

Stepwise assembly and release of Tc toxins from *Yersinia entomophaga*

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a

Protein name	Accession number	Exclusive unique peptide counts
Toxin subunit YenA2	A0A3S6EXX9	126
Toxin subunit YenA1	A0A3S6EWV3	81
Toxin subunit YenB	A0A3S6EX30	77
Toxin subunit YenC1	A0A3S6EXC9	62
Toxin subunit YenC2	A0A3S6EWV9	55
Chitinase 1	A0A3S6EWX1	53
Chitinase 2	A0A3S6EWW7	51
RHS2	A0A2D0TC51	22
sfGFP	A0A059PIQ0	17
Peptidase M66/StcE	A0A3S6EYX4	10

b

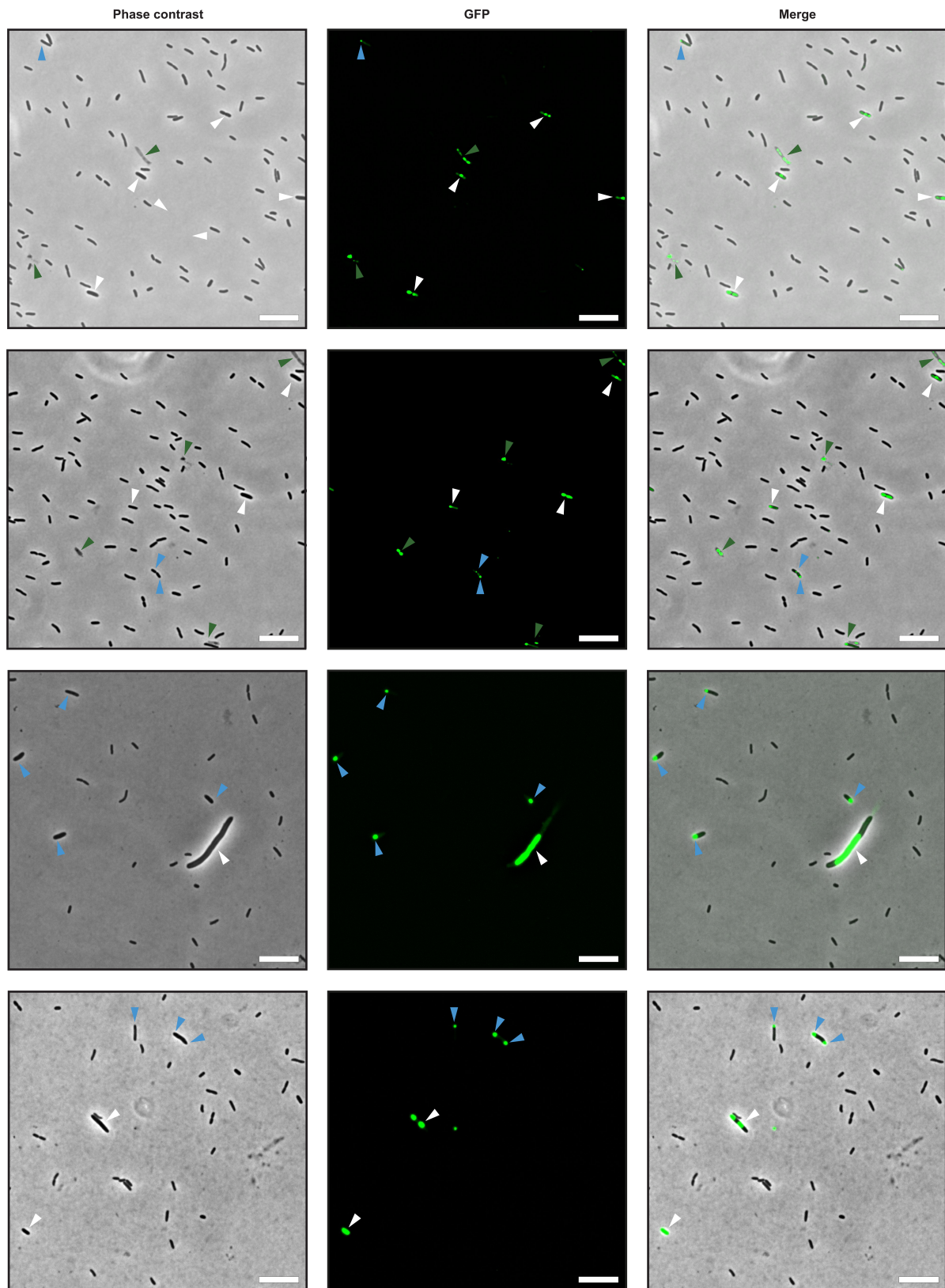


Supplementary Figure 1: Mass Spectrometry and intoxication assays of *Costelytra giveni* indicate that YenTc-sfGFP is active

a: Mass spectrometry analysis of a YenTc purification from *Y. entomophaga chi2-sfGFP* culture identified all YenTc components as well as sfGFP. Note that it also shows the expression of M66/StcE.

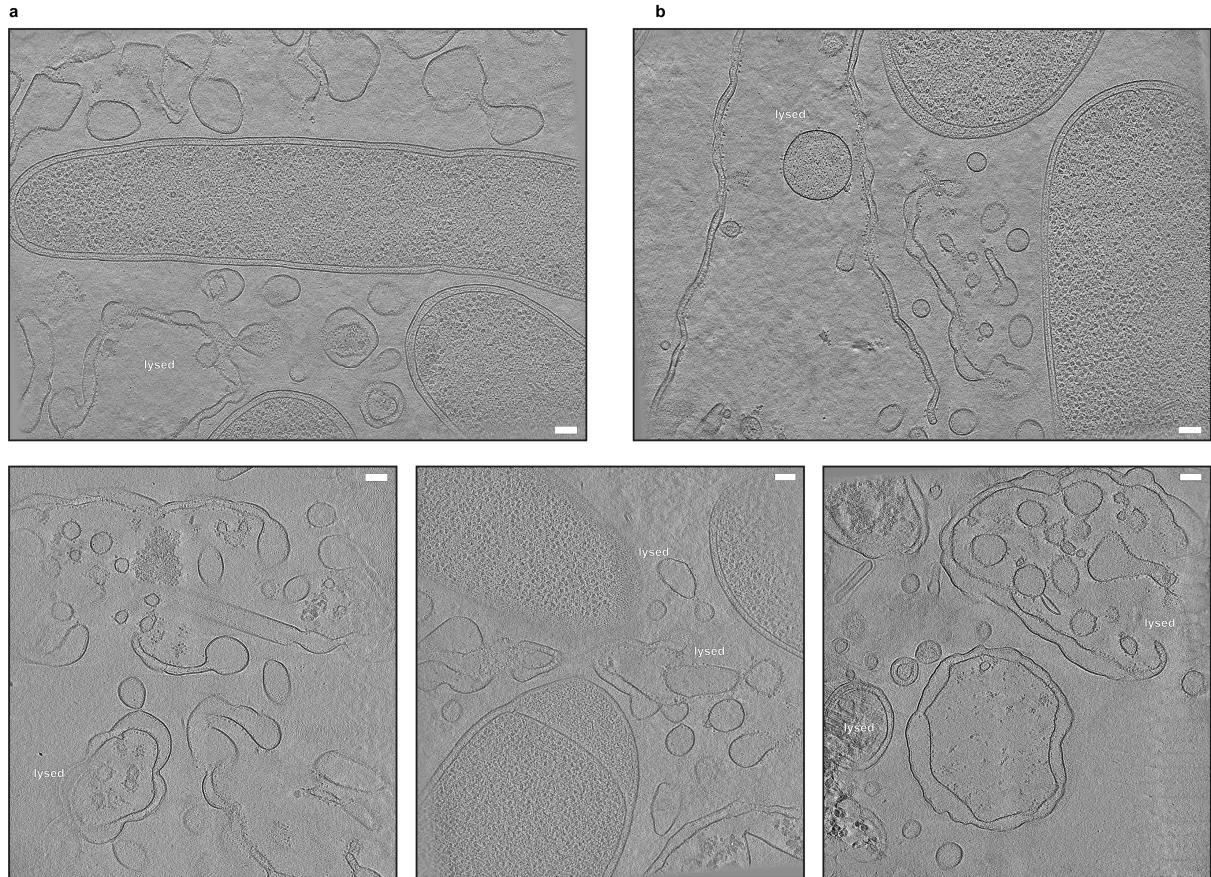
12 **b:** *Costelytra giveni* larvae were fed with carrot food cubes (yellow arrows) with culture
13 filtrates of different *Y. entomophaga* strains or without filtrate (control). The photo was taken
14 three days after feeding. Larvae fed with carrot without filtrate or with Δ YenTc culture filtrate
15 consumed the entire carrot food cube and were of a healthy grey appearance. Larvae fed with
16 carrot with WT or *chi2-sfGFP* culture filtrate showed a cream coloration and reduced feeding
17 activity.

Y. entomophaga chi2-sfGFP



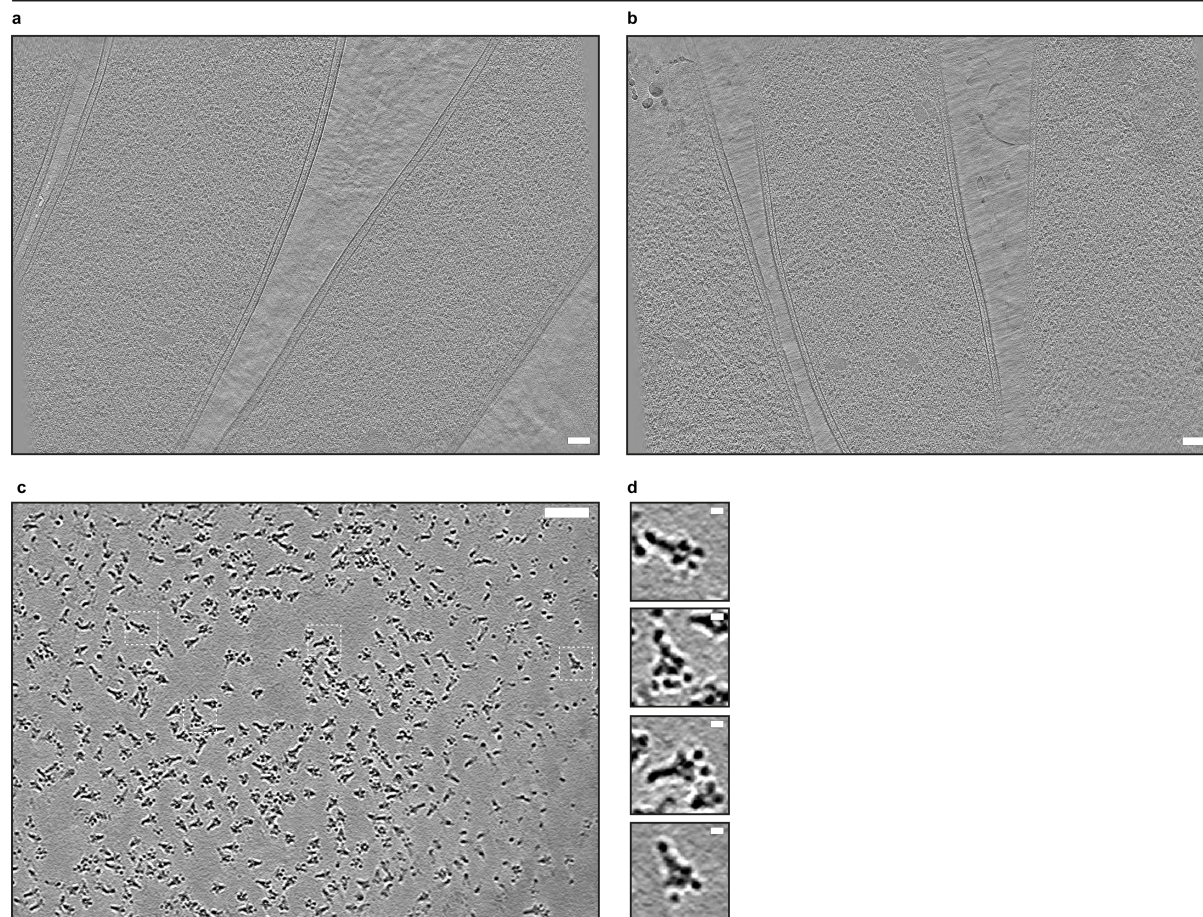
Supplementary Figure 2: More fLM images of *Y. entomophaga* chi2-sfGFP show different morphological categories of Chi2-sfGFP expressing cells

fLM of *Y. entomophaga chi2-sfGFP* grown for 24 h revealed the expression of Chi2-sfGFP only in a subpopulation of cells. Three different morphological classes were detected: Foci at the cell poles (blue arrowheads), distributed signal in somewhat elongated and thicker cells (white arrowheads), and lysing cells (loss of phase contrast, green arrowheads). The experiment was repeated three times for the timepoint 16 h, and four times for the timepoint 24 h with $n_{total, 16h} = 4810$ and $n_{total, 24h} = 7317$ ($n > 1000$ cells for each experiment). Bar: 10 μm .



Supplementary Figure 3: Lysed cells of *Y. entomophaga* Δ YenTc do not reveal any YenTc-like densities

Slices through cryo-tomograms of *Y. entomophaga* Δ YenTc cells showed no apparent YenTc densities inside intact or lysed cells ($n_{\text{lysed}} = 33$). Slice thickness: 18.04 nm. Bar: 100 nm.



Supplementary Figure 4. *Y. entomophaga* Δ LC Δ M66 confirms identity of filaments as M66/StcE and results in holotoxin formation

a/b: Cryo-tomograms of *Y. entomophaga* Δ LC Δ M66 revealed the abolishment of distinct filament bundles in the cytoplasm of the cells ($n = 103$), confirming the identity of the cytoplasmic filaments as M66. Slice thickness: 18.04 nm. Bar: 100 nm.

c: Slice through cryo-tomograms of a purification of YenTc from *Y. entomophaga* Δ LC Δ M66 showed that YenTc can assemble into holotoxins in this mutant. Slice thickness: 18.04 nm. Bar: 100 nm.

d: Close-up views of the boxed YenTc particles from c. Slice thickness: 18.04 nm. Bar: 10 nm.

Supplementary Tables

Supplementary Table 1. Differential expression analysis of Δ LC cells grown at 37 °C compared to 25 °C. FC: fold change, FDR: false discovery rate, Conf.: confidence.

Protein	Accession number	Log ₂ FC	FDR	Conf. low	Conf. high
YenA1	A0A3S6EWV3	-7.2	3.49E-5	-9.28	-5.12
YenA2	A0A3S6EXX9	-6.83	2.51E-4	-9.50	-4.16
YenB	A0A3S6EX30	-5.09	7.76E-8	-5.67	-4.51
YenC1	A0A3S6EXC9	-3.17	6.93E-7	-3.69	-2.65
YenC2	A0A3S6EWV9	-6.06	1.08E-5	-7.52	-4.59
Chi1	A0A3S6EWX1	-7.13	1.74E-7	-7.79	-6.48
Chi2	A0A3S6EWW7	-8.25	1.02E-7	-9.25	-7.25
Cbp	A0A3S6EXR6	-6.41	9.97E-9	-6.91	-5.91
Chi3	A0A3S6F1Q8	-3.99	4.38E-7	-4.60	-3.37
Pil36	A0A3S6F569	-3.75	6.45E-5	-4.94	-2.56
PirA	A0A3S6F007	-4.08	1.94E-6	-4.71	-3.44
NucA	A0A3S6F4M5	-5.13	1.3E-4	-6.67	-3.58
M66/StcE	A0A3S6EYX4	-7.05	1.16E-4	-9.51	-4.6
Tlh	A0A3S6F052	-3.22	4.07E-3	-4.93	-1.51
OmpR/PhoB	A0A3S6F5G2	-3.39	7.34E-5	-4.49	-2.29

Supplementary Table 2. Differential expression analysis of WT cells grown at 37 °C compared to 25 °C. FC: fold change, FDR: false discovery rate, Conf.: confidence.

Protein	Accession number	Log ₂ FC	FDR	Conf. low	Conf. high
YenA1	A0A3S6EWV3	-6.91	5.09E-5	-8.99	-4.83
YenA2	A0A3S6EXX9	-5.81	7.34E-4	-8.48	-3.14
YenB	A0A3S6EX30	-5.64	5.49E-8	-6.21	-5.06
YenC1	A0A3S6EXC9	-3.53	4.07E-7	-4.05	-3.01
YenC2	A0A3S6EWV9	-4.67	6.53E-5	-6.14	-3.21
Chi1	A0A3S6EWX1	-5.67	9.16E-8	-6.33	-5.02
Chi2	A0A3S6EWW7	-6.83	4.07E-7	-7.84	-5.83
Cbp	A0A3S6EXR6	-4.95	5.49E-8	-5.45	-4.45
Chi3	A0A3S6F1Q8	-2.51	1.20E-5	-3.13	-1.9
Pil36	A0A3S6F569	-3.07	2.47E-4	-4.27	-1.88
PirA	A0A3S6F007	-5.37	2.84E-7	-6	-4.74
NucA	A0A3S6F4M5	-7.05	1.02E-5	-8.6	-5.51
M66/StcE	A0A3S6EYX4	-6.57	1.94E-4	-9.02	-4.12
OmpR/PhoB	A0A3S6F5G2	-3.27	9.62E-5	-4.37	-2.17
Endolysin	A0A3S6F4L4	-2.78	2.86E-2	-5.21	-0.36

Supplementary Table 3. List of primers to generate different *Yersinia entomophaga* mutants

Mutant	Name	Sequence (5' to 3')	Purpose
Δ LC Δ YenTc	tc_upstream_F	GCTCCTTCGCATTCAT	Confirmation of plasmid insertion in the genome
Δ LC Δ YenTc	tc_upstream_R	AGGTAGTGCTATTGTGAC	Confirmation of plasmid insertion in the genome
Δ LC Δ YenTc	tc_downstream_F	CTTCCGGCGTAGTGGC	Confirmation of plasmid insertion in the genome
Δ LC Δ YenTc	tc_downstream_R	CCCGCCTGCTATAACTGT	Confirmation of plasmid insertion in the genome
Δ LC Δ YenTc	yenB_F	GTAAGGTGTTCAATGGTG	Confirmation of the deletion of the Tc toxin gene cluster
Δ LC Δ YenTc	yenB_R	AAATCAATTTCTGAAATT AAGAGC	Confirmation of the deletion of the Tc toxin gene cluster
Δ LC Δ YenTc	yentc_f1_sal1	ATATATGTGCGACAGGCAT AATATGTACCTG	Generation of the plasmid for deletion of the gene cluster
Δ LC Δ YenTc	yentc_r1_xho1	ATATATCTCGAGGCGGTT TTCTTTTTTAATCG	Generation of the plasmid for deletion of the gene cluster
Δ LC Δ YenTc	yentc_f2	AACGTTGCGCGCTGGTTT CAGACTCCATTTTTTAA TTA	Generation of the plasmid for deletion of the gene cluster
Δ LC Δ YenTc	yentc_f2	TAATTTAAAAAATGGAGT CTGAAACCAGCGCAAC GTT	Generation of the plasmid for deletion of the gene
Δ LC Δ M66	YenM66_XmaI_F	ATATATCCCGGGATCTTTG TATTGTAATTTTGAGCATC ACAC	Generation of the plasmid for deletion of the gene
Δ LC Δ M66	YenM66_F1	AGATGATAATACGCAAGTT AATATCTGTGGACAGAGTT TGATGCCATACTG	Generation of the plasmid for deletion of the gene
Δ LC Δ M66	YenM66_R1	CAGTATGGCATCAAACCTCT GTCCACAGATATTAACCTG CGTATTATCATCT	Generation of the plasmid for deletion of the gene

Δ LC Δ M66	YenM66_NheI_R	ATATATGCTAGCTTCACTG GACCGACGG	Generation of the plasmid for deletion of the gene
Δ LC Δ M66	Yen_M66_upstre am_F	TTAATGAAAAGCAGCGG	Confirmation of plasmid insertion in the genome
Δ LC Δ M66	Yen_M66_upstre am_R	CATTTTAAATACCTTATTT AATTGATGT	Confirmation of plasmid insertion in the genome
Δ LC Δ M66	Yen_M66_downs tream_F	TCCAACCTGGAATTCA	Confirmation of plasmid insertion in the genome
Δ LC Δ M66	Yen_M66_downs tream_R	CGTACATCCTTTCTGC	Confirmation of plasmid insertion in the genome
Δ LC Δ M66	Yen_Mtp_downst ream_F	TCCAACCTGGAATTCA	Confirmation of the deletion of M66 gene
Δ LC Δ M66	Yen_Mtp_downst ream_R	CGTACATCCTTTCTGC	Confirmation of the deletion of M66 gene
<i>chi2-sfGFP</i>	p443_chi2- sfGFP_A1	TGGGTAAAAAGGATCGA TCCTCTAGAGCGCCGATA CCAGTATTGAAT	Generation of the plasmid for the Chi2- sfGFP fusion
<i>chi2-sfGFP</i>	chi2-sfGFP_B1	CTTCGCCTTTACGCATTCC GCCACCTGCCGCAGCCTT GCTCTTAATTTTCAG	Generation of the plasmid for the Chi2- sfGFP fusion
<i>chi2-sfGFP</i>	chi2-sfGFP_C1	CTGAAATTAAGAGCAAG GCTGCGGCAGGTGGCGG AATGCGTAAAGGCGAAG	Generation of the plasmid for the Chi2- sfGFP fusion
<i>chi2-sfGFP</i>	chi2-sfGFP_D1	TAACAATTGGCGCTGTAA ACGTCATTTGTACAGTTC ATCC	Generation of the plasmid for the Chi2- sfGFP fusion
<i>chi2-sfGFP</i>	chi2-sfGFP_E1	GGATGAACTGTACAAATG ACGTTTACAGCGCCAATT GTTA	Generation of the plasmid for the Chi2- sfGFP fusion
<i>chi2-sfGFP</i>	p443_chi2- sfGFP_F1	GTGAATTCAAAGGGAGA GCTCAGATATCTCGCTCG CCGTAATC	Generation of the plasmid for the Chi2- sfGFP fusion