

1 **Supplementary Material**

2 **Crz1p regulates pH homeostasis in *Candida glabrata* by altering membrane lipid**
3 **composition**

4 **Dongni Yan^{a,b}, Xiaobao Lin^{a,b}, Yanli Qi^{a,b}, Hui Liu^{a,b}, Xiulai Chen^{a,b}, Liming**
5 **Liu^{a,b,c#} and Jian Chen^b.**

6 **Running title:** *CgCrz1p regulates pH homeostasis in C. glabrata*

7 ^a State Key Laboratory of Food Science and Technology, Jiangnan University, 1800
8 Lihu Road, Wuxi, Jiangsu 214122, China

9 ^b The Key Laboratory of Industrial Biotechnology, Ministry of Education, Jiangnan
10 University, 1800 Lihu Road, Wuxi, Jiangsu 214122, China

11 ^c Laboratory of Food Microbial-Manufacturing Engineering, Jiangnan University,
12 1800 Lihu Road, Wuxi, Jiangsu 214122, China

13 [#] Address correspondence to Liming Liu (E-mail address: mingll@jiangnan.edu.cn)

14 Tel: +86-0510-85197875;

15 Fax: +86-0510-85197875.

16 Postal address: State Key Laboratory of Food Science and Technology, Jiangnan
17 University, 1800 Lihu Road, Wuxi 214122, China.

18

19

20

21

22

23 **1. Supplementary Data**

24 **Spreadsheet 1.** Up-regulated genes in the wild-type and *Cgcrz1Δ* strains in YNB-pH
25 2.0 medium, respectively, compared with that of the wild-type and *Cgcrz1Δ* strains in
26 YNB-pH 6.0 medium.

27 **Spreadsheet 2.** Down-regulated genes in the wild-type and *Cgcrz1Δ* strains in
28 YNB-pH 2.0 medium, respectively, compared with that of the wild-type and *Cgcrz1Δ*
29 strains in YNB-pH 6.0 medium.

30 **Spreadsheet 3.** Up-regulated genes in the *Cgcrz1Δ* mutant strain, compared with the
31 wild-type strain in YNB-pH 6.0 and YNB-pH 2.0 medium.

32 **Spreadsheet 4.** Down-regulated genes in the *Cgcrz1Δ* mutant strain, compared with
33 the wild-type strain in YNB-pH 6.0 and YNB-pH 2.0 medium.

34 **Spreadsheet 5.** Regulated differentially genes in the *Cgcrz1Δ* mutant strain compared
35 with the wild-type strain in YNB-pH 2.0 medium in KEGG database (with map).

36 **1.1 Supplementary Description**

37 **The detailed description of the transformation protocol for *C. glabrata*:**

38 *C. glabrata* ATCC 55 competent cells for electroporation were prepared by an
39 improved method that uses an extra treatment with lithium acetate and dithiothreitol
40 (1). The electroporation-competent cells were mixed with 1 μg of the flanking PCR
41 fragment in 10 μL of water, transferred to a 0.2-cm gap cuvette, and incubated for 5
42 min on ice. For construction of $\Delta crz1$ mutants, the flanking PCR fragment was
43 transformed into a Δ HTU strain obtained in this study.

44 The electroporation pulse was applied at 1.5 kV for 5 ms using a GenPulser

45 Xcell™ electroporation system (BioRad, Hercules, CA) (1). The electroporated cells
 46 were immediately diluted with 1 mL of ice-cold 1 mol L⁻¹ sorbitol and cultured under
 47 30 °C for 4 h. For $\Delta crz1$ transformation, 200 μ L aliquots were spread on YNB
 48 supplemented with moderate uracil and tryptophan plates to identify the histidine
 49 marker mutants.

50 2. Supplementary Figures and Tables

51 2.1. Supplementary Tables

52 **Table S1 Strains used in this study**

Strain	Genotype	Source or reference
<i>C.glabrata</i> ATCC 2001	Wild-type strain	(2)
<i>C.glabrata</i> Δ HTU	<i>his3</i> Δ <i>trp</i> Δ <i>ura3</i> Δ	(2)
<i>Cgcrz1</i> Δ	<i>his3</i> Δ <i>trp</i> Δ <i>ura3</i> Δ <i>crz1</i> Δ :: <i>CgHIS3</i>	This study
<i>Cgcrz1</i> Δ / <i>CgCRZ1</i>	<i>his3</i> Δ <i>trp</i> Δ <i>ura3</i> Δ <i>crz1</i> Δ :: <i>CgHIS3</i> <i>CgCRZ1</i> :: <i>URA3</i> (PY26/ <i>Bam</i> HI- <i>Stu</i> I)	This study
Δ HTU/PY26	<i>his3</i> Δ <i>trp</i> Δ <i>ura3</i> Δ :: <i>URA3</i> (PY26)	This study
<i>Cgcrz1</i> Δ /PY26	<i>his3</i> Δ <i>trp</i> Δ <i>ura3</i> Δ <i>crz1</i> Δ :: <i>CgHIS3</i> :: <i>URA3</i> (PY26)	This study

53

54 **Table S2 The lists of primer sequences for gene knock-out frame construction**

Gene	Primer	Sequences (5'→ 3')
<i>Cgcrz1</i> Δ		
CRZ1 Left Arm	Con-LS	ATTAGTAGCGATAACGAGTTGGACG
	Con-LA	<u>ACCCTCTTAACAAACGCCAT</u> TGCTGAATATTGCAAAATCTTGT
Marker HIS3	Con-HS	<u>TACAAGATTTTGCAATATTCAGCAAT</u> GGCGTTTGTTAAGAGGGTT
	Con-HA	ATACTGGAGGTTTGTGTTAATCTATGCTAGGACACCCTTAGTGG
CRZ1 Right Arm	Con-RS	<u>CCACTAAGGGTGTCC</u> TAGCATAGATTAACACAAACCTCCAGTATT
	Con-RA	GCAACCCCTTATTTCCCTTAGAT

55 “—” represented sequences of regions flanking of target gene.

56

57 **Table S3 Primer sequences for gene knock-out verification**

Stains	Primer	Sequences (5' → 3')
<i>Cgcrz1Δ</i>	CRZ1-Ver-S	TGGCACATATGCCTCGATGTA
	CRZ1-Ver-A	TTGTCTTAAATGCGTTGGC

58

59 **Table S4 Primer sequences for the construction of expression plasmids**

Gene	Primer	Sequences (5' → 3')
<i>CgCRZ1</i>	C-Sen	CGGGATCC ATTAGTAGCGATAACGAGTTGGACG (<i>Bam</i> HI)
	C-Anti	AAGGCCTT GCTGAATATTGCAAAATCTTGT (<i>Stu</i> I)

60

61 **Table S5 The number of colonies in *C. glabrata* strains in YNB-pH 2.0 medium**

Strain	0 h	4 h	8h	12h
<i>wt</i>	1.57±0.20×10 ⁷	2.02±0.10×10 ⁷	5.54±0.32×10 ⁷	1.26±0.26×10 ⁸
<i>Cgcrz1Δ</i>	1.27±0.16×10 ⁷	1.96±0.20×10 ⁷	3.20±0.20×10 ⁷	5.15±0.45×10 ⁷
<i>Cgcrz1Δ/CgCRZ1</i>	1.50±0.10×10 ⁷	3.56±0.40×10 ⁷	1.96±0.27×10 ⁸	2.40±0.24×10 ⁹

62

63 **Table S6 Differentially expressed genes associated with membrane lipid**

64 **metabolism**

ORF	Name	Fold change (Log2)		Function
		pH= 6.0	pH= 2.0	
		<i>wt</i> vs <i>Cgcrz1Δ</i>	<i>wt</i> vs <i>Cgcrz1Δ</i>	
CAGL0L10780g	ACC1	-3.45382	-2.77941	Propionyl-CoA carboxylase
CAGL0L10780g	FAS1	-3.45382	-2.77941	Fatty acid synthase subunit
CAGL0D00528g	FAS2	-5.09106	-3.26536	Fatty acid synthase subunit
CAGL0E06138g	FabF	-3.83228	-3.58979	Fatty acid synthase subunit
CAGL0J02970g	FabG	-4.41054	-1.84035	Fatty acid synthase subunit
CAGL0M13013g	FabG	-3.96432	-1.21719	Fatty acid synthase subunit
CAGL0J04774g	FabG	-2.83553	0	Fatty acid synthase subunit
CAGL0H10450g	FabD	-2.21458	-1.53906	Fatty acid synthase subunit
CAGL0L08184g	Elo2	-4.02847	-4.11405	Elongation protein of long chain fatty acids
CAGL0K00583g	Elo3	-1.99207	-3.29584	Elongation protein of long chain fatty acids

65

66

67 **Table S6 Differentially expressed genes associated with membrane lipid**68 **metabolism (continued table)**

ORF	Name	Fold change (Log2)		Function
		pH= 6.0	pH= 2.0	
		<i>wt vs Cgcrz1Δ</i>	<i>wt vs Cgcrz1Δ</i>	
CAGL0G04851g	Elo3	0	1.136983	Elongation protein of long chain fatty acids
CAGL0J10824g	ERG7	-1.66053	0	Lanosterol synthase
CAGL0E04334g	CYP51	1.136591	1.623938	Sterol 14-demethylase
CAGL0E05280g	ERG26	-2.82121	0	Sterol-4 α -carboxylate 3-dehydrogenase
CAGL0E05170g	ERG26	-2.78278	0	Sterol-4 α -carboxylate 3-dehydrogenase
CAGL0M11506g	ERG27	-1.20818	0	3-keto steroid reductase
CAGL0H04653g	ERG6	0	1.137315	Sterol 24-C-methyltransferase
CAGL0L10714g	ERG2	-2.35562	0	C-8 sterol isomerase
CAGL0F01793g	ERG3	1.748376	2.634495	δ -7-sterol 5-desaturase
CAGL0M07656g	ERG5	0	1.096274	Sterol 22-desaturase
CAGL0A00429g	ERG4	-1.14096	1.449763	δ -24(24(1))-sterol reductase
CAGL0C05137g	GPD1	1.650682	2.18454	Glycerol-3-phosphate dehydrogenase
CAGL0I04752g	CDS1	-2.55371	-2.23864	Phosphatidate cytidyltransferase
CAGL0I09812g	CDS1	-3.31574	-2.08754	Phosphatidate cytidyltransferase
CAGL0C03069g	CHO1	-1.6414	-2.54861	CDP-diacylglycerol-serine-O-phosphatidyltransferase
CAGL0I03784g	CLS	0	-1.55532	Cardiolipin synthase A/B
CAGL0M11462g	CLD1	-3.06236	-2.20077	Cardiolipin-specific phospholipase
CAGL0B01969g	CLD1	-5.27799	-1.77761	Cardiolipin-specific phospholipase
CAGL0L04686g	CLD1	-1.41495	-1.03389	Cardiolipin-specific phospholipase
CAGL0J09416g	PISD	-3.33089	-3.26489	Phosphatidylserine decarboxylase
CAGL0I08745g	PISD	-3.17146	-2.56741	Phosphatidylserine decarboxylase
CAGL0K09570g	EPT1	-2.6578	-2.38503	Ethanolamine phosphotransferase
CAGL0L13068g	EPT1	-4.8625	-1.82849	Ethanolamine phosphotransferase
CAGL0M04367g	CKI1	-1.49477	-2.35888	Choline kinase
CAGL0F08723g	ECT1	0	1.093409	Ethanolamine-phosphate cytidyltransferase
CAGL0J11770g	LYPLA1	-1.39527	-1.51419	Lysophospholipase I
CAGL0I06050g	INO1	-3.64446	-5.82456	Myo-inositol-1-phosphate synthase
CAGL0H01089g	INM1	-1.57606	-2.20862	Myo-inositol-1(or 4)-monophosphatase
CAGL0G08360g	VPS34	-2.42441	-1.30054	Phosphatidylinositol 3-kinase
CAGL0K00297g	Ins-1,4,5	-2.86763	-2.02035	Inositol-1,4,5-trisphosphate 5-phosphatase
CAGL0J02134g	Ins-1,4,5	-2.5808	-1.40713	Inositol-1,4,5-trisphosphate 5-phosphatase
CAGL0E03201g	PEMT	-1.68656	-1.43472	Phosphatidylethanolamine N-methyltransferase

69

70

71 **Table S7 Genes used in the validation of RNAseq data by qRT-PCR**

ORF	Gene name	Protein	Fold change ^a	
			qRT-PCR	RNAseq analysis
CAGL0D00528g	<i>FAS1</i>	Fatty acid synthase subunit 1	-3.03	-3.38
CAGL0L08184g	<i>ELO2</i>	Elongation protein of long chain fatty acids	-5.12	-4.11
CAGL0I04752g	<i>CDS1</i>	Phosphatidate cytidylyltransferase	-1.90	-2.24
CAGL0F01793g	<i>ERG3</i>	δ -7-sterol 5-desaturase	1.85	2.63
CAGL0E06138g	<i>FAS2</i>	Fatty acid synthase subunit 2	-2.38	-3.27
CAGL0I06050g	<i>INO1</i>	Myo-inositol-1-phosphate synthase	-4.98	-5.82
CAGL0C03069g	<i>CHO1</i>	CDP-diacylglycerol---serine O-phosphatidyltransferase	-2.01	-2.55
CAGL0J00539g	<i>SLT2</i>	Mitogen-activated protein kinase 7	-1.82	-2.51
CAGL0G08107g	<i>MSN2</i>	Stress transcription factor	-2.92	-3.78
CAGL0M04191g	<i>YPS1</i>	a family of 11 GPI-linked aspartyl proteases	-2.57	-3.78

72 ^a Fold change: values represent fold change when *Cgcrz1Δ* was treated with acid stress for 6 h.

73

74 **Table S8 Primers used in qRT-PCR verification**

Gene	Primer	Sequences (5'→ 3')
<i>FAS1</i>	<i>FAS1-S</i>	GGATGAAGCCTACTGGTT
	<i>FAS1-A</i>	CCTTTCTTGCTGGATTTT
<i>ELO2</i>	<i>ELOVL2-S</i>	TGGTGGAAAGGAATGGGTC
	<i>ELOVL2-A</i>	TGTGGCAGAGTTGGGAAG
<i>CDS1</i>	<i>CDS1-S</i>	GGTGGTTTCTTTGCTTCA
	<i>CDS1-A</i>	ACAATCAACTCTGTCCGTA
<i>ERG3</i>	<i>ERG3-S</i>	CTTGACTGTCCCTTGGTT
	<i>ERG3-A</i>	AAGATGAAAGTGCGTAC
<i>FAS2</i>	<i>FAS2-S</i>	CCTTTCTTGCTGGATTTT
	<i>FAS2-A</i>	GTAAACGGTAAACCAACA
<i>INO1</i>	<i>INO1-S</i>	TATGGACCGCTAACACCG
	<i>INO1-A</i>	ATGGAAGCAGCAGCAAAG
<i>CHO1</i>	<i>CHO1-S</i>	GTCAACTACCCAAGGACG

75

76 **Table S8 Primers used in qRT-PCR verification (continued table)**

Gene	Primer	Sequences (5'→ 3')
<i>CHO1</i>	<i>CHO1-A</i>	GGCAAATATCCTAACAAATC
<i>SLT2</i>	<i>SLT2-S</i>	GCGTGAACGAAATGGAAG
	<i>SLT2-A</i>	TGGGCTTGTCTGTTGGTAAT
<i>MSN2</i>	<i>MSN2-S</i>	GGCAAATATCCTAACAAATC
	<i>MSN2-A</i>	TCTGAGCTTCTTCGCAAC
<i>YPS1</i>	<i>YPS1-S</i>	CGCCCTATTGGACTCTGG
	<i>YPS1-A</i>	TCATCGGACTGGCTTGG
<i>CRZ1</i>	<i>CRZ1-S</i>	GATGCTGAAACGCTACTAAA
	<i>CRZ1-A</i>	AGCCCGATGGTGACGAA
<i>β-ACTIN</i>	<i>β-ACTIN -S</i>	ACCGCTGCTCAATCTTCC
	<i>β-ACTIN -A</i>	TCCTTACGAACATCAACATCAC

77

78

79 **The validation of correct integration of *CgCRZI* fragments by sequencing.**

80 **1. *CgCRZI* gene knock out frame (DNA sequence)**

81 GATGTTTGGCACATATGCCTCGATGTAGATATAGACCACTAGCTATTCATAGGTTACATA

82 CGTATATGCCCATATCCATATAGCAATTCATCAACCTTTGCAGCCAGAGGGACCCTTCCC

83 CCATCCAGTGATCCCACTTTTGCCGAACTTGGTCACTTTTCTTGCAGGAAAAATTATCC

84 CGGTGAGTCCTCCGAAAAGCTCAGTTTCCTTGTAATTACTTGTACAGCACAATGAAGG

85 CTTATAATAGCAAAAACTTCAAATTAGTAGCGATAACGAGTTGGACGCCCTCTTTTG

86 GAAGTCTGTTCTGGTTGCAGATGCTTATAGACCCTGGATCAAGCACTTCATTTCAATGG

87 GATTACAGCTTTTCGTTGTAGAAGAAGGCTTTTAACTAACTTGCAAGAGCAACAACCTC

88 CACTTGGGAAGGGTTTTCTGTCAAACCACTGCTAACCTGGTTTTCTTTTTTTGTAAGCA

89 AACTGGAAATAACATTTGCTGAGTTGCACAACTTCACATTAAGTTTTAGAGTTTGATA

90 GACAGTTTTCTTTTAATCTTTTTTTTCACATTGGGAGCATACTATACAAGATTTTGCAAT

91 ATTCAGCAATGGCGTTTGTTAAGAGGGTTACGCAGGAGACGAATATACAGCTGGCGCT

92 GGATCTTGACGGTGGGTCTGTTTCTGTACGGGAGAGCATACTGGGCAAGGAATATGCT

93 AGTGGTGATGGGCAGACCATCCATGTGCACACTGGAGTTGGGTTTTTGGACCACATGT

94 TGA CTGCGCTGGCGAAGCATGGCGGGTGGTCTCTGATCCTGGAGTGTATAGGGGACTT

95 GCACATCGATGACCACCACACTGTTGAGGACTGTGGGATTGCGCTGGGCCAAGCGTTC

96 AAGGAGGCGCTTGGCTCCGTGCGTGGTATCAAGAGGTTTCGGGCATGGGTTTGCACCAC

97 TGGACGAGGCGCTGAGCCGCGCTGTGGTTGACTTCTCCAATAGGCCTTTCGCCGTGGT

98 GGAGCTGGGCCTGAAGCGAGAACGCATAGGCCAGCTATCCACAGAGATGATCCCGCA

99 CTTCTTGGAGAGTTTCGCCACTGAGGCGCGTATCACCATGCATGTGGACTGTCTGCGG

100 GGCACCAACGACCACCACCGCTCCGAATCAGCTTTC AAGGCGCTCGCCATCGCCATCA

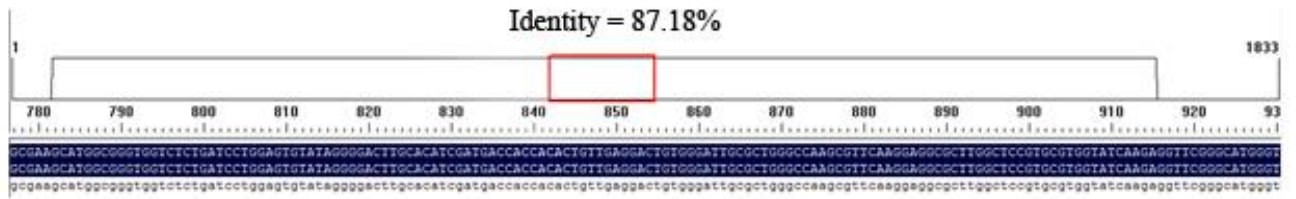
101 GAGAGGCAAGAACACCTACGGGTCGCGATGACGTTCCATCCACTAAGGGTGTCCCTAGC
102 ATAGATTAACACAAACCTCCAGTATTTTTAATGATAAATTTTTTTCTTCAGAATCAAGAT
103 TGTGATGAGATATAAGCATAAAGATCATAAGATAGTTGGTAATTACAAGGAGATTAACAT
104 CCTGATATAAGGCAGTTTTAAATACAAAAGTCTATATAAACTACTTAAATAGAGTTGTAA
105 CAATATTGGGCACTATTACTATAGCAATAGTTATTTATTTTCGAATATTTTTATGTACGGAA
106 AAGATAGGGACTCTAAATATTTCAAACAAATGAAATTTAGTCAAAGCAAGACATGCAT
107 AAGGGCATCGATCACAGTGGCTAGCACGTATGGGTCCTAAATTTATTTCAAATTTGTCA
108 AAAGGTTTAGGAATTAACCACTGAACTGTTGAAAATAATCTAGTCTGTTCCCTAAAGTCA
109 GTTCAATTAGCACTTCCAGTTGTTTGAATCTAAGGAAATAAGGGGTTGCCAACGCATTT
110 AAGACAAATTAAGTAACTATCAGTTAAACAAAATATAGTTCATGATGTCAGCATCTGAT
111 GATCAGTTGCAAGCAGAACTACAAGCCGAATTACAAAGGTTTCAAACCTTTCAGAATG
112 GTTTGTGTA

113 **2. The verified gene sequence of the *CgCRZI* gene deleted mutant strain**

114 TACGTATATGCCATATCCATATAGCAATTCATCAACCTTTGCAGCCAGAGGGACCCTTC
115 CCCCATCCAGTGATCCCCTTTTGCCGAACCTTGGTCACTTTTCTTGCAGGAAAAATTAT
116 CCCGGTGAGTCCTCCGAAAAGCTCAGTTTCCTTGTAATTACTTGTACAGCACAATGAAG
117 GCTTATAATAGCAAAAACTTCAAATTAGTAGCGATAACGAGTTGGACGCCCTCTTTT
118 GGAAGTCTGTTCTGGTTGCAGATGCTTATAGACCCTGGATCAAGCACTTCATTTTCATTG
119 GGATTACAGCTTTTCGTTGTAGAAGAAGGCTTTTAACTAACTTGCAAGAGCAACAAC
120 CCACTTGGAAGGGTTTTCTGTCAAACCACTGCTAACCTGGTTTCTTTTTTTGTAAAGC
121 AAACTGGAAATAACATTTGCTGAGTTGCACAACTTCACATTAAGTTTTAGAGTTTGAT
122 AGACAGTTTTCTTTTAATCTTTTTTTTTCACATTTGGGAGCATACTATACAAGATTTTGCA

123 ATATTCAGCAATGGCGTTTGTAAAGAGGGTTACGCAGGAGACGAATATACAGCTGGCGC
124 TGGATCTTGACGGTGGGTCTGTTTCTGTACGGGAGAGCATACTGGGCAAGGAATATGCT
125 AGTGGTGATGGGCAGACCATCCATGTGCACACTGGAGTTGGGTTTTTGGACCACATGT
126 TGA CTGCGCTGGCGAAGCATGGCGGGTGGTCTCTGATCCTGGAGTGTATAGGGGACTT
127 GCACATCGATGACCACCACACTGTTGAGGACTGTGGGATTGCGCTGGGCCAAGCGTTC
128 AAGGAGGCGCTTGGCTCCGTGCGTGGTATCAAGAGGTTCCGGGCATGGGTTTGCACCAC
129 TGGACGAGGCGCTGAGCCGCGCTGTGGTTGACTTCTCCAATAGGCCTTTCGCCGTGGT
130 GGAGCTGGGCCTGAAGCGAGAACGCATAGGCCAGCTATCCACAGAGATGATCCCGCA
131 CTTCTTGGAGAGTTTCGCCACTGAGGCGCGTATCACCATGCATGTGGACTGTCTGCGG
132 GGCACCAACGACCACCACCGCTCCGAATCAGCTTTCAAGGCGCTCGCCATCGCCATCA
133 GAGAGGCAAGAACACCTACGGGTCGCGATGACGTTCCATCCACTAAGGGTGTCTTAGC
134 ATAGATTAACACAAACCTCCAGTATTTTAAATGATAAATTTTTTCTTCAGAATCAAGAT
135 TGTGATGAGATATAAGCATAAAGATCATAAGATAGTTGGTAATTACAAGGAGATTAACAT
136 CCTGATATAAGGCAGTTTTAAATACAAAAGTCTATATAAACTACTTAAATAGAGTTGTAA
137 CAATATTGGGCACTATTACTATAGCAATAGTTATTTATTTTCGAATATTTTTATGTACGGAA
138 AAGATAGGGACTCTAAATATTTCAAACAAATGAAATTTAGTCAAAGCAAGACATGCAT
139 AAGGGCATCGATCACAGTGGCTAGCACGTATGGGTCCTAAATTTATTTCAA AATTGTCA
140 AAAGGTTTAGGAATTAACCACTGAACTGTTGAAAATAATCTAGTCTGTTCCCTAAAGTCA
141 GTTCAATT
142

143

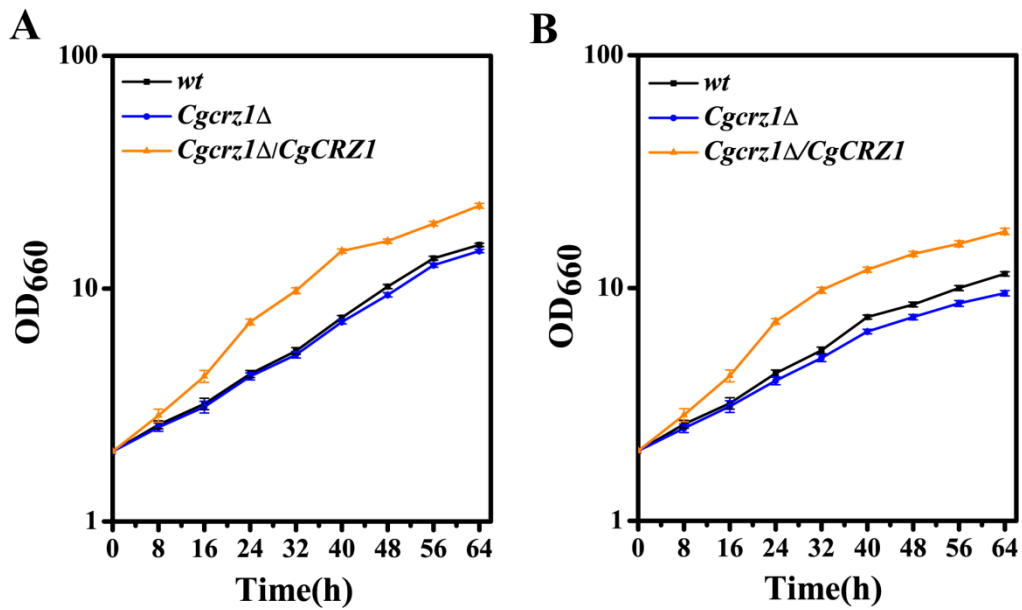


144

145

Figure S1 Correct integration of CRZ1 fragments

146

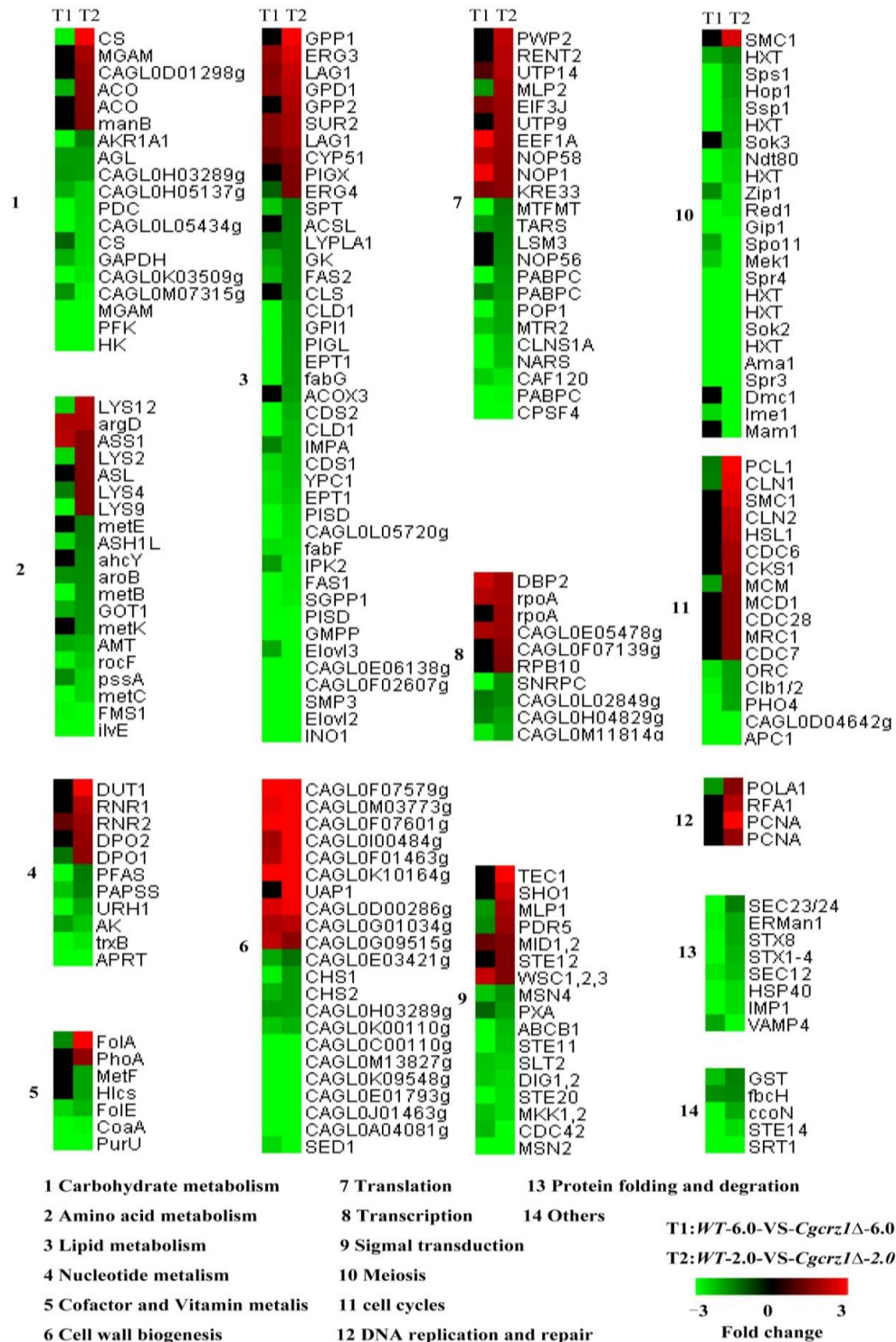


147

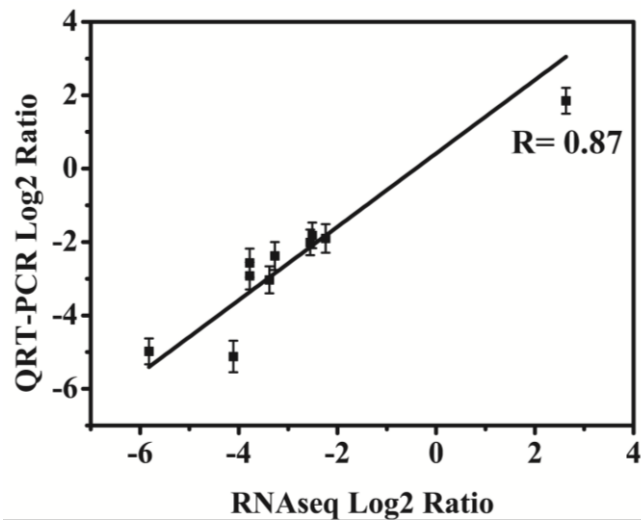
Figure S2 The growth curve of wild type, *Cgcrz1Δ* and *Cgcrz1Δ/CgCRZ1* strains

during pyruvate production (A) with CaCO₃; (B) without CaCO₃. All data are

represented as mean values of three independent experiments.



156



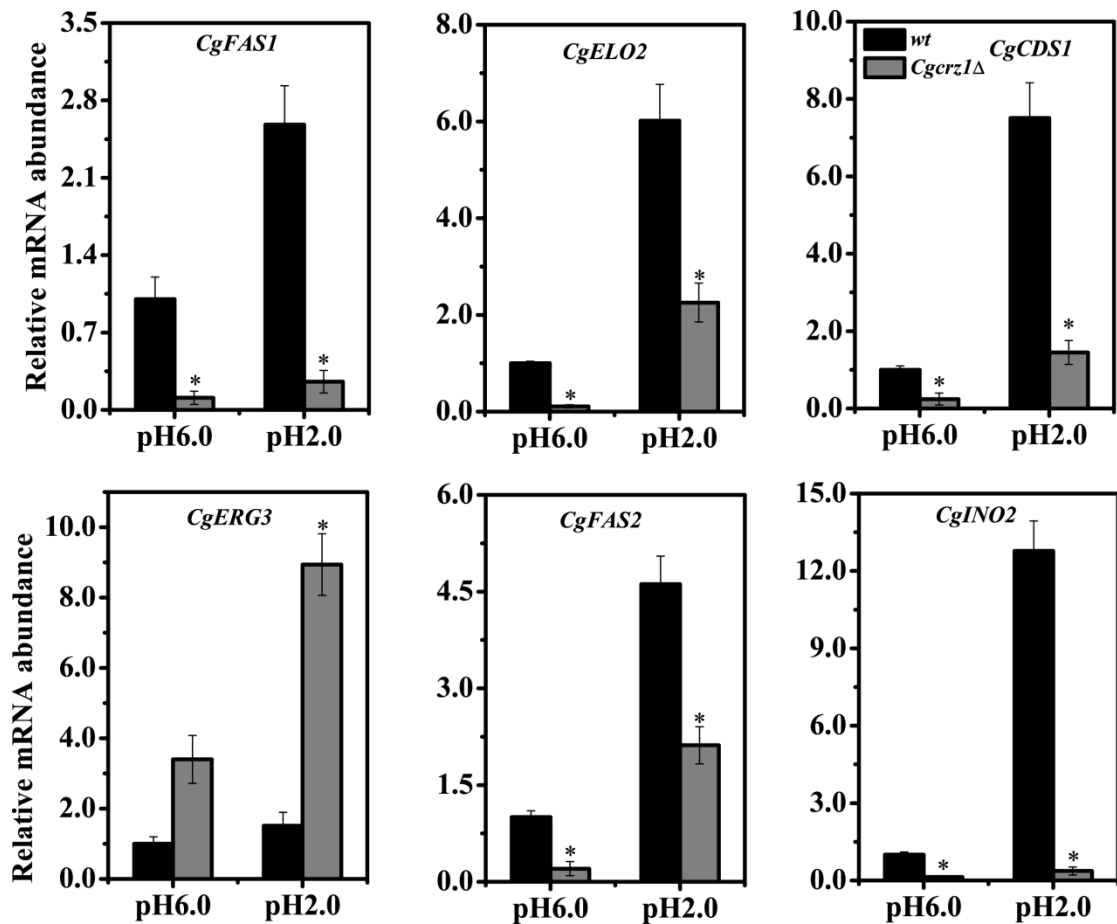
157

158 **Figure S4 qRT-PCR verification of RNA-seq data from *Cgcrz1Δ* strain at**

159

YNB-pH 2.0

160



161

162 **Figure S5 qRT-PCR verification of RNA-seq data involved in plasma membrane**

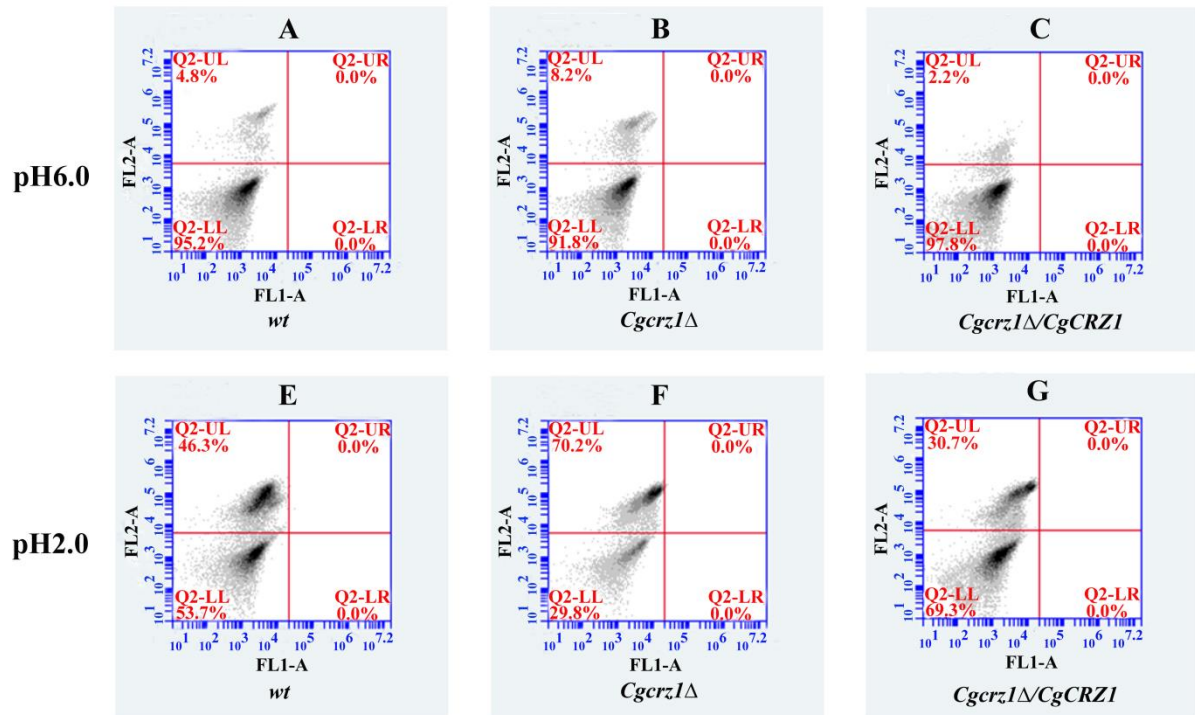
163 **lipid metabolism.** Log-phase *C. glabrata* cells were cultured in YNB-pH6.0 and

164 YNB-pH2.0 medium for 6h. The means and standard deviations for three independent

165 experiments are shown. Error bars represent standard deviations. (*P < 0.05 compared

166 to the corresponding wild-type strain, as determined by t-test).

167



168

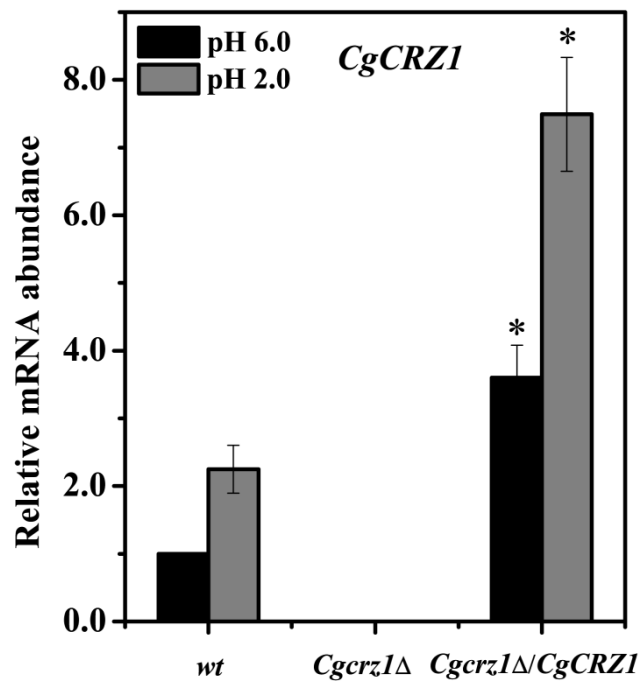
169 **Figure S6** Flow cytometry analyses of *C. glabrata* at pH 6.0 and pH 2.0. (A) the
 170 wide-type strains at pH 6.0; (B) the *Cgcrz1Δ* mutants at pH 6.0; (C) the overexpressed
 171 strain *Cgcrz1Δ/CgCRZI* at pH 6.0; (D) the wide-type strains at pH 2.0; (E) the
 172 *Cgcrz1Δ* mutants at pH 2.0; (F) the overexpressed strain *Cgcrz1Δ/CgCRZI* at pH 2.0.
 173 Q2-UL region: the percentage of PI-stained yeast cell, suggesting that cell membrane
 174 was destroyed by low pH stimulia; Q2-LL region: the percentage of PI-unstained
 175 yeast cell, suggesting that cell membrane was intact in low pH enviroment.

176

177 **The *CgCRZI* expression level in the wide type, *Cgcrz1Δ* and *Cgcrz1Δ/CgCRZI***
 178 **strains**

179 In order to determine the expression level of *CgCRZI* in the wide type, *Cgcrz1Δ*
 180 and *Cgcrz1Δ/CgCRZI* strains the *Cgcrz1Δ/CgCRZI* strains, cells grown in the
 181 logarithmic phase in YNB were extracted and then diluted in fresh medium at pH 6.0
 182 or pH 2.0 to $A_{600}=0.1$. Then the three different type cells were cultured for 4 h, total
 183 RNA was extracted using the MiniBEST Universal RNA Extraction Kit and 1 μ g was

184 used to synthesize cDNA with the Primer Script II 1st Strand cDNA Synthesis Kit
 185 (TaKaRa). The cDNA mixture was diluted to ~100 ng/ μ L and used for qRT-PCR with
 186 SYBR Premix Extaq (TaKaRa) on an iQ5 Continuous Fluorescence Detector System
 187 (Bio-Rad, Hercules, CA, USA). Data were normalized to the actin gene. The primers
 188 used for qRT-PCR verification are given in Table S8 in supplemental material.



189

190 **Figure S7 The expression level of *CgCRZI* in the wide type, *Cgcrz1*Δ and**

191

***Cgcrz1*Δ/*CgCRZI* strains**

192 Log-phase *C.glabrata* cells were cultured in YNB-pH6.0 and YNB-pH2.0 medium for

193 4 h. The means and standard deviations for three independent experiments are shown.

194 Error bars represent standard deviations. (* $P < 0.05$ compared to the corresponding

195 wild-type control, as determined by t-test).

196 **The detailed description of Fig 6A in supplemental material**

197 Phospholipid, glycolipid and cholesterol are the major lipids in the plasma

198 membrane and fatty acid acyl chains serve as precursors for phospho- or glycolipids.

199 Glycerophospholipid and sphingomyelin constitute the phospholipid bilayer, which is
 200 a fundamental membrane structure. Glycolipid signaling chains are embedded in its
 201 surface and ergosterol-enriched membrane domains are distributed within the
 202 transbilayer. Phospholipid molecules included phosphatidylcholine (PC),
 203 phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol,
 204 (PI), phosphatidylglycerol (PG) and phosphatidic acid (PA).

205 **Corrected description of transcriptome data**

206 The accession is [SRP068794](#), for the sample [SAMN04435264](#).

The old library name	the Cgcrz1-pH 2.0-6h	The new (corrected) library name	RNAseq of <i>Candida glabrata</i> under acidic conditions
The old library instrument	Illumina HiSeq 2000	The new (corrected) instrument	Illumina HiSeq 2500
The old library selection	RANDOM	The new (corrected) library selection	RT-PCR
The old Sample Organism	<i>Saccharomyces</i>	The new (corrected) Sample Organism	<i>Candida glabrata</i>
The old Abstract	to study the mechanism that <i>Candida glabrata</i> responds to the acid stimuli at transcriptional level	The new (corrected) Abstract	Identify the function of transcription factor CgCrz1p in regulating pH homeostasis
For corrections of the sample SAMN04435264 in Biosample section			
The old Sample Organism	<i>Saccharomyces</i>	The new (corrected) Sample Organism	<i>Candida glabrata</i>
The old Package	Microbe; version 1.0	The new Package	Pathogen: clinical or host-associated; version 1.0
Attributes			
The old strain	<i>Candida glabrata</i>	The new strain	<i>Candida glabrata</i> gene CgCRZ1 deletion strain

207
208

209 The accession is SRP068794, for the sample SAMN04435263.

The old library name	the Cgcrz1-pH 6.0-6h	The new (corrected) library name	RNAseq of <i>Candida glabrata</i> under acidic conditions
The old library instrument	Illumina HiSeq 2000	The new (corrected) instrument	Illumina HiSeq 2500
The old library selection	RANDOM	The new (corrected) library selection	RT-PCR
The old Sample Organism	<u><i>Saccharomyces</i></u>	The new (corrected) Sample Organism	<u><i>Candida glabrata</i></u>
The old Abstract	to study the mechanism that <i>Candida glabrata</i> responds to the acid stimuli at transcriptional level	The new (corrected) Abstract	Identify the function of transcription factor CgCrz1p in regulating pH homeostasis
For corrections of the sample <u>SAMN04435264</u> in Biosample section			
The old Sample Organism	<u><i>Saccharomyces</i></u>	The new (corrected) Sample Organism	<u><i>Candida glabrata</i></u>
The old Package	<u>Microbe; version 1.0</u>	The new Package	<u>Pathogen: clinical or host-associated; version 1.0</u>
Attributes			
The old strain	<i>Candida glabrata</i>	The new strain	<i>Candida glabrata</i> gene CgCRZ1 deletion strain

210

211

212

213

214

215

216

217

218

219 The accession is SRP068794, for the sample SAMN04435262.

The old library name	WT-pH 2.0	The new (corrected) library name	RNAseq of <i>Candida glabrata</i> under acidic conditions
The old library instrument	Illumina HiSeq 2000	The new (corrected) instrument	Illumina HiSeq 2500
The old library selection	RANDOM	The new (corrected) library selection	RT-PCR
The old Sample Organism	<u><i>Saccharomyces</i></u>	The new (corrected) Sample Organism	<u><i>Candida glabrata</i></u>
The old Abstract	to study the mechanism that <i>Candida glabrata</i> responds to the acid stimuli at transcriptional level	The new (corrected) Abstract	Identify the function of transcription factor CgCrz1p in regulating pH homeostasis
For corrections of the sample <u>SAMN04435264</u> in Biosample section			
The old Sample Organism	<u><i>Saccharomyces</i></u>	The new (corrected) Sample Organism	<u><i>Candida glabrata</i></u>
The old Package	<u>Microbe; version 1.0</u>	The new Package	<u>Pathogen: clinical or host-associated; version 1.0</u>
Attributes			
The old strain	<i>Candida glabrata</i>	The new strain	<i>Candida glabrata</i> ATCC 55

220

221

222

223

224

225

226

227

228

229 The accession is SRP068794, for the sample SAMN04435261.

The old library name	the wide-type strains at pH 6.0	The new (corrected) library name	RNAseq of <i>Candida glabrata</i> under acidic conditions
The old library instrument	Illumina HiSeq 2000	The new (corrected) instrument	Illumina HiSeq 2500
The old library selection	RANDOM	The new (corrected) library selection	RT-PCR
The old Sample Organism	<u><i>Saccharomyces</i></u>	The new (corrected) Sample Organism	<u><i>Candida glabrata</i></u>
The old Abstract	to study the mechanism that <i>Candida glabrata</i> responds to the acid stimuli at transcriptional level	The new (corrected) Abstract	Identify the function of transcription factor CgCrz1p in regulating pH homeostasis
For corrections of the sample <u>SAMN04435264</u> in Biosample section			
The old Sample Organism	<u><i>Saccharomyces</i></u>	The new (corrected) Sample Organism	<u><i>Candida glabrata</i></u>
The old Package	<u>Microbe; version 1.0</u>	The new Package	<u>Pathogen: clinical or host-associated; version 1.0</u>
Attributes			
The old strain	<i>Candida glabrata</i>	The new strain	<i>Candida glabrata</i> ATCC 55

230 1. **Wu SX, Letchworth GJ.** 2004. High efficiency transformation by electroporation of
 231 *Pichia pastoris* pretreated with lithium acetate and dithiothreitol. BioTechniques
 232 **36**:152-154.

233 2. **Roetzer A, Gregori C, Jennings AM, Quintin J, Ferrandon D, Butler G, Kuchler K,**
 234 **Ammerer G, Schueller C.** 2008. *Candida glabrata* environmental stress response
 235 involves *Saccharomyces cerevisiae* Msn2/4 orthologous transcription factors. Mol
 236 Microbiol **69**:603-620.

237