Table S1. Primers used in this study

Primer name	**Sequence (5’ to 3’)**
Cloning	
VciB.2659.for	AAGAGCGCTCGCCGAAACTG
VciB.3658.rev	GCCAAATGGGGATCAATCGTTT
VciB.4337.SO.for	ACGATTGATCCCCATTTGGCTTGACTCCCCACGCGGC
VciB.5336.rev	GTCACGGTTAATTGGCGAGTAA
VciA.596.for	GGTGCTACTGTGCTTTCACAC
VciA.1596.rev	GAAGGA TTAACTCTATTTATTATTGACG
VciA.3639.SO.for	CGTCAATAATAAATAGAGTTAATCCTTCACGATTGATCCCCATTTGGCAT
VciA.4638.rev	ACGGATTGAGAGGTAGCGCA
VciB.3337.EcoRI	ATCGGAATTCCCAACGTATCGCGCCTCAAT
VciB.4336.rev.XbaI	ATGCTCTAGATTAACCAAAGATAGCGAACAGTG
BTvciB.EcoRI.for	GATCGAATTCTCGGAGCCTTGCGGCGC
BTvciB.rev	TCAGGTCAACCCCGCCG
BMvciB.EcoRI.1578.for	GATCGAATTCAGCGGTGAGCGCCTGATG
BMvciB.2524.rev	TCAGGTCAGCCCGGCCG
AHvciB.EcoRI.for	GATGGAATTCGATCGCTTCGCCGACAACG
AHvciB.rev	CTAGAGCAGGGCCACCAG
VciB.V5.for.EcoRI	TTACGAATTCAGGCAAGCCCATCCCCAACCCCTTGCTTGGCTTGGACTCCACCGCCGCAATGCAGCGGCAAGTCAAAGG
VciB.V5fix.for.EcoRV	TATGGATATCGGCAAGCCCATCCCCAAC
VciB.fix.rev.EcoRV	GGTGGATATCCATAGCTGTTTCCTGTGTGAAATT
Nqr.1045.for	GGAACACCATCACGGTTCAGT
Nqr.2250.SO.rev	CAATTAGCCAGTTTGAATCGCGAAAGACACGGATGCA
Nqr.7328.for	TCGCGATTCAAACTGGCTAATTG
Nqr.8338.rev	GCCGGCCTGCGTCCTG
NapC.369.for	GGAATATGGCAGGTTGTGAAC
NapC.1268.SO.rev	GCCCAGTGCAATCAGGGTTACAAACGCACGTCTGGTCATTTT
NapC.3731.for	TAACCCTGATTGCACTGGGC
NapC.4630.rev	GTGCGCGGCGTGCTTGG
pET16b.vciB.NdeI.for	GTACCATATGCAGCGGCAAGTCAAAGGG
pET16b.vciB.XhoI.rev	GATCCTCGAGTTAACCAAAGATAGCGAACAGTG
H8.for	GGAATTGTGAGCGGATAACAATTC
VciB.4336.rev	TTAACCAAAGATAGCGAACAGTGT
SCAM mutagenesis	
VciB.C41S.for	GCCGCCTTCATGCATGGTCCGGCTTCTTTACCCTACTC
VciB.C41S.rev	GAGTAGGGTAAAGAAGCCGGACCATGCATGAAGGCGGC
VciB.S18C.for	CGTTGCATAATCAAGCAGGCCGTTAGGCCAGC
VciB.S18C.rev	GCTGGCCTAACGGCCTGCTTGATTATGCAACG
VciB.I34C.for	TGCCGCCGCCTTCATGCATG
VciB.I34C.rev	CCATTTATTGTTGTGTAGAGGCA
VciB.S60C.for	AGCGCGGTGACATAACCAAAGGCCAGTGAG
VciB.S60C.rev	CTCACTGGCCTTTGGTTATGTCACCGCGCT
VciB.S73C.for	GCCATTCACTTTTGCAGGTGTGTGGCCCGG

VciB.S73C.rev	CCGGGCCACACACCTGCAAAAGTGAATGGC
VciB.S75C.for	CGGGCCACACACCAGCAAATGTGAATGGCAACTCTCGCTCAC
VciB.S75C.rev	GTGAGCGAGAGTTGCCATTCACATTTGCTGGTGTGTGGCCCG
VciB.I106C.for	GGCGAACATGAGTGGACGTGTCGCGAAATCGCCGCGC
VciB.I106C.rev	GCGCGGCGATTTCGCGACACGTCCACTCATGTTCGCC
VciB.A110C.for	TGCGCGCAGCAGTTGCCCA
VciB.A110C.rev	GATTTCGCGTATCGTCCACTC
VciB.S139C.for	GTATTGGGCGCAAAAGGTATCGGTCACGCTGG
VciB.S139C.rev	CCAGCGTGACCGATACCTTTTGCGCCCAATAC
VciB.A160C.for	GCAAGCGGTTAAGCACACAGGCCCAGTTGGCTTTTT
VciB.A160C.rev	AAAAAGCCAACTGGGCCTGTGTGCTTAACCGCTTGC
VciB.T171C.for	TGCCAGCCAAGACCGCATCCCATTCCGCGATGC
VciB.T171C.rev	GCATCGCGGAATGGGATGCGGTCTTGGCTGGCA
VciB.L181C.for	CATGGCAACCGCCGCACAGTCGGAAAACAACTGC
VciB.L181C.rev	GCAGTTGTTTTCCGACTGTGCGGCGGTTGCCATG
VciB.S203C.for	TCGTTTCGGGCAGCCGTGGAGTTTGGTCC
VciB.S203C.rev	GGACCAAACTCCACGGCTGCCCGAAACGA
VciB.S215C.for	GTGTGGTCGCGACACAGCCACCTATCAATAG
VciB.S215C.rev	CTATTGATAGGTGGCTGTGTCGCGACCACAC
VciB.G225C.for	GGCCGCTCTAGATTAACAAAAGATAGCGAACAGTG
VciB.G225C.rev	CACTGTTCGCTATCTTTTGTTAATCTAGAGCGGCC
Conserved residue mutagenesis	
VciB.H38N.for	AATAAATGGATCCGCCGCCTTAATGCATGGTGCGGCTTCTTTAC
VciB.H38N.rev	GTAAAGAAGCCGCACCATGCATTAAGGCGGCGGATCCATTTATT
VciB.H61N.for	AACCGCGCTGTCTGGCCA
VciB.H61N.rev	AGATAACCAAAGGCCAGTGAG
VciB.H166N.for	AATCGCGGAATGGGAACGGG
VciB.H166N.rev	CAAGCGGTTAAGCACAGCG
VciB.W33Y.for	CCTGCCTCTACACAACAATAAATATATCCGCCGCCTTCATGCAT
VciB.W33Y.rev	ATGCATGAAGGCGGCGGATATATTTATTGTTGTGTAGAGGCAGG
VciB.W127Y.for	ATGAAGCGCGCGCCACCAG
VciB.W127Y.rev	AGCGGGCTGGGATCAATAAC
VciB.W197Y.for	CCCTGACCAGTTTACTGATGTATACCAAACTCCACGGCTCCC
VciB.W197Y.rev	GGGAGCCGTGGAGTTTGGTATACATCAGTAAACTGGTCAGGG
VciB.Y142W.for	CGATACCTTTAGCGCCCAATGGGTGCCCGGTACCTTAGTCA
VciB.Y142W.rev	TGACTAAGGTACCGGGCACCCATTGGGCGCTAAAGGTATCG
VciB.D180N.for	AACCTTGCGGCGGTTGCC
VciB.D180N.rev	GGAAAACAACTGCCAGCCAAG

A

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                                                                          ↓    ↓
V.cholerae           ---------------MQ------RQVKGDNHRRAGLTASLIMQRRRLPLHNNKWIRRLHA
A.hydrophila         ------------------------------------MKHRAMKRRKLPLHNNKWVRRIHA
M.tundripaludum      -------------------------------MLQTQNPEAPLNRKSKRIQWLKWLRRIHA
H.massiliense        -------------MNLTQR---DALY---PERETMPAERAADNKRSRRAIFLKWLRKMHG
B.thailandensis      MFCANGSRSVNAPESIEKHSGVVLPYPG-PPRKLDESELAERRRRSRRATFIKWLRKVHG
B.mallei             MFCANGSRSVNAPESIEKHSGVVVPYPG-SPRKLDAFELAERRRRSRRATFIKWLRKVHG
                                                                .::       **:*::*.

                                          ↓
V.cholerae           WCGFFTLLLMLLYGLTGLWLSHRAV-WPLPGPHTSKSEWQLSLTEPIDS-VSTFQAQLNT
A.hydrophila         WAGFATLTLMLLYGLTGLWLQHRAV-LPLPGPHTDKSSEEIVLSAPLTS-PDELKSLLQQ
M.tundripaludum      WVGLWGAALGLLFGVSGILLNHRMV-MKIPAVQMEQSHIEMALPQPLPVDAKALALWLQV
H.massiliense        WIGLWGAVLGLLFGSTGVLLNHRAV-MKIPAAHAQESTLQLPLPDPTPADATEMVEWLQR
B.thailandensis      WIGLWGAVLGLLFGVTGVLLNHRAPPLKISSGEPQVSQIQLALPSPAPATPHAMAAWLRR
B.mallei             WVGLWGAALGLLFGVTGVLLNHRAPPLKISSGEPQVSQLQLALPSPAPATPHAMAAWIRR
                     * *:    * **:* :*: *.**     : . . . *  :: *  *       :   :.

                                                    ↓              ↓
V.cholerae           EYGE--HEWTIREIAAQQLPTPQQPLLIPARWEARATSVTDTFSAQYVPGTLVIQAQHQK
A.hydrophila         RYAAGFEEGRVQLTPPQTLPTPDGPLTLPARWEMRGVTLSQSVAASYVAGTLLVRTELQQ
M.tundripaludum      QLSIDREASKISIDPEKTVTWADRNLQQPSQWRVDFHSPQQSVTAEYWVGNTYVSIKRQD
H.massiliense        ELGFDRPASRVRSEPARPVAWGDKSLKQPARWSAMFSSARMNAQVEYWVGNNFVSVKRSD
B.thailandensis      ELAFDGRLGRVRKDSAQPVAWGDRRVEQPEHWQFGLFGPSRNVQVEYWKGNGYVSVKRTD
B.mallei             ELAFDGRLGRVRKEPAQPVAWGGRRVEQPEHWQFGLFGPRSNVQVEYWKGNGYVSVKRTD
                     . .       :     : :      :  * :*         .  ..*  *.  :  :  .

                               ↓             ↓                ↓
V.cholerae           ANWAAVLNRLHRGMGTGLGWQLFSDLAAVAMLLLALTSLLMWTKLHGSPKRAGWLLIGGS
A.hydrophila         ANFAASLNRLHRGMGTGLPWQLVGDLAALAMLLLALTSLLMWNKLHGPAARGIALLLLGA
M.tundripaludum      ANAFAFITRLHKGVGMGTAWVLLADTLAGGLVFLSLTGLLLWTKLHGSRLAMAGLGLTSL
H.massiliense        NNVFATLNNLHKGVGGSIAWILLVDTLAGSIILLSITGVLLWAMMNRRRMLGAGIGFAGL
B.thailandensis      NAFLTTLNNLHRGVGMNLFWVLLMDTIAGSMVLLSLTGVLLWTELNKRRTVGVILVAGSV
B.mallei             NAFLTALNNLHRGVGMNLFWVLLMDTIAGSMVLLSLTGVLLWTELNKRRTVGVVLVAGSV
                         : :..**:*:* .  * *. *  * .:::*::*.:*:*  ::        :   .

V.cholerae           VATTLFAIFG-
A.hydrophila         LVTVLVALL--
M.tundripaludum      GSMVFITLNSL
H.massiliense        AATVILAMQAM
B.thailandensis      AAALAAGLT--
B.mallei             AAALAAGLT--
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B

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Aeromonas hydrophila BG-2 coding region
ATGAAGCACCGAGCGATGAAACGCAGAAAACTCCCCCTGCACAACAACAAGTGGGTGCGCCGCATCCACGCCTGGG
CCGGCTTTGCCACCCTCACCCTGATGCTGCTCTACGGCCTCACCGGCCTCTGGCTGCAACACAGGGCCGTGCTGCC
GCTGCCCGGCCCCCACACCGATAAATCCAGCGAAGAGATAGTGCTGAGCGCGCCGCTGACCAGCCCGGATGAGCTG
AAAAGCCTGCTGCAACAACGCTATGCAGCAGGGTTCGAGGAGGGGCGCGTCCAGCTCACCCCGCCCCAGACCCTGC
CCACCCCCGACGGCCCGCTCACCCTGCCGGCCCGCTGGGAGATGCGCGGCGTCACCCTCAGCCAGAGCGTGGCCGC
CAGCTATGTGGCGGGCACATTGCTGGTGCGCACCGAGCTGCAGCAAGCCAACTTTGCCGCCAGCCTCAACCGGCTG
CACCGTGGCATGGGCACCGGCCTGCCGTGGCAGCTGGTGGGGGATCTGGCGGCGCTGGCCATGCTGCTGCTGGCCC
TCACCAGCCTGCTGATGTGGAACAAGCTGCACGGCCCGGCCGCGCGTGGCATCGCTCTGCTGCTGCTGGGCGCCCT
GGTCACCGTGCTGGTGGCCCTGCTCTAG
```

Figure S1. Sequence alignment for tested orthologs and *A. hydrophila* isolate BG-2 coding sequence. (A) Alignment of protein sequences was performed using Clustal Omega (43). *Vibrio cholerae*, *Aeromonas hydrophila, Methylobacter tundripaludum*, *Herbaspirillum massiliense*, *Burkholderia thailandensis*, and *Burkholderia mallei* are shown. Arrows indicate residues targeted for mutagenesis, Red arrows indicate essential residues (His38 and His166 in *V. cholerae*). **(B)** The *vciB* locus of the *Aeromonas hydrophila* isolate BG-2 obtained from the Texas Department of Health was sequenced after cloning.

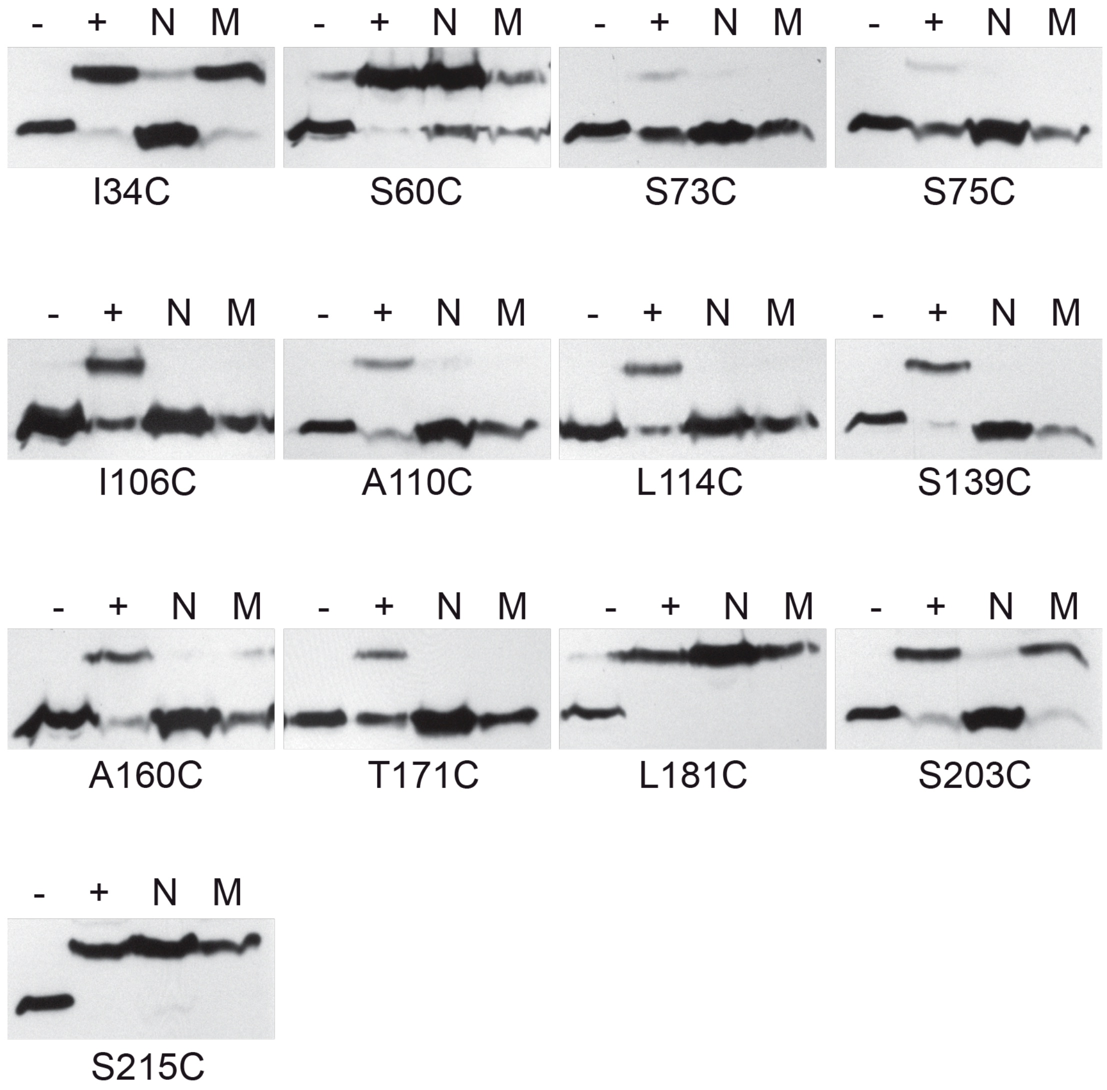

Figure S2. Panel of SCAM Cys mutants. Mutants generated in the V5-tagged, Cys-less (C41S) background were tested for Cysteine accessibility to the solvent using NEM and MTSES reagents. Subsequent treatment with MalPEG was used to visualize initial blocking by NEM and MTSES. – indicates the no treatment negative control, + indicates the MalPEG treated positive control, N indicates samples pretreated with NEM and M indicates samples pretreated with MTSES.