Online Methods

Cloning, expression, and protein purification

Full-length and truncated *K. lactis* Ndc10 constructs, and point-mutants of residues involved in DNA contacts, were cloned, expressed in *E. coli*, and purified as described in Supplementary Methods.

Limited trypsin proteolysis and mass spectrometry

To identify distinct domains of Ndc10, purified full-length *K. lactis* Ndc10 was treated with trypsin at room temperature for 10 min (1:50 (w/w)), and submitted for ion trap mass spectrometry analysis at the HHMI Mass spectrometry laboratory at University of California at Berkeley. We identified three trypsin-sensitive loops in the protein from trypsin cleavage sites C-terminal to Lys410, Lys541, and Lys618 (Fig. 1a, Supplementary Fig. 1a).

Fluorescence polarization (FP)

To detect the minimum length of *K. lactis* CEN1 CDEIII required for Ndc10 binding, 50 nM of 5' fluorescein-labeled 20 bp, 25 bp, 30 bp, 40 bp CDEIII (MWG-biotech AG) were incubated with various concentrations of *K. lactis* Ndc10 full length (10–5,000 nM) at room temperature for 1 hour. FP measurements were performed on an EnVision 2103 Multilabel Reader (PerkinElmer), with excitation at 485 nm and emission at 535 nm. Measured data were used to calculate K_d and B_{max} by fitting (with nonlinear regression) the expression, $Y=B_{max}*X/[K_d+X]$, where X is the protein concentration and Y is the anisotropy, using the program Prism 5.0 (http://www.graphpad.com/prism/prism.htm) (Supplementary Fig. 1d). We also measured K_d's of 30 bp DNA with different G:C content and the K_d of *K. lactis* Ndc10 DI–III with 30bp CDEIII (Supplementary Fig. 1e, f).

Crystallization of *K. lactis* CEN1 CDEIII–Ndc10 DI–II

The complex of *K. lactis* Ndc10 DI–II and 30 bp *K. lactis* CEN1 CDEIII (including an A:T overhang) was purified and crystallized as described in Supplementary Methods. Single-wavelength anomalous scattering (SAD) with SeMet-substituted proteins was used to obtain starting phases. The structure

was refined using PHENIX[54] at 2.8 Å resolution to an *R* factor of 19.3% and R_{free} of 25.1%. Residues 36–39 and 283–292 are disordered in the crystal structure.

Electrophoretic mobility shift assay (EMSA)

DNA and protein at concentrations of about 1 μM were mixed in 30 mM Tris-HCl, pH8.0, 100 mM NaCl, 1 mM tris(2-carboxyethyl)phosphine (TCEP) (buffer A); to 15 μL of the mixture were added 9 μL of the gel-shift assay buffer (20 mM HEPES, pH 7.9, 60 mM KCl, 10 mM DTT, 8 ng μl^{-1} poly(dI–dC), 5 mM $MgCl_2$, 10 % glycerol (v/v)), and the sample was incubated at room temperature for 30 min. A precast 5 % or 10 % polyacrylamide (w/v), TBE-buffered gel (Bio-Rad) was pre-run in 1X TBE at 200 V for 1 hour on ice before loading samples. Electrophoresis was for 1 hour at 200 V. Positions of DNA bands were detected with ethidium bromide (EtBr), and gels were subsequently stained with Coomassie blue to detect protein.

DNA-binding of mutant Ndc10

Site-directed mutagenesis of residues at the DNA contact seen in the crystal strcuture followed a PCR extension strategy. All mutant constructs of the *K.lactis* Ndc10 DI–III were confirmed by DNA sequencing. Mutant proteins were purified as described in Supplementary Methods. To determine binding, 20 pmol DNA (*K. lactis* CEN1 CDEIII) was incubated with a comparable molar quantity of wild-type or mutant Ndc10 in 30 mM Tris-HCl, pH8.0, 100 mM NaCl, 1 mM TCEP (buffer A), and the mixture was subjected to EMSA as described in the preceding paragraph.

Ndc10:DNA ratio in bound complexes

EMSA: 20 pmol of *K. lactis* Ndc10 DI–III was incubated in buffer A with different molar ratios (1:0.5, 1:1, 1:1.5, 1:2, 1:4) of 30 bp *K. lactis* CEN1 CDEIII DNA. Electrophoresis and staining were as described above.

Differentially-labeled DNA pull-down assay: Biotinylated 30 bp CDEIII DNA was synthesized by MWG-Biotech AG; ^{32}P-labeled CDEIII DNA was prepared by *in vitro* labeling using γ-^{32}P-ATP (Roche) and T4 polynucleotide kinase (New England Biolabs). Ndc10 DI-III dimer (20 pmol) was incubated with 25 pmol of biotin-labeled DNA, 5 pmol ^{32}P-labeled DNA, and 20 pmol unlabeled

DNA in buffer A. Biotinylated CDEIII was separated with Dynabeads M-280 Streptavidin (Invitrogen), using a magnetic separator (Invitrogen). Beads were washed at least 5 times with washing buffer (buffer A plus 0.1 % NP-40 (w/v)) and eluted with SDS-PAGE sample buffer containing 300 mM imidazole. Dried gels were scanned with a phophorimager (Bio-Rad). The same protocol was used with Ndc10 DI-II and with DNA alone, as negative controls.

Size-exclusion chromatography of protein-DNA complexes: A fixed amount of Ndc10 DI-III dimer (5 μM) was mixed with 30 bp CDEIII DNA in various molar ratios (1:0, 1:0.5, 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4) and applied to a Superdex200 10/300 GL size exclusion column (GE Healthcare) pre-equilibrated with buffer A. Elution profiles were monitored at 260 nm wavelength to detect both the DNA-protein complex and unbound free DNA. The column profile of protein-free DNA was also obtained.

Ni^{2+}-affinity pull-down assay

To identify binding partners of Ndc10, target proteins were translated *in vitro* using TNT T7 coupled reticulocyte lysate (Promega) with ^{35}S-labeled methionine (Roche). *In vitro* translated target proteins were incubated with His-tagged bait proteins at room temperature for 1 hour. Ni-NTA agarose beads (Qiagen) pre-equilibrated with washing buffer (buffer A plus 0.1 % NP-40 (w/v)) were mixed and incubated at 4°C for 30 min with rotation. The beads were washed 3 times with washing buffer and bead-bound proteins separated by SDS-PAGE. Dried gels were analyzed with overnight exposure of a phosphorimager plate.

Amylose affinity pull-down assay

Ndc10 domain IV-V (residues 532-736) and MBP tagged Scm3 constructs were cloned, expressed in *E. coli*, and purified as described in Supplementary Methods. Ndc10 domains IV-V was incubated in a 1:1 molar ratio with each of the MBP-tagged Scm3 proteins for 1 hour at 4°C. Amylose beads (New England Biolab) pre-equilibrated with washing buffer (buffer A plus 0.1 % NP-40 (w/v)) were then added and the mixtures incubated at 4°C for 30 min with rotation. The

beads were washed 3 times with washing buffer and bead-bound proteins separated by SDS-PAGE.

Methods-only references

54. Adams, P.D. et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 213-221 (2010).