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Supplemental Data

FLO1 Is a Variable Green Beard Gene that Drives Biofilm-like Cooperation in Budding Yeast

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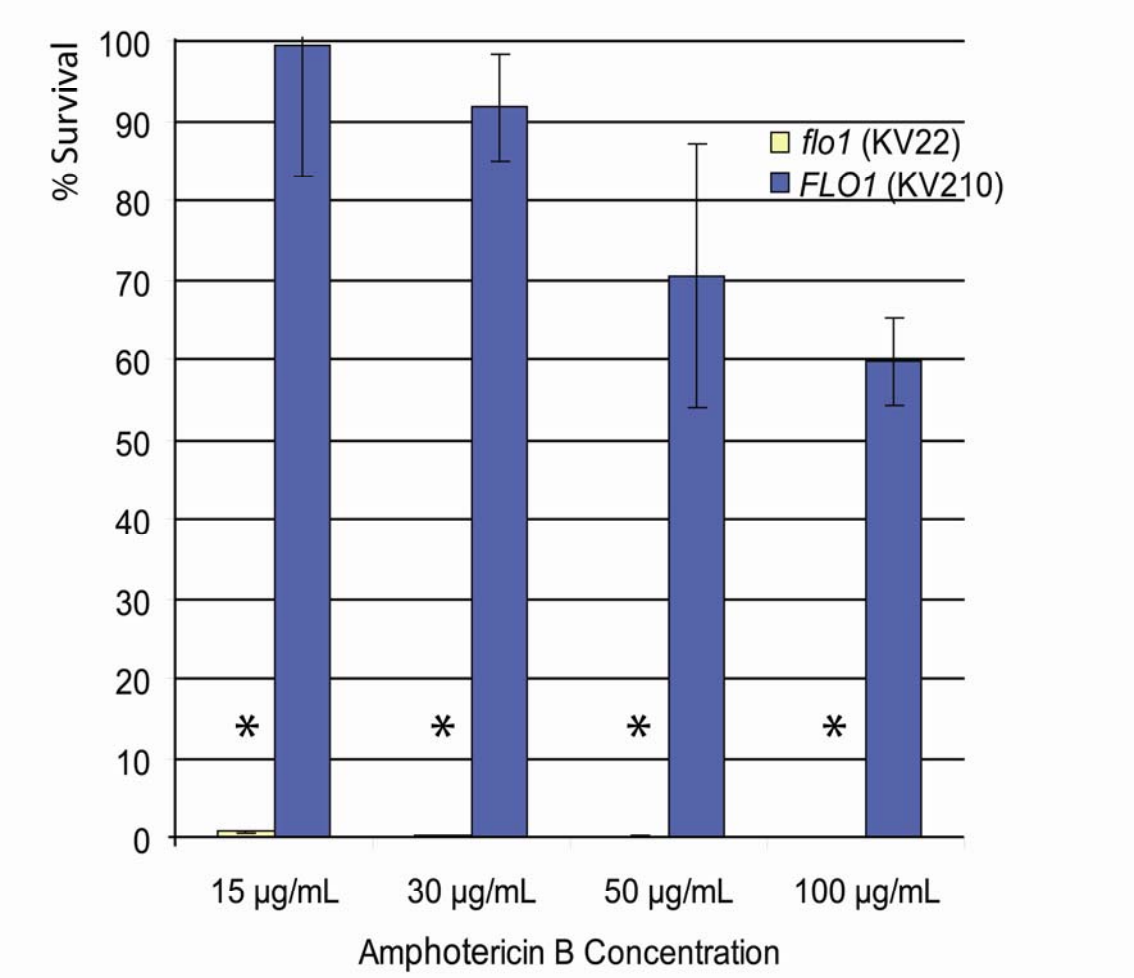
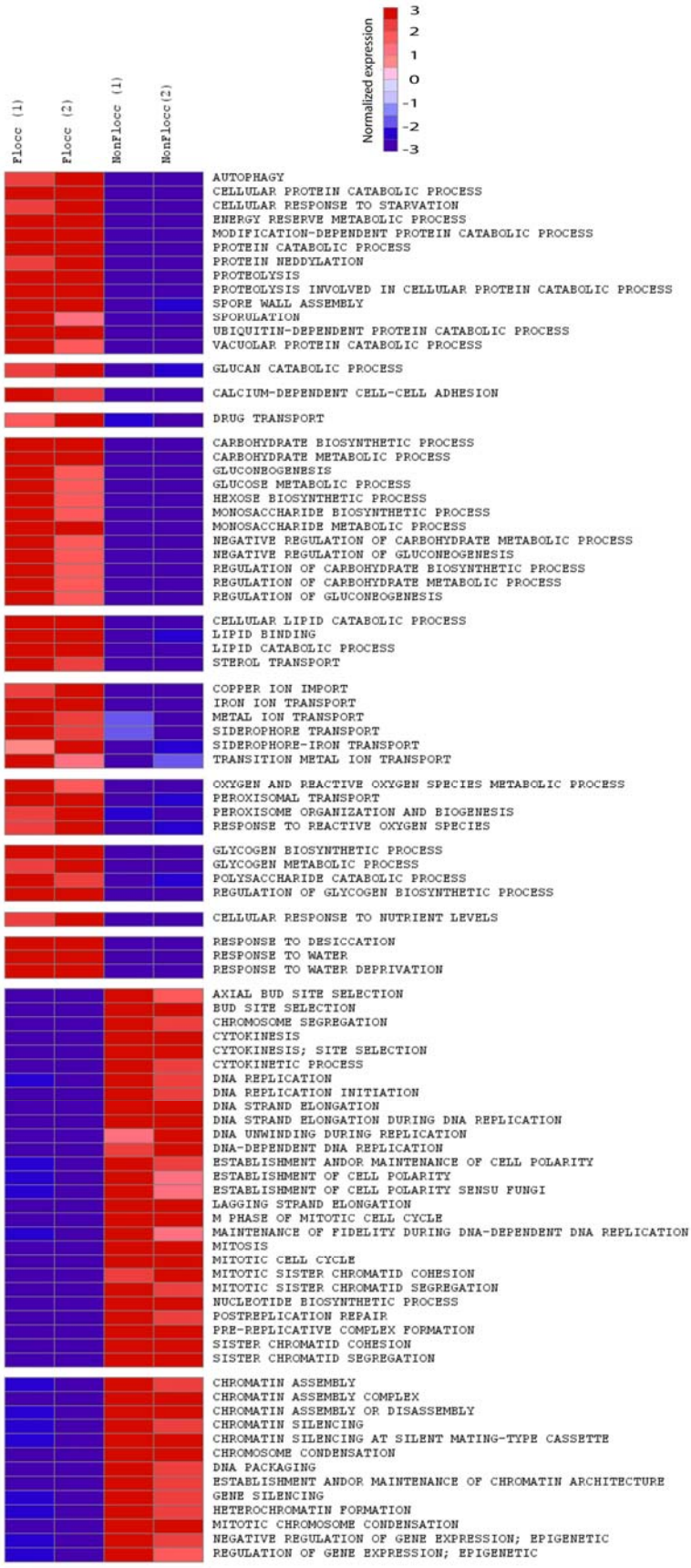


Figure S1. **Flocculent cultures are resistant to extremely high concentrations of amphotericin B.** Flocculent (*FLO1*, KV210) and non-flocculent (*flo1*, KV22) cells were subjected to increasing concentrations of amphotericin B for 4 hours, after which the percentage of surviving cells was determined. Asterisks indicate statistical differences between the flocculating and non-flocculating cultures ($\alpha = 0.05$). Error bars represent standard deviation.


Flocc (1)
Flocc (2)
NonFlocc (1)
NonFlocc(2)
Normalized expression
3
2
1
0
-1
-2
-3
AUTOPHAGY
CELLULAR PROTEIN CATABOLIC PROCESS
CELLULAR RESPONSE TO STARVATION
ENERGY RESERVE METABOLIC PROCESS
MODIFICATION-DEPENDENT PROTEIN CATABOLIC PROCESS
PROTEIN CATABOLIC PROCESS
PROTEIN NEDDYLATION
PROTEOLYSIS
PROTEOLYSIS INVOLVED IN CELLULAR PROTEIN CATABOLIC PROCESS
SPORE WALL ASSEMBLY
SPORULATION
UBIQUITIN-DEPENDENT PROTEIN CATABOLIC PROCESS
VACUOLAR PROTEIN CATABOLIC PROCESS
GLUCAN CATABOLIC PROCESS
CALCIUM-DEPENDENT CELL-CELL ADHESION
DRUG TRANSPORT
CARBOHYDRATE BIOSYNTHETIC PROCESS
CARBOHYDRATE METABOLIC PROCESS
GLUCONEOGENESIS
GLUCOSE METABOLIC PROCESS
HEXOSE BIOSYNTHETIC PROCESS
MONOSACCHARIDE BIOSYNTHETIC PROCESS
MONOSACCHARIDE METABOLIC PROCESS
NEGATIVE REGULATION OF CARBOHYDRATE METABOLIC PROCESS
NEGATIVE REGULATION OF GLUCONEOGENESIS
REGULATION OF CARBOHYDRATE BIOSYNTHETIC PROCESS
REGULATION OF CARBOHYDRATE METABOLIC PROCESS
REGULATION OF GLUCONEOGENESIS
CELLULAR LIPID CATABOLIC PROCESS
LIPID BINDING
LIPID CATABOLIC PROCESS
STEROL TRANSPORT
COPPER ION IMPORT
IRON ION TRANSPORT
METAL ION TRANSPORT
SIDEROPHORE TRANSPORT
SIDEROPHORE-IRON TRANSPORT
TRANSITION METAL ION TRANSPORT
OXYGEN AND REACTIVE OXYGEN SPECIES METABOLIC PROCESS
PEROXISOMAL TRANSPORT
PEROXISOME ORGANIZATION AND BIOGENESIS
RESPONSE TO REACTIVE OXYGEN SPECIES
GLYCOGEN BIOSYNTHETIC PROCESS
GLYCOGEN METABOLIC PROCESS
POLYSACCHARIDE CATABOLIC PROCESS
REGULATION OF GLYCOGEN BIOSYNTHETIC PROCESS
CELLULAR RESPONSE TO NUTRIENT LEVELS
RESPONSE TO DESICCATION
RESPONSE TO WATER
RESPONSE TO WATER DEPRIVATION
AXIAL BUD SITE SELECTION
BUD SITE SELECTION
CHROMOSOME SEGREGATION
CYTOKINESIS
CYTOKINESIS; SITE SELECTION
CYTOKINETIC PROCESS
DNA REPLICATION
DNA REPLICATION INITIATION
DNA STRAND ELONGATION
DNA STRAND ELONGATION DURING DNA REPLICATION
DNA UNWINDING DURING REPLICATION
DNA-DEPENDENT DNA REPLICATION
ESTABLISHMENT AND/OR MAINTENANCE OF CELL POLARITY
ESTABLISHMENT OF CELL POLARITY
ESTABLISHMENT OF CELL POLARITY SENSU FUNGI
LAGGING STRAND ELONGATION
M PHASE OF MITOTIC CELL CYCLE
MAINTENANCE OF FIDELITY DURING DNA-DEPENDENT DNA REPLICATION
MITOSIS
MITOTIC CELL CYCLE
MITOTIC SISTER CHROMATID COHESION
MITOTIC SISTER CHROMATID SEGREGATION
NUCLEOTIDE BIOSYNTHETIC PROCESS
POSTREPLICATION REPAIR
PRE-REPLICATIVE COMPLEX FORMATION
SISTER CHROMATID COHESION
SISTER CHROMATID SEGREGATION
CHROMATIN ASSEMBLY
CHROMATIN ASSEMBLY COMPLEX
CHROMATIN ASSEMBLY OR DISASSEMBLY
CHROMATIN SILENCING
CHROMATIN SILENCING AT SILENT MATING-TYPE CASSETTE
CHROMOSOME CONDENSATION
DNA PACKAGING
ESTABLISHMENT AND/OR MAINTENANCE OF CHROMATIN ARCHITECTURE
GENE SILENCING
HETEROCHROMATIN FORMATION
MITOTIC CHROMOSOME CONDENSATION
NEGATIVE REGULATION OF GENE EXPRESSION; EPIGENETIC
REGULATION OF GENE EXPRESSION; EPIGENETIC

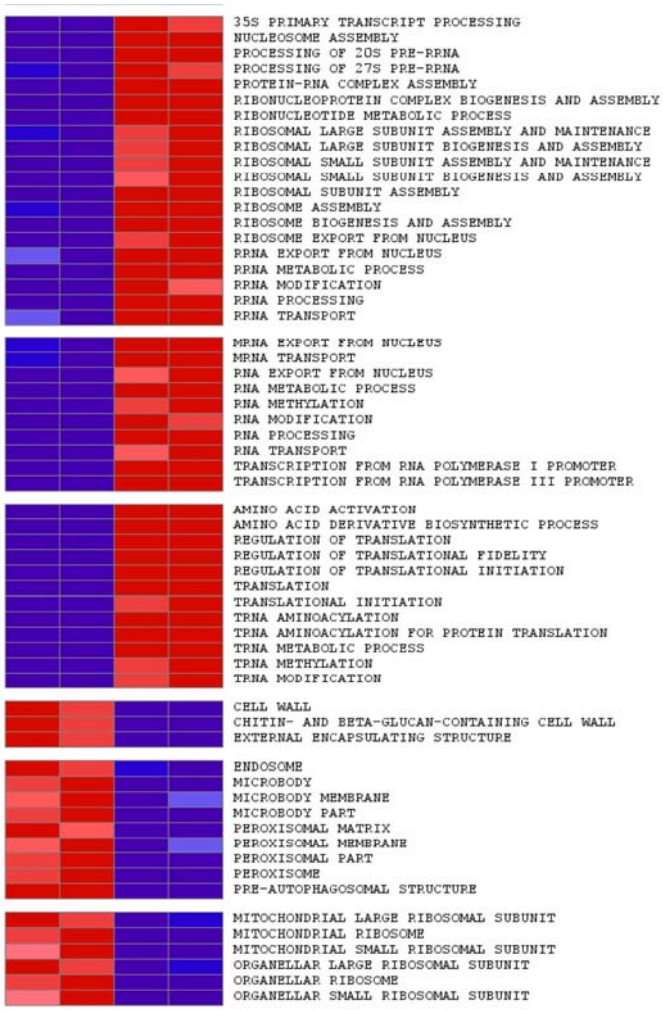


Figure S2. **Flocculating and non-flocculating cells show differential expression of several gene sets.** Gene Ontology (GO) gene sets describing biological processes and cellular components (rows) that differ significantly between flocculating and non-flocculating experiments are shown. The gene sets are grouped according to higher-order categories. For each gene set, the median expression of the leading-edge genes in each experiment from the two conditions is shown (columns). Furthermore, each gene set was normalized by mean centering and unit scaling prior to visualization. Red and blue respectively represent induction and repression as compared to average across all experiments.

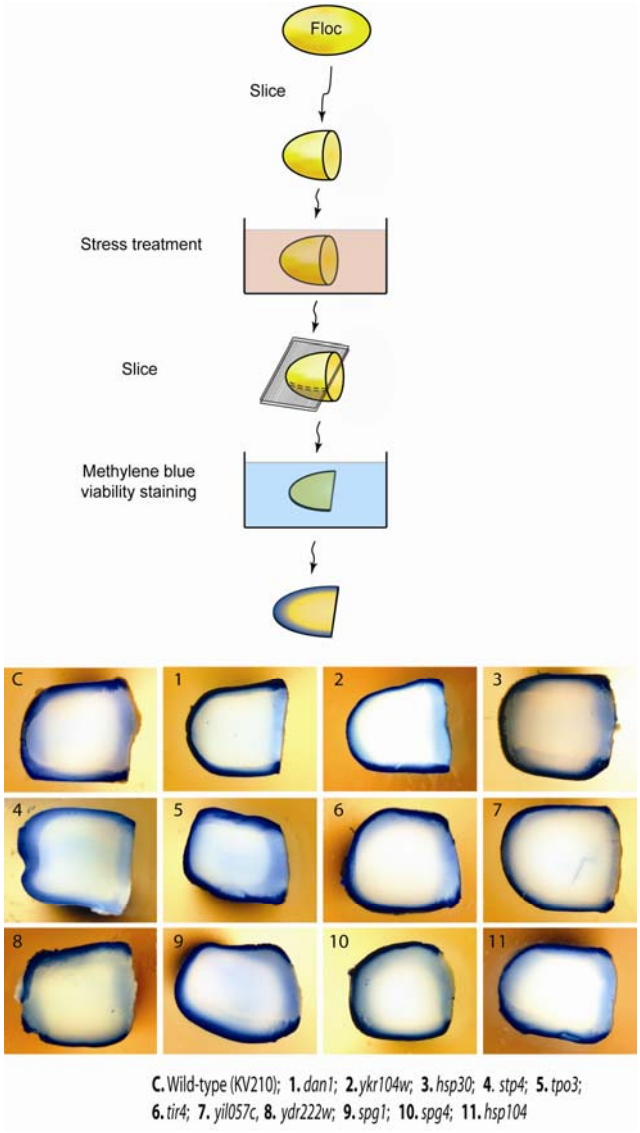


Figure S3. **Deletion of genes that are upregulated in flocculating cultures does not affect resistance to amphotericin B.** We constructed ten mutant strains of the flocculent (*FLO1*$^{+}$) strain KV210. Each of these mutant strains lacks a functional copy of one gene that is upregulated in flocculating cultures (see our gene array analyses). Flocs of these strains were first cut in half before they were submerged into medium containing 100 μg ml^{-1} amphotericin B for 45 minutes. After this treatment, slices of the floc were stained with the methylene blue dye to test for viability (blue cells are dead). Survival was also measured by plating (not shown). No significant differences in amphotericin B resistance were found between any of the mutants and the wild-type control (KV210)

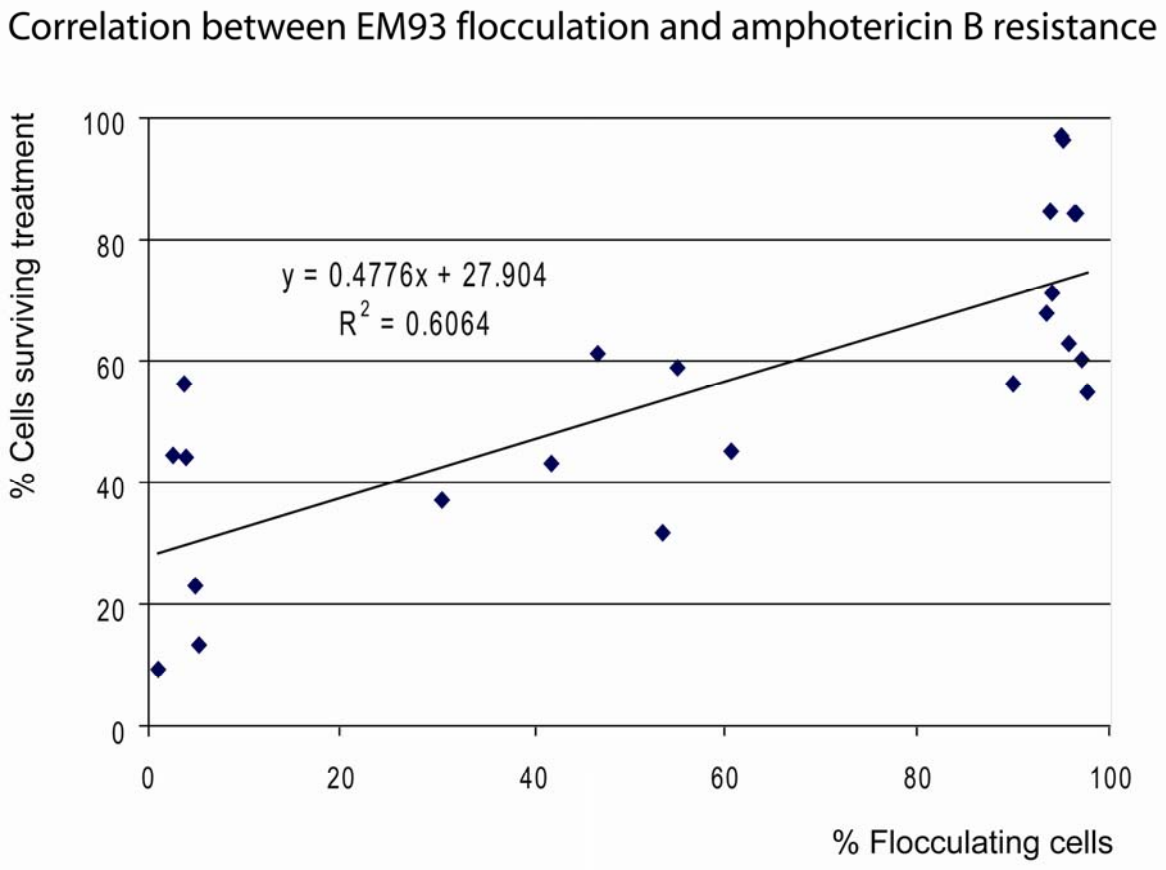


Figure S4. **Correlation between flocculation and amphotericin B resistance in the feral *S. cerevisiae* strain EM93.** Flocculation and % survival after a 4-hour treatment with 100 μg ml^{-1} amphotericin B was measured for 24 haploid derivatives of the feral EM93 strain. The observed positive Pearson correlation between flocculation and amphotericin B resistance indicates that about 60% of the variability in amphotericin B resistance among the 24 strains may be the result of differences in flocculation behavior ($p < 1\ 10^{-5}$).

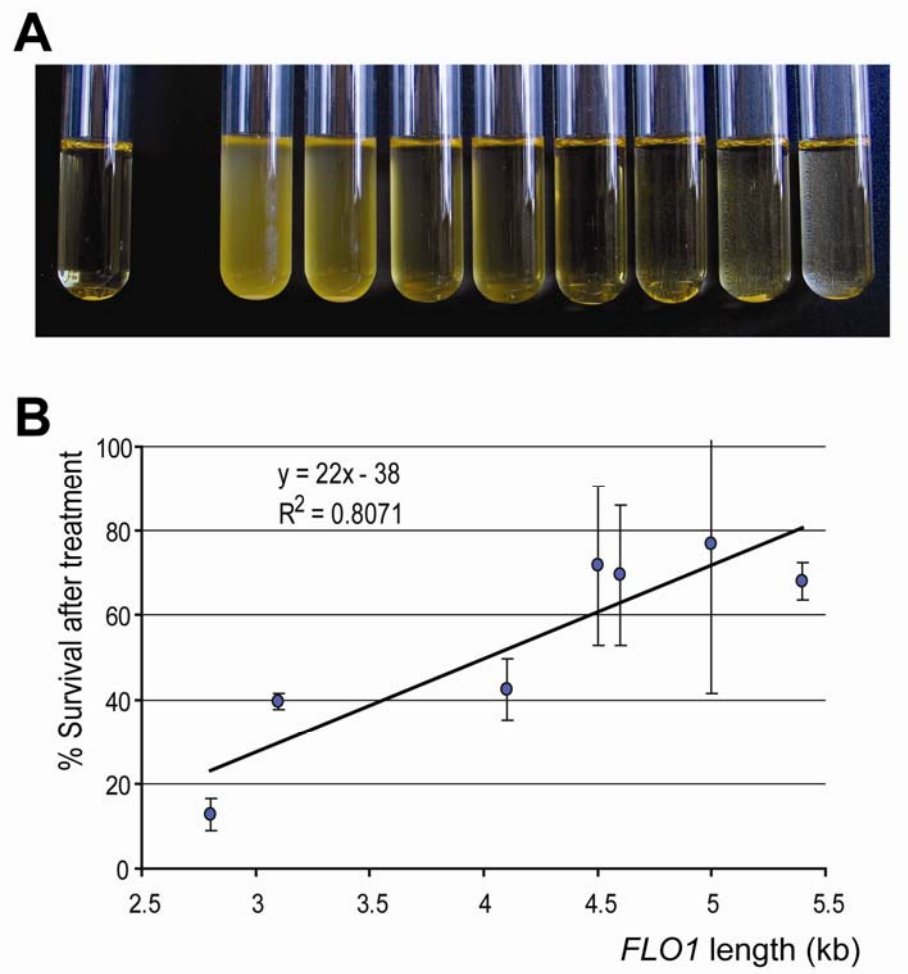


Figure S5. **Flocculation and resistance to amphotericin B correlate with the length of the hypervariable internal repeat region in the *FLO1* gene.** The *S. cerevisiae FLO1* gene contains a stretch of internal tandem repeats. This region is extremely variable, with the number of repeats changing at high rates. **A**. *FLO1*-dependent flocculation gradually increases with an increasing number of internal *FLO1* repeat units (left to right). **B**. Strains overexpressing *FLO1* alleles with an increasing number of internal repeat units show gradually increasing resistance to amphotericin B. Error bars represent standard deviation.

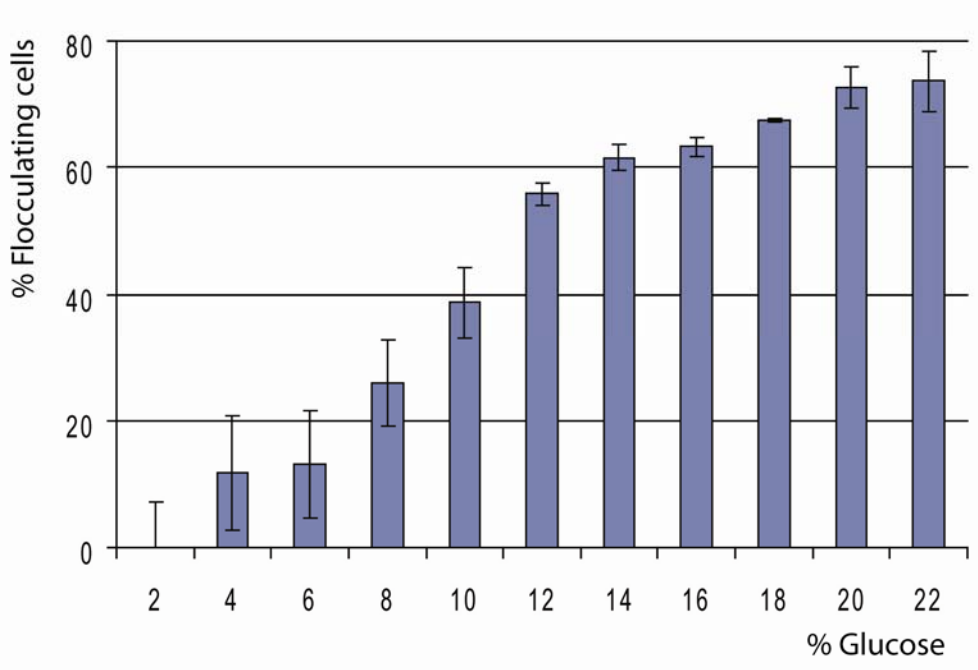


Figure S6. **Flocculation of the feral EM93 strain increases with increasing initial glucose concentration of the growth medium.** Cultures of EM93 cells were grown in YPD medium containing increasing levels of glucose. Flocculation was measured after 24h of growth. Error bars represent standard deviation.

Table S1. Yeast strains

Strain	Relevant Genotype	Reference/ Source
S288C (BY4742)	MATα ; *his3*D1; *leu2*D0; *lys2*D0; *ura3*D0	(Brachmann et al., 1998)
S288C (BY4743)	MAT a/α ; *his3*D1; *leu2*D0; *lys2*D0; *ura3*D0	(Brachmann et al., 1998)
KV210	S288C BY4741 containing *GAL*p-*FLO1* fusion	(Verstrepen et al., 2005)
KV428	S288C BY4741 containing *TEF*p-*FLO1* fusion	This study
KV22	S288C BY4741 *flo1*::*KANMX*	This study
KV211 (KV381); 219 (184); 220 (186); 224 (193); 298 (291), 308 (304), 311 (306), 312 (307)	GAL_P::*FLO1* fusions with various alleles of *FLO1* containing different numbers of internal DNA repeats. Numbers in brackets refer to respective parental strains without GAL_P	(Verstrepen et al., 2005)
EM93	Mat a/α Feral strain, progenitor of S288C	(Mortimer and Johnston, 1986)
KV34	EM93 1A (haploid strain obtained from EM93, 1st tetrad)	This study
KV35	EM93 1B (haploid strain obtained from EM93, 1st tetrad)	This study
KV36	EM93 1C (haploid strain obtained from EM93, 1st tetrad)	This study
KV37	EM93 1D (haploid strain obtained from EM93, 1st tetrad)	This study
KV38	EM93 2A (haploid strain obtained from EM93, 2nd tetrad)	This study
KV39	EM93 2B (haploid strain obtained from EM93, 2nd tetrad)	This study
KV40	EM93 2C (haploid strain obtained from EM93, 2nd tetrad)	This study
KV41	EM93 2D (haploid strain obtained from EM93, 2nd tetrad)	This study
KV42	EM93 5A (haploid strain obtained from EM93, 5th tetrad)	This study
KV43	EM93 5B (haploid strain obtained from EM93, 5th tetrad)	This study
KV44	EM93 5C (haploid strain obtained from EM93, 5th tetrad)	This study
KV45	EM93 5D (haploid strain obtained from EM93, 5th tetrad)	This study
KV46	EM93 8A (haploid strain obtained from EM93, 8th tetrad)	This study
KV47	EM93 8B (haploid strain obtained from EM93, 8th tetrad)	This study
KV48	EM93 8C (haploid strain obtained from EM93, 8th tetrad)	This study
KV49	EM93 8D (haploid strain obtained from EM93, 8th tetrad)	This study
KV50	EM93 9A (haploid strain obtained from EM93, 9th tetrad)	This study
KV51	EM93 9B (haploid strain obtained from EM93, 9th tetrad)	This study
KV52	EM93 9C (haploid strain obtained from EM93, 9th tetrad)	This study
KV53	EM93 9D (haploid strain obtained from EM93, 9th tetrad)	This study
KV54	EM93 10A (haploid strain obtained from EM93, 10th tetrad)	This study
KV55	EM93 10B (haploid strain obtained from EM93, 10th tetrad)	This study
KV56	EM93 10C (haploid strain obtained from EM93, 10th tetrad)	This study
KV57	EM93 10D (haploid strain obtained from EM93, 10th tetrad)	This study
KV478	KV210 *dan1*::*HYGB*	This study
KV479	KV210 *ykr104*::*HYGB*	This study

KV480	KV210 *hsp30*::*HYGB*	This study
KV481	KV210 *stp4*::*HYGB*	This study
KV483	KV210 *tpo3*::*HYGB*	This study
KV485	KV210 *tir4*::*HYGB*	This study
KV486	KV210 *hsp104*::*HYGB*	This study
KV500	KV210 *yil057c*::*HYGB*	This study
KV613	KV210 *ydr222w*::*HYGB*	This study
KV615	KV210 *spg4*::*HYGB*	This study
KV617	KV210 *spg1*::*HYGB*	This study
KV1492	BY4741 *tdh3*::mCherry-*HIS3*	This study
KV1493	BY4741 *tdh3*::yECitrine-*HIS3*	This study
KV1526	BY4741 *TDH3*p::mCherry-*HIS3*	This study
KV1527	BY4741 *TDH3*p::yECitrine-*HIS3*	This study
KV1526	BY4741 *TDH3*p::mCherry-*HYGB*	This study
KV1527	BY4741 *TDH3*p::yECitrine-*HYGB*	This study
KV1579	KV44 *TDH3*p::yECitrine-*HYGB*	This study
KV1581	KV44 *TDH3*p::mCherry-*HYGB*	This study
KV1588	KV48 *TDH3*p::yECitrine-*HYGB*	This study
KV1613	KV48 *TDH3*p::mCherry-*HYGB*	This study
KV1590	KV210 *TDH3*p::yECitrine-*HYGB*	This study
KV1591	KV210 *TDH3*p::mCherry-*HYGB*	This study
KV1557	*Saccharomyces paradoxus*	National Collection of yeast Culture
KV1602	KV1557 GAL_P::*FLO1*	This study
KV1615	KV 1557 *PYK2*p::mCherry-*HYGB*	This study
KV1616	KV 1602 *PYK2*p::yECitrine-*HYGB*	This study
KV1492	BY4741 *tdh3*::mCherry-*HIS3*	This study
KV1493	BY4741 *tdh3*::yECitrine-*HIS3*	This study
KV1526	BY4741 *TDH3*p::mCherry-*HIS3*	This study
KV1527	BY4741 *TDH3*p::yECitrine-*HIS3*	This study
KV1526	BY4741 *TDH3*p::mCherry-*HYGB*	This study
KV1527	BY4741 *TDH3*p::yECitrine-*HYGB*	This study
KV1579	KV44 *TDH3*p::yECitrine-*HYGB*	This study
KV1873	KV52 *flo1*::KAN	This study
KV1875	KV1873 *TDH3*p::yECitrine-*HYGB KANMX*	This study
KV1876	KV1873 *TDH3*p::mCherry-*HYGB KANMX*	This study
KV1877	KV52 *TDH3*p::yECitrine-*HYGB KANMX*	This study
KV1878	KV52 *TDH3*p::mCherry-*HYGB KANMX*	This study

Table S2. Oligonucleotide sequences

Oligo Name	Sequence
28-FLO9-RT-F2	TTATTGTTTACTACTAGCCATCGTCACA
34-FLO10-RT-R2	CGCAATCGTCATTTTCACGTTT
35-FLO11-RT-R2	CTTGCATATTGAGCGGCACTAC
36-ACT1-RT-F1	CTCCACCACTGCTGAAAGAGAA
37-ACT1-RT-R1	CCAAGGCGACGTAACATAGTTTT
38-ACT1-MGB1	TTGTCCGTGACATCAA
43-FLO9-MGB-F1	CATGCCTGCCAGCAA
44-FLO10-MGB-R1	TTTAACGCCACGCTTCA
45-FLO11-MGB-R1	ATCCACACCTGACAGCT
46-FLO1-RT-F3	ATCGCTATATGTTTTTGGCAGTCTTTA
47-FLO5-RT-F3	GCACACCACTGCATATTTTTGGTAA
48-FLO1-5-RT-R3	GTAAGCACGCCTCTGTGGCT
49-FLO9-RT-R3	AAGTTTACATTCATACCATTCTTCCTTGA
50-FLO10-RT-F3	CTGAATATAGCGCTTCCCAGGTT
51-FLO11-RT-F3	CACTTTTGAAGTTTATGCCACACAAG
52-FLO1-MGB-F1	ACTTCTGGCACTAACTAGT
53-FLO5-MGB-F1	CCTTTCTGGCACTAATT
61-FLO1-DEL-F1	AAGCTCTCTTCCGGGTTCTTATTTTTAATTCTTGTCACCAGT AAACAGAACATCC-CGGATCCCCGGGTTAATTAA
63-FLO1-5-9-DEL-R1	TTAGCAAAGAAAAGATACACAGATACGTAAAAAGAACGCG AATTTTATTAAATAATTG-GAATTCGAGCTCGTTTAAAC
91-pTEF-*FLO1*-F1	CTTCCGGGTTCTTATTTTTAATTCTTGTCACCAGTAAACAGA ACATCCAAAAGAATTCGAGCTCGTTTAAAC
92-pTEF-*FLO1*-R1	GTGCCAGAAGTGTAAAGACTGCCAAAAACATATAGCGATG AGGCATTGTCATCATTT TGAGATCCGGGTTTT
443-DAN1-HYG-F1	TTCTTCTTTTTCAGATAAAAGTGTAGCATACTAAATATATAC CCCAAGTATGCCATCTTTGTACAGCTTGCCT
444-DAN1-HYG-R1	TTCAATTATTTTACATCATTTATACAACTGTACAGGGCCGCA CATGATCACGCAGAGCCGTGGCAGG
445-YKR104W-HYG-F1	CTGAAACTGCTCAATGGATCATAAAAGTATTTTCAAGCGTT GAACTTTTGTGCCATCTTTGTACAGCTTGCCT
446-YKR104W-HYG-R1	TACTTCGTAGCTAGAACTGGAATGAATAAAAATAGGAAATT CTAGTTGTCCGCAGAGCCGTGGCAGG
447-HSP30-HYG-F1	CAAGTTTGAGACTTTAATATCTTTTGATTACTAAAAACAAC AAATTTCAATGCCATCTTTGTACAGCTTGCCT
448-HSP30-HYG-R1	TGTTTTGAAATGTGTTAAGCAAAGAATGATTAAGACAATCT CAAGCTGCTCGCAGAGCCGTGGCAGG
449-YDR222W-HYG-F1	CAGTGAGGGCGTATAAGATACATCGTACATACATAGAGACT CATTTAGTGTGCCATCTTTGTACAGCTTGCCT
450-YDR222W-HYG-R1	AAAAAAAAGGACAAAAAACGAATTTCATGTGGAAGTGTTC ACGCTTTTGTCGCAGAGCCGTGGCAGG
451-STP4-HYG-F1	AACACTGGAGCGCTTGGAATATTTGTTACTTCTTTTCTTTGG CTTCCCCTTGCCATCTTTGTACAGCTTGCCT
452-STP4-HYG-R1	ATAAATGTATGTATGTGTGTGTATGTATGAGTCGGTGAATG ACTTTGGTTCGCAGAGCCGTGGCAGG
453-TPO3-HYG-F1	TCATTATTTTAATTTTGCATTAGTACTCCTCTAGCCAAAGAT

	AAACAGAATGCCATCTTTGTACAGCTTGCCT
454-TPO3-HYG-R1	CTACTACTATAATTTTTCATTATTATGCTCGATTCGTAAAAT CGTTACTCCGCAGAGCCGTGGCAGG
455-YIL057C-HYG-F1	TGCAAACGAAACAACGTACAGTATATAACAAAGTATTTTAA ATAATAAGATGCCATCTTTGTACAGCTTGCCT
456-YIL057C-HYG-R1	TTTCGTAAATTCATAAAATTTCGTTAATTCATAAAAACAGCT CCCCAAACCGCAGAGCCGTGGCAGG
457-TIR4-HYG-F1	GAAACCAGCAACAAAAACCTATTCACTCGCTTATTAATACC ATAAAAAATTGCCATCTTTGTACAGCTTGCCT
458-TIR4-HYG-R1	AATAATAATATAATCATAAGCGGAACGAACATTTTCGACAC GTACTAAAACGCAGAGCCGTGGCAGG
459-SPG4-HYG-F1	AGCCACTTCTGTAACAAGATAAATAAAACCAACTAATCGAG ATATCAAATTGCCATCTTTGTACAGCTTGCCT
460-SPG4-HYG-R1	TTAGAATAAATAGACAACACAAGAAAAGACACTATGAATA TCTCCTCCATCGCAGAGCCGTGGCAGG
461-SPG1-HYG-F1	ACAATCAATACAAATATTTAGCGCATAAAATTCAAACAAAG TTTACTGAATGCCATCTTTGTACAGCTTGCCT
462-SPG1-HYG-R1	GAAAACAAAATGCAAAGAACATAAATGCAGGGAACCAAGT ACAAATTCCCCGCAGAGCCGTGGCAGG
463-DAN1-prom-F2	AAGCTCAAAATATCTTTGGAGTTTGACAAT
464-YKR104W-prom-F2	TATGCCAAGTTACGTTTTCATAATGTCACG
465-HSP30-prom-F2	ATCGAAAGCGTGCTTTGTAAGAATATTTG
466-YDR222W-Prom-F2	CGGAGAACTAAGTCATAGACGTAATGCTAA
467-STP4-prom-F2	GTTTTCTACTACTGATAGCTCCCATCCGCA
468-TPO3-Prom-F2	GTTCTCCAAAGTGAATACAATAAGCAGTAT
469-YIL057C-Prom-F2	AACCTTTTCGGCGGTTGGCAATCGTCCGTA
470-TIR4-Prom-F2	CCAGATTCGTGTGTGTGTAATAATTCGTTT
471-SPG4-Prom-F2	GTCATGATTTACGTATAACTAACACATCATG
472-SPG1-Prom-F2	AGAGAAGAATTACGGGATACTGGGATAACA
1751-TDH3-pKT-F	TTTAAAACACCAAGAACTTAGTTTCGAATAAACACACATAA ACAAACAAAGGTGACGGTGCTGGTTTA
1752-TDH3-pKT-R	CTAAGTCATAAAGCTATAAAAAGAAAATTTATTTAAATGCA AGATTTAAATCGATGAATTCGAGCTCG
1921-TDH3-upYRO-F	GTACCGCTTTGGGAGGCCTCATCTTGGTTGTGTCCTCGTATG GCAGCATCTGCCATTGAGTTTATCATTATCAATACTGCCATT TC
1922-TDH3-upYRO-R	GGTGATTTTCGGAAACATGCAGAAGAGTCCTTAGAAATCTT AGGTAAATTCACTCGGCATGACAAGAACAATGCAATAGCG CA
1899-HIS-sub-HYG-F	TCCTTTTAAAATCTTGCTAGGATACAGTTCTCACATCACATC CGAACATATGCCATCTTTGTACAGCTTGCCT
1924-TDH3-upYRO-HYG-R	GGTGATTTTCGGAAACATGCAGAAGAGTCCTTAGAAATCTT AGGTAAATTCACTCGGCATCGCAGAGCCGTGGCAGG
1926-TDH3-PYK-F	CAACTATATTTTACTTTCATCCTCTACGTCCATTGTAAGATT ACAACAAAAGCACTATCGTTTATCATTATCAATACTGCCAT TTC
1929-TDH3-PYK-HYG-R	ACTGACACAATGGACAATTAAATAAAATTAAGAAAAAAAA TAAGGACTTTAATTTTTACGCAGAGCCGTGGCAGG
1942-FLO1-paradoxus-F	TACCGCTTTAAAATGCCTAGTCTTGGGTCGAGGTCTCGTAT GGCAGCATCTGTTATTGAGATCCAAAAGAATTCGAGCTCGT TTAAAC
1943-FLO1-paradoxus-R	GGTGATTTTCGGAAACATGCAGAAGAGTCCAAAGAAATCTT

	AGGTAAATTCACTCGGCATCAATTTGAATATTTGAAAGTAT GGA
2035-KANMX-in-EM93-F	CACAATGTAA ATCTTGCTTT GGGTTGACTG AGGGAAATAA CTATAGACAT – CGGATCCCCGGGTTAATTAA
2036-KANMX-in-EM93-R	ATCACGGAAG TGGTACCAAA ATCGGTAGGT TGTTTTCAAT TTACCCTTTT – GAATTCGAGCTCGTTTAAAC

Supplemental Experimental Procedures

Gene array analysis

Fresh cultures were inoculated for pre-cultures and samples were taken after 24 hours of growth. Total RNA was prepared using a standard phenol-chloroform extraction method. Samples were purified using the RNeasy Mini Kit (Qiagen) and RNase-Free DNase (Qiagen). First- and second-strand synthesis, in vitro transcription, hybridization, and scanning were performed according to the Affymetrix protocol. Samples were probed on Affymetrix Yeast Genome S98 chips. Basic analysis was carried out using the Affymetrix GCOS software package. Raw data and tables with enrichment analysis are available at: http://sysbio.harvard.edu/csb/verstrepen/resources.html. All arrays were scaled to an average intensity of 100 using all measured expression levels. Genes were ranked according to their ability to distinguish between flocculation and non-flocculation. Induction and repression of specific gene groups in flocculating versus non-flocculating conditions was studied using Gene Set Enrichment Analysis (GSEA) as described previously (Subramanian et al., 2005). Significance was estimated based on a gene label permutation. Gene sets with a nominal p-value below 0.01 and a false discovery rate (FDR) below 0.25 were considered to be significant. Expression differences between gene sets were visualized using the GenePattern software (Reich et al., 2006). Expression levels in each gene set were normalized by mean centering and unit scaling prior to visualization.

Fitness measurements

Relative Malthusian fitness was determined as described before (Thompson et al., 2006). Briefly, cultures inoculated with equal numbers of KV210 cells and a nonflocculent reference strain (KV22) were grown for 24 hours and used to inoculate fresh cultures. After 10 transfers (±80 cellular generations), the ratio of flocculent versus non-flocculent cells was determined. The experiment was carried out in YPGal medium (to induce flocculation in the flocculent KV210 strain) and in YPD (control, no *FLO1* expression in either strain). Flocculation was inhibited by adding 0.2 g ml^{-1} mannose (Sigma Aldrich) to the medium. The selective advantage s (fitness factor) was calculated as $s = (\ln (F_f/R_f) - \ln(F_i/R_i))/T$, where F and R are the numbers of flocculent and reference cells, the subscripts refer to final and initial populations, and T is the number of generations that the reference cells have proliferated during the competition.

Supplemental References

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