SUPPLEMENTARY MATERIAL

The cytoplasm-entry domain of antibacterial CdiA is a dynamic α-helical bundle with disulfide-dependent structural features

Nicholas L. Bartelli[a], Sheng Sun[a], Grant C. Gucinski[b], Hongjun Zhou[a], Kiho Song[c], Christopher S. Hayes*[b,c], Frederick W. Dahlquist*[a,b,c]

[a]Department of Chemistry and Biochemistry, University of California, Santa Barbara, CA 93106, United States

[b]Department of Molecular, Cellular and Developmental Biology, University of California, Santa Barbara CA 93106, United States

[c]Biomolecular Science and Engineering Program, University of California, Santa Barbara, CA 93106, United States

*CORRESPONDING AUTHORS

Telephone: +1 805 893-5326, E-mail: dahlquist@ucsb.edu; +1 805 893-2028, E-mail: chayes@lifesci.ucsb.edu

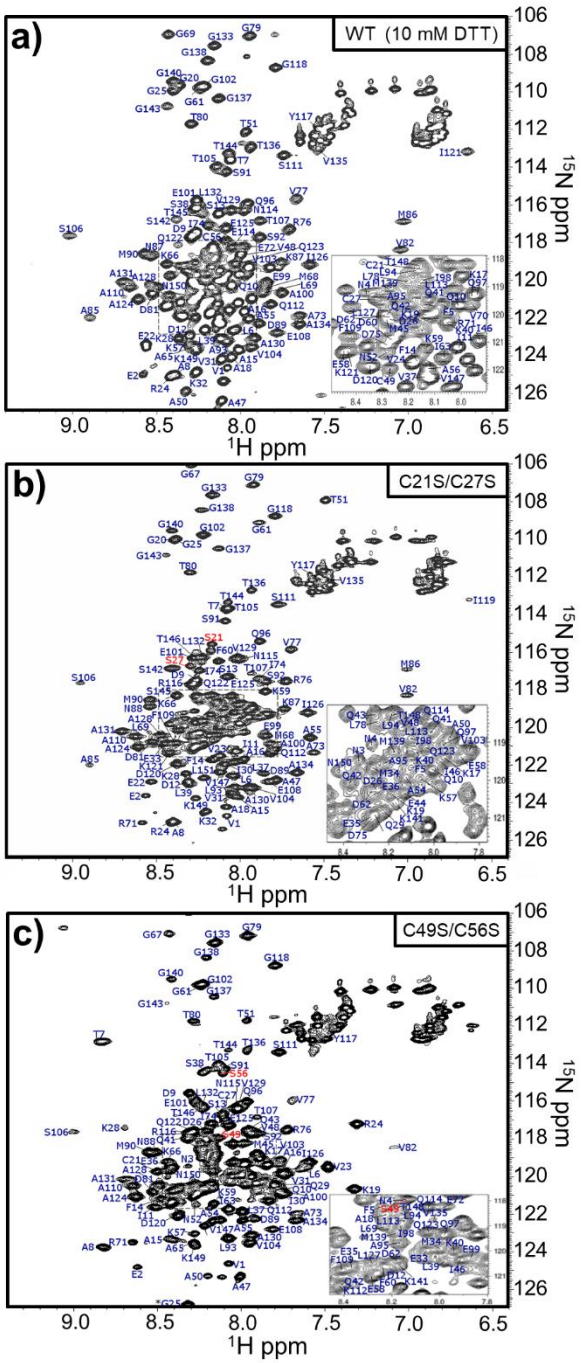

Figure S1. ^{1}H-^{15}N HSQC amide resonance assignments. (**a**) reduced CdiA-CT^{MHI813} cytoplasm-entry domain, (**b**) oxidized C21S/C27S entry domain and (**c**) oxidized C49S/C56S entry domain. The amide resonances for the substituted Ser residues are labeled in red.

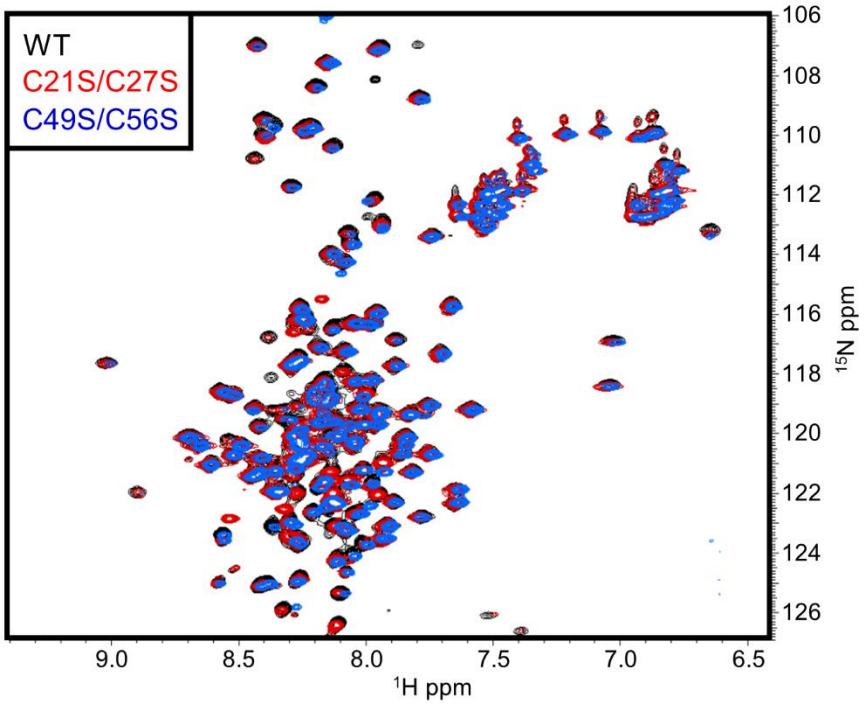

Figure S2. Superimposed ^{1}H-^{15}N HSQC spectra for reduced (10 mM DTT) CdiA-CT^{MHI813} cytoplasm entry domain (black), reduced CdiA-CT^{MHI813} C21S/C27S (red) and reduced CdiA-CT^{MHI813} C49S/C57S (blue).

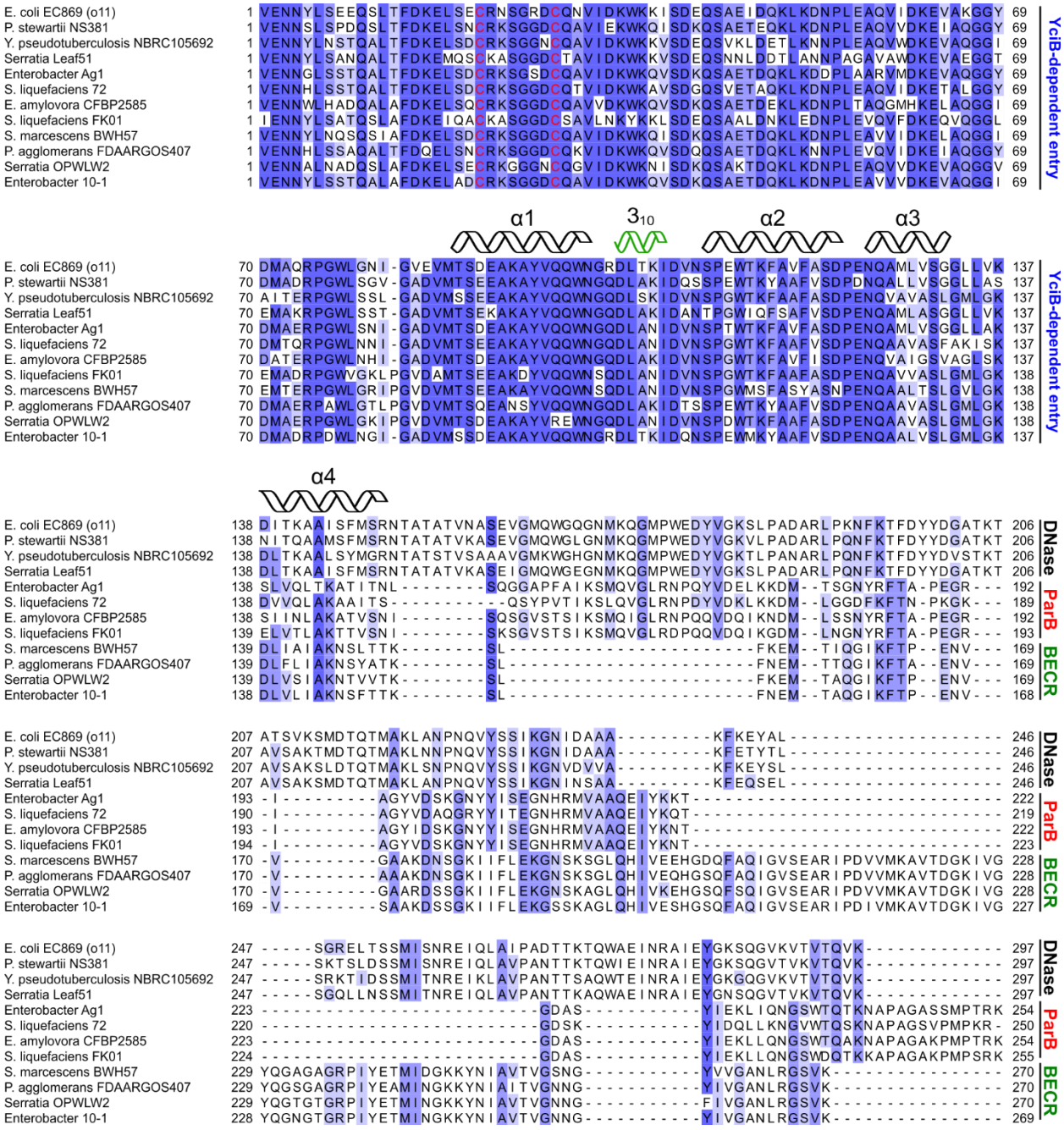

Figure S3. Alignment of CdiA-CT regions that contain YciB-dependent cytoplasm-entry domains. CdiA-CT sequences from *E. coli* EC869 (o11) (NCBI reference sequence: WP_000245723.1), *Pantoea stewartii* NC381 (WP_081316794.1), *Yersinia pseudotuberculosis* NBRC 105692 (WP_024063034.1), *Serratia* sp. Leaf51 (KQN60877.1), *Enterobacter* sp. Ag1 (WP_008454265.1), *Serratia liquefaciens* 72 (OKP23932.1), *Erwinia amylovora* CFBP 2585 (CCO85123.1), *Serratia liquefaciens* FK01 (WP_053225543.1), *Serratia marcescens* BWH57

(WP_060446815.1), *Pantoea agglomerans* FDAARGOS_407 (WP_098052973.1), *Serratia* sp. OPWLW2 (WP_099817977.1) and *Enterobacter* sp. 10-1 (WP_095099049.1) were aligned using Clustal omega and rendered using Jalview with residue colors set at 30% sequence identity [1]. The conserved pair of Cys residues are indicated in red font. The cytoplasm-entry, DNase, ParB and BECR domains are indicated at the right of the alignment. Secondary structure elements from the crystal structure of CdiA-CT_{o11}^{EC869} (PDB: 4G6U) are indicated above the alignment.

[1] Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ. Jalview Version 2--a multiple sequence alignment editor and analysis workbench. Bioinformatics. 2009;25:1189-91.

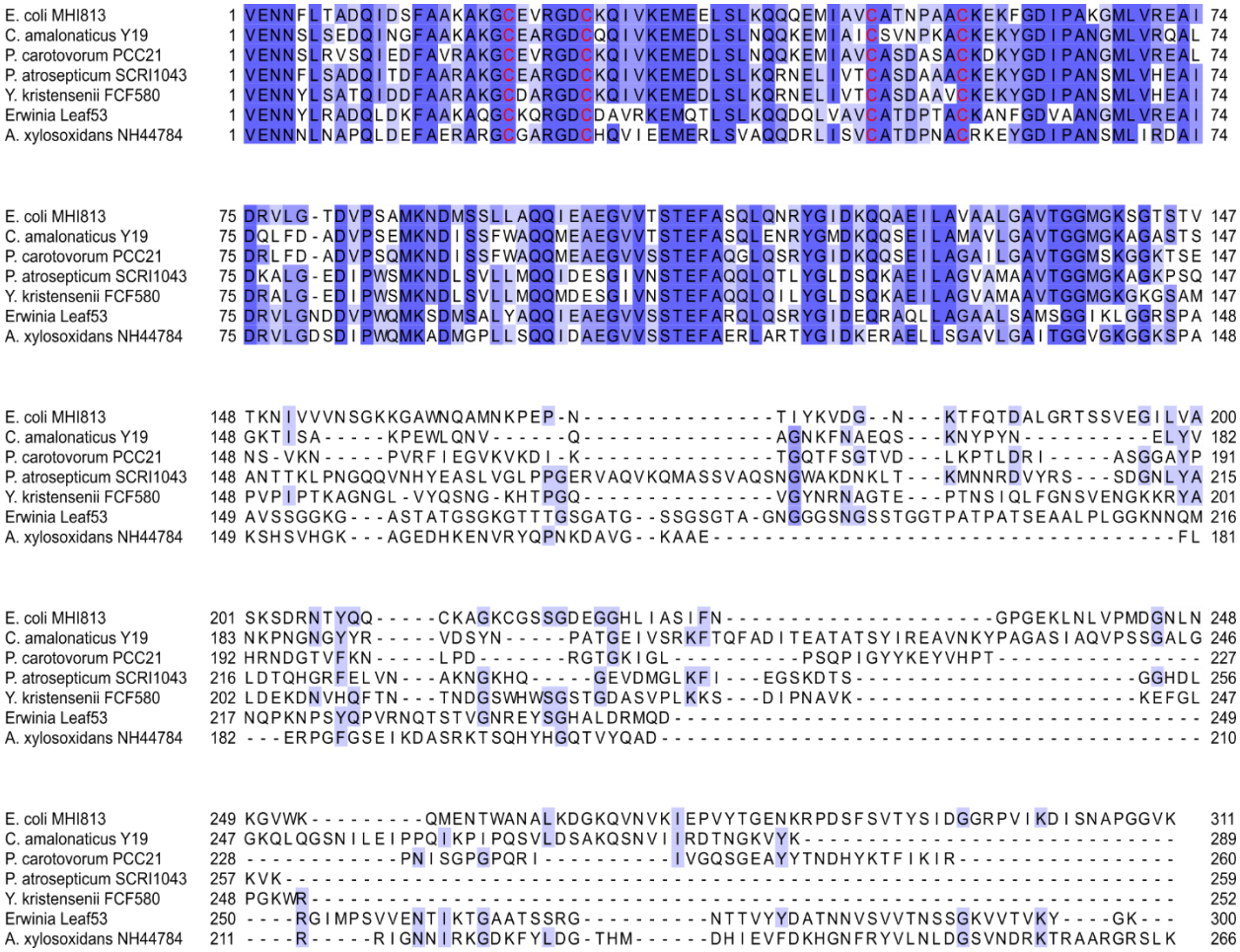

Figure S4. Alignment of CdiA-CT regions that contain MetI-dependent cytoplasm-entry domains. CdiA-CT sequences from *E. coli* MHI813 (WP_001383049.1), *Citrobacter amalonaticus* Y19 (WP_052746947.1), *Pectobacterium carotovorum* PCC21 (WP_014915291.1), *Pectobacterium atrosepticum* SCRI1043 (WP_011093679.1), *Yersinia kristensenii* FCF580 (CNL55446.1), *Erwinia* sp. Leaf53 (WP_056235768.1), and *Achromobacter xylosoxidans* NH44784-1996 (WP_020924618.1) were aligned using Clustal omega and rendered using Jalview with residue colors set at 30% sequence identity [1]. Conserved Cys residues corresponding to Cys21, Cys27, Cys49 and Cys56 of CdiA-CT^{MHI813} are indicated in red font.

[1] Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ. Jalview Version 2--a multiple sequence alignment editor and analysis workbench. Bioinformatics. 2009;25:1189-91.

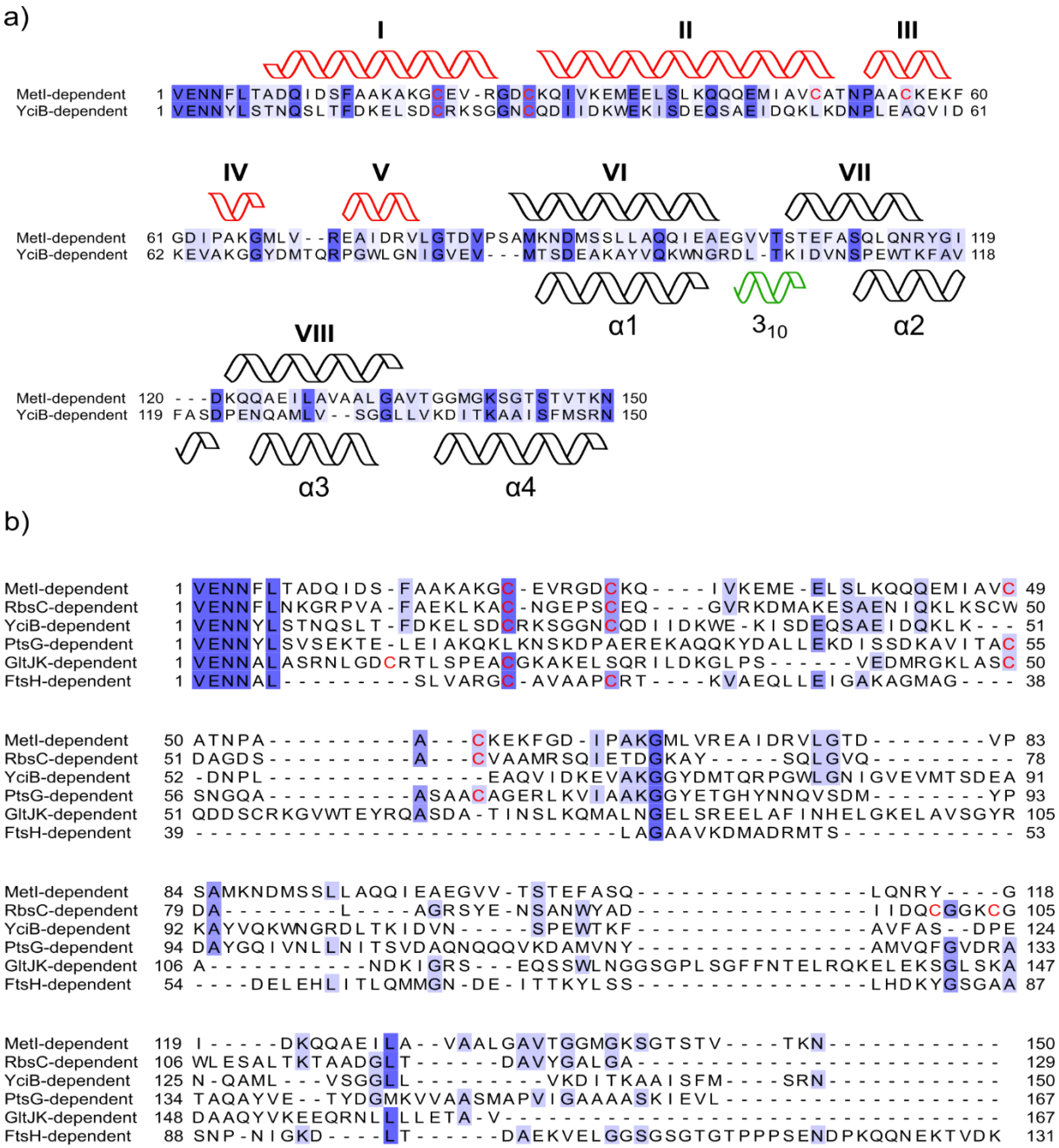

Figure S5. Comparison of characterized CdiA cytoplasm-entry domains. (**a**) The MetI- and YciB-dependent entry domains from CdiA-CT^{MHI813} and CdiA-CT$_{o11}^{EC869}$ (respectively) were aligned using Clustal omega. The paired Cys residues in each domain are shown in red font. Secondary structure elements are indicated above and below the domain sequences. The disulfide-dependent helices of CdiA-CT^{MHI813} are rendered in red. (**b**) CdiA cytoplasm entry domains contain paired Cys residues. Six characterized entry domains from *E. coli* MHI813

(WP_001383049.1), *Dickey dadantii* 3937 (WP_013318031.1), *E. coli* EC869 (o11) (WP_000245723.1), *E. coli* 3006 (EKI34460.1), *Photorhabdus luminescens* TT01 (WP_011144930.1) and *E. coli* 536 (WP_000554175.1) were aligned using Clustal omega and rendered using Jalview with residue colors set at 30% sequence identity [1]. Cys residues are shown in red font.

[1] Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ. Jalview Version 2--a multiple sequence alignment editor and analysis workbench. Bioinformatics. 2009;25:1189-91.

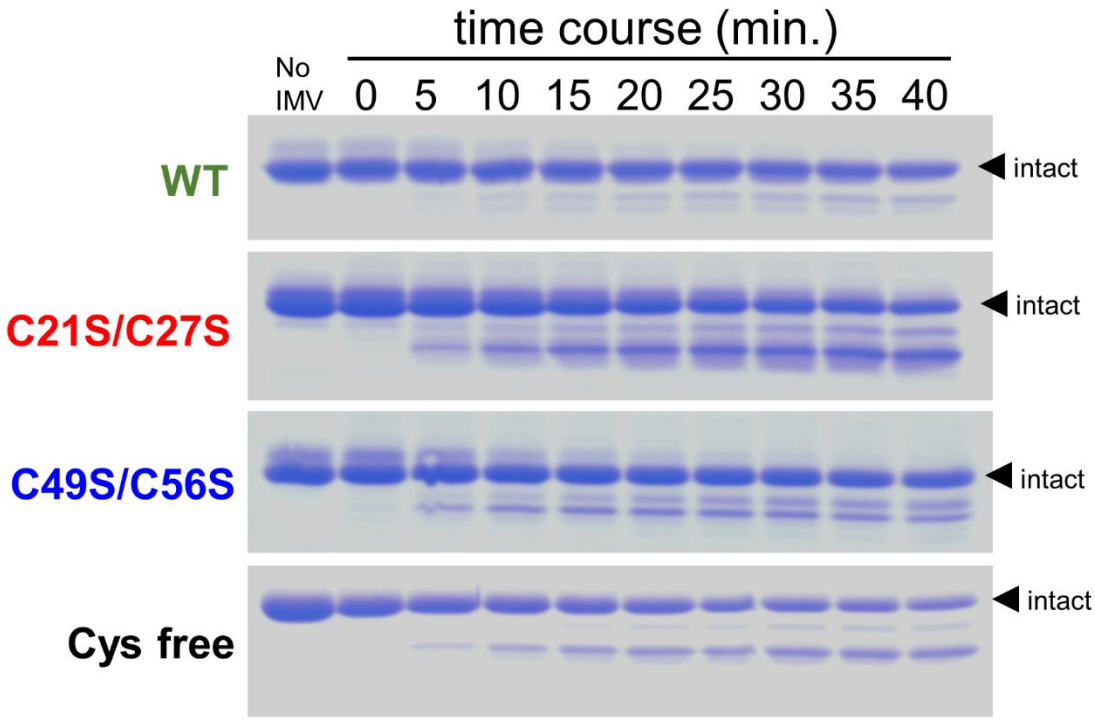

Figure S6. Effect of CdiA-CT^{MHI813} cytoplasm entry domain disulfide formation on protease sensitivity

Purified and oxidized CdiA-CT^{MHI813} cytoplasm entry domain was combined with vesicles isolated from the inner membrane of *E. coli* (IMV). The IMVs exhibit protease activity on the CdiA-CT^{MHI813} cytoplasm entry domain. Samples were removed every 5 minutes and the reaction was quenched via non-reducing SDS-PAGE loading buffer. Protease activity was assessed via SDS-PAGE. The specific CdiA-CT^{MHI813} cytoplasm entry domain employed in each experiment is indicated to the left of each gel image.

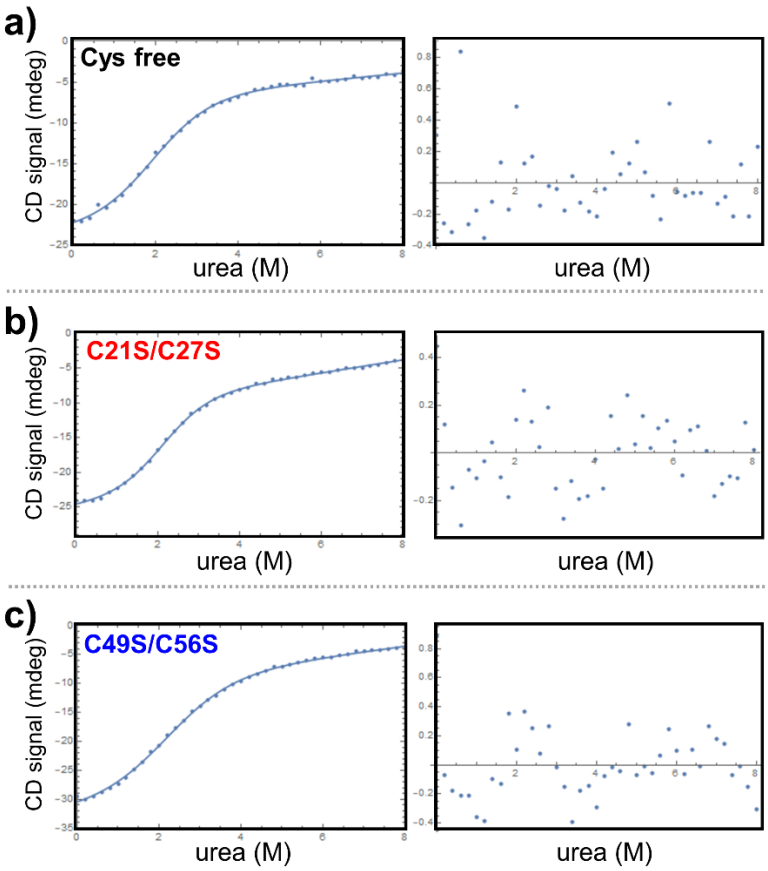

Figure S7. Nonlinear curve fitting of circular dichorism data

Fits and residuals of the observed change in circular dichroism (CD) at 224 nm using a two state model for **a)** Cys free, **b)** oxidized C21S/C27S and **c)** oxidized C49S/C56S CdiA-CT^{MHI813} cytoplasm entry domain. We employed the following function:

func[urea] = (cdf1 + u1*urea) Exp[(g - m*urea2)]/(1 + Exp[(g - m*urea)]) + (cdu + u*urea) 1/(1 + Exp[(g - m*urea)])

Where cdf1 is the CD intensity of the folded state, u1 is the urea dependence of the CD intensity of the folded state, g is the free energy difference in units of RT between the unfolded and folded states in the absence of urea and m is the dependence of the free energy difference on urea in units of RT/Molar.

As can be seen in the overlays of the data (points and fit (solid line) in **Figure S7a, b, & c**, the fits to the two state model are quite good. The residual values are about 2% of the observed and fit values. Visual examination of the residuals suggests some apparent oscillatory quality to the residuals as a function of urea. At least some of this oscillatory pattern in the residual plots reflects the nonlinear fitting procedure we used, the NonLinearModelFit routine in Mathematica.

Table S1. Plasmids

Plasmid	***Description*[a]**	***Reference***
pCH9591	pZS21::*bamA*^{ECL}, Kan^{R}	[1]
pCH9604	pZS21::*bamA*^{Eco}, Kan^{R}	[1]
pCH10163	Cosmid pCdiA-CT/*pheS** that carries a *kan-pheS** cassette in place of the *E. coli* EC93 *cdiA-CT/cdiI* coding sequence. Used for allelic exchange and counter-selection. Cm^{R} Kan^{R}	[2]
pCH11446	Expresses chimeric *cdiA*^{EC93}-*CT*^{MHI813}, Cm^{R}	[3]
pCH12234	pET21d::*cdiA-CT(V1-N150)*^{MHI813}, Amp^{R}	This study
pCH13443	Expresses chimeric *cdiA(C21S/C27S)*^{MHI813}, Cm^{R}	This study
pCH13736	Expresses chimeric *cdiA(C27S/C49S/C56S)*^{MHI813}, Cm^{R}	This study
pCH13737	Expresses chimeric *cdiA(C21S/C27S/C49S/C56S)*^{MHI813}, Cm^{R}	This study
pCH13794	pET21d::*cdiA-CT(V1-N150/C21S/C27S)*^{MHI813}, Amp^{R}	This study
pCH13814	Expresses chimeric *cdiA(C21S/C27S/C56S)*^{MHI813}, Cm^{R}	This study
pCH14122	Expresses chimeric *cdiA(C49S/C56S)*^{MHI813}, Cm^{R}	This study
pCH15122	pET21d::*cdiA-CT(V1-N150/C21S/C27S/C49S/C56S)*^{MHI813}, Amp^{R}	This study
pCH15123	pET21d::*cdiA-CT(V1-N150/C49S/C56S)*^{MHI813}, Amp^{R}	This study

[a]Abbreviations: Amp^{R}, ampicillin-resistance; Cm^{R}, chloramphenicol-resistance; Kan^{R}, kanamycin-resistance.

[1] Ruhe ZC, Wallace AB, Low DA, Hayes CS. Receptor polymorphism restricts contact-dependent growth inhibition to members of the same species. MBio. 2013;4.

[2] Morse RP, Nikolakakis KC, Willett JL, Gerrick E, Low DA, Hayes CS, et al. Structural basis of toxicity and immunity in contact-dependent growth inhibition (CDI) systems. Proc Natl Acad Sci U S A. 2012;109:21480-5.
[3] Willett JL, Gucinski GC, Fatherree JP, Low DA, Hayes CS. Contact-dependent growth inhibition toxins exploit multiple independent cell-entry pathways. Proc Natl Acad Sci U S A. 2015;112:11341-6.

Table S2. Oligonucleotides

Identifier	Oligonucleotide	Sequence	Reference
CH3174	MHI813-CT(OE)-for	5´ - CAG GTA GGA ACT CGG TTG AGA ATA ATT TTT TGA CCG CAG ATC AGA TCG ATA GC	[1]
CH3175	MHI813-CT(OE)-rev	5´ - GGT CTG GTG TCT AAC CTT TGG GTT ATA GTT CAT CAT CAT ATT GAA AGT TTA TGC TAA	[1]
CH3734	MHI813-N150-Xho-rev	5´ - CAC CTC GAG GTT TTT AGT TAC C	This study
CH4041	MHI813-C21A-rev	5´ - AGC CCC TTT CGC TTT TGC C	This study
CH4052	MHI813-C27A-for	5´ - GCC AAA CAG ATT GTA AAG GAG ATG G	This study
CH4053	MHI813-C49A-rev	5´ - GGC GAC AGC TAT CAT TTC CTG C	This study
CH4054	MHI813-C56A-rev	5´ - GGC GGC TGC AGG GTT TGT TG	This study
CH4211	MHI813-Nco-for	5´ - GAC CAT GGT TGA GAA TAA CTT TTT GAC CGC	This study
DL1527	EC93-upstream-for	5´ - GAA CAT CCT GGC ATG AGC G	[2]
DL1663	EC93-downstream-for	5´ - CCC AAA GGT TAG ACA CCA GAC C	[2]
DL2368	EC93-downstream-rev	5´ - GTT GGT AGT GGT GGT GCT G	[3]
DL2470	EC93-upstream-rev	5´ - ATT ATT CTC AAC CGA GTT CCT ACC TG	[3]

[1] Willett JL, Gucinski GC, Fatherree JP, Low DA, Hayes CS. Contact-dependent growth inhibition toxins exploit multiple independent cell-entry pathways. Proc Natl Acad Sci U S A. 2015;112:11341-6.
[2] Poole SJ, Diner EJ, Aoki SK, Braaten BA, t'Kint de Roodenbeke C, Low DA, et al. Identification of functional toxin/immunity genes linked to contact-dependent growth inhibition (CDI) and rearrangement hotspot (Rhs) systems. PLoS Genet. 2011;7:e1002217.
[3] Morse RP, Nikolakakis KC, Willett JL, Gerrick E, Low DA, Hayes CS, et al. Structural basis of toxicity and immunity in contact-dependent growth inhibition (CDI) systems. Proc Natl Acad Sci U S A. 2012;109:21480-5.

Table S3: Selected CdiA-CT NMR resonance data

WT (10 mM DTT)					C21S/C27S (oxidized)					C49S/C56S (oxidized)				
No.	Res.	Amide ^{1}H	Amide ^{15}N	$^{13}C\alpha$	No.	Res.	Amide ^{1}H	Amide ^{15}N	$^{13}C\alpha$	No.	Res.	Amide ^{1}H	Amide ^{15}N	$^{13}C\alpha$
1	Val	8.08	124.6	62.5	1	Val	8.09	124.6	62.5	1	Val	8.08	124.6	62.5
2	Glu	8.58	125.0	56.3	2	Glu	8.57	123.6	56.7	2	Glu	8.62	124.8	56.6
3	Asn	8.54	120.2	53.2	3	Asn	8.34	119.5	53.2	3	Asn	8.32	119.5	53.2
4	Asn	8.36	119.2	53.3	4	Asn	8.28	119.1	53.3	4	Asn	8.13	117.9	53.7
5	Phe	8.09	119.7	57.7	5	Phe	8.11	119.7	57.8	5	Phe	8.25	118.5	58.3
6	Leu	8.03	122.6	55.0	6	Leu	8.05	122.5	55.0	6	Leu	7.64	119.6	54.0
7	Thr	8.06	113.6	61.3	7	Thr	8.08	113.6	61.3	7	Thr	8.84	112.8	60.7
8	Ala	8.39	125.0	53.6	8	Ala	8.41	125.0	53.6	8	Ala	8.82	123.8	55.5
9	Asp	8.26	117.5	55.0	9	Asp	8.27	117.5	55.1	9	Asp	8.31	115.6	56.7
10	Gln	8.01	119.7	56.4	10	Gln	8.02	119.7	56.4	10	Gln	7.78	120.8	58.4
11	Ile	8.01	121.2	62.2	11	Ile	8.03	121.2	62.3	11	Ile	8.51	121.6	65.4
12	Asp	8.30	122.9	54.9	12	Asp	8.32	122.9	55.0	12	Asp	8.21	120.7	57.1
13	Ser	8.13	116.5	59.6	13	Ser	8.14	116.5	59.6	13	Ser	8.11	116.7	61.4
14	Phe	8.17	121.6	59.0	14	Phe	8.18	121.7	59.0	14	Phe	8.48	121.2	62.5
15	Ala	8.01	123.7	53.3	15	Ala	8.03	123.6	53.3	15	Ala	8.41	123.4	55.1
16	Ala	7.98	121.6	53.2	16	Ala	7.98	121.6	53.2	16	Ala	7.76	119.8	54.7
17	Lys	7.95	119.3	56.7	17	Lys	7.96	119.4	56.6	17	Lys	7.89	119.0	58.7
18	Ala	8.05	124.1	52.8	18	Ala	8.09	124.2	52.8	18	Ala	8.23	118.6	53.7
19	Lys	8.13	120.0	56.7	19	Lys	8.19	120.4	56.3	19	Lys	7.32	120.7	58.3
20	Gly	8.37	109.7	45.5	20	Gly	8.40	109.9	45.5	20	Gly	9.29	114.5	46.0
21	Cys	8.16	118.4	58.4	21	Ser	8.18	115.5	58.6	21	Cys	8.50	119.7	58.6
22	Glu	8.56	123.4	56.8	22	Glu	8.54	122.8	56.8	22	Glu	MR	MR	60.3
23	Val	8.18	121.7	62.4	23	Val	8.13	121.5	62.5	23	Val	7.48	119.5	64.6
24	Arg	8.42	125.1	56.0	24	Arg	8.41	125.0	55.8	24	Arg	7.31	117.2	58.2
25	Gly	8.40	109.9	45.4	25	Gly	8.40	110.0	45.4	25	Gly	8.32	126.8	45.7
26	Asp	8.28	120.1	54.1	26	Asp	8.25	120.2	54.1	26	Asp	8.21	117.6	52.8
27	Cys	8.31	119.5	58.9	27	Ser	8.30	116.7	59.1	27	Cys	8.10	117.0	57.8
28	Lys	8.37	123.1	56.9	28	Lys	8.33	122.4	57.0	28	Lys	8.70	117.4	59.2
29	Gln	MR	MR	56.2	29	Gln	8.23	120.8	56.4	29	Gln	7.80	120.5	58.5
30	Ile	8.13	122.3	61.6	30	Ile	8.10	121.9	61.8	30	Ile	7.75	120.9	64.5
31	Val	8.11	124.2	63.0	31	Val	8.06	123.5	63.3	31	Val	7.74	119.9	66.5
32	Lys	8.26	124.9	57.0	32	Lys	8.22	124.5	57.3	32	Lys	7.81	120.4	58.9
33	Glu	MR	MR	MR	33	Glu	8.46	120.8	57.8	33	Glu	8.05	120.0	59.4
34	Met	MR	MR	MR	34	Met	8.28	119.7	56.8	34	Met	8.00	119.9	59.2
35	Glu	MR	MR	MR	35	Glu	8.29	121.0	57.9	35	Glu	8.37	120.7	59.3
36	Glu	MR	MR	57.6	36	Glu	8.25	120.3	57.6	36	Glu	8.41	119.5	59.3
37	Leu	8.14	122.2	56.2	37	Leu	8.04	121.7	56.1	37	Leu	7.99	121.9	57.5
38	Ser	8.28	116.2	59.3	38	Ser	8.42	116.8	59.2	38	Ser	8.21	114.4	61.6

39	Leu	8.12	123.1	56.7	39	Leu	8.30	123.0	57.8	39	Leu	7.98	120.4	57.7
40	Lys	8.06	120.3	57.8	40	Lys	8.11	119.5	58.9	40	Lys	7.94	120.0	59.1
41	Gln	8.13	119.5	57.4	41	Gln	8.02	118.8	58.4	41	Gln	8.32	118.0	58.6
42	Gln	8.28	120.1	57.3	42	Gln	8.38	120.1	58.9	42	Gln	8.40	121.3	58.7
43	Gln	MR	MR	MR	43	Gln	8.27	118.3	58.6	43	Gln	7.96	117.5	57.9
44	Glu	MR	MR	MR	44	Glu	8.14	120.3	58.8	44	Glu	MR	MR	58.1
45	Met	8.29	120.0	56.4	45	Met	MR	MR	58.2	45	Met	7.91	118.8	57.1
46	Ile	7.93	120.9	61.9	46	Ile	7.99	119.5	64.5	46	Ile	7.91	120.4	62.5
47	Ala	8.11	126.4	52.9	47	Ala	7.80	122.8	54.9	47	Ala	8.01	125.3	53.3
48	Val	8.00	118.6	62.9	48	Val	8.15	118.6	65.5	48	Val	7.94	117.7	63.0
49	Cys	8.27	122.0	59.0	49	Cys	MR	MR	58.7	49	Ser	8.14	118.0	58.8
50	Ala	8.33	125.9	53.0	50	Ala	7.93	118.9	54.4	50	Ala	8.20	125.3	53.0
51	Thr	7.97	112.1	61.9	51	Thr	7.50	107.9	63.4	51	Thr	7.97	111.7	61.9
52	Asn	8.28	121.4	51.2	52	Asn	MR	MR	MR	52	Asn	8.27	121.5	51.2
53	Pro	NA	NA	64.0	53	Pro	NA	NA	65.3	53	Pro	NA	NA	63.7
54	Ala	8.13	122.0	52.8	54	Ala	8.13	119.9	55.1	54	Ala	8.17	122.4	52.7
55	Ala	7.95	122.0	52.8	55	Ala	7.59	120.5	53.7	55	Ala	7.99	122.3	52.7
56	Cys	8.09	117.9	58.9	56	Cys	7.89	117.3	58.0	56	Ser	8.11	114.5	58.6
57	Lys	8.30	123.4	57.0	57	Lys	8.07	119.9	58.5	57	Lys	8.28	123.0	56.7
58	Glu	8.44	121.3	57.0	58	Glu	7.89	119.4	58.4	58	Glu	8.29	121.1	56.9
59	Lys	8.14	121.2	56.5	59	Lys	7.86	118.0	57.8	59	Lys	8.16	121.4	56.4
60	Phe	8.24	120.4	57.8	60	Phe	8.18	115.9	58.0	60	Phe	8.23	120.6	57.9
61	Gly	8.25	109.8	45.4	61	Gly	7.90	109.1	45.1	61	Gly	8.25	109.8	45.4
62	Asp	8.17	120.3	54.3	62	Asp	8.29	120.3	56.4	62	Asp	8.18	120.4	54.2
63	Ile	8.07	121.6	59.7	63	Ile	MR	MR	MR	63	Ile	8.07	121.7	59.7
64	Pro	NA	NA	63.6	64	Pro	NA	NA	MR	64	Pro	NA	NA	63.5
65	Ala	8.27	123.5	53.5	65	Ala	MR	MR	54.4	65	Ala	8.28	123.6	53.4
66	Lys	8.44	119.1	58.6	66	Lys	8.39	118.3	59.6	66	Lys	8.44	119.2	58.5
67	Gly	8.43	107.0	46.8	67	Gly	8.30	106.0	47.0	67	Gly	8.43	107.1	46.6
68	Met	MR	MR	57.7	68	Met	7.83	120.8	56.4	68	Met	7.88	120.3	56.8
69	Leu	7.87	120.7	57.3	69	Leu	8.55	120.2	53.3	69	Leu	8.23	119.0	53.1
70	Val	7.96	119.7	66.3	70	Val	MR	MR	62.5	70	Val	MR	MR	62.6
71	Arg	8.05	120.3	58.8	71	Arg	8.59	125.0	56.5	71	Arg	8.65	123.5	56.8
72	Glu	7.98	118.2	59.2	72	Glu	MR	MR	59.6	72	Glu	7.98	118.2	59.1
73	Ala	7.65	121.9	54.9	73	Ala	7.57	121.3	54.9	73	Ala	7.67	122.0	54.9
74	Ile	8.19	117.1	64.5	74	Ile	8.25	116.9	64.7	74	Ile	8.18	117.2	64.6
75	Asp	8.26	120.9	57.6	75	Asp	8.29	121.0	57.6	75	Asp	MR	MR	57.5
76	Arg	7.71	117.3	58.6	76	Arg	7.74	117.5	58.6	76	Arg	7.73	117.5	58.5
77	Val	7.66	115.7	64.1	77	Val	7.71	115.8	64.2	77	Val	7.69	116.0	64.2
78	Leu	8.21	118.8	55.3	78	Leu	8.25	118.5	55.3	78	Leu	MR	MR	55.3
79	Gly	7.95	107.0	45.7	79	Gly	7.93	107.0	45.7	79	Gly	7.96	107.2	45.7

80	Thr	8.29	111.7	62.4	80	Thr	8.31	111.7	62.3	80	Thr	8.29	111.7	62.3
81	Asp	8.52	120.7	55.2	81	Asp	8.53	120.7	55.3	81	Asp	8.52	120.8	55.1
82	Val	7.05	118.4	59.2	82	Val	7.01	118.2	59.2	82	Val	7.09	118.4	59.1
83	Pro	NA	NA	MR	83	Pro	NA	NA	MR	83	Pro	NA	NA	MR
84	Ser	MR	MR	MR	84	Ser	MR	MR	MR	84	Ser	MR	MR	MR
85	Ala	MR	MR	55.3	85	Ala	8.90	121.9	55.4	85	Ala	MR	MR	MR
86	Met	7.03	116.9	58.3	86	Met	7.01	116.9	58.3	86	Met	MR	MR	MR
87	Lys	7.76	119.0	60.5	87	Lys	7.74	118.9	60.5	87	Lys	8.52	118.7	MR
88	Asn	8.53	118.7	55.9	88	Asn	8.55	118.8	55.9	88	Asn	8.52	118.7	55.8
89	Asp	7.89	122.3	57.8	89	Asp	7.87	122.4	57.8	89	Asp	7.92	122.3	57.6
90	Met	8.57	118.6	59.6	90	Met	8.55	118.5	59.5	90	Met	8.55	118.7	59.4
91	Ser	8.09	114.2	62.5	91	Ser	8.10	114.3	62.7	91	Ser	8.10	114.3	62.0
92	Ser	7.89	117.7	61.5	92	Ser	7.85	117.5	61.4	92	Ser	7.91	117.7	61.6
93	Leu	8.08	123.2	58.2	93	Leu	8.04	123.1	58.2	93	Leu	8.08	123.3	58.1
94	Leu	8.18	118.4	57.7	94	Leu	8.20	118.3	57.8	94	Leu	8.17	118.5	57.7
95	Ala	8.20	119.6	55.5	95	Ala	8.22	119.6	55.5	95	Ala	8.17	119.7	55.4
96	Gln	7.96	115.9	58.5	96	Gln	7.89	115.3	58.8	96	Gln	7.97	116.0	58.0
97	Gln	7.96	119.2	58.4	97	Gln	7.96	119.1	58.1	97	Gln	7.98	119.4	58.5
98	Ile	8.16	118.9	64.1	98	Ile	8.11	118.9	63.9	98	Ile	8.16	119.0	64.0
99	Glu	7.84	120.1	58.2	99	Glu	7.86	120.4	57.9	99	Glu	7.87	120.3	58.2
100	Ala	7.75	120.7	53.4	100	Ala	7.80	121.1	53.4	100	Ala	7.77	120.8	53.4
101	Glu	8.26	115.8	56.8	101	Glu	8.28	116.3	56.9	101	Glu	8.27	116.0	56.9
102	Gly	8.22	109.7	45.7	102	Gly	8.23	109.7	45.6	102	Gly	8.23	109.8	45.7
103	Val	7.82	119.3	62.1	103	Val	7.85	119.2	62.3	103	Val	7.84	119.4	62.3
104	Val	7.93	123.5	61.1	104	Val	7.94	123.2	61.4	104	Val	7.95	123.5	61.2
105	Thr	8.14	114.0	60.5	105	Thr	8.08	113.6	61.3	105	Thr	8.14	114.0	60.6
106	Ser	9.02	117.6	62.2	106	Ser	8.96	117.6	61.6	106	Ser	9.01	117.6	MR
107	Thr	7.88	116.8	65.7	107	Thr	7.95	117.1	65.6	107	Thr	7.91	116.8	65.6
108	Glu	7.78	122.8	59.0	108	Glu	7.84	122.8	59.0	108	Glu	7.81	122.8	59.0
109	Phe	8.43	120.8	60.1	109	Phe	8.39	120.8	60.1	109	Phe	8.41	120.8	60.0
110	Ala	8.66	120.3	55.7	110	Ala	8.63	120.4	55.7	110	Ala	8.65	120.5	55.6
111	Ser	7.74	113.4	61.6	111	Ser	7.79	113.4	61.7	111	Ser	7.77	113.4	61.7
112	Gln	7.82	121.3	59.1	112	Gln	7.83	121.3	59.0	112	Gln	7.83	121.3	58.9
113	Leu	8.15	118.8	57.8	113	Leu	8.15	118.7	57.8	113	Leu	8.15	118.8	57.8
114	Gln	8.05	118.2	59.7	114	Gln	8.06	118.3	59.7	114	Gln	8.05	118.3	59.6
115	Asn	7.98	116.3	55.9	115	Asn	8.02	116.3	55.9	115	Asn	8.03	116.4	55.9
116	Arg	8.31	117.8	58.3	116	Arg	8.30	117.7	58.3	116	Arg	8.32	117.7	58.2
117	Tyr	7.52	112.2	57.2	117	Tyr	7.51	112.2	57.3	117	Tyr	7.52	112.5	57.3
118	Gly	7.79	108.7	46.8	118	Gly	7.80	108.7	46.8	118	Gly	7.80	108.8	46.6
119	Ile	6.65	113.2	60.1	119	Ile	6.64	113.2	60.1	119	Ile	MR	MR	60.1
120	Asp	8.34	121.9	54.1	120	Asp	8.39	121.9	54.1	120	Asp	8.38	122.1	54.2

121	Lys	8.42	121.4	60.2	121	Lys	8.43	121.3	60.2	121	Lys	8.42	121.4	60.2
122	Gln	8.29	117.7	59.2	122	Gln	8.29	117.6	59.3	122	Gln	8.29	117.6	59.3
123	Gln	8.03	119.1	58.6	123	Gln	8.04	119.1	58.5	123	Gln	8.05	119.2	58.5
124	Ala	8.61	121.0	55.5	124	Ala	8.62	120.9	55.5	124	Ala	8.63	121.1	55.5
125	Glu	8.09	117.2	60.1	125	Glu	8.08	117.2	60.0	125	Glu	8.08	117.3	60.0
126	Ile	7.59	119.2	64.9	126	Ile	7.61	119.2	64.9	126	Ile	7.59	119.2	64.7
127	Leu	8.26	120.4	57.8	127	Leu	MR	MR	57.9	127	Leu	8.29	120.5	57.8
128	Ala	8.50	120.4	55.8	128	Ala	8.48	120.4	55.8	128	Ala	8.49	120.5	55.7
129	Val	8.05	116.3	66.4	129	Val	8.03	116.2	66.4	129	Val	8.02	116.4	66.4
130	Ala	7.92	123.1	54.8	130	Ala	7.96	123.2	54.8	130	Ala	7.94	123.1	54.7
131	Ala	8.70	120.1	55.1	131	Ala	8.72	120.2	55.2	131	Ala	8.69	120.2	55.1
132	Leu	8.27	116.2	57.3	132	Leu	8.24	116.2	57.3	132	Leu	8.23	116.3	57.2
133	Gly	8.15	107.5	46.6	133	Gly	8.18	107.6	46.6	133	Gly	8.16	107.6	46.5
134	Ala	7.65	122.3	53.7	134	Ala	7.67	122.4	53.5	134	Ala	7.67	122.3	52.8
135	Val	7.54	113.0	63.4	135	Val	7.53	112.2	63.3	135	Val	8.02	118.7	63.3
136	Thr	7.94	112.9	62.9	136	Thr	7.94	112.7	62.8	136	Thr	7.97	113.3	62.8
137	Gly	8.13	110.4	45.9	137	Gly	8.13	110.4	45.9	137	Gly	8.16	110.4	45.9
138	Gly	8.20	108.4	45.5	138	Gly	8.24	108.4	45.5	138	Gly	8.21	108.4	45.5
139	Met	8.17	119.3	55.8	139	Met	8.18	119.3	55.7	139	Met	8.18	119.3	55.8
140	Gly	8.40	109.4	45.5	140	Gly	8.42	109.5	45.5	140	Gly	8.41	109.5	45.4
141	Lys	MR	MR	56.3	141	Lys	8.17	120.6	56.2	141	Lys	8.16	120.6	56.3
142	Ser	8.38	116.8	58.6	142	Ser	8.43	116.8	58.6	142	Ser	8.39	116.8	58.6
143	Gly	8.44	110.8	45.5	143	Gly	8.45	110.8	45.5	143	Gly	8.45	110.8	45.5
144	Thr	8.07	113.3	61.9	144	Thr	8.08	113.3	61.9	144	Thr	8.08	113.3	61.9
145	Ser	8.37	118.1	58.4	145	Ser	8.39	118.2	58.4	145	Ser	8.38	118.1	58.4
146	Thr	8.23	116.5	62.1	146	Thr	8.24	116.5	62.2	146	Thr	8.24	116.5	62.2
147	Val	8.12	122.3	62.5	147	Val	8.13	122.3	62.5	147	Val	8.12	122.3	62.6
148	Thr	8.16	118.2	62.0	148	Thr	8.19	118.3	62.0	148	Thr	8.17	118.2	62.1
149	Lys	8.26	123.7	56.5	149	Lys	8.27	123.7	56.5	149	Lys	8.27	123.7	56.5
150	Asn	8.42	119.8	53.4	150	Asn	8.43	119.8	53.4	150	Asn	8.43	119.7	53.4

Resonance assignments in parts per million (ppm)

MR: missing resonance or assignment

NA: not applicable