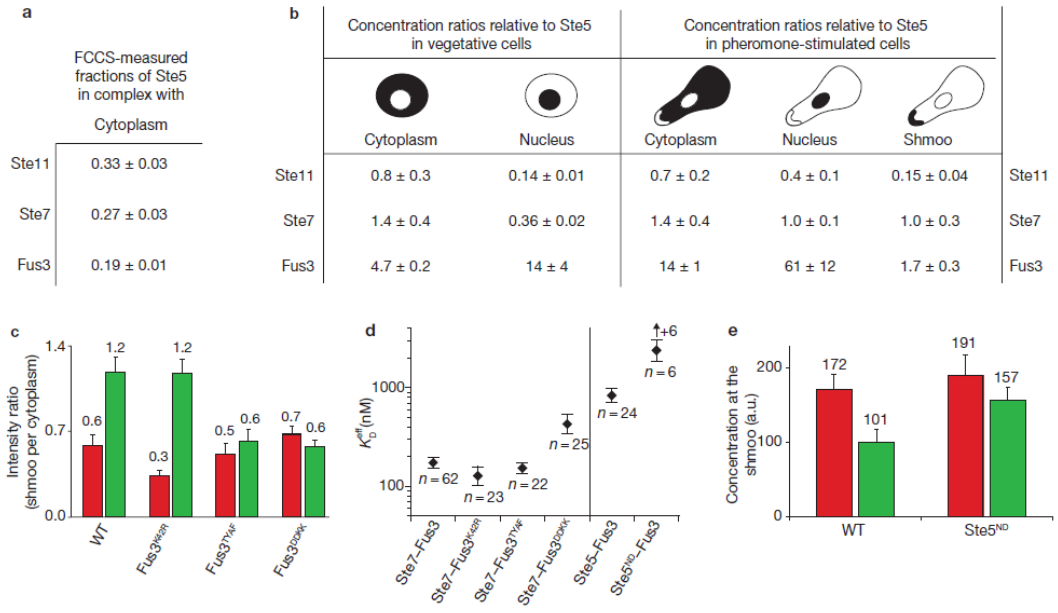

Figure 3 Recruitment of active Fus3^{PP} to the shmoo. (**a**) FCCS-measured fraction of Ste5 in complex with the different MAPKs. (**b**) Quantification of relative MAPK abundances at different sites in yeast cells using APD imaging and image analysis (see Methods). The relative abundance of the three kinases (Ste11, Ste7 and Fus3) versus the scaffold Ste5 in vegetative or pheromone stimulated cells is given at the cellular locations indicated in the schematic representations. Cells were stimulated with α-factor for 2.5–3 h. Error values indicate the s.e.m. Abundances at the shmoo tip were quantified in 20–60 cells. (**c**) Enrichment of Fus3 mutants in the shmoo. Enrichment of wild-type Fus3 (labelled with 3mCherry; red) and Fus3-mutant protein (labelled with 3meGFP; green) in the shmoo in stimulated cells (after 2.5–3 h stimulation). Wild-type Fus3 and mutant Fus3 were expressed in parallel in the same cells. Fus3^{K42R}, kinase dead; Fus3^{TYAF}, non-activatable; Fus3^{DDKK}, substrate docking-site mutant. (**d**) K_D^{eff} of complexes of Fus3 mutants with Ste7 and of the Ste5ND mutant with Fus3 in vegetative cells determined by FCCS. The K_D^{eff} values were calculated in logarithmic units (see Methods). The interaction of Fus3^{DDKK} with Ste7 was significantly weaker (statistical parameters, including *P* values for the comparison of the interactions in different strains, are shown in Supplementary Information, Table S2), but not completely abolished, as the mutations in Fus3 only partially affect the binding to docking peptides[12]. The interaction of Fus3^{DDKK} to Ste5 was not significantly affected (data not shown), probably due to the bipartite nature of this interaction[14]. The interaction of Ste5ND with Fus3 was significantly weaker (see Supplementary Information, Table S2), with no observable level of interaction detected in six cells (indicated by the arrow). These cells could not be used for the calculation of the K_D^{eff} (lower limit). (**e**) Enrichment of Fus3 (red) and Ste5 (green) in the shmoo of wild-type and Fus3-binding impaired Ste5ND cells after 2.5–3 h stimulation. Mean values and s.e.m. for 30–50 cells are shown.