

Supplementary Information

Supplementary Table S1. Strains used in this study.

Strain/plasmid	Genotype/ description	Source
Strains		
Vc O395	<i>V. cholerae</i> Wild-type, Sm ^r	A gift from Dr. Fitnat Yildiz at the University of California Santa Cruz
	Vc 0395 mutant, $\Delta znuA$, Sm ^r	This work
	Vc O395 mutant, $\Delta znuA zrgA \Delta 124-184$, Sm ^r	This work
	Vc O395 mutant, $\Delta znuA, \Delta zrgA$ Sm ^r	This work
Sm10 λ pir	<i>E. coli</i> , with derivative RP4 plasmid integrated, containing the λ pir for replication of R6K suicide vector, for integration into <i>V. cholerae</i> .	A gift from Dr. Fitnat Yildiz at the University of California Santa Cruz
Plasmids		
pCDF-Duet1	Expression vector, Sm ^r	Novagen
	pCDF-Duet1 with <i>V. cholerae zrgA</i> , Sm ^r	This work
	pCDF-Duet1, with <i>V. cholerae zrgA</i> mutation lacking $\Delta 124-184$, Sm ^r	This work
	pCDF-Duet1, with <i>V. cholerae zrgA</i> mutation lacking $\Delta 124-183$, Sm ^r	This work
	pCDF-Duet1, with <i>V. cholerae zrgA</i> mutation lacking $\Delta 124-180$, Sm ^r	This work
pGP704sacB28	pGP704sacB28, R6K suicide plasmid, Ap ^r	A gift from Dr. Fitnat Yildiz at the University of California Santa Cruz

	pGP704sacB28, with upstream and downstream flanking regions comprising 3 amino acids forward and reverse of <i>znuA</i> gene along with 500-600bp flanking gene, Amp ^r	This work
	pGP704sacB28, with upstream and downstream 600bp flanking sequences of <i>zrgA</i> , Amp ^r	This work
	pGP704sacB28, with upstream and downstream 600bp flanking sequences of <i>zrgA</i> flexible loop region 124-184, Amp ^r	This work

Supplementary Table S2. Primers used in this study.

Primer	Primer sequence 5' to 3'
VcholZrgA_pcdf_fow	gtataagaaggagatatacatatgcattaccaacaatgattg
VcholZrgA_pcdf_rev	gtttctttaccagactcgagggttacagttgataagcgttg
ZrgA_Del 123-184 FWD	ggctcattcaccgctcaatatcagttccac
ZrgA_Del 123-184 REV	accataagcgtggctgtgttggtactcttc
ZrgA_del_123-180 FWD	gagcatcaacacggctcattcaccgctcaa
ZrgA_del_123-183 FWD	cacggctcattcaccgctcaatatcagttc
Vc_ZnuA_Del_A	gatccacgaagcttccatggaagcaagtcgggtcttftaataaagc
Vc_ZnuA_Del_B2	taagattgggacaaacacgctctcgataacatggtcgcaaaact
Vc_ZnuA_Del_C2	tggcgaccatgggtatcgagagcgtgttgcctcaatcctaagtcg
Vc_ZnuA_Del_D2	tagaacgggtgacgtcaccacaactcgtgttgctcaaaagcc
Vc_ZrgA_del_A	atccacgaagcttccatggttccaacagtattgcgctttgt
Vc_ZrgA_del_B	tgtttattaggagttttctgccaccgctcggga
Vc_ZrgA_del_C	acctatcccagcgggtggcagaaaaactcctaataaaacaaaaa
Vc_ZrgA_del_D	tatctagaaccggtagctcaccatcaatcattcggggtga
ZrgA_Mut_124-184_A	gatccacgaagcttccatggcaagcggcttaagtcactgc
ZrgA_Mut_124-184_B2	aacacagccacgcttatggtggctcattcaccgctcaatat
ZrgA_Mut_124-184_C2	tattgagcggatgaatgagccaccataagcgtggctgtg
ZrgA_Mut_124-184_D2	tagaacgggtgacgtcaccaccaccacaagcaaggggtga
FWD_Vc_ZnuA_Seq	tgttgataaaaagccagcgtgtctcggc
REV_Vc_ZnuA_Seq	cattagtcgctaaagcccatttcgagact
Vc_ZrgA_Seq_FWD	tcacgggtttattcagtaaagcgttggc

Vc_ZrgA_Seq_REV	cttgattcagcaaaatctgtaatcgcccc
FWD_Vc_ZrgA_mut_Seq	gttggaaaatatagcctatatggtcggcg
REV_Vc_ZrgA_mut_Seq	ggcctaaaaccagtctggtgattgcc

Table S3. Properties of refined zinc sites and ligands.

Site	Chain	Occ.	B-factor	Anom. Density (rmsd, σ)	Ligand* (Distance, Å)
1	A	1.0	32.9	12.1	H35 N ϵ (2.0)
					H36 N ϵ (2.0)
					E66 O ϵ 1 (2.1)
					E66 O ϵ 2 (2.1)
					H ₂ O (2.1)
	B	1.0	40.0	10.5	H35 N ϵ (2.1)
					H36 N ϵ (2.0)
					E66 O ϵ 1 (2.1)
					E66 O ϵ 2 (2.1)
					H ₂ O (2.1)
	C	1.0	31.3	11.5	H35 N ϵ (1.9)
					H36 N ϵ (2.0)
					E66 O ϵ 1 (2.0)
					H ₂ O (2.1)
	D	1.0	43.5	12.1	H35 N ϵ (1.9)
					H36 N ϵ (1.9)
					E66 O ϵ 1 (2.1)
					E66 O ϵ 2 (2.1)
	E	0.43	81.1	4.7	H35 N ϵ (2.3)
					H36 N ϵ (1.8)
E66 O ϵ 1 (2.0)					
E66 O ϵ 2 (2.1)					
F	0.75	43.1	7.9	H35 N ϵ (1.9)	
				H36 N ϵ (2.0)	
				E66 O ϵ 1 (2.1)	
				E66 O ϵ 2 (2.1)	
				H ₂ O (2.1)	
2	A	1.0	33.5	10.3	E83 O ϵ 1 (2.0)
					H86 N ϵ (2.0)
					H87 N ϵ (2.0)
					E79' (D) O ϵ 1 (2.1)
					E79' (D) O ϵ 2 (2.1)
	B	1.0	39.6	9.8	E83 O ϵ 1 (2.0)
					H86 N ϵ (1.9)
					H87 N ϵ (1.9)

					E79'' (F) O ϵ 1 (2.2)
					E79'' (F) O ϵ 2 (2.2)
	C	1.0	38.5	9.0	E83 O ϵ 1 (2.0)
					H86 N ϵ (2.0)
					H87 N ϵ (1.9)
					E79' (E) O ϵ 1 (2.1)
					E79' (E) O ϵ 2 (2.1)
	D	1.0	33.4	11.1	E83 O ϵ 1 (2.1)
					E83 O ϵ 2 (2.1)
					H86 N ϵ (2.0)
					H87 N ϵ (2.0)
					E79' (A) O ϵ 1 (2.1)
					E79' (A) O ϵ 2 (2.1)
	E	1.0	33.2	9.3	E83 O ϵ 1 (2.1)
					H86 N ϵ (1.9)
					H87 N ϵ (2.0)
					E79' (C) O ϵ 1 (2.1)
					E79' (C) O ϵ 2 (2.1)
	F	1.0	49.4	7.2	E83 O ϵ 1 (2.0)
					H86 N ϵ (2.0)
					H87 N ϵ (2.0)
					E79'' (B) O ϵ 1 (1.7)
					E79'' (B) O ϵ 2 (2.7)
3	A	1.0	34.6	10.9	H49 N δ (2.1)
					H194 N ϵ (2.1)
					E196 O ϵ 1 (2.1)
					E196 O ϵ 2 (2.1)
					H118' (D) N δ (2.0)
	B	0.72	51.4	5.5	H49 N δ (2.0)
					H194 N ϵ (2.3)
					E196 O ϵ 1 (2.1)
					H ₂ O (2.1)
	C	0.35	54.0	4.2	H194 N ϵ (2.0)
					E196 O ϵ 1 (2.1)
	D	0.43	52.8	3.0	H194 N ϵ (2.0)
					E196 O ϵ 1 (2.1)
	E	0.57	60.0	5.3	H49 N δ (2.3)
					H194 N ϵ (2.0)
					E196 O ϵ 1 (2.1)
					E196 O ϵ 2 (2.1)
					H118' (C) N δ (2.1)
	F	0.89	63.0	6.6	H49 N δ (2.0)
					H194 N δ (2.0)
					E196 O ϵ 1 (2.0)
3b	C	0.50	55.1	3.4	H194 N δ (2.0)
					D50 O δ 1 (2.1)

					D50 Oδ2 (2.1)
	D	0.67	51.3	7.3	H194 Nδ (1.7)
					D50 Oε1 (2.1)
4	B	0.86	62.2	4.5	H67 Nε (2.1)

*Ligands from another chain in the asymmetric unit are indicated by ('). Those from a symmetry related chain are indicated by ("). The chain ID is indicated in parentheses after the residue name.

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Shewanella_maritima/1-241      1 MNF---K-----VPTLVAAAMICAPT-----AFAADKVVHDQGEVFIINECKQWNVQFNIPAINA 51
Pseudomonas_stutzeri/1-204    1 ---MRHL---L L AV P V L L P L S Y S Q A H E D H H H E H E E A A S L G A H E H G A A R L N A A L D G K R L E L E F I S P A M N L 64
Pseudomonas/1-196             1 ---MRPL---L L A L - V L L P F - A A Q A H D D H D H A - H G S L G K H E H G V A Q L N V A L D G K T L E L E D S P A M N L 60
Pseudomonas_fluorescens/1-202 1 ---MRRL---L L A L P F A L P L A A Q A A Q A H D H E H E - H G S L G A H E H G V G R L N A V L D G Q T L E L E L E S P A M N L 63
Pseudomonas_putida/1-193      1 ---MCRL---L L A L P F A L P L A V A H A H D D H D H A - H G S L G A H E H G V A K L N A V L D G N T L E L E L D S P A M N L 63
Vibrio_caribbeanicus/1-227    1 ---MKNITP I A L S I S L A I S S --- F A Y A --- E E G F R Q H S A H V H G H V E L N I A Q D G K E L L M E I S A P G A D V 58
Photobacterium_lipolyticum/1-193 1 M T F L P R V T T C A L I V S --- S L F S A A A A S --- D E Y H R Q H D A H Q H G I V E F N I A Q D G Q D L L L E I T A P G A D V 61
Vibrio_cholerae/1-242          1 ---MHYPTM I A L M V G A T L S G N V L A D H H S --- D H Q H R O H E A H V H G Q V E L N I A Q D G H D L L E I T A P G A D V 62
Vibrio_paraahaemolyticus/1-209 1 ---MPSKQV L A I V I G L S L --- T V A T --- A E E Y R Q H S A H V H G H V E F N I A Q D G S D L L E I T A P G A D V 57
Pseudoceanicola_batsensis/1-220 1 ---MKP I ---H L A --- L A A S T L A A P A F --- A Q D S R E M D A H V H G V S T A E I A V E H G K V E I N L L S P G M D I 55
Rhodomicrobium_vannielli/1-200 1 ---MK I L ---T T I P L A A L I S A C A L T A S Q --- A E E H R Q L G A H V H G H G K L N I A L E K N I L S I E L E A P G A D I 59
Sulfitobacter_marinus/1-223    1 ---MKQ ---A L S --- L I T L V A A L P A L --- A D H A R Q L D A H E H G V G T L D I A I E C T T V A L S F E A P G A D I 54
Roseobacter_denitrificans/1-218 1 ---MK I L ---P L I --- A I S T L I S S P A L --- A E E V R Q L D A H E H G V G H L D I A F E G Q Q I A M Q L H A P G A D I 55

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Shewanella_maritima/1-241      52 F G F E H R A E D K Q Q Q A A V T K F A K L V Q T A N D V V S L N - - A S K L V S A T D N V E K Q F A L A A H A H D K H D D K H E H H D H 119
Pseudomonas_stutzeri/1-204    65 V G F E H A P A T P A D E E K I A E A R A Q L E Q P A T L F G L P K A A D V V S E Q Q L H G E L L G S A H ----- 118
Pseudomonas/1-196             61 V G F E H A A S T D A D K A A V A K A R A Q L E K P L E L F A L P V T A G C S V A S Q E L R S P L F G D K A ----- 114
Pseudomonas_fluorescens/1-202 64 V G F E H A P S T D A D K A K V A A A R T Q L E Q P L V L F S L P K A A A C V V A K Q E L E S P L F G D K P ----- 117
Pseudomonas_putida/1-193      64 V G F E H A A S S D A D K A K V A A V R Q Q L E Q P L K L F L S A A A D C K E E Q Q A L E S P L F G D A A ----- 117
Vibrio_caribbeanicus/1-227    59 V G F E H A P N T S E Q K H L E N A I T T L K D V N N L F T F P A S A G C V A L S Q S V A H T L E D G D D H H N E H ----- D H 119
Photobacterium_lipolyticum/1-193 62 V G F E H P P T S E Q Q Q A V E R A I E E L K Q A T E L F A F T A S A K Q L T E A F I T E N L I T H D ----- 114
Vibrio_cholerae/1-242          63 V G F E H A P Q D D A Q K A L E K A L E T L H H P E K L F A L S D K A Q C E K R E V L I K H T L G G E E Y Q H S H A Y G E D E E H E H Q H 132
Vibrio_paraahaemolyticus/1-209 58 V G F E H A P E N A E Q E K T L Q H A V A T L E D S N A L F A I N P Q A Q C E I E E V H V E H S L G G Q H E E H E H ----- 115
Pseudoceanicola_batsensis/1-220 56 V G F E Y E A T S A E D K D A V E A I R T M L M P E N I V T L P E A A G C R L T E V L A H L H - S G D H D H D A D ----- A H M - 116
Rhodomicrobium_vannielli/1-200 60 A G F E H E A A T K E D K A L E K A K A T L A K G L E L F T P A S A A G C K Q T S A K V A L E - - A E H E H E G E G K E - - E H E H A - 123
Sulfitobacter_marinus/1-223    55 V G F E Y A A T S E A D L A A I A Q A V K T L G A P L D L F V M P D A A G C A V V D A K A E L E - - G D A H G E H E E H - - G D E H H - 118
Roseobacter_denitrificans/1-218 56 V G F E Y E A K T A E D R T S I D S A V A K L A Q P L T L F V L P E A A Q C S V V Q A S A A L E - - S E E H H D H G A H - - D D H - A - 118

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Shewanella_maritima/1-241      120 D K H D H D K H E H H D H D K H D H D K H D H D K H E H H D H D H D H D H D H D H D H E H H D H D K H D H D K H E H H D H D K - H D E H N H A D A E 188
Pseudomonas_stutzeri/1-204    119 ----- A G Y Q R R D Q D E D H R H E H S H A P A D E Y S G H S D V E 149
Pseudomonas/1-196             115 ----- P A H A H K E K A G - - H E H E H - E H E H E H G H A D I H 141
Pseudomonas_fluorescens/1-202 118 ----- D A D D H D D D H D E D A K D T A - C H E H H H E H S E I H 147
Pseudomonas_putida/1-193      118 ----- K A D D D G D - - - - - E H - E H E H D H Q H S D I G 138
Vibrio_caribbeanicus/1-227    120 D E - - - - H K H - - H D E H D H D K H D H D - - - - - E H K H H D E H D H D E H D H D E H K H H D E H D H D E H S G H G E F N 172
Photobacterium_lipolyticum/1-193 115 ----- S H A G H D H E E H D H D S - - - - - H H E H A T H G G F S 139
Vibrio_cholerae/1-242          133 G E - G E H D H K A H D H K D H D H K G H D H A - - - - - A - D E H A D H D H K E H D H K G H E - H E G H D H D - E H Q H G S F T 188
Vibrio_paraahaemolyticus/1-209 116 ----- H D H E G H D H D - - - - - E H A H D H D K H E H D G H - - E G H D H S E H S D H G E F T 154
Pseudoceanicola_batsensis/1-220 117 ----- D D V H E H E H E D H A E D D H D - - - - - H D H E D H G D D H D H E D H A G D D D H D H G E A Q H S E L H 165
Rhodomicrobium_vannielli/1-200 124 ----- - - - - - A E A K D H D H E K G E H H S E F H 142
Sulfitobacter_marinus/1-223    119 ----- D D H A E E S H D D H G H D D H G - - - - - H D D H C H K D D S H D G H D H D H D H A E D A G H T E F H 167
Roseobacter_denitrificans/1-218 119 ----- A D H G H E D H D H A - - - - - A D H C H K D H D K H G - E D H A H D H D A H A A S H S E F H 160

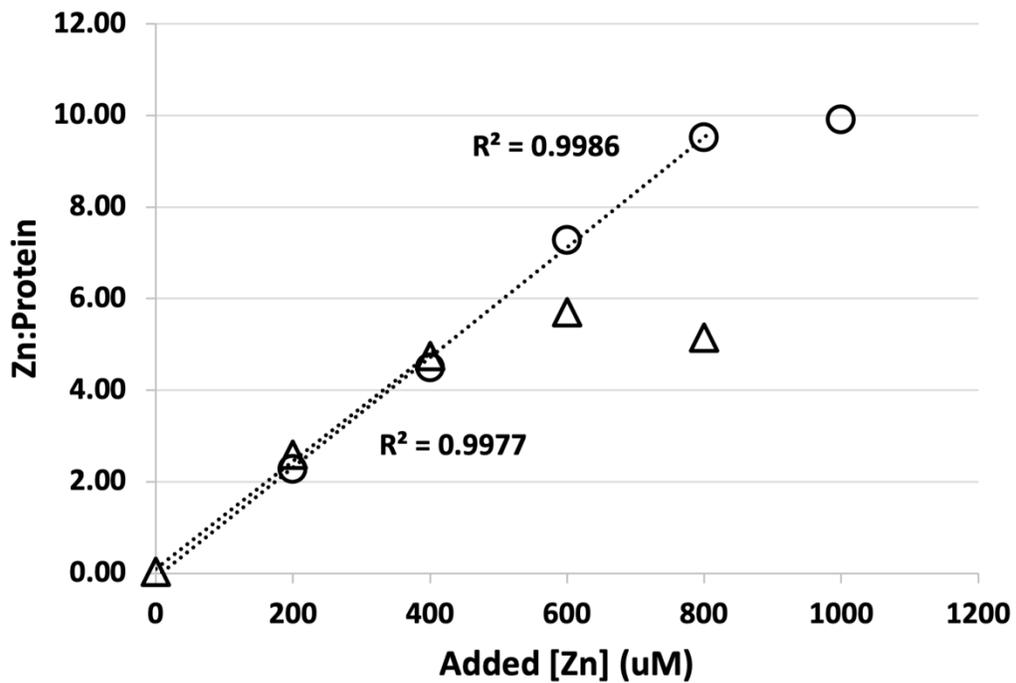
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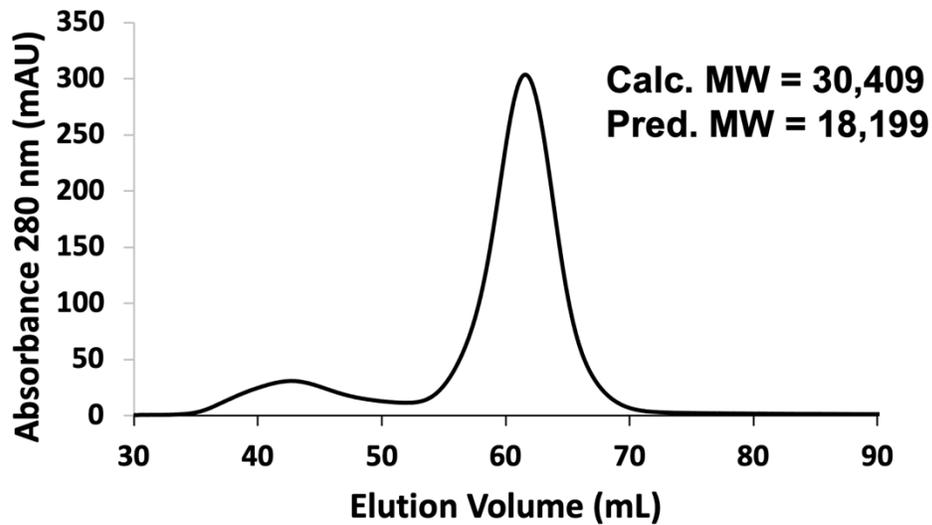
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Pseudomonas_stutzeri/1-204    150 A S Y Q F D C S N P E A L Q A L D L K R L F E H F P G T E K I Q V Q L I G P N G Q Q G - A E L T P A R T Q L P F --- 204
Pseudomonas/1-196             142 A H Y Q L S C E K P E L K L L T L A E F F K R F P A T Q K I Q V Q L I G P D G Q K G - A D L A P A S A E L K L --- 196
Pseudomonas_fluorescens/1-202 148 A H Y Q F T C A K P D A L K T L D L G Q V F K T F P A T Q K I Q V Q L I S P N G Q Q G - V E A S A T A A T L K F --- 202
Pseudomonas_putida/1-193      139 A H Y Q L T C A N P D K L T Q L D L A P L F K A F P A T Q K I N V Q L I G P N G Q K G - V E T T P T K A A V A F --- 193
Vibrio_caribbeanicus/1-227    173 I E Y S Y T C S N I S A L K N I E T - Q W F K H F S N T K S I T V N W L S P D G K I S V V E L G K G D K T I S R --- 227
Photobacterium_lipolyticum/1-193 140 A Q Y A F H C A D I K Q L K D L Q V - S W F K H F S P T E K I A I Q A I T E T S Q K A - E Q L T P T S T L F K F --- 193
Vibrio_cholerae/1-242          189 A Q Y Q F H C E A V D L K Q I D T - Q W F Q Y F P S T E K I Q A N V L T E K Q Q S A - L Q L N A K Q T I L K L --- 242
Vibrio_paraahaemolyticus/1-209 155 V Q Y R F H C A Q V G E L S H I Q T - D W F N Q F P S T E K I Q V N V L F T D T T Q S A - T S L T K S N T Q I A I K --- 209
Pseudoceanicola_batsensis/1-220 166 V S Y A F A C E D E D A L T T I S F - P F D Q F E N A Q E I E A Q Y V T E T G A G Q - A E I T R D A P E L T L E --- 220
Rhodomicrobium_vannielli/1-200 143 V E Y A F E C A A P E K I A S L S F - G Y F K A F P N A Q E L D V T I T P K G Q S S - Y E V T K D K P K L E L K G V M --- 200
Sulfitobacter_marinus/1-223    168 A T Y T L T C D T P D A L T E I N F - A Y F Q T F P N A Q E I D V Q L I S A S G A R A - F E V E R S T P L D L D L E R - --- 223
Roseobacter_denitrificans/1-218 161 A E Y L L T C A D P A A A S E I T F - A Y F D A F P N A R E L E I Q M I S E S G A T A - F E V S R D S P I L D L R G M F --- 218

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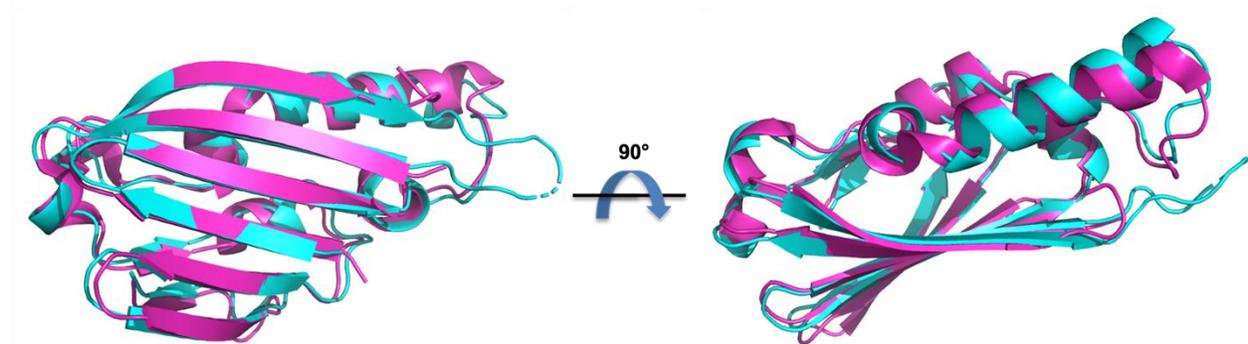
Supplementary Figure S1. Multiple sequence alignment of ZrgA homologues



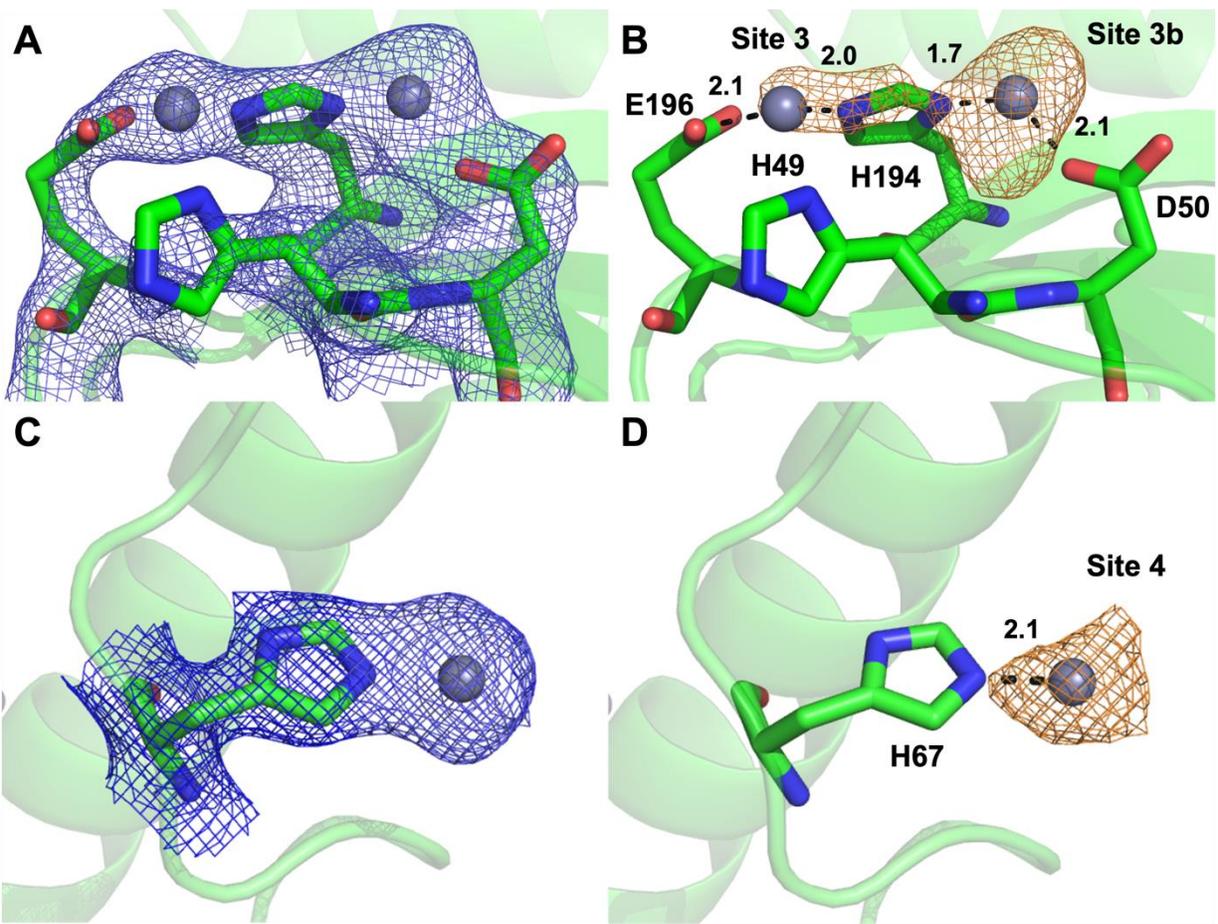
Supplementary Figure S2. Zinc binding to WT (circles) and $\Delta 124-184$ ZrgA (triangles) as determined by incubation of protein with varying concentrations of zinc followed by desalting and zinc quantitation by ICP-OES. Linear fits are applied to the first 5 data points in for WT and the first 3 for $\Delta 124-184$ ZrgA with R^2 values indicated.



Supplementary Figure S3. Size exclusion chromatogram showing purification of ZrgA Δ 124-184 and the molecular weights calculated from the elution time (*calc. MW*) and the primary sequence (*pred. MW*). The elongated structure of ZrgA likely results in a larger apparent MW than expected for a monomer that is nonetheless too small for a dimer.



Supplementary Figure S4. Alignment of crystal structures of Apo PA4063 (PDB ID: 7AHW, magenta) and Apo Δ 124-184 ZrgA (cyan). Figure created with Pymol version 2.5.4, Schrödinger, LLC.



Supplementary Figure S5. Zinc binding sites 3b (A and B) and 4 (C and D) in $\Delta 124-184$ Zrg showing 2Fo-Fc density contoured at 1.0σ (blue mesh, A and C) or anomalous difference density contoured at 2.5σ and zinc ligands with bond distances indicated in Å (orange mesh, B and D). Sites 3 and 3b are shown for the D chain, and site 4 is shown on the B chain. Figure created with Pymol version 2.5.4, Schrödinger, LLC.