

A FEEDBACK MECHANISM REGULATES THE PMK1 MITOGEN-ACTIVATED PROTEIN KINASE PATHWAY IN THE RICE BLAST FUNGUS

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Background

- Yearly 30% of global rice crop yields are lost due to blast disease caused by *Magnaporthe oryzae*
- Pathogenicity Mitogen activated protein Kinase 1 (Pmk1) is essential for *M. oryzae* infection-related development at multiple stages of development
- It is not known how Pmk1 is regulated such that it can perform multiple functions at drastically different stages of development
- In other organisms like *Saccharomyces cerevisiae* Pmk1 orthologues are regulated by feedback phosphorylation which controls the timing of their activation

Big Biological Question

- Determining detailed regulation of Pmk1 at molecular level

Objective

- Identify Pmk1-dependent phosphosites in upstream components of Pmk1 pathway from mass spectrometry data
- Assess biological relevance of phosphosites by computational and genetic approaches

M. oryzae infection cycle

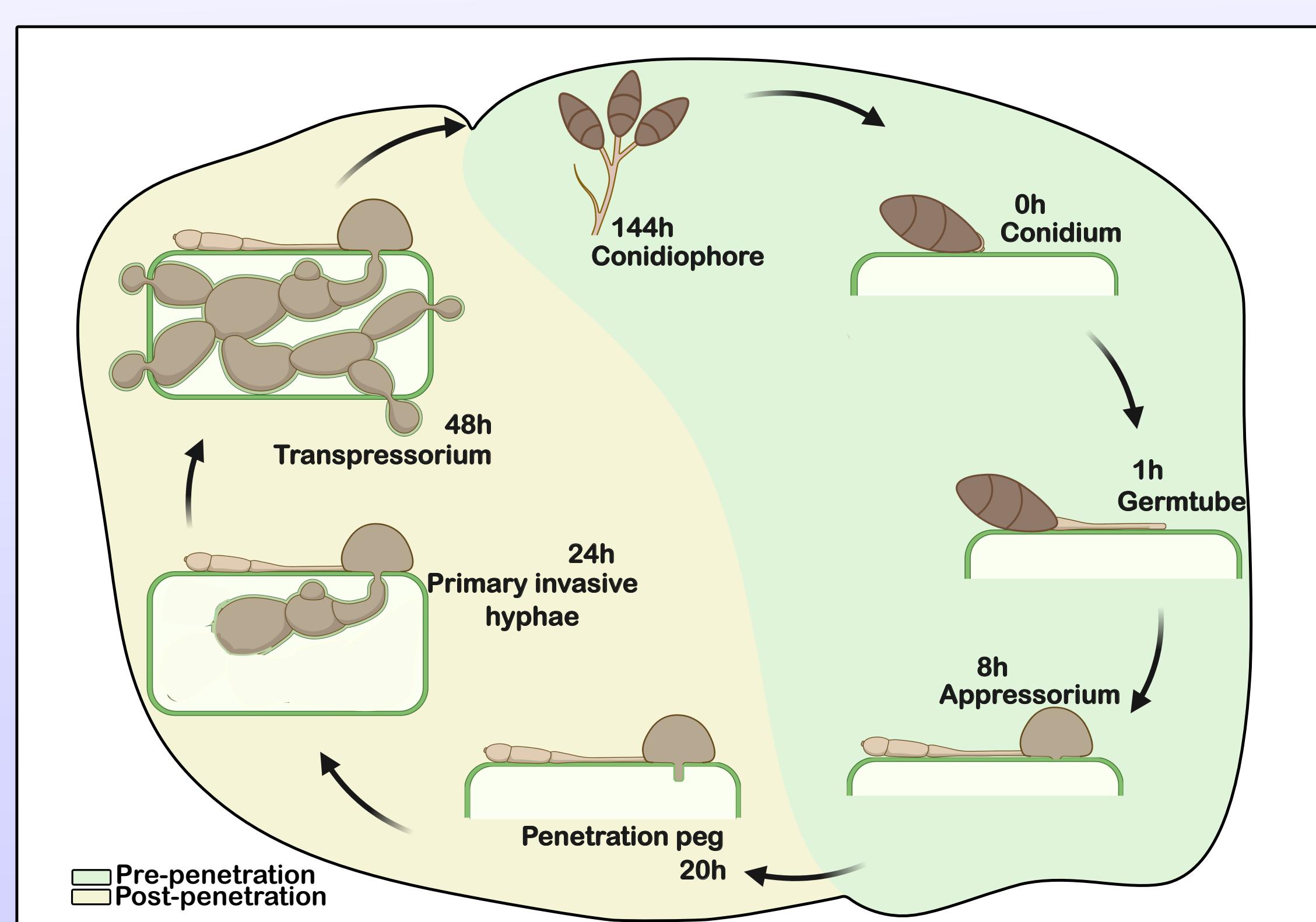


Figure 1. A schematic representation of different developmental stages of the *M. oryzae* life cycle during infection of a rice plant. A specialised cell, called an appressorium, is required for penetration of the leaf surface, essential for successful infection of the host and completing the life cycle.

Phosphorylated residue at position S358 in *M. oryzae* Mst7 is conserved across a range of fungal species

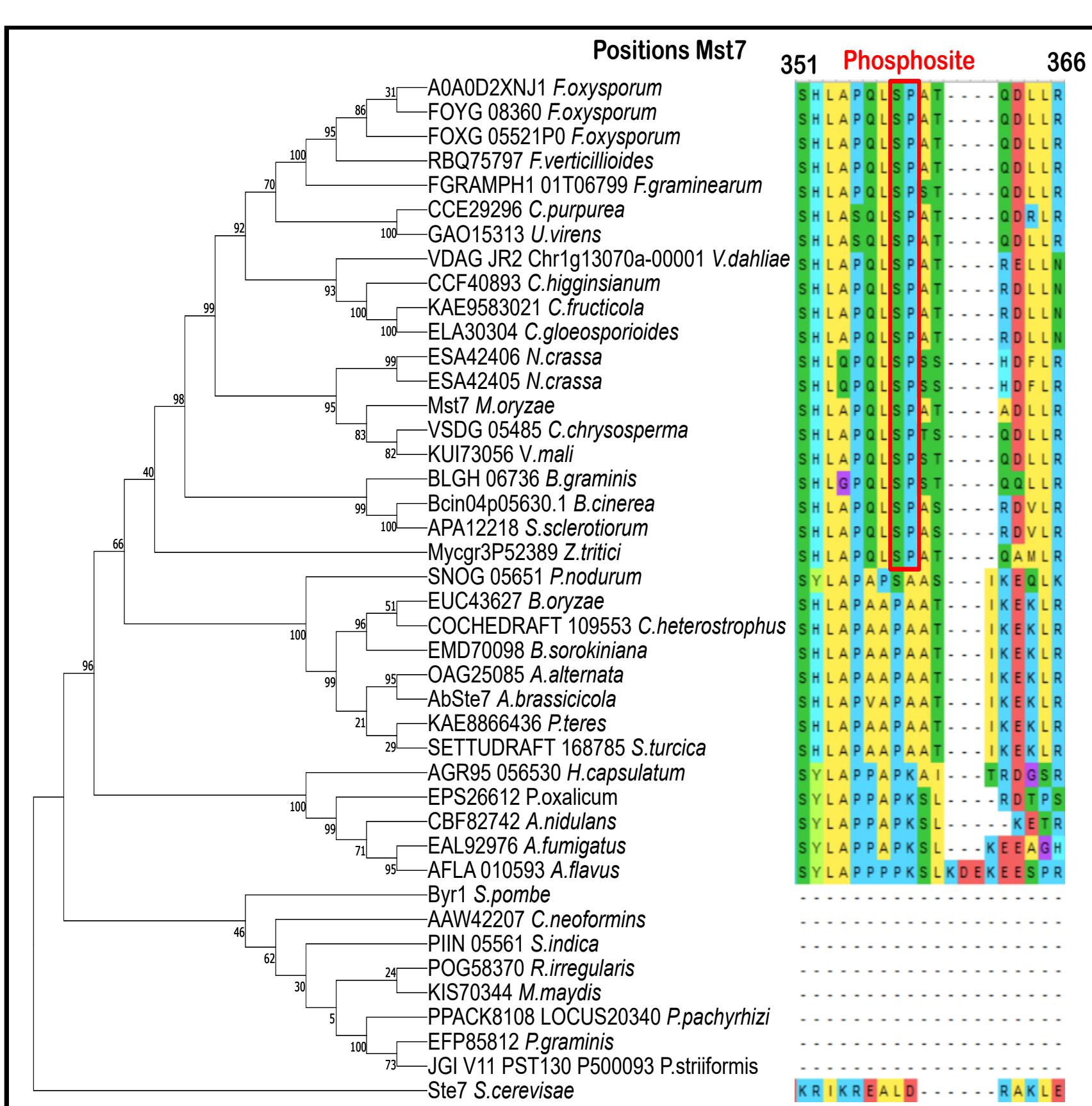


Figure 3. A phylogenetic analysis of Mst7 orthologues shows SP motif is conserved amongst fungal species of class Sordariomycetes and Leotiomycetes. Mst7 orthologues were identified using orthofinder and a phylogenetic tree of these orthologues constructed with MegaX using 1000 bootstraps. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Sequences from proteins used for phylogenetic tree are shown aligned with residues 351 to 366 of Mst7.

Mst7 S358D phosphomimetic allele has reduced appressorium formation

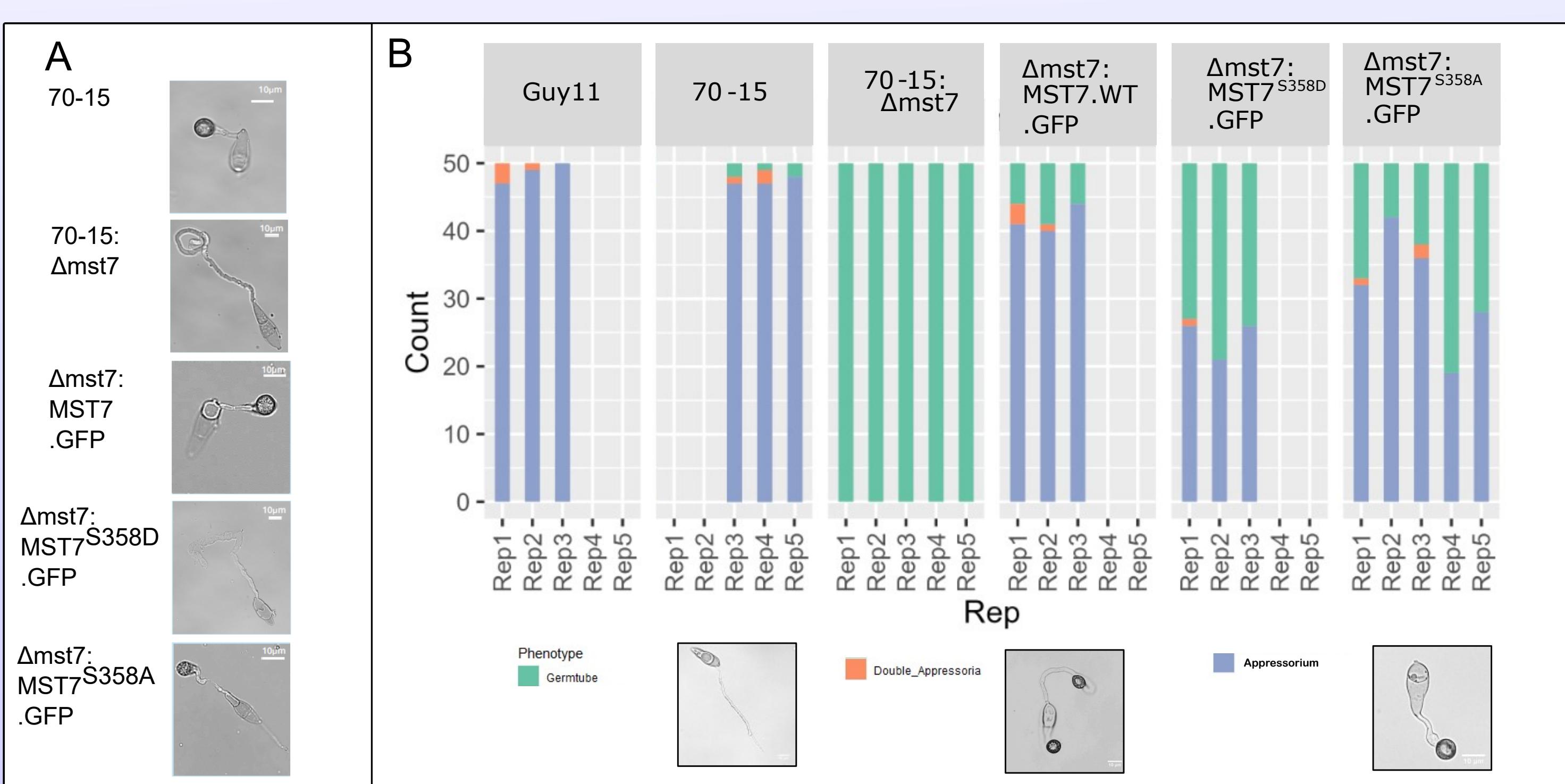


Figure 4. Substitution of serine residue 358 in Mst7 for a negatively charged aspartate residue mimics phosphorylation of the serine residue. A) Introducing this substitution to the Pmk1 dependent phosphosite in Mst7 reduced appressorium formation suggesting it is a negative regulator of the Pmk1 cascade. B) Quantification of appressorium phenotype. Construct expression was confirmed by western blot analysis (data not shown).

Pmk1 is a key regulator of appressorium development

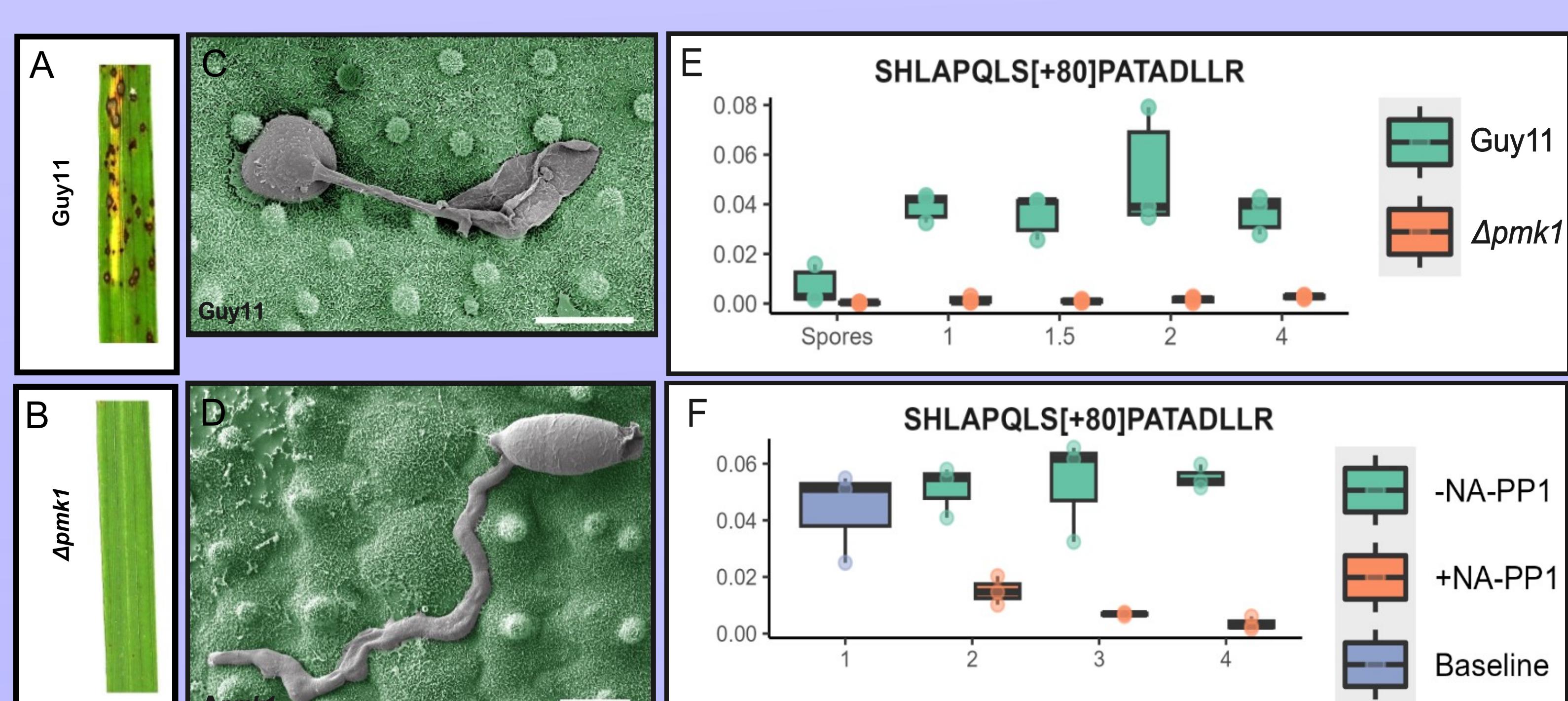
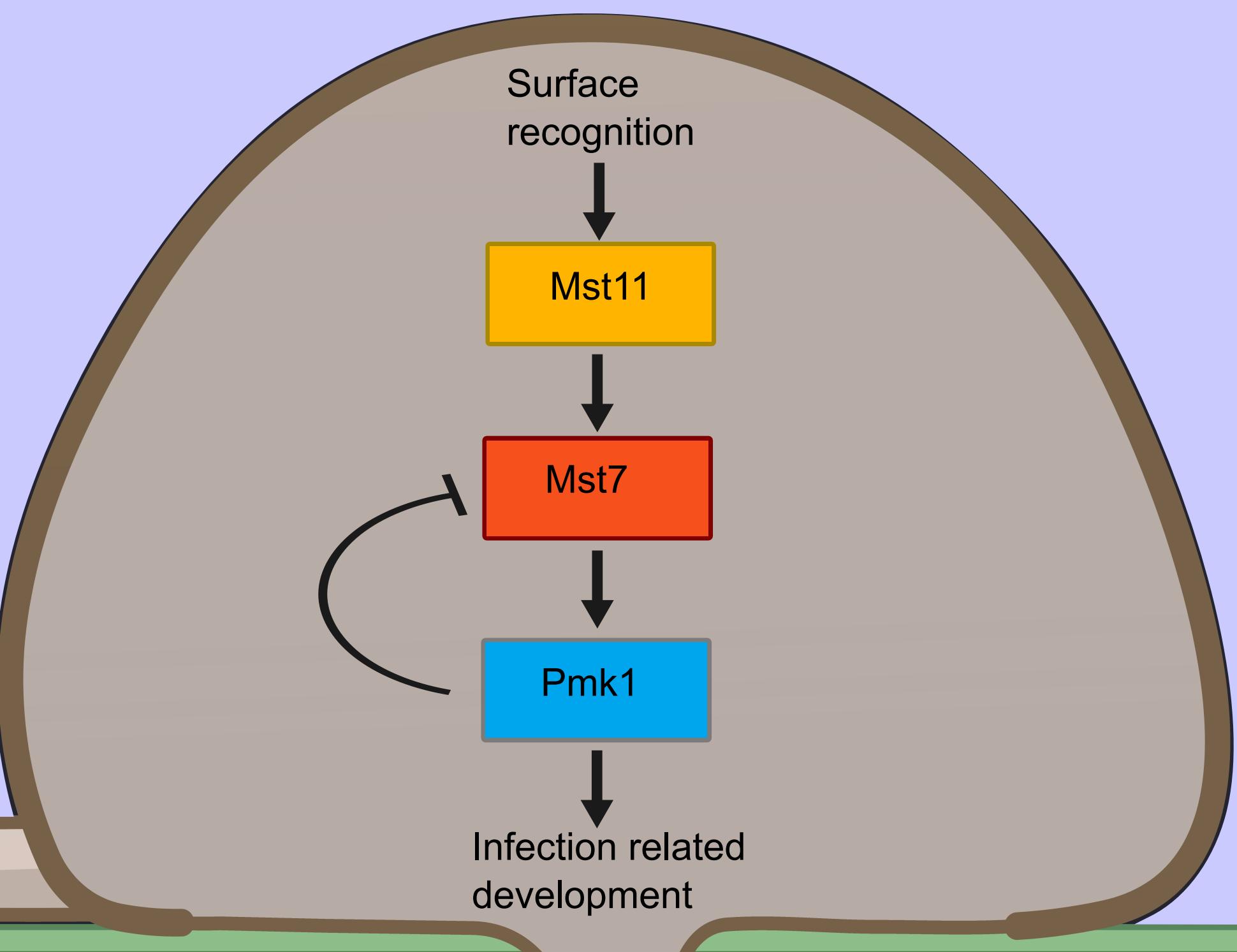


Figure 2. A large scale phosphoproteomic analysis identified Pmk1 dependent phosphosites during infection related development. Wild type *M. oryzae* (Guy11) can infect rice leaf (A) and form appressoria (C). Pmk1 null mutant ($\Delta pmk1$) fails to infect rice leaf (B) or form appressoria (D). A phosphosite on residue S358 in Mst7 is detected in wild type *M. oryzae* but not in $\Delta pmk1$ mutant (E) suggesting this site is a target of Pmk1 phosphorylation (Cruz-Mireles et al., 2024). The same phosphosite was identified by a chemical genetic approach (F). Phosphorylated residue shown in square brackets.

Pmk1 phosphorylation of Mst7 S358 forms a negative feedback loop



Summary
• Phosphorylation of Ser358 in Mst7 is shown to negatively regulate the Pmk1 cascade

Significance

• These results demonstrate a novel mode of regulation for the Pmk1 pathway during infection-related development

Acknowledgments

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