

APPENDIX

DASH/Dam1 complex mutants stabilize ploidy in histone-humanized yeast by weakening kinetochore-microtubule attachments

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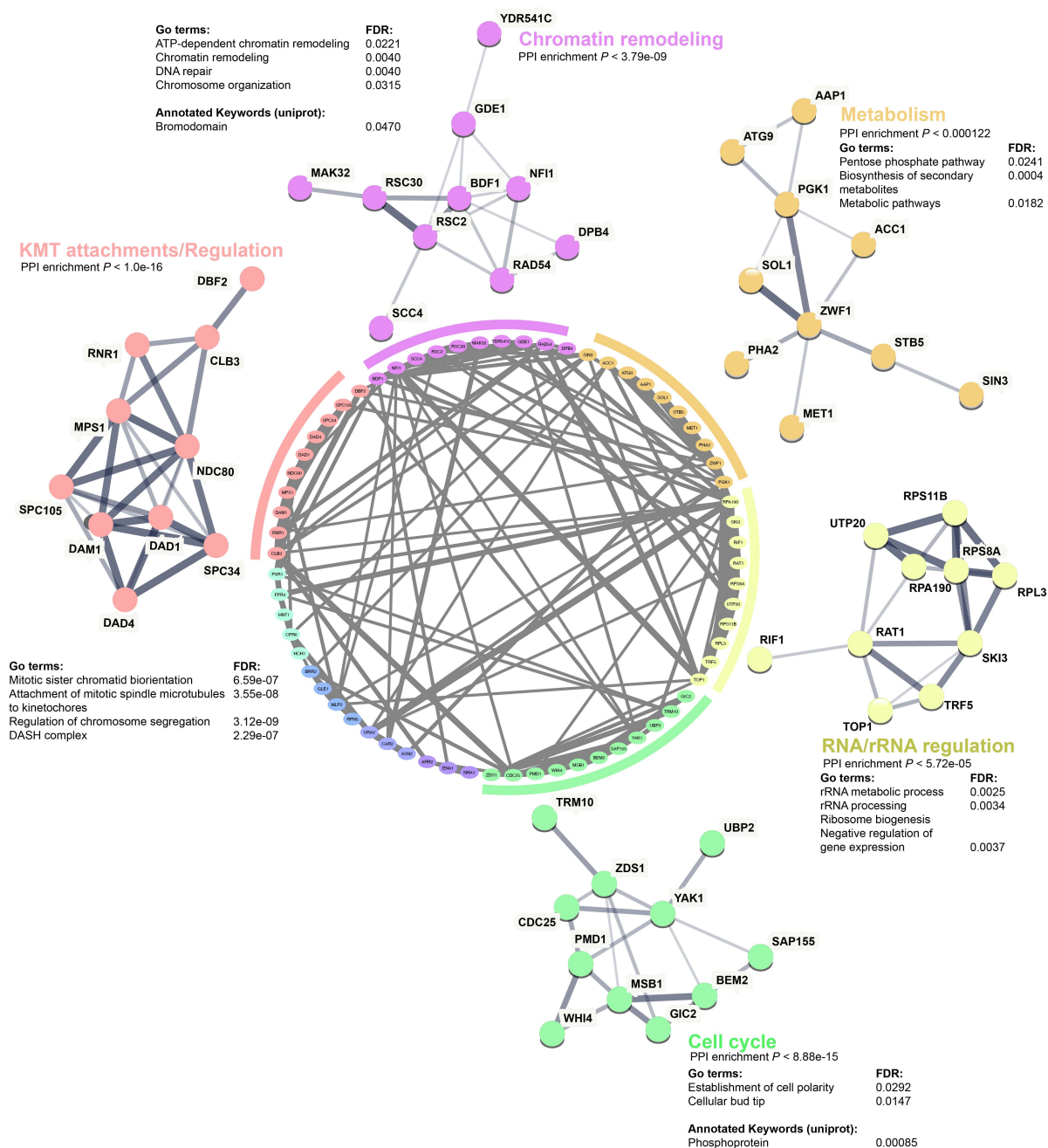
*Corresponding author

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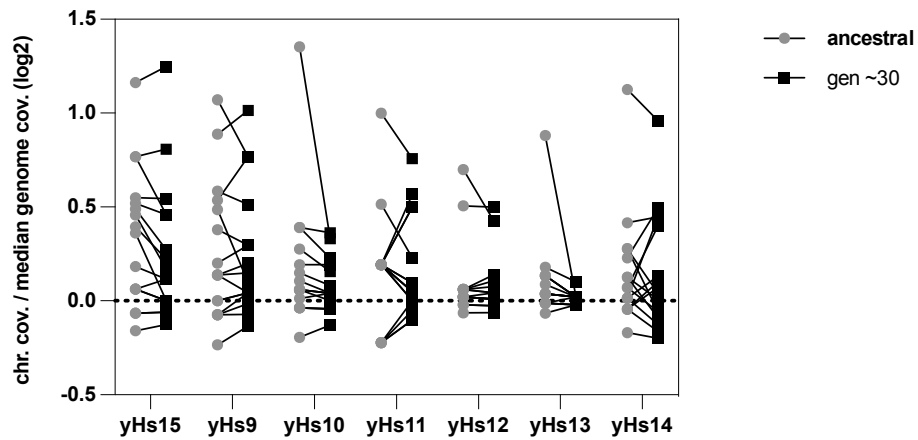
†Equal Contribution

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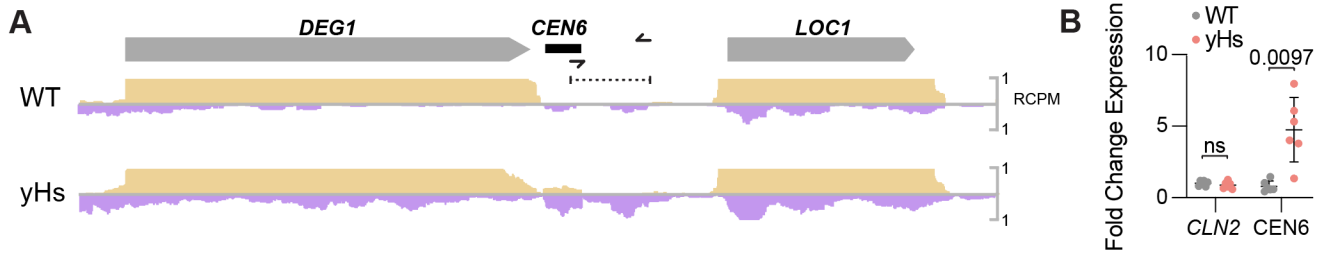
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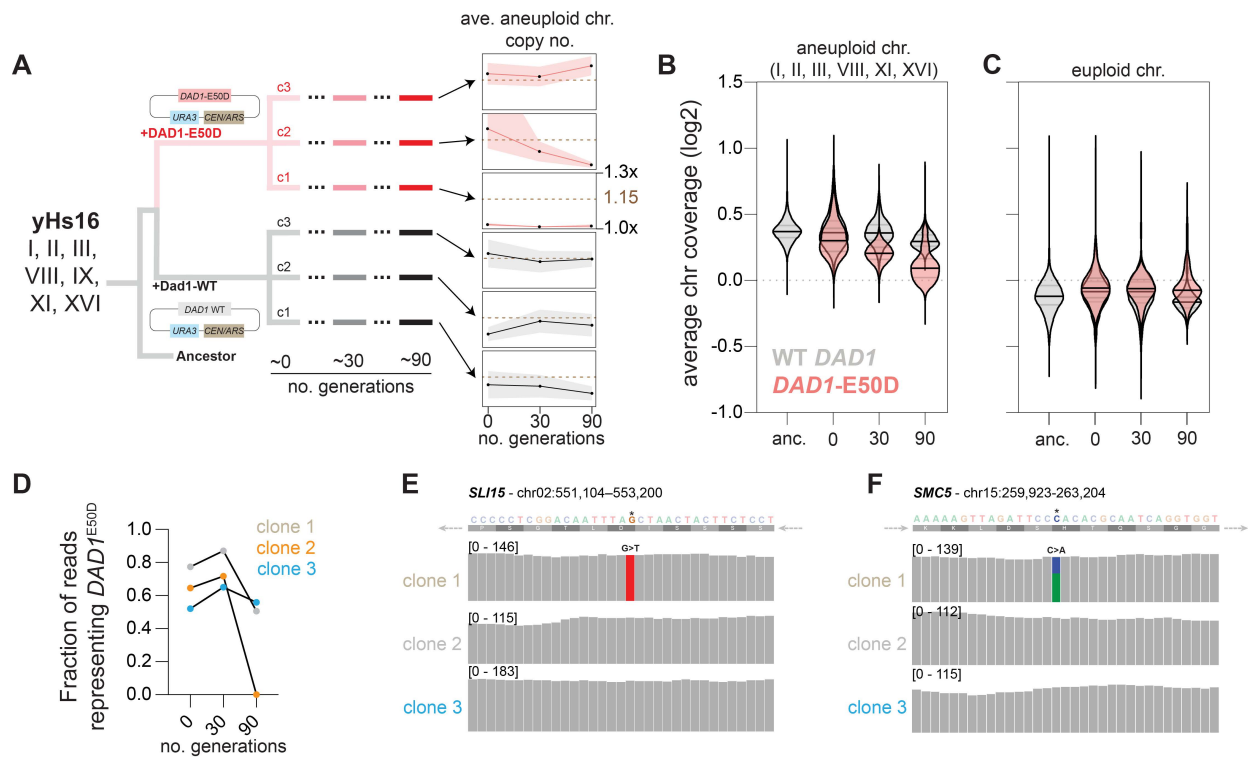
Appendix Figure S1. Protein-protein interaction network of candidate suppressors of human histones. String database analysis of protein-protein interactions (PPI) considering missense, nonsense, and noncoding mutations identified in histone humanized lineages (Table EV1 and Truong and Boeke, 2017). The network was clustered using MCL clustering with the inflation parameter set to 1.8, clusters are colored, with PPI depicted as gray connecting lines. The number of PPI in the global network was significantly enriched from background (PPI enrichment $p = 0.0049$). Local PPI networks are shown for clusters containing more than 10 interactors, with PPI enrichment p -values shown, alongside Go term enrichment terms and Uniprot keywords. Network was constructed using the interaction sources: “textmining”, “experiments”, “databases”, and “co-expression”. The width of each edge represents the strength of interaction between nodes based on available evidence. Disconnected nodes were removed for clarity.



Appendix Figure S2. Chromosome aneuploidy evolution in yHs lineages. For each histone–humanized lineage (ancestral, gray circles; and evolved, black squares) we plot the median log₂ coverage ratio (chromosome divided by median of genome) for each chromosome.

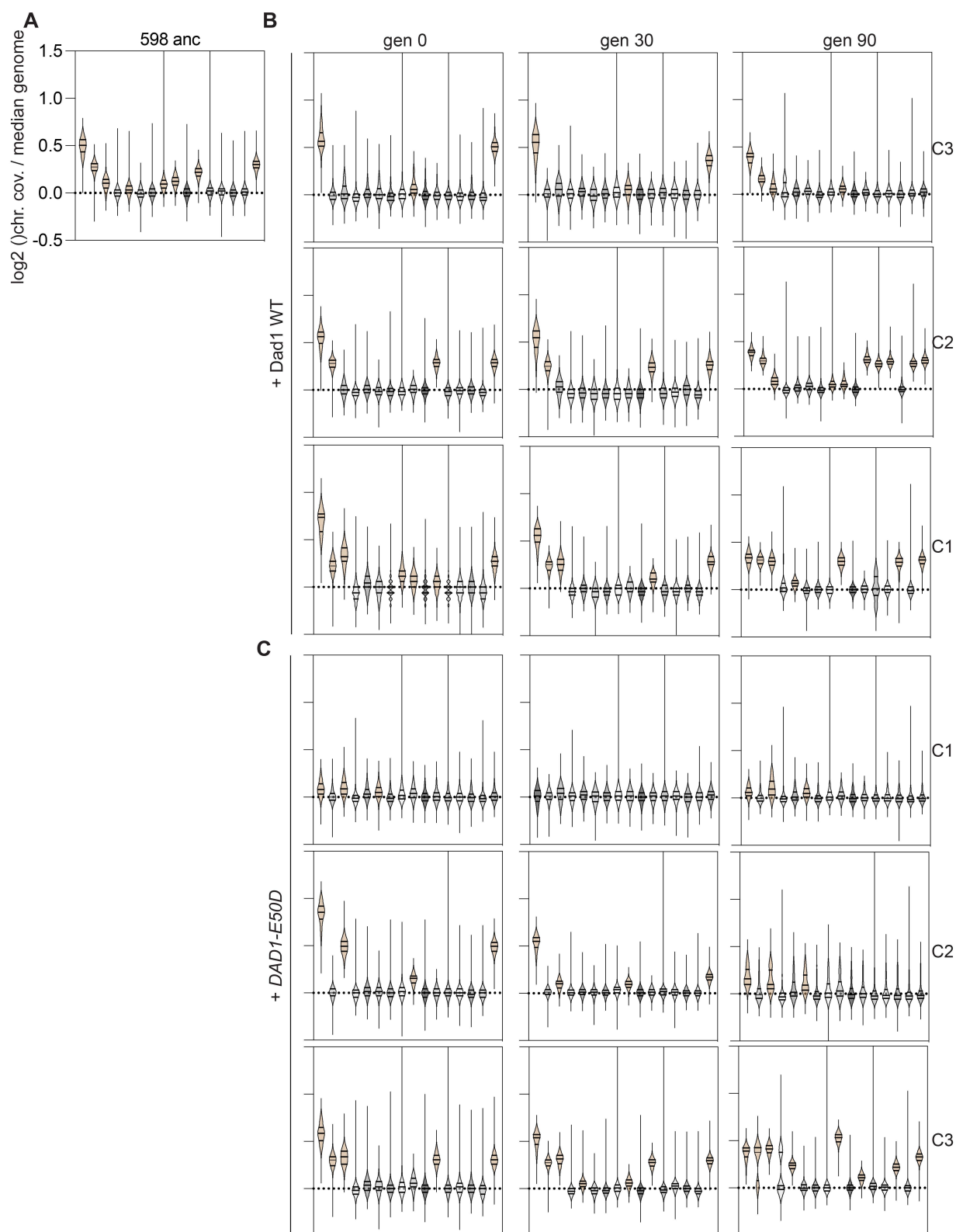


Appendix Figure S3. Transcription at chromosomal CEN6 in wild type and histone-humanized yeasts. (a) The region around centromere VI is shown, with read coverage (read counts per million) for the forward (yellow) and reverse (purple) strands. The region amplified for RT- and qRT-PCR is shown by the two arrow heads and region highlighted in a bracket. (b) Quantitative RT-PCR of CEN6 RNA in WT and histone humanized strains. Expression levels of CLN2 and CEN6 were normalized to the expression level of *UBC6* (see Appendix Table S5).

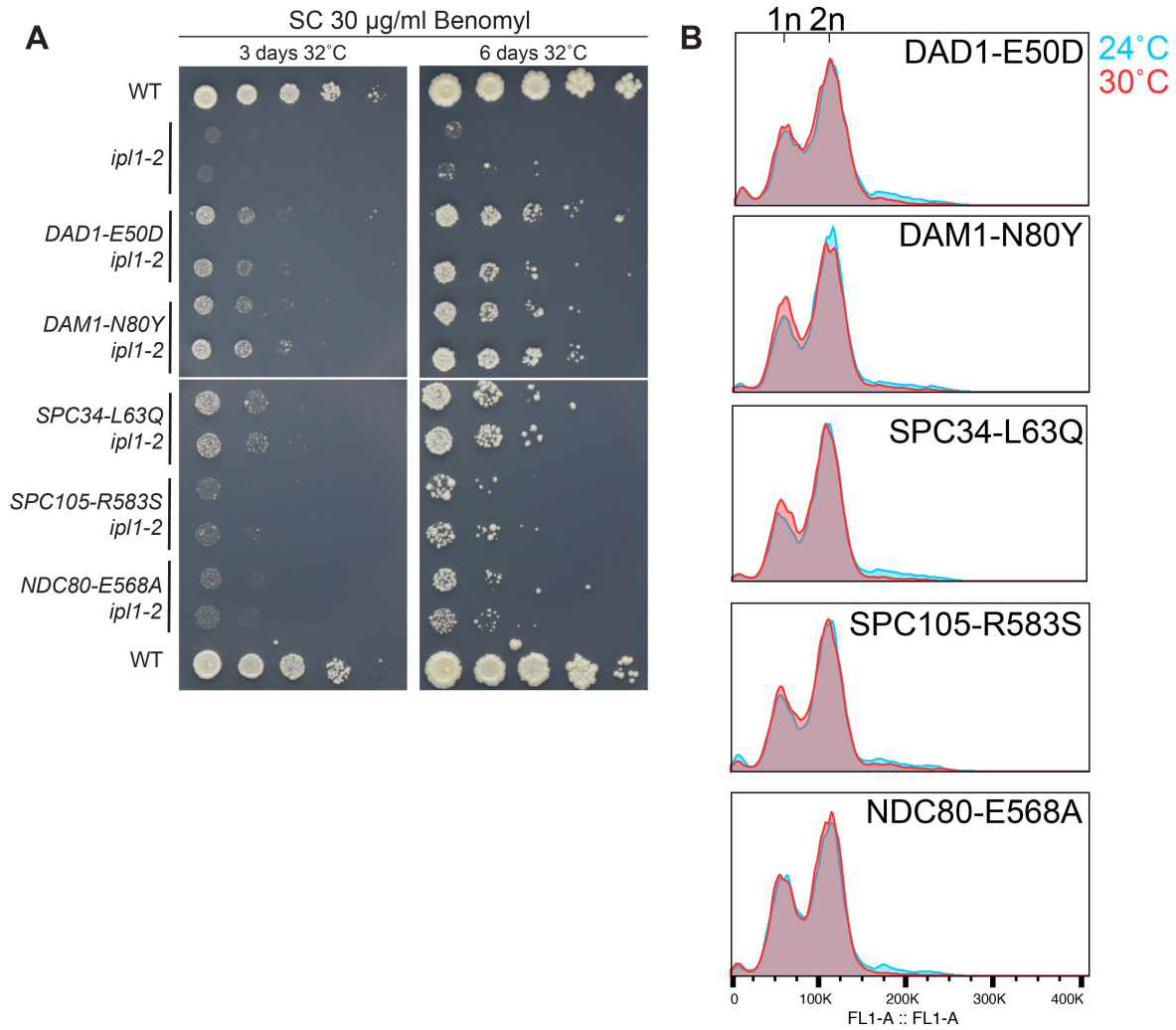


Appendix Figure S4. *DAD1*^{E50D} drives euploidization in a heterozygous state.

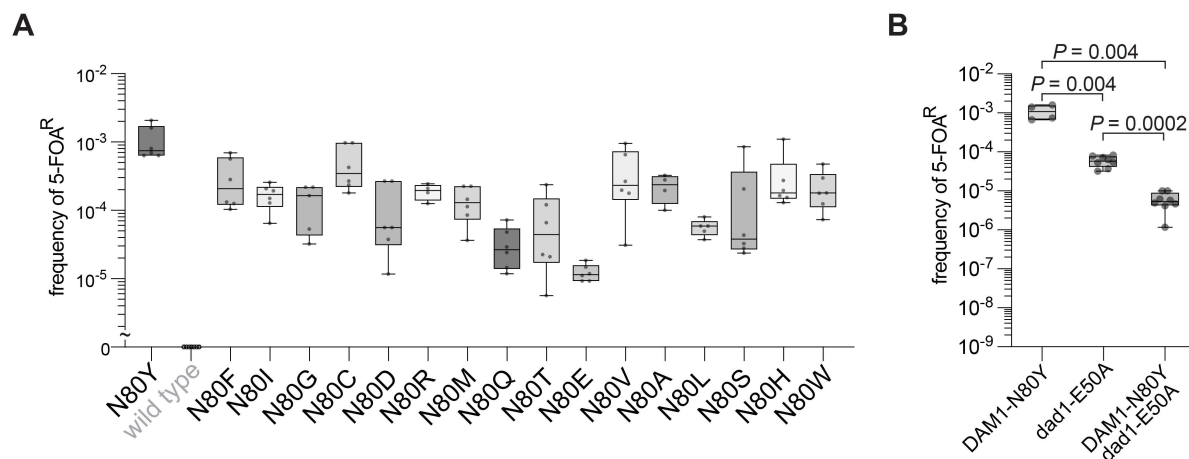
(a) Overview of experimental design and ploidy evolution for each clone. Left, histone-humanized clone yH16 was transformed with a CEN/ARS plasmids encoding either the wild type *DAD1* gene or mutant *DAD1*^{E50D} gene. Three clones were selected and passaged for 90 generations in SC-Ura, to maintain selection of the CEN/ARS plasmid. Genomic DNA was isolated and sequenced to infer chromosome copy numbers. Right, plots of the average chromosome copy number for the ancestral aneuploid chromosomes (I, II, III, VIII, IX, XI, and XVI) at each time point (shaded area represents the standard deviation between the three biological replicates). **(b)** Log2 ratio (chromosome divided by the genome median) chromosomal coverage for the parental aneuploid chromosomes. **(c)** Same as in panel b, however shown for the parental euploid chromosomes. **(d)** Ratio of reads corresponding to the mutant *DAD1*^{E50D} allele for clones transformed with the pRS416-*DAD1*^{E50D} plasmid across each timepoint. **(e)** Genome browser track for the *SLI15* gene, the DNA shown is the Crick strand and represents the template strand of *SLI15* (note the arrow of the direction indicates the orientation of *SLI15*). **(f)** Genome browser track for the *SMC5* gene.



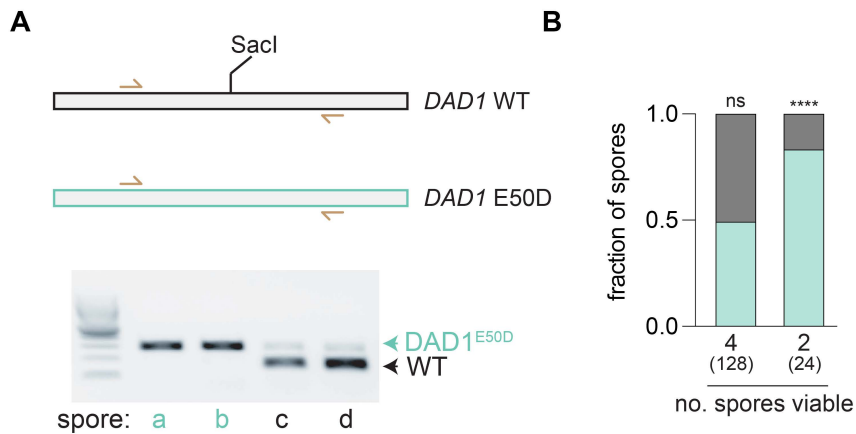
Appendix Figure S5. Chromosome copy number of each clone from aneuploidy reduction experiment. (a) Chromosome coverage for the ancestral strain prior to transformation with the CEN/ARS plasmid. Violin plots shaded brown are indicated to be aneuploid. **(b)** Chromosome coverage for the clones transformed with wild type *DAD1*. **(c)** Chromosome coverage for the clones transformed with mutant *DAD1*^{E50D}.



Appendix Figure S6. **DASH/Dam1c mutants rescue Aurora B kinase *ipl1-2* mutant.** (a) Growth assays of suppressor mutants with the *ipl1-2* temperature sensitive mutant on SC medium with 30 $\mu\text{g/ml}$ of benomyl. (b) Cell cycle analysis of the indicated mutant at 24°C and 30°C. Yeast cultures were grown to logarithmic phase at 24°C and divided and grown at 24°C and 30°C for 6 hours before cells were collected and processed for DNA content analysis using Sytox Green stain.



Appendix Figure S7. **Humanization rates of *dam1* residue 80 mutants and *DAM1*^{N80Y} *dad1*^{E50A} mutant.** (a) Humanization rates (5-FOA^R frequency) for the missense mutants of *dam1* residue 80. (b) Humanization rates (5-FOA^R frequency) of the single and double mutants *dad1*^{E50A} and *DAM1*^{N80Y}.



Appendix Figure S8. PCR genotyping of DAD1 in spores from heterozygous cross. (a) Schematic and example of the PCR digestion genotype assay used. Briefly, the *DAD1* locus is PCR amplified and then digested with the *SacI* enzyme, which only cuts the wild type allele. Digestions are then visualized on a 1% agarose gel. (b) Genotyping of spores isolated from tetrads with either four viable spores or two viable spores. Fraction of spores with the indicated allele (gray *DAD1*WT and cyan *DAD1*E50D) is displayed. The one-tailed binomial test is shown for the probability of observing the frequency of the *DAD1*E50D allele given equal segregation (*DAD1*WT, $P = 0.46$; *DAD1*E50D, $P = 0.0011$). Numbers in parenthesis indicates the total number of spores genotyped.

Appendix Table S1. Candidate Suppressor mutations by lineage.				
Mutant	Isolate	Strain	Mutations	Likely innocuous mutations
yHs9	yHs9evo0_pool	yMAH0344	–	–
	yHs9evo5_pool	yMAH0393	–	–
	yHs9evo5_C1	yMAH531	–	<i>gle1(-8)</i> <i>rif1(-264)</i> <i>car2(-168)</i>
	yHs9evo5_C2	yMAH532	<i>dad4(N61K)</i> <i>YPL216W(K290Stop)</i> <i>gic2(G133S)</i>	<i>gle1(-8)</i> <i>rif1(-264)</i>
	yHs9evo5_C3	yMAH533	<i>gic2(G133S)</i>	–
yHs10	yHs10_evo0_pool	yMAH0345	<i>bdf1(E474D)</i>	<i>trm732(A104A)</i> <i>gde1(R873R)</i>
	yHs10_evo5_pool	yMAH0394	<i>bdf1(E474D)</i>	<i>trm732(A104A)</i> <i>gde1(R873R)</i>
yHs11	yHs11_evo0_pool	yMAH0346	–	–
	yHs11_evo5_pool	yMAH0395	<i>rnr1(P144T)</i> <i>sin3(F470Y)</i>	<i>suf6-1</i> <i>fre1(-183)</i>
	yHs11_evo5_C2	yMAH536	<i>vps8(Q116H)</i> <i>pmd1(V895F)</i> <i>rnr1(P144T)</i> <i>sin3(F470Y)</i> <i>rpn5(E120A)</i>	<i>hch1(-88)</i> <i>suf6-1</i>
yHs12	yHs12_evo0_pool	yMAH0347	–	–
	yHs12_evo5_pool	yMAH0396	<i>ecm4(M84I)</i> <i>rnp1(K115R)</i> <i>aus1(A1189V)</i> <i>rpl3(T296I)</i> <i>rpa190(E23G)</i>	–
	yHs12_evo5_C1	yMAH456	<i>yak1(Q77E)</i> <i>ecm4(M84I)</i> <i>rnp1(K115R)</i> <i>aus1(A1189V)</i> <i>rpl3(T296I)</i> <i>rpa190(E23G)</i>	–
yHs13	yHs13_evo0_pool	yMAH0348	<i>dam1(N80Y)</i>	–
	yHs13_evo5_pool	yMAH0397	<i>rat1(N650K)</i> <i>dam1(N80Y)</i>	–
yHs14	yHs14_evo0_pool	yMAH0349	–	–
	yHs14_evo5_pool	yMAH0398	<i>cdc25(N1574H)</i>	–
	yHs14_evo5_C1	yMAH538	<i>aap1(G226S)</i> <i>cdc25(N1574H)</i>	<i>mne1(V267V)</i> <i>ura2(S1442S)</i> <i>YDR541C(+152)*</i>
yHs15	yHs15_evo0_pool	yMAH0343	–	–

	<i>yHs15_evo5_pool</i>	<i>yMAH0392</i>	–	–
	<i>yHs15_evo5_C1</i>	<i>yMAH528</i>	<i>acc1(Q455R)</i>	<i>rps8A(+39)</i> <i>ts(AGA)D3(C>A;</i> <i>186560)</i>
	<i>yHs15_evo5_C2</i>	<i>yMAH529</i>	<i>acc1(Q455R)</i>	–
	<i>yHs15_evo5_C3</i>	<i>yMAH530</i>	<i>acc1(Q455R)</i>	–
<i>yHs16</i>	<i>yHs16_evo0_pool</i>	<i>yMAH0598</i>	<i>utp20(E1318D) atg9(A546E) avl9(S427C) YLR125W(S82Stop) nha1(L599Stop) ubp2(K405Q)</i>	<i>mps1(–221)</i> <i>ret2(A299A)</i> <i>)</i> <i>YLR123C(L8L)</i>
	<i>yHs16_evo0_C1</i>	<i>yMAH865</i>	<i>utp20(E1318D) atg9(A546E) avl9(S427C) YLR125W(S82Stop) nha1(L599Stop) ubp2(K405Q)</i>	<i>ret2(A299A)</i> <i>)</i> <i>YLR123C(L8L)</i>
	<i>yHs16_evo0_C2</i>	<i>yMAH866</i>	<i>rps11B(Q8H) apl5(S905A) arr2(G59R) utp20(E1318D) atg9(A546E) avl9(S427C) YLR125W(S82Stop) nha1(L599Stop) ubp2(K405Q)</i>	<i>ret2(A299A)</i> <i>)</i> <i>YLR123C(L8L)</i>
	<i>yHs16_evo0_C3</i>	<i>yMAH867</i>	<i>rad54(K588E) mlp2(E377Stop) msb1(D243V) top1(E713stop) utp20(E1318D) avl9(S427C) YLR125W(S82Stop) nha1(L599Stop) ubp2(K405Q)</i>	<i>asn2(–437)</i> <i>tcd2(–37)</i> <i>ret2(A299A)</i> <i>)</i> <i>YLR123C(L8L)</i>
	<i>yHs16_evo0_C4</i>	<i>yMAH868</i>	<i>hnt1(M15I) dpb2(P110T) pxr1(E210stop) YHL017W(E473D) zwf1(G228D) utp20(E1318D) avl9(S427C) YLR125W(S82Stop) nha1(L599Stop) ubp2(K405Q)</i>	<i>met1(V36V)</i> <i>)</i> <i>mag2(T405T) lto1(–222)</i> <i>ret2(A299A)</i> <i>)</i> <i>YLR123C(L8L)</i>
	<i>yHs16_evo0_C5</i>	<i>yMAH869</i>	<i>rad54(K588E) rsc30(D571G) mlp2(E377Stop) msb1(D243V) rsc2(P861T) top1(E713stop) ski3(A1427S) utp20(E1318D) avl9(S427C) YLR125W(S82Stop) nha1(L599Stop) ubp2(K405Q)</i>	<i>mcm6(–1188)</i> <i>asn2(–437)</i> <i>tcd2(–37)</i> <i>ret2(A299A)</i> <i>)</i> <i>YLR123C(L8L)</i>

	<i>yHs16_evo0_C6</i>	<i>yMAH870</i>	<i>rsc3(M641I) arg1(R367stop) bbp1(+230) aos1(Q121R) utp20(E1318D) avl9(S427C)</i> <i>YLR125W(S82Stop) nha1(L599Stop) ubp2(K405Q)</i>	<i>msg5(+292)</i>) <i>ret2(A299A)</i>) <i>YLR123C(L8L)</i>
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Appendix Table S2. <i>Aneuploidy abundance in histone-humanized yeasts.</i>																		
Lineage	Strain	I	II	III	IV	V	VI	VI I	VIII	IX	X	XI	XII	XII I	XI V	X V	XVI	source
yHs1	yHs1-pool												2					Truong and Boeke. Cell 2017
	yHs1C5	1	1	1		1				1		1					2	Truong and Boeke. Cell 2017
	yHs1-evo												2					Truong and Boeke. Cell 2017
	1C5i2												2					Truong and Boeke. Cell 2017
	1PC5i3		1	1									2					Truong and Boeke. Cell 2017
	1PC5i5		1	1									2					Truong and Boeke. Cell 2017
yHs2	yHs2-pool	2	1	1						1							1.5	Truong and Boeke. Cell 2017
	yHs2C5	1	1	1		1				1		1.7					2	Truong and Boeke. Cell 2017
	yHs2-evo	3	1	1		1			1	1		1.5					2	Truong and Boeke. Cell 2017
	2PC6il	2																Truong and Boeke. Cell 2017
	phe2AE	2																Truong and Boeke. Cell 2017
	phe2AF																	Truong and Boeke. Cell 2017
	phe2AH	2		1.7														Truong and Boeke. Cell 2017
	phe2BC	2								1							1.5	Truong and Boeke. Cell 2017
	phe2BD	1.8															1.5	Truong and Boeke. Cell 2017
	phe23C	1.5																Truong and Boeke. Cell 2017
yHs3	yHs3-pool		1.5										2					Truong and Boeke. Cell 2017
	yHs3C5	2																Truong and Boeke. Cell 2017
	yHs3-evo	1	1	1						1	1						1.5	Truong and Boeke. Cell 2017
yHs4	yHs4-pool												2					Truong and Boeke. Cell 2017
	yHs4C5	1		1													1.5	Truong and Boeke. Cell 2017
	yHs4-evo																	Truong and Boeke. Cell 2017
yHs5	yHs5-pool	1	1	1		1				1			1				2	Truong and Boeke. Cell 2017
	yHs5C5	1	1	1		1				1			1				1.5	Truong and Boeke. Cell 2017
	yHs5C10	1	1	1		1				1			1				1.5	Truong and Boeke. Cell 2017
	yHs5-evo	1	1	1		1				1			1				1.5	Truong and Boeke. Cell 2017
yHs6	yHs6-pool		1										2					Truong and Boeke. Cell 2017
	6C5	1		1													2	Truong and Boeke. Cell 2017
	yHs6-evo	1	1	1								1					1.5	Truong and Boeke. Cell 2017

yHs7	yHs7-pool		1.5								1							Truong and Boeke. Cell 2017
	yHs7C5																	Truong and Boeke. Cell 2017
	yHs7C10																	Truong and Boeke. Cell 2017
	yHs7-evo																	Truong and Boeke. Cell 2017
yHs8	yHs8		1	1							1							Truong and Boeke. Cell 2017
yHs9	yHs9-pool	2	1	2		0.7				1	0.2						1	This Study.
	yHs9-evo5	1.2		2	0.2					1.7	0.2						1	This Study.
	yHs9e5C1	1		1						2							1	This Study.
	yHs9e5C2	1		1						1								This Study.
	yHs9e5C3	1		2						1.5							1	This Study.
yHs10	yHs10-pool	2.5		1		0.5	1			0.7							2	This Study.
	yHs10-evo5	1		1		0.2	0.2										1.7	This Study.
yHs11	yHs11-pool	2	0.5	1			0.2										2.2	This Study.
	yHs11-evo5	1.7	1	0.5							1						1	This Study.
	yHs11e5C2	2	1	1		0.5				1	1						1	This Study.
yHs12	yHs12-pool	1.5	1															This Study.
	yHs12-evo5	0.7	1															This Study.
	yHs12e5C1	1	1	1		0.2	0.2			1	0.2						1	This Study.
yHs13	yHs13-pool			0.2													1.7	This Study.
	yHs13-evo5																	This Study.
yHs14	yHs14-pool	2.2	0.5	1			0.2			0.2								This Study.
	yHs14-evo5	2	1	1							1						1	This Study.
	yHs14e5C1	2	1	1						1	1						1	This Study.
yHs15	yHs15-pool	2	1	1			0.2			0.2	0.2						0.7	This Study.
	yHs15-evo5	2	1	1.7							1						1	This Study.
	yHs15e5C1	2	1	2													1	This Study.

	<i>yHs15e5C2</i>	2.5	1	1							1					1	<i>This Study.</i>
	<i>yHs15e5C3</i>	2	1	1.5							1					1.5	<i>This Study.</i>
<i>yHs16</i>	<i>yHs16-_pool</i>	1.7 5	0.7 5						0.2 5		0.5					1	<i>This Study.</i>
	<i>yHs16-evo5C1</i>	2							0.2 5							1.7 5	<i>This Study.</i>
	<i>yHs16-evo5C2</i>	1.7 5	1.5								1					1.2 5	<i>This Study.</i>
	<i>yHs16-evo5C3</i>	2	0.7 5	1					0.2 5							0.7 5	<i>This Study.</i>

yMAH801	NDC80-E568A diploid wild type histone shuffle strain	a / α	his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0/lys2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 met15Δ0/met15Δ0 hta2-htb2Δ0/hta2-htb2Δ0 hta1-htb1Δ0/hta1-htb1Δ0 hht1-hhf1Δ0/hht1-hhf1Δ0 hht2-hhf2Δ0/hht2-hhf2Δ0 NDC80(E568A)/NDC80(E568A)	pDT139	URA3	This study
yMAH810	dad4-N61K diploid wild type histone shuffle strain	a / α	his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0/lys2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 met15Δ0/met15Δ0 hta2-htb2Δ0/hta2-htb2Δ0 hta1-htb1Δ0/hta1-htb1Δ0 hht1-hhf1Δ0/hht1-hhf1Δ0 hht2-hhf2Δ0/hht2-hhf2Δ0 dad4(N61K)/dad4(N61K)	pDT139	URA3	This study
yMAH889	DAD1-E50D diploid wild type histone shuffle strain	a / α	his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0/lys2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 met15Δ0/met15Δ0 hta2-htb2Δ0/hta2-htb2Δ0 hta1-htb1Δ0/hta1-htb1Δ0 hht1-hhf1Δ0/hht1-hhf1Δ0 hht2-hhf2Δ0/hht2-hhf2Δ0 DAD1(E50D)/DAD1(E50D)	pDT139	URA3	This study
yMAH1122	DAM1-N80Y diploid wild type histone shuffle strain	a / α	his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0/lys2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 met15Δ0/met15Δ0 hta2-htb2Δ0/hta2-htb2Δ0 hta1-htb1Δ0/hta1-htb1Δ0 hht1-hhf1Δ0/hht1-hhf1Δ0 hht2-hhf2Δ0/hht2-hhf2Δ0 DAM1(N80Y)/DAM1(N80Y)	pDT139	URA3	This study
yMAH804	SPC34-L63Q het. diploid wild type histone shuffle strain	a / α	his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0/lys2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 met15Δ0/met15Δ0 hta2-htb2Δ0/hta2-htb2Δ0 hta1-htb1Δ0/hta1-htb1Δ0 hht1-hhf1Δ0/hht1-hhf1Δ0 hht2-hhf2Δ0/hht2-hhf2Δ0 SPC34/ SPC34(L63Q)	pDT139	URA3	This study
yMAH802	SPC105-R583S het. diploid wild type histone shuffle strain	a / α	his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0/lys2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 met15Δ0/met15Δ0 hta2-htb2Δ0/hta2-htb2Δ0 hta1-htb1Δ0/hta1-htb1Δ0 hht1-hhf1Δ0/hht1-hhf1Δ0 hht2-hhf2Δ0/hht2-hhf2Δ0 SPC105/SPC105(R583S)	pDT139	URA3	This study
yMAH814	NDC80-E568A het. diploid wild type histone shuffle strain	a / α	his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0/lys2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 met15Δ0/met15Δ0 hta2-htb2Δ0/hta2-htb2Δ0 hta1-htb1Δ0/hta1-htb1Δ0 hht1-hhf1Δ0/hht1-hhf1Δ0 hht2-hhf2Δ0/hht2-hhf2Δ0 NDC80/NDC80(E568A)	pDT139	URA3	This study
yMAH803	dad4-N61K het. diploid wild type histone shuffle strain	a / α	his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0/lys2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 met15Δ0/met15Δ0 hta2-htb2Δ0/hta2-htb2Δ0 hta1-htb1Δ0/hta1-htb1Δ0 hht1-hhf1Δ0/hht1-hhf1Δ0 hht2-hhf2Δ0/hht2-hhf2Δ0 DAD4/dad4(N61K)	pDT139	URA3	This study
yMAH805	DAD1-E50D het. diploid wild type histone shuffle strain	a / α	his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0/lys2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 met15Δ0/met15Δ0 hta2-htb2Δ0/hta2-htb2Δ0 hta1-htb1Δ0/hta1-htb1Δ0 hht1-hhf1Δ0/hht1-hhf1Δ0 hht2-hhf2Δ0/hht2-hhf2Δ0 DAD1/DAD1(E50D)	pDT139	URA3	This study
yMAH813	DAM1-N80Y het. diploid wild type histone shuffle strain	a / α	his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0/lys2Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0 met15Δ0/met15Δ0 hta2-htb2Δ0/hta2-htb2Δ0 hta1-htb1Δ0/hta1-htb1Δ0 hht1-hhf1Δ0/hht1-hhf1Δ0 hht2-hhf2Δ0/hht2-hhf2Δ0 DAM1/DAM1(N80Y)	pDT139	URA3	This study
yMAH1037	scc4-D65Y histone shuffle strain	α	his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 scc4(D65Y)	pDT139	URA3	This study
yMAH1043	scc4-D65Y & DAM1-N80Y histone shuffle strain	α	his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 scc4(D65Y) DAM1(N80Y)	pDT139	URA3	This study
yMAH1047	scc4-D65Y & DAD1-E50D histone shuffle strain	α	his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 scc4(D65Y) DAD1(E50D)	pDT139	URA3	This study
yMAH1331	wild type histone shuffle strain Nuf2-RFP and Spc110-GFP	α	his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 NUF2::ymScarlet SPC110::mNeonGreen	pDT139	URA3	This study
yMAH1337	scc4-D65Y histone shuffle strain Nuf2-RFP and Spc110-GFP	α	his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 NUF2::ymScarlet SPC110::mNeonGreen scc4(D65Y)	pDT139	URA3	This study
yMAH1224	DAD1-E50D histone shuffle strain Nuf2-RFP and Spc110-GFP	α	his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 NUF2::ymScarlet SPC110::mNeonGreen DAD1(E50D)	pDT139	URA3	This study
yMAH1233	SPC105-R583S histone shuffle strain Nuf2-RFP and Spc110-GFP	α	his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 NUF2::ymScarlet SPC110::mNeonGreen SPC105(R583S)	pDT139	URA3	This study
yGOL023	mad3 deletion strain	a	leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 mad3Δ0			This study
yGOL024	mad3 deletion strain	a	leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 mad3Δ0			This study
yGOL027	ipl1-2 ts mutant strain	a	leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 ipl1-2(H352Y)			This study
yGOL028	ipl1-2 ts mutant strain	a	leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 ipl1-2(H352Y)			This study

yGOL029	<i>mad3</i> deletion strain with <i>dad-1E50D</i> mutation	a	<i>leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 DAD1(E50D) mad3Δ0</i>			This study
yGOL030	<i>mad3</i> deletion strain with <i>DAD1-E50D</i> mutation	a	<i>leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 DAD1(E50D) mad3Δ0</i>			This study
yGOL033	<i>ipl1-2 ts</i> mutant strain with <i>DAD1-E50D</i> mutation	a	<i>leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 DAD1(E50D) ipl1-2(H352Y)</i>			This study
yGOL034	<i>ipl1-2 ts</i> mutant strain with <i>DAD1-E50D</i> mutation	a	<i>leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 DAD1(E50D) ipl1-2(H352Y)</i>			This study
yGOL039	<i>ipl1-2 ts</i> mutant strain with <i>NDC80-E568A</i> mutation	a	<i>leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 NDC80-E568A ipl1-2(H352Y)</i>			This study
yGOL040	<i>ipl1-2 ts</i> mutant strain with <i>NDC80-E568A</i> mutation	a	<i>leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 NDC80-E568A ipl1-2(H352Y)</i>			This study
yGOL045	<i>ipl1-2 ts</i> mutant strain with <i>SPC105-R583S</i> mutation	a	<i>leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 SPC105-R583S ipl1-2(H352Y)</i>			This study
yGOL046	<i>ipl1-2 ts</i> mutant strain with <i>SPC105-R583S</i> mutation	a	<i>leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 SPC105-R583S ipl1-2(H352Y)</i>			This study
yGOL051	<i>ipl1-2 ts</i> mutant strain with <i>SPC34-L63Q</i> mutation	a	<i>leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 SPC34-L63Q ipl1-2(H352Y)</i>			This study
yGOL052	<i>ipl1-2 ts</i> mutant strain with <i>SPC34-L63Q</i> mutation	a	<i>leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 SPC34-L63Q ipl1-2(H352Y)</i>			This study
yGOL057	<i>ipl1-2 ts</i> mutant strain with <i>DAM1-N80Y</i> mutation	a	<i>leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 DAM1-N80Y ipl1-2(H352Y)</i>			This study
yGOL058	<i>ipl1-2 ts</i> mutant strain with <i>DAM1-N80Y</i> mutation	a	<i>leu2Δ0 met15Δ0 ura3Δ0 his3Δ1 DAM1-N80Y ipl1-2(H352Y)</i>			This study
yGOL015	<i>mad3</i> deletion shuffle strain	α	<i>his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 mad3Δ</i>	pDT139	URA3	This study
yGOL016	<i>mad3</i> deletion shuffle strain	α	<i>his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 mad3Δ</i>	pDT139	URA3	This study
yMAH955	<i>DAM1</i> residue 80 mutant shuffle strain <i>DAM1-N80C</i>	α	<i>his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 DAM1(N80C)</i>	pDT139	URA3	This study
yMAH958	<i>DAM1</i> residue 80 mutant shuffle strain <i>DAM1-N80G</i>	α	<i>his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 DAM1(N80G)</i>	pDT139	URA3	This study
yMAH961	<i>DAM1</i> residue 80 mutant shuffle strain <i>DAM1-N80I</i>	α	<i>his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 DAM1(N80I)</i>	pDT139	URA3	This study
yMAH964	<i>DAM1</i> residue 80 mutant shuffle strain <i>DAM1-N80M</i>	α	<i>his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 DAM1(N80M)</i>	pDT139	URA3	This study
yMAH967	<i>DAM1</i> residue 80 mutant shuffle strain <i>DAM1-N80R</i>	α	<i>his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 DAM1(N80R)</i>	pDT139	URA3	This study
yMAH969	<i>DAM1</i> residue 80 mutant shuffle strain <i>DAM1-N80D</i>	α	<i>his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 DAM1(N80D)</i>	pDT139	URA3	This study
yMAH971	<i>DAM1</i> residue 80 mutant shuffle strain <i>DAM1-N80S</i>	α	<i>his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 DAM1(N80S)</i>	pDT139	URA3	This study
yMAH972	<i>DAM1</i> residue 80 mutant shuffle strain <i>DAM1-N80A</i>	α	<i>his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 DAM1(N80A)</i>	pDT139	URA3	This study
yMAH973	<i>DAM1</i> residue 80 mutant shuffle strain <i>DAM1-N80V</i>	α	<i>his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 DAM1(N80V)</i>	pDT139	URA3	This study
yMAH975	<i>DAM1</i> residue 80 mutant shuffle strain <i>DAM1-N80L</i>	α	<i>his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 DAM1(N80L)</i>	pDT139	URA3	This study
yMAH976	<i>DAM1</i> residue 80 mutant shuffle strain <i>DAM1-N80W</i>	α	<i>his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 DAM1(N80W)</i>	pDT139	URA3	This study
yMAH978	<i>DAM1</i> residue 80 mutant shuffle strain <i>DAM1-N80H</i>	α	<i>his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 DAM1(N80H)</i>	pDT139	URA3	This study
yMAH980	<i>DAM1</i> residue 80 mutant shuffle strain <i>DAM1-N80F</i>	α	<i>his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hta2-htb2Δ0 hta1-htb1Δ0 hht1-hhf1Δ0 hht2-hhf2Δ0 DAM1(N80F)</i>	pDT139	URA3	This study

yMAH987	DAM1 residue 80 mutant shuffle strain DAM1-N80E	α	his3 Δ 200 leu2 Δ 0 lys2 Δ 0 trp1 Δ 63 ura3 Δ 0 met15 Δ 0 hta2-htb2 Δ 0 hta1-htb1 Δ 0 hht1-hhf1 Δ 0 hht2-hhf2 Δ 0 DAM1(N80E)	pDT139	URA3	This study
yMAH995	DAM1 residue 80 mutant shuffle strain DAM1-N80T	α	his3 Δ 200 leu2 Δ 0 lys2 Δ 0 trp1 Δ 63 ura3 Δ 0 met15 Δ 0 hta2-htb2 Δ 0 hta1-htb1 Δ 0 hht1-hhf1 Δ 0 hht2-hhf2 Δ 0 DAM1(N80T)	pDT139	URA3	This study
yMAH998	DAM1 residue 80 mutant shuffle strain DAM1-N80Q	α	his3 Δ 200 leu2 Δ 0 lys2 Δ 0 trp1 Δ 63 ura3 Δ 0 met15 Δ 0 hta2-htb2 Δ 0 hta1-htb1 Δ 0 hht1-hhf1 Δ 0 hht2-hhf2 Δ 0 DAM1(N80Q)	pDT139	URA3	This study
yMAH1248	DAD1-E50D DAM1-N80Q histone shuffle strain	α	his3 Δ 200 leu2 Δ 0 lys2 Δ 0 trp1 Δ 63 ura3 Δ 0 met15 Δ 0 hta2-htb2 Δ 0 hta1-htb1 Δ 0 hht1-hhf1 Δ 0 hht2-hhf2 Δ 0 DAD1(E50D) DAM1(N80Q)	pDT139	URA3	This study
yMAH1063	DAD1-E50A histone shuffle strain	α	his3 Δ 200 leu2 Δ 0 lys2 Δ 0 trp1 Δ 63 ura3 Δ 0 met15 Δ 0 hta2-htb2 Δ 0 hta1-htb1 Δ 0 hht1-hhf1 Δ 0 hht2-hhf2 Δ 0 DAD1(E50A)	pDT139	URA3	This study
yMAH1074	DAD1-E50A DAM1-N80Y histone shuffle strain	α	his3 Δ 200 leu2 Δ 0 lys2 Δ 0 trp1 Δ 63 ura3 Δ 0 met15 Δ 0 hta2-htb2 Δ 0 hta1-htb1 Δ 0 hht1-hhf1 Δ 0 hht2-hhf2 Δ 0 DAD1(E50A) DAM1(N80Y)	pDT139	URA3	This study
yJL332	SK1 WT diploid	a / α	ho::LYS2/ho::LYS2, lys2/lys2, ura3/ura3, leu2::hisG/leu2::hisG, his3::hisG/his3::hisG, trp1::hisG/his3::hisG			Luo et al.
yMAH1323	SK1 DAD1-E50D/WT het. diploid	a / α	ho::LYS2/ho::LYS2, lys2/lys2, ura3/ura3, leu2::hisG/leu2::hisG, his3::hisG/his3::hisG, trp1::hisG/his3::hisG, DAD1/DAD1(E50D)			This study
yMAH1356	SK1 DAD1-E50D/DAD1-E50D hom. diploid	a / α	ho::LYS2/ho::LYS2, lys2/lys2, ura3/ura3, leu2::hisG/leu2::hisG, his3::hisG/his3::hisG, trp1::hisG/his3::hisG, DAD1(E50D)/DAD1(E50D)			This study
yDT67	wild type histone shuffle strain	α	his3 Δ 200 leu2 Δ 0 lys2 Δ 0 trp1 Δ 63 ura3 Δ 0 met15 Δ 0 hta2-htb2 Δ 0 hta1-htb1 Δ 0 hht1-hhf1 Δ 0 hht2-hhf2 Δ 0	pDT105	TRP1	Truong and Boeke.
yDT180	DAD1(E50D) hH3.1-core histone humanized	α	his3 Δ 200 leu2 Δ 0 lys2 Δ 0 trp1 Δ 63 ura3 Δ 0 met15 Δ 0 hta2-htb2 Δ 0 hta1-htb1 Δ 0 hht1-hhf1 Δ 0 hht2-hhf2 Δ 0 DAD1(E50D)	pDT109	TRP1	Truong and Boeke.
BY4742	Wildtype MAT α	α	MAT α his3 Δ 1 leu2 Δ 0 lys2 Δ 0 ura3 Δ 0			Brachmann CB, et al.
Notes						
	Haase et al.; https://doi.org/10.1534/g3.119.400325					
	Luo et al.; https://doi.org/10.1038/s41586-018-0374-x					
	Truong and Boeke.; https://doi.org/10.1016/j.cell.2017.10.043					
	Brachmann CB, et al.; <a href="https://doi.org/10.1002/(SICI)1097-0061(19980130)14:2<115::AID-YEA204>3.0.CO;2-2">https://doi.org/10.1002/(SICI)1097-0061(19980130)14:2<115::AID-YEA204>3.0.CO;2-2					
	*Detailed genotypes can be found in Supplemental table 2					

Appendix Table S4. Plasmids used in this study.				
Plasmid	Name	markers	Description	Source
yeast histone plasmid	pDT139	KAN URA3	Superloser plasmid with four core histone genes (HTA2-HTB2-HHT1-HHF1)	Haase et al. 2019
human histone plasmid	pDT109	AMP / TRP1	pRS414 with human core histones (H3.1 H4 H2A H2B) with HHT2F2HTA1B1 PROs/TERs	Truong and Boeke. 2017
LEU2 guide RNA expression vector	pNA525	AMP / LEU2	p425-SNR52p-NotI(gRNA)-SUP4t - NotI site for inserting any gRNA using Gibson Assembly.	DiCarlo et al. 2013
Cas9 vector	pNA0519	AMP / HIS	pRS413-TEF1p-Cas9-CYC1t - Cas9 fragment cloned into pRS413	DiCarlo et al. 2013
DAM1 guide RNA plasmid	pMAH298	AMP / LEU2	guide RNA targeting the Dam1 locus at codon 88	this study
SPC34 guide RNA plasmid	pMAH300	AMP / LEU2	guide RNA targeting the Spc34 locus at codon 59	this study
SPC105 guide RNA plasmid	pMAH301	AMP / LEU2	guide RNA targeting the Spc105 locus at codon 583	this study
DAD4 guide RNA plasmid	pMAH302	AMP / LEU2	guide RNA targeting the Dad4 locus at codon 62	this study
NDC80 guide RNA plasmid	pMAH299	AMP / LEU2	guide RNA targeting the Ndc80 locus at codon 618	this study
DAD1 guide RNA plasmid	pMAH604	AMP / LEU2	guide RNA targeting the Dad1 locus at codon 49	this study
SCC4 guide RNA plasmid	pMAH606	AMP / LEU2	guide RNA targeting the Scc4 locus at codon 64	this study
NUF2 guide RNA plasmid	pMAH441	AMP / LEU2	guide RNA targeting the 3' end of the Nuf2 locus	this study
SPC110 guide RNA plasmid	pMAH439	AMP / LEU2	guide RNA targeting the 3' end of the Spc110 locus	this study
SPC110-mNeonGreen repair template	pMAH460	AMP	pUC19-Spc110-mNeonGreen targeting repair template.	this study
NUF2-ymScarlet repair template	pMAH462	AMP	pUC19-NUF2-ymScarlet targeting repair template.	this study
IPL1 guide RNA plasmid	pMAH328	AMP / LEU2	guide RNA targeting the Ipl1 locus at codon 345	this study
MAD3 guide RNA plasmid	pMAH512	AMP / LEU2	guide RNA targeting the Mad3 locus at codon 285	this study
Dam1 complex expression vector	pJT44	AMP	Two polycistrons containing Dad1, Duo1, Spc34-FLAG, Dam1, Hsk3 and Dad4, Dad3, Dad2, Spc19, Ask1	Umbreit et al.2014
Dam1 complex DAD1E50D expression vector	pMAH359	AMP	Two polycistrons containing Dad1(E50D), Duo1, Spc34-FLAG, Dam1, Hsk3 and Dad4, Dad3, Dad2, Spc19, Ask1	this study
pRS416-DAD1-E50D	pMAH490	AMP / URA3	pRS416 with Dad1(E50D) - with native promoter/terminator	this study
pRS416-DAD1	pMAH492	AMP / URA3	pRS416 with Dad1 - with native promoter/terminator	this study

Appendix Table S5. DNA oligos used in this study.				
Name	oligo	sequence 5' -> 3'	notes	source
gRNA-DAM1	oMH876	<u>TGAAAGATAAATGATCTAAGATCAGCTAAACTCTCGGTTTTAGAGCTAGAAA</u>	sgRNA Dam1; flanking homology for subcloning into NotI site on guide expression plasmid	this study
Dam1-N80Y-F	oMH877	CTGAGCGATTCAATCATTACTTTAGATTCTTATTTACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo	this study
Dam1-N80Y-R	oMH878	ACAACAACGAACCTAGGGATTCAATTAAGATCAGCTAAACTCTCGTGATAAAAATTT AAGC	Repair template Reverse oligo	this study
gRNA-NDC80	oMH881	<u>TGAAAGATAAATGATCAACTAAAATTTTCAGATGCTGGTTTTAGAGCTAGAAA</u>	sgRNA Ndc80; flanking homology for subcloning into NotI site on guide expression plasmid	this study
gRNA-SPC34	oMH882	<u>TGAAAGATAAATGATCAAAGGACTGTAACCCAGACGGTTTTAGAGCTAGAAA</u>	sgRNA Spc34; flanking homology for subcloning into NotI site on guide expression plasmid	this study
gRNA-DAD4	oMH905	<u>TGAAAGATAAATGATCTAGTAGCGTCCAATTCAACCGTTTTAGAGCTAGAAA</u>	sgRNA Dad4; flanking homology for subcloning into NotI site on guide expression plasmid	this study
gRNA-DAD1	oMH1392	<u>TGAAAGATAAATGATCGAATGGTCTCAATATATCATGTTTTAGAGCTAGAAA</u>	sgRNA Dad1; flanking homology for subcloning into NotI site on guide expression plasmid	this study
gRNA-SPC105	oMH893	<u>TGAAAGATAAATGATCCAATGGAATGGTATGAATGGGTTTTAGAGCTAGAAA</u>	sgRNA Spc105; flanking homology for subcloning into NotI site on guide expression plasmid	this study
NDC80-E568A-F	oMH879	GCATGACATAAATGAGAAAACCTCAAATTAATGAAAACTTGCATTGG	Repair template Forward oligo	this study
NDC80-E568A-R	oMH880	CTTCAAATTTTAGTTCCGTTGAAGTTACCAATTTTCAGCATCTGAA	Repair template Reverse oligo	this study
SPC34-L63Q-F	oMH883	AGCATTAGAAAAGACATTACTAGACTAATAAAGGACTGTAACCACGACGAAGCGTA TCTT	Repair template Forward oligo	this study
SPC34-L63Q-R	oMH884	TACGTGAAACGGATTGTTTCTCAGGGTTGACTTTGAAGTGAAGATACGCTTCGTCG TGGT	Repair template Reverse oligo	this study
SPC105-R583S-F	oMH894	TGGTCAAAGGATATTCGAAATTGGAGGCAGCAATGGAATGGTATGAATGGAGCAA AAAGC	Repair template Forward oligo	this study
SPC105-R583S-R	oMH895	TTCAAATTTTCTGCCAAAATTAATCCAGACCATTTAGTTGCTTTTTGCTCCATTCAT AC	Repair template Reverse oligo	this study
DAD4-N61K-F	oMH906	ATCATGGGCGCAATTTGTGATAACTACCATAGTAGCGTCCAATTCAAGCTAGAAGC GACC	Repair template Forward oligo	this study
DAD4-N61K-R	oMH907	ATAGAAAATTGGTGAATTAAAGAGGTGGCTTCTTGTATTGGTCGCTTCTAGCTTG AATT	Repair template Reverse oligo	this study
DAD1-E50D-F	oMH636	AAATCAGAAACTGACTGGAATTTCTTCCACAGCTATCGAGCTATCTAATGATAT ATTG	Repair template Forward oligo	this study
DAD1-E50D-R	oMH637	ATGAGACTATGAATCAATCTTGAATGGTCTGAATGGTCTCAATATATCATTAGATA GCT	Repair template Reverse oligo	this study
gRNA-SCC4	oMH1420	<u>TGAAAGATAAATGATCATGTACGCTTTCAGTTCTGGGTTTTAGAGCTAGAAA</u>	sgRNA Scc4; flanking homology for subcloning into NotI site on guide expression plasmid	this study
SCC4-D65Y-F	oMH1418	TATCCAGATGTTTCAGCTATTGAAAACGAAATGTACGCTTTCAGTTCTGGAGTATA GTAA	Repair template Forward oligo	this study
SCC4-D65Y-R	oMH1419	CGTCTCCTGTATCAATAACTCCACCATCTCAAACGTGACCTTACTATACTCCAGAA CTGA	Repair template Reverse oligo	this study
UBC6 F RT-PCR	oMH1571	ACAAGGGCGGTCAATATCACG		Ling and Yuen. PNAS 2019
UBC6 R RT-PCR	oMH1572	TGGGCTTGAAACGTCCATTCCG		Ling and Yuen. PNAS 2019
CLN2 F RT-PCR	oMH1573	ATGCTGCAAGAATACCACCAA		Ling and Yuen. PNAS 2019

CLN2 R RT-PCR	oMH1574	TCATTTTCAGGCTGCTGGTCTA		Ling and Yuen. PNAS 2019
CEN16 F RT-PCR	oMH1577	AAAGGTTGAAGCCGTTATGTTGTCCG		Ling and Yuen. PNAS 2019
CEN16 R RT-PCR	oMH1578	TTAGCCGCTTTGCCGATTTTCGC		Ling and Yuen. PNAS 2019
CEN6 F RT-PCR	oMH1579	TTTTGTTTTCCGAAGATGTAAAATAGGTTG		Ling and Yuen. PNAS 2019
CEN6 R RT-PCR	oMH1580	GTTCTGCTTTCCCTCCCAAACAG		Ling and Yuen. PNAS 2019
CEN1 F RT-PCR	oMH1581	TGTAATGATTTAAGTCTTGTCACATGA		Ling and Yuen. PNAS 2019
CEN1 R RT-PCR	oMH1582	AAAATACTTTGACTGCTTCGGAA		Ling and Yuen. PNAS 2019
CEN7 F RT-PCR	oMH1583	TTCATGGAAATTTGCTTCTTGAGC		Ling and Yuen. PNAS 2019
CEN7 R RT-PCR	oMH1584	TCCAATACTTTGTCGTCAATATTCTTTC		Ling and Yuen. PNAS 2019
CEN3 F RT-PCR	oMH1585	GGAAAATCCACAGAAAGCTATTCA		Ling and Yuen. PNAS 2019
CEN3 R RT-PCR	oMH1586	CCACCAGTAAACGTTTCATATATCCA		Ling and Yuen. PNAS 2019
gRNA-NUF2-3'	oMH1089	<u>TGAAAGATAAATGATCTCGAATATATGCAATAGCAGGTTTTAGAGCTAGAAA</u>	sgRNA Nuf2 (3' end, near stop); flanking homology for subcloning into NotI site on guide expression plasmid	this study
gRNA-SPC110-3'	oMH1098	<u>TGAAAGATAAATGATCATAGAATTGAGAGTAGCAGCGTTTTAGAGCTAGAAA</u>	sgRNA Spc110 (3' end, near stop); flanking homology for subcloning into NotI site on guide expression plasmid	this study
gRNA-IPL1	oMH718	<u>TGAAAGATAAATGATCGATGCATTTTTACGTCTCCAGTTTTAGAGCTAGAAA</u>	sgRNA Ipl1; flanking homology for subcloning into NotI site on guide expression plasmid	this study
IPL1-H352Y-F	oMH719	TTAAACTACTAAAATACGACCCCAAAGATAGAATGCGTCTTGGAGACGTAAAAATG TATC	Repair template Forward oligo	this study
IPL1-H352Y-R	oMH720	CGCTTATTTCCCAAAGGGCTTGTCTTAGTATCCAAGGATACATTTTTACGTCT CCA	Repair template Reverse oligo	this study
gRNA-MAD3	oMH1315	<u>TGAAAGATAAATGATCGAGGTGTTTATAGAGATGGCGTTTTAGAGCTAGAAA</u>	sgRNA Mad3; flanking homology for subcloning into NotI site on guide expression plasmid	this study
MAD3-Δ-F	oMH1316	ATAGTAAACAAAATCATGCGAAAATACAATAAAGACGTTAACTTGATAGAAATAAA ACT	Repair template Forward oligo	this study
MAD3-Δ-R	oMH1317	TGTTTACGATTGGCCAGTATACTTACTCATTGATTGATTGTTTATTTCTATCAA GTT	Repair template Reverse oligo	this study
Dam1-N80A-F	oMH1343	CTGAGCGATTCAATCATTACTTTAGATTCCGCTTTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
Dam1-N80C-F	oMH1344	CTGAGCGATTCAATCATTACTTTAGATTCTGTTTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
Dam1-N80G-F	oMH1345	CTGAGCGATTCAATCATTACTTTAGATTCCGTTTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study

Dam1-N80I-F	oMH1346	CTGAGCGATTCAATCATTACTTTAGATTCCATTTTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
Dam1-N80P-F	oMH1347	CTGAGCGATTCAATCATTACTTTAGATTCCCCATTACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
Dam1-N80S-F	oMH1348	CTGAGCGATTCAATCATTACTTTAGATTCTCTTTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
Dam1-N80T-F	oMH1349	CTGAGCGATTCAATCATTACTTTAGATTCCACTTTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
Dam1-N80V-F	oMH1350	CTGAGCGATTCAATCATTACTTTAGATTCCGTTTTTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
Dam1-N80D-F	oMH1351	CTGAGCGATTCAATCATTACTTTAGATTCCGATTTTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
Dam1-N80E-F	oMH1352	CTGAGCGATTCAATCATTACTTTAGATTCCGAATTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
Dam1-N80K-F	oMH1353	CTGAGCGATTCAATCATTACTTTAGATTCCAAATTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
Dam1-N80L-F	oMH1354	CTGAGCGATTCAATCATTACTTTAGATTCCCTTGTTTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
Dam1-N80M-F	oMH1355	CTGAGCGATTCAATCATTACTTTAGATTCCATGTTTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
Dam1-N80Q-F	oMH1356	CTGAGCGATTCAATCATTACTTTAGATTCCCAATTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
Dam1-N80R-F	oMH1357	CTGAGCGATTCAATCATTACTTTAGATTCCAGATTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
Dam1-N80F-F	oMH1358	CTGAGCGATTCAATCATTACTTTAGATTCTTTTTTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
Dam1-N80H-F	oMH1359	CTGAGCGATTCAATCATTACTTTAGATTCCCATTTTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
Dam1-N80W-F	oMH1360	CTGAGCGATTCAATCATTACTTTAGATTCTGGTTTCACGCGCTTAAATTTTATACAC GAG	Repair template Forward oligo; used with oMH878	this study
DAD1-E50A-F	oMH1393	GAGACTATGAATTC AATCTTGAATGGTCTGAATGGTCTCAATATATCATTAGCTAG CTCG	Repair template Forward oligo	this study
DAD1-E50A-R	oMH1394	ACAAATCAGAAACTGACTGGAATTCCTTCCACAGCTATCGAGCTAGCTAATGAT ATAT	Repair template Reverse oligo	this study
Forward PCRtag for HTA2	oMH308	AGGTGGTAAAGCTGGTTCAGC	histone genotyping	Haase et al. G3 2019
Reverse PCRtag for HTA2	oMH309	CTCAGTTTCTTACAGTTCTTGAGAAGC	histone genotyping	Haase et al. G3 2019
Forward PCRtag for HTB2	oDT133	GGTAACAGCTCTAGTACCTTCAGAG	histone genotyping	Haase et al. G3 2019
Reverse PCRtag for HTB2	oDT134	GCCGAAAAGAAACCAGC	histone genotyping	Haase et al. G3 2019
Forward PCRtag for HHT1	oDT484	GCTGCCAGAAAATCCGCC	histone genotyping	Haase et al. G3 2019
Reverse PCRtag for HHT1	oDT557	GCCAACTTGATATCCTTCTTTGGATAGT	histone genotyping	Haase et al. G3 2019
Forward PCRtag for HHF1	oDT488	AGAGGTAAAGGTGGTAAAGGTCTA	histone genotyping	Haase et al. G3 2019

Reverse PCRtag for HHF1	oDT567	GGATTTCAAGACNGCTCTGAC	histone genotyping	Haase et al. G3 2019
Forward PCRtag for hH2A.1	oMH263	CGAATCTCACCACAAGGCTAAGGG	histone genotyping	Haase et al. G3 2019
Reverse PCRtag for hH2A.1I	oMH243	GTTGTGTGGAATTGTGAGCGGATAAC	histone genotyping	Haase et al. G3 2019
Forward PCRtag for hH2B.3J	oMH264	CTCCAGCTCCAAGAAGGGTTC	histone genotyping	Haase et al. G3 2019
Reverse PCRtag for hH2B.3J	oMH265	GCTTGATATCGAATTCCTGCAGCC	histone genotyping	Haase et al. G3 2019
Forward PCRtag for hH3.1	oMH262	ATGGCTTTGCAAGAGGCCTG	histone genotyping	Haase et al. G3 2019
Reverse PCRtag for hH3.1	oMH242	GCTGGCGAAAGGGGGATG	histone genotyping	Haase et al. G3 2019
Forward PCRtag for hH4	oDT565	GGTGGTAAGGGTTTGGGTAAG	histone genotyping	Haase et al. G3 2019
Reverse PCRtag for hH4	oDT566	GAAAACCTTCAAAACACCTCTGGT	histone genotyping	Haase et al. G3 2019

<i>Appendix Table S6. Sporulation in Dad1-E50D diploids</i>				
Genotype	2 spores	3 spores	Immature	Total
<i>WT</i>	0.85	0.85	3.42	117
<i>HET Dad1-E50D</i>	4.96	5.51	23.69	363
<i>HOMO Dad1-E50D</i>	6.92	6.92	15.38	130

Values are % of total spores after 5 days in sporulation medium.