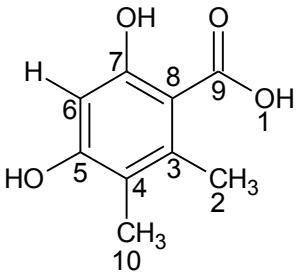
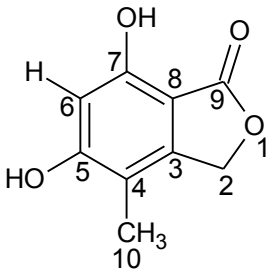
Supplementary Table S1. Top BLAST hits for *P. brevicompactum* MPA gene cluster in *Phaeosphaeria nodorum* and *Talaromyces stipitatus*.

	P. nodorum SN15 (taxid:321614)				*T. stipitatus* ATCC 10500 (taxid:441959)			
Query (*P. brevicompactum*)	Protein ID (top BLAST hit)	BLASTP E-value	BLASTP Identity	Identity (ClustalW)	Protein ID (top BLAST hit)	BLASTP E-value	BLASTP Identity	Identity (ClustalW)
MpaA	SNOG_05304	7.00E-17	30%	17.38%	gi|242821326	1.00E-16	20%	17.12%
MpaB	SNOG_14971	2.00E-89	39%	33.72%	gi|242779340	2.00E-120	50%	42.06%
MpaC (PKS)	SNOG_06682	0	39%	36.90%	gi|242760313	0.00E+00	49%	42.50%
MpaD	SNOG_06679	2.00E-109	47%	31.22%	gi|242760308	1.00E-113	55%	53.13%
MpaE	SNOG_06681	1.00E-81	50%	45.90%	gi|242760293	3.00E-87	50%	47.89%
MpaF (IMPDH-B)	SNOG_00421	0.00E+00	73%	70.45%	gi|242786825	0.00E+00	79%	76.64%
MpaG	SNOG_08626	2.00E-60	31%	30.35%	gi|242781136	0.00E+00	63%	61.85%
MpaH	SNOG_15010	5.00E-50	37%	26.05%	gi|242768952	2.00E-84	39%	36.59%

1. MpaD and MpaE (shown in green) were together identified as a single fusion protein in the current study. BLAST search for the fusion protein MpaDE did not find any good hits. 2. BLASTP search was performed on the NCBI BLAST server (http://blast.ncbi.nlm.nih.gov/Blast.cgi, 16th March 2012) against the two genomes of interest and with the default settings. 4. For MpaC/D/E, amino acid sequence based on the coding region was used for the search. For the rest, protein sequences available in the NCBI gene bank were used.

Supplementary Table S2: ^{1}H and ^{13}C NMR data (extracted from HMBC experiment) for 5-methylorsellinic acid and 5,7-dihydroxy-4-methylphtalide[a]





	5-methylorsellinic acid		5,7-dihydroxy-4-methylphtalide	
Position	δ_C (ppm)	δ_H mult	δ_C (ppm)	δ_H mult
2	18.6	2.28 (3H, s)	67.5	5.11 (3H, s)
3	138.9		149.0	
4	115.1		108.5	
5	159.2		162.1	
6	100.2	6.23 (1H, s)	102.0	6.41 (1H, s)
7	nd		155.6	
8	108.9		nd	
9	172.0		168.6	
10	12.1	1.93 (3H, s)	10.1	1.91 (3H, s)
5-OH		9.81 (1H, s)		10.32 (1H, s)
7-OH		10.92 (1H, bs)		10.18 (1H, s)

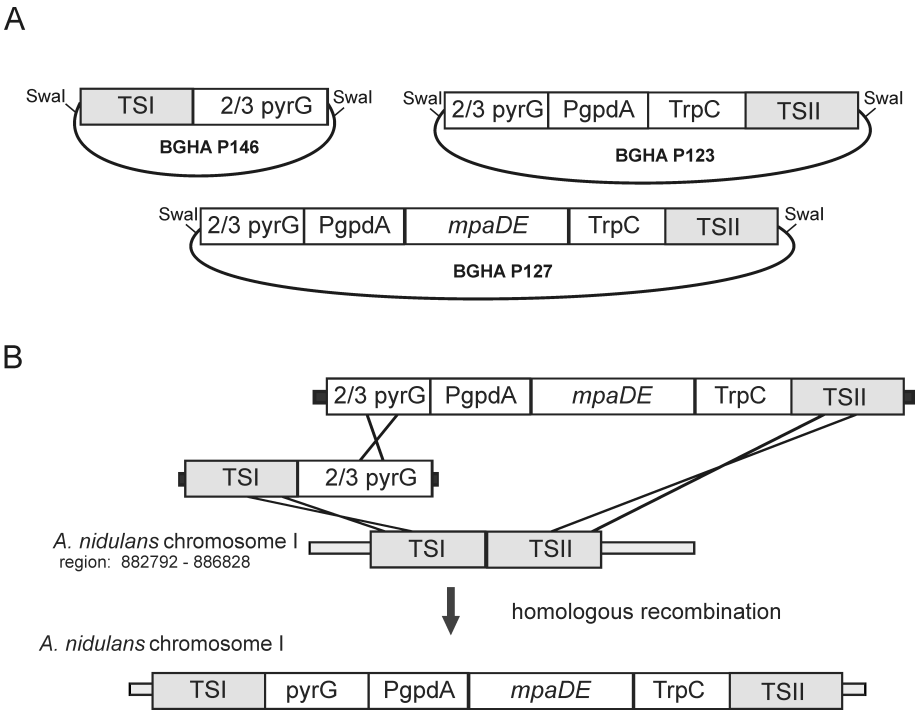
[a] recorded in DMSO-d_6 at 25 °C, referenced to 2.50 ppm

δ_C: carbon chemical shift values

δ_H: proton chemical shift values

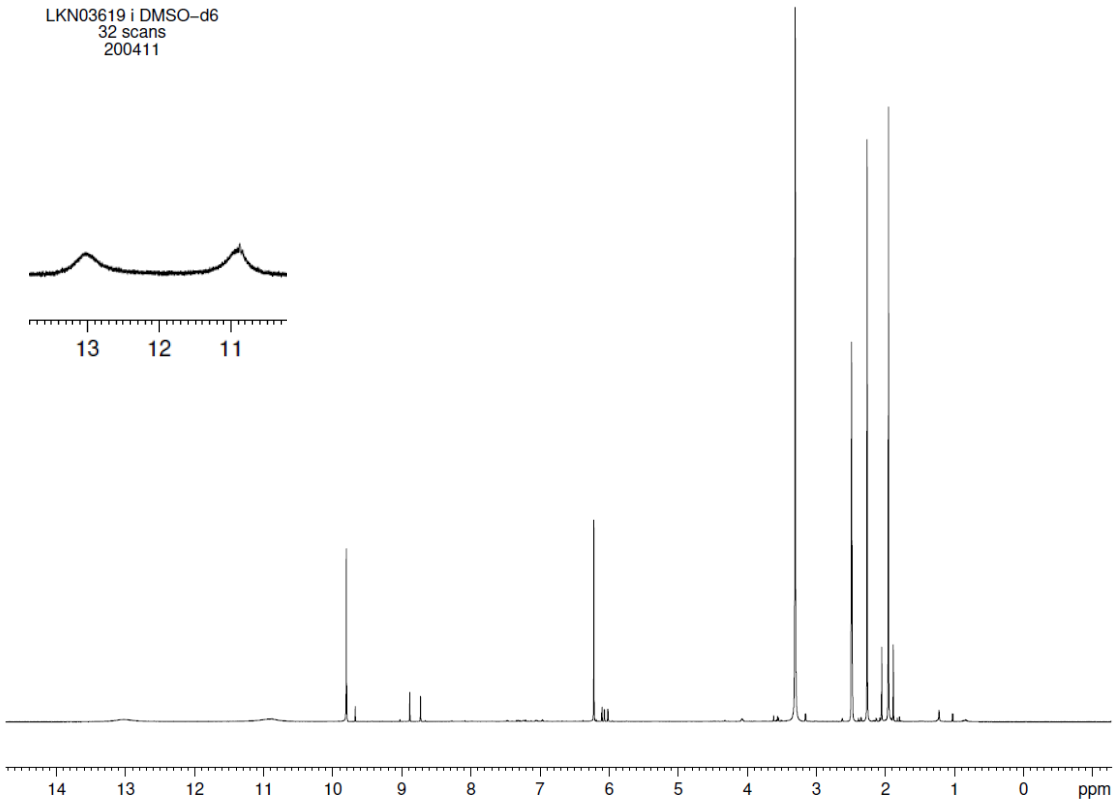
Mult: multiplicity of proton NMR peaks (s: singlet, bs: broad singlet)

HMBC: Heteronuclear Multiple Bond Correlation (nd: not detected via HMBC experiment)



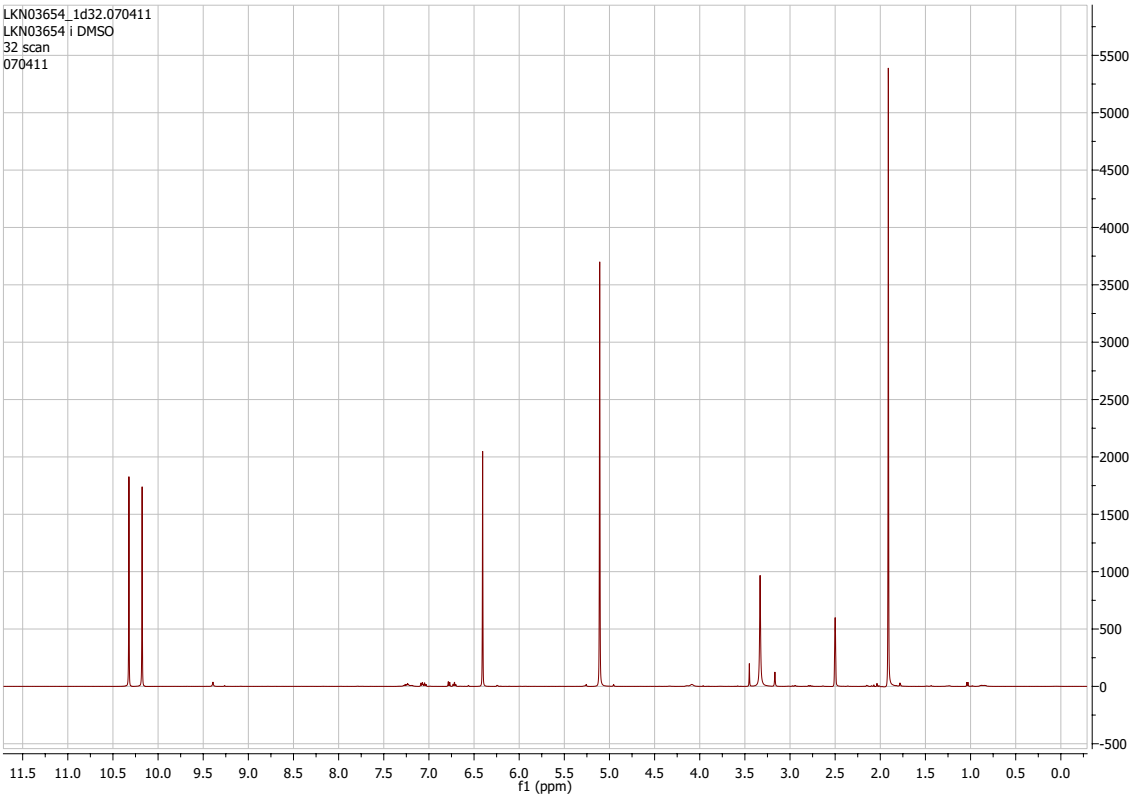
Supplementary Figure S1.

Integration of expression cassette into integration site II on *A. nidulans* chromosome I by homologous recombination using bipartite transformation. A) Schematic drawing of BGHA P127, BGHA P146 and BGHA P123. TSI and TSII are the sequences for homologous integration in IS2. The selection marker is *pyrG* from *A. fumigatus* shown in bipartite gene targeting configuration. PgpdA and TrpC define promoter and terminator of native *gpdA* and *trpC*, respectively, controlling and enabling constitutive *mpaDE* expression. Transformation substrates are generated by SwaI digest of the constructs. B) Integration of the *mpaDE* expression cassette into *A. nidulans* by homologous recombination. Control strain is generated by bipartite transformation with SwaI digested BGHA P146 and BGHA P123. Drawing is not to scale.



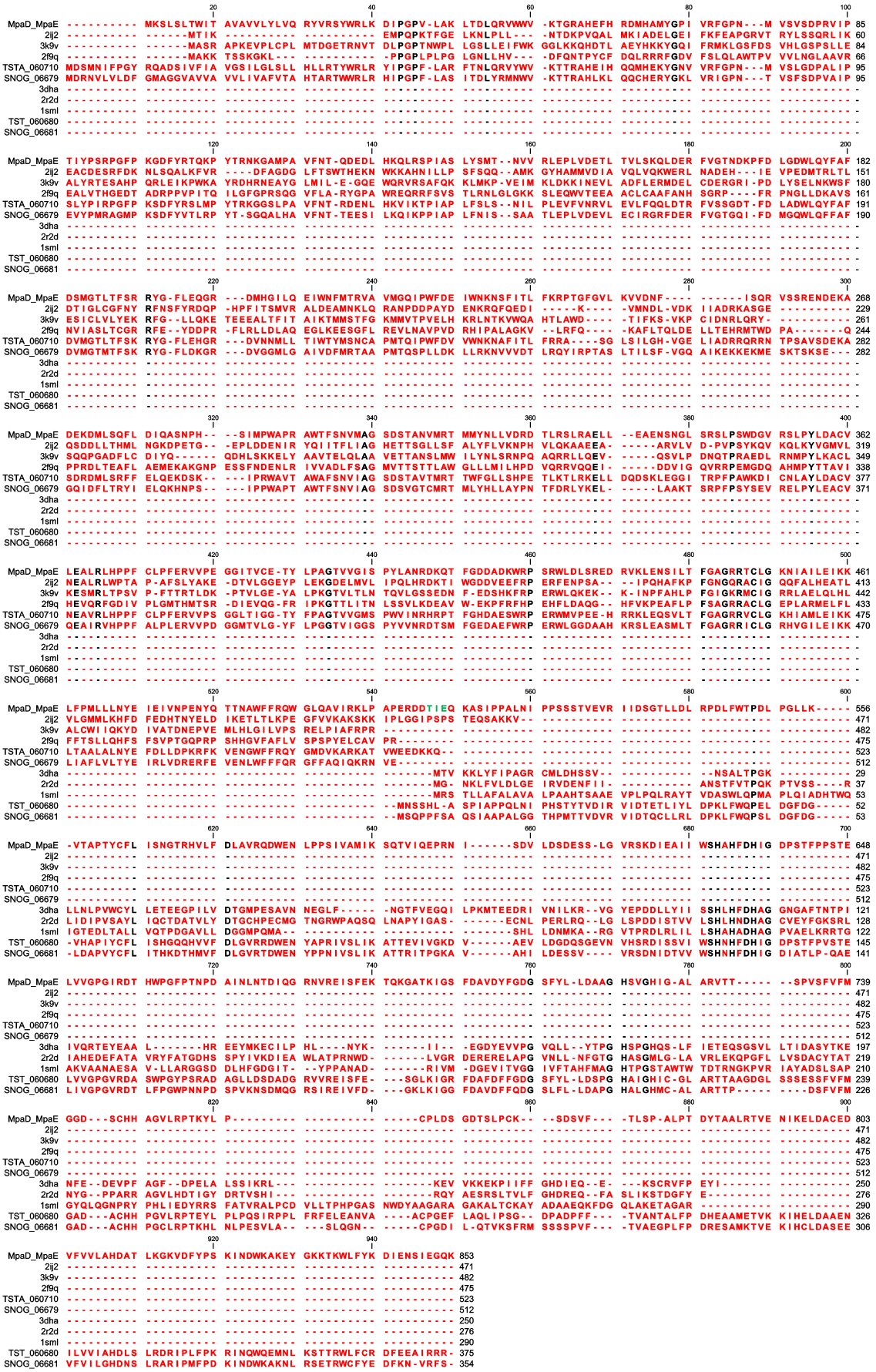
Supplementary Figure S2.

^{1}H NMR spectrum of 5-methylorsellinic acid (5-MOA) measured in DMSO-d_6 (^{1}H at 500 MHz, ^{13}C at 125 MHz) and referenced to solvent residual signals and solvent signals at 2.50 ppm (^{1}H NMR).

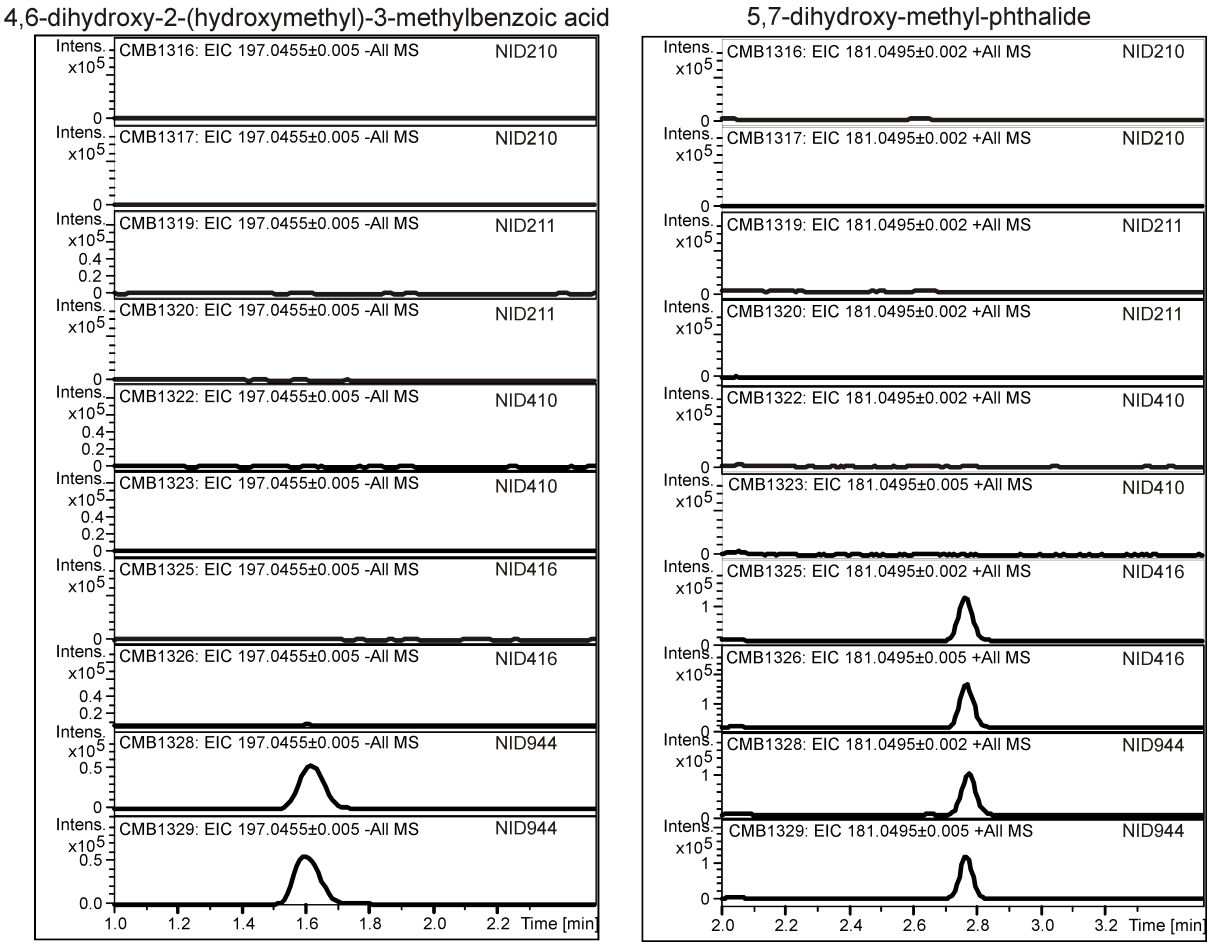


Supplementary Figure S3.

^{1}H NMR spectrum of 5,7-dihydroxy-4-methylphatalide (DHMP) measured in DMSO-d_6 (^{1}H at 500 MHz, ^{13}C at 125 MHz) and referenced to solvent residual signals and solvent signals at 2.50 ppm (^{1}H NMR).



Supplementary Figure S4. Alignment of MpaDE with the CYPs; *Bacillus megaterium* P450 BM3 (2ij2), *Rattus norvegicus* CYP24A1 (PDB 3k9v) , *Homo sapiens* CYP2D6 (PDB 2f9q), *Talaromyces stipitatus* TSTA_060710 and *Phaeosphaeria nodorum* SNOG_06679, *Bacillus thuringiensis serovarkurstaki* lactone hydrolase (PDB 3dha), *Agrobacterium tumefaciens* lactone hydrolase (PDB 2r2d), *Stenotrophomonas maltophilia* lactone hydrolase (PDB 1sml), *Talaromyces stipitatus* TSTA_060680, *Phaeosphaeria nodorum* SNOG_06681. The last three amino acids in the MpaD expressed in this study are marked in green.



Supplementary Figure S5.

MpaD catalyses the conversion of 5MOA to 4,6-dihydroxy-2-(hydroxymethyl)-3-methylbenzoic acid. UHPLC-UV/VIS-HRMS analyses NID210, NID211, NID410, NID416 and NID944. For each strain, two extracts were analysed, resulting in two chromatograms for each strain for each compound. Left column: extracted ion chromatogram, m/z 197.0455±0.005 corresponding to the $[M+H]^+$ ion of 4,6-dihydroxy-2-(hydroxymethyl)-3-methylbenzoic acid. To the right: extracted ion chromatogram, m/z 181.0495 ± 0.002 corresponding to the $[M+H]^+$ ion of 5,7-dihydroxy-methyl-phthalide (DHMP).