2.1 Materials

2.1.1 Strains and plasmids

All *C. glabrata* and bacterial strains, and plasmids used in this study are listed in Table 2.1 and Table 2.2, respectively.

Table 2.1: List of *C. glabrata* and bacterial strains used in the study

Strain	Relevant genotype	Reference
Yeast		
YRK19	*ura3*Δ*::Tn903* G418^R	Cormack & Falkow, 1999
YRK20	*URA3*	De Las Peñas *et al*., 2003
YRK599	*ura3*Δ*::Tn903* G418^R *Cgslt2*Δ*::nat1*	Borah *et al*., 2011
YRK603	*URA3 Cgslt2Δ::nat1*	Borah *et al*., 2011
YRK637	*ura3*Δ*::Tn903* G418^R *Cgslt2*Δ*::nat1*/pRK846	Borah *et al*., 2011
YRK640	*URA3 Cgsit1Δ::nat1*	This study
YRK642	*URA3 Cgfre6*Δ*::nat1*	This study
YRK714	*URA3 Cgftr1*Δ*::nat1*	This study
YRK715	*URA3 Cgyfh1*Δ*::nat1*	This study
YRK716	*URA3 Cgccc2*Δ*::nat1*	This study
YRK720	*URA3 Cgfet5*Δ*::nat1*	This study
YRK728	*URA3 Cgaft2*Δ*::nat1*	This study
YRK731	*URA3 Cgfth1*Δ*::nat1*	This study
YRK749	*URA3 Cgfet4*Δ*::nat1*	This study
YRK750	*URA3 Cgfet3*Δ*::nat1*	This study
YRK762	*ura3*Δ*::Tn903* G418^R *Cgyfh1*Δ*::nat1*	This study
YRK803	*ura3*Δ*::Tn903* G418^R *Cgccw14*Δ*::nat1*	This study
YRK805	*URA3 Cgccw14*Δ*::nat1*	This study
YRK875	*ura3*Δ*::Tn903* G418^R *Cgfet3*Δ*::nat1*	This study
YRK895	*ura3*Δ*::Tn903* G418^R *Cgyfh1*Δ*::nat1*/pRK950	This study
YRK897	*ura3*Δ*::Tn903* G418^R *Cgyfh1*Δ*::nat1*/pRK74	This study
YRK903	*URA3 Cghmx1*Δ*::nat1*	This study

YRK905	*ura3*Δ*::Tn903* G418^R *Cgccw14*Δ*::nat1*/pRK952	This study
YRK909	*ura3*Δ*::Tn903* G418^R *Cgccw14*Δ*::nat1*/pRK74	This study
YRK931	*ura3*Δ*::Tn903* G418^R *Cgftr1*Δ*::nat1*	This study
YRK964	*URA3 Cghog1*Δ*::nat1*	This study
YRK965	*URA3 Cgmam3*Δ*::nat1*	This study
YRK975	*URA3 Cghog1*Δ*::nat1Cgslt2::Tn7*	This study
YRK1024	*ura3*Δ*::Tn903* G418^R *Cghog1*Δ*::nat1*	This study
YRK1026	*ura3*Δ*::Tn903* G418^R *Cgfet3*Δ*::nat1*/pRK1025	This study
YRK1028	*ura3*Δ*::Tn903* G418^R *Cgfet3*Δ*::nat1*/pRK74	This study
YRK1038	*ura3*Δ*::Tn903* G418^R *Cgftr1*Δ*::nat1*/pRK1024	This study
YRK1040	*ura3*Δ*::Tn903* G418^R *Cgftr1*Δ*::nat1*/pRK74	This study
YRK1045	*ura3*Δ*::Tn903* G418^R *Cgmam3*Δ*::nat1*	This study
YRK1047	*ura3*Δ*::Tn903* G418^R *Cghog1*Δ*::nat1*/pRK1027	This study
YRK1051	*ura3*Δ*::Tn903* G418^R *Cgmam3*Δ*::nat1*pRK1026	This study
YRK1053	*ura3*Δ*::Tn903* G418^R *Cgmam3*Δ*::nat1*/pRK74	This study
YRK1178	*ura3*Δ*::Tn903 G418R/pRK999*	This study
YRK1219	*ura3*Δ*::Tn903* G418^R *Cghog1*Δ*::nat1*/pRK999	This study
YRK1221	*ura3*Δ*::Tn903* G418^R *Cghog1*Δ*::nat1*/pRK1054	This study
YRK1223	*ura3*Δ*::Tn903* G418^R *Cghog1*Δ*::nat1*/pRK1056	This study
YRK1225	*ura3*Δ*::Tn903* G418^R *Cghog1*Δ*::nat1*/pRK1057	This study
YRK1227	*ura3*Δ*::Tn903* G418^R *Cghog1*Δ*::nat1*/pRK1058	This study
YRK1229	*ura3*Δ*::Tn903* G418^R *Cgslt2*Δ*::nat1*/pRK999	This study
YRK1231	*ura3*Δ*::Tn903* G418^R *Cgslt2*Δ*::nat1*/pRK1055	This study
YRK1233	*ura3*Δ*::Tn903* G418^R *Cgslt2*Δ*::nat1*/pRK1056	This study
YRK1235	*ura3*Δ*::Tn903* G418^R *Cgslt2*Δ*::nat1*/pRK1057	This study
YRK1237	*ura3*Δ*::Tn903* G418^R *Cgslt2*Δ*::nat1*/pRK1058	This study
YRK1241	*ura3*Δ*::Tn903* G418^R	This study

	*Cghog1*Δ*::nat1*/pRK1055	
YRK1243	*ura3*Δ*::Tn903* G418^R *Cgslt2*Δ*::nat1*/pRK1054	This study
Bacterial		
DH5α	*E. coli DH5α (Δ(argF-lac)U169 supE44 hsdR17 recA1endA1gyrA96 thi-1 relA1(φ80lacZΔM15))*	

Table 2.2: List of plasmids used in the study

Plasmid	Description	Refrence
pRK74	A CEN-ARS plasmid (pGRB2.2) of *C. glabrata* carrying *S. cerevisiae URA3* as a selection marker. MCS sites are flanked by *S. cerevisiae PGK1* promoter at one end and by 3' UTR of *HIS3* at the other end.	Frieman *et al*., 2002
pRK625	pCR2.1 plasmid containing the *nat1* gene	Cormack laboratory
pRK846	*CgSLT2* ORF (1.4 kb) cloned in the pRK74 plasmid	Borah *et al*., 2011
pRK950	*CgYFH1* ORF (0.5 kb) cloned in Xma*I*-Xho*I* sites in the pRK74 plasmid	This study
pRK952	*CgCCW14* ORF (0.6 kb) cloned in BamH*I*-Sal*I* sites in the pRK74 plasmid	This study
pRK999	A CEN-ARS plasmid (Addgene-ID 45323) of *C. glabrata* carrying *S. cerevisiae URA3* as a selection marker. MCS sites are flanked by *S. cerevisiae PDC1* promoter at one end and by 3' UTR of *HIS3* at the other end.	Zordan *et al*., 2013
pRK1024	*CgFTR1* ORF (1.2 kb) cloned in Xma*I*-Xho*I* sites in the pRK74 plasmid	This study
pRK1025	*CgFET3* ORF (1.9 kb) cloned in Xba*I*-Xma*I* sites in the pRK74 plasmid	This study
pRK1026	*CgMAM3* ORF (1.9 kb) cloned in Xma*I*-Xho*I* sites in the pRK74 plasmid	This study

pRK1027	*CgHOG1* ORF (1.3 kb) cloned in BamH*I*-Xma*I* sites in the pRK74 plasmid	This study
pRK1054	*CgSLT2* ORF (1.4 kb) cloned in the pRK999 plasmid	This study
pRK1055	*CgHOG1* ORF (1.3 kb) cloned in BamH*I*-Xma*I* sites in the pRK999 plasmid	This study
pRK1056	*CgMRS3* ORF (0.92 kb) cloned in Xma*I*-Xho*I* sites in the pRK999 plasmid	This study
pRK1057	*CgYAP5* ORF (0.92 kb) cloned in Xba*I*-Xma*I* sites in the pRK999 plasmid	This study
pRK1058	*CgCCC1* ORF (0.92 kb) cloned in BamH*I*-Sal*I* sites in the pRK999 plasmid	This study

2.1.2 Oligonucleotides

Oligonucleotides used for generation of *C. glabrata* deletion strains, for cloning and for quantitative Real time Polymerase Chain Reaction (qPCR) were commercially synthesized either at MWG Biotech Pvt. Limited, Bangalore, India or at Xcelris genomics Pvt. Limited, Ahemdabad, India. All the oligonucleotides used were designed by using freely available online tool Primer 3 plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/) and are listed in Table 2.3.

Table 2.3: List of primers used in the study

Primer Name	**Sequence (5'-3')**	**Target Gene**
For generation and confirmation of *C. glabrata* deletion strains		
OgRK491	GCTAAATTATGCTGTTAGCGGAGCAA	*CgFTR1*
OgRK492	GCGTCGACCTGCAGCGTACGTTGAGCAAAGTTGAATAACACAGTT	*CgFTR1*
OgRK493	CGACGGTGTCGGTCTCGTAGTTTCAAAAAGTATAAGACGTTGGCATG	*CgFTR1*
OgRK494	CTCGATTCAGTAACGAACCTTCGGAAG	*CgFTR1*
OgRK495	CTCACAGACCCTAAAAAGTCATGTTT	*CgFTR1*

OgRK496	TAAGGATCTCAATCAAAGAAACGTC	*CgFTR1*
OgRK502	TGGGAAGGTATTTTCTGTATGATTGC	*CgFTR1*
OgRK503	GTTGCCTTGTTGAATCTGTAGTTTTC	*CgFTR1*
OgRK1193	TGGATAAACAATACGGTTGTGC	*CgFTR1*
OgRK1194	GGTTGTTCAATACCAGGCATATC	*CgFTR1*
OgRK1195	CTCGGGTTCTTTATCTGTTTAGTGA	*CgFTR1*
OgRK1196	GACAGATAACCAGTATTTTGCCATC	*CgFTR1*
OgRK664	CTAGTATCCCGCACAGCTACACTAC	*CgFET3*
OgRK665	GCGTCGACCTGCAGCGTACGTCGTCTATCCTATCT CAAACTTCAAA	*CgFET3*
OgRK666	GAGTTAAATTTACATCCCGTCTCAA	*CgFET3*
OgRK667	CGACGGTGTCGGTCTCGTAGATCTCCCATTAACAA CTCAACTCAA	*CgFET3*
OgRK668	TGGAAATGCAATAGATTATCCCTAA	*CgFET3*
OgRK669	CGTTTACAATCACCTTCTTGTTCTT	*CgFET3*
OgRK670	ATCTTGAGAGACAGGGAGTTTGAT	*CgFET3*
OgRK671	GGCCTTCTAAGTTGAAGAAATCACT	*CgFET3*
OgRK720	ATATAAGAAACAGGTGTTCCCCTTG	*CgCCC2*
OgRK721	GCGTCGACCTGCAGCGTACGTTATCTGTAGCCCTT GATCAGAAAC	*CgCCC2*
OgRK722	TACATTGGCTGAGAACTTTAACAAG	*CgCCC2*
OgRK723	CGACGGTGTCGGTCTCGTAGCCGACATTAGTCAAT TAGAACAACTT	*CgCCC2*
OgRK724	CTATTCATTCATCTTGCAACAGCTC	*CgCCC2*
OgRK725	GAGGTTTTATTGAGATCAGTGGAAG	*CgCCC2*
OgRK726	TATCTTGAAAACAAGGCTAAATCCA	*CgCCC2*
OgRK727	TCTCGCTTCTAGTTAGAGAAGTCCA	*CgCCC2*
OgRK520	GCAATTGCAAGTAGATTTACCC	*CgFRE6*
OgRK521	GCGTCGACCTGCAGCGTACGCACGCTACCGAGAA ATGCTT	*CgFRE6*
OgRK522	CGACGGTGTCGGTCTCGTAGTCATCGCACATAACG GCTAA	*CgFRE6*
OgRK523	ATTCGTTTGTTCCGCTTTGT	*CgFRE6*

OgRK524	CGGGTGTGTAGCATCAAGGT	*CgFRE6*
OgRK525	CTTGGCCTCATCTTCACGTT	*CgFRE6*
OgRK526	TACCACGCGCACAGCTATAA	*CgFRE6*
OgRK527	ACTGGGCATGCTTACCTTTG	*CgFRE6*
OgRK512	TGAGAAAACGACCACATTACCT	*CgSIT1*
OgRK513	GCGTCGACCTGCAGCGTACG TTTAGGATTAGTTGAACAAGAATTGC	*CgSIT1*
OgRK514	CGACGGTGTCGGTCTCGTAGTTATGTCGGCAAAAG TTCGTC	*CgSIT1*
OgRK515	TGGCTTTTCTAAGGAGATTGC	*CgSIT1*
OgRK516	CAATCGTGCACAGACAAACC	*CgSIT1*
OgRK517	CCCAAAGTTGCACAATTCAC	*CgSIT1*
OgRK518	CCGACAAGGATTACGAAGATG	*CgSIT1*
OgRK519	GACCGTAACCGTAACCACAGA	*CgSIT1*
OgRK704	TCTGCTATGTCTATTATGTGGGATG	*CgFET4*
OgRK705	GCGTCGACCTGCAGCGTACGCGATGTGAAATAAT ACTCAATTCCAA	*CgFET4*
OgRK706	ATCACTTTGCTTGAATTTTTATTGC	*CgFET4*
OgRK707	CGACGGTGTCGGTCTCGTAGCCATAATCGTTGGTA GCTTACAAGT	*CgFET4*
OgRK708	AGGTCAAAAATTTCCAATATCCTGT	*CgFET4*
OgRK709	CTCAATTTGTTGTGCTGTTCTTATG	*CgFET4*
OgRK710	TGTATAAAAGGCTCGAAAGAATCAG	*CgFET4*
OgRK711	CTAATGTTTTGGAGAAATGTCGAAC	*CgFET4*
OgRK712	GTTTATCTTTTGAGCGCTATGGATT	*CgFET5*
OgRK713	GCGTCGACCTGCAGCGTACGCAACAGGAATATTTT TCAGCGTAGT	*CgFET5*
OgRK714	TATTCCCCTAAAATCATCACTCAAA	*CgFET5*
OgRK715	CGACGGTGTCGGTCTCGTAGTGACAAGGACTGTAT ATAATTTTACGA	*CgFET5*
OgRK716	GGGATCCAAAATGTTGCAATTA	*CgFET5*
OgRK717	GACATCGACTTTTTGAGATATTTGG	*CgFET5*
OgRK718	AAGAATTATCAATATGGGGCTGTTT	*CgFET5*

OgRK719	GATGTCAGTGCTGTAGTTAATGTGG	*CgFET5*
OgRK736	GAATTCATGAAGAAATACCCACAAC	*CgFTH1*
OgRK737	GCGTCGACCTGCAGCGTACGATGACCTTCCCTTAAGTTTGTCTTT	*CgFTH1*
OgRK738	TGTGCAAGAGTACGACTATTACTGG	*CgFTH1*
OgRK739	CGACGGTGTCGGTCTCGTAGCAGAAAGCTAATGTCATTTCCATGT	*CgFTH1*
OgRK740	GACTACCAATGGCTTTTGAAGATAA	*CgFTH1*
OgRK741	TTCGCAGAAAGAATTACTTAAGACG	*CgFTH1*
OgRK742	TTAAGAGAGAAATTCAGGGTCAAAC	*CgFTH1*
OgRK743	ATAGCTGTTAGAATCATCCATCCAC	*CgFTH1*
OgRK728	TCGGGTATATCCTCTGTTTTGTAAG	*CgYFH1*
OgRK729	GCGTCGACCTGCAGCGTACGATGTAGTGATACAAAGAGCCATGTG	*CgYFH1*
OgRK730	ATGAATTTTAGAGCCATCTCATCTG	*CgYFH1*
OgRK731	CGACGGTGTCGGTCTCGTAGAACAATGTGAAGTAATCATCACACG	*CgYFH1*
OgRK732	AAAAAGCAAAAGATAAAGTCGAATA	*CgYFH1*
OgRK733	GGGCCAGTAAGAGCTATGTAGTAAA	*CgYFH1*
OgRK734	GATGTCAATAAGATTAGCGACCAGT	*CgYFH1*
OgRK735	GCGTCATGTAGCTCTTTGTCTAGTA	*CgYFH1*
OgRK672	AAAGAATGCCAAAAATCTGAAAAAT	*CgAFT2*
OgRK673	GCGTCGACCTGCAGCGTACGCACTATCTAAACTTGGTTTGGGAGA	*CgAFT2*
OgRK674	AATAGCTTTGCAAAAAGAAACTTTG	*CgAFT2*
OgRK675	CGACGGTGTCGGTCTCGTAGAGAGAGACGAGTGAAATGAACAACT	*CgAFT2*
OgRK676	ATCGCAACAAAACCATATAACAAAT	*CgAFT2*
OgRK677	GCAAAAGAAGTGGAAACAGTTACAT	*CgAFT2*
OgRK678	ATTATTTCTTGTGATTGTGGCCTAA	*CgAFT2*
OgRK679	CCTTTAGTGAGACTAGGTTGAGCTG	*CgAFT2*
OgRK1048	AGTATAAGACGTTGGAATGAGTCGT	*CgCCW14*
OgRK1049	GCGTCGACCTGCAGCGTACGTATTATTTTTCCAGC	*CgCCW14*

	GAATTTTTCA	
OgRK1050	AAGAAAATTGGTAAAGAGGATTTCG	*CgCCW14*
OgRK1051	CGACGGTGTCGGTCTCGTAGTCATTACTCCCCTTC CTAATGAAAT	*CgCCW14*
OgRK1052	ATAAGAGTCAGCAACCTAAGCGTAA	*CgCCW14*
OgRK1053	ACAAACCAGAGAGTGAAAATCAAAG	*CgCCW14*
OgRK1054	GTCTCTTCCAGTACTTCCTCTGTTG	*CgCCW14*
OgRK1055	AAGTGATGGTGAAAGTAGAAGATGC	*CgCCW14*
OgRK1185	CAAGTCCGGGTAAATTCAAGTC	*CgHMX1*
OgRK1186	GCGTCGACCTGCAGCGTACGGAAGTTGATCCTGTT GGCTAGG	*CgHMX1*
OgRK1187	ATATAGCCGGGTAAGTACAAGTCC	*CgHMX1*
OgRK1188	CGACGGTGTCGGTCTCGTAGGCTCGTTGTAATTGT TTTTGTATTTG	*CgHMX1*
OgRK1189	GAGTAACCCTTCACCATGTTCTTC	*CgHMX1*
OgRK1190	CATGATCTCAAAGAACTCAACAGTG	*CgHMX1*
OgRK1191	AGATCGTCAAGCAGTTCTGGAT	*CgHMX1*
OgRK1192	AGTTCTTCTTGTACTGCCACTTCAG	*CgHMX1*
OgRK1467	AGTCCAGTCACTTTATCCCAAATTC	*CgMAM3*
OgRK1468	GCGTCGACCTGCAGCGTACGTATGATTGAGGTTTT GACAGGTTGT	*CgMAM3*
OgRK1469	TATACTGGCTTGTAACTGCTATTGG	*CgMAM3*
OgRK1470	CGACGGTGTCGGTCTCGTAGCCTAACCACCTTACT GTTATCATGG	*CgMAM3*
OgRK1471	CGCTTCATATCATGTAAAATAGAAACG	*CgMAM3*
OgRK1472	ACCGTGTTGATGTTATCGATGTATC	*CgMAM3*
OgRK1473	CTTACCAAACGAACCCATGAATTTC	*CgMAM3*
OgRK1474	CTAGGTTTGAAGGAGCTAACTGAAG	*CgMAM3*
OgRK340	CGTACGCTGCAGGTCGACGCCTTCCGCTGCTAGGC GCGCCGTG	*nat1*
OgRK341	CTACGAGACCGACACCGTCGGGCCGCTGACGAAG T	*nat1*
OgRK342	GTCTACTACTTTGGATGATAC	*nat1*

OgRK343	TCTGTTCCAACCAGAATAAG	*nat1*
OgRK344	TGCGCACGTCAAGACTGTCAAGG	*nat1*
OgRK345	TGTGAATGCTGGTCGCTATACTGC	*nat1*
OgRK1451	ATTACTTGATGCCAGAACGTTCC	*CgHOG1*
OgRK1452	GCGTCGACCTGCAGCGTACGCTGTTGTCGCTATTA TCTTTGGGT	*CgHOG1*
OgRK1453	GATGTTCAAAGGATAAACGCGTTC	*CgHOG1*
OgRK1454	CGACGGTGTCGGTCTCGTAGCATTAATACCACATT TTTGTTACCG	*CgHOG1*
OgRK1455	GAAATCTGCATTTATGAGAAAATTG	*CgHOG1*
OgRK1456	ATGTAGAAGAAGTTGCAGACCATG	*CgHOG1*
OgRK1457	TTAACTTGGCAGAAATACGACGTC	*CgHOG1*
OgRK1458	ATAACTCTCCATGTGTCTACTGGT	*CgHOG1*
OgRK1475	CGTGTTCTGATCGGAGTTAGTAAA	*CgHOG1*
OgRK1476	TGGAAAAGAGCCTCAAAAGATATGG	*CgHOG1*
For quantitative Real-time Polymerase Chain Reaction (qPCR)		
OgRK680	TTAGGTTTGATGATGCACGAAG	*CgFTR1*
OgRK681	TGTTGTCCATACGGTTTAGCAG	*CgFTR1*
OgRK682	TATTTCCCCTGCATCCTCTAAA	*CgAFT1*
OgRK683	CAAGAATTCATCTCCAACGTCA	*CgAFT1*
OgRK686	CTACTAAAGCGTGTTGGTGACG	*CgSIT1*
OgRK687	GGCAACAATTGTCTCCAGTCTT	*CgSIT1*
OgRK688	TGTAAAGGTCCCAACTCAAGGT	*CgFET3*
OgRK689	GCGATCATAGTGATACCCAACA	*CgFET3*
OgRK692	GGAGTGTAGTTTGTTCGGTGCT	*CgFET4*
OgRK693	TTCAAGATCAGCTTCGTTGTGT	*CgFET4*
OgRK702	TTCGAACATGAACGAAATTGAT	*CgAFT2*
OgRK703	TGTACAGGCGCCATTAGAAGTT	*CgAFT2*
OgRK89	CTCCACCACTGCTGAAAGAG	*CgACT1*
OgRK90	GGTCAATACCAGCAGATTCTAG	*CgACT1*
OgRK734	GATGTCAATAAGATTAGCGACCAGT	*CgYFH1*
OgRK735	GCGTCATGTAGCTCTTTGTCTAGTA	*CgYFH1*

OgRK1091	TGGGTATCACACAGGTTCCA	*CgGRX4*
OgRK1092	GGGAGTCTTCGTCAAGGTCA	*CgGRX4*
OgRK1572	CTCAGGCAGACGTAGATCTTCT	*CgMSN2*
OgRK1573	TGACGAGTTAGGGCTTATACTTG	*CgMSN2*
OgRK69	GTACGCTATGTTCTCTGAAGA	*CgYPS5*
OgRK70	AACTGCTCTCCTGAGAAACAC	*CgYPS5*
OgRK1183	ACTATGTTACTTTATGGTTA	*CgEPA1*
OgRK1184	TGAGCCCCAGATGGCGTAGG	*CgEPA1*
For cloning		
OgRK1197	CATACCCGGGATGCCTAACAAAGTATTTAAC	*CgFTR1*
OgRK1198	CAGACTCGAGTTAGTCGGAACTGGTAGTGG	*CgFTR1*
OgRK1201	CATATCTAGAATGATGGTACCTTTGCTGCTATCG	*CgFET3*
OgRK1202	CAGACCCGGGTCAGAAAAAGATGAATTTCTTTTTC	*CgFET3*
OgRK1064	CATACCCGGGATGATCAGGAATATTTTACGC	*CgYFH1*
OgRK1065	CAGACTCGAGTCACTGATCTGATTCCACTTTG	*CgYFH1*
OgRK1056	CATAGGATCCATGCGTGCTGCTGTTGTTTCTG	*CgCCW14*
OgRK1057	CAGAGTCGACTCAGATCAATAGAGCAGCGGC	*CgCCW14*
OgRK1481	CATACCCGGGATGAACTCGGTGGCTTACTCCAG	*CgMAM3*
OgRK1482	CAGACTCGAGTCACTTCTTGCTTCTTCTTTTC	*CgMAM3*
OgRK1579	CATAGGATCCATGGCTACTAATGAAGAGTTC	*CgHOG1*
OgRK1580	CATACCCGGGTTATTGTTGGAATTCATTTG	*CgHOG1*
OGRK1753	CATAGGATCCATGGTTTCTTTCCCGCGACAAG	*CgCCC1*
OGRK1754	CAGAGTCGACCTATCCCAAAAGTTTGACG	*CgCCC1*
OGRK1755	CATATCTAGAATGCTGACTGCTCTGGGATC	*CgYAP5*
OGRK1756	CAGACCCGGGCTATGTTCTTTGTCTTTTCGG	*CgYAP5*
OGRK1758	CATACCCGGGATGGAATCATCAGACGTTATAG	*CgMRS3*
OGRK1759	CAGACTCGAGCTAATAATGGAACAAGAAGTG	*CgMRS3*

2.1.3 Antibodies

All antibodies used in this study, their clonality and dilutions used, Manufacturers' details, and catalogue numbers are listed in Table 2.4.

Table 2.4: List of antibodies used in this study

Name	Dilution	Clonality	Manufacturer	Catalogue Number
Primary Antibodies				
Anti-phospho-p38 MAP kinase	1:5,000	Monoclonal	Cell Signalling Technology	4511
Anti-phospho-p44/42 MAP kinase	1:5,000	Monoclonal	Cell Signalling Technology	4370
Anti-GAPDH	1:10,000	Polyclonal	Abcam	ab22555
Secondary Antibodies				
Anti-Rabbit IgG	1:5,000	GeNei		32053

2.1.4 Chemical components, culture medium and kits

All chemicals used in this study were obtained from commercial sources and were of molecular biology grade. The enzymes used for PCR amplification and molecular cloning *viz.*, restriction endonucleases, T4 DNA ligase, Taq DNA polymerase were obtained from New England Biolabs, Fermentas, Sigma and Finnzymes. Kits used for first strand c-DNA synthesis, quantitative real time-PCR were purchased from Invitrogen and Eurogentech, respectively. Kits used for plasmid isolation, PCR product purification, reaction clean up, and gel extraction of DNA fragments were procured from QIAGEN. Agarose, phenol, dimethyl sulphoxide (DMSO), sodium acetate, sodium chloride, sodium hydroxide, sodium carbonate, sodium dodecyl sulphate (SDS), trizma base, bathophenanthrolinedisulfonic acid disodium salt (BPS), ferric chloride, formamide, ethylenediaminetetraacetic acid (EDTA), glycerol, polyethylene glycol, hydroxyurea, methylmethane sulphonate (MMS), ammonium persulphate, acrylamide, bis-acrylamide, N,N,N′,N′-Tetramethylethylenediamine (TEMED), diethylpyrocarbonate (DEPC), lithium acetate, polyethylene glycol (PEG), menadione, phorbol myristate acetate (PMA), isopropanol, tween-20, trypan blue, hydrogen peroxide, uracil and orthrophenylenediamine (OPD) were procured form Sigma Chemicals. INVIVO PROTWIN label mix, S^{35} (Met:Cys-65:25) were obtained from from BRIT-Jonaki, CCMB, Hyderabad. Fluconazole was purchased from Ranbaxy. Ferrozine was purchased from HIMEDIA. Hydrochloric acid, sulphuric acid, acetic acid, methanol, potassium dihydrogen orthrophosphate,

dipotassium hydrogen phosphate, disodium hydrogen orthrophosphate, acetone and citric acid were obtained from Qualigen Chemicals. Kit used for quantitation of histone deacetylase activity was purchased from Cayman Chemical Company (#10011563). Hybond-P membrane for protein transfer and ECL kit for immunoblotting were purchased from Amersham Biosciences. Kits used for estimation of cytokines were procured from BD Biosciences.

Medium components used to culture *C. glabrata* and bacterial strains *viz.*, yeast extract, peptone, dextrose, casamino acid hydrolysate, yeast nitrogen base with amino acids and ammonium sulphate, yeast nitrogen base without amino acids, yeast nitrogen base without amino acids and ammonium sulphate and Luria-Bertani (LB) medium were purchased from BD (Becton, Dickinson and Company, USA). Animal cell culture media RPMI-1640, DMEM and α-MEM were purchased from Hyclone and Gibco-Invitrogen. Fetal bovine serum, glutamine and antibiotics for cell culture medium were procured from Gibco-Invitrogen.

2.1.5 Media

All media and solutions were sterilized by autoclaving at 121°C and 15 psi for 20 min. For preparation of plates, 2% agar was added to the medium before autoclaving. Heat labile components and reagents were filter sterilized by passing them through a 0.22 µm membrane filter.

2.1.5.1 Bacterial media

Luria Bertani (LB)

0.5% Yeast Extract

1% Tryptone

1% NaCl

Super Optimal Broth (SOB)

0.5% Yeast Extract

2% Peptone

10 mM NaCl

2.5 mM KCl

10 mM $MgCl_2$

10 mM $MgSO_4$

SOC

SOB medium was modified to prepare the SOC medium. 20 ml of sterile 1 M glucose solution was added to the autoclaved SOB medium to obtain a final concentration of 20 mM glucose in 1 litre of medium.

Antibiotics

Ampicillin 60 µg/ml

Kanamycin 30 µg/ml

Stock solution of antibiotics (50 mg/ml) were prepared in sterile water. Prior to storage at -20°C, antibiotics were filter sterilized through a 0.22 µm membrane filter. Before pouring the plates, antibiotics were added to moderately warm LB-agar medium.

2.1.5.2 Yeast media

Yeast Extract-Peptone-Dextrose (YPD)

1% Yeast Extract

2% Peptone

2% Dextrose

Yeast Nitrogen Base (YNB)

0.67% Yeast Nitrogen Base

2% Dextrose

For alternate carbon source utilization experiments, dextrose was replaced with other carbon sources *viz.*, ethanol, glycerol, oleic acid and sodium acetate. Ethanol, oleic acid and sodium acetate were used at a final concentration of 2% and glycerol was used at a final concentration of 3%.

Casamino acid (CAA)

0.67% Yeast Nitrogen Base

2% Dextrose

0.6% Casamino acid

2.1.6 Buffers and solutions

2.1.6.1 Common buffers

Phosphate-Buffered Saline (PBS)

137 mM NaCl

2.7 mM KCl

10 mM Na_2HPO_4

2 mM KH_2PO_4

Final pH of the buffer was adjusted to 7.3 with 11.6 N HCl and volume was adjusted to 1 L before autoclaving.

PBS was prepared as a 10X stock solution and diluted to 1 X concentration before autoclaving.

Tris-HCl buffer

0.5 M Trizma Base

Final pH of the buffer was adjusted to 7.6 using 11.6 N HCl.

Tris-EDTA (TE) buffer

10 mM Tris-HCl (pH 8.0)

1 mM EDTA

TE buffer was prepared as a 10 X concentrate and diluted to 1 X concentration before use.

Tris-acetic acid EDTA (TAE) buffer

40 mM Tris Base

0.5 M EDTA

Final pH of the buffer was adjusted to 8.5 with glacial acetic acid.

TAE buffer was prepared as a 50 X concentrate and diluted to 0.5 X concentration prior to use as agarose gel electrophoresis running buffer and to cast agarose gels.

HEPES [4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid] buffer

1 M HEPES

Final pH of the buffer was adjusted to 7.5 with NaOH.

HEPES was used as a buffering agent for preparation of different pH medium. Buffer was filter-sterilized by using a 0.22 µm membrane filter and stored at 4°C.

INOUE transformation buffer

10 mM PIPES

15 mM $CaCl_2.2H_2O$

250 mM KCl

55 mM $MnCl_2.4H_2O$

For preparation of INOUE transformation buffer, above-mentioned solutes were dissolved in appropriate amount in 800 ml of water and then 20 ml of 0.5 M PIPES (piperazine-1,2-bis[2-ethanesulphonic acid]) (pH 6.7) was added. Final volume was adjusted to 1 litre with water, buffer was filter sterilized by using a 0.22 µm membrane filter and stored at -20°C. Stock solution of PIPES was prepared separately by dissolving 15.1 gm of PIPES in 80 ml of water, pH was adjusted to 6.7 using 5 M KOH and volume was adjusted to 100 ml.

Yeast transformation reagents

1 M lithium acetate	36 µl
50 % polyethylene glycol	240 µl

10 mg/ml carrier DNA 5 µl

Above-mentioned reagents were added to prepare the transformation mixture, and the volumes indicated were used per transformation. 500-1,000 ng of desired transforming DNA was added to this transformation mixture and final volume was adjusted to 360 µl with sterile water. Carrier DNA (Sonicated salmon sperm DNA, Stratagene, 201190) was heat denatured at 95°C for 10 min and transferred on ice before addition to the transformation mixture. 43 µl DMSO was added to each transformation mixture before heat shock.

Zymolyase cocktail buffer for yeast colony PCR

2.5 mg/ml zymolyase (MP Biomedicals, 0832092)

1.2 M Sorbitol

The cocktail was prepared in sterile water.

2.1.6.2 Buffers used for nucleic acid extraction

Genomic DNA isolation buffers

Buffer A

50 mM Tris-HCl

10 mM EDTA

150 mM NaCl

1% Triton-X

1% SDS

Buffer B

50 mM Tris-HCl (pH 7.5)

10 mM EDTA

1.1 M Sorbitol

50 mM β-mercaptoethanol (Added fresh before use)

Buffer C

100 mM Tris-HCl (pH 7.5)

10 mM EDTA

10% SDS

Phenol:Chloroform:Isoamyl alcohol (25:24:1) solution

25 ml Tris-equilibrated phenol (pH 8.0)

24 ml Chloroform

1 ml Isoamyl alcohol

DNA sample loading buffer

0.25% Bromophenol blue

0.25% Xylene cyanol

15% Ficoll

Stock solution of the loading buffer was prepared in water as a 6 X concentrate and was added to the sample DNA to the final concentration of 1 X.

RNA isolation buffer

AE buffer

3 M sodium acetate

0.5 M EDTA (pH 8.0)

Reagents used for RNA isolation were prepared in DEPC-treated water and stored at 4°C. For preparation of DEPC-treated water, 0.1 ml DEPC was added to 100 ml water and kept overnight on a magnetic stirrer. Following incubation, the solution was autoclaved to remove any traces of DEPC.

Acid phenol solution

Phenol solution saturated with 0.1 M citrate buffer (pH 4.3 ± 0.2) was procured from Sigma (P4682).

2.1.6.3 Buffers for protein extraction and separation by SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis)

Total cell lysis buffer (Homogenization buffer)

50 mM Tris-HCl (pH 7.5)

2 mM EDTA

10 mM Sodium fluoride*

1 mM Sodium orthovanadate*

1 X protease inhibitor cocktail (Sigma, P 8215)*

* Were added fresh before use.

SDS-PAGE

30% acrylamide solution

29 g Acrylamide

1 g N,N'-Methylenebisacrylamide

Dissolved in 100 ml H_2O

10% Sodium Dodecyl Sulfate (SDS)

10 g SDS in 100 ml H_2O

SDS loading buffer

130 mM Tris-HCl (pH 8.0)

20% (v/v) Glycerol

4.6% (w/v) SDS

0.02% Bromophenol blue

2% DTT

The stock solution of SDS loading buffer was made as a 4 X concentrate and was added to the protein sample to the final concentration of 1 X.

SDS-PAGE running buffer

0.25 M Tris-HCl (pH 8.0)

1.92 M Glycine

1% SDS

The stock solution was prepared as a 10 X concentrate and was diluted to 1 X concentration prior to use.

Resolving gel mix (12%, 10 ml)

3.3 ml H_2O

4 ml 30% Acrylamide:N,N'-Methylenebisacrylamide (29:1) mix

2.5 ml 1.5 M Tris-HCl (pH 8.8)

100 µl 10% SDS

100 µl 10% Ammonium persulfate (APS)

4 µl N,N,N′,N′-Tetramethylethylenediamine (TEMED)

Stacking gel mix (5%, 3 ml)

2.1 ml H_2O

0.5 ml 30% Acrylamide:N,N'-Methylenebisacrylamide (29:1) mix

380 µl 1 M Tris-HCl (pH 6.8)

30 µl 10% SDS

30 µl 10% APS

3 µl TEMED

2.1.6.4 Buffers for western blot experiment

Transfer buffer (10 X stock solution)

0.25 M Tris-HCl (pH 8.0)

1.92 M Glycine

1% SDS

The stock solution was prepared as a 10 X concentrate and was diluted to 1 X concentration prior to use.

1X Transfer buffer (1 litre)

200 ml Methanol

100 ml 10X Transfer buffer

700 ml Water

Tris-Buffered Saline (TBS)

50 mM Tris

150 mM NaCl

Final pH of the buffer was adjusted to 7.4 with HCl.

Blocking buffer

5% Fat-free milk

0.1% Tween-20

Final volume was made to 100 ml with 1 X TBS.

Wash buffer (TBS-T)

TBS (1 X final concentration)

0.1% Tween-20

Final volume was prepared with water.

Stripping buffer

100 mM β-mercaptoethanol

2 % SDS

62.5 mM Tris-HCl (pH 6.7)

Final volume was made to 250 ml with water.

2.2 Methods

2.2.1 Animal cell culture methods

2.2.1.1 Cell lines and culture conditions

Human monocytic cell line THP-1 (ATCC TIB-202^{TM}), derived form 1 year old acute monocytic leukemia patient, was used to perform single cell infection assays. Differentiation of these THP-1 monocytic cells to phagocytic cells was induced by using phorbol myristate acetate (PMA) (16 nM). Lec2 (ATCC CRL-1736^{TM}), an ovary epithelial cell line, which has been derived from the Chinese hamster *Cricetulus griseus*, was used to determine the adherence of *C. glabrata* cells. THP-1 and Lec-2 cells were routinely cultured and maintained in RPMI-1640 and α-MEM media, respectively, supplemented with 10 % heat inactivated fetal bovine serum, 2 mM glutamine, penicillin (100 units/ml) and streptomycin (100 µg/ml). Cultures were maintained in cell culture incubator (Thermo scientific) at 37°C and 5% CO_2. Cultures were split after every 2-3 days and spent medium was replaced with fresh pre-warmed medium. For splitting, cells were harvested at 1,000 rpm for 2-3 min, spent medium was discarded and cell pellet was resuspended in 4-6 ml of pre-warmed medium. Cell density was determined by using hemocytometer. A total of 3-4 million cells were seeded in a 100 mm cell culture dish containing 12 ml fresh medium.

2.2.1.2 Cryopreservation and revival of cell lines

For cryopreservation of THP-1 and Lec-2 cells, 5-6 million cells were resuspended in 0.5 ml of either commercially procured cell preservation medium from GIBCO (12648010) or complete medium supplemented with 10 % fetal bovine serum and 10 % DMSO. Cells were initially kept in an isopropanol bath and were later transferred to -70°C freezer. After 2-3 days, frozen cells were transferred to liquid nitrogen container till further use. To revive the cells, frozen stocks were taken out of the liquid nitrogen container and immediately transferred to water bath set at 37°C for thawing. When freezing medium has thawed completely, cells were transferred to a 100 mm cell culture dish containing 12 ml complete medium and incubated under tissue culture conditions at 37°C and 5% CO_2 for 12 h. After incubation, medium was replaced by 12 ml fresh pre-warmed medium and incubated under tissue culture conditions till they reached 70-80% of confluence before splitting.

2.2.1.3 PMA (Phorbol myristate acetate) treatment of THP-1 monocytic cells

THP-1 monocytes get differentiated into phagocytic macrophages upon treatment with

phorbol myristate acetate (PMA) (Tsuchiya *et al*., 1982). For PMA treatment, THP-1 cells were allowed to grow till 70-80% confluence and were collected in a centrifuge tube by centrifugation at 1,000 rpm for 3 min. THP-1 cell pellets were resuspended in 4-5 ml of pre-warmed complete RPMI-1640 medium, 100 µl of this cell suspension was appropriately diluted in PBS (1X) and viability was determined by counting trypan blue stained cells using hemocytometer. THP-1 cell suspension was diluted appropriately to obtain a final cell density of 10^6 cells/ml with pre-warmed complete RPMI-1640 medium. PMA was added to the THP-1 cell suspension at a final concentration of 16 nM and mixed well by gently inverting the tubes. PMA-treated cells were seeded either in 24-well cell culture plates or in cell culture dishes and allowed to grow for 12 h under tissue culture conditions i.e. at 37°C and 5% CO_2. After 12 h incubation, spent medium was replaced with fresh pre-warmed complete RPMI-1640 medium and cells were allowed to recover for another 12 h.

2.2.1.4 THP-1 macrophage infection assay to monitor the intracellular survival and replication of *C. glabrata*.

To perform survival analysis of *C. glabrata* cells in macrophages, PMA-treated THP-1 cells were seeded to 24-well tissue culture plates to a final cell density of 1 million per well. *C. glabrata* cells were grown in YNB medium for 14-16 h at 30°C and 200 rpm. 1 ml of these *C. glabrata* cells were harvested in 1.5 ml centrifuge tubes, washed twice with 1X sterile PBS and the cell density was adjusted to $2x10^6$ cells/ml. 50 µl of this cell suspension was used to infect PMA-activated macrophages to a MOI (multiplicity of infection) of 0.1. Two hours post infection, THP-1 cells were washed thrice with 1X sterile PBS to remove the non-phagocytosed yeast cells and 1 ml of fresh pre-warmed complete RPMI-1640 medium was added. At different time points, infected THP-1 macrophages were osmolysed with 1 ml sterile water. Post lysis, lysates were collected by scraping the wells using 1 ml microtip. Lysates were diluted in 1X sterile PBS and appropriate dilutions were plated on YPD-agar plates. Plates were incubated at 30°C for 24-48 h and colony forming units (CFUs) were counted. Final CFUs/ml were determined by multiplying CFUs with appropriate dilution factor and percentage phagocytosis was calculated by dividing CFUs obtained at 2 h post infection by total number of yeast cells used for infection. Fold replication was calculated by dividing the CFUs obtained at 24 h post infection by CFUs obtained at 2 h post infection.

2.2.1.5 Estimation of cytokine production by THP-1 macrophages upon infection with *C. glabrata* cells

PMA-treated THP-1 cells were seeded to a 24-well tissue culture plate to a cell density of 1 million cells per well and allowed to grow for 12 h. After 12 h incubation, spent medium was replaced with fresh pre-warmed RPMI-1640 medium and cells were allowed to recover for 12 h before use. *C. glabrata* cells were grown in YNB medium for 14-16 h at 30°C and 200 rpm. 1 ml of these *C. glabrata* cells were harvested in 1.5 ml centrifuge tubes, washed twice with 1X sterile PBS and the cell density was adjusted to $2x10^7$ cells/ml. 50 µl of this cell suspension was used for infection to a MOI of 1:1. Two hours post infection, wells were washed thrice with 1X sterile PBS to remove the non-phagocytosed yeast cells and 1 ml of fresh pre-warmed RPMI-1640 medium was added. Plates were incubated under tissue culture conditions at 37°C and 5% CO_2 for 24 h. Supernatants were collected in 1.5 ml microfuge tubes, centrifuged at 3,000 rpm to remove the particulate matter, if any, and stored at -20°C until use. Estimation of different cytokines were performed using BD OptEIA ELISA kits as per the supplier's instructions.

2.2.2 Microbiological methods

2.2.2.1 Strains and culture conditions

C. glabrata strains were routinely grown in rich YPD medium or synthetically defined YNB medium, or YNB medium supplemented with CAA, unless stated otherwise. To obtain overnight grown liquid cultures, *C. glabrata* cells were inoculated in appropriate medium and incubated at 30°C under constant agitation (200 rpm) to maintain proper aeration. To revive the frozen stocks, about one tipfull of frozen culture was streaked either on YPD-agar or on CAA-agar medium. In general, frozen stocks of *C. glabrata* strains were revived on YPD-agar medium. However, *C. glabrata* strains harbouring plasmids containing *URA3* as a selectable marker were revived on CAA-agar medium. After streaking, plates were allowed to grow for 24-48 h at 30°C and were stored at 4°C for a maximum period of two weeks. For long term storage, freezer stocks of *C. glabrata* strains were prepared in 15% glycerol and stored at -80° C.

Escherichia coli strain DH5α was revived on LB-agar medium from frozen stock and incubated at 37°C for 14-16 h. DH5α strain was used for transformation purpose and maintaining plasmids. Bacterial strains harbouring plasmids containing selection markers were revived on LB-agar medium supplemented with appropriate antibiotics. Bacterial liquid cultures were either grown in LB broth or LB broth containing suitable antibiotics and incubated in a shaker incubator set at 37°C, 200 rpm for 14-16 h. For preparation of bacterial frozen stocks, 1 ml overnight grown bacterial culture was added to 500 µl of 50% glycerol to obtain final concentration of ~16 % glycerol and stored at -80°C until use.

2.2.2.2 Time course growth analysis and determination of generation time

For time course growth analysis, *C. glabrata* cells were inoculated in appropriate medium and grown for 14-16 h. Followed by overnight growth, yeast cells were sub-cultured in test medium at an initial OD_{600} of 0.1 and growth was monitored by recording the absorbance of the culture at 600 nm at regular time-intervals till 72 h. Absorbance values were plotted with respect to time and generation time of yeast strains were calculated between 4-8 h of growth when cells were in logarithmic phase of growth using following equation.

$$\text{Generation time(G)} = \text{T2} - \text{T1 X}\frac{\log 2}{\log \text{Nf/Ni}}$$

G = Generation time (h)

T1 = Initial time point taken for analysis

T2 = Final time point taken for analysis

Nf= Number of cells at time T2 (1 OD_{600} of *C. glabrata* corresponds to 2 X 10^7 cells)

Ni = Number of cells at time T1 (calculated from OD_{600} value as mentioned above)

For CFU-based viability assays, appropriate dilutions of yeast cultures were prepared in 1X sterile PBS and suitable volume of diluted cultures was plated on YPD-agar medium. Plates were incubated at 30°C for 24-48 h and viable colonies that appeared on YPD-agar plate were counted. To obtain logarithmic phase cells, overnight-grown *C. glabrata* cell suspension was inoculated in appropriate medium at an OD_{600} of 0.1 and grown in a shaker incubator set at 30°C, 200 rpm for approximately 4 h till the culture density reached OD_{600} of 0.4-0.6.

2.2.2.3 Serial dilution spot growth assay

To phenotypically characterize *C. glabrata* mutants, serial dilution spot growth assays were performed. Briefly, the optical density of overnight-grown *C. glabrata* cultures was normalized to OD_{600} of 1.0 and normalized cultures were further diluted 10-fold in 1X sterile PBS five times. 3 µl of serially diluted culture were spotted on test plates. Plates were incubated at 30°C (unless mentioned otherwise) for 24-48 h and growth was recorded by capturing plate images. For experiments involving checking the ability of mutants to utilize non-fermentable carbon sources, growth was scored after 6-7 days of incubation.

2.2.2.4 Yeast transformation

Yeast transformation was performed as described previously (Gietz *et al*., 1992) with few modifications. Briefly, overnight grown *C. glabrata* cultures were freshly inoculated in 10

ml YPD broth at an initial OD_{600} of 0.1. Cultures were allowed to grow for 4-5 h in a shaker incubator set at 30°C, 200 rpm until the OD_{600} of the cultures reached 0.4-0.6. Next, cells were harvested in a 15 ml centrifuge tube by centrifugation, washed twice with 10 ml of sterile water, resuspended in 1 ml of sterile water and were transferred to a 1.5 ml microfuge tube. Cells were harvested by centrifugation at 4,000 rpm for 5 min, resuspended in 50 µl of 100 mM lithium acetate solution and transformation mixture was added. Transformation mixture consisted of 240 µl polyethylene glycol (50%), 36 µl of lithium acetate (1 M), 5 µl of heat denatured single stranded carrier DNA (10 mg/ml), 500 ng to 1 µg of transforming DNA and final volume was made to 360 µl with sterile water. The tubes were incubated at 30°C for 45 min. To this, 43 µl of sterile DMSO was added and heat shock was given at 42°C for 15 min. Next, tubes were transferred to ice for 10-15 sec, centrifuged at 4,000 rpm and transformation mixture reagents were removed completely by pipetting. Cells were resuspended in 200 µl of sterile water and spread-plated on appropriate selection medium. Plates were incubated at 30°C for 24-48 h.

2.2.2.5 Preparation of ultra-competent *E. coli* cells

E. coli bacterial strain DH5α was taken out on LB-agar medium from -80°C freezer and incubated at 37°C for 14-16 h. To obtain the starter culture, single bacterial colony was inoculated in 25 ml of SOB medium in a 25 ml flask. The flask was incubated for 6-8 h at 37°C with continuous shaking at 200 rpm. Next, 2, 4 and 10 ml of the starter culture was inoculated in three different 1 litre flasks each containing 250 ml of SOB medium. Cultures were incubated overnight at 18°C with continuous shaking at 200 rpm. After overnight incubation, OD_{600} of all three cultures were monitored after every 45 min interval till OD_{600} of any of the three cultures reached 0.55. These cells were kept on ice for 10 min and the other two cultures were discarded. Cells were harvested by centrifugation at 2,500g in a Sorvall GSA rotor for 10 min at 4°C. Supernatant was poured off completely and cells were gently resuspended in 80 ml of ice-cold Inoue transformation buffer by swirling the tubes (pipetting was avoided at this step). Following resuspension, cells were spun down by centrifugation at 2,500g in a Sorvall GSA rotor for 10 min at 4°C and the supernatant was discarded completely. The cell pellet was resuspended gently in 20 ml of ice-cold Inoue transformation buffer by swirling. 1.5 ml of DMSO was added to the cell suspension and incubated on ice for 10 min. 50 µl aliquots of cell suspension were dispensed in pre-chilled 1.5 ml microfuge tubes, snap-frozen in liquid nitrogen and stored in -80°C freezer till further use.

2.2.2.6 Bacterial transformation

E. coli DH5α ultra-competent cells were used for all bacterial transformations. Briefly, frozen DH5α ultra-competent cells were taken out from -80°C freezer and thawed on ice for 15 min. DNA to be transformed was added to the bacterial cell suspension and incubated on ice for 30 min. For transforming ligation mixtures and plasmids, 5-10 µl and 100-500 ng of DNA was used, respectively. Followed by 30 min incubation on ice, heat shock was given for 60-90 sec at 42°C in a water bath and cells were immediately kept back on ice for 2 min. To this, 1 ml of sterile LB medium was added and tubes were incubated in a shaker incubator set at 37°C, 200 rpm for 45 min. Next, cells were spun down and resuspended in 500 µl of LB medium. About 100-200 µl of resuspended cells were plated on LB-agar medium containing appropriate antibiotics and incubated for 12-16 h at 37°C. Transformants were purified on LB-agar plates containing appropriate antibiotics and positive transformants carrying desired DNA were verified by PCR, restriction digestion and sequencing analyses.

2.2.3 Molecular biology methods

2.2.3.1 Yeast genomic DNA isolation

2.2.3.1.1 Glass bead lysis method

Yeast genomic DNA was isolated by mechanically lysing the yeast cells. Briefly, 10 ml of overnight grown yeast culture was transferred to a 15 ml centrifuge tube and cells were spun down at 4,000 rpm for 5 min. Media was decanted and cells were washed with 10 ml sterile water. Washed cells were resuspended in 500 µl of Buffer A and transferred to a 1.5 ml microcentrifuge tube. Tubes were incubated at 65°C for 15 min. Post incubation, 500 µl of PCI (25:24:1) solution was added. To this, 0.5 g of 0.5 mm glass beads were added and cells were lysed mechanically in a bead-beating homogenizer (MP Biomedicals, FastPrep®-24) thrice, 45 sec each, with intermittent cooling on ice. Tubes were spun at 12,000 rpm for 5 min and the aqueous layer was transferred to a new 1.5 ml microcentrifuge tube. To this, 500 µl of PCI solution was added and mixed gently by inverting the tubes. Tubes were centrifuged again at 12,000 rpm for 5 min and aqueous layer was transferred to another 1.5 ml microcentrifuge tube. Next, 2.5 volume of absolute ethanol was added to the aqueous layer, mixed well and centrifuged at 13,000 rpm for 10 min. Supernatant was decanted and the DNA pellet was washed once with 70% ethanol and centrifuged at 13,000 rpm for 10 min. Washed DNA pellet was air-dried and dissolved in 100-200 µl of 1X TE buffer by gently tapping the tubes.

2.2.3.1.2 Spheroplast lysis method

This method was used to isolate highly pure genomic DNA. Briefly, 10 ml overnight grown *C. glabrata* cultures were spun down and washed with 10 ml sterile water. Washed cells were resuspended in 500 µl sterile water and transferred to a 1.5 ml microcentrifuge tube. Tubes were spun down at 4,000 rpm for 5 min, supernatant was discarded and cell pellet was resuspended in 500 µl of buffer containing 100 mM EDTA and 5% β-mercaptoethanol and incubated at 42°C for 10 min. Post incubation, cells were spun down at 4,000 rpm for 5 min and resuspended in freshly prepared Buffer B. To this, one tip-full of lyticase (Sigma, L4025) was added and incubated at 37°C for 1 h. After incubation, spheroplasts were collected by spinning down tubes at 6,000 rpm for 5 min, supernatant was discarded and the pellet was resuspended in 500 µl of Buffer C. DNA was extracted twice with 500 µl of PCI (25:24:1) solution and the aqueous layer was transferred to a new 1.5 ml microcentrifuge tube. To this, 2.5 volume of absolute ethanol and 1/10th volume of 3 M sodium acetate (pH 5.3) were added. Tubes were spun down at 13,000 rpm for 10 min, DNA pellet was resuspended in 200 µl of 1X TE buffer containing 0.3 µl of RNase cocktail (Ambion) and incubated at 37°C for 30 min. DNA was precipitated again by adding absolute ethanol and sodium acetate as mentioned above. DNA pellet was washed once with 70% ethanol, centrifuged at 13,000 rpm for 10 min, air-dried at room temperature and was resuspended in 100-200 µl of 1X TE buffer by gently tapping the tube. DNA was stored at -20°C until use.

2.2.3.2 Total RNA isolation

All reagents required for RNA extraction were prepared in DEPC-treated water. RNase contamination from non-autoclavable items was removed by wiping them with RNaseZap® (Ambion). Total RNA from yeast cells was extracted using acid phenol extraction method. Briefly, yeast cells were grown under appropriate conditions and at suitable time points, cells were harvested by centrifugation at 4,000 rpm for 5 min. The cell pellet was washed twice with ice-cold DEPC-treated water, resuspended in 350 µl of AE buffer and transferred to a 1.5 ml microcentrifuge tube. To this, 40 µl of 10% SDS and 400 µl of acid phenol (pH 4.3) was added. The cell suspension was mixed well by vortexing thrice, short pulses of 10 seconds each, and incubated at 65°C for 15 min with continuous agitation at 800 rpm. Post incubation, cells were kept on ice for 5 min and centrifuged at 13,000 rpm in a refrigerated centrifuge set at 4°C for 10 min. After centrifugation, aqueous layer was transferred to a new 1.5 ml microcentrifuge tube and 400 µl of chloroform was added. Tubes were mixed well by gently inverting them 4-5 times and centrifuged at 13,000 rpm for 10 min. The aqueous layer was separated and transferred to a new 1.5 ml

microcentrifuge tube. For precipitation of RNA, 1/10th volume of 3 M sodium acetate (pH 5.3) and 2.5 volume of 100% ice-cold ethanol was added. In order to facilitate precipitation, tubes were kept at -20°C for 20 min. Tubes were centrifuged at 13,000 rpm for 10 min in a refrigerated centrifuge. The RNA pellet was washed with 70% ethanol, resuspended in 100-200 µl of nuclease-free water and stored at -20°C until use. Care was taken to keep all reagents and tubes on ice to maintain the cold temperature throughout the RNA extraction process.

2.2.3.3 DNase I digestion

Deoxyribonuclease I (DNase I) enzyme (Invitrogen) was used to remove the DNA contamination from RNA samples, if any. Briefly, 1 µg of RNA was subjected to DNase I digestion by using 1 U of DNase I in a 10 µl reaction mixture which contained 1X DNase I buffer and appropriate volume of water. The reaction mixture was incubated at room temperature for 15 min. Post incubation, to inhibit DNase I enzyme activity, 1 µl of 25 mM EDTA was added to the reaction mixture and tubes were heated at 65°C for 10 min. DNase I-digested RNA samples were used as template to perform PCR for the amplification of *CgACT1* gene and absence of amplification product was used as criterion to confirm proper DNase I digestion and lack of DNA contamination in the RNA sample.

2.2.3.4 Complementary DNA (cDNA) synthesis

Reverse transcriptase "Superscript III" (Invitrogen, 18080-051) was used to perform cDNA synthesis. Briefly, 500 ng of DNase I-digested RNA was incubated with 1 µl of 10 mM dNTP and 50 µM oligo(dT) at 65°C for 5 min in a 10 µl reaction mixture followed by cooling on ice for 5 min. Post incubation, 10 µl of cDNA synthesis mixture was added which contained 2 µl of 10X RT buffer, 4 µl of 25 mM $MgCl_2$, 2 µl of 0.1 M DTT, 1 µl of RNase out (40 units) and 1µl of Superscript III (200 units). Tubes were incubated at 50°C for 1 h and the reaction was terminated at 85°C for 5 min. The quality of synthesized cDNA was checked by using it as a template in a PCR reaction to amplify the housekeeping gene *CgACT1*. Amplification of *CgACT1* was indicative of proper cDNA synthesis.

2.2.3.5 Quantitative Real-time PCR (qPCR)

MESA GREEN qPCR mastermix (RT-SY2X-03+WOULR) supplied by Eurogentech was used in all qPCR experiments. Primers for real-time qPCR experiments were designed by using the Primer3 plus software to obtain 120-200 bp amplification products. Standardization of optimal template and primer concentration conditions was done in a PCR reaction and concentrations resulting in good amplification without primer dimers

were chosen for qPCR. For all qPCR reactions, 0.4 µl of cDNA template was used in a 20 µl reaction volume. Reactions were performed and data were analysed in ABI7500 real-time qPCR machine. Amplified products were run on 2% agarose gel to confirm amplification of the correct size product. C_T values of respective products were normalized with corresponding C_T value of the housekeeping gene *CgACT1*. Relative change in expression was determined by comparative C_T method, also referred as $2^{-\Delta\Delta C_T}$ method, utilizing following equation.

Fold change upon treatment = $2^{-\Delta\Delta C_T}$

$\Delta\Delta C_T = \Delta C_T$ Treated-ΔC_T Untreated

ΔC_T Treated = C_T value for gene of interest upon treatment - C_T value of internal control (*CgACT1*) upon treatment

ΔC_T Untreated = C_T value for gene of interest without treatment - C_T value of internal control (*CgACT1*) without treatment

The reaction cycling conditions were as follows

1) 95°C for 10 min (initial activation)
2) 95°C for 15 sec (denaturation)
3) 55°C for 30 sec (annealing)
4) 72°C for 40 sec (extension)
5) Go to step 2 (40 cycles)
6) 72°C for 10 min (final extension)

2.2.3.6 Plasmid isolation

Bacterial plasmid DNA was isolated using the QIAprep® spin Miniprep kit (QIAGEN, 27106). 10 ml of LB medium supplemented with appropriate antibiotics was inoculated with a single bacterial colony and incubated at 37°C for 12-16 h. Cultures were spun down at 8,000 rpm for 5 min, supernatant was discarded and the cell pellet was processed to isolate plasmid DNA as per instructions given in the kit. DNA was eluted either in nuclease-free water or in elution buffer provided in the kit and stored at -20°C until use.

2.2.3.7 Gel extraction, PCR purification and Reaction clean-up

To extract DNA from agarose gels, the QIAquick® gel extraction kit (QIAGEN, 28706) was used. For purification of PCR amplified DNA products, the QIAquick® PCR purification kit (QIAGEN, 28106) was used. Clean-up of enzymatic reactions was performed using the MinElute® Reaction Cleanup kit (QIAGEN, 28204). All protocols were followed as per manufacturer's instructions.

2.2.3.8 Restriction digestion and ligation

To perform restriction digestion of plasmid DNA and PCR-amplified DNA products, restriction enzymes were procured from *NEW ENGLAND Biolabs* (NEB). Restriction digestion was set in 50 µl reaction volume with appropriate buffer and 1X BSA. For ligation of DNA fragments obtained after restriction digestion, T4 DNA Ligase enzyme (NEB, M0202M) was used. All ligation reactions were set in 20 µl reaction volume containing 1X ligase buffer, 3-10 units of DNA ligase enzyme and vector to insert molar ratio of 1:3. The ligation mixture was either incubated at 16°C for 16 h or at room temperature for 2-3 h. Post incubation, ligation reaction was inhibited by heating tubes at 65°C for 15-20 min. 2-5 µl of ligation mixture was used to transform ultra-competent *E. coli* DH5α cells.

2.2.3.9 Yeast colony PCR

For Yeast colony PCR, yeast cells were subjected to zymolyase (MP Biomedicals, 0832092) digestion to obtain the spheroplast. To perform zymolyase digestion, a digestion cocktail was prepared in 1X PBS consisting of zymolyase (2.5 mg/ml) and sorbitol (1.2 M). The cocktail was dispensed in 0.2 ml PCR tubes in 10 µl aliquots and a tip-full of yeast cells was added to these tubes. Tubes were incubated at 37°C for 2-3 h and 1 µl of digested mixture was used as a template in a PCR reaction.

2.2.3.10 Generation of *C. glabrata* deletion strains

A homologous recombination-based strategy was used to disrupt *C. glabrara* ORFs with a cassette containing the *nat1* gene, which codes for nourseothricin acetyltransferase and imparts resistance to nourseothricin. Briefly, 5'-and 3'-UTR region (nearly 500-700 bp) of the gene to be deleted were amplified by PCR using wild-type genomic DNA as template. Both 5'-and 3'-UTR amplified products were fused to one half each of the *nat1* gene amplified from the plasmid (pRK625). The two *nat1*-amplified fragments share about 300-350 bp complimentary region. To obtain fusion products, primers were designed in such a way that the reverse primer for 5'-UTR and the forward primer for 3'-UTR of the gene of interest share 20 bp complimentary region with the forward primer for 5'*nat1* fragment and the reverse primer for 3'*nat1* fragment amplification, respectively. The fused PCR products were co-transformed in to the wild-type strain and transformants were plated on YPD-agar plates. Plates were incubated at 30°C for 16 h to allow the homologous recombination between *nat1* fragments, and 5'-and 3'-UTR at the genomic loci. Post incubation, cells were replica plated on to YPD-agar plate supplemented with 200 µg/ml nourseothricin and incubated for another 24 h. Nourseothricin-resistant colonies were purified and verified for gene disruption *via* homologous recombination by PCR using appropriate set of primers.

PCR-positive transformants were inoculated in 10 ml YPD medium, allowed to grow for 12 h and genomic DNA was isolated. Another round of PCR was performed using genomic DNA as a template to confirm the gene deletion.

2.2.3.11 Cloning of *C. glabrata* ORFs

For complementation studies, *C. glabrata* ORFs, *CgFTR1* (1.22 kb), *CgFET3* (1.91 kb), *CgYFH1* (0.53 kb), *CgCCW14* (0.64 kb), *CgMAM3* (1.91 kb) and *CgHOG1* (1.34 kb) were PCR amplified from wild-type genomic DNA using Phusion high-fidelity DNA polymerase and cloned down-stream of the *PGK1* promoter into the Xma*I* and Xho*I*, Xba*I* and Xma*I*, Xma*I* and Xho*I*, BamH*I* and Sal*I*, Xma*I* and Xho*I*, and BamH*I* and Xma*I* sites, respectively, in the CEN-ARS containing plasmid pGRB2.2 (pRK74). For over-expression studies, *C. glabrata* ORFs, *CgCCC1* (0.95 kb), *CgYAP5* (1.05 kb) and *CgMRS4* (0.92 kb) were PCR amplified from wild-type genomic DNA using Phusion high-fidelity DNA polymerase and cloned down-stream of the constitutive promoter *PDC1* into the BamH*I* and Sal*I*, Xba*I* and Xma*I*, and Xma*I* and Xho*I* sites, respectively, in the CEN-ARS containing plasmid obtained from Addgene (Addgene-ID 45323). All clones were verified by PCR and sequencing analysis.

2.2.3.12 Total protein extraction and immunoblotting

For protein extraction, cells were spun down at 4,000 rpm for 5 min and washed with ice-cold water. The cell pellet was resuspended in 250-500 µl of homogenisation buffer which contained 50 mM Tris (pH 7.5), 2 mM EDTA, 1 mM phenylmethylsulphonyl fluoride (PMSF) (serine protease inhibitor), 10 mM sodium fluoride (serine/threonine and acid phosphatases inhibitor), 1 mM sodium orthovanadate (Tyrosine and alakaline phosphatases inhibitor) and 1X protease inhibitor cocktail (Sigma, P 8215). The cell suspensison was transferred to a 1.5 ml centrifuge tube and equal amounts of glass beads (0.5 mm size) were added. Cells were lysed mechanically by bead-beating homogenizer (MP Biomedicals, FastPrep®-24) at the maximum speed for 60 seconds, five times each, with intermittent cooling on ice. After lysis, tubes were punctured at the bottom with the help of a surgical needle, and the lysed cell suspension was collected in a fresh microcentrifuge tubes by putting the punctured tubes on top of the fresh tubes and centrifuging them at 3,000 rpm for 10 min. The supernatant was transferred to a fresh microcentrifuge tube and protein concentration was estimated using the BCA protein assay kit (Thermo scientific). Protein preparations were stored at -20°C until use.

To perform immunoblotting or western blotting, appropriate amounts of total protein (ranging from 20-40 µg) were separated on a SDS-PAGE gel of 12% acrylamide concentration in Tris-Glycine-SDS gel running buffer. Protein separation was done at 70-100 V for 2-3 h using a MINI PROTEAN® 3 electrophoresis unit (Bio-Rad). Following separation, proteins were transferred to polyvinylidene difluoride (PVDF) membrane, using a Bio-Rad Mini Trans-Blot electrophoretic transfer unit in Tris-Glycine transfer buffer at 4°C. Before setting transfer assembly, PVDF membrane was first activated in 100% methanol followed by washes in the transfer buffer. The transfer assembly was set in a Bio-Rad Mini gel holder cassette (170-3931) according to manufacturer's instructions. The transfer time and current settings varied depending on the size of the protein of interest. Post transfer, membranes were separated from the assembly and kept for blocking in the blocking buffer (0.1 % Tween-20, 5% w/v fat-free skimmed milk in 1X TBS) for 1 h at room temperature with shaking. Next, membranes were incubated with appropriate dilutions of primary antibodies in the blocking buffer either for 3-4 h at room temperature or overnight at 4°C with gentle shaking. Post incubation, membranes were washed thrice with 1X TBS-T, 10 min each, with constant agitation. After washes, membranes were incubated with appropriate dilutions of secondary antibodies conjugated with horseradish peroxidase (HRP) for 1 h at room temperature with gentle shaking. Next, membranes were washed thrice with 1X TBS-T, 10 min each, with constant agitation. To visualize proteins, membranes were removed from TBS-T, and the HRP substrate ECL plus (Amersham Biosciences, RPN2232) was uniformly added on top of the membrane. Chemiluminescent signals were captured in the western blot imaging system (FluorChem™ E system).

2.2.3.13 Microarray analysis

Log-phase *C. glabrata* cells were grown either in YNB or YNB medium supplemented with either 50 µM BPS (iron limiting) or 500 µM ferric chloride (iron excess) for 2 h. Cells were spun down at 4,000 rpm for 5 min and washed twice with ice-cold DEPC-treated water. Total RNA was extracted using the acid phenol isolation method, resuspended in nuclease-free water and stored at -80°C. The frozen RNA samples were sent to Genotypic Technology Ltd., Bangalore (http://www.genotypic.co.in) wherein quality of RNA samples was determined by examining the RNA integrity number (RIN) before performing microarray analysis. Next, the 8x15 GE Agilent array, comprised of 60mer oligonucleotides representing a total of 5,503 *C. glabrata* ORFs (three replicates of each probe on average), was used for single colour microarray experiments. Data were extracted

using the GENESPRING GX (Version 12.0) software, normalized to 75 percentile shift and represent the average of two hybridizations from biological replicates for each sample. Functional annotation of differentially regulated gene set (≥ 1.5 Fold change with $p \leq 0.05$) was performed using the GENESPRING GX (Version 12.0) software and GO terms with $p < 0.05$ were considered as statistically significant. Using the REVIGO tool (http://revigo.irb.hr), redundant and significantly overlapping GO terms were removed and summarized. In REVIGO analysis, *S. cerevisiae* database was chosen for GO term sizes and the allowed similarity value was set to 0.5 (small). Additionally, to identify the overlap among differentially expressed genes, functional category analysis was performed using the fungal specific annotation tool FUNGIFUN (https://sbi.hki-jena.de/FungiFun/FungiFun.cgi). Significantly enriched FunCat (Functional Catalogue) associated pathways were extracted using the whole *C. glabrata* genome as background and compared across differentially regulated gene sets. The parameters used for FUNGIFUN analysis were cut-off p=0.05; Fisher's exact test; FunCat level 3. Raw data sets for this study are available at the Gene Expression Omnibus database (http://www.ncbi.nlm. nih.gov/geo; accession no. GSE60741).

2.2.4 Other methods

2.2.4.1 Ethics statement

Experiments involving mice were performed at the CDFD animal facility, VIMTA Labs Ltd., Hyderabad, India (www.vimta.com) in strict accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The protocol was approved by Institutional Animal Ethics Committee (IAEC) of the Vimta Labs Ltd. (IAEC protocol approval number: PCD/CDFD/05). Procedures used in this protocol were designed to minimize animal suffering.

2.2.4.2 Mouse infection assay

For mouse infection assay, 10 ml YPD medium was inoculated with different *C. glabrata* strains and allowed to grow at 30°C for 12-16 h. After growth, cultures were washed twice in sterile 1X PBS and the cell pellet was resuspended in appropriate volume of 1X PBS to obtain a cell density corresponding to 20 OD_{600}. 100 µl cell suspension ($4x10^7$ yeast cells) was injected into female BALB/c mice (6-8 weeks old) through tail vein. Seven days post

infection, mice were sacrificed and kidneys, liver, brain and spleen were harvested. Organs were homogenised in 1 ml PBS and appropriate dilutions of tissue homogenate were plated on YPD-agar medium supplemented with penicillin and streptomycin antibiotics (100 units/ml penicillin and 100 µg/ml streptomycin). Plates were incubated at 30°C for 24-48 h and CFUs were counted. Fungal burden in different organs was determined by multiplying the CFUs obtained with an appropriate dilution factor.

2.2.4.3 Adherence assay

Adherence of *C. glabrata* cells to Lec2, Chinese hamster ovarian (CHO) cells, was determined as described previously (Cormack *et al*., 1999). Briefly, Lec2 cells were seeded at a cell density of $5x10^5$ cells per well in a 24-well tissue culture plate. Cells were incubated in a cell culture incubator (Thermo Scientific) set at 37°C and 5% CO_2 for 12 h. Post incubation, the medium was discarded in a reservoir and Lec2 monolayer was washed thrice with sterile 1X PBS without disturbing the monolayer. Lec2 cells were fixed with 3.7% para-formaldehyde for 15 min followed by two PBS washes. 1 ml of 1X PBS containing antibiotics, penicillin (100 units/ml) and streptomycin (100 µg/ml), was added to each well, plates were sealed with PARAFILM, Cole-Parmer (PM-996) and stored at 4°C until use.

C. glabrata cells, to be tested for their adherence potential, were grown in CAA medium for 24 h. 100 µl of 24 h-grown culture was re-inoculated in fresh 5 ml CAA medium containing 200 µCi of S^{35} (Met:Cys-65:25) INVIVO PROTWIN label mix (JONAKI, India) in a 15 ml polypropylene tube. Cultures were allowed to grow for 16-20 h at 30°C with shaking at 200 rpm to radiolabel the cells. Radiolabelled *C. glabrata* cells were harvested by spinning down 1 ml of labelled yeast cultures, and the cell pellet was washed thrice with sterile 1X PBS to remove any residual S^{35} (Met:Cys-65:25) labelling mix from the medium. Post washes, the pellet was resuspended in 1 ml PBS, OD_{600} was measured and cell suspension of 0.4 OD_{600} was prepared. Next, 24 well plates containing fixed Lec2 cells were taken out from 4°C and PBS from the wells was discarded by inverting the plates. Wells were washed once with PBS and $2x10^6$ labelled yeast cells were added to each well, and incubated for 30 min at room temperature. Post incubation, plates were centrifuged at 1,000 rpm and the wells were washed thrice with 1X PBS to remove non-adherent *C. glabrata* cells. Lec2 cells were lysed with 5% SDS in PBS by scraping the wells, lysates were collected and transferred to a vial containing scintillation fluid.

Radioactive counts measured in $2x10^6$ labelled *C. glabrata* cells and lysates were considered as 'input' and 'output' values, respectively. Percentage adherence was calculated by following equation.

$$\% \text{ Adherence} = \frac{\text{Output radioactive counts}}{\text{Input radioactive counts}} \text{ X } 100$$

2.2.4.4 Estimation of total iron

For quantification of intracellular iron content, BPS-based colorimetric method as described by Tamarit *et al*., was used. Briefly, overnight grown *C. glabrata* cells were inoculated in fresh YPD medium and allowed to grow at 30°C and 200 rpm for 6 h. Cultures were spun down and 50 OD_{600} cells were subjected to nitric acid digestion (500 µl, 3%) for 16 h at 96°C. Next, lysates were spun down at 13,000 rpm to remove the cell debris and 400 µl of the lysate was incubated with 126 µl of ammonium acetate (1 M), 320 µl of BPS (1.7 mg/ml) and 160 µl of sodium ascorbate (38 mg/ml) for 5 min at room temperature. Absorbance of the samples was taken against the reagent blank at 535 nm and 680 nm which correspond to BPS-Fe-specific and BPS-Fe-non-specific absorbance, respectively. Non-specific absorbance was subtracted from specific absorbance and the iron content in each sample was calculated from the standard curve prepared using $FeCl_3$ and expressed as µM per OD_{600} cells. In each experiment performed, total iron content of wild-type cells was normalized to 100% and the iron content of mutants were calculated with respect to the iron content of wild-type cells.

2.2.4.5 Measurement of aconitase activity

To determine aconitase activity, mitochondria were isolated as described by Meisinger *et al*. Briefly, YPD-grown *C. glabrata* cells (500 OD_{600}) were subjected to spheroplasting followed by homogenization (15 strokes) with glass Teflon homogenizer. To collect mitochondria, homogenate was centrifuged at 13200 g for 20 min in a refrigerated centrifuge set at 4°C. The mitochondrial pellet was resuspended in SEM buffer (250 mM sucrose, 1 mM EDTA, 10 mM Mops-KOH, pH 7.2) and stored at -80°C until further use. Mitochondrial aconitase activity was estimated by using method as described by Bulteau *et al*. Mitochondrial protein samples (5 µg) were prepared in KH_2PO_4 buffer (25 mM, pH 7.2) containing 0.05 % Triton X-100. The samples were incubated with sodium citrate (1 mM), $MnCl_2$ (0.6 mM), NADP (0.2 mM) and isocitrate dehydrogenase (1 U/ml) for 20 min at room temperature. Isocitrate dehydrogenase catalysed reduction of NADP was recorded

spectro-photometrically at 340 nm. For wild-type cells, mitochondrial aconitae activity was normalized to 100 % and for mutants the relative aconitase activity percentages were calculated.

2.2.4.6 Estimation of histone deacetylase (HDAC) activity

Overnight-grown *C. glabrata* cells were freshly inoculated either in YNB medium or YNB medium supplemented with BPS (50 µM) or $FeCl_3$ (500 µM) and allowed to grow for 4 h at 30°C, 200 rpm. After 4 h growth, cells were spun down at 4,000 rpm for 5 min in a refrigerated centrifuge set at 4°C and total protein was isolated. For estimation of histone deacetylase (HDAC) activity, 40 µg of protein samples were taken and HDAC Fluorometric Activity Assay Kit (#10011563; Cayman Chemical Company, Ann Arbor, MI, USA) was used as per manufacturer's instructions. Fluorescence intensity values obtained in the presence of the HDAC inhibitor, trichostatin A, were subtracted from those of the samples without inhibitor and plotted as relative arbitrary fluorescence units.