Supplemental material

Supplemental 4 figures, 2 tables and 3 movies can be found online.

Supplemental Materials

The internal loop of fission yeast Ndc80 binds Alp7/TACC-Alp14/TOG and ensures proper chromosome attachment

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Supplemental Movies

Movie S1: Mitotic progression of wild type (corresponding to Figure 2C, top, *cen2*-GFP and Sad1-dsRED)

Movie S2: Mitotic arrest of *ndc80-21* (corresponding to Type I, Figure 2C, middle, *cen2*-GFP and Sad1-dsRED)

Movie S3: Mitotic progression and chromosome mis-segregation of *ndc80-21* (corresponding to Type II, Figure 2C, progression, *cen2*-GFP and Sad1-dsRED)

Supplemental Table S1: Fission yeast strains used in this study

Strains	Genotypes	Figures used
513	*h⁻ leu1 ura4*	1B and D, 6A and B
NHT080	*h⁻ ndc80-NH12-kan^r leu1 ura4*	1B, 6A and B, 7A
NHT200	*h⁻ ndc80-NH12-kan^r leu1 ura4* containing pREP1 vector	1B
NHT201	*h⁻ ndc80-NH12-kan^r leu1 ura4* containing pREP1-Ndc80-FL	1B
NHT011	*h⁻ spc25$^+$-YFP-nat^r nuf2$^+$-mCherry-ura4$^+$ cut12$^+$-2CFP-hph^r leu1 ura4*	1C
NHT082	*h⁻ ndc80-NH12-kan^r spc25$^+$-YFP-nat^r nuf2$^+$-mCherry-ura4$^+$ cut12$^+$-2CFP-hph^r leu1 ura4*	1C
NHT099	*h⁻ ndc80-NH12-kan^r kan^r-GFP-atb2$^+$ mis6$^+$-2mRFP-hph^r cut12$^+$-CFP- nat^r leu1 ura4*	1D
NHT339	*h⁻ cen2::hph^r-lacOp his7$^+$::lacI-GFP-ura4$^+$ sad1$^+$-dsRed-leu2$^+$ leu1 ura4*	2A, B and C
NHT344	*h⁻ ndc80-NH12-kan^r cen2::hph^r-lacOp his7$^+$::lacI-GFP-ura4$^+$ sad1$^+$-dsRed-leu2$^+$ leu1 ura4*	2A, B and C
NHT497	*h⁻ mad2::ura4$^+$ cen2::hph^r-lacOp his7$^+$::lacI-GFP-ura4$^+$ sad1$^+$-dsRed-leu2$^+$ leu1 ura4*	2B
NHT522	*h⁻ mad2::ura4$^+$ ndc80-NH12-kan^r cen2::hph^r-lacOp*	2B

	his7^{+}::lacI-GFP-ura4^{+} sad1^{+}-dsRed-leu2^{+} leu1 ura4	
NHT094	*h^{-} kan^{r}-GFP-atb2^{+} cut12^{+}-CFP-nat^{r} mis6^{+}-2mRFP-hph^{r} leu1 ura4*	3
NHT099	*h^{-} ndc80-NH12-kan^{r} kan^{r}-GFP-atb2^{+} cut12^{+}-CFP-nat^{r} mis6^{+}-2mRFP-hph^{r} leu1 ura4*	3
NHT225	*h^{-} dis1^{+}-GFP-ura4^{+} mis6^{+}-2mRFP-hph^{r} cut12^{+}-CFP-nat^{r} leu1 ura4*	4A
NHT275	*h^{-} ndc80-NH12-kan^{r} dis1^{+}-GFP-ura4^{+} mis6^{+}-2mRFP-hph^{r} cut12^{+}-CFP-nat^{r} leu1 ura4*	4A
NHT312	*h^{-} alp7^{+}-3GFP-kan^{r} mis6^{+}-2mRFP-hph^{r} cut12^{+}-CFP-nat^{r} leu1 ura4*	4B
NHT334	*h^{-} ndc80-NH12-kan^{r} alp7^{+}-3GFP-kan^{r} mis6^{+}-2mRFP-hph^{r} leu1 ura4*	4B
NHT222	*h^{-} alp14^{+}-GFP-kan^{r} mis6^{+}-2mRFP-hph^{r} cut12^{+}-CFP-nat^{r} leu1 ura4*	4C
NHT274	*h^{-} ndc80-NH12-kan^{r} alp14^{+}-GFP-kan^{r} mis6^{+}-2mRFP-hph^{r} cut12^{+}-CFP-nat^{r} leu1 ura4*	4C, S4
NHT259	*h- cut9-665 ndc80^{+}-3Flag-kan^{r} leu1 ura4*	5A
NHT358	*h^{-} cut9-665 ndc80^{+}-GFP-kan^{r} leu1 ura4*	5B
NHT367	*h^{-} cut9-665 alp7^{+}-13myc-hph^{r} leu1 ura4*	5B
NHT381	*h^{-} cut9-665 ndc80^{+}-GFP-kan^{r} alp7^{+}-13myc-hph^{r} leu1 ura4*	5B

NHT653	*h⁻ cut9-665 alp7⁺-13myc-hph^r leu1 ura4* containing pREP1-Ndc80	5C
NHT657	*h⁻ cut9-665 alp7⁺-13myc-hph^r leu1 ura4* containing pREP1-Ndc80-F420S	5C
NHT364	*h⁻ ndc80-NH12-kan^r nuf2⁺-alp7 (219-474)-kan^r leu1 ura4*	6A and B
NHT214	*h⁻ ndc80-NH12-kan^r nuf2⁺-alp14⁺-hph^r leu1 ura4*	6A
NHT180	*h⁻ ndc80-NH12-kan^r nuf2⁺-dis1(518-882)-nat^r leu1 ura4*	6A
NHT290	*h⁻ ndc80-NH12-kan^r nuf2⁺-alp14⁺-hph^r leu1 ura4*	6A
NHT353	*h⁻ nuf2⁺-alp7 (219-474)-kan^r leu1 ura4*	6B
NHT211	*h⁺ dis1::ura4⁺ leu1 ura4 his2*	7A
NHT108	*h⁻/ h⁺ leu1/leu1 ura4/ura4 his7/his7 ade6-216/ ade6-210*	7C
NHT306	*h⁻/ h⁺ leu1/leu1 ura4/ura4 his7/his7 ade6-216/ ade6-210 ndc80⁺/ndc80-NH12-kan^r*	7C
NHT475	*h⁻/ h⁺ leu1/leu1 ura4/ura4 his7/his7 ade6-216/ ade6-210 ndc80⁺/ndc80-21-kan^r*	7C
NHT544	*h⁻/ h⁺ leu1/leu1 ura4/ura4 his7/his7 ade6-216/ ade6-210 ndc80-NH12-kan^r/ndc80-NH12-kan^r*	7C
NHT563	*h⁻/ h⁺ leu1/leu1 ura4/ura4 his7/his7 ade6-216/ ade6-210 ndc80-21-kan^r/ndc80-21-kan^r*	7C
NHT545	*h⁻/ h⁺ leu1/leu1 ura4/ura4 his7/his7 ade6-216/ ade6-210 ndc80-NH12-kan^r/ndc80-21-kan^r*	7C

NHT700	h^+ *mis6-2mRFP-hph^r cut12$^+$-CFP-nat^r* containing pREP41-GFP-Ndc80 *leu1 ura4 his2*	S2
NHT603	h^- *ndc80-NH12-kan^r nuf2$^+$-alp7 (219-474)-kan^r alp14$^+$-GFP-kan^r mis6$^+$-2mRFP-hph^r cut12$^+$-CFP-nat^r leu1 ura4*	S4

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*Strains were developed for this study except for NHT011, NHT094, NHT108, NHT211, NHT222, NHT225, NHT259, NHT339 and wild type 513, which are from our lab stock. *his2=his2-245*; his7*=his7-366*; *leu1=leu1-32*; *ura4=ura4-D18.*

Supplemental Table S2: Plasmids used in this study

Name	Gene	Origin*
pREP1-Ndc80	*ndc80*$^{+}$	(Hsu and Toda, 2011)
pREP1-Ndc80-F420S	*ndc80-F420S*	This study
pREP41-GFP-Ndc80	*ndc80*$^{+}$	(Hsu and Toda, 2011)
pGEX-Alp7	*alp7*$^{+}$	This study
pGEX-Alp7N	*alp7N*	This study
pGEX-Alp7C	*alp7C*	This study
pGEX-Alp14	*alp14*$^{+}$	This study

Supplemental Reference

Hsu, K.S., and Toda, T. (2011). Ndc80 internal loop interacts with Dis1/TOG to ensure proper kinetochore-spindle attachment in fission yeast. Curr Biol *21*, 214-220.

Fig S1. Tang et al.

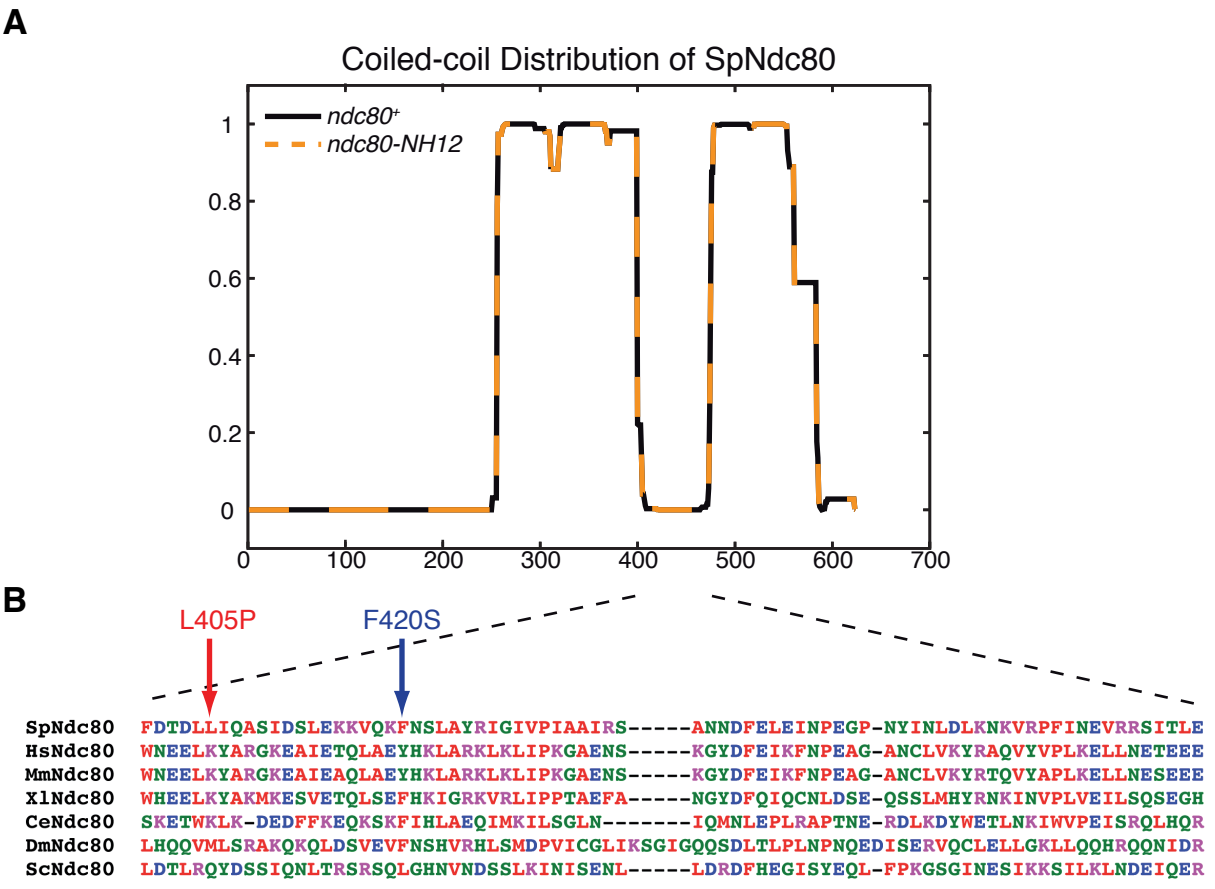

Figure S1: Coiled-coil probability and amino acid sequence of the internal loop including the mutation sites found in the two loop mutants.

(A) Coiled-coil scores (http://embnet.vital-it.ch/software/COILS_form.html) shown in wild type (black) and *ndc80-NH12* (dotted orange). Windows 28 and Matrix MTIDK were used. Note that overall coiled coil patterns are not noticeably affected by the *ndc80-NH12* mutation.

(B) Amino acid residues corresponding to the internal loop of Ndc80 from different species are aligned. The position of F420, which is mutated to S in *ndc80-NH12*, is marked with an arrow. In addition the position of L405P (*ndc80-21*) (Hsu and Toda, 2011) is also pointed. *Sp, Schizosaccharomyces pombe; Hs, Homo sapiens; Mm, Mus musculus; Xl, Xenopus laevis; Ce, Caenorhabditis elegans; Dm, Drosophila melanogaster; Sc, Saccharomyces cerevisiae.*

Fig S2. Tang et al.

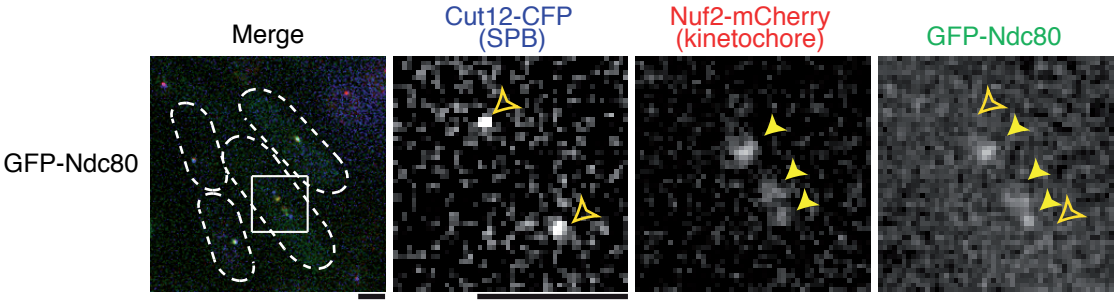

Figure S2: Overproduced Ndc80 does not localize to the mitotic SPB.

Plasmids overproducing GFP-Ndc80 (green) under the thiamine-repressing *nmt41* promoter were introduced into wild type cells containing markers for the SPB (Cut12-CFP) and outer kinetochore (Nuf2-mCherry). The promoter was derepressed by removing thiamine from minimal media, and pictures taken after 20 h incubation at 30°C. Merged images are shown in the panel on the far left (GFP-Ndc80, green; Cut12-CFP, blue; Nuf2-mCherry, red), with the squared region being presented enlarged in the three right panels showing the localization of each protein (positions of the SPBs and kinetochores are marked with open and closed arrowheads, respectively). GFP-Ndc80 precisely co-localizes with Nuf2-mCherry (kinetochore), but not Cut12-CFP (SPB). Bars, 5 µm.

Fig S3. Tang et al.

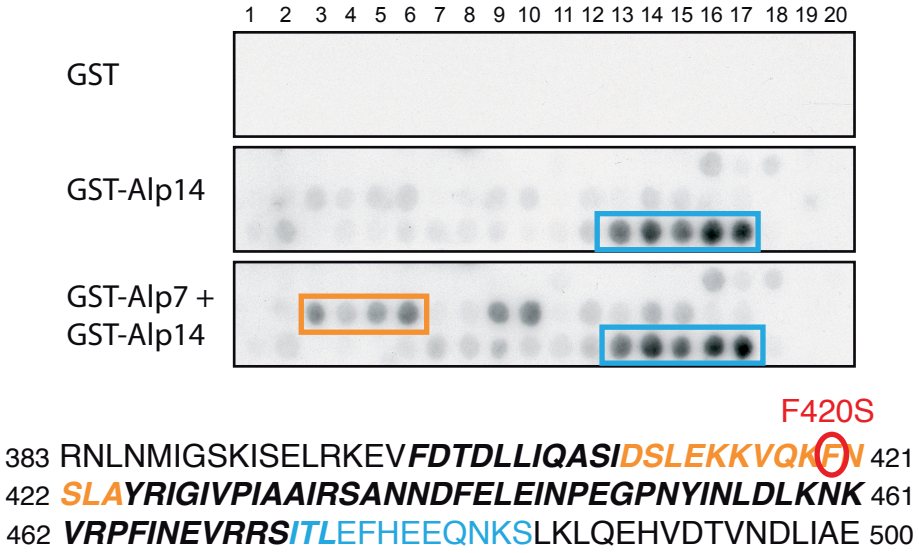

Figure S3: Peptide array assay using GST-Alp7, GST-Alp14 or both.

Peptide array assay for interactions between the loop region of Ndc80 and GST-fused Alp7 and/or GST-Alp14 proteins. GST-Alp14 (middle) or GST-Alp7 and GST-Alp14 (bottom) were used for binding assay. GST (top) acted as a negative control. Peptides that showed positive interactions are boxed in orange and blue. Amino acid sequence of the loop region (bold and italics) is shown on the bottom with two regions that corresponding positive peptides (411-424 in orange and 473-484 in blue). The position of F420 is marked by red circle that is mutated in *ndc80-NH12* (F420S).

Fig S4. Tang et al.

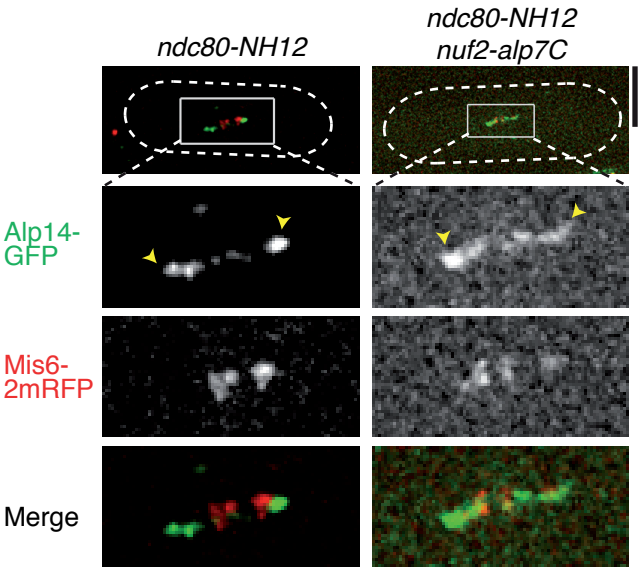

Figure S4: Tethering the C-terminal Alp7 to the outer kinetochore recruits Alp14 to the kinetochore in the *ndc80-NH12* mutant

ndc80-NH12 mutant cells that do not (left) or do carry Nuf2-Alp7C (right, see Figure 6A) were treated with 12.5 mM HU at 25°C for 4 h, filtered and incubated in HU-free media at 36°C for 1 h. These strains contained markers for the kinetochore (Mis6-2mRFP, red) and Alp14-GFP (green). After fixation with methanol, fluorescent images were taken. The top row shows representative images of individual mitotic cells (outlined), and the bottom three rows display enlarged images of the kinetochore region (squares on the top row). The positions of the SPB are marked with arrowheads in the second row. Note that Alp14 co-localizes with Mis6 in *ndc80-NH12* when this mutant contains Nuf2-Alp7C. Bar, 5 μm.