New phytologist **supporting information**

Article title: **Silencing of *AtRAP*, a target gene of a bacteria-induced small RNA, triggers antibacterial defense responses through activation of LSU2 and down-regulation of *GLK1***

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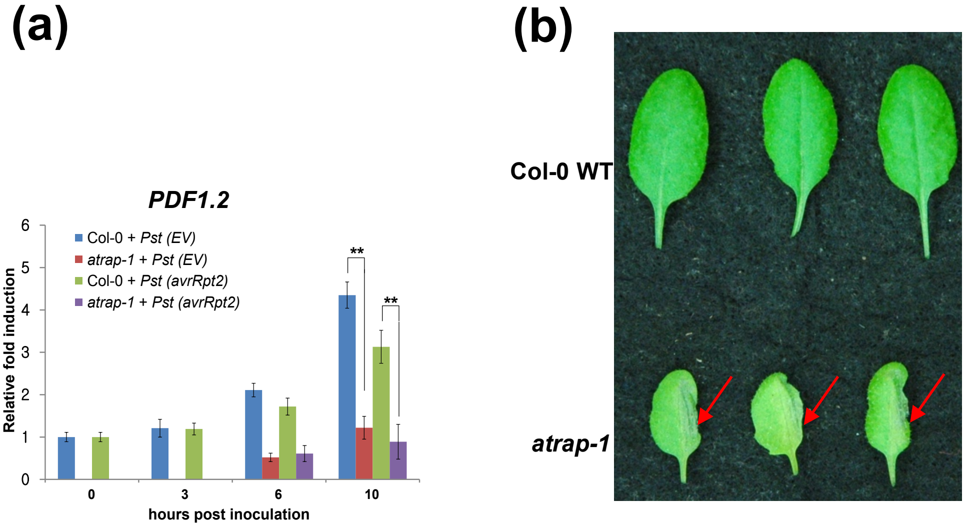

Fig. S1 AtRAP negatively regulates plant defense to *Pst*. (a) Five-week-old *Arabidopsis* Col-0 WT and the *atrap* mutant plants were infiltrated with *Pst* (EV) or *Pst* (*avrRpt2*) at a concentration of 2 x 10^7 cfu/mL. Total RNAs were extracted from the infiltrated leaves at the times indicated on the horizontal axes. Relative accumulation levels of *PDF1.2* transcripts were quantified by quantitative real-time PCR. Error bars represent the SEM from three independent experiments. ** $p < 0.01$ (determined by Student's t test). (b) HR cell death phenotypes in the leaves of *Arabidopsis* Col-0 WT and the *atrap* mutant plants. The leaves were infiltrated with *Pst* (*avrRpt2*) at a concentration of 2 x 10^7 cfu/mL at 8 hours post inoculation (hpi). The red arrowheads indicate collapse of infiltrated leaf areas caused by HR cell death.

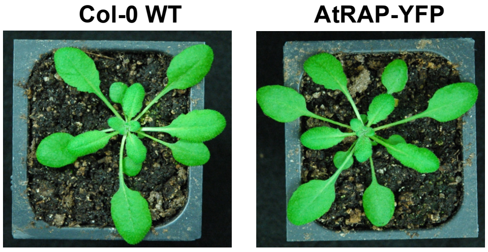

Fig. S2 Phenotype of AtRAP overexpressing plants. 35S::AtRAP-YFP was transformed into the *atrap-1* mutant background. The virescent phenotype and the growth retardation defects of *atrap-1* were complemented.

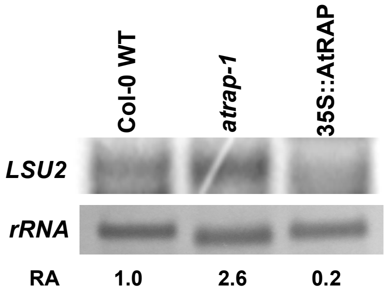

Fig. S3 *LSU2* is negatively regulated by *AtRAP*. Detection of *LSU2* mRNA in the Col-0 WT, the *atrap* mutant and the 35S::AtRAP plants. Five-week-old *Arabidopsis* Col-0 WT, the *atrap* mutant and the 35S::AtRAP plants were collected. Total RNAs were extracted and subjected to Northern blot analysis.

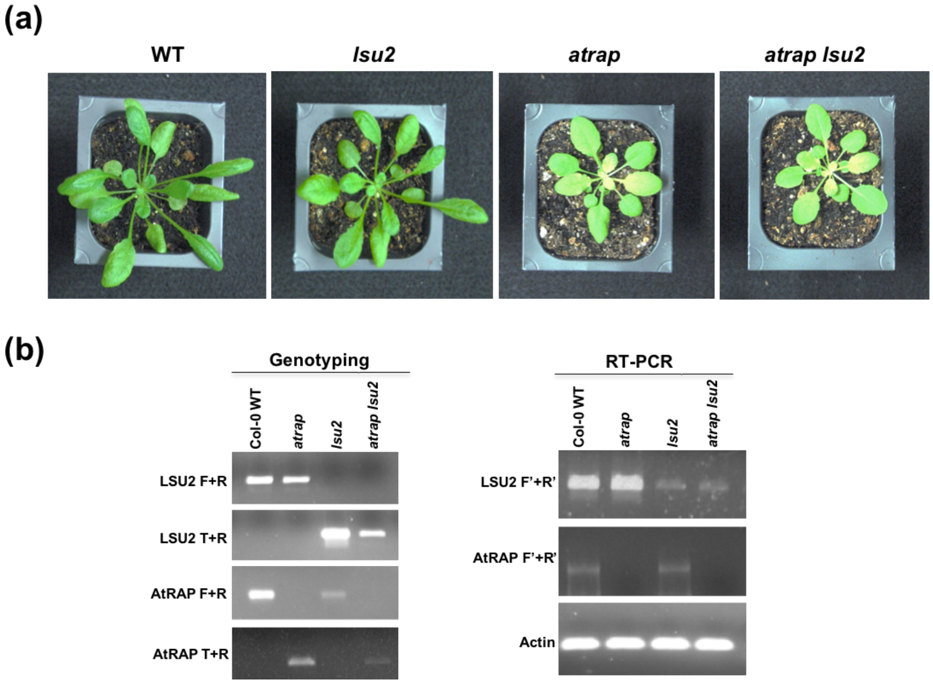

Fig. S4 Characterization of the *atrap lsu2* double mutant. (a) Phenotype of the *atrap lsu2* double mutant. (b) Genotyping and RT-PCR result of the *atrap lsu2* double mutant. F: Genomic DNA forward primer across T-DNA insertion site; R: Genomic DNA reverse primer across T-DNA insertion site; T: T-DNA insertion primer; F': cDNA forward primer; R': cDNA reverse primer. The primers follow previous publication (Katiyar-Agarwal *et al.*, 2006; Ruckle *et al.*, 2012). The *lsu2* T-DNA insertion line (SALK_031648) is a knock-down line within 5' UTR insertion. The double mutant was selected by PCR of the genomic DNA for the homologous T-DNA insertion and confirmed by RT-PCR to show no or very low expression of *AtRAP* and *LSU2*, respectively, in the double mutant.

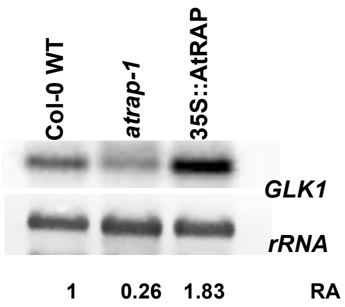

Fig. S5 *GLK1* is positively regulated by *AtRAP*. Detection of *GLK1* mRNA in the Col-0 WT, the *atrap* mutant and the 35S::AtRAP plants. Five-week-old *Arabidopsis* Col-0 WT, the *atrap* mutant and the 35S::AtRAP plants were collected. Total RNAs were extracted and subjected to Northern blot analysis.

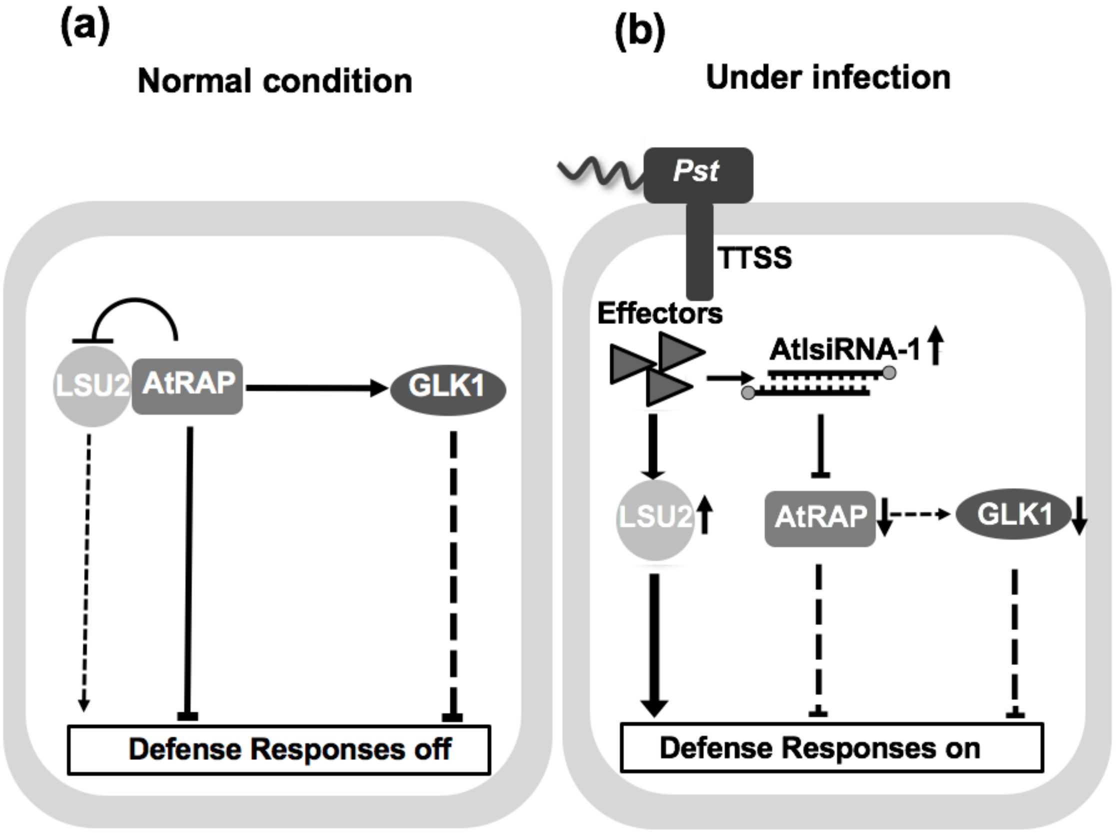

Fig. S6 A hypothetical working model for the regulation of the AtRAP protein in plant antibacterial disease resistance. Under the normal growth condition in the absence of pathogen infection, AtRAP suppresses the positive regulator of plant defense - LSU2 by directly interacting with the LSU2 protein. Upon bacterial infection, AtlsiRNA1 is highly induced and silences the *AtRAP* gene, which quickly activates defense responses by releasing LSU2. Expression of the transcription factor GLK1 is positively regulated by AtRAP protein and acts as a negative regulator of plant antibacterial defense responses.

Table S1. Q-PCR primers used in this study.

Primer ID	**Primer sequence**
PR1 (AT2G14610)-QRT-F	TTCCCTCGAAAGCTCAAGATA
PR1 (AT2G14610)-QRT-R	GGCACATCCGAGTCTCACTGA
PR2 (AT3G57260)-QRT-F	GGCTTGAAGTCAAGGTCTCA
PR2 (AT3G57260)-QRT-R	CCCTGGATCGTTATCAACAG
RBOHd (AT5G47910)-QRT-F	TGCTTTCAGATCAAGCATCA
RBOHd (AT5G47910)-QRT-R	TGAGCTTACGTGTCCAGTCA
RBOHf (AT1G64060)-QRT-F	ACACCGAGGCATACGTGTAA
RBOHf (AT1G64060)-QRT-R	ACATGGACCTTATCAACGCA
PDF1.2 (AT5G44420) QRT-F	TCATCATGGCTAAGTTTGCT
PDF1.2 (AT5G44420) QRT-R	CCTTCAAGGTTAATGCACTG

Table S2. DNA probes used in this study.

Primer ID	**Primer sequence**
LSU2 (AT5G24660) Probe1	ATCTCTTTCTTCATCTCCTCCACAGCTTTCTCCATCTCT
LSU2 (AT5G24660) Probe2	AGCCGCCACCGTCACATAGTTTCCTCCTTTCCCCATTT
GLK1 (AT2G20570) Probe1	AAACGGCGGATTAGGCATGGCGGTAGAAGGCGGAGGTA
GLK1 (AT2G20570) Probe2	CGTCATAAGTCACCGTCTCCGCCGCAACATCGTCTCT

Table S3. Genes down-regulated in the *atrap* mutant.

Affymetrix code	**Relative level (WT/*atrap-1*)**	**AGI code**	**Gene description**
254716_at	0.1347	AT4G13560	UNE15 (unfertilized embryo sac 15)
250824_at	0.1462	AT5G05200	Protein kinase superfamily protein
262357_at	0.3179	AT1G73040	jacalin lectin family protein
260869_at	0.3228	AT1G43800	acyl-(acyl-carrier-protein) desaturase
265948_at	0.3668	AT2G19590	ACC oxidase 1
260004_at	0.4208	AT1G67860	unknown protein
252612_at	0.4555	AT3G45160	unknown protein
254386_at	0.4634	AT4G21960	PRXR1 (Peroxidase superfamily protein)
263715_at	0.4643	AT2G20570	GLK1 (ARABIDOPSIS GOLDEN2-LIKE 1)
255626_at	0.4983	AT4G00780	TRAF-like family protein
245196_at	0.5058	AT1G67750	pectate lyase family protein
259001_at	0.5220	AT3G01960	unknown protein
259535_at	0.5271	AT1G12280	disease resistance protein (CC-NBS-LRR class)
262958_at	0.5329	AT1G54410	dehydrin family protein
264611_at	0.5543	AT1G04680	pectate lyase family protein
262582_at	0.5608	AT1G15410	aspartate-glutamate racemase family
245307_at	0.5688	AT4G16770	2-oxoglutarate and Fe(II)-dependent oxygenase superfamily protein
255578_at	0.5757	AT4G01450	nodulin MtN21 family protein
247685_at	0.5917	AT5G59680	leucine-rich repeat protein kinase
246595_at	0.5983	AT5G14780	Encodes a NAD-dependent formate dehydrogenase
261368_at	0.6011	AT1G53070	legume lectin family protein

Table S4. Genes up-regulated in the *atrap* mutant.

Affimetrix code	**Relative level (WT/*atrap-1*)**	**AGI code**	**Gene description**
253208_at	1.5466	AT4G34830	MRL1 (Pentatricopeptide repeat protein)
264698_at	1.8240	AT1G70200	RNA-binding (RRM)-containing protein
251638_at	1.9411	AT3G57490	RPS2D (40S ribosomal protein S2)
254907_at	2.3464	AT4G11190	disease resistance-responsive family protein
246411_at	2.3464	AT1G57770	FAD/NAD(P)-binding oxidoreductase family protein
244997_at	2.6193	ATCG00170	RNA polymerase beta' subunit-2
249645_at	2.9723	AT5G36910	thionin 2.2

Reference

Katiyar-Agarwal S, Morgan R, Dahlbeck D, Borsani O, Villegas A, Jr., Zhu JK, Staskawicz BJ, Jin H. 2006. A pathogen-inducible endogenous siRNA in plant immunity. *Proc Natl Acad Sci U S A* **103**(47): 18002-18007.

Ruckle ME, Burgoon LD, Lawrence LA, Sinkler CA, Larkin RM. 2012. Plastids are major regulators of light signaling in Arabidopsis. *Plant Physiol* **159**(1): 366-390.