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RESEARCH ARTICLE

# The toxicity of neodymium and genome-scale genetic screen of neodymium-sensitive gene deletion mutations in the yeast *Saccharomyces cerevisiae*

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**Abstract**
The wide usage of neodymium (Nd) in industry, agriculture, and medicine has made it become an emerging pollutant in the environment. Increasing Nd pollution has potential hazards to plants, animals, and microorganisms. Thus, it is necessary to study the toxicity of Nd and the mechanism of Nd transportation and detoxification in microorganisms. Through genome-scale screening, we identified 70 yeast monogene deletion mutations sensitive to Nd ions. These genes are mainly involved in metabolism, transcription, protein synthesis, cell cycle, DNA processing, protein folding, modification, and cell transport processes. Furthermore, the regulatory networks of Nd toxicity were identified by using the protein interaction group analysis. These networks are associated with various signal pathways, including calcium ion transport, phosphate pathways, vesicular transport, and cell autophagy. In addition, the content of Nd ions in yeast was detected by an inductively coupled plasma mass spectrometry, and most of these Nd-sensitive mutants showed an increased intracellular Nd content. In all, our results provide the basis for understanding the molecular mechanisms of detoxifying Nd ions in yeast cells, which will be useful for future studies on Nd-related issues in the environment, agriculture, and human health.

**Keywords** Rare earth element · Sensitive genes · Signaling pathway · Detoxification · Yeast

## Introduction

Rare earth elements include 17 rare earth elements, which are divided as light or heavy rare earth elements according to the atomic mass (Mowafy 2020; Pagano et al. 2019). Rare earth neodymium (Nd) is light rare earth, which is mainly present in the form of oxides, nitrate, and chloride in nature (Thornton and Burdette 2017). Nd is widely used in various fields such as medicine, agriculture, NdFB permanent magnet materials, electronics, and mechanical industries for its excellent performance (Gohda 2021). Moreover, Nd is also applied in non-ferrous metal materials, ceramic, glass materials, and rubber products (Bär et al. 2019; Zhang et al. 2007; Alarfaj et al. 2018).

It has been observed that the concentration of Nd is 0.0071 ~ 6.68 μg/L in the surface water of the Terengganu River Basin in Malaysia (Sultan and Shazili 2009). It is 771 μg/L Nd in streams draining from acid sulfate soils during high-water flow events in autumn in Finland (Åström 2001). In addition, the concentration of Nd is 10.8 μg/L in the surface water of an ex-mining pit lake in Malaysia (Khan et al. 2017), and it is 317 μg/L in a mining pit in Wisniowka (Poland) (Migaszewski et al. 2016). Previously, it was found that $Nd^{3+}$ was a toxic element for the fungi *Penicillium simplicissimum* and *Aspergillus japonicus*. The combinations of $Nd^{3+}$ and $Sm^{3+}$ was the most toxic combination for the tested organisms and fungi (Bergsten-Torralba et al. 2020). The distribution of Nd was studied in the different species of mushrooms collected from unpolluted areas in the province of Ciudad Real, Central Spain, and the maximum absorption of Nd was 7.10 μg/L (Campos et al. 2009). In addition, the significant accumulation of Nd was detected in *Bacillus cereus* isolated from rare earth environments in India (Challaraj Emmanuel et al. 2011).

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In addition, the positive effect of Nd has been observed on seed germination, root development, nutrient absorption, fruit weight, and fruit composition. Thus, it is widely used as a rare earth microfertilizer and plant growth regulator in agriculture (Wang et al. 2003; Ren et al. 2016). However, the wide application of Nd in various fields causes Nd to continuously enter the environment, which will result in soil and water pollution. Furthermore, Nd can be accumulated in the human body (Lemoine et al. 2019). The results of a previous study have shown that the content of Nd in the human blood, urine, and hair samples in the rare earth mining area of Baotou city of China has far exceeded that of the non-mining area (Bao et al. 2018). In addition, Nd can enter the human body through the respiratory system, digestive system, and skin, which will finally damage the lung, liver, central nervous system, and blood system (Zhuang et al. 2017; Wang et al. 2011). However, the mechanism of action and transport of Nd in living organisms have not been fully understood until recently.

As a fully sequencing eukaryotic cell, yeast *Saccharomyces cerevisiae* is a good experimental model for understanding eukaryotic systems, especially metal ion toxicity (Gerwien et al. 2018). Various studies on the genes and signal pathways related to metal ion stress have been investigated, and numerous signaling pathways and ion transporters involved in the metal ion stress have been found (Cyert and Philpott 2013; Bleackley and Macgillivray 2011). To fully understand the mechanism of Nd in eukaryotic cells, we studied the toxicity of Nd and screened the yeast double haploid gene missing library and analyzed the genes affected by Nd in this study. The results will provide the basis for understanding the molecular mechanisms of detoxifying Nd ions in yeast cells, which is useful for future studies on Nd-related issues in the environment, agriculture, and human health.

## Material and methods

### Yeast strains and culture

The diploid *S. cerevisiae* strains were derived from the BY4743 genetic background coming from a public collection. All gene mutant strains had KanMX4 genetic labels with G418 resistant, and the yeast library was purchased from Invitrogen Inc (USA). Yeast was grown at 30 °C in the YPD medium (1% yeast extract, 2% peptone, and 2% glucose, pH 5.6). $Nd(NO_3)_3$ and $NaNO_3$ were purchased from Aladdin Co., Ltd (Shanghai, China).

### Screening Nd-sensitive phenotype of yeast

Based on our preliminary experiments, we firstly tested Nd toxicity on fungi from 1 to 5 mM, then selected 3 to 4 mM during two days according to the method of a previous study (Luo et al. 2016). Finally, 3.7 mM Nd nitrate was used to screen the Nd-sensitive phenotype of yeast. All strains in the library were used for preliminary screening of Nd-sensitive mutations. We copied all strains to a medium containing or not containing 3.7 mM Nd nitrate by using 384 photographs. The tablet was incubated at 30 ℃ for 2 days, photographed, and used to analyze the growth of each mutant. Compared to the other surrounding mutants, it is considered Nd-sensitive if its colony size is reduced by more than 30% on the YPD media containing Nd nitrate based on the analysis of software and observation. Moreover, there is no change of colony size in YPD media containing no Nd.

In addition, the secondary screening was performed for Nd-sensitive mutants by continuous Nd dilution determination according to a previous method (Jiang et al. 2014). The sensitive mutants were streak cultured from the original library to the YPD medium (pH 5.6) and inoculated into the YPD medium for 12 h. A series of culture diluents ($10^{-0} \sim 10^{-4}$) were put on the YPD tablets containing 0, 11.1 mM $NaNO_3$, or 3.7 mM $Nd(NO_3)_3$, respectively. The 11.1 mM sodium nitrate was used for wild-type comparison to confirm that the mutant was sensitive to $Nd^{3+}$ rather than $NO_3^-$ (Luo et al. 2016). The dishes were cultured for 2 ~ 5 days at 30 ℃ and the secondary screening experiment was repeated three times.

### Detecting the intracellular Nd concentration

For 3.7 mM Nd significantly inhibited the yeast growth, logarithmically growing yeast mutant cells and wild-type cells were treated with 1.85 mM Nd $(NO_3)_3$ for 4 h at 30 ℃. Then we collected fungi, measured and recorded its OD value (Zhao et al. 2013). An inductively coupled plasma mass spectrometer is used for detecting the intracellular Nd concentration. Three single colonies of each mutant were measured and wild type BY4743 was used as the control.

### Test the missing strain genome by PCR

The yeast missing strain genome is extracted and the missing strain genome is used as a template for PCR test. Its upstream primer is designed within 100–300 bp upstream of the mutant gene open reading box. The downstream primer is designed at the internal sequence of the KanMX4 knockout box, which is about 1200 bp from the gene initiation

codon. The primers used for testing the missing strain genome are listed in Supplementary Table 1.

### Gene function and localization analysis

The function and localization of the corresponding genes of Nd-sensitive mutants (http://www.yeastgenome.org), MIPS, BioGRID (http://www.thebiogrid.org), and FunSpec (http://funspec.med.utoronto.ca) were annotated using the yeast genome database.

### Data analysis

Data was expressed as mean ± SEM; the statistical software SPSS (16.0) was used for analysis. The difference among the experimental groups was analyzed with the single factor variance analysis (LSD's test) analysis, and the significance level is set at $P < 0.05$.

## Results

### *Sensitive mutants to $Nd^{3+}$ and the associated genes*

In this experiment, we firstly tested the sensitivity of diploid wild type BY4743 to $Nd(NO_3)_3$. After cultivating yeast cells with different concentration gradients of $Nd(NO_3)_3$ medium, we found that yeast cells had specific sensitivity at 3.7 mM $Nd(NO_3)_3$ (Fig. 1). Therefore, we used 3.7 mM $Nd(NO_3)_3$ to screen the diploid yeast mutant library, and we confirmed a total of 70 Nd sensitive mutants (Fig. 2). In addition, the genotype of these mutants was further tested by PCR (Supplementary Fig. 1).

By functional analysis and subcellular localization of the derived genes through *Saccharomyces* Genome Database (SGD) and MIPS-related network resources, we found that the functional classification of these 70 genes includes 10 unknown function genes and 60 known function genes. In addition, 70 genes can be divided into the following 8 categories: A group, transcription (4 genes); B group, protein synthesis (5 genes); C group, protein fate (8 genes); D group, cell cycle and DNA processing (5 genes); E group, cellular transport, transport facilities and transport routes (30 genes); F group, metabolism (6 genes); G group, cell rescue, defense and virulence (2 genes); H group, unknown function genes (10 genes). In addition, the proteins encoded by these 60 genes are mainly localized in the nucleus (10 genes), vacuole (7 genes), and cytoplasm (18 genes). Some of them are present in the endoplasmic reticulum (ER) (6 genes), Golgi body (10 genes), mitochondrial (3 genes), endobody (1 genes), and plasma membrane (7 genes). These genes are listed in Table 1, and the functional notes for each gene are described in Table 2. Functional categories and localization of genes corresponding to sensitive phenotypes are shown in Fig. 3 and Table 3.

Four genes (*BUD31*, *CRZ1*, *PHO4*, and *PHO2*) in the A group participated in cell transcription. Five genes (*RPS1A*, *RPL7A*, *MRT4*, *MSY1*, and *SMM1*) in the B group were involved in regulating protein synthesis, in which *RPS1A* and *RPL7A* are assembly components of the ribosome. The eight genes (*RBL2*, *VMA21*, *GDA1*, *SCJ1*, *SSZ1*, *OLA1*, *CNB1*, and *NCS2*) in the C group were associated with protein fate

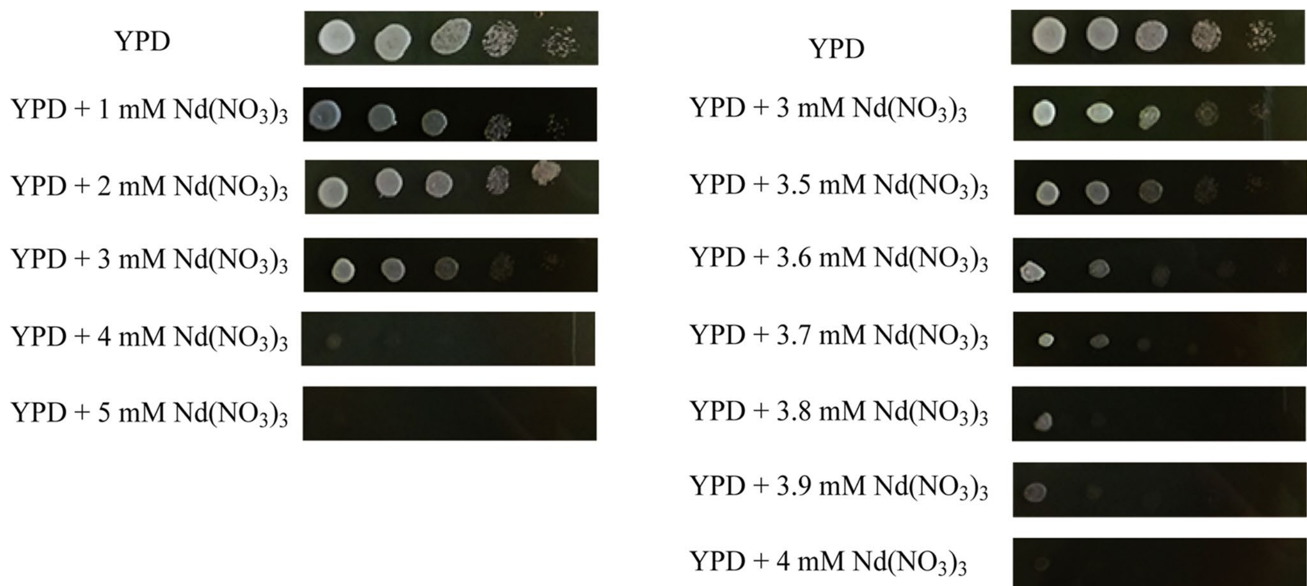
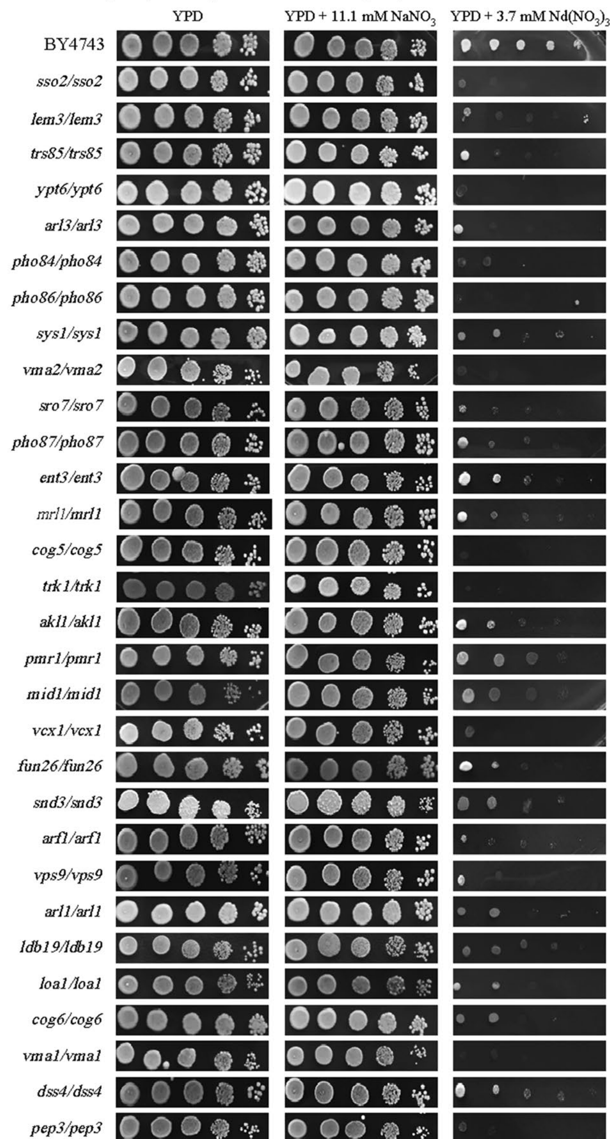
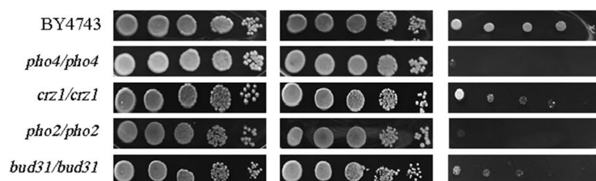
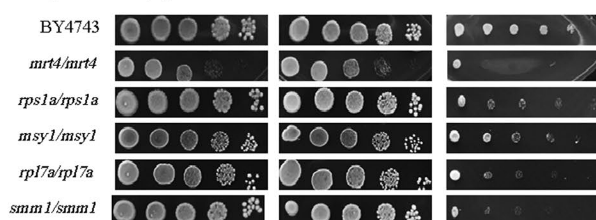
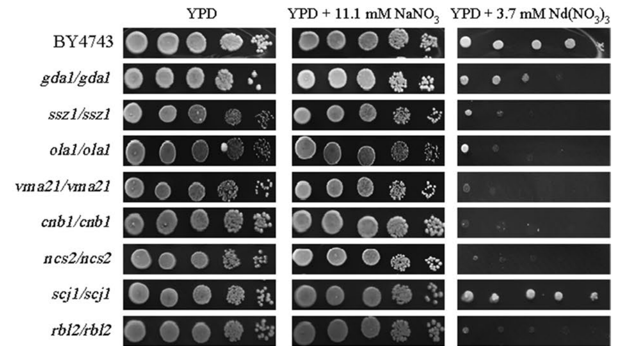


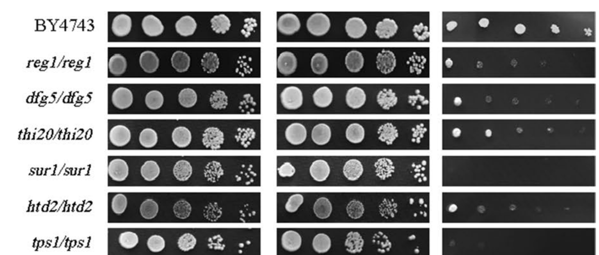

**Fig. 1** Determination of screening concentration of neodymium

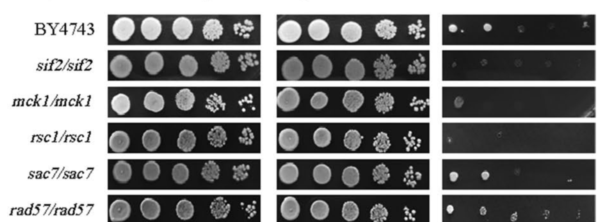

Cellular transport, transport facilities (30)
YPD
YPD + 11.1 mM NaNO3
YPD + 3.7 mM Nd(NO3)3
BY4743
sso2/sso2
lem3/lem3
trs85/trs85
ypt6/ypt6
arl3/arl3
pho84/pho84
pho86/pho86
sys1/sys1
vma2/vma2
sro7/sro7
pho87/pho87
ent3/ent3
mrl1/mrl1
cog5/cog5
trk1/trk1
akl1/akl1
pmr1/pmr1
mid1/mid1
vcx1/vcx1
fun26/fun26
snd3/snd3
arf1/arf1
vps9/vps9
arl1/arl1
ldb19/ldb19
loa1/loa1
cog6/cog6
vma1/vma1
dss4/dss4
pep3/pep3

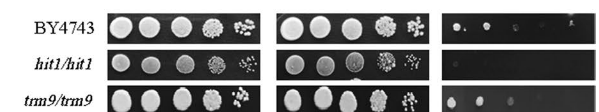

Transcription (4)
BY4743
pho4/pho4
crz1/crz1
pho2/pho2
bud31/bud31

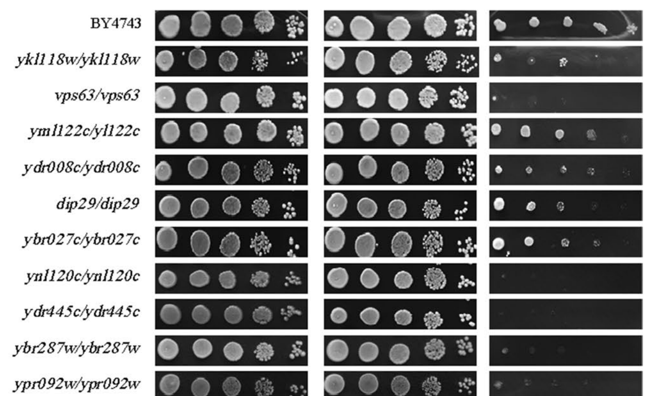

Protein synthesis (5)
BY4743
mrt4/mrt4
rps1a/rps1a
msy1/msy1
rpl7a/rpl7a
smm1/smm1


Protein fate (8)
YPD
YPD + 11.1 mM NaNO3
YPD + 3.7 mM Nd(NO3)3
BY4743
gda1/gda1
ssz1/ssz1
ola1/ola1
vma21/vma21
cnb1/cnb1
ncs2/ncs2
scj1/scj1
rbl2/rbl2


Metabolism (6)
BY4743
reg1/reg1
dfg5/dfg5
thi20/thi20
sur1/sur1
htd2/htd2
tps1/tps1


Cell cycle and DNA processing (5)
BY4743
sif2/sif2
mck1/mck1
rsc1/rsc1
sac7/sac7
rad57/rad57


Cell rescue, defense and virulence (2)
BY4743
hit1/hit1
trm9/trm9


Unclassified proteins (10)
BY4743
ykl118w/ykl118w
vps63/vps63
yml122c/yl122c
ydr008c/ydr008c
dip29/dip29
ybr027c/ybr027c
ynl120c/ynl120c
ydr445c/ydr445c
ybr287w/ybr287w
ypr092w/ypr092w

◂**Fig. 2** Phenotypes of Nd-sensitive gene deletion mutants. Cells of the wild-type BY4743 and 70 gene deletion mutants were identified from the genome-scale screen, which were grown at 30℃ in liquid YPD overnight, serially diluted by 10 times and spotted on YPD plates with or without supplemented reagents as indicated, respectively. Plates were incubated for 1 ~ 5 days at 30℃

(folding, modification, and determination). The D group contains five genes (*SIF2*, *MCK1*, *RSC1*, *RAD57*, and *SAC7*), which regulate cell cycle and DNA processing. The F group is a gene associated with metabolism, including *TPS1*, *SUR1*, and *dfg5p*.

We find that the largest category of Nd-sensitive genes is involved in cell transport, transport facilities, and transport routes. Seventeen genes (*ENT3*, *COG5*, *COG6*, *PEP3*, *SRO7*, *DSS4*, *VPS9*, *ARF1*, *SYS1*, *ARL3*, *AKL1*, *LDB19*, *YPT6*, *ARL1*, *TRS85*, *MRL1*, and *SSO2*) in the E group were involved in multiple processes of vesicular transport in the cell. There are 4 sensitive genes associated with Rab proteins (*VPS9*, *YPT6*, *TRS85*, and *DSS4*). The soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (SNARE) family proteins are a key component of facilitating the specific fusion of transport vesicles with the destination membrane structure. In this study, we identified 3 SNARE proteins, including *PEP3*, *SSO2*, and *SRO7*, which mediate the fusion of the target membrane with transport membrane bubbles. Three genes (*PHO84*, *PHO87*, *PHO86*) are involved in regulating cellular phosphate transport pathways. In addition, the H group includes 10 unknown function genes and the function of these genes is still unknown until now days.

### The content of intracellular Nd ions in yeast mutants

In this study, the yeast was further treated with 1.85 mM Nd. In the 70 missing mutants, compared to the wild type BY4743, the content of intracellular Nd ions was significantly increased in 30 mutants, and it was significantly decreased in 18 mutants. However, no significant difference was found in 22 mutants (Fig. 4). The intracellular Nd content in the other remaining 40 missing strains was near or lower than wild strains. It shows that these genes are not directly involved in affecting the function of intracellular Nd content. These genes may help to relieve Nd toxicity effects and further produce sensitive phenotypes during the loss of cell-related genes, such as repairing DNA damage under Nd stress.

## Discussion

Previously, it was found that $Nd^{3+}$ was a toxic element for the fungi *Penicillium simplicissimum* and *A. japonicus* (Bergsten-Torralba et al. 2020). The mycelium cell membrane permeability of *Fusarium oxysporum* was increased when Nd concentrations ranged from 10 to 400 mg/L (Yufeng et al. 2007). In this study, we identified 70 Nd-sensitive gene deletion mutations from genome-scale genetic screening after fungi, they were treated with 3.7 mM Nd. By functional analysis and subcellular localization of the derived genes through SGD and MIPS-related network resources, we found that the functional classification of these 70 genes includes 60 known function genes and 10 unknown function genes. 70 genes can be divided into the following 8 categories: A ~ H groups.

Four genes (*BUD31*, *CRZ1*, *PHO4*, and *PHO2*) in the A group participated in cell transcription. *PHO4* is an alkaline helix-ring-helix (bHLH) transcription activator in yeast, which is dephosphorylated and transported from the cytoplasm to the nucleus. Then it improves the intracellular phosphate level with the transcription factor *PHO2* (Zhou and O'Shea 2011). The sensitive phenotypes of *PHO4* and *PHO2* demonstrate the importance of intracellular phosphate levels against Nd stress. In addition, *CRZ1* is a transcription factor of the calcium/calcium modulated neurophosphatase signaling pathway. *CRZ1* is responsible for inducing the expression of cytocellular calcium pump genes *PMC1* and *PMR1*, which can be used to decrease the $Ca^{2+}$ concentration in the cytoplasm (Zhao et al. 2013). Thus, it suggests that the maintenance of calcium homeostasis could help cells adapt to Nd stress.

Five genes (*RPS1A*, *RPL7A*, *MRT4*, *MSY1*, and *SMM1*) in the B group were involved in regulating protein synthesis, in which *RPS1A* and *RPL7A* are assembly components of ribosome. *RPS1A* encodes ribosomal protein 10 of 40S small subunits and involves in the mature of small-subunit rRNA (Moteshareie et al. 2018). In addition, the *RPL7A* encodes the L7A protein of the ribosomal 60S large subunit and assembles the 60S subunit (Palumbo et al. 2017). *MRT4* is involved in mRNA conversion and ribosome assembly (Sugiyama et al. 2011). Thus, the ribosomal function appears to play a key role in the sensitivity of yeast cells to Nd. *MSY1* encodes the mitochondrial tyrosine-tRNA synthase and is involved in the assembly of RNA (Rodley et al. 2012). *SMM1* modifies the urosine residue at 20 of cytoplasmic tRNAs location (Rinaldi et al. 2003). The sensitive phenotype of these mutations to Nd suggests that Nd exposure can lead to the decrease of inefficiency and accuracy of the translation.

Eight genes (*RBL2*, *VMA21*, *GDA1*, *SCJ1*, *SSZ1*, *OLA1*, *CNB1*, and *NCS2*) in the C group were associated with protein fate (folding, modification, and determination). It is noteworthy that the absence of regulatory subunit *CNB1* encoding phosphestase makes yeast cells sensitive to Nd, which can coordinate cell response to development cues, environmental stimulation, and intracellular stress. It will finally influence cell proliferation, differentiation, death,

Environmental Science and Pollution Research (2022) 29:41439–41454

**Table 1** Functional categories of 70 genes whose deletion mutants are sensitive to 3.7 mM $Nd^{3+}$

| Systemic name | Standard name | Systemic name | Standard name | Systemic name | Standard name | Systemic name | Standard name |
|---|---|---|---|---|---|---|---|
| Cellular transport, transport facilities, and transport routes (30) | | | | | | | |
| YJR125C | *ENT3* | YJL004C | *SYS1* | YMR183C | *SSO2* | YCR037C | *PHO87* |
| NL051W | *COG5* | YPL051W | *ARL3* | YNL291C | *MID1* | YJL117W | *PHO86* |
| YNL041C | *COG6* | YBR059C | *AKL1* | YGL167C | *PMR1* | YAL022C | *FUN26* |
| YLR148W | *PEP3* | YOR322C | *LDB19* | YDL128W | *VCX1* | YNL323W | *LEM3* |
| YPR032W | *SRO7* | YLR262C | *YPT6* | YDL185W | *VMA1* | YPR139C | *LOA1* |
| YPR017C | *DSS4* | YBR164C | *ARL1* | YBR127C | *VMA2* | YBR106W | *SND3* |
| YML097C | *VPS9* | YDR108W | *TRS85* | YJL129C | *TRK1* | | |
| YDL192W | *ARF1* | YPR079W | *MRL1* | YML123C | *PHO84* | | |
| Metabolism (6) | | | | | | | |
| YDR028C | *REG1* | YOL055C | *THI20* | YMR238W | *DFG5* | YHR067W | *HTD2* |
| YBR126C | *TPS1* | YPL057C | *SUR1* | | | | |
| Transcription (4) | | | | | | | |
| YNL027W | *CRZ1* | YDL106C | *PHO2* | YFR034C | *PHO4* | YCR063W | *BUD31* |
| Cell cycle (5) | | | | | | | |
| YBR103W | *SIF2* | YNL307C | *MCK1* | YGR056W | *RSC1* | YDR004W | *RAD57* |
| YDR389W | *SAC7* | | | | | | |
| Protein synthesis, folding, modification, and destination (8) | | | | | | | |
| YOR265W | *RBL2* | YEL042W | *GDA1* | YHR064C | *SSZ1* | YKL190W | *CNB1* |
| YGR105W | *VMA21* | YMR214W | *SCJ1* | YBR025C | *OLA1* | YNL119W | *NCS2* |
| Protein synthesis (5) | | | | | | | |
| YLR441C | *RPS1A* | YGL076C | *RPL7A* | YKL009W | *MRT4* | YPL097W | *MSY1* |
| YNR015W | *SMM1* | | | | | | |
| Cell rescue, defense and virulence (2) | | | | | | | |
| YJR055W | *HIT1* | YML014W | *TRM9* | | | | |
| Unknown (10) | | | | | | | |
| YBR027C | | YDR008C | | YKL118W | | YML122C | |
| YBR287W | | YDR445C | | YLR261C | *VPS63* | YNL120C | |
| YNR040W | *DPI29* | YPR092W | | | | | |

**Table 2** Function and classification of genes from Nd-sensitive strains

| Systemic name | Standard name | Gene function |
|---|---|---|
| Transcription (4) | | |
| YNL027W | *CRZ1* | Transcription factor of the $Ca^{2+}$-calmodulin-calcium modulation neurophosphatase signaling pathway |
| YDL106C | *PHO2* | Homologous box transcription factor, in response to phosphate hunger, and coordinated with Pho4p to activate transcription |
| YFR034C | *PHO4* | The basic helical-loop-spiral transcription factor, the regulatory targets include genes involved in the phosphate hunger reaction (PHR) |
| YCR063W | *BUD31* | U2 snRNP's component of the SF3b subcomplex |
| Protein synthesis (5) | | |
| YLR441C | *RPS1A* | Ribosomal 40S subunit protein RPS1A |
| YGL076C | *RPL7A* | Ribosomal protein RPL7A of large (60S) subunits |
| YKL009W | *MRT4* | Protein required for ribosomal assembly and mRNA conversion |
| YPL097W | *MSY1* | Tyrosine tRNA synthase in the mitochondria |
| YNR015W | *SMM1* | Dihydrouridine synthase, modifies the urridine residues at 20 cytoplasmic tRNAs |
| Protein synthesis, folding, modification, and destination (8) | | |
| YOR265W | *RBL2* | Protein required for the assembly of the microtubulin complex |
| YGR105W | *VMA21* | Proteins involved in the assembly of vacuole $H^{+}$-ATPase complex |
| YEL042W | *GDA1* | Orniosine diphosphatase in the Gorky tube cavity, involved in the transport of GDP-mannose to the Gorky body cavity |
| YMR214W | *SCJ1* | One of the chaperones involved in the endoplasmic reticulum lumen protein folding |
| YHR064C | *SSZ1* | Hsp70 protein that interacts with Zuo1p (DnaJ homologous); interacts with Zuo1p form ribosome-related complexes |
| YBR025C | *OLA1* | P-ring ATP Enase |
| YKL190W | *CNB1* | Calmodulation neurophosphatase regulation subunit B |
| YNL119W | *NCS2* | tRNA swing position uridine sulfide-related proteins |
| Cell cycle (5) | | |
| YBR103W | *SIF2* | The Set3C histone deacetylase complex subunit containing WD40 repeats |
| YNL307C | *MCK1* | Bispecific serine/threonine and tyrosine protein kinases |
| YGR056W | *RSC1* | The component of the RSC chromatin remodeling complex |
| YDR004W | *RAD57* | Proteins that stimulate chain exchange, participate in recombinant repair of DNA double chain fracture during nutritional growth and meiosis |
| YDR389W | *SAC7* | GTPase activation protein of Rho1p (GAP) |
| Cellular transport, transport facilities, and transport routes (30) | | |
| YJR125C | *ENT3* | Proteins involved in meseshin recruitment and vesicular transport |
| YNL051W | *COG5* | Cell-lysol-binding complex (Cog1p to Cog8p) |
| YNL041C | *COG6* | Cell-lysol-binding complex (Cog1p to Cog8p) |
| YLR148W | *PEP3* | The component of the CORVET membrane-bound complex |
| YPR032W | *SRO7* | effector of Rab GTPase Sec4p |
| YPR017C | *DSS4* | Guanine nucleotide dissociation stimulating factor of sec4p |
| YML097C | *VPS9* | Guanine nucleotide exchange factors (GEF) and ubiquitin receptors |
| YDL192W | *ARF1* | ADP-ribosylation factor |
| YJL004C | *SYS1* | Integrated membrane protein of the Golgi body |
| YPL051W | *ARL3* | The ARF-like small GTP enzyme in the RAS superfamily |
| YBR059C | *AKL1* | Ser-Thr protein kinase |
| YOR322C | *LDB19* | α-inhibitors are involved in ubiquitin-dependent endocytosis |
| YLR262C | *YPT6* | GTP enzyme of Rab family |
| YBR164C | *ARL1* | Soluble GTP enzyme, which regulates the membrane flux |
| YDR108W | *TRS85* | The component of the transport protein particles (TRAPP) complex III |
| YPR079W | *MRL1* | Membrane protein; similar to the mammalian mannose-6-phosphate receptor |
| YMR183C | *SSO2* | Plasma membrane t-SNARE; is involved in the fusion of the plasma membrane secreting vesicles |

**Table 2** (continued)

| Systemic name | Standard name | Gene function |
|---|---|---|
| YNL291C | *MID1* | ER and N-glycosylated integrated membrane protein of ER; as the required tensile-activated $Ca^{2+}$-permeable cation channel for the flow of $Ca^{2+}$ stimulated by pheromone |
| YGL167C | *PMR1* | High-affinity $Ca^{2+}/Mn^{2+}$ P type ATP enzyme; required for the transport of $Ca^{2+}$ and $Mn^{2+}$ to the Golgi body |
| YDL128W | *VCX1* | Fluicular membrane reverse transporters with exchange activity of $Ca^{2+}/H^{+}$ and $K^{+}/H^{+}$, involved in the control of cytosol $Ca^{2+}$ and $K^{+}$ concentrations |
| YDL185W | *VMA1* | Subunit A of the V1 peripheral membrane domain of the V-ATP enzyme |
| YBR127C | *VMA2* | Subunit B of the V1 peripheral membrane domain of the V-ATP enzyme |
| YJL129C | *TRK1* | Part of the Trk1p-Trk2p potassium transport system |
| YML123C | *PHO84* | High-affinity inorganic phosphorus transporters and low-affinity manganese transporters regulated by Pho4p and Spt7p; mutations give resistance to arsenates; Pho86p is withdrawal from ER during maturity |
| YCR037C | *PHO87* | Low-affinity inorganic phosphate transporters |
| YJL117W | *PHO86* | endoplasmic mesh resid protein; ER export of high affinity phosphate transporter Pho84p |
| YAL022C | *FUN26* | High affinity, wide selectivity, nucleoside/nucleobase transporters |
| YNL323W | *LEM3* | The membrane proteins of the plasma membrane and ER specifically interact with the phospholipid transtase (phospholipid transtase) Dnf1p in vivo |
| YPR139C | *LOA1* | Hemolytic phosphatidylate acyltransferase |
| YBR106W | *SND3* | Proteins involved in SRP non-dependent target genes towards ER; components of the alternative ER targeting pathway |
| Metabolism (6) | | |
| YDR028C | *REG1* | Regulatory subunit of the type 1 protein phosphatase Glc7p |
| YBR126C | *TPS1* | Synthase subunit of the alginose-6-P synthase/phosphatase complex |
| YMR238W | *DFG5* | mannosidase |
| YOL055C | *THI20* | Trifunctional enzymes for thiamine biosynthesis, degradation, and repair; with hydroxypyrimidine (HMP) kinase, phosphate acid HMP (hmp-p) kinase, and thiaminase activity |
| YPL057C | *SUR1* | The mannitromannitol phosphate neuramide (MIPC) synthase catalyzes subunits, forming a complex with the regulatory subunit Csg2p |
| YHR067W | *HTD2* | 3-hydroxyacyl-thioester dehydratase in mitochondrion |
| Cell rescue, defense and virulence (2) | | |
| YJR055W | *HIT1* | Participate in the C/D box snoRNP assembly |
| YML014W | *TRM9* | tRNA-methyltransferase |
| Unknown (10) | | |
| YBR027C | | Unknown |
| YBR287W | | Unknown |
| YDR008C | | Unknown |
| YDR445C | | Unknown |
| YKL118W | | Unknown |
| YLR261C | *VPS63* | Unknown |
| YML122C | | Unknown |
| YNL120C | | Unknown |
| YNR040W | *DPI29* | Unknown |
| YPR092W | | Unknown |

and promoting cell survival (Connolly et al. 2018). In addition, *VMA21* is an essential assembly companion for the vesicular ATP enzyme (V-ATPase). Loss of *VMA21* reduces V-ATP enzyme activity and results in the increase of pH in the vacuole (Ramachandran et al. 2013). In the previous studies, V-ATPase was found against the toxic of many metal ions such as cadmium, cobalt, nickel, and calcium (Techo et al. 2020b). Other genes function in this group includes the molecular partners in folding β-tubulin, protein glycosylation, and tRNA mature genes (You et al. 2004; Klassen et al.

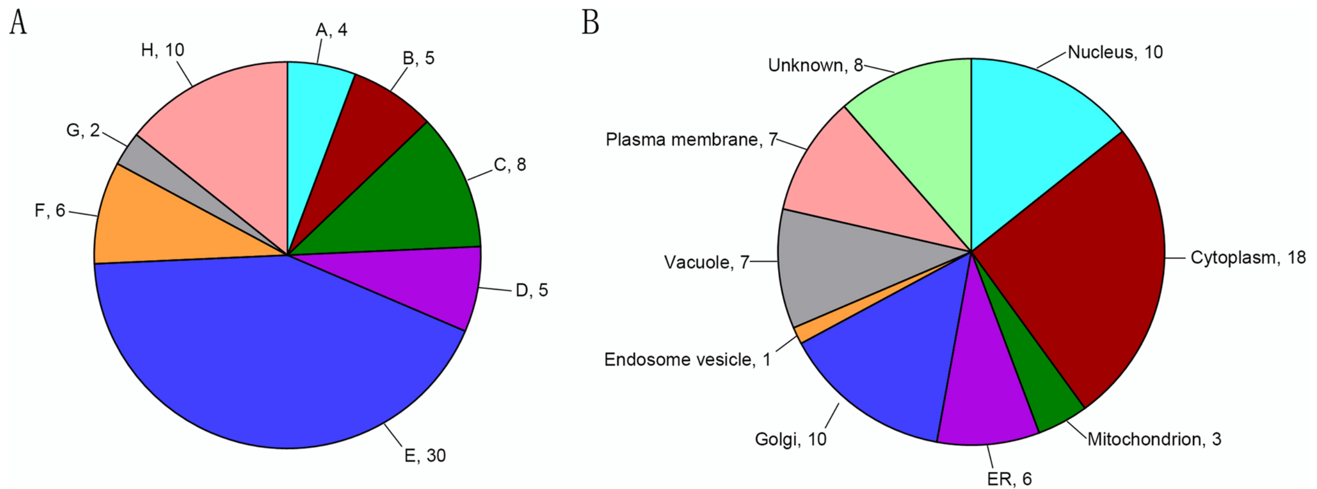



**Fig. 3** Functional categories and subcellular localization of the genes corresponding to the sensitive phenotypes and number of genes associated with each category. **A** Functional categories of the genes in 70 sensitive deletion mutants. The functional classifications are based on gene ontology and molecular function in SGD and MIPS, where A represents genes related to transcription, B for protein synthesis, C for protein fate, D for cell cycle and DNA processing, E for cellular transport, transport facilities, and transport routes, F for metabolism, G for cell rescue, defense and virulence, and H for uncharacterized or dubious ORFs. **B** Subcellular localization of proteins encoded by 70 genes identified from sensitive mutation

**Table 3** Localization of genes related to Nd-sensitive strains in cells

| Subcellular localization | Genes |
|---|---|
| Cell nucleus | *CRZ1, PHO2, PHO4, BUD31, MRT4, SIF2, MCK1, RSC1, RAD57, HIT1* (10) |
| Cytoplasm | *SMM1, RPS1A, RPL7A, RBL2, SSZ1, OLA1, CNB1, NCS2, SAC7, VPS9, AKL1, LDB19, TRS85, REG1, TPS1, THI20, TRM9, SRO7* (18) |
| Mitochondria | *MSY1, HTD2, DPI29* (3) |
| Endoplasmic reticulum | *VMA21, SCJ1, PHO86, LOA1, SND3, YBR287W* (6) |
| Golgi body | *GDA1, COG5, COG6, DSS4, ARF1, SYS1, ARL3, YPT6, ARL1, PMR1* (10) |
| Internal body vesicles | *ENT3* (1) |
| vacuole | *PEP3, MRL1, VCX1, VMA1, VMA2, FUN26, SUR1* (7) |
| plasma membranes | *SSO2, MID1, TRK1, PHO84, PHO87, LEM3, DFG5* (7) |
| Localization unknown | *YBR027C, YDR008C, YDR445C, YKL118W, YML122C, YNL120C, YPR092W,* YLR261C (8) |

2015; Young et al. 2013). In all, the absence of these genes related to protein assembly, protein folding, and modification processes will make cells sensitive to Nd.

The D group contains five genes (*SIF2*, *MCK1*, *RSC1*, *RAD57*, and *SAC7*) that regulate cell cycle and DNA processing. As a member of the family of protein kinase GSK-3, *MCK1* regulates *RCN1*, which can directly bind with caltunphosphaterase. In addition, *MCK1* is involved in regulating the cell wall integrity pathway (Kassir et al. 2006), which is related to the cell sensitivity caused by the absence of caltunphosphaterase pathway genes *CNB1* and *CRZ1*. It was previously found that the loss of *MCK1* also made cells sensitive to lithium and cadmium stress (Lockshon et al. 2012; Jiang et al. 2014). The absence of GTPase-activated protein (GAP) of the core component of the SAC7-coded cell wall integrity (CWI) pathway makes yeast cells sensitive to Nd, which is similar to the result of lithium treatment. It suggests that the regulation of CWI may be associated with the Nd sensitivity of yeast cells. Moreover, the function of the remaining three genes is associated with histone modification, chromatin remodeling, and recombination repairing of DNA double-chain fracture (Chambers et al. 2012; Fung et al. 2009; Baek et al. 2016). The sensitive phenotype of gene deletion mutations supports the conclusion of previous studies that Nd can be genetically toxic and carcinogenic (Chen et al. 2020).

We find that the largest category of Nd-sensitive genes is involved in cell transport, transport facilities, and transport routes. Seventeen genes (*ENT3*, *COG5*, *COG6*, *PEP3*, *SRO7*, *DSS4*, *VPS9*, *ARF1*, *SYS1*, *ARL3*, *AKL1*, *LDB19*, *YPT6*, *ARL1*, *TRS85*, *MRL1*, and *SSO2*) in the E group were involved in multiple processes of vesicular transport in the

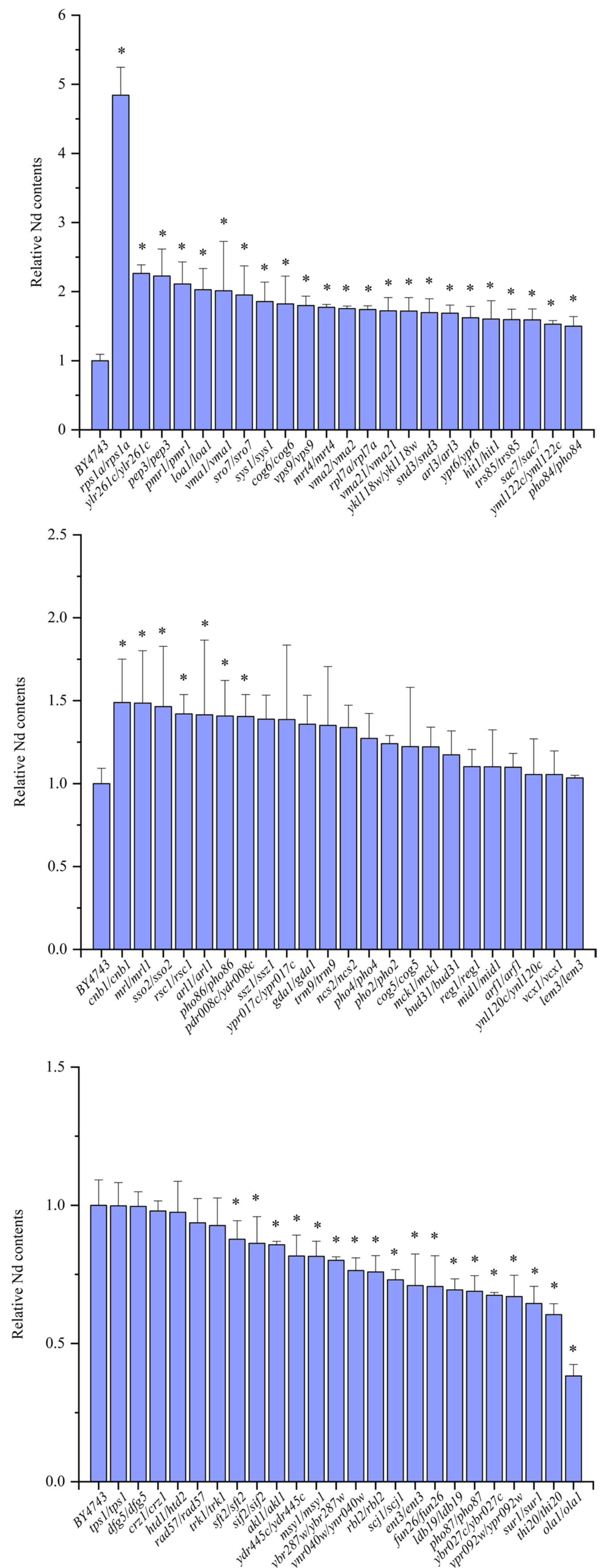



**Fig. 4** Cellular Nd contents of 30 Nd-sensitive gene deletion mutants in response to Nd stress. Log-phase grown cells were treated with 1.85 mM Nd $(NO_3)_3$ for 4 h before they were collected for measurement of intracellular Nd content. The cellular Nd content of the wild type BY4743 is converted as an arbitrary unit of 1, and relative Nd content values of these mutants to that of the wild type are listed according to their functional categories. The value is the average of two independent assays for each strain

cell. The vesicular transport process includes four steps: follicle formation, transport, tying, and fusion. Vesicular transport is a process mediated by intracellular localization through the combination of coated proteins, tethered factors, class Rab/Ypt small G proteins, and SNARE proteins (Guo et al. 2017). Six genes are related to the initiation of vesicles, and the absence of these genes makes cells sensitive to neodymium. During the assembly of the follicles, ARF firstly binds to the mass membrane and then forms vesicles through a series of ARF reactions to the cargo protein and the recruitment and assembly of the cap protein. The genes *ARF1*, *ARL3*, *ARL1*, and *SYS1* are involved in the regulation of the ARF protein that is related to the vesicular initiation assembly. In addition, *ARF1* belongs to the ARF protein and is involved in intracellular transport in Gorky. *ARL1* belongs to the highly similar GTP enzyme ARL protein of ARF and is involved in the assembly of vesicles on the Golgi membrane (Yang and Rosenwald 2016). *ARL3* is responsible for raising *ARL1*, and *SYS1* transports Arf-like GTP enzyme *Arl3p* to the Golgi (Wang et al. 2017). *ENT3* is also a protein associated with the assembly of vesicles, which is involved in Mesin recruitment and transport between the vesicular Golgi body and the inner body (Fang et al. 2010). Moreover, *LDB19* is associated with the late maturation of the trans-Golgi network TGN that is rich in the meseshin cohesion protein complex-1 (AP-1) (Martínez-Márquez and Duncan 2018).

The F group is a gene *TPS1* that is associated with metabolism. *TPS1* is involved in alginose biosynthesis, which plays a role in stress response and inhibition of protein aggregation. It has been demonstrated that *TPS1* is involved in cell resistance to nickel and arsenic as it is upregulated in nickel-resistant yeast mutants, and its deficiency grants sensitivity to As (III) (Johnson et al. 2016a; Bleackley and Macgillivray 2011). The biosynthesis of fatty acid (*HTD2*) and thiamine (*THI20*), as well as the metabolic regulatory process of glucose (*REG1*), exhibit sensitivity to Nd (Kastaniotis et al. 2004; French et al. 2011; Tabba et al. 2010). *SUR1* is associated with the biosynthesis of scabelin (Tanaka and Tani 2018). *SUR1* mutants are also highly sensitive to yttrium and calcium (Grosjean et al. 2018). *dfg5p* is the anchored membrane protein required for the formation of inositol (GPI)-bud to form cell wall biogenesis. The absence of *dfg5p* slows cell growth and cell wall synthesis (Vazquez et al. 2014). In addition, the absence sensitivity of *SUR1* and *DFG5* indicates that the damaged cell walls and membranes make cells sensitive to Nd.

During vesicular transport, there is a small molecule GTP binding protein Rab, which allows the transport vesicular to be anchored on the appropriate target membrane (Li et al. 2019). There are 4 sensitive genes associated with Rab proteins (*VPS9*, *YPT6*, *TRS85*, and *DSS4*). *YPT6* is the GTP enzyme in the Rab family, which is required for retrograde and reverse transport of ER in Gorky. Its absence or dysfunction can cause temperature sensitivity of cell growth (Yang and Rosenwald 2016). *VPS9* is a guanine nucleotide exchange factor (GEF) in yeast *S. cerevisiae*. Membrane transport in the lysosomal pathway can be regulated by activating the Rab5 GTPases (Li et al. 2019). *TRS85* is a specialized subunit of TRAPP complex III, belonging to the polyunit Rab family Ypt1p guanine nucleotide exchange factor. The absence of this gene will inhibit non-specific autophagy (Zou et al. 2015). *DSS4* is a guanine nucleotide dissociation stimulating factor of the Rab protein Sec4p, which is necessary for normal delivery and transporting Gorky posterior vesicles to target mass membrane polarization growth sites (Itzen et al. 2007).

The SNARE family proteins are a key component of facilitating the specific fusion of transport vesicles with the destination membrane structure. In this study, we identified 3 SNARE proteins, namely *PEP3*, *SSO2*, and *SRO7*, which mediate the fusion of the target membrane with transport membrane bubbles. *PEP3* is a component of the CORVET membrane-bound complex (the membrane retention complex), and it promotes the binding of transport vesicles to the vacuole vesicular SNARE protein. It is found that *PEP3* overexpression strains exhibit higher V-ATPase activity (Ding et al. 2015). In addition, *Sec4* and its dual-effect proteins *SRO7* and t-SNARE *Sec9p* form a complex. It is used to butt and fuse the Golgi body vesicles with the plasma membrane (Rossi et al. 2020). *SSO2* is the plasma membrane t-SNARE, and it is directly involved in the fusion of the plasma membrane secretion of vesicles (Yamamoto et al. 2018). Another important factor during vesicular transport is the tethered complex, which interacts with vesicular to promote the formation of the SNARE complex and transports vesicular to the destination. *COG5* and *COG6* are essential components for encoding the oligomeric Gorky complex, which are conserved in eukaryotes and play a role in protein transport. Moreover, *COG5* and *COG6* mediate the fusion of the transport vesicular to the Golgi region chamber (Wang et al. 2017).

In addition, three genes (*PHO84*, *PHO87*, *PHO86*) are involved in regulating cellular phosphate transport pathways. *PHO84* is a high-affinity inorganic phosphate (Pi) transporter and a low-affinity manganese transporter, which is regulated by *Pho4p* and *Spt7p*. The mutations of *PHO84* increase the resistance to arsenates, and the mature endoplasmic reticulum secretion requires *PHO86p*. The low-affinity phosphate transport system *PHO87* uptakes Pi at high phosphate levels (Mouillon and Persson 2006). After metals enter the yeast, cells can reduce metal toxicity through phosphate and polyphosphate cation buffer. The previous studies have shown that most $Zn^{2+}$ can bind to a very rich polyphosphate in the vacuole (MacDiarmid et al. 2000). In addition, three genes (*MID1*, *PMR1*, and *VCX1*) are involved in the

calcium ion homeostasis systems in cells. *MID1* is involved in the mass membrane voltage control (Voltage-gated) $Ca^{2+}$ channel, which is necessary for the internal flow of $Ca^{2+}$. The *PMR1* is a calcium pump, which pumps $Ca^{2+}$ into the endoplasmic network and Golgi body. *VCX1* is a $H^+/Ca^{2+}$ exchange protein localized on the vacuole membrane, which will transport calcium ions from the cytoplasm to the vacuole (Zhao et al. 2013).

Rare-earth ions have similar effects as $Ca^{2+}$ in the cells (Kolouchova et al. 2016). Various reports suggest that yeast uses $Ca^{2+}$ to mediate signals in response to various environmental stimuli. High concentrations of metal exposure transmit signals within the cell through a second messenger (such as $Ca^{2+}$), which enable cells to take the necessary steps for living. It has been found that high concentrations of Cd emit a hazard signal through a sudden increase of cytoplasm $Ca^{2+}$ (Ruta et al. 2014). These results imply the importance of a calcium steady state in combating neodymium toxicity. Moreover, *VMA1* and *VMA2* are two subunits of the V1 outer membrane domain of the V-ATP enzyme, and the vacuole $H^+$-ATPase (V-ATPase) plays a key role in maintaining intracellular pH levels. In addition, V-ATPase maintains intracellular homeostasis by pumping excess protons from the cytoplasm into the vacuole (Hirata et al. 1990).

In the 70 Nd-sensitive gene deletion mutations from genome-scale genetic screening. The proteins encoded by these genes are involved in a number of important cell processes, mainly including transcription, protein synthesis, protein destination, cell cycle, DNA processing, metabolism, and cell transport. The largest number of genes are involved in the process of cell transport, which are associated with 30 genes in 70 Nd-sensitive deletion mutants. It shows that the cell transport process is a key mechanism of Nd ion detoxification in yeast cells. Our results are consistent with the results of previous studies on metal ions (Delorme-Axford and Klionsky 2018; Jiang et al. 2014; Zhao et al. 2013; Grosjean et al. 2018). The functions of these genes are related to cell wall protection, biological synthesis of ribosomes, mRNA/tRNA modification, transcription regulation, V-ATP enzymes, signal transduction, protein transport, and autophagy, which is helpful to cope with Nd toxicity.

Under heavy metal stress, yeast precipitates heavy metals through cell walls, which are the main deposition sites of $Pb^{2+}$, $Cd^{2+}$, and $Zn^{2+}$ (Belde et al. 1988; Suman et al. 2014). It has been demonstrated that the cell wall integrity (CWI) pathway is involved in dealing with cadmium and arsenate-induced cell wall stress (Techo et al. 2020a). The yeast that loss of subunit *SUR1* mannose-mannitol phosphate neuramide (MIPC) synthase is sensitive to Nd, and the biosynthesis of MIPC is necessary for maintaining normal cell wall function (Tanaka and Tani 2018). In addition, we found that GTPase-activating protein *SAC7* of the cell wall integrity pathway and GPI-anchored membrane protein *Dfg5* make yeast sensitive to Nd, which are also necessary for normal biosynthesis of cell walls (Lockshon et al. 2012). Our previous studies found that Nd is mainly bound or deposited in cell wall (Shi et al. 2021). Thus, maintaining the cell wall intact plays a positive role in protecting yeast cells against Nd toxicity.

Rare earth Nd has a certain mutagenic effect on DNA. A certain dose of Nd can cause damage to genetic substances at both DNA level and chromosome level, and it shows the genetic toxic effect (Chen et al. 2020). In this study, we indirectly confirmed the genetic toxicity of Nd by genes related to DNA repair and cell cycle in the sensitive phenotypes. The remodeling complex *RSC1* of *RAD57* and *RSC* chromatin is involved in the repair of double-stranded DNA broken (Chambers et al. 2012; Fung et al. 2009). It has been found that Nd treatment causes the DNA chain breaking in juvenile rainbow trout (Hanana et al. 2021). Thus, the repairing of DNA damage is used by yeast to protect against Nd stress. Gda1 plays an important role in the S phase before subdivision (Wang et al. 2015). The subunits containing the WD40 repeat sequence of the Set3c histone deacetylase (HDAC) complex are involved in regulating spore production (Ryu et al. 2020). The deficiency of *FUN26* nucleoside/nucleobase transporters, which recycle nucleoside and bases from the vacuole and recycle them to the cytoplasm pool, will produce a serious fault of spore formation (Boswell-Casteel et al. 2018). Thus, the spore-producing genes are also important for the survival of yeast cells under Nd stress during cell reproduction.

Autophagy is a highly conservative catabolic pathway, which is essential for metal stress response. Moreover, autophagy plays a role in maintaining intracellular homeostasis and preventing cell damage from heavy metals. When cells are exposed to Cd, *SEC17* expression is induced, which encodes the membrane protein required for vesicular transport and autophagy (Muthukumar and Nachiappan 2013). Autophagy also acts on yeast resisting nickel stress, and a number of nickel-related studies show that loss of autophagy-related genes will lead to the sensitivity to nickel, such as *YVH1* (encoding autophophy structures), *VPS30,* and *VPS38* (encoding the phosphatidylinositol 3-kinase complexes I and II) (Luo et al. 2016). In this study, there are some mutations associated with autophagy. Some studies have also found that rare-earth compounds Nd oxide–induced cancer cell autophagy (Chen et al. 2005). Thus, autophagy plays a potential role in yeast resistance to Nd stress. Cytoplasmic-vacuole targeting (Cvt) pathway is a special biosynthetic form of yeast selective autophagy (Torggler et al. 2017). It has been found that knocking out several genes involved in Cvt and autophagy pathways increased the sensitivity of yeast cerevisiae to $Zn^{2+}$. Microscopic analysis shows that $Zn^{2+}$ partially inhibits the fusion of Cvt vesicles with liquid follicles (Dziedzic and Caplan 2011). In addition, three autophagy-related genes are involved in Cvt

pathways. *YPT6* and *ARL1* are critical for retrograde transport of vesicles from the interior to the trans-Golgi network (TGN) and help form autophagy. The mutations of these genes will lead to severe protein dislocation and growth defects (Yang and Rosenwald 2016). *TRS85* regulates Endo-Golgi transport and is necessary for membrane amplification in the autophagy and Cvt pathway (Zou et al. 2015). Moreover, loss of the subunits of cytosol-binding complex causes yeast sensitivity to Nd, and the subunits of the cytosol-binding complex also showed sensitive phenotypic in the previous genome-wide screening under Al, Pb, Y, and Cr stress (Tun et al. 2014; Johnson et al. 2016b; Du et al. 2015; Grosjean et al. 2018). It is also found that the abnormal COG complex function leads to Cvt pathway defects and non-selective autophagy (Wang et al. 2017). In our study, we also find that the Cvt pathway plays a central role in autophagy against Nd poisoning in yeast cells.

## Conclusion

Through genome-scale screening, we identified 70 yeast monogene deletion mutations sensitive to Nd ions. These genes are involved in metabolism, transcription, protein synthesis, cell cycle, DNA processing, protein folding, modification, and cell transport processes. Furthermore, these data are mapped to the protein interaction group to identify the regulatory networks of Nd toxicity. These networks are associated with various signal pathways, mainly including calcium ion transport, phosphate pathways, vesicular transport, and cell autophagy. In addition, the content of Nd ions in yeast was detected by an inductively coupled plasma mass spectrometry, and most of these Nd-sensitive mutants showed an increased intracellular Nd content. In all, our results provide the basis for understanding the molecular mechanisms of detoxifying Nd ions in yeast cells, which will be useful for future studies on Nd-related issues in the environment, agriculture, and human health.

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**Availability of data and materials** The data obtained during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval** Not applicable. Animals were not used in this study.

**Consent to participate** Not applicable.

**Consent to publication** Not applicable.

**Competing interests** Not applicable.

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