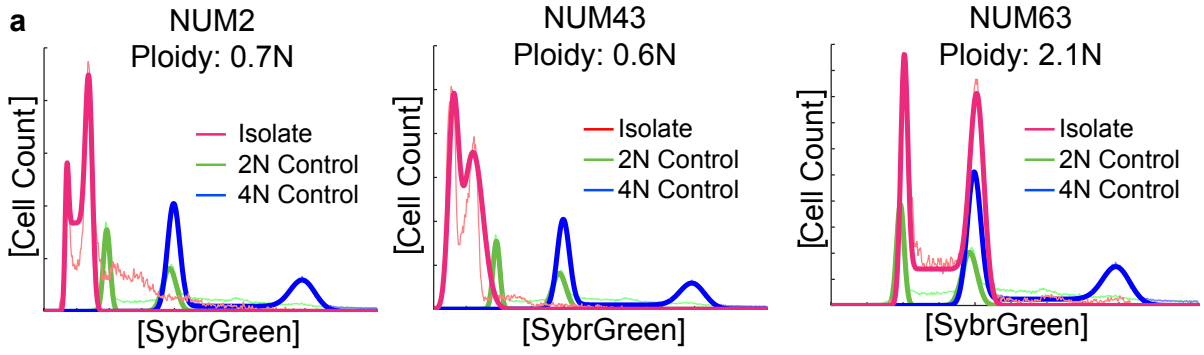
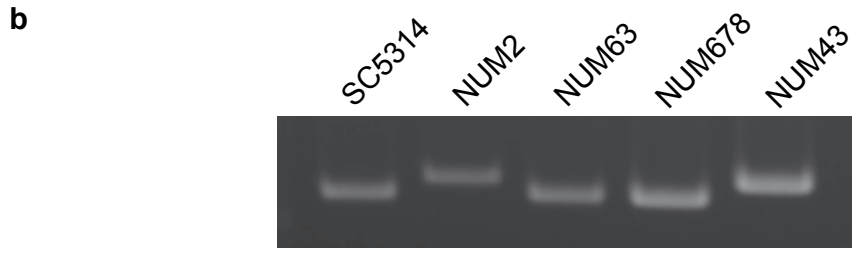
doi:10.1038/nature11865





c

	C. albicans sequence identity	*C.guilliermondii* sequence identity
NUM2	577/690 (84%)	796/800 (99%)
NUM43	580/690 (84%)	799/800 (99%)

Figure S1. a) Flow cytometry DNA content analysis of clinical isolates previously thought to be haploid (Suzuki *et al.,* 1982). NUM2, NUM43 and NUM63 (dark pink) were compared to diploid (SC5314, green) and tetraploid (RBY18, blue) *C. albicans* controls. NUM2 and NUM43 have ploidy levels much lower than the levels in haploid *C. albicans* strains. **b)** PCR amplification of the ITS region (Irobi *et al.,* 1999) from SC5314, NUM2, NUM43, NUM63, NUM678 (a tetraploid clinical isolate from Suzuki *et al.,* 1982). Amplicons from NUM2 and NUM43 are larger than those seen for SC5314 and do not amplify DNA from the mating-type locus (data not shown), indicating they are non-*albicans Candida* species. NUM63 and NUM678 amplicons are very similar in size to SC5314, amplify both the *MTL***a** and *MTL*α alleles of the mating-type locus (data not shown) and are likely to be *C. albicans*. **c)** BLAST sequence analysis (Altschul *et al.,* 1997) revealed that NUM2 and NUM43 are *C. guilliermondii* and not *C. albicans*.

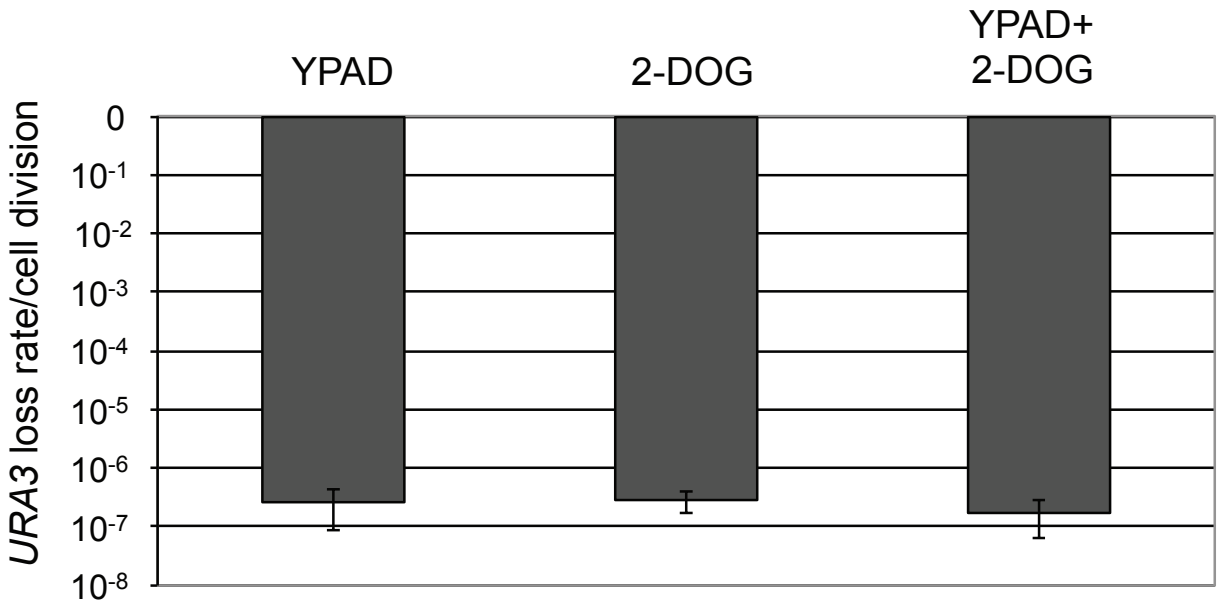


Figure S2. Exposure to 2-deoxygalactose does not increase LOH rates. To ask if 2-deoxygalactose (2-DOG) exposure represents a stress to *C. albicans*, strain YJB8756 (*gal1::URA3/gal1::HIS1*) was streaked for single colonies and grown for two days at 30°C. Eight single colonies each were inoculated into 1 ml of YPAD, 2-DOG (0.1%), or YPAD+ 2-DOG (0.1%). Cultures were grown for 16 hrs at 30°C with shaking. Serial dilutions were plated onto YPAD to obtain total CFUs and onto 5-FOA to select for Ura- mutants. *URA3* loss rates were calculated as described previously (Forche *et al.*, 2011). Loss rates represent the average of two fluctuation analyses.

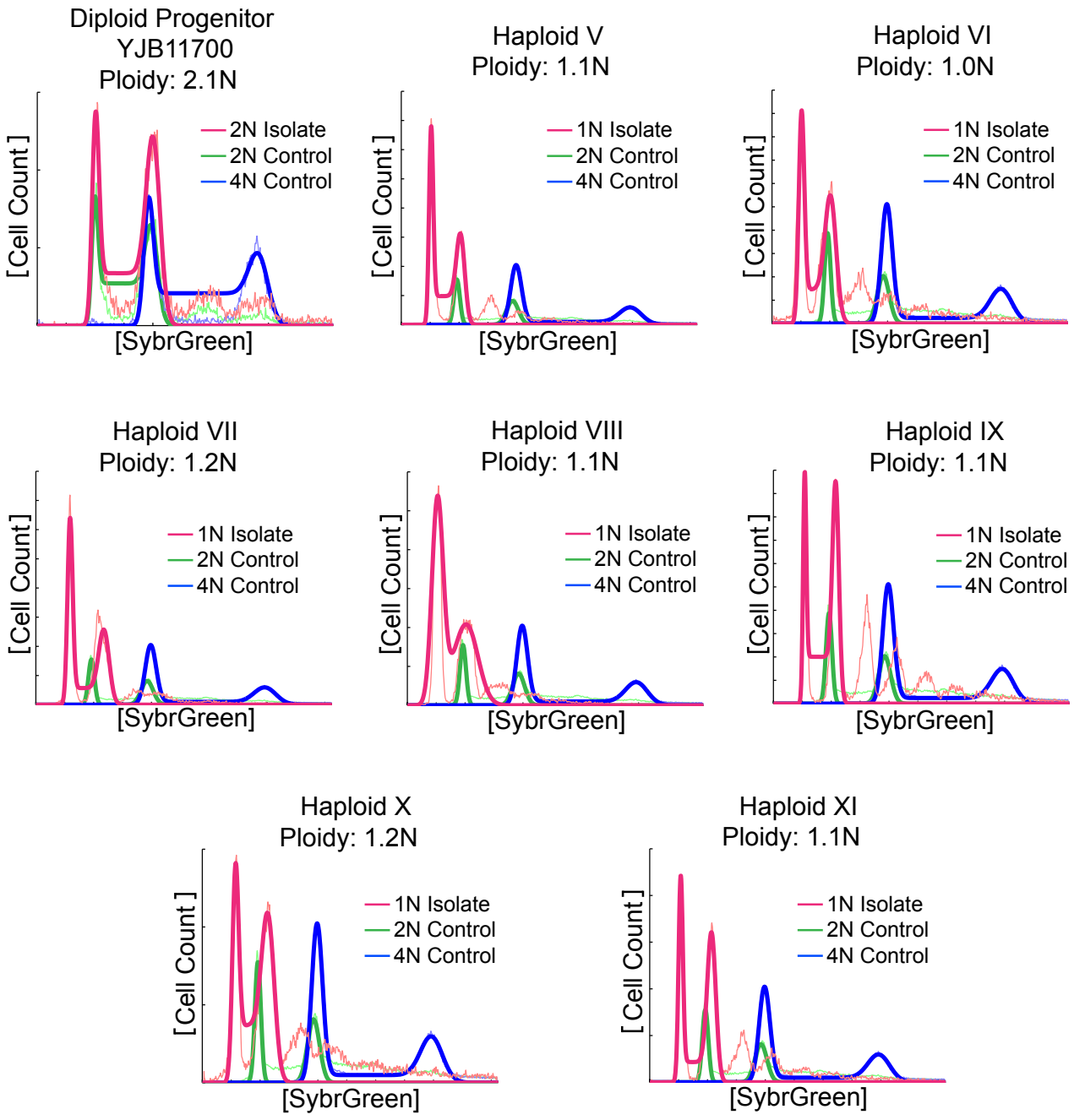


Figure S3. Flow cytometry DNA content analysis of one diploid progenitor strain (YJB11700) and seven additional haploid isolates (Haploids V – XI, dark pink) compared to diploid (SC5314, green) and tetraploid (RBY18, blue) control *C. albicans* strains. Methods and analysis are as in Fig. 1**a**.

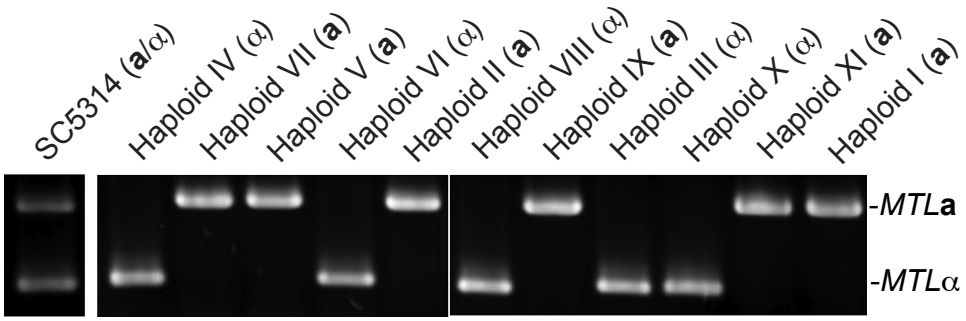


Figure S4. Haploid strains contain a single allele at the mating-type locus. PCR amplification of the mating type locus of haploid isolates (right) and a heterozygous diploid. Amplification was achieved with a multiplex PCR for the *MTL***a** (845 bp) and *MTL*α (516 bp) alleles.

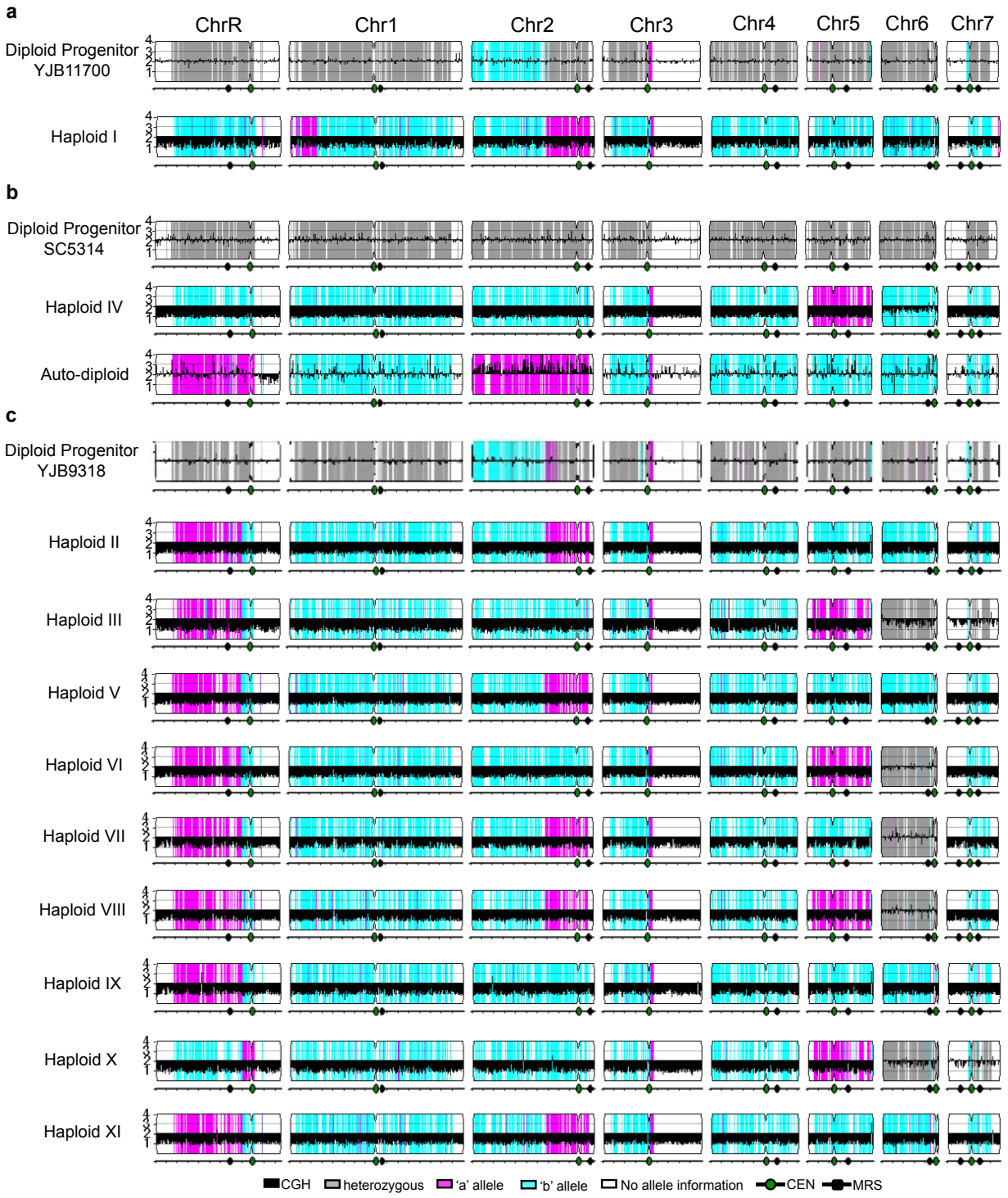


Figure S5. SNP/CGH array analysis of **a)** Haploid I and its diploid progenitor YJB11700 (a derivative of RM1000#2, Alonso-Monge *et al*., 2003), **b)** Haploid IV, Auto-diploid and their diploid progenitor, SC5314 (Gillum *et al*., 1984) and **c)** Haploids II, III, V - XI and their diploid progenitor, YJB9318 (a derivative of RM1000#2) as described in Fig. 1**b**. Copy number variation (ratio, black histogram, normalized using flow cytometry data) and SNP allele ratio information (cyan (allele 'a') and magenta (allele 'b')) for the eight *C. albicans* chromosomes analyzed relative to diploid strain SC5314. Methods have been published previously (Abbey *et al*., 2011). CEN = Centromere, MRS = Major repeat sequence

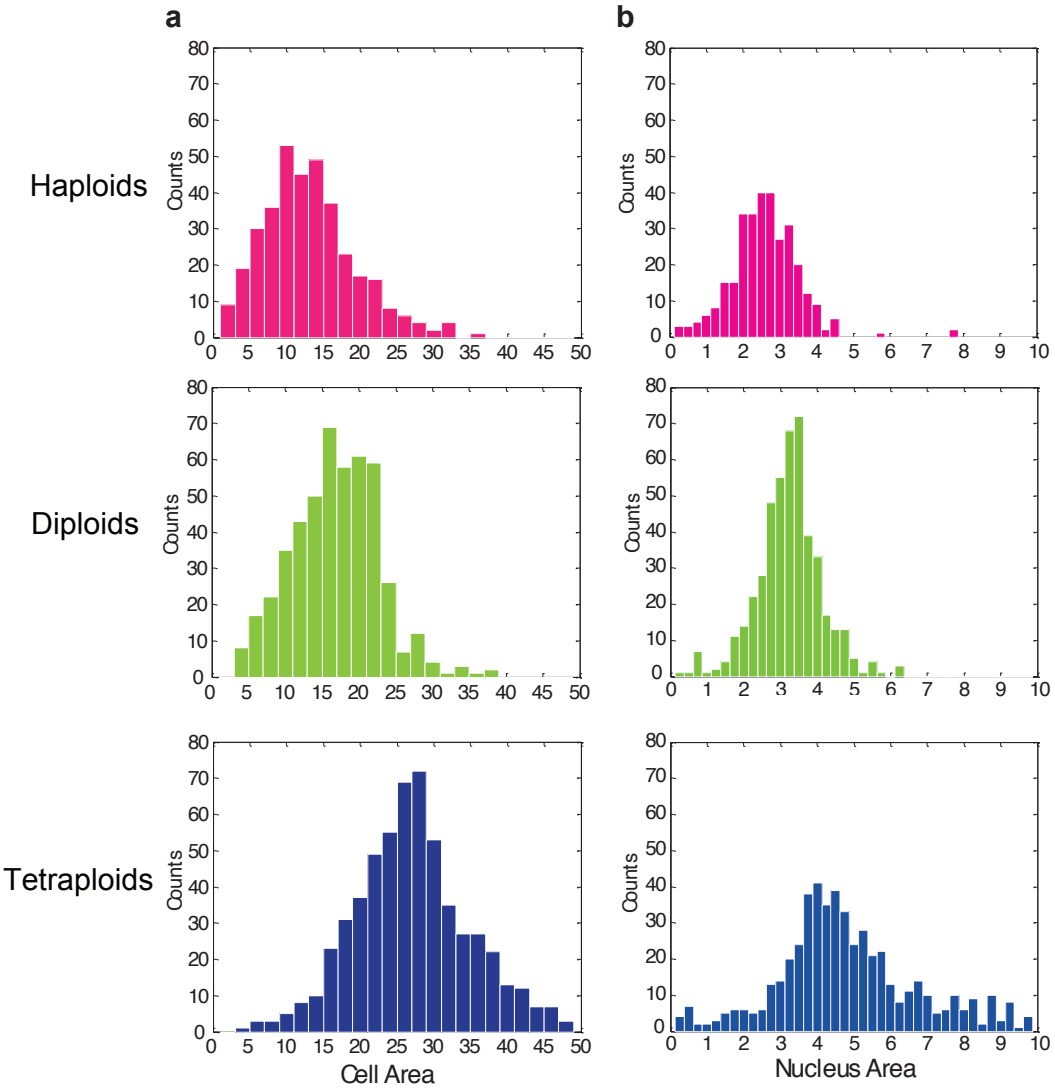


Figure S6. Average yeast cell size and nuclear size. **a)** Cell size distributions for haploid (13.05 μm2, n = 360, pink), diploid (16.85 μm2, n = 478, green), and tetraploid (27.12 μm2, n = 575, blue) yeast cells were determined by measuring cell length and width of cells within the in-focus plane of 11 plane Z-series stacks. Differences in cell area are statistically significant ($p < 0.001$, Student's t-test). **b)** Nuclear size distribution, determined using Eno1-GFP, for populations of haploid (2.60 μm2, n = 311), diploid (3.24 μm2, n = 463), and tetraploid (4.88 μm2, n = 525) cells. Differences in nucleus size are statistically sigificant ($p < 0.001$, Student's t-test).

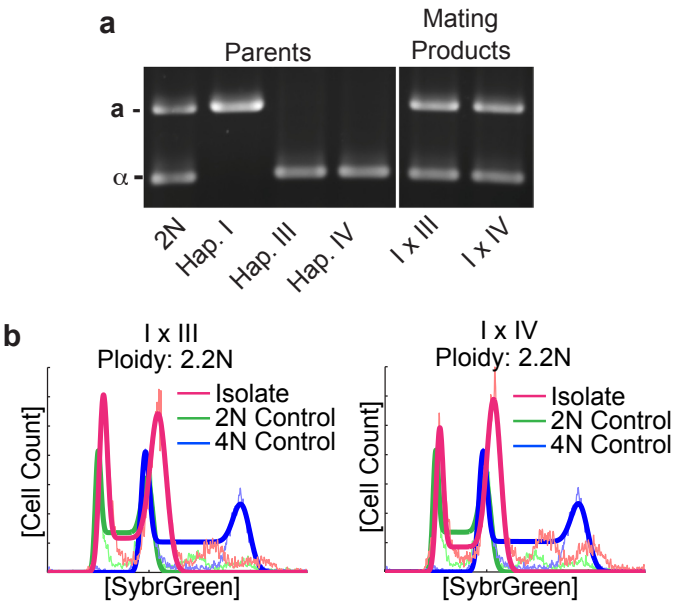


Figure S7. **a)** PCR amplification of *MTL* in parents and mating products shown in Figure 2. **b)** Flow cytometry of mating products.

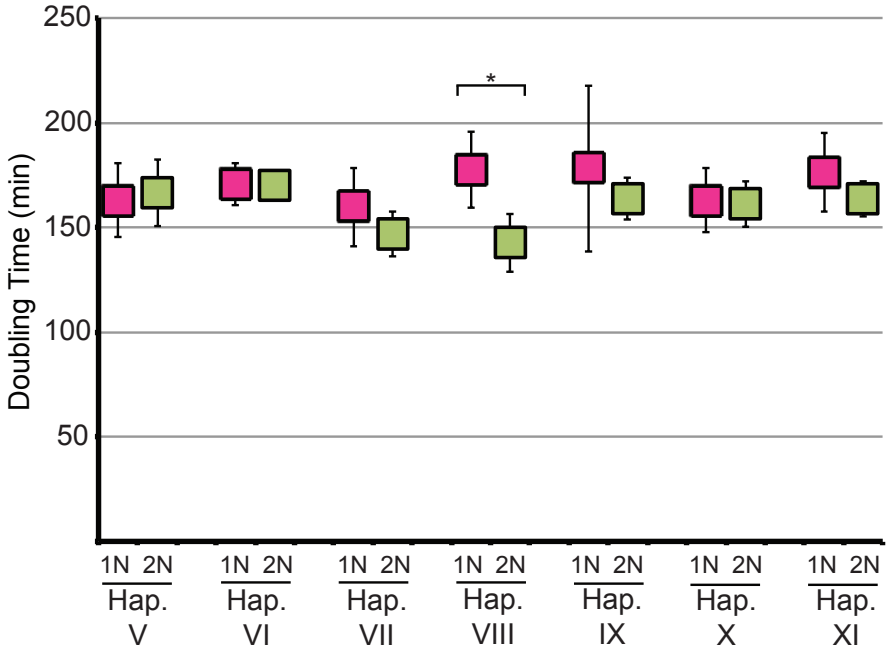


Figure S8. *In vitro* fitness of haploids and auto-diploidized strains based on growth dynamics. Mean doubling times of haploids (dark pink) and their corresponding auto-diploids (green). Growth in YPD supplemented with uridine, adenine and histidine was recorded using a Tecan Sunrise plate reader, and readings were obtained every 15 min for 24 hrs. Doubling time was determined from the slope of the growth during logarithmic phase as previously published in Abbey *et al*., 2011. Error bars represent one standard deviation from the mean. Pairwise comparisons between haploids and their corresponding auto-diploid were performed with Student's tests, (*) p = 0.02. Hap. = Haploid

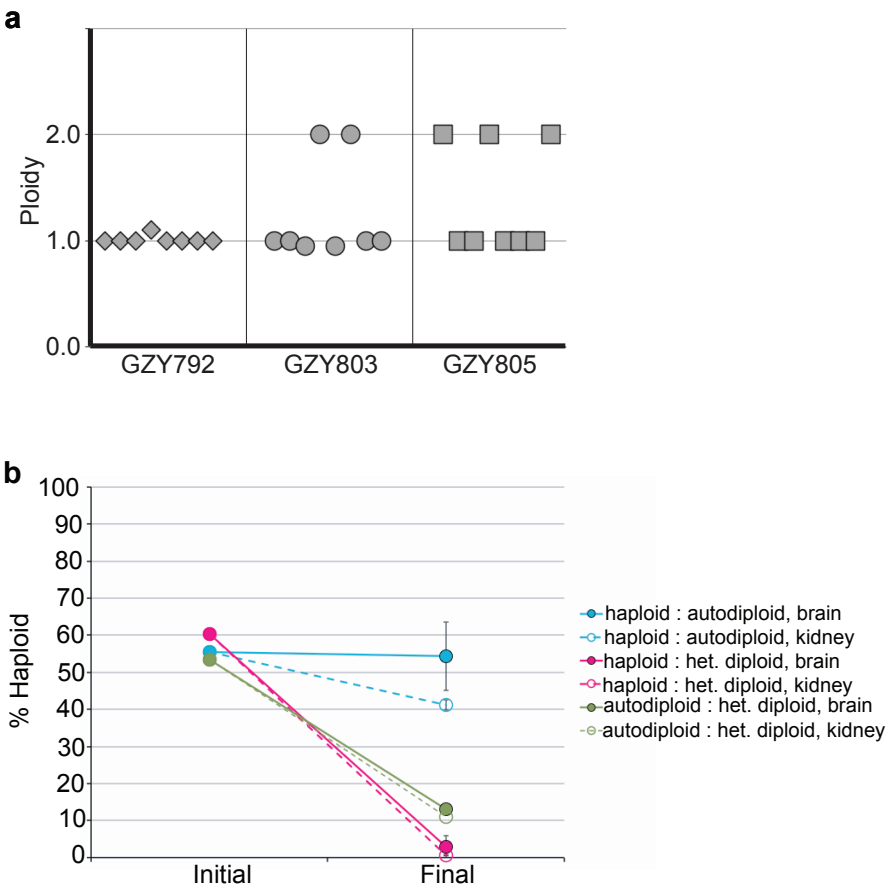


Figure S9: a) Recovery of haploid and auto-diploidized isolates after 48 hrs in a mouse host. Three mice were infected with *C. albicans* haploid strains GZY792, GZY803, and GZY805 (described in Fig. 4 and S10) by injection into the tail vein. The mice were monitored daily and were euthanized after 48 hrs. Kidneys were harvested and the isolates recovered were analyzed for ploidy by flow cytometry. **b)** *In vivo* competition between haploids, autodiploids and heterozygous diploids. Three competition experiments were performed. Blue: Haploid I (NAT resistant) with its autodiploid (YJB12908, NAT sensitive); pink: Haploid I (NAT resistant) and its heterozygous diploid progenitor YJB11700 (NAT sensitive); Green: Autodiploid I (NAT resistant) and its with heterozygous diploid progenitor YJB11700 (NAT sensitive). Two mice per competition experiment were co-inoculated with two different *C. albicans* strains (described above) in a ~50:50 ratio (10e6 total CFU/mouse) by tail vein injection. After 48 hours, the brain (closed circles) and kidneys (open circles) were harvested and CFUs counted by plating on YPD (total growth) and subsquently replica plating to YPAD + NAT media to determine the number of CFUs that were NAT resistant and divided by the total growth to see the percent haploid (or autodiploid, green) after 48 hours in the mouse host. Error bars represent one standard deviation from the mean. The autodiploid:YJB11700 reflects CFUs from only one mouse (due to poor colonization of the second animal).

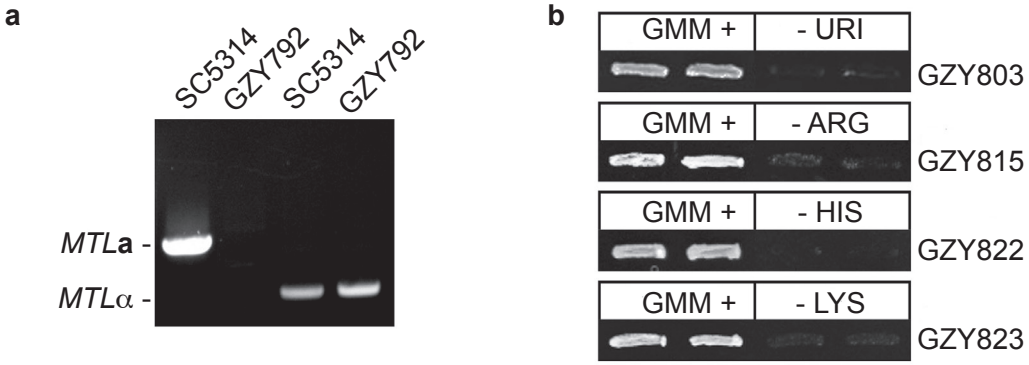


Figure S10. **a)** GZY792 is *MTL*α as detected by PCR using primers specific to the *MTL*α allele. No PCR product was detected using primers specific to the *MTL*a allele. **b**) Confirmation of the auxotrophic phenotypes of GZY803, GZY815, GZY822, and GZY823. Strains grown on glucose minimal media (GMM) (left) supplemented with uridine, arginine, histidine, and lysine, were replica-plated onto GMM plates without (right) one of the four amino acids (as indicated) and incubated at 30° overnight.

Supplemental Table S1: Strains used in this study.

Strains	Alias	Ploidy	Genotype	Source Strain	Condition
SC5314		Diploid	WT	(1)	
YJB9318		Diploid	*ura3Δ::imm434/ura3Δ::imm434 gal1Δ::URA3/GAL1*	RM1000 #2 (2)	
YJB11700		Diploid	*ura3Δ/ura3Δ his1Δ::hisG/his1Δ::hisG ade2/ADE2 gal1Δ::HIS1/GAL1 bud3Δ::URA3/BUD3*	RM1000 #2 (2)	
YJB12801	Haploid I	Haploid	*MTLa his4 galΔ ade2*	YJB11700	Selection for multiple LOH
YJB12908	Auto-Diploid I	Auto-diploid	*MTLa his4 galΔ ade2*	YJB12801	
YJB12814	Haploid II	Haploid	*MTLa his4 galΔ*	YJB9318	*in vivo* passaging
YJB12875	Haploid III	Haploid + Chr6 & Chr7	*MTLα his4 galΔ*	YJB9318	*in vivo* passaging
YJB12804	Haploid IV	Haploid	*MTLα his4 galΔ*	SC5314	fluconazole exposure
YJB12864	Haploid V	Haploid	*MTLa his4 galΔ*	YJB9318	*in vivo* passaging
YJB12812	Haploid VI	Haploid + Chr6	*MTLα his4 galΔ*	YJB9318	*in vivo* passaging
YJB12868	Haploid VII	Haploid + Chr6	*MTLa his4 galΔ*	YJB9318	*in vivo* passaging
YJB12818	Haploid VIII	Haploid + Chr6	*MTLα his4 galΔ*	YJB9318	*in vivo* passaging
YJB12870	Haploid IX	Haploid	*MTLa his4 galΔ*	YJB9318	*in vivo* passaging
YJB12880	Haploid X	Haploid + Chr6 & Chr7	*MTLα his4 galΔ*	YJB9318	*in vivo* passaging
YJB12881	Haploid XI	Haploid	*MTLa his4 galΔ*	YJB9318	*in vivo* passaging
GZY792		Haploid	*MTLα his4*	Haploid IV	*in vitro* passaging
GZY803		Haploid	*MTLα his4 ura3Δ::HIS4*	GZY792	
GZY805		Haploid	*MTLα his4 ura3Δ::HIS4 hgc1Δ::FRT*	GZY803	
GZY806		Haploid	*MTLα his4 ura3Δ::HIS4 hgc1Δ::UFP*	GZY805	
GZY815		Haploid	*MTLα his4 ura3Δ::HIS4 arg4Δ::FRT*	GZY803	
GZY822		Haploid	*MTLα his4 ura3Δ::HIS4 arg4Δ::FRT his1Δ::FRT*	GZY815	
GZY823		Haploid	*MTLα his4 ura3Δ::HIS4 arg4Δ::FRT lys2Δ::FRT*	GZY815	
GZY824		Haploid	*MTLα his4 ura3Δ::HIS4 arg4Δ::FRT his1Δ::FRT sla1Δ::UFP*	GZY822	
GZY825		Haploid	*MTLα his4 ura3Δ::HIS4 arg4Δ::FRT his1Δ::FRT ace2Δ::ARG4*	GZY822	
GZY833		Haploid	*MTLα his4 ura3Δ::HIS4 arg4Δ::FRT his1Δ::FRT sec3Δ::UFP*	GZY822	
GZY834		Haploid	*MTLα his4 ura3Δ::HIS4 arg4Δ::FRT his1Δ::FRT rvs167Δ::ARG4*	GZY822	
YJB12229		Auto-diploid	*MTLα his4*	SC5314	auto-diploidized post fluconazole exposure in transit

1. Gillum, A. M., Tsay, E. Y. & Kirsch, D. R. Isolation of the *Candida albicans* gene for orotidine-5'-phosphate decarboxylase by complementation of *S. cerevisiae ura3* and *E. coli pyrF* mutations. *Mol Gen Genet* **198**, 179–182 (1984).
2. Alonso-Monge, R. *et al.* The Hog1 mitogen-activated protein kinase is essential in the oxidative stress response and chlamydospore formation in Candida albicans. *Eukaryotic Cell* **2**, 351–361 (2003).