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1 **Combined omics approaches reveal distinct responses between light and heavy rare earth**
2 **elements in *Saccharomyces cerevisiae***

3
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26

27 **Highlights**

- 28 • First proteo-transcriptomics analysis revealing yeast molecular response to REEs
29 • Rare earth elements trigger specific responses
30 • Yeast cells react differentially to light vs heavy REEs
31 • Cell wall organization and pheromone signalling pathways are key yeast responses

32

33 **Keywords**

34 Lanthanides; REEs; lanthanum; ytterbium; proteomics; transcriptomics; cell wall

35 **Abstract**

36 The rapid development of green energy sources and new medical technologies contributes to the
37 increased exploitation of rare earth elements (REEs). They can be subdivided into light (LREEs)

38 and heavy (HREEs) REEs. Mining, industrial processing, and end-use practices of REEs has led
39 to elevated environmental concentrations and raises concerns about their toxicity to organisms and
40 their impact on ecosystems. REE toxicity has been reported, but its precise underlying molecular
41 effects have not been well described. Here, transcriptomic and proteomic approaches were
42 combined to decipher the molecular responses of the model organism *Saccharomyces cerevisiae*
43 to La (LREE) and Yb (HREE). Differences were observed between the early and late responses to
44 La and Yb. Several crucial pathways were modulated in response to both REEs, such as oxidative-
45 reduction processes, DNA replication, and carbohydrate metabolism. REE-specific responses
46 involving the cell wall and pheromone signalling pathways were identified, and these responses
47 have not been reported for other metals. REE exposure also modified the expression and abundance
48 of several ion transport systems, with strong discrepancies between La and Yb. These findings are
49 valuable for prioritizing key genes and proteins involved in La and Yb detoxification mechanisms
50 that deserve further characterization to better understand REE environmental and human health
51 toxicity.

52

53 **1. Introduction**

54 The rare earth element (REE) metal group consists of yttrium (Y), scandium (Sc), and 15
55 lanthanides, spanning from lanthanum (La) to lutetium (Lu). REEs can be divided into two different
56 groups known as light REEs (LREEs), which gather La, Ce, Pr, Nd, Pm, Sm, and Eu, and heavy
57 REEs (HREEs), which include Gd, Tb, Dy, Y, Ho, Er, Tm, Yb, and Lu (Cotton, 2006). These
58 elements display unique physical (magnetic) and chemical (catalytic) characteristics that make
59 them essential for advanced technologies (e.g., green energy production, communication, screens,
60 medicines, catalysts) (Ciacci et al., 2015). REEs are also used as fertilizers and food supplements
61 for cattle, pigs, and chickens (He et al., 2001; He and Rambeck, 2000; Hu et al., 2004; Panichev,
62 2015). The expanding exploitation of REEs owing to their increased use and low recyclability
63 (Binnemans et al., 2013; Ciacci et al., 2015) has generated concern regarding their subsequent
64 environmental and toxicological impacts. High REE concentrations are found in the vicinity of
65 REE mining sites and impact the surrounding environment, as well as human health (Lee and Wen,
66 2018; Noack et al., 2014; Wei et al., 2013). In this context, REEs have been categorized as
67 emerging contaminants (Gwenzi et al., 2018; Souza et al., 2021).

68 REEs were initially considered harmless and nonessential elements (Leeuw and Academic, 2000;
69 Setua et al., 2010). However, several recent studies demonstrated the ecotoxicological effect of
70 such elements on both aquatic and terrestrial organisms, including mammals (Gonzalez et al., 2014;
71 Pagano et al., 2015; Rim et al., 2013). REEs have been shown to display antibacterial, antifungal
72 and nematicidal activities (Wakabayashi et al., 2016). They also affect urchin embryogenesis
73 (Pagano et al., 2016; Trifuoggi et al., 2017) and human health (Pagano et al., 2015; Wei et al.,
74 2013). More specifically, it was shown that REEs induced oxidative stress through the production
75 of reactive oxygen species, lipid peroxidation, and the modulation of superoxide dismutase,
76 catalase, and glutathione peroxidase in plants, fish, rats and mice and in animal cell lines (including
77 human cells) (Babula et al., 2015; Gonzalez et al., 2014; Liang and Wang, 2013; Pagano et al.,
78 2015; Xu et al., 2016). Other effects have been observed, such as membrane permeability
79 alterations (El-Ramady, 2008; Gao et al., 2017; Técher et al., 2020; Weiwei et al., 2007; Yufeng et
80 al., 2007), mitotic and chromosomal aberrations (Pagano et al., 2015), and the overproduction of
81 exopolysaccharides (Fitriyanto et al., 2011) in microorganisms. However, these aspects of REE
82 toxicity were investigated in diverse organisms, making it difficult to draw comprehensive

83 conclusions. Moreover, the precise underlying molecular mechanisms are not well described, and
84 only a few studies have hypothesized that LREEs and HREEs might induce different cellular
85 responses as a result of lanthanide contraction (a decreasing ionic radius with an increasing atomic
86 mass) or due to their reactivity and chemical differences (Gonzalez et al., 2014; Wang et al., 2017).
87 For instance, plants that accumulate REEs display specific enrichment of either LREEs or HREEs
88 (Grosjean et al., 2020, 2019). Taken together, these few available studies support the need to
89 elucidate the cellular and molecular mechanisms involved in the response to REEs, similar to work
90 performed on the specific REE yttrium (Grosjean et al., 2018).

91 To decipher the cellular response to new stressors and pollutants such as REEs, combining different
92 and complementary “omics” analyses can offer many benefits (Taymaz-Nikerel et al., 2016). There
93 is a considerable amount of data regarding the exposure of yeast cells to various toxic metals
94 (Wysocki and Tamás, 2010), and large-scale omics methods are powerful approaches for
95 investigating metal stress in yeast (Hosiner et al., 2014; Jin et al., 2008; Taymaz-Nikerel et al.,
96 2016). Such analyses with model organisms are especially relevant as a first step to better
97 comprehend the functions involved in areas that have received little research attention. In our study,
98 we investigated the unicellular eukaryotic model *Saccharomyces cerevisiae*, which has been
99 remarkably informative and extensively used as a proxy for unravelling eukaryotic cellular
100 machinery and mechanisms (Botstein et al., 1997; Kachroo et al., 2017). We hypothesized that
101 REEs would impact the yeast physiology and that LREEs and HREEs would trigger different
102 responses. To test our hypotheses, we chose La and Yb as a representative LREE and HREE,
103 respectively. La was selected as it is the first element of the lanthanide series while Yb is almost
104 the last and La and Yb are among the most extracted and the least recycled LREEs and HREEs,
105 respectively (Ciacci et al., 2015). Additionally, we investigated whether REE-triggered responses
106 would be the same as for other well-known toxic metals.

107 This is the first study that combines an investigation of the transcriptome and proteome of *S.*
108 *cerevisiae* upon exposure to REEs. The transcriptional patterns were compared at two different
109 effective concentrations of two REEs, and time-dependent proteomic profiles were obtained to
110 provide the first insights into REE toxicity in this important eukaryotic model.

111

112 **2. Materials and methods**

113 **2.1. Yeast strain, chemicals and REE toxicity tests**

114 The wild-type strain BY4741 of *S. cerevisiae* (MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0) was
115 used. REEs in their trichloride form were purchased from Sigma-Aldrich (MO, USA). To
116 determine the EC₁₀ (the concentration at which 10% of the population is inhibited) and EC₅₀ (the
117 concentration at which half of the population is inhibited), log-phase cells (OD_{600nm} 0.15) were
118 exposed to increasing REE concentrations in modified YNB-β-glycerophosphate liquid medium
119 (YNB medium lacking KH₂PO₄, substituted by 1 mM β-glycerophosphate to avoid REE
120 precipitation, pH 5.8), and growth (OD_{600nm}) was recorded for 10 h at 28°C under constant shaking
121 (180 rpm). The growth ratio (OD at 9 h over OD at 4 h) was measured in log-phase and plotted
122 against the REE concentrations.

123

124 **2.2. Sample preparation for RNA-seq analysis**

125 The wild-type strain was grown in YNB-β-glycerophosphate liquid medium. Cultures were
126 inoculated at an OD_{600nm} of 0.05, and once the cultures reached an OD_{600nm} of 0.6, the cells were
127 exposed for 1 h to 50 or 160 μM lanthanum or to 6 or 8 μM ytterbium, corresponding to their EC₁₀
128 and EC₅₀, respectively. The cells were then harvested by centrifugation at 4000 rpm for 1 min at
129 room temperature and flash frozen. After cell disruption by bead beating, total RNA was column-

130 purified using an RNeasy mini kit (Qiagen, Les Ulis, France) according to the manufacturer's
131 instructions, and the gDNA was removed using DNase I (Sigma Aldrich). RNA integrity was
132 further assessed using a Bioanalyzer 2100 (Agilent, CA, USA). Strand-specific cDNA libraries
133 were prepared by Eurofins Genomics (Ebersberg, Germany) using the TruSeq-3 library kit.
134 Sequencing was carried out on an Illumina HiSeq 2500 with chemistry v4 applying the high-output
135 run mode and using 2x100 bp paired-end sequencing.

136
137 **2.3. Analysis of differentially expressed (DE) genes**
138 The data obtained were analysed using the online platform Galaxy (<https://usegalaxy.org/>). The
139 reads were first trimmed using the Trimmomatic v0.36.1 tool to remove any remaining Illumina
140 adaptor sequences and low-quality reads, and FastQC v0.67 was used to check read quality before
141 and after the Trimmomatic step. Sorted trimmed reads were mapped to the reference genome
142 (S288C R64-2-1 of 2015-01-31 from <https://www.yeastgenome.org/>) of *S. cerevisiae* using TopHat
143 v2.1.1. Reads that were mapped to the reference genome were quantified using HTSeq-count
144 v0.6.1p1. Finally, differential gene expression analysis between treatments was carried out using
145 DESeq2 v1.14.1. Genes that were differentially expressed among the conditions were identified
146 and expressed as the log₂-fold change, with adjusted p-values calculated via the Benjamini-
147 Hochberg test. A cut-off-adjusted p-value of < 0.01 was applied.

148
149 **2.4. Sample preparation for proteomics analysis**
150 The wild-type strain was grown as mentioned in section 2.1. Cultures were inoculated at an OD_{600nm}
151 of 0.05. Once the cultures reached an OD_{600nm} of 0.6, the REEs were added at the EC₅₀
152 concentration and exposed for 1 or 4 h. The cells were harvested by centrifugation at 4000 rpm at
153 room temperature for 1 min. The cell pellets were washed twice with 50 mM PBS pH 7 and flash
154 frozen. Proteins were extracted from the frozen cells by bead beating with a Precellys 24 instrument
155 (Bertin) after the addition of 1X LDS (Invitrogen) consisting of 106 mM Tris-HCl, 141 mM Tris
156 base, 2% (m/v) lithium dodecyl sulfate, 10% (m/v) glycerol, 0.51 mM EDTA, 0.22 mM SERVA
157 Blue G250, 0.175 mM phenol red, buffered at pH 8.5 and supplemented with 2.5% (v/v) β-
158 mercaptoethanol. The samples were heated for 5 min at 95°C, loaded (25 μL per well) onto a 4-
159 12% gradient 10-well NuPAGE denaturing gel (Invitrogen), and subjected to electrophoresis for 5
160 min as previously recommended (Hartmann et al., 2014). After rapid staining with Coomassie Blue
161 Safe (Invitrogen), polyacrylamide bands corresponding to the whole proteomes were cut out of the
162 gel, destained in ultrapure water, reduced with dithiothreitol, and treated with iodoacetamide before
163 performing proteolysis with Trypsin Gold Mass Spectrometry Grade protease (Promega) in the
164 presence of 0.01% (v/v) ProteaseMAX surfactant (Promega).

165
166 **2.5. Tandem mass spectrometry**
167 Tryptic peptides were subjected to tandem mass spectrometry with a Q-Exactive HF tandem mass
168 spectrometer (Thermo Fisher Scientific, Les Ulis, France) incorporating an ultrahigh field Orbitrap
169 analyser coupled in line to an Ultimate 3000 chromatography system (Thermo Fisher Scientific).
170 The tandem mass spectrometer was operated in the data-dependent acquisition mode as previously
171 described (Klein et al., 2016). Briefly, each sample (4 μL) was injected and desalted on a reverse-
172 phase capillary C18 PepMap™ 100 precolumn (LC-Packing) and then peptides were resolved
173 based on their hydrophobicity on a nanoscale 500-μm C18 PepMap 100 column (5 mm x 300 μm
174 i.d., Thermo Fisher Scientific) using a 90-min gradient from 2.5% to 40% of 80% CH₃CN, 19.9%
175 H₂O, and 0.1% formic acid. Once separated, mass spectra of the peptides were acquired in the full-
176 scan range of 350-1800 *m/z* at a resolution of 60,000 and an AGC target of 3.10⁶. Peptide ions with

177 charges of 2^+ or 3^+ were selected for fragmentation according to a Top20 method consisting of the
178 sequential analysis of each of the 20 most abundant peptide ions. MS/MS mass spectra were
179 acquired with an AGC target set at 10^4 , a loop count of 60 ms, an isolation window of 1.6 m/z , a
180 resolution of 15,000, a dynamic exclusion of 20 s, and a scan performed over a dynamic mass range
181 from the first mass detected up to 15 times the first mass.

182

183 **2.6. Peptide-to-spectrum assignment and protein identification**

184 Peak lists were automatically generated from raw files with an in-house script using the Proteome
185 Discoverer 1.4.1 (Thermo Fisher Scientific) daemon conversion function and the following
186 options: minimum mass (400), maximum mass (5,000), grouping tolerance (0), intermediate scans
187 (0), and threshold (1,000). MS/MS spectra from the resulting *.mgf files were assigned to *S.*
188 *cerevisiae* peptide sequences with Mascot v2.5.1 (Matrix Science) with the following parameters:
189 full trypsin specificity with up to two missed cleavages allowed, static modification of
190 carbamidomethylated cysteine, variable deamidation of asparagine and glutamine, variable
191 oxidation of methionine, mass tolerance of 5 ppm on parent ions and mass tolerance on MS/MS of
192 0.02 Da. Peptide matches with a Mascot peptide score below a p-value of 0.05 were parsed with
193 IRMA (Dupierris et al., 2009). Proteins were validated when at least two different peptides were
194 detected. The false discovery rate for protein identification was less than 1%, as estimated with the
195 reverse database decoy Mascot search option. Spectral counts corresponding to the number of
196 MS/MS spectra assigned per protein were extracted for each sample without applying the
197 parsimony rule to avoid isoform quantitation bias. The normalized spectral abundance factor
198 (NSAF) for each protein was calculated as the total spectral count divided by its molecular mass in
199 kDa and presented as a percentage. Spectral counts were compared between conditions using the
200 T-Fold method in PatternLab (Carvalho et al., 2012) after normalizing the spectral counts. The
201 statistical classes were defined as “Blue” for proteins that satisfied both $|T\text{-fold}| (\geq 1.5)$ and its
202 associated p-value (≤ 0.05), “Orange” for proteins with identifications that did not meet the T-fold
203 criterion (but should be further investigated because they had low p-values), “Green” for proteins
204 that satisfied the fold criteria but were likely false positives, and “Red” for proteins that did not
205 meet both fold and p-value criteria.

206

207 **2.7. GO term enrichment analysis**

208 Overrepresented gene ontology (GO) terms among differentially expressed genes (DEGs) and
209 proteins (DEPs) in each condition tested were obtained using the online Database for Annotation,
210 Visualization and Integrated Discovery (DAVID) tool (Huang et al., 2009a, 2009b). GO terms
211 significantly overrepresented for biological pathways, cellular compartment, and molecular
212 functions were selected with a set p-value cut-off of < 0.05 . Kyoto Encyclopaedia of Genes and
213 Genomes (KEGG) analysis was also performed on DEGs and DEPs using the online DAVID tool
214 with the same cut-off. DEG and DEP heatmaps were obtained using the R package pheatmap
215 v1.0.10 with a complete clustering method. Euclidian clustering distance measurement was
216 selected for rows and columns. Venn diagrams were drawn using the R package Venneuler v1.1-
217 0.

218

219 **2.8. Elemental analyses**

220 Yeast cultures were grown in YNB- β -glycerophosphate as previously mentioned. The cultures
221 were inoculated at an OD_{600nm} of 0.05, and once the cultures reached an OD_{600nm} of 0.6, lanthanum
222 and ytterbium were added at 50 μM and 6 μM , respectively. After 1 h of exposure to the REEs, the
223 yeast cells were harvested by centrifuging at 4000 rpm for 1 min at 4°C. The cell pellets were

224 washed three times with ice-cold MES (20 mM) – EDTA (10 mM) buffer (pH 6), three times with
225 ice-cold ultrapure water, and then dried for 48 h at 70°C. Triplicate samples were prepared.
226 Aliquots (125 mg) were mineralized with 1.75 mL of ultratrace HNO₃ and 0.5 mL of ultratrace
227 hydrogen peroxide in closed tubes placed in a block digestion system (DigiPREP, SCP Sciences,
228 Courtaboeuf, France). Gradual heating was used to achieve a final temperature of 100°C (total
229 heating time of 265 min). Ultrapure water was then added to a final volume of 12.5 mL. Blank and
230 reference materials were digested under the same conditions as the samples. Concentrations of Mn,
231 Mo and Zn were determined using inductively coupled plasma mass spectrometry (ICP-MS, X
232 Series II Model, Thermo Fischer Scientific, Courtaboeuf, France). Other elements were analysed
233 by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Radial ICAP6500 Model,
234 Thermo Fischer Scientific). The validity of the analytical methods was checked using certified
235 reference material (oriental basma tobacco leaves, INCT-OBTL-5, LGC Promochem, Molsheim,
236 France) (Table S1).

237

238 **2.9. Data availability**

239 The mass spectrometry and proteomics datasets are available through the ProteomeXchange
240 Consortium via the PRIDE partner repository (<https://www.ebi.ac.uk/pride/>) under the dataset
241 identifier PXD010700 and project DOI 10.6019/PXD010700. The RNA-seq datasets are available
242 in the NCBI GEO repository (GSE175826).

243

244 **3. Results and discussion**

245 **3.1. REE toxicity assessment**

246 To compare the cellular toxicity of La and Yb, the yeast EC₁₀ and EC₅₀ were first established for
247 the two elements. For this, *S. cerevisiae* was exposed to increasing concentrations of these two
248 REEs, and the cellular density was monitored every hour for nine hours (Fig. 1a,c). Different
249 concentration ranges were used for La and Yb because preliminary tests showed a higher toxicity
250 of Yb than La (data not shown). The growth ratio between 9 and 4 hours was then plotted against
251 the REE concentration to determine the EC₁₀ and EC₅₀ values (Fig. 1b,d). Yb was 20 times more
252 toxic than La, which is in line with observations using *Escherichia coli* (Técher et al., 2020). The
253 respective EC₁₀ and EC₅₀ values for La were calculated to be 50 µM and 160 µM and were 6 µM
254 and 8 µM for Yb (Fig. 1b,d).

255 We also investigated the effect of La and Yb exposure on the elemental composition of the cells
256 (Fig. 1e). We observed an increase in the Mn (18 to 50%), Fe (29 to 42%), Zn (13 to 18%) and Cu
257 (80 to 84%) concentrations under both La and Yb exposures. Under Yb exposure, the Ca (10-fold)
258 and Na (4-fold) concentrations increased, whereas their concentrations were less affected by
259 exposure to La (2-fold increase for Ca at La EC₁₀). The only decreased elemental concentration
260 observed was for K (-20%) under Yb exposure (Fig. 1e). The decrease in K was probably triggered
261 to counterbalance the increase in Ca and Na to maintain the cationic balance in the cells. Another
262 hypothesis is that REEs can induce plasma membrane permeabilization as observed in other
263 organisms (Ramos et al., 2016; Técher et al., 2020). Permeabilization of the plasma membrane
264 would allow the nonspecific influx of ions in the cells, explaining the observed increase of cellular
265 cation content following La and Yb exposure. In addition to these preliminary investigations of
266 REE-dependent ionome alterations and La- and Yb-triggered toxicity, the EC₁₀ and EC₅₀ were
267 chosen to further study the common and potentially specific yeast cell responses to these different
268 lanthanides through transcriptomic and proteomic analyses (Fig. 1f).

269 Prior to describing the cellular mechanisms involved in the response to the different REEs, we first
 270 compared the transcriptome of yeasts exposed to the EC₁₀ and EC₅₀ of the two REEs to test whether
 271 different responses would be observed at these two concentrations. The time-evolving proteome
 272 was also monitored and analysed.

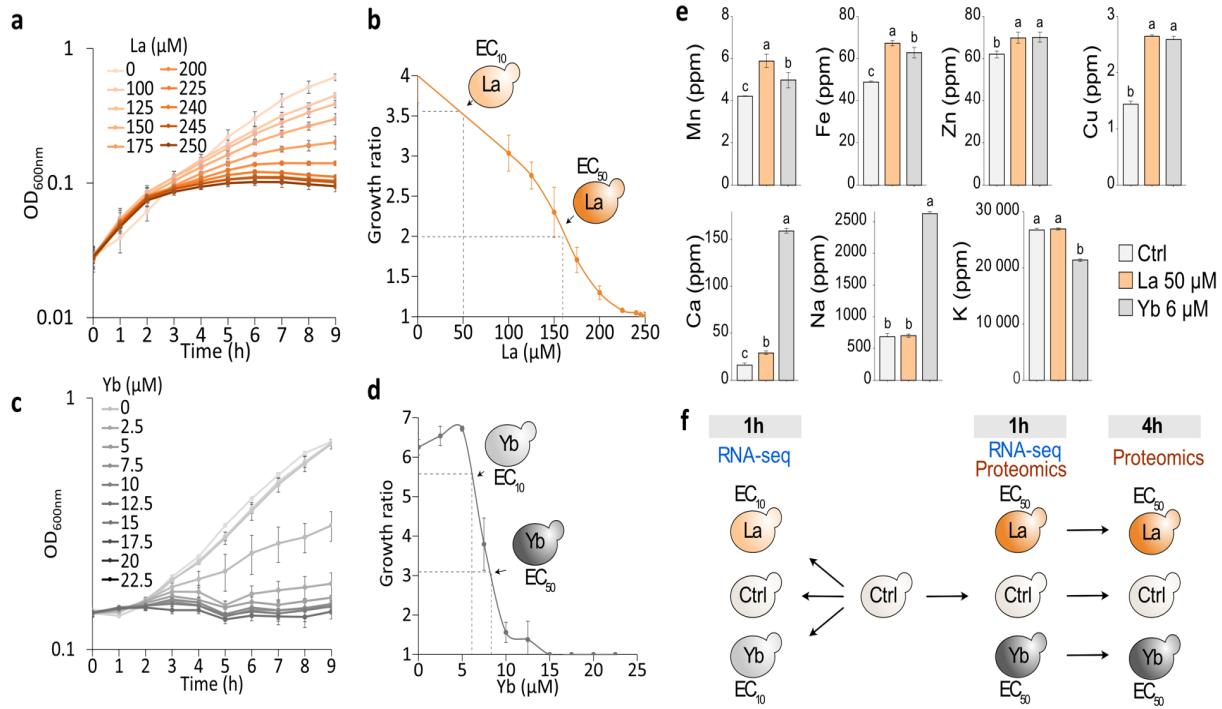


Figure 1. Impact of REEs on *Saccharomyces cerevisiae* growth and ionome and the omics experimental setup approach. (a) Growth of yeast exposed to different La concentrations. (b) Growth ratio (OD at 9 h over OD at 4 h) of yeast exposed to increasing amounts of La, enabling the identification of the EC₁₀ and EC₅₀, the concentrations that inhibited 10% and 50% of the growth, respectively. (c) Growth of yeast exposed to different Yb concentrations. (d) Growth ratio (OD at 9 h over OD at 4 h) of yeast exposed to increasing amounts of Yb, enabling the identification of the EC₁₀ and EC₅₀. (e) Ionome analysis of cells exposed to La at EC₁₀, Yb at EC₁₀ and La at the same concentration as that corresponding to Yb EC₁₀ (6 μM). Significant differences from the wild-type condition are indicated by different letters (ANOVA, Tukey HSD). Data are the means (±SD) of three independent cultures. (f) Experimental setup of yeast cultures used for RNA-seq and proteomic analyses. Cells were exposed to two concentrations of La and Yb (EC₁₀ and EC₅₀) for two exposure durations (1 h and 4 h). Three independent experiments were carried out for each condition.

273
 274 **3.2. Transcriptomic response to different effective concentrations (ECs) of La and Yb**
 275 We first aimed to identify the transcriptomic response of yeast cells to two different conditions of
 276 a high (EC₅₀) and a low (EC₁₀) toxicity concentration. The transcriptome of *S. cerevisiae* exposed
 277 for one hour to La and Yb at their respective EC₁₀ and EC₅₀ values was analysed (Fig. 1f, Fig. 2).
 278 Overall, we identified 1924 differentially expressed genes (DEGs) (applying a fold change (FC)
 279 cut-off of ≥ 1.5 and p < 0.01) in at least one condition (REE-stressed cells vs control condition).

280 The direct comparison of gene expression between EC₅₀ and EC₁₀ for the two REEs identified only
 281 a few DEGs (Fig. 2b). When exposed to La, 10 DEGs showed higher expression at EC₁₀ than at
 282 EC₅₀, while 14 DEGs were upregulated under EC₅₀ conditions (Fig. 2b, Table S2). A similar
 283 observation was made for Yb. Twenty-two DEGs were more abundant under the EC₁₀ condition
 284 than under the EC₅₀ condition, while this was the case for 13 DEGs under the EC₅₀ condition (Fig.
 285 2b, Table S2). We extended the analysis to the entire set of genes that were significantly
 286 differentially expressed relative to the control condition (p < 0.01). Again, a relatively high
 287 similarity of expression patterns existed between the two exposure concentrations, with only a few

288 differences in the transcriptomic profiles between the EC₁₀ and EC₅₀ conditions (Fig. 2a, Table S2).
 289 In line with our observations, few differences in DEGs between EC₁₀ and EC₅₀ exposure were
 290 obtained for other metals such as Zn, Cd, Cu, Cr and Ag (Jin et al., 2008). For instance, when
 291 exposed to these different metals, only 6 DEGs (Cd), 52 DEGs (Cr) and 69 DEGs (Hg) were found
 292 to be different between the EC₁₀ and EC₅₀ conditions.
 293 Given the small number of differences at the transcriptome level between the two tested
 294 concentrations and to optimize the number of changes in protein abundance, only the EC₅₀ for both
 295 La and Yb was used to analyse the time-evolving proteome.

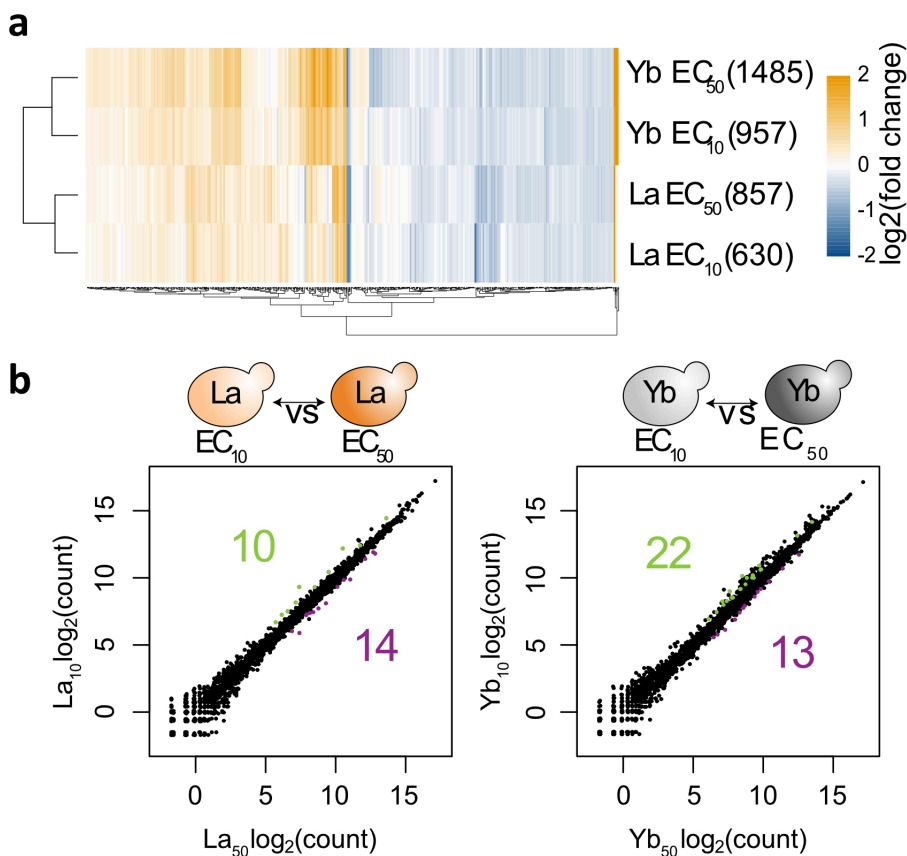


Figure 2. Comparative transcriptomic analyses of yeast exposed to La and Yb showed a similar pattern between the EC₁₀ and EC₅₀ treatments. (a) Heatmap of differentially expressed genes (DEGs) (blue, downregulated; orange, upregulated) under the two different REE concentrations (EC₁₀ and EC₅₀ for both La and Yb) in comparison to the control condition. The total number of DEGs (P ≤ 0.01) is specified in brackets. (b) Scatter plots of DE transcripts under La and Yb exposure. Significantly upregulated and downregulated transcripts (FC ≥ 1.5, P ≤ 0.01) are shown in green and purple, respectively.

296
 297 **3.3. Comparative proteomic analysis for two exposure times**
 298 Temporal response analysis was performed using a high-throughput shotgun proteomic approach
 299 to investigate differences in cellular responses to REEs after short (one hour, representing one
 300 generation) and long (four hours, representing 4 generations) exposure times (Fig. 1f). We
 301 leveraged label-free proteomic as a semi-quantitative method that quantify peptide peak areas,
 302 which allows the direct comparison between samples, unlike label-based methods (iTRAQ, TMT,
 303 SILAC). Combining every condition tested and all replicates, a total of 26,785 peptides were
 304 detected, as supported by the 685,981 MS/MS spectra. This analysis allowed the successful
 305 identification of 2,630 proteins, among which 2,015 were certified and quantified. Identified
 306 proteins with significant changes in abundance (FC ≥ 1.5 and P ≤ 0.05) upon REE exposure were

307 used for the analysis. Many more differentially abundant proteins (DAPs) were found at one hour
 308 than at four hours in both the La and Yb exposure conditions. As shown in Fig. 3a, 2.6 times more
 309 proteins were identified as differentially abundant in the one hour exposure compared to the 4 hour
 310 exposure, with total of 161 DAPs at one hour and 62 at four hours under La stress (Fig. 3a, Table
 311 S3). Similarly, under Yb stress, 226 and 76 DAPs were found after one and four hours (3 times
 312 more), respectively (Fig. 3b, Table S3). Only 10 (La) and 14 (Yb) DAPs were shared between the
 313 two exposure times (Fig. 3a-c). These results suggest the establishment of an intense and faster
 314 response during a short exposure time relative to durable acclimation after a long exposure time.
 315 Similar conclusions were drawn from the direct comparison of DAPs between the one- and four-
 316 hour exposure for both REEs. This was also found in a previous study in which *S. cerevisiae* was
 317 exposed to other metals (Ag, Cd, Hg, Zn) and metalloids (As) and the transcriptomic response
 318 under acute stress (30 min) and sustained stress (2 hours) was compared (Hosiner et al., 2014).
 319 Under exposure to these metals, only 2.4% to 7.9% of the total DEGs were shared between the
 320 acute and sustained stress (Hosiner et al., 2014). These results further support a time-course
 321 adaptation process in yeast cells to cope with toxic metals.

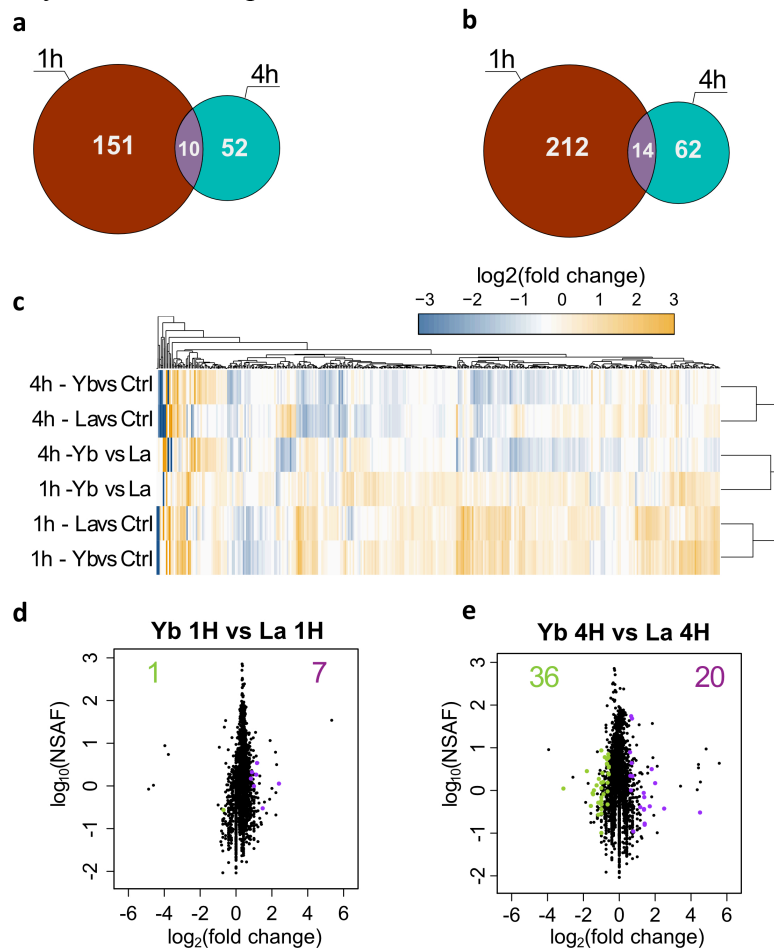


Figure 3. Comparative proteomic analyses of *S. cerevisiae* exposed to La and Yb demonstrate a differential response over time. (a) Venn diagrams of differentially expressed proteins under La exposure vs control conditions after 1 h and 4 h. (b) Same as (a) but for Yb. (c) Heatmap of differentially expressed proteins (blue, downregulated; orange, upregulated) under the two different exposure times (1 h and 4 h). (d) Scatter plot of differentially abundant proteins between Yb and La after 1 hour of exposure. Significantly more abundant proteins under La exposure are shown in green, and those more abundant under Yb are in purple (FC \geq 1.5, $P \leq$ 0.05). (e) Same as (d) but after 4 hours. NSAF = normalized spectral abundance factor.

3.4. Combining transcriptomic and proteomic analyses

The proteome analysis of cells exposed for 1 hour to La or Yb allowed the identification of essential genes and the related pathways that cells first activate upon exposure. There was a relatively low number of downregulated proteins under both conditions, with 12 DAPs for La and 7 DAPs for Yb ($FC \geq 1.5$ and $p \leq 0.05$), and these were mainly shared (Table S3, Fig. 3d). Furthermore, these proteins were all involved in ribosome biogenesis (La) and rRNA processing (La) (Tables S4-S6). For all DAPs with a p -value ≤ 0.05 , there was high representation of the NOP (nucleolar protein), RPL (ribosomal protein of the large subunit) and RPS (ribosomal protein of the small subunit) family proteins (Table S3). These data further support the downregulation of ribosome biogenesis and consequently less protein synthesis under both La and Yb stress. A reduction in ribosome abundance was reported under different metallic stresses as being a rapid, effective, and reversible stress response to prevent misfolded proteins (Guerra-Moreno et al., 2015; Hosiner et al., 2014; Pimentel et al., 2012; Taymaz-Nikerel et al., 2016; White et al., 1990). Guerra-Moreno et al. (2015) suggested that when cellular ribosome levels are reduced, newly synthesized misfolded proteins may be limited. Lower levels of misfolded proteins would enable proteolysis to more effectively eliminate misfolded proteins (Guerra-Moreno et al., 2015).

In line with this observation, and when considering proteins with increased abundance when exposed to REEs, most of the GO terms represented in the two data sets were proteolysis-related (Tables S4 and S5). In the Yb treatment, an increased abundance of transcripts and proteins belonging to ubiquitin-dependent protein catabolism was also found (Table S7). The short-term exposure of yeast cells to toxic ions appears to trigger a rapid detoxification process by degrading proteins that have been damaged by metal stress. Proteolysis induction is a global cellular response to metallic stress and has been observed at toxic concentrations of Al, Ag, Cd, Mn, Ni, Zn, Hg, and As (Guerra-Moreno et al., 2015; Hosiner et al., 2014; Jin et al., 2008). Additionally, under both treatments, proteins involved in carbohydrate metabolism were more abundant (Tables S4 and S5). Jin et al. (2008) grouped metal-induced biological processes under the term ‘common metal responsive’ genes (Jin et al., 2008). It appears that in response to acute stress (i.e., after one hour of exposure), yeast cells preferentially alleviate toxic effects triggered by the accumulated REEs and then establish adaptation responses to sustained stress (Hosiner et al., 2014).

When comparing the transcriptomic and proteomic profiles at one and four hours for both REEs, the highest correlations were observed between transcriptomic profiles obtained after one hour and proteomic profiles obtained after four hours (Fig. 4a,b, Table S8). Strikingly, there was hardly any overlap between differentially expressed mRNA and the corresponding differentially expressed proteins (Fig. 4c,d). For instance, under La exposure, the expression of 21 of the 30 (70%) more abundant proteins and 28 of the 32 less abundant proteins was not altered for the cognate mRNAs (Fig. 4c). The same observation was found for Yb exposure, with as high as 50% of the proteins with increased abundance and 100% of those with decreased abundance for which the corresponding mRNA was not differentially expressed (Fig. 4d). These observations could be explained by the relatively low number of DAPs ($FC \geq 1.5$ and $P \leq 0.05$) in comparison with the number of DEGs ($FC \geq 1.5$ and $P \leq 0.01$). A fourfold higher number of mRNAs than proteins were differentially expressed under La exposure, while it was 6.4-fold greater under Yb exposure (Fig. 4c,d).

Interestingly, several DAPs and DEGs were shared between the Yb and La treatments, but some were specific to one or the other (Fig. 3e). We thus analysed each data set for the enriched gene ontology (GO terms) to identify modulated pathways and functions in response to either La or Yb (Fig. 5a-d, Tables S9-S13).

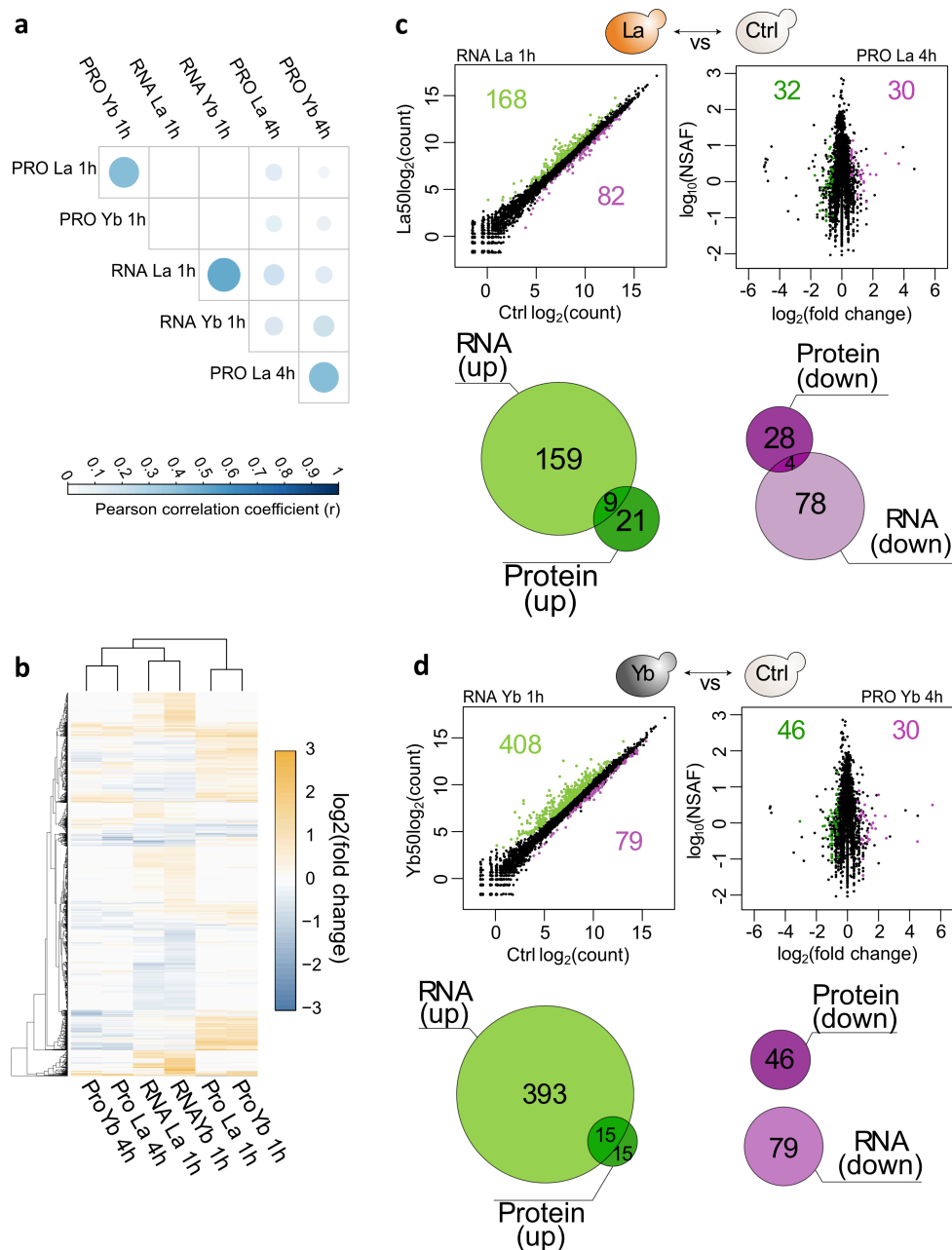


Figure 4. Comparative transcriptomic and proteomic analyses of yeast exposed to REEs at the EC₅₀. (a) Correlation matrix (Pearson's correlation coefficient r) of transcriptomic and proteomic data sets. (b) Heatmap of differentially abundant (DA) proteins (Pro) and differentially expressed (DE) transcripts (RNA) at the different exposure times (1 h and 4 h, as indicated) under La or Yb stress (blue, downregulated; orange, upregulated). (c) Scatter plots and Venn diagrams of DA proteins (after 4 h) and mRNA (after 1 h) under La exposure vs control conditions. Significantly upregulated and downregulated proteins ($FC \geq 1.5$, $P \leq 0.05$) or transcripts ($FC \geq 1.5$, $P \leq 0.01$) are shown in green and purple, respectively. (d) Same legend as in (c) under Yb exposure. NSAF = normalized spectral abundance factor.

370
 371 **3.5. The cell wall as the first barrier to REEs**
 372 Regarding the response to La at the transcriptomic level, upregulated genes were mainly associated
 373 with cell wall biogenesis and organization. These functions were conspicuously represented by
 374 four GO terms and associated with up to 31 different genes (Fig. 5a, Table S12). Accordingly,
 375 fungal-type cell wall organization was also enriched in the differentially expressed proteins (Fig.
 376 5b, Fig. 4c, Table S9). This finding was supported by the enriched GO cellular compartments
 377 (Table S12). Compared to La, Yb exposure upregulated 28 genes involved in fungal-type cell wall

378 organization and cell wall organization (Fig. 5c, Fig. 4c, Table S7). Moreover, these two functions
 379 were also found in the proteomic analysis under Yb stress. This was reinforced by the upregulation
 380 of the chitin cell wall metabolic process and the 1,3- β -D-glucan biosynthesis (Fig. 5d, Table S10).
 381 Mid2p acts as a sensor for cell wall integrity signalling and activates this pathway. Downstream of
 382 the Mid2p signalling pathway, Slt2p, a serine/threonine MAP kinase involved in regulating the
 383 maintenance of cell wall integrity, was also upregulated (Fig. 6a). *GSC2* (*FKS2*) is known to be
 384 triggered by cell wall stress and encodes a catalytic subunit of 1,3- β -glucan synthase (Inoue et al.,
 385 1995), and it was specifically upregulated under Yb stress. *CWP1* and *NCW2* were upregulated
 386 under La and Yb exposure and were identified in the transcriptomic and proteomic analyses (Fig.
 387 6a). Cwp1p is a cell wall mannoprotein linked to a β -1,3- and β -1,6-glucan heteropolymer, while
 388 Ncw2p is thought to aid the organization of the β -1,3-glucan structure of the cell wall. These two
 389 proteins are needed for the proper organization of cell wall constituents (Lesage and Bussey, 2006;
 390 Levin, 2005).

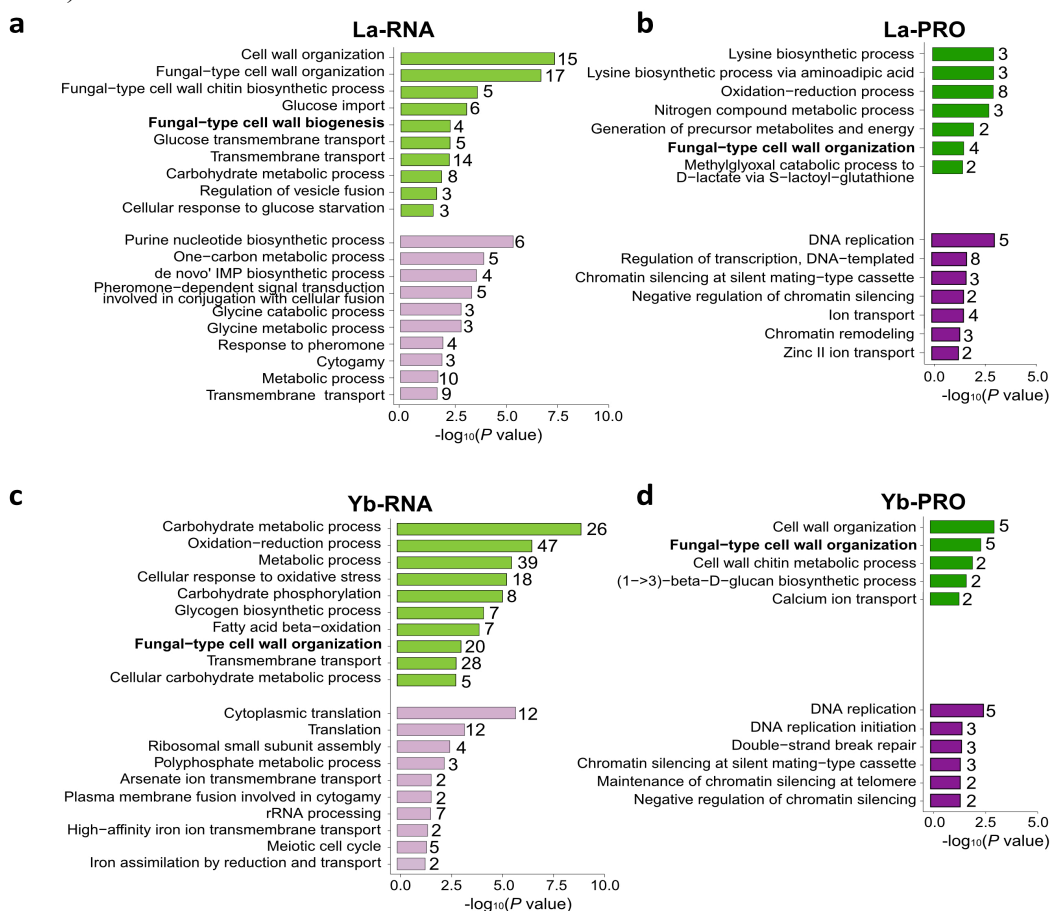


Figure 5. Biological pathway (BP) GO terms overrepresented in the transcriptomic and proteomic analyses of yeast exposed to REEs at the EC₅₀. (a) BP GO terms of DE transcripts under La exposure. (b) BP GO terms of DE proteins under La exposure. (c) BP GO terms of DE transcripts under Yb exposure. (d) BP GO terms of DA proteins under Yb exposure. GO terms significantly enriched in upregulated (green) and downregulated (purple) proteins ($P \leq 0.05$) and transcripts ($P \leq 0.01$). GO terms found in both RNA-seq and proteomic analyses are in bold. Numbers indicate the number of proteins or mRNAs of the specified BP found in the data sets.

391
 392 The overrepresentation of these cell wall-related biological pathways (BPs) supports the role of
 393 this compartment in the first barrier yeast have against REEs. It also provides additional clues about
 394 how the different REEs interact with the cell wall (Fig. 7). Ce (Jiang et al., 2010) and Yb (Jiang et

395 al., 2012) were shown to adsorb to the cell wall of *S. cerevisiae*, *Bacillus subtilis*, and *E. coli*, with
 396 affinity differences between LREEs and HREEs (Takahashi et al., 2010).

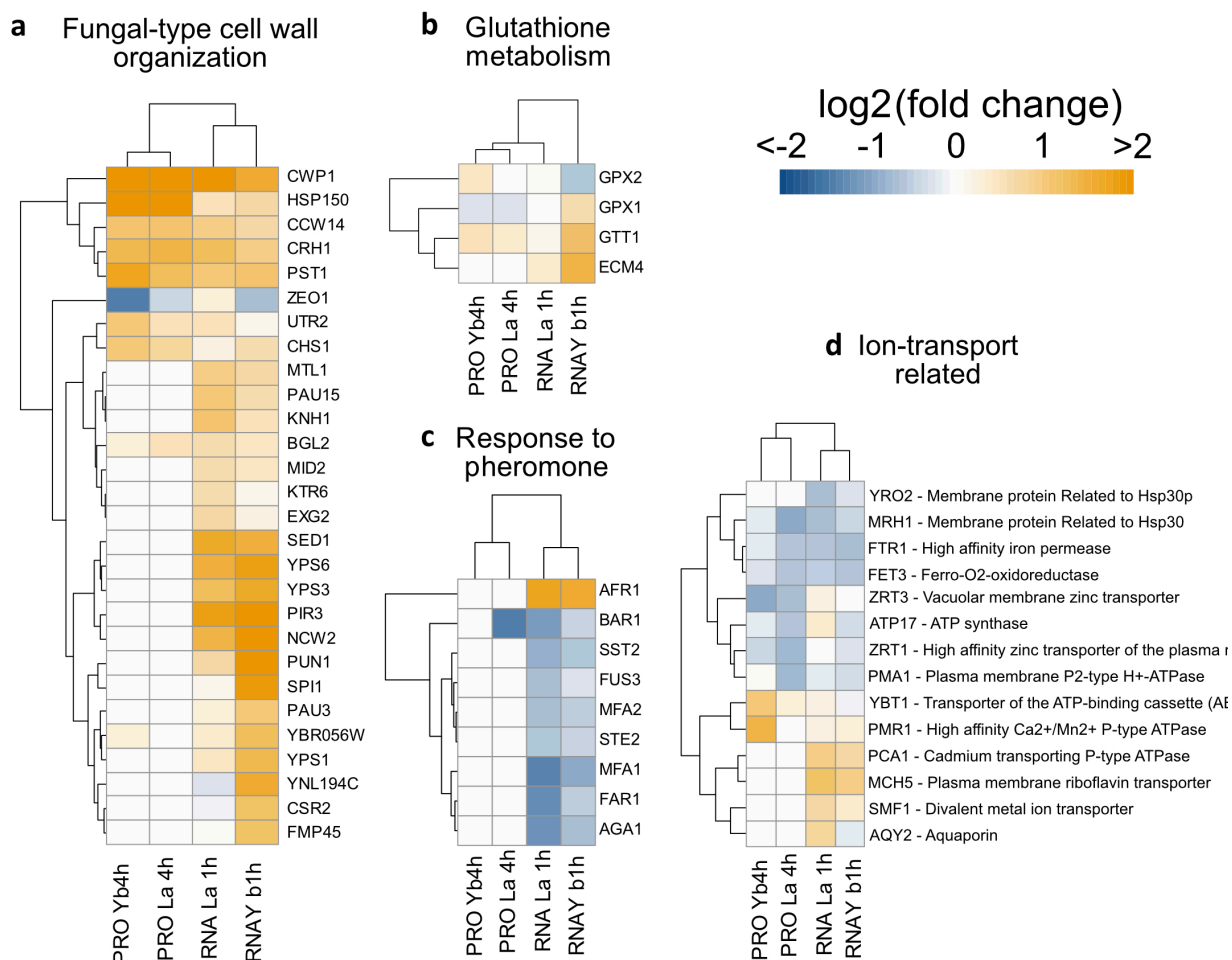


Figure 6. Expression heatmaps of GO terms enriched in proteomic and transcriptomic analyses for both REEs. (a) Fungal-type cell wall organization, (b) Glutathione metabolism, (c) Response to pheromones, (d) Ion transport-related GO terms ($p < 0.05$). The genes and proteins shown were all significantly differentially expressed.

397 Surface complexation modelling showed that LREEs preferentially associated with phosphate
 398 groups, while HREEs associated equally well with carboxyl and phosphate groups. Potentiometric
 399 titration and FTIR spectroscopic analyses of *S. cerevisiae* showed a higher representation of
 400 phosphate groups (68%) on the cell surface, while amino/hydroxyl (16%) and carboxyl groups
 401 (16%) were less represented (Rogowska et al., 2018). The surface of the yeast cell wall, which
 402 bears such groups, has been modelled to bind Ca ions, which could potentially be broadened to
 403 REEs given their similarities (Rogowska et al., 2018). The higher representation of phosphate
 404 groups on the surface and the high affinity of LREEs for these groups could explain the relatively
 405 lower toxicity observed for La compared to Yb (Fig. 1). Thus, the upregulation of cell wall-related
 406 genes appears to be essential in response to REE exposure and could allow the recycling or
 407 selective remodelling of the yeast cell wall to cope with these elements. Modifications of the
 408 exposed groups on the surface could ensue, with possible consequences for REE binding. Taken
 409 together, these results support the involvement of the cell wall as a primary way in which REEs
 410 interact with yeast cells and indicates that there are differences in La- and Yb-triggered effects.
 411

412 **3.6. Involvement of the pheromone signalling pathway**

413 An investigation of the BPs related to sensing the extracellular environment indicated the
414 involvement of pheromone-related GO biological pathways. Indeed, two enriched GO biological
415 pathways were downregulated under La stress, and both were involved in pheromone signalling
416 pheromone-dependent signal transduction (involved with cellular fusion) and the response to the
417 pheromone pathway (Fig. 5a, Fig. 6c, Table S12). Although these GO terms were specifically
418 enriched under La stress, genes belonging to these pathways were also significantly downregulated
419 under Yb stress, but to a lesser extent (Fig. 6c, Table S2). The GO terms included *MFA1* and *MFA2*,
420 the two genes encoding mating pheromone a. The alpha-factor pheromone receptor gene *STE2* and
421 its associated heterotrimeric G protein *SST2* were also downregulated.

422 Two genes downstream of the regulation cascade triggered by *STE2* were also downregulated:
423 *FAR1* and *FUS3*. *FAR1* is phosphorylated by *FUS3* (a mitogen-activated serine/threonine protein
424 kinase involved in mating) in response to mating pheromone. Several other genes modulated by
425 the pheromone response pathways were also downregulated (Fig. 6c). Prm1p is a pheromone-
426 regulated multispanning membrane protein that is involved in membrane fusion during mating, and
427 Bar1p is an aspartyl protease secreted into the periplasmic space of mating type a cells (Mackay et
428 al., 1988). The latter cleaves and inactivates alpha factor, allowing cells to recover from alpha-
429 factor-induced cell cycle arrest.

430 There is a possible link between the pheromone response pathway and the REE-triggered response.
431 It is known that some REEs are Ca analogues, particularly La (Fricker and Fricker, 2006) and can
432 be absorbed through the Ca influx system Cch1-Mid1 in yeast cells (Ene et al., 2015). Ca influx
433 occurs during the mating process and is initiated by mating pheromone detection (Carbó et al.,
434 2016; Cunningham, 2011). The response to mating pheromones notably includes increased
435 expression of the high-affinity Ca channels Cch1p-Mid1p and Ecm7p and increased expression of
436 the putative low-affinity Ca channels (Fig. 1p) (Cunningham, 2011; Muller et al., 2003). Thus,
437 considering that REEs can pass through Ca channels, the downregulation of pathways involved in
438 the transcription of these channels would reduce the amount of REEs in the cytoplasm. However,
439 such a hypothesis requires further investigation of whether REE accumulation modifies pheromone
440 metabolism. In response to pheromones, yeast cells modify their cell wall to allow fusion with cells
441 of the other mating type, but they also induce cell cycle arrest (Chen and Thorner, 2007), thereby
442 leading to a more susceptible cell wall. Therefore, in line with previous observations, the
443 downregulation of genes and proteins involved in the pheromone-dependent signal transduction
444 pathway could reduce cell cycle arrest and cell wall alteration processes, allowing cells to better
445 cope with La and Yb toxicity (Fig. 7).

446 **3.7. Lanthanum and ytterbium impact amino acid, carbohydrate, and DNA metabolism**

447 Under La stress, several metabolic and biosynthetic processes were altered at the transcriptomic
448 and proteomic levels (Fig. 5a, Table S12). The biosynthesis of purine nucleotides, through de novo
449 IMP (Fig. 5a) and AMP biosynthetic processes was significantly downregulated. Additionally
450 downregulated was the one-carbon metabolic process, which is related to glycine metabolism (Fig.
451 5a, Table S12). Conversely, the proteomic analysis demonstrated that lysine biosynthesis was
452 upregulated (10%) under La stress (Fig. 5b, Table S9). Interestingly, other than three upregulated
453 genes involved in proline transmembrane transport, no other amino acid-related GO terms were
454 identified under Yb stress (Fig. 5c, d, Table S7).

455 A relatively high number of upregulated genes were associated with carbohydrate metabolism
456 under both treatments, with eight upregulated genes under La stress and 26 under Yb stress (Fig.
457 5a,c). Many carbohydrate-related GO terms were enriched in the Yb condition, highlighting the
458

459 involvement of this pathway in the response to Yb. However, the induction of carbohydrate
460 metabolism was reported to be a common metal-responsive pathway and was also found when
461 yeast cells were exposed to several other toxic metals (Jin et al., 2008).

462 The two analytical methods identified *INO1* and its encoded protein Ino1p as the most upregulated
463 representatives under both La and Yb exposure (Table S8). The inositol-3-phosphate synthase
464 Ino1p is involved in the synthesis of inositol phosphates and inositol-containing phospholipids.
465 Under phosphate limitation conditions, genes implicated in inositol phosphate uptake and
466 metabolism, phosphate metabolism, and metabolite phosphorylation were induced (Taymaz-
467 Nikerel et al., 2016). It has recently been shown that inositol-3-phosphates are involved in the
468 localization of metal uptake transporters in *Arabidopsis thaliana* (Agorio et al., 2017). Thus, an
469 increase in inositol-3-phosphate through the upregulation of *INO1* could be important for
470 membrane transporter localization in response to REEs. Another explanation would be that
471 increased inositol phosphates help to chelate REEs, thus decreasing their toxicity and reactivity.
472 Indeed, all inositol phosphates are strong chelators of metal ions such as Ca, Mg, Zn, Cu, Fe, Mn
473 and Al (Chastukhina and Sharipova, 2014; Oh et al., 2006).

474 Finally, aside from the ion transport-related GO terms for La, almost all downregulated proteomic
475 pathways for both REEs were related to DNA replication and regulation (Fig. 5b,d, Tables S9,
476 S10). Proteins involved in DNA replication were downregulated under both La and Yb exposure,
477 and proteins involved in transcription regulation were downregulated under La exposure. Yb stress
478 also reduced translation, RNA processing and ribosome small subunit assembly at the
479 transcriptomic level (Fig. 5c). It has been strongly suggested that DNA replication is a target for
480 Cd toxicity (Serero et al., 2008). The downregulation of DNA replication by La and Yb could be
481 evidence of toxicity targets similar to Cd.

482

483 **3.8. Ytterbium triggers oxidative stress response**

484 In Yb-exposed yeast, the most represented process was the GO term oxidation-reduction (47 genes)
485 (Fig. 5c Table S7). In addition, 18 genes involved with the cellular response to oxidative stress
486 were also upregulated, suggesting that Yb triggers oxidative stress and NADPH regeneration (Fig.
487 5c, Table S7). While only seen with the Yb stress in our study, it has been extensively shown that
488 other REEs can also generate oxidative stress (Gonzalez et al., 2014; Liang and Wang, 2013;
489 Pagano et al., 2015). Several genes involved in glutathione (GSH) homeostasis were upregulated
490 specifically under Yb stress. GSH can act at three different levels. First, GSH can chelate toxic
491 metals to form a complex that can subsequently be sequestered into the vacuole. GSH can also act
492 as a protective agent against metal-induced oxidation (Jozefczak et al., 2012). Finally, protein
493 glutathionylation can occur, resulting in a protective role against irreversible metal binding and/or
494 oxidative damage to proteins (Deponce, 2017; Wysocki and Tamás, 2010).

495 Among the upregulated genes involved in GSH homeostasis under Yb stress, we identified *GTT1*
496 (ER) and *ECM4* (*GTO2*, cytoplasm), and, to a lesser extent, *GRX1* (cytosol) and *GRX2* (cytosol
497 and mitochondria) (Fig. 6b, Table S2). Gtt1p and Ecm4p are glutathione-S-transferases (GSTs),
498 while Grx1p and Grx2p are glutaredoxins that can fill the same role as GSTs (Morano et al., 2012).
499 GSTs catalyse the conjugation of GSH with a substrate (e.g., Cd) or the isomerization of GSH to
500 form oxidized glutathione (Daniela et al., 2009). Furthermore, the upregulation of the gene
501 encoding the gamma-glutamyltranspeptidase *ECM38* (Table S2), a major glutathione-degrading
502 enzyme localized into the vacuole, suggests the translocation of oxidized glutathione into the
503 vacuole, with a subsequent need for recycling (Cordente et al., 2015). Thus, along with ROS
504 production inducing oxidative stress, Yb could be internalized through GSH-Yb complexes via the
505 vacuole (Fig. 7). The ABC transporter Ybf1p, which was more abundant under Yb exposure, might

506 be involved in this process (Fig. 6d). This latter hypothesis requires further investigation to verify
 507 the involvement of glutathione in Yb detoxification.

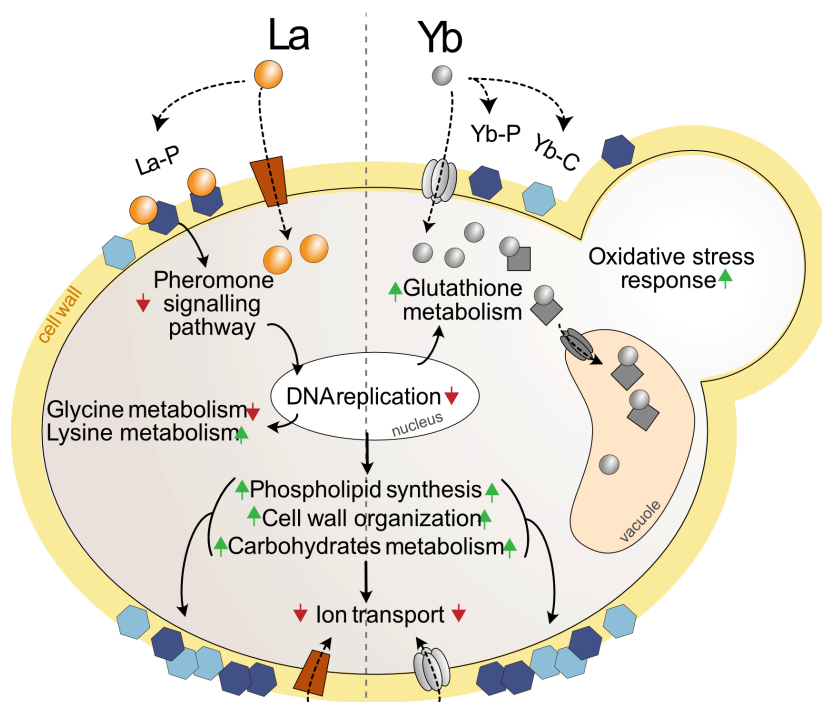


Figure 7. Schematic model of detoxification responses in yeast under acute REE stress. Upregulated functions are indicated by a green arrow, while downregulated functions are indicated by a red arrow. La-P and Yb-P correspond to phosphate-bound REEs, while Yb-C corresponds to carboxylate-bound Yb. Grey squares represent putative ligands that bind HREEs. Dashed arrows indicate REE movement, and plain arrows indicate response mechanisms.

508
 509 **3.9. Lanthanum and ytterbium affect plasma membrane transport**
 510 Reducing the import of toxic ions into the cell is another mechanism by which cells can adapt to
 511 xenobiotic compounds. Of the downregulated genes under Yb and La exposure, several were linked
 512 to plasma membrane ion transport (Fig. 7). Under Yb exposure, three transport-related GO terms
 513 were enriched: arsenate ion transmembrane transport, high-affinity Fe ion transmembrane transport
 514 and Fe assimilation by reduction and transport (Fig. 5c, Table S7). Under La exposure, four
 515 downregulated proteins were involved in ion transport, and two of these were related to Zn ion
 516 transport (Fig. 5b, Table S12). Regarding ion transport GO terms, the two genes encoding the two
 517 components of the high-affinity Fe transport system Fet3p-Ftr1p were downregulated under both
 518 La and Yb exposure (Fig. 6d). A similar finding was identified at the protein level. Fet3p is a
 519 multicopper oxidase that oxidizes ferrous Fe to ferric Fe for subsequent cellular uptake by the
 520 transmembrane permease Ftr1p (Stearman et al., 1996). Other ion transport-related genes and
 521 proteins also had modified expression, as evidenced by transcriptomic or proteomic analyses (Fig.
 522 6d). Along with Fet3-Ftr1, the plasma membrane high-affinity Zn transporter Zrt1p was also
 523 downregulated in the presence of both REEs (Fig. 6d). Also downregulated was the Zn transporter
 524 Zrt3p, which localizes to the vacuolar membrane (Fig. 6d, Table S8). Zrt3p transports Zn ions from
 525 vacuolar storage to the cytoplasm. Conversely, some genes encoding transmembrane ion
 526 transporters were upregulated. Among them were *PCAI*, encoding a cadmium efflux transporter,
 527 and *SMF1*, encoding a divalent metal transporter. A closer investigation of the roles played by
 528 these different metal transport systems would help to elucidate novel REE uptake pathways in yeast
 529 cells. Furthermore, the modulated expression and abundance of these nutrient transporters could

530 be the result of REEs indirectly impacting the overall nutrient balance in cells. Interestingly, we
531 observed an overall increase of the cell content of several cations (Fig. 1e) that might be a result of
532 a partial membrane permeabilization by REEs (Ramos et al., 2016; Técher et al., 2020), triggering
533 a stress response illustrated by the higher abundance of Hog1p, and higher expression of *ENAI*
534 encoding a Na efflux system (Haro et al., 1991) in response to Yb (Tables S2-S3). The Hog1p
535 MAPK pathway is involved in the response to high osmolarity and has been hypothesized to take
536 part in the tolerance to As and other metals (Hosiner et al., 2014). However, this Hog1p mediated
537 stress response is likely due to the plasma membrane permeability induced by REEs rather than an
538 hyperosmolarity given the very low REE concentration used. In response to this uncontrolled ion
539 influx, metal-homeostasis mechanisms would be triggered to reduce metal uptake by inhibiting
540 metal transporters expression and translation. Additionally, high concentration of toxic metals such
541 as Cd downregulate the expression of the zinc transporter Zrt1p to prevent uptake of toxic Cd
542 (Gitan et al., 1998; Wysocki and Tamás, 2010). The high concentrations of REEs could also
543 contribute to this deregulation of membrane influx systems. The altered Fe and Zn homeostasis
544 under La or Yb stresses could rapidly be alleviated by downregulating the corresponding uptake
545 systems (Fet3p-Ftr1p, Zrt1p and Zrt3p) to reduce Fe and Zn loading into the cytosol.
546

547 **4. Conclusion**

548 Here, we report the first cellular and molecular insights into the REE toxicity response in the
549 eukaryotic model organism *S. cerevisiae*. The use of complementary methods at different levels of
550 regulation (mRNA and protein) has furthered our understanding of the potential issues related to
551 these emerging metal contaminants. Some responses have previously been shown for other toxic
552 elements, such as Cd. Among these shared responses, proteolysis, ribosome biogenesis regulation,
553 and oxidative stress were specific to Yb and were not seen for La. However, other pathways and
554 compartments appeared to be important for detoxification and/or the avoidance of the toxic impacts
555 of La and Yb. Unlike the toxicity triggered by other metals, here we found that the cell wall and
556 pheromone signalling pathways were key response pathways for yeast cells exposed to La and Yb
557 (Fig. 7). Interestingly, several differences were observed between La (LREE) and Yb (HREE)
558 exposure, indicating the heterogeneity of impacts and responses triggered by REEs. Other toxic
559 metals, such as Cd, can be taken up through Zrt1p, Smf1p, Fet4p and Mid1p (Wysocki and Tamás,
560 2010). We found in the present study previously unidentified mechanisms of nonspecific REE entry
561 into the cytosol through different transporters. Taken together, these findings provide valuable
562 information at the cellular and molecular levels to better assess the impacts and behaviour of REEs
563 towards eukaryotic organisms.
564

565 **Credit authorship contribution statement**

566 **Nicolas Grosjean:** Methodology, Data acquisition, Data curation and treatment, Data analysis,
567 Writing - original draft, review & editing. **Marie Le Jean, Damien Blaudez:** Conceptualization,
568 Methodology, Formal analysis, Funding acquisition, Supervision, Writing - review & editing. **Jean**
569 **Armengaud:** Proteomics data acquisition. **Michel Chalot:** Data acquisition, Writing - review &
570 editing. **Adam Schikora, Elisabeth M. Gross:** Writing - review & editing.
571

572 **Declaration of Competing Interest**

573 The authors declare that they have no known competing financial interests or personal relationships
574 that could have appeared to influence the work reported in this paper.
575

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580 **Conflicts of interest**

581 Authors have no conflicts of interest to declare.

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801
802

803 **Appendix A. Supplementary material**

804
805 **Table S1: ICP-AES and ICP-MS validity data.** The data include the percentages of recovery of
806 the oriental basma tobacco leaves certified material (INCT-OBTL-5, LGC Promochem, Molsheim,
807 France), the limit of detection (LOD) and limit of quantification (LOQ).
808

809 **Table S2: Summarized RNA-seq results of yeast exposed to La or Yb for 1 h.** Each condition
810 was compared to the non-REE-exposed control condition. Data are the log₂FC expression. The left
811 table presents differentially expressed genes with a padj < 0.01, while the right table includes the
812 fold change values regardless of the padj value.
813

814 **Table S3: Summarized proteomics results of yeast exposed to La or Yb at their respective**
815 **EC₅₀ for 1 h or 4 h.** The left table presents differentially abundant proteins with a padj < 0.05,
816 while the right table includes the fold change values regardless of the padj value.
817

818 **Table S4: GO terms significantly represented in the set of differentially abundant (DA)**
819 **proteins under La vs Ctrl exposure after 1 h.** The tables are separated into biological process
820 (BP), cellular component (CC) and molecular function (MF). Significantly enriched GO terms are
821 identified in the DA downregulated proteins (blue), DA upregulated proteins (orange), or all DA
822 proteins (grey).
823

824 **Table S5: GO terms significantly enriched in the set of differentially abundant (DA) proteins**
825 **under Yb vs Ctrl exposure after 1 h.** The tables are separated into biological process (BP),
826 cellular component (CC) and molecular function (MF). The significantly enriched GO terms are
827 identified in the DA downregulated proteins (blue), DA upregulated proteins (orange) or all DA
828 proteins (grey).
829

830 **Table S6: GO terms significantly enriched in the set of differentially abundant (DA) proteins**
831 **under Yb vs La exposure after 1 h.** The tables are separated into biological process (BP), cellular
832 component (CC) and molecular function (MF). The significantly enriched GO terms are identified
833 in the DA downregulated proteins (blue), DA upregulated proteins (orange) or all DA proteins
834 (grey).
835

836 **Table S7: GO terms significantly enriched in the set of differentially expressed (DE) genes**
837 **under Yb vs Ctrl exposure after 1 h.** The tables are separated into biological process (BP),

838 cellular component (CC) and molecular function (MF). The significantly enriched GO terms are
839 identified in the DE downregulated genes (blue), DE upregulated genes (orange) or all DE genes
840 (grey).

841
842 **Table S8: Summarized RNA-seq and proteomics results.** Each condition was compared to the
843 non-REE-exposed control condition. Cells were exposed to La or Yb at their respective EC₅₀ for 1
844 h or 4 h, as indicated. The left table presents differentially expressed genes with a padj < 0.05
845 (PRO) and < 0.01 (RNA), while the right table includes the fold change values regardless of the
846 padj value.

847
848 **Table S9: GO terms significantly enriched in the set of differentially abundant (DA) proteins**
849 **under La vs Ctrl exposure after 4 h.** The tables are separated into biological process (BP), cellular
850 component (CC) and molecular function (MF). The significantly enriched GO terms are identified
851 in the DA downregulated proteins (blue), DA upregulated proteins (orange) or all DA proteins
852 (grey).

853
854 **Table S10: GO terms significantly enriched in the set of differentially abundant (DA) proteins**
855 **under Yb vs Ctrl exposure after 4 h.** The tables are separated into biological process (BP),
856 cellular component (CC) and molecular function (MF). The significantly enriched GO terms are
857 identified in the DA downregulated proteins (blue), DA upregulated proteins (orange) or all DA
858 proteins (grey).

859
860 **Table S11: GO terms significantly enriched in the set of differentially abundant (DA) proteins**
861 **under Yb vs La exposure after 4 h.** The tables are separated into biological process (BP), cellular
862 component (CC) and molecular function (MF). The significantly enriched GO terms are identified
863 in the DA downregulated proteins (blue), DA upregulated proteins (orange) or all DA proteins
864 (grey).

865
866 **Table S12: GO terms significantly enriched in the set of differentially expressed (DE) genes**
867 **under La vs Ctrl exposure after 1 h.** The tables are separated into biological process (BP), cellular
868 component (CC) and molecular function (MF). The significantly enriched GO terms are identified
869 in the DE downregulated genes (blue), DE upregulated genes (orange) or all DE genes (grey).

870
871 **Table S13: GO terms significantly enriched in the set of differentially expressed (DE) genes**
872 **under La vs Yb exposure after 1 h.** The tables are separated into biological process (BP), cellular
873 component (CC) and molecular function (MF). The significantly enriched GO terms are identified
874 in the DE downregulated genes (blue), DE upregulated genes (orange) or all DE genes (grey).