Plant, Cell and Environment (2017) 40, 2644–2662 doi: 10.1111/pce.12994

Original Article

The dual role of LESION SIMULATING DISEASE 1 as a condition-dependent scaffold protein and transcription regulator

Weronika Czarnocka[1,2], Katrien Van Der Kelen[3,4], Patrick Willems[3,4], Magdalena Szechyńska-Hebda[1,5], Sara Shahnejat-Bushehri[6,7], Salma Balazadeh[6,7], Anna Rusaczonek[1], Bernd Mueller-Roeber[6,7], Frank Van Breusegem[3,4] & Stanisław Karpiński[1]

[1]Department of Plant Genetics, Breeding and Biotechnology, Faculty of Horticulture, Biotechnology and Landscape Architecture, Warsaw University of Life Sciences (SGGW), Nowoursynowska Street 159, 02-776 Warsaw, Poland, [2]Department of Botany, Faculty of Agriculture and Biology, Warsaw University of Life Sciences (SGGW), Nowoursynowska Street 159, 02-776 Warsaw, Poland, [3]Ghent University, Department of Plant Biotechnology and Bioinformatics, Technologiepark 927, 9052 Ghent, Belgium, [4]VIB Center for Plant Systems Biology, Technologiepark 927, 9052 Ghent, Belgium, [5]Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek Street 21, 30-239 Cracow, Poland, [6]Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht Street 24-25, 14476 Potsdam, Germany and [7]Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, 14476 Potsdam, Germany

ABSTRACT

Since its discovery over two decades ago as an important cell death regulator in *Arabidopsis thaliana*, the role of LESION SIMULATING DISEASE 1 (LSD1) has been studied intensively within both biotic and abiotic stress responses as well as with respect to plant fitness regulation. However, its molecular mode of action remains enigmatic. Here, we demonstrate that nucleo-cytoplasmic LSD1 interacts with a broad range of other proteins that are engaged in various molecular pathways such as ubiquitination, methylation, cell cycle control, gametogenesis, embryo development and cell wall formation. The interaction of LSD1 with these partners is dependent on redox status, as oxidative stress significantly changes the quantity and types of LSD1-formed complexes. Furthermore, we show that LSD1 regulates the number and size of leaf mesophyll cells and affects plant vegetative growth. Importantly, we also reveal that in addition to its function as a scaffold protein, LSD1 acts as a transcriptional regulator. Taken together, our results demonstrate that LSD1 plays a dual role within the cell by acting as a condition-dependent scaffold protein and as a transcription regulator.

Key-words: *Arabidopsis thaliana*; dry weight; LSD1; oxidative stress; protein interaction; transcription regulation.

Correspondence: S. Karpiński, Department of Plant Genetics, Breeding and Biotechnology, Faculty of Horticulture, Biotechnology and Landscape Architecture, Warsaw University of Life Sciences (SGGW), Nowoursynowska Street 159, 02-776 Warsaw, Poland. Tel.: +48 22 593 2172; e-mail: stanislaw_karpinski@sggw.pl

INTRODUCTION

Because of their sessile nature, plants growing in a natural environment are continuously exposed to a broad range of both biotic and abiotic stresses. During exposure to a certain unfavourable stimulus or to multiple adverse environmental conditions, plants' optimal metabolic processes get out of balance, which leads to a disturbance of cellular homeostasis that can trigger cell death (CD). Cell death is an organized process of the cells' self-elimination. It plays a crucial role in plant development (Fukuda 2000; Domínguez & Cejudo 2014), immune defence (Jabs, Dietrich, & Dangl 1996) and acclimatory responses (Mühlenbock *et al.* 2007). In this context, CD is not only the ultimate end of the cell life cycle but also maintains cell homeostasis in various plant organs and tissues during unfavourable environmental conditions. While the molecular mechanisms of CD are rather well documented in animal systems, the molecular processes underlying plant CD remain largely enigmatic.

Knowledge gained regarding the molecular, physiological and genetic mechanisms of plant CD at various levels of complexity (cellular or organismal) was facilitated via the identification of various *Arabidopsis thaliana* mutants exhibiting deregulated CD (Dietrich *et al.* 1994; Lorrain *et al.* 2003; Vandenabeele *et al.* 2004; Moeder & Yoshioka 2008; Bruggeman *et al.* 2015). One of the best-studied CD mutants is *lsd1,* which lacks a functional LESION SIMULATING DISEASE 1 protein (LSD1, encoded by AT4G20380). The molecular function of LSD1 is currently unknown, but the phenotype of *lsd1* mutants is characterized by the so-called runaway CD (RCD). Runaway cell death manifests itself as plant leaves' inability to restrict CD propagation once it has been initiated by an external stimulus. Such uncontrolled systemic spread of foliar RCD in *lsd1* can be evoked by factors like excess light (EL) or red light (RL) (Mateo *et al.* 2004; Chai *et al.* 2015), root hypoxia, impeded stomatal conductance

(Mühlenbock *et al.* 2007, 2008), low temperature (Huang *et al.* 2010), drought (Wituszyńska *et al.* 2013; Szechyńska-Hebda *et al.* 2016), UV radiation (Wituszyńska *et al.* 2015) or pathogen infections (Dietrich *et al.* 1994; Rustérucci *et al.* 2001). Therefore, LSD1 is considered to be a negative CD regulator that integrates various signalling pathways in response to both biotic and abiotic stresses (Karpiński *et al.* 2013).

Initially, the uncontrolled spread of CD in *lsd1* was correlated with the accumulation of superoxide ions produced by plasma-membrane-bound NADPH oxidase (Jabs *et al.* 1996). Later, other ROS, such as hydrogen peroxide (H O), were also shown to be engaged in the propagation of *lsd1*-dependent CD during EL, RL, UV, low temperature and plastoquinone reduction (Mateo *et al.* 2004; Mühlenbock *et al.* 2007, 2008; Huang *et al.* 2010; Chai *et al.* 2015; Wituszyńska *et al.* 2015) or after infection with avirulent pathogens (Rustérucci *et al.* 2001; Li *et al.* 2013). Higher ROS levels in the *lsd1* mutant were caused by lower initial activities of antioxidant enzymes (Kliebenstein *et al.* 1999; Mateo *et al.* 2004; Chai *et al.* 2015; Wituszyńska *et al.* 2015). Therefore, LSD1 was suggested to be a positive regulator of antioxidant machinery and to act as a ROS rheostat preventing the pro-death pathways below a certain level of oxidative stress (Wituszyńska *et al.* 2015).

Importantly, the *lsd1* CD phenotype depends on ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1, AT3G48090) and PHYTOALEXIN DEFICIENT 4 (PAD4, AT3G52430), two proteins with triacylglycerol lipase domains that were originally described as components of gene-mediated and basal disease resistance (Parker *et al.* 1996; Glazebrook *et al.* 1997; Falk *et al.* 1999; Jirage *et al.* 1999). Both of these proteins are essential for CD propagation in *lsd1* because in the double loss-of-function mutants *eds1/lsd1* and *pad4/lsd1* RCD was inhibited regardless of the type of stimulus that was imposed on the plants (Rustérucci *et al.* 2001; Mateo *et al.* 2004; Mühlenbock *et al.* 2007, 2008; Wituszyńska *et al.* 2015). Moreover, LSD1 and EDS1/PAD4 elicit opposite effects on ROS and salicylic acid (SA) accumulation under different adverse conditions (Mühlenbock *et al.* 2008; Huang *et al.* 2010; Wituszyńska *et al.* 2013, 2015). Therefore, LSD1 is considered to be a negative regulator of EDS1- and PAD4-dependent cellular pathways that lead to CD. Apart from playing an important role in both abiotic and biotic stress responses, LSD1, together with EDS1 and PAD4, also participates in the conditional regulation of processes that determine plant fitness, for example, photosynthesis, water use efficiency, cellular ROS/hormonal homeostasis and seed yield (Wituszyńska *et al.* 2013, 2015).

Despite its evident importance in plant CD regulation and acclimation, still very little is known about the molecular function of LSD1 and the cellular pathways employing it. It was already suggested that LSD1 might act as a transcription factor (TF) or scaffold protein because it possesses three zinc (Zn)-finger domains that could be responsible for DNA/protein binding (Dietrich *et al.* 1997; Coll *et al.* 2011). The Zn-finger motifs in LSD1 belong to the C2C2 class that are also present in GATA1-type transcription factors containing the conserved consensus sequence: CxxCRxxLMYxxGASxVxCxxC (Dietrich *et al.* 1997; Takatsuji 1998).

When it comes to the protein partners of LSD1, it has been demonstrated that LSD1 interacts with bZIP10 TF, preventing its translocation to the nucleus. Functional bZIP10 is required for *lsd1*-specific RCD during both R-gene-mediated and basal defence responses (Kaminaka *et al.* 2006). A yeast two-hybrid (Y2H) screen revealed 10 additional putative LSD1 interactors (Coll *et al.* 2011); among others, a cysteine-dependent protease, metacaspase 1 (MC1), which is a positive regulator of CD (Coll *et al.* 2010). The second and third Zn-finger domains of LSD1 are responsible for MC1-binding in the Y2H system, and null mutation in MC1 was able to suppress CD in the *lsd1* background (Coll *et al.* 2010). Another protein that interacted with LSD1 in Y2H and the pull-down assays was the GSH-induced LITAF domain protein (GILP), which is a negative regulator of pathogen-induced CD (He, Tan, *et al.* 2011b). Altogether, these results suggest that LSD1 may act as a scaffold protein, bringing together other CD molecular regulators.

However, the assumption that LSD1 acts as a transcriptional regulator by itself cannot be ruled out. In our previous work, we indicated that *LSD1* loss-of-function provokes significant changes in gene expression profiles (Wituszyńska *et al.* 2013). Interestingly, these transcriptional effects were strongly dependent on the growing conditions (laboratory or field). The *lsd1* RCD phenotype did not appear in natural field conditions and correlated with only 105 genes that were deregulated in comparison to wild-type plants. On the other hand, in highly controlled laboratory conditions, *lsd1* displayed significant changes in the expression of 2100 genes and exhibited RCD; only 43 genes were commonly deregulated in both growing conditions (Wituszyńska *et al.* 2013).

Recognizing the LSD1 cellular mode of action and the specific molecular pathways that employ it remains a challenge that would help us understand the mechanism of CD in plants; thus the purpose of this study was to thoroughly investigate the subcellular function of LSD1. In order to accomplish this, we employed different molecular techniques that aimed at defining the subcellular localization of LSD1 and *in-vivo* LSD1-interacting proteins both in non-stress and in oxidative-stress conditions. Our goal was also to identify genes whose expression is regulated by LSD1 and to answer the question if LSD1 can act as a direct transcriptional regulator.

MATERIALS AND METHODS

Plant material and growth conditions

Arabidopsis wild-type plants, accession Wassilewskija (Ws-0), the *lsd1-1* mutant and two lines overexpressing LSD1 fused to a green fluorescent protein (GFP) or a glucocorticoid receptor (GR), both under the control of the cauliflower mosaic virus 35S (CaMV 35S) promoter, were used. For phenotyping purposes, the plants were grown in soil in growth chambers under a 16 h (long day) or 9 h (short day) photoperiod at a photosynthetic photon flux density (PPFD):

100 ± 25 mol m^{-2} s^{-1}, 50% relative air humidity and at a temperature of 22/18 °C (day/night). The plants were cultivated *in vitro* for all of the molecular experiments. Seeds were surface-sterilized by vortexing in 70% (v/v) ethanol for 2 min, followed by 15 min shaking in 5% sodium hypochlorite (v/v) and fourfold rinsing in autoclaved water. Sterile seeds were stratified at 4 °C for 2 d and germinated on a Murashige and Skoog (MS) medium (Duchefa Biochemie, Haarlem, the Netherlands). The plants were grown in a growth chamber under a 16 h photoperiod, PPFD: 100 ± 25 μmol m^{-2} s^{-1}, in 50% relative air humidity and at a temperature of 22/18 °C (day/night). Three-week-old plants were used to determine the dry weight (DW). Individual rosette DW was determined after overnight incubation at 75 °C in a dry oven.

Plasmid construction

Total plant RNA was extracted from 3-week-old Ws-0 rosettes using the TRIzol reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA) and purified from residual DNA with a DNA-free™ DNA Removal Kit (Ambion, Life Technologies, Carlsbad, CA, USA), according to the manufacturer's recommendation. The total RNA concentration was determined at 260 nm using a UV–VIS spectrophotometer (NanoDrop™, Thermo Fisher Scientific, Waltham, MA, USA). cDNA synthesis was performed on 2 μg of RNA using a High Capacity cDNA Reverse Transcription Kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol. Genomic DNA was isolated from 3-week-old Ws-0 rosettes with the use of a DNeasy Plant Mini Kit (Qiagen, Venlo, the Netherlands).

Full-length *LSD1* (AT4G20380), *EDS1* (AT3G48090), *PAD4* (AT3G52430), *DRP3A* (AT4G33650) and *DRP3B* (AT2G14120) coding sequences (CDS) (with or without stop codons) and the *LSD1* promoter sequence were amplified by a polymerase chain reaction (PCR) using a Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. First-strand cDNA or genomic DNA was taken as a template for PCR and amplified with CDS- or promoter-specific primers, respectively, extended with the attB sites for Gateway cloning (Invitrogen, Life Technologies, Carlsbad, CA, USA). For the primer sequence, see Supporting Information Table **S1**. All of the constructs were obtained using recombinational Gateway cloning (Invitrogen, Life Technologies, Carlsbad, CA, USA) and are presented in Supporting Information Table S2. Descriptions of the vectors can be found at http://gateway.psb.ugent.be.

Transient expression in tobacco leaf epidermal cells

All GFP-protein fusion constructs were transiently expressed in the leaf epidermal cells of *Nicotiana benthamiana* by *Agrobacterium tumefaciens* (strain C58C1)-mediated leaf infiltration (Boruc *et al.* 2010). Supporting Information Table S3 presents all of the tagging combinations of nGFP and cGFP used for testing protein localization and interactions in the BiFC assay. Four infiltrated tobacco leaf fragments were analysed per combination in two independent transformation events. The interactions were scored positive if at least 10 fluorescent cells per leaf segment were observed.

Stable transformation of Arabidopsis

For stable transgene expression, the constructs were transformed into Arabidopsis Ws-0 or *lsd1-1* plants using *A. tumefaciens* (strain C58C1) and a floral dip protocol (Clough & Bent 1998). Kanamycin-resistant plants were selected on MS medium supplemented with 50 mg L^{-1} kanamycin (Sigma-Aldrich, St Louis, MO, USA). Taking into account the stress-sensitive phenotype of *lsd1* plants, we could only obtain a few transgenic lines ($35S_{pro}$*:LSD1-GFP* or $35S_{pro}$*:LSD1-GR*) in the *lsd1* background, and in all of them the expression of *LSD1* was at a similar level as in the wild-type plants. However, we managed to obtain several transgenics with stable expression of $35S_{pro}$*:LSD1-GFP* or $35S_{pro}$*:LSD1-GR* in the Ws-0 background. Before a selection of transgenic events for further experiments was made, *LSD1* expression was quantified by quantitative real-time PCR (qPCR) for homozygous transgenic plants (T4 generation).

Quantitative real-time PCR (qPCR)

qPCRs were performed to assess the levels of transgene expression of *LSD1* in stable Arabidopsis transformants. Total RNA extraction and cDNA synthesis were performed as described earlier. Four microlitres of 25-times diluted first-strand cDNA was used as a template in qPCR. Apart from the cDNA, each reaction contained 7.5 μL of Power SYBR® Green PCR Master Mix (Applied Biosystems, Life Technologies, Carlsbad, CA, USA), 0.3 μL of each primer (final concentration of 0.2 μM) and 2.9 μL of sterile MQ water. qPCR reactions were performed in 96-well plates using the ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The specificity of amplified PCR products was verified by melting curve analysis. All reactions were done in triplicate for two biological replicates. Primers were designed with Universal Probe Library Assay Design Center Probe Finder software (Roche; www.roche-applied-science.com) and are shown in Supporting Information Table S4. The AT3G25800 gene encoding PP2AA2 (PROTEIN PHOSPHATASE 2A SUBUNIT A2) was used as a reference. The calculation of reaction efficiency was performed using LinRegPCR software (Ramakers *et al.* 2003), while the calculation of relative gene expression levels and the statistical significance of their differences was done in REST2009 software (Pfaffl *et al.* 2002).

Microscopy and image analysis

The adaxial leaf epidermis of transfected tobacco or transgenic Arabidopsis leaves and roots was assayed for fluorescence with a confocal microscope (LSM510, Zeiss, Jena, Germany)

equipped with 40× and 63× water-corrected objectives. GFP fluorescence was imaged with 488 nm laser excitation. Emission fluorescence was captured in the frame-scanning mode alternating GFP fluorescence via a 500 to 550 nm bandpass emission filter. Palisade and spongy mesophyll cells were imaged in white light with a Nikon Eclipse E-600 microscope equipped with a DXM 1200F digital camera. Cells were counted with CELL COUNTER plugins of ImageJ bundled with 64-bit Java 1.6.0_24. A grid of 6 × 8 squares was used for analysis (related to 100 000 pixels^2 and 0.01 mm^2 per square). The cell area was determined with ImageJ Analysis Tools (Schneider *et al.* 2012).

Tandem affinity purification (TAP)

Wild-type (Ler-0) Arabidopsis cell suspension cultures grown in a 16 h photoperiod were transformed with C- or N-terminal GS-tagged LSD1 constructs (Supporting Information Table S2) by *A. tumefaciens* co-cultivation. Protein extract preparation, Western blot analysis, TAP, proteolysis and mass spectrometry (MS) analysis were performed according to Van Leene *et al.* (2015). In total, four individual TAP experiments were performed; in each of them LSD1-GS recombinant protein and its interacting partners were purified twice and subjected to MS analysis. Proteins recognized by MS in at least two independent experiments were considered as LSD1 interactors.

Hydrogen peroxide (H_2O_2) treatment of cell cultures

Three days after refreshing, dark-grown cell cultures expressing N- or C-terminal GS-tagged LSD1 fusions were treated with 0, 0.1, 1, 5, 10 or 20 mM H_2O_2 for 2 h. Upon harvest, the cells were immediately frozen in liquid nitrogen and further stored at −80 °C until processing.

Protein extractions and Western blot analysis

For the TAP experiments, frozen cell cultures were grinded on ice in the presence of sand and TAP extraction buffer (Van Leene *et al.* 2007) supplemented with 10 mM N-ethylmaleimide (NEM) and 10 mM iodoacetamide (IAM) to prevent de novo oxidation of cysteine residues. Protein concentrations were determined with the Pierce™ Coomassie (Bradford) Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Fifty micrograms of total protein was separated on 12% polyacrylamide gel by Sodium Dodecyl Sulfate Poly-Acrylamide Gel Electrophoresis (SDS-PAGE) with or without β-mercaptoethanol as a reducing agent and transferred to Immobilon-P Membrane (PVDF, 0.45 μm) (Millipore, Merck KGaA, Darmstadt, Germany). In parallel, identical SDS-PAGE gels were loaded and stained with Coomassie Brilliant Blue as a loading control. Membranes were blocked in 3% (v/v) milk powder in 25 mM Tris–Cl (pH = 8), 150 mM NaCl and 0.05% Tween 20 for 1 h at room temperature and incubated overnight at 4 °C with a 1:5000 dilution of a specific peroxidase-antiperoxidase (PAP) soluble complex antibody (Sigma-Aldrich, Saint Louis, MO, USA) to detect the G protein within the GS-tag.

For the anti-GFP immunoblot assay, 3-week-old seedlings grown on MS medium were ground in liquid nitrogen. A total of 100 mg of each sample was re-suspended in 150 μL of phosphate extraction buffer (50 mM NaPO4, 1 mM EDTA, 0.1% Triton X-100, 10 mM β-mercaptoethanol), vortexed and centrifuged at 24 000g at 4° for 10 min. The supernatant was collected, and the amount of protein was quantified using the Bradford protein assay. A total of 20 μg of the protein from each sample was separated by SDS-PAGE, then the proteins were transferred onto a PVDF membrane (Millipore, Merck KGaA, Darmstadt, Germany). The membrane was incubated in blocking solution (phosphate-buffered saline (PBS) solution containing 0.05% TWEEN 20 (PBT) and 3% skimmed milk) for 1 h at room temperature with gentle rocking. After washing in PBT, the membrane was incubated with a 1:1000 dilution of the anti-GFP antibody (Sigma-Aldrich, Saint Louis, MO, USA) for 2 h. Subsequently, the membrane was washed three times in PBT, after which it was incubated in horseradish peroxidase (HRP)-conjugated secondary antibody (1:10000) for 1 h. After washing the membrane for three times in PBT, chemiluminescence was detected using the Pierce™ ECL Plus Western Blotting Substrate (Thermo Fisher Scientific, Waltham, MA, USA) and ChemiDoc Imaging Systems (BioRad, Hercules, CA, USA).

Dexamethasone (DEX) treatment

35S$_{pro}$:LSD1-GR seedlings were grown on a 20 micron nylon mesh (ELKO Filtering Co., Miami, FL, USA) on MS medium for two weeks before being transferred to an MS medium supplemented with 30 μM DEX (Sigma-Aldrich, Saint Louis, MO, USA). After 24 h, the plants were harvested, frozen in liquid nitrogen and further stored at −80 °C.

RNA isolation, library preparation, sequencing and meta-analysis

RNA was extracted from 2-week-old Ws-0, *lsd1-1* mutant, *35S$_{pro}$:LSD1-GFP* and DEX-treated *35S$_{pro}$:LSD1-GR* plants grown on MS medium in a 16 h photoperiod, PPFD: 100 ± 25 μmol m^{-2} s^{-1}, temperature 22/18 °C day/night. Total RNA isolation was performed using the GeneMATRIX Universal RNA Purification Kit (EUR$_X$, Gdansk, Poland) with an additional step of on-column DNAse I treatment on three biological repeats. RNA concentrations, purities and integrities were tested by electrophoretic separation in 1% agarose gel as well as spectrophotometrically using a UV–VIS spectrophotometer (NanoDrop™, Thermo Fisher Scientific, Waltham, MA, USA). RNA samples were sequenced in the VIB Nucleomics Core (Leuven, Belgium) on half a run of the NextSeq500: High 75, single read. Data were preprocessed, and low quality bases/reads and short reads (<36 nt) were removed using Trimmomatic (Bolger *et al.* 2014). Reads passing quality and length thresholds were aligned to the Arabidopsis TAIR10 genome using GSNAP (Wu & Nacu 2010). Read counts for genes were quantified using the HTseq-count (Anders *et al.* 2015) on the resulting alignment files. Differentially expressed genes (DEGs) were identified

using the R software package edgeR (Robinson *et al.* 2010). Differential expression *P*-values were computed using the EdgeR 'ExactTest' method and the common, tagwise dispersion. FDR adjustments of *P*-values were applied using the Benjamini–Hochberg method, and DEGs were selected with a FDR < 0.05 and log2FC > 1 or < -1. Full access to the RNAseq data is available at the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=cranikwarbaxvqf&acc=GSE83262). Functional categorization was done using MapMan 3.5.0 Beta (Thimm *et al.* 2004).

Chromatin immunoprecipitation coupled with quantitative real-time PCR (ChIP-qPCR)

Chromatin immunoprecipitation (ChIP) was performed on 2-week-old Arabidopsis plants overexpressing GFP-tagged LSD1 (*35S$_{pro}$:LSD1-GFP*) and wild-type plants (Ws-0) grown in laboratory (*in vitro*) or greenhouse conditions. In parallel, plants expressing the GFP-tagged JUNGBRUNNEN1 (JUB1, AT2G43000) protein (*35S$_{pro}$:JUB1-GFP*) were used to evaluate the effectiveness of ChIP (Wu *et al.* 2012). Plants were cultivated either on MS medium (laboratory) or in soil (greenhouse) in a 16 h photoperiod, PPFD: 100 ± 25 μmol m^{-2} s^{-1}, temperature 22/18 °C day/night. Chromatin immunoprecipitation was performed according to the protocol by Kaufmann *et al.* (2010) with subsequent modifications. The intensity of sonication was adjusted to obtain chromatin fragments ranging between 500 and 1000 BP. The whole immunoprecipitation procedure was performed using the μMACS Anti-GFP Starting Kit (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), employing the anti-GFP antibody to immunoprecipitate protein–DNA complexes. The ChIP experiment was performed for both laboratory- and greenhouse-grown plants in two independent replicates. Isolated chromatin was used as a template for qPCR in order to verify interaction of LSD1 with the promoter regions of selected genes. Promoter sequences, 1000 BP upstream ATG codon, were retrieved from the Ws-0 genomic sequence. To cover the whole length of the 1000 BP promoter sequence, approximately five pairs of primers were designed in Primer3Plus (Untergasser *et al.* 2007) per each promoter. Each pair yielded ca. 200 BP overlapping products in qPCR. The primer sequences are given in Supporting Information Table S4. qPCR reactions were performed as described previously for four biological replicates, two for laboratory and two for greenhouse conditions. Each qPCR reaction was performed in triplicate. For data normalization, the ChIP-qPCR results were analysed relative to the input (total chromatin isolated from the appropriate genotype). As a negative control, we used primers annealing to a promoter region of MPK9 (AT3G18040) (Wu *et al.* 2012). The amount of genomic DNA coprecipitated by the GFP antibody was calculated in comparison to the total input DNA used for each immunoprecipitation in the following manner: cycle threshold (Ct) = Ct(ChIP) − Ct(Input). To calculate fold enrichment, normalized ChIP signals were compared between the *35S$_{pro}$:LSD1-GFP* line and wild-type plants.

Meta-analysis of LESION SIMULATING DISEASE 1 expression

Analysis of LSD1 expression in different anatomical structures was performed in a Genevestigator (Hruz *et al.* 2008) by choosing all microarray experiments for wild-type Arabidopsis plants (ATH1: 22 k array platform).

RESULTS

LESION SIMULATING DISEASE 1 is localized in the cytoplasm and the nucleus

In order to evaluate the subcellular localization of LSD1, we fused it with GFP under the control of the endogenous (*LSD1$_{pro}$*) or constitutive cauliflower mosaic virus 35S (CaMV 35S) promoter (*35S$_{pro}$*). GFP fusions were prepared in both the N- and C-terminal orientation in order to investigate if the presence of GFP can alter the LSD1 localization (Supporting Information Table S2). The obtained constructs were subsequently used for transient expression assays in the abaxial epidermis cells of *N. benthamiana* leaves via *A. tumefaciens*-mediated transformation. Irrespectively of the GFP orientation, LSD1 fusion proteins expressed from the endogenous promoter were detected in the cytoplasm and nucleus. However, no GFP signal was detectable in the nucleolus (Fig. 1a,b; Supporting Information Table S3).

Because no predicted nuclear localization signal (NLS) was found in the LSD1 protein sequence and the LSD1-GFP fusion protein size (~50 kDa) did not exceed the threshold for passive diffusion through the nuclear pores (Merkle 2003; Mohr *et al.* 2009), we also prepared constructs in which LSD1 was fused with GFP-β-glucuronidase (GUS). This increased the overall mass of the fusion protein to ~110 kDa, which well exceeded the size exclusion limit for passive transport into the nucleus. Nevertheless, the localization of LSD1-GFP-GUS under the control of the endogenous promoter was, again, nucleo-cytoplasmic (Supporting Information Fig. **S1** and Table S3).

We also evaluated the GFP signal in stable transgenic Arabidopsis expressing the LSD1-GFP fusion protein under the control of the CaMV 35S promoter. Moreover, here, a nucleo-cytoplasmic localization of the GFP signal was observed in both the roots and the shoots (Fig. 1c,d). Moreover, the immunoblot assay proved that the fluorescence corresponded to LSD1-GFP fusion, not to the free GFP (Supporting Information Fig. S2).

LESION SIMULATING DISEASE 1 forms homodimers and interacts with ENHANCED DISEASE SUSCEPTIBILITY 1 but not with PHYTOALEXIN DEFICIENT 4 in the cytoplasm and the nucleus

The genetic interdependence of LSD1 and EDS1/PAD4 in CD regulation is well documented (Rustérucci *et al.* 2001; Mateo *et al.* 2004; Mühlenbock *et al.* 2007, 2008; Huang *et al.* 2010; Wituszyńska *et al.* 2015). Therefore, we investigated if LSD1

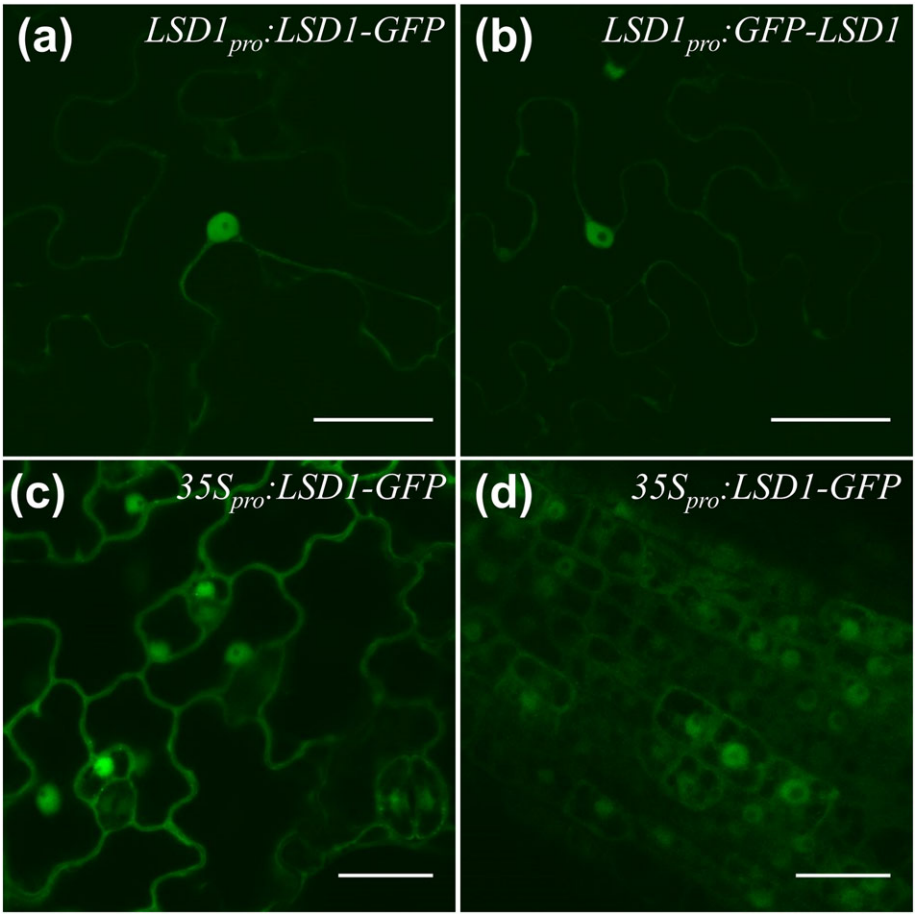

Figure 1. LSD1 localizes in the nucleus and the cytoplasm. (a) Subcellular localization of endogenous promoter-driven C-terminal GFP-tagged LSD1, transiently expressed in tobacco epidermal cells. (b) Subcellular localization of endogenous promoter-driven N-terminal GFP-tagged LSD1, transiently expressed in tobacco epidermal cells. (c) 35S promoter-driven C-terminal GFP-tagged LSD1 in the leaf epidermis of stably transformed Arabidopsis. (d) 35S promoter-driven C-terminal GFP-tagged LSD1 in the root of stably transformed Arabidopsis. Scale bar, 20 μm.

physically interacts with the EDS1 and PAD4 proteins *in vivo* by bimolecular fluorescence complementation (BiFC) experiments. Firstly, we confirmed the previously reported homodimerization of EDS1 and EDS1-PAD4 protein–protein interaction (Feys *et al.* 2001; Zhu *et al.* 2011) (Supporting Information Fig. S4 and Table S3). A strong GFP signal derived from interacting LSD1-split GFP fusion proteins was detected in the nucleus together with a weak GPF signal in the cytoplasm (Fig. 2a; Supporting Information Table S3). This signal most likely reflected a homodimeric complex formation, although complexes of a higher order (e.g. tetramers) cannot be excluded. A similar signal localization was detected when testing the LSD1-EDS1 interaction (Fig. 2b; Supporting Information Table S3). No BiFC complexes were observed between the LSD1 and PAD4 proteins (Supporting Information Table S3). The subcellular localization of CaMV 35S promoter-driven C- and N-terminal full-length GFP fusions with EDS1 and PAD4, performed as the control experiments, showed that both fusions again exhibited a nucleo-cytoplasmic localization (Supporting Information Fig. S3 and Table S3).

Protein interactions with LESION SIMULATING DISEASE 1 are redox dependent

The observed LSD1-EDS1 interaction prompted us to test if LSD1 also interacts with other proteins *in vivo*; therefore, we applied a TAP approach in the Arabidopsis cells. To prepare the genetic constructs for TAP, a GS-tag (Van Leene *et al.* 2008) was fused to the N- or C-terminal end of LSD1 (Supporting Information Table S2). The CaMV 35S promoter-driven constructs were transformed into Arabidopsis cells. Western blot (WB) analysis with a specific PAP antibody demonstrated the transgenic expression of GS-tagged LSD1 in the cell cultures (Supporting Information Fig. S5). Both the *35S$_{pro}$:LSD1-GS* and *35S$_{pro}$:GS-LSD1* constructs were functional because they were able to reverse the RCD phenotype of the *lsd1* plants under non-permissive long day conditions (Supporting Information Fig. S6).

To investigate if the LSD1-interactome is condition dependent, cells expressing the *35S$_{pro}$:LSD1-GS* or *35S$_{pro}$:GS-LSD1* constructs were either incubated in non-oxidative stress conditions or subjected to oxidative stress induced by

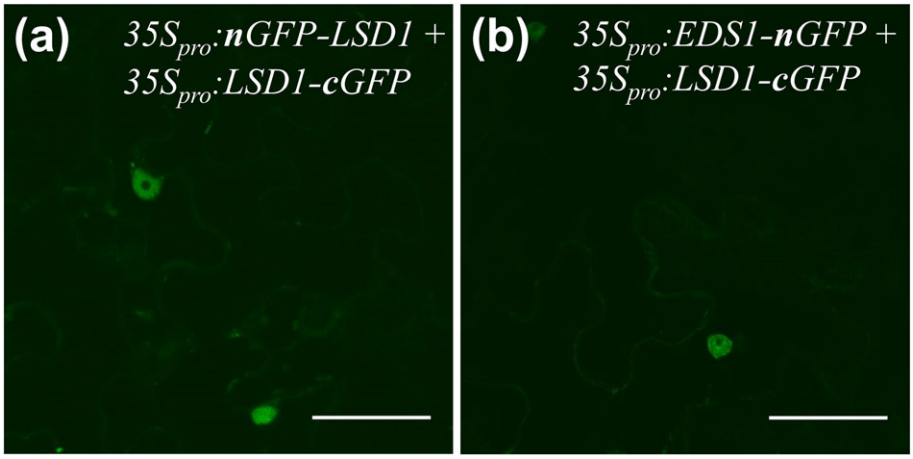

Figure 2. LSD1 forms homodimers and interacts with EDS1 in the nucleus and the cytoplasm. (a) Subcellular localization of LSD1 homodimers, transiently expressed in tobacco epidermal cells. (b) Subcellular localization of the BiFC complex between nGFP-tagged EDS1 and cGFP-tagged LSD1, transiently expressed in tobacco epidermal cells. Scale bar, 20 μm.

hydrogen peroxide. It has been shown that treatment with 10 or 20 mM of H_2O_2 provokes oxidative stress signalling in Arabidopsis cells (Karpinski *et al.* 1999; Desikan *et al.* 2001; Waszczak *et al*. 2014). In our experimental setup, cell cultures were grown to reach their mid-log phase before treatment with 0, 0.1, 1, 5, 10 or 20 mM of H_2O_2. After 2 h of treatment, cells were harvested, and total protein extracts were subjected to TAP. The WB analysis with the PAP antibody demonstrated that a monomeric form of LSD1 fused with the GS-tag migrated as a strong, single band with a molecular mass of ca. 45 kDa, while the LSD1-GS dimers migrated as a thinner band at ca. 90 kDa (Fig. 3a). Generally, increased levels of H_2O_2 treatments resulted in the detection of high-molecular weight LSD1-containing complexes (Fig. 3a). When the GS-tag was fused to the N-terminus of LSD1, larger protein complexes were only detectable at the highest H_2O_2 concentrations (20 mM H_2O_2). In contrast, C-terminal fusions resulted in high molecular weight complex formation already at 5 mM H_2O_2. In these LSD1-GS cell cultures, only few protein complexes were observed within the range of 0 to 1 mM H_2O_2 and they did not exceed 250 kDa. On the contrary, concentrations of H_2O_2 ranging from 5 to 20 mM yielded LSD1-protein complexes that were larger than 250 kDa. Within this concentration range, the band representing the monomeric LSD1-GS fusion protein became very faint (Fig. 3a), while the LSD1-GS dimers were not detectable, thus suggesting that most of the LSD1 had become engaged in interactions with its partners. This WB analysis not only revealed differences in the quantity of proteins interacting with LSD1 depending on the H_2O_2 concentration but also depending on the position of GS-tag fusion (N- or C-terminal).

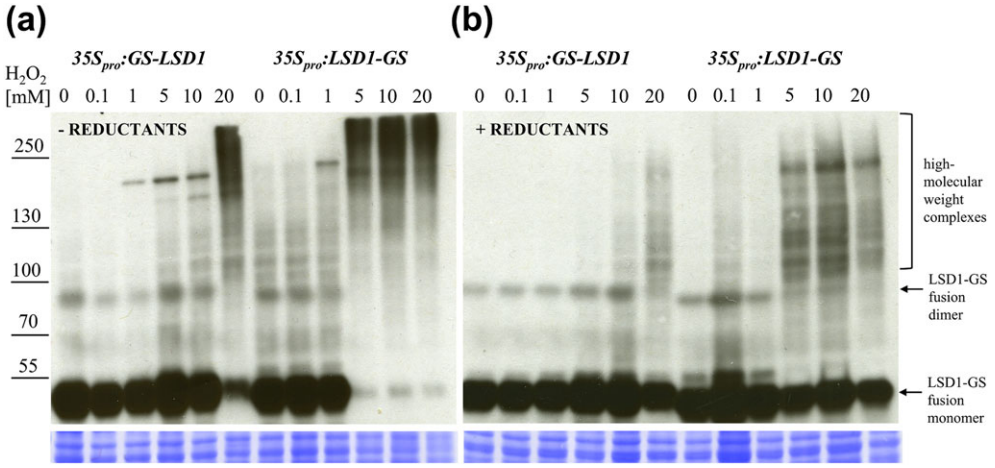

Figure 3. Oxidative stress increases the number of LSD1 heterocomplexes. (a) Immunoblot with the PAP antibody, detecting the GS-tag in the protein extract from cell cultures overproducing LSD1-GS or GS-LSD1, treated with 0, 0.1, 1, 5, 10 or 20 mM H2O2 for 2 h and separated by PAGE in non-reducing conditions. (b) Immunoblot with the PAP antibody, detecting the GS-tag in the protein extract from cell cultures overproducing LSD1-GS or GS-LSD1, treated with 0, 0.1, 1, 5, 10 or 20 mM H_2O_2 for 2 h and separated by PAGE in reducing (β-mercaptoethanol) conditions.

Because LSD1 contains 14 cysteine residues (Cys), of which 13 are located within the Zn-finger domains (Supporting Information Fig. S7) and are highly conserved in LSD1 orthologs, we hypothesized that these Cys are responsible for the oxidation-dependent interaction of LSD1 with its partners. Therefore, we assessed potential disulfide bond formation by protein extraction from TAP cultures in the presence of IAM and NEM to block all free thiols (−SH) and to prevent *de novo* oxidation of Cys residues (Waszczak *et al.* 2014). Next, the isolated proteins were separated by SDS-PAGE in either the absence (Fig. 3a) or presence (Fig. 3b) of β-mercaptoethanol as a reducing agent and blotted, and complexes formed by LSD1 were detected with the PAP antibody. We observed the disappearance of most high-molecular weight complexes after the reduction of Cys (Fig. 3b versus 3a) accompanied by increased levels of LSD1 monomers. However, not all complexes disappeared in β-mercaptoethanol-treated samples, which suggests that LSD1 interacts with its partners not only by intracellular disulfide bond formation.

LESION SIMULATING DISEASE 1-interactome is condition dependent

Based on the previously-described results, indicating that GS-tag fusion to the C-terminus of LSD1 appears to be more effective in facilitating protein interactions, cultures harbouring the *35S$_{pro}$:LSD1-GS* construct were used for further experiments. The TAP analysis was performed on total protein extracts purified from both non-treated (0 mM H_2O_2) and treated (10 mM H_2O_2) cell cultures, representing non-oxidative stress and oxidative stress conditions, respectively. Tandem affinity purification samples were subsequently

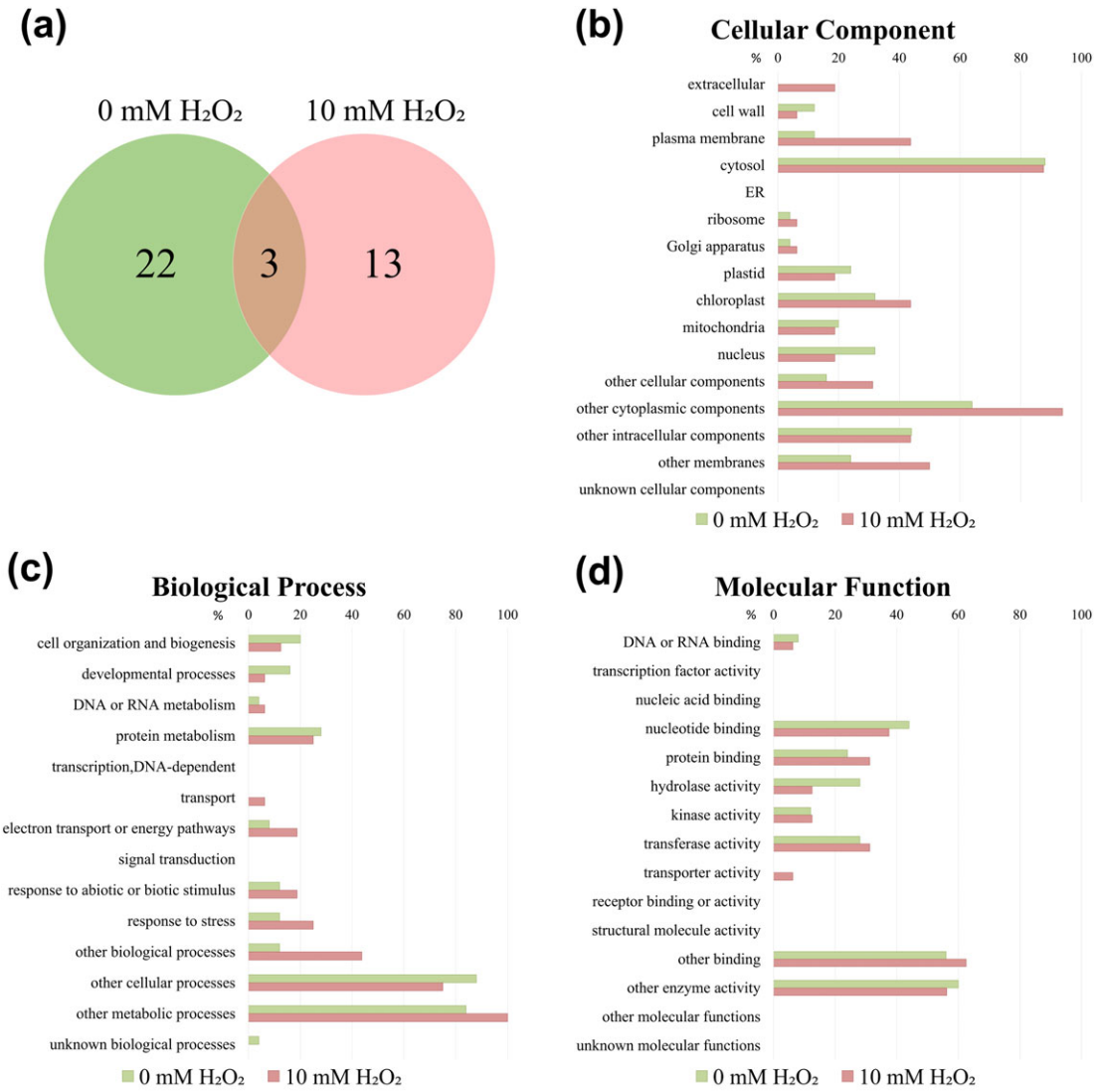

Figure 4. Interactome of LSD1 is condition dependent. (a) Comparison of LSD1-interacting protein number in non-oxidative- and oxidative-stress conditions. (b) Comparison of LSD1-interacting protein subcellular localization in non-oxidative- and oxidative-stress conditions. (c) Comparison of the biological process employing LSD1-interacting proteins in non-oxidative- and oxidative-stress conditions. (d) Comparison of LSD1-interacting protein molecular functions in non-oxidative- and oxidative-stress conditions.

subjected to MS in order to identify the specific proteins that form complexes with LSD1. Four TAP experiments were performed in total, in each of them the LSD1-GS recombinant protein with its interacting partners was purified twice and subjected to MS analysis.

We identified 25 and 16 proteins that interacted with LSD1 in control and oxidative stress conditions, respectively. Three proteins were common for both conditions (Fig. 4a; Table 1). LESION SIMULATING DISEASE 1-interacting proteins proved to be engaged in various molecular pathways such as ubiquitination, methylation, cell cycle control, gametogenesis, embryo development and cell wall formation (Table 1). In oxidative stress, as compared to non-oxidative stress, there was a higher percentage of proteins located in the extracellular space, plasma membrane and chloroplast (Fig. 4b). Under H_2O_2 treatment, LSD1 interacted with a lower percentage of proteins involved in cell organization and biogenesis but with a higher percentage of proteins involved in responses to both biotic and abiotic stresses, transport and energy pathways, in comparison to non-oxidative stress conditions (Fig. 4c,d). This may indicate that in oxidative-stress conditions the function of LSD1 switches towards more intense regulation of the signalling pathways.

Moreover, with the use of BiFC, we assessed and confirmed the *in vivo* interaction of LSD1 with two of the proteins identified by TAP (DRP3A and DRP3B). Both of these dynamin-related proteins play essential roles in mitochondria and peroxisomes fission during cell division (Kang *et al.* 1998;

Table 1. Direct and indirect interactors of LSD1 identified by TAP in either the absence or presence of 10 mM H_2O_2. Proteins marked in bold are present in both non-oxidative- and oxidative-stress conditions

AGI	Protein
0 mM H_2O_2	**25 interactors**
AT5G26830	**threonyl-tRNA synthetase (**addition of amino acids to tRNA during translation)
AT3G11710	lysyl-tRNA synthetase (ATKRS-1) **(**addition of amino acids to tRNA during translation)
AT1G09620	leucyl-tRNA synthetase **(**addition of amino acids to tRNA during translation)
AT4G10320	isoleucyl-tRNA synthetase **(**addition of amino acids to tRNA during translation)
AT3G29360	**UDP-glucose 6-dehydrogenase (UGD2)** (synthesis of nucleotide-sugars)
AT1G64190	6-phosphogluconate dehydrogenase (pentose phosphate pathway)
AT5G41670	6-phosphogluconate dehydrogenase (pentose phosphate pathway)
AT1G12000	pyrophosphate-fructose-6-phosphate 1-phosphotransferase (PFP) (glycolysis)
AT1G06410	trehalose-phosphatase/synthase 7 (TPS7) (sugar metabolism and signalling)
AT2G45300	3-phosphoshikimate 1-carboxyvinyltransferase (EPSP synthase) (chorismate biosynthesis)
AT3G59970	methylenetetrahydrofolate reductase 1 (MTHFR1) (methionine metabolic process, methylation)
AT2G44160	methylenetetrahydrofolate reductase 2 (MTHFR2) (methionine metabolic process, methylation)
AT3G53580	diaminopimelate epimerase (lysine biosynthesis)
AT3G14390	diaminopimelate decarboxylase 1 (lysine biosynthesis)
AT4G33650	dynamin-related protein 3a (DRP3A) (organelle division)
AT2G14120	dynamin-related protein 3b (DRP3B) (organelle division)
AT5G06460	ubiquitin activating enzyme E1 2 (UBA2) (ubiquitination)
AT1G50670	ovarian tumour domain (OTU)-containing deubiquitinating enzyme 2 (OTU2) (deubiquitination)
AT5G12410	THUMP domain-containing protein (RNA modification)
AT3G62310	pre-mRNA-splicing factor ATP-dependent RNA helicase (RNA splicing)
AT3G48750	cell division control 2 (CDC2) (cell division)
AT3G58180	ARM repeat superfamily protein (translation initiation)
AT4G34230	cinnamyl alcohol dehydrogenase 5 (CAD5) (lignin biosynthesis)
AT3G02230	UDP-arabinose mutase 1 (RGP1) (cellulose biosynthesis)
AT5G56350	**pyruvate kinase** (glycolysis)
10 mM H_2O_2	**16 interactors**
AT5G26830	**threonyl-tRNA synthetase (**addition of amino acids to tRNA during translation)
AT5G56680	asparaginyl-tRNA synthetase (SYNC1) **(**addition of amino acids to tRNA during translation)
AT3G62120	class II aaRS and biotin synthetases superfamily protein (tRNA aminoacylation)
AT4G09000	general regulatory G-box factor (GRF1)
AT1G65930	citosolic NADP+ dependent isocitrate dehydrogenase (CICDH) (Krebs cycle)
AT2G47510	fumarate hydratase 1 (FUM1) (Krebs cycle)
AT3G29360	**UDP-glucose 6-dehydrogenase** (synthesis of nucleotide-sugars)
AT3G09820	adenosine kinase 1 (ADK1) (methylation)
AT3G23810	S-adenosyl-l-homocysteine hydrolase 2 (SAHH2) (methylation)
AT4G13930	serine hydroxymethyltransferase 4 (SHM4) (methylation, photorespiration)
AT5G16990	2-alkenal reductase (oxidoreduction)
AT3G48730	glutamate − 1-semialdehyde 2,1-aminomutase 2 (GSA2) (chlorophyll biosynthesis)
AT4G20850	tripeptidyl peptidase II (TPP2) (proteolysis)
AT3G54470	bi-functional orotate phosphoribosyltransferase (nucleotide metabolism)
AT5G56350	**pyruvate kinase** (glycolysis)
AT1G10390	nucleoporin autopeptidase (DRA2) (nuclear pore complex, RNA and protein transport)

Kim *et al.* 2001; Fujimoto *et al.* 2009; Zhang & Hu 2010). Our results of transient expression in tobacco epidermal cells confirmed that both DRP3A- and DRP3B-GFP fusion proteins localized to the peroxisomes and mitochondria (Supporting Information Fig. S8a,c,g). However, the fusion of GFP at the C-terminal end of DRP3B yielded aggregates outside the nucleus (Supporting Information Fig. S8e), similarly to the complexes of LSD1 with both of the tested dynamin-related proteins (Supporting Information Fig. S8i,k).

LESION SIMULATING DISEASE 1 acts as a transcriptional regulator

Our next aim was to identify genes whose expression is regulated by LSD1 and to test if LSD1 can act as a transcriptional regulator in Arabidopsis. To do this, we generated transgenic lines (both in wild-type Ws-0 and the *lsd1* background) harbouring *35S$_{pro}$:LSD1-GFP* or *35S$_{pro}$:LSD1-GR* constructs for LSD1 overexpression or inducible nuclear-translocation, respectively. GR-based fusion together with DEX treatment has successfully been used to control nucleo-cytoplasmic partitioning of proteins in plants (Wagner *et al.* 1999; Samach *et al.* 2000; Levesque *et al.* 2006).

Because the TAP results demonstrated that the free N-terminal side of LSD1 was more efficient in forming complexes with other proteins, as this is the part where three Zn-finger domains are located, functional tags were attached to the LSD1 C-terminus. Both constructs *35S$_{pro}$:LSD1-GFP* and *35S$_{pro}$:LSD1-GR* proved to be functional as they were able to revert the RCD phenotype in *lsd1* plants grown in a long-day photoperiod (Supporting Information Fig. S6). We obtained several transgenics and selected a line that harboured the

Table 2. Functional analysis of 27 genes regulated directly/indirectly by LSD1. Stars after the AGI code indicate genes whose promoters were tested in ChIP-qPCR experiment

AGI	Gene description	Function	*lsd1*	*35S$_{pro}$,LSD1-GR*	*35S$_{pro}$,LSD1-GFP*
Minor CHO metabolism					
AT1G56600 *	GALACTINOL SYNTHASE 2, (**GOLS2**)	galactinol synthesis	1,27	1,20	-1,65
Secondary metabolism					
AT1G67980 *	caffeoyl-CoA 3-O-methyltransferase, (**CCOAMT**)	lignin biosynthesis	1,56	1,34	-1,57
AT5G35940	jacalin-like lectin family protein (**JLP**)	carbohydrate-binding	-1,58	1,32	0,00
AT1G52040	MYROSINASE-BINDING PROTEIN 1 (**MBP1**)	carbohydrate-binding	1,18	1,20	-1,38
AT3G15356	Legume lectin family protein (**LLP**)	carbohydrate binding/abiotic/biotic stress response	1,07	2,24	-1,13
AT3G55970 *	JASMONATE-REGULATED PROTEIN 21 (**JRG21**)	flavonoid biosynthesis	1,90	2,34	-1,59
Hormones metabolism					
AT5G13320 *	GRETCHEN HAGEN 3.12 (**GH3.12**)	benzoate (salicylic acid) conjugation to amino acids	1,98	-1,04	0,00
Stress response					
AT1G13609	defensin-like protein **287** (**DEFL287**)	biotic stress response	-1,02	1,93	-1,42
AT1G13608	defensin-like protein **288** (**DEFL288**)	biotic stress response	-2,08	1,52	-2,00
AT2G14610 *	PATHOGENESIS-RELATED GENE 1 (**PR1**)	abiotic/biotic stress response	1,01	-1,47	-3,20
AT4G33720 *	Cysteine-rich secretory proteins, Antigen 5, and Pathogenesis-related 1 protein (**CAP**)	abiotic/biotic stress response	1,19	-2,23	-2,68
AT2G26020 *	PLANT DEFENSIN 1.2B (**PDF1.2B**)	abiotic/biotic stress response	1,91	3,50	-1,15
AT1G72910	Toll-Interleukin-Resistance domain protein (**TIR**)	abiotic/biotic stress response	1,35	0,00	-1,05
AT3G57260 *	BETA-1,3-GLUCANASE 2 (**BGL2**); PATHOGENESIS-RELATED PROTEIN 2 (PR2)	cellulose degradation, callose deposition	1,06	-2,04	0,00
AT4G11900 *	S-locus lectin protein kinase (**SLPK**)	innate immune response	1,71	0,00	-1,50
AT1G14880	PLANT CADMIUM RESISTANCE 1 (**PCR1**)	heavy metals response	2,91	-1,97	0,00
Development					
AT3G05950 *	germin-like protein subfamily 1 member 7 (**GLP7**)	pollen germination and tube growth	1,67	-1,80	0,00
Protein modification					
AT3G12220 *	SERINE CARBOXYPEPTIDASE-LIKE 16 (**SCPL16**)	protein degradation	1,99	0,00	-1,10
AT4G17470 *	putative palmitoyl-protein thioesterase (**PPT**)	protein-postranslational modification	-1,14	1,64	0,00
Signaling					
AT5G59670	Leucine-rich repeat protein kinase (**LRRK**)	signal transduction	1,48	1,03	-1,60
AT4G04500	CYSTEINE-RICH RLK 37 (**CRK37**)	signal transduction	1,98	-2,51	0,00
AT4G23160 *	CYSTEINE-RICH RLK 8 (**CRK8**)	signal transduction	1,39	-0,92	-1,01
AT1G16260 *	wall-associated receptor kinase-like 8 (**WAKL8**)	receptor kinase	1,15	0,48	-1,57
AT3G50770 *	CALMODULIN-LIKE 41 (**CML41**)	calcium signaling	1,88	1,76	-1,82
AT5G54610 *	ankyrin-repeat transmembrane protein BDA1 (**ANK**)	salicylic acid signaling	1,46	-0,57	-1,24
Transport					
AT2G46450 *	CYCLIC NUCLEOTIDE-GATED CHANNEL 12 (**CNGC12**)	cyclic nucleotide regulated channel transport	1,33	0,00	-1,24
RNA processing					
AT5G06520 *	Suppressor-of-White-Apricot (**SWAP**)	pre-mRNA splicing processes	-2,46	0,00	1,17

35S$_{pro}$:LSD1-GFP construct which overexpressed *LSD1* approximately 280 times higher than in the wild-type plants and a line harbouring the *35S$_{pro}$:LSD1-GR* construct with a 26-fold increase of *LSD1* expression in comparison to wild type, as indicated by qPCR (Supporting Information Fig. S9).

To identify LSD1 potential targets, Arabidopsis lines *35S$_{pro}$:LSD1-GFP* and *35S$_{pro}$:LSD1-GR* (24 h after DEX treatment) together with the wild type (Ws-0) and the *lsd1* mutant were subjected to transcriptional profiling via Next-Generation Sequencing (NGS). The results allowed to identify 27 genes exhibiting opposite expression patterns (up- or down-regulation) depending on either the lack or over-expression of LSD1. These transcripts encoded proteins involved in biotic and abiotic stress responses, signal transduction and cell wall remodelling (Table 2). Some of them, for example, *PATHOGENESIS-RELATED GENE 1* (*PR1*), exhibited the same expression pattern in the *lsd1* background as we described previously (Wituszyńska *et al.* 2013) (Supporting Information Table S5). Eighteen of the genes were tested in a ChIP experiment coupled with quantitative real-time PCR (ChIP-qPCR) analysis as indicated in Table 2.

Chromatin immunoprecipitation was performed on *35S$_{pro}$:LSD1-GFP* plants that were grown in controlled laboratory (*in vitro*) or more variable greenhouse conditions. Wild-type plants (Ws-0) were used for comparison, while the line overexpressing JUNGBRUNNEN1 (JUB1, AT2G43000), *35S$_{pro}$:JUB1-GFP* (Wu *et al.* 2012), was used as a positive control. Under our experimental conditions, JUB1 binding to its known target sequences was confirmed (Supporting Information Table S6; Wu *et al.* 2012). From the 18 promoter sequences derived from the RNA-seq experiment, binding of LSD1 was evidenced for 15 and 13 promoters of plants grown under laboratory and greenhouse conditions, respectively (Table 3). Promoter regions enriched in *35S$_{pro}$:LSD1-GFP* plants preceded *GOLS2*, *PPT*, *CCOAMT*, *JRG21*, *GH3.12*, *PDF1.2B*, *SLPK*, *GLP7*, *SCPL16*, *WAKL8*, *CML41*, *ANK* and *SWAP* genes in plants grown in the greenhouse. In addition, promoter sequences of *PR1* and *CAP* were bound by LSD1 in laboratory-grown plants (Tables 2 & 3). These results indicated that LSD1 influenced the expression of selected genes through either direct or indirect association with their promoters. LESION SIMULATING DISEASE 1 downstream target genes encoded proteins participating in different cellular pathways such as response to stress (PR1, CAP, PDF1.2B, SLPK and WAKL8), pre-mRNA splicing (SWAP), posttranslational protein modification (PPT), proteolysis (SCPL16), SA-, JA-, IAA- and Ca^{2+}-dependent signalling pathways (GH3.12, JRG21, ANK and CML41), cell wall remodelling (CCOAMT), pollen germination and tube growth (GLP7) as well as galactinol synthesis (GOLS2), which demonstrates the protective role during plant oxidative stress (Table 2).

Table 3. Promoter sequences directly or indirectly bound by LSD1 identified in ChIP-qPCR. Numbers after the promoter (1–5) represent the promoter overlapping fragments. Fold enrichment was calculated as described in the Methods section. Values between 1 and 2 mean weak binding to the promoter region. Values higher than 2 mean strong binding and are marked in bold

Promoter region	Fold enrichment LAB	Fold enrichment GREENHOUSE
GOLS *pro*_1	**2.73**	**5.41**
GOLS *pro*_2	**4.38**	**4.26**
GOLS *pro*_3	**5.51**	**5.75**
GOLS *pro*_4	**4.99**	**6.07**
GOLS *pro*_5	**3.17**	**5.19**
PPT *pro*_2	1.12	**3.48**
PPT *pro*_3	1.69	**6.22**
CCOAMT *pro*_2	**2.09**	0.52
CCOAMT *pro*_3	**3.74**	1.75
CCOAMT *pro*_4	**3.58**	1.51
CCOAMT *pro*_5	**2.04**	**2.33**
JRG21 *pro*_1	**3.68**	**2.43**
JRG21 *pro*_2	**6.09**	**2.82**
JRG21 *pro*_3	**5.18**	1.98
GH3.12 *pro*_1	1.45	1.78
PR1 *pro*_2	**2.11**	0.00
CAP *pro*_1	**3.48**	−0.66
CAP *pro*_4	1.42	−0.75
PDF1.2B *pro*_3	1.10	1.23
GLP7 *pro*_1	**3.17**	1.92
GLP7 *pro*_2	1.68	0.53
GLP7 *pro*_3	**3.75**	**3.48**
GLP7 *pro*_4	**3.38**	−2.18
GLP7 *pro*_5	**2.39**	−1.08
SCPL16 *pro*_3	1.41	**4.35**
SLPK *pro*_3	**3.89**	**6.25**
SLPK *pro*_4	**5.69**	1.04
WAKL8 *pro*_2	**2.25**	1.44
CML41 *pro*_2	1.68	**3.14**
CML41 *pro*_4	1.64	**3.80**
ANK *pro*_2	**2.36**	**2.99**
SWAP *pro*_2	1.51	**4.18**

LESION SIMULATING DISEASE 1 regulates cell number and area, and influences plant growth

Our results indicating that LSD1 interacts with proteins involved in cell cycle and cell wall formation as well as controls the expression of some genes engaged in these processes prompted us to assess the impact of LSD1 on leaf growth and development. A microscopic analysis of leaf mesophyll tissues revealed that the *lsd1* mutant leaves contained a significantly higher number of both palisade and spongy mesophyll cells (Fig. 5b,d), and that these cells were generally smaller and more compact in comparison to those of the wild-type plants (Fig. 5b,e). In contrast, the LSD1-OE line (*35S$_{pro}$:LSD1-GFP*) had significantly larger cells (Fig. 5c,e), but the numbers of both palisade and spongy mesophyll cells per unit of leaf area were decreased as compared to the wild type (Fig. 5d). These differences in cell number and area influenced the rosettes' size (Supporting Information Fig. S6) and their DW, both of which were significantly reduced in the *lsd1* mutant and increased in the LSD1-OE plants (Fig. 5f; Supporting Information Fig. S6).

DISCUSSION

Since the discovery of LSD1 as an important negative CD regulator (Jabs *et al.* 1996; Dietrich *et al.* 1997), many attempts

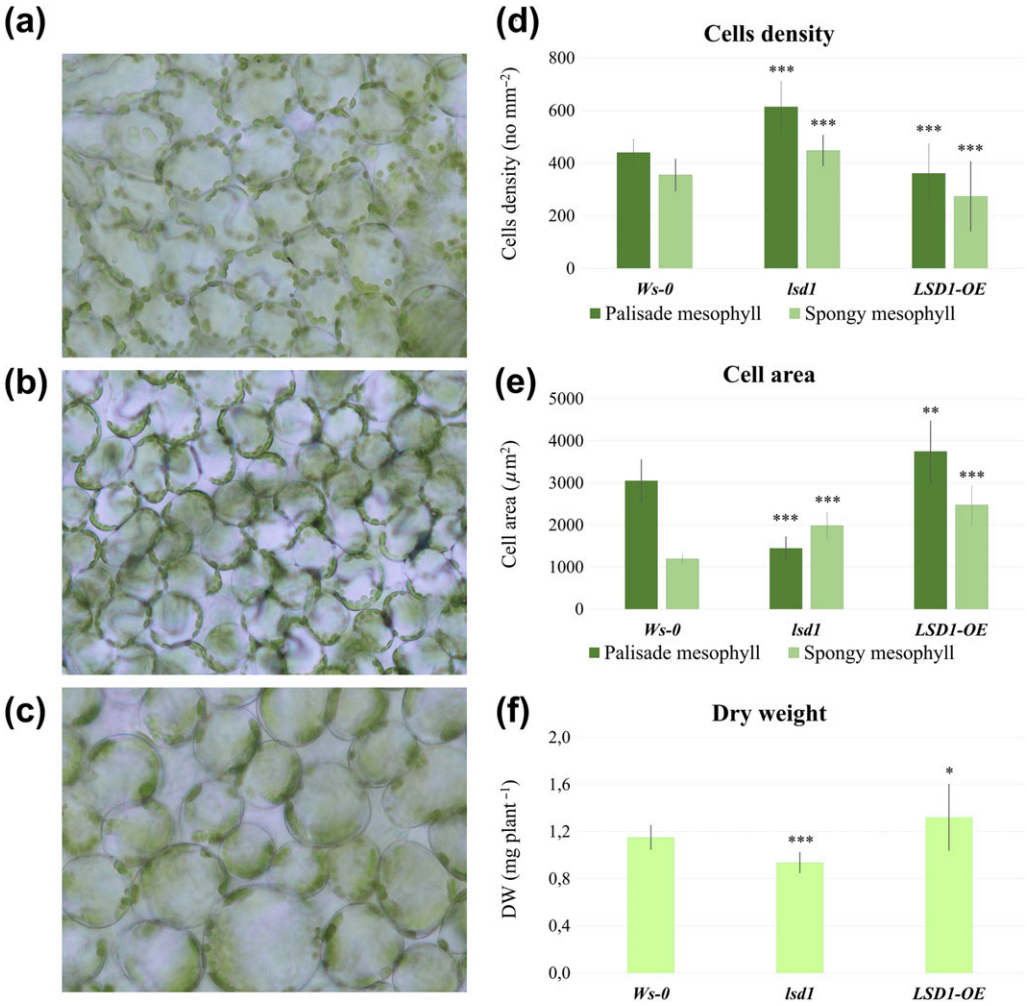

Figure 5. LSD1 affects density and area of palisade and spongy mesophyll cells, and rosettes' dry weight. (a) Mesophyll cells in 3-week-old wild-type plants. (b) Mesophyll cells in lsd1 plants. (c) Mesophyll cells in LSD1-OE (35Spro,LSD1-GFP) plants. (d) Palisade and spongy mesophyll cell density. Values (±SD) are averages of 50–327 leaf fragments (0.01 mm^2) from 9 to 15 different leaves collected in two independent experiments. (e) Palisade and spongy mesophyll cell area. Values (±SD) are averages of 15–38 leaf fragments collected in two independent experiments. (f) Dry weight of rosettes. Values (±SD) are averages of 9–20 rosettes for two independent experiments. Stars above the bars indicate statistically significant differences in comparison to the wild-type, according to the *t*-test at a level of $P < 0.05$ (*),$P < 0.005$ (**) or $P < 0.001$(***).

were undertaken to gain more insight into its mode of action. These efforts indicated that LSD1 controls an integrated response to both biotic and abiotic stresses (Jabs *et al.* 1996; Dietrich *et al.* 1997; Rustérucci *et al.* 2001; Mateo *et al.* 2004; Kaminaka *et al.* 2006; Mühlenbock *et al.* 2007, 2008; Wituszyńska *et al.* 2015). The results of our group proved that LSD1 influences not only plant acclimation to variable environmental conditions but also such important physiological processes as photosynthesis, transpiration, ROS/hormonal homeostasis maintenance, plant biomass and seed yield (Wituszyńska *et al.* 2013; Szechyńska-Hebda *et al.* 2016). However, the molecular LSD1 mode of action has remained largely unknown.

Our first scope was to evaluate the subcellular localization of LSD1. The obtained results proved that it is located in the cytoplasm and nucleus. A similar outcome was presented in previous reports on Arabidopsis (Kaminaka *et al.* 2006) and *Pisum sativa* (He, Huang, *et al.* 2011a). Furthermore, we confirmed the previous results demonstrating that LSD1 forms homodimers (Walter *et al.* 2004; Kaminaka *et al.* 2006) mainly in the nucleus, yet a GFP signal was also present in the cytoplasm.

LESION SIMULATING DISEASE 1 contains three zinc (Zn) finger domains that are located towards the N-terminus. Zinc fingers mediate interaction with other proteins, DNA and/or RNA. Proteins containing Zn-finger domains play an important role in the regulation of signal transduction, development and CD (Ciftci-Yilmaz & Mittler 2008). LSD1 Zn-fingers were classified as C2C2-type, which are distinctive for some TFs (Epple *et al.* 2003; Gupta *et al.* 2012). Although no genuine NLS was found in LSD1, our study indicated that under oxidative stress conditions LSD1 physically interacts

with the nuclear pore complex protein, nucleoporin autopeptidase, which confirms that LSD1 is actively transported through the nuclear pores.

As a negative regulator of CD, LSD1 depends on the activity of two proteins engaged in SA and jasmonic acid (JA)/ethylene (ET) signalling pathways during defence responses – EDS1 and PAD4 (Wiermer *et al.* 2005). The introduction of the *eds1* and *pad4* mutation into the *lsd1* background, yielding *eds1/lsd1* and *pad4/lsd1* double mutants, respectively, inhibited *lsd1*-specific biotic- or abiotic-stress-induced RCD. Therefore, it was concluded that both EDS1 and PAD4 are necessary in CD propagation in the *lsd1* mutant (Rustérucci *et al.* 2001; Mateo *et al.* 2004; Mühlenbock *et al.* 2007, 2008; Huang *et al.* 2010; Wituszyńska *et al.* 2015). Our previous results also proved that LSD1 together with EDS1 and PAD4 participate in the regulation of photosynthesis, water use efficiency, ROS/hormonal homeostasis and seed yield in a condition-dependent manner (Wituszyńska *et al.* 2013). We performed the BiFC experiment to test whether LSD1 physically binds to EDS1 and PAD4, which demonstrated that LSD1 interacts with EDS1 in the cytoplasm and the nucleus. By forming intracellular complexes with EDS1, LSD1 may control EDS1-dependent CD. We also confirmed EDS1 homodimerization and EDS1-PAD4 dimerization, as had already been described in the literature (Feys *et al.* 2001; Zhu *et al.* 2011). On the contrary, no interaction between LSD1 and PAD4 was detected in any combination of the different fusion proteins; thus, the influence of LSD1 on PAD4-regulated CD might be mediated through their common partner – EDS1.

Over the past decade, also other proteins were distinguished as forming complexes with LSD1 in Y2H and pull-down experiments (Kaminaka *et al.* 2006; Coll *et al.* 2010, 2011; He, Tan, *et al.* 2011b; Li *et al.* 2013). In our TAP experiment, we identified 38 novel LSD1 interactors: 25 of them were purified under control and 16 under oxidative stress conditions. No previously described LSD1 interactors, nor EDS1 which was recognized through BiFC in this study, were confirmed using the TAP technique. This might be explained by the temporary nature of LSD1-formed complexes or by their existence only under specific conditions. What is also interesting is that only three proteins were common for non-oxidative and oxidative stress conditions, thus indicating that the LSD1 interactome is strongly condition dependent. Based on these results, we postulate that LSD1 acts as a scaffold protein or a network node facilitating protein–protein interactions. Nodes in protein networks mediate the binding of numerous proteins, thereby regulating various cellular reactions. The absence of a specific node protein is likely to result in severe disturbance of cellular homeostasis (Jeong *et al.* 2001; He & Zhang 2006). This might explain why the *lsd1* phenotype exhibits high instability when exposed to stress in controlled laboratory conditions (Mateo *et al.* 2004; Wituszyńska *et al.* 2013, 2015).

We also suggest that the cellular function of LSD1 is strongly condition dependent because H_2O_2 facilitated the formation of high-molecular weight complexes in a dose-dependent manner and the types of LSD1-formed heterocomplexes differed significantly according to the cellular redox status. The disappearance of LSD1 monomers under oxidative stress may indicate that the ability of LSD1 to interact with its partners is enhanced by intramolecular disulfide bonds that are formed during oxidative conditions. Interestingly, within each of the Zn-finger domains, LSD1 has a thioredoxin-like domain with the canonical CXXC motif. These conserved thioredoxin-like motifs are found in proteins that possess the property of interacting with Cys-containing substrates, catalysing the formation of disulfide bridges or their cleavage (Carvalho *et al.* 2008). In this way, they may confer the LSD1 redox-rheostat property acting as cellular redox switches. The interaction of LSD1 with other proteins through redox-sensing Cys residues seems to be probable because all LSD1-interacting proteins are relatively rich in Cys (Supporting Information Data Set S1). However, further studies are needed to confirm this.

Our results indicate that LSD1 plays some role in ubiquitination and proteolytic processes. We identified three enzymes involved in ubiquitination, that is, UBA2, OTU2 and TPP2, among the LSD1-interacting proteins. Ubiquitination and deubiquitination mechanisms are essential in numerous cellular processes, such as in transcriptional regulation, mRNA splicing, cell division, DNA damage response, intracellular trafficking and signal transduction (Radjacommare *et al.* 2014). Therefore, the ubiquitin (Ub)–proteasome system plays a crucial role in fine-tuning the plant proteome to effectively respond to environmental stresses. Among the LSD1 interactors identified in our experiment, two are considered as targets of metacaspase 9 (MC9) during proteolysis (lysyl-tRNA synthetase and PFP) (Tsiatsiani *et al.* 2013), while a vast number appeared to be targets of polyubiquitin 3 (UBQ3). UBQ3 is attached to proteins that are destined for degradation, and its transcription level is modulated by adverse conditions (Brosché *et al.* 2002). Together, 20 out of 38 proteins (53%) forming complexes with LSD1 have previously been described to bind UBQ3 (Kim *et al.* 2013). This suggests the role of LSD1 in proteolysis, which could be the targeting of polyubiquitinated proteins to the proteasome. Nevertheless, more detailed studies are needed to define the exact role of LSD1 in ubiquitylation and proteolysis processes.

Two proteins forming complexes with LSD1 in control conditions (MTHFR1 and MTHFR2) and three under oxidative-stress conditions (SAHH2, ADK1 and SHM4) are involved in methylation. This indicates that LSD1 affects the methylation processes, irrespectively of the cellular redox status. MTHFRs play an important role in methyl donor formation for DNA methylation (Friso *et al.* 2002). While SHM4 is responsible for methyl donor synthesis, different essential processes such as chromatin modification and mRNA capping rely on transmethylation and thus ADK and SAHH activities. LESION SIMULATING DISEASE 1 interaction with those proteins may suggest the involvement of LSD1 in the regulation of chromatin transcriptional activity. The fact that LSD1 associates with a broad range of promoter sequences can imply that this interaction occurs through enzymes responsible for DNA/histone methylation. Assuming that LSD1 positively regulates enzymes responsible for DNA methylation, thus reducing chromatin transcriptional activity,

could at least partially explain the higher expression of most LSD1-target genes in the *lsd1* mutant and lower expression in LSD1-OE plants. However, further studies are needed to confirm this.

An additional role of ADK1 is the conversion of cytokinin (CK) bases and ribosides to their corresponding nucleotides. Because CK bases and possibly also ribosides are thought to be the active forms of CKs, their conversion to inactive nucleotides may be important for the regulation of these hormones' level in plant cells and thus proper plant development (Moffatt *et al.* 2000, 2002). On the other hand, SAHH2 has been demonstrated to negatively regulate the accumulation of CKs (Li *et al.* 2008). The regulation of ADK1 and SAHH2 activity by LSD1 may influence the CKs' cellular concentration and thus affect cell division. Moreover, among the LSD1 interactors in non-oxidative stress conditions, a couple of proteins were employed in the cell cycle, namely CDC2, RGP1, DRP3A and DRP3B. CDC2 is a key regulator of the cell cycle required for cell division control in leaves, the male gametophyte, embryo and endosperm (Hirayama *et al.* 1991; Verkest *et al.* 2005), while RGP1 is essential in the cell division process during pollen development (Drakakaki *et al.* 2006). The interaction of LSD1 with two dynamin-related proteins (DRP3A and DRP3B) which function as the molecular scissors of mitochondria and peroxisomes during mitotic division (Kang *et al.* 1998; Kim *et al.* 2001; Fujimoto *et al.* 2009; Zhang & Hu 2010) was additionally confirmed by BiFC.

Furthermore, three proteins bound by LSD1 in non-oxidative-stress conditions appeared to be responsible for cell wall formation and modifications, namely CAD5, which is crucial during the lignin biosynthesis pathway (Sibout *et al.* 2005), RGP1, which is a cellulose synthase required for proper cell wall formation (Rautengarten *et al.* 2011) and UGD2, which is common for oxidative and non-oxidative conditions and which provides nucleotide sugars for cell wall pectin synthesis (Reboul *et al.* 2011). Our ChIP-qPCR additionally proved that LSD1 regulated the expression of *CCOAMT*, encoding the enzyme participating in lignin biosynthesis and cell wall reinforcement in response to several biotic and abiotic stresses (Inoue *et al.* 1998).

The fact that LSD1 interacts with proteins engaged in cell cycle and cell wall formation in non-oxidative stress conditions strongly suggests its regulatory function in cell division processes, particularly under optimal growth conditions. LESION SIMULATING DISEASE 1 may modulate their function by binding these cell division regulators. This assumption is supported by our results, which show that the *lsd1* mutant exhibits a smaller area of mesophyll cells in the leaf tissue, which is compensated by their higher density. An exactly opposite pattern was demonstrated in LSD1-OE plants. These results are consistent with our recent study which was performed on two species, Arabidopsis and *Populus tremula x tremuloides*, demonstrating LSD1-dependent changes in cell wall composition and cell division/cell differentiation processes (Szechyńska-Hebda *et al.* 2016).

There was also an overrepresentation of proteins important during gametogenesis and embryo development within the LSD1-interactome. Four aminoacyl-tRNA synthetases (asparaginyl-, threonyl-, leucyl- and lysyl-tRNA synthetase), which are crucial in these processes (Berg *et al.* 2005), formed complexes with LSD1. Moreover, many LSD1 partners are expressed in pollen: TPS7, MTHFR2, diaminopimelate epimerase (AT3G53580), pre-mRNA-splicing factor ATP-dependent RNA helicase, ADK1, SAHH2 (Wang *et al.* 2008), RGP1 (Drakakaki *et al.* 2006), SHM4 and FUM1 (Noir *et al.* 2005). Furthermore, CDC2 is a well-known regulator of pollen development (Nowack *et al.* 2006). These results suggest that LSD1 may be an important regulator during pollen formation and embryogenesis. In fact, LSD1 itself is highly expressed during pollen germination and pollen tube growth (Wang *et al.* 2008). Interestingly, expression analysis performed in the Genevestigator (Hruz *et al.* 2008) proved that LSD1 is particularly highly transcribed in stamen (Supporting Information Fig. S10).

Both gametogenesis and embryogenesis require perfectly synchronized mitotic division and CD cycles. It has been shown that the tapetum undergoes a process of highly regulated CD during microspore maturation (Wilson & Yang 2004). Thus, CD has been suggested to play an important role in the regulation of male gametophyte development (Teng *et al.* 2008; Zhang *et al.* 2011). Successful embryonic development also requires strict coordination of cell proliferation, differentiation and CD processes (Filonova *et al.* 2002; Suarez *et al.* 2004). Thus, LSD1 can be responsible for fine-tuning cell division/death processes during the development of gametes and embryos. Furthermore, in our ChIP-qPCR, we proved that LSD1 either directly or indirectly binds to the promoter regions of *GLP7*, *SLPK* and *WAKL8*, which are expressed in mature pollen grains and pollen tubes (*GLP7*) (Wang *et al.* 2008) or flower buds (*SLPK*, *WAKL8*) (Cao *et al.* 2006). Taking into account all of the above data, we showed here for the first time that LSD1 can be important in such processes as gametogenesis, embryo development and seed germination.

In our TAP experiment, LSD1 also interacted with EPSP synthase, which is a chloroplastic enzyme participating in chorismate production (Klee *et al.* 1987). Chorismate-derived products include primary plant metabolites such as SA and IAA; thus, this interaction could at least partially explain the influence of LSD1 on hormonal homeostasis, which has been well described in the literature (Kliebenstein *et al.* 1999; Mühlenbock *et al.* 2007, 2008; Wituszyńska *et al.* 2013; Chai *et al.* 2015). The involvement of LSD1 in SA-dependent signalling may also be justified by direct or indirect LSD1 association with the promoter of *GH3.12*, which encodes the enzyme required for SA accumulation (Jagadeeswaran *et al.* 2007; Okrent *et al.* 2009).

Apart from the previously mentioned molecular processes, LSD1 also interacted with enzymes involved in such distinct cellular pathways as RNA modification, pentose phosphate pathway, glycolysis, Krebs cycle, sugar and hormone metabolism and tetrapyrrole biosynthesis, which indicates the broad spectrum of LSD1-dependent regulation.

Importantly, we also revealed that the role of LSD1 as a scaffold protein is not its only role because it exhibited transcriptional regulation activity in the nucleus. Within the 27 genes demonstrating an opposite expression pattern

depending on the lack or overexpression of LSD1, some of them (*GOLS2*, *CCOAMT*, *MBP1*, *LLP*, *JRG21*, *PDF1.2B*, *LRRK*, *WAKL8* and *CML41*) exhibited a similar deregulation pattern in the *lsd1* mutant and *35S$_{pro}$:LSD1-GR* line, while an opposite pattern was exhibited in the *35S$_{pro}$:LSD1-GFP* line. This may indicate the high dynamic of LSD1-dependent gene expression regulation or the existence of some LSD1 repressor that is involved in controlling its activity as a transcriptional regulator.

LESION SIMULATING DISEASE 1 target genes encoded proteins participating in different cellular pathways such as response to stress, pre-mRNA splicing, post-translational protein modification, proteolysis, SA-, JA-, IAA- and Ca^{2+}-dependent signalling pathways, cell wall remodelling and pollen germination. Interestingly, most of the genes whose promoters are directly or indirectly bound by LSD1 demonstrated higher expression in the *lsd1* mutant and lower expression in LSD1-OE plants. The broad range of promoter sequences identified in ChIP together with the fact that LSD1 interacts with three enzymes responsible for DNA/histone methylation suggests that it may regulate gene expression by modifying the transcriptional activity of chromatin. By binding these proteins, LSD1 may activate them to methylate specific promoters and thus represses the transcription of specific genes. However, alternative scenarios in which LSD1 either negatively or positively regulates the other specific transcription factor(s) or repressor(s), respectively, cannot be excluded. Therefore, further studies are needed to explain the exact role of LSD1 in transcription regulation.

Surprisingly, the promoters of genes encoding two pathogenesis-related proteins (PR1 and CAP) were enriched in ChIP samples obtained from plants grown in stable laboratory conditions but not in greenhouse-grown plants. These results are consistent with our previous data demonstrating that *PR1* expression was induced in the *lsd1* mutant in laboratory-grown Arabidopsis but not in field-grown plants (Wituszyńska *et al.* 2013). Thus, LSD1 negatively controls the expression of *PR1*, but this regulation depends on ambient conditions. Based on these results, it seems that the activity of LSD1 as a transcriptional regulator is also condition dependent, which further confirms our observations that the LSD1 function strongly depends on the growing conditions, particularly on the plant redox status.

Concluding, this study is the first such comprehensive report on LSD1 molecular and cellular function. We have shown that LSD1 constitutes a condition-dependent molecular regulator

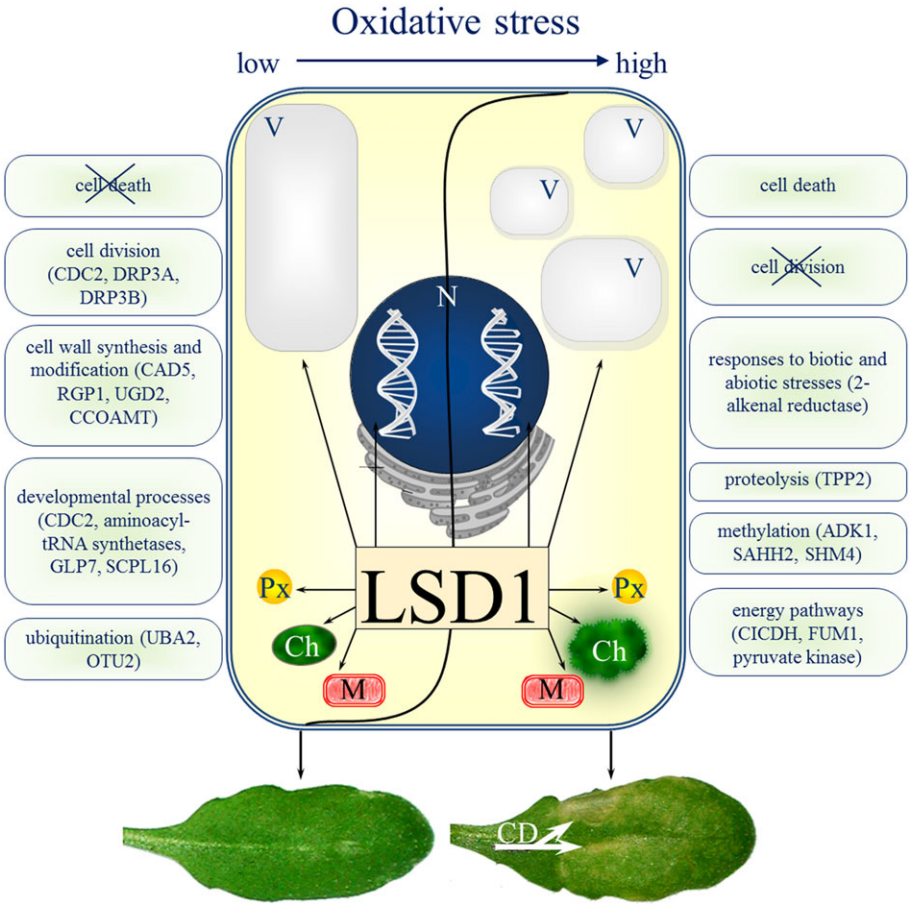

Figure 6. Suggested model of the LSD1 mode of action. Depending on the growth conditions that impose either low or high oxidative stress on the plants, LSD1 conditionally regulates a broad range of diverse molecular processes balancing between cell division and cell death pathways.

of diverse cellular processes by acting as a redox-sensing scaffold protein and transcription regulator. On the basis of the obtained results, we suggest a model of the LSD1 mode of action (Fig. 6) in which, depending on the growth conditions, imposing on plants either low or high levels of oxidative stress, LSD1 conditionally regulates a broad range of diverse molecular processes that balance between cell division and CD pathways. In non-oxidative stress conditions, LSD1 influences the activity of proteins involved in cell division, cell wall modification, ubiquitination and developmental processes, thus promoting plant growth and development. On the other hand, LSD1 regulates stress responses, proteolysis, methylation and energy processes under high oxidative stress, presumably reducing to the necessary minimum those pathways that lead to CD, restricting growth and development. Therefore, LSD1 is an important molecular regulator that is responsible for striking a balance between cell division and CD depending on the actual environmental conditions and associated levels of the plant redox status.

Author Contributions

W.C., S.K., F.V.B. and M.S-H. planned the experiments and postulated the hypotheses tested in this paper; W.C. prepared all of the genetic constructs and performed subcellular localization and BiFC experiments; W.C. performed the immunoblots; W.C. prepared the RNA for NGS sequencing and performed all of the qPCRs; library preparation and sequencing were performed by the VIB Nucleomics Core (www.nucleomics.be); ChIP was done by WC with the help of S.S-B.; K.V.D.K. performed the TAP experiment with the help of WC; P.W. did the annotation and statistical analysis of the RNA sequencing data; M.S-H. performed the microscopic and Genevestigator analysis; A.R. did the photographical documentation and helped in the functional annotations; W.C. wrote the manuscript with the help of S. K. and M.S-H.; S.K., F.V.B., B.M.R. and S.B. reviewed and approved its final version.

ACKNOWLEDGMENTS

We would like to thank Eveline Van De Slijke and Nancy De Winne for their excellent technical assistance. We also thank Cezary Waszczak for substantive consultancy on the TAP experiment. This work was supported by the 'Maestro 6' project (2014/14/A/NZ1/00218) granted to SK by the National Science Centre, by the 'Warsaw Plant Health Initiative' operating within the 7th EU Framework Programme (FP7-REGPOT-2012-1-286093) and by the FWO project (G0D7914N) granted to F.V.B. M.S.H., W.C. and S.K. are also grateful for partial support of this work by the WOODTECH (PBS1/A8/16/2013) and Biostrateg (CROPTECH ID: 298241) projects financed by the National Centre for Research and Development. W.C. was a grant holder of the START program (START 126.2014) for young researchers, financed by the Foundation for Polish Science and a postdoctoral fellow of 'A program to improve the level of the didactic approach to the question of how to obtain raw plant materials for the purposes of energy production in the context of the Europe 2020 Strategy objectives' project (POKL.04.03.00-00-042/12-00). The authors have no conflict of interest to declare.

REFERENCES

Anders S., Pyl P.T. & Huber W. (2015) HTSeq a Python framework to work with high-throughput sequencing data. *Bioinformatics* **31**, 166–169.

Berg M., Rogers R., Muralla R. & Meinke D. (2005) Requirement of aminoacyl-tRNA synthetases for gametogenesis and embryo development in Arabidopsis. *The Plant Journal* **44**, 866–878.

Bolger A.M., Lohse M. & Usadel B. (2014) Trimmomatic, a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120.

Boruc J., Van den Daele H., Hollunder J., Rombauts S., Mylle E., Hilson P., … Russinova E. (2010) Functional modules in the Arabidopsis core cell cycle binary protein–protein interaction network. *The Plant Cell* **22**, 1264–1280.

Brosché M., Schuler M.A., Kalbina I., Connor L. & Strid A. (2002) Gene regulation by low level UV-B radiation, identification by DNA array analysis. *Photochemical & Photobiological Sciences* **1**, 656–664.

Bruggeman Q., Raynaud C., Benhamed M. & Delarue M. (2015) To die or not to die? Lessons from lesion mimic mutants. *Frontiers in Plant Science* **6**, 24.

Cao D., Cheng H., Wu W., Soo H.M. & Peng J. (2006) Gibberellin mobilizes distinct DELLA-dependent transcriptomes to regulate seed germination and floral development in Arabidopsis. *Plant Physiology* **142**, 509–525.

Carvalho A.T.P., Swart M., van Stralen J.N.P., Fernandes P.A., Ramos M.J. & Bickelhaupt F.M. (2008) Mechanism of thioredoxin-catalyzed disulfide reduction. *Activation of the buried thiol and role of the variable active-site residues. The Journal of Physical Chemistry B* **112**, 2511–2523.

Chai T., Zhou J., Liu J. & Xing D. (2015) LSD1 and HY5 antagonistically regulate red light induced-programmed cell death in Arabidopsis. *Frontiers in Plant Science* **6**, 292.

Ciftci-Yilmaz S. & Mittler R. (2008) The zinc finger network of plants. *Cellular and Molecular Life Sciences: CMLS* **65**, 1150–1160.

Clough S.J. & Bent A.F. (1998) Floral dip, a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. *The Plant Journal* **16**, 735–743.

Coll N.S., Epple P. & Dangl J.L. (2011) Programmed cell death in the plant immune system. *Cell Death and Differentiation* **18**, 1247–1256.

Coll N.S., Vercammen D., Smidler A., Clover C., Van Breusegem F., Dangl J.L. & Epple P. (2010) Arabidopsis type I metacaspases control cell death. *Science* **330**, 1393–1397.

Desikan R., A-H-Mackerness S., Hancock J.T. & Neill S.J. (2001) Regulation of the Arabidopsis transcriptome by oxidative stress. *Plant Physiology* **127**, 159–172.

Dietrich R.A., Delaney T.P., Uknes S.J., Ward E.R., Ryals J.A. & Dangl J.L. (1994) Arabidopsis mutants simulating disease resistance response. *Cell* **77**, 565–577.

Dietrich R.A., Richberg M.H., Schmidt R., Dean C. & Dangl J.L. (1997) A novel zinc finger protein is encoded by the Arabidopsis LSD1 gene and functions as a negative regulator of plant cell death. *Cell* **88**, 685–694.

Domínguez F. & Cejudo F.J. (2014) Programmed cell death (PCD), an essential process of cereal seed development and germination. *Frontiers in Plant Science* **28**, 366.

Drakakaki G., Zabotina O., Delgado I., Robert S., Keegstra K. & Raikhel N. (2006) Arabidopsis reversibly glycosylated polypeptides 1 and 2 are essential for pollen development. *Plant Physiology* **142**, 1480–1492.

Epple P., Mack A.A., Morris V.R.F. & Dangl J.L. (2003) Antagonistic control of oxidative stress-induced cell death in Arabidopsis by two related, plant-specific zinc finger proteins. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 6831–6836.

Falk A., Feys B.J., Frost L.N., Jones J.D., Daniels M.J. & Parker J.E. (1999) EDS1, an essential component of R gene-mediated disease resistance in Arabidopsis has homology to eukaryotic lipases. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 3292–3297.

Feys B.J., Moisan L.J., Newman M.-A. & Parker J.E. (2001) Direct interaction between the Arabidopsis disease resistance signaling proteins, EDS1 and PAD4. *The EMBO Journal* **20**, 5400–5411.

Filonova L.H., von Arnold S., Daniel G. & Bozhkov P.V. (2002) Programmed cell death eliminates all but one embryo in a polyembryonic plant seed. *Cell Death and Differentiation* **9**, 1057–1062.

Friso S., Choi S.W., Girelli D., Mason J.B., Dolnikowski G.G., Bagley P.J., … Selhub J. (2002) A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 5606–5611.

Fujimoto M., Arimura S., Mano S., Kondo M., Saito C., Ueda T., … Tsutsumi N. (2009) Arabidopsis dynamin-related proteins DRP3A and DRP3B are functionally redundant in mitochondrial fission, but have distinct roles in peroxisomal fission. *The Plant Journal* **58**, 388–400.

Fukuda H. (2000) Programmed cell death of tracheary elements as a paradigm in plants. *Plant Molecular Biology* **44**, 245–253.

Glazebrook J., Zook M., Mert F., Kagan I., Rogers E.E., Crute I.R., … Ausubel F. M. (1997) Phytoalexin-deficient mutants of Arabidopsis reveal that PAD4 encodes a regulatory factor and that four PAD genes contribute to downy mildew resistance. *Genetics* **146**, 381–392.

Gupta S.K., Rai A.K., Kanwar S.S. & Sharma T.R. (2012) Comparative analysis of zinc finger proteins involved in plant disease resistance. *PloS One* **7**, e42578.

He S., Huang K., Zhang X., Yu X., Huang P. & An C. (2011a) The LSD1-type zinc finger motifs of *Pisum sativa* LSD1 are a novel nuclear localization signal and interact with importin alpha. *PloS One* **6**, e22131.

He S., Tan G., Liu Q., Huang K., Ren J., Zhang X., … An C. (2011b) The LSD1-interacting protein GILP is a LITAF domain protein that negatively regulates hypersensitive cell death in Arabidopsis. *PloS One* **6**, e18750.

He X. & Zhang J. (2006) Why do hubs tend to be essential in protein networks? *PLoS Genetics* **2**, e88.

Hirayama T., Imajuku Y., Anai T., Matsui M. & Oka A. (1991) Identification of two cell-cycle-controlling cdc2 gene homologs in *Arabidopsis thaliana*. *Gene* **105**, 159–165.

Hruz T., Laule O., Szabo G., Wessendorp F., Bleuler S., Oertle L., … Zimmermann P. (2008) Genevestigator v3, a reference expression database for the meta-analysis of transcriptomes. *Advances in Bioinformatics* **2008**, 420747.

Huang X., Li Y., Zhang X., Zuo J. & Yang S. (2010) The Arabidopsis LSD1 gene plays an important role in the regulation of low temperature-dependent cell death. *The New Phytologist* **187**, 301–312.

Inoue K., Sewalt V.J., Murray G.B., Ni W., Stürzer C. & Dixon R.A. (1998) Developmental expression and substrate specificities of alfalfa caffeic acid 3-O-methyltransferase and caffeoyl coenzyme A 3-O-methyltransferase in relation to lignification. *Plant Physiology* **117**, 761–770.

Jabs T., Dietrich R.A. & Dangl J.L. (1996) Initiation of runaway cell death in an Arabidopsis mutant by extracellular superoxide. *Science* **273**, 1853–1856.

Jagadeeswaran G., Raina S., Acharya B.R., Maqbool S.B., Mosher S.L., Appel H.M., … Raina R. (2007) Arabidopsis GH3-LIKE DEFENSE GENE 1 is required for accumulation of salicylic acid, activation of defense responses and resistance to *Pseudomonas syringae*. *The Plant Journal* **51**, 234–246.

Jeong H., Mason S.P., Barabási A.L. & Oltvai Z.N. (2001) Lethality and centrality in protein networks. *Nature* **411**, 41–42.

Jirage D., Tootle T.L., Reuber T.L., Frost L.N., Feys B.J., Parker J.E., … Glazebrook J. (1999) *Arabidopsis thaliana* PAD4 encodes a lipase-like gene that is important for salicylic acid signaling. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 13583–13588.

Kaminaka H., Näke C., Epple P., Dittgen J., Schütze K., Chaban C., … Dangl J.L. (2006) bZIP10-LSD1 antagonism modulates basal defense and cell death in Arabidopsis following infection. *The EMBO Journal* **25**, 4400–4411.

Kang S.G., Jin J.B., Piao H.L., Pih K.T., Jang H.J., Lim J.H. & Hwang I. (1998) Molecular cloning of an Arabidopsis cDNA encoding a dynamin-like protein that is localized to plastids. *Plant Molecular Biology* **38**, 437–447.

Karpinski S., Reynolds H., Karpinska B., Wingsle G., Creissen G. & Mullineaux P. (1999) Systemic signaling and acclimation in response to excess excitation energy in Arabidopsis. *Science* **284**, 654–657.

Karpiński S., Szechyńska-Hebda M., Wituszyńska W. & Burdiak P. (2013) Light acclimation, retrograde signalling, cell death and immune defences in plants. *Plant, Cell & Environment* **36**, 736–744.

Kaufmann K., Muiño J.M., Østerås M., Farinelli L., Krajewski P. & Angenent G. C. (2010) Chromatin immunoprecipitation (ChIP) of plant transcription factors followed by sequencing (ChIP-SEQ) or hybridization to whole genome arrays (ChIP-CHIP). *Nature Protocols* **5**, 457–472.

Kim Y.W., Park D.S., Park S.C., Kim S.H., Cheong G.W. & Hwang I. (2001) Arabidopsis dynamin-like 2 that binds specifically to phosphatidylinositol 4-phosphate assembles into a high-molecular weight complex in vivo and in vitro. *Plant Physiology* **127**, 1243–1255.

Kim D.-Y., Scalf M., Smith L.M. & Vierstra R.D. (2013) Advanced proteomic analyses yield a deep catalog of ubiquitylation targets in Arabidopsis. *The Plant Cell* **25**, 1523–1540.

Klee H.J., Muskopf Y.M. & Gasser C.S. (1987) Cloning of an *Arabidopsis thaliana* gene encoding 5-enolpyruvylshikimate-3-phosphate synthase, sequence analysis and manipulation to obtain glyphosate-tolerant plants. *Molecular & General Genetics* **210**, 437–442.

Kliebenstein D.J., Dietrich R.A., Martin A.C., Last R.L. & Dangl J.L. (1999) LSD1 regulates salicylic acid induction of copper zinc superoxide dismutase in *Arabidopsis thaliana*. *Molecular Plant-Microbe Interactions* **12**, 1022–1026.

Levesque M.P., Vernoux T., Busch W., Cui H., Wang J.Y., Blilou I., … Benfey P.N. (2006) Whole-genome analysis of the SHORT-ROOT developmental pathway in Arabidopsis. *PLoS Biology* **4**, e143.

Li Y., Chen L., Mu J. & Zuo J. (2013) LESION SIMULATING DISEASE1 interacts with catalases to regulate hypersensitive cell death in Arabidopsis. *Plant Physiology* **163**, 1059–1070.

Li C.-H., Yu N., Jiang S.-M., Shangguan X.-X., Wang L.-J. & Chen X.-Y. (2008) Down-regulation of S-adenosyl-L, -homocysteine hydrolase reveals a role of cytokinin in promoting transmethylation reactions. *Planta* **228**, 125–136.

Lorrain S., Vailleau F., Balagué C. & Roby D. (2003) Lesion mimic mutants, keys for deciphering cell death and defense pathways in plants? *Trends in Plant Science* **8**, 263–271.

Mateo A., Mühlenbock P., Rustérucci C., Chang C.C.-C., Miszalski Z., Karpinska B., … Karpinski S. (2004) LESION SIMULATING DISEASE 1 is required for acclimation to conditions that promote excess excitation energy. *Plant Physiology* **136**, 2818–2830.

Merkle T. (2003) Nucleo-cytoplasmic partitioning of proteins in plants, implications for the regulation of environmental and developmental signalling. *Current Genetics* **44**, 231–260.

Moeder W. & Yoshioka K. (2008) Lesion mimic mutants, A classical, yet still fundamental approach to study programmed cell death. *Plant Signaling & Behavior* **3**, 764–767.

Moffatt B.A., Stevens Y.Y., Allen M.S., Snider J.D., Pereira L.A., Todorova M.I., … Wagner C. (2002) Adenosine kinase deficiency is associated with developmental abnormalities and reduced transmethylation. *Plant Physiology* **128**, 812–821.

Moffatt B.A., Wang L., Allen M.S., Stevens Y.Y., Qin W., Snider J. & von Schwartzenberg K. (2000) Adenosine kinase of Arabidopsis. Kinetic properties and gene expression. *Plant Physiology* **124**, 1775–1785.

Mohr D., Frey S., Fischer T., Güttler T. & Görlich D. (2009) Characterisation of the passive permeability barrier of nuclear pore complexes. *The EMBO Journal* **28**, 2541–2553.

Mühlenbock P., Plaszczyca M., Plaszczyca M., Mellerowicz E. & Karpinski S. (2007) Lysigenous aerenchyma formation in Arabidopsis is controlled by LESION SIMULATING DISEASE1. *The Plant Cell* **19**, 3819–3830.

Mühlenbock P., Szechyńska-Hebda M., Płaszczyca M., Baudo M., Mateo A., Mullineaux P.M., … Karpiński S. (2008) Chloroplast signaling and LESION SIMULATING DISEASE1 regulate crosstalk between light acclimation and immunity in Arabidopsis. *The Plant Cell* **20**, 2339–2356.

Noir S., Bräutigam A., Colby T., Schmidt J. & Panstruga R. (2005) A reference map of the *Arabidopsis thaliana* mature pollen proteome. *Biochemical and Biophysical Research Communications* **337**, 1257–1266.

Nowack M.K., Grini P.E., Jakoby M.J., Lafos M., Koncz C. & Schnittger A. (2006) A positive signal from the fertilization of the egg cell sets off endosperm proliferation in angiosperm embryogenesis. *Nature Genetics* **38**, 63–67.

Okrent R.A., Brooks M.D. & Wildermuth M.C. (2009) Arabidopsis GH3.12 (PBS3) conjugates amino acids to 4-substituted benzoates and is inhibited by salicylate. *The Journal of Biological Chemistry* **284**, 9742–9754.

Parker J.E., Holub E.B., Frost L.N., Falk A., Gunn N.D. & Daniels M.J. (1996) Characterization of eds1, a mutation in Arabidopsis suppressing resistance to Peronospora parasitica specified by several different RPP genes. *The Plant Cell* **8**, 2033–2046.

Pfaffl M.W., Horgan G.W. & Dempfle L. (2002) Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Research* **30**, e36.

Radjacommare R., Usharani R., Kuo C.-H. & Fu H. (2014) Distinct phylogenetic relationships and biochemical properties of Arabidopsis ovarian tumor-related deubiquitinases support their functional differentiation. *Frontiers in Plant Science* **5**, 84.

Ramakers C., Ruijter J.M., Deprez R.H.L. & Moorman A.F.M. (2003) Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neuroscience Letters* **339**, 62–66.

Rautengarten C., Ebert B., Herter T., Petzold C.J., Ishii T., Mukhopadhyay A., … Scheller H.V. (2011) The interconversion of UDP-arabinopyranose and UDP-arabinofuranose is indispensable for plant development in Arabidopsis. *The Plant Cell* **23**, 1373–1390.

Reboul R., Geserick C., Pabst M., Frey B., Wittmann D., Lütz-Meindl U., … Tenhaken R. (2011) Down-regulation of UDP-glucuronic acid biosynthesis leads to swollen plant cell walls and severe developmental defects associated with changes in pectic polysaccharides. *The Journal of Biological Chemistry* **286**, 39982–39992.

Robinson M.D., McCarthy D.J. & Smyth G.K. (2010) edgeR, a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139–140.

Rustérucci C., Aviv D.H., Holt B.F., Dangl J.L. & Parker J.E. (2001) The disease resistance signaling components EDS1 and PAD4 are essential regulators of the cell death pathway controlled by LSD1 in Arabidopsis. *The Plant Cell* **13**, 2211–2224.

Samach A., Onouchi H., Gold S.E., Ditta G.S., Schwarz-Sommer Z., Yanofsky M. F. & Coupland G. (2000) Distinct roles of CONSTANS target genes in reproductive development of Arabidopsis. *Science* **288**, 1613–1616.

Schneider C.A., Rasband W.S. & Eliceiri K.W. (2012) NIH Image to ImageJ, 25 years of image analysis. *Nature Methods* **9**, 671–675.

Sibout R., Eudes A., Mouille G., Pollet B., Lapierre C., Jouanin L. & Séguin A. (2005) CINNAMYL ALCOHOL DEHYDROGENASE-C and -D are the primary genes involved in lignin biosynthesis in the floral stem of Arabidopsis. *The Plant Cell* **17**, 2059–2076.

Suarez M.F., Filonova L.H., Smertenko A., Savenkov E.I., Clapham D.H., von Arnold S., … Bozhkov P.V. (2004) Metacaspase-dependent programmed cell death is essential for plant embryogenesis. *Current Biology* **14**, R339–R340.

Szechyńska-Hebda M., Czarnocka W., Hebda M. & Karpiński S. (2016) PAD4, LSD1 and EDS1 regulate drought tolerance, plant biomass production, and cell wall properties. *Plant Cell Reports* **35**, 527–539.

Takatsuji H. (1998) Zinc-finger transcription factors in plants. *Cellular and Molecular Life Sciences* **54**, 582–596.

Teng C., Dong H., Shi L., Deng Y., Mu J., Zhang J., … Zuo J. (2008) Serine palmitoyltransferase, a key enzyme for de novo synthesis of sphingolipids, is essential for male gametophyte development in Arabidopsis. *Plant Physiology* **146**, 1322–1332.

Thimm O., Bläsing O., Gibon Y., Nagel A., Meyer S., Krüger P., … Stitt M. (2004) mapman, a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *The Plant Journal* **37**, 914–939.

Tsiatsiani L., Timmerman E., De Bock P.-J., Vercammen D., Stael S., van de Cotte B., … Van Breusegem F. (2013) The Arabidopsis metacaspase9 degradome. *The Plant Cell* **25**, 2831–2847.

Untergasser A., Nijveen H., Rao X., Bisseling T., Geurts R. & Leunissen J.A.M. (2007) Primer3Plus, an enhanced web interface to Primer3. *Nucleic Acids Research* **35**, W71–W74.

Van Leene J., Eeckhout D., Cannoot B., De Winne N., Persiau G., Van De Slijke E., … De Jaeger G. (2015) An improved toolbox to unravel the plant cellular machinery by tandem affinity purification of Arabidopsis protein complexes. *Nature Protocols* **10**, 169–187.

Van Leene J., Stals H., Eeckhout D., Persiau G., Van De Slijke E., Van Isterdael G., … De Jaeger G. (2007) A tandem affinity purification-based technology platform to study the cell cycle interactome in *Arabidopsis thaliana*. *Molecular & Cellular Proteomics* **6**, 1226–1238.

Van Leene J., Witters E., Inzé D. & De Jaeger G. (2008) Boosting tandem affinity purification of plant protein complexes. *Trends in Plant Science* **13**, 517–520.

Vandenabeele S., Vanderauwera S., Vuylsteke M., Rombauts S., Langebartels C., Seidlitz H.K., … Van Breusegem F. (2004) Catalase deficiency drastically affects gene expression induced by high light in *Arabidopsis thaliana*. *The Plant Journal* **39**, 45–58.

Verkest A., de Manes C.-L.O., Vercruysse S., Maes S., Van Der Schueren E., Beeckman T., … De Veylder L. (2005) The cyclin-dependent kinase inhibitor KRP2 controls the onset of the endoreduplication cycle during Arabidopsis leaf development through inhibition of mitotic CDKA;1 kinase complexes. *The Plant Cell* **17**, 1723–1736.

Wagner D., Sablowski R.W. & Meyerowitz E.M. (1999) Transcriptional activation of APETALA1 by LEAFY. *Science* **285**, 582–584.

Walter M., Chaban C., Schütze K., Batistic O., Weckermann K., Näke C., .., Kudla J. (2004) Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation. *The Plant Journal* **40**,428–438.

Wang Y., Zhang W.-Z., Song L.-F., Zou J.-J., Su Z. & Wu W.-H. (2008) Transcriptome analyses show changes in gene expression to accompany pollen germination and tube growth in Arabidopsis. *Plant Physiology* **148**, 1201–1211.

Waszczak C., Akter S., Eeckhout D., Persiau G., Wahni K., Bodra N., … Van Breusegem F. (2014) Sulfenome mining in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 11545–11550.

Wiermer M., Feys B.J. & Parker J.E. (2005) Plant immunity, the EDS1 regulatory node. *Current Opinion in Plant Biology* **8**, 383–389.

Wilson Z.A. & Yang C. (2004) Plant gametogenesis, conservation and contrasts in development. *Reproduction (Cambridge, England)* **128**, 483–492.

Wituszyńska W., Ślesak I., Vanderauwera S., Szechyńska-Hebda M., Kornaś A., Kelen K.V.D., … Karpiński S. (2013) LESION SIMULATING DISEASE1, ENHANCED DISEASE SUSCEPTIBILITY1, and PHYTOALEXIN DEFICIENT4 conditionally regulate cellular signaling homeostasis, photosynthesis, water use efficiency, and seed yield in Arabidopsis. *Plant Physiology* **161**, 1795–1805.

Wituszyńska W., Szechyńska-Hebda M., Sobczak M., Rusaczonek A., Kozłowska-Makulska A., … Karpiński S. (2015) LESION SIMULATING DISEASE 1 and ENHANCED DISEASE SUSCEPTIBILITY 1 differentially regulate UV-C-induced photooxidative stress signalling and programmed cell death in *Arabidopsis thaliana*. *Plant, Cell & Environment* **38**, 315–330.

Wu A., Allu A.D., Garapati P., Siddiqui H., Dortay H., Zanor M.-I., … Balazadeh S. (2012) JUNGBRUNNEN1, a reactive oxygen species-responsive NAC transcription factor, regulates longevity in Arabidopsis. *The Plant Cell* **24**, 482–506.

Wu T.D. & Nacu S. (2010) Fast and SNP-tolerant detection of complex variants and splicing in short reads. *Bioinformatics* **26**, 873–881.

Zhang X. & Hu J. (2010) The Arabidopsis chloroplast division protein DYNAMIN-RELATED PROTEIN5B also mediates peroxisome division. *The Plant Cell* **22**, 431–442.

Zhang J., Teng C. & Liang Y. (2011) Programmed cell death may act as a surveillance mechanism to safeguard male gametophyte development in Arabidopsis. *Protein & Cell* **2**, 837–844.

Zhu S., Jeong R.-D., Venugopal S.C., Lapchyk L., Navarre D., Kachroo A. & Kachroo P. (2011) SAG101 forms a ternary complex with EDS1 and PAD4 and is required for resistance signaling against turnip crinkle virus. *PLoS Pathogens* **7**, e1002318.

Received 19 December 2016; received in revised form 14 May 2017; accepted for publication 15 May 2017

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Figure S1. Subcellular localization of endogenous promoter-driven N-terminal GFP-GUS-tagged LSD1, transiently expressed in tobacco epidermal cells.

Supporting Information Figure S2. Immunoblot of the 35S promoter-driven recombinant LSD1-GFP fusion protein from three biological replicates of stably transformed Arabidopsis.

Figure S3. Subcellular localization of EDS1 and PAD4 transiently expressed in tobacco epidermal cells.

Figure S4. Subcellular localization of EDS1 homodimers and EDS1–PAD4 BiFC complexes in tobacco epidermal cells.

Figure S5. Immunoblot of 35S promoter-driven recombinant LSD1-GS-tag fusion proteins from transformed Arabidopsis cell cultures.

Figure S6. Morphology of three-week-old Arabidopsis plants grown in long day conditions.

Figure S7. Primary structure of the LSD1 protein.

Figure S8. Subcellular localization of DRP3A and DRP3B, and their BiFC complexes with LSD1, transiently expressed in tobacco epidermal cells.

Figure S9. Expression level of LSD1 in Ws-0, lsd1 and two transgenic lines: harbouring 35Spro:LSD1-GFP or 35Spro: LSD1-GR genetic constructs.

Figure S10. LSD1 expression potential in different Arabidopsis anatomical structures.

Table S1. List of attB-flanked primers used for coding and promoter sequence amplification prior to Gateway cloning.
Table S2. Genetic constructs obtained using Gateway recombination cloning technology.
Table S3. Tagging combinations of full-length GFF, nGFP (N-terminal moiety of GFP) and cGFP (C-terminal moiety of GFP) used for testing the protein subcellular localization and interactions in the BiFC assay.
Table S4. Primers used for qPCR expression analysis of LSD1 and promoter sequences after ChIP.
Table S5. Expression pattern of genes deregulated in the lsd1 mutant (Wituszyńska *et al.* 2013) found also within the list of 27 genes oppositely regulated depending on either the presence or absence of LSD1 in the RNAseq experiment.
Table S6. Control of the ChIP experiment – fold enrichment of promoter sequences either interacting or non-interacting with JUB1.
Data Set 1. Amino acid sequences of LSD1-interacting proteins.