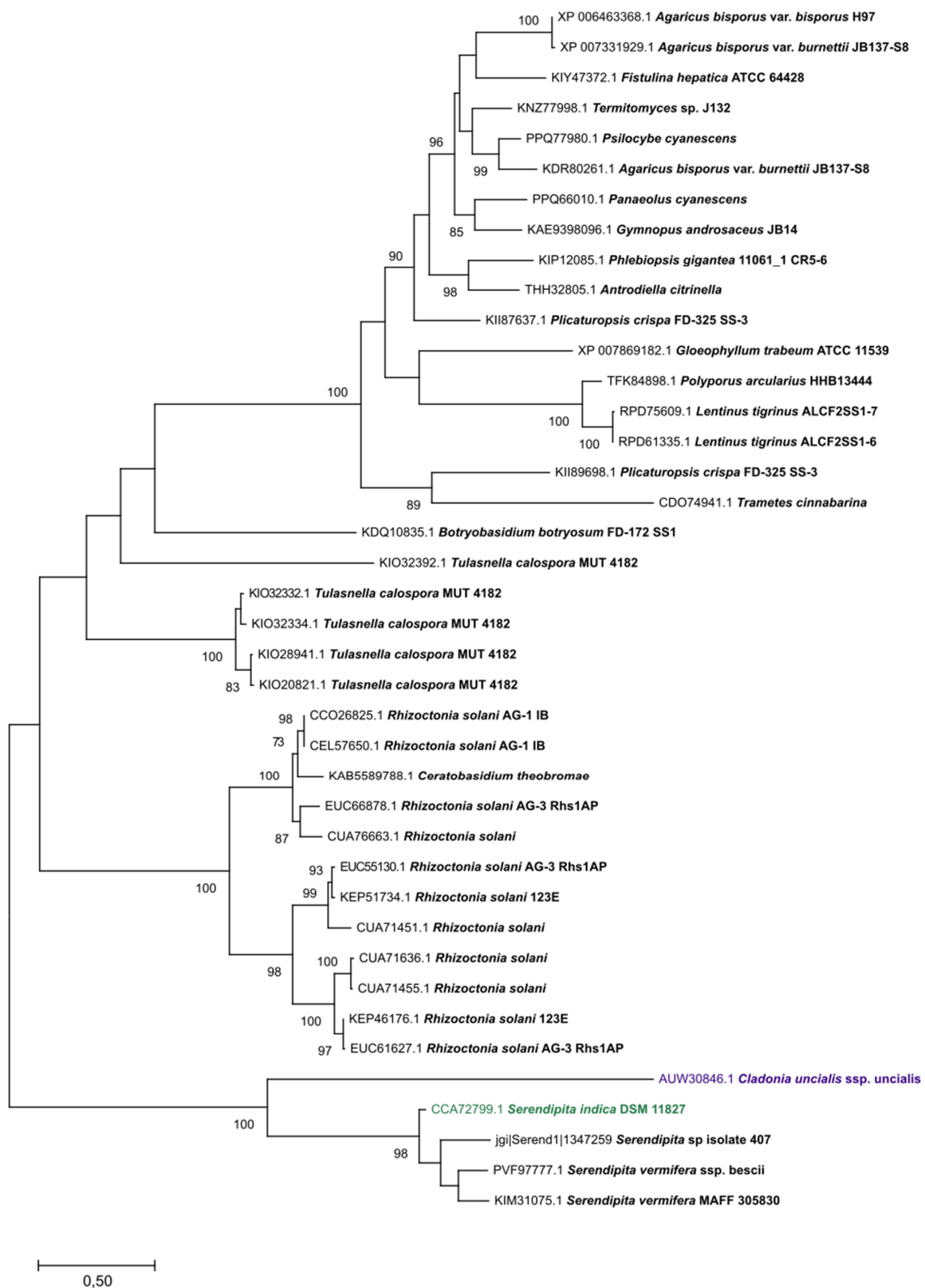


**A sesquiterpene synthase from the endophytic fungus *Serendipita indica* catalyses formation of viridiflorol**

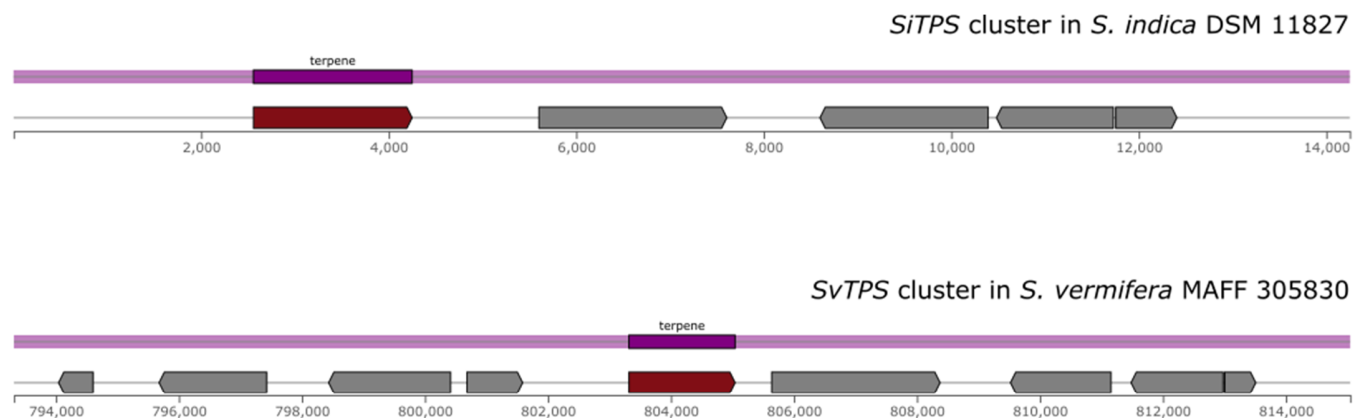
**Fani Ntana <sup>1</sup>, Wajid W. Bhat <sup>2</sup>, Sean R. Johnson <sup>3</sup>, Hans J. L. Jørgensen <sup>4</sup>, David B. Collinge <sup>4</sup>, Birgit Jensen <sup>4</sup> and Björn Hamberger <sup>2,\*</sup>**

## SUPPLEMENTARY FIGURES

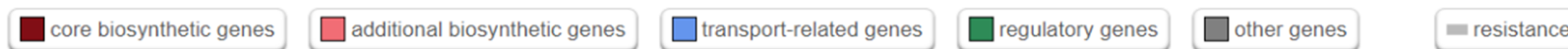


**Figure S1.** Phylogenetic analysis of SiTPS and related sequences. A Maximum-likelihood tree was constructed (bootstrap of 500), using a selection of proteins (NCBI NR, threshold of E-value  $2e-67$ ) similar

to SiTPS (shown here in green) and all the putative TPSs from other *Serendipita* species. The TPS from *Cladonia unciallis* (AUW30846.1) is marked in purple. The scale bar indicates a genetic distance of 0.50 and the bootstrap values are shown below the branches.

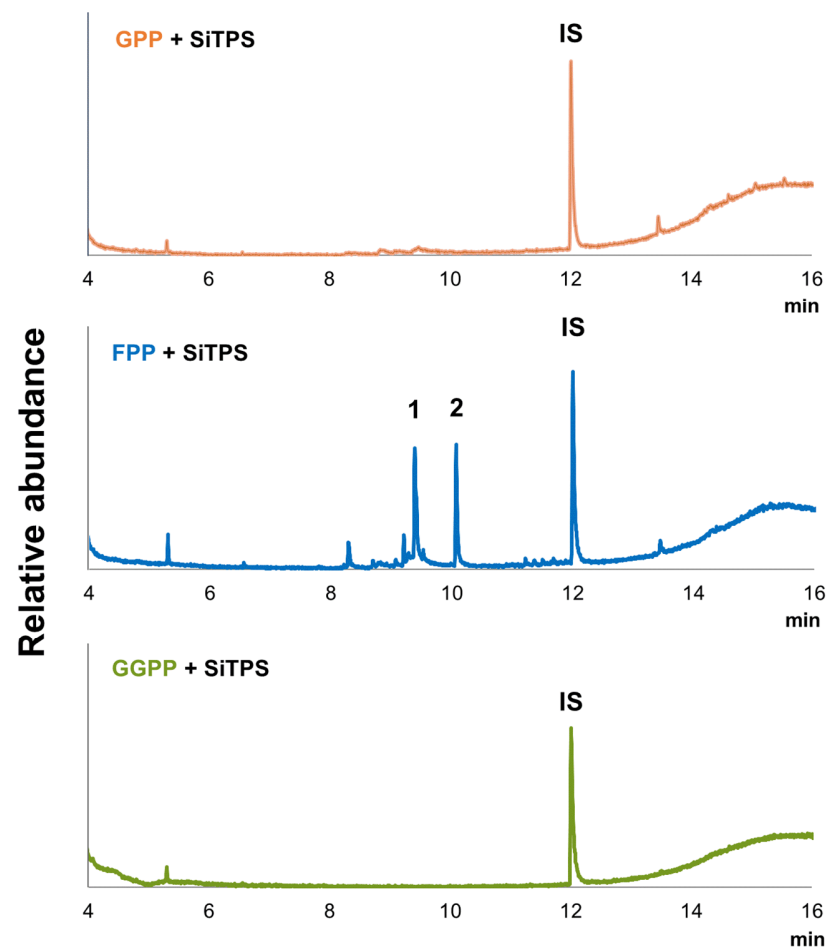


**Legend:**



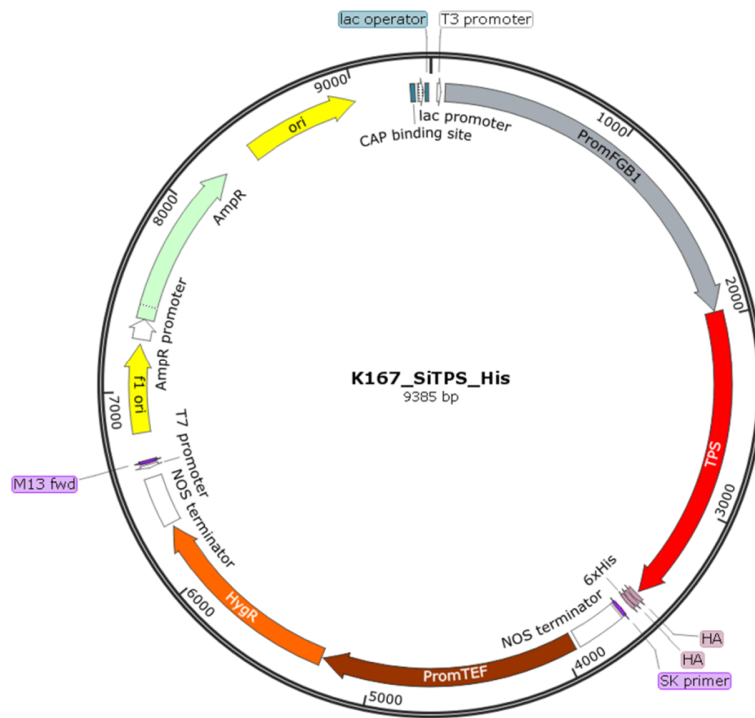
**Figure S2.** Terpene biosynthetic clusters of *SiTPS* and *SvTPS* in *S. indica* and *S. vermifera*, respectively. No other relevant to terpenoid biosynthesis or transport-related gene is located close to the two *TPS* genes. Results displayed have been generated after analysis of the two fungal genomes with the antiSMASH fungal version.



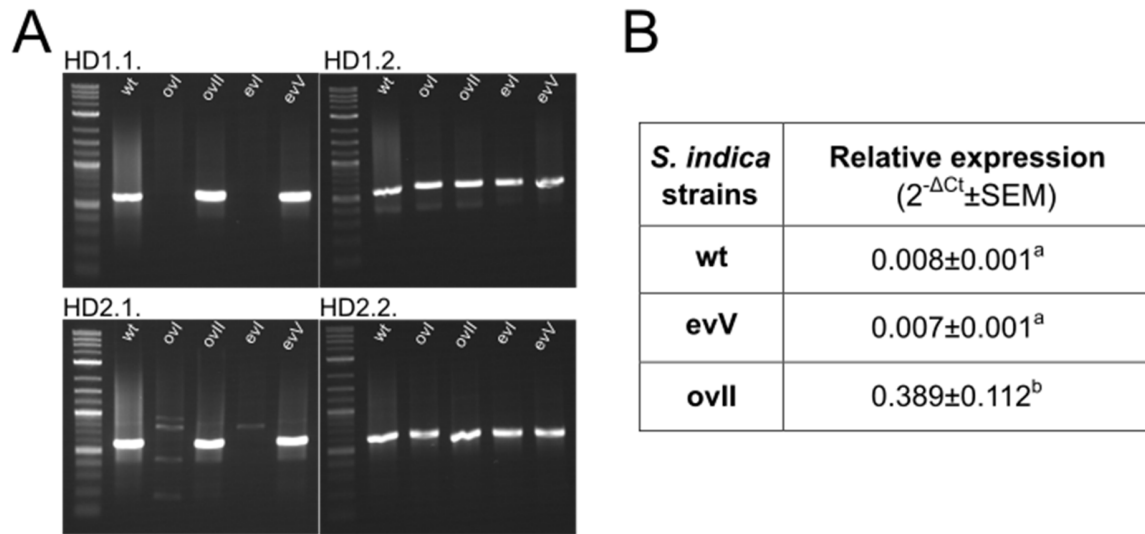


**Figure S3.** GC-MS chromatograms (total ion count, TIC) from hexane extracts of the *in vitro* enzyme assays of SiTPS with GPP, FPP (*E,E*-FPP) and GGPP (*E,E,E*-GPP) (IS, Internal Standard). GC-MS analysis was done on an Agilent 7890A GC with an Agilent VF-5ms column (30 m × 250 μm × 0.25 μm, with 10m EZ-Guard) and an Agilent 5975C detector. The inlet was set to 280°C splitless injection of 1 μl, helium carrier gas with column flow of 13,8 ml/min. The detector was activated after a four-minute solvent delay. The oven temperature ramp started at 35°C hold for 1 min, increase 20°C/min to 300°C

and hold for 3 min. Obtained spectra from the assay with *E,E*-FPP were compared with NIST17 Mass Spectral Database, suggesting peak 1 is viridiflorene and peak 2 viridiflorol.



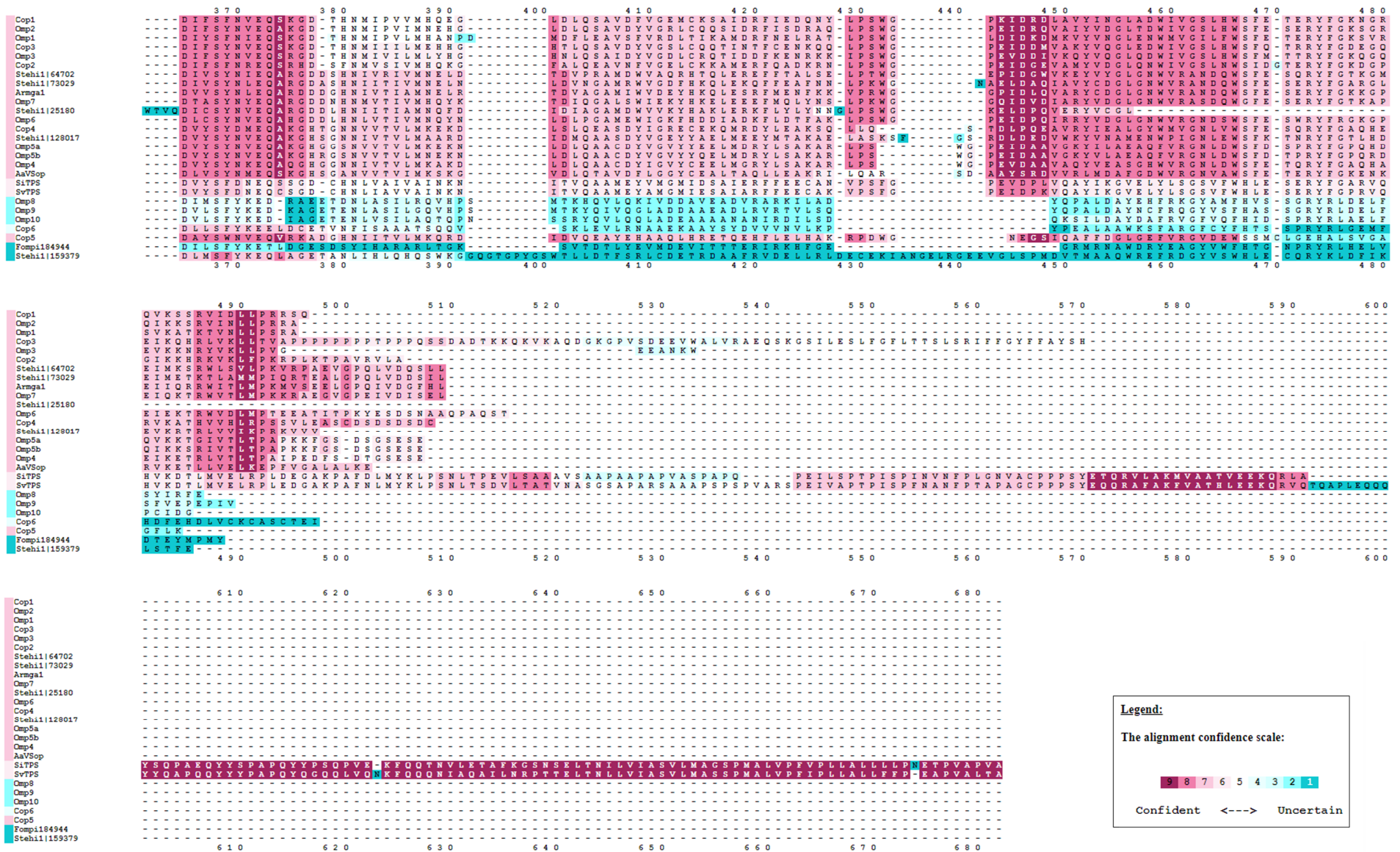
**Figure S4.** The plasmid K167\_PromFCGB1\_SiTPS\_His [S. Wawra and H. Widmer, unpublished; modified from [46]] used for making the *SiTPS*-overexpressing mutants. The vector backbone is comprised of a hygromycin marker cassette and an ampicillin-resistance gene. *SiTPS* sequence (shown in red) is HA- and His-tagged. Gene expression is under the control of the promoter FCGB1 of *S. indica*.



**Figure S5.** *S. indica* transformants used in colonisation studies were selected based on: **A.** their mating type; gel electrophoresis showing products resulting from the amplification of the four mating type loci HD1.1, HD1.2, HD2.1, HD2.2 performed on gDNA templates extracted from the regenerated transformants. Only heterokaryotic transformants, with all four mating type genes, were used. **B.** *SiTPS* relative expression in transformants grown on CM agar plates for 14 days (wt, wild type; evV, empty vector strain-transformant V; ovll, *SiTPS*-overexpressing strain-transformant II). Relative expression was normalised using *SiGAPDH* (GenBank: FJ810523.1) expression levels and calculated with the  $2^{-\Delta C_t}$  method [1,2]. Standard error of the mean (SEM) is also presented ( $n = 4$ ) and different letters indicate statistical differences (one-way ANOVA in R-studio) at  $P \leq 0.05$ .







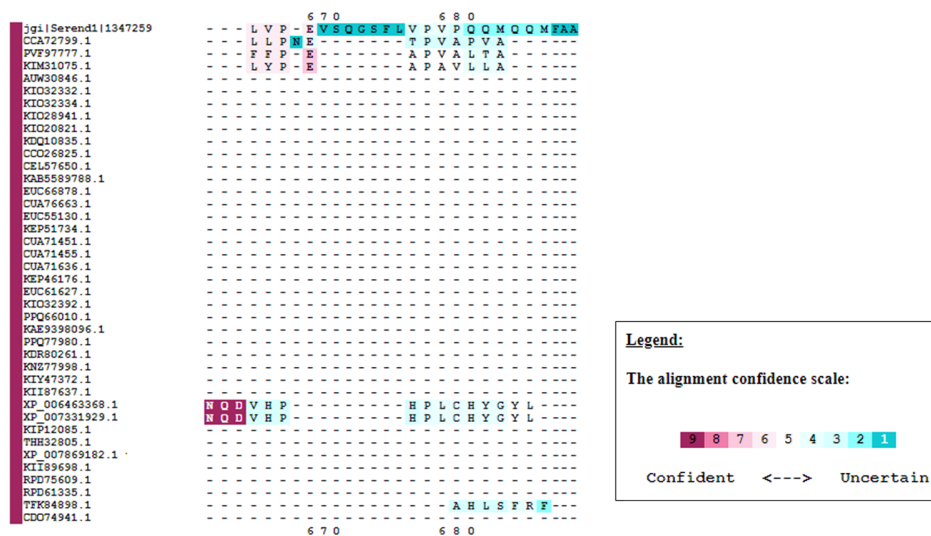
**Figure S6.** Multiple Sequence Alignment (MSA) of Basidiomycota STSs, coloured according to the confidence score generated from GUIDANCE2 Server (CLUSTALW). This is a projection of the confidence scores of each residue onto the MSA, using a colour-scale. Shades of magenta indicate confidently aligned residues while shades of blue indicate uncertainly aligned residues (see legend).





jgi Serendil 1347259	VSLRRDTSALIKVTYACIEYCKLNDIVPDEAFYHPSVLAALQEAAGNDILSWANDVYSFDNEQCSGDCCHNLVAVVAINKNITVVOAAMEYVMSMIDSAIERFFFECEKVPSPSGPGE	230	240	250	260	270	280	290	300	310	320	330
CCA72799.1	VSLRRDTSALIKVTYACIEYCKLNDIVPDEAFYHPSVLAALQEAAGNDILSWANDVYSFDNEQCSGDCCHNLVAVVAINKNITVVOAAMEYVMSMIDSAIERFFFECEKVPSPSGPGE											
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jgi Serendil 1347259	450	460	470	480	490	500	510	520	530	540	550
CCA72799.1	-	-	-	-	-	-	-	-	-	-	-
PVF97777.1	-	-	-	-	-	-	-	-	-	-	-
KIM31075.1	-	-	-	-	-	-	-	-	-	-	-
AUM30846.1	-	-	-	-	-	-	-	-	-	-	-
KIO32332.1	-	-	-	-	-	-	-	-	-	-	-
KIO32334.1	-	-	-	-	-	-	-	-	-	-	-
KIO28941.1	-	-	-	-	-	-	-	-	-	-	-
KIO20821.1	-	-	-	-	-	-	-	-	-	-	-
KDQ10835.1	-	-	-	-	-	-	-	-	-	-	-
CCO26825.1	-	-	-	-	-	-	-	-	-	-	-
CEL57650.1	-	-	-	-	-	-	-	-	-	-	-
KAB5589788.1	-	-	-	-	-	-	-	-	-	-	-
EUC66878.1	-	-	-	-	-	-	-	-	-	-	-
CUA76663.1	-	-	-	-	-	-	-	-	-	-	-
EUC55130.1	-	-	-	-	-	-	-	-	-	-	-
KEP51734.1	-	-	-	-	-	-	-	-	-	-	-
CUA71451.1	-	-	-	-	-	-	-	-	-	-	-
CUA71455.1	-	-	-	-	-	-	-	-	-	-	-
CUA71636.1	-	-	-	-	-	-	-	-	-	-	-
KEP46176.1	-	-	-	-	-	-	-	-	-	-	-
EUC61627.1	-	-	-	-	-	-	-	-	-	-	-
KIO32392.1	-	-	-	-	-	-	-	-	-	-	-
PPQ66010.1	-	-	-	-	-	-	-	-	-	-	-
KAE3398096.1	-	-	-	-	-	-	-	-	-	-	-
PPQ77980.1	-	-	-	-	-	-	-	-	-	-	-
KDR80261.1	-	-	-	-	-	-	-	-	-	-	-
KNE77998.1	-	-	-	-	-	-	-	-	-	-	-
KIY47372.1	-	-	-	-	-	-	-	-	-	-	-
KII87637.1	-	-	-	-	-	-	-	-	-	-	-
XP_006463368.1	-	-	-	-	-	-	-	-	-	-	-
XP_007331929.1	-	-	-	-	-	-	-	-	-	-	-
KIP12085.1	-	-	-	-	-	-	-	-	-	-	-
TRH32805.1	-	-	-	-	-	-	-	-	-	-	-
XP_007869182.1	-	-	-	-	-	-	-	-	-	-	-
KII89698.1	-	-	-	-	-	-	-	-	-	-	-
RPD75609.1	-	-	-	-	-	-	-	-	-	-	-
RPD61335.1	-	-	-	-	-	-	-	-	-	-	-
TFK84898.1	-	-	-	-	-	-	-	-	-	-	-
CDQ74941.1	-	-	-	-	-	-	-	-	-	-	-
jgi Serendil 1347259	560	570	580	590	600	610	620	630	640	650	660
CCA72799.1	-	-	-	-	-	-	-	-	-	-	-
PVF97777.1	-	-	-	-	-	-	-	-	-	-	-
KIM31075.1	-	-	-	-	-	-	-	-	-	-	-
AUM30846.1	-	-	-	-	-	-	-	-	-	-	-
KIO32332.1	-	-	-	-	-	-	-	-	-	-	-
KIO32334.1	-	-	-	-	-	-	-	-	-	-	-
KIO28941.1	-	-	-	-	-	-	-	-	-	-	-
KIO20821.1	-	-	-	-	-	-	-	-	-	-	-
KDQ10835.1	-	-	-	-	-	-	-	-	-	-	-
CCO26825.1	-	-	-	-	-	-	-	-	-	-	-
CEL57650.1	-	-	-	-	-	-	-	-	-	-	-
KAB5589788.1	-	-	-	-	-	-	-	-	-	-	-
EUC66878.1	-	-	-	-	-	-	-	-	-	-	-
CUA76663.1	-	-	-	-	-	-	-	-	-	-	-
EUC55130.1	-	-	-	-	-	-	-	-	-	-	-
KEP51734.1	-	-	-	-	-	-	-	-	-	-	-
CUA71451.1	-	-	-	-	-	-	-	-	-	-	-
CUA71455.1	-	-	-	-	-	-	-	-	-	-	-
CUA71636.1	-	-	-	-	-	-	-	-	-	-	-
KEP46176.1	-	-	-	-	-	-	-	-	-	-	-
EUC61627.1	-	-	-	-	-	-	-	-	-	-	-
KIO32392.1	-	-	-	-	-	-	-	-	-	-	-
PPQ66010.1	-	-	-	-	-	-	-	-	-	-	-
KAE3398096.1	-	-	-	-	-	-	-	-	-	-	-
PPQ77980.1	-	-	-	-	-	-	-	-	-	-	-
KDR80261.1	-	-	-	-	-	-	-	-	-	-	-
KNE77998.1	-	-	-	-	-	-	-	-	-	-	-
KIY47372.1	-	-	-	-	-	-	-	-	-	-	-
KII87637.1	-	-	-	-	-	-	-	-	-	-	-
XP_006463368.1	-	-	-	-	-	-	-	-	-	-	-
XP_007331929.1	-	-	-	-	-	-	-	-	-	-	-
KIP12085.1	-	-	-	-	-	-	-	-	-	-	-
TRH32805.1	-	-	-	-	-	-	-	-	-	-	-
XP_007869182.1	-	-	-	-	-	-	-	-	-	-	-
KII89698.1	-	-	-	-	-	-	-	-	-	-	-
RPD75609.1	-	-	-	-	-	-	-	-	-	-	-
RPD61335.1	-	-	-	-	-	-	-	-	-	-	-
TFK84898.1	-	-	-	-	-	-	-	-	-	-	-
CDQ74941.1	-	-	-	-	-	-	-	-	-	-	-



**Figure S7.** Multiple Sequence Alignment (MSA) of similar to SiTPS proteins, coloured according to the confidence score generated from GUIDANCE2 Server (MAFFT). This is a projection of the confidence scores of each residue onto the MSA, using a colour-scale. Shades of magenta indicate confidently aligned residues while shades of blue indicate uncertainly aligned residues (legend).

**Table S1.** Primer list

No	Name	Sequence 5'-3'	Used for	Primer efficiency (%)
1	SiTPSinf-F	AGAAGGAGATATACCATGCCA TCTGTCTCTCCTGCCAC	In-fusion® cloning pET_SiTPS	
2	SiTPSinf-R	GGTGGTGGTGCTCGAACGCAAC GGGAGCCACTGGG	In-fusion® cloning pET_SiTPS	
3	TPSovinf-F	CTCCAAAAACAGTCGATGCCAT CTGTCTCTCCTGC	In-fusion® cloning K167_SiTPS_His	
4	TPSovinfHA-R	TAGATATCGTAGTTTGCAACGG GAGCCACTGGGGT	In-fusion® cloning K167_SiTPS_His	
5	HD1.1-F	CGATACCTACCCGCCTACAA	<i>S. indica</i> mating type locus HD 1.1 (PIIN_09915)	
6	HD1.1-R	CTTTTAAGCGGTGCTGGAG	<i>S. indica</i> mating type locus HD 1.1 (PIIN_09915)	
7	HD2.1-R	ATGAGTACGATTGCCCAAGG	<i>S. indica</i> mating type locus HD 2.1 (PIIN_09916)	
8	HD2.1-F	TCGTCTCGTAGGCGACTTTT	<i>S. indica</i> mating type locus HD 2.1 (PIIN_09916)	
9	HD1.2-R	AGATATCCGGAGGCGAGTTT	<i>S. indica</i> mating type locus HD 1.2 (PIIN_09977)	
10	HD1.2-F	CCTGAATCTGCTGTTTCGTCA	<i>S. indica</i> mating type locus HD 1.2 (PIIN_09977)	
11	HD2.2-F	ACATCTGGCTCCCATTTACG	<i>S. indica</i> mating type locus HD 2.2 (PIIN_09978)	
12	HD2.2-R	GTTGAGCTTTGGCTCGTTTC	<i>S. indica</i> mating type locus HD 2.2 (PIIN_09978)	
13	SiGAPDH-F	TCGGTCGTATTGGCCGTATC	<i>SiTPS</i> relative expression- Normalization	
14	SiGAPDH-R	CTCGATAACATAGTCGACACCT	<i>SiTPS</i> relative expression- Normalization	110
15	SiTPS-F	CAACAGCTACAACATGATGC	<i>SiTPS</i> relative expression	
16	SiTPS-R	GTCCAGAAGTCATGGAAAGC	<i>SiTPS</i> relative expression	100
13	SiITS-F	CAACACATGTGCACGTCGAT	<i>S. indica</i> ITS estimation of fungal biomass	
14	SiITS-R	CCAATGTGCATTGAGAACGA	<i>S. indica</i> ITS estimation of fungal biomass	
15	SITUB-F	AACCTCCATTGAGGAGATGTTT	<i>Solanum lycopersicum</i> $\beta$ -tubulin estimation of plant biomass	
16	SITUB-R	TGCTGTAGCATCCTGGTATT	<i>Solanum lycopersicum</i> $\beta$ -tubulin estimation of plant biomass	

