A chimeric IDD4 repressor constitutively induces immunity in *Arabidopsis* via the modulation of salicylic- and jasmonic acid homeostasis.

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Abstract:

INDETERMINATE DOMAIN (IDD)/BIRD proteins belong to a highly-conserved plant-specific group of transcription factors with dedicated functions in plant physiology and development. Here, we took advantage of the chimeric repressor gene-silencing technology (CRES-T, SRDX) to widen our view on the role of *IDD4/IMPERIAL EAGLE* and *IDD* family members in plant immunity. The hypomorphic *idd4SRDX* lines are compromised in growth and show a robust auto-immune phenotype. Hormonal measurements revealed the concomitant accumulation of salicylic acid and jasmonic acid suggesting that IDDs are involved in regulating the metabolism of these biotic-stress hormones. The analysis of immunity-pathways showed enhanced activation of immune MAP kinase-signaling pathways, the accumulation of hydrogen-peroxide and spontaneous programmed cell-death. The transcriptome of non-elicited *idd4SRDX* lines can be aligned to approximately 40% of differentially expressed genes (DEGs) in flg22-treated wild-type plants. The pattern of DEGs implies IDDs as pivotal repressors of flg22-dependent gene induction. Infection experiments showed the increased resistance of *idd4SRDX* lines to *Pseudomonas syringae* and *Botrytis cinerea* implying a function of IDDs in defense adaptation to hemibiotrophs and necrotrophs. Genome-wide *IDD4* DNA binding studies (DAP-SEQ) combined with DEG analysis of *idd4SRDX* lines identified *IDD4*-

regulated functional gene clusters that contribute to plant growth and development. In summary, we discovered that the expression of *idd4SRDX* activates a wide range of defense-related-traits opening up the possibility to apply *idd4SRDX* as a powerful tool to stimulate innate immunity in engineered crops.

Keywords: Arabidopsis, IDD4, immunity, MAPK, transcription factor, salicylic acid, jasmonic acid

Introduction:

Plant responses to pathogens require the tight control of immune mechanisms to favor defense on the expense of growth and development. Thus, plants can trigger specific differentiation programs or promote defense strategies instead of growth depending on the pathogenic intruder. Although the molecular mechanisms by which plants integrate environmental and endogenous cues are not entirely understood, there is a high degree of collaboration between the various elements of plant MAP kinase signaling pathways (Marin-de la Rosa et al. 2015). Similar to animals, plants use pattern recognition receptors (PRRs) to rapidly activate defense signaling pathways and immune responses upon pathogen attack (Kaisho and Akira 2006; Zipfel 2008). PRRs and their associated signaling components possess a wide range of similarities in mammals, plants, and invertebrates (Mushegian and Medzhitov 2001; Shiu and Bleecker 2001). Plant immunity relies on the recognition of pathogen-derived molecules to activate pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) (Jones and Dangl 2006). The PTI is initiated after the perception of pathogen-associated molecular patterns (PAMPs) of characteristic pathogen components. FLAGELLIN22 (flg22), a 22 amino acid peptide naturally occurring in bacterial flagella, represents one crucial PAMP to trigger PTI in plants. Flg22 is perceived by the plasma membrane-localized receptor FLAGELLIN-INSENSITIVE2 (FLS2) which associates with BRI1-ASSOCIATED RECEPTOR KINASE (BAK1) to rapidly stimulate two mitogen-activated protein kinase (MAPK) cascades. MAPK cascades consist of three sequentially activated kinase modules composed of a MAPK kinase kinase, a MAPK kinase and eventually a MAPK, thereby linking upstream signals to downstream targets.

Throughout the plant kingdom, the MAPK orthologues of MPK3, MPK4, and MPK6 represent the final step in the two flg22-activated MAP kinase cascades and transmit signals to respective target proteins by phosphorylation (Bigeard et al. 2015; Tsuda and Katagiri 2010). MPK3, MPK4, and MPK6 are required for the comprehensive activation of defense genes (Frei dit Frey et al. 2014). To reset the immune signaling system and readjust the trade-off between growth and defense, the *Arabidopsis* Ser/Thr phosphatase of type 2C (AP2C1) functions as a stress-signaling repressor and inactivates MPK4 and MPK6 via dephosphorylation (Schweighofer et al. 2007). Mutant *ap2c1* plants generate higher amounts of jasmonates and show, together with enhanced activation of MAPKs, an elevated resistance against phytophagous mites (*Tetranychus urticae*) (Schweighofer et al. 2007). By contrast, overexpression of *AP2C1* hampers the flg22-induced phosphorylation of MPK3 and MPK6 as well as the ethylene (ET) production and increases the susceptibility to *Botrytis cinerea* (Galletti et al. 2011).

The three immune phytohormones salicylic acid (SA), jasmonic acid (JA) and ET are the canonical immunity agents that coordinate defense depending on the sort of biotic threat. SA biosynthesis and signaling are induced after direct exposure to a wide range of biotrophic pathogens such as the oomycete *Hyaloperonospora arabidopsidis* and hemibiotrophic pathogens, like *Pseudomonas syringae*, while JA and ET signaling are generally important for immunity against necrotrophs including the fungal pathogen *Alternaria brassicicola* (Li et al. 2019). SA plays a crucial role in local and systemic acquired resistance (Chen et al. 1993). Recent studies have shown that SA signaling is an integral part of PTI and ETI (Chen et al. 1993; Dempsey and Klessig 1994; Durrant and Dong 2004; Gaffney et al. 1993). Furthermore, SA contributes to stomatal immunity upon flg22 perception by FLS2, which results in the stomatal closure in order to avoid the entry of bacterial invaders (Song 2006; Zeng and He 2010). Pathogen-induced SA biosynthesis mainly takes place in chloroplasts by *ISOCHORISMATE SYNTHASE 1 (ICS1)* (Strawn et al. 2007; Wildermuth et al. 2001). Several transcription factors (TFs) have been reported to be directly recruited to the *ICS1* promoter in order to regulate *ICS1* expression, such as the two closely related pathogen-induced

TFs *SYSTEMIC ACQUIRED RESISTANCE DEFICIENT 1 (SARD1)* and *CALMODULIN-BINDING PROTEIN 60g (CBP60g)* which are essential for *ICS1* induction and SA accumulation after pathogen perception (Wang et al. 2011; Zhang et al. 2010). In *sard1 cbp60g* double mutants, *ICS1* expression is not induced, SA biosynthesis is interrupted, and plants are more susceptible to *Psm* ES4326 whereby *SARD1* overexpression results in constitutive activation of defense responses and increased SA levels (Zhang et al. 2010). *SARD1* and *CBP60g* modulate the expression of signaling components of PTI and contribute to flg22-induced resistance to *Pst* DC3000 (Sun et al. 2015). The *WRKY* family members *WRKY28* and *WRKY46* have also been shown to promote induction of *ICS1*, partly in complex with *SARD1* (van Verk et al. 2011; Wang et al. 2015). A hub in SA signaling is formed by *NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1)* and its paralogs *NPR3* and *NPR4* (Cao et al. 1994; Cao et al. 1997; Fu et al. 2012; Kuai et al. 2015; Zhang and Cai 2005). These factors act as SA receptors (Canet et al. 2010; Fu et al. 2012; Manohar et al. 2014; Wu et al. 2012), whose function is mediated by members of the TGA family (Despres et al. 2000; Zhou et al. 2000), TCP transcription factors (Li et al. 2018) and by the NIM1-INTERACTING (NIMIN) proteins (Hermann et al. 2013). However, *NPR1* and *NPR3/4* exert opposite transcriptional regulation on plant immunity in a SA-dependent manner (Ding et al. 2018). Several studies demonstrated that the SA signaling pathway predominantly acts antagonistically to JA-mediated signaling (Robert-Seilaniantz et al. 2011; Van der Does et al. 2013). In general, these tradeoffs are confined to the tissues adjacent to the infection sites, and systemic tissues are omitted (Spoel et al. 2007).

The phytohormones JA and Gibberellin (GA) regulate the signal integration at the growth-defense conjunction and coordinate the allocation of resources to balance the metabolic cost in an environment-dependent manner. GA influences plant growth and defense by targeting for degradation a class of GA-controlled inhibitory proteins called DELLAs (Cheng et al. 2004; Dill et al. 2001). DELLAs can act as key negative regulatory switches at the junction of growth and defense by simultaneously integrating light and JA signals (Daviere and Achard 2013; Navarro et al. 2008). JA positively regulates defense response

by controlling JASMONATE-ZIM-DOMAIN PROTEIN (JAZ) protein degradation to promote JASMONATE INSENSITIVE 1 (MYC2)-controlled transcription (Chini et al. 2007; Fernandez-Calvo et al. 2011; Niu et al. 2011; Zhang et al. 2015). The transcriptional hub composed of PHYTOCHROME-INTERACTING FACTORS (PIFs), DELLA, JAZ, and MYC2 negotiate the growth-defense trade-off in dependence on the environmental condition and biotic stresses, like the perception of a pathogen (Smakowska et al. 2016). Auxin, JA, GA, and brassinosteroids, as well as abiotic stimuli like light-intensity and temperature, modulate the activity of the helix-loop-helix TF PIF4 (Gangappa et al. 2017). PIF4 acts as a positive regulator of cell elongation and stem growth but suppressor of immunity (Choi and Oh 2016; Leivar and Monte 2014; Oh et al. 2012; Yamashino et al. 2013). Furthermore, PIF4 transcriptionally regulates biosynthesis and signaling genes of auxin (Franklin et al. 2011; Sun et al. 2013), the central hormone for growth and differentiation (Bhatia et al. 2016; Ljung 2013; Schlereth et al. 2010) and ethylene (Sakuraba et al. 2014) which conditionally contributes both to particular defense reactions and tissue differentiation programs (Pre et al. 2008). Previous studies have shown that DELLAs can interact with PIFs, JAZ and MYC in a stress-dependent manner while JAZ interacts with MYC (Li et al. 2016). The increase in GA concentrations leads to the degradation of DELLAs (Murase et al. 2008), which triggers the dissociation of PIFs, JAZ, and MYCs. PIFs bind on their target promoters to activate the expression of genes for optimal GA-regulated growth and development (Marin-de la Rosa et al. 2015; Oh et al. 2012). Meanwhile, JAZs sequester MYC proteins to block their DNA binding and transcriptional activities to inhibit defense-related processes (Melotto et al. 2008). In this way, plants weight GA signals to promote growth over defense. However, when a plant perceives pathogens in their ambient environment, then JA levels increase, which, in turn, leads to the degradation of JAZs, which triggers the dissociation of DELLAs, PIFs, and MYCs. MYCs bind at their target promoters to activate the expression of genes required for JA-regulated defense. In the meanwhile, the sequestration and degradation of PIFs by DELLAs prevent the unfolding of GA-controlled growth programs

(Zheng et al. 2016). Thus, plants prioritize defense over growth by integrating JA signals (Huot et al. 2014; Smakowska et al. 2016).

The *INDETERMINATE DOMAIN (IDD)/BIRD* family of plant-specific transcription factors is highly conserved in both monocots and dicots (Colasanti et al. 2006). The high degree of sequence similarity amongst all IDD genes from *Arabidopsis*, *Oryza sativa* and *Zea mays* suggest that they are derived from a common ancestor (Colasanti et al. 2006). To date, at least 16 *IDD* genes have been found in the *Arabidopsis* genome (*AtIDD*), 15 in the *Oryza sativa* genome (*OsIDD*) and 21 in the *Zea mays* genome (*ZmIDD*).

In *Arabidopsis*, each of the *IDDs* contains a conserved N-terminal ID domain composed of four zinc fingers (ZFs) and a long sequence for protein interactions (Colasanti et al. 2006; Yoshida et al. 2014). The four ZFs can be subdivided into the C₂H₂ type ZF1 and ZF2, which are assigned to DNA interaction, and the C₂HC type ZF3 and ZF4, which are essential for the interaction of *IDD3/MAGPIE* and *IDD10/JACKDAW* with the transcription factors *SHORT-ROOT (SHR)* and *SCARECROW (SCR)* (Hirano et al. 2017). *IDDs* have been assigned to function in multiple developmental processes. In monocots, the maize *INDETERMINATE1 (ID1)* (Colasanti et al. 2006; Coneva et al. 2007) and the rice *Ehd2* (Matsubara et al. 2008) act as key regulators of flowering time. In *Arabidopsis*, *IDD8* regulates photoperiodic flowering by modulating sugar transport and metabolism (Jeong et al. 2015; Seo et al. 2011b). Furthermore, it was reported that *IDD3*, *IDD4*, *IDD6*, *IDD8*, and *IDD10* organize and are expressed in the root ground tissue thereby coordinating the differentiation of the endodermis initial stem cell niche in order to give rise to cortex and endodermis cells (Long et al. 2015; Moreno-Risueno et al. 2015; Yoshida et al. 2014). So far, it was reported that *IDD4*, *IDD5* are expressed in the root ground tissue of the basal meristem. *IDD1* promotes the transition to germination by regulating light and hormonal signaling during seed maturation (Feurtado et al. 2011). In a distant subclade of the *IDD* taxonomy *IDD14*, *IDD15* and *IDD16* mutually regulate lateral organ morphogenesis and gravitropism by supporting auxin biosynthesis and transport (Cui et al. 2013). Moreover, two splice variants of *IDD14* competitively form nonfunctional heterodimers which may

regulate starch metabolism (Seo et al. 2011a). Intriguingly, the IDD family members 2, 3, 4, 5, 9 and 10 serve as transcriptional scaffolds to enable transactivation activity of the gibberellin-inhibitor DELLA/RGA proteins in association with the transcriptional regulator SCARCROW-like 3 (SCL3) (Fukazawa et al. 2014; Yoshida et al. 2014; Yoshida and Ueguchi-Tanaka 2014).

In order to overcome the redundancy problem of TF family members and to generate multiple-gene-knockdown mutants, the chimeric repressor gene-silencing technology (CRES-T) was developed (Hiratsu et al. 2003). The CRES-T system has been widely used in functional studies in plants (Mahfouz et al. 2012; Oh et al. 2014; Volz et al. 2013), generating a chimeric repressor by the fusion of a transcription factor to a modified ERF-associated amphiphilic repression-(EAR)-motif as plant-specific repression domain (SRDX). The EAR motif was previously identified as a repressor domain in plants. The core sequence consists of only six amino acids (aa) which following the conserved order LXLXLX (X can be any aa) in various plant TFs. The dominant-SRDX peptide (LDLDLELRGFA) bears the conserved EAR-motif core sequence and was studied on the imposed repressive-dominate effect on transcriptional activators (Hiratsu et al. 2003). The fusion of this amphiphilic peptide is sufficient to turn a transcriptional activator into a strong repressor (Hiratsu et al. 2003; Hiratsu et al. 2004). The exact mode of action depends on the corresponding TF serving as DNA-binding module for the amphiphilic repressor and might involve the association of co-repressors. In general, the fusion protein dominantly suppresses the expression of target genes over the activity of the endogenous and functionally redundant transcription factors (Mitsuda and Ohme-Takagi 2009). The TF-SRDX fusion protein binds to the *cis*-regulatory elements in the promoter sequences of target genes. However, the transcriptional activity is suppressed, and the failure in target gene expression cannot be complemented by redundant proteins since corresponding promoter elements are occupied by the chimeric TF-SRDX protein. It was reported that transgenic plants expressing the SRDX fused to a transcriptional activator exhibit phenotypes likewise to those of the corresponding loss-of-function mutant but unlike to plants that overexpress the factor itself. In accordance with this finding, the fusion

of SRDX to ETHYLENE-INSENSITIVE 3 (EIN3) converts this central factor in the ethylene signaling cascade into a dominant repressor thereby resulting in an ethylene-insensitive phenotype corresponding to the allelic *ein3-1* mutant (Volz et al. 2013). The usage of CRES-T to studying IDD function in plant physiology has been demonstrated previously. A repressive effect of SRDX linked on IDD3 was shown in the regulation of GA signaling pathways (Yoshida et al. 2014). The *IDD3SRDX* plants exhibit a dwarf-like phenotype with smaller leaves and shorter roots and show a delay in flowering initiation thereby mimicking the phenotype of a GA-signaling and –deficient mutant.

In this study, we report on the expression of the chimeric *idd4SRDX* construct in *Arabidopsis*. We show that the expression globally alters the transcriptome composition towards an activated immune system and immune MAP kinase signaling pathways, while factors that promote growth and development are repressed. Furthermore, in accordance with hormonal measurements, pivotal factors that coordinate SA and JA metabolism are supported. *idd4SRDX* lines show an elevated resistance to *Pseudomonas syringae* and *Botrytis cinerea* suggesting that the canonical antagonistic nature of SA vs. JA in defense adaptation to hemibiotrophs and necrotrophs is bypassed by *IDD4SRDX* expression.

Results:

Dominant-negative IDD4 constitutively activates innate immunity without bacterial infection

We sought to find new players involved in the regulation of innate immunity and the coordination of defense hormone homeostasis. Therefore, we generated dominant-negative versions of transcription factors that were previously introduced as positive regulators of abiotic stress response and coordinators of plant development. One of the candidate genes that emerged from this study was IDD4. We took advantage of the chimeric repressor gene-silencing technology (CRES-T) and linked IDD4 with the ERF-

associated amphiphilic repression-(EAR)-motif (SRDX). The expression of *idd4SRDX* under the control of the *UBIQUITIN10* promoter in WT plants showed a striking phenotype in 19 out of 23 T1 plants, with a reduced fresh and dry weight and plant size (**Figure 1A, S1A-B**). Two representative lines *idd4SRDX1* and *idd4SRDX2* were selected to analyze the phenotype in more detail. Notably, non-elicited *idd4SRDX* lines form aberrant lesions on leaf surfaces, reminiscent of a hypersensitive response reaction to avoid pathogen spreading in leaf tissues (**Figure 1B**). In order to evaluate the lesion phenotype, 3,3'-diaminobenzidine (DAB) staining was performed on non-elicited plants to detect the reactive oxygen species H₂O₂ and lactophenol trypan blue (LTB) staining to examine the live/dead cell ratio in leaves. After DAB staining, untreated *idd4SRDX* lines showed elevated basal hydrogen-peroxide levels, especially in the lesion-forming areas (**Figure 1C**). This staining correlated with strong LTB staining (**Figure 1D**) which is indicative of cell death and lesion formation. ROS production and release are among the first immune responses to the sensing of PAMPs and are a benchmark for the strength of the plant defense reactions. Therefore, we evaluated the ROS burst after PAMP perception. The *idd4SRDX* lines showed already much higher ROS levels than WT at 1 min after flg22 application, and the levels of ROS release could not be increased further (**Figure 1H, S1D**). In this regard, the ROS burst 10 min after the application was still two-fold higher in the two independent *idd4SRDX* lines, but lower than at the very beginning of the measurements.

Another plant strategy to avoid pathogen infection is the deposition of callose at the respective infection sites. Without flg22-treatment and under sterile conditions on sugar-free MS medium, *idd4SRDX* lines exhibited spontaneous callose depositions on the leaf surfaces (**Figure 1E, S1C**). Moreover, a number of innate immunity and PTI-marker genes such as *FLG22-INDUCED RECEPTOR-LIKE KINASE 1 (FRK1)* (Asai et al. 2002), *WRKY33* (Mao et al. 2011), *WRKY22* (Asai et al. 2002) and *PATHOGENICITY-RELATED FACTORS PR2* (Petersen et al. 2000) were consistently expressed at higher levels in the *idd4SRDX* lines without pathogen infection (**Figure 1J, K**). The expression of these genes demonstrates an auto-immune activated

transcriptome in *idd4SRDX* plants. Stomata are entry points of pathogens and are under tight control mediated by the stomatal immunity response (Melotto et al. 2006). Stomatal closure in response to pathogens is mediated by salicylic acid (SA) (Montillet and Hirt 2013) and is counteracted by the bacterial toxin coronatine that provokes the reopening of the stomata in order to facilitate the access of the bacterial intruders (Abulfaraj et al. 2018; Zheng et al. 2012). The question of the stomatal response in *idd4SRDX* lines is an interesting aspect that might affect the bacterial invasion. Therefore, we analyzed the stomatal aperture at 1h and 4h after bacterial infection by using the hemibiotrophic pathogen *PstDC3000*. Interestingly, in the absence of infection, the stomatal aperture in *idd4SRDX* lines was already reduced when compared to WT plants (**Figure 1F-G**). However, 1 hr after pathogen perception, the stomata in *idd4SRDX* and WT are consistently closed as part of the stomata immune response. Intriguingly, 4 hrs after infection, the bacterial strategy to reopen the stomata by the release of the polyketide toxin coronatine does not efficiently reopen the stomata in *idd4SRDX* when compared to WT. The compromised reopening process might help to prevent effective colonization of the *idd4SRDX* plants.

To evaluate whether the MAP kinase signaling cascades are affected in the *idd4SRDX* lines, we analyzed their activation after flg22-application in comparison to WT and *GFP:IDD4* lines (*IDD4ox*). The expression of the *GFP:IDD4* fusion construct is driven by the *UBIQUITIN10* promoter. MAPK activation was assessed by pTpY antibody-based immunoprecipitation that targets the phosphorylated MAPKs. Before PAMP-perception, MPK3 and 6 activations (at 0 min) were below the detection levels in WT, *idd4SRDX* and *IDD4ox* lines. However, already 5min after flg22-treatment in *idd4SRDX*, MPK3 and 6 were much more strongly activated than in WT and *IDD4ox* plants (**Figure 1I**). The distinct activation of MPK3 and 6 in the *idd4SRDX* line suggests a function of IDD4 in the regulation of the flg22-induced MAP kinase signaling cascade. Intriguingly, our findings show that particular defense-reactions in *idd4SRDX* such as the MAP kinase-activation and the stomatal immune response (**Fig.1G**) are exclusively triggered by PAMP/pathogen-perception in *idd4SRDX* plants and thus cannot be considered as an auto-immune phenotype

per se. All in all, the high ROS levels together with the lesion phenotype, the up-regulation of PTI marker genes, the callose deposition and the reduced stomatal aperture, indicate a constitutive activation of defense responses in *idd4SRDX* and suggest a role of IDD4 and redundant family members as inhibitors of defense in the absence of a pathogenic challenge.

Salicylic acid levels are significantly elevated in *idd4SRDX*

Innate immunity of plants is controlled by a complicated hormonal regulatory network involving the phytohormones SA, jasmonic acid (JA) and ethylene. Depending on the biotic challenge, synergistic and antagonistic interactions between these stress hormones coordinate an adequate immune response. First, we analyzed the free SA levels in 14 day-old plants grown on sugar-free MS medium. *idd4SRDX* lines 1 and 2 showed a massive increase of SA in comparison to WT (**Figure 2A**). To evaluate the high SA levels at the molecular level, we analyzed the expression of genes involved in SA-metabolism and signaling. The level of *ICS1* (Wildermuth et al. 2001) which encodes an enzyme catalyzing the rate-limiting step in the SA biosynthesis is strikingly increased as well as its transcriptional activator *SARD1* (Sun et al. 2015; Wang et al. 2011) (**Figure 2B**). Furthermore, *WRKY28* (**Figure 2B**) and *WRKY46* (**Figure 2C**) that mutually contribute to SA biosynthesis (van Verk et al. 2011), and *WRKY38* (**Figure 2C**) (Xie et al. 2010), involved in SA perception, are up-regulated. The high SA levels in *idd4SRDX* lines are also accompanied by the differential expression of genes involved in the SA catabolism. One of the SA catabolism genes is SA 3-hydroxylase (*S3H*). *S3H* is up-regulated in *idd4SRDX* lines (**Figure 2B**) and readily inducible after 6h of SA treatment with similar expressions patterns like *PR1* (Zhang et al. 2013). The *S3H* enzyme converts SA to 2,3-dihydroxybenzoic acid (2,3-DHBA) *in vivo* and to 2,5-DHBA *in vitro*, and both compounds are considered as precursors of SA's major storage form 2,3-DHBA sugar conjugates. The up-regulation of *S3H* corresponds to an accumulation of 2,3-DHBA sugar conjugates, which was recently shown to be enriched in infected or aged *Arabidopsis* leaves (Bartsch et al. 2010). The elevated SA levels in *idd4SRDX* lines

demonstrate a function of IDD4 in the regulation of SA biosynthesis to avoid SA-dependent defense response activation in the absence of a pathogen.

Jasmonic acid homeostasis is elevated in *idd4SRDX*

In addition to SA, we measured the concentration of JA in the two independent *idd4SRDX* lines. JA was shown to coordinate immunity against necrotrophic pathogens and herbivory. We detected 10-15 fold higher levels of JA (**Figure 2D**) in both *idd4SRDX* lines indicating a function of IDD4 in the regulation of JA metabolism. The increased basal level of JA is in accordance with the up-regulation of the JA biosynthesis gene *ACO2* (**Figure 2E**). Furthermore, the JA response genes *PDF1.2*, *ERF1* and *ORA59* (**Figure 2E**) are more highly expressed in *idd4SRDX* than WT. Interestingly, the expression of these three genes is synergistically activated by JA and ethylene (Pre et al. 2008) which might also indicate altered ethylene homeostasis in *idd4SRDX*.

Dominant-negative *IDD4* lines show enhanced resistance against *Pst DC3000* and *Botrytis cinerea*

To study the impact of a dominant-negative IDD4 version on plant defense, at first, *idd4SRDX* lines were treated with the virulent pathogen *Pst DC3000* and *Pst DC3000 hrcC-*. The *Pst hrcC-* strain is impaired in virulence due to its incapacity to deliver any bacterial effector owing to a non-functional type III-secretion system. The bacterial growth for both strains was monitored after spray-infection in five-week-old plants grown under short-day conditions. 72 hrs after plant infection, the proliferation levels of the bacteria were approximately 1.5-orders of magnitude reduced in the *idd4SRDX* lines compared to WT (**Figure 3A, B**). The results demonstrate that the ectopic expression of *idd4SRDX* in stable transgenic *Arabidopsis* lines increases the resistance against *Pst DC3000*, implying a function of IDD4 and closely related-family members in basal immunity.

The increased levels of JA in *idd4SRDX* plants also suggested that defense responses to necrotrophic pathogens might differ from WT. Therefore, we challenged the *idd4SRDX* lines with the necrotrophic

fungus *Botrytis cinerea* and analyzed lesion formation on the leaf surface at 30 hpi. Lesion formation around the fungal infection sites was significantly reduced in both *idd4SRDX* lines when compared to WT (**Figure 3C**). These findings indicate that the expression of *idd4SRDX* also increases resistance against necrotrophs. We reason that IDD4 and closely related family members influence immunity against necrotrophs via its regulation of JA metabolism.

Reduction of SA levels restores WT-like defense response in *idd4SRDX*

To test whether a reduction of the elevated free SA levels could suppress the phenotype of the *idd4SRDX* lines, we expressed the catabolic enzyme *SAGT1* in the *idd4SRDX* background. *SAGT1* converts SA to SA-2-l- β -D-glucoside (SAG) and SA-glucose-ester (SGE) that are deemed as biologically inactive storage forms of SA (Song et al. 2009). Overexpression of *SAGT1* (*SAGT1ox*) in the *idd4SRDX* lines partially reconstituted the WT phenotype (**Figure 3E, S2A**) and restored susceptibility to *Pst DC3000 hrcC*- at WT levels, 72 hours after *Pst hrcC*- infection (**Figure 3D**). Furthermore, the level of reactive oxygen species H_2O_2 was significantly diminished in *idd4SRDX/SAGT1ox* compared to *idd4SRDX* after DAB staining evaluation (**Figure 3E, S2B**), implying a reduced SA-mediated H_2O_2 accumulation. To assure that the restored phenotype in *idd4SRDX/SAGT1ox* is not provoked by the silencing of the *IDD4SRDX* transgene, we confirmed its expression by RT-PCR (**Figure S2C**). Finally, to discerning whether the compromised pathogen resistance of the *idd4SRDX/SAGT1ox* was caused by changes in the SA hormonal levels, the concentration of free SA was determined by LC-MS/MS. Unlike the *idd4SRDX* line, which possessed strongly increased SA levels, the amounts of free SA in the *idd4SRDX/SAGT1ox* line were as low as in WT (**Figure 3F**). These results demonstrate the biological significance of the IDD4-mediated transcriptional control of SA biosynthesis genes in plant immunity and proper plant growth.

Transcriptome reprogramming by *idd4SRDX* towards defense adaptation

To identify differentially regulated genes in *idd4SRDX*, we performed RNA-SEQ analysis of 14 day-old independent transgenic *idd4SRDX* lines. To determine whether in *idd4SRDX* the whole transcriptome or a distinct subset of genes is affected, we calculated the Pearson correlation of differentially expressed genes. Furthermore, we determined the correlation coefficient for WT plants treated by the PAMP flg22. A close to a linear correlation coefficient of WT versus *idd4SRDX* (0.958), and WT versus WT (flg22) (0.927) was obtained after taking into account the expression profiles for all transcripts (FPKM) (**Figure 4A-B**). The strict correlation suggests that *idd4SRDX* does not affect general gene expression, but rather influences subsets of genes of particular biological processes. At a stringency of $p \leq 0.01$, 2362 differentially regulated genes (DEGs) (**Table S2**) could be identified that show a $\log_2$ -fold change from 0.4 to 5.07 of positively regulated genes and from -3.00 to -0.39 of negatively regulated genes. Among these 2362 genes, 1520 genes are up- and 842 genes are down-regulated (**Figure 4C**). To categorize DEGs in functional modules, we determined the gene ontology (GO) terms by using the AgriGO (Tian et al. 2017) platform. The up-regulated genes can be categorized in GO terms for innate immune response, salicylic acid, and jasmonic acid metabolic process. Furthermore, the PTI-receptor components *FLS2*, *BIR1*, *SOBIR1*, and *RPL23* are up-regulated as the signaling factors *MPK3*, *MPK11* and *MAP3K16* (**Table S5A**) (Bigeard et al. 2015; Meng and Zhang 2013). The transcriptome composition of the up-regulated genes in *idd4SRDX* lines showed a high overlap of 23.6% with the genome-wide up-regulated genes of 14 day-old WT plants that were treated with flg22 for 1h (2553 genes, $p \leq 0.05$, **Figure 4D**, **Table S4**). Among the overlapping set of up-regulated genes (602 genes), the most significantly enriched GO categories belong to innate immune response, SA-, JA- and ethylene biosynthetic process (**Figure 4F**, **Table S3**). If we align all differentially regulated genes in *idd4SRDX* (2362 genes) and flg22-treated WT plants (4020 genes, $p \leq 0.05$), then an overlap of 41.3% emerged. Regarding the hypersensitive response phenotype observed in the *idd4SRDX* lines, the up-regulated genes can be grouped into modules for oxygen and reactive oxygen species metabolic process

and plant-type hypersensitive response (**Figure 4E, Table S1**). Furthermore, the up-regulated genes can be grouped in GO terms for SA-, JA- and ethylene biosynthetic process corresponding to the hormonal measurements. Taken together, this comparison illustrates the reprogramming of the transcriptome in the *idd4SRDX* lines towards a plant that is challenged by flg22 and suggest IDD4 as a key mediator of the immune response in *Arabidopsis*.

Regulation of SA metabolism and signaling

The comparison of the up-regulated genes of *idd4SRDX* versus WT emphasizes GO terms particularly for hormonal control and defense response (**Figure 4E-F, Table S1**). Notably, pivotal factors involved in SA biosynthesis and signaling are up-regulated in *idd4SRDX* lines (**Table S5B-C**). The level of the key SA biosynthesis gene *ICS1* that catalyzes the conversion of chorismate to isochorismate is strikingly increased in the *RNA*-SEQ analysis (**Table S5B, Figure 2B**). Moreover, the expressions of *SARD1*, *WRKY28* and *WRKY46* (**Table S5B, Figure 2B-C**) that cooperatively mediate the expression of *ICS1* are up-regulated in the *idd4SRDX* lines. The SA receptor components and *NPR1*-interaction factors *NIMIN1/2/3* and *NPR3* are also among the up-regulated genes (**Table S5B**) as well as *PKS5*, *WRKY38* (**Figure 2C**) and the *PATHOGENICITY-RELATED FACTORS* *PR2* and *PR5* whose expression directly depends on SA-action (Seo et al. 2008) (**Table S5A-B, Figure 1J**). Furthermore, the PAMP-triggered SA-dependent immunity marker *FRK1* is highly expressed (**Table S5A, Figure 1I**). All in all, the up-regulation of SA metabolic and signaling genes in the *idd4SRDX* lines confirms the role of *IDD4* in mediating SA-dependent innate immunity.

Regulation of jasmonic acid and ethylene metabolism and signaling

In addition to up-regulated SA-biosynthesis genes, the *RNA*-SEQ-based transcriptome study of *idd4SRDX* lines revealed the elevated expression of genes that contribute to JA- and ethylene metabolism. Intriguingly, the elevated JA levels are accompanied by GO terms highlighting genes involved in JA biosynthesis process and JA -mediated signaling pathway (**Figure 4E, Table S1**).

We detected stronger expression of *PR3* and *PR4* whose expression directly depends on JA (Seo et al. 2008) and of *ALLENE OXIDE CYCLASE 1* (*AOC1*) and *AOC2* (**Table S5D**) which code for enzymes, whose catalyze the rate-limiting step in octadecanoid and JA-biosynthesis (Stenzel et al. 2003). Similarly, the genes of the chloroplast-located LIPOXYGENASE 1 (*LOX1*), *LOX3*, *LOX4*, and *LOX6* are higher expressed than in WT and are required for wound-induced JA accumulation (**Table S5D**). Interestingly, *ERF1*, *ORA59*, and *PDF1.2*, whose expression is synergistically promoted by JA and ethylene, are up-regulated (**Table S5D-E**) thereby suggesting increased levels of ethylene in *idd4SRDX* (Pre et al. 2008). In this context, the conversion of S-adenosyl-L-methionine to 1-aminocyclopropane-1-carboxylate (ACC) is the first committed and generally rate-limiting step in ethylene biosynthesis and is catalyzed by the enzyme ACC synthase (ACS). We found the ACS family members *ACS4*, *5*, *8* and *11* were up-regulated in the *idd4SRDX* lines. Furthermore, factors that contribute to ethylene signaling like the *ETHYLENE RESPONSE SENSOR 2* (*ERS2*) and several further *ETHYLENE-RESPONSE FACTORS* show an increased expression (**Table S5E**). Taken together, these results show that apart of SA and JA, genes are expressed that promote ethylene biosynthesis thereby suggesting an elevated level of this biotic stress hormone in *idd4RDX*.

Functional clusters contributing to plant growth and development are compromised in *idd4SRDX* plants

To decide whether the differentially regulated genes in *idd4SRDX* that can be considered as major IDD4 targets, we took into account genome-wide binding studies of IDD4 of non-elicited plants (O'Malley et al. 2016). The association of IDD4 to particular chromatin regions was gained after DNA affinity purification sequencing (DAP-SEQ). In that study, the *Arabidopsis* cistrome was defined to unveil the binding motifs and peaks for 529 TFs. Moreover, a genome-wide overview of primary target genes was provided for these TFs (O'Malley et al. 2016). One of the analyzed TFs in this study was IDD4 that we also confirmed to be localized to the nucleus (Moreno-Risueno et al. 2015) in *Arabidopsis* (**Figure 5A**). The most frequent binding site of IDD4 was determined to carry the core-sequence A/C/GGACAA (nucleotides 8-13) (**Figure**

5B), also called *ID1-cis* motif. Based on the analysis of the distribution of significant IDD4 binding sites (blue arrows) containing the *ID1-cis* motif in the proximate sequence upstream of the transcriptional start sequence (TSS), 444 repressed genes in *idd4SRDX* plants could be assigned as primary downstream targets (**Table S6**). To arrange these genes in functional clusters, we used the STRING 10 database. This web resource critically evaluates and integrates information from numerous sources, including experimental repositories, computational prediction methods and public text collections, consequently acting as a meta-database that plots all protein-protein interactions, including direct (physical) as well as indirect (functional) associations (Jensen et al. 2009; Szklarczyk et al. 2015). By using STRING 10, we identified functional groups among others for Gibberellin-signaling/-response, Stomatal-patterning/-aperture and Auxin-transport/-signaling (**Figure 5I, 5N, 6E**). Major IDD4 target genes such as *GA INSENSITIVE DWARF1B (GID1B)* (Ueguchi-Tanaka et al. 2005), *SCARECROW-LIKE 3 (SCL3)* (Zhang et al. 2011), *GIBBERELLIN 3-OXIDASE (GA3OX) 1* and *HOMEODOMAIN GLABRA 1 (ATH1)* (Garcia-Martinez and Gil 2001) that promote gibberellin signaling and biosynthesis are reduced in their expression (**Figure 5C-I**). Furthermore, *GID1B* expression is compromised in *idd4SRDX* before and after flg22-treatment and IDD4 binds to several regions upstream of the transcriptional start sequence (TSS), in which multiple *ID1-cis* motifs can be found. Likewise, the binding of IDD4 to the *ID1-cis* motif-containing sequences upstream of the TSS could be confirmed for *GA3OX1*, *ATH1*, and *SCL3* (**Figure 5C-F**). The downregulation of these genes in *idd4SRDX* indicates IDD4 as a positive regulator of their expression and function. By contrast, we found genes that promote GA catabolism such as *GIBBERELLIN 2-OXIDASE (GA2OX) 2*, *GA2OX6*, *GA2OX8* (Singh et al. 2002) and those that repress GA-response such as *REPRESSOR OF GA-like (RGL) 1* and *RGL3* (Daviere and Achard 2013) as being more highly expressed in *idd4SRDX* than in WT (**Figure 5H**). These results suggest that IDD4 functions in promoting GA-biosynthesis and -signaling.

Furthermore, we identified several genes that can be clustered to function in stomatal patterning and aperture. In this context, we identified *STOMAGEN* (Pillitteri et al. 2007) and *SPEECHLESS* (Lampard et al.

2008) that control stomata development, as two major IDD4 binding targets in the DAP-SEQ as being down-regulated in *idd4SRDX* (**Figure 5K-N**). In addition, further genes such as *STOMATAL DENSITY AND DISTRIBUTION 1 (SDD1)* (Yoo et al. 2010), and *MUTE*, a basic helix-loop-helix (bHLH) protein that controls meristemoid differentiation during stomatal development (Pillitteri et al. 2007) are compromised in their expression in *idd4SRDX*. In **Figure 1G**, we showed that the stomatal aperture in *idd4SRDX* is constitutively diminished and the bacterial-triggered reopening process is impaired. In this regard, we found several differentially regulated genes that have been shown to determine stomatal aperture such as *POTASSIUM CHANNEL IN ARABIDOPSIS THALIANA 2 (KAT2)*, that is critical for the stomatal opening induced by blue light and circadian clock (Zhang et al. 2016), *EMBRYO SAC DEVELOPMENT ARREST 39 (EDA39)* (Zhou et al. 2012), *NITRATE TRANSPORTER 1.1 (NRT1.1)* (Guo et al. 2003), the protein kinase phosphatase *PP2C5* (Brock et al. 2010) and *SYP121*, that encodes a trafficking protein (Eisenach et al. 2012) (**Figure 5M**). Altogether, these findings suggest that IDD4 contributes directly to the regulation of genes that govern stomatal development. In addition, the transcriptional deregulation of genes involved in stomatal patterning, movement and responsiveness to biotic stress responses implies a genetic interaction of IDD4 with these factors.

Moreover, the plant hormone auxin is essential for coordinating plant growth and differentiation by controlling cell division/expansion/differentiation, embryogenesis, flowering and lateral root formation (Ljung 2013; Schlereth et al. 2010). *idd4SRDX* plants are affected in growth and immune-repression that might be caused by the deregulation of genes that coordinate auxin-transport and signaling. Indeed, we identified several genes that were shown to be directly bound by IDD4 in the DAP-SEQ study (O'Malley et al. 2016). *PIN5* and *PIN-LIKES 3* are down-regulated in *idd4SRDX* and identified as direct targets of IDD4 (**Figure 6A-C**). Both genes code for proteins that belong to the auxin efflux carrier family that are involved in auxin transport from the cytosol to the lumen of the endoplasmatic reticulum and mediate the intracellular auxin homeostasis (Barbez et al. 2012; Mravec et al. 2009). Furthermore, the DNA-binding

study and *idd4SRDX* RNA-SEQ data suggest *INDOLE-3-ACETIC ACID INDUCIBLE 24/ MONOPTEROS (IAA24)* as a direct target gene of IDD4 (**Figure 6A, D**). The biological function of *IAA24* has been intensively studied, and it was shown to be essential for embryo-axis formation, cell polarity, pattern phyllotaxis and vascular development (Bhatia et al. 2016; Schlereth et al. 2010). In addition, we identified several genes that are up-regulated in *idd4SRDX* and contribute to auxin-metabolism, such as *WES* and *DFL1*. Moreover, *ARF19*, *IAA19*, and *PIN19* involved in auxin signaling and transport show an elevated expression (**Figure 6A**). The cluster analysis revealed a direct relationship between those genes and suggests IDD4 as a general up-stream regulator of these deregulated targets (**Figure 6E**). Our findings show that several genes that contribute to auxin biosynthesis and transport are differentially expressed thereby suggesting that the auxin-signaling system and associated developmental processes are compromised in *idd4SRDX* plants

Immune-modulators are affected in their expression

The auto-immune phenotype in *idd4SRDX* might be caused by the transcriptional repression of factors that suppress the plant defense system. Indeed, we identified factors that impact the activation of MAP kinase-signaling cascade while others compromise immunity upon target gene expression. In this context, we found the *PP2C-type Phosphatase AP2C1*, that dephosphorylates and consequently deactivates MPK3, 4 and 6 (Brock et al. 2010; Schweighofer et al. 2007) as being downregulated in the *idd4SRDX* lines with a $\log_2(\text{FC})$ of -0.45 (**Figure 5M**). By qPCR, we confirmed its downregulation in *idd4SRDX* and could show an up-regulation in the *IDD4ox* lines (**Figure 6F**). Interestingly, as shown in **Figure 1H**, the activation of MPK3 and MPK6 in *idd4SRDX* lines after flg22 treatment was enhanced after flg22-application when compared to WT and *IDD4ox*. This finding suggests that the reduced expression of *AP2C1* results in the attenuated dephosphorylation of MPK3 and MPK6 in *idd4SRDX*.

Furthermore, WRKY27 was reported to contribute to disease symptom development in response to the bacterial wilt pathogen *Ralstonia solanacearum* (Mukhtar et al. 2008). The *wrky27-1* plants showed

delayed disease symptom generation and did not show *PR* marker gene expression. In our RNA-SEQ study, we found *WRKY27* as being differentially expressed in the *idd4SRDX* lines (**Figure 5M**). Interestingly, as shown in **Figure 5J**, IDD4 binds to the promoter region of *WRKY27* and a specific area in the gene body with an accumulation of the IDD4 binding motifs AGACAA and GGACAA thereby suggesting *WRKY27* as a direct target of IDD4. The differential expression of *WRKY27* might contribute to the enhanced disease symptoms and the auto-immune phenotype of *idd4SRDX* plants.

Stress-related deadenylation of mRNA is a control mechanism in response to biotic and abiotic stresses (Gutierrez et al. 2002). In yeast, the CCR4 associated factor 1 (CAF1) mediates deadenylation as part of the well-characterized CCR4-NOT complex (Denis and Chen 2003). The *Arabidopsis* homologs AtCAF1a of the yeast *CAF1* gene complement the yeast *caf1* mutant and exhibit deadenylation activity *in vivo* and a transcriptional induction by stress hormones (Liang et al. 2009; Walley et al. 2010). Furthermore, AtCAF1a mutant plants are compromised in resistance to *Pst DC3000* (Liang et al. 2009). The genome-wide binding study showed IDD4-binding close to the *CAF1* gene-body and implies *CAF1* as a direct target (**Figure 6H**) (O'Malley et al. 2016). Furthermore, *CAF1a* was differentially regulated in the *idd4SRDX* lines, while the expression in *IDD4ox* lines was indistinguishable from WT (**Figure 5M, 6G**) suggesting a transcriptional regulation of *CAF1a* by IDD4.

Taken together, we showed that genes and associated functional clusters which promote growth and development are compromised in *idd4SRDX* plants. However, activators of immune-response and associated phytohormone pathways are constitutively up-regulated. These data suggest that IDD4 and closely related family members are repressors of plant defense-processes including the biosynthesis of SA and JA.

Discussion

In the present study, we showed that the expression of an *IDD4-amphiphilic repressor* construct in *Arabidopsis* activates the metabolism of SA, JA, and ethylene, in accordance with hormonal measurements and marker gene expression. These key-biotic stress hormones coordinate pivotal and distinct response pathways to cope with different sorts of pathogenic challenges.

Regulation of SA-biosynthesis

The biosynthesis of SA is induced upon challenge by various pathogens to establish systemic acquired resistance (SAR) and local resistance. A variety of genes essential for SA biosynthesis is up-regulated in *idd4SRDX* lines. The key biosynthesis gene *ICS1* and its main upstream regulator *SARD1*, *WRKY28* and *WRKY46*, as well as *EDS1* and *PAD4* are highly expressed. Notably, *CCA1 HIKING EXPEDITION (CHE)* that regulates systemic induction of *ICS1* by promoting *SARD1* (Zheng et al. 2015) is enriched (**Table S5B**). To shed light on factors that control systemic immunity and PAMP-triggered immunity, the up-regulation of *SARD1* in the *idd4SRDX* lines could indeed play a critical role. Recently *SARD1* and *CBP60g* were shown to be master regulators of the plant immune response that bind to the promoter region of several defense regulators that function upstream of SA biosynthesis (Sun et al. 2015). In this context, *SARD1* has been demonstrated to target and exert transcriptional control of *EDS1*, *PAD4*, *ADR1-L1* and *ADR1-L2* factors (**Table S5B**) that promote SA biosynthesis during pathogen infection. Negative regulators of plant immunity whose expression depends on *SARD1*-mediated induction comprise *NUDT6*, *BAP1*, and *WRKY40* (**Table S2**) (Sun et al. 2015). To balance high SA levels, *S3H* is up-regulated and catabolizes free SA in an inactive storage form (Zhang et al. 2013). These findings demonstrate that the core network regulating SA biosynthesis and catabolism is subject to IDD regulation. Loss of IDD function in *idd4SRDX* provokes the pathogen-independent activation and accumulation of SA as seen in *idd4SRDX* lines.

The interplay of the biotic-stress hormones

SA acts antagonistically to JA and ethylene action and biosynthesis, which work synergistically to set up adaptation to wounding and the infection by necrotrophic pathogens. Elevated SA levels are a core element in innate immunity in response to biotrophic and hemibiotrophic phytopathogens. The SA-mediated hypersensitive response is a critical instrument in the defense response to avoid spreading of the pathogen in the host tissue and establishing a long-term response to the pathogen. Surprisingly, the SA and JA levels are consistently increased in the *idd4SRDX* lines which concomitantly show increased resistance against *PstDC3000* and *Botrytis cinerea*. Moreover, marker gene analysis of *ERF1*, *ORA59*, *PDF1.2* indicates that the ethylene homeostasis is as well promoted. The increased levels of these three biotic stress hormones refer to IDD function as a general repressor of their hormonal biosynthesis pathways. Furthermore, elevated resistance against *PstDC3000* and *Botrytis cinerea* suggest that the canonical antagonistic nature of SA vs JA in defense adaptation (Robert-Seilaniantz et al. 2011) to hemi-/biotrophs and necrotrophs might be abrogated by the expression of *idd4SRDX*. Reminiscent of our findings, Lui and colleagues discovered a non-canonical pathway showing the induction of JA-responsive genes and *de novo* JA biosynthesis in response to SA accumulation (Liu et al. 2016) which indicates that JA contributes positively to ETI (Tsuda and Katagiri 2010). Moreover, it was reported that the JA rise is mediated by the SA receptors NPR3 and NPR4 which interact and degrade the JA signaling repressors JAZs in a SA-dependent manner. In this way, JA signaling regulates positively the early stage of RPS2-mediated ETI and enables the plant to take advantage of the hypersensitive response (HR) to fight against biotrophic pathogens without giving the plant more susceptibility against necrotrophic pathogen growth in tissues undergoing HR cell death. In this context, it was shown that the JA signaling pathway is activated in the surrounding cells adjacent to the SA accumulating cells around the infection site after avirulent *Pseudomonas* infection (Betsuyaku et al. 2018; Spoel et al. 2007). Recent findings suggest that the degree of synergistic interaction of SA and JA in *Arabidopsis* depends further on their concentrations and the sort

of pathogenic intruder. Interestingly, the transient application of low concentrations of JA and SA provoked the expression of a wide set of common target genes, whereas SA-JA antagonism was observed at prolonged treatments and higher concentrations (Mur et al. 2006). Furthermore, JA signaling promotes resistance to the biotrophic pathogen *Xanthomonas oryzae* (Yamada et al. 2012) and, unlike the virulent *PstDC3000* strain, the avirulent *PstDC3000* strains *avrRpt2* and *avrRpm1* do not suppress JA-dependent defense which concurrently supports the resistance against *Alternaria brassicicola* (Spoel et al. 2007).

IDDs and DELLAs - two sides of the same medal

IDDs act as a DNA-scaffold protein of the transcriptional co-activators DELLAs (Fukazawa et al. 2014; Yoshida and Ueguchi-Tanaka 2014), which cannot bind directly to DNA, due to the lack of a DNA-binding domain. GA-mediated degradation of DELLA is delayed by flg22-treatment which suggests a function of IDD4 to stabilize DELLAs under particular stress conditions (Navarro et al. 2008). GA stimulates cell division/expansion in response to light or darkness (photomorphogenesis and skotomorphogenesis) and regulates flower initiation and its development and is essential for male and female fertility. The DELLA quadruple loss-of-function mutant *gai-t6 rga-t2 rgl1-1 rgl2-1* displayed a reduction to *Pst DC3000* infection accompanied by an enrichment of free SA (Navarro et al. 2008). In accordance, *idd4SRDX* lines are also significantly less susceptible to *Pst DC3000* and accumulate free SA. Together, these findings suggest that IDD4 and DELLAs synergistically repress plant responses to bacterial infection and suppress SA-biosynthesis. The *idd4SRDX* transcriptome revealed elevated expression of the GA catabolic genes *GIBBERELLIN 2-OXIDASE 2*, 6 and 8 that converts the active form of GA into an inactive version and of *GIBBERELLIC ACID METHYLTRANSFERASE 2* (*GAMT2*), a repressor of Gibberellin production (**Figure 5H**). Additionally, *RGL1* and *RGL3*, negative regulators of GA responses, are up-regulated. On the other hand, *GID1B*, encoding a gibberellin (GA) receptor and also *GIBBERELLIN 3-OXIDASE 1* (*GA3OX1*) involved in the production of bioactive GA are down-regulated and considered as direct target genes of IDD4. Likewise, *SCL3*, a GA response factor, whose induction depends on the synergistic function of IDD4 and DELLA/RGA,

is compromised in its expression (**Figure 5H**). The co-repressor SCL3 competes with DELLAs for the binding to IDD proteins that eventually results in the antagonistic regulation of GA signaling pathways (Yoshida and Ueguchi-Tanaka 2014). The deregulation of these genes in *idd4SRDX*, by taking into account their status as direct target genes, suggest IDD4 as an activator of GA metabolism and function. Moreover, the IDD4 family member GAI-ASSOCIATED FACTOR1/IDD2 regulates GA homeostasis and signaling in *Arabidopsis* by exerting transcriptional control on genes which are part of GA feedback regulation. IDD2 forms a complex with DELLA/GAI and TOPLESS RELATED and GA converts the trimeric complex from a transcriptional activator to a repressor upon the degradation of DELLA (Fukazawa et al. 2014). The IDD2/GAI complex also binds to the 5'-sequence of the TSS of *GA3ox2*, *GID1b* and *RGL1* and exerts transcriptional control as shown in trans-activation assays. This example illustrates the redundant function of IDD members in the GA-feedback regulation. Interestingly, besides the role of DELLAs as co-activators, they can also inhibit transcriptional activation of, e.g., PIFs in the low-light response by preventing their DNA binding or with JAZ to promote JA-signaling. These two functions of DELLA working mechanisms can be considered as a key element in GA-signaling. Low GA levels suppress the expression of genes involved in growth and development by the titration of transcriptional activators (PIFs) and concomitantly promote genes dedicated to GA-feedback regulation (Fukazawa et al. 2014; Li et al. 2016). It is thus conceivable that IDD4 and other IDD family members redundantly activate GA biosynthesis after PAMP perception, to promote plant growth and to reset the plant immune system.

Future perspectives

The *IDD* gene family is highly conserved in the plant kingdom, and closely related members were identified in monocots and dicots. Coelho and colleagues (Coelho et al. 2018) explored the phylogenetic relationships among grass *IDDs* for which reference genome sequences were available. In this way, *IDD* sequences were identified in *Setaria viridis*, *Brachypodium distachyon*, *Oropetium thomaeum*, *Sorghum bicolor* and as previously described *Oryza sativa* and *Zea mays* (Colasanti et al. 2006). The *IDD* sequences were identified

by their conserved features like the N-terminal nuclear localization signal and most importantly the four zinc finger-containing ID1 domains. The ID1 domain determines the DNA-binding to conserved *cis*-regulatory sequences and the high degree of conservation suggests that, across the plant kingdom, *IDDs* control similar biological processes by exerting transcriptional control on primary target genes (Colasanti et al. 2006). Notably, *IDD* proteins occupy multiple functions by integrating metabolism with development and are proposed as good targets for genetic modification through either classical breeding or genome editing/ engineering technologies (Coelho et al. 2018). In this regard, recent publications describe *IDD* function to regulate C_4 Kranz anatomy in maize and imply them in engineering C_4 traits into C_3 crops (Coelho et al. 2018; Wang et al. 2014). The directive autoimmune phenotype and the consistently increased hormonal levels provoked by the expression of the chimeric *idd4SRDX* construct bear the capacity for the generation of genetically improved crop plant. By the use of an inducible system for the *idd4SRDX* expression, the transgene could be conditionally activated instantly after pathogen attack to induce a stronger plant defense response in engineered crops. Alternatively, we suspect that a constant, slight expression of *idd4SRDX* might have a sustainable, supportive influence on the plant health and immunity and future research on this potential resource is warranted. Given the unambiguous resistance phenotype in *idd4SRDX* lines, this chimeric construct may serve as a valuable tool for the genetic activation of a wide collection of defense-associated traits to cope with harsh biotic stress condition in agricultural farming.

Materials and methods

Plant Material and Growth Conditions

Experiments were performed by the use of *Arabidopsis thaliana* (Columbia accession) grown on soil in plant growth chambers (Percival Scientific) under short-day conditions (8h light/ 16 h dark) and long-day condition (16h light/8h dark) at 22°C, respectively.

Plasmid construction

Whole seedling Arabidopsis cDNA library was used to amplify the coding sequence (CDS) of *SAGT1* and *IDD4* by using oligonucleotides that add the *SRDX* coding sequence on the 3'-terminus of the *IDD4* fragment. Subsequently, the entry clone was generated by introducing the CDS either in the *pENTR*-vector or *pCR[®]8/GW/TOPO[®]* using the *pENTR[™] Directional TOPO[®] Cloning Kit* by INVITROGEN according to manufactory's instruction. Subsequently, *IDD4* and *idd4SRDX* were cloned into destination vectors expressing the cDNA under *UBIQUITIN10* promoter (Grefen et al. 2010). *SAGT1* was cloned in the *pGWB5* vector (Invitrogen).

Overview of used oligo-nucleotides

Primer	Sequence	Description
SAGT1 5'	ATGGAGCATAAGAGAGGACA	<i>cDNA SAGT1</i>
SAGT1 3'	TTTGCTCTGAACCTTGATA	<i>cDNA SAGT1</i>
P81	<u>CACCATGTCGTCATCATATATAACAC</u>	<i>cDNA IDD4, AT2G02080</i>
P82	TC <u>W</u> ACCTCTTCCAAATGGATAATTTTG	<i>cDNA IDD4, AT2G02080</i>
352s	GGTGGAGGACGAATTATGGA	<i>cDNA IDD4, AT2G02080</i>
251as	AATCTCAACTCCAAATCCAAATCC	<i>IDD4-SRDX</i>
105as	<u>TCAAGCGAATCCCAATCTCAACTCCAAATCCAAATCCAAACCTCTTCCAAAT</u> GGATAA	<i>IDD4-SRDX-STOP</i>
RV16s	accacctgaaaggaagtacagtg	<i>Actin AT3G18780, genomic: 210bp cDNA: 124bp</i>
RV16as	ctgtgaacgattcctggacctgc	<i>Actin AT3G18780, genomic: 210bp cDNA: 124bp</i>
PDF1.2a-F	AGAAGTTGTGCGAGAAGCCAAG	<i>qPCR PDF1.2</i>
PDF1.2a-R	GTGTGCTGGGAAGACATAGTTGC	<i>qPCR PDF1.2</i>
193s	AGACATCGTTGCTCTTCACG	<i>qPCR SARD1</i>
193as	CACACTTACTTCTCCGGCAA	<i>qPCR SARD1</i>
175s	ACGGGCATAGTCCACAAAG	<i>qPCR FRK1</i>
175as	CGTCAAAAGAACGACGATGA	<i>qPCR FRK1</i>
P926	ACTAGCAAACCCAGTGGCTC	<i>qPCR WRKY22</i>
P927	CATGCCCAGACATCGGAGTT	<i>qPCR WRKY22</i>
196s	GACGGGTATCTTTGGAGGAA	<i>qPCR WRKY38</i>
196as	AAACCGGAGGATTGTCTTTG	<i>qPCR WRKY38</i>
198s	TCCAGCAGCGTATGAATCTC	<i>qPCR WRKY28</i>
198as	GCACCACCATTTGATTACGTC	<i>qPCR WRKY28</i>

199s	GAGCAATCGGAAGACATGAA	<i>qPCR WRKY46</i>
199as	TTCCACACACGTTCTCAAT	<i>qPCR WRKY46</i>
87s	GAATCGGTGCTTTGGTTGCTA	<i>qPCR GADPH</i>
87as	TTAACAGCGACGAGCTCAACAT	<i>qPCR GADPH</i>
ksk-145	TATGGGGGATAAGGGGTTCT	<i>qPCR ICS1</i>
ksk-146	GCCCTAGTTACAACCCGAAA	<i>qPCR ICS1</i>
ksk-155	ATCGTTGGAAATCGTGGTGT	<i>qPCR PR2</i>
ksk-156	TCGGTGATCCATTCTTACA	<i>qPCR PR2</i>
ksk-170	TCCCAACACTTTCATCGTCA	<i>qPCR DLO1/S3H</i>
ksk-171	ACGGCTCTATGGAGCACACT	<i>qPCR DLO1/S3H</i>
ksk-123	GTACCGCGCTAAGGACAT	<i>qPCR AOC2</i>
ksk-124	CACAGCGATACGAGAAACATT	<i>qPCR AOC2</i>
167s	GAGGATGGTTGTTCTCCGGT	<i>qPCR ERF1</i>
167as	AGACCCCAAAAGCTCCTCAA	<i>qPCR ERF1</i>
P808	ATTCGCGGCCGAGATAAGAG	<i>qPCR ORA59</i>
P809	GGCTGCCTTTCAAAGCGAAA	<i>qPCR ORA59</i>
267s	CGCTCCTGGTCCATTATCTT	<i>qPCR AP2C1</i>
267as	CAAATCCAGCAACACCAATC	<i>qPCR AP2C1</i>
361s	ATGCGTTTCAGAGGATGAGG	<i>qPCR CAF1a</i>
361as	CAAAACCCAGCATGTTTCT	<i>qPCR CAF1a</i>
335s	TTGCGGTGTTGTTGTGTCT	<i>qPCR GID1B</i>
335as	GTTGAGAGCGTTCCATCCAT	<i>qPCR GID1B</i>

RNA Extraction and real-time quantitative PCR analysis

Total RNAs were extracted from 14 day-old seedlings, grown on half-strength Murashige and Skoog (MS) plates, with the NucleoSpin® RNA Plant (MACHEREY-NAGEL), according to the manufacturer's instructions. First strand cDNA was synthesised from 5µg of total RNAs using SuperScript® First-Strand Synthesis System for RT-PCR (Lifetechnology) according to the manufacturer's instructions. The cDNA stock was diluted to a final concentration of 25ng/ul. Subsequently, 500nM of each primer was applied and mixed with LightCycler® 480 Sybr Green I Master mix (Roche Applied Science) for quantitative PCR analysis, according to the manufacturer's instructions. Products were amplified and fluorescent signals acquired with a LightCycler® 480 detection system. The specificity of amplification products was determined by melting curves. *GADPH* was used as internal control for signals normalization. Exor4

relative quantification software (Roche Applied Science) automatically calculates the relative expression level of the selected genes with algorithms based on the $\Delta\Delta C_t$ method. Data generated from duplicates of at least three biological replicates (bioRep) were used.

Bioinformatics analysis of RNA-SEQ data

Sequencing was performed on each library to generate 101-bp paired-end reads on the Illumina HiSeq2500 Genome Analyzer platform. Read quality was checked using FastQC and low-quality reads were trimmed using the Trimmomatic version 0.32 (<http://www.usadellab.org/cms/?page=trimmomatic>) with the following parameters: Minimum length of 36 bp; Mean Phred quality score higher than 30; Leading and trailing bases removal with base quality below 3; Sliding window of 4:15. After pre-processing the Illumina reads, the transcript structures were reconstructed using a series of programs, namely, TopHat (ver. 2.1.1; <http://tophat.cbcb.umd.edu/>) for aligning with the genome, and Cufflinks (ver. 2.2.1; <http://cufflinks.cbcb.umd.edu/>) for gene structure predictions. For TopHat, the Reference-*Arabidopsis thaliana* (TAIR10) genome (<https://www.arabidopsis.org>) was used as the reference sequences with a maximum number of mismatches as 2. To identify the differentially expressed genes, the following parameters were used: p-value of 0.05 with a statistical correction using Benjamini Hochberg FDR of 0.05 in cuffdiff. A cut-off of 2 fold up- or down-regulation has been chosen to define the differential expression. After processing the data, visualization of differential expression was done using cummeRbund v2.14.0 (<http://bioconductor.org/packages/release/bioc/html/cummeRbund.html>). Differentially regulated genes that are common among the samples were identified using Venny. The complete RNA-SEQ data is available at the GEO repository (GEO accession GSE120791).

GO term analysis

For the GO term analysis, AGRIGO analysis tool was used (<http://bioinfo.cau.edu.cn/agriGO/>, (Tian et al. 2017) by using significantly differentially expressed genes between the tested conditions (p -value ≤ 0.05 for WT and P value ≤ 0.01 for *idd4SRDX*)

Quantification of SA and JA

Plant materials were lyophilized and ground in a bead beater (Biospec Products, Bartlesville, Okla., USA). Aliquots (about 5 mg dry weight) of powdered tissues were extracted with 400 μ L of 10% methanol containing 1% acetic acid and internal standards (11.1 ng of $^2\text{H}_4$ -SA and 0.25 ng of $^2\text{H}_2$ -JA, purchased from OlchemIm Ltd., Olomouc, Czech Republic). The samples were extracted in the bead beater for 1 min, placed in ice for 30 min, and then centrifuged at 13,000 g for 10 min at 4 °C (Eppendorf centrifuge 5424, Hamburg, Germany). The supernatant was carefully removed and the pellet re-extracted with 400 μ L of 10% methanol containing 1% acetic acid. Following further 30 min incubation in ice, the extracts were centrifuged and the supernatants combined. The samples were filtered through 0.22 μ m PTFE filters before LC-MS/MS analysis. Analysis of SA and JA was performed by comparing retention times, and mass transitions with the standards using an Agilent 1200 HPLC (Agilent Technologies, Waldbronn, Germany) coupled to a Q-TRAP 5500 MS (AB SCIEX, MA, USA) with an electrospray source. Chromatographic separation was carried out at 35 °C on a Phenomenex (Torrance, CA, USA) Gemini C18 (150 $\times$ 2.0 mm, 5 μ m) column with the solvent system formic acid/acetonitrile/water (0.1/94.9/5, v/v/v; mobile phase A) and formic acid/ acetonitrile/water (0.1/5/94.9, v/v/v; mobile phase B). The gradient used was 0-20 min, 0%-100% A; 20-25 min, 100%A; 25-26 min, 100%-0% A; 26-36 min, 0% A. To reduce contamination of the MS, the first 5 min of the run was directed to waste using the inbuilt Valco valve. Analysis of SA and JA was based on appropriate Multiple Reaction Monitoring (MRM) of ion pairs for labeled and endogenous SA and JA using the following mass transitions: $^2\text{H}_4$ SA 141>97, SA 137>93; $^2\text{H}_2$ JA 211>61, JA 209>59. The MS was operated in negative ionization mode. The conditions were as follows: Temperature 500 °C, Ion source gas 1 50 psi, Ion source gas 2 60 psi, Ion Spray Voltage -4500 V, curtain gas 40 psi, Collision Gas

Medium; DP (-25 V), EP (-9) and CXP (-2) were the same for all compounds. DT (50) for $^2\text{H}_4\text{SA}$ and SA; CE (-25), and DT (100) for $^2\text{H}_2\text{JA}$ and JA. Data of 3 bioReps (consisting of 3 technical replicates per bioRep) were acquired and analyzed using Analyst 1.4 software (Applied Biosystems).

Quantitation of immunoblot membranes

Bradford assays were used to quantify protein levels in extracts and ensure equal loading of total proteins for gels used for immunoblot analysis and EMSA.

Immunoblot

Nuclear proteins were extracted from 14 day-old seedlings grown on half MS-medium. After quantification with the Bradford method, equal amounts of proteins were resolved by SDS-PAGE and then transferred to a polyvinylidene difluoride membrane (Bio-Rad) using a Mini-Protean 3 Cell (Bio-Rad). Immunoblot analysis was performed using 1 $\mu\text{g}/\text{mL}$ primary polyclonal pTpY antibodies and then with secondary antibodies conjugated to alkaline phosphatase. Antibody complexes were detected by chemiluminescence using the Immun-Start AP Substrate kit (Bio-Rad). Three bioReps were performed and shown is a representative result.

***Pseudomonas syringae* and *Botrytis cinerea* infections**

Plants were spray-inoculated with *Pseudomonas syringae* DC3000 and *Pseudomonas syringae* DC3000 *hrcC*- at $\text{OD}_{600} = 0.2$ and sampled 2 h and 72 h after inoculation to determine the level of colonization as described previously (Jelenska et al. 2010). The density of colony-forming units (CFU) was analyzed 2 and 72 hours post inoculation (hpi). In three bioReps, a total of 30 plants was sampled for each plant genotype by each taking three leaf discs per plant. The *Botrytis cinerea* inoculum was freshly prepared for each experiment from a spore stock stored at -80°C in 25% glycerol. Spores were evenly spread on PDA medium (200 g peeled potato slice, 17 g agar, 20 g dextrose, 1 L distilled water) and incubated at 24°C for 14 days. Afterward, spores were harvested and their concentration determined by the use of a

hemocytometer. Leaves were inoculated with a 2µl droplet of a spore suspension (5×10^6 spores/ml) and analyzed 30 hpi. In three bioReps, a total of at least 30 plants was sampled for each plant genotype.

Tryphan blue staining and DAB staining

Stainings were conducted by using 14 day-old seedlings grown under sterile conditions on half MS medium, in accordance with (Genot et al. 2017). Experiments were repeated three times with similar results and shown is a representative result.

Callose deposition assay

Leaves of 10 day-old seedlings grown under sterile conditions on half MS medium were incubated in acetic acid: ethanol (1:3 v/v) for 8 h to remove the chlorophyll and subsequently rehydrated by a series of ethanol dilutions (from 70%-0% v/v). Leaves were incubated in the dark in 0.01% aniline blue in 150 mM K_2HPO_4 (pH9.5) and subsequently analyzed by epifluorescence microscopy. Experiments were repeated three times with similar results.

ROS burst assay

ROS burst assay was performed as described by (Smith and Heese 2014). Data of 3 bioReps (consisting of 16 technical replicates per bioRep) were acquired, and the average value is presented.

Stomatal Aperture assay

The stomatal assay was performed as described by (Zheng et al. 2015) of 3 week-old plants and subsequently analyzed by microscopy to determine the stomatal opening by calculating the area of the enclosed ellipse $a = \pi * r1 * r2$. Presented are the results of three bioReps.

Author Contribution

R.V., S.K., H.H designed/ performed and analyzed most of the experiments. K.M. conducted *RNA*-SEQ data processing. J. M. and S.A.B carried out the hormonal measurements. A.S analyzed data and performed microscopy. R.V. wrote the manuscript. H.H, S.K helped to prepare the manuscript.

Supplementary data

Supplemental information is online available and includes two figures and six tables. The complete *RNA*-SEQ data is available at the GEO repository (GEO accession GSE120791).

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Figure legends

Figure 1: Dominant-negative *idd4SRDX* lines exhibit defense-related traits without infection.

- (A) Overview of 4 week-old WT, *idd4SRDX1* and *idd4SRDX2* line. Shoot fresh and dry weight are depicted in Fig. S1A-B.
- (B) Lesion formation on the leaf surface of 14 day-old untreated *idd4SRDX* plants compared to WT
- (C) 3,3'-Diaminobenzidine staining (DAB) to evaluate H_2O_2 levels in *idd4SRDX* lines in comparison to WT without infection/ treatment
- (D) Lactophenol trypan blue (LTB) cell death staining of leaves without infection/ treatment
- (E) Callose deposition assays on leaves of untreated *idd4SRDX* lines, orange arrows indicate callose spots (Fig. S1C). Scale bars=3 mm (B-E).
- (F) Overview of untreated stomata in WT, *idd4SRDX1* and *idd4SRDX2* line. Scale bar=100µm.
- (G) Stomatal aperture was determined in untreated *idd4SRDX* lines and at 1hr and 4hr after *PstDC3000* infection. Box plots show the interquartile range 25 to 75, and the inner square highlights the median, whiskers show the SEM, Statistical significance was analyzed by Student's test. Asterisks indicate significant differences n.s., non-significant, ** $p \leq 0.01$, *** $p \leq 0.001$.
- (H) flg22-dependent ROS production is significantly enhanced in the *idd4SRDX* lines. 36 leaf discs from 4 week-old plants were treated with water (mock-treated samples Fig. S1D) or 1µM flg22 over 45 min. The data are shown as mean $\pm$ SE.
- (I) flg22-induced MAP kinase-activation assays in *idd4SRDX1*, *GFP-IDD4 (IDD4ox)* and WT plants. 14 day-old seedlings were treated with 1µM flg22 and samples were harvested at the indicated time points. Activation of MAP kinases 3 and 6 were analyzed by immunoblot using pTpY-antibody recognizing the MAPKs in their activated form. Protein loading control was performed by Ponceau S staining for Rubisco (RBC).
- (J) Expression of the PTI-/ Defense markers *FRK1* in *idd4SRDX* and *IDD4ox* lines without infection.
- (K) Expression of *WRKY33*, *WRKY22* and *PR2* in the *idd4SRDX* lines without infection. Statistical significance was analyzed by Student's test. Letters above bars represent significance groups, $p \leq 0.05$.

Figure 2: Hormonal levels of salicylic acid and jasmonic acid are elevated in *idd4SRDX* lines

- (A, D) Quantitative analysis of free salicylic acid (SA) (A) and jasmonic acid (JA) (D) by using LC-MS/MS. Box plots are depicted for *idd4SRDX* lines and WT, boxes showing the interquartile range (IQR) 25st to

75th percentiles, inner ellipse representing the median, whiskers show the SEM, outliers are depicted by dots (Min/Max range).

(B-C) Genes involved in SA biosynthesis (*S3H*, *ICS1*, *WRKY28*, *WRKY46*, *SARD1*) and signaling (*WRKY38*) are up-regulated in untreated *idd4SRDX* lines compared to WT.

(E) Genes involved in JA-biosynthesis (*ACO2*) and response (*PDF1.2*, *ERF1*, *ORA59*) are up-regulated in untreated *idd4SRDX* lines compared to WT. Statistical significance was analyzed by Student's test. Letters above bars represent significance groups, $p \leq 0.05$.

Figure 3: Dominant-negative *idd4SRDX* mutants are more resistant to *PstDC3000* and *Botrytis cinerea* infection.

(A-B) WT plants and *idd4SRDX* lines challenged by *PstDC3000* **(A)** and *PstDC3000 hrcC-* **(B)**, respectively. Plants, of three biological replicates ($n=30$), were spray-inoculated with a bacterial suspension at OD_{600} 0.2, the density of colony-forming units (cfu) was analyzed 2 and 72 hours post inoculation (hpi).

(C) WT plants and *idd4SRDX* lines challenged by *Botrytis cinerea*. Plants, of three biological replicates, were droplet-inoculated with a concentration of 5×10^6 spores/ml. Boxes represent the 25th and 75th percentiles, and the inner rectangle highlights the median, and outliers are depicted by dots (Min/Max range), whiskers show the SEM, and letters above boxes represent significance groups as determined by multiple comparison Student's test $p \leq 0.001$.

(D) WT plants, *idd4SRDX1*, *idd4SRDX1/ SAGT1ox*, and *SAGT1ox* lines were treated by *Pst DC3000 hrcC-*. Expression of *SAGT1ox* in *idd4SRDX1* lines reconstitutes WT-like susceptibility to infection. Plants were spray-inoculated with a bacterial suspension at OD_{600} 0.2. Statistical significance was analyzed by Student's test. Letters above bars represent significance groups, $p \leq 0.05$.

(E) Overview of WT plants, *idd4SRDX1*, *idd4SRDX1/ SAGT1ox*, and *SAGT1ox* lines. DAB-staining shows that the hydrogen peroxide levels are significantly reduced in *idd4SRDX2/SAGT1ox* in comparison to *idd4SRDX* lines (quantification **Figure S2 B**). Scale bar, 7.5 mm.

(F) Quantitative analysis of salicylic acid by LC-MS/MS depicted as box plots for WT, *idd4SRDX1*, *idd4SRDX1/SAGT1ox*, and *SAGT1ox*, boxes representing the 25th and 75th percentiles and the inner rectangle highlights the median, whiskers show the SEM, and outliers are depicted by dots (Min/Max range).

Figure 4: Transcriptome composition of *idd4SRDX* lines.

(A-B) Depicted are scatter plots of whole-genome transcript fragments per kilobase of transcript per million mapped reads (FPKM) in WT (Col0) vs. *idd4SRDX* **(A)** or WT (mock) vs. WT (1h, flg22) **(B)**.

(C) Log₂ distribution of up- and down-regulated genes in *idd4SRDX* lines, $p \leq 0.01$ (**Table S2**)

(D) Transcriptome composition of up-regulated genes in *idd4SRDX* shows high overlap with genome-wide transcriptome data of 14 day-old WT plants treated with flg22 for 1h. 39,6 % of up-regulated genes in *idd4SRDX* overlap with those in flg22-treated WT plants.

(E) Significantly enriched GO terms of *idd4SRDX* up-regulated genes (**Table S1**)

(F) Significantly enriched GO categories among up-regulated genes of the overlap between *idd4SRDX* and flg22-treated WT plants (**Table S3**).

Figure 5: Functional categorization of primary IDD4 target genes and differentially regulated genes in *idd4SRDX*.

(A) Subcellular localization of GFP:IDD4:SRDX in the nucleus of *Arabidopsis*.

(B) Genome-wide binding study of IDD4, provided by O'Malley et al. 2016, revealed the most frequent enrichment of the *ID1-cis* motif in the IDD4 target sequences. The *ID1* motif consists of the core sequence A/C/GGACAA.

(C-F, J-L) Genome-wide binding study of IDD4 (O'Malley et al. 2016) revealed binding profiles of IDD4 to selected target sequences of *GA3OX1* **(C)**, *ATH1* **(D)**, *GID1B* **(E)**, *SCL3* **(F)**, *WRKY27* **(J)**, *STOMAGEN* **(K)** and *SPEECHLESS* **(L)**. The blue arrows depict significant IDD4 binding regions. The distribution of

the variable core *ID1*-motif in the gene-body and the 5' upstream and 3'-downstream sequence of each gene is indicated in a color-code. The TAIR annotations of the genomic loci are shown at the bottom of each panel. The genomic locus indicated above the scale represents forward orientation, while the one below represents reverse orientation.

(G) Expression of *GID1B* in *idd4SRDX*, without treatment and 1hr after flg22 application. Error bars show $\pm$ SEM; statistical significance was analyzed by Student's test, letters above bars represent significant groups $p \leq 0.05$.

(H, M) Transcriptome composition of differentially-regulated genes in *idd4SRDX* ($p < 0.01$) contribute to Gibberellin-signaling/ response (H) and Stomatal patterning/ aperture (M).

(I, N) Protein-interaction networks derived from the genome-wide binding study of IDD4 (O'Malley et al. 2016) and differentially-regulated genes in *idd4SRDX*. All significant target genes were pooled and used to generate a network using STRING (version 10.0). Meaning of network edges "evidence".

Figure 6: Functional categorization of differentially regulated genes in *idd4SRDX* by taking into account primary IDD4 target genes.

(A) Transcriptome composition of differentially-regulated genes in *idd4SRDX* ($p < 0.01$) contribute to Auxin transport/ signaling.

(B-D, H) Genome-wide binding study of IDD4 (O'Malley et al. 2016) revealed binding profiles of IDD4 to selected target sequences of *PIN5* (B), *PIN-LIKES 3* (C), *IAA24* (D) and *CAF1a* (H). The blue arrows depict significant IDD4 binding regions. The distribution of the variable core *ID1*-motif in the gene-body and the 5'-upstream and 3'-downstream sequence of each gene is indicated in a color-code. The genomic locus indicated above the scale represents forward orientation, while the one below represents reverse orientation.

(E) Protein interaction networks derived from the genome-wide binding study of IDD4 (O'Malley et al. 2016) and differentially-regulated genes in *idd4SRDX*. All significant target genes were pooled and used to generate a network using STRING (version 10.0). Meaning of network edges "evidence".

(F-G) Expression of *AP2C1* (F) and *CAF1a* (G) in *idd4SRDX* and *IDD4ox* lines. Error bars show $\pm$ SEM; statistical significance was analyzed by Student's test against WT. Asterisks indicate significant differences * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$; n.s, non-significant.

Figure S1. *idd4SRDX* lines have reduced fresh and dry weight and exhibit callose spots without infection.

(A-B) Fresh and dry weight is significantly reduced in *idd4SRDX1*, -2 compared to WT.

(C) Without flg22-treatment and under sterile conditions on sugar-lacking MS medium, *idd4SRDX* lines exhibit spontaneous callose depositions on the leaf surface. Statistical significance was analyzed by using Student's t-test against WT control *** $P \leq 0.001$ (Figure 3E).

(D) Mock-treated ROS burst assay of WT, *iddSRDX1*, *idd4SRDX2* lines (refers to Figure 3H). Statistical significance was analyzed by Student's test. Asterisks indicate significant differences compared to WT, *** $p \leq 0.001$.

Figure S2: Characterization of *idd4SRDX2/SAGT1ox* lines.

(A) The perimeter of WT, *idd4SRDX1*, *idd4SRDX1/SAGT1ox* and *SAGT1ox* lines of 4 week-old plants. Notch boxes show the interquartile range 25 to 75, and the inner rectangle highlights the median, whiskers show the SEM, and outliers are depicted by dots (Min/Max range)

(B) DAB staining intensity of WT, *idd4SRDX1*, *idd4SRDX1/SAGT1ox* and *SAGT1ox* lines of 14 day-old plants. Notch boxes show the interquartile range 10 to 90, and the inner rectangle highlights the median, whiskers show the SEM, and outliers are depicted by dots (Min/Max range).

(C) Expression of *IDD4SRDX* in *idd4SRDX1* and *idd4SRDX1/SAGT1ox* lines. The expression was tested by the use of the primer 352s, which binds on *IDD4*, and primer 251as that binds on *SRDX* sequence.

(A-B) Statistical significance was analyzed by Student's test. Letters above bars represent significance groups, $p \leq 0.05$.

Table S1: GO term analysis of up- and down-regulated genes in *idd4SRDX* lines

Table S2: Overview of differentially regulated genes in *idd4SRDX* line

Table S3: Overview of commonly up-regulated genes and corresponding GO term analysis of untreated *idd4SRDX* lines and flg22-treated WT plants.

Table S4: Differentially expressed genes in WT plants, 1 hr after flg22-treatment.

Table S5: Components of the SA, JA and ethylene metabolism and signaling pathway are differentially expressed in *idd4SRDX* lines.

Table S6: Differentially expressed genes in *idd4SRDX* that are considered as major IDD4 downstream targets by O'Malley, Huang, et al. 2016

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