Supporting Information (SI)

Cu(I)-mediated allosteric switching in a copper-sensing operon repressor (CsoR)

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Table S1. Biochemically and structurally characterized DUF156 (CsoR/RcnR) proteins used to interpret the multiple sequence alignment and the phylogenetic tree shown in **Fig. 1** (main text). The CsoR structurally characterized here is highlighted in red.

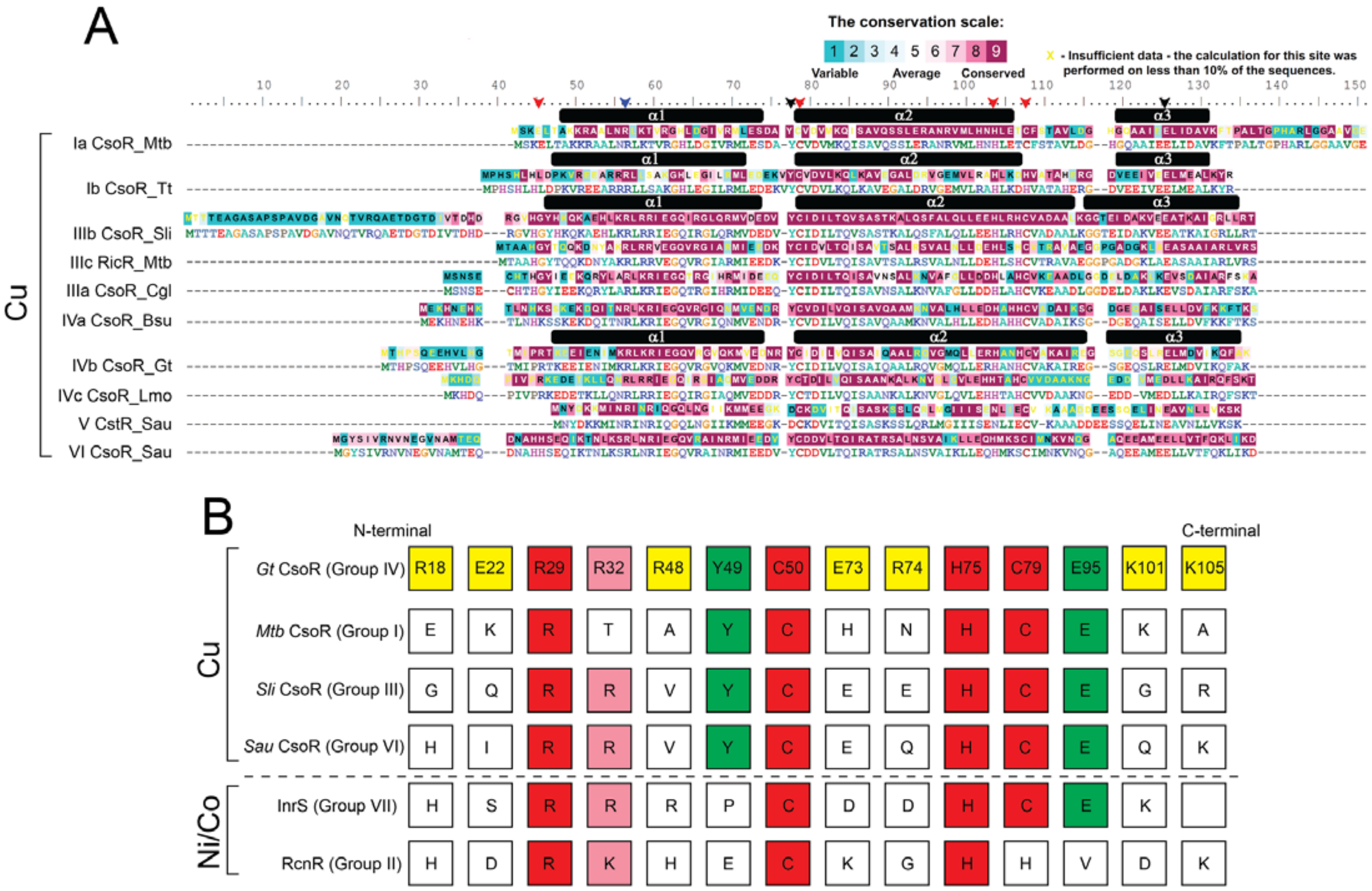
Protein ID	Clade ID	UniProt number	Organism	Locus tag	Inducer(s)	Structure of apo/holo-form	PDB ID	References
CsoR_Mtb	Ia	P71543	*Mycobacterium tuberculosis*	Rv0967	Cu(I)	Homotetramer	2HH7	(1)
CsoR_Tt	Ib	Q5SHL1	*Thermus thermophilus*	TTHA1719	Cu(I)		3AAI	(2)
NcrB_Lf	Ic	Q06VT2	*Leptospirillum ferriphilum*	ncrB	Ni(II)/Co(II)			(3)
RcnR	IIa	P64530	*Escherichia coli*	yohL	Ni(II)/Co(II)	Homotetramer		(4,5)
FrmR	IIb	P0AAP3	*Escherichia coli*	yaiN	Formaldehyde			(6)
CsoR_Cg	IIIa	A4QB25	*Corynebacterium glutamicum*	cgR_0458	Cu(I)			(7)
CsoR_Sli	IIIb	Q9KZW5	*Streptomyces lividans*	SCO4136	Cu(I)	Homotetramer	4ADZ	(8)
RicR_Mtb	IIIc	O07434	*Mycobacterium tuberculosis*	MT0200	Cu(I)	Homotetramer[a]		(9)
CsoR_Bsu	IVa	O32222	*Bacillus subtilis*	BSU33520	Cu(I)	Homotetramer		(10,11)
CsoR_Gt	IVb	A4INJ9	*Geobacillus thermodenitrificans*	GTNG_1533	Cu(I)	Homotetramer	4M1P	This work
CsoR_Lmo	IVc	Q8Y646	*Listeria monocytogenes*	Lmo1854	Cu(I)			(12)
CstR_Sau	V		*Staphylococcus aureus*	NWMN_26.5	HS^-/SO_3^{-2}			(13)
CsoR_Sau	VI	A6QIT1	*Staphylococcus aureus*	NWMN_1991	Cu(I)			(13)
InrS_Syn	VII	Q55554	*Synechocystis* PCC 6803	sll0176	Ni(II)	Homotetramer		(14)

[a] B. Kester, F. Chang, D. Giedroc, unpublished data.

Table S2. UniProt numbers for all nonredundant 227 DUF156 sequences used to infer the phylogenetic analysis (see **Fig. 1**, main text). Representative proteins for each subgroup are highlighted in the *red* text while *Gt* CsoR is highlighted in *green*.

Clade ID	UniProt number	Organism
Ia	B2HRV7	*Mycobacterium marinum*
	D5P3A4	*Mycobacterium parascrofulaceum*
	G0TG78	*Mycobacterium canettii*
	P71543	***Mycobacterium tuberculosis***
	D6FPL0	*Mycobacterium tuberculosis*
	E2UJB5	*Mycobacterium tuberculosis*
	F4CKQ7	*Pseudonocardia dioxanivorans*
	B2HH23	*Mycobacterium marinum*
	A0PLB8	*Mycobacterium ulcerans*
Ib	Q7NE35	*Gloeobacter violaceus*
	F0RPY0	*Deinococcus proteolyticus*
	H8GUG6	*Deinococcus gobiensis*
	Q1J3E5	*Deinococcus geothermalis*
	C1D2I3	*Deinococcus deserti*
	D7CRA7	*Truepera radiovictrix*
	H1NWI8	*Holophaga foetida*
	E4UA60	*Oceanithermus profundus*
	D3PLW2	*Meiothermus ruber*
	D7BGS1	*Meiothermus silvanus*
	F2NR74	*Marinithermus hydrothermalis*
	G8NB26	*Thermus*
	E8PKY0	*Thermus scotoductus*
	H7GHY2	*Thermus sp. RL*
	Q5SHL1	***Thermus thermophilus***
	H5SN98	*uncultured Thermus/Deinococcus group bacterium*
	B7A606	*Thermus aquaticus*
Ic	Q88IN0	*Pseudomonas putida*
	Q44590	*Alcaligenes xylosoxydans xylosoxydans*
	A1WLJ0	*Verminephrobacter eiseniae*
	C7RW62	*Accumulibacter phosphatis*
	Q0F1N3	*Mariprofundus ferrooxydans*
	B9Z0S0	*Pseudogulbenkiania ferrooxidans*
	G0C7G9	*Serratia*
	D4BYS5	*Providencia rettgeri*
	H3MEZ5	*Klebsiella oxytoca*
	Q1KLR1	*Serratia marcescens*
	H3MVD2	*Klebsiella oxytoca*
	Q06VT2	***Leptospirillum ferriphilum***
	Q6RUG2	*Klebsiella oxytoca*
	Q93M45	*Legionella pneumophila*
	Q12AN4	*Polaromonas sp.*
	H0HUQ8	*Mesorhizobium alhagi*
	G9AHM6	*Rhizobium fredii*
	Q2K0K6	*Rhizobium etli*
	B3PWC0	*Rhizobium etli*
IIa	A9MRK3	*Salmonella arizonae*
	D2TJK2	*Citrobacter rodentium*
	G9Z4D7	*Yokenella regensburgei*
	C1MB27	*Citrobacter sp.*
	F7MY94	*Escherichia coli*
	E7T4V7	*Shigella boydii*
	B1EK43	*Escherichia albertii*
	H5QVT8	*Escherichia coli*
	H5NB78	*Escherichia coli*
	E3PCP9	*Escherichia coli*
	F4VH74	*Escherichia coli*
	C0ST98	*Escherichia coli*
	H4VJ75	*Escherichia coli*
	E2X0R0	*Escherichia coli*
	P64530	***Escherichia coli***
	G1YBA7	*Escherichia coli*
IIb	B5JUQ3	*gamma proteobacterium*
	D8A2B2	*Escherichia coli*
	F4VAD6	*Escherichia coli*
	D7ZJL9	*Escherichia coli*
	H5H2W4	*Escherichia coli*
	P0AAP3	***Escherichia coli***
	H4ZQC4	*Escherichia coli*
	I0QLA2	*Serratia sp.*
	H5VPY7	*Salmonella enterica*
	Q8KKB0	*Proteus vulgaris*
	D1P3L2	*Providencia rustigianii*
	G7LSK1	*Brenneria sp.*
	B2SZZ0	*Burkholderia phytofirmans*
	Q1IAA5	*Pseudomonas entomophila*
	B9BFA7	*Burkholderia multivorans*
	F0DZ53	*Pseudomonas sp.*
	Q8UGZ5	*Agrobacterium tumefaciens*
IIIa	G0HDW3	*Corynebacterium variabile*
	G8RI37	*Mycobacterium rhodesiae*
	C7MDY5	*Brachybacterium faecium*
	G7GSB0	*Gordonia amarae*
	E2SCC2	*Aeromicrobium marinum*
	A1RCP4	*Arthrobacter aurescens*
	D2PLJ6	*Kribbella flavida*
	H5U7Y6	*Gordonia terrae*
	F9VS64	*Gordonia alkanivorans*
	D0LEV5	*Gordonia bronchialis*
	E6J497	*Dietzia cinnamea*
	C2GID9	*Corynebacterium glucuronolyticum*
	C0XQS9	*Corynebacterium lipophiloflavum*
	A4QB25	***Corynebacterium glutamicum***
	Q8FSI5	*Corynebacterium efficiens*
IIIb	F3NEI7	*Streptomyces griseoaurantiacus*
	D5ZS95	*Streptomyces ghanaensis*
	D9XRU9	*Streptomyces griseoflavus*
	D9WXW3	*Streptomyces viridochromogenes*
	D6B9L0	*Streptomyces albus*
	B5HLZ2	*Streptomyces sviceus*
	G2NCY4	*Streptomyces sp.*
	G0PV66	*Streptomyces griseus*
	D6APN9	*Streptomyces roseosporus*
	Q82G20	*Streptomyces avermitilis*
	G2GAM7	*Streptomyces zinciresistens*
	C9ZFK1	*Streptomyces scabies*
	H1QSR5	*Streptomyces coelicoflavus*
	Q9KZW5	***Streptomyces lividans***
	D6EK73	*Streptomyces lividans*
IIIc	B8ZTH5	*Mycobacterium leprae*
	D5P6F4	*Mycobacterium parascrofulaceum*
	I0RZC1	*Mycobacterium phlei*
	I0RNH6	*Mycobacterium xenopi*
	O07434	***Mycobacterium tuberculosis***
	D5YB34	*Mycobacterium tuberculosis*
	H6S5C9	*Mycobacterium tuberculosis*

	H8JM13	*Mycobacterium intracellulare*
	B2HMW1	*Mycobacterium marinum*
	F9QKS4	*Mycobacterium colombiense*
	F7P7F4	*Mycobacterium avium*
	D5PAQ8	*Mycobacterium parascrofulaceum*
	A1U967	*Mycobacterium sp.*
	A3PSS4	*Mycobacterium sp.*
	G4HYH3	*Mycobacterium rhodesiae*
	H1JWI1	*Mycobacterium tusciae*
IVa	I0UKK4	*Bacillus licheniformis*
	F4EN13	*Bacillus amyloliquefaciens*
	A7Z8S5	*Bacillus amyloliquefaciens*
	E1UQL4	*Bacillus amyloliquefaciens*
	E3DV80	*Bacillus atrophaeus*
	D5MXP8	*Bacillus subtilis*
	O32222	***Bacillus subtilis***
	D4G1E3	*Bacillus subtilis*
	G4P0R1	*Bacillus subtilis*
IVb	C3AZB3	*Bacillus mycoides*
	C3BFU7	*Bacillus pseudomycoides*
	D5TZD1	*Bacillus thuringiensis*
	A4INJ9	***Geobacillus thermodenitrificans***
	I0U905	*Geobacillus thermoglucosidans*
	F8D055	*Geobacillus thermoglucosidasius*
	C5D2A2	*Geobacillus sp.*
	G9QHY1	*Bacillus smithii*
	A4INS6	*Geobacillus thermodenitrificans*
	C5D228	*Geobacillus sp.*
	D7D180	*Geobacillus sp.*
	G8MY26	*Geobacillus thermoleovorans*
	B7GJE1	*Anoxybacillus flavithermus*
IVc	D7UY58	*Listeria grayi*
	Q4EH65	*Listeria monocytogenes*
	H1G8K0	*Listeria innocua*
	E4A0I5	*Listeria seeligeri*
	D3UPF2	*Listeria seeligeri*
	E3ZHR5	*Listeria ivanovii*
	Q92AF4	*Listeria innocua serovar 6a*
	D3KLX9	*Listeria monocytogenes*
	E3Z8U6	*Listeria innocua*
	D4PPT3	*Listeria monocytogenes*
	Q8Y646	***Listeria monocytogenes***
	C8K240	*Listeria monocytogenes*
V	B1YM25	*Exiguobacterium sibiricum*
	F0P9Q3	*Staphylococcus pseudintermedius*
	Q8VUY1	*Staphylococcus hominis*
	H3V9C4	*Staphylococcus epidermidis*
	D2N3A3	*Staphylococcus aureus*
	I0TVN3	*Staphylococcus epidermidis*
	F3TW60	*Staphylococcus epidermidis*
	C5QR26	*Staphylococcus caprae*
	C8L5G8	*Staphylococcus aureus*
	D9N347	*Staphylococcus fleurettii*
	Q0ZKM1	*Staphylococcus sp.*
	D1R191	*Staphylococcus aureus*
	C8LCP6	*Staphylococcus aureus*
	H3XIT8	*Staphylococcus aureus*
	H0CST2	*Staphylococcus aureus*
	CstR_Sau	***Staphylococcus aureus***
VI	F3SRX6	*Staphylococcus warneri*
	C4WBP1	*Staphylococcus warneri*
	G5JKX9	*Staphylococcus simiae*
	C8MC25	*Staphylococcus aureus*
	D2G328	*Staphylococcus aureus*
	H7G5D5	*Staphylococcus aureus*
	E1E205	*Staphylococcus aureus*
	C5N2P9	*Staphylococcus aureus*
	E0P3L8	*Staphylococcus aureus*
	D6LZU9	*Staphylococcus aureus*
	A6QIT1	***Staphylococcus aureus***
	C8MRJ4	*Staphylococcus aureus*
	H3YH46	*Staphylococcus aureus*
	Q2YUG7	*Staphylococcus aureus*
	F0DDT2	*Staphylococcus aureus*
	F0D6X0	*Staphylococcus aureus*
VII	**Q55554**	***Synechocystis sp.***
	F7URS5	*Synechocystis sp.*
	Q8DIK8	*Thermosynechococcus elongatus*
	B7KLC9	*Cyanothece sp.*
	Q8YTG1	*Nostoc sp.*
	B4VKE0	*Coleofasciculus chthonoplastes*
	B0JIQ2	*Microcystis aeruginosa*
	B1WW97	*Cyanothece sp.*
	Q4C3T0	*Crocosphaera watsonii*
	C7QUT0	*Cyanothece sp.*
	E0UGY4	*Cyanothece sp.*
	F5UE85	*Microcoleus vaginatus*
	D8G9T7	*Oscillatoria sp.*
	D5A592	*Arthrospira platensis*
	B5VUT3	*Arthrospira maxima*
	H1W7C2	*Arthrospira sp.*
unassigned	C0GHC4	*Dethiobacter alkaliphilus*
	F8IBQ9	*Sulfobacillus acidophilus*
	H5SUX9	*uncultured candidate division OP1 bacterium*
	I0I1Y4	*Caldilinea aerophila*
	F7Q2B9	*Haloplasma contractile*
	C2QFM2	*Bacillus cereus*
	C3DNB1	*Bacillus thuringiensis*
	C2XF89	*Bacillus cereus*
	B9IV31	*Bacillus cereus*
	H0NR67	*Bacillus cereus*
	C2YDY1	*Bacillus cereus*
	C2U1D4	*Bacillus cereus*
	E3PTG5	*Clostridium sticklandii*
	I0JMB3	*Halobacillus halophilus*
	E5WPT7	*Bacillus sp.*
	Q8UGZ5	*Agrobacterium tumefaciens*



Gt CsoR (Group IV)	R18	E22	R29	R32	R48	Y49	C50	E73	R74	H75	C79	E95	K101	K105
Mtb CsoR (Group I)	E	K	R	T	A	Y	C	H	N	H	C	E	K	A
Sli CsoR (Group III)	G	Q	R	R	V	Y	C	E	E	H	C	E	G	R
Sau CsoR (Group VI)	H	I	R	R	V	Y	C	E	Q	H	C	E	Q	K
InrS (Group VII)	H	S	R	R	R	P	C	D	D	H	C	E	K	
RcnR (Group II)	H	D	R	K	H	E	C	K	G	H	H	V	D	K

Fig. S1. (*A*) ConSurf analysis of full-length sequences for Cu(I)-sensing CsoR groups. The A-[W-X-Y-Z]-B fingerprints (15) are indicated by *red* (W-Y-Y-Z) and *black* (A,B) arrows (top of the figure), the R residue conserved along all DUF156 proteins (R29 in *Gt* CsoR) is indicated by a *blue* arrow. Secondary structures of CsoRs with defined crystal structure are labeled in black (see Table S1 for pdb codes). (*B*) Schematic representation of the residue positions that are conserved across all Cu(I)-sensing CsoRs (shaded *red*) vs. group IV clade-specific residue positions (shaded *yellow*, *Gt* CsoR residue number indicated). Those residue positions shaded *pink* are conserved in three of four Cu(I)-containing CsoR groups. Cu(I) second coordination shell ligands (A and B in the fingerprint) are shaded *green* (16).

Supplementary References

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