

Supplementary Material for:

Forward genetics in *Candida albicans* that reveals the Arp2/3 complex is required for hyphal formation, but not endocytosis

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Supplementary Text:

Assessing the efficiency of obtaining diploid homozygous UAU1 insertion mutants

Given that the efficiency of recovering auxotrophs, glycerol sensitive or hyphal deficient mutants was not that high (one in 300, one or two in about 4700, respectively), we assessed the frequency of obtaining homozygous diploid *orf::UAU1/orf::URA3* mutants by arbitrarily selecting 24 mutants from the pool of 4700 and using inverse PCR to identify the site of the transposon insertion. We found that in six of these 24 mutants the transposon had inserted directly in an open reading frame (ORF), in a further six the transposon had inserted in the promoter region (< 1 kb upstream of an ORF), in four transformants the insertion was less than 1 kb downstream of an ORF, while six transposons had inserted in a clear intergenic region (> 1 kb away from any ORF) and two insertions could not be mapped. One of the two unmapped insertions was flanked by an *E. coli* sequence, but how this transposon construct was inserted into the *C. albicans* genome along with *E. coli* coding sequence is unclear. The other unmapped insertion generated an unusually large, very faint band after inverse PCR that gave ambiguous sequence. We used *Mbol* (restriction digestion site: GATC) to generate the inverse PCR template, so it is possible that this insertion falls in a region with infrequent *Mbol* sites and thus was difficult to amplify. Next, we checked the six ORF and the six promoter insertion mutants for aneuploidy. PCR analysis showed that in only one instance was the WT gene clearly missing (Figure S2E, left). Thus, while recombination of a transposon insertion into a gene or potential regulatory region is efficient, insertions that ultimately create a clean homozygous diploid *orf::UAU1/orf::URA3* mutant can be as low as one out of 24 after random selection.

mutant name	phenotype			systematic name	CGD description	site of insertion relative to start (insertion/ORF length)
	YPD	10% FBS	Spider			
BWP17					WT	
<i>Tn::orf19.5984</i>				<i>orf19.5984</i>	uncharacterized	657/1002
<i>Tn::orf19.7227</i>				<i>orf19.7227</i>	mutation confers hypersensitivity to toxic ergosterol analog	122/375
<i>Tn::orf19.6868</i>				<i>orf19.6868</i>	uncharacterized	585/1017
<i>Tn::orf19.7212</i>				<i>orf19.7212</i>	uncharacterized	1011/2304
<i>Tn::orf19.2761</i>				<i>orf19.2761</i>	uncharacterized	472/798
<i>Tn::orf19.1300</i>				<i>orf19.1300</i>	uncharacterized	953/1335
<i>Tn::orf19.2397.3</i>				<i>orf19.2397.3</i>	uncharacterized	153/1082
<i>Tn::orf19.6719</i>				<i>orf19.6719</i>	uncharacterized	3677/4796
<i>Tn::orf19.860</i>				<i>orf19.860</i>	uncharacterized	-553/2085

Figure S1. Potential hypo-filamentation phenotypes recovered from the screen

Hyphal formation was assessed as described in Figure legend 1. The mutants listed here showed reduced wrinkle formation on Spider media, YPD + 10% FBS or on both, but all of these mutants could still form true hyphae under liquid inducing conditions (not shown). For each mutant, a description from CGD (www.candidagenome.org) and the site of insertion relative to the start codon of the coding sequence is given in the last column.

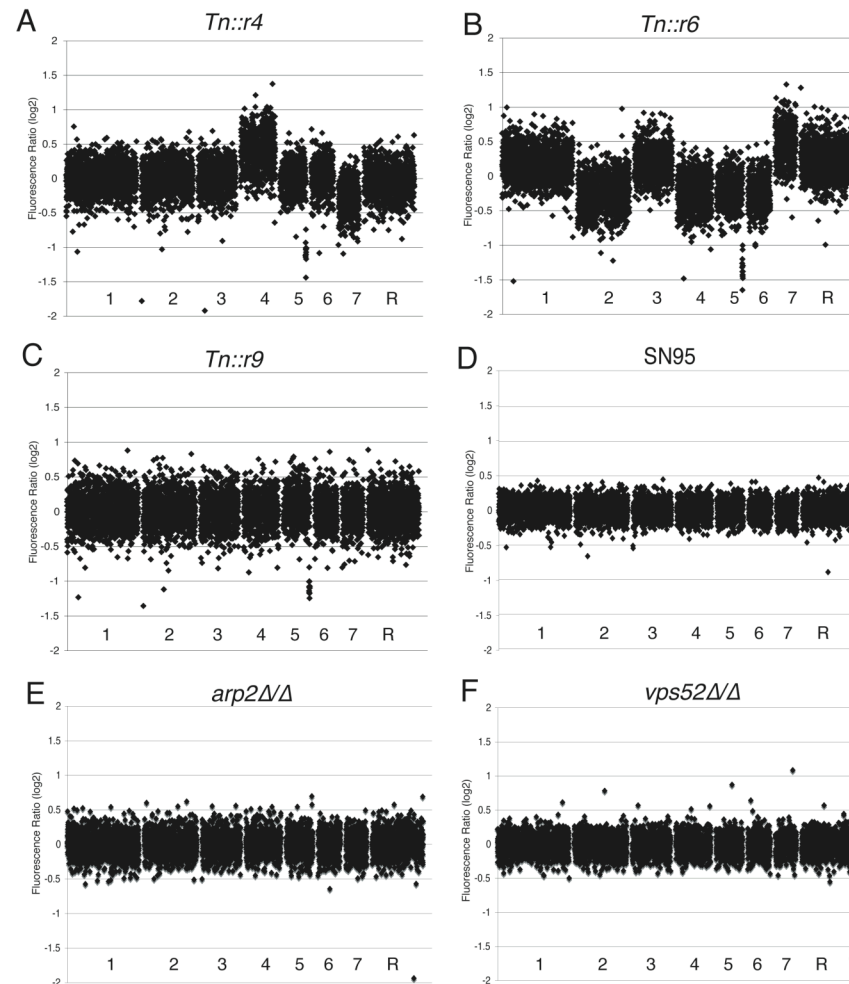


Figure S3. The UAU1 system can introduce major chromosomal rearrangements

Cy3 labeled genomic DNA from either three randomly chosen UAU1 mutants (*Tn::r4*, *Tn::r6* and *Tn::r9*) or from strain SN95 was hybridized to DNA microarrays with Cy5 labeled genomic DNA from the reference strain SC5314. Shown are plots of CGH (comparative genome hybridization) analyses, where the y-axis shows the log₂ fluorescence ratio of the mutant strains versus SC5314 and the x-axis shows all chromosomes (1 to R). A single black rhombus represents the log₂ fluorescence ratio plotted as a function of its position in the *C. albicans*' assembly 21. In this representation, a 1.5-fold increase in fluorescence ratio (i.e. three chromosome copies versus two) equals a log₂ ratio of ~ 0.58 . **A.** Mutant *Tn::r4* has at least one more copy of chromosome four and one copy less of chromosome seven compared to SC5314. The UAU1 cassette integrated on chromosome three. **B.** Mutant *Tn::r6* has acquired at least one copy each for chromosomes one, three, seven and R, while chromosomes two, four, five and six have lost at least one copy each. The UAU1 cassette integrated on chromosome R. **C - F.** No aneuploidy was detected in mutant *Tn::r9*, SN95 and the deletion mutants for *arp2Δ/Δ* and *vps52Δ/Δ*.

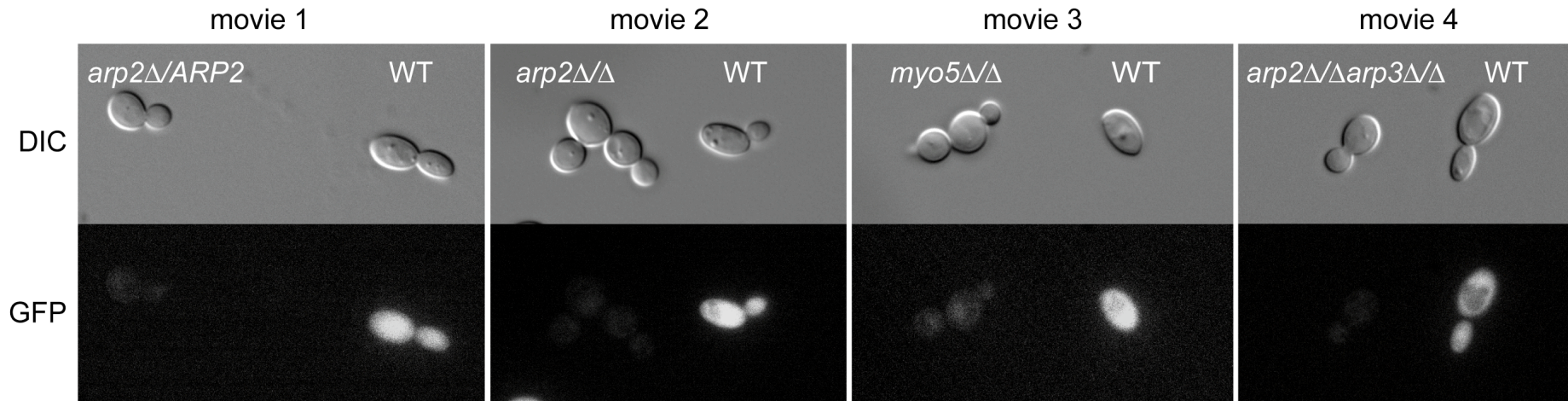


Figure S4. Coincubation of mutant and WT strains during time-lapse analysis. A GFP-labeled WT strain was coincubated with different mutant strains to simulate equal conditions for time-lapse analysis. The pictures shown here represent images taken at the beginning of time-lapse movies to distinguish between WT and mutant strains. Movie 1 shows the *arp2Δ/ARP2* heterozygous mutant and WT cells, movie 2 shows the *arp2Δ/Δ* deletion mutant and WT cells, movie 3 shows the *myo5Δ/Δ* deletion mutant and WT cells, and movie 4 shows the *arp2Δ/Δarp3Δ/Δ* double deletion mutant and WT cells. Movies can be found in the supplementary material.

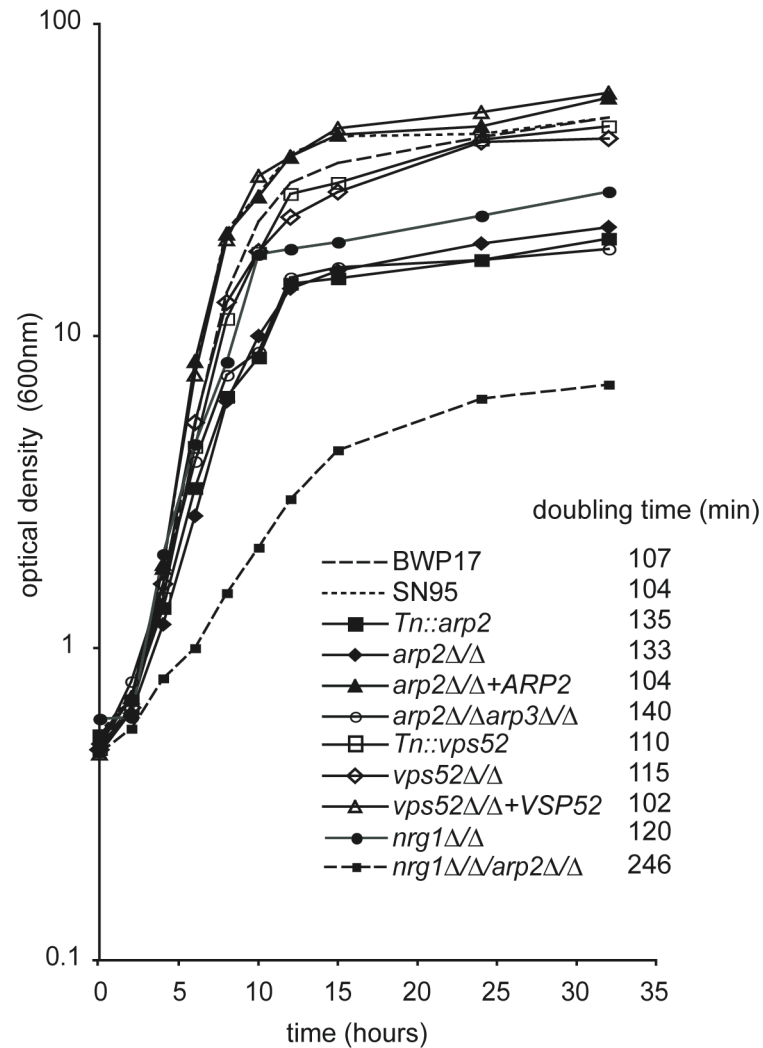


Figure S5. Growth properties of VSP52, NRG1 and Arp2/3 complex mutants. To measure growth, an overnight culture of each strain was diluted to an OD_{600} of 0.5 in YPD media and incubated at 30°C with constant shaking. OD_{600} measurements were taken at indicated times. The doubling time was equal to $\ln 2 / [(\ln(A/A_0))/t]$ where A is the OD_{600} at time t (12 hours), A_0 is the initial OD_{600} (2 hours) and t is the time (10 hours).

Table S1.

Pearson correlation of the entire *ARP2* microarray data set and the *MYO5/SLA2* microarray data sets.

	sla2-H	myo5-H	arp2-H	sla2-Y	myo5-Y	arp2-Y	sla2-HY	myo5-HY	arp2-HY	HY- (Oberholzer)
WT-HY (Epp)	0.126	0.067	-0.112	0.213	0.152	0.246	0.578	0.308	0.273	0.631
WT-HY (Oberholzer)	0.080	0.008	-0.058	0.081	0.094	0.071	0.813	0.400	0.266	
arp2-HY	-0.070	-0.132	0.146	-0.069	-0.088	-0.247	0.230	0.143		
myo5-HY	0.069	0.020	-0.147	0.061	0.008	0.025	0.498			
sla2-HY	0.239	0.178	-0.010	0.162	0.171	0.114				
arp2-Y	0.584	0.667	0.345	0.597	0.693					
myo5- Y	0.812	0.721	0.404	0.666						
sla2-Y	0.559	0.734	0.358							
arp2-H	0.397	0.470								
myo5-H	0.627									

The values reported here were used to visualize Figure 5A using TreeView version 1.6 (<http://rana.lbl.gov/EisenSoftware.htm>).

Table S2.
Hyphae specific genes.

Hyphae specific genes are color coded:
Red indicates transcript levels > 4.0; orange indicates transcript levels < 4.0 and > 1.5;
pale green indicates transcript levels < 0.7 and > 0.3; dark green indicates < 0.5

Systematic name	Description	Common name	WT-HY (Epp)	WT-HY (Oberholzer)	arp2-HY	myo5-HY	sla2-HY	arp2-Y	myo5-Y	sla2-Y	arp2-H	myo5-H	sla2-H
orf19.1816	agglutinin-like protein	ALS3-1	31.1		1.8			1.0			0.0		
orf19.3374	secreted cell elongation protein	ECE1	30.4	81.2	6.2	3.3	18.9	1.0	1.1	1.0	0.2	0.2	0.7
orf19.4082	flocculent specific protein;contains NNDDNSYG motif	DDR48	26.7	4.4	0.9	0.7	1.6	29.5	6.1	6.1	1.1	3.7	3.0
orf19.5760	Induced in Hyphal Development;membrane protein	IHD1	24.4	10.6	2.5	5.6	5.0	0.9	0.3	0.7	0.1	0.5	0.6
orf19.4975	hyphally regulated protein	HYR1	20.2	1.3	1.6	1.0	1.1	1.0	1.2	1.0	0.1	0.9	1.0
orf19.5636	repressed by TUP1 protein 5	RBT5	19.9		0.6			3.9			0.5		
orf19.1327	repressed by Tup1, related to HWP1	RBT1*	17.2		7.4			1.3			0.6		
orf19.5585	secreted aspartyl proteinase 5	SAP5	17.2	19.1	1.2	1.5	3.1	1.0	1.3	1.1	0.1	0.7	1.5
orf19.1321	hyphal wall protein	HWP1*	16.4	25.6	3.5	9.5	9.1	1.5	0.9	0.8	0.5	0.6	1.5
orf19.4215	multicopper ferro-O2-oxidoreductase involved in high-affinity iron uptake	FET35*	13.7		4.0			2.5			0.3		
orf19.5674	glycosyl-phosphatidylinositol protein;similar to RBT5	RBT8*	12.0	13.8	1.2	2.5	9.2	0.8	0.8	1.0	0.1	1.4	0.8
orf19.251	conserved hypothetical protein	-	11.7	4.8	3.0	1.1	2.2	5.7	2.7	2.4	1.6	1.7	3.3
orf19.2355			9.4		1.3			1.2			0.1		
orf19.4943	mannose-1-phosphate guanyltransferase	PSA2*	9.0	2.0	3.0	1.9	1.2	1.8	1.0	1.0	0.3	1.0	1.3

orf19.3829	pH regulated GPI-anchored membrane protein that is required for morphogenesis	PHR1	8.9	5.0	0.9	3.8	2.6	3.1	2.0	5.5	0.6	1.1	2.4
orf19.2210	hypothetical protein	-	8.6	1.4	1.0	1.2	1.1	1.3	1.1	1.3	0.3	1.0	1.1
orf19.4980	heat shock protein 70	HSP70*	8.5	1.5	1.4	1.2	1.5	2.6	1.2	0.9	0.3	1.2	1.1
orf19.5542	candidapepsin 6 precursor	SAP6	8.0	3.7	1.5	1.5	1.5	0.7	1.3	1.4	0.2	0.7	1.2
orf19.4527	hexose transporter	HGT1	8.0	4.6	2.2	2.1	3.3	1.2	1.2	1.1	0.4	0.9	1.0
orf19.1691	conserved hypothetical protein	-	7.2	3.9	3.9	1.2	1.3	0.8	0.7	1.0	0.1	0.5	0.6
orf19.5265	kinesin heavy chain homolog	KIP4*	6.7	1.3	1.8	1.2	1.2	1.4	1.0	1.0	0.3	1.0	1.0
orf19.7610	protein tyrosine phosphatase	PTP3*	6.6	1.7	1.7	2.1	1.5	2.3	1.0	1.2	0.6	1.1	1.0
orf19.716	similar to pore-forming bacterial Septicolysin	-	5.8	1.1	1.7	1.1	1.0	1.2	1.0	1.1	0.6	0.9	1.0
orf19.2241	1,4-benzoquinone reductase;brefeldin A resistance protein;Protoplast-Secreted protein	PST1*	5.8	1.2	1.6	1.0	1.0	1.3	1.6	1.7	0.5	1.0	1.0
orf19.113	closely related to cadmium-induced protein CIP1	CIP1*	5.8	1.2	1.2	0.9	1.0	1.0	1.1	1.1	0.4	0.8	1.1
orf19.3384			5.7	3.0	1.1	1.5	1.5	1.2	1.4	1.1	0.6	0.6	1.1
orf19.6202	pathogenesis related protein, repressed by TUP1 protein 4	PRY4*	5.3	5.6	0.4	1.4	2.0	14.5	6.3	1.2	1.3	1.4	4.3
orf19.85	glutathione peroxidase	GPX1*	4.9	3.6	1.7	1.1	1.8	0.3	0.9	0.6	0.1	0.3	0.7
orf19.815	DOCK180 protein	-	4.6	2.1	2.6	1.4	1.7	1.3	1.0	1.0	0.3	0.9	1.0
orf19.1996	catabolic serine/threonine dehydratase	CHA2*	4.5	3.1	1.3	1.4	2.0	2.5	2.9	2.4	1.6	1.8	2.7
orf19.2952	exo-1,3-beta-glucanase	EXG1*	4.4	1.3	2.3	1.3	1.4	1.4	1.2	1.5	0.8	1.1	1.5
orf19.5645	O-acetylhomoserine sulfhydrylase	MET15	4.4		0.4			1.1			0.2		
orf19.2125	hypothetical protein	-	4.3	2.9	0.9	0.8	1.4	19.9	17.9	6.4	3.9	5.1	6.7
orf19.4477	aryl-alcohol dehydrogenase	IFD4*	4.1		1.5			8.0			4.2		
orf19.7531	conserved hypothetical protein	-	4.1	1.9	1.3	1.9	2.0	1.8	1.9	1.7	1.0	1.7	1.8
orf19.4590	similar to DNA-binding protein but may be missing DNA-binding domain	RFX1*	4.0	1.2	1.3	1.0	1.0	1.4	1.0	1.0	0.4	0.9	1.0
orf19.6608	transposase	-	4.0	2.4	1.4	2.4	1.8	2.0	0.9	1.3	0.7	0.9	0.7

orf19.4530.1	hypothetical protein	-	4.0	1.0	1.6	1.0	1.1	1.2	1.3	1.2	0.4	1.0	1.1
orf19.2959.1	conserved hypothetical protein	-	3.9		1.1			4.1			6.0		
orf19.7323	corticosteroid- binding protein	CBP1	3.9		3.6			0.6			0.4		
orf19.675	hypothetical protein	-	3.9	4.8	0.2	1.1	2.4	23.9	7.1	1.7	2.8	3.9	7.1
orf19.7053	regulatory subunit for phosphoprotein phosphatase type 1 (PP-1)	GAC1*	3.9		1 .1			1.4			0.4		
orf19.4749	hypothetical membrane protein	-	3.8	1.7	2.2	1.1	1.1	0.8	1.3	1.3	0.6	1.0	0.9
orf19.6420	hypothetical membrane protein with repeated hydrophobic/hydrophilic domains	-	3.8	4.2	0.4	3.2	2.5	7.5	1.8	1.3	1.3	1.6	6.5
orf19.6287	aspartate aminotransferase	AAT2*	3.8	1.2	1.5	1.0	1.3	1.0	1.1	1.1	0.5	1.5	1.4
orf19.1799	general amino acid permease	GAP6*	3.7	1.0	1.3	1.0	1.1	0.9	1.1	1.1	0.5	0.9	1.0
orf19.1655	fatty-acyl coenzyme A oxidase	POX3*	3.7	1.0	0.7	1.0	1.0	1.5	1.2	0.9	0.5	0.9	1.0
orf19.5614	ribonuclease H	RNH2*	3.6	2.0	1.3	1.2	2.4	6.5	4.0	1.5	1.5	2.7	2.8
orf19.3746	oligopeptide transporter protein	OPT2	3.5	1.2	0.9	1.0	1.0	1.1	0.9	1.0	0.4	1.0	0.9
orf19.6705	conserved hypothetical protein	-	3.5	1.8	1.3	1.4	1.6	1.2	0.8	1.0	0.4	0.8	0.8
orf19.7362	glucan synthase subunit involved in cell wall assembly	SKN1	3.4	2.3	2.2	1.7	2.5	1.0	1.6	2.0	1.2	1.3	2.2
orf19.2372			3.3	1.3	1.8	1.6	1.3	0.9	0.8	1.1	0.3	1.0	0.5
orf19.5373			3.3	1.8	2.1	1.6	1.4	0.9	0.6	0.8	0.3	0.7	0.5
orf19.7304	hypothetical protein	-	3.3	2.9	2.4	1.7	2.5	0.8	1.0	0.9	0.9	1.2	1.2
orf19.6021	Induced in Hyphal Development	IHD2	3.3	1.3	1.2	1.1	1.1	1.3	1.6	1.1	0.8	1.1	1.1
orf19.7504	conserved hypothetical protein	-	3.2	2.1	0.8	1.1	1.4	3.9	3.0	1.7	1.3	1.4	2.4
orf19.715	hypothetical protein	-	3.2	1.2	1.9	0.9	1.0	1.5	0.9	1.0	2.1	0.7	1.1
orf19.5025	ATP sulfurylase, Amino acid metabolism	MET3*	3.2	1.3	1.2	0.9	1.0	0.5	1.0	1.0	0.2	0.9	0.8
orf19.7214	glucan 1,3-beta-glucosidase	-	3.1	1.9	1.3	0.9	1.6	2.9	2.8	2.7	1.8	2.3	3.9
orf19.682			3.1	1.0	0.6	1.0	1.1	2.3	1.0	1.0	0.6	1.2	1.0

orf19.6079	hypothetical protein	-	3.0		1.1			1.0	1.0	1.0	0.8	1.2	
orf19.6601.1	non-native actin binding complex polypeptide	YKE2*	3.0	0.9	2.0	1.0	1.2	1.3	1.2	1.0	1.6	1.0	1.1
orf19.2685	hypothetical protein	-	2.9	1.7	0.6	1.1	1.5	3.8	1.9	1.6	0.7	1.5	1.8
orf19.7350	highly charged conserved protein of unknown function	-	2.9	2.0	1.1	1.1	0.9	1.9	6.7	1.7	0.6	2.5	1.9
orf19.7219	plasma membrane iron permease	FTR1	2.9	2.3	2.4	1.2	3.8	1.9	2.3	1.2	0.7	1.8	1.2
orf19.4056	GATA-family DNA binding proteins	-	2.9	2.5	0.8	2.1	1.2	4.4	1.0	1.0	0.5	1.0	0.8
orf19.5615	1-acyl dihydroxyacetone phosphate reductase	AYR1*	2.8	1.6	1.7	1.0	1.2	0.8	1.1	1.0	0.6	1.0	1.0
orf19.5619	hypothetical protein	-	2.8	1.6	0.6	1.5	1.3	1.9	1.5	1.0	0.6	1.2	1.2
orf19.1690	conserved hypothetical protein	TOS1*	2.8	1.4	0.6	1.2	1.1	2.8	1.4	1.1	0.6	1.2	1.3
orf19.2947	stationary phase protein	SNZ1*	2.8	2.7	1.6	1.9	1.2	0.7	0.6	1.3	0.4	0.8	1.4
orf19.6741	coneseved hypothetical protein	-	2.8	1.3	1.8	1.3	1.1	0.9	1.5	1.4	0.7	1.3	1.2
orf19.13			2.7	3.9	2.1	0.9	1.4	0.6	1.4	1.1	0.2	0.9	0.8
orf19.413	hypothetical protein	-	2.7	2.1	1.6	2.3	1.7	1.0	1.4	1.0	0.6	1.1	1.0
orf19.7324	pyrimidine precursor biosynthesis enzyme	THI13*	2.6	1.1	1.4	1.1	1.0	1.0	1.1	1.5	0.7	1.3	1.1
orf19.2292			2.6		1.2			0.8			0.2		
orf19.2886	MAP kinase involved in pheromone signal transduction	CEK1	2.6	1.3	1.1	1.0	1.0	3.7	1.4	2.4	1.8	1.8	1.4
orf19.2903	conserved hypothetical protein	-	2.5	1.7	1.0	0.9	1.3	1.8	1.5	1.2	0.6	1.2	1.0
orf19.3219	phosphohydrolase	-	2.5	1.0	1.7	1.1	0.9	1.1	0.6	1.0	0.8	0.8	0.9
orf19.3483	possible glycerophosphoryl phosphodiesterase	-	2.5	1.3	2.0	1.2	1.4	2.1	1.2	1.0	2.0	1.1	1.3
orf19.1964	conserved hypothetical protein	-	2.5	1.8	0.8	1.5	1.7	0.7	0.4	0.9	0.2	0.8	0.8
orf19.1408			2.5	3.9	2.2	0.9	1.4	0.6	1.4	1.1	0.2	0.8	0.8
orf19.1654	hypothetical protein	-	2.5	1.5	1.5	1.1	1.2	1.0	1.0	1.1	0.4	0.9	1.3
orf19.6028	G1 cyclin	HGC1	2.4	1.3	0.9	1.1	1.1	1.1	1.0	1.1	0.4	0.9	1.0
orf19.7308	alpha-1 tubulin	TUB1	2.4	1.6	0.5	1.2	1.1	1.3	1.0	1.4	0.3	1.3	1.1
orf19.2777			2.4		0.5			2.1			0.3		
orf19.1652	fatty-acyl coenzyme A oxidase	POX1*	2.3	1.0	0.8	0.7	1.0	2.1	1.1	1.2	0.5	1.1	1.1
orf19.4607	conserved hypothetical	-	2.3	0.9	1.5	0.9	1.0	1.6	1.2	1.1	0.9	1.0	1.0

	protein												
orf19.7583	potential fungal Zn(2)-Cys(6) binuclear cluster domain	-	2.3	1.0	1.6	1.0	1.0	1.9	1.5	1.2	0.8	1.1	1.0
orf19.6116	aldohexose specific glucokinase	GLK4*	2.3	2.0	2.4	0.9	1.2	0.6	1.2	1.1	0.2	0.7	0.9
orf19.2762	alkyl hydroperoxide reductase	AHP1*	2.3	1.5	1.5	1.1	1.2	1.0	1.1	1.2	0.6	0.8	1.1
orf19.7021	glycogen phosphorylase	GPH1*	2.3	4.6	2.0	1.5	1.9	1.6	1.6	1.1	0.6	1.0	1.2
orf19.4033	RNA-dependent ATPase	PRP22*	2.3	1.0	0.7	1.1	1.0	1.0	0.8	1.0	0.3	0.9	1.0
orf19.1153	glutamate decarboxylase	GAD1*	2.3	1.7	1.1	1.3	1.2	1.9	1.2	1.3	0.6	1.2	1.2
orf19.467	hypothetical protein	-	2.3	1.5	1.0	1.4	0.9	1.1	0.9	0.9	0.6	0.9	0.8
orf19.4263	hypothetical protein	-	2.2		1.4			1.3			0.8		
orf19.1847	pyruvate decarboxylase	ARO10*	2.2	3.5	2.3	1.2	2.0	0.6	1.6	1.5	1.1	1.2	1.2
orf19.7657	RNase P and RNase MRP subunit	POP3*	2.2	0.9	1.1	1.0	0.9	1.0	0.9	0.9	0.7	0.8	0.9
orf19.3150	similar to plant dihydroflavonol 4-reductase	GRP4*	2.2	2.0	1.3	0.8	1.2	1.3	1.8	1.1	0.9	1.0	1.8
orf19.5799	conserved hypotheticalprotein	-	2.2	1.6	0.5	1.1	1.8	3.4	2.9	2.5	1.2	1.8	5.1
orf19.4349.6			2.2		1.1			1.4			1.3		
orf19.6402	cystathionine gamma-lyase	CYS3*	2.2	1.1	0.9	0.9	1.3	1.1	1.0	0.9	0.3	1.1	1.2
orf19.1592	conserved hypothetical protein	-	2.2		1.1			1.0			0.5		
orf19.5620	conserved hypothetical protein	-	2.1	2.0	2.2	0.9	1.0	0.5	1.0	1.1	0.7	0.9	1.0
orf19.1995	alpha-1,2-mannosyltransferase	MNN5*	2.1	1.2	1.4	1.0	1.1	1.2	1.1	1.0	1.3	1.2	1.2
orf19.7635	ATP dependent RNA helicase	DRS1*	2.1	1.0	1.2	1.1	1.0	1.4	0.9	1.0	0.9	1.0	1.0
orf19.5451	hypothetical protein	-	2.1		1.4			1.3			0.9		
orf19.3013	septin	CDC12	2.1	1.1	1.3	1.0	1.2	1.9	1.3	1.1	1.0	1.2	1.1
orf19.2721	highly conserved hypothetical protein	CDC123*	2.1	1.0	1.5	1.0	1.0	0.7	1.0	1.0	1.0	1.1	1.0
orf19.4433	transcription factor modulated by MAP kinase cascade and involved in filamentous growth;homolog of budding yeast Ste12p	CPH1	2.1	0.8	0.8	1.2	0.8	1.3	0.8	0.9	0.6	0.8	0.8
orf19.4649	zinc finger transcription	-	2.1	0.9	1.3	1.0	1.0	1.1	1.0	1.0	0.8	1.0	1.0

	factor												
orf19.6073	heme binding protein	HMX1*	2.1	1.1	1.3	0.8	1.1	1.2	1.2	1.1	1.1	1.1	1.2
orf19.4666	hypothetical protein	-	2.1	1.7	1.1	1.5	1.7	1.9	2.3	1.4	1.4	2.0	1.7
orf19.3941	CTP synthase	URA7*	2.0	0.9	1.4	1.3	1.5	1.1	0.8	1.0	0.5	1.1	1.0
orf19.156	hypothetical protein	-	2.0		2.2			1.2			1.2		
orf19.2346	highly conserved hypothetical protein	-	2.0	1.0	1.3	1.0	1.0	1.4	0.9	1.0	1.0	1.0	1.1
orf19.3984	conserved hypothetical protein	-	2.0		0.9			1.8			1.4		
orf19.6864	conserved hypothetical protein	-	2.0	1.2	1.2	0.9	1.0	1.7	1.8	1.1	1.4	1.0	1.3
orf19.6724	mitochondrial fumarate hydratase, 5-prime end	FUM2*	0.5		2.0			0.6			0.6		
orf19.4318	transcriptional regulator	MIG1	0.5	1.0	0.9	1.1	1.1	1.1	1.0	0.9	0.9	0.8	0.9
orf19.868	putative adenosine deaminase;transcriptional regulation	-	0.5	1.2	0.7	0.9	0.8	1.3	1.3		1.4	1.2	1.3
orf19.1067	phosphoglycerate mutase	GPM2*	0.5	1.0	2.2	1.0	0.9	0.6	1.0	1.0	1.1	0.7	0.9
orf19.4167	hypothetical protein	-	0.5		1.2			0.4			1.1		
orf19.1776	pantotheine-phosphate adenylyltransferase	-	0.5	0.9	1.1	0.9	1.0	0.5	0.8	0.9	1.4	1.0	0.9
orf19.4577.3	involved in import of mitochondrial protein	TIM10*	0.5	1.1	0.9	0.8	1.0	0.7	1.0	1.1	1.9	0.8	1.1
orf19.2116	N-acetyltransferase for N-terminal methionine	NAT2*	0.5	1.0	0.5	1.1	1.3	1.0	1.2	1.0	0.6	1.5	1.2
orf19.7498	3-isopropylmalate dehydratase	LEU1*	0.5	1.0	0.4	0.8	0.6	0.9	0.7	0.9	0.6	0.8	0.7
orf19.6645	high mobility group-like protein	HMO1*	0.5	0.6	0.6	0.7	0.7	1.0	1.1	1.0	1.0	1.1	0.7
orf19.4676	conserved hypothetical protein	-	0.5	0.7	1.1	0.9	0.8	0.5	0.8	1.0	1.3	0.9	0.9
orf19.5911	Ca2+/calmodulin-dependent protein kinase	CMK1*	0.5	0.9	0.8	1.0	0.8	1.0	1.1	0.9	0.5	0.7	0.6
orf19.1857	histone acetyltransferase and transcription regulatory protein	SPT11*	0.5	0.9	1.2	0.6	0.7	0.6	1.2	1.3	1.4	0.9	1.2
orf19.3893	Soluble Cell Wall protein	SCW11*	0.5		1.4			0.9			1.7		
orf19.3526	myo-inositol transporter	ITR2*	0.5	0.7	0.6	0.7	0.9	1.2	0.8	0.9	1.0	1.0	1.0
orf19.4444	4-nitrophenyl phosphatase	PHO15*	0.5	1.5	1.2	1.1	0.9	0.7	1.0	1.0	0.9	0.9	1.0
orf19.2655	cell cycle arrest protein	BUB3*	0.5	1.3	2.1	1.0	1.1	0.5	0.6	1.0	0.7	1.0	0.9
orf19.1485	mitochondrial ribosomal	MRPL31*	0.5	0.7	1.4	0.8	0.6	0.4	0.8	0.9	2.2	0.9	0.7

	protein												
orf19.4952.1	FK506-binding protein 2 precursor	FKB2*	0.5	1.0	1.1	0.7	0.8	0.5	1.2	1.0	1.8	0.9	1.2
orf19.2896	peroxisomal 2,4- dienoyl-CoA reductase, and sorbitol utilization protein	SOU1*	0.4	0.9	0.6	0.5	0.7	1.4	2.7	0.9	3.2	1.1	1.8
orf19.5228	3,4-dihydroxy-2-butanone 4- phosphate synthase, riboflavin biosynthesis	RIB3*	0.4	1.2	1.3	0.9	0.7	0.6	0.8	0.9	0.7	0.7	0.8
orf19.1340	aldose reductase	-	0.4	0.9	1.0	0.9	1.0	0.7	0.9	1.0	1.9	1.0	1.1
orf19.6660	unknown protein	-	0.4	1.3	0.9	1.9	1.1	1.2	1.2	1.1	1.4	1.0	1.5
orf19.4602	mitochondrial malate dehydrogenase	MDH1*	0.4	0.8	0.4	1.1	0.9	1.1	1.1	1.5	1.1	1.9	1.3
orf19.254	hypothetical protein	-	0.4	0.9	0.6	0.8	0.9	1.1	0.9	0.8	0.7	0.9	0.9
orf19.2977	conserved hypothetical protein	-	0.4	0.6	0.9	0.9	0.5	0.6	1.0	0.9	1.2	0.9	0.9
orf19.3803	Golgi alpha-1,2-mannosyltransferase	MNN22*	0.4	0.8	1.0	1.1	1.1	0.7	0.8		0.9	1.3	1.1
orf19.4393	nuclear citrate synthase	CIT1*	0.4	0.7	0.2	0.9	1.1	1.4	1.3	1.1	1.1	1.1	1.6
orf19.2398	hypothetical protein	-	0.4	1.2	0.4	1.7	0.9	1.3	0.8	1.0	0.8	1.0	1.0
orf19.6874	myb-like transcription factor	-	0.4	0.9	0.3	1.0	1.0	1.7	1.2	0.9	1.2	1.1	0.9
orf19.7218	homology to plant PR-1 class of proteins	PRY2*	0.4	1.0	1.0	1.1	1.1	0.7	0.9	0.6	1.1	0.9	0.6
orf19.2846	hypothetical protein	-	0.4	1.0	1.2	1.1	1.1	2.2	1.3	1.0	5.5	1.1	1.2
orf19.2397.3	conserved hypothetical protein	-	0.4	1.1	1.3	1.1	1.0	0.6	0.8	1.0	1.1	1.0	1.2
orf19.780	urea amidolyase	DUR1*	0.4	1.2	0.8	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0
orf19.5118	spindle-pole body protein	SDS24	0.4	1.0	0.3	1.4	1.1	1.2	1.1	1.0	0.9	1.0	1.0
orf19.633	putative methyltransferase	-	0.4	1.1	1.3	0.7	0.9	0.4	0.9	1.0	1.0	0.7	0.9
orf19.3869	hypothetical protein	-	0.4	0.7	0.5	0.8	0.9	1.4	1.1	0.7	0.8	1.3	0.8
orf19.5626	conserved hypothetical protein	-	0.3	3.1	1.3	3.4	1.2	1.0	0.8	0.9	1.3	0.8	0.7
orf19.4195.1	cytosine deaminase	FCY1*	0.3	0.8	1.5	0.8	0.7	0.3	0.9	0.8	1.9	0.7	0.9
orf19.5917	zinc finger protein involved in pre-tRNA splicing	STP3*	0.3	0.5	0.9	0.7	0.9	0.6	0.6	0.8	1.0	1.0	0.9
orf19.6514	copper homeostasis, peptide import regulation	CUP9*	0.3	0.9	0.7	0.8	1.0	1.1	0.8	1.0	0.9	1.1	1.2
orf19.1614	low affinity high capacity ammonium permease	MEP1	0.3	0.9	1.1	0.9	0.7	0.2	0.4	1.0	0.8	1.0	0.9
orf19.2876	centromere-binding kinetochore protein	CBF1	0.3	0.5	0.8	1.0	0.5	0.5	0.6	1.1	0.8	0.8	0.4

orf19.2173	nuclear-cytoplasmic transport;negative effector of Pol III synthesis	MAF1*	0.3	1.2	1.6	1.1	1.1	0.4	0.8	0.9	0.9	0.8	0.9
orf19.6937	peptide transporter	PTR2*	0.3	0.6	0.4	1.3	0.7	0.7	0.8	0.6	0.9	1.0	0.5
orf19.449	predicted phosphatidyl synthase	-	0.2	1.1	1.4	1.3	1.0	0.6	0.7	0.9	1.1	1.0	1.0
orf19.3895	chitinase 2 precursor	CHT2	0.2	0.9	0.3	0.6	1.0	0.3	0.3	0.9	0.4	0.2	0.6
orf19.4716	NADP-glutamate dehydrogenase	GDH3*	0.2	0.6	0.3	0.4	0.8	0.2	0.4	0.9	0.3	0.7	0.5
orf19.5282	hypothetical protein	-	0.2	1.2	1.0	0.9	0.9	0.4	0.7	1.1	0.9	0.9	1.0
orf19.5267	hypothetical protein	-	0.2	0.7	1.2	0.9	1.1	0.4	0.4	0.9	1.4	0.9	0.8
orf19.7150	transcriptional repressor	NRG1	0.2	1.2	0.3	2.0	1.1	1.1	0.6	0.7	1.9	1.1	0.8
orf19.5288	Zn-containing alcohol dehydrogenase	YAL60*	0.2	1.4	1.9	1.1	1.0	0.1	0.4	0.9	0.3	0.7	0.8
orf19.6570	purine nucleoside permease	NUP3	0.2	0.9	0.9	1.2	0.8	0.3	0.9	1.0	0.9	1.0	0.8
orf19.4737	membrane transporter of the MFS-MDR family	DHA12*	0.1	1.0	0.6	1.1	1.1	0.4	0.4	0.5	0.9	0.7	1.0
orf19.5305	conserved protein repressed in hyphal development	RHD3	0.1	0.7	0.7	0.9	0.6	0.3	0.3	1.2	0.9	0.6	0.7
orf19.4211	multicopper ferro-O ₂ -oxidoreductase involved in high-affinity iron uptake	FET3*	0.1	0.4	0.3	0.7	0.7	1.2	1.9	1.4	1.8	2.4	2.1
orf19.54	Repressed in Hyphal Development;family of conserved protein of unknown function	RHD1	0.1	0.3	0.5	0.4	0.5	0.5	1.2	1.1	2.7	1.5	1.5
orf19.3618	putative cell wall protein	YWP1	0.0	0.7	0.1	0.8	0.5	0.6	0.9	0.9	2.0	0.7	0.7

Table S3.

Relative induction of upregulated hyphae specific genes in *myo5Δ/Δ* and *arp2Δ/Δ* mutants.

Green indicates hyphae specific genes are less upregulated in *arp2Δ/Δ* mutants compared to *myo5Δ/Δ* mutants.

Red indicates hyphae specific genes are more upregulated in *arp2Δ/Δ* mutants compared to *myo5Δ/Δ* mutants.

	column B	column C	column D	column E	column F	column G	
Systematic name	WT-HY (Epp)	arp2-HY	WT-HY (Oberholzer)	myo5-HY	ratio of columns C over B	ratio of columns E over D	ratio of columns G over F
orf19.1816	31.09	1.80			0.06		
orf19.3374	30.38	6.15	81.22	3.33	0.20	0.04	4.9
orf19.4082	26.66	0.91	4.36	0.70	0.03	0.16	0.2
orf19.5760	24.42	2.51	10.56	5.58	0.10	0.53	0.2
orf19.4975	20.21	1.60	1.25	1.03	0.08	0.82	0.1
orf19.5636	19.92	0.57			0.03		
orf19.1327	17.25	7.42			0.43		
orf19.5585	17.16	1.16	19.10	1.53	0.07	0.08	0.8
orf19.1321	16.43	3.50	25.56	9.55	0.21	0.37	0.6
orf19.4215	13.68	3.98			0.29		
orf19.5674	11.96	1.18	13.82	2.45	0.10	0.18	0.6
orf19.251	11.74	3.02	4.85	1.08	0.26	0.22	1.2
orf19.2355	9.40	1.33			0.14		
orf19.4943	9.00	2.96	1.99	1.86	0.33	0.93	0.4
orf19.3829	8.89	0.87	5.04	3.85	0.10	0.76	0.1
orf19.2210	8.55	1.02	1.41	1.17	0.12	0.83	0.1
orf19.4980	8.53	1.41	1.48	1.18	0.17	0.79	0.2
orf19.5542	8.05	1.50	3.74	1.51	0.19	0.40	0.5
orf19.4527	7.96	2.17	4.63	2.07	0.27	0.45	0.6
orf19.1691	7.18	3.89	3.91	1.23	0.54	0.31	1.7
orf19.5265	6.73	1.77	1.33	1.22	0.26	0.92	0.3
orf19.7610	6.62	1.66	1.66	2.09	0.25	1.26	0.2
orf19.716	5.84	1.69	1.09	1.07	0.29	0.98	0.3
orf19.2241	5.82	1.57	1.17	1.00	0.27	0.86	0.3
orf19.113	5.79	1.24	1.21	0.86	0.21	0.71	0.3
orf19.3384	5.68	1.14	3.00	1.51	0.20	0.50	0.4
orf19.6202	5.31	0.39	5.58	1.44	0.07	0.26	0.3
orf19.85	4.94	1.74	3.58	1.11	0.35	0.31	1.1

orf19.815	4.56	2.58	2.08	1.35	0.56	0.65	0.9
orf19.1996	4.51	1.28	3.09	1.40	0.28	0.45	0.6
orf19.2952	4.41	2.28	1.35	1.27	0.52	0.94	0.6
orf19.5645	4.37	0.35			0.08		
orf19.2125	4.35	0.89	2.95	0.82	0.20	0.28	0.7
orf19.4477	4.09	1.48			0.36		
orf19.7531	4.09	1.26	1.86	1.89	0.31	1.02	0.3
orf19.4590	4.05	1.26	1.22	1.01	0.31	0.83	0.4
orf19.6608	3.99	1.35	2.40	2.38	0.34	0.99	0.3
orf19.4530.1	3.96	1.64	1.01	0.98	0.42	0.97	0.4
orf19.2959.1	3.93	1.07			0.27		
orf19.7323	3.91	3.55			0.91		
orf19.675	3.90	0.21	4.83	1.11	0.05	0.23	0.2
orf19.7053	3.85	1.09			0.28		
orf19.4749	3.77	2.24	1.72	1.09	0.59	0.63	0.9
orf19.6420	3.76	0.42	4.18	3.17	0.11	0.76	0.1
orf19.6287	3.75	1.54	1.18	0.96	0.41	0.81	0.5
orf19.1799	3.70	1.27	1.04	0.99	0.34	0.95	0.4
orf19.1655	3.67	0.71	1.04	0.98	0.19	0.95	0.2
orf19.5614	3.59	1.31	1.96	1.23	0.36	0.63	0.6
orf19.3746	3.50	0.94	1.18	1.05	0.27	0.89	0.3
orf19.6705	3.49	1.28	1.84	1.39	0.37	0.76	0.5
orf19.7362	3.40	2.25	2.32	1.68	0.66	0.73	0.9
orf19.2372	3.34	1.83	1.28	1.63	0.55	1.27	0.4
orf19.5373	3.29	2.11	1.84	1.56	0.64	0.85	0.8
orf19.7304	3.28	2.45	2.91	1.67	0.75	0.57	1.3
orf19.6021	3.27	1.20	1.26	1.13	0.37	0.90	0.4
orf19.7504	3.23	0.83	2.14	1.14	0.26	0.53	0.5
orf19.715	3.22	1.93	1.17	0.91	0.60	0.78	0.8
orf19.5025	3.21	1.21	1.35	0.89	0.38	0.66	0.6
orf19.7214	3.14	1.33	1.93	0.87	0.42	0.45	0.9
orf19.682	3.10	0.62	1.02	1.01	0.20	0.99	0.2
orf19.6079	2.98	1.13			0.38		
orf19.6601.1	2.96	1.97	0.92	0.97	0.67	1.06	0.6
orf19.2685	2.94	0.58	1.65	1.10	0.20	0.66	0.3
orf19.7350	2.91	1.06	2.01	1.08	0.36	0.54	0.7
orf19.7219	2.90	2.42	2.34	1.23	0.84	0.53	1.6
orf19.4056	2.88	0.76	2.49	2.10	0.26	0.84	0.3
orf19.5615	2.81	1.75	1.59	1.00	0.62	0.63	1.0

orf19.5619	2.81	0.60	1.64	1.53	0.21	0.93	0.2
orf19.1690	2.78	0.57	1.36	1.25	0.20	0.91	0.2
orf19.2947	2.78	1.56	2.69	1.89	0.56	0.70	0.8
orf19.6741	2.76	1.78	1.32	1.34	0.64	1.01	0.6
orf19.13	2.71	2.11	3.89	0.92	0.78	0.24	3.3
orf19.413	2.67	1.59	2.06	2.33	0.60	1.13	0.5
orf19.7324	2.62	1.44	1.15	1.12	0.55	0.98	0.6
orf19.2292	2.56	1.24			0.48		
orf19.2886	2.56	1.05	1.31	0.95	0.41	0.73	0.6
orf19.2903	2.53	1.00	1.75	0.89	0.39	0.51	0.8
orf19.3219	2.52	1.66	1.02	1.08	0.66	1.06	0.6
orf19.3483	2.51	1.96	1.30	1.19	0.78	0.91	0.9
orf19.1964	2.50	0.78	1.83	1.47	0.31	0.81	0.4
orf19.1408	2.48	2.20	3.90	0.90	0.89	0.23	3.9
orf19.1654	2.46	1.52	1.46	1.10	0.62	0.76	0.8
orf19.6028	2.42	0.87	1.27	1.06	0.36	0.84	0.4
orf19.7308	2.38	0.53	1.59	1.18	0.22	0.74	0.3
orf19.2777	2.36	0.54			0.23		
orf19.1652	2.33	0.83	1.02	0.71	0.36	0.69	0.5
orf19.4607	2.32	1.55	0.93	0.93	0.67	1.00	0.7
orf19.7583	2.32	1.65	1.01	1.01	0.71	1.00	0.7
orf19.6116	2.32	2.45	1.95	0.91	1.06	0.47	2.3
orf19.2762	2.31	1.49	1.48	1.07	0.64	0.72	0.9
orf19.7021	2.30	1.97	4.56	1.47	0.85	0.32	2.6
orf19.4033	2.30	0.66	1.04	1.09	0.29	1.05	0.3
orf19.1153	2.26	1.06	1.71	1.26	0.47	0.73	0.6
orf19.467	2.25	1.01	1.51	1.43	0.45	0.94	0.5
orf19.4263	2.24	1.44			0.64		
orf19.1847	2.23	2.31	3.52	1.18	1.04	0.34	3.1
orf19.7657	2.23	1.10	0.94	0.96	0.49	1.02	0.5
orf19.3150	2.19	1.33	2.03	0.82	0.61	0.40	1.5
orf19.5799	2.17	0.51	1.65	1.08	0.24	0.66	0.4
orf19.4349.6	2.17	1.08			0.50		
orf19.6402	2.16	0.95	1.07	0.94	0.44	0.88	0.5
orf19.1592	2.16	1.10			0.51		
orf19.5620	2.13	2.22	1.97	0.87	1.04	0.44	2.3
orf19.1995	2.13	1.37	1.16	1.04	0.64	0.90	0.7
orf19.7635	2.13	1.21	0.96	1.06	0.57	1.10	0.5
orf19.5451	2.12	1.44			0.68		

orf19.3013	2.11	1.31	1.05	1.01	0.62	0.96	0.6
orf19.2721	2.08	1.47	1.04	1.04	0.71	1.00	0.7
orf19.4433	2.07	0.80	0.82	1.17	0.38	1.43	0.3
orf19.4649	2.07	1.30	0.88	1.02	0.63	1.16	0.5
orf19.6073	2.06	1.31	1.11	0.79	0.64	0.71	0.9
orf19.4666	2.05	1.12	1.66	1.45	0.54	0.87	0.6
orf19.3941	2.05	1.35	0.85	1.33	0.66	1.56	0.4
orf19.156	2.05	2.24			1.09		
orf19.2346	2.02	1.30	1.02	1.01	0.64	0.99	0.6
orf19.3984	2.02	0.95			0.47		
orf19.6864	2.01	1.20	1.25	0.93	0.60	0.74	0.8

Table S4.
NRG1 dependent hyphae specific genes.

This gene list shows the 28 *NRG1*-dependent hyphae specific genes as described in Kadosh et al. (2005).

Note that 'nrg1/arp2-H' represents the microarray data for the comparison of the *nrg1ΔΔ/arp2ΔΔ* mutant grown under hyphal conditions to WT cells grown under hyphal conditions.

Note that 'nrg1/arp2-H vs arp2-H' represents the microarray data for the comparison of the *nrg1ΔΔ/arp2ΔΔ* mutant grown under hyphal conditions to the *arp2ΔΔ* mutant grown under hyphal conditions.

Systematic name	Common name	nrg1/arp2-H Normalized log2 ratio	nrg1/arp2-H vs arp2-H Normalized log2 ratio	Description
orf19.4975	HYR1	0.9	10.9	Nonessential, GPI anchored, predicted cell wall protein; macrophage-induced protein; induced in hyphae; induction associated with azole resistance; regulated by Rfg1p, Efg1p, Nrg1p, Tup1p, Cyr1p
orf19.5585	SAP5	0.2	5.9	Secreted aspartyl proteinase; sap4 sap5 sap6 triple mutant has defects in virulence and utilization of protein as nitrogen source; expressed in mucosal & systemic infections, oral carriage; absent from less virulent species, <i>C. dubliniensis</i>
orf19.5716	SAP4	0.3	5.5	Secreted aspartyl proteinase; sap4 sap5 sap6 triple mutant has defects in systemic virulence (mouse, guinea pig), survival in macrophage, and protein utilization for nitrogen; expressed during mucosal and systemic infections, oral carriage
orf19.5265	KIP4	0.9	3.3	Transposon mutation affects filamentous growth; filament induced; shows Mob2p-dependent hyphal regulation; regulated by Nrg1p, Tup1p
orf19.6079		2.2	2.6	Predicted ORF in retrotransposon Tca8 with similarity to the Gag region encoding nucleocapsid-like protein; transcription is downregulated in response to treatment with ciclopirox olamine; filament induced; regulated by Rfg1p, Tup1p
orf19.1816	ALS3	0.2	2.4	Adhesin; ALS family; role in epithelial adhesion, endothelial invasiveness; allelic variation in adhesiveness; immunoprotective in mice; predicted GPI-anchor cell wall protein; macrophage, hyphal induced, pH, Nrg1p, Rfg1p, Tup1p regulated
orf19.1822	UME6	1.8	2.2	Transcription factor; required for wild-type hyphal extension, virulence; zinc cluster DNA-binding motif; similar to <i>S. cerevisiae</i> Ume6p, which is a meiotic regulator; alkaline upregulated; filament induced; regulated by Nrg1p, Tup1p, RFG1p
orf19.5760	IHD1	1.0	1.9	Putative GPI-anchored protein of unknown function; alkaline upregulated; greater transcription in hyphal form than yeast form; regulated by Nrg1p, Rfg1p, Tup1p; regulated by Tsa1p, Tsa1Bp in minimal media at 37; not essential for viability

orf19.7323	CBP1	0.8	1.7	Corticosteroid binding protein; transcription induced at late log-phase or upon adherence to polystyrene; not induced by corticosterone; contains a possible NAD/FAD binding region; regulated by Nrg1p, Tup1p
orf19.6705		0.9	1.6	Putative guanyl nucleotide exchange factor with Sec7 domain; transcriptionally regulated upon yeast-hyphal switch; filament induced; regulated by Nrg1p, Tup1p; shows Mob2p-dependent hyphal regulation
orf19.2355	ALS10	0.5	1.5	ALS family protein; ALS family includes cell-surface glycoproteins, some with adhesin function; putative GPI-anchor; filament induced; regulated by Nrg1p, Rfg1p, Tup1p; merged with orf19.1816 in Assembly 20
orf19.3374	ECE1	0.4	1.4	Protein comprising eight 34-residue repeats; hyphal-specific expression increases with extent of elongation of the cell; regulated by Rfg1p, Nrg1p, Tup1p, Cph1p, Efg1p, Hog1p, farnesol, phagocytosis; may contribute to biofilm formation
orf19.1691		0.1	1.4	Predicted ORF in Assemblies 19, 20 and 21; fluconazole-induced; filament induced; Hog1p-induced; regulated by Nrg1p, Tup1p; increased expression in response to prostaglandins
orf19.207	PGA55	1.3	1.3	Putative GPI-anchored protein of unknown function; filament induced; regulated by Nrg1p, Tup1p; possibly transcriptionally regulated upon hyphal formation
orf19.2060	SOD5	1.0	1.2	Copper- and zinc-containing superoxide dismutase; protective role against oxidative stress; induced by neutrophil contact, hyphal growth, caspofungin, osmotic or oxidative stress; member of a gene family including SOD1, SOD4, SOD5, and SOD6
orf19.6582	PRE10	1.1	1.1	Alpha7 (C8) subunit of the 20S proteasome; multiple phosphorylated residues; transcription is induced upon filamentous growth
orf19.5076	PFY1	1.0	1.1	Profilin, functional homolog of <i>S. cerevisiae</i> Pfy1p; hyphal-induced; macrophage/pseudohyphal-induced; regulated by Nrg1p, Tup1p; gene lacks intron (unlike <i>S. cerevisiae</i> PFY1); complements growth of <i>S. cerevisiae</i> srv2 mutant; nonessential
orf19.7561	DEF1	1.0	1.1	Protein required for filamentous growth and for escape from epithelial cells and dissemination in an RHE model; transcription induced in oral candidiasis clinical isolates; induced by fluconazole, high cell density; hyphally regulated
orf19.1120	FAV2	1.1	1.1	Induced by mating factor in MTLa/MTLa opaque cells; greater mRNA abundance observed in a <i>cyr1</i> homozygous null mutant than in wild type; filament induced; regulated by Nrg1p, Rfg1p, Tup1p; possibly spurious ORF (AWG prediction)
orf19.5968	RDI1	1.1	1.1	Protein similar to <i>S. cerevisiae</i> Rdi1p, an inhibitor of Rho-GDP dissociation; transposon mutation affects filamentous growth; farnesol-, filament-induced; regulated by Nrg1p, Tup1p
orf19.3829	PHR1	1.1	1.1	Glycosidase of cell surface; may act on cell-wall beta-1,3-glucan prior to beta-1,6-glucan linkage; E169 and E270 at active site; role in systemic but not vaginal virulence (neutral, not low pH); high pH or filamentation induced
orf19.4255	ECM331	1.9	1.0	GPI-anchored protein; mainly at plasma membrane, also at cell wall; caspofungin induced; Plc1p-regulated; repressed by Rim101p, Hog1p; colony morphology-related gene regulation by Ssn6p; induced in <i>cyr1</i> homozygous null mutant yeast-form cel

orf19.1321	HWP1	1.0	1.0	Hyphal cell wall protein; covalently crosslinked to epithelial cells by host transglutaminase; opaque- and a-specific, alpha-factor induced; at MTL _a side of conjugation tube; assessment of virulence role complicated by URA3 effects
orf19.3380	HWP2	1.0	1.0	Putative GPI-anchored protein; cell wall localization predicted; hyphal-specific expression; regulated by Efg1p, Tup1p; has similarity to Hwp1p, Ece2p, Rbt1p domains; predicted signal sequence and O-glycosylation
orf19.1860	LSC2	1.4	1.0	Protein described as beta subunit of succinate-CoA ligase; transcription regulated by Mig1p and Tup1p; transcriptionally regulated by iron; expression greater in high iron; filament induced
orf19.1825		1.1	0.9	Protein not essential for viability; filament induced; regulated by Nrg1p, Rfg1p, Tup1p
orf19.3698		1.2	0.9	Predicted ORF in Assemblies 19, 20 and 21; filament induced
orf19.5567	POP4	1.0	0.7	Protein likely to be essential for growth, based on an insertional mutagenesis strategy; similar to <i>S. cerevisiae</i> Pop4p, which is a subunit of both RNase MRP and nuclear RNase P; filament induced; regulated by Nrg1p, Tup1p

Table S5.

Ergosterol genes that are downregulated (< 2 fold change) in either "arp2-H" or "arp2-Y".

systematic name	common name	WT-HY vs arp2-HY		WT-Y vs arp2-Y		Description
		Normalized log2 ratio	t-test P-value	Normalized log2 ratio	t-test P-value	
orf19.922	ERG11	0.49	2.7E-01	0.48	5.4E-04	cytochrome P450 lanosterol 14a-demethylase
orf19.406	ERG1	0.19	1.7E-02	0.37	2.6E-02	squalene epoxidase(monooxygenase), ergosterol biosynthesis
orf19.5178	ERG5	0.44	2.5E-02	0.71	1.4E-01	cytochrome P450
orf19.3240	ERG27	0.47	1.8E-02	0.96	6.2E-01	3-keto sterol reductase
orf19.1591	ERG10	0.24	4.0E-02	1.47	1.8E-01	acetyl-CoA acetyltransferase;acetoacetyl CoA thiolase
orf19.1631	ERG6	0.75	4.7E-01	0.48	1.2E-01	S-adenosyl-methionine delta-24- sterol-c-methyltransferase
orf19.4631	ERG252	0.29	1.0E-01	0.60	4.3E-02	C-4 sterol methyl oxidase;membrane-bound non-heme di-iron oxygenase involved in lipid metabolism

Table S6.

Strains used in this study.

name	description	parent strain	relavent genotype	reference
SC5314	WT strain	clinical isolate	prototroph	Gillum et al. (1984)
BWP17	WT strain	RM1000	arg4/arg4 his1/his1 ura3/ura3	Wilson et al. (1999)
SN95	WT strain	SN76	arg4/arg4 his1/his1	Noble et al. (2005)
SN148	WT strain	SN76	arg4/arg4 his1/his1 ura3/ura3 leu2/leu2	Noble et al. (2005)
nrg1	nrg1 deletion strain	CAI4	nrg1::HisG/nrg1::HisG	Murad et al. (2001)
Tn:trp1	transposon mutant	BWP17	trp1::UAU1/trp1::URA3	This study
Tn::cdc39	transposon mutant	BWP17	cdc39::UAU1/cdc39::URA3	This study
Tn::arp2	transposon mutant	BWP17	arp2::UAU1/arp2::URA3	This study
Tn::vps52	transposon mutant	BWP17	vps52::UAU1/vps52::URA3	This study
Tn::875	transposon mutant	BWP17	orf19.875:UAU1/orf19.875::URA3	This study
arp2 heterozygote 1		SN95	arp2::HIS1/ARP2	This study
arp2 heterozygote 2		SN95	arp2::HIS1/ARP2	This study
CaEE86	arp2 deletion mutant	arp2 heterozygote 1	arp2::ARG4/arp2::HIS1	This study
CaEE87	arp2 deletion mutant	arp2 heterozygote 1	arp2::ARG4/arp2::HIS1	This study
CaEE88	arp2 deletion mutant	arp2 heterozygote 2	arp2::ARG4/arp2::HIS1	This study
CaEE89	arp2 deletion mutant	arp2 heterozygote 2	arp2::ARG4/arp2::HIS1	This study
CaEE193	arp2 revertant 1	CaEE86	ARP2::SAT1-FLIP/arp2::HIS1	This study
CaEE195	arp2 revertant 2	CaEE86	arp2::ARG4/ARP2::SAT1-FLIP	This study
CaEE196	arp2 revertant	CaEE193	ARP2::FRT/arp2::HIS1	This study
CaEE198	arp2 revertant	CaEE195	arp2::ARG4/ARP2::FRT	This study
CaEE63	vps52 heterozygote 1	SN95	vps52::HIS1/VPS52	This study
CaEE64	vps52 heterozygote 2	SN95	vps52::HIS1/VPS52	This study
CaEE105	vps52 deletion mutant	CaEE63	vps52::HIS/vps52::ARG4	This study
CaEE120	vps52 deletion mutant	CaEE64	vps52::HIS/vps52::ARG4	This study
CaEE191	vps52 revertant	CaEE120	vps52::HIS1/VPS52::SAT1-FLIP	This study
CaEE192	vps52 revertant	CaEE191	vps52::HIS1/VPS52::FRT	This study
orf19.875 heterozygote 1		SN95	orf19.875::HIS1/ORF19.875	This study

orf19.875 heterozygote 2		SN95	orf19.875::HIS1/ORF19.875	This study
CaEE90	orf19.875 deletion mutant	orf19.875 heterozygote 1	orf19.875::HIS1/orf19.875::ARG4	This study
CaEE91	orf19.875 deletion mutant	orf19.875 heterozygote 1	orf19.875::HIS1/orf19.875::ARG4	This study
CaEE92	orf19.875 deletion mutant	orf19.875 heterozygote 2	orf19.875::HIS1/orf19.875::ARG4	This study
CaEE93	orf19.875 deletion mutant	orf19.875 heterozygote 2	orf19.875::HIS1/orf19.875::ARG4	This study
CaEE175	orf19.875 revertant 1	CaEE90	orf19.875::HIS1/ORF19.875::SAT1-FLIP	This study
CaEE176	orf19.875 revertant 2	CaEE90	ORF19.875::SAT1-FLIP/orf19.875::ARG4	This study
CaEE182	orf19.875 revertant	CaEE175	orf19.875::HIS1/ORF19.875::FRT	This study
CaEE183	orf19.875 revertant	CaEE176	ORF19.875::FRT/orf19.875::ARG4	This study
CaEE218	arp2 heterozygote	SN148	arp2::LEU2/ARP2	This study
CaEE221	arp3 heterozygote	CaEE218	arp2::LEU2/ARP2 arp3::URA3/ARP3	This study
CaEE224	arp3 deletion mutant	CaEE221	arp2::LEU2/ARP2 arp3::URA3/arp3::ARG4	This study
CaEE227	arp2/arp3 double KO	CaEE224	arp2::LEU2/arp2::HIS1 arp3::URA3/arp3::ARG4	This study
TEF1-GFP	TEF1 tagged with GFP	BWP17	BWP17, tef1::TEF1p-GFP-HIS1/TEF1, ura3, arg4	This study
CaEE329	arp2 heterozygote	nrg1 deletion strain	arp2/ARP2 nrg1/nrg1	This study
CaEE335	nrg1/arp2 deletion mutant	arp2 deletion in nrg1 mutant	arp2/arp2::SAT1-FLIP nrg1/nrg1	This study

Table S7.

Plasmids used in this study.

name	description
pEE18	<i>VPS52</i> cloned in pSFS2A
pEE20	downstream <i>VPS52</i> sequence cloned in pEE18
pEE22	<i>ARP2</i> cloned in pSFS2A
pEE23	<i>ORF19.875</i> cloned in pSFS2A
pEE30	downstream <i>ORF19.875</i> sequence cloned in pEE23
pEE33	downstream <i>ARP2</i> sequence cloned in pEE22
pEE59	upstream <i>ARP2</i> sequence cloned into KpnI/NotI digested pEE33

Table S8.
Primers (oligonucleotids) used in this study.

name	sequence ¹	description
MR1538/9	GTTTCCACTCACCGAGAAAACCAGCAAGAGAAAATAGAAAAGTGAAAGCTGGGTTGGACACTAGTATCTTTT TTTGCTGTGTTGACACATTCATAGTAAAGCAATTAATATATCTATATCgaagcttcgtacgctgcaggtc	ARP2 upstream flanking primer
MR1540/1	GTGATAGATATGATGCTTTGGTTGACTCTGTAATGAAACAAATACAAAACCTTAAACTACAGCTAGTGTA TAAAATATTTATATCTATGTTAATGAAAATACAAAATCATATATGTAAACCctcgaattcatcgatgatcaga	ARP2 downstream flanking primer
MR1546/7	TTTGTTGTATATGTTCAAATCGTAGATTGTATAGTATATTTTGCTTCAACTAGATATGAGTGAGTGGGAA TGTTTCCATTTTGTAAATCATCGACTTTTTTTTTTTGGCCAACCCTTTAgaagcttcgtacgctgcaggtc	ORF19.875 upstream flanking primer
MR1548/9	GGCGAAACAGACCTAATAATAAATAGTTTATGTATACTTTGTATATTCTGCTTATTTACAAGTCAACTATTA AGTAGTTTACGGATATATGTATACCTACAATAAGGAAAAAAAAAAACgaagcttcgtacgctgcaggtc	ORF19.875 downstream flanking primer
MR1322	GGAGTTCCATTTAGAGAAACTCATC	pRS-ARG F
MR1588	ACGAGGATCAAGAGTCTCCTCTCAC	ORF19.875 external R
MR1324	GAAGTTGTGTGGGAAAAATCTTCAC	pGEM-HIS F
MR1587	TTTACGAAAACGATTAGACCCTGTG	ORF19.875 external F
MR1589	TTTCTTAATGGGATCAACGCCACTTC	ORF19.875 internal R
MR1582	TACACCGTGACCCAAACGATAGATG	ARP2 external R
MR1581	AGTCACACTTCATACTTGACGTTTGC	ARP2 external F
MR1583	GGGTTCATAGGTGGTTCTGTTAACAAG	ARP2 internal R
oEE58	ACGAGGAAAAAAAAAATAAAAGTTAGATCAACTCAATATATATTTTTTATACAGATTGGTAACAAACCACCACGT TGACTTAACTTTTGTGATAATCATGgaagcttcgtacgctgcaggtc	VPS52 upstream flanking primer
oEE59	TGGATGTGATCAAAGAAATTGGGTTTGCCCATATGCGGGTGTGGAGGGTGTGGCTACATATTTACAATT GTGTGGTTTGTACGCAAAGATATGTGACCTTCTGgaagcttcgtacgctgcaggtc	VPS52 downstream flanking

		primer
oEE42	GTACAAAAGTCACAGGCTAAATTGC	VPS52 internal F
oEE43	TCTCATCAAATAGCTCTCGTACTCG	VPS52 internal R
oEE199	GCTAAGACCAATAAGGTGGAACC	ORF19.875 internal F
oEE200	GTATCAAATCATCAGCCCAACC	ORF19.875 internal R
oEE166	CGGGGTACCCCGCAAATGGACGCAAGACAGG	ARP2 Kpn1
oEE167	TCCCGCGGGGATAATGCTGTACCTGACCACACC	ARP2 SacII
oEE169	ATAAGAATGCGGCCGCTAAACTATCATAGATATAAATATTTTATACACTAGC	ARP2 Not1
oEE170	CGGGGTACCCCGAGGTGCTACTGGAACATCTGTCTG	ORF19.875 Kpn1
oEE174	CGGGGTACCCCGACCTTCTCTAACCTCCAGTAATGC	ORF19.7660 Kpn1
oEE228	CCGCTCGAGCGGGTGCCTGTTAGTGCTATCG	Arp2 Xho1
oEE229	CCGCTCGAGCGGGCGTCTTGTTCCAAGCTATGC	ORF19.875 Xho1
oEE230	AAGGAAAAAAGCGGCCGCAAAGGAAAAGATACAGAAATCCACAAAAGACG	ORF19.875 Not1
oEE231	TCCCGCGGGGATGTGTCGAGTGACATTACCG	ORF19.875 SacII
oEE232	CCGCTCGAGCGGGGACACTGACATTCAAAGTGGC	VPS52 Xho1
oEE233	AAGGAAAAAAGCGGCCGCAAAGGAAAATTCCAAACGTTGACAGATGC	VPS52 Not1
oEE234	TCCCGCGGGGACTTTGCAGTCACGTTGTGCG	VPS52 SacII
oEE5	TGTTGAAAGTTGCTGTAGTG	inverse PCR F
oEE6	CCAACCAGATAAGTGAAATC	inverse PCR R
oEE9	TATTAGCTTACGACGCTACAC	sequencing F after inverse PCR
oEE23	CTAATTCTGTATTAGTCATTCC	sequencing R after inverse PCR
oEE3	GTTACGAATCAATGGCACTAC	check for Tn presence F

oEE4	GTGGAAATGGAGTTTTTAAGG	check for Tn presence R
oEE282	AAAATGGACGCAAGACAGG	GSP1 ARP2
oEE283	cacggcgcgcctagcagcgg GGAAGGCGAAGTTGAGAAATCAACA	GSP2 ARP2
oEE284	gtcagcggccgcatccctgc CATAGATATAAATATTTTATACACTAGC	GSP3 ARP2
oEE285	TAATGCTGTACCTGACCACACC	GSP4 ARP2
oEE181	GTTGAAGTGGCGGCTAAACG	ARP2 F
oEE182	GAAATTGGATGCTCCGTTGG	ARP2 R
oEE197	GTGATGGTGTGACTCATATTGTGC	ARP2 internal F
oEE198	ACACTCTTGGTCCTTGTTCTTCC	ARP2 internal R
oEE201	TCTCGTAAGGGAATAATGATATG	GSP1 ARP3
oEE202	cacggcgcgcctagcagcgg AACTGATTGTGATTGCTGATTTCTC	GSP2 ARP3
oEE203	gtcagcggccgcatccctgc AAAATTGCTTAGATTGGAGTTGTCG	GSP3 ARP3
oEE204	GCTAACTTACCCAAAGTACGAATAGC	GSP4 ARP3
oEE205	CGAAGGTATCAGAGCATTACACG	ARP3 F
oEE206	GGCTGCATGTCTTCTATTCTCG	ARP3 R
oEE207	GCCATTAAGAATATCCCCTCG	ARP3 internal F
oEE208	ATCATAGTCGCTCTTCGTGTAGC	ARP3 internal R
oEE24	ATAACTACATCACCTGTATCACCTC	VPS52 external F
oEE72	ACACATGTTGTGATAGATTTTAC	pFA-HIS1 F
oEE73	AAGAGGACTGGGTTGCCATCTCATC	pFA-HIS1 R
oEE25	ACATTACCATCAAGCTACTATGGAG	VPS52 external R
oEE57	GAATTATGTCGGTCAATATGTC	pFA-ARG4 R
oEE56	AGAGATGCTATTGGTGGTACTGC	pFA-ARG4 F
oEE315	GTGTTACGAATCAATGGCACT	pFA-URA3 R
oEE316	GTGAAGGGGGAGATTTTCACT	pFA-URA3 F
oEE289	ATTGAACCCGGCTGCGGAACCC	pFA-CmLeu R
oEE290	CGAATATTATTAATACAAAATGAACACCG	pFA-CmLeu F
oEE329	CATCTTCGTCGTCACTGTGTTTATCC	CdHIS1 R

oEE194	GATACGTTGGTGGTTCAGTTGAGG	pSN52 F
oEE80	GGTTCTAATGCTAGTCAAGCTAATGC	ORF19.875 F
oEE81	ATATTCGTCTTGTTCCAAGCTATGC	ORF19.875 R
oEE78	TGACGTTTGCTTGTAAGATTCC	ORF19.7292 F
oEE79	CCGCATATAAAGATTTCTTCC	ORF19.7292 R
oEE92	GCCACTGAACATGAATTACG	ORF19.1084 F
oEE93	TAGCAACTTCTGCATCTTGG	ORF19.1084 R
oEE142	TCAATGCTGCCAATAGAACG	Tn r1 F
oEE143	TCAACTCCAGTGACTTGTTCCG	Tn r1 R
oEE144	GATTATCCACCACCCAATTACC	Tn r5 F
oEE145	GCAAAGTTGGACCTAAGAATCG	Tn r5 R
oEE146	ACGGTTCTATCACCCAAGTGC	Tn r6 F
oEE147	CAGGTCGTTCTGAGTTCTACCC	Tn r6 R
oEE148	CTCCTCGATGTCGTATTTTCG	Tn r8 F
oEE149	TCATCTTCCTCCGAAGTTAAGC	Tn r8 R
oEE382	<u>CCGCTCGAGCGGGATATAGATATATTAATTGCTTTAC</u>	ARP2 NotI 2nd

¹ Lower-case letters (in blue) indicate annealing sequences for auxotrophic marker amplification or, as in the case for Gene Specific Primers (GSP), annealing sequences for fusion PCR (Noble et al. Strains and Strategies for Large-Scale Gene Deletion Studies of the Diploid Human Fungal Pathogen *Candida albicans*. Eukaryotic Cell (2005). Underlined sequences indicate restriction recognition sites. PCR verification of mutant constructs was done as previously described (Schaub et al. New pFA-cassettes for PCR-based gene manipulation in *Candida albicans*. J Basic Microbiol (2006).