

Table S1. Strains used in this study

Strain	Genotype	Figure
AP152	<i>mat1M-smf0 KΔ::ade6⁺ his2 leu1-32 ade6-210 ura4-D18</i>	4A
SP1152	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ ade6-210 ura4-D18</i>	2C; S2D
SPA03	<i>mat1M-smf0 his2 leu1-32 ura4 ade6-216 otr1R:ura4⁺ pds5::KAN</i>	3A, B; S3
SPA10	<i>h⁺ leu1-32 ade6 ura4-D18 otr1R:ura4⁺ pds5::KAN ago1::KAN</i>	S3
SPA13	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-216 ura4-D18 pds5::KAN</i>	2B; 3C; 7B
SPA15	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-210 ura4-D18 rpl42(sP56Q)-CYH pds5::KAN</i>	1D, E; 2B, C; 3C; 6A; 7A, D, F; S2D; S5
SPA17	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-216 ura4 rpl42(sP56Q)-CYH pds5::KAN ago1::KAN</i>	S2D
SPA20	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-210 ura4-D18 rpl42(sP56Q)-CYH pds5::KAN</i>	2C
SPA21	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-210 ura4-D18 rpl42(sP56Q)-CYH pds5::KAN</i>	2C
SPA24	<i>mat1M-smf0 his2 leu1-32 ura4-DSE ade6-210 otr1R:ura4⁺ pds5-GFP>KAN</i>	5; S4
SPA52	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT rpl42(sP56Q)-CYH ade6-216 ura4-D18 psc3-4T-GFP>KAN</i>	7A
SPA61	<i>mat1M-smf0 his2 leu1-32 ura4-DSE ade6-216 otr1R:ura4⁺ pds5-GFP>KAN swi6::NAT</i>	5
SPA71	<i>mat1M-smf0 his2 leu1-32 ura4-DSE ade6-210 otr1R:ura4⁺ pds5-GFP>KAN clr4::KAN</i>	5
SPA78	<i>h90 his2 leu1-32 ade6-216 ura4 Kint2:ura4⁺ pds5::KAN</i>	S2A, C
SPA79	<i>h90 his2 leu1-32 ade6-216 ura4 Kint2:ura4⁺ pds5::KAN</i>	S2A
SPA82	<i>h90 his2 leu1-32 ade6-216 ura4 Kint2:ura4⁺</i>	S2A, C
SPA83	<i>h90 his2 leu1-32 ade6-210 ura4 Kint2:ura4⁺</i>	S2A
SPDF662	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT ura4-D18 ade6-210</i>	2B; 3C; 7B
SPDF734	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT rpl42(sP56Q)-CYH ade6-210 ura4-D18</i>	1D, E; 2A, B; 3C; 6A; 7A, C, E; S1B; S5
SPDF735	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT rpl42(sP56Q)-CYH ade6-210 ura4</i>	2B; 3C; 7E
SPDF1223	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ ura4-D18 ade6-210 dhml1::HYG</i>	1D, E; S1B
SPDF1417	<i>mat1M-smf0 his2 leu1 ura4 otr1R:ura4⁺ rad21-GFP<LEU2</i>	S4
SPDF2044	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ leu1-32 his2::NAT rpl42(sP56Q)-CYH ade6-216 ura4-D18 rpn10::KAN</i>	1D, E
SPDF2068	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ leu1-32 his2::NAT rpl42(sP56Q)-CYH ade6-216 ura4-D18 rpl39::KAN</i>	1D, E; S1B
SPDF2080	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-216 ura4-D18 rpl2102::KAN</i>	1D, E; S1B
SPDF2084	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-210 ura4-D18 rpl502::KAN</i>	1D, E
SPDF2110	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT ura4-D18 ade6-210 dhm2::KAN</i>	1D, E
SPDF2117	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT ura4-D18 ade6-210 yap18::KAN</i>	1D, E
SPDF2154	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-210 Padh1-rec8-HA>ura4⁺ rad21::ura4⁺ psc3::KAN Padh41-rec11>ura4⁺</i>	7C, D, E
SPDF2157	same as SPDF2154	7B, E
SPDF2176	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-216 ura4-D18 Padh1-rec8-HA>ura4⁺</i>	7C
SPDF2208	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-210 ura4-D18 Padh1-rec8-HA>ura4⁺ rad21::ura4⁺ psc3::KAN Padh41-rec11>ura4⁺ pds5::HYG</i>	7F
SPDF2209	same as SPDF2208	7D, E, F
SPDF2210	same as SPDF2208	7E, F
SPDF2234	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-216 ura4-D18 hrk1::KAN</i>	6A
SPDF2244	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT leu1-32 ade6-210 ura4-D18 rpl42(sP56Q)-CYH pds5::HYG wpl1::KAN</i>	S5
SPDF2246	<i>mat1M-smf0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-210 ura4-D18 pds5::HYG</i>	2B; 3C; 7E, F
SPDF2247	<i>h⁺ leu1-32 ade6-210 ura4-D18 pds5::HYG</i>	4A

SPDF2250	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ his2::NAT rpl42(sP56Q)-CYH ade6-216 ura4-D18 rad21-K1>ura4::HYG</i>	7A
SPDF2257	<i>h90 his2 leu1-32 ade6-210 ura4-DSE Kint2::ura4⁺ ago1::KAN pds5::HYG</i>	S2C
SPDF2293	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ his2::NAT leu1-32 ade6-210 ura4-D18 pds5::HYG</i>	2A; 7E, F
SPDF2294	<i>mat1M-smΔ0 leu1-32 ade6-210 ura4-D18 KΔ::ura4⁺</i>	S2B
SPDF2295	<i>mat1M-smΔ0 leu1-32 ade6-216 ura4-D18 KΔ::ura4⁺ pds5::HYG</i>	S2A, B
SPDF2296	<i>mat1M-smΔ0 leu1-32 ade6-210 ura4-D18 KΔ::ura4⁺ pds5::HYG</i>	S2A, B
SPDF2297	<i>mat1M-smΔ0 leu1-32 ade6-216 ura4-D18 KΔ::ura4⁺</i>	S2A, B
SPDF2298	<i>mat1M-smΔ0 leu1-32 ade6-210 ura4-D18 KΔ::ura4⁺</i>	S2A, B
SPDF2299	<i>mat1M-smΔ0 leu1-32 ade6-216 ura4-D18 KΔ::ura4⁺ pds5::HYG</i>	S2B
SPDF2326	<i>mat1M-smΔ0 KΔ::ade6⁺ his2 leu1-32 ade6-210 ura4-D18</i>	4B
SPDF2327	<i>mat1M-smΔ0 KΔ::ade6⁺ his2 leu1-32 ade6-210 ura4-D18 pds5::HYG</i>	4B
SPDF2328	<i>mat1M-smΔ0 KΔ::ade6⁺ his2 leu1-32 ade6-210 ura4-D18 pds5::HYG</i>	4B
SPDF2329	<i>mat1M-smΔ0 KΔ::ade6⁺ his2 leu1-32 ade6-210 ura4-D18</i>	4B
SPDF2330	<i>mat1M-smΔ0 KΔ::ade6⁺ his2 leu1-32 ade6-210 ura4-D18 pds5::HYG</i>	4B
SPDF2331	<i>mat1M-smΔ0 KΔ::ade6⁺ his2 leu1-32 ade6-210 ura4-D18</i>	4B
SPDF2354	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-210 ura4-D18 psm3(KKQQ)>NAT</i>	6B
SPDF2357	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ his2::NAT leu1 ade6-210 ura4-D18 rpl42(sP56Q)-CYH wpl1::KAN esol::LEU2 psm3(KKQQ)>NAT</i>	6B
SPDF2359	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ his2::NAT leu1 ade6-210 ura4-D18 pds5::HYG esol::LEU2</i>	S5
SPDF2361	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ his2::NAT leu1 ade6-210 ura4-D18 pds5::HYG wpl1::KAN esol::LEU2</i>	S5
SPDF2363	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ his2::NAT leu1 ade6-210 ura4-D18 esol::LEU2 psm3(KKQQ)>NAT</i>	6B
SPDF2390	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ his2::NAT leu1 ade6-210 ura4-D18 wpl1::KAN</i>	6A
SPDF2391	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ his2::NAT leu1 ade6-210 ura4-D18 esol::LEU2 wpl1::KAN</i>	6A, B
SPDF2412	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-210 ura4-D18 pds5::KAN</i>	6B, D
SPDF2413	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ his2::NAT leu1 ade6-210 ura4-D18 rpl42(sP56Q)-CYH pds5::KAN</i>	6C, D
SPDF2414	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-210 ura4-D18 rpl42(sP56Q)-CYH pds5::KAN</i>	6D
SPDF2415	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-210 ura4-D18 pds5::KAN psm3(KKQQ)>NAT</i>	6B, D
SPDF2416	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-210 ura4-D18 pds5::KAN psm3(KKQQ)>NAT</i>	6D
SPDF2417	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-210 ura4-D18 pds5::KAN psm3(KKQQ)>NAT</i>	6C, D
SPF223	<i>mat1M-smΔ0 his2 leu1-32 ura4-DSE ade6-210 otr1R:ura4⁺ ago1::KAN</i>	7B
SPHC221	<i>mat1M-smΔ0 his2 leu1-32 ura4-DSE ade6-210 otr1R:ura4⁺ clr4::KAN</i>	S2D; S3
SPHC316	<i>mat1M-smΔ0 his2 leu1-32 ura4-DSE ade6-210 otr1R:ura4⁺ ago1::KAN</i>	S3
SPK423	<i>h90 his2 leu1-32 ade6-210 ura4-DSE Kint2::ura4⁺ clr4::KAN</i>	S2C
SPNT572	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ ade6-210 ura4 leu1-32 clr4::KAN</i>	1D, E; 2A; 6A; 7A; S1B; S5
SPR156	<i>mat1M-smΔ0 his2 leu1-32 ura4-DSE ade6-210 otr1R:ura4⁺</i>	3A, B; S2D; S3
SPT1294	<i>h90 his2 leu1-32 ade6-210 ura4-DSE Kint2::ura4⁺ ago1::KAN</i>	S2C
SPTA256	<i>mat1M-smΔ0 his2 leu1-32 ura4 ade6 psc3-GFP>KAN</i>	S4
SPVC566	<i>mat1M-smΔ0 REIIΔ mat2P::ura4⁺ his2::NAT ade6-216 ura4-D18 rpl42(sP56Q)-CYH ago1::KAN</i>	S2D

Table S2. Single deletion strains selected for further validation

Plate #	Gene ID	Name	Score ≥ 2	Unpublished	Genotype	Haploid Meiosis $\geq 2\%$	Variegation
V4-P01-35	SPAC10F6.06	<i>vip1</i>	NO				
V4-P01-44	SPAC110.02	<i>pds5</i>	YES	YES	YES	YES	YES
V4-P04-16	SPAC17C9.15c	<i>dhm2</i>	YES	YES	YES	YES	NO
V4-P05-50	SPAC1B3.17	<i>clr2</i>	YES	NO			
V4-P05-73	SPAC1F7.01c	<i>spt6</i>	NO				
V4-P09-03	SPAC31G5.07	<i>dni1</i>	NO				
V4-P10-94	SPAC589.02c	<i>med13</i>	YES	YES	YES	NO	
V4-P11-39	SPAC637.10c	<i>rpn10</i>	YES	YES	YES	YES	YES
V4-P11-48	SPAC664.01c	<i>swi6</i>	YES	NO			
V4-P11-49	SPAC664.02c	<i>arp8</i>	NO				
V4-P11-67	SPAC694.06c	<i>mrc1</i>	YES	NO			
V4-P13-19	SPAC959.08	<i>rpl2102</i>	YES	YES	YES	YES	YES
V4-P13-60	SPAP27G11.10c	<i>nup184</i>	YES	YES	NO		
V4-P14-23	SPAPJ691.02		NO				
V4-P14-47	SPBC1105.02c	<i>lys4</i>	NO				
V4-P14-56	SPBC115.02c	<i>afg1</i>	NO				
V4-P14-83	SPBC11C11.09c	<i>rpl502</i>	YES	YES	YES	YES	YES
V4-P15-78	SPBC15D4.03	<i>slm9</i>	YES	NO			
V4-P16-39	SPBC16C6.10	<i>chp2</i>	YES	NO			
V4-P16-50	SPBC16E9.13	<i>ksp1</i>	NO				
V4-P17-25	SPBC1773.08c	<i>omh4</i>	NO				
V4-P17-96	SPBC19C7.02	<i>ubr1</i>	NO				
V4-P18-09	SPBC19F8.03c	<i>yap18</i>	YES	YES	YES	YES	NO
V4-P19-94	SPBC2D10.17	<i>clr1</i>	YES	NO			
V4-P20-05	SPBC2F12.11c	<i>rep2</i>	NO				
V4-P20-11	SPBC2G2.03c	<i>sbh1</i>	NO				
V4-P20-48	SPBC31F10.13c	<i>hip1</i>	YES	NO			
V4-P21-70	SPBC428.07	<i>meu6</i>	YES	NO			
V4-P21-71	SPBC428.08c	<i>clr4</i>	YES	NO			
V4-P22-44	SPBC609.05	<i>pob3</i>	YES	NO			
V4-P23-09	SPBC800.03	<i>clr3</i>	YES	NO			
V4-P24-03	SPBP35G2.10	<i>mit1</i>	YES	NO			
V4-P24-11	SPBP4H10.05c	<i>spe2</i>	NO				
V4-P24-87	SPCC11E10.08	<i>rik1</i>	YES	NO			
V4-P25-47	SPCC1393.09c	<i>gir2</i>	YES	YES	NO		
V4-P27-34	SPCC338.16	<i>pof3</i>	YES	NO			
V4-P28-12	SPCC63.06		YES	YES	YES	NO	
V4-P28-23	SPCC663.04	<i>rpl39</i>	YES	YES	YES	YES	YES
V4-P29-18	SPCP1E11.10	<i>dhm1</i>	YES	YES	YES	YES	YES
V4-P29-60	SPAC12G12.15	<i>sif3</i>	NO				
V4-P30-77	SPAC25A8.01c	<i>fft3</i>	YES	NO			
V4-P30-78	SPAC25B8.05	<i>deg1</i>	NO				

V4-P31-02	SPAC29A4.18	<i>prw1</i>	NO		
V4-P31-62	SPAC4F10.13c	<i>mpd2</i>	NO		
V4-P32-67	SPBC1347.02	<i>ani1</i>	YES	NO	
V4-P33-51	SPBC21D10.10	<i>bdc1</i>	NO		
V4-P34-25	SPBC3H7.05c		NO		
V4-P34-46	SPBC56F2.02	<i>rpl1901</i>	NO		
V4-P35-10	SPCC1223.15c	<i>spc19</i>	YES	YES	NO
V4-P35-73	SPCC24B10.04		NO		
V4-P36-45	SPCC613.12c	<i>raf1</i>	YES	NO	
V4-P36-69	SPCC970.07c	<i>raf2</i>	YES	NO	

Table S3. Results of validation assays

Plate	ID	Name	Score	Iodine staining*		Growth	
				5S	–URA	+FOA**	–URA***
V4-P05-50	SPAC1B3.17	<i>clr2</i>	4	1	1	1	1
V4-P11-48	SPAC664.01c	<i>swi6</i>	4	1	1	1	1
V4-P16-39	SPBC16C6.10	<i>chp2</i>	4	1	1	1	1
V4-P19-94	SPBC2D10.17	<i>clr1</i>	4	1	1	1	1
V4-P21-71	SPBC428.08c	<i>clr4</i>	4	1	1	1	1
V4-P23-09	SPBC800.03	<i>clr3</i>	4	1	1	1	1
V4-P24-03	SPBP35G2.10	<i>mit1</i>	4	1	1	1	1
V4-P24-87	SPCC11E10.08	<i>rik1</i>	4	1	1	1	1
V4-P36-45	SPCC613.12c	<i>raf1</i>	4	1	1	1	1
V4-P36-69	SPCC970.07c	<i>raf2</i>	4	1	1	1	1
V4-P13-19	SPAC959.08	<i>rpl2102</i>	3.5	1	1	0.5	1
V4-P21-70	SPBC428.07	<i>meu6</i>	3.5	1	1	0.5	1
V4-P29-18	SPCP1E11.10	<i>dhm1</i>	3.5	1	1	0.5	1
V4-P04-16	SPAC17C9.15c	<i>dhm2</i>	3	1	1	0	1
V4-P28-23	SPCC663.04	<i>rpl39</i>	3	0.5	1	1	0.5
V4-P30-77	SPAC25A8.01c	<i>fft3</i>	3	0.5	1	0.5	1
V4-P01-44	SPAC110.02	<i>pds5</i>	2.5	0.5	1	0.5	0.5
V4-P11-67	SPAC694.06c	<i>mrc1</i>	2.5	0.5	1	0	1
V4-P15-78	SPBC15D4.03	<i>slm9</i>	2.5	0.5	0.5	1	0.5
V4-P20-48	SPBC31F10.13c	<i>hip1</i>	2.5	0.5	1	0.5	0.5
V4-P28-12	SPCC63.06		2.5	0.5	1	0	1
V4-P22-44	SPBC609.05	<i>pob3</i>	2.5	0.5	0.5	0.5	1
V4-P32-67	SPBC1347.02	<i>ani1</i>	2.5	0.5	1	0	1
V4-P10-94	SPAC589.02c	<i>med13</i>	2	0	1	0	1
V4-P11-39	SPAC637.10c	<i>rpn10</i>	2	0	1	0	1
V4-P14-83	SPBC11C11.09c	<i>rpl502</i>	2	0	1	0.5	0.5
V4-P18-09	SPBC19F8.03c	<i>yap18</i>	2	0.5	1	0	0.5
V4-P27-34	SPCC338.16	<i>pof3</i>	2	0.5	0.5	0	1

NB: Only factors with scores ≥ 2 are shown.

* Iodine staining in PMG 5S and PMG –URA was scored as follows: 1=black; 0.5=brown/variegated; 0=yellow.

** Growth in PMG +FOA was scored as follows: 1=none; 0.5=medium; 0=full.

** Growth in PMG –URA was scored as follows: 1=full; 0.5=medium; 0=none.

Table S4. Oligonucleotides used in this study

PCR product	Sequence 5'–3'	Application
<i>act1</i>	TCATCAGCCATTCTTTTCGTC ACACATTCAACCATTTGGCTTC	RT-qPCR
<i>ade6</i>	CCTGCTCGTCTTCCCTTCTC CGAGGAGCAATTCGTTGAG	ChIP-qPCR
<i>cenH</i>	AGTAATTCTATGTACCCGTGC CGTCGTCACCAATCGATCTT	ChIP-qPCR & RT-qPCR
<i>cc</i>	CAGACAATCGCATGGTACTATC AGGTGAAGCGTAAGTGAGTG	ChIP-qPCR
<i>chrIII-0.36Mb</i>	TAGCTTCGGAAGGATGGAAC TTTCGGTACGCGACACTCTC	ChIP-qPCR
<i>dg</i>	AATTGTGGTGGTGTGGTAATAC GGGTTCATCGTTTCCATTGAG	ChIP-qPCR & RT-qPCR
<i>fbp1</i>	AATGACAATTCCCCACTAGCC ACTTCAGCTAGGATTCACCTGG	ChIP-qPCR
<i>leu1</i>	CTGGCAAGGGCATTGTTAAT CCACGTCCACCAATTGAAGT	RT-qPCR
<i>mat2-Pc</i>	ATCAGGAGATTGGGCAGGTG TGCGCTCTAACTTGGAATT	ChIP-qPCR & RT-qPCR
<i>mat2P-up</i>	ACAAATAACATGTTTCCTTCGCCT TCCGTTGTTGTATGGGTCCT	ChIP-qPCR
<i>mat2P-down</i>	GCATTGTAGATCATATCCGTCGA ATACAGATGCCAATAACTTCGTG	ChIP-qPCR
<i>ura4</i>	TTGGCTACTGGTTCCTACACAGAG TCGCTACCGCAGTTTACAATCACTTC	ChIP-qPCR & RT-qPCR