

# Supporting Information

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## SI Materials and Methods

**Plant Growth Conditions for Microbe-Associated Molecular Pattern Assays.** For the microbe-associated molecular pattern (MAMP)-anthocyanin assays, seedlings were grown under constant light in liquid media containing 0.5× MS medium for 3 d and then for a further 3 d with or without the addition of 100 mM sucrose and MAMPs at the indicated concentrations. For immunoblot and gene expression analysis, seedlings were grown on agar plates containing 0.5× MS and 25 mM sucrose for 5 to 6 d under 12-h light/12-h dark conditions and then transferred to liquid media with 0.5× MS and 25 mM sucrose for an additional 5 to 6 d. For MAPK activity assays and callose deposition assays, seedlings were grown on agar plates with 0.5× MS and 25 mM sucrose for 12–14 d under 12-h light/12-h dark conditions. For reactive oxygen species (ROS) and bacterial inoculation assays plants were grown on soil under 10-h light/14-h dark conditions for 4 to 5 wk.

**Positional Cloning of PRIORITY IN SWEET LIFE6 (PSL6).** We identified the causative mutation in *psl6* by positional cloning. The mutation was linked to a 1,200-kb region on chromosome V, in which *ETHYLENE-INSENSITIVE2 (EIN2) (At5g03280)* has been earlier described as a genetic requirement for *FLAGELLIN-SENSING2 (FLS2)* expression and the receptor function (Fig. S1A) (1, 2). Sequencing of the *EIN2* locus in *psl6* mutants revealed a point substitution inducing a stop codon at the amino acid position 1140 (Fig. S1B). Consistent with the predicted truncation in the C-terminal stretch (154-aa residues) of the EIN2 protein, *psl6* as well as severely dysfunctional *ein2-1* seedlings fail to exhibit the triple response to ethylene (ET) that is characterized by root/hypocotyl elongation arrest, exaggerated apical hook tightening, and hypocotyl swelling (3) (Fig. S1C). We verified that *ein2-1* seedlings also allow an increase of anthocyanin accumulation in the presence of flg22 and elf18 (Fig. S1D). Moreover, the point substitution in *EIN2* locus cosegregates with the *psl6* mutant phenotype in a backcrossed F2 population as below. Thus, we conclude that PSL6 identifies EIN2.

### Cosegregation test of the *psl* phenotype and the corresponding mutant genotype in F2 plants of the *psl6* × Col-0 backcrossed line

Cross	Phenotype	No. of plants tested	Genotype		
			+/+	+/-	-/-
<i>psl6</i> × Col-0	<i>psl</i>	30	0	0	30
	WT	32	18	14	0

F2 plants were tested with the MAMP–sucrose cross-talk assay, as described in Fig. 1A, with the following modification: seedlings were treated with 100 mM sucrose and 200 nM flg22 for 2 d, before scoring the phenotype.

**Gene Expression Analysis.** Total RNA was isolated from whole seedlings using TRI reagent, according to the manufacturer's instruction (Ambion). cDNA was generated using oligo dT primer and used as a template for quantitative PCR analysis by a Bio-Rad iQ5 multicolor real-time PCR detection system (Bio-Rad). The expression level of genes of interest was normalized to the reference gene At4g26410 (4).

**Microarray Analysis.** Total RNA was isolated with the RNeasy Mini kit supplied with RNase-Free DNase (Qiagen) according to the manufacturer's instructions. RNA quality was assessed with RNA Nanochips on a Bioanalyzer (Agilent). Biotinylated cRNA was prepared according to a standard Ambion protocol from 1 µg total

RNA (MessageAmp II-Biotin Enhanced Kit; Ambion). After amplification and fragmentation, 12.5 µg of cRNA was hybridized for 16 h at 45 °C on GeneChip ATH1-121501 Genome Array. GeneChips were washed and stained with Fluidics Script FS450-004 in the Affymetrix Fluidics Station 450 and scanned using a GeneChip Scanner 3000 7G. The data were analyzed with Affymetrix GeneChip Operating Software version 1.4 using Affymetrix default analysis settings and global scaling for signal normalization. Probe signal values were subjected to the quantile normalization (5) and summarization using the GeneChip robust multiarray average algorithm (6), and then the expression values of the genes were determined. The results were analyzed by the following linear model using the lmFit function in the limma package in the R environment:  $\log_2(\text{expression level value}) \sim \text{sample} + \text{replicate}$ . The eBayes function in the limma package was used for variance shrinkage in calculation of the *P* values. The *q* value function in the *q* value package was used in calculation of the Storey's *q* values (7). All of the microarray data obtained in this study were submitted to the Gene Expression Omnibus (accession no. GSE40354).

Elf18-responsive genes in WT seedlings were selected based on the following criteria: at 2 h upon elf18 treatment, transcript levels were normalized to their levels at 0 h. Genes showing  $\geq$ twofold change in transcript levels ( $q \leq 0.05$ ) are defined as elf18-induced/repressed genes (Dataset S2). At 10 h upon elf18 treatment, transcript levels were normalized to those of equally treated *efr* seedlings. Genes showing  $\geq$ twofold change in transcript levels ( $q \leq 0.05$ ) are shown in Dataset S2.

EIN2-dependent genes were selected based on the following criteria: genes showing  $\geq$ twofold higher or lower transcript levels in *ein2* plants compared with those in WT plants are defined as EIN2-dependent genes at the indicated times and are listed in Dataset S3.

Hierarchical clustering analysis was performed with Genesis software (8), using complete linkage mode and default settings. Clustering was performed on 466 genes that showed EIN2-dependent regulation at 10 h after elf18 treatment. Genes that belong to the six defined clusters and those that do not belong to any of these clusters are shown in Dataset S4. Cross-referencing the selected genes to public transcriptome database was performed with Genevestigator v3 [AtGenExpress (9)].

**Immunoblot Analysis.** Total protein was extracted from whole seedlings in a lysis buffer containing 50 mM Tris-HCl (pH 7.0), 2% (wt/vol) SDS, 2 mM DTT, 1 mM 4-(2-aminoethyl) benzene-sulfonyl fluoride hydrochloride, and 1× Protease Inhibitor Mixture (Roche) and subjected to immunoblot analysis using anti-*EF-TU RECEPTOR (EFR)* or anti-*FLS2* antibodies as previously described (10). Anti-phospho p44/p42 MAPK antibody that specifically recognizes an active MAPK form was purchased from Cell Signaling Technology. The signal identity of active MAPK3 (MPK3) and MPK6 forms has been verified (10).

**Triple Response Assay.** Surface-sterilized seeds were stratified for 3–5 d at 4 °C and then planted on half-strength MS agar plates with or without 10 µM 1-aminocyclopropane-1-carboxylate (ACC). Plates were placed vertically in darkness at 22 °C for 4–5 d before taking photos of representative seedlings.

**Measurement of Ethylene Biosynthesis.** Leaf disks (1-mm thickness) sliced from mature leaves of eight 4-wk-old plants were randomly mixed and separated into three pools (three disks per

pool). The leaf preparations were kept on water overnight before incubation in a 0.4-mL solution containing elf18 or flg22 at the indicated concentrations. Ethylene concentrations were determined 4 h after elicitation by gas chromatography (Shimazu GC-14A).

**Plant Materials.** *A. thaliana* mutants for UDP-glucose glycoprotein glycosyltransferase (*uggt*) and PSL4 were previously described (10,

11). The mutant alleles *salicylic acid induction deficient2-1* (*sid2-1*), *nonexpressor of PR genes1-1* (*npr1-1*), *phytoalexin deficient4-1* (*pad4-1*), *enhanced disease susceptibility5-1* (*eds5-1*), *jasmonate resistant1-1* (*jar1-1*), *ein2-1*, *coronatine-insensitive1-1* (*coi1-1*), and the transgenic line expressing the bacterial salicylate hydroxylase NahG were used (12–19). The *ein3 ein1* mutants used were described previously (20).

#### Primers used for PCR

Gene	Forward primer	Reverse primer	Purpose
<i>PATHOGENESIS-RELATED GENE1</i> ( <i>PR1</i> )	GGTAGCGGTGACTTGTCTGG	AAGCCCACCAGAGTGTATG	Real-time RT-PCR
<i>EFR</i>	CGGATGAAGCAGTACGAGAA	CCATTCTCTGAGGAGAACCTTG	Real-time RT-PCR
<i>FLS2</i>	GCGAAACAGAGCTTTGAACC	GTGTCGTAACGAACCGATGA	Real-time RT-PCR
<i>ETHYLENE-RESPONSIVE FACTOR1</i> ( <i>ERF1</i> )	TCGGCGATTCTCAATTTTTT	ACAACCGGAGAACAACCATC	Real-time RT-PCR
<i>MYB DOMAIN PROTEIN51</i> ( <i>MYB51</i> )	ACAAATGGTCTGCTATAGCT	CTTGTGTGTAAGTGGATCAA	Real-time RT-PCR
<i>ELICITOR PEPTIDE PRECURSOR2</i> ( <i>PROPEP2</i> )	AGAAAAGCCTAGTTCAGGTCGTC	CTCCTTATAAAGTGTATTGCCGC	Real-time RT-PCR
<i>PROPEP3</i>	GTTCCGGTCTCGAAGTTCATC	ATCTTCTCGCTGTGTGATGAC	Real-time RT-PCR
<i>VEGETATIVE STORAGE PROTEIN2</i> ( <i>VSP2</i> )	TCAGTGACCGTTGGAAGTTGTG	GTTCGAACCATTAGGCTTCAATATG	Real-time RT-PCR
<i>PEP1 RECEPTOR1</i> ( <i>PEPR1</i> )	CAACAACAATGTGGAGGATA	AACGAGATTACCGAAGTCAA	Real-time RT-PCR
<i>AT4G26410</i>	GAGCTGAAGTGGCTTCCATGAC	GGTCCGACATACCCATGATCC	Real-time RT-PCR
<i>PSL6</i>	AAGCTGAAGCTCGAGAAATAAACC	TGAGAGACAAGTCAAGGACACGGTGAATGCAT	CAPS-marker for <i>psl6</i> genotyping; restriction enzyme: MseI

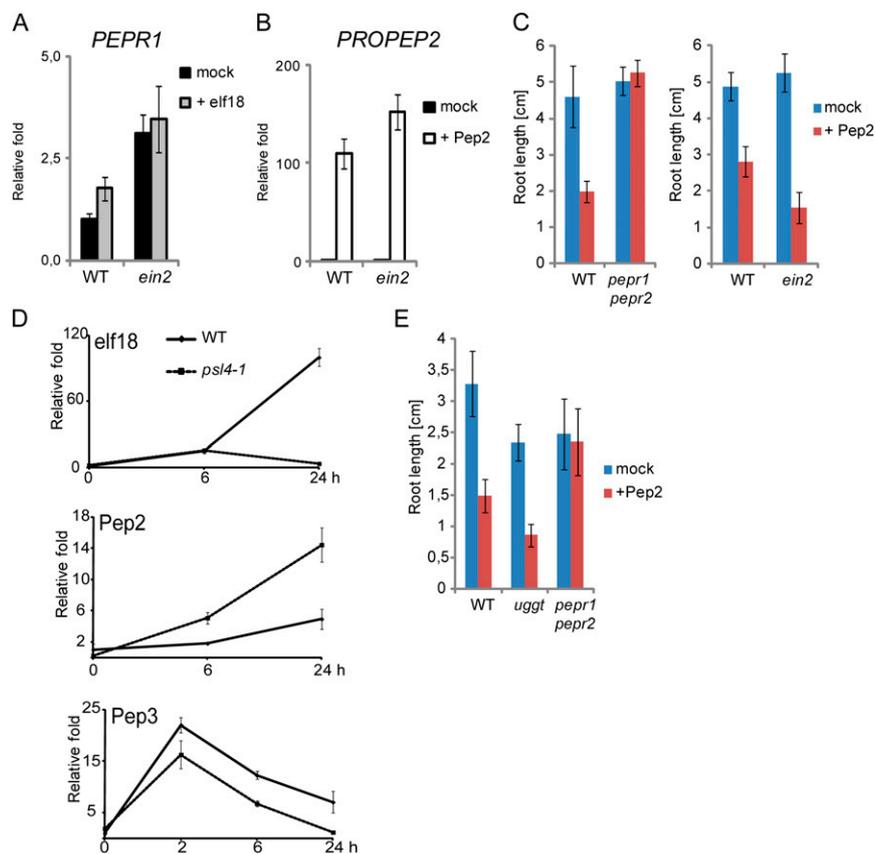
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**Fig. S6.** Retention of PEPR-triggered outputs in *ein2* and two endoplasmic reticulum (ER) folding pathway mutants. (A) *PEPR1* expression in seedlings treated with water (mock) or 1  $\mu$ M elf18 for 24 h. (B) *PROPEP2* expression in seedlings treated with water (mock) or 1  $\mu$ M Pep2 for 1 h. (C) Pep2-mediated root growth inhibition in seedlings exposed to 100 nM Pep2 for 7 d. The SD of  $n \geq 15$  is shown. (D) Ten-day-old WT and *psl4-1* seedlings treated with 1  $\mu$ M of the indicated elicitors were tested at the indicated time points for *PROPEP3* expression. Quantitative RT-PCR results are shown as described in Fig. S3 A, B, and D. (E) Pep2-mediated root growth inhibition in seedlings upon treatment with 100 nM Pep2 for 7 d. The SD of  $n \geq 15$  is shown. The *psl4-1* and *uggt* mutants carry severely dysfunctional alleles for the ER lumen enzymes glucosidase II ( $\beta$ -subunit) and UDP-glucose:glycoprotein glycosyltransferase, respectively (1, 2). The *uggt* mutant (SALK008557) carries a T-DNA insertion in the gene body and was selected on the basis of elf18-insensitive anthocyanin accumulation in seedlings. *ein2-1* and *pepr1-1 pepr2-3* were used.

1. Saijo Y, et al. (2009) Receptor quality control in the endoplasmic reticulum for plant innate immunity. *EMBO J* 28(21):3439–3449.
2. Lu X, et al. (2009) Uncoupling of sustained MAMP receptor signaling from early outputs in an *Arabidopsis* endoplasmic reticulum glucosidase II allele. *Proc Natl Acad Sci USA* 106(52): 22522–22527.

**Table S1. Responsiveness of EFR-regulated genes to SA, JA (MeJA) or ET (ACC) application**

Parameter	No. genes	Data source
EFR activated	1,932	This study
EFR repressed	2,195	This study
SA induced (3 h)	1,201	Genevestigator v3 (Exp. ID AT-00113)
SA induced (24 h)	2,051	Genevestigator v3 (Exp. ID AT-00320)
SA repressed (3 h)	648	Genevestigator v3 (Exp. ID AT-00113)
SA repressed (24 h)	2,040	Genevestigator v3 (Exp. ID AT-00320)
SA induced (3 h)	262	Genevestigator v3 (Exp. ID AT-00110)
SA induced (24 h)	232	Genevestigator v3 (Exp. ID AT-00110)
EFR activated and SA induced (3 h)	632	$P < 2.2e-16$
EFR activated and SA induced (24 h)	976	$P < 2.2e-16$
EFR activated and SA repressed (3 h)	37	$P = 0.9971$
EFR activated and SA repressed (24 h)	79	$P = 1$
EFR repressed and SA induced (3 h)	29	$P = 1$
EFR repressed and SA induced (24 h)	40	$P = 1$
EFR repressed and SA repressed (3 h)	203	$P < 2.2e-16$
EFR repressed and SA repressed (24 h)	920	$P < 2.2e-16$
EFR activated and ACC induced	88	$P < 2.2e-16$
EFR activated and ACC repressed	23	$P = 0.2442$
EFR repressed and ACC induced	12	$P = 0.9992$
EFR repressed and ACC repressed	93	$P < 2.2e-16$
EFR activated and MeJA induced	184	$P < 2.2e-16$
EFR activated and MeJA repressed	158	$P < 2.2e-16$
EFR repressed and MeJA induced	111	$P = 4.369e-4$
EFR repressed and MeJA repressed	177	$P < 2.2e-16$
Cluster 1 (127 genes)		
SA induced (3 h)	26	$P = 2.06e-09$
SA induced (24 h)	73	$P < 2.2e-16$
Cluster 2 (58 genes)		
SA induced (3 h)	21	$P = 6.138e-13$
SA induced (24 h)	20	$P = 7.051e-8$
MeJA induced	13	$P = 1.521e-7$
Cluster 3 (30 genes)		
ACC induced	18	$P < 2.2e-16$
Cluster 4 (36 genes)		
ACC induced	6	$P = 3.164e-6$
MeJA repressed	18	$P < 2.2e-16$
Cluster 5 (125 genes)		
ACC repressed	35	$P < 2.2e-16$
MeJA induced	26	$P = 6.454e-13$
Cluster 6 (60 genes)		
ACC repressed	13	$P = 3.012e-14$
MeJA induced	10	$P = 6.486e-5$

Elf18, SA, MeJA, or ACC responsive genes were selected by setting a cutoff of twofold induction or repression compared with a negative control (*efr* seedlings or mock treatment). The number of total genes (gene probes) tested is 22,810 for all these studies.  $P$  values calculated with Fisher exact test.

