SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Structure of Ku-TLC1_{KBS} and Comparison with Human Ku-dsDNA, Related to Figure 1.

(A) Electron density map of TLC1_{KBS}. Stereo view of the Sigma-A weighted 2Fo-Fc map that shows nucleotides 288-312 of TLC1_{KBS} are ordered in the crystal. Refined model of TLC1_{KBS} is superimposed on the electron density map. Contours are drawn at the 1.0 σ level. (B) Bar diagram and the structure of Ku70. From the N- to C-termini, the vWA domain, the BBD domain, the insertion, and the CTD motif are colored in light green, green, pink, and dark green, respectively. (C) Bar diagram and the structure of Ku80. From the N- to C-termini, the vWA domain, the N- to C-termini, the vWA domain, the CTD motif are colored in light blue, blue, purple, and dark blue, respectively. (D and E) Structural comparison of yeast Ku70/80-TLC1_{KBS} complex and human Ku70/80-dsDNA complex. Two orthogonal views of the structure of human Ku70/80-dsDNA complexes (D) and yeast Ku70/80-TLC1_{KBS} complexes (E), respectively.

Figure S2. ITC Measurement of the Interactions between Ku70/80 and Wildtype and Mutant $TLC1_{KBS}$ and Proposed Secondary Structures of $TLC1_{KBS}$ Orthologs, Related to Figure 2.

(A) TLC1_{KBS} (nt 288-312) binds to Ku70/80 equally well as KBS-48nt (nt 278-325) and the mutants of TLC1_{KBS} disrupt or weaker its interaction with Ku70/80, consistent with the Ku70/80-TLC1_{KBS} complex structure analyses. (B) Sequence analysis of predicted TLC1_{KBS} RNAs from different yeast species clearly showed that, except for the terminal loop, the sequence of TLC1_{KBS} is highly conserved in the *Saccharomyces* clade.

Figure S3. Structural Analyses of the Ku80_{vWA}-Sir4_{KBM} Interaction, Related to Figure 3.

(A and B) Characterization of the interaction between Ku80 and Sir4, and analysis of the mutant Ku80_{vWA}-Sir4_{KBM} interactions by yeast two-hybrid assay. Data are average of three independent β -galactosidase measurements. Error bars in the graph represent standard errors of the mean (SEM). (C) Electron density map of the Sir4_{KBM} peptide. The Sigma-A weighted 2Fo-Fc map that shows residues 100-115 of Sir4_{KBM} are ordered in the crystal. The refined model of Sir4_{KBM} is superimposed on the electron density map. Contours are drawn at the 1.0 σ level. (D) Sir4_{KBM}, TLC1_{KBS} and Ku70/80 form a quaternary complex. GST-Sir4_{KBM} was used to pull down Ku70/80 and TLC1_{KBS}. Proteins were visualized on SDS-PAGE by Coomassie blue staining, and the TLC1_{KBS} RNA were visualized on 8 M urea PAGE by SYBR[®] Gold staining. (E) ITC measurements of the interactions between wild-type and mutant Ku80_{vWA} and Sir4_{KBM}.

Figure S4. Functional Analysis of the Ku80_{vWA}-Sir4_{KBM} Interaction, Related to Figure 4.

(A and B) Mutants that disrupt Ku80-Sir4 interaction do not show senescence phenotype. A *sir4-null* strain was transformed with *CEN* plasmids expressing either WT or mutant Sir4. The cells were serially re-streaked four times and plated at streak 5 (A). A ku80-null strain was transformed with *CEN* plasmids expressing either wild-type or mutant Ku80. The cells were serially re-streaked for 4 times and plated at streak 5 (B). (C) The interaction between Ku80 and Sir4 is required for telomere silencing. Top: Wild-type telomere end protection in strains bearing mutations in

KU80 integrated at the endogenous locus that disrupt the Ku80-Sir4 interaction, as revealed by normal growth at restrictive temperature (37 °C). Shown are five-fold serial dilutions of wild-type, mutant, or null strains plated on YPD at 28 °C (to monitor plating efficiency) and 37 °C. Bottom: Derepression of *URA3* telomeric reporters in strains bearing mutations in *KU80* that disrupt the Ku80-Sir4 interaction integrated at the endogenous locus. Shown are five-fold serial dilutions of wild-type, mutant, or null strains on media lacking uracil (-Ura; to monitor plating efficiency) or containing 5-fluorotic acid (5-FOA), as indicated. **(D)** The interaction between Ku80 and Sir4 is not required for Ku's role in telomere end protection or NHEJ. Wild-type NHEJ in strains bearing mutations in *KU80* that disrupt the Ku80-Sir4 interaction integrated at the endogenous locus, as revealed by growth in the presence of constitutively expressed HO endonuclease (+HO). Shown are five-fold serial dilutions of wild-type, mutant, or null strains plated on YPD (to monitor plating efficiency) and YPGal, as indicated.

Figure S5. Primary Sequence Analysis of Est1 Proteins and Characterization of *KI*Cdc13_{EBM}, Related to Figure 5.

(A) Multiple sequence alignment of Est1 family members. The secondary structures (α , α -helix; β , α -strand; η , 3_{10} -helix) of *Kl*Est1 are labeled on the top of each bars. Conserved residues are boxed and highlighted in red. (**B**) Protein folding analysis of *Sc*Est1 and *Kl*Est1 by the PONDR-Fit program. The analysis results show that the C-terminal regions of most Est1 proteins are disordered. (**C**) ITC measurement of the interactions between *Kl*Est1 and different fragments of *Kl*Cdc13 within *Kl*Cdc13_{RD}.

Figure S6. Crystallographic and mutational analyses of the $K/Est1-K/Cdc13_{EBM}$ interaction, Related to Figure 5.

(A) Electron density map of the $K/Cdc13_{EBM}$ peptide in the $K/Est1-K/Cdc13_{EBM}$ complex. Stereo view of the Sigma-A weighted 2Fo-Fc map that shows of $K/Cdc13_{EBM-N}$ (residues 213-222) and $K/Cdc13_{EBM-C}$ (residues 232–238) of $K/Cdc13_{EBM}$ are ordered in the crystal. Refined models of $K/Cdc13_{EBM-N}$ and $K/Cdc13_{EBM-C}$ are superimposed on the electron density map. Contours are drawn at the 1.0 σ level. (B) ITC measurement of the wild-type and mutant $K/Est1-K/Cdc13_{EBM}$ interactions.

Figure S7. Functional Analysis of the *KI*Est1-*KI*Cdc13_{EBM} Interaction, Related to Figure 6.

(A) Mutations in Cdc13_{EBM-N} do not show senescence phenotype. A *CDC13/cdc13A* diploid strain was transformed with *CEN CDC13* (or mutant *cdc13*), and then was sporulated and dissected. Cells were taken from serial streaks 2, 4, 6 and 8. (B) Cdc13-F237A and -E252K mutant proteins are expressed at the wild-type level. Upper panel: Myc-tagged Cdc13-WT, -F237A, and -E252K proteins were ectopically expressed under control of the native Cdc13 promoter using a *CEN* vector introduced into the *cdc13A* strain. The expression level was verified by quantitative western blot using anti-Myc antibody and IRDye conjugated secondary antibody. Wild-type Cdc13 protein with identical 18xMyc tag expressed from native genomic locus served as endogenous control. Fluorescence signal in the linear range was normalized to that of the loading control. Lower panel: Mean expression levels for three independent clones of each strain are plotted, the error bars are SEM. (C) Cdc13-F237A and -E252K Mutant Proteins Exhibit Nearly Wild-type Level of Telomere Binding. Cdc13-

Myc ChIP-qPCR assay performed with asynchronous yeast cultures using the complementation system described in (B). The mean values of % input recovered for three independent clones for each strain are plotted. The error bars are SEM. ARO1 is a non-telomeric locus control. IgG control for non-specific binding was performed with the CDC13-WT strain. (D) Disruption of both the TLC1-Ku70/80-Sir4 and the Est1-Cdc13 pathways resulted in critically short telomeres. Measurement of average telomere length of yeast strains shown in Figure 6C and 6D taken from streak 6 (wildtype, $cdc13^{F237A}$ and $tlc1^{\Delta AU}$) or streak 4 (for strains with senescence phenotype). Telomere length was calculated from three independent experiments (mean \pm SEM).

Figure S1

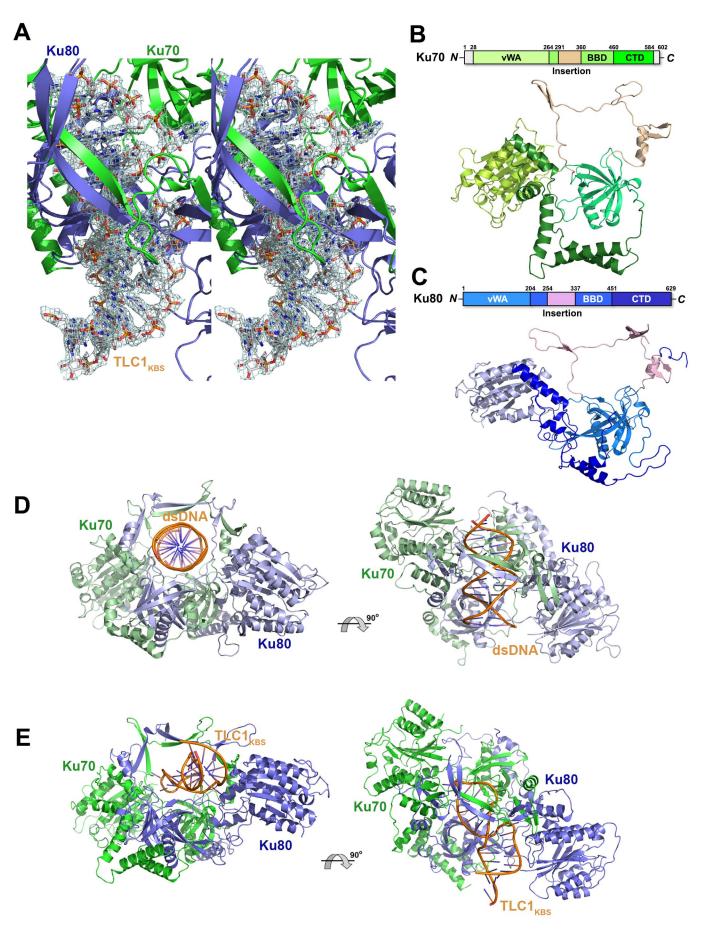
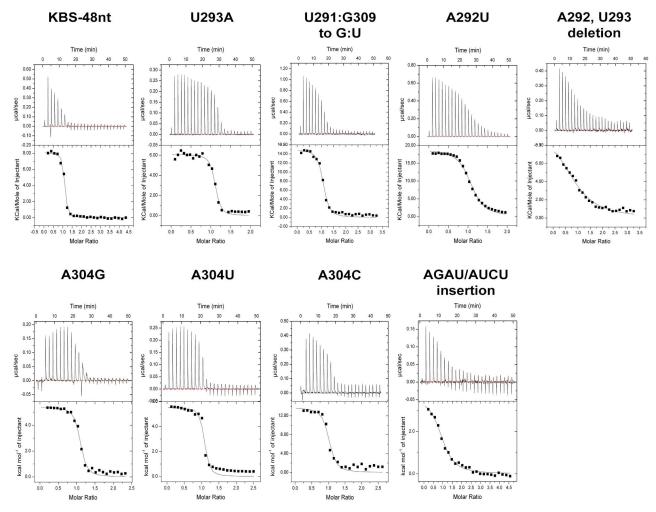
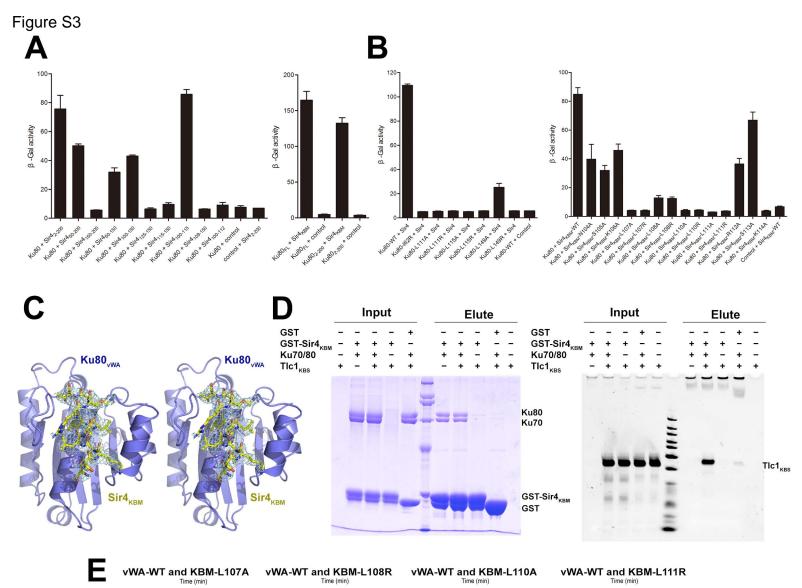


Figure S2





30 0.5 0.40 0.30 pcal/sec 0.20 0.10 0.00 0.90 kcal mol.¹ 0.80 kcal mol.¹ 0.60 kcal mol.¹ 0.0 0.5 10 Molar Ratio vWA-I62A and KBM-WT

30 40

Molar Ratio

0.40

0.30

0.20

0.10

0.00

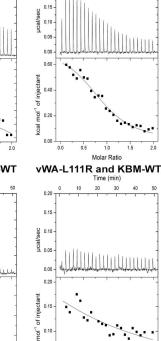
0.60 upiectant 0.40 0.20

kcal r

0.00

0.0 0.5 1.0 1.5 2.0

pcal/sec



kcal

0.05

0.0 0.5 1.0 1.5 2.0

Molar Ratio

20 30

2.0

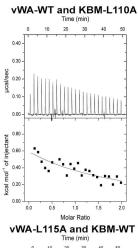
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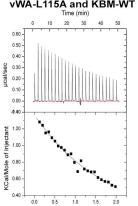
pcal/sec

40

0.25

0.20





Molar Ratio

20 30

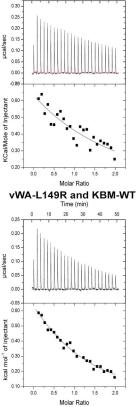
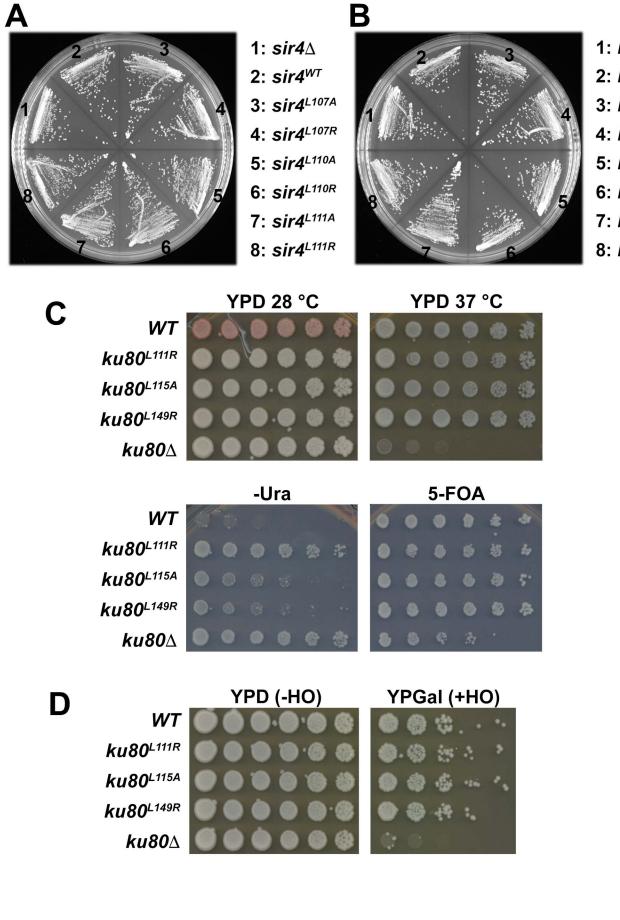
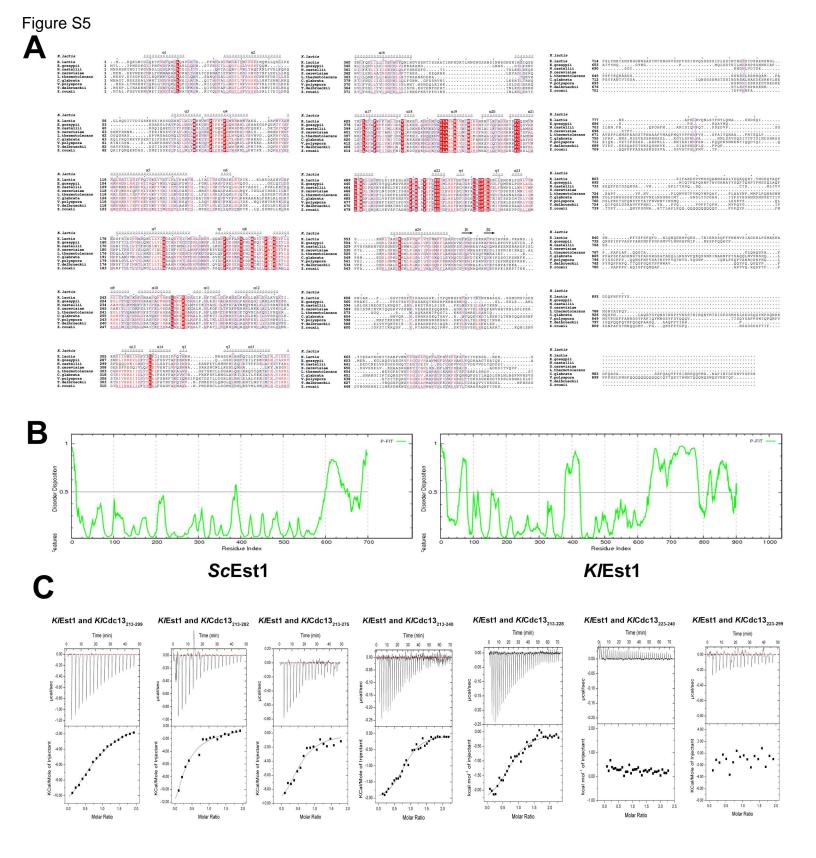
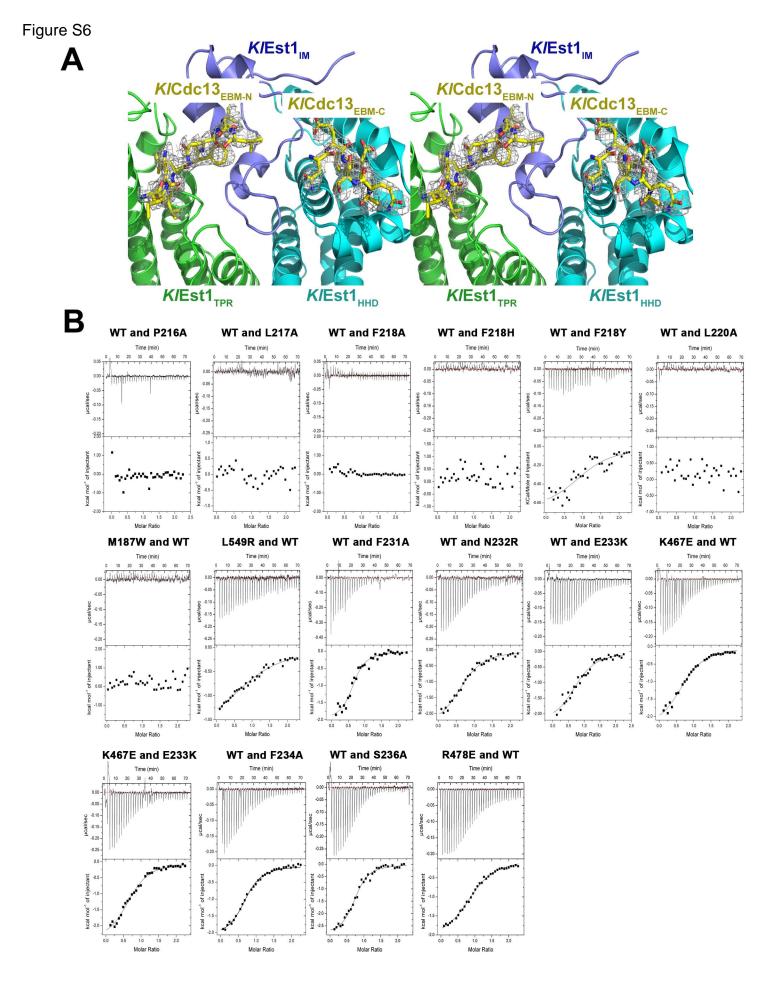


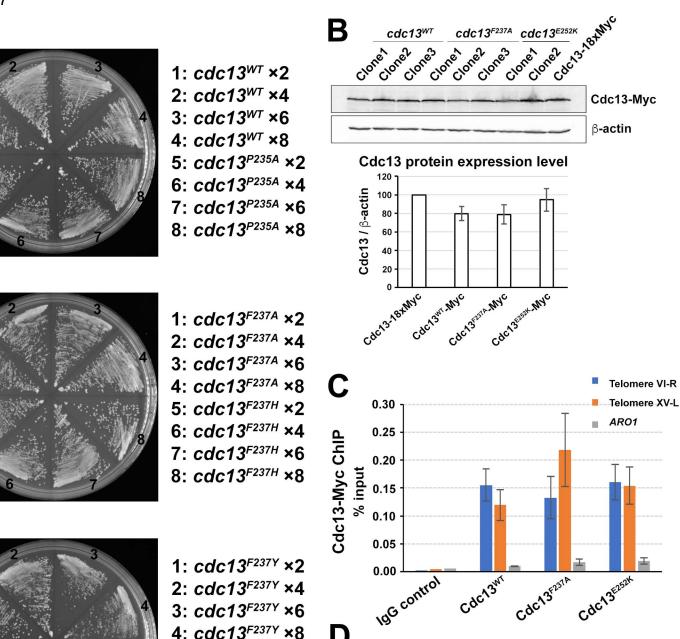
Figure S4



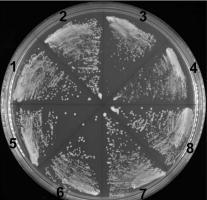
- 1: *ku80*∆
- 2: *ku80^{w⊤}*
- 3: *ku80*′^{62R}
- 4: ku80^{L111A}
- 5: *ku80*^{L111R}
- 6: *ku80*^{L115A}
- 7: *ku80^{L115R}*
- 8: *ku80*^{L149R}







D



- 4: cdc13^{F237Y} ×8 5: cdc13P239A ×2
- 6: cdc13P239A ×4
- 7: cdc13P239A ×6
- 8: cdc13P239A ×8

Construct	Decrease in
Genotype	telomere length (bp)
WT	0
cdc13 ^{F237A}	~220
tic1 ^{^AU}	~130
cdc13 ^{F237A} + tlc1∆AU	~300 (senesced)
cdc13 ^{F237A} + sir4∆	~300 (senesced)
cdc13 ^{F237A} + sir4 ^{WT}	~280
cdc13 ^{F237A} + sir4 ^{L107A}	~290 (senesced)
cdc13 ^{F237A} + sir4 ^{L111R}	~290 (senesced)
cdc13 ^{F237A} + ku80 ^{WT}	~220
cdc13 ^{F237A} + ku80 ^{L111R}	~290 (senesced)
cdc13 ^{F237A} + ku80 ^{L115A}	~290 (senesced)

Α

	Ku70/80_TLC1 _{KBS} (Native)	Ku70/80_TLC1 _{KBS} (SeMet-SAD)
Data collection		
Wavelength (Å)	0.97853	0.97853
Space group	P1	P1
Cell dimensions	1 1	1 1
a, b, c (Å)	116.3, 115.2, 115.8	115.4, 114.2, 115.1
	77.4, 78.4, 63.7	77.6, 78.7, 63.9
α, β, γ (°) P araplution (Å)		, ,
Resolution (Å)	2.8	3.2
R_{merge} (%)	7.6 (55.6) *	9.3 (61.3) *
$I / \sigma I$	16.3(2.6) *	12.6 (2.0) *
Completeness (%)	93.9 (96.0) *	98.8 (98.6) *
Redundancy	3.8 (3.8) *	23.5 (4.5) *
Refinement		
Resolution (Å)	44.11-2.80	
No. of reflections	118,137	
$R_{\rm work} / R_{\rm free}$ (%)	21.5/25.8	
No. of atoms		
Ku70	13,647	
Ku80	13,444	
Tlc1 _{KBS}	1,923	
Water	306	
<i>B</i> -factors (Å ²)		
Ku70	61.1	
Ku80	77.7	
Tlc1 _{KBS}	63.9	
Water	40.9	
R.m.s. deviations		
Bond lengths (Å)	0.003	
Bond angles (°)	0.587	
Ramanchandran plot		
Favored region	97.3%	
Allowed region	100.0%	
Outlier region	0.0%	
Outlier region	0.070	

 Table S1. Crystal Data Collection and Refinement Statistics, Related to Figure 1

*Highest resolution shell is shown in parenthesis

	Ku80 _{vWA} Sir4 _{KBM}	Ku80 _{vWA} _Sir4 _{KBM}
	(Native)	(SeMet-SAD)
Data collection		
Wavelength (Å)	0.97851	0.97851
Space group	<i>P</i> 3 ₁ 21	$P3_{2}21$
Cell dimensions		
a, b, c (Å)	79.5, 79.5, 82.9	79.2, 79.2, 165.7
α, β, γ (°)	90.0, 90.0, 120.0	90.0, 90.0, 120.0
Resolution (Å)	2.4	2.8
R_{merge} (%)	7.1 (88.8) *	10.9 (76.9) *
Ι/σΙ	48.0 (1.8) *	24.6 (2.0) *
Completeness (%)	99.6 (96.2) *	99.7 (99.9) *
Redundancy	18.0 (11.4) *	19.1 (12.0) *
Refinement		
Resolution (Å)	27.64-2.40	
No. of reflections	10,957	
$R_{\text{work}} / R_{\text{free}}$ (%)	20.1/24.8	
No. of atoms	20.1/21.0	
Ku80 _{vWA}	1,498	
Sir4 _{KBM}	94	
Ion	5	
Water	35	
<i>B</i> -factors ($Å^2$)	55	
Ku80 _{vWA}	28.7	
Sir4 _{KBM}	44.4	
Ion	66.3	
Water	23.9	
R.m.s. deviations	-0.7	
Bond lengths (Å)	0.010	
Bond angles (°)	1.306	
Ramanchandran plot	1.500	
Favored region	98.4%	
Allowed region	100.0%	
Outlier region	0.0%	
Outlier region	0.070	

 Table S2. Crystal Data Collection and Refinement Statistics, Related to Figure 3

*Highest resolution shell is shown in parenthesis

	<i>Kl</i> Est1_ <i>Kl</i> Cdc13 _{EBM}	<i>Kl</i> Est1_ <i>Kl</i> Cdc13 _{EBM}
	(Native)	(SeMet-SAD)
Data collection		
Wavelength (Å)	0.97860	0.97860
Space group	<i>C</i> 2	<i>C</i> 2
Cell dimensions		
a, b, c (Å)	108.6, 41.7, 147.1	112.7, 42.5, 150.9
α, β, γ (°)	90.0, 97.5, 90.0	90.0, 98.0, 90.0
Resolution (Å)	2.2	2.5
R_{merge} (%)	10.1 (59.4) *	11.3 (76.0) *
Ι/σΙ	18.2 (1.9) *	33.3 (2.2) *
Completeness (%)	91.8 (93.6) *	94.7 (95.3) *
Redundancy	6.3 (5.8) *	13.3 (11.8) *
Refinement		
Resolution (Å)	40.86-2.20	
No. of reflections	30,684	
$R_{\text{work}} / R_{\text{free}}$ (%)	17.6/22.5	
No. of atoms		
Est1	4,233	
Cdc13 _{EBM}	136	
Water	73	
<i>B</i> -factors ($Å^2$)		
Est1	65.4	
$Cdc13_{EBM}$	89.0	
Water	53.8	
R.m.s. deviations		
Bond lengths (Å)	0.005	
Bond angles (°)	0.852	
Ramanchandran plot		
Favored region	99.0%	
Allowed region	100.0%	
Outlier region	0.0%	

 Table S3. Crystal Data Collection and Refinement Statistics, Related to Figure 5

*Highest resolution shell is shown in parenthesis

Plasmid Name	Description	Source
pRS315	CEN LEU2	Gift from JQ Zhou
pRS316	CEN URA3	Gift from JQ Zhou
pRS415	CEN LEU2	Gift from JQ Zhou
pRS303- <i>est1∆</i>	YIp <i>HIS3 est1∆</i>	Gift from JQ Zhou
pVL1107	CEN LEU2 CDC13-EST2 fusion	Gift from JQ Zhou
pCH249	CEN LEU2 cdc13-2-EST2 fusion	pVL1107
pCH253	CEN URA3 ESTI	This study
pCH254	CEN URA3 est1-60	pCH253
pRS305- <i>tlc1∆</i>	YIp <i>LUE2 tlc1</i> ∆	Gift from JQ Zhou
pRS303- <i>tlc1∆</i>	YIp <i>HIS3 tlc1</i> ∆	pRS305- <i>tlc1</i> ∆
pRS316-TLC1	CEN URA3 TLC1	Gift from JQ Zhou
pRS316- <i>tlc1∆48</i>	$CEN URA3 tlc 1\Delta 48$	Gift from JQ Zhou
pCH414	$CEN URA3 tlc 1 \Delta AU$	pRS315-TLC1
pCH198	CEN URA3 CDC13	This study
pCH323	CEN LUE2 CDC13	This study
pCH324	CEN LEU2 cdc13-P235A	pCH323
pCH325	CEN LEU2 cdc13-F237A	pCH323
pCH326	CEN LEU2 cdc13-E252K	pCH323
pCH327	CEN LEU2 cdc13-F253A	pCH323
pCH328	CEN LEU2 cdc13-S255A	pCH323
pCH395	CEN LEU2 cdc13-F237H	pCH323
pCH396	CEN LEU2 cdc13-F237Y	pCH323
pCH397	CEN LEU2 cdc13-P239A	pCH323
pCH407	YIp <i>HIS3 sir4</i> ∆	This study
pCH408	CEN URA3 SIR4	This study
pCH416	CEN URA3 sir4-L107R	pCH408
pCH420	CEN URA3 sir4-L111R	pCH408
pRS303- <i>ku80∆</i>	YIp <i>HIS3 ku80∆</i>	Gift from JQ Zhou
pCH410	CEN LEU2 KU80	This study
pCH427	CEN LEU2 ku80-L111R	pCH410
pCH428	CEN LEU2 ku80-L115A	pCH410
pMLW323	CEN LEU2 CDC13	This study
pMLW324	CEN LEU2 cdc13-F237A	pMLW323
pMLW325	CEN LEU2 cdc13-E252K	pMLW323

 Table S4. Plasmids Used for This Study, Related to STAR Methods

Strain Name	Genotype	Source
	$MATa/\alpha$ his3 $\Delta 1$ /his3 $\Delta 1$ leu2 $\Delta 0$ /leu2 $\Delta 0$	
yCH001	$LYS2/lys2\Delta0$ met $15\Delta0/MET15$ ura $3\Delta0/ura3\Delta0$	Euroscarf, SRD
	CDC13/cdc13A::Kanr	GmbH
	MATa/α his $3\Delta 1$ /his $3\Delta 1$ leu $2\Delta 0$ /leu $2\Delta 0$	
yCH002	LYS2/lys2 Δ 0 met15 Δ 0/MET15 ura3 Δ 0/ura3 Δ 0	yCH001
	CDC13/cdc13A::kanr EST1/est1A::HIS3	
	MATα (or MATa) his $3\Delta 1$ leu $2\Delta 0$ lys $2\Delta 0$ (or	
yCH005	$met15\Delta 0$) $ura3\Delta 0$ $cdc13\Delta$:: $kanr pCH198(CEN)$	yCH001
	URA3 CDC13)	
011051	$MATa/\alpha$ his $3\Delta 1/h$ is $3\Delta 1$ leu $2\Delta 0/leu2\Delta 0$	CI I O O I
yCH051	$LYS2/lys2\Delta0 met15\Delta0/MET15 ura3\Delta0/ura3\Delta0$	yCH001
	$CDC13/cdc13\Delta$::kanr KU80/ku80 Δ ::HIS3 MATa/ α his3 Δ 1/his3 Δ 1 leu2 Δ 0/leu2 Δ 0	
yCH052	$LYS2/lys2\Delta0$ met15 $\Delta0/MET15$ ura3 $\Delta0/ura3\Delta0$	yCH001
yC11052	CDC13/cdc13A::kanr TLC1/tlc1A::HIS3	yCII001
	$MAT\alpha$ his $3\Delta 1$ leu $2\Delta 0$ lys $2\Delta 0$ ura $3\Delta 0$	Euroscarf, SRD
yCH133	ku804::kanr	GmbH
	$MAT\alpha$ his 3 $\Delta 1$ leu 2 $\Delta 0$ lys 2 $\Delta 0$ ura 3 $\Delta 0$	Euroscarf, SRD
yCH134	sir4 <i>∆</i> ::kanr	GmbH
	MATα (or MATa) his $3\Delta 1$ leu $2\Delta 0$ lys $2\Delta 0$ (or	
yCH143	$met15\Delta 0$) $ura3\Delta 0$ $cdc13\Delta$:: $kanr sir4\Delta$:: $HIS3$	yCH005
	pCH198(CEN URA3 CDC13)	-
	MATα (or MATa) his $3\Delta 1$ leu $2\Delta 0$ lys $2\Delta 0$ (or	
yDZ651	met15 Δ 0) ura3 Δ 0 cdc13 Δ ::kanr sir4 Δ ::HIS3	yCH143
<i>yD2031</i>	pCH198(CEN URA3 CDC13) EST2-Gly8-	yennis
	myc13::LYS2	
	MATa ura3-52 lys2-801 ade2-101 trp1- Δ 63	Singer and
UCC3505	$his3-\Delta 200 \ leu2-\Delta 1 \ ppr1::HIS3 \ adh4::URA3-$	Gottschling,
	(URA3 at TEL VIIL) DIA5-1 (ADE2 at TEL	1994
	VR) MATα yku80-Δ::kan ^r ura3-52 lys2-801 ade2-	
	$101 \text{ trp1-}\Delta63 \text{ his3-}\Delta200 \text{ leu2-}\Delta1 \text{ ppr1::HIS3}$	Bertuch and
YVL885	adh4::URA3-(URA3 at TEL VIIL) DIA5-1	Lundblad, 2003
	(ADE2 at TEL VR)	Eulidolud, 2005
M VAB1010	MATa yku80-L111R ura3-52 lys2-801 ade2-101	
	$trp1-\Delta 63$ his3- $\Delta 200$ leu2- $\Delta 1$ ppr1::HIS3	11002505
	adh4::URA3-(URA3 at TEL VIIL) DIA5-1	UCC3505
	(ADE2 at TEL VR)	
	MATα yku80-L115A ura3-52 lys2-801 ade2-101	
YAB1011	$trp1-\Delta 63 his3-\Delta 200 leu2-\Delta 1 ppr1::HIS3$	UCC3505
17101011	adh4::URA3-(URA3 at TEL VIIL) DIA5-1	0003303
	(ADE2 at TEL VR)	
YAB1012	MATα yku80-L149R ura3-52 lys2-801 ade2-101	
	$trp1-\Delta 63$ his $3-\Delta 200$ leu $2-\Delta 1$ ppr $1::HIS 3$	UCC3505
	adh4::URA3-(URA3 at TEL VIIL) DIA5-1	
	(ADE2 at TEL VR)	

 Table S5. Yeast Strains Used for This Study, Related to STAR Methods

	MATα hml- Δ ::ADE1 hmr- Δ ::ADE1 ade1-110	
JKM139	leu2,3-112 lys5 trp1-∆∷hisG ura3-52	Lee et al., 1998
	ade3::GAL10 HO	
	MATα yku80-Δ::kan ^r YKU70-TAF hml-	
YAB795	Δ ::ADE1 hmr- Δ ::ADE1 ade1-110 leu2,3-112	JKM139
	<i>lys5 trp1-</i> Δ :: <i>hisG ura3-52 ade3</i> :: <i>GAL10 HO</i>	
	MATα yku80-L111R hml-Δ::ADE1 hmr-	
YAB1013	Δ :: <i>ADE1 ade1-110 leu2</i> , <i>3-112 lys5 trp1-</i> Δ :: <i>hisG</i>	JKM139
	ura3-52 ade3::GAL10 HO	
	MATα yku80-L115A hml-Δ::ADE1 hmr-	
YAB1014	Δ :: <i>ADE1 ade1-110 leu2</i> , <i>3-112 lys5 trp1-</i> Δ :: <i>hisG</i>	JKM139
	ura3-52 ade3::GAL10 HO	
	MATα yku80-L149R hml-Δ::ADE1 hmr-	
YAB1015	Δ :: <i>ADE1 ade1-110 leu2</i> , <i>3-112 lys5 trp1-</i> Δ :: <i>hisG</i>	JKM139
	ura3-52 ade3::GAL10 HO	
	MATa his $3\Delta 1$ leu $2\Delta 0$ LYS2 ura $3\Delta 0$	
	cdc13 <i>A</i> ::Kanr pMLW323 (CEN LEU2 CDC13)	yCH001
	Est1-13Myc::TRP1	
	MATa his3∆1 leu2∆0 LYS2 ura3∆0	
	cdc13 <i>A</i> ::Kanr pMLW325	yCH001
	(CEN LEU2 cdc13-F237A) Est1-13Myc::TRP1	
	MATa his $3\Delta 1$ leu $2\Delta 0$ LYS2 ura $3\Delta 0$	
	cdc13∆::Kanr pMLW326	yCH001
	(CEN LEU2 cdc13-E252K) Est1-13Myc::TRP1	
	MATa his $3\Delta 1$ leu $2\Delta 0$ LYS2 ura $3\Delta 0$	yCH001
	cdc13∆::Kanr pMLW323	
	(CEN LEU2 CDC13) Est2-13Myc::TRP1	
	MATa his $3\Delta 1$ leu $2\Delta 0$ LYS2 ura $3\Delta 0$	
	cdc13∆::Kanr pMLW325	yCH001
	(CEN LEU2 cdc13-F237A) Est2-13Myc::TRP1	
	$MATa his 3\Delta 1 leu 2\Delta 0 LYS2 ura 3\Delta 0$	
	1	yCH001
	(CEN LEU2 cdc13-E252K) Est2-13Myc::TRP1	