Deletion of *BAR1* induces autocrine pheromone signaling in P37005 isolates

To confirm that loss of Bar1 activity results in autocrine signaling in strain backgrounds other than SC5314, the *BAR1* gene was deleted in P37005, a natural **a**/**a** isolate that has previously been used for mating studies[12, 21]. Both copies of the *BAR1* gene were targeted using the pDS1 plasmid, as described in Methods. Cells were switched to the opaque state and analyzed for cell and colony morphology. As shown in Supplemental Figure 1, P37005 Δ*bar1* cells produced the wrinkled colony morphology characteristic of cells undergoing a mating response. Microscopic analysis of cells from these colonies confirmed that they were producing polarized mating projections (Suppl. Fig. 1B). These experiments demonstrate that loss of *BAR1* activity results in autocrine signaling and self-mating in both SC5314 and P37005 strain backgrounds of *C. albicans*.

Halo formation in *C. albicans* is enhanced by autocrine pheromone signaling

The halo assay is an established, semi-quantitative method for determining the strength of the mating response to pheromones[9, 10]. The present study indicates that *C. albicans* opaque **a** cells can be induced to express high levels of α-pheromone, either when challenged with exogenous α-pheromone, or when lacking the Δ*bar1* gene so that autocrine stimulation of α-pheromone production occurs. We therefore utilized the halo assay to further compare the pheromone response of three different opaque **a** strains: wildtype, Δ*bar1*, and Δ*bar1* Δ*mfα* (each derived from SC5314). For these experiments, α-pheromone was spotted onto a nascent lawn of opaque cells, and the lawn allowed to grow to maturity (3 days on Spider medium[10]). Those cells that responded strongly to pheromone ceased cell division and formed mating projections, as evident by a halo of growth inhibition in the plate[10].

As shown in Supplemental Figure 2, wildtype *C. albicans* **a** cells did not produce a clear zone of growth arrest in response to exogenous α-pheromone, in agreement with published observations[10, 13, 35]. In contrast, Δ*bar1* **a** cells were highly sensitized to the presence of pheromone, producing larger and clearer halos than that exhibited by the wildtype strain[10]. Interestingly, Δ*bar1* Δ*mfα* **a** cells produced an intermediate phenotype, with a larger and more distinct halo than that seen with wildtype cells, but a smaller halo than with the

Δ*bar1* strain. Quantification of the halo response confirmed that wildtype halos (16 mm) were smaller than Δ*bar1* Δ*mfα* halos (21 mm), which were themselves smaller than Δ*bar1* halos (29 mm) (Supplemental Figure 2D).

These results demonstrate that the large, clear halos formed by Δ*bar1* strains are due, at least in part, to secretion of α-pheromone by these cells. This provides independent verification that α-pheromone secretion occurs in Δ*bar1* **a** cells, and that the level of secretion is sufficient that activity can be readily detected in halo assays.

Biofilm formation is enhanced in *bar1* mutants

Recent studies have implicated phenotypic switching and pheromone signaling as playing a role in biofilm formation by *C. albicans* strains[36, 37]. Phenotypic switching refers to an alternation between different inheritable states and is exemplified by the white-opaque switch in *C. albicans*[38, 39]. Opaque phase cells are the mating competent form of *C. albicans*, able to both secrete and respond to mating pheromones and to undergo cell-cell fusion, and are therefore the form used predominantly in this study. White phase cells are the alternative phenotypic form and are mating incompetent, but can still respond to mating pheromones by undergoing increased cell-cell cohesion. The latter has been shown to increase biofilm formation *in vitro*, as white cells responding to pheromones exhibited increased adhesion to a synthetic substrate and greater biofilm mass[36, 37]. The response by white cells can promote mating between rare opaque cells by generating a three-dimensional matrix across which pheromone gradients can be maintained, and thus opaque cells can find one another by chemotropism[36].

To determine if autocrine pheromone signaling can similarly enhance *C. albicans* adhesion and biofilm formation, wildtype and Δ*bar1* strains were compared in two *in vitro* biofilm assays. First, adherence to a plastic substratum was monitored in white and opaque cells from P37005 (natural **a**/**a** isolate). As shown in Supplementary Figure 3, both white and opaque cells adhered to the plastic surface in the presence of α-pheromone, although opaque adherence was more fragile than that of white cells, in keeping with published reports[36, 37]. However, opaque cells from Δ*bar1* mutants were able to strongly adhere to the plastic even without the addition of exogenous α-pheromone (Supplementary Figure 3B). Significantly,

adherence required pre-culture of the Δ*bar1* strain in Spider medium, which is necessary for upregulation of the mating response[17].

A second *in vitro* assay was used in which biofilm formation on silicone elastomer was evaluated, as this is a natural substrate (e.g. catheter material) for biofilm formation in the clinic[40, 41]. Again, white and opaque forms from wildtype and Δ*bar1* strains of P37005 were compared, as well as cells from SC5314-derived **a** strains. Supplemental Figure 3C shows that wildtype white and opaque cells, as well as white cells from Δ*bar1* mutants, showed relatively small biofilms (0.9 -2.0 mg). Opaque cells derived from Δ*bar1* mutants, however, produced significantly larger biofilms in this assay (2.7 and 4.9 mg in SC5314 and P37005, respectively). While we consider it unlikely, we note that a potential role for contaminating white cells in promoting biofilm formation by opaque cells cannot be ruled out. Again, biofilm formation was greatest when opaque Δ*bar1* mutants were pre-grown in Spider medium to initiate autocrine signaling and self-mating (Supp. Fig. 3C and data not shown).

Taken together, these assays demonstrate that autocrine signaling in Δ*bar1* mutants can have a significant effect on adhesion to synthetic substrates and biofilm formation. It is not known what factors mediate this increased adhesion, although opaque cells responding to pheromone are known to upregulate factors (e.g., Hwp1) implicated in cell-cell cohesion and biofilm formation[11, 42-45].

Mating efficiencies in different genetic crosses in *C. albicans*

To further characterize same-sex mating in *C. albicans* a number of additional mating crosses were performed. Again, all experiments involved opaque phase cells as these represent the mating competent form of the organism[6]. In addition, control experiments confirmed that no detectable same-sex mating occurred when using strains grown in the white phase (data not shown). Unless stated otherwise, mating crosses were performed on Spider medium for 3 days prior to analysis of mating products, as described in Methods Summary.

First, we compared unisexual mating efficiencies between different clinical isolates of *C. albicans*. These experiments establish that same-sex mating can occur in populations from multiple strain backgrounds. For example, Δ*bar1* **a** strains derived from P37005 were tested for their ability to mate with four clinical **a** strains as well as an **a** strain derived from the laboratory isolate, SC5314 (Supplemental Table 3A). Same-sex **a**-**a** mating was observed in

all five crosses with frequencies ranging from nearly 7% in the P37005 x J981315 **a cross** to 0.23% in the P37005 x L1086 **a** cross. In general, it appeared that the mating efficiencies of P37005 Δ*bar1* mutants were slightly lower than those of SC5314 Δ*bar1* mutants crossed to the same strains (compare Figure 3 and Supplemental Table 3A). However, in both cases a similar trend in mating efficiencies was observed. For example, J981315 **a** (DSY932) acted as an efficient mating partner with both Δ*bar1* mutants of P37005 (7%) and SC5314 (16%). These trends also correlated with **a**-α mating efficiencies; SC5314 α cells mated with J981315 **a** cells at nearly 100% efficiency, SC5314 α x L1086 **a** mating occurred at 27% efficiency, while SC5314 α x 12C **a** mating only at 0.4% (**a**-α mating efficiencies determined after 1 d on Spider medium). These results demonstrate that *C. albicans* mating efficiencies can vary dramatically in both **a**-**a** and **a**-α crosses depending on the strain background.

Ménage à trois matings were similarly performed using several different combinations of *C. albicans* isolates. In these experiments, α cells were used as surrogates to induce mating between genetically marked populations of wildtype **a** cells. Such three-way matings resulted in 0.22% same-sex mating between SC5314 **a** cells, and 0.11-0.14% mating between RIH09 **a** cells, depending on whether SC5314 or 19F was present as the α strain (Supp. Table 3B). Note that these experiments underestimate the total frequency of same-sex mating (by ~2-fold) as only mating between the two differently marked **a** strains can be followed. These studies establish that pheromone secretion from a surrogate α strain can drive same-sex **a**-**a** mating in different wildtype strains of *C. albicans*. This contrasts with what was observed in *S. cerevisiae*, where the presence of α cells did not stimulate same-sex mating between populations of **a** cells[46].

Mating experiments were also carried out to further examine the specificity of unisexual cell fusion events. Using SC5314, Δ*bar1* **a** cells mated with wildtype **a** cells with an efficiency of 0.88%, while Δ*bar1*/Δ*ste2* **a** cells did not undergo detectable mating with other **a** cells (Figure 3). Furthermore, Δ*bar1* **a** cells did not mate with Δ*ste2* **a** cells (<0.00002%) while mating with Δ*ste3* **a** cells (0.24%) was similar to that observed with wildtype **a** cells (Supp. Table 3C). These experiments demonstrate that same-sex fusion products are due to pheromone signaling and mating amongst these populations, and not non-specific fusion of Δ*bar1* cells with other cells. Furthermore, we can conclude that (i)

autocrine signaling is required to amplify α-pheromone secretion by Δ*bar1* **a** cells, and (ii) detection of α-pheromone secreted by Δ*bar1* cells requires the Ste2 receptor on partner **a** cells. Thus, same-sex fusion requires cell-cell signaling for successful mating and tetraploid formation.

Mating crosses were also performed to establish that even limiting numbers of α cells can potentially drive same-sex mating amongst populations of **a** cells. Two different auxotrophic **a** strains (RBY1118 and RBY1179) were co-incubated with varying percentages (0.01-33%) of α cells (RBY1138). While the presence of a high concentration of α cells (33%) induced significant levels of **a-a** mating (0.27%), lower numbers of α cells could also induce same-sex mating (Supplemental Table 3D). For example, 1% of α cells in a mating mix produced 0.05% **a-a** mating, and even 0.01% of α cells produced detectable levels of **a-a** fusion products. In addition, α cells placed adjacent to populations of **a** cells could also induce low levels of **a-a** mating. As shown in Supplemental Figure 4, α cells induced wrinkling (a mating response) in colonies of neighboring **a** cells when cultured adjacently on YPD medium. Subsequent analysis of genetically marked **a** cells revealed that same-sex mating had taken place under these conditions, albeit at low frequency (0.0005%). (Note again, these experiments underestimate the total frequency of same-sex mating as only mating between differently marked **a** strains is detected.) Taken together, these experiments establish that pheromone secretion from α cells is sufficient to drive **a-a** mating, even if α cells are present at low frequency or at a distance from **a** cells.

Zygote formation in unisexual mating experiments

Mating zygotes demonstrating fusion events between two fluorescently labeled nuclei are shown in Supplemental Figure 5. In these experiments, an SC5314 Δ*bar1* **a** strain expressing *HTB-RFP* (CAY589) was co-incubated with a wildtype **a** strain expressing *HTB-YFP*, derived either from SC5314 (RBY1009, top 3 panels in Figure 4) or IHEM16614 **a/a** (DSY908, bottom 4 panels). In both cases, arrows indicate nuclei expressing both YFP and RFP demonstrating that successful mating and nuclear fusion (karyogamy) has taken place between the two **a** strains.

The products of same-sex mating are tetraploid cells

To confirm that the majority of same-sex mating products are tetraploid cells, flow cytometric analysis was performed on multiple isolates. Altogether, 75 isolates were analyzed and confirmed to be tetraploid. Supplemental Figure 6 shows products from three different mating crosses that generated stable tetraploid strains. These included mating between two Δ*bar1* **a** strains, mating between a Δ*bar1* **a** strain and a wildtype **a** strain, and mating between two wildtype **a** strains (mating driven by a surrogate α strain). Thus, the products of same-sex mating are tetraploid cells similar to those generated by classical **a**-α mating in *C. albicans*.

A parasexual mating cycle can be completed following unisexual conjugation

During **a**-α mating in *C. albicans*, diploid strains fuse to form a tetraploid **a**/α mating product[4, 5]. No meiotic program has been observed in *C. albicans*, yet ploidy reduction back to the diploid state can be completed by induction of chromosome instability by culturing on certain media. Thus, growth of tetraploid strains on sorbose medium causes chromosome loss from the tetraploid and the formation of diploid, or near-diploid, progeny strains[47, 48]. This process can therefore complete an alternative parasexual mating cycle in *C. albicans*[47, 48].

To test if tetraploid **a** strains could also undergo the parasexual program of chromosome loss, tetraploids were cultured on sorbose medium at 30°C for 1 week. Cells were re-streaked for single colonies on YPD medium and subsequently tested to see if they had undergone loss of genetic markers (*ARG4*, *LEU2*, or *SAT1*) that were heterozygous in the tetraploid strains. The latter would indicate that they had potentially lost the chromosome(s) encoding these genes and had therefore undergone a reduction in ploidy. A subset of these strains were tested by flow cytometry to determine cell ploidy, as previously described[4]. As shown in Supplemental Figure 7, many of the products analyzed by flow cytometry were diploid, or close to diploid, in ploidy (C-F). In addition, some progeny produced profiles that were clearly intermediate between diploid and tetraploid (G-J), indicating that while some chromosome loss had occurred, cells had not returned all the way to diploid. This result resembles the pattern of chromosome loss observed in **a**/α tetraploid strains[47, 48].

These experiments establish that the products of same-sex mating can undergo the parasexual cycle. Furthermore, these results demonstrate that *C. albicans* parasexual chromosome loss is not dependent on cells being genotypically **a**/α, a result that contrasts with that of meiosis in *S. cerevisiae* which is positively regulated by the **a**1/α2 heterodimeric complex[49].

Supplemental strain construction

Gene knockouts in clinical strains were generated using the *SAT1* flipper method described by Reuss[28]. All strains are listed in Supplemental Table 1. The plasmid pDS1 was digested with *Apa*I/*Sac*I and transformed into strain P37005 to generate the *bar1* mutant DSY929/930. The plasmid pDS4 was generated by first PCR amplifying the 5' end of *GAL1* using oligos 44/45, digesting the product with *Apa*I/*Xho*I, and cloning into plasmid pSFS2a. Subsequently, oligos 46/47 were used to PCR amplify the 3' of *GAL1* that was digested with *Sac*I and *Sac*II and ligated into the vector to generate plasmid pDS4. *Apa*I/*Sac*I digested pDS4 was then used to transform the clinical strains RIH09, AM2005/0377, DSY164 and DSY167 to generate the *gal1-/- SAT1*^{R} derivatives DSY940, DSY941, DSY933 and DSY932, respectively. Additionally, RBY1113 was also transformed to generate the *gal1-/- SAT*^{R} strain DSY1034. *Apa*I/*Sac*I digested pDS4 was also used to transform the clinical strain 19F to generate the *gal1-/- SAT1*^{S} strain DSY981. All construct integrations were confirmed by PCR using oligos 48/49 paired with oligos internal to pSFS2a, and disruption of the *GAL1* ORF confirmed by PCR using oligos 50/51.

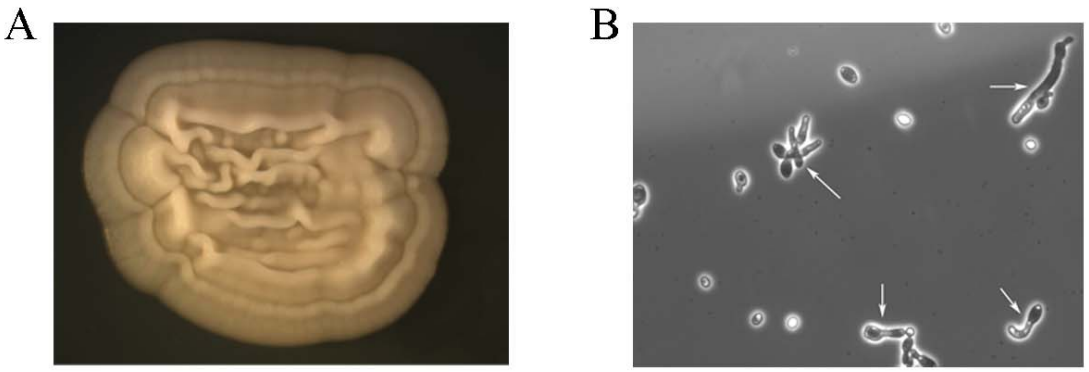
Supplemental methods

Halo assays were performed as described previously by Schaefer *et al*[10]. Quantification of the halo response was carried out by measuring the distance from the spot of the pheromone to where larger colonies (only weakly responsive to pheromone) were evident.

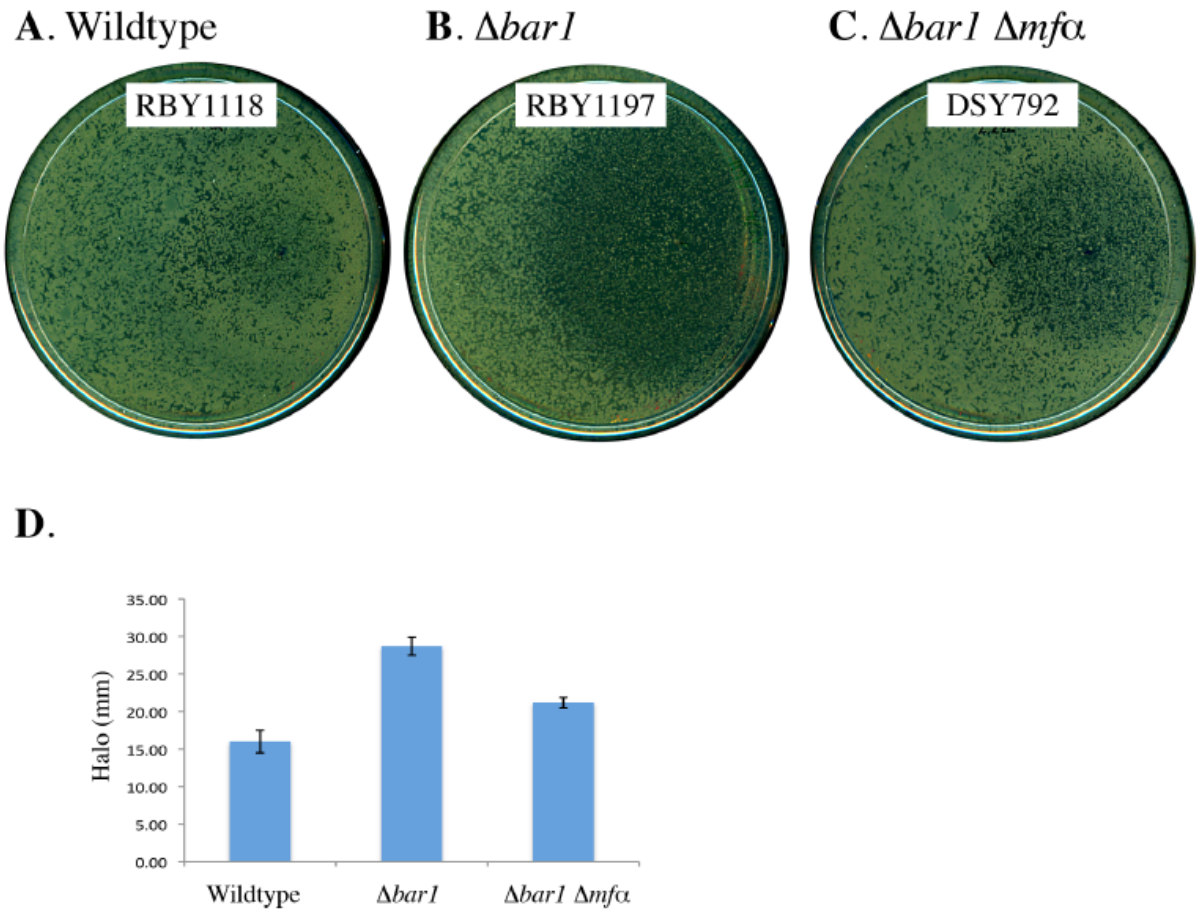
Biofilm assays were performed by modification of established protocols[36, 40]. Briefly, adherence to plastic surfaces was analyzed by growing overnight white or opaque phase cultures in SCD or Spider medium, washed, and 4 x 10^7 cells inoculated into six-cluster well plates containing 2 ml of modified Lee's culture medium[36]. Pheromone was added to indicated wells at 10 μg/ml and plates grown for 24 h at 30°C. Wells were then gently rinsed

with phosphate-buffered saline (PBS) and photographed. For the silicone elastomer model of biofilm formation[50], strains were grown overnight in Spider medium, cells washed, and 8 x 10^7 cells added to a well of a sterile 12-well plate containing pre-weighed 1.5 x 1.5 cm silicone squares (Cardiovascular Instrument) and 2 ml Spider medium. The inoculated plates were incubated for 90 min at 29°C with gentle agitation (150 rpm) for adherence to occur, excess cells removed and squares washed with 2 ml PBS. 2 ml of fresh Spider medium was added to the squares and incubation continued for 72 h at 29°C with agitation. Media was removed, squares allowed to dry overnight and then re-weighed to determine the mass of *C. albicans* cells attached to them.

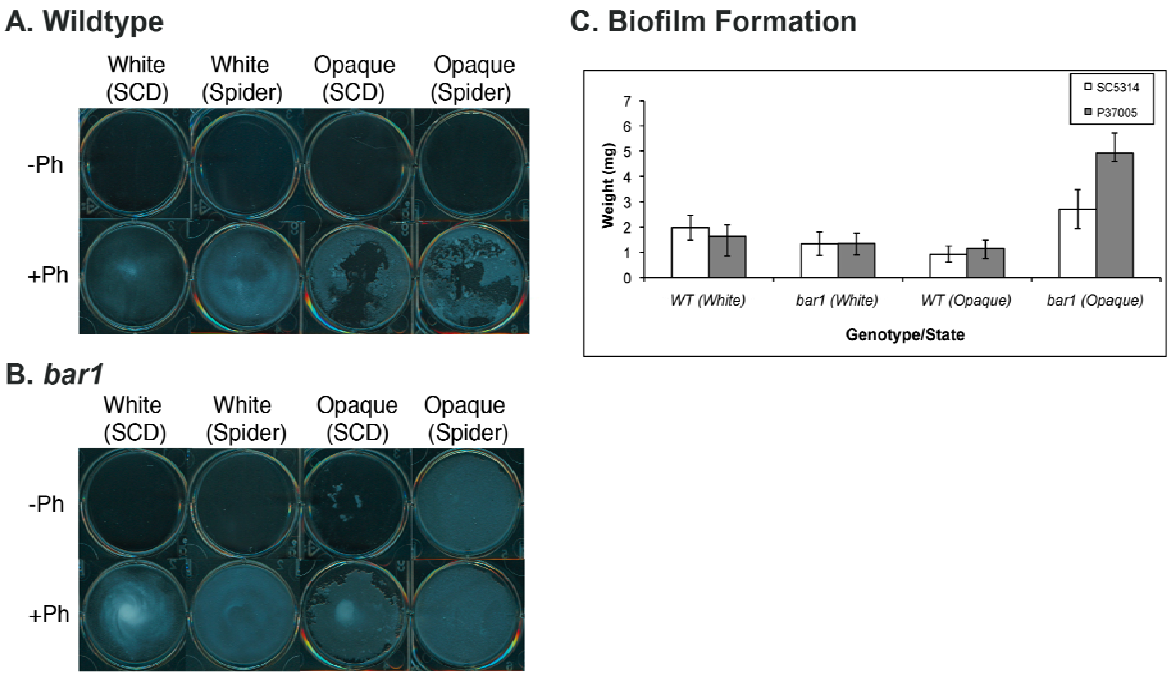
Parasexual chromosome loss in *C. albicans* tetraploid strains was carried out by culturing of tetraploids on sorbose medium at 30°C for 1 week, as previously described[47]. Colonies were re-streaked on YPD medium and single colonies analyzed for *leu-*, *arg-* or SAT^S phenotypes. Colonies that had one or more of these phenotypes (and hence had potentially undergone loss of chromosomes encoding *LEU2*, *ARG4* or *SAT1* genes), were analyzed by flow cytometry to determine their ploidy as described[4].



Supplementary Figure 1. Deletion of *BAR1* results in autocrine pheromone signaling in the clinical isolate, P37005. To establish that autocrine pheromone signaling occurs in strain backgrounds other than SC5314, the *BAR1* gene was deleted in the natural homozygous **a**/**a** isolate, P37005. (**A**) Colony of Δ*bar1* P37005 opaque cells showing the characteristic wrinkled phenotype of cells undergoing autocrine pheromone signaling (see also Figure 1). Colony was grown for 4 d on YPD medium and photographed. (**B**) Many cells taken from the wrinkled colony exhibit mating projections (arrows) indicating that they are undergoing a mating response to autocrine pheromones.

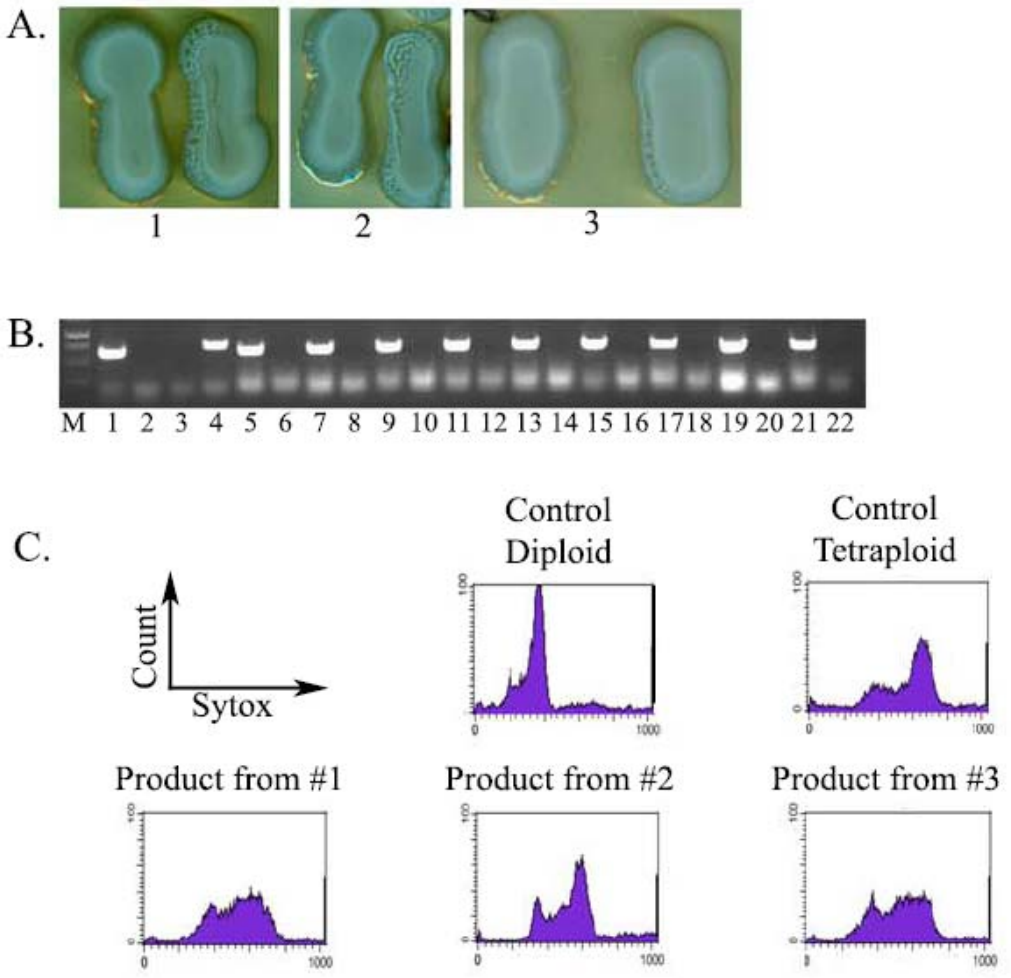


Supplementary Figure 2. Halo formation is enhanced in strains undergoing autocrine pheromone signaling. Halo assays were performed by plating a lawn of opaque **a** cells from each test strain onto Spider medium and spotting α-pheromone (2 μg) on the right-hand side of each plate, as previously described[10]. Plates were incubated at room temperature for 3 days to allow the lawn of cells to grow up and plates photographed. (**A**) Wildtype (RBY1118), (**B**) Δ*bar1* (RBY1197) and (**C**) Δ*bar1* Δ*mfα* (DSY792) strains were tested in this assay. (**D**) Quantification of halo formation. Halos formed by wildtype, Δ*bar1*, and three independent Δ*bar1* Δ*mfα* strains (DSY792, DSY793 and DSY794) were used to quantify halo formation. Deletion of *MFα* from Δ*bar1* strains significantly reduced the size and clarity of the halo formed. These experiments demonstrate that halo formation in Δ*bar1* strains is significantly enhanced by α-pheromone production in these cells and subsequent autocrine signaling. Three independent experiments were performed for this analysis.

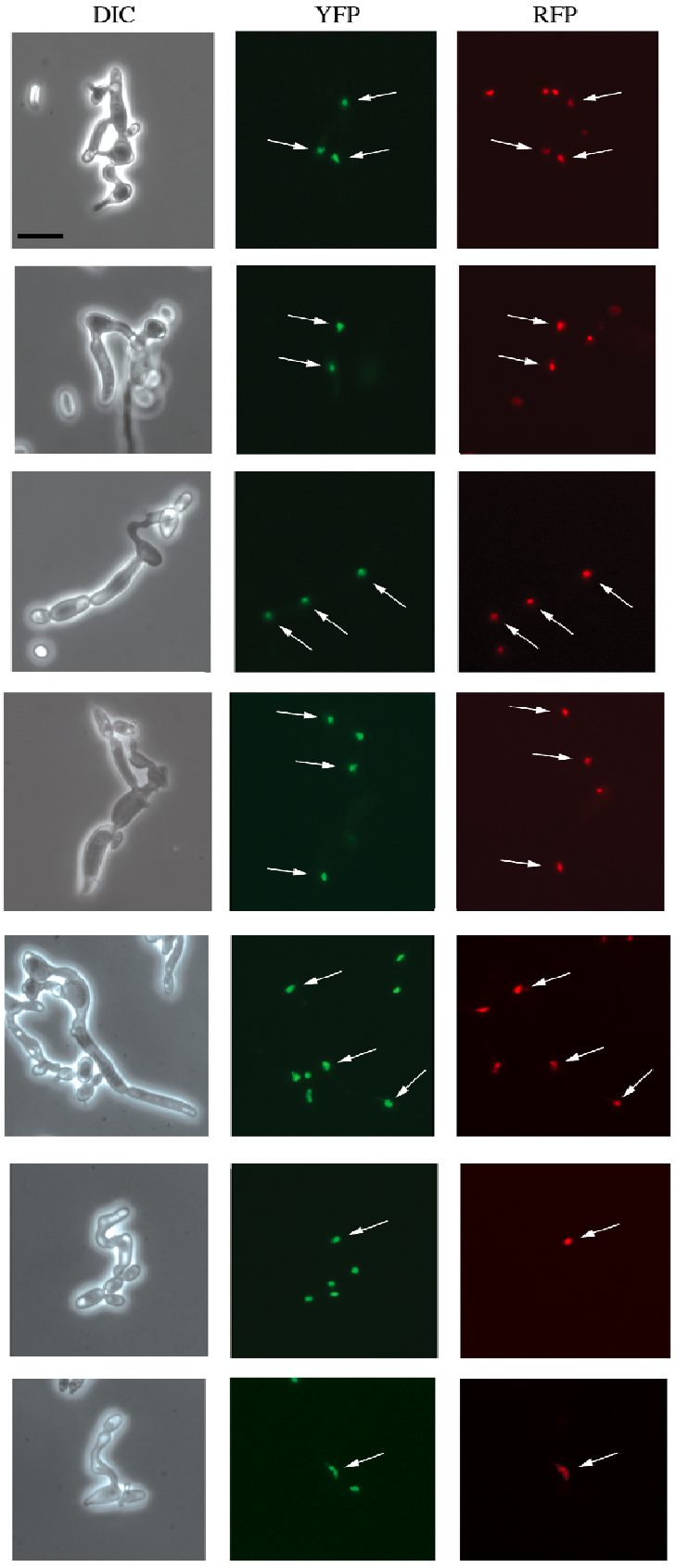


Supplementary Figure 3. Biofilm formation is increased in *Δbar1* opaque cells. Cells from wildtype (**A**) and *Δbar1* (**B**) P37005 strains were tested for adherence to plastic in multi-well dishes. Both white and opaque phase **a** cells were examined in the presence (+Ph) and absence (-Ph) of exogenous α-pheromone (10 μg/ml). White and opaque cells were found to adhere to plastic in the presence of pheromone, although *Δbar1* opaque cells also adhered to plastic even in the absence of exogenous pheromone if pre-cultured in Spider medium to upregulate autocrine pheromone signaling. Cells were cultured in six-well cluster plates for 24 h at 30°C without agitation before being gently washed with buffered saline and photographed.

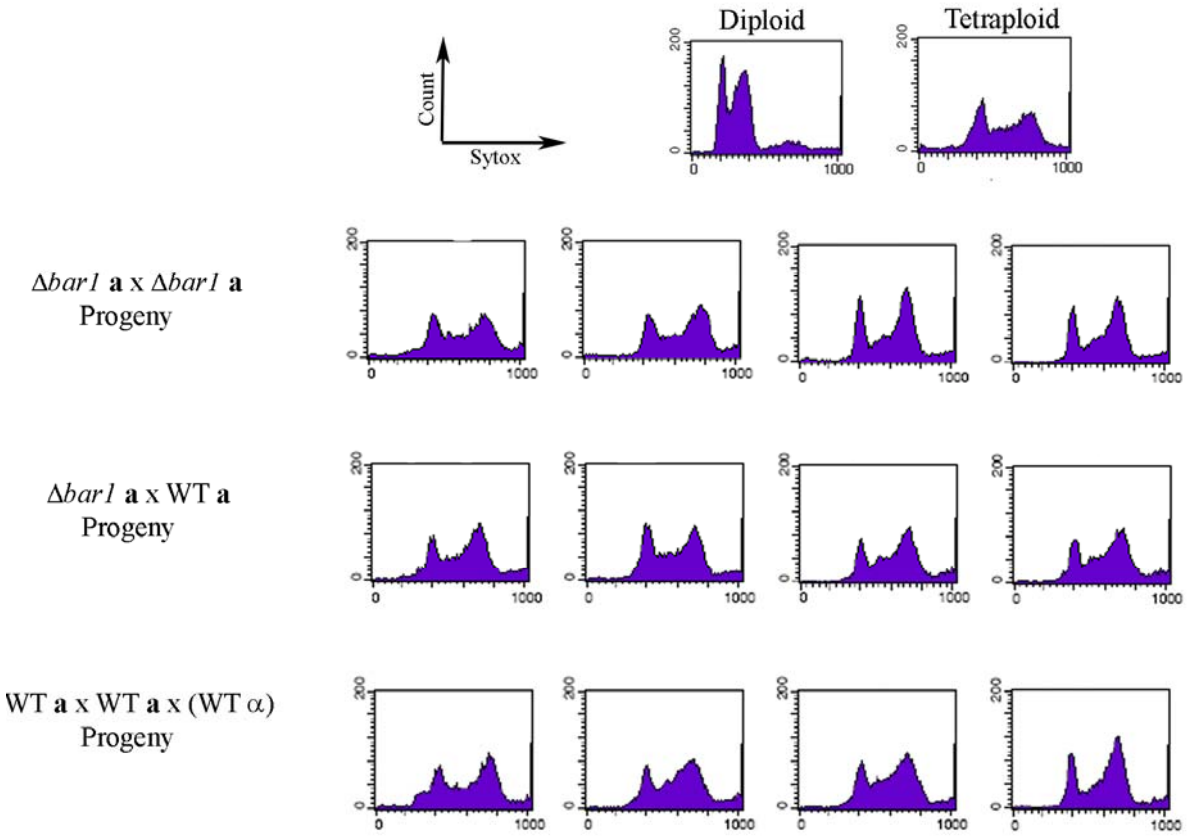
Wildtype and *Δbar1* **a** cells derived from SC5314 and P37005 were also compared in a silicone elastomer model of biofilm formation (**C**). Cells were grown overnight in Spider medium and then cultured with silicone elastomer squares for 90 min in fresh medium. Squares were gently rinsed to remove non-adherent cells and further cultured in Spider medium for 72 h. Elastomers were dried and weighed to determine the mass of *C. albicans* cells adhered to the surface, as previously described[50].



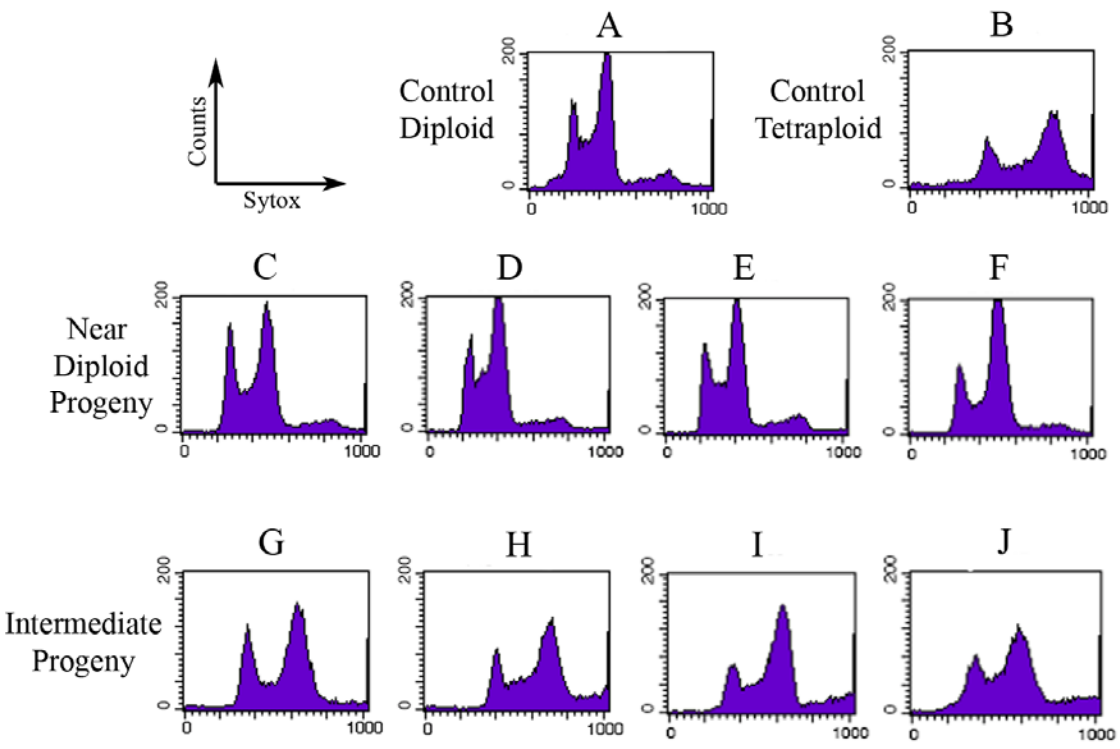
Supplemental Figure 4. Mating at a distance in *C. albicans*. (**A**) Patches of opaque α cells (RBY1138) were placed adjacent to a mixed colony of two differentially marked opaque **a** strains (RBY1118 and RBY1179) on YPD medium (pH 7.5). Colonies were allowed to grow at room temperature for 7 days before being photographed. Opaque α cells were observed to induce a mating response (wrinkling) in neighboring opaque **a** cells, even without touching of the **a** cells. Cells were taken from these wrinkled regions of the colonies and **a**-**a** mating products identified by selection on –arg -his medium. Each mating pair shown in (A) resulted in the production of same-sex mating products. Mating products were confirmed to be **a**-type tetraploids by PCR of the *MTL* (mating type-like) locus using primers directed against *OBP***a** (odd lanes) and *OBP*α genes (even lanes). (**B**) and by flow cytometric analysis (**C**).



Supplemental Figure 5. Zygotes formed by same-sex mating in *C. albicans*. A Δ*bar1* opaque **a** strain (CAY589) expressing a nuclear HTB-RFP fusion protein was mated with a wildtype opaque **a** strain (RBY1009) expressing HTB-YFP (top three panels). In this experiment both strains are derived from SC5314. Alternatively, CAY589 was mated with a clinical opaque **a** strain (IHEM16614) expressing HTB-YFP (bottom four panels). In each case, cultures were co-incubated for 2-3 d on Spider medium and mating zygotes containing fused nuclei (arrows) detected by fluorescent microscopy. Note that the bottom two panels show zygotes that have yet to undergo nuclear division and daughter cell budding. Scale bar, 15 μm.



Supplemental Figure 6. The products of *C. albicans* same-sex **a**-**a** mating are tetraploid cells. Same-sex mating products from Δ*bar1* **a** x Δ*bar1* **a** (row 2), Δ*bar1* **a** x wildtype **a** (row 3), or wildtype **a** x wildtype **a** (row 4) crosses were analyzed by DNA staining and flow cytometry to determine cell ploidy. Mating products were tetraploid strains as shown by comparison with representative diploid and tetraploid control strains (top row).



Supplemental Figure 7. The products of same-sex mating are competent to undergo the parasexual mating cycle. Same-sex mating produces tetraploid products that are stable when propagated on most media. Previously, **a**/α tetraploids were shown to become unstable when cultured on sorbose medium, undergoing chromosome loss and returning to a diploid or aneuploid state[47, 48]. To examine if **a**/**a**/**a**/**a** tetraploids were also competent to undergo a parasexual program of chromosome loss, tetraploid strains (e.g. DSY739 and DSY748) were cultured on sorbose medium for 1 week at 30°C and potential parasexual progeny strains identified (see Methods). Flow cytometric analysis revealed that many of these progeny strains had been reduced in ploidy to that of diploid or near diploid strain (C-F) while others had an intermediate ploidy between that of diploid and tetraploid (G-J). These experiments confirm that *C. albicans* tetraploids can undergo a parasexual mating cycle whether the products of same-sex **a**-**a** mating or traditional **a**-α mating.

Supplemental Table 1

Strains used in this Study

Strain(s)	Genotype	Mating Type	Source
RBY1009	*HTB::HTB-YFP:URA3*	a/a	22
RBY1081/1082	*ste3::LEU2/ste3::HIS1*	a/a	This Study
RBY1107/1108	*ste2::LEU2/ste2::HIS1*	a/a	This Study
RBY1113/1114	*mfα::LEU2/mfα::HIS1*	a/a	This Study
RBY1117/1118	*leu2/leu2 his1/his1*	a/a	10
RBY1119/1120	*leu2/leu2 his1/his1*	α/α	10
RBY1133/1134	*leu2/leu2 his1/his1 arg4/arg4*	α/α	10
RBY1177	*leu2::hisG/leu2::hisG his1::hisG/HIS1*	a/a	10
RBY1178	*leu2::hisG/leu2::hisG his1::hisG/HIS1*	α/α	10
RBY1179	*arg4::hisG/arg4::hisG his1::hisG/HIS1*	a/a	10
RBY1180	*arg4::hisG/arg4::hisG his1::hisG/HIS1*	α/α	10
RBY1197/1220	*leu2::hisG/leu2::hisG his1::hisG/his1::hisG arg4::hisG/arg4::hisG bar1::LEU2/bar1::HIS1*	a/a	10
DSY20	*leu2::hisG/leu2::hisG his1::hisG/his1::hisG arg4::hisG/arg4::hisG bar1::LEU2/bar1::HIS1/BAR1::SAT1*	a/a	10
DSY159	Sorbose Selected L1086 Clinical Isolate	α/α	This Study
DSY161	Sorbose Selected YSU751 Clinical Isolate	α/α	This Study
DSY162	Sorbose Selected AM2003/0191 Clinical Isolate	a/a	This Study
DSY164	Sorbose Selected L1086 Clinical Isolate	a/a	This Study
DSY165	Sorbose Selected Hun92 Clinical Isolate	α/α	This Study
DSY167	Sorbose Selected J981315 Clinical Isolate	α/α	This Study
DSY168	Sorbose Selected IHEM16614 Clinical Isolate	a/a	This Study
DSY170/171	*leu2::hisG/leu2::hisG his1::hisG/his1::hisG* $SAT1^R$	a/a	This Study
DSY211	*leu2::hisG/leu2::hisG his1::hisG/his1::hisG* $SAT1^R$	α/α	This Study
DSY183	Sorbose Selected IHEM16614 Clinical Isolate	α/α	This Study
DSY194	Sorbose Selected J981315 Clinical Isolate	a/a	This Study
DSY690	*leu2::hisG/leu2::hisG his1::hisG/his1::hisG arg4::hisG/arg4::hisG bar1::LEU2/bar1::HIS1 FUS1::GFP:SAT1*	a/a	This Study
DSY696	*leu2::hisG/leu2::hisG his1::hisG/his1::hisG arg4::hisG/arg4::hisG bar1::LEU2/bar1::HIS1 FIG1::GFP:SAT1*	a/a	This Study
DSY700	*leu2/leu2 his1/his1 FIG1::GFP:SAT1*	a/a	This Study
DSY702	*leu2/leu2 his1/his1 FUS1::GFP:SAT1*	a/a	This Study
DSY739	RBY1134 x RBY1118 x KAY17 mating product	a/a/a/a	This Study

DSY748	CAY303 x CAY320 mating product	a/a/a/a	This Study
DSY753	CAY303 x RBY1118 mating product	a/a/a/a	This Study
DSY760/761	*ste3::LEU2/ste3::HIS1 bar1::SAT1/bar1::SAT1*^{S}	a/a	This Study
DSY762/768	*ste2::LEU2/ste2::HIS1 bar1::SAT1/bar1::SAT1*^{S}	a/a	This Study
DSY792/793/ 794	*mfα::LEU2/mfα::HIS1 bar1::SAT1/bar1::SAT1*^{S}	a/a	This Study
DSY906	*leu2::hisG/leu2::hisG his1::hisG/his1::hisG arg4::hisG/arg4::hisG bar1::LEU2/bar1::HIS1 HTB::HTB-RFP:SAT1*	a/a	This Study
DSY908	Sorbose Selected IHEM16614 Clinical Isolate *HTB::HTB-YFP:SAT1*	a/a	This Study
DSY929/930	P37005 *bar1::SAT1/bar1::SAT1*^{S}	a/a	This Study
DSY932	J981315 *gal1::SAT1/gal1::SAT*^{R}	a/a	This Study
DSY933	L1086 *gal1::SAT1/gal1::SAT*^{R}	a/a	This Study
DSY940	RIH09 *gal1::SAT1/gal1::SAT*^{R}	a/a	This Study
DSY941	AM2005/0377 *gal1::SAT1/gal1::SAT*^{R}	a/a	This Study
DSY981	19F *gal1::SAT1/gal1::SAT*^{S}	α/α	This Study
DSY1034	*mfα::LEU2/mfα::HIS1 gal1::SAT1/gal1::SAT*^{R}	a/a	This Study
CAY303	*leu2::hisG/leu2::hisG his1::hisG/his1::hisG arg4::hisG/arg4::hisG bar1::LEU2/bar1::HIS1 WOR1::WOR1-YFP:SAT1*	a/a	This Study
CAY320/321	*leu2::hisG/leu2::hisG his1::hisG/his1::hisG arg4::hisG/arg4::hisG bar1::LEU2/bar1::ARG4*	a/a	This Study
CAY371	*leu2::hisG/leu2::hisG his1::hisG/his1::hisG arg4::hisG/arg4::hisG bar1::LEU2/bar1::ARG4 WOR1::WOR1-YFP:SAT1*	a/a	This Study
KAY17	*arg4::hisG/arg4::hisG his1::hisG/HIS1 WOR1::WOR1-YFP:SAT1*	a/a	32
	12C Clinical Isolate	a/a	30
	19F Clinical Isolate	α/α	30
	78048 Clinical Isolate	α/α	30
	AM2003/0165 Clinical Isolate	α/α	**
	AM2005/0377 Clinical Isolate	a/a	**
	L26 Clinical Isolate	a/a	30
	P37005 Clinical Isolate	a/a	30
	RIH09 Clinical Isolate	a/a	**
	T101 Clinical Isolate	a/a	**
	WO-1 Clinical Isolate	α/α	30

**These strains were generous gifts of the Aberdeen Fungal Group.

Supplemental Table 2

Oligonucleotides used in this study. Underlined sequences denote restriction sites.

Oligo	Name	Sequence
1	*STE2*-oligo1	ATCTGTACGCAATAACCACA
2	*STE2*-oligo3	cacggcgcgcctagcagcggCGCCTGGTTTATCAGGTATG
3	*STE2*-oligo4	gtcagcggccgcatccctgcGGAATACAATTCCTCGTCTT
4	*STE2*-oligo6	GTTCCAAGTGCCTCCAAACT
5	*STE2*-5' check	ATGACACCAATTATCCCTCC
6	*STE2*-3' check	TACCACTCCCATTGCCATTC
7	*STE2*-5' orf	GGTTGGTATGATGGATCATC
8	*STE2*-3' orf	ATCAGACAGATGATGACGAG
9	*STE3*-oligo1	CTCTTTGTTTCGGAAATTGG
10	*STE3*-oligo3	cacggcgcgcctagcagcggCCATTCCAGCTGCTCCTTTG
11	*STE3*-oligo4	gtcagcggccgcatccctgcTCTTTTGCGTGTACTTTACC
12	*STE3*-oligo6	CCTGATTCTGTGCTTGATAG
13	*STE3*-5' check	TGAAATCTGTCGTCGCTTAG
14	*STE3*-3' check	GATACGTTCCAGATTCAGAG
15	*STE3*-5' orf	GATGCTGGTTCTTCTGTTGG
16	*STE3*-3' orf	AATGTCACGCATTGTTGAGG
17	*MFα*-oligo1	ACCAAAGCCATACTTTTACC
18	*MFα*-oligo3	cacggcgcgcctagcagcggAGCAGTCAATAAAGTTAAGG
19	*MFα*-oligo4	gtcagcggccgcatccctgcAGAAGATGGAAAGCATACTG
20	*MFα*-oligo6	GATAGGCAAATGCAGAAGTA
21	*MFα*-5' check	CGCAGTTCATTTATGCACGTC
22	*MFα*-3' check	GCTTATGGTACGTGAATGTG
23	*MFα*-5' orf	GCCACTATTGTTGCTGCTGC
24	*MFα*-3'orf	GTCTAAAACCGGCTTCAGCA
25	*BAR1*-5' for (*Apa*I)	ggcgccgggcccGTATCGAACCGTGAGAGAAAC
26	*BAR1*-5' rev (*Xho*I)	gccggcctcgagGGAAATTCAAGCAGTTTATCTAG
27	*BAR1*-3' for (*Sac*II)	gccggcccgcggAGTTACCGGTACCCACTGTT
28	*BAR1*-3' rev (*Sac*I)	ggcgccgagctcTCGAGGACACTCGTCGGTAT
29	*BAR1*-5' check	GCTTACTGTGTACTGTGTATC
30	*BAR1*-3' check	GGCGAAAATGACAAGTACTGG
31	*BAR1*-5' orf	GATCTACAACTTCTCGGCTT
32	*BAR1*-3' orf	TGGTACAAATCCGTTGGTGC
33	*GFP*-5' (*Apa*I)	ggcgccgggcccATGTCTAAAGGTGAAGAATTATTCACTGG
34	*GFP*-3' (*Sal*I)	gccggcgtcgacCTATTTGCTTATTTACTGGTGTCC
35	*FUS1*-5' (*Kpn*I)	gccggcggtaccGAGTTTAAATGGAGTCATCACTG
36	*FUS1*-3' (*Apa*I)	gccggcgggcccTATTTATTGTTGACGTGATAAAGAATATGC
37	*FIG1*-5' (*Kpn*I)	gccggcggtaccGTAGTTCTACCACAAACACAG

38	*FIG1*-3' (*Apa*I)	gccggcgggcccTTTATATCTCTTTTTTGAAGAATGGAGGAT
39	*FUS1*-for check	CAAGCTTTGCATTGCTTGCA
40	*GFP*-rev check	CAAGGGTATCACCTTCAAAC
41	*FIG1*-for check	TGGAGTTCAATTGATAGGCAG
42	*SAT1* Chr5 for	CCTTCCCAATCGCACCATCCATAACATCTTATCTCCTATCCCCCAAAATATCCTCTCCTACACCAAGTATGATCTAAATTCTGGAAATCTGG
43	*SAT1* Chr5 rev	GCCCAATCAATATCAAATACAATCCATTTCATAAATTAAAGCCCCATGCTGATGCTGCTGTCAAAATTTGCGTCACGGGTATTTTCTCTTGT
44	*GAL1*-5' for (*Apa*I)	gcggccatgggcccTCCCGACACCAAAATCATAATT
45	*GAL1*-5' rev (*Xho*I)	caggcgcgctcgagTCAAACGTAGGAACTGACATGG
46	*GAL1*-3' for (*Sac*II)	gcggccaccgcggCCATCTATGGGTAGTTGTATTG
47	*GAL1*-3' rev (*Sac*I)	caggcgcggagctcGACTTGTATAAGCCTACTTTGC
48	*GAL1*-5' check	GGGAATCTTCGGATTAATTTATG
49	*GAL1*-3' check	TTTGCTTGGGTCCGGAATAG
50	*GAL1*-5' orf	TTGACCAACAACAACGATGG
51	*GAL1*-3' orf	ATACGTGTTTGGCACGTTGG

Supplemental Table 3

A. Unisexual mating between a P37005 Δ*bar1* **a** strain and alternative wildtype **a** strains.

Mating	Strain	Mating Frequency (%)
1	SC5314 **a**	2.85 ± 1.06
2	L1086 **a**	0.23 ± 0.23
3	J981315 **a**	6.95 ± 1.63
4	RIH09 **a**	1.32 ± 1.03
5	AM2005/0377 **a**	0.68 ± 0.56

B. Comparing ménage à trois matings.

Mating	Strains Crossed	Mating Frequency (%)
1	SC5314 **a** x SC5314 **a** x (SC5314 α)	0.22 ± 0.04
2	RIH09 **a** x RIH09 **a** x (SC5314 α)	0.14 ± 0.03
3	RIH09 **a** x RIH09 **a** x (19F α)	0.11 ± 0.03

C. Unisexual mating between SC5314-derived mutant strains.

Mating	Genotypes Crossed	Mating Frequency (%)
1	*Δbar1* **a** x *Δste2* **a**	<0.00002
2	*Δbar1* **a** x *Δste3* **a**	0.24 ± 0.30
3	*Δbar1* **a** x *Δste2/Δste3* **a**	<0.0002

D. Mating between two wildtype **a** isolates in the presence of limiting α cells (ménage à trois matings).

Mating	Percentage α's	Mating Frequency (%)
1	0.01%	0.00023 ± 0.00026
2	0.1%	0.00025 ± 0.00030
3	1%	0.052 ± 0.060
4	10%	0.071 ± 0.061
5	33%	0.27 ± 0.18

Supplemental References

35. Dignard, D. & Whiteway, M. SST2, a regulator of G-protein signaling for the Candida albicans mating response pathway. *Eukaryotic cell* **5**, 192-202 (2006).
36. Daniels, K.J., Srikantha, T., Lockhart, S.R., Pujol, C. & Soll, D.R. Opaque cells signal white cells to form biofilms in Candida albicans. *Embo J* **25**, 2240-2252 (2006).
37. Sahni, N., Yi, S., Pujol, C. & Soll, D.R. The white cell response to pheromone is a general characteristic of Candida albicans strains. *Eukaryot Cell* **8**, 251-256 (2009).
38. Johnson, A. The biology of mating in Candida albicans. *Nat Rev Microbiol* **1**, 106-116 (2003).
39. Soll, D.R. Mating-type locus homozygosis, phenotypic switching and mating: a unique sequence of dependencies in Candida albicans. *Bioessays* **26**, 10-20 (2004).
40. Nobile, C.J. & Mitchell, A.P. Regulation of cell-surface genes and biofilm formation by the C. albicans transcription factor Bcr1p. *Curr Biol* **15**, 1150-1155 (2005).
41. Nobile, C.J. & Mitchell, A.P. Genetics and genomics of Candida albicans biofilm formation. *Cell Microbiol* **8**, 1382-1391 (2006).
42. Daniels, K.J., Lockhart, S.R., Staab, J.F., Sundstrom, P. & Soll, D.R. The adhesin Hwp1 and the first daughter cell localize to the a/a portion of the conjugation bridge during Candida albicans mating. *Mol Biol Cell* **14**, 4920-4930 (2003).
43. Lockhart, S.R., Zhao, R., Daniels, K.J. & Soll, D.R. Alpha-pheromone-induced "shmooing" and gene regulation require white-opaque switching during Candida albicans mating. *Eukaryot Cell* **2**, 847-855 (2003).
44. Nobile, C.J., Nett, J.E., Andes, D.R. & Mitchell, A.P. Function of Candida albicans adhesin Hwp1 in biofilm formation. *Eukaryot Cell* **5**, 1604-1610 (2006).
45. Nobile, C.J. *et al.* Complementary adhesin function in C. albicans biofilm formation. *Curr Biol* **18**, 1017-1024 (2008).
46. Bender, A. & Sprague, G.F., Jr. Pheromones and pheromone receptors are the primary determinants of mating specificity in the yeast Saccharomyces cerevisiae. *Genetics* **121**, 463-476 (1989).
47. Bennett, R.J. & Johnson, A.D. Completion of a parasexual cycle in *Candida albicans* by induced chromosome loss in tetraploid strains. *Embo J* **22**, 2505-2515 (2003).
48. Forche, A. *et al.* The parasexual cycle in Candida albicans provides an alternative pathway to meiosis for the formation of recombinant strains. *PLoS Biol* **6**, e110 (2008).
49. Mitchell, A.P. Control of meiotic gene expression in Saccharomyces cerevisiae. *Microbiol Rev* **58**, 56-70 (1994).
50. Nobile, C.J. *et al.* Critical role of Bcr1-dependent adhesins in C. albicans biofilm formation in vitro and in vivo. *PLoS Pathog* **2**, e63 (2006).