Histone H4R3 Methylation Catalyzed by SKB1/PRMT5 Is Required for Maintaining Shoot Apical Meristem

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Abstract

The shoot apical meristem (SAM) is the source of all of the above-ground tissues and organs in post-embryonic development in higher plants. Studies have proven that the expression of genes constituting the WUSCHEL (WUS)-CLAVATA (CLV) feedback loop is critical for the SAM maintenance. Several histone lysine acetylation and methylation markers have been proven to regulate the transcription level of WUS. However, little is known about how histone arginine methylation regulates the expression of WUS and other genes. Here, we report that H4R3 symmetric dimethylation (H4R3sme2) mediated by SKB1/PRMT5 represses the expression of CORYNE (CRN) to maintain normal SAM geometrics. SKB1 lesion results in small SAM size in Arabidopsis, as well as down-regulated expression of WUS and CLV3. Up-regulation of WUS expression enlarges SAM size in skb1 mutant plants. We find that SKB1 and H4R3sme2 associate with the chromatin of the CRN locus to down-regulate its transcription. Mutation of CRN rescues the expression of WUS and the small SAM size of skb1 mutant. Thus, SKB1 and SKB1-mediated H4R3sme2 are required for the maintenance of SAM in Arabidopsis seedlings.

Introduction

The shoot apical meristem (SAM) is a self-maintaining structure harboring stem cells which give rise to all of the above-ground tissues and organs in higher plants. The stem cells reside at the center of the SAM, known as the central zone (CZ). The SAM also possesses two other zones, the peripheral zone (PZ) and the rib zone (RZ), which are comprised by the descendant stem cells. The PZ is the source of lateral organs, while the RZ gives rise to the pith of stem [1-3].

Underlying the CZ, there is a region containing about ten cells termed the organizing center (OC), in which a homeodomain transcription factor WUSCHEL (WUS) is expressed to maintain the number of stem cells [4]. In wus mutants, SAM is not properly organized in the embryo. In the postembryonic phase, the defective SAM terminates prematurely in flat structures, and new SAMs are initiated and terminated repeatedly [5]. Mature WUS protein migrates to the CZ to activate the transcription of CLAVATA3 (CLV3), which is a marker gene for stem cell identity, by binding to its promoter [6]. CLV3 encodes a 96-amino acid protein with an 18-amino acidic peptide secretory signal in its N-terminal region [7]. After synthesis in the stem cells, CLV3 is processed to a 13-amino-acid arabinosylated glycopeptide, and then secreted into the extracellular space [8]. clv3 mutants develop enlarged SAMs during both the embryonic and postembryonic phases, and show an enlarged WUS expressing domain in the SAM [9,10]. Overexpression of CLV3 results in a wus-like phenotype [11]. Thus, CLV3 forms a negative feedback loop with WUS to maintain the constant number of stem cells. Mutation screening and genetic analysis have identified several receptor-like kinases that play important roles in transmitting CLV3 signal for repression of WUS transcription. CLV1 encodes a transmembrane protein which is comprised of an extracellular leucine-rich repeat domain, a transmembrane domain, and an intracellular Ser/Thr kinase domain [12]. CLV2 has a similar structure but lacks the kinase domain [13]. Both clv1 and clv2 mutants have similar phenotypes and WUS expression patterns with clv3 mutants [10]. CLV3 can form a 185 kD...
complex with CLV1 and bind to the ectodomain of CLV1 directly [14,15].

CORYNE (CRN) is another receptor-like kinase that transmits CLV3 signal. It comprises an N terminal signal peptide, a transmembrane domain, and a Ser/Thr kinase domain. CRN defect in Arabidopsis results in large SAM size, increased floral organ number, abnormal siliques, etc. [16]. Genetic and biochemical analyses indicate that CRN forms a heterodimer with CLV2 to transmit CLV3 signal, not only because that CRN lacks the extracellular receptor domain and CLV2 lacks the kinase domain, but also because they require each other to localize to the membrane [17-19].

CRN also mediates the interaction of CLV1 with CLV2, CRN, and RECEPTOR-LIKE PROTEIN KINASE 2 (RPK2) / TOADSTOOL 2 (TOAD2). RPK2/TOAD2 is an additional receptor kinase that functions in transmitting the CLV3 signal [20,21]. RPK2 doesn’t interact with CLV1 or CLV2 in the absence of CRN, while CLV1 only interacts weakly with CLV2 in the presence of CRN [18,19].

SKB1/PRMT5 is a member of the type II arginine methyltransferase family, which is conserved among many eukaryotic species [22-26]. In human, SKB1/PRMT5 was found to mediate symmetric dimethylation of arginine residues of histone and non-histone protein to regulate chromatin remodeling, gene transcription, pre-mRNA splicing, and protein stability [27-31]. In Arabidopsis, we and others have found that SKB1 promotes flowering and responses to salt stress through controlling both gene transcription and pre-mRNA splicing [32-35], and regulates circadian rhythms by regulating alternative splicing [36].

In this study, we show that the seedlings of skb1 mutant plants grew slower and smaller than WT plants. A detailed morphological analysis revealed that the SAM size of skb1 was significantly reduced. We found that the expression levels of WUS and CLV3 were down-regulated in skb1, and up-regulated WUS expression could rescue the small SAM size of skb1. We also found that SKB1 and histone H4R3me2 were associated with the chromatin of the membrane kinase gene CRN, and did not repress WUS expression directly. Introduction of CRN mutation into the skb1 mutant could rescue both WUS expression and the small SAM size.

Results

SAM defects of skb1 mutants

skb1 mutants display many characteristics during their development, including curled and darker-colored leaves, late flowering, hypersensitivity to salt stress, alteration of circadian clock, etc. [32-36]. We also found that the skb1 seedling size was smaller when compared with the wild type Col-0 seedlings. At 10 days after germination (DAG), the size of skb1 seedlings was significantly smaller than that of Col-0. Interestingly, the difference between seedling sizes at 5 DAG was minimal (Figure 1A). We compared mature seeds of skb1 and Col-0 and found that there were no obvious morphological defects, nor was the mass of the skb1 seeds decreased (Figure S1). This indicates that the small seedling was a growth defect in skb1 mutants, rather than a developmental defect of the seeds. Moreover, the skb1 plants grew to a normal size at later developmental stages (Figure S2).

To fully characterize the growth defect of skb1 seedlings, we examined the leaf initiation rates and found that skb1 leaf initiation was decreased about 50% compared with that of Col-0 (Figure 1B and 1C, Figure S3). This decrease suggests that the skb1 mutants may have lower SAM activity than Col-0. To verify this notion, we measured the SAM size of the seedlings at different post-embryonic stages to see whether the size was relative to the activity of SAM. We found that the SAM size in skb1 plants was smaller than in Col-0 at 6 to 12 DAG (Figure 1D and 1E). Under microscope, a typical three layers of SAM outer cells (L1, L2, L3) could be observed clearly in structure without difference between Col-0 and skb1 mutant. Consistent with the SAM size, L1 cell number in skb1 plants was also less than in Col-0 at different post-embryonic stage (Figure 1E, lower panel). When we introduced a 35S::SKB1 construct into the skb1 mutants, both the leaf initiation rate and SAM size phenotypes were reversed (Figure 1B to 1D). These data indicate that SKB1 plays an important role in maintaining SAM size and in regulating SAM activity in Arabidopsis seedlings.

Genetic interaction between SKB1 and CLV genes

Unlike the small SAM size of skb1 mutants, clv mutants showed enlarged SAMs. To investigate whether a genetic interaction exist between SKB1 and CLV genes, we crossed skb1 (Ler background) with clv1-1 and clv3-2. The SAM sizes of clv1 skb1 and clv3 skb1 at 9 DAG were much larger than skb1 single mutants, while they showed only a slight difference from clv1 and clv3, respectively (Figure 2A to 2G). The sizes of the inflorescence meristems of clv1 skb1 and clv3 skb1 were also increased markedly compared with skb1 (Figure 2H to 2M). We also over-expressed SKB1 in clv1-1 and clv3-2 by driving SKB1 under the control of the cauliflower mosaic virus 35S promoter. The defects of the inflorescences, flowers, and siliques of clv3 didn’t show any detectable rescue (Figure S4 and Data not shown).

In addition to the stem cell defects of clv1 skb1 and clv3 skb1, we noticed that these plants exhibit late flowering. This late flowering phenotype is similar to skb1 and distinct from clv1 and clv3 (Figure 2N). Thus, SKB1 appears to maintain the shoot meristem upstream of CLAVATA genes, or regulate the process in a parallel pathway.

SKB1 is required for the expression of WUS and CLV3

SAM and shoot apical stem cell maintenance is primarily regulated through two pathways. WUS and SHOOT MERISTEMLESS (STM) play the primary roles in each pathway [10,37,38]. To investigate the molecular mechanism underlying the SAM phenotype in skb1 mutants, we examined the transcript levels of key genes regulating shoot meristem maintenance. Among the genes we tested, WUS and CLV3 were found down-regulated in the skb1 mutants, but STM and other genes were not altered significantly (Figure 3A and 3B). We also used the GUS assay to investigate the localization and dynamic transcription levels of WUS and CLV3. At 3 DAG, the expression of WUS and CLV3 was quite low in both Col-0 and
At 6 DAG and during a few following days, the expression of *WUS* and *CLV3* was lower in *skb1* than in Col-0 (Figure 3C and 3D), but the localizations of *WUS* and *CLV3* were not changed in the *skb1* mutants. These data suggest that *SKB1* is involved in the WUS-CLV feedback loop to maintain the stem cells.

In *Arabidopsis*, flowering is associated with the transition of SAMs into inflorescence and floral meristems. Flowering time is regulated mainly by four pathways. These are photoperiod pathway, vernalization pathway, gibberellins (GA) pathway, and autonomous pathway [39]. FLOWERING LOCUS C (FLC) is a key regulator of flowering time control, and integrates the autonomous pathway and vernalization pathway. As a member of autonomous pathway genes, *SKB1* works upstream of *FLC* and regulates the transcription of *FLC* directly [33]. We wondered whether the increased expression level of *FLC* in *skb1* mutants was responsible for the small SAM size, so we introduced pWUS::GUS and pCLV3::GUS to flc and ffc *skb1* mutants. We found that the expression levels of both *WUS* and *CLV3* in flc *skb1* double mutants were similar to those in *skb1* and much lower than in flc or Col-0 plants (Figure 3E and 3F). These results suggest that *SKB1* regulates the expression of *WUS* and *CLV3* in an FLC-independent manner.

To explore whether a low expression level of *WUS* is the major cause for a small SAM size in *skb1*, we crossed *skb1* with the *pga6-1* mutant to generate a *pga6-1 skb1* double mutant. *pga6-1* is a gain-of-function mutant in which the expression of *WUS* can be induced by application of 17-β-estradiol [40]. In the absence of the inducer compound 17-β-estradiol, the growth of *pga6-1 skb1* seedlings was almost the same as that of *skb1* (Figure 1A and 4A). While in the presence of 17-β-estradiol, the growth of *pga6-1 skb1* seedlings was slightly repressed but not as repressed as in

**Figure 1. Phenotypes of growth rates and SAM size in *skb1* seedlings.** (A) Comparison of seedling size of Col-0 and *skb1* grown on MS medium at 5 DAG and 10 DAG. Scale bar = 1 cm. (B) and (C) Comparison of rosette leaf growth rate of Col-0, *skb1* and 35S::SKB1 *skb1* plants grown on MS medium. Leaf initiation rate is calculated as the number of new leaves produced per day between 6 DAG and 10 DAG. Data represent the means ± SE (n=36). (D) Comparison of SAM size of Col-0, *skb1* and 35S::SKB1 *skb1* plants grown on MS medium at 6 DAG. Scale bar = 20 μm. (E) Quantitative analysis of SAM size (upper panel) and Layer 1 cell number (lower panel) of Col-0 and *skb1* plants grown on MS medium at 6 DAG, 9 DAG and 12 DAG. L1 means layer 1 of SAM. More than 13 SAM sections were measured for size and counted for L1 cell number under microscope for each data point. Data represent the means ± SE.

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Interestingly, the SAM size was much larger in pga6-1 skb1 and, even larger than that of Col-0 (Figure 4C and 4D). We examined the expression of WUS, and found that it was increased to a much greater extent in pga6-1 and pga6-1 skb1 in the presence of 17-β-estradiol than Col-0 and skb1 (Figure 4B). In the presence of the inducer, the expression level of CLV3 in pga6 skb1 double mutant was also higher than in Col-0 (Figure 4E). These results imply that the SAM defect in skb1 seedlings was correlated with the reduction of the WUS expression level.

**SKB1 is required for the deposition of histone H4R3 symmetric dimethylation in CRN chromatin**

To explore whether WUS is a direct target of SKB1, we performed chromatin immunoprecipitation (ChIP) assays using 12-day-old seedlings as materials with an anti-SKB1 antibody. Ten regions covering the promoter and the gene body of WUS were tested by PCR. Region c of WUS contains the critical cis element for the expression boundaries of WUS transcription in the shoot apical stem cell niche [41]. The ChIP results revealed that SKB1 could not pull down any regions of the promoter or the gene body of WUS (Figure S5). Given that the WUS expression cells were less than 1% of the whole seedling, we...
also collected the inflorescence tip of clv1 and clv1 skb1 which have enlarged SAM and much more apical stem cells to perform the experiment, and we got the same results (data not shown). Thus, SKB1 does not regulate the transcription level of WUS directly.

Through ChIP-sequencing experiments using anti-SKB1 antibody (data not shown), we found that a receptor-like kinase gene named CRN could be a direct target of SKB1. Previous studies have proven that CRN forms a heterodimer with CLV2 to transmit CLV3 signal [18,19]. Mutation of CRN resulted in a larger SAM [16]. To confirm the ChIP-seq data, we performed a ChIP (-qPCR) analysis with anti-SKB1 and anti-H4R3sme2 antibodies. SKB1, as well as H4R3sme2 was significantly enriched in the promoter and first exon but not the 3’ end of CRN (Figure 5A and 5B). To date, H3R3sme2 was found as a suppressor code for transcription in Arabidopsis [33,34]. To confirm that CRN was a target gene of SKB1, we checked the expression level of CRN in skb1 mutants, and found that CRN was up-regulated in skb1 (Figure 5C). These results indicate that SKB1 regulates the expression of CRN through altering the modification levels of histone mark H4R3sme2 in CRN chromatin.

To confirm that the increased expression of CRN was the direct cause of the decreased SAM size in skb1, we quantified SAM sizes from the crn skb1 double mutant. At 9 DAG, the SAM size of crn skb1 was comparable to that of crn, providing direct evidence that the small SAM size of skb1 was rescued by crn mutation (Figure 5D and 5E). We also determined WUS and CLV3 expression levels in crn skb1 and found that both were higher than in the skb1 single mutant, even than in Col-0, though not as high as in crn (Figure 5F). Thus, SKB1 regulates

Figure 3. Differences in expression profiles between Col-0 and skb1 plants. (A) Semi-quantitative RT–PCR analysis of known meristem regulators. TUBULIN (TUB) was the loading control. Three biological replicates were performed with similar results. (B) Quantitative real time PCR analysis of the expression of WUS and CLV3 in Col-0 and skb1 seedlings and normalized with ACTIN2 expression. Data represent the means ± SE of three independent experiments. (C) and (D) Staining results of GUS activity assays of pWUS::GUS (C) and pCLV3::GUS (D) transgenic lines in Col-0 and skb1 backgrounds, respectively. Materials used were seedlings grown to 3, 6, 9 and 12 DAG on MS medium. (E) and (F) Staining results of GUS activity assays of pWUS::GUS (E) and pCLV3::GUS (F) transgenic lines in Col-0, skb1, flc, and flc skb1 backgrounds, respectively. Materials used were seedlings grown to 5 DAG on MS medium. Scale bar = 20 μm.

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SAM size by methylating H4R3 and controlling the expression of CRN.

Discussion

In this study, we found that the protein arginine methyltransferase SKB1 was functionally involved in the maintenance of SAM in Arabidopsis. SKB1 associated with the chromatin of the gene CRN, mediated the symmetric dimethylation of histone H4R3, and repressed the transcription of CRN. The WUS and CLV3 expression were down-regulated in skb1 mutants because of the increased CRN level, and thus resulted in the smaller SAM size. Our results reveal a previously unknown function of SKB1 in regulating SAM size through the major WUS-CLV signaling pathway.

SKB1 is important for the maintenance of the SAM

To date, two parallel pathways have been identified to play critical functions in maintaining shoot meristems. The homeodomain transcription factors WUS and STM regulates each pathway, respectively [10,37,38]. It is thought that the WUS-CLV pathway maintains a constant stem cell number, while STM pathway prevents differentiation. WUS is required for the expression of the stem cell marker gene CLV3. CLV3 works as a ligand, binding to the membrane localized receptor-like kinases to restrict the expression of WUS. Expression level changes of these genes alter the SAM and seedling phenotypes. We found that the expression of WUS was decreased in the skb1 mutant in which the modification levels of H4R3sme2 reduced, which is not consistent with that histone H4R3sme2 is a suppressor code for transcription if WUS is a direct target of SKB1 [33,34]. Further experiments found that SKB1 did not associate with the chromatin of WUS (Figure S5), and SKB1-mediated H4R3sme2 directly repressed the
transcription level of CRN, which is an important member of WUS-CLV pathway. In skb1 mutants, the expression level of CRN was increased, resulting in decreased WUS and CLV3 transcription levels, and small SAM size. It has been reported previously that the WUS expression was regulated by several histone modifications, such as H3K27me3, H3K4me3, H3K9me2 and H3K9ac [42,43], our data highlighted the function of histone arginine methylation in controlling the WUS expression and SAM size maintenance.

Although skb1 can regulate alternative RNA splicing [32,34,36], we did not find any significant pre-mRNA splicing defects of WUS, CLV3 or CRN (data not shown). Rather, we noted the transcription level alternation.

Another major pathway that control SAM maintenance is the homeobox genes of the KNOX family. One of these, STM is reported to distinguish the SAM from lateral organs. STM is expressed throughout most of the SAM. It prevents differentiation by restricting the expression of ASYMMETRIC...
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**LEAVES1 (AS1) and ASYMMETRIC LEAVES2 (AS2) to the domain where lateral organs will initiate [44,45]. AS1 and AS2 are necessary to leave organ initiation, and have the proper structure and shape [46,47]. In our study, we noticed that the rosette leaf initiation rate of skb1 was slower than that of WT plants, while the expression of KNOX genes (such as STM, KNAT1, KNAT2, and KNAT6) as well as AS1, AS2 were not altered. Further study will elucidate how the lower rosette leaf initiation rate occurred in skb1 mutants.**

**Relationship between seedling and SAM size**

In this paper and our previously reports, we noticed the mutation of SKB1 result in smaller organ size and reduced growth rate in Arabidopsis (Figure 1 and Figure S2) [33,34]. Similar growth defects were reported in other eukaryotic species. In fission yeast, Schizosaccharomyces pombe, cells carrying a skb1 null mutant were less elongated than WT and exhibit a moderate growth defect [48]. Further studies show that SKB1 is a component of the morphology control branch of the Ras signaling cascade, and negatively regulates mitosis [48,49]. In animals, SKB1/PRMT5 interacts with different transcription factors and chromatin modifying enzymes and mediates methylation of nuclear and cytoplasm proteins to modulate cell growth, transformation, apoptosis, and organelle biogenesis [50]. These results indicate that SKB1/PRMT5 plays a conserved role in regulating growth among different species.

We obtain several lines of evidence that CRN is an important target of SKB1 in regulating SAM maintenance in Arabidopsis. Mutant of CRN could rescue specifically the small size of skb1 mutants, but not seedling size (Figure S6), which indicates that in addition to the smaller SAM size, there might be other factors that lead to the smaller size of the seedlings. Firstly, though the SAM size of crn skb1 was almost the same as crn, the expression level of WUS and CLV3 was lower than that of crn, which may impact seedling growth. Secondly, the root length of skb1 mutants was reduced compared with Col-0 [34]. The shorter roots caused by SKB1 lesion might create a deficit in providing sufficient nutrients. Thirdly, skb1 mutants are hypersensitive to salt stress [34] and the root stem cells are hypersensitive to DNA damage (unpublished data), which may also impact seedlings grow and render the plants much weaker and smaller. Collectively, our studies revealed a SKB1–CRN regulatory mechanism mediated by H4R3me2 modification that plays an important role in SAM geometry.

**SAM maintenance and flowering-time control**

The SAM is formed during seed development, and gives rise to all of the above-ground tissues and organs including leaves, stems, flowers, and so on. With transition from vegetative to reproductive growth, the SAM transitions to become the inflorescence and floral meristems. Floral meristem identity factors such as LEAFY (LFY) and APETALA1 (API) are activated by flowering-time control genes such as SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1) and FLOWERING LOCUS T (FT) [39]. Previous studies reported that the floral homeotic gene AGAMOUS (AG) repressed WUS expression resulted in termination of meristem growth during flower formation [51]. This indicates that flowering is connected with SAM termination. In this study, we noticed that flowering time suppressor FLC did not regulate the expression of WUS and CLV3 (Figure 3E and 3F), in spite of the pivotal role of the FLC in regulating flowering in skb1 mutants [33]. In addition, mutation of the CLV genes in skb1 mutant background did not change the flowering time of skb1 (Figure 2N). This means that flowering-time control is connected with the formation of inflorescence and floral meristems and termination of the SAM, but does not regulate SAM maintenance genes like WUS or CLVs directly.

**Materials and Methods**

**Plant material and growth conditions**

The Arabidopsis thaliana ecotypes Col-0 and Ler were used as wild type plants in this study. skb1 (Salk_065814), 35S::SKB1 skb1, ftc (ftc-3), ftc skb1, clv1-1, clv3-2, cm (so-2-1), paga-6, pWUS::GUS Col-0, and pCLV3::GUS Col-0 were described previously [9,33,52,53]. skb1 (Ler background) was generated by crossing skb1 with Ler and back crossing to Ler three times. 35S::SKB1 Ler, 35S::SKB1 clv1-1, 35S::SKB1 clv3-2 were transformed using construct 35S::SKB1, as reported previously [33].

Plants were grown on MS medium or in soil. Seeds were surface sterilized with 70% ethanol for 1 minute, 15% sodium hypochlorite for 15 minutes, and washed four times with sterile water. Sterilized seeds were sown on MS medium. To promote germination, seeds on MS medium were stratified at 4 °C in darkness for 3 days and then transferred to a culture room at 22 °C under long-day (16 hours light/ 8 hours dark) conditions.

**RNA extraction and RT-PCR analysis**

Total RNA was extracted using Trizol reagent (Invitrogen) from the SAM material grown in soil to 5 DAG (Figure 3B), or grown on MS medium to 6 DAG (Figure 4) or 9 DAG (Figure 3A and 5C). cDNA synthesized using a template of 4 μg RNA using Super Script III (Invitrogen) reverse transcriptase. Semi-quantitative and quantitative real-time PCR were performed, TUBULIN and ACTIN2 were used as endogenous expression control in semi-quantitative and quantitative real-time PCR, respectively. The primers used are listed in Table S1.

**GUS staining**

β-glucuronidase (GUS) activity detection was performed following Scarcella et al. [54]. With modification that both potassium ferro- and ferricyanide to 5 mM were added and incubation time of 6 hours was used in all experiments except in Figure 3F, which had a 1 hour incubation time.

**SAM observation and measurement**

For SAM size measurement, seedlings with indicated age and genotype were fixed with 4% PFA for 15 minutes, washed by PBS, then were incubated overnight at 4°C in 10% glycerol. Seedlings were embedded in O.C.T. Compound (Tissue-Tek) and were longitudinally sectioned into 20 μm serial slices. These slices were then observed under a differential interference contrast (DIC) microscope. The middle slice for
each seedling which had the clearest SAM shape was taken to measure SAM size and L1 cell numbers.

**ChIP and ChIP-sequencing**

12-day-old seedlings grown in soil were analyzed in ChIP assays as described previously [33], and inflorescence tips for ChIP-PCR analysis with antibodies anti-SKB1 and anti-Histone H4 (symmetric di methyl R3) antibody (ab5823). Primers used for PCR are listed in Table S1.

For ChIP-sequencing, 2 g of 12-day-old Arabidopsis whole seedlings were used. Polyclonal anti-SKB1 antibody was used with BSA-blocked Protein G agarose beads (Millipore) to immunoprecipitate the SKB1-DNA complex. skb1 mutant seedlings grown under the same condition were used as negative control following the same assay procedure.

The ChIP-sequencing library was constructed according to Illumina’s instructions (www.illumina.com). Sequence reads were mapped to the unmasked Arabidopsis genome (TAIR8 build) using the SOAP2.20 software, allowing at most two mismatches at any position. Enrichment was then calculated in each valid base pair by comparing using UCSC Genome Browser. Peak region was valuated using the MACS 1.3.6 software.

**Accession Numbers**

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: SKB1, At4G31120; WUS, At2G17950; CLV1, At1G75820; CLV3, At2G27250; STM, At1G62360; KNAT1, At4G08150; KNAT2, At1G70510; KNAT6, At1G23380; AG, At4G18960; AP3, At3G54340; AS1, At2G37630; AS2, At1G65620; POL, At2G46920; TUB, At5G62690; FLC, At5G10140; CRN, At5G13290; ACTIN2, At3G18780.

**Supporting Information**

**Figure S1.** Comparison of skb1 and Col-0 seeds. (A) Morphology of skb1 and Col-0 seeds. (B) Comparison of weight per thousand seeds of skb1 and Col-0.

**Figure S2.** Phenotypes of skb1 in different growth stage. (A) to (D) Sizes of skb1 compared with Col-0 at 25 DAG (A), 33 DAG (B), 41DAG (C) and 59 DAG (D).

**Figure S3.** Rosette leaf number calculation. The top views of Col-0, skb1, 35S::SKB1 skb1 seedlings under a dissecting microscope at 6 DAG and 10 DAG. All mature rosette leaves as well as young leaves were counted. The arrowhead indicates a rosette leaf and c indicates a cotyledon.

**Figure S4.** Phenotypes of 35S::SKB1 clv3-2 and 35S::SKB1 clv1-1. (A) Top views of inflorescences of Ler, 35S::SKB1 Ler, clv3-2, 35S::SKB1 clv3-2, clv1-1, 35S::SKB1 clv1-1. (B) Phenotypes of flowers in 35S::SKB1 clv3-2 and 35S::SKB1 clv1-1 compared with Ler, clv3-2 and clv1-1. (C) Phenotypes of whole plants of 35S::SKB1 clv3-2 and 35S::SKB1 clv1-1 compared with Ler, clv3-2 and clv1-1.

**Figure S5.** ChIP analysis of Col-0 and skb1 at the WUS locus. (A) A diagram of the WUS gene structure, with bars representing the a-i regions examined by ChIP. White boxes indicate WUS open reading frame. (B) The ChIP assay was performed with antibody against SKB1.

**Figure S6.** Comparison of size of crn and crn skb1 seedlings at 9DAG. Scale bar = 1 cm.

**Table S1.** primers used in the paper.

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**Author Contributions**

Conceived and designed the experiments: SB MY Y. Zhang. Performed the experiments: MY Y. Zhao QL Y. Zhang ZZ. Analyzed the data: SB MY Y. Zhang. Contributed reagents/materials/analysis tools: SB MY Y. Zhang Y. Zhao ZZ. Wrote the manuscript: MY SB QL.
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