SUPPLEMENTARY FIGURES

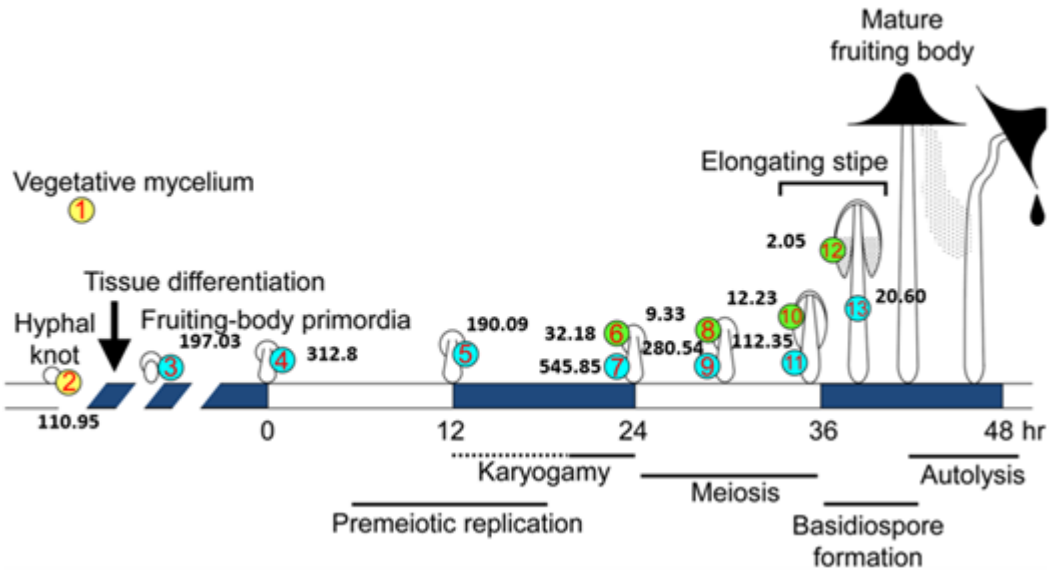

Figure S1. Expression of the *cml1* gene during fruiting body formation of *C. cinerea* AmutBmut. Black numbers indicate fold changes relative to vegetative mycelium according to the S1 dataset of (Muraguchi et al., 2015). The figure has been adapted from Figure 1 from this paper. The numbers in the circles indicate different fruiting body stages and tissues. Cap tissue is indicated by green and stipe tissue by turquoise shading of the circles. Yellow shading of the circles indicates mycelial tissue.

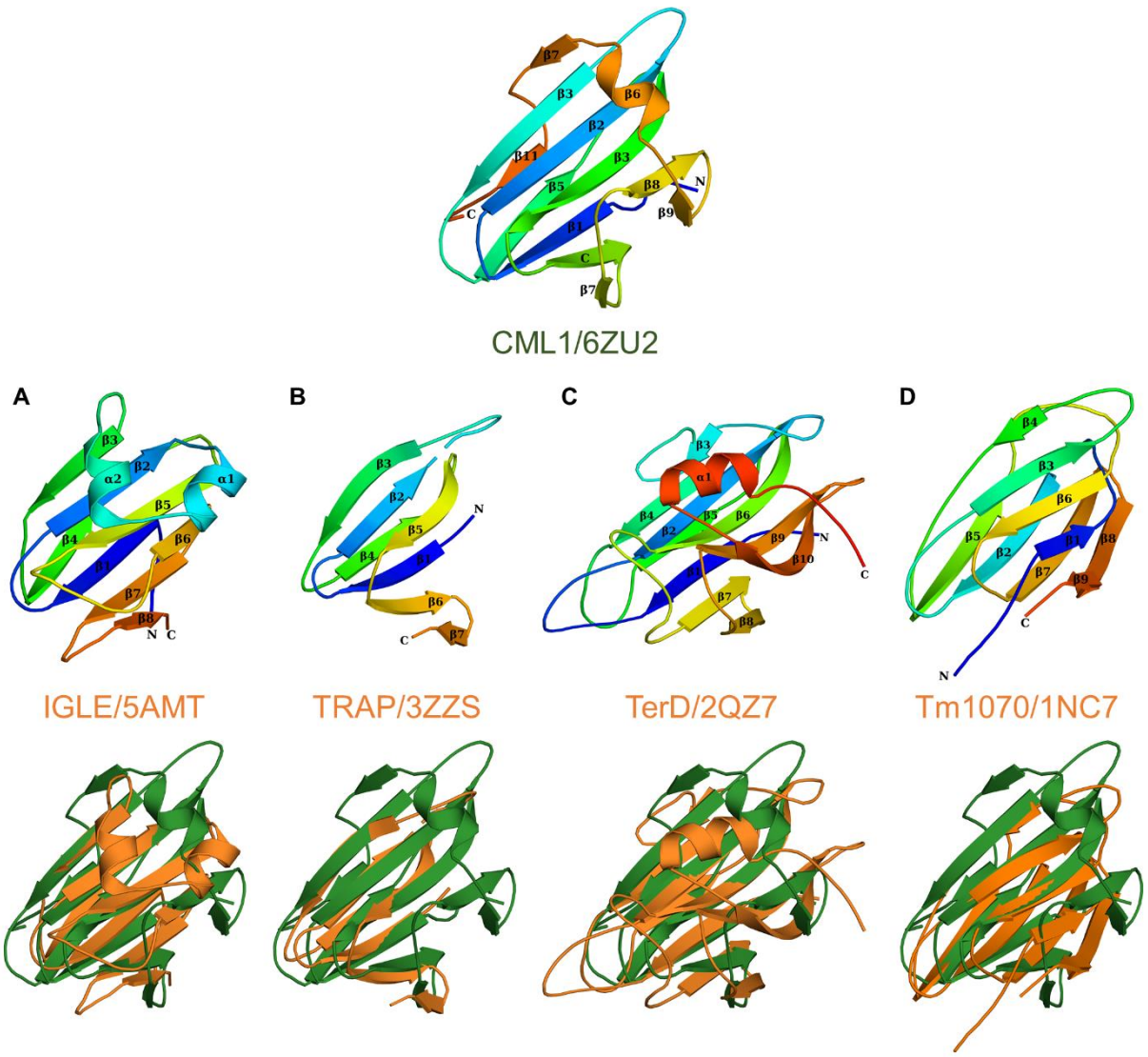

Figure S2. Structural alignment of CML1 with other proteins. Cartoon representation of CML1 (top), *Francisella tularensis* intracellular growth loci protein E (IGLE, A, PDB ID: 5AMT), *Bacillus stearothermophilus* TRP RNA-binding attenuation protein (TRAP, B, PDB ID:3ZZS), telluride resistance protein (C, PDB ID:2QZ7) and hypothetical protein TM1070 from *Thermotoga maritima* (D, PDB ID:1NC7) colored from blue to red from N to C-terminus with secondary structure elements labelled. Overlay of CML1 colored in dark green with other proteins colored in orange is represented in the bottom panels.

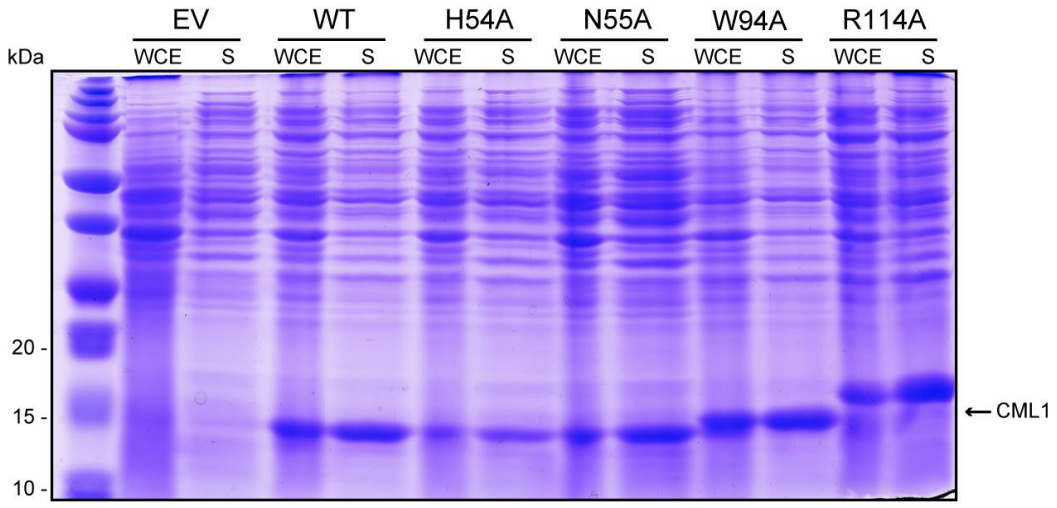

Figure S3. Expression levels of CML1 wildtype (WT) and variants thereof (H54A, N55A, W94A, R114A) in the respective *E. coli* BL21 transformants used for the nematotoxicity assays (Figure 10). Bacterial whole cell extracts (WCE) and supernatants (S) after high spin centrifugation thereof (Kunzler et al., 2010) were loaded on an SDS-PAGE gel and stained with Coomassie Blue. BL21 containing empty vector (EV) served as negative control. The protein sizes of the marker and the position of the CML1 band are indicated.

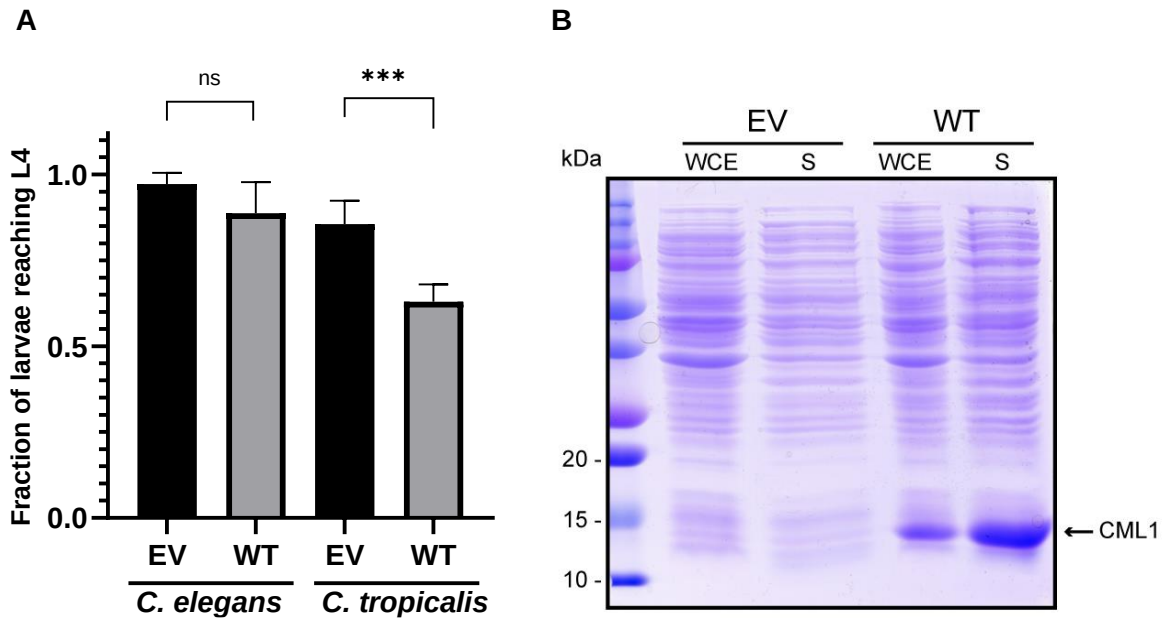

Figure S4. Toxicity of CML1 towards bacterivorous nematodes *C. elegans and C tropicalis*. (A) The development of L1 larvae feeding on *E. coli* expressing CML1 wildtype protein (WT) or empty vector (EV) to the L4 stage was assessed. The error bars indicate the standard deviation of five replicates. Comparisons between conditions were performed using Dunnett's Multiple Comparison Test (ns, not significant; *, P<0.05;**, P<0.01,***, P<0.001;****, P<0.0001). (B) Expression levels of CML1 wildtype (WT) in the respective *E. coli* BL21 transformants used for the nematotoxicity assay compared to BL21 containing empty vector (EV). Bacterial whole cell extracts (WCE) and high spin centrifugation supernatants (S) thereof (Kunzler et al., 2010) were loaded on an SDS-PAGE gel and stained with Coomassie Blue. Protein sizes of the marker and the position of the CML1 band are indicated.

SUPPLEMENTARY TABLES

Table SI. Oligonucleotides used in this study. Restrictions sites are underlined and substituted codons for site-directed mutagenesis are in bold.

Oligonucleotide	Sequence (5' → 3')
locus-fwd	GGAACTCTGCGGCACATGATGAAGG
locus-rev	AGGAATCCTGTGGACCGCTTGATTG
NdeI-CML1-N	CATATGGCCATCTTTCACACCGGCAGCGAGCTCTTC
BamHI-CML1-C	GGATCCTCAAAGGTTATCCGGGAGGGCATAAGCGG
NdeI-CML1-NHis	CATATGGCCCATCATCATCATCATCATCACATCTTTCA CACCGGCAGCGAGCTCTTC
CML1-H54A-fwd	CGTCTCCTTCAGC**GCC**AACTACACCCGCTTC
CML1-H54A-rev	GAAGCGGGTGTAGTT**GGC**GCTGAAGGAGACG
CML1-N55A-fwd	CTCCTTCAGCCAC**GCC**TACACCCGCTTCTCC
CML1-N55A-rev	GGAGAAGCGGGTGTA**GGC**GTGGCTGAAGGAG
CML1-W94A-fwd	CGAGCACTATCCAT**GCC**GGATCGTCGACTG
CML1-W94A-rev	CAGTCGACGATCC**GGC**ATGGATAGTGCTCG
CML1-R114A-fwd	TTCCGGGGGCGGTTAAT**GCC**GATAAGGTCACTA
CML1-R114A-rev	GTAGTGACCTTATC**GGC**ATTAACCGCCCCCGGA

Table SII. Plasmids used in this study.

Plasmid	Description	Source
PMA64	pET22-NHisCML1	This study
PMA78	pET22-CML1	This study
PMA123	pET24-CML1	This study
PMA360	pET22-NHisCML1(H54A)	This study
PMA361	pET22-NHisCML1(N55A)	This study
PMA362	pET22-NHisCML1(W94A)	This study
PMA363	pET22-NHisCML1(R114A)	This study
PMA857	pET22-CML1(H54A)	This study
PMA858	pET22-CML1(N55A)	This study
PMA859	pET22-CML1(W94A)	This study
PMA860	pET22-CML1(R114A)	This study

Table SIII. CML1 homologues found by a BLAST search in the Mycocosm database (Grigoriev et al., 2014).

Name in Figure	Mycocosm Protein Id	Species name	Residue range	NCBI entry	% identity
Coprinopsis cinerea AmutBmut	439037	*Coprinopsis cinerea* AmutBmut1 pab1-1 v1.0	1-127	XP_001832882.2	–
Coprinopsis cinerea O7	8993	*Coprinopsis cinerea* okayama7	1-127		100
Volvariella volvacea 1	121128	*Volvariella volvacea* V23	1-127		57
Volvariella volvacea 2	121154		1-126		53
Coprinopsis sclerotiger	521131	*Coprinopsis C. sclerotiger* v1.0	1-128		54
Volvariella volvacea 3	121144	*Volvariella V. volvacea* V23	1-126		48
Coprinus phaeopunctatus 1	398102	*Coprinus phaeopunctatus* MPI-PUGE-AT-0042 v1.0	1-128		52
Coprinus phaeopunctatus 2	398089		1-128		48
Agaricus bisporus	127454	*Agaricus bisporus var. burnettii* JB137-S8	1-127	XP_007329114.1	47
Gymnopus luxurians	46110	*Gymnopus luxurians* v1.0	1-127	KIK57536.1	43
Amanita muscaria	346422	*Amanita muscaria Koide* v1.0	1-128	KIL57993.1	51
Clitocybe sp.	68350	*Clitocybe sp*. CONT 1119283 v1.0	1-127		49
Piloderma croceum 1	75139	*Piloderma P. croceum* F 1598 v1.0	1-125	KIM78688.1	44
Piloderma croceum 2	824131		1-125		43
Russula earlei 1	1201497	Russula *R. earlei* BPL698 v1.0	1-127		44
Russula earlei 2	1201446	*R. earlei* BPL698 v1.0	1-127		43
Russula earlei 3	1227043		1-126		40
Russula earlei 4	1242301		1-126		40
Laccaria bicolor 1	328965	*Laccaria bicolor* v2.0	1-128	XP_001883034.1	45
Laccaria bicolor 2	630915	*L. bicolor* v2.0	111-238	XP_001883043.1	44
Laccaria amethystina 1	13511	*Laccaria amethystina* LaAM-08-1 v2.0	1-128	KIJ92571.1	44
Laccaria amethystina 2	1673216		1-127	KIJ96346.1	42
Laccaria amethystina 3	7479		1-127	KIK00628.1	44
Laccaria amethystina 4	686406		1-127	KIJ90915.1	44
Rhodocollybia butyracea 1	520169	*Rhodocollybia butyracea* CCBAS 279 v1.0	1-127		42
Rhodocollybia butyracea 2	1360276	*R. butyracea* AH 40177 v1.0	1-127		41
Rhodocollybia butyracea 3	134408	*R. butyracea* CCBAS 279 v1.0	1-128		41
Rhodocollybia butyracea 4	298035	*R. butyracea* AH 40177 v1.0	1-128		41
Galerina marginata 1	1352495	*Galerina marginata* v1.0	1-128	KDR69481.1	34
Galerina marginata 2	411187		1-131	KDR77697.1	31
Hebeloma cylindrosporum 1	444533	*Hebeloma cylindrosporum* h7 v2.0	279-412	KIM42913.1	32
Hebeloma cylindrosporum 2	444533	*H. cylindrosporum* h7 v2.0	145-279	KIM42913.1	30
Sphaerobolus stellatus 1	42948	*Sphaerobolus stellatus* v1.0	1-132	KIJ53189.1	32
Sphaerobolus stellatus 2	42925		1-130	KIJ53140.1	31
Sphaerobolus stellatus 3	775828		1-131[a]	KIJ53146.1	27
Sphaerobolus stellatus 4	155324		1-139[a]	KIJ53139.1	28
Sphaerobolus stellatus 5	85738		1-129	KIJ53145.1	27
Mycena rebaudengoi 1	1514180	*Mycena rebaudengoi* CBHHK068 v1.0	1-129		32
Mycena rebaudengoi 2	1071743		1-129		31
Mycena rebaudengoi 3	1090025		1-129		31
Mycena latifolia	1243747	*Mycena latifolia* 10383 v1.0	1-127		33
Mycena epipterygia	676034	*Mycena epipterygia* CBHHK145m v1.0	1-127		28
Roridomyces roridus	803218	*Roridomyces roridus* 9284 v1.0	1-130		25

[a] contains C-terminal extension

Table SIV. Statistics on Data Collection and Refinement

Structure	***Apo CML1 Platinium derivative***	***CML1 - H-type I complex***	***CML1 – Lewis A complex***
Data collection			
Space Group	$P2_1$	$P3_1$	$P3_1$
Unit cell a, b, c (Å) α, β, γ (°)	44.50, 73.63, 111.96 90, 101.03, 90	74.04, 74.04, 119.89 90, 90, 120	73.925, 73.925, 119.59 90, 90, 120
Beamline	X06DA/SLS	PX1/ SOLEIL	PX1/ SOLEIL
Wavelength (Å)	1.07210	0.97856	0.97856
Resolution limits (Å)*	61.17-1.35 (1.43-1.35)	37.02-1.55 (1.58-1.55)	43.7-1.95 (2.0-1.95)
Reflections: measured unique	2256301 303404	974517 106704	222807 52412
Completeness (%)*	98.9 (95.2)	100 (100)	98.4 (99.4)
R_{meas} (%)	7.6 (150.3)	5.6 (60.5)	6.4 (57.5)
Mean I / σI*	17.3 (1.34)	21.6 (3.7)	13.0 (2.5)
Multiplicity*	7.4 (6.8)	9.1 (9.3)	4.3 (4.2)
$CC_{1/2}$*	100 (63.4)	99.9 (89.9)	99.9 (85.1)
Wilson B factor	14.2	21.8	29.5
Refinement			
Resolution (Å)	61.17-1.35	37.02-1.55	43.7-1.95
R_{work}/R_{free} (%)†	14.6/ 17.6	15.1 / 18.1	18.7 / 23.9
Nb reflections R_{work}/R_{free}	153851 / 7693	101249 / 5401	49818 / 2590
CC(work) / CC(Free)	0.97 / 0.96	0.98 / 0.97	0.97 / 0.95
No. of atoms/ Bfac ($Å^2$)			
-Protein	5935 / 17.5	5878/ 22.2	5772 /33.0
-Sugar	NA	216 / 28.0	238 / 43.9
-Waters	885 / 32.5	917 / 34.0	708 / 38.4
R.m.s.d. bond lengths (Å)	0.006	0.015	0.015
R.m.s.d. bond angles (°)	1.38	1.89	1.73
R.m.s.d. chiral ($Å^3$)	0.086	0.102	0.099
Clashscore	3	1	1
Ramachandran plot (%)			
-Allowed regions	99.2	99.7	100
-Favored regions	96.5	96.7	97.2
-Outliers	6	2	0
PDB Code	6ZRW	6ZU2	6ZV5

* Values in parentheses correspond to high resolution shell in data collections.

SUPPLEMENTARY REFERENCES

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Kunzler, M., Bleuler-Martinez, S., Butschi, A., Garbani, M., Luthy, P., Hengartner, M.O., and Aebi, M. (2010). Biotoxicity assays for fruiting body lectins and other cytoplasmic proteins. Methods Enzymol *480*, 141-150.
Muraguchi, H., Umezawa, K., Niikura, M., Yoshida, M., Kozaki, T., Ishii, K., Sakai, K., Shimizu, M., Nakahori, K., Sakamoto, Y.*, et al.* (2015). Strand-Specific RNA-Seq Analyses of Fruiting Body Development in Coprinopsis cinerea. PLoS One *10*, e0141586.