

1 **Supplementary Material**

2 ***CgHog1-mediated CgRds2 phosphorylation alters glycerophospholipid composition to***  
3 ***coordinate osmotic stress in Candida glabrata***

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5 **Running title:** *CgHog1 phosphorylates CgRds2 to resist osmotic stress*

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16 **1. Supplementary Data**

17 **Spreadsheet 1.** Upregulated genes in the wild-type and *Cgrds2Δ* strains at 1.5 M NaCl, respectively,  
18 compared with that of the wild-type and *Cgrds2Δ* strains at 0 M NaCl.

19 **Spreadsheet 2.** Downregulated genes in the wild-type and *Cgrds2Δ* strains at 1.5 M NaCl, respectively,  
20 compared with that of the wild-type and *Cgrds2Δ* strains at 0 M NaCl.

21 **Spreadsheet 3.** Upregulated genes in the *Cgrds2Δ* strain, compared with the wild-type strain at 0 M  
22 NaCl and at 1.5 M NaCl.

23 **Spreadsheet 4.** Downregulated genes in the *Cgrds2Δ* strain, compared with the wild-type strain at 0  
24 M NaCl and at 1.5 M NaCl.

25 **Spreadsheet 5.** Regulated differentially genes in the *Cgrds2Δ* strain compared with the wild-type  
26 strain at 1.5 M NaCl in KEGG database.

27

28 **2. Supplementary Tables and Figures**

29 **2.1 Supplementary Tables**

30 **Table S1 List of genes that played potential roles in response to stress conditions.**

| Number | Gene         | ORF                  |                    | Function in <i>S. cerevisiae</i>  |
|--------|--------------|----------------------|--------------------|---|
|        |              | <i>S. cerevisiae</i> | <i>C. glabrata</i> |   |
| 1      | <i>ARG80</i> | YMR042W              | CAGL0F06259g       | Transcription factor involved in regulation of arginine-responsive genes  |
| 2      | <i>ASG1</i>  | YIL130W              | CAGL0G08844g       | Zinc cluster protein proposed to function as a transcriptional regulator involved in the stress response  |
| 3      | <i>CST6</i>  | YIL036W              | CAGL0I05170g       | Basic leucine zipper (bZIP) transcription factor of the ATF/CREB family, proposed to be a regulator of oleate responsive genes; involved in utilization of non-optimal carbon sources and chromosome stability                                |
| 4      | <i>CRZ1</i>  | YNL027W              | CAGL0M06831g       | Transcription factor that activates transcription of genes involved in stress response  |
| 5      | <i>GAT1</i>  | YFL021W              | CAGL0K07634g       | Transcriptional activator of genes involved in nitrogen catabolite repression; contains a GATA-1-type zinc finger DNA-binding motif; activity and localization regulated by nitrogen limitation   |
| 6      | <i>GLN3</i>  | YER040W              | CAGL0C02277g       | Transcriptional activator of genes regulated by nitrogen catabolite repression (NCR), localization and activity regulated by quality of nitrogen source   |
| 7      | <i>GSM1</i>  | YJL103C              | CAGL0L03674g       | Putative zinc cluster protein of unknown function; proposed to be involved in the regulation of energy metabolism   |
| 8      | <i>HAL6</i>  | YOR028C              | CAGL0M08800g       | Basic leucine zipper (bZIP) transcription factor of the yAP-1 family; mediates pleiotropic drug resistance and salt tolerance; nuclearly localized under oxidative stress and sequestered in the cytoplasm by Lot6p under reducing conditions |
| 9      | <i>MED2</i>  | YDL005C              | CAGL0C04477g       | Subunit of the RNA polymerase II mediator complex; associates with core polymerase subunits to form the RNA polymerase II holoenzyme; essential for transcriptional regulation  |

|    |              |         |              |  |
|----|--------------|---------|--------------|--|
| 10 | <i>MED3</i>  | YGL025C | CAGL0A01408g | Subunit of the RNA polymerase II mediator complex; associates with core polymerase subunits to form the RNA polymerase II holoenzyme; essential for basal and activated transcription                                      |
| 11 | <i>MED20</i> | YHR041C | CAGL0D04136g | Subunit of the RNA polymerase II mediator complex; associates with core polymerase subunits to form the RNA polymerase II holoenzyme; general transcription factor involved in telomere maintenance                        |
| 12 | <i>MED31</i> | YGL127C | CAGL0I00308g | Subunit of the RNA polymerase II mediator complex; associates with core polymerase subunits to form the RNA polymerase II holoenzyme; involved in telomere maintenance   |
| 13 | <i>RDS2</i>  | YPL133C | CAGL0M02651g | Transcription factor involved in regulating gluconeogenesis and glyoxylate cycle genes; member of the zinc cluster family of proteins; confers resistance to ketoconazole  |
| 14 | <i>RLM1</i>  | YPL089C | CAGL0H05621g | MADS-box transcription factor, component of the protein kinase C-mediated MAP kinase pathway involved in the maintenance of cell integrity   |
| 15 | <i>USV1</i>  | YPL230W | CAGL0E06116g | Putative transcription factor containing a C2H2 zinc finger; mutation affects transcriptional regulation of genes involved in growth on non-fermentable carbon sources, response to salt stress and cell wall biosynthesis |

31 **Table S2 Glycerophospholipid content of various strains at both 0 M NaCl and 1.5 M NaCl**

| Glycerophospholipid | Conditions | Strains     |                |                       |                            |
|---------------------|------------|-------------|----------------|-----------------------|----------------------------|
|                     |            | <i>wt</i>   | <i>Cgrds2Δ</i> | <i>Cgrds2Δ/CgRDS2</i> | <i>Cgrds2<sup>2A</sup></i> |
| PA (μg/g)           | 0 M NaCl   | 2411.05±245 | 1474.08±29     | 2950.34±29            | 1622.04±50                 |
|                     | 1.5 M NaCl | 1910.24±69  | 1220.60±34     | 2576.94±176           | 1279.98±37                 |
| PE (μg/g)           | 0 M NaCl   | 2188.78±128 | 1760.18±82     | 2835.66±51            | 1699.80±44                 |
|                     | 1.5 M NaCl | 2704.75±94  | 2315.72±89     | 3340.17±71            | 1869.43±53                 |
| PC (μg/g)           | 0 M NaCl   | 6876.65±135 | 5639.09±185    | 9409.02±291           | 6442.94±117                |

|           |            |             |             |              |             |
|-----------|------------|-------------|-------------|--------------|-------------|
|           | 1.5 M NaCl | 9336.44±192 | 6444.75±202 | 11538.15±268 | 8247.68±251 |
|           | 0 M NaCl   | 955.66±32   | 1064.19±46  | 1536.92±63   | 975.71±46   |
| PI (µg/g) | 1.5 M NaCl | 1084.97±54  | 663.45±12   | 1848.61±69   | 745.49±65   |
|           | 0 M NaCl   | 1164.81±27  | 955.41±20   | 1264.33±19   | 1092.12±51  |
| PS (µg/g) | 1.5 M NaCl | 1455.03±39  | 1058.82±27  | 1755.01±43   | 1344.17±23  |
|           | 0 M NaCl   | 1078.92±44  | 526.14±51   | 1035.63±59   | 1076.71±54  |
| PG (µg/g) | 1.5 M NaCl | 1081.14±73  | 549.26±21   | 1302.03±53   | 1002.33±56  |

32 **Table S3 Genes linked to the typical osmotic stress response were downregulated in the *Cgrds2Δ***  
33 **strain compared with the wild-type strain at 1.5 M NaCl.**

| Gene name   | ORF          | Fold change | Log <sub>2</sub> Fold change | Gene description                         |
|-------------|--------------|-------------|------------------------------|--|
| <i>ENA1</i> |              |             |                              | Na(+)-exporting P-type ATPase            |
| <i>ENA2</i> | CAGL0K12034g | -4.33       | -2.11                        |  |
| <i>ENA5</i> |              |             |                              |  |
| <i>GPD1</i> | CAGL0K01683g | -5.83       | -2.54                        | glycerol-3-phosphate dehydrogenase       |
| <i>GPD2</i> | CAGL0C05137g | -4.29       | -2.10                        | glycerol-3-phosphate dehydrogenase       |
| <i>TPS1</i> | CAGL0J09812g | -4.83       | -2.27                        | alpha,alpha-trehalose-phosphate synthase |
| <i>TPS2</i> | CAGL0G05335g | -2.43       | -1.28                        | trehalose-phosphatase                    |
| <i>TPS3</i> | CAGL0H02387g | -3.51       | -1.81                        | trehalose 6-phosphate synthase           |

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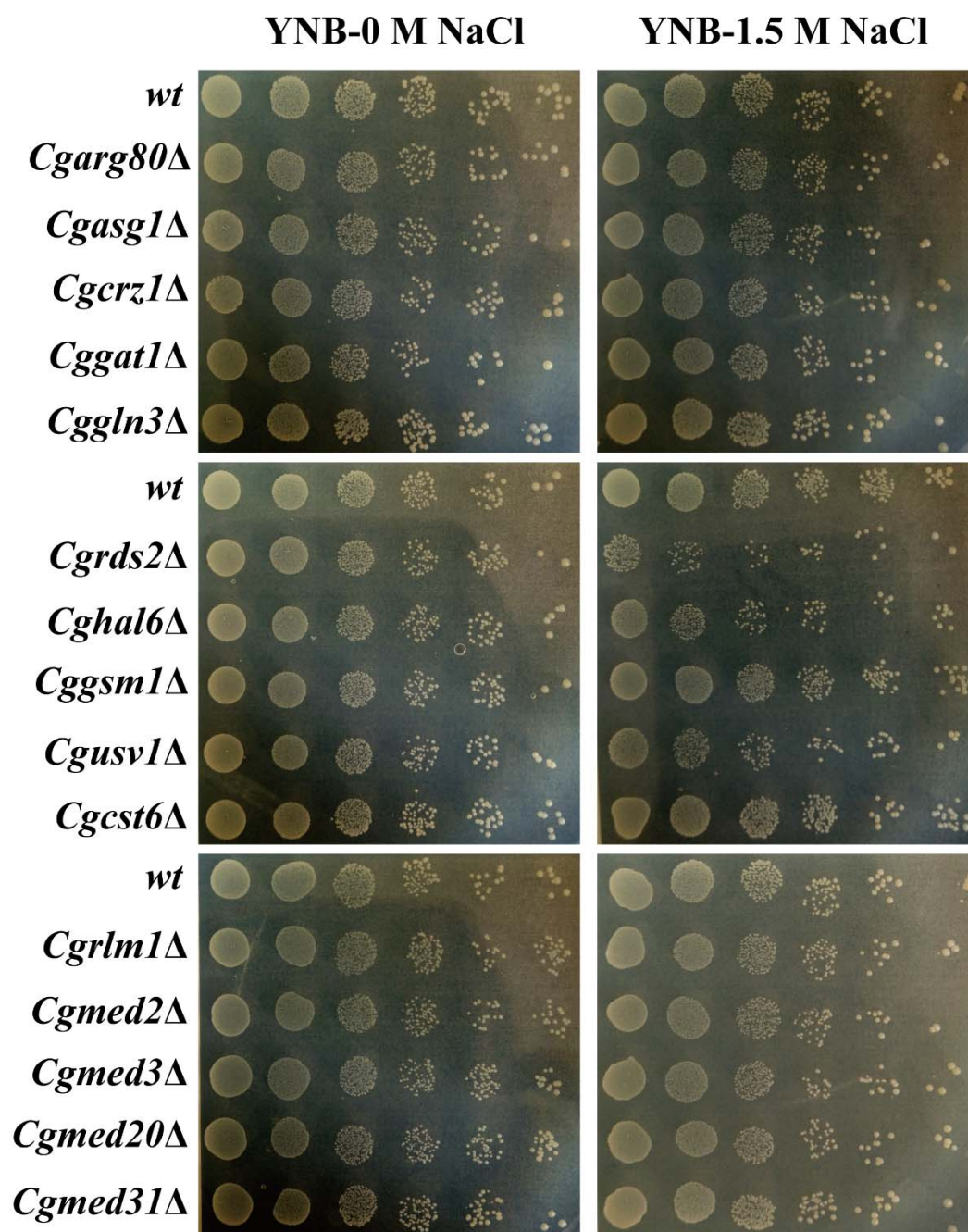
35 **Table S4 Comparison of osmotic stress response genes in *S. cerevisiae* and *C. glabrata* from the**  
 36 **aspects of carbohydrate transport, glycerol metabolism, trehalose metabolism and glycogen**  
 37 **metabolism. FC represents fold change. NS represents no significant.**

| Gene name                     | <i>S. cerevisiae</i> |                     | <i>C. glabrata</i> |                     | Gene description                              | Ref. |
|-------------------------------|----------------------|---------------------|--------------------|---------------------|---|------|
|                               | ORF                  | Log <sub>2</sub> FC | ORF                | Log <sub>2</sub> FC |   |      |
| <b>Carbohydrate transport</b> |                      |                     |                    |                     |   |      |
| <i>GLK1</i>                   | YCL040W              | 5.71                | CAGL0F00605g       | 1.46                | glucose-inactivated glycerol proton symporter | (1)  |
| <i>MAL11</i>                  | YGR289C              | 3.55                | CAGL0I03872g       | 1.45                | alpha-glucoside permease                      | (2)  |
| <i>HXK2</i>                   | YGL253W              | 2.93                | CAGL0H07579g       | 1.61                | hexokinase 2                                  | (1)  |
| <i>HXT1</i>                   | YHR094C              | 3.19                | CAGL0A01804g       | NS                  | hexose transporter                            | (1)  |
| <i>HXT2</i>                   | YMR011W              | 3.84                | CAGL0I00286g       | 3.10                | hexose transporter                            | (2)  |
| <i>HXT5</i>                   | YHR096C              | 2.32                | CAGL0A01826g       | 1.84                | hexose transporter                            | (2)  |
| <i>HXT6</i>                   | YDR343C              | 5.14                | CAGL0A02211g       | 2.37                | hexose transporter                            | (2)  |
| <i>HXT7</i>                   | YDR342C              | 5.14                | CAGL0A02211g       | 2.37                | hexose transporter                            | (2)  |
| <i>HXT10</i>                  | YFL011W              | 5.54                | CAGL0I00286g       | 3.10                | hexose transporter                            | (1)  |
| <i>HXT15</i>                  | YDL245C              | 5.25                | CAGL0A01804g       | NS                  | hexose transporter                            | (2)  |
| <i>HXT16</i>                  | YJR158W              | 5.01                | CAGL0A01804g       | NS                  | hexose transporter                            | (2)  |
| <i>HXT17</i>                  | YNR072W              | 3.87                | CAGL0A00737g       | NS                  | hexose transporter                            | (2)  |
| <i>STL1</i>                   | YDR536W              | 3.29                | CAGL0A02211g       | 2.37                | glucose-inactivated glycerol proton symporter | (2)  |
| <b>Glycerol metabolism</b>    |                      |                     |                    |                     |   |      |

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|                             |         |      |              |      |   |     |
|-----------------------------|---------|------|--------------|------|---|-----|
| <i>GPD1</i>                 | YDL022W | 6.26 | CAGL0K01683g | 3.35 | glycerol-3-phosphate dehydrogenase          | (1) |
| <i>GPD2</i>                 | YOL059W | 4.03 | CAGL0C05137g | 3.10 | glycerol-3-phosphate dehydrogenase          | (1) |
| <i>DAK1</i>                 | YML070W | 3.23 | CAGL0L11374g | 2.29 | dihydroxyacetone kinase                     | (1) |
| <b>Trehalose metabolism</b> |         |      |              |      |   |     |
| <i>TPS1</i>                 | YBR126C | 5.03 | CAGL0J09812g | 2.90 | alpha,alpha-trehalose-phosphate synthase    | (1) |
| <i>TPS2</i>                 | YDR074W | 2.74 | CAGL0G05335g | 1.99 | trehalose-phosphatase                       | (2) |
| <i>TPS3</i>                 | YMR261C | 3.87 | CAGL0H02387g | 2.81 | trehalose 6-phosphate synthase              | (1) |
| <i>TSL1</i>                 | YML100W | 4.93 | CAGL0H02387g | 2.81 | trehalose 6-phosphate synthase              | (2) |
| <i>NTH1</i>                 | YDR001C | 3.58 | CAGL0M10439g | 2.66 | alpha,alpha-trehalase                       | (1) |
| <b>Glycogen metabolism</b>  |         |      |              |      |   |     |
| <i>GLC3</i>                 | YEL011W | 3.60 | CAGL0M03377g | 2.18 | 1,4-alpha-glucan branching enzyme           | (2) |
| <i>GSY1</i>                 | YFR015C | 3.78 | CAGL0K10626g | 2.76 | glycogen (starch) synthase                  | (2) |
| <i>GSY2</i>                 | YLR258W | 2.77 | CAGL0F04719g | 1.70 | glycogen (starch) synthase                  | (2) |
| <i>GAC1</i>                 | YOR178C | 2.96 | CAGL0F04917g | NS   | protein phosphatase regulator               | (2) |
| <i>GDB1</i>                 | YPR184W | 2.44 | CAGL0G09977g | 1.86 | 4-alpha-glucanotransferase                  | (1) |
| <i>GLG1</i>                 | YKR058W | 2.32 | CAGL0H02695g | 1.28 | glycogenin glucosyltransferase              | (1) |
| <i>UGP1</i>                 | YKL035W | 4.38 | CAGL0L01925g | NS   | UTP glucose-1-phosphate uridylyltransferase | (1) |
| <i>GPH1</i>                 | YPR160W | 3.19 | CAGL0F04895g | NS   | glycogen phosphorylase                      | (1) |
| <i>PGM1</i>                 | YKL127W | 2.70 | CAGL0K07480g | NS   | phosphoglucomutase                          | (1) |

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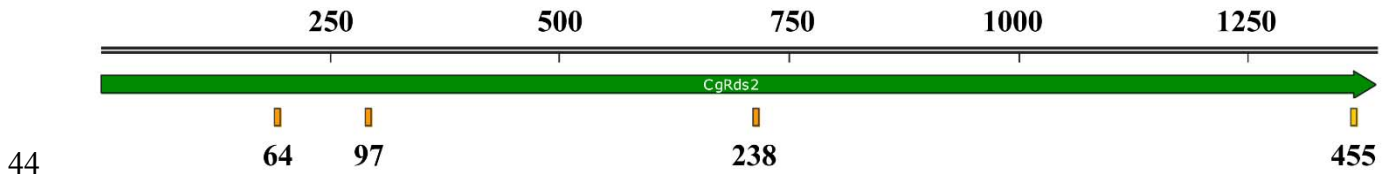
40

41 **Figure S1 Growth profile of the wild-type (*wt*) and 15 mutant strains grown on YNB medium**

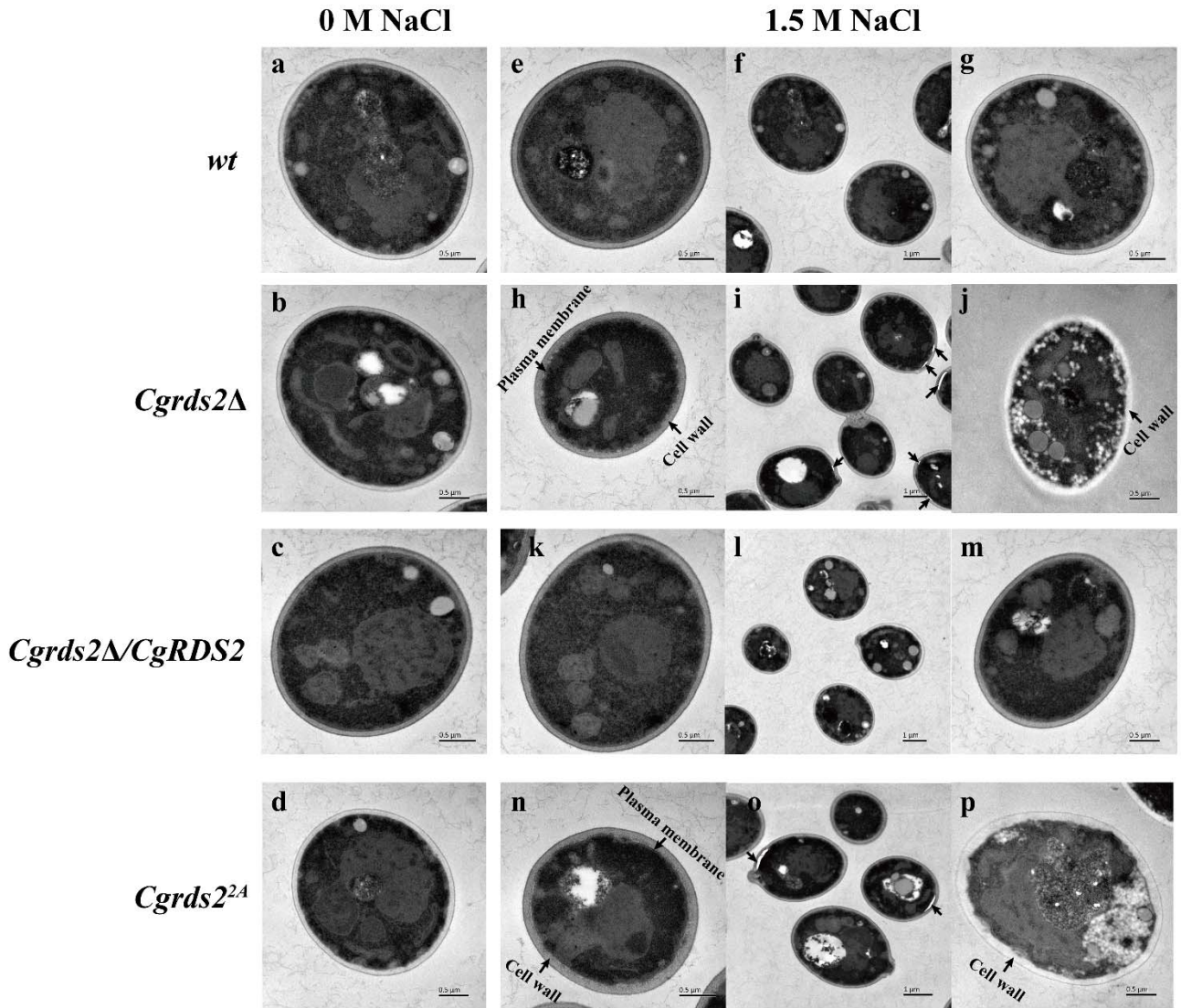
42 **with or without 1.5 M NaCl.**

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45 **Figure S2** Sketch map of the consensus phosphorylation sites for *CgHog1* in the *CgRds2* protein.



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47 **Figure S3** Transmission electron microscopy analysis of membrane integrity in the wild-type (*wt*),

48 *Cgrds2Δ*, *Cgrds2Δ/CgRDS2* and *Cgrds2<sup>2A</sup>* strains at 0 M NaCl and 1.5 M NaCl. The arrows in the (i)

49 and (o) referred to the separation of cell membrane from cell wall.

50

51 **3. *CgRds2* sequence**

52 **3.1 *CgRDS2* nucleotide sequence (NCBI-GeneID: 2891470)**

53 atggaagaaccagcagccaagaagcagaagaagctgtacaaatcatgcataatggccgaggtcgcattggaattgtgatcatcagcgacctgt  
54 tcgcggtgtatcaagaggagataggccatttatgtgttctgatgagaatgtctctaatatacaaaagccaataccaatataagaatagtagtgcgc  
55 aatagggagcacagaaatcccaatgctgagatcaatataggtgatgaaaccctgcaattcagggtcgaatagcagcgtatatggtacagaaa  
56 caccaatatcatcctctaacaataacctattattacccccacaacaaactttgatcggaaaatgtaggatccgagtttagttcactcaatgaattct  
57 gatgatgttgagaatccaataccgggtgaatcagaaaataccagccaaagtaataaagagcaagaacgtacagctcactccaacatggaagat  
58 agcatagagaatacgaagagcagaccgataacaatgaaggtattataggaagtgttagtgaaccaggaaatctgcaggatataaacgaag  
59 caagtagtaaagctaaccgtaccaagacagtcaatgatggacaagggaagaacaagaccaggagcataatagacaaaaactgaataaaca  
60 gagagcagcaattctaaagctcaaagtgacaaatgatgacgatacaccacatcaaaggaacagttcttctaacgctgctgatccgtccaccgaa  
61 atgaatcccgtagacagactgaagctagttatcaacgcgaagctagaagcagggtattaaaacatgatgactacgccaagggtacgcaagatt  
62 acaagaatatatggataagtacatgaacgacacgaataagcaagaatgtgaaaccattaatgagcatacagacctgcatttaggaccatagccaa  
63 gtcgttaaaggatgtagacttggttttagtagaggagagctttgagaggatgttactatcatatgatcagatattcacttcgatgagtagcctgcatgc  
64 ctctggagacgtactggtgaaatataataggccaacaaagagttgcatcgtgtagactgcacgggtgatgatctgcgcgatggtaaactgccc  
65 atttacgagttgatgactgaagaaagcgcagtaaattctgggagaagtatggttcattgcctttgataagggccaaaaagctgtactcaccagttg  
66 ctgctgagaaccaaagacggctgaagaaaaggcctgtgtttcagttcacaattagacgtgatcggtaacaatccccatctgcattgttgta  
67 actttatcccactatctcctacaagagatcattccaactaa

68 **3.2 *CgRds2* amino acid sequence (NCBI-ProteinID: XP\_449462).** The consensus phosphorylation

69 sites for *CgHog1* in the *CgRds2* protein were highlighted in yellow.

70 MEEPAAKKQKKLYKSCIFCRRSHVNC DHQRPCSRCIKREIGHL CVADENVSNIQSQYQYKNS

71 MSPIGSTEIPNAEINIGDETRAIQGSNSSVYGTETPISSNNLLPPQPNFVSENVGSEFSSLNE

72 FLMMLNPIPGESENTSQSNKEQERTAHSNMEDSIENTQEQT DNNEGIIGSVSVNQEISQDINE

73 ASSKANGTKTVNDGQGQEQDQEHNRQKLNKQESSNSKAQSDKYDDTP TSKEQFFLTAADP  
74 STEMNPVDRLKLVINAKLEAGLLKPYDYAKGYARLQEYMDKYMNDTNKQRILKPLMSIRPA  
75 FRTIAKSLKDVDLVLVEESFERMLLSYDRVFTSMSMPACLWRRTGEIYRANKEFASLVDCTV  
76 DDLRDGKLAIYELMTEESAVNFWEKYGSIAFDKGQKAVLTSCSLRRTKDGLKKRPCCFSFTIR  
77 RDRYNIPICIVGNFIPLSPTRDHSN

78 **Reference**

- 79 1. **Posas F, Chambers JR, Heyman JA, Hoeffler JP, de Nadal E, Arino J.** 2000. The  
80 transcriptional response of yeast to saline stress. *J Biol Chem* **275**:17249-17255.
- 81 2. **Tekarslan-Sahin SH, Alkim C, Sezgin T.** 2018. Physiological and transcriptomic analysis of  
82 a salt-resistant *Saccharomyces cerevisiae* mutant obtained by evolutionary engineering.  
83 *Bosnian J Basic Med* **18**:55-65.

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