Supplementary information

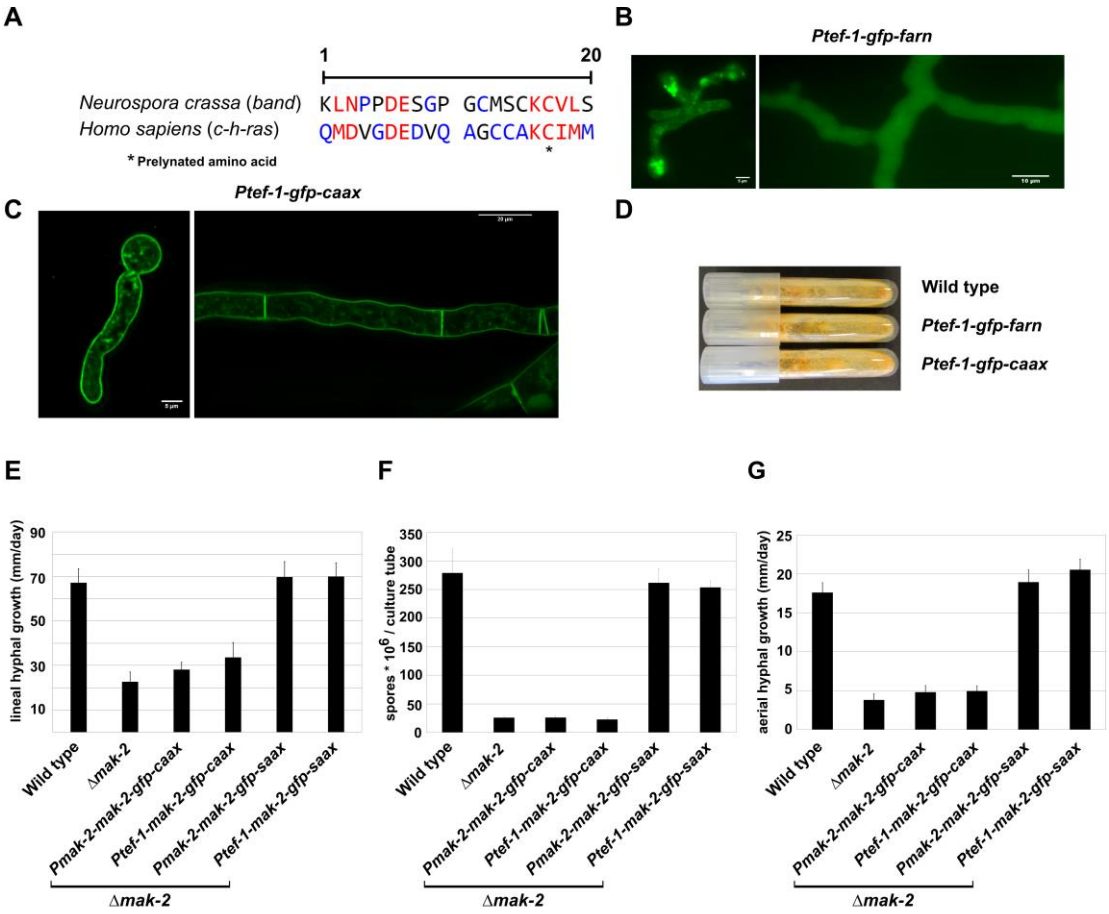


Figure S1. Localization of the GFP-CAAX variants and phenotypical analyses of the membrane tethered MAK-2. (A) Alignment of the -CAAX domains from the BAND-1 protein from *N. crassa* and the C-H-R protein from humans. **(B)** Subcellular localization of GFP fused to the CAAX domain from human Ras (strain 37). Similar observations were made for multiple samples (n=10) **(C)** Localization of GFP with the C-terminal CAAX domain from the BAND gene (strain 800). Similar observations were made for multiple samples (n=10) **(D)** Macroscopic phenotype of the wild type (FGSC 2489), 37 (*his3::Ptef-1-gfp-farn*) and 800 (*his3::Ptef-1-gfp-caax*). **(E)** Quantification of the lineal hyphal growth of the following strains: wild type (FGSC 2489), Δ*mak-2*, 642 (*mak-2::hph;his3::Pmak-2-mak-2-gfp-caax*), 640 (*mak-2::hph;his3::Ptef-1-mak-2-gfp-caax*), 404 (*mak-2::hph;his3::Pmak-2-mak-2-gfp-saax*) and 381 (*mak-2::hph;his3::Ptef-1-mak-2-gfp-saax*). Errors bars represent the standard deviation calculated from 5 race tubes per strain. **(F)** Quantification of spore production of the strains tested above. Errors bars represent the standard deviation calculated from 5 tubes per strain. **(G)** Quantification of aerial hyphae of the strains tested above. Errors bars represent the standard deviation calculated from 5 tubes per strain

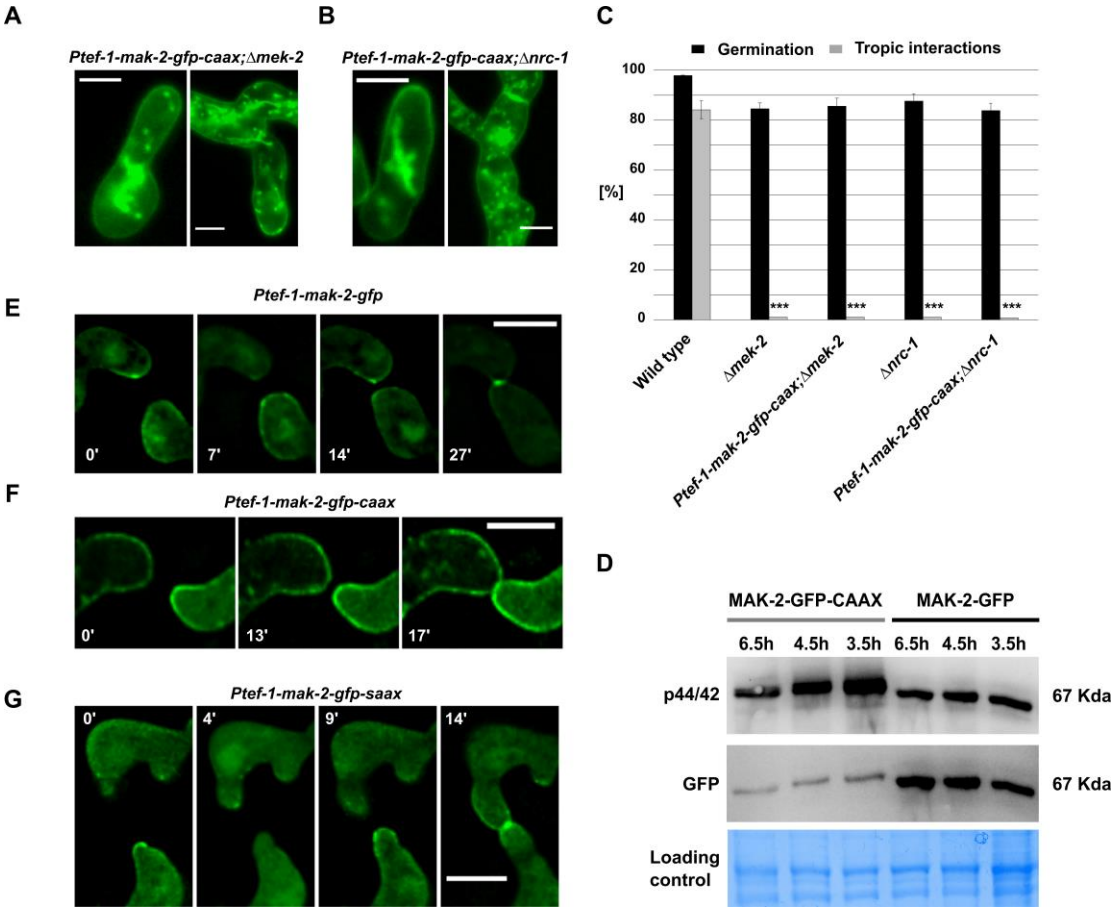


Figure S2. Localization of different MAK-2 variants (membrane-tethered and untethered in the wild-type background) and phosphorylation of MAK-2/MAK-2-CAAX during germling fusion. (A) The permanent membrane localization of MAK-2-CAAX is unaffected in the absence of MEK-2. Strain 384 (*mek-2::hph;his3::Ptef-1-mak-2-gfp-caax*). Scale bars: 5 µm. Similar observations were made for multiple samples (n=5)**. (B)** Similarly, the localization remains unaffected in the absence of NRC-1. Strain 670 (*nrc-1::hph;his3::Ptef-1-mak-2-gfp-caax*). Scale bars: 5 µm. Similar observations were made for multiple samples (n=5) **(C)** Quantification of tropic interactions and germination of the following strains: wild type (FGSC 2489), Δ*mek-2*, 384 (*mek-2::hph;his3::Ptef-1-mak-2-gfp-caax*), Δ*nrc-1* and 670 (*nrc-1::hph;his3::Ptef-1-mak-2-gfp-caax*). Errors bars represent the standard deviation calculated from approximately 100 observed germlings per strain. *** indicates statistically significant differences to the wild type with $p < 0.01$. **(D)** Phospho-western blot analysis testing MAK-2 activation during early colony establishment in strains 267 (*his3::Ptef-1-mak-2-gfp-caax*) and 665 (*his3::Ptef-1-mak-2-gfp*). Comparable observations were made in 3 independent western blots. **(E)** Oscillatory recruitment of MAK-2 to the cell tips of interacting cells in strain 665 (*his3::Ptef-1-mak-2-gfp*). Scale bar: 5 µm Comparable observations were made for multiple samples (n=10)**. (F)** Localization of membrane-tethered MAK-2 during cell interactions in strain

267 (*his3::Ptef-1-mak-2-gfp-caax*). Scale bar: 5 µm. Comparable observations were made for multiple samples (n=10). **(G)** Subcellular oscillatory recruitment of the -SAAX control, strain 361 (*his3::Ptef-1-mak-2-gfp-saax*). Scale bar: 5 µm. Comparable observations were made for multiple samples (n=10).

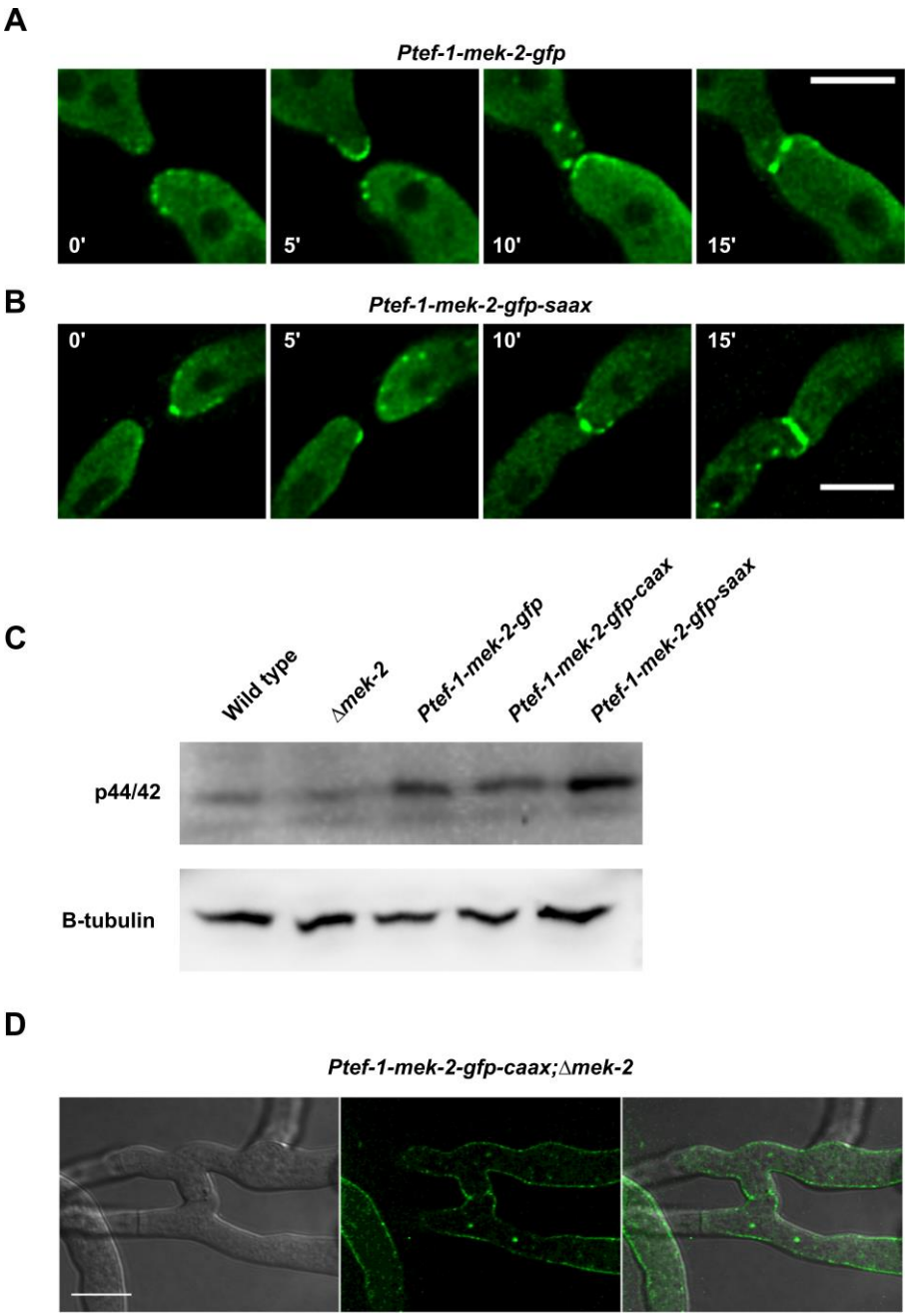


Figure S3. Localization of MEK-2 and complementation of the hyphal fusion defect of the Δ*mek-2* by MEK-2-GFP-CAAX. (A) Oscillatory recruitment of MEK-2-GFP during cell interactions. Strain 406 (*his3::Ptef-1-mek-2-gfp*). Images were acquired every 5 minutes. Scale bar: 5 µm. Comparable observations were made for multiple samples (n=5)**. (B)** Localization of MEK-2-GFP-SAAX during cell fusion. Strain 549 (*his3::Ptef-1-mek-2-gfp-saax*). Images were acquired every 5 minutes. Scale bar: 5 µm. Comparable observations were made for multiple samples (n=5)**. (C)** Phospho-western blot analyses of the following strains: wild type (FGSC 2489), Δ*mek-2*, 406 (*his3::Ptef-1-mek-2-gfp*), 330 (*his3::Ptef-1-mek-2-gfp-caax*) and 549 (*his3::Ptef-1-mek-2-gfp-saax*). Comparable observations were made in 3 independent western blots**. (D)** Hyphal fusion observed in the *Δmek*-2, strain 279 (*mek-2::hph;his3::Ptef-1-mek-2-gfp-caax*). Scale bar: 10 µm.

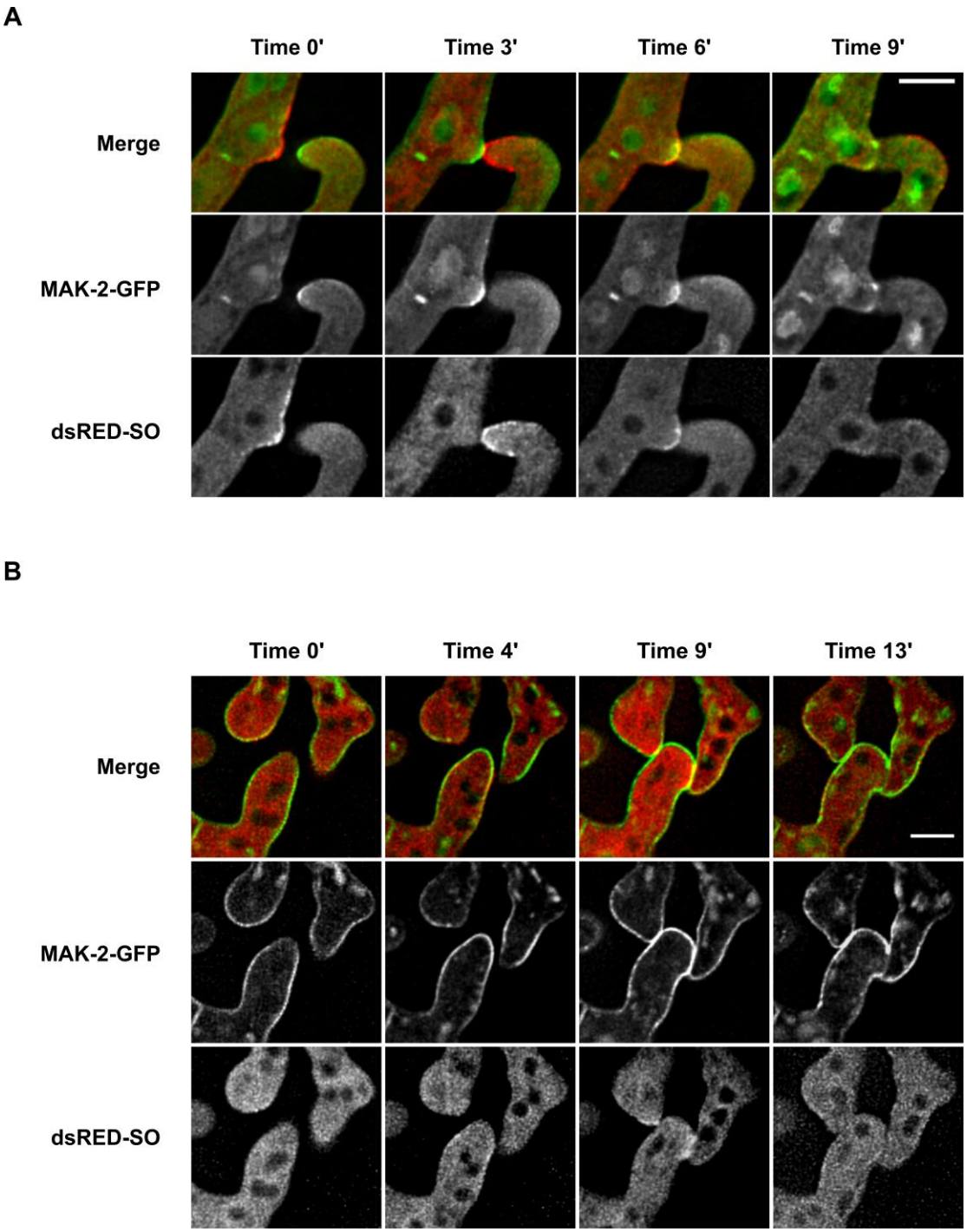
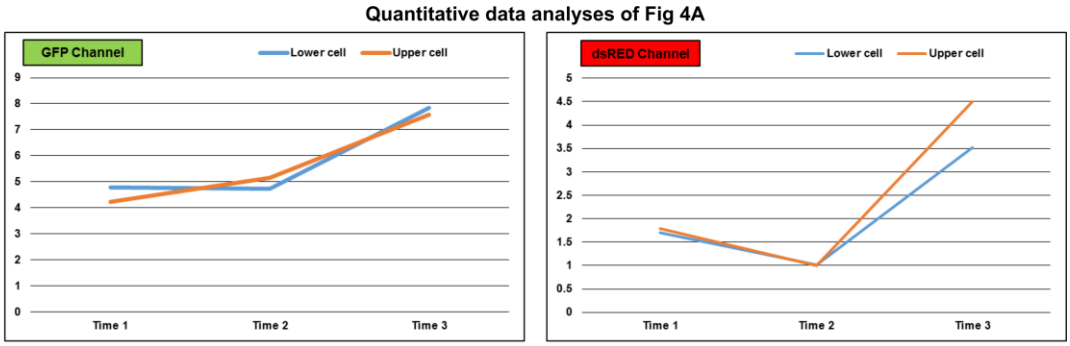
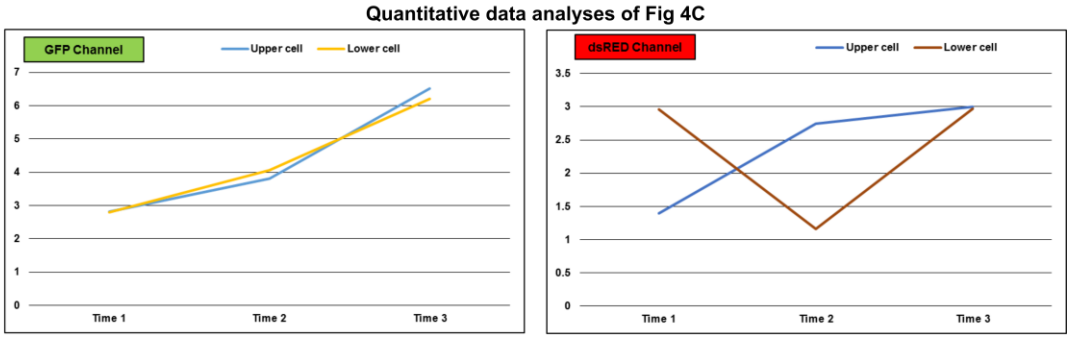


Figure S4. Subcellular localization of MAK-2 and SO, and MAK-2-CAAX and SO during hyphal fusion in the inner parts of a mature colony. (A) Localization of MAK-2-GFP and dsRed-SO in the heterokaryon formed by the strains 665 (*his3::Ptef-1-mak-2-gfp*) and 843 (*his3::Ptef-1-dsRED-so*) during hyphal fusion. Scale bar: 5 µm. **(B)** Localization of the MAK-2-GFP-CAAX and dsRed-SO during hyphal fusion. The heterokaryon w created with the strains 267 (*his3::Ptef-1-mak-2-gfp-caax*) and 843 (*his3::Ptef-1-dsred-so*). Scale bar: 5 µm. Comparable observations in hyphal fusion were made for multiple samples (n=5)**.**

A



B



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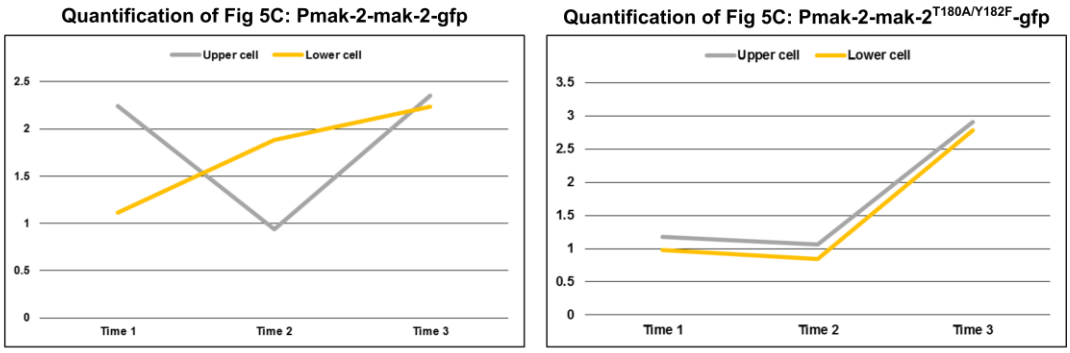
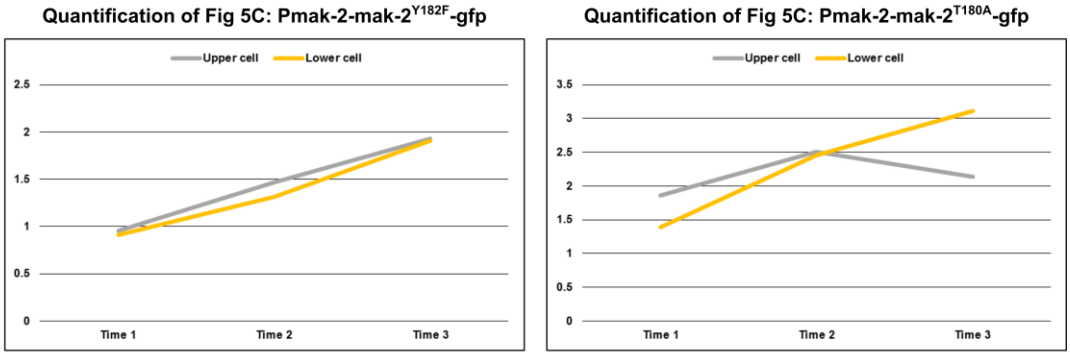




D

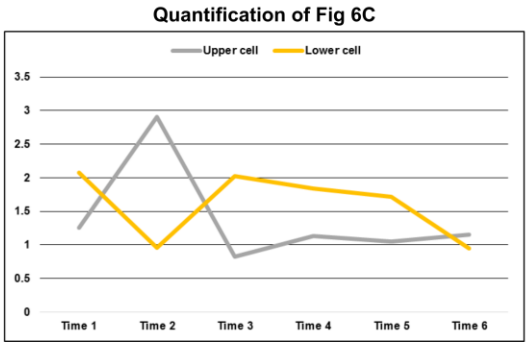
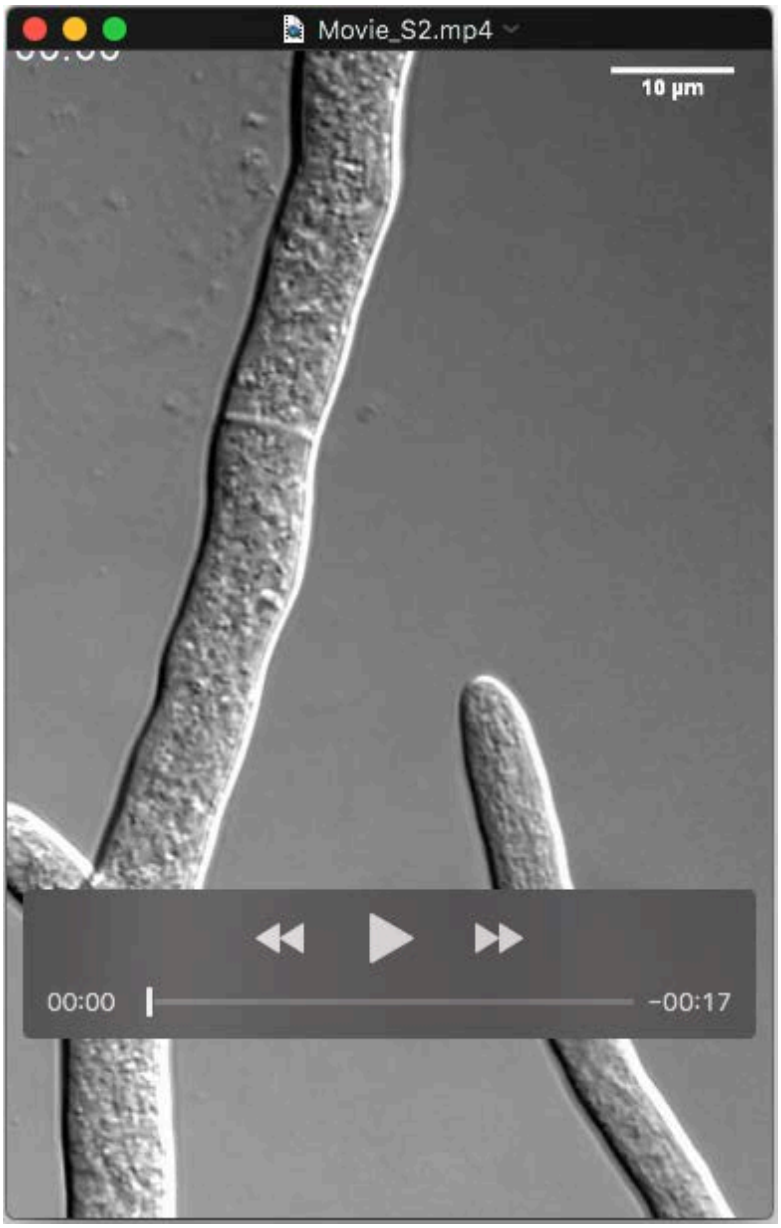


Figure S5. Quantitative analyses of the fluorescence pictures shown in the main figures. (A) Quantitative data of the relative fluorescence at the cell tips normalized with the cytoplasmic fluorescence. MAK-2-GFP-CAAX is represented in the GFP channel and dsRed-SO in the dsRed channel. **(B)** Quantitative data of the relative fluorescence at the cell tips normalized with the cytoplasmic fluorescence. MAK-2$^{T180A/Y182F}$-GFP is represented in the GFP channel and dsRed-SO in the dsRed channel. **(C)** Quantification of the GFP fluorescence intensity of the cell tips normalized with the cytoplasmic fluorescence. **(D)** Quantification of the relative fluorescence of the cell tips normalized with the cytoplasmic fluorescence during the time course represented in Fig 6C.

Movie S1. Membrane-tethering of MEK-2 results in a cell polarity defect during spore germination. The strain used is 286 (*mek-2::hph;his3::Ptef-1-mek-2-gfp-caax*). Spores were incubated for 90 minutes on solid minimal medium. Time is indicated in minutes. Scale bar: 5 µm.



Movie S2. Cellular interaction and fusion between two hyphae in the inner part of the colony. The strain used is the wild type (FGSC 2489). Spores were incubated for 60 minutes in solid minimal medium before the images were acquired. Time is indicated in minutes. Scale bar: 10 µm.

Table S1. *N. crassa* strains used in this study

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