

**Supplementary Table 1. *Saccharomyces cerevisiae* strains used in this work.**

| <b>Strain</b>                               | <b>Genotype</b>                                                                                           | <b>Reference</b>       |
|---------------------------------------------|-----------------------------------------------------------------------------------------------------------|------------------------|
| <b>MPY17</b>                                | <i>MAT<math>\alpha</math> ctr1::ura3::Kan<sup>r</sup> ctr3::TRP1 his3 lys2-801 CUP1<sup>R</sup></i>       | (Peña et al., 1998)    |
| <b>MPY17 <i>ctr2</i><math>\Delta</math></b> | <i>MAT<math>\alpha</math> ctr1::ura3::Kan<sup>r</sup> ctr3::TRP1 ctr2::HIS3 lys2-801 CUP1<sup>R</sup></i> | (Rees et al., 2004)    |
| <b>WYT</b>                                  | <i>MAT<math>\alpha</math> his3 can1-100 ade2 leu2 trp1 ura3 yap1:: TRP1</i>                               | (Kuge and Jones, 1994) |

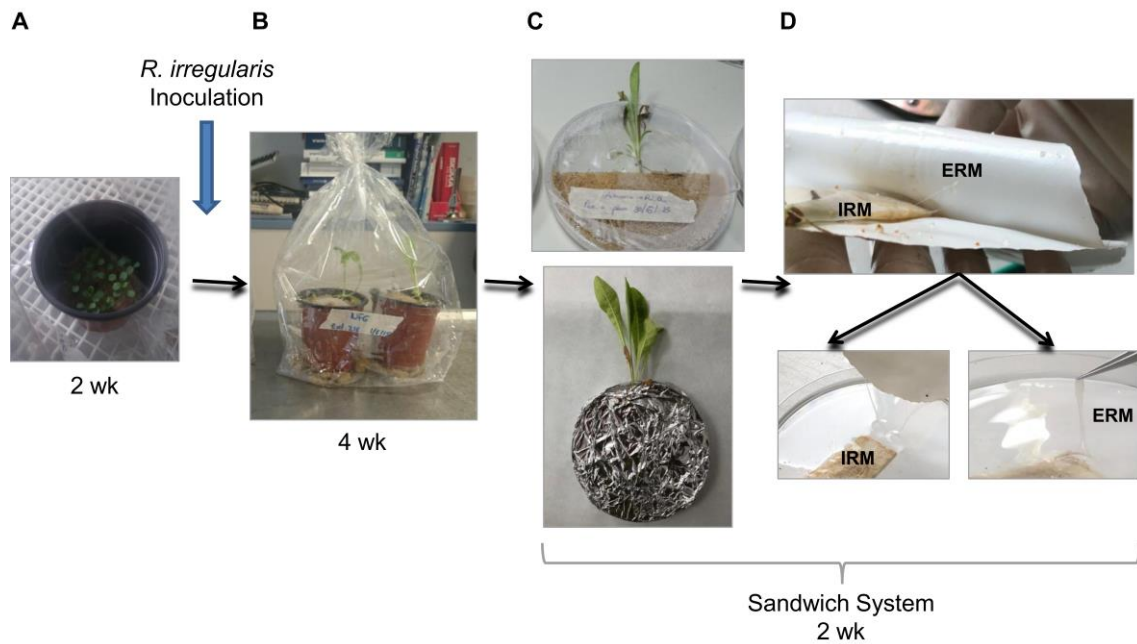
**Supplementary Table 2. Primers used in experimental procedures.** Overlaps with the corresponding cloning vectors and restriction sites are highlighted in bold or underlined, respectively. w/o: without

| Primer ID        | Sequence (5'-3')                                    | Purpose                                                       |
|------------------|-----------------------------------------------------|---------------------------------------------------------------|
| RiCTR1R.5r       | ATCAATTGTCCTGTAGTCAAGCCCGTC                         | 5'RACE of <i>RiCTR1</i>                                       |
| RiCTR2R.5r       | CATGCATCGCTATCATGACTACCACCATG                       | 5'RACE of <i>RiCTR2</i>                                       |
| RiCTR3R.5ra      | AGTATGAAATGAGGTAACCTTGAACCCCT                       | 5'RACE of <i>RiCTR3</i>                                       |
| RiCTR3R.5rb      | CCCTGCATAAGTAAATGTCCTTATTACAACC                     | 5'RACE of <i>RiCTR3</i>                                       |
| RiCTR3R.5rc      | <b>GATTACGCCAAGCTT</b> GGAGATCATCATCTTGCGAAATTGGAAC | 5'RACE of <i>RiCTR3</i>                                       |
| RiCTR1F.3r       | ACAGAACATACAACCCCAGCTCACTTCG                        | 3'RACE of <i>RiCTR1</i>                                       |
| RiCTR3F.3r       | CAGGGTTCCAAGTTACCTCATTTTCATACT                      | 3'RACE of <i>RiCTR3</i>                                       |
| RiCTR1FL.F       | TCCCCCGGGATGTCAGAGACAATGACAGAAC                     | Cloning of <i>RiCTR1</i> CDS into pDR196                      |
| RiCTR1FL.R       | CCGCCTCGAGTTATGTCCTCTTTTCCTCCTTG                    | Cloning of <i>RiCTR1</i> CDS into pDR196                      |
| RiCTR2FL.F       | AACTGCAGATGAATTATCAACAATTT                          | Cloning of <i>RiCTR2</i> CDS into pDR196                      |
| RiCTR2FL.R       | ACGCGTCGACTTAATGGCAAGTTATTGA                        | Cloning of <i>RiCTR2</i> CDS into pDR196                      |
| RiCTR3AFL.F      | AACTGCAGATGCTTTATTTTACGAGTGATTTG                    | Cloning of <i>RiCTR3A</i> CDS into pDR196                     |
| RiCTR3BFL.F      | <u>AACTGCAGATGCT</u> ATTAATAATGTCGATGAATTC          | Cloning of <i>RiCTR3B</i> CDS into pDR196                     |
| RiCTR3FL.R       | ACGCGTCGACTCATTTATTAACGAGACTTTGCTG                  | Cloning of <i>RiCTR3A/B</i> CDS into pDR196                   |
| RiCTR3FLg-F      | ATATACGCAGCCTCATAAATTC                              | <i>RiCTR3</i> genomic clone                                   |
| RiCTR1gwF        | <b>CACC</b> ATGTCAGAGACAATGACAGAAC                  | Cloning of <i>RiCTR1</i> CDS into pENTR/D-TOPO                |
| RiCTR1gwR        | TTATGTCCTCTTTTCCTCCTTG                              | Cloning of <i>RiCTR1</i> CDS into pENTR/D-TOPO                |
| RiCTR1gwNoSTOP-R | TGTCCTCTTTTCCTCCTTG                                 | Cloning of <i>RiCTR1</i> CDS w/o stop codon into pENTR/D-TOPO |
| RiCTR2gwF        | <b>CACC</b> ATGAATTATCAACAATTTATCATAAAAG            | Cloning of <i>RiCTR2</i> CDS into pENTR/D-TOPO                |
| RiCTR2gwR        | TTAATGGCAAGTTATTGATTTACC                            | Cloning of <i>RiCTR2</i> CDS into pENTR/D-TOPO                |
| RiCTR2gwNoSTOP-R | ATGGCAAGTTATTGATTTACC                               | Cloning of <i>RiCTR2</i> CDS w/o stop codon into pENTR/D-TOPO |
| RiCTR3AgwF       | <b>CACC</b> ATGCTTTATTTTACGAGTGATTTG                | Cloning of <i>RiCTR3A</i> CDS into pENTR/D-TOPO               |

| Primer ID         | Sequence (5'-3')              | Purpose                                                        |
|-------------------|-------------------------------|----------------------------------------------------------------|
| RiCTR3AgwR        | TCATTTATTAACGAGACTTTGCTG      | Cloning of <i>RiCTR3A</i> CDS into pENTR/D-TOPO                |
| RiCTR3AgwNoSTOP-R | TTTATTAACGAGACTTTGCTGC        | Cloning of <i>RiCTR3A</i> CDS w/o stop codon into pENTR/D-TOPO |
| RiCTR1Fq          | TATAGTGGTGACGGGCTTGACT        | Real-Time qPCR of <i>RiCTR1</i>                                |
| RiCTR1Rq          | GAAGGGTGAATTGTATATTGTTGG      | Real-Time qPCR of <i>RiCTR1</i>                                |
| RiCTR2Fq          | GGTGGTAGTCATGATAGCGATG        | Real-Time qPCR of <i>RiCTR2</i>                                |
| RiCTR2Rq          | CTATTTACGCGCCACCAAGT          | Real-Time qPCR of <i>RiCTR2</i>                                |
| RiCTR3AFq         | GCTTTATTTTACGAGTGATTTG        | Real-Time qPCR of <i>RiCTR3A</i>                               |
| RiCTR3ARq         | TTGTCAATCCAGTTACCACCAC        | Real-Time qPCR of <i>RiCTR3A</i>                               |
| RiCTR3BFq         | TGGTAAAATATTTCACTAGTTACTCTGAC | Real-Time qPCR of <i>RiCTR3B</i>                               |
| RiCTR3BRq         | ATCTCAAAATTGTTAACCCTGCAT      | Real-Time qPCR of <i>RiCTR3B</i>                               |
| RiMST2Fq          | GGCAGGATATTTGTCTGATAG         | Real-Time qPCR of <i>RiMST2</i>                                |
| RiMST2Rq          | GCAATAACTCTTCCCGTATAC         | Real-Time qPCR of <i>RiMST2</i>                                |
| RiSOD1Fq          | GTAATACTTTTCATTCAGGA          | Real-Time qPCR of <i>RiSOD1</i>                                |
| RiSOD1Rq          | AGTTCATGACCACCTTTACCAA        | Real-Time qPCR of <i>RiSOD1</i>                                |
| RiEF1αFq          | GCTATTTTGATCATTGCCGCC         | Real-Time qPCR of <i>RiEF1α</i>                                |
| RiEF1αRq          | TCATTAACGTTCTTCCGACC          | Real-Time qPCR of <i>RiEF1α</i>                                |
| CiEF1αFq          | CATGCGTCAGACGGTTGCTGT         | Real-Time qPCR of <i>CiEF1α</i>                                |
| CiEF1αRq          | CTTCACTCCCTTCTTGGCTGC         | Real-Time qPCR of <i>CiEF1α</i>                                |
| DcEF1αFq          | TCAAGGATCTCAAGCGTGGTTATGT     | Real-Time qPCR of <i>DcEF1α</i>                                |
| DcEF1αRq          | CAGCAATGTGGCAAGTGTGACAAT      | Real-Time qPCR of <i>DcEF1α</i>                                |

**Supplementary Table 3. Comparison among the predicted amino acid sequence of the *R. irregularis* CTR family members.** Percentages of identity/similarity are indicated.

|         | RiCTR1  | RiCTR2  | RiCTR3A | RiCTR3B |
|---------|---------|---------|---------|---------|
| RiCTR1  | 100/100 | 16/24   | 39/53   | 21/27   |
| RiCTR2  |         | 100/100 | 11/20   | 4/9     |
| RiCTR3A |         |         | 100/100 | 55/55   |
| RiCTR3B |         |         |         | 100/100 |



**Supplementary Figure 1. Scheme of the *in vivo* whole plant bidimensional experimental system described by Pepe et al. (2017), with some modifications. (A)** Surface-sterilized chicory (*Cichorium intybus* L.) seeds were germinated for two weeks. **(B)** Seedlings were inoculated with spores, ERM and colonized roots obtained from *R. irregularis* monoxenic cultures and transplanted into 50 mL pots filled with sterile sand. Pots were placed in sun-transparent bags and maintained during one month in a growth chamber (24°C/21°C day/night, 16 h light photoperiod). **(C)** A sandwich culture system was established as detailed in Materials and Methods and maintained during two weeks in a growth chamber under the conditions described above. **(D)** ERM spreading from the nylon net onto the membranes was collected with tweezers, while mycorrhizal roots were wrapped in the nylon net.

|         |                                                            |    |
|---------|------------------------------------------------------------|----|
| RiCTR1  | -----                                                      | 0  |
| RiCTR3A | -----                                                      | 0  |
| RiCTR3B | -----                                                      | 0  |
| ScCTR1  | MEGMNMGSSMNDAMSSASKTVASSMASMSMDAMSSASKTILSSSSMSMEAMSSASKTL | 60 |
| ScCTR3  | -----                                                      | 0  |
| ScCTR2  | -----MDDKKTWSTVTLR-----                                    | 13 |
| RiCTR2  | -----                                                      | 0  |

|         |                                                        |     |
|---------|--------------------------------------------------------|-----|
| RiCTR1  | -----MSE-----TMTEHT                                    | 9   |
| RiCTR3A | -----                                                  | 0   |
| RiCTR3B | -----                                                  | 0   |
| ScCTR1  | ASTMSMASMSGSSMSGSMSSSTPTSSASAQTSDSSMSGMSGSSSDNSSSSSGM- | 119 |
| ScCTR3  | -----MNMGG-----SSSTAA                                  | 11  |
| ScCTR2  | --TFNQ-----LVTSSLIG-----YSKMDSMNHKMEGNAGHDHSDMHMGD     | 52  |
| RiCTR2  | -----MN-----YQFI--IKAM--NYD--HGGSH                     | 19  |

|         |                                                           |     |
|---------|-----------------------------------------------------------|-----|
| TM1     |                                                           |     |
| RiCTR1  | TPAHFDHNKL---YFSDDLISKISL---LQVNSIFS-----FVLASIF--V---TL  | 48  |
| RiCTR3A | -----ML--YFTSDLSKVVLFSGFQVTSFHT-----FVLATLF--V---IS       | 35  |
| RiCTR3B | -----                                                     | 0   |
| ScCTR1  | --DMDMSGMNYYLTPYKNYPVLFHHLHANNSG---KAFGIFLLFVVAAFVYKLLLF  | 172 |
| ScCTR3  | KKATCKISMLWNWYTI---DTCFIARSWRNDTKGKFAGSCIGCFALVVAQWL---TR | 63  |
| ScCTR2  | GDDTCSMMLFWSYK---NTCVVFEWWHIKTLPLGLILSCLAIFGLAYLYEYL---KY | 104 |
| RiCTR2  | DSDACNMMLFNWDTE---KLCIIFTWVRVNSIGFLILSCFIIILLATSEFEL---RF | 71  |

|         |                                                             |     |
|---------|-------------------------------------------------------------|-----|
| RiCTR1  | LCWCERFLN-----YYYHK---KSK-----                              | 65  |
| RiCTR3A | LCWCERLFT-----YYYEN---AG-----                               | 51  |
| RiCTR3B | -----                                                       | 0   |
| ScCTR1  | VSWCLEVHWFKWKDKQNKYSTLPANSKDEGKHYDTENNFEIQGLPKLPNLLSDIF---  | 228 |
| ScCTR3  | FSRQFDVELLKRQKIKHLASYSPEEYVVKCGEEDAKSDIEELQGFYNPSWKTTLISLQK | 123 |
| ScCTR2  | CVHKKQLSQ-----RVLLPN-----                                   | 119 |
| RiCTR2  | ALRKYDTKI-----QVSYSQLQ---EGRD-AN---SEEVA-----               | 99  |

|         |                                                             |     |
|---------|-------------------------------------------------------------|-----|
| TM2     |                                                             |     |
| RiCTR1  | -----YDRNQKRFRSIAVKTLISYGILTTLRLS                           | 92  |
| RiCTR3A | -----YEGQKRFRKRVVIRTFTYAGLTILRFF                            | 78  |
| RiCTR3B | -----                                                       | 0   |
| ScCTR1  | -----VPS--LMDLFHDIIIRAFLVFTSTMIIM                           | 254 |
| ScCTR3  | SFIYSFVVGPRRLNEPEDDLLKKVLSCTLTITPVDLYPTFLDHMIRVTIFVLQWGLSYI | 183 |
| ScCTR2  | -----RSLTKINQADKVSNSILYGLQVGFSEFM                           | 146 |
| RiCTR2  | -----T-----QSNANRITYVQQLVRTLHTSQVFLSFF                      | 128 |

|                |                                                           |     |
|----------------|-----------------------------------------------------------|-----|
| TM3            |                                                           |     |
| RiCTR1         | YLLIISMNSQIFIIIVVTTLTQLLIVEYIRSIPIYQYTIIPSEITPNTIEGSLVMGN | 152 |
| RiCTR3A        | YLLIISMNSQIFIVVVTTLTQLLIVEYVVISVYSTQLVPISQDD-----DLPTL    | 131 |
| RiCTR3B        | YLLIISMNSQIFIVVVTTLTQLLIVEYVVISVYSTQLVPISQDD-----DLPTL    | 52  |
| ScCTR1         | LILATMSFVLTYVFAVITLALSEVFFNRKIAMLKRWD-----IQREIQKAKSPGPF  | 307 |
| ScCTR3         | IILLFYNGYIIISCLIAIVRFICYEPLGSLGANGSAQGTVSYDKESDDRKCSL--   | 241 |
| ScCTR2         | LILVFITYNGWMLAVVCAIWNYSWTSYSPEIDSSSLACH                   | 189 |
| RiCTR2         | LILVFITYNGFLMISVIIAATSYVFSSSV-IDFGGKSITCH                 | 170 |
| ** * . : : * . |                                                           |     |

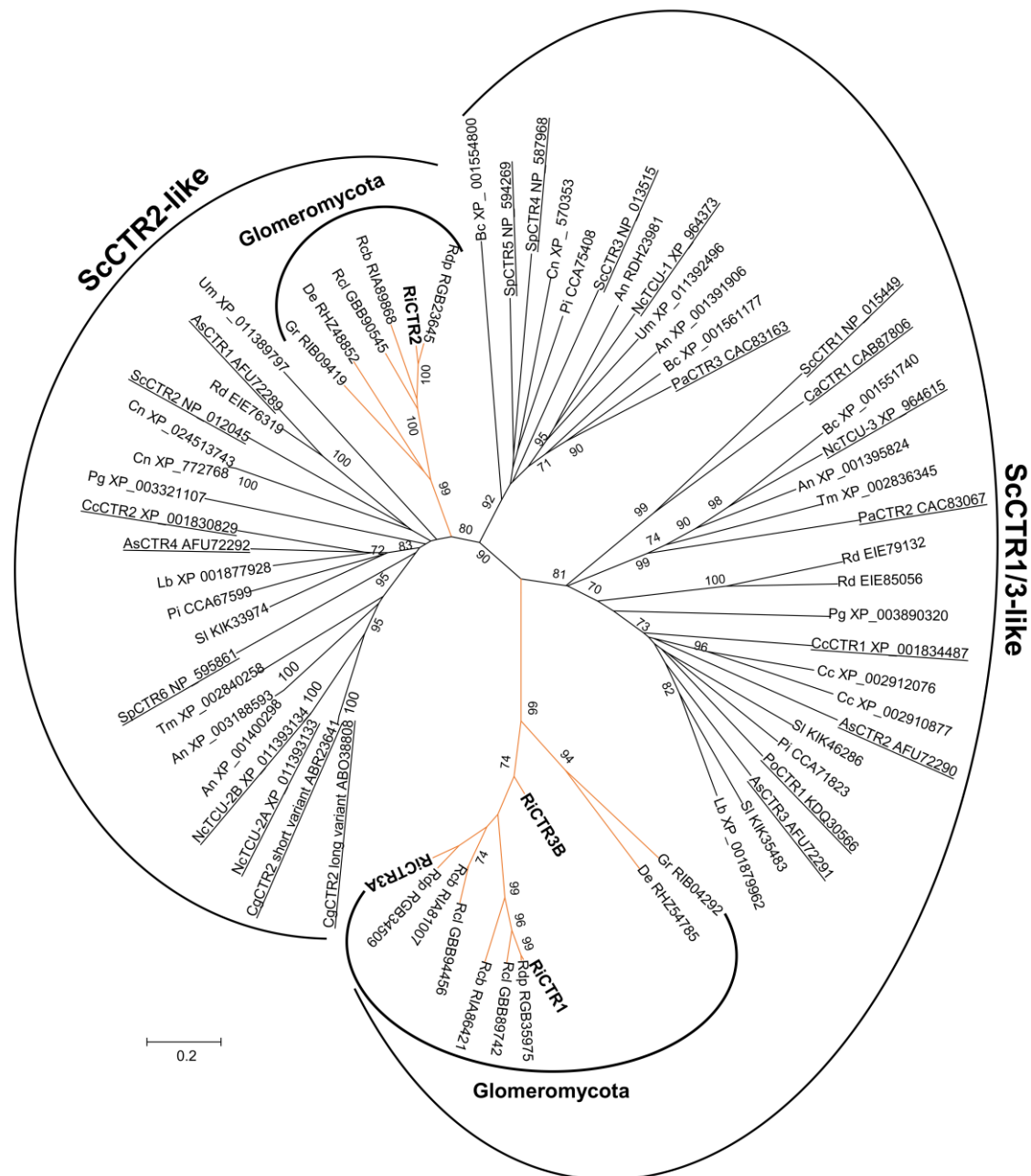
  

|         |                                                          |     |
|---------|----------------------------------------------------------|-----|
| RiCTR1  | NNNY-RDEIELESRNRFVNIENINNETKEE-----                      | 179 |
| RiCTR3A | ANKL-DDELELQPQSLIENVNSDDEYD-----                         | 158 |
| RiCTR3B | ANKL-DDELELQPQSLIENVNSDDEYD-----                         | 79  |
| ScCTR1  | GNCGGRPEPSPDPIAVADTTSGSDQSTREKNNESKVAISENNQKKTPTQEEGNCAT | 367 |
| ScCTR3  | -----                                                    | 241 |
| ScCTR2  | -----                                                    | 189 |
| RiCTR2  | -----                                                    | 170 |

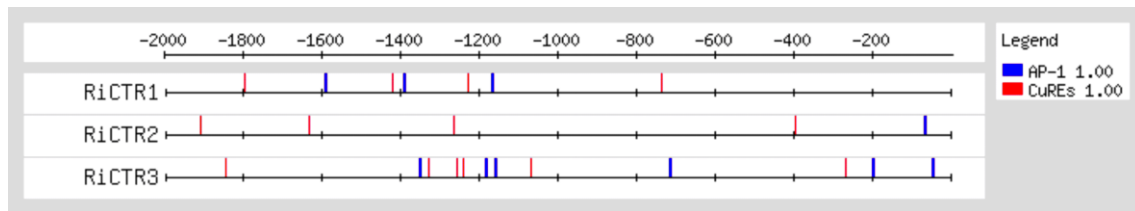
|         |                                         |     |
|---------|-----------------------------------------|-----|
| RiCTR1  | -----KRT-----                           | 182 |
| RiCTR3A | -----YQINDIVKYE---QOSLVNK-----          | 176 |
| RiCTR3B | -----YQINDIVKYE---QOSLVNK-----          | 97  |
| ScCTR1  | DSGKNQANIERDILENSKLQEQSGNMDQNLLPAEKFTIN | 406 |
| ScCTR3  | -----                                   | 241 |
| ScCTR2  | -----                                   | 189 |
| RiCTR2  | -----                                   | 170 |

**Supplementary Figure 2. Alignment of the deduced amino acid sequences of the *R. irregularis* CTRs with the amino acid sequences of the *S. cerevisiae* CTR proteins.** Conserved features of this family are highlighted in different colors. In blue: Met residues located in the N terminal region, in red the Met and Gly residues included in the MetXXXMet-X<sub>12</sub>-GlyXXXGly signature, in green: Cys and His residues located in the C terminus and in gray: predicted transmembrane domains (TM).



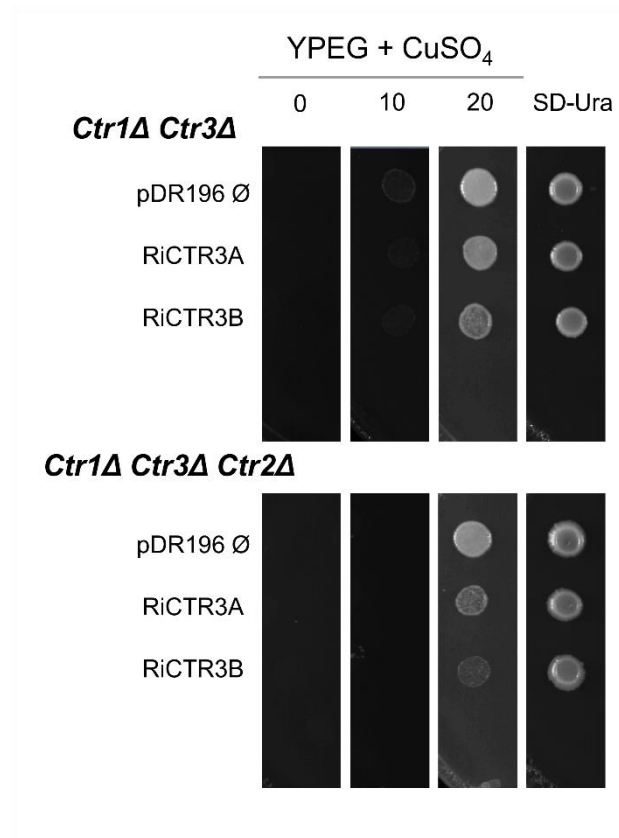
### Supplementary Figure 3. Phylogenetic relationships between fungal CTR proteins.

The unrooted Neighbor-joining tree was generated using MEGA v. 6 with 1,000 bootstrap replicates. *R. irregularis* CTR proteins are in bold. Functionally characterized CTRs are underlined. GenBank Accession numbers are provided. Organisms: As, *Amanita strobiliformis*; Bc, *Botrytis cinerea*; Ca, *Candida albicans*; Cc, *Coprinopsis cinerea*; Cg, *Colletotrichum gloeosporioides*; Cn, *Cryptococcus neoformans*; De, *Diversispora epigaea*; Gr, *Gigaspora rosea*; Lb, *Laccaria bicolor*; Nc, *Neurospora crassa*; Pa, *Podospira anserina*; Pi, *Piriformospora indica*; Pg, *Puccinia graminis*; Po, *Pleurotus ostreatus*; Sc, *Saccharomyces cerevisiae*; Sl, *Suillus luteus*; Sp, *Schizosaccharomyces pombe*; Tb, *Tuber melanosporum*; Rcb, *Rhizophagus cerebriforme*; Rcl, *Rhizophagus clarus*; Rdp, *Rhizophagus diaphanous*; Ri, *Rhizophagus irregularis*; Rd, *Rhizopus delemar*; Um, *Ustilago maydis*. Bootstrap values above 70 and supporting a node used to define a cluster are indicate.

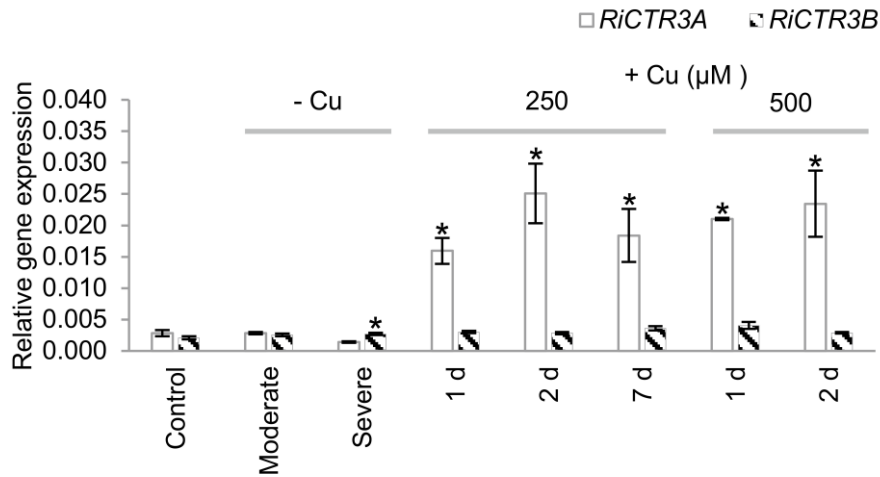


**Supplementary Figure 4. Putative *cis*-regulatory elements in the promoter region of *Rhizophagus irregularis* CTRs genes.** *Cis*-regulatory elements were screened in the *Saccharomyces cerevisiae* Promoter Database SCPD (<http://rulai.cshl.edu/SCPD/>) and specific Cu responsive elements (CuREs) and AP-1 sites were further screened through DNA pattern matching analyses in the fungal RSAT server without allowing any nucleotide substitution (<http://rsat-tagc.univ-mrs.fr/rsat/>).

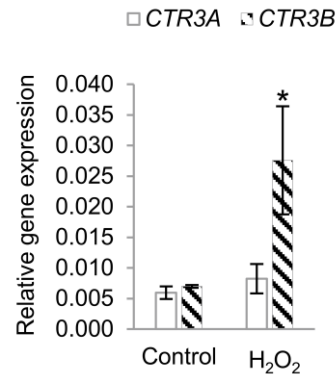




**Supplementary Figure 5. Analysis of functionality of RiCTR3 splicing variants in *Saccharomyces cerevisiae* CTR mutants.** *ctr1Δctr3Δ* and *ctr1Δctr2Δctr3Δ* yeast cells transformed with the empty vector or expressing *RiCTR3A* or *RiCTR3B* were plated on YPEG media supplemented with Cu (0, 10 or 20 μM CuSO<sub>4</sub>) or on SD medium without uracil. *ctr1Δctr3Δ* and *ctr1Δctr2Δctr3Δ* plated cells were incubated at 30 °C for 4 and 7 days, respectively.



**Supplementary Figure 6. Regulation of *RiCTR3A* and *RiCTR3B* expression by Cu availability.** *R. irregularis* ERM was grown in monoxenic cultures in M media containing 0.5  $\mu\text{M}$   $\text{CuSO}_4$  (control) or in M media lacking Cu in plates started with roots and inoculum previously grown either in M media containing 0.5  $\mu\text{M}$   $\text{CuSO}_4$  (moderate Cu deficiency) or in M media without Cu (severe Cu deficiency). For the Cu toxicity treatments, the ERM grown in optimal M media was exposed for 1, 2 and 7 days to 250  $\mu\text{M}$   $\text{CuSO}_4$  or for 1 and 2 days to 500  $\mu\text{M}$   $\text{CuSO}_4$ . Relative expression levels were calculated by the  $2^{-\Delta\text{CT}}$  method using *RiEF1 $\alpha$*  as a normalizer. Bars represent standard error. Asterisks show statistically significant differences ( $P < 0.05$ ;  $n = 3$ ) in comparison to the corresponding control value.



**Supplementary Figure 7. Regulation of *RiCTR3A* and *RiCTR3B* expression by oxidative stress.** *R. irregularis* ERM grown in monoxenic cultures in M-C medium was exposed or not (control) for 1h to 1 mM H<sub>2</sub>O<sub>2</sub>. Relative expression levels were calculated by the  $2^{-\Delta CT}$  method using *RiEF1 $\alpha$*  as a normalizer. Bars represent standard error. Asterisks show statistically significant differences ( $P < 0.05$ ;  $n = 3$ ) in comparison to the corresponding control value.