Fig. S1. The rescuing effect of *dnt1Δ* on temperature-sensitive SIN mutant *sid2-250* and actomyosin ring formation mutant *cdc8-110* is reversed by higher level of Cdc25 induced by gain-of-function version of *cdc25*$^{+}$ (*cdc25-D1*). Serial dilutions (10-fold) of the indicated strains were spotted on EMM plus or without thiamine after being grown in YE and then washed by EMM liquid and incubated for 3–5 days at the indicated temperatures before being photographed.

Fig. S2. Quantification of cell lengths at cell division in double mutants between *dnt1Δ* and known cell cycle regulator mutants. Cells were grown in EMM at 25°C and *n*>200 cells with septa were measured for each genotype. Data are presented as in Fig. 1E. ***P<0.001.

Fig. S3. Dnt1 regulates G2/M transition independently of G2 DNA damage checkpoint and RACK1 kinase Cpc2. (A) Quantification of cell lengths at cell division in double mutants between *dnt1Δ* and G2 DNA damage checkpoint mutants. Cells were grown in EMM at 25°C and *n*>200 cells with septa were measured for each genotype. ***P<0.001. (B) Quantification of cell lengths at cell division in double mutants between *dnt1Δ* and *cpc2Δ* mutant. Cells were grown in EMM at 30°C and *n*>200 cells with septa were measured for each genotype. ***P<0.001. (C) *cpc2Δ* further enhanced the rescue of SIN mutant *cdc14-118* by *dnt1Δ*, showing additive effect. Serial dilutions (10-fold) of the indicated strains were performed as described in Fig. 1B.

Fig. S4. Cut12 and Dma1 are not involved in Dnt1 regulation of G2/M transition. (A) Quantification of cell lengths at cell division in double mutants between *dnt1Δ* and *dma1Δ* or *cut12-S11*. Cells were grown in YE at 25°C and *n*>200 cells with septa were measured for each genotype. ***P<0.001. **P<0.01. (B) Negative genetic interaction between *dnt1Δ* and *cdc25-22* cannot be rescued by *dma1Δ* or gain-of-function mutant *cut12-S11*. Serial dilutions (10-fold) of the indicated strains were spotted on YE plates and incubated for 3–5 days at the indicated temperatures before being photographed.

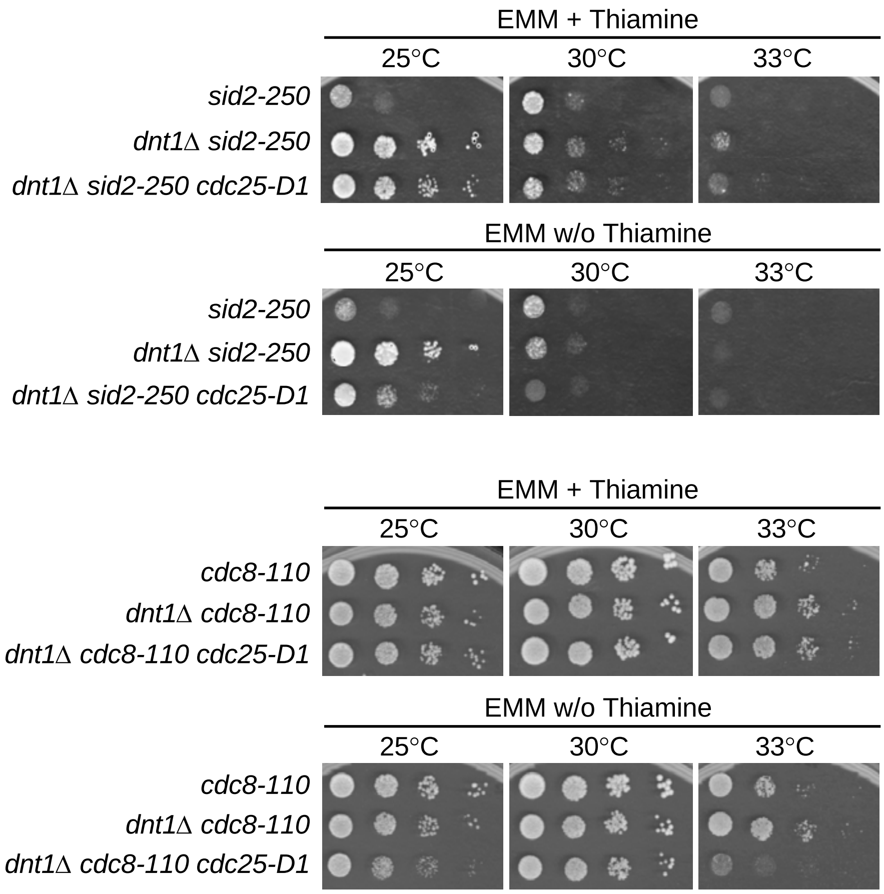
Fig. S5. Protein levels of other Cdk1 regulators, such as Cdc25, Cdc13 (cyclin B) or Cdr2 are not altered in *dnt1Δ* mutant. Protein samples were prepared from wild type and *dnt1Δ* strains with or without *cdc25-12myc*, *cdc13-HA* or *cdr2-2HA-6His*. Immunoblotting was performed with anti-myc or anti-HA antibodies depending the tagged proteins to be detected. The levels of each Cdk1 regulator protein were normalized to those of total Cdc2 in each strain. *n*=3. P>0.05.

Fig. S6. *wee1Δ* completely abolished rescuing capability of *dnt1Δ* on actomyosin ring formation mutant *cdc8-110*. (A) Serial dilutions (10-fold) of the indicated strains were spotted on YE plates and incubated for 3–5 days at the indicated temperatures before being photographed. (B) The frequencies of cells with multiple nuclei (as indication of cytokinetic defects) were quantified in single, double and triple mutants with indicated genotypes, as described in Fig. 4C.

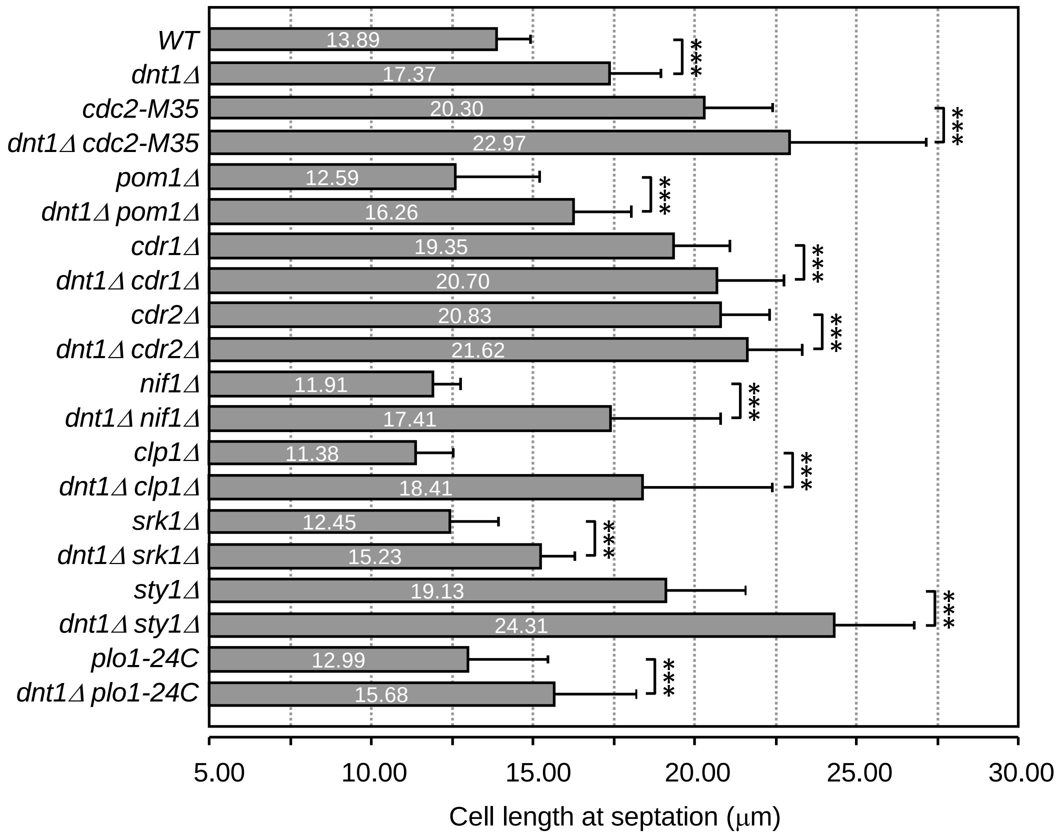
Fig. S7. Dma1 and Cut12 are not involved in SIN regulation by Dnt1. (A and B) Deletion of *dma1*$^{+}$ does not affect the rescue of SIN mutant *cdc14-118* by *dnt1Δ*. (A) Serial dilutions (10-fold) of the indicated strains were spotted on YE plates and incubated for 3–5 days at the indicated temperatures before being photographed. (B) The frequencies of cells with multiple nuclei (as indication of cytokinetic defects) were quantified in single, double and triple mutants with indicated genotypes, as described in Fig. 4C. (C and D) The gain-of-function mutant *cut12-S11* does not affect the rescue of SIN mutant *cdc14-118* by *dnt1Δ*. Drop test of serial dilutions and quantification of multiple nuclei were analyzed as for *dma1Δ* mutants in A and B.

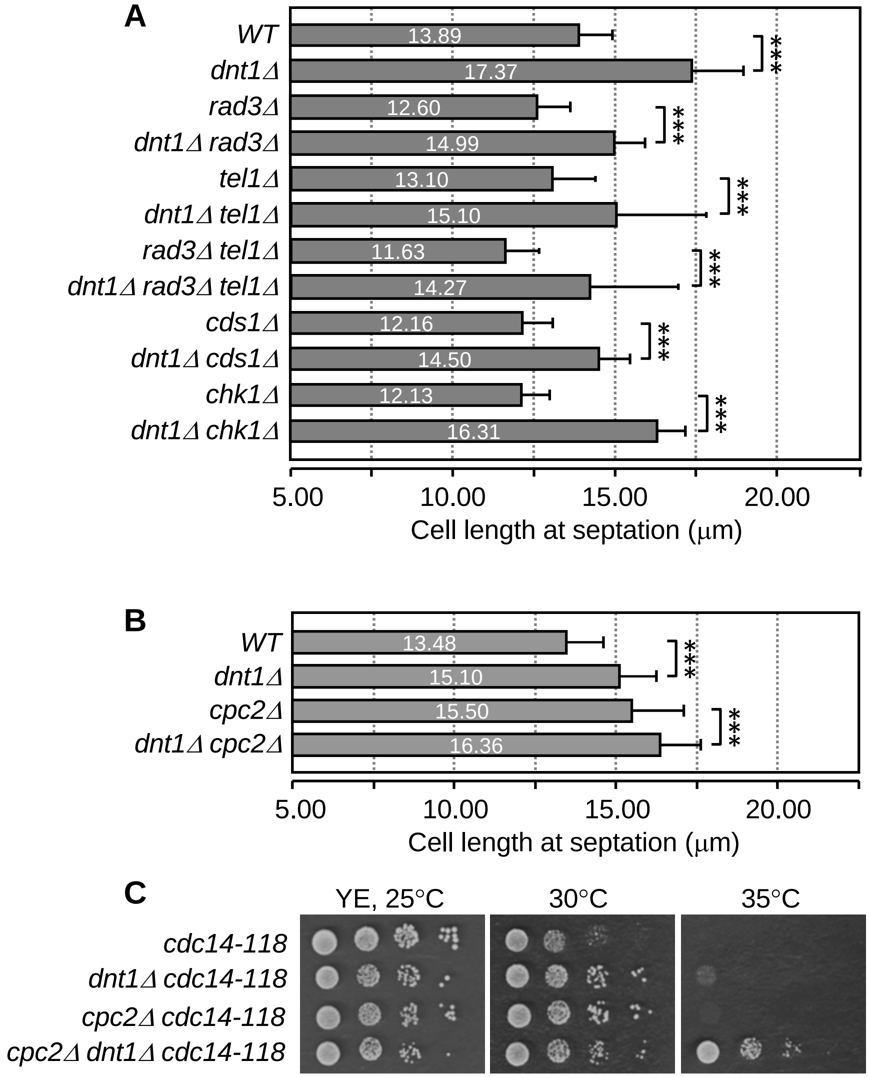
Table S1. Yeast strains used in this study.

Yu et al., Supplementary Figure S1.

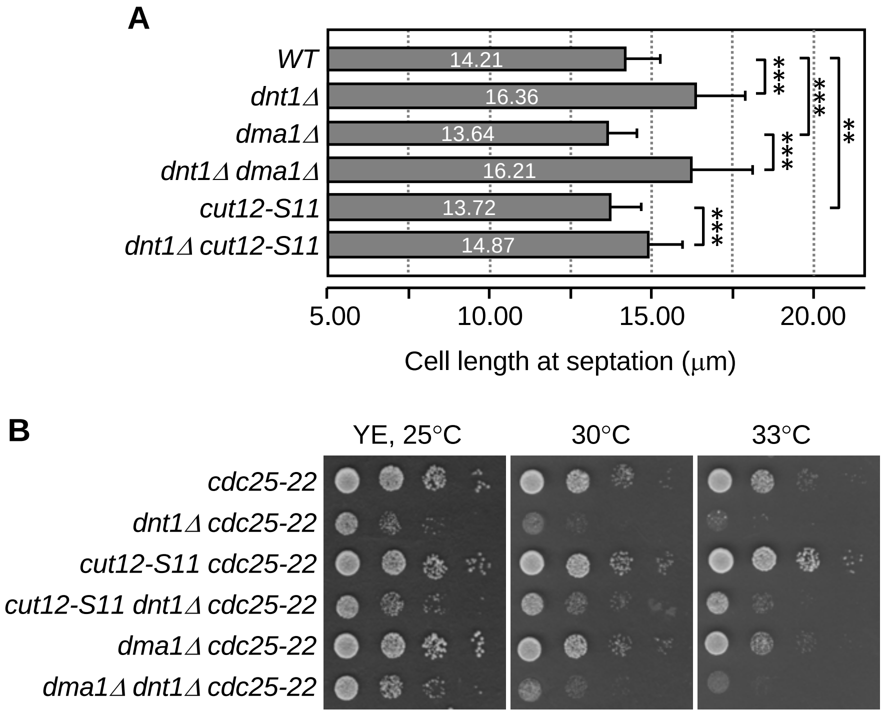


Yu et al., Supplementary Figure S2.

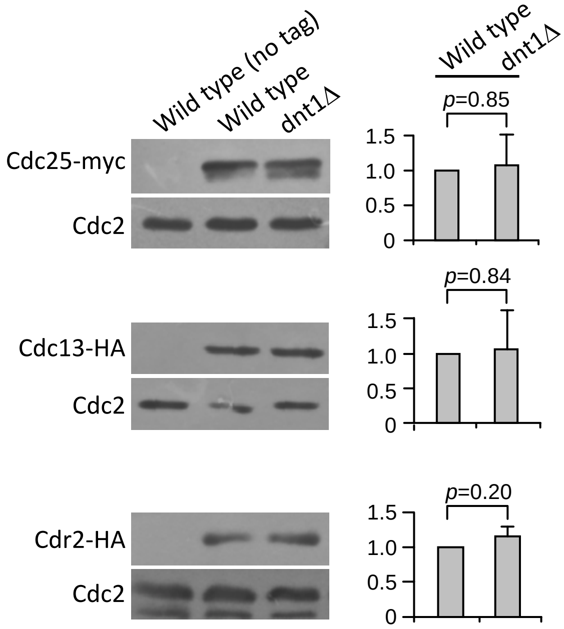



A
WT
dnt1Δ
rad3Δ
dnt1Δ rad3Δ
tel1Δ
dnt1Δ tel1Δ
rad3Δ tel1Δ
dnt1Δ rad3Δ tel1Δ
cds1Δ
dnt1Δ cds1Δ
chk1Δ
dnt1Δ chk1Δ
13.89
17.37
12.60
14.99
13.10
15.10
11.63
14.27
12.16
14.50
12.13
16.31
***
5.00
10.00
15.00
20.00
Cell length at septation (μm)
B
WT
dnt1Δ
cpc2Δ
dnt1Δ cpc2Δ
13.48
15.10
15.50
16.36
***
5.00
10.00
15.00
20.00
Cell length at septation (μm)
C
YE, 25°C
30°C
35°C
cdc14-118
dnt1Δ cdc14-118
cpc2Δ cdc14-118
cpc2Δ dnt1Δ cdc14-118

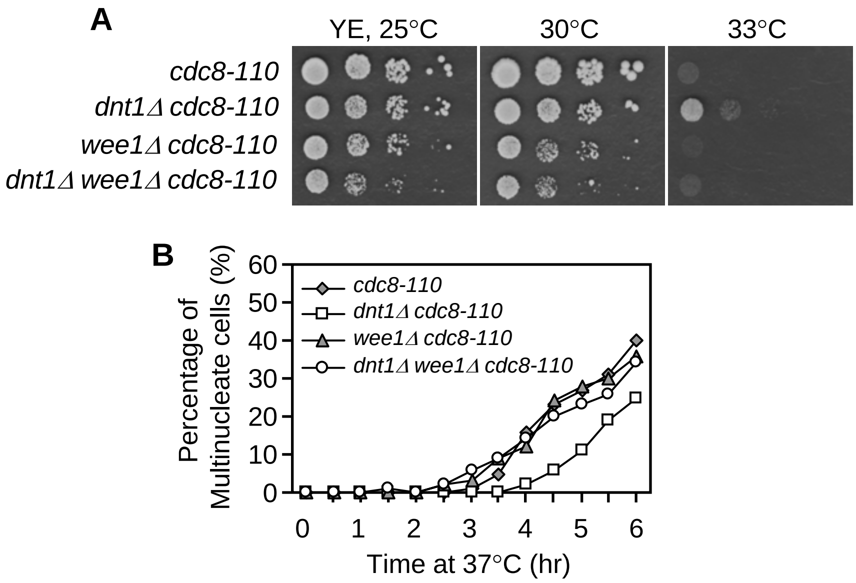
Yu et al., Supplementary Figure S4.



Yu et al., Supplementary Figure S5.



Yu et al., Supplementary Figure S6.



Yu et al., Supplementary Figure S7.

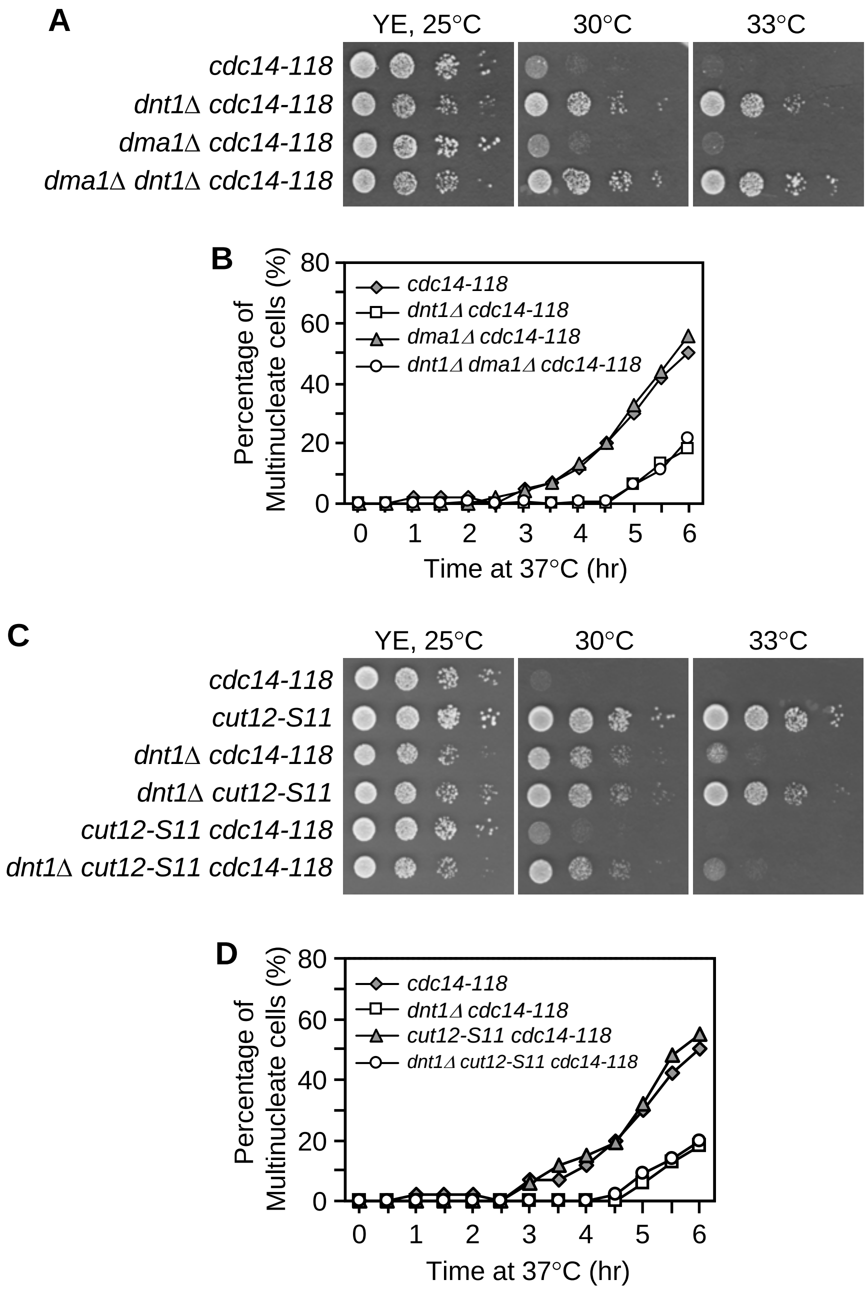


Table S1. Yeast strains used in this study

Strain Name	Genotype
JY1	*h^{-} leu1-32 ura4-D18 ade6-210*
JY2	*h^{+} leu1-32 ura4-D18 ade6-210*
JY109	*h^{-} dnt1Δ::ura4^{+} ade6-210 leu1-32 ura4-D18*
JY145	*h^{+} dnt1Δ::kanR ade6-210 leu1-32 ura4-D18*
JY2166	*h^{90} cdc2-M35 ura4-D18 leu1-32*
JY2281	*$h^{?}$ dnt1Δ::ura4^{+} cdc2-M35 ura4-D18 leu1-32*
JY2423	*h^{+} cdr1Δ::kanR his2 leu1-32 ura4-D18*
JY2470	*$h^{?}$ dnt1Δ::ura4^{+} cdr1Δ::kanR his2 leu1-32 ura4-D18*
JY 2155	*h^{-} cdr2Δ::ura4^{+} ura4-D18*
JY 2204	*$h^{?}$ dnt1Δ::kanR cdr2Δ::ura4^{+} ura4-D18*
JY 2159	*h^{-} cmk2Δ::ura4^{+} ura4-D18*
JY 2278	*$h^{?}$ dnt1Δ::kanR cmk2Δ::ura4^{+} ura4-D18*
JY 2165	*h^{-} cdc2-1w leu1*
JY 2229	*$h^{?}$ dnt1Δ::kanR cdc2-1w leu1*
JY 2137	*h^{+} cpc2Δ::kanR ade^{-} leu^{-} ura^{-}*
JY 2194	*h^{+} cpc2Δ::kanR dnt1Δ::ura4^{+} ade^{-} leu^{-} ura^{-}*
JY125	*h^{-} dnt1-13myc::kanR ade6-210 leu1-32 ura4-D18*
JY2136	*h^{+} cpc2-GFP::kanR ade^{-} leu^{-} ura^{-}*
JY2493	*$h^{?}$ dnt1-13myc::kanR cpc2-GFP::kanR ade^{-} leu^{-} ura^{-}*
JY2263	*$h^{?}$ cpc2-GFP::kanR dnt1Δ::ura4^{+} ade^{-} leu^{-} ura^{-}*
JY2357	*h^{-} nif1Δ::ura4^{+} ura4-D18*
JY2409	*$h^{?}$ dnt1Δ::kanR nif1Δ::ura4^{+} ura4-D18*
JY18	*h^{-} wee1-50 ade6-21x his3-D1 leu1-32 ura4-D18*
JY2395	*$h^{?}$ dnt1Δ::kanR wee1-50 ade6-21x his3-D1 leu1-32 ura4-D18*
JY2558	*h^{+} wee1-50 mik1Δ::ura4^{+} leu1-32 ura4-D18*
JY2562	*$h^{?}$ dnt1Δ::kanR wee1-50 mik1Δ::ura4^{+} leu1-32 ura4-D18*
JY2160	*h^{-} srk1Δ::ura4^{+} ura4-D18*
JY2267	*$h^{?}$ dnt1Δ::kanR srk1Δ::ura4^{+} ura4-D18*
JY2162	*h^{-} sty1Δ::ura4^{+}*
JY2333	*$h^{?}$ dnt1Δ::kanR sty1Δ::ura4^{+} ura4-D18*
JY14	*h^{-} plo1-24C ade6-210 leu1-32 ura4-D18*
JY439	*h^{-} dnt1Δ::kanR plo1-24C ade6-21x leu1-32 ura4-D18*
JY492	*h^{-} pom1Δ::ura4^{+} leu^{-} ura^{-}*

JY2084	h$^{?}$ dnt1Δ::kanR pom1Δ::ura4^{+} leu^{-} ura^{-}
JY1694	h^{+} dnt1Δ::ura4^{+} cdc8-110 leu1-32 ura4-D18
JY1849	h$^{?}$ dnt1Δ::kanR cdc8-110 leu1-32::P$_{nmt1}$-cdc25-D1::leu1^{+} ura4-D18
JY2758	h$^{?}$ dnt1Δ::kanR wee1Δ::ura4^{+} cdc8-110 ura4-D18
JY2869	h^{+} cdc8-110 wee1Δ::ura4^{+} ura4-D18
JY47	h^{+} cdc14-118 ade6-210 leu1-32 ura4-D18
JY2211	h$^{?}$ cdc14-118 leu1-32::P$_{nmt1}$-cdc25-D1::leu1^{+} ade6-21x ura4-D18
JY2212	h$^{?}$ cdc14-118 leu1-32::P$_{nmt1}$-cdc25-D1::leu1^{+} ura4-D18
JY1110	h^{-} dnt1Δ::kanR cdc14-118 ade6-210 ura4-D18
JY2177	h$^{?}$ dnt1Δ::kanR cdc14-118 leu1-32::P$_{nmt1}$-cdc25-D1::leu1^{+} ade6-210 ura4-D18
JY2867	h$^{?}$ wee1Δ::ura4^{+} cdc14-118 ura4-D18
JY2441	h$^{?}$ cpc2Δ::kanR cdc14-118 ade^{-} leu^{-} ura^{-}
JY2504	h$^{?}$ cpc2Δ::kanR dnt1Δ::ura4^{+} cdc14-118 ade- leu- ura-
JY2662	h^{-} wee1-3HA6H leu1-32 ura4-D18
JY2715	h$^{?}$ dnt1Δ::ura4^{+} wee1-3HA6H leu1-32 ura4-D18
JY22	h^{+} cdc25-22 ura4-D18
JY1845	h$^{?}$ dnt1Δ::kanR cdc25-22 ura4-D18
JY7	h^{-} cdc8-110 leu1-32 his3-D1 ura4-D18
JY2361	h^{-} cdr2-2HA-6His::ura4^{+} leu1-32 ura4-D18
JY2412	h$^{?}$ dnt1Δ::kanR cdr2-2HA-6His::ura4^{+} leu1-32 ura4-D18
JY270	h^{-} cdc13-HA::ura4^{+} leu1 ura4-D18
JY1836	h$^{?}$ dnt1Δ::kanR cdc13-HA::ura4^{+} leu1-32 ura4-D18
JY2156	h^{-} wee1Δ::ura4^{+}
JY2270	h$^{?}$ dnt1Δ::kanR wee1Δ::ura4^{+} ura4-D18
JY1652	h^{-} leu1-32::P$_{nmt1}$-cdc25-D1::leu1^{+} ura^{-}
JY1800	h^{-} dnt1Δ::kanR leu1-32::P$_{nmt1}$-cdc25-D1::leu1^{+} ade6-210 ura4-D18
JY61	h^{+} clp1Δ::ura4^{+} ade6-216 leu1-32 ura4-D18
JY130	h^{-} dnt1Δ::ura4^{+} clp1Δ::ura4^{+} ade6-21x leu1-32 ura4-D18
JY2943	h^{-} rad3Δ::ura4^{+} leu1-32 ura4-D18
JY2984	h^{+} dnt1Δ::kanR rad3Δ::ura4^{+} leu1-32 ura4-D18
JY2947	h^{-} chk1Δ::ura4^{+} ade6-704 leu1-32 ura4-D18
JY2991	h^{+} dnt1Δ::kanR chk1Δ::ura4^{+} leu1-32 ura4-D18
JY2944	h^{-} cds1Δ::ura4^{+} leu1-32 ura4-D18
JY2993	h^{-} dnt1Δ::kanR cds1Δ::ura4^{+} leu1-32 ura4-D18
JY2163	h^{-} cdc2-3w cdc25Δ::ura4^{+} leu1-32 ura4-D18
JY2327	h^{+} dnt1Δ::kanR cdc2-3w ade6-21x ura4-D18

JY1693	h^{-} *cdc25-12myc::*$ura4^{+}$ *leu1-32 ura4-D18*
JY1744	$h^{?}$ *dnt1Δ::kanR cdc25-12myc::*$ura4^{+}$ *leu1-32 ura4-D18*
JY44	h^{+} *sid2-250 ade6-21x leu1-32 ura4-D18*
JY2576	h^{+} *dnt1Δ::*$ura4^{+}$ *sid2-250 ade6-21x leu1-32 ura4-D18*
JY3047	$h^{?}$ *wee1Δ::*$ura4^{+}$ *sid2-250 ura4-D18*
JY3049	$h^{?}$ *dnt1Δ::kanR wee1Δ::*$ura4^{+}$ *sid2-250 ura4-D18*
JY2762	$h^{?}$ *dnt1Δ::kanR sid2-250 ade6-21x leu1-32 ura4-D18*
JY2765	$h^{?}$ *sid2-250 leu1-32::*P_{nmt1}*-cdc25-D1::*$leu1^{+}$ *ade6-21x ura4-D18*
JY3507	h^{-} *ade4-luc::kanR wee1-R.luc::natR leu1-32 ura4-18*
JY3583	h^{+} *dnt1Δ::*$ura4^{+}$ *ade4-luc::kanR wee1-R.luc::natR leu1-32 ura4-D18*
JY3576	h^{+} *mts3-1 ade4-luc::kanR wee1-R.luc::natR leu1-32 ura4-D18*
JY3626	h^{+} *skp1-A7 ade4-luc::kanR wee1-R.luc::natR leu1 ura4*
JY3640	h^{-} *dnt1Δ::*$ura4^{+}$ *skp1-A7 ade4-luc::kanR wee1-R.luc::natR leu1 ura4*
JY3514	h^{+} *tel1Δ::*$LEU2^{+}$ *ade6-210 leu1-32 ura4-D18*
JY3595	h^{+} *dnt1Δ::kanR tel1Δ::*$LEU2^{+}$ *ade6-210 leu1-32 ura4-D18*
JY3597	h^{-} *dnt1Δ::kanR rad3Δ::*$ura4^{+}$ *tel1Δ::*$LEU2^{+}$ *ade6-210 leu1-32 ura4-D18*
JY3649	h^{-} *rad3Δ::*$ura4^{+}$ *tel1Δ::*$LEU2^{+}$ *leu1-32 ura4-D18*
JY3269	h^{-} *skp1-A7 leu1 ura4*
JY3418	h^{+} *dnt1Δ::kanR skp1-A7 leu1 ura4*
JY3480	h^{-} *skp1-A7 cdc14-118 leu1 ura4*
JY3482	h^{+} *dnt1Δ::kanR skp1-A7 cdc14-118 leu1 ura4*
JY2580	$h^{?}$ *dma1Δ::*$ura4^{+}$ *cdc14-118 leu1-32 ura4-D18*
JY2582	$h^{?}$ *dma1Δ::*$ura4^{+}$ *dnt1Δ::kanR cdc14-118 leu1-32 ura4-D18*
JY2499	h^{+} *kanR::*P_{81nmt1}*-GFP-wee1*
JY4328	h^{-} *kanR::*P_{81nmt1}*-GFP-wee1 cdc14-118*
JY2312	h^{+} *cut12-S11 leu1-32*
JY2125	h^{-} *dnt1Δ::kanR cut12-S11 leu1-32*
JY2594	h^{+} *cut12-S11 cdc25-22 ade6-210 leu1-32 ura4-D18*
JY2589	h^{+} *dnt1Δ::kanR cdc25-22 cut12-S11 leu1-32 ura4-D18*
JY2595	$h^{?}$ *dnt1Δ::kanR dma1Δ::*$ura4^{+}$ *cdc25-22 leu1-32 ura4-D18*
JY2596	$h^{?}$ *dma1Δ::*$ura4^{+}$ *cdc25-22 leu1-32 ura4-D18*
JY4364	h^{-} *cut12-S11 cdc14-118 ade6-21x leu1-32* $ura4^{-}$
JY4365	h^{+} *dnt1Δ::kanR cut12-S11 cdc14-118 ade6-21x leu1-32*