Supplementary Information for
Chromatin-level regulation of biosynthetic gene clusters

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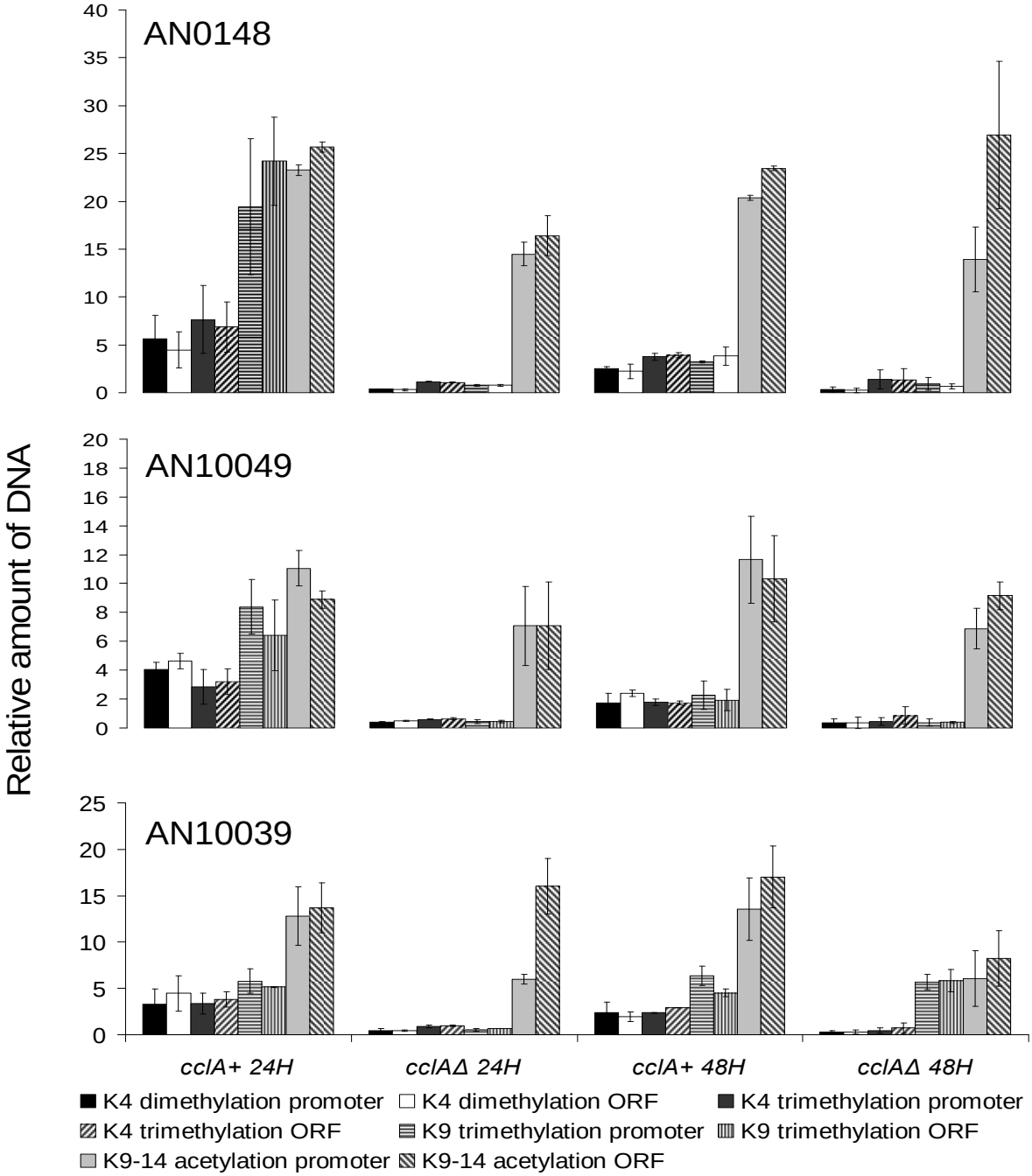
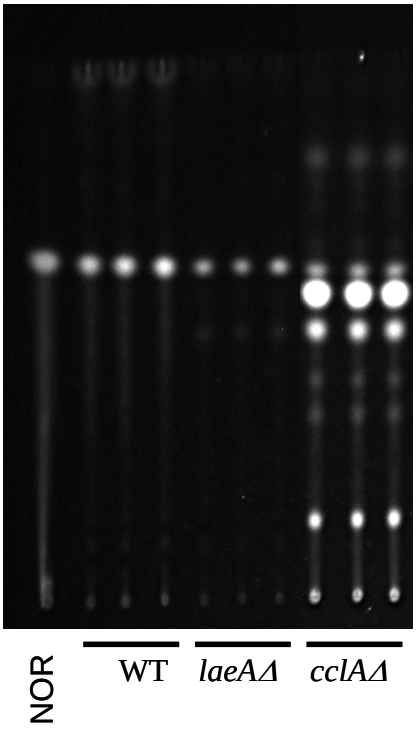


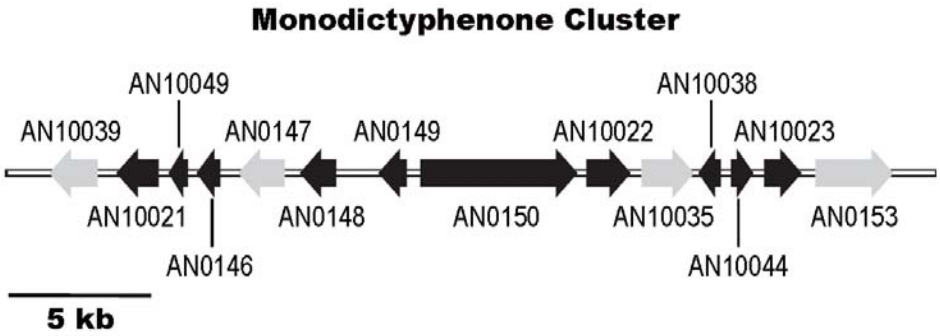
Supplementary Figure 1. Results of chromatin immunoprecipitation (ChIP) analysis of two genes up-regulated in the *cclA*Δ strain and proposed to be involved in the biosynthesis of monodictyphenone (AN0148 and AN10049) and one gene residing near the proposed biosynthesis cluster (AN10039) but lacking activation in *cclA*Δ, and most probably not involved in the biosynthetic pathway (see also Figure 1). The ChIP procedure is detailed in Supplementary Materials and Methods. Antibodies used for IP were specific to histone H3 lysine 4 in the dimethylated (K4 dimethylation) or the trimethylated (K4 trimethylation) state, or specific to histone H3 lysine 9 in the trimethyl state (K9 trimethylation). Analysis of histone H3 acetylation state was carried out using an antibody recognizing histone H3 acetylated at lysine 9 and lysine 14 (K9-14 acetylation). For each gene precipitated DNA was amplified with one primer set immediately upstream (promoter) and downstream (ORF) of the predicted start codon. "Relative amount of DNA" given on the y-axis indicates the level of the specific modification in histone H3 and is calculated from the ratio of the DNA amount obtained from modified H3 IPs (methylated or acetylated at the indicated lysine residues) to the DNA amount obtained from total H3 IPs at the amplified locus. We compare the isogenic wild type strain (*cclA*$^{+}$) or the *cclA* deletion strain (*cclA*Δ). ChIPs were performed after 24 hours (24H) and 48 hours (48H) of cultivation in glucose minimal medium (GMM). Transcriptional activation of genes AN0148 and AN10049 in the *cclA*Δ strain after 48 hours of growth is correlated with hypomethylated K4 and K9 at H3, as well as with high levels of K9 and K14 acetylation, whereas the promoter and ORF of AN10039, the gene lacking activation under the same conditions, is characterized by high levels of trimethylated H3K9 and reduced levels of H3K9-K14 acetylation.

Supplementary Figure 2



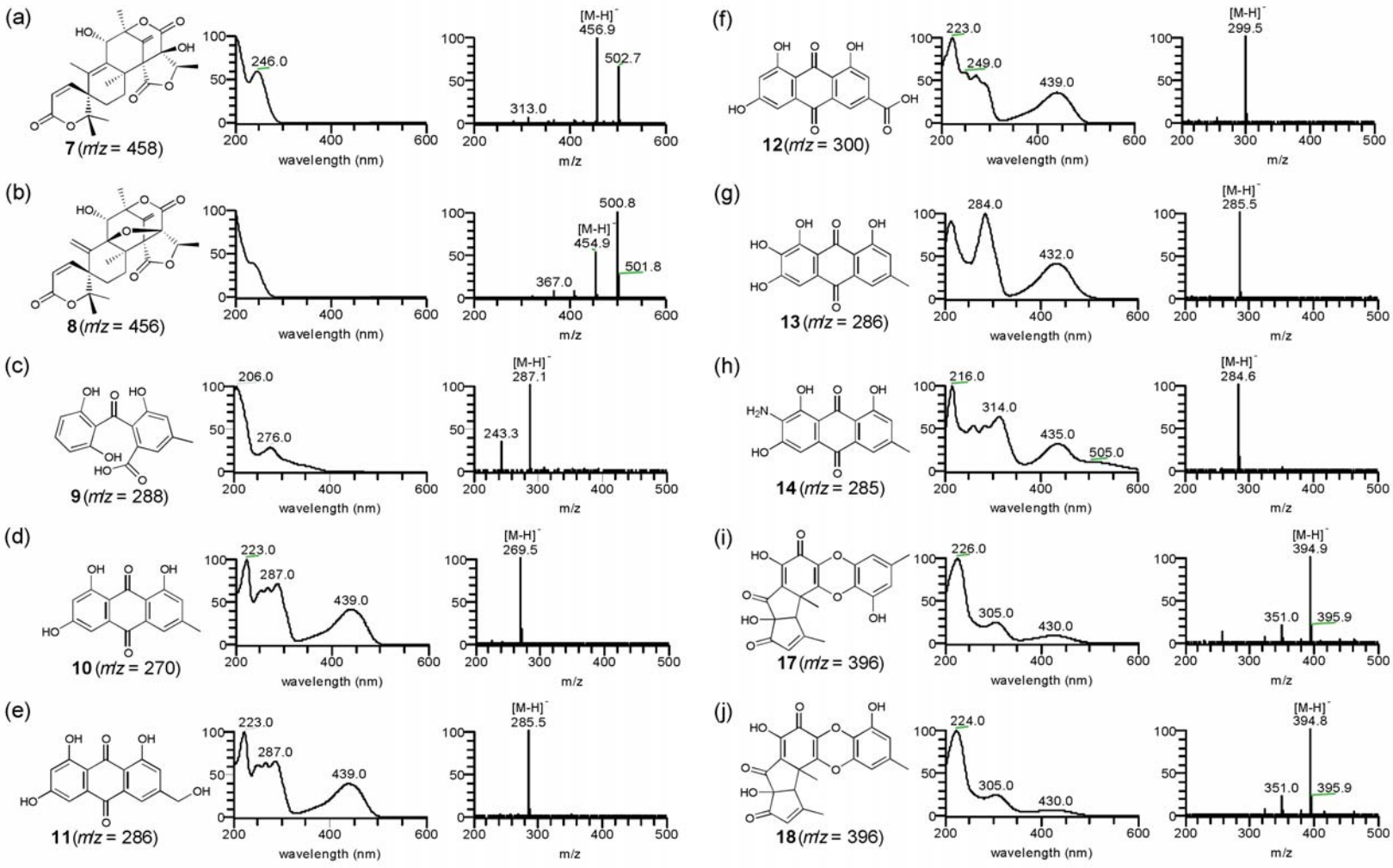
Supplementary Figure 2. Thin layer chromatography of chloroform extracts from three *A. nidulans* strains differing only in presence of *cclA* and *laeA*. All strains contain the allele *stcEΔ::argB* which results in the production of norsolorinic acid (**19**, NOR) instead of sterigmatocystin (**2**). NOR = norsolorinic acid standard, WT = RJW65, *laeAΔ* = RJW81.1, *cclAΔ* = RJW75.2.

Supplementary Figure 3



Supplementary Figure 3. Order of genes in the monodictyphenone gene cluster. The monodictyphenone PKS (AN0150, MdpG) contains starter unit ACP transacylase (SAT), ketosynthase (KS), acyltransferase (AT), product template (PT) and acyl carrier protein (ACP) domains as is typical for a NR-PKS. BLAST analysis indicates that AN0149 (MdpF) is a lactamase or hydrolase and that AN0147 (MdpD) is a monooxygenase (**Supplementary Table 1).** We propose that after polyketide formation by monodictyphenone PKS (MdpG), this polyketide is subsequently hydrolyzed from the PKS by the action of the hydrolase (AN0149, MdpF) to endocrocin-9-anthrone (**20**). AN0147 (MdpD) might be responsible for the oxidation of endocrocin-9-anthrone (**20**) to endocrocin (**21**). Endocrocin (**21**) is likely to decarboxylate spontaneously to form emodin (**10**). AN0147 (MdpD) might also oxidize emodin (**10**) to produce emodin analogs **11** – **13**. Emodin analog **14** may be derived from **13** by a transaminase activity (See Figure 1d). It should be noticed that AN0147 (MdpD) mRNA is not up-regulated during the time course studied. The endogenous level of AN0147 is possibly enough to catalyze all oxidation steps. However, the possibility that other monooxygenases not in the monodictyphenone cluster catalyzes the monodictyphenone pathway cannot be excluded. AN10049 (scytalone dehydratase), AN0146 (StcU homolog), AN10038 (StcT homolog), AN10044 (StcO homolog), and AN10023 (AflY homolog) might also be involved in catalyzing emodin (**10**) to form monodictyphenone (**9**)

Supplementary Figure 4



Supplementary Figure 4. UV/Vis and ESI-MS in negative mode of (a) austinol (**7**), (b) dehydroaustinol (**8**), (c) monodictyphenone (**9**), (d) emodin (**10**) (e) ω-hydroxyemodin (**11**), (f) emodic acid (**12**), (g) 2-hydroxyemodin (**13**), (h) 2-aminoemodin (**14**), (i) F9775B (**17**), (j) F9775A (**18**).

Supplementary Table 1. Gene identities in two novel CclA regulated secondary metabolite gene clusters.

Gene [a]	Putative function [b]	Highest Blast/E-value [c]	Organism [d]
Monodictyphenone cluster			
AN10039	histidine acid phosphatase	putative, $5e^{-151}$	*Aspergillus clavatus* NRRL1
AN10021, mdpA	regulatory gene	*aflJ* homolog, $3e^{-69}$	*Aspergillus flavus*
AN10049, mdpB	scytalone dehydratase	*scd1*, $7e^{-54}$	*Colletotrichum lagenarium*
AN0146, mdpC	versicolorin ketoreductase	*stcU* homolog, $5e^{-96}$	*Aspergillus nidulans*
AN0147, mdpD	flavin-containing mono-oxygenase	MAK1, $7e^{-79}$	*Nectria haematococca*
AN0148, mdpE	regulatory gene	*aflR* homolog, $8e^{-34}$	*Aspergillus flavus*
AN0149, mdpF	Zn-dependent hydrolase metallo-beta-lactamase	putative, $1e^{-119}$	*Neosartorya fischeri*
AN0150, mdpG	polyketide synthase	putative, 0.0	*Aspergillus sp.*
AN10022, mdpH	DUF 1772 superfamily	putative, $3e^{-49}$	*Neosartorya fischeri*
AN10035, mdpI	AMP binding, acyl-coA synthase	putative, $4e^{-17}$	*Corynebacterium efficiens*
	O-succinylbenzoic acid--CoA ligase	putative, $2e^{-18}$	*Bacillus sp.*
	cinnamyl alcohol dehydrogenase	putative, $2e^{-16}$	*Arabidopsis*
AN10038, mdpJ	glutathione S transferase [e]	*stcT* homolog, $2e^{-30}$	*Aspergillus nidulans*
AN10044, mdpK	oxidoreductase	*stcO* homolog, $3e^{-78}$	*Aspergillus nidulans*
AN10023, mdpL	Baeyer-Villiger oxidase	*aflY*, (stcR) $2e^{-83}$	*Aspergillus parasiticus*
AN0153	MYB DNA binding protein	*trf1* homolog, $3e^{-33}$	*Schizosaccharomyces pombe*
F9775 cluster			
AN7907	glyoxylate-bleomycin resistance protein	putative, $2e^{-24}$	*Xanthobacter autotrophicus*
AN7908	a-L-arabinosidase	putative, $3e^{-140}$	*Aspergillus sojae*
AN7909	polyketide synthase	putative, 0.0	*Aspergillus spp.& Ascomycete*
AN7910	hypothetical	predicted	
AN7911	amidohydrolase	putative, $7e^{-59}$	*Mycobacterium smegmatis*
AN7912	tyrosinase	putative, $2e^{-62}$	*Aspergillus clavatus*
AN7913	hypothetical	predicted	
AN7914	alcohol dehydrogenase	*cipB*, $2e^{-69}$	*Pyrenophora tritici-repentis*
AN7915	β-glucosidase 2	putative, $1e^{-127}$	*Pyrenophora tritici-repentis*
AN7916	AA transporter	putative, $6e^{-77}$	*Aspergillus fumigatus*

[a] *A. nidulans* genome annotation (http://www.broad.mit.edu/annotation/genome/aspergillus_nidulans/MultiHome.html).
[b] Putative function was based on BLAST search at NCBI and the putative domains were from the Pfam database.
[c] Best match from BLAST search at NCBI.
[d] Organism of best BLAST match.
[e] This gene also has homologies to translation elongation factor.

Supplementary Table 2. Fungal strains used in this study. For polyketide synthase deletions, multiple identical transformants were tested for secondary metabolite production. LO2154-LO2156 are, for example, sister transformants with the same genotype. AN0148::*AfpyrG-alcA*(p)-AN0148 is a replacement of the endogenous promoter of AN0148 with the *alcA* promoter and the *A. fumigatus pyrG* gene (*AfpyrG*). *AfpyroA* is the *A. fumigatus pyroA* gene and *AfriboB* is the *A. fumigatus riboB* gene.

Strain	Genotype	Source
RJW33.1	*wA3; argB2; metG1; stcE::argB, trpC801, laeA::metG, veA1*	This study
TJW73	*wA3; argB2; metG1; stcE::argB, trpC801, laeA::metG, veA1; cclA::pyroA*	This study
RJW65	*stcE::argB; veA1*	This study
RJW75.2	*cclA ::pyroA; pyroA4; stcE::argB; veA1*	This study
RJW76.1	*cclA ::pyroA; pyroA4; stcE::argB; laeA::metG, veA1*	This study
RJW81.1	*stcE::argB; laeA::metG, veA1*	This study
LO2026	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*AfriboB*	This study
LO2051	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*AfriboB*; *cclA*::*AfpyroA*	This study
LO2149	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; *cclA*::*AfpyroA;* AN0150::*AfpyrG*	This study
LO2154-LO2156	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; *cclA*::*AfpyroA;* AN0523::*AfpyrG*	This study
LO2159, LO2161, LO2163	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; *cclA*::*AfpyroA;* AN1034::*AfpyrG*	This study
LO2165-LO2167	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; *cclA*::*AfpyroA;* AN2032::*AfpyrG*	This study
LO2169, LO2170, LO2173	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; *cclA*::*AfpyroA;* AN3230::*AfpyrG*	This study
LO2174, LO2176, LO2177	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; *cclA*::*AfpyroA;* AN3386::*AfpyrG*	This study
LO2179-LO2181	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; *cclA*::*AfpyroA;* AN6000::*AfpyrG*	This study
LO2184-LO2186	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; *cclA*::*AfpyroA;* AN6448::*AfpyrG*	This study
LO2189-LO2191	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; *cclA*::*AfpyroA;* AN7071::*AfpyrG*	This study
LO2194	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB*; *cclA*::*AfpyroA;* AN7909::*AfpyrG*	This study
LO2333	*pyrG*89; *pyroA*4, *nkuA::argB*; *riboB*2, *stcJ*::*riboB;* AN0148::*AfpyrG-alcA*(p)-AN0148	This study

Supplementary Table 3. Primers used in this study.

Gene	Primer	Sequence
stcJ		
AN7815	LIZP575ES	5'AGCATGGTGTGACTCCAGC
	LIZP576ES	5'CAAAACGACAGTTGCAGGG
	LIZP577ES	5'GACTCCTGAACGGCCTCTTTAAGCGTCTTGGCTGGACC
	LIZP578ES	5'TTCTGCAGCGCAAACGTTCTCAACGGCTCCAGACATGTC
	LIZP579ES	5'TACCAGGAGCTGATACTACC
	LIZP580ES	5'AAGTACTATGGGTCATCAAGC
cclA		
AN9399	Ccl5F	5'TCGTCCCATTGATCCTCATTGAAC
	Ccl5R	5'GCGAAAGCTTTGCGGATAGGTATTTCACCG
	Ccl3F	5'GACCGGATCCTGAATGGGGTTGTACTTTCAGCTG
	Ccl3R	5'TCGAGTATAACATCTCTGAACTCG
	CclIF	5'ATCGCCAAACGGATGTTCCTCC
	CclIR	5'GACAAGCATTGCTTCGACCTCG
	LIZP587ES	5'TCCTCATTGAACTTGCCTTCG
	LIZP588ES	5'TCCGAAAATACATCTTCATCC
	LIZP589ES	5'GTAATCCAGCATCTGATGTCCTTTGCGGATAGGTATTTCACC
	LIZP590ES	5'CTTCATTATGTAGACACTCGCTGGGGTTGTACTTTCAGCTG
	LIZP591ES	5'CCTGGTGTCACTCCTTGG
	LIZP592ES	5'ACATCTCTGAACTCGCCCC
Monodictyphenone cluster		
AN10039	10039F	5'TTTCGCACTCCTGAGCCTTCTG
	10039R	5'CGCAGCTCTACGTTCG ATTCCT
	10039_prom_F	5'TTTCCGAACAGTAATACGCTCG
	10039_prom_R	5'GGGATGCATTCTTCACGTTTC
	10039_ORF_F	5'TCCTTATCTGGTGGACAACGAC
	10039_ORF_R	5'GCCGACGGTAACAATCGAAG
AN10021	10021F	5'TAGTCTATCCGACCTTGAAACCC
	10021R	5'GTAGCCACAAACACGACGACCT
AN10049	10049F	5'ACGCTGCAGCCAACATTTGAAGG
	10049R	5'TCAGACGGACCTGACCTCAACC
	10049_prom_F	5'GTCGTAGCCAGAACAATAAAATGGC
	10049_prom_R	5'TGTGCCGTCCCTGTTTCTCTAC
	10049_ORF_F	5'GGTAACCCACTCCTCAAAACG
	10049_ORF_R	5'CAGTCCATCGTATATTCGGTTC
AN0146	0146F	5'CGAAGGCAAAGTTGCCCTTGTG
	0146R	5'GTGGGTGAACGGAAAGATCATT
AN0147	0147F	5'CATTGTCATGCAGCCTTCTGCC
	0147R	5'GTGGAGGATTTGTTGGATGGTGA
AN0148	0148F	5'TCTTCAGAGGGTCCAGGTATCC
	0148prom_F	5'CCCACTCTGGGCATCAGCTC
	0148prom_R	5'GATTGATATCGCCTCACTCCCACT
	0148_ORF_F	5'CCACGTCTATCAACAAAGATGATG
	0148_ORF_R	5'GAAGCAAGATAACCGTCGTAGTC
	0148R	5'CGCACAGCTAGTTCTCAGCGAA
AN0149	0149F	5'TCAGCCGCAGCAGCATAAAGGC
	0149R	5'GCGAGGAGGTGTAAAACGATGG
AN0150	0150F	5'TGATACTGACATGGTCGTCGCC

	0150R	5’CGCCTTTACAGGTCAAGGTGCA
AN10022	10022F	5’AAAATTGCTCACAGCCTAGCAGG
	10022R	5’CTAGAGTCGCTTCGGGACATCA
AN10035	10035F	5’AAATCGATGGTGGGCACCTACC
	10035R	5’CGATTGTTTCCGAAAGCGAGAGT
AN10038	10038F	5’GTCATTCGGAACACTCTACACCC
	10038R	5’GGGACTTGAGTTAGAGATTCCAG
AN10044	10044F	5’AATACTGGCACGGCTCTGATCC
	10044R	5’GGGGTTGCATCGATTTCTGCCA
AN10023	10023F	5’TCTCTCAACCAAACTCGGCCAG
	10023R	5’GTGTACCCGAGCTTGATTTCGAA
AN0153	0153F	5’TTCAGAGCCAGTTTCCTGTCGC
	0153R	5’GGGAAAGGAAGTCCATGGAGAG

F9775 cluster

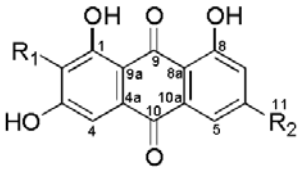
AN7907	7907F	5’ACTGAAAACAACCCACCTCCCC
	7907R	5’CACCTTGCCGAGATTGCGTCGA
AN7908	7908F	5’TGAGAAACCTCAGTACCTGGCC
	7908R	5’CGGCTACGACCTTCTGCCATAT
AN7909	7909F	5’AACGTCGTACAGCACTGGACAG
	7909R	5’AGAAGTCATCGACATTGAACCG
AN7910	7910F	5’CTACTGAGATTGCCGAGATCACC
	7910R	5’GTGGTGCCGAGGATATAGTTGAA
AN7911	7911F	5’AGAACACTACTACTCGACCGCC
	7911R	5’GGTGCATATGAAGAAGGAGCAGT
AN7912	7912F	5’ATTTGCTGACCGGTTTGCCAGG
	7912R	5’CGAGCTGACAGTCTGCGATGAT
AN7913	7913F	5’AAGATCCTCGAGTACGACGGTC
	7913R	5’GAAACAGCGTCCTTGC GGAGAT
AN7914	7914F	5’TGGCTCACCAAGGCAGGCAATG
	7914R	5’CCAAGCTGGTTGTGGAGTTGTAA
AN7915	7915F	5’TTTAGCTCTAGTGTCCCTGGGG
	7915R	5’CGCAACACCGATCAGGACAATC
AN7916	7916F	5’GACTTGCTACAAACCCATCGGC
	7916R	5’CTCGAACCTGGTAAGAAAGACTC

Polyketide synthases

AN0150	AN0150.3P1	5’TGG TGT GAA TTC AGC TTT CG
	AN0150.3P2	5’ACA TAT GGT CAT GCG AGT GC
	AN0150.3P3	5’CGAAGAGGGTGAAGAGCATTGAAGTGACAAGCGTCAGATCG
	AN0150.3P4	5’GCATCAGTGCCTCCTCTCAGACAGCCCATCCTCACTCATCAA CC
	AN0150.3P5	5’TTG ACT GAA CCC TGC TAG GC
	AN0150.3P6	5’TAC TGG AAG CGC TGA TAT GC
AN0523	AN0523.3P1	5’TCC GAG GTA GAA ATC GTT GG
	AN0523.3P2	5’CAG TGG GTT GAC TCT GTT GC
	AN0523.3P3	5’CGAAGAGGGTGAAGAGCATTGAAGGATTGTCCGGCTTATGG
	AN0523.3P4	5’GCATCAGTGCCTCCTCTCAGACAGGTCCAGTAAGGCCAAGTT CG
	AN0523.3P5	5’GAA TGA GGG CTG GTT ACT GG
	AN0523.3P6	5’GAT CTG CGA AGA CTG GAA GG
AN1034	AN1034.3P1	5’CAC CCG CTT CCT TAC TAT GC
	AN1034.3P2	5’GCT TGA GAA GGT CCA GAA CG
	AN1034.3P3	5’CGAAGAGGGTGAAGAGCATTGATTGGAGCCGGAGATTAAGG
	AN1034.3P4	5’GCATCAGTGCCTCCTCTCAGACAGCCTAACCAGCTCCAGTGA CC
	AN1034.3P5	5’CCT GTT TCG TAC CCA ATT CC
	AN1034.3P6	5’TGA TAA TGC GGA CAG AGA CG
AN2032	AN2032.3P1	5’GAC TGA AAC CCT GGA ACA CC

	AN2032.3P2	5’CTT CGA CCG TCA TCC TAT CG
	AN2032.3P3	5’CGAAGAGGGTGAAGAGCATTGTGAAGGCTGAACATGCTAGG
	AN2032.3P4	5’GCATCAGTGCCTCCTCTCAGACAGCCTCGGAAGGTACAATGT GG
	AN2032.3P5	5’CCA TGG ACA TGC ACT GTA GG
	AN2032.3P6	5’TCT GTT GGA CCA CTG TCT CG
AN3230	AN3230.3P1	5’GCA TCT TGC AGG GTT CTA CC
	AN3230.3P2	5’ACA TTC ACC AAG CTG GTT CC
	AN3230.3P3	5’CGAAGAGGGTGAAGAGCATTGTGGATTACGTCCAGCTAGGG
	AN3230.3P4	5’GCATCAGTGCCTCCTCTCAGACAGAAGCCAGATGGGACAATA CG
	AN3230.3P5	5’CGT CTA TGC AGT CGT GTT CC
	AN3230.3P6	5’ATC AAG ATT CGG GAG ACA CG
AN3386	AN3386.3P1	5’GTT GAT GTT CGC TCG TAT CG
	AN3386.3P2	5’TGC TTG AGT CGA TCA CTT GG
	AN3386.3P3	5’CGAAGAGGGTGAAGAGCATTGACCTCTCACCGAGACTTTGC
	AN3386.3P4	5’GCATCAGTGCCTCCTCTCAGACAGACATCCCCACTGTCATTT CG
	AN3386.3P5	5’CCT GTG TGA TTG GAC TGT GG
	AN3386.3P6	5’AAG CGG GTT GTT TGT TAT CG
AN6000	AN6000.3P1	5’TTT TAT CCG GAA GGT CAT CG
	AN6000.3P2	5’TCT TCC ACC ACC GAG TAT CC
	AN6000.3P3	5’CGAAGAGGGTGAAGAGCATTGGTTCAATGAGGGCAAAATGC
	AN6000.3P4	5’GCATCAGTGCCTCCTCTCAGACAGTCAATTCATTTCGATGGT TCC
	AN6000.3P5	5’AGA TGG TGG GTG AGT CAA GG
	AN6000.3P6	5’GAG CTG TCA CTA CGC TCT GC
AN6448	AN6448.3P1	5’CAG ATG CGG TGT AGA TGT CG
	AN6448.3P2	5’TGG ATT GAG CAG AGA CAA CG
	AN6448.3P3	5’CGAAGAGGGTGAAGAGCATTGGAGGCAGGACAAAACACAGG
	AN6448.3P4	5’GCATCAGTGCCTCCTCTCAGACAGTATGTTGCCTGCATTTCAGC
	AN6448.3P5	5’CAC CGG GAG TCA ATA CAT GG
	AN6448.3P6	5’CCG TTG TGC ATG ATT TCT CC
AN7071	AN7071.3P1	5’CTT GCT GGA GGA AGA GAA CG
	AN7071.3P2	5’TGG CTA CAG TGT TGC TGA GG
	AN7071.3P3	5’CGAAGAGGGTGAAGAGCATTGCCCAGCTGGCTTATACTTGG
	AN7071.3P4	5’GCATCAGTGCCTCCTCTCAGACAGCGGAAGTGAGGGAGTGTA GC
	AN7071.3P5	5’CCT GCA TCC TAC AGG TAG CC
	AN7071.3P6	5’CCC AGC TGA ATC AGA ACT CC
AN7079	AN7079.3P1	5’CAT GAC AAA GGC CAT ACA GC
	AN7079.3P2	5’GGA AAG GAG CAT ACC AGT CG
	AN7079.3P3	5’CGAAGAGGGTGAAGAGCATTGGTTGATGTCAGCCCAGAAGC
	AN7079.3P4	5’GCATCAGTGCCTCCTCTCAGACAGCAGGTATAATGTGCACGT CTCC
	AN7079.3P5	5’GAG GAT CCA GAC ATC CTT GG
	AN7079.3P6	5’CAG GTA GGA AAA TGC AGT AGG G

Underlined sequences show the placement of restriction sites *Hind*III (Br5R) & *BamH*I (Br3F). Blue and red sequences are tails that anneal to the *A. fumigatus pyrG* fragment (AfpyrG) during fusion PCR.

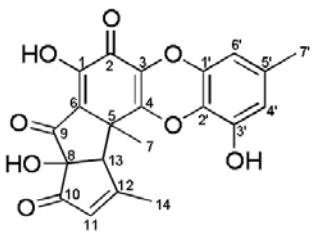


10 R_1 = H, R_2 = CH_3
11 R_1 = H, R_2 = CH_2OH
12 R_1 = H, R_2 = COOH
13 R_1 = OH, R_2 = CH_3
14 R_1 = NH_2, R_2 = CH_3

Supplementary Table 4. NMR data for compounds **10** – **14** (400 and 100 MHz in DMSO-d_6)

	11	**12**	**13**	**14**		**10**
position	δ_C	δ_C	δ_C	δ_C	δ_H	δ_C
1	166.0, qC	166.5, qC	151.4, qC	148.7[a], qC	—	166.0, qC
1-OH	—	—	—	—	12.07[a] (1H, br s)	—
2	108.0, CH	108.0, CH	139.2, qC	131.8, qC	—	107.9, CH
2-NH_2	—	—	—	—	5.51 (2H, br s)	—
3	164.5, qC	164.8, qC	152.5, qC	148.6[a], qC	—	164.5, qC
3-OH	—	—	—	—	11.12 (1H, s)	—
4	109.1, CH	109.4, CH	109.3, CH	108.6, CH	7.27 (1H, s)	109.0, CH
4a	135.1, qC	133.4, qC	124.6, qC	120.8, qC	—	132.6, qC
5	117.1, CH	135.0, CH	120.1, CH	120.0, CH	7.42 (1H, s)	120.4, CH
6	152.9, qC	138.4, qC	148.2, qC	148.3[a], qC	—	148.1, qC
7	120.8, CH	124.2, CH	123.4, CH	123.0, CH	7.06 (1H, s)	124.0, CH
8	161.5, qC	161.1, qC	161.3, qC	161.3, qC	—	161.4, qC
8-OH	—	—	—	—	12.01[a] (1H, br s)	—
8a	114.1, qC	118.2, qC	113.4, qC	113.5, qC	—	113.2, qC
9	189.7, qC	189.1, qC	190.4, qC	191.1, qC	—	189.5, qC
9a	108.9, qC	109.1, qC	109.7, qC	109.5, qC	—	108.6, qC
10	181.5, qC	180.9, qC	180.4, qC	179.7, qC	—	181.2, qC
10a	132.9, qC	118.9, qC	133.0, qC	133.8, qC	—	134.9, qC
11	62.0, CH_2	165.6, qC	21.6, CH_3	21.6, CH_3	2.38 (3H, s)	21.5, CH_3

[a] Values bearing the same superscript in the same column may be interchanged.



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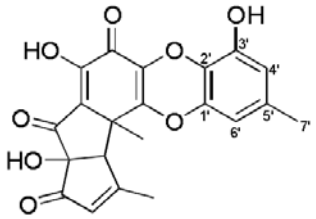

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Supplementary Table 5. NMR data for compound **17** and **18** (400 and 100 MHz in DMSO-d_6)

	17		**18**	
position	δ_C	δ_H	δ_C	δ_H
1	148.8	—	148.1	—
2	174.6	—	174.4	—
3	130.9	—	131.3	—
4	155.1	—	154.4	—
5	44.1	—	43.9	—
6	118.9	—	119.2	—
7	29.0	1.76 (3H, s)	28.6	1.74 (3H, s)
8	87.3	—	87.2	—
8-OH	—	6.75 (1H, s)	—	6.84 (1H, s)
9	194.2	—	194.6	—
10	199.7	—	199.4	—
11	131.4	6.05 (1H, br s)	131.6	6.07 (1H, br s)
12	174.9	—	174.9	—
13	59.3	3.46 (1H, s)	59.4	3.36 (1H, s)
14	19.4	2.23 (3H, s)	19.6	2.24 (3H, s)
1′	141.5	—	139.8	—
2′	125.8	—	127.4	—
3′	145.4	—	145.6	—
3′-OH	—	9.92 (1H, s)	—	10.0 (1H, s)
4′	113.5	6.42 (1H, br s)	114.6	6.46 (1H, br s)
5′	135.4	—	133.8	—
6′	107.4	6.29 (1H, br s)	107.4	6.39 (1H, br s)
7′	20.7	2.13 (3H, s)	20.5	2.14 (3H, s)

Supplementary Methods

Strains.

Supplementary Table 2 lists all of the fungal strains used for this study. Some strains are not discussed in the text but were used for sexual crosses to obtain the strains of interest. All strains were maintained as silica or glycerol stocks and were grown at 37°C on glucose minimal medium (GMM) [1] or solid YAG plates (5 g/l yeast extract, 15 g/l agar, 20 g/l *d*-glucose, and 1 ml/l trace element solution). All media contained appropriate supplements to maintain auxotrophs.

Gene deletions or *alcA* promoter exchange of *cclA* (AN9399), *stcJ* (AN7815), *mdpG* (AN0150), *mdpE* (AN0148), AN7909 and other polyketides.

All genes are designated by using the Broad Institute designations (AN numbers). All primers are listed on **Supplementary Table 3.**

cclA: *Aspergillus nidulans cclA* was disrupted in the wild-type strain RJW33.1 by replacement of *cclA* with the *A. nidulans pyroA* marker gene obtained from plasmid p14 [2]. An *A. nidulans cclA* gene disruption vector, pJW81.6, was constructed by insertion of a 1 kb DNA fragment upstream of the *cclA* start codon (primers Ccl5F and Ccl5R) and a 1 kb DNA fragment downstream of the *cclA* stop codon (primers Ccl3F and Ccl3R) on either side of the *pyroA* marker gene (*Hind*III and *BamH*I). Fungal protoplasts were transformed by the polyethylene glycol method as previously described [3]. Homologous single-gene replacement of *cclA* was confirmed by Southern blot analysis. One of the resultant deletants, TJW73, was crossed with RJW65 to generate RJW75.2.

***stcJ/cclA double mutant*:** *stcJ* was replaced with the *A. fumigatus riboB* gene (*AfriboB*) [4] as follows. A linear construct consisting of *AfriboB* flanked by 1000 bp upstream of the *stcJ* coding sequence and 1000 bp downstream of the *stcJ* coding sequence was generated by fusion PCR using the protocol of Szewczyk *et al.*[5]. The construct was transformed into strain TN02A7 [4] using the procedures of Szewczyk *et al.*[5]. Four transformants were chosen and all were determined by diagnostic PCR and Southern hybridizations to have a correct replacement of *stcJ* with *AfriboB* and no heterologous integration of transforming DNA. Metabolite profiles revealed that these transformants did not produce sterigmatocystin (**2**). One of these

transformants, LO2026, was chosen for deletion of *cclA*. *cclA* was replaced with *A. fumigatus pyroA* [4] using the same approach and procedures as were used for the *stcJ* replacement. Four transformants were analyzed by diagnostic PCR and Southern hybridizations. All had a correct replacement of *cclA* with *AfpyroA*, but one had an additional heterologous integration. All of the three correct transformants showed similar metabolite profiles and one of them (LO2051) was used for subsequent experiments.

***Deletion of polyketide synthase genes*:** The aromatic nature of monodictyphenone (**9**), emodin (**10**) and related compounds (**11** – **14**), and F9775A (**18**) and B (**17**) suggested that these compounds should be catalyzed by nonreduced fungal type I PKSs. Non-reduced (NR) type I PKSs contains the minimally necessary ketosynthase (KS), acyltransferase (AT), and acyl carrier protein (ACP) domains. Highly reduced (HR) type I PKSs contain additional modification domains such as methylation (CMe), ketoreductase (KR), enoylreductase (ER) and/or dehydratase (DH). We analyzed and grouped the 27 PKSs in *A. nidulans* into NR PKS and HR PKS by examining the PKS domain structure using the bioinformatic tools provided by the MIT Broad Institute (http://www.broad.mit.edu/annotation/genome/aspergillus_group/MultiHome.html). The genes targeted were AN0150, AN0523, AN1034, AN2032, AN3230, AN3386, AN6000, AN6448, AN7071, and AN7909. These ten NR-PKS genes were selected because they encompass every NR-PKS in the *A. nidulans* genome where the products are still unknown. All ten NR-PKS were disrupted by replacement, in LO2051, with the *A. fumigatus pyrG* gene (*AfpyrG*) [6] using the same approach and procedures as for *stcJ* and *cclA*. Mutants were examined for lack of production of the compounds in question. The relevant deletants were AN0150*Δ* (*mdpGΔ*) for emodin (**10**) and related molecules (**11** – **14**), and AN7909*Δ* deletants for F9775A (**18**) and B (**17**).

Diagnostic PCR of the deletant and wild type strains was performed using the external primers used in the first round of the PCR. The difference in the sizes between the gene replaced by the selective marker and the native gene allowed the determination of correct gene replacement. In cases when the sizes of both the wild-type and deletant products were similar, diagnostic PCR was performed using one of the external primers and a primer located inside the marker gene. In

those cases, the deletant gave the PCR product of the expected size whereas no product was present in non-deletants.

***stcJ/cclA/mdpG(AN0150) triple mutant*:** LO2051 was chosen for deletion of *mdpG* (AN0150). *mdpG* was replaced with *AfpyrG* using the same approach and procedures as were used for the *stcJ* replacement. Five transformants were analyzed by diagnostic PCR. All had a correct replacement of *mdpG* with *AfpyrG*. All three correct transformants showed similar metabolite profiles and one of them (LO2149) was used for subsequent experiments.

***Over expression mdpE (AN0148) mutant*:** LO2026 was used for over expression of *mdpE* (AN0148). 153 bp upstream of the start codon were replaced with *AfpyrG* followed by a 401 bp fragment containing the *A. nidulans alcA* promoter [7] using the procedure of Szewczyk *et al.*[5].

***stcJ/cclA/AN7909 triple mutant*:** LO2051 was chosen for deletion of AN7909. AN7909 was replaced with *AfpyrG* using the same approach and procedures as were used for the *stcJ* replacement. Three transformants were analyzed by diagnostic PCR. All had a correct replacement of AN7909 with *AfpyrG*. All three transformants showed similar metabolite profiles and one of them (LO2194) was used for subsequent experiments.

Nucleic Acid Manipulations.

The extraction of DNAs from fungi and bacteria, restriction enzyme digestion, gel electrophoresis, blotting, hybridization, and probe preparation were performed by standard methods [8]. *Aspergillus* DNA for diagnostic PCR was isolated using the method of Lee and Taylor (1990)[9]. RNA was extracted from *Aspergillus* strains by use of Trizol reagent (Invitrogen) according to the manufacturer's instructions. RNA blots were hybridized with 1 kb primers amplified by PCR for AN10039, AN10021, AN10049, AN0146, AN0147, AN0148, AN0149, AN0150, AN10022, AN10035, AN10038, AN10044, AN10023, AN0153, AN7907, AN7908, AN7909, AN7910, AN7911, AN7911, AN7912, AN7913, AN7914, AN7915, and AN7916 using primers listed on **Supplementary Table 3**.

ChIP coupled to quantitative PCR analysis.

ChIP was carried out as described previously [10]. Antibodies used for ChIP were from Abcam: rabbit polyclonal to human C-terminus Histone H3 antibody (AB1791), rabbit polyclonal to Histone H3-tri methyl K9 (AB8898), rabbit monoclonal to Histone H3-di methyl K4 (AB32356), mouse monoclonal to Histone H3-tri methyl K4 (AB1229), and from Upstate Biotechnology, rabbit polyclonal to histone H3 acetyl K9-K14 (06-599). 2 µl of antibody were used per reaction of 200 µg total protein.

Wild type and *cclAΔ* were grown for 24 and 48 hr as above. Amplification and detection of precipitated DNA in real-time quantitative PCR was performed with Platinum SYBR Green qPCR SuperMix UDG (Invitrogen) following the instructions of the provider. The relative amounts of DNA were calculated by dividing the immunoprecipitated DNA by the input DNA. Each PCR reaction was replicated. To normalize the amount of DNA precipitated with the antibodies recognizing histone H3 modifications (H3K4me2, H3K4me3, H3K9me2/3, H3K9acK14ac), the resulting ratio of the precipitation with these antibodies was divided by the precipitate:input ratio of the antibody recognizing the H3 C-terminal tail. The resulting numbers represent the relative level of H3 modification and are expressed as relative DNA amounts (**Supplementary Fig. 1**). Two biological repeats were performed for each condition and standard deviation was calculated upon these.

To amplify the promoter and ORF regions of AN0148, AN10039 and AN10049 the primers listed on **Supplementary Table 3** were used.

Fermentation and LC/MS analysis.

Aspergillus nidulans controls and deletant strains were cultivated at 37°C on solid YAG plates at 22.5×10^6 spores/15 cm plate (~ 40 ml medium/plate) with required supplements. After 5 days, agar was chopped into small pieces, and the material was extracted with 50 ml of MeOH, followed by 50 ml of 1:1 CH_2Cl_2/MeOH, with each sonicated for 1 hr. The combined extracts were evaporated *in vacuo* to yield a residue, which was suspended in H_2O (50 ml) and partitioned with ethyl acetate (50 ml × 2). The combined EtOAc layers were evaporated *in vacuo*, re-dissolved in MeOH (1 mg/ml) and injected with 10 µL for HPLC-DAD-MS analysis. LC/MS was carried out in negative mode using a ThermoFinnigan LCQ Advantage ion trap mass

spectrometer with an RP C_{18} column (Alltech Prevail C18 3 μm 2.1 × 100mm) at a flow rate of 125 μL/min. The solvent gradient for HPLC was 95% MeCN/H_2O (solvent B) in 5% MeCN/H_2O (solvent A), both containing 0.05% formic acid: 0% B from 0 to 5 min, 0 to 100% B from 5 to 35 min, maintained at 100% B from 35 to 40 min, 100 to 0% B from 40 to 45 min, and re-equilibration with 0% B from 45 to 50 min. Conditions for MS included a capillary voltage 5.0 kV, a sheath gas flow rate at 60 arbitrary units, an auxiliary gas flow rate at 10 arbitrary units, and the ion transfer capillary temperature at 350°C.

For *alcA*(p) inducing conditions, 50×10^6 spores of strain LO2333 (**Supplementary Table 2**) was grown in 50 ml liquid LMM medium (15 g/l lactose instead of 10 g/l glucose in GMM medium) supplemented with pyridoxine (0.5 mg/l) at 37ºC with shaking at 200 rpm. Cyclopentanone at a final concentration of 30 mM was added to the medium after 18 hr of inoculation. Culture medium was collected 48 hr after cyclopentanone induction by filtration and extracted with the same volume of EtOAc twice. The combined EtOAc layers were evaporated *in vacuo* and analyzed by HPLC-DAD-MS as described above.

Isolation and identification of secondary metabolites.

For structural elucidation, 20 YAG plates inoculated with wild-type *A. nidulans* were grown for 5 days at 37ºC and extracted with EtOAc as described above. The crude extract was applied to a SiO_2 gel column (Merck 230-400 mesh, ASTM) and eluted with $CHCl_3$-MeOH mixtures of increasing polarity (fraction A, 1:0, 300 ml; fraction B, 19:1, 300 ml; fraction C, 9:1, 300 ml; fraction D, 7:3, 300 ml). Fraction B was further purified by normal phase HPLC [Phenomenex Luna 5 μm Si (2), 250 × 10 mm] with a flow rate of 5.0 ml/min and measured by a UV detector at 254 nm using isocratic 1:1 ethyl acetate/hexane to afford austinol (**7**, 16.2 mg t_R = 9.4 min) and dehydroaustinol (**8**, 24.7 mg, t_R = 8.5 min).

Twenty-five YAG plates inoculated with double *stcJΔ*, *cclAΔ A. nidulans* (LO2051, **Supplementary Table 2**) were used to purify monodictyphenone (**9**), emodin and its derivatives (**10 – 14**) as described above. The crude extract was applied to a COSMOSIL 75C_{18}-OPN reverse phase gel (Nacalai USA) and eluted with H_2O-MeCN mixtures of decreasing polarity (fraction A, 1:9, 500 ml; fraction B, 3:7, 500 ml; fraction C, 7:3, 500 ml; fraction D, 0:1, 500 ml).

Fraction A, which contained monodictyphenone (**9**), was further purified by reverse phase HPLC [Phenomenex Luna 5μm C18 (2), 250 × 21.2 mm] with a flow rate of 10.0 ml/min and measured by a UV detector at 254 nm. The gradient system was MeCN (solvent B) in 5 % MeCN/H_2O (solvent A) both containing 0.05 % TFA: 20 to 45 % B from 0 to 25 min, 45 to 100% B from 25 to 26 min, maintained at 100 % B from 26 to 31 min, 100 to 20% B from 31 to 32 min, and re-equilibration with 20 % B from 32 to 37 min. Monodictyphenone (**9**, 3.2 mg) eluted at 17.3 min. Fractions B and C, which contained emodin and its derivatives (**10** – **14**), were combined and purified by reverse phase HPLC [Phenomenex Luna 5μm C18 (2), 250 × 10 mm] with a flow rate of 5.0 ml/min and measured by a UV detector at 254 nm. The gradient system was MeCN (solvent B) in 5 % MeCN/H_2O (solvent A) both containing 0.05 % TFA: 30 to 60 % B from 0 to 25 min, 60 to 100% B from 25 to 26 min, maintained at 100 % B from 26 to 31 min, 100 to 30% B from 31 to 32 min, and re-equilibration with 30 % B from 32 to 37 min. ω-Hydroxyemodin (**11**, 25.5 mg), emodic acid (**12**, 18.6 mg), 2-hydroxyemodin (**13**, 46.8 mg), 2-aminoemodin (**14**, 26.0 mg), and emodin (**10**, 48.3 mg) were eluted at 10.8, 12.5, 15.2, 20.0, and 25.0 min, respectively. In some cases, TFA could not be removed from evaporation and was removed by elution of compound through COSMOSIL 75C_{18}-OPN reverse phase gel (Nacalai USA).

One liter of Czapek's medium (30 g/l sucrose, 1 g/l K_2HPO_4, 0.5 g/l KCl, 3 g/l $NaNO_3$, 0.5 g/l MgSO4·7H_2O, 0.01 g/l FeSO4·7H_2O) supplemented with pyridoxine (0.5 mg/l), uracil (1 mg/ml) and uridine (10 mM) was inoculated with 1000 × 10^6 spores of *stcJΔ A. nidulans* (LO2026, **Supplementary Table 2**). This was than aliquoted to 20 of 125 ml flasks and grown at 37ºC with shaking at 200 rpm. After 4 days, culture medium was collected by filtration and acidified to pH = 2 followed by EtOAc extraction twice. The crude extract was applied to a COSMOSIL 75C_{18}-OPN reverse phase gel (Nacalai USA) and eluted with H_2O-MeOH mixtures of decreasing polarity (fraction A, 7:3, 100 ml; fraction B, 1:1, 100 ml; fraction C, 0:1, 50 ml). Fraction A which contained F9775B (**17**) and A (**18**) was further purified by reverse phase HPLC [Phenomenex Luna 5 μm C18 (2), 250 × 21.2 mm] with a flow rate of 10.0 ml/min and measured by a UV detector at 254 nm. The gradient system was MeCN (solvent B) in 5 % MeCN/H_2O (solvent A) both containing 0.05 % TFA: 30 to 70 % B from 0 to 20 min, 70 to 100% B from 20 to 21 min, 100 to 30% B from 21 to 22 min, and re-equilibration with 30 % B from 22 to 27 min. F9775B (**17**, 5.0 mg) and A (**18**, 4.2 mg) were eluted at 11.5 and 13.3 min, respectively.

Compound identification.

All compounds were characterized mainly by NMR (**Supplementary Tables 4 and 5**), UV and Mass (**Supplementary Figure 4**) spectroscopic methods, as well as comparison of the spectra data with literature values. Compound **14** has the molecular formula of $C_{15}H_{11}NO_5$ based on the high-resolution time-of-flight mass spectrometry (HRTOFMS) and ^{13}C NMR data (**Supplementary Table 4**). The ^{1}H NMR data of compound **14** were similar to those of 2-hydroxyemodin (**13**). The only difference was an addition of two D_2O exchangeable protons (δ_H 5.51) in compound **14**. These data suggest that one of the phenol hydroxyl groups in 2-hydroxyemodin (**13**) is replaced by aniline amino group in compound **14**. ^{13}C NMR data suggest the aniline amino group should locate either to C-1, C-2, or C-3 since the chemical shifts of carbons on the ring C are almost identical between **13** and **14** (**Supplementary Table 4**). An upshift of C-2 from δ_C 139.2 in **13** to δ_C 131.8 in **14** suggests the aniline amino group locates to C-2. 2D NMR (gHMQC and gHMBC) spectral data also confirmed the assigned structure. Thus, compound **14** was identified as 2-aminoemodin.

Optical rotations were measured on a JASCO P-1010 digital polarimeter, IR spectra were recorded on a Perkin–Elmer 983G spectrophotometer. NMR spectra, detailed in supplementary data, were run on a Varian Mercury Plus 400 spectrometer, and HRESIMS spectrum was obtained on Waters LCT Premier XE electrospray time-of-flight mass spectrometer.

Compound spectral data.

Austinol (7): colorless solid; $[\alpha]_D^{23}$ +218.7° (MeOH, c 0.1); IR (ZnSe) cm^{-1} 3368, 1778, 1726, 1688, 1538, 1292, 1217; For UV and ESI-MS data, see **Supplementary Figure 4**; ^{1}H and ^{13}C NMR data, in good agreement with the published data [11].

Dehydroaustinol (8): colorless solid; $[\alpha]_D^{23}$ +321.5° (MeOH, c 0.1); IR (ZnSe) cm^{-1} 3400, 1748, 1711, 1378, 1275, 1119, 1065; For UV and ESI-MS data, see **Supplementary Figure 4**; ^{1}H and ^{13}C NMR data, in good agreement with the published data [12].

Monodictyphenone (9): yellow powder, IR (ZnSe) cm^{-1} 3421, 1682, 1644, 1608, 1400, 1206, 1139; For UV and ESI-MS data, see **Supplementary Figure 4**; ^{1}H and ^{13}C NMR data, in good agreement with the published data [13].

Emodin (10): yellow powder, IR (ZnSe) cm^{-1} 3339, 1651, 1627, 1264, 1220, 1171; For UV and ESI-MS data, see **Supplementary Figure 4**; ^{1}H NMR data, in good agreement with the published data [14]; ^{13}C NMR data, see **Supplementary Table 4**.

ω-Hydroxyemodin (11): yellow powder, IR (ZnSe) cm^{-1} 3404, 1682, 1628, 1434, 1206, 1137; For UV and ESI-MS data, see **Supplementary Figure 4**; ^{1}H NMR data, in good agreement with the published data [14]; ^{13}C NMR data, see **Supplementary Table 4**.

Emodic acid (12): yellow powder, IR (ZnSe) cm^{-1} 3422, 1682, 1628, 1436, 1206, 1143; For UV and ESI-MS data, see **Supplementary Figure 4**; ^{1}H NMR data, in good agreement with the published data [14]; ^{13}C NMR data, see **Supplementary Table 4**.

2-Hydroxyemodin (13): brown powder, IR (ZnSe) cm^{-1} 3384, 1621, 1471, 1371, 1278, 1235; For UV and ESI-MS data, see **Supplementary Figure 4**; ^{1}H NMR data, in good agreement with the published data [14]; ^{13}C NMR data, see **Supplementary Table 4**.

2-Aminoemodin (14): brown powder, IR (ZnSe) cm^{-1} 3382, 1621, 1470, 1372, 1283, 1215; For UV and ESI-MS data, see **Supplementary Figure 4**; ^{1}H and ^{13}C NMR data, see **Supplementary Table 4**. HRTOFMS, $[M+H]^{+}$ *m/z* found 286.0710; calc. for $C_{15}H_{12}NO_5$: 286.0715.

F9775B (17): yellow powder; $[\alpha]_D^{25}$ 0.0° (MeOH, c 0.7); IR (ZnSe) cm^{-1} 3306, 1732, 1638, 1527, 1512, 1322, 1230, 1084; For UV and ESI-MS data, see **Supplementary Figure 4**; ^{1}H and ^{13}C NMR data (DMSO-d_6), see **Supplementary Table 5**. ^{1}H and ^{13}C NMR data (CD_3OD) are in good agreement with the published data [15] except that we detected a resonance (δ_H = 6.27) not previously reported. The structures presented in the patent predict three olefinic and aromatic proton resonances, in accordance with our NMR data. Also our ^{13}C resonances are down shifted 2.5 ppm with respect to every resonance previously reported.

F9775A (18): yellow powder; $[\alpha]_D^{25}$ 0.0° (MeOH, c 0.3); IR (ZnSe) cm^{-1} 3306, 1733, 1637, 1530, 1512, 1322, 1230, 1084; For UV and ESI-MS data, see **Supplementary Figure 4**. ^{1}H and ^{13}C NMR data (DMSO-d_6), see **Supplementary Table 5**; ^{1}H and ^{13}C NMR data (CD_3OD), in good agreement with the published data [15].

References

1. Shimizu, K., & Keller, N.P. *Genetics* **157,** 591-600 (2001).
2. Osmani, A.H., May, G.S. & Osmani, S.A. *J. Biol. Chem.* **274,** 23565-23569 (1999).
3. Bok, J.W. & Keller, N.P. *Eukaryot. Cell* **3,** 527-535 (2004).
4. Nayak, T. *et al. Genetics* **172,** 1557-1566 (2006).
5. Szewczyk, E. *et al. Nat. Protoc.* **1,** 3111-3120 (2006).
6. Weidner, G., d'Enfert, C., Koch, A., Mol, P.C. & Brakhage, A.A. *Curr. Genet.* **33,** 378-385 (1998).
7. Waring, R. B., May, G. S. and Morris, N. R. *Gene* **79,** 119-130 (1989).
8. Sambrook, J., Fritsch, E. F. & Maniatis, T. Cold Spring Harbor Laboratory Press (Cold Spring Harbor, New York, USA, 1989).
9. Lee, S.B. & Taylor, J.W. In *PCR protocols: A guide to methods and applications*. (ed. M.A. Innis) 282-287 (Academic Press, San Diego; USA, 1990)
10 Bernreiter, A. *et al. Mol. Cell. Biol.* **27,** 791-802 (2007).
11. Simpson, T.J., Stenzel, D.J., Bartlett, A.J., O'Brien, E. & Holker, J.S.E. *J. Chem. Soc. Perkin Trans.* **1**, 2687-2692 (1982).
12. Marquez-Fernandez, O. *et al. Eukaryot. Cell* **6**, 710-720 (2007).
13. Krick, A. *et al. J. Nat. Prod*. **70,** 353-360 (2007).
14. Morooka, N., Nakano, S., Itoi, N. & Ueno, Y. *Agric. Biol. Chem.* **54,** 1247-1252 (1990).
15. Sato, S., Morishita, T., Hosoya, T. & Ishikawa, Y. (ed. Koho, J.K.T.) 11 Japan; 1999).