

***Arabidopsis* LBP/BPI related-1 and -2 bind to LPS directly and regulate *PR1* expression**

Sayaka Iizasa*^{1, 2}, Ei'ichi Iizasa³, Sawako Matsuzaki^{1, 4}, Hiroyuki Tanaka⁵, Yutaka Kodama⁵, Keiichi Watanabe^{2, 4}, and Yukio Nagano*^{1, 2}

¹Analytical Research Center for Experimental Sciences, Saga University, 1 Honjo-machi, Saga 840-8502, Japan. ²Department of Biological Science and Technology, The United Graduate School of Agricultural Sciences, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-8580, Japan. ³Department of Immunology, Graduate School of Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan. ⁴Department of Applied Biochemistry and Food Science, Faculty of Agriculture, Saga University, 1 Honjo-machi, Saga 840-8502, Japan. ⁵Center for Bioscience Research and Education, Utsunomiya University, Tochigi 321-8505, Japan. Correspondence and requests for materials should be addressed to I. S. (k8930172@kadai.jp) or N. Y. (nagano@cc.saga-u.ac.jp)

Supplemental Table S1. Primers set used in this study.

For AtLBRs cloning and plasmid construction.

Set	F/R	Sequence
ATLBR-1n	F	5'-TTCGAGCTCCGTCGAACCGATTCATTCACATCGGT-3'
	R	5'-AGCAGCCGGATCTCATTATATAGGATCACTGGTGA-3'
ATLBR-2n	F	5'-TTCGAGCTCCGTCGAAACAATGGCGGTCACATTTC-3'
	R	5'-AGCAGCCGGATCTCATTAGACAGGGTTGCCTGTAA-3'
Nes1n	F	5'-CCAAGTTTCTTCTTCTTGCC-3'
	R	5'-GTGAAAACCTCTAACTACCGA-3'
Nes2n	F	5'-TCTTCGTCTCGGTGTCATCG-3'
	R	5'-GCCAACTTCAACTGTAGTTA-3'
pSU2amp	F	5'-GGCCATGGCTGATATCGGATCCGAATTCGAGCTCCGTCGA-3'
	R	5'-ACTCAGCTTCCTTTCGGGCTTTGTTAGCAGCCGGATCTCA-3'

For semiquantitative RT-PCR.

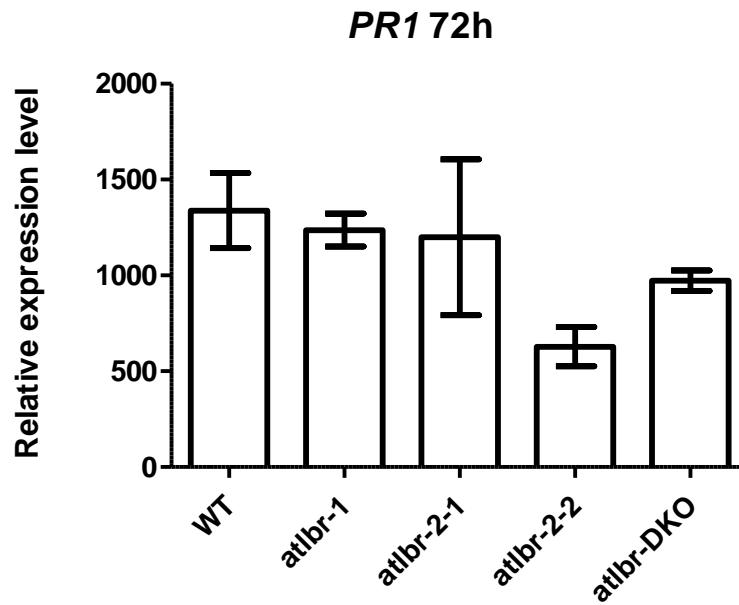
Set	F/R	Sequence
RT- AtLBR1	F	5'-GCACCGATTCATTCACATCG-3'
	R	5'-CCTTTGGAAGGCTTTGTAGG-3'
RT- AtLBR2	F	5'-GGTGGTTGATGCATTTCAA-3'
	R	5'-CTTCAGGCTTACGTACATGC -3'
RT-Actin	F	5'-TCTTGATCTTGCTGGTCGTG-3'
	R	5'-GAGCTGGTTTTGGCTGTCTC-3'

For quantitative RT-PCR (qRT-PCR).

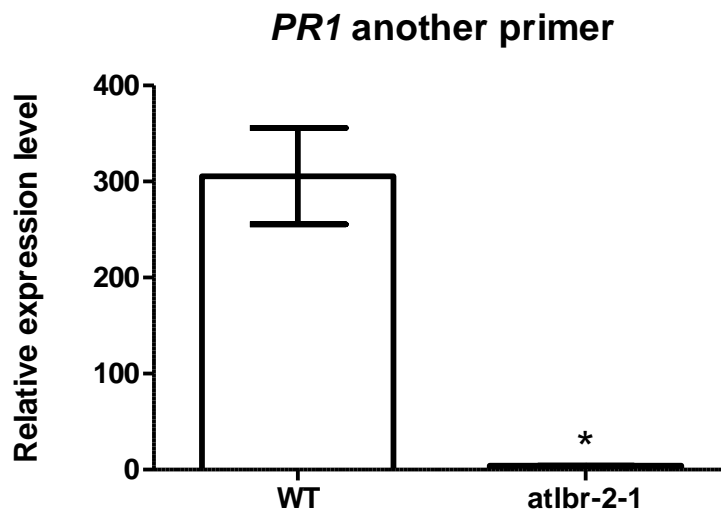
Set	F/R	Sequence
qAtLBR-1	F	5'- CCATTGAGTTGGAAGGAGGA-3'
	R	5'- TGGCAATGGTACTTTCCACA-3'
qAtLBR-2	F	5'- CGGGTCCATTCTAAGCACAT-3'
	R	5'- ATCGTCGCATCAATTCCATT-3'
qPR1	F	5'- GGAGCTACGCAGAACAATAAGA-3'
	R	5'- CCCACGAGGATCATAGTTGCAACTGA-3'
qPR4	F	5'- TTGCTCCACGTGGGATGCTGAT-3'
	R	5'- AGCTCATTGCCACAGTCGACAA-3'
qPR5	F	5'- CGGTACAAGTGAAGGTGCTCGTT-3'
	R	5'- GCCTCGTAGATGGTTACAATGTCA-3'
q β - Tubulin4	F	5'- GAGGGAGCCATTGACAACATCTT-3'
	R	5'- GCGAACAGTTCACAGCTATGTTCA-3'

For Supplementary Fig. S1 (another set of *PR1* primers).

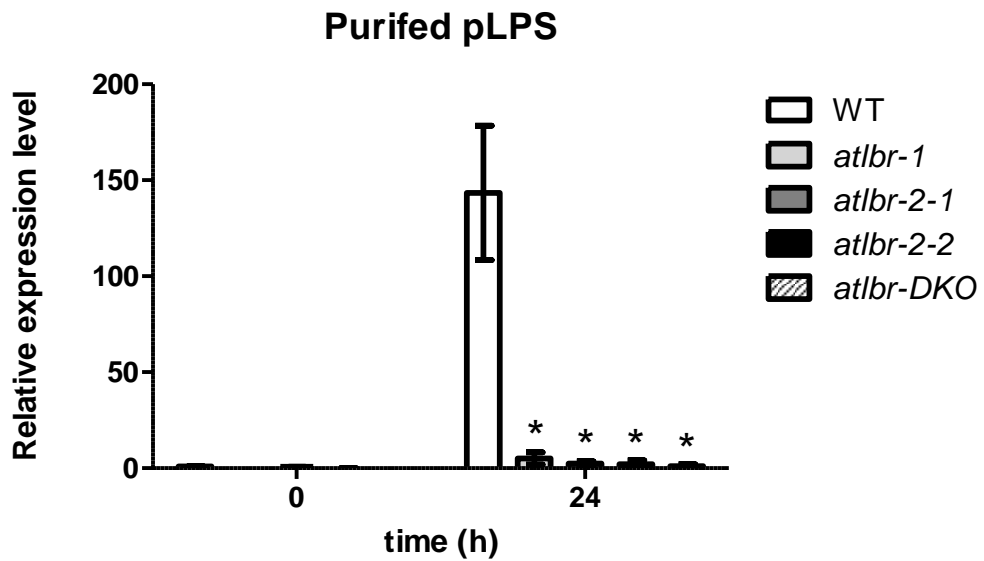
Set	F/R	Sequence
qPR1-2	F	5'- GTCTTTGTAGCTCTTGTAGGTG-3'
	R	5'- CAACCCTCTCGTCCCCTGTCAT-3'



Supplementary Fig. S1. *PR1* mRNA levels in WT and *atlbr* mutant *Arabidopsis* seedlings treated with 100 µg/ml pLPS during 72 h were detected by qRT-PCR. Mean expression values were calculated from the results of three independent experiments. Means \pm standard errors are presented. Significant differences among means compared with WT plants were evaluated by one-way ANOVA followed by Tukey's multiple comparison test.



Supplementary Fig. S2. *PR1* mRNA levels in WT and *atlbr-2* *Arabidopsis* seedlings treated with 100 μ g/ml pLPS during 24 h were detected by qRT-PCR using another set of primers (Supplementary Table S1). Mean expression values were calculated from the results of three independent experiments. Means \pm standard errors are presented. The significant difference between means was evaluated with a *t*-test; * $P < 0.05$.



Supplementary Fig. S3. Purchased *P. aeruginosa* LPS was purified by degradation of nucleic acids and proteins with DNase I, RNase, and proteinase K. *PR1* mRNA levels in WT and *atlbr* mutant *Arabidopsis* seedlings treated with 100 µg/ml purified pLPS during 24 h were detected by qRT-PCR. Mean expression values were calculated from the results of three independent experiments. Means ± standard errors are presented. Significant differences among means compared to WT plants were evaluated by two-way ANOVA followed by *post hoc* Bonferroni tests; * $P < 0.001$.