

Fig. S1. PEF1 is dispensable for general growth and development of *N. crassa*

Phenotypical characterization of the *pef1* gene deletion strain (NCU02738).

Quantification of the colony's linear extension rate rate per day (**A**), the formation of aerial hyphae (**B**) and the amount of produced spores (**C**) of the wild type (strain FGSC 988), the $\Delta pef1$ mutant (strain FGSC 15890) and the $\Delta pef1$, *pef1-gfp* complemented strain (strain GN3-17). Error bars indicate the standard deviation calculated from four independent experiments. **D**: Macroscopic phenotype of the wild type (FGSC 988) (left), the $\Delta pef1$ mutant (FGSC 15890) (middle) and the complemented strain (GN3-17). The mutant exhibits a characteristic brown pigmentation at the glass/hyphal interphase (arrow). Measurement of the amount of extracellular protein recovered from the culture tubes. **E**, **F**: Comparison of the spore germination rate (**E**) and germling interaction rate (**F**) of wild type (FGSC 988), $\Delta pef1$ (FGSC 15890) and the complemented strain (GN3-17). Error bars indicate the standard deviation calculated from three independent experiments (n=100 each).

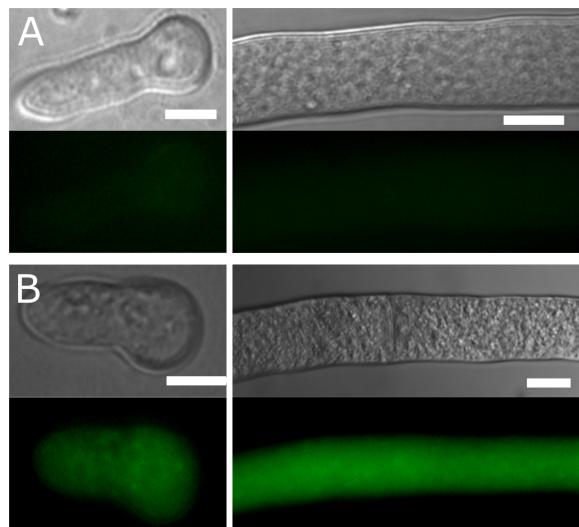


Fig. S2. PEF1-GFP localization in strains expressing the *pef1-gfp* construct under control of the native and the over expression promotores. **A:** Strain GN9-3 expressing *pef1-gfp* under control of the native promoter. Top: DIC image; bottom: GFP fluorescence. Left: germling; right: mature hypha.
B: Strain GN3-17 expressing *pef1-gfp* under control of the *ccg-1* promoter. Top: DIC image; bottom: GFP fluorescence. Left: germling; right: mature hypha. A, B: Scale bar = 10 μ m in hyphae, 5 μ m in germlings.

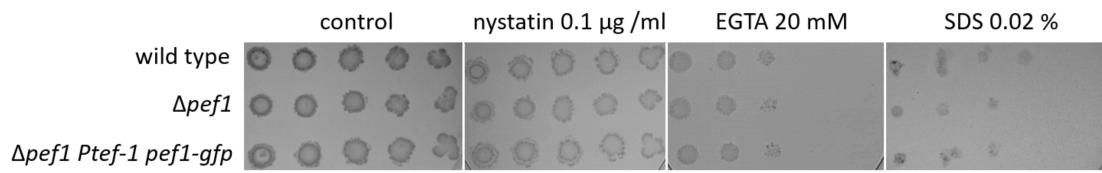


Fig. S3. Lack of PEF1 does not result in growth defects in the presence of nystatin, EGTA, or SDS.

Fivefold serial dilutions of wild type (FGSC 988), $\Delta pef1$ (FGSC 15890) and the complemented strain $\Delta pef1$, *Ptef-1-pef1-gfp* (GN9-22) were spotted on BDES medium (control) and BDES media containing nystatin, EGTA, or SDS. Plates were incubated at 30 °C and growth was recorded after four days.

Tab. S1: Oligonucleotide primers used in this study.

No.	Nucleotide sequence (5'-3')
317	TCGTCCGAGGGCAAAGGAATAGAG
356	GATTATTATCTAGAATGCAGCAAGGACCACCAACCAGACCG
357	GAATATATTAATTAACCTCAACTGGCGCAGAATTCCG
374	CAAAATGAATTCTGAGGGTTAGGG
595	TCAGCTTGGACGCGTCAACAACGCAC
596	GTGCGTTGTTGAACGCGTCCAAGCTGA
597	GTAACGCCAGGGTTTCCCAGTCACGACGTCTAGATGGCCTACAACAGATCC
598	GCGGATAACAATTCACACAGGAAACAGCTTAATTAACTTCAACTGGCGCAGAATTCC
633	GATTATTAGCGGCCGCCCTCACGAAACGTAGAGTT
944	CAGCTCTCCGCACGAGCACTCTCCGGCG
945	CGCCGCGGAGAGTGCTCGTGCGGAGAGCTG
1106	AATGTATCTAGAATGGCGAACCGACAGCTTGC
1107	AATGTATTAATTAAAGCCGCAAAGCCGCAAAC
1110	AATGTATCTAGAATGGTCCGGTTGAGAGAGATC
1111	AATGTATTAATTAAAGGAGTGGCCTGCTCATGC