Supplementary Data

In order to determine the optimal concentrations for pheromone as well as cAMP treatment we performed dose response experiments (Supplementary Fig. 1). In case of synthetic a2 pheromone a concentration of 2.5 µg/ml resulted in maximal response. This holds true for reporter gene expression stimulating FB1mfa1-egfp (Supplementary Fig. 1A) as well as β gene expression in strains expressing prf1 constitutively (Supplementary Fig. 1C). The optimal concentration for cAMP is 6 mM, because higher concentrations lead to an increase of cells with defects in cytokinesis, a response not observed during mating (Supplementary Fig. 1B).

Putative phosphorylation sites in the central part of Prf1 are functionally important

To eliminate possible complications that might arise from autoregulation of prf1 (Hartmann et al., 1999) we performed mating assays with strains expressing prf1 alleles under control of the constitutively active tef1 promoter (Spellig et al., 1996). Crosses of strains expressing prf1eM1-6d or prf1eP1-5 exhibited reduced formation of dikaryotic hyphae in contrast to the prf1e expressing control verifying that mutation of PKA or MAPK phosphorylation sites caused reduced mating (Supplementary Fig. 2).

In a more sensitive assay for Prf1 activity we selected several strains harbouring mutant alleles for plant infection experiments (Supplementary Table 1). As expected, crosses of wild type strains and prf1e expressing strains formed tumours efficiently (94 % and 78 % plants with tumours, respectively). prf1eM1-6d expressing strains were nonpathogenic and prf1eM345 mutants were reduced in tumour formation (11 %). Strains harbouring prf1eP1-5 were severely reduced in pathogenicity (2 % tumour formation) and strains carrying prf1eP34 were slightly reduced in their ability to induce tumours (61 %). Testing strains that express prf1 alleles
constitutively revealed that in contrast to the control allele prf1<sup>ce</sup> (76 %), strains containing either prf1<sup>ceM1-6d</sup> or prf1<sup>ceP1-5</sup> were unable to form tumours. This stresses the necessity for posttranscriptional activation of Prf1 during tumour formation.

**Supplementary Fig. 1.** Effect of increasing amounts of either synthetic a2 pheromone (A) or cAMP (B) on reporter gene expression in strain FB1mfa1-egfp. Relative fluorescence units (RFU) were measured and normalized to optical density (OD<sub>600</sub>). Cells were stimulated with
increasing amounts of synthetic a2 pheromone [µg/ml, closed circles] for 6 h or cAMP [mM, closed circles] for 18 h in A and B, respectively. In addition the dependency between cAMP concentration and cells that are defective in cytokinesis is given in B (open circles). The stippled lines in A and B indicate the concentrations of pheromone (2.5 µg/ml) and cAMP (6 mM) that have been used throughout this work. In C, FB1 derivatives indicated at the top were incubated for 6 hours either in absence or presence 2.5 or 5 µg/ml synthetic a2 pheromone. 10 µg of total RNA was loaded and the same RNA was analysed with probes indicated on the right.

**Supplementary Fig. 2.** Mating assays on plates containing activated charcoal. White, fuzzy colonies reflect the formation of b-dependent aerial hyphae. Respective FB2 (a2b2) derivatives labelled on the left were either inoculated alone, with FB1 (a1b1), with FB1 derivatives carrying identical prf1 alleles, or with pheromone tester strain CL13 (a1bW2bEl) given on the top. prf1ceM1-6d indicate that respective alleles were under control of the constitutively active tef1 promoter (Spellig et al., 1996).
### Supplementary Table I. Pathogenicity assays

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<td>12</td>
<td>11</td>
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### Supplementary Methods

#### Plasmids and plasmid constructions

Standard molecular techniques were followed (Sambrook et al., 1989). Plasmids pSP72 (Promega), pCR2.1-Topo (Invitrogen), pBluescriptSKII (Stratagene), pGEX-2T (Amersham Biosciences), pEG202 (pLexA), and pJG4-5 (Gyuris et al., 1993) were used as cloning vehicles. Plasmid pmfa1-egfp-cbx (pUMa12) was constructed by inserting a 723 bp NcoI-NotI fragment encoding the enhanced version of the green fluorescent protein from *Aequorea victoria* (pEGFP-N1; Clontech) into plasmid pMFA1-SG (Spellig et al., 1996). To generate pmfa1PREA-egfp-cbx (pUMa172) a 646 bp PvuII-BstEII fragment was deleted from pmfa1-egfp-cbx. Plasmid pdik6-egfp-cbx (pUMa181) was constructed by inserting a 303 bp NcoI-Acc65I fragment containing the dik6 basal promoter (Bohmann, 1996) in the pmfa1-egfp-cbx backbone. To construct pPREdik6-egfp-cbx (pUMa182) a 145 bp PacI-Acc65I fragment,
derived from PRE6-topo (pUMa65) was inserted into pdik6-egfp-cbx. In PRE6-topo a PCR product with primers MF60 (5’-ACTTAATTAAACAAAGGGATGGTATAC- AAAGGGATAGTCCACAAAGGGATGTCGG-3’) and MF61 (5’-CCTCCCTTTGTGATA- ACTCCCTTTGTCCCATAGTCCCTTTGTCCGACATCCCCCTTTGTGAG-3’) was inserted in pCR2.1-TOPO (Invitrogen).

Plasmid pkpp2K50R-nat has been published elsewhere (Müller et al., 2003). Plasmid pprf1^{EM1-6}-hyg (pUMa185) was generated by replacing a 420 bp MscI-BamHI fragment from pprf1^{EM1-6d}-hyg (Müller et al., 1999) with the wild type sequence from pprf1^{E}-hyg (Müller et al., 1999). Plasmid pprf1^{EM2}-hyg (pUMa186) was generated by replacing a 420 bp MscI-BamHI fragment from pprf1^{E2}-hyg with the mutated sequence from pprf1^{EM1-6d}-hyg. Plasmid pprf1^{EM126}-hyg (pUMa187) was constructed by replacing a 780 bp BsiWI-BamHI fragment from pprf1^{EM1-6d}-hyg with the wild type sequence from pprf1^{E}-hyg. Plasmid pprf1^{EM345}-hyg (pUMa188) was generated by replacing a 780 bp BsiWI-BamHI fragment from pprf1^{E}-hyg with the mutated sequence from pprf1^{EM1-6d}-hyg. Plasmid pprf1^{EM34}-hyg (pUMa190) was constructed by replacing a 839 bp MluI-SacII fragment from pprf1^{E}-hyg with the mutated sequence from pprf1^{EM345}-hyg. We generated plasmid pprf1^{EM5}-hyg (pUMa189) by replacing a 661 bp SacII-BamHI fragment from pprf1^{E}-hyg with the mutated sequence from pprf1^{EM345}-hyg. We constructed plasmid pprf1^{EM3}-hyg (pUMa96) and pprf1^{EM4}-hyg (pUMa97) by replacing a 358 bp BsiWI-MscI fragment from pprf1^{EM}-hyg with respective mutated sequences from pprf1^{EM345}-hyg. Plasmid pprf1^{E1-5}-hyg (MF28) was generated by replacing a 1500 bp MluI-BamHI fragment from pprf1^{E}-hyg with mutated sequences, according to the construction of pprf1^{EM1-6d}-hyg (Müller et al., 1999). The following oligonucleotide megaprimers were used to substitute the indicated amino acid by alanine: T253, 5’-GGCA-CGCAAGGCGGCGCCGCTTCCGTGGC-3’, S366, 5’-TGACCGGTACAGACGCGGACGC- CGGC-3’, S511S512, 5’-AGATCTGAGGAGACGCAGTGCACCTTGACAGATCTGGTC-3’, S659, 5’-GCTAGCATGAAAAGGCGTCGGGCCCCAAATGGGACAGACGC-3’. Plasmid
ppr1<sup>ΔP125</sup>-hyg (pUMa78) was constructed by replacing a 358 bp BsiWI-MscI fragment from ppr1<sup>ΔP1-5</sup>-hyg with wild type sequence from ppr1<sup>ε</sup>-hyg. Plasmid ppr1<sup>ΔP34</sup>-hyg (pUMa79) was constructed by replacing a 358 BsiWI-MscI fragment from ppr1<sup>ε</sup>-hyg with mutated sequences from ppr1<sup>ΔP1-5</sup>-hyg. For the construction of ppr1<sup>ε-C</sup>-hyg, five fragments were combined in pSP72 (Promega). Three fragments were derived from ppr1<sup>ε</sup>-hyg, a 1675 bp SphI-XbaI fragment, a 1165 bp Ncol-AgeI fragment, and a 2361 bp AgeI-SacI fragment. An additional 2772 bp AvrII-XhoI fragment contained the hygromycin resistance cassette from pSL-hyg (Müller et al., 1999) and the last 291 bp XhoI-Ncol fragment included the tef1 promoter from pSGFP-tef (Spellig et al., 1996). Plasmids ppr1<sup>εM1-6d</sup>-hyg (pUMa184) and ppr1<sup>εP1-5</sup>-hyg (pUMa183) were constructed by replacing a 2046 bp MluI-Acc65I fragment from ppr1<sup>ε</sup>-hyg with mutated sequences from ppr1<sup>εM1-6d</sup>-hyg and ppr1<sup>εP1-5</sup>-hyg, respectively. pPr1<sup>ε-NLS</sup> (pUMa58) was constructed by inserting a 2712 bp EcoRI-Acc65I fragment containing an epitope-tagged Prf1<sup>ε</sup> ORF in plasmid pJG4-5 linearized with Acc65I and XhoI by oligonucleotide adapters (Gyuris et al., 1993). Thereby the SV40 large T antigen nuclear localisation signal was N-terminally fused to Prf1<sup>ε</sup>. pLexA-Kpp2 (pUMa76) was generated by inserting a 1090 bp EcoRI-PstI fragment containing the kpp2 ORF (Müller et al., 1999) in pEG202 (Gyuris et al., 1993). pLexA-Adr1 (pUMa521) was constructed by replacing kpp2 from pLexA-Kpp2 with a 1280 bp Ncol-BamHI fragment encoding Adr1 (Dürenberger et al., 1998). We constructed pGST-Prf1<sup>140-565</sup> (pUMa98) by inserting a 390 bp Ncol-EcoRI fragment in pGEX-2T (Amersham Biosciences). The Ncol site was introduced using oligonucleotide 5’-GCCCATGGCCTCGTACGACGACCAGTCAC-3’ in a PCR reaction with ppr1<sup>ε</sup>-hyg as template. For cloning compatibility the Ncol site from the prf1 insert and the BamHI site from pGEX-2T were both converted to blunt ends by Klenow polymerase. pGST-Prf1<sup>140-565M345</sup> (pUMa404) and pGST-Prf1<sup>140-565P34</sup> (pUMa403) were constructed by replacing a 381 bp BsiWI-EcoRI fragment from pGST-Prf1<sup>140-565</sup> with mutated sequences from ppr1<sup>εM1-6d</sup>-hyg and ppr1<sup>εP1-5</sup>-hyg, respectively. Plasmids pGST-Kpp2
(MF32) and pGST-Adr1 (MF35) were generated by inserting NcoI-EcoRI fragments in pGST-Prf1\textsuperscript{440-565} that contained complete open reading frames of \textit{kpp2} (1364 bp) and \textit{adr1} (1601 bp), respectively (Dürrenberger \textit{et al.}, 1998; Müller \textit{et al.}, 1999). All constructions were confirmed by sequencing and all plasmid sequences are available upon request.

Expression of proteins used in \textit{in vitro} kinase assays

Protein expression was performed as follows. BL21(DE3)pLysS cells harbouring plasmids pGEX-Prf1\textsuperscript{440-565}, pGEX-Prf1\textsuperscript{440-565M345}, pGEX-Prf1\textsuperscript{440-565P34}, pGEX-Kpp2, or pGEX-Adr1 were grown in dYT medium and ampicillin (100 µg/ml) at 37°C. At OD\textsubscript{600} of 1 cells were induced with 0.2 mM IPTG for 16 h at 20°C. 50 ml cells were harvested and washed once with sterile water and resuspended in 2 ml buffer A (100 mM NaPi, pH 8; 150 mM NaCl; 5 mM DTT) containing protease inhibitor cocktail (Complete, Roche 1873580). Cells were sonicated and insoluble components were removed by centrifugation (4°C, 30 min, 50000 g). The supernatant was incubated with 50 µl of GSH-sepharose beads (Amersham Biosciences) 20 min at 4°C. Beads were washed five times with buffer A. Elution of GST fusion proteins was performed using 20 mM reduced glutathione. Protein concentration was determined according to Bradford, 1976.

\textit{Supplementary References}


