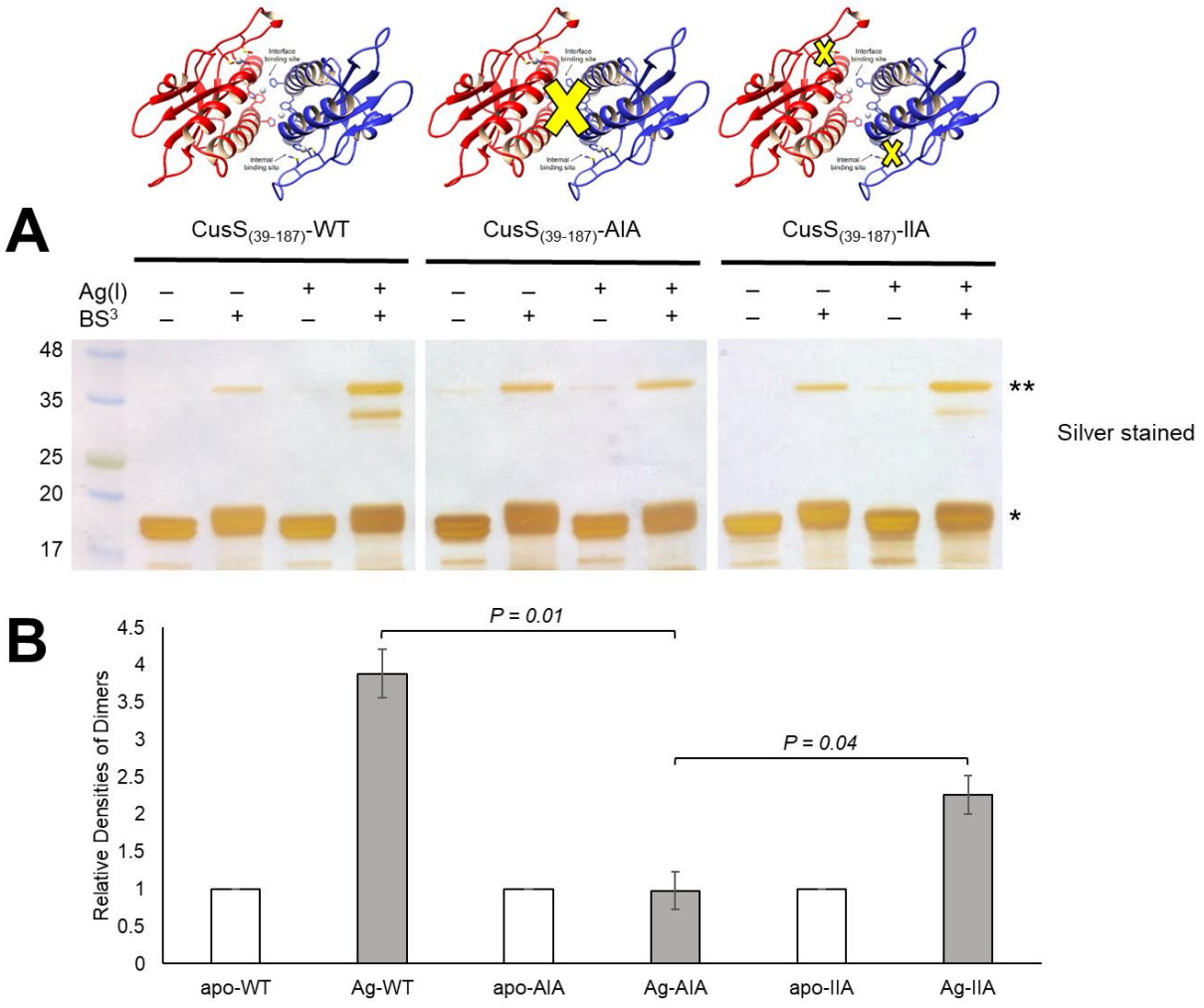
Supplemental Table S1. Plasmids used in this study

Plasmid	Genotype	Source/Reference
pTXB3	pBR322 derivative, *amp*^{R}	NEB
pcusS$_{(39\text{-}187)}$	pTXB3/*cusS*$_{(39\text{-}187)}$	Previous work (20)
pStrep-cusS$_{(39\text{-}187)}$*-WT*	pTXB3/*cusS*$_{(39\text{-}187)}$ with N-terminal Strep tag and S192A	This work
pStrep-cusS$_{(39\text{-}187)}$*-AIA*	*pStrep-cusS*$_{(39\text{-}187)}$*-WT* + H42A/F43I/H176A	This work
pStrep-cusS$_{(39\text{-}187)}$*-IIA*	*pStrep-cusS*$_{(39\text{-}187)}$*-WT* + M133I/M135I/H145A	This work
pET21b(+)	pBR322 ori, *lac* coding sequence, *amp*^{R}	Novagen
pcusS-WT	pET21b(+)/*cusS* with C-terminal 6xHis tag	Previous work (22)
pcusS-H271A	pET21b(+)/*cusS-H271A*	This work
pcusS-AIA	pET21b(+)/*cusS-H42A/F43I/H176A*	Previous work (22)
pcusS-IIA	pET21b(+)/*cusS-M133I/M135I/H145A*	Previous work (22)
pcusS$_{cp}$*-short*	pET21b(+)/*cusS*$_{cp}$*-6xHis*	This work
pcusS$_{cp}$*-short-H271A*	pET21b(+)/*cusS*$_{cp}$*-H271A*	This work
pcusS$_{cp}$*-short-AA*	pET21b(+)/*cusS*$_{cp}$*-N386A/N414A*	This work
pcusS$_{cp}$*-short-AAA*	pET21b(+)/*cusS*$_{cp}$*-H271A/N386A/N414A*	This work
pET28b(+)	pBR322 ori, *lac* coding sequence, *kan*^{R}	Bio Basic
pcusS$_{cp}$*-long*	pET28b(+)/*cusScp-Strep-Myc-Strep-FLAG-HA-Strep*	This work
pcusS$_{cp}$*-long-H271A*	pET28b(+)/*cusS*$_{cp}$*-H271A*	This work
pcusS$_{cp}$*-long-AA*	pET28b(+)/*cusS*$_{cp}$*-N386A/N414A*	This work
pcusS$_{cp}$*-long-AAA*	pET28b(+)/*cusS*$_{cp}$*-H271A/N386A/N414A*	This work
pET22b(+)	pBR322 ori, *lac* coding sequence, *amp*^{R}	Novagen
pcusR	pET22b(+)/*cusR-6xHis*	This work
pcusR-D51A	pET22b(+)/*cusR-D51A-6xHis*	This work

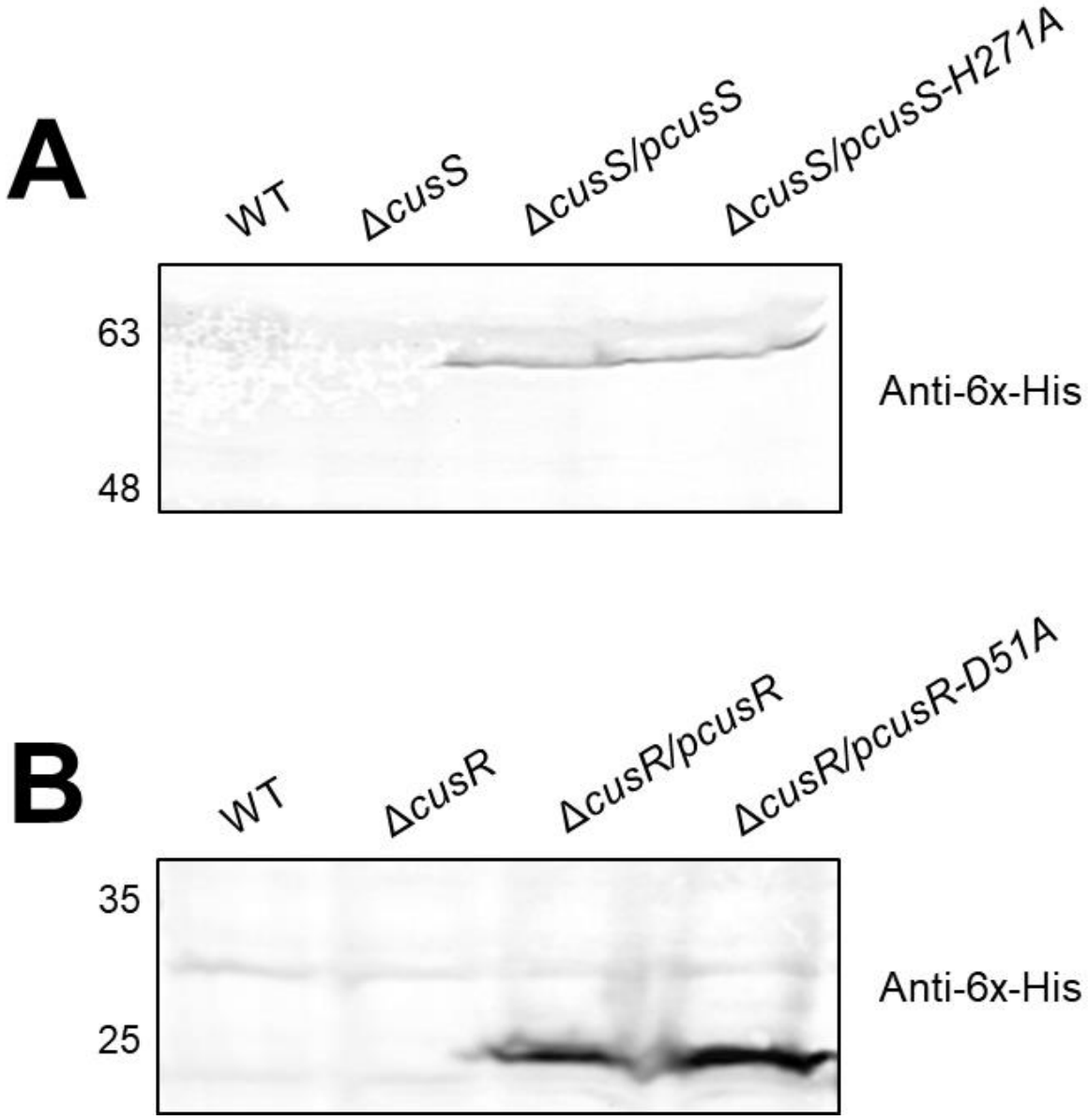
Supplemental Table S2. Bacterial strains used for *in vivo* complementation assay

	Genotype	Source/Reference
Strain		
BW25113	*lacI*^{q}*,rrnB*$_{T14}$*, ΔlacZ*$_{WJ16}$*, hsdR514, ΔaraBAD*$_{AH33}$*, ΔrhaBAD*$_{LD78}$	Datsenko & Wanner (35), Baba et al (34)
JW5082-1	BW25113/*ΔcusS::kan*^{R}	Baba et al (34)
JW0560-1	BW25113/*ΔcusR::kan*^{R}	Baba et al (34)
WT	BW25113/*ΔcueO::cat*^{R}/pET21b(+) or pET22b(+)	Previous work (22); This work
ΔcusS	BW25113/*ΔcueOΔcusS*/pET21b(+)	Previous work (22)
ΔcusR	BW25113/*ΔcueOΔcusR*/pET22b(+)	This work

Supplemental Figure S1. *In vitro* crosslinking reactions of apo and Ag(I)- $CusS_{(39-387)}$ wild-type and mutants. (A) Apo- and Ag(I)- $CusS_{(39-387)}$ samples (10 μM) in the presence and absence of BS^3 crosslinker were analyzed by SDS-PAGE followed by silver staining. The expected monomer (~18 kDa) $CusS_{(39-387)}$ is indicated by (*) and the expected dimer (~36 kDa) is indicated by (**). Experiment was repeated twice; a representative experiment is shown. (B) The relative densities of the dimers (**) were calculated and normalized to the apo proteins. Student's t test was used to calculate the *P* value. Error bars indicate standard deviations of duplicate experiments.



Supplemental Figure S2. Relative expression levels of (A) CusS-WT/H271A from pET21b(+) plasmids and (B) CusR-WT/D51A from pET22b(+) plasmids. Cells were grown at 37˚C for 2 hr, then cells were induced with 1 mM IPTG and 0.5 mM $CuSO_4$ and continued shaking at 37˚C for 6 hr. Cells were normalized to the same OD_{600} before collecting the pellets. Anti-His tag antibody was used to probe the expressed proteins.



Supplemental Figure S3. (A) Sequence alignment of the entire cytoplasmic domain of *E. coli* CusS ($CusS_{cp}$, residues 208-480) and *E. coli* CpxA ($CpxA_{HDC}$, residues 188-455). The conserved catalytic His residues are indicated with white text and highlighted in black. Two residues (S238 and P253) that are responsible in causing helical bending in $CpxA_{HDC}$ structure are highlighted in green. Conserved residues are labeled with asterisks (*). (B) 3D structure of the cytoplasmic domain of *E. coli* CusS (residues 208-480) predicted by Robetta (60). This structure was predicted based on the structure of the cytoplasmic domain of *E. coli* CpxA (PDB entry 4BIU (54)), which shares significant sequence homology with $CusS_{cp}$ (31.1% identity, 47.9% similarity, calculated by EMBOSS Needle). The catalytic H271 residue is shown in magenta stick representation. Two residues (R261 and P276) that correspond to S238 and P253 on $CpxA_{HDC}$ are shown in green sticks. An ADP molecule adapted from PDB entry 4BIU is shown in line representation in the CA domain. The domains of CusS are labeled as HAMP, histidine kinase, adenylyl cyclases, methyl-accepting proteins, phosphatases; DHp, dimerization and histidine phosphotransfer; CA, catalytic and ATP binding domain.

A

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CusScp      KGHAPIRSVSRQIQNITSKDLDVRLDPQTVPIELEQLVLSFNHMIERIEDVFTRQSNFSA 267
CpxAHDC     K---PARKLKNAADEVAQGNLRQHPELEAGPQEFLAAGASFNQMVTALERMMTSQQRLLS 244
            *...* * .      .    *  . .    * *      *** *.  .* . * *

CusScp      DIAHEIRTPITNLITQTEIALSQSRSQKELEDVLYSNLEELTRMAKMVSDMLFLAQA-DN 326
CpxAHDC     DISHELRTPLTRLQLGTALLRRRSGESKELERIE----TEAQRLDSMINDLLVMSRNQQK 300
            ** **.***.* *   * .    *   **** . .... *  *.  *. *.* .   .

CusScp      NQLIPEKKMLN-LADEVGKVFDFFEALAEDRGVELRFVGDKCQVAGDPLMLRRALSNLLS 385
CpxAHDC     NALVSETIKANQLWSEVLDN-AAFEAEQMGKSLTVNFPPGPWPLYGNPNALESALENIVR 359
            * *. *    *.*  **   .  ***    . . . *      . * *  *  ** *..

CusScp      NALRYTPTGETIVVRCQTVDHLVQVIVENPGTPIAPEHLPRLFDRFYRVDPSRQRKGEGS 445
CpxAHDC     NALRYSHTK--IEVGFAVDKDGITITVDDDGPGVSPEDREQIFRPFYRTDEARDRESGGT 417
            *****. * ..* *        . . *.  *  . **    .*  *** *  * *   *.

CusScp      GIGLAIVKSIVVAHKGTVAV-TSDARGTRFVITLPA-- 480
CpxAHDC     GLGLAIVETAIQQHRGWVKAEDSPLGGLRLVIWLPLYK 455
            *.***** . .  *.* *  . *   * * ** ** ..
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