Supplemental Material for:

Structure-function analysis of the LytM domain of EnvC, an activator of cell wall remodeling at the *Escherichia coli* division site

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Supplemental Methods and Materials

Plasmid construction

Plasmids used in this study are listed below. In all cases PCR was performed using KOD polymerase (Novagen) according to the manufacturer's instructions. Unless otherwise indicated, MG1655 chromosomal DNA was used as the template. Restriction sites for use in plasmid constructions are bold, italicized and underlined in the primer sequences given below. Plasmid DNA and PCR fragments were purified using the Qiaprep spin miniprep kit (Qiagen) or the Qiaquick PCR purification kit (Qiagen), respectively.

pBD4:

The plasmid pBD4 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(Y350A)] was constructed as follows. The primer 5'-CTCAACCACCACCACCAGAC**CG**GCGCCTTGCAGCCAGTCAGCCAG-3' was used for single oligonucleotide mutagenesis with pTU115 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*] (Uehara *et al.*, 2010).

pDY280:

The plasmid pDY280 [*bla* P_{T7}::*H-SUMO*-^{lyt}*envC*] was constructed by digesting the ^{lyt}*envC* fragment from pNP33 using *Bam*HI and *Hind*III and ligating the resulting fragment to pTD68 [*bla* P_{T7}::*H-SUMO*] (Uehara *et al.*, 2010) digested with the same enzymes.

pDY281:

The plasmid pDY281 [*bla* P_{T7}::*H-SUMO*-^{lyt}*envC*(K321E)] was constructed by digesting the ^{lyt}*envC*(K321E) fragment from pNP34 using *Bam*HI and *Hind*III and ligating the resulting fragment to pTD68 [*bla* P_{T7}::*H-SUMO*] (Uehara *et al.*, 2010) digested with the same enzymes.

pDY282:

The plasmid pDY282 [*bla* P_{T7}::*H-SUMO*-^{lyt}*envC*(R405H)] was constructed by digesting the ^{lyt}*envC*(R405H) fragment from pNP35 using *Bam*HI and *Hind*III and ligating the resulting fragment to pTD68 [*bla* P_{T7}::*H-SUMO*] (Uehara *et al.*, 2010) digested with the same enzymes.

pDY283:

The plasmid pDY283 [*bla* P_{T7}::*H-SUMO*-^{lyt}*envC*(Y350A)] was constructed by digesting the ^{lyt}*envC*(Y350A) fragment from pNP36 using *Bam*HI and *Hind*III and ligating the resulting fragment to pTD68 [*bla* P_{T7}::*H-SUMO*] (Uehara *et al.*, 2010) digested with the same enzymes.

pDY286:

The plasmid pDY286 [*bla* P_{T7}::*H-SUMO*-^{lyt}*envC*(V353A)] was constructed by digesting the ^{lyt}*envC*(V353A) fragment from pNP39 using *Bam*HI and *Hind*III and ligating the resulting fragment to pTD68 [*bla* P_{T7}::*H-SUMO*] (Uehara *et al.*, 2010) digested with the same enzymes.

pFC2:

The plasmid pFC2 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(K321E)] was constructed as follows. The primer 5'-GAAGCACCGATAACCATACCT**T**CCCAGCGTAATTCACCCTGTA-3' was used for single oligonucleotide mutagenesis on pTU115 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*] (Uehara *et al.*, 2010).

pNP31:

The plasmid pNP31 [*tetA* P_{lac}::^{fl}*envC*] was constructed as follows. The *envC* containing *Xba*I/*Hind*III fragment from pTD25 [*cat* P_{ara}::^{fl}*envC*] was used to replace the corresponding fragment of pMM60 [*tetA* P_{lac}::*ycfM*-^{sf}*gfp*] (Peters *et al.*, 2011).

pNP33:

The plasmid pNP33 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*] was constructed as follows. The primers 5'-GTCA***GGATCC***GGTACCGAAAGCGAAAAATCGCTGATG-3' and 5'-GCAT***AAGCTT***TTATTATCTTCCCAACCACGGCTG-3' were used to amplify ^{lyt}*envC* from pTU115 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*] and eliminate GTTTTGTTTCCATTTCGTCGTAACGTTCTTGCATTTGCCGCTCTGTTGGCGCTCTCC, which was native *envC* sequence downstream of the stop codon. The resulting PCR product was purified and digested with *Bam*HI and *Hind*III and ligated into pTU115. pNP33 is identical to pTU115 except that it lacks chromosomal sequence downstream of the *envC* stop codon present in pTU115.

pNP34:

The plasmid pNP34 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(K321E)] was constructed as follows. The primers 5'-GTCA***GGATCC***GGTACCGAAAGCGAAAAATCGCTGATG-3' and 5'-GCAT***AAGCTT***TTATTATCTTCCCAACCACGGCTG-3' were used to amplify ^{lyt}*envC* from pFC2 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(K321E)] and eliminate

GTTTTGTTTCCATTTCGTCGTAACGTTCTTGCATTTGCCGCTCTGTTGGCGCTCTCC, which was native *envC* sequence downstream of the stop codon. The resulting PCR product was purified and digested with *Bam*HI and *Hind*III and ligated into pTU115.

pNP35:

The plasmid pNP35 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(R405H)]was constructed as follows. The primers 5'-GTCA***GGATCC***GGTACCGAAAGCGAAAAATCGCTGATG-3' and 5'-GCAT***AAGCTT***TTATTATCTTCCCAACCACGGCTG-3' were used to amplify ^{lyt}*envC* from pTU206-63 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(R405H)] and eliminate GTTTTGTTTCCATTTCGTCGTAACGTTCTTGCATTTGCCGCTCTGTTGGCGCTCTCC, which was native *envC* sequence downstream of the stop codon. The resulting PCR product was purified and digested with *Bam*HI and *Hind*III and ligated into pTU115.

pNP36:

The plasmid pNP36 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(Y350A)] was constructed as follows. The primers 5'-GTCA***GGATCC***GGTACCGAAAGCGAAAAATCGCTGATG-3' and 5'-GCAT***AAGCTT***TTATTATCTTCCCAACCACGGCTG-3' were used to amplify ^{lyt}*envC* from pBD4 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(Y350A)] and eliminate GTTTTGTTTCCATTTCGTCGTAACGTTCTTGCATTTGCCGCTCTGTTGGCGCTCTCC, which was native *envC* sequence downstream of the stop codon. The resulting PCR product was purified and digested with *Bam*HI and *Hind*III and ligated into pTU115.

pNP37:

The plasmid pNP37 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(N412K)] was constructed as follows. The primers 5'-GTCA***GGATCC***GGTACCGAAAGCGAAAAATCGCTGATG-3' and 5'-GCAT***AAGCTT***TTATTATCTTCCCAACCACGGCTG-3' were used to amplify ^{lyt}*envC* from pTU206-2 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(N412K)] and eliminate GTTTTGTTTCCATTTCGTCGTAACGTTCTTGCATTTGCCGCTCTGTTGGCGCTCTCC, which was native *envC* sequence downstream of the stop codon. The resulting PCR product was purified and digested with *Bam*HI and *Hind*III and ligated into pTU115.

pNP38:

The plasmid pNP38 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(L400P)] was constructed as follows. The primers 5'-GTCA***GGATCC***GGTACCGAAAGCGAAAAATCGCTGATG-3' and 5'-GCAT***AAGCTT***TTATTATCTTCCCAACCACGGCTG-3' were used to amplify ^{lyt}*envC* from pTU206-5 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(L400P)] and eliminate GTTTTGTTTCCATTTCGTCGTAACGTTCTTGCATTTGCCGCTCTGTTGGCGCTCTCC, which was native *envC* sequence downstream of the stop codon. The resulting PCR product was purified and digested with *Bam*HI and *Hind*III and ligated into pTU115.

pNP39:

The plasmid pNP39 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(V353A)] was constructed as follows. The primers 5'-GTCA***GGATCC***GGTACCGAAAGCGAAAAATCGCTGATG-3' and 5'-GCAT***AAGCTT***TTATTATCTTCCCAACCACGGCTG-3' were used to amplify ^{lyt}*envC* from pTU206-6 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(V353A)] and eliminate

GTTTTGTTTCCATTTCGTCGTAACGTTCTTGCATTTGCCGCTCTGTTGGCGCTCTCC, which was native *envC* sequence downstream of the stop codon. The resulting PCR product was purified and digested with *Bam*HI and *Hind*III and ligated into pTU115.

pNP46: The plasmid pNP46 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{35\text{-}419}$*envC*] was constructed as follows. The *envC* containing *Asc*I/*Hind*III fragment of pNP33 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*] was used to replace the corresponding fragment of pTU113 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{35\text{-}419}$*envC*], eliminating GTTTTGTTTCCATTTCGTCGTAACGTTCTTGCATTTGCCGCTCTGTTGGCGCTCTCC, which was native *envC* sequence downstream of the stop codon.

pNP52:

The plasmid pNP52 [*bla* P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*(Y350A)] was constructed as follows. The ^{lyt}*envC*(Y350A) containing *Asc*I/*Hind*III fragment of pNP36 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(Y350A)] was used to replace the corresponding fragment of pTU153 [*bla* P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*], thus including the mutation while removing GTTTTGTTTCCATTTCGTCGTAACGTTCTTGCATTTGCCGCTCTGTTGGCGCTCTCC, native *envC* sequence downstream of the stop codon in pTU153.

pNP53:

The plasmid pNP53 [*bla* P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*(R405H)] was constructed as follows. The ^{lyt}*envC*(R405H) containing *Asc*I/*Hind*III fragment of pNP35 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(R405H)] was used to replace the corresponding fragment of pTU153 [*bla* P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*], thus including the mutation while removing

GTTTTGTTTCCATTTCGTCGTAACGTTCTTGCATTTGCCGCTCTGTTGGCGCTCTCC, native *envC* sequence downstream of the stop codon in pTU153.

pNP54:

The plasmid pNP54 [*bla* P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*(K321E)] was constructed as follows. The ^{lyt}*envC*(K321E) containing *Asc*I/*Hind*III fragment of pNP34 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(K321E)] was used to replace the corresponding fragment of pTU153 [*bla* P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*], thus including the mutation while removing GTTTTGTTTCCATTTCGTCGTAACGTTCTTGCATTTGCCGCTCTGTTGGCGCTCTCC, native *envC* sequence downstream of the stop codon in pTU153.

pNP55:

The plasmid pNP55 [*bla* P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*] was constructed as follows. The ^{lyt}*envC* containing *Asc*I/*Hind*III fragment of pNP33 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*] was used to replace the corresponding fragment of pTU153 [*bla* P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*], removing GTTTTGTTTCCATTTCGTCGTAACGTTCTTGCATTTGCCGCTCTGTTGGCGCTCTCC, which was native *envC* sequence downstream of the stop codon in pTU153.

pNP56:

The plasmid pNP56 [*bla* P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*(V353A)] was constructed as follows. The ^{lyt}*envC*(V353A) containing *Asc*I/*Hind*III fragment of pNP39 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(V353A)] was used to replace the corresponding fragment of pTU153 [*bla* P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*], thus including the mutation while removing

GTTTTGTTTCCATTTCGTCGTAACGTTCTTGCATTTGCCGCTCTGTTGGCGCTCTCC, native *envC* sequence downstream of the stop codon in pTU153.

pNP60:

The plasmid pNP60 [*tetA* P_{lac}::^{fl}*envC*(R405H)] was constructed as follows. The ^{lyt}*envC*(R405H) containing *Asc*I/*Hind*III fragment of pNP35 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(R405H)] was used to replace the corresponding fragment of pNP31 [*tetA* P_{lac}::^{fl}*envC*].

pNP61:

The plasmid pNP59 [*tetA* P_{lac}::^{fl}*envC(V353A)*] was constructed as follows. The ^{lyt}*envC*(V353A) containing *Asc*I/*Hind*III fragment of pNP39 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(V353A)] was used to replace the corresponding fragment of pNP31 [*tetA* P_{lac}::^{fl}*envC*].

pNP63:

The plasmid pNP63 [*tetA* P_{lac}::^{fl}*envC*(Y350A)] was constructed as follows. The ^{lyt}*envC*(Y350A) containing *Asc*I/*Hind*III fragment of pNP36 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(Y350A)] was used to replace the corresponding fragment of pNP31 [*tetA* P_{lac}::^{fl}*envC*].

pNP65:

The plasmid pNP65 [*tetA* P_{lac}::^{fl}*envC*(K321E)] was constructed as follows. The ^{lyt}*envC(K321E)* containing *Asc*I/*Hind*III fragment of pNP34 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(K321E)] was used to replace the corresponding fragment of pNP31 [*tetA* P_{lac}::^{fl}*envC*].

pNP99: The plasmid pNP99 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{35\text{-}419}$*envC*(Y401E)] was constructed as follows. The internal primers 5'-GCCTTCACTC**GAG**TTCGAAATTCGCCGCCAGGG-3', 5'-GAATTTCGAA**CTC**GAGTGAAGGCCGACCCTGAC-3' and external primers 5'-GCAT***GGATCC***GGTACCGAAAGCGA-3', 5'-TGAGTGACACAGGAACACTTAACGGCTG-3' were used for overlap extension site directed mutagenesis of pNP46 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{35\text{-}419}$*envC*]. The resulting $^{35\text{-}419}$*envC*(Y401E) *Bam*HI/*Hind*III fragment was used to replace the corresponding fragment of pTU121 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{27\text{-}379}$*nlpD*].

pNP100: The plasmid pNP100 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{35\text{-}419}$*envC*(V324E)] was constructed as follows. The internal primers 5'-GAAAGGTATG**GAG**ATCGGTGCTTCTGAAGGTAC-3', 5'-AAGCACCGAT**CTC**CATACCTTTCCAGCGTAATT-3' and external primers 5'-GCAT***GGATCC***GGTACCGAAAGCGA-3', 5'-TGAGTGACACAGGAACACTTAACGGCTG-3' were used for overlap extension site directed mutagenesis of pNP46 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{35\text{-}419}$*envC*]. The resulting $^{35\text{-}419}$*envC*(V324E) *Bam*HI/*Hind*III fragment was used to replace the corresponding fragment of pTU121 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{27\text{-}379}$*nlpD*].

pNP106: The plasmid [*bla* P_{lac}::^{ss}*dsbA-mCherry*-$^{35\text{-}419}$*envC*(Y401E)] was constructed as follows. The $^{35\text{-}419}$*envC(Y401E)* containing *Asc*I/*Hind*III fragment of pNP99 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{35\text{-}419}$*envC*(Y401E)] was used to replace the corresponding fragment of pTU153 [*bla* P_{lac}::^{ss}*dsbA-mCherry*-$^{35\text{-}419}$*envC*].

pNP107: The plasmid [*tetA* P_{lac}::^{fl}*envC*(V324E)] was constructed as follows. The $^{35\text{-}419}$*envC*(V324E) containing *Asc*I/*Hind*III fragment of pNP100 [*bla* P_{lac}::^{ss}*dsbA*-

^{sf}*gfp*-$^{35\text{-}419}$*envC*(V324E)] was used to replace the corresponding fragment of pNP31 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{35\text{-}419}$*envC*(Y401E)].

pNP108: The plasmid [*bla* P_{lac}::^{ss}*dsbA-mCherry*-$^{35\text{-}419}$*envC*(V324E)] was constructed as follows. The $^{35\text{-}419}$*envC*(V324E) containing *Asc*I/*Hind*III fragment of pNP100 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{35\text{-}419}$*envC*(V324E)] was used to replace the corresponding fragment of pTU153 [*bla* P_{lac}::^{ss}*dsbA-mCherry*-$^{35\text{-}419}$*envC*].

pNP109: The plasmid [*tetA* P_{lac}::^{fl}*envC*(Y401E)] was constructed as follows. The $^{35\text{-}419}$*envC*(Y401E) containing *Asc*I/*Hind*III fragment of pNP99 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{35\text{-}419}$*envC*(Y401E)] was used to replace the corresponding fragment of pNP31 [*tetA* P_{lac}::^{fl}*envC*].

pTB280: The plasmid pTB280 [*bla*(M182T)] was constructed as follows. The primers 5'-GAGCGTGACACC**ACG**ACGCCTGTAGCAATGGCA-3' and 5'-TGCCATTGCTACAGG**CGT**CGTGGTGTCACGCTC-3' were used for site directed mutagenesis to create the (M182T) mutation in the beta lactamase gene on the template plasmid pTD16 [*bla*].

pTD50: The plasmid pTD50 [*cat* P_{ara}:: ^{ss}*dsbA-bla*] was constructed as follows. The *bla*(M182T) containing *Avr*II/*Hind*III fragment of pTB280 [*bla*(M182T)] was used to replace the corresponding fragment of pTB286 [*cat* P_{ara}:: ^{ss}*dsbA*-^{sf}*gfp*].

pTD51: The plasmid pTD51 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*] was constructed as follows. The primers 5'-GCAGTATGAAGATCT**G**GAGGGTCCGGCTGGTC-3' and 5'-GACCAGCCGGACCCTC**C**AGATCTTCATACTGC-3' were used for site directed mutagenesis to eliminate the *Xho*I restriction site at the junction between the ^{ss}*dsbA* and the linker.

pTD52: The plasmid pTD52 [*cat* P_{ara}:: ^{ss}*dsbA*-^{sf}*gfp-bla*] was constructed as follows. The *Xba*I/*Hind*III containing fragment of pTD51 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*] was used to replace the corresponding fragment of pTD50 [*cat* P_{ara}:: ^{ss}*dsbA-bla*].

pTD70: The plasmid pTD70 [*cat* P_{ara}:: ^{ss}*dsbA*-^{sf}*gfp-bla*] was constructed as follows. The primers 5'-GATCCCAGTAATAACAGCAG**GCATGC**CAGCAGCAGC-3' and 5'-TCGAGCTGCTGCTG**GCATGC**CTGCTGTTATTACTGG-3' were annealed to pTD52 to add a *Sph*I site in the linker portion of the ^{ss}*dsbA*-^{sf}*gfp-bla* fusion.

pTU115: The plasmid pTU115 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*] was constructed as follows. The primers 5'-GTCA***GGATCC***GGTACCGAAAGCGAAAAATCGCTGATG-3' and 5'-GTCA***AAGCTT***GGAGAGCGCCAACAGAGCGGC-3' were used to create an $^{278\text{-}419}$*envC* fragment (^{lyt}*envC*) from MG1655 adding *Bam*HI/*Hind*III restriction sites. The resulting fragment replaced the corresponding fragment from pTB263 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*].

pTU121: The plasmid pTU121 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{27\text{-}379}$*nlpD*] was constructed as follows. The primers *5'-GTCA**GGATCC**TCTGACACTTCAAATCCACCGGC-3'* and 5'-GTCA***AAGCTT***CCGCCGATTTATCGCTGC-3' were used to create an $^{27\text{-}419}$*nlpD* fragment from

MG1655 adding *Bam*HI/*Hind*III restriction sites. The resulting fragment replaced the corresponding fragment from pTB263 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*].

pTU153: The plasmid pTU153 [*bla* P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*] was constructed as follows. The $^{35\text{-}419}$*envC* containing *Bam*HI/*Hind*III fragment from pTU113 [*bla* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{35\text{-}419}$*envC*] was used to replace the corresponding fragment from pTU136 [*bla* P_{lac}::^{ss}*dsbA*-*mCherry*].

pTU206-2: The plasmid pTU206-2 [*cat* P_{ara}:: ^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(N412K)-*bla*] was constructed as follows. Mutagenic PCR was performed using MG1655 genomic DNA and primers 5'-GTCA***GGATCC***GGTACCGAAAGCGAAAAATCGCTGATG-3' and 5'-GTCA***CTCGAG***TCTTCCCAACCACGGCTGTGG-3'. The primers also created *Bam*HI and *Xho*I restrictions sites, used to incorporate the mutagenized fragment into pTD70.

pTU206-5: The plasmid pTU206-2 [*cat* P_{ara}:: ^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(L400P)-*bla*] was constructed as follows. Mutagenic PCR was performed using MG1655 genomic DNA and primers 5'-GTCA***GGATCC***GGTACCGAAAGCGAAAAATCGCTGATG-3' and 5'-GTCA***CTCGAG***TCTTCCCAACCACGGCTGTGG-3'. The primers also created *Bam*HI and *Xho*I restrictions sites, used to incorporate the mutagenized fragment into pTD70.

pTU206-6: The plasmid pTU206-2 [*cat* P_{ara}:: ^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(V353A)-*bla*] was constructed as follows. Mutagenic PCR was performed using MG1655 genomic DNA and primers 5'-GTCA***GGATCC***GGTACCGAAAGCGAAAAATCGCTGATG-3' and 5'-

GTCA***CTCGAG***TCTTCCCAACCACGGCTGTGG-3'. The primers also created *Bam*HI and *Xho*I restrictions sites, used to incorporate the mutagenized fragment into pTD70.

pTU206-63: The plasmid pTU206-2 [*cat* P_{ara}:: ^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(R405H)-*bla*] was constructed as follows. Mutagenic PCR was performed using MG1655 genomic DNA and primers 5'-GTCA***GGATCC***GGTACCGAAAGCGAAAAATCGCTGATG-3' and 5'-GTCA***CTCGAG***TCTTCCCAACCACGGCTGTGG-3'. The primers also created *Bam*HI and *Xho*I restrictions sites, used to incorporate the mutagenized fragment into pTD70.

Table S1. Data collection and refinement statistics

Data collection	
X-ray source	ESRF beamline ID14EH1
Scan range (°)	360
Oscillation (°)	0.5
Space group	P31
Unit-cell parameters	
a, Å	57.4
b, Å	57.4
c, Å	129.3
α, °	90
β, °	90
γ, °	120
Resolution (last shell), Å	1.56 (1.66-1.56)
Completeness (last shell), %	96.4 (73.0)
$I/\sigma(I)$ (last shell)	15.9 (2.8)
Rsym† (last shell), %	7.6 (49.3)
No of unique reflections	64864
Wilson B factor, (Å²)	19.02
Refinement and model statistics	
Resolution (last shell), Å	1.57 (1.65-1.57)
R-factor‡, R-free§(last shell)	0.166, 0.207 (0.266, 0.315)
Molecules/asymmetric unit	4
rmsd from target[i]	
Bond lengths, Å	0.0135
Bond angle, °	1.639
Average B-factor, Å²	14.9
Mean B factor (Å²)	14.897
Ramachandran plot**	
Core, %	91.2
Additionally allowed, %	8.8
Generously allowed, %	0
Disallowed, %	0

†Rsym = (Σ(ABS(*I*(h,i)-(*I*(h)))) / (Σ(*I*(h,i))).
‡R-factor = ΣjjFoj - jFcjj/ΣjFoj where Fo and Fc are the observed and calculated structure factor amplitudes, respectively.
§R-free is the R-factor calculated with 5% of the reflections chosen at random and omitted from refinement.
[i]rmsd of bond lengths and bond angles from ideal geometry.
**Performed by Procheck.

Table S2. Strains used in this study.

Strain	Genotype[a]	Source/Reference[b]
DH5α	*F– hsdR17 deoR recA1 endA1 phoA supE44 thi-1 gyrA96 relA1 Δ(lacZYA-argF)U169 ϕ80dlacZΔM15*	Gibco BRL
BL21(λDE3)	*ompT rB– mB– (PlacUV5::T7gene1)*	Novagen
BW25113	*Δ(araD-araB)567 ΔlacZ4787(::rrnB-3) rph-1 Δ(rhaD-rhaB)568 hsdR514*	(Baba *et al.*, 2006)
TB10	*rph1 ilvG rfb-50 λΔcro-bio nad::Tn10*	(Johnson *et al.*, 2004)
TB28	MG1655 *ΔlacIZYA*::*frt*	(Bernhardt and de Boer, 2004)
TB134	TB28 *ΔenvC*::Kan^r	(Bernhardt and de Boer, 2004)
TB145	TB28 *ΔnlpD::frt*	(Peters *et al.*, 2011)
HC260	TB10 *zapA*-GFP Cam^r	(Peters *et al.*, 2011)
HC262	TB28 *envC::frt zapA*-GFP Cam^r	(Peters *et al.*, 2011)
NP1	TB28 *zapA*-GFP *frt*	(Peters *et al.*, 2011)
NP32	TB28 *envC::frt zapA*-GFP *frt*	(Peters *et al.*, 2011)
NP130	TB28 *ΔnlpD::frt ΔenvC::Kan^r*	P1(TB145) xTB134

[a] The Kan^R cassette is flanked by *frt* sites for removal by FLP recombinase. An *frt* scar remains following removal of the cassette using FLP expressed from pCP20.

[b] Strain constructions by P1 transduction are described using the shorthand: P1(donor) x recipient. In all cases transductants were selected on LB Kan plates.

Table S3. Plasmids used in this study.

Plasmid	Genotype	Origin	Source or reference
pBD4	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(Y350A)	R6K	This Study
pCP20	*bla cat c*I875 *repA*(Ts) PR::*flp*	pSC101	(Datsenko and Wanner, 2000)
pDY280	*bla lacI*^{q} P_{T7}*::H-SUMO*-^{lyt}*envC*	pBR/colE1	This Study
pDY281	*bla lacI*^{q} P_{T7}*::H-SUMO*-^{lyt}*envC*(K321E)	pBR/colE1	This Study
pDY282	*bla lacI*^{q} P_{T7}*::H-SUMO*-^{lyt}*envC*(R405H)	pBR/colE1	This Study
pDY283	*bla lacI*^{q} P_{T7}*::H-SUMO*-^{lyt}*envC*(Y350A)	pBR/colE1	This Study
pDY286	*bla lacI*^{q} P_{T7}*::H-SUMO*-^{lyt}*envC*(V353A)	pBR/colE1	This Study
pFC2	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(K321E)	R6K	This Study
pINT-ts	*bla c*I875 *repA*(Ts) P_{R}::*intλ*	pSC101	(Haldimann and Wanner, 2001)
pMM60	*att*HK022 *tetA tetR lacI*^{q} P_{lac}::*ycfM*-^{sf}*gfp*	R6K	(Peters *et al.*, 2011)
pNP31	*att*HK022 *tetA tetR lacI*^{q} P_{lac}::^{fl}*envC*	R6K	This Study
pNP33	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*	R6K	This Study
pNP34	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(K321E)	R6K	This Study
pNP35	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(R405H)	R6K	This Study
pNP36	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(Y350A)	R6K	This Study
pNP37	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(N412K)	R6K	This Study
pNP38	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(L400P)	R6K	This Study
pNP39	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(V353A)	R6K	This Study

pNP46	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{35\text{-}419}$*envC*	R6K	This Study
pNP52	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*(Y350A)	R6K	This Study
pNP53	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*(R405H)	R6K	This Study
pNP54	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*(K321E)	R6K	This Study
pNP55	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*	R6K	This Study
pNP56	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*(V353A)	R6K	This Study
pNP60	*att*HK022 *tetA tetR lacI*^{q} P_{lac}::^{fl}*envC*(R405H)	R6K	This Study
pNP61	*att*HK022 *tetA tetR lacI*^{q} P_{lac}::^{fl}*envC*(V353A)	R6K	This Study
pNP63	*att*HK022 *tetA tetR lacI*^{q} P_{lac}::^{fl}*envC*(Y350A)	R6K	This Study
pNP65	*att*HK022 *tetA tetR lacI*^{q} P_{lac}::^{fl}*envC*(K321E)	R6K	This Study
pNP99	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{35\text{-}419}$*envC*(Y401E)	R6K	This Study
pNP100	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{35\text{-}419}$*envC*(V324E)	R6K	This Study
pNP106	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*(Y401E)	R6K	This Study
pNP107	*att*HK022 *tetA tetR lacI*^{q} P_{lac}::^{fl}*envC*(V324E)	R6K	This Study
pNP108	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-*mCherry*-$^{35\text{-}419}$*envC*(V324E)	R6K	This Study
pNP109	*att*HK022 *tetA tetR lacI*^{q} P_{lac}::^{fl}*envC*(Y401E)	R6K	This Study
pTB263	*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA*-^{sf}*gfp*	R6K	(Uehara *et al.*, 2009)
pTB280	*att*HK022 *bla*(M182T) *lacI*^{q}	R6K	This Study
pTB286	*attλ cat araC* P_{ara}:: ^{ss}*dsbA*-^{sf}*gfp*	R6K	(Cho *et al.*, 2011)
pTD16	*att*HK022 *bla lacI*^{q}	R6K	(Uehara *et al.*, 2009)

pTD25	*attλ cat araC* P_{ara}::^{fl}*envC*	R6K	(Uehara *et al.*, 2009)
pTD50	*attλ cat araC* P_{ara}:: ^{ss}*dsbA-bla*	R6K	This Study
pTD51	*att*HK022 *bla lacI^{q}* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*	R6K	This Study
pTD52	*attλ cat araC* P_{ara}:: ^{ss}*dsbA*-^{sf}*gfp-bla*	R6K	This Study
pTD68	*bla* P_{T7}*-H-SUMO*	pBR/colE1	(Yang *et al.*, 2012)
pTD70	*attλ cat araC* P_{ara}::^{ss}*dsbA*-^{sf}*gfp-bla*	R6K	This Study
pTU113	*att*HK022 *bla lacI^{q}* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{35\text{-}419}$*envC*	R6K	(Uehara *et al.*, 2010)
pTU115	*att*HK022 *bla lacI^{q}* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*	R6K	This Study
pTU121	*att*HK022 *bla lacI^{q}* P_{lac}::^{ss}*dsbA*-^{sf}*gfp*-$^{27\text{-}379}$*nlpD*	R6K	This Study
pTU136	*att*HK022 *bla lacI^{q}* P_{lac}::^{ss}*dsbA-mCherry*	R6K	(Uehara *et al.*, 2009)
pTU153	*att*HK022 *bla lacI^{q}* P_{lac}::^{ss}*dsbA-mCherry*-$^{35\text{-}419}$*envC*	R6K	This Study
pTU206-2	*att*HK022 *bla lacI^{q}* P_{ara}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(N412K)-*bla*	R6K	This Study (mutant isolate)
pTU206-5	*att*HK022 *bla lacI^{q}* P_{ara}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(L400P)-*bla*	R6K	This Study (mutant isolate)
pTU206-6	*att*HK022 *bla lacI^{q}* P_{ara}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(V353A)-*bla*	R6K	This Study (mutant isolate)
pTU206-63	*att*HK022 *bla lacI^{q}* P_{ara}::^{ss}*dsbA*-^{sf}*gfp*-^{lyt}*envC*(R405H)-*bla*	R6K	This Study (mutant isolate)

[a] A 6xHis tag for purification is indicated by the letter *H*. ^{ss}*dsbA* corresponds to the first 24 codons of *dsbA* encoding its export signal. P_{lac}, P_{T7}, P_R, and P_{ara} indicate the lactose, phage T7, λR and arabinose promoters, respectively. Numbers in parenthesis indicate the codons included in the relevant clones.

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Supplemental Figure Legends

Figure S1. Superimposition of ^{Lyt}EnvC and ^{Sa}LytM. Shown is are additional views of the superimposition of ^{Lyt}EnvC (cyan) with truncated ^{Sa}LytM (green) (PDB ID: 2b13) (Firczuk et al., 2005) highlighting the zinc ion present in the ^{Sa}LytM active site as a grey sphere and the residues involved in Zn coordination as sticks. Note that none of the Zn-coordinating residues of ^{Sa}LytM (H210, D214 and H293) are conserved in ^{Lyt}EnvC (W320, V324, Y401). Residue labels are colored the same as their corresponding structure, ^{Lyt}EnvC (cyan) and ^{Sa}LytM (green).

Figure S2. Steady state accumulation of defective ^{SF}GFP-^{Lyt}EnvC variants. Protein extracts from cultures producing ^{SF}GFP-^{Lyt}EnvC variants were prepared and subjected to immunoblotting for the detection of the fusions as described in the legend to Figure 3.

Figure S3. Amidase activation function of ^{FL}EnvC variants. Cells of strain NP130 [Δ*envC* Δ*nlpD*] producing the indicated ^{FL}EnvC variants under control of the arabinose promoter were grown and imaged as described in the legend to Figure 5.

Figure S4. Recruitment of mCherry-^{FL}EnvC variants to the septal ring. Cells of strain NP32 [Δ*envC zapA*-GFP] producing the indicated mCherry-^{FL}EnvC variant were grown and imaged as described in the legend to Figure 5.

Figure S5. Rigid-body docking of the AmiB autoinhibitory helix with ^{Lyt}EnvC. Ribbon (A, C) and surface (B, D) representations of the best 10 rigid-body docking orientations of a model of AmiB autoinhibitory helix with ^{Lyt}EnvC. Models were generated by FTDock (Gabb *et al.*,

1997) and evaluated by pyDock scoring function (Cheng *et al.*, 2007). Scoring values are tabulated in (E) and include electrostatics (Ele), desolvation (Desolv) energy and limited van der Waals (VDW) contribution. All energy values are given in kcal/mol. The number of hydrogen bonds (Hb) between AmiB α3 helix and EnvC are also given. The color of the rows in the table corresponds with that of the amidase autoinhibitory helix docking model in A-D.

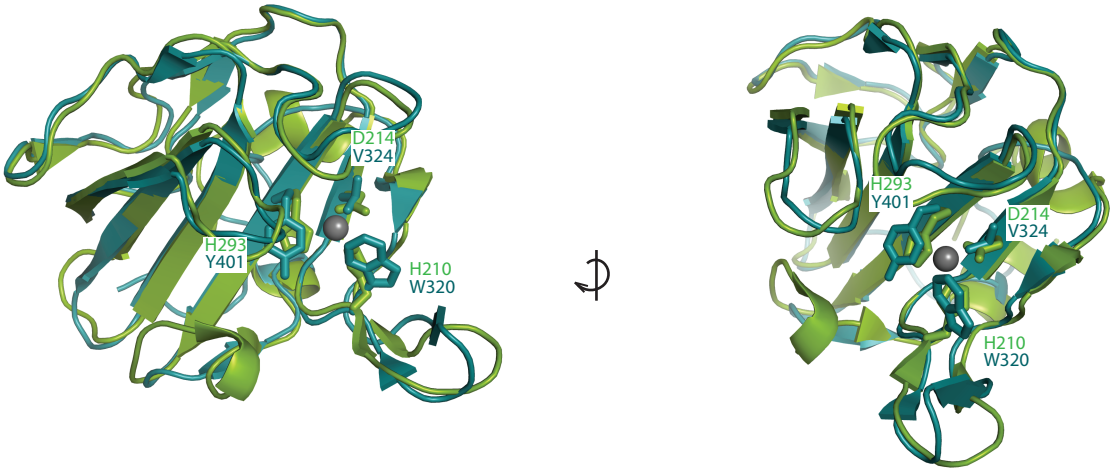
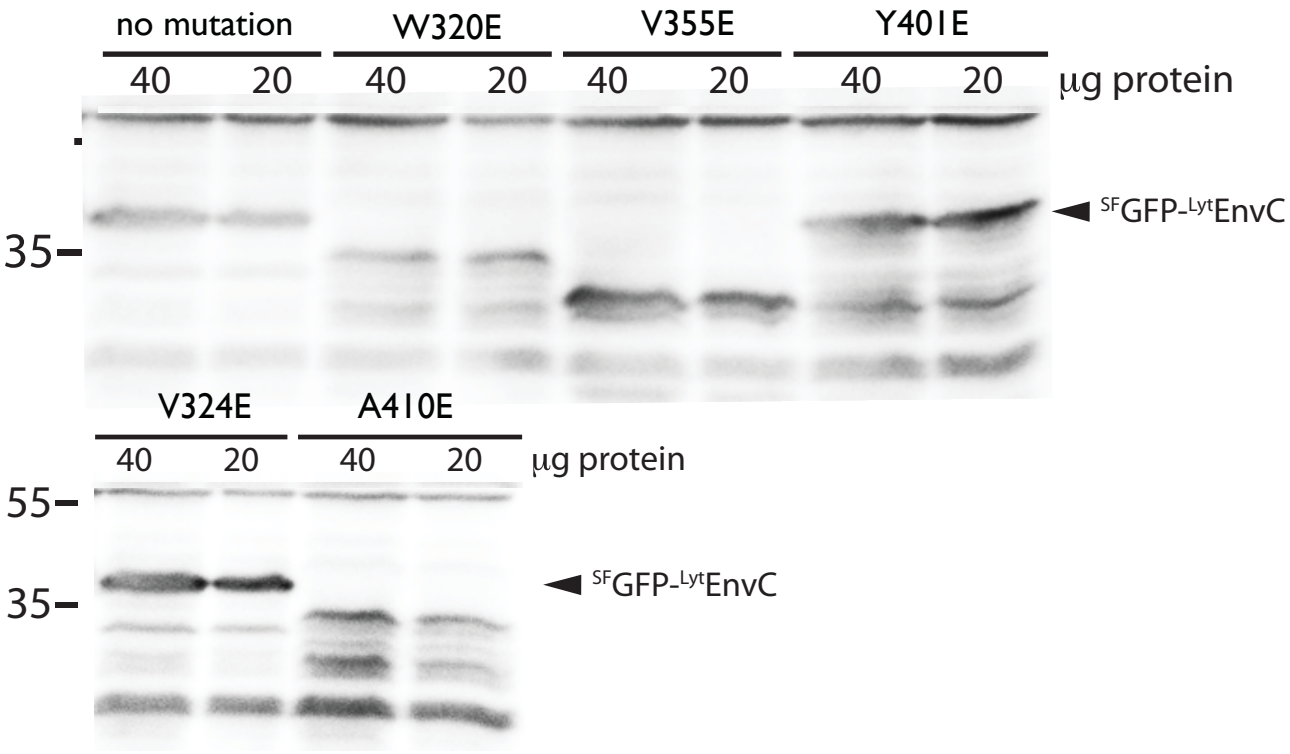


Figure S1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W320E
V355E
Y401E
40
20
40
20
40
20
40
20
µg protein
35
SFGFP-LytEnvC
V324E
A410E
40
20
40
20
µg protein
55
35
SFGFP-LytEnvC

Figure S2

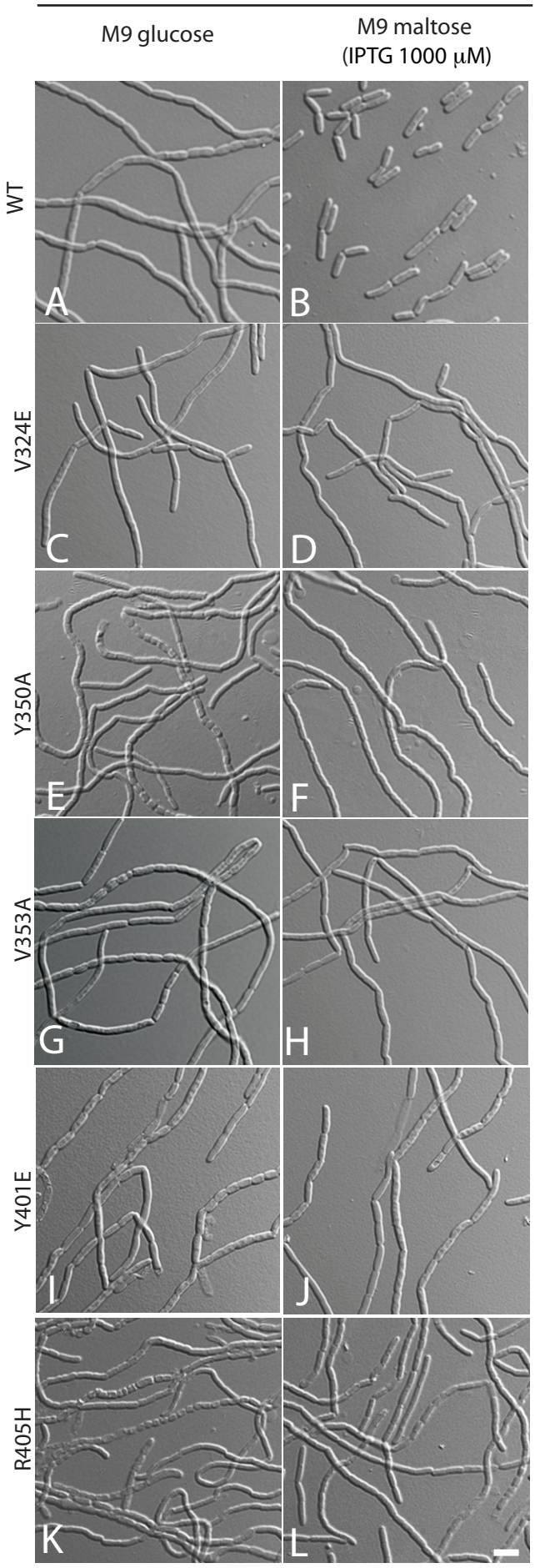

ΔenvC ΔnlpD P lac ::fl envC
M9 glucose
M9 maltose
(IPTG 1000 μM)
WT
V324E
Y350A
V353A
Y401E
R405H
A
B
C
D
E
F
G
H
I
J
K
L

Figure S3

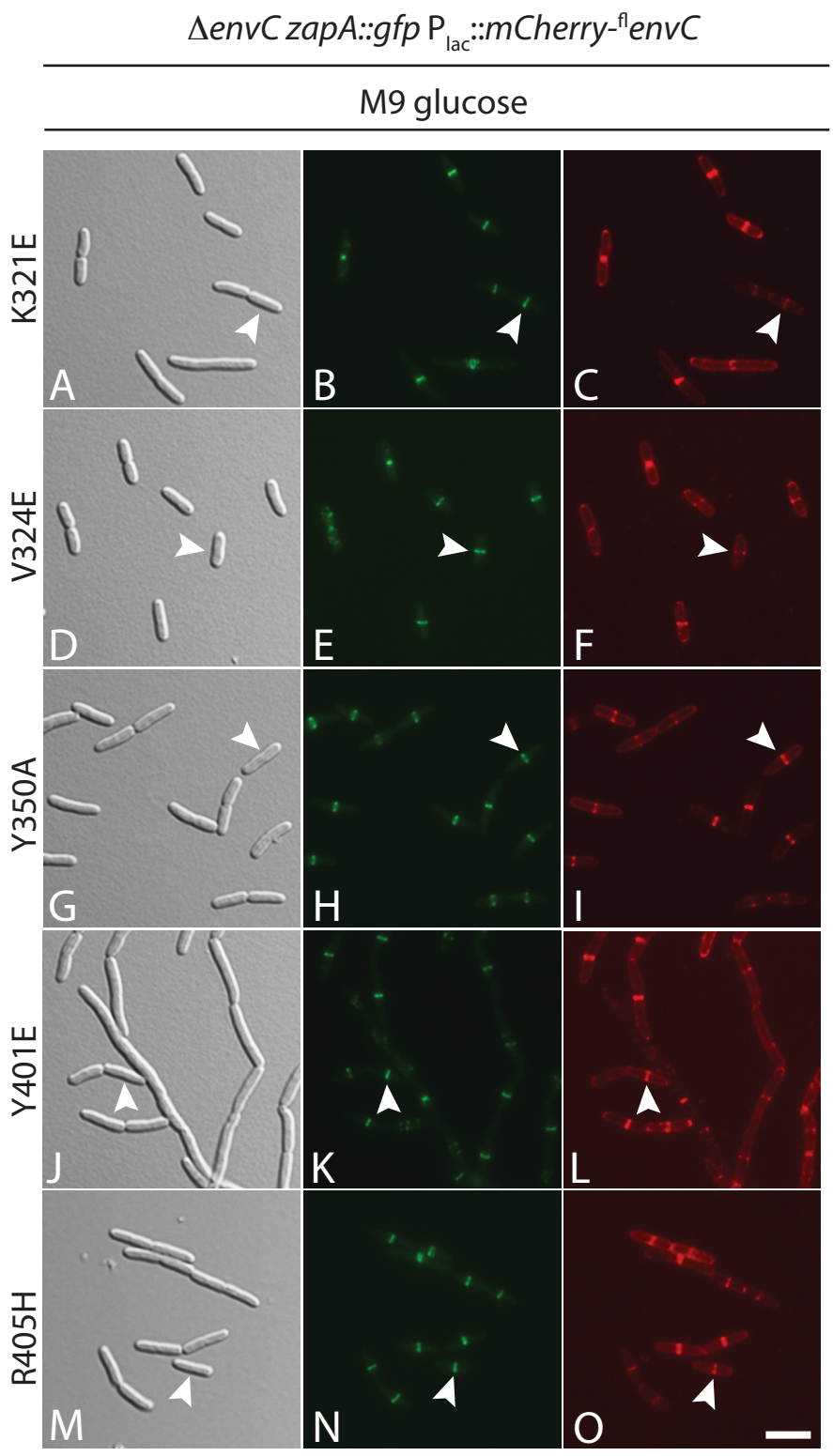
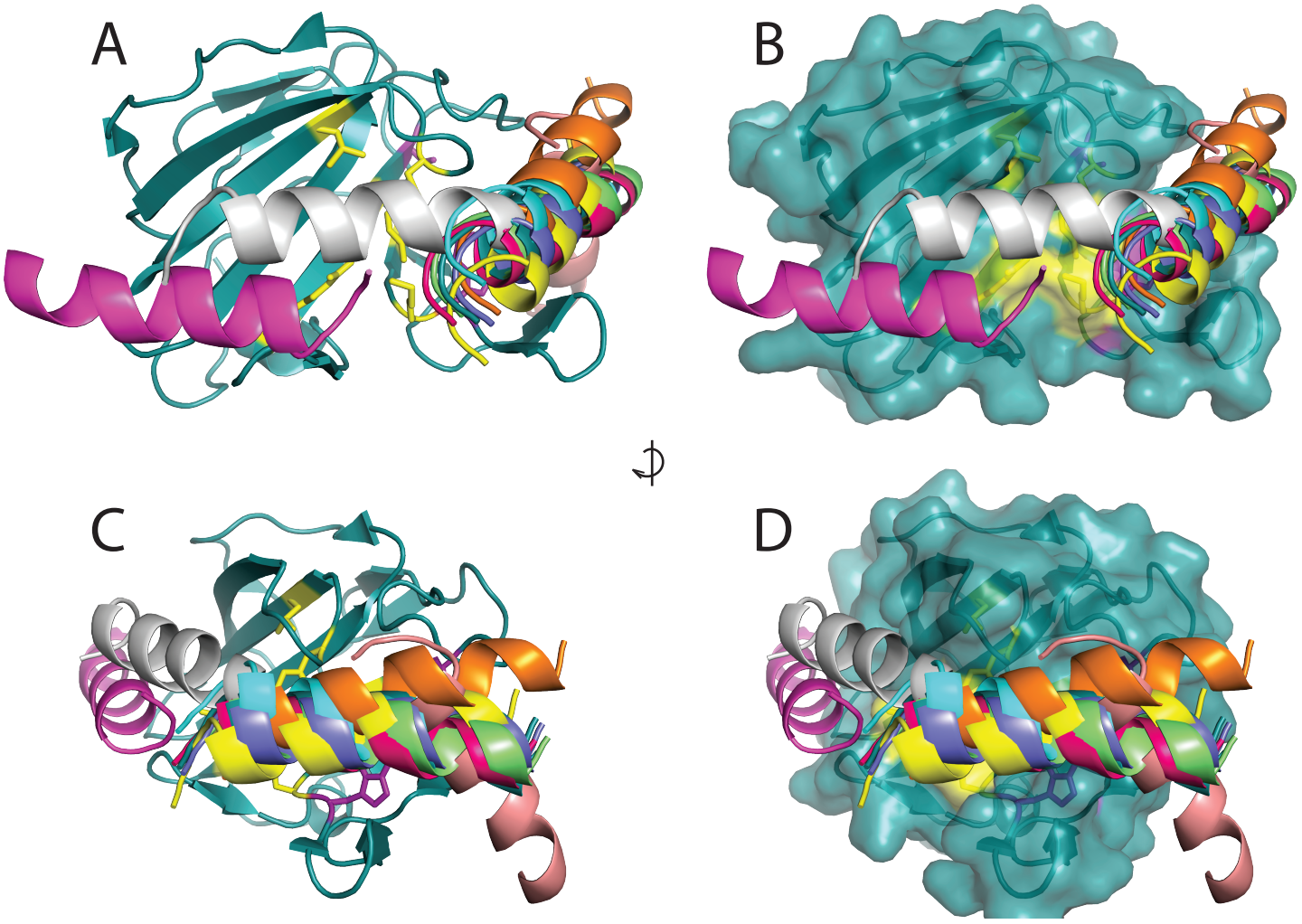

ΔenvC zapA::gfp P lac ::mCherry-fl envC
M9 glucose
K321E
V324E
Y350A
Y401E
R405H
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O

Figure S4



E

Model Rank	Ele	Desolv	VDW	Total	Hb (AmiB-EnvC)
1	-18.505	-15.196	31.715	-30.529	0
2	-22.907	-7.198	11.904	-28.915	2
3	-14.245	-14.573	36.666	-25.152	1
4	-14.558	-12.017	14.656	-25.110	2
5	-16.279	-11.110	22.837	-25.105	1
6	-14.542	-14.617	43.777	-24.781	1
7	-12.947	-16.792	50.025	-24.736	4
8	-17.791	-13.601	68.299	-24.563	2
9	-15.606	-15.377	65.556	-24.428	0
10	-19.444	-13.237	82.895	-24.392	2

Figure S5