Supplementary Information

Leucine-rich repeat receptor-like gene screen reveals that *Nicotiana* RXEG1 regulates glycoside hydrolase 12 MAMP detection

Wang et al.



Supplementary Figure 1. Phylogenetic analysis of LRR-RLKs in *Nicotiana benthamiana.* LRR-RLKs in *Arabidopsis thaliana*, which represent the 20 subgroups^{1,2}, were used as references. Different colors indicate different subgroups. Phylogenetic maximum-likelihood (ML) tree was constructed using the predicted kinase domain sequences and PhyML implemented in SEAVIEW software (<u>http://doua.prabi.fr/software/seaview</u>). The phylogenetic trees were displayed using iTOL (http://itol.embl.de).

а



TRV:GFP

T106

TRV:BAK1



b

	0					100)	
Construct	Time	Torrete	No. of	Sequence Similarity				
Construct	туре	Targets	LRRs	S. lycopersicum	S. tuberosum	C. annuum	G. max	A. thalian
T106	RD-LRR-RLK	Niben101Scf01683g11003.1	21	88.58	88.82	88.23	69.86	66.69
	RD-LRR-RLK	Niben101Scf13404g00002.1	22	88.58	88.73	88.31	69.96	66.61
T123	GH-LRR-RLK	Niben101Scf11153g00012.1	5	77.96	78.85	76.61	48.3	50.23
	LRR-RLP	Niben101Scf02298g01015.1	5	76.1	44.5	63.4	49.5	30.8
T136	RD-LRR-RLK	Niben101Scf10608g00008.1	9	78.06	87.91	86.38	65.73	61.41
	RD-LRR-RLK	Niben101Scf03888g01021.1	9	78.05	87.15	85.27	65.13	60.71
T140	GN-LRR-RLK	Niben101Scf04292g00003.1	9	77.6	77.59	68.02	67.2	61.6
	GN-LRR-RLK	Niben101Scf10152g04001.1	9	77.85	79.59	68.53	67.88	62.81
T211	GN-LRR-RLK	Niben101Scf06204g00001.1	3	73.68	75.53	76.4	46.55	32.18
	LRR-RLP	Niben101Scf00683g02006.1	4	71.8	12.6	79.2	43.2	23.5

Supplementary Figure 2. Several conserved LRR-RLKs play a potential role in plant development.

a, Morphology of TRV construct-treated *N. benthamiana* plants. Plants were photographed five weeks after silencing.

b, Conservation of LRR receptor-like genes with a potential role in plant development among different plant species. The number indicates the similarity with their orthologs in indicated plant species.

RXE	31	RXE	3L1	RXEG	L2
SP	MGKREYPSSAHFLVTLSLLLLQAAFG	SP	MDKRKYPRLDHFLVTWSLLLLQTALG	NT	MRGPLPDLALFPSLRELHLGFQWRI
NT	LTLCIEKERDALLEFKRGLSD	NT	LTSREVNKTMCIOKERDALLEFKRGLID	NT	PQEFWVSSNRLEGLPKS
NT	NFGQLSTWGDEEDKKECCKWKGIECNKTTGH	NT	DFDRLSTWGDEEDKKECCKWKGIECDKRSGH	1	MGOLSNIESTDASYNVLKGTTTESH
NT	VIVLDLHNAFTCSASACFAPRLTGKLSPS	NT	VTVLDLHTEVSCPVRSCFAPMLTGKLSPS	2	LSNLSSLVDLDLSFNSLALKTSFD
1	LLELEYLNFLDLSVNEFERSEIPRF	1	LLELHHLNYLDLSHNGFDKIEIPRF	3	WLPPFQLQFINLPSCNLGPSFPKW
2	ICSFKRLEYLNLSSSFFSGLIPTO	2	ISSLKRLEYLNLSSSDFSGVIPTO	4	LHSQNNCTVLEISLANLSDALPSWF
3	FKNLTSLRILDLGYNNLIVKDLTW	3	LKNLTSLRILDLGNNNOLIVKDLGW	5	SDLPLNLKILTLSNNHISGRVSEL
4	LSHLSSLELLSLGGSDFQVKNWFQE	4	LSYLSSLEILRLGGNDFQASNWFQE	6	IVNKQDYMVIDLSSNNFSGPLP
5	ITKLPLLKELDLSLCGLSKLVPSPAEIA	5	ITKVPSLKELDLSVCGLSKFVPSPADLV	7	QVPTNVRIFYLHKNKFSGSTSSIC
6	NSSLISLSVLHLCCNEFSSSAKYSWL	6	KSSLISLSVLHLCCNQFTSSAEYSWL	8	KSTTGAATSLDLSHNLFSGELPDC
7	FNFSTSLTSIDLSNNQLDGQIDDR	7	FNFSTSLTSIDLSNNQLDGPIDDR	9	WMNMSNLVVLNLAFNNFSGKLPHGILPS
8	FGNLMYLEHLNLANELNLKGGIPSS	8	FGSLMYLEHLKLADQFNLKGVGVPSS	10	FSQCQLLQILDLGGNKLTGRIPAWI
9	FGNLTRLRYLDMSNTRTYQWLPELFVRL	9	FGNLTRLRYLDISSTRTYQWLPELFLRL	11	GTDLLNLRILSLRENKEYGSILEI
10	SGSRKTLEVLGLNDNSMFGSLVD	10	SGSRKTLEVLGLNDNSMFGSLVN	12	TCOPOLIOIPPRANGT2EOC
11	VTRFSALKRLYLQKNVLNGFFMER	11	VTRFSALKRLYLQNNVLNGFFMER	ID	FNNFTLLHQENGSGE SMNFSVQY
12	FGQVSSLEYLDLSDNQMRGPLPD	12	FGQVSSLEYLDLSDNQMRGSLPD	ID	DYMPRSYLYIGNLLVQWKNQEAEY
13	LALFPSLRELHLGSNHFNGRIPQG	13	LALFPSMRELHLGSNQFQGRIPQG	13	KNPLLYLKAIDLSSNKLVGGIPKE
14	IGKLSQLKILDVSSNRLEGLPES	14	IGKLSQLRILDVSSNRLEGLPES	14	IAEMRGLKSLNLSRNDLNGSIIEG
15	MGQLSNLESFDASYNVLKGTITESH	15	MGQLSNLESFDASYNALKGTITESH	15	IGQMKMLESLDLSRNQLSGMIPKD
16	LSNLSSLVDLDLSFNSLALKTSID	16	LSNLSSLVYLDLSFNSLALKTSFD	16	LANLTFIGVLDLSNNHLSGRIPSS
17	WLPPFQLQVINLPSCNLGPSFPKW	17	WLPPFQLQFINLPSCNLGPSFPKW	.TM	TOLOTEERSSYSCNAKLCOPPLOECPG
18	LQSQNNYTVLDISLANISDALPSWF	18	LQSQNNYTVLDISLANLSDALPSWF	JM	YAPPSPRIDHNSNMNPOELDDDDDFPSLE
19	SGLPPDIKILNLSNNQISGRVSDL	19	SDLPLNLKILNLSNNHISGRVSEF	_	
20	IENAIDIMVIDLSSNNFSGPLP	20	IVNKQDIMVIDLSSNNFSGPLP	TW	FYISMVLGFFVAFWGILGSLIVN
21	LVPTNVQIFILHKNQFFGSISSIC	21	LVPINVRIFILHRNKFSGSTSSIC	CT	HSWRNAYFIFLMDVKNWLAMISRV
22	KSTTGATSLDLSHNQFSGELPDC	22	KSTTGGATSVDLSHNQFSGELPDC		
23	LOST INTER LANDONG BOOM DC	23	I COLECTED INTERING SCREPUS		
25	LOCOSIOIIDI CONVITORIDANI	24	ECOCOSI OTI DI CONVENCENTE		
26	GTDLLNLDILSLDENKEYGSISPI	25	CNDLINIDII.CLDENKEY/CCIDCI		
27	ICOLOFLOILDISANGLAGKIPOC	27	TCOLOFIOLIDISANGLSGKIPOC		
		- /	Keeks and a spectrum of the Kee		
ID	FNNFTLLHQENGLGEPMEFLVQGFY	ID	FNNFTLLHQENGSGESMEFLVQLD		
ID	GKYPRHYSYLGNLLVQWKNQEAEY	ID	YLPRSYLYIGNLLVQWKNQEAEY		
28	KNPLTYLKTIDLSSNKLVGGIPKE	28	KNPLLYLKAIDLSSNKLVGNIPKE		
29	MAEMRGLKSLNLSRNDLNGSIIKG	29	IAEMRGLKSLNLSRNDLNGSIIEG		
30	IGQMKMLESLDLSRNQLSGMIPKD	30	IGQMKMLESLDLSRNQLSGMIPKG		
31	LANLTFIGVLDLSNNHLSGRIPSS	31	LANLTFIGVLDLSNNHLSGRIPSS		
ЛМ	TQLQTFERSSYSGNAQLCGPPLQECPG	JM	TOLOTFETSSYSGNAOLCGPPLEECPG		
ЛМ	YAPPSPRIDHNSNMNPQELDVDDDFPSLE	JM	FAPPSPRINHGSNINPQELGDDDEFPSLE		
тм	FYISMVLGFFVAFWGILGSLIVN	TM	FYISMVLGFFVAFWGILGCLIVN		
СТ	HSWRNAYFIFLMDTKNWLAMISRVCCSRLKRKLRA	CT	RAWRNAYFTFLMDTKNWLAMISRVCFTRLKGKLRAS		

Supplementary Figure 3. The domain compositions of RXEG1, RXEGL1 and RXEGL2. The LRRs were aligned and determined according to Jehle et al. 2013³ and Zipfel et al. 2006⁴. SP, signal peptide; NT, N-terminus of the LRR domain; ID, island domain; JM, juxtamembrane domain; TM, transmembrane domain; CT, cytoplasmic tail.



Supplementary Figure 4. Expression of RXEG1^(S) in *Nicotiana benthamiana* does not induce plant cell death.

a, *Nicotiana benthamiana* leaves (n=6) expressing RXEG1^(S)-eGFP or eGFP photographed 3 days after infiltration (dai). RXEG1^(S)-eGFP or eGFP was co-expressed with P19 in *N. benthamiana* and protein accumulation was monitored 3 dai with anti-GFP antibody.

b, Representive *Nicotiana benthamiana* leaves expressing RXEG1^(S)-eGFP or eGFP stained with trypan blue.

c, Relative ion leakage in leaves expressing RXEG1^(S)-eGFP or eGFP. Leaf discs were collected 3 dai. Bars represent average value (\pm s.e.m) of six replicates.



Supplementary Figure 5. Morphology of *N. benthamiana* plants treated with TRV: *GFP* or *RXEG1*-silencing constructs. Plants were photographed five weeks after silencing.



Supplementary Figure 6. Flg22-induced plant immune responses in TRV:*GFP* and TRV:*RXEG1-3*-treated *N. benthamiana*.

a, Production of ROS in *RXEG1*-silencing *N*. *benthamiana* treated with 100 nM flg22 or H_2O . Mean values (± s.e.m) of four replicates are shown. Experiment was repeated three times with similar results.

b, Relative expression of defense-related marker gene *CYP71D20* in *RXEG1*-silencing *N*. *benthamiana* leaves 3 h after treatment with 100 nM flg22 or H₂O. Transcript levels were quantified by qRT-PCR and normalized to EF-1 α . Bars represent the mean fold changes (± s.e.m) of the flg22-treated leaves relative to the value in H₂O-treated leaves, which was set as 1. Experiment was repeated twice with similar results.



Supplementary Figure 7. Representative leaves showing the disease symptoms of *SOBIR1*-silencing, *RXEG1*-silencing (a) or overexpressing (b) *N. benthamiana* leaves upon infection with *Phytophthora parasitica*. *N. benthamiana* leaves (silencing, n=12; overexpressing, n=16) were inoculated with *P. parasitica* mycelial plugs and photographed at 3 days post inoculation (dpi). Experiments were repeated twice with similar results. Protein accumulation was monitored 2 dai with anti-GFP antibody. Protein loading is indicated by Ponceau S staining.

RXEG1 LeEix1 LeEix2 L DL SVNEFERSEIPRFICSFKRLEYLNLS L DL SVNEFERSEIPRFIGSLKRLEYLNLS RXEG1 LeEix1 RXEG1 LeEix1 LeEix2 RXEG1 LeEix1 LeEix2 RXEG1 LeEix1 LeEix2 RXEG1 LeEix1 RXEG1 LeEix1 RXEG1 LeEix1 LeEix2 B40 850 870 880 870</th RXEG1 LeEix1 LeEix2 RXEG1 LeEix1 LeEix2 1010 1020 1030 WRNAYFIFLMDTKNWLAMISRVCCSRLKRKLRA--WRNAYFKFLTDTTSWLDMISRVWFARLKKKLRRAR RXEG1 LeEix1



Supplementary Figure 8. Phylogenetic analysis of RXEG1 with homologs in *N. benthamiana* and tomato.

a, Sequence alignment of RXEG1 with LeEix1 and LeEix2 using ClustalW.

b, A phylogenetic tree was built using tomato LeEIX1/2 and all the identified *N. benthamiana* RLPs containing the C3-D domain⁵ following the method described by Fritz-Laylin et al. 2005⁶ and Petre et al. 2014⁷. The C3-D domain of tomato Cf9⁵ was used as the reference.

b



Supplementary Figure 9. Schematic representation of RXEG1, RXEGL1, *Arabidopsis* RLP23, and derivatives. Amino acids of each domain are indicated.



Supplementary Figure 10. Representative leaves showing the disease symptoms of *BAK1*-silencing and *SOBIR1*-silencing *N. benthamiana* leaves upon infection with *Phytophthora parasitica*.

a, *N. benthamiana* leaves (n=12) were inoculated with *P. parasitica* mycelial plugs and photographed at 3 dpi. Experiments were repeated twice with similar results.

b, Relative quantification of *P. parasitica* biomass in TRV:*GFP*, TRV:*BAK1* and TRV:*SOBIR1*-treated *N. benthamiana* leaves using qPCR. Infected leaves (n=12) were collected at 3 dpi and used for DNA isolation and qPCR analysis. *, significant differences (P < 0.05, Student's *t*-test). Experiments were repeated twice with similar results.



Supplementary Figure 11. Interactions of RLP23 or RXEG1 with BAK1 or SOBIR1 *in planta*.

a, RLP23 interacts with BAK1 in a nlp20-dependent manner while interacts constitutively with SOBIR1. *N. benthamiana* leaves were agro-infiltrated to express RLP23-eGFP, BAK1-mRFP, or SOBIR1-HA and collected 2 dai after treatment with 1 μ M nlp20 (*Pp*NLP) or H₂O for 5 min. Extracted proteins were subjected to Co-IP using GFP-trap® A beads and western blotting with anti-GFP, anti-RFP, and anti-HA antibodies.

b, SOBIR1-HA interacts constitutively with RXEG1-eGFP but not with eGFP. SOBIR1-HA was co-expressed with RXEG1-eGFP or eGFP in *N. benthamiana* leaves. Infiltrated leaves were collected at 2 dai and treated with 1 μ M XEG1 or EV for 10 min before protein isolation. Extracted proteins were subjected to Co-IP using GFP-trap® A beads and western blotting with anti-GFP and anti-HA antibodies.



(refers to 6f)

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Supplementary Figure 12. Original western-blots shown in main Figure. * pointing to the band with expected sizes. – labeling the same blot visualized in different contrasts.

Primers	Sequences 5'-3'	Used for		
pBin(c)GFP-RXEG1-F	TTACGAACGATAGCCGGTACCATGGGCAAAAGGGAATATCCAAG	cloning full length RXEG1 in N. benthamiana		
pBin(c)GFP-RXEG1-R	GCTCACCATCCCGGGGGTACCAGCCCTTAACTTTCTCTTCAGTC			
pBin(c)GFP-RXEG1 ^{SP+LRR} -R	GCTCACCATCCCGGGGGTACCGGATGATCTCTCAAAAGTTTGG	cloning RXEG1 ^{SP+LRR} domain		
pBin(c)GFP-RXEG1 ^{SP+LRR(s)} -R	GCTCACCATCCCGGGGGTACCCGATGACCTCTCAAATGTCTGG	cloning synthesized RXEG1 ^{SP+LRR} domain		
pBin(c)GFP-RXEG1 ^{SP+TM} -R(F)	GCTTGATGGAATTCTCCCTGATCCAAAAGCTGCTTGTAGGAG	cloning RXEG1-∆LRR domain		
pBin(c)GFP-RXEG1 ^{SP+TM-tail} -F(R)	CTCCTACAAGCAGCTTTTGGATCAGGGAGAATTCCATCAAGC	cloning RXEG1-∆LRR domain		
pBin(c)GFP-RXEGL1-F	TTACGAACGATAGCCGGTACCATGGATAAAAGGAAATATCCAAG	cloning full longth DVECI 4 in N honthermines		
pBin(c)GFP-RXEGL1-R	GCTCACCATCCCGGGGGTACCTGAGGCCCTTAGCTTTCCCT	cioning fuil length RAEGLT IN N. benthamlana		
pBin(c)GFP-RXEGL1 ^{SP+LRR} -R	GCTCACCATCCCGGGGGTACCGGATGATGTCTCAAAAGTTTGGAG	cloning RXEGL1-SP+LRR domain		
pBin(c)GFP-RXEGL1 ^{SP+LRR(s)} -R	GCTCACCATCCCGGGGGTACCCGAAGATGTTTCAAATGTTTGC	cloning synthesized RXEGL1-SP+LRR domain		
pBin(c)GFP-RXEGL1 ^{SP+TM} -R(F)	GCTTGATGGAATTCTCCCTGATCCTAAAGCTGTCTGTAAGAGC	cloning RXEGL1-∆LRR domain		
pBin(c)GFP-RXEGL1 ^{™-tail} -F(R)	GCTCTTACAGACAGCTTTAGGATCAGGGAGAATTCCATCAAGC	cloning RXEGL1-∆LRR domain		
pBin(c)HA-SOBIR1-F	TTACGAACGATAGCCGGTACCATGGCCTTCACTGCTTCAC	cloning full longth SORID1 in N. honthorsing		
pBin(c)HA-SOBIR1-R	AACATCGTATGGGTACCCGGGATGCTTGATCTGAGTTAACATACACC	Cioning fuil length SOBIRT in IV. benthamlaha		
pBin(c)mRFP-BAK1-F	TTACGAACGATAGCCGGTACCATGGATCAATGGCTATTGGGGATC	cloning full longth PAK1 in AL boothomics		
pBin(c)mRFP-BAK1-R	AACATCGTATGGGTACCCGGGTCTTGGCCCTGATAACTCATC	Coming fuil length DART IN N. Denthamiana		
pBin(c)GFP-RXEGL2-F	TTACGAACGATAGCCGGTACCATGAGAGGGCCATTACCAG	cloning full length RXEGL2		
pBin(c)GFP-RXEGL2-R	GCTCACCATCCCGGGGGTACCGACTCTTGATATCATAGCGAGC			
pBin(c)GFP-AtRLP23-F	TTACGAACGATAGCCGGTACCATGTCAAAGGCGCTTTTGCAT	cloning full length AtPL P23		
pBin(c)eGFP-AtRLP23-R	GCTCACCATCCCGGGGGTACCACGCTTTCTGCGTTTATTCAG			
pBin(c)GFP-RLP23 ^{SP+LRR} -R	GCTCACCATCCCGGGGGTACCGTCTTCTTGCTTTTGGTGATAC	cloning AtRLP23-SP+LRR domain		
pGR107-PsXEG1-F CCATCGATATGAAGGGATTCTTCGCCG		cloning full length XEG1 in Phytophtohra sojae		

pGR107-PsXEG1-R	TCCCCCGGGGTTGACCGCAGCCGAGAAC					
pGR107-ps119627-F	GCACCAGCTAGCATCATGAAGGTTGCGTTCGCTACTG	cloning full length XEG1 homolgue Ps119627 in				
pGR107-ps119627-R	CTCTAGAGGATCCCCGACGCGACGAACACTGCGG	Phytophtohra sojae				
pGR107-ps138787-F	GCACCAGCTAGCATCATGAAGGTTCTGTTCGCCAC	cloning full length XEG1 homolgue Ps138787 in				
pGR107-ps138787-R	CTCTAGAGGATCCCCCTCGCGACGAACGCGACGGA	Phytophtohra sojae				
pGR107-PPTG_16272-F	CCATCGATATGAAGCTTTCCTTCGC	cloning full length PPTG_16272 in <i>Phytophtohra parasitica</i>				
pGR107-PPTG_16272-R	TCCCCCGGGAACTGTGTGGACAGCGGCC					
pGR107-PITG_06962-F	TCAGCACCAGCTAGCATCGATATGAAGGTGTTCTTCGCTGCTG	cloning full length PITG_06962 in Phytophtohra				
pGR107-PITG_06962-R	AATCTCTAGAGGATCCCCGGGTCGTCCCCTGAAGAAGTCGG	infestans				
pGR107-VDAG_07406-F	TCAGCACCAGCTAGCATCGATATGAAGTTCATCACAGTCCTTAC	cloping full length VDAG 07406 in Verticilium dalia				
pGR107-VDAG_07406-R	AATCTCTAGAGGATCCCCGGGACTGCACTGGGAGTACCACTG					
pGR107-NPP1-F	CCATCGATATGAACGTCCTTACATTCCTC	cloning full length NPP1 in Phytophtohra parasitica				
pGR107-NPP1-R	TCCCCCGGGAGCGTAGTAAGCGTTGCCT					
NbCYP71D20-RT-F	CCGCACCATGTCCTTAGAG	Antje Heese et al., 2007				
NbCYP71D20-RT-R	CTTGCCCCTTGAGTACTTGC					
NbEF1a-RT-F	GTATGCCTGGGTGCTTGAC	Antia Haasa at al. 2007				
NbEF1a-RT-R	ACAGGGACAGTTCCAATACCA	- Anije neese et al., 2007				
NbSOBIR1-RT-F	CTTAGAAAAACTCTCTTTAGC	Lichrond et al. 2012				
NbSOBIR1-RT-R	TATGGATTGGAGTGACATTATG					
NbSERK3-RT-F	ATTGCTGGAGGAGTTGCTGCCGG	PT shock expression of PAK1 hemologues				
NbSERK3-RT-R	CCACGTACAGCAGTGGTAACAT	KI CHECK EXPRESSION OF BAKT NOMOLOGUES				
PAR-F	ATGAACTTCCGCGCTCTGTT	Dhutanhtahra naraaitiga hismoos analusia				
PAR-R	CAGTGACGCGCACGTAGAC	rnytophonia parasitica biomass analysis				

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