SUPPLEMENTARY INFORMATION

A CRISPR Cas9-based gene drive platform for genetic interaction analysis in *Candida albicans*

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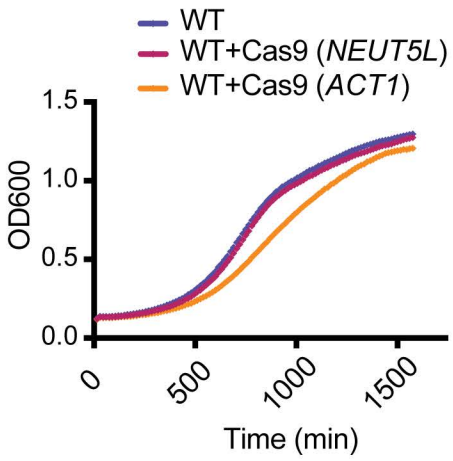
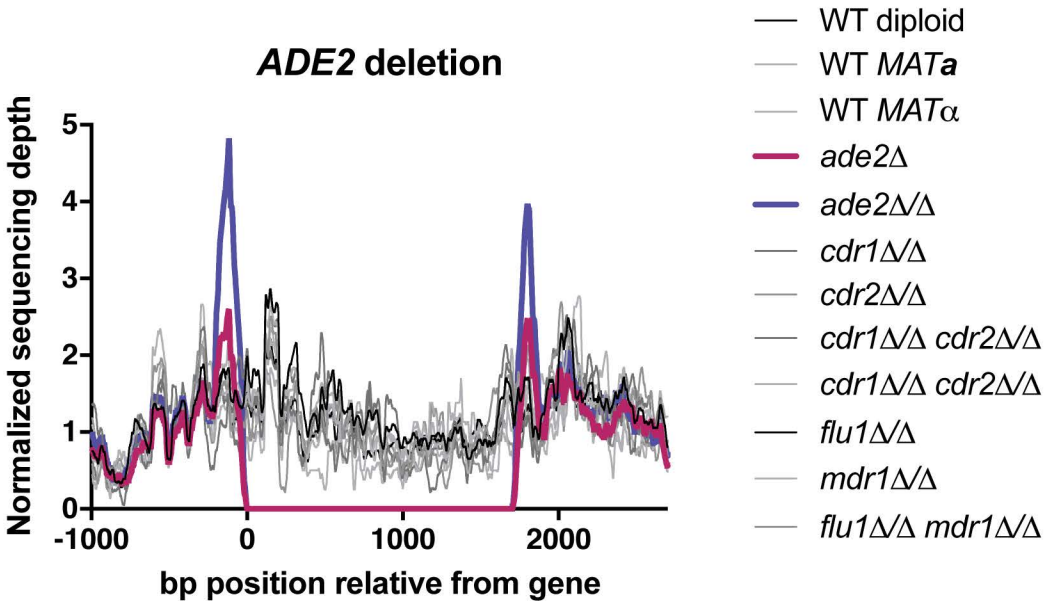
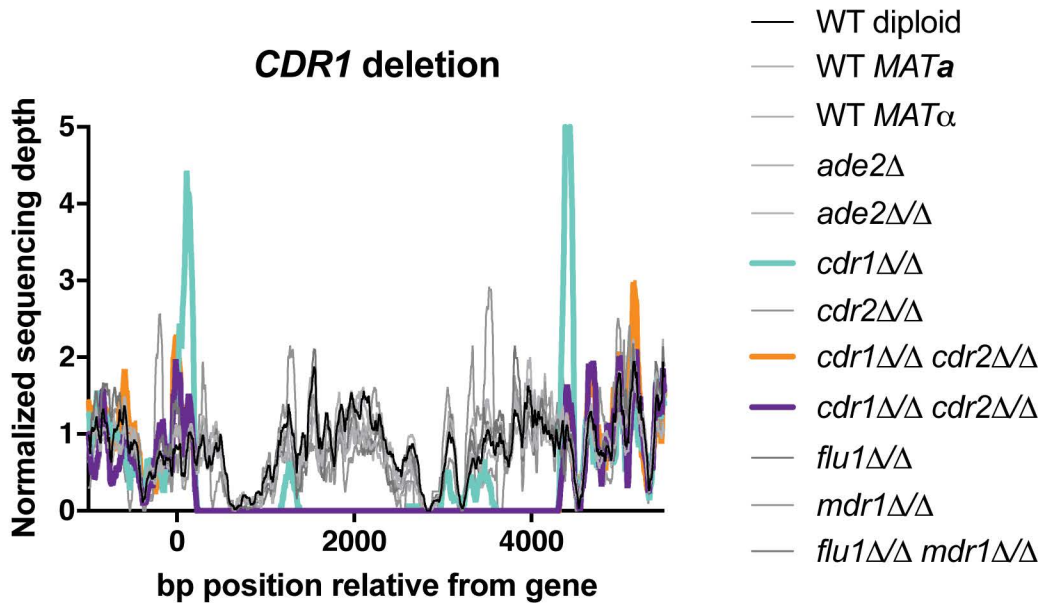
S1

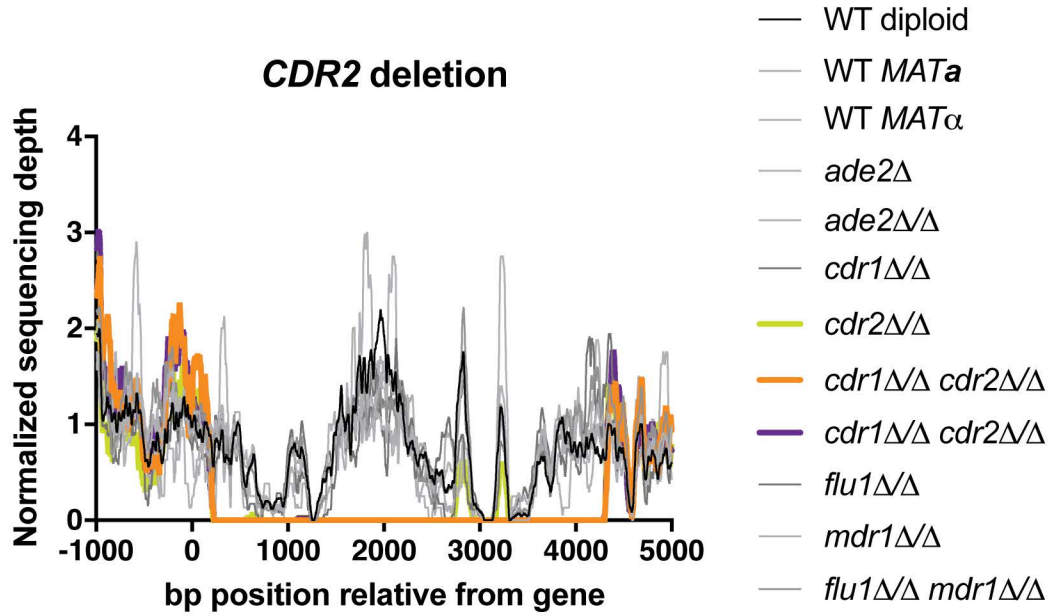

WT
WT+Cas9 (NEUT5L)
WT+Cas9 (ACT1)
1.5
1.0
0.5
0.0
OD600
0
500
1000
1500
Time (min)

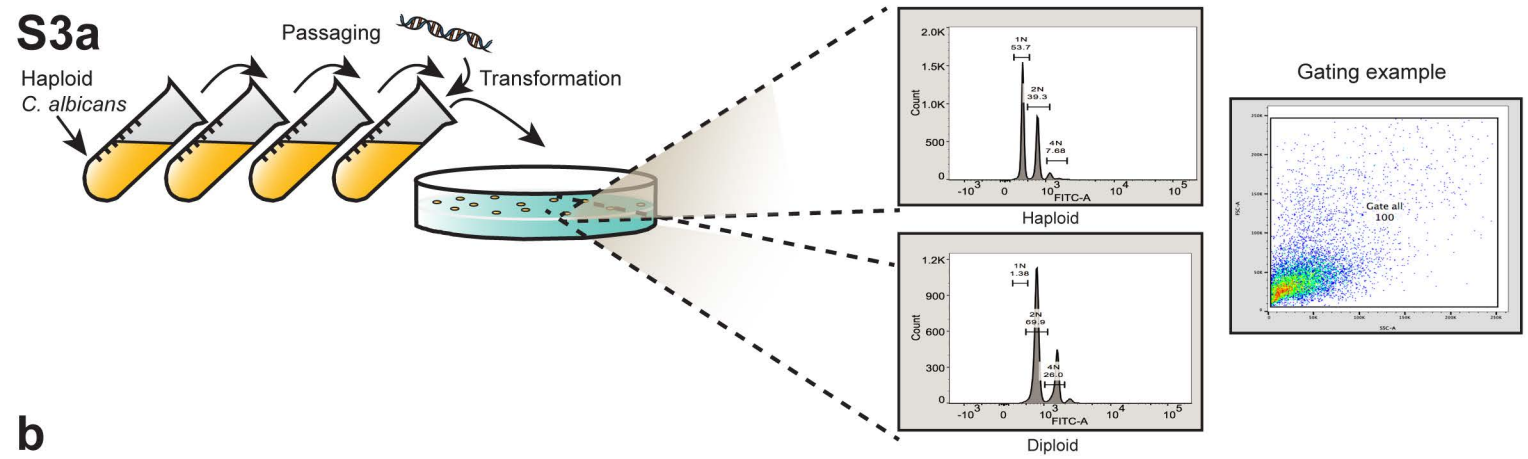
S2a


ADE2 deletion
Normalized sequencing depth
0
1
2
3
4
5
-1000
0
1000
2000
bp position relative from gene
WT diploid
WT MATa
WT MATα
ade2Δ
ade2Δ/Δ
cdr1Δ/Δ
cdr2Δ/Δ
cdr1Δ/Δ cdr2Δ/Δ
cdr1Δ/Δ cdr2Δ/Δ
flu1Δ/Δ
mdr1Δ/Δ
flu1Δ/Δ mdr1Δ/Δ

b

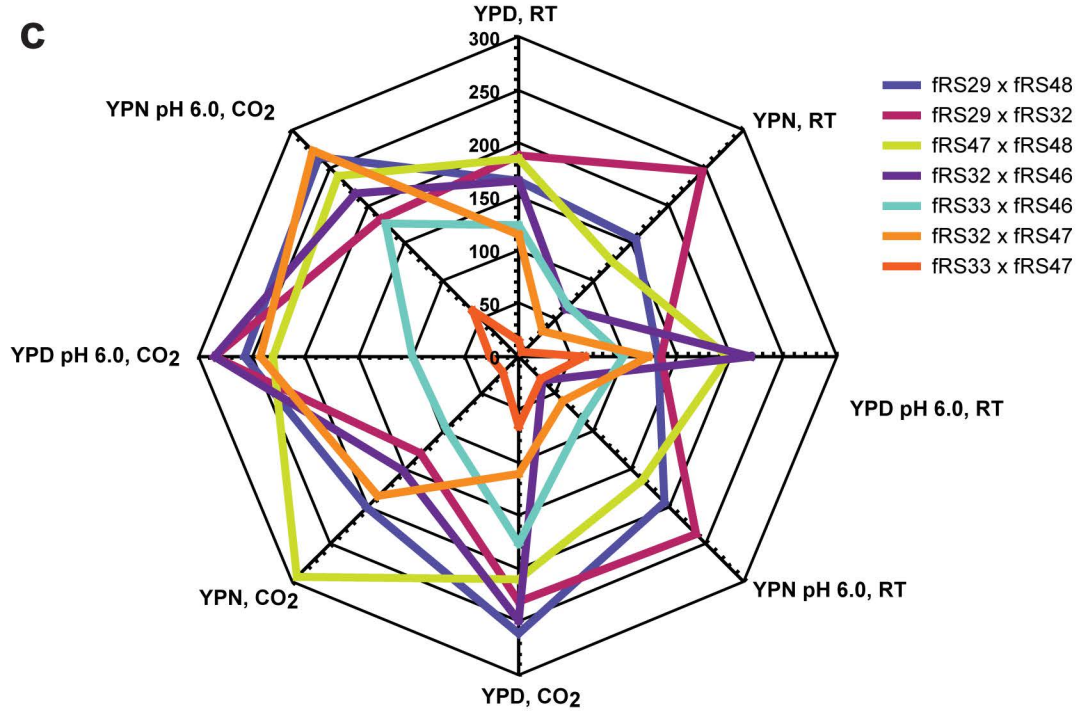

CDR1 deletion
Normalized sequencing depth
0
1
2
3
4
5
0
2000
4000
bp position relative from gene
WT diploid
WT MATa
WT MATα
ade2Δ
ade2Δ/Δ
cdr1Δ/Δ
cdr2Δ/Δ
cdr1Δ/Δ cdr2Δ/Δ
cdr1Δ/Δ cdr2Δ/Δ
flu1Δ/Δ
mdr1Δ/Δ
flu1Δ/Δ mdr1Δ/Δ

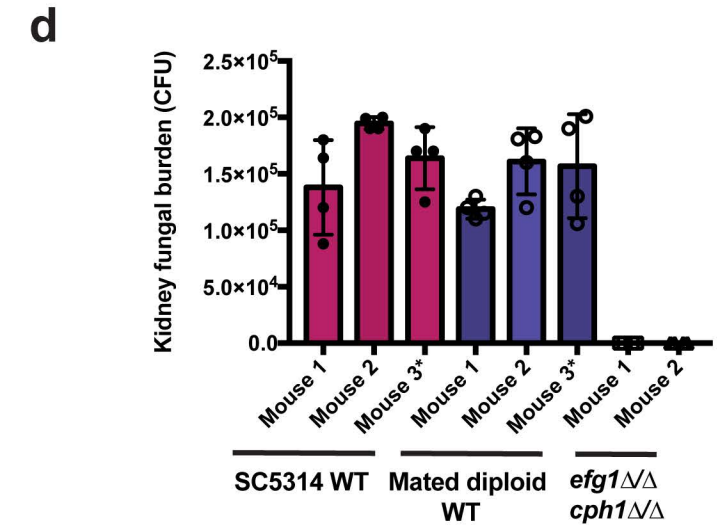

CDR2 deletion
Normalized sequencing depth
0
1
2
3
4
-1000
0
1000
2000
3000
4000
5000
bp position relative from gene
WT diploid
WT MATa
WT MATα
ade2Δ
ade2Δ/Δ
cdr1Δ/Δ
cdr2Δ/Δ
cdr1Δ/Δ cdr2Δ/Δ
cdr1Δ/Δ cdr2Δ/Δ
flu1Δ/Δ
mdr1Δ/Δ
flu1Δ/Δ mdr1Δ/Δ

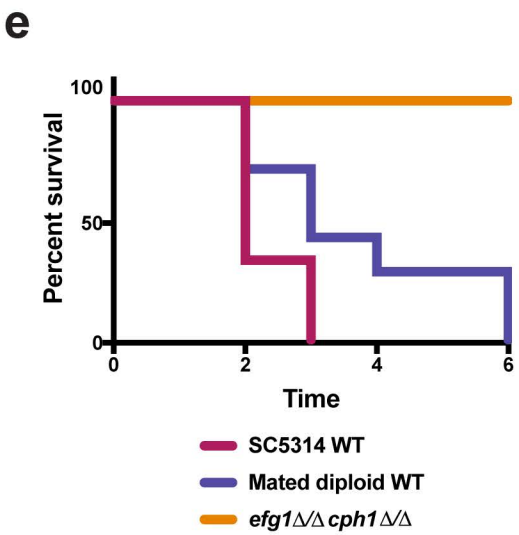


b

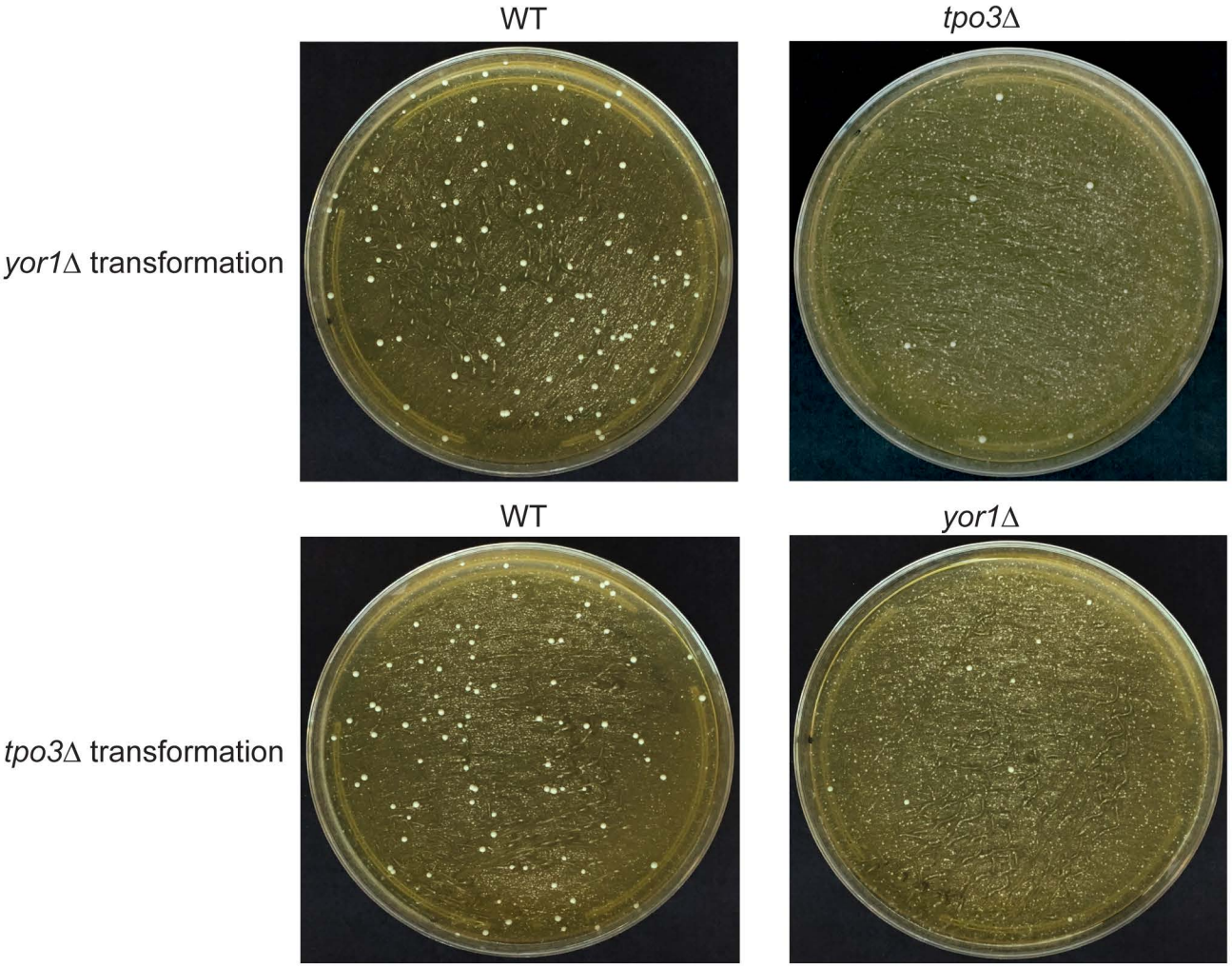
Strain	fRS28	fRS29	fRS30	fRS31	fRS46	fRS47	fRS32	fRS33	fRS48
Mating type	*MATα*	*MATα*	*MATα*	*MATα*	*MATα*	*MATα*	*MAT**a***	*MAT**a***	*MAT**a***
Auto-diploidization frequency	4/24	0/24	9/24	13/24	2/24	3/24	0/24	2/24	2/24
Auto-diploidization rate	0.16667	0	0.375	0.54167	0.08333	0.125	0	0.08333	0.08333



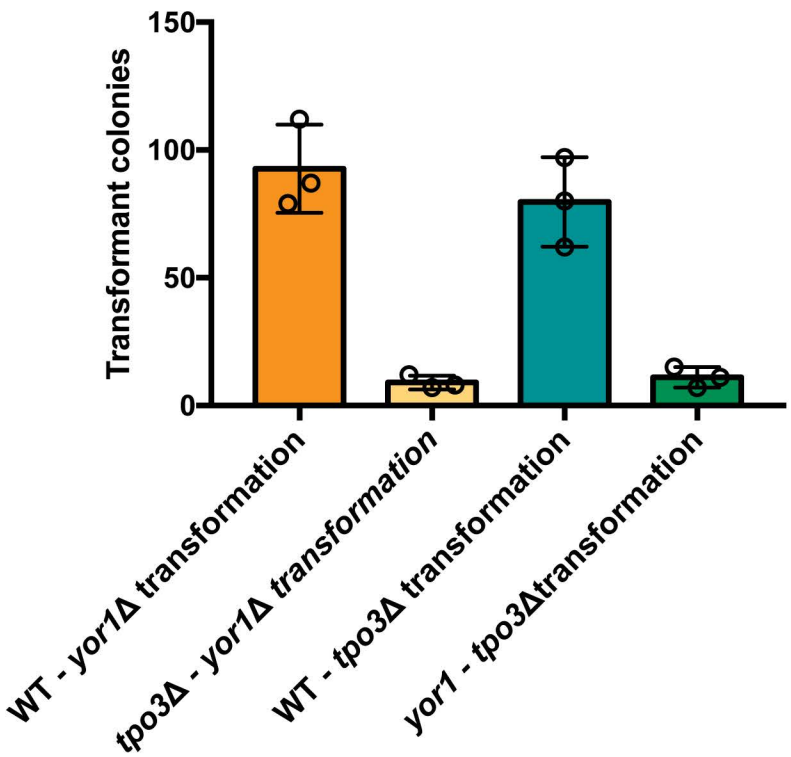




S4a


WT
tpo3Δ
yor1Δ transformation
WT
yor1Δ
tpo3Δ transformation

b


Transformant colonies
150
100
50
0
WT - yor1Δ transformation
tpo3Δ - yor1Δ transformation
WT - tpo3Δ transformation
yor1 - tpo3Δtransformation

SUPPLEMENTAL FIGURE LEGENDS

Supplementary Figure 1. Integration of *CAS9* at the *NEUT5L* locus has no identifiable fitness cost in *C. albicans*. *Related to Figure 2.* The *C. albicans* optimized *CAS9* gene was integrated in the *C. albicans* genome at either the *NEUT5L* or *ACT1* gene locus, and growth of these strains was monitored relative to a wild-type strain. The strain containing Cas9 at the *NEUT5L* site grows in a manner that is not significantly different from wild-type. Each strain was grown in YPD over 24 hours, with OD600 measurements taken every 15 minutes. Each strain was grown in triplicate.

Supplementary Figure 2. *C. albicans* gene drive deletions are specific to the targeted gene. *Related to Figure 2.* **(a)** Whole-genome sequencing reveals that deletion of *ADE2* is only identified in the haploid strain transformed with the *ADE2* gene drive, and the diploid strain resulting from mating of this haploid to a wild-type strain. **(b)** Sequencing further reveals that closely related genes (*CDR1* and *CDR2*) are specifically deleted in the appropriate strains, and not deleted in any other transporter deletion strains. In all cases, no additional deletions, aside from those specifically introduced into the genome via gene drives, were identified. We found small peaks in regions for deleted genes (*CDR1* or *CDR2*), but identified these as regions that are 100% identical between *CDR1* and *CDR2*, and are thus a remnant of sequence identity between *CDR1* and *CDR2* that is not able to be distinguished by the Illumina sequencing. The lack of peaks in the double mutant further confirms this.

Supplementary Figure 3. Optimizing conditions for *C. albicans* haploid stability and mating, and control virulence assays. *Related to Figure 2.* **(a)** To identify *C. albicans* haploid lineages that remain stably diploid over serial passaging and transformation, haploid cells were grown to saturation, subcultured, passaged three times, and then subjected to DNA transformation. Then, 24 transformants from each haploid background were selected and

screened for ploidy status via DNA SYBR staining and FACS analysis. All cells were including in the gating; an example of the gating strategy is depicted. **(b)** Quantification of the frequency and rate of auto-diploidization of each haploid lineage after passaging and transformation. **(c)** Different combinations of *C. albicans MAT**a*** and *MAT*α haploids were grown under different growth conditions to stimulate white-opaque switching, and then mated. The number of resultant diploid progeny were quantified and plotted. Different growth conditions for white-opaque switching included growth on YPD (yeast-peptone-dextrose) or YPN (yeast-peptone-*N*-acetylglucosamine) media, growth on media that was not tested for pH, or media that was adjusted to pH 6.0, and incubation at room temperature (RT) at ambient CO_2 levels, or at 28˚C with 5% CO_2. **(d)** Kidney fungal burden following murine tail vein injection with *C. albicans* confirms virulence of control mated diploid strain. CFUs were counted for mice injected with the SC5314 wild-type (WT) strain, our mated diploid WT strain, or an *efg1*Δ/Δ *cph1*Δ/Δ strain as an avirulent control. Two mice in each group were sacrificed after 48h to monitor kidney fungal burden. For both WT strains, CFU from one additional mice that had died at 48h was also counted (depicted with an *). Individual data points represent two technical replicates at two different dilutions. Error bars are standard deviation. **(f)** Mouse survival data confirms virulence of control mated diploid WT strain. Mouse survival following tail vein injection was monitored over fourteen days (n = 8 mice per strain).

Supplementary Figure 4. Evidence for a synthetic lethal interaction between *C. albicans TPO3* and *YOR1* genes. *Related to Figure 3.* **(a)** Reciprocal transformation of *YOR1* knock-out construct into wild-type or *tpo3*Δ haploid strain, and *TPO3* knock-out construct into wild-type or *yor1*Δ haploid strain, yields more colonies on wild-type compared to deletion mutant plates. **(b)** Quantification of three independent transformations of wild-type, *tpo3*Δ, and *yor1*Δ strains. All transformant colonies on *tpo3*Δ and *yor1*Δ mutant plates were confirmed to have wild-type

loci of either *TPO3* or *YOR1*. No double mutants were able to be generated. Data depicts mean values from three transformations; error bars are standard deviation.

SUPPLEMENTAL FILES

Supplementary Table 1. Gene drive construct variants. ***Related to Figure 2.*** This table summarizes the different gene drive construct variants used as part of the optimziation of the *C. albicans* gene drive system. (Format .xls; 57 KB)

Supplementary Table 2. ***C. albicans*** **efflux and adhesin genes targeted for deletion, and library matrix summary.** ***Related to Figure 3.*** This table summarizes the different *C. albicans* adhesin and efflux genes targeted for deletion, and lists each single and double gene deletion strains generated as part of this study. (Format .xls; 40 KB)

Supplementary Table 3. Whole-genome sequencing summary of gene drive deletion strains. ***Related to Figure 2, Figure 3.*** This table summarizes the results of whole-genome sequencing, and lists each gene found to be deleted in different strain backgrounds, as well as seqence coverage information. (Format .xls; 16 KB)

Supplementary Table 4. Genetic interaction scores and significant genetic interactions for double gene deletion libraries. ***Related to Figure 2, Figure 3, and Figure 4.*** This table lists genetic ineractions scores (calculated using a multiplicative model) and significant positive and negative genetic interactions for both *C. albicans* double gene deletion libraries (efflux and adhesin mutants). (Format .xls; 109 KB)

Supplementary Table 5. Summary of antifungal perturbations for drug efflux pump deletion screening. ***Related to Figure 4.*** This table lists all perturbation conditions used for screening the *C. albicans* efflux pump library, including the concentration of drug tested in the screen. (Format .xls; 13 KB)

Supplementary Note 1:

Candida albicans contains >6000 coding genes (http://www.candidagenome.org/cache/C_albicans_SC5314_genomeSnapshot.html). In order to create a comprehensive library of double mutants using conventional CRISPR-Cas9 approaches a set of 6000 initial knockout strains would need to be generated. Each of these knockouts would then need to have all other genes knocked out from them (rough estimate 6000 x 6000). Because a cell in which gene A is first deleted followed by gene B is the same as a cell in which gene B is initially removed followed by gene A, only half as many transformations need to occur at the second stage (rough estimate 6000 x 6000)/2. This leads to 18 million total transformations required to generate a comprehensive double mutant library.

If instead a gene drive approach is used an initial library of 6000 knockout strains would have to be created within both the *MAT**a*** and *MAT*α backgrounds (6000+6000) a total of 12,000 transformations. With these mutant libraries in hand any double mutant of interest can be readily generated by simply mating the corresponding haploid cells together.