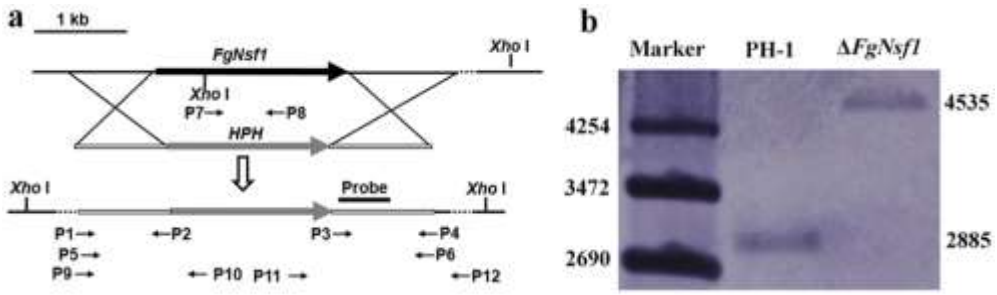

Appendix A Generation and identification of *FgNSF1* deletion mutant. **a**. Gene deletion strategy used for *FgNSF1*. The gene replacement cassette contains a hygromycin-phosphotransferase (*HPH*) gene. **b**. Southern blot analysis of *FgNSF1* deletion mutant. Genomic DNA was digested with *Xho* I.

Appendix B Oligonucleotide primers used in this study

Primers	Sequence (5'-3')	Relevant characteristics
P1	GTCGAACTGTGATCAGTC	PCR primers to amplify *FgNSF1* upstream fragment for construction of the gene deletion vector
P2	GGAGGTCAACACATCAATGATGCAATACTACTGACAC	
P3	GGCAAAGGAATAGAGTAGCACCCTTTCCAAACATC	PCR primers to amplify *FgNSF1* downstream fragment for construction of the gene deletion vector
P4	GCTCATCTACCATGTCAC	
P5	GACTCCACAGAGACAATCG	PCR primers to amplify the 3.04kb deletion vector
P6	GATCATGAGAATCCTGAG	
P7	CGACGACGGCGTTGGATCTG	PCR primers for identification of *FgNSF1* deletion mutants
P8	GACATCGACTCTCTGCGTGG	
P9	CTGTGCGACTCGAATCAGTC	PCR primers for identification of *FgNSF1* deletion transformants at the left junction
P10	CTCGCCGATAGTGGAAA	
P11	TTTGGATGCTTGGGTAGA	PCR primers for identification of *FgNSF1* deletion transformants at the right junction
P12	GGATCATACTGTTACAGCTG	
P13	GCGTCTTCCTTGTTTACAAT	PCR primers to amplify 656 bp probe for southern blotting
P14	GCTGCCCATAACAAGGAAAG	
P15	GGAGGTCAACACATCAAT	PCR primers to amplify the 1.35kb *HPH* fragment
P16	CTACTCTATTCCTTTGCC	
P17	TTTCGTAGGAACCCAATCTTCAAAATGGCGGCCGCTTTTCGACC	PCR primers to amplify the full *FgNSF1* fragment for construction of the FgNSF1-GFP vector
P18	CACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACGTA	

	CGCTTTAGCGGTTGAGC	
P19	CACCTGTTGTCCGTTTCG	PCR primers for the identification of the in-frame FgNSF1-GFP fusion vector
P20	CGTGCTGCTTCATGTGGTCG	
P21	AAAAAGCAGCCAAGGAGCAT	Quantitative real-time PCR primers for analysis of *AurJ* expression
P22	TTCTGATGACACGCTCCCGTA	
P23	ATCTTCAGTCTTGACCATCCC	Quantitative real-time PCR primers for analysis of *AurF* expression
P24	TACCCAAGATGTTCTGGCAA	
P25	TCGGCACATCAGTATCTCCAA	Quantitative real-time PCR primers for analysis of *AurO* expression
P26	CAATACTATCGCCTGTCGCTT	
P27	AGGTCGTTGACACGGCAT	Quantitative real-time PCR primers for analysis of *AurR2* expression
P28	TGTGCCAGGAGTAAACTTTGA	
P29	GAGTGTTTCATGCATGGCTACGTC	Quantitative real-time PCR primers for analysis of *TRI5* expression
P30	CTGAGCCTCCTTCACATCGTCC	
P31	TGCGTTCTTTGGCCTCTAC	Quantitative real-time PCR primers for analysis of *TRI6* expression
P32	TACGACGGATGTTGGTGTAATC	
P33	ATCCACGTCACCACTTTCAA	Quantitative real-time PCR primers for analysis of actin gene expression
P34	TGCTTGGAGATCCACATTTG	
P35	GATCGAATCCTTTGACCCTCTT	Quantitative real-time PCR primers for analysis of *FgNSF1* gene expression
P36	CTCGGGAAGATGTGGTTGATAG	
P37	GTGATGGGTTTGAAATCGCTG	Quantitative real-time PCR primers for analysis of FGSG_08691 expression
P38	GTTGGTTCGGGCTATACTGG	
P39	CAAGCAAAACCAAAGGAGACG	Quantitative real-time PCR primers for analysis of FGSG_00408 expression
P40	ATGAAATGCTGTAGTCCGAGG	
P41	AAGTTGATATCTGGAGTGCTGG	Quantitative real-time PCR primers for analysis of FGSG_09612 expression
P42	GATGGAGAATTGGTTGACGTG	
P43	GAAGGATGTCTGGGCTAATCG	Quantitative real-time PCR primers for analysis of FGSG_06326 expression
P44	GTCTCTGAACTCTCGGCAATC	
P45	GCCACTATCTCTATCGCCATG	Quantitative real-time PCR primers for analysis of FGSG_ 07295 expression
P46	CTCAGCAATTTTACCCAGCAC	
P47	GATGCTAGGTGGGTTTCGAG	Quantitative real-time PCR primers for analysis of FGSG_10313 expression
P48	GAAATGAAGATGAGGGAAACGC	

Appendix C Conidial germination experiments on water agar plates for 12 h. Both the normal and malformed conidia could germinate successfully on the water agar plates, though the germinal tube (12 h) of Δ*FgNsf1* is much shorter.

Appendix D DON production, and expression levels of *TRI* genes in *F. graminearum* strains

Strain	DON production (μg g^{-1} dry mycelia)*	Relative expression levels†	
		TRI5	*TRI6*
PH-1	5.42±0.86a	1±0.21a	1±0.18a
Δ*FgNsf1*	0.31±0.03b	0.35±0.1b	0.48±0.15b
Δ*FgNsf1-C*	5.18±0.37a	NA	NA

Different letters for each treatment indicate significant difference at $P < 0.05$ by LSD test.

*Deoxynivalenol production in TBI inoculated with 5×10^4 conidia ml^{-1} for 7 days at 28 ℃. Data were collected from three independent experiments.

†Relative gene expression levels of *TRI5* and *TRI6*. Actin gene was used as internal control. The expression levels of *TRI* genes in PH-1 were set to 1.0. NA means not analysis.