Table S1. List of the various fungal strains used in this study.

Strain	Genotype description	Reference
PH-1	Wild type	(Cuomo et al., 2007)
Δ*Fggyp1*	FGSG_17336 deletion mutant in PH-1	(Zheng et al., 2020)
Δ*Fggyp1-C*	Δ*Fggyp1* strain expressing the FgGyp1-GFP construct	This study
FgGyp1-GFP+ FgKex2-mCherry	Δ*Fggyp1* strain expressing the FgGyp1-GFP and FgKex2-mCherry constructs	This study
FgGyp1-GFP+ FgBet3-mCherry	Δ*Fggyp1* strain expressing the FgGyp1-GFP and FgBet3-mCherry constructs	This study
FgGyp1-GFP+ mCherry-FgGos1	Δ*Fggyp1* strain expressing the FgGyp1-GFP and mCherry-FgGos1 constructs	This study
PH-1+FgTri1-GFP	PH-1 strain expressing the FgTri1-GFP construct	This study
Δ*Fggyp1*+ FgTri1-GFP	Δ*Fggyp1* strain expressing the FgTri1-GFP construct	This study
PH-1+FgTri4-GFP	PH-1 strain expressing the FgTri4-GFP construct	This study
Δ*Fggyp1*+ FgTri4-GFP	Δ*Fggyp1* strain expressing the FgTri4-GFP construct	This study
Δ*TBC*	Δ*Fggyp1* strain expressing the FgGyp1$^{\Delta TBC}$ -GFP construct	This study
Δ*N*	Δ*Fggyp1* strain expressing the FgGyp1$^{\Delta N}$-GFP construct	This study
Δ*C*	Δ*Fggyp1* strain expressing the FgGyp1$^{\Delta C}$-GFP construct	This study
R284K	Δ*Fggyp1* strain expressing the FgGyp1^{R284K}-GFP construct	This study
R357K	Δ*Fggyp1* strain expressing the FgGyp1^{R357K}-GFP construct	This study
PH-1+GFP-FgSnc1	PH-1 strain expressing the GFP-FgSnc1 construct	(Zheng et al., 2020)
Δ*Fggyp1*+ GFP-FgSnc1	Δ*Fggyp1* strain expressing the GFP-FgSnc1 construct	This study

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W.H., Quesneville, H., Roncero, M.I.G., Seong, K.Y., Tetko, I.V., Urban, M., Waalwijk, C., Ward, T.J., Yao, J.Q., Birren, B.W., and Kistler, H.C. (2007). The Fusarium graminearum genome reveals a link between localized polymorphism and pathogen specialization. *Science* 317**,** 1400-1402.

Zheng, H., Li, L., Yu, Z., Yuan, Y., Zheng, Q., Xie, Q., Li, G., Abubakar, Y.S., Zhou, J., Wang, Z., and Zheng, W. (2020). FgSpa2 recruits FgMsb3, a Rab8 GAP, to the polarisome to regulate polarized trafficking, growth and pathogenicity in Fusarium graminearum. *New Phytol* 10.1111/nph.16935.

Table S2. List of all primers involved in this study.

Primer	Sequence (5′→3′)	Application
FGSG_17336AF	GCGAGTTAGCGGACAACC	*FgGYP1* deletion
FGSG_17336AR	TTGACCTCCACTAGCTCCAGCCAAGCCTCATCATCCTCATAGCCCATC	
FGSG_17336BF	GAATAGAGTAGATGCCGACCGCGGGTTTGTTCCTCCAGAGCCTTCC	
FGSG_17336BR	CCCGTTTACTACACCACCC	
FGSG_17336OF	GCTCGCTCCTCGCATAAT	
FGSG_17336OR	ACCACTTGCTGGGTGTCG	
FGSG_17336UA	TTTTCAGCGTCCTATGTAGCC	
H853	GACAGACGTCGCGGTGAGTT	
Fg17336CF	agggaacaaaagctgggtaccTGCCTCTGATGACAGAACCT	FgGyp1-GFP
Fg17336GR	GCCGCCGCCGCCGCCAAGCTTTAGTTGTAAATTCGTGACGG	
FgGyp1-R284K-R1	AGCAGCAGTTGCCAGGTAATGGCCTTAACCTCTTGCGGCACACCAG	For R284K mutation
FgGyp1-R284K-F1	TGGTCTGGTGTGCCGCAAGAGGTTAAGGCCATTACCTGGCAACTGCTG	
FgGyp1-R357K-R1	TACAGCTCAATATGCGGGTTGGTCTTGGGTACATCGATGCTGATTT	For R357K mutation
FgGyp1-R357K-F1	ACCAAATCAGCATCGATGTACCCAAGACCAACCCGCATATTGAGCT	
17336-TBC-R1	CGGCACACCAGACCAGGCGA	For TBC domain deletion
17336-TBC-F1	GGCAACTCGCCTGGTCTGGTGTGCCGGACATGGATTTCCAGGAGAT	
17336ZR	CATGGTTCGATGACGAGAAGTGA	
17336-N-F	TCACTTCTCGTCATCGAACCATGCAAGAGGTTCGCGCCATTAC	For C/N-terminal deletion of FgGyp1
17336queC-R	GCCGCCGCCGCCGCCAAGCTTGAGGAGCTTATCGGACCATTT	
FgBet3CF	agggaacaaaagctgggtaccTCCCTTTCTCAGCCGTAG	FgBet3-mCherry
FgBet3CR	gcccttgctcaccataagcttGTCATCCTCAGGTGGCAGTT	
FgGyp1-MBP-F	ATCGAGGGAAGGATTTCAGAATTCATGTGGTCATCTTCTGGAAG	For MBP-FgGyp1
FgGyp1-MBP-R	GCCAGTGCCAAGCTTGCCTGCAGTCATAGTTGTAAATTCGTGA	
FgGyp1-TBC-MBP-F	ATCGAGGGAAGGATTTCAGAATTCATGCAAGAGGTTCGCGCCATTAC	For MBP-TBC

FgGyp1-TBC-MBP-R	GCCAGTGCCAAGCTTGCCTGCAGTCAGAGGAGCTTATCGGACC

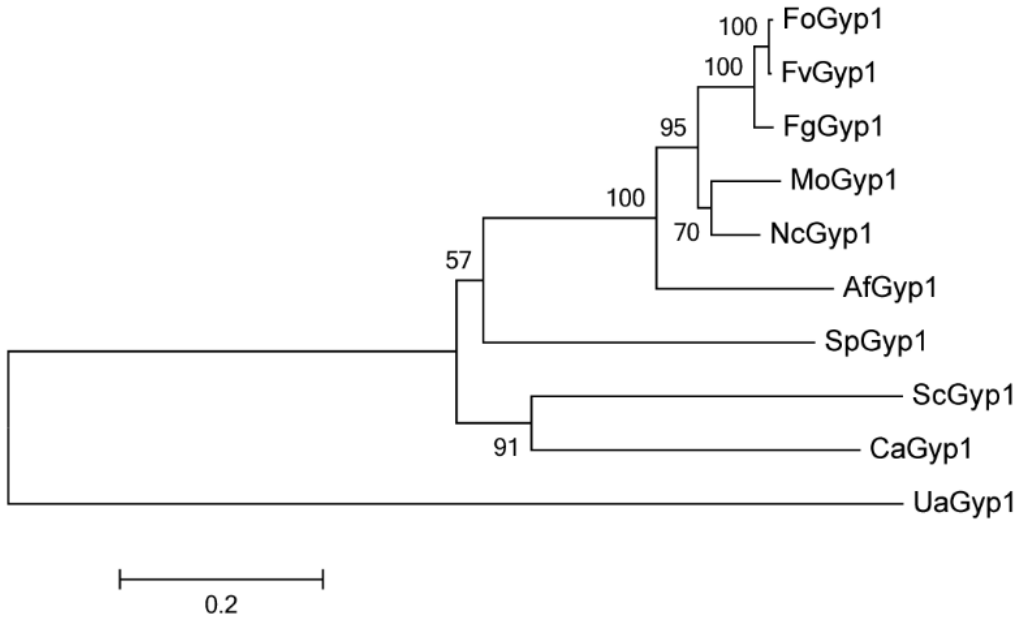

Figure S1. Phylogenetic relationship of Gyp1 proteins from different organisms
Sequence alignments were performed using the Clustal X 1.83 program and the calculated phylogenetic tree was viewed using Mega 6 program. A neighbour-joining tree with 10000 bootstrap replicates between the Gyp1 homologs in the different organisms.

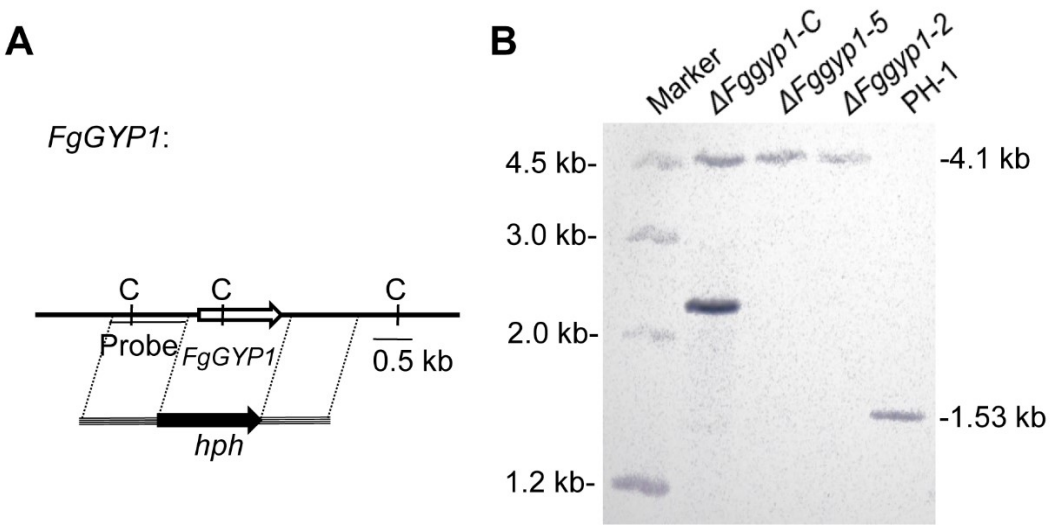

Figure S2. Generation of *FgGYP1* deletion mutants and confirmation by Southern blot analysis

(A) The split-marker approach was used to delete the *FgGYP1* gene. Genomic DNAs were extracted from PH-1 and the putative transformants. Schematic diagram of the genomic regions of *FgGYP1* and *hph* genes

(B) Targeted gene deletion of *FgGYP1, Cla*I (C) digested DNAs showed a 1.53 kb band in PH-1 and a 4.10 kb band in the mutants.

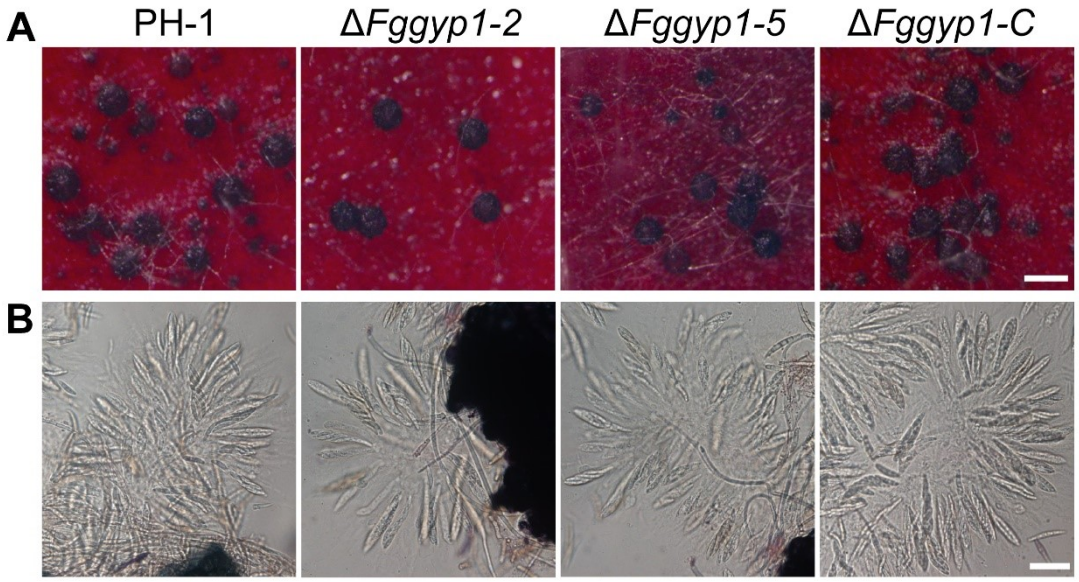

Figure S3. FgGyp1 is dispensable for sexual reproduction

(A) FgGyp1 is not required for perithecium formation. Bar = 200 μm.

(B) FgGyp1 is not required for ascospore formation. Bar = 50 μm.

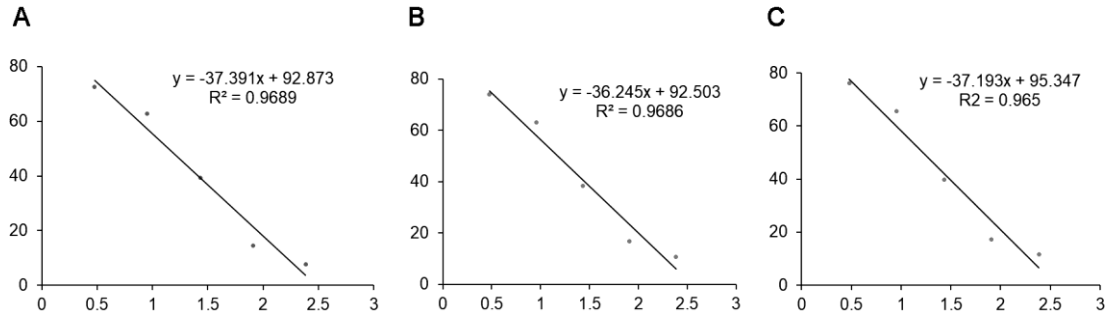

Figure S4. The standard curves for quantification of the DON

(A-C) The standard curves of three independent experiments for quantification of the DON. x: logarithm of the standard solution concentration. y: percentage of the standard solution absorbance.