SUPPLEMENTAL DATA

Cdc13 N-terminal dimerization, DNA binding and telomere length regulation

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SUPPLEMENTAL TABLES

Table S1: Data collection, phasing and refinement statistics

	Native 1	Native 2	Hg1	Hg2
Data collection				
Space group	P1	$P2_12_12$	$P2_12_12$	$P2_12_12$
Cell dimensions				
a, *b*, *c* (Å)	62.0 66.9 68.1	62.0 70.2 53.5	61.6 71.6 53.7	61.5 70.3 53.4
α, β, γ (º)	116.2 90.3 94.7			
Resolution (Å)	20-2.5 (2.54-2.50)	20-2.70 (2.85-2.70)*	50-3.30 (3.48-3.30)	40-3.5 (3.69-3.5)
R_{sym}	5.7 (39.6)	5.9 (41.6)	8.8 (43.4)	16.2 (58.6)
$I/\sigma I$	13.8 (2)	14.5 (2.5)	13.6 (3.2)	10.6 (2.5)
Completeness (%)	93.1 (94.2)	96.5 (98.1)	99.6 (99.9)	98.4 (98.5)
Redundancy	1.9 (1.9)	4.0 (4.1)	4.4 (4.6)	5.7 (5.9)
Phasing Analysis				
Resolution (Å)			40-3.5	40-3.8
Phasing Power (acent/cent)			2.23/1.51	1.92/1.33
R_{cullis} (acent/cent)			0.59/0.63	0.63/0.68
R_{cullis} (anom)			0.85	0.91
Number of sites			5	5
Mean figure of merit (FOM)			0.53	0.49
Refinement				
Resolution (Å)	20-2.5	20-2.70		
No. reflections	29636	6168		
R_{work}/R_{free}	24.5/28.7	22.9/27.2		
No. atoms				
Protein	6376	1551		
Water	20	47		
B-factors				
Protein	54.7	67.9		
Water	27	79.3		
R.m.s deviations				
Bond lengths (Å)	0.014	0.009		
Bond angles (°)	1.295	1.279		
Ramachandran plot (%)				
Most favored	84.8	82.2		
Allowed	12.4	16.1		
Generously allowed	2.8	1.7		

*Highest resolution shell is shown in parenthesis.

Table S2: DNA oligonucleotides used in this study

Name	Sequence
11mer	GTGTGGGTGTG
18mer	GTGTGGTGTGGGTGTGGG
26mer	GTGGGTGTGGTGGGTGTGTGGGTGTG
37mer	GTGTGGGTGTGGTGTGGGTGGGTGTGGTGTGGGTGTG
43mer	GTGGTGGGTGGGTGTGTGTGGGTGTGGTGGGTGTGTGGGTGTG
Poly(dT) (50mer)	(dT)50
Random ssDNA (43mer)	TATGAATGAGTTCAAATATTGCACATTGAAATTATATTTTACG
Random dsDNA	GGCACAGTCACGTCGATGCACTAGTCGAGTTTCTTCGGAAACTCGACTAGTGCATCGACGTGACTGTGCC
Telomeric dsDNA	CACACCCACACGTCGATGCACTAGTCGAGTTTCTTCGGAAACTCGACTAGTGCATCGACGTGTGGGTGTG
Telomeric dsDNA with 11mer overhang	GTCGATGCACTAGTCGAGTTTCTTCGGAAACTCGACTAGTGCATCGACGTGTGGGTGTG

Table S3: Yeast strains used in this study

Strain	Genotype	Reference
YBJ254	BY4743: *MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0*	Brachmann et al., 1998.
YBJ641	YBJ254 *cdc13Δ::kanMX/CDC13*	This study
JSY0225	YBJ641 + pRS415-*CDC13*	This study
JSY0226	YBJ641+ pRS415	This study
JSY0227	YBJ641+ pRS415-*K73A*	This study
JSY0228	YBJ641+ pRS415-*K75A*	This study
JSY0229	YBJ641+ pRS415-*L84A*	This study
JSY0230	YBJ641+ pRS415-*I87A*	This study
JSY0231	YBJ641+ pRS415-*L91A*	This study
JSY0232	YBJ641+ pRS415-*V133A*	This study
JSY0233	YBJ641+ pRS415-*T140A*	This study
JSY0234	YBJ641+ pRS415-*F142A*	This study
JSY0235	YBJ641+ pRS415-*L84A/I87A*	This study
JSY0236	*cdc13Δ::kanMX* + pRS415-*CDC13*	This study
JSY0238	*cdc13Δ:: kanMX* + pRS415-*K73A*	This study
JSY0240	*cdc13Δ:: kanMX* + pRS415-*K75A*	This study
JSY0242	*cdc13Δ:: kanMX* + pRS415-*L84A*	This study
JSY0244	*cdc13Δ:: kanMX* + pRS415-*I87A*	This study
JSY0246	*cdc13Δ:: kanMX* + pRS415-*L91A*	This study
JSY0248	*cdc13Δ:: kanMX* + pRS415-*V133A*	This study
JSY0250	*cdc13Δ:: kanMX* + pRS415-*T140A*	This study
JSY0252	*cdc13Δ:: kanMX* + pRS415-*F142A*	This study
JSY0254	*cdc13Δ:: kanMX* + pRS415-*L84A/I87A*	This study

SUPPLEMENTAL FIGURES

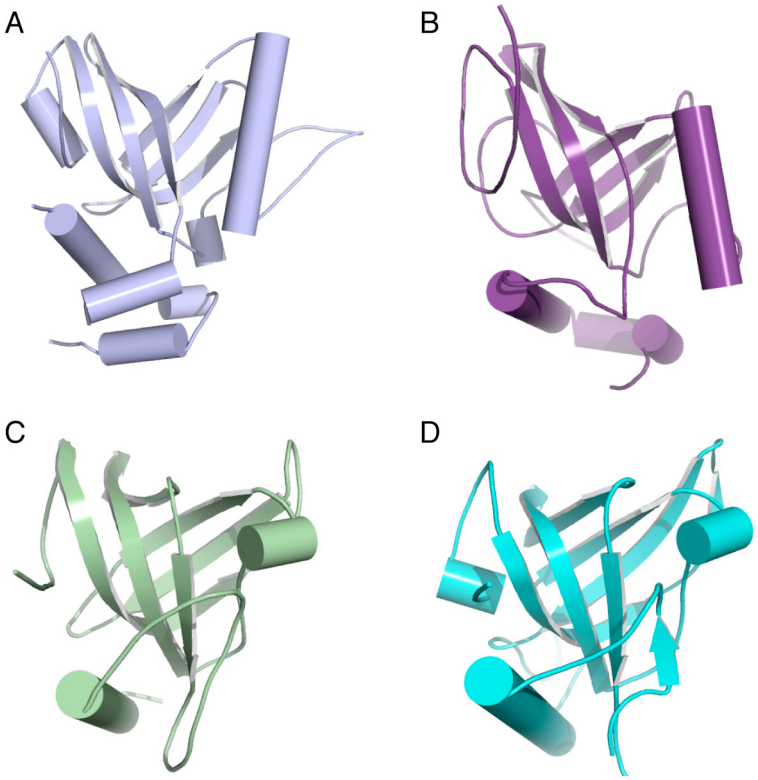

Fig. S1. Comparison of the Cdc13N OB-fold with structural homologues (A) Cdc13N, OB-fold 1 (blue). (B) Cdc13 DNA Binding Domain (DBD, OB-fold 2), (purple) (PDB ID: 1S40). (C) Human Pot1 OB-fold (green), (PDB ID: 1XJV). (D) *on*TEBP OB-fold (cyan), (PDB ID: 1KIX)

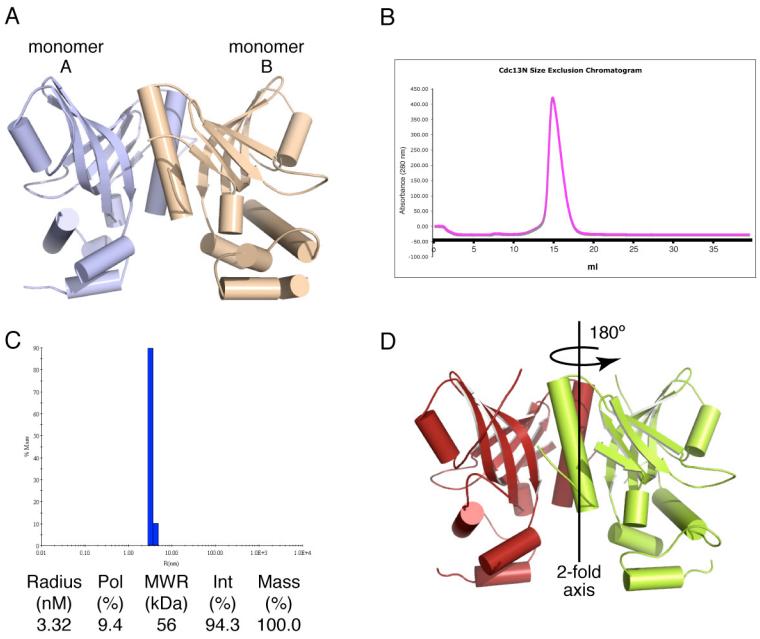

Fig. S2. Cdc13N dimerization data (A) Cartoon representation of the Cdc13N dimer showing monomer A in blue and monomer B in wheat color. (B) Size exclusion data from a Superdex™ S200 column (GE Healthcare). The protein elutes at 14.8 ml, which corresponds to a ~55 kDa molecule, which corresponds to a Cdc13N dimer. (C) Dynamic light scattering data of Cdc13N carried out at two different temperatures (4 and 22 ºC) and in size exclusion buffer (see methods for buffer details). The results show a ~56 kDa particle in solution with 9% polydispersity, which corresponds to a Cdc13N dimer. (D) The Cdc13N dimer of the orthorhombic crystal form ($P2_12_12$) is shown for comparison with the Cdc13N dimer of the P1 crystal form of panel (A). The $P2_12_12$ crystal form contains a monomer in the asymmetric unit. Crystal symmetry analysis of Cdc13N shows that the two fold symmetry axis of the Cdc13N dimer coincides with the two-fold crystallographic axis.

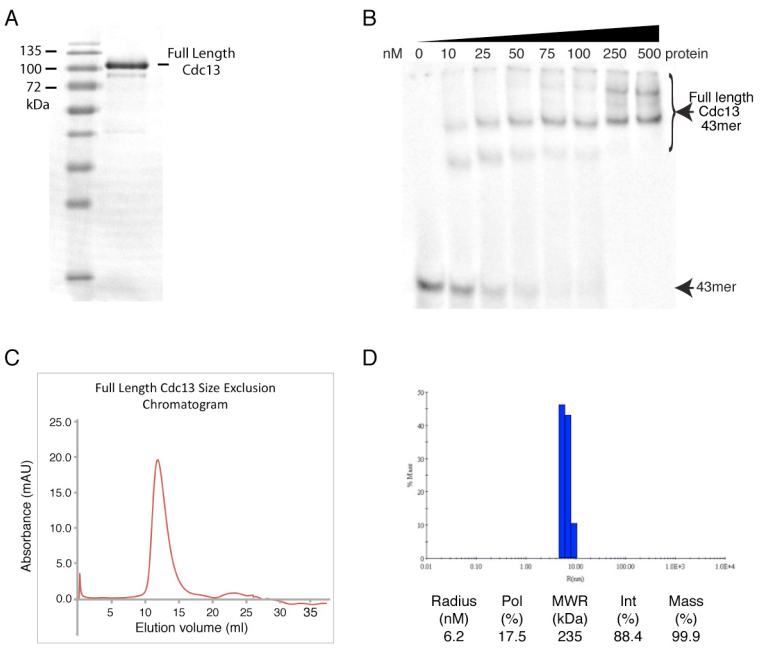

Fig. S3. Full length Cdc13 (flCdc13) dimerization and DNA binding data (A) 12% SDS page gel of full length Cdc13. (B) EMSA of the flCdc13 (concentration varies 0-500 nM) with the 43mer (concentration constant at 1nM) yeast telomeric DNA. (C) Size exclusion data from a Superdex™ S200 column (GE Healthcare). The protein elutes at 11.5 ml, which corresponds to a ~220 kDa molecule, which corresponds to a Cdc13 dimer. (D) Dynamic light scattering data of the full length Cdc13 carried out at two different temperatures (4 and 22 ºC) and in size exclusion buffer (see methods for buffer details). The results show a 235 kDa particle in solution with 17.5% polydispersity, which corresponds to a Cdc13 dimer.

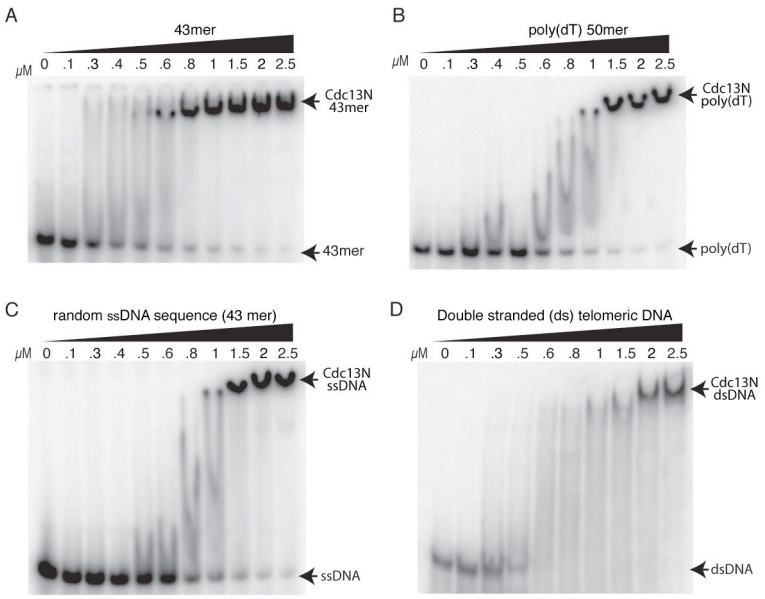

Fig. S4. Wild type Cdc13N DNA binding specificity (A) Electrophoretic mobility shift assay (EMSA) of Cdc13N with the 43mer (Table S2) (same as Fig. 3D). (B) EMSA of Cdc13N with poly(dT) 50mer. (C) EMSA of Cdc13N with a random single-stranded DNA sequence (43 base long). (D) EMSA of Cdc13N with double stranded telomeric DNA.

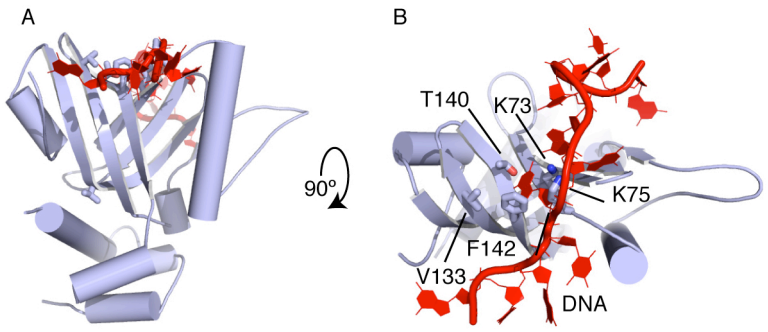

Fig. S5. Model of Cdc13N – DNA monomer complex (A) Model of Cdc13N monomer (blue cartoon) bound to single-stranded telomeric DNA (red stick). The model was created by overlaying the DBD of Cdc13 bound to single-stranded telomeric DNA (PDB ID: 1S40) with the Cdc13N structure solved here. (B) Orthogonal view of panel (A) showing residues K73, K75, V133, T140 and F142 (blue stick) that affect DNA-binding.

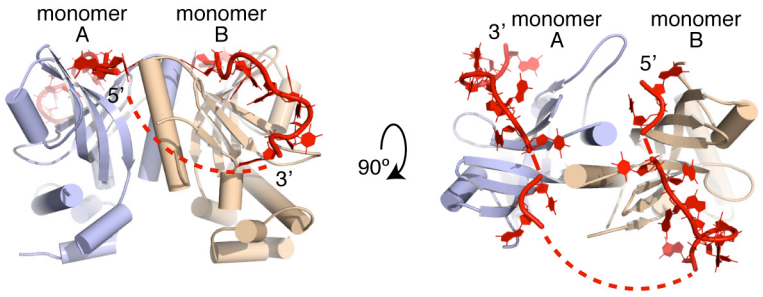

Fig. S6: Model of Cdc13N dimer ((monomers A (blue) and B (wheat)) bound to single-stranded telomeric DNA (red stick). The 5' and 3' ends of the two DNA strands are shown. The missing single-stranded DNA that connects the DNA bound to each monomer is shown as a red dashed line.