Supplementary Information

(9 Figures and 3 Tables)

Prolyl isomerization of the CENP-A N-terminus regulates centromeric integrity in fission yeast

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Supplementary Tables

Supplementary Table S1 List of strains ectopically expressing alanine point-mutation constructs, single copy integration strains and epitope-tagged strains.

Overexpression strains - Site directed amino acids substitution							
Name	**Host Strain**	**Transform plasmid**	***his***	***lys***	***leu***	***ura***	***ade***
K3A	*HF123*	*pREP41-K3A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
K4A	*HF123*	*pREP41-K4A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
S5A	*HF123*	*pREP41-S5A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
L6A	*HF123*	*pREP41-L6A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
M7A	*HF123*	*pREP41-M7A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
E9A	*HF123*	*pREP41-E9A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
P10A	*HF123*	*pREP41-P10A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
G11A	*HF123*	*pREP41-G11A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
D12A	*HF123*	*pREP41-D12A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
P13A	*HF123*	*pREP41-P13A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
I14A	*HF123*	*pREP41-I14A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
P15A	*HF123*	*pREP41-P15A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
R16A	*HF123*	*pREP41-R16A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
P17A	*HF123*	*pREP41-P17A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
R18A	*HF123*	*pREP41-R18A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
K19A	*HF123*	*pREP41-K19A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
K20A	*HF123*	*pREP41-K20A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
R21A	*HF123*	*pREP41-R21A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
Y22A	*HF123*	*pREP41-Y22A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
R23A	*HF123*	*pREP41-R23A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
P24A	*HF123*	*pREP41-P24A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
G25A	*HF123*	*pREP41-G25A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
T26A	*HF123*	*pREP41-T26A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*

T27A	*HF123*	*pREP41-T27A*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
4PA-GFP	*HF123*	*pREP41-4PA-GFP*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
FL-GFP	*HF123*	*pREP41-FL-GFP*	*his+*	*lys+*	*leu+*	*ura+*	*ade+*
Overexpression strains - Suppression							
Name	**Host Strain**	**Transform plasmid**	***his***	***lys***	***leu***	***ura***	***ade***
Δani1-vector	*Δani1*	*pREP41*	*his+*	*-*	*leu+*	*-*	*ade-*
Δani1-FL	*Δani1*	*pREP41-ani1-FL*	*his+*	*-*	*leu+*	*-*	*ade-*
Δani1-ΔPPIase	*Δani1*	*pREP41-ani1-ΔPPIase*	*his+*	*-*	*leu+*	*-*	*ade-*
Δani2-vector	*Δani2*	*pREP41*	*his+*	*-*	*leu+*	*ura+*	*ade+*
Δani2-FL	*Δani2*	*pREP41-ani2FL*	*his+*	*-*	*leu+*	*ura+*	*ade+*
Δani2- ΔPPIase	*Δani2*	*pREP41-ani2-ΔPPIase*	*his+*	*-*	*leu+*	*ura+*	*ade+*
WT-vector	*HF123*	*pREP41*	*his+*	*-*	*leu+*	*ura+*	*ade+*
WT-sim3⁺	*HF123*	*pREP41-sim3HIS*	*his+*	*-*	*leu+*	*ura+*	*ade+*
P13A-vector	*P13A*	*pREP41*	*his+*	*-*	*leu+*	*ura+*	*ade+*
P13A-sim3⁺	*P13A*	*pREP41-sim3HIS*	*his+*	*-*	*leu+*	*ura+*	*ade+*
P15A-vector	*P15A*	*pREP41*	*his+*	*-*	*leu+*	*ura+*	*ade-*
P15A-sim3⁺	*P15A*	*pREP41-sim3HIS*	*his+*	*-*	*leu+*	*ura+*	*ade-*
P13AP15A-vector	*P13AP15A*	*pREP41*	*his+*	*-*	*leu+*	*ura+*	*ade+*
P13AP15A-sim3⁺	*P13AP15A*	*pREP41-sim3HIS*	*his+*	*-*	*leu+*	*ura+*	*ade+*
Δani1Δani2-vector	*Δani1Δani2*	*pREP41*	*his+*	*-*	*leu+*	*ura+*	*ade+*
Δani1Δani2-sim3⁺	*Δani1Δani2*	*pREP41-sim3HIS*	*his+*	*-*	*leu+*	*ura+*	*ade+*

Single Copy Integrant Strain / Deletion strains					
Name	**Locus of integration**	**Mutation**	***lys***	***leu***	***ura***

cnp1-4PA	*cnp1*$^{+}$	P10A,P13A,P15A,P17A	*lys+*	*leu-*	*ura+*
cnp1-P13A	*cnp1*$^{+}$	P13A	-	*leu-*	*ura+*
cnp1-P15A	*cnp1*$^{+}$	P15A	*lys+*	*leu-*	*ura+*
cnp1-P13AP15A	*cnp1*$^{+}$	P13A,P15A	-	*leu-*	*ura-*
cnp1-WT	*cnp1*$^{+}$	-	-	*leu-*	*ura+*
cnp1-E9A	*cnp1*$^{+}$	E9A	*lys-*	*leu-*	*ura-*
cnp1-P10A	*cnp1*$^{+}$	P10A	*lys-*	*leu-*	*ura-*
cnp1-G11A	*cnp1*$^{+}$	G11A	*lys-*	*leu-*	*ura-*
cnp1-D12A	*cnp1*$^{+}$	D12A	*lys-*	*leu-*	*ura-*
cnp1-I14A	*cnp1*$^{+}$	I14A	*lys-*	*leu-*	*ura-*
cnp1-R16A	*cnp1*$^{+}$	R16A	*lys-*	*leu-*	*ura-*
cnp1-P17A	*cnp1*$^{+}$	P17A	*lys-*	*leu-*	*ura-*
cnp1-R18A	*cnp1*$^{+}$	R18A	*lys-*	*leu-*	*ura-*
cnp1-K19A	*cnp1*$^{+}$	K19A	*lys-*	*leu-*	*ura-*
cnp1-K20A	*cnp1*$^{+}$	K20A	*lys-*	*leu-*	*ura-*
cnp1-R21A	*cnp1*$^{+}$	R21A	*lys-*	*leu-*	*ura-*
cnp1-Y22A	*cnp1*$^{+}$	Y22A	*lys-*	*leu-*	*ura-*
Δani1	*ani1*$^{+}$	*ani1*$^{+}$*::kanR*$^{+}$	-	*leu-*	-
Δani2	*ani2*$^{+}$	*ani2*$^{+}$*::kanR*$^{+}$	-	*leu-*	*ura+*
Δani1	*ani1*$^{+}$	*ani1*$^{+}$*::kanR*$^{+}$	*lys+*	*leu+*	*ura+*
Δani2	*ani2*$^{+}$	*ani2*$^{+}$*::kanR*$^{+}$	*lys+*	*leu+*	*ura+*
Δani1Δani2	*ani1*$^{+}$*ani2*$^{+}$	*ani1*$^{+}$*::kanR*$^{+}$, *ani2*$^{+}$*::kanR*$^{+}$	*lys+*	*leu+*	*ura+*
Δani1Δani2	*ani1*$^{+}$*ani2*$^{+}$	*ani1*$^{+}$*::kanR*$^{+}$, *ani2*$^{+}$*::kanR*$^{+}$	*lys+*	*leu-*	*ura+*

Epitope-tagged Strains

Name	Tagged Gene/locus	Tag	Gene	Mutations
WT CEN1-GFP	centromere I	GFP	*cnp1*$^{+}$	
4PA CEN1-GFP	centromere I	GFP	*cnp1*$^{+}$	P10A,P13A,P15A,P17A
WTGFP-cnp1HA	*cnp1* 3' end, lys^{-}	GFP,HA	*cnp1*$^{+,}$ *cnp1*$^{+}$	
4PAGFP-cnp1HA	*cnp1* 3' end, lys^{-}	GFP,HA	*cnp1*$^{+,}$ *cnp1*$^{+}$	P10A,P13A,P15A,P17A
Ani1GFP	*ani1* 3' end	GFP	*ani1*$^{+}$	
Ani2GFP	*ani2* 3' end	GFP	*ani2*$^{+}$	
Ani1GFP+Cbh1HA	*ani1* 3' end,*cbh1* 3' end	GFP,HA	*ani1*$^{+}$, *cbh1*$^{+}$	
Ani2GFP+Cbh1HA	*ani2* 3' end,*cbh1* 3' end	GFP,HA	*ani2*$^{+}$, *cbh1*$^{+}$	
Ani1FLAG+SpCENP-A-GFP	*ani1* 3'end, *cnp1* 3' end	FLAG, GFP	*ani1*$^{+}$, *cnp1*$^{+}$	
Ani2FLAG+SpCENP-A-GFP	*ani2* 3'end, *cnp1* 3' end	FLAG, GFP	*ani2*$^{+}$, *cnp1*$^{+}$	
ani1GFP+Sim3FLAG	*ani1* 3' end, *sim3* 3'end	GFP, FLAG	*ani1*$^{+}$, *sim3*$^{+}$	
ani2GFP+Sim3FLAG	*ani2* 3' end, *sim3* 3'end	GFP, FLAG	*ani2*$^{+}$, *sim3*$^{+}$	
Δani1Δani2+Sim3FLAG +Cbh1-HA	*ani1*$^{+}$*::kanR*$^{+}$, *ani2*$^{+}$*::kanR*$^{+}$, *sim3* 3'end, *cbh1* 3'end	FLAG,HA	*sim3*$^{+}$, *cbh1*$^{+}$	*Δani1Δani2*
Δani1+Sim3FLAG+Cbh 1-HA	*ani1*$^{+}$*::kanR*$^{+}$, *sim3* 3'end, *cbh1* 3'end	FLAG, HA	*sim3*$^{+}$, *cbh1*$^{+}$	*Δani1*
Δani2+Sim3FLAG+Cbh 1-HA	*ani2*$^{+}$*::kanR*$^{+}$, *sim3* 3'end, *cbh1* 3'end	FLAG,HA	*sim3*$^{+}$, *cbh1*$^{+}$	*Δani2*
Δani1Δani2+SpCenp-A-HA	*ani1*$^{+}$*::kanR*$^{+}$, *ani2*$^{+}$*::kanR*$^{+}$,	HA	*cnp1*$^{+}$	*Δani1Δani2*

	cnp1^{+} 3'end *lys loci*			
Δani1+ SpCenp-A-HA	*ani1*$^{+}$*::kanR*$^{+}$, *cnp1*$^{+}$ 3'end *lys loci*	HA	*cnp1*$^{+}$	*Δani1*
Δani2+ SpCenp-A-HA	*ani2*$^{+}$*::kanR*$^{+}$, *cnp1*$^{+}$ 3'end *lys loci*	HA	*cnp1*$^{+}$	*Δani2*

Supplementary Table S2 List of primers employed for making alanine site-directed mutations, 6× His epitope-tagging of Ani1 and Ani2 and for PCR detection of ChIP signals at the centromere.

Site-Mutated Primers	
Site mutated (Overlapping primers)	**Forward 5'- 3'**
Cnp-1 K3A	GAACAACTTAATATGGCAGCGAAATCTTTAATGGCTGAG
Cnp-1 K4A	CAACTTAATATGGCAAAGGCATCTTTAATGGCTGAGCCT
Cnp-1 S5A	CTTAATATGGCAAAGAAAGCTTTAATGGCTGAGCCTGGA
Cnp-1 L6A	AATATGGCAAAGAAATCTGCAATGGCTGAGCCTGGAGAT
Cnp-1 M7A	ATGGCAAAGAAATCTTTAGCGGCTGAGCCTGGAGATCCT
Cnp-1 E9A	AAGAAATCTTTAATGGCTGCGCCTGGAGATCCTATTCCA
Cnp-1 P10A	AAATCTTTAATGGCTGAGGCTGGAGATCCTATTCCACGG
Cnp-1 G11A	TCTTTAATGGCTGAGCCTGCAGATCCTATTCCACGGCCA
Cnp-1 D12A	TTAATGGCTGAGCCTGGAGCTCCTATTCCACGGCCACGT
Cnp-1 P13A	ATGGCTGAGCCTGGAGATGCTATTCCACGGCCACGTAAA
Cnp-1 I14A	GCTGAGCCTGGAGATCCTGCTCCACGGCCACGTAAAAAG
Cnp-1 P15A	GAGCCTGGAGATCCTATTGCACGGCCACGTAAAAAGAGA
Cnp-1 R16A	CCTGGAGATCCTATTCCAGCGCCACGTAAAAAGAGATAT
Cnp-1 P17A	GGAGATCCTATTCCACGGGCACGTAAAAAGAGATATCGT

Cnp-1 R18A	GATCCTATTCCACGGCCAGCTAAAAAGAGATATCGTCCA
Cnp-1 K19A	CCTATTCCACGGCCACGTGCAAAGAGATATCGTCCAGGT
Cnp-1 K20A	ATTCCACGGCCACGTAAAGCGAGATATCGTCCAGGTACT
Cnp-1 R21A	CCACGGCCACGTAAAAAGGCATATCGTCCAGGTACTACG
Cnp-1 Y22A	CGGCCACGTAAAAAGAGAGCTCGTCCAGGTACTACGGCG
Cnp-1 R23A	CCACGTAAAAAGAGATATGCTCCAGGTACTACGGCGTTA
Cnp-1 P24A	CGTAAAAAGAGATATCGTGCAGGTACTACGGCGTTAAGA
Cnp-1 G25A	AAAAAGAGATATCGTCCAGCTACTACGGCGTTAAGAGAG
Cnp-1 T26A	AAGAGATATCGTCCAGGTGCTACGGCGTTAAGAGAGATT
Cnp-1 T27A	AGATATCGTCCAGGTACTGCGGCGTTAAGAGAGATTCGA
Cnp-1 P10.13.15.17A (4PA)	GCTGGAGATGCTATTGCACGGGCACGTAAAAAGAGATATCGT
Cnp1 P13A	ATGGCTGAGCCTGGAGATGCTATTCCACGGCCACGTAAA
Cnp-1 P15A	GAGCCTGGAGATCCTATTGCACGGCCACGTAAAAAGAGA
Cnp-1 P13.15A	GAGCCTGGAGATGCTATTGCACGGCCACGTAAAAAGAGA
Site mutated (Overlapping primers)	**Reverse 5'- 3'**
Cnp-1 K3A	CTCAGCCATTAAAGATTTCGCTGCCATATTAAGTTGTTC
Cnp-1 K4A	AGGCTCAGCCATTAAAGATGCCTTTGCCATATTAAGTTG
Cnp-1 S5A	TCCAGGCTCAGCCATTAAAGCTTTCTTTGCCATATTAAG
Cnp-1 L6A	ATCTCCAGGCTCAGCCATTGCAGATTTCTTTGCCATATT
Cnp-1 M7A	AGGATCTCCAGGCTCAGCCGCTAAAGATTTCTTTGCCAT
Cnp-1 E9A	TGGAATAGGATCTCCAGGCGCAGCCATTAAAGATTTCTT
Cnp-1 P10A	CCGTGGAATAGGATCTCCAGCCTCAGCCATTAAAGATTT
Cnp-1 G11A	TGGCCGTGGAATAGGATCTGCAGGCTCAGCCATTAAAGA
Cnp-1 D12A	ACGTGGCCGTGGAATAGGAGCTCCAGGCTCAGCCATTAA
Cnp-1 P13A	TTTACGTGGCCGTGGAATAGCATCTCCAGGCTCAGCCAT

Cnp-1 I14A	CTTTTTACGTGGCCGTGGAGCAGGATCTCCAGGCTCAGC
Cnp-1 P15A	TCTCTTTTTACGTGGCCGTGCAATAGGATCTCCAGGCTC
Cnp-1 R16A	ATATCTCTTTTTACGTGGCGCTGGAATAGGATCTCCAGG
Cnp-1 P17A	ACGATATCTCTTTTTACGTGCCCGTGGAATAGGATCTCC
Cnp-1 R18A	TGGACGATATCTCTTTTTAGCTGGCCGTGGAATAGGATC
Cnp-1 K19A	ACCTGGACGATATCTCTTTGCACGTGGCCGTGGAATAGG
Cnp-1 K20A	AGTACCTGGACGATATCTCGCTTTACGTGGCCGTGGAAT
Cnp-1 R21A	CGTAGTACCTGGACGATATGCCTTTTTACGTGGCCGTGG
Cnp-1 Y22A	CGCCGTAGTACCTGGACGAGCTCTCTTTTTACGTGGCCG
Cnp-1 R23A	TAACGCCGTAGTACCTGGAGCATATCTCTTTTTACGTGG
Cnp-1 P24A	TCTTAACGCCGTAGTACCTGCACGATATCTCTTTTTACG
Cnp-1 G25A	CTCTCTTAACGCCGTAGTAGCTGGACGATATCTCTTTTT
Cnp-1 T26A	AATCTCTCTTAACGCCGTAGCACCTGGACGATATCTCTT
Cnp-1 T27A	TCGAATCTCTCTTAACGCCGCAGTACCTGGACGATATCT
Cnp-1 P10.13.15.17A (4PA)	ACGATATCTCTTTTTACGTGCCCGTGCAATAGCATCTCCAGC
Cnp1 P13A	TTTACGTGGCCGTGGAATAGCATCTCCAGGCTCAGCCAT
Cnp-1 P15A	TCTCTTTTTACGTGGCCGTGCAATAGATCTCCAGGCTC
Cnp-1 P13.15A	TCTCTTTTTACGTGGCCGTGCAATAGCATCTCCAGGCTC

Site mutated (Primers with cloning sites)	**5'- 3'**
Cnp-1 NdeI K3A For	GAACAACTTCATATGGCAGCGAAATCTTTAATGGCTGAG
Cnp-1 NdeI K4A For	GAACAACTTCATATGGCAAAGGCATCTTTAATGGCTGAG
Cnp-1 NdeI S5A For	GAACAACTTCATATGGCAAAGAAAGCTTTAATGGCTGAG
Cnp-1 NdeI L6A For	GAACAACTTCATATGGCAAAGAAATCTGCAATGGCTGAG

Cnp-1 NdeI M7A For	GAACAACTTCATATGGCAAAGAAATCTTTAGCGGCTGAG
cnp-1 flank For 2	GCTTGGGTTCATATGATTAATTTCTCTTACC
cnp-1 flank Rev	CGGTTAACGGATCCAGTAGATGAATTC
WT(NdeI*)* For	TGAATTCCATATGGCAAAGAAATCTTTAATGG
CNB(BamHI) Rev	ACGGGATCCTCAAGCACCACGAATCCTC
CNN(NotI) Rev	TGAATTCTCGAGCGGCCGCGAGCACCACGAATCCTC
ani1(NdeI) For	GGGAATTCCATATGTCTCTTCCAATTGCT
ani2(NdeI) For	GGGAATTCCATATGAGTAAGGAAGAGACC
ani1-PPIase (BamHI) Rev	GGAATTCCGGATCCTTAGGGACTAGAAGGTGCATT
ani2-PPIase (SacI) Rev	GGAATTCCGAGCTCTTAATAAGTTTTTGTAGACTT
ani1(BamHI) Rev	CGCGGATCCTTAGTGAACGCGAACAAGC
ani2(SacI) Rev	GGAATTCCGAGCTCTTAATTAACCGCTAAAAG
sim3(NdeI) For	GTTACGCGAcatATGTCTTCTGATACGAAAACACTG
sim3 x6HIS (BamHI) Rev	ACTAATTGTGGATCCTTAGTGGTGATGGTGATGATGATCCTTCTTTTTCTTATCTTTAGG

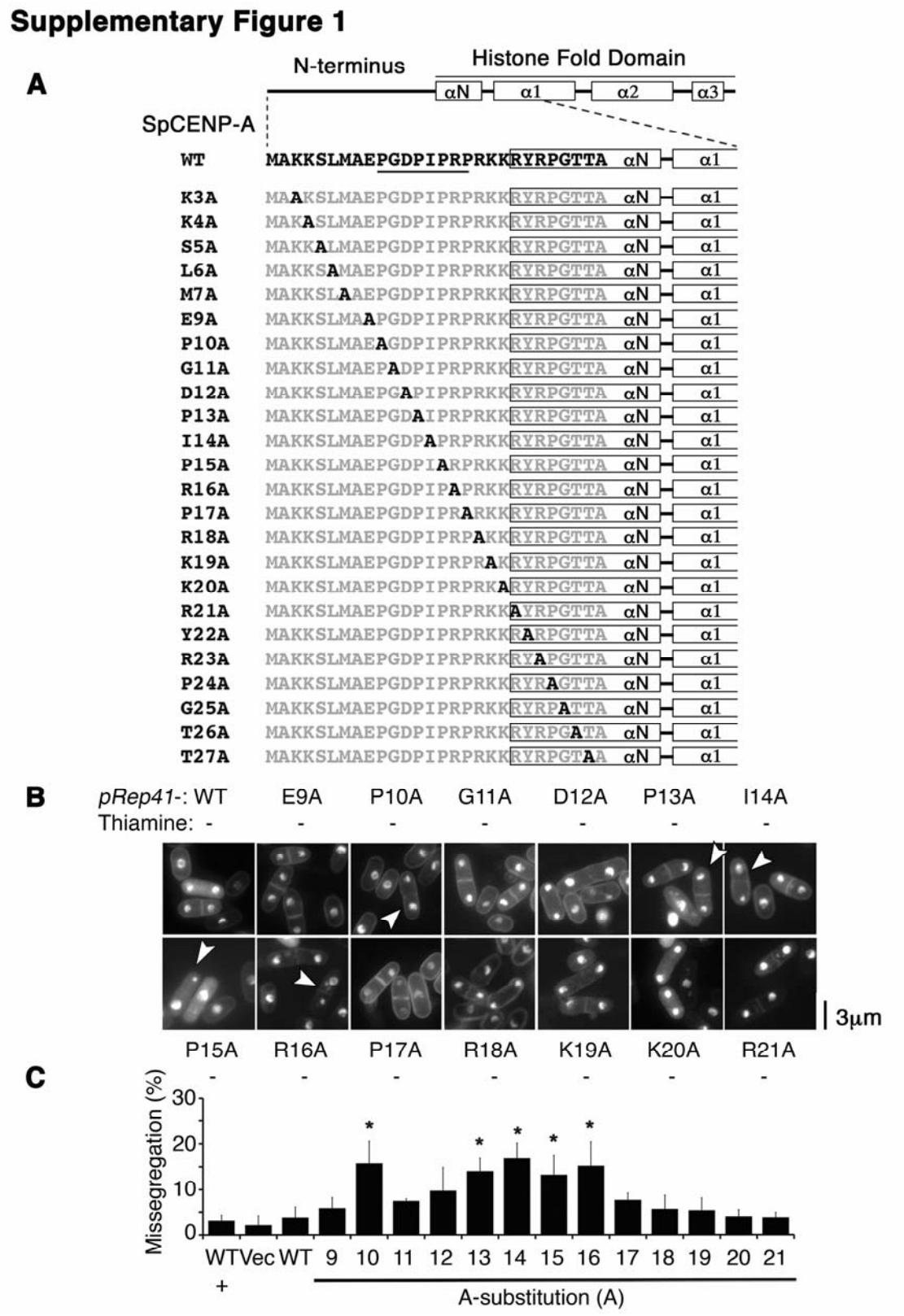
Centromere-Specific Primers	
	Forward 5'- 3'
cnt G	CGTAAATGATAATTTCAACCCG
cnt T	AACAATAAACACGAATGCCTC
act1	GAGTCCAAGACGATACCAGTG
NDR	TGATCTATCGATCGAGAGC
	Reverse 5'- 3'
cnt G	CAGACGACTTAAATGGATTGG

cnt T	ATAGTACCATGCGATTGTCTG
act1	GGCATCACACTTTCTACAACG
NDR	TGAGATGCTGAGAAGGTAG

6×His-Tagged primers	
	Forward 5'-3'
ani1 (EcoRI*)*	GGATGTCGATATCATGTCTCTTCCAATTGCTGTTTATAG
ani2 *(*EcoRV*)*	GGATGTCGATATCATGAGTAAGGAAGAGACCCTTTACAG
Sim3 F(EcoRV)	GGATGTCGATATCATGTCTTCTGATACGAAAACACTG
	Reverse 5'-3'
ani1 *(*BamHI*)*	CGCGGATCCTTAGTGAACGCGAACAAGC
ani2 *(*EcoRI*)*	CCGGAATTCTTAATTAACCGCTAAAAGCTTTAC
Sim3 R(BamHI)	CGCGGATCCATCCTTCTTTTTCTTATCTTTAGGACC

Supplementary Table S3 Sequences of peptides.

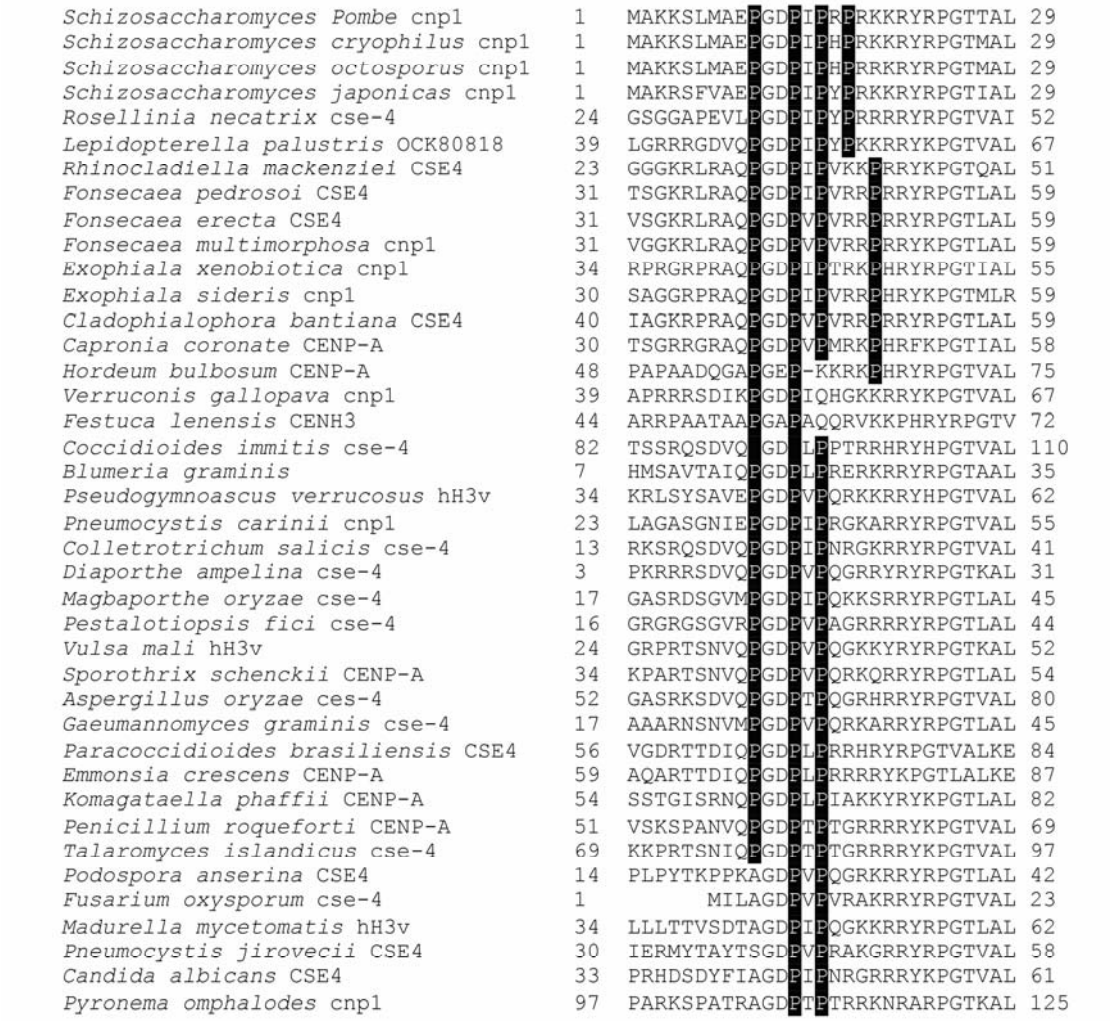
Peptide Sequences for Peptide Pull-Down Experiments	
WT/FL	MAKKSLMAEPGDPIPRPRKKRYRPGTTALRK[BIOTIN]
4PA	MAKKSLMAEAGDAIARARKKRYRPGTTALRK[BIOTIN]
H3 NTD	MARTKQTARKSTGGKAPRKQLASKAARKAAPATGGVKKPHRK[BIOTIN]
H3 PA	MARTKQTARKSTGGKAARKQLASKAARKAAAATGGVKKAHRK[BIOTIN]
Peptide Sequence for NMR Experiments	
P15FR16K	SLMAEPGDPIFKPR
P17FR18K	SLMAEPGDPIPRFK
WT	SLMAEPGDPIPRPR



Supplementary Figure 1 Chromosome missegregation induced by the ectopic expression of amino-terminal domain (NTD) alanine-substitution mutant *cnp1* genes. (A) 24 site-directed, single-alanine substitution *cnp1* constructs. "A" in black

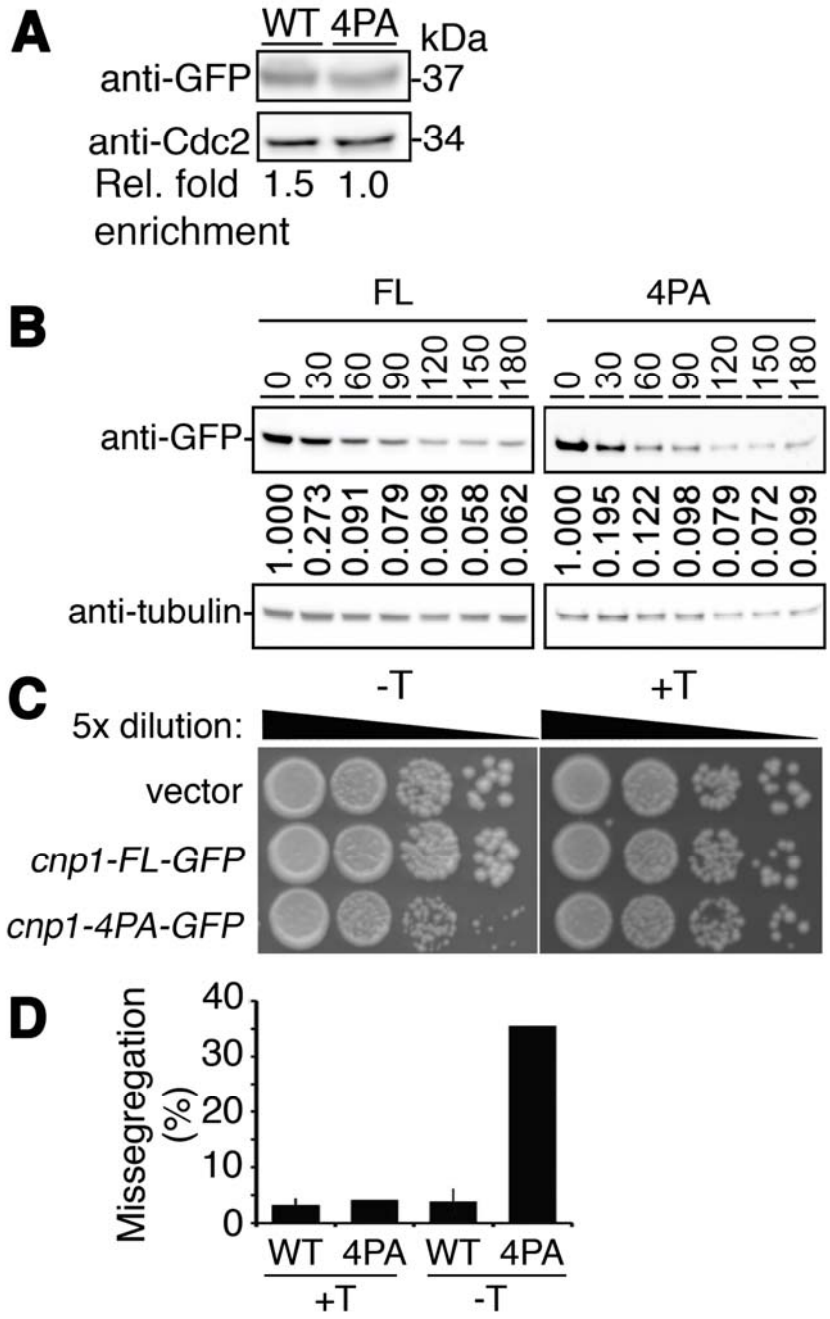
indicates the position of the alanine mutation, whereas the non-mutated residues are in grey. Constructs were expressed with the *nmt41* promoter. Wild-type (WT) cells transformed with full length, vector (vec) and *cnp1*-N-terminal single amino acid mutant constructs (*pRep41*-E3A to T27A). The "GRANT" (Genomic stability-Regulating site within CENP-A N-Terminus) residues (P10 to P17) are underlined. (B) Phenotype of WT cells expressing the constructs shown in part (A). Numerous cells show unequal chromosome segregation. Arrowhead: Unequal chromosome segregation. (C) Quantification of phenotype in part (B). + and -, + thiamine and - thiamine. Two tailed *t-test*. *$p<0.05$; N=200. Bar: S.D. Bar plot indicates mean of three biological replicates.

Supplementary Figure 2



Supplementary Figure 2 GRANT motif is conserved. Alignment of the CENP-A NTD highlighting the prolines corresponding to the "GRANT" (Genomic stability-Regulating site within CENP-A N-Terminus) motif in *Schizosaccharomyces pombe* (S. pombe) that contains prolines at positions 10, 13, 15 and 17.

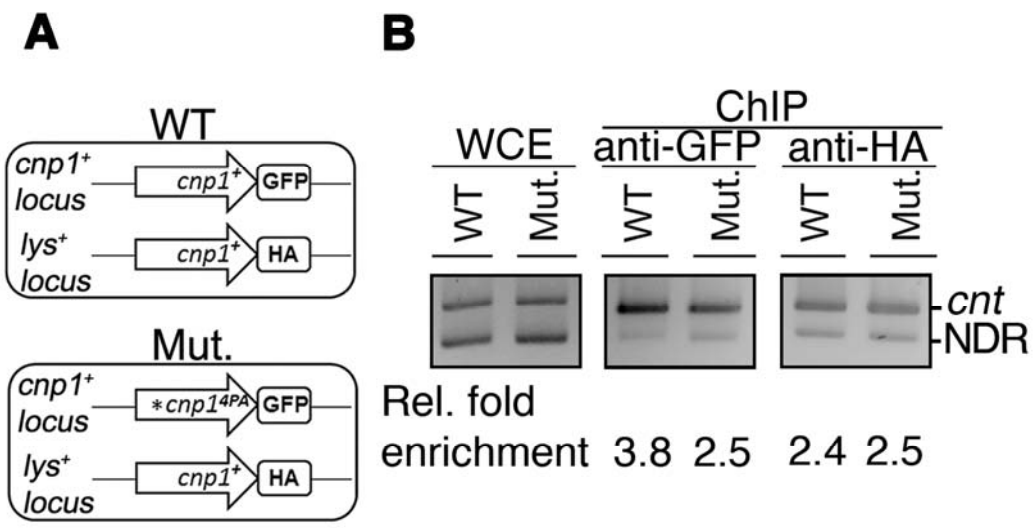
Supplementary Figure 3



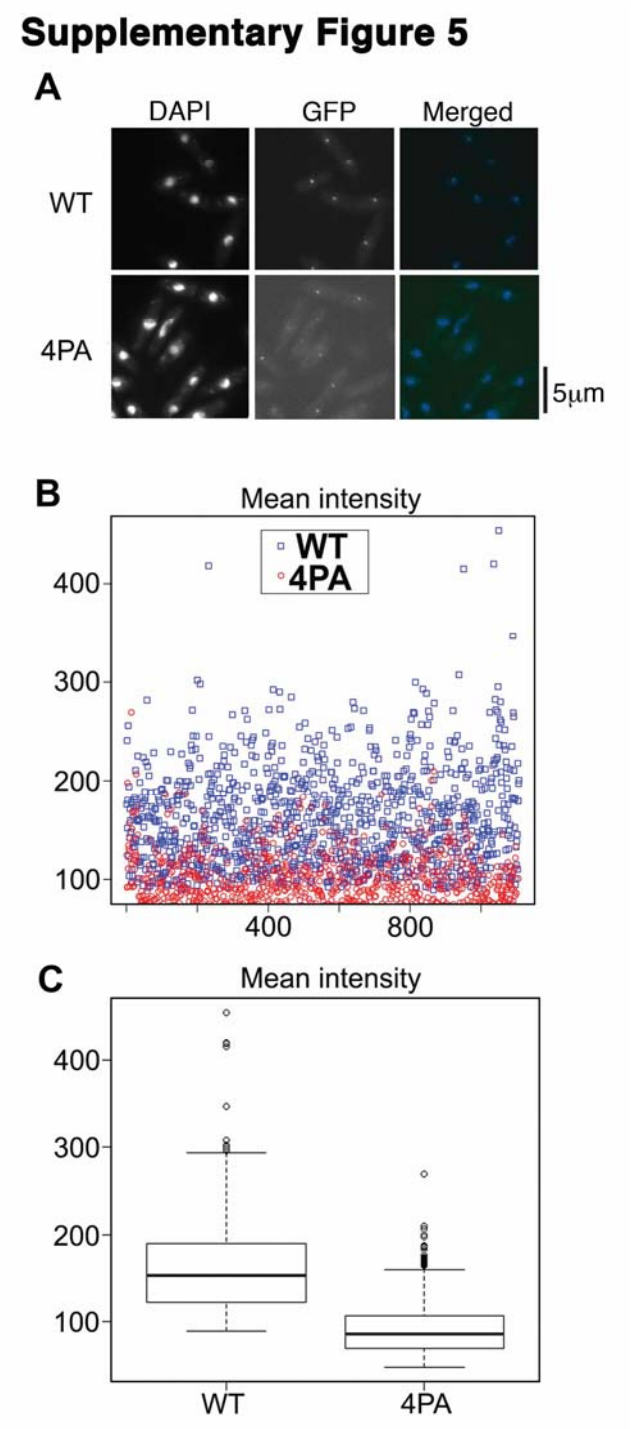
Supplementary Figure 3 *cnp1-4PA* (P10AP13AP15AP17A) mutation-induced chromosome missegregation. (A) Protein level of GFP-tagged SpCENP-A (wild type, WT) and SpCENP-A-4PA constructs expressed in WT cells 18 h after the removal of thiamine from the culture media. Level of Cdc2 (detected with anti-

PSTAIRE/Cdc2) was included as a loading control. Rel. fold enrichment, relative fold of protein level to WT.(B) Protein turnover of GFP-tagged full length (FL) versus 4PA SpCENP-A at 30-min intervals after treatment with 100 μg/ml cycloheximide (CHX), 18 h after protein induction at 30°C in EMM-leucine, thiamine-free media. The level of α-tubulin was employed as a loading control. Values represent the relative protein levels with respect to that at 0 min. Results shown represent one of two repeated experiments. (C) Growth of cells expressing vector, *cnp1-FL-GFP*, and *cnp1-4PA-GFP* constructs in WT cells at 30ºC, day 2. +T and –T: with or without thiamine; *nmt41* promotor. (D) Frequency of bi-nucleated cells exhibiting a chromosome missegregation phenotype in WT cells expressing *cnp1*$^{+}$ (WT) and *cnp1-4PA* (4PA) constructs. +T and –T: with or without thiamine, 18 h, 30°C; *nmt41* promoter. N=200, Error: S.D. Bar plot indicates the mean of at least two biological replicates.

Supplementary Figure 4

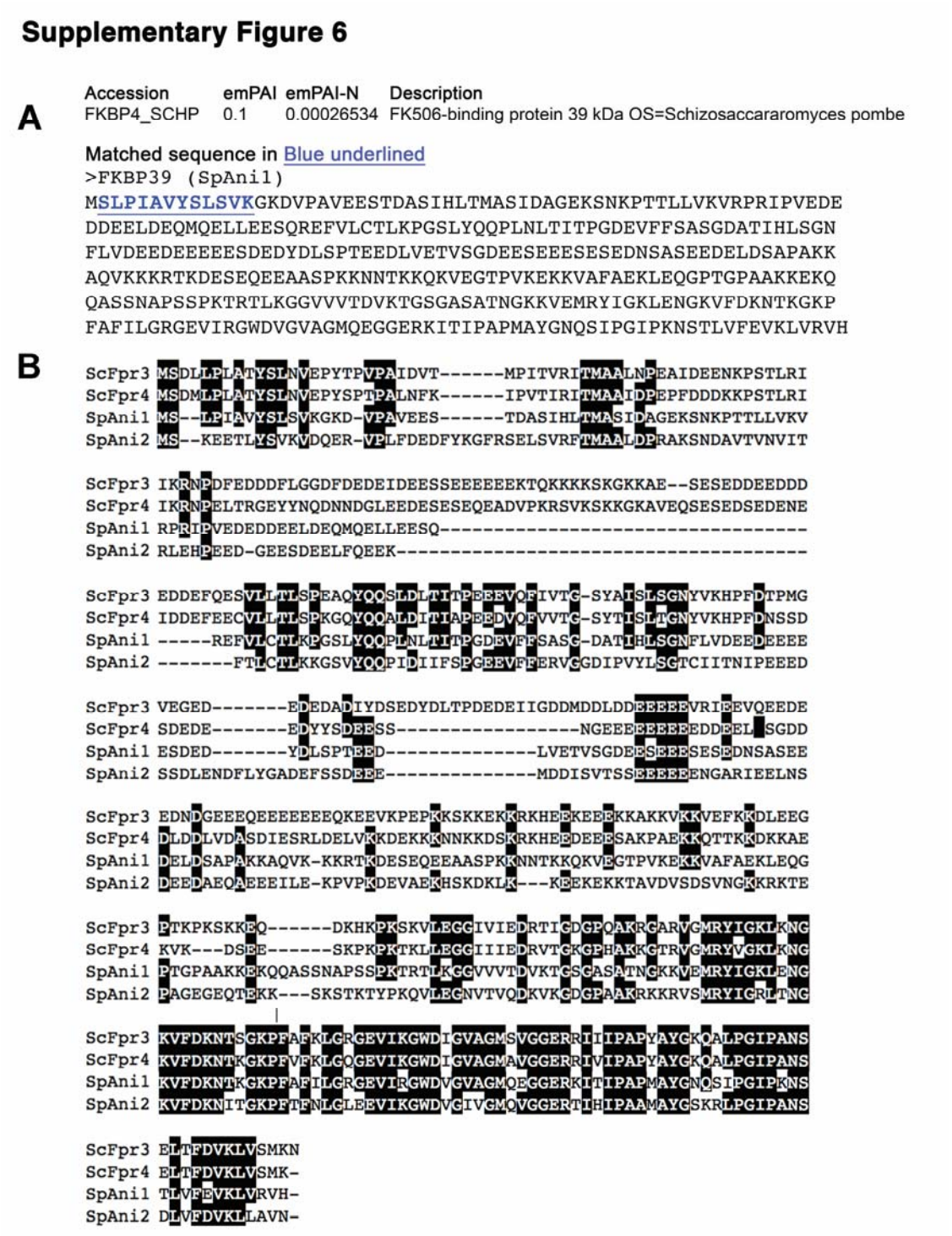


Supplementary Figure 4 Mutation of GRANT-prolines disrupts the localization of SpCENP-A to the centromere. (A) Genotype of the two strains for which chromatin immunoprecipitation (ChIP) was performed. These two strains contained two copies of *cnp1*$^+$ genes at the original as well as *lys1*$^+$ loci. Wild type (WT) strain (top) hosted a non-mutated *cnp1*$^+$ gene at the original gene locus, whereas the mutant (Mut.) strain contained *cnp1-4PA*. **cnp1*^{4PA} indicates a mutated *cnp1*$^+$ gene. HA- and GFP-tags were linked to the *cnp1*$^+$ copy at the *lys1*$^+$ and the original loci, respectively. (B) ChIP assay for WT and 4PA-mutated (Mut.) SpCENP-A at the central core region of centromere 1 (*cnt*). Anti-HA antibodies detected ChIP signals of WT SpCENP-A-HA expressed from the *lys1*$^+$ locus, whereas anti-GFP antibodies detected ChIP signals of WT or mutant SpCENP-A-GFP signals expressed from the authentic *cnp1*$^+$ chromosomal locus. WCE, Whole cell extract; NDR, nucleosome-depleted region as normalization control. Representative image of three biological replicates.



Supplementary Figure 5 Mutation of GRANT-prolines disrupts the localization of SpCENP-A to the centromere. (A) Localization of wild-type SpCENP-A-GFP (WT; top) and 4PA-mutated SpCENP-A-GFP (4PA; bottom), produced from GFP-fused *cnp1*$^{+}$ genes at the original locus. The strain used is depicted in Supplementary Figure 4A. DAPI, GFP and merged signals are shown. Bar: 5 μm. (B) Scatter plot

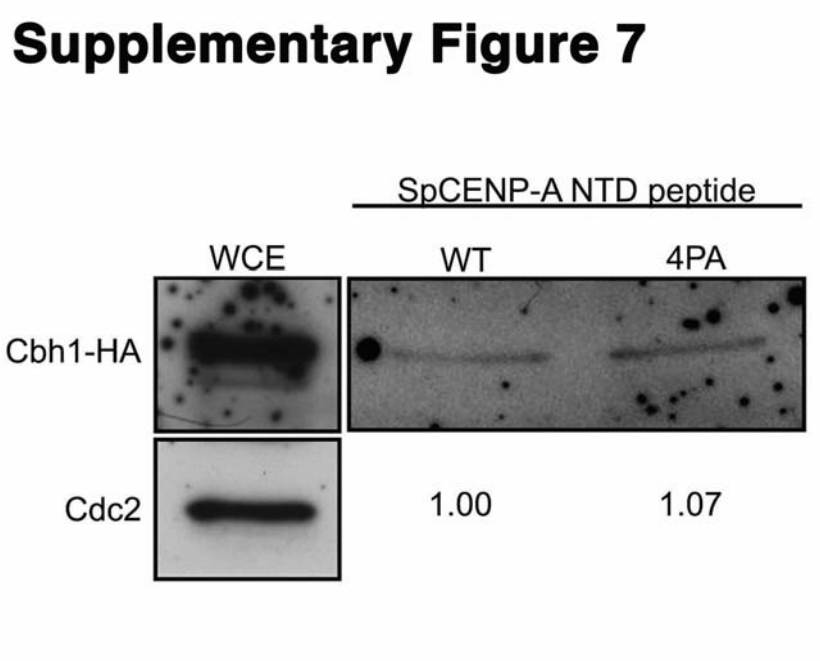
showing the mean intensity distribution of the WT (blue) and 4PA (red) SpCENP-A-GFP spots, obtained by dividing the sum pixel intensity in each GFP spot by the total area of the spot. N=1000. (C) Box plot for mean GFP intensity signal in part (B). Maximum and minimum values within the interquartile range and multiplied by 1.5 are represented by the ends of the whiskers. Outliers are denoted by points beyond whisker ends. The boxes indicate the 25^{th}, 50^{th} (mean) and 75^{th} percentiles. A statistical comparison of the differences in the variances between WT and 4PA was performed with a F-test, followed by a comparison with the Welch *t*-test; the variance is estimated separately for each dataset using Welch modification of the degrees of freedom. A statistical significant difference between WT and 4PA GFP intensity ($p<2.2\times10^{-16}$) was detected.



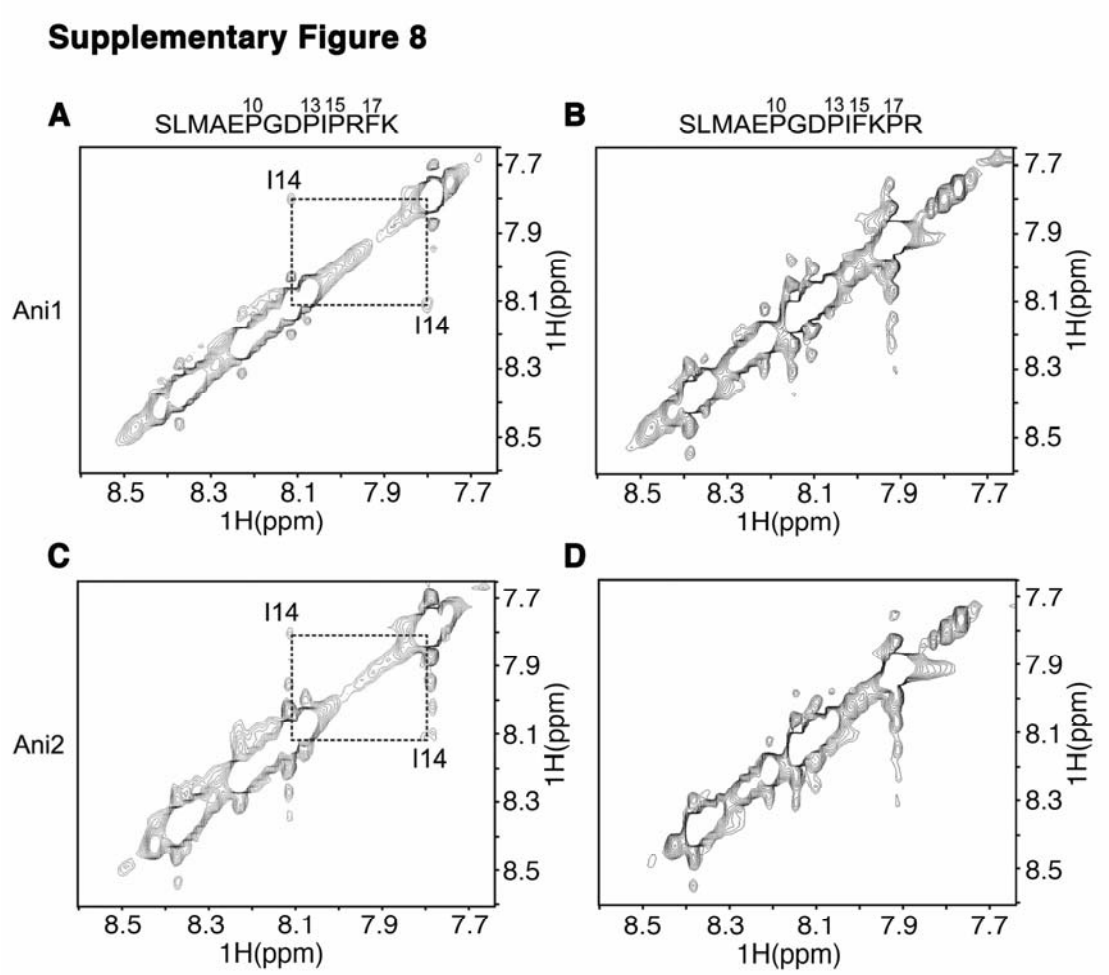
Supplementary Figure 6 An FKBP-type *cis-trans* peptidyl prolyl isomerase affinity purified with wild type (WT) SpCENP-A N-terminal domain (NTD). (A) Mass spectrometry identified a novel coding sequence, which was predicted to be a 39-kDa FK506-binding protein from fission yeast. The peptide isolated using mass spectrometry is highlighted in blue. This putative isomerase has been named

SpCENP-A NTD Isomerase 1 (Ani1). (B) Alignment revealed that the fission yeast genome contains another unknown gene, SPAC27F1.06c, which encodes an Ani1-like protein (named Ani2). Ani1 and Ani2 share high sequence similarity with the Fpr3 and Fpr4 proline *cis-trans* isomerases from budding yeast across the isomerase catalytic domain. Sp: *Schizosaccharomyces pombe* and Sc: *Saccharomyces cerevisiae*. | indicates the point of C-terminal truncation.

Supplementary Figure 7

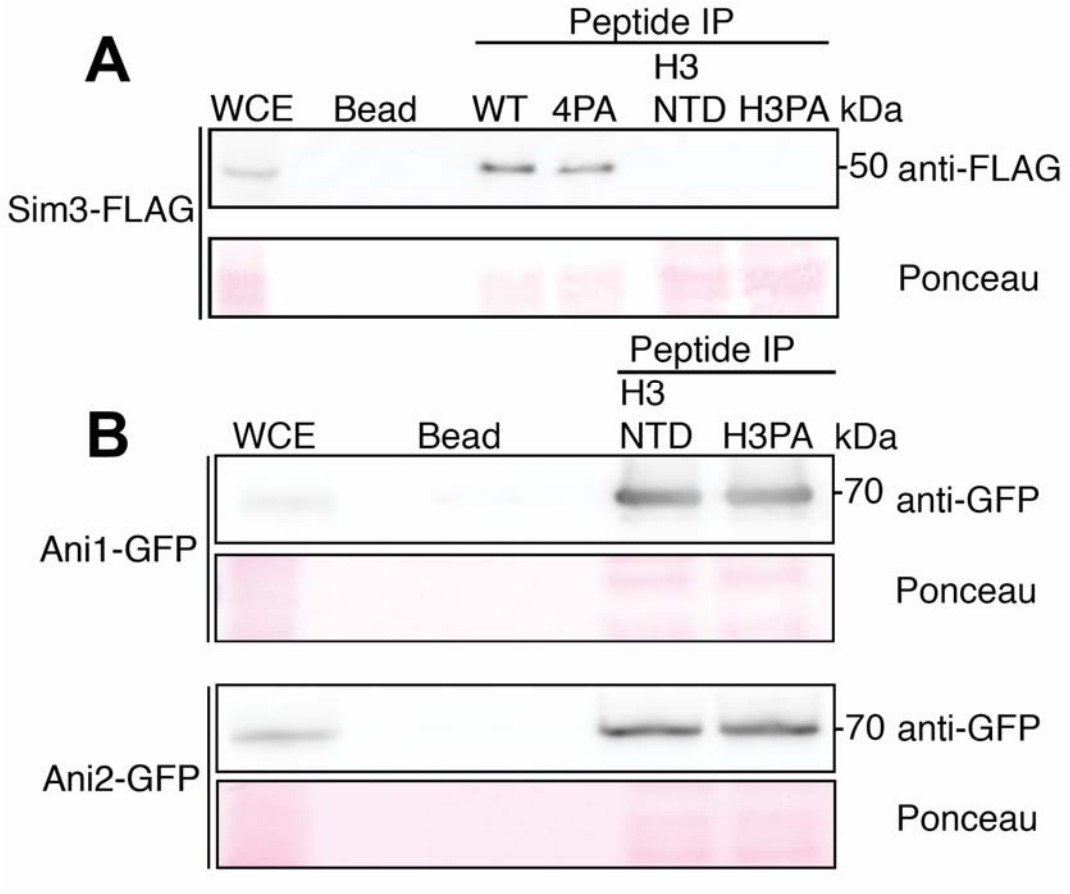


Supplementary Figure 7 SpCENP-A N-terminal domain (NTD) interacts with Cbh1-HA independent of proline residues. Peptide pull-down of wild type (WT) and mutant 4PA SpCENP-A and Cbh1-HA. WCE, Whole cell extract. Cdc2, input.



Supplementary Figure 8 Proline-15 within the SpCENP-A N-terminal domain (NTD) is the target of prolyl isomerization by Ani1 and Ani2. (A–D) Rotating frame Overhauser effect spectroscopy (ROESY) nuclear magnetic resonance (NMR) profiles obtained by incubating recombinant Ani1 and Ani2 with peptides SLMAEPGDPIPRFK (which hosts a P17FR18K mutation) and SLMAEPGDPIFKPR (which hosts a P15FR16K mutation). A cross peak at isoleucine (I)-14 was observed both in the case of (A) Ani1 and (C) Ani2 for peptide SLMAEPGDPIPRFK but not SLMAEPGDPIFKPR (B, D, respectively). Proline (P) and arginine (R) were mutated to phenylalanine (F) and lysine (K), respectively, to preserve the aromatic and basic nature of the original amino acid residues. Ani, SpCENP-A NTD-isomerase.

Supplementary Figure 9



Supplementary Figure 9 Physical interaction for wild type (WT) and mutant histone H3 N-terminus with Ani1-GFP, Ani2-GFP or Sim3, as examined by biotinylated peptide pull-down assays. (A) Peptide immunoprecipitation (IP) of Sim3-FLAG with SpCENP-A WT and 4PA mutant peptides, WT histone H3 N-terminal domain (NTD) and H3 "PA" (proline → alanine) peptides. WCE, Whole cell extract.(B) Peptide IP of Ani1-GFP and Ani2-GFP with H3 NTD and H3 PA peptides. WCE, Whole cell extract; H3 NTD, WT histone H3 N-terminus (NTD) peptide; H3PA, Mutant H3 NTD peptide, in which all proline residues were mutated to alanine residues. Ponceau staining was used to confirm equal loading. Representative blot of three experimental replicates.