Figure S1. Schematic representation of the BFP/GFP-module series.

(A) Chromosome 4 map. BFP/GFP-expressing cassettes were integrated on both homologs of Ch4 in the *PGA59-PGA62* intergenic region. **(B) The different BFP and GFP cassettes produced in this study**. Expression of BFP or GFP is controlled by the *TDH3* promoter and is associated to different selectable markers.

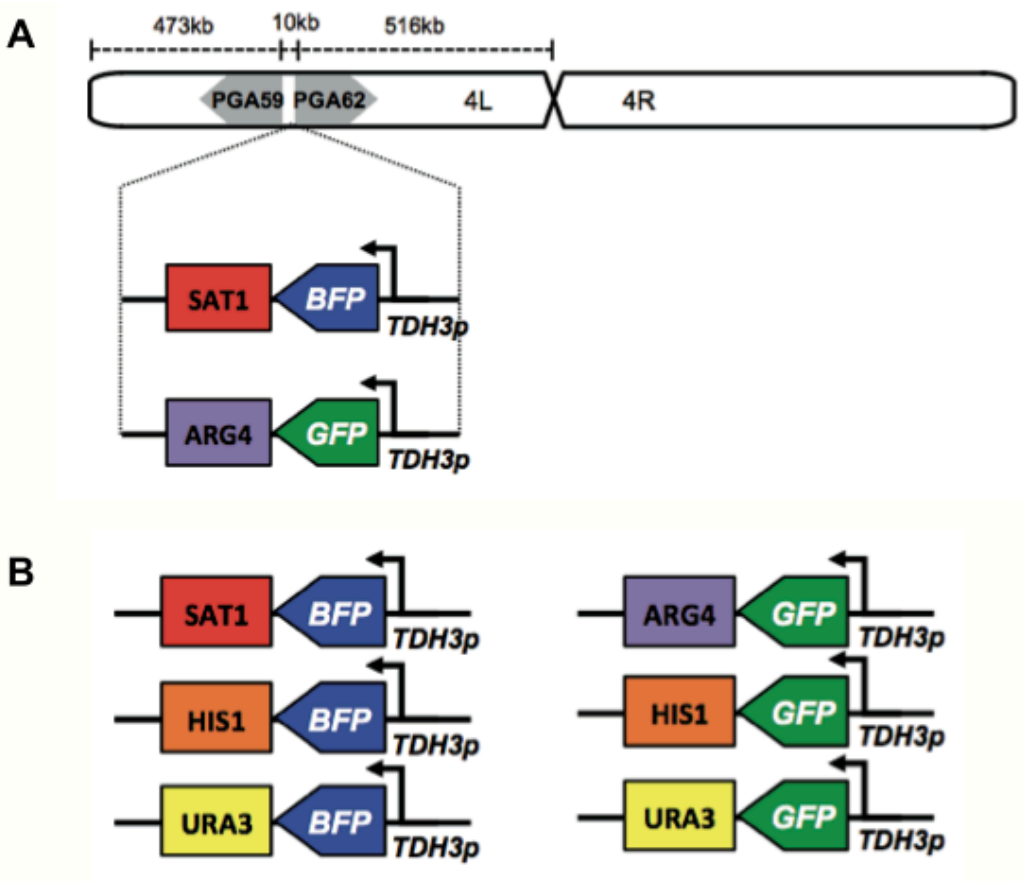
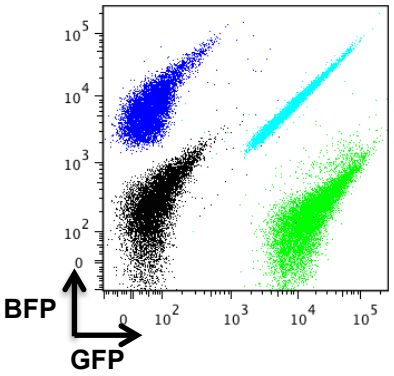


Figure S2. BFP/GFP plots of control strains after exposure or not to a heat stress.

A. BFP/GFP plots of control strains in absence of stress. Strains SN100 (unlabeled), CEC4007 (mono-BFP), CEC3998 (mono-GFP) and CEC3989 (BFP/GFP) were grown overnight in YPD at 30°c. Cells were diluted at 1:1000 in 1X PBS and analyzed by flow cytometry. 200,000 events are displayed.

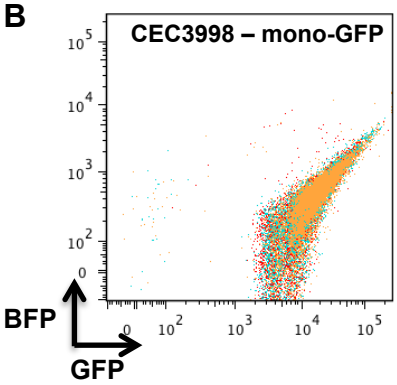
B. BFP/GFP plots of control strains after exposure to elevated temperatures. Strains SN100 (unlabeled), CEC4007 (mono-BFP), and CEC3998 (mono-GFP) were grown overnight in YPD at 30°c, 37°c and 39°c and re-inoculated in YPD alone to recover overnight at 30°c. Cells were diluted at 1:1000 in 1X PBS and analyzed by flow cytometry. 200,000 events are displayed.

A

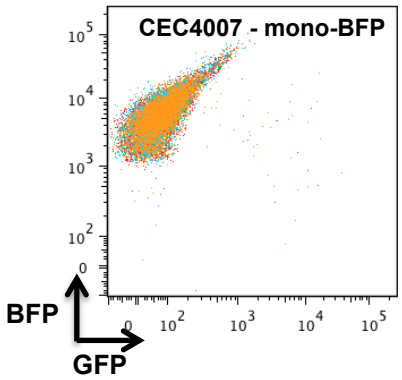


SN100 - unlabeled
CEC4007 - mono-BFP
CEC3998 – mono-GFP
CEC3989 – BFP/GFP

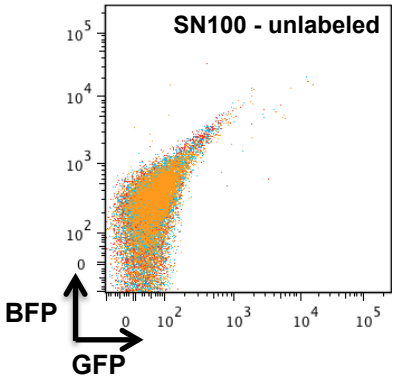
B



30°C heatshock + recovery
37°C heatshock + recovery
39°C heatshock + recovery



30°C heatshock + recovery
37°C heatshock + recovery
39°C heatshock + recovery



30°C heatshock + recovery
37°C heatshock + recovery
39°C heatshock + recovery

Figure S3. Primary LOH screen to identify genes whose overexpression results in an increase in LOH at the BFP/GFP locus. After an overnight induction in presence of ATc 3ug/ml, the 124 overexpression mutants and the control strain were individually screened by flow cytometry to identify genes whose overexpression resulted in an increase in the proportion of Gfp^+ Bfp^- mono-labelled cells. 10^6 cells were analyzed**.** The graph shows the standard deviation distribution of the mutants. This screen identified 33 candidate genes with a proportion of Gfp^+ Bfp^- cells that differed from the one observed in the control strain with a Z-score of 5. The 4 candidate genes validated in the secondary LOH screen are shown in red.

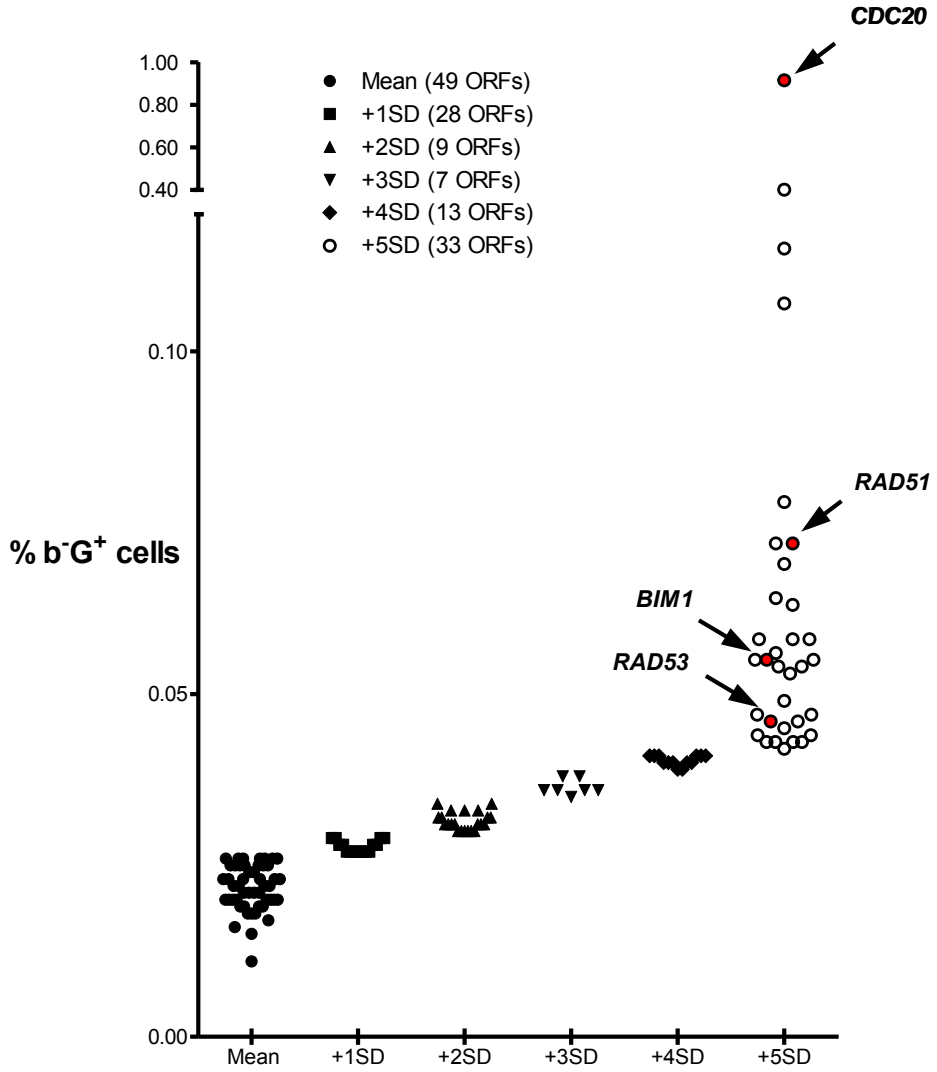


Table S1. Oligonucleotides used in this study

Primer name	Sequence (5' → 3')	Use
TDH-XHO-UP	AATCCGCTCGAGCACACACATACAGACAATTGCG	*TDH3* promoter
TDH-ECORV-DWN	CCGGATATCGATTGTAAAGTTTGTTGATGTTAATTG	*TDH3* promoter
TDH3p-F-XhoI	GAGAGACTCGAGCACACACATACAGACAATTG	Yeast Fluorescent Protein (yFP) cassette
CYC1t-R-XhoI	AGAGAGCTCGAGAATTTGAAATATAAATAACG	Yeast Fluorescent Protein (yFP) cassette
TDH3p-BFP-R	TCTTTAATCAATTCAGACATTTTAATAAAGCTTCTGCAGG	Yeast Fluorescent Protein (yFP) cassette
TDH3p-BFP-F	CCTGCAGAAGCTTTATTAAAATGTCTGAATTGATTAAAGA	Yeast Fluorescent Protein (yFP) cassette
BFP-Cyc1t-R-XhoI	GAGAGACTCGAGAATTTGAAATATAAATAACGTTCTTAATACTAACATAACTATAA AAAAATAAATAGGGACGCTAGCTTAGTTCAATTTGTGTCCTA	Yeast Fluorescent Protein (yFP) cassette
K7 yFP-Fwd	TCTGATAGTGTCAAAAATGAGGATGTTAAGAAATCTATTATTAGTTACAATAGTTCT ACTAGTAAACATTGATCGAGAACAAGTAGAGTCAAAGGTAGTAgttttcccagtcacgacg	BFP/GFP system
K7 yFP-Rev	ATAGTTAGTAACTATTTACTGTTGTTATTGTTACAATTACATAAATTAGAGAAAAGC AATTATCATGGTGAAATTGTTTGGTTTAAAAGGAGAATTCTCTcaggaaacagctatgacc	BFP/GFP system

Underlining indicates the restriction sites as indicated in the primer name
Lowercase lettering indicates the vector sequences from the plasmids containing the YFP cassettes

Table S3. Plasmids used in this study

Plasmid	LabID	Use	Reference
pBluescript-KS	-	Yeast Fluorescent Protein (yFP) cassette	Stratagene
pKS-P_{TDH3}	ECC517	Yeast Fluorescent Protein (yFP) cassette	This study
pGFP	ECC112	Yeast Fluorescent Protein (yFP) cassette	[1]
CIp10-P_{TDH3}-GFP	ECC570	Yeast Fluorescent Protein (yFP) cassette	This study
pCR-Blunt-II-TOPO	-	Yeast Fluorescent Protein (yFP) cassette	Invitrogen
pCRBlunt-GFP	ECC590	Yeast Fluorescent Protein (yFP) cassette	This study
pFA-ARG4	ECC75	Yeast Fluorescent Protein (yFP) cassette	[2]
pFA-URA3	ECC68	Yeast Fluorescent Protein (yFP) cassette	[2]
pFA-SAT1	ECC338	Yeast Fluorescent Protein (yFP) cassette	[3]
pECC596-GA	ECC596	Yeast Fluorescent Protein (yFP) cassette	This study
TagBFP-AS-C	-	Yeast Fluorescent Protein (yFP) cassette	Evrogen
pCRBlunt-BFP	ECC690	Yeast Fluorescent Protein (yFP) cassette	This study
pFA-HIS1	ECC76	Yeast Fluorescent Protein (yFP) cassette	[2]
pECC727-BH	ECC727	Yeast Fluorescent Protein (yFP) cassette	This study
pECC640-GH	ECC640	Yeast Fluorescent Protein (yFP) cassette	This study
pECC817-GU	ECC817	Yeast Fluorescent Protein (yFP) cassette	This study
pECC818-BU	ECC818	Yeast Fluorescent Protein (yFP) cassette	This study
pECC729-BS	ECC729	Yeast Fluorescent Protein (yFP) cassette	This study
CIp10-P_{TET}-GTW	ECC289	Yeast Fluorescent Protein (yFP) cassette	[4, 5]
pDONR207	-	3R overexpression collection	Invitrogen
pNIMX	ECC675	Tetracycline-inducing system	[5]
CIp10	ECC86	*URA3* complementation	[6]
CIp30	ECC208	*URA3, ARG4, HIS1* complementation	[7]

REFERENCES

1. **Barelle CJ, Manson CL, MacCallum DM, Odds FC, Gow NA, Brown AJ.** 2004. GFP as a quantitative reporter of gene regulation in Candida albicans. Yeast **21:**333-340.
2. **Gola S, Martin R, Walther A, Dunkler A, Wendland J.** 2003. New modules for PCR-based gene targeting in Candida albicans: rapid and efficient gene targeting using 100 bp of flanking homology region. Yeast **20:**1339-1347.
3. **Schaub Y, Dunkler A, Walther A, Wendland J.** 2006. New pFA-cassettes for PCR-based gene manipulation in Candida albicans. J. Basic Microbiol. **46:**416-429.
4. **Cabral V, Chauvel M, Firon A, Legrand M, Nesseir A, Bachellier-Bassi S, Chaudhari Y, Munro CA, d'Enfert C.** 2012. Modular gene over-expression strategies for Candida albicans. Methods Mol. Biol. **845:**227-244.
5. **Chauvel M, Nesseir A, Cabral V, Znaidi S, Goyard S, Bachellier-Bassi S, Firon A, Legrand M, Diogo D, Naulleau C, Rossignol T, d'Enfert C.** 2012. A Versatile Overexpression Strategy in the Pathogenic Yeast Candida albicans: Identification of Regulators of Morphogenesis and Fitness. PloS one **7:**e45912.
6. **Murad AM, Lee PR, Broadbent ID, Barelle CJ, Brown AJ.** 2000. CIp10, an efficient and convenient integrating vector for Candida albicans. Yeast **16:**325-327.
7. **Dennison PM, Ramsdale M, Manson CL, Brown AJ.** 2005. Gene disruption in Candida albicans using a synthetic, codon-optimised Cre-loxP system. Fungal Genet. Biol. **42:**737-748.

SUPPLEMENTAL MATERIALS AND METHODS

Plasmid constructions

Plasmids for the BFP/GFP system:

• P_{TDH3}-*GFP-ARG4* cassette

The promoter region (850bp) of *TDH3* (P_{TDH3}) has been amplified by PCR with modified oligonucleotides TDH-XHO-UP and TDH-ECORV-DWN. The PCR product was digested with *Xho*I and *Eco*RV and cloned in *Xho*I, *Eco*RV-digested pBluescript-KS, yielding plasmid pKS-P_{TDH3}. P_{TDH3} was excised by *Xho*I and *Pst*I digest of pKS-P_{TDH3} and ligated in *Xho*I, *Pst*I-digested pGFP plasmid (1), yielding plasmid CIp10-P_{TDH3}-GFP. A P_{TDH3}-GFP cassette was PCR-amplified from plasmid Cip10-P_{TDH3}-GFP with oligonucleotides TDH3p-F-XhoI and CYC1t-R-XhoI and cloned in the pCR-Blunt-II-TOPO plasmid (Invitrogen), yielding plasmid pCRBlunt-GFP. The *ARG4* marker was excised from plasmid pFA-ARG4 (2) by *Not*I digest and ligated in *Not*I-cut pCRBlunt-GFP, yielding plasmid pECC596-GA (ECC596) that harbors a P_{TDH3}-GFP-*ARG4* cassette.

• P_{TDH3}-*BFP-HIS1* cassette

The TagBFP-AS-C entry plasmid encoding BFP was obtained from Evrogen. The P_{TDH3}-BFP cassette was generated by fusion PCR. P_{TDH3} was amplified from plasmid Cip10-P_{TDH3}-GFP with oligonucleotides TDH3p-F-XhoI and TDH3p-BFP-R. The BFP gene was amplified from the TagBFP-AS-C entry plasmid (Evrogen) with oligonucleotides TDH3p-BFP-F and BFP-CYC1t-R-XhoI. The two PCR products were mixed in a third PCR with oligonucleotides TDH3p-F-XhoI and BFP-Cyc1t-R-XhoI. The resulting PCR product was cloned in the pCR-Blunt-II-TOPO plasmid (Invitrogen), yielding plasmid pCRBlunt-BFP. The *HIS1* marker was excised from plasmid pFA-HIS1 (2) by *Not*I digest and ligated in *Not*I-digested pCRBlunt-BFP, yielding plasmid pECC727-BH (ECC727) that harbors a P_{TDH3}-BFP-*HIS1* cassette.

• Additional BFP- and GFP-expressing cassettes

The *HIS1*, *URA3* and *SAT1* markers were excised by *Not*I digest from the pFA plasmids (2,3) and ligated in *Not*I-cut pCRBlunt-GFP or *Not*I-cut pCRBlunt-BFP, yielding plasmids pECC640-GH (ECC640), pECC817-GU (ECC817), pECC818-BU (ECC818) and pECC729-BS (ECC729), that harbors a P_{TDH3}-GFP-*HIS1,* P_{TDH3}-GFP-*URA3*, P_{TDH3}-BFP-*URA3* or P_{TDH3}-BFP-*SAT1* cassette, respectively.

Overexpression plasmids

Overexpression plasmids were constructed using the Gateway™ Technology (Invitrogen-GTW) as described in Chauvel et al. (4). Briefly, ORFs for 204 selected genes were PCR amplified using oligonucleotides listed in Table S3 in the supplemental material and cloned in pDONR207, yielding 151 pDONR207 derivatives. All plasmids were subjected to Sanger sequencing from the 5-prime end of the ORF to verify cloning of the expected ORF. Furthermore, a pool of all pDONR207 derivatives was subjected to Solexa sequencing in order to check the integrity of the cloned ORFs. Subsequently, ORFs were transferred into plasmid Cip10-P_{TET}-GTW (4), yielding 147 Cip10-P_{TET}-GTW derivatives.

REFERENCES

1. Barelle, C.J., Manson, C.L., MacCallum, D.M., Odds, F.C., Gow, N.A. and Brown, A.J. (2004) GFP as a quantitative reporter of gene regulation in Candida albicans. *Yeast*, **21**, 333-340.
2. Gola, S., Martin, R., Walther, A., Dunkler, A. and Wendland, J. (2003) New modules for PCR-based gene targeting in Candida albicans: rapid and efficient gene targeting using 100 bp of flanking homology region. *Yeast*, **20**, 1339-1347.
3. Schaub, Y., Dunkler, A., Walther, A. and Wendland, J. (2006) New pFA-cassettes for PCR-based gene manipulation in Candida albicans. *J Basic Microbiol*, **46**, 416-429.

4. Chauvel, M., Nesseir, A., Cabral, V., Znaidi, S., Goyard, S., Bachellier-Bassi, S., Firon, A., Legrand, M., Diogo, D., Naulleau, C. *et al.* (2012) A Versatile Overexpression Strategy in the Pathogenic Yeast Candida albicans: Identification of Regulators of Morphogenesis and Fitness. *PloS one*, **7**, e45912.