

Expanded View Figures

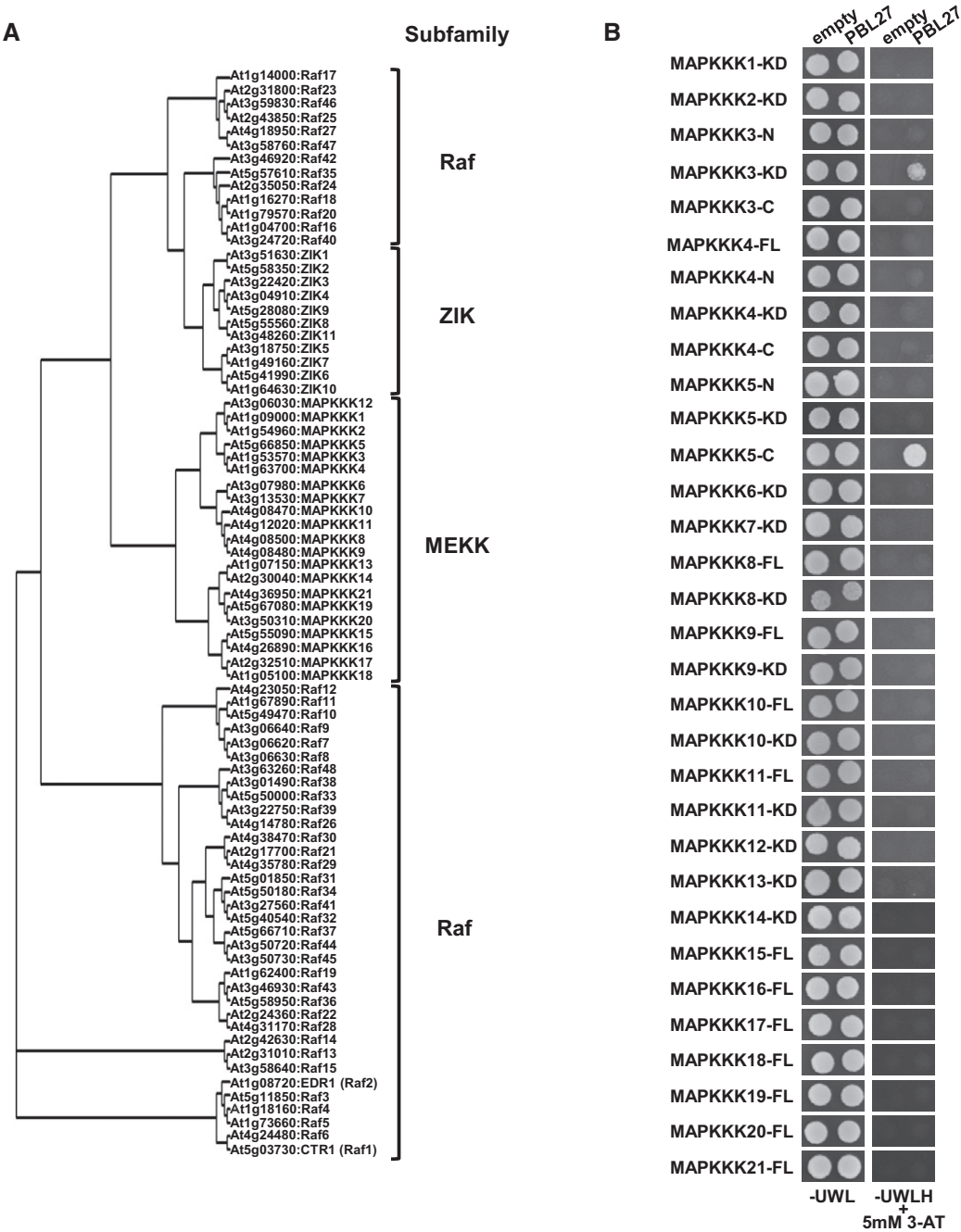


Figure EV1. Phylogenetic tree of *Arabidopsis* MAPKKK family and interaction of PBL27 and MEKK subfamily in yeast two-hybrid assay.

A The neighbor-joining phylogenetic tree was created using ClustalW on the DNA Data Bank of Japan (<http://www.ddbj.nig.ac.jp/>). The full-length protein sequence for each MAPKKK was used for the alignment. The phylogenetic tree was generated from the modified alignment with Treeview X software.

B The growth of yeast colonies on plates (-ULWH) lacking uracil (U), leucine (L), tryptophan (W), and histidine (H) with 5 mM 3-aminotriazole (3-AT) indicates a positive interaction. The 3-AT is a competitive inhibitor of the *HIS3* gene product (histidine synthase), which is the reporter gene for the interaction in the yeast two-hybrid assay. FL: full length, N: N-terminal domain, KD: kinase domain, C: C-terminal domain.

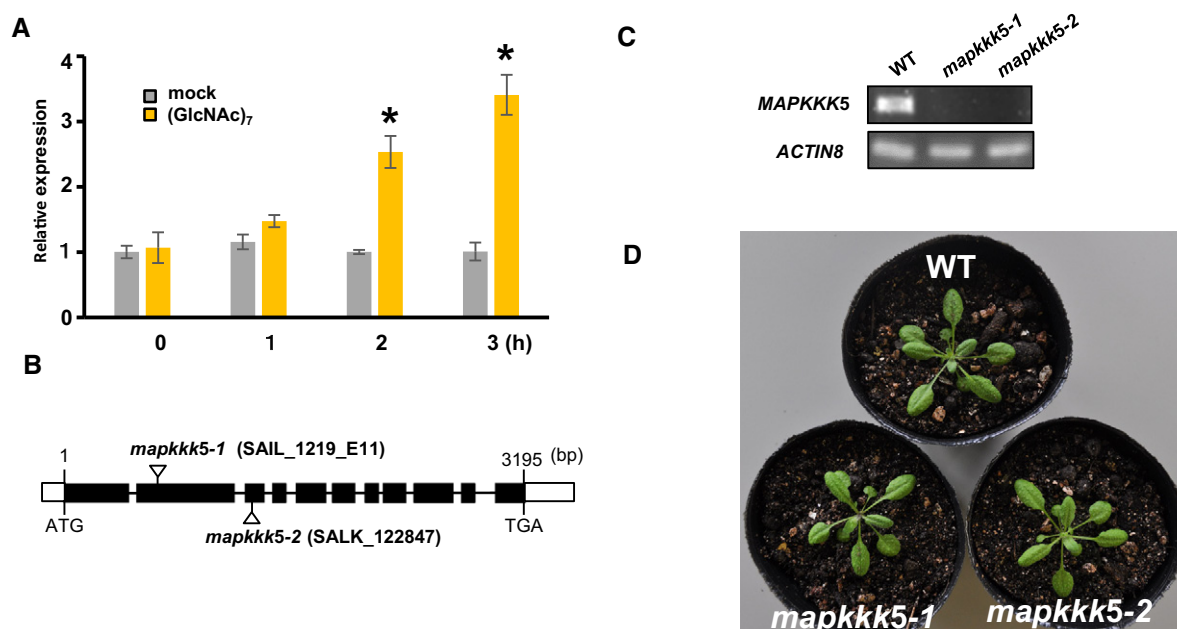


Figure EV2. Expression of *MAPKKK5*.

- A Chitin-induced expression of *MAPKKK5*. Expression of *MAPKKK5* was analyzed by real-time RT-PCR using specific primers for *MAPKKK5*. Bars represent means $\pm$ SD calculated from three independent experiments. The asterisks indicate statistically significant differences from the mock treatment by Student's *t*-test ($P < 0.05$).
- B Gene structure of *MAPKKK5* and the T-DNA insertion sites. Exons are indicated by black boxes. Arrows indicate each T-DNA insertion site.
- C Expression of *MAPKKK5* in two T-DNA insertion mutants was analyzed by semiquantitative RT-PCR.
- D The phenotype of *mapkkk5* homozygous mutant plants was similar to that of wild-type (Col-0) plants. *Arabidopsis* plants were grown in soil in a growth chamber at 23°C under 16-h light–8-h dark cycle. Plants were photographed 3 weeks after germination.

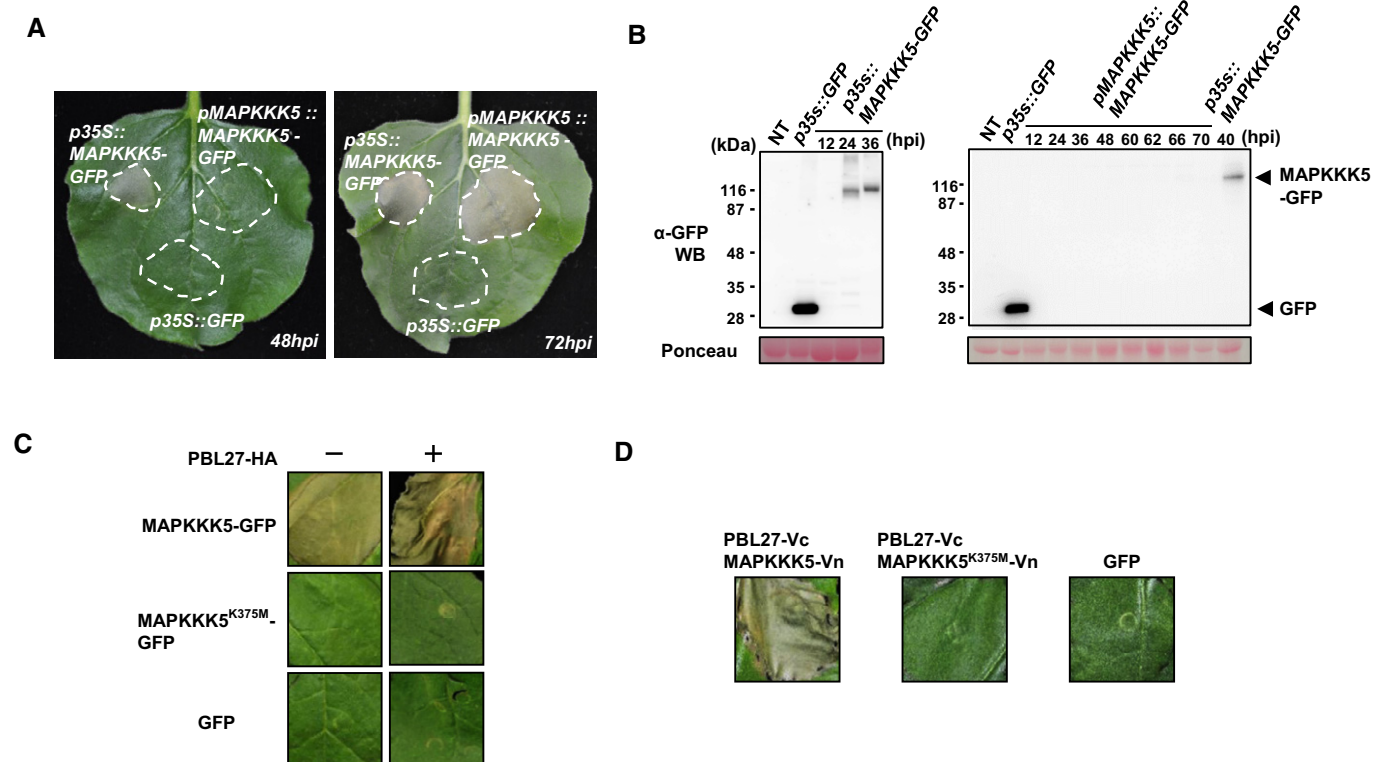


Figure EV3. MAPKKK5 induces cell death in *Nb* leaves.

- A Agroinfiltration assays using native or 35S promoters in *Nb* leaves show that MAPKKK5 induces cell death. The results were photographed at 48 or 72 h postinoculation (hpi).
- B Detection of the MAPKKK5-GFP proteins in *Nb* leaves by immunoblots with α -GFP.
- C The kinase-dead MAPKKK5^{K375M} with or without PBL27 does not induce cell death in *Nb* leaves. The results were photographed at 72 hpi.
- D MAPKKK5 induces cell death in the BiFC assay. The results were photographed at 72 hpi.

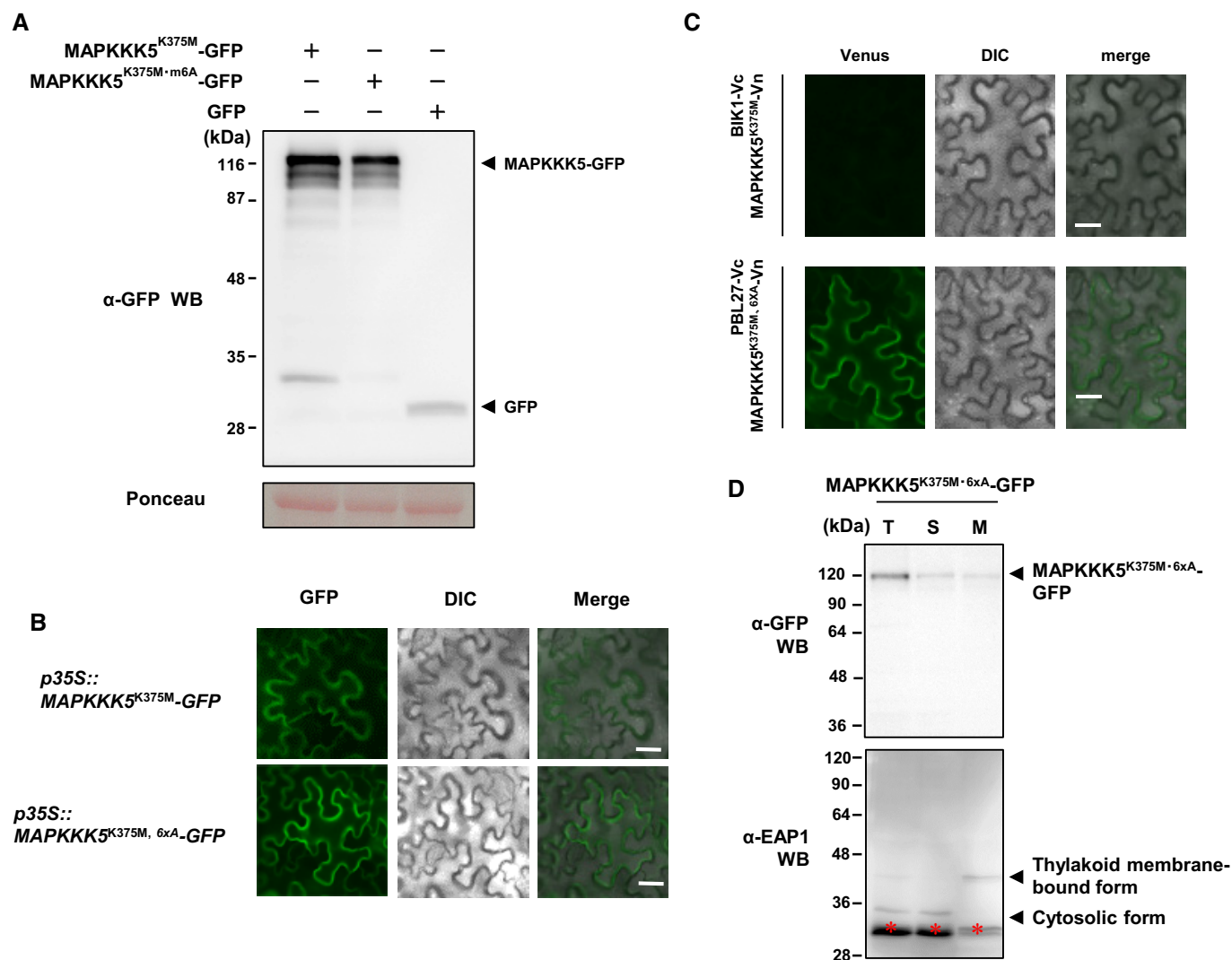


Figure EV4. MAPKKK5^{K375M, 6xA} interacts with PBL27 in *Nb* leaves.

- A MAPKKK5^{K375M} and MAPKKK5^{K375M, 6xA} were transiently expressed in *Nb* leaves. Total proteins were prepared from the leaves at 48 hpi. The protein levels were analyzed by immunoblots with α-GFP.
- B Subcellular localization of MAPKKK5^{K375M} and MAPKKK5^{K375M, 6xA} in *Nb* leaves. Microscopic observation was done at 48 hpi. Scale bars = 50 μm.
- C Visualization of the interaction between PBL27 and MAPKKK5^{K375M, 6xA} using bimolecular fluorescence complementation in *Nb* leaves. Venus fluorescence indicates the interaction between PBL27 and MAPKKK5^{K375M, 6xA}. Scale bars = 50 μm.
- D The MAPKKK5^{K375M, 6xA}-GFP protein was detected in the membrane fraction. MAPKKK5^{K375M, 6xA}-GFP was expressed in *Nb* leaves. Total proteins were prepared from *Nb* leaves at 40 hpi. Immunoblots with α-GFP and α-EAP1 were carried out using total (T), soluble (S), and microsomal membrane (M) fractions. α-EAP1, the antibody against *Euglena* ascorbate peroxidase, cross-reacts with cytosolic and thylakoid membrane-bound AsAPs of *N. bensamiana*. Asterisks indicate artificial bands.

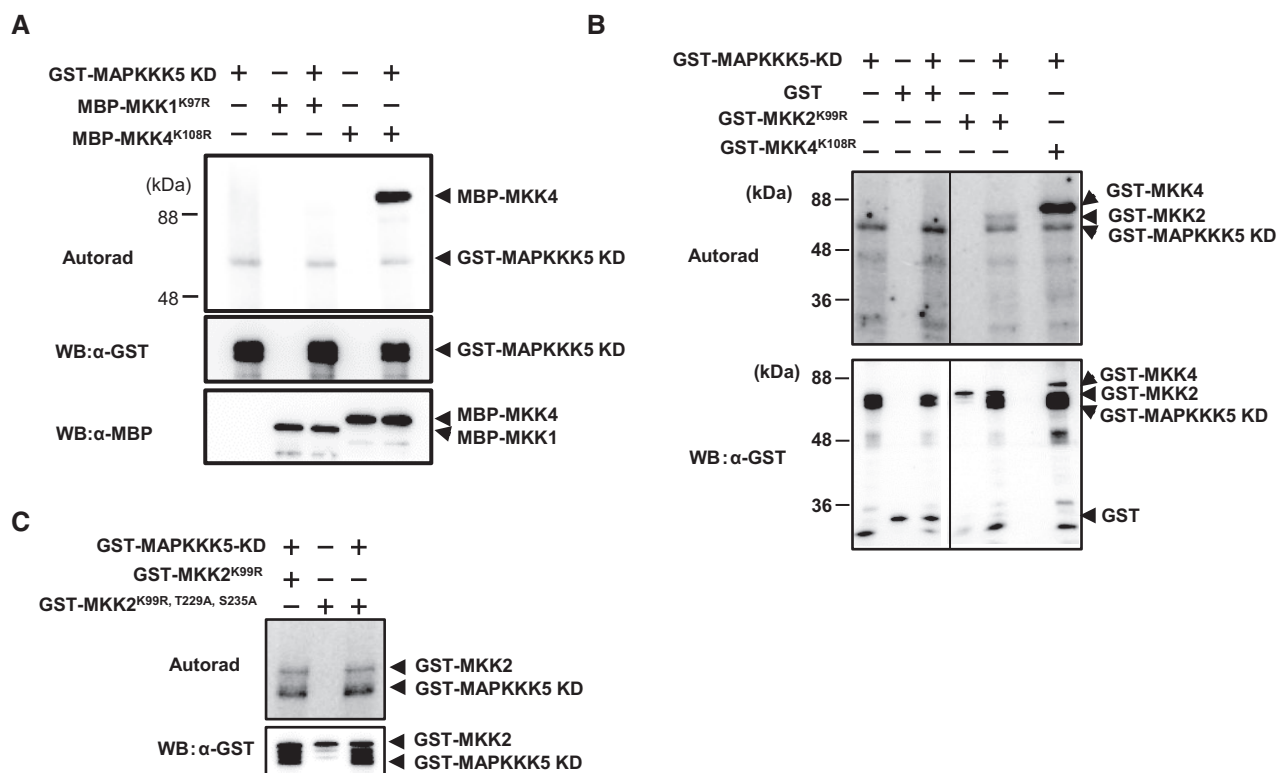


Figure EV5. MAPKKK5-KD does not phosphorylate the activation loops of MKK1 and MKK2.

A MAPKKK5-KD does not phosphorylate MKK1^{K97R} *in vitro*. The *in vitro* phosphorylation reaction was carried out using MBP-fused MKK1 and MKK4, and the phosphorylated proteins were detected by autoradiography. MKK4^{K108R} was used as a positive control.

B MAPKKK5-KD weakly phosphorylates GST-MKK2^{K99R} *in vitro* phosphorylation assay

C The T229A and S235A mutations of MKK2 did not reduce phosphorylation of MKK2^{K99R}.

Data information: The above experiments were performed three times with similar results.

Source data are available online for this figure.