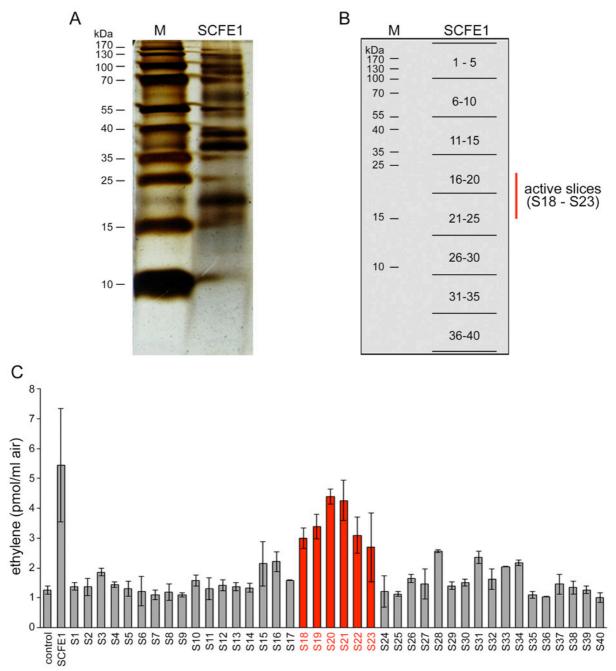


#### Supplemental Figure 1. Isolation and physico-chemical properties of the SCFE1containing fraction.

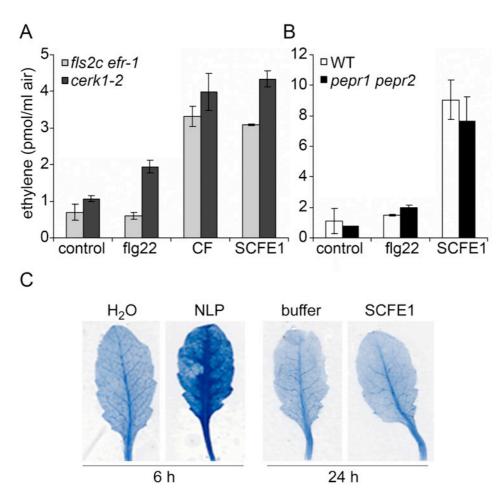
(A) SDS-PAGE of 2.5  $\mu$ g *S. sclerotiorum* culture filtrate proteins (CF), 0.4  $\mu$ g proteins eluted from a Sepharose SP cation exchange chromatography (S1), 0.3  $\mu$  g proteins rechromatographed on Source 15S cation exchange FPLC (SCFE1). Standard Protein Molecular Mass Marker (M). Proteins were visualized by silver staining. (B) Fractions from *S. sclerotiorum* were tested for ethylene-inducing activity on *Arabidopsis* Col-0 leaf pieces: buffer (control), 6  $\mu$  g/ml culture filtrate proteins (CF), 0.5  $\mu$  g/ml proteins eluted from Sepharose SP (S1), 0.35  $\mu$ g/ml proteins eluted from Source 15S (SCFE1). Ethylene production was measured by gas chromatography 3 h after incubation. Bars represent average values ± S.D. (n=2). The experiment was repeated at least three times with similar results. (C) The SCFE1-containing fraction (0.5  $\mu$ g/ml) was incubated with different proteases (+) - proteinase K (Prot K), AspN, trypsin, GluC, or a commercial deglycosylation mixture (degluc) - or the respective enzyme buffers (-) and tested for ethylene-inducing activity on

*Arabidopsis* Col-0 leaf pieces. Untreated leaf pieces were used as a control (c). Bars represent average values  $\pm$  S.D. (n=2). The experiment was performed two times with similar results. **(D)** Before addition to *Arabidopsis* Col-0 leaf pieces, the SsE1-containing fraction was either left untreated (-), heated for 10 min at 95°C (heat) or incubated in 0.1% SDS for 1 h at room temperature (SDS). Untreated leaf pieces were used as a control (c). Bars represent average values  $\pm$  S.D. (n=2). The experiment was performed two times with similar results. **(E)** Dose-response relationship for SCFE1-induced ethylene production in *Arabidopsis* Col-0 leaves. Represented are average values  $\pm$  S.D. (n=2). The experiment was repeated three times with similar results.

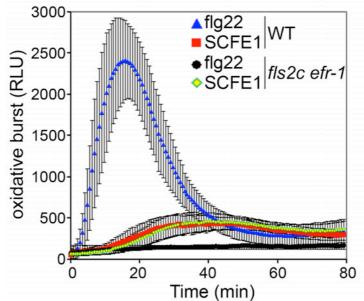


# Supplemental Figure 2. Elicitor activity of SCFE1 can be purified from SDS-PAGE.

(A) SDS-PAGE of 8  $\mu$ g of SCFE1-containing fractions that were pooled and freeze-dried after elution of the Source 15S cation exchange FPLC. Standard Protein Molecular Mass Marker (M). Proteins were visualized by silver staining. (B) 50  $\mu$ g of SCFE1-containing fractions that were pooled and freeze-dried after elution of the Source 15S cation exchange FPLC were separated on a tricine-SDS PA gel and the lane was cut into 2 mm large segments (S1-S40) as depicted in the scheme. Each segment was incubated overnight in 100  $\mu$ l 100 mM Mes buffer pH 5.4 containing 0.1% SDS for protein elution. (C) 20  $\mu$ l of eluted proteins were tested for ethylene-inducing activity on *Arabidopsis* Col-0 leaf pieces. SCFE1 activity was recovered from gel segments S18-S23 (red) corresponding to proteins with a molecular mass of 16-22 kDa. Buffer (control), 0.35  $\mu$ g/ml proteins eluted from Source 15S (SCFE1). Ethylene production was measured by gas chromatography 3 h after incubation. Bars represent average values ± S.D. (n=2). The experiment was repeated three times with similar results.

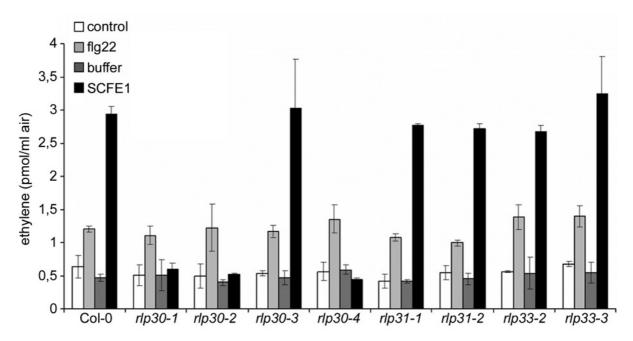


Supplemental Figure 3. The SCFE1-containing fraction does not induce necrosis and triggers ethylene production independently of known MAMP or DAMP receptors. (A, B) Leaf pieces of *Arabidopsis* Col-0 wild type plants (WT), the *cerk1* single mutant or the *fls2c efr-1* and *pepr1pepr2* double mutant were treated with *S. sclerotiorum* culture filtrate (CF), 0.5 µg/ml of the SCFE1-containing fraction, 500 nM flg22 or left untreated (control) prior to measurement of ethylene production. Bars represent average values  $\pm$  S.D. (n=2). The experiment was repeated two times with similar results. (C) The SCFE1-containing fraction was tested for cell death-inducing activity. 0.5 µg/ml SCFE1 or 3 µM purified NLP from *P. parasitica* were infiltrated into *Arabidopsis* Col-0 leaves. Cell death was visualized by trypan blue staining.

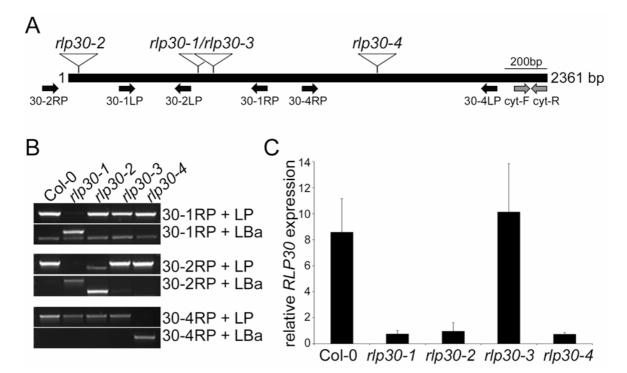


Supplemental Figure 4. The SCFE1-containing fraction triggers ROS production independently of known MAMP receptors.

Oxidative burst triggered by 0.12  $\mu$ g/ml of the SCFE1-containing fraction in *Arabidopsis* Col-0 (red) and the fls2c efr-1 double mutant (green) leaf discs or by 100 nM flg22 in *Arabidopsis* Col-0 (blue) and the fls2c efr-1 double mutant (black) leaf discs, measured in relative light units (RLU). Results are means ± S.D. (n = 6).

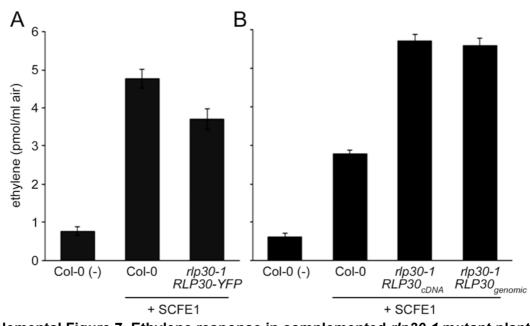


Supplemental Figure 5. SCFE1-triggered ethylene production in candidate *rlp* mutants. Ethylene assay in *Arabidopsis* Col-0 plants compared to *rlp* mutants representing candidate genes in the chromosome region mapped for SCFE1 sensitivity. Leaf pieces were treated with 0.25  $\mu$ g/ml of the SCFE1-containing fraction or as a control with 500 nM flg22 or buffer prior measurement of ethylene production. Bars represent average values ± S.D. (n=2). The experiment was repeated three times with similar results.

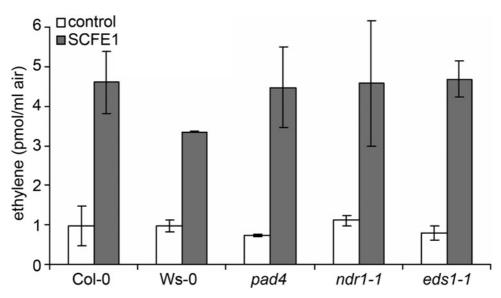


#### Supplemental Figure 6. Analysis of *Arabidopsis rlp30* mutants.

(A) Schematic representation of the *RLP30* gene with the positions of the four *rlp30* T-DNA insertions and the primers used for genotyping (B) and transcript analysis (C). (B) Genomic DNA from Col-0 wild type seedlings or the indicated *rlp30* mutant lines was subjected to PCR using the indicated primer pairs (RP, right border primer; LP, left border primer; LBa, left border primer from the T-DNA insertion). Positions of the primers are depicted in (A). (C) Transcriptional profiling of *RLP30* in the different *rlp30* T-DNA insertion lines by quantitative real-time PCR (qRT-PCR). Total RNA from seedlings was subjected to qRT-PCR using *RLP30* specific primers cyt-F and cyt-R, of which positions are depicted in (A). (C) expression was normalized to the levels of *EF-1* $\alpha$  transcript. Error bars, S.D. (n=3).

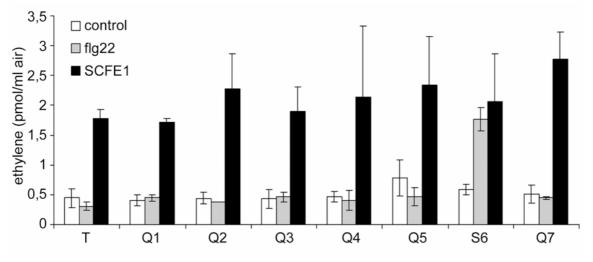


**Supplemental Figure 7. Ethylene response in complemented** *rlp30-1* **mutant plants.** Ethylene assay in *Arabidopsis* Col-0 plants and *rlp30-1* mutant lines complemented with constructs *p35S:RLP30-YFP* (**A**) *p35S:RLP30<sub>cDNA</sub>* and *p35S:RLP30<sub>genomic</sub>* (**B**). Leaf pieces were treated with 0.25 µg/ml SCFE1-containing fraction or left untreated (-) prior to measurement of ethylene production. Bars represent average values  $\pm$  S.D. (n=2). The experiment was repeated with similar results.



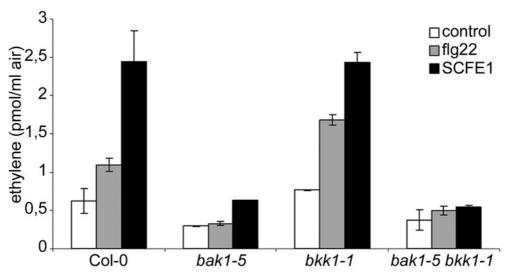
Supplemental Figure 8. The activity of the SCFE1-containing fraction does not depend on known ETI components.

Leaf pieces of *pad4*, *ndr1-1* plants (in the Col-0 background) or the *eds1-1* single mutant (in the Ws-0 background) were treated with 0.25  $\mu$  g/ml of the SCFE1-containing fraction or water as a control prior to measurement of ethylene production. Bars represent average values ± S.D. (n=2). The experiment was repeated two times with similar results.



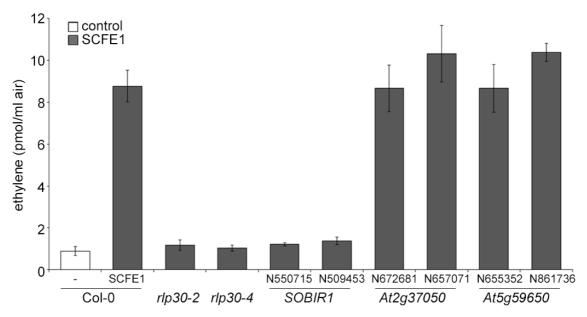
Supplemental Figure 9. SCFE1 perception does not require LRR-RLK XII family members.

The SCFE1-containing fraction (0.8  $\mu$ g/ml) was tested for ethylene-inducing activity on leaf pieces of indicated *Arabidopsis* mutants. The mutants were the following: T (*fls2 efr cerk1* triple mutant), Q1 (*fls2 efr cerk1 xii1* quadruple mutant), Q2 (*fls2 efr cerk1 xii2*), Q3 (*fls2 efr cerk1 xii3*), Q4 (*fls2 efr cerk1 xii4*), Q5 (*fls2 efr cerk1 xii5*), S6 (*xii6* single mutant), Q7 (*fls2 efr cerk1 xii7*). Nomenclature of the LRR-RLK XII family members was according to Shiu and Bleecker (Shiu and Bleecker, 2001). Bars represent average values  $\pm$  S.D. (n=2). The experiment was performed two times with similar results.



Supplemental Figure 10. The activity of the SCFE1-containing fraction does not depend on BKK1.

Leaf pieces of *Arabidopsis* Col-0 wild type plants, *bak1-5* and *bkk1-1* single mutants or the *bak1-5 bkk1-1* double mutant were treated with 0.25  $\mu$ g/ml of the SCFE1-containing fraction, 500 nM flg22 or left untreated (control) prior to measurement of ethylene production. Bars represent average values ± S.D. (n=2). The experiment was repeated three times with similar results.



Supplemental Figure 11. The activity of the SCFE1-containing fraction depends on SOBIR1/EVR.

Ethylene assay in *Arabidopsis* Col-0 plants compared to *rlk* mutants identified in Y2H as candidate interactors of RLP30. Leaf pieces were treated with 0.25  $\mu$  g/ml of the SCFE1-containing fraction or as a control with 500 nM flg22 or buffer prior measurement of ethylene production. Bars represent average values ± S.D. (n=2).

Supplemental Table 1. SCFE1 sensitivity is controlled by a single recessive gene locus in different *Arabidopsis* accessions.

Plants	Sensitivity	Insensitivity
F1 (Mt-0 X Col-0)	10	0
F1 (Sq-1 X Col-0)	10	0
F1 (Lov-1 X Col-0)	10	0
F1 (Lov-1 X Mt-0)	0	10
F1 (Lov-1 X Sq-1)	0	10
F2 (Lov-1 X Col-0)	206	64

The SCFE1-containing fraction was tested for ethylene-inducing activity on leaf pieces of indicated *Arabidopsis* plants (number of plants as shown). Sensitivity: induction level was above the untreated control. Insensitivity: induction level was similar to the untreated control. F2 populations from the Lov-1 x Col-0 cross showed a segregation ratio of 3:1 (206 sensitive versus 64 insensitive plants,  $X^2 = 0.178$ , p-value = 0.67309, using  $X^2$  test and after Yates's correction).

	ble 2. Putative interactors of RLP30.
AGI ID	Functional Description
<u>AT1G07860</u>	Unknown: BEST Arabidopsis thaliana protein match is: Protein
	kinase superfamily protein (TAIR:AT1G07870.2)
AT1G12670	Unknown protein
AT1G13770	RUS3, Protein of unknown function, DUF647
AT1G14020	O-fucosyltransferase family protein
AT1G14360	ATUTR3, UTR3, UDP-galactose transporter 3
AT1G17280	UBC34, ubiquitin-conjugating enzyme 34
AT1G19570	DHAR1, ATDHAR1, DHAR5, dehydroascorbate reductase
AT1G21240	WAK3, wall associated kinase 3 RLK/Pelle
AT1G21870	GONST5, golgi nucleotide sugar transporter 5
AT1G23300	MATE efflux family protein
AT1G27290	Unknown protein
AT1G29060	Target SNARE coiled-coil domain protein
AT1G31812	ACBP6, ACBP, acyl-CoA-binding protein 6
AT1G34640	peptidases
AT1G45145	ATTRX5, ATH5, LIV1, TRX5, thioredoxin H-type 5
AT1G63110	GPI transamidase subunit PIG-U
AT1G63120	ATRBL2, RBL2, RHOMBOID-like 2
AT1G65690	Late embryogenesis abundant (LEA) hydroxyproline-rich
<u>////000000</u>	glycoprotein family
AT1G77350	Unknown protein
AT1G78240	TSD2, QUA2, S-adenosyl-L-methionine-dependent
111010210	methyltransferases superfamily protein
AT1G78380	ATGSTU19, GST8, GSTU19, glutathione S-transferase TAU 19
AT2G22425	Microsomal signal peptidase 12 kDa subunit (SPC12)
AT2G26180	IQD6, IQ-domain 6 (Calcium binding)
AT2G27290	Protein of unknown function (DUF1279)
AT2G28315	Nucleotide/sugar transporter family protein
AT2G31880	SOBIR1, EVR, Leucine-rich repeat protein kinase family
	protein
AT2G36305	RCE1, ATFACE-2, ATFACE2, FACE2, farnesylated protein-
	converting enzyme 2
AT2G37050	Leucine-rich repeat protein kinase family protein
AT2G41490	GPT, UDP-glcnac-adolichol phosphate glcnac-1-p-transferase
AT3G01360	Family of unknown function (DUF716)
AT3G03210	Unknown protein
AT3G10640	VPS60.1, SNF7 family protein
AT3G11550	Uncharacterised protein family (UPF0497)
AT3G12180	Cornichon family protein
AT3G13175	Unknown protein
AT3G17000	UBC32, ubiquitin-conjugating enzyme 32
AT3G17210	ATHS1, HS1, heat stable protein 1
AT3G18800	Unknown protein
AT3G20600	NDR1, Late embryogenesis abundant (LEA) hydroxyproline-rich
	glycoprotein family
AT3G25805	Unknown protein
AT3G26020	Protein phosphatase 2A regulatory B subunit family protein
AT3G57650	LPAT2, lysophosphatidyl acyltransferase 2
AT3G62560	Ras-related small GTP-binding family protein
AT3G66654	Cyclophilin-like peptidyl-prolyl cis-trans isomerase family protein
AT4G14455	ATBET12, BET12, ATBS14B, BS14B, Target SNARE coiled-coil
<u> </u>	

Supplemental Table 2. Putative interactors of RLP30.

	domain protein		
AT4G20790	Leucine-rich repeat protein kinase family protein		
AT4G23010	ATUTR2, UTR2, UDP-galactose transporter 2		
AT4G23400	PIP1D, PIP1;5, plasma membrane intrinsic protein 1;5		
AT4G27780	ACBP2, acyl-CoA binding protein 2		
AT4G29330	DER1, DERLIN-1		
AT4G30500	Protein of unknown function (DUF788)		
AT4G30850	HHP2, heptahelical transmembrane protein2		
AT4G37370	CYP81D8, cytochrome P450, family 81, subfamily D, polypeptide		
	8		
AT4G37680	HHP4, heptahelical protein 4		
<u>AT4G38690</u>	PLC-like phosphodiesterases superfamily protein		
<u>AT4G39890</u>	AtRABH1c, RABH1c, RAB GTPase homolog H1C		
AT5G06320	NHL3, NDR1/HIN1-like 3		
AT5G11890	Unknown: BEST Arabidopsis thaliana protein match is: Late		
	embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein		
	family (TAIR:AT1G17620.1)		
AT5G21920	YLMG2, ATYLMG2, YGGT family protein		
AT5G35460	Protein of unknown function DUF2838		
AT5G42980	ATTRX3, ATH3, ATTRXH3, TRXH3, TRX3, thioredoxin 3		
AT5G49540	Rab5-interacting family protein		
AT5G52240	MSBP1, membrane steroid binding protein 1		
AT5G52420	Unknown protein		
AT5G59650	Leucine-rich repeat protein kinase family protein		
AT5G63030	Thioredoxin superfamily protein		

Original data retrieved from the membrane-based Interactome Network Database (http://www.associomics.org/Associomics/Home.html). RLK-type proteins are indicated in bold.

Supplemental Table 3. Arabidopsis mutant and transgenic lines used in this study

		-	nt and transgenic line	
AGI	Gene	Mutant name	Stock name	Reference
	name			
At3g05360	RLP30	rlp30-1	SALK_122528	(Wang et al.,
		rlp30-2	SALK_008911	2008)
		rlp30-3	SALK_122536	
		rlp30-4	SALK_145342	
At3g05370	RLP31	rlp31-1	SALK_058586	(Wang et al.,
		rlp31-2	SALK_094160	2008)
At3g05660	RLP33	rlp33_2	SALK_087631	(Wang et al.,
		rlp33_3	SALK_085252	2008)
At5g20480	EFR	efr-1	SALK_044334	(Zipfel et al., 2006)
At5g46330	FLS2	fls2c	SAIL_691C4	(Zipfel et al., 2004)
At4g33430	BAK1	bak1-3	SALK_034532	(Schwessinger
		bak1-4	SALK_116202	et al., 2011)
		bak1-5	EMS-mutant	
At2g13790	BKK1	bkk1-1	SALK_057955	(Roux et al., 2009)
At1g73080	PEPR1	pepr1	SALK_059281	(Krol et al., 2010)
At3g21630	CERK1	cerk1-2	GK_096F09	(Miya et al 2007)
At1g17750	PEPR2	pepr2	SALK_098161	(Krol et al., 2010)
At3g52430	PAD4	pad4-1	EMS-mutant	(Jirage et al., 1999)
At3g20600	NDR1	ndr1-1	Fast-neutron-Mutant	(Century et al., 1995)
At3g48090	EDS1	eds1-1	EMS-mutant	(Aarts et al., 1998)
At1g35710	XII1	xii1-2	GK-031G02	This study
At2g24130	XII2	xii2-1	SAIL_373_E04	
At3g47090	XII3	xii3-1	SALK 101474	
At3g47110	XII4	xii4-1	SALK_101668	
At3g47570	XII5	xii5-1	GK-415H04	
At3g47580	XII6	xii6-1	SAIL_31_F02	
At4g08850	XII7	xii7-1	SALK_061769	
At2g31880	SOBIR1	sobir1-12	SALK_050715	(Gao et al.,
	(EVR)	(evr-3)		2009)
		sobir1-13	SALK_009453	(Leslie et al.,
		(evr-4)		2010)
At2g37050	LRR-	Irr-kinase	SALK_143700C	This study
	KINASE		SALK_071422C	
At5g59650	LRR-	Irr-kinase	SALK_022711C	This study
	KINASE		SAIL_1297_H07	

Marker name	Polymorphism Col-0/Lov-1	Primer name	Primer sequence (5' – 3')
F11A12	Rsal digest	F11A12-F	GATATGCAGCTGATTGCAGAAG
		F11A12-R	TCACGTCATCGACTAGCTGTTT
MSJ11	442bp/262bp	MSJ11-F	GTGCGACGTGCAAAAACTTAAA
			AG
		MSJ11-R	GAGTTGTAGATAGAGACATCAT
			GG
F21O3	Alul digest	F21O3-F	GGTGAGTTTTCATCACCAACAT
		F21O3-R	ATCAAATGGCCGTCTTTGTG
T9J14	ScrFI digest	T9J14-F	CGGAGCTGATCTCGAATTGT
		T9J14-R	ATCGGTGGTCTCTGATGGAC

# Supplemental Table 4. Primers used for map-based cloning.

Supplemental Table 5. Primers used in qPCR analyses.

		in qr on analyses.
Gene name	Primer name	Primer sequence (5' – 3')
At3g05360	RLP-fd	CATGACCAAAGGGATGCTCT
(RLP30)	RLP30-rv	ACCTCGCCAGATTCATCATC
	RLP30cyt-F	ACTGCACACAAACACGAG
	RLP30cyt-R	TCAACGAGCACTTGTGGTG
At2g19190 (FRK1)	FRK1-100-F	AGCGGTCAGATTTCAACAGT
	FRK1-100-R	AAGACTATAAACATCACTCT
At3g26830 (PAD3)	PAD3-F	CTTTAAGCTCGTGGTCAAGGAGAC
	PAD3-R	TGGGAGCAAGAGTGGAGTTGTTG
At3g04720	PR4-F	GCAAGTATGGCTGGACCGCCT
(PR-4)	PR4-R	CCAAGCCTCCGTTGCTGCATTG
At2g30770	CYP71A13-F	GTGCTTCGGTTGCATCCTTCTC
(CYP71A13)	CYP71A13-R	CGCCCAAGCATTGATTATCACCTC
At1g07920/30/40	EF1a-F	TCACATCAACATTGTGGTCATTGG
(EF-1α)	EF1a-R	TTGATCTGGTCAAGAGCCTACAG
At5g09810	Actin-fd	AGTGGTCGTACAACCGGTATTGT
(Actin)	Actin-rv	GAGGAAGAGCATACCCCTCGTA
Atcg00490	AtRubisco-QF	GCAAGTGTTGGGTTCAAAGCTGGTG
(Rubisco)	AtRubisco-QR	CCAGGTTGAGGAGTTACTCGGAATG CTG
KC748491	Scl-qPCR-F	GGATCTCTTGGTTCTGGCAT
(Sclerotinia ITS)	Scl-qPCR-R	GCAATGTGCGTTCAAAGATT
BC1G_08198	Bc_actin_qF	CCTCACGCCATTGCTCGTGT
– (Botrytis Actin)	Bc_actin_qR	TTTCACGCTCGGCAGTGGTGG

# **Supplemental Methods**

## Enzymatic, chemical and thermal treatments of the SCFE1-containing fraction

To characterize the chemical nature of elicitor activity, the SCFE1-containing fraction was incubated with different proteases. For complete protein digestion, proteinase K (Roche) was added to SCFE1-containing fractions to a final concentration of  $0.25 \ \mu g/\mu l$ . Samples were incubated of 1 h at 37 °C. For partial protein digestion, fractions were treated with the endoproteinases AspN, GlucC or trypsin (New England Biolabs) according to the manufacturer's protocols. AspN digestion was performed at 37 °C for 1 h, whereas GluC and trypsin digestions were performed at 25 °C for 1 h. To test whether glycosylation is important for elicitor activity, the SCFE1-containing fraction was incubated with a deglycosylation enzyme cocktail (New England Biolabs) in non-denaturing reaction conditions for 4 h at 37 °C according to the manufacturer's instructions. The importance of the native structure of SCFE1 for elicitor activity was investigated either by addition of SDS to a final concentration of 0.1 % or by heating at 95 °C for 10 min.

### Tricine SDS-PAGE of the SCFE1-containing fraction

Compared to the conventional Laemmli SDS-PAGE system, the Tricine SDS-PAGE is more suitable for the separation of small proteins (Schagger, 2006). Tricine SDS-PAGE was carried out by using the Mini–PROTEAN 3 system (Biorad) and discontinuous polyacrylamide gels were prepared as described (Schagger, 2006). In this study, a 12% running gel and a 4% stacking gel were used. The upper buffer chamber was filled with cathode buffer (500 ml: 6.055 g Tris base, 8.96 g Tricine and 0.5 g SDS), and the lower chamber was filled with anode buffer (500 ml: 12.11 g Tris base, pH 8.9). Active fractions containing SCFE1 were pooled and freeze-dried before being resuspended in NOVEX 2X Tricine-SDS sample buffer (10 ml: 3.0 ml 3.0 M Tris-HCl, pH 8.45, 2.4 ml glycerol, 0.8 g SDS, 1.5 ml 0.1% Coomassie Brilliant Blue G-250, 0.5 ml 0.1% Phenol Red). The SCFE1-containing sample was heated for 5 min at 95 °C and subsequently electrophoresed at 15-30 mA until the dye was eluted from the gel.

## Plant material

The transgenic *pPR-1:GUS* and *p35S:RLP30-YFP* lines and the T-DNA insertion lines for *RLP 30* (*rlp30-1*, SALK\_122528; *rlp30-2* SALK\_008911; *rlp30-3*, SALK\_122536; *rlp30-4*, SALK\_145342), *RLP31* (*rlp31-1*, SALK\_058586; *rlp31-2*, SALK\_094160), *RLP33* (*rlp33-2*, SALK\_087631; *rlp33-3*, SALK\_085252), *BAK1* (*bak1-3*, SALK\_034523; *bak1-4*, SALK\_116202; *bak1-5*, EMS-mutant), *BKK1* (*bkk1-1*, SALK\_057955), *SOBIR1* (*sobir1-12*, SALK\_009453; *sobir1-13*, SALK\_009453), *CERK1* (*cerk1-2*, GABI\_096F09) and the double mutants *bak1-5 bkk1-1*, *fls2c efr-1 and pepr1 pepr2* have been characterized previously (Shapiro and Zhang, 2001; Chinchilla et al., 2007; Miya et al., 2007; Wang et al., 2008; Gao et al., 2009; Nekrasov et al., 2009; Krol et al., 2010; Leslie et al., 2010; Roux et al., 2011; Schwessinger et al., 2011)

To generate *fls2 efr cerk1 xii* quadruple mutants, *Arabidopsis xii* single mutants in the Col-0 background were crossed with the *fls2c efr-1 cerk1-2* triple mutant (Gimenez-Ibanez et al., 2009). T-DNA insertion in *fls2c* confers resistance to BASTA and T-DNA insertion in *cerk1-2* confers resistance to sulfadiazine. F2 seeds were surface-sterilized and germinated on MS medium supplemented with 10 mg/L phosphinotricine (Duchefa) and 5.25 mg/L sulfadiazine sodium salt (Sigma). 50 mg/L kanamycin was added as a third antibiotic for the selection of seedlings carrying *xii(x-x)*. Seedlings were assayed for their responses to elf18 (growth inhibition assay, (Zipfel et al., 2006)), flg22 and chitin (Albrecht et al., 2012). Insensitive plants were subsequently genotyped for the T-DNA insertions in *fls2c*, *efr-1*, *cerk1-2* and the mutation of the family *XII* gene of interest using the primers indicated below. Primers were

designed with the iSect tool (<u>http://signal.salk.edu/tdnaprimers.2.html</u>) and Primer 3 (http://primer3.wi.mit.edu/).

Gene name	Mutant name	Mutant-ID	Primer name	Primer sequence (5' - 3')
RLP30	rlp30-1	SALK_122528	30-1LP	GAATCTGGCGTGGTGGTTCAC
(At3g05360)			30-1RP	GCCCAACTAAGTTGTTGTGG
	rlp30-2	SALK_008911	30-2LP	TCCCGACAAATGAATTCTCAC
			30-2RP	TGTCGACGAGAAGCTTAGCTC
	rlp30-3	SALK_122536	30-1LP	GAATCTGGCGTGGTGGTTCAC
			30-1RP	GCCCAACTAAGTTGTTGTGG
	rlp30-4	SALK_145342	30-4LP	TCAACTTGTTCTTCTGGTTCCG
			30-4RP	TGGACTTGGTTCGAATTCAC
FLS2	fls2c	SAIL_691_C4	FLS2-LP	GGAGACAGAACACCTTCAAGT
( <u>At5g46330</u> )			FLS2-RP	TGACCAGATTCCTCAATAGTC
EFR	efr-1	SALK_044334	EFR-LP	CCATCCCTCGCTTACATAGATTTGTC
( <u>At5g20480</u> )			EFR-RP	GCTGCAGCCACATATCCAGAC
CERK1 ( <u>At3g21630</u> )	cerk1-2	GK-096F09	CERK1- LP	AGCAACTCGGGGTGCAATGGGT
			CERK1- RP	CCTAGTCTAGACCGGCCGGACATAAG ACTGACTAAATCTTCG
XII1	xii1-2	GK-031G02	XII1-LP	GCTTTTCGAAACGTTGGAGT
( <u>At1g35710</u> )			XII1-RP	CGCCAGGAGTAGCAAACTCT
XII2	xii2-1	SAIL_373_E04	XII2-LP	TCAAGTTACACACGCGTTCAC
( <u>At2g24130</u> )			XII2-RP	ACTGAAGTTGTGGCATCTTGC
XII3	xii3-1	SALK_101474	XII3-LP	CCTGCAAAGTTTGAGAAGAAC
( <u>At3g47090</u> )			XII3-RP	GGGAGCAATTAGTCAAAGCATC
XII4 ( <u>At3g47110</u> )	xii4-1	SALK_101668	XII4-LP	ACCCCTTCCGTTGGCACAGC
			XII4-RP	GGTACCATTCCAGAGACACTTTCCA
XII5 ( <u>At3g47570</u> )	xii5-1	GK-415H04	XII5-LP	TAAACGAGATGAATGCTTCTTCACCAC AGA
			XII5-RP	GAGAACAATCTGACAGGAAGTATTCCT AC

XII6 ( <u>At3g47580</u> )	xii6-1	SAIL_31_F02	XII6-LP	AATGCTTTGTCTCCACTGGTG
			XII6-RP	AATGGTGGGTCTTGGGTTATC
XII7 ( <u>At4g08850</u> )	xii7-1	SALK_061769	XII7-LP	AACGGATCGATTCCTTCTGA
			XII7-RP	TTTTGCCTGATAGCCGATTC
SALK			LBa	TGGTTCACGTAGTGGGCCATCG
SALK			LBb1.3	ATTTTGCCGATTTCGGAAC
SAIL			LB3SAIL	TAGCATCTGAATTTCATAACCAATCTCG ATACAC
GABI			08409	ATATTGACCATCATACTCATTGC

#### Cloning of RLP30 constructs for complementation in the rlp30 mutant

*RLP30* is predicted to be a single exon, This was confirmed by sequencing full-length cDNA from Col-0 amplified using RT-PCR. The resulting cDNA was cloned into the gateway entry vector pDONR/Zeo using BP clonase (Invitrogen) and subsequently transferred to the gateway compatible binary vector pEarleyGate101 (Earley et al., 2006) using LR clonase (Invitrogen). This resulted in a plasmid where *AtRLP30* was fused to YFP-HA and expression was driven by the CaMV 35S promoter. The *rlp30-1* mutant was transformed with this plasmid using the floral dip method (Clough and Bent, 1998). Transformed plants were selected on soil soaked with 150mg/I Basta herbicide (glufosinate-ammonium, Bayer CropScience) and confirmed by PCR. Plants were checked for fluorescence using an Olympus IX70 microscope equipped with a Fluroview 300 confocal laser scanning unit. RLP30-YFP-HA fluorescence was excited with a 488nm argon laser and fluorescence detected between 510nm and 530nm (Wang et al., 2008).

*RLP30* cDNA was also cloned in the same way into the gateway compatible binary vector pEarleyGate100 (Earley et al., 2006) using LR clonase (Invitrogen). This resulted in a plasmid (RLP30<sub>cDNA</sub>) where *RLP30* was driven by the CaMV 35S promoter without any fusion for complementation study. Similarly RLP30 genomic DNA was cloned into the vector pEarleyGate100 where the gene was driven by the native promoter. Both constructs were also transformed into the *rlp30-1* and the transgenic lines were selected using Basta and confirmed by PCR.

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