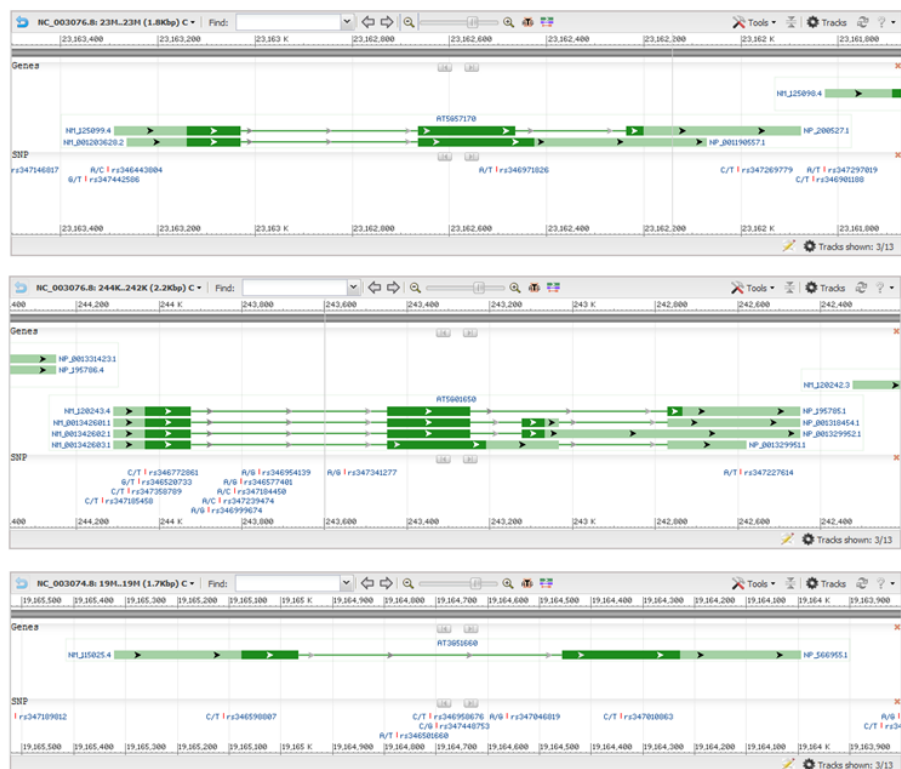


## Supplementary Figure 1:

**A**



**MDL1.1**  
**MDL1.2**

MDL2.1  
MDL2.2  
MDL2.3  
MDL2.4

MDL3

**B**

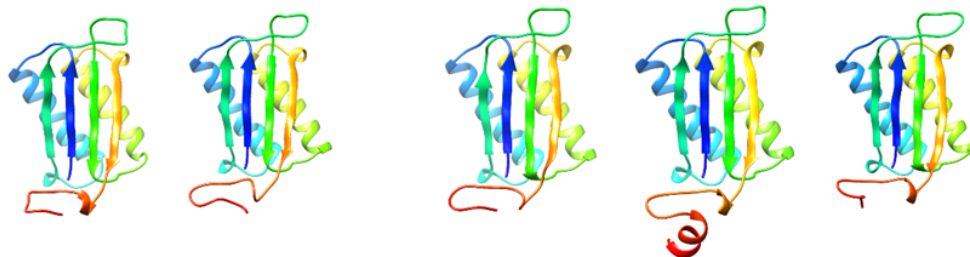
## MDL1.1

## MDL1.2

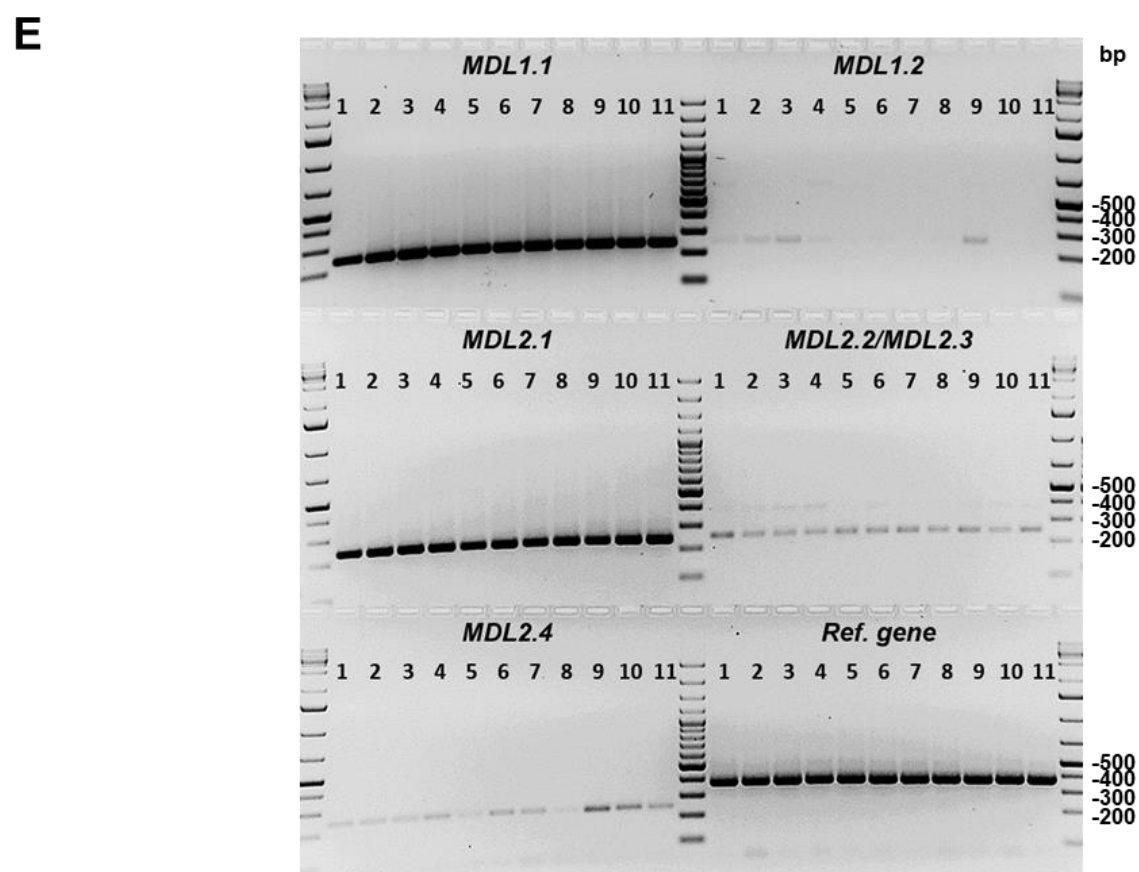
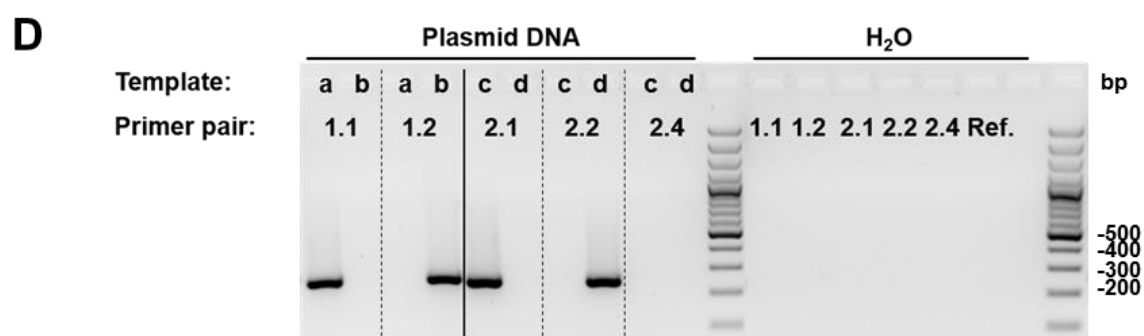
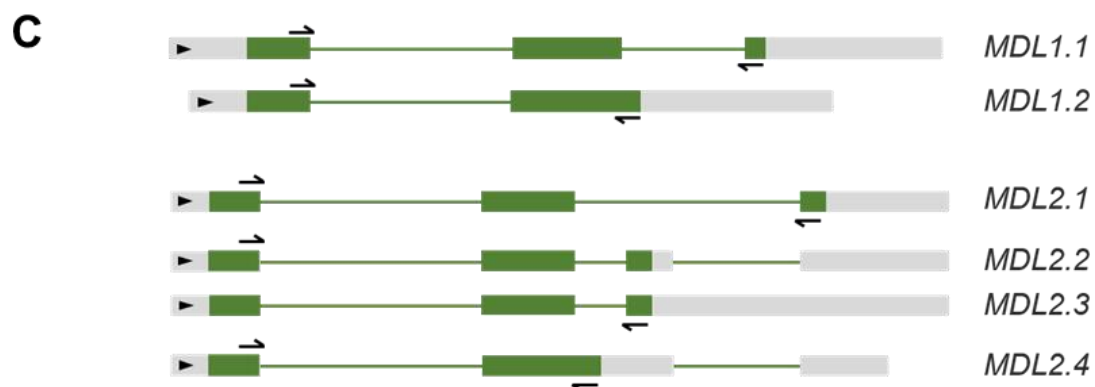
MDL2.1

MDL2.2

MDL2.4



## Supplementary Figure 1 continued:



### **Supplementary Figure 1: Validation and expression of *MDL* splice variants.**

**(A)** Screenshot indicating splice variants of *MDL1*, *MDL2*, and *MDL3* suggested by the NCBI database (<https://www.ncbi.nlm.nih.gov/gene/>) (accessed in April 2017). *MDL1* and *MDL2* are supposed to occur in different splice variants designated as *MDL1.1* and *MDL1.2* as well as *MDL2.1*, *MDL2.2*, *MDL2.3*, and *MDL2.4*, respectively. Note that *MDL2.2* and *MDL2.3* transcripts differ only in the 3' untranslated region and code for the same protein variant (in our study designated MDL2.2).

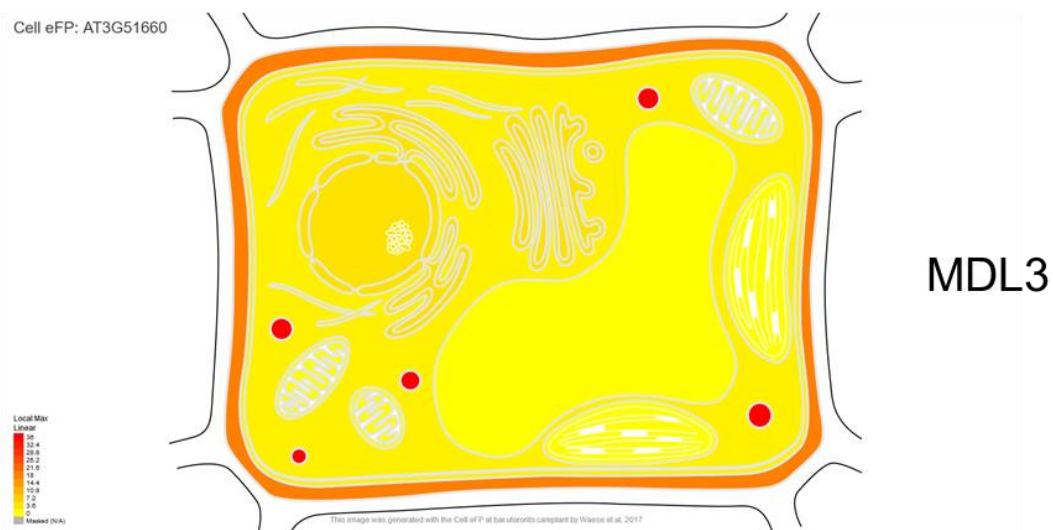
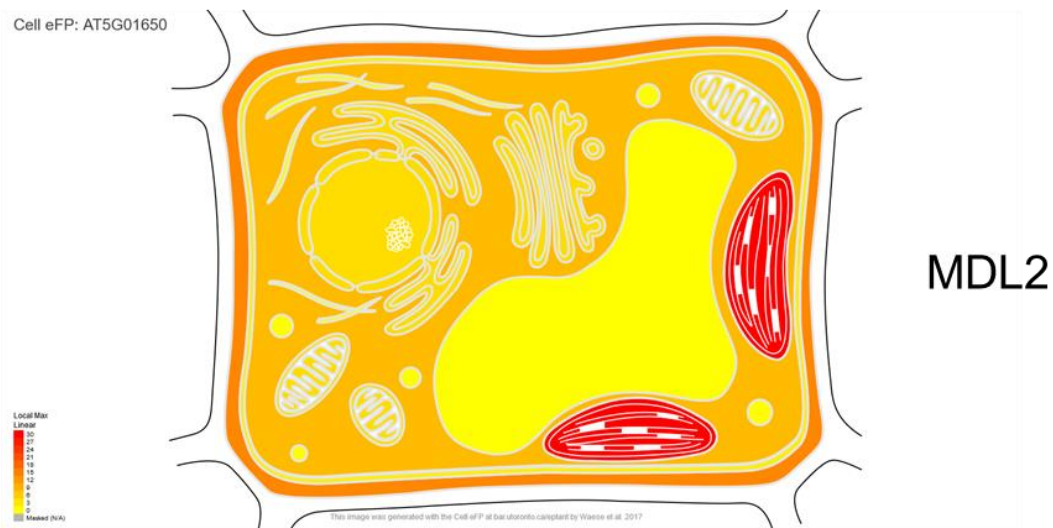
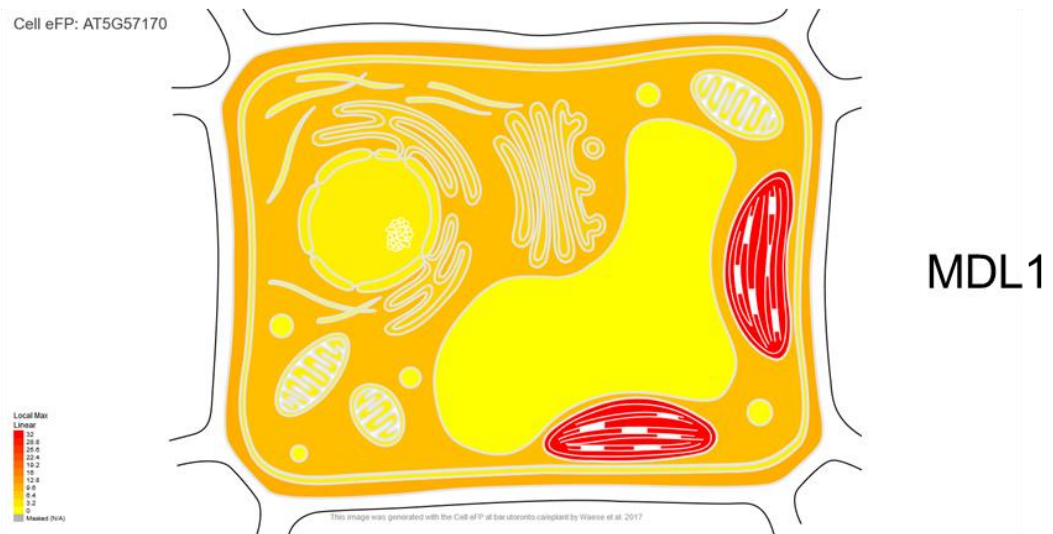
**(B)** Protein structure prediction of *MDL1* and *MDL2* splice variants generated with I-TASSER (<https://zhanglab.ccmb.med.umich.edu/I-TASSER/>).

**(C)** Positioning of primer pairs specific for the amplification of *MDL1* and *MDL2* splice variants by RT-PCR. Note that transcripts *MDL2.2* and *MDL2.3* are co-amplified by the same primer pair.

**(D)** PCR to validate the specificity of primer pairs for the amplification of *MDL1* and *MDL2* splice variants using cloned *MDL* versions (plasmid DNA) as a template. Plasmid template DNA is as follows: a, pDONR207-MDL1.1; b, pDONR207-MDL1.2; c, pDONR207-MDL2.1; d, pDONR207-MDL2.2. The right side of the gel shows water controls (no template DNA) for the various primer pairs.

**(E)** Semiquantitative RT-PCR analysis applying the splice variant-specific primer pairs and a primer pair for a reference gene (*AT4G26410*) as indicated on template cDNA from different plant organs of Arabidopsis Col-0 at different ages: 1: roots; 2-7: rosette leaves of different plant age (14-50 days-old); 8-11: floral structures (cauline leaves, flowers, siliques) of different plant age (42-50 days-old). Primer sequences are listed in Table S3.

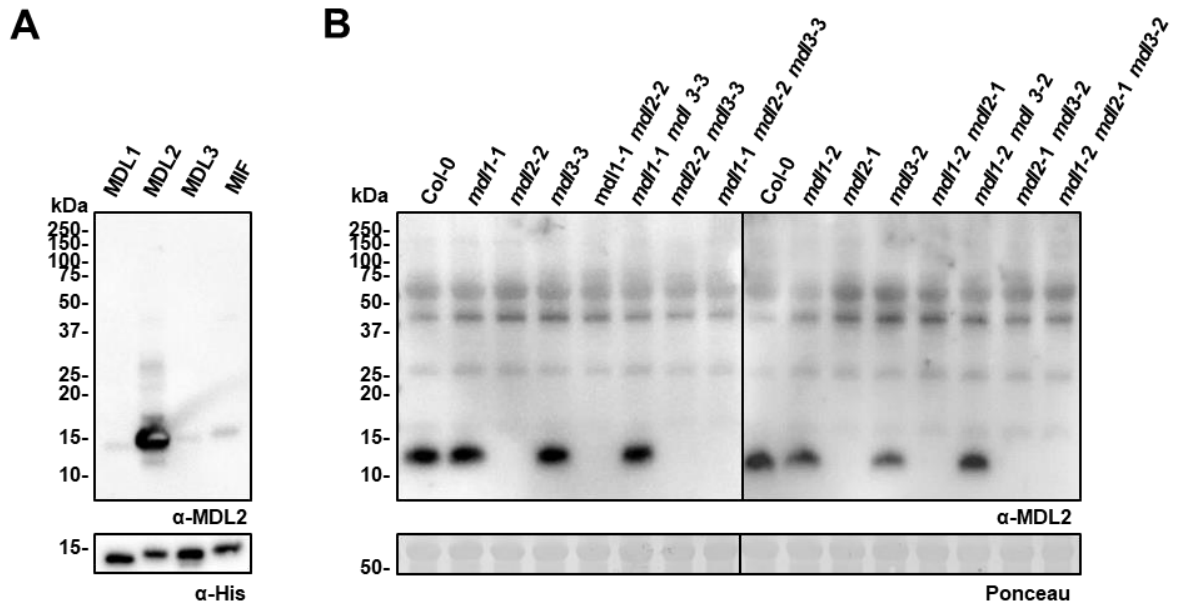
## Supplementary Figure 2:



## **Supplementary Figure 2: Predicted subcellular localization of MDL proteins.**

Screenshots of predictions of the Arabidopsis Cell electronic Fluorescent Pictograph (eFP) Browser at BAR ePlant (<https://bar.utoronto.ca/eplant/>) for MDL1, MDL2, and MDL3 (accessed on 10<sup>th</sup> July 2020). Color-coding according to the scales on the left indicate a score (presumed reliability) for the predicted subcellular localization. Organelles shown in red (i.e., highest score) represent chloroplasts for MDL1 and MDL2 and peroxisomes for MDL3.

## Supplementary Figure 3:

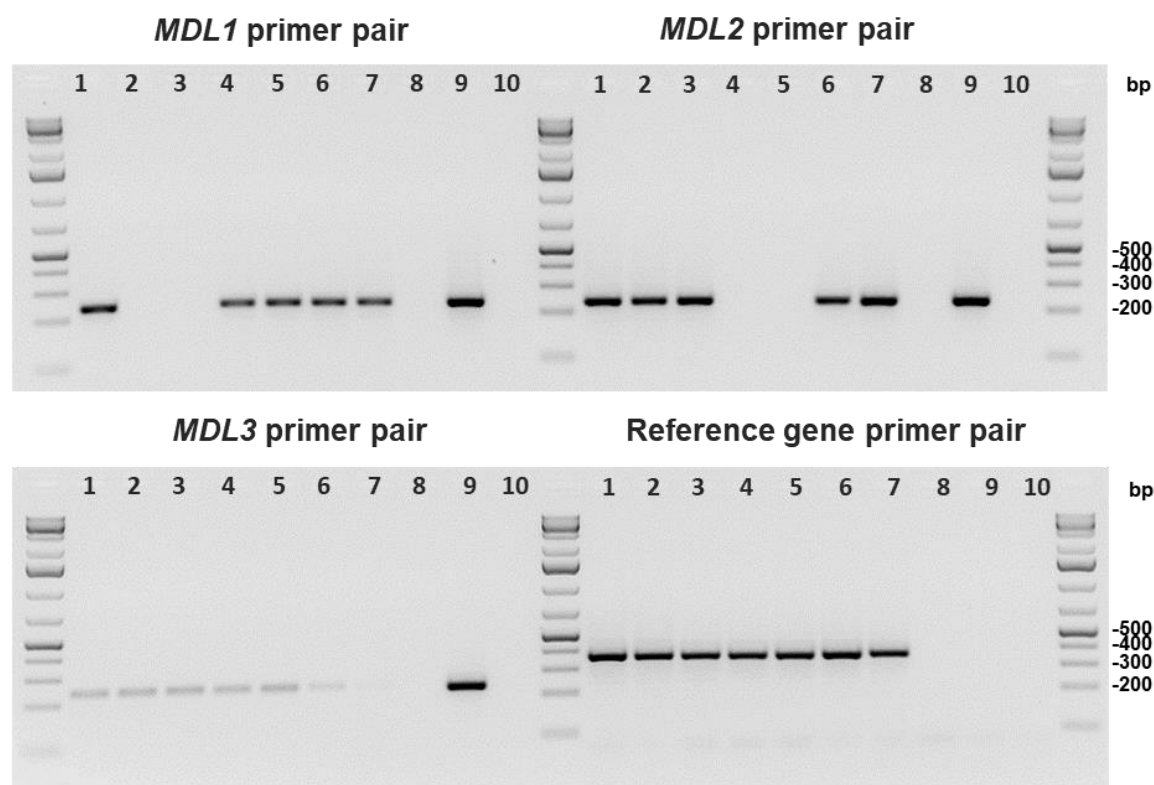


**Supplementary Figure 3: Specificity of the monoclonal  $\alpha$ -MDL2 antibody.**

**(A)** Bacterial lysates of *Escherichia coli* expressing 6xHis-MDL1, -MDL2, -MDL3, or -MIF (human MIF; control) proteins (21, 52) were used to test the specificity of the monoclonal  $\alpha$ -MDL2 antibody ATM 20C8. Proteins were separated by SDS-PAGE, transferred onto a nitrocellulose membrane, and recombinant MDL2 was detected using the monoclonal  $\alpha$ -MDL2 antibody ATM 20C8 (upper panel). Equal loading of 6xHis-MDLs/MIF was confirmed by immunodetection of the hexahistidine tag with an  $\alpha$ -His antibody (lower panel).

**(B)** MDL2 accumulation in leaves of five-week-old Arabidopsis Col-0 wild-type and *mdl* single, double, and triple mutant plants was analyzed with the monoclonal  $\alpha$ -MDL2 antibody ATM 20C8. Total protein (10  $\mu$ g) of leaf extracts was separated by SDS-PAGE, transferred onto a nitrocellulose membrane, and MDL2 was detected using the monoclonal  $\alpha$ -MDL2 antibody ATM 20C8 (upper panel). Equal loading was confirmed by Ponceau S staining (lower panel).

## Supplementary Figure 4:

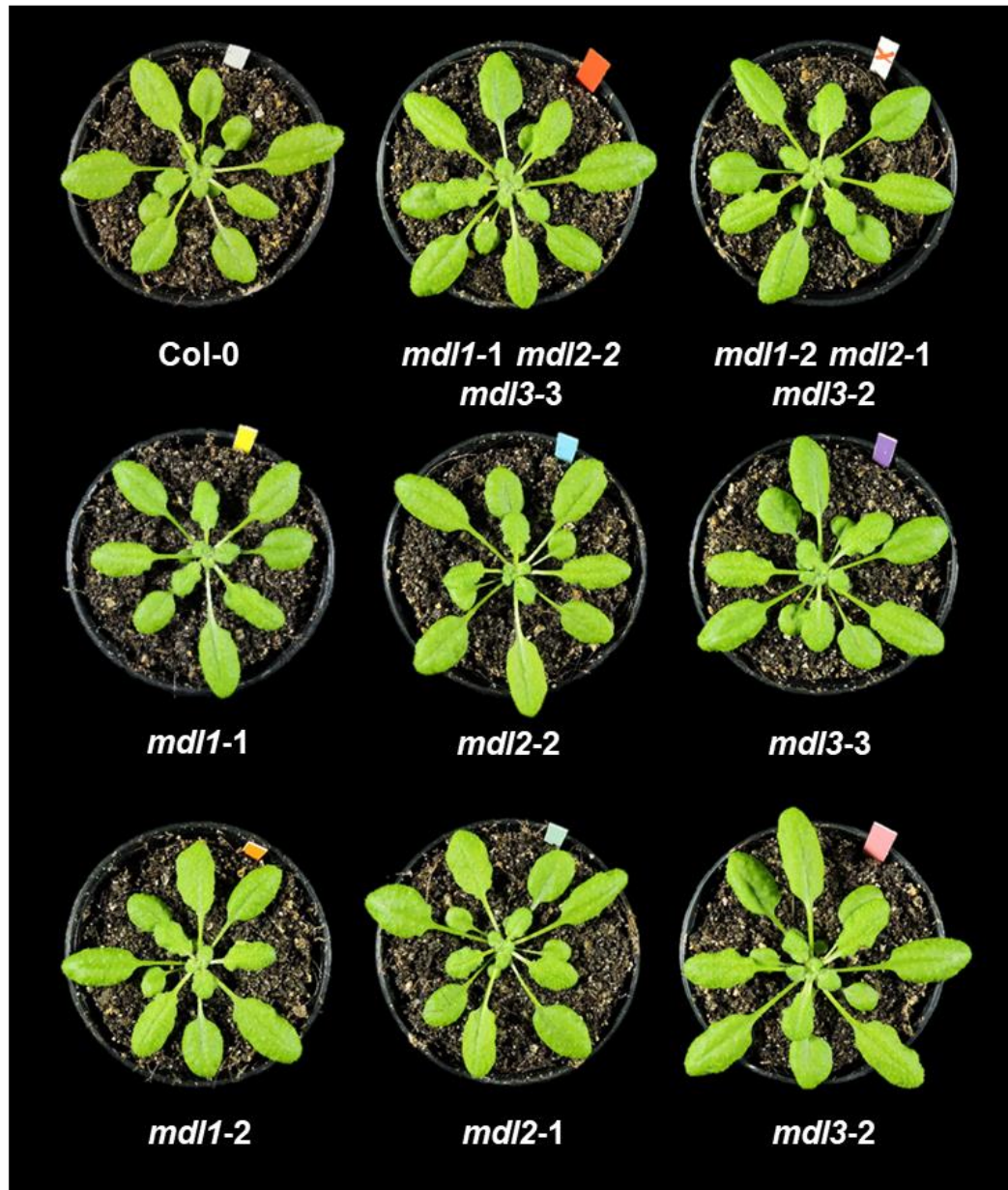


### Supplementary Figure 4: Characterization of *mdl* T-DNA insertion mutants.

Semi-quantitative RT-PCR analysis of cDNA from 1: Col-0 wild-type, 2: *mdl1-1*, 3: *mdl1-2*, 4: *mdl2-1*, 5: *mdl2-2*, 6: *mdl3-2*, 7: *mdl3-3*, 8: genomic DNA of Col-0 wild-type, 9: plasmid DNA pDONR207-MDL1/-MDL2/-MDL3, and 10: ddH<sub>2</sub>O with primer pairs specific for *MDL1*, *MDL2*, *MDL3*, or a reference gene (*AT4G26410*). Primer sequences are listed in Table S3.



## Supplementary Figure 5:



Supplementary Figure 5: Naïve *mdl* mutants do not show obvious abnormalities during vegetative growth.

Habitus of five-week-old naïve *mdl* single and triple mutants in comparison to Col-0 wild-type.

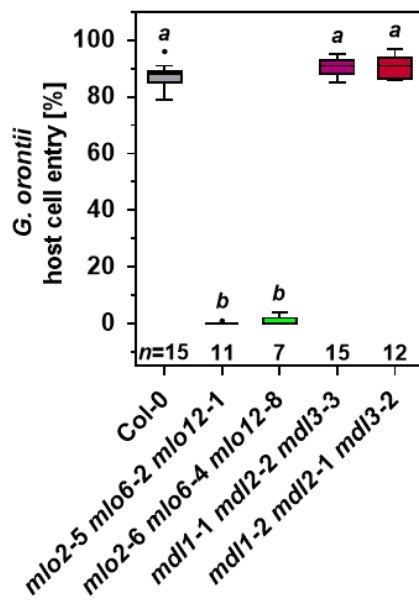


## Supplementary Figure 6:

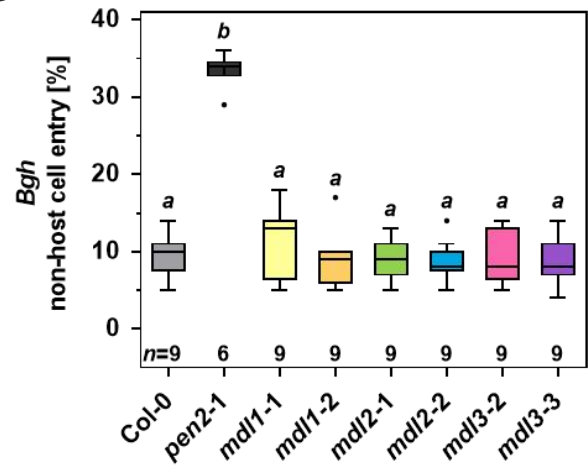
**A**



**B**



**C**



**Supplementary Figure 6: *mdl* mutants show unaltered infection phenotypes upon challenge with *G. orontii* and *Bgh*.**

Four-week-old Arabidopsis plants were inoculated with conidiospores of (A-B) *G. orontii* or (C) *Bgh*.

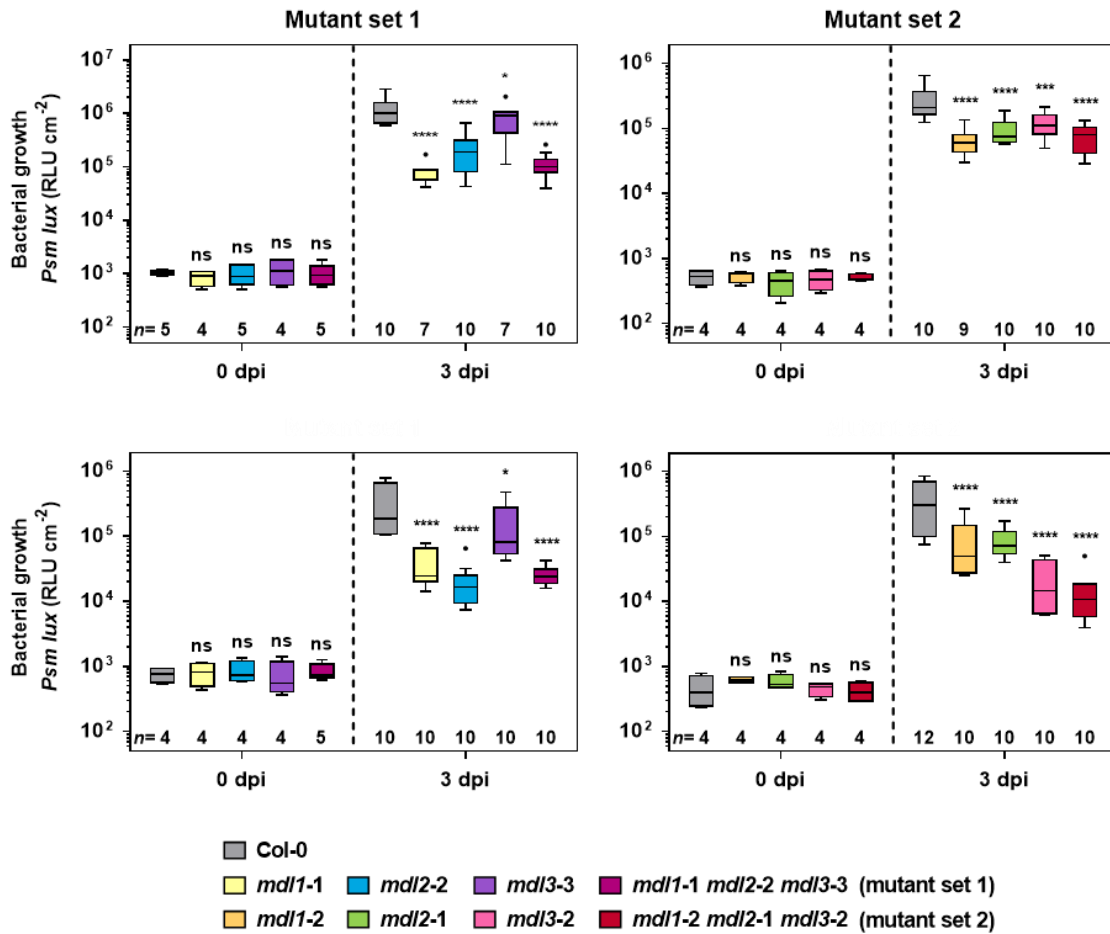
**(A)** Representative macroscopic disease phenotypes of various *mdl* single and triple mutants in comparison to Col-0 wild-type and the powdery mildew-resistant *mlo2 mlo6 mlo12* mutant at 7 dpi with *G. orontii*.

**(B)** Boxplots represent microscopically assessed host cell entry rates of *G. orontii* at 2 dpi in leaf epidermis cells of two *mdl1 mdl2 mdl3* triple mutants in comparison to Col-0 wild-type and two powdery mildew resistant *mlo2 mlo6 mlo12* triple mutants. The number of evaluated plants per genotype in a total of four independent biological replicates is given as *n* above the x-axis with each plant value representing the evaluation of three to four leaves and a minimum of 100 evaluated interaction sites per leaf.

**(C)** Boxplots represent microscopically assessed host cell entry rates of *Bgh* at 2 dpi in leaf epidermis cells of various *mdl* single mutants in comparison to Col-0 wild-type and the susceptible *pen2-1* mutant. The number of evaluated plants per genotype in a total of three independent biological replicates is given as *n* above the x-axis with a minimum of 100 inspected interaction sites per leaf.

Statistical significance between the plant genotypes was determined with a one-way multi-paired ANOVA test. Letters above the boxplots in **(B)** and **(C)** denote different significance groups ( $p < 0.05$ ). Raw data and exact statistical values for these two graphs can be found in in the Source data file.

## Supplementary Figure 7:



**Supplementary Figure 7: *mdl* mutants exhibit enhanced basal resistance toward *Psm* – additional experimental data to Figure 4.**

Shown are experimental repetitions additional to Fig. 4.

Three mature leaves of five-week-old Arabidopsis plants were infiltrated with *Psm lux* (OD<sub>600</sub> 0.0005). Boxplots represent bacterial titers of *Psm lux* at 0 dpi and 3 dpi in leaves from the indicated *mdl* single and triple mutants of the two *mdl* mutant sets in comparison to Col-0 wild-type. Titters were recorded via bacterial bioluminescence and are given in RLU cm<sup>-2</sup>. The number of evaluated plants per genotype and time point is given as *n* above the x-axis with each plant value representing the mean of three leaves. Statistical significance between the plant genotypes was determined for the 0 dpi and 3 dpi datasets, each in comparison to the respective Col-0 control, with a two-way multi-paired ANOVA test (\*\*\*\* *p*<0.0001, \*\*\*

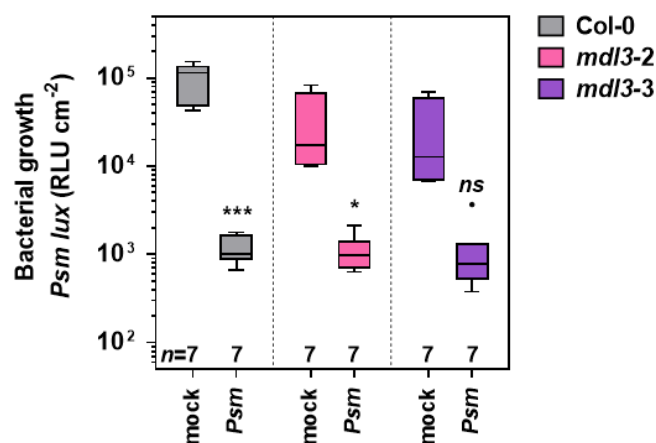
$p < 0.001$ , \*  $p < 0.05$ , ns = not significant). Raw data and exact statistical values for these four graphs can be found in in the Source data file.

## Supplementary Figure 8:

**A**

	Name		MDL3	MDL1	MDL2
	AGI code		AT3G51660	AT5G57170	AT5G01650
Mean expression values	Col-0	H <sub>2</sub> O	3.0	22.5	48.4
		Pip	24.4	14.8	46.9
	fmo1	H <sub>2</sub> O	1.9	19.6	44.9
		Pip	2.3	19.5	46.1
Fold-change (log <sub>2</sub> )	Rank		144	1046	5235
	Col-0	Pip/H	2.7**	-0.6	0.0
	fmo1	Pip/H	0.2	0.0	0.0
	Pip gene		+		
Mean expr. value	Col-0	mock	5.2	14.7	40.5
		Psm	152.7	3.2	43.5
	Rank		75	5824	25937
Fold-change (log <sub>2</sub> )	Col-0	Psm/m	4.6**	-1.9**	0.1
	SAR gene		+	-	

**B**



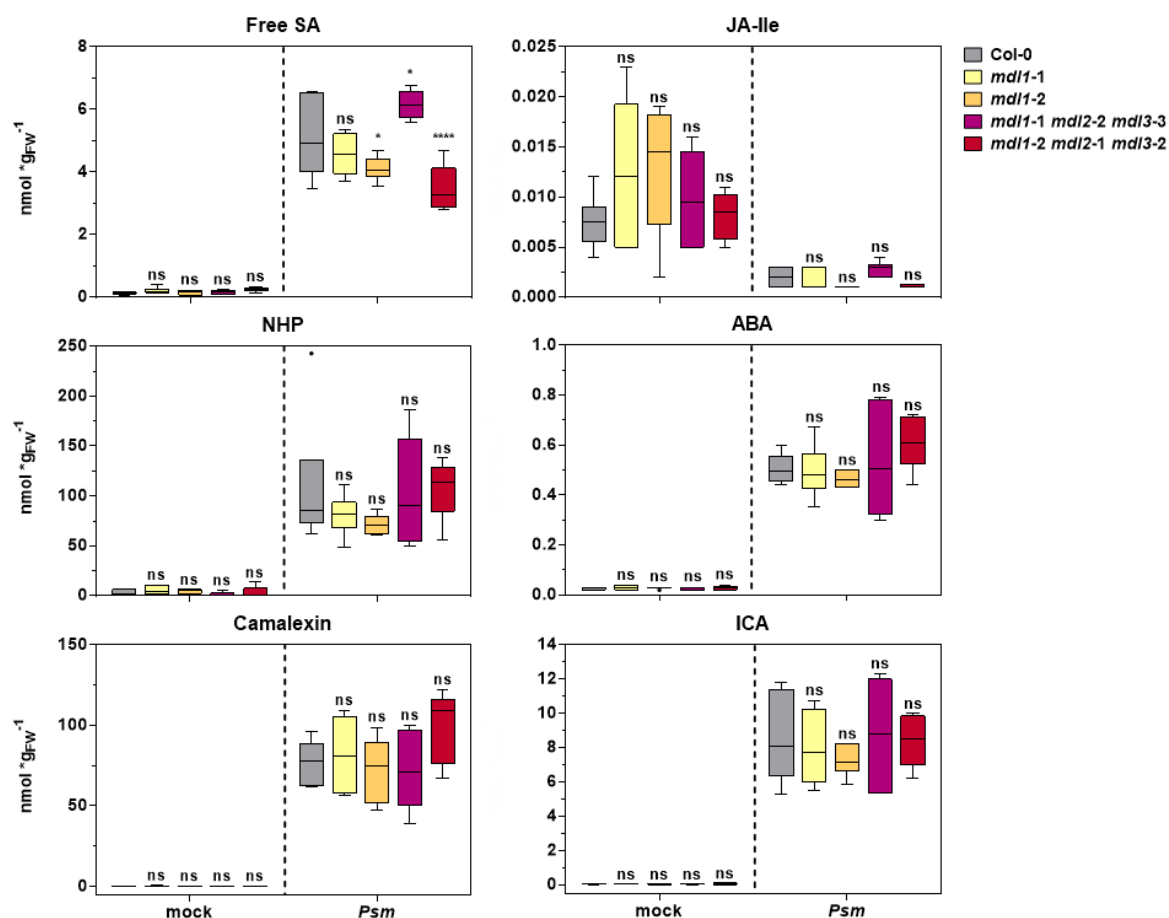
**Supplementary Figure 8: *mdl3* mutants are SAR-competent.**

**(A)** *MDL3* is systemically upregulated in pipecolic acid (Pip)- and SAR-induced Arabidopsis Col-0 plants. The Table was compiled based on published whole transcriptome shotgun sequencing (RNA-seq) data (42, 43). Mean expression values include three replicates per treatment and genotype (Col-0 and *fmo1*). In addition, fold-changes are depicted by mean log<sub>2</sub>-transformed Pip/H- or *Psm*/m-ratios. A positive value denotes significant up- and a negative value downregulation of the gene in the respective treatment. Expression values are given as reads per million (RPM) and asterisks indicate significant expression differences. (\*\*: false discovery rate < 0.001). H: H<sub>2</sub>O; m: mock.

**(B)** SAR response of two independent *mdl3* mutants was monitored by a primary infiltration of three lower leaves of five-week-old plants with *Psm* (OD<sub>600</sub> 0.005) or 10 mM MgCl<sub>2</sub> (mock), followed by a secondary inoculation of three systemic leaves with *Psm lux* (OD<sub>600</sub> 0.0005) 2 days later. Bacterial titers of *Psm lux* were measured as bacterial bioluminescence at 3 dpi and are expressed as RLU cm<sup>-2</sup>. The number of evaluated plants per genotype and treatment is given as *n* above the x-axis with each plant value representing the mean of three leaves. Statistical significance between mock-treated and *Psm*-challenged plants was determined with a two-way multi-paired ANOVA test (\*\*\* *p*<0.001, \* *p*<0.05, ns = not significant). Raw data and exact statistical values for this graph can be found in in the Source data file.



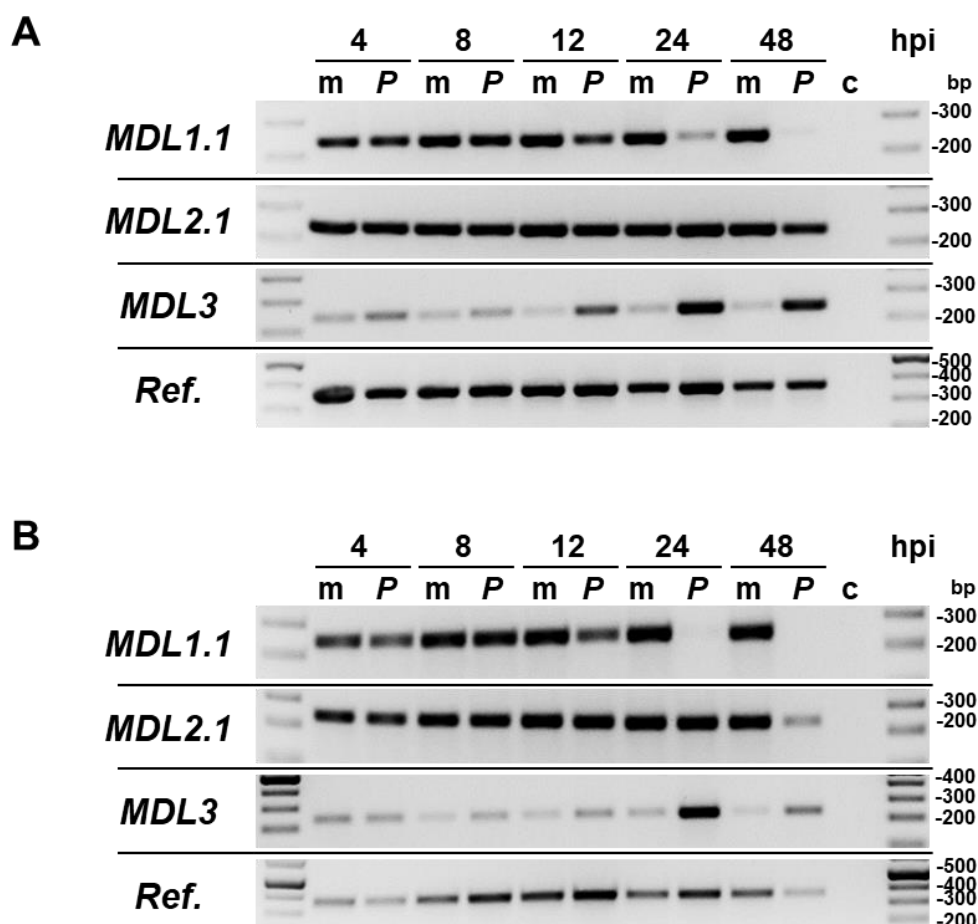
## Supplementary Figure 9:



**Supplementary Figure 9: Unaltered phytohormone and defense metabolite accumulation in leaves of *mdl* mutants in response to *Psm* infection.**

**(A)** Local levels of common phytohormones and defense-associated metabolites - free SA, JA-Ile, NHP, ABA, camalexin, and ICA – determined at 32 hpi of two *mdl1* single and two *mdl1 mdl2 mdl3* triple mutants, and *Col-0* wild-type with *Psm* (OD<sub>600</sub> 0.005) or buffer (10 mM MgCl<sub>2</sub>; mock control). Boxplots represent data from two independent biological replicates with three technical replicates each (six values in total). Statistical significance between the plant genotypes was determined for the mock-treated and *Psm*-challenged plants, each in comparison to the respective *Col-0* control, with a two way multi-paired ANOVA test (\*\*\*\*  $p < 0.0001$ , \*  $p < 0.05$ , ns = not significant). Raw data for these six graphs can be found in the Source data file.

## Supplementary Figure 10:



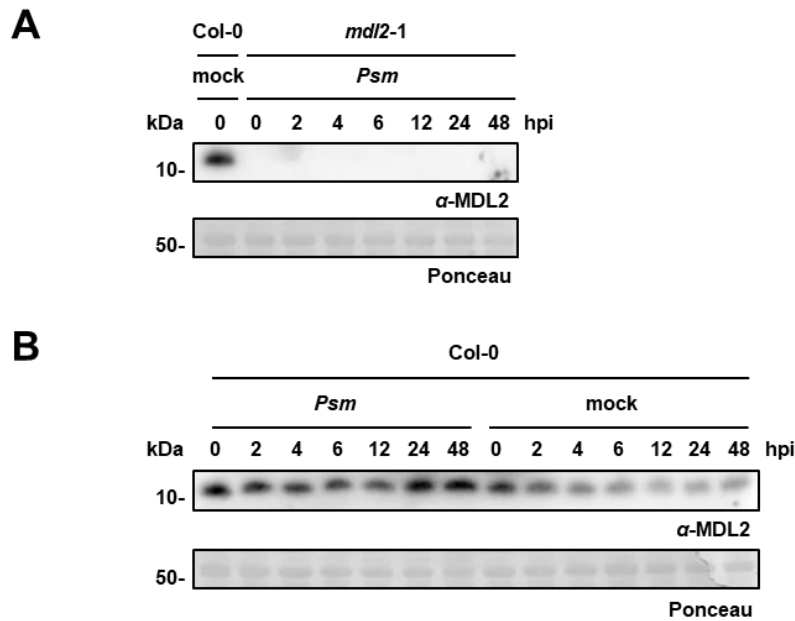
**Supplementary Figure 10: Differential transcript accumulation of *MDL1.1*, *MDL2.1*, and *MDL3* in response to *Psm* infection.**

Three mature leaves of five-week-old *Arabidopsis* Col-0 wild-type plants were either infiltrated with *Psm* (P; OD<sub>600</sub> 0.005) or mock-treated (m; 10 mM MgCl<sub>2</sub>). Infiltrated leaves were sampled at the indicated time points (hpi). After extraction of total RNA, cDNA was generated via reverse transcription. The resulting cDNA was applied as a template for an *MDL* splice variant-specific PCR amplification with 35 cycles and an annealing temperature of 57 °C. The reference gene (*Ref.*) used in these experiments is *AT4G26410*. Primer sequences are listed in Table S3.

**(A)** and **(B)** show two independent biological replicates. In each experiment, the cDNA of every sample is based on the pooled RNA from a total of six plants. m = mock, P = *Psm*, c = control

(ddH<sub>2</sub>O instead of cDNA). The Figure shows the relevant cropped areas of the four original gels.

## Supplementary Figure 11:



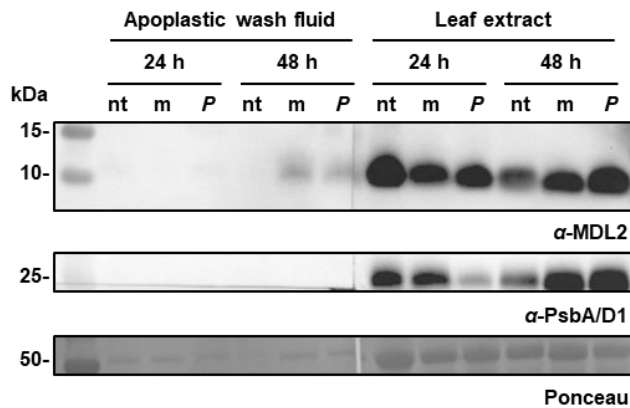
### Supplementary Figure 11: MDL protein accumulation during bacterial pathogenesis.

Three mature leaves of five-week-old *Arabidopsis* Col-0 wild-type and *mdl2-1* mutant plants were infiltrated with *Psm* (OD<sub>600</sub> 0.005) or 10 mM MgCl<sub>2</sub> (mock control) and leaves were sampled at the indicated time points. Total protein (10 µg) extracted from the leaf samples was separated by SDS-PAGE and proteins were transferred onto a nitrocellulose membrane. MDL2 was detected using the monoclonal  $\alpha$ -MDL2 antibody ATM 20C8 (upper panels). Equal loading was confirmed by Ponceau S staining (lower panels).

**(A)** The specificity of the monoclonal  $\alpha$ -MDL2 antibody ATM 20C8 and the absence of cross-reaction with bacterial proteins were tested with *mdl2-1* mutant plants inoculated with *Psm* and samples harvested at the indicated time points.

**(B)** Accumulation of MDL2 in Col-0 after inoculation with *Psm* in comparison to mock-treatment in samples harvested at the indicated time points. The experiment was repeated twice with similar results.

## Supplementary Figure 12:



### Supplementary Figure 12: No evidence for pathogen-triggered accumulation of MDL2 in leaf AWF.

MDL2 does not accumulate in apoplastic wash fluid of five-week-old Arabidopsis Col-0 wild-type plants that were spray-inoculated with *Psm* (OD<sub>600</sub> 0.2), mock-treated, or left untreated. Displayed is an immunoblot of AWF and leaf extract at 24 and 48 hpi *Psm* (P), mock (m), and no treatment (nt). In the case of the AWF, 10  $\mu$ L per sample was loaded. Leaf extract was isolated from two leaves per sample after AWF extraction, and 5  $\mu$ g per sample were loaded. MDL2 was detected using the monoclonal  $\alpha$ -MDL2 antibody ATM 20C8 and the chloroplast protein-specific antibody  $\alpha$ -PsbA/D1 was used as a control for cytosolic contamination. Equal loading was confirmed by Ponceau S staining. The experiment was performed three times with similar results.