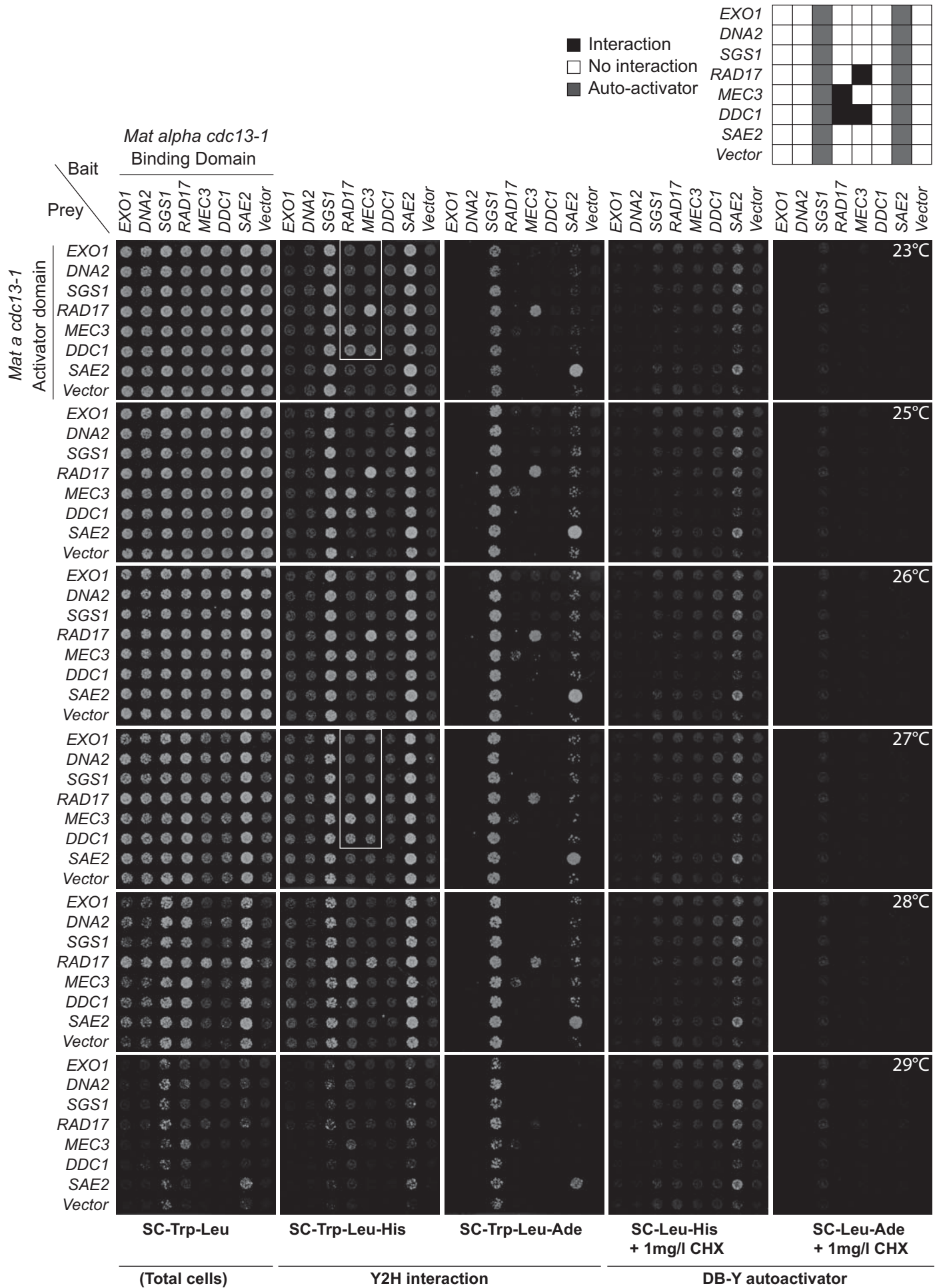


Table S1

Strains	Genotype	Source
DLY7830	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1</i>	This work, Fig.3
DLY7834	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 rad9Δ::HIS3</i>	This work, Fig.3
DLY7960	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 mec3Δ::TRP1</i>	This work, Fig.3
DLY7964	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 rad9Δ::HIS3 mec3Δ::TRP1</i>	This work, Fig.3
DLY7115	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 dna2-1</i>	This work, Fig.5
DLY7813	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 rad9Δ::HIS3 dna2-1</i>	This work, Fig.5
DLY7967	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 mec3Δ::TRP1 dna2-1</i>	This work, Fig.5
DLY7973	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 rad9Δ::HIS3 mec3Δ::TRP1 dna2-1</i>	This work, Fig.5
DLY7837	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 rad9::HIS3 exo1Δ::LEU2</i>	This work, Fig.5
DLY8080	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 rad9::HIS3 exo1Δ::LEU2 mec3Δ::TRP1</i>	This work, Fig.5
DLY5267	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 rad9::HIS3 sgs1Δ::KanMX</i>	This work, Fig.5
DLY8547	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 rad9::HIS3 mec3::TRP1 sgs1Δ::KanMX</i>	This work, Fig.5
DLY5273	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 rad9::HIS3 sgs1Δ::KanMX exo1Δ::LEU2</i>	This work, Fig.5
DLY8615	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 rad9::HIS3 sgs1Δ::KanMX exo1Δ::LEU2 mec3::TRP1</i>	This work, Fig.5
DLY8019	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Dna2-Myc-KanMX</i>	This work, Fig.6
DLY8022	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Dna2-Myc-KanMX rad9Δ::LEU2</i>	This work, Fig.6
DLY8024	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Dna2-Myc-KanMX mec3Δ::TRP1</i>	This work, Fig.6
DLY8027	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Dna2-Myc-KanMX rad9Δ::LEU2 mec3Δ::TRP1</i>	This work, Fig.6
DLY8587	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Sgs1-Myc-HphNT1</i>	This work, Fig.6
DLY8589	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Sgs1-Myc-HphNT1 rad9Δ::LEU2</i>	This work, Fig.6
DLY8591	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Sgs1-Myc-HphNT1 mec3Δ::TRP1</i>	This work, Fig.6
DLY8593	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Sgs1-Myc-HphNT1 rad9Δ::LEU2 mec3Δ::TRP1</i>	This work, Fig.6
DLY8033	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Exo1-Myc-HIS3</i>	This work, Fig.6
DLY8036	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Exo1-Myc-HIS3 rad9Δ::LEU2</i>	This work, Fig.6
DLY8039	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Exo1-Myc- HIS3 mec3Δ::TRP1</i>	This work, Fig.6
DLY8042	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Exo1-Myc-HIS3 rad9Δ::LEU2 mec3Δ::TRP1</i>	This work, Fig.6
DLY8045	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Dna2-Myc-KanMX rad24Δ::TRP1</i>	This work, Fig.S4
DLY8048	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Dna2-Myc-KanMX rad9::HIS3 rad24::TRP1</i>	This work, Fig.S4

Table S2

Strains	Genotype	Source
DLY7833	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 exo1Δ::LEU2</i>	This work, Fig.4
DLY8478	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 exo1Δ::LEU2 mec3Δ::TRP1</i>	This work, Fig.4
DLY5123	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 sgs1Δ::KanMX</i>	This work, Fig.4
DLY8544	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 sgs1Δ::KanMX mec3Δ::TRP1</i>	This work, Fig.4
DLY5269	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 sgs1Δ::KanMX exo1Δ::LEU2</i>	This work, Fig.4
DLY8548	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 sgs1Δ::KanMX exo1Δ::LEU2 mec3::TRP1</i>	This work, Fig.4
DLY7870	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Ddc1-Myc-HIS3MX6</i>	This work, Fig.4
DLY8971	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Mec3-3HA-KanMX</i>	This work, Fig.6
DLY8989	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Mec3-3HA-KanMX Ddc1-Myc-HIS3MX6</i>	This work, Fig.6
DLY7929	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Ddc1-Myc-his3MX6 Dna2-3HA-KanMX</i>	This work, Fig.6
DLY8983	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Ddc1-Myc-HIS3MX6 Sgs1-3HA-KanMX</i>	This work, Fig.6
DLY8985	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Ddc1-Myc-HIS3MX6 Exo1-3HA-KanMX</i>	This work, Fig.6
DLY8963	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Sgs1-3HA-KanMX</i>	This work, Fig.6
DLY8965	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Dna2-3HA-KanMX</i>	This work, Fig.6
DLY8967	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Exo1-3HA-KanMX</i>	This work, Fig.6
Y8800	<i>Mata leu2-3,112 trp1-901 his3-200 ura3-52 gal4Δ gal80Δ GAL2-ADE2 LYS2::GAL1-HIS3 MET2::GAL7-LacZ cyh2R</i>	Dreze et.al, Fig.6
Y8930	<i>Matalpha leu2-3,112 trp1-901 his3-200 ura3-52 gal4Δ gal80Δ GAL2-ADE2 LYS2::GAL1-HIS3 MET2::GAL7-LacZ cyh2R</i>	Dreze et.al, Fig.6
DLY7451	<i>Mata leu2-3,112 trp1-901 his3-200 ura3-52 gal4Δ gal80Δ GAL2-ADE2 LYS2::GAL1-HIS3 MET2::GAL7-LacZ cyh2R cdc13-1</i>	This work, Fig.6
DLY7452	<i>Matalpha leu2-3,112 trp1-901 his3-200 ura3-52 gal4Δ gal80Δ GAL2-ADE2 LYS2::GAL1-HIS3 MET2::GAL7-LacZ cyh2R cdc13-1</i>	This work, Fig.6
DLY7909	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Mec3-Myc-HIS3MX6</i>	This work, Fig.S4
DLY7925	<i>MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5 cdc13-1 cdc15-2 bar1 Ddc1-Myc-HIS3MX6</i>	This work, Fig.S4

Table S3

Primer	Length (nts)	Sequence
<u>Upstream</u>		
		<u>Listed 5' to 3'</u>
U1	26	CGCCAGGGTTTTCCCAGTCACGACCA
<u>Downstream</u>		
		<u>Listed 5' to 3'</u>
D1	53	TTCACGCCTGTTAGTTAATTCACTGGCCGTCGTTTTACAACGACGTGACTGG G
D2	55	CGACCGTGCCAGCCTAAAACACTTGCCCGTGCCACCATCCCGACGCCACCT CCTG
D3	60	AGACGAATTCGGATACGACGGCAGTGCCGACCGTCCAGCCTAAATTCAATC CACCC
<u>Template</u>		
		<u>Listed 3' to 5'</u>
T1	49	GCGGTCCCAAAGGGTCAGTGCTGGGCAAATGTTGCTGCACTGACCCG
T2	56	GCTGGCACGGTCGGATTTGTGAACGGGCACGGTGGTAGGGCTGCGGTGG AGGACG
T3	110	CAGGTGGGCGCGGTGGAGGACGAAGTTACACGACCTAGGATGTTGTTCTG CTTAAGCCTATGCTCCGGTCACGGCTGCACGGTCGGATTAAGTTAGGTGG

Table S4

Primer	Sequence 5'-3'	Description
M2588	aagataccccaccaaaccacaaaaaaagaggggtgggGGTATCCAAGGTCTTCTTCC	<i>AD-EXO1</i>
M2589	aaggtaaacagacagttgactgtatcgtcgaggtcg GGTATCCAAGGTCTTCTTCC	<i>DB-EXO1</i>
M2590	cttacttagagctcgacgtcttacttacttagcggccgc TTTACCTTTATAAACAAATTGGG	R- <i>EXO1</i>
M3100	aagataccccaccaaaccacaaaaaaagaggggtggg CCCGGAACGCCACAGAAG	<i>AD-DNA2</i>
M3099	aaggtaaacagacagttgactgtatcgtcgaggtcgCCCGGAACGCCACAGAAG	<i>DB-DNA2</i>
M3101	cttacttagagctcgacgtcttacttacttagcggccgcACTTTCATACTCTTGTAGAA	R-DNA2
M3104	aagataccccaccaaaccacaaaaaaagaggggtgggGTGACGAAGCCGTCACATAACTTA	<i>AD-SGS1</i>
M3103	aaggtaaacagacagttgactgtatcgtcgaggtcgGTGACGAAGCCGTCACATAACTTA	<i>DB-SGS1</i>
M2626	TTAAAACCTTTGTTTTAGGAAAACAG	<i>DB-Rev-SGS1</i>
M2625	CCTTGGTCTGATGAAGTTTTATAT	<i>DB-Fwd-SGS1</i>
M2596	cttacttagagctcgacgtcttacttacttagcggccgcTTAAAACCTTTGTTTTAGGAAAACAG	R-SGS1
M2600	aagataccccaccaaaccacaaaaaaagaggggtgggTCATTTAAGGCAACTATCACC	<i>AD-DDC1</i>
M2602	cttacttagagctcgacgtcttacttacttagcggccgcGTCAAATATACCCCTTGGC	R-DDC1
M2603	aagataccccaccaaaccacaaaaaaagaggggtgggCGAATCAACAGTGAGCTAG	<i>AD-RAD17</i>
M2604	aaggtaaacagacagttgactgtatcgtcgaggtcgCGAATCAACAGTGAGCTAG	<i>DB-RAD17</i>
M2605	cttacttagagctcgacgtcttacttacttagcggccgcAAAAATATAGGAATATCCTTTGTTG	R-RAD17
M2606	aagataccccaccaaaccacaaaaaaagaggggtgggAAATTTAAATGATAGTAAATGGTTGT	<i>AD-MEC3</i>
M2607	aaggtaaacagacagttgactgtatcgtcgaggtcgAAATTTAAATGATAGTAAATGGTTGT	<i>DB-MEC3</i>
M2608	cttacttagagctcgacgtcttacttacttagcggccgcCAAGCCCTTCGATCTT	R- <i>MEC3</i>
M3106	aagataccccaccaaaccacaaaaaaagaggggtgggGTGACTGGTGAAGAAAATGTGT	<i>AD-SAE2</i>
M3105	aaggtaaacagacagttgactgtatcgtcgaggtcgGTGACTGGTGAAGAAAATGTGT	<i>DB-SAE2</i>
M3107	C cttacttagagctcgacgtcttacttacttagcggccgcACATCTAGCATATATCTGCAATAA	R-SAE2

Forward primers generate the fusion of the Gal4 activation domain (*AD-X*) or the Gal4 DNA binding domain (*DB-Y*) with the protein of interest. Each reverse primer (R) can be used with either the *AD-X* or the *DB-Y* primers. *DB-SGS1* was cloned using two PCR fragments: M3103-M2626 and M2625-M2596.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1 (A) Helicase assay testing purity of the 9-1-1 and PCNA protein preparations. **(B,C)** DNA2 cleavage activities on a dsDNA substrate and a gap substrate in the presence of the 9-1-1 complex or PCNA. Substrates used are depicted on the top of the gel with the asterisk indicating the site of the ^{32}P label.

Figure S2 (A) The loci studies are shown on the map of chromosome V and VI. **(B)** Analyses of 3'ssDNA accumulation following telomere uncapping in *cdc13-1 cdc15-2 bar1* strains. **(C)** Analyses of 5'ssDNA accumulation following telomere uncapping in *cdc13-1 cdc15-2 bar1* strains. **(D)** Analyses of DNA amount by qPCR at the indicated loci in *cdc13-1 cdc15-2 bar1* background strains. *PAC2* is a control locus located 400kb away from the right arm of chromosome V. The data and error bars plotted in B are means and standard deviations from two independent experiments, and the data and error bars in C,D are the group means and standard deviations from individual samples measured in triplicate.

Figure S3 (A) Analyses of 3'ssDNA accumulation in *cdc13-1 cdc15-2 bar1* background strains at 32°C. The data and error bars are the means and standard deviations from individual samples measured in triplicate. **(B)** Comparison of relevant genotypes from Figure 4 and 5.

Figure S4 (A) Co-immunoprecipitation experiment to detect interaction between 9-1-1 and Dna2/Sgs1/Exo1 following *cdc13-1* induced telomere uncapping at 36°C. Protein extract from cells expressing (+) or not expressing (-) the indicated epitope tagged proteins were subjected to immunoprecipitation with anti-HA (left panel) or anti-Myc (right panel) antibodies, before probing with anti-HA (top panels) or anti-Myc (bottom panels) antibodies. **(B)** ChIP analyses of Dna2-Myc binding to *DUG1* and *PDA1* following telomere uncapping. **(C)** ChIP analyses of Mec3-Myc and Ddc1-Myc binding to *PDA1* following telomere uncapping in *cdc13-1 cdc15-2 bar1* strains. The data and error bars plotted in B and C are means and standard deviations from individual samples measured in triplicate.

Figure S5 The yeast two-hybrid system used *cdc13-1* reporter strains to test for protein interactions between Rad17, Mec3, Ddc1, Dna2, Sgs1 and Exo1. The genes of interest were fused to the Gal4 activator domain (left) or the Gal4 DNA binding domain (top) to observe interaction. Interactions were analysed using *HIS3*, *ADE2* and cyclo-hexamide (CHX) reporter genes at the temperatures indicated. Boxes highlight panels presented in Fig. 6B.

Table S1 and S2 Yeast strains used in the study.

Table S3 Oligonucleotides used for making substrates for nuclease assays.

Table S4 Oligonucleotides used for making substrates for two-hybrid experiments.

SUPPLEMENTARY METHOD

Helicase Assays: Five fmol of experimental substrate was incubated with 250, 500 fmol of either 9-1-1 complex or PCNA, in a reaction volume of 20 μ l, on ice for 25 min. The reaction buffer contained 50 mM Tris-HCl (pH 7.5), 25 mM NaCl, 2 mM dithiothreitol, 0.25 mg/ml bovine serum albumin, 4 mM $MgCl_2$, and 8 mM ATP. Lane 6 containing 100 fmol of DNA2 alone contained 2mM $MgCl_2$ in the reaction buffer to minimize the nuclease activity of the protein. The reactions were terminated using 6X helicase dye (50 mM EDTA, 0.9% SDS, 0.125% bromphenol blue, 0.125% xylene cyanole, 30% glycerol). After termination, samples were loaded on a pre-run 5% native polyacrylamide gel and resolved by electrophoresis for 1.5 h at 150 V. Helicase products are indicated on the gel.