Histone Lysine Methyltransferase SDG8 Is Involved in Brassinosteroid-Regulated Gene Expression in Arabidopsis thaliana

Xiaolei Wang, Jiani Chen, Zhouli Xie, Sanzhen Liu, Trevor Nolan, Huaxun Ye, Mingcai Zhang, Hongqing Guo, Patrick S. Schnable, Zhaohu Li, and Yanhai Yin

Published Version Information

Citation: Wang, X., Chen, J., Xie, Z., Liu, S., Nolan, T., Ye, H., et al. (2014). Histone lysine methyltransferase SDG8 is involved in brassinosteroid- regulated gene expression in arabidopsis thaliana. Molecular Plant, 7(8), 1303-1315.

Digital Object Identifier (DOI): 10.1093/mp/ssu056

Publisher's Link: http://mplant.oxfordjournals.org/content/7/8/1303.abstract

Histone Lysine Methyltransferase SDG8 Is Involved in Brassinosteroid Regulated Gene Expression in *Arabidopsis thaliana*

Xiaolei Wang[1, 2], Jiani Chen[2], Zhouli Xie[2], Sanzhen Liu[3§], Trevor Nolan[2], Huaxun Ye[2‡], Mingcai Zhang[1], Hongqing Guo[2], Patrick S. Schnable[3,4], Zhaohu Li[1*] & Yanhai Yin[2*]

[1]College of Agronomy, China Agricultural University, Beijing, China

[2]Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011

[3]Department of Agronomy, Iowa State University, Ames, IA 50011

[4]Data2Bio LLC, Ames IA 50011-3650

[§]Current Address, Department of Plant Pathology, Kansas State University, Manhattan KS 66506-5502

[‡]Current Address: Du Pont Pioneer Inc. Johnston, Iowa.

* Corresponding Authors:

Department of Genetics, Development and Cell Biology

Iowa State University, Ames, Iowa 50011-3650

Tel: (515) 294-4816;

Fax: (515) 294-5256

yin@iastate.edu

Running title: SDG8 in brassinosteroid regulated gene expression

Abstract

The plant steroid hormones, brassinosteroids (BRs), play important roles in plant growth, development and responses to environmental stresses. BRs signal through receptors localized to the plasma membrane and other signaling components to regulate the BES1/BZR1 family of transcription factors, which modulates the expression of 4,000-5,000 genes. How BES1/BZR1 and their interacting proteins function to regulate the large number of genes are not completely understood. Here we report that histone lysine methyltransferase SDG8, implicated in Histone 3 lysine 36 di- and tri-methylation (H3K36me2 and me3), is involved in BR-regulated gene expression. BES1 interacts with SDG8, directly or indirectly through IWS1, a transcription elongation factor involved in BR-regulated gene expression. The knockout mutant *sdg8* displays a reduced growth phenotype with compromised BR responses. Global gene expression studies demonstrated that SDG8 plays a major role in BR-regulated gene expression as more than half of BR-regulated genes are differentially affected in *sdg8* mutant. A Chromatin Immunoprecipitation (ChIP) experiment showed that H3K36me3 is reduced in BR-regulated genes in the *sdg8* mutant. Based on these results, we propose that SDG8 plays an essential role in mediating BR-regulated gene expression. Our results thus reveal a major mechanism by which histone modifications dictate hormonal regulation of gene expression.

Key words: Brassinosteroid, histone modifications, gene regulation

Introduction

Understanding how histone modifications and chromatin structure modulate gene expression is an important aspect of regulatory biology. In response to various stimuli such as hormones, pathway-specific transcription factors can recruit chromatin-remodeling complexes and histone-modifying enzymes to regulate chromatin structure and gene expression (Li et al., 2007a). Histones can be modified at multiple positions, which forms a "histone code" that determines gene activity (Jenuwein and Allis, 2001). The hormonal regulation of histone modifications is not well understood in plant systems.

Plant steroid hormones, brassinosteroids (BRs), regulate many processes including cell elongation, cell division, leaf senescence, vascular differentiation, flowering time control, male reproduction, photomorphogenesis, and responses to both biotic and abiotic stresses (Divi and Krishna, 2009; Hao et al., 2013; Yang et al., 2011; Zhu et al., 2013). BRs signal through the plasma membrane-localized receptor kinase BRI1 to regulate BES1 and BZR1 (BES1/BZR1) family transcription factors, which in turn control the target gene expression (Clouse, 2011; Guo et al., 2013; Hothorn et al., 2011; Kim and Wang, 2010; Li, 2010; Li and Chory, 1997; She et al., 2011).

In the absence of BRs, several mechanisms function together to reduce basal BR signaling. A negative regulator, BKI1, binds and inhibits BRI1 function (Wang and Chory, 2006). BES1/BZR1 are phosphorylated and inhibited by negative regulator BIN2 kinase (Choe et al., 2002; He et al., 2002; Li and Nam, 2002; Perez-Perez et al., 2002; Yin et al., 2002; Zhao et al., 2002). Moreover, a Leucine C-terminal Methyltransferase (LCMT) activates PP2A, which dephosphorylates BRI1 and turns off BR signaling (Di

Rubbo et al., 2011; Wu et al., 2011). Autophosphorylation of BRI1 also leads to its self-deactivation (Oh et al., 2012).

Binding of BRs to BRI1 leads to the release of BKI1; BKI1 then sequesters 14-3-3s that keep phosphorylated BES1/BZR1 in the cytoplasm (Jaillais et al., 2011; Wang et al., 2011). BRs also promote the association of BRI1 with co-receptor BAK1 and the activation of BRI1 kinase through sequential phosphorylation (Gou et al., 2012; Li et al., 2002; Nam and Li, 2002; Oh et al., 2009; Russinova et al., 2004; Wang et al., 2005; Wang et al., 2008). Activated BRI1 and BAK1 act through BSK kinase, CDG kinase, BSU1 phosphatase and other components to inhibit BIN2 kinase activity (Kim et al., 2011; Kim et al., 2009; Mora-Garcia et al., 2004; Tang et al., 2008). The inhibition of BIN2 kinase and action of PP2A phosphatase allow the accumulation of BES1/BZR1 in the nucleus to regulate gene expression (He et al., 2005; Ryu et al., 2010a; Ryu et al., 2010b; Tang et al., 2011; Yin et al., 2005).

In the nucleus, BES1 interacts with other transcription factors and regulators to control target gene expression (Guo et al., 2013; Li, 2010). EARLY FLOWERING 6 (ELF6) and RELATIVE OF ELF6 (REF6) were originally identified as two related proteins that function to regulate flowering time by affecting the function of the flowering repressor FLC (Flowering Locus C) (Noh et al., 2004). The REF6 and ELF6 proteins have conserved Jumonji domains (JmjN and JmjC), which function as histone demethylases (Klose and Zhang, 2007). REF6 is a histone 3 lysine 27 (H3K27) demethylase (Lu et al., 2011), which removes methylation groups from di- or tri-methylated H3K27 (H3K27me2 or H3K27me3), a transcriptional repression mark (Turck et al., 2007; Zhang et al., 2007). REF6 and ELF6 interact with BES1 and are involved in

BR regulated gene expression (Yu et al., 2008). It is therefore conceivable that BES1 recruits REF6 to remove H3K27me3 and thus allow the expression of target genes.

Additionally, it has been shown that IWS1 (Interacts With Spt6) is involved in BR-regulated gene expression via a forward genetic approach (Li et al., 2010b). The *iws1* mutant has semi-dwarf phenotype with reduced BR responses and global reduction in BR-induced gene expression. IWS1 interacts with BES1 and is recruited by BES1 to target genes. Moreover , IWS1 (also named SPN1 in yeast) is part of the Pol II complex and implicated in transcription elongation (Fischbeck et al., 2002; Krogan et al., 2002; Lindstrom et al., 2003; Ling et al., 2006; Mayer et al., 2010; Yoh et al., 2007). Studies in yeast and mammalian systems suggested that IWS1 is involved in chromatin remodeling and histone modifications. In yeast, IWS1 recruits transcription elongation factor Spt6 and chromatin remodeling complex SWI/SNF to induce *CYC1* gene expression (Zhang et al., 2008). In cultured human cells, IWS1, Spt6 and HKMT HYPB/Setd2 form a protein complex on the C-terminal domain (CTD) of the largest subunit of RNA Polymerase II (Yoh et al., 2008). HYPB/Setd2 is a SET domain (Su(var)3-9, E(z) and Trithorax), the core catalytic motif conferring histone lysine methyltransferase (HKMT) activity and catalyzes the formation of histone 3 lysine 36 trimethylation (H3K36me3). H3K36me3 displays a positive correlation with transcription rate, likely by preventing non-specific transcription initiations in the coding regions through histone deacetylation (Li et al., 2007b; Pokholok et al., 2005).

In *Arabidopsis*, there are 43 SET Domain Group (SDG**)** containing proteins with conserved SET domains. Of the 43, SDG8 exhibits the highest sequence similarity (44% overall and 52% in SET domain) to HYBP/Setd2 (Xu et al., 2008). The

loss-of-function mutant, *sdg8*, exhibits a global reduction in H3K36me2 and H3K36me3, and an increase in H3K36me1, which is consistent with the hypothesis that SDG8 targets H3K36 modifications (di- and tri-methylation, H3K36me2 and H3K36me3) (Xu et al., 2008; Zhao et al., 2005). *SDG8* was also identified as *EARLY FLOWERING IN SHORT DAYS* (*EFS*) in Arabidopsis, mutation of which leads to an early flowering phenotype due to altered chromatin modifications at the *FLC* locus and reduced expression of *FLC* (Kim et al., 2005). Chromatin Immunoprecipitation (ChIP) studies demonstrated that H3K36me3 is enriched in coding regions of a majority of genes on Chromosome 4 in *Arabidopsis* (Roudier et al., 2011), suggesting that this modification can affect many biological processes. Indeed, more recent studies showed that SDG8 is involved in many other biological processes such as shoot branching (Dong et al., 2008), ovule and anther development (Grini et al., 2009), carotenoid composition (Cazzonelli et al., 2009; Cazzonelli et al., 2010), seed gene expression (Tang et al., 2012), fungal defense related to jasmonic acid and ethylene regulated gene expression (Berr et al., 2010) and innate immunity (Palma et al., 2010). Rice SDG725, an H3K36 HMT, was recently shown to be involved in BR signaling as well as flowering time control (Sui et al., 2012; Sui et al., 2013). Knockdown of SDG725 gene results in BR loss-of-function phenotypes such as dwarfism, shortened internodes, erect leaves, and small seeds. Gene expression and ChIP analyses revealed that *D11* (*DWARF11*), *BRI1*, and *BU1* (*BRASSINOSTEROID-UPREGULATED 1*), involved in BR biosynthesis, perception, and downstream signaling in rice, respectively, are down-regulated in the mutant (Sui et al., 2012). A recent study showed that two of the SDG8-regulated microRNAs are regulated by BRs and are

implicate in BR-regulated gene expression (Dong and Li, 2013). These results further support the role of H3K36 methylation in BR-regulated gene expression and signaling.

In this study, we investigate the function of Arabidopsis H3K36 HKMT SDG8 in BR signaling and the potential mechanisms of its action. We found that SDG8 is likely recruited to BR target genes through interactions with both IWS1 and BES1. The knockout mutant of SDG8 displays a BR-response phenotype and many BR-regulated genes are no longer regulated or misregulated in the *sdg8* mutant. Based on our genetic and genomic data, we propose that SDG8 play an important role in BR-regulated gene expression and BR signaling.

Results

SDG8 interacts with IWS1 and BES1

Previous results indicated that the BR-specific transcription factor, BES1, recruits the transcription elongation factor IWS1 to control BR-regulated gene expression (Li et al., 2010). Since human IWS1 forms a complex with H3K36 histone methyltransferase HYPB/Setd2 (Yoh et al., 2008), we tested whether Arabidopsis IWS1 directly interacts with H3K36 histone lysine methyltransferase SDG8 using yeast two-hybrid assays. The SDG8 protein is predicated to have 1805 amino acid residues and contains several domains, including an amino-terminal domain, a CW domain (cystein and tryptophan conserved, amino acid or aa 864-910), AWS (associated with SET, aa 974-1,025), the SET domain (aa 1,025-1,147), and a cystein-rich domain (C, aa 1152-1168) and carboxyl-terminal domain (Xu et al., 2008; Zhao et al., 2005). Several overlapping

fragments of SDG8 (F1-6) were cloned into pGBKT7 vector to examine their potential interactions with full length or C-terminal conserved domain of IWS1 (Fig.1A). Fig.1B showed that two regions of SDG8, F2 (aa 304–663) and F4 (aa 823–974, including the CW domain), interact with IWS1. The C-terminal conserved domain of IWS1 was sufficient for the interaction with SDG8 (Li et al., 2010a).

We also tested the possible direct interaction between BES1 and SDG8. We found that the first part of N-terminal (F1, amino acids 1–329) of SDG8 interacts with BES1 through its central part (P and S domains of BES1, aa 99-197, and 150-267, respectively). The interactions between SDG8/BES1 and SD8/IWS1 were further confirmed *in vitro* by GST pull-down assays (Fig.1D). GST-SDG8-F2 and GST-SDG8-F4 interact with MBP-IWS1C (upper panel); and GST-SDG8-F1 interacts with MBP-BES1 (lower panel).

Bimolecular Fluorescence Complementation (BiFC) assays were used to confirm BES1-SDG8 and SDG8-IWS1 interactions in plants (Fig. 2 and Fig. S1). Full-length clones of BES1, SDG8 or IWS1 were fused in-frame with cYFP (C-terminal of YFP) or nYFP (N-terminal of YFP) and coexpressed in tobacco leaves mediated by Agrobacteria. Coexpression of SDG8-cYFP and BES1-nYFP led to the complementation of YFP and strong fluorescence signals in the nucleus (Fig. 2A and B). In contrast, coexpression of cYFP and BES1-nYFP did not produce any fluorescence signal (Fig. 2D) and coexpression of SDG8-cYFP and nYFP produced some background signals, which happened with less frequency and intensity than those observed with coexpression of SDG8-cYFP and BES1-nYFP (Fig. 2C). Interactions between SDG8 and IWS1 were also observed in plants by BiFC (Fig. S1). Taken together, these studies suggest that BES1

form a trimeric complex with IWS1 and SDG8 to regulate BR-regulated gene expression (Fig 1. E).

The *sdg8* mutant shows altered BR response phenotype

To determine if SDG8 is involved in BR signaling, we obtained a T-DNA insertional line, SALK_036941 with the T-DNA insertion between aa 1058 and 1059 was used in the study. The allele was previously designated as *sdg8-4* (Dong et al., 2008; Tang et al., 2012) and is referred as *sdg8* in this study for simplicity. The *sdg8* mutant plants displayed a reduced growth phenotype at both vegetative and reproductive stages (Fig. 3A and Fig. 3B). The *sdg8* mutant plants had smaller leaves, shorter inflorescences, more branches and smaller siliques, compared to wild-type (WT) plants (Fig. 3B), similar to other reported loss-of-function alleles of *sdg8* (Cazzonelli et al., 2009; Dong et al., 2008; Grini et al., 2009; Tang et al., 2012; Xu et al., 2008; Zhao et al., 2005).

To investigate how SDG8 is involved in the BR response, we determined the response of the mutant to brassinazole (BRZ), an inhibitor of BR biosynthesis in dark-grown seedlings (Asami and Yoshida, 1999). As shown in Fig. 3C, *sdg8* mutants, like *iws1* (Li et al., 2010a), were more sensitive to BRZ than WT. In the presence of brassinolide (BL, the most active BR), *sdg8* mutant also had clearly reduced BR response than WT (Fig. 3D). These results indicated that SDG8 is required for BR-regulated plant growth.

SDG8 differentially modulates BR-regulated gene expression

To investigate how SDG8 modulates BR-regulated gene expression, we performed global gene expression study with 4-week-old WT and *sdg8* mutant plants in the absence or presence of BR. While BR regulates about 5,000 genes (2,678 induced and 2,376 repressed, Table S1 and Table S2, respectively) in WT plants, it regulates only about 676 genes (Table S3) in the *sdg8* mutant (Fig. 4A). The results demonstrate that SDG8 plays a critical in BR-regulation of gene expression.

We also compared BR-regulated genes in WT with genes affected in *sdg8* mutant. Consistent with the strong mutant phenotype, about 1/3 of the genes detected by RNA-seq are either up- (4,240) or down (4,078)-regulated in the *sdg8* mutant as compared to WT (Table S4 and Table S5). Both BR-induced and BR-repressed genes significantly overlap with genes affected by *sdg8*, which are designated as Groups A-D (Fig. 4B). More than half of the BR-induced genes are differentially expressed in the *sdg8* mutant (N=550 [Group A] + 794 [Group B], Fig 4B). Similarly, about half of the BR-repressed genes are differentially expressed in the *sdg8* mutant (851 [Group C] + 401 [Group D]). These results show that SDG8 can function to either activate (Groups A and C) or repress (Groups B and D) BR-regulated-gene expression, directly or indirectly. Gene ontogeny analysis indicated genes affected by BR and SDG8 can regulate many cellular, metabolic and signaling processes as well as cellular responses to stress and other stimuli (Fig S2).

To further reveal how SDG8 controls BR-regulated genes, we generated heatmaps for the 4 groups of genes (A, B, C and D) in WT and *sdg8* mutants with or without BR treatment (Fig 5). Group A genes are down-regulated in *sdg8* under both BR-treated and

untreated conditions, but their induction by BR is not affected in the mutant (Fig. 5A and Fig S3). In contrast, other BR-induced genes (Group B) are up-regulated in *sdg8* and BR has little, if any, effects on their expression (Fig. 5B and Fig. S3). These results suggest that SDG8 may function, directly or indirectly, to repress some BR-induced genes in the absence of BR; and BR reverses the "repression" activity of SDG8, thereby de-repressing (i.e., activating) these BR-induced genes.

On the other hand, a significant portion of BR-repressed genes (Group C) is down-regulated in the *sdg8* mutant; and BR has little effect on these genes in the mutant (Fig. 5C and Fig. S3). These results suggest that SDG8 activate these BR-repressed genes in the absence of the hormone and BR inhibits the "activation" activity of SDG8. As in the case of BR-induced genes, SDG8 acts to represses some BR-repressed genes (Group D) as they are up-regulated in *sdg8* mutant and still repressed by BR (Fig. 5D and Fig. S3).

BR can induce many genes encoding cell wall loosening enzymes required for cell elongation such as expansins, xyloglucan endotransglucosylase/hydrolases (XTHs), and pectin lyases (Darley et al., 2001). We examined the regulation of the cell wall-modifying genes by BR and SDG8 (Table 1). Most of the BR-regulated cell wall-related genes belong to Group A (induced by BR and reduced in *sdg8* mutant), which is consistent with the reduced growth phenotype of the mutant.

Histone 3 Lysine 36 trimethylation (H3K36me3) is reduced in BR-regulated genes

To investigate how H3K36 trimethylation affects BR-regulated gene expression, we examined four BR-induced genes that are either down-regulated (At3g16240 and At3g61430 from Group A) or up-regulated (At3g57260 and At2g24850 from Group B) in *sdg8* mutant by ChIP analysis. These four genes were chosen because they were clearly regulated by BR and affected in *sdg8* (Fig. 6A). ChIP assays were performed with H3K36me3 (H3K36 trimethylation) antibody using chromatin prepared from WT and *sdg8* mutant plants. Since it is well established that H3K36me3 mostly accumulates in transcribed regions (Mayer et al., 2010), we examined H3K36me3 accumulation in coding regions of the four selected genes. While H3K36me3 is enriched in all 4 genes, it is largely abolished in *sdg8* mutant (Fig. 6B), consistent with previous findings that H3K36me3 is globally reduced in *sdg8* mutant (Xu et al., 2008; Zhao et al., 2005).

Discussion

In this study, we revealed the function of SDG8, a H3K36 HKMT, in BR-regulated gene expression and BR response in Arabidopsis. We provided evidence to show that SDG8 is recruited to BR target genes by interacting with both BES1 and IWS1. Genetics studies indicated that SDG8 is required for BR-regulated growth as *sdg8* mutant has much reduced growth and compromised BR responses. Our global gene expression study supports a complex role of SDG8 and its corresponding H3K36 methylation in BR-

regulated gene expression. It also provides previous unknown functions of H3K36 methylation in the hormonal regulation of gene expression.

Our global gene expression study demonstrated that SDG8 plays a critical role in BR-regulated gene expression because only about 10% BR-regulated genes are still regulated by the hormone in *sdg8* mutant and more than half BR-regulated genes are affected in *sdg8* mutant (Fig. 4). We also examined ~4500 BES1 and BZR1 target genes identified by ChIP-chip (Sun et al., 2010; Yu et al., 2011) and found that about 40% are either up- or down-regulated in *sdg8* (Fig S4), which reinforces the idea that SDG8 affects BR target gene expression.

The function of SDG8 in BR-regulated gene expression appears to be mediated by its interaction with BES1, a transcription factor mediating BR-regulated gene expression (Yin et al., 2005; Yin et al., 2002; Yu et al., 2011). The results raise the possibility that BR signaling functions to recruit SDG8 to target genes to modulate BR regulated gene expression. Consistent with the finding in human cells that IWS1 interacts with H3K36 HMT HYPB/Setd2 to form a protein complex (Yoh et al., 2008), we found that Arabidopsis IWS1, previously shown to be involved in modulating BR-regulated gene expression (Li et al., 2010a), also interacts with SDG8. Interestingly, the interaction between IWS1 and SDG8 is partially mediated through CW domain in SDG8 (Fig 1). The CW domain of SDG8 recognizes H3K4 methylation (He et al., 2010; Hoppmann et al., 2011). It's possible that the CW domain of SDG8 (implicated in H3K36 methylation) can both recognize another histone mark (H3K4 methylation) and interact with different proteins involved in gene expression, consistent with the notion that combination of

modifications (histone code) dictates specific gene expression and biological processes (Shafiq et al., 2014).

Another interesting observation from the gene expression study is that 4,240 genes are up-regulated and 4,078 are down-regulated in *sdg8* mutant. It was also found that many seed-related genes are up-regulated in *sdg8* mutant, although it's not known if these up-regulated genes are directly or indirectly affected by SDG8 (Tang et al., 2012). Similarly, both BR-induced and BR-repressed genes are affected in the *sdg8* mutants (Fig. 3B). Since H3K36 trimethylation is generally believed to be involved in gene activation, the large number of genes up-regulated in *sdg8* mutant may be caused by the pleiotropic phenotypes of the mutant. Alternatively, SDG8 may be involved in transcriptional repression through functions either related or unrelated to H3K36 methylation. This possibility is supported by the fact that among H3K36 target genes on chromosome 4 (Roudier et al., 2011), 396 are up-regulated and 475 are down-regulated in *sdg8* mutant (Fig. S5). Further genomic and functional studies can help distinguish between these two possibilities.

Our gene expression analysis also provides a more complete picture on the phenotype of *sdg8* mutant. Some BR-induced (Group A) genes are down-regulated and some BR-repressed genes (Group D) are up-regulated in *sdg8* mutant, which predicts a reduced BR-regulated growth. On the other hand, some other BR-induced (Group B) genes are up-regulated and some BR-repressed genes (Group C) are down-regulated in *sdg8* mutant, which should lead to constitutive BR responses in the mutant. The *sdg8* phenotype thus may represent the combined effects of all 4 groups of BR-regulated genes.

The fact that more cell elongation related genes are reduced in *sdg8* mutant than those up-regulated in the mutant (Table 1) explain the overall reduced growth phenotype and BR responses of *sdg8* (Fig. 3).

In summary, our genetic and genomic studies provide evidence that SDG8 is implicated in BR-regulated gene expression and BR responses. The results suggest that BR-regulated transcription factors such as BES1 recruit many general transcription regulators including those implicated in chromatin modifications (REF6, IWS1 and SDG8) to control BR-regulated gene expression. Our results also indicate a complex and critical role of SDG8 and H3K36 trimethylation in hormone-regulation of gene expression. Further functional characterization of SDG8 in BR-regulated gene expression and its regulation should facilitate the understanding on how chromatin modifications and hormone signaling interact in the regulation of plant growth and development.

Material and Methods

Plant materials growth conditions

Arabidopsis thaliana ecotype *Columbia (Col-0)* was used as the wild type control. T-DNA knockout mutants *sdg8* and *iws1* were obtained from ABRC (Arabidopsis Biological Resource Center), corresponding to lines SALK_036941 and SALK_056238, respectively (Alonso et al., 2003). The seeds were germinated and grown on half-strength MS plates for 10 days before transferred to soil and grown under long day conditions (16h light/ 8h dark) at 22°C.

Hypocotyl length assays

BRZ and BL (Wako Chemicals USA, Inc.) were added to the half-strength MS agar medium. The seeds were germinated and grown on the medium for 5 days in the dark (BRZ response) or under light (BL response). The average hypocotyl lengths were calculated using 10 to 20 samples. The experiments have been repeated at least three times with similar results.

Plasmid construction

All primers used in this study are listed in Table S6. For recombinant protein and GST pull-down assays, SDG8 coding regions were amplified from Col-0 cDNA and incorporated into the pET42a(+) (Novagen). IWS1, BES1 and various deletion constructs were cloned into pETMALc-H vector to generate MBP-fusion proteins.

Yeast Two-Hybrid and LacZ Assays.

Matchmaker two-hybrid system (Clontech) was used to test protein-protein interactions. Yeast strain Y187 (*MAT*α, *ura3-52*, *his3-200*, *ade2-101*, *trp1-901*, *leu2-3, 112*, *gal4*Δ, *met–*, *gal80*Δ, *MEL1*, *URA3*::*GAL1UAS -GAL1TATA-lacZ*) transformed with bait (pGBKT7) and prey (pGADT7) constructs was grown in media lacking Trp and Leu. The interactions between proteins were detected by LacZ reporter assay using X-gal (5-bromo-4-chloro-3-indolyl-b-Dgalactopyranoside; Sigma).

GST Pull-Down Assays

SDG8 fragments fused with GST were cloned into pET42a and purified with glutathione agarose beads (Sigma). IWS1, BES1 and their fragments fused with maltose binding protein (MBP) were purified with amylose resin (NEB). GST pull-down assays were performed as described (Yin et al., 2002). The pull-down products were detected by Western blotting using antibody against MBP (NEB). Pull-down assays were repeated twice with similar results.

BiFC Assays

The constructs of N or C-terminus of EYFP were described previously (Yu et al., 2008). The coding regions of IWS1 and BES1 were subcloned in-frame into upstream of the nYFP vector. The full-length coding region of SDG8 (Xu et al., 2008) was subcloned in-frame into cYFP vector. All constructs were confirmed by DNA sequencing and transformed into *Agrobacterium tumefaciens* strain GV3101.

Cultures of Agrobacterium tumefaciens strain GV3101 were grown overnight in TY medium containing 200μM acetosyringone. After washed with infiltration medium (1/2 MS medium, pH 5.6) and resuspended to an OD600 of 0.5 with 200 μM acetosyringone. Agrobacterium carrying nYFP and cYFP contructs were mixed in equal ratios along with Agrobacteria expressing p19 protein of tomato bushy stunt virus to suppress gene silencing (Voinnet et al., 2003). The Agrobacterium mixtures were infiltrated into the lower surface of *N. benthamiana* leaves and infiltrated areas were examined for YFP signals 3 days post-infiltration. Detection of YFP was performed using a Leica SP5 X MP confocal microscope equipped with an HCS PL APO CS 20.0x0.70 oil objective. YFP was excited with a 514nm laser line and detected from 530-560nm. Images were acquired with LAS AF software (Leica Microsystems) using identical settings for all samples.

RNA-seq and data analysis

Two biological replications of *sdg8* and the wild-type control plants were grown in soil under long-day conditions for 4 weeks, after which time they were sprayed with either 1000 nM BL in water with 0.1% Triton or water with 0.1% Triton only. Three hours after treatment, duplicate samples were collected and processed for RNA extraction. Total RNA was extracted and purified from rosette leaves of different genotypes and treatments using the RNeasy Mini Kit (Qiagen) with on-column DNase digestion, following the manufacturers' instructions.

Following RNA high-through-put sequencing on an Illumina instrument, raw RNA-seq reads were subjected to quality checking and trimming and then aligned to the

Arabidopsis reference genome (TAIR10) using the Genomic Short-read Nucleotide Alignment Program (GSNAP) (Wu and Nacu, 2010). The alignment coordinates of uniquely aligned reads from each sample were used to calculate the number of reads per annotated gene. These values were used to detect differential expression. The negative binomial QLSpline method as implemented in the QuasiSeq package (http://cran.r-project.org/web/packages/QuasiSeq) was used to compute a p-value for each gene having a minimum of one average read across all the samples in the comparison. The 0.75 quantile of reads from each sample was used as the normalization factor (Bullard et al., 2010). Adjusted q-values were generated from p-values using an approach that controls for multiple testing (Benjamini and Hochberg, 1995; Nettleton et al., 2006). To control the false discovery rate at the 5% level, genes with q-values smaller than 0.05 were considered to be differentially expressed. Average reads per million (RPM) for each gene were used to generate heatmaps using the Heatplus package (http://www.bioconductor.org/packages/2.12/bioc/html/Heatplus.html), in which average RPM were scaled to the same level across genes and plotted using a log2 scale. The GO analysis is performed as previously described (Li et al., 2010a; Yu et al., 2011).

ChIP-PCR

For ChIP-PCR experiments, the *sdg8* and the wild-type control plants were grown together on soil under long-day conditions for 4 weeks and the rosette leaves were used for ChIP. ChIP using anti-trimethyl-H3K36 (ab9050) from abcam (http://www.abcam.com) was performed as previously described (Li et al., 2010a; Yu et al., 2011).

Acknowledgements

We are grateful to Dr. Wen-Hui Shen (Universit e de Strasbourg, France) and Aiwu Dong (Fudan University, China) for providing SDG8 full-length clone. We thank Dr. Eddy Yeh at Iowa State University for helping with RNA-seq analysis and Hui-Hsien Feng for editing the manuscript. XW, JC and XZ were partially supported by fellowships from CSC (Chinese Scholarship Council). The work is supported by grant from NSF (IOS-1257631) and by Plant Science Institute at Iowa State University to YY.

Figure legends

Figure 1. SDG8 interacts with IWS1 and BES1.

(A) SDG8 protein structure and fragments used in protein-protein interaction experiment. Predicted SDG8 contains an amino terminal domain, a CW domain, an AWS, a SET domain involved in histone lysine methyltransferase activity, a cystein-rich (C) and a carboxyl domain. The fragments used in yeast two-hybrid and GST pull-down assays and the amino acid positions are indicated.

(B) SDG8 interacts with IWS1 in yeast two-hybrid assay. SDG8 fragments (F1-F6) were cloned into pGBKT7 and full-length IWS1 or C-terminal of IWS1 (IWS1-C) was cloned into pGADT7. The yeast strains harboring indicated plasmids were used for LacZ assays. SDG8 F2 and F4 interact with both IWS1-C and IWS1. The SET domain itself auto-activates the reporter gene expression and was not shown.

(C) SDG8 interacts with BES1 in yeast two-hybrid assay. SDG8 fragments were tested for interactions with BES1 (C: aa 268-335, S: aa 150-267 or P: aa 99-197). SDG8 F1 interacts with BES1 S and P domains.

(D) GST pull-down assays confirmed the interactions between SDG8 and IWS1/BES1. GST, GST-SDG8-F2 and GST-SDG8-F4 were used to pull-down MBP-IWS1C. GST and GST-SDG8-F1 were used to pull-down MBP-BES1. The IWS1 and BES1 proteins were detected with anti-MBP antibody (NEB).

(E) A model for SDG8 functions in the BR pathway: SDG8 may act in a complex with IWS1 and BES1 in the control of BR regulated-genes expression.

Fig. 2. SDG8 interacts with BES1 in plants as revealed by BiFC assays in tobacco leaves.

(A-B) Coexpression with 35S:SDG8:cYFP and 35S:BES1:nYFP led to the reconstitution of YFP activity in the nuclei.

(C) Coexpression of 35S:SDG8:cYFP and 35S:nYFP showed a lower level of fluorescence with less frequency compared with (A-B).

(D) Coexpression of 35S:cYFP and 35S:BES1:nYFP did not produce any positive signals.

For each panel (A-D), YFP fluorescence (left), bright-field differential interference contrast (DIC, middle) and overlay images (right) were observed with a confocal microscope. The YFP signals are indicated by arrows. The bar in each image represents 20μm. The experiments were repeated 3 times with similar results.

Figure 3. *SDG8* knockout mutant plants show altered BR response phenotypes.

(A) *sdg8* mutant plants display a reduced growth phenotype. Three-week-old plants are shown. The bar represents 2 cm.

(B) *sdg8* mutant plants have a dwarf phenotype with reduced inflorescence stems. Mature plants are shown. The bar represents 5 cm.

(C) *sdg8* and *iws1* mutants are hypersensitive to BR biosynthesis inhibitor brassinazole (BRZ). Hypocotyl elongation assay with 5-day-old dark-grown seedlings of WT, *sdg8* and *iws1* grown on half strength MS medium with 0, 100, or 1000 nM BRZ.

(D) *sdg8* and *iws1* mutants have reduced BR response. Hypocotyl elongation assay with 7-day-old light grown seedlings of WT, *sdg8* grown on half strength MS medium with 0, or 100 nM BL. In (C) and (D), averages and standard deviations were calculated from 10-

20 samples. The significance of difference between mutant and WT at each concentration was determined by student's t-test: **P<0.01, and ***P<0.001.

Figure 4. SDG8 is involved in BR-regulated gene expression.

(A) BR-regulated gene expression is compromised in *sdg8* mutants. Global gene expression profiles were determined with WT and *sdg8* mutants treated with or without BL by RNA-seq. Differentially expressed genes were identified by statistical analysis (see Materials and Methods section). The overlaps among BR-induced genes in WT (2678, Table S1), BR-repressed genes in WT (2376, Table S2), and BR-regulated genes in *sdg8* (676, Table S3) are analyzed by Venny program (http://bioinfogp.cnb.csic.es/tools/venny/index.html).

(B) BR-regulated genes significantly overlap with genes affected in *sdg8* mutant. Overlaps among BR-regulated genes and genes up-regulated in *sdg8* (4240, Table S4) or down-regulated in *sdg8* (4078, Table S5) are analyzed. BR-induced genes that are down-regulated in *sdg8* (550) are designated as Group A (A). BR-induced genes that are up-regulated in *sdg8* (794) are designated as Group A (B). BR-repressed genes that are down-regulated in *sdg8* (851) are designated as Group C (C). BR-repressed genes that are up-regulated in *sdg8* (401) are designated as Group D (D).

Figure 5. BR-regulated genes are differentially affected in *sdg8* mutant.

Heatmaps of BR-regulated genes in WT and *sdg8* mutant treated with or without BRs. Four groups of BR-regulated genes (A, B, C and D) defined in Fig. 4B were used to generate the heatmaps. For each gene, average reads per million (RPM) were used for the

heatmap plotting. Note that the RPM data were log2-scaled to the same level between genes.

Figure 6. Histone 3 lysine 36 trimethylation (H3K36me3) is reduced in BR-regulated genes in *sdg8* mutant.

(A). The expression levels of 4 representative BR-induced genes. The expression levels were derived from RNA-seq data. At3g16240 and At3g61430 are from Group A (BR-induced, down-regulated in *sdg8* mutant); At3g57260 and At2g24850 are from Group B (BR-induced, up-regulated in *sdg8* mutant).

(B) H3K36me3 is reduced in four tested BR-regulated genes. Chromatin prepared from WT and *sdg8* mutant plants were immunoprecipitated with anti-H3K36me3 antibody or control IgG. The ChIP products were used for quantitative PCR analysis with various primers. The relative enrichment of H3K36me3 in the coding regions of indicated genes was calculated compared to control IgG.

Fig. S1. SDG8 interacts with IWS1 in plants as revealed by BiFC assays in tobacco leaves.

(A-B) Coexpression with 35S:SDG8:cYFP and 35S:IWS1:nYFP led to the reconstitution of YFP activity in the nuclei.

(C) Coexpression of 35S:SDG8:cYFP and 35S:nYFP showed a lower level of fluorescence with less frequency compared with (A-B).

(D) Coexpression of 35S:cYFP and 35S:IWS1:nYFP did not produce any positive signals.

For each panel (A-D), YFP fluorescence (left), bright-field differential interference contrast (DIC, middle) and overlay images (right) were observed with a confocal microscope. The YFP signals are indicated by arrows. The bar in each image represents 20μm. The experiments were repeated twice with similar results.

Figure S2. Genes involved in many biological and cellular processes are affected by BR-regulated and SDG8 modulated genes. Gene Ontology analysis of cellular functions represented by BR-induced and BR-repressed genes in each gene Class (A-D) show in Fig 4. All genes detected in RNA-Seq samples were used as control.

Figure S3. The average expression levels of four groups of BR-regulated genes. The average expression levels of four groups of BR-regulated genes defined in Fig. 4B are calculated to roughly determine if each group is still regulated by BR in *sdg8* mutant. While Group A and D genes are still somewhat regulated by BR, Group B and C genes are mostly not regulated by BR in *sdg8* mutant.

Figure S4. Overlaps between BES1/BZR1 target genes and genes affected in *sdg8* mutant.

Overlaps between BES1/BZR1 target genes (BES1/BZR1 Tgt) (Sun et al., 2010; Yu et al., 2011) and genes up-regulated in *sdg8* (4240, Table S4) or down-regulated in *sdg8* (4078, Table S5) are analyzed are analyzed by Venny program (http://bioinfogp.cnb.csic.es/tools/venny/index.html).

Figure S5. Overlaps between genes with H3K36 trimethylation on Arabidopsis chromosome 4 and genes affected in *sdg8* mutant.

Overlaps between 2433 genes with H3K36me3 mark on Arabidopsis chromosome 4 (Chr 4 H3K36) (Roudier et al., 2011) and genes up-regulated in *sdg8* (4240, Table S4) or down-regulated in *sdg8* (4078, Table S5) are analyzed are analyzed by Venny program (http://bioinfogp.cnb.csic.es/tools/venny/index.html).

References

Alonso JM, et al. Genome-wide insertional mutagenesis of Arabidopsis thaliana. Science (2003) 301:653-657.

Asami T, Yoshida S. Brassinosteroid biosynthesis inhibitors. Trends Plant Sci (1999) 4:348-353.

Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Roy. Statist. Soc. Ser. B (1995) 57:289-300.

Berr A, McCallum EJ, Alioua A, Heintz D, Heitz T, Shen WH. Arabidopsis histone methyltransferase SET DOMAIN GROUP8 mediates induction of the jasmonate/ethylene pathway genes in plant defense response to necrotrophic fungi. Plant Physiol (2010) 154:1403-1414.

Bullard JH, Purdom E, Hansen KD, Dudoit S. Evaluation of statistical methods for normalization and differential expression in mRNA-Seq experiments. BMC Bioinformatics (2010) 11:94.

Cazzonelli CI, et al. Regulation of carotenoid composition and shoot branching in Arabidopsis by a chromatin modifying histone methyltransferase, SDG8. Plant Cell (2009) 21:39-53.

Cazzonelli CI, Roberts AC, Carmody ME, Pogson BJ. Transcriptional control of SET DOMAIN GROUP 8 and CAROTENOID ISOMERASE during Arabidopsis development. Mol Plant (2010) 3:174-191.

Choe S, et al. Arabidopsis brassinosteroid-insensitive dwarf12 mutants are semidominant and defective in a glycogen synthase kinase 3beta-like kinase. Plant Physiol (2002) 130:1506-1515.

Clouse SD. Brassinosteroid signal transduction: from receptor kinase activation to transcriptional networks regulating plant development. Plant Cell (2011) 23:1219-1230.

Darley CP, Forrester AM, McQueen-Mason SJ. The molecular basis of plant cell wall extension. Plant Mol Biol (2001) 47:179-195.

Di Rubbo S, Irani NG, Russinova E. PP2A phosphatases: the "on-off" regulatory switches of brassinosteroid signaling. Sci Signal (2011) 4:pe25.

Divi UK, Krishna P. Brassinosteroid: a biotechnological target for enhancing crop yield and stress tolerance. N Biotechnol (2009) 26:131-136.

Dong G, Li J. MicroRNAs Profiling Reveals a Potential Link between the SDG8 Methyltransferase and Brassinosteroid-Regulated Gene Expression in Arabidopsis. J. Data Mining Genomics Proteomics (2013) 4:e110.

Dong G, Ma DP, Li J. The histone methyltransferase SDG8 regulates shoot branching in Arabidopsis. Biochem Biophys Res Commun (2008) 373:659-664.

Fischbeck JA, Kraemer SM, Stargell LA. SPN1, a conserved gene identified by suppression of a postrecruitment-defective yeast TATA-binding protein mutant. Genetics (2002) 162:1605-1616.

Gou X, et al. Genetic evidence for an indispensable role of somatic embryogenesis receptor kinases in brassinosteroid signaling. PLoS Genet (2012) 8:e1002452.

Grini PE, et al. The ASH1 HOMOLOG 2 (ASHH2) histone H3 methyltransferase is required for ovule and anther development in Arabidopsis. PLoS One (2009) 4:e7817.

Guo H, Li L, Aluru M, Aluru S, Yin Y. Mechanisms and networks for brassinosteroid regulated gene expression. Curr Opin Plant Biol (2013) 16:545-553.

Hao J, Yin Y, Fei SZ. Brassinosteroid signaling network: implications on yield and stress tolerance. Plant Cell Rep (2013) 32:1017-1030.

He F, et al. Structural insight into the zinc finger CW domain as a histone modification reader. Structure (2010) 18:1127-1139.

He JX, et al. BZR1 is a transcriptional repressor with dual roles in brassinosteroid homeostasis and growth responses. Science (2005) 307:1634-1638.

He JX, Gendron JM, Yang Y, Li J, Wang ZY. The GSK3-like kinase BIN2 phosphorylates and destabilizes BZR1, a positive regulator of the brassinosteroid signaling pathway in Arabidopsis. Proc Natl Acad Sci U S A (2002) 99:10185-10190.

Hoppmann V, et al. The CW domain, a new histone recognition module in chromatin proteins. EMBO J (2011) 30:1939-1952.

Hothorn M, et al. Structural basis of steroid hormone perception by the receptor kinase BRI1. Nature (2011) 474:467-471.

Jaillais Y, et al. Tyrosine phosphorylation controls brassinosteroid receptor activation by triggering membrane release of its kinase inhibitor. Genes Dev (2011) 25:232-237.

Jenuwein T, Allis CD. Translating the histone code. Science (2001) 293:1074-1080.

Kim SY, He Y, Jacob Y, Noh YS, Michaels S, Amasino R. Establishment of the vernalization-responsive, winter-annual habit in Arabidopsis requires a putative histone H3 methyl transferase. Plant Cell (2005) 17:3301-3310.

Kim TW, Guan S, Burlingame AL, Wang ZY. The CDG1 kinase mediates brassinosteroid signal transduction from BRI1 receptor kinase to BSU1 phosphatase and GSK3-like kinase BIN2. Mol Cell (2011) 43:561-571.

Kim TW, et al. Brassinosteroid signal transduction from cell-surface receptor kinases to nuclear transcription factors. Nat Cell Biol (2009) 11:1254-1260.

Kim TW, Wang ZY. Brassinosteroid signal transduction from receptor kinases to transcription factors. Annu Rev Plant Biol (2010) 61:681-704.

Klose RJ, Zhang Y. Regulation of histone methylation by demethylimination and demethylation. Nat Rev Mol Cell Biol (2007) 8:307-318.

Krogan NJ, et al. RNA polymerase II elongation factors of Saccharomyces cerevisiae: a targeted proteomics approach. Mol Cell Biol (2002) 22:6979-6992.

Li B, Carey M, Workman JL. The role of chromatin during transcription. Cell (2007a) 128:707-719.

Li B, Gogol M, Carey M, Lee D, Seidel C, Workman JL. Combined action of PHD and chromo domains directs the Rpd3S HDAC to transcribed chromatin. Science (2007b) 316:1050-1054.

Li J. Regulation of the nuclear activities of brassinosteroid signaling. Curr Opin Plant Biol (2010) 13:540-547.

Li J, Chory J. A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell (1997) 90:929-938.

Li J, Nam KH. Regulation of brassinosteroid signaling by a GSK3/SHAGGY-like kinase. Science (2002) 295:1299-1301.

Li J, Wen J, Lease KA, Doke JT, Tax FE, Walker JC. BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. Cell (2002) 110:213-222.

Li L, Ye H, Guo H, Yin Y. Arabidopsis IWS1 interacts with transcription factor BES1 and is involved in plant steroid hormone brassinosteroid regulated gene expression. Proc Natl Acad Sci U S A (2010a) 107:3918-3923.

Li L, Ye H, Guo H, Yin Y. Arabidopsis IWS1 interacts with transcription factor BES1 and is involved in plant steroid hormone brassinosteroid regulated gene expression. Proc Natl Acad Sci U S A (2010b) 107:3918-3923.

Lindstrom DL, et al. Dual roles for Spt5 in pre-mRNA processing and transcription elongation revealed by identification of Spt5-associated proteins. Mol Cell Biol (2003) 23:1368-1378.

Ling Y, Smith AJ, Morgan GT. A sequence motif conserved in diverse nuclear proteins identifies a protein interaction domain utilised for nuclear targeting by human TFIIS. Nucleic Acids Res (2006) 34:2219-2229.

Lu F, Cui X, Zhang S, Jenuwein T, Cao X. Arabidopsis REF6 is a histone H3 lysine 27 demethylase. Nat Genet (2011) 43:715-719.

Mayer A, Lidschreiber M, Siebert M, Leike K, Soding J, Cramer P. Uniform transitions of the general RNA polymerase II transcription complex. Nat Struct Mol Biol (2010) 17:1272-1278.

Mora-Garcia S, Vert G, Yin Y, Cano-Delgado A, Cheong H, Chory J. Nuclear protein phosphatases with Kelch-repeat domains modulate the response to brassinosteroids in Arabidopsis. Genes Dev (2004) 18:448-460.

Nam KH, Li J. BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell (2002) 110:203-212.

Nettleton D, Hwang JTG, Caldo RA, Wise RP. Estimating the number of true null hypotheses from a histogram of p-values. J. Agricultural, Biological, and Environmental Statistics (2006) 11:337-356.

Noh B, et al. Divergent roles of a pair of homologous jumonji/zinc-finger-class transcription factor proteins in the regulation of Arabidopsis flowering time. Plant Cell (2004) 16:2601-2613.

Oh MH, Wang X, Clouse SD, Huber SC. Deactivation of the Arabidopsis BRASSINOSTEROID INSENSITIVE 1 (BRI1) receptor kinase by autophosphorylation within the glycine-rich loop. Proc Natl Acad Sci U S A (2012) 109:327-332.

Oh MH, Wang X, Kota U, Goshe MB, Clouse SD, Huber SC. Tyrosine phosphorylation of the BRI1 receptor kinase emerges as a component of brassinosteroid signaling in Arabidopsis. Proc Natl Acad Sci U S A (2009) 106:658-663.

Palma K, et al. Autoimmunity in Arabidopsis acd11 is mediated by epigenetic regulation of an immune receptor. PLoS Pathog (2010) 6:e1001137.

Perez-Perez JM, Ponce MR, Micol JL. The UCU1 Arabidopsis gene encodes a SHAGGY/GSK3-like kinase required for cell expansion along the proximodistal axis. Dev Biol (2002) 242:161-173.

Pokholok DK, et al. Genome-wide map of nucleosome acetylation and methylation in yeast. Cell (2005) 122:517-527.

Roudier F, et al. Integrative epigenomic mapping defines four main chromatin states in Arabidopsis. EMBO J (2011) 30:1928-1938.

Russinova E, et al. Heterodimerization and endocytosis of Arabidopsis brassinosteroid receptors BRI1 and AtSERK3 (BAK1). Plant Cell (2004) 16:3216-3229.

Ryu H, Cho H, Kim K, Hwang I. Phosphorylation dependent nucleocytoplasmic shuttling of BES1 is a key regulatory event in brassinosteroid signaling. Mol Cells (2010a) 29:283-290.

Ryu H, Kim K, Cho H, Hwang I. Predominant actions of cytosolic BSU1 and nuclear BIN2 regulate subcellular localization of BES1 in brassinosteroid signaling. Mol Cells (2010b) 29:291-296.

Shafiq S, Berr A, Shen WH. Combinatorial functions of diverse histone methylations in Arabidopsis thaliana flowering time regulation. New Phytol (2014) 201:312-322.

She J, et al. Structural insight into brassinosteroid perception by BRI1. Nature (2011) 474:472-476.

Sui P, et al. H3K36 methylation is critical for brassinosteroid-regulated plant growth and development in rice. Plant J (2012) 70:340-347.

Sui P, Shi J, Gao X, Shen WH, Dong A. H3K36 methylation is involved in promoting rice flowering. Mol Plant (2013) 6:975-977.

Sun Y, et al. Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in Arabidopsis. Dev Cell (2010) 19:765-777.

Tang W, et al. BSKs mediate signal transduction from the receptor kinase BRI1 in Arabidopsis. Science (2008) 321:557-560.

Tang W, et al. PP2A activates brassinosteroid-responsive gene expression and plant growth by dephosphorylating BZR1. Nat Cell Biol (2011) 13:124-131.

Tang X, et al. Synergistic repression of the embryonic programme by SET DOMAIN GROUP 8 and EMBRYONIC FLOWER 2 in Arabidopsis seedlings. J Exp Bot (2012) 63:1391-1404.

Turck F, et al. Arabidopsis TFL2/LHP1 specifically associates with genes marked by trimethylation of histone H3 lysine 27. PLoS Genet (2007) 3:e86.

Voinnet O, Rivas S, Mestre P, Baulcombe D. An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. Plant J (2003) 33:949-956.

Wang H, et al. Dual role of BKI1 and 14-3-3 s in brassinosteroid signaling to link receptor with transcription factors. Dev Cell (2011) 21:825-834.

Wang X, Chory J. Brassinosteroids regulate dissociation of BKI1, a negative regulator of BRI1 signaling, from the plasma membrane. Science (2006) 313:1118-1122.

Wang X, et al. Identification and functional analysis of in vivo phosphorylation sites of the Arabidopsis BRASSINOSTEROID-INSENSITIVE1 receptor kinase. Plant Cell (2005) 17:1685-1703.

Wang X, et al. Sequential transphosphorylation of the BRI1/BAK1 receptor kinase complex impacts early events in brassinosteroid signaling. Dev Cell (2008) 15:220-235.

Wu G, Wang X, Li X, Kamiya Y, Otegui MS, Chory J. Methylation of a phosphatase specifies dephosphorylation and degradation of activated brassinosteroid receptors. Sci Signal (2011) 4:ra29.

Wu TD, Nacu S. Fast and SNP-tolerant detection of complex variants and splicing in short reads. Bioinformatics (2010) 26:873-881.

Xu L, et al. Di- and tri- but not monomethylation on histone H3 lysine 36 marks active transcription of genes involved in flowering time regulation and other processes in Arabidopsis thaliana. Mol Cell Biol (2008) 28:1348-1360.

Yang CJ, Zhang C, Lu YN, Jin JQ, Wang XL. The mechanisms of brassinosteroids' action: from signal transduction to plant development. Mol Plant (2011) 4:588-600.

Yin Y, Vafeados D, Tao Y, Yoshida S, Asami T, Chory J. A new class of transcription factors mediates brassinosteroid-regulated gene expression in Arabidopsis. Cell (2005) 120:249-259.

Yin Y, et al. BES1 accumulates in the nucleus in response to brassinosteroids to regulate gene expression and promote stem elongation. Cell (2002) 109:181-191.

Yoh SM, Cho H, Pickle L, Evans RM, Jones KA. The Spt6 SH2 domain binds Ser2-P RNAPII to direct Iws1-dependent mRNA splicing and export. Genes Dev (2007) 21:160-174.

Yoh SM, Lucas JS, Jones KA. The Iws1:Spt6:CTD complex controls cotranscriptional mRNA biosynthesis and HYPB/Setd2-mediated histone H3K36 methylation. Genes Dev (2008) 22:3422-3434.

Yu X, Li L, Guo M, Chory J, Yin Y. Modulation of brassinosteroid-regulated gene expression by Jumonji domain-containing proteins ELF6 and REF6 in Arabidopsis. Proc Natl Acad Sci U S A (2008) 105:7618-7623.

Yu X, et al. A brassinosteroid transcriptional network revealed by genome-wide identification of BESI target genes in Arabidopsis thaliana. Plant J (2011) 65:634-646.

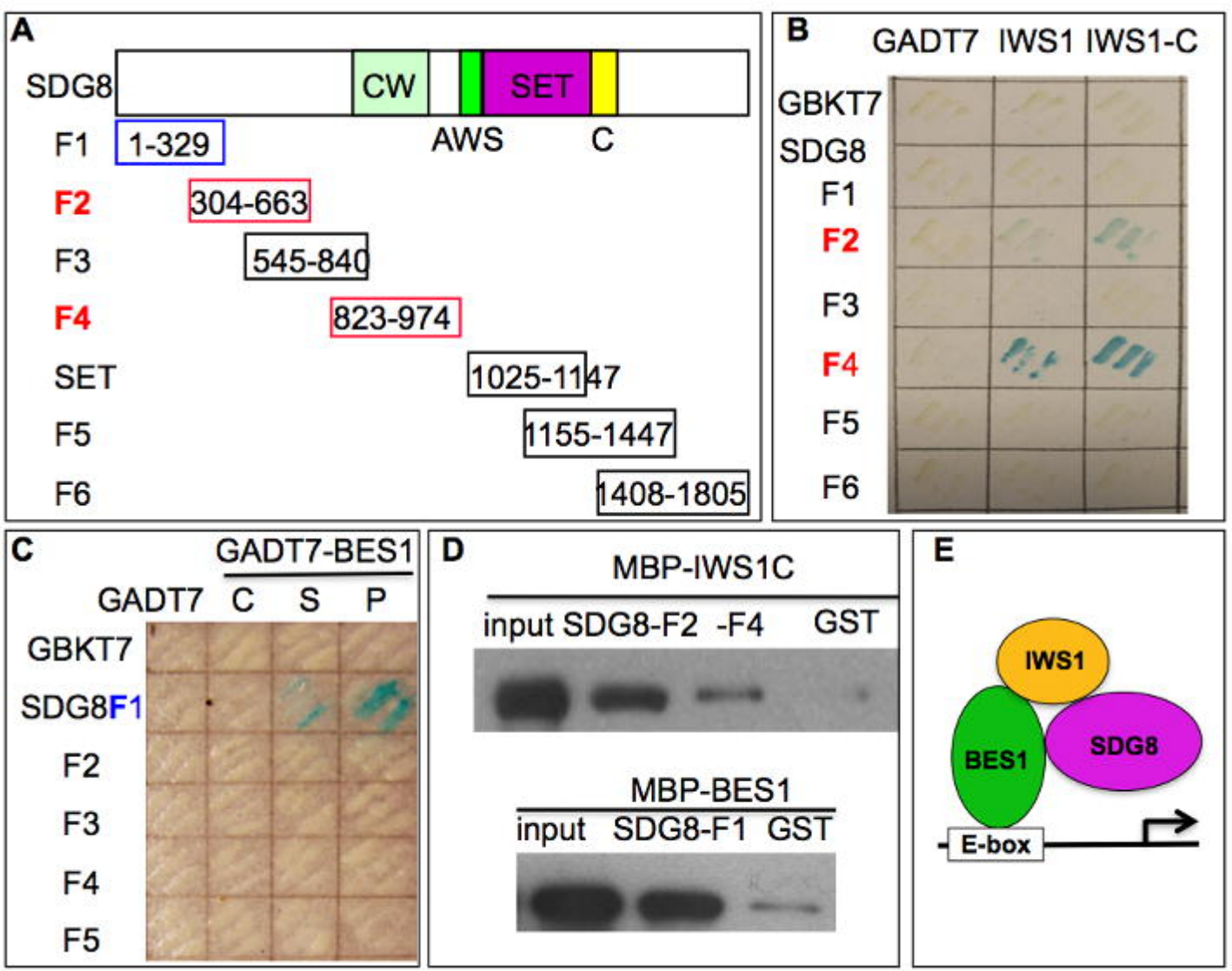
Zhang L, Fletcher AG, Cheung V, Winston F, Stargell LA. Spn1 regulates the recruitment of Spt6 and the Swi/Snf complex during transcriptional activation by RNA polymerase II. Mol Cell Biol (2008) 28:1393-1403.

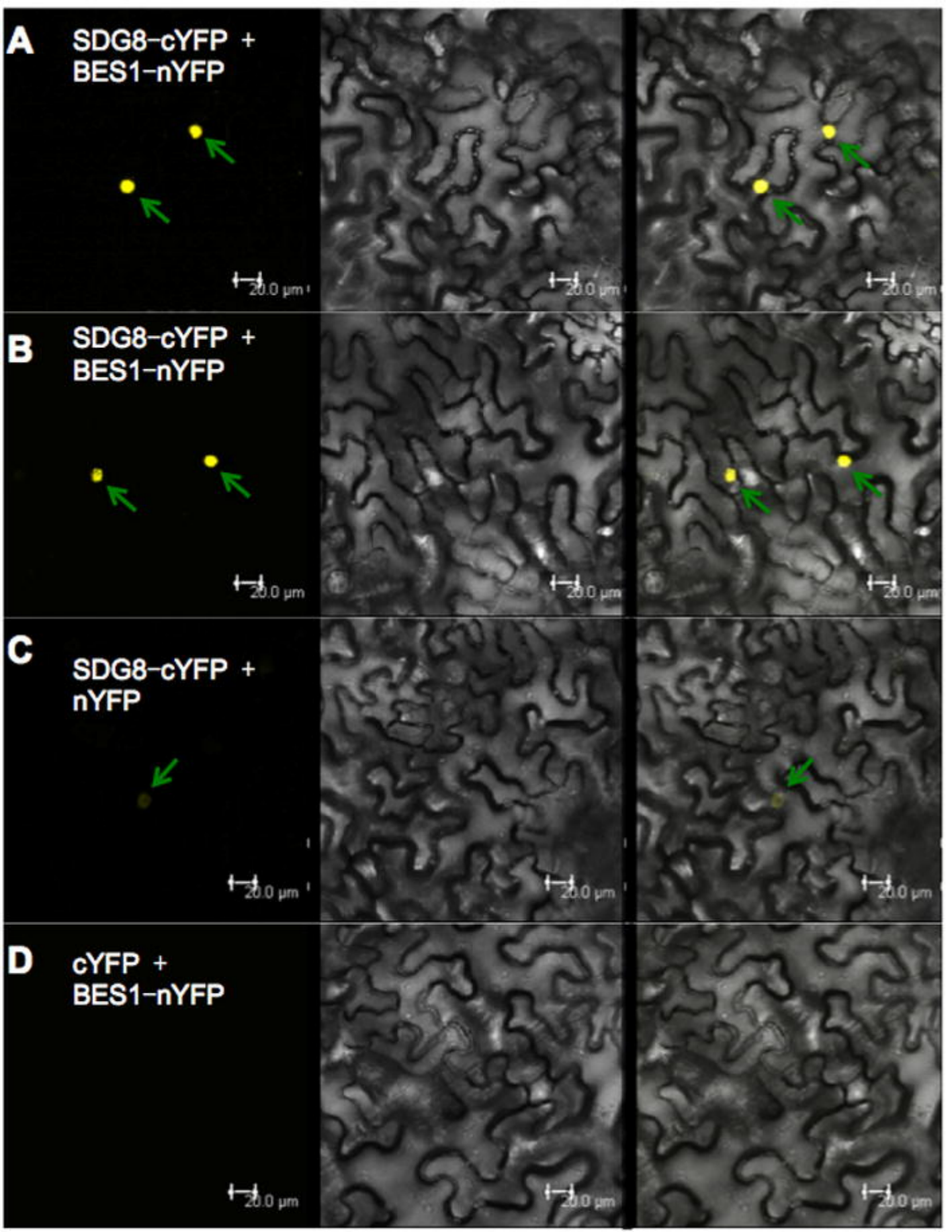
Zhang X, Germann S, Blus BJ, Khorasanizadeh S, Gaudin V, Jacobsen SE. The Arabidopsis LHP1 protein colocalizes with histone H3 Lys27 trimethylation. Nat Struct Mol Biol (2007) 14:869-871.

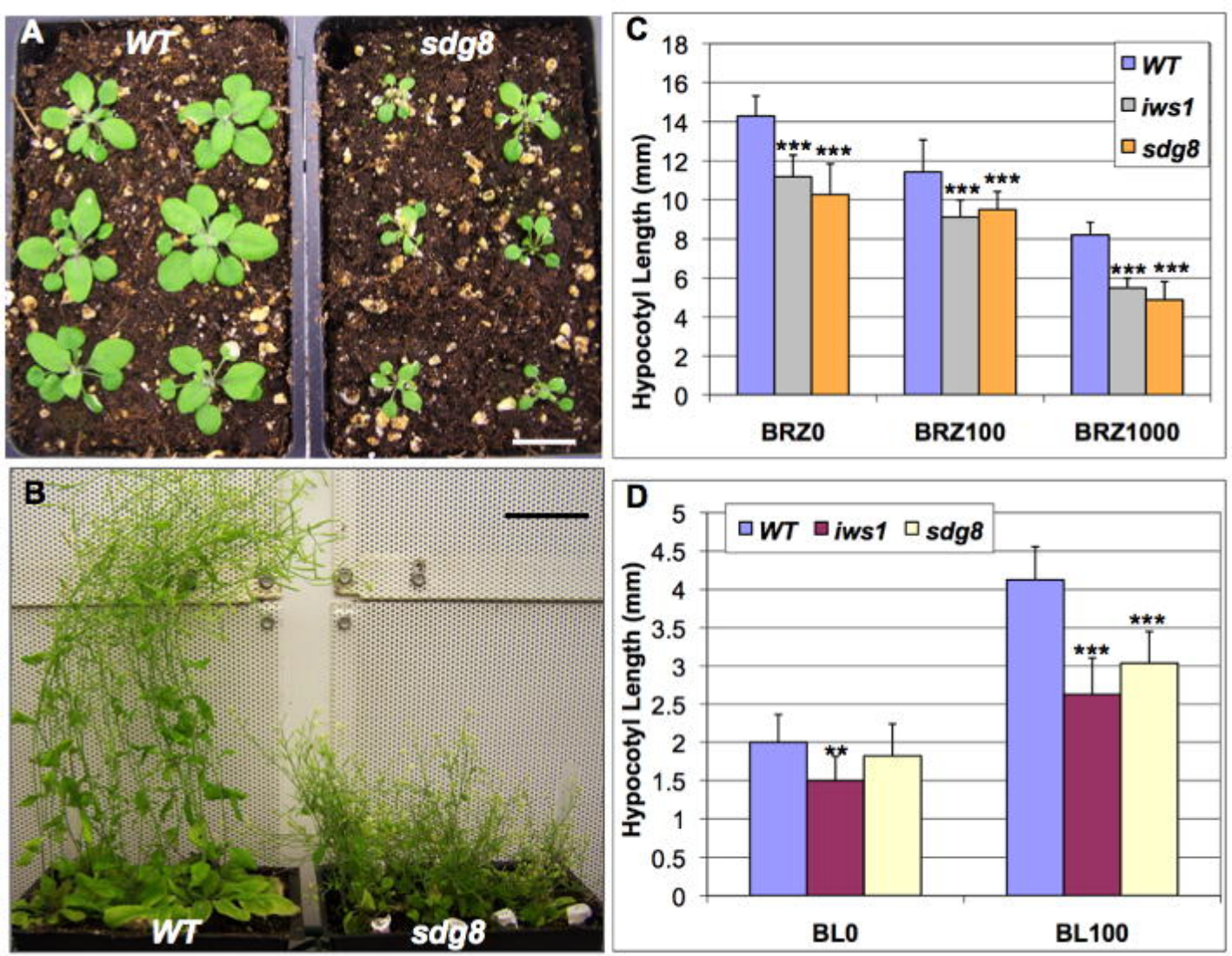
Zhao J, Peng P, Schmitz RJ, Decker AD, Tax FE, Li J. Two putative BIN2 substrates are nuclear components of brassinosteroid signaling. Plant Physiol (2002) 130:1221-1229.

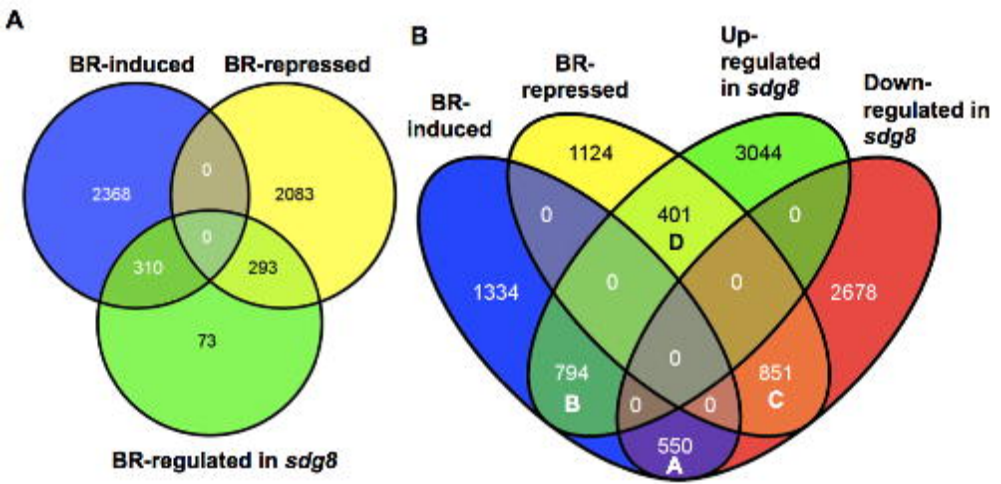
Zhao Z, Yu Y, Meyer D, Wu C, Shen WH. Prevention of early flowering by expression of FLOWERING LOCUS C requires methylation of histone H3 K36. Nat Cell Biol (2005) 7:1256-1260.

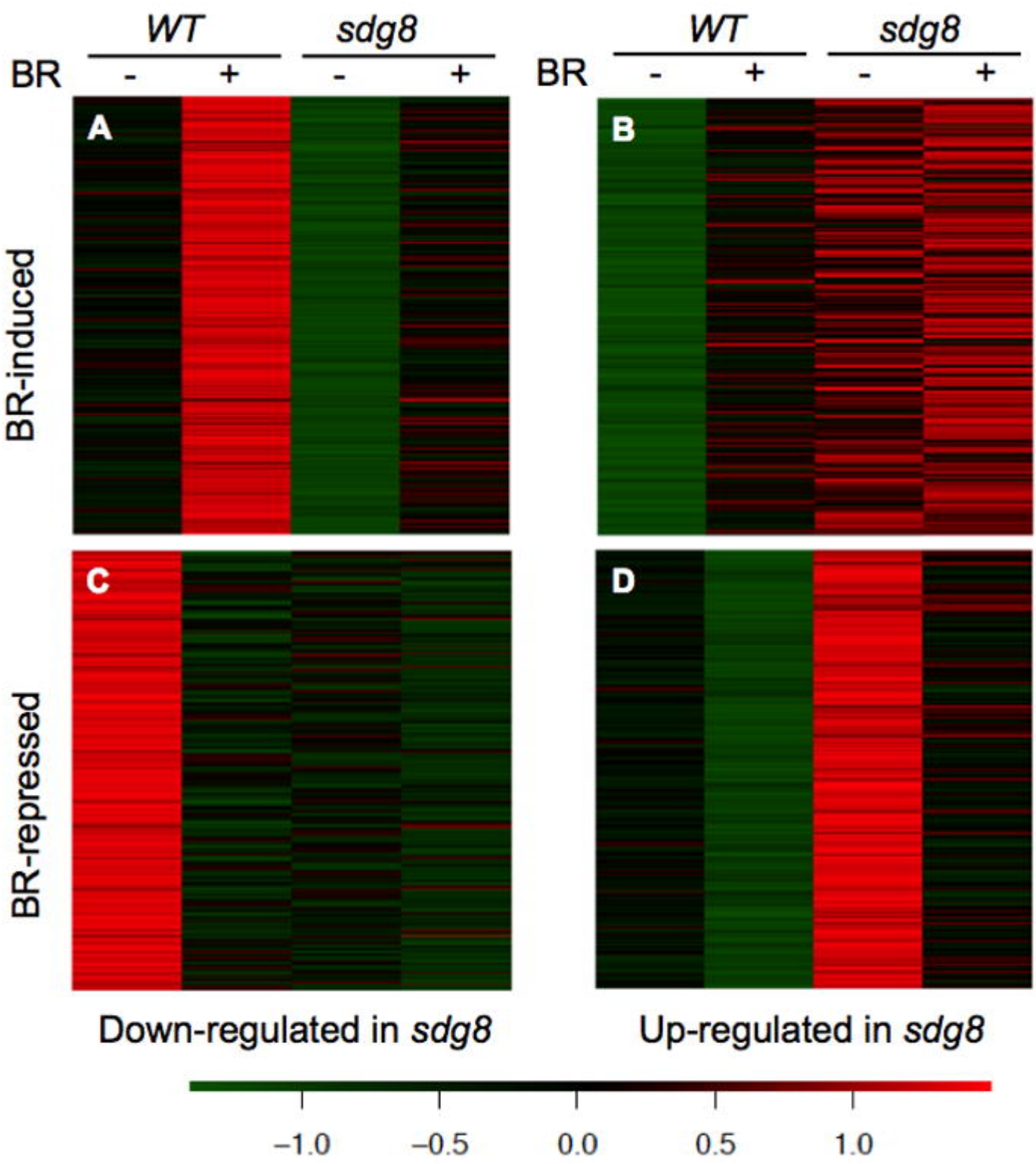
Zhu JY, Sae-Seaw J, Wang ZY. Brassinosteroid signalling. Development (2013) 140:1615-1620.


A
SDG8
CW
SET
AWS
C
F1
1-329
F2
304-663
F3
545-840
F4
823-974
SET
1025-1147
F5
1155-1447
F6
1408-1805
B
GADT7
IWS1
IWS1-C
GBKT7
SDG8
F1
F2
F3
F4
F5
F6
C
GADT7-BES1
GADT7
C
S
P
GBKT7
SDG8F1
F2
F3
F4
F5
D
MBP-IWS1C
input
SDG8-F2
-F4
GST
MBP-BES1
input
SDG8-F1
GST
E
IWS1
BES1
SDG8
E-box


A
SDG8-cYFP +
BES1-nYFP
20.0 μm
B
SDG8-cYFP +
BES1-nYFP
20.0 μm
C
SDG8-cYFP +
nYFP
20.0 μm
D
cYFP +
BES1-nYFP
20.0 μm


A
WT
sdg8
B
WT
sdg8
C
Hypocotyl Length (mm)
WT
iws1
sdg8
***
BRZ0
BRZ100
BRZ1000
D
Hypocotyl Length (mm)
WT
iws1
sdg8
**
***
BL0
BL100


A
BR-induced
BR-repressed
2368
0
2083
0
310
293
73
BR-regulated in sdg8
B
BR-induced
BR-repressed
Up-regulated in sdg8
Down-regulated in sdg8
1124
3044
0
401
D
0
1334
0
0
2678
0
794
B
851
C
0
0
550
A


WT
sdg8
WT
sdg8
BR
-
+
-
+
BR
-
+
-
+
A
B
C
D
BR-induced
BR-repressed
Down-regulated in sdg8
Up-regulated in sdg8
−1.0
−0.5
0.0
0.5
1.0

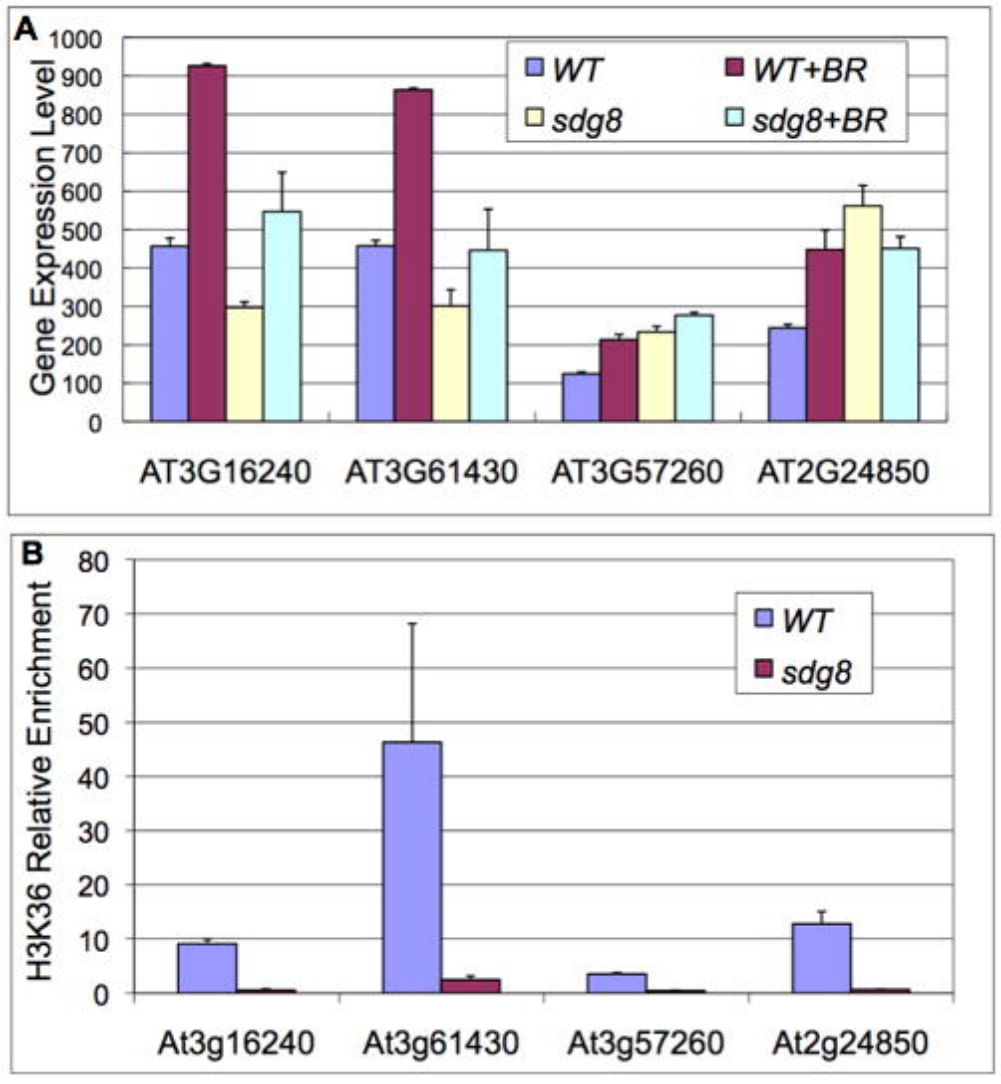

A
Gene Expression Level
1000
900
800
700
600
500
400
300
200
100
0
WT
WT+BR
sdg8
sdg8+BR
AT3G16240
AT3G61430
AT3G57260
AT2G24850
B
H3K36 Relative Enrichment
80
70
60
50
40
30
20
10
0
WT
sdg8
At3g16240
At3g61430
At3g57260
At2g24850

Table 1. Expression levels of cell elongation related genes in WT and sdg8 mutant

	Gene No.	Gene Name	*WT*	*WT+BR*	*sdg8*	*sdg8+BR*
BR-repressed & down-regulated in sdg8	AT5G65730	Xth6	95.8	64.0	49.7	36.7
	AT4G30270	Xth24	136.8	52.7	82.5	39.1
	AT5G57550	Xth25	6.5	1.6	2.6	1.0
	AT2G01850	Exta3	166.0	118.2	138.5	100.9
BR-induced & down-regulated in sdg8	AT1G69530	Expansin A1	391.2	465.5	186.8	354.9
	AT1G20190	Expansin 11	19.7	36.2	9.8	19.7
	AT3G29030	Expansin A5	82.4	116.1	45.4	76.6
	AT3G45970	Expansin-like A1	25.3	36.3	9.0	12.4
	AT4G37800	Xth7	102.4	147.2	44.5	69.6
	AT1G11545	Xth8	24.7	50.0	17.6	42.6
	AT3G23730	Xth16	36.1	46.4	28.6	48.0
	AT1G65310	Xth17	2.0	6.6	0.9	5.0
	AT4G38400	Expansin-like A2	8.6	18.9	5.3	12.8
	AT2G06850	Xth4	189.7	379.0	108.3	249.2
	AT3G44990	Xtr8	11.2	15.9	7.6	10.0
	AT1G10550	Xet33	1.8	4.4	0.7	1.6
	AT3G07010	Pectin lyase-like superfamily protein	48.2	83.3	34.7	68.9
	AT4G24780	Pectin lyase-like superfamily protein	121.7	199.4	96.3	160.3
	AT5G48900	Pectin lyase-like superfamily protein	30.7	45.8	22.9	32.2
	AT2G20750	Expansin B1	1.2	2.3	2.5	2.4
BR-induced & up-regulated in sdg8	AT4G30290	Xth19	5.5	31.2	8.0	34.5
	AT1G67750	Pectate lyase family protein	28.9	66.1	42.5	73.4
	AT3G27400	Pectin lyase-like superfamily protein	11.8	19.3	20.7	29.5
	AT3G53190	Pectin lyase-like superfamily protein	37.5	48.2	67.2	66.3

The gene expression levels (RPM) are from RNA-seq data. The average from two replicates are shown. The standard deviations are not shown.