FIGURE LEGENDS

FIG S1 Analysis of caspofungin or anidulafungin susceptibility of the mutants lacking *CHT2*, *CSU51* or *PGA4* by spot assay. (A and B) Shown are two independent repeats for growth of all single- or double-deletion mutants vs their parental strain CAF4-2 on medium supplemented with caspofungin (CAS) and for growth of representative double-deletion mutants vs their parental strain CAF4-2 on medium supplemented with anidulafungin (ANI). Strains are indicated on the left. From left to right, the number of cells that were spotted onto each plate, were 10^4, 10^3, 10^2, and 10^1. Plates were incubated at 37°C.

FIG S2 Analysis of caspofungin (CAS) susceptibility of the representative double-deletion mutants NAC4 a.k.a. *cht2*-/-, NACS1 a.k.a. *csu51*-/-, NAP88 a.k.a. *pga4-/-* by standard broth microdilution assay. (A and B) Shown are two independent repeats for growth of mutants vs their parental strain CAF4-2 in different concentration of caspofungin as indicated. For more explanations see the legend of Fig. 3B. Data are presented as the best fit curve (see Materials and Methods).

FIG S3 Analysis of anidulafungin (ANI) susceptibility of the representative double-deletion mutants NAC4 a.k.a. *cht2*-/-, NACS1 a.k.a. *csu51*-/-, NAP88 a.k.a. *pga4-/-* by standard broth microdilution assay. (A and B) Shown are two independent repeats for growth of mutants vs their parental strain CAF4-2 in different concentration of caspofungin as indicated. For more explanations see the legend of Fig. S3. Note that experiment was conducted on the same microtiter plate.

FIG S4 Analysis of caspofungin susceptibility of *pga4*-/- and FJS5 mutants with both copies of either *PGA4* (1) or *CHT2* (2), respectively, disrupted with the *Tn7*-UAU1 cassette, as well as of the mutant DSY1768 (3) having incomplete deletion of ORF of *CHT2*. Shown is the spot assay for growth of the mutant FJS5 vs the control strain DAY286 (and also vs the

parental strain BWP17) or of the mutant DSY1768 or the mutant *pga4*-/- vs the control strain CAF2-1 on control YPD medium supplemented with arginine, histidine and uridine, as well as on YPD medium supplemented with the aforementioned aminoacids and caspofungin (CAS), as indicated. Strains are indicated on the left. From left to right, 10^4, 10^3, 10^2, and 10^1 cells were spotted on each plate and incubated for 3 days at 37°C.

FIG S5 Example of semiquantitative RT-PCR analysis of products amplified from total RNA from deletion strain JMC200-3-3 and its parental strain JRCT1. The *CHT2*, *CSU51* or *PGA4* gene was amplified and ran on a conventional electrophoresis gel, which was ethidium bromide stained and photographed and the imaged was processed, as described in Materials and Methods. Lanes show products sampled at increasing cycles. Values for the *CHT2*, *CSU51* or *PGA4* gene were normalized against control *REX2*. *CHT2*, *CSU51* or *PGA4* monosomic to disomic (JMC200-3-3/JRCT1) ratio was calculated, as averaged from three consecutive cycles.

TABLE S1 Calculated values[a] of caspofungin MICs (ng/ml) for double-deletion mutants and CAF4-2 (2nd and 3rd set of experiments)

		NAC4		NACS1		NAP88
	CAF4-2	a.k.a. *cht2*-/-	CAF4-2	a.k.a. *csu51*-/-	CAF4-2	a.k.a. *pga4*-/-
MIC_{50}	91 ± 4	122 ± 1	31 ± 1	63 ± 14	45 ± 2	123 ± 1
MIC_{70}	105 ± 5	125 ± 1	39 ± 1	107 ± 26	59 ± 3	123 ± 1
MIC_{80}	115 ± 6	128 ± 1	46 ± 1	150 ± 36	70 ± 4	124 ± 1
MIC_{90}	139 ± 1	171 ± 1	58 ± 1	248 ± 59	90 ± 5	124 ± 6

[a] MIC_{50}, MIC_{70}, MIC_{80}, or MIC_{90} refers to the concentration of caspofungin at which 50%, 70%, 80%, or 90% of growth is inhibited. See Materials and Methods for the calculation of MIC values. The differences between mutants and CAF4-2 were evaluated with Student's *t* test and all p-values were <0.05.

		NAC4		NACS1		NAP88
	CAF4-2	a.k.a. *cht2*-/-	CAF4-2	a.k.a. *csu51*-/-	CAF4-2	a.k.a. *pga4*-/-
MIC_{50}	117 ± 5	206 ± 11	96 ± 6	154 ± 25	60 ± 1	157 ± 4
MIC_{70}	146 ± 6	233 ± 13	103 ± 6	260 ± 56	75 ± 1	193 ± 5
MIC_{80}	167 ± 7	254 ± 14	107 ± 6	362 ± 101	87 ± 1	220 ± 6
MIC_{90}	206 ± 9	284 ± 15	113 ± 6	331 ± 26	108 ± 1	267 ± 7

[a] MIC_{50}, MIC_{70}, MIC_{80}, or MIC_{90} refers to the concentration of caspofungin at which 50%, 70%, 80%, or 90% of growth is inhibited. See Materials and Methods for the calculation of MIC values. The differences between mutants and CAF4-2 were evaluated with Student's *t* test and all p-values were <0.05.

41 TABLE S2 Calculated values[a] of anidulafungin MICs (ng/ml) for double-deletion

42 mutants and CAF4-2 (2nd and 3rd set of experiments)

	CAF4-2	NAC4 a.k.a. *cht2-/-*	NACS1 a.k.a. *csu51-/-*	NAP88 a.k.a. *pga4-/-*
MIC_{50}	8.5 ± 1	25 ± 2.1*	11.5 ± 1.2	64.5 ± 1.2*
MIC_{70}	12 ± 1.4	22.7 ± 1.8*	14.6 ± 1.5	71.5 ± 3.1*
MIC_{80}	15 ± 1.8	24.3 ± 1.9*	16.9 ± 1.8	68.8 ± 1.4*
MIC_{90}	20.8 ± 2.5	27.0 ± 2.2	21.3 ± 2.2	73.4 ± 1.8*

[a] MIC_{50}, MIC_{70}, MIC_{80}, or MIC_{90} refers to the concentration of caspofungin at which 50%, 70%, 80%, or 90% of growth is inhibited. See Materials and Methods for the calculation of MIC values. The differences between mutants and CAF4-2 were evaluated with Student's *t* test and p-values indicated as an asterisk (*) were <0.001.

	CAF4-2	NAC4 a.k.a. *cht2-/-*	NACS1 a.k.a. *csu51-/-*	NAP88 a.k.a. *pga4-/-*
MIC_{50}	9.1 ± 1.0	21.6 ± 0.9*	13.6 ± 0.8*	31.9 ± 1.6*
MIC_{70}	11.1 ± 1.3	24.5 ± 1.0*	15.1 ± 0.9*	35.6 ± 1.8*
MIC_{80}	12.6 ± 1.4	26.6 ± 1.1*	16.2 ± 1.0	38.1 ± 1.9*
MIC_{90}	15.2 ± 1.7	30.1 ± 1.3*	17.9 ± 1.1	42.3 ± 2.1*

[a] MIC_{50}, MIC_{70}, MIC_{80}, or MIC_{90} refers to the concentration of caspofungin at which 50%, 70%, 80%, or 90% of growth is inhibited. See Materials and Methods for the calculation of MIC values. The differences between mutants and CAF4-2 were evaluated with Student's *t* test and all p-values indicated as an asterisk (*) were <0.05.

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1. **Plaine A, Walker L, Da Costa G, Mora-Montes HM, McKinnon A, Gow NA, Gaillardin C, Munro CA, Richard ML.** 2008. Functional analysis of *Candida albicans* GPI-anchored proteins: roles in cell wall integrity and caspofungin sensitivity. Fungal Genet Biol **10:**1404-1414.
2. **Nobile CJ, Andes DR, Nett JE, Smith FJ, Yue F, Phan QT, Edwards JE, Filler SG, Mitchell AP.** 2006. Critical role of Bcr1-dependent adhesins in *C. albicans* biofilm formation in vitro and in vivo. PLoS Pathog **2:**e63.
3. **Selvaggini S, Munro CA, Paschoud S, Sanglard D, Gow NA.** 2004. Independent regulation of chitin synthase and chitinase activity in *Candida albicans* and *Saccharomyces cerevisiae*. Microbiology **150:**921-928.

Fig. S1

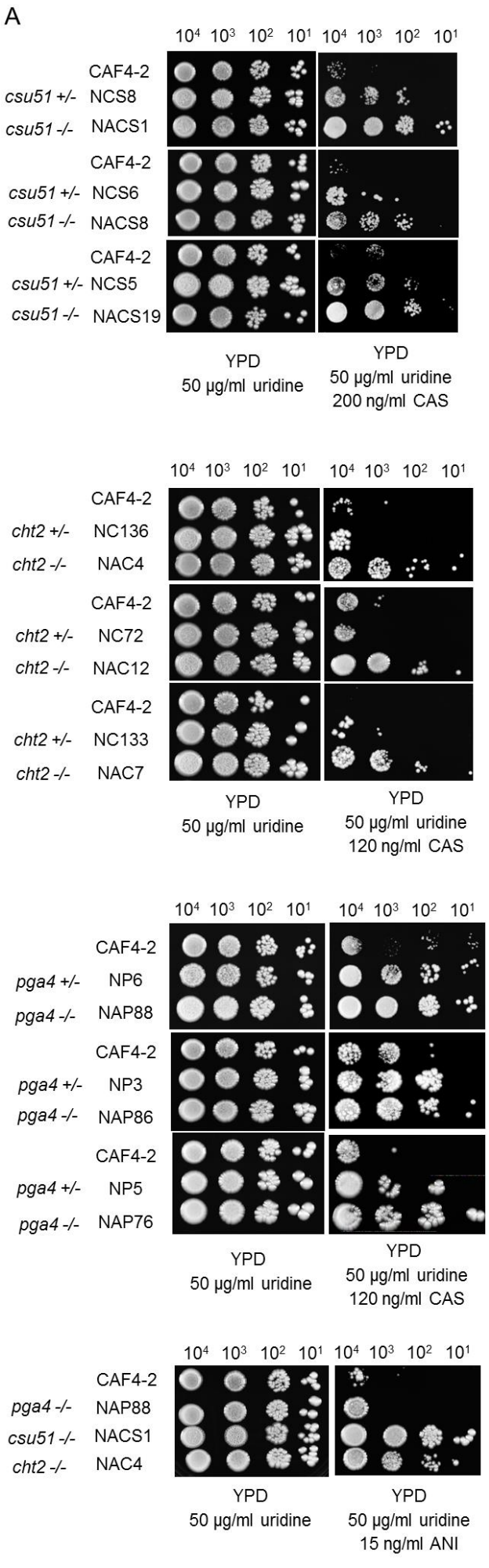
A
10⁴ 10³ 10² 10¹ 10⁴ 10³ 10² 10¹
CAF4-2
csu51 +/- NCS8
csu51 -/- NACS1
CAF4-2
csu51 +/- NCS6
csu51 -/- NACS8
CAF4-2
csu51 +/- NCS5
csu51 -/- NACS19
YPD
50 µg/ml uridine
YPD
50 µg/ml uridine
200 ng/ml CAS
10⁴ 10³ 10² 10¹ 10⁴ 10³ 10² 10¹
CAF4-2
cht2 +/- NC136
cht2 -/- NAC4
CAF4-2
cht2 +/- NC72
cht2 -/- NAC12
CAF4-2
cht2 +/- NC133
cht2 -/- NAC7
YPD
50 µg/ml uridine
YPD
50 µg/ml uridine
120 ng/ml CAS
10⁴ 10³ 10² 10¹ 10⁴ 10³ 10² 10¹
CAF4-2
pga4 +/- NP6
pga4 -/- NAP88
CAF4-2
pga4 +/- NP3
pga4 -/- NAP86
CAF4-2
pga4 +/- NP5
pga4 -/- NAP76
YPD
50 µg/ml uridine
YPD
50 µg/ml uridine
120 ng/ml CAS
10⁴ 10³ 10² 10¹ 10⁴ 10³ 10² 10¹
CAF4-2
pga4 -/- NAP88
csu51 -/- NACS1
cht2 -/- NAC4
YPD
50 µg/ml uridine
YPD
50 µg/ml uridine
15 ng/ml ANI

Fig. S1

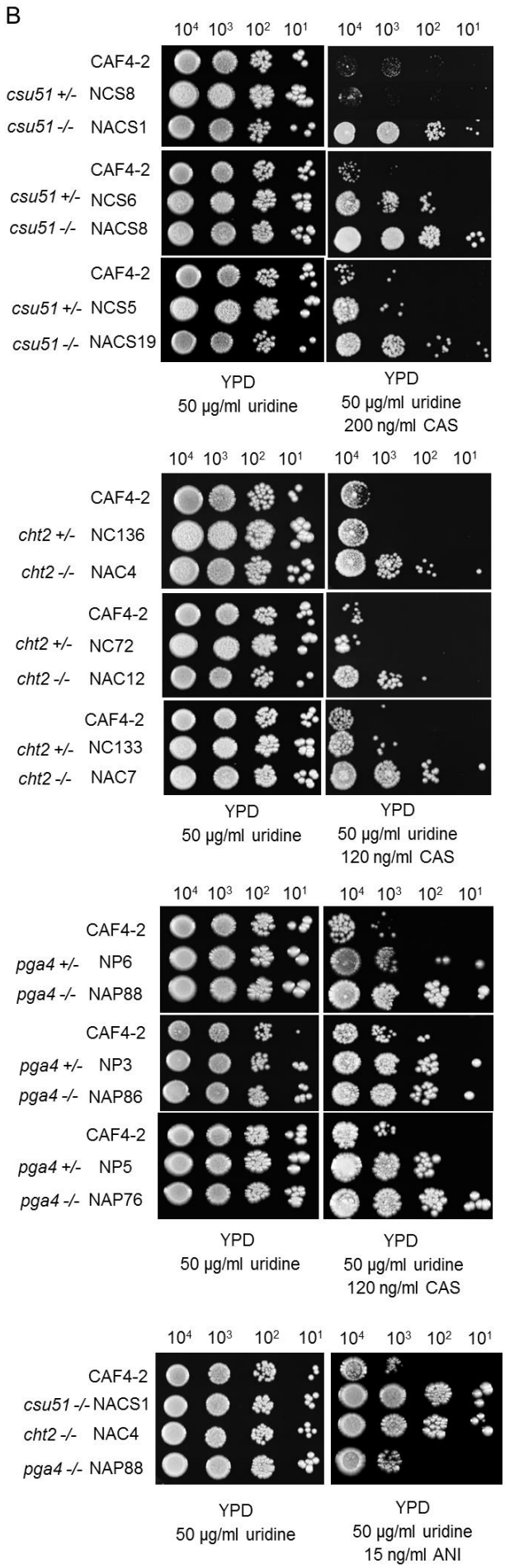
B
10⁴ 10³ 10² 10¹ 10⁴ 10³ 10² 10¹
CAF4-2
csu51 +/- NCS8
csu51 -/- NACS1
CAF4-2
csu51 +/- NCS6
csu51 -/- NACS8
CAF4-2
csu51 +/- NCS5
csu51 -/- NACS19
YPD
50 µg/ml uridine
YPD
50 µg/ml uridine
200 ng/ml CAS
10⁴ 10³ 10² 10¹ 10⁴ 10³ 10² 10¹
CAF4-2
cht2 +/- NC136
cht2 -/- NAC4
CAF4-2
cht2 +/- NC72
cht2 -/- NAC12
CAF4-2
cht2 +/- NC133
cht2 -/- NAC7
YPD
50 µg/ml uridine
YPD
50 µg/ml uridine
120 ng/ml CAS
10⁴ 10³ 10² 10¹ 10⁴ 10³ 10² 10¹
CAF4-2
pga4 +/- NP6
pga4 -/- NAP88
CAF4-2
pga4 +/- NP3
pga4 -/- NAP86
CAF4-2
pga4 +/- NP5
pga4 -/- NAP76
YPD
50 µg/ml uridine
YPD
50 µg/ml uridine
120 ng/ml CAS
10⁴ 10³ 10² 10¹ 10⁴ 10³ 10² 10¹
CAF4-2
csu51 -/- NACS1
cht2 -/- NAC4
pga4 -/- NAP88
YPD
50 µg/ml uridine
YPD
50 µg/ml uridine
15 ng/ml ANI

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Fig. S2

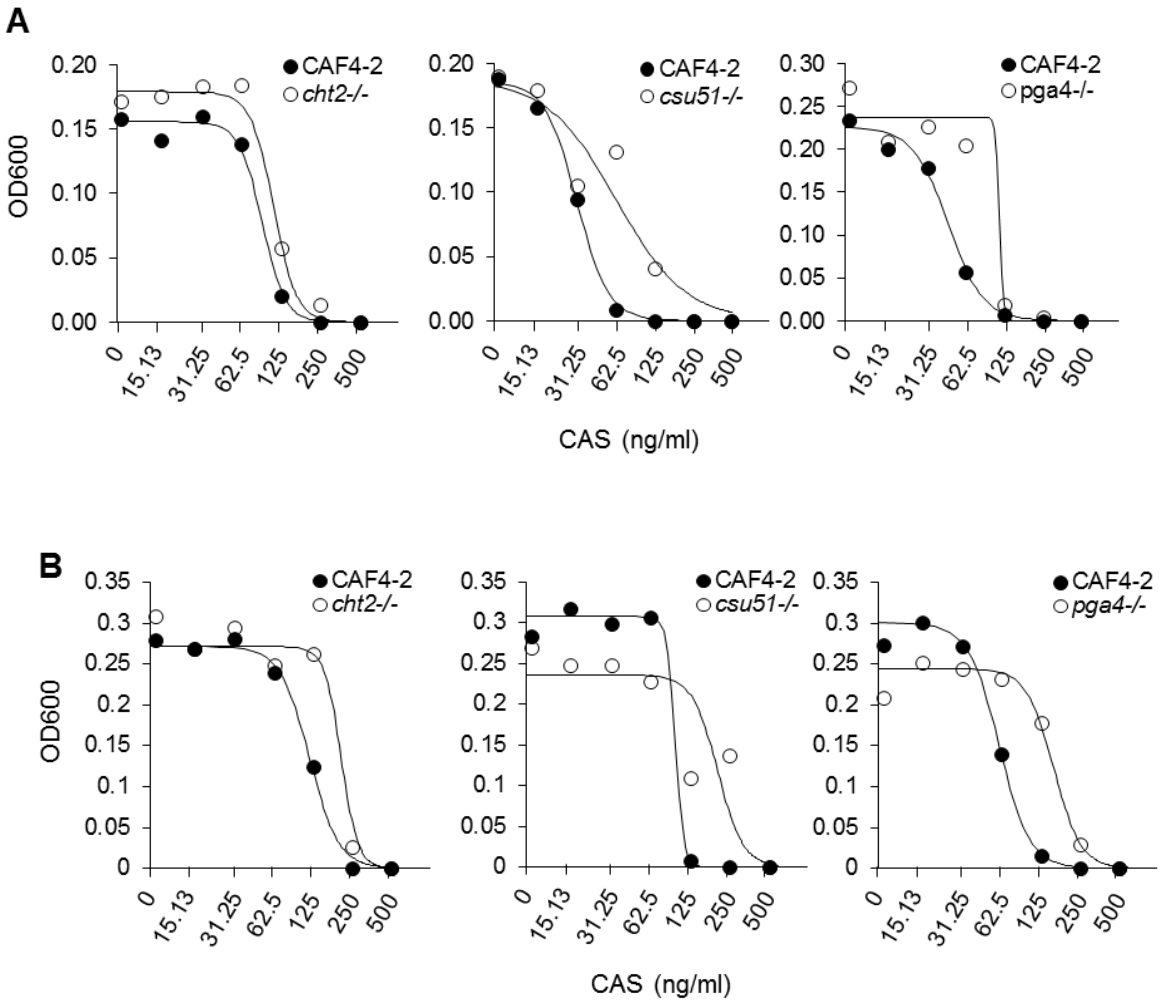

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Fig. S3

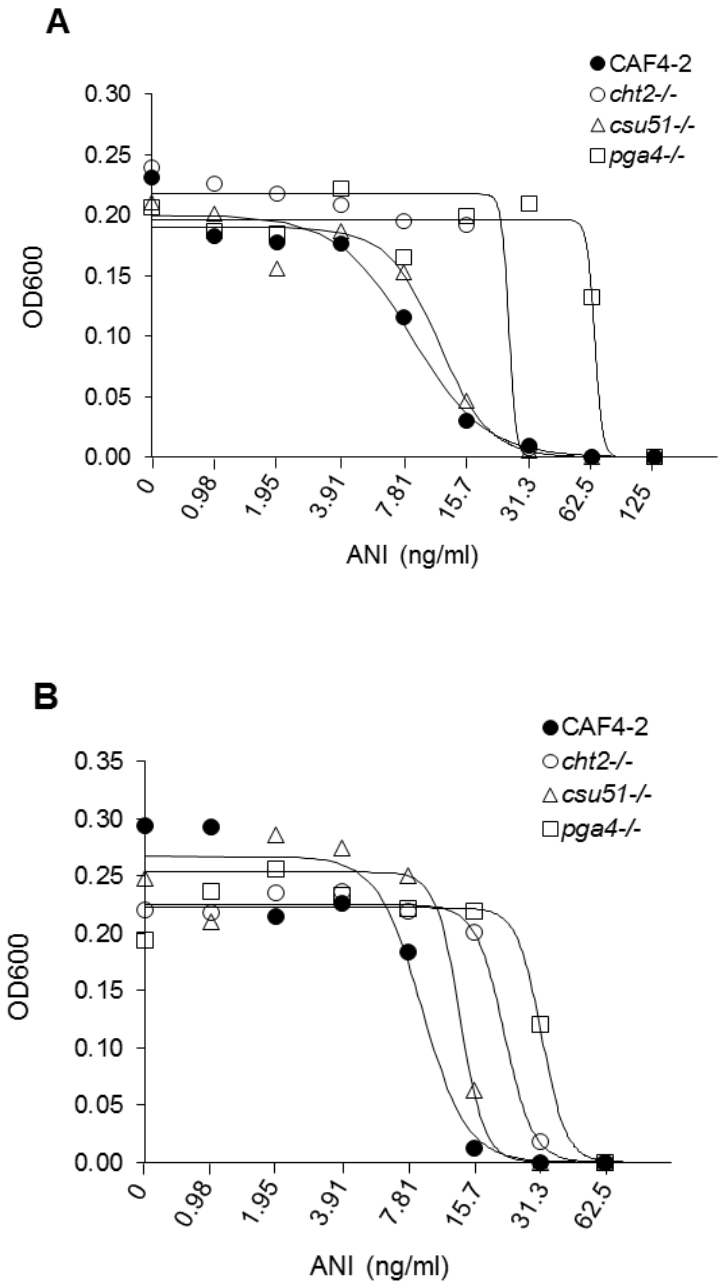

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Fig. S4

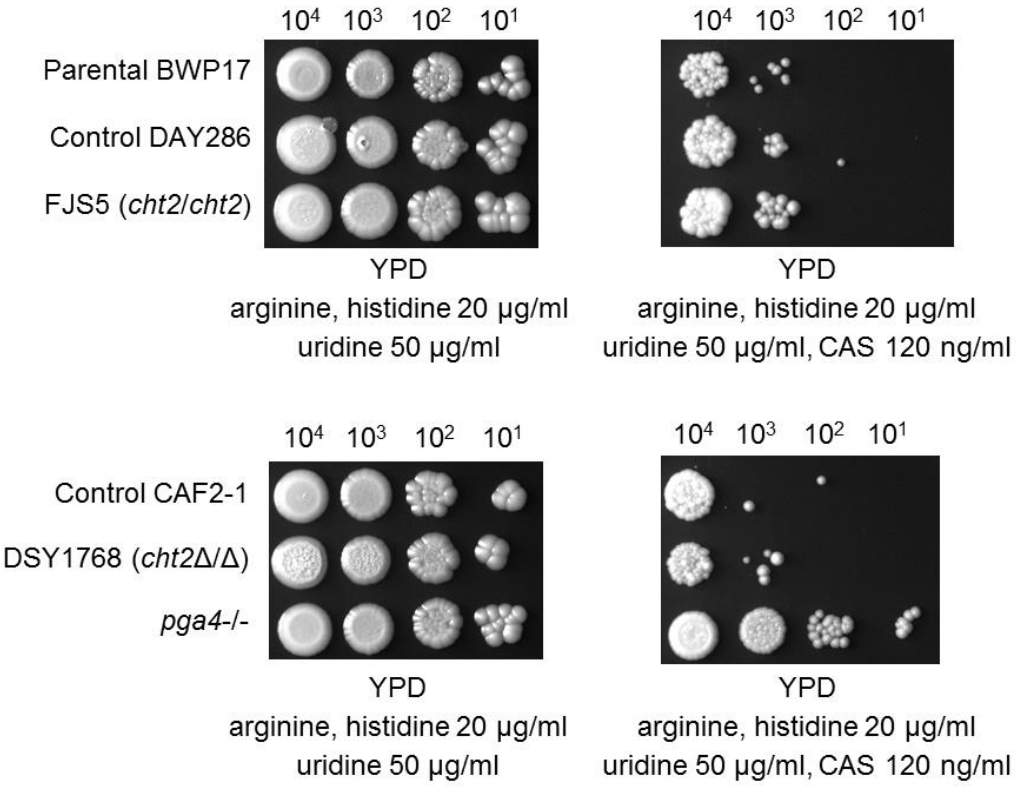

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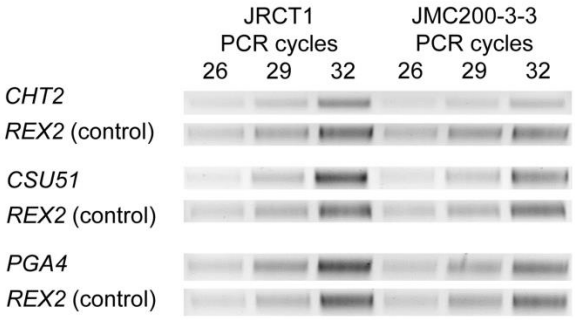
Fig. S5



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