Supplemental Material:

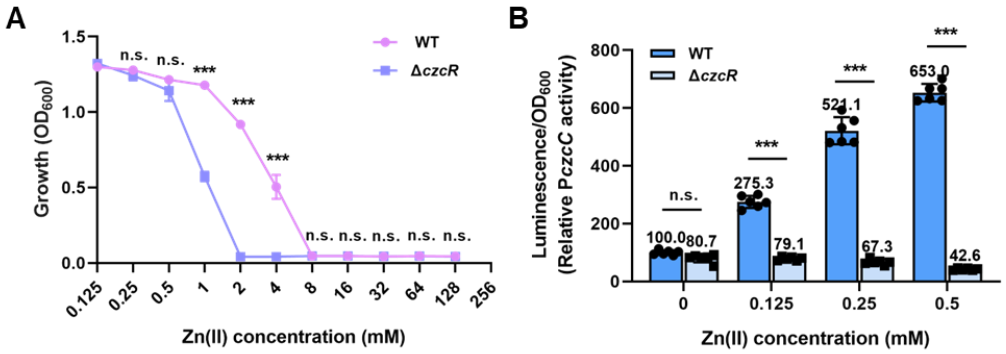

Figure S1. Growth of the PAO1 WT and Δ*czcR* strains and the activity of the P*czcC* promoter during treatments with different concentrations of Zn. (**A**) Final values of OD_{600} were measured for the PAO1 WT and Δ*czcR* strains when they were cultured in the presence of Zn at different concentrations. (**B**) Expression of the P*czcC-lux* was measured in the WT and Δ*czcR* strains when they were cultured in the presence of Zn at different concentrations. Statistical significance was calculated compared to the WT group based on two-way ANOVA (n.s., not significant; ***, $P< 0.001$).

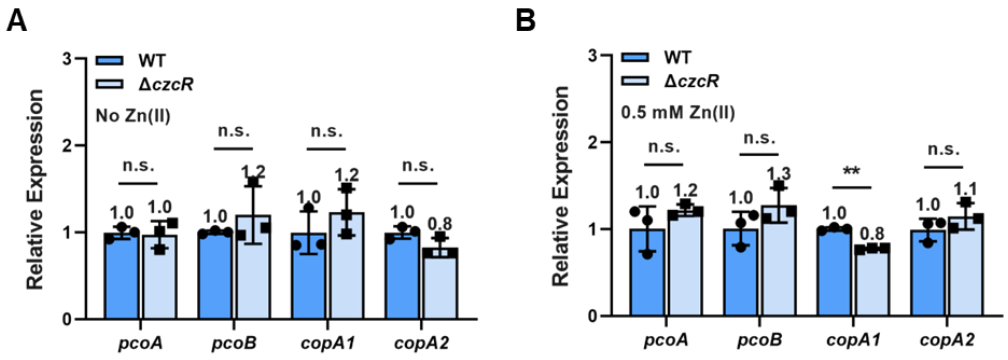

Figure S2. CzcS/CzcR did not cause obvious expression changes of other major genes involved in Cu tolerance besides *ptrA* and *PA2807*. Relative expression of the *pcoA*, *pcoB*, *copA1*, and *copA2* in the Δ*czcR* strain compared to the WT strain when they were cultured in the absence (**A**) or presence (**B**) of Zn. Statistical significance was calculated compared to the WT group based on Student's *t*-test (n.s., not significant; **, $P< 0.01$).

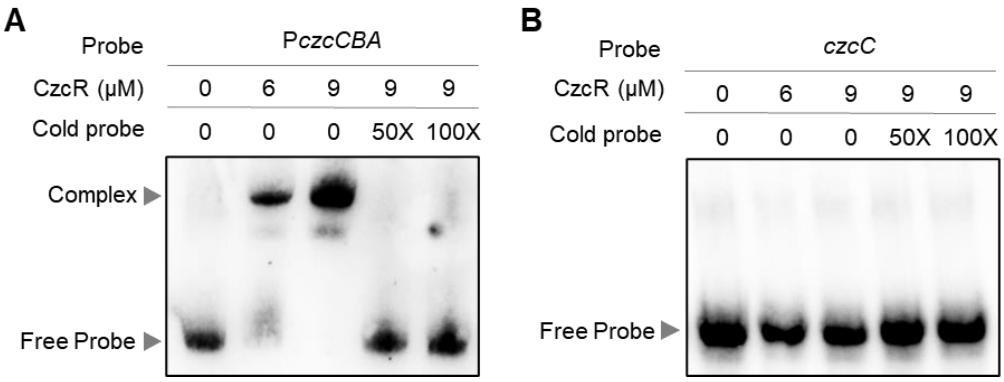

Figure S3. Positive and negative controls to determine specific interactions between CzcR and target DNA probes. EMSAs showed the binding ability of CzcR at the promoter of *czcCBA* (**A**) but not the coding region of *czcC* (**B**).

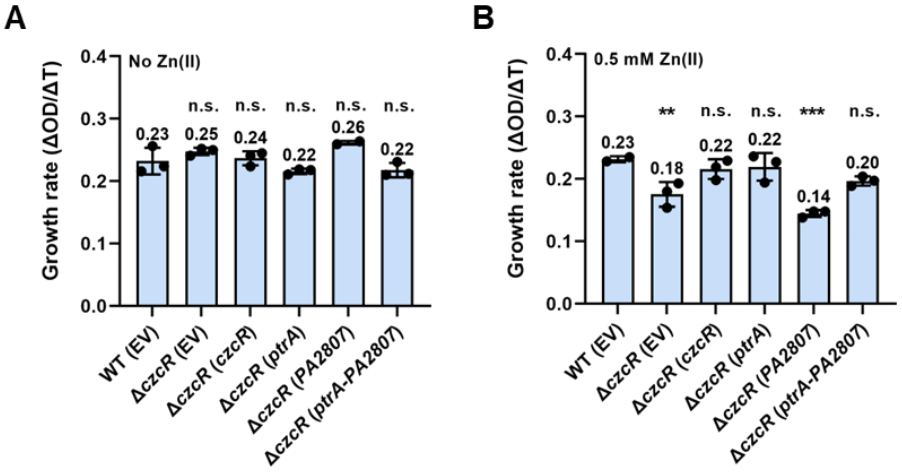

Figure S4. CzcS/CzcR maintained bacterial tolerance to Cu *via* PtrA during Zn excess. (**A**) Growth rates of the WT strain, Δ*czcR* mutant, and the Δ*czcR* mutant with the overexpression of *ptrA*, *PA2807*, or *ptrA-PA2807* in the presence of 2 mM $CuSO_4$ when the strains were not pretreated with Zn. (**B**) Growth rates of the WT strain, Δ*czcR* mutant, and the Δ*czcR* mutant with the overexpression of *ptrA*, *PA2807*, or *ptrA-PA2807* in the presence of 2 mM $CuSO_4$ after the strains were pretreated with Zn. Statistical significance was calculated compared to the WT (EV) group based on one-way ANOVA (n.s., not significant; **, $P< 0.01$; ***, $P< 0.001$).

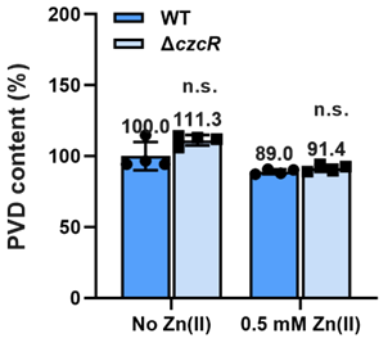

Figure S5. Production of pyoverdine (PVD) was measured in WT and Δ*czcR* strains when they were cultured in the absence or presence of Zn. Statistical significance was calculated compared to the WT group based on Student's *t*-test (n.s., not significant).

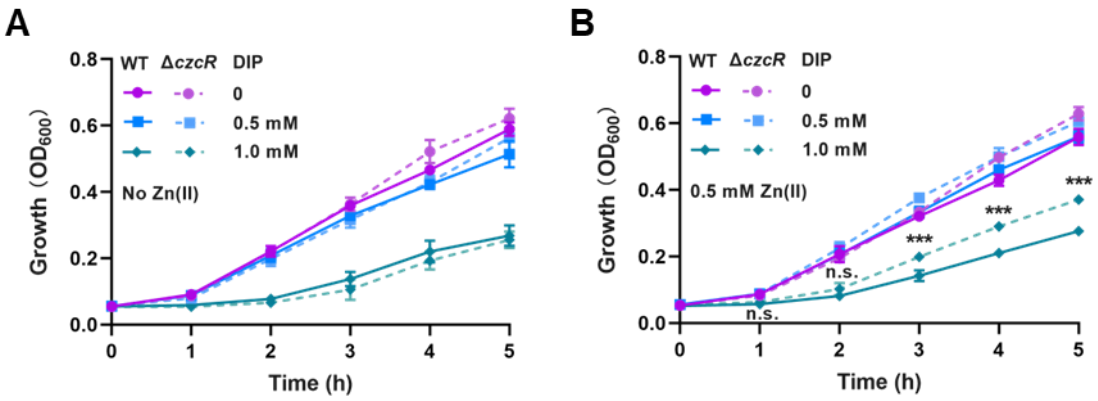

Figure S6. Deletion of *czcR* promoted cell growth under Fe depletion and Zn excess conditions. (**A**) Growth of the WT strain and the Δ*czcR* mutant in the absence or presence of 0.5 mM and 1.0 mM DIP when two strains were not pretreated with Zn. (**B**) Growth of the WT strain and the Δ*czcR* mutant in the absence or presence of 0.5 mM and 1.0 mM DIP when two strains were pretreated with Zn. Statistical significance was calculated compared to the WT group based on two-way ANOVA (n.s., not significant; ***, $P< 0.001$).

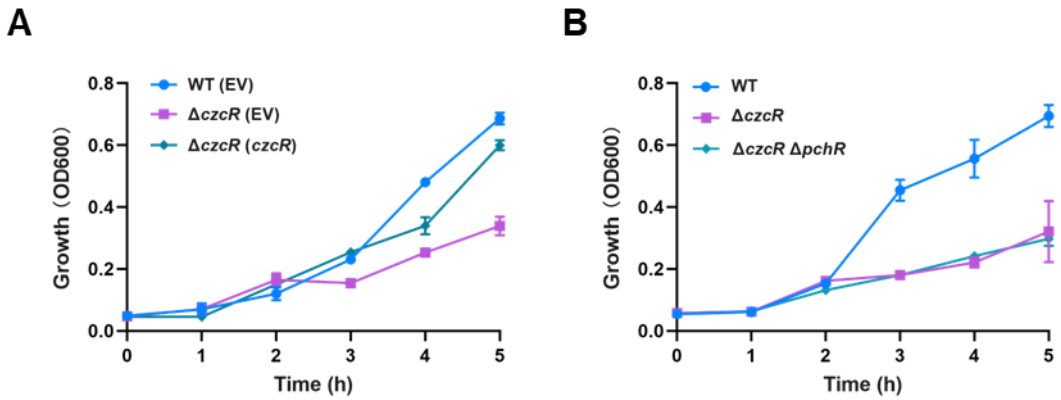

Figure S7. Deletion of *czcR* increased cell susceptibility to exogenous H_2O_2. (**A**) Growth of the WT strain, Δ*czcR* mutant, and the Δ*czcR* mutant with the complementation of *czcR* in the presence of 0.75 mM H_2O_2 when the strains were pretreated with Zn. (**B**) Growth of the WT strain, Δ*czcR* and Δ*czcR* Δ*pchR* mutants in the presence of 0.75 mM H_2O_2 when the strains were pretreated with Zn.

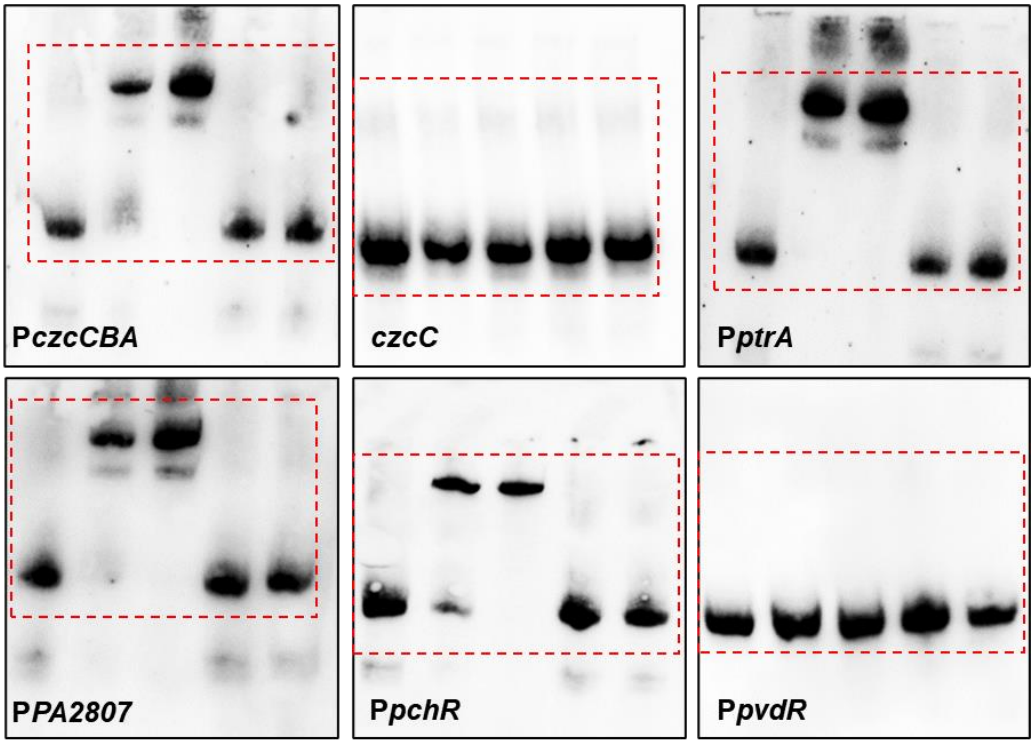

Figure S8. Uncropped gel images of the EMSA results obtained from this study. P*czcCBA* and *czcC* were used in Figure S3A and Figure S3B, P*ptrA* and P*PA2807* were used in Figure 2C, P*pchR* and P*pvdR* were used in Figure 5F.

Table S2. Bacterial strains, plasmids, and primers used in this study

1. Strains	
Name	**Description**
DH5α	*E. coli* strain used for plasmid construction and propagation
BL21(DE3)	*E. coli* strain used for protein expression and purification
SM10	*E. coli* strain used for conjugation
PAO1 WT	*P. aeruginosa* PAO1 wild-type strain from lab collection
Δ*czcR*	PAO1 with the deletion of *czcR*
Δ*czcR* (*czcR*)	PAO1 Δ*czcR* with the *in trans* expression of *czcR*
Δ*pchR*	PAO1 with the deletion of *pchR*
Δ*czcR* Δ*pchR*	PAO1 with the double deletion of *czcR* and *pchR*
Δ*czcR* (*ptrA*)	PAO1 Δ*czcR* with the *in trans* expression of *ptrA*
Δ*czcR* (*2807*)	PAO1 Δ*czcR* with the *in trans* expression of *PA2807*
Δ*czcR* (*ptrA-2807*)	PAO1 Δ*czcR* with the *in trans* expression of *ptrA* and *PA2807*
Δ*pchR* (*pchR*)	PAO1 Δ*pchR* with the *in trans* expression of *pchR*
Δ*czcR* Δ*pchR* (*pchR*)	PAO1 Δ*czcR* Δ*pchR* with the *in trans* expression of *pchR*
2. Plasmids	
Name	**Description**
mini-CTX-P*ptrA-lux*	mini-CTX-*lux* containing promoter of *ptrA*
mini-CTX-P*PA2807-lux*	mini-CTX-*lux* containing promoter of *PA2807*
mini-CTX-P*pchD-lux*	mini-CTX-*lux* containing promoter of *pchD*
mini-CTX-P*pchR-lux*	mini-CTX-*lux* containing promoter of *pchR*
mini-CTX-P*pchE-lux*	mini-CTX-*lux* containing promoter of *pchE*
pBBR1-*czcR*	pBBR1-MCS5 to express the *czcR* gene
pBBR1-*ptrA*	pBBR1-MCS5 to express the *ptrA* gene
pBBR1-*PA2807*	pBBR1-MCS5 to express the *PA2807* gene
pBBR1-*ptrA-PA2807*	pBBR1-MCS5 to express the *ptrA* and *PA2807* genes
pBBR1-*pchR*	pBBR1-MCS5 to express the *pchR* gene
pET28a-*czcR*	For CzcR expression and purification

3. Primer sequences		
Name	**Sequence (5' to 3')**	**Description**
lux-P*ptrA*-F	GGTCGACGGTATCGATAAGCTTGAAGCCTGCCTCGGCG	Amplify the *ptrA* promoter to construct P*ptrA-lux* reporter
lux-P*ptrA*-R	TCTAAAGAAGAATTGGGGATCCGGGAAGTCTCCTCGAA	
lux-P*PA2807*-F	GGTCGACGGTATCGATAAGCTTCGACGAAGAAGGACAAGG	Amplify the *PA2807* promoter to construct P*PA2807-lux* reporter
lux-P*PA2807*-R	TCTAAAGAAGAATTGGGGATCCTACATCTTTGTCAGCTTGCCG	
lux-P*pchD*-F	GGTCGACGGTATCGATAAGCTTCAGGTTTTCCTGTAGCCCGG	Amplify the *pchD* promoter to construct P*pchD-lux* reporter
lux-P*pchD*-R	TCTAAAGAAGAATTGGGGATCCGCGATCTCCGTGGATGCGGT	
lux-P*pchE*-F	GGTCGACGGTATCGATAAGCTTCCGCTGAGTCTCCGCG	Amplify the *pchE* promoter to construct P*pchE-lux* reporter
lux-P*pchE*-R	TCTAAAGAAGAATTGGGGATCCGGGGGCTCCCTAGGGC	
lux-P*pchR*-F	GGTCGACGGTATCGATAAGCTTATTGTTGGGAAATGAGATTT	Amplify the *pchR* promoter to construct P*pchR-lux* reporter
lux-P*pchR*-R	TCTAAAGAAGAATTGGGGATCCCAGGTTTTCCTGTAGCCC	
lux-P*pvdR*-F	GGTCGACGGTATCGATAAGCTTGTGGACCTGGAACATCGCGA	Amplify the *pvdR* promoter to construct P*pvdR-lux* reporter
lux-P*pvdR*-R	TCTAAAGAAGAATTGGGGATCCCGTCTCATTCGGGAATCCGG	
CTX-*lux*-veri-F	CGCGCGTAATACGACTCACTA	Verify the construction of promoter-*lux* reporter
CTX-*lux*-veri-R	GCAATCTAATTTTTACCGGCAG	
P*pchR*-F	TCAGGTTTTCCTGTAGCCCGG	For EMSA assay
P*pchR*-R	GGAAGTCATGCGATCTCCGT	

P*pvdR*-F	CATCCGCAGCCTGGT	For EMSA assay
P*pvdR*-R	GGTTCGTCTCATTCGGGA	
P*czcC*-F	GTTCCGCTCCTCGTCT	For EMSA assay
P*czcC*-R	CGTGAGGGGCAATGCC	
P*ptrA*-F	GGTACGCATGGGAAGTCTCC	For EMSA assay
P*ptrA*-R	GAAGCCTGCCTCGGCG	
P*PA2807*-F	CGGGAGCATTACATCTTTGT	For EMSA assay
P*PA2807*-R	CGACGAAGAAGGACAAGGAA	
P*czcC*(in)-F	CCAGGGAGGTCTTCGCCA	For EMSA assay
P*czcC*(in)-R	CCAGACCAGCGTCAGCAT	
pBBR-*czcR*-F	GGTCGACGGTATCGATAAGCTTATGCGCATCCTTATTATC	Construct pBBR1-*czcR*
pBBR-*czcR*-R	GCCGCTCTAGAACTAGTGGATCCTCATCGGCGCGCTTCCAG	
pBBR-*ptrA*-F	GGTCGACGGTATCGATAAGCTTATGCGTACCTTCACTG	Construct pBBR1-*ptrA*
pBBR-*ptrA*-R	GCCGCTCTAGAACTAGTGGATCCTCAGCAGTTTTCCTTGT	
pBBR-*PA2807*-F	GAGGTCGACGGTATCGATAAGCTTATGCTCCCGACAGCCA	Construct pBBR1-*PA2807*
pBBR-*PA2807*-R	GCCGCTCTAGAACTAGTGGATCCTCAGGGCTGCACGGTC	
pBBR-*ptrA-PA2807*-F	AGGTCGACGGTATCGATAAGCTTATGCGTACCTTCACTGC	Construct pBBR1-*ptrA-PA2807*
pBBR-*ptrA-PA2807*-R	GGCCGCTCTAGAACTAGTGGATCCTCAGGGCTGCACGGTC	
pBBR-*pchR*-F	AGGTCGACGGTATCGATAAGCTTATGACCATCACCATCA	Construct pBBR1-*pchR*
pBBR-*pchR*-R	CCGCTCTAGAACTAGTGGATCCTCAGCGGATCTCGCTG	
pBBR-veri-F	TACGCAAACCGCCTCTCCCC	Verify the construction of plasmids for *in trans* expression
pBBR-veri-R	GCTGCGCAACTGTTGGGAAG	
pET28a-*czcR*-F	ACAGCAAATGGGTCGCGGATCCATGCGCATCCTTATTATCGAAG	Construct the plasmid pET28a-*czcR* for CzcR expression
pET28a-*czcR*-R	GGTGGTGGTGGTGGTGCTCGAGTCATCGGCGCGCTTCC	
pET28a-veri-F	CGAAGCAGCGCAACGATAT	Verify the construction of pET28a-*czcR*
pET28a-veri-R	TTCCAGTGCGCCATCGC	
czcB-RT-F	GCGCAGAGCACCTACAA	qPCR
czcB-RT-R	GATCTCGGCTTCCTGCAAA	
ptrA-RT-F	CATCGTCTTCGAGCGCAT	qPCR
ptrA-RT-R	TTGTCCTTCTTCGTCGCTTC	
PA2807-RT-F	GGCGATATGTACTTCAAGCCT	qPCR
PA2807-RT-R	CATCTCCAGCATCTCCTTCTG	
pcoA-RT-F	ACACCTATACCTACCTGCTCAA	qPCR
pcoA-RT-R	GGAATGCGGACGTCGAAATA	
pcoB-RT-F	TGAACAGCTTCTTCCTGCTC	qPCR
pcoB-RT-R	CCACAGGCGGTTGATGT	
copA1-RT-F	GTTCTGGGCCTTCATCTACAA	qPCR
copA1-RT-R	CGCTGACGCTGGAGAAG	
copA2-RT-F	CTGATGATCGAAGGCATCAGTT	qPCR
copA2-RT-R	CGATGGTTGGACAGGTTGAG	
czcR-UF	CCCTTTCGTCTTCACAGTATCGGCATGTTCCGCTC	Amplify upstream and downstream donor sequences for *czcR* deletion
czcR-UR	GACAGTCGGCCTGGTGGCGGTTCGCCCCTATATAAAGTA	
czcR-DF	TACTTTATATAGGGGCGAACCGCCACCAGGCCGACTGTC	
czcR-DR	GGATCAGGAATACCCCAATGGCAGGAGGAAGGGCAG	
pchR-UF	CCCTTTCGTCTTCACGCAGGTCTCGACGAAGGCGA	Amplify upstream and downstream donor sequences for *pchR* deletion
pchR-UR	TCGGGGGTCGTCGCGGAGACCAGGTTTTCCTGTAGCCCGG	
pchR-DF	CCGGGCTACAGGAAAACCTGGTCTCCGCGACGACCCCCGA	
pchR-DR	GGATCAGGAATACCCCGCACGGAAGACAGCTCGAAC	

czcR-T	AGGGCCTGACCGAAAGCGGCTACATCGTCGAC	Spacer sequence for *czcR* deletion
pchR-T	GAACATGAAGCTGGTGACCGGAACCTTCTGTT	Spacer sequence for *pchR* deletion