Supplementary data

Material and method for supplementary material

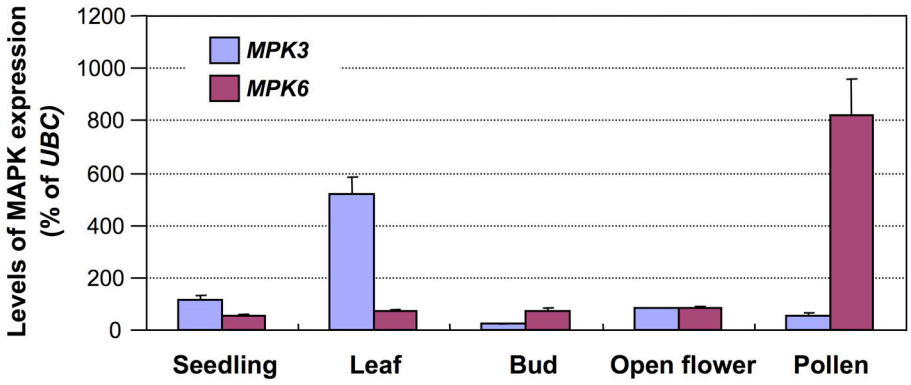
For quantitative RT-PCR analysis, total RNA was extracted from pollen using RNAqueous (Ambion Inc) and other tissues using Trizol (Invitrogen Inc) according to the manufacturer's instructions. After DNase treatment, 1 μg of total RNA was reverse transcribed and quantitative PCR analysis was performed using an Optican 2 real-time PCR machine (Bio-Rad). Relative levels of each transcript were calculated after being normalized to the *UBC21* control. Primers used in quantitative PCR were: *MPK3* (5'TGACGTTTGACCCCAACAGA3' and 5'CTGTTCCTCATCCAGAGGCTG3'), *MPK6* (5'TGTTTGGTCTGTAGGCTGTATT3' and 5'GCTGTCTTATGTATCGCTTTGC3'), *UBC21* (5'CTGCGACTCAGGGAATCTTCTAA3' and 5'TTGTGCCATTGAATTGAACCC3').

To generate the *LAT52* promoter driven *YFP-MPK3* and *YFP-MPK6* fusion constructs, we first replaced the *35S* promoter in a modified *pBI121* with the *LAT52* promoter. *YFP* cDNA was then PCR cloned and insert into the Xho I and Spe I sites to make the *pBI-LAT52:YFP* intermediate construct. *MPK3* and *MPK6* cDNA was then cloned into the Spe I and EcoCR I sites to generate the final constructs. For subcellular localization, pollen was germinated in liquid pollen germination medium, and fluorescence microscopy was performed 2 hours after germination.

Table S1. Distribution of *mpk3 mpk6* fertilized seeds in siliques from wild type pistils pollinated with pollen grains from *mpk3*$^{-/-}$ *mpk6*$^{+/-}$ plants.

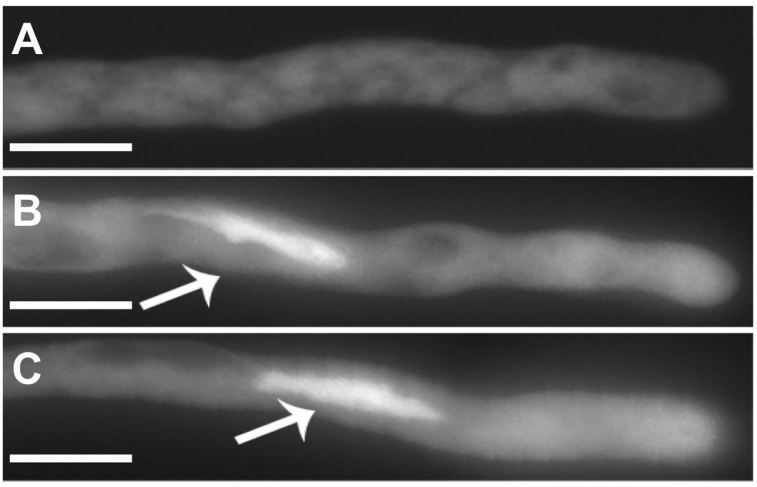
Pistil #	Segregation (*mpk3 mpk6* : *mpk3 MPK6* fertilized seeds)		
	Upper half	Lower half	Total
1	1:19	1:25	2:44
2	2:20	1:20	3:40
3	2:22	2:23	4:45

Genotypes of male parent, *mpk3*$^{-/-}$ *mpk6*$^{+/-}$; and female parent, wild type. Segregation, transmission of *mpk3 mpk6* and *mpk3 MPK6* pollen based on the genotypes of the progenies.



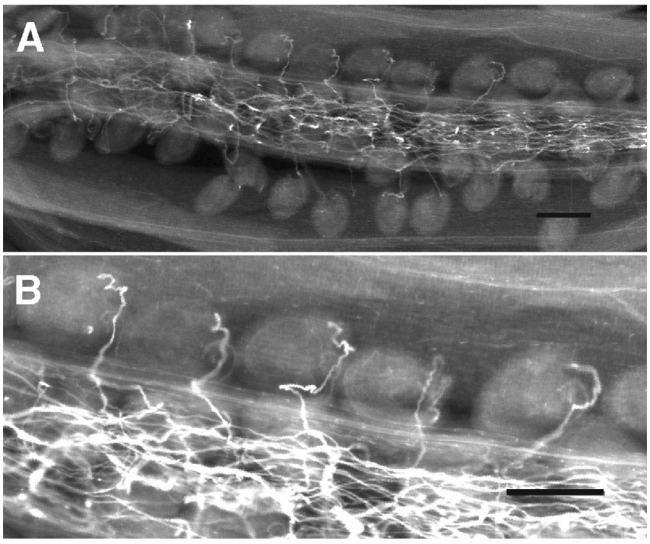
Supplemental Figure S1. *MPK6* expression is higher than *MPK3* in pollen.

Expression of *MPK3* and *MPK6* in different tissues is quantified by the percentage of reference gene, *UBC21*. Error bar=s.d.



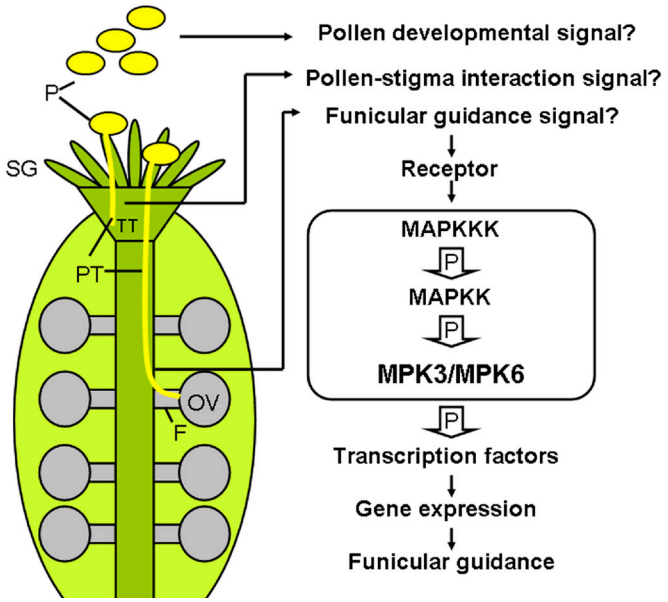
Supplemental Figure S2. Subcellular localization of MPK3 and MPK6 fusion protein.

(A) Subcellular localization of free YFP protein in pollen tube. (B) Subcellular localization of YFP-MPK3 fusion protein in pollen tube. (C) Subcellular localization of YFP-MPK6 fusion protein in pollen tube. Arrows indicate the localization of YFP-tagged MAPKs in the nuclei. Bar=10 μm.



Supplemental Figure S3. *In vivo* tube growth of pollen grains from *mpk3*$^{-/-}$ *mpk6*$^{+/-}$ plants in excessive pollination.

(A) Aniline blue staining of a pistil pollinated with pollen grains from *mpk3*$^{-/-}$ *mpk6*$^{+/-}$ plants. (B) Close up view of pistil in (A). Note only one pollen tube landed on each funiculus and fertilized the ovule. Bar=100 μm.



Supplemental Figure S4. Proposed model for MPK3/MPK6 signaling in pollen tube guidance.

MPK3/MPK6 signaling cascade may be activated in developing or mature pollen, during pollen tube germination and penetration of pistils, or by funicular guidance cues during pollen tube guidance. MPK3/MPK6 in turn activate downstream transcription factors to regulate the expression of funicular guidance related genes.