Supplementary Material

Supplementary Results

Magnesium and iron. The cellular iron content was unchanged in all of the studied mutants. The cellular magnesium content was unaltered in the CH34, AE104, Δ*zupT* and Δ*zur* mutants (Suppl. Table S3); however, the number of cell-bound magnesium atoms was nearly 4-fold increased in the Δe4 strain as published (1). While an additional Δ*zupT* deletion did not alter this increased content (1), deletion of *zur* or any *cobW* decreased the Mg content back to the wild type level (Suppl. Table S2). In the absence of the four metal efflux systems ZntA, CadA, DmeF and FieF, all three CobWs were required to maintain a cellular magnesium level of about 10 million atoms per cell.

Cadmium resistance. In liquid TMM, the IC_{50} of strain CH34 was only about 100 µM (Suppl. Table S2) and much lower than the MIC on solid medium (Table 2). As published (2), the influence of the plasmids on cadmium resistance was lower in liquid medium than on agar. Cadmium resistance in liquid medium decreased with a ΔW2 ΔW3 deletion in strains CH34 and AE104, ΔW1-cluster in AE104, deletion of *zupT,* of the four efflux systems in Δe4, or by most additional deletions in the Δe4 strain (Suppl. Table S2), indicating a role of all these factors in cadmium resistance. Cadmium resistance even increased in the CH34 ΔW1-cluster ΔW3 double and the ΔW1-cluster ΔW2 ΔW3 triple mutant, AE104 Δ*zur* compared to AE104, and AE104 Δ*zupT* ΔW1-cluster compared to AE104 Δ*zupT* (Suppl. Table S2). In AE104 Δ*zupT*, this increase in the ΔW1-cluster mutant was CobW3-dependent because it was absent in the ΔW1-cluster ΔW3 double mutant and, as in the case of zinc resistance, linked to the considered action of CobW3 on the activity of metal uptake systems. In the case of the Δ*zur* mutant, the ΔW1-cluster or ΔW3 single deletions did not alter cadmium resistance again but cadmium resistance of the three Δ*zur* ΔW double mutants and the ΔW2 single mutant was not different from that of the parent strain AE104. Increased cadmium resistance, supposedly by enhanced zinc-handling abilities, needed up-regulation of *zupT* and CobW2 or CobW1 plus CobW3. The very low cadmium resistance level of the Δe4 strain (22 µM) decreased even further with ΔW deletions (Suppl. Table S2) with the lowest cadmium resistance levels reached (1.6 µM) by the Δe4 ΔW1-cluster, Δe4 ΔW3, the Δe4 Δ*zupT* ΔW3, the Δe4 Δ*zur* and the Δe4 Δ*zur* ΔW2 mutants, indicating that the full set of CobWs was able to protect the cell against cadmium even in the absence of efflux systems. In contrast to the effect of the Δ*zupT* deletion in

the strain AE104, a resistance decrease by 2/3, deletion of *zupT* did not decrease cadmium resistance of ∆e4 further so that the efflux systems plus the Zur regulon components were able to handle the imbalanced metal ion uptake that results in part from the *zupT* deletion (1).

While the plasmid pMOL30 with the *czc* determinant, and CobW2 were needed for high-level cadmium resistance (MIC 2.2 mM, Table 2) on solid growth medium, resistance to cadmium in liquid culture (IC_{50} = 0.1 mM, Suppl. Table S2) required the four chromosomal efflux systems, most probably CadA and ZntA, and additionally the complete Zur regulon components ZupT plus the three CobWs. Controlled import of zinc, its cytoplasmic handling by the CobWs, and controlled efflux by ZntA and CadA was important for the cells to reach full cadmium resistance.

Nickel. Deletion of *cobW2* or of the *cobW1* cluster but not of *cobW3* increased the cellular nickel content in the CH34 background (Suppl. Table S4). The nickel content of the CH34 ∆W1-cluster ∆W2 mutant was higher than that of the two ∆W1-cluster and ∆W2 single mutants and that of the ∆W2 mutant higher than that of the ∆W1-cluster mutant. CobW2 and to some degree the CobW1 system controlled the cellular nickel content in strain CH34. The cellular nickel content was not much affected in strains AE104 or ∆*zupT,* decreased in some ∆*zur* mutants and all ∆e4 mutants. CobW2 and the CobW1 system were only required for control of the cytoplasmic nickel content in the presence of the plasmid-encoded metal resistance factors.

The ∆W3 deletion, which did not change the cellular nickel content, decreased the IC_{50} for nickel (2.6 mM) in CH34 strains by half (Suppl. Table S2). Loss of the plasmids decreased the nickel resistance of the resulting strain AE104 down to 226 µM (Suppl. Table S2) and now the ∆W3 deletion had no effect. Loss of *zupT* or of *zur* decreased nickel resistance of strain AE104 only slightly but again, additional loss of CobW3 decreased nickel resistance by half. Loss of the four efflux systems, in this case mainly DmeF, decreased nickel resistance of AE104 to 25 µM and each ∆W deletion decreased it further. The lowest nickel resistance of all tested strain with IC_{50} = 3.7 µM displayed the strain ∆e4 ∆*zupT* ∆W2, ∆W3.

This indicated the importance of all CobWs but especially of CobW3 in nickel resistance. Since the ∆W3 mutant displayed no altered cellular nickel content, nickel toxicity was not connected to increased nickel accumulation but rather to enhanced toxicity of the nickel ions present in the cell. Because the cellular iron content was unchanged, the 4,000 cell-bound nickel

ions may have interfered with the metabolism of the 64,000 zinc atoms or 1,200 cobalt atoms per cell in the absence of CobW3.

Cobalt. Deletion of *cobW2* or of the Δ*cobW1* cluster, which both led to an increased nickel level, decreased the cellular cobalt in the CH34 background (Suppl. Table S4). A ΔW3 deletion also caused no change in the cobalt content of strain CH34. In most of the AE104 derivatives, the cobalt content was increased but to a lower extent in AE104 Δ*zur* ΔW mutants. The cobalt content was back to the wild type level of strain CH34 in the Δe4 Δ*zur* ΔW2 mutant, indicating that the plasmid-encoded and chromosomal efflux systems, the CobW1 system and especially CobW2 were also involved in control of the cellular cobalt level.

The IC_{50} of 1.5 mM Co(II) of strain CH34 decreased with the ΔW3 deletion but no other single ΔW deletion (Suppl. Table S2), increased to 2.2 mM with an additional ΔW1-cluster deletion and went back to the parent strain level in the ΔW1-cluster ΔW2 ΔW3 triple mutant of strain CH34. While CobW3 was the major player in nickel resistance of strain CH34, the ΔW3 deletion in strain CH34 caused the interference of CobW2 and the CobW1 system, which was evident in EDTA resistance, to appear also in cobalt resistance.

The IC_{50} for Co(II) decreased with loss of the plasmids in strain AE104 to 135 µM, further down to 85 µM in strain AE104 Δ*zupT* and to 4.7 µM in the Δe4 mutant (Suppl. Table S2). It was not influenced by the Δ*zur* deletion. All ΔW double mutants of strains AE104, AE104 Δ*zupT* and AE104 Δ*zur* displayed a decreased cobalt resistance level compared to their respective parent strains. Of the single mutants, ΔW1-cluster decreased cobalt resistance in strain AE104, ΔW2 and ΔW3 but not ΔW1-cluster in AE104 Δ*zupT*, indicating a function of the three CobWs in cobalt resistance. The Δe4 Δ*zupT* ΔW2 ΔW3 mutant with an IC_{50} of 1.6 µM had the lowest cobalt resistance level of all tested strains (Suppl. Table S2).

As in the case of nickel, the decrease of cobalt resistance in the ΔW mutant strains was not connected to a reciprocal increase in cell-bound cobalt but instead should have been mediated by an increased toxicity of the cobalt ions already present in the cell. This indicated that a main function of the three CobWs might be to shield the cellular zinc homeostasis against the competing ions Co(II), Ni(II) and Cd(II), although a role in cobalt and nickel homeostasis could not be excluded at this stage.

Expression of genes for efflux systems in Δ*cobW3* mutant strains. CobW3 seems to control metal accumulation under certain conditions. This could occur at the transcriptional or post-transcriptional level, and at the level of uptake or efflux systems. To examine the effect of CobW3 on the expression of the genes for the four metal efflux systems deleted in the Δe4 mutant, *lacZ*-fusions were constructed with the genes *zntA, cadA, dmeF,* and *fief* in CH34, AE104, AE104 Δ*zupT*, and its respective Δ*cobW3* mutants.

The basic expression level of *zntA* for the main zinc-exporting inner membrane efflux system was twice as high in AE104 as in CH34, probably because the plasmid-encoded *czc* system was absent (Suppl. Table S5). This level decreased by half with the Δ*zupT* deletion, appeared to increase again with the ΔW3 deletion but the values 37.4±7.5 and 50.7±8.4 U/mg were not significantly different from each other. There was also no difference between the EDTA-, zinc-, or cadmium-induced up-regulation of *zntA-lacZ* in the presence or absence of *cobW3*. Only in strain AE104 Δ*zupT*, additional deletion of *cobW3* caused a stronger cobalt-induced up-regulation of *zntA-lacZ* (Suppl. Table S5). CobW3 had no influence of *zntA* expression except that CobW3 was required to quench a cobalt-dependent gratuitous induction of *zntA* in strain AE104 Δ*zupT*.

The basic expression level of *cadA-lacZ* was down-regulated by the ΔW3 deletion in strains CH34 and AE104 but not in AE104 Δ*zupT* (Suppl. Table S5). The presence of cadmium compensated this effect, so that the cells did not suffer from decreased cadmium resistance (Suppl. Table S2). The influence of CobW3 on expression of *dmeF* was very small, and absent in the case of *fieF*, which encodes the Fe(II) efflux pump FieF (3, 4). Together, CobW3 influenced the expression of genes of metal efflux systems only to a small degree, however, some interaction at the post-transcriptional, -translational, activity level or delivery of surplus zinc to these exporters could not be ruled out. Since absence of CobW3 also increased the zinc content of the Δe4 Δ*zupT* mutant, which had no metal efflux systems, no ZupT but at least 9 other zinc uptake systems, CobW3 may interfere with metal uptake by these remaining metal import systems of *C. metallidurans*.

Expression of genes for metal import systems in Δ*cobW3* mutant strains. Ranked by the activity of the *lacZ*-fusions in AE104 cells grown in unamended medium, the expression levels of the 9 metal

importers in this strain should be PitA >> CorA1 > CorA2 > CorA3 >> ZupT > MgtB > ZntB > MgtA >>> HoxN, with the latter not being expressed in strain AE104 (0.87±1.68 U/mg, Suppl. Table S6). In addition to HoxN, in strain CH34 ZntB, CorA3 and MgtA were also repressed (Suppl. Table S6, areas in grey fields), so that the wild-type strain should use only 5 of the 9 known importers, PitA for metal-phosphate uptake, CorA1, CorA2 for divalent metal cations including Mg^{2+}, ZupT and the MgtB Ca^{2+}/Mg^{2+} P-type ATPase (5, 6).

The MIT protein ZntB was activated in AE104 and even more in its Δ*zupT* mutant, and repressed by zinc in these strains (Suppl. Table S6). CobW3 was responsible for the complete repression of *zntB-lacZ* in strain CH34. Expression of *zupT, pitA, corA2, mgtA* or *mgtB* was not influenced by CobW3. As in the case of *zntB*, repression of *corA3* in strain CH34 was ameliorated in the CH34 ΔW3 mutant. Expression of *corA1* in the Δ*zupT* strain was strongly repressed by zinc but not so in the Δ*zupT* ΔW3 mutant, while the gene was strongly repressed by EDTA in the Δ*zupT* ΔW3 mutant compared to the Δ*zupT* parent or the other strains. CobW3 was needed to repress ZntB and CorA3 in CH34, for a zinc-dependent repression of *corA1* in the absence of *zupT*, and for an EDTA-dependent expression of *corA1* in the same strain (Suppl. Table S6).

Since the CorA systems are known since a long time to be involved in cobalt import (7, 8), the influence of CobW3 on the metal content of cobalt-treated cells was analyzed. As in the presence of zinc and EDTA, the Mg content of the Δe4 mutant was increased but was reduced back down to the AE104 parent level again when *cobW3* was deleted. This also occurred in the Δe4 Δ*zupT* mutant (Suppl. Table S7). The phosphate content was also elevated by more than 50%. Otherwise, strain AE104 treated with 25 µM $CoCl_2$ contained more Co per cell than CH34, as expected in the absence of the cobalt efflux systems Czc and Cnr. A major difference in the absence of CobW3 was a significantly increased cellular cobalt content in the Δ*zupT* strain, and a decreased nickel and even iron content, with the iron content of cobalt-treated Δ*zupT* ΔW3 cells not different from the Δ*zupT* parent cells. Consequently, the missing repression of *corA1* in the Δ*zupT* strain (Suppl. Table S6) coincided by an increased cellular cobalt and decreased nickel content (Suppl Table S7) in cobalt-treated but not in zinc-, EDTA- or un-treated cells (Suppl. Table S4), reversion of the decreased zinc content of Δ*zupT* cells (Table 2), and a decreased cobalt and nickel resistance (Suppl. Table S2).

Supplementary Table S1. RT-PCR Results[a]

Primer pair	Position	%DNA	Description
zur-region			
Gene position	1149-1658		*zur* gene
a	1166-1427	6.6%±6.3%	*zur* internal
7	1354-1708	7.7%±9.0%	*zur-cobW2*
Gene position	1709-2800		*cobW2 gene*
9	1354-1988	1.6%±1.2%	*zur-cobW2*
10	1354-2425	1.3%±0.9%	*zur-cobW2*
8	1659-1988	29.5%±14.5%	*zur-cobW2*
b	2128-2425	81.6%±10.7%	*cobW2* internal
17	2128-3564	0.6%±0.6%	*cobW2-dksA1*
3	2128-3118	19.4%±9.9%	*cobW2-dksA1*
24	2532-3328	9.8%±4.5%	*cobW2-dksA1*
1	2801-3118	71.1%±10.0%	*cobW2-dksA1*
2	2801-3564	0.8%±1.0%	*cobW2-dksA1*
Gene position	3119-3808		*dksA1* gene
c	3287-3564	30.3%±14.6%	*dksA1* internal
6	3287-4147	2.1%±3.4%	*dksA1-cobW3*
4	3669-4147	2.6%±3.0%	*dksA1-cobW3*
5	3809-4603	2.7%±2.8%	*dksA1-cobW3*
Gene position	4001-5176		*cobW3* gene
d	4300-4603	5.1%±2.3%	*cobW3* internal
19	4300-5487	0.2%±2.2%	*cobW3-Rmet_0124*
25	4835-5487	3.6%±1.6%	
Gene position	5166-5564		*Rmet_0124* gene
18	5176-5487	5.1%±3.5%	*Rmet_0124* internal

cobW1-region			
14	9616-10000	2.5%±1.6%	*cobW1* upstream
15	9616-10717	*0.4%±1.8%*	*cobW1* upstream
26	9735-10000	5.5%±3.8%	*cobW1* upstream
27	9735-10717	*1.8%±0.6%*	*cobW1* upstream
Gene position	10001-11194		*cobW1* gene
a	10364-10717	83.8%±9.9%	*cobW1* internal
16a	10364-11212	31.0%±5.6%	*cobW1-folEIB2*
16	10364-11561	2.1%±1.4%	*cobW1-folEIB2*
12	10922-11561	17.0%±3.9%	*cobW1-folEIB2*
Gene position	11210-12184		*folEIB2* gene
b	11195-11561	79.0%±1.5%	*folEIB2* internal
28	11889-12495	48.2%±32.3%	*folEIB2-cycS*
Gene position	12181-13599		*cysS* gene *Rmet_1100*
11	12178-12495	87.7%±3.4%	*folEIB2-cycS*
13	12495-11195	10.3%±9.1%	*folEIB2-cycS*
20	13324-13913	6.8%±2.4%	*cycS-Rmet_1101*
Gene position	13609-14190		*Rmet_1101* gene
21	13880-14505	69.8%±13.8%	*Rmet_1101-Rmet_1102*
Gene position	14210-14770		*Rmet_1102* gene
22	14473-15144	12.1%±5.6%	*Rmet_1102-Rmet_1103*
Gene position	14828-16225		*allB* gene *Rmet_1103*
23	15923-16587	-0.2%±3.8%	*Rmet_1103-Rmet_1104*
Gene position	16291-18477		*Rmet_1104 (other strand)*

[a]RNA was isolated and reverse-transcribed using random priming. The resulting cDNA was amplified by PCR. The product was visualized on an agarose gel with ethidium bromide with DNA as positive and water as negative control. All bands were scanned using ImageJ (9), the gel background intensity directly above the band subtracted and the signal value of the negative water control subtracted. The resulting value was divided by the signal value of the positive DNA control. Three biological repeats, mean %DNA value with deviations shown. No RT-PCR was done for *Rmet_1104* on the other DNA strand.

Supplementary Table S2. Metal resistance of mutant strains in liquid culture

Bacterial strain	% IC_{50} values				
	EDTA	$ZnCl_2$	$NiCl_2$	$CoCl_2$	$CdCl_2$
CH34	100%	100%	100%	100%	100%
CH34 ΔW1-cluster	**145%**	105%	81%	107%	123%
CH34 ΔW2	**60%**	100%	73%	100%	125%
CH34 ΔW3	100%	95%	**50%**	**67%**	103%
CH34 ΔW1-cl. ΔW2	103%	85%	69%	93%	119%
CH34 ΔW1-cl. ΔW3	**142%**	105%	**65%**	**147%**	**141%**
CH34 ΔW2 ΔW3	75%	80%	**65%**	**53%**	**44%**
CH34 ΔW1-cl. ΔW2 ΔW3	107%	**65%**	**58%**	100%	**146%**
AE104	**53%**	**25%**	**8.7%**	**9.0%**	116%
AE104 ΔW1-cluster	**47%**	100%	n.d.	**65%**	**51%**
AE104 ΔW2	75%	120%	n.d.	92%	100%
AE104 ΔW3	128%	120%	123%	95%	96%
AE104 ΔW1-cl. ΔW3	**19%**	100%	78%	**50%**	91%
AE104 ΔW2 ΔW3	84%	100%	96%	**40%**	**61%**
ΔzupT	**56%**	**27%**	72%	**63%**	**34%**
ΔzupT ΔW1-cl.-cluster	**267%**	115%	n.d.	106%	**185%**
ΔzupT ΔW2	89%	110%	n.d.	**42%**	100%
ΔzupT ΔW3	83%	110%	**49%**	**20%**	118%
ΔzupT ΔW1-cl. ΔW3	**33%**	96%	96%	**35%**	98%
ΔzupT ΔW2 ΔW3	72%	100%	**45%**	**31%**	80%
Δzur	**56%**	97%	86%	101%	**135%**
Δzur ΔW1-cluster	106%	106%	94%	99%	103%
Δzur ΔW2	89%	90%	92%	88%	78%
Δzur ΔW3	72%	106%	**64%**	74%	102%
Δzur ΔW1-cl. ΔW2	89%	**57%**	79%	**46%**	84%
Δzur ΔW1-cl. ΔW3	78%	92%	77%	**57%**	75%
Δzur ΔW2 ΔW3	100%	92%	96%	**46%**	78%
Δe4	**53%**	**1.5%**	**11%**	**3.5%**	**18%**
Δe4 ΔW1-cluster	88%	70%	**27%**	98%	**9.0%**
Δe4 ΔW3	88%	74%	**23%**	100%	**7.1%**
Δe4 ΔW1-cl. ΔW2	94%	70%	**23%**	98%	n.d.
Δe4 ΔW1-cl. ΔW3	100%	**21%**	**23%**	94%	67%
Δe4 ΔW2 ΔW3	88%	71%	**19%**	**64%**	**14%**
Δe4 Δ*zupT*	112%	75%	85%	87%	105%
Δe4 Δ*zupT Δcobw3*	94%	74%	54%	98%	**8.1%**
Δe4 Δ*zupT* ΔW1-cl. ΔW3	100%	83%	81%	98%	**11%**

Δe4 Δ*zupT* ΔW2 ΔW3	94%	72%	**15%**	**34%**	**13%**
Δe4 Δ*zur*	94%	**62%**	69%	**157%**	**7.1%**
Δe4 Δ*zur* ΔW2	94%	**64%**	**62%**	121%	**7.6%**

Metal resistance of mutants carrying a deletion of the complete *cobW1* cluster, of the *cobW3* gene, or disruptions (dis) of *cobW1* or *cobW2,* respectively, was tested in dose-response experiments and the IC (concentration of half-maximum growth inhibition) was calculated. These values were compared for: (i) CH34 mutants including the plasmid-free strain AE104 (shaded box) to CH34 wild type cells (bold box at the top line); (ii) AE104 mutants including Δ*zupT*, Δ*zur* and Δe4 (Δ*cadA* Δ*zntA* Δ*fieF* Δ*dmeF*)(boxes) to AE104 cells (shaded box); (iii) mutants of Δ*zupT*, Δ*zur* and Δe4 to these respective parents (boxes directly above). If D > 1 (n>3, deviation bars of the data points do not touch or overlap), an IC_{50} ratio < 67% is in red, an IC_{50} ratio > 133% in green. 100% values for CH34 cells TMM: 6.0±1.13 mM EDTA, 2.0±0.1 mM $ZnCl_2$, 2.6±0.2 mM $NiCl_2$, 1.5±0.2 mM $CoCl_2$, 103±9 µM $CdCl_2$. n.d., not determined.

Supplementary Table S3. Mg an Fe content of mutant strains

Addition	none		100 µM EDTA		10/100 µM Zn	
Bacterial strain	**Mg**	**Fe**	**Mg**	**Fe**	**Mg**	**Fe**
CH34	100%	100%	96%	107%	99%	99%
CH34 ΔW1-cluster	88%	113%	84%	84%	79%	99%
CH34 ΔW2	98%	108%	94%	84%	102%	112%
CH34 ΔW3	94%	111%	94%	89%	103%	97%
CH34 ΔW1-cl. ΔW2	92%	140%	90%	98%	91%	122%
CH34 ΔW1-cl. ΔW3	92%	125%	103%	98%	92%	117%
CH34 ΔW2 ΔW3	85%	106%	97%	107%	92%	117%
CH34 ΔW1-cl. ΔW2 ΔW3	92%	131%	97%	119%	90%	118%
AE104	97%	121%	95%	94%	97%	73%
AE104 ΔW1-cluster	123%	83%	124%	89%	106%	62%
AE104 ΔW2	102%	97%	115%	99%	112%	76%
AE104 ΔW3	92%	77%	108%	104%	120%	108%
AE104 ΔW1-cl. ΔW3	96%	89%	101%	92%	115%	97%
AE104 ΔW2 ΔW3	97%	93%	101%	93%	122%	100%
ΔzupT	88%	87%	95%	94%	103%	95%
ΔzupT ΔW1-cluster	120%	88%	105%	97%	99%	105%
ΔzupT ΔW2	102%	94%	101%	100%	101%	78%
ΔzupT ΔW3	137%	98%	127%	81%	144%	93%
ΔzupT ΔW1-cl. ΔW3	133%	98%	112%	83%	111%	102%
ΔzupT ΔW2 ΔW3	134%	91%	122%	82%	113%	93%
Δzur	93%	99%	102%	113%	118%	113%
Δzur ΔW1-cluster	100%	90%	102%	90%	96%	100%
Δzur ΔW2	90%	77%	85%	66%	90%	73%
Δzur ΔW3	115%	105%	101%	89%	99%	99%
Δzur ΔW1-cl. ΔW2	85%	71%	93%	69%	92%	75%
Δzur ΔW1-cl. ΔW3	82%	69%	94%	92%	87%	86%
Δzur ΔW2 ΔW3	95%	94%	96%	70%	83%	71%
Δe4	**373%**	99%	**343%**	103%	**261%**	134%
Δe4 ΔW1-cluster	**24%**	73%	**25%**	73%	**37%**	84%
Δe4 ΔW3	**25%**	81%	**24%**	71%	**27%**	77%
Δe4 ΔW1-cl. ΔW2	**24%**	75%	**26%**	55%	**29%**	69%
Δe4 ΔW1-cl. ΔW3	**24%**	80%	**25%**	67%	**25%**	79%
Δe4 ΔW2 ΔW3	**21%**	67%	**24%**	55%	**28%**	80%
Δe4 Δ*zupT*	88%	93%	97%	85%	92%	86%
Δe4 Δ*zupT* Δ*cobW3*	**25%**	70%	**29%**	79%	**35%**	80%
Δe4 Δ*zupT* ΔW1-cl. ΔW3	**23%**	62%	**25%**	75%	**27%**	83%
Δe4 Δ*zupT* ΔW2 ΔW3	**24%**	64%	**25%**	62%	**27%**	55%
Δe4 Δ*zur*	**23%**	80%	**25%**	80%	**28%**	75%
Δe4 Δ*zur* ΔW2	**24%**	64%	**24%**	69%	**30%**	83%

The mutants carrying a deletion of the complete *cobW1* cluster, of the *cobW3* gene, or disruptions (dis) of *cobW1* or *cobW2*. The metal content was measured in cells grown in TMM with 100 µM EDTA, 100 µM $ZnCl_2$, or no addition (10 µM in case of the Δe4 strains). The metal content was compared: (i) CH34 cells grown in amended medium (bold boxes) to CH34 cells in unamended

medium (bold shaded box); (ii) CH34 mutants including the plasmid-free strain AE104 (shaded boxes) to CH34 cells cultivated under the same conditions (bold boxes in the top line); (iii) AE104 mutants including Δ*zupT*, Δ*zur* and Δe4 (Δ*cadA* Δ*zntA* Δ*fieF* Δ*dmeF*) (boxes) to AE104 cells cultivated under the same conditions (shaded boxes); (iv) mutants of Δ*zupT*, Δ*zur* and Δe4 to these respective parents grown under the same conditions. If D > 1 (n>4, deviation bars of the data points do not touch or overlap), a metal content < 50% is in red, a metal content > 200% in green. 100% values for CH34 cells in non-amended TMM: $(11.9\pm1.0)\cdot10^6$ Mg, $(636\pm65)\cdot10^3$ Fe atoms per cell.

Supplementary Table S4. Ni and Co content of *C. metallidurans* derivatives

	Nickel			Cobalt		
Bacterial strain	none	EDTA	Zn	none	EDTA	Zn
CH34	**1.00**	1.33	*1.80*	1.00	1.25	1.00
CH34 ΔW1-cluster	*2.23*	**3.02**	*2.06*	1.00	*0.67*	*0.50*
CH34 ΔW2	**2.84**	**3.33**	**2.95**	*0.67*	*0.50*	*0.50*
CH34 ΔW3	1.32	0.91	*1.56*	*1.67*	1.17	**2.17**
CH34 ΔW1-cl. ΔW2	**4.29**	**3.48**	**3.72**	0.83	*0.50*	*0.50*
CH34 ΔW1-cl. ΔW3	**3.18**	**2.58**	**2.52**	0.75	**0.42**	**0.42**
CH34 ΔW2 ΔW3	**2.99**	**3.18**	**3.80**	*1.42*	0.92	0.83
CH34 ΔW1-cl. ΔW2 ΔW3	**4.03**	**3.06**	**4.42**	0.83	*0.50*	**0.42**
AE104	1.26	0.72	1.06	**2.75**	*2.00*	**2.92**
AE104 ΔW1-cluster	0.84	0.77	1.05	**5.17**	**2.50**	**3.75**
AE104 ΔW2	*0.64*	*1.38*	0.88	**2.08**	*1.33*	**2.58**
AE104 ΔW3	*0.53*	0.73	1.25	1.25	**0.42**	**2.42**
AE104 ΔW1-cl. ΔW3	*1.37*	*1.41*	1.24	**2.17**	*1.42*	**2.33**
AE104 ΔW2 ΔW3	*1.73*	*1.40*	1.24	**2.58**	*1.58*	**2.83**
ΔzupT	0.91	1.15	*1.55*	**4.58**	**4.00**	**4.92**
ΔzupT ΔW1-cluster	1.16	1.13	1.23	**4.25**	**3.08**	**3.58**
ΔzupT ΔW2	0.87	0.73	*1.37*	**4.17**	**2.33**	**3.83**
ΔzupT ΔW3	0.77	0.71	*1.42*	**3.50**	*1.92*	**3.42**
ΔzupT ΔW1-cl. ΔW3	*1.46*	1.28	1.14	**4.08**	**2.50**	**3.58**
ΔzupT ΔW2 ΔW3	*1.84*	1.30	1.13	**4.33**	**2.83**	**3.58**
Δzur	1.24	*0.54*	*1.51*	**2.33**	**2.67**	**2.83**
Δzur ΔW1-cluster	*1.41*	0.86	0.82	*1.42*	1.25	*1.50*
Δzur ΔW2	0.87	*0.56*	*0.59*	*1.58*	1.25	**1.92**
Δzur ΔW3	*1.59*	1.26	1.09	**1.58**	0.92	*1.50*
Δzur ΔW1-cl. ΔW2	*0.64*	0.77	*0.45*	*1.50*	*1.42*	*1.50*
Δzur ΔW1-cl. ΔW3	0.68	*0.53*	0.99	*1.50*	1.00	*1.92*
Δzur ΔW2 ΔW3	0.82	*0.60*	*0.54*	*1.58*	1.00	**1.75**
Δe4	*0.41*	*0.57*	0.84	**3.25**	**2.83**	**3.08**
Δe4 ΔW1-cluster	*0.56*	*0.52*	*0.54*	**1.92**	**3.00**	*1.50*
Δe4 ΔW3	*0.52*	*0.62*	*0.50*	**3.58**	*2.08*	*1.67*
Δe4 ΔW1-cl. ΔW2	**0.35**	*0.43*	*0.50*	**2.83**	**2.33**	**2.42**
Δe4 ΔW1-cl. ΔW3	*0.51*	*0.39*	*0.52*	**3.17**	**2.25**	**2.50**
Δe4 ΔW2 ΔW3	*0.49*	*0.34*	*0.36*	**2.83**	**2.17**	**2.33**
Δe4 Δ*zupT*	*0.46*	*0.37*	0.86	**4.33**	**3.17**	**2.17**
Δe4 Δ*zupT* Δ*cobW3*	*0.43*	*0.49*	*0.49*	**3.33**	**3.00**	**3.33**
Δe4 Δ*zupT* ΔW1-cl. ΔW3	*0.36*	*0.46*	*0.55*	**2.67**	**2.42**	**2.33**
Δe4 Δ*zupT* ΔW2 ΔW3	**0.29**	*0.30*	*0.39*	**2.92**	*1.83*	*2.83*
Δe4 Δ*zur*	*0.48*	**0.36**	*0.39*	**3.33**	1.25	*1.33*
Δe4 Δ*zur* ΔW2	**0.31**	**0.28**	**0.40**	1.00	0.92	*1.33*

The mutants carrying a deletion of the complete *cobW1* cluster, of the *cobW3* gene, or disruptions (dis) of *cobW1* or *cobW2*. The metal content was measured in cells grown in TMM with 100 µM EDTA, 100 µM $ZnCl_2$, or no addition (10 µM $ZnCl_2$ in case of the Δe4 mutant) The metal content was compared to that of CH34 cells in unamended medium. If D

> 1 (n>4, deviation bars of the data points do not touch or overlap), a metal content < 67% is in red, a metal content > 133% in green. For nickel, also non-significant ratios are indicated in italics. 100% value for CH34 cells in non-amended TMM: 1200±500 Co, 4010±2190 Ni atoms per cell.

Supplementary Table S5. Expression of reporter fusions of efflux systems[a]

Bacterial strain	Basic act.	EDTA		$ZnCl_2$		$CoCl_2$		$CdCl_2$	
	(U/mg dw)	1 mM	5 mM	750 µM	2 mM	0.1 mM	1 mM	10 µM	100 µM
ϕ(*zntA-lacZ*)									
CH34	21.0±7.7	0.97	1.01	**2.13**	***0.15***	1.05	1.06	**3.55**	**4.72**
CH34 ΔW3	21.1±5.8	0.81	0.88	**2.33**	0.54	1.08	1.43	**4.56**	**6.51**
AE104	**50.4±5.8**	0.63	0.62	**7.33**	**5.95**	**3.10**	1.02	**6.80**	**5.79**
AE104 ΔW3	**53.2±8.6**	0.65	0.65	**9.42**	**3.23**	**2.09**	**1.42**	**8.37**	**5.03**
Δ*zupT*	37.4±7.5	0.50	0.55	**10.3**	**5.48**	**1.65**	0.91	**6.29**	**15.7**
Δ*zupT* ΔW3	**50.7±8.4**	0.72	0.61	**8.33**	**2.63**	**4.64**	**2.14**	**7.92**	**8.76**
ϕ(*cadA-lacZ*)									
CH34	20.8±5.3	1.39	1.28	1.27	0.70	1.29	1.03	**3.04**	**9.44**
CH34 ΔW3	***7.2±1.7***	1.06	1.09	**2.48**	0.75	0.95	1.02	**7.07**	**17.5**
AE104	28.5±4.7	0.88	0.86	**1.70**	0.77	1.07	1.01	**8.30**	**20.9**
AE104 ΔW3	***15.0±2.6***	0.68	0.73	**2.34**	**1.67**	1.24	0.87	**12.3**	**40.2**
Δ*zupT*	25.7±5.0	0.65	0.61	**1.40**	**0.35**	1.14	0.91	**6.70**	**20.7**
Δ*zupT* ΔW3	25.2±7.5	0.75	0.73	**1.82**	0.82	0.97	0.68	**9.64**	**2.77**
ϕ(*dmeF-lacZ*)									
CH34	54.9±4.1	1.77	0.81	0.55	***0.17***	1.61	0.84	0.87	0.86
CH34 ΔW3	35.6±4.0	**2.05**	0.94	0.40	***0.02***	**2.08**	1.40	1.03	1.10
AE104	33.4±6.7	1.43	1.69	1.52	0.58	**2.13**	**2.53**	1.48	**3.43**
AE104 ΔW3*3*	31.7±7.9	1.47	**2.79**	1.53	0.55	**2.45**	**3.92**	1.53	**3.91**
Δ*zupT*	34.3±6.7	1.27	1.29	1.25	***0.29***	**4.59**	**3.31**	1.34	**3.12**
Δ*zupT* ΔW3	***25.5±4.6***	1.06	1.94	1.79	***0.59***	**3.82**	**3.12**	1.05	**2.60**
ϕ *fieF-lacZ*)									
CH34	18.3±1.8	1.20	1.06	0.55	0.27	1.00	0.85	0.97	0.96
CH34 ΔW3	15.1±4.0	1.29	1.22	0.58	0.29	1.50	1.27	1.35	1.41
AE104	20.1±4.4	1.15	0.99	1.29	0.50	1.19	0.98	1.13	0.83
AE104 ΔW3	23.6±4.7	1.14	1.22	1.20	0.33	1.20	1.06	1.22	1.09
Δ*zupT*	25.3±3.3	1.05	0.98	0.97	0.25	1.06	0.93	1.07	0.83
Δ*zupT* ΔW3	31.0±4.8	1.03	0.93	0.72	0.27	0.95	0.84	1.07	0.88

[a] Chromosomal *lacZ* fusions were constructed downstream of *zntA* and *cadA*, leaving both functional. Parent strains were *C. metallidurans* CH34 wild type (top), AE104 (bottom), and the Δ*zupT* mutant. Early exponential-phase cells of these strains were cultivated for 3 h with shaking at 30°C in TMM without or with the indicated additions, and β-galactosidase activity was determined. The specific activity of cells grown in TMM without additions is provided, the remaining –fold up-regulation values refer to these values listed in the subsequent rows. Bold-faced numbers indicate significant up-regulation of the basic value of a fusion compared to non-amended medium (D > 1 and ratio > 2-fold, n ≥ 3), bold-faced values in italics down-regulation

Supplementary Table S6. Expression of reporter fusions of import systems[a]

	Bacterial strain					
Fusion	AE104	AE104 ΔW3	Δ*zupT*	Δ*zupT* ΔW3	CH34	CH34 ΔW3
ϕ(*zntB-lacZ)*	6.97±1.11	1.10	**5.45**	**5.17**	***0.00***	0.80
0.5 mM Zn(II)	0.56	0.58	**2.87**	**2.22**	***0.00***	**0.48**
5 mM EDTA	0.79	1.07	**4.59**	**4.72**	***0.05***	1.13
ϕ(*zupT-lacZ)*	12.5±2.9	1.13	n.d.	n.d.	1.34	1.12
0.5 mM Zn(II)	0.65	0.61	n.d.	n.d.	0.81	0.75
5 mM EDTA	**3.42**	**5.72**	n.d.	n.d.	**5.66**	**4.99**
ϕ(*hoxN-lacZ)*	0.87±1.68	0.00	5.13	0.00	0.00	0.00
0.5 mM Zn(II)	0.49	0.11	1.64	0.00	0.25	0.86
5 mM EDTA	1.65	0.00	**10.5**	0.00	0.35	0.00
ϕ(*pitA-lacZ)*	157±35	1.63	1.03	1.36	1.27	0.98
0.5 mM Zn(II)	***0.49***	0.76	0.71	0.73	0.52	***0.48***
5 mM EDTA	0.63	1.15	0.85	1.28	1.23	0.84
ϕ(*corA1-lacZ)*	59.0±10.0	0.79	***0.33***	***0.42***	***0.43***	***0.37***
0.5 mM Zn(II)	***0.45***	***0.38***	***0.04***	***0.25***	***0.19***	***0.22***
5 mM EDTA	0.80	*0.63*	***0.38***	***0.08***	*0.67*	*0.65*
ϕ(*corA2-lacZ)*	44.0±7.2	1.11	0.95	0.82	1.21	0.86
0.5 mM Zn(II)	*0.67*	*0.62*	***0.50***	***0.39***	***0.47***	*0.52*
5 mM EDTA	*0.68*	*0.87*	*0.83*	***0.50***	1.17	1.04
ϕ(*corA3-lacZ)*	32.7±6.0	1.09	0.91	0.90	***0.09***	*0.59*
0.5 mM Zn(II)	***0.49***	***0.41***	***0.24***	***0.28***	***0.06***	***0.39***
5 mM EDTA	*0.56*	*0.62*	*0.66*	*0.74*	***0.15***	0.83
ϕ(*mgtA-lacZ)*	5.57±1.17	1.15	1.00	0.79	***0.00***	***0.00***
0.5 mM Zn(II)	***0.26***	***0.29***	***0.47***	***0.20***	***0.00***	***0.00***
5 mM EDTA	1.19	0.76	0.94	1.06	***0.00***	***0.00***
ϕ(*mgtB-lacZ)*	10.3±2.6	0.95	**0.49**	0.59	1.01	0.65
0.5 mM Zn(II)	0.61	0.63	**0.24**	**0.29**	**0.51**	0.54
5 mM EDTA	0.66	0.95	**0.54**	0.77	1.20	0.95

[a] Chromosomal *lacZ* fusions were constructed. Parent strains were *C. metallidurans* CH34 wild type, AE104, AE104 Δ*zupT*, and their respective ΔW3 mutants. Early exponential phase cells of these strains were cultivated for 3 h with shaking at 30°C in TMM without or with the indicated additions, and β-galactosidase activity was determined. The specific activity of cells grown in TMM without additions is given in the boxed areas in U/mg dry mass, the remaining –fold up-regulation values refer to these values listed in the subsequent cells. Bold-faced numbers indicate significant up-regulation of the basic value of a fusion compared to non-amended medium (D > 1 and ratio > 2-fold, n ≥ 3), bold-faced values in italics down-regulation. Since some values for CH34 strains were zero, AE104 was used for reference in this experiment. Shaded areas indicate interesting results.

Supplementary Table S7. Cellular metal content of cobalt-treated ΔW3 mutants

Bacterial strain	Mg, -fold	P, -fold	Co, -fold	Ni, -fold	Fe, -fold
25 µM Co					
CH34	1.00	1.00	1.00	1.00	1.00
CH34 ΔW3	0.84	0.89	0.84	2.08	1.19
AE104	0.89	0.84	**2.25**	0.73	0.80
AE104 ΔW3	0.79	0.79	**2.19**	0.46	0.67
Δ*zupT*	0.74	0.76	**2.66**	0.94	0.70
Δ*zupT* ΔW3	1.00	0.85	**3.45**	**0.37**	**0.59**
Δ*zur*	0.82	0.83	**2.91**	0.79	0.81
Δ*zur* ΔW3	0.93	0.84	**2.68**	1.46	1.00
1 µM Co					
Δe4	**2.67**	**1.58**	1.00	0.72	0.88
Δe4 Δ*zupT*	**2.78**	**1.54**	1.16	0.56	1.05
Δe4 ΔW3	0.92	0.87	1.73	0.78	1.01
Δe4 Δ*zupT* ΔW3	0.98	0.89	1.25	0.58	0.97

100% values for CH34: 13.1±1.4 x 10^6 Mg, 120±10 x 10^6 P, 732±106 x 10^3 Fe, 64.3±6.5 x 10^3 Zn, 10.8±4.3 x 10^3 Cu, 45.4±8.6 x 10^3 Co, 4.53±1.53 x 10^3 Ni, and for Δe4 at 1 µM cobalt 33.6±1.4 x 10^3 Co. No change in the number of Cu atoms per cell. Bold-faced, if [(Q<0.66 OR Q>1.5) AND D >1], underlined value significantly different (54% more Co) than the AE104 parent level. Interesting results are boxed. The underlined cobalt content of Δ*zupT* ΔW3 is significantly different from the cobalt content of the Δ*zupT* parent.

Supplementary Table S8. Bacterial strains and primers

Strain	Plasmids	Characteristics	Referenz
Escherichia coli			
Rosetta (DE3) pLysS	pLysSRARE	*F-*, *ompT*, *gal, dcm, lon, hsdS$_B$*, *(r$_B^-$ m$_B^-$)λ*(DE3), pLysSRARE; cam^R; BL21 (DE3)	Stratagene GmbH, Heidelberg
S17/1		*pro*, Tra$^+$ *recA*	(10)
VS208	pASK-IBA7	plasmid for expression with N-terminal Strep-tag®II, amp^R	IBA-GmbH, Göttingen
VS218	pCM157	plasmid for expression of *Cre*-recombinase; tet^R	(11)
VS585	pECD794-1	pLO2::*lacZ* for transcriptional fusions; kan^R	(2)
VS600	pRHB152	pET28A TEV, kan^R	Novagen, Californien (USA)
VS624	pTH24::*tev*	pET24 for Tev expression with C-terminal His-tag; Amp^R	(12)
ECA61	pECD795	*znt*A in pECD1794-1; *lacZ*-operon fusion *φ*(*zntA-lacZ*), kan^R	(13)
ECA62	pECD796-1	*cad*A in pECD1794-1; *lacZ*-operon fusion *φ*(*cadA-lacZ*), kan^R	(13)
ECA410	pECD986	*pitA* in pECD1794-1; *lacZ*-operon fusion *φ*(*pitA-lacZ*), kan^R	(17)
ECA411	pECD987	*zupT* in pECD1794-1; *lacZ*-operon fusion *φ*(*zupT-lacZ*), kan^R	(17)
ECA412	pECD988	*corA$_1$* in pECD1794-1; *lacZ*-operon fusion *φ*(*corA$_1$-lacZ*), kan^R	(17)
ECA413	pECD989	*corA$_2$* in pECD1794-1; *lacZ*-operon fusion *φ*(*corA$_2$-lacZ*), kan^R	(17)
ECA414	pECD990	*corA$_3$* in pECD1794-1; *lacZ*-operon fusion *φ*(*corA$_3$-lacZ*), kan^R	(17)
ECA410	pECD986	*pitA* in pECD1794-1; *lacZ*-operon fusion *φ*(*pitA-lacZ*), kan^R	(17)
ECA483	pECD1003	*lacZ, sacB;* km^R, tet^R, amp^R; pCM184 with mutations of *loxP66* & *loxP71*	(2)
ECA779	pECD1204	pECD794-1 with *Rmet_1098* as *Pst*I/*Xba*I fragment *φ*(*cobW$_1$'*::*lacZ*)354, kan^R	(14)
ECA814	pECD1239	pET28A TEV with *cobW$_1$* as *Sac*I/*Bam*HI-fragment, kan^R	This publication
ECA815	pECD1240	pET28A TEV with *cobW$_2$* as *Sac*I/*Bam*HI-fragment, kan^R	This publication
ECA816	pECD1241	pET28A TEV with *cobW$_3$* as *Sac*I/*Bam*HI-fragment, kan^R	This publication
ECA819	pECD1244	pECD794-1 with *cobW$_2$* as *Pst*I/*Xba*I fragment *φ*(*cobW$_2$'*::*lacZ*)299, kan^R	(15)
ECA914	pECD1339	pASK-IBA7 with *zur* as *Sal*I/*Xba*I fragment, amp^R	(16)

ECB018	pECD1442	*fieF* in pECD1794-1; *lacZ*-operon fusion *φ*(*fieF-lacZ*), kan^R	This publication
ECB062	pECD1486	*zntB* in pECD1794-1; *lacZ*-operon fusion *φ*(*zntB-lacZ*), kan^R	(5)
ECB063	pECD1487	*hoxN* in pECD1794-1; *lacZ*-operon fusion *φ*(*hoxN-lacZ*), kan^R	(5)
ECB064	pECD1488	*mgtA* in pECD1794-1; *lacZ*-operon fusion *φ*(*mgtA-lacZ*), kan^R	(6)
ECB065	pECD1489	*mgtB* in pECD1794-1; *lacZ*-operon fusion *φ*(*mgtB-lacZ*), kan^R	(6)
ECB188	pECD1606	pECD1003 for *cobW$_3$*-deletion; *cre-lox*-System	This publication
ECB189	pECD1607	pECD1003 for *cobW$_1$*-Cluster (*Rmet_1098* bis *Rmet_1103*)-deletion; *cre-lox*-System	This publication
ECB190	pECD1608	*cobW$_3$* in pECD1794-1; *lacZ*-operon fusion *φ*(*cobW$_3$-lacZ*), kan^R	(15)
ECB191	pECD1609	*cobW$_2$* in pECD1794-1; *lacZ*-operon fusion *φ*(*cobW$_2$-lacZ*), kan^R	(15)
ECB195	pECD1612	*dmeF* in pECD1794-1; *lacZ*-operon fusion *φ*(*dmeF-lacZ*), kan^R	This publication
ECB194	pECD1611	pASK-IBA3 with FolE$_{IB2}$ (*Rmet_1099*) as *Sac*I/NcoI fragment, amp^R	This publication
Cupriavidus metallidurans			
CH34	pMOL28		(17)
	pMOL30		
AE104			(17)
DN515		AE104 Δ*zupT*	(18)
DN578		AE104Δ*zntA*Δ*cadA*Δ*fieF*Δ*dmeF* (Δe4)	(2)
DN579		AE104Δ*zntA*Δ*cadA*Δ*fieF*Δ*dmeF*Δ*zupT* (Δe4Δ*zupT*)	(1)
DN780		Δe4Δ*zur*	This publication
DN818		AE104Δ*cobW$_3$*	This publication
DN819	pMOL28	CH34Δ*cobW$_3$*	This publication
	pMOL30		
DN820	pMOL28	CH34Δ*cobW$_1$*-Cluster	This publication
	pMOL30		
DN821		AE104Δ*zur*Δ*cobW$_3$*	This publication
DN822		AE104Δ*zur*Δ*cobW$_1$*-Cluster	This publication
DN823	pMOL28	CH34Δ*cobW$_1$*-ClusterΔ*cobW$_3$*	This publication
	pMOL30		
DN824		AE104Δ*zupT*Δ*cobW$_3$*	This publication
DN825	pECD1204	AE104Δ*zur*Δ*cobW$_3$* *φ*(*cobW$_2$*'::*lacZ*)299	This publication
DN826	pECD1204	AE104Δ*zur*Δ*cobW$_1$*-Cluster *φ*(*cobW$_2$*'::*lacZ*)299	This publication
DN827	pECD1204	Δe4Δ*zur* *φ*(*cobW$_2$*'::*lacZ*)299	This publication
DN828		Δe4Δ*cobW$_1$*-Cluster	This publication
DN829	pMOL28	CH34 *φ*(*cobW$_2$*'::*lacZ*)299	This publication
	pMOL30		
	pECD1204		
DN830	pMOL28	CH34Δ*cobW$_3$* *φ*(*cobW$_2$*'::*lacZ*)299	This publication

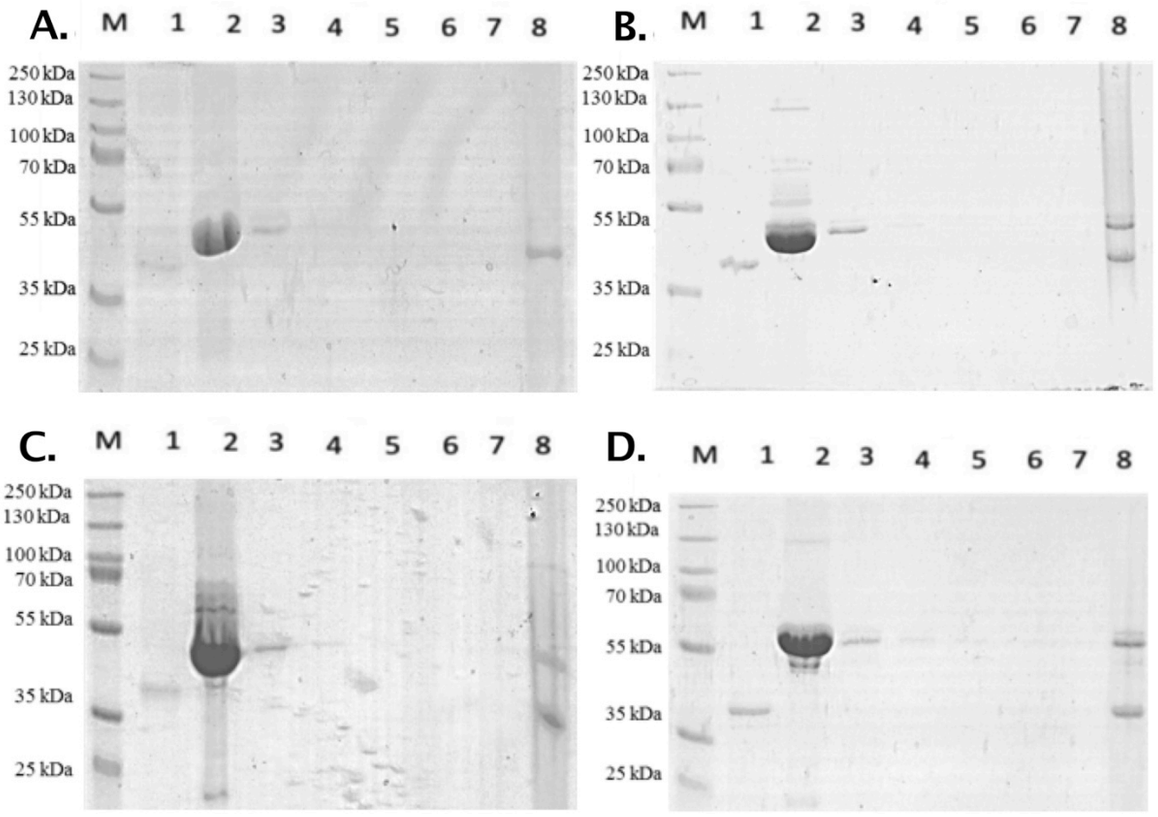
	pMOL30 pECD1204		
DN831	pMOL28 pMOL30 pECD1204	CH34Δ*cobW*$_1$-Cluster *φ*(*cobW*$_2$'::*lacZ*)299	This publication
DN832	pMOL28 pMOL30 pECD1204	CH34Δ*cobW*$_1$-ClusterΔ*cobW*$_3$ *φ*(*cobW*$_2$'::*lacZ*)299	This publication
DN833		Δe4Δ*cobW*$_3$	This publication
DN834		Δe4Δ*zupT*Δ*cobW*$_3$	This publication
DN835	pECD1204	AE104Δ*zupT*Δ*cobW*$_3$ *φ*(*cobW*$_1$'::*lacZ*)354	This publication
DN836	pECD1244	AE104Δ*zupT*Δ*cobW*$_3$ *φ*(*cobW*$_2$'::*lacZ*)299	This publication
DN837	pECD1204	AE104Δ*cobW*$_3$ *φ*(*cobW*$_1$'::*lacZ*)354	This publication
DN838	pECD1244	AE104Δ*cobW*$_3$ *φ*(*cobW*$_2$'::*lacZ*)299	This publication
DN839	pECD1244	Δe4Δ*cobW*$_1$-Cluster *φ*(*cobW*$_2$'::*lacZ*)299	This publication
DN840	pECD1204	AE104Δ*zur*Δ*cobW*$_3$ *φ*(*cobW*$_1$'::*lacZ*)354	This publication
DN841	pECD1204	Δe4Δ*cobW*$_3$ *φ*(*cobW*$_1$'::*lacZ*)354	This publication
DN842	pECD1204	Δe4Δ*zupT*Δ*cobW*$_3$ *φ*(*cobW*$_1$'::*lacZ*)354	This publication
DN843	pECD1244	Δe4Δ*cobW*$_3$ *φ*(*cobW*$_2$'::*lacZ*)299	This publication
DN844	pECD1244	Δe4Δ*zupT*Δ*cobW*$_3$ *φ*(*cobW*$_2$'::*lacZ*)299	This publication

Primers

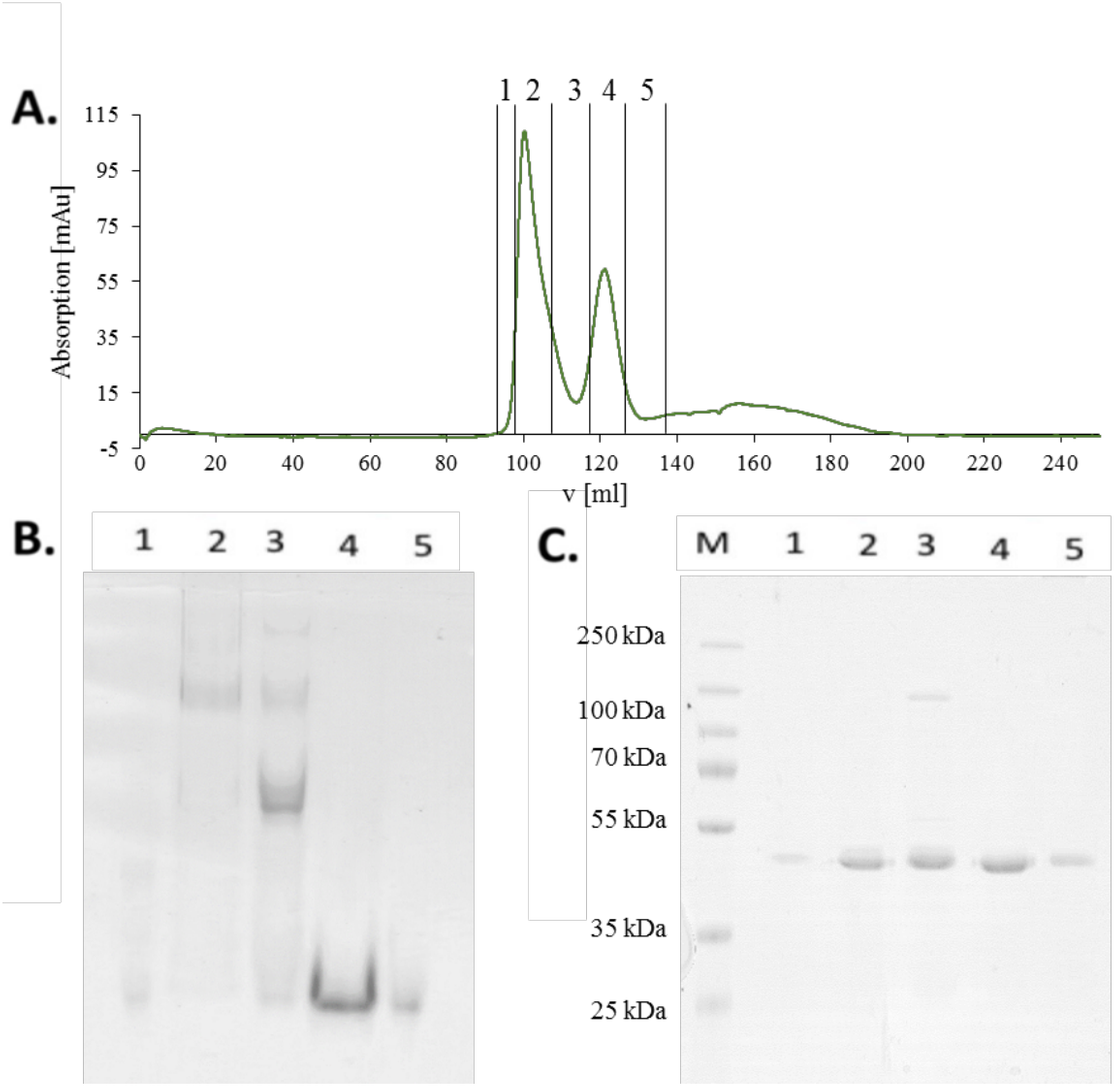
name/orientation →/←	5'→3' sequence	position
gene disruption		
*Rmet_0127 Pst*I Dis →	AAACTGCAGGCTCGACAAGCAGGAAGAAG	binds 412 bp downstream of ATG$_{Rmet_0127}$
*Rmet_0127 Xba*I Dis ←	AAATCTAGAGCAATCGGTGCCGCAGTGT	binds 376 bp upstream of TGA$_{Rmet_0127}$
*Rmet_1098 Pst*I Dis →	AAACTGCAGGGCCGCAGTCTCAATGAGG	binds 270 bp downstream of ATG$_{Rmet_1098}$
*Rmet_1098 Xba*I Dis ←	AAATCTAGAGGGCGCTTTCGATGCTTCC	binds 550 bp upstream of TGA$_{Rmet_5377}$
cre-lox		
Cre 0125 Age →	AAAACCGGTTCGAATCCGGCGACTATGGCTG	binds 332 bp upstream of ATG$_{cobW3}$
Cre 0125 Apa ←	AAAGGGCCCGATGATCGATGTTGATGCAACAAAG	binds directly upstream of ATG$_{cobW3}$
*Cre 0125 Not*I →	AAAGCGGCCGCAGTCCTGCACCCTCTCTC	binds directly downstream TGA$_{cobW3}$
*Cre 0125 Nco*I →	AAACCATGGCCAGGTTGGCTGGCTTGAC	binds 311 bp downstream of TGA$_{cobW3}$
Cre 1098 *Mun* →	AAACAATTGGTTACCCACTTCGGATACG	binds 385 bp upstream of ATG$_{cobW1}$
Cre 1098 Not ←	AAAGCGGCCGCGGGGATTTGGTTTGCCCG	binds directly upstream of ATG$_{cobW1}$
Cre cobW$_1$-Cluster *Apa*→	AAAGGGCCCAATCAGGCGGCAGGGG	binds directly downstream of TGA$_{Rmet_1103}$
Cre cobW$_1$-Cluster *Age*←	AAAACCGGTAAGGTAACCGGCGATATG	binds 296 bp downstream of TGA$_{Rmet_1103}$
Cre 0128 Age →	AAAACCGGTTCTCGCGCTTGCTGTAGG	binds 399 bp upstream of ATG$_{zur}$
Cre 0128 Apa ←	AAAGGGCCCGCGAAGGATTTAACCATAGG	binds directly upstream of ATG$_{zur}$

Cre 0128 NcoI	*AAACCATGG-CGAACGCGATCTTGCCTTC*	*binds 330 bp downstream of TGAzur*
*Cre 0128 Not*I	AAAGCGGCCGCCACATCCAGACACCTTTAG	binds directly downstream of TGA_{zur}
fusions His-/Strep-tag		
*1098 Bam*HI His →	AAAGGATCCCTTCCAGCCAAGCTTCCTG	5‘ part of *cobW1* without ATG
*1098 Sac*I His ←	AAAGAGCTCAGTGTCAGGGCCAGTCAGG	3‘ part of *cobW1* with TGA
pRHB152 *0127 Bam*HI →	AAAGGATCCTCCAAACTGATTCCGGTCACG	5‘ part of *cobW2* without ATG
pRHB152 *0127 Sac*I ←	AAAGAGCTCTAAAGTCAGGCGAGGCAGGC	3‘ part of *cobW2* with TGA
pRHB152 *0125 Bam*HI →	AAAGGATCCGCCGTTCGTCTGCCCGTCA	5‘ part of *cobW3* without ATG
pRHB152 *0125 Sac*I ←	AAAGAGCTCTCAGTGTGCATGTCCGCAATC	3‘ part of *cobW3* with TGA
pASK3 *Rmet_1099* SacI→	AAAGAGCTCCGCCCAGGACATTGGGGATGC	5‘ part of $folE_{IB2\ 5}$ without ATG
pASK3 *Rmet_1099 Nco*I ←	AAACCATGGGCGGCCACCTCCCGTGAATG	3‘part of $folE_{IB2\ 5}$ without TGA
***lacZ*-fusions**		
*dmeF-lacZ Pst*I →	AAACTGCAGAGCGGGACGGTGCTGCTC	binds 315 bp upstream of TAG_{dmeF}
*dmeF-lacZ Xba*I →	AAATCTAGATTGCCGCCTAGTGACGGTG	binds directly upstream of TAG_{dmeF}
RT-PCR *zur* region		
Rmet_0125 PstI Dis h →	AAACTGCAGCCGCTTCTTGCAGGACTAC	4300
Rmet_0125 XbaI Dis h ←	AAATCTAGATTGCCAGCCGGCGGCGTT	4603
Rmet_0126 PstI Dis h →	AAACTGCAGGCCAGCCAGAGTGAAACAG	3298
Rmet_0126 XbaI Dis h ←	AAATCTAGATCCGGCACGATGACCGTTT	3564
Rmet_0127 PstI Dis h →	AAACTGCAGGCTCGACAAGCAGGAAGAAG	2128
Rmet_0127 XbaI Dis h ←	AAATCTAGAGCAATCGGTGCCGCAGTGT	2425
Rmet_0128 PstI Dis h →	AAACTGCA**G**ACATGCGGCTGCGTCCGA	1166
Rmet_0128 XbaI Dis h ←	AAATCTAGAGTCGTTGCCGGCGCGTTT	1427
Cre 0125 Age →	AAAACCGGTTCGAATCCGGCGACTATGGCTG	3669
Cre 0125 Not →	AAA GCG GCC GC AGTCCTGCACCCTCTCTC	5176
Cre 0125 Nco ←	AAACCATGGCCAGGTTGGCTGGCTTGAC	5487
Cre 0125 Apa ←	AAA GGG CCC GC GATGATCGATGTTGATGCAACAAAG	4000
Cre 0126 Mun ←	AAA CAA TTG CAGCCGTTCGACTTCGGCAAGC	4147
Cre 0126 Apa ←	AAAGGGCCCGCGGGGACACCTCTCTACG	3118
Cre 0126 Not →	AAA GCG GCC GC TTCGATCCTTCGAGAGGCGGAAA	3809
Cre 0126 Age →	AAAACCGGT CGTTTAGTGCGCCATCAGAC	2806
Cre 0127 Age →	AAAACCGGTACCGCGTGACCGTGTACCG	1354
Cre 0127 Not →	AAA GCG GCC GC CTTTACGTTTAGTGCGCCATC	2801
Cre 0127 Nco ←	AAACCATGGCTCTCTACGCGTCGAAGAGTG	3110
Cre 0127 Apa ←	AAA GGG CCC GC GTTGTGGTACTCCGAGAAAA	1708
Cre 0128 Nco ←	AAACCATGGCGAACGCGATCTTGCCTTC	1988
Cre 0128 Age →	AAAACCGGTTCTCGCGCTTGCTGTAGG	749
Cre 0128 Apa ←	AAA GGG CCC GC GCGAAGGATTTAACCATAGG	1148
Cre 0128 Not →	AAA GCG GCC GC CACATCCAGACACCTTTAG	1659
cobW2→dksA 2532up →	GCA GCG ACA AGC CGT TCC	2532
cobW2→dksA3328down ←	GTC CGC TCG GGT GCA GTG	3328
cobW3→0124 4835 up →	GCA TGG TGG GCA GCG ATC	4835
RT-PCR *cobW1* region		
Rmet_1098 PstI Dis h →	AAACTGCAGGGCCGCAGTCTCAATGAGG	10364
Rmet_1098 XbaI Dis h ←	AAATCTAGAGGGCGCTTTCGATGCTTCC	10717
Rmet_1098 Mun1 →	AAACAA TTGGTTACCCACTTCGGATACG	9616
Rmet_1098 Not2 ←	AAA GCG GCC GC GGGGATTTGGTTTGCCCG	10000

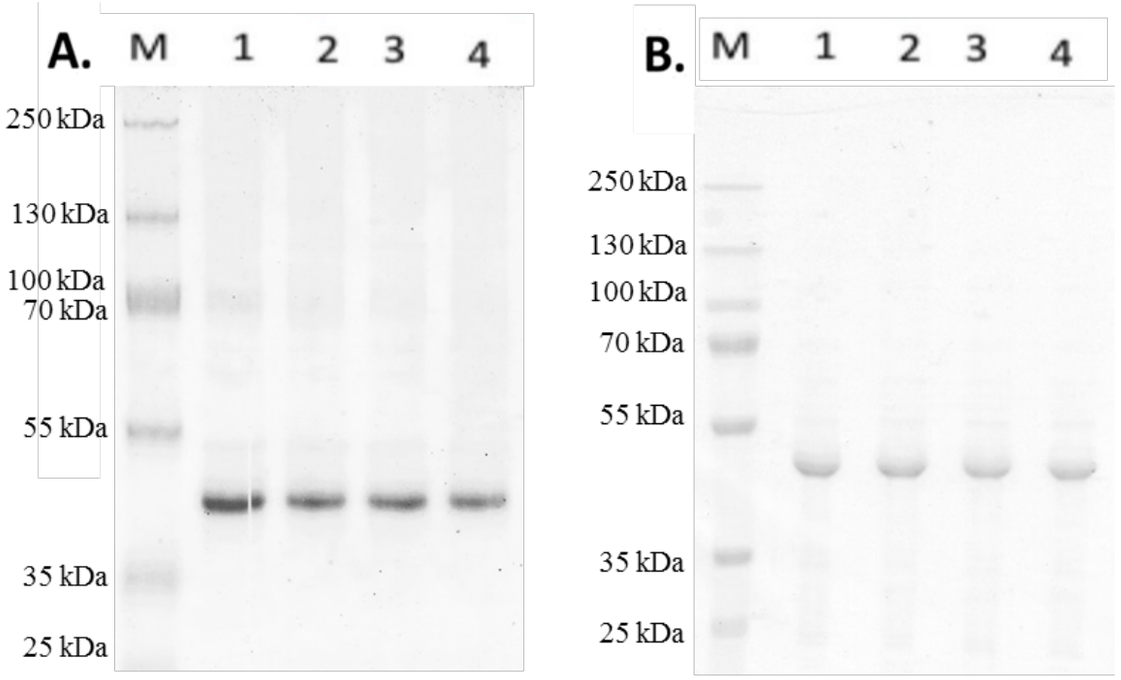
Rmet_1098 Apa3	→	AAA GGG CCC GCCACTTTGGGCTCCCCATG	11195
Rmet_1098 Age4	←	AAAACCGGTCGCTGCCTGTCTCGCAATC	11561
Crelox F1 1099 Mun	→	AAA CAA TTG GGCGCCGTTGCCAGGCAC	10922
Crelox F1 1099 Nco	←	AAACCATGG CATGGGGAGCCCAAAGTGT	11212
Crelox F2 1099 Apa	→	AAA GGG CCC GC CGCATGACTTCCGCGC	12178
Crelox F2 1099 Age	←	AAAACCGGTCGCGCGCATGTCTTCGTGG	12495
Rm1100→13324	→	CGG ATG ATG GCG TCG GGA	13324
Rm1101→13913	←	GTT CGG CGC TTG GCG ACA	13913
Rm1101→13880	→	GCC AGC ATT CAG AGC GTT	13880
Rm1102→14505	←	TAA ACA CCC CAT TGC CGA	14505
Rm1102→14473	→	GCG TCG TGC CAT CGT CCA	14473
Rm1103→15144	←	GCG TGT TCG GCA TCT CGA	15144
Rm1103→15923	→	GAC CTG ACA AGC GCC GGA	15923
Rm1104→16587	←	TTG CCA CGC CGA GCT ACA	16587
166bp vor Zurbox 9735 up	→	TCG CCT GGC AGT ACG GGA	9735
folE2→cysS 11889up	→	TTG CCG TCG ATT TGC CGA	11889



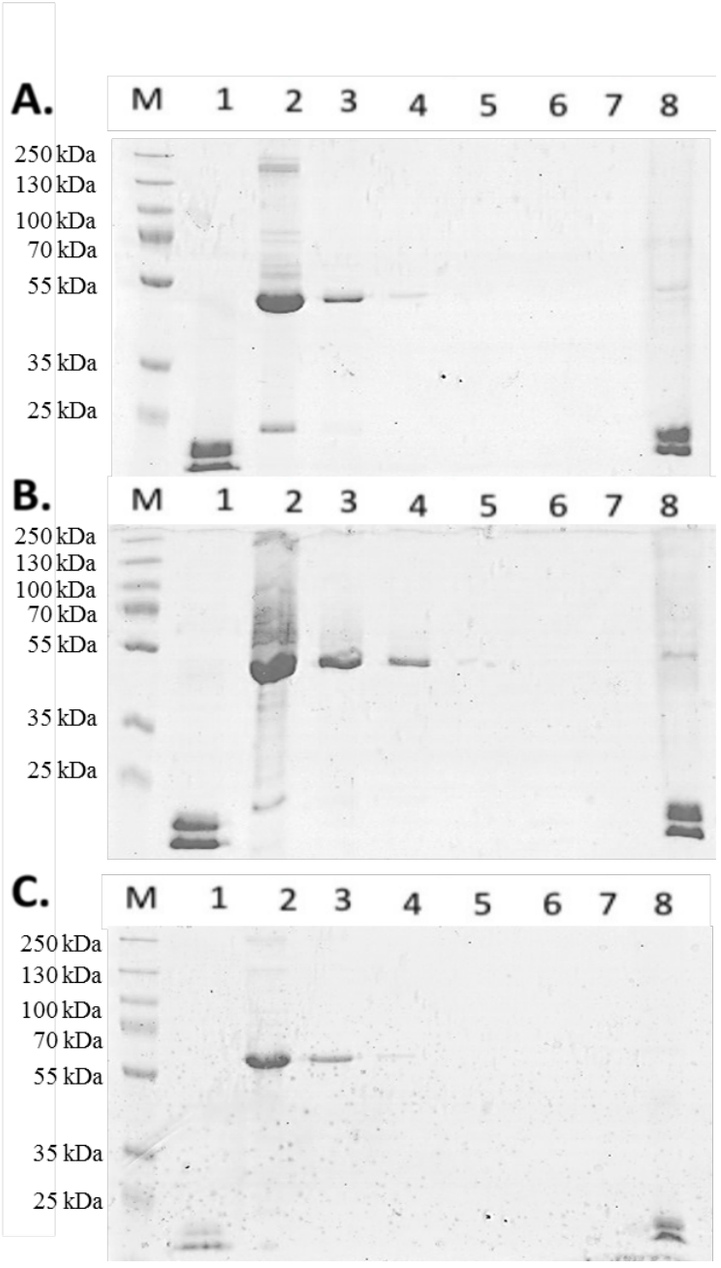
Supplementary Figure S1. Interaction of CobWs with FolE$_{IB2}$. A pull-down assay was performed using magnetic MagStrep XT beads washed three times in buffer W (50 mM TrisHCl, pH 8, 150 mM NaCl) to bind 10 µg of FolE$_{IB2}$-strep-tag for 30 min at 4 °C in buffer W (50 mM TrisHCl, pH 8, 150 mM NaCl) additionally containing 500 µM $MgCl_2$, 10 µM GTP and 10 µM $ZnCl_2$ (Panels B, C, D) or no zinc (Panel A). The supernatant (Lane 1) was removed and 100 µg CobW1-His-tag (Panels A and B), CobW2-His-tag (Panel C) or CobW3-His-tag (Panel D) were added. The mixture was incubated for 3 h at 4 °C. Again, the supernatant was removed (Lane 2) and the beads washed with buffer W five times (Lanes 3 to 7). Finally, the bound proteins were removed using two-fold concentrated SDS sample buffer (Lane 8). The Panels show Coomassie-stained SDS polyacrylamide gels.



Supplementary Figure S2. Dimerization of CobW1. An amount of 5 mg of apo-CobW1 was analyzed by size exclusion chromatography using a HiPrep Sephacryl S-100 column and Tris buffer (50 mM, pH = 8) containing 150 mM NaCl. Panel A shows the elution profile at 280 nm. Samples corresponding to 5 µg were applied to a non-denaturing (Panel B) and SDS polyacrylamide gel (Panel C). The additional band in Panel C lane 3 was identified by MALDI-TOF-MS as contaminating pyruvate kinase from *E. coli*. Presence of this protein resulted in a complicated pattern in the non-denaturing polyacrylamide gel (lane B3). Coomassie-staining.



Supplementary Figure S3. CobW1 did not form dimers when zinc was added. An amount of 5 µg of CobW1 monomers as purified by size exclusion chromatography was incubated with 1 mM $ZnCl_2$ (Lane 2), 1 mM MgGTP (Lane 3), both (Lane 4) or without additions for 2 h at 4°C. Subsequently, the samples were applied to a non-denaturing (Panel A) and SDS polyacrylamide gel (Panel B). Coomassie-staining.



Supplementary Figure S4. Interaction of CobWs with Zur. A pull-down assay was performed using magnetic MagStrep XT beads washed three times in buffer W (50 mM TrisHCl, pH 8, 150 mM NaCl) to bind 10 µg of Zur-strep-tag for 30 min at 4 °C in buffer W (50 mM TrisHCl, pH 8, 150 mM NaCl) additionally containing 500 µM $MgCl_2$ and 100 µM $ZnCl_2$. The supernatant (Lane 1) was removed and 100 µg CobW1-His-tag (Panel A), CobW2-His-tag (Panel B) or CobW3-His-tag (Panel C) were added. The mixture was incubated for 3 h at 4 °C. Again, the supernatant was removed (Lane 2) and the beads washed with buffer W five times (Lanes 3 to 7). Finally, the bound proteins were removed using two-fold concentrated SDS sample buffer (Lane 8). The Panels show Coomassie-stained SDS polyacrylamide gels

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