

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	<i>his</i>	<i>lys</i>	<i>leu</i>	<i>ura</i>	<i>ade</i>
K3A	HF123	pREP41-K3A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
K4A	HF123	pREP41-K4A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
S5A	HF123	pREP41-S5A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
L6A	HF123	pREP41-L6A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
M7A	HF123	pREP41-M7A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
E9A	HF123	pREP41-E9A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
P10A	HF123	pREP41-P10A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
G11A	HF123	pREP41-G11A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
D12A	HF123	pREP41-D12A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
P13A	HF123	pREP41-P13A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
I14A	HF123	pREP41-I14A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
P15A	HF123	pREP41-P15A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
R16A	HF123	pREP41-R16A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
P17A	HF123	pREP41-P17A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
R18A	HF123	pREP41-R18A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
K19A	HF123	pREP41-K19A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
K20A	HF123	pREP41-K20A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
R21A	HF123	pREP41-R21A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
Y22A	HF123	pREP41-Y22A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
R23A	HF123	pREP41-R23A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
P24A	HF123	pREP41-P24A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
G25A	HF123	pREP41-G25A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +
T26A	HF123	pREP41-T26A	<i>his</i> +	<i>lys</i> +	<i>leu</i> +	<i>ura</i> +	<i>ade</i> +

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
<i>Δani1</i> -vector	<i>Δani1</i>	pREP41	his+	-	leu+	-	ade-
<i>Δani1</i> -FL	<i>Δani1</i>	pREP41- <i>ani1</i> -FL	his+	-	leu+	-	ade-
<i>Δani1</i> - <i>ΔPPIase</i>	<i>Δani1</i>	pREP41- <i>ani1</i> - <i>ΔPPIase</i>	his+	-	leu+	-	ade-
<i>Δani2</i> -vector	<i>Δani2</i>	pREP41	his+	-	leu+	ura+	ade+
<i>Δani2</i> -FL	<i>Δani2</i>	pREP41- <i>ani2</i> FL	his+	-	leu+	ura+	ade+
<i>Δani2</i> - <i>ΔPPIase</i>	<i>Δani2</i>	pREP41- <i>ani2</i> - <i>ΔPPIase</i>	his+	-	leu+	ura+	ade+
WT-vector	HF123	pREP41	his+	-	leu+	ura+	ade+
WT- <i>sim3</i> ⁺	HF123	pREP41- <i>sim3</i> HIS	his+	-	leu+	ura+	ade+
P13A-vector	P13A	pREP41	his+	-	leu+	ura+	ade+
P13A- <i>sim3</i> ⁺	P13A	pREP41- <i>sim3</i> HIS	his+	-	leu+	ura+	ade+
P15A-vector	P15A	pREP41	his+	-	leu+	ura+	ade-
P15A- <i>sim3</i> ⁺	P15A	pREP41- <i>sim3</i> HIS	his+	-	leu+	ura+	ade-
P13AP15A-vector	P13AP15A	pREP41	his+	-	leu+	ura+	ade+
P13AP15A- <i>sim3</i> ⁺	P13AP15A	pREP41- <i>sim3</i> HIS	his+	-	leu+	ura+	ade+
<i>Δani1Δani2</i> -vector	<i>Δani1Δani2</i>	pREP41	his+	-	leu+	ura+	ade+
<i>Δani1Δani2</i> - <i>sim3</i> ⁺	<i>Δani1Δani2</i>	pREP41- <i>sim3</i> HIS	his+	-	leu+	ura+	ade+

Single Copy Integrant Strain / Deletion strains

Name	Locus of integration	Mutation	lys	leu	ura
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<i>cnp1-4PA</i>	<i>cnp1</i> ⁺	P10A,P13A,P15A,P17A	<i>lys</i> ⁺	<i>leu</i> ⁻	<i>ura</i> ⁺
<i>cnp1-P13A</i>	<i>cnp1</i> ⁺	P13A	-	<i>leu</i> ⁻	<i>ura</i> ⁺
<i>cnp1-P15A</i>	<i>cnp1</i> ⁺	P15A	<i>lys</i> ⁺	<i>leu</i> ⁻	<i>ura</i> ⁺
<i>cnp1-P13AP15A</i>	<i>cnp1</i> ⁺	P13A,P15A	-	<i>leu</i> ⁻	<i>ura</i> ⁻
<i>cnp1-WT</i>	<i>cnp1</i> ⁺	-	-	<i>leu</i> ⁻	<i>ura</i> ⁺
<i>cnp1-E9A</i>	<i>cnp1</i> ⁺	E9A	<i>lys</i> ⁻	<i>leu</i> ⁻	<i>ura</i> ⁻
<i>cnp1-P10A</i>	<i>cnp1</i> ⁺	P10A	<i>lys</i> ⁻	<i>leu</i> ⁻	<i>ura</i> ⁻
<i>cnp1-G11A</i>	<i>cnp1</i> ⁺	G11A	<i>lys</i> ⁻	<i>leu</i> ⁻	<i>ura</i> ⁻
<i>cnp1-D12A</i>	<i>cnp1</i> ⁺	D12A	<i>lys</i> ⁻	<i>leu</i> ⁻	<i>ura</i> ⁻
<i>cnp1-I14A</i>	<i>cnp1</i> ⁺	I14A	<i>lys</i> ⁻	<i>leu</i> ⁻	<i>ura</i> ⁻
<i>cnp1-R16A</i>	<i>cnp1</i> ⁺	R16A	<i>lys</i> ⁻	<i>leu</i> ⁻	<i>ura</i> ⁻
<i>cnp1-P17A</i>	<i>cnp1</i> ⁺	P17A	<i>lys</i> ⁻	<i>leu</i> ⁻	<i>ura</i> ⁻
<i>cnp1-R18A</i>	<i>cnp1</i> ⁺	R18A	<i>lys</i> ⁻	<i>leu</i> ⁻	<i>ura</i> ⁻
<i>cnp1-K19A</i>	<i>cnp1</i> ⁺	K19A	<i>lys</i> ⁻	<i>leu</i> ⁻	<i>ura</i> ⁻
<i>cnp1-K20A</i>	<i>cnp1</i> ⁺	K20A	<i>lys</i> ⁻	<i>leu</i> ⁻	<i>ura</i> ⁻
<i>cnp1-R21A</i>	<i>cnp1</i> ⁺	R21A	<i>lys</i> ⁻	<i>leu</i> ⁻	<i>ura</i> ⁻
<i>cnp1-Y22A</i>	<i>cnp1</i> ⁺	Y22A	<i>lys</i> ⁻	<i>leu</i> ⁻	<i>ura</i> ⁻
Δ <i>ani1</i>	<i>ani1</i> ⁺	<i>ani1</i> ⁺ :: <i>kanR</i> ⁺	-	<i>leu</i> ⁻	-
Δ <i>ani2</i>	<i>ani2</i> ⁺	<i>ani2</i> ⁺ :: <i>kanR</i> ⁺	-	<i>leu</i> ⁻	<i>ura</i> ⁺
Δ <i>ani1</i>	<i>ani1</i> ⁺	<i>ani1</i> ⁺ :: <i>kanR</i> ⁺	<i>lys</i> ⁺	<i>leu</i> ⁺	<i>ura</i> ⁺
Δ <i>ani2</i>	<i>ani2</i> ⁺	<i>ani2</i> ⁺ :: <i>kanR</i> ⁺	<i>lys</i> ⁺	<i>leu</i> ⁺	<i>ura</i> ⁺
Δ <i>ani1Δ<i>ani2</i></i>	<i>ani1</i> ⁺ <i>ani2</i> ⁺	<i>ani1</i> ⁺ :: <i>kanR</i> ⁺ , <i>ani2</i> ⁺ :: <i>kanR</i> ⁺	<i>lys</i> ⁺	<i>leu</i> ⁺	<i>ura</i> ⁺
Δ <i>ani1Δ<i>ani2</i></i>	<i>ani1</i> ⁺ <i>ani2</i> ⁺	<i>ani1</i> ⁺ :: <i>kanR</i> ⁺ , <i>ani2</i> ⁺ :: <i>kanR</i> ⁺	<i>lys</i> ⁺	<i>leu</i> ⁻	<i>ura</i> ⁺

Epitope-tagged Strains

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

	<i>cnp1</i> ⁺ 3'end <i>lys loci</i>			
$\Delta ani1$ + <i>SpCenp-A-HA</i>	<i>ani1</i> ⁺ :: <i>kanR</i> ⁺ , <i>cnp1</i> ⁺ 3'end <i>lys loci</i>	HA	<i>cnp1</i> ⁺	$\Delta ani1$
$\Delta ani2$ + <i>SpCenp-A-HA</i>	<i>ani2</i> ⁺ :: <i>kanR</i> ⁺ , <i>cnp1</i> ⁺ 3'end <i>lys loci</i>	HA	<i>cnp1</i> ⁺	$\Delta ani2$

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	GAACAACCTTAATATGGCAGCGAAATCTTTAATGGCTGAG
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	CCTGGAGATCCTATTCCACGGCCACGTAAAAAGAGATAT
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	AGATATCGTCCAGGTACTGCGGGCGTTAAGAGAGATTCGA
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	CTCAGCCATTAAGATTTTCGCTGCCATATTAAGTTGTTCC
Cnp-1 K4A	AGGCTCAGCCATTAAGATGCCTTTGCCATATTAAGTTG
Cnp-1 S5A	TCCAGGCTCAGCCATTAAGCTTTCTTTGCCATATTAAG
Cnp-1 L6A	ATCTCCAGGCTCAGCCATTGCAGATTTCTTTGCCATATT
Cnp-1 M7A	AGGATCTCCAGGCTCAGCCGCTAAAGATTTCTTTGCCAT
Cnp-1 E9A	TGGAATAGGATCTCCAGGCGCAGCCATTAAGATTTCTT
Cnp-1 P10A	CCGTGGAATAGGATCTCCAGCCTCAGCCATTAAGATTT
Cnp-1 G11A	TGGCCGTGGAATAGGATCTGCAGGCTCAGCCATTAAGA
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	GAACAACCTTCATATGGCAGCGAAATCTTTAATGGCTGAG
Cnp-1 NdeI K4A For	GAACAACCTTCATATGGCAAAGGCATCTTTAATGGCTGAG
Cnp-1 NdeI S5A For	GAACAACCTTCATATGGCAAAGAAAGCTTTAATGGCTGAG
Cnp-1 NdeI L6A For	GAACAACCTTCATATGGCAAAGAAATCTGCAATGGCTGAG

Cnp-1 Ndel M7A For	GAACAACTTCATATGGCAAAGAAATCTTTAGCGGCTGAG
cnp-1 flank For 2	GCTTGGGTTTCATATGATTAATTTCTCTTACC
cnp-1 flank Rev	CGGTTAACGGATCCAGTAGATGAATTC
WT(Ndel) For	TGAATTCCATATGGCAAAGAAATCTTTAATGG
CNB(BamHI) Rev	ACGGGATCCTCAAGCACCACGAATCCTC
CNN(NotI) Rev	TGAATTCTCGAGCGGCCGCGAGCACCACGAATCCTC
ani1(Ndel) For	GGAATTCCATATGTCTCTTCCAATTGCT
ani2(Ndel) For	GGAATTCCATATGAGTAAGGAAGAGACC
ani1-PPIase (BamHI) Rev	GGAATTCCGGATCCTTAGGGACTAGAAGGTGCATT
ani2-PPIase (Sacl) Rev	GGAATTCCGAGCTCTTAATAAGTTTTGTAGACTT
ani1(BamHI) Rev	CGCGGATCCTTAGTGAACGCGAACAAGC
ani2(Sacl) Rev	GGAATTCCGAGCTCTTAATTAACCGCTAAAAG
sim3(Ndel) For	GTTACGCGAcatATGTCTTCTGATACGAAAACACTG
sim3 x6HIS (BamHI) Rev	ACTAATTGTGGATCCTTAGTGGTGATGGTGATGATGATCCTTCTTTTTCTTATCT TTAGG

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

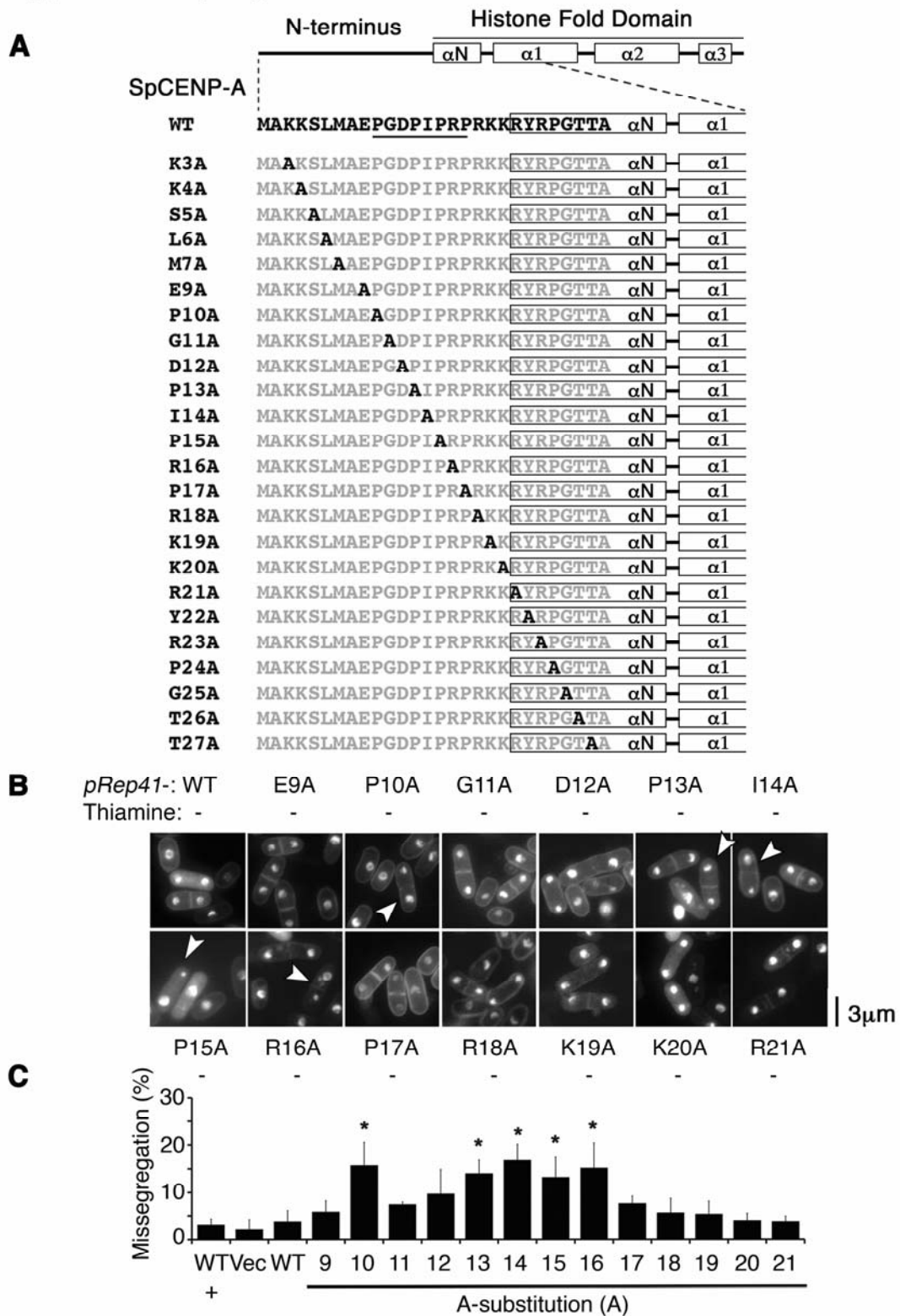
cnt T	ATAGTACCATGCGATTGTCTG
act1	GGCATCACACTTTCTACAACG
NDR	TGAGATGCTGAGAAGGTAG

6xHis-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



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

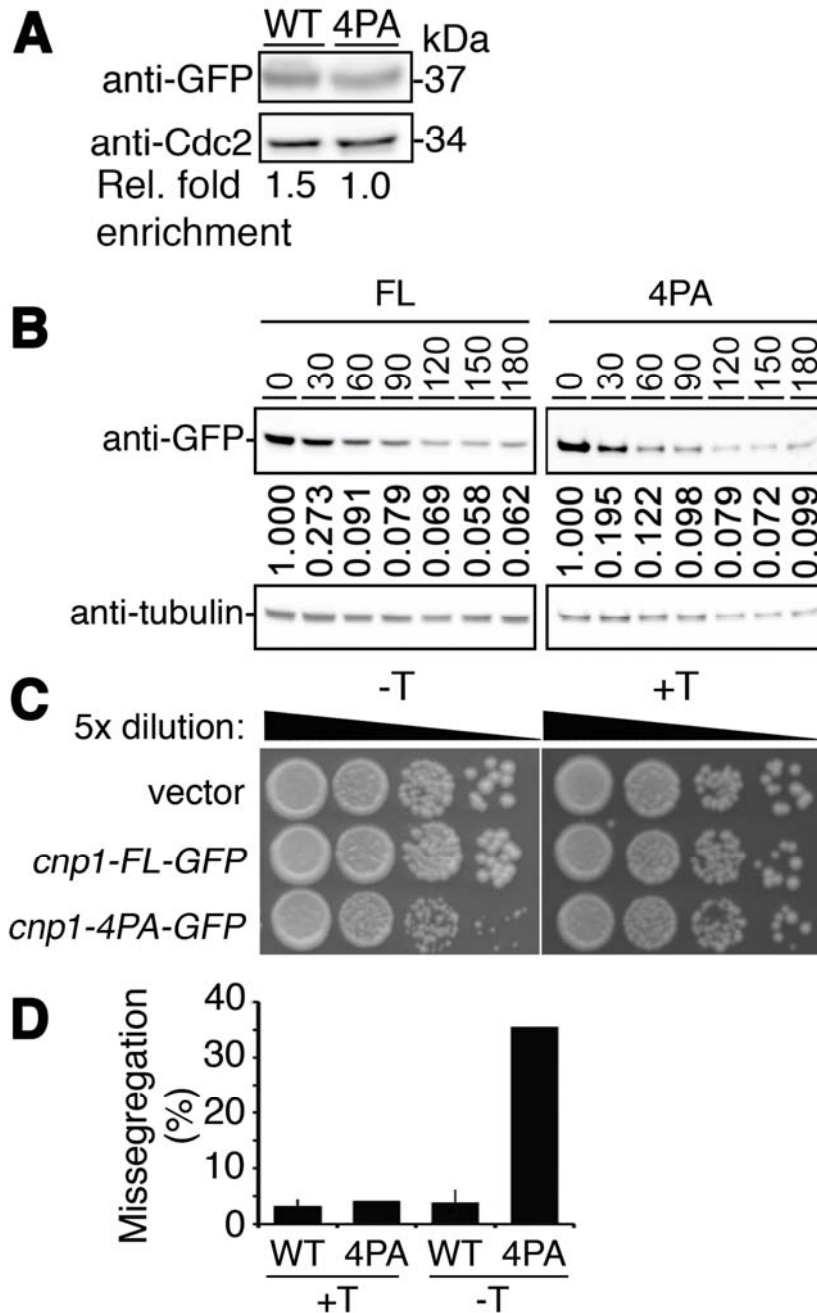
indicates the position of the alanine mutation, whereas the non-mutated residues are in grey. Constructs were expressed with the *nmt41* promoter. Wild-type (WT) cells transformed with full length, vector (vec) and *cnp1*-N-terminal single amino acid mutant constructs (*pRep41*-E3A to T27A). The “GRANT” (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

<i>Schizosaccharomyces Pombe</i> cnp1	1	MAKKSLMAE	PGDPT	PHPRKKRYR	PGTTAL	29
<i>Schizosaccharomyces cryophilus</i> cnp1	1	MAKKSLMAE	PGDPT	PHPRKKRYR	PGTMAL	29
<i>Schizosaccharomyces octosporus</i> cnp1	1	MAKKSLMAE	PGDPT	PHPRKKRYR	PGTMAL	29
<i>Schizosaccharomyces japonicas</i> cnp1	1	MAKRSFVAE	PGDPT	PHYPRKKRYR	PGTIAL	29
<i>Rosellinia necatrix</i> cse-4	24	GSGGAPEVL	PGDPT	PHYPRRRRYR	PGTVAI	52
<i>Lepidopterella palustris</i> OCK80818	39	LGRRRGDVQ	PGDPT	PHYPKKRRYK	PGTVAL	67
<i>Rhinochlamydia mackenziei</i> CSE4	23	GGGKRLRAQ	PGDPT	PEVKKP	RRYKPGTQAL	51
<i>Fonsecaea pedrosoi</i> CSE4	31	TSGKRLRAQ	PGDPT	PEVRRP	RRYRPGTLAL	59
<i>Fonsecaea erecta</i> CSE4	31	VSGKRLRAQ	PGDPE	PEVRRP	RRYRPGTLAL	59
<i>Fonsecaea multimorphosa</i> cnp1	31	VGGKRLRAQ	PGDPE	PEVRRP	RRYRPGTLAL	59
<i>Exophiala xenobiotica</i> cnp1	34	RPRGRPRAQ	PGDPT	PETRKPE	RRYRPGTIAL	55
<i>Exophiala sideris</i> cnp1	30	SAGGRPRAQ	PGDPT	PEVRRP	RRYKPGTMLR	59
<i>Cladophialophora bantiana</i> CSE4	40	IAGKRPRRAQ	PGDPE	PEVRRP	RRYRPGTLAL	59
<i>Capronia coronate</i> CENP-A	30	TSGRRGRAQ	PGDPE	PEMRKPE	HRFKPGTIAL	58
<i>Hordeum bulbosum</i> CENP-A	48	PAPAADQGA	PGEP	PKKRP	PHRYRPGTVAL	75
<i>Verruconis gallopava</i> cnp1	39	APRRRSDIK	PGDPT	IQHGKKRRYK	PGTVAL	67
<i>Festuca lenensis</i> CENH3	44	ARRPAATAA	PGA	PAQQRVKK	PHRYRPGTV	72
<i>Coccidioides immitis</i> cse-4	82	TSSRQSDVQ	GD	LEP	TRRHHYHPGTVAL	110
<i>Blumeria graminis</i>	7	HMSAVTAIQ	PGDPT	PERERKRRYR	PGTAAL	35
<i>Pseudogymnoascus verrucosus</i> hH3v	34	KRLSYSAVE	PGDPE	PEQRKKRRYH	HPGTVAL	62
<i>Pneumocystis carinii</i> cnp1	23	LAGASGNIE	PGDPT	PEFRGKARRYR	PGTVAL	55
<i>Colletotrichum salicis</i> cse-4	13	RKSRQSDVQ	PGDPT	PENRGKRRYR	PGTVAL	41
<i>Diaporthe ampelina</i> cse-4	3	PKRRRSDVQ	PGDPE	PEQGRRYR	YRPGTKAL	31
<i>Magbaporthe oryzae</i> cse-4	17	GASRDSGVMP	PGDPT	PEQKKSRRYR	PGTLAL	45
<i>Pestalotiopsis fici</i> cse-4	16	GRGRGSGVR	PGDPE	PEAGRRRRYR	PGTLAL	44
<i>Vulsa mali</i> hH3v	24	GRPRTSNVQ	PGDPE	PEQGGKRYR	YRPGTKAL	52
<i>Sporothrix schenckii</i> CENP-A	34	KPARTSNVQ	PGDPE	PEQRKQRRYR	PGTLAL	54
<i>Aspergillus oryzae</i> ces-4	52	GASRKSVDVQ	PGDPT	PEQGRHRRYR	PGTVAL	80
<i>Gaeumannomyces graminis</i> cse-4	17	AAARNSNVM	PGDPE	PEQRKARRYR	PGTLAL	45
<i>Paracoccidioides brasiliensis</i> CSE4	56	VGDRTTDIQ	PGDPE	PEFRRHHYR	YRPGTVALKE	84
<i>Emmonsia crescens</i> CENP-A	59	AQARTTDIQ	PGDPT	PERRRRYK	PGTLALKE	87
<i>Komagataella phaffii</i> CENP-A	54	SSTGISRNQ	PGDPE	PEFAKKRYR	KPGTLAL	82
<i>Penicillium roqueforti</i> CENP-A	51	VSKSPANVQ	PGDPT	PEFTGRRRRYK	PGTVAL	69
<i>Talaromyces islandicus</i> cse-4	69	KKPRTSNIQ	PGDPT	PEFTGRRRRYK	PGTVAL	97
<i>Podospora anserina</i> CSE4	14	PLPYTKPKAGD	PE	PEQGRKRRYR	PGTLAL	42
<i>Fusarium oxysporum</i> cse-4	1	MILAGDPE	PEV	PEVRAKRRYR	PGTVAL	23
<i>Madurella mycetomatis</i> hH3v	34	LLLTTVSDTAGD	PE	PEQGGKRRYR	PGTLAL	62
<i>Pneumocystis jirovecii</i> CSE4	30	IERMYTAYTSGD	PE	PEVRAKRRYR	PGTVAL	58
<i>Candida albicans</i> CSE4	33	PRHDSDFIAGD	PE	PENRGRRYK	PGTVAL	61
<i>Pyronema omphalodes</i> cnp1	97	PARKSPATRAGD	PE	PETRRKNRAR	PGTKAL	125

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.

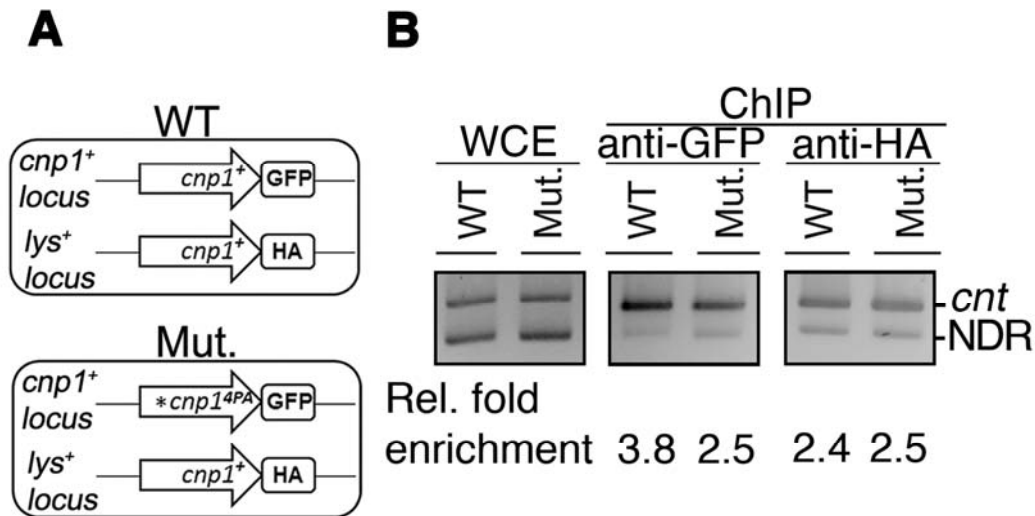
Supplementary Figure 3



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-

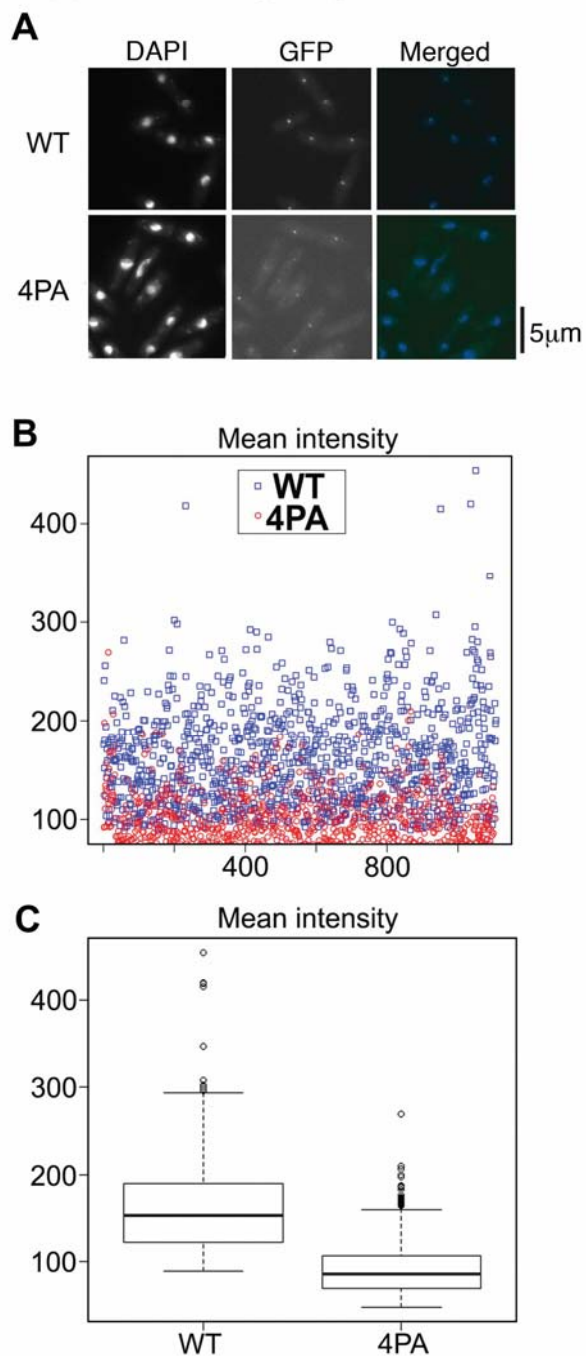
PSTAIRES/Cdc2) was included as a loading control. Rel. fold enrichment, relative fold of protein level to WT.(B) Protein turnover of GFP-tagged full length (FL) versus 4PA SpCENP-A at 30-min intervals after treatment with 100 μ g/ml cycloheximide (CHX), 18 h after protein induction at 30°C in EMM-leucine, 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 30°C, day 2. +T and -T: with or without thiamine; *nmt41* promoter. (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



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 μ m. (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 25th, 50th (mean) and 75th 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 ($p < 2.2 \times 10^{-16}$) was detected.

Supplementary Figure 6

A

Accession	emPAI	emPAI-N	Description
FKBP4_SCHP	0.1	0.00026534	FK506-binding protein 39 kDa OS=Schizosaccaromyces pombe

Matched sequence in Blue underlined
 >FKBP39 (SpAni1)
MSLPIAVYLS**SVK**GKDVPAVEESTDASIHLMASIDAGEKSNKPTLLVKVRPRIPVEDE
 DDEELDEQMQELLEESQREFVLCTLKPGLYQQPLNLTITPGDEVFFSASGDATIHLSGN
 FLVDEEDEEEEESEDEDYDLSPTTEEDLVETVSGDEESESESESEEDNSASEEDELDSAPAKK
 AQVKKKRTKDESEQEEAASPKNNTKKQKVEGTPVKEKKVAFAEKLEQGPTGPAKKEKQ
 QASSNAPSSPKTRTLKGGVVVTDVKTGSGASATNGKKVEMRYIGKLENGKVFDKNTKGGP
 FAFILGRGEVIRGWDVGVAGMQEGGERKITIPAPMAYGNQSIPIGIPKNSTLVFEVKLVRVH

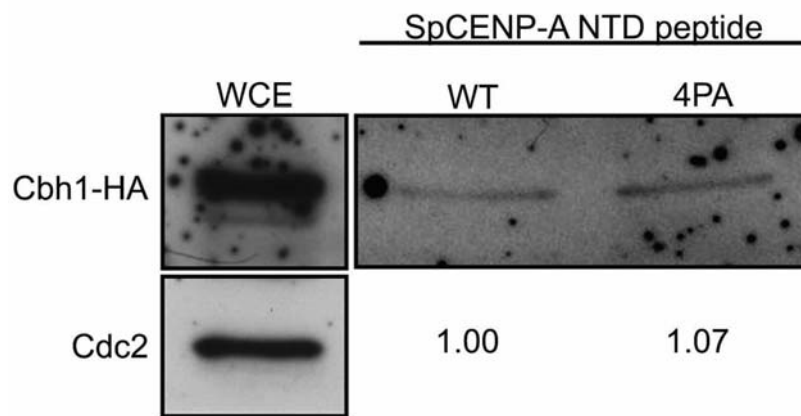
B

ScFpr3	MSDLLPLATYSLNVEPYTPVPAIDVT-----MPITVRITMAALNPEAIDEENKPTLRI
ScFpr4	MSDMLPLATYSLNVEPYSPALNFK-----IPVTIRITMAALDPEPFDDDKKPTLRI
SpAni1	MS--LPTAVYLSVKGKD-VPAVEES-----TDASIHLMASIDAGEKSNKPTLLVKV
SpAni2	MS--KEETLYSVKVDQER-VPLFDEDFYKGRSELSVRFTMAALDPRAKSNDAVTVNVIT
ScFpr3	IKRNPDEFDDDFLGGDFDEDEIDEESEEEEEKTKKKKSKGKAE--SESEDEEDDD
ScFpr4	IKRNPDELTRGEYQNDDNGLEDESESEQEAADVPKRSVSKKGGKAVEQSESEDESE
SpAni1	RPRIPVEDEDEELDEQMQELLEESQ-----
SpAni2	RLEHPEED-GEESDEELFQEEK-----
ScFpr3	EDEFQESVLLTLSPEAQYQQLDITIPPEEVEQFIVTG--SYATSLSGNYVKHPFDTPMG
ScFpr4	IDDEFEECVLLTSPKGYQQALDITIAPEEVEQFVVVG--SYTISLTGNVYKHPFDNSSD
SpAni1	----REFVLCNLKPGSLYQQPLNLTITPGDEVFFSASG-DATIHLSGNFLVDEEDEEEE
SpAni2	-----FTLCTLKKGSVYQQPIDIIFSEGEVFFERVCGDIPVYLSGTCIITNIPPEED
ScFpr3	VEGED-----EDEDADIYDSEDYDLTPDEDEIIGDDMDDLDEEEEEEVRIEVEQEEDE
ScFpr4	SDEDE-----EDYSDDESS-----NGEEEEEEEPEDDEEL_SGDD
SpAni1	ESDED-----YDLSPTTEED-----LVETVSGDEESESESEEDNSASEE
SpAni2	SSDLENDFLYGADEFSSDEEE-----MDDISVTSSEEEEEEENGARIEELNS
ScFpr3	EDNDGEEEEEEEEEQKEEVKPEPKKSKKEKRRKHEEKEEKKAKKVKVVEFKKDLEEG
ScFpr4	DLDDLVDASDIESRLDELVKKDEKKNNKDSKRRKHEEDEESAKPAEKKQTTKKDKKAE
SpAni1	DELDSAPAKKAQVK-KKRTKDESEQEEAASPKNNTKKQKVEGTPVKEKKVAFAEKLEQG
SpAni2	DEEDAEOAEEIILE-KVPVKDEVAEKHSKDKLK---KEEKEKKTAVDVSDSVNGKRRKTE
ScFpr3	PTKPKSKKEQ-----DKHKPKSKVLEGGIVIEDRTIGDGPQAKRGRVGMRYIGKLNK
ScFpr4	KVK---DSEE-----SKPKPKTKLLEGGIIEEDRVTKGPHAKKGRVGMRYIGKLNK
SpAni1	PTGPAAKKEKQASSNAPSSPKTRTLKGGVVVTDVKTGSGASATNGKKVEMRYIGKLENG
SpAni2	PAGEGEQTEKK---SKSTKTYPKQVLEGNVTVQDKVKGDGPAKRKRKRVSRYIGRLTNG
ScFpr3	KVFDKNTSGKPFPAFKLGRGEVIKWDIGVAGMSVGGERRIIPAPYAYGKQALPGIPANS
ScFpr4	KVFDKNTKPKPFVFKLGOGEVIKWDIGVAGMAVGGERRIVIPAPYAYGKQALPGIPANS
SpAni1	KVFDKNTKPKPFAFILGRGEVIRGWDVGVAGMQEGGERKITIPAPMAYGNQSIPIGIPKNS
SpAni2	KVFDKNTKPKPFTFNLGLEEVIKWDVGVGMVGGERRIIPAPMAYGSKRLPGIPANS
ScFpr3	ELTFDVKLVSMKN
ScFpr4	ELTFDVKLVSMK-
SpAni1	TLVFEVKLVRVH-
SpAni2	DLVFDVKLLAVN-

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

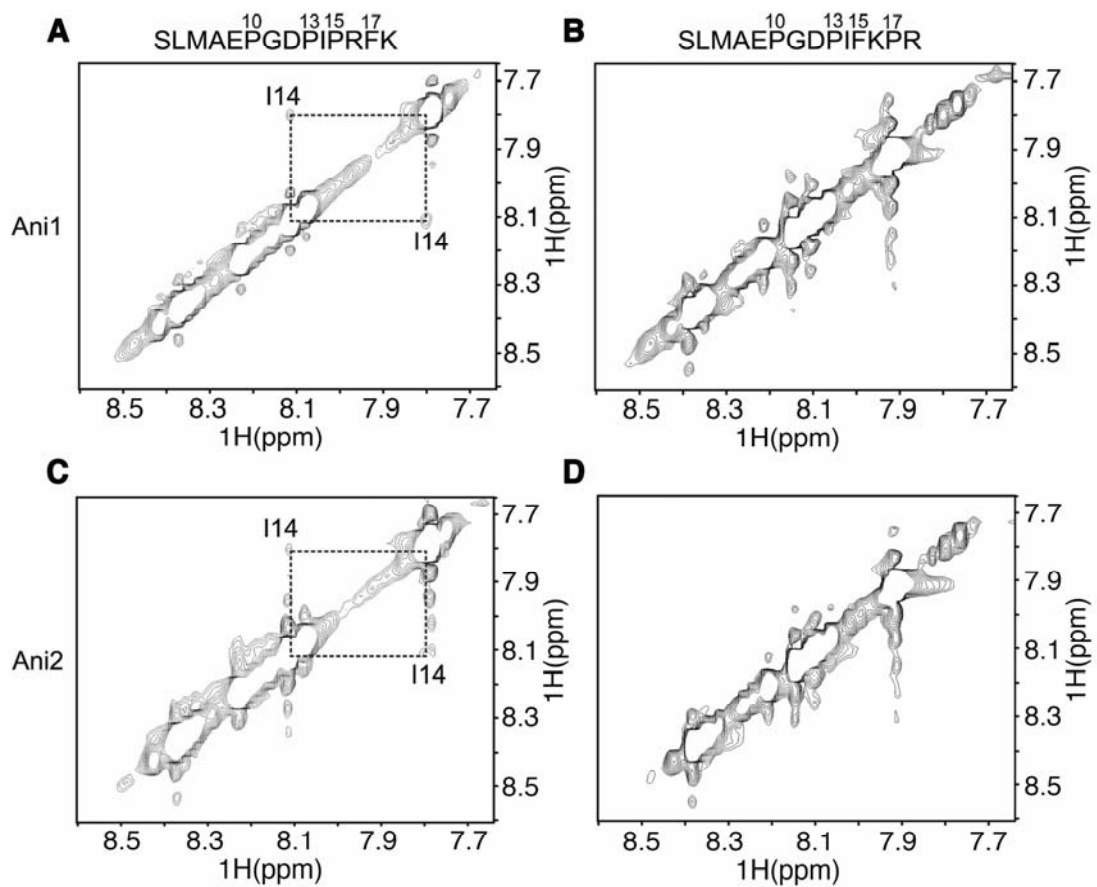
SpCENP-A NTD Isoomerase 1 (Ani1). (B) Alignment revealed that the fission yeast genome contains another unknown gene, SPAC27F1.06c, which encodes an Ani1-like protein (named Ani2). Ani1 and Ani2 share high sequence similarity with the Fpr3 and Fpr4 proline *cis-trans* isomerases from budding yeast across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

Supplementary Figure 7



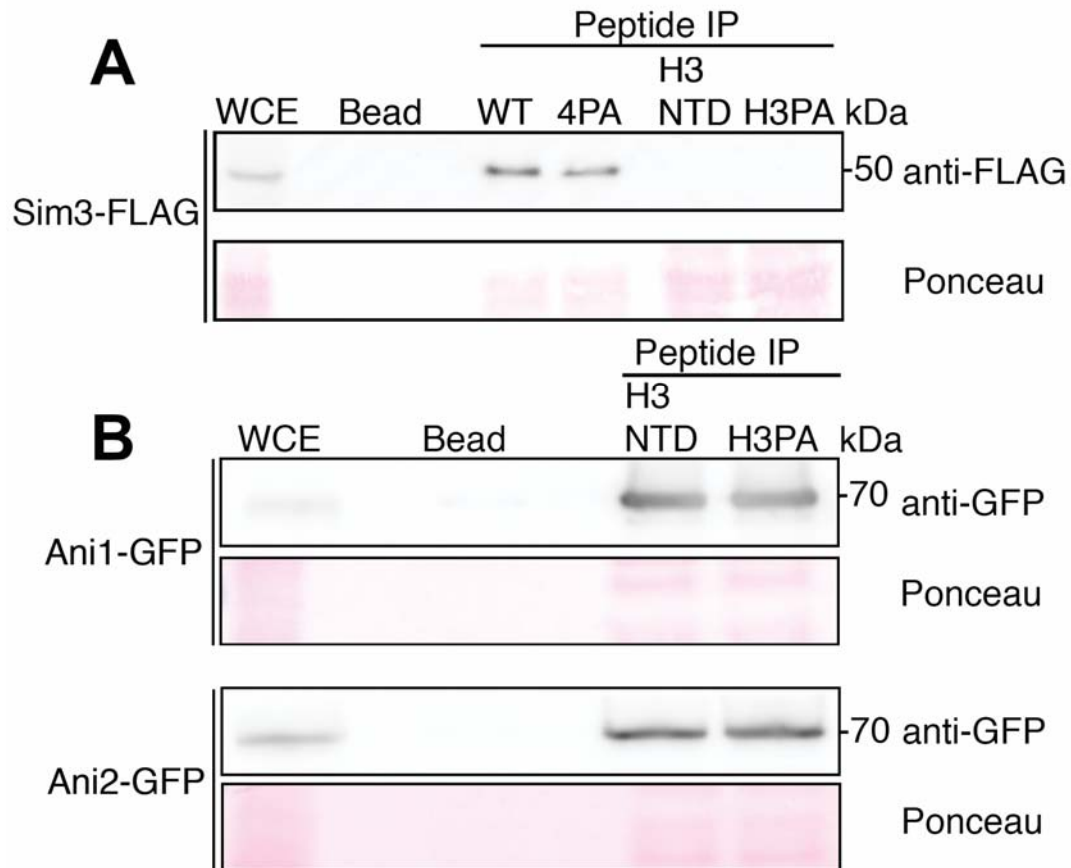
Supplementary Figure 7 SpCENP-A N-terminal domain (NTD) interacts with Cbh1-HA independent of proline residues. Peptide pull-down of wild type (WT) and mutant 4PA SpCENP-A and Cbh1-HA. WCE, Whole cell extract. Cdc2, input.

Supplementary Figure 8



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.