

Supplementary Figure 1. Targeted disruption of the *F. oxysporum fhk1* gene.

A. Physical maps of the *fhk1* locus and the gene replacement construct obtained by PCR fusion ($\Delta fhk1$ allele). **B**. Southern hybridization analysis of wild type strain 4287 (1) and transformants *efhk1-1* (2), $\Delta fhk1-2$ (3), $\Delta fhk1-4$ (4), $\Delta fhk1-7$ (5), $\Delta fmk1$ (6) and $\Delta fmk1\Delta fhk1$ (7). Genomic DNA treated with *PstI* was hybridized with the probe indicated in A. **C.** PCR amplification of genomic DNA of the wild-type strain 4287 (1) knockout mutant $\Delta fhk1-2$ (2), and complemented strains $\Delta fhk1+fhk1$ 1 (3) and $\Delta fhk1+fhk1$ 2 (4), using primers Fhk1-1 and Fhk1-2 (*fhk1*) or M13 Forward and PHL (*phleo*).



Supplementary Figure 2. Fhk1 is not required for vegetative hyphal growth.

Conidia of the indicated strains were spotted onto plates with synthetic medium (SM) buffered at the indicated pH values and grown at 28°C for 3 days.



Supplementary Figure 3. Fhk1 is not required for invasive growth.

Microconidial suspensions of the indicated strains were applied for the different invasive growth assays. **A.** Invasive growth on apple slices inoculated with microconidia and incubated at 28°C for 4 days. **B.** Penetration of cellophane sheets. Colonies were grown for 4 days on a plate with minimal medium covered by a cellophane sheet (before), then the cellophane with the colony was removed and plates were incubated for an additional day (after).