Supplementary Information

Fatal attraction of *Caenorhabditis elegans* to predatory fungi through 6-methyl-salicylic acid

Xi Yu[1, 4], Xiaodi Hu[1], Maria Mirza[1], Nicole Wernet[1], Frank Kirschhöfer[2], Gerald Brenner-Weiß[2], Julia Keller[3], Mirko Bunzel[3] and Reinhard Fischer[1]*

running head: Methyl salicylate as morphogen and quorum sensing molecule in *D. flagrans*

Address: [1]Karlsruhe Institute of Technology (KIT) - South Campus
Institute of Applied Biosciences
Dept. of Microbiology
Fritz-Haber-Weg 4
D-76131 Karlsruhe, Germany
Phone: +49-721-6084-4630
E-mail: reinhard.fischer@KIT.edu
Homepage: www.iab.kit.edu

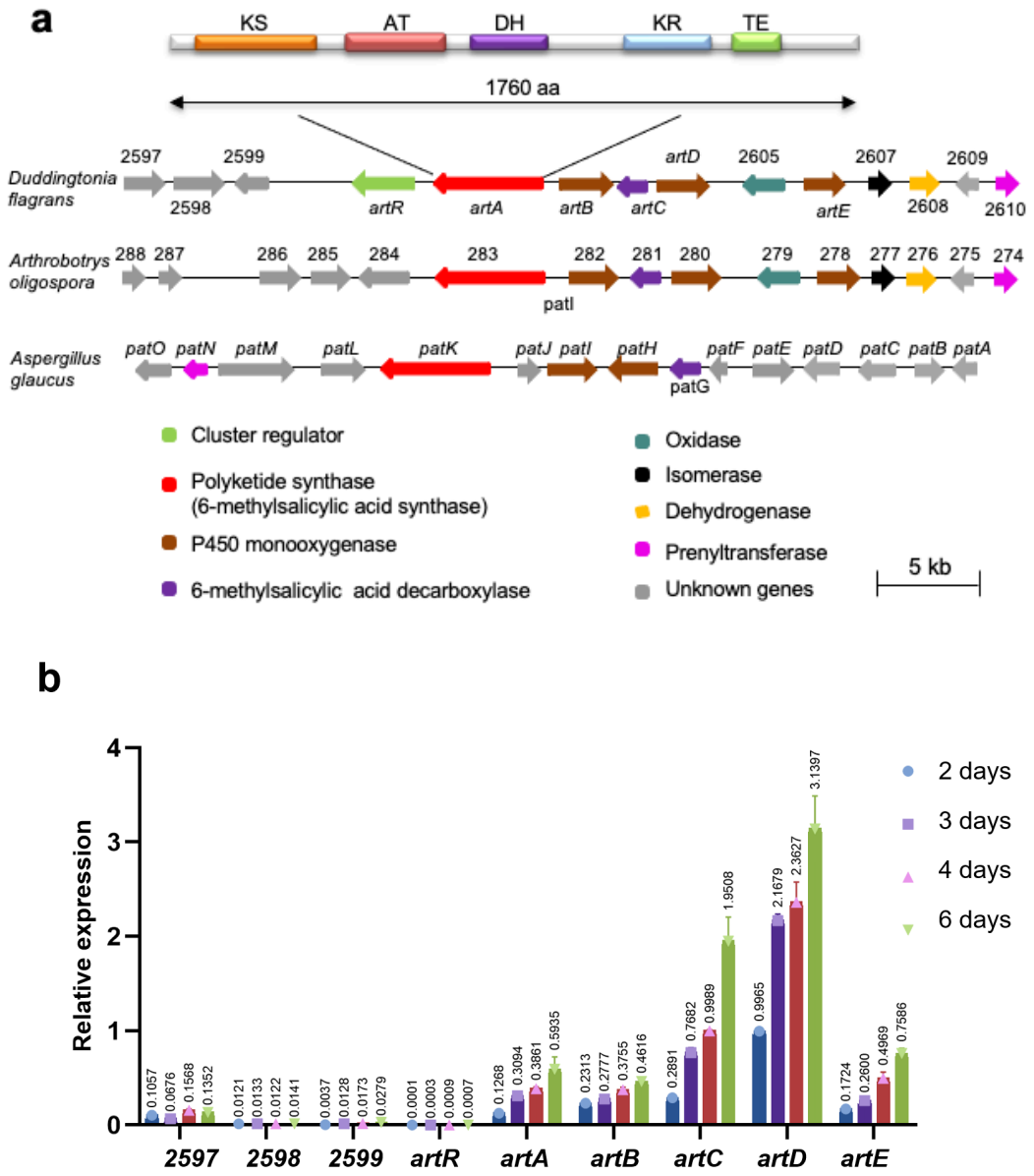
[2] Karlsruhe Institute of Technology (KIT) - North Campus
Institute of Functional Interfaces
Bioengineering and Biosystems
Hermann-von-Helmholtz-Platz 1
D-76344 Eggenstein Leopoldshafen

[3] Karlsruhe Institute of Technology (KIT) - South Campus
Institute of Applied Biosciences
Dept. of Food Chemistry and Phytochemistry
Adenauerring 20A
D-76131 Karlsruhe

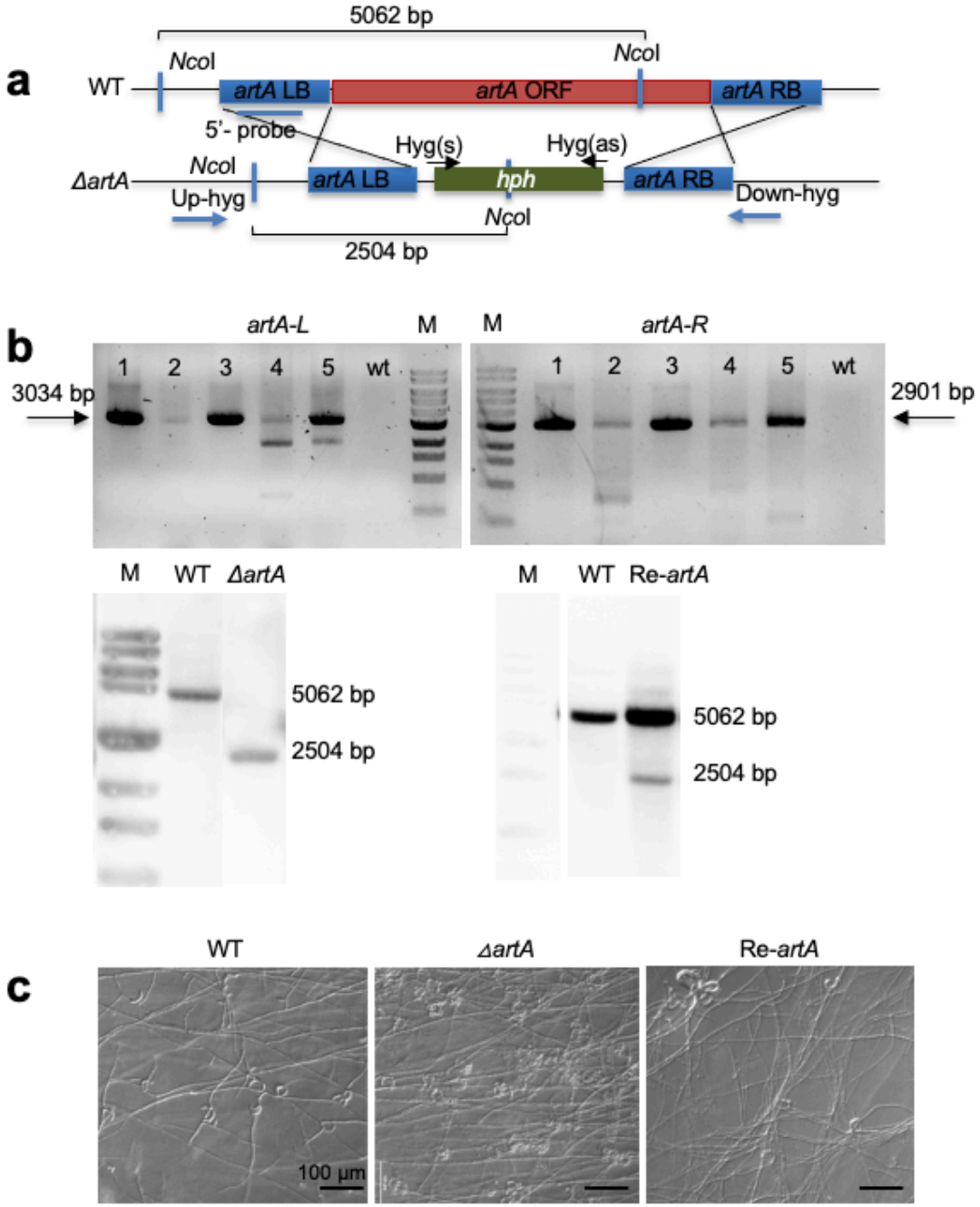
[4] present address:
Shanghai Engineering Research Centre of Hadal Science and Technology, College of Marine Sciences, Shanghai Ocean University
Huchenghuan Road 999
201306, Shanghai, China

* corresponding author

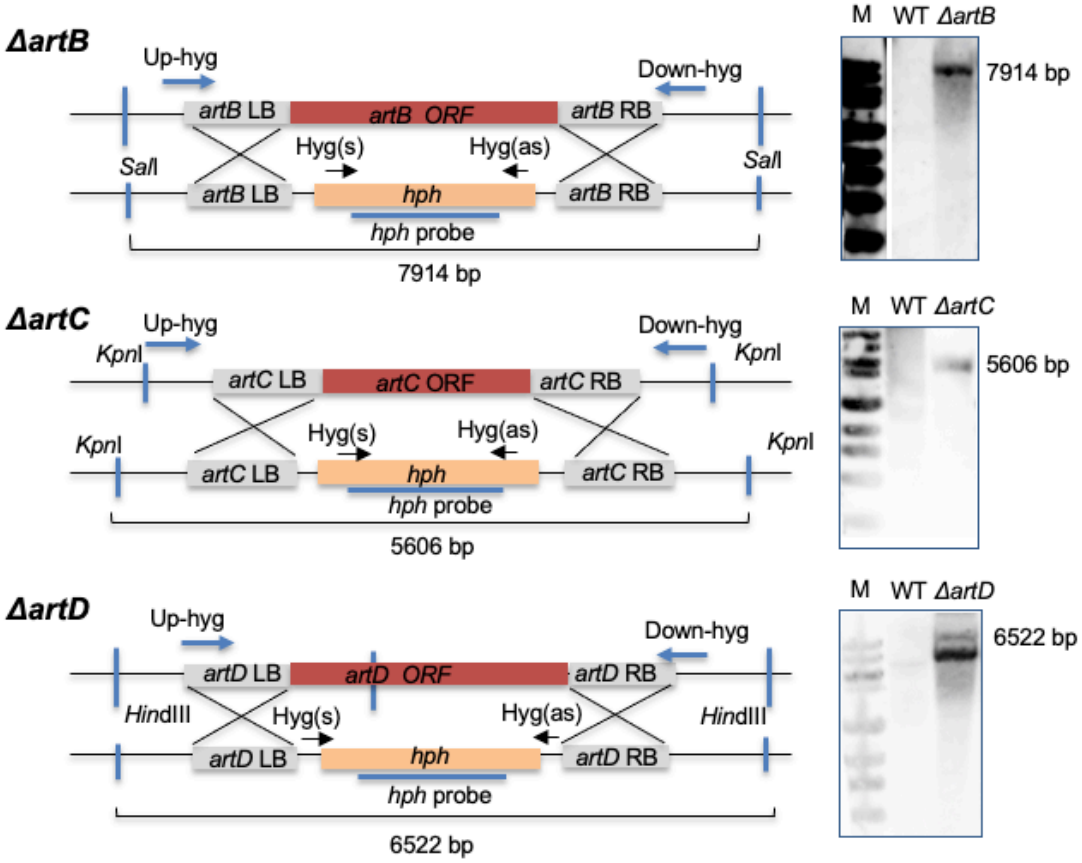
Suppl. Figures



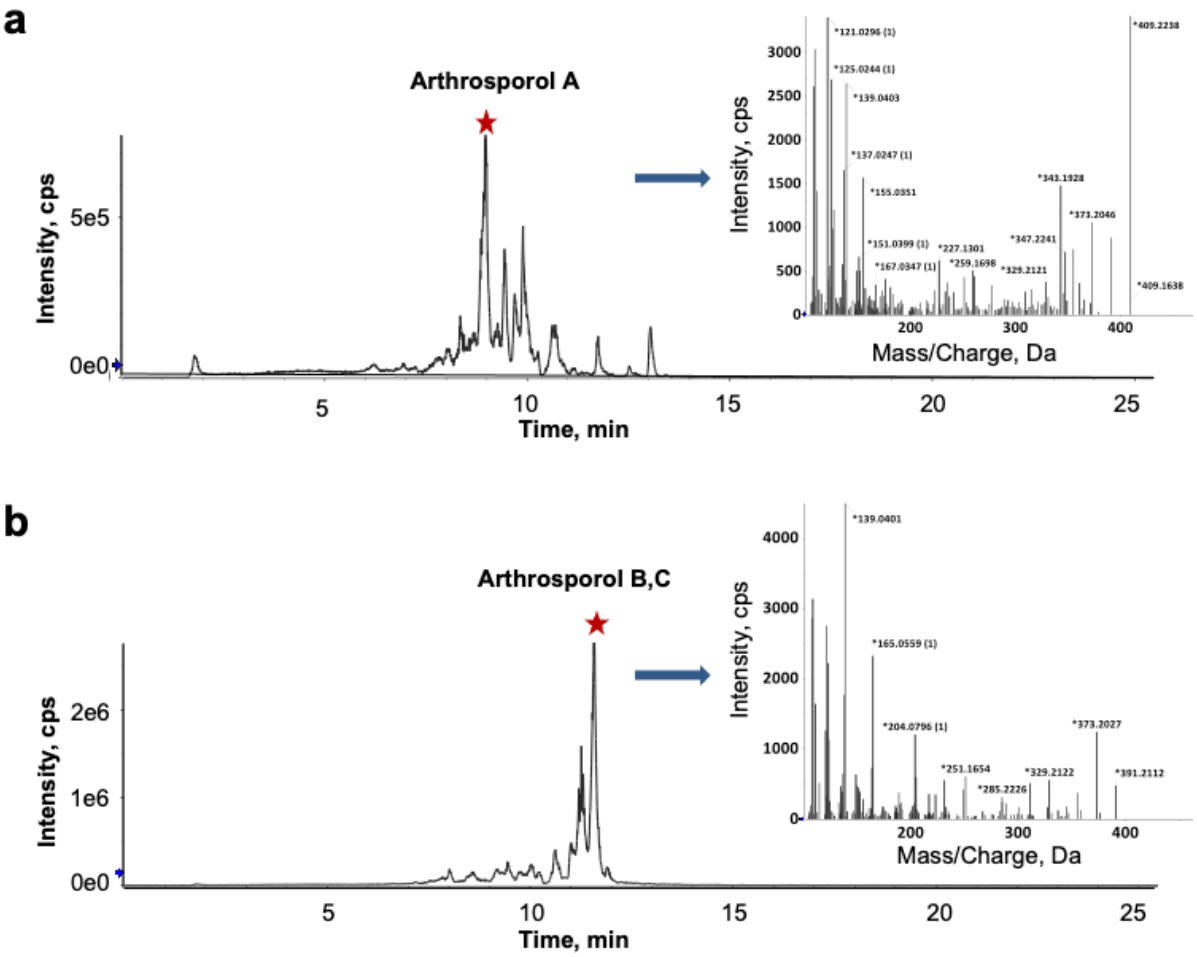
Suppl. Fig. S1: Characterization of the *artA*-gene cluster. (a) Bioinformatic analysis and comparison with the corresponding clusters in *Arthrospora oligospora* and the patulin gene cluster of *Aspergillus glaucus*. The *artA* gene (DFL_002601) is 5363 bp in length and encodes a 1760-amino acid long protein. It contains five typical catalytic domains, including a Keto Synthase (KS), an acyltransferase (AT), a dehydratase (DH), a β-ketoacylreductase domain (KR), and an acyl carrier protein (ACP) for the PKS. **(b)** Expression analysis of several genes indicated in **(a)**. RNA was extracted after two, three, four and six days and analyzed by quantitative real time RT PCR. Data are presented as mean values +/- SD, *n* = 3 biological with 3 technical replicates. Source data are provided as a Source Data file.



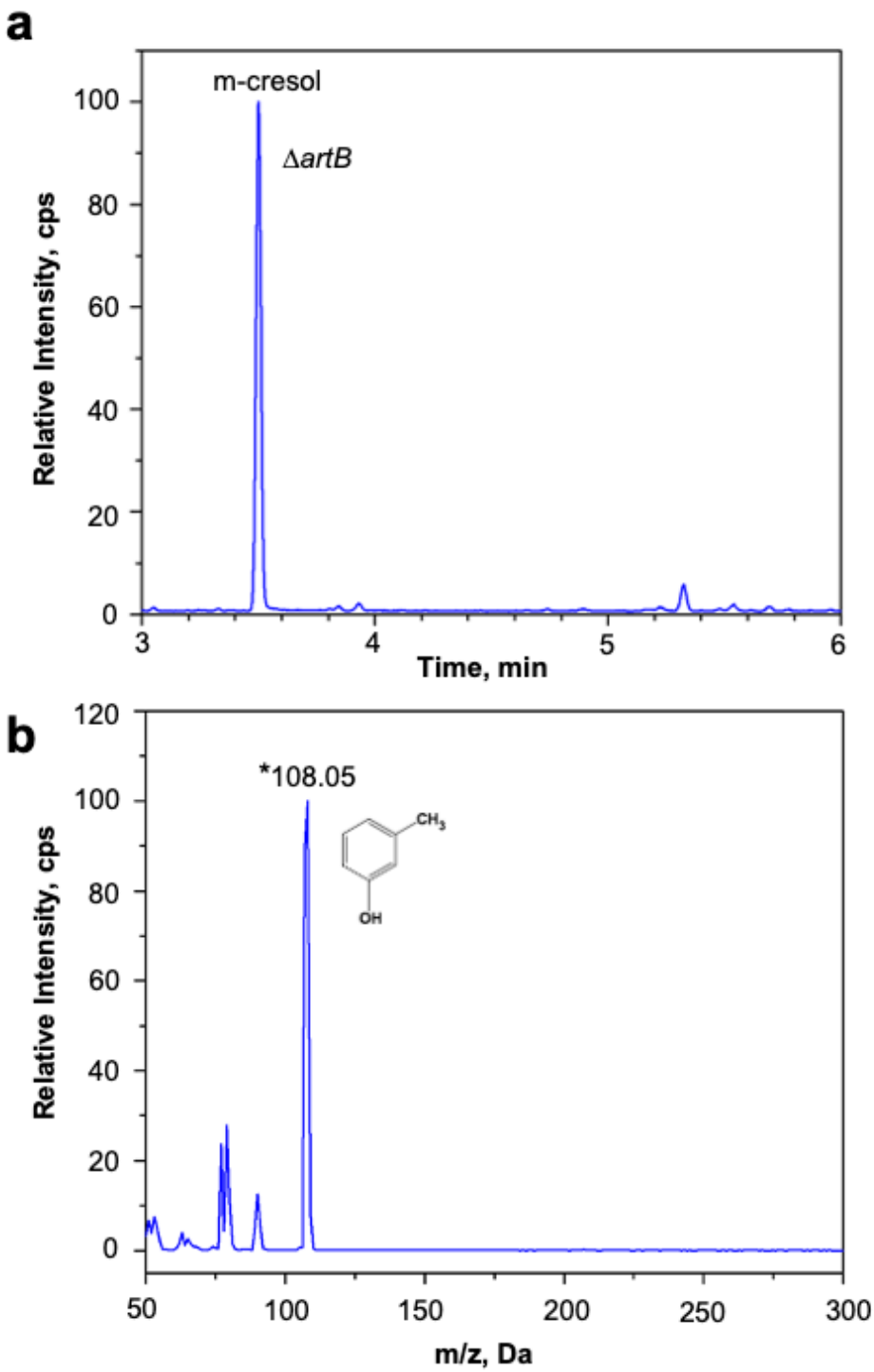
Suppl. Fig. S2: **(a)** Scheme of the deletion strategy for *artA*. **(b)** (upper panels) PCR analysis of WT and five independent transformants. The left part (*artA-L*) was amplified with the Up-hyg and Hyg(as), and the right part (*artA-R*) with Hyg(s) and Down-hyg. (lower panels) Southern blot analysis of the WT and Δ*artA*-deletion strain (SXY05) and a re-complemented strain (SXD05). Genomic DNA was digested and hybridized as indicated in **(a)**. **(c)** Overview of traps formed in colonies on agar plates (9 cm diameter) of the strains described in **(b).** Scale bar, 100 µm. Source data are provided as a Source Data file.



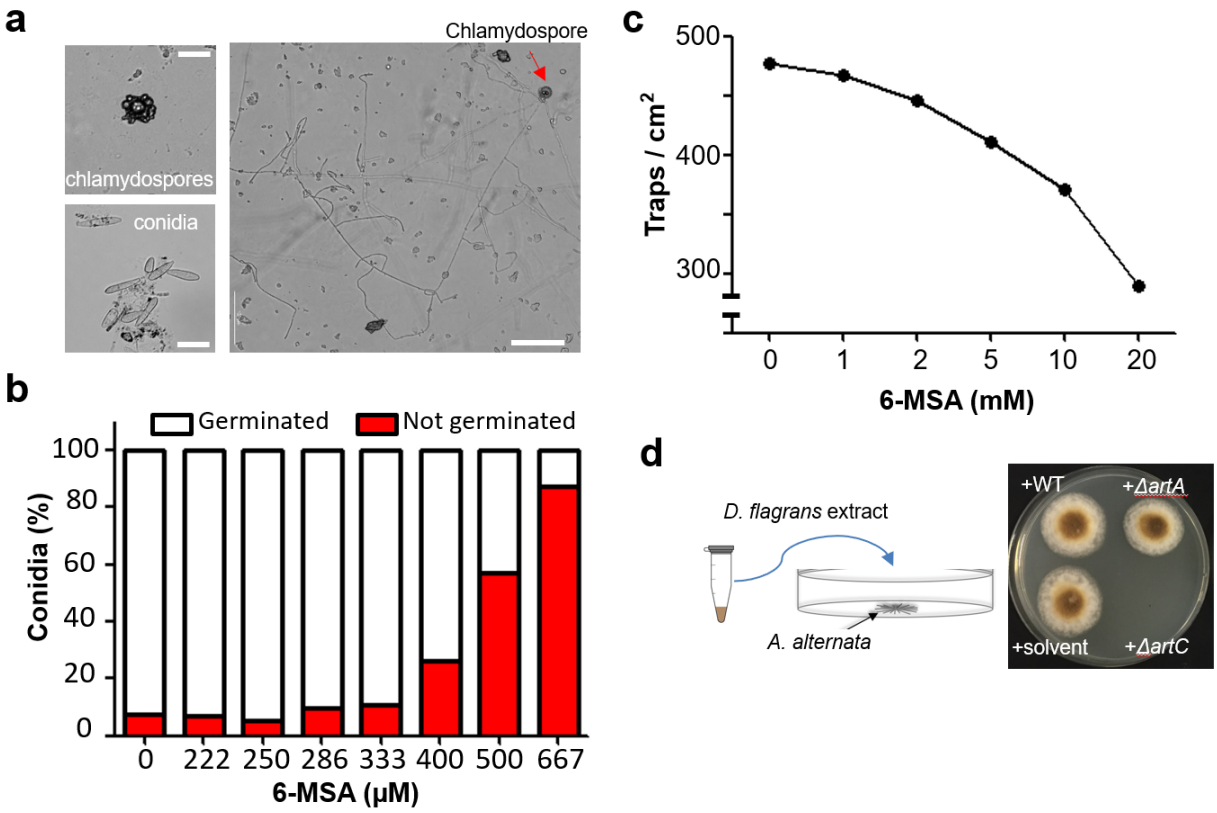
Suppl. Fig. S3: Schemes for the deletion of *artB-D* and results of the Southern hybridization using the *hph* gene as probe. Strains were SXY15 (Δ*artB*), SXY14 (Δ*artC*) and SXY16 (Δ*artD*). Source data are provided as a Source Data file.



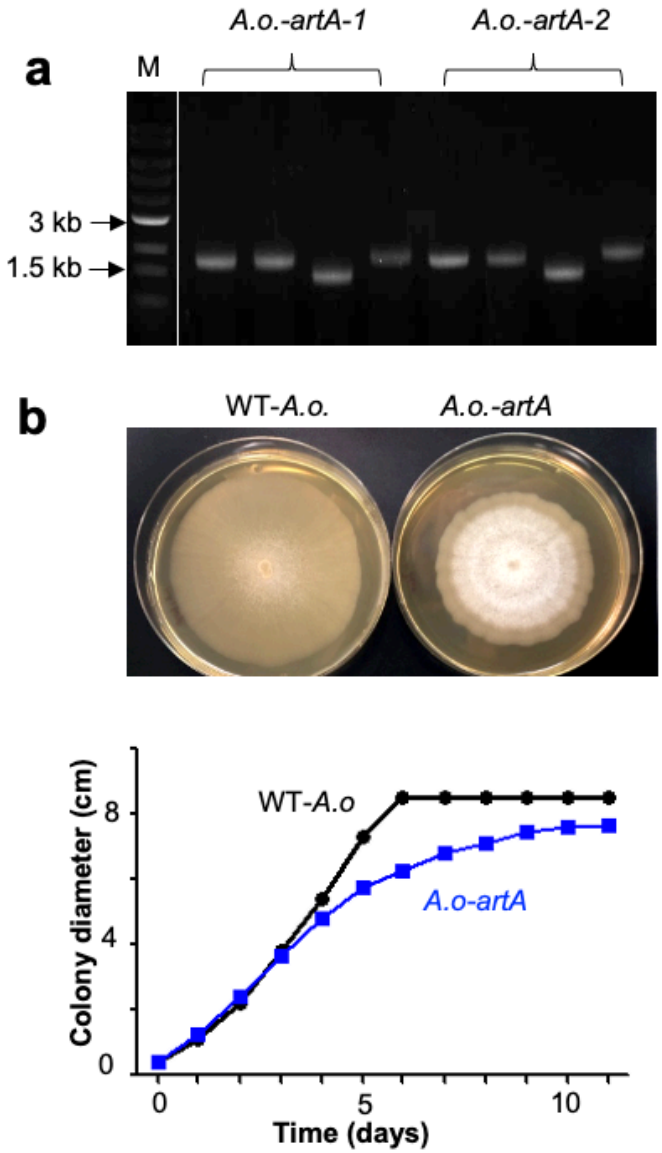
Suppl. Fig. S4: (a) LC-MS/MS characterization of arthrosporol A of the culture broths of wild type and the *ΔartA*-deletion strain. IDA TOF MS (100-1000) from 6.821 min, 409.223 +/-0.005 Da, noise filtered (noise multiplier=1.5), Gaussian smoothed (0.5 points). (**b)** Quantitative product ion; IDA TOF MSMS (70-1000) from 6.825 min. Precursor 409.2 Da, +1, noise filtered (noise multiplier=1.5), Gaussian smoothed (0.5 points). Quantitative and Qualitative ions m/z MS/MS of 409.223, 139.1, 155.0, 391.4. Source data are provided as a Source Data file.



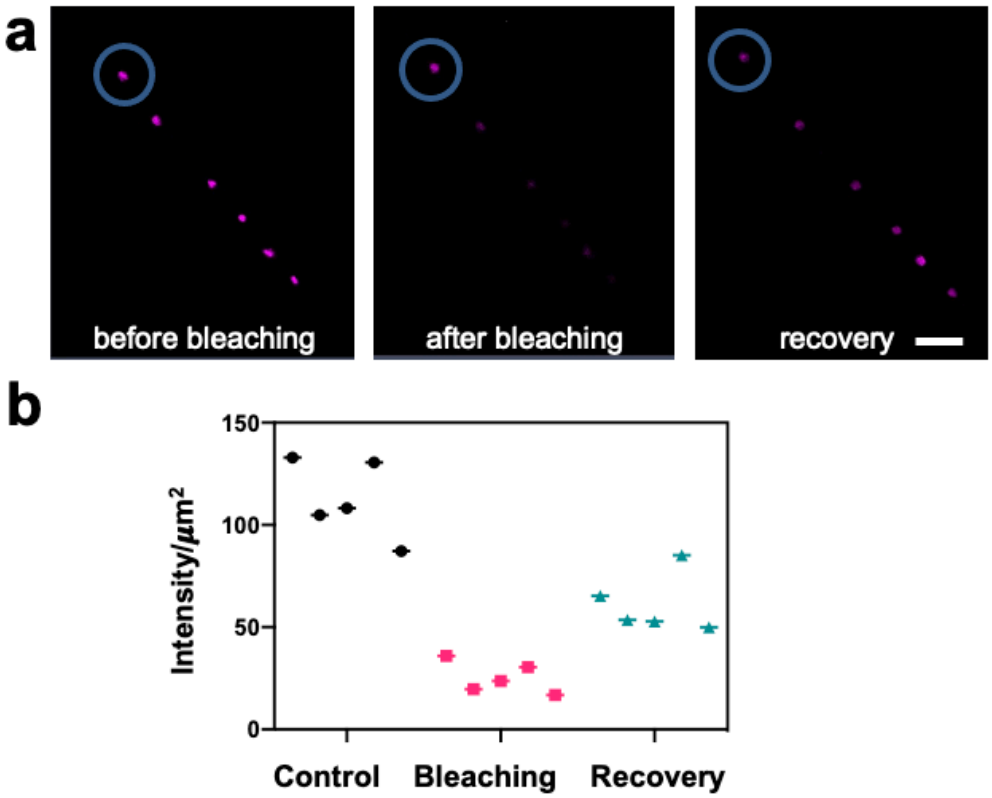
Suppl. Fig. S5: (a) GC-MS/MS characterization of m-cresol of the cultural broths of the *ΔartB*-deletion strain. Retention time of m-cresol: 3.5 min. **(b)** MS spectrum of the cultural broth of *ΔartB* for m-cresol: Raw spectrum, 3.470 -> 3.542 (scan: 283 -> 326); base peak, m/z 108.05 (Inten: 2,106,625). Source data are provided as a Source Data file.



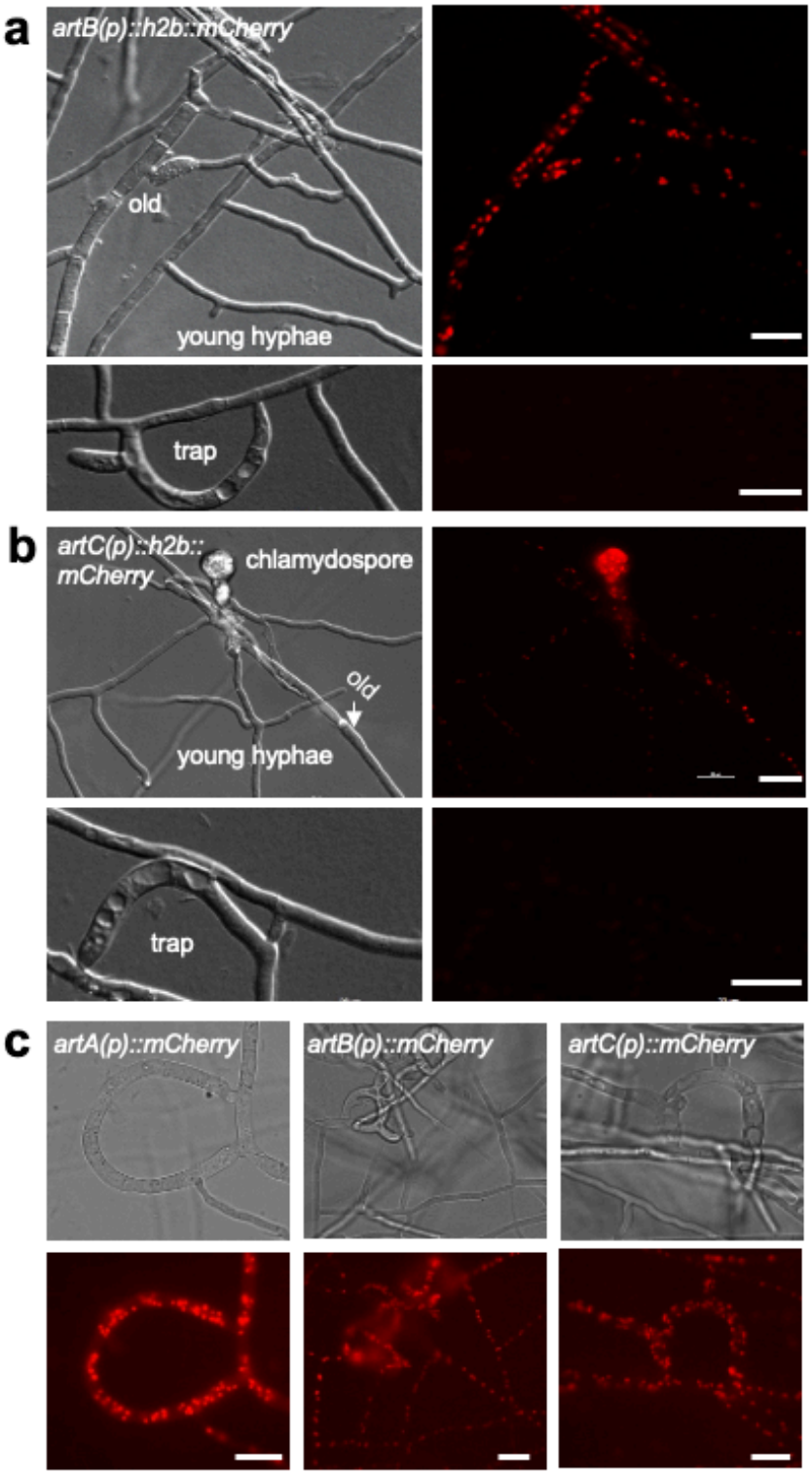
Suppl. Fig. S6: (a) Incubation of chlamydospores and conidia in the presence of 10 µl (undiluted)(left, Scale bar, 20 µm) and 10 µl (diluted 1:10 with the solvent) (right, Scale bar, 100 µm) extract. **(b)** Germination of spores was determined at different concentrations of 6-MSA. **(c)** Quantification of the number of traps a different 6-MSA concentrations. **(d)** Effect of *D. flagrans* extracts of cultural broth from WT and the Δ*artA* and Δ*artC* mutants on colony growth of *Alternaria alternata*. Source data are provided as a Source Data file.



Suppl. Fig. S7: Heterologous expression of *artA* in *A. oryzae.* (a) (left) PCR analysis of different transformants. Left lane: Two transformants *artA-1* and *artA-2* and WT were chosen for further analysis. Four fragments were amplified with primers pksA_Ao_transform_for and pksA_AO_tf_up_rev (1639 bp), pksA_AO_tf_up_for and PksA _recomp_mid_rev (1623 bp), PksA _recomp_mid_for and pksA_AO_tf_down_rev (1343 bp), pksA_AO_tf_down_for and pksA_Ao_PCRconfirm_rev (1719 bp). (right) Two colonies of WT and the *artA*-expressing strain (SXD01) after 11 days of growth on PDA medium. **(b)** Quantification of fungal growth by measuring the colony diameter. Source data are provided as a Source Data file.



Suppl. Fig. S8: Promoter activity of *artA* in *D. flagrans.* The promoter of *artA* was fused to *h2b* and *mCherry* (strain SXY17). (**a)** Fluorescence recovery after photobleaching (FRAP). The sample was bleached for 5 seconds and recovered for 14 minutes. (Scale bar, 10 µm) **(b)** Quantification of CTCF intensity value of 5 different nuclei (left) after bleaching 5 s and recovery for 14 min. Scale bar, 20 µm. Source data are provided as a Source Data file.



Suppl. Fig. S9: Expression analysis of *artA-C.* **(a)** Expression analysis of *artB* (strain SXY20). **(b)** Expression analysis of *artC* (strain SXY19). **(c)** Analysis of *artA-C* expression in old traps. Scale bar, 20 µm.

Suppl. Table S1: *D. flagrans* and *A. oryzae* strains used in this study.

Strain	Genotype	Reference
D. flagrans **CBS 349.94**	wild type (WT)	CBS-KNAW culture collection
SXY05	WT transformed with PXY09, Δ*artA::hyg*	This work
SXY14	WT transformed with PXY15, Δ*artC:*hyg,	This work
SXY15	WT transformed with PXY16, Δ*artB:*hyg	This work
SXY16	WT transformed with PXY17, Δ*artD:*hyg	This work
SXY17	WT transformed with PXY18, hyg, expression of *artA*	This work
SXY20	WT transformed with PXY21, hyg, expression of *artB*	This work
SXY19	WT transformed with PXY20, hyg, expression of *artC*	This work
SXD05 ***Re-artA***	*artA* re-complementation; SXY05 transformed with pXD6	This work
SXD53	Co-localization of ArtA-GFP and ArtB-mCherry *in locus*, WT transformed with pXD88 and pXD89	
A. oryzae **Wild type**	Wild type	Russel J. Cox, Leibniz University
SXD01 ***A.o-artA***	Heterologous expression of *artA* in WT, with pXD1	This work

Suppl. Table S2: Plasmids used in this study.

Name	Description	Reference
pJET1.2	ampR	Thermo Fisher
pXY09	*artA(l)::trpC(p)::hph::trpC(t):: artA(r); ampR;* plasmid for the deletion of *artA*	This work
pXY15	*artC(l)::trpC(p)::hph::trpC(t):: artC(r); ampR;* plasmid for the deletion of *artC*	This work
pXY16	*artB(l)::trpC(p)::hph::trpC(t):: artB(r); ampR* plasmid for the deletion of *artB*	This work
pXY17	*artD(l)::trpC(p)::hph::trpC(t):: artD(r); ampR* plasmid for the deletion of *artD*	This work
pXY18	*artA(p)::h2b::mCherry::TubT;* expression of *artA*	This work
pXY21	*artB(p)::h2b::mCherry::TubT* expression of *artB*	This work
pXY20	*artC(p)::h2b::mCherry::TubT* expression of *artC*	This work
pXD6	*pksA(p)::artA::artA(t); ampR; adeA* *artA* re-complementation	This work
pXD1	*amyB(p)::pksA::amyB(T); ampR; adeA* Heterologous expression of *artA* in *A. oryzae*	This work
pNH30	*gpdA(p)::mCherry::TubT*	[1]
pXD88	*artA::GFP::trpC(p)::hph::trpC(t)::artA(t)*	This work
pXD89	*artB::mCherry::trpC(p)::hph::trpC(t)::artB(t)*	This work

Suppl. Table S3: Oligonucleotides used in this study.

Name	Sequence (from 5' to 3')	Description
***artA* olup_fw**	CCGGATGGCTCGAGTTTTTCAGCAAGA TACATCCAATCCCCCCTAAC	*artA*-knockout-plasmid
***artA* olup_rev**	TCCACTAGCATTACACTTGGATCCcgAT CCCACCCATCCCAATTC	*artA*-knockout-plasmid
hygolup_fw	GGATCCAAGTGTAATGCTAGTGGA	*artA*-knockout-plasmid
hygolup_rev	CTGCAGTGGGGGGAGTTTAGGGAAA	*artA*-knockout-plasmid
***artA*oldown_fw**	TTTCCCTAAACTCCCCCCACTGCAGaa GCCAAAGAAGTCAAGTCAAAAG	*artA*-knockout-plasmid
***artA*oldown_rev**	TATTGTAGGAGATCTTCTAGAAAGATAC ATAACCGAACACCAAACAG	*artA*-knockout-plasmid
artBolup_fw	TGGCTCGAGTTTTTCAGCAAGATGTGG GCGAATAAGAATGAAAAG	*artB*-knockout-plasmid
artBolup_rev	TCCACTAGCATTACACTTGGATCCcgGA GCAAAGCGGAAACATAAAAG	*artB*-knockout-plasmid
artBoldown_fw	TTTCCCTAAACTCCCCCCACTGCAGaaA AGGAATACACGGGGCAAG	*artB*-knockout-plasmid
artBoldown_rev	TATTGTAGGAGATCTTCTAGAAAGATTA CACGAACAGCGATGGAC	*artB*-knockout-plasmid
artColup_fw	TGGCTCGAGTTTTTCAGCAAGATCTCG AACCCAAGCGGTAGAG	*artC*-knockout-plasmid
artColup_rev	CCTCCACTAGCATTACACTTCGGATTT GGGGCACAGTCTT	*artC*-knockout-plasmid
artColdown_fw	TCTTTCCCTAAACTCCCCCCATTCCGTT CCGATCGACTTCGT	*artC*-knockout-plasmid
artColdown_rev	TATTGTAGGAGATCTTCTAGAAAGATTG ACTTTGCGGCTCTTCCA	*artC*-knockout-plasmid
artDolup_fw	GATGGCTCGAGTTTTTCAGCAAGATTA	*artD*-knockout-plasmid

	CAAAGACAACGGCGGAG	
artDolup_rev	CTCCACTAGCATTACACTTGGATCCcgC CCCAAAGGTAACAAGAGAAG	*artD*-knockout-plasmid
artDoldown_fw	TTTCCCTAAACTCCCCCCACTGCAGaa CGCCCCCTTAGTCTATTTTT	*artD*-knockout-plasmid
artDoldown_rev	GTAGGAGATCTTCTAGAAAGATTCTTG CTCTCCTTTTTCTGTC	*artD*-knockout-plasmid
H2b-FW	GAAGAAGGCAGGAAAGAAGAC	House-keeping gene
H2b-Rev	TTTGGCAGACGAGGAAGAG	real-time PCR
2597-Fw	CTGTCATCAACACCCTGAACC	2597 real-time PCR
2597-Rev	TCGCTTCTCTCGCTCTATCC	2597 real-time PCR
2598-Fw	GACCCCGCTACATTGGAAAC	2598 real-time PCR
2598-Rev	TACCCTTCACTCCCTCCTCTAC	2598 real-time PCR
2599-Fw	CAACCATACCTCCTCCAACCTC	2599 real-time PCR
2599-Rev	ACTTCCATCGCTCTGACCTG	2599 real-time PCR
artA-fw	AACATCTCATCTCCCTCACC	*artA* real-time PCR
artA-Rev	AACTCCCATCAGTCAAGCC	*artA* real-time PCR
artB-FW	GACCCCAAGATTGAAAGAGCC	*artB* real-time PCR
artB-Rev	CTACCACCTTCCAACTGAACAC	*artB* real-time PCR
artC -fw	GTCGTTCTACTTCGATCTCG	*artC* real-time PCR
artC -fw	GTACTCTATACCCTTTGTCG	*artC* real-time PCR
artD-Fw	CGAAACTACTTGATGAGCCTCC	*artD* real-time PCR
artD-Rev	TCCGCTTTGCCACTTGTTTAC	*artD* real-time PCR
2605-Fw	GTTCTCCAGACCAAGTTCAGC	2605 real-time PCR
2605-Rev	TCCCCATCTATTTCCCACACC	2605 real-time PCR
artE-FW	CGATGATCCCGAAGCACAAG	*artE* real-time PCR
artE-REV	CGCAAGAAGGAAGATGGCAC	*artE* real-time PCR
pjetBB_rekom_rev	ATCTTGCTGAAAAACTCGAGC	*artA* re-complementation
trpcP_for	AAGTGTAATGCTAGTGGAGGT	*artA* re-complementation

PksA_LB_recomp_ for	GATGGCTCGAGTTTTTCAGCAAGATTC TCATGACAAGGTTGCGCT	*artA* re-complementation
PksA_recomp _rev	CGCTCTCGGAAAACTACACT	*artA* re-complementation
PksA_recomp_for	AGTGTAGTTTTCCGAGAGCG	*artA* re-complementation
PksA_RB_recomp_ rev	GTTGACCTCCACTAGCATTACACTTAC ATAACCGAACACCAAACAG	*artA* re-complementation
pksA_Ao_for	AAGAGTCAGTCAGTCTTAATATGACTTC TACGCCAGCGA	Heterologous expression of *artA* in A.o
PksA_recomp_for	AGTGTAGTTTTCCGAGAGCG	Heterologous expression of *artA* in A.o
PksA_recomp _rev	CGCTCTCGGAAAACTACACT	Heterologous expression of *artA* in A.o
pksA_Ao_rev	AGATCGACTGACTGACTTTATCAGACC TTCTCTTCAAACCA	Heterologous expression of *artA* in A.o
artA(p)-h2b-fw	CTCTTTCCCTAAACTCCCCCCACCTCA ACGAACCGCTTACA	fluorescent signal of *artA*
artA(p)-h2b-rev	CGGCGGCTTTTGGTGGCATGGCAGGC CAAAGAAGTCAAG	fluorescent signal of *artA*
artC(p)-h2b-fw	CTCTTTCCCTAAACTCCCCCCAAGGCA AAAGTCGTAACCATATC	fluorescent signal of *artC*
artC(p)-h2b-rev	CGGCGGCGGCTTTTGGTGGCATTTTGT GAATTGTTTAAAATTAA	fluorescent signal of *artC*
artB(p)-h2b-fw	CTCTTTCCCTAAACTCCCCCCAAAGGA TGAGAGGAAAGCGG	fluorescent signal of *artB*
artB(p)-h2b-rev	GCGGCGGCTTTTGGTGGCATTTTTTGC GCATACAAAAGAGAG	fluorescent signal of *artB*
artB(p)-h2b-rev	GCGGCGGCTTTTGGTGGCATTTTTTGC GCATACAAAAGAGAG	fluorescent signal of *artB*
ArtAend_pjet_ol_fo	GGATGGCTCGAGTTTTTCAGCAAGATC	ArtA-GFP tag *in locus*

r	CAACGACGATTCAGCTATC	
ArtAend_gfp_ol_rev	TACTTACCTCACCCTTGGAAACCATGACCTTCTCTTCAAACCATTTAA	ArtA-GFP tag *in locus*
Gfp_ArtAend_ol_for	GGTTAAATGGTTTGAAGAGAAGGTCATGGTTTCCAAGGGTGAGGT	ArtA-GFP tag *in locus*
Gfp_H_ol_rev	GTTGACCTCCACTAGCATTACACTTCTAAGCGGCCGCTTTGTAAA	ArtA-GFP tag *in locus*
H_Gfp_ol_for	TGAACTTTACAAAGCGGCCGCTTAGAAGTGTAATGCTAGTGGAGGT	ArtA-GFP tag *in locus*
H-ArtARB-ol-rev	AGAAAAACCCATACCAACACTTCCCTGGGGGGAGTTTAGGGAAA	ArtA-GFP tag *in locus*
ArtARB_hph_ol_for	ATGCTCTTTCCCTAAACTCCCCCCAGGGAAGTGTTGGTATGGGTT	ArtA-GFP tag *in locus*
ArtARB_pjet_ol_rev	ATTGTAGGAGATCTTCTAGAAAGATCAGTATCACCAATTCTCATCTG	ArtA-GFP tag *in locus*
ArtBend_pJet_ol_for	GATGGCTCGAGTTTTTCAGCAAGATCGCGATATGGAGCGATTGTA	ArtB-mCherry tag *in locus*
ArtBend_mCherry_ol_rev	tacttacCTCGCCCTTGCTTACCATTATCTTGGCCTCAACTTGCC	ArtB-mCherry tag *in locus*
mCherry_artBend_ol_for	CACGGGGCAAGTTGAGGCCAAGATAATGGTAAGCAAGGGCGAG	ArtB-mCherry tag *in locus*
mCherry_H_ol_rev	GTTGACCTCCACTAGCATTACACTTttaTTTGTAGAGTTCATCCATTC	ArtB-mCherry tag *in locus*
H_mCherry_ol_for	TGGAATGGATGAACTCTACAAAtaaAAGTGTAATGCTAGTGGAGGT	ArtB-mCherry tag *in locus*
H_artBRB_ol_rev	CAGTACAAGATATCGGCAAAACACATGGGGGGAGTTTAGGGAAA	ArtB-mCherry tag *in locus*
ArtBRB_H_ol_for	ATGCTCTTTCCCTAAACTCCCCCCATGTGTTTTGCCGATATCTTGTA	ArtB-mCherry tag *in locus*

ArtBRB_pJet_ol_rev	ATTGTAGGAGATCTTCTAGAAAGATAC GAGACTACACGAACAGC	ArtB-mCherry tag *in locus*

Reference

1 Youssar, L. *et al.* Intercellular communication is required for trap formation in the nematode-trapping fungus *Duddingtonia flagrans*. *PLoS Genet* **15**, e1008029 (2019).