

Genetic interactions derived from high-throughput phenotyping of 6,589 yeast cell cycle mutants

Supplemental Methods and Figures

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Supplementary Methods

Parent Strain Construction

We used several strategies to generate the eight sets of parent strains used in this study (Table S1). We obtained most of the Set 1 and Set 3 strains by sporulation and tetrad analysis of the heterozygous diploid commercial collection of *kanMX* strains^{1,2}, but we made some by *de novo kanMX* PCR-mediated gene deletions in BY4741 or BY4742 (using pFA6a-*kanMX* as the template and listed primers³⁻⁶, Table S2). We obtained most of the Set 2 and Set 4 strains by transformation of the heterozygous diploid strains with a *natMX* PCR product (using MX.for and MX.rev primers in Table S2 with pAG25 template. pAG25 and its sequences are available from Addgene) to switch markers, followed by selection of nourseothricin-resistant/G418-sensitive transformants, sporulation, and tetrad analysis. We made the rest of sets 2 and 4 by *de novo natMX* PCR-mediated gene deletions in BY4741 or BY4742 (using pAG25 as the template and listed primers). We made most of Set 7 and Set 8 by *de novo* PCR-mediated gene deletions in the SGA strain Y8205⁷, but we made some of these strains by crossing one of the BY4741-derived gene deletion strain with Y8205, followed by tetrad dissection. We then used the SGA method to cross these strains to BY4741 and obtain *MATa* versions of these strains for Set 5 and Set 6. All strains were confirmed by PCR of genomic DNA using one set of test primers for the gene deletion and another for the wild-type gene⁸ (Table S2). All strains (parents and progeny) are available upon request.

Double Mutant Progeny Construction

All crosses followed a standard format in which the *MATα* strains (Sets 3, 4, 7, and 8) which we will call the “hit” strains, were arrayed alphabetically by gene name so that each strain was a single well in a 36-well block, with two replicate *MATα* blocks per plate, leaving the first and fifth rows empty for the addition of the wild-type parents during phenotyping. If a deletion strain was missing in a *MATα* set, we left the position empty. We arrayed the *MATa* strains, which we will call the query or “bait” strains, so that each *MATa* strain in the set fills a block of 36 wells at the same positions as one of the two blocks of *MATα* strains (i.e., in rows 2-4 or 6-8).

Before crossing, each set of parent strains was arrayed in 96-well microtiter PlusPlates (Singer Instruments, Somerset, UK) containing YPD broth and pinned onto YPD+G418 (300 µg/ml; odd numbered sets) or YPD+nat (150 µg/ml; even numbered sets) and grown for 3-4 days at 30 °C.

For the crosses, we used a Rotor HDA (Singer Instruments, Somerset, UK) to replica-pin each *MATα* plate to 12-18 YPD plates using 96 long repads with 6 wet mix cycles and 4 dry mix cycles to ensure robust inoculation of each plate. Visual inspection of each plate ensured proper transfer of cells. We then pinned each *MATa* plate on top of one *MATα* plate using the same conditions to ensure good mixing of the two parent strains on the YPD plate. Matings were performed on YPD at 30 °C for two days.

Diploids were selected on YPD + G418/nat (300/150 µg/ml) at 30 °C for two days. Diploids were sporulated on enriched sporulation media (1% potassium acetate, 0.1% yeast extract, 0.05% glucose, 0.1 g of his/leu/lys/ura supplement) at 24 °C for five days.

Haploid progeny were selected as described in Tong et al. (2006) ⁷, except that we separately selected for both *MATa* and *MATα* progeny. We first selected haploids from the sporulation plates by replica-pinning on SD-arg/his/lys+canavanine/thialysine (100/100 µg/ml) for *MATa* progeny and SD-arg/leu/lys+canavanine/thialysine (100 µg/ml) for *MATα* progeny. For the second round of haploid selection, we added G418 (300 µg/ml) to these plates. For the final haploid selection, we added both G418 (600 µg/ml) and nourseothricin (150 µg/ml) to obtain double mutant haploids.

Halo Assays

Halo assays were used to confirm the mating type of the parents and progeny and identify potential cell signaling and chromosome segregation defects. We performed halo assays as described ⁹ using Y955 lawns to test for *a*-halos, and Y991 to test for *α*-halos. We prepared halo assay plates by growing Y955 and Y991 in YPD broth at 30 °C overnight in a shaking incubator. The next morning, we diluted each strain 1/5 in YPD broth, vortexed the tubes, and used sterile glass beads to spread 500 µl of the dilution per plate onto YPD PlusPlates. We allowed these plates to dry before pinning each plate of parent strains or haploid progeny (at 96 colony densities) onto both a Y955 lawn (to test for *a*-factor secretion) and a Y991 lawn (to test for *α*-factor secretion). We imaged halo assay plates after 48 h of growth (see Additional Data). Individual lines that did not behave as expected (most likely due to isolated genetic mishaps) were not excluded, as the consensus between multiple biological replicates was used to draw the conclusions presented.

Identifying Curation Errors

For several of the manually curated synthetic lethal interactions on the Saccharomyces Genome Database, we found that the listed SL interaction was not supported by the cited paper. In some cases, what was curated as synthetic lethal was in fact not lethal but exhibited some other kind of growth defect. In other cases, the interaction was lethal, but was tested in a mutant background in which one or more additional cell cycle genes were knocked out. Those synthetic lethal interactions that we found to be curation errors are listed, along with their references, below:

*clb5Δ clb6Δ*¹⁰⁻¹², *cdc55Δ cln1Δ*¹³, *cdc55Δ cln2Δ*¹³, *cln1Δ cln2Δ*¹⁴⁻²⁰, *cln1Δ cln3Δ*^{16,17}, *cln1Δ msn5Δ*²¹, *cln2Δ cln3Δ*^{16,17}, *cln2Δ msn5Δ*²¹, *fkh1Δ fkh2Δ*²², *ssa1Δ ydj1Δ*²³

Tetrad Analysis

To identify synthetic lethality by tetrad analysis, several tetrads (usually 12) were dissected for one or more biological replicates of each of the 58 gene combinations listed in Figure 4. The surviving spores were patched onto YPD plates, and then replica plated onto YPD+G418 (600ug/ml), YPD+nat (150ug/ml), and Y955 and Y991 lawn plates (as described above but using 300ul diluted culture).

Dissections from which we could recover very few live spores of any genotype were identified as having a likely meiotic defect (MD). Recovery of a live spore that was resistant to both antibiotics was considered evidence for viability (V). Cases where a dead spore in a tetrad could be inferred to have the double mutant phenotype based on allele segregation were considered evidence for synthetic lethality (SL). If the ratio of spores supporting synthetic lethality to spores supporting viability (SL:V) was 4:1 or greater, we considered the gene combination SL. If the SL:V ratio was between 4:1 and 1:1, we considered the gene combination to have reduced viability (RV). If the SL:V ratio was less than 1:1, we considered the gene combination to be viable (V). Cases where we were able to identify fewer than two spores as potentially SL or V (due to low viability overall) were also designated MD.

Supplementary Figures

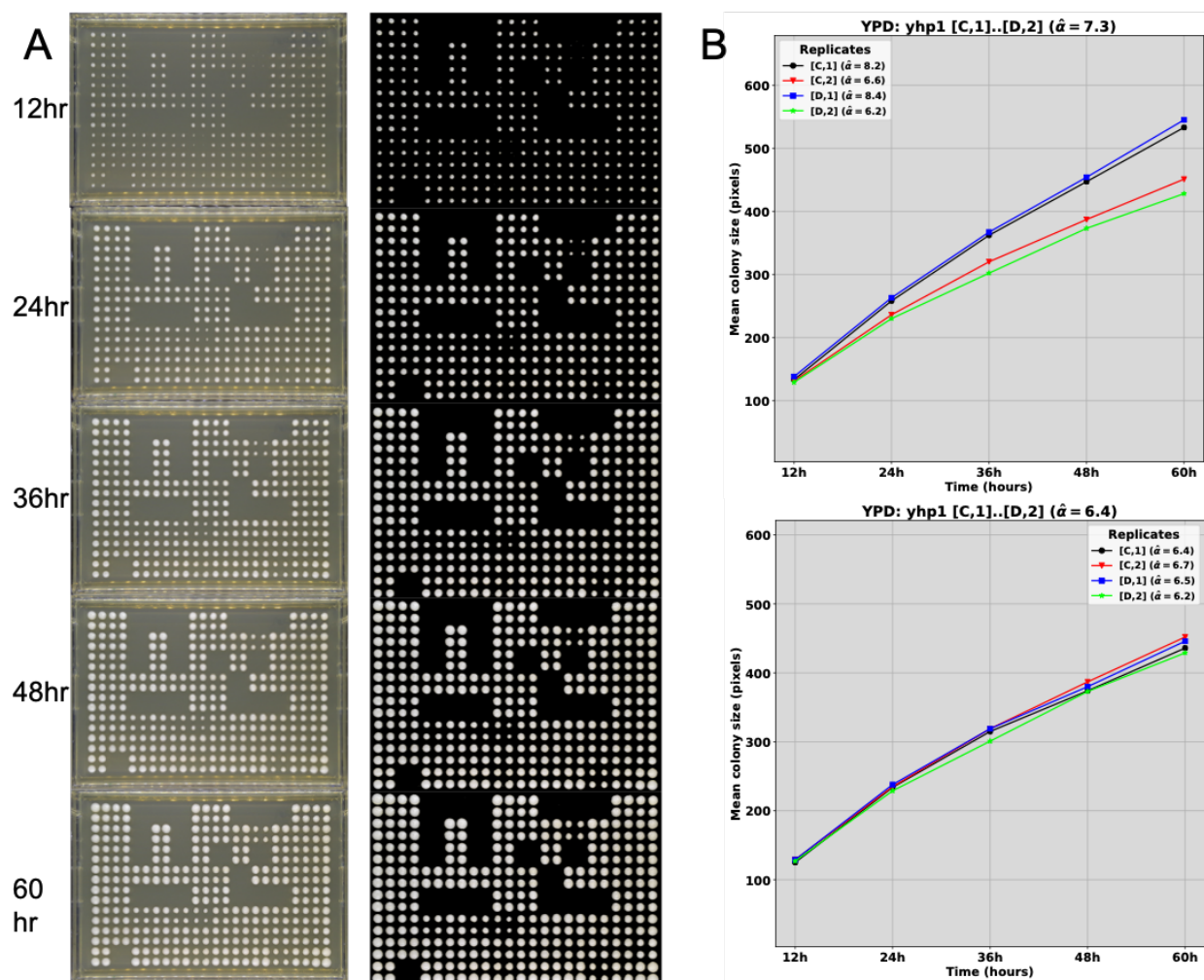


Figure S1. Example of plate images and growth curves. (A) Unprocessed (left) and processed (right) images of a phenotyping plate across the 5 time points. **(B)** Growth curves for one of the quadruplicates shown in A before (top) and after (bottom) normalization. Each colored line represents one of the colonies in the quadruplicate. The black and blue lines plot edge colonies (positions C1 and D1), while the red and green lines plot non-edge colonies (C2 and D2).

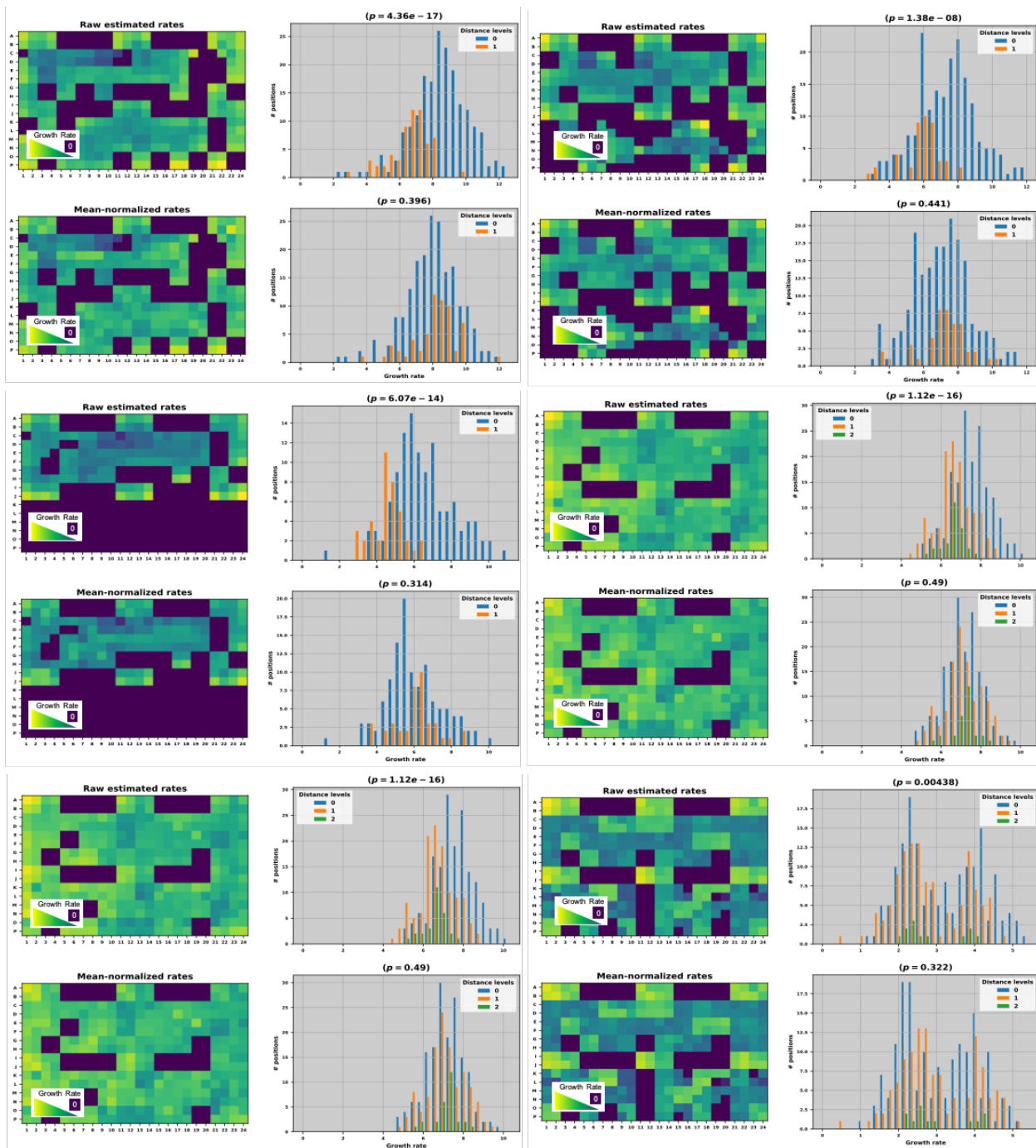


Figure S2. Normalizations for six representative phenotyping plates. Normalizations based on the mean growth rate of the wildtype controls on each plate were used to account for edge effects. Heat maps show a visual representation of growth rates across each plate. The X and Y axis are the coordinates for the 384 positions where a colony may appear. In every case, wild-type controls are in rows A, B, I, and J, columns 1-4, 11-14, and 21-24. Histograms compare the growth rate of colonies that are on the edge of the plate or adjacent to an empty position (distance level 0) with those that are one or more positions away from an edge (non-zero distance levels). The p-value reported above the histogram marks the significance of the difference between the growth rate of edge-adjacent colonies and internal colonies. In each case, raw, unnormalized heat maps, histograms, and p-values are shown just above their normalized counterparts.

Figure S3: Basal parameter values for wild-type cells in the 2020 model. Parameter values differing from the 2015 model are highlighted.

Rate constants (min^{-1}) (subscripts: “s” for synthesis, “d” for degradation)							
$k_{s,\text{bck2}}$	0.13	$k_{d,\text{bck2}}$	0.25				
$k_{s,\text{bud,e}}$	1.3	$k_{d,\text{bud}}$	0.01				

$k_{s,cdc20}$	0.0008	$k_{s,cdc20,m1}$	0.2	$k_{d,cdc20}$	0.32		
$k_{s,cki}$	0.00124	$k_{s,cki,swi5}$	0.02745	$k_{d,cki}$	0.0153	$k_{d,ckip}$	0.5
$k_{s,clb2}$	0.0052	$k_{s,clb2,m1}$	0.19772				
$k_{d,clb2}$	0.0086	$k_{d,clb2,20}$	1.1642	$k_{d,clb2,20,i}$	0.15	$k_{d,clb2,h1}$	0.6
$k_{s,clb5}$	0.0008	$k_{s,clb5,mbf}$	0.0156				
$k_{d,clb5}$	0.035	$k_{d,clb5,20}$	0.8	$k_{d,clb5,20,i}$	0.25		
$k_{s,cln2}$	0	$k_{s,cln2,sbf}$	0.1	$k_{d,cln2}$	0.135		
$k_{s,cln3}$	0.1	$k_{d,cln3}$	0.2				
$k_{s,ori,e}$	2	$k_{d,ori}$	0.06				
$k_{s,pds1}$	0.03	$k_{s,pds1,mbf}$	0.03				
$k_{d,pds1}$	0.01	$k_{d,pds1,20}$	2.5	$k_{d,pds1,20,i}$	1.7		
$k_{s,po1o}$	0	$k_{s,po1o,m1}$	0.22	$k_{d,po1o}$	0.3	$k_{d,po1o,h1}$	1.5
$k_{s,swi5}$	0.005	$k_{s,swi5,m1}$	0.03	$k_{d,swi5}$	0.08		
$k_{s,spn}$	0.24	$k_{d,spn}$	0.03				
$k_{s,swel}$	0.001	$k_{s,swel,sbf}$	0.007				
$k_{d,swel}$	0.01	$k_{d,swel,h1}$	0.5	$k_{d,swel,p}$	0.2	$k_{d,swel,p,h1}$	0.7
Other time-scale factors (min⁻¹)							
μ	0.0077	(mdt = 90 min in glucose medium)					
γ	1	γ_{cki}	10	γ_{apc}	0.5	γ_{tem1}	0.1
Interaction coefficients (dimensionless) (subscripts: “a” for activation, “i” for inactivation, “p” for phosphorylation, “dp” for dephosphorylation)							
$\omega_{a,apc,b2}$	0.625	$\omega_{i,apc}$	0.7				
$\omega_{a,bub2,14}$	0.05	$\omega_{a,bub2}$	2.71	$\omega_{a,bub2,c55}$	0.8	$\omega_{i,bub2,lo}$	4.4
$\omega_{i,bub2,lte,lo}$	6.7						
$\omega_{a,cdc15,14}$	0.85	$\omega_{i,cdc15}$	0.23	$\omega_{i,cdc15,b2}$	0.0149		
$\omega_{i,cdc55,p1}$	0.981						
$\omega_{a,cdh1,14}$	1.1	$\omega_{a,cdh1}$	0.032				
$\omega_{i,cdh1,n2}$	0.202	$\omega_{i,cdh1,b5}$	8.76	$\omega_{i,cdh1,b2}$	0.162		
$\omega_{p,cki,n2}$	1.15	$\omega_{p,cki,b5}$	9.5	$\omega_{p,cki,b2}$	1.65		
$\omega_{dp,cki}$	0.7	$\omega_{dp,cki,14}$	1.747				
$\omega_{dp,clb2}$	1.5	$\omega_{p,clb2,we}$	1.05				
$\omega_{a,ma2}$	30	$\omega_{i,ma2}$	0.6				
$\omega_{a,mcm1,b2}$	10	$\omega_{i,mcm1}$	1.7				
$\omega_{p,net1,b2}$	0.0225	$\omega_{p,net1,en}$	6.6	$\omega_{p,net1,15}$	0.288	$\omega_{p,net1}$	0.22
$\omega_{dp,net1}$	0.055	$\omega_{dp,net1,14}$	2.51	$\omega_{dp,net1,c55}$	1.0		
$\omega_{a,po1o,b2}$	4.8	$\omega_{i,po1o}$	0.2				
$\omega_{p,s6s4pw,n3}$	8.43	$\omega_{p,s6s4pw,n2}$	0.01	$\omega_{p,s6s4pw,k2}$	2.1	$\omega_{p,s6s4pw,b5}$	0.01

$\omega_{i,s6s4pw}$	0.8	$\omega_{p,s6s4pw,b2}$	2.87				
$\omega_{p,s6s4,n3}$	3.6	$\omega_{p,s6s4,n2}$	0.28	$\omega_{p,s6s4,k2}$	0.6	$\omega_{p,s6s4,b5}$	4.62
$\omega_{i,s6s4}$	0.5	$\omega_{p,s6s4,b2}$	0.035	$\omega_{p,s6s4np,b2}$	0.4		
$\omega_{p,s4s4p,k2}$	8.5	$\omega_{i,s4s4p}$	0.5	$\omega_{p,s4s4p,b2}$	4.5		
$\omega_{p,s6mb,n3}$	3.1	$\omega_{p,s6mp,n2}$	0.2	$\omega_{p,s6mb,k2}$	1.25	$\omega_{p,s6mb,b5}$	6.2
$\omega_{i,s6mb}$	0.5	$\omega_{dp,s6mb,b2}$	0.73				
$\omega_{p,ssa1,b2}$	2.0	$\omega_{dp,ssa1}$	1.0				
$\omega_{p,swel,b2}$	1.5	$\omega_{dp,swel}$	0.5				
$\omega_{a,swi5,14}$	5.1	$\omega_{a,swi5}$	0.2	$\omega_{i,swi5,b2}$	1.0		
$\omega_{a,tem1,lo}$	1.1	$\omega_{a,tem1}$	0.5	$\omega_{i,tem1,bub2}$	2.5		
Total concentrations (dimensionless)							
[APC _T]	25	[Bub2 _T]	1	[Cdc14 _T]	2	[Cdc15 _T]	1
[Cdc55 _T]	1	[Cdh1 _T]	1	[Esp1 _T]	0.5	[Mad2 _T]	25
[Mbp1 _T]	5.5	[Mcm1 _T]	1	[Net1 _T]	3.55	[Pr ₂]	2
[Pr ₅]	2	[Ssa1 _T]	1	[Swi4 _T]	5.5	[Swi6 _T]	30
[Tem1 _T]	2	[Whi5 _T]	10				
Other parameters (dimensionless)							
$e_{bud,b5}$	0.38	$e_{bud,n2}$	0.45	$e_{bud,n3}$	0.3	$e_{ori,b2}$	0.35
$e_{ori,b5}$	0.5	J_{spn}	0.14	$k_{5,s6m}$	0.16	k_{s4}	0.91
k_{s6m}	0.63	k_{s4s6}	0.3	$k_{5,s6s4}$	1.1	k_{ydjl}	2.3
$r_{cdc14,n \rightarrow c}$	13	$r_{cdc14,c \rightarrow n}$	0.022	v_{bck2}	3	v_{clb2}	1.4
v_{clb5}	0.2	v_{cln3}	12	v_{ydjl}	2.9	θ_{cd}	0.2
θ_{cleave}	0.05	θ_{rl}	0.199	σ	10	$\rho_{14,net1}$	1

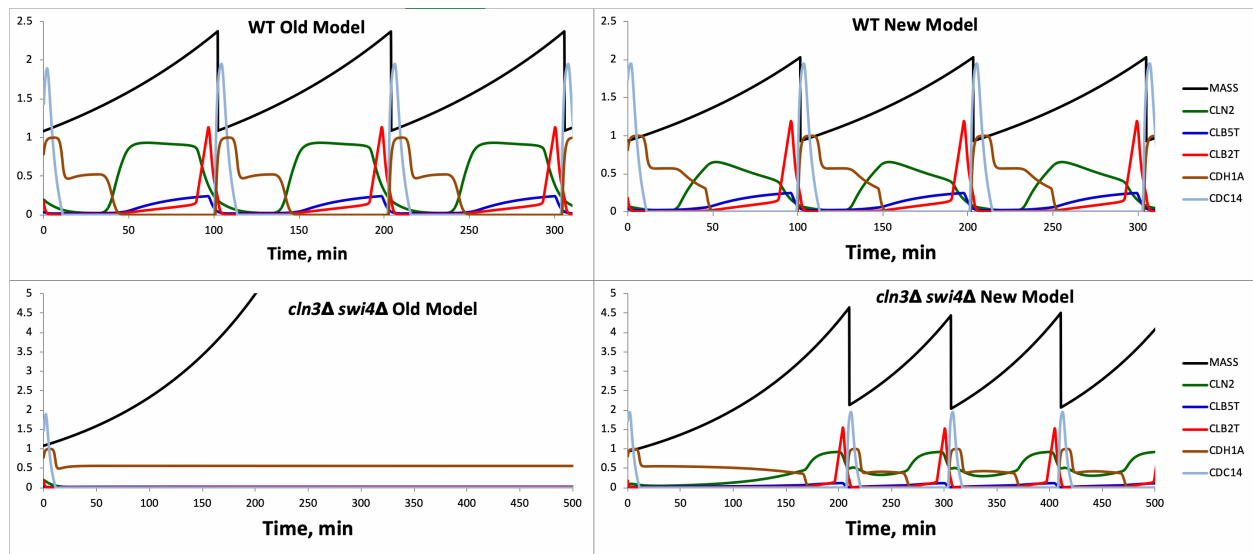


Figure S4: Comparison of the 2015 and 2020 model. Adjusting 13 parameters of the 2015 model does not significantly affect the dynamics of the model in the WT cells as shown by representing the time-evolution of variables (top half of the figure). However, the adjusted parameter values result in qualitative changes of the dynamics in selected mutants as shown in the bottom half of the figure.

Figure S5: List of mutant simulations. We have simulated all the mutants in this figure. We compared simulations and published phenotypes when possible. Mutants highlighted in red indicate inconsistencies between simulation and phenotypes. Mutants we have simulated for which no observed phenotype has been published are indicated with *. Mutants highlighted in bold have different phenotypes in the 2015 and 2020 models. New mutant simulations reported in the “New Data” column on the left.

New Data <i>bck2Δ cdh1Δ</i> <i>bck2Δ cln3Δ</i> <i>bck2Δ swi4Δ</i> <i>bck2Δ swi6Δ</i> <i>bfa1Δ lte1Δ</i> <i>bub2Δ cdc55Δ</i> <i>cdc55Δ cdh1Δ</i> <i>cdc55Δ clb5Δ</i> <i>cdc55Δ lte1Δ</i> <i>cdc55Δ swi4Δ</i> <i>cdh1Δ clb5Δ</i> <i>cdh1Δ cln3Δ</i> <i>cdh1Δ lte1Δ</i> <i>cdh1Δ mad2Δ</i> <i>cdh1Δ sic1Δ</i> <i>cdh1Δ ssa1Δ</i> <i>cdh1Δ swi4Δ</i> <i>cdh1Δ swi5Δ</i> <i>cdh1Δ swi6Δ</i> <i>cdh1Δ whi5Δ</i> <i>cdh1Δ ydj1Δ</i> <i>clb1Δ clb2Δ</i> <i>clb2Δ CLB1 mad2Δ</i> <i>clb2Δ CLB1 swi6Δ</i> <i>clb2Δ CLB1 sic1Δ</i> <i>clb5Δ sic1Δ</i> <i>clb5Δ swi4Δ</i> <i>clb5Δ swi6Δ</i> <i>cln3Δ swi4Δ</i> <i>lte1Δ mad2Δ</i> <i>lte1Δ sic1Δ</i> <i>lte1Δ swi6Δ</i> <i>lte1Δ ydj1Δ</i> <i>mad2Δ sic1Δ</i> <i>mad2Δ whi5Δ</i> <i>mbp1Δ swi4Δ</i> <i>swi4Δ ydj1Δ</i> <i>swi4Δ swi6Δ</i>	Wild-type In glucose In galactose Start –mutants <i>mbp1Δ</i> <i>ssa1Δ</i> <i>swi4Δ</i> <i>swi6Δ</i> <i>swi6Δ</i> in galactose <i>whi5Δ</i> <i>ydj1Δ</i> <i>WHI5^{OP}</i> <i>bck2Δ mbp1Δ</i> <i>bck2Δ whi5Δ</i> <i>cln3Δ mbp1Δ</i> <i>cln3Δ ssa1Δ</i> <i>cln3Δ swi6Δ</i> <i>cln3Δ whi5Δ</i> <i>cln3Δ whi5Δ</i> <i>cdh1Δ ydj1Δ</i> <i>mbp1Δ swi6Δ</i> <i>mbp1Δ whi5Δ</i> <i>swi4Δ whi5Δ</i> <i>swi6Δ whi5Δ</i> <i>whi5Δ GAL-BCK2</i> <i>bck2Δ cln3Δ swi6Δ</i> <i>bck2Δ cln3Δ whi5Δ</i> <i>bck2Δ swi6Δ sic1Δ</i> <i>bck2Δ swi6Δ GAL-CLB5*</i> <i>bck2Δ swi6Δ whi5Δ</i> <i>cln1Δ cln2Δ mbp1Δ*</i> <i>cln1Δ cln2Δ swi4Δ</i> <i>cln1Δ cln2Δ swi6Δ</i> <i>cln3Δ mbp1Δ swi6Δ*</i> <i>cln3Δ mbp1Δ multi-copy BCK2*</i> <i>cln3Δ mbp1Δ whi5Δ*</i> <i>cln3Δ swi4Δ sic1Δ*</i> <i>cln3Δ swi4Δ multi-copy BCK2*</i> <i>cln3Δ swi4Δ GAL- BCK2*</i> <i>cln3Δ swi4Δ whi5Δ*</i> <i>mbp1Δ swi4Δ GAL- BCK2</i> <i>mbp1Δ swi4Δ GAL- CLB5*</i> <i>mbp1Δ swi4Δ GAL-CLN2</i> <i>mbp1Δ swi4Δ GAL-CLN3*</i> <i>mbp1Δ swi4Δ cdh1Δ*</i> <i>mbp1Δ swi4Δ sic1Δ*</i> <i>mbp1Δ swi4Δ whi5Δ*</i> <i>swi4Δ swi6Δ sic1Δ</i> <i>swi4Δ swi6Δ GAL- BCK2</i> <i>swi4Δ swi6Δ GAL-CLB5*</i> <i>swi4Δ swi6Δ GAL-CLN2</i> <i>swi4Δ swi6Δ GAL-CLN3</i> <i>swi4Δ swi6Δ whi5Δ*</i> <i>bck2Δ cln3Δ mbp1Δ whi5Δ*</i> <i>bck2Δ cln3Δ swi4Δ whi5Δ</i> <i>bck2Δ cln3Δ swi6Δ GAL-CLN3</i> <i>cln1Δ cln2Δ cln3Δ whi5Δ</i> <i>cln3Δ swi4Δ whi5Δ sic1Δ*</i> Bck2 mutants <i>bck2Δ</i> Multi-copy BCK2 <i>bck2Δ cln3Δ cdh1Δ*</i> <i>bck2Δ cln3Δ sic1Δ</i> <i>bck2Δ cln3Δ GAL-CLB5*</i> <i>bck2Δ cln3Δ multi-copy CLN2</i> <i>bck2Δ cln3Δ GAL-CLN3</i> <i>bck2Δ cln3Δ cdc6Δ sic1Δ*</i> <i>bck2Δ cln3Δ GAL-CLN2</i> Cln mutants <i>cln3Δ</i> <i>GAL-CLN2</i> <i>GAL-CLN3</i>	<i>cln1Δ cln2Δ</i> <i>cln1Δ cln2Δ bck2Δ</i> <i>cln1Δ cln2Δ cdh1Δ</i> <i>cln1Δ cln2Δ cln3Δ</i> <i>cln1Δ cln2Δ sic1Δ</i> <i>cln1Δ cln2Δ GAL-SIC1</i> <i>cln1Δ cln2Δ cdc6Δ sic1Δ</i> <i>cln1Δ cln2Δ bck2Δ cdh1Δ*</i> <i>cln1Δ cln2Δ cdh1Δ GAL-CLN2</i> <i>cln1Δ cln2Δ cdh1Δ GAL-SIC1</i> <i>cln1Δ cln2Δ cln3Δ apc^Δ</i> <i>cln1Δ cln2Δ cln3Δ cdh1Δ</i> <i>cln1Δ cln2Δ cln3Δ sic1Δ</i> <i>cln1Δ cln2Δ cln3Δ multi-copy BCK2</i> <i>cln1Δ cln2Δ cln3Δ GAL-CLB2</i> <i>cln1Δ cln2Δ cln3Δ GAL-CLB5</i> <i>cln1Δ cln2Δ cln3Δ multi-copy CLB5</i> <i>cln1Δ cln2Δ cln3Δ GAL-CLN2</i> <i>cln1Δ cln2Δ cln3Δ GAL-CLN3</i> <i>cln1Δ cln2Δ GAL-SIC1 GAL-CLN2</i> <i>cln1Δ cln2Δ cln3Δ sic1Δ cdc6Δ*</i> <i>cln1Δ cln2Δ GAL-SIC1 GAL-CLN2 cdh1Δ</i> <i>cln1Δ cln2Δ cln3Δ bck2Δ GAL-CLN2</i> Cdh1, Sic1 mutants <i>cdh1Δ</i> <i>cdc6Δ</i> <i>sic1Δ</i> <i>swi3Δ</i> <i>GAL-CDC6</i> Multi-copy CDC6 CDH1 constitutively active <i>SIC1-4A</i> <i>GAL-SIC1</i> Multi-copy <i>SIC1</i> Multi-copy <i>SIC1-high</i> <i>GAL-SIC1-dbΔ</i> <i>GAL-SIC1-4A</i> <i>cdc6Δ sic1Δ</i> <i>sic1Δ GAL-CLB2</i> <i>cdh1Δ GAL-CLB2</i> <i>swi5Δ GAL-CLB2</i> <i>cdh1Δ GAL-CLB5</i> <i>sic1Δ GAL-CLB5</i> <i>sic1Δ CLB5-dbΔ</i> <i>cdc6Δ sic1Δ cdh1Δ</i> <i>sic1Δ cdh1Δ GAL-CDC20</i> <i>swi5Δ cdh1Δ GAL-SIC1</i> <i>SIC1-4A clb5Δ clb6Δ</i> <i>sic1Δ cdc6Δ cdh1Δ GAL-CDC20</i> Clb5 Clb6 mutants <i>clb5Δ</i> <i>GAL-CLB5</i> Multi-copy CLB5 <i>CLB5-dbΔ</i> <i>GAL-CLB5-dbΔ</i> <i>clb5Δ clb6Δ</i> <i>clb5Δ pds1Δ</i> <i>CLB5-dbΔ pds1Δ</i> <i>clb5Δ pds1Δ + multi-copy SIC1-high</i> <i>clb5Δ clb6Δ cln1Δ cln2Δ</i> <i>clb5Δ clb6Δ pds1Δ + multi-copy SIC1-high</i> Clb1 Clb2 mutants <i>GAL-CLB2</i> <i>CLB2-dbΔ</i> <i>CLB2-dbΔ in galactose</i> Multi-copy <i>GAL-CLB2</i> <i>clb2Δ CLB1</i> <i>CLB2-dbΔ clb5Δ</i> <i>CLB2-dbΔ clb5Δ in gal</i> <i>CLB2-dbΔ GAL-SIC1</i> <i>CLB2-dbΔ multi-copy SIC1</i> <i>clb2Δ CLB1 cdh1Δ</i>	<i>clb1Δ clb2Δ clb5Δ</i> <i>clb2Δ CLB1 pds1Δ</i> <i>CLB2-dbΔ clb5Δ clb6Δ</i> <i>CLB2-dbΔ clb5Δ clb6Δ in gal*</i> <i>clb1Δ clb2Δ clb5Δ clb6Δ</i> Cdc20 mutants <i>cdc20^{ts}</i> <i>GAL-CDC20</i> <i>cdc20Δ clb5Δ</i> <i>cdc20Δ pds1Δ</i> <i>cdc20Δ GAL-SIC1-4A</i> <i>cdc20Δ clb5Δ pds1Δ</i> <i>cdc20Δ pds1Δ cdh1Δ</i> <i>cdc20Δ pds1Δ CLB5-dbΔ</i> <i>cdc20Δ pds1Δ SIC1-4A*</i> <i>cdc20Δ pds1Δ + multi-copy SIC1-high</i> <i>cdc20Δ clb5Δ clb6Δ pds1Δ</i> <i>cdc20Δ pds1Δ clb5Δ cdh1Δ</i> <i>cdc20Δ pds1Δ cdh1Δ + multi-copy SIC1-high</i> <i>cdc20Δ pds1Δ clb5Δ + multi-copy SIC1-high</i> <i>cdc20Δ pds1Δ clb5Δ cdh1Δ + multi-copy SIC1-high</i> APC mutants <i>APC-A</i> <i>APC-A cdh1Δ</i> <i>APC-A cdh1Δ in galactose</i> <i>APC-A sic1Δ</i> <i>APC-A GAL-CLB2</i> <i>APC-A cdc6Δ sic1Δ</i> <i>APC-A cdh1Δ GAL-CDC6</i> <i>APC-A cdh1Δ multi-copy CDC6</i> <i>APC-A cdh1Δ GAL-SIC1</i> <i>APC-A cdh1Δ multi-copy SIC1</i> <i>APC-A chd1Δ multi-copy CDC20</i> Pds1/Esp1 interaction <i>cdc55Δ</i> <i>cdc55Δ in galactose</i> <i>esp1^{ts}</i> <i>pds1Δ</i> <i>pds1Δ in galactose</i> <i>GAL-CDC55</i> <i>PDS1-dbΔ</i> <i>GAL-PDS1-dbΔ</i> <i>GAL-ESP1 cdc20^{ts}</i> <i>GAL-PDS1-dbΔ esp1^{ts}</i> <i>GAL-ESP1 GAL-PDS1-dbΔ</i> <i>cdc20Δ GAL-PDS1-dbΔ GAL-TEV CDC20-back</i> <i>GAL-TEV cdc20Δ</i> <i>GAL-ESP1 cdc20Δ clb5Δ</i> <i>GAL-PDS1-dbΔ GAL-TEV cdc20-back</i> <i>GAL-TEV cdc20Δ clb5Δ</i> <i>GAL-TEV cdc20Δ GAL- SIC1</i> Checkpoint mutants <i>WT</i> in hydroxyurea <i>WT</i> in nocodazole <i>bub2Δ</i> <i>bub2Δ in nocodazole</i> <i>bub2Δ in hydroxyurea-arrested cells</i> <i>mad2Δ</i> <i>mad2Δ in nocodazole</i> <i>net1^{ts}</i> in nocodazole <i>pds1Δ in nocodazole</i> <i>bub2Δ cdc20^{ts}</i> <i>bub2Δ pds1Δ in nocodazole</i> <i>mad2Δ bub2Δ</i> <i>mad2Δ bub2Δ in nocodazole</i> <i>mad2Δ cdc20^{ts}</i> <i>mad2Δ pds1Δ in nocodazole</i> <i>mad2Δ GAL-TEM1 in nocodazole</i> MEN pathway mutants <i>cdc15Δ</i> <i>cdc15^{ts}</i> <i>lte1Δ</i> <i>tem1Δ</i> <i>GAL-CDC15</i> in hydroxyurea-arrested cells <i>GAL-CDC15</i> Multi-copy <i>CDC15</i>	<i>GAL-TEM1</i> multi-copy <i>TEM1</i> <i>cdc15Δ net1^{ts}</i> <i>cdc15Δ cdc20-back</i> <i>lte1Δ bub2Δ</i> <i>lte1Δ esp1^{ts}</i> <i>tem1Δ net1^{ts}</i> <i>cdc15^{ts} multi-copy CDC14</i> <i>cdc15Δ GAL-SIC1</i> <i>cdc15^{ts} multi-copy TEM1</i> <i>cdc15Δ multi-copy SWI5</i> <i>tem1^{ts} GAL-CDC15</i> <i>tem1Δ multi-copy CDC14</i> <i>tem1^{ts} multi-copy CDC15</i> <i>cdc15Δ net1^{ts} cdh1Δ</i> <i>cdc15^{ts} cdc20Δ pds1Δ</i> <i>cdc15^{ts} cdc20Δ pds1Δ clb5Δ</i> <i>cdc15^{ts} cdc20Δ pds1Δ clb5Δ clb6Δ</i> Exit-from-mitosis mutants <i>cdc5Δ</i> <i>cdc14^{ts}</i> <i>cdc14^{ts} in galactose</i> <i>net1^{ts}</i> <i>swe1Δ</i> <i>CDC5-dbΔ</i> <i>GAL-CDC5</i> Nocodazole then <i>GAL-CDC5</i> Hydroxyurea then <i>GAL-CDC5</i> <i>GAL-CDC14</i> <i>GAL-NET1</i> <i>TAB6-1</i> <i>cdc5Δ bub2Δ</i> <i>cdc5Δ cdc15Δ</i> <i>cdc5Δ net1Δ</i> <i>cdc5Δ tem1Δ</i> <i>cdc14^{ts} cdh1Δ non-permissive temperature</i> <i>cdc14^{ts} sic1Δ non- permissive temperature</i> <i>net1^{ts} cdc20^{ts}</i> <i>cdc5Δ GAL-CDC15</i> <i>cdc5Δ GAL-SIC1</i> <i>cdc5Δ multi-copy SWI5</i> <i>cdc14-1 GAL-CDC15</i> <i>bub2Δ GAL-CDC5 in hydroxyurea</i> <i>GAL-CDC5 cdc15Δ</i> <i>cdc14^{ts} GAL-CLN2 non-permissive temp.</i> <i>cdc14^{ts} GAL-SIC1</i> <i>TAB6-1 cdc15^{ts}</i> <i>TAB6-1 clb5Δ</i> <i>GAL-CDC5 cdc20Δ</i> <i>GAL-CDC14 GAL-NET1</i> <i>TAB6-1 clb5Δ clb6Δ</i> <i>TAB6-1 clb2Δ CLB1</i> <i>GAL-CDC5 GAL-PDS1 cdc20Δ*</i> <i>cdc20Δ then GAL-PDS1 dbΔ GAL-CDC5</i> <i>cdc20Δ pds1Δ cdh1Δ then URL-cdc5</i> Cdc14- oscillating mutants <i>GAL-CLB2-dbΔ</i> <i>GAL-CLB2nd</i> <i>GAL-CLB2-dbΔ cdc5-1</i> <i>GAL-CLB2-dbΔ CDC5-dbΔ</i> <i>GAL-CLB2-dbΔ cdc14-1</i> <i>GAL-CLB2-dbΔ cdc15^{ts}</i> <i>GAL-CLB2-dbΔ cdc20-3</i> <i>GAL-CLB2-dbΔ cdh1Δ</i> <i>GAL-CLB2-dbΔ sic1Δ</i> <i>GAL-CLB2-dbΔ swi5Δ</i> <i>GAL-CLB2-dbΔ cdc6Δ sic1Δ*</i> <i>GAL-CLB2nd cdc15^{ts}</i> <i>GAL-CLB2nd cdc5-1</i> <i>GAL-CLB2nd CDC5-dbΔ</i> <i>GAL-CLB2nd cdc14-1</i> <i>GAL-CLB2nd cdc15^{ts}</i> <i>GAL-CLB2nd cdc20-3</i> <i>GAL-CLB2nd cdh1Δ</i> <i>GAL-CLB2nd sic1Δ</i> <i>GAL-CLB2nd swi5Δ</i> <i>GAL-CLB2nd cdc6Δ sic1Δ*</i> <i>GAL-CLB2nd cdc20Δ clb5Δ pds1Δ*</i>
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MAT α parents

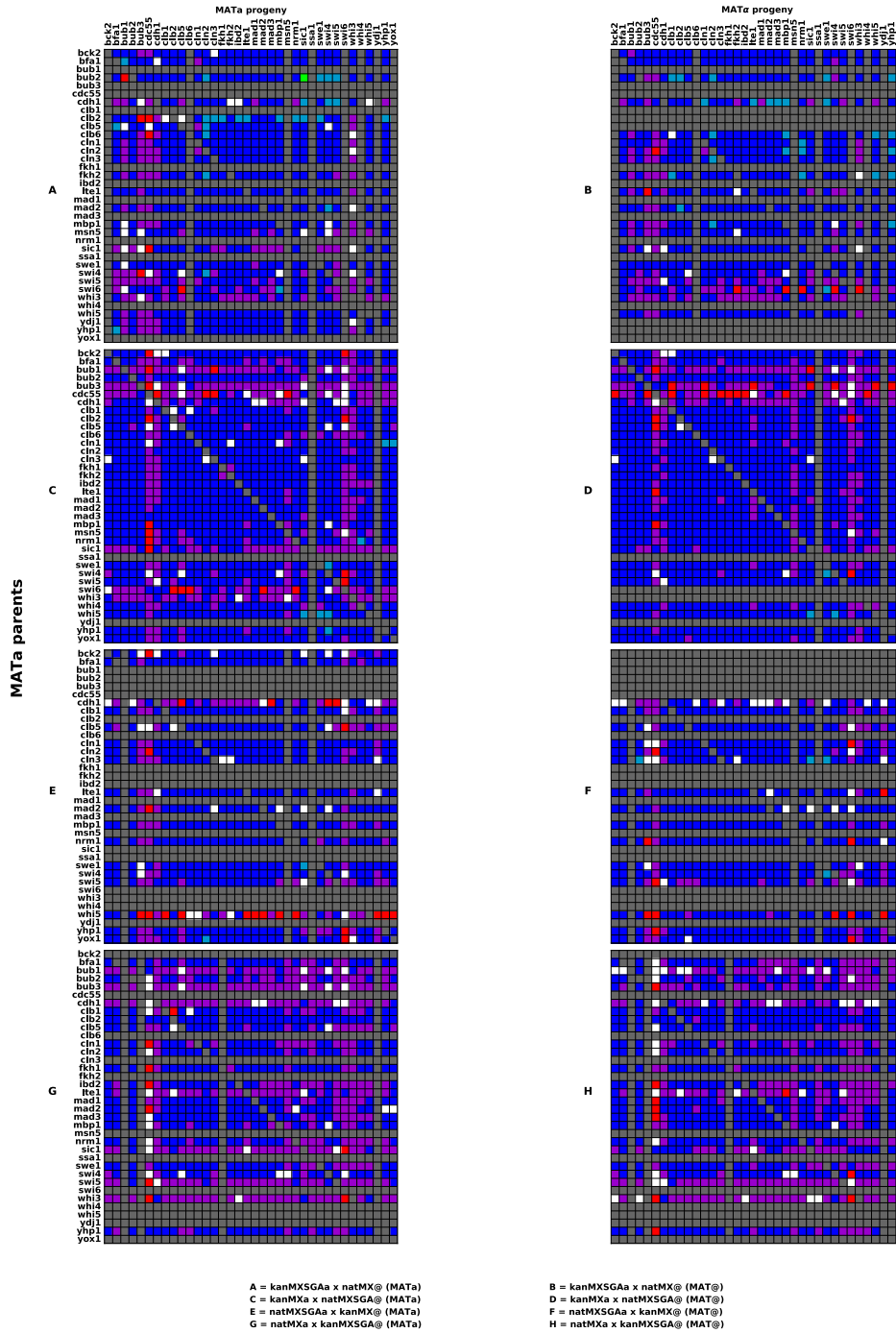


Figure S6. Comparison of fitness scores for double mutants in all four sets of crosses on YPR media. White cells indicate zero growth and grey cells indicate missing or excluded data. Royal blue is used to designate fitness scores that differ from WT by fewer than 2 standard deviations. Cyan and green indicate fitness scores that are greater than WT by up to or more than 6 standard deviations respectively. Magenta and red indicate fitness scores that are less than WT by up to or more than 6 standard deviations respectively. **A & B) Cross 1. C & D) Cross 2. E & F) Cross 3. G & H) Cross 4.**

MAT α parents

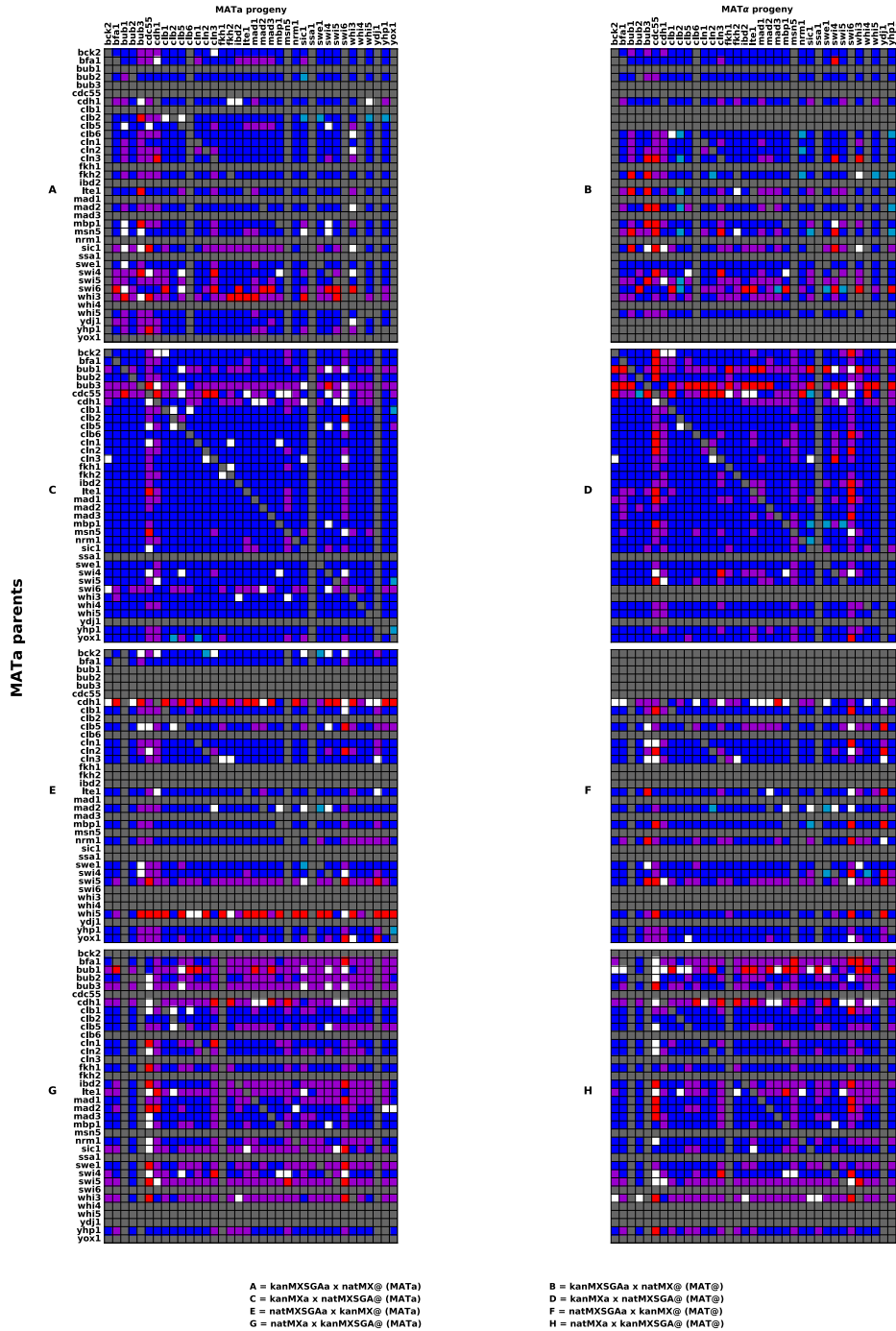


Figure S7. Comparison of fitness scores for double mutants in all four sets of crosses on YPG media. White cells indicate zero growth and grey cells indicate missing or excluded data. Royal blue is used to designate fitness scores that differ from WT by fewer than 2 standard deviations. Cyan and green indicate fitness scores that are greater than WT by up to or more than 6 standard deviations respectively. Magenta and red indicate fitness scores that are less than WT by up to or more than 6 standard deviations respectively. A & B) Cross 1. C & D) Cross 2. E & F) Cross 3. G & H) Cross 4.

MAT α parents

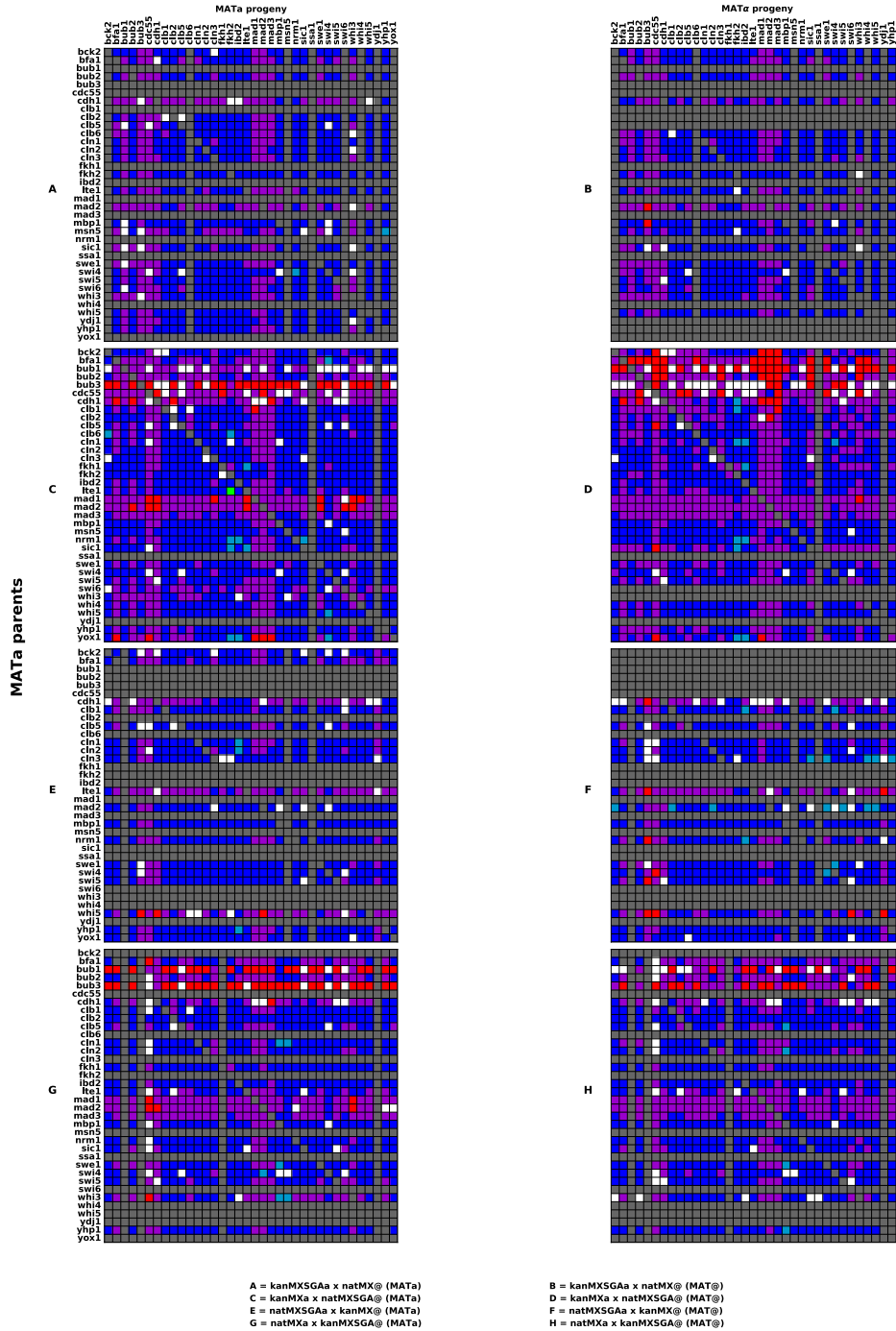


Figure S8. Comparison of fitness scores for double mutants in all four sets of crosses on YPD-Ben media. White cells indicate zero growth and grey cells indicate missing or excluded data. Royal blue is used to designate fitness scores that differ from WT by fewer than 2 standard deviations. Cyan and green indicate fitness scores that are greater than WT by up to or more than 6 standard deviations respectively. Magenta and red indicate fitness scores that are less than WT by up to or more than 6 standard deviations respectively. **A & B) Cross 1. C & D) Cross 2. E & F) Cross 3. G & H) Cross 4.**

MAT α parents

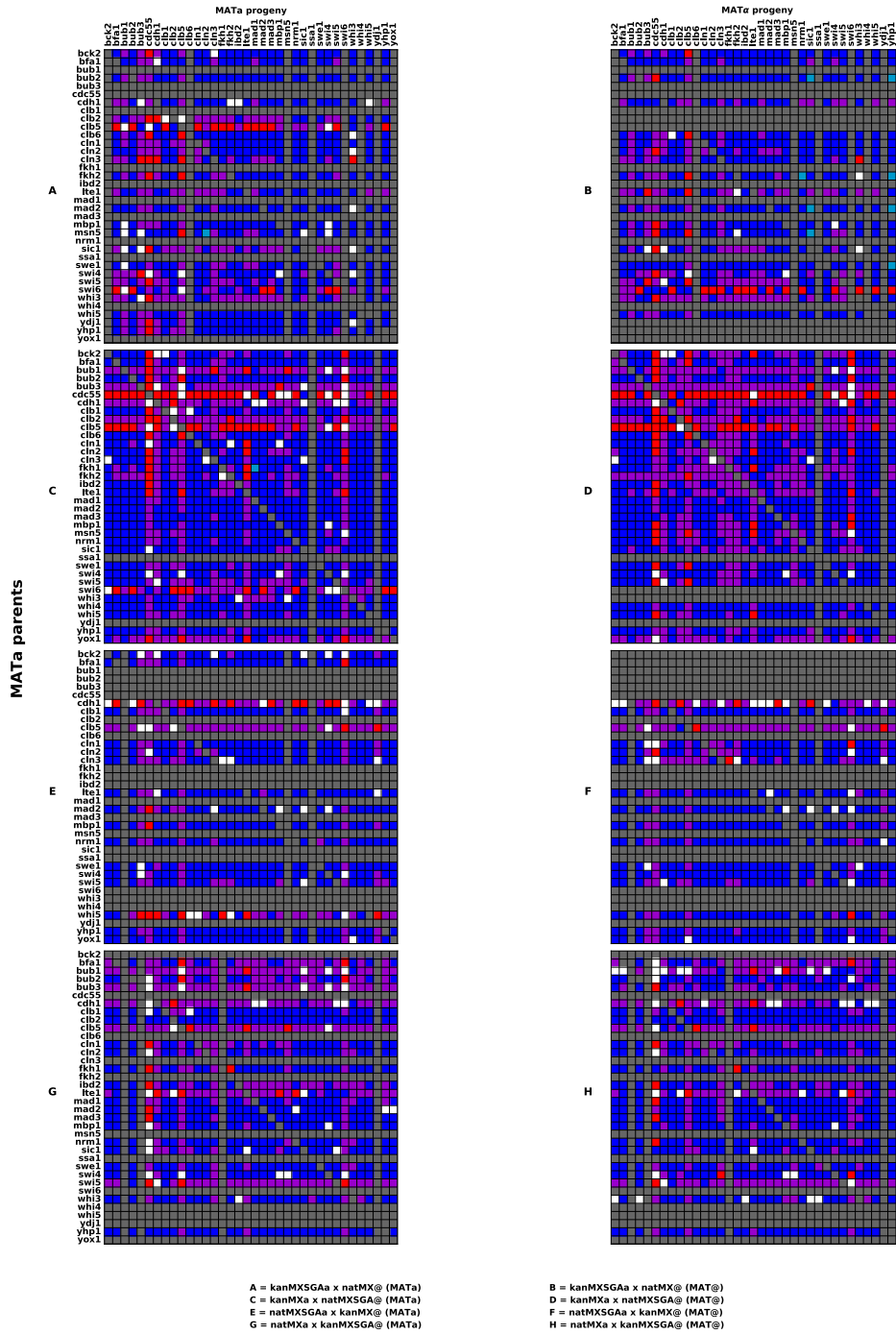


Figure S9. Comparison of fitness scores for double mutants in all four sets of crosses on YPD-CPT media. White cells indicate zero growth and grey cells indicate missing or excluded data. Royal blue is used to designate fitness scores that differ from WT by fewer than 2 standard deviations. Cyan and green indicate fitness scores that are greater than WT by up to or more than 6 standard deviations respectively. Magenta and red indicate fitness scores that are less than WT by up to or more than 6 standard deviations respectively. **A & B) Cross 1. C & D) Cross 2. E & F) Cross 3. G & H) Cross 4.**

MAT α parents

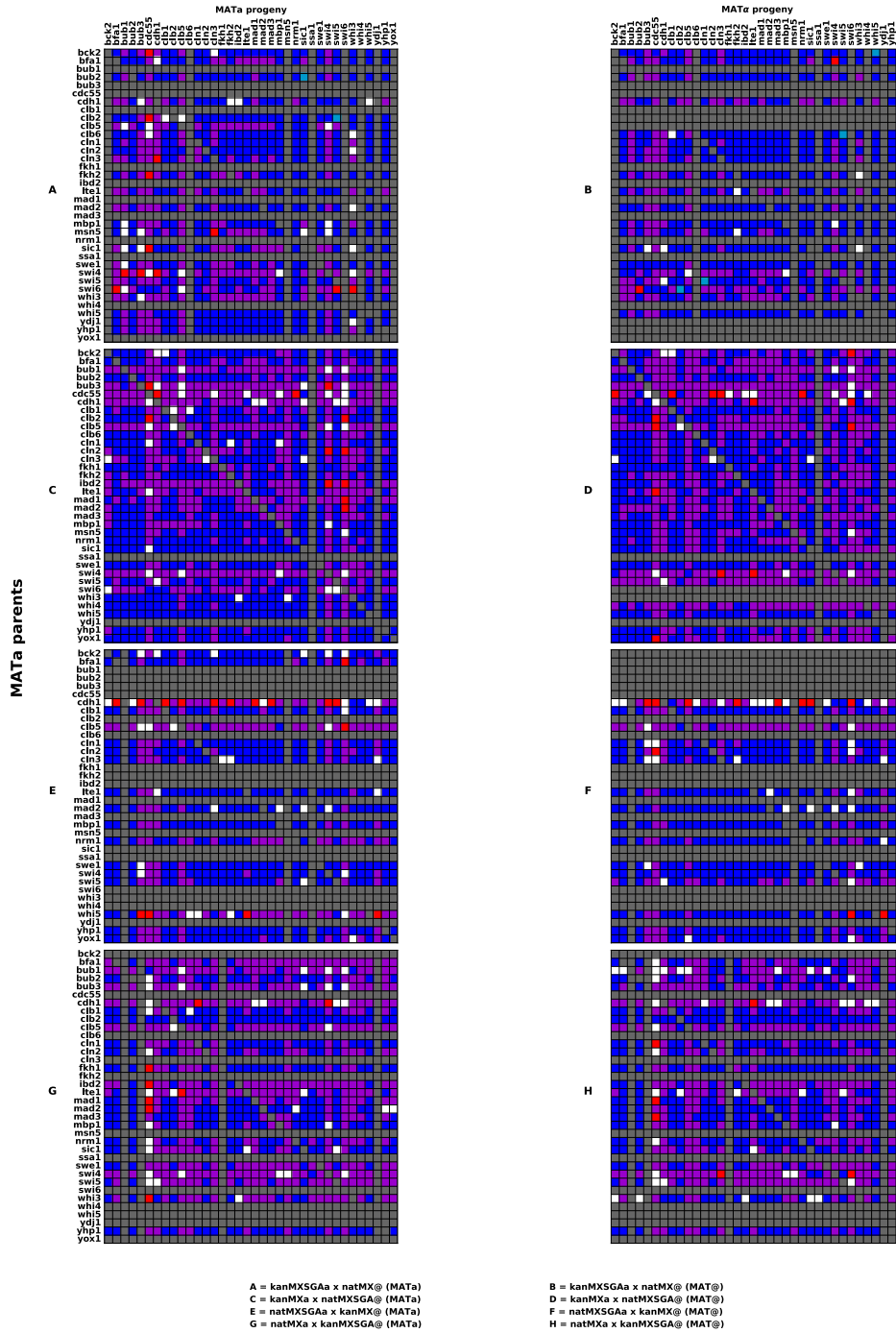


Figure S10. Comparison of fitness scores for double mutants in all four sets of crosses on YPD-HU media. White cells indicate zero growth and grey cells indicate missing or excluded data. Royal blue is used to designate fitness scores that differ from WT by fewer than 2 standard deviations. Cyan and green indicate fitness scores that are greater than WT by up to or more than 6 standard deviations respectively. Magenta and red indicate fitness scores that are less than WT by up to or more than 6 standard deviations respectively. **A & B) Cross 1. C & D) Cross 2. E & F) Cross 3. G & H) Cross 4.**

Supplementary Tables

Supplementary tables are provided as Excel files in the Supplementary Data file.

Table S1. Parent Strains used in this study

Table S2. Primers used in this study

Table S3. Potential sources of false negatives

Table S4. Replicates supporting synthetic lethal (SL)

Table S5. Colony size and growth rate variability across biological replicates of *cdh1Δ swi4Δ*

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