New Phytologist

***New Phytologist* Supporting Information**

Article title: **A *Phytophthora* effector protein promotes symplastic cell-to-cell trafficking by physical interaction with plasmodesmata-localized callose synthases**
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Fig. S2 Relative *RxLR3* expression during *P. brassicae* infection.

Fig. S3 Ectopically expressed RxLR3 interferes with Arabidopsis development.

Fig. S4 Effect of RxLR3 expression on disease resistance towards *P. brassicae*. Arabidopsis plants (Ws-0) containing the empty vector (WT) and two lines constitutively expressing FLAG-RxLR3 were inoculated with a zoospore suspension of *P. brassicae* isolate CBS179.87.

Fig. S5 GFP-RxLR3 localizes to dotlike structures at the cell periphery.

Fig. S6 RxLR3 co-localizes with CalS3. Localization of GFP-CalS3 and RFP-RxLR3 in agroinfiltrated *N. benthamiana* epidermal cells.

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Fig. S8 Effect of *Agrobacterium* density on expression pattern of RxLR3 in agroinfiltrated *N. benthamiana*.

Fig. S9 Assessment of GFP tracer diffusion assay in agroinfiltrated *N. benthamiana* epidermal cells.

Fig. S10 The PD callose quantification method in epidermal cells of Arabidopsis WT and RxLR3 transgenic lines after infection with *P. brassicae* zoospores.

Fig. S11 The overview: examples of Arabidopsis epidermal cells of (a) WT and (b) RxLR3

transgenic lines scored as showing "induced" or "non induced" PD callose in cells invaded by *P. brassicae* zoospore.

Table S1 List of primers.

Table S2 List of constructs.

Table S3 List of top candidates of RxLR3 interacting proteins identified with Co-IP/MS with FLAG-RxLR3 as a bait.

Fig. S1 Amino acid sequence of the RxLR3 effector of *P. brassicae* (GenBank MN708974). The signal peptide is underlined and sequences representing the RxLR and dEER motifs are highlighted in bold.

MRLSYVFLVAAATTLFASGSAVPITGKRTTEDSLMVSPVLAVSPAVEK**RALR**IRNAEEKEKE
N**DEEE**KTFTEATKLLDDVMELQKLNKSYSQGNKDFVAKLLHPAVLEKAAAARSTREELEQLF
WLMNHHYIVPDNLGRGKALDTYYSQYLNGRWKRSEG

Fig. S2 Relative *RxLR3* expression during *P. brassicae* infection. The susceptible Arabidopsis mutant *cyp79B2cyp79B3* was inoculated with zoospores of *P. brassicae* isolate CBS179.87 and the kinetics of *RxLR3* transcript accumulation was determined by qPCR. No qPCR product was obtained with uninoculated plant material. *β-tubulin* and *actin* of *P. brassicae* were used as reference transcripts. The relative expression of *RxLR3* at 12hpi was set at 1. Error bars represent mean values (± SE) of six biological replicates. Transcript level of *RxLR3* at 12 hpi is equal to 20% of *β-tubulin* expression level. Calculation of the relative expression values was done as described (Vandesompele *et al*., 2002).

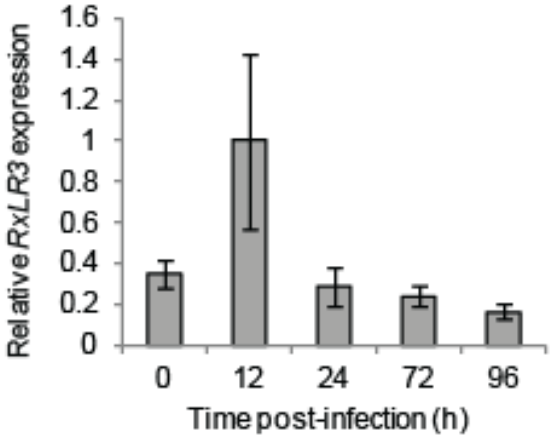

Fig. S3 Ectopically expressed RxLR3 interferes with Arabidopsis development. **(**a) Growth phenotype of representative individuals of 3-week-old Arabidopsis lines expressing FLAG-RxLR3 compared to Ws-0 wild type plant (WT). (b) Quantification of plant size based on rosette diameter. Diagram shows mean values ±SD of six plants per line. The size of the wild type plants corresponds to 100%. (c) Immunoblot analysis comparing FLAG-RxLR3 protein accumulation of different lines. Rubisco served as loading control. Experiments were performed twice with similar results.

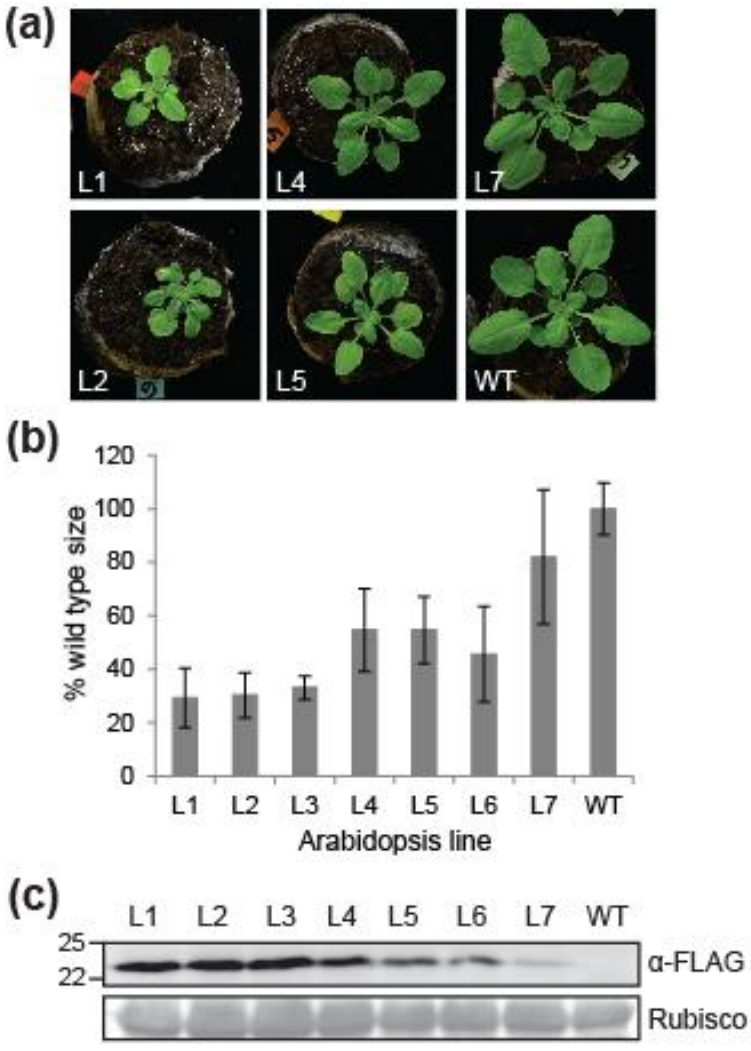

Fig. S4 Effect of RxLR3 expression on disease resistance towards *P. brassicae*. Arabidopsis plants (Ws-0) containing the empty vector (WT) and two lines constitutively expressing FLAG-RxLR3 were inoculated with a zoospore suspension of *P. brassicae* isolate CBS179.87. Relative distribution of susceptibility scores based on lesion size was scored 7 dpi (0 = fully susceptible; 4 = fully resistant; Schlaeppi *et al*., 2010). The results are based on two experiments each including 40 fully developed leaves of 10 plants per line. Statistically significant differences labelled with a and b based on multiple pairwise comparison using Fisher's exact test ($p<0.05$; p-values adjusted for multiple testing by the method of Benjamini and Hochberg).

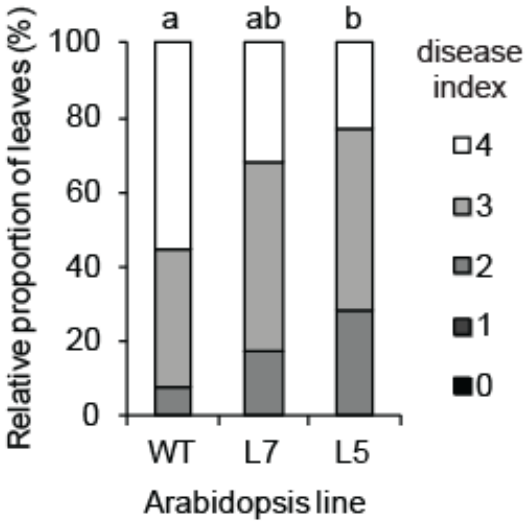

Fig. S5 GFP-RxLR3 localizes to dotlike structures at the cell periphery. GFP-RxLR3 was expressed via agroinfiltration in leaves of *N. benthamiana*. Chloroplast autofluorescence indicated in yellow. Picture is a z-stack consisting of 6 images with a voxel depth of 0.8µm. Scale bar 20µm. Picture taken 24h after agroinfiltration.

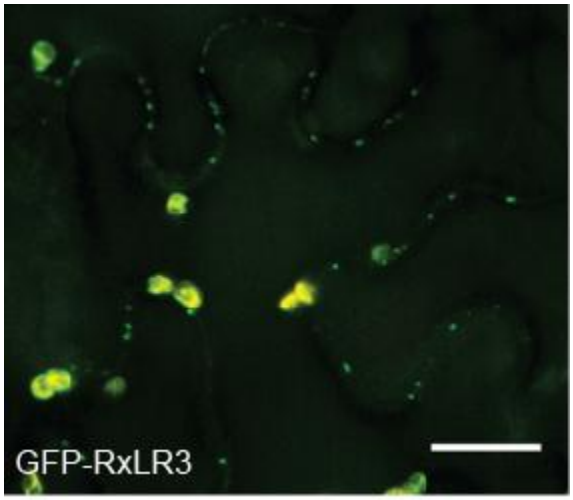

Fig. S6 RxLR3 co-localizes with CalS3. Localization of GFP-CalS3 and RFP-RxLR3 in agroinfiltrated *N. benthamiana* epidermal cells. Co-localization of both proteins at PD varies from green-yellow to orange color due to different intensities of the channels and is marked with arrowheads. All pictures are single images taken 24h after agroinfiltration. Scale bar: 5µm. Fluorescence intensity plot shows fluorescence profile overlap between both signals. The length of x-axis in the graph correspond to the white line in the merged picture.

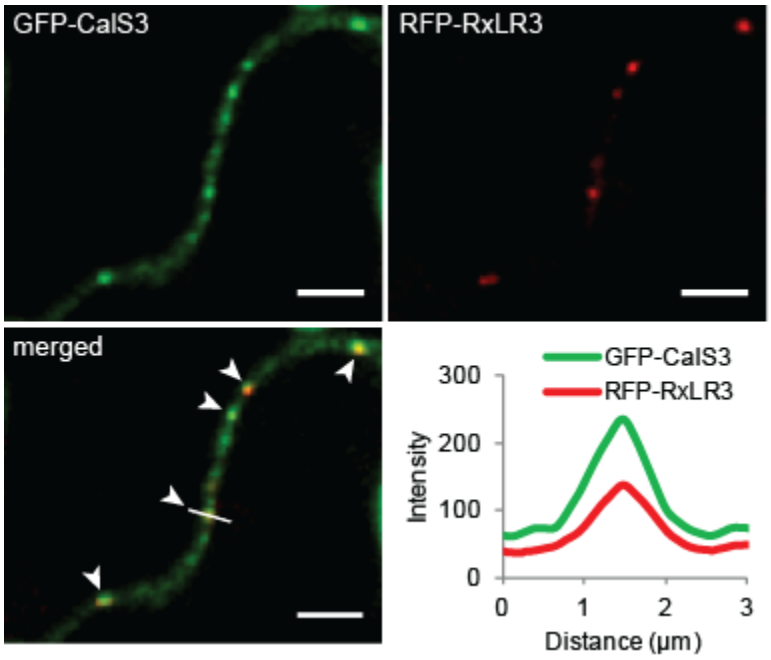

Fig. S7 RxLR3 causes reduced callose deposition at PD in *N.benthamiana* epidermal cells. (a) Callose at PD stained with aniline blue in *N. benthamiana* leaves expressing empty vector control or FLAG-RxLR3. Fluorescent signals represent plasmodesmal callose deposits. Observations were performed three days after agroinfiltration. Each picture represents a z-stack consisting of 11 images with a voxel depth of 1µm. Scale bar: 20µm. (b) Signal intensity quantification of aniline blue staining at PD in leaves expressing empty vector control or FLAG-RxLR3. Calculation is based on 96 pictures taken at random places in two independent experiments. Asterisk indicates statistical significance between the mean values (±SE) determined by t-test, $p<0.001$. (c) Immunoblot analysis of FLAG-RxLR3 accumulation in tissues used for microscopical analysis.

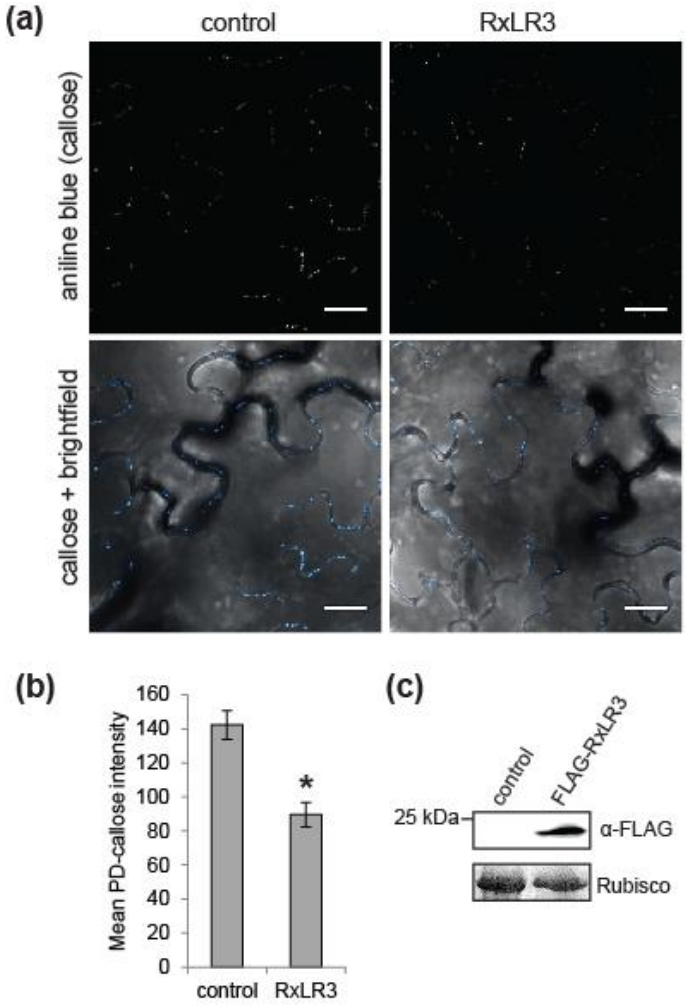

Fig. S8 Effect of *Agrobacterium* density on expression pattern of RxLR3 in agroinfiltrated *N. benthamiana*. Leaves of *N. benthamiana* were agroinfiltrated with different concentrations of Agrobacteria containing a GFP-RxLR3 construct. (a) Infiltration with an *Agrobacterium* suspension of an OD_{600}=0.15. (b) Infiltration with an *Agrobacterium* suspension of an OD_{600}=0.0005. Pictures taken three days after agroinfiltration. Scale bar: 200µm.

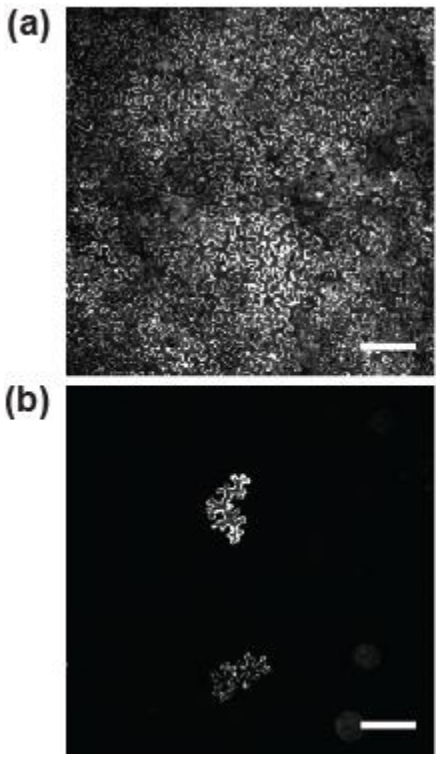

Fig. S9 Assessment of GFP tracer diffusion assay in agroinfiltrated *N. benthamiana* epidermal cells. (a) Diffusion layers were counted as a maximal number of rows of cells with detectable GFP signal on a straight line starting at the initially transformed cell expressing ER-CFP (considered as single foci/single transformation event). (b) The initially transformed cell layer (layer 0) is marked in cyan and the subsequent diffusion layers in yellow, red, orange and pink lines, respectively. Scale bar: 100µm.

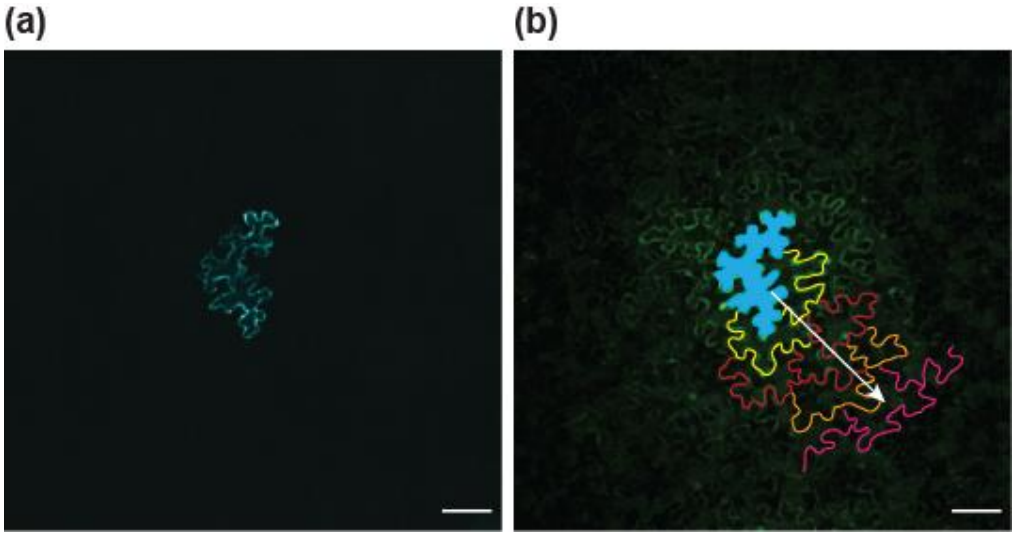

Fig. S10 The PD callose quantification method in epidermal cells of Arabidopsis WT and RxLR3 transgenic lines after infection with *P. brassicae* zoospores. (a) The scheme of single infection site considered for assessment. Orange shape: the cell with the site of attempted penetration and dotted lines represent callose at PD in cell invaded by a zoospore. Green shape: neighboring, non-infected cells and dotted green line represent PD callose deposits in non-infected cells. Blue squares mark the areas considered for assessment. Representative pictures taken 7h after infection for WT (b) and for RxLR3 lines (c) in the experiment of PD callose quantification after infection. Left panels show aniline stained callose and panels on the right show callose on the brightfield background. Orange asterisk indicates the area of invaded cell and green asterisk - neighboring cells. Orange arrows mark induced PD callose in invaded cells, green arrows mark the PD callose spots (if visible) in the neighboring cells. Pictures are z-stack images consisting of 5 images taken with an EMCCD camera and 60 x lens. All z-stack images were prepared with maximal projection type, voxel depth 1µm. Scale bar: 5µm.

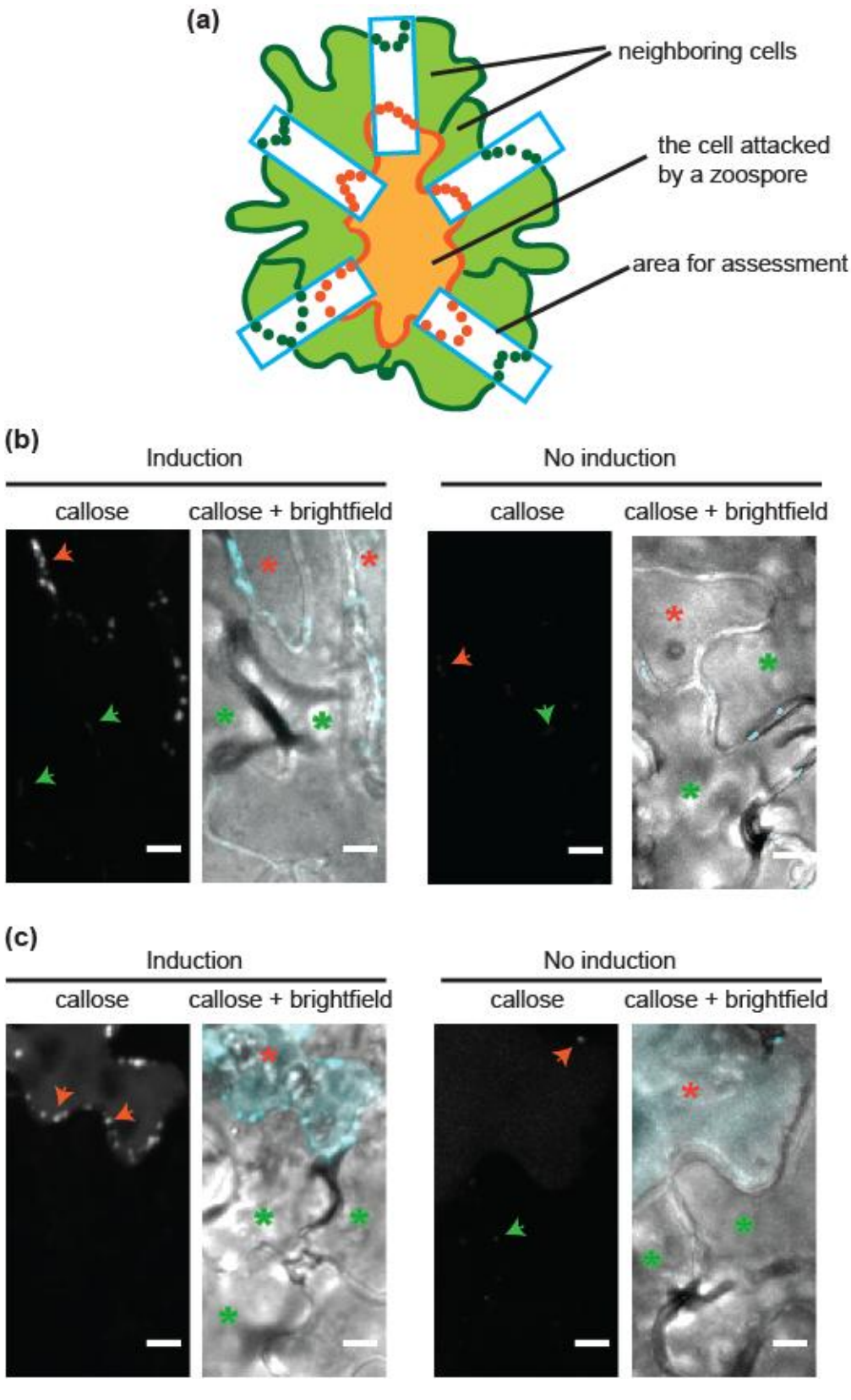
(a)
neighboring cells
the cell attacked
by a zoospore
area for assessment
(b)
Induction
No induction
callose
callose + brightfield
callose
callose + brightfield
(c)
Induction
No induction
callose
callose + brightfield
callose
callose + brightfield

Fig. S11 The overview: examples of Arabidopsis epidermal cells of (a) WT and (b) RxLR3 transgenic lines scored as showing "induced" or "non induced" PD callose in cells invaded by *P. brassicae* zoospore. The cells with induced PD callose marked with red asterisk and yellow asterisk when callose deposition at PD was quantified as unchanged. Orange arrows mark induced PD callose in attacked cells, green arrows mark the PD callose spots in the neighboring cells (if visible). Pictures taken 7hai. Pictures are z-stack images consisting of 17 (a, right), 20 (a, left), 15 (b, left) and 15 single images (b, right). All z-stack images were prepared with maximal projection type, voxel depth 1µm. Scale bar 20µm.

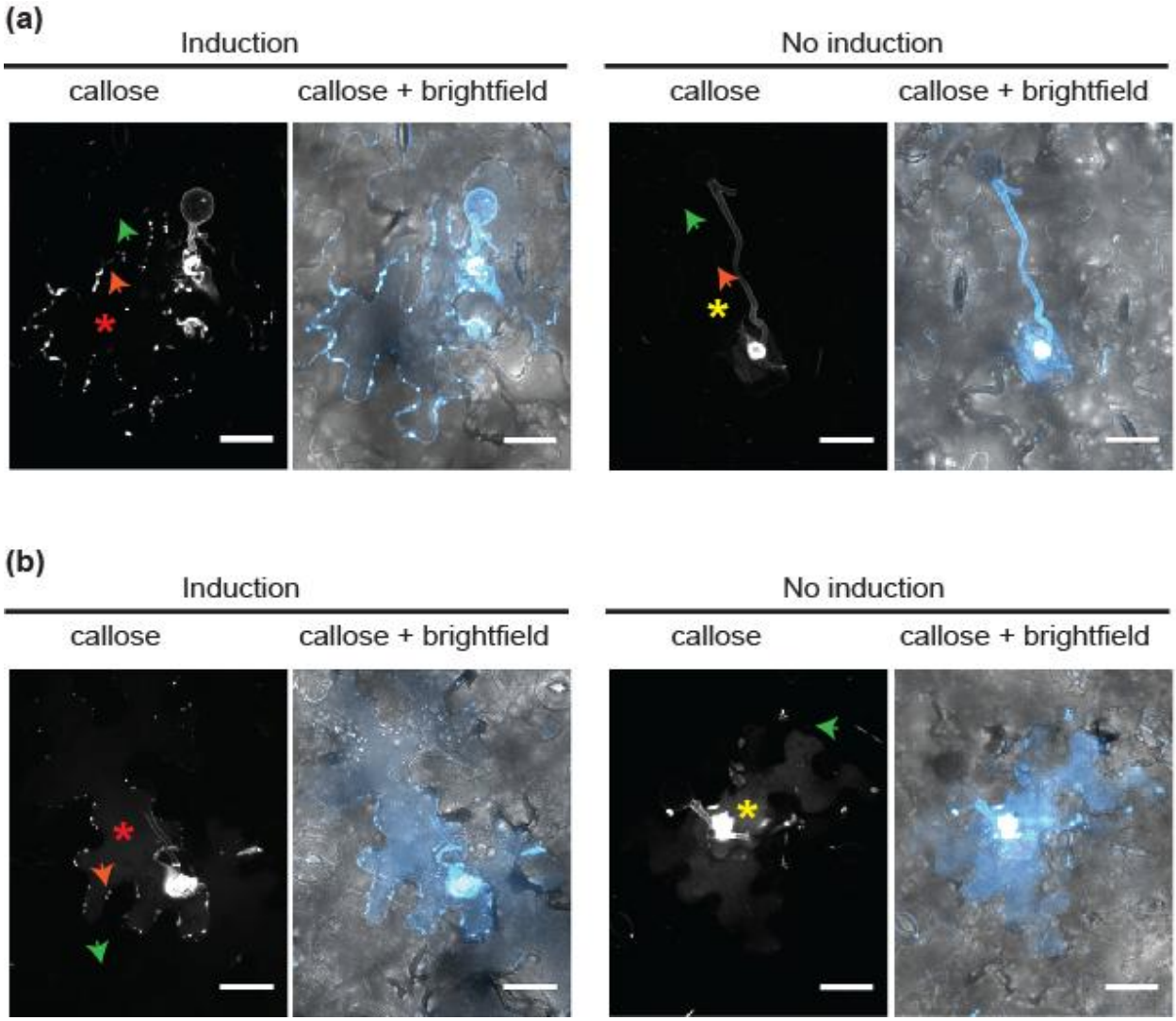

Table S1 List of primers.

Gene	Primer forward	Primer reverse
PDLP5	CACCATGATCAAGACAAAGACGACGTCC	TTTACACCATTTCTCATCTTGTAATTTTCTAC
RxLR3 (without signal peptide)	CACCATGAGCGCAGTCCCGATCACTGGC	GGTTCCTAGCCCTCACTGCG

Table S2 List of constructs.

Construct	Gene	GenBank number	Plasmid
PDLP5-mCherry-HA	PDLP5	BT006325 (At1g70690)	pEarleyGate101 (Earley *et al.*, 2006)
3xFLAG-RxLR3	RxLR3	MN708974	pFASTR02
YFP-RxLR3			pEarleyGate104
RFP-RxLR3			pB7WGR2
GFP-RxLR3			pB7WGF2
ER-CFP	CFP		ER-CFP in CD3-742
GFP tracer	GFP		GFP in pB7WGF2
GFP-CALS3	CalS3	At5g13000	(Vatén *et al.*, 2011)

Table S3 List of top candidates of RxLR3 interacting proteins identified with Co-IP/MS with FLAG-RxLR3 as a bait. 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 total unique peptides matched per protein within a protein cluster. Peptides with post-translational modifications were counted separately. In brackets number of peptides exclusively matching individual proteins. In parallel with PbRxLR3 (GenBank MN708974), three non-related FLAG-tagged RxLR effectors of P. brassicae (PbRxLR5, GenBank MN708975; PbRxLR19, GenBank MN708976; PbRxLR29, GenBank MG489829) served as negative controls.

Annotation for proteins identified as potential RxLR3 targets		TAIR accession number	Subcellular location	Number of identified peptides			
				RxLR3	RxLR5	RxLR19	RxLR29
Members of the callose synthase (GLUCAN SYNTHASE-LIKE) family	CALS3 (GSL12)	At5g13000	plasma membrane	53 (41)	0	0	0
	CALS2 (GSL3)	At2g31960	plasma membrane	38 (13)	0	0	0
	CALS1 (GSL6)	At1g05570	plasma membrane	33 (9)	0	0	0
	CALS5 (GSL2)	At2g13680	plasma membrane	2 (0)	0	0	0
NITRILE-SPECIFIER PROTEIN 1		At3g16400	unknown	16 (4)	3 (1)	0	4 (1)
PROTEIN ARGONAUTE 1		At1g48410	cytoplasm	11 (10)	3 (3)	2 (2)	0
ACETOACETYL-COA THIOLASE		At5g48230	unknown	11 (11)	3 (3)	0	0

Earley KW, Haag JR, Pontes O, Opper K, Juehne T, Song K, Pikaard CS. 2006. Gateway-compatible vectors for plant functional genomics and proteomics. *The Plant Journal* **45**(4): 616-629.

Schlaeppi K, Abou-Mansour E, Buchala A, Mauch F. 2010. Disease resistance of Arabidopsis to *Phytophthora brassicae* is established by the sequential action of indole glucosinolates and camalexin. *The Plant Journal* **62**(5): 840-851.

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology* **3**(7): 1.

Vatén A, Dettmer J, Wu S, Stierhof Y-D, Miyashima S, Yadav SR, Roberts CJ, Campilho A, Bulone V, Lichtenberger R. 2011. Callose biosynthesis regulates symplastic trafficking during root development. *Developmental Cell* **21**(6): 1144-1155.