Supplementary Material

Med15B regulates acid stress response and tolerance in *Candida glabrata*

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Running title: Med15B regulates *C. glabrata* acid tolerance

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17 **1.Datasets**

18 **Datasets S1.** Up-regulated genes in the *med15B*Δ mutant strain, compared with the

19 parent strain in YNB medium at pH 6.0 and pH 2.0.

20 **Datasets S2.** Down-regulated genes in the *med15B*Δ mutant strain, compared with the

21 parent strain in YNB medium at pH 6.0 and pH 2.0.

22 **Datasets S3.** Metabolic Pathways of regulated differentially genes in the *med15B*Δ

23 mutant strain compared with the parent strain in YNB medium at pH 2.0 medium base

24 on KEGG database (with map).

25 **Datasets S4.** The fold change and functions of differentially expressed genes related

26 to membrane lipid metabolism in the *med15B*Δ strain compared with that of the parent

27 strain at pH 6.0 and pH 2.0.

28 **2. Supporting information description**

29 **1.1 The detailed protocol of the deletion strain:**

30 The *Cgmed15B*Δ deletion strain was generated from the *C. glabrata* ATCC 55

31 (*HTU*Δ) using genomic integration of a *CgHIS3* marker in the *CgMED15B* locus. The

32 *CgHIS3* marker gene was amplified from the genome of the *C. glabrata* ATCC2001

33 strain by PCR and fused with 5' and 3' flanking regions corresponding to *CgMED15B*.

34 10 μL fusion fragments (1 μg/μL) were mixed with and transformed into 80 μL *C.*

35 *glabrata* ATCC55 competent cells, which were prepared as described previously that

36 uses an extra treatment with lithium acetate (1 M) and dithiothreitol (1 M) (1), by

37 electroporation. The electroporated competent cells were immediately diluted with 1

38 ml of ice-cold sorbitol (1 M) and incubated at 30 °C for 1-2 h. For *Cgmed15B*Δ

39 transformation, 200 μL aliquots were spread on YNB solid medium without histidine.
40 The appeared colonies aftera 3-5 day incubation at 30 °C may be the correct
41 homologous recombination strains that the fusion fragment was correct integrated into
42 the genome. These strain were verified by genomic PCR and DNA sequencing.
43 Primer sequences were listed in Table 2.

44 The electroporation-competent cells were transferred to a 0.2-cm gap cuvette,
45 and incubated on ice for 5 min. The electroporation pulse was applied at 1.5 kV for 5
46 ms using a GenPulser Xcell™ electroporation system (BioRad, Herculus, CA) (1).

47 **1.2 The detailed protocol of the overexpression strain:**

48 The *HTUΔ/CgMED15B* mutant was obtained using the method as described
49 previously (2). The *CgMED15B* gene was amplified from the genome of *C. glabrata*
50 ATCC2001 by PCR, using the primers *Not*I-5' *CgMED15B* and 3' *CgMED15B*- *Sac*II.
51 First, the PCR fragments were cut at the introduced *Not*I and *Sac*II sites and ligated to
52 the shuttle plasmid pY26, which was digested with *Not*I and *Sac*II, resulting in
53 pY26-*CgMED15B*. DNA polymerase (Takara), restriction endonucleases (Takara),
54 and the DNA ligase Kit Ver.2.0 (Takara) were used to prepare the PCR fragments and
55 plasmid. DNA fragments were separated by electrophoresis in a 0.8% (w/v) agarose
56 gel in Tris-borate/EDTA and extracted with the Column DNA Gel Extraction Kit
57 Ver.2.0 (Takara) according to the instructions supplied by the manufacturer. Next,
58 Plasmids were amplified in *Escherichia coli* JM109 and isolated with the MiniBEST
59 Plasmid Purification Kit Ver.2.0 (Takara) according to the instructions supplied by the
60 manufacturer. Plasmids were transformed into *E. coli* JM109 as described previously

61 (3). Plasmids pY26 and pY26-*CgMED15B* were transformed into *C. glabrata HTU*Δ
62 by electroporation. After transformation with the plasmids, the *C. glabrata* strains
63 were spread on YNB solid medium without uracil. The appeared colonies aftera 3-5
64 day incubation at 30 °C were verified by colony PCR and yielded strains *HTU*Δ/pY26
65 and *HTU*Δ/*CgMED15B*, respectively. Primer sequences were listed in Table 2.
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67 **3. Tables**
68 **Table S1 The number of colonies in *C. glabrata* strains in YNB-pH 2.0 medium**
69 The viability of *C. glabrata* at pH 2.0 was determined by counting CFUs.
70 Log-phase *C. glabrata* cells of the *HTU*Δ, *med15B*Δ and *HTU*Δ/*CgMED15B* strains
71 were inoculated into fresh YNB medium at pH 2.0 with an initial A_{660} of 1.0. Cultures
72 were taken at regular time intervals and appropriately diluted. The dilutions of 200
73 μLwere spread on YNB solid medium and cultured at 30 °C for 2 days. Total
74 colony-forming units (CFUs) were calculated by counting viable colonies and the
75 detail values were presented here.

Strain	0 h	2h	4 h	6h	8h	10	12h
*HTU*Δ	1.29± 0.21×10^6	1.50± 0.30×10^6	2.15± 0.37×10^6	2.46± 0.57×10^6	1.14± 0.47×10^6	1.47± 0.76×10^7	2.43± 0.11×10^7
*med15B*Δ	1.13± 0.17×10^6	1.33± 0.22×10^6	1.49± 0.30×10^6	1.55± 0.45×10^6	3.10± 0.78×10^6	9.4± 0.65×10^6	1.56± 0.13×10^7
*HTU*Δ/ *CgMED15B*	2.40± 0.19×10^6	3.03± 0.26×10^6	8.05± 0.37×10^6	1.11± 0.46×10^6	3.25± 0.49×10^7	5.98± 0.69×10^7	1.50± 0.16×10^8

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78 **References**

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