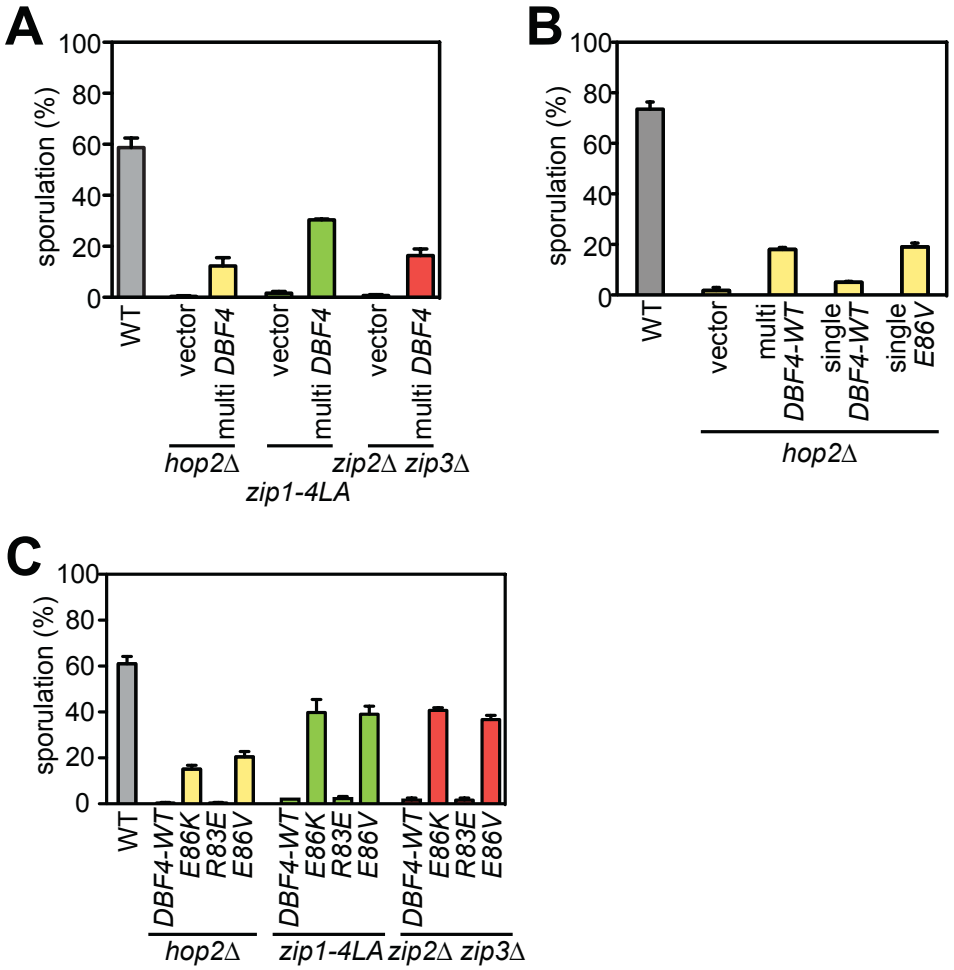
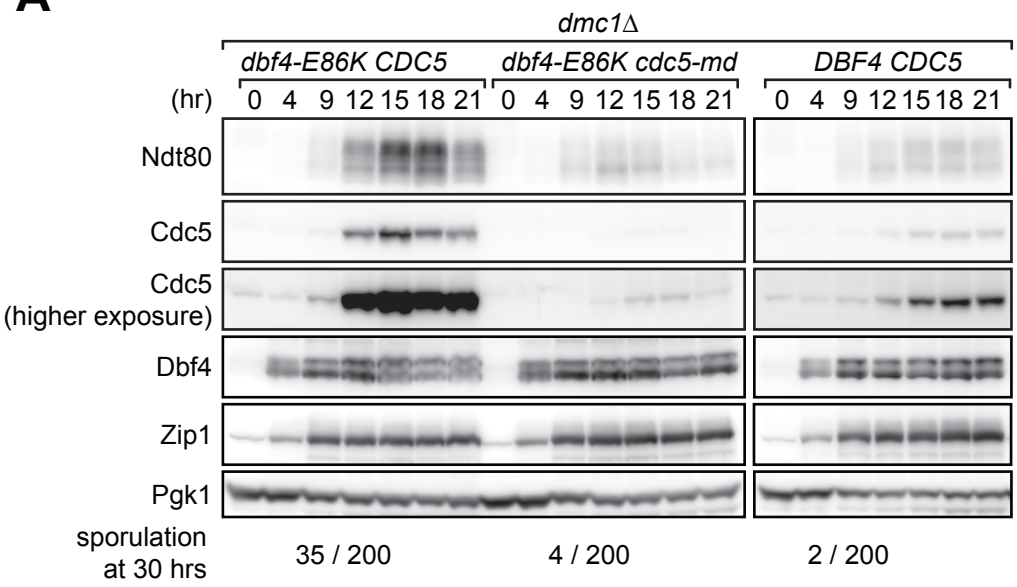
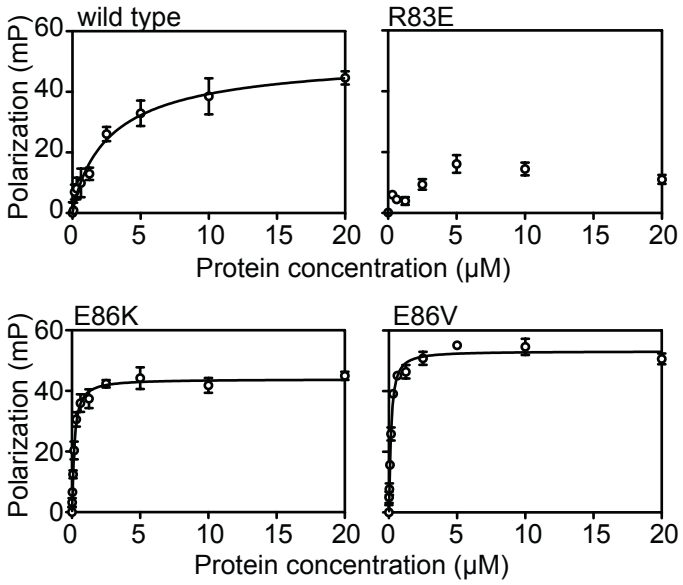
Appendix: Contents


A
sporulation (%)
100
80
60
40
20
0
WT
vector
multi DBF4
vector
multi DBF4
vector
multi DBF4
hop2Δ
zip1-4LA
zip2Δ zip3Δ
B
sporulation (%)
WT
vector
multi DBF4-WT
single DBF4-WT
single E86V
hop2Δ
C
sporulation (%)
WT
DBF4-WT
E86K
R83E
E86V
DBF4-WT
E86K
R83E
E86V
DBF4-WT
E86K
R83E
E86V
hop2Δ
zip1-4LA
zip2Δ zip3Δ

A


dmc1Δ
dbf4-E86K CDC5
dbf4-E86K cdc5-md
DBF4 CDC5
(hr)
0 4 9 12 15 18 21
0 4 9 12 15 18 21
0 4 9 12 15 18 21
Ndt80
Cdc5
Cdc5
(higher exposure)
Dbf4
Zip1
Pgk1
sporulation
at 30 hrs
35 / 200
4 / 200
2 / 200

B


wild type
R83E
E86K
E86V
Polarization (mP)
0
20
40
60
0 5 10 15 20
Protein concentration (μM)

Appendix figure legends

Figure S1. Enhanced Dbf4-Cdc5 Interaction Suppresses the Cell Cycle Arrest Associated with Defects in Meiotic HR Factors

(A-C) Strains in the BR1919 background were incubated for 3 days on sporulation plates and sporulation percentage was determined by light microscopy. These mutant backgrounds were employed as they show pachytene arrest as a result of meiotic recombination initiation in the BR1919 background. multi, multicopy vector; single, single copy vector; WT, wild type.

Data are represented as means ± SEM from three experiments. At least 100 cells were scored per experiment.

Figure S2. Dbf4-Mediated Suppression of Pachytene Arrest Requires the Dbf4-Cdc5 Interaction

(A) Strains were induced to synchronously enter meiosis. Cells were harvested at the indicated time points and proteins were detected as in Fig 1B. An overexposed immunoblot of Cdc5 is shown to illustrate the presence of basal levels of Cdc5 before, or in the absence of, pachytene exit.

(B) Plotted data of fluorescence polarization measurements. These data were used to calculate dissociation constants (K_d) for the interaction between Dbf4 peptides and Cdc5-PBD (see Fig 1C).

Supplementary Methods

Strains and plasmids

The genotypes of all strains used are listed in Appendix Table S1. Strains and plasmids used in each Figure are as follows. Figure 1B: TBR6887, TBR7552, TBR7553, TBR7483. Figure 1C: p1351. Figure 1D: BA081, BA0133, BA0135, BA0137. Figure 2A: TBR6887, TBR7483, TBR8454, TBR8450, TBR9176, TBR9121. Figure 2B: TBR6621, TBR6887, TBR8454, TBR8450, TBR8372, TBR9121. Figure 3A: TBR6887, TBR7483, TBR8672, TBR9175, TBR9237. Figure 3B-3C: TBR10060, TBR10062, TBR10080, TBR10078, TBR10076. Figure 4A: TBR9695, TBR9693, TBR9697, TBR9699, TBR9701. Figures 4B-4C: TBR8673, TBR8674, TBR8764, TBR8765, TBR9107. Figure 4D: TBR9367, TBR9533, TBR9747, TBR9749. Figures 5A-5B: TBR10575. Figure 5C: TBR10060, TBR10062, TBR10080, TBR10190, TBR10192. Figure 5D: TBR10060, TBR10190, TBR10192. Figure 5E: TBR9695, TBR9693. Figure 6A: TBR10582, TBR10656, TBR10603. Figure 6B: TBR10696, TBR10697. Figure 6C: TBR10699. Figure 6D: TBR9693, TBR10798, TBR10840. Figure 6E: TBR10816, TBR10800, TBR10842. Figure 6F: TBR10718, TBR10131, TBR10129. Figure 7A: TBR10843, TBR11031. Figure 7B: TBR10843, TBR10990, TBR10991. Figure EV1A: TBR2065 without vector, along with TBR310, TBR2434, TBR2780, carrying YEplac33 (vector) or p587 (multicopy *DBF4*). Figure EV1B: TBR2065 without vector, TBR310 carrying YCplac22 (vector), p587 (multicopy *DBF4-WT*), p586 (singlecopy *DBF4-WT*), p698 (singlecopy *E86V*). Figure EV1C: TBR2065, TBR310, TBR6448, TBR6449, TBR6450, TBR2434, TBR6451, TBR6505, TBR6506, TBR2780, TBR6507, TBR6508, TBR6557. Figure EV2A: TBR7552, TBR11165, TBR6887. Figure EV3A-3B: TBR310, TBR4711, TBR6448, TBR6449, TBR6450. Figure EV4A: TBR6621, TBR6887, TBR7483, TBR8672. Figure EV4C-4D: TBR11138, TBR11161, TBR11163. Figure EV5A: TBR9697, TBR9701. Figure EV5B: TBR8673, TBR8674, TBR8764, TBR8765, TBR8766. Figure EV5C: TBR6621, TBR9488, TBR7464. Figures EV5D-F: TBR10575. Figure EV6A: TBR10582, TBR10732. Figure EV6B: TBR10603, TBR10733. Figure EV7A:

TBR10101, TBR10105, TBR10119. Figure EV7B: TBR10129. Figure EV7C-D: TBR10843, TBR11143. Figure EV7E: TBR10843, TBR10995. Figure EV7F: TBR10843, TBR11028.

Gene deletions and C-terminus epitope tagging were performed using PCR-mediated gene replacement and tagging techniques as described previously (Longtine et al., 1998). *NDT80-6xHA* was described previously (Tung et al., 2000).

In order to create the *CDC5-DBF4* fusion gene under the control of the *DBF4* promoter, the *DBF4* promoter (536 nucleotides before the start codon), *CDC5,* and *DBF4*/*dbf4-R83E*, were individually amplified and assembled by PCR, with a sequence encoding five consecutive alanines placed between the two ORFs as a linker. The assembled construct was cloned at the EcoRI and PstI sites of YIplac211 (Gietz and Sugino, 1988). The resultant plasmids were linearized by digestion with NcoI and integrated at the *URA3* locus.

To identify Ser/Thr residues in Dbf4 that might be phosphorylated, we focused on residues within or near motif M (Masai and Arai, 2000) that are conserved among *Saccharomyces* species (*cerevisiae*, *bayanus*, *mikatae*, *paradoxus*, *castellii*, *kluyveri*). These residues were subsequently changed to Ala and the phosphorylation of Dbf4 was monitored by electrophoretic mobility, leading to identification of the *dbf4-4A* mutant, in which S318, S319, S374 and T375 were changed to Ala.

Measuring sporulation

Single colonies were patched onto YPD or selective plates and incubated at 30°C overnight. These were replica plated onto sporulation medium and incubated at 30°C and sporulation was examined. Incubation was for 48 hours, unless indicated otherwise. For each strain, spore formation was measured in three independent experiments, with at least 100 cells scored in each experiment. Measurements of spore viability came from the dissection of at least 40 tetrads.

Protein purification

A fragment of *CDC5* encoding a part of the protein including the PBD, from amino acid 357 to the C-terminus, was cloned at the BamHI and XhoI sites of pGEX-6P-1 (GE Healthcare Life Sciences) to construct p1351, which was used to produce the GST-CDC5-PBD fusion protein. GST-CDC5-PBD was induced in Rosetta2 (DE3) at 18°C overnight with 0.3 mM of IPTG. The induced cells were sonicated in breaking buffer (50 mM TrisCl (7.5), 10% glycerol, 0.5 mM EDTA, 250 mM NaCl, 0.01% igepal, 1 mM β-MeOH and 100 ug/ml PMSF). The GST-CDC5-PBD fusion in the cleared lysate was bound to glutathione agarose beads (Qiagen), and the bound protein was eluted in the elution buffer (25 mM TrisCl (7.5), 10% glycerol, 0.5 mM EDTA, 250 mM NaCl, 0.01% igepal, 1 mM β-MeOH and 20 mM glutathione). The eluate was fractionated in a gel filtration column with the column buffer (20 mM HEPES pH 7.5, 250 mM NaCl, 5 mM EDTA, 0.5 mM TCEP, 10% glycerol). Peak fractions were combined and concentrated using a microconcentrator (vivaspin-30). The concentrated protein solution was immediately used for measurements.

Fluorescence polarization assay

All peptides used were purchased from Peptide Protein Research (Fareham, UK). Amino acid sequences of the used peptides are as follows:

wild type	Flu-GGEKKRARIERARSIEGAVQVSKGTG
R83E	Flu-GGEKKRARIERAESIEGAVQVSKGTG
E86K	Flu-GGEKKRARIERARSIKGAVQVSKGTG
E86V	Flu-GGEKKRARIERARSIVGAVQVSKGTG

(Flu, Fluorescein. Two Gs were placed between Flu and the Dbf4 peptide as a linker).

Fluorescein-labelled peptides (WT and mutant Dbf4 peptides) at 100 nM were incubated at room temperature with increasing concentrations of Cdc5-PBD in 20 mM HEPES pH 7.5, 250 mM NaCl, 5 mM EDTA, 0.5 mM TCEP, 10% glycerol and 0.05% tween 20. The sample mixtures, 50 µl per sample, were transferred to a black 96-well polypropylene plate for measurement of fluorescence polarization in a POLAR star Omega multimode microplate reader (BMG LABTECH). Emission signals from 50 flashes, with

excitation/emission wavelengths of 485 and 520 nm, respectively, were collected and averaged in endpoint mode for each well with either parallel or perpendicular polarizers in-line. Background fluorescence in samples carrying only peptides was subtracted from all values obtained for the other samples. Polarization data were analysed using GraphPad Prism 5.0 by non-linear fitting with a one-site total binding model. The non-specific binding component was then subtracted from the data for presentation purposes. All data represent the mean of three separate experiments, and error bars represent one standard deviation.

Immunoprecipitation (IP)

100 mL of cells were harvested from sporulating cultures (OD_{600} = 1.9) and supplemented with 2 mM PMSF. Cells were washed with ice-cold water containing 2 mM PMSF and cell pellets were frozen in liquid nitrogen and stored at -80˚C until required. Pellets were thawed on ice and resuspended in 300 µL of buffer KA70 (number indicates concentration of KOAc in mM; 50 mM HEPES-KOH pH 7.5, 70 mM KOAc, 5 mM MgOAc, 0.1% NP-40, 10% glycerol, 1 mM DTT, 0.5 mM PMSF, 20 mM β-glycerophosphate, 1 mM NaF and 2x Roche protease inhibitor cocktail). An equal volume of 500 micron glass beads was added and cells were disrupted with a ribolyser (Yasui Kikai). The lysate was stored on ice. Residual lysate was recovered by briefly washing the glass beads with 500 µL of buffer KA70. The combined lysate was centrifuged twice (20000x*g*, 5 mins, 2˚C). Cleared lysate was then split into two equal aliquots. Protein A or Protein G-conjugated magnetic beads, which had been incubated in PBS 0.1% Tween with or without antibody (overnight, 4˚C), were then added to these aliquots and incubated with gentle agitation (3 hrs, 4˚C). Beads were washed with buffer KA70 once and buffer KA100 twice. Proteins were eluted on a thermomixer (65˚C, 1300rpm) with 100 µL of urea loading buffer (8 M urea, 5% SDS, 200 mM Tris-Cl pH 6.8, 1 mM EDTA, 0.01% BPB) freshly supplemented with 0.1 mM DTT and 0.2 M Tris. Proteins were resolved by SDS-PAGE and transferred to PVDF membranes for immunoblot analysis. Protein A beads were used for IP with anti-V5 antibody (mouse, Bio-Rad) and Protein G beads were used for IP with anti-Dbf4 antibody (goat, Santa Cruz yA-16).

Cytology

Meiotic chromosomes were surface spread, and immunostaining was carried out as described previously (Humphryes et al., 2013). Antibodies used are: Rad51, Zip1, and Red1 (rabbit, 300-fold dilution, Shirleen Roeder; Sym et al., 1993; Smith and Roeder, 1997), Rfa1 (rabbit, 300-fold dilution, Bruce Stillman; Zou and Stillman, 2000), tubulin (rat 200-fold dilution, Abcam, ab6161), and HA (mouse, 300-fold dilution, Covance MMS-101R). Images were captured on the Deltavision IX70 system (Applied Precision, Olympus) with a 100x objective lens (NA 1.40) and a camera (CoolSNAP HQ2, Photometrics) using *softWoRx* software at room temperature. Images were deconvolved using the constrained iterative deconvolution algorithm within *softWoRx*, and appropriate consecutive deconvolved z-slices were projected together to form the final processed image.

Anchor-away technique

The Anchor-away technique was performed as described previously (Haruki et al., 2008) Briefly, FRB-tagged strains were constructed in a *tor1-1 fpr1* background to confer resistance to rapamycin and prevent competition between Fpr1 and FKBP12 for FRB binding. Rapamycin (Sigma) was added to cultures at the indicated time points at a final concentration of 1 µg/ml. *DBF4* and *CDC7* were FRB-tagged at their C-termini.

Appendix references

Gietz RD, Sugino A (1988) New yeast-*Escherichia* coli shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. *Gene* 74, 527-534.

Longtine MS, McKenzie A, Demarini DJ, Shah NG, Wach A, Brachat A, Philippsen P, Pringle JR (1998) Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast* 14, 953-961.

Masai H, Arai K (2000) Dbf4 motifs: conserved motifs in activation subunits for Cdc7 kinases essential for S-phase. *Biochem Biophys Res Commun* 275, 228-232.

Sym M, Engebrecht JA, Roeder GS (1993) Zip1 is a synaptonemal complex protein required for meiotic chromosome synapsis. *Cell* 72, 365-378.

Zou L, Stillman B (2000) Assembly of a complex containing Cdc45p, replication protein A, and Mcm2p at replication origins controlled by S-phase cyclin-dependent kinases and Cdc7p-Dbf4p kinase. *Mol Cell Biol* 20, 3086-096.

Table S1. Genotypes of yeast strains used in this study.

Strain	Genotype[1,2]	Background
TBR310	*hop2::ADE2*	BR1919
TBR2065	wild type	BR1919
TBR2434	*zip1-4LA*	BR1919
TBR2780	*zip2::kanMX4 zip3::hphMX4*	BR1919
TBR4711	*hop2::ADE2 rad17::LEU2*	BR1919
TBR6448	*hop2::ADE2 dbf4-E86K*	BR1919
TBR6449	*hop2::ADE2 dbf4-R83E*	BR1919
TBR6450	*hop2::ADE2 dbf4-E86V*	BR1919
TBR6451	*zip1-4LA dbf4-E86K*	BR1919
TBR6505	*zip1-4LA dbf4-R83E*	BR1919
TBR6506	*zip1-4LA dbf4-E86V*	BR1919
TBR6507	*zip2::LEU2 zip3::URA3 dbf4-E86K*	BR1919
TBR6508	*zip2::LEU2 zip3::URA3 dbf4-R83E*	BR1919
TBR6557	*zip2::LEU2 zip3::URA3 dbf4-E86V*	BR1919
TBR6621	wild type	SK1
TBR6887	*dmc1::natMX4*	SK1
TBR7464	*dbf4-E86V*	SK1
TBR7483	*dmc1::natMX4 dbf4-E86V*	SK1
TBR7552	*dmc1::natMX4 dbf4-E86K*	SK1
TBR7553	*dmc1::natMX4 dbf4-R83E*	SK1
TBR8372	*dmc1::natMX4 P_{DBF4}-CDC5-DBF4-URA3 dbf4-R83E*	SK1
TBR8450	*dmc1::natMX4 P_{DBF4}-CDC5-URA3 dbf4-R83E*	SK1
TBR8454	*dmc1::natMX4 P_{DBF4}-CDC5-URA3*	SK1
TBR8672	*dmc1::natMX4 dbf4-E86V dbf4-E86V-URA3*	SK1
TBR8673	*ndt80::LEU2 ER-GAL-URA3 / ura3-1*	BR1919
TBR8674	*ndt80::LEU2 ER-GAL-URA3 / P_{GAL}-CDC5-URA3*	BR1919
TBR8764	*ndt80::LEU2 ER-GAL-URA3 / P_{GAL}-CDC5-URA3 dbf4-E86K*	BR1919
TBR8765	*ndt80::LEU2 ER-GAL-URA3 / P_{GAL}-CDC5-URA3 dbf4-R83E*	BR1919
TBR9107	*ndt80::LEU2 ER-GAL-URA3 / P_{GAL}-CDC5-URA3 dbf4-E86V*	BR1919
TBR9121	*dmc1::natMX4 P_{DBF4}-CDC5-dbf4-R83E-URA3 dbf4-R83E*	SK1
TBR9175	*dmc1::natMX4 rad51::hisGURA3hisG dbf4-E86V*	SK1
TBR9176	*dmc1::natMX4 P_{DBF4}-CDC5-dbf4-R83E-URA3*	SK1
TBR9237	*dmc1::natMX4 rad51::kanMX4 dbf4-E86V dbf4-E86V-URA3*	SK1
TBR9367	*NDT80-6HA*	BR1919
TBR9488	*dbf4-R83E*	SK1
TBR9533	*NDT80-6HA dbf4-R83E*	BR1919
TBR9693	*ndt80::LEU2 ER-GAL-URA3 / P_{GAL}-CDC5-URA3*	SK1
TBR9695	*ndt80::LEU2 ER-GAL-URA3 / ura3*	SK1
TBR9697	*ndt80::LEU2 ER-GAL-URA3 / P_{GAL}-CDC5-URA3 dbf4-E86K*	SK1
TBR9699	*ndt80::LEU2 ER-GAL-URA3 / P_{GAL}-CDC5-URA3 dbf4-R83E*	SK1
TBR9701	*ndt80::LEU2 ER-GAL-URA3 / P_{GAL}-CDC5-URA3 dbf4-E86V*	SK1
TBR9747	*NDT80-6HA DBF4 / dbf4::kanMX4 CDC5 / cdc5::natMX4*	BR1919
TBR9749	*NDT80-6HA dbf4-R83E / dbf4::kanMX4 CDC5 /*	BR1919

	cdc5::natMX4	
TBR10060	*ndt80::LEU2 dmc1::natMX4 ER-GAL-URA3 / ura3*	SK1
TBR10062	*ndt80::LEU2 dmc1::natMX4 ER-GAL-URA3 / P_{GAL}-CDC5-URA3*	SK1
TBR10076	*ndt80::LEU2 dmc1::natMX4 ER-GAL-URA3 / P_{GAL}-CDC5-URA3 dbf4-E86V*	SK1
TBR10078	*ndt80::LEU2 dmc1::natMX4 ER-GAL-URA3 / P_{GAL}-CDC5-URA3 dbf4-R83E*	SK1
TBR10080	*ndt80::LEU2 dmc1::natMX4 ER-GAL-URA3 / P_{GAL}-CDC5-URA3 dbf4-E86K*	SK1
TBR10101	*MATα tor1-1::HIS3 fpr1::hphMX4 RPL13A-2xFKBP12::TRP1 dmc1::natMX4 CDC7-FRB*	SK1
TBR10105	*MATα tor1-1::HIS3 fpr1::hphMX4 RPL13A-2xFKBP12::TRP1 dmc1::natMX4 DBF4-FRB*	SK1
TBR10119	*MATα tor1-1::HIS3 fpr1::hphMX4 RPL13A-2xFKBP12::TRP1 dmc1::natMX4*	SK1
TBR10129	*ndt80::LEU2 tor1-1::HIS3 fpr1::hphMX4 RPL13A-2xFKBP12::TRP1 ER-GAL-URA3 / P_{GAL}-CDC5-URA3 DBF4-FRB-kanMX4*	SK1
TBR10131	*ndt80::LEU2 tor1-1::HIS3 fpr1::hphMX4 RPL13A-2xFKBP12::TRP1 ER-GAL-URA3 / P_{GAL}-CDC5-URA3 CDC7-FRB-kanMX4*	SK1
TBR10190	*ndt80::LEU2 dmc1::natMX4 ER-GAL-URA3 / P_{GAL}-cdc5-N209A-URA3*	SK1
TBR10192	*ndt80::LEU2 dmc1::natMX4 ER-GAL-URA3 / P_{GAL}-cdc5-N209A-URA3 dbf4-E86K*	SK1
TBR10575	*ndt80::LEU2 rad51::hisGURA3hisG dmc1::natMX4 ER-GAL-URA3 / P_{GAL}-CDC5-URA3*	SK1
TBR10582	*cdc28-as1 P_{CLB2}-cdc20*	SK1
TBR10603	*cdc28-as1 P_{CLB2}-cdc20 P_{CLB2}-cdc5*	SK1
TBR10656	*cdc28-as1 P_{CLB2}-cdc20 dbf4-R83E*	SK1
TBR10696	*cdc28-as1 ndt80::LEU2*	SK1
TBR10697	*cdc28-as1 ndt80::LEU2 P_{CLB2}-cdc5*	SK1
TBR10699	*cdc28-as1 ndt80::LEU2 P_{CLB2}-cdc5 ER-GAL-URA3 / P_{GAL}-CDC5-URA3*	SK1
TBR10718	*ndt80::LEU2 tor1-1::HIS3 fpr1::hphMX4 RPL13A-2xFKBP12::TRP1 ER-GAL-URA3 / P_{GAL}-CDC5-URA3*	SK1
TBR10732	*cdc28-as1*	SK1
TBR10733	*cdc28-as1 P_{CLB2}-cdc5*	SK1
TBR10798	*ndt80::LEU2 ER-GAL-URA3 / P_{GAL}-CDC5-URA3 P_{CLB2}-dbf4*	SK1
TBR10800	*ER-GAL-URA3 / P_{GAL}-CDC5-URA3 P_{CLB2}-dbf4*	
TBR10816	*ER-GAL-URA3 / P_{GAL}-CDC5-URA3*	SK1
TBR10840	*ndt80::LEU2 ER-GAL-URA3/ P_{GAL}-CDC5-URA3 P_{CLB2}-dbf4 MCM5-bob1*	SK1
TBR10842	*ER-GAL-URA3/ P_{GAL}-CDC5-URA3 P_{CLB2}-dbf4 MCM5-bob1*	SK1
TBR10843	*ndt80::LEU2 ER-GAL-URA3-DBF4-TRP1 / P_{GAL}-CDC5-*	SK1

	URA3-DBF4-TRP1 P_{CLB2}-dbf4	
TBR10990	*ndt80::LEU2 ER-GAL-URA3-dbf4^{S374A,T375A}-TRP1 / P_{GAL}-CDC5-URA3- dbf4^{S374A,T375A} -TRP1 P_{CLB2}-dbf4*	SK1
TBR10991	*ndt80::LEU2 ER-GAL-URA3- dbf4^{S374A}-TRP1 / P_{GAL}-CDC5-URA3- dbf4^{S374A}-TRP1 P_{CLB2}-dbf4*	SK1
TBR10995	*ndt80::LEU2 ER-GAL-URA3- dbf4^{T375A}-TRP1 / P_{GAL}-CDC5-URA3- dbf4^{T375A}-TRP1 P_{CLB2}-dbf4*	SK1
TBR11028	*ndt80::LEU2 ER-GAL-URA3- dbf4^{S318A,S319A}-TRP1 / P_{GAL}-CDC5-URA3- dbf4^{S318A,S319A}-TRP1 P_{CLB2}-dbf4*	SK1
TBR11031	*ndt80::LEU2 ER-GAL-URA3- dbf4-4A-TRP1/ P_{GAL}-CDC5-URA3- dbf4-4A-TRP1 P_{CLB2}-dbf4*	SK1
TBR11138	*HIS4::LEU2-(NBam_mom) / HIS4:LEU2-(NBam_dad)*	SK1
TBR11143	*ndt80::LEU2 ER-GAL-URA3- dbf4-4A-TRP1/ P_{GAL}-CDC5-URA3- dbf4-4A-TRP1 P_{CLB2}-dbf4 P_{DMC1}-dbf4-4A-HIS6/HIS6*	SK1
TBR11161	*dmc1::natMX4 dbf4-E86V HIS4::LEU2-(NBam_mom) / HIS4:LEU2-(NBam_dad)*	SK1
TBR11163	*dmc1::natMX4 dbf4-E86V URA3-dbf4-E86V HIS4::LEU2-(NBam_mom) / HIS4:LEU2-(NBam_dad)*	SK1
TBR11165	*dmc1::natMX4 dbf4-E86K P_{CLB2}-cdc5*	SK1
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TBR11165	*dmc1::natMX4 dbf4-E86K P_{CLB2}-cdc5*	SK1
BA081	*P_{CLB2}-cdc20 CDC7-9xV5*	SK1
BA0133	*P_{CLB2}-cdc20 CDC7-9xV5 dbf4-E86K*	SK1
BA0135	*P_{CLB2}-cdc20 CDC7-9xV5 dbf4-R83E*	SK1
BA0137	*P_{CLB2}-cdc20 CDC7-9xV5 dbf4-E86V*	SK1

[1]BR1919 strains are in the following genetic background:
ho leu2-3, 112 his4-260 ura3-1 ade2-1 thr1-4 trp1-289 lys2

[2]SK1 strains are in the following genetic background:
ho::LYS2 lys2 ura3 leu2::hisG his3::hisG trp1::hisG

All listed strains are isogenic diploids, unless indicated otherwise, derived from either BR1919 or SK1. All loci are homozygous, unless indicated otherwise by a forward slash symbol (/), in which case heterozygosity is described. Strains carrying point mutations in *DBF4* at R83 or E86 also contain a silent G to C transversion at nucleotide 285 of the open-reading frame, resulting in the generation of a KpnI site. This restriction site was used for genotyping purposes. *P_{CLB2}* denotes that a gene was placed under the control of the *CLB2* promoter. *CLB2* is not expressed in meiotic cells. This is the equivalent of a meiotic depletion mutant (*-md*; Lee and Amon, 2003).