

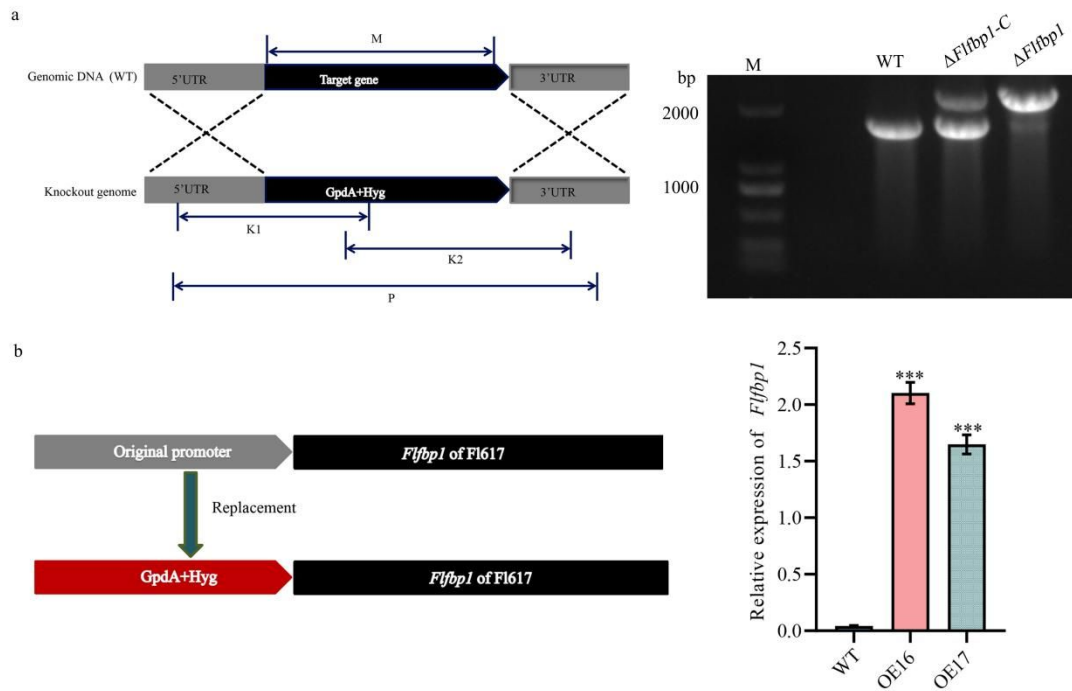


Fig. S3. Construction and verification of *Flfbp1* knockout and overexpression mutants in *F. lateritium* F1617. (a) Knockout strategy: The *Flfbp1* coding region was replaced with a selection cassette containing the constitutive strong promoter GpdA and the hygromycin phosphotransferase resistance gene (HYG). Diagnostic PCR with primers K1, K2, and P was performed to confirm successful homologous recombination. The right panel shows agarose gel electrophoresis confirming the genotypes of WT, $\Delta Flfbp1$ knockout mutant, and $\Delta Flfbp1$ -C complementation strain. M, DNA molecular weight marker (bp). Complementation strategy: Homozygous knockout mutants verified by genotyping were used as recipient strains. A complementation construct harboring the full-length ORF of *Flfbp1* fused to its native 1.5–2.0 kb promoter was introduced into protoplasts via PEG-mediated transformation to obtain complemented strains. Specific diagnostic PCR primers (K1, K2, P, M) were used to verify the correctness of both homologous recombination and complementation events. (b) Construction strategy of *Flfbp1* overexpression mutant (*Flfbp1*^{OE}). The native promoter of *Flfbp1* was replaced with GpdA to drive constitutive high-level expression. The right panel shows RT-qPCR analysis of transcript levels in two independent overexpression lines (OE16, OE17), presented as fold change relative to WT. Data are mean $\pm$ SD (n = 3). ** indicating significantly upregulated expression compared with WT, (P < 0.01).