

## Supplemental Materials

### Supplemental Figure Legends

**Supplemental Figure 1** Transcript levels of *AtTLP* genes in *attlp* mutants.

mRNA transcription was determined in respective mutants by semi-quantitative PCR. The expression of housekeeping gene *Ubiquitin 5 (Ubi5)* served as control. Number of cycles is indicated underneath each gel image.

**Supplemental Figure 2** Unaltered expression of selected *AtTLP* genes during *Arabidopsis* root colonization by *P. indica*.

Three-week-old *Arabidopsis* plants (Col-0) were mock-treated or inoculated with *P. indica* and harvested at 1, 3, and 7 days after treatments. None of the genes showed an altered expression in response to *P. indica*. Expression values were calculated by the  $2^{-\Delta Ct}$  method by relating Ct thresholds of candidates to those of the housekeeping gene *AtUbiquitin 5*. Data are means from two to three independent experiments and bars represent standard deviation.

**Supplemental Figure 3** flg22-induced seedling growth inhibition is unaltered in *attlp* mutants.

flg22 or mock treatment was applied to two-week-old plants non-colonized or colonized by *P. indica* (5 days after inoculation). flg22 treatment resulted in growth inhibition in all mutants and *P. indica* was able to abolish this response. Data are averages from two independent experiments with at least 15 plants per line and treatment. Bars represent standard deviations.

**Supplemental Figure 4** Unaltered colonization of *attlp* mutants by *E. cruciferarum*, *B. cinerea*, and *P. parasitica*.

(A) Leaves of Col-0, *attlp2-1*, *3-1*, *5-1*, and *8-1* were inoculated with *Botrytis cinerea* and lesion diameter was determined at 4 dai. The variation between mutants and Col-0 were statistically insignificant. Data are averages from at least ten plants of one biological experiment and bars represent standard errors. The experiment was repeated four times with similar results. (B) Roots of Col-0, *attlp2-1*, *3-1*, *5-1* and *9-1* were inoculated with *Phytophthora parasitica* and disease index was determined at 7, 8, 10, 11, 14, 15, 17, and 17 days after inoculation. Differences in disease indices between Col-0 and mutants were statistical not significant. Bars represent standard deviations from one biological. The

experiment was repeated two times with similar results. (C) Leaves of Col-0, *attlp3-1*, *3-2*, *5-2* were inoculated with *Erysiphe cruciferarum*. Images present leaves overgrown with the fungus at 11 dai. The experiment was repeated two times with similar results.

**Supplemental Figure 5** Marker genes for MAMP-triggered immunity and abiotic stress do not show an elevated expression in *attlp3* mutant during *P. indica* colonization.

Three-week-old *Arabidopsis* plants (Col-0, *attlp3*) were mock-treated or inoculated with *P. indica* and harvested at 0, 1, 3, and 7 days after treatments. None of the genes showed an altered expression due to the lack of AtTLP3. Minor changes were observed for *WRKY29* expression at 1, 3, and 7 dai or mock treatment and for *At5G10695* at 7 days after mock treatment. Expression values were calculated by the  $2^{-\Delta Ct}$  method by relating Ct thresholds of candidates to those of the housekeeping gene *AtUbiquitin 5*. Standard errors are from three technical replicates of one biological experiment. Experiments were repeated at least twice with similar results.

**Supplemental Figure 6** Separate localization of F-box and Tubby domain of AtTLPs.

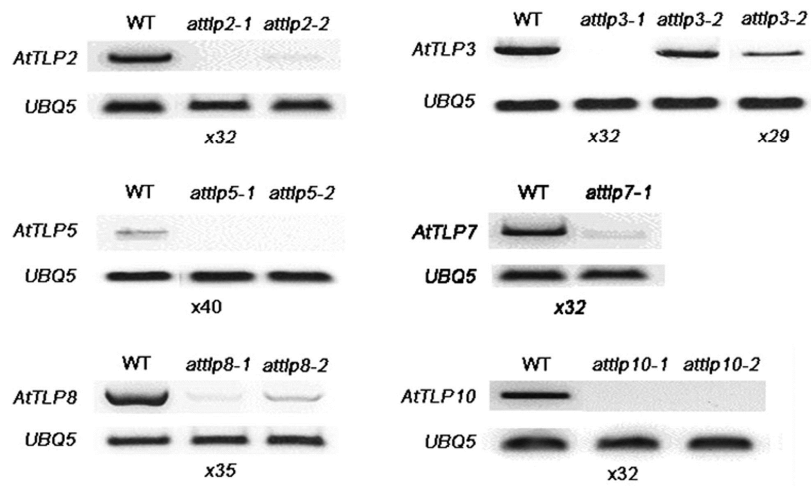
(A) Model of AtTLPs with the F-box (FB) domain at the N-terminus (NT) and the Tubby domain at the C-terminus (CT). Scale bar indicates the length of AtTLPs in amino acids (aa). For all experiments, leaf cells were co-transformed with the *NT-AtTLPs-GFP* (B, E, H, K) or *GFP-CT-AtTLPs* (N, Q, T, W) fusions under control of the 35S promoter and cytosolic and nucleoplasmic marker *mCherry* (C, F, I, L, O, R, U, X) by biolistic transformation. (D, G, J, M, P, S, V, Y) Merged images indicate subcellular localization of domains. Constructs used for transformation are imaged on the left. (B-D) Plastidial and nucleo-cytosolic localization of NT-AtTLP2-GFP ( $\Delta 112-394$ ). (E-G) Plastidial localization of NT-AtTLP7-GFP ( $\Delta 108-379$ ). (H-J) Cytosolic and nucleoplasmic localization of NT-AtTLP8-GFP ( $\Delta 150-397$ ). (K-M) Plastidial and nucleo-cytosolic localization of NT-AtTLP10-GFP ( $\Delta 123-445$ ). (N-P) Plasma membrane (PM) localization of GFP-CT-AtTLP2 ( $\Delta 1-111$ ). (Q-S) PM localization of GFP-CT-AtTLP7 ( $\Delta 1-107$ ). (T-V) Cytosolic and nucleoplasmic localization of GFP-CT-AtTLP8 ( $\Delta 1-149$ ). (W-Y) PM localization of GFP-CT-AtTLP10 ( $\Delta 1-122$ ). Bars = 20 $\mu$ m.

**Supplemental Figure 7** Effects of H<sub>2</sub>O<sub>2</sub> on relative ion leakage in leaf discs of control and mutant plants. Bars represent standard deviations from three independent measurements of one biological experiment. The experiment was performed twice with similar results.

**Supplemental Figure 8** Subcellular localization of truncated versions of AtTLP3 in *Nicotiana benthamiana*.

*Nicotiana benthamiana* leaf cells were co-transformed with the *GFP-CT-AtTLP3* ( $\Delta 1-115$ ) (A), *NT-AtTLP3-GFP* ( $\Delta 116-406$ ) (D) or *GFP-CT-AtTLP3* ( $\Delta 1-115$ , K187A, R189A) (G) fusions under control of the 35S promoter and cytosolic and nucleoplasmic marker *mCherry* (B, E, H) by biolistic transformation. Amino acids eliminated in truncated versions used for transformation and treatments are drafted on the left. (A) *GFP-CT-AtTLP3* ( $\Delta 1-115$ ) displayed plasma membrane (PM) localization. (D) Plastidial and nucleo-cytosolic localization of *NT-AtTLP3-GFP*. (G) *GFP-CT-AtTLP3* ( $\Delta 1-115$ ), in which K187 and R189 had been converted to alanine (A), has lost its PM binding ability and shows cytosolic and nucleoplasmic localization. (C, F, I) Merged images indicate subcellular localization of truncated domain versions. Yellow colour indicates co-localization of green and red fluorescing proteins. Bars = 20  $\mu\text{m}$ .

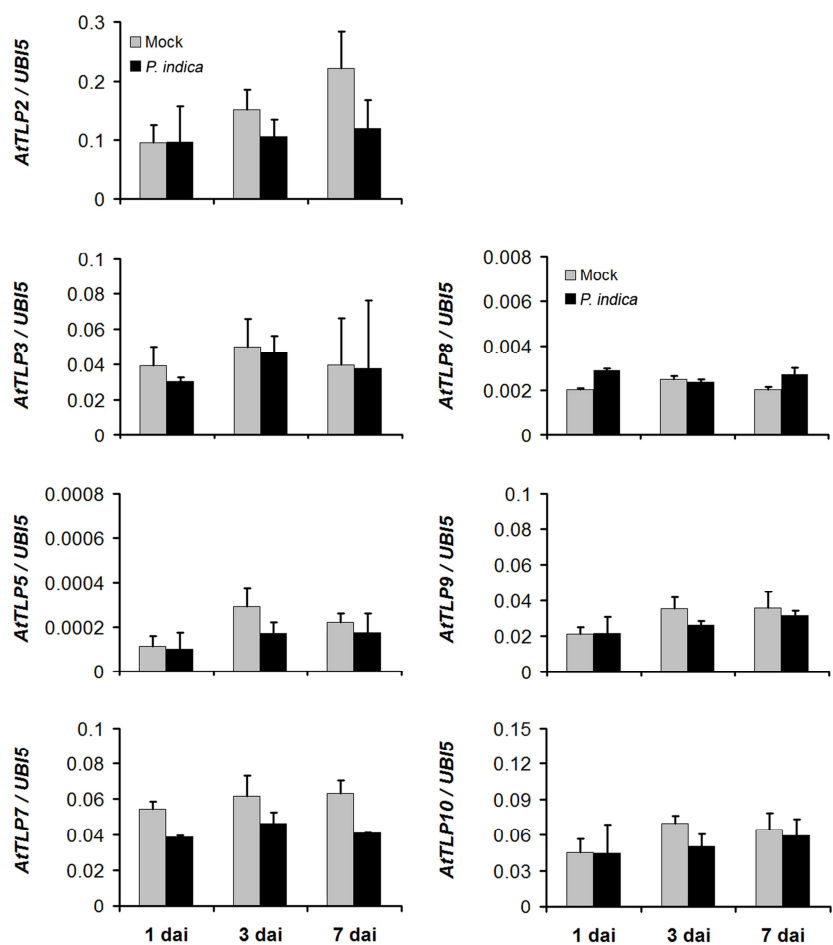
## Supplemental Figure 1



Supplemental Figure 1 Transcript levels of AtTLP genes in *attlp* mutants.

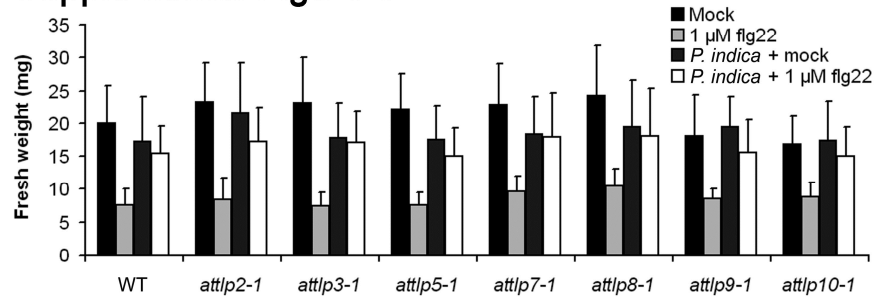
mRNA transcription was determined in respective mutants by semi-quantitative PCR. The expression of housekeeping gene Ubiquitin 5 (Ubi5) served as control. Number of cycles is indicated underneath each gel image.

## Supplemental Figure 2



**Supplemental Figure 2 Unaltered expression of selected AtTLP genes during Arabidopsis root colonization by *P. indica*.** Three-week-old Arabidopsis plants (Col-0) were mock-treated or inoculated with *P. indica* and harvested at 1, 3, and 7 days after treatments. None of the genes showed an altered expression in response to *P. indica*. Expression values were calculated by the  $2^{-\Delta Ct}$  method by relating Ct thresholds of candidates to those of the housekeeping gene AtUbiquitin 5. Data are averages from two to three independent experiments and bars represent standard deviation.

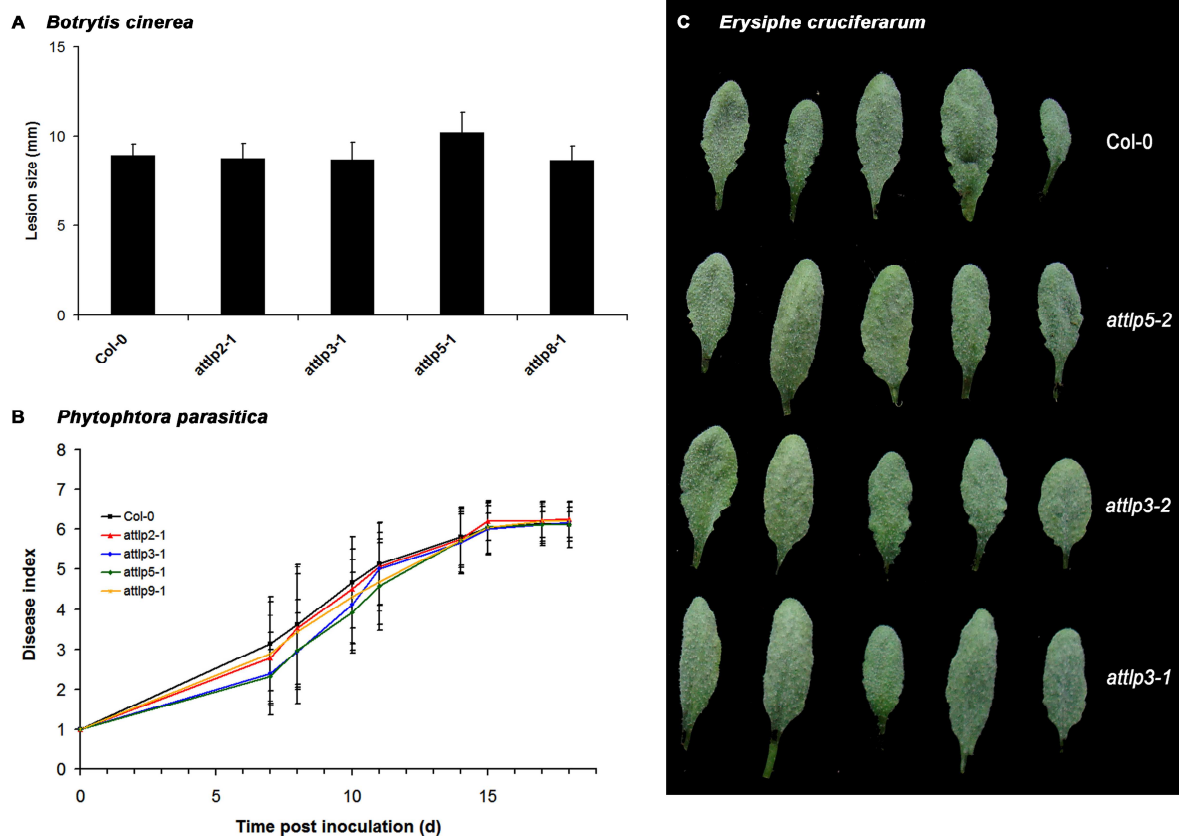
### Supplemental Figure 3



**Supplemental Figure 3 flg22-induced seedling growth inhibition is unaltered in attlp mutants.**

flg22 or mock treatment was applied to two-week-old plants non-colonized or colonized by *P. indica* (5 days after inoculation). flg22 treatment resulted in growth inhibition in all mutants and *P. indica* was able to abolish this response. Data are averages from two independent experiments with at least 15 plants per line and treatment. Bars represent standard deviations.

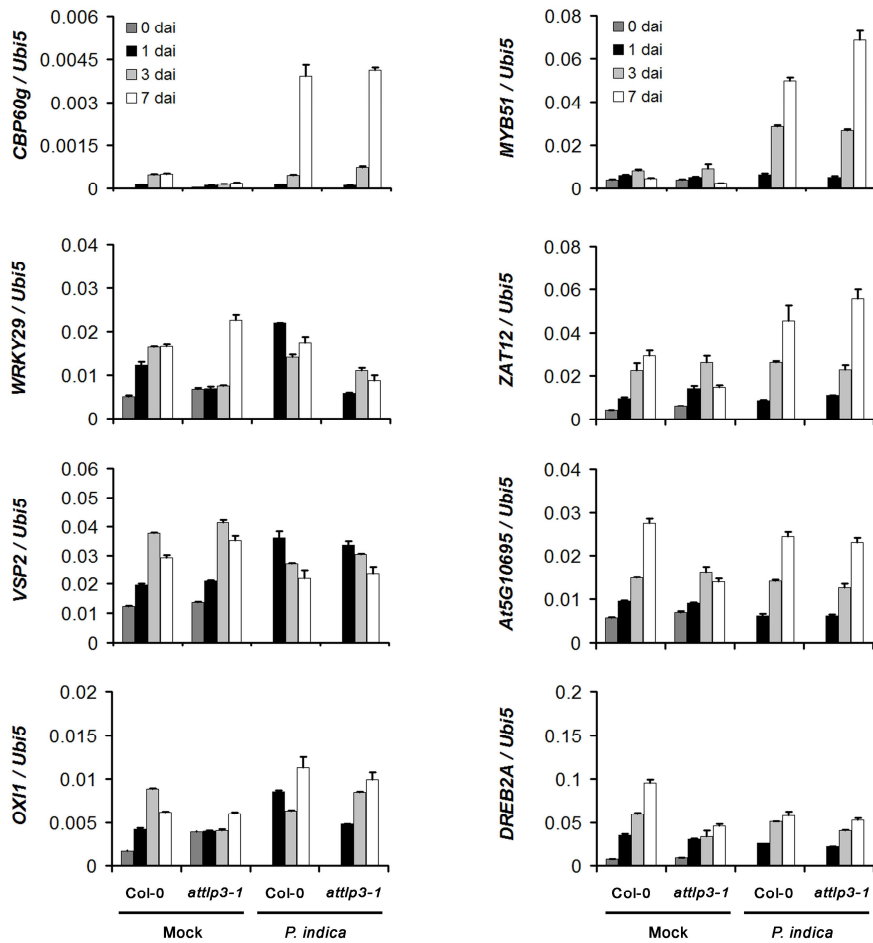
## Supplemental Figure 4



Supplemental Figure 4 Unaltered colonization of attlp mutants by *E. cruciferarum*, *B. cinerea*, and *P. parasitica*.

(A) Leaves of Col-0, attlp2-1, 3-1, 5-1, and 8-1 were inoculated with *Botrytis cinerea* and lesion diameter was determined at 4 dai. The variation between mutants and Col-0 were statistically insignificant. Data are averages from at least ten plants of one biological experiment and bars represent standard errors. The experiment was repeated four times with similar results. (B) Roots of Col-0, attlp2-1, 3-1, 5-1 and 9-1 were inoculated with *Phytophthora parasitica* and disease index was determined at 7, 8, 10, 11, 14, 15, 17, and 17 days after inoculation. Differences in disease indices between Col-0 and mutants were statistical not significant. Bars represent standard deviations from one biological. The experiment was repeated two times with similar results. (C) Leaves of Col-0, attlp3-1, 3-2, 5-2 were inoculated with *Erysiphe cruciferarum*. Images present leaves overgrown with the fungus at 11 dai. The experiment was repeated two times with similar results.

## Supplemental Figure 5

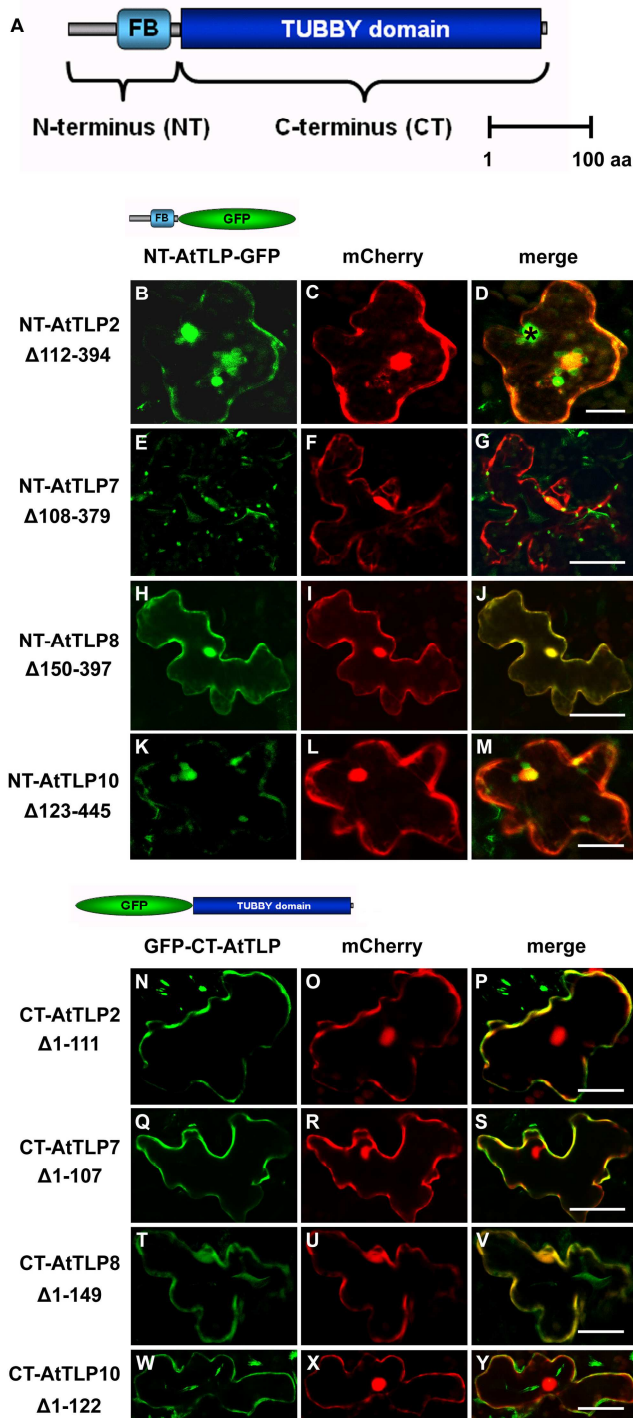


Supplemental Figure 5 Marker genes for MAMP-triggered immunity and abiotic stress do not show an elevated expression in *attlp3* mutant during *P. indica* colonization.

Three-week-old *Arabidopsis* plants (Col-0, *attlp3*) were mock-treated or inoculated with *P. indica* and harvested at 0, 1, 3, and 7 days after treatments. None of the genes showed an altered expression due to the lack of AtTLP3. Minor changes were observed for WRKY29 expression at 1, 3, and 7 dai or mock treatment and for At5G10695 at 7 days after mock treatment. Expression values were calculated by the  $2^{-\Delta Ct}$  method by relating Ct thresholds of candidates to those of the housekeeping gene AtUbiquitin 5. Standard errors are from three technical replicates of one biological experiment. Experiments were repeated at least twice with similar results.



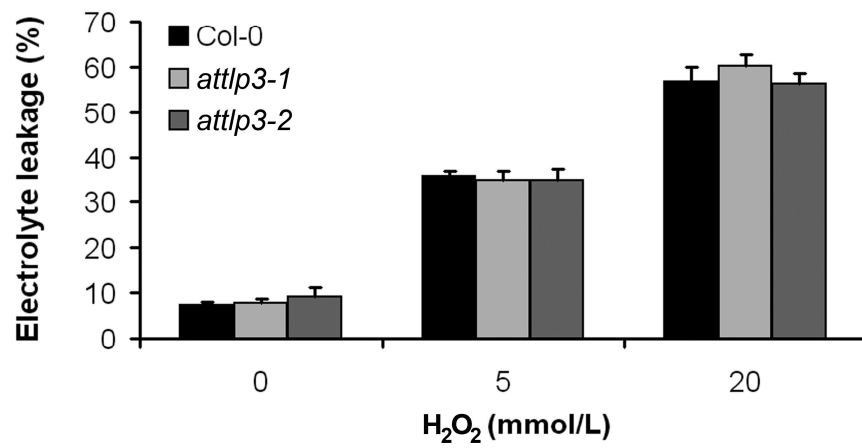
Supplemental Figure 6



Supplemental Figure 6 Separate localization of F-box and Tubby domain of AtTLPs.

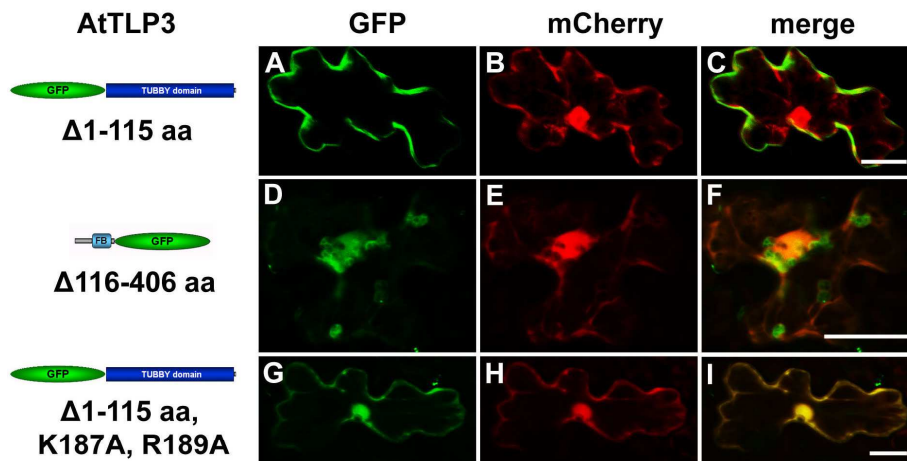
(A) Model of AtTLPs with the F-box (FB) domain at the N-terminus (NT) and the Tubby domain at the C-terminus (CT). Scale bar indicates the length of AtTLPs in amino acids (aa). For all experiments, leaf cells were co-transformed with the NT-AtTLPs-GFP (B, E, H, K) or GFP-CT-AtTLPs (N, Q, T, W) fusions under control of the 35S promoter and cytosolic and nucleoplasmic marker mCherry (C, F, I, L, O, R, U, X) by biolistic transformation. (D, G, J, M, P, S, V, Y) Merged images indicate subcellular localization of domains. Constructs used for transformation are imaged on the left. (B-D) Plastidial and nucleo-cytosolic localization of NT-AtTLP2-GFP ( $\Delta$ 112-394). (E-G) Plastidial localization of NT-AtTLP7-GFP ( $\Delta$ 108-379). (H-J) Cytosolic and nucleoplasmic localization of NT-AtTLP8-GFP ( $\Delta$ 150-397). (K-M) Plastidial and nucleo-cytosolic localization of NT-AtTLP10-GFP ( $\Delta$ 123-445). (N-P) Plasma membrane (PM) localization of GFP-CT-AtTLP2 ( $\Delta$ 1-111). (Q-S) PM localization of GFP-CT-AtTLP7 ( $\Delta$ 1-107). (T-V) Cytosolic and nucleoplasmic localization of GFP-CT-AtTLP8 ( $\Delta$ 1-149). (W-Y) PM localization of GFP-CT-AtTLP10 ( $\Delta$ 1-122). Bars = 20 $\mu$ m.

## Supplemental Figure 7



Supplemental Figure 7 Effects of H<sub>2</sub>O<sub>2</sub> on relative ion leakage in leaf discs of control and mutant plants. Bars represent standard deviations from three independent measurements of one biological experiment. The experiment was performed twice with similar results.

## Supplemental Figure 8



Supplemental Figure 8 Subcellular localization of truncated versions of AtTLP3 in *Nicotiana benthamiana*. *Nicotiana benthamiana* leaf cells were co-transformed with the GFP-CT-AtTLP3 ( $\Delta 1-115$ ) (A), NT-AtTLP3-GFP ( $\Delta 116-406$ ) (D) or GFP-CT-AtTLP3 ( $\Delta 1-115$ , K187A, R189A) (G) fusions under control of the 35S promoter and cytosolic and nucleoplasmic marker mCherry (B, E, H) by biolistic transformation. Amino acids eliminated in truncated versions used for transformation and treatments are drafted on the left. (A) GFP-CT-AtTLP3 ( $\Delta 1-115$ ) displayed plasma membrane (PM) localization. (D) Plastidial and nucleo-cytosolic localization of NT-AtTLP3-GFP. (G) GFP-CT-AtTLP3 ( $\Delta 1-115$ ), in which K187 and R189 had been converted to alanine (A), has lost its PM binding ability and shows cytosolic and nucleoplasmic localization. (C, F, I) Merged images indicate subcellular localization of truncated domain versions. Yellow colour indicates co-localization of green and red fluorescing proteins. Bars = 20  $\mu\text{m}$ .

## Supplemental Tables

**Supplemental Table 1** Prediction of the subcellular localization of AtTLPs by WoLF PSORT.

WoLF PSORT			
Prediction of subcellular localization of AtTLP proteins			
Name	AGI	Amino acids	WoLF PSORT
AtTLP1	AT1G76900	1-120	Chloroplast (7.0), cytosol (3.0), nucleus (2.0), mitochondrion (1.0)
AtTLP2	AT2G18280	1-111	Cytosol (6.0), chloroplast (5.0), nucleus (2.0)
AtTLP3	AT2G47900	1-115	Chloroplast (12.0), cytosol (1.0)
AtTLP5	AT1G43640	1-118	Chloroplast (9.0), cytosol (3.0), plasma membrane (1.0)
AtTLP6	AT1G47270	1-132	Chloroplast (7.0), cytosol/nucleus (3.0), nucleus (2.5), cytosol (2.5), extracellular (1.0)
AtTLP7	AT1G53320	1-107	Mitochondrion (6.0), nucleus (3.5), cytosol/nucleus (2.5), chloroplast (2.0), extracellular (2.0)
AtTLP8	AT1G16070	1-149	Nucleus (13)
AtTLP9	AT3G06380	1-97	Chloroplast (7), mitochondrion (7)
AtTLP10	AT1G25280	1-122	Chloroplast (10), nucleus (4)
AtTLP11	AT5G18680	1-103	Chloroplast (7.5), chloroplast/mitochondrion (6.0), mitochondrion (3.5), cytosol (2.0)

**Supplemental Table 2** List of primers used in this study.

Please see supplemental excel sheet.