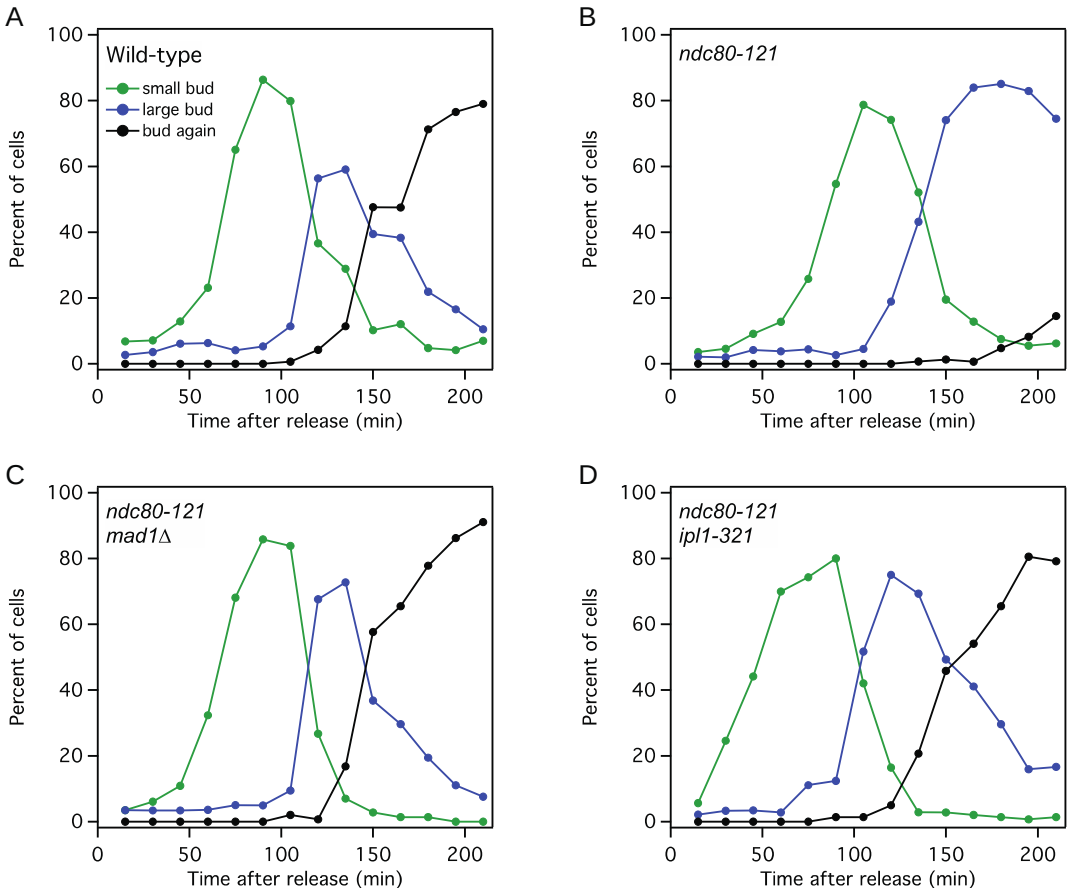

Figure S1 The mitotic arrest of *ndc80-121* cells is dependent on Ipl1 activity and the spindle checkpoint. Budding indices for (A) wild-type, (B) *ndc80-121*, (C) *ipl1-321*, and (D) *ndc80-121 ipl1-321* cells after synchronization at G1 and release into 37˚C medium. At 37˚C, large-budded *ndc80-121* cells exhibit broken spindles and fail to undergo anaphase (Figure 2A and B).

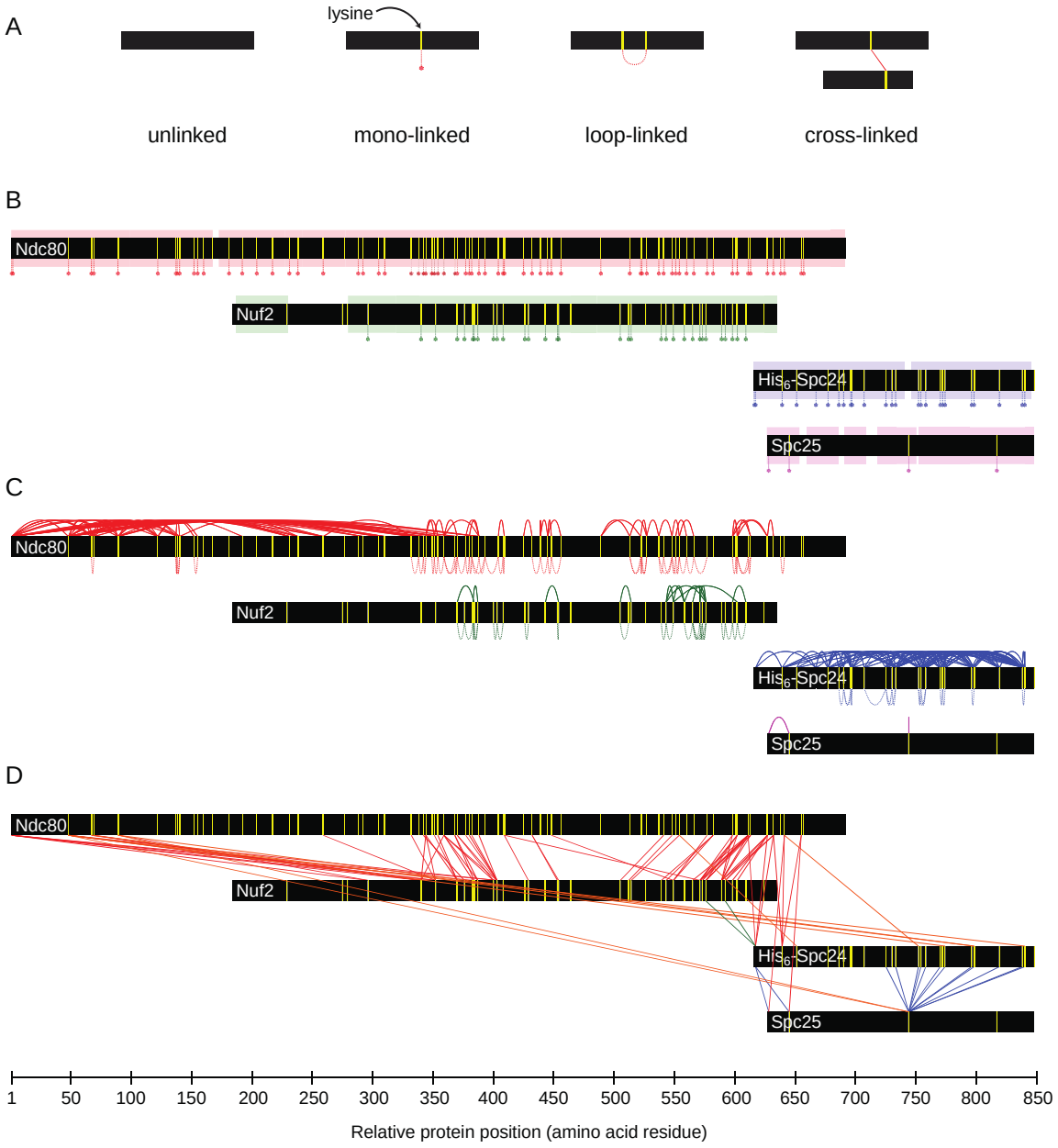

Figure S2 Summary of cross-linking study on wild-type recombinant *S. cerevisiae* Ndc80 complex. (A) After cross-linking and trypsin digestion, four possible peptides were identified by mass spectrometry. (B-D) Diagram representation of cross-linking results for Ndc80, Nuf2, His_6-Spc24, and Spc25 (black bars). (B) Peptide sequence coverage (colored boxes) and mono-links (dotted vertical lines and circles). (C) Loop-links (dotted lines) and self cross-links (solid lines, cross-links between two peptides from the same protein). (D) Cross-links between different proteins (solid line). Vertical yellow lines denote positions of lysines. Full lists of cross-linking results are shown in Tables S4 and S5.

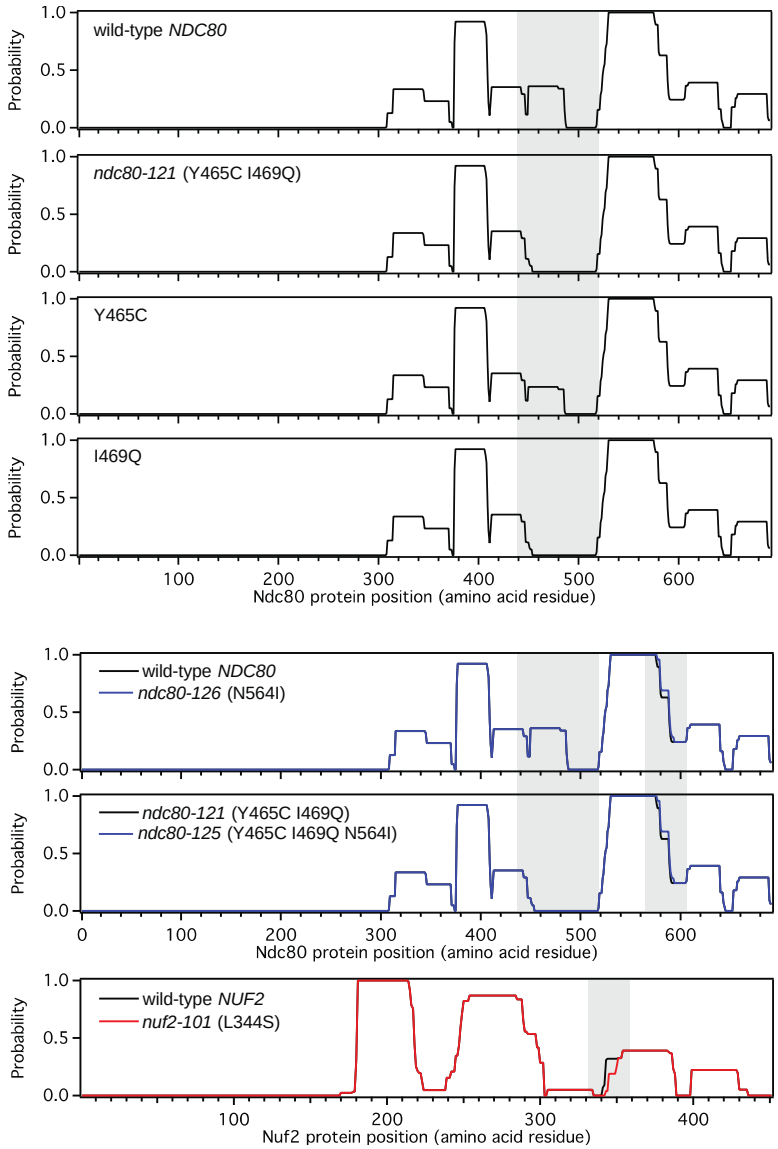

Figure S3 The effects of mutations in Ndc80 and Nuf2 on predicted coiled-coil formation. The probabilities of coiled-coil formation, as predicted by Paircoil2 (McDonnell *et al.* 2006), for Ndc80 or Nuf2 containing the indicated mutations. Where genotypes are shown (in italics), the mutations in the translated protein sequences are shown in brackets. Areas of interest are highlighted in grey boxes.

Table S1 Yeast strains used in this study[a]

Strain	Genotype	Reference
W303	*ade2-1oc can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	
CRY1	MAT**a**	GEISER *et al.* (1993)
JTY14	MAT**a** *ade3Δ-100 lys2Δ::HIS3 ndc80(Y465C)*	This study
JTY23	MAT**a** *ade3Δ-100 lys2Δ::HIS3 ndc80(I469Q)*	This study
JTY8	MAT**a** *ade3Δ-100 lys2Δ::HIS3 ndc80-121*	This study
JTY13	MAT**a** *ade3Δ-100 lys2Δ::HIS3 ndc80(S467A S468A)*	This study
JTY17	MAT**a** *ade3Δ-100 lys2Δ::HIS3 ndc80(S467D S468D)*	This study
JTY18	MAT**a** *ade3Δ-100 lys2Δ::HIS3 ndc80(D466G)*	This study
JTY30-4A	MAT**a** *ade3Δ-100 NUF2-TAP::KanMX NDC80*	This study
JTY30-1A	MAT**a** *ade3Δ-100 NUF2-TAP::KanMX ndc80-121*	This study
JTY58-2D	MAT**a** *ade3Δ-100 lys2Δ::HIS3 SPC110-mCherry::hphMX NDC80*	This study
JTY59-8D	MAT**a** *ade3Δ-100 cyh2^r lys2Δ::HIS3 SPC110-mCherry::hphMX ndc80-121*	This study
JTY96-14C	MAT**a** *ade3Δ-100 lys2Δ::HIS3 SPC110-mCherry::hphMX ndc80-121 mad1Δ::URA3*	This study
JTY98-4B	MAT**a** *ade3Δ-100 SPC110-mCherry::hphMX ndc80-121 ipl1-321*	This study
JTY9-4A	MAT**a** *ade3Δ-100 URA3::TUB1-GFP SPC110-mCherry::hphMX NDC80*	This study
JTY9-10D	MAT**a** *ade3Δ-100 lys2Δ::HIS3 URA3::TUB1-GFP SPC110-mCherry::hphMX ndc80-121*	This study
JTY59-12D	MAT**a** *ade3Δ-100 lys2Δ::HIS3 STU2-GFP::NatMX SPC110-mCherry::hphMX NDC80*	This study
JTY59-7A	MAT**a** *ade3Δ-100 lys2Δ::HIS3 STU2-GFP::NatMX SPC110-mCherry::hphMX ndc80-121*	This study
JTY73-17C	MAT**a** *ade3Δ-100 lys2Δ::HIS3 STU2-GFP::NatMX SPC110-mCherry::hphMX CDC20-AID::KanMX ura3::pADH1-OsTIR1-9myc::URA3 NDC80*	This study
JTY73-2A	MATα *ADE3 STU2-GFP::NatMX SPC110-mCherry::hphMX CDC20-AID::KanMX ura3::pADH1-OsTIR1-9myc::URA3 ndc80-121*	This study
MMWY61#2	MAT**a** *ade3Δ-100 pCUP1-GFP12LacI12::HIS3 CEN3-LacO33array::KanMX SPC110-mCherry::hphMX NDC80*	WARGACKI *et al.* (2010)
JTY65-16B	MAT**a** *ade3Δ-100 pCUP1-GFP12LacI12::HIS3 CEN3-LacO33array::KanMX SPC110-mCherry::hphMX ndc80-121*	This study
JTY112-58A	MAT**a** *ade3Δ-100 lys2Δ::HIS3 pCUP1-GFP12-LacI12::HIS3 CEN3::33LacO::KanMX SPC110-mCherry::hphMX CDC20-AID::KanMX ura3::pADH1-OsTIR1-9myc::URA3 NDC80*	This study
JTY112-20B	MAT**a** *ADE3 lys2Δ::HIS3 pCUP1-GFP12-LacI12::HIS3 CEN3::33LacO::KanMX SPC110-mCherry::hphMX CDC20-AID::KanMX ura3::pADH1-OsTIR1-9myc::URA3 ndc80-121*	This study
JTY11-5A	MAT**a** *ade3Δ-100 NUF2-GFP::HIS3 SPC110-mCherry::hphMX NDC80*	This study
JTY11-16A	MAT**a** *ade3Δ-100 NUF2-GFP::HIS3 SPC110-mCherry::hphMX ndc80-121*	This study
MSY284-8D	MAT**a** *ade3Δ-100 NUF2-GFP::HIS3 SPC110-mCherry::hphMX ipl1-321*	Michelle M. Shimogawa
JTY98-13D	MAT**a** *ade3Δ-100 NUF2-GFP::HIS3 SPC110-mCherry::hphMX ndc80-121 ipl1-321*	This study
KGY315	MAT**a**/MATα *ADE3/ade3Δ-100 cyh2^r/CYH2^s*	GREENLAND *et al.* (2010)
JTY82	MAT**a**/MATα *ade3Δ-100/ade3Δ-100 cyh2^r/cyh2^r lys2Δ::HIS3/lys2Δ::HIS3 ndc80-121/ndc80-121*	This study
JTY74	MAT**a**/MATα *ade3Δ-100/ADE3 cyh2^r/CYH2^s lys2Δ::HIS3/LYS2 ndc80-121/NDC80*	This study
JTY83	MAT**a** *ade3Δ-100 lys2Δ::HIS3 ndc80-125*	This study
JTY81	MAT**a**/MATα *ade3Δ-100/ade3Δ-100 cyh2^r/cyh2^r lys2Δ::HIS3/lys2Δ::HIS3 ndc80-121/ndc80-125*	This study

Strain	Genotype	Reference
JTY102	MAT**a**/MATα *ade3Δ-100/ade3Δ-100 cyh2^r/cyh2^r lys2Δ::HIS3/lys2Δ::HIS3 ndc80-125/ndc80-125*	This study
JTY79	MAT**a**/MATα *ade3Δ-100/ADE3 cyh2^r/CYH2^s lys2Δ::HIS3/LYS2 ndc80-125/NDC80*	This study
JTY114	MAT**a** *ade3Δ-100 cyh2^r lys2Δ::HIS3 ndc80-126*	This study
JTY116	MAT**a**/MATα *ade3Δ-100/ade3Δ-100 cyh2^r/cyh2^r lys2Δ::HIS3/lys2Δ::HIS3 ndc80-126/ndc80-121*	This study
JTY84	MAT**a** *ade3Δ-100 lys2Δ::HIS3 ndc80-121 nuf2-101*	This study
JTY86-5C	MAT**a** *ade3Δ-100 cyh2^r lys2Δ::HIS3 NDC80 nuf2-101*	This study
JTY88	MAT**a**/MATα *ade3Δ-100/ade3Δ-100 cyh2^r/cyh2^r lys2Δ::HIS3/lys2Δ::HIS3 ndc80-121/ndc80-121 nuf2-101/NUF2*	This study
JTY101-6B	MAT**a** *ade3Δ-100 cyh2^r lys2Δ::HIS3 ndc80-125 nuf2-101*	This study
JTY101	MAT**a**/MATα *ade3Δ-100/ade3Δ-100 cyh2^r/cyh2^r lys2Δ::HIS3/lys2Δ::HIS3 ndc80-121/ndc80-125 nuf2-101/NUF2*	This study

[a]All strains have the same markers as W303 except as noted

Table S2 Temperature-sensitive mutants in a lethal insertion cluster identified by linker-scanning mutagenesis

Insertion (first mutation) or Mutation	Sequence[a]	Growth		
		25˚C	30°C	37°C
CGRRQ (Y465C)	TLRQ**CGRRQ**YDSS	++	-	-
CGRKY (D466C)	LRQY**CGRKY**DSSI	+++	+	-
CGRND (S467C)	RQYD**CGRND**SSIQ	+++	+	-
CGRNS (S468C)	QYDS**CGRNS**SIQN	+++	++	-
MRPQS (I469M)	YDSS**MRPQS**IQNL	+++	+++	-
Wild-type	TLRQYDSSIQNL	+++	+++	+++
Y465C	TLRQ**C**DSSIQNL	+++	+++	+++
I469Q	TLRQYDSS**Q**QNL	+++	+++	+++
Y465C I469Q[b]	TLRQ**C**DSS**Q**QNL	+++	+++	-
S467A S468A	TLRQYD**AA**IQNL	+++	+++	+++
S467D S468D	TLRQYD**DD**IQNL	+++	+++	+++
D466G	TLRQY**G**SSIQNL	+++	+++	+++

[a]Insertions and mutations are in bold text
[b]*ndc80-121* allele

Table S3 Immunoprecipitation of Ndc80 complex from wild-type and *ndc80-121* cells

Hit Protein	Wild-type[a]			*ndc80-121*[a]		
	Sequence coverage	Spectrum count (SC)	Normalized SC	Sequence coverage	Spectrum count (SC)	Normalized SC
Ndc80	54%	242	2.18	75%	523	1.10
Spc24	85%	268	2.41	81%	394	0.83
Nuf2-TAP	48%	111	1	57%	475	1
Spc25	50%	92	0.83	55%	284	0.60

[a]Asynchronous cultures were shifted to 37˚C for 100 min. Immunoprecipitated proteins, from a Nuf2-TAP pull-down, were identified by mass spectrometry.

Table S4 Recombinant Ndc80 complex cross-links

Protein 1	Position 1[a]	Protein 2	Position 2[a]	No. PSMs[b]	No. Peptides	Best Peptide *q*-value
Ndc80	1	Ndc80	48	62	1	0
Ndc80	1	Ndc80	67	15	2	0
Ndc80	1	Ndc80	69	7	1	0
Ndc80	1	Ndc80	89	21	1	0
Ndc80	1	Ndc80	122	4	2	0
Ndc80	1	Ndc80	140	11	1	0
Ndc80	1	Ndc80	259	4	1	0.001
Ndc80	1	Ndc80	292	3	1	0
Ndc80	1	Ndc80	305	32	1	0
Ndc80	1	Ndc80	310	6	1	0
Ndc80	1	Ndc80	332	3	1	0
Ndc80	1	Ndc80	338	27	1	0
Ndc80	1	Ndc80	342	1	1	0.008
Ndc80	1	Ndc80	344	14	1	0
Ndc80	1	Ndc80	354	1	1	0.006
Ndc80	1	Ndc80	359	24	1	0
Ndc80	1	Ndc80	370	16	1	0
Ndc80	1	Ndc80	388	2	1	0.001
Ndc80	1	Nuf2	113	3	1	0.004
Ndc80	1	Nuf2	157	19	1	0
Ndc80	1	Nuf2	169	4	1	0
Ndc80	1	Nuf2	200	1	1	0.013
Ndc80	1	Nuf2	220	11	1	0
Ndc80	48	Ndc80	67	21	2	0
Ndc80	48	Ndc80	69	3	1	0.008
Ndc80	48	Ndc80	89	14	1	0
Ndc80	48	Ndc80	122	2	1	0.001
Ndc80	48	Ndc80	138	3	1	0.004
Ndc80	48	Ndc80	140	2	1	0
Ndc80	48	Ndc80	231	5	1	0
Ndc80	48	Ndc80	238	2	1	0.004
Ndc80	48	Ndc80	259	5	1	0
Ndc80	48	Ndc80	292	2	1	0.002
Ndc80	48	Ndc80	305	12	1	0
Ndc80	48	Ndc80	310	1	1	0.043
Ndc80	48	Ndc80	332	1	1	0.005
Ndc80	48	Ndc80	338	12	1	0
Ndc80	48	Ndc80	354	4	1	0.001
Ndc80	48	Ndc80	388	2	1	0.001
Ndc80	48	Nuf2	157	12	1	0
Ndc80	48	Nuf2	169	6	2	0
Ndc80	48	Nuf2	220	1	1	0
Ndc80	48	His_6-Spc24	159	1	1	0.023
Ndc80	48	His_6-Spc24	225	1	1	0.006
Ndc80	67	Ndc80	89	21	2	0
Ndc80	67	Ndc80	122	1	1	0.037
Ndc80	67	Nuf2	157	6	2	0

Protein 1	Position 1[a]	Protein 2	Position 2[a]	No. PSMs[b]	No. Peptides	Best Peptide *q*-value
Ndc80	67	His_6-Spc24	183	2	1	0.004
Ndc80	69	Ndc80	89	5	1	0
Ndc80	69	Ndc80	122	1	1	0.002
Ndc80	69	Ndc80	231	1	1	0.036
Ndc80	69	Nuf2	157	3	1	0.001
Ndc80	89	Ndc80	122	11	2	0
Ndc80	89	Ndc80	138	8	1	0
Ndc80	89	Ndc80	140	2	1	0
Ndc80	89	Ndc80	192	2	1	0.001
Ndc80	89	Ndc80	231	10	2	0
Ndc80	89	Ndc80	238	1	1	0.004
Ndc80	89	Ndc80	259	1	1	0
Ndc80	89	Nuf2	113	4	1	0
Ndc80	89	His_6-Spc24	183	1	1	0.003
Ndc80	122	Ndc80	152	28	4	0
Ndc80	122	Ndc80	155	40	2	0
Ndc80	137	Ndc80	140	18	3	0
Ndc80	140	Ndc80	238	1	1	0.003
Ndc80	259	Ndc80	332	13	1	0
Ndc80	259	Nuf2	169	38	4	0
Ndc80	332	Nuf2	169	3	1	0
Ndc80	342	Nuf2	193	3	1	0.003
Ndc80	344	Ndc80	351	8	1	0.012
Ndc80	344	Ndc80	354	14	1	0.001
Ndc80	344	Nuf2	157	3	1	0.002
Ndc80	344	Nuf2	169	23	4	0
Ndc80	344	Nuf2	187	1	1	0.03
Ndc80	351	Ndc80	359	21	2	0
Ndc80	351	Nuf2	187	62	4	0.001
Ndc80	354	Nuf2	187	52	4	0
Ndc80	359	Ndc80	370	66	2	0
Ndc80	359	Ndc80	388	1	1	0.004
Ndc80	359	Nuf2	157	12	1	0
Ndc80	359	Nuf2	200	20	3	0
Ndc80	359	Nuf2	204	1	1	0
Ndc80	359	Nuf2	217	3	1	0
Ndc80	359	Nuf2	220	7	1	0.001
Ndc80	368	Nuf2	201	2	1	0.003
Ndc80	368	Nuf2	204	40	2	0
Ndc80	370	Nuf2	217	40	2	0.001
Ndc80	370	Nuf2	220	118	2	0
Ndc80	377	Ndc80	388	73	3	0.001
Ndc80	377	Nuf2	220	70	1	0.001
Ndc80	380	Ndc80	388	7	1	0.003
Ndc80	380	Nuf2	220	9	1	0.002
Ndc80	382	Nuf2	220	12	1	0.001
Ndc80	388	Nuf2	220	5	1	0.002
Ndc80	404	Ndc80	409	11	1	0

Protein 1	Position 1[a]	Protein 2	Position 2[a]	No. PSMs[b]	No. Peptides	Best Peptide *q*-value
Ndc80	409	Nuf2	246	5	2	0
Ndc80	409	Nuf2	388	1	1	0.012
Ndc80	425	Ndc80	432	6	2	0.003
Ndc80	432	Nuf2	270	8	1	0.002
Ndc80	432	Nuf2	271	1	1	0.009
Ndc80	439	Ndc80	439	1	1	0.011
Ndc80	439	Ndc80	445	2	1	0.023
Ndc80	445	Ndc80	448	51	3	0
Ndc80	445	Ndc80	456	1	1	0.032
Ndc80	448	Nuf2	366	2	1	0.01
Ndc80	489	Ndc80	513	6	2	0
Ndc80	489	Ndc80	522	1	1	0.003
Ndc80	513	Ndc80	527	8	2	0
Ndc80	522	Ndc80	527	6	1	0.001
Ndc80	527	Ndc80	537	1	1	0.005
Ndc80	537	Ndc80	548	14	1	0.008
Ndc80	541	Nuf2	322	8	2	0
Ndc80	548	Ndc80	554	34	2	0
Ndc80	548	Nuf2	329	1	1	0.004
Ndc80	551	Ndc80	560	2	1	0.009
Ndc80	554	Ndc80	566	1	1	0.004
Ndc80	554	Nuf2	331	205	1	0
Ndc80	554	His_6-Spc24	36	1	1	0.022
Ndc80	577	Nuf2	366	23	4	0
Ndc80	582	Nuf2	356	5	1	0.001
Ndc80	582	Nuf2	360	33	3	0
Ndc80	582	Nuf2	366	6	1	0.001
Ndc80	598	Ndc80	602	15	1	0.001
Ndc80	598	Ndc80	613	1	1	0.02
Ndc80	598	Ndc80	627	2	1	0.013
Ndc80	598	Nuf2	388	6	1	0
Ndc80	598	Nuf2	406	2	1	0.001
Ndc80	602	Ndc80	613	56	3	0
Ndc80	602	Ndc80	627	2	1	0.001
Ndc80	602	Nuf2	388	20	1	0.001
Ndc80	602	Nuf2	390	3	1	0
Ndc80	602	Nuf2	393	12	2	0
Ndc80	602	Nuf2	406	2	1	0
Ndc80	611	Nuf2	382	68	2	0
Ndc80	611	Nuf2	388	13	3	0
Ndc80	611	Nuf2	390	107	4	0
Ndc80	611	Nuf2	393	76	3	0
Ndc80	611	Nuf2	406	3	1	0
Ndc80	611	His_6-Spc24	2	5	1	0
Ndc80	613	Nuf2	388	27	2	0
Ndc80	613	Nuf2	393	4	1	0
Ndc80	613	Nuf2	406	3	1	0.001
Ndc80	627	Ndc80	632	10	1	0

Protein 1	Position 1[a]	Protein 2	Position 2[a]	No. PSMs[b]	No. Peptides	Best Peptide *q*-value
Ndc80	627	Nuf2	406	1	1	0.009
Ndc80	627	Nuf2	409	14	4	0
Ndc80	627	Nuf2	415	76	6	0
Ndc80	627	Nuf2	419	14	1	0.001
Ndc80	627	His_6-Spc24	2	2	1	0
Ndc80	632	Nuf2	406	20	2	0
Ndc80	632	Nuf2	409	30	4	0
Ndc80	632	His_6-Spc24	2	2	1	0.005
Ndc80	632	His_6-Spc24	24	20	1	0
Ndc80	641	His_6-Spc24	24	64	2	0
Ndc80	641	His_6-Spc24	137	3	1	0
Ndc80	655	His_6-Spc24	24	23	1	0
Nuf2	187	Nuf2	201	1	1	0.002
Nuf2	200	Nuf2	204	19	1	0
Nuf2	260	Nuf2	271	11	1	0
Nuf2	322	Nuf2	331	1	1	0
Nuf2	360	Nuf2	366	1	1	0.047
Nuf2	360	Nuf2	375	2	1	0.013
Nuf2	360	Nuf2	393	1	1	0.011
Nuf2	366	Nuf2	375	7	1	0.001
Nuf2	366	Nuf2	419	4	1	0.003
Nuf2	375	Nuf2	393	1	1	0.044
Nuf2	382	Nuf2	393	3	1	0
Nuf2	388	Nuf2	388	1	1	0
Nuf2	388	Nuf2	393	12	2	0
Nuf2	393	His_6-Spc24	2	3	1	0.011
Nuf2	409	His_6-Spc24	2	1	1	0.001
Nuf2	415	Nuf2	426	16	1	0
His_6-Spc24	2	His_6-Spc24	24	50	1	0
His_6-Spc24	2	His_6-Spc24	52	11	1	0
His_6-Spc24	24	His_6-Spc24	52	1	1	0.014
His_6-Spc24	24	His_6-Spc24	118	2	1	0.006
His_6-Spc24	24	His_6-Spc24	143	1	1	0.003
His_6-Spc24	24	His_6-Spc24	183	2	1	0.002
His_6-Spc24	36	His_6-Spc24	62	1	1	0.002
His_6-Spc24	36	His_6-Spc24	118	5	1	0.001
His_6-Spc24	36	His_6-Spc24	225	4	1	0.003
His_6-Spc24	52	His_6-Spc24	62	16	1	0
His_6-Spc24	52	His_6-Spc24	118	1	1	0.009
His_6-Spc24	62	His_6-Spc24	71	7	2	0
His_6-Spc24	62	His_6-Spc24	75	4	1	0.009
His_6-Spc24	62	His_6-Spc24	81	1	1	0.009
His_6-Spc24	62	His_6-Spc24	82	1	1	0.004
His_6-Spc24	62	His_6-Spc24	137	1	1	0.001
His_6-Spc24	62	His_6-Spc24	183	4	1	0.002
His_6-Spc24	62	His_6-Spc24	225	1	1	0.013
His_6-Spc24	71	His_6-Spc24	81	12	1	0.014
His_6-Spc24	71	His_6-Spc24	82	6	1	0.002

Protein 1	Position 1[a]	Protein 2	Position 2[a]	No. PSMs[b]	No. Peptides	Best Peptide *q*-value
His_6-Spc24	71	His_6-Spc24	118	1	1	0.027
His_6-Spc24	71	His_6-Spc24	137	4	1	0.039
His_6-Spc24	75	His_6-Spc24	82	23	1	0.005
His_6-Spc24	82	His_6-Spc24	92	18	2	0
His_6-Spc24	82	His_6-Spc24	110	4	1	0
His_6-Spc24	82	His_6-Spc24	118	4	1	0.002
His_6-Spc24	82	His_6-Spc24	137	6	1	0.004
His_6-Spc24	82	His_6-Spc24	225	5	1	0.01
His_6-Spc24	92	His_6-Spc24	118	5	2	0
His_6-Spc24	92	His_6-Spc24	155	1	1	0.004
His_6-Spc24	92	His_6-Spc24	225	1	1	0.001
His_6-Spc24	110	His_6-Spc24	118	425	1	0
His_6-Spc24	110	His_6-Spc24	137	2	1	0
His_6-Spc24	110	His_6-Spc24	139	6	1	0
His_6-Spc24	110	His_6-Spc24	143	5	1	0
His_6-Spc24	110	His_6-Spc24	155	5	1	0.004
His_6-Spc24	110	His_6-Spc24	204	1	1	0.002
His_6-Spc24	110	His_6-Spc24	225	5	1	0
His_6-Spc24	115	His_6-Spc24	225	1	1	0.001
His_6-Spc24	118	His_6-Spc24	139	4	1	0.016
His_6-Spc24	118	His_6-Spc24	143	6	1	0.001
His_6-Spc24	118	His_6-Spc24	155	11	1	0.002
His_6-Spc24	118	His_6-Spc24	159	6	1	0
His_6-Spc24	118	His_6-Spc24	181	3	1	0.001
His_6-Spc24	118	His_6-Spc24	183	8	1	0.018
His_6-Spc24	118	His_6-Spc24	204	6	1	0
His_6-Spc24	118	His_6-Spc24	223	7	2	0.001
His_6-Spc24	118	His_6-Spc24	225	12	1	0.001
His_6-Spc24	137	His_6-Spc24	143	10	1	0.002
His_6-Spc24	137	His_6-Spc24	155	14	1	0.001
His_6-Spc24	137	His_6-Spc24	159	5	1	0
His_6-Spc24	137	His_6-Spc24	183	7	1	0.017
His_6-Spc24	137	His_6-Spc24	204	2	1	0.005
His_6-Spc24	137	His_6-Spc24	225	5	1	0.003
His_6-Spc24	139	His_6-Spc24	155	10	1	0.002
His_6-Spc24	139	His_6-Spc24	159	12	1	0
His_6-Spc24	139	His_6-Spc24	181	2	1	0.001
His_6-Spc24	139	His_6-Spc24	204	6	1	0
His_6-Spc24	139	His_6-Spc24	223	2	1	0.001
His_6-Spc24	139	His_6-Spc24	225	5	1	0.013
His_6-Spc24	143	His_6-Spc24	159	23	1	0
His_6-Spc24	143	His_6-Spc24	181	1	1	0.001
His_6-Spc24	143	His_6-Spc24	183	19	1	0
His_6-Spc24	143	His_6-Spc24	204	6	1	0
His_6-Spc24	143	His_6-Spc24	223	3	1	0.002
His_6-Spc24	143	His_6-Spc24	225	2	1	0.007
His_6-Spc24	155	His_6-Spc24	159	4	1	0.001
His_6-Spc24	155	His_6-Spc24	181	6	1	0.001

Protein 1	Position 1[a]	Protein 2	Position 2[a]	No. PSMs[b]	No. Peptides	Best Peptide *q*-value
His_6-Spc24	155	His_6-Spc24	183	6	1	0.001
His_6-Spc24	155	His_6-Spc24	204	6	1	0.002
His_6-Spc24	155	His_6-Spc24	223	3	1	0.015
His_6-Spc24	155	His_6-Spc24	225	8	1	0.002
His_6-Spc24	159	His_6-Spc24	159	1	1	0.004
His_6-Spc24	159	His_6-Spc24	183	19	1	0
His_6-Spc24	159	His_6-Spc24	204	7	1	0
His_6-Spc24	159	His_6-Spc24	223	1	1	0
His_6-Spc24	159	His_6-Spc24	225	8	1	0
His_6-Spc24	181	His_6-Spc24	204	5	1	0
His_6-Spc24	181	His_6-Spc24	223	1	1	0.001
His_6-Spc24	181	His_6-Spc24	225	4	1	0
His_6-Spc24	183	His_6-Spc24	204	23	1	0
His_6-Spc24	183	His_6-Spc24	223	4	2	0.002
His_6-Spc24	183	His_6-Spc24	225	15	1	0.007
His_6-Spc24	204	His_6-Spc24	225	11	1	0
His_6-Spc24	223	His_6-Spc24	225	1	1	0.002
His_6-Spc24	225	His_6-Spc24	225	6	1	0.003
Spc25	2	Spc25	19	4	1	0
Spc25	2	Ndc80	641	37	1	0
Spc25	2	His_6-Spc24	2	15	1	0
Spc25	19	Ndc80	655	36	3	0
Spc25	19	His_6-Spc24	2	23	1	0.001
Spc25	118	Spc25	118	5	1	0
Spc25	118	Ndc80	48	4	1	0
Spc25	118	Ndc80	89	4	1	0
Spc25	118	His_6-Spc24	110	3	1	0
Spc25	118	His_6-Spc24	118	3	1	0.002
Spc25	118	His_6-Spc24	137	15	1	0
Spc25	118	His_6-Spc24	139	8	1	0.004
Spc25	118	His_6-Spc24	143	25	1	0
Spc25	118	His_6-Spc24	155	17	1	0.001
Spc25	118	His_6-Spc24	159	19	1	0
Spc25	118	His_6-Spc24	181	1	1	0.002
Spc25	118	His_6-Spc24	183	10	1	0.004
Spc25	118	His_6-Spc24	204	13	1	0
Spc25	118	His_6-Spc24	223	4	2	0.001
Spc25	118	His_6-Spc24	225	8	1	0

[a]Wild-type sequence of Spc24 starts at residue 21 due to presence of His_6 tag
[b]PSM, peptide spectrum match

Table S5 Recombinant Ndc80 complex loop-links

Protein	Position 1[a]	Position 2[a]	No. PSMs[b]	No. Peptides	Best Peptide *q*-value
Ndc80	67	69	19	1	0
Ndc80	137	138	41	4	0
Ndc80	137	140	8	2	0
Ndc80	138	140	18	2	0
Ndc80	152	155	25	2	0
Ndc80	332	338	6	1	0
Ndc80	338	342	20	3	0
Ndc80	342	344	7	1	0.002
Ndc80	349	351	3	2	0.002
Ndc80	351	354	37	6	0
Ndc80	354	359	14	2	0
Ndc80	359	368	4	2	0.002
Ndc80	359	370	8	2	0.002
Ndc80	368	370	2	1	0
Ndc80	370	377	8	1	0.002
Ndc80	377	380	32	1	0.001
Ndc80	380	382	2	1	0.001
Ndc80	382	388	7	1	0
Ndc80	382	393	1	1	0.002
Ndc80	388	393	1	1	0.001
Ndc80	393	404	28	3	0
Ndc80	404	408	20	1	0
Ndc80	408	409	24	1	0
Ndc80	432	439	1	1	0.001
Ndc80	439	445	14	2	0
Ndc80	445	448	15	3	0
Ndc80	448	456	7	1	0
Ndc80	513	522	8	1	0
Ndc80	513	523	9	1	0
Ndc80	522	523	14	3	0
Ndc80	522	527	1	1	0.001
Ndc80	523	527	20	1	0
Ndc80	537	541	5	1	0.001
Ndc80	537	548	1	1	0.023
Ndc80	541	548	3	2	0.001
Ndc80	548	551	26	4	0
Ndc80	548	554	2	2	0.001
Ndc80	551	554	60	4	0
Ndc80	554	560	6	1	0
Ndc80	560	566	9	3	0
Ndc80	566	577	4	1	0.002
Ndc80	598	601	18	2	0
Ndc80	601	602	12	2	0
Ndc80	602	611	1	1	0.002
Ndc80	602	613	2	2	0.002
Ndc80	611	613	12	3	0
Ndc80	638	641	60	1	0

Protein	Position 1[a]	Position 2[a]	No. PSMs[b]	No. Peptides	Best Peptide *q*-value
Nuf2	187	193	2	1	0.005
Nuf2	193	200	30	1	0
Nuf2	200	201	62	3	0
Nuf2	200	204	1	1	0.002
Nuf2	201	204	23	1	0
Nuf2	217	220	97	1	0
Nuf2	220	225	3	1	0
Nuf2	243	246	76	3	0
Nuf2	270	271	7	1	0
Nuf2	322	329	52	2	0
Nuf2	356	360	48	2	0
Nuf2	360	366	1	1	0.001
Nuf2	375	382	24	1	0
Nuf2	382	388	12	2	0
Nuf2	382	390	13	2	0
Nuf2	382	393	3	1	0.001
Nuf2	388	390	112	3	0
Nuf2	388	393	18	2	0
Nuf2	390	393	96	2	0
Nuf2	406	409	39	2	0
Nuf2	409	415	1	1	0.003
Nuf2	415	419	39	2	0
Nuf2	419	426	15	1	0
His_6-Spc24	71	75	26	2	0
His_6-Spc24	75	81	3	2	0.002
His_6-Spc24	75	82	2	1	0.004
His_6-Spc24	81	82	15	2	0
His_6-Spc24	92	110	10	2	0.001
His_6-Spc24	110	115	20	2	0
His_6-Spc24	110	118	21	1	0
His_6-Spc24	115	118	30	2	0
His_6-Spc24	137	139	13	2	0
His_6-Spc24	137	143	8	1	0
His_6-Spc24	139	143	33	1	0
His_6-Spc24	155	157	22	3	0
His_6-Spc24	157	159	33	1	0
His_6-Spc24	181	183	18	1	0
His_6-Spc24	223	225	17	2	0

[a]Wild-type sequence of Spc24 starts at residue 21 due to presence of His_6 tag
[b]PSM, peptide spectrum match

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