Zinc excess increases cellular demand for iron and decreases tolerance to copper in *Escherichia coli*

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SUPPORTING INFORMATION:

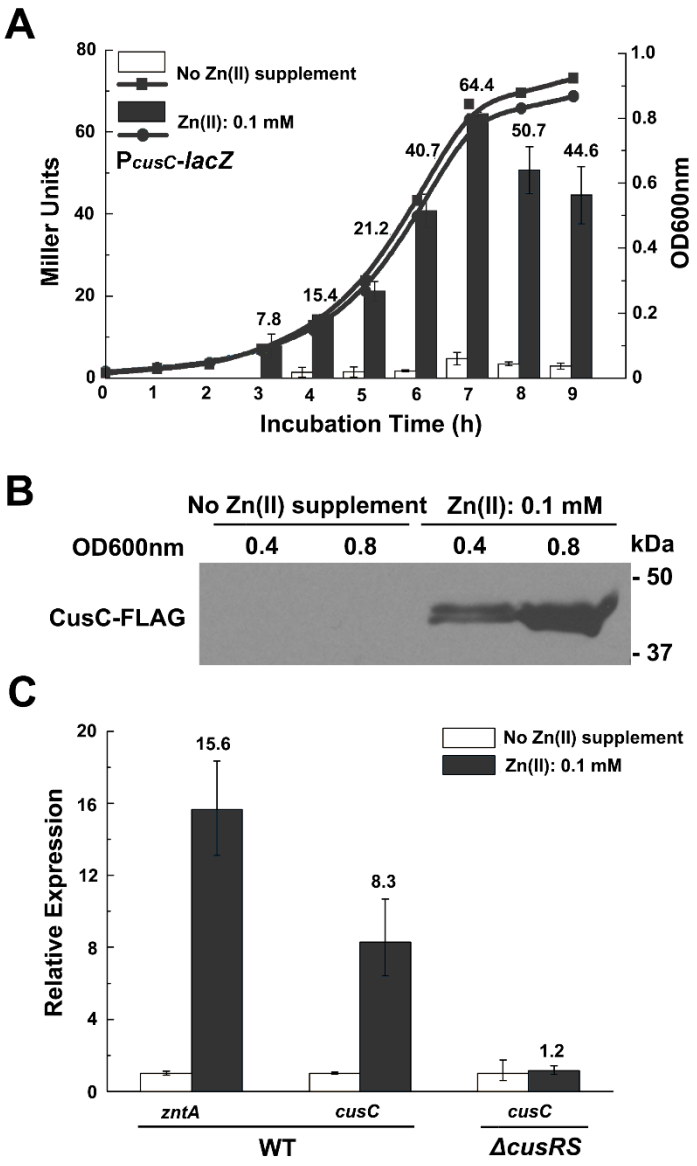

Figure S1. *cusCFBA* system is induced in a CusRS dependent manner during Zn excess. (A) Induction of P_{cusC}-*lacZ* during the growth of *E. coli* cells with Zn excess. **(B)** Western blot analysis to detect the production of chromosomal FLAG-tagged CusC protein during Zn excess at OD_{600} value of 0.4 and 0.8. **(C)** Transcripts of *cusC* in wild-type *E. coli* and Δ*cusRS* mutant during Zn excess measured by RT-qPCR. Expression of *zntA* served as the positive control.

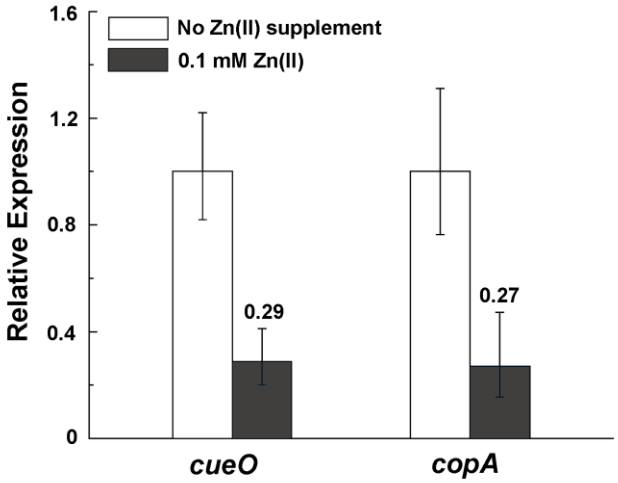

Figure S2. RT-qPCR analysis of the transcripts of *cueO* and *copA* during Zn excess in *E. coli* cells.

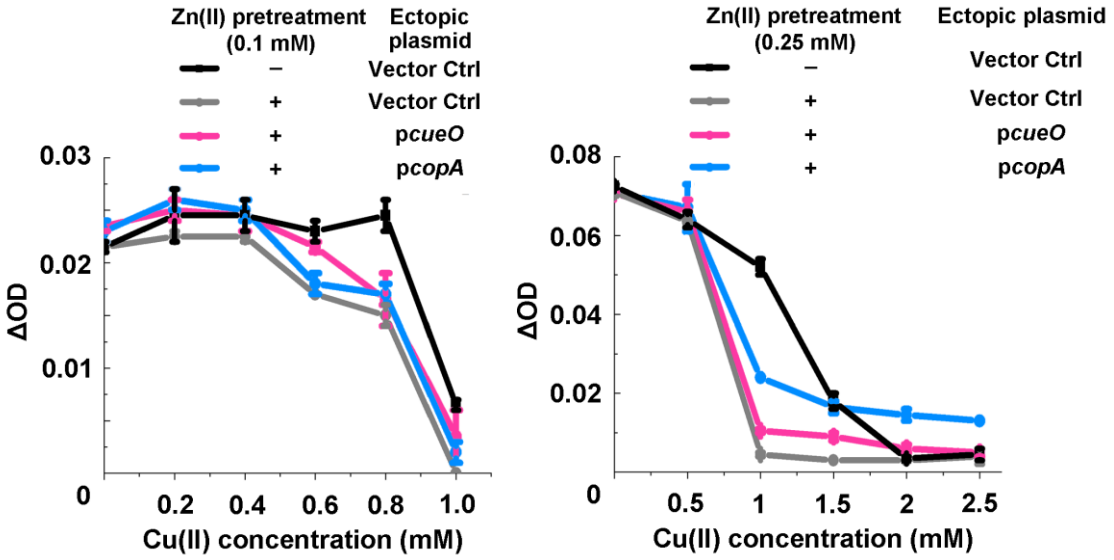

Figure S3. Copper sensitivity of wild-type *E. coli* strain with pET11a vector (Vector Ctrl), or *E. coli* MG1655 with ectopic over-expression of *cueO* (pET11a-*cueO*, p*cueO*) or *copA* (pET11a-*copA*, p*copA*) in the presence of 0.1 mM or 0.25 mM Zn pretreatment.

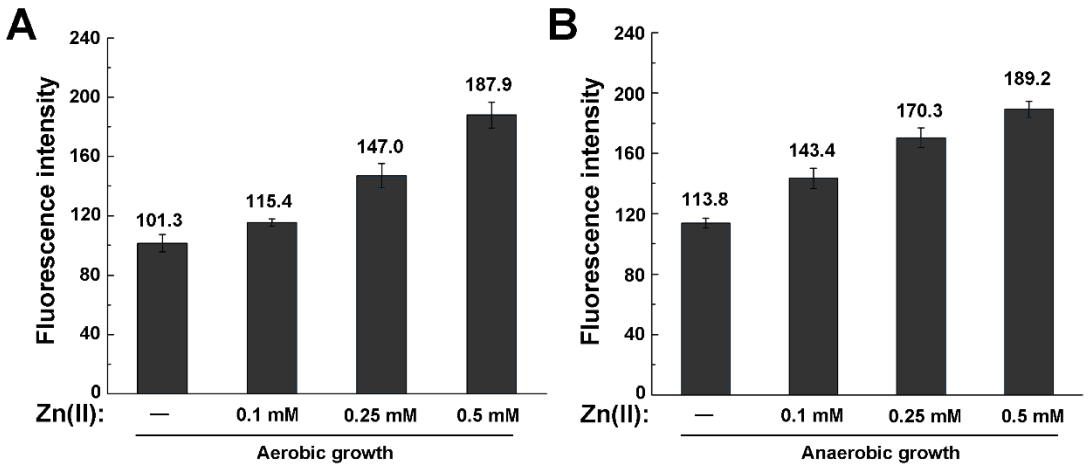

Figure S4. Intracellular Fe(II) content measured using the probe HMRhoNox-M during Zn excess under aerobic **(A)** and anaerobic **(B)** conditions.

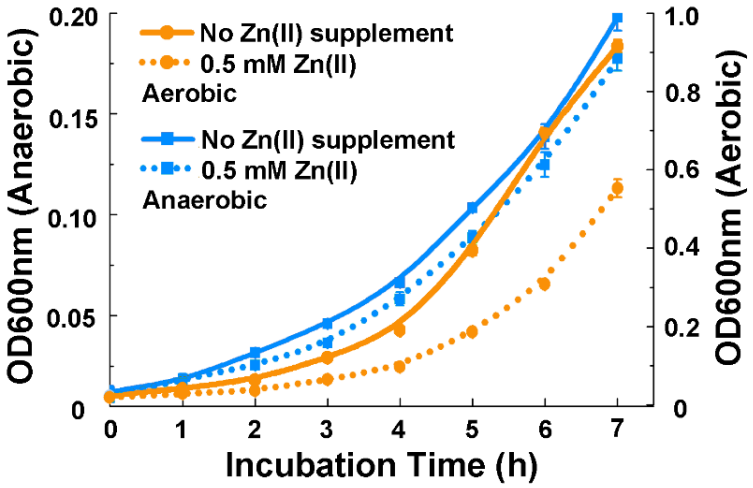

Figure S5. Growth of *E. coli* cells in the presence or absence of Zn excess (0.5 mM) under aerobic or anaerobic conditions. Error bars represent standard deviations.

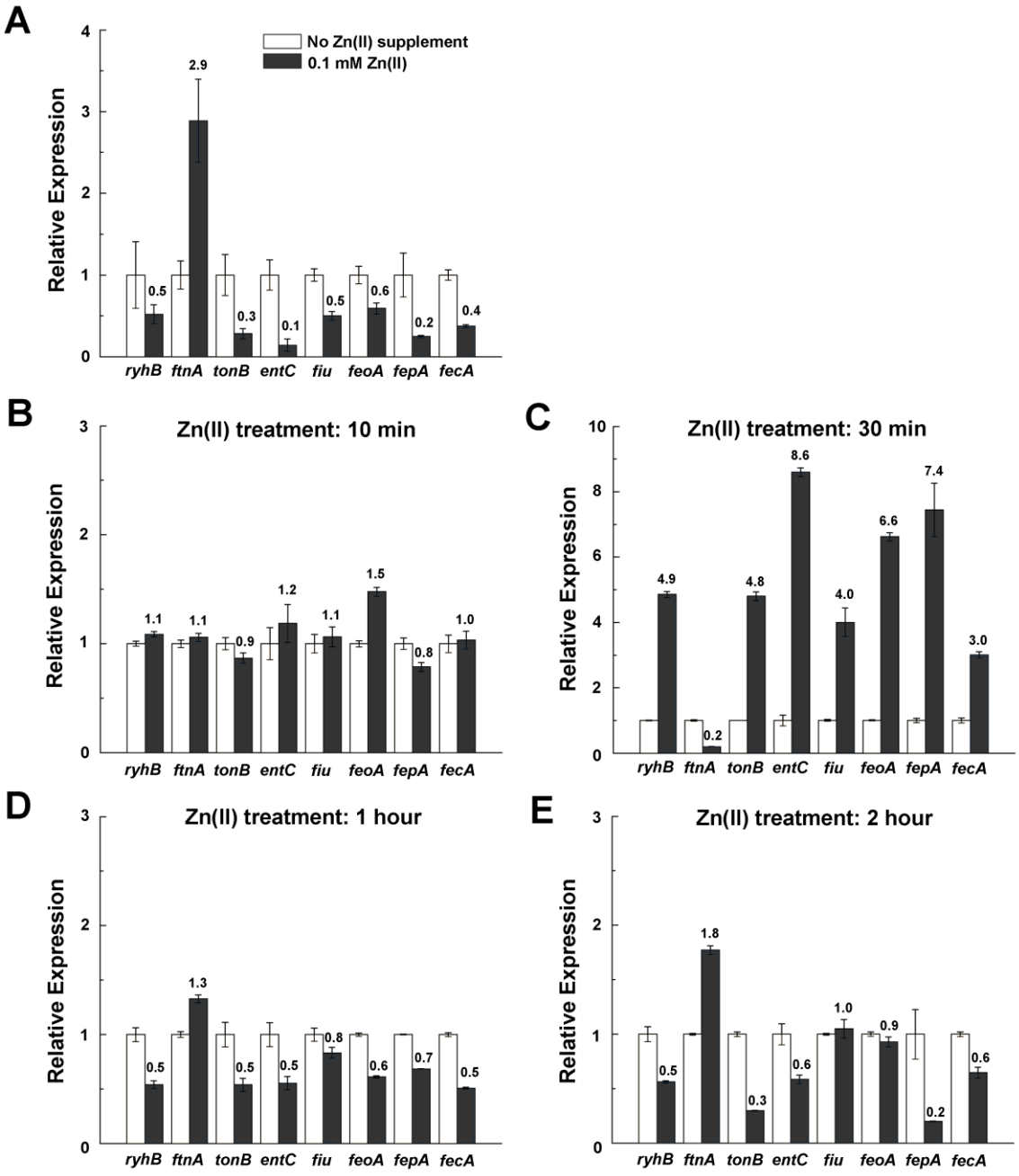

Figure S6. Expression of Fur regulon genes involved in Fe uptake (*tonB*, *entC*, *fiu*, *feoA*, *fepA*, *fecA*) and storage (*ftnA*) under Zn excess condition and during the adaption to Zn stress. **(A)** Cells were inoculated in Zn excess condition and grown to OD_{600} of 0.4. **(B-E)** Cells were grown to OD_{600} of 0.3 under non-Zn stressed condition and were subject to Zn stress for 10 min, 30 min, 1 hour, and 2 hour, respectively.

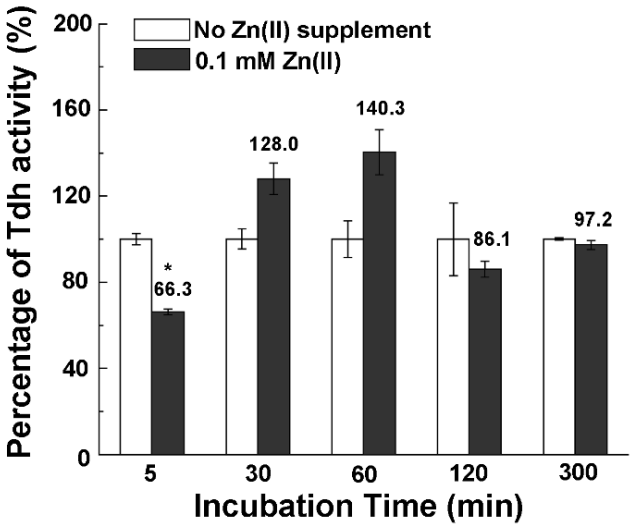

Figure S7. Activity of Tdh in *E. coli* cells at different time points following the switch to Zn excess condition. *, $P<0.05$; versus no Zn treatment (Based on student's *t* test).

Table S1. Bacterial strains used in this study

Strain No.	Genotype/plasmid	Source
MG1655	*E. coli* F^- λ^- *ilvG rfb-50 rph-1*	Lab collection
AY1026	MG1655 *cusC*-FLAG::Kn^R	Lab collection
AY1036	MG1655 Δ*cusRS*::CM *cusC*-FLAG::Kn^R	Lab collection
AY1038	MG1655 Δ*cusRS*::Cm^R	Lab collection
AY1040	MG1655 Δ*cusC*::Kn^R	Lab collection
AY1047	MG1655 Δ*cusCFBA*::Kn^R	Lab collection
AY1051	MG1655 Δ*copA* Δ*cueO*::Kn^R	Lab collection
AY1005	MG1655 Δ*lacZ*::Kn^R, P_{cusC}-lacZ	Lab collection
AY1089	MG1655 Δ*cusRS* ΔlacZ:Kn^R, P_{cusC}-lacZ	Lab collection
AY1883	MG1655 Δ*lacZ*::Kn^R, P_{copA}-lacZ	Lab collection
AY3616	MG1655 Δ*lacZ*::Kn^R, P_{cueO}-lacZ	This study
AY3619	*E. coli* BL21, pET28a-His_6-CueR	This study
AY3660	*E. coli* BL21, pET28a-CueR-F58TAG and PEvol-CouRS	This study
UTI-1	uropathogenic *E. coli* clinical isolate	Queen Mary Hospital (HONG KONG)

Table S2. Primers (for RT-qPCR) used in this study

zntA-F	GAATCAACATGCGCCGCTGG
zntA-R	TCTGTGCCGCTACCCATTGC
cusC-F	CTATCAGAACGCGGGCTGGC
cusC-R	GCACTTTCAGCGTCGCCATG
cueO-F	TGGGCTGGTTTGGCGATACG
cueO-R	GCCATTGAGCAAACGCAGGC
copA-F	TGCGTAGTGATAGCGTGGCG
copA-R	TGGCGGTGGTTGGGTTATCC
ryhB-F	CCCTCGCGGAGAACCTGAAAG
ryhB-R	CCCGGCTGGCTAAGTAATACTGG
ftnA-F	CGCCCAGGAAGAGATGACGC
ftnA-R	TCAGCAAACGGAGATTCAACGG
tonB-F	TCATGGTGCTGTTGTGGCGG
tonB-R	TGTGGCGGTTCGAGATCAGC
entC-F	GACTCTGGCCTGTCTGCTGC
entC-R	CCCACAATGCCGCCAAACAG
fiu-F	GGCGCTGTATCACCTGACGG
fiu-R	GTTGGCACTGTTACCGCTGC
feoA-F	TCTTGGCATGTTACCTGGCTCC
feoA-R	AGGCTCACACGACGGGTTTC
fepA-F	CAACCAGGGCCATCAGTCCG
fepA-R	CAATGGCGCGAAATCCCAGC
fecA-F	CCAGTTCCAGCCAGACAGCC
fecA-R	CCCAGTAGTTACGCGGCGAG