Figure S1



С







Α

В



Figure S4



EXO1

Strains	Genotype	Source
	MAT a ade2-1 trp1-1 can1-100 leu2-3 112 his3-11 15 ura3 GAI + psi+ ssd1-d2 RAD5	
DLY7830	cdc13-1 cdc15-2 har1	This work, Fig.3
	MAT a ade $2.1$ trn1-1 can1-100 leu $2.3$ 112 bis $3.11$ 15 ura $3$ GAL + nsi+ scd1-d2 RAD5	
DLY7834	ade12 1 ade15 2 bert red0	This work, Fig.3
	MAT = ada2.1 trat.1 cont.100 low 2.2.112 bio2.11.15 ura2.CAL + asi + cont. d2 DADE	
DLY7960	MAT a ade2-1 (1)1-1 car11-100 leu2-3, 112 11183-11, 15 uras GAL+ psi+ sso1-o2 RADS	This work, Fig.3
DLY7964	MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5	This work. Fig.3
	cdc13-1 cdc15-2 bar1 rad9∆:::HIS3 mec3∆:::TRP1	
DLY7115	MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5	This work, Fig.5
	cdc13-1 cdc15-2 bar1 dna2-1	, <b>g</b>
DLY7813	MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5	This work Fig 5
	cdc13-1 cdc15-2 bar1 rad9∆:::HIS3 dna2-1	This work, Fig.o
DI Y7967	MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5	This work Fig 5
BEITOOT	cdc13-1 cdc15-2 bar1 mec3∆:::TRP1 dna2-1	This work, Fig.o
	MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5	This work Fig F
DLY/9/3	cdc13-1 cdc15-2 bar1 rad9∆:HIS3 mec3∆:TRP1 dna2-1	This work, Fig.5
	MAT a ade2-1 trn1-1 can1-100 leu2-3 112 bis 3-11 15 $\mu$ ra3 GAI + nsi+ ssd1-d2 RAD5	
DLY7837	$dc13_1$ $dc2_2$ $hp17$ $cdn17$ $roto rc2_2$ $c,r12$ $rc2$	This work, Fig.5
	$M\Delta T$ a de 2.1 trn 1.1 can 1.100 lev 2.3 112 his 3.11 15 ura 3 GAL + nsi + csd 1.d2 RAD5	This work Fig F
DLY8080	ddd2 = 1 (p) $ddd2 = 1$ (p) $ddd1 = 100$ (dd $2 = 0, 112$ (list = 1, 10 (list = 0, 112) (dd 2 (list = 0, 112)) (	This work, Fig.5
	$MAT = ade_{2.1} trn_{1.1} can_{1.100} lev_{2.3} 112 bis_{2.11} 15 ura_{2.6} l + nsi + scd_{2.6} RAD_{5}$	
DLY5267	do12.1 do15.2 bor1 rod0::HIS2 cos14:::KonMV	This work, Fig.5
	COUTS-T COUTS-Z Datit Taug. HISS Sys TA Katilitik	
DLY8547	MAT a adez-1 trp1-1 can1-100 leu2-3,112 nis3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5	This work, Fig.5
	cdc13-1 cdc15-2 bar1 rad9::HIS3 mec3::TRP1 sgs1∆:::KanMX	
DLY5273	MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5	This work, Fig.5
	cdc13-1 cdc15-2 bar1 rad9::HIS3 sgs1∆:::KanMX exo1∆:::LEU2	-
DI Y8615	MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5	This work Fig 5
DETOOTO	cdc13-1 cdc15-2 bar1 rad9::HIS3 sgs1∆:::KanMX exo1∆:::LEU2 mec3::TRP1	THIS WORK, TIG.5
	MAT a ade 2-1 trn1-1 can1-100 leu 2-3 112 his 3-11 15 ura3 GAI + nsi+ ssd1-d2 RAD5	
DLY8019	cdc13-1 cdc15-2 bar1 Dna2-Myc-KanMY	This work, Fig.6
DLY8022	MAT a ade2-1 trp1-1 can1-100 leu2-3,112 nls3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5	This work Fig 6
	cdc13-1 cdc15-2 bar1 Dna2-Myc-KanMX rad9∆:::LEU2	The Work, Tig.o
	MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5	This work Fig 6
DL10024	cdc13-1 cdc15-2 bar1 Dna2-Myc-KanMX mec3∆:::TRP1	11113 WOIK, 1 Ig.0
	MAT a ade2-1 trp1-1 can1-100 leu2-3.112 his3-11.15 ura3 GAL+ psi+ ssd1-d2 RAD5	This work Fig 6
DLY8027	cdc13-1 cdc15-2 bar1 Dna2-Mvc-KanMX rad9A:::LEU2 mec3A:::TRP1	THIS WORK, TIG.0
	MAT a ade2-1 trp1-1 can1-100 leu2-3 112 his3-11 15 ura3 GAI + psi+ ssd1-d2 RAD5	This work Fig.6
DLY8587	cdc13-1 cdc15-2 har1 Sas1-Mvc-HnhNT1	This work, Fig.o
	MAT a side 2-1 trol 1 cap 1-100 leu 2-3 112 bis 3-11 15 ura 3 GAL + psi+ scd1-d2 PAD5	
DLY8589	$ddc13.1$ $ddc2=1$ $dp1=1$ $cdc15.2$ $bar1.$ San $Myc$ HabNIT1 $rad0A \cdots$ EU2	This work, Fig.6
	$M\Delta T$ a de 2.1 tro 1.1 can 1.100 leu 2.3 112 his 3.11 15 ura 3 GAL + nsi + scd 1.d2 RAD5	
DLY8591	cdc13-1 $cdc15-2$ har1 Sas1-Myc-HnhNT1 mec3 $h$ ···TRP1	This work, Fig.6
	MAT = ado2 1 trat 1 can1 100 lou2 2 112 bio2 11 15 ura2 CAL + aci + ccd1 d2 BADE	
DLY8593	ade12 1 ade15 2 here Sect Mue HabNT1 red04 wit EU2 mee24 wTDD1	This work, Fig.6
DLY8033	MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5	This work, Fig.6
	cdc13-1 cdc15-2 bar1 Exo1-Myc-HIS3	
DLY8036	MAT a ade2-1 trp1-1 can1-100 leu2-3.112 his3-11.15 ura3 GAI + psi+ ssd1-d2 RAD5	This work, Fig.6
DETODOO	$cdc13-1$ $cdc15-2$ har1 Exo1-Myc-HIS3 rad9 $\Lambda$ ····I EI 12	, <b>G</b>
DLY8039	MAI a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5	This work, Fig.6
	cdc13-1 cdc15-2 bar1 Exo1-Myc- HIS3 mec3∆:::TRP1	
DLY8042	MAT a ade 2-1 trn1-1 can1-100 leu 2-3 112 his 3-11 15 ura 3 GAL + nsi+ scd1-d2 DAD5	This work Fig 6
	cdc13.1 cdc15.2 har1 Evo1.Muc.HIS2 rad0IEII2 mac2TDD1	THIS WORK, FIG.0
DLY8045	MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5	This work. Fig.S4
	cdc13-1 cdc15-2 bar1 Dna2-Myc-KanMX rad24∆.::TRP1	
	MAT a ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL+ psi+ ssd1-d2 RAD5	This work. Fia.S4
	cdc13-1 cdc15-2 bar1 Dna2-Mvc-KanMX rad9::HIS3 rad24::TRP1	

Strains	Genotype	Source
DLY7833	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∆:::LEU2	This work, Fig.4
DLY8478	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∆:::LEU2 mec3∆:::TRP1	This work, Fig.4
DLY5123	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	This work, Fig.4
DLY8544	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	This work, Fig.4
DLY5269	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 sqs1	This work, Fig.4
DLY8548	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 sgs14:::KanMX exo14:::LEU2 mec3::TRP1	This work, Fig.4
DLY7870	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	This work, Fig.4
DLY8971	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	This work, Fig.6
DLY8989	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	This work, Fig.6
DLY7929	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	This work, Fig.6
DLY8983	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	This work, Fig.6
DLY8985	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	This work, Fig.6
DLY8963	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	This work, Fig.6
DLY8965	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	This work, Fig.6
DLY8967	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	This work, Fig.6
Y8800	Mata leu2-3,112 trp1-901 his3-200 ura3-52 gal4∆ gal80∆ GAL2-ADE2 LYS2::GAL1-HIS3 MET2::GAL7-LacZ cyh2R	Dreze et.al, Fig.6
Y8930	Matalpha leu2-3,112 trp1-901 his3-200 ura3-52 gal4∆ gal80∆ GAL2-ADE2 LYS2::GAL1-HIS3 MET2::GAL7-LacZ cyh2R	Dreze et.al, Fig.6
DLY7451	Mata leu2-3,112 trp1-901 his3-200 ura3-52 gal4Δ gal80Δ GAL2-ADE2 LYS2::GAL1-HIS3 MET2::GAL7-LacZ cyh2R cdc13-1	This work, Fig.6
DLY7452	Matalpha leu2-3,112 trp1-901 his3-200 ura3-52 gal4Δ gal80Δ GAL2-ADE2 LYS2::GAL1-HIS3 MET2::GAL7-LacZ cyh2R cdc13-1	This work, Fig.6
DLY7909	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	This work, Fig.S4
DLY7925	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	This work, Fig.S4

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Primer	Length	Sequence
	(nts)	
<u>Upstream</u>		Listed 5' to 3'
U1	26	CGCCAGGGTTTTCCCAGTCACGACCA
<u>Downstream</u>		Listed 5' to 3'
D1	53	TTCACGCCTGTTAGTTAATTCACTGGCCGTCGTTTTACAACGACGTGACTGG
		G
D2	55	CGACCGTGCCAGCCTAAAACACTTGCCCGTGCCACCATCCCGACGCCACCT
		CCTG
D3	60	AGACGAATTCGGATACGACGGCAGTGCCGACCGTCCAGCCTAAATTCAATC
		CACCC
<u>Template</u>		Listed 3' to 5'
T1	49	GCGGTCCCAAAAGGGTCAGTGCTGGGCAAAATGTTGCTGCACTGACCCG
T2	56	GCTGGCACGGTCGGATTTTGTGAACGGGCACGGTGGTAGGGCTGCGGTGG
		AGGACG
ТЗ	110	CAGGTGGGCGCGGTGGAGGACGAAGTTACACGACCTAGGATGTTGTTCTG
10	110	

Primer	Sequence 5'-3'	Description
M2588 M2589	aagataccccaaccaaacccaaaaaaagagggtgggGGTATCCAAGGTCTTCTTCC aaggtcaaagacagttgactgtatcgtcgaggtcg GGTATCCAAGGTCTTCTTCC	AD-EXO1 DB-EXO1
M2590	cttacttagagctcgacgtcttacttacttagcggccgc TTTACCTTTATAAACAAATTGGG	R- <i>EX01</i>
M3100	aagataccccaaccaaaacccaaaaaaagagggtggg CCCGGAACGCCACAGAAG	AD-DNA2
M3099	aaggtcaaagacagttgactgtatcgtcgaggtcgCCCGGAACGCCACAGAAG	DB-DNA2
M3101	cttacttagagctcgacgtcttacttacttagcggccgcACTTTCATACTCTTGTAGAA	R-DNA2
M3104	aagataccccaaccaaaacccaaaaaaagagggtgggGTGACGAAGCCGTCACATAACTTA	AD-SGS1
M3103	aaggtcaaagacagttgactgtatcgtcgaggtcgGTGACGAAGCCGTCACATAACTTA	DB-SGS1
M2626	TTAAAACTTTGTTTTAGGAAAACAG	DB-Rev-SGS1
M2625	CCTTGGTCTGATGAAGTTTTATAT	DB-Fwd-SGS1
M2596	cttacttagagctcgacgtcttacttacttagcggccgcTTAAAACTTTGTTTTAGGAAAACAG	R-SGS1
M2600	aagataccccaaccaaaacccaaaaaagagggtgggTCATTTAAGGCAACTATCACC	AD-DDC1
M2602	cttacttagagctcgacgtcttacttacttagcggccgcGTCAAATATACCCCTTGGC	R-DDC1
M2603	aagataccccaaccaaaacccaaaaaaagagggtgggCGAATCAACAGTGAGCTAG	AD-RAD17
M2604	aaggtcaaagacagttgactgtatcgtcgaggtcgCGAATCAACAGTGAGCTAG	DB-RAD17
M2605	cttacttagagctcgacgtcttacttacttagcggccgcAAAAAATATAGGAATATCCTTTGTTG	R- <i>RAD17</i>
M2606	aagataccccaaccaaacccaaaaaaagagggtgggAAATTAAAATTGATAGTAAATGGTTGT	AD-MEC3
M2607	aaggtcaaagacagttgactgtatcgtcgaggtcgAAATTAAAATTGATAGTAAATGGTTGT	DB-MEC3
M2608	cttacttagagctcgacgtcttacttacttagcggccgcCAAGCCCTTCGATCTT	R- <i>MEC3</i>
M3106	aagataccccaaccaaacccaaaaaaagagggtgggGTGACTGGTGAAGAAAATGTGT	AD-SAE2
M3105	aaggtcaaagacagttgactgtatcgtcgaggtcgGTGACTGGTGAAGAAAATGTGT	DB-SAE2
M3107	C cttacttagagctcgacgtcttacttagcggccgcACATCTAGCATATATCTGCAATAA	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 <sup>32</sup>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  $\mu$ l, on ice for 25 min. The reaction buffer contained 50 mM Tris-HCI (pH 7.5), 25 mM NaCl, 2 mM dithiothreitol, 0.25 mg/ml bovine serum albumin, 4 mM MgCl<sub>2</sub>, and 8 mM ATP. Lane 6 containing 100 fmol of DNA2 alone contained 2mM MgCl<sub>2</sub> 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.