Signalling of *Arabidopsis* Response to *Pieris brassicae* eggs shares similarities with PAMP-triggered immunity.

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Supplementary Data

Figure S1. EDS1 is required for egg-induced expression of SA-signalling genes. Relative expression levels of *ICS1* (At1g74710), *TRX-H5* (At1g45145), *TI* (At1g73260), and *CHIT* (At2g43570) were analysed in *Arabidopsis* by QPCR. Leaves of Col-0 and *eds2-1* plants were treated with 2 μl of *Pieris brassicae* egg extract for 24 h before RNA extraction. Expression levels were normalized with respect to the housekeeping gene *EIF4A* (At3g13920). Data bars represent the mean (±SE) of three technical repeats. This experiment was repeated once with similar results.

Figure S2. Egg-induced ROS and cell death accumulation in *rbohD* and *rbohF* mutants. Leaves from Col-0, *rbohD*, *rbohF*, and the double mutant *rbohD/F* were treated with 2 μl of *P. brassicae* egg extract for 72 h. Histochemical staining of leaves with nitroblue tetrazolium (NBT) to detect O_2^- (A), 3,3-diaminobenzidine (DAB) to detect H_2O_2 (B), and trypan blue to detect cell death (D) was performed. Control plants (CTL) were not treated and stained after 72 h. For all stainings, leaves from three to five different plants per genotype were used. Panels are close-up photographs of the spotted area. This experiment was repeated twice with similar results. All images were taken with the same magnification. Bar = 1 mm.

Figure S3. Quantification of ROS and cell death accumulation in response to egg extract treatment. Different plant genotypes were treated with 2 μl of *P. brassicae* egg extract for 72 h. Histochemical staining of leaves with nitroblue tetrazolium (NBT) to detect O_2^-, 3,3-diaminobenzidine (DAB) to detect H_2O_2, and trypan blue (TB) to detect cell death was performed. Microscope images were saved as TIFF files and the stained area was measured with ImageJ software. For each genotype, at least 5 leaves were analyzed. Error bars represent SE. Asterisks indicate statistically significant differences compared with Col-0 (Student's *t* test, *P<0.05, **P<0.01, ***P<0.001)

Figure S4. Purified egg extracts activate the expression of reporter genes. Two µl of crude egg extract, total egg lipids, and a fraction eluting at 100% MeOH from a from solid phase extraction (SPE) column were applied onto leaves of *Arabidopsis* promoter::GUS lines. DMSO (10%) was used as a control. GUS expression was analysed by histochemical staining 72 h after treatment. *PR-1*, *TI* (trypsin inhibitor, At1g73260), and *SAG13* (At2g29350) are egg-inducible genes. All images were taken with the same magnification. Bar = 1 mm

Figure S5. Purified egg extracts activate the expression of early PAMP-responsive genes. Plants were treated with two µl of a fraction eluting at 100% MeOH from a from solid phase extraction (SPE) column for 3 and 9 h before RNA extraction. Relative expression levels of *FRK1* (At2g19190), *CYP81F2* (At5g57220), *NHL10* (At2g35980), and *PHI1* (At1g35140) were analysed in *Arabidopsis* by QPCR. Expression levels were normalized with respect to the housekeeping gene *EIF4A* (At3g13920). Data bars represent the mean (±SE) of three technical repeats. This experiment was repeated once with similar results.

Figure S6. Egg viability in SA signalling mutants. (A) Hatching rate was monitored five days after *P. brassicae* oviposition on different *Arabidopsis* genotypes. Data represent the mean (±SE) of three independent experiments. The average number of eggs laid on each genotype was 681 ± 210 (Col-0), 532 ± 195 (*eds1-2*), and 369 ± 52 (*nudt7-1*). (B) Thirty freshly hatched *P. brassicae* larvae originating form eggs laid on Col-0, *eds2-1* and *nudt7-1* plants were placed on five-week-old Col-0 plants and larval weight (mean ±SE) was measured after 7 days of feeding. This experiment was repeated twice with similar results.

Figure S1

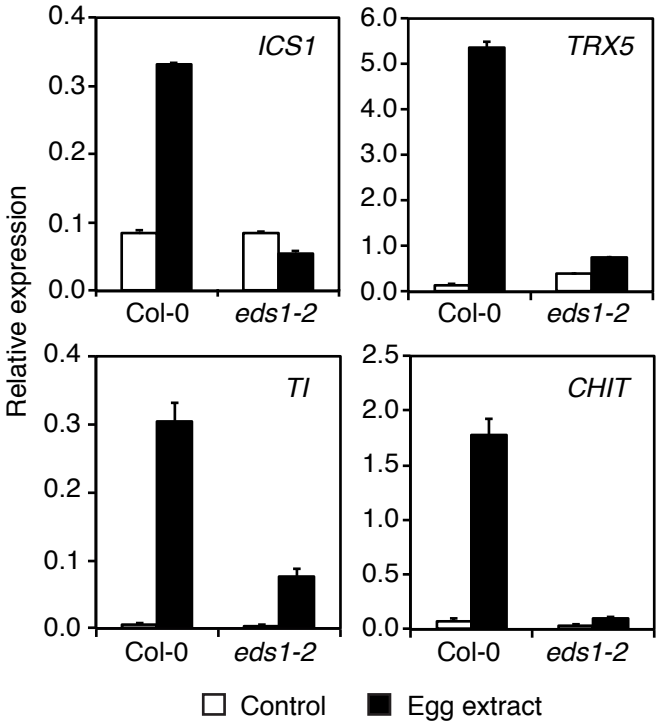
Relative expression
ICS1
TRX5
TI
CHIT
Col-0
eds1-2
Control
Egg extract

Figure S2

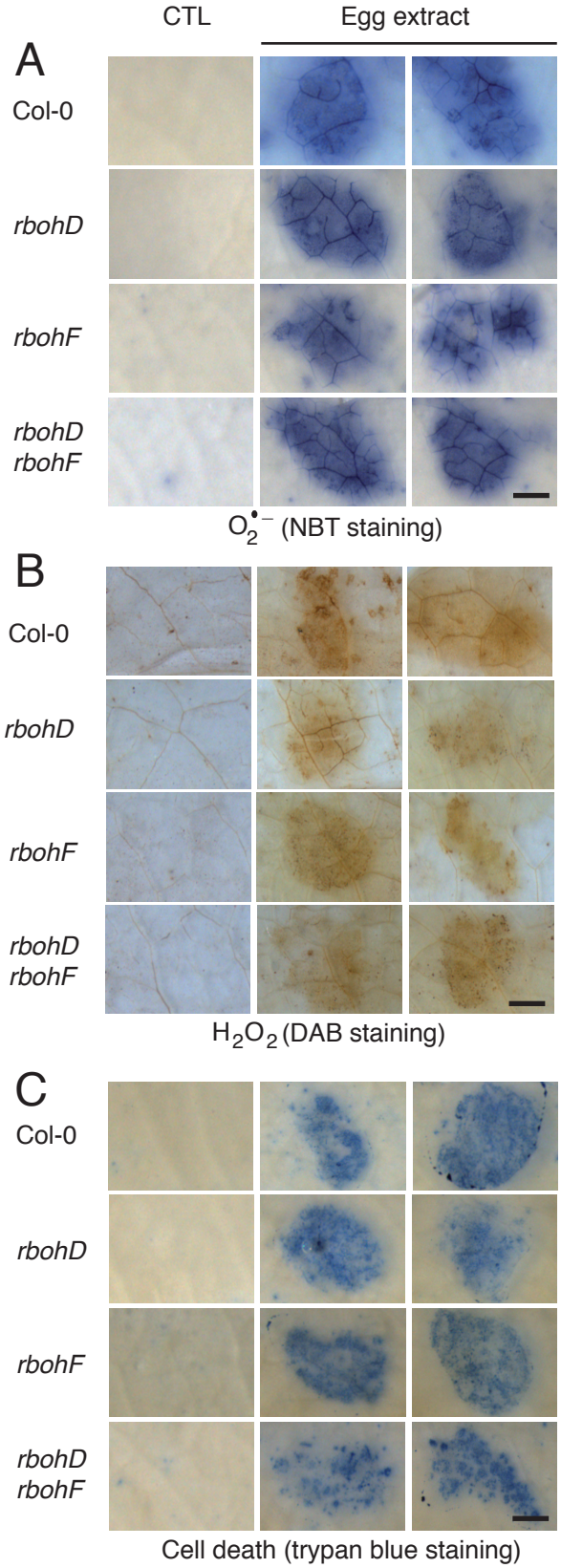
CTL
Egg extract
A
Col-0
rbohD
rbohF
rbohD
rbohF
$O_2^{\bullet -}$ (NBT staining)
B
Col-0
rbohD
rbohF
rbohD
rbohF
H_2O_2 (DAB staining)
C
Col-0
rbohD
rbohF
rbohD
rbohF
Cell death (trypan blue staining)

Figure S3

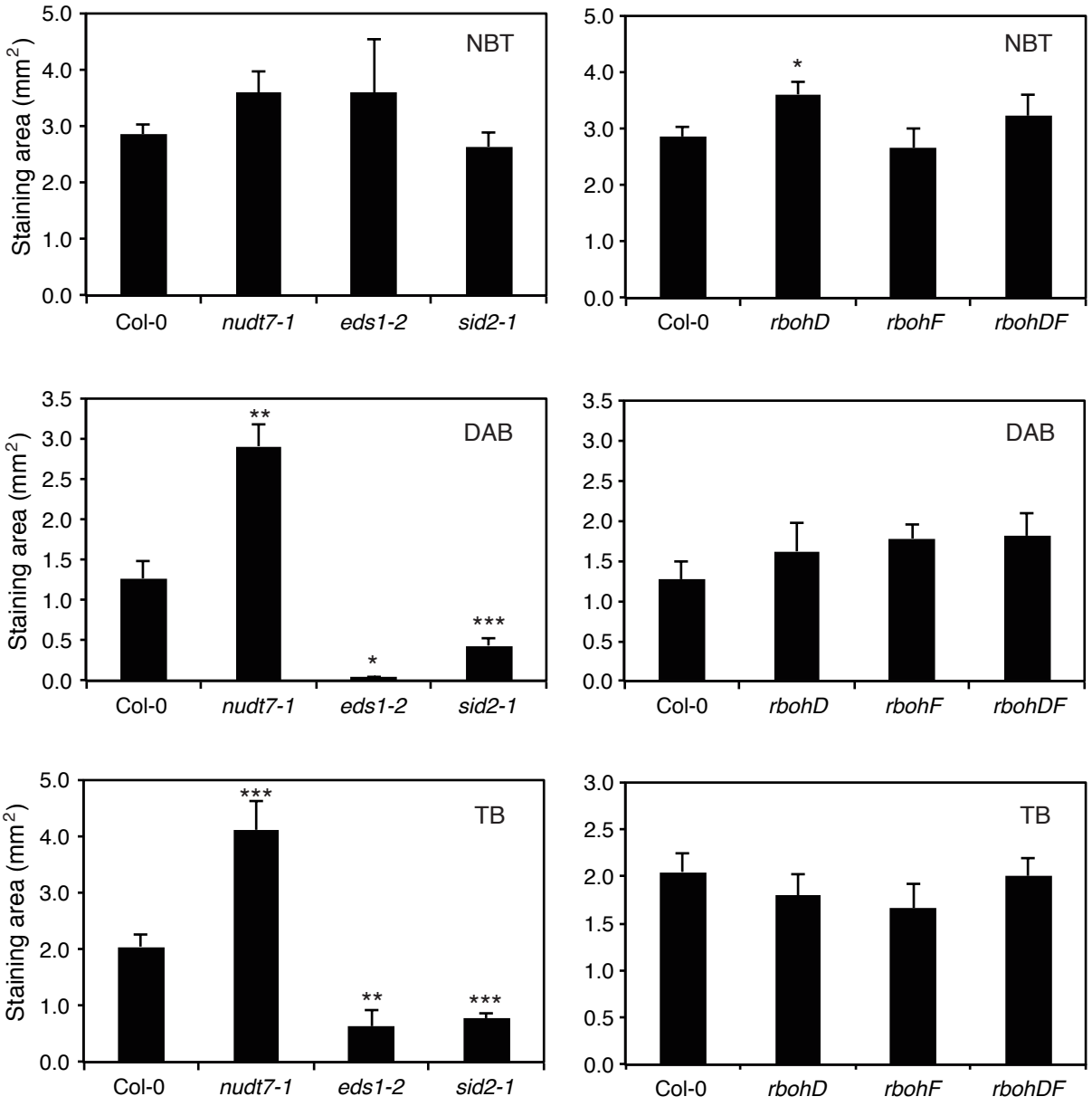
NBT
Staining area (mm2)
5.0
4.0
3.0
2.0
1.0
0.0
Col-0
nudt7-1
eds1-2
sid2-1
*
rbohD
rbohF
rbohDF
DAB
3.5
3.0
2.5
2.0
1.5
1.0
0.5
**
***
TB

Figure S4

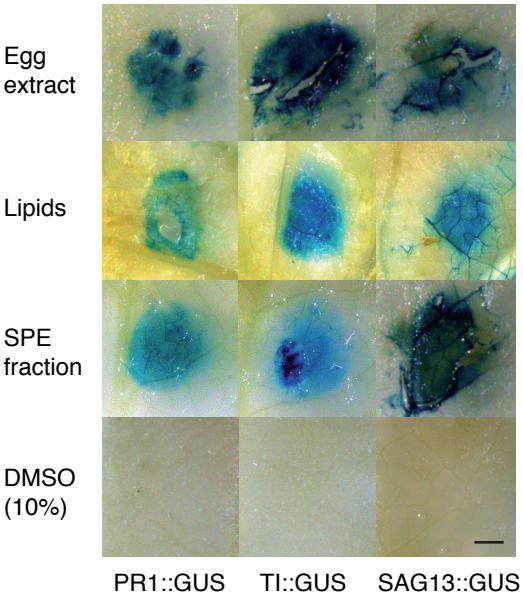
Egg extract
Lipids
SPE fraction
DMSO (10%)
PR1::GUS
TI::GUS
SAG13::GUS

Figure S5

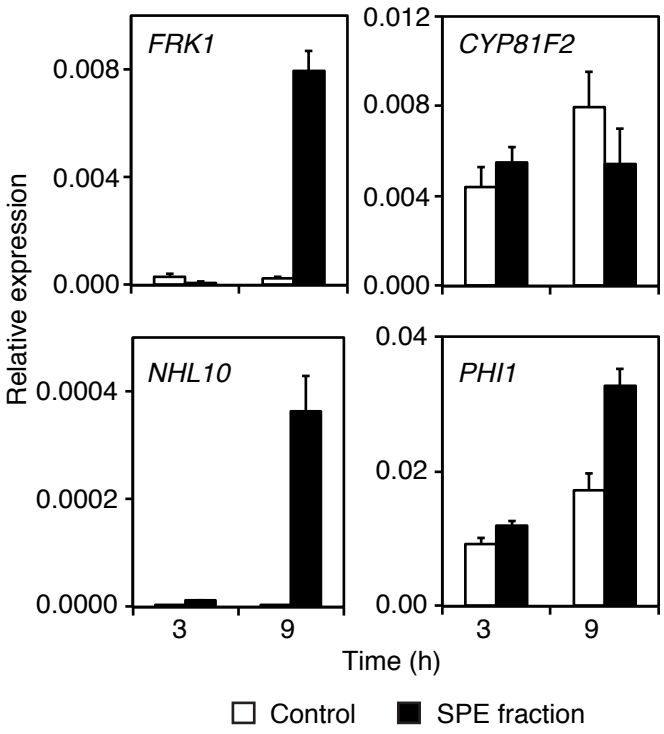
FRK1
CYP81F2
NHL10
PHI1
Relative expression
0.008
0.004
0.000
0.012
0.008
0.004
0.000
0.0004
0.0002
0.0000
0.04
0.02
0.00
3
9
3
9
Time (h)
Control
SPE fraction

Figure S6

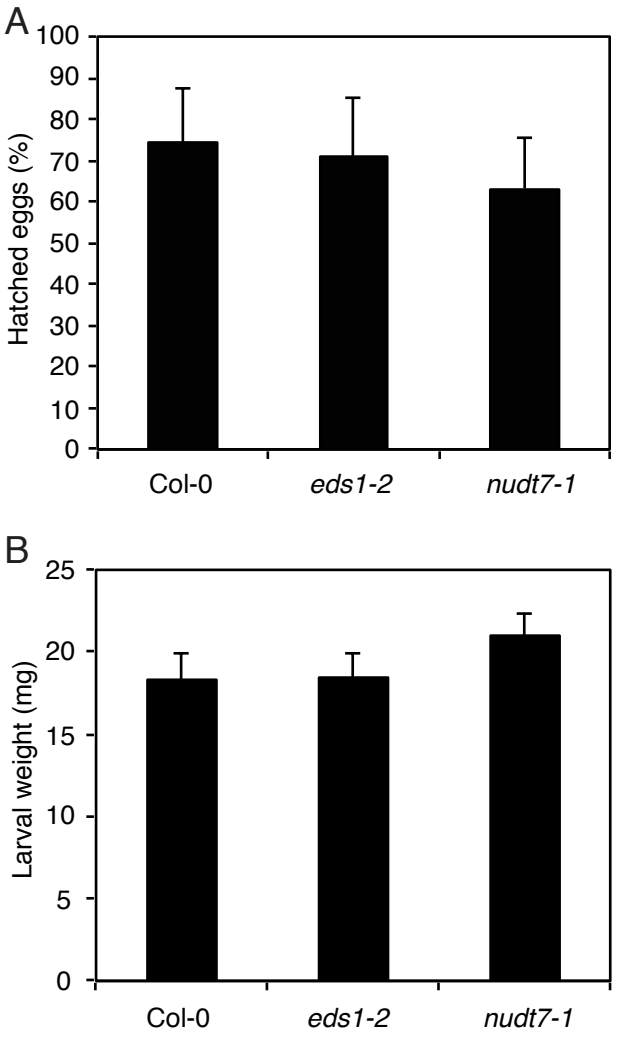
A
Hatched eggs (%)
100
90
80
70
60
50
40
30
20
10
0
Col-0
eds1-2
nudt7-1
B
Larval weight (mg)
25
20
15
10
5
0
Col-0
eds1-2
nudt7-1

Table S1. List of primers used in this study

Gene	AGI	Primer	Sequence (5' - 3')
CHIT	At2g43570	CHIT-Fw	GGAGAGTACTGCGACACAGAGAAA
		CHIT-Rev	GGCAGGAACCTGGTCTTGAGCAA
CYP81F2	At5g57220	CYP81F2-Fw	TATTGTCCGCATGGTCACAG
		CYP81F2-Rev	CTCGCACCACTGTTGTCATT
EIF4A	At3g13920	EIF4A-Fw	CCAGAAGGCACACAGTTTGAT
		EIF4A-Rev	AGACTGAGCCTGTTGAATCAC
FRK1	At2g19190	FRK1-Fw	TACGGCTCTTGTTGAACACT
		FRK1-Rev	TCACTATACGCGGTGTCCAT
ICS1	At1g74710	SID2c-Fw	GGACTCAATTAGGTGTCTGC
		SID2c-Rev	AAGCCTTGCTTCTTCTGCTG
NHL10	At2g35980	NHL10-Fw	GCTGCTGAACAACCTCTCAA
		NHL10-Rev	CTACGCCGAGGATGACAATA
PHI1	At1g35140	PHI1-Fw	TGTGTTTGGATGCGAGAACG
		PHI1-Rev	ATCGGTTACGATTGCACGCT
PR1	At2g14610	PR1-Fw	GTGGGTTAGCGAGAAGGCTA
		PR1-Rev	ACTTTGGCACATCCGAGTCT
TI	At1g73260	TI-Fw	CCTCGTGGTTGCTGGTCCAAA
		TI-Rev	CCTCTCACATAGTCTTGGACGAAA
TRX-H5	At1g45145	TRXh5-Fw	AGTGATTGCTTGCCATACCC
		TRXh5-Rev	CTGCAAACACTGGTGCAATG
LecRK-I.8	At5g60280	LP	ACACCACAACCAACAGGTCTC
		RP	CAGAAGCTGTTTCTCAATCGG