Supporting Legends

Supplemental Figure S1. Amino acid sequence alignment of RxLR24 effector homologs. Identical amino acids are highlighted in black and similar residues are shown as grey boxes. Sequences encoding signal peptides (SP) are not included. RxLR and dEER motifs are marked with asterisks. Sequence accessions: *P. brassicae* RxLR24 (MG489826), *P. infestans* RxLR24 (XP002997548/ PITG 18405), *P. parasitica* RxLR24 (XP008915662), *P. sojae* RxLR24 (XP009516297) and *P. nicotianae* RxLR24 (KUF87794).

Supplemental Figure S2. Relative RxLR24 expression of *P. brassicae* during infection of Arabidopsis. Leaves of the susceptible Arabidopsis mutant *cyp79B2cyp79B3* were drop-inoculated with a zoospore suspension and *PbRxLR24* transcript levels were determined by qPCR. β -tubulin and actin of *P. brassicae* served as reference transcripts. The relative expression of RxLR24 at 96 hpi was set at 1. Error bars represent mean values (± SD) of two biological replicates.

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Supplemental Figure S4. High magnification pictures of GFP-RxLR24 positive vesicles. RxLR24 positive vesicles observed under confocal microscope in the cells of transgenic Arabidopsis leaf (upper panel) and root (lower panel). White rectangles mark the fragments which are zoomed in the panel on the right side. The approximate size of vesicles varies from 0.8 to 2 μ m. Scale bar represents 2 μ m.

Supplemental Figure S5. RxLR24 accumulates in the membrane fraction. *N. benthamiana* leaves expressing FLAG-RxLR24 or FLAG-RxLR24 Δ C, respectively, were used for cell fractionation. Fractions representing 40 mg of leaf tissue were analyzed by SDS-PAGE and immunoblotting with anti-FLAG antibodies. The FLAG-RxLR24 protein accumulated in the membrane fraction (MF). In contrast, the truncated FLAG-RxLR24 Δ C was present exclusively in the soluble fraction (SF). Ponceau-S (PS) stained Rubisco large subunit served as a loading control for membrane purity.

Supplemental Figure S6. Intensity profiles of the co-localization pictures shown in Figure 4B-E. White arrows indicate locations analyzed by fluorescence intensity plots shown in graphs to the right side of each image. The length of the arrows corresponds to the x-axis in the graphs.

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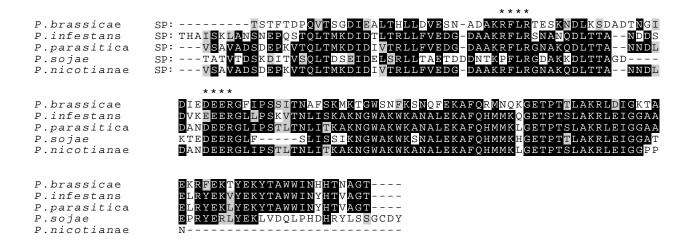
Supplemental Table S4. List of primers used for qPCR analysis.

Supplemental File S1. List of peptide sequences derived from proteins associated with *Pb*RxLR24 in Co-IP experiment.

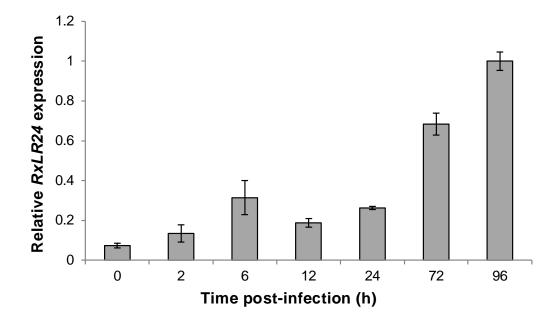
Supplemental Movie S1. Movement of GFP-RxLR24 positive vesicles in leaf cells. Movie (15 fps) shows vesicle movement observed with confocal microscopy in epidermal leaf cells. The movie consists of 100 time points and was recorded with use of EMCCD camera and 60 x magnification. Exposure time was 200 ms.

Supplemental Movie S2. Movement of GFP-RxLR24 positive vesicles in leaf cells. Movie (15 fps) shows vesicle movement observed with confocal microscopy in the root cells. The movie consists 300 time points and was recorded with use of EMCCD camera and 60x magnification. Exposure time was 500 ms.

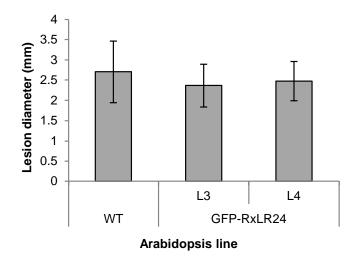
SUPPORTING FIGURES



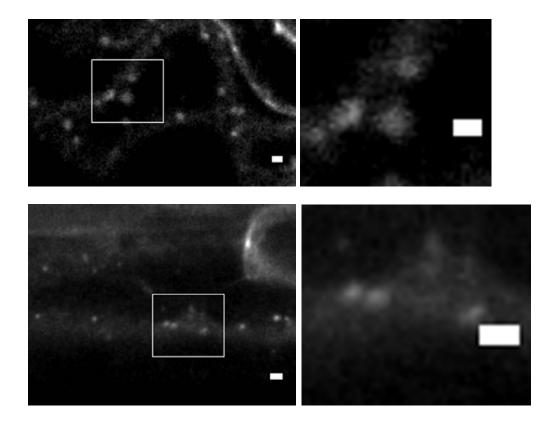
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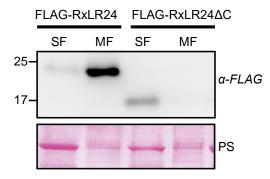
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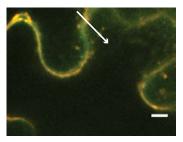
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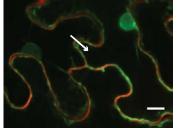
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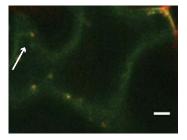
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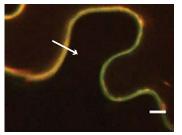
Merged: FM4-64 + GFP-RxLR24 scale bar 5 µm



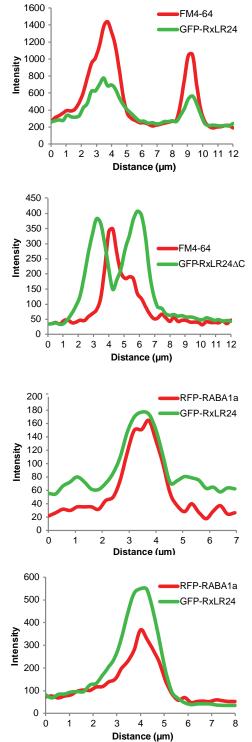
Merged: FM4-64 + GFP-RxLR24 Δ C scale bar 10 μ m



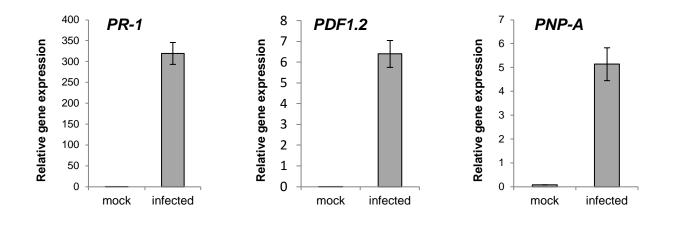
Merged: RFP-RABA1a + GFP-RxLR24 scale bar 5 µm



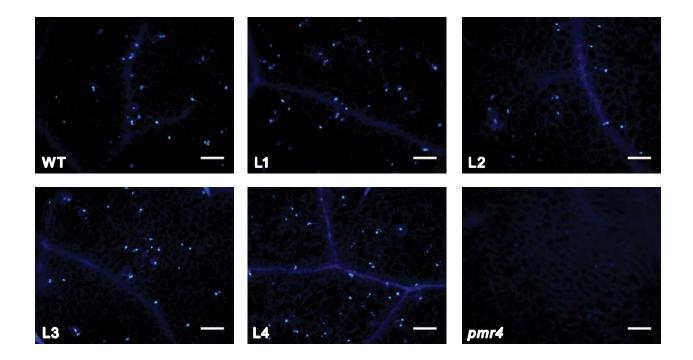
Merged: RFP-RABA1a + GFP-RxLR24 scale bar 5 µm



Supplemental Figure S6. Intensity profiles of the co-localization pictures shown in Figure 4B-E. White arrows indicate locations analyzed by fluorescence intensity plots shown in graphs to the right side of each image. The length of the arrows corresponds to the x-axis in the graphs.



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SUPPORTING TABLES

Supplemental Table S1. Sequence comparison of RxLR24 homologs of selected *Phytophthora* species. Percentage of identity (similarity) between RxLR24 protein sequences of five different *Phytophthora* species. Sequences encoding signal peptides were excluded from the comparison. The compared sequences correspond to the sequences listed in Figure S1.

	P. brassicae	P. infestans	P. parasitica	P. sojae	P. nicotianae
P. brassicae		51.0% (64.6%)	54.3% (66.4%)	41.4% (55.2%)	40.0% (50.7%)
P. infestans	51.0% (64.6%)		82.3% (90.8%)	50.0% (62.5%)	64.5% (72.3%)
P. parasitica	54.3% (66.4%)	82.3% (90.8%)		53.7% (65.1%)	81.2% (81.2%)
P. sojae	41.4% (55.2%)	50.0% (62.5%)	53.7% (65.1%)		48.3% (58.0%)
P. nicotianae	40.0% (50.7%)	64.5% (72.3%)	81.2% (81.2%)	48.3% (58.0%)	

Supplemental Table S2. Top target candidates identified by untargeted Co-IP/mass spectrometry. Mass spectrometry data of *Arabidopsis thaliana* proteins with the strongest association to the RxLR24 effector after co-immunoprecipitation. Predicted protein localization based on UniProt database. Peptide spectrum matching results were from Mascot (Matrix Science) searches and only those matching with a probability score >95% are shown. Numbers reflect the number of total unique peptides matched per protein. In parallel with *Pb*RxLR24, three non-related FLAG-tagged RxLR effectors of *P. brassicae* were used as negative controls. Proteins marked with asterisks were chosen to verify the interaction with the RxLR24 in reciprocal Co-IP experiments. Accession numbers: *Pb*RxLR23 (MG489827), *Pb*RxLR24 (MG489826), *Pb*RxLR27 (MG489828) and *Pb*RxLR29 (MG489829).

Annotation for proteins identified as potential RxLR24 targets		TAIR accession	Subcellular location	plant prote	ein identified	eptides matche for each RxLR	effector
	,	number		RxLR24	RxLR23	RxLR27	RxLR29
	RABA1a*	AT1G06400		9	0	0	0
RAB GTPases subfamilly	RABA1b	AT1G16920		9	0	0	0
RAB GIFASES Sublamily RABA1	RABA1c	AT5G45750		8	0	0	0
RADAT	RABA1d	AT4G18800		8	0	0	0
	RABA1f	AT5G60860	a se da a a se a	9	0	0	0
	RABA2a*	AT1G09630	endosome membrane, plasma membrane	9	0	0	0
RAB GTPases subfamilly	RABA2c	AT3G46830		10	0	0	0
RABA2	RABA2d	AT5G59150		12	0	0	0
	RABA4a*	AT5G65270		12	0	0	0
RAB GTPases subfamilly RABA4	RABA4b	AT4G39990		5	0	0	0
KADA4	RABA4c	AT5G47960		5	0	0	0
RAB GTPases subfamilly RABG3	RABG3f*	AT3G18820		3	0	0	0
Magnesium-protoporphyrin IX monomethyl ester [oxidative] cyclase		AT3G56940	chloroplast	8	2	0	0
AT2G30950 protei	n	AT2G30950	chloroplast	6	0	0	3

Supplemental Table S3. List of primers used for PCR amplification of cDNAs and plasmids used for the generation of

fusion proteins. † Obtained from gene synthesis service (GenScript).

Gene	Primer forward	Primer reverse	Destination plasmid	Tagged fusion protein
<i>Pb</i> RxLR24 (without SP)	CACCACCTCGACGTTCACC	TTACGTTCCAGCATTCGTGT	pB2GW7 (Karimi <i>et al</i> ., 2002)	3xFLAG-RxLR24
(without SF)	GACCCC	GG	pFAST-R06 (Shimada <i>et al.</i> , 2010)	GFP-RxLR24
<i>Pb</i> RxLR24∆C	CACCACCTCGACGTTCACC	TTAAAATTGGTTCGATTTGAA	pB2GW7 (Karimi <i>et al</i> ., 2002)	3xFLAG-RxLR24∆C
(truncated)	GACCCC	GTTGG	pFAST-R06 (Shimada <i>et al</i> ., 2010)	GFP-RxLR24
<i>Pi</i> RxLR24† (without SP)			pB2GW7 (Karimi <i>et al</i> ., 2002)	3xFLAG-PiRxLR24
RABA1a	CACCGCTGGTTACAGAGCC	CTAGTTAGAGCAGCAACCCA	pEarleyGate203 (Earley <i>et al</i> ., 2006)	Myc-RABA1a
(A. thaliana)	GATGAAG	TTC	pB7WGF2 (Karimi <i>et al</i> ., 2002)	RFP-RABA1a
RABA2a (A. thaliana)	CACCGCGAGAAGACCGGA CGAAGAATAC	TCAAGACGATGAGCAACAAG GCTTC	pEarleyGate203 (Earley <i>et al.</i> , 2006)	Myc-RABA2a
RABA4a † (A. thaliana)			pEarleyGate203 (Earley <i>et al</i> ., 2006)	Myc-RABA4a
RABG3f (A. thaliana)	CACCCCGTCCCGTAGACGT ACCCTCC	TTAGCATTCACACCCTGTAGA CCTC	pEarleyGate203 (Earley <i>et al</i> ., 2006)	Myc-RABG3f
RABA1a (S. tuberosum)	GCAGGTTATAGAGGTGATG ATGAG	CTAGCTCGAACAGCACCCAA AC	pEarleyGate203 (Earley <i>et al.</i> , 2006)	Myc-RABA1a _{potato}
RABA2a (S. tuberosum)	GCGAGAAGAGCGGAAGAG GAG	CTATGCAGAGCAACATGCCT TC	pEarleyGate203 (Earley <i>et al.</i> , 2006)	Myc-RABA2a _{potato}
RABA4a (S. tuberosum)	GCAAGTGGGGGTGGGTAT G	TTAAGAACTACAGCATGCCTT C	pEarleyGate203 (Earley <i>et al</i> ., 2006)	Myc-RABA4a _{potato}
ph-dependent secGFP			pRB35S (Bartetzko <i>et al</i> ., 2009)	secGFP
PR-1 (A. thaliana)	TCAGTCGACATGAATTTTAC TGGCTATTCTCG	AGTGCGGCCGCGAGTATGG CTTCTCGTTCACA	pB2GW7 (Karimi <i>et al.</i> , 2002)	PR-1-phGFP
PDF1.2 (A. thaliana)	TCAGTCGACATGGCTAAGT TTGCTTCCATCATC	AGTGCGGCCGCGAACATGG GACGTAACAGATAC	pB2GW7 (Karimi <i>et al</i> ., 2002)	PDF1.2-phGFP
PNP-A (A. thaliana)	TCAGTCGACATGATAAAAAT GGCAGTAAAATTTG	AGTGCGGCCGCGATATCGGT GTGTATACGACAC	pB2GW7 (Karimi <i>et al.</i> , 2002)	PNP-A-phGFP

Supplemental Table S4. List of primers used for qPCR analysis.

Gene	Organism	Forward sequence	Reverse sequence
RxLR24		AGAAGGGAGAGACGCCAAC	ATTCGTGTGGTGGTTGATCC
Actin	P. brassicae	GCAGATGTGGATCTCGAAGG	CAGCAGCGATGTATCTCCAG
Beta-tubulin		CCAAGGGACTGAAGATGAGC	AGCCTTACGACGGAACATTG
PR1		ACTACAACTACGCTGCGAACAC	GTTACACCTCACTTTGGCACATC
PDF1.2	Anabidanaia	ATCACCCTTATCTTCGCTGCTC	ACTTGGCTTCTCGCACAACTTC
PNP-A	Arabidopsis	CCGTAGACGTGAAGGTAGTTGA	CGAATGTTACCGGCATCAGTAT
ExpG		GAGCTGAAGTGGCTTCCATGAC	GGTCCGACATACCCATGATCC

SUPPORTING TABLES

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potontial riveriz r targ	3010	number	location	RxLR24	RxLR23	RxLR27	RxLR29
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RAB GTPases subfamilly	RABA1b	AT1G16920		9	0	0	0
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RADAT	RABA1d	AT4G18800		8	0	0	0
	RABA1f	AT5G60860	andaaana	9	0	0	0
	RABA2a*	AT1G09630	endosome membrane, plasma membrane	9	0	0	0
RAB GTPases subfamilly RABA2	RABA2c	AT3G46830		10	0	0	0
RADA2	RABA2d	AT5G59150		12	0	0	0
	RABA4a*	AT5G65270		12	0	0	0
RAB GTPases subfamilly RABA4	RABA4b	AT4G39990		5	0	0	0
KADA4	RABA4c	AT5G47960		5	0	0	0
RAB GTPases subfamilly RABG3	RABG3f*	AT3G18820		3	0	0	0
Magnesium-protoporphyrin IX monomethyl ester [oxidative] cyclase		AT3G56940	chloroplast	8	2	0	0
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Gene	Primer forward	Primer reverse	Destination plasmid	Tagged fusion protein
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(A. thaliana)	GATGAAG	TTC	pB7WGF2 (Karimi <i>et al</i> ., 2002)	RFP-RABA1a
RABA2a (A. thaliana)	CACCGCGAGAAGACCGGA CGAAGAATAC	TCAAGACGATGAGCAACAAG GCTTC	pEarleyGate203 (Earley <i>et al.</i> , 2006)	Myc-RABA2a
RABA4a† (A. thaliana)			pEarleyGate203 (Earley <i>et al.</i> , 2006)	Myc-RABA4a
RABG3f (A. thaliana)	CACCCCGTCCCGTAGACGT ACCCTCC	TTAGCATTCACACCCTGTAGA CCTC	pEarleyGate203 (Earley <i>et al.</i> , 2006)	Myc-RABG3f
RABA1a (S. tuberosum)	GCAGGTTATAGAGGTGATG ATGAG	CTAGCTCGAACAGCACCCAA AC	pEarleyGate203 (Earley <i>et al</i> ., 2006)	Myc-RABA1a _{potato}
RABA2a (S. tuberosum)	GCGAGAAGAGCGGAAGAG GAG	CTATGCAGAGCAACATGCCT TC	pEarleyGate203 (Earley <i>et al.</i> , 2006)	Myc-RABA2a _{potato}
RABA4a (S. tuberosum)	GCAAGTGGGGGGGGGGTAT G	TTAAGAACTACAGCATGCCTT C	pEarleyGate203 (Earley <i>et al</i> ., 2006)	Myc-RABA4a _{potato}
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Beta-tubulin		CCAAGGGACTGAAGATGAGC	AGCCTTACGACGGAACATTG
PR1		ACTACAACTACGCTGCGAACAC	GTTACACCTCACTTTGGCACATC
PDF1.2	Anabidanaia	ATCACCCTTATCTTCGCTGCTC	ACTTGGCTTCTCGCACAACTTC
PNP-A	Arabidopsis	CCGTAGACGTGAAGGTAGTTGA	CGAATGTTACCGGCATCAGTAT
ExpG		GAGCTGAAGTGGCTTCCATGAC	GGTCCGACATACCCATGATCC