

FIGURES

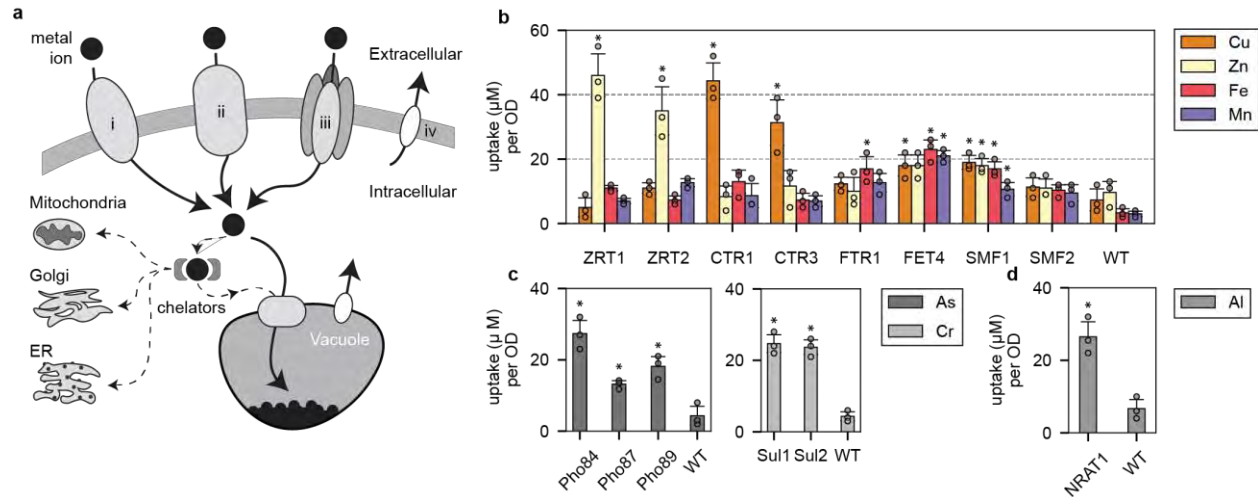


Figure 1 | Metal transporters were used to selectively internalize heavy metals into yeast. a)

A simplified schematic of metal transport in a eukaryotic cell. Membrane transporters can be divalent metal transporters (i), permeases (ii), metal transporters that are modified or found to have auxiliary metal transport function (iii), or exporters which are used to remove excess metals out of the cell (iv). **b)** Bar coloring indicates metal measured, with over-expressed transporter labeled on the x-axis. Values are reported in μM of metal uptake normalized per yeast culture density ($\mu\text{M}/\text{OD}$). Yeast metal transporters for zinc (ZRTs), copper (CTRs), iron (FTRs and FETs), and manganese (SMFs) were overexpressed and studied for metal hyperaccumulation. A WT strain was also tested in parallel as a control. **c)** The same study was performed for phosphate and sulfate permeases (PHOs, and SULs) which showed transport of arsenate and chromate, respectively. **d)** The Nr1t1 transporter, previously shown to uptake trivalent metals in certain strains of rice, was expressed and showed aluminum(III) transport. Asterisk above bar charts represent significance increase in uptake compared to WT ($p < .05$) for strains mentioned in the text. For all data, the mean \pm s.d. of three replicates are shown.

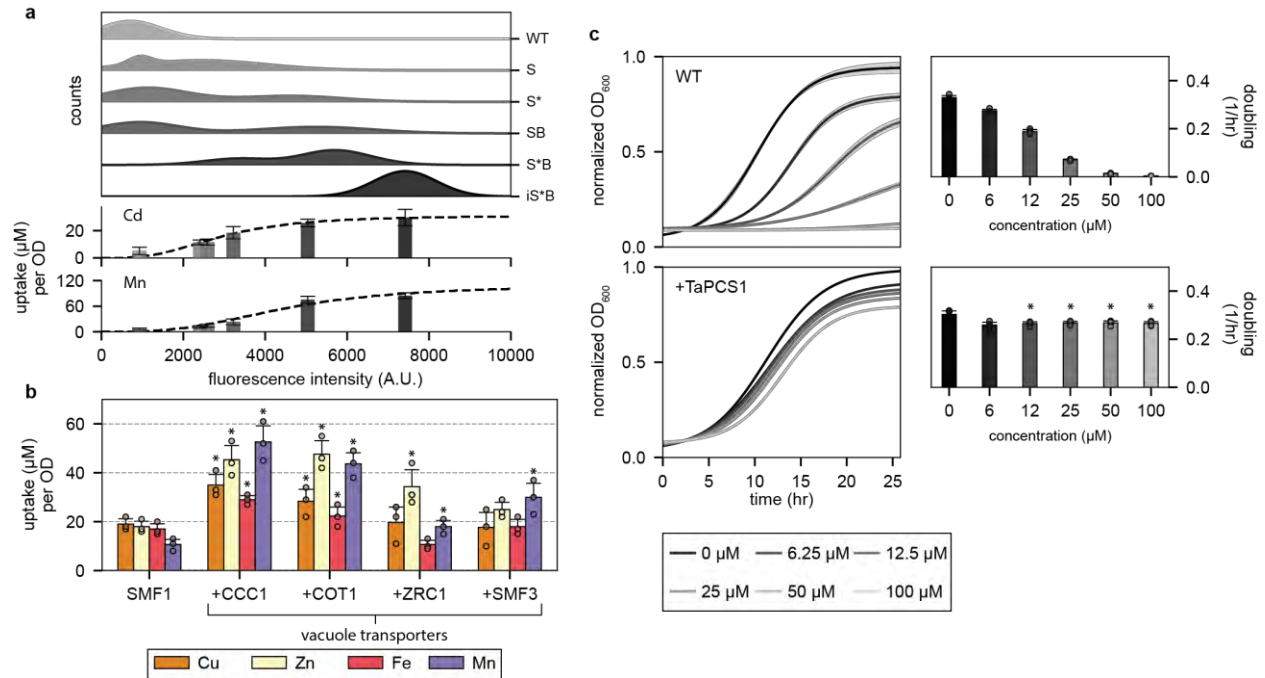


Figure 2 | Modifying yeast metal trafficking pathways improved metal uptake and

tolerance. **a)** Top subpanel shows the population distribution of SMF1 variants measured with fluorescently labelled V5-tag using flow cytometry. The weighted average of the fluorescent intensity corresponds to the placement of the lower subpanel bar charts which represent the level of metal uptake for that strain. Increasing expression levels of SMF1 correlated to increased metal uptake of cadmium or manganese; however, up to a certain point indicated by the plateau in uptake. **b)** Expression of vacuole transporters CCC1, COT1, ZRC1, and SMF3 in addition to SMF1 enhanced metal uptake. Asterisk above bar charts represent significant increase in uptake compared to SMF1 ($p < .05$). **c)** Constitutively expressing wheat phytochelatin synthase, TaPCS1, conferred heavy metal tolerance against cadmium. Asterisk above bar charts represent significant changes in growth rates compared to WT ($p < .01$). For all data, the mean \pm s.d. of three replicates are shown.

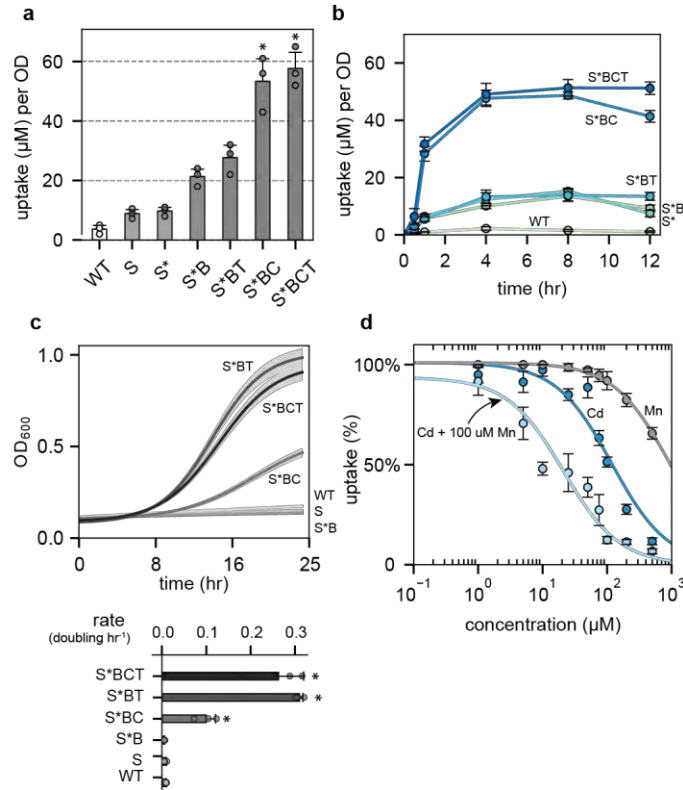


Figure 3 | Combining SMF1, CCC1, and TaPSC1 improved metal uptake capacity and tolerance. **a)** SMF1 (S) and its modifications (S* and Δ BSD2 as B) along with vacuole transporter CCC1 (C) and metal resistance enzyme TaPCS1 (T) incrementally enhanced cadmium uptake. Asterisk above bar charts represent significant increase in cadmium uptake when compared to WT ($p < .01$). **b)** Combinations of S*, B, C, and T showed changes in uptake rate, capacity, and metal retention over 12 hours of metal incubation. **c)** In the presence of 100 μ M cadmium, the growth rate is rescued with the addition of CCC1 and furthermore with TaPCS1. Subfigure below represents the doubling time of each strain. Asterisk to the side of bar charts represent significant increase in growth rate compared to WT ($p < .01$). **d)** S*BCT strain was titrated against cadmium, manganese, or cadmium in the presence of 100 μ M manganese (x-axis). Metal uptake experiments were performed at varying concentrations from 1 μ M to 1 mM, metal content analyzed using ICP, and values reported as percent uptake. S*BCT showed a

higher preference for manganese than cadmium, with cadmium uptake being dramatically reduced in the background presence of 100 μ M manganese (light blue curve). For all data, the mean \pm s.d. of three replicates are shown.

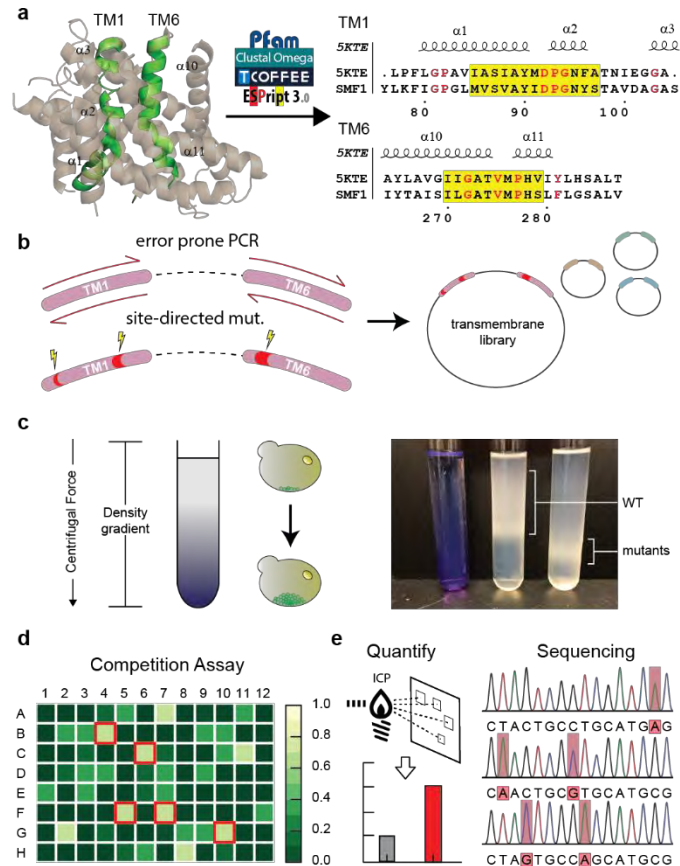


Figure 4 | A developed high throughput screen to systematically engineer selective metal transporters. a) Pfam protein database and clustering services such as ClustalΩ, TCOFFEE, and ESript3 were used to align SMF1 with referenced protein crystal structure 5KTE³³. Through literature searches and multi-alignments, transmembrane 1 and 6 (TM1, 6) were found to be the most significant regions for mutagenesis. The alignment comparing 5KTE with SMF1 shows the TM1 and TM6 region, where yellow highlights indicate conserved regions, and red text indicate highly conserved residues (similarity score > 0.7). **b)** Mutations cited to enhance or decrease metal transport were selectively mutated using site-directed mutagenesis. Libraries were then generated on top of these mutations through error-prone PCR. **c)** An initial screen was performed through rate-zonal density gradient centrifugation. **d)** Fractionated layers were plated, picked, and assayed for metal uptake. A competition assay of the desired metal versus the native metal

(e.g. manganese) was performed calorimetrically. Wells with the least amount of native metal uptake (highest signal) were selected and **e)** quantitatively measured for metal uptake using ICP. Mutations were sequenced and reintroduced in the pipeline to generate better performing mutants.

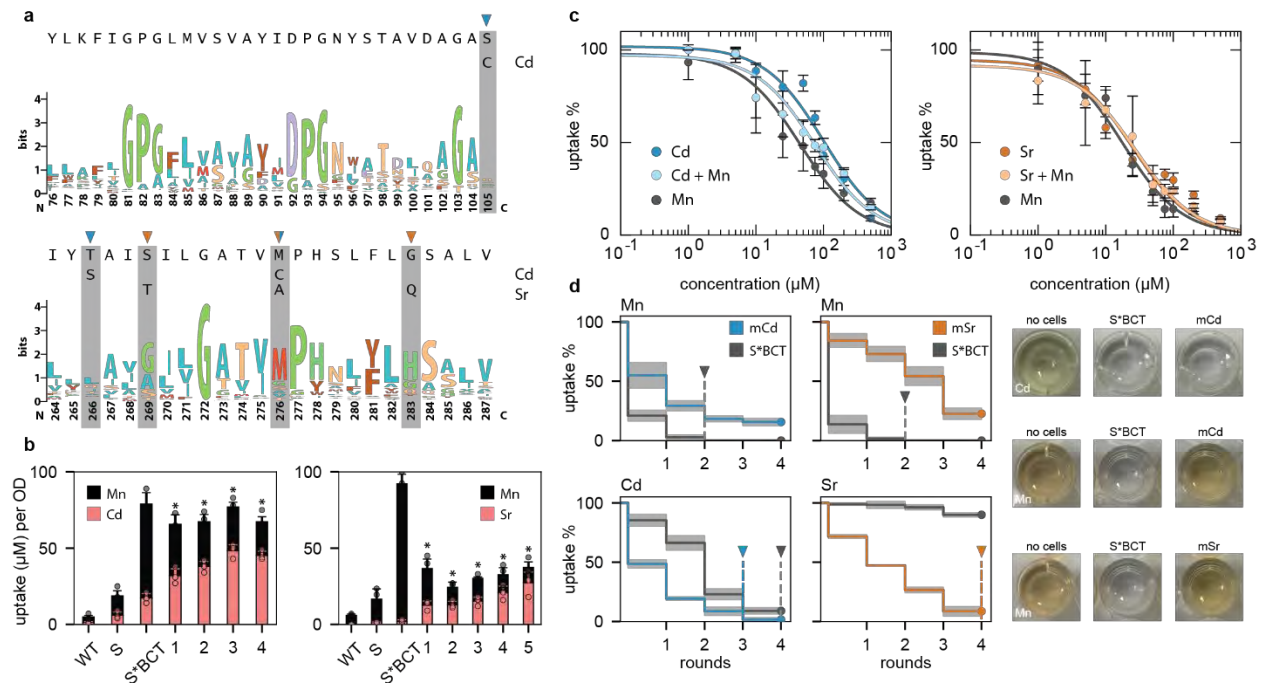


Figure 5 | Creation of a cadmium and strontium metal transporter after 4-5 rounds of screens. **a)** Weblogos of SMF1 TM1,6 from Nramp multi-alignment are displayed, with cadmium and strontium mutations highlighted. Cadmium mutants had S105C, T266S, and M276C. Strontium mutants had G189R, S269T, M276A, and G283Q. **b)** Total metal uptake of 100 μM cadmium and manganese were measured to assess manganese interference. Cadmium mutant labeling corresponds to 1=M276C, 2=M276C+S105C, 3=M276C+T266S, and 4=M276C+S105C+T266S. Strontium mutant labeling corresponds to 1=M276A, 2=M276A+G189R, 3=M276A+G189R+S269T, 4=M276A+G189R+G283Q, and 5=M276A+G189R+S269T+G283Q. Strain background for all mutants were BCT. Asterisk above bar charts represent significant changes in both Cd and Mn uptake compared to unmutated S*BCT (p < .05). **c)** Titration curves of fully mutated cadmium and strontium transporters in strain BCT were performed for Cd or Sr, respectively, with or without 100 μM Mn; x-axis represents the concentration of either Cd, Sr, or Cd, Sr with Mn. **d)** Sequential uptake

experiments, up to 4 rounds, were performed to measure the amount of iterations required for complete elimination of 100 μM cadmium or strontium in a mixture of 100 μM manganese.

Images on the right are colorimetric detection of cadmium and manganese (there are no available colorimetric assays for strontium at this concentration) showing selective preference for cadmium (no coloration) against native metal manganese (darkened well). For all data, the mean \pm s.d. of three replicates are shown.

