

## Supporting Information

### The Sesquiterpene Synthase from the Botrydial Biosynthetic Gene Cluster of the Phytopathogen *Botrytis cinerea*

Cristina Pinedo,<sup>1</sup>† Chieh-Mei Wang,<sup>2</sup>† Jean-Marc Pradier,<sup>3</sup> Bérengère Dalmais,<sup>3</sup> Mathias Choquer,<sup>3</sup> Pascal Le Pêcheur,<sup>3</sup> Guillaume Morgant,<sup>3</sup> Isidro G. Collado,<sup>1</sup>\* David E. Cane,<sup>2</sup>\* Muriel Viaud<sup>3</sup>\*

<sup>1</sup>Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, 11510 Puerto Real, Spain. <sup>2</sup>Department of Chemistry, Box H, Brown University, Providence, Rhode Island 02912-9108 USA. <sup>3</sup>UMR BIOGER, INRA, Route de Saint-Cyr, 78086 Versailles, France

†These authors contributed equally to this work.

\*To whom correspondence should be addressed. E-mail: [isidro.gonzalez@uca.es](mailto:isidro.gonzalez@uca.es); [David\\_Cane@brown.edu](mailto:David_Cane@brown.edu); [viaud@versailles.inra.fr](mailto:viaud@versailles.inra.fr)

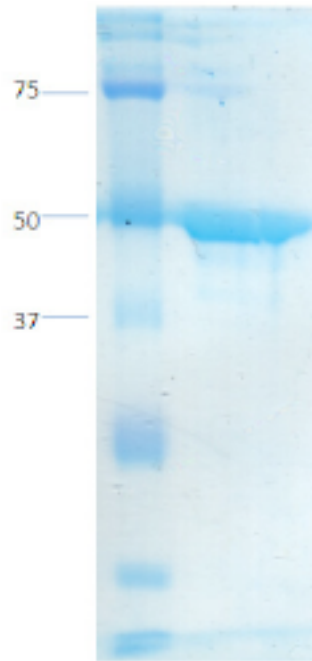
**Supplementary Table 1.** Primers used in quantitative RT-PCR

Gene	Forward primer	Sequence 5'>3'	Reverse primer	Sequence 5'>3'	Size (bp)
<i>BcBOT4</i>	BD1	GGGAACCGAGGTCAATGTCA	BD13	GCTTGAATTCCCAGGGATCTG	77
<i>BcBOT5</i>	BD28	CACGAACTGGACAAAGGCTAAA	BD29	ACCACCCGCAATCAAAGC	70
<i>BcBOT3<sup>a</sup></i>	BD12	CTAAGGGCTCGCGGAGTTG	BD24	GCATTGCGCTGGCAAGA	69
<i>BcBOT2<sup>b</sup></i>	BD5	CAGGTTATCCCTTTGCATGAGTAGT	BD17	TTACTACTGGTGAATGATGTTTTGTCTT	95
<i>BcBOT1<sup>c</sup></i>	BD8	GGCTCCCCTGCTTGCA	BD20	GCGAGGTGAAGAAGTTAGAGAAGGT	76
<i>EF1B</i>	BcEF1b-F	GCTGCCAAGTCTGTTGTCACA	BcEF1b-R	CAATGCTACCATGTCCGGTCTCA	66

<sup>a</sup>Formerly *CND11*. <sup>b</sup>Formerly *CND15*. <sup>c</sup>Formerly *CND5*.

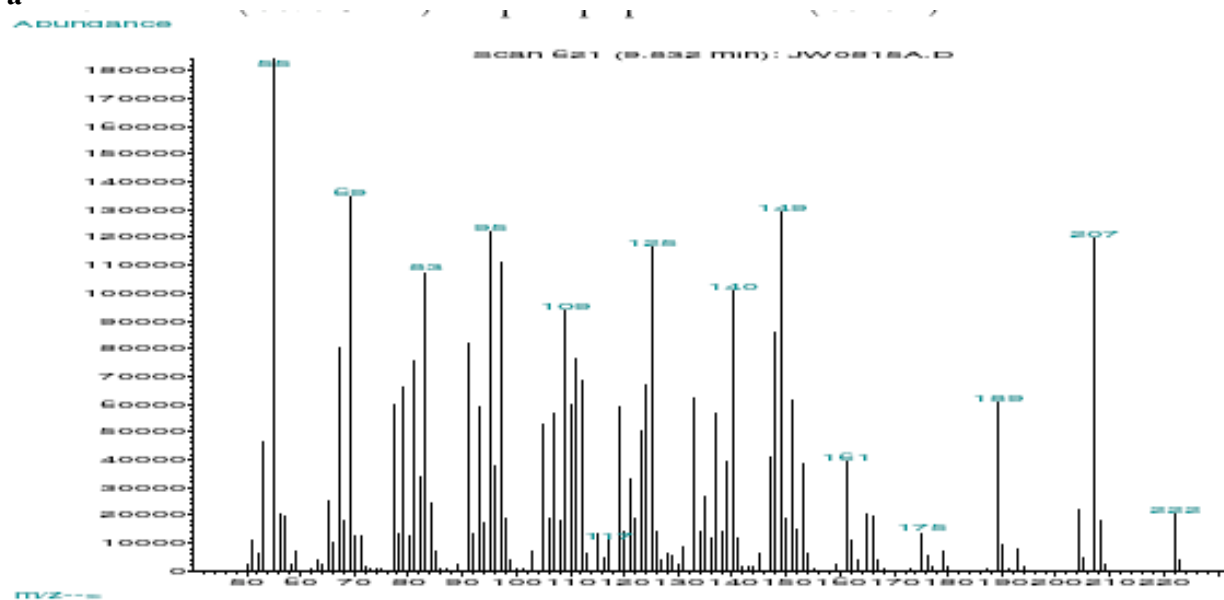
**Supplementary Table 2.** Primers used for gene inactivation

Primer	Sequence 5'>3' (restriction sites indicated in bold)
Cyc1	GAA GCG TGC CTA GCA CTT GT
Cyc2	TGA TCA TCC TGG AGG GAT TC
CycA-seam	AGT TAC <b>TCT TCA</b> CAC GCG ATC AGC CTT AGC GAG TA
CycB-seam	AGT TAC <b>TCT TCA</b> TGG GTC CGG AAC ACG AAC GAA TG
Bar-seam-up	TTA <b>CTC TTC</b> ACC ACC TGA ATG GCG AAT GGA AAT
Bar-seam-low	TTA <b>CTC TTC</b> AGT GCA CGG AAA TGT TGA ATA CTC
Nat1-F-NN	GCG GCC GCC <b>CAT GGG</b> AAC TCT CTA GAG CCG C
Nat1-R-SS	ACT AGT <b>CCG CGG</b> AAT TCC TGC AGG CCG CTC
Cyc5	GGC CAG GTA TCT TGG ACA GA
Bar547	CAT GCGCAC GCT CGG GTC GTT

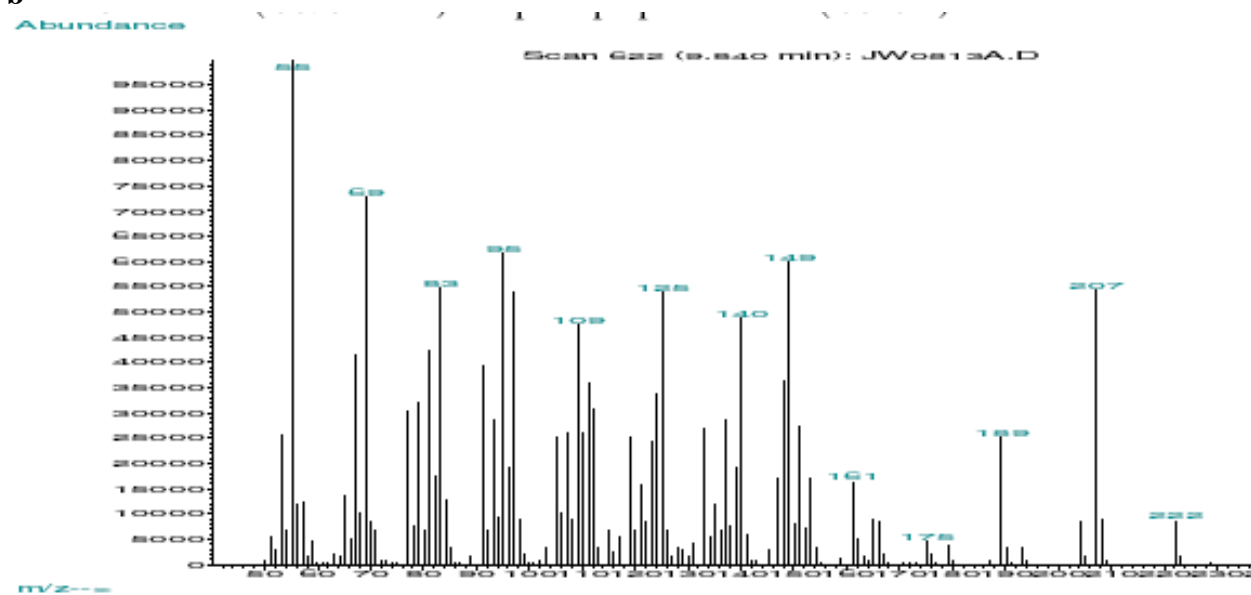


**Supplementary Figure 1.** SDS-PAGE Analysis. Left lane, molecular weight markers. Right lane, recombinant His<sub>6</sub>tag-BcBOT2 protein

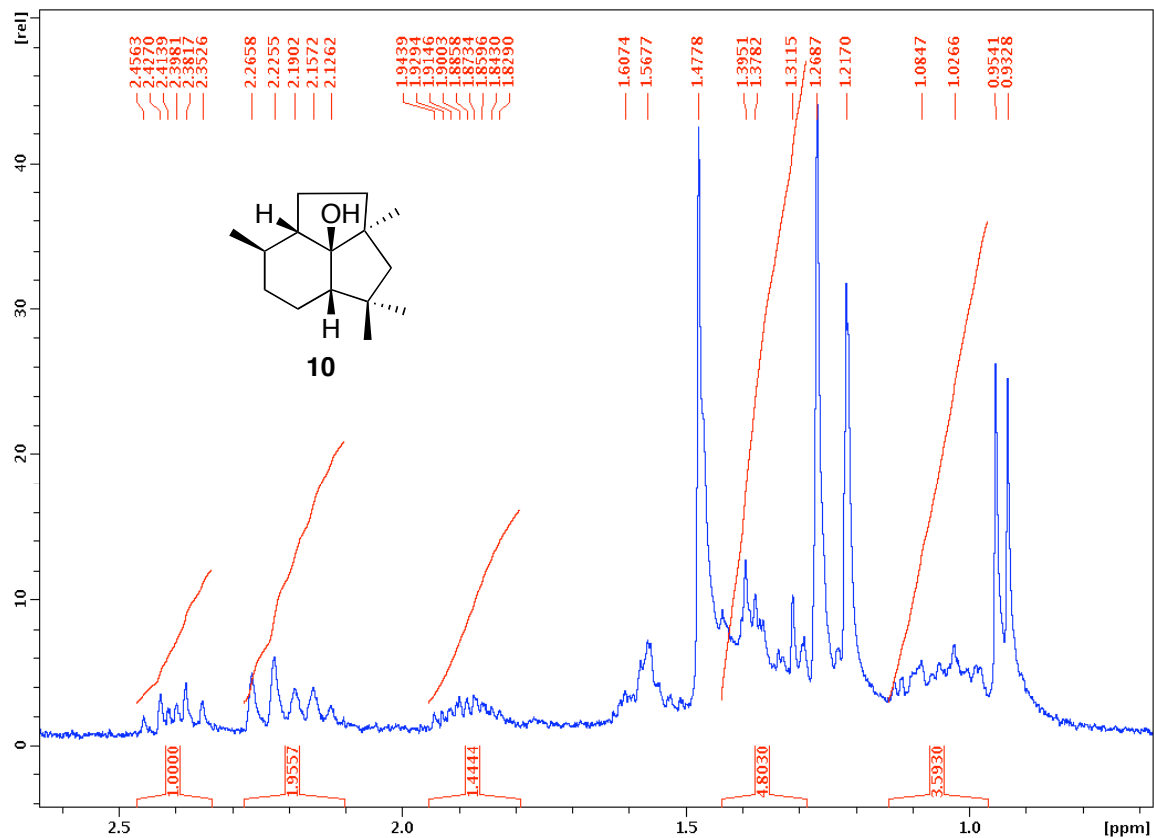
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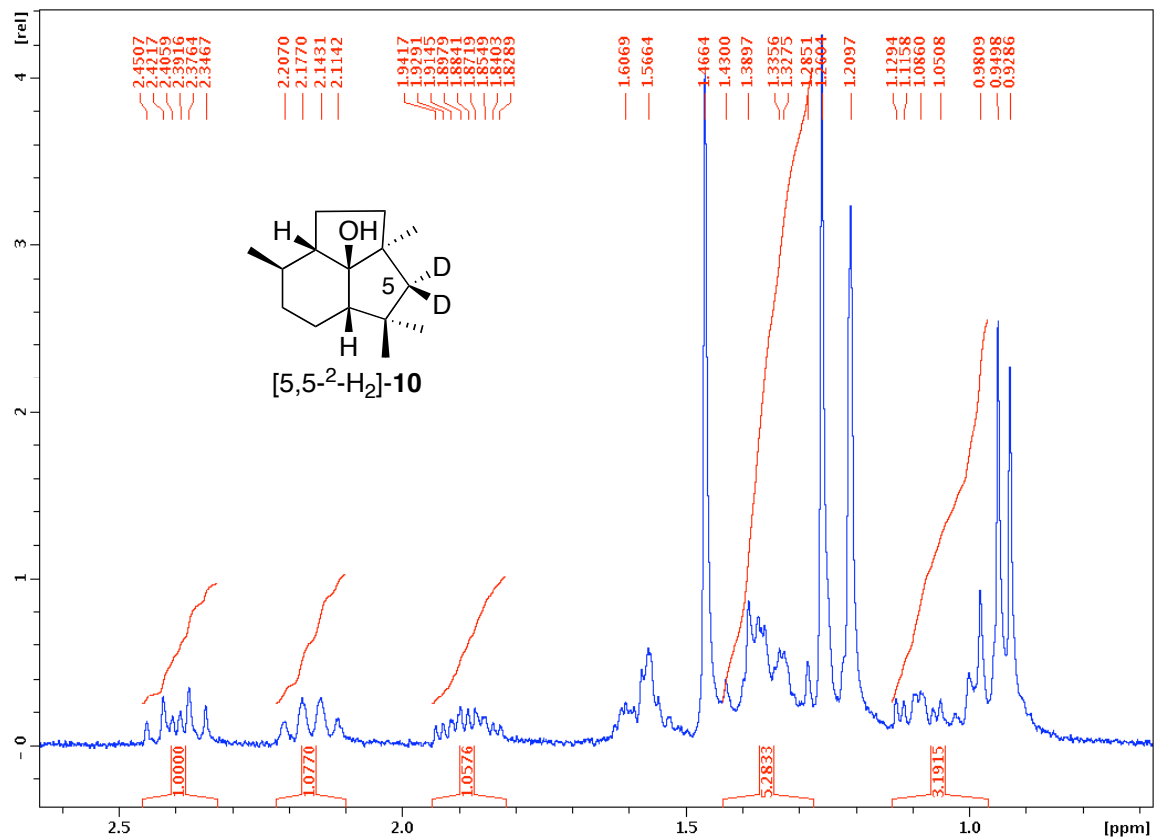
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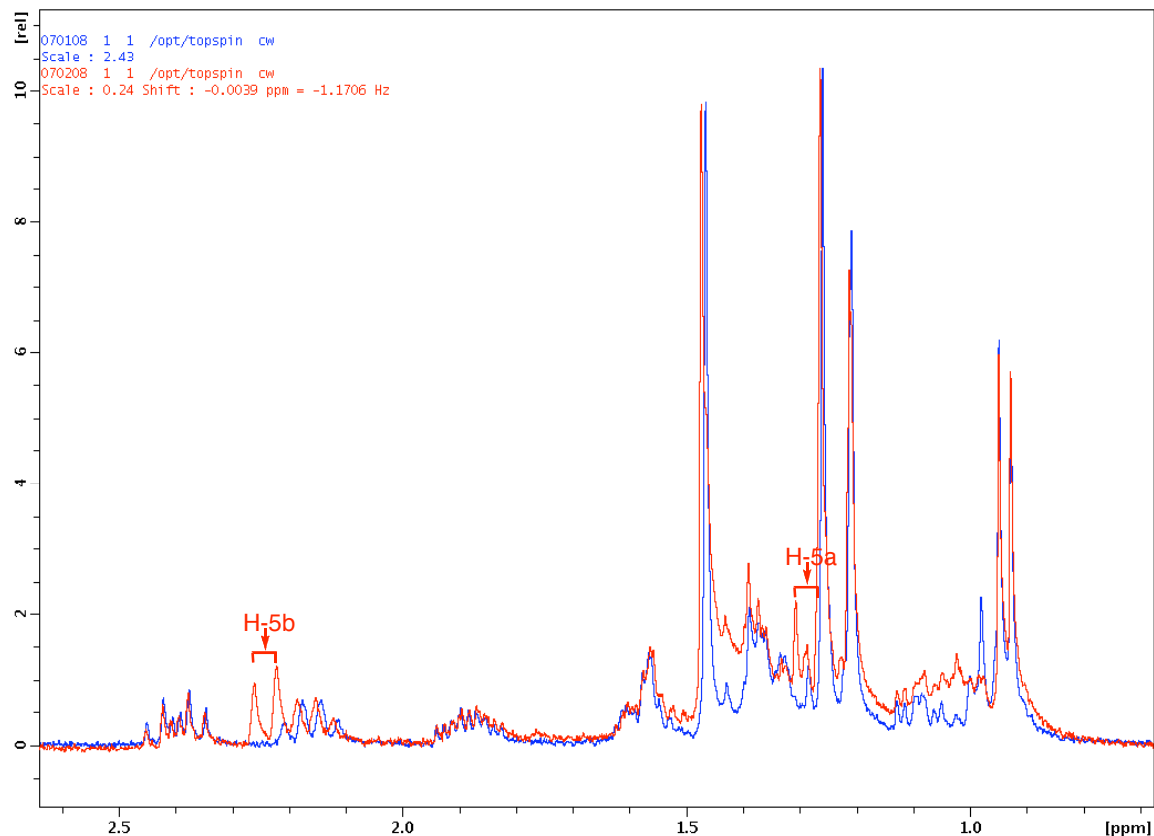
**Supplementary Figure 2.** GC-MS analysis of the enzymatic cyclization of FPP by BcBOT2 protein. A. MS of authentic presilphiperfolan-8-ol (**10**). B. MS of presilphiperfolan-8-ol (**10**) from incubation of FPP (60  $\mu$ M) with BcBOT2 protein (1  $\mu$ M) at 30 °C for 1 h.



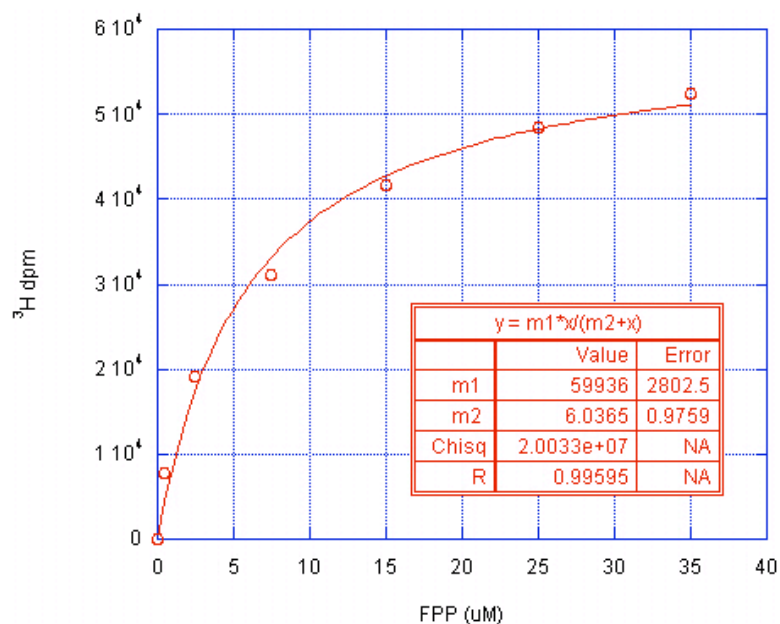
**Supplementary Figure 3.**  $^1\text{H}$  NMR ( $\text{C}_6\text{D}_6$ , 300 MHz) spectrum of the enzymatically generated presilphiperfolan-8-ol (**10**) from the incubation of FPP ( $60\ \mu\text{M}$ ) with BcBOT2 protein ( $1\ \mu\text{M}$ ) at  $30\ ^\circ\text{C}$  for 2 h.



**Supplementary Figure 4.**  $^1\text{H}$  NMR ( $\text{C}_6\text{D}_6$ , 300 MHz) spectrum of the enzymatically generated  $[5,5\text{-}^2\text{H}_2]$ -presilphiperfolan-8-ol (**10**) from the incubation of  $[1,1\text{-}^2\text{H}_2]$ -FPP ( $60\ \mu\text{M}$ ) with BcBOT2 protein ( $1\ \mu\text{M}$ ) at  $30\ ^\circ\text{C}$  for 2 h.



**Supplementary Figure 5.** Superposition of  $^1\text{H}$  NMR spectra of **10** (red) and  $[5,5\text{-}^2\text{H}_2]\text{-10}$  (blue).



**Supplementary Figure 6.** Michaelis-Menten plot of the reaction velocity of the formation of presilphiperfolan-8 $\beta$ -ol (**10**) as a function of the concentration of FPP.