

The zinc repository of *Cupriavidus metallidurans*

Electronic Supplementary Information

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Nies^{1*}**

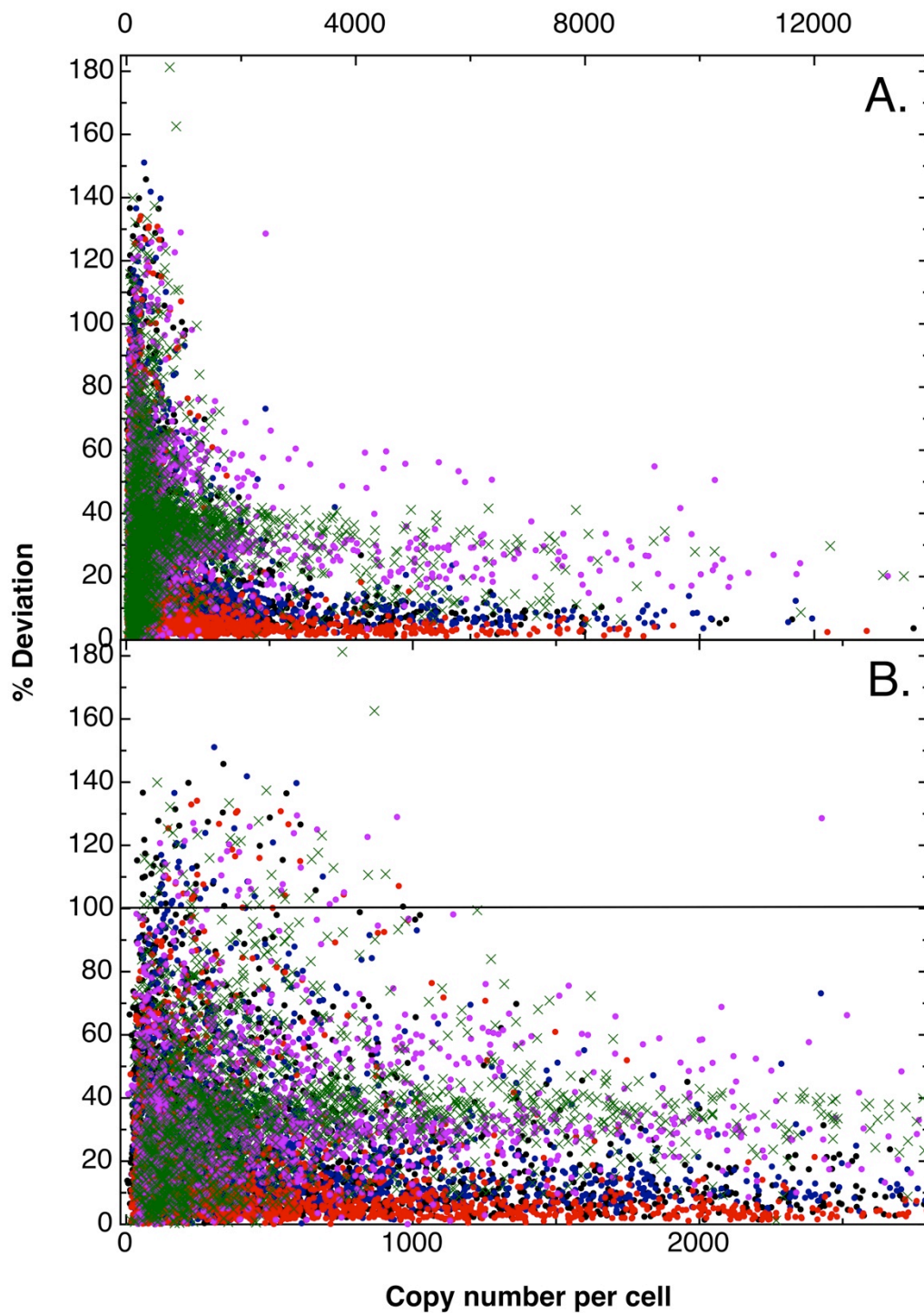
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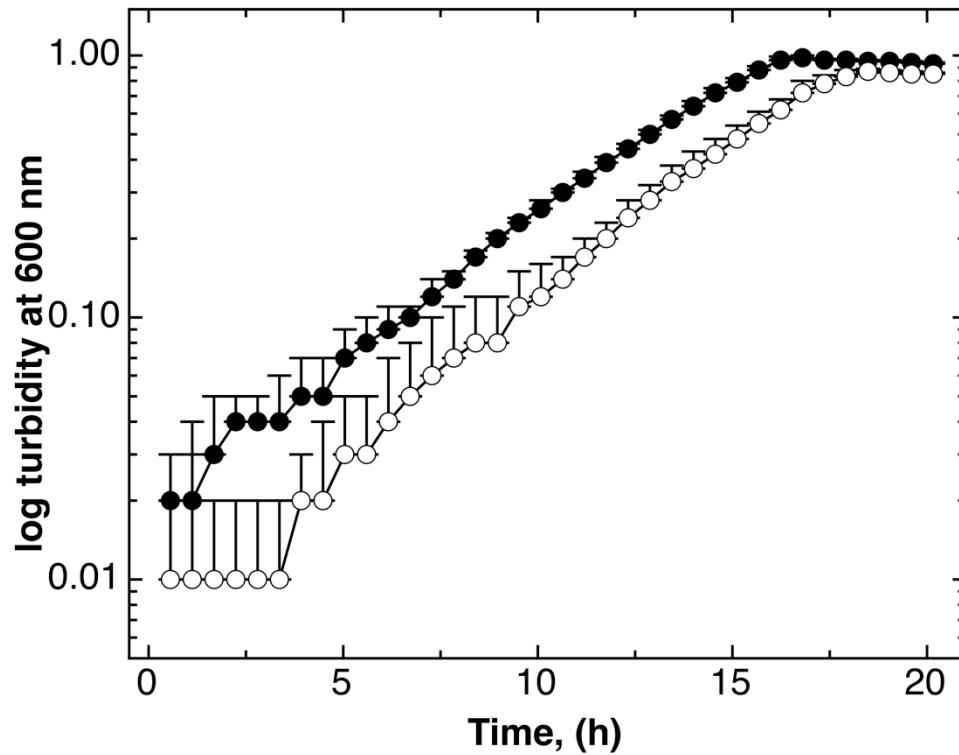
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Supplementary Figure S1. Deviation of the results. The deviation of the number of individual proteins per cell was plotted against the number per cell obtained in a biological repeat with the differently colored symbols indicating the experiment. Run 1 not plotted. Panel B gives a 5-fold expansion of Panel A.



Supplementary Figure S2. Growth rate of *C. metallidurans* strain AE104 and its $\Delta zupT$ mutant. The growth curve shows the increase in turbidity of a AE104 (filled circles) and a $\Delta zupT$ (open circles) culture at 30°C in Tris-buffered mineral salts medium with sodium gluconate as the carbon source. At least three experiments, deviation bars shown only to the top due to the logarithmical scale.

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ABF08170 1 MCVVCGC-----SDNSPTHARHVQQASGEPGVITVN 31
ABF08419 1 MCTICGCGAGETRIEGQELHTEHVHADGTVHAHAHPPHEQDHHYADHDHDHAHAHRAV 60
      CxxCGC motif

ABF08170 32 PANGDLHFGAGVARVSVPGMSQERAIKLETDILGANNRVAQQNRAHFELHGVTALNVVSS 91
ABF08419 61 RGTDHLHYGHGPAGAHAPGMSQARMVKIEQDILGKNNAYAAQNRWFDEHGVFALNFVSS 120

ABF08170 92 PGSGKTTLLCATIEALKARQPGLHVAVIEGDQQTSFDADRIRATGAPAIQVNTGKGCHLD 151
ABF08419 121 PGSGKTTLLVRTIEAL---QSTCGLAVIEGDQQTSFDAERIRATGVRALQINTGKGCHLD 177
      GxGKTT (Walker A motif)

ABF08170 152 APMVAEAFAKLHTEHADGHSHDHVDRHGHHHGHGHGHEHDHHHHGDAHSLLFIENVGN 211
ABF08419 178 AYMVGHALEQLRPQD-----ESVLLIENVGN 203
      (Walker B motif) ExxG

ABF08170 212 LVCPAMWDLGEAAKVAILSSVTEGEDKPLKYPDMFAASQLMILNKDLLPHVKFDVARCIA 271
ABF08419 204 LVCPSAFDLGEAHKVVILSVTEGEDKPLKYPDMFRAASLMLLNKCDLLPHLSFDVERTIE 263
      (Base recognition motif) NKxD

ABF08170 272 LARRVNPAIEVIQLSATTGEGMDAWLHWDHAMSTHHHHHDETVSEEAALRERVKQLEAE 331
ABF08419 264 YARRVNPALHVIRTSSTTGEGFAEWLAWITEGLVQRAKRSQNVD---LLRARVAELEAE 320

ABF08170 332 LARAKAVLSAKTPI 345
ABF08419 321 LAVLKR----- 326

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Supplementary Figure S3. Alignment of the two HypB GTPases and nickel chaperones required for synthesis of the membrane-bound hydrogenase (AB08170, HypB1 Rmet_1285) and the soluble NAD-reducing hydrogenase (AB08419, HypB2, Rmet_1536). Alignment performed with Geneious (www.geneious.com), histidine- and cysteine-rich regions underlined, and conserved motifs indicated.

Supplementary Table S1. Number of identified proteins in the SYNAPT experiment. Three biological repeats were performed to identify and quantify the proteins in the supernatant or solubilized sediment of ultracentrifuged crude extract from mid-exponential cells of *C. metallidurans* AE104, its $\Delta zupT$ mutant, and in one experiment only the plasmid-containing *C. metallidurans* wild type strain CH34(pMOL28, pMOL30). The data base contained 5804 proteins. Of these, 3469 proteins (60%) were identified in at least one experiment, the other 2335 proteins were not further evaluated.

Run	Strain	Fraction	Not found ^a	Not quantified ^b	One value ^c	Large deviation ^d	Lower deviation ^e
1	AE104	Supern.	2807	8	210	312	132
1	AE104	Membr.	2769	4	650	26	20
1	$\Delta zupT$	Supern.	2776	3	639	26	25
1	$\Delta zupT$	Membr.	2618	5	281	54	511
2	AE104	Supern.	1366	23	360	154	1566
2	AE104	Membr.	1167	43	412	361	1486
2	$\Delta zupT$	Supern.	1570	29	292	112	1466
2	$\Delta zupT$	Membr.	1772	29	342	129	1197
3	AE104	Supern.	1425	17	380	197	1450
3	AE104	Membr.	1840	26	302	192	1109
3	$\Delta zupT$	Supern.	1711	28	337	503	890
3	$\Delta zupT$	Membr.	1861	22	409	325	852
3	CH34	Supern.	1474	25	331	202	1437
3	CH34	Membr.	1316	28	441	929	755

^aNot found in at least three technical repeats of this biological experiment. ^bAt least one specific tryptic peptide of this protein has been identified but the SYNAPT algorithm quantifies the protein only if at least three peptides were unambiguously found. ^cA protein could be quantified in one of the technical repeats but not in the other technical repeats. ^dThe deviation of the calculated fmol quantities of the protein from the technical repeats was above 50%, or ^ebelow this value, usually in the range between 10% and 20%. Supern., supernatant of the ultracentrifugation step; Membr., solubilized sediment.

Supplementary Table S2. Number of proteins in the parent strain AE104 and the $\Delta zupT$ mutant sorted into the KEGG orthology system^a

AE104	$\Delta zupT$	KEGG Orthology Description
612,625±72,441	662,067±93,313	Metabolism
<u>200,869±23,125</u>	<u>241,419±34,575</u>	<u>Carbohydrate Metabolism</u>
68,733±4,997	72,589±5,924	Citrate cycle (TCA cycle) {PATH:ko00020}
36,266±4,969	42,806±5,546	Glycolysis / Gluconeogenesis {PATH:ko00010}
30,210±4,314	34,284±5,509	Pyruvate metabolism {PATH:ko00620}
23,434±2,468	25,812±4,313	Butanoate metabolism {PATH:ko00650}
12,398±2,083	14,953±3,091	Pentose phosphate pathway {PATH:ko00030}
10,092±1,406	11,209±2,407	Propanoate metabolism {PATH:ko00640}
6,005±1,300	24,129±5,929	Glyoxylate and dicarboxylate metabolism {PATH:ko00630}
4,747±302	4,957±515	Nucleotide sugars metabolism {PATH:ko00520}
4,529±455	5,144±601	Aminosugars metabolism {PATH:ko00530}
2,504±391	3,059±462	Pentose and glucuronate interconversions {PATH:ko00040}
688±23	704±153	Inositol phosphate metabolism {PATH:ko00562}
400±76	594±5	Galactose metabolism {PATH:ko00052}
400±174	531±55	Starch and sucrose metabolism {PATH:ko00500}
362±149	490±64	Ascorbate and aldarate metabolism {PATH:ko00053}
101±20	158±0	Fructose and mannose metabolism {PATH:ko00051}
<u>94,065±11,188</u>	<u>86,568±10,497</u>	<u>Energy Metabolism</u>
56,941±6,825	42,808±5,914	Oxidative phosphorylation {PATH:ko00190}
22,209±2,408	26,567±2,863	Nitrogen metabolism {PATH:ko00910}
8,565±873	10,513±1,156	Methane metabolism {PATH:ko00680}
5,727±763	5,585±541	Sulfur metabolism {PATH:ko00920}
554±300	516±0	ATPases
68±19	47±0	Pyruvate/Oxoglutarate oxidoreductases
0±0	531±23	Carbon fixation {PATH:ko00710}
<u>34,683±4,743</u>	<u>33,887±7,043</u>	<u>Lipid Metabolism</u>
21,258±2,793	21,710±4,688	Fatty acid biosynthesis {PATH:ko00061}
5,329±962	5,039±1,319	Biosynthesis of steroids {PATH:ko00100}
3,472±127	4,021±297	Fatty acid metabolism {PATH:ko00071}
2,536±378	1,921±504	Arachidonic acid metabolism {PATH:ko00590}
1,293±164	756±98	Glycerophospholipid metabolism {PATH:ko00564}
543±249	351±127	Androgen and estrogen metabolism {PATH:ko00150}
252±68	88±9	Glycerolipid metabolism {PATH:ko00561}
<u>61,782±6,450</u>	<u>67,541±8,705</u>	<u>Nucleotide Metabolism</u>
44,287±4,777	48,445±6,359	Purine metabolism {PATH:ko00230}
17,495±1,673	19,096±2,346	Pyrimidine metabolism {PATH:ko00240}
<u>151,834±15,392</u>	<u>164,823±20,872</u>	<u>Amino Acid Metabolism</u>
31,496±2,253	28,867±2,888	Methionine metabolism {PATH:ko00271}
25,163±2,342	27,051±3,671	Glycine, serine and threonine metabolism {PATH:ko00260}
17,669±1,746	20,003±2,580	Valine, leucine and isoleucine biosynthesis {PATH:ko00290}
14,857±2,196	16,954±2,557	Phenylalanine, tyrosine and tryptophan biosynthesis {PATH:ko00400}
11,334±997	13,804±1,801	Lysine biosynthesis {PATH:ko00300}

9,985±1,574	11,470±2,339	Histidine metabolism {PATH:ko00340}
9,669±854	10,079±1,210	Alanine and aspartate metabolism {PATH:ko00252}
		Urea cycle and metabolism of amino groups
8,739±956	10,169±1,127	{PATH:ko00220}
6,544±526	7,221±393	Glutamate metabolism {PATH:ko00251}
5,883±526	6,190±380	Tyrosine metabolism {PATH:ko00350}
4,049±649	4,854±593	Arginine and proline metabolism {PATH:ko00330}
2,719±352	3,574±775	Valine, leucine and isoleucine degradation {PATH:ko00280}
1,300±44	1,708±162	Cysteine metabolism {PATH:ko00272}
1,057±212	1,010±181	Phenylalanine metabolism {PATH:ko00360}
961±29	1,277±84	Tryptophan metabolism {PATH:ko00380}
409±138	591±131	Lysine degradation {PATH:ko00310}
<u>9,885±1,592</u>	<u>10,807±2,028</u>	<u>Metabolism of Other Amino Acids</u>
5,299±695	5,645±853	Glutathione metabolism {PATH:ko00480}
1,696±128	1,876±441	Aminophosphonate metabolism {PATH:ko00440}
1,471±548	1,467±488	D-Glutamine and D-glutamate metabolism {PATH:ko00471}
628±30	894±60	D-Alanine metabolism {PATH:ko00473}
605±187	783±141	beta-Alanine metabolism {PATH:ko00410}
131±2	69±39	Taurine and hypotaurine metabolism {PATH:ko00430}
55±1	73±6	Selenoamino acid metabolism {PATH:ko00450}
<u>8,279±1,844</u>	<u>6,242±1,006</u>	<u>Glycan Biosynthesis and Metabolism</u>
7,465±1,652	5,811±0,960	Lipopolysaccharide biosynthesis {PATH:ko00540}
814±191	431±46	Peptidoglycan biosynthesis {PATH:ko00550}
<u>41,595±6,539</u>	<u>41,738±7,571</u>	<u>Metabolism of Cofactors and Vitamins</u>
9,311±1,779	9,149±2,026	Biotin metabolism {PATH:ko00780}
7,232±1,262	7,416±1,312	Porphyrin and chlorophyll metabolism {PATH:ko00860}
4,976±964	4,521±1,302	Folate biosynthesis {PATH:ko00790}
4,615±443	5,495±738	Riboflavin metabolism {PATH:ko00740}
4,384±630	3,510±358	Nicotinate and nicotinamide metabolism {PATH:ko00760}
4,118±506	4,579±710	Pantothenate and CoA biosynthesis {PATH:ko00770}
2,822±241	3,344±248	Thiamine metabolism {PATH:ko00730}
2,413±407	2,146±483	Ubiquinone biosynthesis {PATH:ko00130}
1,725±309	1,579±393	Vitamin B6 metabolism {PATH:ko00750}
<u>2,855±599</u>	<u>2,548±428</u>	<u>Biosynthesis of Secondary Metabolites</u>
2,031±445	1,638±139	Penicillins and cephalosporins biosynthesis {PATH:ko00311}
622±109	443±16	Flavonoid biosynthesis {PATH:ko00941}
203±045	467±274	Terpenoid biosynthesis {PATH:ko00900}
<u>6,778±970</u>	<u>6,493±587</u>	<u>Xenobiotics Biodegradation and Metabolism</u>
4,328±574	3,913±300	gamma-Hexachlorocyclohexane degradation
859±167	1,260±190	{PATH:ko00361}
725±98	268±033	Benzoate degradation via hydroxylation {PATH:ko00362}
425±99	477±42	Toluene and xylene degradation {PATH:ko00622}
278±32	230±23	Caprolactam degradation {PATH:ko00930}
122±0	201±0	2,4-Dichlorobenzoate degradation {PATH:ko00623}
42±0	145±0	Benzoate degradation via CoA ligation {PATH:ko00632}
		Carbazole degradation {PATH:ko00629}

536,214±72,575	577,255±73,912	Genetic Information Processing
<u>31,993±2,625</u>	<u>35,432±6,312</u>	<u>Transcription</u>
20,644±1,668	23,181±5,295	RNA polymerase {PATH:ko03020}
11,349±957	12,251±1,017	Other transcription related proteins
<u>331,138±45,010</u>	<u>360,607±44,054</u>	<u>Translation</u>
306,099±41,078	333,597±38,425	Other translation proteins
23,961±3,815	25,696±5,446	Other translation proteins
1,078±117	1,314±183	Aminoacyl-tRNA biosynthesis {PATH:ko00970}
<u>130,497±19,153</u>	<u>134,839±16,789</u>	<u>Folding, Sorting and Degradation</u>
102,051±13,660	117,933±13,506	Protein folding and associated processing
14,593±2,589	10,452±1,991	Protein export {PATH:ko03060}
13,455±2,890	5,789±1,149	Type II secretion system {PATH:ko03090}
398±14	665±143	Type IV secretion system {PATH:ko03080}
<u>42,586±5,786</u>	<u>46,377±6,757</u>	<u>Replication and Repair</u>
31,713±4,623	37,071±5,528	Other replication, recombination and repair proteins
5,908±777	5,761±796	Replication complex
4,965±386	3,545±432	DNA polymerase {PATH:ko03030}
95,811±17,990	67,397±13,440	Environmental Information Processing
<u>84,682±13,886</u>	<u>61,389±12,200</u>	<u>Membrane Transport</u>
66,640±10,709	51,326±9,900	ABC transporters {PATH:ko02010}
7,114±1,329	3,679±799	Pores ion channels
7,091±1,080	3,172±859	Other ion-coupled transporters
2,153±404	1,391±384	Other transporters
1,601±364	1,822±258	Phosphotransferase system (PTS) {PATH:ko02060}
84±0	0±0	Electron transfer carriers
<u>11,129±4,104</u>	<u>6,008±1,240</u>	<u>Signal Transduction</u>
11,129±4,104	6,008±1,240	Two-component system {PATH:ko02020}
15,906±1,791	11,866±1,776	Cellular Processes
<u>3,104±610</u>	1,354±427	<u>Cell Motility</u>
1,734±431	1,115±427	Bacterial chemotaxis {PATH:ko02030}
1,370±179	239±0	Flagellar assembly {PATH:ko02040}
<u>12,718±1,150</u>	<u>10,512±1,348</u>	<u>Cell Growth and Death</u>
12,718±1,150	10,512±1,348	Cell division
<u>83±31</u>	0±0	<u>Endocrine System</u>
83±31	0±0	PPAR signaling pathway {PATH:ko03320}
164±0,043	98±12	Human Diseases
164±0,043	98±12	<u>Neurodegenerative Disorders</u>
164±0,043	98±12	Amyotrophic lateral sclerosis (ALS) {PATH:ko05030}
659,445±109,092	617,497±120,010	Not in KEGG orthology

^aThe number of proteins in each KEGG orthology group ¹ is indicated. Level 2 groups are underlined and contain the sum of the numbers in each of its level 3 groups, the level 1 groups

(bold) respectively those of their level 2 groups. Red letter indicate a decreased number in the mutant, green letters an increased number. Number in italics indicate no significance.

Supplementary Table S3. The cluster of genes for autotrophic metabolism and its gene products

Name	WT, cell ⁻¹	Mut, cell ⁻¹	Description
Rmet_1497	NF	144±10	Q1LN96 Putative uncharacterized protein
Rmet_1498, CbbO	NF	169±38	Q1LN95 Rubisco activation protein
Rmet_1499, CbbQ	NF	433±315	Q1LN94 ATPase associated with Rubisco activation
Rmet_1500, CbbS	NF	1689±1619	Q1LN93 Ribulose biphosphate carboxylase small chain
Rmet_1501, CbbL	70	6269±139	Q1LN92 Ribulose biphosphate carboxylase large chain
Rmet_1502, CbbR2	NF	290	Q1LN91 Transcriptional regulator, LysR family
Rmet_1503, TnpA	NFF	NQ	Q9RBF5 Insertion sequence IS1090
Rmet_1504			Q1LN89 Putative uncharacterized protein
Rmet_1505			Q1LN88 ISSod11, transposase
Rmet_1506, TnpA	NQ	NF	Q6SKD6 Transposase
Rmet_1507, CbbF1	NeF	NeF	Q1LN86 D-fructose 1,6-bisphosphatase
Rmet_1508, CbbR2	NF	47	Q1LN85 Transcriptional regulator, LysR family
Rmet_1510, CbbE1	NF	32	Q1LN83 Ribulose-5-phosphate 3-epimerase
Rmet_1511, CbbF2	NF	237±200	Q1LN82 D-fructose 1,6-bisphosphatase
Rmet_1512, CbbT	NF	531±23	Q1LN81 Phosphoribulokinase
Rmet_1513, CbbT1	NF	327±151	Q1LN80 Transketolase
Rmet_1514, CbbZ1	163	120±24	Q1LN79 Phosphoglycolate phosphatase
Rmet_1515, CbbG1	62±16	573±497	Q1LN78 Glyceraldehyde-3-phosphate dehydrogenase
Rmet_1516, Pgl1	277±190	151±28	Q1LN77 Phosphoglycerat-Kinase
Rmet_1517, CbbY	NF	61	Q1LN76 HAD-superfamily hydrolase subfamily IA, variant 3
Rmet_1518, CbbA2	68±33	191±130	Q1LN75 Fructose-bisphosphate aldolase
Rmet_1519, PykA2	NeF	NeF	Q1LN74 Pyruvate kinase
Rmet_1520, CbbJ1	NF	101±13	Q1LN73 Triosephosphate isomerase
Rmet_1521, CbbI1	NF	76±14	Q1LN72 Ribose-5-phosphate isomerase
Rmet_1522, HoxF	NF	1694±1662	Q1LN71 NAD-reducing hydrogenase large subunit, 51 kDa subunit
Rmet_1523, HoxU	NF	1098±989	Q1LN70 NAD-reducing hydrogenase small subunit
Rmet_1524, HoxY	NF	1855±67	Q1LN69 NAD-reducing hydrogenase hoxS, 20 kDa delta subunit
Rmet_1525, HoxH	34	4611±84	Q1LN68 Nickel-dependent hydrogenase, large subunit

Rmet_1526, HoxW	NF	1766±37	Q1LN67 HoxW, highly specific carboxyl-terminal protease involved in hydrogenase maturation
Rmet_1527, HoxI	NF	8605±319	Q1LN66 Cyclic nucleotide-binding domain (CNMP-BD) protein
Rmet_1528, PntAA'			Q1LN65 Alanine dehydrogenase/PNT-like protein, interrupted
Rmet_1529	NF	373±49	Q1LB62 Integrase, catalytic region
Rmet_1530, PntAA''			Q1LN63 Alanine dehydrogenase/PNT-like protein, interrupted
Rmet_1531, PntAB			Q1LN62 Pyridine nucleotide transhydrogenase subunit alpha, truncated
Rmet_1532, PntB	NeF	NeF	Q1LN61 NAD(P) transhydrogenase, beta subunit
Rmet_1533, HoxN	NeF	NeF	Q1LN60 High-affinity nickel-transporter
Rmet_1534, HoxV			Q1LN59 Putative uncharacterized protein
Rmet_1535, HypA2	NF	622±77	Q1LN58 Hydrogenase nickel insertion protein HypA
Rmet_1536, HypB2	NF	5295±249	Q1LN57 Hydrogenase accessory protein HypB
Rmet_1537, HypF2	NF	504±33	Q1LN56 (NiFe) hydrogenase maturation protein HypF, carbamoyl phosphate phosphatase
Rmet_1538, HypC2	NeF	NeF	Q1LN55 Hydrogenase assembly chaperone hypC/hupF
Rmet_1539, HypD2	NF	493±29	Q1LN54 Hydrogenase expression/formation protein HypD
Rmet_1540, HypE2	NF	2965±149	Q1LN53 Hydrogenase expression/formation protein HypE
Rmet_1541, HoxX	NF	371±61	Q1LN52 Formyl transferase-like protein
Rmet_1542, HoxA	608±41	240	Q1LN51 hydrogenase transcriptional regulatory protein

^aThe protein numbers were taken from Supplementary Data Base. Colored numbers or letter for strain AE104 show difference in number compared to CH34 wild type, for the $\Delta zupT$ strain for the comparison $\Delta zupT$ /AE104. Red indicates down- and green up-regulation. Numbers in italics show a non significant difference. Descriptions on a green field show that the corresponding genes are transcribed in the forward orientation, yellow reverse. Two insertion elements are boxed. “NF”, not found; “NeF”, never found in any strain.

Supplementary Table S4. The predicted zinc and cobalt proteome of the strain AE104 and $\Delta zupT^a$

Name	WT, cell ⁻¹	Mut, cell ⁻¹	Description
Predicted zinc-binding proteins:			
TOTAL	109,839±10,662	122,837±13,608	
Environ.			
Inform.			
Proces. (0.05%)	60±32	1018	
Membrane Transport	60±32	1018	
ABC transporters	60±32	1018	
Rmet_1412	60±32	1018	Q1LNI1 D-aminopeptidase DppA. Metallo peptidase.
Pores ion channels	0	0	MEROPS family M55
Rmet_2621, ZupT	NeF	NeF	Q1LK28 Zinc/iron permease
Genetic Information			
Processing (50.4%)	55,305±4510	61,220±6109	
Folding. Sorting and			
Degradation	3254±319	4447±225	
Protein folding and			
associated processing	3254±319	4447±225	
Rmet_2922	3040±256	3720±115	O33522 Chaperone protein DnaK
Rmet_3563	143±33	105±33	Q1LHE1 HtpX-2 peptidase. Metallo peptidase. MEROPS family M48B
Rmet_3464	70±30	NF	Q1LHP0 HtpX peptidase. Metallo peptidase. MEROPS family M48B
Rmet_1286	NF	NF	Q1LNV5 Hydrogenase nickel insertion protein HypA
Rmet_1535	NF	622±77	Q1LN58 Hydrogenase nickel insertion protein HypA
Replication and Repair	2358±325	2188±267	
Replication. recomb..			
repair	741±152	442±282	
Rmet_4549	545±111	376±282	Q1LEL2 UvrA family protein

Rmet_3328	196±41	66	Q1LI26 ATP-dependent DNA helicase RecQ
Replication complex	1617±174	1746±267	
Rmet_2298	NeF	NeF	Q8GQ10 DNA topoisomerase
Rmet_3569	1120±88	1042±4	Q1LHD5 DNA topoisomerase
Rmet_2519	335±24	390±52	Q1LKD0 DNA topoisomerase IV subunit A
Rmet_2607, DnaG	161±61	314±211	Q1LK42 DNA primase
Transcription	16,512±966	18,626±3315	
RNA polymerase	16,512±966	18,626±3315	
Rmet_3291, RpoA ²	6895±498	8414±1248	Q1LI63 DNA-directed RNA polymerase subunit alpha
Rmet_3334, RpoB ³	4908±340	5411±962	Q1LI20 DNA-directed RNA polymerase subunit beta
Rmet_3333, RpoC ⁴	4709±128	4801±1105	Q1LI21 DNA-directed RNA polymerase subunit beta'
Translation	33,182±2899	35,959±2301	
Other translation proteins	2297±221	2685±502	
Rmet_0049, GatA	1246±134	1435±272	Q1LSE1 Glutamyl-tRNA(Gln) amidotransferase subunit A
Rmet_0046, GatB	1051±87	1250±230	Q1LSE4 Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B
Ribosome	30,884±2678	33,274±1800	
Rmet_3314, RplB	8352±824	8668±102	Q1LI40 50S ribosomal protein L2
Rmet_0411, RplM	7992±1046	8961±1057	Q1LRC9 30S ribosomal protein S9
Rmet_1435, RpsB	6441±349	6218±114	Q1LNF8 30S ribosomal protein S2
Rmet_0921, RpsO	4847±392	5282±473	Q1LPW9 30S ribosomal protein S15
Rmet_3308, RpsQ	3251±67	4144±55	Q1LI46 30S ribosomal protein S17

Human Diseases**(0.15%)****164±43****98±12****Neurodegenerative Disorders****164±43****98±12****Amyotrophic lateral sclerosis (ALS)****164±43****98±12**

Rmet_2757	164±43	98±12	Q1LJP6 Superoxide dismutase (Cu-Zn)
<u>Metabolism (37.5%)</u>	<u>41,195±4379</u>	<u>43,768±5669</u>	
<u>Amino Acid Metabolism</u>	<u>18,399±1700</u>	<u>19,383±3279</u>	
Alanine and aspartate	1794±284	1624±248	
Rmet_0831, AlaS	1794±284	1624±248	Q1LQ59 Alanyl-tRNA synthetase
Cysteine	748±32	935±24	
Rmet_1100	NeF	NeF	Q1LPE0 Cysteinyl-tRNA synthetase
Rmet_1085, CysS	748±32	935±24	Q1LPF5 Cysteinyl-tRNA synthetase
Glutamate metabolism (PATH:ko00251)	1047±149	1160±42	
Rmet_2140, GltX	1047±149	1160±42	Q1LLF7 Glutamyl-tRNA synthetase
Glycine. serine. threonine	1119±45	1126±223	
Rmet_1160, ThrS	1119±45	1126±223	Q1LP80 Threonyl-tRNA synthetase / Ser-tRNA(Thr) hydrolase
Lysine	859±42	1113±338	
Rmet_1420	859±42	1113±338	Q1LNH3 Succinyldiaminopimelate desuccinylase
Methionine	11,055±930	11,402±1986	
Rmet_4564	3887±412	4022±138	Q1LEK0 Methionine synthase (B12-independent)
Rmet_5072	3925±194	4886±907	Q1LD42 Methionine synthase (B12-independent)
Rmet_2769, MetG	2075±222	1576±614	Q1LJN4 Methionyl-tRNA synthetase
Rmet_0087	664±15	628±243	Q1LSA3 Methionine synthase (B12-dependent)
Rmet_0088	504±87	290±84	Q1LSA2 Methionine synthase (B12-dependent)
Urea cycle. amino group	516±149	657±227	
Rmet_1956	516±149	657±227	Q1LLZ1 Acetylornithine deacetylase / acetylornithine deacetylase ArgE. Metallo peptidase. MEROPS family M20A
Rmet_1610	NeF	NeF	Q1LMY3 Acetylornithine deacetylase or succinyl-diaminopimelate desuccinylase

Valine.	leucine.		
isoleucine		1261±69	1365±190
Rmet_2885, IleS		1261±69	1365±190 Q1LJB8 Isoleucyl-tRNA synthetase
<u>Carbohydrate</u>			
<u>Metabolism</u>		5355±514	6415±756
Fructose and mannose:		61±19	77
Rmet_4175		61±19	77 Q1LFN4 N-acetylglucosamine 2-epimerase
Glycolysis	/		
Gluconeogenesis		4488±356	5145±437
Rmet_1492	NeF	NeF	Q1LNA1 Fructose-bisphosphate aldolase
Rmet_5102	NeF	NeF	Q1LD15 Alcohol dehydrogenase. zinc-binding
Rmet_0503	3706±119	3909±69	Q1LR37 Fructose-bisphosphate aldolase
Rmet_3189	714±204	1046±238	Q1LIG5 Alcohol dehydrogenase. zinc-binding
Rmet_1518	68±33	191±130	Q1LN75 Fructose-bisphosphate aldolase
Pentose	phosphate		
pathway		467±111	493±99
Rmet_2980		467±111	Q1LJ23 Transketolase
Rmet_1513	NF	327±151	Q1LN80 Transketolase
Pyruvate metabolism		338±28	372±69
Rmet_0562		338±28	Q1LQX8 Acetate kinase
<u>Energy Metabolism</u>		7473±883	8106±184
ATPases		478±288	NF
Rmet_4594, ZntA		396±288	NF Q1LEH0 Heavy metal translocating P-type ATPase
Rmet_2303, CadA		82	NF A7HYL0 Heavy metal translocating P-type ATPase
Methane	metabolism		
(PATH:ko00680)		5999±507	6981±169
Rmet_2680		4867±392	5474±143 Q1LJX3 Serine hydroxymethyltransferase
Rmet_4784		934±79	1310±2 Q1LDY0 Alcohol dehydrogenase. zinc-binding
Rmet_0545		197±35	197±24 Q1LQZ5 Alcohol dehydrogenase. zinc-binding
Nitrogen	metabolism		
(PATH:ko00910)		995±87	1125±14

Rmet_5185	NeF	NeF	Q1LCT2 Carbonic anhydrase
Rmet_5605	NF	NF	Q1LBL2 Carbonic anhydrase
Rmet_0105	962±87	1125±14	Q1LS85 Carbonic anhydrase
Rmet_2078	33	NF	Q1LLL9 Carbonic anhydrase
<u>Glycan Biosynthesis and Metabolism</u>	<u>598±65</u>	<u>630±79</u>	
<u>Lipopolysaccharide biosynthesis</u>	<u>598±65</u>	<u>630±79</u>	
Rmet_1445, LpxD	506±26	556±27	Q1LNE8 UDP-3-O-(3-hydroxymyristoyl) glucosamine N-acyltransferase
Rmet_3121, LpxC	92±39	74±52	Q1LIN3 UDP-3-O-(3-hydroxymyristoyl) N-acetylglucosamine deacetylase
<u>Metabolism of Cofactors and Vitamins</u>	<u>5184±723</u>	<u>4382±837</u>	
<u>Folate biosynthesis</u>	<u>1583±189</u>	<u>456±257</u>	
Rmet_4085	17	NF	Q1LFX4 Alkaline phosphatase
Rmet_3990	181±112	246±72	Q1LG68 GTP cyclohydrolase
Rmet_2441, MoaA	137±77	73±33	Q1LKK8 GTP cyclohydrolase subunit MoaA
Rmet_4084	1248	137±152	Q1LFX5 Alkaline phosphatase
<u>Porphyrin and chlorophyll</u>	<u>1824±168</u>	<u>2012±2</u>	
Rmet_3286	1824±168	2012±2	Q1LI68 Delta-aminolevulinic acid dehydratase
<u>Riboflavin</u>	<u>190±47</u>	<u>252±107</u>	
Rmet_0194	190±47	252±107	Q1LRZ6 GTP cyclohydrolase-2
<u>Ubiquinone</u>	<u>665±38</u>	<u>581±169</u>	
Rmet_0711	599±11	540±156	Q1LQH9 3-demethylubiquinone-9 3-methyltransferase
Rmet_3848	66±27	41±13	Q1LGL0 3-demethylubiquinone-9 3-methyltransferase
<u>Vitamin B6</u>	<u>922±281</u>	<u>1081±303</u>	
Rmet_2415	648±155	880±276	Q1LKN4 Pyridoxal phosphate biosynthetic protein PdxJ
Rmet_0438	274±126	201±27	Q1LRA2 4-hydroxythreonine-4-phosphate dehydrogenase
<u>Nucleotide Metabolism</u>	<u>4186±494</u>	<u>4853±534</u>	

**Purine metabolism
(PATH:ko00230)**

	833±140	1072±110	
Rmet_2624	323±22	414±20	Q1LK25 DNA polymerase I
Rmet_0889	247±25	265±12	Q1LQ01 Guanine deaminase
Rmet_3443	171±89	228±30	Q1LHR1 Amidase. hydantoinase/carbamoylase
Rmet_3722	93±4	165±49	Q1LGY2 CMP/dCMP deaminase. zinc-binding

**Pyrimidine metabolism
(PATH:ko00240)**

	3352±354	3781±425	
Rmet_1103	NeF	NeF	Q1LPD7 Dihydroorotase
Rmet_4568	NeF	NeF	Q1LEJ6 Amidohydrolase 3
Rmet_2740, PyrB	1099±106	1152±219	Q1LJR3 Aspartate carbamoyltransferase
Rmet_2739	1166±95	1281±111	Q1LJR4 Dihydroorotase
Rmet_0406	618±114	760±62	Q1LRD4 Dihydroorotase
Rmet_2889	469±39	588±33	Q1LJB4 Deoxyuridine 5'-triphosphate nucleotidohydrolase

**Not assigned to KO
(11.9%)**

	13,115±1698	16,732±1818	
Rmet_1436, Tsf	4224±164	4521±129	Q1LNF7 Elongation factor Ts
Rmet_0127, CobW2	1755±195	2129±10	Q1LS63 Cobalamin synthesis protein. P47K
Rmet_0924	1244±293	1329±361	Q1LPW6 Alcohol dehydrogenase. zinc-binding
Rmet_1191	1185±66	1523±221	Q1LP49 Oligopeptidase A. Metallo peptidase.
Rmet_0432	1121±160	1477±194	Q1LRA8 Aminopeptidase P. Metallo peptidase.
Rmet_0197	794±122	882±80	Q1LRZ3 Alcohol dehydrogenase. zinc-binding
Rmet_1849	410±101	487±94	Q1LM98 Alcohol dehydrogenase. zinc-binding
Rmet_5925	396±140	572±10	Q1LAP2 3-demethylubiquinone-9 3-methyltransferase
Rmet_3442	384±9	537±33	Q1LHR2 Acetylornithine deacetylase
Rmet_2119	359±158	417±145	Q1LLH8 Alcohol dehydrogenase. zinc-binding
Rmet_5645	286±61	621±259	Q1LBH2 Alcohol dehydrogenase. zinc-binding
Rmet_0125, CobW3	263±68	324±132	Q1LS65 Cobalamin synthesis CobW-like protein
Rmet_3067	164±31	101±61	Q1LIT7 Zinc finger/thioredoxin putative
Rmet_1573	111±77	41±9	Q1LN20 tRNA-adenosine deaminase

Rmet_1442	90±20	61±7	Q1LNF1 RseP peptidase. Metallo peptidase.
Rmet_4467	81±13	251±8	Q1LEU4 3-demethylubiquinone-9 3-methyltransferase
Rmet_0128, FurC	77±17	102±26	Q1LS62 Putative ferric uptake regulator. FUR family
Rmet_5912	59	490	Q1LAQ5 Transcriptional regulator Ada
Rmet_4804	58	NF	Q1LDW0 L-threonine 3-dehydrogenase
Rmet_4293	32	70	Q1LFB7 Alcohol dehydrogenase. zinc-binding
Rmet_4439	321	0	Q1LEX2 Dihydroorotase
Rmet_4628	183	0	Q1LED6 Alcohol dehydrogenase. zinc-binding
Rmet_5746	23	50	Q1LB71 Ferric uptake regulator. Fur family
Rmet_2583	NF	727	Q1LK66 Alkaline phosphatase
Rmet_4293	32	70	Q1LFB7 Alcohol dehydrogenase. zinc-binding
Rmet_5285	NF	20	Q1LCI2 Alcohol dehydrogenase. zinc-binding
Rmet_0293	NeF	NeF	Q1LRP7 Formamidopyrimidine-DNA glycosylase
Rmet_0551	NeF	NeF	Q1LQY9 Hycolysin. Metallo peptidase. MEROPS family M30
Rmet_1102	NF	NF	Q1LPD8 Carbonic anhydrases/acetyltransferase
Rmet_1716	NeF	NeF	Q1LMM9 Alcohol dehydrogenase. zinc-binding
Rmet_1909	NeF	NeF	Q1LM38 Alcohol dehydrogenase. zinc-binding
Rmet_2057	NeF	NeF	Q1LLP0 Hycolysin. Metallo peptidase. MEROPS family M30
Rmet_2613	NeF	NeF	Q1LK36 Alkaline phosphatase
Rmet_3085	NeF	NeF	Q1LIR9 Alcohol dehydrogenase. zinc-binding
Rmet_3150	NeF	NeF	Q1LIK4 Putative zinc protease protein
Rmet_3352	NeF	NeF	Q1LI02 Cytidine/deoxycytidylate deaminase family protein
Rmet_3354	NeF	NeF	Q1LI00 CMP/dCMP deaminase. zinc-binding
Rmet_4281	NeF	NeF	Q1LFC9 Alcohol dehydrogenase. zinc-binding
Rmet_4406	NeF	NeF	Q1LF05 Zinc finger. CDGSH-type
Rmet_4572	NeF	NeF	Q1LEJ2 GTP cyclohydrolase II
Rmet_4852	NeF	NeF	Q1LDR2 Alcohol dehydrogenase. zinc-binding

Rmet_5495	NeF	NeF	Q1LBX2 Alcohol dehydrogenase. zinc-binding
Rmet_5498	NeF	NeF	Q1LBW9 Oxidoreductase. zinc-binding

CONTROL: Co-binding proteins

TOTAL	1302±169	1035±413	
Rmet_0087	664±15	628±243	Q1LSA3 Methionine synthase (B12-dependent)
Rmet_0088	504±87	290±84	Q1LSA2 Methionine synthase (B12-dependent)
Rmet_0210	134±67	118±86	Q1LRY0 Methylmalonyl-CoA mutase
Rmet_5902	NeF	NeF	Q1LAR5 Ethanolamine ammonia-lyase heavy chain

^aAll homologs to zinc- and cobalt-binding proteins from *E. coli*⁵ in the *C. metallidurans* proteome (WT, AE104; Mut, $\Delta zupT$) were identified and their numbers sorted into the KEGG orthology groups¹. “NeF”, never, “NF” not found in the proteome analysis. Numbers in red indicate down-regulation, in green up-regulation, bold numbers significant differences, italics not significant differences between CH34/AE104 (AE104 data) or AE104/ $\Delta zupT$ ($\Delta zupT$ data).

Supplementary Literature

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