

1 ***Local jasmonic acid cues drive systemic acquired resistance signal generation***

2

3 Jennifer Sales¹, Elisabeth Pabst¹, Marion Wenig¹, Heiko H. Breitenbach¹, Gerardo
4 Perez¹, Claudia Knappe¹, Richard Hammerl², Jinghui Liu³, Wilfried Rozhon⁴, Brigitte
5 Poppenberger⁴, Erwin Grill³, Corinna Dawid², and A. Corina Vlot^{1*}

6

7 ¹Helmholtz Zentrum Muenchen, Department of Environmental Science, Institute of
8 Biochemical Plant Pathology, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany;

9 ²Technical University Munich, Chair of Food Chemistry and Molecular Sensory
10 Science, Lise-Meitner-Str. 34, 85354 Freising; ³Technical University Munich, Chair of
11 Botany, Emil-Ramann-Str. 4, 85354 Freising; ⁴Technical University Munich, TUM
12 School of Life Sciences Weihenstephan, Biotechnology of Horticultural Crops, Liesel-
13 Beckmann-Str. 1, 85354 Freising

14

15 *To whom correspondence should be addressed: [corina.vlot@helmholtz-
muenchen.de](mailto:corina.vlot@helmholtz-
16 muenchen.de), +49-89-31873985

17

18 Running title: SA-JA cross talk drives systemic immunity

19

20 Key words: plant defence, systemic acquired resistance, salicylic acid, jasmonic acid,
21 cross talk

22

23 Word counts:

24 Abstract: 148

25 Main text: 2999

26 Methods: 1495

27

1 **Abstract**

2 The phytohormones salicylic acid (SA) and jasmonic acid (JA) promote two, mutually
3 antagonistic immune pathways respectively protecting plants from biotrophic
4 pathogens and necrotrophic pathogens or insects. This trade-off largely precludes
5 the exploitation of SA and JA immune components for crop protection, raising the
6 interest in immune signalling components that disrupt SA-JA antagonism. A local
7 pathogen infection primes SA-dependent immunity in systemic tissues. This so-called
8 systemic acquired resistance (SAR) ensures a long-lasting, broad-spectrum disease
9 resistance that is not subject to SA-JA antagonism. Here, we show that two
10 sequence-related LEGUME LECTIN-LIKE PROTEINs (LLPs) promote SAR through
11 spatially separated functions with JA promoting local SAR signal generation through
12 LLP3. In concert with LLP1, which is important for systemic recognition and
13 propagation of SAR signals, LLP3 promotes both SA-dependent SAR and JA-
14 mediated immunity. Thus, exploitation of LLP-associated signalling cues might allow
15 application of plant innate immune signals to promote (crop) plant health.

16

17 **Introduction**

18 As plants lack the dedicated immune cells and complex homeostatic systems that are
19 found in animals, they developed alternative strategies of dealing with stress. An
20 important aspect of this is the action of phytohormones and their associated
21 signalling pathways. A key threat to plants comes from biotrophic or hemibiotrophic
22 pathogens, which are fended off through salicylic acid (SA)-dependent responses
23 induced at the site of infection. Pathogen-associated molecular patterns (PAMPs)
24 from virulent pathogens are recognised by Pattern Recognition Receptors (PRRs)
25 that are localised at the plasma membrane and initiate PAMP-triggered immunity
26 (PTI)¹. Effectors from avirulent pathogens are recognised by intracellular nucleotide-
27 binding domain and leucine-rich repeat proteins (NLRs), and initiate the relatively
28 stronger effector-triggered immunity (ETI)². Both PTI and ETI responses rely on SA.
29 *ENHANCED DISEASE SUSCEPTIBILITY1* (*EDS1*) acts as a central regulator
30 upstream of SA driving a positive feedback loop with SA to fortify defence^{3,4}. Once
31 local SA cascades are triggered, a systemic signal is generated to upregulate
32 defence in distal plant parts. This broad spectrum response is known as Systemic
33 Acquired Resistance (SAR). *EDS1* is required for a successful SAR response, as

1 evidenced by defects in both SAR signal generation and recognition in *eds1* mutant
2 plants⁵.

3 During ETI, LEGUME LECTIN-LIKE PROTEIN1 (LLP1) accumulates in apoplast-
4 enriched extracts of the model plant *Arabidopsis thaliana* in an EDS1-dependent
5 manner⁵. *LLP1* is essential for SAR, primarily functioning in the systemic tissue in
6 SAR signal perception or propagation^{5,6}. LLP2 (At3g16530), which shares 66%
7 similarity at the amino acid (AA) level with LLP1, was identified as a possible SAR-
8 associated protein along with LLP1⁵. LLP1 and LLP2 respectively share 61% and
9 87% AA similarity with LLP3 (At3g15356; LECTIN in⁷). LLP2 and LLP3 are induced
10 at the transcriptional level by chitin and jasmonic acid (JA), respectively^{7,8}, but their
11 physiological roles remain unknown.

12 For a functional SAR response to occur, two interconnected signalling pathways are
13 required^{9,10}. The first of these pathways is primarily associated with SA^{4,11}. The
14 second involves pipecolic acid (Pip), and its presumed bioactive derivative *N*-
15 hydroxy-pipecolic acid (NHP)¹²⁻¹⁴. LLP1 has a key role in the latter cascade acting
16 downstream of Pip and upstream of SAR-associated volatile signals to propagate
17 SAR-associated immunity in systemic tissues⁶.

18 When considering plants in complex natural systems, biotrophic defence signalling
19 cascades associated with SA interact with other stress response pathways. These
20 include abiotic stress responses associated with abscisic acid (ABA) and defence
21 against necrotrophic pathogens and insects controlled by JA¹⁵⁻¹⁷. In order to fine tune
22 defence, and optimise resource allocation, there is general antagonism between the
23 three pathways¹⁸. Studies in *Arabidopsis*, for example, showed that after SA
24 defences were activated by a hemibiotrophic pathogen, *Alternaria brassicicola*, plants also became more
25 susceptible to a necrotrophic pathogen, *Alternaria brassicicola*, pointing towards a
26 down-regulation in JA-mediated defences^{19,20}. Interestingly, the same studies found
27 that this antagonism was restricted to the infected tissues and did not spread
28 systemically during SAR. Recent evidence further convolutes the role of JA in SA-
29 mediated defence which appears to be highly dependent on concentration, spatial
30 distribution, and circadian rhythm²¹⁻²³.

31 It is common for plants to be challenged by abiotic factors at the same time that they
32 are under pathogen attack. Indeed, as climates shift, not only are traditional crops
33 placed under greater stress from factors such as drought and salinity, but also from

1 emerging infectious diseases and existing pathogens that have expanded their
2 geographical range^{24,25}. This threat to crop security increases the necessity for
3 knowledge of interactions between the different stress pathways. Here we show that
4 *LLP1* and *LLP3* have novel functions in multiple stress responses, and harbor
5 significant potential for engineering multi-stress tolerance in plants.

6

7 **Results**

8 *LLP3* is essential for local SAR signal generation

9 SA is a key component for plant defence against biotrophic pathogens, in both local
10 and systemic tissues. Here, both Col-0 wild type and *eds1-2* knockout plants were
11 spray-treated with 1 mM SA. After 24 h, the levels of *LLP1* transcripts were induced,
12 and this effect was not dependent on *EDS1* (Fig. 1A;⁵). In contrast to Lyou et al.⁷,
13 who detected a slight reduction in *LLP3* transcript abundance after treatment of
14 plants with 50 µM SA, we did not observe a reproducible down regulation of *LLP3*.
15 Similarly, *LLP2* transcript levels did not significantly change in response to SA in
16 either genotype. Similar results were seen if plants were treated with the SA
17 analogue 1,2,3-benzothiadiazole-7-carbothioic acid S-methyl ester (BTH;
18 Supplementary Fig. 1). Thus, *LLP1* transcript accumulation, and not that of its
19 homologues *LLP2* and *LLP3*, is regulated by SA, and this regulation is independent
20 of *EDS1*.

21 Reduced transcript accumulation of *LLP1*, *LLP2*, and *LLP3* in *RNAi:LLP1-3* plants
22 compromises the ability of the plants to generate or transmit phloem-mobile SAR
23 signal(s)⁶. Because we have so far been unable to generate viable *llp2* mutant plants,
24 we focused on *LLP3*, whose transcript accumulation was reduced in an *llp3* T-DNA
25 mutant (Supplementary Fig. 2). To analyze SAR, these plants were initially infiltrated
26 in two leaves with either *Pst* *AvrRpm1* or a 10 mM MgCl₂ mock control solution.
27 Three days later, two leaves distal to the initial infection were infiltrated with virulent
28 *Pst*. After another four days, the resulting *in planta* *Pst* titres were determined. In
29 wildtype plants, a local *Pst* *AvrRpm1* infection reduced *Pst* growth in the systemic
30 tissues compared to that in mock-treated plants, indicating the establishment of SAR
31 (Fig. 1B). SAR was fully abolished in *llp3* mutant plants (Fig. 1B). Ectopic expression
32 of a wildtype copy of *LLP3* driven by its native promoter in the *llp3* mutant
33 background (*llp3-LLP3:LLP3*) raised *LLP3* transcript accumulation to intermediate

1 levels between that of wildtype and *llp3* mutant plants (Supplementary Fig. 2). This
2 complemented the SAR-defective phenotype of the *llp3* mutant (Fig. 1B), ascribing
3 *LLP3* an essential role in SAR.

4 We next tested if *LLP3* acts locally or systemically in SAR by using petiole exudates
5 (PEX) from *Pst AvrRpm1*-inoculated and mock-treated plants. 24 Hours (h) after
6 infiltration of these PEX in recipient plants, the treated leaves were inoculated with
7 *Pst* and the resulting *Pst* titers monitored at 4 days post-inoculation (dpi). PEX from
8 infected wildtype plants reduced *Pst* growth in wildtype recipient plants as compared
9 to PEX from mock-treated wildtype plants (Fig. 1C). Similarly, *llp3* recipient plants
10 responded with reduced *Pst* growth to PEX from infected wildtype plants, suggesting
11 that *LLP3* is not involved in systemic recognition or propagation of SAR signal(s). In
12 contrast, PEX from infected *llp3* donor plants did not reduce *Pst* growth in wildtype
13 recipient plants (Fig. 1C), suggesting that *LLP3* is necessary for local SAR signal
14 generation or transmission. This spatially separates the role in SAR of *LLP3* from that
15 of its sequence-related homolog *LLP1*, which acts systemically in SAR⁶.

16

17 *LLP1-3* influence responses to abiotic stress

18 Because *LLP3* did not show a significant response to SA treatment, but nevertheless
19 influenced SAR, we questioned if *LLP3* might be regulated by phytohormones other
20 than SA. Yasuda et al.²⁶ showed that ABA and ABA-dependent responses to salinity
21 stress compromised SA signalling and potentially SAR. In order to investigate
22 whether ABA had an impact upon the transcript levels of *LLP1*, *LLP2*, and *LLP3*,
23 plants were spray-treated with 100μM ABA and tissues were harvested 24 h later. In
24 both Col-0 wild type and *eds1-2* plants, *LLP1* transcript levels were significantly
25 downregulated, while there was no change in transcript levels of either *LLP2* or *LLP3*
26 (Figure 2A). Thus, ABA downregulates *LLP1* transcript accumulation independently
27 of *EDS1*.

28 ABA is an important phytohormone in abiotic stress signalling. Therefore, it seemed
29 possible that if *LLP1* was transcriptionally regulated by ABA, *llp1-1* mutant and
30 *RNAi:LLP1-3* plants may show an altered phenotype under abiotic stress. To test for
31 aberrant reactions to high salinity, seedlings were germinated and after 6 days
32 transferred to treatment plates with 100mM NaCl. The length of the primary roots was
33 measured at 6 and 12 days post transfer and normalised to those on control plates

1 (to which seedlings had also undergone transfer). While *lfp1-1* plants had marginally
2 longer roots than wildtype on control plates (Supplementary Fig. 3), the plants of all
3 genotypes showed a significant reduction in root length when grown on salt
4 compared to control conditions. Notably, both *lfp1-1* and *RNAi:LLP1-3* plants showed
5 more pronounced salt-induced root growth inhibition as compared to wildtype plants,
6 and *RNAi:lfp1-3* was significantly more affected than *lfp1-1* (Fig. 2B).

7 To test for a possible contribution of *LLP3* to salt-induced root shortening, we
8 transferred *lfp3* seedlings and those of two *lfp3-LLP3:LLP3* complementation lines to
9 treatment plates with 100 mM NaCl. Similar to *lfp1-1*, *lfp3* mutants displayed
10 exaggerated root growth inhibition under salt stress, and this phenotype was
11 complemented by ectopic expression of *LLP3:LLP3* (Fig. 2C). This might be
12 associated with changes in ABA responses, because ABA-induced root shortening
13 was also exaggerated in the *lfp3* mutant, but not in *lfp3-LLP3:LLP3* complementation
14 lines (Fig. 2D). However, ABA-induced root shortening was only moderately changed
15 in *lfp1-1* and not changed in *RNAi:LLP1-3* plants compared to wild type
16 (Supplementary Fig. 4A). Also, ABA-induced transcript accumulation of the ABA
17 marker gene *RAB18* was the same in all genotypes (Supplementary Fig. 4B/C).
18 Therefore, a contribution of ABA to *LLP*-associated root shortening is probably minor.
19 The absence of ABA-associated phenotypes was further supported by the response
20 of *RNAi:LLP1-3* plants to progressive drought. There was no physiologically relevant
21 or significant difference between *RNAi:LLP1-3* and wild type plants when water
22 consumption and water use efficiency (WUE) under progressive drought, were
23 examined (Supplementary Fig. 5). We therefore posit that the increased sensitivity of
24 the *RNAi:LLP1-3* lines to high salinity is most likely mechanistically independent of
25 ABA.

26

27 *LLP3* responds to MeJA and affects JA-mediated responses

28 Another candidate pathway that has been shown to be involved in both biotic
29 defence and salt stress tolerance is the JA pathway²⁷. Also, MeJA treatment has
30 been associated with increased *LLP3* transcript levels⁷. Here, spray treatment of
31 Arabidopsis with 100 µM MeJA increased accumulation of *LLP3* transcripts in both
32 Col-0 and *eds1-2* plants, suggesting that MeJA induces *LLP3* in an *EDS1*-
33 independent manner (Fig. 3A). The accumulation of *LLP1* and *LLP2* transcripts was

1 not significantly changed by MeJA. We next investigated whether the reduction of
2 LLP3 in *RNAi:LLP1-3* plants would affect JA-mediated defence against a
3 necrotrophic pathogen. Indeed, lesions induced by *A. brassicicola*, a necrotrophic
4 fungus, were larger in *RNAi:LLP1-3* plants than in wildtype plants and similar
5 phenotypes were observed in *lfp1-1* and *lfp3* mutant plants (Fig. 3B/C). This shows
6 that LLP1, LLP2, and/or LLP3 promote defence against necrotrophic pathogens and
7 thus potentially normal JA signalling under biotic stress.

8 We subsequently tested if compromised JA signalling could have been responsible
9 for the root growth inhibition phenotype of the *RNAi:LLP1-3* seedlings on salt. To this
10 end, we again used the root growth inhibition assay, but this time the treatment plates
11 were supplemented with 40 μ M MeJA. This treatment induced similar results as
12 treatment with NaCl. Both the *lfp1-1* mutant and *RNAi:LLP1-3* seedlings showed
13 significantly enhanced root length inhibition compared to wildtype (Fig. 3D). Similarly,
14 *lfp3* mutant seedlings displayed exaggerated root growth inhibition on MeJA and this
15 phenotype was complemented by ectopic expression of *LLP3:LLP3* (Fig. 3E). JA
16 downstream signalling pathways in the seedlings after 12 days on MeJA-
17 supplemented plates were also aberrant in the *RNAi:LLP1-3* seedlings. Transcript
18 accumulation of the JA marker gene *PDF1.2* was increased 12 days after transfer of
19 wildtype, *lfp1-1*, and *lfp3* seedlings from control to MeJA plates (Fig. 3F and
20 Supplementary Fig. 6A). By contrast, the induction of *PDF1.2* transcript accumulation
21 was compromised in *RNAi:LLP1-3* plants (Fig. 3F), while the transcript accumulation
22 of *VSP2* remained unchanged (Supplementary Fig. 6B).

23 To determine whether the loss of *LLP1-3* was affecting gene expression through
24 changes in hormone biosynthesis, or through downstream interactions, the net
25 content of SA, JA and ABA was measured in *RNAi:LLP1-3* plants after treatment with
26 salt. Although there was an increase in net JA and ABA content after salt treatment,
27 there was no significant difference between wildtype and *RNAi:LLP1-3* plants
28 (Supplementary Fig. 7). This indicates that signalling aberration does not occur in the
29 biosynthesis of these phytohormones, and that any crosstalk occurs in the pathways
30 downstream of phytohormone biosynthesis. Thus, the three LLP proteins appear to
31 simultaneously promote JA-associated defence against necrotrophic *A. brassicicola*
32 and JA-associated salt tolerance in a process that is occurring downstream of JA
33 accumulation.

1
2 *Crosstalk between JA and SA signalling pathways is misregulated in RNAi:LLP1-3*
3 *plants*

4 From the above experiments, the *RNAi:LLP1-3* plants show a different level of JA
5 marker gene transcript accumulation under abiotic stress. The SA and JA signalling
6 pathways have multiple points of interaction, normally resulting in antagonistic cross
7 talk¹⁸. We therefore investigated *PR1* transcript accumulation as a marker of SA
8 signalling after watering of mature plants with salt. Whereas *PR1* transcript levels
9 were reduced in Col-0 wild type after salt treatment when compared to a mock
10 control, possibly due to the antagonistic relationship between SA and either ABA or
11 MeJA, *PR1* transcript levels remained unchanged in *llp1-1* and were upregulated by
12 ~80-fold in salt- compared to mock-treated *RNAi:LLP1-3* plants (Fig. 4A). Hence,
13 LLP1, LLP2, and/or LLP3 might co-operate in compromising responses to salt or
14 associated JA-SA crosstalk events resulting in enhanced SA-associated responses in
15 *RNAi:LLP1-3* plants in response to salinity stress.

16 As MeJA-associated stress was able to induce SA-dependent gene expression in
17 *LLP1-3*-compromised plants, we investigated whether a local application of MeJA
18 would be sufficient to reconstitute a systemic defence response in the same lines.
19 Using a similar experimental setup to a classical SAR assay, two lower leaves were
20 infiltrated with 100 uM MeJA, and systemic leaves were infiltrated with virulent *Pst*
21 three days later. The bacterial titres in the systemic leaves at 4 dpi indicated that
22 MeJA, while not affecting bacterial titres in wildtype, was able to reconstitute a SAR
23 response in *llp1-1* mutant plants, but not in *llp3* or *RNAi:LLP1-3* lines (Fig. 4B).

24

25 **Discussion**

26 In this paper we show that LLP3 acts locally in SAR signal generation. *LLP3*
27 expression is induced by MeJA, and *llp3* mutant plants display JA-associated biotic
28 and abiotic stress tolerance phenotypes (Fig. 3). This implies that local JA responses
29 contribute to SAR signal generation or transmission. Until now, a potential role of JA
30 in SAR has been under debate^{28,29}, and JA has been believed to be subject to
31 antagonistic control in local infected tissues undergoing ETI^{19,20}. However, during
32 RPS2-mediated ETI the accumulation of SA and downstream signalling through the
33 NPR3 and NPR4 receptors initiates *de novo* JA synthesis³⁰, and the SA sector in

1 Arabidopsis immune networks activated during PTI is dependent upon the JA
2 sector³¹. Given that PTI and ETI have some convergent signalling pathways,
3 including through SA accumulation^{32,33}, it is likely that JA may have a more important
4 role in biotrophic immunity than has been traditionally recognized.

5

6 SA and Pip are thought to function via interconnected signalling pathways during
7 SAR^{9,10}. *LLP1* transcript accumulation is increased in response to SA (Fig. 1A), but is
8 dispensable for SA-induced immunity⁵. *LLP1* further promotes systemic SAR signal
9 recognition or propagation downstream of Pip and drives a positive feedback loop
10 propagating volatile monoterpene emissions as airborne SAR cues⁶. Notably, MeJA-
11 induced root growth inhibition was only marginally, if at all, exaggerated in Pip-
12 deficient *ald1*³⁴ plants and in different monoterpene emission-compromised⁶ mutants
13 (Supplementary Fig. 8). Therefore, *LLP1* might interact with an *LLP3*-associated SAR
14 signalling component in a pathway that is mostly separate from its role in Pip
15 signalling and monoterpene transmission.

16

17 JA activates two separate signalling pathways, depending upon which other
18 signals/factors are detected at the same time. This allows the plant to use JA to fine-
19 tune responses to multiple stresses³⁵. The exaggerated root shortening and
20 enhanced *A. brassicicola* susceptibility phenotypes of the *llp1-1*, *llp3*, and
21 *RNAi:LLP1-3* plants suggest that there is a misregulation in signalling at a point
22 upstream of one of JA's two key pathway regulators, MYC2 and ERF1³⁶. Transcript
23 levels of the ERF1 pathway marker gene *PDF1.2* were reduced in MeJA-treated
24 *RNAi:LLP1-3* plants, whereas the MYC2 pathway marker gene *VSP2* was not
25 misregulated in the same plants (Fig. 3 and Supplementary Fig. 6). *ERF1* is a key
26 transcription factor activated in conjunction with ethylene signalling, which is
27 implicated in defence against necrotrophic pathogens^{37,38}, and is strongly induced in
28 response to salt stress³⁹. The susceptibility of *llp1-1*, *llp3*, and *RNAi:LLP1-3* plants to
29 the pathogen *A. brassicicola* thus further supports a misregulation of the ERF1-
30 regulated branch of JA signalling in these mutants. Together, the data suggest that
31 *LLP1*, 2, and/or 3 influence JA responses through ERF1 (Fig. 4C).

32

1 Although observed physiologically in all genotypes tested, misregulated SA-JA cross
2 talk events were observed at the molecular level, *i.e.* *PDF1.2* and *PR1* transcript
3 accumulation changes, only in *RNAi:LLP1-3* plants. This hints at possible additive
4 roles of *LLP1*, *LLP2*, and *LLP3* in this process. During SAR, however, the roles of
5 *LLP1* and *LLP3* appear to be spatially separated with *LLP1* acting systemically and
6 *LLP3* promoting local SAR signal generation. This might explain why MeJA enhanced
7 the systemic resistance of *llp1-1*, but not *RNAi:LLP1-3* plants against *Pst* if *LLP3* is
8 required locally to drive JA-associated SAR signal generation or transmission through
9 ERF1 (Fig. 4C). During SAR, SA-JA antagonism is observed locally, but not in the
10 systemic tissues^{19,20}. Perhaps, *LLP1* fine-tunes incoming signals to avoid
11 antagonistic trade-offs between SA- and JA-mediated defences in the systemic tissue
12 during SAR (Fig. 4C).

13

14 The recently suggested spatial role of JA signalling in the perimeter of SA-induced
15 HR lesions²¹ might explain how JA locally influences SAR signal generation. High SA
16 levels in the core of the lesion promote *LLP1* transcript accumulation, while JA
17 accumulation in the rim of the lesion drives up *LLP3* expression. We hypothesize that
18 this signal is then relayed through the *ERF1* pathway affecting salt tolerance,
19 defence against necrotrophic pathogens, and also driving SAR signal emission from
20 this site (Fig. 4C). The role of *LLP1* allows this pathway to act synergistically with
21 both the SA- and Pip-dependent systemic defence signals, creating an interwoven
22 network necessary for SAR-associated defence priming. Priming of SA defences in
23 the absence of deleterious effects on JA defences further assigns a high potential to
24 LLP-associated SAR signalling components for application in future durable plant
25 protection strategies. A possible exploitation of LLP-associated signaling moieties
26 towards resource-efficient defence priming will be subject of further study.

27

28 **Methods**

29 *Plant materials and growth conditions*

30 *A. thaliana* ecotype Columbia-0 (Col-0) was used as the wild type control throughout
31 all experiments. Transgenic lines *llp1-1*, *eds1-2*, *ald1*, *ggpps12*, *tps24-1*, *tps24-2*, and
32 *RNAi:LLP1-3* have been described previously^{5,6,34,40,41}. *RNAi:LLP1-3* line C3 13-1⁶
33 was used for all experiments. SALK_030762 with a T-DNA insertion in *LLP3*

1 (At3G15356) was obtained from the Nottingham Arabidopsis Stock Center⁴², and
2 propagated to homozygosity. Plants that were homozygous for the T-DNA insertion
3 were used for all experiments and as the parental line for generating *l/p3-LLP3:LLP3*
4 complementation lines 3.02 and 4.01. For the latter, *LLP3:LLP3* constructs were
5 generated from Col-0 wild type genomic DNA. The native promoter was chosen from
6 ~2 kilo base pairs upstream to the *LLP3* transcriptional start site, and the *LLP3:LLP3*
7 target sequence was isolated by PCR using the primers *LLP3:LLP3-F* and
8 *LLP3:LLP3-R* (Supplementary Table 1). The resulting DNA fragment was cloned into
9 pENTR™/ D-TOPO® (Invitrogen) and sequenced. The resulting construct was
10 transferred to the binary Gateway® cloning vector pBGWFS7,0⁴³, with the *GUS*
11 sequence removed using the restriction enzyme *Nru*1 (pBGWFS7,0Δ*GUS*). The
12 resulting binary vector was transformed into *Agrobacterium tumefaciens* strain
13 GV3101 and used for plant transformation by floral dip⁴⁴. Transgenic T1 plants were
14 selected via 200g/L BASTA spray (Hoechst, Germany). Experiments were performed
15 in T3 plants. *LLP3* transcript levels were determined by RT-qPCR as described below
16 with the *LLP3* primer sets c1 or c2 (Supplementary Table 1).
17 Plants were grown on potting soil (without fertilizer) mixed with sand in 5:1 ratio, and
18 kept under short day conditions (10 hours (h) light with an intensity of 100 μ E $m^{-2} s^{-1}$
19 at 22°C and 14 h dark at 18°C, 70% relative humidity).

20

21 *Phytohormone treatments*

22 To analyse *LLP1-3* transcript accumulation in response to phytohormone treatment,
23 green tissues of 2- to 3-week old plants were sprayed until drop-off with 1 mM SA
24 (Sigma Aldrich), 100 μ M MeJA (Sigma Aldrich), or 100 μ M ABA (Sigma Aldrich)
25 dissolved in 0.1% $MgCl_2$, 0.01% Tween® 20, and 0.025% MeOH. Plants of the same
26 age were sprayed with 0.1% $MgCl_2$, 0.01% Tween® 20, 0.025% MeOH as the mock
27 control treatment. Leaf samples were taken at 8 and 24 h after treatment and flash
28 frozen in liquid N₂.

29

30 *Pathogen infection assays*

31 *Pseudomonas syringae* pathovar *tomato* (*Pst*) and *Pst AvrRpm1* were maintained as
32 described⁵. To induce a SAR response, plants were infiltrated in their first two true
33 leaves with 1×10^6 CFU/mL of *Pst/AvrRPM1*. Three days later, two systemic leaves

1 were infiltrated with 1×10^5 CFU/mL of *Pst*. The resulting *in planta* bacterial titres were
2 determined at 4 dpi as described⁶. *A. brassicicola* was maintained on malt medium
3 (3% malt extract (Merck), 1.5% agar-agar (Roth)) and transferred to oat plates (oats
4 (Alnatura) in 1.5% w/v agar-agar) before experiments. Mycelium was solved in MKP
5 buffer (62mM KH₂PO₄, 0.01% glucose, 0.01% Tween[®] 20) until a concentration of
6 200 spores/μL was achieved. Plants were inoculated by placing 3 μL droplets onto
7 the third and fourth true leaf. The resulting lesion sizes were determined at 5 dpi
8 using ImageJ. Cell death was visualized using trypan blue staining as described⁴⁵.

9

10 *Petiole exudate experiments*

11 Petiole exudate experiments were performed as described⁶. In short, *Pst AvrRpm1*-
12 inoculated leaves were cut in the middle of the rosette at 24 h post-inoculation, and
13 incubated with their petioles in 1 mM EDTA. After 1 h 6 leaves per exudate were
14 transferred to 2.0 mL of sterilized water and allowed to exude for 48 h. The resulting
15 PEX solutions were filter-sterilized (Millipore, 0.22 μm) and supplemented with MgCl₂
16 to a final concentration of 1 mM. 24 h after syringe infiltration of the PEX in leaves of
17 naïve recipient plants, the infiltrated leaves were inoculated with 10^5 cfu mL⁻¹ of *Pst*,
18 *in planta* titres of which were determined at 4 dpi as described above.

19

20 *Root length inhibition assays*

21 For root growth inhibition measurements, seedlings were sterilised in 75% followed
22 by 100% EtOH (Merck), dried, and sown on 1x Murashige Skoog medium including
23 vitamins (Duchefa) with 0.1% cefotaxim (Acros Organics) and 0.25% Carbenicillin
24 (Roth). Seedlings were transferred after 6 days to treatment plates containing either
25 10 μM ABA, 100 mM NaCl, or 40 μM MeJA (Sigma-Aldrich), or to control MS plates.
26 All plates were placed upright in the growth chamber under long day conditions, and
27 the seedlings were photographed 6 and 12 days post-transfer. Root length was
28 measured using ImageJ. The seedlings were harvested, pooled per genotype and
29 treatment, and flash frozen in N₂ for RNA extraction.

30

31 *Phytohormone content measurements*

32 ABA in seedlings was measured as described⁴⁶. In short, the frozen material was
33 spiked with 10 ng ABA-d₆ and incubated with 40% acetonitrile (ACN) for 30 min prior

1 acidification with phosphoric acid and extraction with *tert*-butyl methyl ether. The
2 organic extract was passed over a Chromabond NH2 500 mg solid phase extraction
3 column (Macherey-Nagel, Düren, Germany). The eluate was diluted with distilled
4 water and passed over a Chromabond C18ec 100 mg solid phase extraction column
5 (Macherey-Nagel). The eluate was evaporated in a vacuum concentrator, dissolved
6 and fractionated by RP-HPLC using a Nucleodur 100-5 C18ec 125x4.6 mm column
7 (Macherey-Nagel). The ABA-containing fraction was collected, evaporated to dryness
8 and methylated with 2 M (trimethylsilyl)diazomethane/methanol = 1:19. After
9 evaporation, the residue was dissolved in ACN and analysed by gas
10 chromatography-mass spectrometry using a VF-5ms column (Agilent, St. Louis, MO,
11 USA) and helium as carrier gas at a flow rate of 1.5 ml/min. The ions with *m/z* 190
12 (ABA) and 194 (ABA-d₆) were used for quantification and the ions at 134 and 162
13 (ABA) and 138 and 166 (ABA-d₆) were used as qualifiers.

14 The phytohormones ABA, SA and JA were measured in mature plants according to⁴⁶
15 using a versatile UHPLC-MS/MS_{MRM} system. The plant material (50-250 mg) was
16 placed in 2 mL bead beater tubes (CKMix-2 mL, Bertin Technologies,
17 Montigny-le-Bretonneux, France). An aliquot of the internal standard (20 μ L),
18 containing ABA-d₆ (2.5 μ g/mL), SA-d₄ (2.5 μ g/mL), and JA-d₅ (25 μ g/mL) in
19 acetonitrile was added to the plants and incubated for 30 min at room temperature.
20 After extractive grinding with ethyl acetate (1 mL) in a bead beater (Precellys
21 Homogenizer, Bertin Technologies, Montigny-le-Bretonneux, France) the supernatant
22 was membrane filtered (0.45 μ m), evaporated to dryness, resolved in acetonitrile
23 (70 μ L) and injected into the LC-MS/MS-system (2 μ L).

24 For LC-MS/MS analysis a QTRAP 6500⁺ mass spectrometer (Sciex, Darmstadt,
25 Germany) was used to acquire electrospray ionization (ESI) mass spectra and
26 product ion spectra. Negative and positive ions were detected in the scheduled
27 multiple reaction monitoring (MRM) mode.

28 For analysis of ABA, SA and JA, the MS/MS parameters were tuned to achieve
29 fragmentation of the [M-H]⁻ and [M+H]⁺ molecular ions into specific product ions to
30 receive a qualifier and a quantifier transition for every compound.

31 Chromatography was performed by means of an ExionLC UHPLC system (Shimadzu
32 Europa GmbH, Duisburg, Germany) equipped with a Kinetex F5 column
33 (100 x 2.1 mm, 100 Å, 1.7 μ m, Phenomenex, Aschaffenburg, Germany). Operated

1 with a flow rate of 0.4 mL/min using 0.1% formic acid in water (v/v) as solvent A and
2 0.1% formic acid in acetonitrile (v/v) as solvent B, chromatography was performed
3 with the following gradient: 0% B held for 2 min, increased in 1 min to 30% B, in
4 12 min to 30% B, increased in 0.5 min to 100% B, held 2 min isocratically at 100% B,
5 decreased in 0.5 min to 0% B, held 3 min at 0% B. Data acquisition and instrumental
6 control were performed using Analyst 1.6.3 software (Sciex, Darmstadt, Germany).

7

8 *Salt pouring experiments*

9 Plants were watered with distilled water or 300 mM NaCl three times with four day
10 intervals starting from 4 weeks after germination. Leaf tissue was harvested 4 days
11 after the final salt treatment, weighed, and flash frozen in liquid N₂.

12

13 *RNA isolation and RT-qPCR*

14 Total RNA was extracted from leaves and seedlings using TriReagent (Sigma-
15 Aldrich) following the manufacturer's instructions. cDNA was synthesized using
16 SuperScriptII reverse transcriptase (Invitrogen). Real-time quantitative PCR was
17 performed using the Sensimix SYBR low-rox kit (Bioline) and the primers in
18 Supplementary Table 2, with *UBIQUITIN* as the reference gene. Endogenous *LLP3*
19 transcript accumulation was determined with the primers LLP3-F and LLP3-R. qPCR
20 was performed on a 7500 real-time PCR system (Applied Biosystems). Transcript
21 accumulation was analysed using the 7500 Fast System Software 1.3.1.

22

23 *Drought assay*

24 The progressive drought experiment was performed as described⁴⁷. In brief,
25 Arabidopsis plantlets were exposed to a slowly increasing water deficit by minimizing
26 evaporation and withholding watering under short day conditions (8 h light). Water
27 consumption per plant was recorded from 18 to 73 days after seeding. Above-ground
28 material was used for determining the dry-weight biomass and WUE was expressed
29 as the ratio of biomass to consumed water.

30

31 *Statistics*

32 Data was analysed in GraphPad Prism 8 for Windows. If necessary, outliers were
33 removed using a Grubbs' test ($\alpha=0.05$). Normal distribution of the data was checked

1 using D'Agostino Pearson ($\alpha=0.01$). Data that showed normal distribution was tested
2 for significance using an unpaired one-way ANOVA with Tukey's multiple comparison
3 test, and data that was not normally distributed was tested using a Kruskal-Wallis test
4 with a Dunn's multiple comparison test.

5

6 **Supplementary Material**

7 **Supplementary Table 1** Primers for *LLP3:LLP3* construct generation and qPCR

8 **Supplementary Table 2** Primers for qPCR

9 **Supplementary Figure 1** BTH induces transcript accumulation of *LLP1*.

10 **Supplementary Figure 2** *LLP3* transcript levels in *llp3* and *llp3-LLP3:LLP3*
11 complementation lines

12 **Supplementary Figure 3** *LLP1* moderately influences primary root growth.

13 **Supplementary Figure 4** *llp1-1* and *RNAi:LLP1-3* lines do not show an altered
14 response to ABA

15 **Supplementary Figure 5** *LLP1-3* do not affect the response to drought stress.

16 **Supplementary Figure 6** JA-associated marker gene expression

17 **Supplementary Figure 7** *LLP1-3* do not influence phytohormone accumulation in
18 response to salt

19 **Supplementary Figure 8** Salt- and MeJA-induced primary root growth inhibition in
20 seedlings of SAR-associated mutant lines

21

22 **Acknowledgments**

23 We thank Miriam Lenk for critically reading the manuscript. This work was funded by
24 the Deutsche Forschungsgemeinschaft (DFG) as part of SFB924 (projects A12 (BP),
25 B01 (EG), B12 (CD), and project B06 to ACV).

1 **References**

- 2 1. Boutrot, F. & Zipfel, C. Function, Discovery, and Exploitation of Plant Pattern Recognition
3 Receptors for Broad-Spectrum Disease Resistance. *Annu Rev Phytopathol* **55**, 257-286 (2017).
- 4 2. Jones, J.D. & Dangl, J.L. The plant immune system. *Nature* **444**, 323-9 (2006).
- 5 3. Cui, H., Tsuda, K. & Parker, J.E. Effector-triggered immunity: from pathogen perception to
6 robust defense. *Annu Rev Plant Biol* **66**, 487-511 (2015).
- 7 4. Vlot, A.C., Dempsey, D.A. & Klessig, D.F. Salicylic Acid, a multifaceted hormone to combat
8 disease. *Annu Rev Phytopathol* **47**, 177-206 (2009).
- 9 5. Breitenbach, H.H. *et al.* Contrasting Roles of the Apoplastic Aspartyl Protease APOPLASTIC,
10 ENHANCED DISEASE SUSCEPTIBILITY1-DEPENDENT1 and LEGUME LECTIN-LIKE PROTEIN1 in
11 Arabidopsis Systemic Acquired Resistance. *Plant Physiol* **165**, 791-809 (2014).
- 12 6. Wenig, M. *et al.* Systemic acquired resistance networks amplify airborne defense cues. *Nat
13 Commun* **10**, 3813 (2019).
- 14 7. Lyou, S.H. *et al.* The Arabidopsis AtLEC gene encoding a lectin-like protein is up-regulated by
15 multiple stimuli including developmental signal, wounding, jasmonate, ethylene, and chitin
16 elicitor. *Mol Cells* **27**, 75-81 (2009).
- 17 8. Ramonell, K. *et al.* Loss-of-function mutations in chitin responsive genes show increased
18 susceptibility to the powdery mildew pathogen *Erysiphe cichoracearum*. *Plant Physiol* **138**,
19 1027-36 (2005).
- 20 9. Kim, Y., Gilmour, S.J., Chao, L., Park, S. & Thomashow, M.F. Arabidopsis CAMTA Transcription
21 Factors Regulate Pipecolic Acid Biosynthesis and Priming of Immunity Genes. *Mol Plant* **13**,
22 157-168 (2020).
- 23 10. Sun, T. *et al.* Redundant CAMTA Transcription Factors Negatively Regulate the Biosynthesis
24 of Salicylic Acid and N-Hydroxypipecolic Acid by Modulating the Expression of SARD1 and
25 CBP60g. *Mol Plant* **13**, 144-156 (2020).
- 26 11. Gao, Q.M., Zhu, S., Kachroo, P. & Kachroo, A. Signal regulators of systemic acquired
27 resistance. *Front Plant Sci* **6**, 228 (2015).
- 28 12. Chen, Y.C. *et al.* N-hydroxy-pipecolic acid is a mobile metabolite that induces systemic
29 disease resistance in Arabidopsis. *Proc Natl Acad Sci U S A* **115**, E4920-E4929 (2018).
- 30 13. Hartmann, M. *et al.* Flavin Monooxygenase-Generated N-Hydroxypipecolic Acid Is a Critical
31 Element of Plant Systemic Immunity. *Cell* **173**, 456-469 e16 (2018).
- 32 14. Wang, C. *et al.* Pipecolic acid confers systemic immunity by regulating free radicals. *Sci Adv* **4**,
33 eaar4509 (2018).
- 34 15. Ellis, C. & Turner, J.G. The Arabidopsis mutant cev1 has constitutively active jasmonate and
35 ethylene signal pathways and enhanced resistance to pathogens. *Plant Cell* **13**, 1025-33
36 (2001).
- 37 16. Farmer, E.E. & Ryan, C.A. Octadecanoid Precursors of Jasmonic Acid Activate the Synthesis of
38 Wound-Inducible Proteinase Inhibitors. *Plant Cell* **4**, 129-134 (1992).
- 39 17. Saijo, Y. & Loo, E.P. Plant immunity in signal integration between biotic and abiotic stress
40 responses. *New Phytol* **225**, 87-104 (2020).
- 41 18. Pieterse, C.M., Van der Does, D., Zamioudis, C., Leon-Reyes, A. & Van Wees, S.C. Hormonal
42 modulation of plant immunity. *Annu Rev Cell Dev Biol* **28**, 489-521 (2012).
- 43 19. Spoel, S.H., Johnson, J.S. & Dong, X. Regulation of tradeoffs between plant defenses against
44 pathogens with different lifestyles. *Proc Natl Acad Sci U S A* **104**, 18842-7 (2007).
- 45 20. Wittek, F. *et al.* Folic acid induces salicylic acid-dependent immunity in Arabidopsis and
46 enhances susceptibility to *Alternaria brassicicola*. *Mol Plant Pathol* **16**, 616-22 (2015).
- 47 21. Betsuyaku, S. *et al.* Salicylic Acid and Jasmonic Acid Pathways are Activated in Spatially
48 Different Domains Around the Infection Site During Effector-Triggered Immunity in
49 *Arabidopsis thaliana*. *Plant Cell Physiol* **59**, 8-16 (2018).

1 22. Karapetyan, S. & Dong, X. Redox and the circadian clock in plant immunity: A balancing act.
2 *Free Radic Biol Med* **119**, 56-61 (2018).

3 23. Mur, L.A., Kenton, P., Atzorn, R., Miersch, O. & Wasternack, C. The outcomes of
4 concentration-specific interactions between salicylate and jasmonate signaling include
5 synergy, antagonism, and oxidative stress leading to cell death. *Plant Physiol* **140**, 249-62
6 (2006).

7 24. Anderson, P.K. *et al.* Emerging infectious diseases of plants: pathogen pollution, climate
8 change and agrotechnology drivers. *Trends Ecol Evol* **19**, 535-44 (2004).

9 25. Bebber, D.P. Range-expanding pests and pathogens in a warming world. *Annu Rev
10 Phytopathol* **53**, 335-56 (2015).

11 26. Yasuda, M. *et al.* Antagonistic interaction between systemic acquired resistance and the
12 abscisic acid-mediated abiotic stress response in Arabidopsis. *Plant Cell* **20**, 1678-92 (2008).

13 27. Valenzuela, C.E. *et al.* Salt stress response triggers activation of the jasmonate signaling
14 pathway leading to inhibition of cell elongation in Arabidopsis primary root. *J Exp Bot* **67**,
15 4209-20 (2016).

16 28. Attaran, E., Zeier, T.E., Griebel, T. & Zeier, J. Methyl salicylate production and jasmonate
17 signaling are not essential for systemic acquired resistance in Arabidopsis. *Plant Cell* **21**, 954-
18 71 (2009).

19 29. Truman, W., Bennett, M.H., Kubigsteltig, I., Turnbull, C. & Grant, M. Arabidopsis systemic
20 immunity uses conserved defense signaling pathways and is mediated by jasmonates. *Proc
21 Natl Acad Sci U S A* **104**, 1075-80 (2007).

22 30. Liu, L. *et al.* Salicylic acid receptors activate jasmonic acid signalling through a non-canonical
23 pathway to promote effector-triggered immunity. *Nat Commun* **7**, 13099 (2016).

24 31. Hillmer, R.A. *et al.* The highly buffered Arabidopsis immune signaling network conceals the
25 functions of its components. *PLoS Genet* **13**, e1006639 (2017).

26 32. Peng, Y., van Wersch, R. & Zhang, Y. Convergent and Divergent Signaling in PAMP-Triggered
27 Immunity and Effector-Triggered Immunity. *Mol Plant Microbe Interact* **31**, 403-409 (2018).

28 33. Sun, T. *et al.* ChIP-seq reveals broad roles of SARD1 and CBP60g in regulating plant immunity.
29 *Nat Commun* **6**, 10159 (2015).

30 34. Navarova, H., Bernsdorff, F., Doring, A.C. & Zeier, J. Pipecolic acid, an endogenous mediator
31 of defense amplification and priming, is a critical regulator of inducible plant immunity. *Plant
32 Cell* **24**, 5123-41 (2012).

33 35. Kazan, K. & Manners, J.M. JAZ repressors and the orchestration of phytohormone crosstalk.
34 *Trends Plant Sci* **17**, 22-31 (2012).

35 36. Kazan, K. Diverse roles of jasmonates and ethylene in abiotic stress tolerance. *Trends Plant
36 Sci* **20**, 219-29 (2015).

37 37. Berrocal-Lobo, M., Molina, A. & Solano, R. Constitutive expression of ETHYLENE-RESPONSE-
38 FACTOR1 in Arabidopsis confers resistance to several necrotrophic fungi. *Plant J* **29**, 23-32
39 (2002).

40 38. Lorenzo, O., Piqueras, R., Sanchez-Serrano, J.J. & Solano, R. ETHYLENE RESPONSE FACTOR1
41 integrates signals from ethylene and jasmonate pathways in plant defense. *Plant Cell* **15**,
42 165-78 (2003).

43 39. Cheng, M.C., Liao, P.M., Kuo, W.W. & Lin, T.P. The Arabidopsis ETHYLENE RESPONSE
44 FACTOR1 regulates abiotic stress-responsive gene expression by binding to different cis-
45 acting elements in response to different stress signals. *Plant Physiol* **162**, 1566-82 (2013).

46 40. Bartsch, M. *et al.* Salicylic acid-independent ENHANCED DISEASE SUSCEPTIBILITY1 signaling in
47 Arabidopsis immunity and cell death is regulated by the monooxygenase FMO1 and the
48 Nudix hydrolase NUDT7. *Plant Cell* **18**, 1038-51 (2006).

49 41. Riedlmeier, M. *et al.* Monoterpenes Support Systemic Acquired Resistance within and
50 between Plants. *Plant Cell* **29**, 1440-1459 (2017).

1 42. Alonso, J.M. *et al.* Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science*
2 **301**, 653-7 (2003).

3 43. Karimi, M., Inze, D. & Depicker, A. GATEWAY vectors for *Agrobacterium*-mediated plant
4 transformation. *Trends Plant Sci* **7**, 193-5 (2002).

5 44. Logemann, E., Birkenbihl, R.P., Ulker, B. & Somssich, I.E. An improved method for preparing
6 *Agrobacterium* cells that simplifies the *Arabidopsis* transformation protocol. *Plant Methods*
7 **2**, 16 (2006).

8 45. Pogany, M. *et al.* Dual roles of reactive oxygen species and NADPH oxidase RBOHD in an
9 *Arabidopsis*-*Alternaria* pathosystem. *Plant Physiol* **151**, 1459-75 (2009).

10 46. Avramova, V. *et al.* Carbon isotope composition, water use efficiency, and drought sensitivity
11 are controlled by a common genomic segment in maize. *Theor Appl Genet* **132**, 53-63 (2019).

12 47. Yang, Z. *et al.* Leveraging abscisic acid receptors for efficient water use in *Arabidopsis*. *Proc*
13 *Natl Acad Sci U S A* **113**, 6791-6 (2016).

14

15

1 **Figure captions**

2

3 **Figure 1** *LEGUME LECTIN-LIKE PROTEIN3 (LLP3)* promotes systemic acquired
4 resistance (SAR) signal generation/transmission. (A) *LLP3* transcript accumulation is
5 not affected by salicylic acid (SA). 4-week-old Col-0 wild type and *eds1-2* *Arabidopsis*
6 plants were spray-treated with 1mM SA, and 24 hours (h) later *LLP1*, *LLP2*, and
7 *LLP3* transcript accumulation was determined by RT-qPCR. Transcript accumulation
8 was normalized to that of *UBIQUITIN* and is shown relative to the normalized
9 transcript levels in the appropriate mock controls. Bars represent the $\log_2(\text{mean}) \pm$
10 SEM of four biologically independent replicates. The letters above the bars indicate
11 statistically significant differences (one-way ANOVA, n=4, P<0.05, F=4.623, DF=22).
12 (B) *LLP3* is required for SAR. Plants were infiltrated locally with either *Pst AvrRpm1*
13 (SAR) or with 10 mM MgCl₂ as the mock control (M). To monitor SAR, 3 days after
14 the primary treatment leaves distal to the initial treatment site were infiltrated with *Pst*.
15 Plant lines included *llp3* mutants and 2 independently transformed complementation
16 lines carrying a transgene driving *LLP3* expression from its native promoter (*I3-L3:L3*
17 3.02 and 4.01). Box plots represent average *Pst* titres in systemic leaves at 4 days
18 post-inoculation (dpi) from 4 biologically independent experiments, including 3
19 replicates each \pm min and max values. The letters above the box plots indicate
20 statistically significant differences (Kruskal-Wallis test, P<0.05, n=12, KW
21 statistic=101.4). (C) *LLP3* is required to send, but not to receive phloem-mobile SAR
22 signals. Leaves of donor plants were inoculated with *Pst AvrRpm1* (S) or with the
23 appropriate mock control (M). After 24 h, petiole exudates were collected from the
24 donor plants and infiltrated into leaves of naïve recipient plants. 24h later, the treated
25 leaves were challenged with *Pst*. Bars represent average *Pst* titres at 4 dpi from 3
26 biologically independent experiments, including 3 replicates each \pm SD. The letters
27 above the bars indicate statistically significant differences (one-way ANOVA, n=9,
28 P<0.05, F=6.258, DF=35).

29

30 **Figure 2** *LLP1*, *LLP2*, and/or *LLP3* compromise *Arabidopsis* responses to salt stress.
31 (A) *LLP1* transcript accumulation is reduced after ABA treatment. Col-0 wild type and
32 *eds1-2* plants were spray-treated with 100 μ M ABA, and after 24 h *LLP1*, *LLP2*,
33 and/or *LLP3* transcript accumulation was determined by RT-qPCR. Transcript

1 accumulation was normalized to that of *UBIQUITIN* and is shown relative to the
2 normalized transcript levels in the appropriate mock controls. Bars represent the
3 $\log_2(\text{mean}) \pm \text{SEM}$ of three biologically independent replicates. The letters above the
4 bars indicate statistically significant differences (one-way ANOVA, $n=3$, $P<0.05$,
5 $F=6.291$, $DF=40$). (B/C) *LLP1*, *LLP2*, and/or *LLP3* compromise salt-associated root
6 growth inhibition. Seedlings of Col-0 wild type, *llp1-1*, and *RNAi:LLP1-3* (B) and of
7 *llp3* and two *llp3-LLP3:LLP3* complementation lines (*I3-L3:L3 3.02* and *4.01*; C) were
8 germinated on control MS plates, and after 6 days transferred to either further control
9 plates, or to plates supplemented with 100 mM NaCl. Primary root length was
10 measured at 6 and 12 days post transfer and normalized to that of the same
11 genotype on control plates. Box plots represent average normalized root length \pm min
12 and max values. The letters above the box plots indicate statistically significant
13 differences (B: one-way ANOVA, $P=<0.05$, $F=30.70$, $DF=233$, for day 6, Col-0 $n=48$,
14 *llp1-1* $n=38$, *RNAi:LLP1* $n=40$, for day 12 Col-0 $n=48$, *llp1-1* $n=29$, *RNAi:LLP1-3*
15 $n=21$; C: Day 6: Kruskal-Wallis test, $P=<0.05$, KW test statistic =165.5, Col-0 $n=83$,
16 *llp3* $n=86$, *I3-L3:L3 3.02* $n=96$, *I3-L3:L3 4.01* $n=89$. Day 12: one-way ANOVA,
17 $P=<0.05$, $F=25.08$, $DF=519$, Col-0 $n=81$, *llp3* $n=84$, *I3-L3:L3 3.02* $n=94$, *I3-L3:L3 4.01*
18 $n=84$). These experiments were repeated 3 (C) to 4-8 times (B) with comparable
19 results. (D) *LLP3* compromises root growth inhibition on 10 μM ABA. Col-0 wild type,
20 *llp3* and two *llp3-LLP3:LLP3* complementation lines were treated as described in
21 (B/C), and the treatment plates were supplemented with 10 μM ABA. Box plots
22 represent average normalized root length \pm min and max values. The letters above
23 the box plots indicate statistically significant differences (Day 6: one-way ANOVA,
24 $P=<0.05$, $F=76.10$, $DF=538$, Col-0 $n=76$, *llp3* $n=86$, *3.01* $n=89$, *I3-L3:L3 3.02* $n=98$,
25 *I3-L3:L3 4.01* $n=94$, *8.01* $n=96$. Day 12: Kruskal-Wallis test, $P=<0.05$, KW test
26 statistic =121.1, Col-0 $n=85$, *llp3* $n=90$, *I3-L3:L3 3.02* $n=100$, *I3-L3:L3 4.01* $n=97$).
27 This experiment was repeated 3 times with comparable results.
28

29 **Figure 3** *LLP1*, *LLP2*, and/or *LLP3* differentially affect jasmonic acid (JA)-associated
30 responses in *Arabidopsis*. (A) *LLP3* transcript accumulation is induced by methyl
31 jasmonate (MeJA). Col-0 wild type and *eds1-2* plants were spray-treated with 100 μM
32 MeJA, and after 24 h *LLP1*, *LLP2*, and/or *LLP3* transcript accumulation was
33 determined by RT-qPCR. Transcript accumulation was normalized to that of

1 *UBIQUITIN* and is shown relative to the normalized transcript levels in the
2 appropriate mock controls. Bars represent the $\log_2(\text{mean}) \pm \text{SEM}$ of four biologically
3 independent replicates. The letters above the bars indicate statistically significant
4 differences (one-way ANOVA, $n=4$, $P<0.05$, $F=4.493$, $DF=45$). (B/C) *LLP1*, *LLP2*,
5 and/or *LLP3* promote JA-associated defence against *Alternaria brassicicola*. Droplets
6 containing spores of the necrotrophic fungus *A. brassicicola* were placed on the
7 leaves of four-week-old Col-0 wild type, *llp1-1*, *llp3*, and *RNAi:LLP1-3* plants.
8 Resulting lesions were photographed (B) and measured (C) 5 days later. Box plots in
9 (C) represent mean lesion diameters from 4 biologically independent experiments
10 including 15 replicates each \pm min and max values. The letters above the box plots
11 indicate statistically significant differences (Kruskal-Wallis test, $P=<0.05$, KW test
12 statistic =24.10, $n=60$ for all genotypes). (D/E) *LLP1*, *LLP2*, and/or *LLP3* compromise
13 JA-associated root growth inhibition. Seedlings of Col-0 wild type, *llp1-1*, and
14 *RNAi:LLP1-3* (D) and of *llp3* and two *llp3-LLP3:LLP3* complementation lines (*I3-L3:L3*
15 3.02 and 4.01; E) were germinated on control MS plates, and after 6 days transferred
16 to either further control plates, or to plates supplemented with 40 μM MeJA. Primary
17 root length was measured at 6 and 12 days post transfer and normalized to that of
18 the same genotype on control plates. Box plots represent average normalized root
19 length \pm min and max values. The letters above the box plots indicate statistically
20 significant differences (D: Day 6: one-way ANOVA, $F=44.87$, $DF=147$, Col-0 $n=29$,
21 *llp1-1*, *RNAi:LLP1-3* $n=30$. Day 12: one-way ANOVA, $F=74.62$, $DF=175$, Col-0 $n=29$,
22 *llp1-1* $n=30$, *RNAi:LLP1-3* $n=28$; E: Day 6: one-way ANOVA, $P<0.05$, $F=61.40$,
23 $DF=541$, Col-0 $n=71$, *llp3* $n=85$, *I3-L3:L3* 3.02 $n=94$, *I3-L3:L3* 4.01 $n=97$. Day 12:
24 Kruskal-Wallis test, $P=<0.05$, KW test statistic =140.7, Col-0 $n=63$, *llp3* $n=73$, *I3-*
25 *L3:L3* 3.02 $n=94$, *I3-L3:L3* 4.01 $n=97$, 8.01 $n=95$). These experiments were repeated
26 (E) to 5 times (D) with comparable results. (F) *LLP1*, *LLP2*, and/or *LLP3*
27 compromise MeJA-induced *PDF1.2* transcript accumulation. *PDF1.2* transcript
28 accumulation was monitored by qRT-PCR in seedlings from (D). Transcript
29 accumulation was normalized to that of *UBIQUITIN* and is shown relative to the
30 normalized transcript levels in the appropriate mock controls. Bars represent the
31 $\log_2(\text{mean}) \pm \text{SEM}$ of biologically independent replicates. The letters above the bars
32 indicate statistically significant differences (one-way ANOVA, $P=<0.05$, $F=14.93$,
33 $DF=7$, $n=3$ for Col-0 and *RNAi:LLP1-3*, $n=2$ for *llp1-1*).

1
2 **Figure 4** *LLP1*, *LLP2*, and/or *LLP3* dampen antagonistic SA-JA cross talk between
3 defence pathways. (A) Exposure to salt drives up *PR1* transcript levels when *LLP1*,
4 *LLP2*, and/or *LLP3* transcript levels are reduced. Four-week-old Col-0 wild type, *llp1-1*,
5 and *RNAi:LLP1-3* plants were irrigated with 300 mM NaCl three times over the
6 course of 9 days. Three days later, *PR1* transcript accumulation in the leaves was
7 determined by qRT-PCR. Transcript accumulation was normalized to that of
8 *UBIQUITIN* and is shown relative to the normalized transcript levels in the
9 appropriate mock controls. Bars represent the $\log_2(\text{mean}) \pm \text{SEM}$ of four biologically
10 independent replicates. The letters above the bars indicate statistically significant
11 differences (one-way ANOVA, n=4, P<0.05, F=12.23, DF=11). (B) In the absence of
12 functional *LLP1*, MeJA triggers SAR-like resistance in distal tissues. Col-0 wild type,
13 *llp1-1*, *llp3*, and *RNAi:LLP1-3* plants were treated locally with 100 μM MeJA by leaf
14 infiltration. To monitor systemic SA-associated defence responses, leaves distal to
15 the site of the initial treatment were inoculated with *Pst* 3 days after the primary
16 treatment. Box plots represent average *Pst* titres in systemic leaves at 4 dpi from 4-5
17 biologically independent experiments, including 3-4 replicates each \pm min and max
18 values. The letters above the box plots indicate statistically significant differences
19 (Kruskal-Wallis test, P<0.05, KW test statistic=20.61, Col-0 mock n=17, MeJA n=20,
20 *llp1-1* mock n=18, MeJA n=19, *llp3* mock n=11, MeJA n=11, *RNAi:LLP1-3* mock
21 n=19, MeJA n=19). (C) *LLP3* promotes local SAR signal generation downstream of
22 (Me)JA accumulating in the perimeter of HR (hypersensitive response) lesions.
23 Elevated *LLP3* expression promotes *PDF1.2* expression and defence against
24 necrotrophic pathogens through ERF1 (ETHYLENE RESPONSE FACTOR 1) as well
25 as salt stress tolerance. In parallel with EDS1-dependent, SA-associated long
26 distance signals, *LLP3* promotes accumulation or transmission of a long distance
27 SAR signal downstream of (Me)JA. Systemically, *LLP1* balances incoming signals
28 promoting SAR while restricting deleterious effects of SA-associated SAR on JA-
29 associated defence responses. Abbreviations: NPR3/4, NON-EXPRESSOR OF PR
30 GENES3/4

31
32

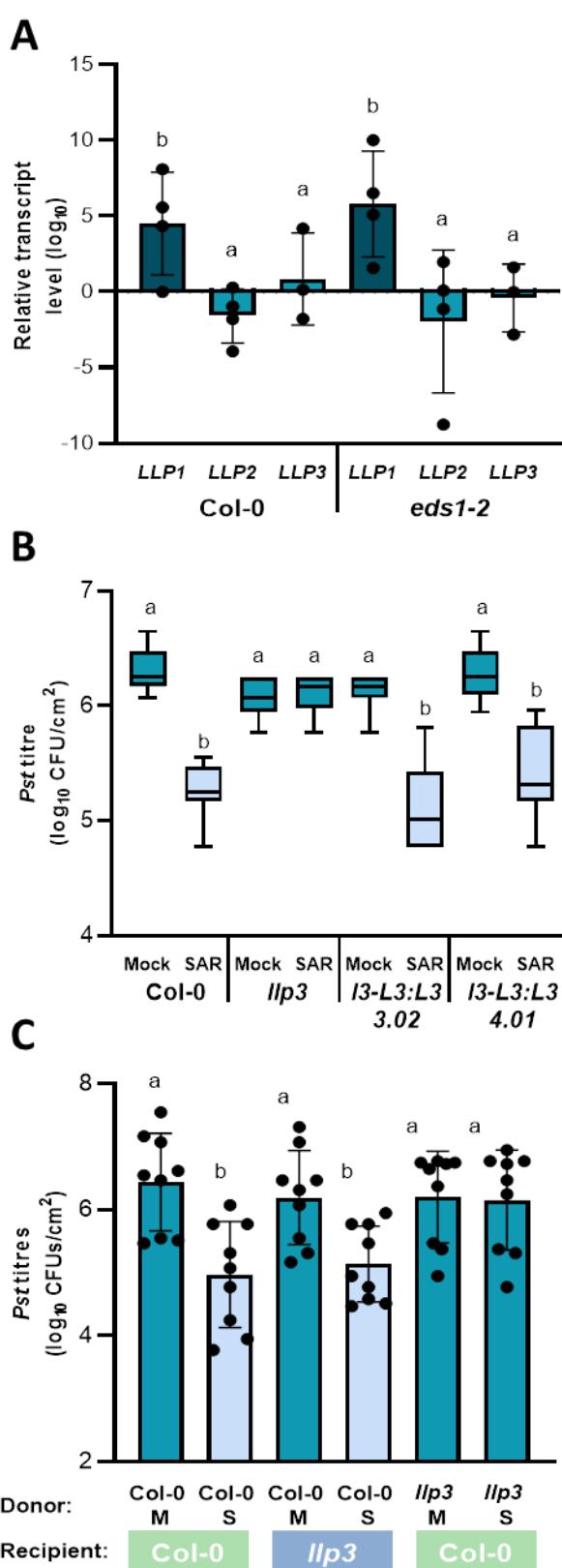
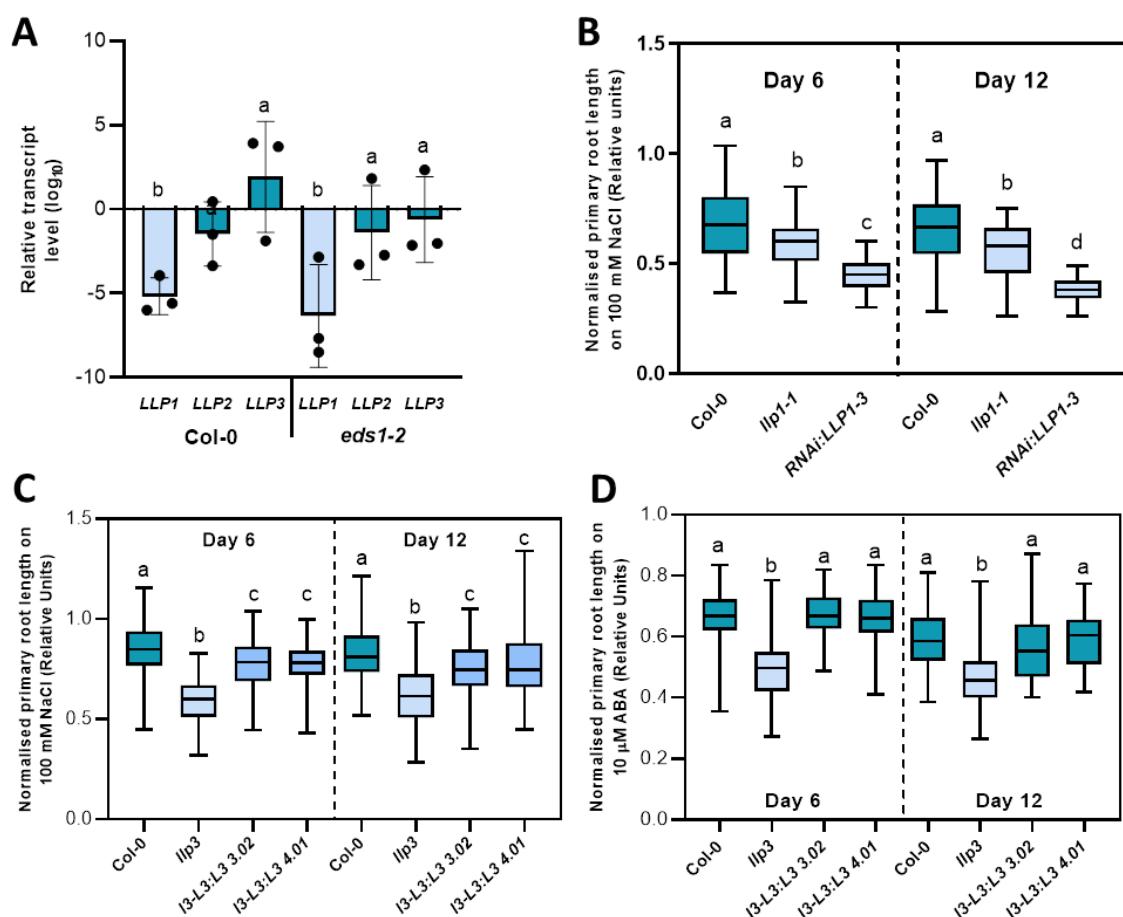


Figure 1 LEGUME LECTIN-LIKE PROTEIN3 (LLP3) promotes systemic acquired resistance (SAR) signal generation/transmission. (A) *LLP3* transcript accumulation is not affected by salicylic acid (SA). 4-week-old Col-0 wild type and *eds1-2* Arabidopsis plants were spray-treated with 1mM SA, and 24 hours (h) later *LLP1*, *LLP2*, and *LLP3* transcript accumulation was determined by RT-qPCR. Transcript accumulation was normalized to that of *UBIQUITIN* and is shown relative to the normalized transcript levels in the appropriate mock controls. Bars represent the log₂(mean) ± SEM of four biologically independent replicates. The letters above the bars indicate statistically significant differences (one-way ANOVA, n=4, P<0.05, F=4.623, DF=22). (B) *LLP3* is required for SAR. Plants were infiltrated locally with either *Pst AvrRpm1* (SAR) or with 10 mM MgCl₂ as the mock control (M). To monitor SAR, 3 days after the primary treatment leaves distal to the initial treatment site were infiltrated with *Pst*. Plant lines included *Iip3* mutants and 2 independently transformed complementation lines carrying a transgene driving *LLP3* expression from its native promoter (*I3-L3:L3* 3.02 and 4.01). Box plots represent average *Pst* titres in systemic leaves at 4 days post-inoculation (dpi) from 4 biologically independent experiments, including 3 replicates each ± min and max values. The letters above the box plots indicate statistically significant differences (Kruskal-Wallis test, P<0.05, n=12, KW statistic=101.4). (C) *LLP3* is required to send, but not to receive phloem-mobile SAR signals. Leaves of donor plants were inoculated with *Pst AvrRpm1* (S) or with the appropriate mock control (M). After 24 h, petiole exudates were collected from the donor plants and infiltrated into leaves of naïve

recipient plants. 24h later, the treated leaves were challenged with *Pst*. Bars represent average *Pst* titres at 4 dpi from 3 biologically independent experiments, including 3 replicates each ± SD. The letters above the bars indicate statistically significant differences (one-way ANOVA, n=9, P<0.05, F=6.258, DF=35).



1
2 **Figure 2** *LLP1*, *LLP2*, and/or *LLP3* compromise Arabidopsis responses to salt stress. (A) *LLP1* transcript
3 accumulation is reduced after ABA treatment. Col-0 wild type and *eds1-2* plants were spray-treated with 100 μ M
4 ABA, and after 24 h *LLP1*, *LLP2*, and/or *LLP3* transcript accumulation was determined by RT-qPCR. Transcript
5 accumulation was normalized to that of *UBIQUITIN* and is shown relative to the normalized transcript levels in the
6 appropriate mock controls. Bars represent the \log_2 (mean) \pm SEM of three biologically independent replicates. The
7 letters above the bars indicate statistically significant differences (one-way ANOVA, $n=3$, $P<0.05$, $F=6.291$,
8 $DF=40$). (B/C) *LLP1*, *LLP2*, and/or *LLP3* compromise salt-associated root growth inhibition. Seedlings of Col-0
9 wild type, *lfp1-1*, and *RNAi:LLP1-3* (B) and of *lfp3* and two *lfp3-LLP3:LLP3* complementation lines (*I3-L3:L3 3.02*
10 and *4.01*; C) were germinated on control MS plates, and after 6 days transferred to either further control plates, or
11 to plates supplemented with 100 mM NaCl. Primary root length was measured at 6 and 12 days post transfer and
12 normalized to that of the same genotype on control plates. Box plots represent average normalized root length \pm
13 min and max values. The letters above the box plots indicate statistically significant differences (B: one-way
14 ANOVA, $P<0.05$, $F=30.70$, $DF=233$, for day 6, Col-0 $n=48$, *lfp1-1* $n=38$, *RNAi:LLP1* $n=40$, for day 12 Col-0 $n=48$,
15 *lfp1-1* $n=29$, *RNAi:LLP1-3* $n=21$; C: Day 6: Kruskal-Wallis test, $P<0.05$, KW test statistic =165.5, Col-0 $n=83$, *lfp3*
16 $n=86$, *I3-L3:L3 3.02* $n=96$, *I3-L3:L3 4.01* $n=89$. Day 12: one-way ANOVA, $P<0.05$, $F=25.08$, $DF=519$, Col-0
17 $n=81$, *lfp3* $n=84$, *I3-L3:L3 3.02* $n=94$, *I3-L3:L3 4.01* $n=84$). These experiments were repeated 3 (C) to 4-8 times
18 (B) with comparable results. (D) *LLP3* compromises root growth inhibition on 10 μ M ABA. Col-0 wild type, *lfp3* and
19 two *lfp3-LLP3:LLP3* complementation lines were treated as described in (B/C), and the treatment plates were
20 supplemented with 10 μ M ABA. Box plots represent average normalized root length \pm min and max values. The
21 letters above the box plots indicate statistically significant differences (Day 6: one-way ANOVA, $P<0.05$,
22 $F=76.10$, $DF=538$, Col-0 $n=76$, *lfp3* $n=86$, *3.01* $n=89$, *I3-L3:L3 3.02* $n=98$, *I3-L3:L3 4.01* $n=94$, *8.01* $n=96$. Day 12:
23 Kruskal-Wallis test, $P<0.05$, KW test statistic =121.1, Col-0 $n=85$, *lfp3* $n=90$, *I3-L3:L3 3.02* $n=100$, *I3-L3:L3 4.01*
24 $n=97$). This experiment was repeated 3 times with comparable results.
25

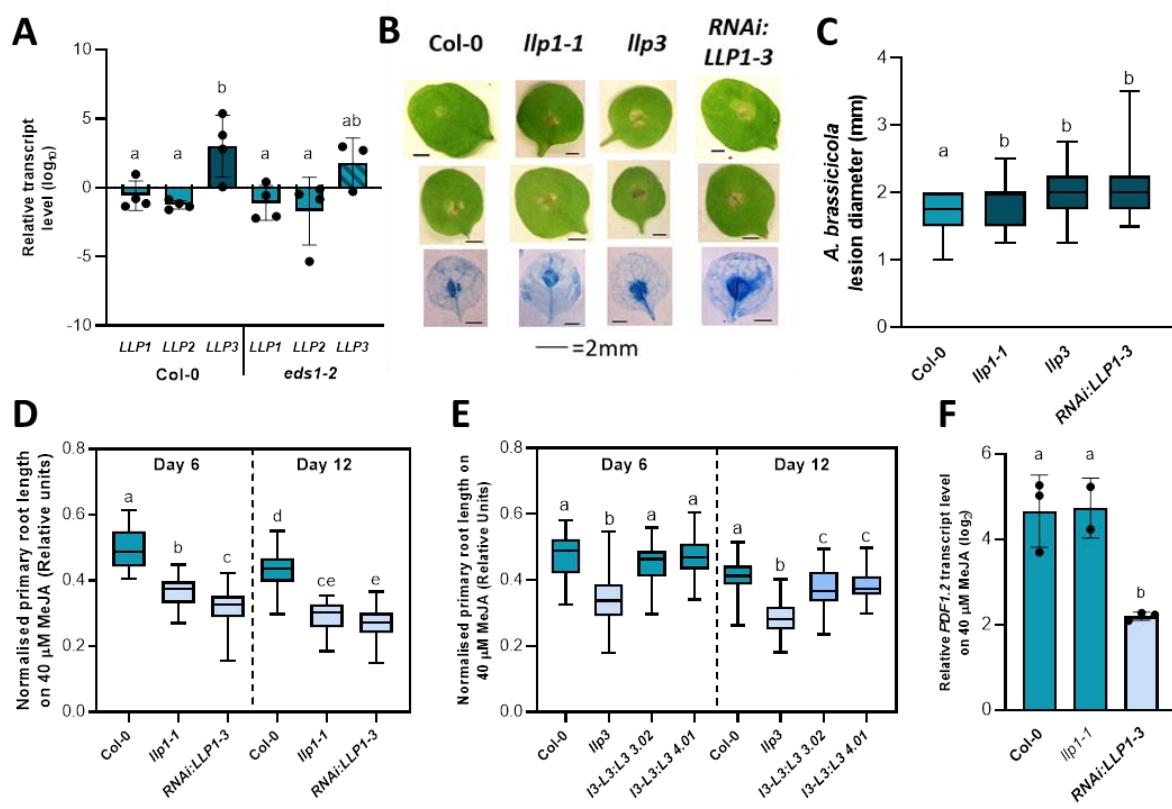


Figure 3 *LLP1*, *LLP2*, and/or *LLP3* differentially affect jasmonic acid (JA)-associated responses in *Arabidopsis*. (A) *LLP3* transcript accumulation is induced by methyl jasmonate (MeJA). *Col-0* wild type and *eds1-2* plants were spray-treated with 100 μ M MeJA, and after 24 h *LLP1*, *LLP2*, and/or *LLP3* transcript accumulation was determined by RT-qPCR. Transcript accumulation was normalized to that of *UBIQUITIN* and is shown relative to the normalized transcript levels in the appropriate mock controls. Bars represent the \log_2 (mean) \pm SEM of four biologically independent replicates. The letters above the bars indicate statistically significant differences (one-way ANOVA, $n=4$, $P<0.05$, $F=4.493$, $DF=45$). (B/C) *LLP1*, *LLP2*, and/or *LLP3* promote JA-associated defence against *Alternaria brassicicola*. Droplets containing spores of the necrotrophic fungus *A. brassicicola* were placed on the leaves of four-week-old *Col-0* wild type, *llp1-1*, *llp3*, and *RNAi:LLP1-3* plants. Resulting lesions were photographed (B) and measured (C) 5 days later. Box plots in (C) represent mean lesion diameters from 4 biologically independent experiments including 15 replicates each \pm min and max values. The letters above the box plots indicate statistically significant differences (Kruskal-Wallis test, $P=<0.05$, KW test statistic =24.10, $n=60$ for all genotypes). (D/E) *LLP1*, *LLP2*, and/or *LLP3* compromise JA-associated root growth inhibition. Seedlings of *Col-0* wild type, *llp1-1*, and *RNAi:LLP1-3* (D) and of *llp3* and two *llp3-LLP3:LLP3* complementation lines (*I3-L3:L3* 3.02 and 4.01; E) were germinated on control MS plates, and after 6 days transferred to either further control plates, or to plates supplemented with 40 μ M MeJA. Primary root length was measured at 6 and 12 days post transfer and normalized to that of the same genotype on control plates. Box plots represent average normalized root length \pm min and max values. The letters above the box plots indicate statistically significant differences (D: Day 6: one-way ANOVA, $F=44.87$, $DF=147$, *Col-0* $n=29$, *llp1-1*, *RNAi:LLP1-3* $n=30$. Day 12: one-way ANOVA, $F=74.62$, $DF=175$, *Col-0* $n=29$, *llp1-1* $n=30$, *RNAi:LLP1-3* $n=28$; E: Day 6: one-way ANOVA, $P<0.05$, $F=61.40$, $DF=541$, *Col-0* $n=71$, *llp3* $n=85$, *I3-L3:L3* 3.02 $n=94$, *I3-L3:L3* 4.01 $n=97$. Day 12: Kruskal-Wallis test, $P=<0.05$, KW test statistic =140.7, *Col-0* $n=63$, *llp3* $n=73$, *I3-L3:L3* 3.02 $n=94$, *I3-L3:L3* 4.01 $n=97$, 8.01 $n=95$). These experiments were repeated 3 (E) to 5 times (D) with comparable results. (F) *LLP1*, *LLP2*, and/or *LLP3* compromise MeJA-induced *PDF1.2* transcript accumulation. *PDF1.2* transcript accumulation was monitored by qRT-PCR in seedlings from (D). Transcript accumulation was normalized to that of *UBIQUITIN* and is shown relative to the normalized transcript levels in the appropriate mock controls. Bars represent the \log_2 (mean) \pm SEM of biologically independent replicates. The letters above the bars indicate statistically significant differences (one-way ANOVA, $P=<0.05$, $F=14.93$, $DF=7$, $n=3$ for *Col-0* and *RNAi:LLP1-3*, $n=2$ for *llp1-1*).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

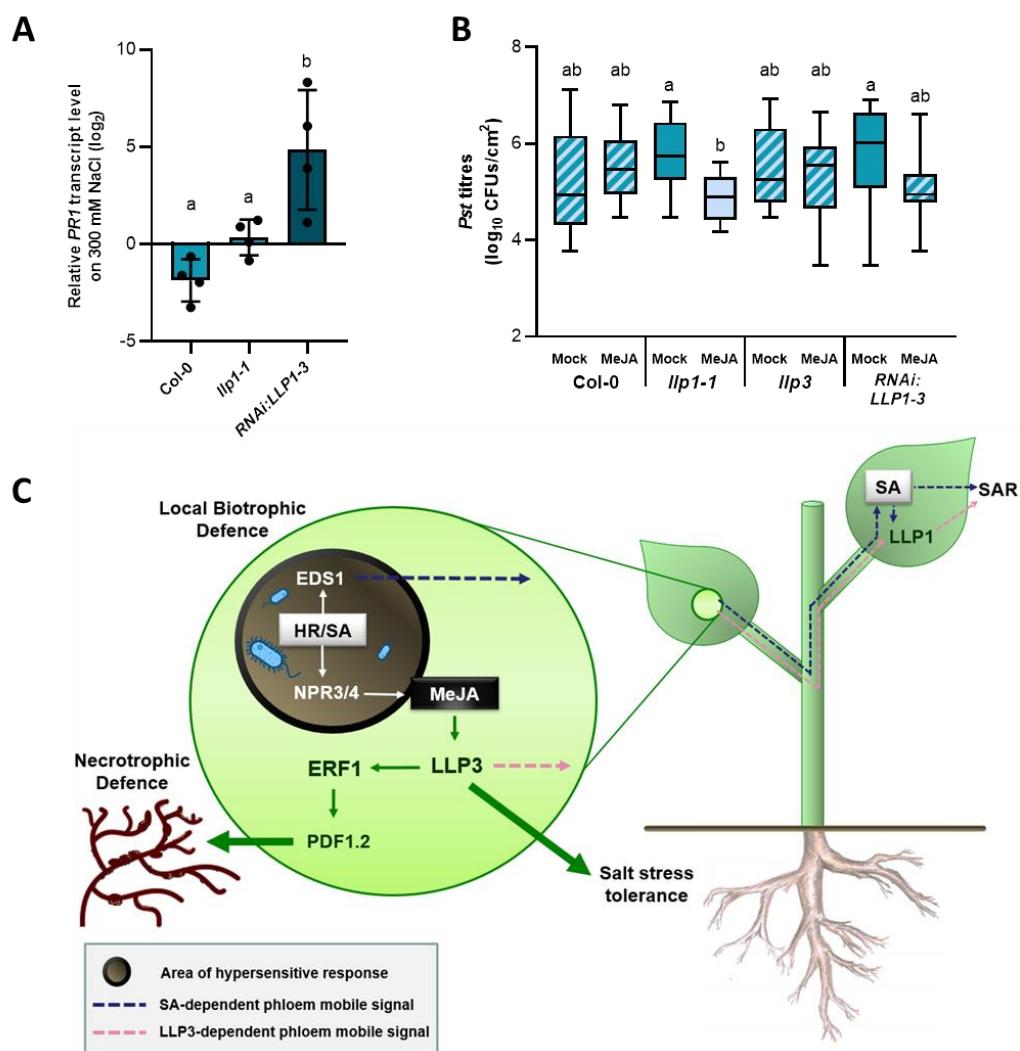


Figure 4 *LLP1*, *LLP2*, and/or *LLP3* dampen antagonistic SA-JA cross talk between defence pathways. (A) Exposure to salt drives up *PR1* transcript levels when *LLP1*, *LLP2*, and/or *LLP3* transcript levels are reduced. Four-week-old Col-0 wild type, *llp1-1*, and *RNAi:LLP1-3* plants were irrigated with 300 mM NaCl three times over the course of 9 days. Three days later, *PR1* transcript accumulation in the leaves was determined by qRT-PCR. Transcript accumulation was normalized to that of *UBI/QUITIN* and is shown relative to the normalized transcript levels in the appropriate mock controls. Bars represent the $\log_2(\text{mean}) \pm \text{SEM}$ of four biologically independent replicates. The letters above the bars indicate statistically significant differences (one-way ANOVA, $n=4$, $P<0.05$, $F=12.23$, $DF=11$). (B) In the absence of functional *LLP1*, MeJA triggers SAR-like resistance in distal tissues. Col-0 wild type, *llp1-1*, *llp3*, and *RNAi:LLP1-3* plants were treated locally with 100 μM MeJA by leaf infiltration. To monitor systemic SA-associated defence responses, leaves distal to the site of the initial treatment were inoculated with *Pst* 3 days after the primary treatment. Box plots represent average *Pst* titres in systemic leaves at 4 dpi from 4-5 biologically independent experiments, including 3-4 replicates each \pm min and max values. The letters above the box plots indicate statistically significant differences (Kruskal-Wallis test, $P<0.05$, KW test statistic=20.61, Col-0 mock $n=17$, MeJA $n=20$, *llp1-1* mock $n=18$, MeJA $n=19$, *llp3* mock $n=11$, MeJA $n=11$, *RNAi:LLP1-3* mock $n=19$, MeJA $n=19$). (C) *LLP3* promotes local SAR signal generation downstream of (Me)JA accumulating in the perimeter of HR (hypersensitive response) lesions. Elevated *LLP3* expression promotes *PDF1.2* expression and defence against necrotrophic pathogens through *ERF1* (ETHYLENE RESPONSE FACTOR 1) as well as salt stress tolerance. In parallel with EDS1-dependent, SA-associated long distance signals, *LLP3* promotes accumulation or transmission of a long distance SAR signal downstream of (Me)JA. Systemically, *LLP1* balances incoming signals promoting SAR while restricting deleterious effects of SA-associated SAR on JA-associated defence responses. Abbreviations: NPR3/4, NON-EXPRESSOR OF PR GENES3/4