



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

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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
  - Rare earth elements trigger specific responses
  - Yeast cells react differentially to light vs heavy REEs
  - 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; Trifuggi 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

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

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

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

## 2. Materials and methods

### 2.1. Yeast strain, chemicals and REE toxicity tests

The wild-type strain BY4741 of *S. cerevisiae* (MAT $\alpha$ ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0) was used. REEs in their trichloride form were purchased from Sigma-Aldrich (MO, USA). To determine the EC<sub>10</sub> (the concentration at which 10% of the population is inhibited) and EC<sub>50</sub> (the concentration at which half of the population is inhibited), log-phase cells (OD<sub>600nm</sub> 0.15) were exposed to increasing REE concentrations in modified YNB-β-glycerophosphate liquid medium (YNB medium lacking KH<sub>2</sub>PO<sub>4</sub>, substituted by 1 mM β-glycerophosphate to avoid REE precipitation, pH 5.8), and growth (OD<sub>600nm</sub>) was recorded for 10 h at 28°C under constant shaking (180 rpm). The growth ratio (OD at 9 h over OD at 4 h) was measured in log-phase and plotted against the REE concentrations.

### 2.2. Sample preparation for RNA-seq analysis

The wild-type strain was grown in YNB-β-glycerophosphate liquid medium. Cultures were inoculated at an OD<sub>600nm</sub> of 0.05, and once the cultures reached an OD<sub>600nm</sub> of 0.6, the cells were exposed for 1 h to 50 or 160 μM lanthanum or to 6 or 8 μM ytterbium, corresponding to their EC<sub>10</sub> and EC<sub>50</sub>, respectively. The cells were then harvested by centrifugation at 4000 rpm for 1 min at 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 log<sub>2</sub>-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<sub>600nm</sub>  
151 of 0.05. Once the cultures reached an OD<sub>600nm</sub> of 0.6, the REEs were added at the EC<sub>50</sub>  
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-mm 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% CH<sub>3</sub>CN, 19.9%  
175 H<sub>2</sub>O, 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 3.10<sup>6</sup>. 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           **2.6. Peptide-to-spectrum assignment and protein identification**

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

### 206           **2.7. GO term enrichment analysis**

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

### 218           **2.8. Elemental analyses**

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

washed three times with ice-cold MES (20 mM) – EDTA (10 mM) buffer (pH 6), three times with ice-cold ultrapure water, and then dried for 48 h at 70°C. Triplicate samples were prepared. Aliquots (125 mg) were mineralized with 1.75 mL of ultratrace HNO<sub>3</sub> and 0.5 mL of ultratrace hydrogen peroxide in closed tubes placed in a block digestion system (DigiPREP, SCP Sciences, Courtaboeuf, France). Gradual heating was used to achieve a final temperature of 100°C (total heating time of 265 min). Ultrapure water was then added to a final volume of 12.5 mL. Blank and reference materials were digested under the same conditions as the samples. Concentrations of Mn, Mo and Zn were determined using inductively coupled plasma mass spectrometry (ICP-MS, X Series II Model, Thermo Fischer Scientific, Courtaboeuf, France). Other elements were analysed by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Radial ICAP6500 Model, Thermo Fischer Scientific). The validity of the analytical methods was checked using certified reference material (oriental basma tobacco leaves, INCT-OBTL-5, LGC Promochem, Molsheim, France) (Table S1).

## 2.9. Data availability

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

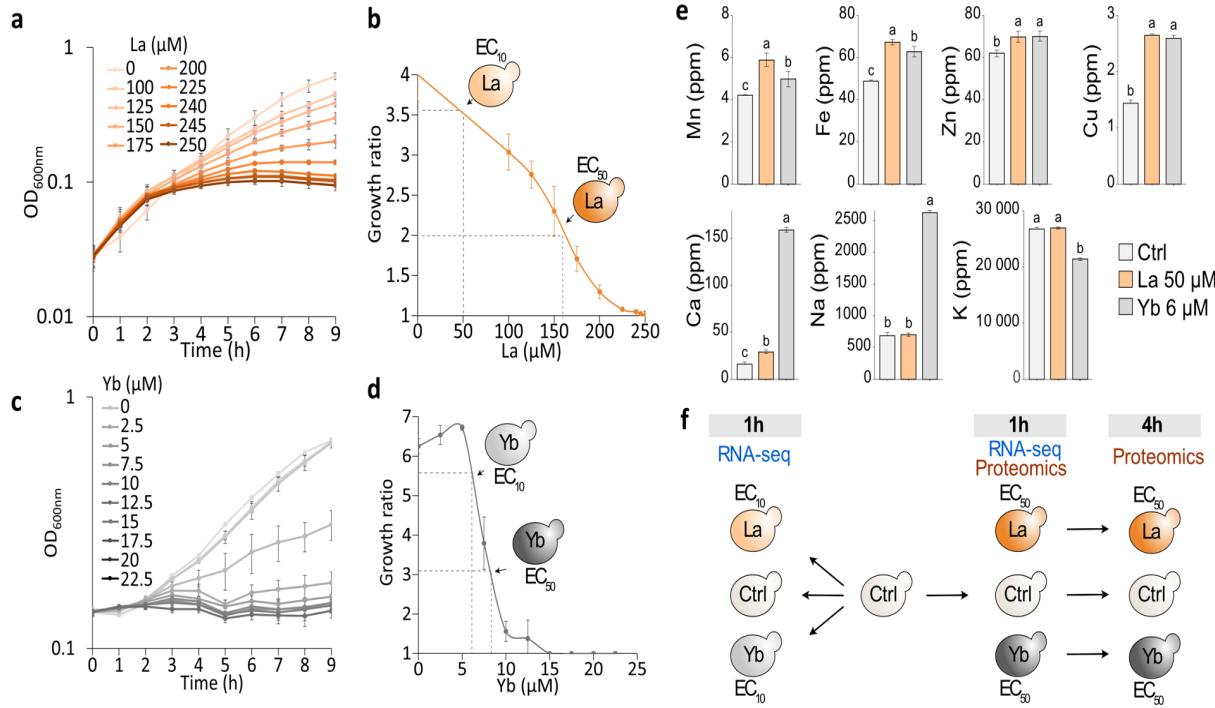
## 3. Results and discussion

### 3.1. REE toxicity assessment

To compare the cellular toxicity of La and Yb, the yeast EC<sub>10</sub> and EC<sub>50</sub> were first established for the two elements. For this, *S. cerevisiae* was exposed to increasing concentrations of these two REEs, and the cellular density was monitored every hour for nine hours (Fig. 1a,c). Different concentration ranges were used for La and Yb because preliminary tests showed a higher toxicity of Yb than La (data not shown). The growth ratio between 9 and 4 hours was then plotted against the REE concentration to determine the EC<sub>10</sub> and EC<sub>50</sub> values (Fig. 1b,d). Yb was 20 times more toxic than La, which is in line with observations using *Escherichia coli* (Técher et al., 2020). The respective EC<sub>10</sub> and EC<sub>50</sub> values for La were calculated to be 50 µM and 160 µM and were 6 µM and 8 µM for Yb (Fig. 1b,d).

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

Prior to describing the cellular mechanisms involved in the response to the different REEs, we first compared the transcriptome of yeasts exposed to the EC<sub>10</sub> and EC<sub>50</sub> of the two REEs to test whether different responses would be observed at these two concentrations. The time-evolving proteome was also monitored and analysed.

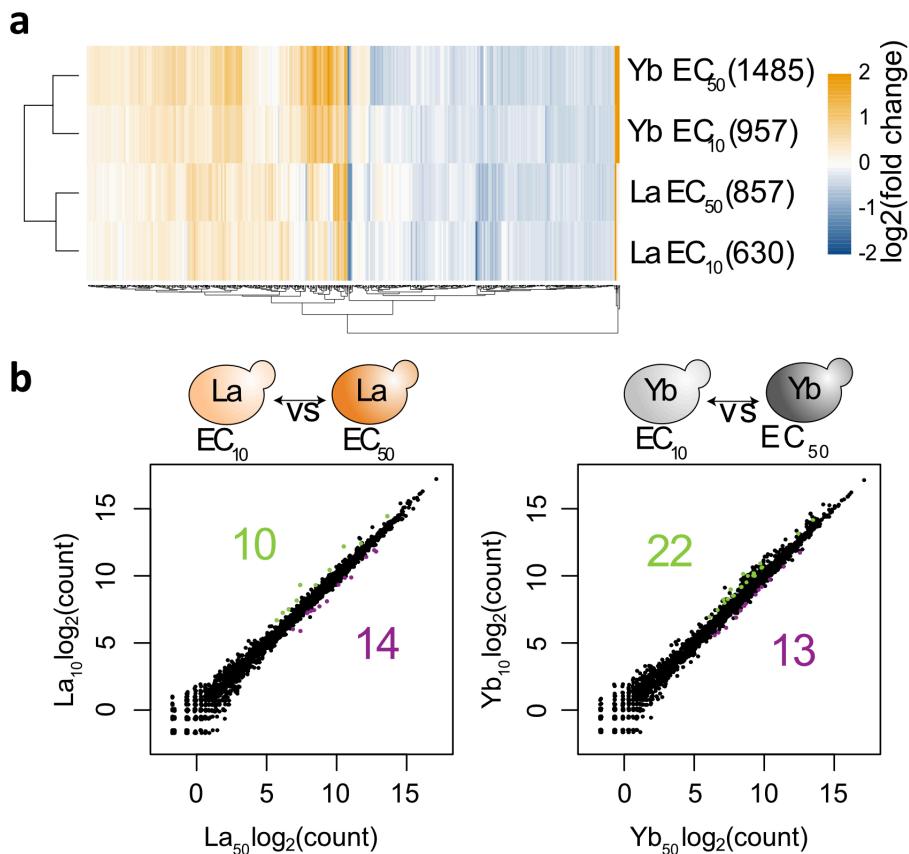


**Figure 1. Impact of REEs on *Saccharomyces cerevisiae* growth and ionome and the omics experimental setup approach.** (a) Growth of yeast exposed to different La concentrations. (b) Growth ratio (OD at 9 h over OD at 4 h) of yeast exposed to increasing amounts of La, enabling the identification of the EC<sub>10</sub> and EC<sub>50</sub>, 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 EC<sub>10</sub> and EC<sub>50</sub>. (e) Ionome analysis of cells exposed to La at EC<sub>10</sub>, Yb at EC<sub>10</sub> and La at the same concentration as that corresponding to Yb EC<sub>10</sub> (6  $\mu$ M). Significant differences from the wild-type condition are indicated by different letters (ANOVA, Tukey HSD). Data are the means ( $\pm$ 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 (EC<sub>10</sub> and EC<sub>50</sub>) for two exposure durations (1 h and 4 h). Three independent experiments were carried out for each condition.

**3.2. Transcriptomic response to different effective concentrations (ECs) of La and Yb**  
We first aimed to identify the transcriptomic response of yeast cells to two different conditions of a high (EC<sub>50</sub>) and a low (EC<sub>10</sub>) toxicity concentration. The transcriptome of *S. cerevisiae* exposed for one hour to La and Yb at their respective EC<sub>10</sub> and EC<sub>50</sub> values was analysed (Fig. 1f, Fig. 2). Overall, we identified 1924 differentially expressed genes (DEGs) (applying a fold change (FC) cut-off of  $\geq 1.5$  and  $p < 0.01$ ) in at least one condition (REE-stressed cells vs control condition).

The direct comparison of gene expression between EC<sub>50</sub> and EC<sub>10</sub> for the two REEs identified only a few DEGs (Fig. 2b). When exposed to La, 10 DEGs showed higher expression at EC<sub>10</sub> than at EC<sub>50</sub>, while 14 DEGs were upregulated under EC<sub>50</sub> conditions (Fig. 2b, Table S2). A similar observation was made for Yb. Twenty-two DEGs were more abundant under the EC<sub>10</sub> condition than under the EC<sub>50</sub> condition, while this was the case for 13 DEGs under the EC<sub>50</sub> condition (Fig. 2b, Table S2). We extended the analysis to the entire set of genes that were significantly differentially expressed relative to the control condition ( $p < 0.01$ ). Again, a relatively high similarity of expression patterns existed between the two exposure concentrations, with only a few

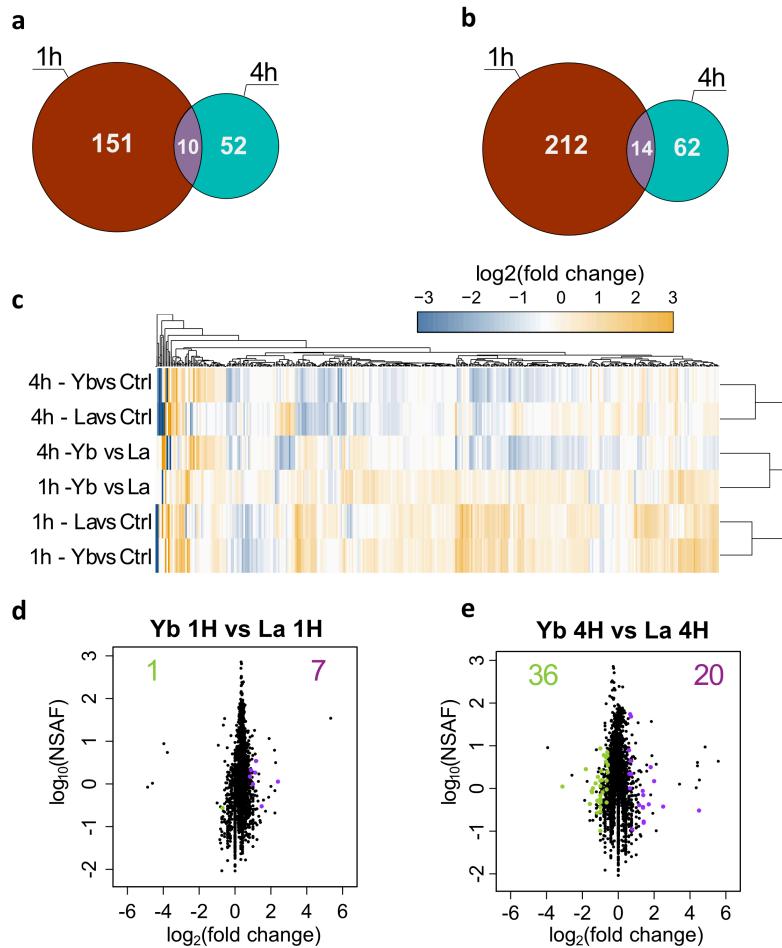
288 differences in the transcriptomic profiles between the EC<sub>10</sub> and EC<sub>50</sub> conditions (Fig. 2a, Table S2).  
 289 In line with our observations, few differences in DEGs between EC<sub>10</sub> and EC<sub>50</sub> exposure were  
 290 obtained for other metals such as Zn, Cd, Cu, Cr 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 EC<sub>10</sub> and EC<sub>50</sub> 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 EC<sub>50</sub> 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 EC<sub>10</sub> and EC<sub>50</sub> treatments.** (a) Heatmap of differentially expressed genes (DEGs) (blue, downregulated; orange, upregulated) under the two different REE concentrations (EC<sub>10</sub> and EC<sub>50</sub> for both La and Yb) in comparison to the control condition. The total number of DEGs ( $P \leq 0.01$ ) is specified in brackets. (b) Scatter plots of DE transcripts under La and Yb exposure. Significantly upregulated and downregulated transcripts ( $FC \geq 1.5$ ,  $P \leq 0.01$ ) 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 ( $FC \geq 1.5$  and  $P \leq 0.05$ ) 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.

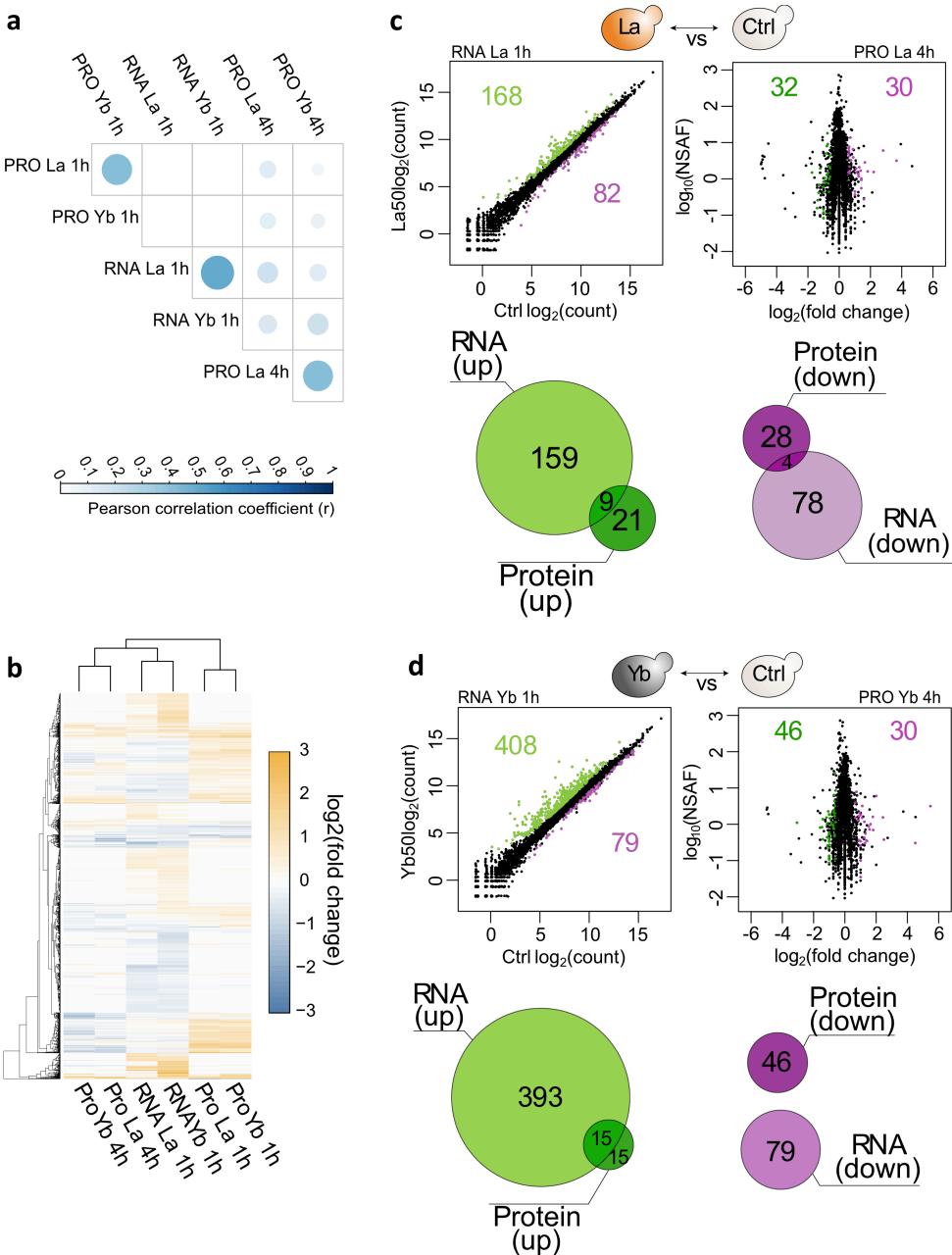
323      **3.4. Combining transcriptomic and proteomic analyses**

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

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

352  
353      When comparing the transcriptomic and proteomic profiles at one and four hours for both REEs,  
354      the highest correlations were observed between transcriptomic profiles obtained after one hour and  
355      proteomic profiles obtained after four hours (Fig. 4a,b, Table S8). Strikingly, there was hardly any  
356      overlap between differentially expressed mRNA and the corresponding differentially expressed  
357      proteins (Fig. 4c,d). For instance, under La exposure, the expression of 21 of the 30 (70%) more  
358      abundant proteins and 28 of the 32 less abundant proteins was not altered for the cognate mRNAs  
359      (Fig. 4c). The same observation was found for Yb exposure, with as high as 50% of the proteins  
360      with increased abundance and 100% of those with decreased abundance for which the  
361      corresponding mRNA was not differentially expressed (Fig. 4d). These observations could be  
362      explained by the relatively low number of DAPs ( $FC \geq 1.5$  and  $P \leq 0.05$ ) in comparison with the  
363      number of DEGs ( $FC \geq 1.5$  and  $P \leq 0.01$ ). A fourfold higher number of mRNAs than proteins were  
364      differentially expressed under La exposure, while it was 6.4-fold greater under Yb exposure (Fig.  
365      4c,d).

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



**Figure 4. Comparative transcriptomic and proteomic analyses of yeast exposed to REEs at the EC<sub>50</sub>.** (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.

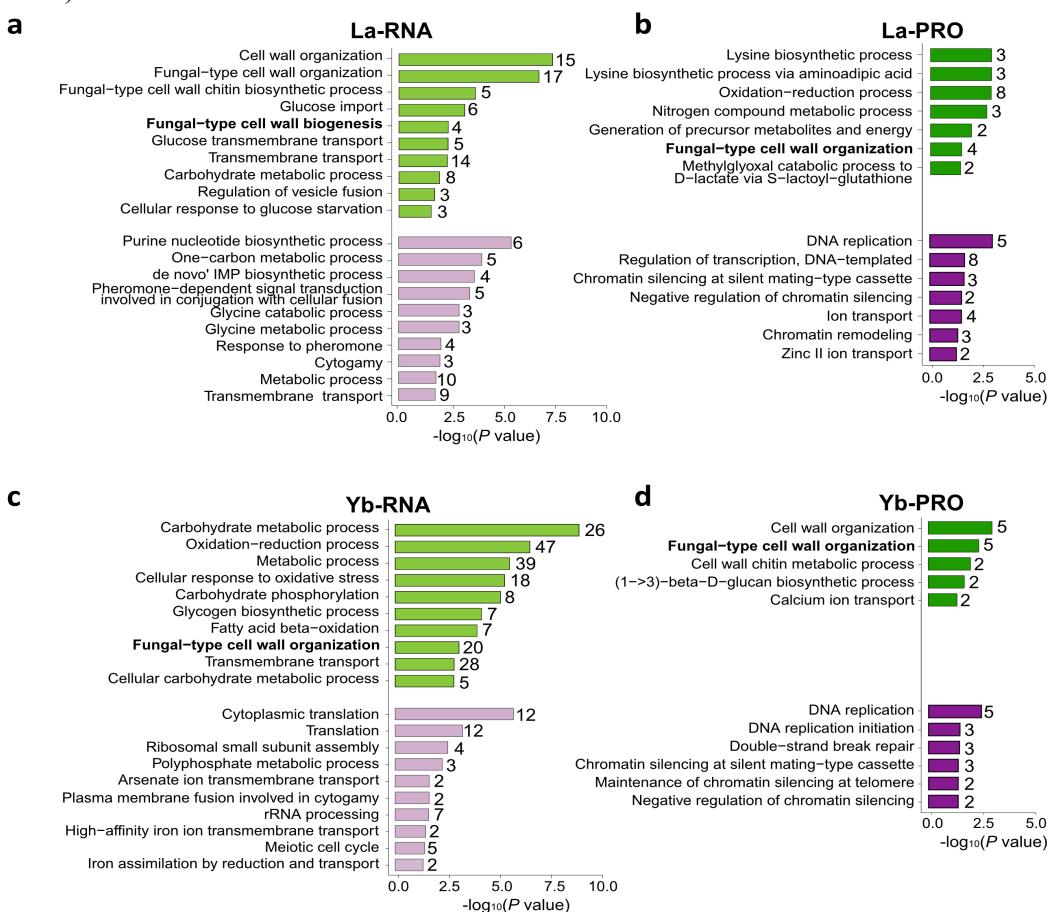
370

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 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

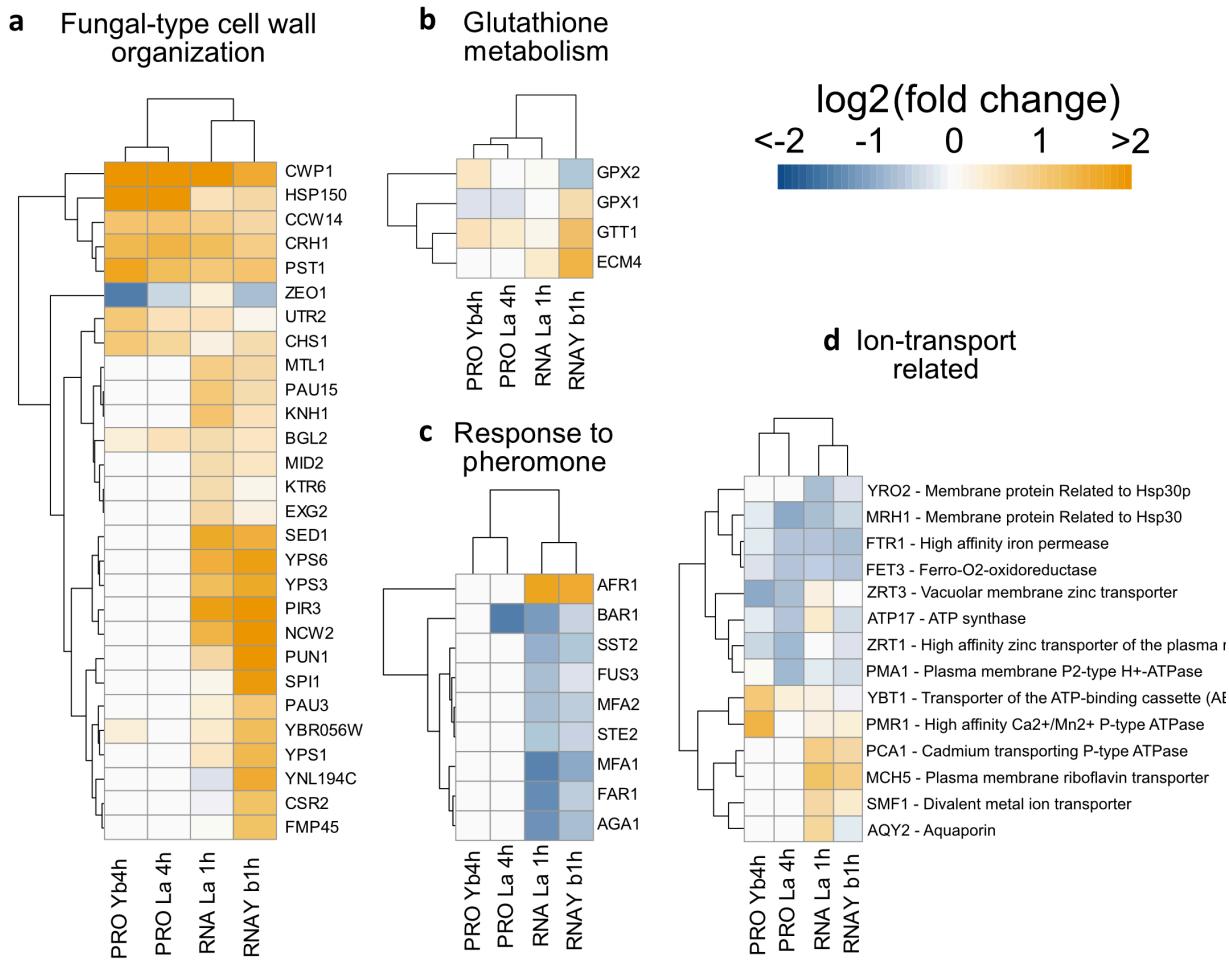
organization and cell wall organization (Fig. 5c, Fig. 4c, Table S7). Moreover, these two functions were also found in the proteomic analysis under Yb stress. This was reinforced by the upregulation of the chitin cell wall metabolic process and the 1,3- $\beta$ -D-glucan biosynthesis (Fig. 5d, Table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



**Figure 5. Biological pathway (BP) GO terms overrepresented in the transcriptomic and proteomic analyses of yeast exposed to REEs at the EC<sub>50</sub>.** (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.

The overrepresentation of these cell wall-related biological pathways (BPs) supports the role of this compartment in the first barrier yeast have against REEs. It also provides additional clues about 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 multispansing 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

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.

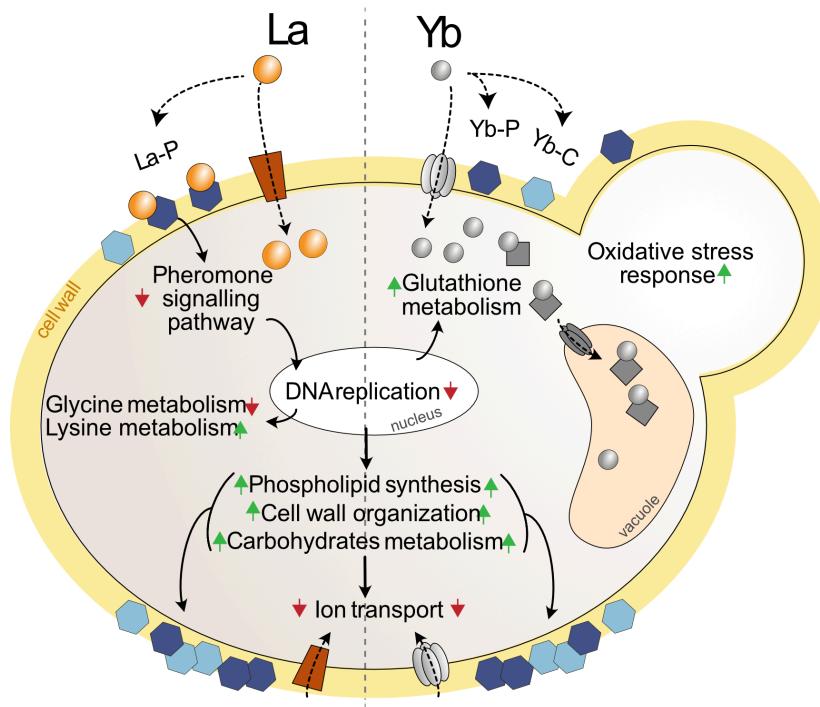
482

### 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 (Deponte, 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 *PCA1*, 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 *ENA1*  
534 encoding a Na<sup>+</sup> 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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## 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       **EC<sub>50</sub> 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 EC<sub>50</sub> 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  
849 **Table S9: GO terms significantly enriched in the set of differentially abundant (DA) proteins**  
850 **under La vs Ctrl exposure after 4 h.** The tables are separated into biological process (BP), cellular  
851 component (CC) and molecular function (MF). The significantly enriched GO terms are identified  
852 in the DA downregulated proteins (blue), DA upregulated proteins (orange) or all DA proteins  
853 (grey).

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

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

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

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