SUPPLEMENTARY INFORMATION

A Scalable Platform to Identify Fungal Secondary Metabolites and Their Gene Clusters

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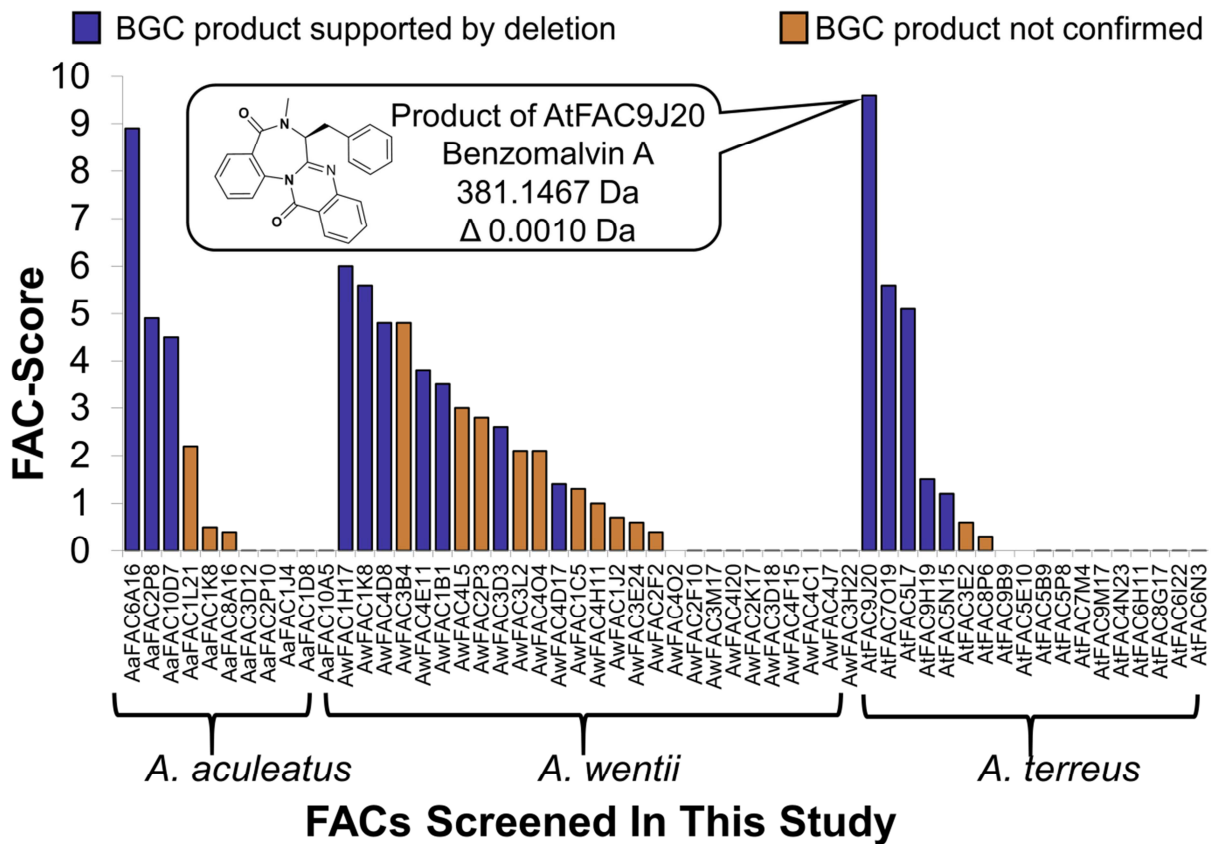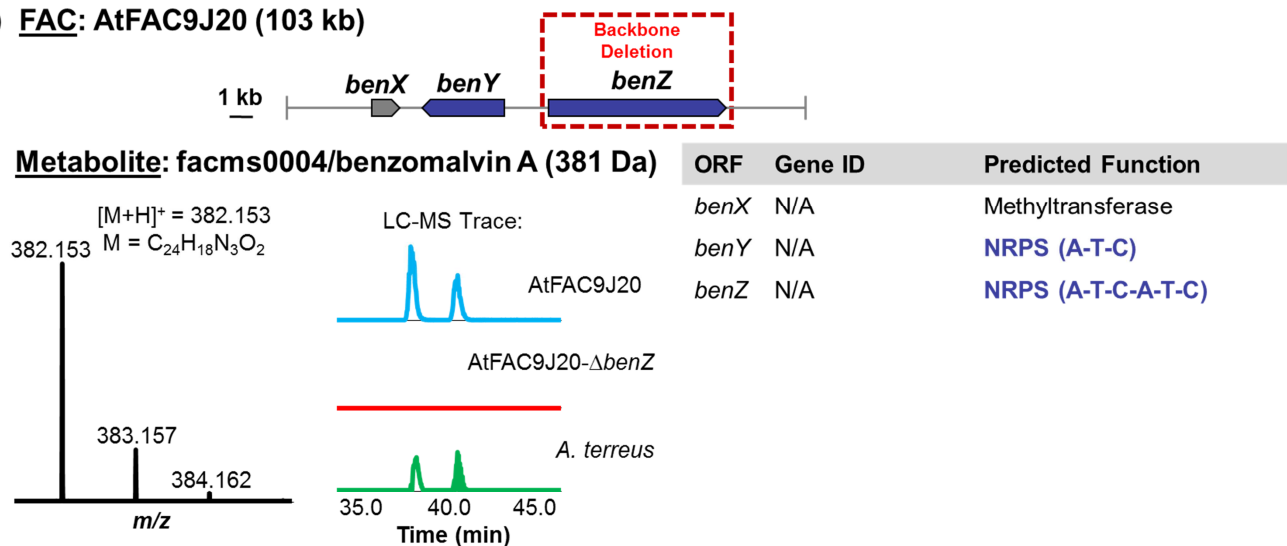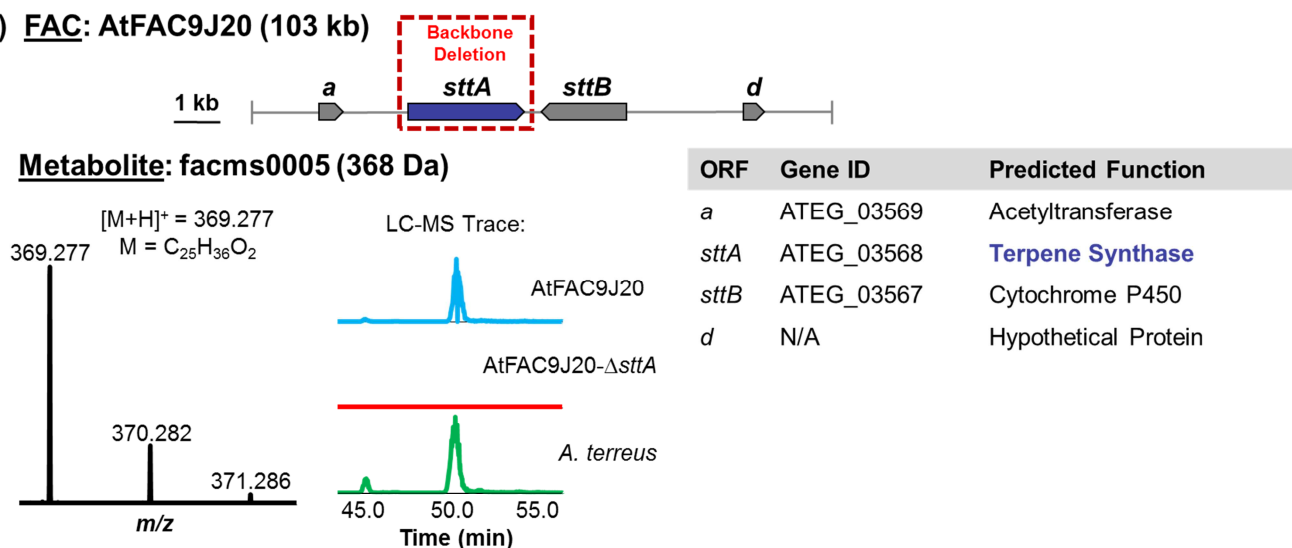
Supplementary Figure 1. **FACs screened in this study.** Bars show the FAC-Score of the top scoring compound from each FAC, reflecting a ~30% success rate (15/56 FACs) for expression and detection of the heterologous metabolite of FAC-strains in this study. Brown bars represent high scoring compounds which were present at trace levels in negative controls (or for one case was refuted by backbone deletions within the FAC). Blue bars represent FAC-encoded secondary metabolites, confirmed by manual analysis of controls and validation by genetic deletions. FACs without visible bars lacked compounds with positive FAC-Scores. The inset highlights the deorphanized and overexpressed secondary metabolite, benzomalvin A, which is encoded by AtFAC9J20.

Supplementary Figure 2. FAC produced metabolites and their clusters. Confirmatory data are shown for the 17 unique compounds from 15 FACs reported to produce unique SM products. For each FAC in panels **a-q**, the gene cluster diagram for the cluster contained in the FAC is shown, along with a table of ORFs with predicted biosynthetic functions. The MS[1] of each FAC metabolite is also shown, along with the selected ion chromatogram for that metabolite from the FAC, its deletant, and its parent strain. The backbone gene subjected to deletion for empirical validation is highlighted by a red box in each case. Panels **c-q** are continued below.
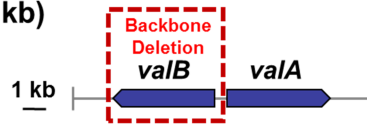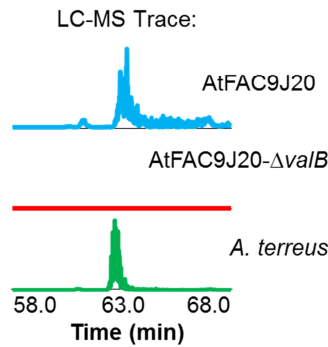
A) <u>FAC</u>: AtFAC9J20 (103 kb)

<u>Metabolite</u>: facms0004/benzomalvin A (381 Da)

$[M+H]^+ = 382.153$
$M = C_{24}H_{18}N_3O_2$

ORF	Gene ID	Predicted Function
benX	N/A	Methyltransferase
benY	N/A	NRPS (A-T-C)
benZ	N/A	NRPS (A-T-C-A-T-C)

B) <u>FAC</u>: AtFAC9J20 (103 kb)

<u>Metabolite</u>: facms0005 (368 Da)

$[M+H]^+ = 369.277$
$M = C_{25}H_{36}O_2$

ORF	Gene ID	Predicted Function
a	ATEG_03569	Acetyltransferase
sttA	ATEG_03568	Terpene Synthase
sttB	ATEG_03567	Cytochrome P450
d	N/A	Hypothetical Protein

3

C) FAC: AtFAC9J20 (103 kb)

Backbone Deletion

valB   valA

1 kb

Metabolite: facms0017/valactamide A (506 Da)

$[M+H]^+ = 507.416$
$M = C_{30}H_{54}N_2O_4$

507.416

508.418

509.421

m/z

LC-MS Trace:

AtFAC9J20

AtFAC9J20-ΔvalB

A. terreus

58.0   63.0   68.0
Time (min)
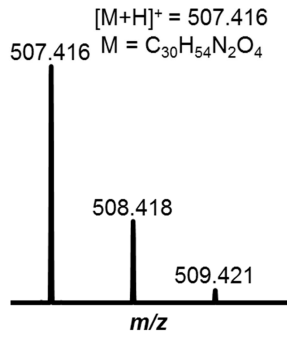
ORF	Gene ID	Predicted Function
valB	ATEG_03576	NRPS (C-A-T-C-A-T-C)
valA	ATEG_03575, ATEG_03574	PKS (KS-AT-DH-MT-ER-KR-T)

D) FAC: AtFAC9H19 (96 kb)

Backbone Deletion

1 kb   a b c   d   e   f g h   i   j   k l m n o

Metabolite: facms0007 (610 Da)

$[M+H]^+ = 610.925$

610.925

m/z

LC-MS Trace:

AtFAC9H19

AtFAC9H19-Δj

A. terreus

0.0   3.5   7.0
Time (min)
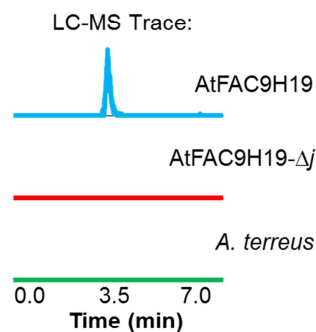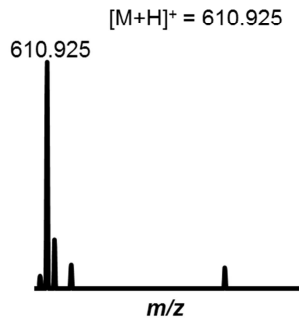
ORF	Gene ID	Predicted Function
a	ATEG_07076	MFS Transporter
b	ATEG_07075	Kinesin-Like
c	ATEG_07074	Muramidase
d	ATEG_07073	LysM Domain-Containing
e	ATEG_07072	Glycosyl Hydrolase
f	ATEG_07071	Tetratricopeptide Repeat
g	ATEG_07070	WD Domain-Containing
h	ATEG_07069	Hypothetical Protein
i	ATEG_07068	F-Box Domain Protein
j	ATEG_07067	PKS (KS-AT-DH-KR-T)
k	ATEG_07066	DUF341 Domain
l	ATEG_07065	NRPS (A)
m	ATEG_07064	Integral Membrane Protein
n	ATEG_07063	Nitrite Reductase
o	ATEG_07062	Alcohol Dehydrogenase

E) FAC: AtFAC5N15 (101 kb)

1 kb   a b c d e   f g h i j   k   l m   **Backbone Deletion** n   o   p q r s t
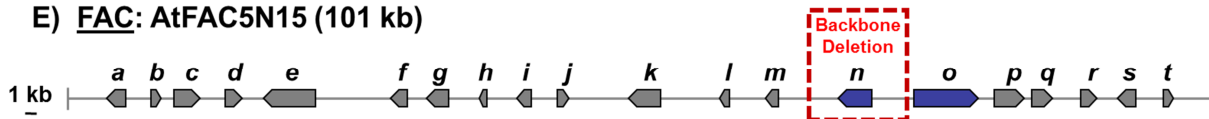
Metabolite: facms0008 (277 Da)

$[M+H]^+ = 278.212$
$M = C_{17}H_{27}NO_2$
278.212
279.215
280.218
m/z

LC-MS Trace:
AtFAC5N15
AtFAC5N15-Δn
A. terreus
51.0   56.0   61.0
Time (min)
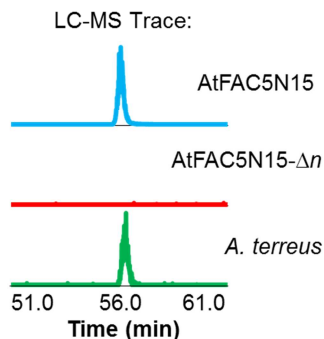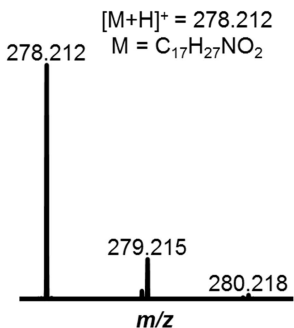
ORF	Gene ID	Predicted Function
a	ATEG_07393	Aldehyde Dehydrogenase
b	ATEG_07392	Short Chain Dehydrogenase
c	ATEG_07391	Transcription Factor
d	ATEG_07390	FAD-Binding Domain
e	ATEG_07389	C6 Finger Domain
f	ATEG_07388	Alkaline Peptidase
g	ATEG_07387	Beta-Lactamase
h	ATEG_07386	Resistance Protein
i	ATEG_07385	Aldo-Keto Reductase
j	ATEG_07384	Alpha/Beta Hydrolase
k	ATEG_07383	Beta-Xylosidase
l	ATEG_07382	Short Chain Dehydrogenase
m	ATEG_07381	Alpha/Beta Hydrolase
n	ATEG_07380	**NRPS-Like (A-T-R)**
o	ATEG_07379	**PKS (KS-AT-DH-T-T)**
p	ATEG_07378	FAD-Binding Domain
q	ATEG_07377	O-Methyltransferase
r	ATEG_07376	Myb-Like DNA-Binding
s	ATEG_07375	MFS Transporter
t	ATEG_07374	3-Oxo-Acyl-ACP Reductase

F) FAC: AtFAC7O19 (28 kb)

1 kb   a   b   c d   e   **Backbone Deletion** f   g   h   i   j
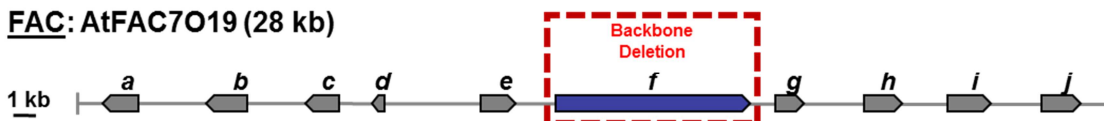
Metabolite: facms0016 (309 Da)

$[M+H]^+ = 310.119$
$M = C_{17}H_{15}N_3O_3$
310.119
311.123
312.125
m/z

LC-MS Trace:
AtFAC7O19
AtFAC7O19-Δf
A. terreus
25.0   30.0   35.0
Time (min)
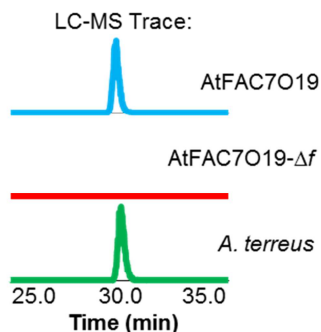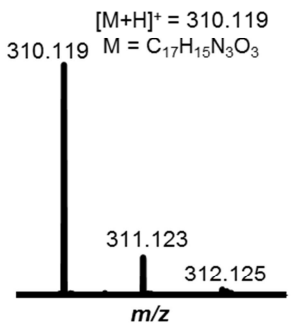
ORF	Gene ID	Predicted Function
a	ATEG_07363	MFS Transporter
b	ATEG_07362	Cytochrome P450
c	ATEG_07361	Integral Membrane Protein
d	ATEG_07360	Thaumatin Domain
e	ATEG_07359	Indoleamine 2,3-Dioxygenase
f	ATEG_07358	**NRPS (A-T-C-A-T-C-T)**
g	ATEG_07357	Transcription Factor
h	ATEG_07356	Glycoside Hydrolase
i	ATEG_07355	Sugar Transporter
j	ATEG_07354	Zinc Dehydrogenase

G) FAC: AaFAC6A16 (93 kb)

Backbone Deletion

1 kb

a *b* *c* *d* *e* *f* *g* *h*
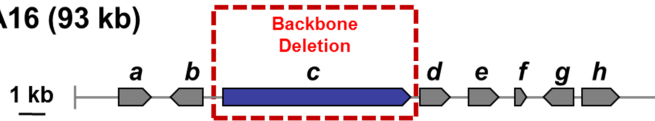
Metabolite: facms0001 (402 Da)

$[M+H]^+ = 403.202$
$M = C_{25}H_{26}N_2O_3$

403.202

404.204

405.209

m/z

LC-MS Trace:

AaFAC6A16

AaFAC6A16-Δ*c*

A. aculeatus

37.0   42.0   47.0
Time (min)
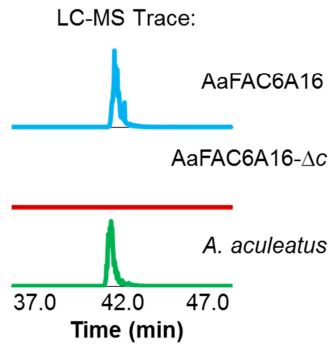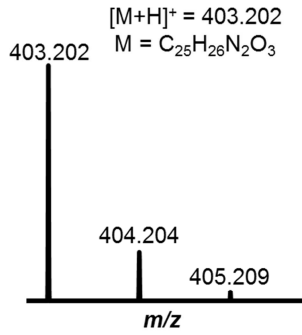
ORF	Gene ID	Predicted Function
a	AACU_46597	Tryptophan DMAT
b	AACU_46596	Aldehyde Reductase
c	AACU_46595	NRPS (A-T-C-A-T-C)
d	AACU_34424	Alcohol Dehydrogenase
e	AACU_63363	Integral Membrane Protein
f	AACU_46592	Hypothetical Protein
g	AACU_46591	Arrestin-Like
h	AACU_46590	C6 Transcription Factor

H) FAC: AaFAC10D7 (122 kb)

Backbone Deletion

1 kb

a *b* *c* *d* *e* *f* *g* *h*
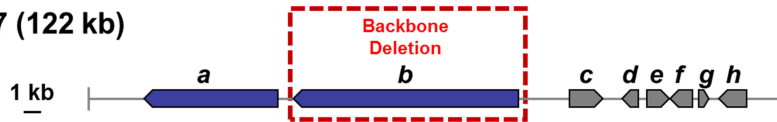
Metabolite: facms0003 (273 Da)

$[M+H]^+ = 274.036$
$M = C_{13}H_7NO_6$

274.036

275.039

276.042

m/z

LC-MS Trace:

AaFAC10D7

AaFAC10D7-Δ*b*

A. aculeatus

36.0   41.0   46.0
Time (min)
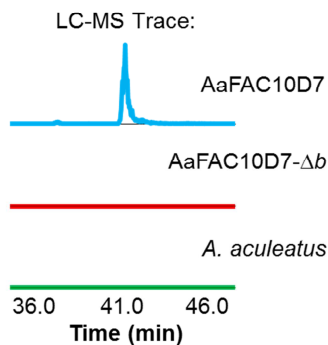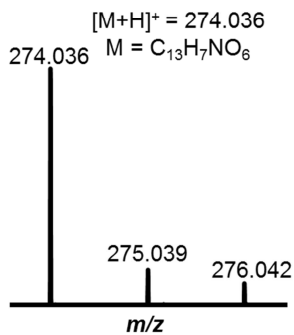
ORF	Gene ID	Predicted Function
a	AACU_26431	PKS (KS-AT-DH-MT-ER-KR-T)
b	AACU_51108	NRPS/PKS (KS-AT-DH-MT-KR-C-A-T-R)
c	AACU_51107	Cytochrome P450
d	AACU_08044	Hypothetical Protein
e	AACU_41415	Alcohol Dehydrogenase
f	AACU_26007	Alpha/Beta-Hydrolase
g	AACU_58887	Hypothetical Protein
h	AACU_26403	Cytochrome P450

6

I) <u>FAC</u>: AaFAC2P8 (141 kb)

Backbone Deletion

1 kb

a b c d e f g h i j k l m n o p q r s t u v w x
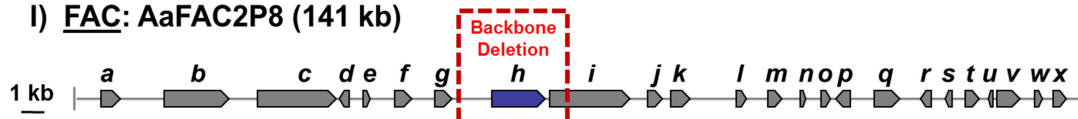
Metabolite: facms0002 (558 Da)

$[M+H]^+ = 559.234$
$M = C_{29}H_{30}N_6O_6$

559.234

560.237

561.240

m/z

LC-MS Trace:

AaFAC2P8

AaFAC2P8-Δ*h*

A. aculeatus

34.0   39.0   44.0

Time (min)
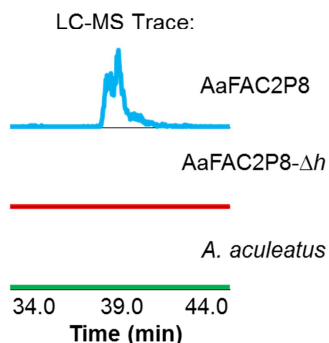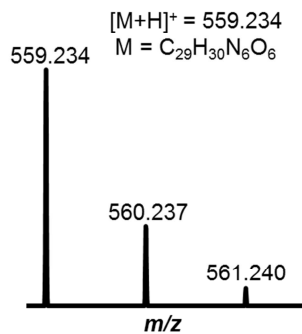
ORF	Gene ID	Predicted Function
a	AACU_58519	ToxD-Like Oxidoreductase
b	AACU_25249	WD40 Repeat-Like
c	AACU_50845	Tetratricopeptide-Like
d	AACU_40975	Hypothetical Protein
e	AACU_40974	Cytochrome P450
f	AACU_40973	Formate/Nitrite Transporter
g	AACU_40972	Hypothetical Protein
h	AACU_59515	**NRPS-like (A-T-R)**
i	AACU_50843	ABC Transporter
j	AACU_40969	Zinc Finger Protein
k	AACU_25059	Aorisin Protease
l	AACU_40967	Acyl-CoA N-Acyltransferase
m	AACU_58511	Extracellular Dioxygenase
n	AACU_40965	GNAT N-Acetyltransferase
o	AACU_40964	Short Chain Dehydrogenase
p	AACU_40963	Beta-Lactamase
q	AACU_40962	C2H2 Transcription Factor
r	AACU_40961	Cytochrome P450
s	AACU_40960	Alpha/Beta-Hydrolase
t	AACU_40959	FAD Monooxygenase
u	AACU_40958	Hypothetical Protein
v	AACU_40957	C6 Transcription Factor
w	AACU_50839	14-Alpha Sterol Demethylase
x	AACU_58505	Cinnamoyl-CoA Reductase

J) FAC: AwFAC1K8 (114 kb)
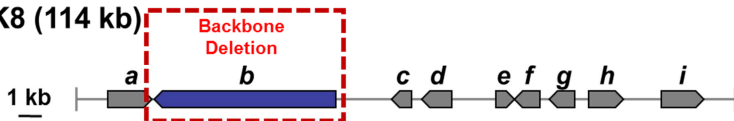

Metabolite: facms0010 (987 Da)

$[M+H]^+ = 988.557$
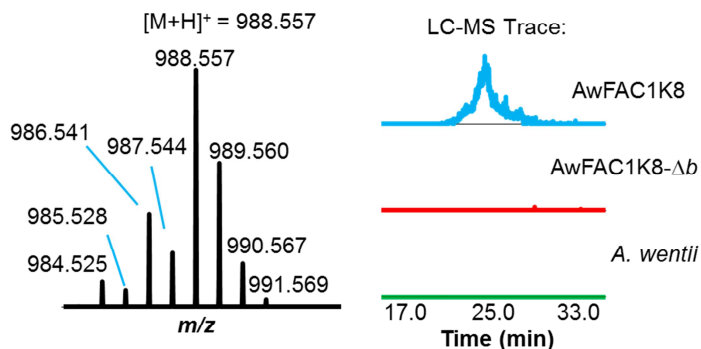
ORF	Gene ID	Predicted Function
a	ASPWE_182850	Acetyltransferase
b	ASPWE_027400	**PKS (KS-AT-DH-MT-ER-KR-T)**
c	ASPWE_110131	NADP Oxidoreductase
d	ASPWE_027397	Lipase
e	ASPWE_182848	NAD Oxidoreductase
f	ASPWE_027395	Inositol Dehydrogenase
g	ASPWE_112338	ArcA-Like Protein
h	ASPWE_112121	MFS Transporter
i	ASPWE_154084	Sugar Transporter

K) FAC: AwFAC4E11 (105 kb)
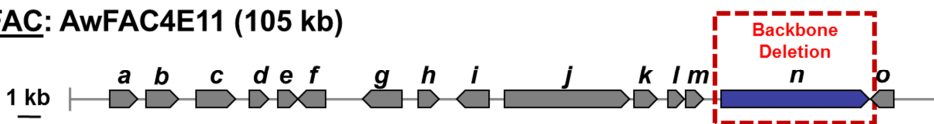
Metabolite: facms0012 (576 Da)

$[M+H]^+ = 577.317$
$M = C_{35}H_{44}O_7$
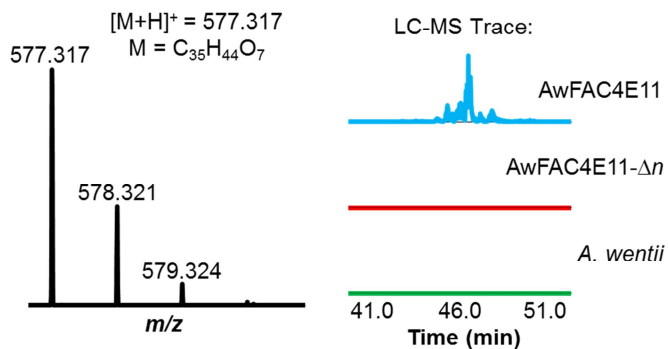
ORF	Gene ID	Predicted Function
a	ASPWE_022952	C6 Transcription Factor
b	ASPWE_022951	Enoyl-CoA Hydratase
c	ASPWE_034287	O-Methyltransferase
d	ASPWE_034285	Hydrophobin
e	ASPWE_064169	Lysophospholipase-Like
f	ASPWE_034283	Acetyltransferase
g	ASPWE_178631	ZIP Zinc Transporter
h	ASPWE_166859	Major Allergen
i	ASPWE_056119	Short Chain Dehydrogenase
j	ASPWE_099620	Transcription Factor
k	ASPWE_166855	Gluconolaconase
l	ASPWE_034275	Monooxygenase
m	ASPWE_233835	Methyltransferase
n	ASPWE_034272	**PKS (AT-KS-AT-DH-T-T)**
o	ASPWE_102062	Beta-Lactamase

8

L) FAC: AwFAC4D17 (96 kb)

1 kb

Backbone Deletion

a b c d e f g h i j k l m n o p q
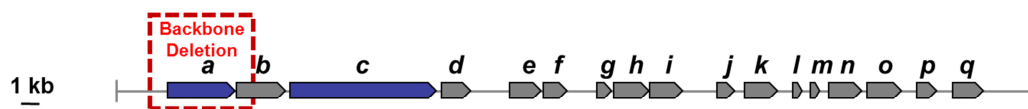
Metabolite: facms0015 (541 Da)

$[M+H]^+ = 542.386$
$M = C_{33}H_{51}NO_5$

542.386

543.389

544.392

m/z

LC-MS Trace:

AwFAC4D17

AwFAC4D17-Δa

A. wentii

34.0   39.0   44.0

Time (min)
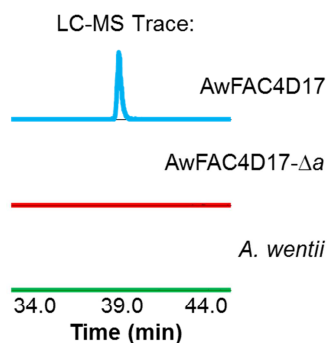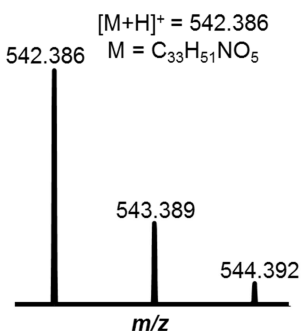
ORF	Gene ID	Predicted Function
a	ASPWE_042597	NRPS-like (A-T-R)
b	ASPWE_042596	MFS Transporter
c	ASPWE_042595	PKS (KS-AT-DH-MT-KR-T)
d	ASPWE_052779	Cytochrome P450
e	ASPWE_586808	Zinc Dehydrogenase
f	ASPWE_743260	Cytochrome P450
g	ASPWE_113704	Oxidoreductase
h	ASPWE_042592	C6 Transcription Factor
i	ASPWE_042591	Glycosyltransferase
j	ASPWE_042589	Transcription Factor
k	ASPWE_112861	MFS Transporter
l	ASPWE_185079	Ankyrin
m	ASPWE_042586	Zinc Finger, C2H2-Like
n	ASPWE_070267	Dethiobiotin Synthase
o	ASPWE_060925	MFS Transporter
p	ASPWE_136814	Dioxygenase
q	ASPWE_052774	MFS Transporter

9

M) <u>FAC</u>: AwFAC4D8 (80 kb)

Backbone Deletion

1 kb   *a*   *b c d e f g   h i   j k l m n o   p q r*
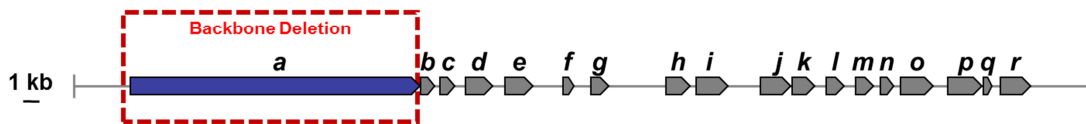
<u>Metabolite</u>: facms0011 (679 Da)

$[M+H]^+ = 680.356$
$M = C_{38}H_{45}N_7O_5$

680.356

681.359

682.361

m/z

LC-MS Trace:

AwFAC4D8

AwFAC4D8-Δ*a*

A. wentii

19.0   24.0   29.0
Time (min)
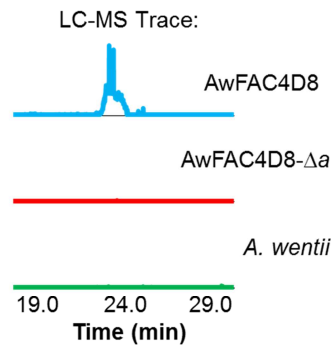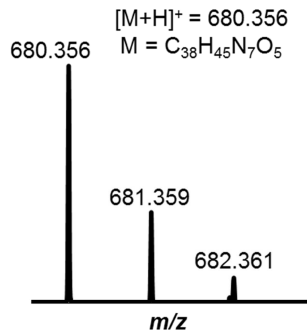
ORF	Gene ID	Predicted Function
a	ASPWE_044725	**NRPS (A-T-T-C-A-T-E-C-A-T-C-A-T-C)**
b	ASPWE_670242	N-Methyltransferase
c	ASPWE_031465	N-Acetyltransferase
d	ASPWE_044723	Hypothetical Protein
e	ASPWE_118134	MFS Transporter
f	ASPWE_116569	Hypothetical Protein
g	ASPWE_670176	C6 Transcription Factor
h	ASPWE_044719	C6 Transcription Factor
i	ASPWE_162202	MFS Transporter
j	ASPWE_162198	Cytochrome P450
k	ASPWE_186729	Terpene Synthase
l	ASPWE_031457	Enoyl Reductase
m	ASPWE_175848	Arabinitol Dehydrogenase
n	ASPWE_062177	Short Chain Dehydrogenase
o	ASPWE_175846	C6 Transcription Factor
p	ASPWE_175845	Hypothetical Function
q	ASPWE_175844	Glutamine Amidotransferase
r	ASPWE_162187	MFS Transporter

N) <u>FAC</u>: AwFAC1H17 (87 kb)

Backbone Deletion

1 kb   *a*   *b*   *c*   *d e f*   *g*   *h*   *i j*   *k*   *l*   *m*

<u>Metabolite</u>: facms0009 (422 Da)

$[M+H]^+ = 423.182$
$M = C_{25}H_{26}O_6$

423.182

424.185

425.188

m/z

LC-MS Trace:

AwFAC1H17

AwFAC1H17-Δ*g*

A. wentii

30.0   35.0   40.0
Time (min)
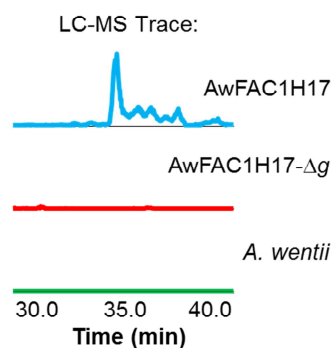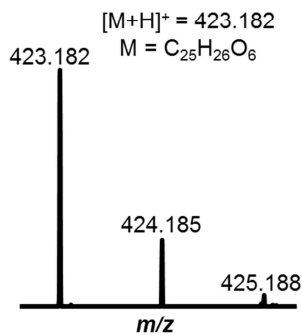
ORF	Gene ID	Predicted Function
a	ASPWE_182811	FAD Monooxygenase
b	ASPWE_110653	Glucose Oxidase
c	ASPWE_110954	Alpha/Beta Hydrolase
d	ASPWE_050976	Metalloprotease
e	ASPWE_109715	Ankyrin
f	ASPWE_096725	Ankyrin
g	ASPWE_085322	**NRPS (C-A-T-C-A-T-C)**
h	ASPWE_739335	Aminotransferase
i	ASPWE_171489	C6 Transcription Factor
j	ASPWE_068122	Oxidase
k	ASPWE_027350	Hypothetical Protein
l	ASPWE_171486	Hemeolysin E
m	ASPWE_132289	MFS Transporter

O) FAC: AtFAC5L7 (124 kb)

Backbone Deletion

a b c d e f g h i j k l m n o p q r

1 kb
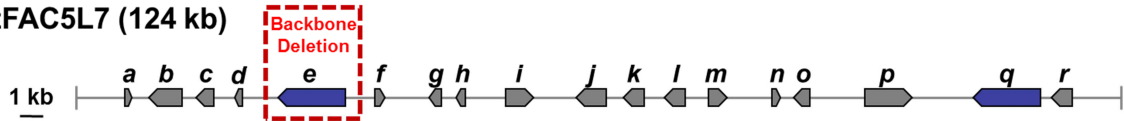
Metabolite: facms0006 (673 Da)

$[M+H]^+ = 674.269$
$M = C_{36}H_{39}N_3O_{10}$

674.269

675.272

676.275

m/z

LC-MS Trace:

AtFAC5L7

AtFAC5L7-Δe

A. terreus

18.0   23.0   28.0
Time (min)
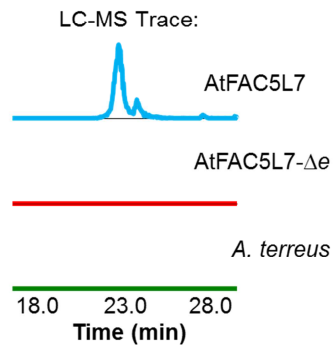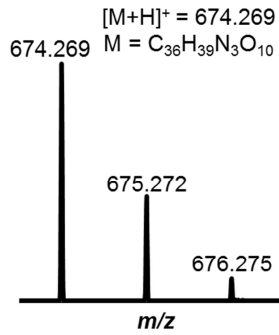
ORF	Gene ID	Predicted Function
a	ATEG_07504	Transporter
b	ATEG_07503	N,N-Dimethylglycine Oxidase
c	ATEG_07502	Monooxygenase
d	ATEG_07501	O-Methytransferase
e	ATEG_07500	**PKS (KS-AT-DH-T-T-TE)**
f	ATEG_07499	C6 Transcription Factor
g	ATEG_07498	Dioxygenase
h	ATEG_07497	Chitin Binding Domain
i	ATEG_07496	C6 Zinc Finger Domain
j	ATEG_07495	Hypothetical Protein
k	ATEG_07494	Transporter
l	ATEG_07493	Exoglucanase
m	ATEG_07492	Arylsulfotransferase Domain
n	ATEG_07491	N-Acetyltransferase
o	ATEG_07490	Oxidoreductase
p	ATEG_07489	ABC Transporter
q	ATEG_07488	**NRPS (A-T-C-T-C)**
r	ATEG_07487	Iron Transporter

P) <u>FAC</u>: AwFAC3D3 (109 kb)

Backbone Deletion

1 kb

a b c d e f g   h i   j k l m n o p q r s t
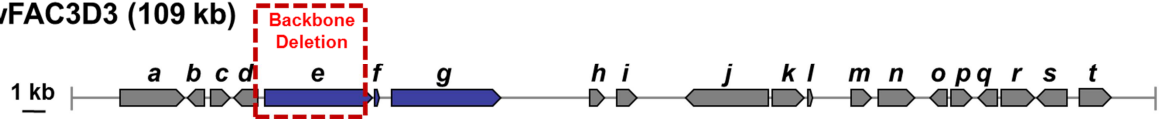
<u>Metabolite:</u> facms0014 (735 Da)

$[M+H]^+ = 736.205$
$M = C_{41}H_{29}N_5O_9$

736.205

737.208

738.211

m/z

LC-MS Trace:

AwFAC3D3

AwFAC3D3-Δe

A. wentii

27.0   32.0   37.0
Time (min)
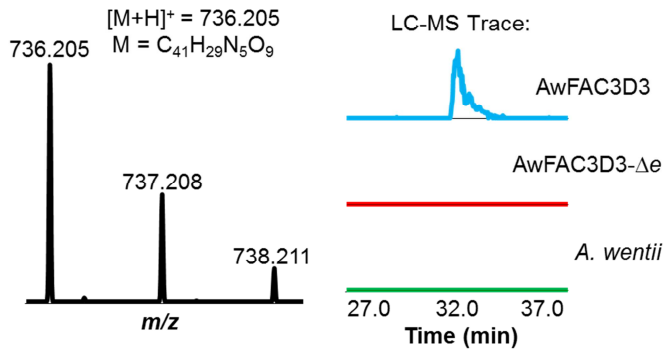
ORF	Gene ID	Predicted Function
a	ASPWE_169905	ABC Transporter
b	ASPWE_169904	Histidine Kinase
c	ASPWE_405376	Pyrroline-5-Carboxylate Reductase
d	ASPWE_037945	Protein Kinase
e	ASPWE_151732	**NRPS (A-T-C)**
f	ASPWE_080973	**NRPS (A)**
g	ASPWE_151729	**NRPS (A-T-E-C-A-T-TE)**
h	ASPWE_169900	Chitinase
i	ASPWE_025854	Hypothetical Protein
j	ASPWE_108842	Chitin-Binding
k	ASPWE_737166	Chitin-Binding
l	ASPWE_107897	Chitin-Binding
m	ASPWE_037942	Hypothetical Protein
n	ASPWE_169895	Transcription Factor
o	ASPWE_049886	Glycoside Hydrolase
p	ASPWE_037937	Glycoside Hydrolase
q	ASPWE_058281	Fructose-Bisphosphate Aldolase
r	ASPWE_107812	Transcription Factor
s	ASPWE_151707	MFS Transporter
t	ASPWE_049881	NADP Dehydrogenase

Q) <u>FAC</u>: AwFAC1B1 (90 kb)

Backbone Deletion

1 kb

a b c d e f g h i j
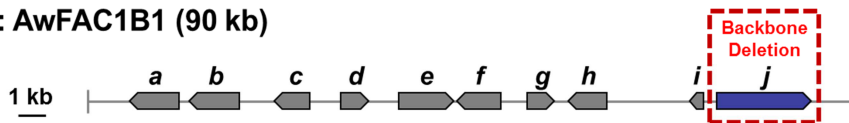
<u>Metabolite:</u> facms0013 (471 Da)

$[M+H]^+ = 472.209$
$M = C_{24}H_{29}N_3O_7$

472.209

473.213

474.217

m/z

LC-MS Trace:

AwFAC1B1

AwFAC1B1-Δj

A. wentii

23.0   26.0   29.0
Time (min)
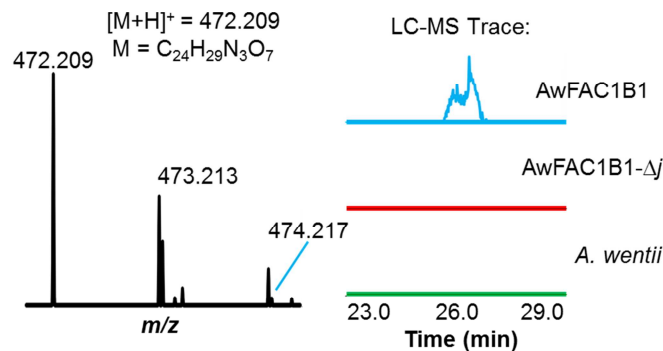
ORF	Gene ID	Predicted Function
a	ASPWE_163811	FAD Oxidase
b	ASPWE_698138	Integral Membrane Protein
c	ASPWE_045595	Hypothetical Protein
d	ASPWE_163803	2-Hydroxyisobutyrate Dehydrogenase
e	ASPWE_747605	Transcription Factor
f	ASPWE_119056	MFS Transporter
g	ASPWE_045592	Beta-Galactosidase
h	ASPWE_054731	O-Methyltransferase
i	ASPWE_045587	Hypothetical Protein
j	ASPWE_163793	**NRPS-Like (A-T-R)**
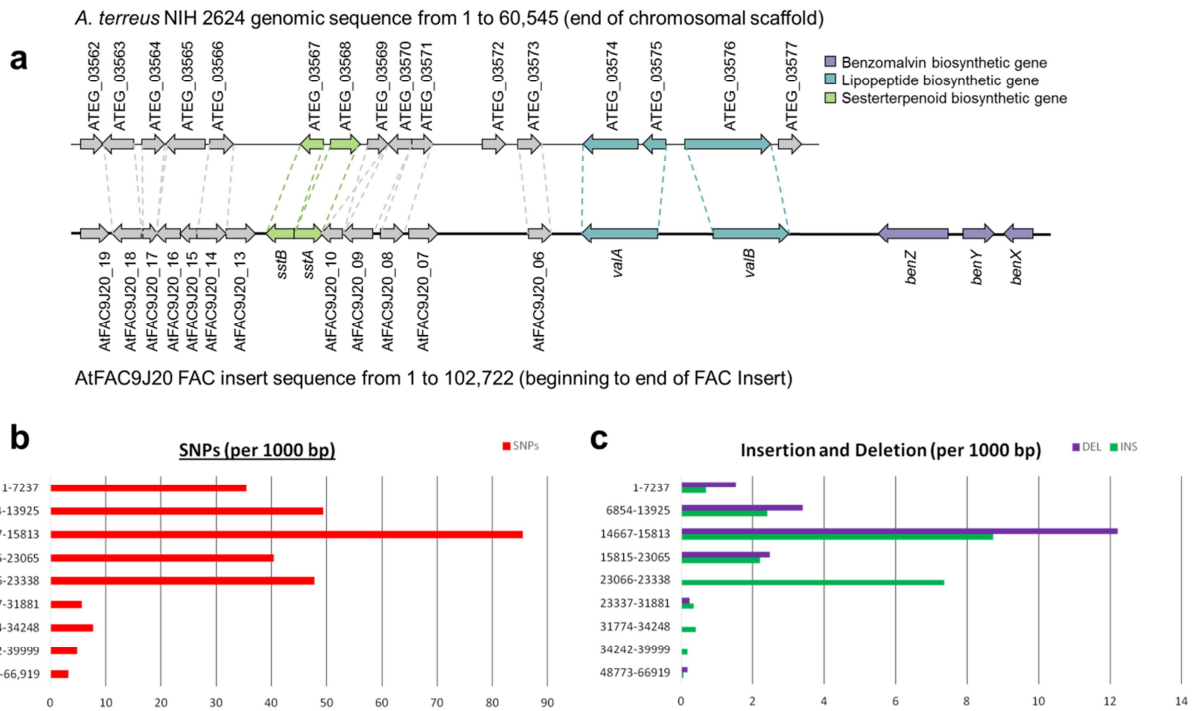
**Supplementary Figure 3. Alignment of the seqeunced AtFAC9J20 insert with *A. terreus* strain NIH 2624 reference genome. (a)** linear alignment of *A. terreus* strain NIH 2624 reference sequence (top) and the sequence of AtFAC9J20 derived from *A. terreus* strain ATCC 20542 (bottom). AtFAC9J20 BGCs for the sesterterpenoid (green), valactamide (teal), and benzomalvin (purple) are shown; the middle panel of *a* shows sequence alignment with large deletions, insertions, inversions and conserved sequences. **(b)** SNPs found in AtFAC9J20 insert relative to *A. terreus* strain NIH 2624. **(c)** Insertions and deletions found in AtFAC9J20 insert relative to *A. terreus* strain NIH 2624.
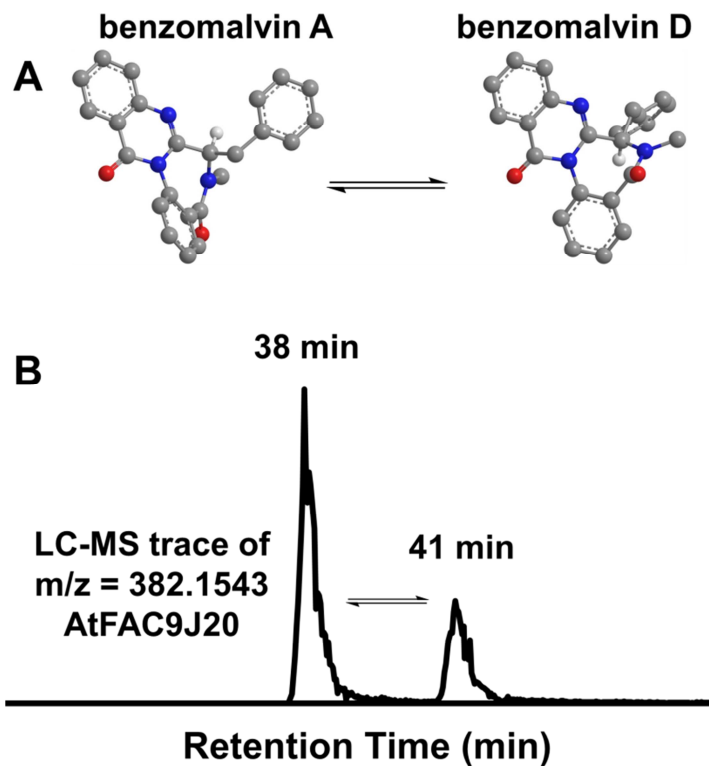
Supplementary Figure 4. AtFAC9J20 produces atropisomeric pair benzomalvin A and benzomalvin D. (a) Benzomalvin A is in equilibrium with its atropisomer, benzomalvin D. **(b)** Peaks corresponding to each atropisomer are shown a 38 and 41 minutes. Based on relative peak abundance, it is expected that benzomalvin A is the peak at 38 min and benzomalvin D at 41 min.
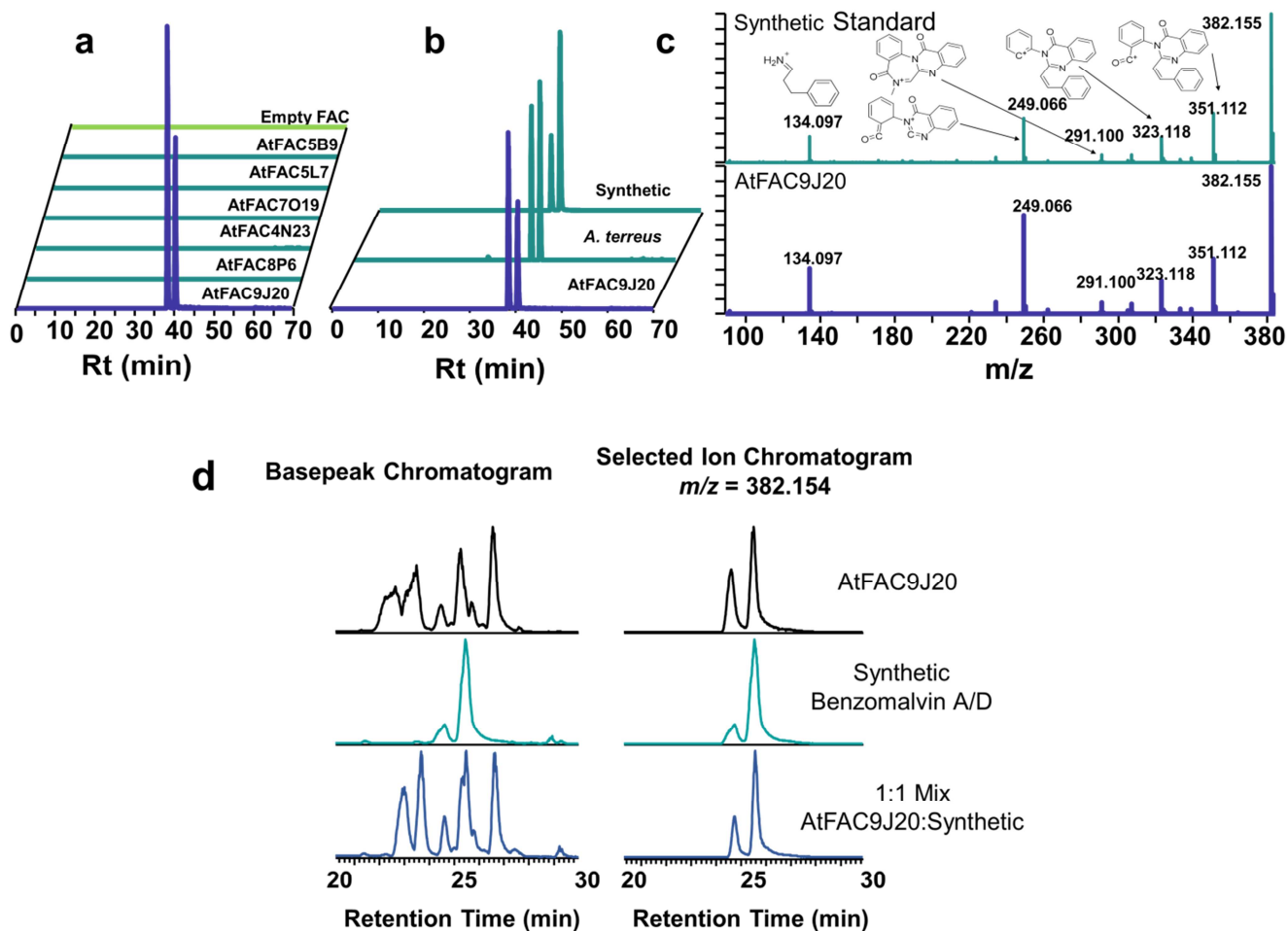
Supplementary Figure 5. AtFAC9J20 encodes the BGC for benzomalvin A/D. (a) Selected ion chromatograms of 382.1545 ±5 ppm reveal an unknown, highly abundant ion unique to extracts from a strain harboring FAC AtFAC9J20. **(b)** Benzomalvin A/D was synthesized and analyzed by LC-MS/MS. Retention time and *m/z* of the synthetic standard matches the ion detected at 382.1545, found in both AtFAC9J20 extract and extract from *A. terreus* ATCC 20524. **(c)** MS[2] of ion at *m/z* = 382.1545 of the synthetic standard matches that of AtFAC9J20, confirming the identity of the unknown ion as benzomalvin A/D. **(d)** Comparison of benzomalvin from AtFAC9J20, from the synthetic standard, and coinjection of the two as a 1:1 mixture confirms their homogeneity.
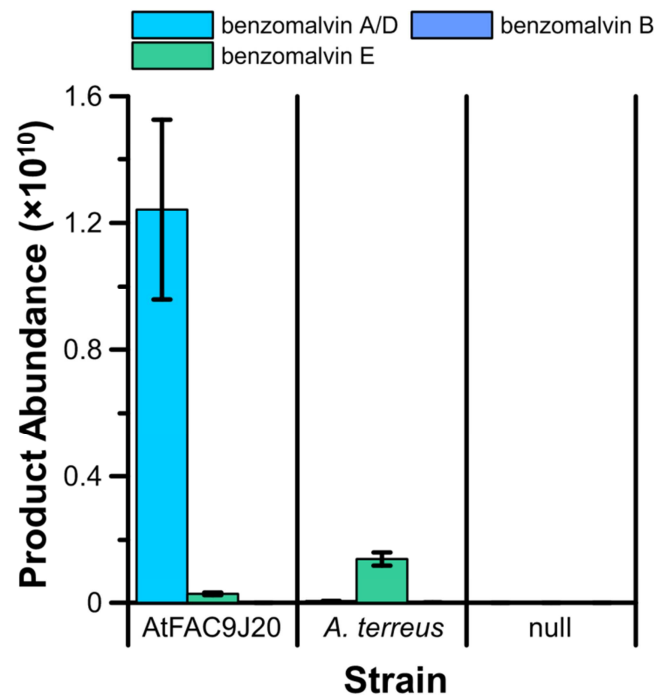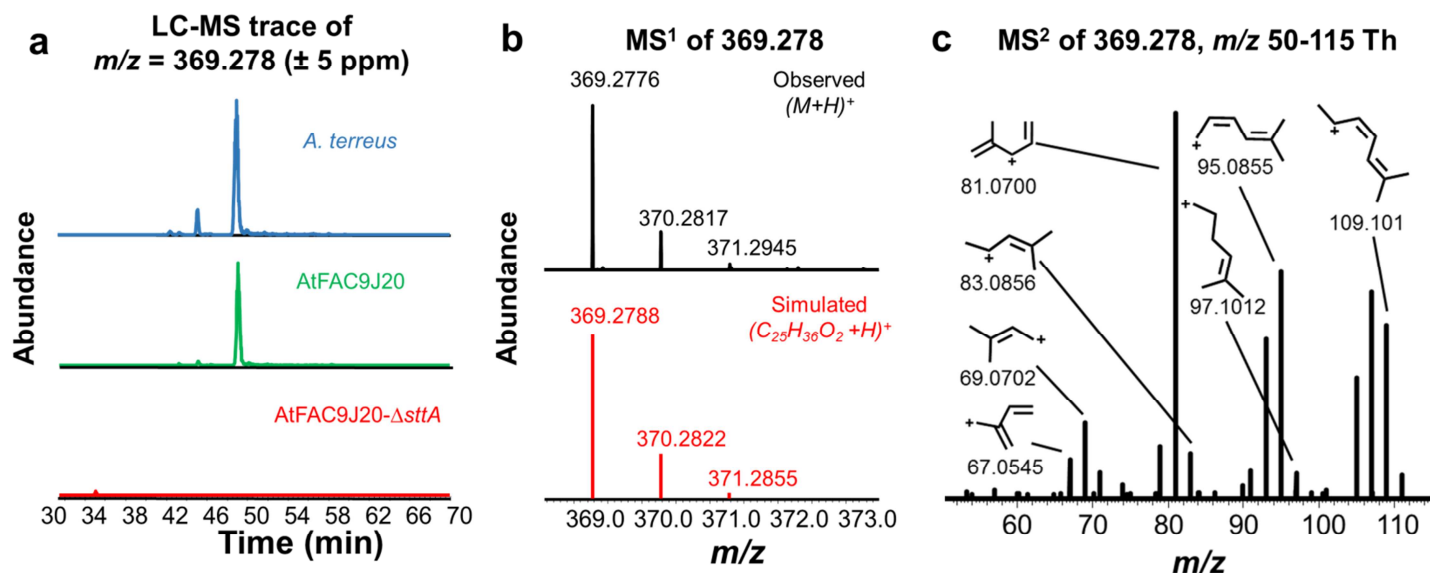
Supplementary Figure 6. Abundance of benzomalvins in AtFAC9J20-transformed strain.
Amount of benzomalvin A/D, E, and B produced by AtFAC9J20, *A. terreus* strain ATCC 20524, and AtFAC9J20-null.
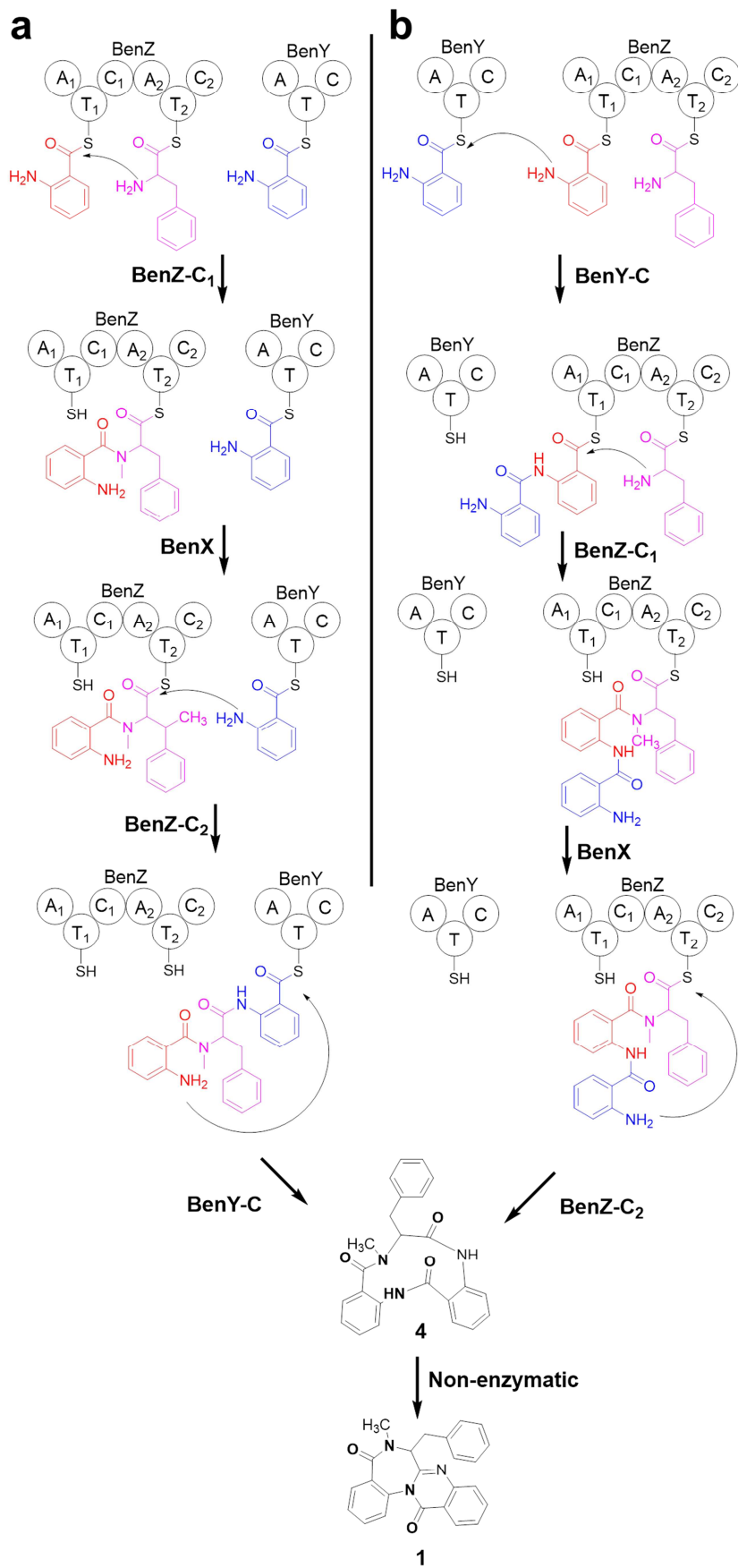
Supplementary Figure 7. Possible models of benzomalvin biosynthesis. Two potential models (**a** and **b**) of benzomalvin biosynthesis are shown, which fit the data presented in this study. **a**) The pathway begins with the loading of amino acid precursors onto the A domains of BenY and BenZ. BenY and the A_1 domain of BenZ are loaded with Anth, while the A_2 domain of BenZ is loaded with Phe. N-methylation of Phe by BenX may happen before loading of Phe onto BenZ, after loading of Phe, or after dipeptide formation. Condensation of Anth with the secondary amine of NmPhe or Phe is catalyzed by the C_1 domain of BenZ, forming a dipeptide intermediate, which is observed to accumulate in the *benY* deletant due to spontaneous hydrolysis. This is followed by *in trans* condensation of the Anth-NmPhe dipeptide with Anth bound to the T domain of BenY by the C_2 domain of BenZ to form the linear tripeptide Anth-NmPhe-Anth. Cyclization and release of the tripeptide is then catalyzed by the C_T domain of BenY and the resulting 11-member macrocyclic intermediate is expected to spontaneously collapse to form the benzodiazepine core, as previously reported for the anthranilate containing tripeptide secondary metabolite scaffold, asperlicin[15]. Alternatively, the model represented in **b)** is also possible based on the current evidence, where the observed accumulation of the Anth-NmPhe dipeptide would be an off-pathway product resulting from the absence of BenY activity in the *benY* deletant. In this pathway, the linear tripeptide Anth-Anth-NmPhe is formed attached to BenZ-T_2 and BenZ-C_2 acts as the terminal, cyclizing C domain to release the macrocyclic tripeptide. Sequence alignment is not able to predict which of the two C domains act as the terminal cyclizing C-domain, and future studies will seek to investigate the roles of the C domains of BenZ and BenY in order to determine which biosynthetic pathway occurs.

a LC-MS trace of m/z = 369.278 (± 5 ppm)

A. terreus

AtFAC9J20

AtFAC9J20-ΔsttA

30 34 38 42 46 50 54 58 62 66 70
Time (min)

b MS^1 of 369.278

369.2776 — Observed (M+H)$^+$

370.2817
371.2945

369.2788 — Simulated (C$_{25}$H$_{36}$O$_2$ +H)$^+$

370.2822
371.2855

369.0 370.0 371.0 372.0 373.0
m/z

c MS^2 of 369.278, m/z 50-115 Th

81.0700
83.0856
69.0702
67.0545
95.0855
109.101
97.1012

60 70 80 90 100 110
m/z

Supplementary Figure 8. Detection of sesterterpenoid product. (a) The ion with m/z = 369.2783 is present in extracts of *A. terreus* strains ATCC 20524, AtFAC9J20, but not in the AtFAC9J20-Δ*sttA* deletant. **(b)** The simulated formula C$_{25}$H$_{37}$O$_2$ matches the observed MS^1 spectrum within 3 ppm. **(c)** Analysis of the low-mass region (m/z 50 to 115) of the MS^2 spectrum of parent ion 369.278 confirms terpenoid structure, with terpenoid diagnostic fragments shown.

Fungal compounds matching:
C$_{25}$H$_{36}$O$_2$, 368.2715 Da

21-hydroxy-Ophiobolin G
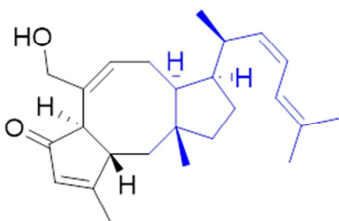
Ophiobolin V
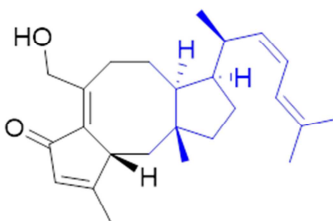
Variecolin
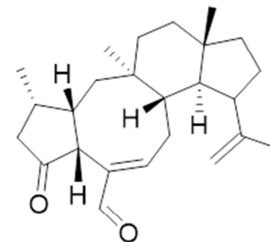
6-Epiophiobolin N
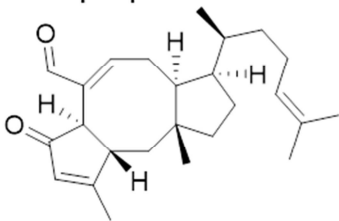
Ophiobolin U
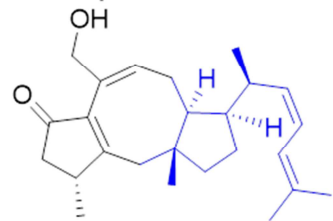
Supplementary Figure 9. Structures of sesterterpenoids. Structures of known fungal sesterterpenoids with the molecular formula of C$_{25}$H$_{36}$O$_2$, matching the unknown ion with m/z = 369.2710.
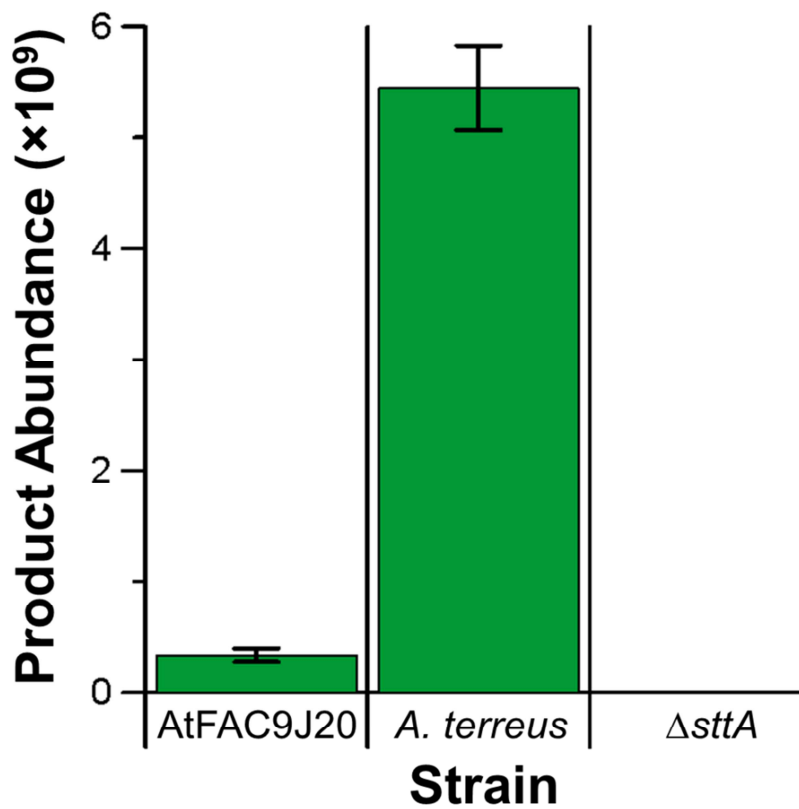
Supplementary Figure 10. Sesterterpenoid production by AtFAC9J20, *A. terreus* ATCC 20524, and AtFAC9J20-Δ*sttA*. The sesterterpenoid is produced by AtFAC9J20 at lower levels than by *A. terreus*. Deletant AtFAC9J20-Δ*sttA* eliminates production of the sesterterpenoid.
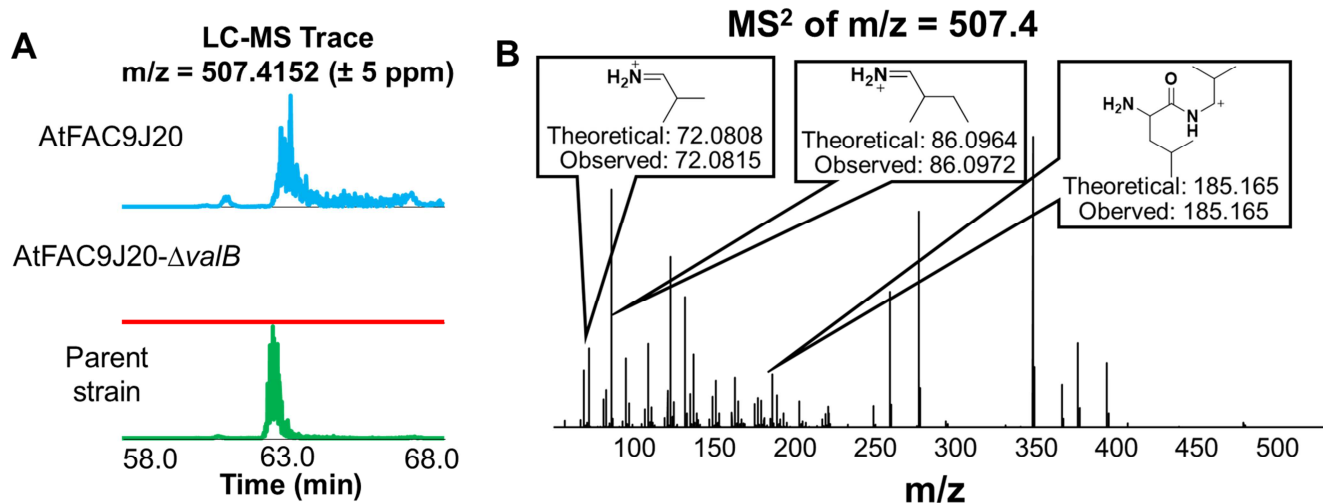
Supplementary Figure 11. Valactamide A produced by AtFAC9J20. (a) An ion with $m/z =$ 507.4152, corresponding to the newly discovered metabolite valactamide A, was detected in extracts from AtFAC9J20 and *A. terreus* strain ATCC 20542, but not AtFAC9J20-Δ*valB*. **(b)** The MS[2] spectrum reveals immonium ions consistent with Val (obs. m/z = 72.0815) and either Ile or Leu residues (obs. m/z = 86.0972). Also an ion characteristic of a dipeptide of Ile and/or Leu with Val was observed (obs. m/z = 185.165).
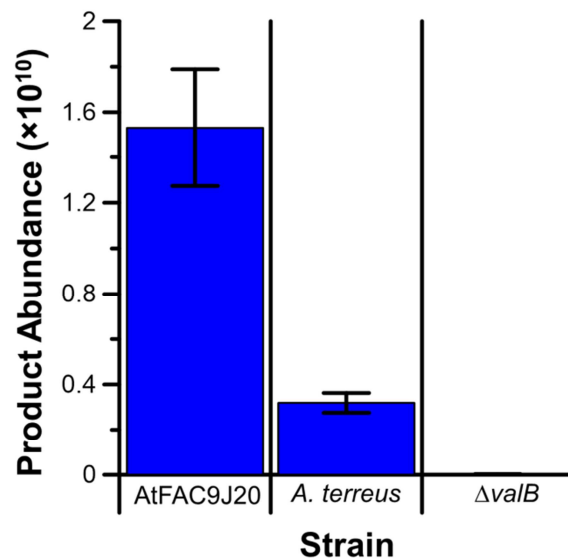
Supplementary Figure 12. **Valactamide A production by AtFAC9J20,** *A. terreus* **strain ATCC 20542, and AtFAC9J20-Δ*valB*.** Valactamide A production is increased in AtFAC9J20 relative to *A. terreus* and is abolished by the AtFAC9J20-Δ*valB* deletant.
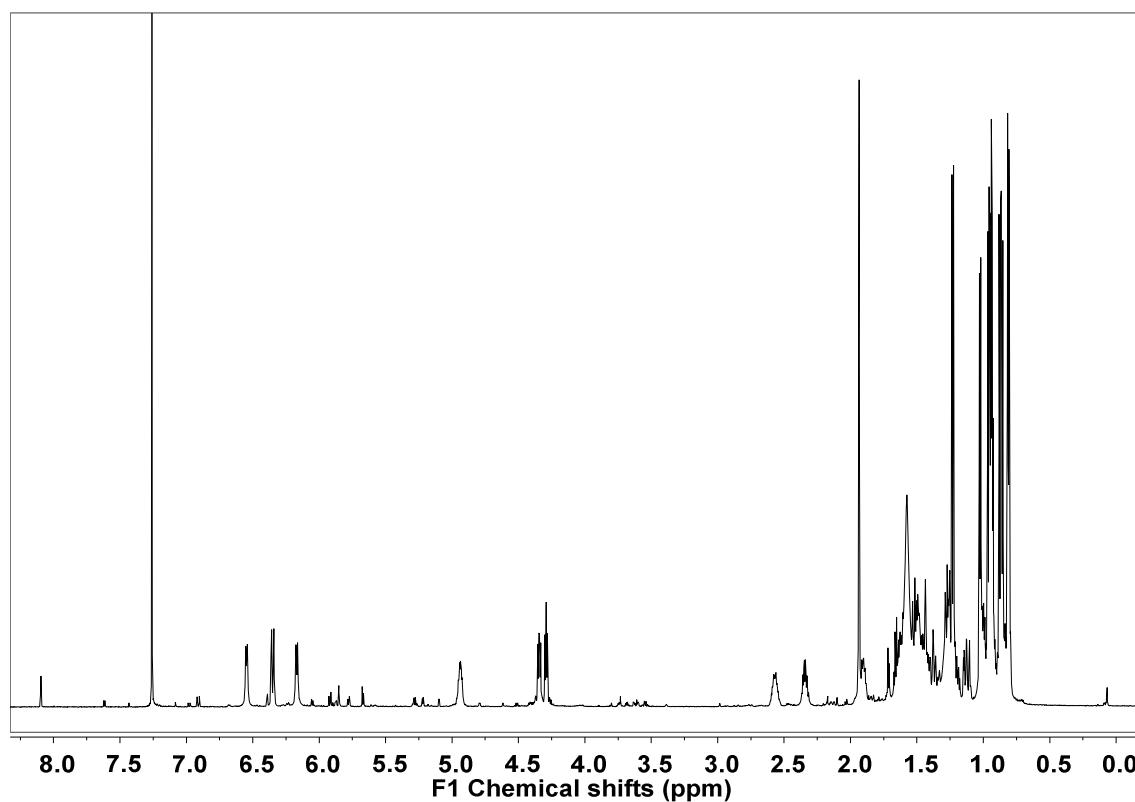
Supplementary Figure 13. ^{1}H NMR spectrum of valactamide A (CDCl₃, 600 MHz).
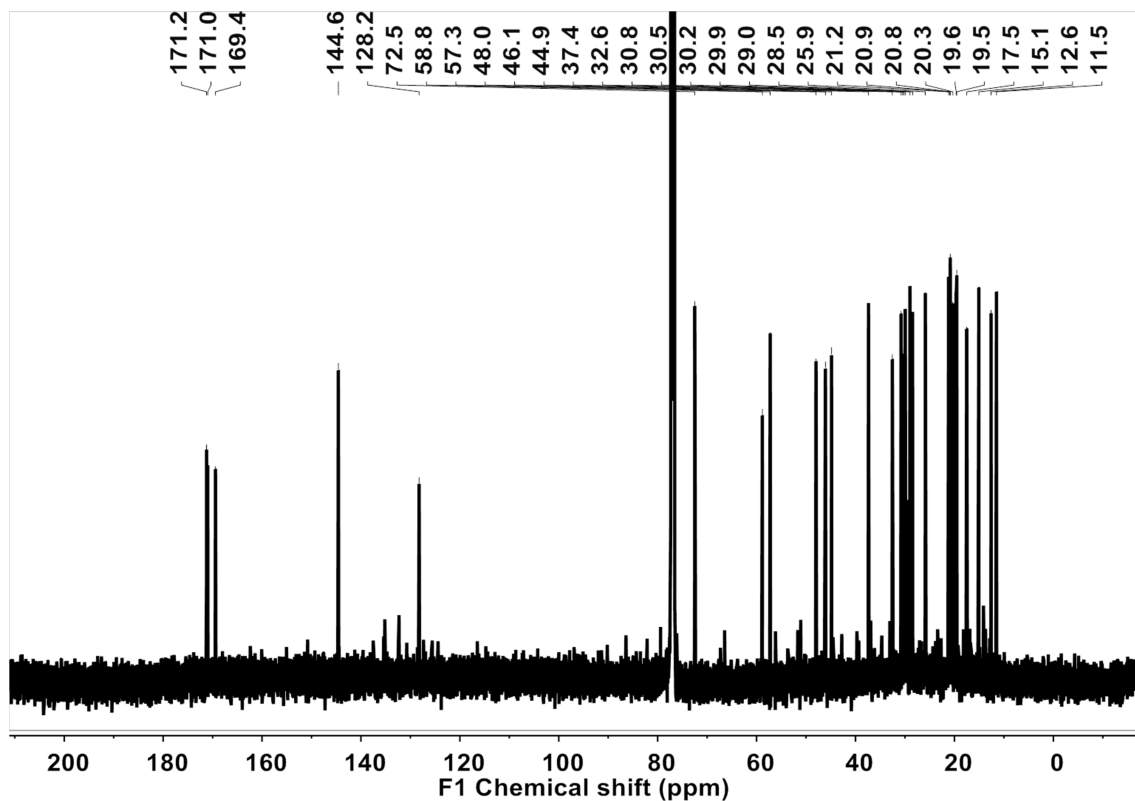
Supplementary Figure 14. ^{13}C NMR spectrum of valactamide A (CDCl$_3$, 126 MHz).
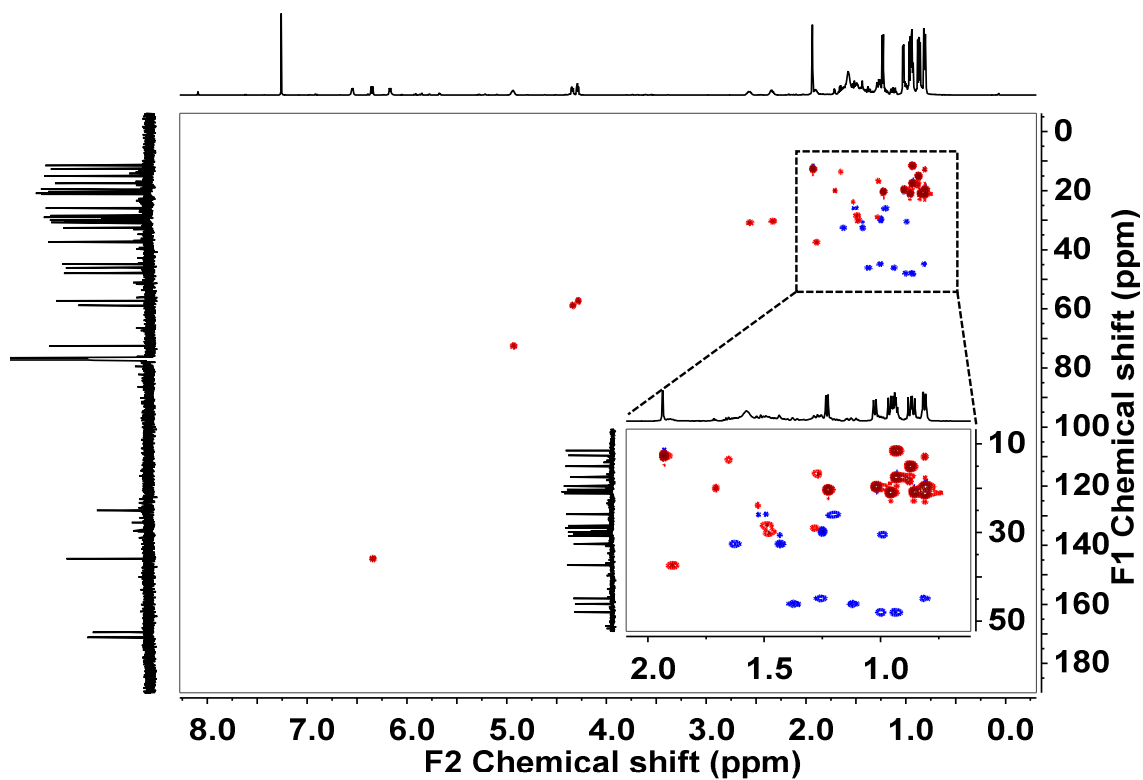
Supplementary Figure 15. HSQC spectrum of valactamide A (CDCl$_3$, 600 MHz).
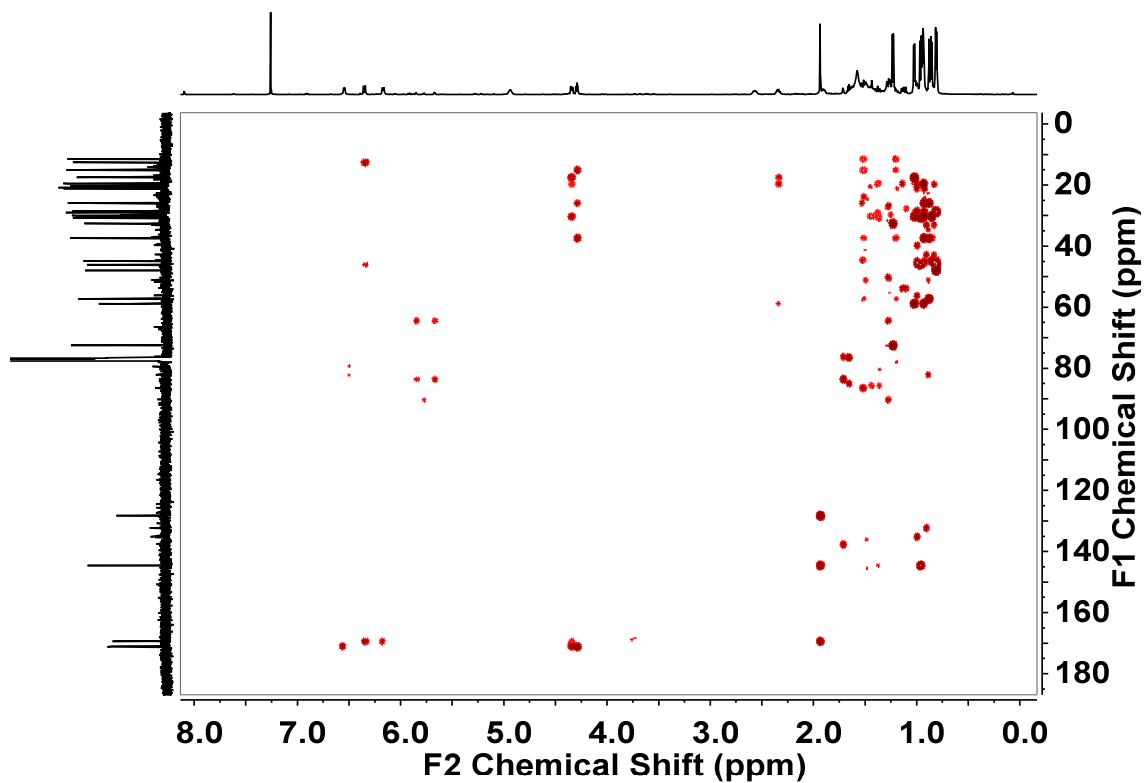
Supplementary Figure 16. HMBC spectrum of valactamide A (CDCl$_3$, 600 MHz).
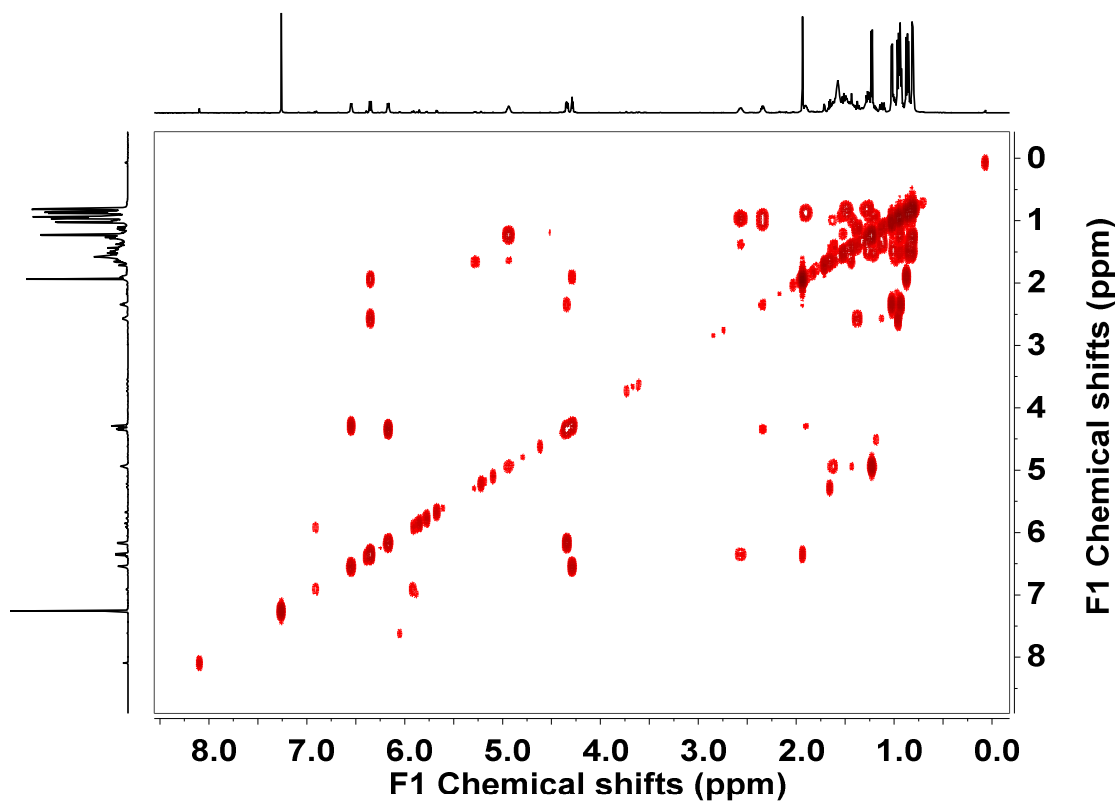
Supplementary Figure 17. gCOSY spectrum of valactamide A (CDCl$_3$, 600 MHz).
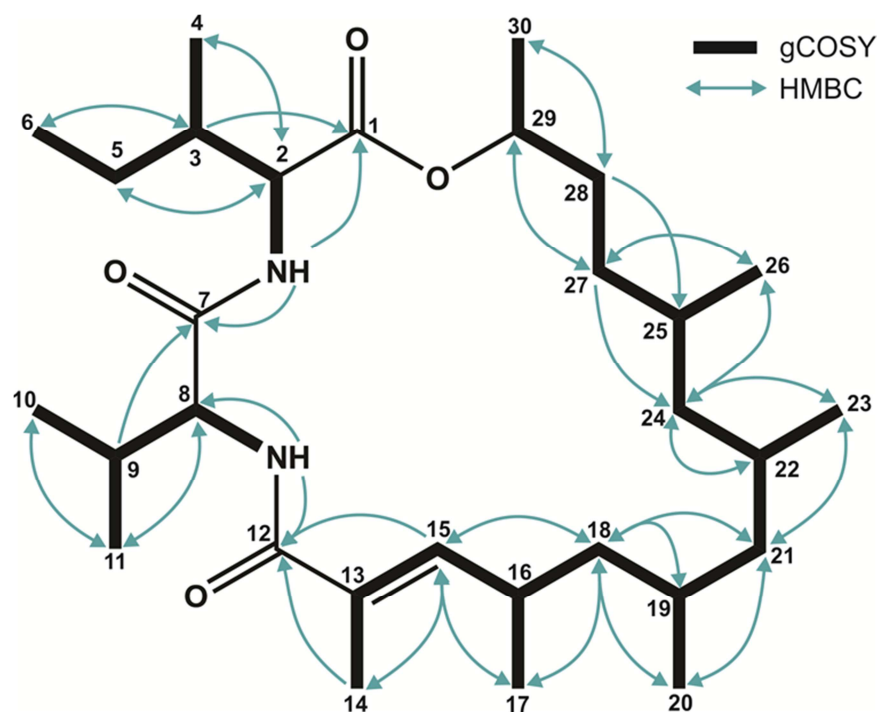
Supplementary Figure 18. **Main HMBC (blue) and gCOSY (bold bonds) correlations used for the structure elucidation of valactamide A.** COSY correlations are shown as thick lines and HMBC correlations are shown by blue arrows.
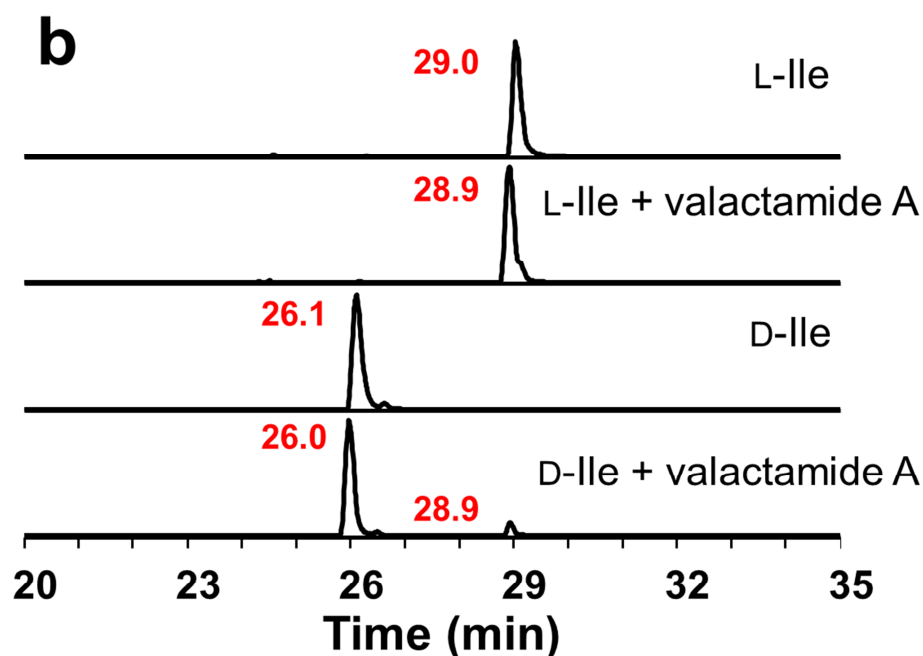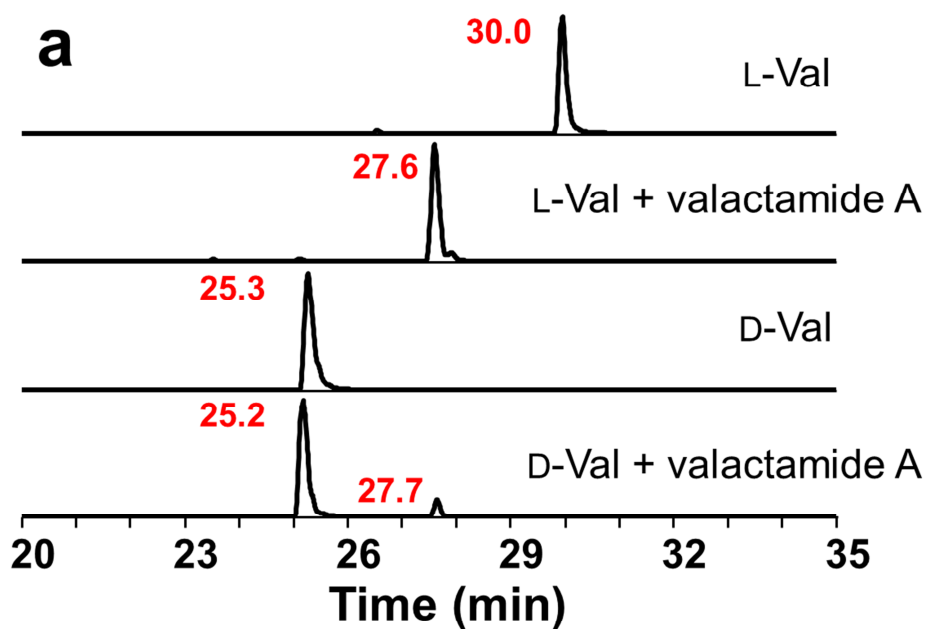
Supplementary Figure 19. Valactamide A contains L-Val and L-Ile. Marfey's reagent was used to derivatize free D and L amino acid standards and amino acids released by hydrolysis of valactamide A. Chromatographic retention times of each peak are shown in red. (**a**) Selected ion chromatogram of m/z = 412.183 ± 5 ppm, corresponding to derivatized valine. Coinjection of derivatized L-Val and valactamide A hydrolysate gives one peak at 27.6 min (2nd *from top*). Coinjection using D-Val gives two peaks, one for D-Val at 25.2 min and one for L-Val at 27.2 min (*bottom*). (**b**) Selected ion chromatogram of m/z = 426.198 ± 5 ppm, corresponding to derivatized isoleucine. Coinjection of L-Ile and valactamide A gives one peak at 28.9 min (2nd from top). Coinjection of D-Ile and valactamide A gives two peaks, one at 26.0 min for D-Ile and one at 28.9 min for L-Ile (bottom).

valactamide F valactamide E valactamide B valactamide C

valactamide A valactamide D valactamide G valactamide H
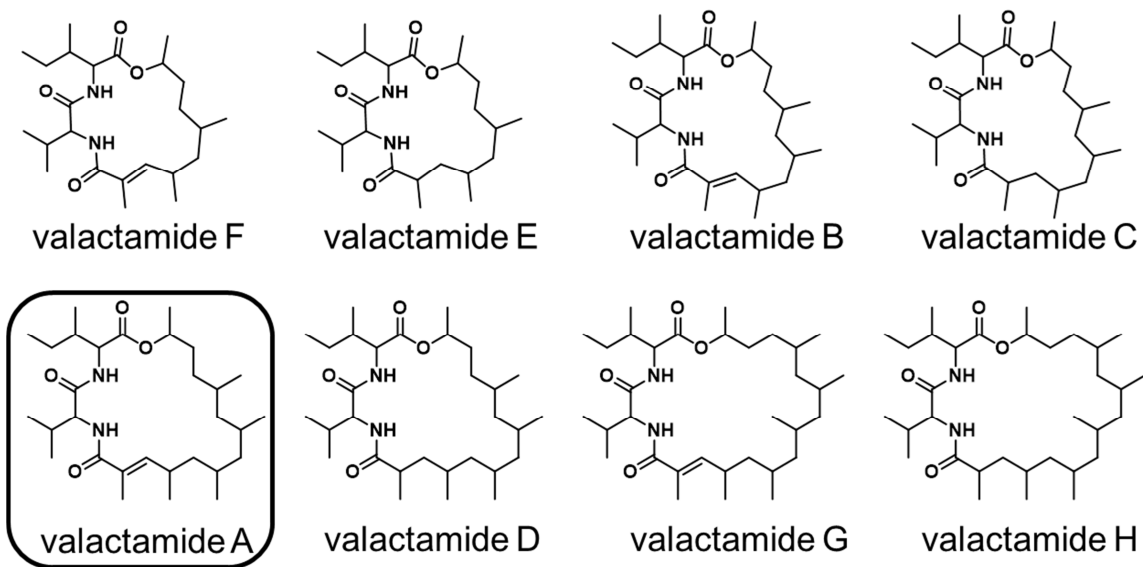
Supplementary Figure 20. **Valactamides A-H.** The family of valactamide metabolites proposed to be produced by the valactamide gene cluster is shown. The structure of valactamide A (shown in black box) was determined by NMR. The structures of the other compounds are proposed based on similarity of MS^1 and MS^2 data and molecular genetic evidence (see **Supplementary Fig. 21** and **22**).

Supplementary Figure 21. **Effect of *valB* deletion on valactamide metabolites.** Each proposed valactamide metabolite is eliminated by deletion of *valB.* The blue box denotes valactamide A.
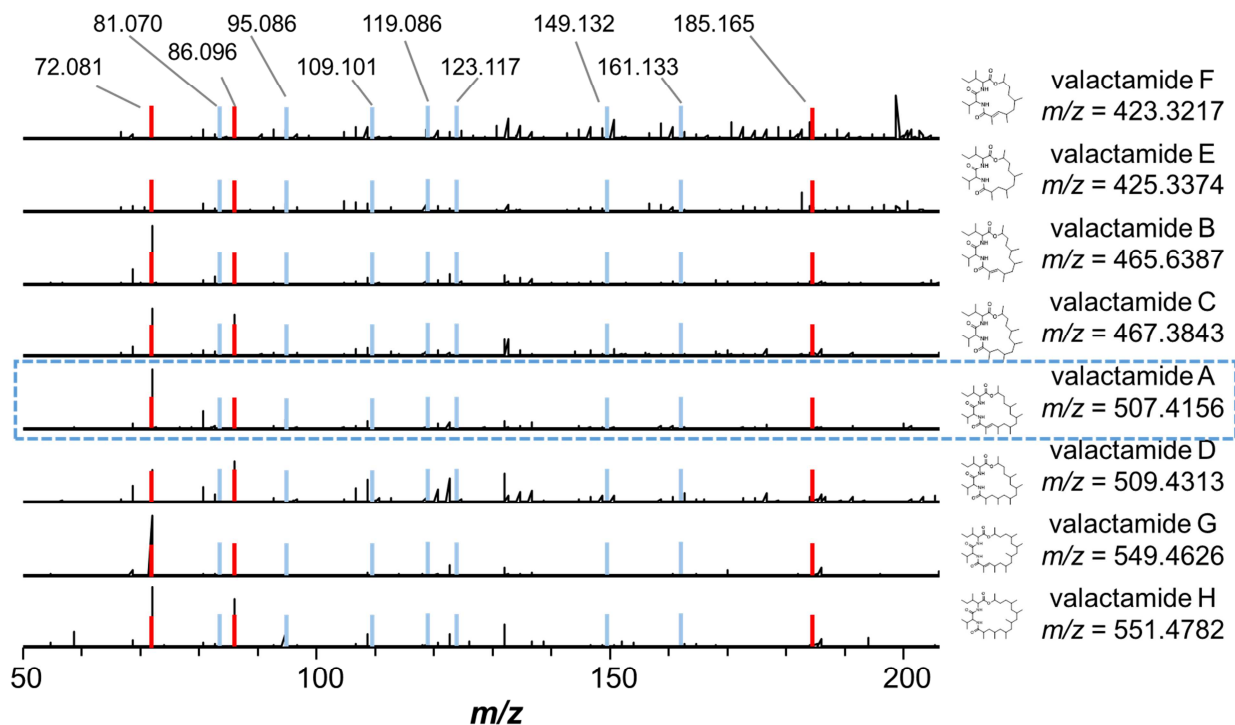
Supplementary Figure 22. MS^2 of the valactamide family. The 50 to 200 *m/z* region from MS^2 for valactamides A-H is shown. Selected fragments which are the same within <1 ppm across each family member are highlighted with sticks. Red sticks correspond to diagnostic fragment ions for Val, Ile, and the Val-Ile dipeptide (amino acid derived fragment structures shown in **Supplementary Figure 11**). Valactamide A is highlighted with a blue box.

```
x1 <- xcmsSet(method="centWave", ppm=3, peakwidth=c(20,100), snthresh=10, prefilter=c(5,10000),
mzCenterFun="wMean", integrate=1, mzdiff=0.001, fitgauss=FALSE, noise=1000, sleep=0,
verbose.columns=FALSE, nSlaves=4)
#Grouping
x2 <- group(x1, bw=30, minfrac=0.5, mzwid=0.01)
#wrting grouping output
dat <- groupval(x2, "medret", "into")
rownames(dat) <- groupnames(x2, mzdec=4, rtdec=0)
dat <- rbind(group=as.character(phenoData(x2)$class), dat)
write.csv(dat, file="groupedoutput.csv")
#retention time correction
x3 <-retcor(x2, family="s", plottype="m")
#regroup after retcor
x4 <- group(x3, bw=30, minfrac=0.5, mzwid=0.01)
#re-write file after retcor
dat2 <- groupval(x4, "medret", "into")
rownames(dat2) <- groupnames(x4, mzdec=4, rtdec=0)
dat2 <- rbind(group=as.character(phenoData(x4)$class), dat2)
write.csv(dat2, file="groupedoutput.csv")
# peakfilling
pkfill <-x4
pkfill@profinfo$step <- 0.001
pkfill2 <-fillPeaks(pkfill)
#load camera
library(CAMERA)
#running camera
x5 <-xsAnnotate(pkfill2)
x6 <-groupFWHM(x5, perfwhm=0.6)
x7 <-groupCorr(x6)
x8 <-findIsotopes(x7)
x9 <-findAdducts(x8, polarity="positive")
write.csv(getPeaklist(x9), file="resultCAMERA.csv")
```

Supplementary Figure 23. XCMS Commands. Commands used in R for feature detection and annotation of untargeted LC-MS data using XCMS and CAMERA.

Supplementary Table 1. AtFAC9J20 sequence comparison with genomic reference strain *A. terreus* NIH 2624. The strain actually used to generate AtFAC9J20 was ATCC20542.

AtFAC9J20	NIH 2624	Large Variations	Location
102,551 -102,722 (Telomere)	no data	NA	NA
66,920 - 102,550	no data	NA	NA
48,773 - 66,919	42,395 - 60,545	10bp duplication in this strain	intergenic
40,000 - 48,772	missing	(+)8,773 bp	intergenic
34,242 - 39,999	36,648 - 42,404		
missing	36,304 - 36,647	(-)344bp	intergenic
31,774 - 34,248	33,830 - 36,303	7bp duplication in the NIH strain	
missing	33,724 - 33,829	(-)106bp	intergenic
23,337 - 31,881	25,175 - 33,723	2bp duplication in this strain	intergenic
missing	24,806 - 25,174	(-)369bp	intergenic
23,066 - 23,338	24,805 - 24,541	265bp Inversion	intergenic
missing	24,445 - 24,540	(-)96bp	intergenic
15,815 - 23,065	17,183 - 24,444		
missing	15,332 - 17,182	(+)2bp and (-)1,851bp	intergenic
14,667 - 15,813	14,166 - 15,331		
13,926 -14,666	missing	(+)741bp and (-)8bp	A10 (14,101 - 14,925)
6854-13925	7072-14159	207 or 225bp duplication in the NIH strain	A11 (7,736 - 6,762)
1-7237	1-7248		

Supplementary Table 2. Summary of open reading frames on the FAC, AtFAC9J20.

Gene Name	*A. terreus* NIH2624 homolog	Start	Stop	Predicted domains (CDD)	Deletant Made?
benX	None	91734	90495	SAM binding	Yes
benY	None	86362	89695	NRPS: A-T-C	Yes
benZ	None	84859	77576	NRPS: A-T-C-A-T-C	Yes
valB	ATEG_03576	58960	66874	NRPS: C-A-T-C-A-T-C	Yes
valA	ATEG_03575 and ATEG_03574[a]	57999	50187	PKS: KS-AT-DH-Mtase-ER-KR-PP	Yes
AtFAC9J20_6	ATEG_03573	37549	36224	TRI7-like toxin biosynthesis protein	No
AtFAC9J20_7	ATEG_03571	28172	27526	None	No
AtFAC9J20_8	None	33718	34061	None	No
AtFAC9J20_9	ATEG_03570	25661	25386	BBE domain	No
AtFAC9J20_10	ATEG_03569	24016	24968	acetyltransferase	No
sttA	ATEG_03568	20124	22740	Isoprenoid C1 superfamily	Yes
sttB	ATEG_03567	19694	17673	p450 superfamily	Yes
AtFAC9J20_13	None	14107	14931	None	Yes
AtFAC9J20_14	ATEG_03566	10961	12219	SUR7 – transmembrane	No
AtFAC9J20_15	None	10154	9035	None	No
AtFAC9J20_16	None	7254	6768	None	No
AtFAC9J20_17	ATEG_03564	5109	6241	Tyrosinase superfamily	No
AtFAC9J20_18	ATEG_03563	3898	1120	NRPS: A-T-TE	No
AtFAC9J20_19	None	153	611	None	No

[a] Previously mis-annotated as two genes, correctly annotated here as one gene, *valA*

Supplementary Table 3. AtFAC9J20 Deletants.

Deletant Name	Genes Deleted	Benzomalvin A/D Detected?	Terpenoid Detected?	Valactamide A Detected?
null	*benX, benY, benZ, valB, valA*	No	Yes	No
ΔbenX	*benX*	Yes	Yes	Yes
ΔbenY	*benY*	No	Yes	Yes
Δbenz	*benZ*	No	Yes	Yes
ΔvalB	NRPS *valB*	Yes	Yes	No
ΔvalA	PKS *valA*	Yes	Yes	No
ΔsttA	Terpene synthase *sttA*	Yes	No	Yes
ΔsttB	Cytochrome P450 *sttB*	Yes	No	Yes

Supplementary Table 4. Extracted Adenylation Domain Signatures of NRPS genes encoded by AtFAC9J20.

Domain	Extracted A-domain Signature	Predicted Amino Acid
BenY-**A**TC	GMFIVGLGMK	Anth
BenZ-**A**TCATC	GINFIGAGTK	Anth
BenZ-ATC**A**TC	DMNVMGGVTK	Phe, NmPhe, Tyr
ValB- C**A**TCATC	DALLLGITIK	Branched Aliphatic
ValB- CATC**A**TC	DLGFSGPIIK	Branched Aliphatic

Supplementary Table 5. Annotated ^{1}H and ^{13}C chemical shifts for valactamide A (CDCl$_3$, ^{a}600 MHz and ^{b}150 MHz).

Position	$^a\delta_C$, mult.	$^b\delta_H$, mult. (J in Hz)
1	171.2, C	
2	57.3, CH	4.28, dd (6.5, 6.2)
3	37.4, CH	1.90, m
4	15.1, CH$_3$	0.87, d (6.9)
5	25.9, CH$_2$	1.20, m; 1.51, m
6	11.5, CH$_3$	0.94, dd (7.4, 7.3)
7	170.9, C	
8	58.8, CH	4.33, dd (7.6, 5.0)
9	30.2, CH	2.33, ddd (6.9, 6.8, 5.00
10	17.5, CH$_3$	0.93, d (6.8)
11	19.6, CH$_3$	1.02, d (6.9)
12	169.4, C	
13	128.2, C	
14	12.6, CH$_3$	1.93, d (0.9)
15	144.6, CH	6.34, dq (10.2, 0.9)
16	30.8, CH	2.57, dddd (10.6, 10.2, 6.7, 2.6)
17	20.9, CH$_3$	0.96, d (6.7)
18	46.1, CH$_2$	1.12, ddd (13.0, 10.6, 3.4); 1.37, ddd (13.0, 10.9, 2.6)
19	29.0, CH	1.28, m
20	19.5, CH$_3$	0.80, d (6.3)
21	48.0, CH$_2$	0.94, m; 1.00, ddd (13.4, 7.6, 5.1)
22	28.4, CH	1.49, m
23	21.2, CH$_3$	0.81, d (6.5)
24	44.8, CH$_2$	0.82, m; 1.26, ddd (13.6, 9.4, 3.3)
25	29.9, CH	1.48, m
26	20.8, CH$_3$	0.86, d (6.6)
27	30.5, CH$_2$	0.99, m; 1.44, m
28	32.6, CH$_2$	1.43, m; 1.62, m

29	72.5, CH	4.93, ddd (7.0, 6.4, 4.4)
30	20.3, CH$_3$	1.23, d (6.4)
HN-I		6.56, d (6.2)
HN-V		6.18, d (7.5)

Supplementary Table 6. Relative Abundance of valactamide metabolites.

Metabolite	Molecular Formula	Exact Mass	# ketides	Abundance
valactamide A	C$_{30}$H$_{54}$N$_2$O$_4$	506.4084	7	94 %
valactamide B	C$_{27}$H$_{48}$N$_2$O$_4$	464.3614	6	3.0%
valactamide C	C$_{27}$H$_{50}$N$_2$O$_4$	466.3771	6	1.4%
valactamide D	C$_{30}$H$_{56}$N$_2$O$_4$	508.4240	7	0.94%
valactamide E	C$_{24}$H$_{44}$N$_2$O$_4$	424.3301	5	0.099%
valactamide F	C$_{24}$H$_{42}$N$_2$O$_4$	422.3145	5	0.027%
valactamide G	C$_{33}$H$_{60}$N$_2$O$_4$	548.4553	8	0.018%
valactamide H	C$_{33}$H$_{62}$N$_2$O$_4$	550.4710	8	0.00043%

Supplementary Table 7. FAC clones containing 56 full-length BGCs.

Fungal species	Cluster No.	FAC Name	FAC Location (Chr: position)	FAC Size (bp)
A. *aculeatus*	9	AaFAC1K8	7:483343-596987	113,644
A. *aculeatus*	19	AaFAC1J4	25:37-110883	110,846
A. *aculeatus*	21_22	AaFAC1D8	1:78626-186378	107,752
A. *aculeatus*	1_25	AaFAC2P10	8:211037-336059	125,022
A. *aculeatus*	30	AaFAC6A16	16:18608-112106	93,498
A. *aculeatus*	32	AaFAC8A16	1:2370038-2483583	113,545
A. *aculeatus*	34	AaFAC10A5	4:1981-104478	102,497
A. *aculeatus*	35	AaFAC10D7	5:13457-135109	121,652
A. *aculeatus*	39	AaFAC1L21	18:355848-455803	99,955
A. *aculeatus*	40	AaFAC3D12	22:235518-338083	102,565
A. *aculeatus*	41	AaFAC2P8	4:870643-1011680	141,037
A. *terreus*	7	AtFAC8P6	2:37278-154428	117,150
A. *terreus*	8	AtFAC5P8	2:175456-236080	60,624
A. *terreus*	10	AtFAC6H11	3:7234-82997	75,763
A. *terreus*	12	AtFAC8G17	3:1480896-1564240	83,344
A. *terreus*	20	AtFAC9J20	4:2151734-the missing telomeric end	102,722
A. *terreus*	29	AtFAC3E2	8:1151835-1242603	95,425
A. *terreus*	31	AtFAC9M17	8:1557569-1681014	123,445
A. *terreus*	35	AtFAC9B9	10:388955-491178	102,223
A. *terreus*	36	AtFAC9H19	10:590841-687211	96,370
A. *terreus*	37	AtFAC4N23	10:1162818-1274448	111,630
A. *terreus*	38	AtFAC7O19	10:1344783-1469927	125,144
A. *terreus*	39	AtFAC5N15	10:1464244-1565353	101,109
A. *terreus*	40	AtFAC5L7	11:189737-313873	124,136
A. *terreus*	46	AtFAC5B9	13:398169-522253	124,084
A. *terreus*	48	AtFAC5E10	14: 6368-97532	91,164
A. *terreus*	49	AtFAC6I22	14:10646-148251	137,605
A. *terreus*	51	AtFAC7M4	14:179105-281612	102,507
A. *terreus*	53	AtFAC6N3	15:324192-439862	115,670
A. *wentii*	1	AwFAC4O2	2:748867-861001	112,134
A. *wentii*	2	AwFAC1K8	4:96694-210549	113,855
A. *wentii*	3	AwFAC2F10	10:572788-655030	82,242
A. *wentii*	4	AwFAC4E11	1:2038648-2143968	105,320
A. *wentii*	5	AwFAC4L5	2:1829642-1920197	90,555
A. *wentii*	6	AwFAC2P3	4:186740-312513	125,773
A. *wentii*	7	AwFAC4I20	4:3165620-3255977	90,357
A. *wentii*	8	AwFAC4D17	5:2466262-2562334	96,072
A. *wentii*	10	AwFAC4D8	7:2270034-2350260	80,226
A. *wentii*	11	AwFAC3M17	10:471140-562395	91,255
A. *wentii*	13	AwFAC3D18	7:2189142-2288310	99,168
A. *wentii*	19	AwFAC1H17	4:1-87455	87,454
A. *wentii*	20	AwFAC2K17	1:3915914-4008958	93,044

A. *wentii*	25	AwFAC3E24	8:318322-407365	89,043
A. *wentii*	26	AwFAC3L2	9:672348-770093	97,745
A. *wentii*	27	AwFAC3B4	9:1507369-1620289	112,920
A. *wentii*	29	AwFAC4J7	2:84403-199331	114,928
A. *wentii*	30	AwFAC2F2	2:2776606-2876645	100,039
A. *wentii*	31	AwFAC3D3	3:179174-288015	108,841
A. *wentii*	32	AwFAC1B1	8:2015243-2105484	90,241
A. *wentii*	34	AwFAC1C5	1:4290614-4374135	83,521
A. *wentii*	38	AwFAC4H11	7:1563096-1662018	98,922
A. *wentii*	39	AwFAC4F15	10:118366-207802	89,436
A. *wentii*	40	AwFAC3H22	1:2934184-3041025	106,841
A. *wentii*	43	AwFAC1J2	10:1-87900	87,899
A. *wentii*	44	AwFAC4O4	2:2094226-2208140	113,914
A. *wentii*	47	AwFAC4C1	4:1016467-1115739	99,272

Supplementary Table 8. PCR primers used for FAC engineering in this study.

Name	Sequence
ΔAtFAC9J20Ben-lpt-clusters (AtFAC9J20-null**)**	
Pben-KF	TCTTTAAGACCAGCTTGAGCAGTACCTTTAGAATGCAACGGCTTTTAACCCCTGTTGACAATTAATCATCGGCA
Pben-KR	ACTAAAGTTTGGAGCTTGTAAGTTAATTTTTGCGTTGTTACTATTGCCTCTCAGCACTGTCCTGCTCCTT
ΔAtFAC9J20Sesterterpenoid-cluster (AtFAC9J20-null2**)**	
Poph-KF	GTGGTGGCAATCCTATCCCCGATCCTGCAGGCTCTGGAAGCTCGGGATCCCCTGTTGACAATTAATCATCGGCA
Poph-KR	TTAGGAGGCTTATGCTTCTTACCGGATGTTAATAAGACTGCTTTGTTAGCTCAGCACTGTCCTGCTCCTT
ΔAtFAC9J20BenX	
Mtase-KF	ATGTCCGCGGTCGAGATTCCTCACCCCTCCGGCTGCCGAGTATACGATATCCTGTTGACAATTAATCATCGGCA
Mtase-KR	TCACCGATGTGCGACACTTACTCTCTCGTTCCCGGACGTGAACGTCTGGGTCAGCACTGTCCTGCTCCTT
ΔAtFAC9J20BenY	
NRPS1-KF	ATGGTCTCACGTAAACCAGCACTGGCAGTCAAAGAATTGGGATGCATCAGCCTGTTGACAATTAATCATCGGCA
NRPS1-KR	CTCATGGGGCCATTATGTTGACCAGAGCTTGTTCCGGGTGCCTCGCGAGCTCAGCACTGTCCTGCTCCTT
ΔAtFAC9J20BenZ	
NRPS2-KF	TCACACTTTGATCTTGTCCAACAGCTCTCCAGGCTGACTAGCAAACGCCGCCTGTTGACAATTAATCATCGGCA
NRPS2-KR	CATGACAAGGATAGGCTTCCAGCAGCATATCACTGGGGAGGATACTGGGATCAGCACTGTCCTGCTCCTT

ΔAtFAC9J20BenY-C-domain	
At20BenYCkan-F	GTGAGCTCTGTCTGTCGCATCTTCGGGAACAGACATCGTTGCTGGACTCTCGACCTGCAGCCTGTTGACAA
At20BenYCkan-R	GAACGCGACCCCATACGATCAATCAAAGCACCCTTCCAATCACCATCTCAGTCGAGGCTGATCAGCGA
ΔAtFAC9J20BenZ-C-domain	
At20BenZCkan-F	ATGATACATTCTGGGTGGTGGATGTACCCTCCTCCGGAAGAGATAGATCACGACCTGCAGCCTGTTGACAA
At20BenZCkan-R	TTGACGGTGAGCTCCGTGCAGTGGCAACGCGCGAGCTAGTCAGCCGCGGCGTCGAGGCTGATCAGCGA
ΔAtFAC9J20valB	
NRPS3-KF	ATGGCGGACGGAGCTGACTATACCCAACGCAATATCCATTCTCTCGACTCCCTGTTGACAATTAATCATCGGCA
NRPS3-KR	TCATGATGCTGCTTCGATGATGAATCTGCCAGTGCCGGCGTTGTTATGCGTCAGCACTGTCCTGCTCCTT
ΔAtFAC9J20valA	
PKS-KF	ATGAGCCTTCCAGCACAGTCCCCAATTGCCGTCGTGGGCGTTGCATACAGCCTGTTGACAATTAATCATCGGCA
PKS-KR	TCAAGAGACGCATGTTTCCTTCCCCTTTAACCCTGAGCCACGATCCGACTTCAGCACTGTCCTGCTCCTT
ΔAtFAC9J20-ATEG_03569	
20t03569-kF	CTCTTCTCCCGCTTAGAGCTTATACCAAGCACTGTCTGTTCCTGCCCAAACGACCTGCAGCCTGTTGA
20t03569-kR	GAAACCTTCCAATACAGTATGGGTGATCAAACAACTGTGCATTCTATATAGTCGAGGCTGATCAGCGA
ΔAtFAC9J20-ATEG_03568	

20DMAT-kF	TCAGTACCATAACAAGGTGATAACATTTTCAATTTGGCTGGCTCGTCAAACGACCTGCAGCCTGTTGA
20DMAT-kR	CCTACATTAAGAACTAACAAACACATGCACCAAAATATTACCACAGCAGAGTCGAGGCTGATCAGCGA
ΔAtFAC9J20-ATEG_03567	
20DMAT2-kF	GTTGAAGCACAACCTTCGTACACCTGCCAATAGCATGATAATCGTCTCCTCGACCTGCAGCCTGTTGA
20DMAT2-kR	AGACATACGCATATGTCCTGCCGGAACGATACCTGGAGAAAATCAAGAACGTCGAGGCTGATCAGCGA
ΔAtFAC9J20-ATEG_03573	
At20-71Kan-F	ATGAGCTTATTGCATCCACTTTTAGTGCAGCTGATTGCTTTGGCACTTCCCGACCTGCAGCCTGTTGACAA
At20-71Kan-R	TCAGATGTTTATACCAATGTGACGCCAAATAAGCAGTGTTCCTGTCGCCAGTCGAGGCTGATCAGCGA
ΔAtFAC36-9H19-ATEG_07067	
At36-7kan-F	AGTTTATCGCCTCGTCCGATTTCCTGTTTGCGGCCTACACTCCACATATACGACCTGCAGCCTGTTGACAA
At36-7kan-R	CATATAATATGGCACTTAATAAATTCTAGAGTACAGGCTGTCTGTCAGGCGTCGAGGCTGATCAGCGA
ΔAtFAC38-7O19-ATEG_07358	
At38NRPS-kF	CTCATAATCGGAAGTATCATTGCTTGCATCTCAGCCACTAGCTCGTCTATCGACCTGCAGCCTGTTGA
At38NRPS-kR	GATTCATCAGAATACCTCTTTCGCCCAGCTCTCATTGTCGCGAAATCAATGTCGAGGCTGATCAGCGA
ΔAtFAC39-5N15-ATEG_07380	
At39-80kan-F	CTGTTGCAAGAAACCTTGACCCGTCTTGAAGTGTTCTGAGTCTACTCACCCGACCTGCAGCCTGTTGACAA

At39-80kan-R	ATGTCAATTGGGAGCCACGAGAAGGACTGCCACTTCGTCAGCTGCGTTCCGTCGAGGCTGATCAGCGA
ΔAaFAC30-6A16-Aacu16872_046595	
Aa30NRPSkan-F	GACTAAAGATTCAAGGATTGAGGGAAATAGACTCATAAAACTCAGTTGTCCGACCTGCAGCCTGTTGACAA
Aa30NRPSkan-R	CCTTCATATATATCGTATCATCCTGAGGTTCTATGACCTCTGTGGGGCCTGTCGAGGCTGATCAGCGA
ΔAaFAC35-10D7-Aacu16872_51108	
Aa35PKSkan-F	ACTATCCTCATATCCAACAGTGCCATTCTGGTTAAAGAGATAATCTCCACCGACCTGCAGCCTGTTGACAA
Aa35PKSkan-R	GATCGAGCATTGGATTGTTTACAAACACAGCATAGAGGTAAGATAATAGAGTCGAGGCTGATCAGCGA
ΔAaFAC39-1L21-Aacu16872_054820	
Aa39PKSkan-F	GTCAGGATGACCACCTATCGGGAACATTCAGTCCTACCGCAGTATGTGGCCGACCTGCAGCCTGTTGACAA
Aa39PKSkan-R	AACTCCAAAGCACATCCCGCGCAGCTTCATGCCCAACCTTAACAACCAACGTCGAGGCTGATCAGCGA
ΔAaFAC41-2P8-Aacu16872_058515	
Aa41NRPSkab-F	TCAAAACCGAACGAAATAGCTTGAGATCTTATTTCAGACCGACATCGAAACGACCTGCAGCCTGTTGACAA
Aa41NRPSkab-R	CAAACCCCATCCGTTTCCCCGCCCCATTCCGCTATCAGCCCTAAGCCGCCGTCGAGGCTGATCAGCGA
ΔAwFAC2-1K8-Aspwe1_0027400	
Aw2-400kan-F	GTTTCCCTTTTTTTCCAACATTAACCAACTTTCTGACCAATAACACCAATCGACCTGCAGCCTGTTGACAA
Aw2-400kan-R	TATTCAATGTGTCATCGTGGATTGTTCCTGGTTACTTCATTCAGATGCATGTCGAGGCTGATCAGCGA

ΔAwFAC4-4E11-Aspwe1_0034272	
Aw4-72kan-F	TCTAGTGGATCACATGTACCAGCTGAACCTGAAGCTGAATGCGGTCCAGGCGACCTGCAGCCTGTTGACAA
Aw4-72kan-R	CCCCAACATGAAAACAATCTAGCTGTAGTACTCCTCCATCCAAGACCTCAGTCGAGGCTGATCAGCGA
ΔAwFAC8-4D17-Aspwe1_0042597	
Aw8-97kan-F	ACGTCAAGTGCGATACAAACAAGAGCAAAACTAACGACAGCGGAATCATCCGACCTGCAGCCTGTTGACAA
Aw8-97kan-R	CATCGCATAAGACATTATCCATAAATACTACCAACTTGATACCAATTCACGTCGAGGCTGATCAGCGA
ΔAwFAC10-4D8-Aspwe1_0044725	
Aw10-25kan-F	GCAGCCCCTGCGTACTCTGTACGCCGCAGATTCACCCCAAAGCACGCAAGCGACCTGCAGCCTGTTGACAA
Aw10-25kan-R	GAATAGCGCTTTGCAAACACTTTCAACAGAACTGTCAGTGCTACACCCCAGTCGAGGCTGATCAGCGA
ΔAwFAC19-1H17-Aspwe1_0085322	
Aw19-22kan-F	ACGATCTCTCTCTCCTGCAGTCCAAGTATCTGCCTGAAGCAGGGATCAAACGACCTGCAGCCTGTTGACAA
Aw19-22kan-R	GCTTGATATCTGAAGATCCGTTGAAACAGACATCCAACGTCTGGCGGAACGTCGAGGCTGATCAGCGA
ΔAwFAC27-3B4-Aspwe1_0121409	
Aw27-09kan-F	GCTCGTATTTGCTATTATGTAACAGTTGCTAAAGCTCTTGCTCCCTTGTACGACCTGCAGCCTGTTGACAA
Aw27-09kan-R	GTATTGTACACAGGTATCCTTAAACAATAGTAACACAAACAGACGATCCAGTCGAGGCTGATCAGCGA

ΔAwFAC31-3D3-Aspwe1_0151732	
Aw31-32kan-F	TGGGCATCGATTCAATTGCTTGATTGCAAAAGCTTGTGGACGCAAGAACTCGACCTGCAGCCTGTTGACAA
Aw31-32kan-R	ATGCATTCAAGATAGAGTCCTTGTCCAAACTCAACTCGACCGTGTTGATCGTCGAGGCTGATCAGCGA
ΔAwFAC32-1B1-Aspwe1_0163793	
Aw32-93kan-F	TAGCACGGGTTTTTGTTGATCTCTGTGTCTGGCTGTATAATATTCATAGTCGACCTGCAGCCTGTTGACAA
Aw32-93kan-R	ACGAATCAATATATACAAGGTCAAGAGGCAGGCCTGCCATCTTATACGTTGTCGAGGCTGATCAGCGA
ΔAwFAC43-1J2-Aspwe1_0294248	
Aw43-48kan-F	ATGCCATTCTTGCGGTGCACAACACCGGCTGTAGTCCGGCTTTAATAGCCCGACCTGCAGCCTGTTGACAA
Aw43-48kan-R	TTCGGTGGCTTTGAGGGAGTCAACTGTGACATGTTAGCATACATCATATGGTCGAGGCTGATCAGCGA