Fig S1.

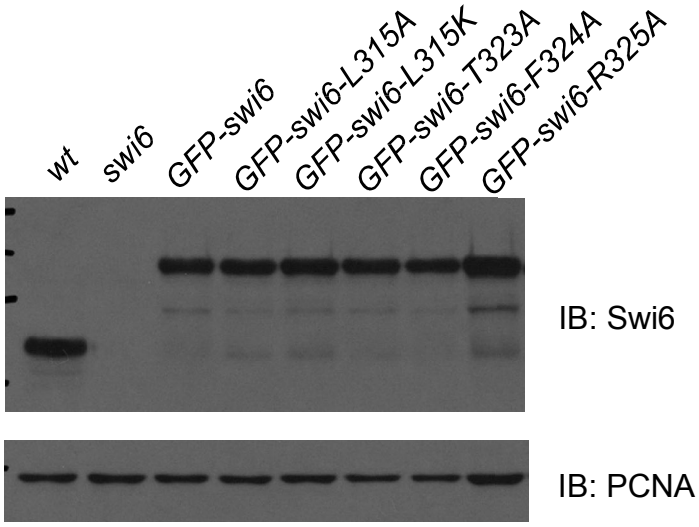

Fig S1: **Protein expression of GFP-Swi6 constructs is similar to endogenous Swi6 determined by western blot.** Mid-log phase cells were grown in EMM medium and protein lysates were prepared. 1:5000 dilution of antibody to endogenous Swi6 [37] was used. PCNA was detected as the loading control.

Fig S2.

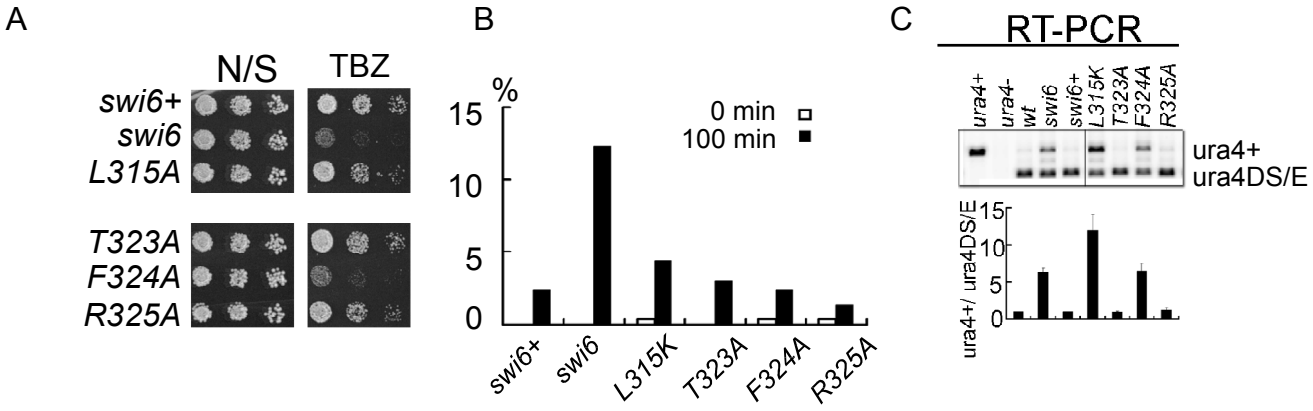

Fig S2: Chromosome segregation phenotypes of the *swi6* mutants. (**A**) TBZ sensitivity of mutants was measured by serial dilution on 17 µg/ml TBZ in EMM medium. (**B**) Quantitation of lagging chromosomes. Cells were arrested in 15 mM hydroxyurea at 32ºC for 4 hours and released into the cell cycle. At 100 min, the fraction of cells with >2 DAPI staining bodies was determined. 100 cells were counted in two independent experiments; the average is presented. **(C)** *ura4*$^{+}$ expression in *cen1L(dh)* measured using RT-PCR. Signals were normalized to expression of the *ura4-DS/E* minigene at the normal (euchromatic) locus.

Fig S3.

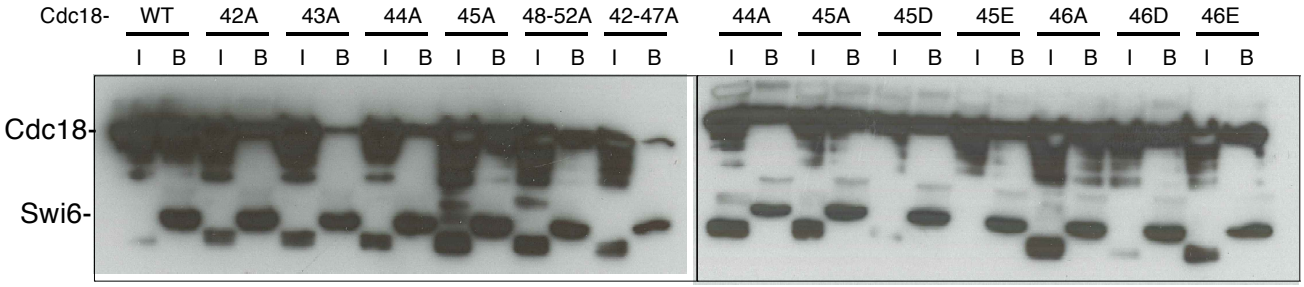

Fig S3: analysis of additional cdc18 mutations. *In vitro* binding studies were performed as in Fig 4B, by immunoprecipitation of recombinant MBP-Myc Cdc18 wild-type or fragments or mutants in presence of bacterially produced recombinant His6FLAG-Swi6 wild-type on FLAG M2-agarose conjugate beads. Cdc18 binding was measured by western blotting. Immunoblotting was performed with anti-Myc antibodies for cdc18 or anti-FLAG antibodies for Swi6. I *input*; B *beads*.

Fig S4

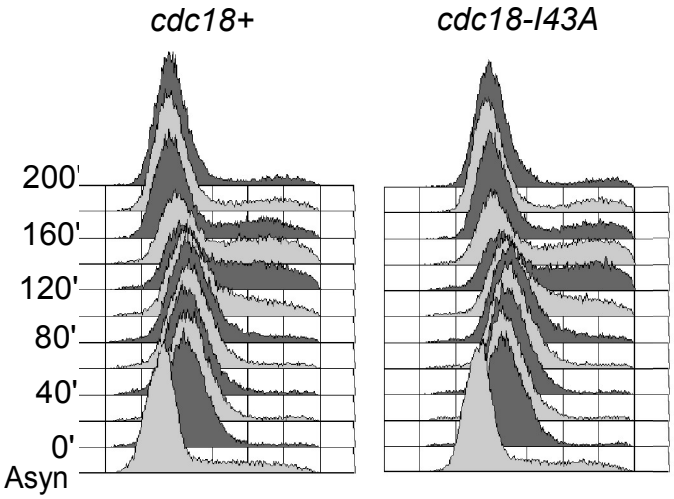

Fig S4: Cell cycle progression of *cdc18*$^{+}$ and *cdc18-I43A* determined by flow cytometry. Cells were synchronized at G2 by *cdc25-22* shifting to 36˚C for 4 hours and released at 25˚C. Samples were collected every 20 min for 200 min and fixed in 70% ethanol.

Fig S5

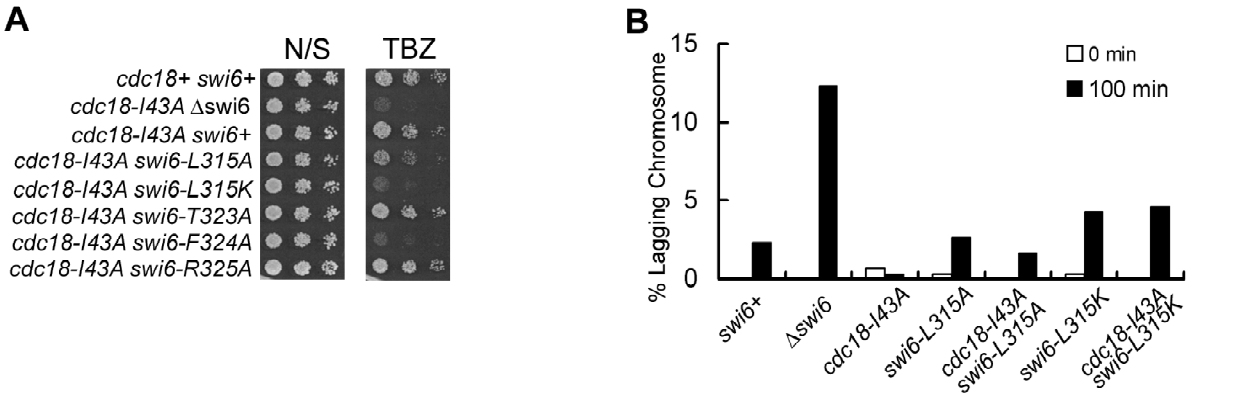

Fig S5: The phenotype of *cdc18-I43A* and *swi6* double mutants. (**A**) Centromere integrity and gene silencing were assayed by TBZ sensitivity, and cell viability on FOA medium. (**B**) Lagging chromosomes determined as Fig **S2.**

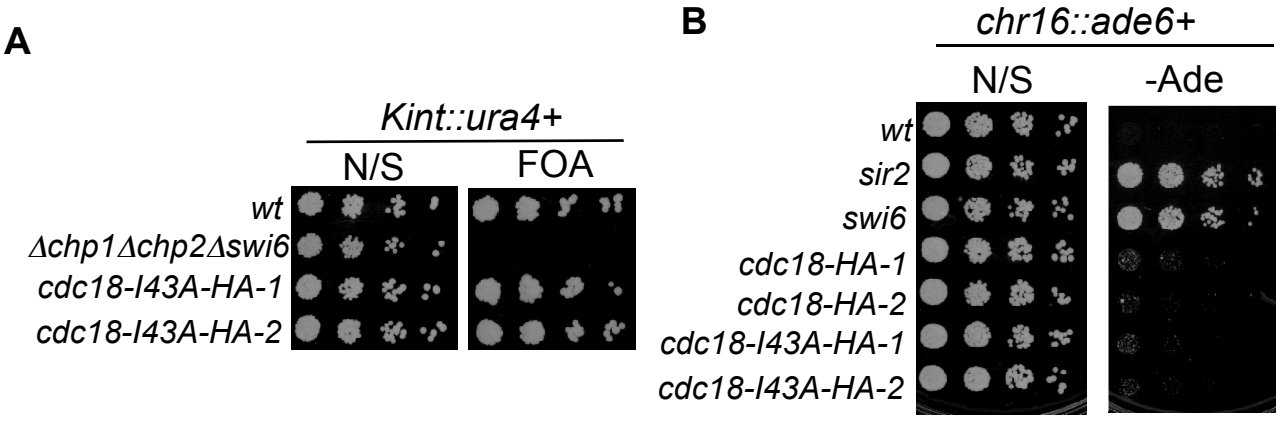

Fig S6: **Gene silencing of *cdc18-I43A* at different regions** was tested using a *ura4*$^{+}$ transgene at the mating locus (**A**), telomere (**B**). Normal silencing results in viability on FOA medium or Ade minus medium.

Table S1. Strains list

Number	Genotype	
254	h- can1-1 leu1-32 ade6-M210 ura4-D18	
2228	h- ∆swi6::his1+ otr1L(dh/HindIII)::ura4+ ade6-M210 leu1-32 ura4-DS/E his1-102	1
3386	h- ∆swi6::his1+ otr1L(dh/HindIII)::ura4+ ade6-M210 leu1-32::[leu1+ nmt 81-GFP-swi6] ura4-DS/E his1-102	This Study
3387	h- ∆swi6::his1+ otr1L(dh/HindIII)::ura4+ ade6-M210 leu1-32::[leu1+ nmt 81-GFP-swi6L315A] ura4-DS/E his1-102	This Study
3388	h- ∆swi6::his1+ otr1L(dh/HindIII)::ura4+ ade6-M210 leu1-32::[leu1+ nmt 81-GFP-swi6-L315K] ura4-DS/E his1-102	This Study
3389	h- ∆swi6::his1+ otr1L(dh/HindIII)::ura4+ ade6-M210 leu1-32::[leu1+ nmt 81-GFP-swi6-T323A] ura4-DS/E his1-102	This Study
3390	h- ∆swi6::his1+ otr1L(dh/HindIII)::ura4+ ade6-M210 leu1-32::[leu1+ nmt 81-GFP-swi6-F324A] ura4-DS/E his1-102	This Study
3391	h- ∆swi6::his1+ otr1L(dh/HindIII)::ura4+ ade6-M210 leu1-32::[leu1+ nmt 81-GFP-swi6-R325A] ura4-DS/E his1-102	This Study
3716	h- cdc18-I43A-HA ∆swi6::his1+ otr1L(dh/HindIII)::ura4+ ade6-M210 leu1-32::[leu1+ nmt 81-GFP-swi6] ura4-DS/E his1-102	This Study
3717	h- cdc18-I43A-HA ∆swi6::his1+ otr1L(dh/HindIII)::ura4+ ade6-M210 leu1-32::[leu1+ nmt 81-GFP-swi6-L315A] ura4-DS/E his1-102	This Study
3718	h- cdc18-I43A-HA ∆swi6::his1+ otr1L(dh/HindIII)::ura4+ ade6-M210 leu1-32::[leu1+ nmt 81-GFP-swi6-L315K] ura4-DS/E his1-102	This Study
3719	h- cdc18-I43A-HA ∆swi6::his1+ otr1L(dh/HindIII)::ura4+ ade6-M210 leu1-32::[leu1+ nmt 81-GFP-swi6-T323A] ura4-DS/E his1-102	This Study
3720	h- cdc18-I43A-HA ∆swi6::his1+ otr1L(dh/HindIII)::ura4+ ade6-M210 leu1-32::[leu1+ nmt 81-GFP-swi6-F324A] ura4-DS/E his1-102	This Study
3721	h- cdc18-I43A-HA ∆swi6::his1+ otr1L(dh/HindIII)::ura4+ ade6-M210 leu1-32::[leu1+ nmt 81-GFP-swi6-R325A] ura4-DS/E his1-102	This Study
3722	h- cdc18-I43A-HA ∆swi6::his1+ otr1L(dh/HindIII)::ura4+ ade6-M210 leu1-32::[leu1+ nmt 81-GFP] ura4-DS/E his1-102	This Study
2221	h+ swi6::his1 ade6-210 his1-102 leu1-32 ura4-DS/E otr1R (dg-glu) Sph1::ade6+	Robin Allshire
4008	h+ cdc18::cdc18-HA ade6∆N/N otr1R (dg-glu) Sph1:: ade6+ leu1	This Study
4009	h- cdc18::cdc18-I43A-HA ade6∆N/N otr1R (dg-glu) Sph1:: ade6+ leu1-32(PCL061209)	This Study
3324	h+ ort1L(dh/HindIII)::ura4+ leu1-32 ura4∆S/E ade6∆N/N [ch16 M23::LEU2+ tel::ade6+]	This Study
3384	h90 ∆sir2::KanMx6 cent::ura4+ leu1-32 ura4dS/E ade6dN/N [ch16 LEU2 tel::ade6+]	This Study
3385	h+ ∆swi6::his1+ c ent::ura4+ leu1-32 his1-102? ura4dS/E ade6dN/N ch16[LEU2 tel::ade6+]	This Study
3709	h+ cdc18-HA ura4DS/E otr1L::ura4+ ade6∆N/N [ch16 M23::LEU2+ tel::ade6+]	This Study
3710	h- cdc18-I43A-HA ura4DS/E otr1L::ura4+ ade6∆N/N [ch16 M23::LEU2+ tel::ade6+]	This Study
3472	h90 ade6-M216 his2 leu1-32 ura4DS/E Kint::ura4+ chp2+-13myc-kanMX6	2
3474	h90 ade6-M216 his2 leu1-32 ura4DS/E Kint2::ura4+ Dchp1::kanMX6 Dchp2::kanMX6 Dswi6::kanMX6	2
3723	h? cdc18-I43A-HA chp2-myc-kan uraDS/E kint::ura4+ leu1-32 ade?	This Study
3898	h+ leu1-32 ade6-M216 ura4DS/E imr1R::ura4+ ori1 chp1::TAP-kan^R ∆dcr1::nat^R	3
4010	h- cdc18::cdc18-HA imr1L(NcoI)::ura4+ ade6-M210 leu1-32 ura4-DS/E (PCL061209)	This Study
4011	h- cdc18::cdc18-I43A-HA imr1L(NcoI)::ura4+ ade6-M210 leu1-32 ura4-DS/E(PCL061209)	This Study
4233	h+ cdc25-22 cdc18::cdc18-HA leu1-32 ura4-D18 a de6-M210	This Study
4234	h- cdc25-22 cdc18::cdc18-I43A-HA leu1-32 ura4-D18 ade6-M210	This Study
4359	h- cdc18::cdc18-I43A-HA otr2 K" HindIII::ura4 oriII ade6-M210 leu1-32 ura4-DS/E	This Study
4360	h- cdc18::cdc18-I43A-HA otr2 K" HindIII::ura4 oriII ade6-M210 leu1-32 ura4-DS/E	This Study
4361	h- cdc18::cdc18-HA otr3 K" HindIII::ura4 ori1 ade6-M210 leu1-32 ura4-DS/E	This Study
4362	h+ ∆swi6::kanMX otr2 K" HindIII::ura4 ori1 ade6-M210 leu1-32 ura4-DS/E	This Study
4413	h+ ∆swi6::kanMX otr3 K" HindIII::ura4 ori1 ade6-M210 leu1-32 ura4-DS/E	This Study
4414	h+ cdc18::cdc18-I43A-HA otr3 K" HindIII::ura4 ori1 ade6-M210 leu1-32 ura4-DS/E	This Study
2318	h+ cdc10-V50 leu1-32::hENT1-leu1+(pJAH29) his7-366::hsv-tk-his7+(pJAH31) ade6-M216 ura4-D18	This Study

3812	h+ cdc10-V50 Dswi6::ura4+ leu1-32::hENT1-leu1+(pJAH29) his7-366::hsv-tk-his7+(pJAH31) ade6-M216 ura4-D18	This Study
3813	h+ cdc10-V50 cdc18::cdc18-I43A-HA leu1-32::hENT1-leu1+(pJAH29) his7-366::hsv-tk-his7+(pJAH31) ade6-M216 ura4-D18	This Study
4354	h- otr1Ldh(HindIII)::ura4+ leu1::hENT-Leu1+ his7-366::hsv-tk-his7+ ura4DS/E ade6-M210	This Study
4355	h+ cdc18::cdc18-I43A-HA otr1Ldh(HindIII)::ura4+ leu1::hENT-Leu1+ his7-366::hsv-tk-his7+ ura4DS/E ade6-M210	This Study
4356	h90 ∆swi6::kanMX otr1Ldh(HindIII)::ura4+ leu1::hENT-Leu1+ his7-366::hsv-tk-his7+ ura4DS/E ade6-M210	This Study

Table S2. Primers list

Genes	No.	Sequence
dh-F	1033	GTAAGTATGAGCAACTGGCG
dh-R	1034	GGAACAAATCAGGAAACCGAG
dg-F	1041	TTTTCAGCGAGACATGTACC
dg-R	1042	TCATAAAGCAACACTGGGTG
cnt-F	978	AGTTAAGCGGTATTATCACG
cnt-R	979	GAATTGACATATACTCTGTC
ura4-F	796	GAGGGGATGAAAAATCCCAT
ura4-R	797	TTCGACAACAGGATTACGACC
act1-F	1315	GAG TCC AAGACGATACCAGTG
act1-R	1316	GGCATCACACTTTCTACAACG
imr1-F	1039	CACATACCAAAAAGTCTGG
imr1-R	1040	GCTGAGGCTAAGTATCTGTT
ars2004-F	1374	ATGGTAGATGGAGAAACGGG
ars2004-R	1375	CACGGCATCTTTCTTCACGA
Non-ars-F	1376	TCGAAGATCCTACCGCTTTC
Non-ars-R	1377	GATTCACATAACCCGCTAGC

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2. Sadaie M, Iida T, Urano T, Nakayama J. A chromodomain protein, Chp1, is required for the establishment of heterochromatin in fission yeast. EMBO J 2004; 23:3825-35.

3. Motamedi MR, Verdel A, Colmenares SU, Gerber SA, Gygi SP, Moazed D. Two RNAi complexes, RITS and RDRC, physically interact and localize to noncoding centromeric RNAs. Cell 2004; 119:789-802.