Supplemental Material

Double tubular contractile structure of the type VI secretion system displays striking flexibility and elasticity

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Table S1

Organism-strain	Genotype	Description	Reference
V. cholerae-V52	Wilde-type, Str^r	Serotype 37 clinical isolated from Sudan. Parental strain.	(1)
	Δ*vasK (tssM)*, Str^r	T6SS negative mutant, deletion of VCA0120 gene.	(1)
	vipA-sfGFP, Str^r	C-terminal chromosomal fusion of sfGFP to *vipA*.	(2)
	vipA-mCherry2, Str^r	C-terminal chromosomal fusion of mCherry2 to *vipA*.	(2)
	clpV-sfGFP, Str^r	C-terminal chromosomal fusion of sfGFP to *clpV*.	(2)
	vipA-mCherry2, clpV-sfGFP, Str^r	C-terminal chromosomal fusions of mCherry2 to *vipA* and sfGFP to *clpV*.	(2)
	Δ*mrcA*, Str^r	Chromosomal deletion of VC2635 to induce spheroplasts.	This study
	vipA-sfGFP, *ΔmrcA,* Str^r	Chromosomal deletion of VC2635 in VipA-sfGFP background.	This study
	vipA-N3-sfGFP, Str^r	Chromosomal VipA-sfGFP fusion with 3 amino acids (codons 26-28) duplicated. Non-contractile sheaths.	This study
	ΔvipB, Str^r	Chromosomal deletion of VC0108. Negative control donor strain for delivery assay.	(3)
	Δhcp1/2, vipA-sfGFP, Str^r	Chromosomal deletion of VC1415 and VCA0017 in VipA-sfGFP background. Recipient strain for delivery assay.	(4)
	ΔvgrG2, vipA-sfGFP, Str^r	Chromosomal deletion of VCA0018 in VipA-sfGFP background. Recipient strain for delivery assay.	(4)
	Δhcp1/2, vipA-mCherry2, Str^r	Chromosomal deletion of VC1415 and VCA0017 in VipA-mCherry2 background. Negative control for PAO1 reporter assay.	(4)
	ΔtagA	Chromosomal deletion of VCA0121.	(3)
	ΔtagA, vipA-sfGFP, Str^r	Chromosomal deletion of VCA0121 in VipA-sfGFP background.	This study
P. aeruginosa-PAO1	tssB-sfGFP, Irg^r	C-terminal chromosomal fusion of sfGFP to PA0083. Reporter strain for functionality assay.	This study
E. coli-DH5α λ pir	F- , endA1, glnV44, thi-1, recA1, relA1, gyrA96 deoR, nupG, Φ80dlacZΔM15, (lacZYA-argF)U169, hsdR17(rKmK+), λ-	Strain used for cloning	New England Biolabs
E. coli-SM10 λ pir	Km^r , thi-1, thr, leu, tonA, lacY, supE, recA::RP4-2- Tc::Mu, pir	Strain used for conjugation	John Mekalanos lab
E. coli MG1655	Gen^r	Random transposon mutant strain. Prey used for T6SS killing	John Mekalanos lab

Note: Str, Streptomycin; Irg, Irgasan; Gen, Gentamycin; r, Resistance.

Table S2.

Plasmid	Description	Source
pDS132, Cm^r	Suicidal conjugation backbone vector for making in-frame deletions in *V. cholerae*	John Mekalanos lab
pDS132-vipA-mCherry2	Suicidal vector to create chromosomal insertion VipAmCherry2 in strain V52	This study
pDS132-vipA-sfGFP	Suicidal vector to create chromosomal insertion VipAmsfGFP in strain V52	This study
pDS132-N3-vipA-sfGFP	Suicidal vector to create chromosomal VipA-N3-sfGFP	This study
pBAD18, Km^r	Arabinose-inducible expression backbone vector, conferring kanamycin resistance	John Mekalanos lab
pBAD18-*tagA*, Km^r	Arabinose inducible expression of VCA0121	This study
pEXG2.0, Gen^r	Suicidal conjugation backbone vector for making in-frame deletions in *P.aeruginosa*	John Mekalanos lab
pEXG2.0-PA0083-sfGFP	Suicidal vector to create chromosomal insertion of tssB-sfGFP in strain PAO1	This study

Note: Str, Streptomycin; Irg, Irgasan; Cm, Chloramphenicol; Km, Kanamycin; Gen, Gentamycin. R, Resistance.

Table S3

Primer name	Sequence (5' to 3')	Description
VCA2635-ko1-XbaI*	GATA**TCTAGA**AAAGCGCGTCCTCTAAACCA	To create deletion mutant of *mrcA* V52
VCA2635-ko2	GTTTACTAGAACAGCTCTTCTAGTTGAGGCTGCACTAATG	To create deletion mutant of *mrcA* V52
VCA2635-ko3	CATTAGTGCAGCCTCAACTAGAAGAGCTGTTCTAGTAAAC	To create deletion mutant of *mrcA* V52
VCA2635-ko4-XbaI*	GATA**TCTAGA**TTTGTCAGCCAACCCACCTT	To create deletion mutant of *mrcA* V52
VCA2635-ko5	AGGCAGTGACAAGCGTTGAT	To confirm deletion mutant of *mrcA* V52
VCA2635-ko6	GCAGAAGCGTGCTTTGTCAG	To confirm deletion mutant of *mrcA* V52
VipA-N3-F**	CAGGCTGAGGTTGCTGAGGTTGAGCTACCACTCAAAACCC	For inserting N3 (AEV) after codon 25 of VipA V52
VipA-N3-R**	TCAACCTCAGCAACCTCAGCCTGTGCATCCCCCGTCGCCG	For inserting N3 (AEV) after codon 25 of VipA V52
pBAD-*tagA*-F-NheI*	ATA**GCTAGC**AGGAGGAAACGATGTCCAATGTGATTTTTATTG	For cloning *tagA* (VCA0121) into pBAD18
pBAD-*tagA*-R-XbaI*	TCAG**TCTAGA**TCACTGCAACAAAGCCGCAT	For cloning *tagA* (VCA0121) into pBAD18
PA0083-sfGFP-1-XbaI*	GAGA**TCTAGA**AGTACTGGGACGGCGTCTAT	To create chromosomal sfGFP insertion into *tssB* PAO1
PA0083-sfGFP-2	TCCTCCTCCTGCGGCCGCGGCCGCCTGCGGCTCGTCGTCT	To create chromosomal sfGFP insertion into *tssB* PAO1
PA0083-sfGFP-3	AGACGACGAGCCGCAGGCGGCCGCGGCCGCAGGAGGAGGA	To create chromosomal sfGFP insertion into *tssB* PAO1
PA0083-sfGFP-4	TCGGCCATGCTGGAATCCTCTTATTTGTAGAGCTCATCCA	To create chromosomal sfGFP insertion into *tssB* PAO1
PA0083-sfGFP-5	TGGATGAGCTCTACAAATAA GAGGATTCCAGCATGGCCGA	To create chromosomal sfGFP insertion into *tssB* PAO1
PA0083-sfGFP-6-XbaI*	GAGA**TCTAGA**CACCAGCCGTAGAGCTTGAA	To create chromosomal sfGFP insertion into *tssB* PAO1

Note: *letter in bold indicate enzyme restriction site, ** Gibson assembly cloning

Supplemental figures.

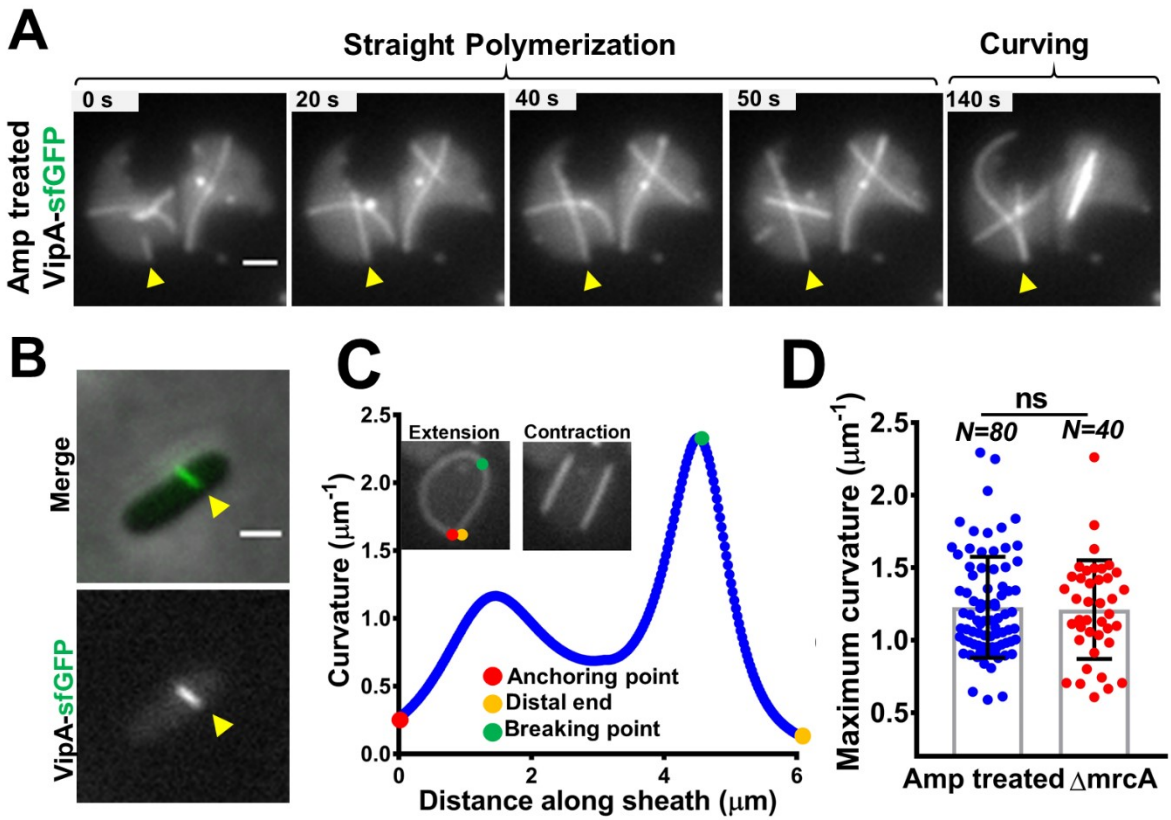

FIG S1. Deformation of the T6SS sheath in spheroplasts. A. An examples of curved sheath polymerization guided by contact with cell membrane. Image sequence corresponds to VipA-sfGFP labeled ampicillin treated cell. The GFP (green) fluorescence channel is shown in grayscale. Yellow arrows indicate anchoring point. Full video is 5 min time-lapse, 10 sec per frame. Scale bar, 1 μm. **B.** Fully extended sheath in rod cell. Top image is a merge of phase and GFP (green) channel. Bottom image is GFP only in grayscale. Scale bar, 1 μm. Yellow arrowhead indicates extended sheath. **C.** Sheath curvature (*y*, axes) was measured at each point along the sheath (*x*, axes) in VipA-sfGFP labeled spheroplasts. 3 x 3 μm insets show the extended and contracted state of the sheath at the top left. The sheath anchoring, distal end and breaking point are shown in red, yellow and green dots, respectively. **D.** The curvature at each point along the sheath was measured for curved sheaths formed in ampicillin treated (Mean=1.22 μm^{-1} ± 0.34; N=80, blue dots) and *mrcA* deletion (Mean=1.21 μm^{-1} ± 0.33; N=40, red dots) cells using Kappa, as shown in panel C. The maximum value, Maximum Curvature (μm^{-1}), for each sheath is plotted. Each data point represents a sheath, *N* indicates number of analysed sheaths. Significance was calculated using a two-tailed Student's *t*-test. ns, non-significant. Measurements were taken from at least three biological replicates.

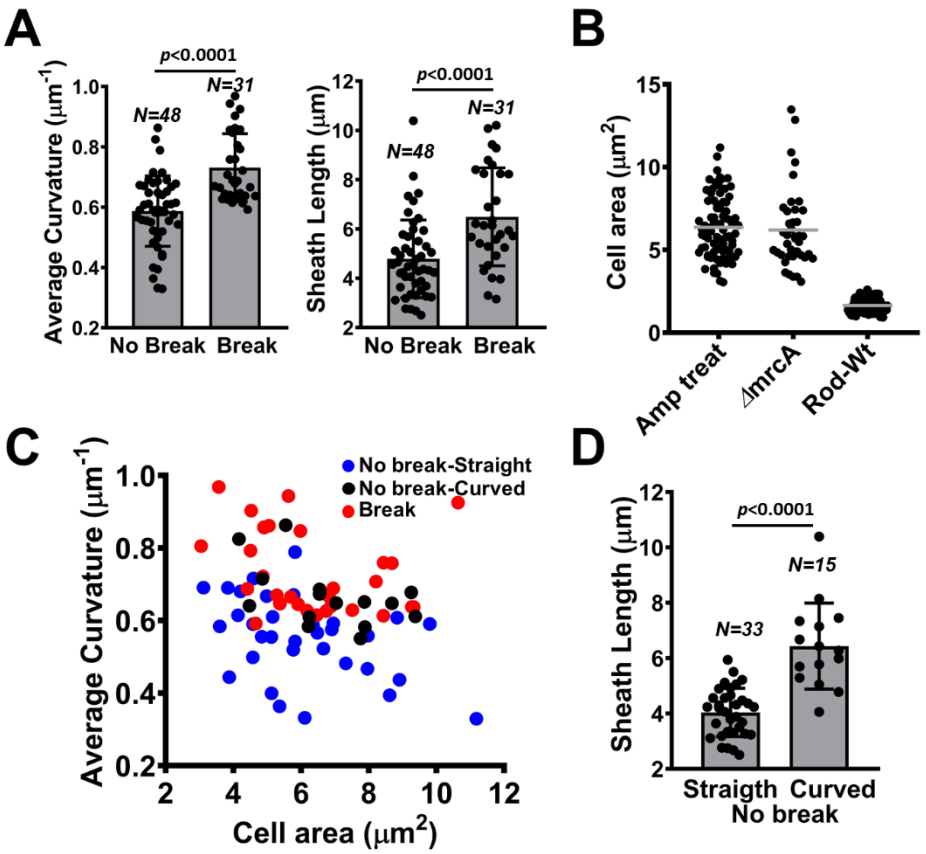

FIG S2. Contraction events of curved sheaths. A. Average curvature (μm^{-1}) and curve length (μm) of the extended sheaths and their *no break* and *break* contraction events are plotted. **B**. Cell area (μm^2) measured for VipAsfGFP labeled ampicillin treated and *mrcA* spheroplasts and for rod cells. Each data point represents a cell. Mean value is indicated by gray line. **C.** Scatter plot shows the dispersion of the cell area (μm^2) and the average curvature (μm^{-1}) (*x*, *y*) for extended sheaths that showed: normal contraction straightened (*No break-Straight*, blue dots, N=33), normal contraction slightly curved (*No break-Curved*, black dots, N=15) and sheaths that break into pieces after contraction (*Break*, red dots, N=31). Each data point represents a sheath. **D.** Curve length (μm) of contracted sheaths that straighten or remain slightly curved after contraction. In all graphs, scatter plots show mean value in gray bars±SD (black lines). Significance was calculated using a two-tailed Student's *t*-test: *p* values are indicated in the graph. Measurements were taken from at least three biological replicates.

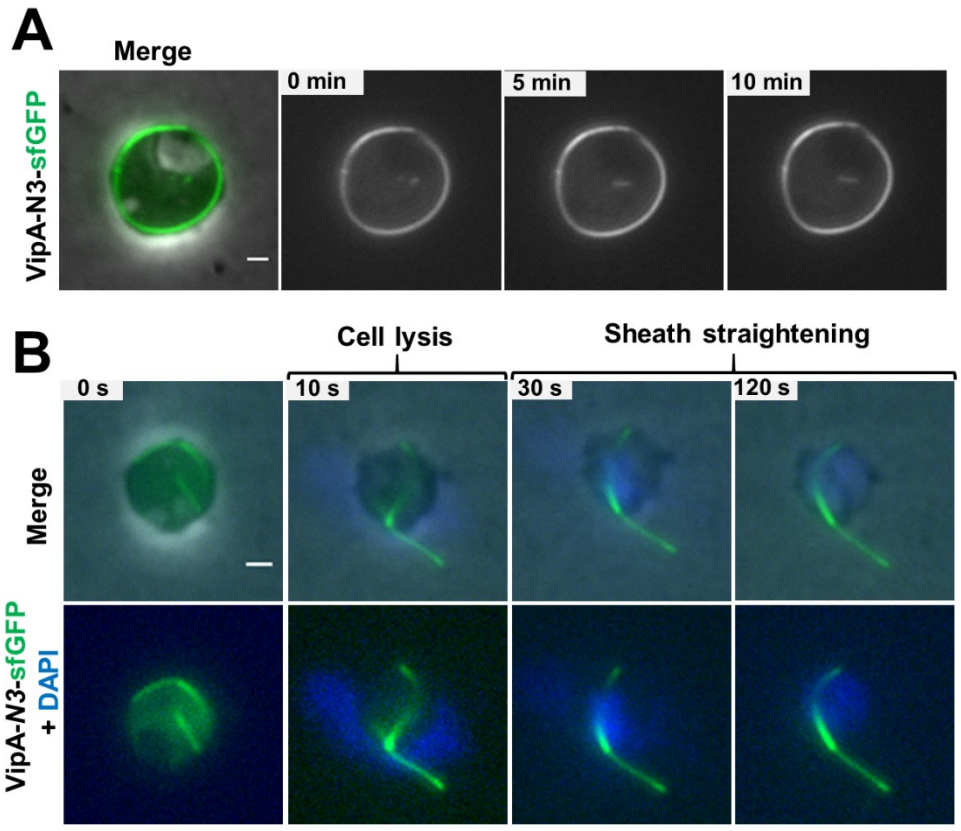

FIG S3. Elasticity of curved sheaths. Related to Fig. 2. **A.** Image sequence shows non-contractile curved sheath assembled in the VipA-N3-sfGFP ampicillin treated strain. Image on the left is a merge of phase and green (GFP) channels. **B.** Additional example of straightening after cell lysis (related to Fig. 2C) induced by 80 µg/mL colistin-0.1% triton X-100. Top row, merge of phase, green (VipA-sfGFP) and blue (DAPI) channels. Bottom row, green and blue channels only. Original video is 8 min time-lapse, frame rate 10 sec per frame. Full video is included in Movie S3, cell 2. All scale bars, 1 µm.

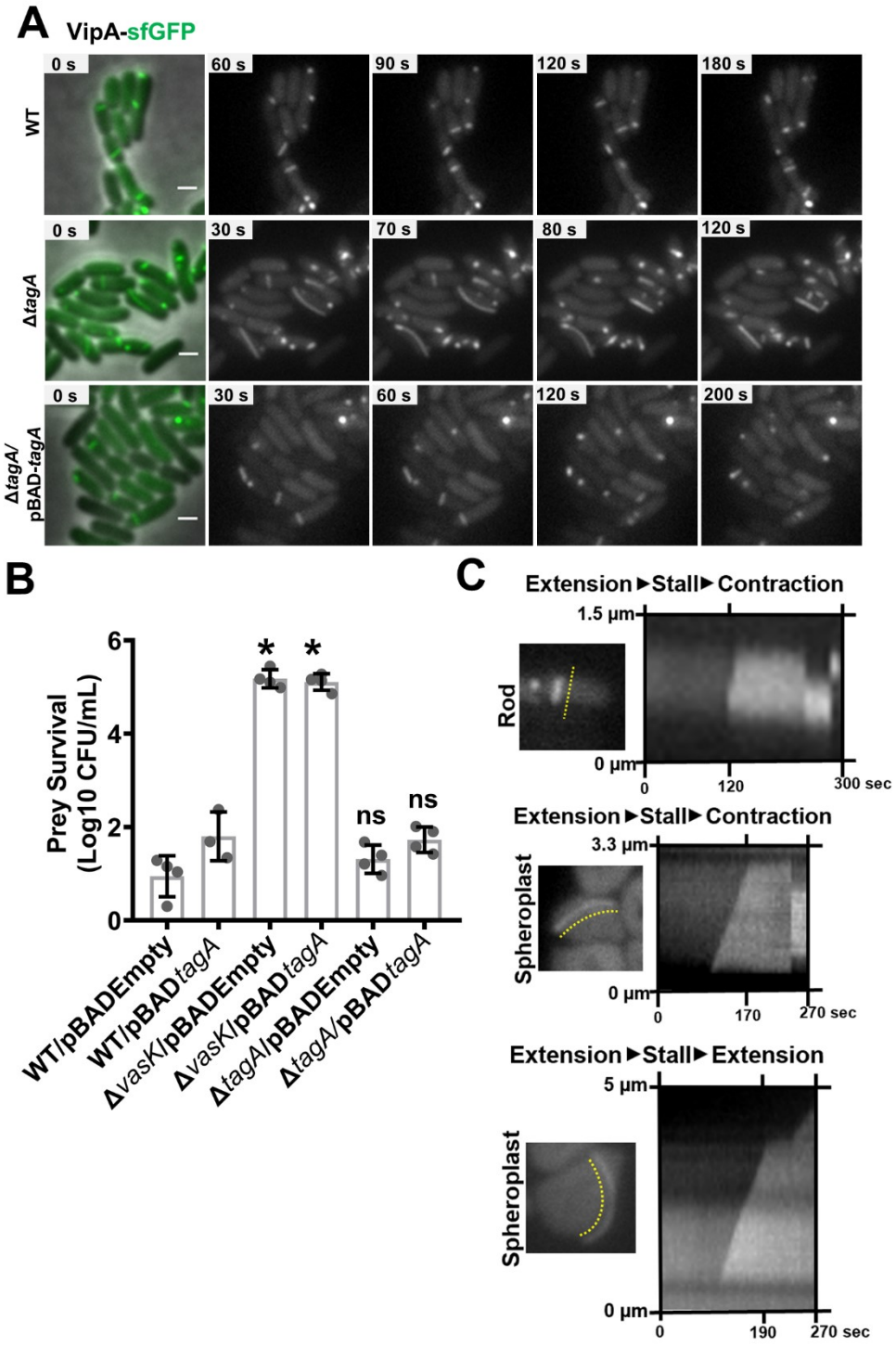

FIG S4. Evaluation of curved sheaths in rod cells. Related to Fig. 3. **A.** Image sequence of rod shaped-VipA-sfGFP wild type (WT), *tagA* deletion (Δ*tagA*), and the corresponding complemented strain (Δ*tagA*/pBAD-*tagA*) induced with 0.01% L-arabinose. The left image is a merge of the phase and GFP (green) channels in all rows. Image series correspond to a 5 min time-lapse, 10 sec per frame. Scale bars, 1 µm. **B.** Quantification of prey survival (*E. coli* MG1655) after 3 h co-incubation with killer cells: wild type (WT), T6SS negative control (Δ*vasK*), or TagA deletion (Δ*tagA*) carrying pBAD empty plasmid as

control or the expression plasmid pBAD-*tagA*. Scatter plots show mean value in gray bars±SD (black lines). Each data point represents an independent biological replicate. Significance was calculated using a two-tailed Student's *t*-test: (*) $p < 0.05$; ns, not significant, when compared to prey cell counts after co-incubation with the wild type strain (WT) carrying the same plasmid. **C.** Kymographs correspond to the area of the sheath indicated by yellow dash lines. 5 min time-lapse videos of 5.5 sec (for spheroplasts) and 10 sec (for rod) interval per frame were used to build the kymographs. Top and middle images show examples of sheath stall stage before contraction in, spheroplast (=70 sec) and rod (= 90 sec) cells. Bottom image shows example of sheath stall stage (= 45 sec) in spheroplast before re-starting polymerization.

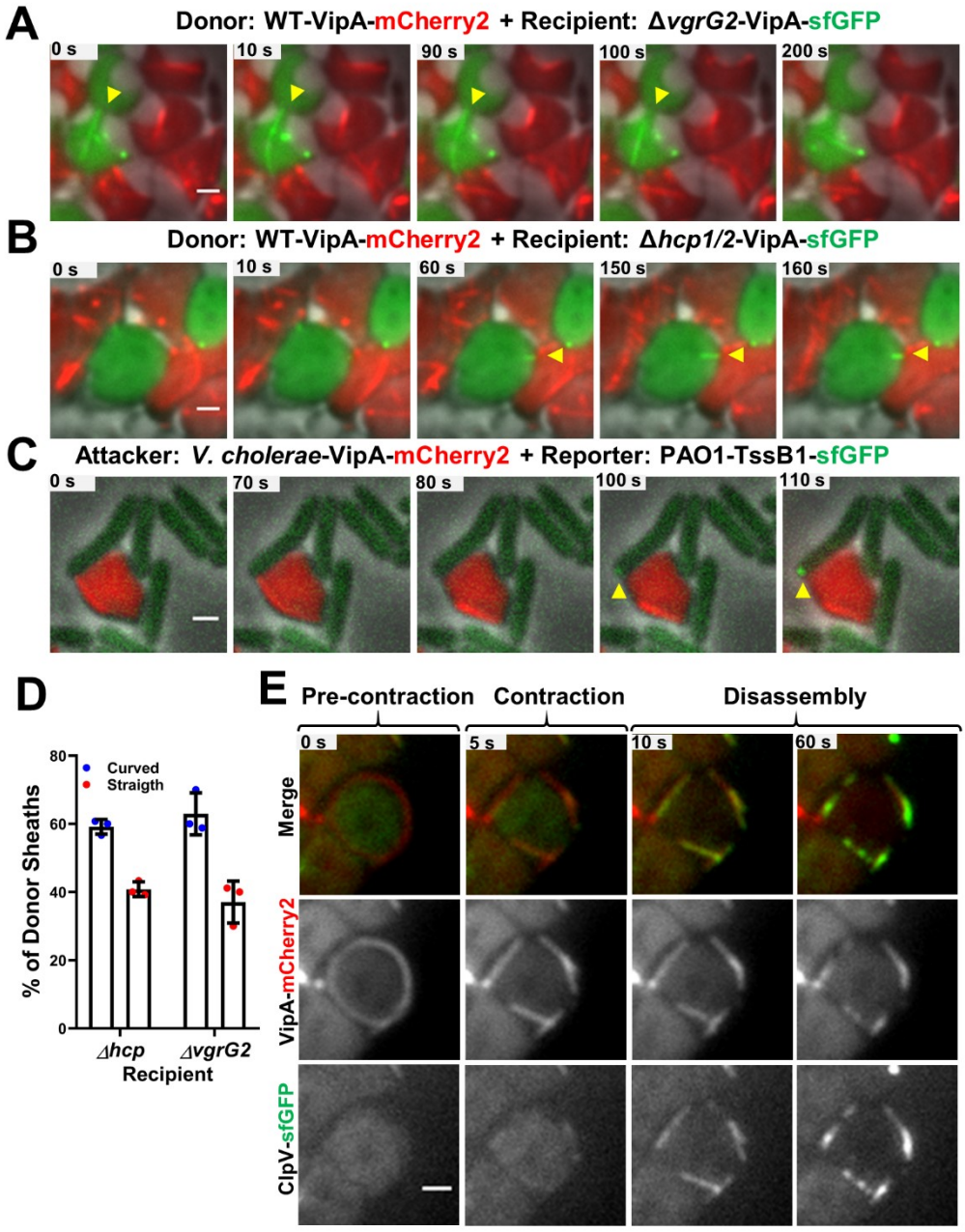

FIG S5. Disassembly of curved sheaths by ClpV. Related to Fig. 4. Image sequences show representative examples of sheath formation in the recipient cells, Δ*vgrG2*-VipA-sfGFP (**A**) and *Δhcp*1/2-VipA-sfGFP (**B**) co-incubated with the donor strain T6SS+ VipA-mCherry2. Both recipient and donor cells were treated with 500 µg/mL ampicillin for 45 min, mixed at a 3:1 ratio (donor to recipient) and incubated onto an agarose pad for 3 h before imaging. Each image is a merge of phase, mCherry2 (red) and GFP (green) channels. Sheath formation in the recipient cell is indicated by yellow arrows. **C.** A representative example of a focus formed (yellow arrows at 100 and 110 sec time points) in the reporter strain *P. aeruginosa* PAO1-TssB-sfGFP after sheath contraction in the attacker cell, *V. cholerae* T6SS+ VipA-mCherry2, is shown. All scale bars, 1 µm. **D.** Bar graph shows the percentage of curved and straight sheaths assembled in the donor strain T6SS+ VipA-mCherry2 surrounding Δ*vgrG2 or Δhcp*1/2-

VipA-sfGFP recipient cells. **E.** Additional example of a curved circular sheath assembled in a double labeled VipA-mCherry2; ClpV-sfGFP ampicillin treated cell. Pre-contraction, contraction and disassembly by ClpV are shown. Top row corresponds to a merge of mCherry2 (red) and GFP (green) fluorescence channels. Middle and bottom rows show VipA-mCherry2 and ClpV-sfGFP signal only, respectively in grayscale. 5 minutes time-lapse, frame rate of 5.5 sec per frame. All scale bars, 1 μm.

Supplemental Movie legends.

Movie S1. Curved sheath formation in *V. cholerae* spheroplasts. Related to Fig. 1 and Fig. S1. VipA-sfGFP labelled cells were treated with 500 μg/mL ampicillin for 45 min and incubated on agarose pad before imaging. Two representative fields of view (FOV) of 40 x 40 μm acquired during 15 min time-lapse with a 10 sec frame rate are shown. Video plays at a rate of 10 frames per second. Left field shows a merge between phase (pseudo-colored as blue) and GFP fluorescence channels; right field shows only GFP fluorescence channel in grayscale.

Movie S2. Non-contractile VipA-N3 curved sheaths. Related to Fig. S3A. The VipA-N3-sfGFP strain was treated with ampicillin for 45 min to induce spheroplast formation. Cells were imaged after 3 h incubation on agarose pad. Three representative videos acquired during 10 min time-lapse with a 10 sec frame rate are shown. Field of view (FOV) is 40 x 40 μm. Video plays at a rate of 10 frames per second. Left field shows a merge between phase and GFP fluorescence channels; right field shows only GFP fluorescence channel in grayscale.

Movie S3. Cell lysis and sheath straightening. Related to Fig. 2C and Fig. S3B. The VipA-N3-sfGFP strain was treated with ampicillin and incubated on agarose pad for 3 h. Cell lysis was induced by addition of 80 μg/mL colistin-0.1% Triton X to the agarose pad. To stain nucleic acid, 10 μg/mL DAPI were added. Two representative cell lysis events are shown. Field of view is 10 x 10 μm. Image series were taken during 8 min time-lapse at a 10 sec frame rate. Video plays at a rate of 10 frames per second. Left field shows a merge of phase, GFP (green) and DAPI (blue) fluorescence channels. On the right panel, a merge of only GFP and DAPI fluorescence channels is shown.

Movie S4. Overexpression of TagA in spheroplasts assembling curved sheaths. Related to Fig. 3A, B and C. Wild type VipA-sfGFP labeled cells carrying plasmid pBAD-*tagA* were treated with ampicillin for curved sheath formation on an agarose pad, at this point 1 µL of 0.05% L-arabinose (+ Ara, left panel) or PBS (- Ara, right panel) was added to the cells and immediately imaged during 20 minute time-lapse at a frame rate of 10 sec per frame. Each field of view is 30 x 30 µm. Video plays at a rate of 10 frames per second. GFP signal is shown in gray scale. Scale bars, 5 µm.

Movie S5. Curved sheaths are disassembled by ClpV upon contraction. Related to Fig. 4D and Fig. S5E. The VipA-mCherry2 ClpV-sfGFP double labeled strain was used to induce spheroplasts by ampicillin-treatment. Image series were taken after 3 h incubation on an agarose pad. Three representative fields of view (FOV) acquired during 5 min time-lapse with a 5.5 sec frame rate are shown. Field of view is 40 x 40 µm. Video plays at a rate of 10 frames per second. Left field shows a merge of phase channel, mCherry2 (red) and GFP (green) fluorescence channels; middle field shows only mCherry2 channel in gray scale and right field shows only GFP fluorescence channel in gray scale.

References

1. Pukatzki S, Ma AT, Sturtevant D, Krastins B, Sarracino D, Nelson WC, Heidelberg JF, Mekalanos JJ. 2006. Identification of a conserved bacterial protein secretion system in *Vibrio cholerae* using the Dictyostelium host model system. Proc Natl Acad Sci 103:1528–1533.
2. Basler M, Mekalanos JJ. 2012. Type 6 Secretion Dynamics Within and Between Bacterial Cells. Science (80-) 337:815–815.
3. Zheng J, Ho B, Mekalanos JJ. 2011. Genetic analysis of anti-amoebae and anti-bacterial activities of the type vi secretion system in *Vibrio cholerae*. PLoS One 6.
4. Ma AT, McAuley S, Pukatzki S, Mekalanos JJ. 2009. Translocation of a *Vibrio cholerae* Type VI Secretion Effector Requires Bacterial Endocytosis by Host Cells. Cell Host Microbe 5:234–243.