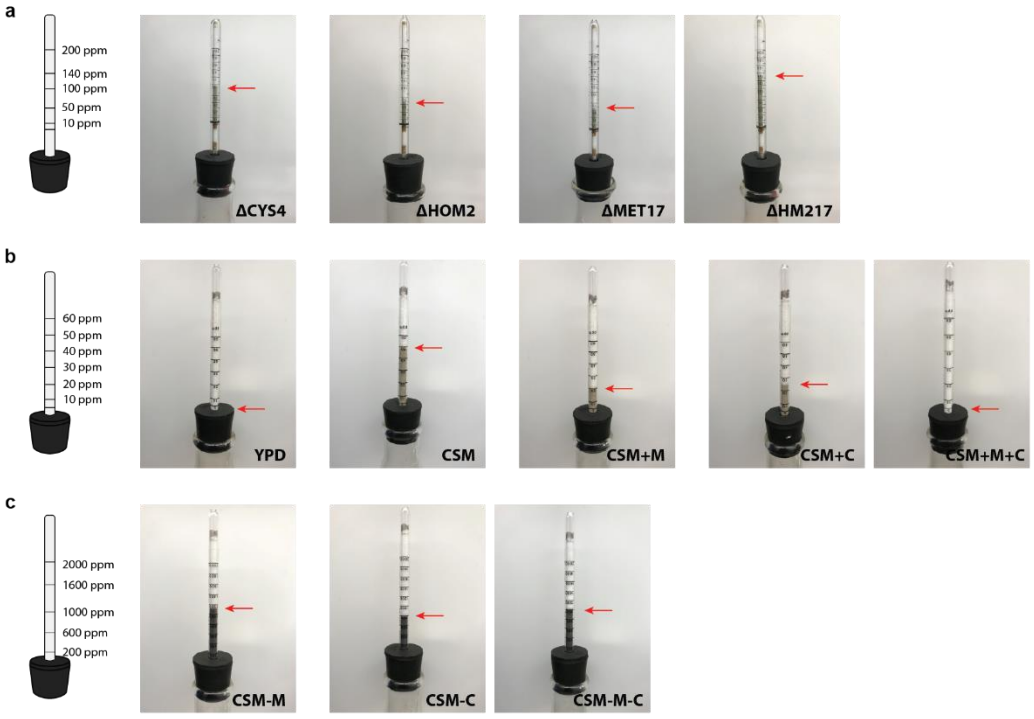
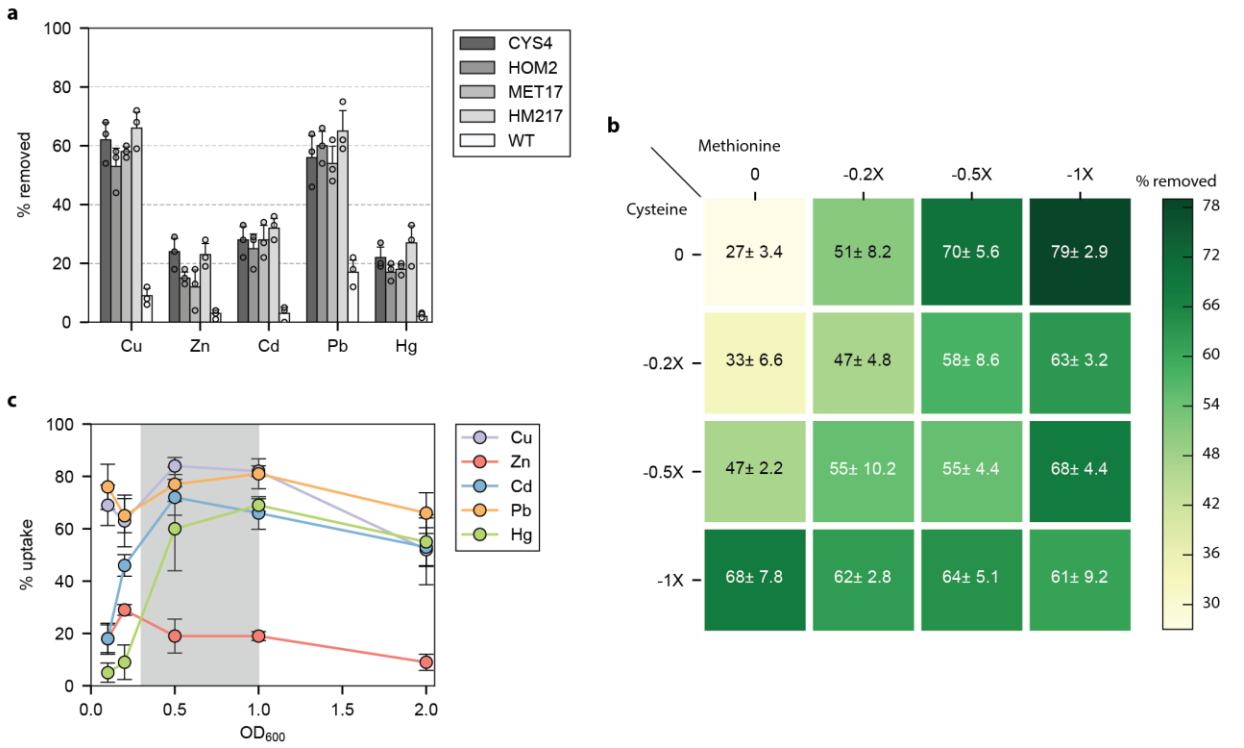
Using yeast to sustainably remediate and extract heavy metals from wastewaters

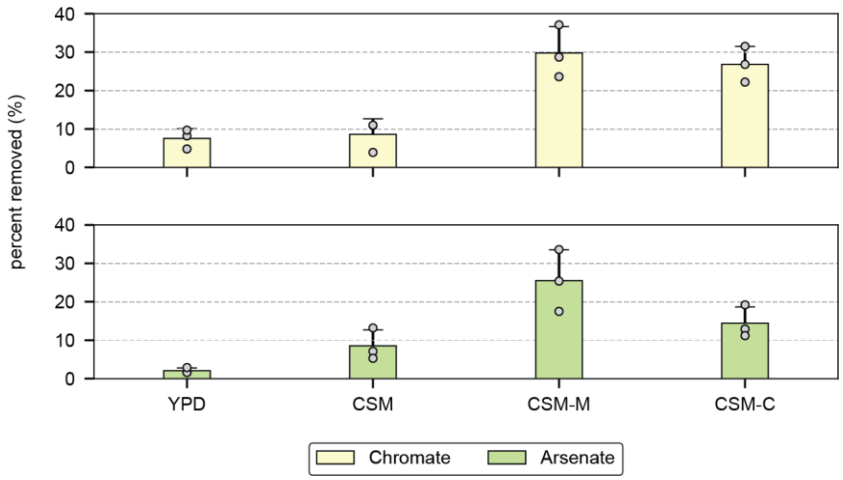
Supplementary Figures



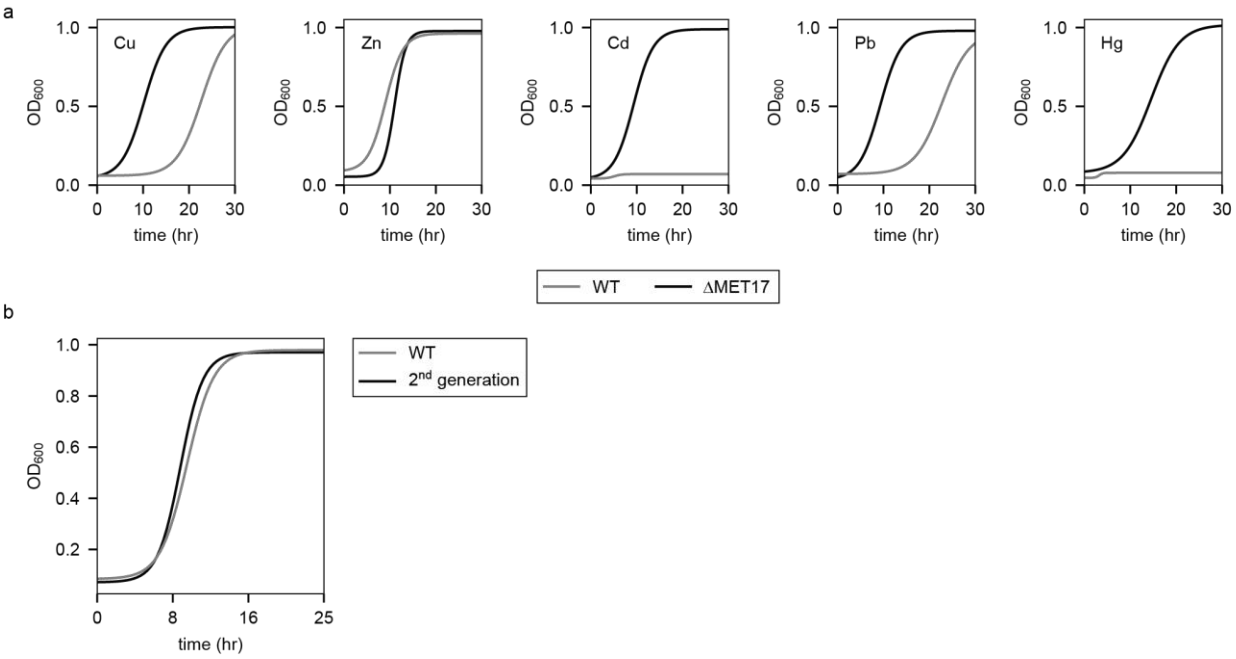
Supplementary Figure 1 | Measuring yeast H_2S production. Illustrations left of the images represent H_2S detection columns with tick marks indicating the level of sulfide measured in ppm. (**a**) Sulfide detection using 200 pm columns for mutants ΔCYS4, ΔHOM2, ΔMET17, and ΔHM217. (**b**) Sulfide detection using 60 ppm columns for ΔMET17 in cultures of YPD, CSM, and CSM with the addition (+) of methionine (M) or cysteine (C). (**c**) Sulfide detection using 2000 ppm columns for ΔMET17 in CSM cultures lacking (-) methionine or cysteine, or both.



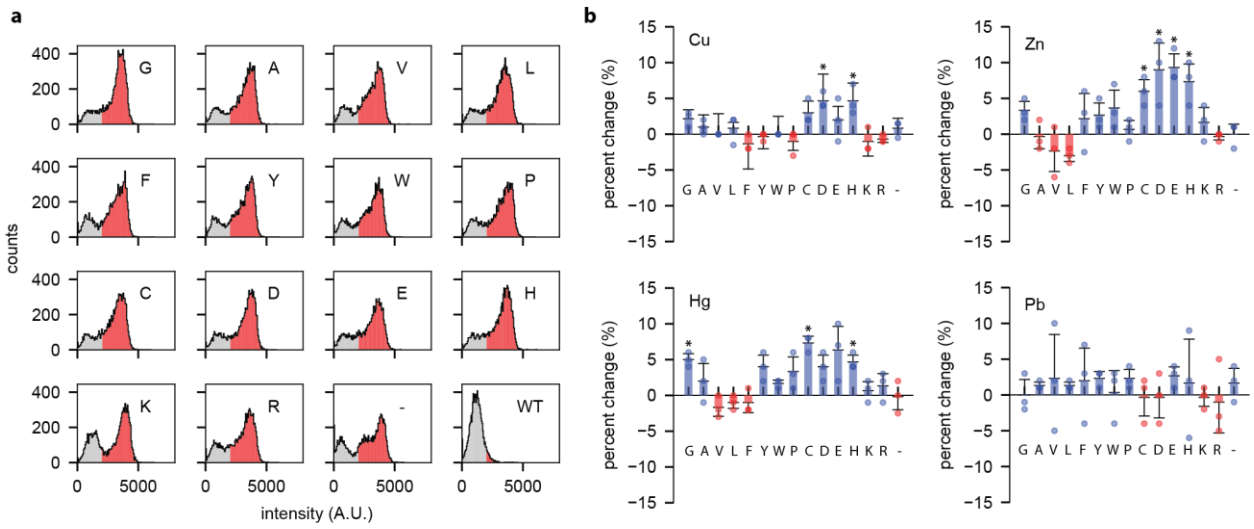
Supplementary Figure 2 | Strain, culture density (OD_{600}), and media composition effects on metal precipitation. (**a**) Precipitation of copper, zinc, cadmium, lead, and mercury with mutants ΔCYS4, ΔHOM2, ΔMET17, and ΔHM217, and WT as a control, in CSM. (**b)** Effects of removing methionine (M) and/or cysteine (C) from CMS on precipitation efficacy using ΔMET17. Columns represent removal of M while rows represent removal of C from CSM. 1X stands for 100% removal (i.e. 0.2X = 20% and 0.5X=50%). Annotated values per cell grid represent the percent cadmium removed and standard error. (**c**) Optimal culture density (marked within grey bounds) was determined by titrating cultures of ΔMET17 at different OD_{600} with copper, zinc, cadmium, lead, and mercury. Metal color coding matches those used in the main text. For all data, the mean ±s.d. of three replicates were taken for each data point.



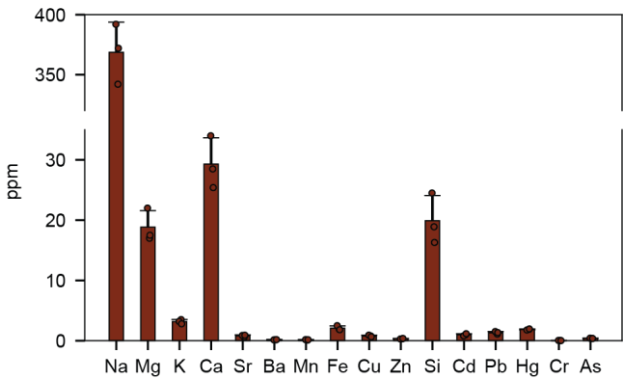
Supplementary Figure 3 | Removal of polyatomic anions chromate (CrO_4^{3-}) and arsenate (AsO_4^{3-}) using sulfide-producing yeast. Chromate and arsenate were removed through sulfur-mediated metal reduction by conversion of Cr(IV) to Cr(III) and As(V) to As(III). The reaction mechanism differed from the transition metal sulfide precipitation reactions in that sulfide acts as a reducing agent to convert chromate and arsenate into reduced insoluble oxides. For all data, the mean $\pm$s.d. of three replicates were taken for each data point.



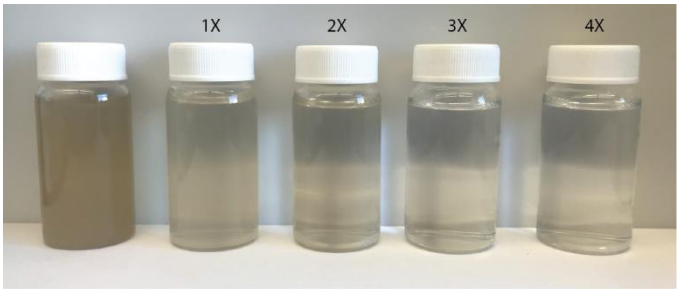
Supplementary Figure 4 | Growth curve comparisons of ΔMET17 and WT in metal containing cultures. (**a**) Growth curves of ΔMET17 and WT grown in CSM with 100 μM metals specified. (**b**) ΔMET17 was used to precipitate 100 μM cadmium overnight. Cells were then diluted 1 to 100 and grown again (2nd generation) and compared to WT. All data points were measured in a BioTek Synergy 2 plate reader with 100 μL cultures shaken at 30ºC. All data was normalized to 1 within each experiment.



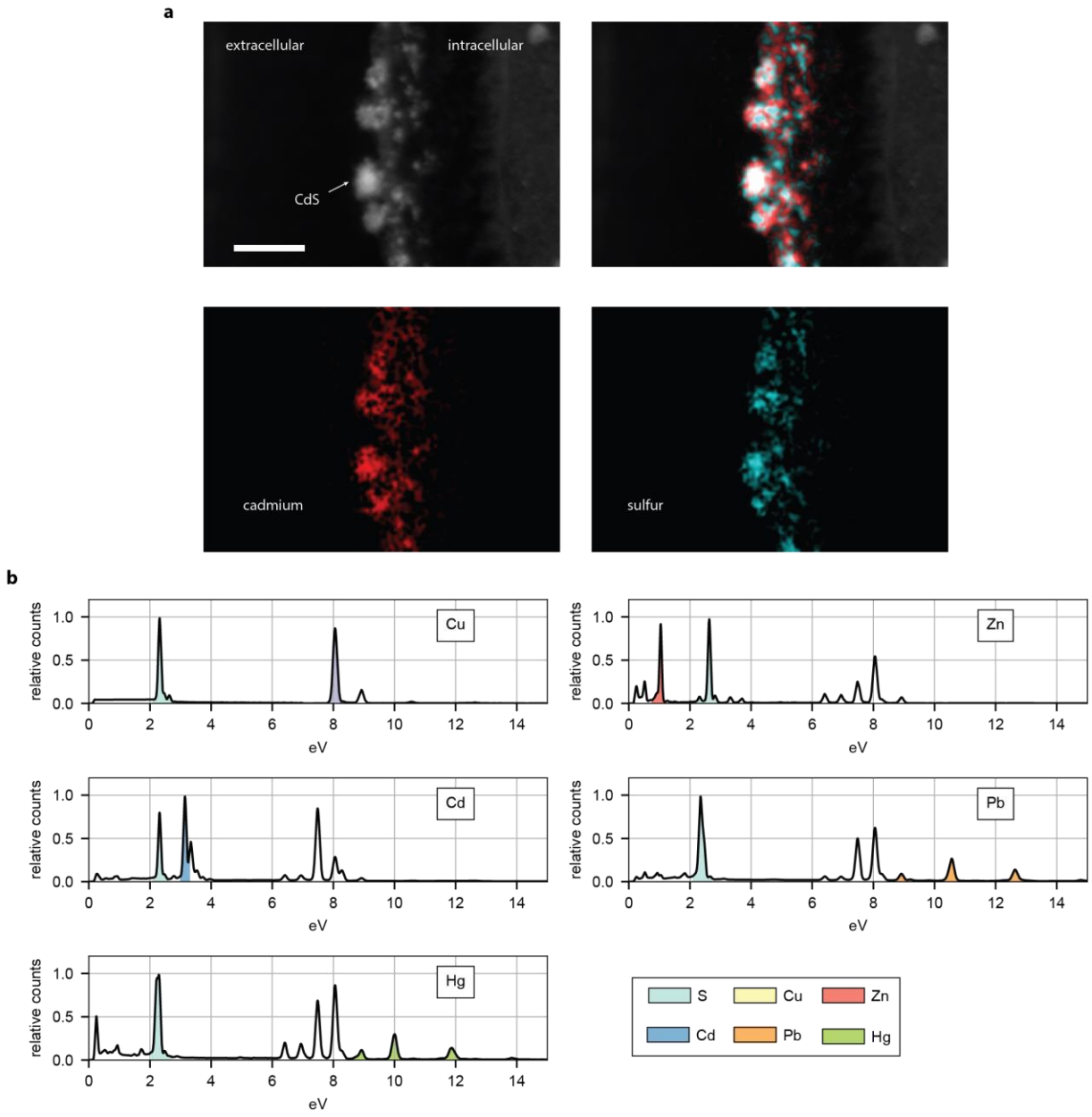
Supplementary Figure 5 | Effects of yeast displayed on metal precipitation. (**a**) Flow cytometry data showing fluorescence intensity of labelled C-terminus V5 tag on hexa-amino acid repeats. Expression was compared against an empty displaying pYAGA vector (-) and non-displaying WT for controls. Positive expression was cutoff at 2000 A.U. for segregating expressing versus non-expressing populations (grey and red, respectively). (**b**) ΔMET17 transformed with pYAGA with the hexa-amino acid motif (specified on the x-axis) was used to change the amount of precipitation of copper, zinc, lead, and mercury.



Supplementary Figure 6 | Profile of metal content of effluent taken from the Canadian Athabasca Oil sands. Group I and II elements, such as Na, Mg, K, and Ca, in addition to silicon were strongly present. Heavier and toxic elements such as cadmium, mercury, lead, and arsenic were appreciably present and 1-3 orders of magnitude greater than EPA standards.

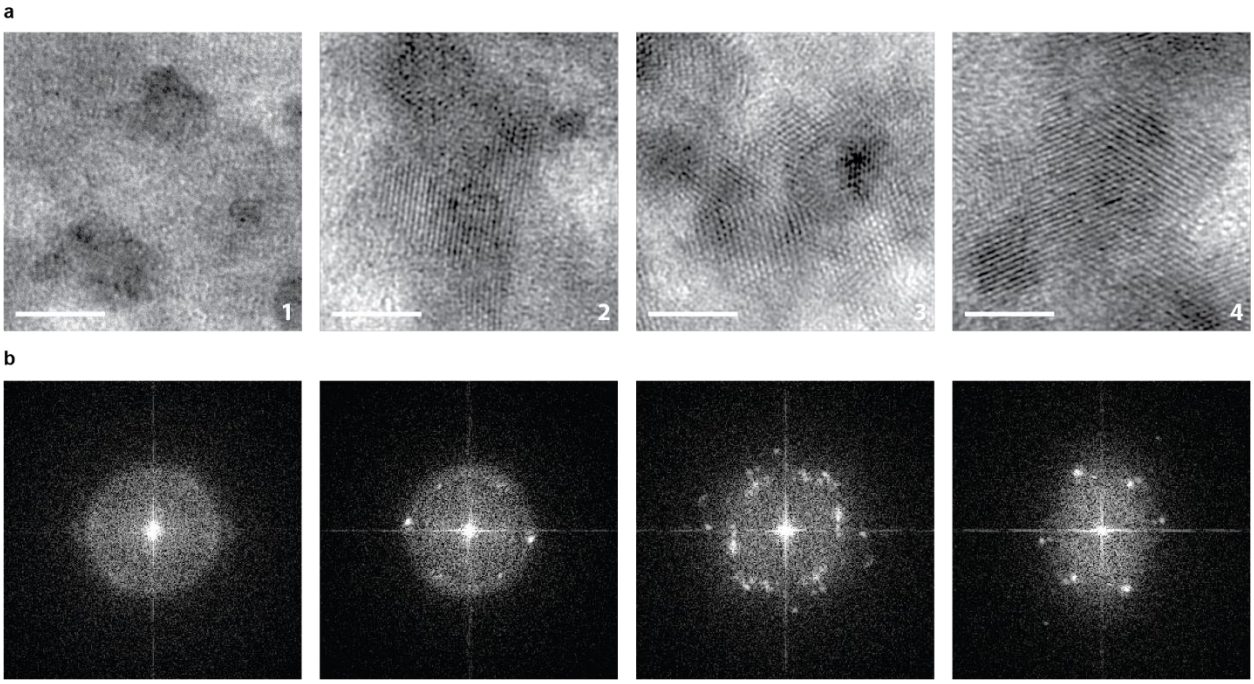


Supplementary Figure 7 | Visual representation of remediated effluent taken from oil sands after several rounds with ΔMET17. Numbers at the top (1X, 2X, etc.) of each sample indicate the number of precipitation rounds performed.

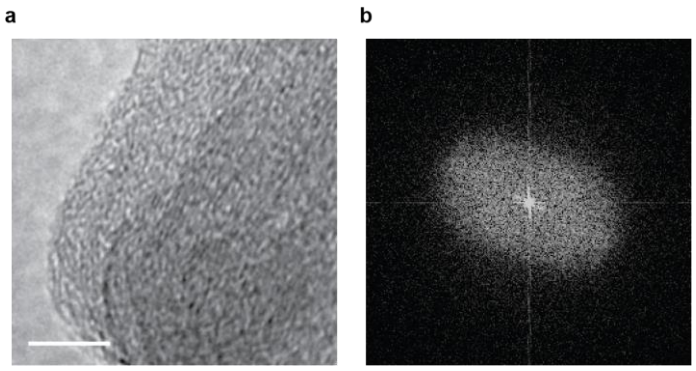


Supplementary Figure 8 | Elemental mapping of precipitated metal sulfide particles. (**a**) Elemental mapping of HRTEM images of cadmium sulfide nanoparticles deposited on the cell wall of ΔMET17. Cadmium was false colored as red, sulfide as blue. Scale bars represent 50 nm. (**b**) Elemental dispersive X-ray (EDX) spectroscopy was performed on purified precipitated copper, cadmium, lead, mercury, and zinc sulfide particles under TEM. Elemental Kα peaks

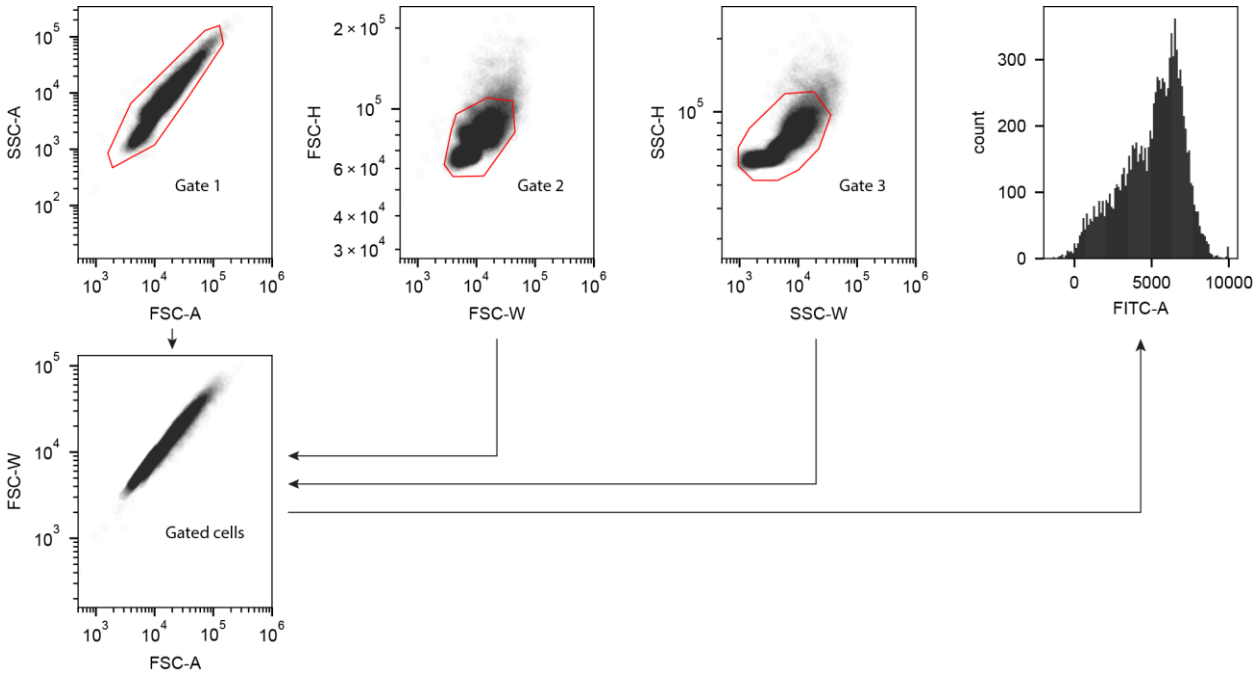
were colored and highlighted as areas under the curve for qualitative comparisons. Metal color coding of spectral plots match those used in the main text.



Supplementary Figure 9 | Enlarged HRTEM images from Error! Reference source not found.**a.** (**a**) 1=GGGGGG, 2=CCCCCC, 3=GGCGGC, 4=GCCGCC. (**b**) Fourier transform of image (a)**.** Scale bars represent 5 nm.



Supplementary Figure 10 | HRTEM image of purified cadmium sulfide particles from ΔMET17 without displayed peptides (control). (**a**) High resolution image of precipitated particle edge. (**b**) Fourier transform of image (a). Scale bar represents 5 nm.



Supplementary Figure 11 | Yeast gating strategy for flow cytometry measurements. FSC-A and SSC-A gated cells. FSC-W and FSC-H gated vertically oriented cells (vertical singlets). SSC-W and SSC-H gated horizontally oriented cells (horizontal singlets). Gating on these 3 plots, single cells were measured for fluorescence.

Supplementary Tables

Name	direction	primer
pRS303 (HIS)	fwd	TATTACTCTTGGCCTCCTCT
	rev	CCTGATGCGGTATTTTCTC
pRS305 (LEU)	fwd	AACTGTGGGAATACTCAG
	rev	GGTCAGGTCATTGAGTG

Supplementary Table 1 | Primers used to amplify HIS and LEU auxotrophic cassettes from the pRS303 and 305 vector. Primers were used to append overhangs for homologous recombination of W303α strains to delete specific sulfate assimilation genes.

Supplementary Table 2 can be found in the attached excel document along with this publication.

Name	AA	fwd	rev
mut1	*GCCGCC*	GGTTGTTGTGGATGCTGT	ACAGCATCCACAACAACC
mut2	*CCGCCG*	TGCTGTGGGTGCTGTGGC	GCCACAGCACCCACAGCA
mut3	*CGGCGG*	TGTGGCGGCTGTGGCGGC	GCCGCCACAGCCGCCACA
mut4	*GCGGCG*	GGATGCGGAGGCTGCGGA	TCCGCAGCCTCCGCATCC
mut5	*GGCGGC*	GGTGGTTGCGGTGGGTGC	GCACCCACCGCAACCACC

Supplementary Table 3 | Amino acid and DNA sequences used for cloning glycine-cysteine motifs for yeast display. Columns AA, fwd, and rev stand for the hexa-amino acid sequence, forward (5`-3`) oligo, and reverse (3`-5`) oligo, respectively. Reverse oligos were followed with a TAG stop codon. Forward and reverse oligos were appended with BamHI and PmeI sticky ends, respectively. Oligos were ordered from IDT and annealed to form duplex DNA strands which were then ligated into pYAGA digested with BamHI and PmeI.