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## Global deletome profile of *Saccharomyces cerevisiae* exposed to lithium

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1 **Global deletome profile of *Saccharomyces cerevisiae* exposed to lithium**

2

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17

## 18 Abstract

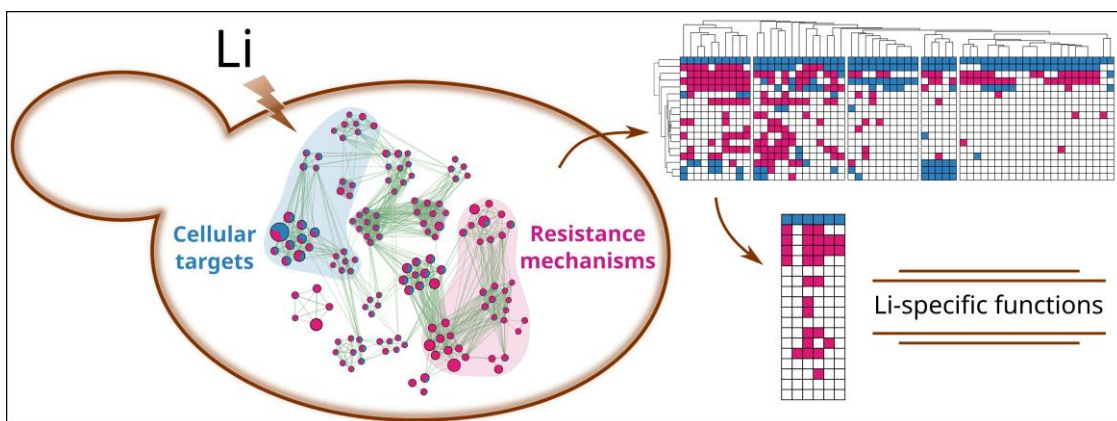
19 The increasing use of lithium (Li) in new technologies raises the question of its impact on  
20 living microorganisms. In the present study, we aimed to identify putative Li targets and  
21 resistance mechanisms in the yeast model *Saccharomyces cerevisiae* using a deletomic  
22 approach based on the screening of a collection of 4,733 knockout mutants under Li exposure.  
23 This screening highlighted 60 mutants resistant to Li and 124 mutants sensitive to Li.  
24 Through functional enrichment analyses, transport systems and catabolite repression were  
25 identified as playing a central role in cell resistance to toxic concentrations of Li. In contrast,  
26 the AKT/protein kinase B family, signal transduction or cell communication were identified  
27 as potential toxic targets of Li. The majority of the mutants with a Li-sensitive phenotype  
28 were also sensitive to other alkali and alkaline-earth metals, whereas the Li-resistance  
29 phenotype was mostly resistant to Na but poorly resistant to other metals. A comparison with  
30 the results of deletomics studies carried out in the presence of other metals highlighted Li-  
31 specific phenotypes. Three genes (*NAM7*, *NMD2*, *UPF3*) of the nonsense-mediated decay  
32 pathway were specifically involved in resistance to Li. In contrast, mutants with the *NCA2*,  
33 *SPT20*, *GCN5*, *YOR376W*, *YPK3*, and *DCW1* genes deleted were specifically sensitive to Li.  
34 These genes encode various functions from putative mannosidase to constitution of the Spt-  
35 Ada-Gcn5 acetyltransferase complex. This work provides a better understanding of potential  
36 specific resistance mechanisms and cellular targets of Li in yeast.

37

38 Keywords: genome-wide screening, lithium-specific phenotypes, transport systems,  
39 signalling, yeast.

## 40 Graphical abstract

41 Yeast deletomics under lithium stress.



42

## 43 **Introduction**

44 Lithium (Li) has become an essential element in industry due to many of its physical and  
45 chemical properties (*e.g.*, low density, high specific heat capacity, high redox potential value)  
46 [1]. It is used in various fields, such as medicine (< 4%), ceramics and glass (14%), polymers  
47 (2%) and especially the manufacture of batteries (74%) [2]. The use of Li for batteries has  
48 almost doubled in 6 years [1] due to the growing demand for electric vehicles [2]. In addition,  
49 a forecast study estimated a 250% increase in Li demand by 2035 [3]. The exponential  
50 demand associated with a low recycling rate (expensive, energy consuming and polluting  
51 methods for the most part) [4,5] leads to significant Li dissemination in the environment [6,7].  
52 Currently, Li is present in relatively low concentrations in soil and sea water (~ 40 ppm and ~  
53 150 µg/L, respectively) [6,8,9], but high concentrations can be found, especially near  
54 extraction areas, from 200 to 1000 ppm [6].

55 Li is not an essential element for living organisms, which may explain the absence of  
56 Li-specific transport systems. However, Li is able to cross biological membranes through Na  
57 or K transporters. In *Saccharomyces cerevisiae*, ENA (Exitus NAtru, a subfamily of P-type  
58 ATPase) transporters allow the export of Li<sup>+</sup> as well as Na<sup>+</sup> [10]. Nha1p, a Na<sup>+</sup>/H<sup>+</sup> antiporter,  
59 is also involved in the efflux of Li<sup>+</sup> [11]. Conversely, Marquina et al., (2012) highlighted that  
60 the K<sup>+</sup>-transporter Trk1,2p can import Li<sup>+</sup> and consequently trigger intracellular toxic effects  
61 on yeast cells. Li has been shown to inhibit Hal2p and the RNase MRP, impacting the  
62 maturation of RNA [13], delaying sporulation [14], increasing protein aggregation and  
63 reducing cell volume [15]. Furthermore, Li can inhibit Pgm2p, a phosphoglucomutase, by  
64 competing with its cofactor Mg<sup>2+</sup> [16]. In conclusion, literature data related to Li cellular  
65 targets and resistance mechanisms remain fragmentary and deserve more genome-wide  
66 investigation.

67 The yeast model *S. cerevisiae* has been extensively used to study the tolerance  
68 mechanisms and cellular targets of various toxic compounds, mainly by screening collections  
69 of nonessential mutants [17–24]. In a previous work, genome-wide screening in the presence  
70 of LiCl identified 114 Li-sensitive mutants, highlighting the involvement of meiosis,  
71 sporulation and vacuoles in the tolerance of yeast to Li [25]. However, since high  
72 concentrations of LiCl were used for the screening of Li-responsive mutants, it could be  
73 hypothesized that high concentrations of the counteranion (Cl<sup>-</sup>) could trigger false positives.  
74 In the same study, six Li-resistant mutants were reported; however, this limited number of

75 mutants prevented a thorough analysis of Li toxicity targets. Therefore, the first objective of  
76 our study was to revisit the search for Li-responsive genes in yeast by performing a series of  
77 screening steps including the use of two Li salts to eliminate false positives. A deep functional  
78 analysis was then performed to elucidate the functions involved in Li sensitivity or resistance.  
79 The second objective was to study the specificity of these functions between alkali metals and  
80 alkaline earth metals. To this aim, the Li-specific functions were investigated by exposing the  
81 retained mutants to four alkali and alkaline earth metals (Na, Mg, K and Ca). By combining  
82 these results, we provide evidence of specific phenotypes to Li and those shared among alkali  
83 metals and alkaline-earth metals.

84

## 85 **Materials and Methods**

### 86 **Yeast strains and medium**

87 A collection of KO deletion mutants for each of the 4,733 nonessential genes of  
88 *Saccharomyces cerevisiae* was used (Euroscarf; haploid BY4741 background; MATa *his3Δ1*  
89 *leu2Δ0 met15Δ0 ura3Δ0*). All experiments were performed with YPD medium at pH 6.5 (per  
90 litre: 10 g yeast extract, 20 g peptone, 20 g glucose, 15 g agar if required).

### 91 **Genome-wide screening with Li**

92 The library screening consisted of a three-step approach to identify and validate Li-sensitive  
93 and Li-resistant mutants. The primary mutant screening consisted of a qualitative assessment  
94 of their growth response on Li-agar plates. For this purpose, the individual deleted mutants  
95 were first grown in 200  $\mu$ L of YPD in 96-well microplates at 28°C with agitation (160 rpm)  
96 from stationary phase cultures. The mutants were subsequently replicated on YPD agar plates  
97 supplemented or not supplemented with lithium chloride (LiCl, Sigma Aldrich, Saint-  
98 Quentin-Fallavier, France) by using a Thermo Scientific Nunc Replication System. Four  
99 replicas were performed per mutant. Two ranges of Li concentrations were first tested on a  
100 sub-set of the library: 600, 650, and 700 mM and 850, 900, 950, and 1000 mM to find the best  
101 conditions to identify the sensitive and resistant mutants, respectively. We found that the 600  
102 mM condition was the best option to identify sensitive mutants. Similarly, 900 and 1000 mM  
103 Li were chosen to select resistant mutants. In conclusion, the 4,733 mutants were tested in the  
104 presence of these three Li concentrations as well as on Li-free YPD. The plates were  
105 photographed and analysed after 5 days of growth at 28°C. Li-sensitive and Li-resistant  
106 mutants were identified by visual inspection as decreases or increases in their colony size,

107 respectively, compared to that of the WT on Li-amended medium. Li-free plates were also  
108 used as growth controls.

109 A secondary screening was performed with the previously identified sensitive and  
110 resistant mutants to confirm the phenotypes obtained. The mutants were grown overnight in  
111 200  $\mu$ L of YPD in a 96-well microplate at 28°C with agitation (160 rpm). Tenfold serial  
112 dilutions were carried out, and 5  $\mu$ L drops of each dilution were spotted on plates with two  
113 ranges of LiCl: 300 to 600 mM and 650 to 1000 mM (by 50 mM increment steps in both  
114 cases) to validate the phenotype of sensitive and resistant mutants, respectively. After 5 days  
115 of growth, the mutants were considered sensitive when they exhibited no growth for at least  
116 one order of dilution relative to the WT. Conversely, the mutants were considered resistant  
117 when they showed additional growth for at least one order of dilution compared to the WT.

118 The tertiary screening consisted of a validation screening in liquid cultures. The  
119 mutants validated from the secondary screening were grown overnight in 5 mL of YPD and  
120 then inoculated ( $OD_{600nm} = 0.05$ ) in the presence of a range of LiCl concentrations in 96-well  
121 microplates: 0 to 400 mM (by 50 mM increments) or 0 to 600 mM (by 100 mM increments)  
122 to confirm the sensitivity or resistance of the mutants, respectively. The microplates were  
123 incubated for 24 hours before measuring  $OD_{600nm}$  with a spectrophotometer (Xenius SAFAS,  
124 Monaco). As an additional validation step, a quaternary screening was performed. It consisted  
125 of the same strategy as in the tertiary screening but with another Li salt ( $Li_2SO_4 \cdot H_2O$ , Sigma  
126 Aldrich). To this end, a concentration range of 50, 100 to 500 mM Li (by 100 mM increments)  
127 was used. The data were normalized to those obtained at 0 mM Li, and a growth percentage in  
128 relation to the WT was calculated.

129 Finally, for both the tertiary and quaternary screenings, the mutants were classified  
130 according to their percentage of sensitivity or resistance compared to the WT. Mutants  
131 exhibiting less than 50% growth at 50 mM Li were classified as high-sensitivity (HS)  
132 mutants. Between 50% and 90% growth at 50 mM Li, the mutants were classified as medium-  
133 sensitivity (MS) mutants. The sensitive mutants exhibiting growth above 90% at both 100  
134 mM and 150 mM Li were grouped in the low-sensitivity (LS) category. Mutants exhibiting  
135 growth above 150% at 100 mM Li were classified as high-resistance (HR) mutants. Mutants  
136 exhibiting growth between 110% and 150% at 100 mM Li were classified as medium-  
137 resistance (MR) mutants. Finally, the resistant mutants displaying growth below 110% at 200  
138 mM were grouped in the low-resistance (LR) category.

### 139 **Cross-comparison of Li-responsive mutants with other metals**

140 To investigate the potential Li-specific phenotypes of the mutants, liquid growth assays were  
141 performed in the presence of toxic concentrations of other alkali or alkaline-earth metals:  
142  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  (0.8 to 1.8 M); KCl (1 to 2 M); NaCl (0.8 to 1.7 M);  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (100 to 500  
143 mM). For these four compounds, 100 mM increments were tested.

144 The mutants identified in the present study were also compared with those reported  
145 from other screenings carried out on yeast KO mutant libraries on Al [23,26], As [21,22,27–  
146 29], Cd [28–31], Co [17], Cr [28,32], Cu [17,24,28,33], Fe [17,24,33], Mn [17,24,34], Ni  
147 [17,30,35], Zn [17,24,28,36], Y [19], Yb and La [18]. When similar independent studies on  
148 the same stressor were performed, the corresponding lists of mutants were combined.  
149 Clustering and heatmaps were generated using the R package pheatmap.

### 150 **EC50 determination**

151 For the Li-specific responsive mutants obtained from the quaternary screening, their  $\text{EC}_{50}$   
152 values for Li were determined. The mutants and WT were inoculated ( $\text{OD}_{600\text{nm}} = 0.05$ ) with a  
153 range (0 to 500 mM by 50 mM increments) of LiCl in 96-well microplates. Biological  
154 triplicates were performed. The microplates were incubated for 24 hours at 28°C and 160  
155 rpm. The  $\text{OD}_{600\text{nm}}$  was recorded, and the  $\text{EC}_{50}$  values were calculated using R software with  
156 the ec50estimator package.

### 157 **Gene function and functional enrichment analysis**

158 Gene descriptions of the sensitive and resistant mutants were retrieved from the  
159 *Saccharomyces* Genome Database (SGD). Biological process ( $p$  value < 0.01) and cellular  
160 compartment ( $p$  value < 0.05) were analysed using MIPS and FunSpec [37], respectively. The  
161 functions involved in the mechanisms of sensitivity and resistance to Li were determined with  
162 functional enrichment. This analysis was carried out on the mutants from the quaternary  
163 screening. The enrichment was calculated using Metascape software ( $p$  value < 0.01) [38] and  
164 visualized with Cytoscape v3.9.1 software [39] with default settings (maximum of 10  
165 functions visualized per cluster).

### 166 **Determination of cellular Li concentrations**

167 The mutants and WT were grown in liquid YPD medium (28°C, 160 rpm). Cultures were  
168 inoculated at an  $\text{OD}_{600\text{nm}}$  of 0.1 and incubated until reaching an  $\text{OD}_{600\text{nm}}$  of 1. The cells were  
169 then exposed for 1 hour to 100 mM LiCl, a concentration corresponding to the  $\text{EC}_{10}$  of the  
170 WT. The cells (from 100 mL cultures) were harvested by centrifugation (4000 rpm, 1 min, 4  
171 °C) and washed three times with ice-cold MES (20 mM)-EDTA (10 mM) buffer (pH 6) and  
172 then three times with ice-cold ultrapure water. The pellets were dried at 60 °C for 24 h. Five

173 and three independent biological replicates were prepared for the WT and the mutants,  
174 respectively. Concentrations of Li, macroelements and microelements were determined using  
175 inductively coupled plasma–mass spectrometry (Thermo iCap-TQ-ICP–MS, Thermo Fisher  
176 Scientific, Courtaboeuf, France). Cell pellets (approximately 60 mg DW) were mineralized  
177 with 1.75 mL HNO<sub>3</sub> and 0.5 mL H<sub>2</sub>O<sub>2</sub> in DigiPREP tubes placed in a block digestion system  
178 (DigiPREP, SCP Sciences, Courtaboeuf, France). The samples were gradually heated to 100  
179 °C over a 180 min period. The volume was completed to 12.5 mL with ultrapure water  
180 followed by filtration to 1 µm. The results are expressed as micrograms of element per gram  
181 of cell DW. The validity of the analytical method was verified by a standard reference  
182 material (oriental basma tobacco leaves, INCT-OBTL-5).

### 183 **Statistical analysis**

184 The statistical tests were performed using R software (version 4.1.2). Two-tailed Student's *t*  
185 *test* to compare the different conditions to WT was performed. EC<sub>50</sub> values are the means ±  
186 standard deviations of biological triplicates. Values of Li concentrations in cells are the means  
187 ± standard errors of three or five independent replicates for the mutants and the WT,  
188 respectively.

189

## 190 **Results and Discussion**

### 191 **Identification of a large set of Li-responsive genes**

192 The screening of the 4,733 mutants was performed by four successive steps under Li exposure  
193 to identify sensitive and resistant mutants (Supplemental Figure S1). The primary screening  
194 identified a total of 327 sensitive mutants and 240 resistant mutants (Figure 1). Therefore, a  
195 secondary screening was carried out with the drop test method (Supplemental Figure S1) to  
196 refine the results and confirmed 155 (47%) sensitive and 96 (40%) resistant mutants (Figure  
197 1). To further eliminate remaining putative false positives possibly arising from unequal  
198 amounts of cell inoculum, a tertiary screening consisting of liquid cultures exposed to Li was  
199 performed by individually normalizing the initial OD of all liquid cultures (Supplemental  
200 Figure S1). The mutants were then classified into six categories according to their level of  
201 sensitivity and resistance, from high sensitivity (HS) to high resistance (HR) (Tables 1&2).  
202 On the one hand, 59 low sensitivity (LS), 50 moderate sensitivity (MS), 31 HS and 15 false-  
203 positive mutants were identified. On the other hand, 18 low-resistance (LR), 35 medium-  
204 resistance (MR), 28 HR and 15 false-positive mutants were identified. Finally, to exclude  
205 sensitive/resistant phenotypes stemming from the accompanying chloride anion of the LiCl



206 salt used, the same test was repeated in a quaternary screening with  $\text{Li}_2\text{SO}_4$ . This comparison  
207 confirmed a large majority (89%) of the Li-sensitive mutants ( $n=124$ ) (Figure 1, Table 1).  
208 Mutants that were lost (sensitive to LiCl but not to  $\text{Li}_2\text{SO}_4$ ) belonged essentially to the LS  
209 category. Indeed, 76, 96 and 100% of LS, MS and HS mutants from the tertiary screening in  
210 the presence of LiCl were also sensitive to  $\text{Li}_2\text{SO}_4$  (Supplemental Figure S2A). Similarly, but  
211 to a lesser extent, 70% of the LiCl-resistant mutants were also resistant to  $\text{Li}_2\text{SO}_4$  ( $n=60$ )  
212 (Figure 1, Table 2). In more detail, 56, 74 and 86% of LR, MR and HR mutants were resistant  
213 to both Li salts, respectively (Supplemental Figure S2B). The high conservation rate of the  
214 HS and HR mutants after this quaternary screening suggests functions that are critical to the  
215 cell response in the presence of Li.

216 Variable results can be encountered when screening experiments of the same metal are  
217 performed in yeast. For example, for Cu screenings, Jo et al., (2008) found 89 sensitive  
218 mutants, compared to three for Wang et al., (2007) and 170 for Bleackley et al., (2011).  
219 Similarly, 18 [24], 89 [36] and 130 [17] sensitive mutants were retrieved for Zn genome-wide  
220 screenings, and 67 [34] versus 141 [17] sensitive mutants were found in two Mn deletomics  
221 screenings. The differences that were observed could originate from variations in the yeast  
222 mutant collection, medium composition, culture conditions, screening method and/or the  
223 arbitrary threshold used to define a sensitivity/resistance phenotype. In the case of Li, a  
224 similar screening to the one described here was performed with LiCl and reported 114 Li-  
225 sensitive and 6 Li-resistant mutants [25]. Seventy-three sensitive and all (6) resistant mutants  
226 were found in common with our primary screening. Since the same medium (YPD) was used  
227 in both studies, the difference could be explained by the fact that a slightly different yeast  
228 mutant collection was used in this study (BY4743 homozygous diploid mutants versus  
229 BY4741 haploid mutants in our study). However, the differences we observed are more likely  
230 the result of using a different screening method. Zhao et al., (2010) screened their collection  
231 on agar plates with a replicator, followed by colony streaking to confirm sensitive and  
232 resistant mutants. However, this screening used a single concentration of Li, and there was no  
233 mention about cell quantity normalization when the confirmation screening on agar plates was  
234 performed. Moreover, high concentrations (several hundreds of mM) of LiCl were used to  
235 achieve toxicity in yeast cells. As an adverse consequence, the Li false positives that arose in  
236 both studies could be due to the toxic effects of the counteranion (chloride). This problem was  
237 circumvented in the present study by using another Li salt ( $\text{Li}_2\text{SO}_4$ ). Finally, 40 (35%)  
238 sensitive and 4 (7%) resistant mutants from Zhao et al., (2010) were found in our quaternary

239 screening. Among these mutants, 24, 22 and 45% of LS, MS and HS mutants were identified  
240 in the present study, respectively (Supplemental Figure S3). Another yeast mutant screening  
241 was performed with LiPF<sub>6</sub>, a Li-ion battery electrolyte [20], but identified only four and five  
242 mutants in common with the study of Zhao et al., (2010) and the present study, respectively  
243 (Supplemental Figure S3). This high discrepancy is very likely due to the extreme  
244 cytotoxicity of LiPF<sub>6</sub>, where a concentration of 3-5 mM was sufficient to inhibit yeast growth  
245 in YPD medium. The authors concluded that the LiPF<sub>6</sub>-mediated toxicity was not due to Li  
246 but to the LiPF<sub>6</sub> compound itself. In conclusion, and compared to these two previous reports,  
247 the screening performed here allowed the identification of a consequent batch (80) of new Li-  
248 sensitive mutants (Supplemental Figure S3). These mutants belong in similar proportions to  
249 the LS, MS and HS categories, notably highlighting new proteins playing an important role in  
250 Li resistance in yeast. Moreover, this conclusion is all the more true for Li-resistant mutants  
251 (60 mutants; Table 2), for which 10 times more mutants were reported and confirmed here  
252 with two lithium salts.

253

#### 254 **Conserved functions involved in yeast resistance to alkali and alkaline-earth metals**

255 The study of the sensitive mutants allowed us to highlight the functions involved in yeast  
256 resistance under Li stress. These functions were revealed by a functional enrichment analysis  
257 (Figure 2). Whereas most of the clusters grouped functions that gathered both sensitive and  
258 resistant mutants, the endosomal transport, cytosolic transport, endocytosis, and cellular  
259 response to abiotic stimulus clusters gathered nearly exclusively sensitive mutants (Figure  
260 2A). However, among the different clusters, some corresponded to functions for which the  
261 majority of sensitive mutants was found and for which the enrichment ratio (k/f) was the  
262 highest (Supplemental Table S1). This was the case for endocytosis ( $p$  value = 1.70E-03)  
263 endosomal transport ( $p$  value = 9.14E-09), cytosolic transport ( $p$  value = 6.48E-05), vacuole  
264 organization ( $p$  value = 2.95E-05), and vacuolar transport clusters ( $p$  value = 4.81E-08)  
265 composed of 91, 89, 89, 88 and 82% sensitive mutants, of which 50, 36, 50, 40 and 34% were  
266 HS, respectively (Figures 2A & 2B, Supplemental Table S1).

267 The majority of the 124 mutants were found to show a phenotype sensitive to alkali  
268 metals and alkaline earth metals (Figure 3). A similarity of 94, 84, 85 and 72% with the Li  
269 dataset was indeed found for Mg, K, Na and Ca, respectively (Supplemental Table S2). More  
270 globally, 75 of the 124 mutants (60%) exhibited sensitivity to the five alkali/alkaline-earth  
271 metals (Supplemental Figure S4A). Sixteen other mutants (13%) also shared a sensitive

272 phenotype but were not sensitive to Ca. The functional enrichment analysis revealed that three  
273 of the clusters described above were also retrieved for the other alkali and alkaline-earth  
274 elements (Figure 2B). The clusters endosomal transport ( $p$  value =  $9.14E-09$ ), cytosolic  
275 transport ( $p$  value =  $9.37E-05$ ), endocytosis ( $p$  value =  $1.70E-03$ ) (Supplemental Table S1)  
276 were indeed represented under at least three other metals. Negative regulation of cellular  
277 process, regulation of cellular component organization, and viral life cycle – HIV-1 clusters  
278 were identified in the presence of five metals. Conversely, the AKT family and cell  
279 communication clusters were evidenced under Mg and K, and Na and K, respectively. Three  
280 other functional clusters (regulation of nucleobase metabolism, regulation of biological  
281 quality and chromatin organization with a  $p$  value of  $7.46E-08$ ,  $2.37E-05$  and  $3.18E-04$ ,  
282 respectively) (Supplemental Table S1) were less represented but were found for the three  
283 alkali metals and Mg. Similarly, the clusters macromolecule catabolic process, regulation of  
284 transport, and signal transduction were only identified under Na or Mg stress.  
285 Interestingly, four other clusters were Li-specific and included vacuolar transport ( $p$  value =  
286  $4.81E-08$ ), vacuole organization ( $p$  value =  $2.95E-05$ ), cellular response to abiotic stimulus ( $p$   
287 value =  $2.36E-04$ ), and transcription process ( $p$  value =  $2.74E-04$ ) (Figure 2B). These clusters  
288 were composed of 34, 40, 12 and 42% HS mutants, respectively (Figure 2B). The high  
289 representation of HS mutants suggests that the genes belonging to these clusters could play a  
290 key role in the Li-specific resistance of yeast.

291 The high concentrations of Li salts used in the media could trigger osmotic stress in  
292 yeast cells. To investigate the extent to which osmotic stress favoured the retrieval of sensitive  
293 mutants, we compared our sensitive mutant dataset with that obtained from a study focusing  
294 on osmotic stress mediated by high Na [40] (Supplemental Figure S5). Out of the 124 Li-  
295 sensitive mutants, only nine mutants were found in common with those identified in the  
296 previous study. Therefore, the high majority of the sensitive mutants found in the present  
297 study are very likely Li-responsive and not osmotic stress-responsive.

298 As described above, several functional clusters involved in metal transport were  
299 retrieved. Notably, the endosomal transport cluster was found for the five metals tested but  
300 was threefold larger in diameter for Li (15%) than for the other metals (6 to 9%). Similarly,  
301 the vacuolar transport cluster was Li-specific and the cluster regulation of transport was only  
302 common between Li and Na. Combined with the high number of HS mutants found in the  
303 transport functions (>30%), our data confirm the central role of transport systems in Li  
304 resistance. Li entry into yeast cells can be nonspecifically handled by  $K^+$  [41] or  $Na^+$  [11]

305 transporters. As reported by Zhao *et al.* (2010), we found that the *TRK1* mutant was HS to Li  
306 (Table 1), Moreover, the *HAL5* mutant was also sensitive to Li in our study. Hal5p, the  
307 halotolerance protein kinase C, is a regulator of the K transporter Trk1p [11] and was shown  
308 to be a high-copy suppressor of numerous Li-sensitive mutations of genes involved in  
309 sporulation, meiosis and biosynthesis of ergosterol [25], a major compound of membranes in  
310 yeast. Conversely, Ena5p and Nha1p allow the efflux of both Na and Li out of the cells  
311 [10,11]. The *ENA* mutants are not included in the EUROSCARF library and were therefore  
312 not tested. However, several *ENA* genes (*ENA1*, *ENA2*, *ENA5*) are present in the BY4741  
313 strain and have a very similar function (salt tolerance, and possibly Li tolerance). Nha1p is a  
314 Na<sup>+</sup>/H<sup>+</sup> antiport whose activity varies according to the external pH. At pH 7, the quantity of  
315 lithium that was effluxed was 4 and 6 times lower than those at pH 5.5 and 3.5, respectively  
316 [42]. Our screening was performed at pH 6.5, therefore in this condition, the role of Nha1p in  
317 Li efflux could be minor compared to that played by the ENA proteins, explaining why this  
318 mutant was not found as sensitive. Li and Na ions have a very similar ionic radius [43], most  
319 likely explaining this nonspecificity. Li accumulation in the vacuole also occurs [44], but the  
320 transport system has not yet been identified. Under Li stress, yeast could partly internalize Li  
321 in vacuoles, thus limiting its toxicity. In line with this hypothesis, 14 mutants involved in  
322 vacuolar protein sorting (VPS) [45,46] were retrieved in the present study.

323 Finally, a comparative analysis of sensitive mutants was derived from an *in silico*  
324 analysis using the experimental data described above for alkali metals and alkaline earth  
325 metals as well as from the literature data reporting similar yeast mutant screenings with  
326 transition metals and lanthanides (Figure 3, Supplemental Table S3). The sensitive phenotype  
327 was poorly conserved when considering other elements (Figure 3). Three clusters (#4, #6, #8)  
328 out of the eight that were identified were characterized by rare mutants exhibiting a phenotype  
329 sensitive to transition metals and lanthanides. In these clusters, functions involved in  
330 nucleotide binding, RNA degradation or modification by phosphorylation could be listed.  
331 However, a high similarity of the response was found for As (41%) and Cd (52%). This was  
332 the case for a majority of mutants retrieved within clusters #1, #2, #3, #5, #7 that showed a  
333 predominance of the phenotype sensitive to As and Cd (Figure 3) and gathering  
334 vacuolar/lysosomal transport (*VAM7*, *VPS25*, *VPS1*, *VPS51*, *SNF7*, *PEP3*, *VPS34*, *VPS33*,  
335 *ARP5*, *VPS75*, *VPS27*, *PEP12*, *SNF8*, *VPS16*, *VPS28*, *BRO1*, *VMA11*, *VPS4*), regulation of C-  
336 compound and carbohydrate metabolism (*CCR4*, *PTC1*, *PBS2*, *VPS25*, *SNF7*, *HOG1*, *PFK2*,  
337 *SNF8*, *TAF14*) or actin cytoskeleton (*SLA1*, *SAC6*, *MNN10*, *VPS52*, *PBS2*, *VRP1*, *END3*).  
338 Yeast tolerance to As and Cd was reported to be managed through vacuolar internalization

339 [47,48]. Furthermore, Perkins and Gadd (1993) reported that Li was mainly  
340 compartmentalized in the yeast vacuole. Taken together, these data reinforce the idea that  
341 vacuolar transport is an important mechanism in the resistance of *S. cerevisiae* to Li. Another  
342 cluster (#2) showed a particular pattern. The 12 mutants from this cluster exhibited a sensitive  
343 phenotype to most of the metals, except Al, for which a very low number of mutants were  
344 retrieved from the screening [23,26]. This cluster was composed of 50% HS mutants, and  
345 vacuolar/lysosomal transport was the main function identified. This cluster therefore groups  
346 mutants for which the deleted genes likely play a common role in mediating resistance to a  
347 large set of metals, including Li, possibly by sequestering metals into vacuoles. However,  
348 taking all the results together, the present data indicate that the sensitive phenotype of the  
349 mutants is highly conserved for alkali metals and alkaline earth metals but poorly conserved  
350 with transition metals and lanthanides.

351

### 352 **Proteins of the NMD pathway are specifically involved in Li resistance**

353 Considering the whole set of Li-sensitive mutants and all metals tested, we investigated  
354 mutants specifically sensitive to Li. Only three mutants specifically sensitive to Li were  
355 identified: *NAM7* (alias *UPF1*), *NMD2* (alias *UPF2*) and *UPF3*. The mutants for the two first  
356 genes were categorized as LS, whereas the third one was classified as MS from the quaternary  
357 screening. The encoded UPF proteins are part of the NMD (nonsense-mediated mRNA decay)  
358 pathway that recognizes and degrades mRNAs containing premature termination codons [49–  
359 52].

360 To quantify the tolerance of the WT and the UPF mutants, EC<sub>50</sub> values were  
361 determined from liquid cultures and a range of Li concentrations. The three mutants  
362 harboured significantly lower EC<sub>50</sub> values compared to that of the WT, approximately 140  
363 mM Li for the *UPF* mutants versus 175 mM for the WT (Figure 4A), confirming the Li-  
364 sensitive phenotype of these mutants. Yeast sensitivity to metals is often related to higher  
365 metal accumulation by cells. Therefore, we investigated the Li content of the mutants in the  
366 presence of a nonlethal concentration (EC<sub>10</sub>) of Li. There was no significant difference in Li  
367 accumulation between *UPF3* and WT cells (Figure 4B). Conversely, the *NMD2* and *NAM7*  
368 mutants significantly accumulated 1.9 and 1.7 times more Li than the WT strain. This higher  
369 accumulation of Li very likely induced a concomitant higher toxicity for cells. We  
370 hypothesize that this Li overaccumulation phenotype could be due either to a higher Li entry  
371 into cells or to a lower efflux out of cells. However, a Li efflux experiment was performed

372 and revealed that Li efflux was similar for the three mutants and the WT (data not shown),  
373 refuting the second hypothesis. Therefore, Li entry into cells is likely to be more important in  
374 NMD mutants, explaining the higher toxicity that was observed.

375 The NMD pathway could modulate metal ion homeostasis and detoxification. Indeed,  
376 Zhang and Kebaara (2022) reported that the transport of Mg, Zn, Cu, Cd and Fe was regulated  
377 by the NMD pathway, either by acting directly on the transporter or indirectly through a  
378 regulator. Mutating one of the three UPF genes increased yeast tolerance to Cu by regulating  
379 COX mRNAs in the mitochondria [54]. Conversely, if lactate, a nonfermentable carbon  
380 source, was used, the single *UPF* mutants had a growth response similar to that of the WT  
381 under Cu stress [55]. In the case of Li, how the NMD pathway interacts with Li homeostasis  
382 remains an open question. However, considering the opposite phenotype of the *UPF* mutants  
383 under Cu and Li stresses, the mRNA targets are likely different under Cu and Li exposure.  
384 Since the NMD pathway regulates hundreds of genes in yeast [56], detailed investigations are  
385 needed to decipher which UPF gene(s) are involved and identify their targets under Li stress.  
386 Some genes may be specifically regulated by only one of the three UPF proteins. Notably, it  
387 was reported that *NAM7* affects the translation of the phosphoglucomutase *PGM2* by  
388 interacting with its 5' UTR, explaining the high sensitivity to Li of the *PGM2* mutant in a  
389 medium containing galactose as the sole source of C [57]. Nevertheless, the majority of the  
390 mRNAs that are targets of NMD are regulated by the three UPF proteins [56]. As the three  
391 UPF mutants were all sensitive to Li in the present study, their targets under Li stress could be  
392 the same. However, this point deserves further attention, especially since *UPF* mutants were  
393 the only Li-specific mutants. Moreover, as functions of the NMD pathway are conserved in  
394 eukaryotic organisms [58], this pathway could also participate in Li resistance in other  
395 eukaryotes by eliminating abnormal mRNAs produced under Li stress.

396

397

### 398 **Li-resistant mutants are mostly resistant to Na but show dissimilar behaviour toward** 399 **other metals**

400 The study of resistant mutants allowed us to identify the functions involved in yeast  
401 sensitivity under Li stress. A comparative mutant clustering analysis showed that the patterns  
402 were globally conserved under Li and Na (Figure 5, Supplemental Table S4). Indeed, 41  
403 mutants out of 60 were both Li- and Na-resistant (Supplemental Figure S4B, Supplemental  
404 Table S2). Conversely, among the Li-resistant mutants, only 16 and 13 mutants were resistant  
405 to Ca and K, respectively. Moreover, only two resistant mutants were found for Mg. One

406 cluster (#1) out of five grouped 10 Li-resistant mutants that were all sensitive to all other  
407 alkali metals and alkaline earth metals. Interestingly, four out of the 10 mutants corresponded  
408 to deleted genes encoding transcription regulators (e.g., Gzf3p, Spt20p, Gcn5p and Ngg1p).  
409 Other clusters (#2-5) grouped mutants that were mostly resistant to both Li and Na but rarely  
410 to other metals.

411 Unlike sensitive mutants, as revealed by the functional enrichment analysis (Figure 2A  
412 & 2B), no cluster was uniquely involved in the resistance phenotype (Figure 2A). This could  
413 be due to a twofold lower number of resistant mutants obtained from the quaternary screening  
414 (Figure 1). However, among the clusters where the proportion of resistant mutants was the  
415 highest, chromatin organization ( $p$  value = 3.18E-04), signal transduction ( $p$  value = 5.23E-  
416 04), AKT family ( $p$  value = 2.08E-05), cell communication ( $p$  value = 3.05E-05) or  
417 transcription process ( $p$  value = 9.97E-06) were found and composed of 38, 37, 36, 35 and  
418 34% resistant mutants, of which 25, 17, 50, 44 and 40% were HR, respectively (Figure 2B,  
419 Supplemental Table S1). The main cluster gathering the functions involved in yeast sensitivity  
420 was the AKT family cluster and was also retrieved, but to a lesser extent, from the functional  
421 enrichment analysis in the presence of Mg and K (36% for Li against 21% and 24% for Mg  
422 and K, respectively) (Figure 2B). Similarly, the four other clusters described above also had a  
423 proportion of resistant mutants that was higher under Li stress than under other alkali and  
424 alkaline-earth metals (Figure 2B). A functional analysis of the genes found in these five  
425 clusters indicated that they were mainly related to phosphorylation/dephosphorylation and  
426 acetylation/deacetylation activities ( $p$  value = 8.28E-12). Protein synthesis initiation is a target  
427 of salt toxicity [11]. Indeed, mutation of the protein kinase Gcn2p improves salt tolerance  
428 [59]. This kinase regulates the initiation of protein synthesis by phosphorylating the initiation  
429 factor eIF2 [60]. Gcn2p is overactivated during Na stress, which is deleterious for cell growth.  
430 Given the high similarity of responses between Na and Li found in the present study, we  
431 could hypothesize a similar impact of Li on protein translation mediated by  
432 phosphorylation/dephosphorylation (Npr1p, Sky1p, Ypk3p, Ypk1p) and  
433 acetylation/deacetylation (Spt20p, Gcn5p, Ngg1p, Spt8p, Pdb1p) activities of proteins found  
434 within these clusters, which would consequently affect yeast survival under Li stress.  
435 Altogether, our data highlight that the potential toxicity targets are similar for Li and Na but  
436 dissimilar for other alkali, alkaline earth, and transition metals.

437

438 **A small set of genes are specifically involved in Li cytotoxicity**

439 The screening allowed the identification of six mutants specifically resistant to Li (Figure 5).  
440 These mutants were *YOR376W* (MR), *DCW1* (LR), *NCA2* (HR), *YPK3* (MR), *GCN5* (LR),  
441 and *SPT20* (LR). The Li tolerance level of the WT and the six resistant mutants was further  
442 examined by determining the EC<sub>50</sub> values. With EC<sub>50</sub> varying between 189 mM and 315  
443 mM for the mutants against 175 mM Li for WT, all the mutants had a higher EC<sub>50</sub> than the  
444 WT, although for *GCN5*, the difference was not significant (Figure 6A). These results  
445 therefore confirmed the Li-resistance phenotype of these mutants.

446 Among the six Li-specific resistant mutants, *SPT20*, *GCN5* and *YPK3* were grouped in  
447 the peptidyl-amino acid modification cluster (Figure 2A, Supplemental Table S1). Spt20p and  
448 Gcn5p are part of the SAGA (Spt-Ada-Gcn5 acetyltransferase) complex [61], which regulates  
449 approximately 10% of the genes in *S. cerevisiae* [62]. It is a multifunctional complex involved  
450 in histone acetylation and deubiquitination [63,64] or in mRNA export [65]. Ypk3p is an  
451 AGC kinase (protein kinase A/protein kinase G/protein kinase C family) essential to the  
452 TORC1 pathway [66]. TORC1 denotes Target of Rapamycin Complex 1, one of the two  
453 complexes of the TOR pathway [67]. Genetic interactions between the SAGA complex and  
454 the TORC1 pathway have been demonstrated in *Schizosaccharomyces pombe* [68].  
455 Interestingly, the SAGA complex was shown to control the expression of key genes needed  
456 for Cd tolerance in *S. pombe* [69]. The *GCN5* and *SPT20* KO mutants of *S. pombe* were  
457 among the most Cd-sensitive mutants. Similarly, in *S. cerevisiae*, these mutants were also  
458 reported as Cd-sensitive [31] (Figure 5). The components and functions of the SAGA  
459 complex are highly conserved between many species [70,71], which explains why the SAGA  
460 pathway likely plays a role in Cd resistance in both yeast species. However, in the present  
461 study, the opposite phenotype for Li was found for the *GCN5* and *SPT20* mutants (resistant  
462 mutants). We can therefore hypothesize that Gcn5p and Spt20p might regulate key genes  
463 other than those involved in the Cd response. This hypothesis could be extended to other  
464 alkali metals and alkaline earth metals since they also induced a sensitive phenotype for the  
465 *GCN5* and *SPT20* mutants (Figure 5). The SAGA complex is mainly considered an activator  
466 complex, but it can also inhibit some genes through Gcn5p, for instance [72]. In conclusion,  
467 our data suggest that the SAGA complex could (i) be involved in the activation of gene(s)  
468 responsible for Li sensitivity and/or (ii) repress gene(s) promoting Li resistance in yeast. We  
469 further investigated whether the Li resistance phenotype of these mutants could result from  
470 reduced Li accumulation in cells. The ICP-MS results obtained for Li accumulation in these  
471 three mutants were variable (Figure 6B). Indeed, the *YPK3* and *GCN5* mutants accumulated



472 1.7 and 1.2 times more Li than the WT, respectively, although it was not significant for  
473 *GNC5*. Conversely, *SPT20* accumulated 0.6 times less Li than the WT. These results  
474 demonstrate a different accumulation capacity for these Li-specific resistant mutants. Since Li  
475 accumulation was not reduced in *GNC5* and *YPK3* mutant cells, their Li-resistant phenotype  
476 is likely independent of Li accumulation and can be better explained if Gcn5p and Ypk3p are  
477 targets of Li.

478 The second group of Li-specific resistant mutants contained *DCW1* and *NCA2*, which  
479 belong to the macromolecule catabolic process cluster, and *YOR376W*, a gene encoding a  
480 protein of unknown function (Figure 2A, Supplemental Table S1). *DCW1* encodes a putative  
481 mannosidase that is essential for cell wall biogenesis. A mutation of *DCW1* causes cell wall  
482 malformations [73]. *NCA2* regulates the expression of the F0-F1 ATP synthase subunits [74].  
483 In a genome-wide screening, the three mutants were found to affect vacuole membrane  
484 fragmentation under NaCl stress [75]. We hypothesized that a similar phenotype occurs for  
485 these mutants under Li stress. Induction of fragmentation would produce several small  
486 vacuoles, therefore diminishing the volume for storing Li and contributing to enhanced  
487 cytotoxicity. However, the three mutants harboured the opposite phenotype of resistance to  
488 Li, which is in disagreement with the given hypothesis. Moreover, the three mutants had a  
489 higher cellular Li content compared to the WT, although the data were only significant for the  
490 *YOR376W* mutant (Figure 6B). Altogether, our data suggest the existence of other, yet  
491 unknown, mechanisms. As described above for Gcn5p and Ypk3p, the resistant phenotypes of  
492 *DCW1*, *NCA2* and *YOR376W* were not accompanied by a reduction in Li content in cells and  
493 therefore can be better explained if these proteins are targets of Li.

494

## 495 **Conclusion**

496 An in-depth study of the results of Li screenings of a collection of KO mutants identified  
497 potential cellular targets and mechanisms of resistance to Li in yeast. Transport systems are  
498 involved in resistance to Li and other alkali/alkaline earth metals. The NMD pathway is a  
499 resistance mechanism specific to Li, a pathway generally involved in sensitivity to other  
500 metals. In contrast, the AKT family, signal transduction, chromatin organization, transcription  
501 process or cell communication are the main functions involved in Li sensitivity, with the  
502 SAGA complex as a Li-specific target. The majority of putative cellular targets are shared  
503 between Na and Li. Proteomic or transcriptomic studies should be designed to investigate  
504 these data further, notably to reveal the details of the roles played by the NMD pathway and

505 the SAGA complex under Li. In conclusion, the present data obtained on this eukaryotic  
506 model species suggest new perspectives to better understand the environmental impact of Li,  
507 a major emerging metallic contaminant, on living organisms.

508

### 509 **Author contributions**

510 Conceptualization: NF, PBa, PBi and DB, data analysis: NF, writing and editing the  
511 manuscript: NF, PBa, PBi and DB. Project management and financial support: PBa, DB. All  
512 the authors have read and approved the final manuscript.

513

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524

### 525 **Conflict of interest**

526 The authors declare no conflict of interest.

527

### 528 **Data availability**

529 All the data described is available in the online Supplemental material.

530

531

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719 *Saccharomyces cerevisiae*. *PLoS One* 2013;**8**:e54160.

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721

722 **Table 1: List of the 124 lithium-sensitive mutants identified from the quaternary**  
 723 **screening.**

724 The mutants were classified according to their level of sensitivity compared to the WT after  
 725 exposure to a range of Li concentrations. Mutants inhibited by more than 50% at 50 mM Li  
 726 were classified as highly sensitive. Mutants that had residual growth between 50% and 90% at  
 727 50 mM Li were classified as medium sensitive. Low-sensitivity mutants were those for which  
 728 growth was below 90% at 100 mM and 150 mM Li.

Systematic name	Standard name	Systematic name	Standard name	Systematic name	Standard name	Systematic name	Standard name
<b>High sensitive mutants (31/124)</b>							
YCR008W	<i>SAT4</i>	YFR040W	<i>SAP155</i>	YKR001C	<i>VPS1</i>	YLR396C	<i>VPS33</i>
YDR069C	<i>DOA4</i>	YGL038C	<i>OCH1</i>	YKR020W	<i>VPS51</i>	YML008C	<i>ERG6</i>
YDR195W	<i>REF2</i>	YGL246C	<i>RAI1</i>	YLR025W	<i>SNF7</i>	YNL294C	<i>RIM21</i>
YDR207C	<i>UME6</i>	YGR056W	<i>RSC1</i>	YLR048W	<i>RPS0B</i>	YPL002C	<i>SNF8</i>
YDR245W	<i>MNN10</i>	YGR285C	<i>ZUO1</i>	YLR148W	<i>PEP3</i>	YPL045W	<i>VPS16</i>
YDR484W	<i>VPS52</i>	YHR141C	<i>RPL42B</i>	YLR192C	<i>HCR1</i>	YPL129W	<i>TAF14</i>
YDR532C	<i>KRE28</i>	YJL129C	<i>TRK1</i>	YLR226W	<i>BUR2</i>	YPL271W	<i>ATP15</i>
YER083C	<i>GET2</i>	YJR043C	<i>POL32</i>	YLR240W	<i>VPS34</i>		
<b>Medium sensitive mutants (48/124)</b>							
YBL006C	<i>LDB7</i>	YDR364C	<i>CDC40</i>	YLR056W	<i>ERG3</i>	YNL170W	-
YBL007C	<i>SLA1</i>	YEL050C	<i>RML2</i>	YLR113W	<i>HOG1</i>	YNL171C	-
YBL025W	<i>RRN10</i>	YGL012W	<i>ERG4</i>	YLR235C	-	YNL298W	<i>CLA4</i>
YBR082C	<i>UBC4</i>	YGL045W	<i>RIM8</i>	YLR262C	<i>YPT6</i>	YOR030W	<i>DFG16</i>
YBR200W	<i>BEM1</i>	YGL173C	<i>XRN1</i>	YLR322W	<i>VPS65</i>	YOR080W	<i>DIA2</i>
YCR094W	<i>CDC50</i>	YGR072W	<i>UPF3</i>	YLR330W	<i>CHS5</i>	YPL065W	<i>VPS28</i>
				YMR031W-			
YDL006W	<i>PTC1</i>	YHR205W	<i>SCH9</i>	A	-	YPL084W	<i>BRO1</i>
YDL213C	<i>NOP6</i>	YJL006C	<i>CTK2</i>	YMR075W	<i>RCO1</i>	YPL157W	<i>TGS1</i>
YDR049W	<i>VMS1</i>	YJL075C	<i>APQ13</i>	YMR205C	<i>PFK2</i>	YPL234C	<i>VMA11</i>
YDR137W	<i>RGP1</i>	YJL165C	<i>HAL5</i>	YNL016W	<i>PUB1</i>	YPR045C	<i>THP3</i>
YDR138W	<i>HPR1</i>	YJR102C	<i>VPS25</i>	YNL059C	<i>ARP5</i>	YPR163C	<i>TIF3</i>
YDR293C	<i>SSD1</i>	YKL158W	<i>APE2</i>	YNL084C	<i>END3</i>	YPR173C	<i>VPS4</i>
<b>Low sensitive mutants (45/124)</b>							
YAL021C	<i>CCR4</i>	YDR389W	<i>SAC7</i>	YKL190W	<i>CNB1</i>	YNR006W	<i>VPS27</i>
YAR020C	<i>PAU7</i>	YFL013C	<i>IES1</i>	YKR035C	<i>OPI8</i>	YOR006C	<i>TSR3</i>
YBL024W	<i>NCL1</i>	YGL003C	<i>CDH1</i>	YKR072C	<i>SIS2</i>	YOR036W	<i>PEP12</i>
YBR036C	<i>CSG2</i>	YGL019W	<i>CKB1</i>	YKR073C	-	YOR141C	<i>ARP8</i>
YBR231C	<i>SWC5</i>	YGL046W	-	YLR039C	<i>RIC1</i>	YPL194W	<i>DDC1</i>
YBR246W	<i>RRT2</i>	YGL212W	<i>VAM7</i>	YLR234W	<i>TOP3</i>	YPR023C	<i>EAF3</i>
YDL001W	<i>RMD1</i>	YGR122W	-	YLR337C	<i>VRP1</i>	YPR135W	<i>CTF4</i>
YDL020C	<i>RPN4</i>	YHL027W	<i>RIM101</i>	YML057W	<i>CMP2</i>	YPR160W	<i>GPH1</i>
YDR072C	<i>IPT1</i>	YHR077C	<i>NMD2</i>	YMR080C	<i>NAM7</i>	YPR194C	<i>OPT2</i>
YDR127W	<i>ARO1</i>	YHR200W	<i>RPN10</i>	YNL064C	<i>YDJ1</i>		
YDR129C	<i>SAC6</i>	YIL076W	<i>SEC28</i>	YNL246W	<i>VPS75</i>		
YDR136C	<i>VPS61</i>	YJL128C	<i>PBS2</i>	YNL307C	<i>MCK1</i>		



730

731 **Table 2: List of the 60 lithium-resistant mutants identified from the quaternary**  
 732 **screening.**

733 The mutants were classified according their level of resistance compared to the WT after  
 734 exposure to a range of Li concentrations. Mutants harbouring a growth above 150% at 100  
 735 mM Li were classified as high resistance. Mutants exhibiting between 110% and 150%  
 736 growth at 100 mM Li were classified as medium resistance. Low-resistance mutants had  
 737 growth above 110% at 200 mM Li.

Systematic name	Standard name	Systematic name	Standard name	Systematic name	Standard name	Systematic name	Standard name
<b>High resistant mutants (24/60)</b>							
YBR085c-A	-	YER167W	<i>BCK2</i>	YJR078W	<i>BNA2</i>	YNL323W	<i>LEM3</i>
YBR121C	<i>GRS1</i>	YGL066W	<i>SGF73</i>	YKL126W	<i>YPK1</i>	YOR241W	<i>MET7</i>
YDL197C	<i>ASF2</i>	YGL095C	<i>VPS45</i>	YLR244C	<i>MAP1</i>	YOR375C	<i>GDH1</i>
YDR043C	<i>NRG1</i>	YGL168W	<i>HUR1</i>	YMR216C	<i>SKY1</i>	YPL062W	-
YDR199W	-	YIL130W	<i>ASG1</i>	YNL164C	<i>IBD2</i>	YPL137C	<i>GIP3</i>
YEL045C	-	YJL190C	<i>RPS22A</i>	YNL229C	<i>URE2</i>	YPR155C	<i>NCA2</i>
<b>Medium resistant mutants (26/60)</b>							
YAL049C	<i>AIM2</i>	YDR176W	<i>NGG1</i>	YLR391W	-	YOL114C	<i>PTH4</i>
YBR028C	<i>YPK3</i>	YDR297W	<i>SUR2</i>	YLR455W	<i>PDP3</i>	YOR220W	<i>RCN2</i>
YBR044C	<i>TCM62</i>	YJL024C	<i>APS3</i>	YML006C	<i>GIS4</i>	YOR376W	-
YCL001w-B	-	YKR055W	<i>RHO4</i>	YML016C	<i>PPZ1</i>	YPL061W	<i>ALD6</i>
YDL081C	<i>RPP1A</i>	YLR055C	<i>SPT8</i>	YMR116C	<i>ASC1</i>	YPL138C	<i>SPPI</i>
YDL133C-A	<i>RPL41B</i>	YLR374C	-	YMR269W	<i>TMA23</i>		
YDR112W	<i>IRC2</i>	YLR375W	<i>STP3</i>	YNL183C	<i>NPR1</i>		
<b>Low resistant mutants (10/60)</b>							
YBR221C	<i>PDB1</i>	YGR252W	<i>GCN5</i>	YKL046C	<i>DCW1</i>	YNR052C	<i>POP2</i>
YDR200C	<i>VPS64</i>	YJL028W	<i>YJL028W</i>	YMR172W	<i>HOT1</i>	YOL148C	<i>SPT20</i>
YEL046C	<i>GLY1</i>	YJL110C	<i>GZF3</i>				

738

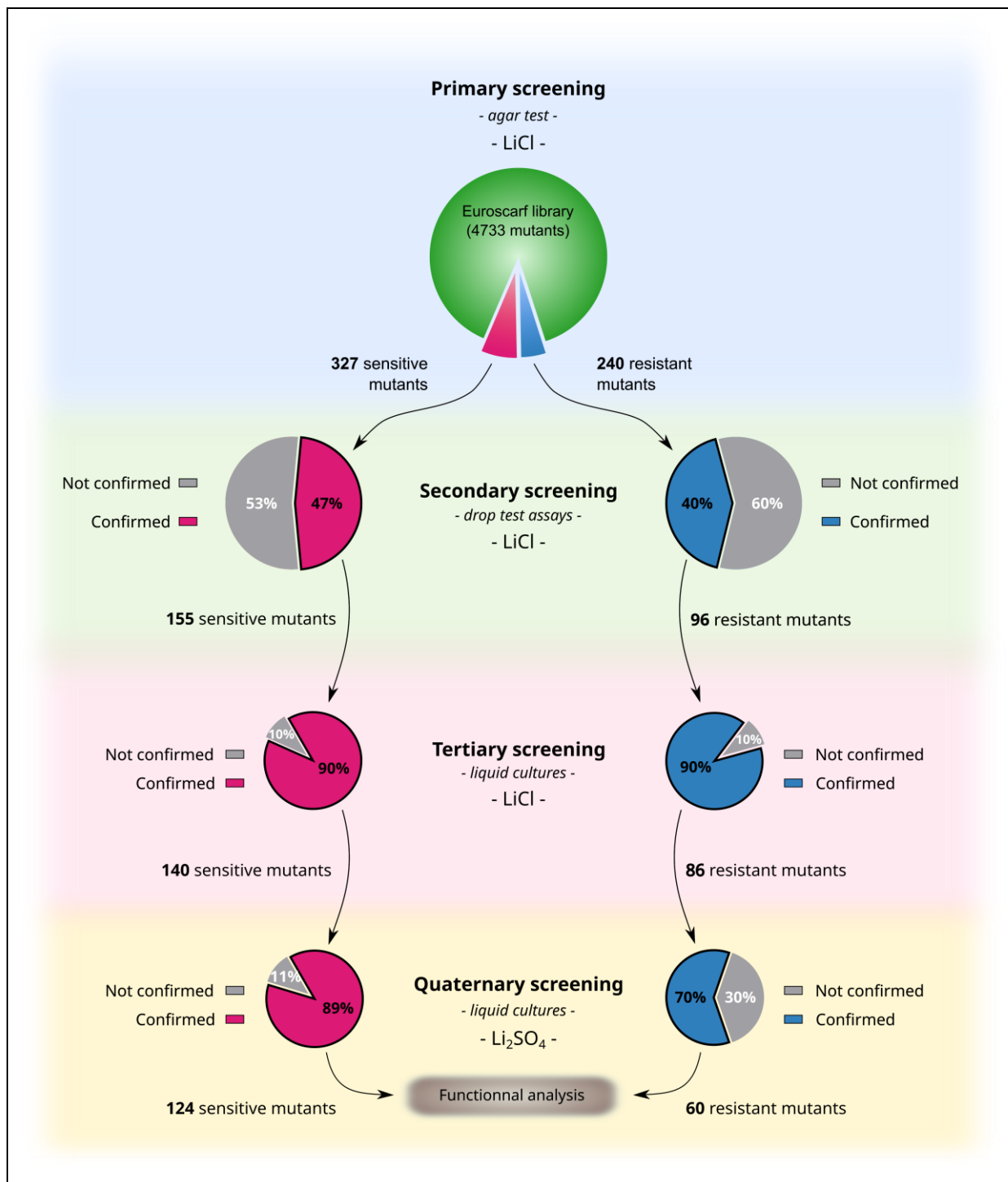
739

740 **Table 3: MIPS subcellular compartments involved in Li stress.** k is the number of mutants  
741 found from the quaternary screening. f is the total number of genes in a given function. NS:  
742 not significant; ER: Endoplasmic reticulum.

Subcellular compartment	All mutants			Sensitive mutants		Resistant mutants	
	f	<i>p</i> value	k	<i>p</i> value	k	<i>p</i> value	k
Endosome	57	2.17E-05	9	8.88E-06	8	NS	-
Cytoskeleton	107	2.65E-03	9	1.56E-04	9	NS	-
ER	537	6.29E-03	25	2.02E-03	20	NS	-
Golgi	125	7.40E-03	9	5.01E-04	9	NS	-
Cytoplasm	2879	1.63E-02	94	NS	-	NS	-
Nucleus	1976	2.56E-02	67	NS	-	1.45E-02	26
Vacuole	224	4.48E-02	11	8.71E-03	10	NS	-
Vacuolar membrane	98	NS	-	9.66E-03	6	NS	-
Actin cytoskeleton	54	NS	-	1.77E-02	4	NS	-
Golgi membrane	95	NS	-	3.17E-02	5	NS	-
ER membrane	131	NS	-	3.51E-02	6	NS	-
Nuclear envelope	167	NS	-	3.59E-02	7	NS	-

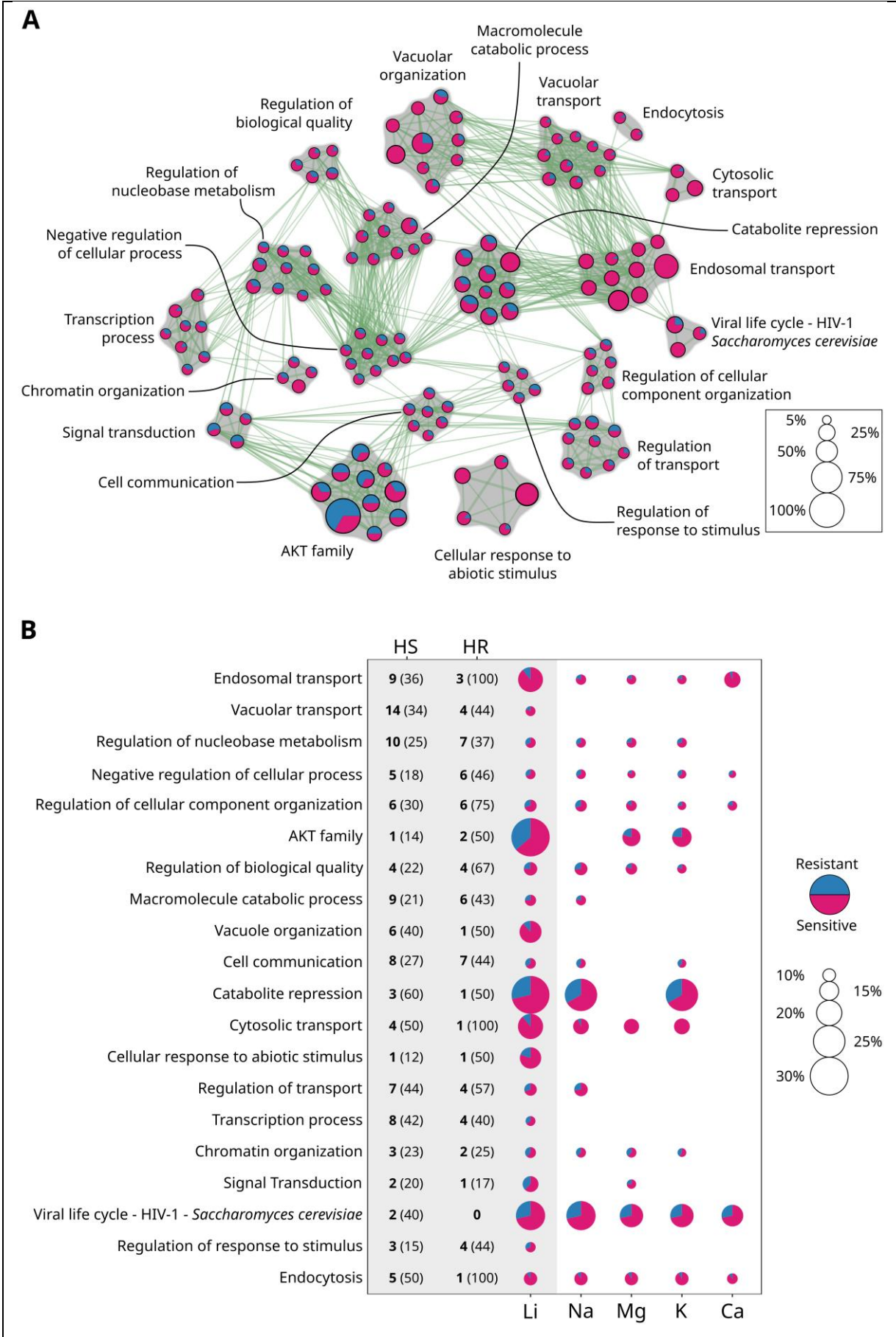
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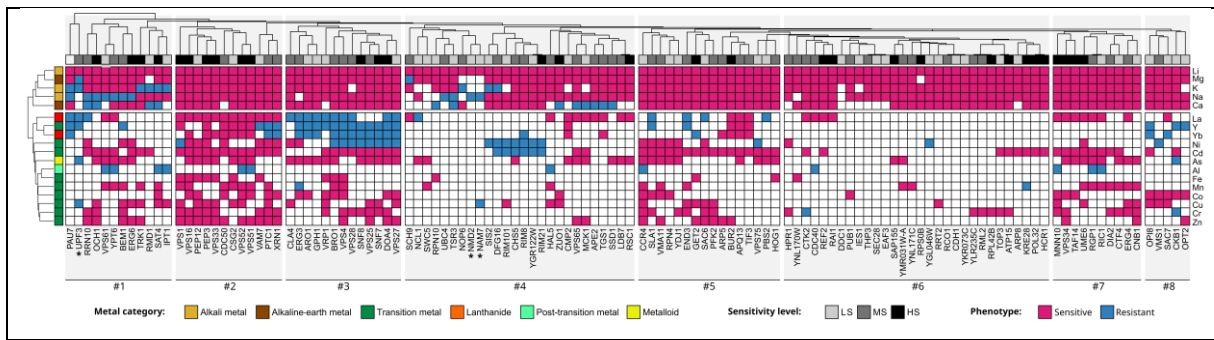


746 **Figure 1: Overview of the different steps of the deletomics screening to identify the**  
 747 **mutants sensitive and resistant to lithium.** The number of selected mutants is indicated for  
 748 each step of the screening. The percentage of false positives is also denoted within the pie  
 749 charts. Representative data from the primary, secondary, tertiary and quaternary screenings  
 750 are presented in Supplemental Figure S1.

751



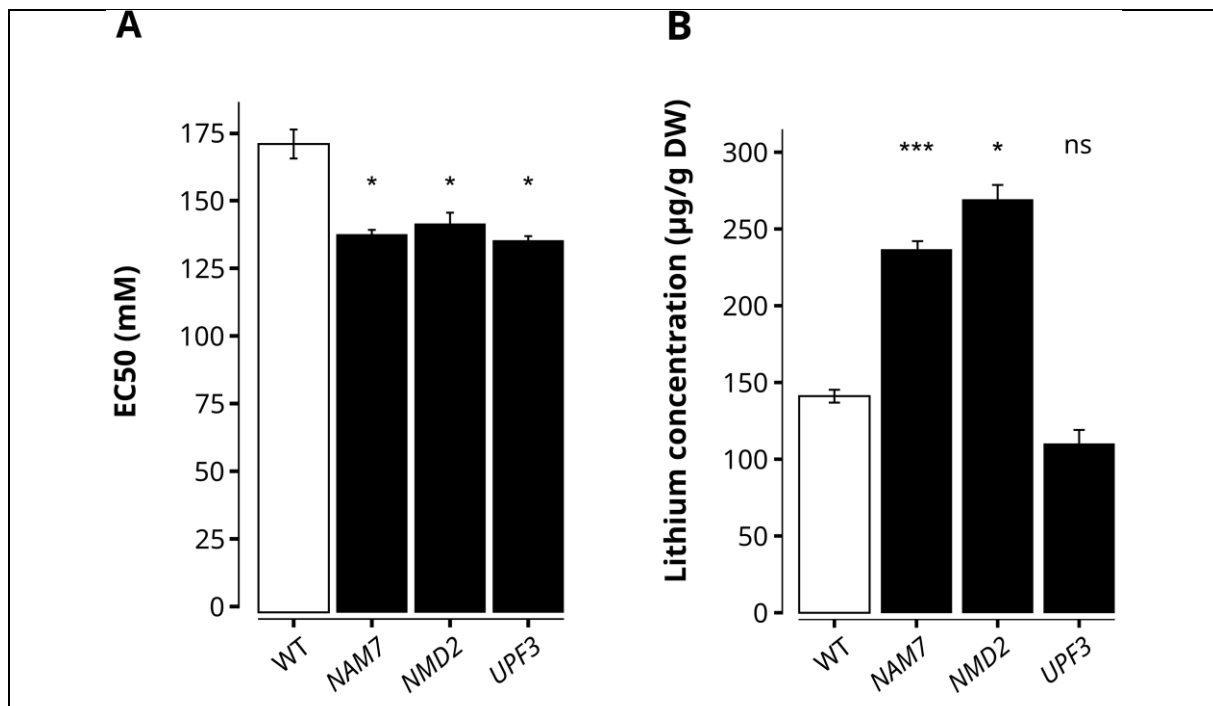
753 **Figure 2: Functional enrichment analyses of resistant and sensitive mutants to lithium**  
754 **(A) or to alkali and alkaline-earth metals (B).** The proportion of genes involved in the  
755 resistance (blue) and sensitivity (magenta) phenotypes is represented by pie charts inside each  
756 node (function) (A) or each functional cluster (B). (A) The size of each node/functional  
757 cluster corresponds to the number of genes found in a given category divided by the total  
758 number of genes in this category and expressed as a percentage. Green lines represent  
759 gene overlaps between two functions, and the edge width is proportional to the number of  
760 shared genes. (B) The number of Li high-sensitivity (HS) and high-resistance (HR) mutants  
761 are displayed on the left. Values in brackets denote the percentage of HS and HR mutants  
762 among the total number of mutants found in the functional analysis. The enrichment maps  
763 were built using Metascape and visualized with either Cytoscape (A) or the Scatterpie  
764 package in R (B).  
765



767

768 **Figure 3: Cross-comparison of lithium-sensitive mutants versus other metals.** The y-axis  
 769 corresponds to metallic stressors. The x-axis represents a hierarchical clustering of the genes  
 770 deleted that confer a sensitive phenotype to Li. Mutants exhibiting a sensitivity or  
 771 resistance phenotype are shown in magenta and blue, respectively. Clusters 1 to 8 include  
 772 mutants with a similar profile of sensitivity or resistance to metals. Asterisks indicate the three  
 773 mutants that are specifically sensitive to Li. LS: low sensitivity; MS: medium sensitivity; HS:  
 774 high sensitivity.

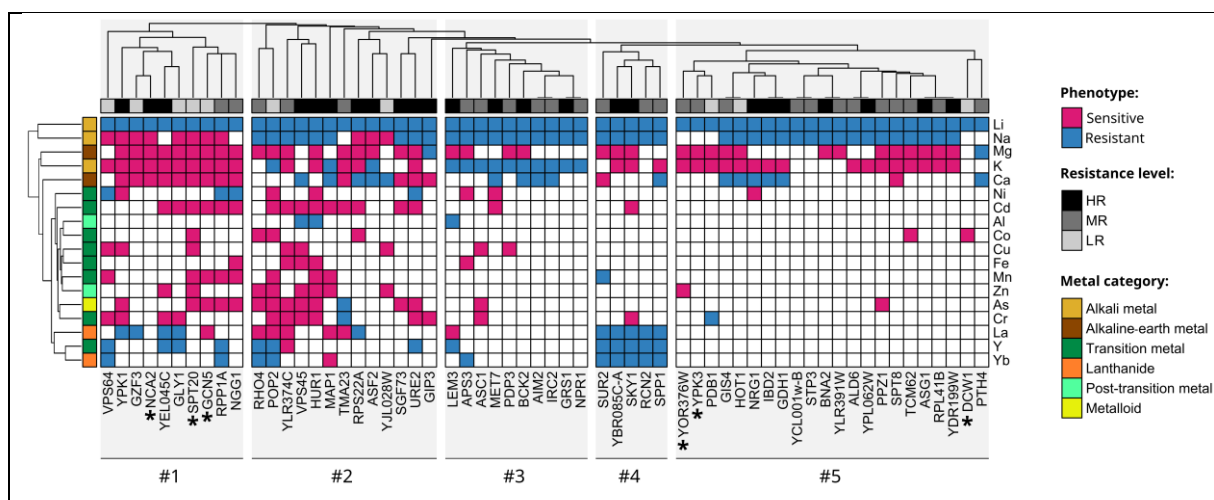
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777

778 **Figure 4: Sensitivity level and lithium accumulation by lithium-specific sensitive**  
 779 **mutants.** (A) EC50 values for LiCl for the three mutants and the WT. Data are the means (+/-  
 780 SE) of three independent liquid cultures. A *t* test comparison between the mutants and the  
 781 WT was carried out. (B) Yeast Li concentrations after one hour of exposure to 100 mM LiCl.  
 782 Data are the means of five and three independent cultures for the WT and the  
 783 mutants, respectively. Two-tailed Student's *t* tests to compare the different mutants to WT  
 784 were performed. For all experiments: \* $<0.05$ ; \*\*\* $<0.001$ ; ns: not significant.

785

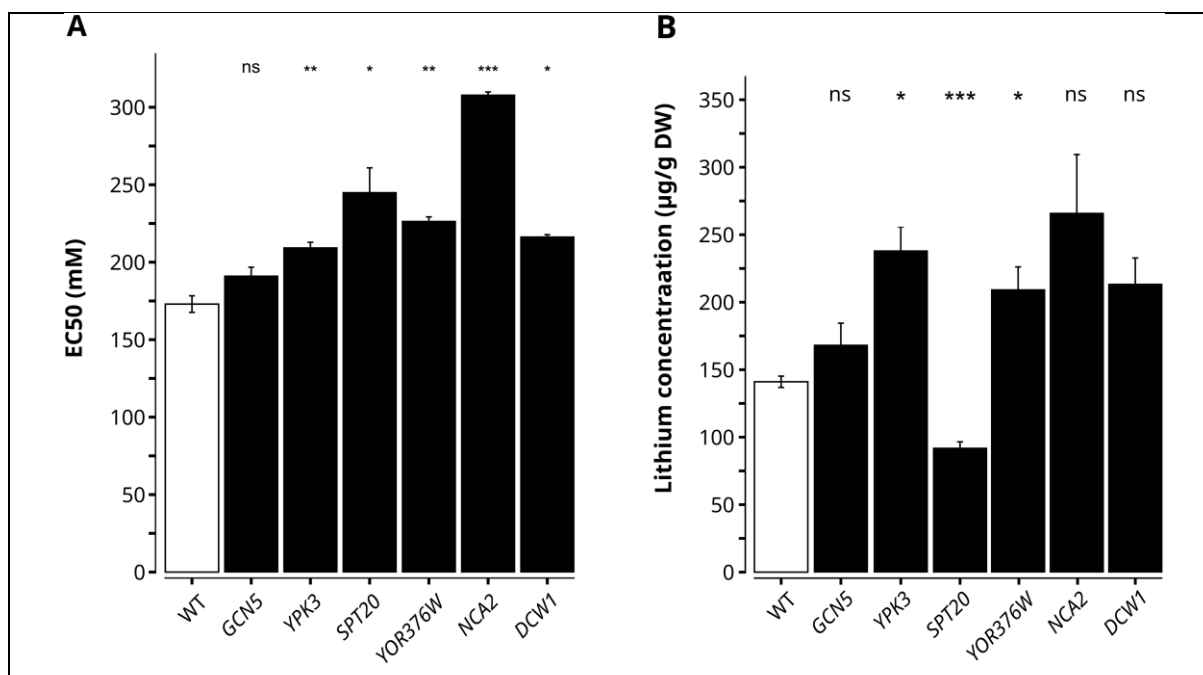


787

788 **Figure 5: Cross-comparison of lithium-resistant mutants versus other metals.** The y-axis  
 789 corresponds to metallic stressors. The x-axis represents a hierarchical clustering of the genes  
 790 deleted that confer a resistance phenotype to Li. Mutants exhibiting a sensitivity or  
 791 resistance phenotype are shown in magenta and blue, respectively. Clusters 1 to 5 gather  
 792 mutants with a similar profile of sensitivity or resistance to metals. Asterisks denote the six  
 793 mutants that are specifically resistant to Li. LR: low resistance; MR: medium resistance; HR:  
 794 high resistance.

795





797

798 **Figure 6: Resistance level and lithium accumulation by lithium-specific resistant**799 **mutants.** (A) EC50 values for LiCl for the six mutants and the WT. Data are the means (+/-800 SE) of three independent liquid cultures. A *t* test comparison between the mutants and the

801 WT was carried out. (B) Yeast Li concentrations after one hour of exposure to 100 mM LiCl.

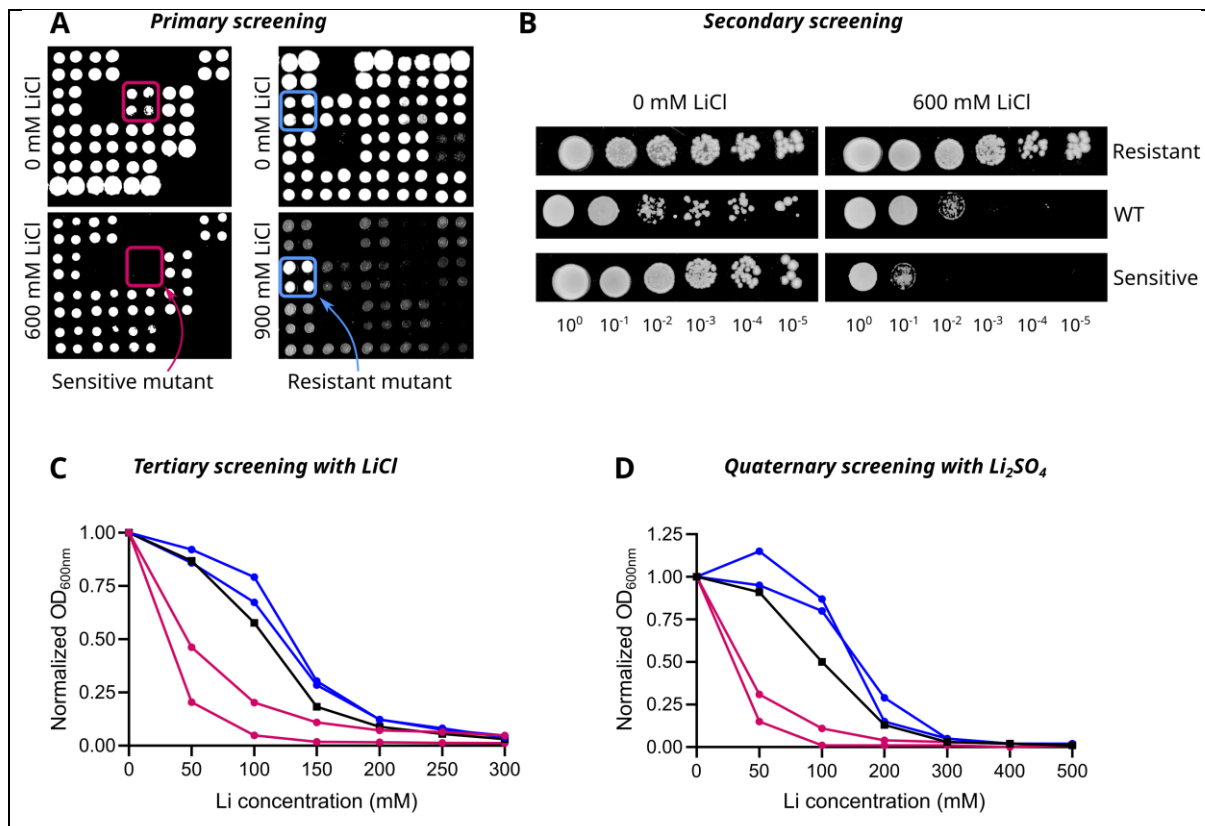
802 Data are the means of five and three independent cultures for the WT and the

803 mutants, respectively. Two-tailed Student's *t* tests to compare the different mutants to WT804 were performed. For all experiments: \* $<0.05$ ; \*\* $<0.01$ ; \*\*\* $<0.001$ ; ns: not significant.

805

806 **Supplemental data**

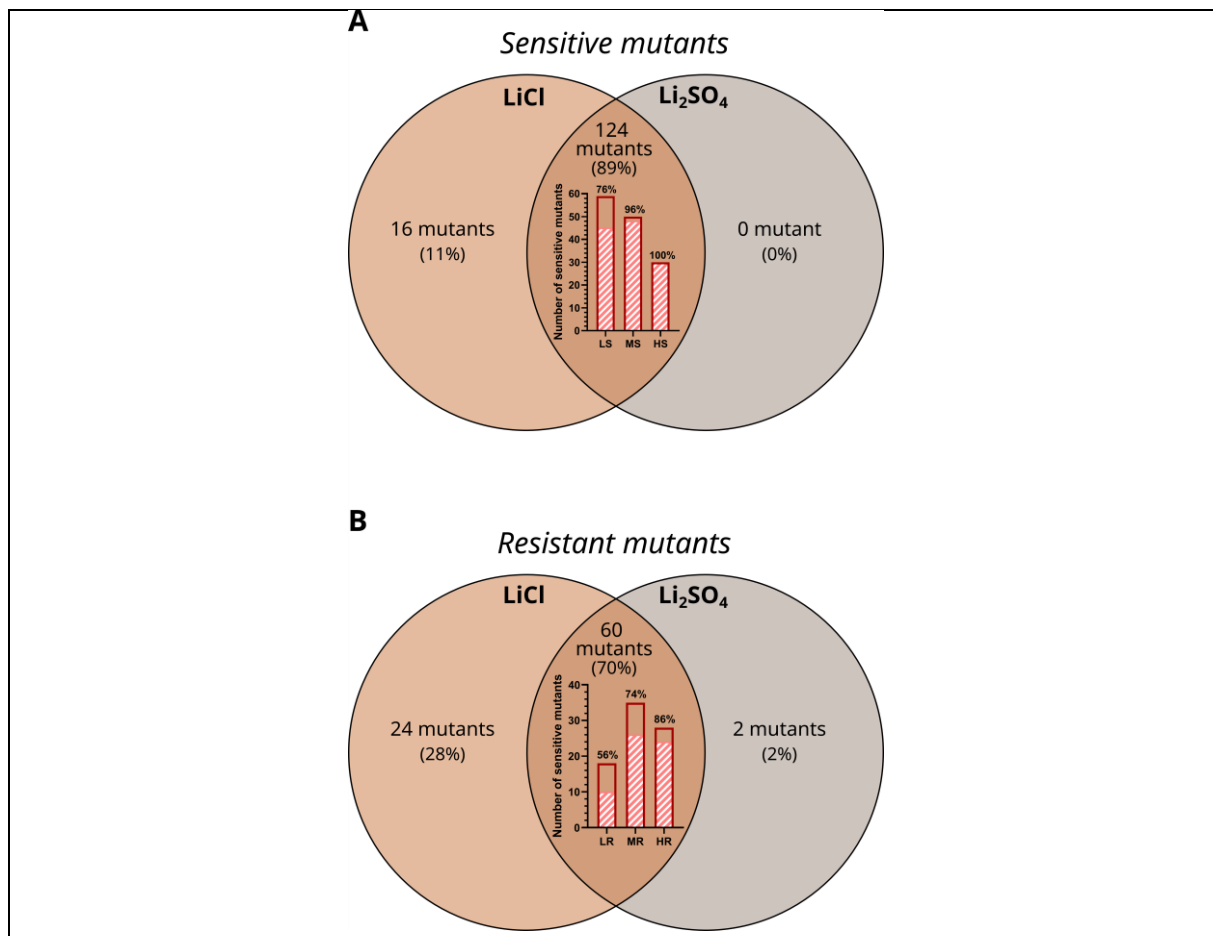
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809 **Supplemental Fig. S1: Representative data from the four screens of the Euroscarf**  
 810 **deletion mutant library in the presence of Li.** (A) Representative growth test of the primary  
 811 screening. Four replicas per mutant were performed on the same plate. Putative Li-sensitive  
 812 (magenta) and Li-resistant (blue) mutants are highlighted. (B) Drop test confirmation of the  
 813 sensitive and resistant phenotypes by a secondary screening. Tenfold serial dilutions of yeast  
 814 saturation cultures were deposited on the medium. The mutants were considered sensitive  
 815 when they exhibited less colony-forming ability than WT. Conversely, the mutants were  
 816 considered resistant when they exhibited better colony-forming ability than WT. (C) LiCl  
 817 concentration-dependent growth of the mutants (tertiary screening). (D) Li concentration-  
 818 dependent growth of the mutants under Li<sub>2</sub>SO<sub>4</sub> (quaternary screening). The OD data were  
 819 normalized to those obtained in Li-free medium, and a growth percentage in relation to the  
 820 WT was calculated. (C-D) Blue: resistant mutant; magenta: sensitive mutant; black: WT.

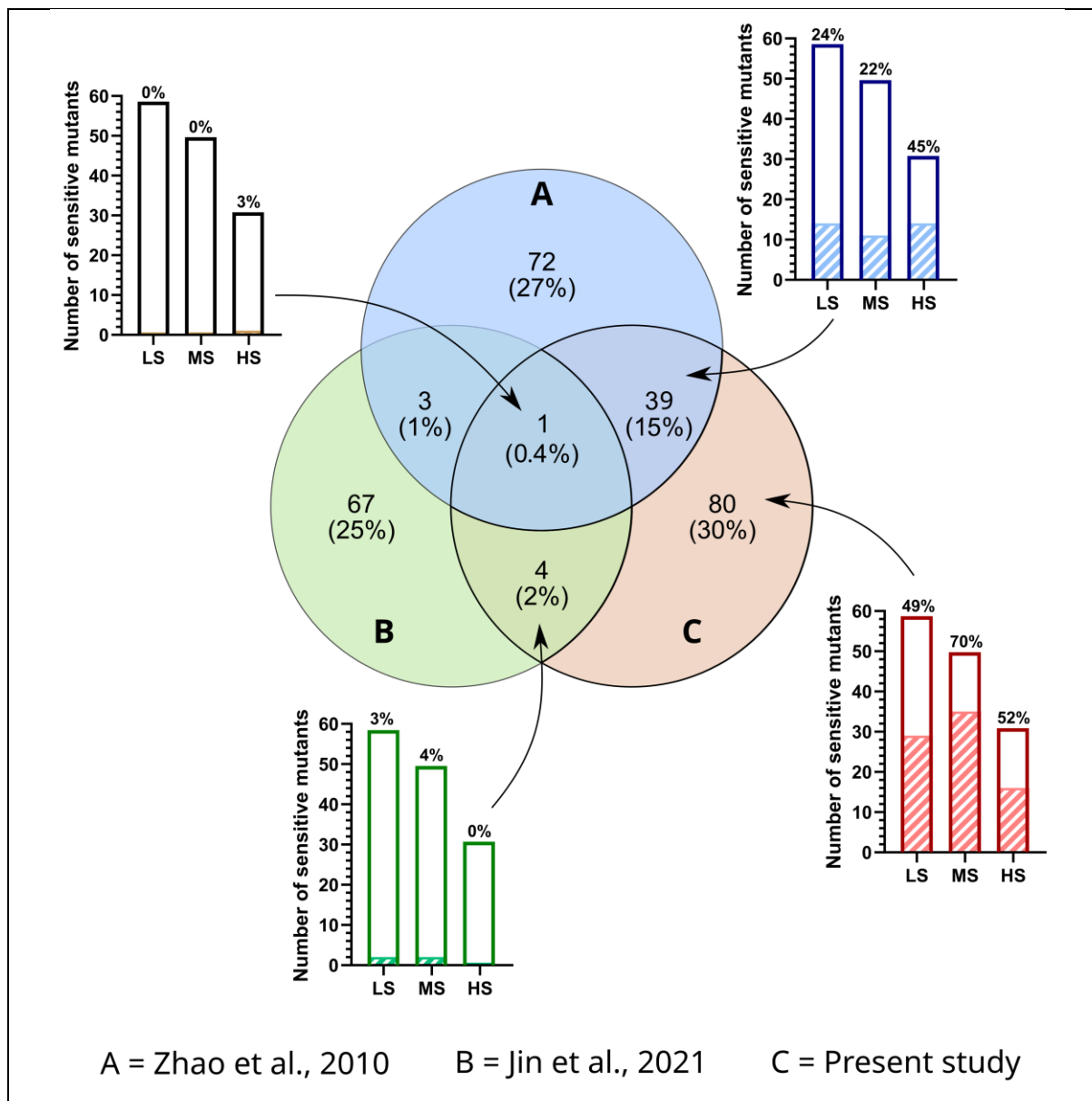
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823

824 **Supplemental Fig. S2: Venn diagram presenting the number of mutants sensitive (A) or**  
 825 **resistant (B) to LiCl (red) and Li<sub>2</sub>SO<sub>4</sub> (grey).** The distribution of mutants in each category is  
 826 denoted in parentheses. The numbers of low-sensitivity (LS), medium-sensitivity (MS), high-  
 827 sensitivity (HS), low-resistance (LR), medium-resistance (MR) and high-resistance (HR)  
 828 mutants are shown by hatching within the bars. The red bars represent the total number of  
 829 mutants in each category identified in the tertiary screening with LiCl. The proportion of  
 830 mutants in each category that were conserved after quaternary screening with Li<sub>2</sub>SO<sub>4</sub> is  
 831 displayed above the bars.

832



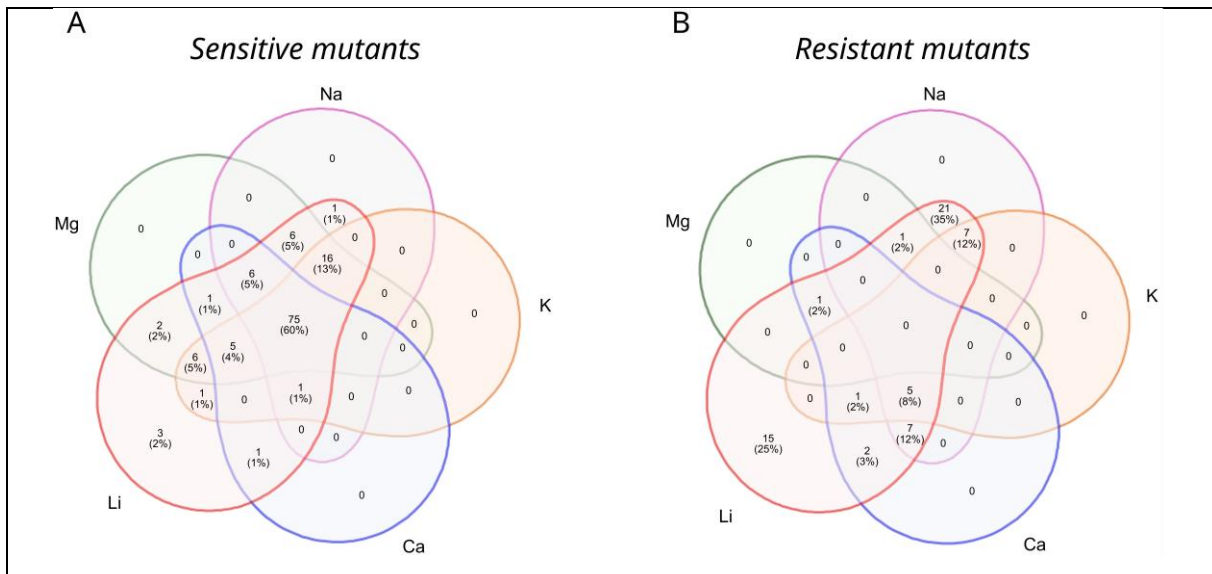
834

835 **Supplemental Fig. S3: Venn diagram comparing the number of lithium-sensitive**  
 836 **mutants identified from three lithium screening studies of yeast deletion mutant**  
 837 **libraries.** (A) Jin et al., (2021) in the presence of  $\text{LiPF}_6$ , (B) Zhao et al., (2010) in the  
 838 presence of  $\text{LiCl}$  and (C) our study after quaternary screening in the presence of  $\text{LiCl}$  and  
 839  $\text{Li}_2\text{SO}_4$ . The percentage distribution of mutants in each category is denoted in parentheses.

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843

844 **Supplemental Fig. S4: Venn diagram presenting the number of mutants sensitive (A) or**  
845 **resistant (B) to lithium and alkali metals or alkaline earth metals. The percentage**  
846 **distribution of mutants in each category is given in parentheses.**