Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Information for

Human Calprotectin Affects the Redox Speciation of Iron

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This Supplementary Information includes:

Table S1. Nomenclature of Human Calprotectin Variants

Protein	S100A8 Mutation(s)	S100A9 Mutation(s)
CP	N/A	N/A
CP-Ser	C42S	C3S
CP-Ser ΔHis_3Asp	C42S, H83A, H87A	C3S, H20A, D30A
CP-Ser ΔHis_4	C42S, H17A, H27A	C3S, H91A, H95A
CP-Ser $\Delta\Delta$	C42S, H17A, H27A, H83A, H87A	C3S, H20A, D30A, H91A, H95A
CP-Ser-AAA	C42S	C3S, H103A, H104A, H105A

Table S2. Metal Analysis of Bacterial Growth Media [a]

	Concentration (μM)		
Element	TSB [b]	BHI [c]	LB [d]
Mg	684 ± 67	157 ± 49	1190 ± 90
Ca	332 ± 15	231 ± 12	337 ± 12
Mn	0.566 ± 0.040	0.818 ± 0.61	0.893 ± 0.043
Fe	13.8 ± 1.0	11.7 ± 1.6	14.5 ± 0.8
Co	0.175 ± 0.016	0.366 ± 0.087	0.923 ± 0.051
Ni	0.843 ± 0.121	0.227 ± 0.144	0.442 ± 0.401
Cu	0.366 ± 0.028	0.419 ± 0.131	1.14 ± 0.08
Zn	19.2 ± 1.0	24.0 ± 2.4	41.4 ± 1.5

[a] Four independent media preparations were analyzed (mean ± SDM, n = 4). [b] Tryptic soy broth. [c] Blood heart infusion medium. [d] Luria-Bertani medium.

Table S3. Fe Dissociation Constant and Reduction Potential Values of Small-Molecule Chelators [a]

Chelator	$K_{d,Fe(III)}$ (M)	$K_{d,Fe(II)}$ (M)	E^o (V vs. SHE)	References
Ent	10^{-49}	10^{-22}	–0.75	1, 2, 3
DFO	10^{-31}	10^{-9}	–0.48	4, 5
EDTA	10^{-26}	10^{-15}	+0.12	6
DP	—	10^{-18}	+0.82	7, 8, 9, 10
Phen	—	10^{-25}	+0.82	7, 8, 10

[a] Ent, DFO, and EDTA form 1:1 Fe(II):ligand complexes. DP and Phen form 1:3 Fe(II):ligand complexes. The K_d and E^o values listed here are representative of the trends discussed in the main text and were obtained under various experimental conditions as detailed in the references.

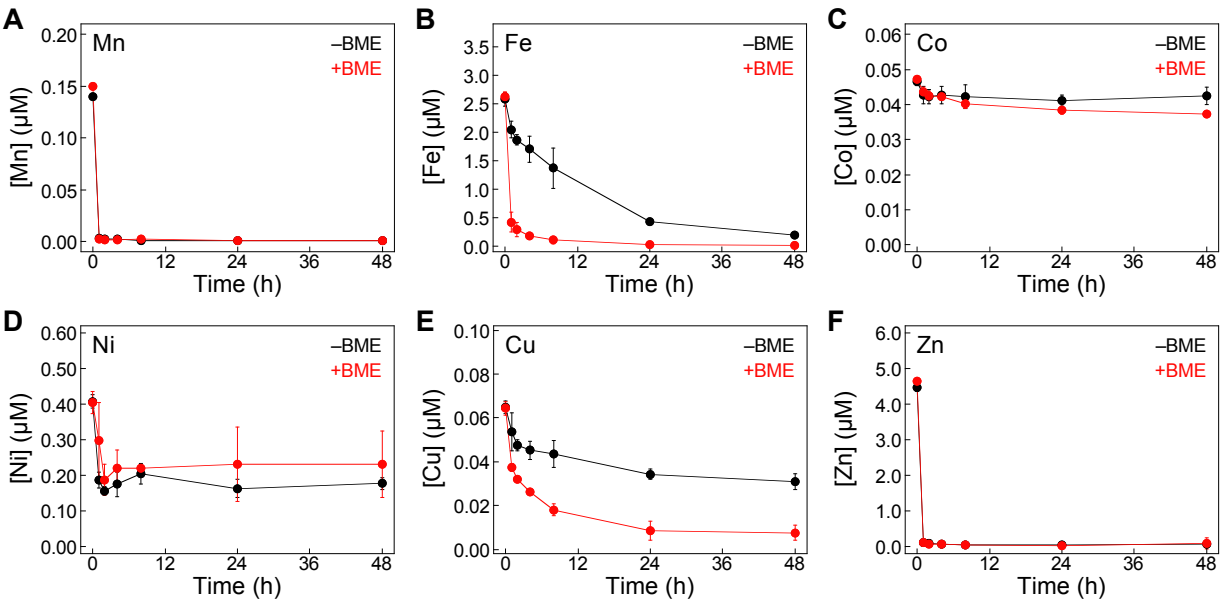

Fig. S1. Metal depletion by CP-Ser under aerobic conditions. Tris:TSB was treated with 10.5 μM (250 μg/mL) CP-Ser in the absence (black) or presence (red) of ≈3 mM BME at 30 ºC, 150 rpm. The Mn (A), Fe (B, reproduced from Fig. 2B of the main text), Co (C), Ni (D), Cu (E), and Zn (F) in the CP-treated medium was analyzed by ICP-MS at t = 0, 1, 2, 4, 8, 24, and 48 h (mean ± SDM, *n* = 3).

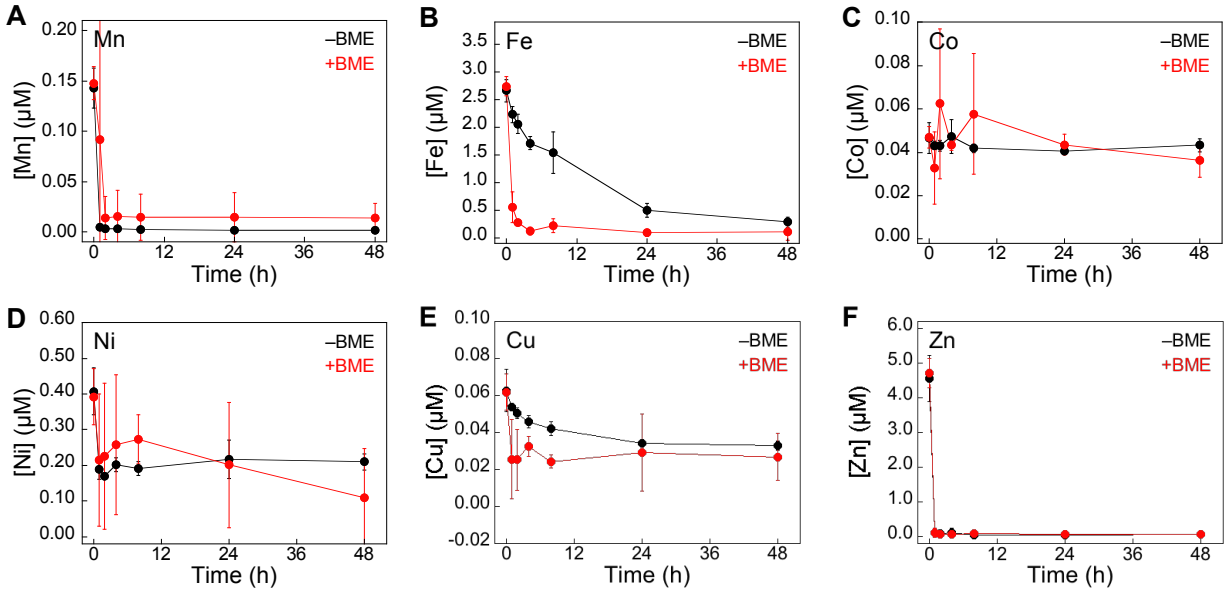

Fig. S2. Metal depletion by ΔHis$_3$Asp under aerobic conditions. Tris:TSB was treated with 10.5 μM (250 μg/mL) CP-Ser in the absence (black) or presence (red) of ≈3 mM BME at 30 ºC, 150 rpm. The Mn (A), Fe (B, reproduced from Fig. 2B of the main text), Co (C), Ni (D), Cu (E), and Zn (F) in the CP-treated medium was analyzed by ICP-MS at t = 0, 1, 2, 4, 8, 24, and 48 h (mean ± SDM, *n* = 3).

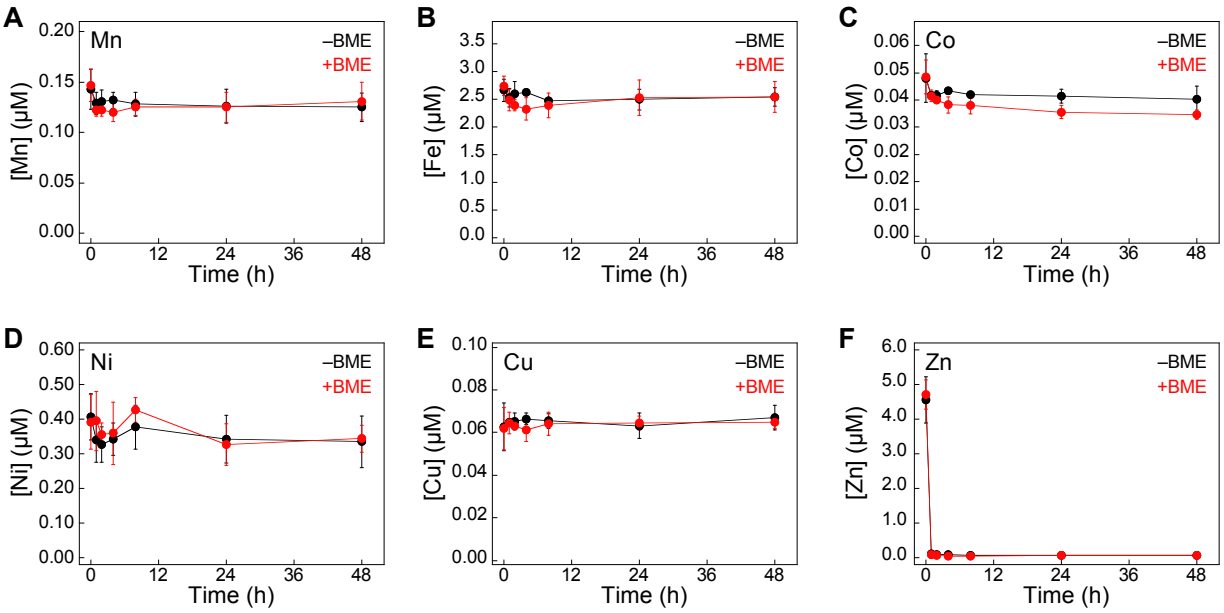

Fig. S3. Metal depletion by ΔHis$_4$ under aerobic conditions. Tris:TSB was treated with 10.5 μM (250 μg/mL) CP-Ser in the absence (black) or presence (red) of ≈3 mM BME at 30 ºC, 150 rpm. The Mn (A), Fe (B, reproduced from Fig. 2B of the main text), Co (C), Ni (D), Cu (E), and Zn (F) in the CP-treated medium was analyzed by ICP-MS at t = 0, 1, 2, 4, 8, 24, and 48 h (mean ± SDM, *n* = 3).

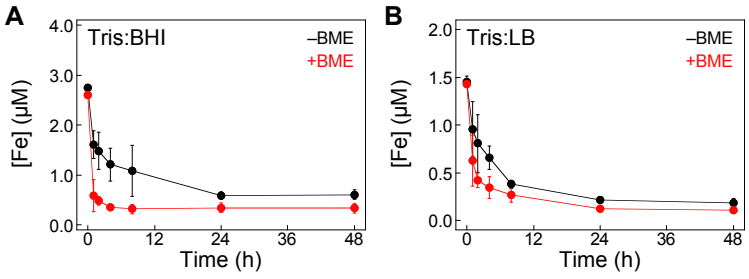

Fig. S4. Iron depletion of bacterial growth media by CP-Ser. (A) Tris:BHI and (B) Tris:LB were treated with 10.5 μM (250 μg/mL) CP-Ser in the absence (black) or presence (red) of ≈3 mM BME at 30 ºC, 150 rpm. The metal content of Fe was analyzed by ICP-MS at t = 0, 1, 2, 4, 8, 24, and 48 h (mean ± SDM, *n* = 3).

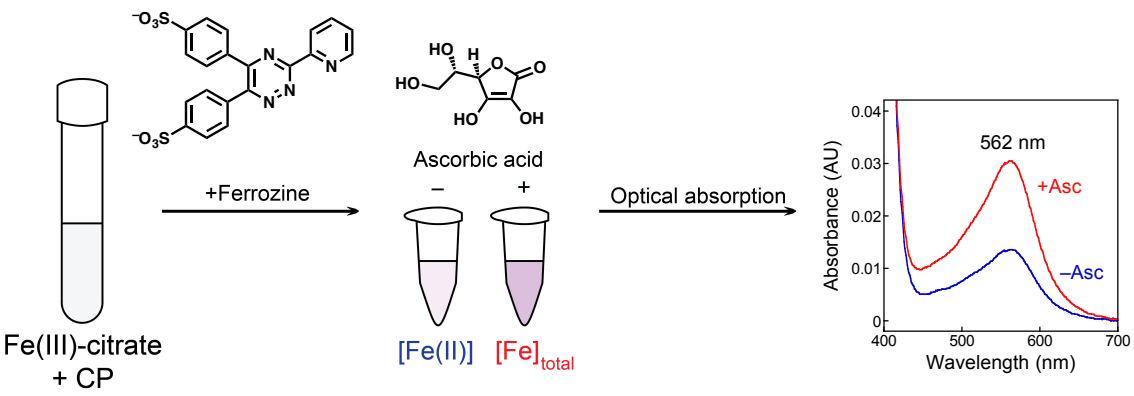

Fig. S5. Schematic cartoon of the ferrozine assay quantifying Fe(II) and Fe(III). A solution of 10 μM Fe(III) citrate in 75 mM HEPES, 100 mM NaCl, 2 mM $CaCl_2$, pH 7.0 was incubated with 10.5 μM CP. At each time point, aliquots of the mixture were transferred to microcentrifuge tubes and treated with ferrozine in the absence (Fe(II), blue) and presence (total Fe, red) of ascorbic acid. The optical absorption spectrum of each sample was collected, and Fe concentration was quantified using a calibration curve.

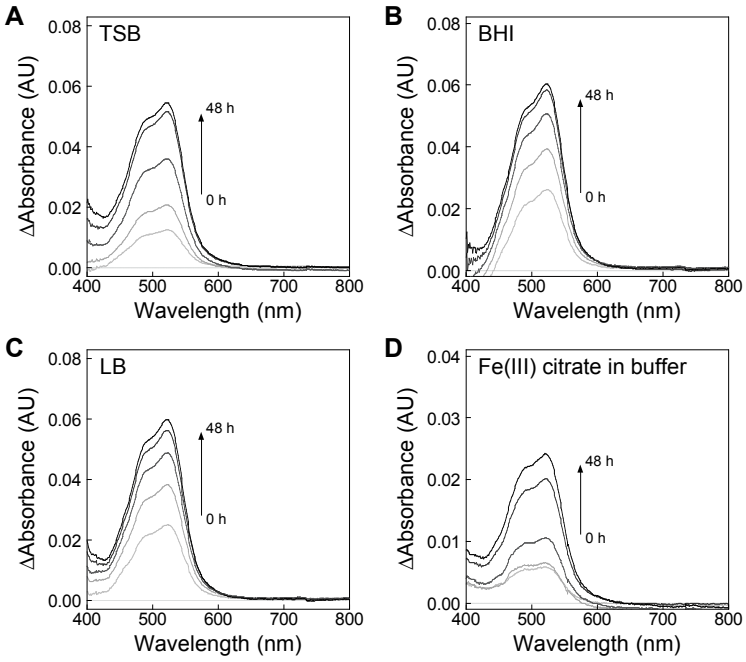

Fig. S6. Optical absorption spectroscopy of 2',2'-dipyridyl (DP) incubated in bacterial growth medium and buffer solutions. Representative optical absorbance difference spectra of (A) TSB, (B) BHI, (C) LB, and (D) 10 μM Fe(III) citrate in 75 mM HEPES, 100 mM NaCl, 2 mM $CaCl_2$, pH 7.0 incubated with 1.0 mM DP at 30 ºC, 150 rpm. The spectrum collected at t = 0 h was subtracted from those of other time points (t = 2, 4, 8, 24, 48 h).

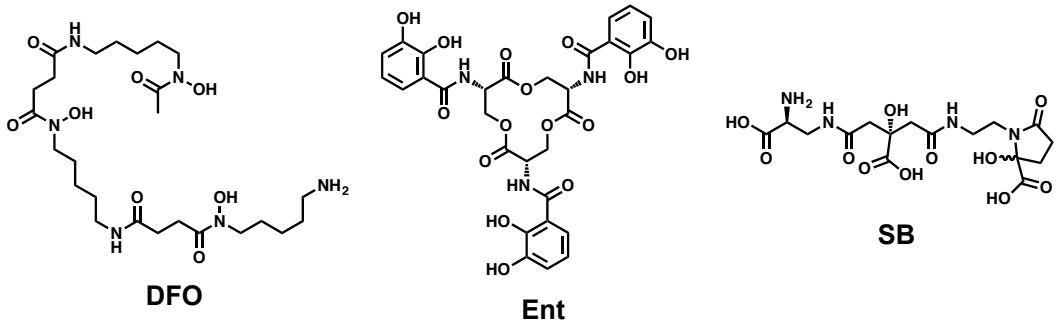

Fig. S7. Chemical structures of the siderophores employed in this study.

Supplementary References

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