

Supplementary Information

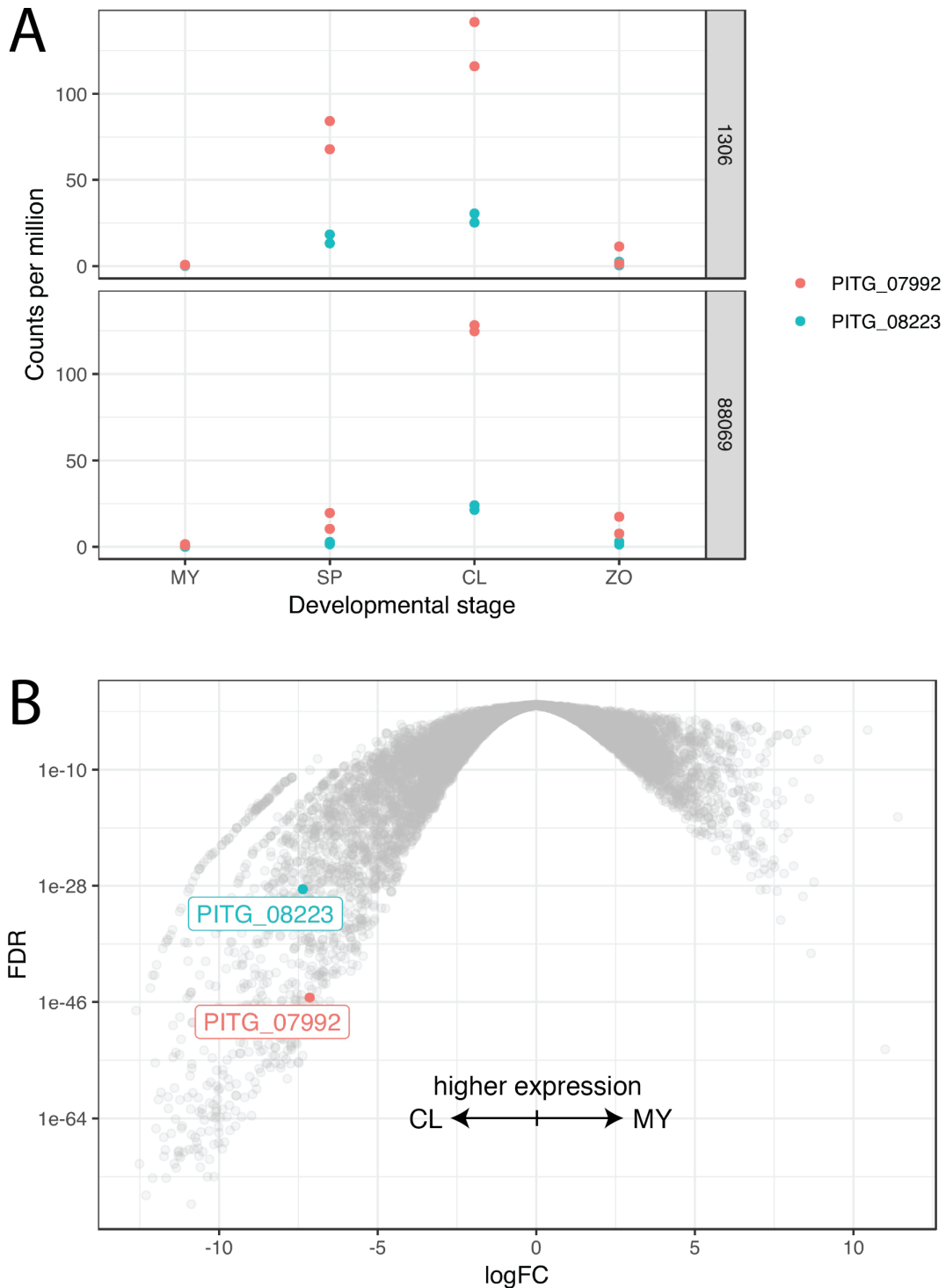
The structure of bactofilin filaments reveals the mode of membrane binding and lack of polarity

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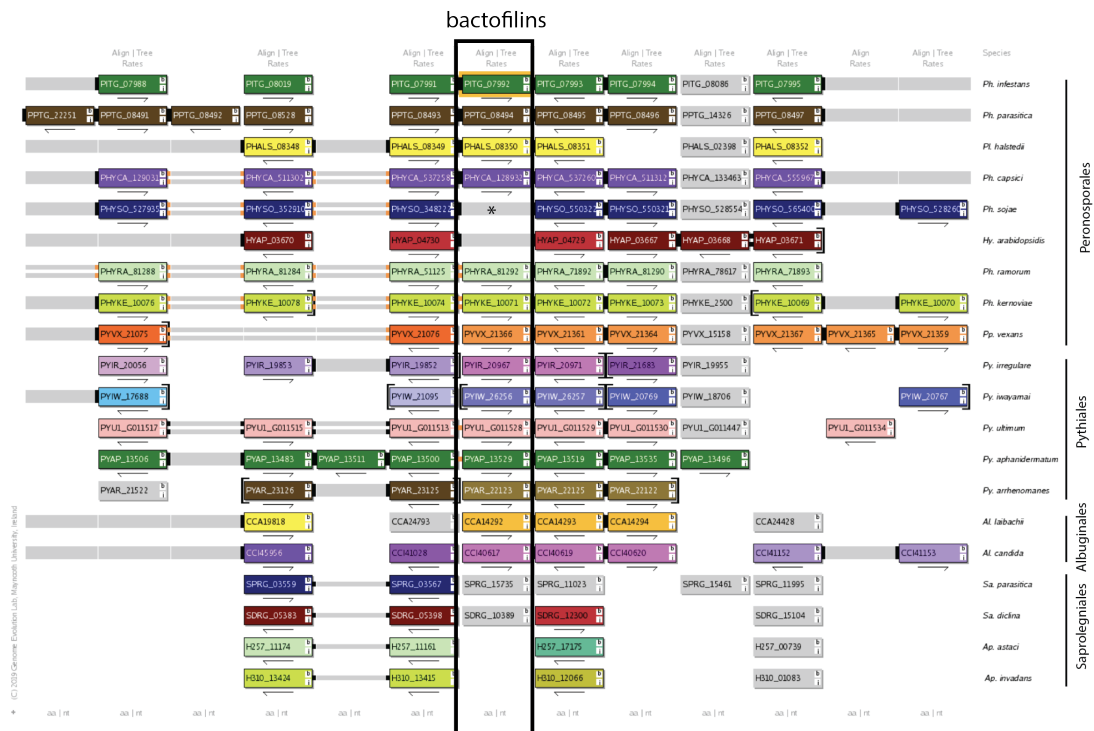
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Supplementary Figure S1. A) Developmental changes in relative abundance (counts per million) of bactofilin mRNA. Data from Ah-Fong *et al.*¹. Upper and lower panels (datasets 1306 and 88069) correspond to independent experiments. Development stages: non-sporulating mycelia (MY), purified sporangia (SP), sporangia chilled in water to induce the cleavage of sporangia into zoospores (CL), zoospores released from the sporangia (ZO), and germinated cysts (GC). B) Volcano plot showing differential expression between

MY and CL life stages in dataset 88069, data taken again from Ah-Fong *et al.* ¹. Bactofilin genes are highlighted by colour and labels. Fold changes in expression of both bactofilin genes are in the 5th percentile of all genes.



Gene Box

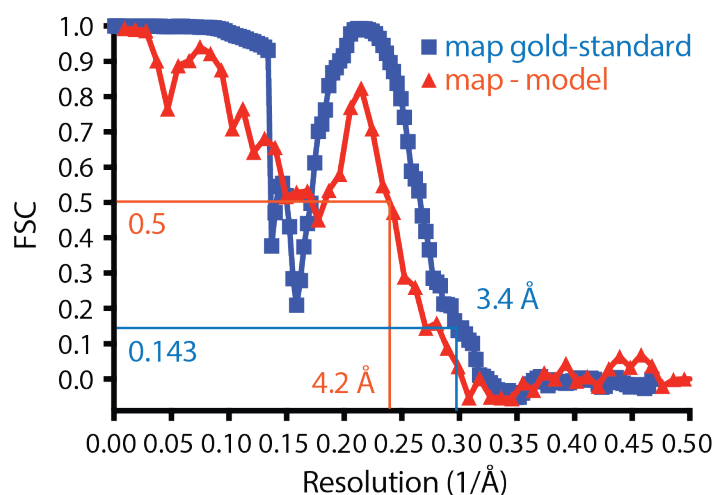
Genes are represented by coloured boxes that show its gene ID. Horizontal tracks correspond to chromosome / scaffold segments. Each genome has a colour palette that distinguishes different chromosomes or scaffolds. Genes that are in the same vertical column (homology pillar) are orthologs. Genes that are coloured grey are not syntenic.

Connectors

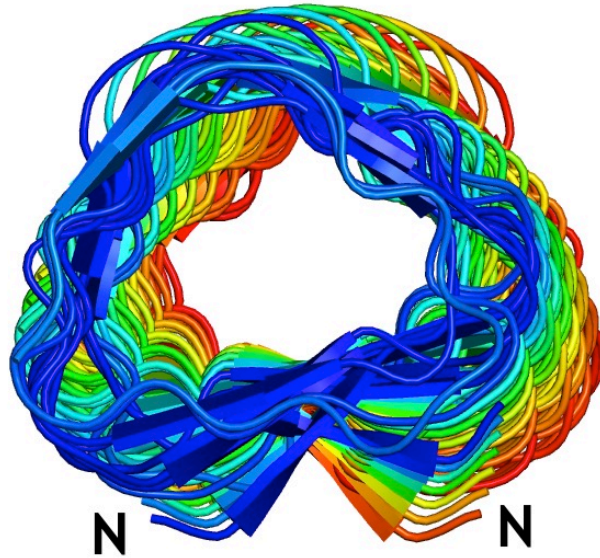
Connectors join nearby genes. A solid connector between two genes indicates that the two genes are adjacent in the genome, two small bars indicate that they are within 5 genes of each other and one small bar if they are within 20 genes. Connectors that are coloured orange denote an inversion. The connectors are continued over any intervening space between genes with grey extensions. Arrows: The arrows under each gene box denote the gene's relative orientation (i.e. Watson or Crick strand). Brackets: A bracket around a gene box indicates the end of a chromosome or scaffold.

From https://ogob.ie/gob/OGO_help.pdf

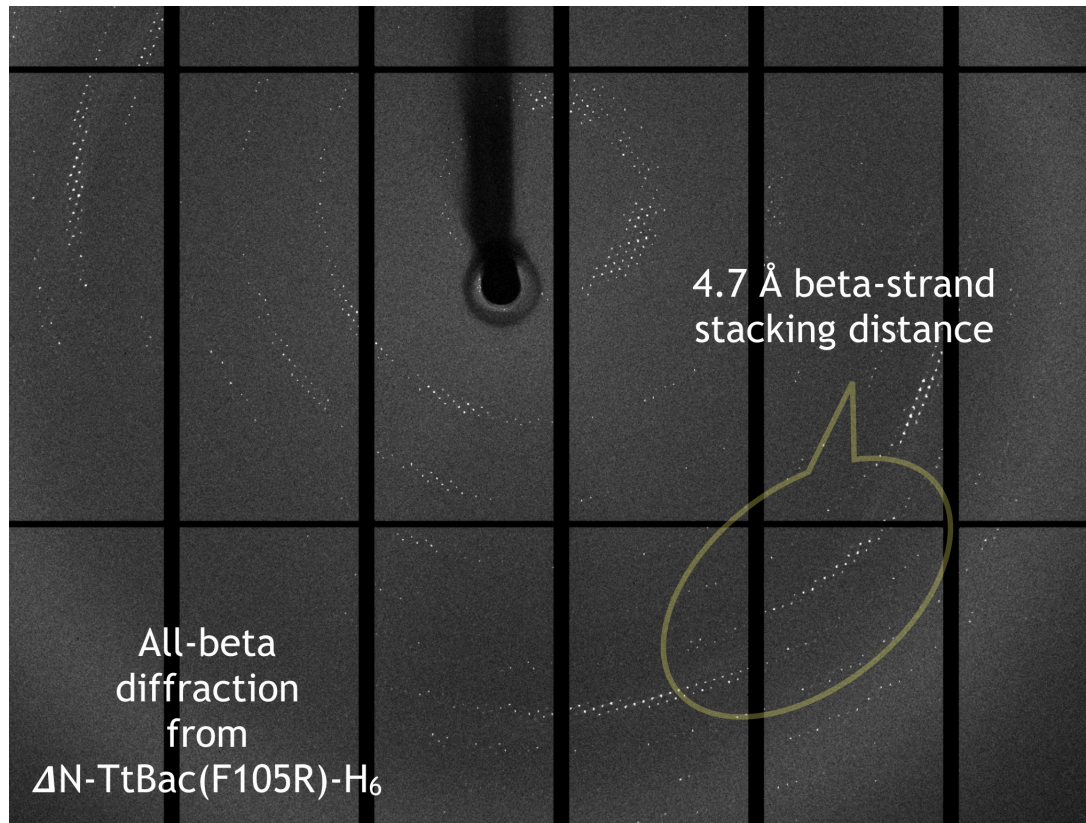
Supplementary Figure S2. Output from the Oomycete Genome Order Browser² for PITG_07992 (Uniprot D0N980). Orthologues are found and at least some synteny of the region is present across the Peronosporales (with the exception of the non-spore forming *H. Arabidopsis*, which does not have a bactofilin), Pythiales and Albuginales. Orthologues are also found in Saprolegniales, but gene order is not conserved (Aphanomyces orthologues not shown in this view, and SDRG_10389 should be SDRG_10390). A star indicates the annotation in the *P. sojae* genome of PHYSO_348222 as a gene fusion, the downstream part is a bactofilin domain.



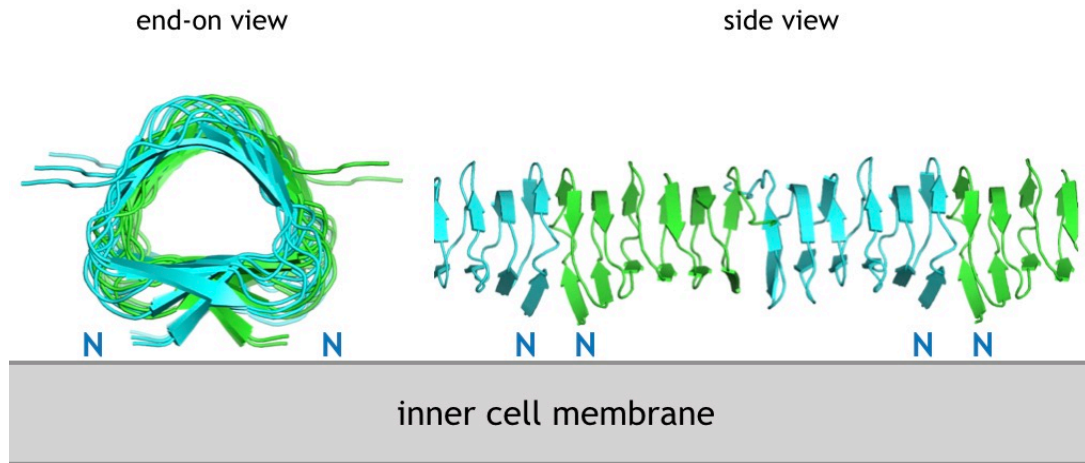
Supplementary Figure S3. Fourier Shell Correlation (FSC) analysis of the final cryo-EM TtBac bactofilin map and model. FSC 0.143³ leads to a nominal resolution of the map of 3.4 Å, but comparison of the map to the model indicates lower resolution around 4.2 Å, most likely caused by severe anisotropy of the cryo-EM map due to the beta stacking along the filament. This can be seen by the dip of the FSC curves before the peak at 4.7 Å, corresponding to the distance between the windings of the beta helix of bactofilin that dominates the Fourier transform of the filaments.



Supplementary Figure S4. In our cryo-EM structure, TtBac-WT protofilaments twist only very slightly, roughly 5° per 57 \AA rise (see also Supplementary Table T1 for exact values, the rise corresponding to two bactofilin subunits because of the antiparallel arrangement of the subunits).



Supplementary Figure S5. All-beta diffraction from a bactofilin crystal similar to the ones used for structure determination (Supplementary Table T1). Diffraction is anisotropic and strongest around 4.7 Å, caused by the dominant beta-stacking interactions along the beta helical fold.



Supplementary Figure S6. Model of membrane binding by bactofilin filaments. The N-terminal tails containing a conserved membrane-targeting sequence (Figure 6A) bind to lipid membrane, utilising avidity that allows each interaction to be rather weak. The lack of significant twist of the filament (Supplementary Figure S4) allows the filament to bind over long distances.

Supplementary Table T1. Cryo-EM data

<i>Thermus thermophilus</i> bactofofilin	
Protein Database ID: WP_011173792.1	
Construct: 1-123	
Structure Database IDs: EMDB-4887, PDB 6RIB	

Data collection and processing	
Magnification	75,000 x
Voltage (kV)	300 kV
Electron exposure (e-/Å ²)	40 e-/Å ²
Defocus range (µm)	-1.0 - -3 µm
Pixel size (Å)	1.07 Å ²
Symmetry imposed	helical (4.89° twist, 57.46 Å rise) and D1 (C2 along X-axis)
Initial particle images (no.)	456k helical segments, 57 Å apart, from 2130 movies
Final particle images (no.)	346k
Map resolution (Å)	3.4 Å
FSC threshold	0.143
Map resolution range (Å)	100 to 3.4 Å
Refinement	
Initial model used (PDB code)	2N3D
Model resolution (Å)	4.2 Å
FSC threshold	0.5
Model resolution range (Å)	100 to 4.0 Å
Map sharpening <i>B</i> factor (Å ²)	-127.67 Å ²
Model composition	
Non-hydrogen atoms	1488
Protein residues	2 chains, 12-112, 202 in total
Ligands	-
<i>B</i> factors (Å ²)	
Protein	122.93 Å ²
Ligand	-
R.m.s. deviations	
Bond lengths (Å)	0.007 Å
Bond angles (°)	1.041°
Validation	
MolProbity score	96 th percentile
Clashscore	6.94
Poor rotamers (%)	9.1 %
Ramachandran plot	
Favored (%)	89.9 %
Allowed (%)	10.1 %
Disallowed (%)	0 %

Supplementary Table T2. Crystallography data

	ΔN-TtBac(F105R)-H₆ native	ΔN-TtBac(F105R)-H₆ SeMet
	Protein Database ID: WP_011173792.1 Construct: 11-123-GSHHHHHH, F105R mutation, native	Protein Database ID: WP_011173792.1 Construct: 11-123-GSHHHHHH, F105R mutation, SeMet
	Structure Database IDs: PDB 6RIA	
Data collection	Diamond I03	Diamond I03
Space group	I2 ₁ 2 ₁ 2 ₁	I2 ₁ 2 ₁ 2 ₁
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	191.9 Å, 244.9 Å, 505.9 Å	198.0, 247.3, 504.5
α , β , γ (°)	90°, 90°, 90°	90°, 90°, 90°
		<i>selenium peak wavelength</i>
Wavelength	0.97623 Å	0.97928 Å
Resolution (Å)	3.5 Å	4.0 Å
<i>R</i> _{merge} *	0.123 (0.541)	0.418 (2.086)
<i>I</i> / σ <i>I</i>	7.1 (2.5)	10.8 (2.9)
Completeness (%)*	99.2 % (100 %)	99.9 % (100.0 %)
Redundancy*	3.8 (3.9)	40.4 (40.7)
Refinement		
Resolution (Å)	3.5 Å	
No. reflections	147,502	
<i>R</i> _{work} / <i>R</i> _{free}	0.283 (0.307)	
No. atoms	32 chains, each amino acids 11-100	
Protein	21663	
Ligand/ion	-	
Water	-	
<i>B</i> -factors		
Protein	113.45 Å ²	
Ligand/ion	-	
Water	-	
R.m.s deviations		
Bond lengths (Å)	0.003	
Bond angles (°)	0.682	

Supplementary Table T3. Proteins used in this study

PiBac: *Phytophthora infestans* bactofilin, NCBI ref: EEY54368.1

TtBac: *Thermus thermophilus* bactofilin, NCBI ref: WP_011173792.1

PiBac-WT 1-203 negative stain EM

MEEAPVPRNPPPKPKRSNVPSAPADYPDDTYSDQDYNMSPIRRGRQHNR
QSGSPMTPPYTVPHQAKVPIIDAEPETTIGAAVKMKGELSFERLLRIEG
EFEGKLNSKGSVLIGTRGALIGNVDNMKEVYITGGRIVGNVNVEKLVLRD
KAQIFGNIIAKSVKIEPECIVVGRINVNPQAPERINEKGEIVKDDAPDGT
PSS

H₆-TtBac 1-123 negative stain EM, cryo-EM

MGSSHHHHHMGRMLGRKERTLTYLGPDTEVLGDMRAKGQVRIDGLVRGS
VLVEGELEVGPTGRVEGERVEARSVLIHGEVKAELTAEKVVLSKTARFTG
QLKAQALEVEAGAVFVGQSVAGEHKALEAPKEA

TtBac-WT 1-123 cryo-EM, membrane binding studies

MGRMLGRKERTLTYLGPDTEVLGDMRAKGQVRIDGLVRGSVLVEGELEVG
PTGRVEGERVEARSVLIHGEVKAELTAEKVVLSKTARFTGQLKAQALEVE
AGAVFVGQSVAGEHKALEAPKEA

Nanobody NB4-mut2-(L13S, Q15D, K45D, K66D) cryo-EM

MAQVQLQESGGGSVDAGGSLRLSCAASGRTFGASLMGWFRQAPGDEREFV
AAINWTGKIWYTDSVDGRFTISRDNKNTANLQMNNLPEDTAIYYCAAR
LGIGFAPSSVEYDYWGQGTQVTVSSAAASSHHHHH

Δ N-TtBac(F105R)-H₆ 11-123 crystallography, polymerisation-impaired

MTLTYLGPDTEVLGDMRAKGQVRIDGLVRGSVLVEGELEVGPTGRVEGER
VEARSVLIHGEVKAELTAEKVVLSKTARFTGQLKAQALEVEAGAVRVGQS
VAGEHKALEAPKEAGSHHHHH

Δ N-TtBac 11-123 membrane binding studies

MTLTYLGPDTEVLGDMRAKGQVRIDGLVRGSVLVEGELEVGPTGRVEGER
VEARSVLIHGEVKAELTAEKVVLSKTARFTGQLKAQALEVEAGAVFVGQS
VAGEHKALEAPKEA

Supplementary Movie M1-M4 legends

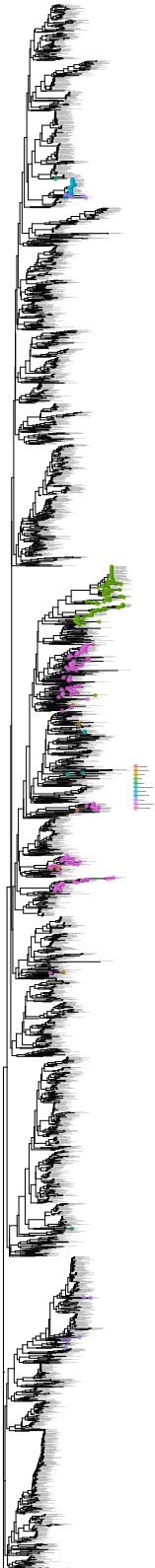
Supplementary Movie M1. Cryo-EM density after helical reconstruction of TtBac filament bound by nanobody NB4-mut2. Boundaries of the bactofilin monomers are clearly visible, as is their antiparallel arrangement in each protofilament. An image from this movie is shown in Figure 3F.

Supplementary Movie M2. Overview of TtBac bactofilin cryo-EM structure, providing a better 3D impression of the assembly.

Supplementary Movie M3. Electron cryotomography (cryo-ET) of an *E. coli* cell with TtBac-WT overexpressed. Note that filament bundles are arranged all around the cell's periphery, under the inner membrane and are particularly obvious when the movie goes through the upper and lower cellular envelope where the bactofilin bundles run at roughly 45° angles to the long cell axis. Images from the tomogram are shown in Figure 6E, left.

Supplementary Movie M4. Same as Movie M3, but Δ N-TtBac has been overexpressed. Because the filaments no longer bind to the inner membrane of the *E. coli* cells, a very large bactofilin bundle runs along the long cell axis, also inhibiting cell division at the septum site. An image from this tomogram is shown in Figure 6E, right.

Supplementary Dataset D1



Supplementary References

1. Ah-Fong, A. M., Kim, K. S. & Judelson, H. S. RNA-seq of life stages of the oomycete *Phytophthora infestans* reveals dynamic changes in metabolic, signal transduction, and pathogenesis genes and a major role for calcium signaling in development. *BMC Genomics* **18**, 198 (2017).
2. McGowan, J., Byrne, K. P. & Fitzpatrick, D. A. Comparative Analysis of Oomycete Genome Evolution Using the Oomycete Gene Order Browser (OGOB). *Genome Biol Evol* **11**, 189-206 (2019).
3. Rosenthal, P. B. & Henderson, R. Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. *J Mol Biol* **333**, 721-745 (2003).