Supplementary Material

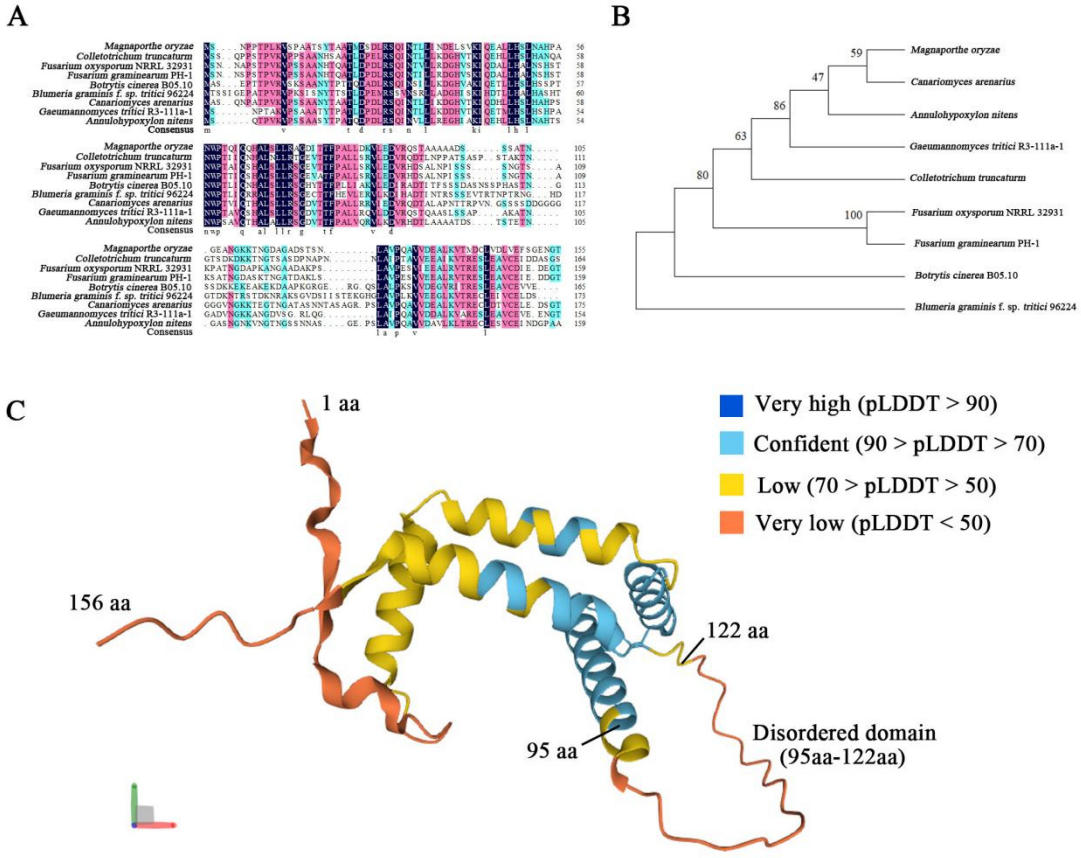

Fig. S1. Identification of MoLfa1 in *M. oryzae*. (A) CLUSTALW was used to align the amino acids of Lfa1 in *M. oryzae* (MoLfa1) with those of the homologous proteins in *C. truncatum* (XP_036575343.1), *F. oxysporum* NRRL 32931 (EWY86874.1), *F. graminearum* PH-1 (XP_011324077.1), *B. cinerea* B05.10 (XP_001553949.2), *B. graminis* f. sp. *tritici* 96224 (EPQ63018.1), *C. arenarius* (KAK4115313.1), *G. tritici* R3-111a-1 (XP_009217046.1), and *A. nitens* (KAI0901762.1). (B) MoLfa1 homology analysis in different species. (C) Molecular structure prediction of MoLfa1 protein. AlphaFold produces a per-residue confidence score (pLDDT) between 0 and 100. Some regions with low pLDDT may be unstructured in isolation. 95-122aa is a disordered region.

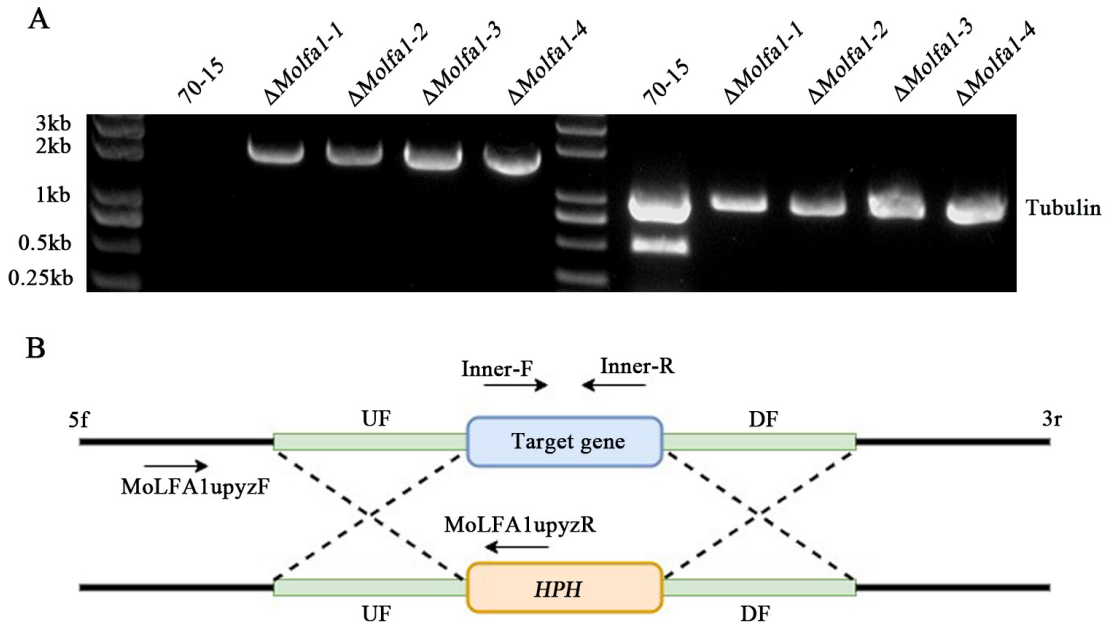

Fig. S2. The targeted gene in the transformant was screened by PCR using the tubulin gene as a positive control. A 0.5 kb short band was amplified from the wild-type strain and the ectopic transformant, indicating that the target gene was contained, whereas the 0.5 kb short band was not found in the null mutant. A unique recombinant DNA fragment labeled as a knockout event in the transformant was screened by PCR. A 1.5-2.5 kb long band was amplified from the null mutant, whereas the wild-type strain and the ectopic transformant did not have the long band. (B) The knockout model in *M. oryzae*.

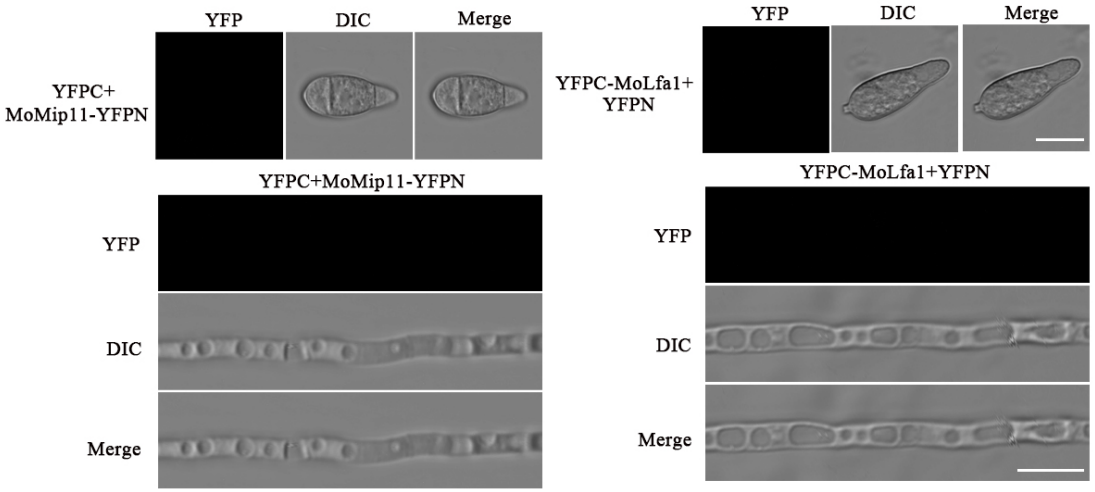

Fig. S3. A negative control of the interaction between MoLfa1 and MoMip11 in vivo.

Construct pairs of YFPC-MoLfa1 and YFPN, MoMip11-YFPN, and YFPC were co-transformed into wild-type 70-15 by ATMT to serve as negative controls. Bar, 10 μm.

Name	Sequence (5’ - 3’)
Primers used for gene knockout	
HPH-F	TAGTGGAGGTCAACAATGAATG
HPH-R	CATCTACTCTATTCCTTTGCCC
MoLFA1up-F	AGGCTAACTGACACTCTAGAATGAGATGGAGGCGGTAT

Table S1 Primers in this study

MoLFA1up-R	TGTTGACCTCCACTAACAGAAAGAGGAAGGCAGT
MoLFA1dn-F	GGAATAGAGTAGATGACCTCATTCGAGGCCCTATGC
MoLFA1dn-R	CGACGGCCAGTGCCAAGCTTGATGAAGAATGCTGGCCC
MoLFA1inner-F	TCCGGCGGCGACAAGTTAT
MoLFA1inner-R	CGACTAAATCGACTAAGCAG
MoLFA1upyz-F	CCGACAAAGCTTTCCTGCAC
MoLFA1upyz-R	GTCGGAGACGCTGTCGAACTT
Tubulin-F	CCATCCCGAGCTTGTTGATA
Tubulin-R	GTAGTTCAGGTCACCGTATGAG
Primers used for complementation	
pKD5-MoLFA1-GFP-F	ATCACAATGGCCGGATCCATGTCAAACCCACCGACACCG
pKD5-MoLFA1-GFP-R	CTTGCTCACCATCCCGGGAGTAGTGCCATTCTCCCCCGA
pKD5-MoLFA1-N1-GFP-F	ATCACAATGGCCGGATCCATGTCAAACCCACCGACACC
pKD5-MoLFA1-N1-GFP-R	CTTGCTCACCATCCCGGGCGCAGTGCTCTGCCTGACGT
pKD5-MoLFA1-N2-GFP-F	ATCACAATGGCCGGATCCGCGGCGGCGGCGGATTCATC
pKD5-MoLFA1-N2-GFP-R	CTTGCTCACCATCCCGGGAGTAGTGCCATTCTCCCCCGA
Primers used in fluorescent observation	
MoLfa1-mCherry-F	ATCACAATGGCCGGATCCATGTCAAACCCACCGACACCG
MoLfa1-mCherry-R	CTTGCTCACCATCCCGGGAGTAGTGCCATTCTCCCCCGA
Primer used for Pull-down	
MoLfa1-GST-F	CTGGTTCCGCGTGGATCCATGTCAAACCCACCGACACCG
MoLfa1-GST-R	CGGGAATTCCGGGGATCCAGTAGTGCCATTCTCCCCCGA
MoMip11-His-F	CAAGGTCGACAAGCTTATGGCTGAGCAGCTCATT
MoMip11-His-R	GTGCGGCCGCAAGCTTTGCCCTAGACATGACGCC
Primer used for BiFC	
YFPC-MoLfa1-F	GAGCTGTACAAGTCTAGAATGTCAAACCCACCGACACCG
YFPC-MoLfa1-R	CGCTTACTGCAGGTCGACAGTAGTGCCATTCTCCCCCGA
MoMip11-YFPN-F	GTCAAAATGGTCGGATCCATGGCTGAGCAGCTCATT
MoMip11-YFPN-R	GGCGATGGAGCGCCCGGGTGCCCTAGACATGACGCC