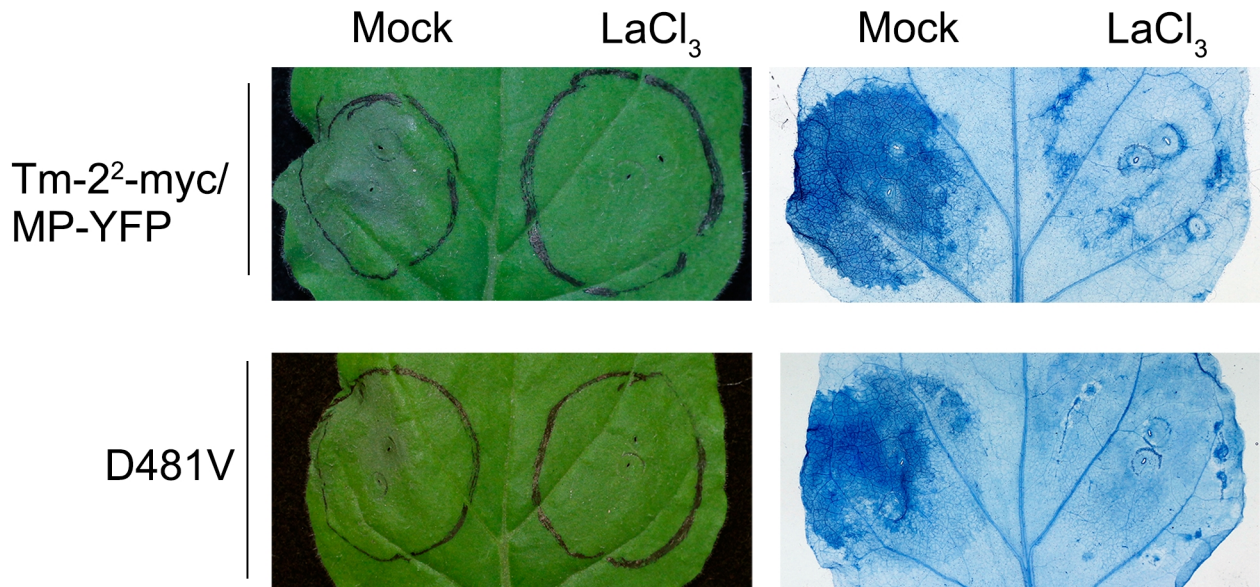


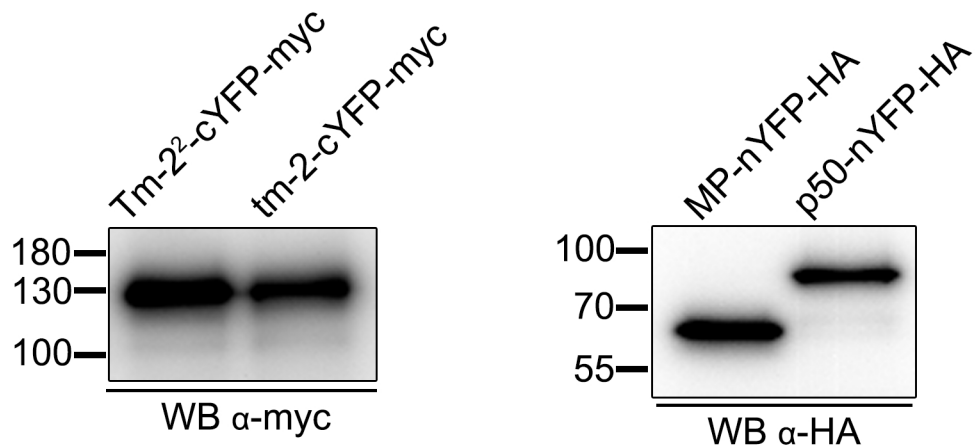
Supplemental Figure S1



Supplemental Figure S1. LaCl₃ inhibits Tm-2²-mediated Cell death.

Tm-2² and TMV MP were transiently expressed in *N. benthamiana* leaves, and leaves were treated with water (mock) or LaCl₃ (Upper panel). D481V autoactive mutant was agroinfiltrated in wild-type *N. benthamiana* leaves with the same treatments (lower panel). Leaves were photographed before (left column) and after trypan blue staining (right column). Circles indicate the leaf areas infiltrated with agrobacteria.

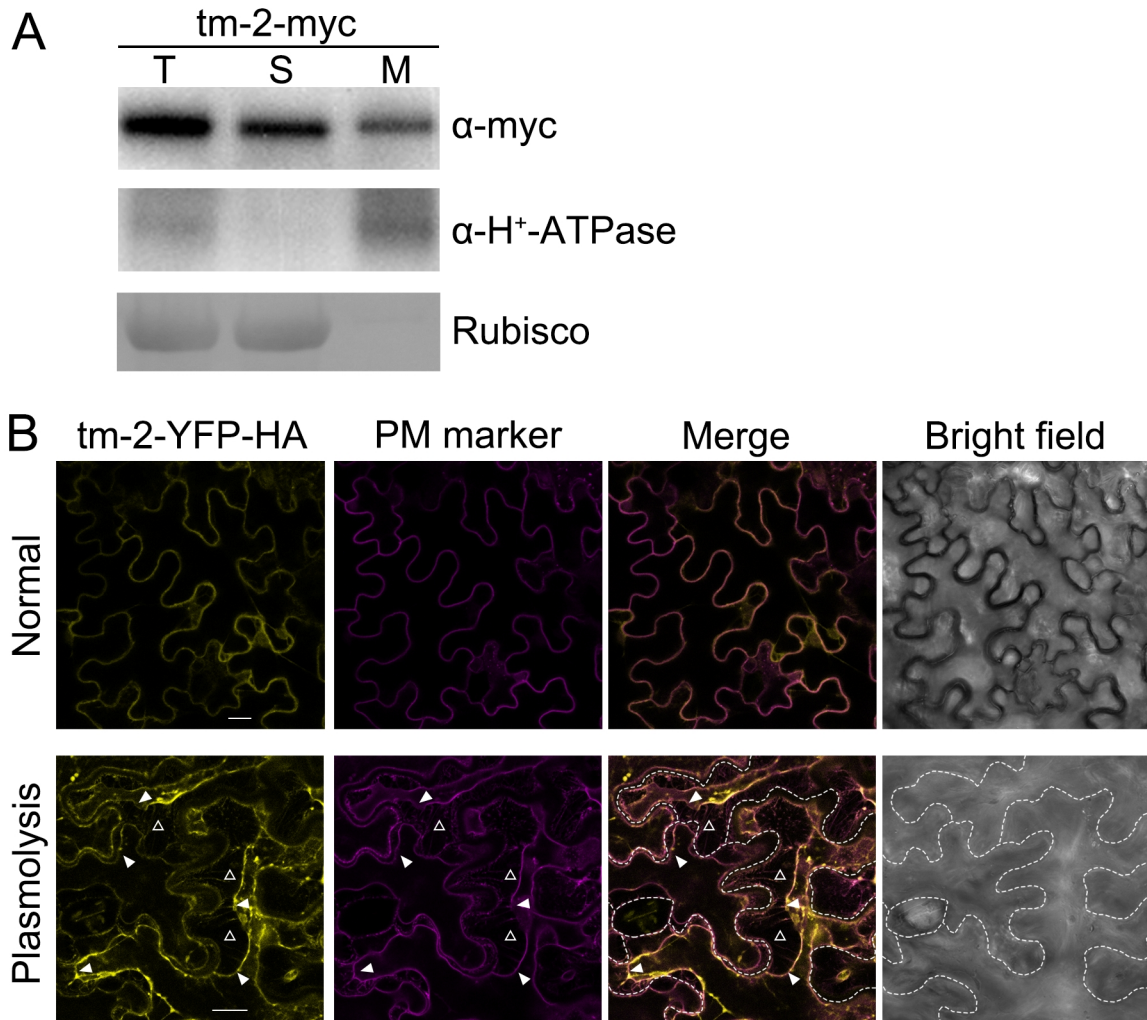
Supplemental Figure S2



Supplemental Figure S2. Expression of fusion proteins for BiFC

Tm-2²-cYFP-myc, tm-2-cYFP-myc, MP-nYFP-HA and p50-nYFP-HA were transiently expressed, and extracted from *N. benthamiana* leaves at 36 hpi. Tm-2²-cYFP-myc and tm-2-cYFP-myc were detected by anti-myc, and MP-nYFP-HA and p50-nYFP-HA by anti-HA.

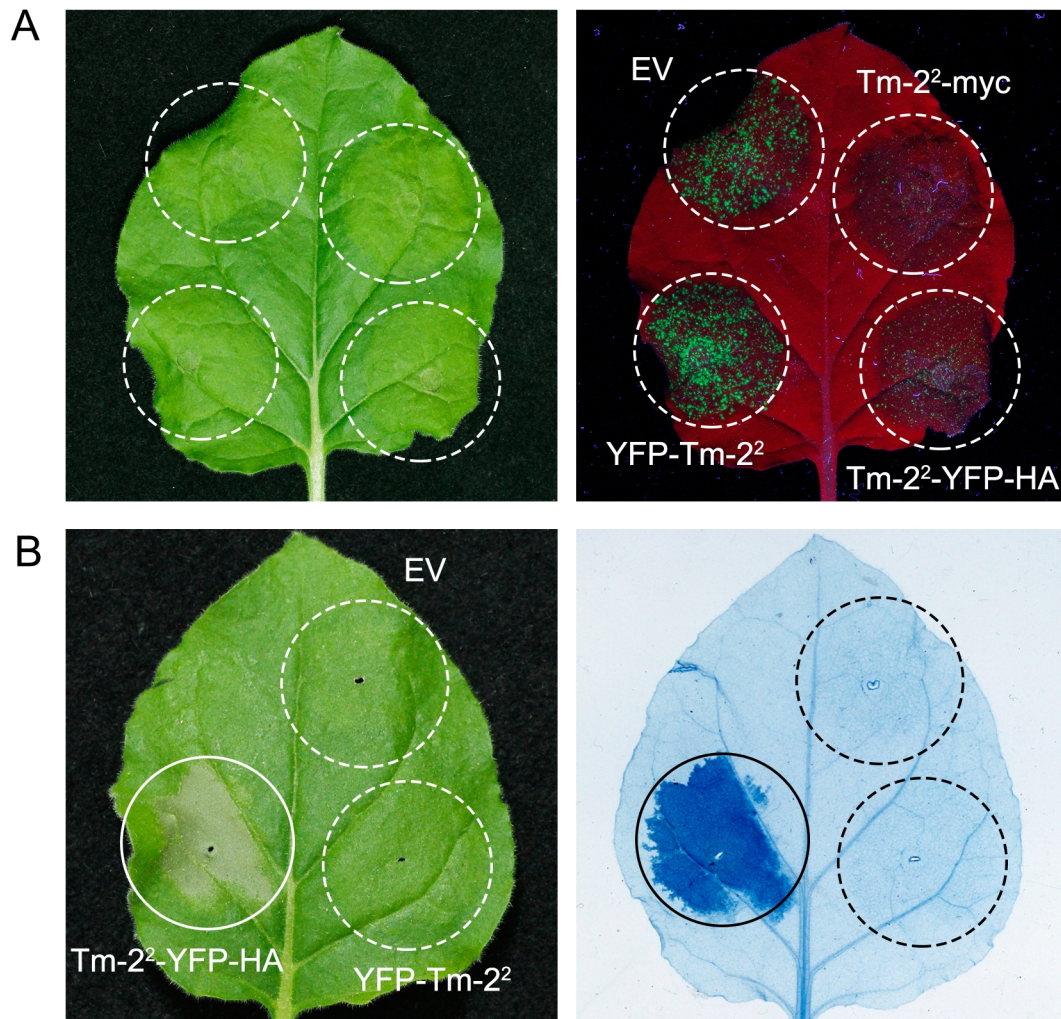
Supplemental Figure S3



Supplemental Figure S3. tm-2 is localized in the cytoplasm and at the PM.

A, Cell fractionation of tm-2-myc. tm-2 appeared in both soluble and membrane fraction. B, Confocal images of tm-2-YFP-HA and PM marker in normal condition or after cell plasmolysis. RFP-AtRop10 was expressed as a PM marker. Outlined triangles indicate Hechtian strands, and solid triangles indicate the retracted PM. Cell wall is labeled by dotted lines. Bars = 20 μ M.

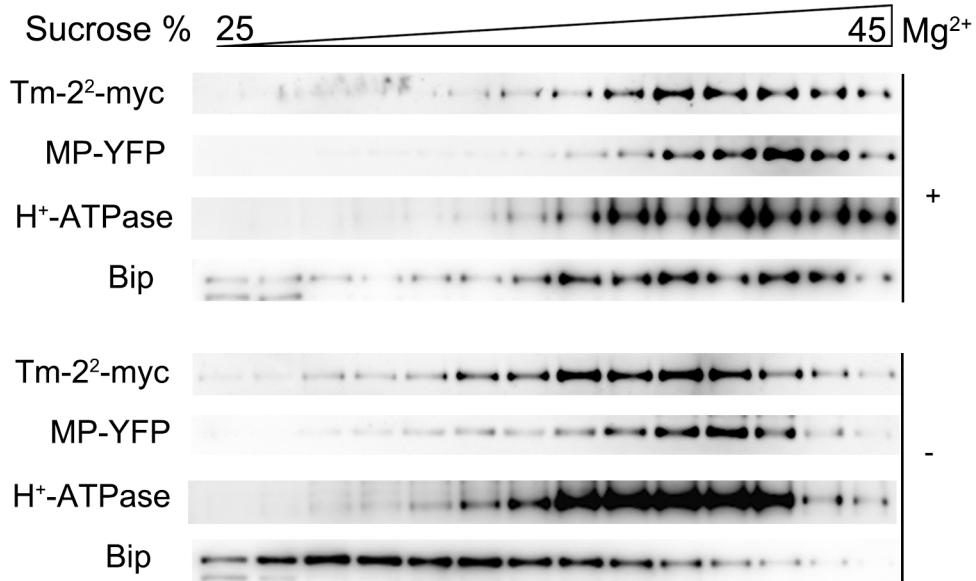
Supplemental Figure S4



Supplemental Figure S4. N-terminal tag affects Tm-2² function.

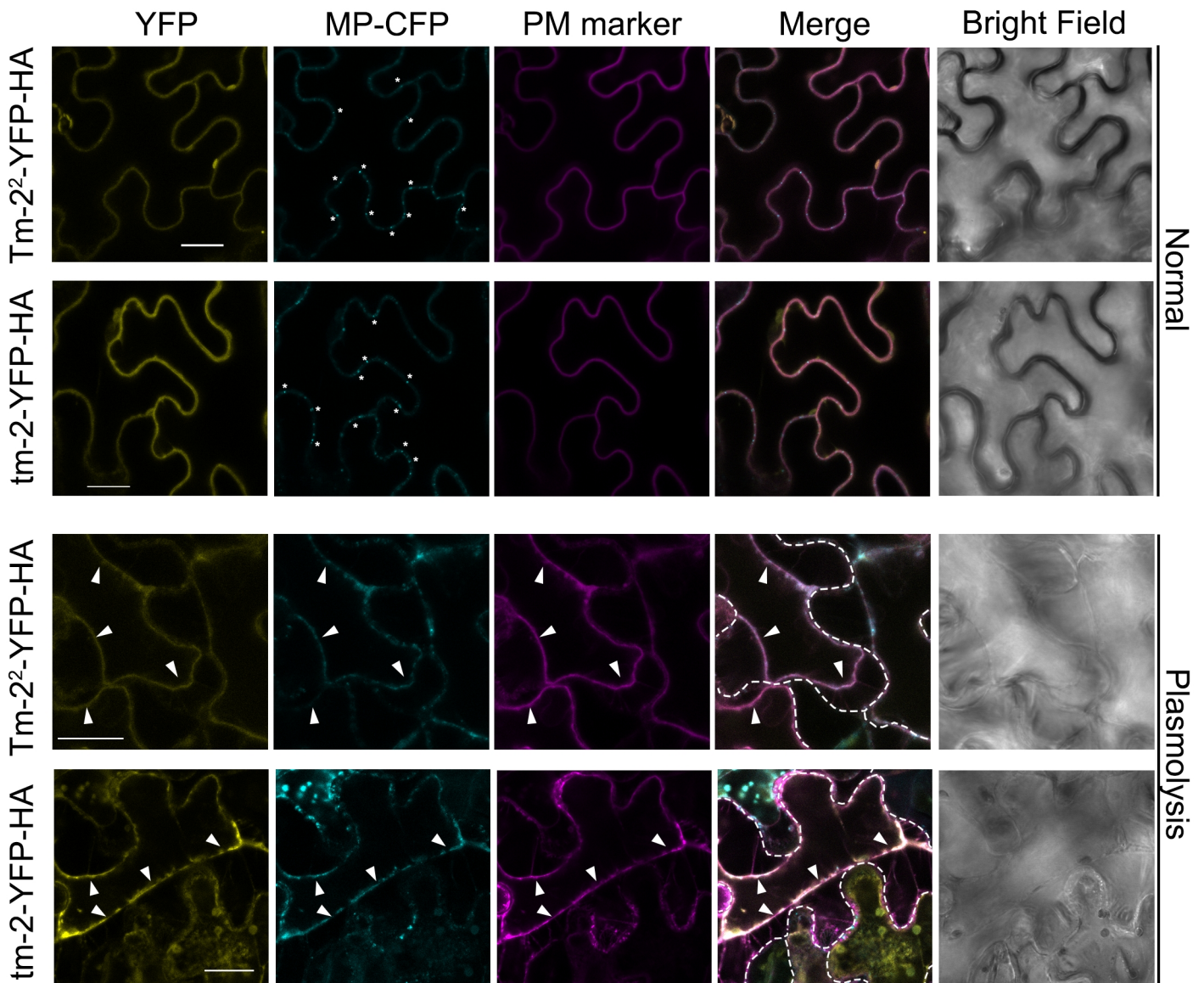
The N-terminal and C-terminal fusion proteins of Tm-2² were agroinfiltrated into *N. benthamiana* leaves with either TMV-GFP (A) or MP-YFP (B). A, leaves were photographed under normal light (left column) and UV light (right column). B, leaves were photographed before (left column) and after trypan blue staining (right column). EV: empty vector.

Supplemental Figure S5



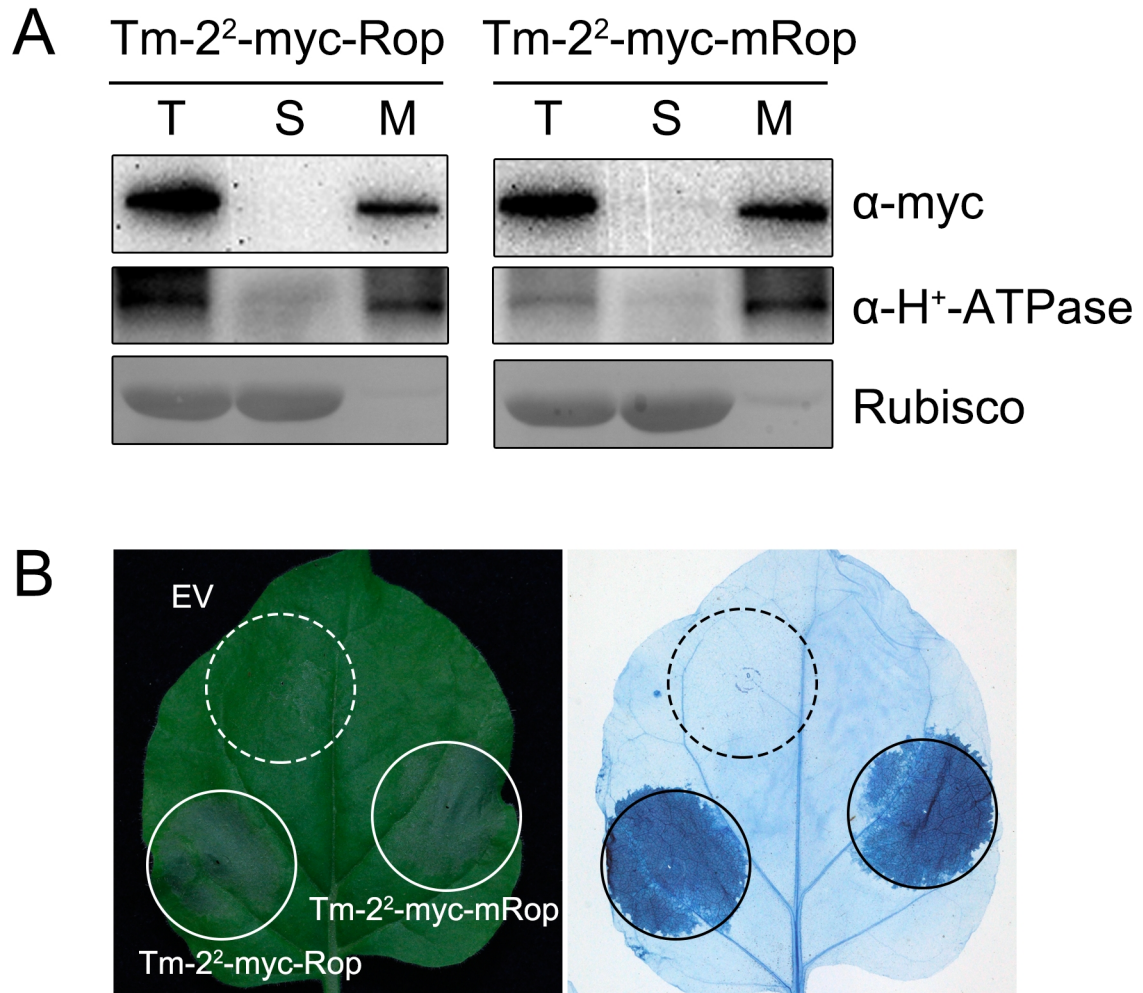
Supplemental Figure S5. Sucrose gradient analysis of Tm-2² in the presence of MP. Tm-2² in the presence of MP co-fractionated with the PM marker. Sucrose gradients were used to fractionate the microsomal membrane purified from plants co-expressing Tm-2² and MP. Fractions from low density to high density were analyzed by western blot using antibodies against myc epitope, GFP, H⁺-ATPase (the PM marker), Bip (ER marker) and V-ATPase (tonoplast marker).

Supplemental Figure S6



Supplemental Figure S6. MP and Tm-2² or tm-2 are co-localized at the PM. Tm-22-YFP-HA or tm-2-YFP-HA co-expressed with MP-CFP and RFP-tagged PM marker. The confocal images of epidermal cells were acquired in normal condition or after cell plasmolysis. MP PD accumulation is indicated by stars. Solid triangles indicate the retracted PM and cell wall is labeled by dotted lines. Bars = 20 μ M.

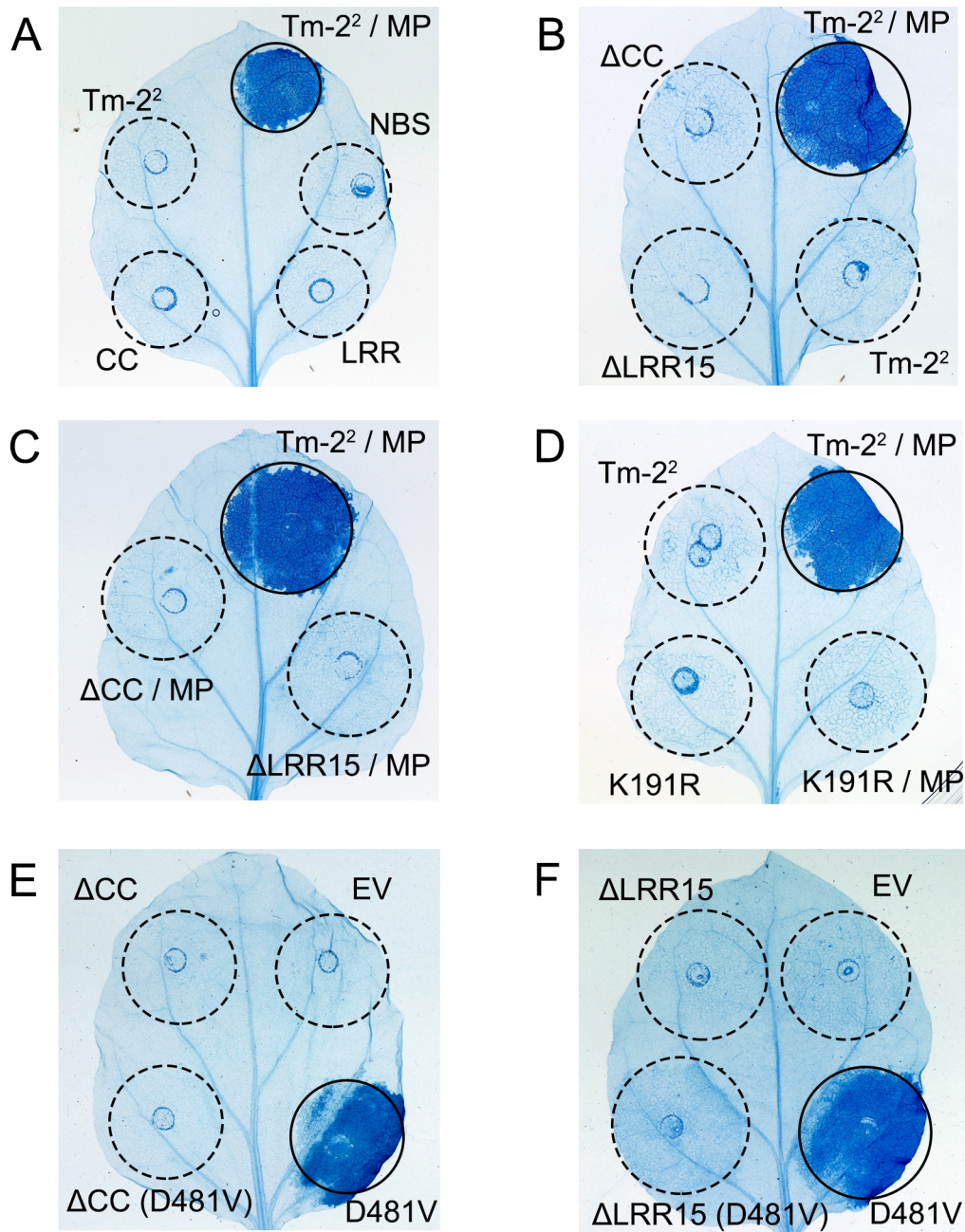
Supplemental Figure S7



Supplemental Figure S7. Rop tag does not affect Tm-2² function.

A, Cell fractionation of Tm-2²-myc-Rop or Tm-2²-myc-mRop. B, Cell death of Tm-2²-myc-Rop or Tm-2²-myc-mRop in the presence of MP. All constructs were co-expressed with MP. EV: empty vector.

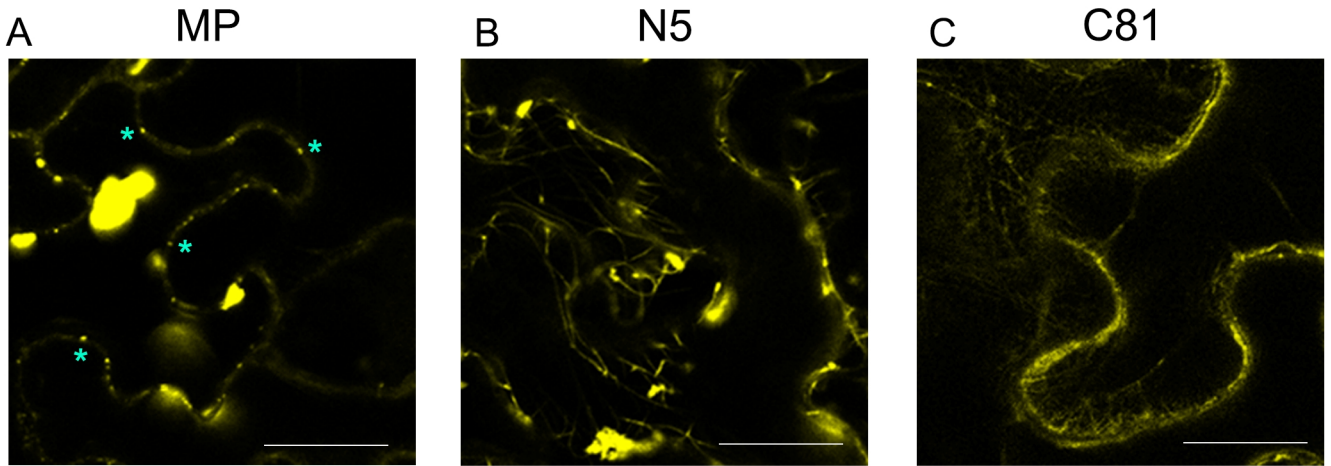
Supplemental Figure S8



Supplemental Figure S8. Tm-2² mutants failed to induce cell death in the presence or absence of MP.

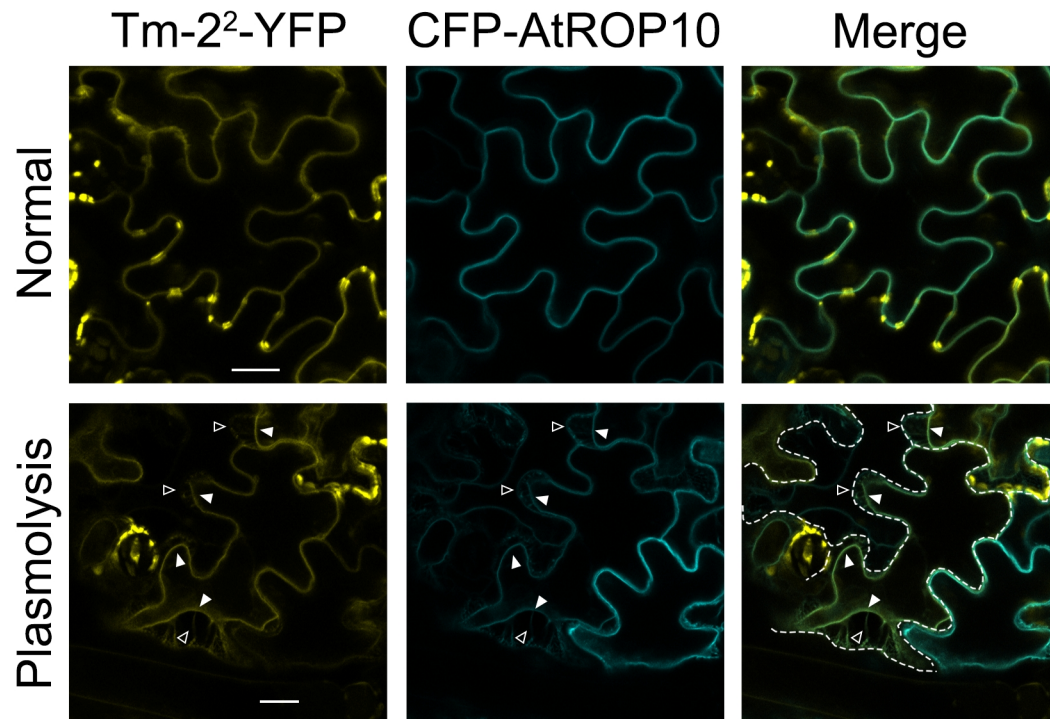
A, Expression of CC, NBS, or LRR domain alone does not trigger cell death. B, Δ CC or Δ LRR15 mutant triggers no HR. C, Δ CC or Δ LRR15 mutant in the presence of MP does not induce cell death. D, P-loop (K191R) mutant does not trigger MP-dependent HR. E, Deletion of CC domain in D481V mutant compromises constitutive activation of D481V mutant. F, The constitutive activation of D481V mutant is abolished by the deletion of last LRR motif. Leaves were stained by trypan blue. Solid line circles indicate cell death; dashed line circles indicate no obvious cell death.

Supplemental Figure S9



Supplemental Figure S9. Subcellular localization of TMV MP and mutants. The subcellular localization of MP-YFP (A) and mutants N5-YFP (B) and C81-YFP (C) in leaf epidermal cells was monitored at 36 hpi. MP PD accumulation is indicated by stars. Bars = 20 μ M.

Supplemental Figure S10



Supplemental Figure S10. Confocal images of Tm-2²-YFP Tm-2²-YFP (used in Du et al., 2013) was co-expressed with CFP-AtRop10 as the PM marker. The confocal images of normal or plasmolyzed epidermal cells were acquired. Outlined triangles indicate Hechtian strands, and solid triangles indicate the retracted PM. Cell wall is labeled by dotted lines. Bars = 20 μ M.