Table S1. Bacterial strains and plasmids

Bacteria	**Description**	**Reference/Source**
V. cholerae O395*	Classical Ogawa, Sm^{R}	(Taylor *et al.*, 1987)
V. cholerae O395, pMT5	pMT5::*toxT*	This study
V. cholerae C6706	El Tor Inaba, Sm^{R}	(Hase *et al.*, 1994)
V. cholerae C6706, pMT5	pMT5::*toxT*	(DiRita *et al.*, 1996)
V. cholerae RT4340	El Tor C6706 *tcpA* in classical O395	This study
V. cholerae RT4524	*tcpA*::*lacZ*, pMIN, Gm^{R}	This study
V. cholerae LC1	TcpA A138E	This study
V. cholerae LC6	TcpA K187T	This study
V. cholerae LC7	TcpA A156D/E158A	This study
V. cholerae LC9	TcpA A138E/A156D/E158A	This study
V. cholerae LC10	TcpA A138E/A156D/E158A/K172A	This study
V. cholerae LC11	TcpA A138E/A156D/E158A/K187T	This study
V. cholerae LC12	TcpA A156D/E158A/K172A/D175N	This study
V. cholerae LC13	TcpA A138E/A156D/E158A/K172A/D175N	This study
V. cholerae LC15	TcpA A156D/E158A/K172A/D175N/K187T	This study
V. cholerae LC16	TcpA A138E/A156D/E158A/K172A/D175N/K187T	This study
V. cholerae ML9	TcpA D113A	This study
V. cholerae ML10	TcpA C120A	This study
V. cholerae ML12	TcpA D175N	This study
V. cholerae ML14	TcpA K172A/D175N	This study
V. cholerae ML15	TcpA A156D	This study
V. cholerae ML16	TcpA A138E/A156D/E158A/K172A/K187T	This study
V. cholerae ML17	TcpA E158A	This study
V. cholerae ML18	TcpA K172A	This study
V. cholerae ML25	LC16 with D156A	This study
V. cholerae ML28	TcpA D113G	This study
V. cholerae CL101	O395, pCTX-Knϕ	(Kirn *et al.*, 2000)
E. coli S17	λpir	(Skorupski and Taylor, 1996)
E. coli RT4024	*E. coli* S17 with pTK1	This study
E. coli KSK580	X90 with pMT5	This study
E. coli KSK575	MM294 with pRK2013, a helper plasmid for pMT5	(Figurski and Helinski, 1979)
pMT5	*toxT*, Ap^{R}	(DiRita et al., 1996)
pTK1	pKAS32::*tcpA*^{Cl}-*rpsL* Ap^{R}	(Kirn et al., 2000)
pMIN1	pACYC184 Gm^{R} cassette	(Nye *et al.*, 2000)

*All *tcpA* mutation strains are derived from *V. cholerae* O395 and carry pMT5, which has the *toxT* gene under control of the *lac* promoter.

Table S2. Primers

Primer	Sequence
D113A	F[1]: 5’ GCATTTGCAATTTCAGTGGCTGGTCTGACACAGGC
	R[2]: 5’ GCCTGTGTCAGACCAGCCACTGAAATTGCAAATGC
C120A	F: 5’ GGTCTGACACAGGCTCAAGCCAAGACACTTATTACCAG
	R: 5’ CTGGTAATAAGTGTCTTGGCTTGAGCCTGTGTCAGACC
D175N	F: 5’ TCGCTCCCGCTAGTAAGAATTTAAATCTAACGAACATCACTCACG
	R: 5’ CGTGAGTGATGTTCGTTAGATTTAAATTCTTACTAGCGGGAGCGA
K172A/D175N	F: 5’ TCGCTCCCGCTAGTGCGAATTTAAATCTAACGAACATCAC
	R: 5’ GTGATGTTCGTTAGATTTAAATTCGCACTAGCGGGAGCGA
A156D	F: 5’ GAGAATTCTGCAGCAGACGCTGAGACAGGCGT
	R: 5’ ACGCCTGTCTCAGCGTCTGCTGCAGAATTCTC
E158A	F: 5’ CTGCAGCAGCGGCTGCGACAGGCGTTGGTGT
	R: 5’ ACACCAACGCCTGTCGCAGC CGCTGCTGCAG
K172A	F: 5’ TCGCTCCCGCTAGTGCGAATTTAGATCTAACG
	R: 5’ CGTTAGATCTAAATTCGCACTAGCGGGAGCGA
D156A/E158A	F: 5’ TTGAGAATTCTGCAGCAGCGGCTGCGACAGGCGTTGG
	R: 5’ CCAACGCCTGTCGCAGCCGCTGCTGCAGAATTCTCAA
D113G	F: 5’ GCATTTGCAATTTCAGTGGGTGGTCTGACACAGGC
	R: 5’ GCCTGTGTCAGACCACCCACTGAAATTGCAAATGC
A138E	F: 5’ ATATTGCAATCAAAGAAGGTGGCGCAGTAGC
	R: 5’ GCTACTGCGCCACCTTCTTTGATTGCAATAT
K187T	F: 5’ GTTGAGAAATTATGTACAGGTACTGCTCCATTC
	R: 5’ GAATGGAGCAGTACCTGTACATAATTTCTCAAC
A156D/E158A	F: 5’ GAGAATTCTGCAGCAGACGCTGCGACAGGCGTTGGTGTG
	R: 5’ CACACCAACG CCTGTCGCAG CGTCTGCTGC AGAATTCTC
Sequencing primer	F: 5’ CGCATTTCCTTTAAACACGAGTAAAATG
	R: 5’ GCCCATTATTTAATGGGCAACGTT

[1] F, forward primer

[2] R, reverse primer

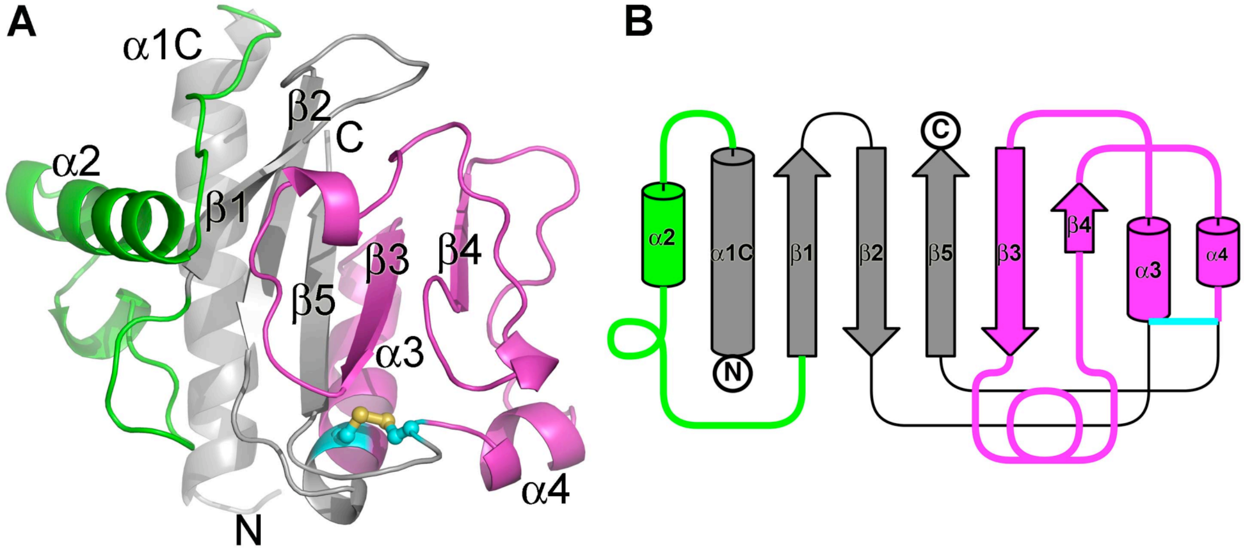

Fig. S1. Atomic structure of the N-terminally-truncated El Tor Type IVb pilin, ΔN-TcpA. ΔN-TcpA^{ET} is a globular protein with an N-terminal α-helical spine (α1C, residues 30-53) embedded in a twisted antiparallel β-sheet. This αβ-roll fold is seen in all the Type IV pilin structures solved to date, and is referred to as the conserved structural core of the pilin subunit (Craig *et al.*, 2004). TcpA^{ET} has the canonical fold of the Type IVb pilin subclass, having a discontinuous, non-nearest-neighbor connectivity for the β-sheet, with the most C-terminal segment of the protein forming the central (β5) strand. In TcpA^{ET}, the αβ-loop (residues 54-93, shown in green), which lies between α1 and the β-sheet, forms one edge of the globular domain. The αβ-loop begins with an irregular loop followed by a four-turn α-helix, α2, that lies at right angles to α1C. Following α2 there is a single helical turn then an irregular β-hairpin that feeds into the β-sheet at β1. The polypeptide chain exits the β-sheet after the first two strands (β1 and β2) to form an α-helix, α3, that runs under and parallel to the β-sheet then re-enters the β-sheet as its fourth strand (called β3 since it follows β2 in the polypeptide). The chain again leaves the sheet to form an irregular loop that winds around the front of the β-sheet then folds into a very short terminal strand (β4) of the sheet. From there the chain follows another irregular loop that runs along the edge of the globular domain, forms a short α-helix, α4, and then inserts into the β-sheet as the central strand (β5), which is also the TcpA C-terminus. The D-region, which is the TcpA segment that spans the conserved, disulfide-bonded cysteines (C120 and C186, colored magenta) comprises a large portion of the globular domain and includes most of α3, α4, the irregular loop structures and the two terminal strands (β3 and β4) of the β-sheet. Thus, the αβ-loop and D-region form the edges and much of one face of the globular domain. These regions display a high degree of sequence and structural variation among the Type IV pilins and are implicated in a number of T4P functions (Craig et al., 2004).

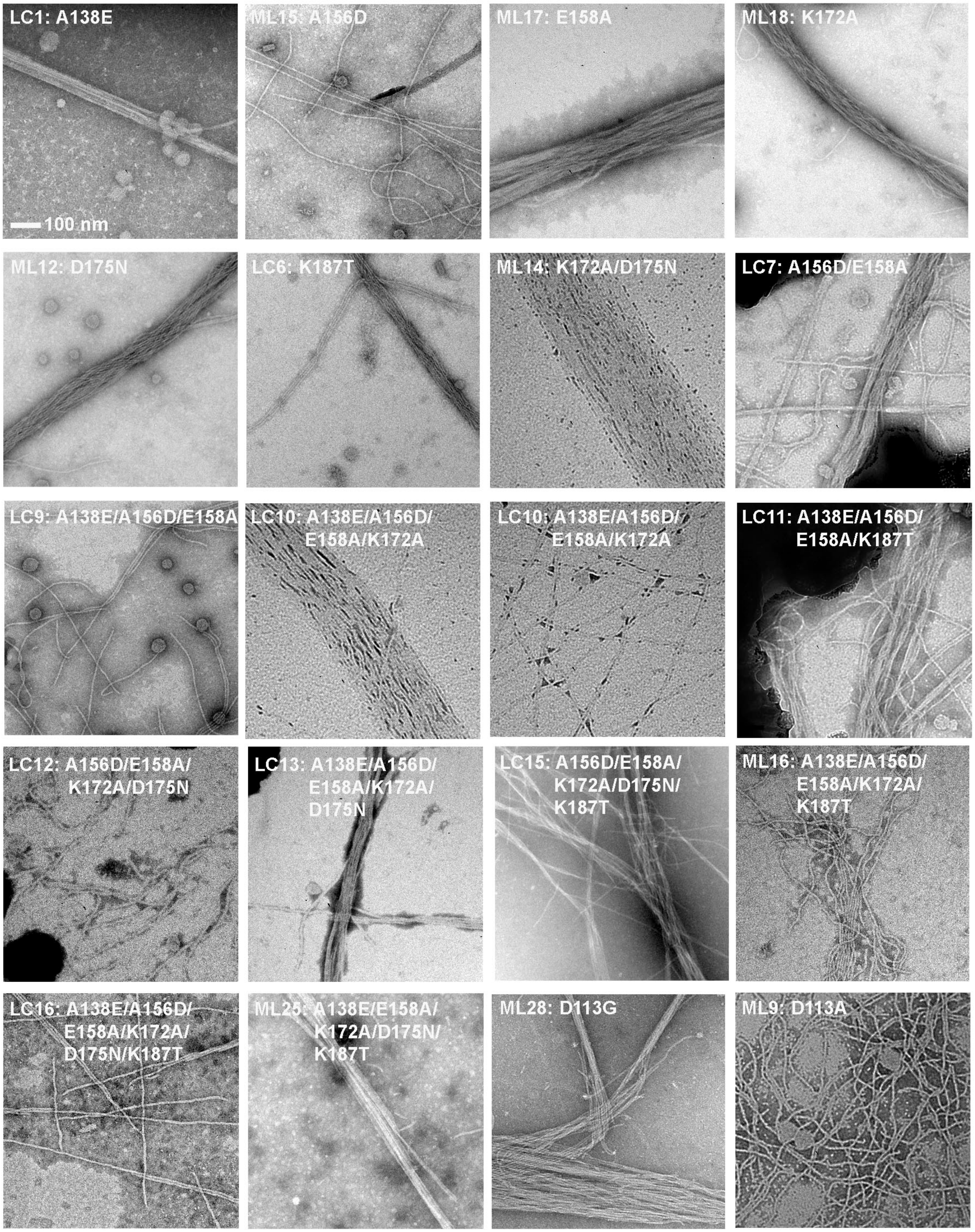

Fig. S2. TEM images of TCP from whole cell cultures of classical-to-El Tor mutant *V. cholerae*. Scale bar for all panels, 100 nm.

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