**TABLE S1. PCR primers used in this study**

| Primer | Sequence (5’ → 3’) |
|---|---|
| 3’-Int1s | CATTGGACCTCGCAACCCTAG |
| 3’-Int3s | CACGAACTTCCGGGACGCCTC |
| 3’-Int4as | CCTCCGCCCTTTCTTCTTCGC |
| 5’-Int1s | ATGTCGCACTATTAGAAGGG |
| 5’-Int3s | ATAATCTTGATGGCAGTTCG |
| 5’-Int2as | CCGGTCCCGGATTAATAATC |
| Fusion-primer1s | CAACAGCAGTCAACAGATCA |
| Fusion-primer2as | GCATGCCAGAAAGAGTCACCATCGTCTTCTGCAATCGGTA |
| Fusion-primer3s | TTCATTTTCGGGAGACGAGATAACGCGAGAGGTAGAAAAG |
| Fusion-primer4as | CGCTGACAACTTCCGTGGTA |
| Fusion-primer5s | TACCGATTGCAGAAGACGATGGTGACTCTTTCTGGCATGC |
| Fusion-primer6as-2 | CCTCTAAGTTCTTCATCATCTCTCGTCTCCCGAAAATGAA |
| Fusion-primer7s | TTCATTTTCGGGAGACGAGAGATGAAGAACTTAGAGG |
| Fusion-primer8as | GATTTCAAATGTTGCAGA |
| GFP-Fus-HPH1-2s | GACCCGGGTACAGTGACCGGTGACTCTTTC |
| GFP-Fus-HPH2as | GCTCTAGATCTCTTCGCCGGAGCCTG |
| GPD-cDNA-test-s | GAACGATCCATTCATTGA |
| GPD-cDNA-test-as | GCTTGCCGTTGAGCTCTGGG |
| GPD-RTs | TCAAGGGCACGATCGAGGCT |
| GPD-RTas | TCGCTCCATGGGATCTCGGA |
| HO05J24F | GTGCGCTGTGGTCAAGATGTT |
| HO05J24R | TAGTGATATCGCTCATGCCGG |
| HPH1s | AAAGTTCGACAGCGTCTCCG |
| HPH200-f | GAGCGGGTTCGGCCCATTCG |
| HPH CDS2s | CAC TCG TCC GAG GGC AAA GG |
| $LB20_{57}WT16bis$ | GAGTAAGTCTGCAACAGCAG |
| $LB20_{57}WT17$ | GTCAACAGATGAATTCGAGC |
| Oligo-dT-spec | $TGTAATACGACTCACTATAGGGCGAGAATTCAACG(T)_{18}$ |
| Oligo-dT-out | TGTAATACGACTCACTATAGGGCG |
| Oligo-dT-in | TCACTATAGGGCGAGAATTCAACG |
| PFP-ESTs | ATAAAGCGGAACTACTATAA |
| PFP-RTs | GATCCCCAGGTGAAGCCCGA |

| | |
|---|---|
| PFP-RTas | GATTCGCTTCCCCAGACGCC |
| PFP2as | ATACCGCGTCCAATTAACTG |
| PFP3s | TGATATGGACGAGCAGGATG |
| PFP13s | GTTCTAGAGAAGTATAGTTTCGACATCGTGGAG |
| PFP19s | CTAGCGGTTCTTGACGGTAC |
| PFP21as | CCTTGTCTTCGAATCGAGCCAG |
| PFP23as | AGCAGGCTCGCTTCTTTTAC |
| PFP27s | ACTGCTGGCCAAGATTCAATTG |
| PFP48s | TCGGGCCCCGTATCTTGGCTTCCCAA |
| PFP49as | GAGGGCCCAAAGATTTCAAATGTTGC |
| pSS2-ble3s | GCCAAGTTGACCAGTGCCGT |
| pSS2-ble4as | CAGTCCTGCTCCTCGGCCAC |

**TABLE S2.**

**(A) Total amino acid sequence comparison**

| | | Identity [%] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PFP1 | NTO1 | BRPF1[a] | BRPF2[b] | BRPF3 | JADE-1 | JADE-2 | JADE-3 | EPL1 |
| Similarity [%] | PFP1[IV c] | - | 18.6 | 22.3 | 19.3 | 22.1 | 19.0 | 18.4 | 18.1 | 13.6 |
| | NTO1[IV] | 29.4 | - | 15.1 | 17.2 | 20.0 | 17.3 | 18.9 | 18.0 | 13.4 |
| | BRPF1[III] | 33.4 | 24.0 | - | 49.7 | 45.7 | 19.6 | 19.0 | 19.0 | 12.4 |
| | BRPF2[III] | 28.3 | 28.1 | 62.1 | - | 50.4 | 21.6 | 19.8 | 19.5 | 14.1 |
| | BRPF3[III] | 34.2 | 32.9 | 56.7 | 62.3 | - | 21.9 | 22.1 | 22.9 | 16.2 |
| | JADE-1[IV] | 30.0 | 30.4 | 29.0 | 33.0 | 33.9 | - | 46.9 | 42.5 | 12.7 |
| | JADE-2[IV] | 28.8 | 29.2 | 28.6 | 29.8 | 33.0 | 58.9 | - | 38.8 | 12.5 |
| | JADE-3[IV] | 28.8 | 30.2 | 28.5 | 29.3 | 34.5 | 56.1 | 52.5 | - | 14.0 |
| | EPL1[II] | 24.3 | 25.8 | 23.9 | 25.2 | 27.7 | 22.4 | 23.5 | 25.7 | - |

[a] Alternative names: BR140, peregrin. [b] alternative names: BRD1, BRL. [c] Roman numbers indicate Epc-N classes. Proteins belonging to mainly histone 3-acetylating HAT complexes are marked in grey. GenBank accession numbers: NTO1, NP_015356; BRPF1, NP_001003694; BRPF2, XP_005261529; BRPF2, AAI17388; JADE-1, NP_955352; JADE-2, XP_005272000; JADE-3, NP_055550; EPL1, EEU04231. Sequences were aligned pairwise using the matrix BLOSUM62 of the European Molecular Biology Open Source Software Suite (www.ebi.ac.uk/tools/psa/emboss_needle/).

**(B) Comparison of Epc-N domains**

| | | Identity [%] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | PFP1 | NTO1 | BRPF1[a] | BRPF2[b] | BRPF3 | JADE-1 | JADE-2 | JADE-3 |
| Similarity [%] | PFP1[IV c] | - | 29.0 | 37.1 | 29.6 | 28.8 | 24.3 | 27.7 | 27.3 |
| | NTO1[IV] | 42.4 | - | 29.6 | 29.8 | 28.1 | 28.3 | 25.9 | 28.5 |
| | BRPF1[III] | 51.8 | 44.5 | - | 58.9 | 55.4 | 29.9 | 28.3 | 29.5 |
| | BRPF2[III] | 39.2 | 46.1 | 67.7 | - | 59.4 | 30.1 | 28.5 | 30.8 |
| | BRPF3[III] | 41.9 | 43.7 | 66.1 | 73.5 | - | 28.5 | 28.0 | 29.9 |
| | JADE-1[IV] | 36.4 | 45.3 | 40.5 | 44.3 | 42.0 | - | 62.0 | 61.7 |
| | JADE-2[IV] | 41.6 | 39.0 | 39.4 | 40.2 | 40.5 | 72.0 | - | 56.1 |
| | JADE-3[IV] | 39.0 | 45.4 | 43.0 | 44.8 | 40.0 | 76.3 | 69.6 | - |

[a] Alternative names: BR140, peregrin. [b] alternative names: BRD1, BRL. [c] Roman numbers indicate Epc-N classes. Proteins belonging to mainly histone 3-acetylating HAT complexes are marked in grey. GenBank accession numbers: NTO1, NP_015356; BRPF1, NP_001003694; BRPF2, XP_005261529; BRPF2, AAI17388; JADE-1, NP_955352; JADE-2, XP_005272000; JADE-3, NP_055550. Sequences were aligned pairwise using the matrix BLOSUM62 of the European Molecular Biology Open Source Software Suite (www.ebi.ac.uk/tools/psa/emboss_needle/).

**(C) Comparison of PHD-like domains**

| | | Identity [%] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | PFP1 | NTO1 | BRPF1[a] | BRPF2[b] | BRPF3 | JADE-1 | JADE-2 | JADE-3 |
| Similarity [%] | PFP1$^{IV}$ [c] | - | 43.1 | 52.5 | 51.2 | 50.8 | 42.9 | 41.2 | 46.3 |
| | NTO1$^{IV}$ | 62.6 | - | 47.5 | 48.4 | 47.5 | 45.9 | 43.4 | 42.6 |
| | BRPF1$^{III}$ | 68.0 | 68.9 | - | 81.7 | 81.7 | 46.3 | 44.6 | 49.6 |
| | BRPF2$^{III}$ | 64.8 | 70.5 | 90.0 | - | 75.0 | 47.9 | 43.8 | 49.6 |
| | BRPF3$^{III}$ | 68.0 | 68.0 | 90.8 | 88.3 | - | 45.6 | 42.4 | 47.2 |
| | JADE-1$^{IV}$ | 58.8 | 58.2 | 55.4 | 55.4 | 55.2 | - | 82.3 | 78.8 |
| | JADE-2$^{IV}$ | 57.1 | 56.6 | 55.4 | 55.4 | 53.6 | 90.3 | - | 76.1 |
| | JADE-3$^{IV}$ | 57.9 | 56.6 | 61.2 | 58.7 | 56.0 | 87.6 | 87.6 | - |

[a] Alternative names: BR140, peregrin. [b] alternative names: BRD1, BRL. [c] Roman numbers indicate Epc-N classes. Proteins belonging to mainly histone 3-acetylating HAT complexes are marked in grey. GenBank accession numbers: NTO1, NP_015356; BRPF1, NP_001003694; BRPF2, XP_005261529; BRPF2, AAI17388; JADE-1, NP_955352; JADE-2, XP_005272000; JADE-3, NP_055550. Sequences were aligned pairwise using the matrix BLOSUM62 of the European Molecular Biology Open Source Software Suite (www.ebi.ac.uk/tools/psa/emboss_needle/).

Fig. S1

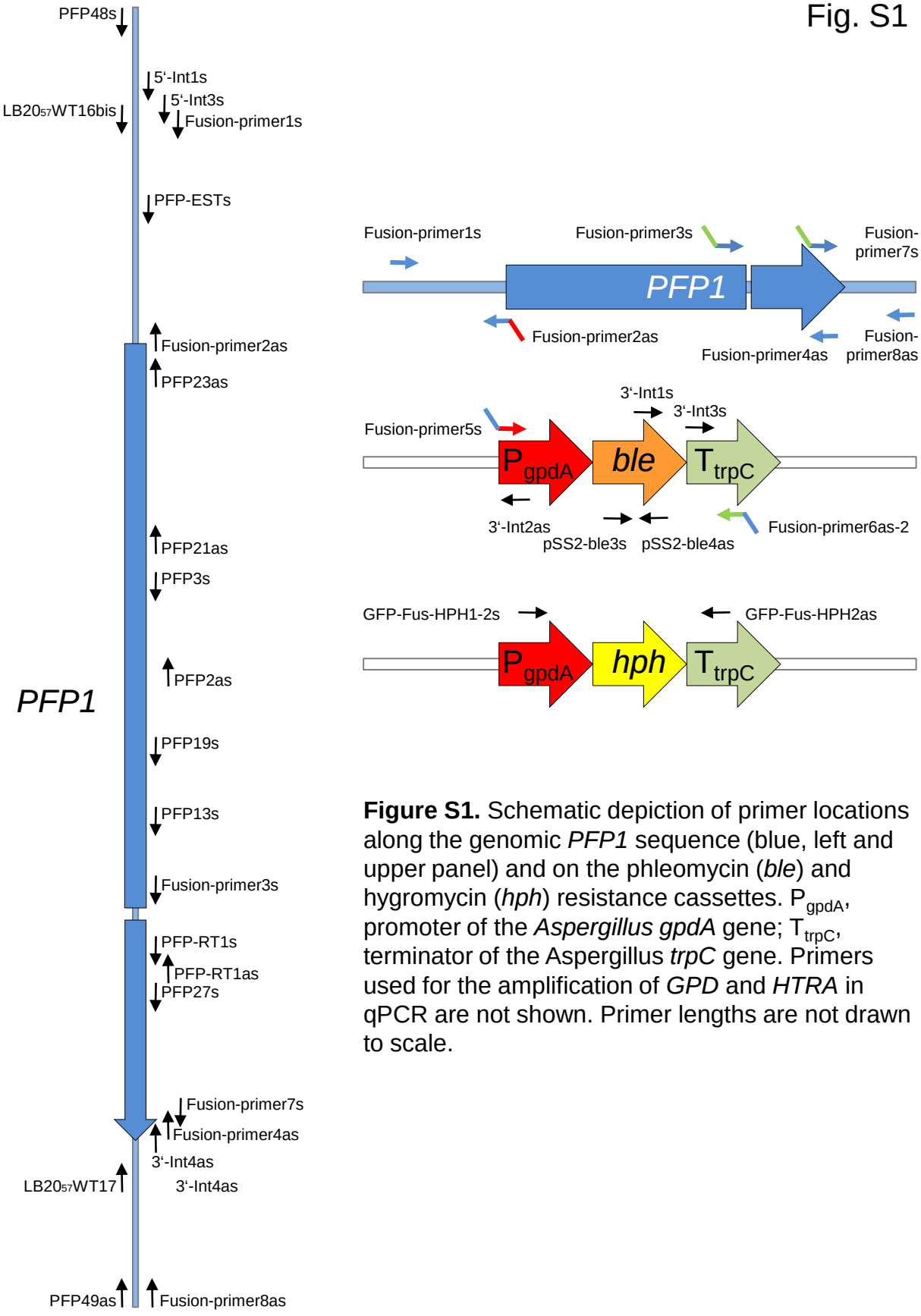


**Figure S1.** Schematic depiction of primer locations along the genomic *PFP1* sequence (blue, left and upper panel) and on the phleomycin (*ble*) and hygromycin (*hph*) resistance cassettes. $P_{gpdA}$, promoter of the *Aspergillus gpdA* gene; $T_{trpC}$, terminator of the Aspergillus *trpC* gene. Primers used for the amplification of *GPD* and *HTRA* in qPCR are not shown. Primer lengths are not drawn to scale.

Fig. S2

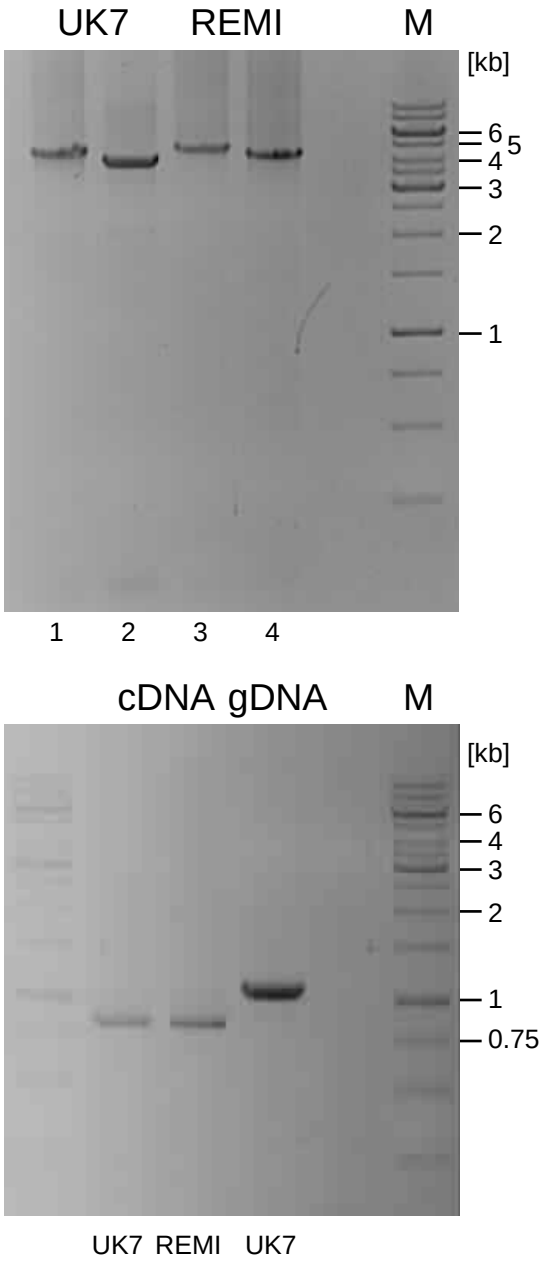


**Figure S2.** Determination of PFP1 mRNA length. **Upper panel:** RT-PCR products obtained with RNA from fungal wild-type isolate UK7 and from the REMI mutant, respectively. Fusion-primer4as was combined with four 5' primers: 1, Fusion-primer1s (4628 bp); 2, PFP1 EST s (4218 bp); 3, HPH1s (4878 bp); 4, HPH-200f (4539 bp). **Bottom panel:** The cDNA was tested for the presence of genomic DNA by amplifying the GPD sequence (cDNA: 861 bp, gDNA: 1115 bp) using the primers GPD-cDNA-test-s and GPD-cDNA-test-as.

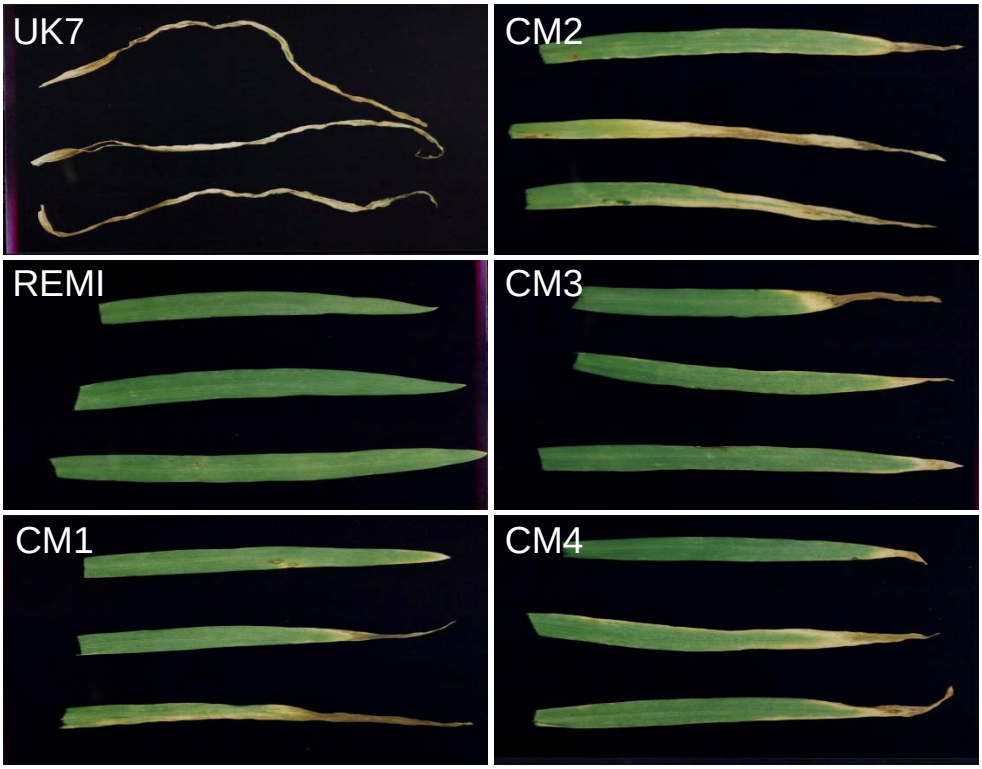


**Figure S3.** Disease phenotype caused by wild-type strain UK7, the REMI mutant and four independent REMI complementation mutants (CM1-CM4) and on susceptible barley cultivar ‘Sloop’ 28 dpi.

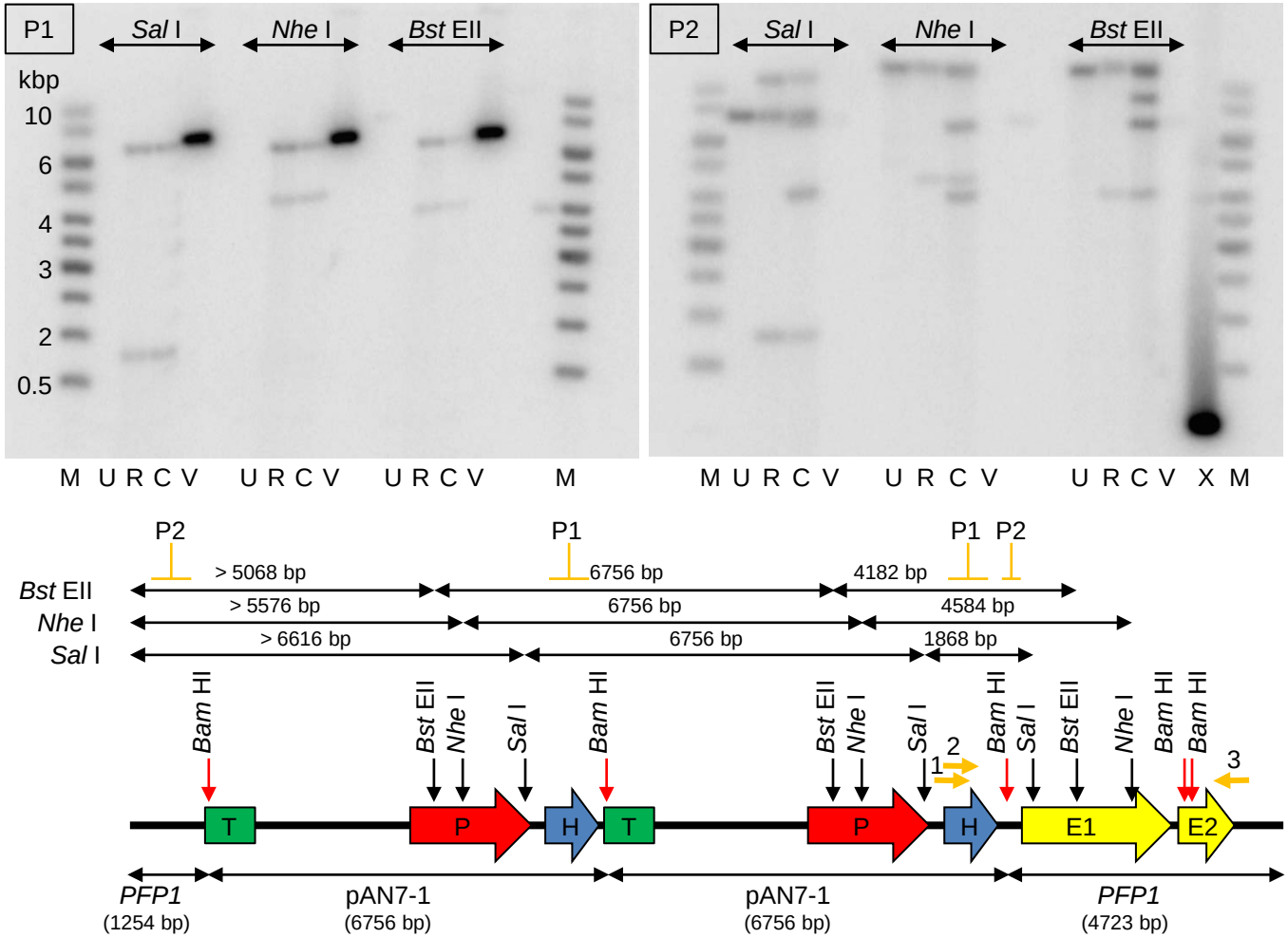


**Figure S4.** The plasmid pAN7-1 was integrated in tandem in a head-to-tail manner. **Upper panels:** DNA hybridization using DNA cleaved with the restriction enzymes *Sal* I, *Nhe* I and *Bst* EII, respectively, and probes from the *hygromycin B phosphotransferase* (*hph*) sequence (P1) or from the *PFP1* upstream region (P2). Lanes: M, size marker ladder; U, wild-type strain UK7; R, REMI mutant; C, REMI complementation mutant; V, linearized pAN7-1 as vector control; X, *Eco* RI-cleaved *PFP1* 5' UTR cloned into pCR2.1. **Bottom panel:** Schematic depiction of the plasmid integration site in the 5977-bp *PFP1* genomic sequence. E1, E2, *PFP1* exons 1 and 2; H, *hph* gene; P, promoter of the *GPDA* gene from *Aspergillus nidulans*; T, termination sequence of the *trpC* gene from *A. nidulans*. P1 and P2 indicate the probe hybridization regions. *Sal* I, *Nhe* I and *Bst* EII restriction sites are marked by black arrows, *Bam* HI sites by red arrows. Upper double-headed arrows show the position and size of the fragments identified after DNA restriction by Southern hybridization, bottom double-headed areas mark the two pAN7-1 copies and the flanking *PFP1* sequence. Orange arrows: 1, HPH1s; 2, HPH200-f; 3, Fusion-primer4as.

Fig. S4

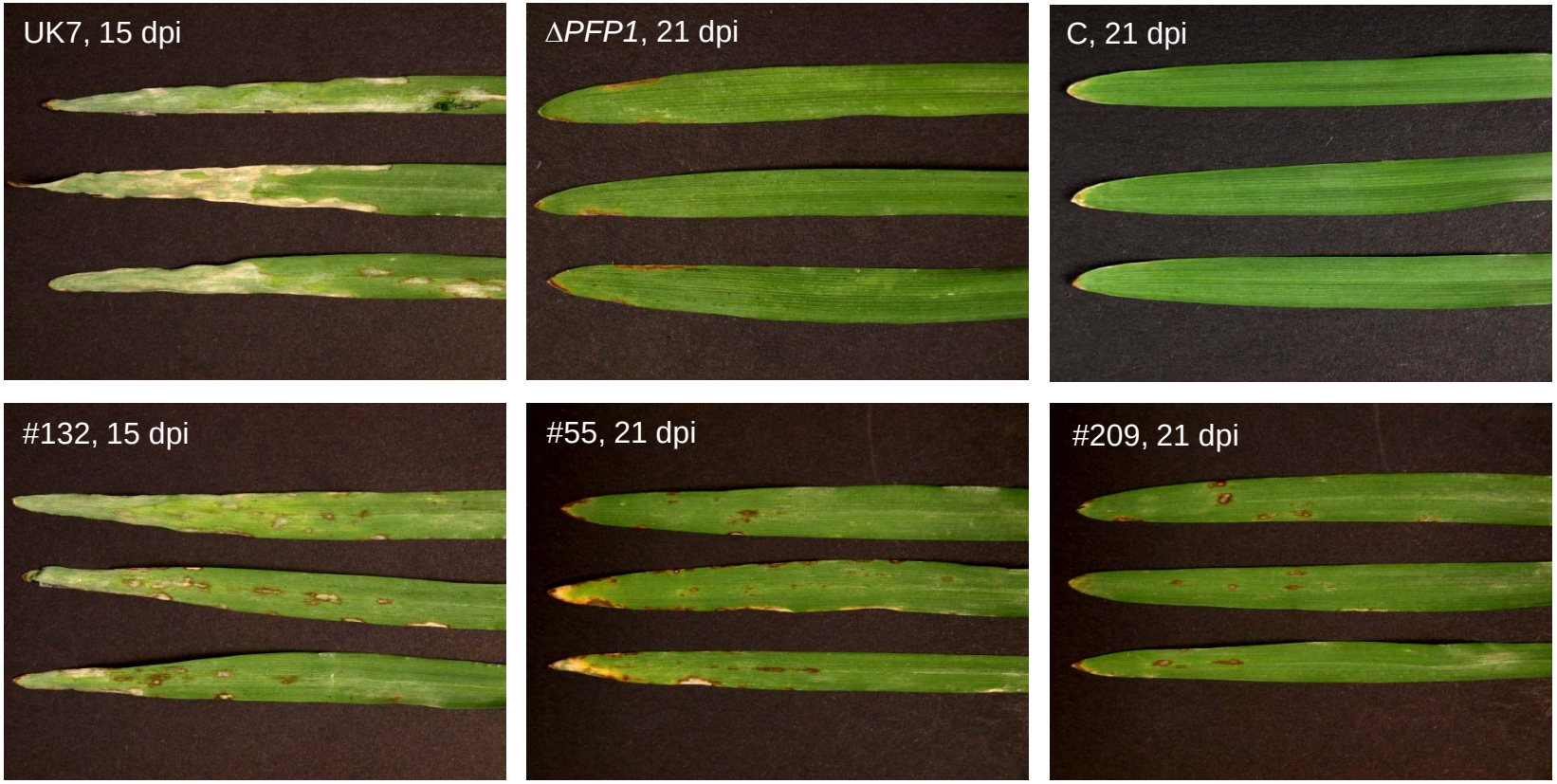


**Figure S5.** Disease phenotype on susceptible barley cultivar 'Ingrid' of fungal wild-type strain UK7, of the deletion mutant UK7D*PFP1* (D*PFP1*) and of three UK7D*PFP1* complementation mutants (#55, #132, #209). Disease symptoms were photographed at 15 dpi for wild-type strain UK7 and the most virulent complementation mutant #132, at 21 dpi for the less virulent mutants #55 and #209. C, non-inoculated control leaves.

Fig. S5