Article type : Original Article

ORIGINAL ARTICLE

Identifying the target genes of SUPPRESSOR OF GAMMA RESPONSE 1, a master transcription factor controlling DNA damage response in *Arabidopsis*

Nobuo Ogita[1], Yoko Okushima[1], Mutsutomo Tokizawa[2,†], Yoshiharu Y. Yamamoto[3], Maho Tanaka[4], Motoaki Seki[4,5], Yuko Makita[4], Minami Matsui[4], Kaoru Okamoto-Yoshiyama[1,‡], Tomoaki Sakamoto[1,‡], Tetsuya Kurata[1,§], Kei Hiruma[1], Yusuke Saijo[1], Naoki Takahashi[1], Masaaki Umeda[1,6,*]

[1] Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Nara 630-0192, Japan

[2] The United Graduate School of Agricultural Science, Gifu University, Gifu, Gifu 501-1193, Japan

[3] Faculty of Applied Biological Sciences, Gifu University, Gifu, Gifu 501-1193, Japan

[4] RIKEN Center for Sustainable Resource Science, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan

[5] JST, CREST, Kawaguchi, Saitama 332-0012, Japan

[6] JST, CREST, Ikoma, Nara 630-0192, Japan

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/tpj.13866

[†] Present address: Global Institute for Food Security, University of Saskatchewan, Saskatoon, SK S7N 4J8, Canada

[‡] Present address: Department of Bioresource and Environmental Sciences, Kyoto Sangyo University, Kita-ku, Kyoto, Kyoto 603-8555, Japan

[§] Present address: EditForce Inc., Chuo-ku, Fukuoka, Fukuoka 810-0001, Japan

[*] Correspondence author: Masaaki Umeda, Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192 Japan, E-mail: mumeda@bs.naist.jp, TEL: +81-743-72-5592, FAX: +81-743-72-5599

RUNNING TITLE: SOG1 target genes in *Arabidopsis*

KEYWORDS: *Arabidopsis thaliana*, ChIP-Seq, *cis*-element, DNA damage response, immune response, NAC transcription factor, p53, SOG1

SUMMARY

In mammalian cells, the transcription factor p53 plays a crucial role in transmitting DNA damage signals to maintain genome integrity. However, in plants, orthologous genes for p53 and checkpoint proteins are absent. Instead, the plant-specific transcription factor SUPPRESSOR OF GAMMA RADIATION 1 (SOG1) controls most of the genes induced by gamma irradiation and promotes DNA repair, cell cycle arrest, and stem cell death. Thus far, the genes directly controlled by SOG1 remain largely unknown, limiting the understanding of DNA damage signaling in plants. Here, we conducted a microarray analysis and chromatin

immunoprecipitation (ChIP)-sequencing, and identified 146 *Arabidopsis* genes as direct targets of SOG1. By using the ChIP-sequencing data, we extracted the palindromic motif [CTT$(N)_7$AAG] as a consensus SOG1-binding sequence, which mediates target gene induction in response to DNA damage. Furthermore, DNA damage-triggered phosphorylation of SOG1 is required for efficient binding to SOG1-binding sequence. Comparison between SOG1 and p53 target genes showed that both transcription factors control genes responsible for cell cycle regulation, such as CDK inhibitors, and DNA repair proteins, whereas SOG1 preferentially targets genes involved in homologous recombination. We also found that defense-related genes were enriched in the SOG1 target genes. Consistent with this, SOG1 is required for resistance against the hemi-biotrophic fungus *Colletotrichum higginsianum*, suggesting that SOG1 has a unique function in controlling immune response.

INTRODUCTION

Maintenance of genome integrity is crucial for organisms to survive under various environmental conditions. DNA damage, such as single-strand breaks (SSBs) and double-strand breaks (DSBs), occurs during chromosomal replication but also originates from endogenous (*e.g.* reactive oxygen species and metabolic byproducts) and exogenous factors (*e.g.* ultraviolet and ionizing radiation) (Jackson and Bartek, 2009). DNA damage triggers cell cycle arrest and, in severe cases, cell death, thereby ensuring DNA repair and preventing carcinogenesis (Jackson and Bartek, 2009). In eukaryotes, DNA lesions are sensed by the cellular kinases ATAXIA-TELANGIECTASIA MUTATED (ATM) and ATM AND RAD3-RELATED (ATR) (Sancar et al., 2004; Hu et al., 2016). ATM is activated by DSBs,

whereas ATR responds to SSBs and stalled replication forks (Sancar et al., 2004; Hu et al., 2016). In animals, ATM and ATR phosphorylate and activate checkpoint kinase 2 (Chk2) and Chk1, respectively. The signals are subsequently transmitted to the tumor suppressor protein p53, a transcription factor governing DNA damage response (Meek, 2009). In the absence of DNA damage, p53 is actively degraded through ubiquitin-mediated proteolysis. In the presence of DNA damage, p53 is phosphorylated and stabilized, inducing the expression of genes for apoptosis, cell cycle regulation, DNA repair, and senescence (Kruse and Gu, 2009; Meek, 2009; Bieging et al., 2014).

Although p53 orthologues can be traced back to the unicellular Choanoflagellates (Bely et al., 2010), it is missing in plants (Yoshiyama et al., 2014). Instead, the plant-specific transcription factor SUPPRESSOR OF GAMMA RESPONSE 1 (SOG1) plays a key role in transmitting DNA damage signals (Preuss and Britt, 2003; Yoshiyama et al., 2009). SOG1 is a member of NAC [NO APICAL MERISTEM (NAM), *ARABIDOPSIS* TRANSCRIPTION ACTIVATION FACTOR (ATAF), CUP-SHAPED COTYLEDON (CUC)] transcription factors, which are known to have diverse functions in controlling plant development, such as stem cell maintenance, cell wall formation and senescence, and abiotic and biotic stress responses (Aida et al., 1997; Kubo et al., 2005; Puranik et al., 2012; Kim et al., 2016). While the amino acid sequences of SOG1 and p53 display no similarity to each other, SOG1 plays a crucial role in DNA damage response as p53 does in animals (Yoshiyama et al., 2014). The *Arabidopsis sog1-1* mutant, which carries a missense mutation in the conserved NAC domain, was originally isolated as a suppressor of the gamma-sensitive phenotype observed in the DNA repair-defective *xpf* mutant (Preuss and Britt, 2003). Among 282 genes that were induced by gamma-irradiation in the wild-type, 249 genes did not show any change in mRNA

levels in *sog1-1* (Yoshiyama et al., 2009), suggesting that SOG1 is a key transcription factor governing DNA damage response. Other studies also showed that SOG1 is involved in a variety of responses, such as cell cycle arrest, DNA repair, early onset of endoreplication, and induction of cell death (Furukawa et al., 2010; Adachi et al., 2011; Weimer et al., 2016; Chen et al., 2017).

Phosphoregulation of p53 is crucial for the DNA damage response in mammals; ATM and CHK2 phosphorylate p53 in the presence of DSBs, whereas ATR and CHK1 render p53 phosphorylation in response to SSBs and replication stress (Banin et al., 1998; Canman et al., 1998; Tibbetts et al., 1999; Hirao et al., 2000; Shieh et al., 2000; Sancar et al., 2004). In *Arabidopsis*, SOG1 is also phosphorylated by ATM and ATR. It was reported that human ATM phosphorylates five serine-glutamine (SQ) motifs in the C-terminal region of SOG1 *in vitro* and that SOG1 is phosphorylated *in vivo* upon treatment with DSB-inducing agents (Yoshiyama et al., 2013). The recent study indicated that the number of phosphorylated sites is associated with the transcriptional activity of SOG1 (Yoshiyama et al., 2017). ATR also phosphorylates SOG1 *in vitro*, although the phosphorylation sites have not been identified thus far (Sjogren et al., 2015). Such phosphoregulation of SOG1 is important for transmitting DNA damage signals. DNA damage-induced root growth inhibition, stem cell death, and transcriptional induction were rescued by introducing the intact *SOG1* gene into *sog1-1*, but not by introducing *SOG1* carrying alanine substitutions at the five SQ motifs (Yoshiyama et al., 2013; Yoshiyama et al., 2014). These observations indicate that, despite amino-acid sequence dissimilarity between SOG1 and p53, the regulatory system for the two key transcription factors is likely similar to each other.

SOG1 was shown to directly control several cell cycle- and DNA repair-related genes. For example, SOG1 binds to the promoters of genes for B1-type cyclin *CYCB1;1* (Weimer et al., 2016), CDK inhibitors *SMR5* and *SMR7* (Yi et al., 2014), and the DNA-repair protein *AtBRCA1* (Sjogren et al., 2015), and thereby upregulates their expression. We recently found that *FMO1*, which encodes a flavin-containing monooxygenase that is associated with the production of reactive oxygen species (ROS), is also directly controlled by SOG1 under DNA damage conditions (Chen and Umeda, 2015). However, no comprehensive identification of SOG1 target genes has been conducted so far, thereby restricting the understanding of SOG1-mediated control of DNA damage response. Here we identified *Arabidopsis* genes that were directly controlled by SOG1 in response to DSBs. Comparison of target genes between SOG1 and mammalian p53 indicated that cell cycle regulators, such as CDK inhibitors, are commonly induced by these key transcription factors. On the other hand, defense-related genes were also enriched in the SOG1 target genes, suggesting the involvement of SOG1 in plant immunity.

RESULTS

Genome-wide identification of SOG1 target genes

SOG1 is known to control the expression of almost all genes that respond to gamma irradiation (Yoshiyama et al., 2009), indicating that SOG1 plays a crucial role in the DSB response. To identify the SOG1 target genes and uncover the downstream events, microarray and chromatin immunoprecipitation (ChIP)-sequence analyses were performed. To collect samples for microarray analysis, two-week-old wild-type and *sog1-1* seedlings

were treated with 15 μM zeocin, which induces DSBs (Chankova et al., 2007), for 2 h. Total RNA was extracted from whole seedlings. Two independent experiments using the Agilent Custom Microarray, which covers all annotated *Arabidopsis* genes, showed that transcript levels of 442 genes changed more than two-fold by zeocin treatment in the wild-type, of which 342 and 100 genes were increased and decreased, respectively. In the *sog1-1* mutant, 332 out of 342 up-regulated genes were not induced by zeocin, and the mRNA levels of all down-regulated genes were not reduced. This indicates that the expression of 432 genes (332 up- and 100 down-regulated genes) is regulated by SOG1 (Figure 1a and Table S1). To identify the SOG1-binding genomic regions, we conducted ChIP-sequence analysis. We used wild-type plants harboring the transgene *ProSOG1:SOG1-Myc*, which expresses the *SOG1-Myc* fusion gene under the 2-kb *SOG1* promoter. It was previously demonstrated that *SOG1-Myc* can complement the *sog1-1* mutation (Yoshiyama et al., 2013). After treatment of seedlings with 15 μM zeocin for 2 h, chromatin fragments bound to SOG1-Myc were immunoprecipitated with the anti-Myc antibody. The immuno-precipitated DNA was then subjected to next generation sequencing. We identified 778 SOG1-binding peak summits, which displayed peak values 16-fold higher than those in wild-type plants without the transgene. The genomic regions between 5-kb upstream and downstream of each peak summit included 1514 genes (Figure 1a), among which we found *SMR5*, *SMR7*, and *CYCB1;1* that were previously identified as SOG1 target genes (Yi et al., 2014; Weimer et al., 2016) (Figure S1).

Among 1514 genes identified by ChIP-sequencing, 146 genes overlapped with the SOG1-regulated genes extracted from the microarray data (Figure 1a); therefore, they were considered candidates for SOG1 target genes. It is noteworthy that all of them were upregulated by zeocin treatment (Table S2). To validate whether SOG1 directly controls their transcription, two putative target genes, *AtRAD51* and *AtBRCA1*, which displayed significant peaks around the transcription start sites (TSS) in ChIP-sequencing (Figure S1), were individually analyzed by ChIP-PCR. The results showed that, for both genes, SOG1-Myc bound to genomic regions near the TSS in the presence of zeocin; the highest binding was detected for the P2 region (Figure 1b). However, when plants were not treated with zeocin, a significant level of SOG1 binding was not detected (Figure 1b), suggesting that DNA damage signals are required for SOG1 to bind to target sites. qRT-PCR and expression analysis using the *promoter:GUS* reporter lines showed that both genes were induced by zeocin in wild-type, but not in the *sog1-1* mutant (Figures 1c,d and S2). Similar results were obtained for 15 genes that were randomly selected from the 146 candidate genes (Figure S3), suggesting that most of them are directly controlled by SOG1. Therefore, we hereafter name them as SOG1 target genes (Table S2).

As described above, our results showed that SOG1 binds to target gene promoters only under DNA damage conditions (Figures 1b and S3a). It was previously shown that ATM and ATR phosphorylate and activate SOG1 in response to DNA damage (Yoshiyama et al., 2013; Sjogren et al., 2015); therefore, we speculated that phosphorylated SOG1 efficiently binds to target sites. To examine this possibility, we conducted ChIP-PCR using *sog1-1* plants expressing SOG1-Myc or SOG1(5A)-Myc under the *SOG1* promoter. SOG1(5A) carries serine-to-alanine substitutions at five serine-glutamine (SQ) motifs, which are

potential phosphorylation sites by ATM, and is unable to complement the *sog1-1* mutation (Yoshiyama et al., 2013). When plants were treated with zeocin, SOG1-Myc bound to the promoters of *SMR7*, *AtRAD51*, and *AtBRCA1*, whereas no significant binding was detected for SOG1(5A)-Myc (Figure 2). This suggests that DNA damage-triggered phosphorylation of SOG1 is required for its efficient binding to target sites.

SOG1 target genes respond to abiotic stresses and pathogen infection

To characterize the SOG1 target genes, we performed the gene ontology (GO) analysis using the agriGO program (Du et al., 2010). Genes related to DNA damage response but also to abiotic stress were highly enriched in the SOG1 target genes (Figure S4a and Table S3). Therefore, we examined their response to different types of abiotic stress using AtGenExpress public microarray datasets. About 80% of the SOG1 target genes displayed more than a two-fold increase in transcript levels by treatment with bleomycin and mitomycin C (MMC) that cause DSBs (Figure S4b). Moreover, a significant number of SOG1 target genes are up-regulated in response to various abiotic stresses, such as cold stress, osmotic stress, salt stress, drought stress, oxidative stress, wounding stress, and heat stress (Figure S4b). Missirian et al. (2014) reported that a part of genes induced by HZE (high atomic weight, high-energy radiation) and gamma radiation also respond to a wide variety of abiotic stresses (*e.g.* cold stress, drought stress, salt stress, osmotic stress, UV-B, and wounding stress). However, the response to other stresses occurs at later time points; thus, they suggested that these transcriptional changes may reflect downstream effects caused by the stresses. Regarding the SOG1 target genes, some are upregulated at later time points;

others are induced soon after stress treatments (Figure S4b). Indeed, in the SOG1 targets, we found early response genes such as *DEHYDRATION RESPONSE ELEMENT-BINDING PROTEIN 19* (*DREB19*) for salt, heat, and drought stress responses (Krishnaswamy et al., 2011), *NAC WITH TRANSMEMBRANE MOTIF 1-LIKE 4* (*NTL4*)/*ANAC053* for heat and drought stress response (Lee et al., 2012), and *EARLY RESPONSE TO DEHYDRATION 14* (*ERD14*) for cold, salt and drought stress responses (Kiyosue et al., 1994). Therefore, it is likely that at least some of the SOG1 target genes respond to abiotic stresses other than DNA damage.

Moreover, GO analysis revealed that genes related to 'response to chitin', 'immune effector process', and 'response to fungus' were significantly enriched in the SOG1 target genes (Figure S4a). Indeed, defense-related genes, such as *SENESCENCE-ASSOCIATED GENE101* (*SAG101*), *OXIDATIVE SIGNAL-INDUCIBLE1* (*OXI1*), *AtMYB44, WRKY50*, and *FMO1*, were included in the target genes (Table S2) (Rentel et al., 2004; Feys et al., 2005; Mishina and Zeier, 2006; Gao et al., 2011; Shim et al., 2013). Recent studies demonstrated that DNA damage occurs during infection of microbial pathogens, including bacteria, fungi, and oomycetes (Song and Bent, 2014), and when salicylic acid (SA), which accumulates after bacterial and fungal infections, is present at high levels in *Arabidopsis* leaves (Yan et al., 2013). Moreover, AtGenExpress public microarray datasets indicate that a part of the SOG1 target genes are induced by challenge with bacterial and fungal pathogens, such as *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000), *Pst avrRpt2*, and *Botrytis cinerea*, and elicitor treatments (Figure S4c). These results suggest that SOG1 is activated in response to pathogen attack and controls immune response.

Comparison between SOG1 and p53 target genes

In mammals, the transcription factor p53 plays a central role in controlling DNA damage response (Kruse and Gu, 2009). Previous study on mouse embryo fibroblasts showed that, in response to doxorubicin-induced DSBs, p53 directly controls the expression of 432 genes, of which 365 and 67 genes were up- and down-regulated, respectively (Kenzelmann Broz et al., 2013). We next examined how many SOG1 target genes are overlapped with the p53 target genes. Our BLAST analysis of 146 SOG1 targets identified 66 genes with high similarities to *Mus musculus* genes (BLASTP 2.7.1, *E*-value < 1e-10) (Altschul et al., 1997). Among them, only two genes are included in the p53 targets; namely, genes for Ribonucleotide reductase M2 (Rrm2) and DNA polymerase kappa (Polk) in the p53 target genes were orthologous to those for TSO2 (AT3G27060) and AtPOLK (AT1G49980) in the SOG1 target genes, respectively (Table S2). In addition, one of the SOG1 targets, KIP-RELATED PROTEIN 6 (KRP6), is known to have a limited similarity to mammalian $p21^{Cip1/Waf1}$, which is directly regulated by p53 (Wang et al., 1997; De Veylder et al., 2001; Kenzelmann Broz et al., 2013). The low percentage of overlapping genes between the SOG1 and p53 targets is likely due to low amino acid similarity between plant and mammalian factors involved in DNA damage response. We therefore performed GO analysis by using the GO program BiNGO (ver. 3.03) (Maere et al., 2005) (Tables S4 and S5). Overrepresented GO terms were visualized on the Cytoscape (ver. 3.3) platform, which enables the integration and visualization of interaction networks in terms of biological information (Smoot et al., 2011). SOG1 target genes displayed three major clusters of 'cell cycle/checkpoint', 'DNA repair/DNA replication', and 'response to abiotic stress' (Figure 3a). On the other hand, p53 target genes showed ten clusters (Figure 3b), supporting the previously proposed idea that

p53 has multiple roles in intracellular signaling including DNA damage response (Bieging et al., 2014). Among 154 GO terms enriched with the SOG1 target genes, 81 overlapped with those enriched with the p53 target genes; the shared GO terms include 'cell cycle' (p = 1.4 x 10^{-7} and 1.6 x 10^{-7} for the SOG1 and p53 target genes, respectively), 'cell cycle arrest' (p = 7.2 x 10^{-3} and 3.1 x 10^{-6}) and 'response to abiotic stimulus' (p = 7.1 x 10^{-3} and 9.7 x 10^{-4}) (Figure 3, Tables S4 and S5).

On the other hand, GO terms 'DNA repair', 'double-strand break repair', and 'double-strand break repair via homologous recombination' were enriched only with the SOG1 target genes (Table S4). For example, *RAD51, BRCA1, RAD17*, and *REPLICATION PROTEIN A s* (*RPAs*) are targeted by SOG1, but not by p53. This suggests that, unlike mammals, homologous recombination (HR) is directly induced through the ATM–SOG1 pathway in plants. It is noteworthy that the GO terms 'apoptosis process', 'programmed cell death', and 'apoptotic signaling pathway' were enriched only in the p53 target genes (Table S5). However, plants do not have a conserved set of genes involved in apoptosis (Bonneau et al., 2008), making it difficult to conclude that SOG1 does not directly control cell death.

Identification of the SOG1-binding motif

Our analysis of the peak summits obtained by ChIP sequencing showed that the majority of SOG1-binding sites were located near the TSS (Figure 4a). We therefore searched for the consensus binding motif within 1 kb upstream of the TSS. We first used the MEME program that identifies ungapped motifs (Bailey et al., 2006), but no consensus sequence was found. Next we used the Regulatory Sequence Analysis Tools (RSAT)-spaced dyad tool that

extracts motifs composed of a pair of highly conserved trinucleotides separated by non-conserved nucleotides with fixed width (Helden et al., 2000). As a result, we found the palindromic sequence CTT$(N)_7$AAG as a putative SOG1-binding motif (Figure 4b). Of the 146 target genes, 51% possess single or multiple CTT$(N)_7$AAG motifs (Figures 4c and S5). This motif was significantly enriched with the SOG1 target genes within 400 bp upstream of the TSS as compared to the total *Arabidopsis* genes ($p < 0.05$) (Figure 4d). GO categories for the target genes with or without the CTT$(N)_7$AAG motif showed that similar GO terms, such as 'double-strand break repair', 'cell cycle', and 'response to stress', were enriched irrespective of the presence of the motif (Figure S6).

To test whether SOG1 can recognize this motif *in vitro*, we monitored the interaction between *in vitro*-translated SOG1 protein and synthesized biotin-labeled double-strand DNA (dsDNA) by AlphaScreen binding assay. This assay detects chemiluminescence from SOG1-fused acceptor beads that bind to dsDNA fused to donor beads, which transfer singlet oxygen to acceptor beads upon laser excitation (Tokizawa et al., 2015). We used 30-bp dsDNA corresponding to -80 to -51 bp upstream of the TSS in the *AtRAD51* promoter, which contains a sequence of the SOG1-binding motif CTTGTTGAAGAAG (Figure 4e, Region A). The result showed that SOG1 binds to this dsDNA, but not to another promoter region from -1160 to -1131 (Figure 4f, Region B). The AlphaScreen signal representing the interaction with Region A disappeared when non-biotin-labeled dsDNA of Region A, but not Region B, was added to the reaction (Figure 4g). These results suggest that SOG1 specifically recognizes genomic regions containing the CTT$(N)_7$AAG motif.

To test the requirement of each nucleotide in the consensus sequence, we introduced point mutations in Region A of *AtRAD51*. Since the nucleotides flanking both sides of the consensus motif were also partially conserved (Figure 4b), we introduced mutations into the 17-bp sequence GACTTGTTGAAGAAGCC. We found that the competition activity was significantly reduced by substitutions at positions 3, 4, and 5 (CTT) and at positions 12, 13, 14, and 15 (GAAG) (Figure 5a). When either one of the two palindromic trinucleotides, CTT and AAG, was substituted with TCC and GGA, respectively, the competition activity was partially suppressed (Figure 5b, TCC and GGA). Moreover, replacement of both CTT and AAG led to a complete loss of competition activity, regardless of the resultant palindromic TCC/GGA sequence (Figure 5b). These results suggest that both CTT and AAG trinucleotides, rather than the palindromic nature, are important for recognition by SOG1. Deletion of three out of seven intervening nucleotides, which are not conserved among the SOG1 target genes, partially suppressed the competition activity (Figure 5b, Deletion), suggesting that the distance between the CTT and AAG trinucleotides is also important for SOG1 binding.

The consensus motif is required for SOG1-mediated induction of target genes *in vivo*

To investigate whether the consensus motif identified above functions *in vivo*, we first established a transient transactivation assay system using *Arabidopsis* protoplasts. The 2-kb *AtRAD51* promoter, which contains the consensus sequence, was fused to the *luciferase* gene to generate the reporter construct. First, protoplasts isolated from leaf mesophyll cells of wild-type and *sog1-1* seedlings were transfected with only the reporter construct. As shown

in Figure S7a, luciferase activity was four-fold higher in wild-type protoplasts as compared with that in *sog1-1*, suggesting that SOG1 is already activated in wild-type protoplasts in the absence of exogenous genotoxic treatment. One possibility is that enzymatic degradation of the cell wall during protoplasting might cause DNA damage, thereby activating SOG1. We then introduced the effector *35S:SOG1* as well as the reporter construct into protoplasts prepared from *sog1-1* seedlings that were treated with or without 15 μM zeocin for 2 h. The result showed that exogenously expressed SOG1 can also induce the reporter gene in protoplasts in the absence of genotoxic treatment (Figure S7b).

When *sog1-1* protoplasts were transfected with *35S:SOG1(5A)* instead of *35S:SOG1*, no increase in luciferase activity was observed (Figure 6a), supporting the above-mentioned idea that phosphorylation is required for SOG1's function. We then introduced mutations in the consensus CTTGTTGAAGAAG sequence in the *AtRAD51* promoter. We substituted both sets of conserved trinucleotides and inner single nucleotides, which are also partially conserved (Figure 4b), to generate the mutated sequence TCCATTGAAAGGA. When this mutated sequence was included in the reporter construct, luciferase activity was not induced by co-transfection with *35S:SOG1* (Figure 6a), suggesting the essential function of the consensus motif in plant cells. Next, to examine whether the consensus CTT$(N)_7$AAG sequence is sufficient for transcriptional activation, we fused four tandem repeats of the consensus sequence derived from the *AtRAD51* promoter (CTTGTTGAAGAAG) and the minimal cauliflower mosaic virus (*CaMV*) *35S* promoter (*35Smini*) to the luciferase gene, generating the reporter construct (Figure 6b). When co-transfected with *35S:SOG1*, luciferase activity increased 75-fold as compared to the negative control *35Smini:LUC*, and the induction was rather higher than that observed for the

AtRAD51 promoter (Figure 6b). However, when base substitutions were included in the consensus sequence (TCCGTTGAAGGGA), no significant increase in luciferase activity was observed (Figure 6b). These results suggest that the CTT$(N)_7$AAG sequence is sufficient for SOG1-mediated induction of target genes.

We then generated transgenic plants expressing the *GUS* reporter gene under the 2-kb *AtRAD51* promoter with or without nucleotide substitutions, as described above. Zeocin treatment highly induced the *GUS* expression in transgenic lines carrying the native *AtRAD51* promoter, but not in those with substitutions in the consensus motif (Figure 6c). Similar results were also obtained for the *AtBRCA1* promoter (Figure S8). These results suggest that the CTT$(N)_7$AAG motif is crucial for the DNA damage-induced expression of SOG1 target genes *in planta*.

SOG1 contributes to plant resistance against fungal pathogens

As described above, GO terms 'response to chitin', 'immune effector process', and 'response to fungus' were significantly enriched with the SOG1 target genes (Figure S4a). This prompted us to test whether SOG1 is involved in immune response. First, we examined possible alterations in defense responses of *sog1* mutants to bacterial pathogens. In addition to *sog1-1*, we used another allele *sog1-101* (GABI_602B10), which has a T-DNA insertion in the third intron (Figure S9a). Quantitative RT-PCR showed that the *SOG1* expression was missing in *sog1-101* (Figure S9b). Moreover, the expression of *AtRAD51* and *AtBRCA1* in *sog1-101* was not induced by zeocin treatment, and root growth was tolerant to zeocin as

observed in *sog1-1* (Yoshiyama et al., 2013; Yoshiyama et al., 2014) (Figure S9c and S9d), indicating that *sog1-101* is a null allele.

We first tested bacterial resistance in these *sog1* mutant plants. *sog1* plants showed wild-type-like resistance against *Pst* DC3000, whereas *pad4-1* plants that are defective in SA signaling showed high susceptibility as described previously (Jirage et al., 1999) (Figure S10). These results imply that bacterial resistance is largely retained in the absence of SOG1.

Next, we examined the response of *sog1* mutants to the hemi-biotrophic fungus *Colletotrichum higginsianum* (*Ch*), which infects *Brassicaceae* species including *Arabidopsis thaliana* and generates anthracnose lesions on the infected tissues (O'Connell et al., 2012). When true leaves were inoculated with *Ch* spores, the anthracnose lesion area was significantly larger in *sog1-1* and *sog1-101* plants than that in wild-type (Figure 7a and 7b). Determination of the mRNA levels for *Ch ACTIN* (*ChACT*) revealed a significant increase in the fungal biomass in *sog1-101* plants as compared to the wild-type plants (Figure 7c). These results demonstrate that SOG1 contributes to *Ch* resistance.

DISCUSSION

In animals, p53 plays a central role in cell cycle regulation, DNA repair, cellular homeostasis, and apoptosis in response to DNA damage (Bieging et al., 2014). However, plants have no p53 orthologue; instead, the plant-specific NAC-type transcription factor SOG1 triggers DNA damage response (Yoshiyama et al., 2014). In this study, we identified 146 *Arabidopsis* genes that are responsive to DSBs and directly controlled by SOG1.

Among the SOG1 target genes, there were negative regulators of the cell cycle, such as *KRP6*, *SMR4*, *SMR5*, *SMR7*, and *WEE1*. *KRP* and *SMR* encode CDK inhibitors that bind to CDK-cyclin complexes and inhibit kinase activity (Nakai et al., 2006; Van Leene et al., 2010; Guérinier et al., 2013; Yi et al., 2014). WEE1 kinase phosphorylates and inactivates CDKs (De Schutter et al., 2007). Therefore, it is likely that DNA damage immediately reduces CDK activities under the control of SOG1. However, we have recently reported that such early responses to DNA damage are not sufficient to arrest the cell cycle. This is because *Arabidopsis* mutants with defects in repressing the G2/M-specific genes were tolerant to DSBs, regardless of high induction of *SMR*s (Chen et al., 2017). In the list of SOG1 target genes, there are 21 transcription factors; thus, it is probable that they control other cell cycle regulators and trigger cell cycle arrest. p53 also induces the expression of negative cell cycle regulators, such as the CDK inhibitor $p21^{Cip1/Waf1}$ (El-Deiry et al., 1993; Harper et al., 1993; Kenzelmann Broz et al., 2013). Moreover, p53 downregulates the genes for Cdc25C phosphatases, which antagonize inactivating phosphorylation by WEE1 (Clair et al., 2004). Although plants lack functional orthologues of Cdc25 (Boudolf et al., 2006), these observations suggest that SOG1 and p53 have a similar function in inducing CDK inhibitors and enhancing inactivating phosphorylation on CDKs in response to DNA damage.

SOG1 also targets DNA repair-related genes, such as those for Retinoblastoma binding protein 8 (Rbbp8), Replication factor C (RFC), RPA, BRCA1, RAD51, and the orthologue of *Schizosaccharomyces pombe* RAD17. In DSB repair, Rbbp8 resects DNA to generate ssDNA; RPA then binds to ssDNA to coat the exposed DNA (Schwertman et al., 2016). RFC and RAD17 are recruited to RPA-coated ssDNA and stimulate ATR activity to promote the HR pathway (Jazayeri et al., 2006). BRCA1 is important for the exchange of

RPA with RAD51 on ssDNA (Prakash et al., 2015), leading to the activation of RAD51/RAD54-facilitated DNA repair by HR (Sugawara et al., 2003). The enrichment of HR-related factors in the SOG1 target genes suggests that SOG1 preferentially activates HR in response to DSBs. Indeed, the number of cells indicating RAD54 foci where HR might occur in chromosomes was increased after DSB treatment in the SOG1-dependent manner (Hirakawa et al., 2017). On the other hand, p53 target genes include those involved in other DNA repair pathways, such as genes for MutS homolog 6 (Msh6), Msh2, Excision repair cross-complementation group 5 (Ercc5), Damage-specific DNA binding protein 2 (Ddb2), xeroderma pigmentosum, complementation group C (Xpc), and Ku86 (Kenzelmann Broz et al., 2013; Bieging et al., 2014). Msh6 and Msh2 are crucial for DNA mismatch repair (MMR), and Ercc5, Ddb2, and Xpc are required for nucleotide excision repair (NER) (Bouwman and Jonkers, 2012). Ku86 functions in non-homologous end-joining (NHEJ) (Myung et al., 1998; Bouwman and Jonkers, 2012). Considering that SOG1 and p53 regulate different sets of DNA repair-related genes, it is probable that plants and animals have distinct preferences for activating DNA repair pathways.

In mammals, severe DNA damage usually causes cell death (Jackson and Bartek, 2009). Caspases are endoproteases involved in DNA damage-induced cell death (Tait and Green, 2010). It was demonstrated that p53 plays a crucial role in caspase-mediated programmed cell death through the induction of several key pro-apoptotic factors, such as BCL2 associated X protein (Bax), BCL2 binding component 3 (Bbc3/Puma), Phorbol-12-myristate-13-acetate-induced protein 1 (Pmaip1/Noxa), and Fas cell surface death receptor (Fas) (Chipuk and Green, 2006; Kenzelmann Broz et al., 2013). On the other hand, plants possess no orthologous gene encoding caspases. However, plants also undergo cell

death in response to DNA damage, though specifically in stem cells and their daughter cells, and this response is dependent on SOG1 (Fulcher and Sablowski, 2009; Furukawa et al., 2010; Yoshiyama et al., 2013). Therefore, it is conceivable that the SOG1-mediated pathway controls the expression of key factors triggering cell death. However, in the present study, we could not find any apoptosis-related gene in the SOG1 target genes, probably because SOG1-dependent cell death occurs only in stem cells and their daughters. Nonetheless, there is another possibility that apoptosis-related genes are regulated indirectly from SOG1. Metacaspases share structural similarities to caspases but lack the endoprotease activity (Vercammen et al., 2004; Watanabe and Lam 2005). Among nine metacaspase genes in *Arabidopsis*, *AtMC8* encoding a type II metacaspase is highly induced by oxidative stress, and its knockout mutant exhibited a reduced level of cell death after UVC radiation or hydrogen peroxide treatment (He et al., 2008). However, DSB-induced cell death occurred in the *atmc8* mutant similarly to the wild-type (Fulcher and Sablowski, 2009), suggesting that other metacaspases are involved in the response to DSBs. This possibility is supported by the public microarray data showing that several metacaspase genes are induced 12 h after bleomycin and MMC treatment (Table S6). We could not find any metacaspase gene in the SOG1 target genes, suggesting a possibility that metacaspase gene(s) are indirectly regulated by SOG1 and involved in DNA damage-induced stem cell death.

Previous studies showed that ANAC019, ANAC055, and ANAC072 recognize the core sequence CGT[G/A] (Tran et al., 2004), while several other NAC-type transcription factors bind to different sequences (Zhong et al., 2010; Wu et al., 2012; Sakuraba et al., 2015). In this study, we found that 286 DSB-induced genes are indirectly regulated by SOG1 (Figure 1a). Moreover, we showed that over half of the SOG1 target genes possessed single

or multiple CTT$(N)_7$AAG consensus sequences within 1-kb promoter regions, whereas the motif was missing in 72 target genes (Figure 4c and Table S2), suggesting a possibility that they are also indirectly regulated by SOG1. It is known that NAC-type transcription factors form homo- and heterodimers (Xie et al., 2000; Ernst et al., 2004; Mitsuda et al., 2004; Yamaguchi et al., 2008; Gladman et al., 2016). Therefore, it is likely that SOG1 forms heterodimers with other NAC proteins which recognize DNA sequences distinct from the SOG1-binding motif. Several NAC proteins are included in the SOG1 target genes; thus, they are good candidates for SOG1 partners that mediate SOG1 binding to promoters without the consensus motif.

Consistent with previous studies that DNA damage substantially occurs during pathogen challenge and that ATM and ATR are required for pathogen resistance (Song and Bent, 2014), this study revealed that defense-related genes represent a large portion of the SOG1 target genes. In line with this, we presented evidence that SOG1 contributes to fungal resistance against *C. higginsianum*. However, we did not detect significant alterations in bacterial resistance in the absence of SOG1. In this respect, previous studies reported that bacterial resistance is compromised in the absence of ATM and/or ATR (Song and Bent, 2014; Yan et al., 2013). The apparent discrepancy might reflect the existence of a SOG1-independent branch, which also mediates DNA damage responses and bacterial resistance downstream of ATM and ATR. Indeed, ATM and ATR phosphorylate not only SOG1 but also the proteins involved in DNA repair (LIG4 and MRE11), chromatin remodeling (PIE1 and SDG26), DNA replication (PCNA1, WAPL, and PDS5), and meiosis (ASK1 and HTA1) (Roitinger et al., 2015). Some of the DSB repair proteins and chromatin remodeling factors are known to participate in plant immunity (Fu and Dong, 2013; Berriri et

al., 2016), raising the possibility that SOG1-independent pathways are involved in bacterial resistance. Nevertheless, our findings strengthen the view that DNA damage induced during pathogen challenge involves SOG1 to directly establish defense-related transcriptome that underlies effective pathogen resistance.

EXPERIMENTAL PROCEDURES

Plant materials and growth conditions

The Col-0 accession of *Arabidopsis thaliana* was used in this study. *sog1-1* (Yoshiyama et al., 2009), *pad4-1* (Jirage et al., 1999), *proSOG1:SOG1-MYC*, *sog1-1 proSOG1:SOG1-MYC*, and *sog1-1 proSOG1:SOG1*(*5A*)*-MYC* (Yoshiyama et al., 2013) were described previously. *sog1-101* (GABI_602B10) was obtained from The Nottingham *Arabidopsis* Stock Centre (NASC) (http://arabidopsis.info). Homozygous *sog1-101* plants were identified by PCR using the primers listed in Table S7. Plants were grown in Murashige and Skoog (MS) medium [0.5 x MS salts, 0.5 g/l 2-(N-morpholino)ethanesulfonic acid (MES), 1% sucrose, and 1 x vitamin (pH 6.3)]. To make the transcriptional reporter constructs, the 2-kb promoter regions of *AtRAD51* and *AtBRCA1* were PCR-amplified from *Arabidopsis* genomic DNA and cloned into pDONR221 (Thermo Fisher Scientific) by BP recombination reaction according to the manufacturer's instructions (Thermo Fisher Scientific). Primers used for PCR are listed in Table S7. Nucleotide substitutions on the *AtRAD51* promoter were introduced by PCR using primers listed in Table S7. To generate the fusion construct with *GUS*, an LR reaction (Thermo Fisher Scientific) was performed with the destination vector pGWB3 (Nakagawa et al., 2007). All constructs were transferred into

the *Agrobacterium tumefaciens* GV3101 strain harboring the plasmid pMP90. The obtained strains were used to generate stably transformed *Arabidopsis* by the floral dip transformation method (Clough and Bent, 1998).

Chromatin immunoprecipitation

ChIP was performed as previously described with minor modifications (Gendrel et al., 2005). *proSOG1:SOG1-MYC* seeds were germinated in 100 mL of liquid MS medium, and cultured under continuous light at 23 ℃ with gentle shaking (50 rpm). After a 14-d culture period, zeocin was added to the medium giving a final concentration of 15 μM, and the seedlings were cultured for 2 h. Chromatin bound to the SOG1-Myc fusion protein was precipitated with anti-Myc antibody (clone 4A6, Millipore). Library preparation for the sequencing of precipitated DNAs was performed using NEBNext Ultra DNA Library Prep Kit (New England BioLabs) and a Genomic DNA Sample Prep Oligo Only Kit (Illumina). Prepared libraries were deep-sequenced using an Illumina Genome Analyzer IIx, and then 33-bp reads were output. Obtained reads were used for following Chip-seq analysis under StrandNGS (Starnd) environment. Reads were mapped to Arabidopsis thaliana TAIR10 reference genome using COBWeb algorithm. Peak calling was taken place using MACS peak detection algorithm with following setting: Average Fragment Length (bp) = 150 p-value, Cutoff = 9.999999747378752E-6, Enrichment Factor = 16, Detect local biases = true. And then genes located within 5 kb from detected peaks were listed. The data files are available on the Gene Expression Omnibus (GEO) website (https://www.ncbi.nlm.nih.gov/geo/) (GEO

accession number GSE106415). The processed data were visualized with The Integrative Genome Browser software (Robinson et al., 2011).

ChIP-qPCR was performed using immunoprecipitated DNA. Three independent ChIP experiments were conducted to validate the identified SOG1 target genes. To quantify the precipitated chromatin, real-time qPCR was performed using gene-specific primers listed in Table S7. The primer set for *Mutator-like transposon* (*Mut*) was used as negative control (Sauret-Güeto et al., 2013). PCR reactions were conducted with the LightCycler 480 Real-Time PCR system (ROCHE) under the following conditions: 95 ℃ for 5 min; 60 cycles at 95 ℃ for 10 sec, at 58 ℃ for 10 sec, and at 72 ℃ for 15 sec.

Microarray

The seeds were germinated in 100 mL of liquid MS medium, and cultured under continuous light at 23 ℃ with gentle shaking (50 rpm). After a 14-d culture period, zeocin was added to the medium giving a final concentration of 15 μM, and the seedlings were cultured for 2 h. Total RNA was extracted using the Plant Total RNA Mini Kit (FAVORGEN). Microarray analysis was performed using an Agilent *Arabidopsis* microarray platform as previously described. Cyanine-3 (Cy3) labeled cDNA, obtained from total RNA, was hybridized to Agilent-034592 *Arabidopsis* Custom Microarray. The slide scanning was performed by Agilent DNA Microarray Scanner (G2539A ver.C). Our microarray data are available on the GEO website (GEO accession number GSE106154).

Microarray data raw files were obtained from AtGenExpress consortium (Kilian et al., 2007). Affymetrix Gene Chip-based raw data were analyzed using Robust Multichip Average (RMA) algorithms (Irizarry et al., 2003) with R (ver. 3.0.1) (R Core Team, 2013). The expression patterns were clustered with Cluster 3.0 (De Hoon et al., 2004), and visualized on Java TreeView (ver. 1.1.6r4) (Saldanha, 2004).

Gene ontology analysis

GO analysis was performed using agriGO (ver. 1.2) program (Du et al., 2010). BiNGO (ver. 3.03) (Maere et al., 2005) plugin in Cytoscape (ver. 3.3) platform (Smoot et al., 2011) was used to compare the target genes of SOG1 and p53. Gene ontology and annotation files for *Arabidopsis thaliana* and *Mus musculus* were obtained from the Gene Ontology Consortium (http://www.geneontology.org). The statistical enrichment analysis for BiNGO was based on a hypergeometric test, and *p*-values were given by Benjamini & Hochberg false discovery rate (FDR) correction.

Quantitative RT-PCR

Total RNA was extracted using the Plant Total RNA Mini Kit (FAVORGEN). First-strand cDNA was prepared from total RNA using ReverTra Ace® (Toyobo) according to the manufacturer's instructions. Quantitative RT-PCR was performed with a THUNDERBIRD SYBR qPCR Mix (Toyobo) containing 100 nM primers and 0.1 µg of first-strand cDNAs. Primer sequences are listed in Table S7. PCR reactions were conducted

with the LightCycler 480 Real-Time PCR system (ROCHE) under the following conditions: 95 °C for 5 min; 55 cycles at 95 °C for 10 sec, at 58 °C for 10 sec, and at 72 °C for 15 sec.

GUS staining

Seedlings were incubated in GUS staining solution [100 mM sodium phosphate, 1 mg/ml 5-bromo-4-chloro-3-indolyl ß-D-glucuronide, 0.5 mM ferricyanide, and 0.5 mM ferrocyanide (pH 7.4)] for 9 h at 37 °C in the dark. Shoots were fixed with 90 % (v/v) acetone for 1 h and then incubated in GUS staining solution as describe above. The samples were cleared with a transparent solution [chloral hydrate, glycerol and water (8 g:1 ml:1 ml)] and observed under a light microscope, Axioakop 2 Plus (Zeiss) and SZX16 (OLYMPUS).

Prediction of SOG1-binding consensus sequence

The sequences within 1 kb upstream from the TSS of SOG1 target genes were analyzed using R (ver. 3.0.1), and then subjected to the MEME program (Bailey et al., 2006) and the Regulatory Sequence Analysis Tools (RSAT)-spaced dyad tool (Helden et al., 2000). The putative SOG1-binding sequence was visualized by WebLogo 3 software (Crooks et al., 2004).

In vitro dsDNA-protein interaction assay

In vitro interaction between dsDNA and the SOG1 protein was performed as previously described (Tokizawa et al., 2015). The FLAG-tagged SOG1 proteins were synthesized using an *in vitro* transcription/translation system (BioSieg). Both biotinylated and nonbiotinylated DNA oligos used in assays are listed in Table S8. The donor and acceptor beads for the AlphaScreen detection were coated with the anti-FLAG antibody and streptavidin, respectively. The beads were labeled with the FLAG-tagged SOG1 protein or the biotinylated dsDNA-oligo(s) using the AlphaScreen FLAG (M2) Detection Kit (PerkinElmer). Competition assays to identify the SOG1 binding motif were performed by adding mutated dsDNA-oligos to the reaction buffer containing the biotinylated dsDNA-oligo-labeled acceptor beads. The AlphaScreen signals which is intensity of chemiluminescence by the binding between the donor and the acceptor beads were determined with the Enspire Multimode plate reader (PerkinElmer).

Protoplast transactivation assay

To make the reporter construct, the 2 kb *AtRAD51* promoter with or without nucleotide substitutions of SOG1-binding motif was cloned into the pAGL vector (Endo et al., 2015) by LR recombination reaction (Thermo Fisher Scientific) to generate a transcriptional fusion gene with *firefly luciferase* (*fLUC*). Oligonucleotides consisting of four tandem repeats of $CTT(N)_7AAG$ or $TCC(N)_7GGA$ were synthesized and annealed by heating and subsequent gradual cooling (Table S7). Then the double-stranded DNA was cloned into the GAL4 UAS:TATA:LUC reporter plasmid (Ohta et al., 2000), by *Hind*III and *Xba*I

digestion followed by ligation, to generate *4x[CTT(N)$_7$AAG]-35Smini:fLUC* and *4x[TCC(N)$_7$GGA]-35Smini:fLUC*. To make *35Smini:LUC*, the reporter plasmid GAL4 UAS:TATA:LUC (Ohta et al., 2000) was digested with *Hin*dIII and *Xba*I to remove GAL4UAS, followed by blunting and self-ligation. To generate the effector constructs, the coding regions of *sGFP* (negative control), *SOG1*, and *SOG1(5A)* (Yoshiyama et al., 2013) were cloned into the pA35S vector (Endo et al., 2015) to generate transcriptional fusion genes with the cauliflower mosaic virus (CaMV) 35S promoter. Protoplasts prepared from 6-week-old wild-type and *sog1-1* leaves were cotransfected with the reporter plasmid, the effector plasmids, and the normalization construct carrying the *Renilla reniformis luciferase* (*rLUC*) gene under the *CaMV 35S* promoter (Ohta et al., 2000). Protoplast transfection was performed as previously described (Yoo et al., 2007; Wu et al., 2009). After transfection, protoplasts were incubated at 22 °C for 15 h, and fLUC and rLUC activities were measured with the Dual-Luciferase reporter system (Promega) according to the manufacturer's instructions using a luminometer TriStar^2 LB942 (Berthold).

Pathogen infection assay

Inoculation with bacterial pathogens was performed as previously described (Lu et al., 2009). True leaves of five-week-old plants grown under short-day conditions (10 h light/14 h dark) at 22°C were syringe-infiltrated with *Pst* DC3000 suspension (OD_{600} = 0.0002). After 3 days, leaf discs were collected and ground in 10 mM $MgCl_2$. The extracts were cultured on NYGA plates [0.5 % peptone, 0.3 % yeast extract, 2 % glycerol, and 1.5 % agar] with 50 µg/mL rifampicin for 2 days at 28 ˚C, and then the number of colonies was

recorded. Infection assays with the fungal pathogen *C. higginsianum* were performed as previously described (Hiruma et al. 2013). Spore suspension (2.5×10^5) was dropped onto leaves of five-week-old plants. The inoculated plants were then grown under high humidity conditions for five to seven days. The lesion areas were measured using ImageJ software (Schneider et al., 2012). For the *C. higginsianum* biomass assay, total RNA was isolated from inoculated leaves at 5 and 7 days post-inoculation using the Plant Total RNA Mini Kit (FAVORGEN). First-strand cDNA was prepared from total RNA using ReverTra Ace® (Toyobo). Fungal biomass was assessed with the mRNA levels of *C. higginsianum ACTIN* gene relative to that of *Arabidopsis ACTIN2* gene. Primer sequences are listed in Table S7. PCR was conducted with the LightCycler 480 Real-Time PCR system (ROCHE) under the following conditions: 95 °C for 5 min; 55 cycles at 95 °C for 10 sec, at 58 °C for 10 sec, and at 72 °C for 15 sec.

ACCESSION NUMBERS

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: *SOG1* (AT1G25580), *AtRAD51* (AT5G20850), *AtBRCA1* (AT4G21070), *SMR7* (AT3G27630), *AtRAD17* (AT5G66130), *AtPARP2* (AT4G02390), *OXI1* (AT3G25250), *TRFL10* (AT5G03780), *SYN2* (AT5G40840), *DREB19* (AT2G38340), *CYP96A11* (AT4G39500), *POLD4* (AT1G09815), *AGO2* (AT1G31280), *TFIIB* (AT2G41630), *KRP6* (AT3G19150), *SAG101* (AT5G14930), *UBP21* (AT5G46740), *AtRPA1A* (AT2G06510), *ACT2* (AT3G18780) and *ChACT*

(CH63R_04240). Raw data of ChIP-Seq and microarray has been deposited in the GEO database under accession number GSE106415 and GSE106154, respectively.

ACKNOWLEDGMENTS

We would like to thank Anne B. Britt for *sog1-1*. We would also like to thank Ryosuke Sano for ChIP-Seq data management. This work was supported by MEXT KAKENHI (Grant numbers 16H01243, 17H03965, 17H06477, and 17K15141) and JST, CREST (Grant numbers JPMJCR12B2 and JPMJCR13B4).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

SHORT LEGENDS FOR SUPPORTING INFORMATION

Figure S1. ChIP-sequencing signal in *SMR5*, *SMR7*, *CYCB1;1, AtRAD51,* and *AtBRCA1* loci.

Figure S2. SOG1 is required for the activation of *AtRAD51* and *AtBRCA1* promoters upon zeocin treatment.

Figure S3. Validation of SOG1 target genes identified by ChIP-sequencing and microarray analysis.

Figure S4. SOG1 target genes respond to abiotic and biotic stresses.

Figure S5. The positions of the CTT(N)$_7$AAG motif in the SOG1 target promoters.

Figure S6. Overrepresented GO terms in SOG1 target genes with or without the CTT(N)$_7$AAG motif.

Figure S7. SOG1 is activated in wild-type protoplasts

Figure S8. The CTT(N)$_7$AAG motif is important for the activation of *AtBRCA1* promoter upon zeocin treatment.

Figure S9. Characterization of the *sog1-101* mutant.

Figure S10. Susceptibility of *sog1* mutants to bacterial pathogen.

Table S1. List of SOG1-regulated genes.

Table S2. List of SOG1 target genes.

Table S3. Overrepresented GO terms identified in SOG1 target genes by agriGO.

Table S4. Overrepresented GO terms identified in SOG1 target genes by BiNGO.

Table S5. Overrepresented GO terms identified in p53 target genes by BiNGO.

Table S6. Expression profiles of *Arabidopsis* metacaspase genes

Table S7. List of primers used in this study.

Table S8. List of oligos used in *in vitro* protein-dsDNA interaction assay.

REFERENCES

Adachi, S., Minamisawa, K., Okushima, Y., Inagaki, S., Yoshiyama, K., Kondou, Y., Kaminuma, E., Kawashima, M., Toyoda, T., Matsui, M., Kurihara, D., Matsunaga, S., and Umeda, M. (2011) Programmed induction of endoreduplication by DNA double-strand breaks in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA*, **108**, 10004–10009.

Aida, M., Ishida, T., Fukaki, H., Fujisawa, H., and Tasaka, M. (1997) Genes involved in organ separation in Arabidopsis: an analysis of the *cup-shaped cotyledon* mutant. *Plant Cell*, **9**, 841–857.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res*. **25**, 3389–3402.

Bailey, T.L., Williams, N., Misleh, C., and Li, W.W. (2006) MEME: discovering and analyzing DNA and protein sequence motifs. *Nucleic Acids Res*. **34**, W369–W373.

Banin, S., Moyal, L., Shieh, S.Y., Taya, Y., Anderson, C.W., Chessa, L., Smorodinsky, N.I., Prives, C., Reiss, Y., Shiloh, Y., and Ziv, Y. (1998) Enhanced phosphorylation of p53 by ATM in response to DNA damage. *Science*, **281**, 1674–1677.

Belyi, V.A., Ak, P., Markert, E., Wang, H., Hu, W., Puzio-Kuter, A., and Levine, A. J. (2010) The origins and evolution of the p53 family of genes. *Cold Spring Harb. Perspect. Biol*. **2**, a001198.

Berriri, S., Gangappa, S.N., and Kumar, S.V. (2016) SWR1 Chromatin-Remodeling Complex Subunits and H2A. Z Have Non-overlapping Functions in Immunity and Gene Regulation in *Arabidopsis*. *Mol. Plant*, **9**, 1051–1065.

Bieging, K.T., Mello, S.S., and Attardi, L.D. (2014) Unravelling mechanisms of p53-mediated tumour suppression. *Nat. Rev. Cancer*, **14**, 359–370.

Bonneau, L., Ge, Y., Drury, G.E., and Gallois, P. (2008) What happened to plant caspases? *J. Exp. Bot*. **59**, 491–499.

Boudolf, V., Inzé, D., and De Veylder, L. (2006) What if higher plants lack a CDC25 phosphatase? *Trends Plant Sci*. **11**, 474–479.

Bouwman, P., and Jonkers, J. (2012) The effects of deregulated DNA damage signalling on cancer chemotherapy response and resistance. *Nat. Rev. Cancer*, **12**, 587–598.

Canman, C.E., Lim, D.S., Cimprich, K.A., Taya, Y., Tamai, K., Sakaguchi, K., Appella, E., Kastan, M.B., and Siliciano, J.D. (1998) Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. *Science*, **281**, 1677–1679.

Chankova, S.G., Dimova, E., Dimitrova, M., and Bryant, P.E. (2007) Induction of DNA double-strand breaks by zeocin in *Chlamydomonas reinhardtii* and the role of increased DNA double-strand breaks rejoining in the formation of an adaptive response. *Radiat. Environ. Biophys*. **46**, 409–416.

Chen, P., and Umeda, M. (2015) DNA double□strand breaks induce the expression of flavin□containing monooxygenase and reduce root meristem size in *Arabidopsis thaliana*. *Genes Cells*, **20**, 636–646.

Chen, P., Takatsuka, H., Takahashi, N., Kurata, R., Fukao, Y., Kobayashi, K., Ito M., and Umeda M. (2017) *Arabidopsis* R1R2R3-Myb proteins are essential for cell cycle arrest in response to DNA damage. *Nat. Commun*. **8**, 635.

Chipuk, J.E., and Green, D.R. (2006) Dissecting p53-dependent apoptosis. *Cell Death Differ*. **13**, 994–1002.

Clair, S.S., Giono, L., Varmeh-Ziaie, S., Resnick-Silverman, L., Liu, W.J., Padi, A., Dastidar, J., DaCosta, A., Mattia, M., and Manfredi, J.J. (2004) DNA damage-induced downregulation of Cdc25C is mediated by p53 via two independent mechanisms: one involves direct binding to the *cdc25C* promoter. *Mol. Cell*, **16**, 725–736.

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

Crooks, G.E., Hon, G., Chandonia, J.M., and Brenner, S.E. (2004) WebLogo: a sequence logo generator. *Genome Res*. **14**, 1188–1190.

De Hoon, M.J., Imoto, S., Nolan, J., and Miyano, S. (2004) Open source clustering software. *Bioinformatics*, **20**, 1453–1454.

De Schutter, K., Joubès, J., Cools, T., Verkest, A., Corellou, F., Babiychuk, E., Van Der Schueren, E., Beeckman, T., Kushnir, S., Inzé, D., and De Veylder, L. (2007) *Arabidopsis* WEE1 kinase controls cell cycle arrest in response to activation of the DNA integrity checkpoint. *Plant Cell*, **19**, 211–225.

De Veylder, L., Beeckman, T., Beemster, G.T., Krols, L., Terras, F., Landrieu I, van der Schueren E, Maes S, Naudts M, and Inzé, D. (2001) Functional analysis of cyclin-dependent kinase inhibitors of Arabidopsis. *Plant Cell*, **13**, 1653–1668.

Du, Z., Zhou, X., Ling, Y., Zhang, Z., and Su, Z. (2010) agriGO: a GO analysis toolkit for the agricultural community. *Nucleic Acids Res*. **38**, W64–W70.

El-Deiry, W.S., Tokino, T., Velculescu, V.E., Levy, D.B., Parsons, R., Trent, J.M., Lin, D., Mercer, W.E., Kinzler, K.W., and Vogelstein, B. (1993) WAF1, a potential mediator of p53 tumor suppression. *Cell*, **75**, 817–825.

Endo, H., Yamaguchi, M., Tamura, T., Nakano, Y., Nishikubo, N., Yoneda, A., Kato, K., Kubo, M., Kajita, S., Katayama, Y., Ohtani, M., and Demura, T. (2015) Multiple classes of transcription factors regulate the expression of *VASCULAR-RELATED NAC-DOMAIN7*, a master switch of xylem vessel differentiation. *Plant Cell Physiol*. **56**, 242–254.

Ernst, H.A., Olsen, A.N., Skriver, K., Larsen, S., and Leggio, L.L. (2004) Structure of the conserved domain of ANAC, a member of the NAC family of transcription factors. *EMBO Rep*. **5**, 297–303.

Feys, B.J., Wiermer, M., Bhat, R.A., Moisan, L.J., Medina-Escobar, N., Neu, C., Cabral, A., and Parker, J.E. (2005) *Arabidopsis* SENESCENCE-ASSOCIATED GENE101 stabilizes and signals within an ENHANCED DISEASE SUSCEPTIBILITY1 complex in plant innate immunity. *Plant Cell*, **17**, 2601–2613.

Fu, Z.Q., and Dong, X. (2013) Systemic acquired resistance: turning local infection into global defense. Annu. Rev. Plant Biol. **64**, 839-863.

Fulcher, N., and Sablowski, R. (2009) Hypersensitivity to DNA damage in plant stem cell niches. *Proc. Natl. Acad. Sci. USA*, **106**, 20984–20988.

Furukawa, T., Curtis, M.J., Tominey, C.M., Duong, Y.H., Wilcox, B.W., Aggoune, D., Hays, J.B., and Britt, A.B. (2010) A shared DNA-damage-response pathway for induction of stem-cell death by UVB and by gamma irradiation. *DNA repair*, **9**, 940–948.

Gao, Q.M., Venugopal, S., Navarre, D., and Kachroo, A. (2011) Low oleic acid-derived repression of jasmonic acid-inducible defense responses requires the WRKY50 and WRKY51 proteins. *Plant Physiol.* **155**, 464–476.

Gendrel, A.V., Lippman, Z., Martienssen, R., and Colot, V. (2005) Profiling histone modification patterns in plants using genomic tiling microarrays. *Nat. Methods*, **2**, 213–218.

Gladman, N.P., Marshall, R.S., Lee, K.H., and Vierstra, R.D. (2016) The proteasome stress regulon is controlled by a pair of NAC transcription factors in Arabidopsis. *Plant Cell*, **28**, 1279–1296.

Guérinier, T., Millan, L., Crozet, P., Oury, C., Rey, F., Valot, B., Mathieu, C., Vidal, J., Hodges, M., Thomas, M., and Glab, N. (2013) Phosphorylation of $p27^{KIP1}$ homologs KRP6 and 7 by SNF1□related protein kinase–1 links plant energy homeostasis and cell proliferation. *Plant J.* **75**, 515–525.

Harper, J.W., Adami, G.R., Wei, N., Keyomarsi, K., and Elledge, S.J. (1993) The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell*, **75**, 805–816.

He, R., Drury, G.E., Rotari, V.I., Gordon, A., Willer, M., Farzaneh, T., Woltering, E.J., and Gallois, P. (2008) Metacaspase-8 modulates programmed cell death induced by ultraviolet light and H_2O_2 in *Arabidopsis*. *J. Biol. Chem*. **283**, 774–783.

Helden, J.V., Rios, A., and Collado-Vides, J. (2000) Discovering regulatory elements in non-coding sequences by analysis of spaced dyads. *Nucleic Acids Res*. **28**, 1808–1818.

Hirakawa, T., Hasegawa, J., White, C.I., and Matsunaga, S. (2017) RAD54 forms DNA repair foci in response to DNA damage in living plant cells. *Plant J*. **90**, 372–382.

Hirao, A., Kong, YY., Matsuoka, S., Wakeham, A., Ruland, J., Yoshida, H., Liu, D., Elledge, S.J., and Mak, T.W. (2000) DNA damage-induced activation of p53 by the checkpoint kinase Chk2. *Science*, **287**, 1824–1827.

Hiruma, K., Fukunaga, S., Bednarek, P., Piślewska-Bednarek, M., Watanabe, S., Narusaka, Y., Shirasu, K., and Takano, Y. (2013) Glutathione and tryptophan metabolism are required for *Arabidopsis* immunity during the hypersensitive response to hemibiotrophs. *Proc. Natl. Acad. Sci. USA*, **110**, 9589–9594.

Hu, Z., Cools, T., and De Veylder, L. (2016) Mechanisms used by plants to cope with DNA damage. *Annu. Rev. Plant Biol*. **67**, 439–462.

Irizarry, R.A., Hobbs, B., Collin, F., Beazer-Barclay, Y.D., Antonellis, K.J., Scherf, U., and Speed, T.P. (2003) Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics*, **4**, 249–264.

Jackson, S.P., and Bartek, J. (2009) The DNA-damage response in human biology and disease. *Nature*, **461**, 1071–1078.

Jazayeri, A., Falck, J., Lukas, C., Bartek, J., Smith, G.C., Lukas, J., and Jackson, S.P. (2006) ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. *Nat. Cell Biol*. **8**, 37–45.

Jirage, D., Tootle, T.L., Reuber, T.L., Frost, L.N., Feys, B.J., Parker, J.E., Ausubel, F.M., and Glazebrook, J. (1999) *Arabidopsis thaliana PAD4* encodes a lipase-like gene that is important for salicylic acid signaling. *Proc. Natl. Acad. Sci. USA*, **96**, 13583–13588.

Kenzelmann Broz, D., Spano Mello, S., Bieging, K.T., Jiang, D., Dusek, R.L., Brady, C.A., Sidow, A., and Attardi, L.D. (2013) Global genomic profiling reveals an extensive p53-regulated autophagy program contributing to key p53 responses. *Genes Dev*. **27**, 1016–1031.

Kilian, J., Whitehead, D., Horak, J., Wanke, D., Weinl, S., Batistic, O., D'Angelo, C., Bornberg-Bauer, E., Kudla, J., and Harter, K. (2007) The AtGenExpress global stress expression data set: protocols, evaluation and model data analysis of UV□B light, drought and cold stress responses. *Plant J*. **50**, 347–363.

Kim, H.J., Nam, H.G., and Lim, P.O. (2016) Regulatory network of NAC transcription factors in leaf senescence. *Curr. Opin. Plant Biol*. **33**, 48–56.

Kiyosue, T., Yamaguchi-Shinozaki, K., and Shinozaki, K. (1994) Characterization of two cDNAs (ERD10 and ERD14) corresponding to genes that respond rapidly to dehydration stress in *Arabidopsis thaliana*. *Plant Cell Physiol*. **35**, 225–231.

Krishnaswamy, S., Verma, S., Rahman, M.H., and Kav, N.N. (2011) Functional characterization of four APETALA2-family genes (*RAP2.6, RAP2.6L, DREB19 and DREB26*) in *Arabidopsis*. *Plant Mol. Biol*. **75**, 107–127.

Kruse, J.P., and Gu, W. (2009) Modes of p53 regulation. *Cell*, **137**, 609–622.

Kubo, M., Udagawa, M., Nishikubo, N., Horiguchi, G., Yamaguchi, M., Ito, J., Mimura, T., Fukuda, H., and Demura, T. (2005) Transcription switches for protoxylem and metaxylem vessel formation. *Genes Dev*. **19**, 1855–1860.

Lee, S., Seo, P.J., Lee, H.J., and Park, C.M. (2012) A NAC transcription factor NTL4 promotes reactive oxygen species production during drought-induced leaf senescence in Arabidopsis. *Plant J*. **70**, 831–844.

Lu, X., Tintor, N., Mentzel, T., Kombrink, E., Boller, T., Robatzek, S., Robatzek, S., Schulze-Lefert, P., and Saijo, Y. (2009) Uncoupling of sustained MAMP receptor signaling from early outputs in an Arabidopsis endoplasmic reticulum glucosidase II allele. *Proc. Natl. Acad. Sci. USA*, **106**, 22522–22527.

Maere, S., Heymans, K., and Kuiper, M. (2005) BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. *Bioinformatics*, **21**, 3448–3449.

Meek, D.W. (2009) Tumour suppression by p53: a role for the DNA damage response? *Nat. Rev. Cancer*, **9**, 714–723.

Mishina, T.E., and Zeier, J. (2006) The Arabidopsis flavin-dependent monooxygenase FMO1 is an essential component of biologically induced systemic acquired resistance. *Plant Physiol.* **141**, 1666–1675.

Missirian, V., Conklin, P.A., Culligan, K.M., Huefner, N.D., and Britt, A.B. (2014) High atomic weight, high-energy radiation (HZE) induces transcriptional responses shared with conventional stresses in addition to a core "DSB" response specific to clastogenic treatments. *Front. Plant Sci*. **5**, 364.

Mitsuda, N., Hisabori, T., Takeyasu, K., and Sato, M.H. (2004) VOZ; isolation and characterization of novel vascular plant transcription factors with a one-zinc finger from *Arabidopsis thaliana*. *Plant Cell Physiol*. **45**, 845–854.

Myung, K., Braastad, C., He, D.M., and Hendrickson, E.A. (1998) KARP-1 is induced by DNA damage in a p53-and ataxia telangiectasia mutated-dependent fashion. *Proc. Natl. Acad. Sci. USA*, **95**, 7664–7669.

Nakagawa, T., Kurose, T., Hino, T., Tanaka, K., Kawamukai, M., Niwa, Y., Toyooka, K., Matsuoka, K., Jinbo, T., and Kimura, T. (2007) Development of series of gateway

binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. *J. Biosci. Bioeng*. **104**, 34–41.

Nakai, T., Kato, K., Shinmyo, A., and Sekine, M. (2006) *Arabidopsis* KRPs have distinct inhibitory activity toward cyclin D2-associated kinases, including plant-specific B-type cyclin-dependent kinase. *FEBS Lett*. **580**, 336–340.

O'Connell, R.J., Thon, M.R., Hacquard, S., Amyotte, S.G., Kleemann, J., Torres, M.F., Damm, U., Buiate, E.A., Epstein, L., Alkan, N., Altmüller, J., Alvarado-Balderrama, L., Bauser, C.A., Becker, C., Birren, B.W., Chen, Z., Choi, J., Crouch, J.A., Duvick, J.P., Farman, M.A., Gan, P., Heiman, D., Henrissat, B., Howard, R.J., Kabbage, M., Koch, C., Kracher, B., Kubo, Y., Law, A.D., Lebrun, M.H., Lee, Y.H., Miyara, I., Moore, N., Neumann, U., Nordström, K., Panaccione, D.G., Panstruga, R., Place, M., Proctor, R.H., Prusky, D., Rech, G., Reinhardt, R., Rollins, J.A., Rounsley, S., Schardl, C.L., Schwartz, D.C., Shenoy, N., Shirasu, K., Sikhakolli, U.R., Stüber, K., Sukno, S.A., Sweigard, J.A., Takano, Y., Takahara, H., Trail, F., van der Does, H.C., Voll, L.M., Will, I., Young, S., Zeng, Q., Zhang, J., Zhou, S., Dickman, M.B., Schulze-Lefert, P., Ver Loren van Themaat, E., Ma, L.J., and Vaillancourt, L.J. (2012) Lifestyle transitions in plant pathogenic *Colletotrichum* fungi deciphered by genome and transcriptome analyses. *Nat. Genet*. **44**, 1060–1065.

Ohta, M., Ohme□Takagi, M., and Shinshi, H. (2000) Three ethylene-responsive transcription factors in tobacco with distinct transactivation functions. *Plant J*. **22**, 29–38.

Prakash, R., Zhang, Y., Feng, W., and Jasin, M. (2015) Homologous recombination and human health: the roles of BRCA1, BRCA2, and associated proteins. *Cold Spring Harb. Perspect. Biol*. **7**, a016600.

Preuss, S.B., and Britt, A.B. (2003) A DNA-damage-induced cell cycle checkpoint in Arabidopsis. *Genetics*, **164**, 323–334.

Puranik, S., Sahu, P.P., Srivastava, P.S., and Prasad, M. (2012) NAC proteins: regulation and role in stress tolerance. *Trends Plant Sci*. **17**, 369–381.

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rentel, M.C., Lecourieux, D., Ouaked, F., Usher, S.L., Petersen, L., Okamoto, H., Knight, H., Peck, S.C., Grierson, C.S., Hirt, H., and Knight, M.R. (2004) OXI1 kinase is necessary for oxidative burst-mediated signalling in *Arabidopsis*. *Nature*, **427**, 858–861.

Robinson, J.T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E.S., Getz, G., and Mesirov, J.P. (2011) Integrative genomics viewer. *Nat. Biotechnol*. **29**, 24–26.

Roitinger, E., Hofer, M., Köcher, T., Pichler, P., Novatchkova, M., Yang, J., Schlögelhofer, P., and Mechtler, K. (2015) Quantitative phosphoproteomics of the ataxia telangiectasia-mutated (ATM) and ataxia telangiectasia-mutated and rad3-related (ATR) dependent DNA damage response in *Arabidopsis thaliana*. *Mol. Cell. Proteomics*, **14**, 556-571.

Sakuraba, Y., Kim, Y.S., Han, S.H., Lee, B.D., and Paek, N.C. (2015) The Arabidopsis transcription factor NAC016 promotes drought stress responses by repressing *AREB1* transcription through a trifurcate feed-forward regulatory loop involving NAP. *Plant Cell*, **27**, 1771–1787.

Sancar, A., Lindsey-Boltz, L.A., Ünsal-Kaçmaz, K., and Linn, S. (2004) Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu. Rev. Biochem*. **73**, 39–85.

Sauret-Güeto, S., Schiessl, K., Bangham, A., Sablowski, R., and Coen, E. (2013) *JAGGED* controls *Arabidopsis* petal growth and shape by interacting with a divergent polarity field. *PLoS Biol*. **11**, e1001550.

Saldanha, A.J. (2004) Java Treeview—extensible visualization of microarray data. *Bioinformatics*, **20**, 3246–3248.

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

Schwertman, P., Bekker-Jensen, S., and Mailand, N. (2016) Regulation of DNA double-strand break repair by ubiquitin and ubiquitin-like modifiers. *Nat. Rev. Mol. Cell Biol*. **17,** 379–394.

Shieh, S.Y., Ahn, J., Tamai, K., Taya, Y., and Prives, C. (2000) The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites. *Genes Dev*. **14**, 289–300.

Shim, J.S., Jung, C., Lee, S., Min, K., Lee, Y.W., Choi, Y., Lee, J.S., Song, J.T., Kim, J.K., and Choi, Y.D. (2013) *AtMYB44* regulates *WRKY70* expression and modulates antagonistic interaction between salicylic acid and jasmonic acid signaling. *Plant J*. **73**, 483–495.

Sjogren, C.A., Bolaris, S.C., and Larsen, P.B. (2015) Aluminum-dependent terminal differentiation of the Arabidopsis root tip is mediated through an ATR-, ALT2-, and SOG1-regulated transcriptional response. *Plant Cell*, **27**, 2501–2515.

Smoot, M.E., Ono, K., Ruscheinski, J., Wang, P.L., and Ideker, T. (2011) Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics*, **27**, 431–432.

Song, J., and Bent, A.F. (2014) Microbial pathogens trigger host DNA double-strand breaks whose abundance is reduced by plant defense responses. *PLoS Pathog*. **10**, e1004030.

Sugawara, N., Wang, X., and Haber, J.E. (2003) In vivo roles of Rad52, Rad54, and Rad55 proteins in Rad51-mediated recombination. *Mol. Cell*, **12**, 209–219.

Tait, S.W., and Green, D.R. (2010) Mitochondria and cell death: outer membrane permeabilization and beyond. *Nat. Rev. Mol. Cell Biol*. **11**, 621–632.

Tibbetts, R.S., Brumbaugh, K.M., Williams, J.M., Sarkaria, J.N., Cliby, W.A., Shieh, S.Y., Taya, Y., Prives, C., and Abraham, R.T. (1999) A role for ATR in the DNA damage-induced phosphorylation of p53. *Genes Dev*. **13**, 152–157.

Tokizawa, M., Kobayashi, Y., Saito, T., Kobayashi, M., Iuchi, S., Nomoto, M., Tada, Y., Yamamoto, Y.Y., and Koyama, H. (2015) Sensitive to proton rhizotoxicity1,

calmodulin binding transcription activator2, and other transcription factors are involved in aluminum-activated malate transporter1 expression. *Plant Physiol*. **167**, 991–1003.

Tran, L.S., Nakashima, K., Sakuma, Y., Simpson, S.D., Fujita, Y., Maruyama, K., Fujita, M., Seki, M., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2004) Isolation and functional analysis of Arabidopsis stress-inducible NAC transcription factors that bind to a drought-responsive *cis*-element in the *early responsive to dehydration stress 1* promoter. *Plant Cell*, **16**, 2481–2498.

Vercammen, D., Van De Cotte, B., De Jaeger, G., Eeckhout, D., Casteels, P., Vandepoele, K., Vandenberghe, I., Van Beeumen, J., Inzé, D., and Van Breusegem, F. (2004) Type II metacaspases Atmc4 and Atmc9 of *Arabidopsis thaliana* cleave substrates after arginine and lysine. *J. Biol. Chem*. **279**, 45329–45336.

Van Leene, J., Hollunder, J., Eeckhout, D., Persiau, G., Van De Slijke, E., Stals, H., Van Isterdael, G., Verkest, A., Neirynck, S., Buffel, Y., De Bodt, S., Maere, S., Laukens, K., Pharazyn, A., Ferreira, P.C., Eloy, N., Renne, C., Meyer, C., Faure, J.D., Steinbrenner, J., Beynon, J., Larkin, J.C., Van de Peer, Y., Hilson, P., Kuiper, M., De Veylder, L., Van Onckelen, H., Inzé, D., Witters, E., and De Jaeger, G. (2010) Targeted interactomics reveals a complex core cell cycle machinery in *Arabidopsis thaliana*. *Mol. Syst. Biol*. **6**, 397.

Watanabe, N., and Lam, E. (2005) Two *Arabidopsis* metacaspases AtMCP1b and AtMCP2b are arginine/lysine-specific cysteine proteases and activate apoptosis-like cell death in yeast. *J. Biol. Chem*. **28**, 14691–14699.

Wang, H., Fowke, L.C., and Crosby, W.L. (1997) A plant cyclin-dependent kinase inhibitor gene. *Nature*, **386**, 451–452.

Weimer, A.K., Biedermann, S., Harashima, H., Roodbarkelari, F., Takahashi, N., Foreman, J., Guan, Y., Pochon, G., Heese, M., Van Damme, D., Sugimoto, K., Koncz, C., Doerner, P., Umeda, M., and Schnittger, A. (2016) The plant□specific CDKB1□CYCB1 complex mediates homologous recombination repair in *Arabidopsis*. *EMBO J*. **5**, 2068–2086.

Wu, F.H., Shen, S.C., Lee, L.Y., Lee, S.H., Chan, M.T., and Lin, C.S. (2009) Tape-*Arabidopsis* Sandwich - a simpler *Arabidopsis* protoplast isolation method. *Plant Methods*, **5**, 16.

Wu, A., Allu, A.D., Garapati, P., Siddiqui, H., Dortay, H., Zanor, M.I., Asensi-Fabado, M.A., Munné-Bosch, S., Antonio, C., Tohge, T., Fernie, A.R., Kaufmann, K., Xue, G.P., Mueller-Roeber, B., and Balazadeh, S. (2012) *JUNGBRUNNEN1*, a reactive oxygen species-responsive NAC transcription factor, regulates longevity in *Arabidopsis*. *Plant Cell*, **24**, 482–506.

Xie, Q., Frugis, G., Colgan, D., and Chua, N.H. (2000) *Arabidopsis* NAC1 transduces auxin signal downstream of TIR1 to promote lateral root development. *Genes Dev*. **14**, 3024–3036.

Yamaguchi, M., Kubo, M., Fukuda, H., and Demura, T. (2008) Vascular-related NAC-DOMAIN7 is involved in the differentiation of all types of xylem vessels in Arabidopsis roots and shoots. *Plant J*. **55**, 652–664.

Yan, S., Wang, W., Marqués, J., Mohan, R., Saleh, A., Durrant, W.E., Song, J., and Dong, X. (2013) Salicylic acid activates DNA damage responses to potentiate plant immunity. *Mol. Cell*, **52**, 602–610.

Yi, D., Alvim Kamei, C.L., Cools, T., Vanderauwera, S., Takahashi, N., Okushima, Y., Eekhout, T., Yoshiyama, K.O., Larkin, J., Van den Daele, H., Conklin, P., Britt, A., Umeda, M., and De Veylder, L. (2014) The *Arabidopsis* SIAMESE-RELATED cyclin-dependent kinase inhibitors SMR5 and SMR7 regulate the DNA damage checkpoint in response to reactive oxygen species. *Plant Cell*, **26**, 296–309.

Yoo, S.D., Cho, Y.H., and Sheen, J. (2007) *Arabidopsis* mesophyll protoplasts: a versatile cell system for transient gene expression analysis. *Nat. Protoc*. **2**, 1565–1572.

Yoshiyama, K., Conklin, P.A., Huefner, N.D., and Britt, A.B. (2009) Suppressor of gamma response 1 (*SOG1*) encodes a putative transcription factor governing multiple responses to DNA damage. *Proc. Natl. Acad. Sci. USA*, **106**, 12843–12848.

Yoshiyama, K.O., Kobayashi, J., Ogita, N., Ueda, M., Kimura, S., Maki, H., and Umeda, M. (2013) ATM□mediated phosphorylation of SOG1 is essential for the DNA damage response in *Arabidopsis*. *EMBO Rep*. **14**, 817–822.

Yoshiyama, K.O., Kimura, S., Maki, H., Britt, A.B., and Umeda, M. (2014) The role of SOG1, a plant-specific transcriptional regulator, in the DNA damage response. *Plant Signal. Behav*. **9**, e28889.

Yoshiyama, K.O., Kaminoyama, K., Sakamoto, T., and Kimura, S. (2017) Increased phosphorylation of Ser-Gln sites on SUPPRESSOR OF GAMMA RESPONSE1

strengthens the DNA damage response in *Arabidopsis thaliana*. *Plant Cell*, **29**, 3255–3268.

Zhong, R., Lee, C., and Ye, Z.H. (2010) Global analysis of direct targets of secondary wall NAC master switches in *Arabidopsis*. *Mol. Plant*, **3**, 1087–1103.

FIGURE LEGENDS

Figure 1. Identification of SOG1 target genes.

(a) Venn diagram shows overlap among SOG1-regulated genes and genes that are identified by ChIP-sequencing. A total of 146 genes are identified as potential target genes of SOG1. **(b)** (Upper panel) Schematic representation of the *AtRAD51* and *AtBRCA1* loci highlighting the amplified regions in ChIP-qPCR (P1–P3). (Middle and lower panel) Chromatins bound with *AtRAD51*, *AtBRCA1*, and *Mutator-like transposon* (*Mut*; negative control) loci were collected by immunoprecipitation with anti-Myc antibodies from two-week-old wild-type (WT) and *ProSOG1:SOG1-Myc* seedlings treated with (lower panel) or without 15 μM zeocin (middle panel) and subjected to qPCR analysis. The average enrichment of qPCR products from immunoprecipitated DNA is normalized against the corresponding input DNA. Data are represented as mean ± SD (n = 3). Significant differences from the wild-type control were determined by Student's *t*-test: **, $P < 0.01$, ***, $P < 0.001$. **(c)** Transcript levels of *AtRAD51* and *AtBRCA1*. Two-week-old wild-type (WT) and *sog1-1* seedlings were transferred to a control medium (- Zeocin) or a medium containing 15 μM zeocin (+ Zeocin) for 2 h. The mRNA levels were normalized to that of *ACTIN2* and are indicated as relative

values, with that of the control set to 1. Data are presented as mean ± SD (n = 3). Significant differences from the control were determined by Student's *t*-test: ***, $P < 0.001$. **(d)** GUS staining of roots harboring *ProRAD51:GUS* and *ProBRCA1:GUS* reporter constructs introduced into wild-type (WT) and *sog1-1* mutant backgrounds. Seven-day-old seedlings grown in MS medium were transferred to new medium containing 15 μM zeocin and grown for 2 hr. Bar = 100 μm.

Figure 2. DNA damage-triggered SOG1 phosphorylation is required for the binding to target sites.

Chromatins bound with *Mutator-like transposon* (*Mut*, negative control) and the promoter regions of *SMR7*, *AtRAD51*, and *AtBRCA1* were collected using immunoprecipitation with anti-Myc antibodies from two-week-old *sog1-1*, *sog1-1* harboring *ProSOG1:SOG1-Myc*, or *ProSOG1:SOG1*(*5A*)*-Myc* seedlings treated with 15 μM zeocin for 2 h, and subjected to qPCR analysis. The average enrichment of qPCR products from immunoprecipitated DNA is normalized against the corresponding input DNA. Data are represented as mean ± SD (n = 3). Significant differences from the control were determined by Student's *t*-test: **, $P < 0.01$, ***, $P < 0.001$.

Figure 3. Comparison between SOG1 and p53 target genes.

(a, b) GO analysis of the SOG1 **(a)** and p53 target genes **(b)**. The yellow-to-red color of circles corresponds to the level of significance of the overrepresented GO category according to a multiple *t*-test with false discovery rate–corrected *P* value ($P < 0.01$). The size of the nodes is proportional to the number of genes in the GO category. The related GO categories are encompassed with same clusters.

Figure 4. Identification of SOG1-binding motif.

(a) Locations of SOG1 binding peak maxima within the 10 kb surrounding transcription start site (TSS). **(b)** Sequence logo of the overrepresented motif found in the set of 146 direct SOG1 target genes. The logo was created using WebLogo. The overall height of each stack indicates the sequence conservation at that position. The height of symbols within the stack indicates the relative frequency of the corresponding nucleic acid at that position. **(c)** Proportion of the number of $CTT(N)_7AAG$ motifs within 1 kb upstream from TSS in 146 SOG1 target genes. **(d)** Distribution of the $CTT(N)_7AAG$ motif in the promoter regions of SOG1 target genes. White bars represent the relative frequency of the $CTT(N)_7AAG$ motif in 100 bp intervals upstream from the TSS for promoter sequences on all annotated *Arabidopsis* genes, whereas black bars represent the relative frequency of the consensus motif in the promoter regions of 146 SOG1 target genes. The $CTT(N)_7AAG$ motif is significantly enriched with SOG1 target genes (**, $P < 0.01$, Fisher's exact test). **(e)** Schematic representation of the *AtRAD51* locus highlighting the position of $CTT(N)_7AAG$ sequence (red letters). Exons are shown as black boxes, and probes for AlphaScreen system are

represented as A and B. **(f)** *In vitro* binding assay of dsDNA and the SOG1 protein using an AlphaScreen system. *In vitro*-translated SOG1 proteins labeled with the acceptor beads of the AlphaScreen system were co-incubated with dsDNA probes. Relative AlphaScreen signals were calculated as a ratio of signals of the biotin-labeled probe to those of the nonbiotin-labeled probe. Data are presented as mean ± SD (n = 3). Significant differences from the AlphaScreen signals were determined by Student's *t*-test: ***, $P < 0.001$. **(g)** Competition assays of the probe A region with nonbiotin-labeled probes. The biotin-labeled probe A was incubated with SOG1 proteins in the presence of nonbiotin-labeled probe A or probe B. Relative values were calculated as the ratio of the value obtained from the absence of the competitor (None). Data are presented as mean ± SD (n = 3). Significant differences from the AlphaScreen signals in the absence of the competitor were determined by Student's *t*-test: ***, $P < 0.001$.

Figure 5. SOG1 binds CTT(N)$_7$AAG motif.

(a) Competition assays of the probe A region with the single nucleotide mutagenized probes. The biotin-labeled probe A was incubated with SOG1 proteins in the presence of nonbiotin-labeled probe A or the probe carrying a single-nucleotide mutation. Relative values were calculated as the ratio of the value obtained from the absence of the competitor (None). Data are presented as mean ± SD (n = 3). Significant differences from the AlphaScreen signals in the presence of nonbiotin-labeled probe A were determined by Student's *t*-test: **, $P < 0.01$, ***, $P < 0.001$. **(b)** Competition assays of the probe A region with the nucleotides mutagenized or deleted probes. The biotin-labeled probe A was incubated with SOG1

proteins in the presence of nonbiotin-labeled probe A, probe B, or the probe carrying nucleotide substitutions or deletions. Relative values were calculated as the ratio of the value obtained from the absence of the competitor (None). Data are presented as mean ± SD (n = 3). Different letters represent significant differences from one another ($P < 0.05$; ANOVA followed by Turkey's HSD test).

Figure 6. The CTT(N)$_7$AAG motif is crucial for *RAD51* induction by SOG1 *in planta.*

(a) Transactivation analysis showing the SOG1-activated expression of the *fLUC* reporter gene driven by the corresponding wild-type *RAD51* promoter (*proAtRAD51:fLUC*) or the mutated promoter, carrying nucleotide substitutions from consensus CTTGTTGAAGAAG sequence to TCCATTGAAAGGA (*proAtRAD51m:fLUC*). The *sog1-1* protoplasts were cotransfected with the reporter, effector and normalization plasmids. Luciferase activity was normalized by *35S:rLUC* and is indicated as relative values. Data are represented as mean ± SD (n = 5). Significant differences from the control (*35S:GFP*) were determined by Student's *t*-test: **, $P < 0.01$. **(b)** (Left panel) Schematic representation of the luciferase reporter constructs containing *35S* minimal promoter (*35Smini*; black boxes) (*35Smini:fLUC*), four-tandem repeat of consensus CTT(N)$_7$AAG sequence (red circles) (*4x[CTT(N)$_7$AAG]-35Smini:fLUC*), four-tandem repeat of mutated consensus sequence (X mark) (*4x[TCC(N)$_7$GGA]-35Smini:fLUC*), and wild-type *RAD51* promoter. (Right panel) Luciferase activity by co-transfection with *35S:SOG1* in *sog1-1* protoplasts. Luciferase activity was normalized to that of *35S:GFP* (negative control) and indicated as relative values. Data are presented as mean ± SD (n = 3). Different letters represent significant

differences from one another ($P < 0.05$; ANOVA followed by Turkey's HSD test). **(c)** GUS staining of wild-type (WT) roots carrying the wild-type *RAD51* promoter or mutated promoter carrying nucleotide substitutions in the consensus motif. Seven-day-old seedlings grown in MS medium were transferred to new medium containing 15 μM zeocin and grown for 2 hr. Bar = 100 μm.

Figure 7. SOG1 is involved in immune response to fungal pathogens.

(a) Five-week-old wild type (WT), *sog1-1*, *sog1-101*, and *pad4-1* leaves were inoculated with *C. higginsianum*, and then incubated for six days. Bar = 1 cm. **(b)** Anthracnose lesion area six days after *C. higginsianum* inoculation. Data are presented as mean ± SD (n > 96). Significant differences from wild-type were determined by Student's *t*-test: ***, $P < 0.001$. **(c)** Biomass of *C. higginsianum* in the inoculated leaves at 0, 5, and 7 days post-inoculation. The mRNA levels of *C. higginsianum ACTIN* relative to that of *A. thaliana ACTIN2* (relative fungal levels) are shown. Data are presented as mean ± SD (n = 3). Significant differences from wild-type at the corresponding times were determined by Student's *t*-test: *, $P < 0.05$.