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Ranf S, Gisch N, Schäffer M, Illig T, Westphal L, Knirel YA, Sánchez-Carballo PM, Zähringer U, Hückelhoven R, Lee J, Scheel D (2015). A lectin S-domain receptor kinase mediates lipopolysaccharide sensing in *Arabidopsis thaliana*. *Nature Immunology* 16(4): 426–433.

Version of record: <https://www.nature.com/articles/ni.3124>

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A lectin S-domain receptor kinase mediates lipopolysaccharide sensing in *Arabidopsis thaliana*

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ABSTRACT

Microbe-associated molecular pattern (MAMP) sensing triggers innate immunity in animals and plants. Lipopolysaccharide (LPS) of Gram-negative bacteria is a potent MAMP in mammals with the lipid A moiety activating proinflammatory responses via Toll-like receptor 4. Here, we show that *Arabidopsis thaliana* specifically senses LPS of *Pseudomonas* and *Xanthomonas*. We isolated LPS-insensitive mutants defective in the bulb-type lectin S-domain-1 receptor-like kinase LORE (LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION, AT1G61380) *alias* SD1-29, which are hypersusceptible to *Pseudomonas syringae* infection. Chemical dissection of *Pseudomonas* LPS suggests that LORE primarily detects the lipid A moiety of LPS. LORE confers LPS sensitivity to tobacco upon transient expression, which demonstrates a key function in LPS sensing and provides the prospect of engineering resistance to bacteria in crop species.

INTRODUCTION

Yield losses due to infestation with pathogenic microorganisms are of major agro-economical concern. Plant breeding aims at producing resistant varieties by exploiting the plant's natural

defense repertoire to reduce the application of chemical pesticides. Lacking an adaptive immune system, plants predominantly rely on innate immunity^{1,2}. They evolved diverse immune receptors to detect microbial invaders through microbe-associated molecular patterns (MAMPs), microbial effectors, and microbe-induced cellular perturbations. MAMPs, generally common to a wide range of pathogens and conserved due to their essential functions for the pathogen, are detected by specific pattern-recognition receptors (PRRs) at the cell surface and induce pattern-triggered immunity (PTI). To infect host plants, some pathogens have evolved effectors to subvert PTI.

Interestingly, genome analysis revealed a disproportionate increase of the family of plant cell surface receptor genes – with >400 receptor-like kinases (RLKs) and >170 receptor-like proteins (RLPs) in *A. thaliana*^{3,4}. RLKs are membrane-spanning proteins with a cytoplasmic kinase domain for intracellular signaling and diverse extracellular domains with ligand-binding potential. To date, the biological function of only a few RLK-ligand-pairs has been elucidated, several of which are involved in PTI. The leucine-rich-repeat (LRR)-RLKs, FLAGELLIN SENSING 2 (FLS2) and EF-TU RECEPTOR (EFR) sense bacterial flagellin-derived flg22 and elongation factor Tu (EF-Tu)-derived elf18 peptide MAMPs, which also requires the LRR-RLK, BAK1 (BRASSINOSTEROID-INSENSITIVE-1-ASSOCIATED KINASE 1)⁵. Similarly, the CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1, *alias* LYK1) acts in concert with other LysM-RLKs, LYK4 and LYK5, or LysM-RLPs, LYM1 and LYM3, to sense fungal chitin oligomers or bacterial peptidoglycan, respectively⁵⁻⁷. Generally, different MAMPs activate a common set of signaling and defense responses. Rapid changes in the cytosolic concentration of the secondary messenger Ca^{2+} ($[\text{Ca}^{2+}]_{\text{cyt}}$) are crucial for downstream responses. Subsequent generation of reactive oxygen species (ROS) by the NADPH oxidase, RBOHD, directly confines pathogen spread *via* toxic effects and contributes to signaling. Deposition of the β -1,3-glucan callose leads to cell wall fortification. Activation of calcium-dependent and mitogen-activated protein kinases (MPK) switches metabolic processes and gene expression to a defensive state².

In mammals, several LRR-type Toll-like receptors (TLRs) sense MAMPs such as flagellin or lipopolysaccharide (LPS). LPS, abundant in the Gram-negative bacteria outer cell envelope, consists of lipid A and a core oligosaccharide region that is mostly decorated with an O-polysaccharide (OPS) consisting of many oligosaccharide repeats⁸. The variable OPS exhibits specific antigenic activities in the adaptive immune system^{8,9}. The outer core oligosaccharide of *Pseudomonas aeruginosa* LPS is recognized by cystic fibrosis transmembrane conductance regulator (CFTR)^{10,11}. The lipid A moiety is sensed as a MAMP by the innate immune system

via TLR4-MD2 (Toll-like receptor 4, myeloid differentiation factor 2) and activates proinflammatory responses¹². An exaggerated, uncontrolled immune reaction to LPS, also called endotoxin, can result in sepsis and life-threatening septic shock¹². Additionally, certain mammalian caspases have been identified as TLR4-independent intracellular LPS sensors¹³.

LPS also acts as a MAMP in different plants species^{14,15}. In the model plant *Arabidopsis thaliana*, for instance, nitric oxide production is stimulated by LPS from different bacteria as well as enterobacterial lipid A¹⁶, while defense gene expression is induced by core oligosaccharides and lipid A from *Xanthomonas campestris*, lipid A from *Burkholderia cepacia* as well as by OPS rhamnans, found in many phytopathogenic bacteria¹⁶⁻¹⁹. Thus, plants are capable of sensing all three parts of LPS. However, the underlying plant sensing mechanisms for the different LPS components are yet unknown¹⁴.

Here, we report that *A. thaliana* sensitively detects LPS of *Pseudomonas* spp. and *X. campestris* via the bulb-type lectin S-domain-1 receptor-like kinase LORE (*alias* SD1-29). *lore* mutants are impaired in LPS-triggered PTI to *Pseudomonas syringae* infection. Phylogeny of LORE-like SD-RLKs suggests that LORE-mediated LPS sensing is restricted to the plant family of *Brassicaceae*. Transient expression of LORE confers LPS sensitivity to solanaceous tobacco, indicating that LORE is an LPS immune receptor. Finally, we demonstrate that the lipid A moiety is required and sufficient for LORE-dependent sensing of LPS.

RESULTS

Pseudomonas* LPS acts as MAMP in *A. thaliana

Using *A. thaliana* plants carrying the calcium reporter aequorin (Col-0^{AEQ}), it was shown that MAMPs rapidly induce characteristic $[Ca^{2+}]_{cyt}$ elevations²⁰. LPS extracted from several *Pseudomonas* spp. induced $[Ca^{2+}]_{cyt}$ elevations in *A. thaliana* seedlings (**Fig. 1a**). Sources of LPS included phytopathogenic *P. syringae*, *P. fluorescens*, and *P. alcaligenes*, common soil and water bacteria, as well as human-pathogenic *P. aeruginosa* (**Table 1**). LPS-induced $[Ca^{2+}]_{cyt}$ elevations were not altered in several RLK mutants (*cerk1*, *lyk4*, *fls2*, *efr*, and *bak1*) or after treatment of LPS preparations with proteinase K (**Supplementary Fig. 1a,b**). The Ca^{2+} channel blocker $LaCl_3$ or the protein kinase inhibitor K-252a inhibited the LPS-induced $[Ca^{2+}]_{cyt}$ elevations (**Supplementary Fig. 1c**). To determine the LPS structural regions crucial for sensing by *A. thaliana*, we used *P. aeruginosa* mutants defective in LPS core oligosaccharide biosynthesis. The PAN1 mutant strain, a so-called rough-type strain, lacks the OPS²¹. The phosphoglucomutase $\Delta algC$ mutant or phage-resistant strains H4 and R5, further lack most of the outer core region²² (**Supplementary Fig. 1d**). LPS from these mutants

triggered $[Ca^{2+}]_{cyt}$ elevations similar to smooth-type OPS-containing LPS e.g. from *P. aeruginosa* PAO1 or Fisher-type 1 (F1) (**Fig. 1a**). LPS concentrations $<0.5 \mu\text{g/ml}$ induced $[Ca^{2+}]_{cyt}$ elevations in *A. thaliana* (**Fig. 1b**), which were substantially lower than LPS elicitor concentrations currently reported for plants^{14,15}. The size heterogeneity of the repetitive OPS in smooth-type LPS precludes estimation of molar concentrations. However, for rough-type LPS such as that from *P. aeruginosa* H4 (main component MW $\sim 2800 \text{ Da}$)²³ a concentration of $0.5 \mu\text{g/ml}$ can be estimated to correspond to 150-200 nM – a concentration typically used for other MAMPs in plants. Thus, *A. thaliana* sensitively detects the conserved inner core-lipid A moiety of *Pseudomonas* LPS^{8,22}. Besides $[Ca^{2+}]_{cyt}$ elevations, *Pseudomonas* LPS triggered ROS accumulation via NADPH oxidase RBOHD, activation of mitogen-activated protein kinases, MPK3 and MPK6, as well as callose deposition (**Supplementary Fig. 2**). Therefore, *Pseudomonas* LPS initiates MAMP-responses typically mediated by PRRs in plants^{2,20}.

***Pseudomonas* LPS sensing requires the RLK LORE**

Screening for mutants with altered MAMP-induced $[Ca^{2+}]_{cyt}$ elevations proved suitable for identifying components of PRR complexes²⁴. In a genetic screen for mutants that are impaired in *Pseudomonas* LPS-induced $[Ca^{2+}]_{cyt}$ elevations, we isolated four allelic *A. thaliana* mutants that were specifically insensitive to *Pseudomonas* LPS and named *lore-1* to *lore-4* for *lipooligosaccharide-specific reduced elicitation* (**Fig. 2a-c** and **Supplementary Fig. 3a**). Genetic mapping identified LORE as S-domain-1 receptor-like kinase 29 (SD1-29, AT1G61380), a bulb-type (B-type) lectin RLK (**Supplementary Fig. 3b**). LORE is strongly up-regulated by MAMP treatment and pathogen infection and co-expressed with the PRR genes, *FLS2* and *EFR*, supporting a potential role in PTI²⁵. *A. thaliana* S-domain-RLKs are encoded by a multi-gene family, with the ‘SD1’ subfamily consisting of 32 members that share a similar domain structure⁴ (**Supplementary Fig. 3c**). A signal peptide, together with a transmembrane domain, suggests plasma membrane localization of LORE. An N-terminal B-type lectin domain, followed by an S-locus glycoprotein or epidermal growth factor (EGF) domain and a plasminogen-apple-nematode (PAN) domain comprise the extracellular putative ligand-binding region. The cytoplasmic serine-threonine protein kinase is a so-called ‘RD kinase’, i.e. an arginine precedes the conserved aspartate proton acceptor within the kinase domain². A domain of unknown function, DUF3403, constitutes the very C-terminal end. Mutations in *lore-2* and *lore-3* lead to amino acid exchanges within the PAN and kinase domain, whereas *lore-1* and *lore-4* are truncated through premature stop codons after the EGF domain or within the kinase domain, respectively (**Supplementary Fig. 3c**). At the functional level all *lore* mutants showed

greatly abolished LPS-triggered $[Ca^{2+}]_{cyt}$ elevations (**Fig. 2a-c** and **Supplementary Fig. 3a**). In addition, a T-DNA insertion line of *SD1-29* (*sd1-29*, SAIL_857_E06) was obtained, which lacks detectable full-length *SD1-29* transcripts (**Supplementary Fig. 3b,d**). Aequorin-expressing *sd1-29^{AEQ}* plants, generated by crossing of *sd1-29* and Col-0^{AEQ} plants, also showed strongly diminished LPS-triggered $[Ca^{2+}]_{cyt}$ elevations (**Fig. 2b** and **Supplementary Fig. 3e**). A survey with eleven different smooth- and rough-type *Pseudomonas* LPS confirmed diminished $[Ca^{2+}]_{cyt}$ elevations in *lore* and *sd1-29^{AEQ}* but all mutants responded normally to chitin, flg22 and elf18 (**Fig. 2b**). Whereas $[Ca^{2+}]_{cyt}$ measurements using the ‘native’ aequorin luminophore, coelenterazine, showed little $[Ca^{2+}]_{cyt}$ elevations above the resting level in *lore* mutant lines (**Fig. 2a** and **Supplementary Fig. 3a**), the more sensitive coelenterazine derivative ‘h’ revealed some marginal but variable $[Ca^{2+}]_{cyt}$ fluctuations in the mutant lines in response to individual LPS preparations (**Fig. 2b** and **Supplementary Fig. 3e**). Additionally, introduction of a genomic DNA fragment, covering the *SD1-29* open reading frame and a 1 kb upstream cis-regulatory region, in *lore-1* (complementation lines, CL) restored the $[Ca^{2+}]_{cyt}$ response to *Pseudomonas* LPS (**Fig. 2c**). In line with the strongly diminished $[Ca^{2+}]_{cyt}$ elevations, LPS-triggered ROS accumulation, MPK3 and MPK6 activation and expression of PTI response genes, e.g. *FRK1* and *GST1*, were also largely abolished in *lore* and *sd1-29* mutants (**Fig. 2d-g** and **Supplementary Fig. 3f**). The closest *LORE* paralog, *SD1-23* (AT1G61390; compare **Supplementary Fig. 5a**) lies in tandem with *SD1-29*. It shares overall 77% amino acid identity with *SD1-29* and the potential ligand-binding ectodomain is even 81% identical. However, *SD1-23* appeared not to be required for LPS sensing, as a T-DNA insertion mutant (*sd1-23*, GABI_079G10), lacking detectable full-length *SD1-23* transcripts (**Supplementary Fig. 3b,d**), responded to LPS treatment with PTI responses comparable to the Col-0 control (**Fig. 2d,f,g** and **Supplementary Fig. 3f**). In conclusion, *LORE* is required for sensing of the conserved inner core-lipid A moiety of *Pseudomonas* LPS.

LPS sensing modulates immunity to *Pseudomonas* infection

In line with the diminished MAMP responses upon treatment with *Pseudomonas* LPS, *lore* and *sd1-29* mutants were more susceptible to virulent *Pst* DC3000 than the respective Col-0 or Col-0^{AEQ} controls, *sd1-23* mutants or two independent complementation lines (CL) upon spray inoculation (**Fig. 3a** and **Supplementary Fig. 4a**). MAMP application induces PTI resulting in pathogen resistance in *A. thaliana*^{2,26}. Accordingly, Col-0 and Col-0^{AEQ} control plants pre-treated with *Pseudomonas* LPS were more resistant to subsequent post-invasive *Pst* DC3000 infection but this LPS-induced resistance was lost in *lore* and *sd1-29* mutants (**Fig. 3b** and

Supplementary Fig. 4b,c). These results highlight an important role of LORE in plant LPS-triggered immunity.

LORE is also required for sensing of *Xanthomonas* LPS

LPS from *Xanthomonas* spp. and *Burkholderia* spp. have been shown to induce defense responses in *A. thaliana*¹⁴. To further determine the specificity of LORE-mediated LPS sensing, LPS preparations from these bacteria as well as enterobacterial LPS were tested for LORE-dependent early PTI responses (**Fig. 4a-c**). *X. campestris* LPS triggered $[Ca^{2+}]_{cyt}$ elevations and ROS production in Col-0^{AEQ} and Col-0 control plants (**Fig. 4a,c**). By contrast, no obvious $[Ca^{2+}]_{cyt}$ elevations or ROS production was detectable upon application of LPS from *B. cepacia* or *B. pseudomallei* as well as from enterobacteria such as *Escherichia coli* or *Salmonella enterica* (**Fig. 4a-c**). *X. campestris* LPS-triggered $[Ca^{2+}]_{cyt}$ elevations were greatly diminished in *lore* and *sd1-29*^{AEQ} mutants but restored in two complementation lines (CL) (**Fig. 4d**). Likewise, *X. campestris* LPS-triggered ROS production was LORE-dependent (**Fig. 4e**). While pre-treatment with *Pa* H4 and *Pst* LPS led to resistance to subsequent *Pst* DC3000 infection in Col-0 and Col-0^{AEQ} control plants, only a tendency toward higher resistance was observed with *X. campestris* LPS (**Supplementary Fig. 4b,c**). Pre-treatment with *E. coli* LPS also induced resistance to *Pst* DC3000 infection in several experiments (**Fig. 4f** and **Supplementary Fig. 4b,c**). However, in contrast to resistance induced by *Pseudomonas* LPS, *E. coli* LPS-induced resistance was independent of LORE. Taken together, this suggests that a pattern common to LPS of both *Pseudomonas* spp. and *X. campestris* but differing in LPS of *E. coli*, *S. enterica*, and *Burkholderia* spp. is specifically sensed in a LORE-dependent manner in *A. thaliana*.

LORE-mediated LPS sensing is limited to the *Brassicaceae*

SD-RLKs are widespread in land plants, especially in flowering plants (Angiosperms)²⁷. Comparison of *A. thaliana* and rice *SD-RLKs* shows a rapid increase of different subgroups, where *LORE* belongs to a subgroup containing mainly *A. thaliana* *SD-RLKs*^{25,27}. Further phylogenetic analysis of LORE-related proteins of diverse plant families suggested that LPS sensing via this class of RLKs is restricted to the *Brassicaceae* (**Supplementary Fig. 5a**). Genome analysis of the *Brassicaceae* species *Eutrema salsugineum* and *Capsella rubella* revealed LORE homologs with 81% and 92% overall amino acid identity to *A. thaliana* LORE (AtLORE), respectively. Indeed, both species reproducibly generated ROS in response to *Pseudomonas* and *X. campestris* LPS but not *E. coli* LPS (**Supplementary Fig. 5b**). Thus, the

similar LPS sensing specificity to that of AtLORE strongly suggests that the closest homologs described above may be LORE orthologs mediating LPS sensing.

LORE confers LPS sensitivity to solanaceous tobacco

According to the phylogenetic analysis no LORE orthologs are predicted in solanaceous tobacco. Therefore, we tested the response of tobacco to LPS. The tested LPS preparations did not consistently trigger ROS production in *Nicotiana tabacum* or *Nicotiana benthamiana* leaf discs (**Supplementary Fig. 5c**). Besides slight and variable elevations of the resting $[Ca^{2+}]_{cyt}$ concentrations with some of the LPS preparations, we also did not observe typical MAMP-triggered $[Ca^{2+}]_{cyt}$ elevation kinetics in *N. tabacum* seedlings (**Supplementary Fig. 5d,e**). Thus, we examined if transient expression of LORE in tobacco can confer LPS responsiveness. In support of the predicted localization, transiently expressed fluorescent LORE-eGFP fusion proteins are detected at the plasma membrane in tobacco (**Fig. 5a** and **Supplementary Fig. 5f**). Furthermore, LORE expression in tobacco led to a rapid *Pseudomonas* LPS-induced ROS production in contrast to control leaves expressing just mCherry, or a LORE-K516A variant, mutated in the conserved ATP binding site of the kinase domain (**Fig. 5b**). The latter supports that *LORE* encodes a functional kinase²⁸ whose activity is required for LPS-triggered ROS accumulation. In addition to LPS of diverse *Pseudomonas* spp., LPS of *X. campestris* but not *B. cepacia* or *E. coli* elicited ROS production in LORE-eGFP-expressing tobacco (**Fig. 5c**). Hence, the LPS specificity of LORE-mediated LPS sensing is maintained upon heterologous expression in tobacco. Taken together, our data prove that a functional LORE is required and decisive for conferring sensitivity to *Pseudomonas* and *X. campestris* LPS to plants.

Lipid A is sufficient for LORE-mediated LPS sensing

Further genetic dissection of the MAMP-active LPS motif is not feasible in *Pseudomonas* because deep-rough mutations of the inner core are lethal²². Therefore, we used rough-type *Pa* H4 LPS to further analyze the MAMP activity of the distinct LPS moieties upon chemical modification and separation. Hydrazine treatment removed all ester-bound fatty acids resulting in LPS-OH carrying only the two primary amide-bound fatty acids attached to the lipid A-backbone. LPS-OH did not trigger $[Ca^{2+}]_{cyt}$ elevations or ROS accumulation in Col-0^{AEQ} and Col-0 plants, respectively (**Fig. 6a,b**). Mild acid hydrolysis separated the lipid A moiety from the inner core oligosaccharide²³. Further fractionation by gel permeation chromatography (GPC) and high performance liquid chromatography (HPLC) yielded pure core oligosaccharide and lipid A, respectively. We obtained two major lipid A fractions that contained mainly penta-

acylated lipid A species with marginal modifications in their fatty acid composition (**Supplementary Fig. 6a,b**). Strikingly, these lipid A fractions both elicited $[Ca^{2+}]_{cyt}$ elevations and ROS production, albeit to a lower extent than rough-type LPS, whereas the isolated core oligosaccharide did not induce $[Ca^{2+}]_{cyt}$ elevations (**Fig. 6a,b**). Moreover, the lipid A-induced $[Ca^{2+}]_{cyt}$ elevations were LORE-dependent as they were diminished in *lore* mutants but restored in complementation lines (**Fig. 6c** and **Supplementary Fig. 6c**). Likewise, lipid A-induced ROS production required LORE (**Fig. 6d**). In agreement with the results obtained with *E. coli* LPS, HPLC-purified Kdo₂-lipid A (hexa-acylated) from the deep-rough *E. coli* strain KPM 53²⁹ did not trigger $[Ca^{2+}]_{cyt}$ elevations or ROS production. Finally, these results demonstrate that in *A. thaliana* *Pseudomonas* LPS is specifically sensed through its lipid A moiety and that the lipid A moiety is sufficient for immune sensing in a LORE-dependent manner.

DISCUSSION

LPS preparations from various bacteria induce defense responses in different plant species suggesting that plants possess LPS sensing systems with species-specific recognition of distinct structural determinants¹⁴. To date, however, the plant LPS sensing components remained unknown. In this study, we provide evidence for a sensitive sensing system for *Pseudomonas* LPS in the plant family of *Brassicaceae*. Smooth- and rough-type LPS as well as isolated lipid A act as MAMPs and trigger PTI in *A. thaliana* via the B-type lectin S-domain-1 RLK LORE (*alias* SD1-29). *X. campestris* LPS is likewise sensed in a LORE-dependent manner, whereas LPS from *E. coli*, *S. enterica*, or *Burkholderia* spp. do not activate typical early PTI responses in *A. thaliana*. In mammals, TLR4-MD2 is strongly activated by the prototypical enterobacterial hexa-acylated lipid A of *E. coli*³⁰, while other lipid A variants from diverse bacteria including *Pseudomonas* are less potent agonists due to different acylation, phosphorylation and other structural modifications^{30,31}. Penta-acylated lipid A from *P. aeruginosa* only weakly activates human TLR4 but strongly activates murine TLR4, demonstrating host species-specificity in sensing lipid A^{8,31}. Similarly, *A. thaliana* appears to sense a specific structural LPS variant occurring in both *Pseudomonas* and *X. campestris*. Here, the lipid A part is indispensable because neither the isolated core oligosaccharide nor LPS with only two fatty acid residues (LPS-OH) triggers $[Ca^{2+}]_{cyt}$ elevations. Indeed, two HPLC-purified *Pseudomonas* lipid A fractions comprising mainly penta-acylated lipid A are sufficient to trigger $[Ca^{2+}]_{cyt}$ elevations in *A. thaliana* in a LORE-dependent manner. Similarly, isolated *X. campestris* lipid A induces defense gene expression in *A. thaliana*¹⁷. However, as $[Ca^{2+}]_{cyt}$

elevations triggered by *Pseudomonas* lipid A are apparently lower than that induced by the respective rough-type LPS (although the LPS has a higher molecular weight and the same concentration (w/v) was used), the presence of the core oligosaccharide in the LPS molecule enhances sensitivity of *A. thaliana* to LPS. The inner core of *Pseudomonas* LPS, which is highly negatively charged due to phosphorylation^{8,22}, possibly contributes to its higher activity compared to the isolated lipid A as it might promote receptor interaction through specific ionic interactions. Along this line, the core oligosaccharide of *Capnocytophaga canimorsus* LPS was shown to boost its binding to mammalian MD2^{32,33}. Similarly, substitution of the lipid A phosphate groups with phospho-ethanolamine as well as de-phosphorylation of *X. campestris* LPS impairs its ability to induce defense gene expression in *A. thaliana*^{17,34}. Since LORE also senses LPS from human-pathogenic *P. aeruginosa*, mechanistic insights into this plant LPS sensing opens new perspectives for development of anti-bacterial therapies to combat bacterial infections or sepsis-related complications.

Loss-of-function *lore* mutants are greatly diminished in typical PTI responses upon elicitation with several *Pseudomonas* and *X. campestris* LPS. However, individual LPS preparations cause a slight response in *lore* mutants and in tobacco. Pretreatment with *E. coli* LPS enhances resistance to bacterial infection in *A. thaliana* but apparently these LPS do not trigger the typical early signaling reactions regularly observed with *Pseudomonas* LPS and other MAMPs. These LORE-independent responses may be due to low levels of contaminants. Alternatively, there may be (an)other LPS - possibly low sensitivity - sensing system(s) or direct LPS incorporation into membranes *via* its lipid A moiety may initiate 'non-canonical' LPS recognition^{35,36}. Such different sensing systems might account for the discrepancies to, and also between, previous studies reporting immune responses for different LPS, including *E. coli* and *B. cepacia* LPS, in *A. thaliana* as well as other plant species^{14,16,19}. We consistently observed rapid and specific $[Ca^{2+}]_{cyt}$ elevations and other typical PRR-mediated PTI responses with all *Pseudomonas* and *X. campestris* LPS tested as well as with HPLC-purified lipid A of *P. aeruginosa* in *A. thaliana*. Related species such as *C. rubella* and *E. salsugineum* that have LORE homologs, also rapidly produce ROS upon treatment with *Pseudomonas* and *X. campestris* but not *E. coli* LPS. Taken together, our data suggest that LORE is an immune receptor for *Pseudomonas* and structurally related LPS. Future studies will reveal if LPS directly binds to LORE and if accessory LPS binding proteins, potentially analogous to mammalian LPS sensing^{12,37}, are required. However, since heterologous LORE expression in *N. benthamiana* led to LPS sensitivity with the same specificity as in *A. thaliana*, this points to a key role for LORE in LPS binding.

LORE is also interesting with respect to evolutionary aspects of eukaryotic MAMP sensing. Although the mammalian TLR5 and plant FLS2 flagellin receptors both contain LRR-ectodomains, they evolved independently and recognize distinct flagellin epitopes^{2,38}. By contrast, mammals and plants evolved structurally different receptor types for LPS sensing, i.e. an LRR-ectodomain for TLR4³⁷ but a plant-specific class of B-type lectin domain for LORE²⁵. Strikingly, although the lipid A moiety is sufficient to confer immune recognition in plants and animals, they differ in their preference for detecting distinct acylation patterns. It will be interesting to see if more phytopathogenic bacteria are sensed through their lipid A moiety and if this requires LORE. Future studies may reveal the different selective factors driving the evolution of distinct lipid A sensing preferences between mammals and plants.

The name ‘S-domain (SD)’ originates from the roles of plant B-type lectin RLKs in *Brassica* mating self-incompatibility^{27,39,40} – a process that can be described as an endogenous defense strategy to prevent inbreeding. However, LORE is not closely related to these *Brassica* SRKs (S-LOCUS RECEPTOR KINASE). Since SD-RLKs are widespread in the plant kingdom, they probably fulfil diverse functions²⁷. Several of the *SD-RLKs* in *A. thaliana* are induced by biotic stresses, suggesting that further SD-RLKs might be involved in innate immunity²⁵. Given the common occurrence of SD-RLKs in land plants, downstream signaling components may be sufficiently conserved to allow inter-family receptor transfer. As proof-of-concept, we conferred LPS sensing *via* LORE to tobacco. Thus, transfer of LORE to crops like tomato, potato or cereals might enable engineering of bacterial resistance traits, for instance to *Pseudomonas* and *Xanthomonas*, which include major plant pathogens.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

We thank Wilbert Bitter, Boris A. Dmitriev, Erich Glawischnig, Kazuyoshi Kawahara, Marc Knight, Uwe Mamat, Marcel Quint, Klaus Rudolph, Sof’ya N. Senchenkova and Gary Stacey for providing material, Christoph Hemetsberger for assistance with confocal imaging as well as Nicole Bauer, Birte Buske, Bastian Kölbl, Brigitte Kunz, Hermann Moll and Ursula Schombel

for technical assistance, Harald Schempp for fruitful discussions and Claus Schwechheimer for critical reading of the manuscript. This work is supported by a German Research Foundation through Priority Program SPP1212 (J.L. and D.S.) and Collaborative Research Centre SFB924/TP-B10 (S.R.).

AUTHOR CONTRIBUTIONS

S.R., M.S., T.I., L.W. and J.L. performed experiments. P.S.-C., S.R., Y.A.K. and U.Z. prepared LPS. S.R. and D.S. initiated the project, S.R. designed plant experiments, N.G. and U.Z. designed LPS purification and chemical dissection experiments, S.R., N.G., and J.L. analyzed the data, and S.R., N.G., J.L. and R.H. interpreted data. S.R., N.G., J.L., R.H., U.Z. and D.S. wrote the manuscript. All authors discussed the results and approved the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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FIGURES

Figure 1 $[Ca^{2+}]_{cyt}$ elevations in *A. thaliana* seedlings treated with *Pseudomonas* LPS. **(a)** Col-0^{AEQ} seedlings were treated with LPS (15 µg/ml) from different *Pseudomonas* spp. (**Table 1**). $[Ca^{2+}]_{cyt}$ levels one minute before elicitation (grey bars) are shown compared to maximum $[Ca^{2+}]_{cyt}$ elevation after LPS application (black bars) (mean ± s.d.; n = 6, 10, 8, 8, 12, 8, 8, 8, 8, 8, 8 individual seedlings per LPS sample (left to right); aequorin reconstitution with 5 µM coelenterazine-h). **(b)** $[Ca^{2+}]_{cyt}$ elevations in Col-0^{AEQ} seedlings in response to different LPS concentrations (0.2 – 50 µg/ml) of *P. aeruginosa* smooth-type (PAO1) or rough-type (H4) LPS (mean of n = 4 individual seedlings per concentration; 20 µM coelenterazine-native). **(a,b)** Experiments were repeated twice with similar results.

Figure 2 LPS-triggered PTI responses in *lore*, *sd1-29* and *sd1-23* *A. thaliana* mutants. **(a)** $[Ca^{2+}]_{cyt}$ elevation kinetics in Col-0^{AEQ} control and *lore-1* mutant seedlings induced by *Pa* H4 LPS (50 µg/ml) (mean ± s.d. of n = 7 individual seedlings per genotype; 20 µM coelenterazine-native). **(b)** Maximum $[Ca^{2+}]_{cyt}$ elevation after treatment with flg22 (1 µM), elf18 (1 µM), chitin (100 µg/ml) or different LPS preparations (15 µg/ml) in *sd1-29*^{AEQ}, *lore* and Col-0^{AEQ} seedlings (mean ± s.d.; n = 6,5,6,6; 6,5,6,6; 10,7,10,10; 6,4,6,6; 10,2,10,4; 8,7,8,8; 8,8,8,8; 12,11,12,12; 8,5,8,8; 8,4,8,8; 8,5,8,8; 8,8,8,8; 8,6,8,8; 8,5,8,8 individual seedlings per genotype and treatment (left to right); 5 µM coelenterazine-h). **(c)** Maximum $[Ca^{2+}]_{cyt}$ elevation after treatment with LPS samples (15 µg/ml) in Col-0^{AEQ}, *lore-1* or complementation lines (CL) (mean ± s.d., n = 6,6,7,8; 14,14,14,14; 10,10,10,10; 8,8,8,8; 14,14,14,14; 12,12,12,12 individual seedlings per genotype and treatment (left to right); 5 µM coelenterazine-h). **(d)** ROS accumulation kinetics in leaf discs of control and different mutant lines induced by *Pa* H4 LPS (10 µg/ml). **(e)** Maximum ROS accumulation in leaf discs of Col-0 control and *sd1-29* mutant plants induced by different *Pseudomonas* LPS (15 µg/ml). **(d,e)** Data are depicted after normalization to ROS levels before LPS application and subtraction of untreated controls (mean ± s.e.m.; n = 16 **(d)** and n = 8 **(e)** leaf discs from 8 individual plants per genotype). **(f)** Activation of MPK6, MPK3 and MPK4 (indicated by arrowheads) triggered by *Pa* H4 LPS (20 µg/ml) or chitin (50 µg/ml) was detected at indicated time points (min) by anti-phospho-MPK immunoblot. Amido black staining shows total protein loading. **(g)** Expression of PTI response genes *FRK1* and *GST1* compared to *EF1a* control four hours after elicitation with *Pa* H4 or *Pst* LPS (10 µg/ml) or chitin (50 µg/ml). **(a-g)** Experiments were repeated twice with similar results.

Figure 3 *Pseudomonas syringae* infection in *lore*, *sd1-29* and *sd1-23 A. thaliana* mutants. **(a)** Spray inoculation with *Pst* DC3000 (1×10^8 CFU/ml inoculum). Bacterial growth was measured after the indicated days (mean $\pm$ s.d., $n = 4$ for Col-0 (day 2), all others $n = 6$ leaf samples randomly obtained from three individual plants per genotype; different letters indicate different statistical significance groups according to two-way analysis of variance (ANOVA; genotype $\times$ treatment) and Bonferroni's multiple-comparison post-test, $P < 0.05$; statistical analysis was performed separately for day 2 (small letters) and day 4 (capital letters) samples and separately for non-reporter (a,b or A,B) and aequorin-expressing (x,y or X,Y) plant lines). **(b)** LPS-induced resistance to subsequent *Pst* DC3000 infection. Plants were treated with 25 μ g/ml *Pa* H4 LPS one day before *Pst* DC3000 infiltration (1×10^5 CFU/ml inoculum). Bacterial growth was measured after three days (mean $\pm$ s.d., $n = 3$ (day 0) or $n = 6$ (day 3) leaf samples randomly obtained from three individual plants per genotype and treatment; different letters indicate different statistical significance groups according to one-way analysis of variance (ANOVA) and Tukey's multiple-comparison post-test, $P < 0.001$; statistical analysis was performed separately for non-reporter (a,b) and aequorin-expressing (x,y) plant lines). **(a,b)** Experiments were repeated four times **(a)** or twice **(b)** with similar results.

Figure 4 PTI responses to LPS from different bacteria in *A. thaliana*. **(a,b)** Col-0^{AEQ} seedlings were treated with 25 μ g/ml **(a)** or 50 μ g/ml **(b)** LPS from different bacteria (**Table 1**). $[Ca^{2+}]_{cyt}$ one minute before elicitation (grey bars) are shown compared to maximum $[Ca^{2+}]_{cyt}$ after LPS application (black bars) (mean $\pm$ s.d., $n = 20, 12, 9, 12, 12, 3, 3$ **(a)** and $n = 18, 12, 12, 6, 12$ **(b)** individual seedlings per LPS sample (left to right); 5 μ M coelenterazine-h). **(c)** Maximum ROS accumulation in Col-0 leaf discs induced by different LPS (25 μ g/ml). Data are depicted after normalization to ROS levels before LPS application and subtraction of untreated controls (mean $\pm$ s.e.m.; $n = 16$ leaf discs from 8 individual plants per genotype). **(d)** Maximum $[Ca^{2+}]_{cyt}$ elevations after treatment with *X. campestris* LPS (25 μ g/ml) in Col-0^{AEQ}, *lore* and *sd1-29*^{AEQ} mutants and complementation lines (CL) (mean $\pm$ s.d., $n = 14, 7, 14, 8, 14, 14; 12, 9, 12, 12, 12, 12; 8, 7, 8, 8, 8, 8; 18, 9, 17, 12, 18, 18$ individual seedlings per genotype and treatment (left to right); 5 μ M coelenterazine-h). **(e)** Maximum ROS accumulation in leaf discs of Col-0 and *sd1-29* plants induced by different *X. campestris* LPS (25 μ g/ml). Data are depicted after normalization to ROS levels before LPS application and subtraction of untreated controls (mean $\pm$ s.e.m., $n = 8$ leaf discs from 8 individual plants per genotype). **(a-e)** Experiments were repeated twice

with similar results. **(f)** Col-0 and *sd1-29* plants were treated with the indicated LPS (25 µg/ml) one day before *Pst* DC3000 infiltration (1×10^5 CFU/ml). Bacterial growth was measured after three days (mean $\pm$ s.d., $n = 6$ leaf samples randomly obtained from three individual plants per genotype and treatment; different letters indicate different statistical significance groups according to one-way analysis of variance (ANOVA) and Tukey's multiple-comparison post-test, $P < 0.05$). Independent experiments are shown in **Supplementary Figure 4b,c**.

Figure 5 Transient expression of LORE in tobacco. **(a)** Localization of LORE-Km-eGFP fusion proteins transiently expressed in *N. tabacum* was analyzed by confocal imaging (LORE-Km, K516A kinase mutant of LORE; scale bar = 30 µm). Co-expression of mCherry was used as a cytoplasmic and nucleoplasmic control. Shown is a representative picture of three epidermal cells which strongly express either LORE-Km-eGFP, mCherry or both. Co-expression experiments were performed in *N. tabacum* (once with LORE-Km-eGFP and mCherry) as well as in *N. benthamiana* (twice with LORE-eGFP and mCherry) with similar results. **(b)** ROS accumulation kinetics in *N. benthamiana* (left) or *N. tabacum* (right) transiently transformed with the indicated constructs upon application of *Pa* H4 LPS (20 µg/ml). Data are depicted after subtraction of untreated controls (mean $\pm$ s.e.m., $n = 12$ leaf discs from three individual plants per construct). **(c)** ROS accumulation in *N. benthamiana* transiently transformed with LORE-eGFP or LORE-Km-eGFP 10 min after elicitation with the indicated LPS preparations (25 µg/ml). Data are depicted after normalization to ROS levels before LPS application and subtraction of untreated controls (mean $\pm$ s.e.m., $n = 8$ leaf discs from four individual plants; each construct was infiltrated into one half of the same leaf). **(b,c)** Experiments were repeated three times with similar results.

Figure 6 PTI responses to different part structures of *Pseudomonas* LPS in *A. thaliana*. **(a)** $[Ca^{2+}]_{\text{cyt}}$ elevation kinetics in Col-0^{AEQ} seedlings induced by different LPS fragments (25 µg/ml) (mean $\pm$ s.d. of pooled data from two experiments, $n = 25, 11, 13, 13, 12, 4$ individual seedlings per treatment (top down); 10 µM coelenterazine-h). **(b)** ROS accumulation kinetics in Col-0 leaf discs induced by different LPS fragments (25 µg/ml). Data are depicted after normalization to ROS levels before elicitation and subtraction of untreated controls (mean $\pm$ s.e.m. of pooled data from two experiments, $n = 24, 12, 12, 12, 12$ leaf discs from 12 individual plants (top down)). **(c)** Maximum $[Ca^{2+}]_{\text{cyt}}$ after treatment with the indicated LPS fragments (25 µg/ml) in *lore-1* compared to Col-0^{AEQ} seedlings (mean $\pm$ s.d. of pooled data from two experiments, $n =$

25,25; 11,13; 13,13; 13,13; 12,13; 4,4 individual seedlings per genotype and treatment (left to right); 10 μ M coelenterazine-h). **(d)** Maximum ROS accumulation in leaf discs of Col-0 and *sd1-29* plants induced by different LPS fragments (25 μ g/ml). Data are depicted after normalization to ROS levels before elicitation and subtraction of untreated controls (mean $\pm$ s.e.m. of pooled data from two experiments, n = 24,24; 12,12; 12,12; 12,12; 12,12 leaf discs from 12 individual plants per genotype and treatment (left to right)). **(a-d)** Data in **(a)** and **(c)** as well as data in **(b)** and **(d)** are from the same experiments, respectively. Whereas **(a)** and **(b)** show the kinetics of the response in the controls, **(c)** and **(d)** show the comparison of the maximum response between control and mutant lines. LA (1) and LA (2), HPLC lipid A fractions (1) and (2); LPS-OH, hydrazine-treated LPS carrying only the two amide-bound fatty acids; core OS, isolated core oligosaccharide.

ONLINE METHODS

Plant material and growth conditions

A. thaliana Col-0 expressing pCaMV35S-apoaequorin in the cytosol (Col-0^{AEQ}) was obtained from M. Knight⁴¹. T-DNA insertion lines *cerk1-2* (*lyk1*, At3G21630, GK_096F09) and *lyk4* (At2G23770, WiscDsLox297300_01C) in Col-0^{AEQ} background were provided by G. Stacey⁶. *fls2-26* (At5G46330), *efr-1* (At5G20480, SALK_044334), *bak1-4* (At4G33430, SALK_116202) in Col-0^{AEQ} background, *rbohD* (At5G47910, dSpm transposon), *mpk3-1* (At3g45640, SALK_151594) and *mpk6-3* (At2G43790, SALK_127507) mutants were described previously^{20,24}. *sd1-29* (At1G61380, SAIL_857_E06) and *sd1-23* (At1G61390, GK_079G10) mutants were obtained from the European Arabidopsis Stock Centre (NASC) and genotyped with primers listed in **Supplementary Table 2**. *sd1-29*^{AEQ} was generated by crossing of *sd1-29* and Col-0^{AEQ} plants. For growth in liquid medium, *A. thaliana* seeds were surface-sterilized²⁴, stratified at 4 °C for ≥ 2 days and grown in liquid MS medium (0.5 x Murashige & Skoog medium including vitamins (Duchefa), 0.25% sucrose, 1 mM MES, pH 5.7) in 24-well plates (~ 15 seedlings/well) under long day (16 h light) conditions at 20-22 °C. Experimental *A. thaliana* and *C. rubella* Reut. (N22697, obtained from M. Quint) were grown on soil in climate chambers with 8 h light, *E. salsugineum* ecotype Shandong plants (obtained from E. Glawischnig), *N. tabacum* var. Samsun and *N. benthamiana* with 10 h light at 20-22 °C.

MAMPs

Flg22 (QRLSTGSRINSAKDDAAGLQIA)⁴² and elf18 (ac-SKEKFERTKPHVNVGTIG)⁴³ peptides were synthesized on an Abimed EPS221 (Abimed) system. Shrimp shell chitin (Sigma #C9752) was ground to fine powder, resuspended in water and the soluble fraction used for experiments. LPS preparations are summarized in **Supplementary Table 1**. Some LPS preparations are kind donations (as indicated) and unpublished LPS preparations are described below. These LPS preparations were digested overnight with proteinase K (2 mg/ml LPS; 10 µg/ml proteinase K, 50 °C) prior to use in bioassays. *E. coli* K12 and O111:B4 as well as *S. enterica* sv. Thypimurium and sv. Minnesota R595 LPS were obtained from InvivoGen or Enzo Life Sciences.

Preparation of LPS of Pseudomonas syringae pv. *tomato* DC3000. Bacteria were grown in King's B medium at 26 °C under shaking (230 rpm) to an optical density (OD₆₀₀) of ~0.8, harvested by centrifugation (3,000 × *g*, 4 °C, 20 min), washed with ethanol, acetone (twice), diethyl ether, and dried (recovery: 10.79 g). Hot phenol-water-extraction (PW-extraction)⁴⁴ was performed by stirring bacteria in 45% aqueous phenol (18.5 ml/g bacteria) for 20 min at 68 °C. After centrifugation (5,500 × *g*, 4 °C, 30 min) the upper water phase was collected (~60 ml). The extraction was repeated with the same volume of water. Combined water phases and the phenolic phase were dialyzed against water (4 °C, 12-16 kDa cut-off, Biomol, Germany) and lyophilized. By contrast to the usual PW-extraction, the water phase was found to contain almost no LPS. Therefore, a phenol-chloroform-petroleum ether extraction (PCP)⁴⁵ was performed with the material of the phenolic phase (7.1 g). It was resuspended in 90% phenol/chloroform/petroleum ether (40-60 °C) 2:5:8 (v/v/v) (80 mg/ml) using an Ultra-Turrax, stirred for 30 min at RT, and centrifuged (5,800 × *g*, 20 °C, 20 min). The supernatant was collected and the extraction repeated twice. Combined supernatants were evaporated in vacuum until phenol crystallization started. LPS was precipitated with 11.8 ml water (overnight, 4 °C) and 68 ml ethanol (3 d, 4 °C). After centrifugation (3,000 × *g*, 4 °C, 20 min), the precipitate was washed three times with acetone (5-10 ml; 3,000 × *g*, 4 °C, 15 min) and dried. LPS (14.6 mg) was resuspended in water (5 mg/ml) and sequentially treated with DNase and RNase (~10 µg/ml, 37 °C, overnight) and proteinase K (~10 µg/ml, 50 °C, overnight).

P. syringe pv. *apii* (bacterial mass provided by K. Rudolph, Göttingen, Germany) and *P. alcaligenes* 537 (bacterial mass provided by W. Bitter, Utrecht, The Netherlands) were isolated by hot PW-extraction as described above with following modifications. For *P. syringe* pv. *apii* 9.5 ml 45% aqueous phenol/g dried bacterial mass were used (centrifugation: 2,800 × *g*, 0 °C, 20 min). Three extraction steps were performed to gain acceptable yields. The water phase

of the third extraction was processed separately and contained ~80% of the total LPS. After dialysis (4 d, 4 °C) and lyophilization, the pellet was resuspended in water (55 mg/ml) and ultracentrifuged three times ($\sim 200,000 \times g$, 4 °C, 4 h). The LPS pellet (10 mg/ml in water) was sequentially treated with DNase, RNase and proteinase K (overnight; enzymes $\sim 60 \mu\text{g/ml}$) at RT, dialysed and lyophilized. For *P. alcaligenes* 537 enzymatic treatment (enzymes $\sim 35 \mu\text{g/ml}$) was performed prior to PW-extraction. After enzymatic treatment the sample was dialyzed (5 d, 4 °C), centrifuged ($14,300 \times g$, 0 °C, 20 min) and the pellet washed twice with acetone. LPS was extracted with 14 ml 45% aqueous phenol/g dried bacterial (centrifugation: $4,300 \times g$, 4 °C, 30 min) and the combined water phases were dialyzed and lyophilized.

E. coli KPM 53²⁹ was grown overnight at 37 °C. Cells were washed twice with ethanol and acetone ($18,000 \times g$, 4 °C, 15 min), once with diethyl ether. The dried pellet was resuspended in water (30 mg/ml), sequentially treated with DNase and RNase and proteinase K (overnight at RT; enzymes $\sim 30 \mu\text{g/ml}$), dialyzed against water (2 d, 4 °C) and lyophilized. LPS extraction was achieved by PCP extraction⁴⁵. Briefly, the pellet was suspended in phenol (90%)/chloroform/petroleum ether (40-60 °C) 2:5:8 (v/v/v) (120 mg/ml) using an Ultra-Turrax, stirred for 30 min at RT, and the supernatant was collected after centrifugation ($5,800 \times g$, 4 °C, 20 min). The extraction was repeated three times, the supernatants were combined and evaporated in vacuum (50 °C) until phenol crystallization started. LPS was precipitated by adding water (phenol concentration >80%) in the cold, centrifuged ($5,800 \times g$, 25 °C, 20 min), and washed two times with 80% phenol and three times with acetone. The triethylamine (TEN)-salt of the LPS was formed⁴⁶ and purified by RP-HPLC as described³² with the following modifications: the initial solvent system (2% B) was maintained for 20 min, followed by a linear two step gradient raising from 2 to 17% B (20-50 min) and 17 to 27% B (50-85 min). The solvent was held at 27% B for 25 min, the column re-equilibrated in 10 min to 2% B and was held there for 10 min before the next injection. The pool used for the experiments contained solely hexa-acylated LPS with a monoisotopic MW of 2237.337 Da (2 x GlcN, 2 x P, 2 x Kdo, 4 x 14:0(3-OH), 1 x 14:0, 1 x 12:0). For the injection methanol-chloroform-water (57:12:31, v/v/v) containing 10 mM NH₄OAc/0.1 M Na-EDTA (pH 7) 4:1 (v/v) (sample concentration: 8 mg/ml) was used.

Preparation and purification of lipid A and core oligosaccharide of Pa H4 after acid hydrolysis
— *Pa* H4 LPS (104.7 mg) was dissolved in 14 ml water, 1.75 ml of a 10% SDS-solution, and

1.75 ml acetate buffer (1 M NaOAc, pH 4.4), heated for 3 h at 100 °C with occasional mixing and lyophilized. SDS was removed by four washes with 60 ml 2 M HCl/ethanol (1:99, v/v) ($5,800 \times g$, 20 °C, 20 min). The dried pellet was resuspended in 5 ml water. Afterwards, 5 ml $\text{CHCl}_3\text{:CH}_3\text{OH}$ 4:1 (v/v) were added, the suspension mixed vigorously, and centrifuged ($5,800 \times g$, 4 °C, 10 min). The organic phase was collected, the water phase (incl. interphase) was extracted again successively with 5 ml $\text{CHCl}_3\text{:CH}_3\text{OH}$ 9:1 (v/v) and CHCl_3 . All organic phases (containing the lipid A) were combined, evaporated in vacuum, and dried (recovery: 21.5 mg). The core oligosaccharide-containing water phase was lyophilized. Core oligosaccharides were GPC purified on a Sephadex G-50 column (2.5 x 80 cm, GE Healthcare), eluted with pyridine/acetic acid/water (8/20/2000, by vol., pH ~4.7). Oligosaccharides were monitored by a Knauer differential refractometer. Fractions containing the core sugars (detected as per-acetylated methyl glycosides by GLC-MS) were collected and lyophilized. Besides the pure core oligosaccharide (10.3 mg), a second, earlier eluting core-oligosaccharide containing pool was obtained (15.9 mg), in which a polyamine of yet unknown structure was present. Only the polyamine-free core-oligosaccharide was used for biological experiments. The lipid A was further purified by RP-HPLC (**Supplementary Figure 6**) essentially as described³².

Preparation of the core-lipid A backbone oligosaccharide with amide-bound fatty acids (LPS-OH) – Pa H4 LPS (50 mg) was de-O-acylated by mild hydrazinolysis (2.0 ml hydrazine, 37 °C, 2 h). The product was precipitated from ice-cold acetone (20 ml, 30 min at -20 °C), centrifuged ($3,800 \times g$, 4 °C, 20 min), washed four times with ice-cold acetone, and centrifuged ($47,800 \times g$, 4 °C, 30 min). The dried pellet was resuspended in water (20 mg/ml) and acidified to pH 5-6 with 10 μl of 4 M HCl. Acetone (-20 °C) was added, centrifuged as before, the pellet washed once with acetone, and dried (26.8 mg LPS-OH).

A. *thaliana* mutant screen

The Col-0^{AEQ} mutant population and screening procedure was described in detail previously²⁴. In short, Col-0^{AEQ} seeds were mutagenized with 0.4% ethyl methane sulfonate (EMS) for 8 h at RT with subtle shaking, washed with water and dried. M₁ plants were grown on soil in a greenhouse and seeds harvested from individual M₁ plants. M₂ seedlings (≥ 12 per M₁ plant line) were grown in sterile MS medium, transferred individually to wells of 96-well plates and reconstituted with 10 μM coelenterazine-h overnight in the dark. Aequorin luminescence measurements were performed as described below but without discharge. Seedlings showing strongly reduced aequorin luminescence elevations upon application of 5 $\mu\text{g/ml}$ *Pa* H4 LPS

were rescued from the 96-well plates and grown on soil for seed setting. The phenotype of putative mutants was verified by testing the corresponding M₃ offspring with quantitative [Ca²⁺]_{cyt} measurements as described below by estimating L_{max}. We obtained four *lore* mutants that were insensitive to *Pseudomonas* LPS and allelic in a complementation test, i.e. F1 hybrids resulting from reciprocal crosses failed to complement each other for LPS-induced [Ca²⁺]_{cyt} elevations.

Mapping and sequencing of candidate genes

Mapping populations were generated by crossing *lore-1* and *lore-4* with *Ler-0*. M₂ offspring seedlings of these crosses showing strongly reduced aequorin luminescence elevations upon application of 5 µg/ml *Pa* H4 LPS were rescued and grown on soil for seed setting. This resulted in 134 plants for *lore-1* x *Ler-0*, 82 for *lore-4* x *Ler-0* and 101 for *lore-4* x *Ler-0*^{AEQ}. The analysis of PCR-based markers localized *LORE* between the INDEL markers 469696 and CER460336⁴⁷ on chromosome 1. For sequence analysis *LORE* was amplified from genomic DNA by PCR with gene-specific primers SD1-29-F and -R using Phusion® Hot Start High-Fidelity DNA Polymerase (Thermo Scientific) and purified PCR products were sequenced using the indicated primers (**Supplementary Table 2**). *LORE* of all four *lore* mutants was sequenced as described above and a single nucleotide exchange was detected in each *lore* mutant. An independently obtained T-DNA insertion line of SD1-29 (see above) was found to be allelic to all four *lore* mutants in a complementation test, i.e. F1 hybrids resulting from reciprocal crosses failed to complement each other for LPS-induced [Ca²⁺]_{cyt} elevations.

Molecular cloning and generation of transgenic lines

Full-length *SD1-29* coding sequence was amplified by PCR using primers SD129-START, SD129-STOP and SD129-EspMut-F and -R (to mutate internal Esp3I-site) and cloned into pUC18 modified for GoldenGate-based cloning procedure similar as described⁴⁸. Site-directed mutagenesis (*LORE*-Km, K516A) was performed by whole plasmid amplification with Phusion® Hot Start High-Fidelity DNA polymerase (Thermo Scientific) and subsequent re-circulation using GoldenGate methods. The coding sequences were combined with CaMV35S promoter and terminator and C-terminal eGFP or mCherry epitope tag with a (glycine)₁₀-linker using GoldenGate techniques. Expression cassettes were transferred into GoldenGate-modified binary plasmid pCB302. For complementation of *lore-1*, a genomic *SD1-29* fragment including the putative promoter was amplified by PCR by using primers SD129-PROM, SD129-STOP

and SD129-EspMut-F and -R and cloned into GoldenGate-modified pCB302. After transfer of the constructs into *Agrobacterium tumefaciens* GV3101, *A. thaliana* plants were transformed by floral-dip transformation. Transgenic plants were selected by spraying with BASTA[®] (glufosinat-ammonium; Bayer).

Aequorin luminescence measurements

For aequorin luminescence measurements, 8- to 10-days-old liquid-grown apoaequorin-expressing seedlings were placed individually in 96-well plates in coelenterazine/water (coelenterazine-native or -h, P.J.K.) in the dark overnight. Luminescence was recorded by scanning 1 or 2 rows in 6 or 10 sec intervals, respectively, using a Luminoskan Ascent 2.1 (Thermo Scientific) or a Tecan F200 (Tecan) luminometer. Remaining aequorin was discharged by addition of 150 µl 2 M CaCl₂ with 20% EtOH per well. [Ca²⁺]_{cyt} concentrations were calculated as $L/L_{\max}$ (luminescence counts per sec/total luminescence counts remaining) as described in detail previously²⁴.

ROS detection in *A. thaliana* leaves

For ROS detection 3 mm leaf discs from 6-8 weeks-old soil-grown plants were floated overnight in 200 µl water in 96-well plates in the dark. Shortly before the assay, water was replaced with 100 µl 5 µM L-012 (WAKO chemicals) and 2 µg/ml horseradish peroxidase (Type II, Roche). Luminescence was recorded as relative light units (RLU) in 1 min intervals (Tecan F200). After 10 min background reading, elicitors were added to the final concentrations indicated and luminescence readings continued over 60 min. Data are depicted after normalization to average ROS levels 5 min before LPS application and subtraction of untreated controls that were included for each genotype on the same plate.

Immunoblot analysis of MPK activation

14-days-old liquid-grown seedlings were equilibrated in fresh MS medium for 24 h. Medium was discarded and after 30 min recovery, MAMPs in MS medium were added at the indicated concentrations. Seedlings were harvested at indicated time points. Protein extraction and immunoblot with anti-phospho-MPK (anti-phospho-p44/42-ERK; Cell Signaling Technology #9101) were performed as described²⁰.

Gene expression analysis

12-days-old liquid-grown seedlings were equilibrated in fresh MS medium for two days before treatment with the indicated elicitors in MS medium or medium as control. Whole seedlings were harvested at zero or four hours and frozen in liquid nitrogen. Total RNA was extracted using the conventional Trizol method followed by DNaseI treatment (Thermo Scientific) and reverse-transcribed with oligo(dT)₁₈ and Revert Aid[®] reverse transcriptase (Thermo Scientific) according to the manufacturers' instructions. Expression of defense-related genes was determined by semi-quantitative PCR with primers listed in **Supplementary Table 2**. The cDNA was diluted 1:10 in water and a 2 µl aliquot was used in a 20 µl standard PCR reaction. Amplification conditions were 1 min at 95 °C; x cycles of 10 sec at 95 °C, 10 sec at 54 °C and 45 sec at 72 °C, followed by 5 min at 72 °C. Optimal PCR cycle numbers determined to be in the non-saturated range have been established previously for each primer pair and are indicated in **Supplementary Table 2**. PCR products were analyzed on 1% TBE agarose gels containing ethidium bromide. Quantitative real-time PCR was performed on a MxPro3005P (Agilent Technologies Inc.) according to manufacturer's instructions (40 cycles of 30 sec at 95 °C, 60 sec at 60 °C, 25 sec at 72 °C) using the Maxima SYBR Green-ROX qPCR Master Mix (Thermo Scientific) and analyzed using the MxPro-Mx3005P Software (Agilent). The expression level of *SD1-29* was normalized to ubiquitin. *SD1-29* expression in mutant lines is depicted relatively to the *SD1-29* expression of the Col-0 control. Amplification specificity was analyzed by non-template controls and primer dissociation curves. All primer pairs used for gene expression analysis were chosen to span at least one intron if possible.

Histochemical staining of callose

10-days-old liquid-grown seedlings were equilibrated in fresh MS medium 24 h before application of LPS in MS medium or medium as control. After 24 hours incubation under light, seedlings were cleared in 95% ethanol overnight, washed with 80% ethanol, twice with 50% ethanol, twice with water and equilibrated in 100 mM Na₂HPO₄ (pH 9) for 1 hour. Seedlings were stained with 0.01% aniline blue in 100 mM Na₂HPO₄ (pH 9) for 1 hour in the dark and examined for fluorescence using a Zeiss Axioplan epifluorescence microscope.

Bacterial infection assay

Spray inoculation: Leaves of 6-week-old plants were spray-inoculated with a suspension of 1×10^8 CFU/ml of *P. syringae* pv. *tomato* DC3000 (*Pst* DC3000) containing 0.04% Silwet-L77 (Lehle seeds). Infected leaves were harvested 3-4 hours (day 0) and 3 days after inoculation. Bacterial growth was determined by colony counting of serial dilutions on LB plates supplemented with 75 µg/ml rifampicin as described²⁶. Statistical analysis (genotype x treatment two-way analysis of variance, ANOVA) was performed with GraphPad Prism 5.0.

Pressure infiltration: 25 µg/ml LPS or water as control was infiltrated into *A. thaliana* leaves using a needleless syringe. After 24 h *Pst* DC3000 was infiltrated at 1×10^5 CFU/ml. Bacterial growth was determined as described above. Statistical analysis (one-way analysis of variance, ANOVA) was performed with GraphPad Prism 5.0.

Transient expression in tobacco and confocal microscopy

Overnight cultures (supplemented with 100 µM acetosyringone) of *A. tumefaciens* GV3101 carrying the indicated constructs were harvested by centrifugation ($1,000 \times g$, RT, 5 min) and washed twice with infiltration medium (10 mM MgCl₂, 10 mM MES pH 5.7, 150 µM acetosyringone). Bacteria were resuspended in infiltration medium to an OD₆₀₀ of 1.0, incubated at room temperature for 4-6 h and pressure infiltrated into leaves of 6-8 weeks-old tobacco plants. For expression in *N. tabacum* bacteria were diluted to an OD₆₀₀ of 0.1, and for expression in *N. benthamiana* bacteria (OD₆₀₀ of 0.05) were mixed 1:1 with *A. tumefaciens* GV3101 carrying the p19 suppressor of silencing⁴⁹. For co-expression, bacteria carrying the indicated constructs were mixed 1:1 prior to infiltration. Leaf discs (3 mm) were cut and used for ROS detection two days after infiltration as described above. Detection of eGFP and mCherry fluorescence was performed with a Leica TCS SP5 confocal microscope using standard settings. Image processing was performed using Leica LAS AF software.

Supplementary figure legends

Supplementary Figure 1 LPS-induced $[Ca^{2+}]_{cyt}$ elevations in *cerk1*, *lyk4*, *fls2*, *efr*, and *bak1* mutant lines and after treatment with proteinase K, calcium channel blocker or protein kinase inhibitor. **(a)** $[Ca^{2+}]_{cyt}$ elevations in Col-0^{AEQ} control and indicated mutant seedlings treated with *Pa* PAO1 (left) or *Pa* H4 (right) LPS (50 µg/ml). Black bars represent LPS-induced maximum $[Ca^{2+}]_{cyt}$ elevations in the indicated mutant line, while grey bars represent maximum LPS-induced $[Ca^{2+}]_{cyt}$ elevations in the respective Col-0^{AEQ} control (mean $\pm$ s.d., n = 6,6; 4,6; 4,6; 4,9; 6,4 (left panel) and n = 9,9; 6,6; 6,6; 6,9; 9,5 (right panel) individual seedlings per genotype

and treatment (left to right); aequorin reconstitution with 5 μ M coelenterazine-h). **(b)** Col-0^{AEQ} seedlings were treated with LPS (50 μ g/ml) or flg22 and elf18 peptides (1 μ M each). LPS preparations (30 μ l of 2 mg/ml) and peptide solutions (30 μ l of 40 μ M) were incubated overnight at 60 °C with proteinase K (10 μ g) or without as control and heat-inactivated for 15 min at 95 °C. Maximum $[Ca^{2+}]_{cyt}$ elevations induced by control-treated sample are shown in comparison to $[Ca^{2+}]_{cyt}$ elevations induced by proteinase K-treated sample at peak maximum (LPS) or at time of peak maximum in control (flg22 and elf18). The experiment was repeated twice with similar results (mean $\pm$ s.d., n = 4 individual seedlings per treatment; aequorin reconstitution with 5 μ M coelenterazine-h). **(c)** Col-0^{AEQ} seedlings were treated with 10 mM LaCl₃, 10 μ M K-252a or DMSO as control for 30 min before application of *Pa* PAO1 or *Pa* H4 LPS (50 μ g/ml). Maximum LPS-induced $[Ca^{2+}]_{cyt}$ elevations in DMSO-treated controls are shown in comparison to LPS-induced $[Ca^{2+}]_{cyt}$ elevations at the same time in inhibitor-treated samples (mean $\pm$ s.d., n = 4 individual seedlings per treatment; aequorin reconstitution with 10 μ M coelenterazine-native). **(d)** Scheme of LPS structure showing a representative core region and lipid A of *Pseudomonas aeruginosa*. Black dashed lines indicate truncations in rough mutants, grey dotted lines non-stoichiometric modifications. Ara4N, 4-amino-4-deoxy-L-arabinose; Cm, carbamoyl; Etn, ethanolamine; GalN, galactosamine; Glc, glucose; GlcN, glucosamine; Hep, L-glycero-D-manno-heptose; Kdo, 3-deoxy-D-manno-oct-2-ulosonic acid; LOS, lipooligosaccharide; OPS, O-polysaccharide; P, phosphate; Rha, L-rhamnose.

Supplementary Figure 2 ROS production, MPK activation and callose deposition in response to LPS in *A. thaliana*. **(a)** ROS accumulation triggered by *Pa* H4 (left) or *Pst* (right) LPS (10 μ g/ml) in leaf discs of Col-0 control and *rbohD* mutant plants. Data are depicted after subtraction of untreated controls (mean $\pm$ s.e.m., n = 8 leaf discs from four individual plants per genotype). The experiment was repeated twice with similar results. **(b)** MPK activation in Col-0 control compared to *mpk3-1* and *mpk6-3* mutant seedlings treated with LPS (50 μ g/ml), chitin (50 μ g/ml) or flg22 (100 nM) was detected 5 and 10 min after elicitation by anti-phospho-MPK immunoblot. Amido black staining shows total protein loading. The experiment was repeated twice with similar results. **(c)** Col-0 seedlings were treated for 24 hours with *Pa* H4 LPS or water as control. Callose deposition was visualized by fluorescence microscopy after aniline blue staining. Four individual cotyledons per treatment are shown (scale bar = 200 μ M). The experiment was repeated three times with similar results.

Supplementary Figure 3 LPS-induced $[Ca^{2+}]_{cyt}$ elevations and MPK activation in *lore*, *sd1-29* and *sd1-23 A. thaliana* mutants. **(a)** $[Ca^{2+}]_{cyt}$ elevation kinetics in Col-0^{AEQ} control and different *lore* mutant seedlings in response to *Pa* H4 LPS (50 μ g/ml). The experiment was repeated twice

with similar results (mean $\pm$ s.d., $n = 7,7; 7,3; 7,7; 7,9$ (left to right); Col-0^{AEQ} controls are identical in all graphs; aequorin reconstitution with 20 μ M coelenterazine-native). Full figure of **Figure 2a**. **(b)** Schematic representation of *SD1-29* and *SD1-23* gene structure. Black boxes indicate exons and white boxes indicate untranslated regions (UTR). Arrowheads show location of *lore* point mutations and white arrows T-DNA insertion sites. **(c)** LORE protein scheme showing predicted conserved domains and location of *lore* loss-of-function mutations. SP, signal peptide; TM, transmembrane; EGF, epidermal growth factor; PAN, plasminogen-apple-nematode; DUF, domain of unknown function. **(d)** Analysis of *SD1-29* and *SD1-23* expression by quantitative real-time RT-PCR (before and after the T-DNA insertion site) in two Col-0 control, *sd1-29* and *sd1-23* seedling pools each (labelled as cDNA (1) and (2)) or by semi-quantitative RT-PCR (across the T-DNA insertion site) using the primers (**Supplementary Table 2**) indicated by colored arrows. Expression of *EF1a* served as constitutive control. Semi-quantitative RT-PCR experiments were repeated twice with similar results. **(e)** $[Ca^{2+}]_{cyt}$ elevations in Col-0^{AEQ}, *lore-1* and *sd1-29*^{AEQ} seedlings treated with *Pa* H4 LPS (15 μ g/ml). Experiment was repeated twice with similar results (mean $\pm$ s.d., $n = 10, 7, 10$ (top down) individual seedlings per genotype; aequorin reconstitution with 5 μ M coelenterazine-h). **(f)** MPK activation in seedlings treated with the indicated LPS preparations (20 μ g/ml), chitin (50 μ g/ml) or water as control was detected at indicated time points (min) after elicitation by anti-phospho-MPK immunoblot. Amido black staining shows total protein loading. Full version of **Figure 2f**. The experiment was repeated twice with similar results.

Supplementary Figure 4 *Pst* DC3000 infection in *lore* and *sd1-29* mutants. **(a)** *Pst* DC3000 infection of Col-0^{AEQ} control, *lore-1* mutant and complementation lines (CL) by spray inoculation (1×10^8 CFU/ml inoculum). Bacterial growth was measured after three days (mean $\pm$ s.d., $n = 12$ leaf samples randomly obtained from four individual plants per genotype; different letters indicate different statistical significance groups according to two-way analysis of variance (ANOVA; genotype x treatment) and Bonferroni's multiple-comparison post-test, $P < 0.05$). The experiment was repeated three times with similar results. **(b,c)** Plants were treated with 25 μ g/ml of the indicated LPS or water as control one day before *Pst* DC3000 infiltration (1×10^5 CFU/ml inoculum). Bacterial growth was measured after three days (mean $\pm$ s.d., **(b)** $n = 6$ and **(c)** $n = 4$ for *sd1-29* (day 3), all others $n = 6$ leaf samples randomly obtained from three individual plants per genotype; different letters indicate different statistical significance groups according to one-way analysis of variance (ANOVA) and Tukey's multiple-comparison post-test, $P < 0.05$).

Supplementary Figure 5 Phylogenetic distribution of LORE and functional analysis of LPS sensing in selected plant species. **(a)** Phylogram of SD-RLKs from *A. thaliana* and diverse other plant species. Protein BLAST was performed against diverse plant species using LORE amino acid sequence as query. Sequence data sources: TAIR10, Ensembl Plants, BOLbase, Sol Genomics Network and NCBI databases. Plant families (species): *Brassicaceae* (*Arabidopsis thaliana*, *Arabidopsis lyrata*, *Brassica rapa*, *Brassica oleracea*, *Capsella rubella*, *Eutrema salsugineum*), *Fabaceae* (*Glycine max*, *Medicago truncatula*), *Solanaceae* (*Nicotiana benthamiana*, *Nicotiana tabacum*, *Solanum lycopersicum*, *Solanum tuberosum*), *Poaceae* (*Hordeum vulgare*, *Oryza sativa*, *Zea mays*), *Salicaceae* (*Populus trichocarpa*). Sequences of *A. thaliana* SD-1 RLKs⁴, RLKs FLS2 and CERK1, and the best LORE BLAST hits (max. 3 from each species) were aligned using ClustalW. A phylogenetic tree was constructed using the Maximum Likelihood method implemented in MEGA6. Bootstrapping values (1000 replicates; >50%) are shown next to the branches. Branch lengths indicate the number of substitutions per site. LORE (marked with red filled dot) and its putative orthologs are highlighted in red. A subclade apparently specific to the *Brassicaceae* is highlighted in blue. The closest LORE paralog, SD1-23, is marked with a blue diamond, *Brassica* SRKs (Self-incompatibility Receptor Kinases) with black triangles, and tobacco SD-RLKs with grey squares. **(b)** ROS accumulation kinetics in leaf discs of *E. salsugineum* and *C. rubella* (marked with red open circles in **(a)**) induced by 25 µg/ml of the indicated LPS samples (mean ± s.e.m., n = 8 leaf discs from 4 individual plants). Experiments were repeated at least three times with similar results. **(c)** ROS accumulation in leaf discs of *N. tabacum* (left) and *N. benthamiana* (right) treated with 50 µg/ml of the indicated LPS preparations or 1 µM flg22. Data are depicted after normalization to ROS levels before LPS application and subtraction of untreated controls (mean ± s.e.m.; n = 8 leaf discs from three individual plants). The experiment was repeated twice with similar results. **(d)** $[Ca^{2+}]_{cyt}$ elevations kinetics in aequorin-expressing *N. tabacum* seedlings treated with 50 µg/ml of the indicated LPS or 1 µM flg22 after 2 min measurement of the resting $[Ca^{2+}]_{cyt}$. **(e)** Maximum $[Ca^{2+}]_{cyt}$ elevations (black bars) in aequorin-expressing *N. tabacum* seedlings induced by 50 µg/ml of the indicated LPS preparations, 1 µM flg22 or water as control are shown compared to the resting $[Ca^{2+}]_{cyt}$ at 1 min (grey bars). **(d,e)** Data in **(d)** and **(e)** are from the same experiment. Whereas **(d)** shows the kinetics of the response, **(e)** shows the comparison of the maximum $[Ca^{2+}]_{cyt}$ elevations compared to the resting $[Ca^{2+}]_{cyt}$ (mean ± s.d.; n = 3 for *Pst* LPS all other treatments n = 4 individual seedlings). **(d)** Epidermal *N. benthamiana* cell expressing LORE-Km-mCherry before and ~15 min after treatment with

1 M NaCl for plasmolysis (scale bar = 25 μ M). Due to water loss in the hypertonic NaCl solution, the protoplast including the plasma membrane is retracted from the cell wall.

Supplementary Figure 6 HPLC purification and analysis by mass spectrometry of *Pa* H4 lipid

A. (a) Reversed phase HPLC profile obtained from the *Pa* H4 LPS after mild acetate buffer hydrolysis. A semi-preparative Kromasil C18 column (5 μ m, 100 Å, 20 x 250 mm, MZ Analysentechnik) was used and the sample (4 mg/ml in CHCl₃/CH₃OH 4:1, v/v) was eluted using a gradient that consisted of methanol-chloroform-water (57:12:31, v/v/v) containing 10 mM NH₄OAc as mobile phase A and chloroform-methanol (70.2:29.8, v/v) with 50 mM NH₄OAc as mobile phase B. The initial solvent system consisted of 2% B and maintained for 40 min, followed by a linear three step gradient raising from 2 to 17% B (40-100 min), 17 to 27% B (100-170 min), and 27 to 80% B (170-314 min). The solvent was held at 80% B for 20 min, the column re-equilibrated in 20 min to 2% B and held there for additional 21 min before the next injection. The flow rate was 4 ml/min using a splitter between the evaporative light-scattering detector (Sedex model 75C ELSD, S.E.D.E.R.E., France) equipped with a low-flow nebulizer recording the chromatogram and the fraction collector (fractions in 1 min intervals). Nitrogen (purity 99.996%) was used as gas to nebulize the post column flow stream at 3.5 bar into the detector at 50 °C setting the photomultiplier gain to 11. The detector signal was transferred to the Gilson HPLC Chemstation (Trilution LC, version 2.1, Gilson) for detection and integration of the ELSD signal. The major lipid A containing pools are indicated as LA (1) and (2), respectively. (b) The corresponding mass spectra of the two lipid A containing HPLC pools. Electrospray Ionization Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FT-ICR-MS) was performed on a 7 Tesla APEX Qe instrument (Bruker Daltonics, USA). Samples were dissolved in a water/propan-2-ol/7 M triethylamine/acetic acid mixture (50:50:0.06:0.02, v/v/v/v) and spectra were acquired in the negative ion mode with nano-ESI using the Triversa Nanomate (Advion, USA) as ion source with a spray voltage set to -1.1 kV. Mass spectra were recorded in broad band mode and the mass scale was externally calibrated with glycolipids of known structure. Spectra were charge deconvoluted; given mass numbers refer to the monoisotopic mass of neutral molecules. Mass peaks representing proton adducts of lipid A species are assigned in bold style, the other assigned mass peaks are belonging to sodium adducts (Δ = +22 u) of these molecules. The major species (VI) in *Pa* H4 LA (1) could be assigned to a penta-acylated lipid A composed of 2 x GlcN, 2 x P, 4 x 12:0(OH), and 1 x 10:0(OH) (monoisotopic MW_{calc}: 1462.816 u). Minor molecule species with a third phosphate moiety ((VII), MW_{calc}: 1542.777 u) or with an additional Ara4N residue ((IX), MW_{calc}: 1593.869 u) are present as well. The major species in *Pa* H4 LA (2), molecule (V), could be assigned to

a penta-acylated lipid A as well, but with one 12:0 instead of a 12:0(OH) (composition: 2 x GlcN, 2 x P, 3 x 12:0(OH), 1 x 12:0, and 1 x 10:0(OH); MW_{calc}: 1446.821 u). (XI) corresponds to (V) + P (MW_{calc}: 1526.782 u) and (VIII) to (V) + Ara4N (MW_{calc}: 1577.874 u), (X) corresponds to (VIII) – P (MW_{calc}: 1497.910 Da). Mainly in pool (1), minor occurring tetra-acylated lipid A variants could be identified as well ((III) = (V) - 10:0(OH) (MW_{calc}: 1276.696 u); (IV) = (VI) - 10:0(OH) (MW_{calc}: 1292.691 u); (I) (MW_{calc}: 1196.735 u) and (II) (MW_{calc}: 1212.730 u) are the mono-phosphorylated forms of (III) and (IV), respectively). (c) Maximum [Ca²⁺]_{cyt} after treatment with the indicated LPS fragments (25 µg/ml) in Col-0^{AEQ} controls, *lore-1* or *sd1-29*^{AEQ} mutants and complementation lines (CL) (mean ± s.d., n = 4,4,4,6,4; 4,6,4,6,4; 4,6,4,6,4; 4,4,4,6,4; 4,6,4,6,3; 4,5,4,6,3 individual seedlings per genotype and treatment (left to right); 10 µM coelenterazine-h). LA (1) and LA (2), HPLC lipid A fractions (1) and (2); LPS-OH, hydrazine-treated LPS carrying only the two amide-bound fatty acids; core OS, isolated core oligosaccharide.

Table 1 Bacterial strains and abbreviations used

Bacterial strain	Abbreviation
<i>Pseudomonas aeruginosa</i> Fisher-type 1 (Habs 6)**	<i>Pa</i> F1
<i>Pseudomonas aeruginosa</i> PA01	<i>Pa</i> PAO1
<i>Pseudomonas aeruginosa</i> PA01 Δ algC	<i>Pa</i> PAO1 Δ algC
<i>Pseudomonas aeruginosa</i> H4	<i>Pa</i> H4
<i>Pseudomonas aeruginosa</i> R5	<i>Pa</i> R5
<i>Pseudomonas aeruginosa</i> PAN1	<i>Pa</i> PAN1
<i>Pseudomonas aeruginosa</i> PAC 1R Δ algC	<i>Pa</i> PAC1R Δ algC
<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	<i>Pst</i>
<i>Pseudomonas syringae</i> pv. <i>apii</i>	<i>Psa</i>
<i>Pseudomonas alcaligenes</i> 537	<i>Palc</i>
<i>Pseudomonas fluorescens</i> ATCC49271	<i>Pfluor</i>
<i>Xanthomonas campestris</i> pv. <i>malvacearum</i> GSPB1386	<i>Xcm</i> (1)
<i>Xanthomonas campestris</i> pv. <i>malvacearum</i> HSV GSPB2388	<i>Xcm</i> (2)
<i>Xanthomonas campestris</i> pv. <i>phaseoli</i> var. <i>fuscans</i> GSPB271	<i>Xcph</i>
<i>Xanthomonas campestris</i> pv. <i>begoniae</i> GSPB525	<i>Xcb</i>
<i>Burkholderia cepacia</i> (GIFU12046/3P-62)	<i>Bcp</i>
<i>Burkholderia pseudomallei</i> (GIFU645/ATCC25416)	<i>Bpm</i>
<i>Escherichia coli</i> K12	<i>Ec</i> K12
<i>Escherichia coli</i> O111:B4	<i>Ec</i> O111
<i>Escherichia coli</i> KPM 53	<i>Ec</i> Kdo ₂ -LA
<i>Salmonella enterica</i> sv. Typhimurium	<i>Sm</i> Typh
<i>Salmonella enterica</i> sv. Minnesota R595	<i>Sm</i> R595

Figure 1

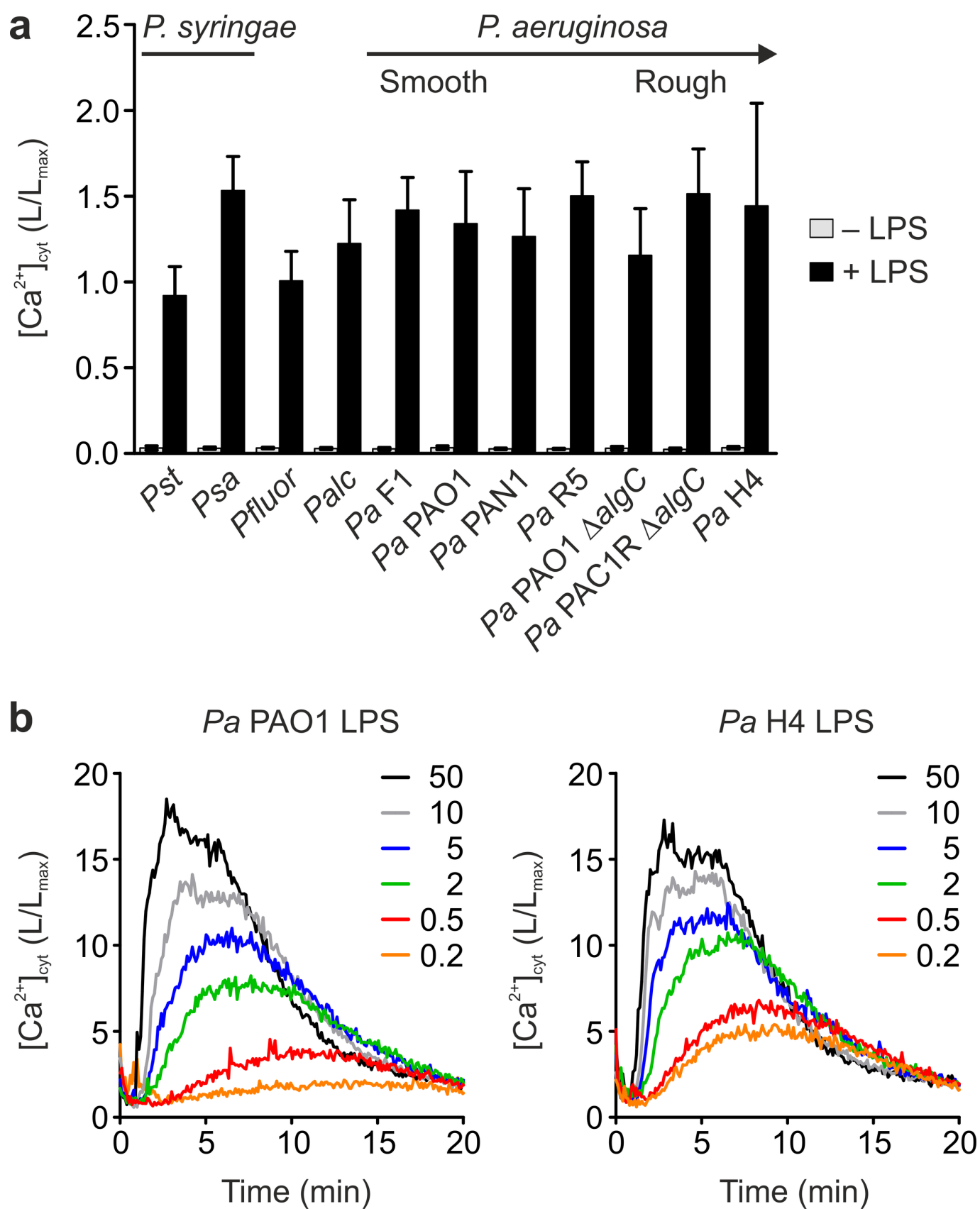


Figure 2

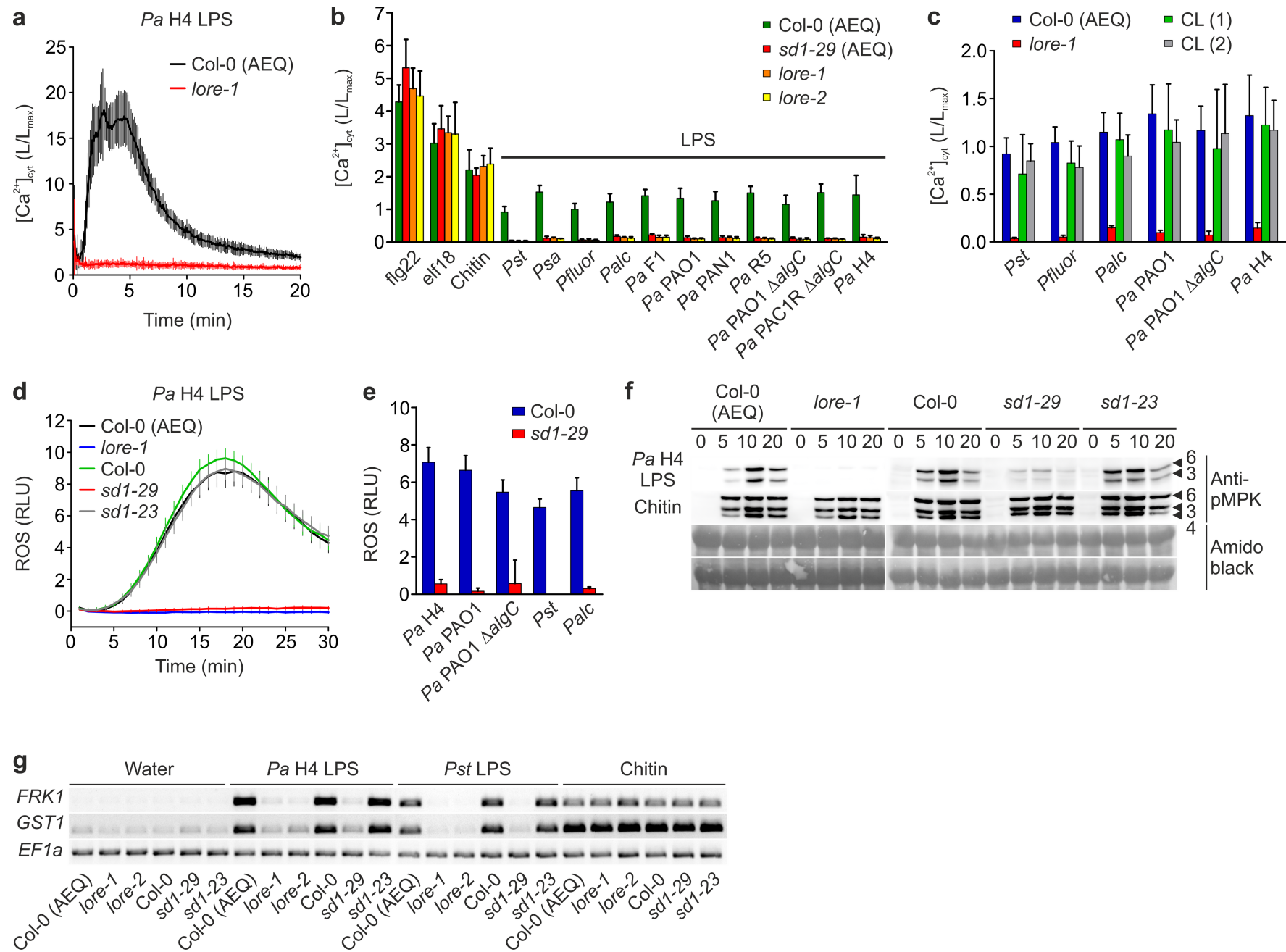


Figure 3

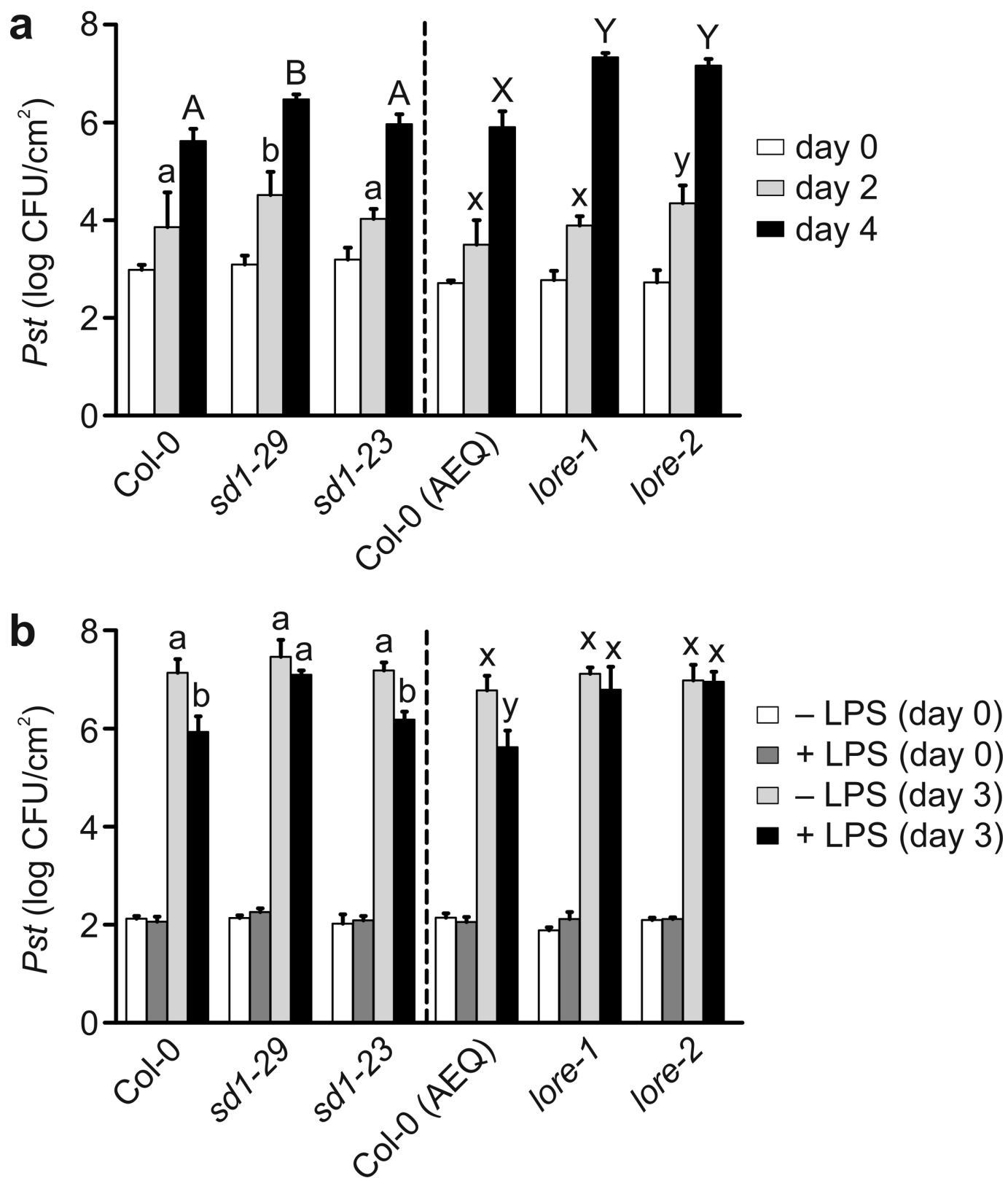


Figure 4

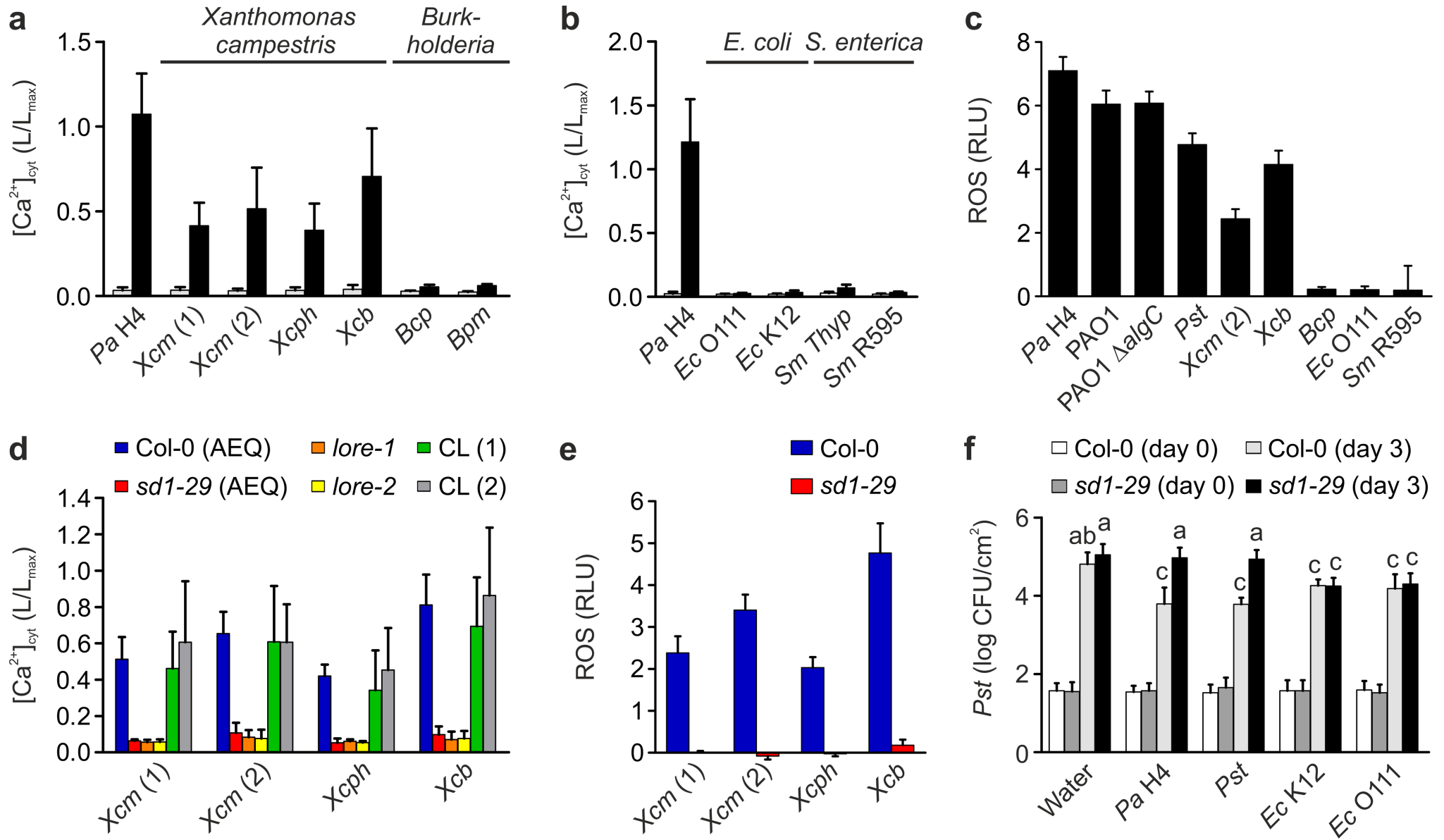


Figure 5

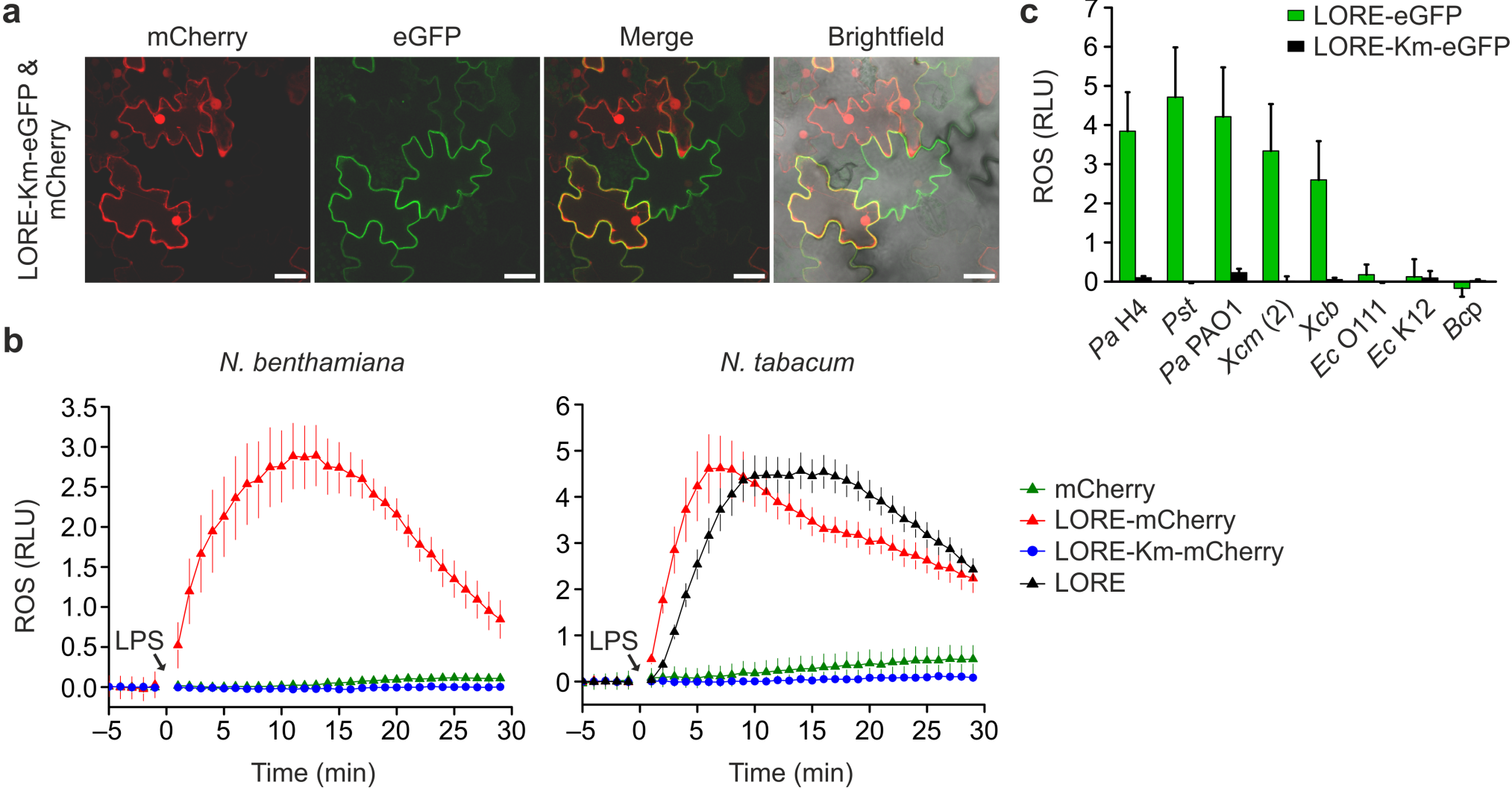
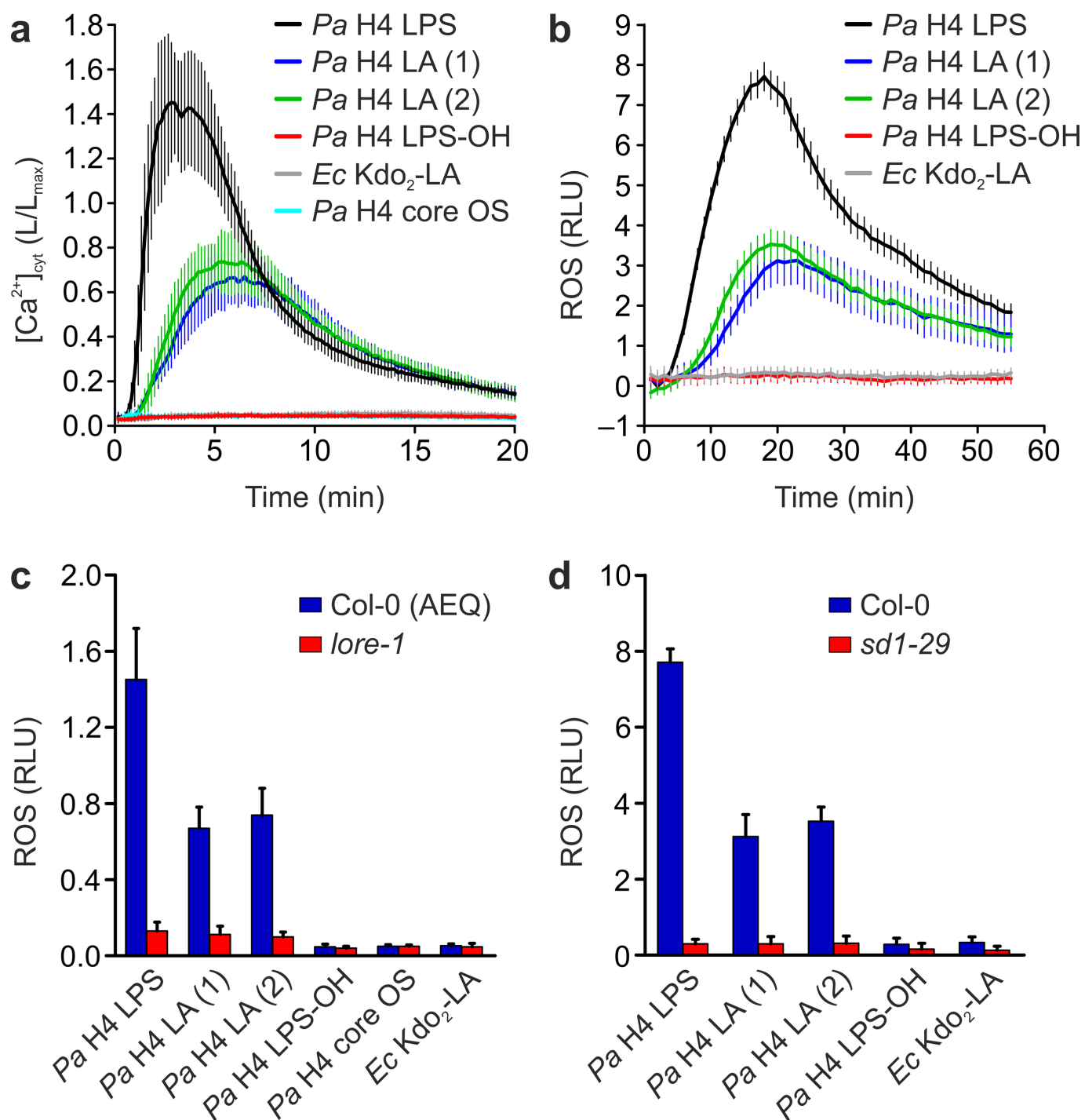
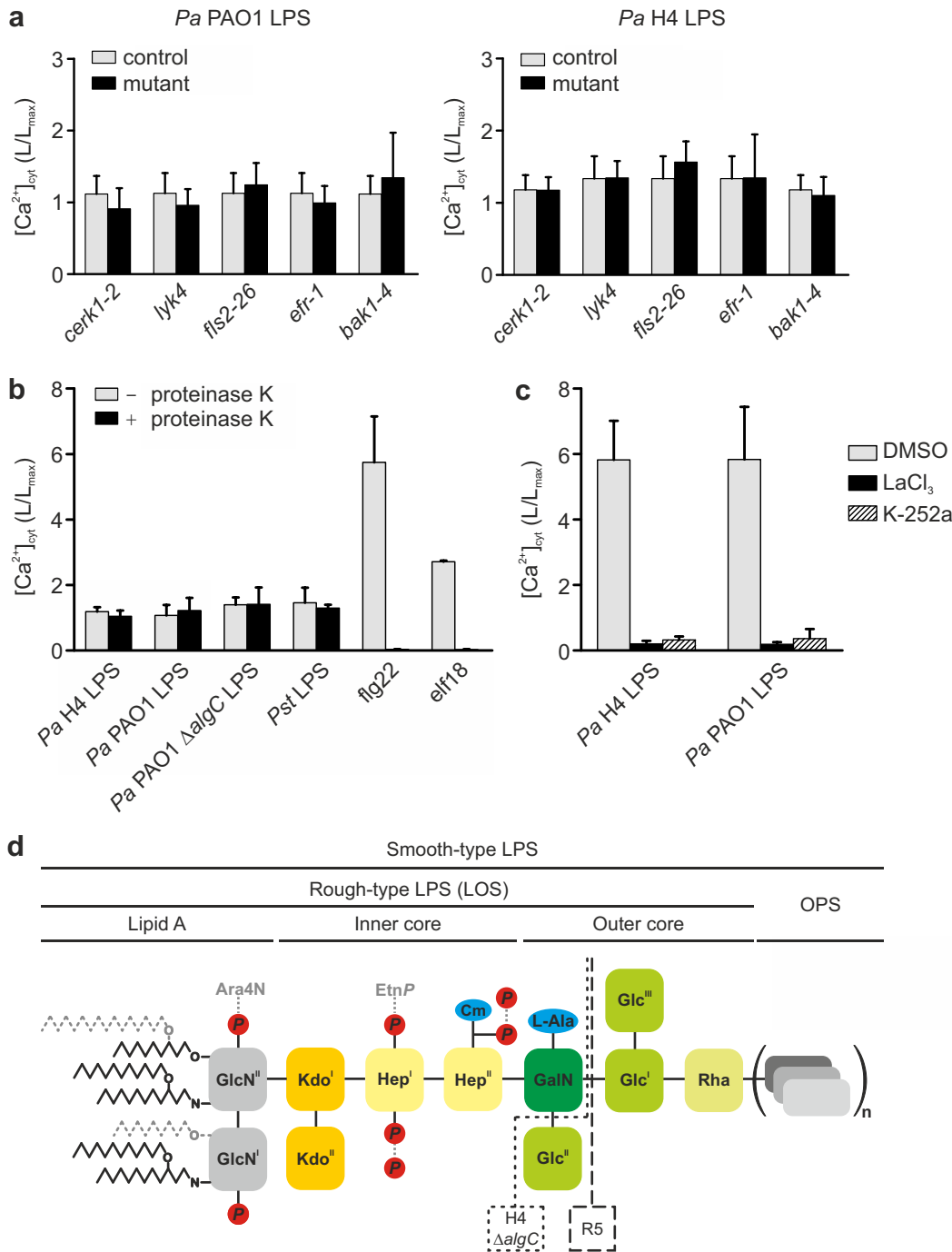
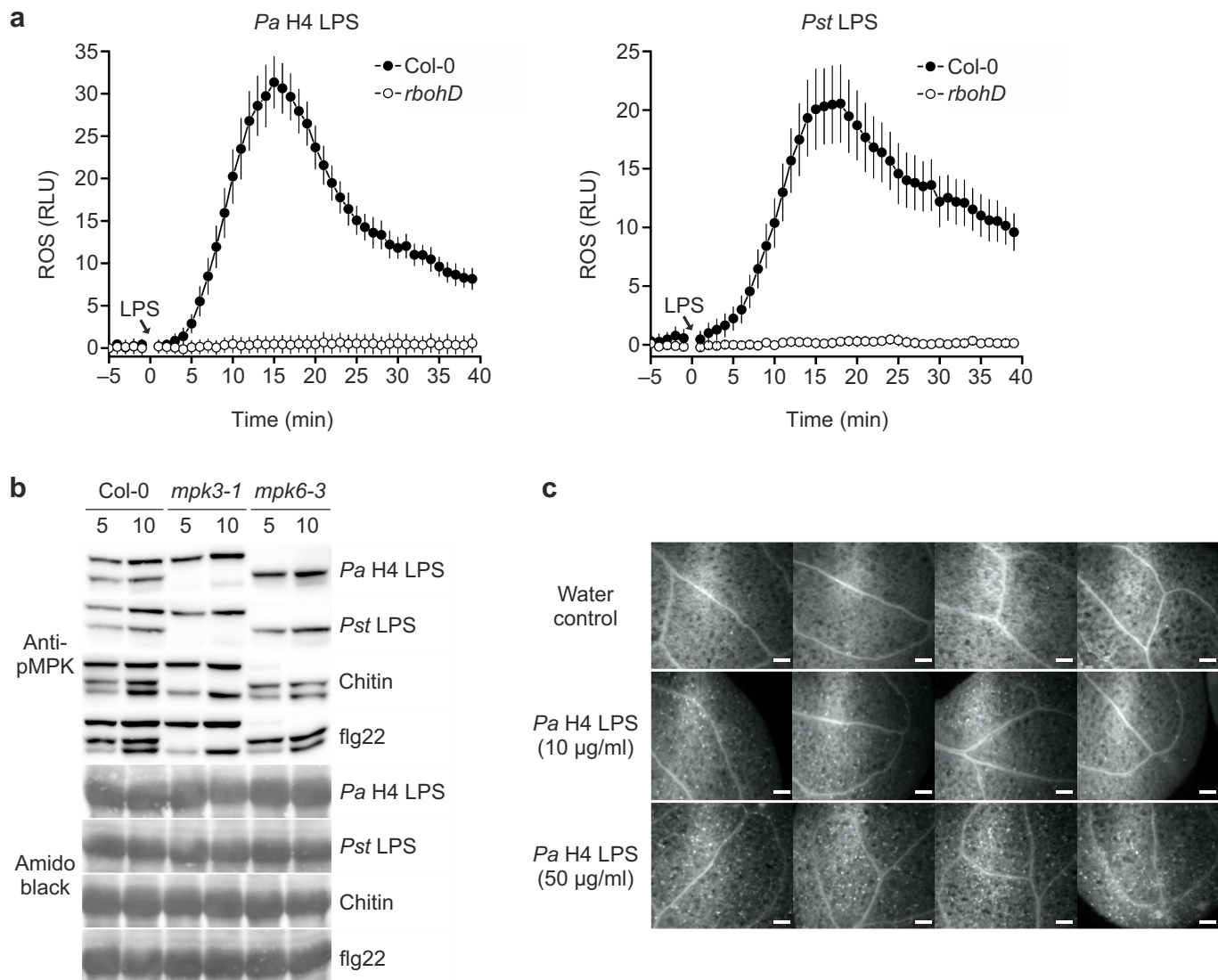


Figure 6

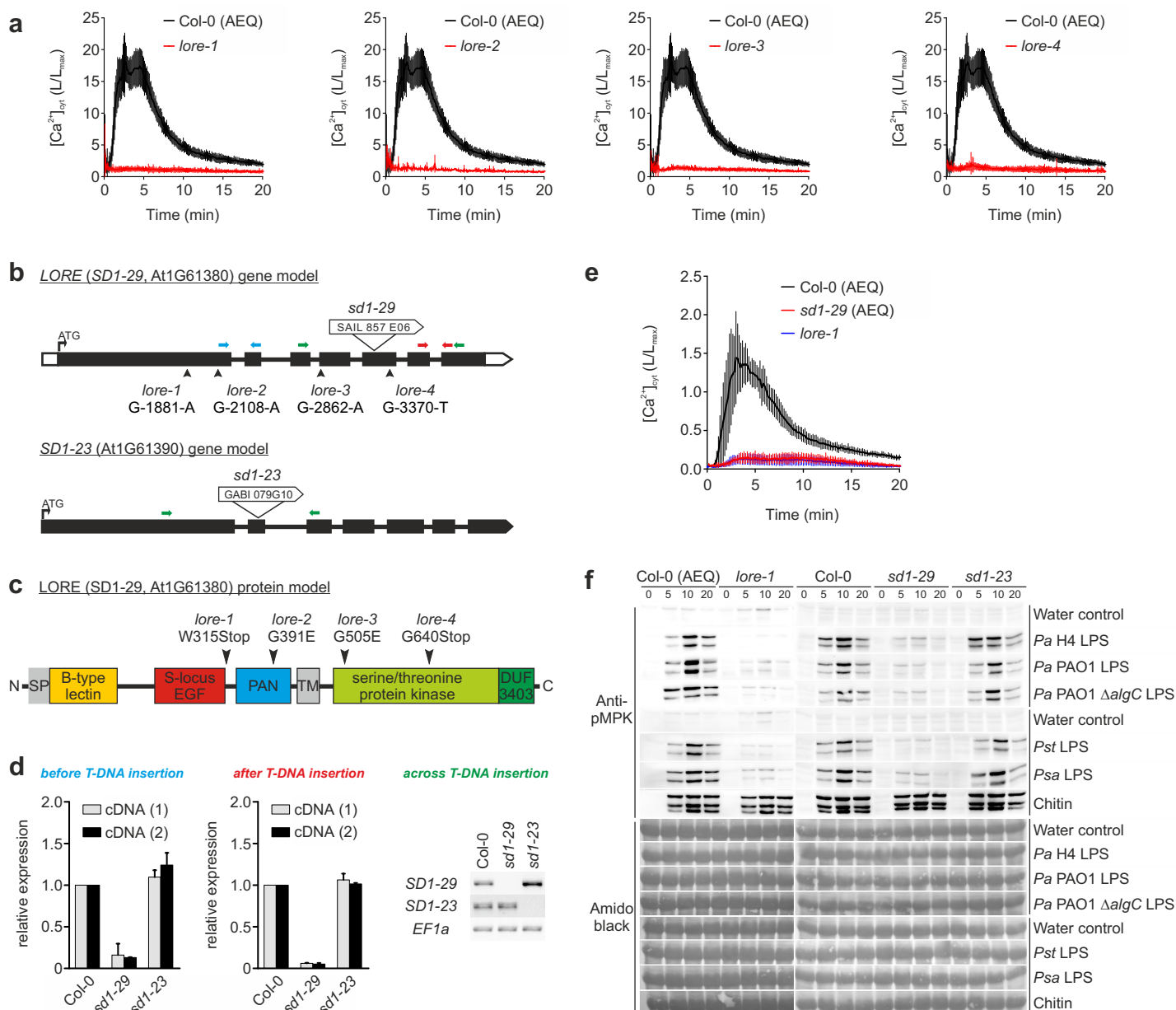




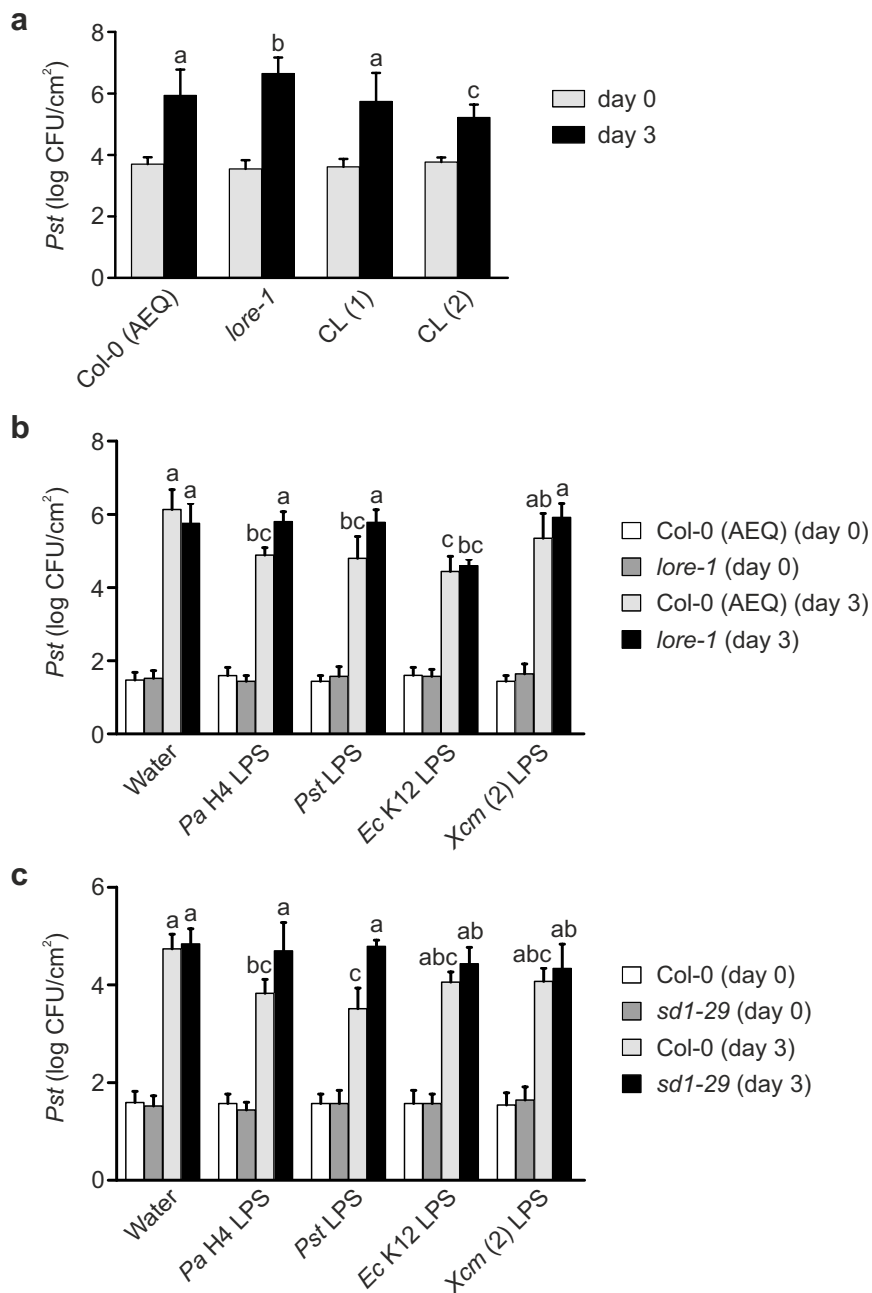
Supplementary Figure 1 LPS-induced $[Ca^{2+}]_{cyt}$ elevations in *cerk1*, *lyk4*, *fls2*, *efr*, and *bak1* mutant lines and after treatment with proteinase K, calcium channel blocker or protein kinase inhibitor. **(a)** $[Ca^{2+}]_{cyt}$ elevations in Col-0^{AEQ} control and indicated mutant seedlings treated with *Pa* PAO1 (left) or *Pa* H4 (right) LPS (50 μ g/ml). Black bars represent LPS-induced maximum $[Ca^{2+}]_{cyt}$ elevations in the indicated mutant line, while grey bars represent maximum LPS-induced $[Ca^{2+}]_{cyt}$ elevations in the respective Col-0^{AEQ} control (mean $\pm$ s.d., $n = 6,6; 4,6; 4,6; 4,9; 6,4$ (left panel) and $n = 9,9; 6,6; 6,6; 6,9; 9,5$ (right panel) individual seedlings per genotype and treatment (left to right); aequorin reconstitution with 5 μ M coelenterazine-h). **(b)** Col-0^{AEQ} seedlings were treated with LPS (50 μ g/ml) or flg22 and elf18 peptides (1 μ M each). LPS preparations (30 μ l of 2 mg/ml) and peptide solutions (30 μ l of 40 μ M) were incubated over night at 60 $^{\circ}$ C with proteinase K (10 μ g) or without as control and heat-inactivated for 15 min at 95 $^{\circ}$ C. Maximum $[Ca^{2+}]_{cyt}$ elevations induced by control-treated sample are shown in comparison to $[Ca^{2+}]_{cyt}$ elevations induced by proteinase K-treated sample at peak maximum (LPS) or at time of peak maximum in control (flg22 and elf18). The experiment was repeated twice with similar results (mean $\pm$ s.d., $n = 4$ individual seedlings per treatment; aequorin reconstitution with 5 μ M coelenterazine-h). **(c)** Col-0^{AEQ} seedlings were treated with 10 mM LaCl₃, 10 μ M K-252a or DMSO as control for 30 min before application of *Pa* PAO1 or *Pa* H4 LPS (50 μ g/ml). Maximum LPS-induced $[Ca^{2+}]_{cyt}$ elevations in DMSO-treated controls are shown in comparison to LPS-induced $[Ca^{2+}]_{cyt}$ elevations at the same time in inhibitor-treated samples (mean $\pm$ s.d., $n = 4$ individual seedlings per treatment; aequorin reconstitution with 10 μ M coelenterazine-native). **(d)** Scheme of LPS structure showing a representative core region and lipid A of *Pseudomonas aeruginosa*. Black dashed lines indicate truncations in rough mutants, grey dotted lines non-stoichiometric modifications. Ara4N, 4-amino-4-deoxy-L-arabinose; Cm, carbamoyl; Etn, ethanolamine; GalN, galactosamine; Glc, glucose; GlcN, glucosamine; Hep, L-glycero-D-manno-heptose; Kdo, 3-deoxy-D-manno-oct-2-ulonic acid; LOS, lipooligosaccharide; OPS, O-polysaccharide; P, phosphate; Rha, L-rhamnose.



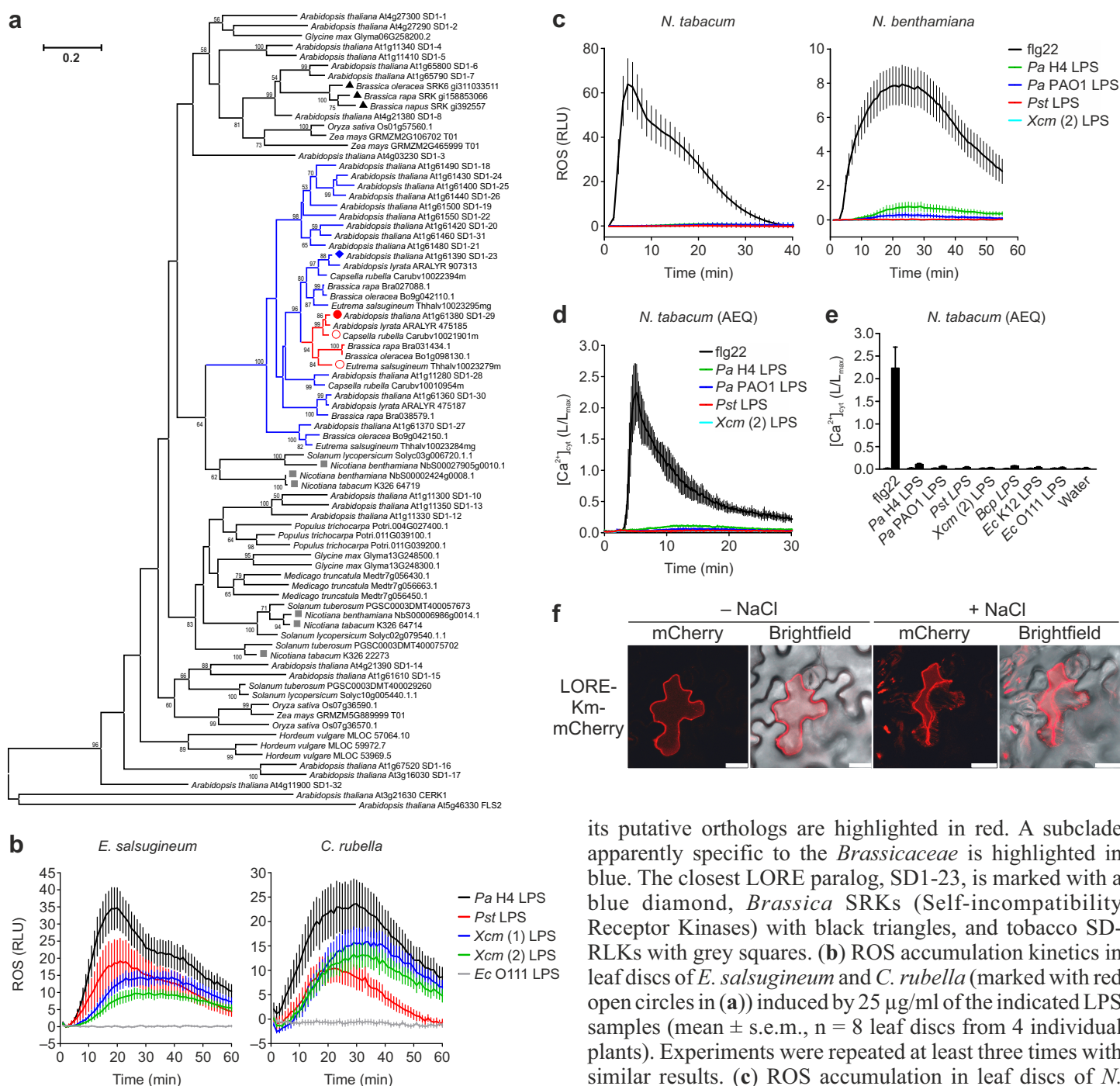
Supplementary Figure 2 ROS production, MPK activation and callose deposition in response to LPS in *A. thaliana*. **(a)** ROS accumulation triggered by *Pa* H4 (left) or *Pst* (right) LPS (10 μ g/ml) in leaf discs of Col-0 control and *rbohD* mutant plants. Data are depicted after subtraction of untreated controls (mean $\pm$ s.e.m., $n = 8$ leaf discs from four individual plants per genotype). The experiment was repeated twice with similar results. **(b)** MPK activation in Col-0 control compared to *mpk3-1* and *mpk6-3* mutant seedlings treated with LPS (50 μ g/ml), chitin (50 μ g/ml) or flg22 (100 nM) was detected 5 and 10 min after elicitation by anti-phospho-MPK immunoblot. Amido black staining shows total protein loading. The experiment was repeated twice with similar results. **(c)** Col-0 seedlings were treated for 24 hours with *Pa* H4 LPS or water as control. Callose deposition was visualized by fluorescence microscopy after aniline blue staining. Four individual cotyledons per treatment are shown (scale bar = 200 μ M). The experiment was repeated three times with similar results.



Supplementary Figure 3 LPS-induced $[Ca^{2+}]_{cyt}$ elevations and MPK activation in *lore*, *sd1-29* and *sd1-23* *A. thaliana* mutants. **(a)** $[Ca^{2+}]_{cyt}$ elevation kinetics in Col-0^{AEQ} control and different *lore* mutant seedlings in response to *Pa* H4 LPS (50 μ g/ml). The experiment was repeated twice with similar results (mean $\pm$ s.d., $n = 7, 7; 7, 3; 7, 7; 7, 9$ (left to right); Col-0^{AEQ} controls are identical in all graphs; aequorin reconstitution with 20 μ M coelenterazine-native). Full figure of **Figure 2a**. **(b)** Schematic representation of *SD1-29* and *SD1-23* gene structure. Black boxes indicate exons and white boxes indicate untranslated regions (UTR). Arrowheads show location of *lore* point mutations and white arrows T-DNA insertion sites. **(c)** LORE protein scheme showing predicted conserved domains and location of *lore* loss-of-function mutations. SP, signal peptide; TM, transmembrane; EGF, epidermal growth factor; PAN, plasminogen-apple-nematode; DUF, domain of unknown function. **(d)** Analysis of *SD1-29* and *SD1-23* expression by quantitative real-time RT-PCR (before and after the T-DNA insertion site) in two Col-0 control, *sd1-29* and *sd1-23* seedling pools each (labelled as cDNA (1) and (2)) or by semi-quantitative RT-PCR (across the T-DNA insertion site) using the primers (**Supplementary Table 2**) indicated by colored arrows. Expression of *EF1a* served as constitutive control. Semi-quantitative RT-PCR experiments were repeated twice with similar results. **(e)** $[Ca^{2+}]_{cyt}$ elevations in Col-0^{AEQ}, *lore-1* and *sd1-29*^{AEQ} seedlings treated with *Pa* H4 LPS (15 μ g/ml). Experiment was repeated twice with similar results (mean $\pm$ s.d., $n = 10, 7, 10$ (top down) individual seedlings per genotype; aequorin reconstitution with 5 μ M coelenterazine-h). **(f)** MPK activation in seedlings treated with the indicated LPS preparations (20 μ g/ml), chitin (50 μ g/ml) or water as control was detected at indicated time points (min) after elicitation by anti-phospho-MPK immunoblot. Amido black staining shows total protein loading. Full version of **Figure 2f**. The experiment was repeated twice with similar results.

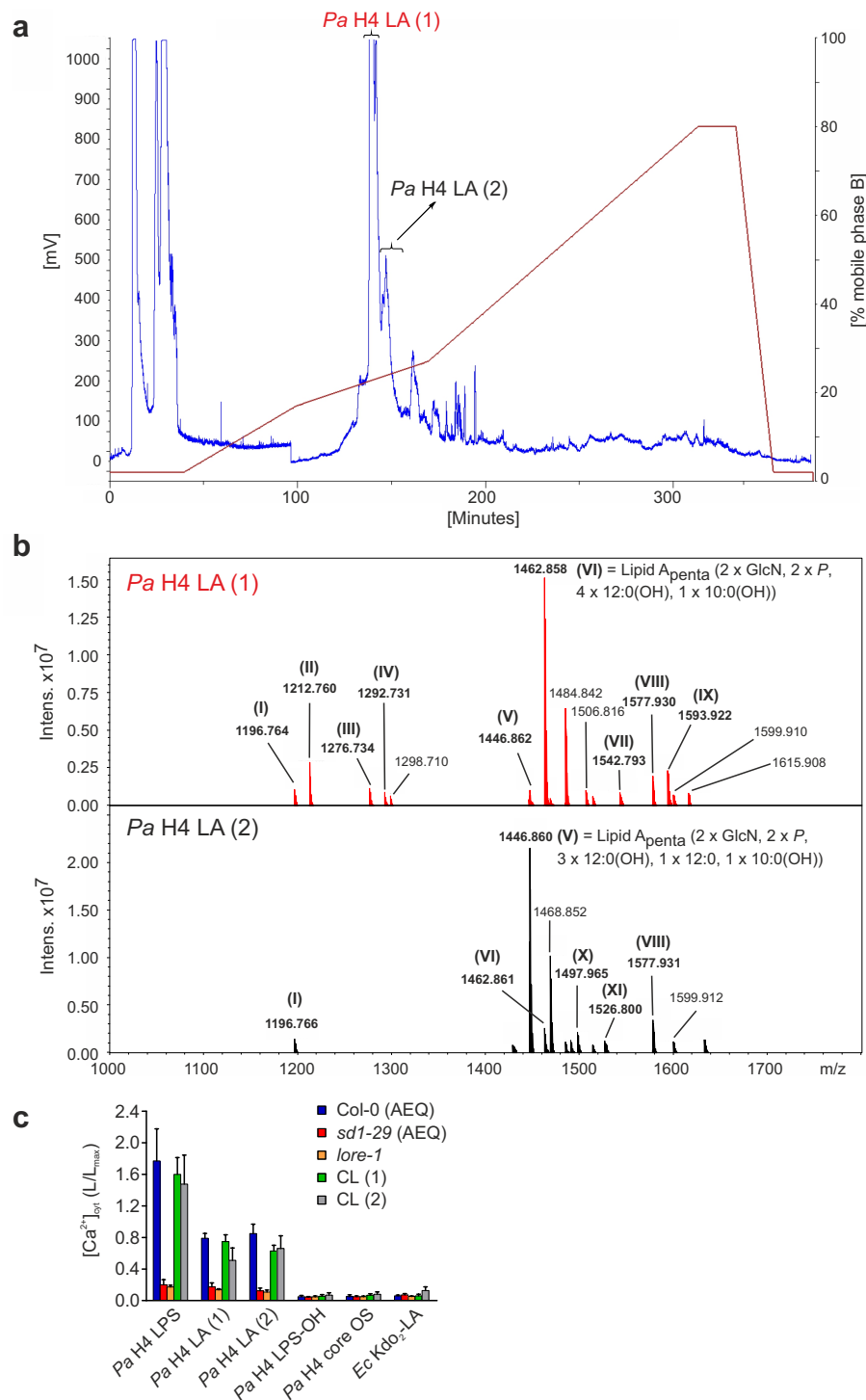


Supplementary Figure 4 *Pst* DC3000 infection in *lore* and *sd1-29* mutants. **(a)** *Pst* DC3000 infection of Col-0^{AEQ} control, *lore-1* mutant and complementation lines (CL) by spray inoculation (1×10^8 CFU/ml inoculum). Bacterial growth was measured after three days (mean $\pm$ s.d., $n = 12$ leaf samples randomly obtained from four individual plants per genotype; different letters indicate different statistical significance groups according to two-way analysis of variance (ANOVA; genotype $\times$ treatment) and Bonferroni's multiple-comparison post-test, $P < 0.05$). The experiment was repeated three times with similar results. **(b,c)** Plants were treated with 25 μ g/ml of the indicated LPS or water as control one day before *Pst* DC3000 infiltration (1×10^5 CFU/ml inoculum). Bacterial growth was measured after three days (mean $\pm$ s.d., **(b)** $n = 6$ and **(c)** $n = 4$ for *sd1-29* (day 3), all others $n = 6$ leaf samples randomly obtained from three individual plants per genotype; different letters indicate different statistical significance groups according to one-way analysis of variance (ANOVA) and Tukey's multiple-comparison post-test, $P < 0.05$).



Supplementary Figure 5 Phylogenetic distribution of LORE and functional analysis of LPS sensing in selected plant species. **(a)** Phylogram of SD-RLKs from *A. thaliana* and diverse other plant species. Protein BLAST was performed against diverse plant species using LORE amino acid sequence as query. Sequence data sources: TAIR10, Ensembl Plants, BOLbase, Sol Genomics Network and NCBI databases. Plant families (species): *Brassicaceae* (*Arabidopsis thaliana*, *Arabidopsis lyrata*, *Brassica rapa*, *Brassica oleracea*, *Capsella rubella*, *Eutrema salsugineum*), *Fabaceae* (*Glycine max*, *Medicago truncatula*), *Solanaceae* (*Nicotiana benthamiana*, *Nicotiana tabacum*, *Solanum lycopersicum*, *Solanum tuberosum*), *Poaceae* (*Hordeum vulgare*, *Oryza sativa*, *Zea mays*), *Salicaceae* (*Populus trichocarpa*). Sequences of *A. thaliana* SD-1 RLKs⁴, RLKs FLS2 and CERK1, and the best LORE BLAST hits (max. 3 from each species) were aligned using ClustalW. A phylogenetic tree was constructed using the Maximum Likelihood method implemented in MEGA6. Bootstrapping values (1000 replicates; >50%) are shown next to the branches. Branch lengths indicate the number of substitutions per site. LORE (marked with red filled dot) and

its putative orthologs are highlighted in red. A subclade apparently specific to the *Brassicaceae* is highlighted in blue. The closest LORE paralog, SD1-23, is marked with a blue diamond, *Brassica* SRKs (Self-incompatibility Receptor Kinases) with black triangles, and tobacco SD-RLKs with grey squares. **(b)** ROS accumulation kinetics in leaf discs of *E. salsugineum* and *C. rubella* (marked with red open circles in (a)) induced by 25 μ g/ml of the indicated LPS samples (mean $\pm$ s.e.m., n = 8 leaf discs from 4 individual plants). Experiments were repeated at least three times with similar results. **(c)** ROS accumulation in leaf discs of *N. tabacum* (left) and *N. benthamiana* (right) treated with 50 μ g/ml of the indicated LPS preparations or 1 μ M flg22. Data are depicted after normalization to ROS levels before LPS application and subtraction of untreated controls (mean $\pm$ s.e.m.; n = 8 leaf discs from three individual plants). The experiment was repeated twice with similar results. **(d)** $[Ca^{2+}]_{cyt}$ elevations kinetics in aequorin-expressing *N. tabacum* seedlings treated with 50 μ g/ml of the indicated LPS or 1 μ M flg22 after 2 min measurement of the resting $[Ca^{2+}]_{cyt}$. **(e)** Maximum $[Ca^{2+}]_{cyt}$ elevations (black bars) in aequorin-expressing *N. tabacum* seedlings induced by 50 μ g/ml of the indicated LPS preparations, 1 μ M flg22 or water as control are shown compared to the resting $[Ca^{2+}]_{cyt}$ at 1 min (grey bars). **(d,e)** Data in (d) and (e) are from the same experiment. Whereas (d) shows the kinetics of the response, (e) shows the comparison of the maximum $[Ca^{2+}]_{cyt}$ elevations compared to the resting $[Ca^{2+}]_{cyt}$ (mean $\pm$ s.d.; n = 3 for *Pst* LPS all other treatments n = 4 individual seedlings). **(d)** Epidermal *N. benthamiana* cell expressing LORE-Km-mCherry before and ~15 min after treatment with 1 M NaCl for plasmolysis (scale bar = 25 μ M). Due to water loss in the hypertonic NaCl solution, the protoplast including the plasma membrane is retracted from the cell wall.



Supplementary Figure 6 HPLC purification and analysis by mass spectrometry of *Pa* H4 lipid A. **(a)** Reversed phase HPLC profile obtained from the *Pa* H4 LPS after mild acetate buffer hydrolysis. A semi-preparative Kromasil C18 column (5 μ m, 100 $\text{\AA}$, 20 x 250 mm, MZ Analysentechnik) was used and the sample (4 mg/ml in $\text{CHCl}_3/\text{CH}_3\text{OH}$ 4:1, v/v) was eluted using a gradient that consisted of methanol-chloroform-water (57:12:31, v/v/v) containing 10 mM NH_4OAc as mobile phase A and chloroform-methanol (70.2:29.8, v/v) with 50 mM NH_4OAc as mobile phase B. The initial solvent system consisted of 2% B and maintained for 40 min, followed by a linear three step gradient raising from 2 to 17% B (40-100 min), 17 to 27% B (100-170 min), and 27 to 80% B (170-314 min). The solvent was held at 80% B for 20 min, the column re-equilibrated in 20 min to 2% B and held there for additional 21 min before the next injection. The flow rate was 4 ml/min using a splitter between the evaporative light-scattering detector (Sedex model 75C ELSD, S.E.D.E.R.E., France) equipped with a low-flow nebulizer recording the chromatogram and the fraction collector (fractions in 1 min intervals). Nitrogen (purity 99.996%) was used as gas to nebulize the post column flow stream at 3.5 bar into the detector at 50 $^\circ\text{C}$ setting the photomultiplier gain to 11. The detector signal was transferred to the Gilson HPLC Chemstation (Trilution LC, version 2.1, Gilson) for detection and integration of the ELSD signal. The major lipid A containing pools are indicated as LA (1) and (2), respectively. **(b)** The corresponding mass spectra of the two lipid A containing HPLC pools. Electrospray Ionization Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FT-ICR-MS) was performed on a 7 Tesla APEX Qe instrument (Bruker Daltonics, USA). Samples were dissolved in a water/propan-2-ol/7 M triethylamine/acetic acid mixture (50:50:0.06:0.02, v/v/v/v) and spectra were acquired in the negative ion mode with nano-ESI using the Triversa Nanomate (Advion, USA) as ion source with a spray voltage set to -1.1 kV. Mass spectra were recorded in broad band mode and the mass scale was externally calibrated with glycolipids of known structure. Spectra were charge deconvoluted; given mass numbers refer to the monoisotopic mass of neutral molecules. Mass peaks representing proton adducts of lipid A species are assigned in bold style, the other assigned mass peaks are belonging to sodium adducts ($\Delta = +22$ u) of these molecules. The major species (VI) in *Pa* H4 LA (1) could be assigned to a penta-acylated lipid A composed of 2 x GlcN, 2 x P, 4 x 12:0(OH), and 1 x 10:0(OH) (monoisotopic MW_{calc} : 1462.816 u). Minor molecule species with a third phosphate moiety ((VII), MW_{calc} : 1542.777 u) or with an additional Ara4N residue ((IX), MW_{calc} : 1593.869 u) are present as well. The major species in *Pa* H4 LA (2), molecule (V), could be assigned to a penta-acylated lipid A as well, but with one 12:0 instead of a 12:0(OH) (composition: 2 x GlcN, 2 x P, 3 x 12:0(OH), 1 x 12:0, and 1 x 10:0(OH); MW_{calc} : 1446.821 u). (XI) corresponds to (V) + P (MW_{calc} : 1526.782 u) and (VIII) to (V) + Ara4N (MW_{calc} : 1577.874 u), (X) corresponds to (VIII) - P (MW_{calc} : 1497.910 Da). Mainly in pool (1), minor occurring tetra-acylated lipid A variants could be identified as well ((III) = (V) - 10:0(OH) (MW_{calc} : 1276.696 u); (IV) = (VI) - 10:0(OH) (MW_{calc} : 1292.691 u); (I) (MW_{calc} : 1196.735 u) and (II) (MW_{calc} : 1212.730 u) are the mono-phosphorylated forms of (III) and (IV), respectively). **(c)** Maximum $[\text{Ca}^{2+}]_{\text{cyt}}$ after treatment with the indicated LPS fragments (25 $\mu\text{g}/\text{ml}$) in Col-0^{AEQ} controls, *lore-1* or *sd1-29*^{AEQ} mutants and complementation lines (CL) (mean $\pm$ s.d., n = 4,4,4,6,4; 4,6,4,6,4; 4,6,4,6,4; 4,4,4,6,4; 4,6,4,6,3; 4,5,4,6,3 individual seedlings per genotype and treatment (left to right); 10 μM coelenterazine-h). LA (1) and LA (2), HPLC lipid A fractions (1) and (2); LPS-OH, hydrazine-treated LPS carrying only the two amide-bound fatty acids; core OS, isolated core oligosaccharide.

Supplementary Table 1 Bacterial strains, LPS isolation methods and references

Bacterial strain	Isolation procedure*	Reference/ source
<i>P. aeruginosa</i> Fisher-type 1 (F1) (Habs 6)**	Phenol/water	1, 2
<i>P. aeruginosa</i> PA01	Phenol/water	3
<i>P. aeruginosa</i> PA01 Δ algC	PCP	3
<i>P. aeruginosa</i> H4	PCP	4
<i>P. aeruginosa</i> R5	PCP	5
<i>P. aeruginosa</i> PAN1	PCP	6
<i>P. aeruginosa</i> PAC 1R Δ algC	PCP	3
<i>P. syringae</i> pv. <i>tomato</i> DC3000	1) Phenol/water 2) PCP	this study***
<i>P. syringae</i> pv. <i>apii</i>	Phenol/water	this study***
<i>P. alcaligenes</i> 537	Phenol/water	this study
<i>P. fluorescens</i> ATCC49271	PCP	7
<i>X. campestris</i> pv. <i>malvacearum</i> GSPB1386	Phenol/water	8
<i>X. campestris</i> pv. <i>malvacearum</i> HSV GSPB2388	Phenol/water	8
<i>X. campestris</i> pv. <i>phaseoli</i> var. <i>fuscans</i> GSPB271	Phenol/water	8
<i>X. campestris</i> pv. <i>begoniae</i> GSPB525	Phenol/water	9
<i>B. cepacia</i> (GIFU12046/3P-62)****	Phenol/water	10
<i>B. pseudomallei</i> (GIFU645/ATCC25416; LPS-1)****	Phenol/water	11
<i>E. coli</i> K12 (Ultrapur)	-	InvivoGen
<i>E. coli</i> O111:B4 (Ultrapur)	-	InvivoGen
<i>E. coli</i> KPM 53 (HPLC-purified Kdo ₂ -lipid A)	PCP	this study, 12
<i>S. enterica</i> sv. Typhimurium (TLRgrade)	-	Enzo Life Sciences
<i>S. enterica</i> sv. Minnesota R595 (Ultrapur)	-	InvivoGen

*LPS preparations were generated using phenol/water extraction¹³, phenol-chloroform-petroleum ether (PCP) extraction¹⁴ or a combination of the two protocols in the indicated order. **Kind gift of B. A. Dmitriev. ***The inner core of *P. syringae* pv. *phaseolicola* has been investigated¹⁵ and proven to be identical with the published inner core structures of *P. aeruginosa* and *P. fluorescens*. ****Kind gift of K. Kawahara.

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Supplementary Table 2 Primers used in this study

Gene AGI code	Primer	Sequence (5' -> 3')	used for	sqPCR cycle #
At1G61380	SD1-29-F	TCTCTTTGCTGGTCAATAGGG	PCR	
At1G61380	SD1-29-R	CGCAATATTGGGGCTTTATG	PCR	
At1G61380	SD1-29-F1	TTCAAAGAAGTAGTGTGAACCAAGA	Sequencing	
At1G61380	SD1-29-F2	CTGGGGAGTTCTCACTGGAG	Sequencing	
At1G61380	SD1-29-F3	CGGAGAGTTTCTCTTCATTCG	Sequencing	
At1G61380	SD1-29-F4	GTTCCGGTCAGGGTACAGAG	Sequencing	
At1G61380	SD1-29-F5	ATGGGCAGGATTGTTCTCTG	Sequencing	
At1G61380	SD1-29-F2b	CACAAGGCAAAGACACAGACA	Sequencing	
At1G61380	SD1-29-F3b	TGCATGGAAGAATGGTTTTG	Sequencing	
At1G61380	SD129-START	tcgtctctaATGGGTATGGTTTTATTTGCTTGC	Cloning	
At1G61380	SD129-STOP	tcgtctctaCTACCTTCCTTGGATCATAGATTCTG	Cloning	
At1G61380	SD129-PROM	tcgtctctCGAGAAACAGTGTGTGATCGAAATTTG	Cloning	
At1G61380	SD129-EspMut-F	tcgtctctGTtAGACGTACAAAATTATCTTGCC	Cloning	
At1G61380	SD129-EspMut-R	tcgtctccTaACACACCCACTTGTCCAG	Cloning	
At1G61380	SD129-K516A-F	tgaagactTgcaCGCCTTGCTAGTAGTTCC	Mutagenesis	
At1G61380	SD129-K516A-R	tgaagactTgcaACACCTATTTCTTCCCATC	Mutagenesis	
At1G61380	before_SAIL857E06-F	GCGGAGAGTTTCTCTTCATTCG	qRT-PCR	
At1G61380	before_SAIL857E06-R	GTTTCGCTCTGTATCTCCACAAC	qRT-PCR	
At1G61380	after_SAIL857E06-F	AGCGGAAAGAGAATTTACAG	qRT-PCR	
At1G61380	after_SAIL857E06-R	ACTTCGAATGCTTGGCATGT	qRT-PCR	
AT3G62250	UBQ5-F	CCAAGCCGAAGAAGATCAA	qRT-PCR	
AT3G62250	UBQ5-R	ACTCCTTCCTCAAACGCTGA	qRT-PCR	
At1G02930	GST1-F	TGTCGAGCTCAAAGATGGTG	sqRT-PCR	23x
At1G02930	GST1-R	GGACTCACCAAGCCTGTGTT	sqRT-PCR	
At2G19190	FRK1-F	TGAAGGAAGCGGTCAGATTT	sqRT-PCR	30x
At2G19190	FRK1-R	CTGACTCATCGTTGGCCTCT	sqRT-PCR	
At1G07920	EF1a-F	TCACATCAACATTGTGGTCATTGGC	sqRT-PCR	26x
At1G07920	EF1a-R	TTGATCTGGTCAAGAGCCTCAAG	sqRT-PCR	
At1G61380	SAIL857E06-LP	TGCCACCAATAACTTCAGTCC	Genotyping sqRT-PCR	34x
At1G61380	SAIL857E06-RP	ACCAATCTGAACACATCTCGC	Genotyping sqRT-PCR	
At1G61390	GABI079G10-LP	GGGAAAATGAAGATCCTCTGG	Genotyping sqRT-PCR	44x (Phusion)
At1G61390	GABI079G10-RP	CATGCATCTTGTGAACTGTGG	Genotyping sqRT-PCR	
---	GABI-o8474	ATAATAACGCTGCGGACATCTACATTTT	Genotyping	
---	SAIL-LB1short	TGGATAAATAGCCTTGCTTCC	Genotyping	