SUMO modification of LBD30 by SIZ1 regulates secondary cell wall formation in Arabidopsis thaliana

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Abstract

A wide range of biological processes are regulated by sumoylation, a post-translational modification involving the conjugation of SUMO (Small Ubiquitin-Like Modifier) to protein. In Arabidopsis thaliana, AtSIZ1 encodes a SUMO E3 ligase for SUMO modification. siz1 mutants displayed defective secondary cell walls (SCWs) in inflorescence fiber cells. Such defects were caused by repression of SND1/NST1-mediated transcriptional networks. Yeast two-hybrid assay indicated that SIZ1 interacts with the LBD30 C-terminal domain, which was further confirmed using bimolecular fluorescence complementation and immunoprecipitation. Mass spectrometry and co-immunoprecipitation indicated that SIZ1 mediates SUMO conjugation to LBD30 at the K226 residue. Genes controlling SCW formation were activated by the overexpression of LBD30, but not in the LBD30(K226R) mutant. LBD30 enhancement of SCW formation resulted from upregulation of SND1/NST1-mediated transcriptional networks. This study presents a mechanism by which sumoylation of LBD30, mediated by SIZ1, regulates SCW formation in A. thaliana.
Introduction

Plant cells are surrounded by walls that provide structural support and regulate growth. All plant cells form primary cell walls, which are synthesized during cell expansion and differentiation, while specialized cell types can also deposit a secondary wall on the inside of the primary wall once cell elongation has finished. Examples of the SCW are found in vascular tissues, such as in fiber cells and tracheary elements, as well as in other mechanically important tissues, for example, collenchyma cells. The major constituents of the SCW are cellulose, non-cellulosic polysaccharides and lignin. These polymers are cross-linked, providing cell walls with both mechanical strength and hydrophobic properties. Such characteristics are needed for upright growth, long-distance transport of solutes[1], selectivity of nutrient and water transport in root endodermis[2], defense against pathogens[3], and phenomena such as pod shattering[4], anther dehiscence[5] and flower abscission [6].

In the cells undergoing SCW biosynthesis, SCW cellulose synthase complexes in the plasma membrane produce β-(1–4) glucan chains that assemble into microfibrils in the orientation guided by cortical microtubules [7]. The microfibrils are extruded into the cell wall matrix and interact with Golgi-synthesized hemicellulose, generally xylan and mannan, to form a stable network [8]. Lignin monomers are transported to the space within the polysaccharide network where they are oxidized and polymerized to make matured SCW [9]. Genes responsible for the SCW biosynthesis process are regulated by a group of transcriptional activators and repressors, which constitute a hierarchical regulatory network controlling SCW formation in various locations [10]. For example, SND1 and NST1 control SCW deposition in fiber cells [11–13] while VND6 and VND7 are responsible for vessel cells SCW formation in A.thaliana [14, 15].

Increasingly, post translational regulation of SCW formation is also being studied. For example, N-glycosylation regulates the enzyme activity of PtrMAN6 in suppression of SCW formation in Populus [16]. The phosphorylation of cellulose synthase AtCesA7 affected SCW cellulose biosynthesis in A.thaliana [17].

Sumoylation, conjugation of SUMO to substrate proteins, is a reversible and dynamic protein modification that regulates a range of biological processes [18]. SUMO conjugation forms a covalent bond between the C-terminal glycine carboxyl group of SUMO and the ε-amino group of a lysine residue, mostly occurring at the consensus motif PXXD/E (P, hydrophobic amino acid; K, lysine for conjugation; X, any amino acid; D/E, acidic amino acids) of target proteins [19]. Completion of sumoylation requires an enzymatic cascade of SUMO E1 activating enzyme, SUMO E2 conjugating enzyme and SUMO E3 ligase [18]. This process can be reversed through desumoylating proteases [20]. Generally sumoylation results in either stabilization of the target protein by protecting it against ubiquitylation [21, 22] or destabilization by promoting the sumoylated protein for proteasomal degradation [23]. Sumoylation can also alter protein cellular localization and modulate protein function or enzymatic activity [24]. In plants sumoylation plays a variety of roles in stress responses, growth, flowering, photomorphogenesis, nutrient homeostasis, and other biological processes [25, 26].

AtSIZ1 is an SP-RING (SIZ/PIAS-type) E3 ligase identified from Arabidopsis thaliana. It contains five structural domains including SAP (Scaffold attachment factor A/B/acinus/PIAS) domain, PINIT domain, SP-RING (SIZ/PIAS-RING) domain, SXS domain and PHD (Plant Homeodomain) [27]. These domains determine AtSIZ1 subcellular localization, enzyme activity, and action in responding to biotic and abiotic stresses [27]. AtSIZ1 plays various roles in growth [28], flowering[29, 30], light response[31, 32], immunity[33, 34] and metabolism of nutrient elements, such as phosphate[35], nitrogen [36] and copper[37]. AtSIZ1 is also implicated in sugar signaling [38]. Recent studies have shown that AtSIZ1 mediated sumoylation is involved in plant response to various stresses[26], including cold[39], heat stress[40], drought.
stress[41] as well as in signaling processes such as abscisic acid[42, 43], salicylic acid[44], auxin[45] and gibberellin signaling pathways[46].

In this study, we observed SCW defects in the *A. thaliana* *siz1* mutants. Genetic and biochemical analyses indicate that the SCW defects were caused by failure of the LBD30 sumoylation which was mediated by SIZ1. The study reveals a mechanism that sumoylation functions as a regulatory expedient in SCW formation in *A. thaliana*.

**Results**

*siz1* mutants display SCW defects in inflorescence fiber cells

We screened an *A. thaliana* T-DNA insertion pool (Col-0 background) for the phenotypic abnormality of SCW formation in the inflorescence stem through microscopy observation. Two T-DNA insertion alleles, *siz1*-2 and *siz1*-3, which impair AtSIZ1 SUMO E3 ligase function [35] (Fig 1A), displayed morphological

The *siz1* mutant plants were smaller with shorter inflorescence stems compared to WT (Fig 1A). To determine whether SIZ1 directly affects SCW formation, we employed an RNAi strategy to inhibit AtSIZ1 expression specifically in the cells forming secondary walls. The promoter of the fiber cell-specific SND1 was used to drive SIZ1-RNAi in *P. S. SIZ1-i* transgenic

![Image](https://doi.org/10.1371/journal.pgen.1007928.g001)

Fig 1. Inhibition of secondary cell wall thickening in *siz1* mutants. (A) Wild type (WT, Col-0), *siz1*-2 and *siz1*-3 mutant plants. (B), (F) and (K) Cross section of the interfascicular region of WT (B), *siz1*-2 mutant (F) and *siz1*-3 mutant (K) stems. (C), (G) and (L) Cross sections of the vascular bundle region of WT (C), *siz1*-2 mutant (G) and *siz1*-3 mutant (L) stems. (D), (H) and (M) Transmission electron micrographs of interfascicular fiber cells of WT (D), *siz1*-2 mutant (H) and *siz1*-3 mutant (M) plants. (E), (I) and (N) Transmission electron micrographs of xylem cells of WT (E), *siz1*-2 mutant (I) and *siz1*-3 mutant (N) plants. (J) Wall thickness of vessels and fibers in the inflorescence stems of WT and *siz1* mutants. Data represent average values±SD (n = 30 cells from 3 independent plants). **P < 0.01(Student’s t-test). co: cortex, if: interfascicular fiber, ph: phloem, ve: vessel, xf: xylary fiber. Scale bars = 10mm in (A), 20μm in (B), (C), (F), (G), (K) and (L), 2μm in (D), (E), (H), (I), (M) and (N), defects in SCWs. Specifically, interfascicular fiber cells and xylary fiber cells from the inflorescence stems of *siz1*-2 (Fig 1F and 1G) and *siz1*-3 (Fig 1K and 1L) showed a significant reduction in wall thickness compared to the wild-type (WT) (Fig 1B and 1C). Transmission electron microscopy analysis confirmed that *siz1* mutants form much thinner cell walls in the fiber cells (Fig 1D, 1E, 1H, 1I, 1M and 1N), while the wall thickness of vessel cells showed little difference between the *siz1* mutants and WT (Fig 1J).
plants (S1A Fig). In transgenic lines, expression of AtSIZ1 was suppressed by about 50% (S1E Fig). The wall thickness of the fiber cells in inflorescence stem was reduced compared to WT (S1B, S1C and S1F Fig). These suggest that SIZ1 plays a role in SCW formation in inflorescence fiber cells.

To investigate how SCW formation is changed in the siz1 mutants, we analyzed the chemical composition of their cell walls and examined expression of the SCW-related genes. In inflorescence stem crystalline cellulose and lignin were reduced by more than 20% in siz1 plants compared to WT (Fig 2A and 2B).

The xylose from non-cellulosic polysaccharides was also significantly decreased in siz1 plants (Fig 2C). Expression of the genes responsible for SCW formation was significantly suppressed in siz1 plants. These genes included transcription factor genes (SND1, NST1, MYB46 and MYB103)[1] (Fig 2D), SCW cellulose synthase genes (CesA4, CesA7 and CesA8)[1] (Fig 2E), lignin biosynthesis genes (PAL1, CCoAOMT and 4CL1)[1] (Fig 2F) and xylan biosynthesis genes (IRX8, IRX9 and IRX14)[1] (Fig 2G). These results indicated that AtSIZ1 is involved in regulating the transcriptional network that controls SCW formation.

Fig 2. Reduction of SCW components and suppressed expression of genes related to SCW synthesis in siz1 mutants. (A) Crystalline cellulose contents in wild type (WT) and siz1 stems. (B) Lignin contents of WT and siz1 stems. (C) Monosaccharide composition of cell wall residues from WT and siz1 stems. (D)-(G) Expression level of SCW synthesis associated genes in the basal first and second internodes of inflorescence stem of WT and siz1 plants. Relative levels of the indicated transcripts are normalized to ACT2. The WT transcript level of genes of interest was set to 1. Data represent average values±SD (n = 4 replicates in A-C, 3 in D-G). * P < 0.05, ** P < 0.01 (Student’s t-test). AIR: Alcohol-insoluble residues.

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**SIZ1 interacts with LBD30**

AtSIZ1 promoter was active in cortex cells and interfascicular fibers of the inflorescence stem undergoing SCW formation (S2 Fig). SIZ1 is a nuclear-localized protein [27] and functions in facilitating SUMO conjugation to target proteins [47]. Using AtSIZ1 as the bait against a cDNA library made from *A. thaliana* inflorescence stem undergoing SCW formation, we conducted yeast two-hybrid (Y2H) screening to identify its target proteins for sumoylation. Among 191 identified candidates, four were found to be different parts from the ASYMMETRIC LEAVES2/LATERAL ORGAN BOUNDARIES DOMAIN (AS2/LBD) protein, LBD30, encoded by At4g00220 locus [48, 49]. We examined LBD30 expression in public databases and found that it is highly expressed in the inflorescence stem (S3 Fig). LBD/AS2 family proteins have a characteristic LOB domain at N terminus that possess DNA-binding ability [49, 50]. We re-examined the interaction between AtSIZ1 and LBD30 in an Y2H system and found that AtSIZ1 interacted with LBD30 through its C-terminus (LBD30-C, amino acids 121–228) (Fig 3A). This interaction was verified through

**AtSIZ1 mediates SUMO1 modification of LBD30**

SIZ1 interacted with LBD30, but expression of LBD30 was not altered in siz1 mutants (S4 Fig). LBD30 is predicted to contain a sumoylation motif (ΨKXE) with K226 as a potentialSUMO conjugation residue (S1 Table) and showed a high possibility to be sumoylated among a list of SCW formation-related proteins [1] (S1 Table). Then we examined whether LBD30 could be SUMO conjugated at the ΨKXE motif. Using tandem mass spectrometry analysis, a mutant AtSUMO1_{T91R} protein, which allows production of a signature peptide containing a diglycine remnant at the sumoylation site [51], was identified at K226 in LBD30 (Fig 4A). To verify this sumoylation, recombinant LBD30 was generated and the sumoylated LBD30 was detected in sumoylation assay (Fig 4B). When LBD30 was mutated to generate a K226R variant (LBD30_{K226R}), the substitution of K226 to R resulted in failure of SUMO1 conjugation to LBD30 (Fig 4C) without affecting its nuclear localization (S5 Fig). Furthermore, we examined if the mutant LBD30 can be sumoylated by AtSIZ1 in planta. By combinational expression of LBD30 or LBD30_{K226R} - AtSIZ1 and AtSUMO1 in tobacco leaves, immunoblotting indicated only LBD30 is SUMO-conjugated (S6 Fig). Next, AtSUMO1 and LBD30 or LBD30_{K226R} were co-expressed in siz1-2 and WT *A. thaliana*. AtSUMO1 conjugation to LBD30 was only detected in the transgenic plants with WT background expressing LBD30 and AtSUMO1 (Fig 4D). These demonstrated that SIZ1 mediates LBD30 sumoylation at the K226 residue.

**AtSIZ1-mediated sumoylation of LBD30 affects SCW formation and development**

We investigated the effect of LBD30 sumoylation in the transgenics overexpressing LBD30 and LBD30_{K226R}. Overexpression of LBD30 caused drastic phenotypic changes, severe dwarfism, short petioles and downward curled leaves. Ectopic lignin deposition was detected in cotyledons in 24 out of 28 T1 transgenic plants (Fig 5A–5C). In contrast, overexpression of LBD30_{K226R} showed little phenotypic changes (Fig 5A and 5D). Similarly, expression of LBD30 in siz1-2 caused no phenotypic change in 28 transgenic plants out of 36 T1 plants and minor changes in remaining 8 plants compared to the siz1-2 plants (Fig 5A, 5E and 5F). LBD30 sumoylation played a role in development and secondary cell wall biosynthesis. Then, we investigated whether the SCW defects in siz1 plants is caused by failure of LBD30 SUMO modification. We examined the transcripts of SND1 and NST1 in the transgensics over-expressing LBD30 (S7A Fig). The transgenic plants were unable to develop normal
inflorescence stem (Fig 5A) but expression of SND1 and NST1 was drastically up-regulated in the 2 weeks-old seedlings (Fig 5G). This upregulation of SND1 and NST1 expression was insignificant in the transgenics carrying LBD30K226R or in the transgenics overexpressing LBD30 in siz1 mutant background (Fig 5G). When LBD30 was overexpressed in nst1/snd1 double

Fig 3. AtSIZ1 interacts with LBD30. (A) AtSIZ1 directly interacted with LBD30 through its C-terminus. Full-length AtSIZ1 and various lengths of LBD30 cDNA were fused to the Gal4 activation domain (AD) and the Gal4 DNA-binding domain (BD) and transformed into yeast cells in different combinations of empty vectors and recombinant plasmids. The upper panel indicates LBD30 and its derivatives showing the conserved LOB domain (yellow). (B) In vitro pull-down of LBD30 with AtSIZ1. His<sub>6</sub>-LBD30 fusion protein pulled down with GST or GST-AtSIZ1 fusion protein was detected by immunoblotting using an anti-His antibody. (C) Bimolecular fluorescence complementation (BiFC) analysis of the interaction between AtSIZ1 and LBD30. Full-length AtSIZ1 and LBD30 cDNAs were fused to the N-terminal part of yellow fluorescent protein (YFP) and the C-terminal part of YFP, respectively. Infiltration of target protein with an empty vector was used as the negative control. DAPI (4',6-diamidino-2-phenylindole) signal indicates nuclei. Scale bars = 20 μm. (D) Co-immunoprecipitation of AtSIZ1 and LBD30. Myc-tagged AtSIZ1 and HA-tagged LBD30 were expressed or coexpressed in tobacco leaves. Proteins were detected by immunoblotting with an anti-Myc antibody and an anti-HA antibody in crude lysates and in protein extracts after immunoprecipitation with an anti-Myc antibody and an anti-HA antibody, respectively. Co-precipitation (Fig 3B) and bimolecular fluorescence complementation (BiFC) assays (Fig 3C). In addition, by co-expression of LBD30 and AtSIZ1 in tobacco (N. benthamiana) leaves, the two proteins interacted with each other (Fig 3D). Together, these results demonstrated that AtSIZ1 interacts with LBD30 at C-terminal.

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mutant[11–13], ectopic lignin deposition in cotyledons was not detected in the transgenics (31/31 T1 plants) and the ns1/nsd1 double mutant (S7B and S7C Fig). On the other hand, we evaluated the effect of LBD30 sumoylation on SND1 and NST1 expression using a dual luciferase assay in A. thaliana protoplasts. The effector was constructed by using a 35S promoter to drive expression of LBD30 and LBD30(K226R). A firefly luciferase driven by SND1 or NST1 promoter was used as a reporter (Fig 5H and 5I). LBD30 showed a significantly higher activity in activation of SND1 or NST1 promoter than LBD30(K226R) (Fig 5H and 5I), suggesting that LBD30 SUMO conjugation affected SND1 and NST1 expression. Thus, the SUMO modification of LBD30 played a role in regulating SCW formation through the SND1/NST1-directed transcriptional network.

**Discussion**

In higher plants all cells form primary cell wall. In some type cells, additional SCWs are formed inside the primary wall, providing plants with mechanical support for erect growth and channels for long-distance transportation of water, nutrients, and photosynthetic products. Formation of the SCWs in various type cells need to be precisely regulated in a spatio-temporal manner during growth and development [52]. To ensure a precise deposition of SCWs in some type cells, multiple levels of regulation have to be developed in plants. Disturbance of the regulatory networks causes abnormal growth and development [1]. At the transcriptional level, complex regulatory networks are involved in SCW formation [8, 53]. SCW formation in
different cell types is initiated through cell type-specific transcription regulators [11–15]. Many signaling molecules regulating SCW formation have yet-to-be characterized [54].

At the protein level, post-translation modifications, such as protein phosphorylation and N-glycosylation [16, 17], are also being studied for their roles in regulating SCW formation. While a large number of proteins are modified with SUMO-conjugation and such modification affects a variety of biological processes [18], this study presents a detailed picture of how sumoylation can lead to the upregulation of SCW formation. Specifically, we found LBD30 sumoylation is required for activation of the SND1/NST1-mediated transcriptional networks in SCW formation.

AtSIZ1-mediated sumoylation is involved in a variety of growth and development processes such as flowering, response to light, immunity and nutrient element metabolisms in A. thaliana [31–34, 36, 55]. In this study, we observed that sizl mutants displayed defective SCWs in

Fig 5. Overexpression of sumoylated LBD30 affects plant development and induce ectopic deposition of lignified SCWs. (A) 2-weeks old seedlings of the wild type (WT, Col-0), an LBD30 overexpressing transgenic plant, an LBD30(K226R) overexpressing transgenic plant, an LBD30 overexpressing transgenic plant in the siz1-2 mutant background and the siz1-2 mutant (Col-0). (B-F) Lignin autofluorescent signals of cotyledons of plants corresponding to (A). White arrowheads show ectopic secondary wall thickening in the cotyledon of the LBD30 overexpressing transgenic plant. White dotted box shows a higher magnification picture. mv, middle vein. (G) Expression level of SND1 and NST1 in whole seedlings of WT, mutants and transgenic Arabidopsis thaliana plants. Relative levels are normalized to ACT2. The WT transcript level of genes of interest were set to 1. Data represent average values ±SD (n = 3 replicates). Lowercase letters indicate significant differences at p < 0.01 by ANOVA. (H-I) Transcriptional activation analysis of SND1 (H) and NST1 (I) by LBD30 or LBD30 (K226R) in A. thaliana protoplasts. Data represent average values ±SD (n = 3 replicates). **P < 0.01(Student’s t-test). Bars = 10 mm in (A), 200 μm in (B to F).
interfascicular fiber cells. Analysis indicated that SIZ1 interacted with LBD30 and catalyzed its sumoylation at K226 position in the sumoylation motif.

LBD30 is a transcription factor belonging to the Lateral Organ Boundaries Domain (LBD) family\[^{56, 57}\]. LBD30 and its homolog LBD18 in \(A. \text{thaliana}\) were preferentially expressed in vascular tissues and LBD18 played a role in regulating tracheary element differentiation\[^{57}\].

Defective SIZ1 or mutated LBD30 at K226 position led to loss of LBD30 function during the formation of SCW in interfascicular fiber cells. The evidence indicated that LBD30, when it was sumoylated by SIZ1, played a role in activating the \(\text{SND1}/\text{NST1}\)-mediated transcriptional networks (Fig 6) which regulate SCW formation in the fiber cells of inflorescence stem.

Generally, stress conditions cause activation of SCW formation \[^{58, 59}\]. Several transcription factors sumoylated by \(\text{AtSIZ1}\) are related to stress responses, including ICE1 in freezing stress\[^{39}\], HsfA2 in heat stress\[^{60}\], PHR1 in phosphate (Pi) deficiency\[^{35}\], MYB30 and ABI5 in the abscisic acid-dependent drought stress\[^{42, 43}\]. It is worthy of further study whether LBD30 sumoylation acts as a linking device between stress responses and SCW formation.

Generally LBD family proteins regulate plant development through interaction with other transcription factors \[^{50}\]. A number of transcription factors have been identified to bind to \(\text{SND1}\) and \(\text{NST1}\) promoters to activate their expression\[^{1, 61}\]. In this study, we found that transcription factor LBD30 was sumoylated by SIZ1 and such protein modification affected activation of the \(\text{SND1}/\text{NST1}\)-mediated transcriptional networks for SCW formation in fiber cells. Though it remains to be investigated how LBD30 sumoylation performs its function in

![Fig 6. A model of the role of sumoylation in the regulation of SCW formation.](https://doi.org/10.1371/journal.pgen.1007928.g006)
activation of the transcriptional networks, one possibility is that LBD30 sumoylation may affect the transcription factor interactions that are necessary for activation of SND1/NST1 expression. This possibility might justify the observation that LBD30 sumoylation showed different strength of effect on SND1 and NST1 expression between transgenics and protoplast system. Interaction of LBD30 with other factors in planta affected the SND1 and NST1 promoter activity.

The finding that LBD30 sumoylation acts as another layer of regulation to aid in the precise control of SCW formation provides additional insight into a key process that is essential for upright growth and the long-distance transport of water and solutes in plants and has implications in cell wall modification via regulation of LBD30 sumoylation in crop improvement.

Materials and methods

Plant materials and culture conditions

The A. thaliana Col-0 ecotype (WT) and the T-DNA insertion mutant lines, siz1-2 (SALK_065397) [35], siz1-3 (SALK_034008) [35] and snd1/nst1 double mutant (CS67921) [11–13], were grown in a phytotron at 22°C with a photoperiod of 16 h of light and 8 h of darkness. Transformation of A. thaliana was performed using the Agrobacterium tumefaciens-mediated floral dip method [62]. Transgenic plants were selected on MS medium containing 50 μg/ml hygromycin. Positive T2 transgenic plants were used for further analysis, with the exception of LBD30 overexpressing plants in the Col-0 background, where T1 plants were used because the T1 transgenic displayed severe growth defects and hardly produced seeds.

Gene cloning and plasmid construction

cDNAs for AtSIZ1 (At5g60410), LBD30 (At4g00220), AtSUMO1 (At4g26840) and the promoter regions of AtSIZ1(3535bp), SND1(2858bp) and NST1(2913bp) were PCR-amplified from a cDNA pool of A. thaliana as well as from genomic DNA with specific primers listed in S2 Table. For the Y2H assay, the coding region of LBD30 and AtSIZ1 were inserted respectively into the pGBK7T and pGADT7 plasmids (Clontech) and introduced into AH109 yeast cells (Clontech) following the manual. For BiFC analysis, LBD30-YC and YN-AtSIZ1 were constructed as previously described [63] and mobilized into A. tumefaciens strain GV3101 and transformed into Nicotiana benthamiana tobacco leaf cells [63]. For purification of recombinant proteins, the LBD30, a mutated LBD30(K226R) and AtSIZ1 coding regions were cloned into the pET-28b (Novagen) and pGEX-4T-1 (GE Healthcare) plasmids to produce the His6-LBD30, His6-LBD30(K226R) and GST-AtSIZ1 fusion proteins, respectively. The site directed mutagenesis of LBD30(K226R) was generated according to Hieff Mut™ Site-Directed Mutagenesis Kit (Yeasen Biotech). For protein expression in plants, the full coding regions of AtSIZ1, LBD30, LBD30(K226R) and AtSUMO1 were subcloned into the binary pCambia 1300 vector to produce chimeric MYC-AtSIZ1, LBD30-HA, LBD30(K226R)-HA and FLAG-AtSUMO1 fusions under the control of the constitutive CaMV 35S promoter. These constructs were coexpressed in Nicotiana benthamiana tobacco leaves for transient expression, and transformed into A. thaliana protoplasts. For transcriptional activation analysis, the coding regions of LBD30 and LBD30(K226R) and the promoter regions of the SND1 and NST1 genes were cloned into the effector (35S-transcription factor) and reporter (firefly luciferase) vectors (pGreenII vector, Promega) and then coexpressed in A. thaliana protoplasts [64]. For analysis of AtSIZ1 expression, an AtSIZ1
promoter fragment was cloned and fused to a β-glucuronidase (GUS) reporter gene in the pCambia1301 vector for A. thaliana transformation. To investigate the function of AtSIZ1 in inflorescence stems, two different genomic DNA fragments specific to AtSIZ1 were amplified separately to form hairpin structures under the control of the SND1 gene promoter. These constructs were designed to cause RNAi suppression (SND1promoter-AtSIZ1RNAi1 and SND1promoter-AtSIZ1RNAi2) specifically in A. thaliana inflorescence stems.

Microscopy analyses

The basal internodes of inflorescence stems of 8-week-old plants with the same flowering date were collected as described before. Briefly, the internodes were fixed in FAA overnight and embedded in paraffin (Sigma-Aldrich 18635) after dehydration through a graded ethanol series. Ten-micrometer-thick sections were cut and stained with toluidine blue for light microscopy. Free-hand cross sections of A. thaliana inflorescence stems were stained with 0.5% phloroglucinol (Sigma-Aldrich P3502) (w/v) in 12% HCl for 3 min, and immediately observed under a bright-field microscope (OLYMPUS BX53). For transmission electron microscopy, ultrathin sections were cut and observed as described [65]. To visualize lignin auto-fluorescence under UV light and the sub-cellular localization of GFP-fusion proteins, A. thaliana cotyledons were grown on MS plates and A. thaliana leaf protoplasts were observed using a fluorescent microscope (OLYMPUS BX53). For the BiFC analysis, tobacco leaf cells were stained with DAPI [66] and visualized using a confocal microscope (LSM 510 META; Zeiss).

Analysis of cell wall components

Fluorescence stems from at least three independent 8-week-old A. thaliana WT or mutant plants were collected and ground in liquid nitrogen to a fine powder to prepare alcohol insoluble residue (AIR) as previously described [67]. After the de-starched procedure [67], the crystalline cellulose content and monosaccharide composition were analyzed according to a previously published protocol [68]. The lignin content was determined following the methods in [69].

Gene expression analysis

Total RNA isolated from the lower center part of the inflorescence stem of 4-week-old A. thaliana plants and whole seedlings of 2-week-old WT, mutants and transgenic plants were extracted using the E.Z.N.A. Total RNA Kit (Omega) according to the manufacturer’s instructions. cDNA was synthesized by treatment with reverse transcriptase and oligo (dT) primer (TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix, Transgene Biotech) and quantitative PCR assays were conducted with a MyiQ real-time PCR detection system (Bio-Rad) using SYBR Green (TransStart Top Green qPCR MIX) following the user manual. The A. thaliana ACT2 gene (AT3G18780) was used as an internal control to normalize the data. The mathematical analysis for qPCR quantification was delta-delta Ct method [70]. The quantitative PCR (qPCR) experiment was performed in biological triplicates.

GUS staining assay

Free-hand cross-sections of the lower internodes of the inflorescence stems from 4 week old AtSIZ promoter-GUS transgenic A. thaliana were examined for GUS activity as previously described [71].
Protein-protein interaction assay

To identify AtSIZ1 interacting proteins, a Y2H library was generated using cDNA derived from 4-week-old A. thaliana inflorescence stems and used to screen for target proteins, using the Make Your Own Mate & Plate Library System (Clontech), according to the manufacturer’s directions. For the BiFC analysis, the constructs were transformed into Agrobacterium strain GV3101, and the resulting strains were used to transform N. benthamiana leaf cells, either individually or in combination. The leaves were examined after 48 h of incubation. To investigate the physical interaction between AtSIZ1 and LBD30 in vitro, recombinant His$_6$-LBD30 and GST-AtSIZ1 proteins were expressed in Escherichia coli and purified with Ni-NTA Agarose (Qiagen) and Pierce GST Agarose (Thermo Scientific), according to the manufacturer’s instructions. GST and GST-tagged AtSIZ1 proteins from the cell lysates were first immobilized on the GST Agarose (Thermo Scientific). After washing away unbound proteins with 1×PBS, the immobilized GST and GST-AtSIZ1 proteins were incubated with the cell lysate of Escherichia coli expressing His$_6$-LBD30. After several washing steps with 1×PBS, the complexes were eluted with 2×SDS loading buffer and boiled at 100˚C for 5 min. The eluted proteins were separated by SDS-PAGE, transferred to a PVDF membrane and the protein was immunoblotted with an anti-His antibody (1:5000 dilution, Abmart).

In vivo AtSIZ1 and LBD30 interactions were analyzed by co-immunoprecipitation (co-IP). Myc-tagged AtSIZ1 and HA-tagged LBD30 were expressed transiently in tobacco leaf cells. Proteins were extracted by grinding the leaves in liquid nitrogen and thawed in extraction buffer [50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1mM EDTA, 0.2% Triton X-100 (Sigma Aldrich), 10% glycerol, 1mM PMSF, 2% PVPP (polyvinylpolypyrrolidone) and 1x concentration of protease inhibitor cocktail (Roche)] for 30 min. The homogenate was then filtered through a 0.22 μm filter membrane (Millipore) and 1mL of the filtrate was incubated with 50 μL agarose conjugated anti-Myc mouse monoclonal antibody (Abmart) or 50 μL anti-HA rat monoclonal antibody Affinity Matrix (Roche) for 3 h at 4˚C. The beads were washed three times with wash buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.2% Triton X-100), and the bound proteins were eluted with 2×SDS-PAGE loading buffer and boiled at 100˚C for 5 min. The eluted proteins were immunoblotted as above and incubated with anti-HA mouse monoclonal antibody (1:3000 dilution, Abmart) or anti-Myc mouse monoclonal antibody (1:3000 dilution, Abmart).

Sumoylation assay

The in vitro sumoylation was performed using the SUMOlink™ SUMO-1 Kit (Active Motif). Briefly, recombinant His$_6$-LBD30 and His$_6$-LBD30(K226R) proteins were expressed in E. coli and purified. A total of 3 μg of target protein was added to 20 μl reaction buffer and incubated at 30˚C for 3 h. The reaction was stopped by adding 10 μl of 2×SDS-PAGE loading buffer. Sumoylated of His$_6$-LBD30 was detected by immunoblot analysis using an anti-His mouse monoclonal antibody (1:5000 dilution, Abmart) and a SUMO-1 rabbit antibody (1:2000 dilution, Active Motif).

The reaction mixture was also separated by SDS-PAGE. After staining with Coomassie Blue R-250, the sumoylated His$_6$-LBD30 protein band was cut into 1 mm wide pieces for digestion and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of the LBD30 sumoylation site. Protein digestion for LC-MS/MS analysis was performed by the Beijing Protein Institute. Briefly, the protein bands were destained with 50% v/v acetonitrile (ACN) [72] and 25 mM ammonium bicarbonate and dried in 100% ACN and the gel slices were incubated with a 10 ng μl$^{-1}$ trypsin solution in 25 mM ammonium bicarbonate at 37˚C for 12h. The extracts were then dried in a stream of N$_2$ and resuspended in 5% ACN in 0.1% v/v formic acid.
acid FA. LC-MS/MS analysis was performed using an Ultimate3000 liquid chromatography system (Dionex) connected to a Q Exactive mass spectrometer (Thermo Scientific) as described previously [72] with modifications. The extracts were separated by a C18 reverse-phase column with a 1 hour gradient of mobile phase (phase A, 5% ACN in 0.1% FA; phase B, 95% CAN in 0.1% FA) at a flow rate of 300 nL/min. The separated sample was then injected into the mass spectrometer and a method of full scans were acquired with AGC target value of 1E6, resolution of 70,000 FWHM at 200 m/z, and maximum ion injection time (IT) of 100 ms. The mass spectra were extracted by BioWork version 3.3.1 sp1 (Thermo Fisher). All MS/MS samples were analyzed using Mascot software (Matrix Science).

For the in vivo sumoylation assay, the Myc tagged AtSIZ1, the FLAG-tagged AtSUMO1 and the HA-tagged LBD30 or LBD30 (K226R) were expressed in tobacco leaves. The FLAG-AtSUMO1 transgenic A. thaliana plants (Col-0 background) were crossed with LBD30-HA or LBD30 (K226R)-HA transgenic A. thaliana plants (siz1-2 background). F2 progeny of transgenic plants with WT and siz1-2 background overexpressing FLAG-AtSUMO1 and LBD30-HA or LBD30 (K226R)-HA were obtained. Total proteins were extracted and immunoprecipitated with an anti-FLAG mouse monoclonal M2 affinity gel (Sigma-Aldrich). The sumoylated LBD30 was detected by immunoblotting with an anti-HA Rat monoclonal high-affinity antibody (1:2000 dilution, Roche) after IP.

**Dual luciferase assay**

Protoplasts used in the transient effector-reporter analysis were isolated from 2-week-old A. thaliana seedlings as previously described [64]. The coding sequences of LBD30 and LBD30 (K226R) were cloned into the effector plasmid. The promoters of SN1 and NST1 were cloned into the firefly luciferase reporter vector (pGreenII, Promega). The Renilla luciferase gene driven by the CaMV 35S promoter served as a control to normalize for transformation efficiency. Luciferase activities were measured with a dual-luciferase reporter assay system (Promega).

**Supporting information**

S1 Fig. Reduction in SCW thickness by RNAi repression of SIZ1 in cell types that undergo SCW thickening. (A) Wild type (WT, Col-0) plant (left), transgenic Arabidopsis thaliana plants with SND1 promoter controlled RNAi inhibition of SIZ1 (middle) and siz1 mutants (right). (B-D) Cross sections of WT and SIZ1 RNAi transgenic plant stems stained with phloroglucinol-HCl. if: interfascicular fiber, ve: vessel, xf: xylary fiber. Scale bars = 10 mm in (A), 20 μm in (B-D). (E) Quantitative PCR analysis showing a reduction in the mRNA levels of SIZ1 in the stems of two independent SND1 promoter-SIZ1 RNAi lines. The expression level of SIZ1 in Col-0 was set to 1. Data represent average values±SD (n = 3). **P < 0.01(Student’s t-test). (F) Wall thickness of vessels and fibers in the inflorescence stems of WT and transgenic plants. Data represent average values±SD (n = 30 cells from 3 independent plants). **P < 0.01 (Student’s t-test).

(TIF)

S2 Fig. SIZ1 promoter activity in inflorescence stems. (A) SIZ1 promoter-GUS (β-glucuronidase) expression in a cross-section of an internode near the cessation of elongation in an inflorescence stem of a 4 weeks old transgenic Arabidopsis thaliana plant. (B) High magnification of a stem section of a SIZ1 promoter-GUS transgenic plant. co: cortex, if: interfascicular fiber, vb: vascular bundle. Scale bars = 100 μm in (A), 20 μm in (B).

(TIF)
S3 Fig. LBD30 expression profile during *Arabidopsis thaliana* development. The region indicated by the black line is the second internode of the inflorescence stem. Data were obtained from the AtGenExpress Visualization Tool (http://jsp.weigelworld.org/expviz/expviz.jsp).
(TIF)

S4 Fig. The LBD30 expression level in the basal first and second internodes of inflorescence stems of wild-type (WT) and *siz1* plants. Relative levels were normalized to ACT2. The transcript level of LBD30 in WT was set to 1.0. Data represent average values±SD (n = 3 replicates).
(TIF)

S5 Fig. Fluorescent signals of green fluorescent protein (GFP) fused LBD30 proteins in *Arabidopsis thaliana* leaf protoplasts. (A) A protoplast expressing GFP alone. (B, C) Protoplasts expressing GFP tagged LBD30. (D, E) Protoplasts expressing GFP tagged LBD30 (K226R).
(TIF)

S6 Fig. Examination of LBD30 sumoylation in tobacco. Myc tagged AtSIZ1, FLAG-tagged AtSUMO1, and HA-tagged LBD30 or LBD30 (K226R) were expressed in tobacco leaves as indicated. Expression of the proteins was detected by anti-Myc, anti-HA and anti-FLAG antibodies, respectively. After immunoprecipitation with an anti-FLAG antibody, sumoylated LBD30 was detected by immunoblotting with an anti-HA antibody. Black arrows indicate Myc-AtSIZ1.
(TIF)

S7 Fig. Immunoblot detection of LBD30-cHA expression. (A) The protein expression level of LBD30-cHA. ACTIN was used as an internal control, detected by an anti-ACTIN antibody (1:3000 dilution, Abmart). (B,C) Lignin autofluorescent signals of cotyledons of LBD30 over-expressing transgenic plant in the *nst1/snd1* double mutant background and the *nst1/snd1* double mutant (Col-0). mv, middle vein. Bars = 200 μm in (B to F).
(TIF)

S1 Table. Predicted sumoylation sites in SCW related proteins by GPS-SUMO.
(XLS)

S2 Table. Primers used in this study.
(DOCX)

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References

1. Zhong R, Ye ZH. Secondary cell walls: biosynthesis, patterned deposition and transcriptional regulation. Plant & cell physiology. 2015; 56(2):195–214. Epub 2014/10/09. https://doi.org/10.1093/pcp/pcu140 PMID: 25294860.

2. Kamiya T, Borghi M, Wang P, Danku JM, Kalmbach L, Hosmani PS, et al. The MYB36 transcription factor orchestrates Casparian strip formation. Proceedings of the National Academy of Sciences of the United States of America. 2015; 112(33):10533–8. https://doi.org/10.1073/pnas.1507691112 PMID: 26124109; PubMed Central PMCID: PMCPMC4547244.

3. Miedes E, Vanholme R, Boerjan W, Molina A. The role of the secondary cell wall in plant resistance to pathogens. Frontiers in plant science. 2014; 5:358. Epub 2014/08/28. https://doi.org/10.3389/fpls.2014.00358 PMID: 25161657; PubMed Central PMCID: PMCPMC4122179.

4. Dong Y, Yang X, Liu J, Wang BH, Liu BL, Wang YZ. Pod shattering resistance associated with domestication is mediated by a NAC gene in soybean. Nature communications. 2014; 5:3352. https://doi.org/10.1038/ncomms4352 PMID: 24549030.

5. Yang C, Song J, Ferguson AC, Klsch D, Simpson K, Mo R, et al. Transcription Factor MYB26 is Key to Spatial Specificity in Anther Secondary Thickening Formation. Plant physiology. 2017; 175(1):333–50. https://doi.org/10.1104/pp.17.00719 PMID: 28724622; PubMed Central PMCID: PMCPMC5580765.

6. Lee Y, Yoon TH, Lee J, Jeon SY, Lee JH, Lee MK, et al. A Lignin Molecular Brace Controls Precision Processing of Cell Walls Critical for Surface Integrity in Arabidopsis. Cell. 2018; 173(6):1468–80 e9. https://doi.org/10.1016/j.cell.2018.03.060 PMID: 29731167.

7. Meents MJ, Watanabe Y, Samuels AL. The cell biology of secondary cell wall biosynthesis. Ann Bot. 2018; 121(6):1107–25. https://doi.org/10.1093/aob/mcy005 PMID: 29415210; PubMed Central PMCID: PMCPMC5946954.

8. Kumar M, Campbell L, Turner S. Secondary cell walls: biosynthesis and manipulation. Journal of experimental botany. 2016; 67(2):515–31. Epub 2015/12/15. https://doi.org/10.1093/jxb/erv533 PMID: 26663392.

9. Barros J, Serk H, Granlund I, Pesquet E. The cell biology of lignification in higher plants. Ann Bot. 2015; 115(7):1053–74. https://doi.org/10.1093/aob/mcv046 PMID: 25878140; PubMed Central PMCID: PMCPMC4648457.

10. Nakano Y, Yamauchi M, Endo H, Rejaib NA, Ohtani M. NAC-MYB-based transcriptional regulation of secondary cell wall biosynthesis in land plants. Frontiers in plant science. 2015; 6:288. Epub 2015/05/23. https://doi.org/10.3389/fpls.2015.00288 PMID: 25999964; PubMed Central PMCID: PMCPMC4419676.

11. Zhong R, Demura T, Ye Z-H. SND1, a NAC domain transcription factor, is a key regulator of secondary wall synthesis in fibers of Arabidopsis. The Plant cell. 2006; 18(11):3158–70. https://doi.org/10.1105/tpc.106.047399 PMID: 17114348

12. Zhong R, Richardson EA, Ye Z-H. Two NAC domain transcription factors, SND1 and NST1, function redundantly in regulation of secondary wall synthesis in fibers of Arabidopsis. Planta. 2007; 225 (6):1603–11. https://doi.org/10.1007/s00425-007-0498-y PMID: 17332520

13. Mitsuda N, Iwase A, Yamamoto H, Yoshida M, Seki M, Shinozaki K, et al. NAC transcription factors, NST1 and NST3, are key regulators of the formation of secondary walls in woody tissues of Arabidopsis. The Plant cell. 2007; 19(1):270–80. https://doi.org/10.1105/tpc.106.047043 PMID: 17237351; PubMed Central PMCID: PMCPMC1820955.

14. Yamaguchi M, Goue N, Igarashi H, Ohtani M, Nakano Y, Mortimer JC, et al. VASCULAR-RELATED NAC-DOMAIN6 and VASCULAR-RELATED NAC-DOMAIN7 effectively induce transdifferentiation into xylem vessel elements under control of an induction system. Plant physiology. 2010; 153(3):906–14. Epub 2010/05/22. https://doi.org/10.1104/pp.110.154013 PMID: 20488698; PubMed Central PMCID: PMCPMC2899331.

15. Ohashi-Ito K, Oda Y, Fukuda H. Arabidopsis VASCULAR-RELATED NAC-DOMAIN6 directly regulates the genes that govern programmed cell death and secondary wall formation during xylem...
differentiation. The Plant cell. 2010; 22(10):3461–73. https://doi.org/10.1105/tpc.110.075036 PMID:20952636; PubMed Central PMCID: PMCPMC2990123.

16. Zhao Y, Song D, Sun J, Li L. Populus endo-beta-mannanase PtrMAN6 plays a role in coordinating cell wall remodeling with suppression of secondary wall thickening through generation of oligosaccharide signals. The Plant journal: for cell and molecular biology. 2013; 74(3):473–85. Epub 2013/02/07. https://doi.org/10.1111/tpj.12137 PMID:23380457.

17. Taylor NG. Identification of cellulose synthase AtCesA7 (IRX3) in vivo phosphorylation sites—a potential role in regulating protein degradation. Plant molecular biology. 2007; 64(1–2):161–71. https://doi.org/10.1007/s11103-007-9142-2 PMID:17427041.

18. Miura K, Hasegawa PM. Sumoylation and other ubiquitin-like post-translational modifications in plants. Trends Cell Biol. 2010; 20(4):233–32. https://doi.org/10.1016/j.tcb.2010.01.007 PMID:20189809.

19. Cappadocia L, Lima CD. Ubiquitin-like Protein Conjugation: Structures, Chemistry, and Mechanism. Chem Rev. 2018; 118(3):889–918. https://doi.org/10.1021/acs.chemrev.6b00737 PMID:28234446; PubMed Central PMCID: PMCPMC5815371.

20. Castro PH, Bachmair A, Bejarano ER, Coupland G, Lois LM, Sadanandom A, et al. Revised nomenclature and functional overview of the ULP gene family of plant deSUMOylating proteases. Journal of experimental botany. 2018; 69(19):4505–9. https://doi.org/10.1093/jxb/ery301 PMID:30124991; PubMed Central PMCID: PMCPMC6117577.

21. Jentsch S, Psakhye I. Control of nuclear activities by substrate-selective and protein-group SUMOylation. Annu Rev Genet. 2013; 47:167–86. https://doi.org/10.1146/annurev-genet-112112-133453 PMID:24016193.

22. Wilkinson KA, Henley JM. Mechanisms, regulation and consequences of protein SUMOylation. The Biochemical journal. 2010; 428(2):133–45. https://doi.org/10.1042/BJ20100158 PMID:20462400; PubMed Central PMCID: PMCPMC3310159.

23. Perry JJ, Tainer JA, Boddy MN. A SIM-ultaneous role for SUMO and ubiquitin. Trends Biochem Sci. 2008; 33(5):201–8. https://doi.org/10.1016/j.tibs.2008.02.001 PMID:18403209.

24. Park HJ, Yun DJ. New Insights into the Role of the Small Ubiquitin-like Modifier (SUMO) in Plants. Int Rev Cel Mol Bio. 2013; 45(Pl A):143–54. https://doi.org/10.1016/j.pbi.2018.06.006 PMID:30014889.

25. Elrouby N. Regulation of Plant Cellular and Organisal Development by SUMO. Adv Exp Med Biol. 2017; 963:227–47. https://doi.org/10.1007/978-3-319-50044-7_14 PMID:28197916.

26. Augustine RC, Vierstra RD. SUMOylation: re-wiring the plant nucleus during stress and development. Curr Opin Plant Biol. 2018; 45(Pl A):143–54. https://doi.org/10.1016/j.pbi.2018.06.006 PMID:30014889.

27. Cheong MS, Park HC, Hong MJ, Lee J, Choi W, Jin JB, et al. Specific domain structures control abscisic acid-, salicylic acid-, and stress-mediated SIZ1 phenotypes. Plant physiology. 2009; 151(4):1930–42. Epub 2009/10/20. https://doi.org/10.1104/pp.109.143719 PMID:19837819; PubMed Central PMCID: PMCPMC2785975.

28. Elrouby N. Analysis of Small Ubiquitin-Like Modifier (SUMO) Targets Reflects the Essential Nature of Protein SUMOylation and Provides Insight to Elucidate the Role of SUMO in Plant Development. Plant physiology. 2013; 159(1):339–51. https://doi.org/10.1104/pp.112.208072 PMID:23856819; PubMed Central PMCID: PMCPMC3883301.

29. Lin XL, Niu D, Hu ZL, Kim DH, Jin YH, Cai B, et al. An Arabidopsis SUMO E3 Ligase, ATSI2, regulates flowering by controlling a salicylic acid-mediated floral promotion pathway and through affects on FLC chromatin structure. The Plant journal: for cell and molecular biology. 2008; 53(3):330–40. https://doi.org/10.1111/j.1365-313X.2007.03359.x PMID:18069938; PubMed Central PMCID: PMCPMC2258419.

30. Son GH, Park BS, Song JT, Seo HS. FLC-mediated flowering repression is positively regulated by sumoylation. Journal of experimental botany. 2014; 65(1):339–51. https://doi.org/10.1093/jxb/ert383 PMID:24218331; PubMed Central PMCID: PMCPMC3883301.

31. Lin XL, Niu D, Hu ZL, Kim DH, Jin YH, Cai B, et al. An Arabidopsis SUMO E3 Ligase, SI2, Negatively Regulates Photomorphogenesis by Promoting COP1 Activity. PLoS genetics. 2016; 12(4):e1006016. Epub 2016/04/30. https://doi.org/10.1371/journal.pgen.1006016 PMID:27125446; PubMed Central PMCID: PMCPMC4581335.

32. Sadanandom A, Adam E, Orosa B, Viczian A, Klose C, Zhang C, et al. SUMOylation of phytochrome-B negatively regulates light-induced signaling in Arabidopsis thaliana. Proceedings of the National Academy of Sciences of the United States of America. 2015; 112(35):11109–13. Epub 2015/08/19. https://doi.org/10.1073/pnas.1415260112 PMID:26283376; PubMed Central PMCID: PMCPMC4568247.

33. Gou M, Huang Q, Qian W, Zhang Z, Jia Z, Hua J. Sumoylation E3 Ligase SIZ1 Modulates Plant Immunity Partly through the Immune Receptor Gene SNC1 in Arabidopsis. Molecular plant-microbe
interactions: MPMI. 2017; 30(4):334–42. Epub 2017/04/15. https://doi.org/10.1094/MPMI-02-17-0041-R

34. Hammoudi V, Vokkens L, Beens B, Vlachakis G, Chatterjee S, Arroyo-Mateos M, et al. The Arabidopsis SUMO E3 ligase SI1 mediates the temperature dependent trade-off between plant immunity and growth. PLoS genetics. 2018; 14(1):e1007157. Epub 2018/01/23. https://doi.org/10.1371/journal.pgen.1007157 PMID: 29357355; PubMed Central PMCID: PMC5794169.

35. Miura K, Rus A, Sharkhauu A, Yokoi S, Karthikeyan AS, Raghothama KG, et al. The Arabidopsis SUMO E3 ligase SI1 controls phosphate deficiency responses. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102(21):7760–5. https://doi.org/10.1073/pnas.0500778102 PMID: 15894620; PubMed Central PMCID: PMC1104025.

36. Park BS, Song JT, Seo HS. Arabidopsis nitrate reductase activity is stimulated by the E3 SUMO ligase AtSI1. Nature communications. 2011; 2:400. Epub 2011/07/21. https://doi.org/10.1038/ncomms1408 PMID: 21772271; PubMed Central PMCID: PMC3160146.

37. Chen CC, Chen YY, Yang IC, Liang HM, Lai CC, Chieu JM, et al. Arabidopsis SUMO E3 ligase SI1 is involved in excess copper tolerance. Plant physiology. 2011; 156(4):2225–34. https://doi.org/10.1104/pp.111.178996 PMID: 21632972; PubMed Central PMCID: PMC3149952.

38. Castro PH, Verde N, Lourenco T, Magalhaes AP, Tavares RM, Bejarano ER, et al. SI1-Dependent Post-Translational Modification by SUMO Modulates Sugar Signaling and Metabolism in Arabidopsis thaliana. Plant & cell physiology. 2015; 56(12):2297–311. https://doi.org/10.1093/pcp/pcv149 PMID: 26468507.

39. Miura K, Jin JB, Lee J, Yoo CY, Stirm V, Miura T, et al. SI1-mediated sumoylation of ICE1 controls CBF3/DEBA1 expression and freezing tolerance in Arabidopsis. The Plant cell. 2007; 19(4):1403–14. https://doi.org/10.1105/tpc.106.048397 PMID: 17416732; PubMed Central PMCID: PMC1913760.

40. Rytz TC, Miller MJ, Mcloughlin F, Augustine RC, Marshall RS, Juan Y-t, et al. SUMOylation Reveals a Diverse Array of Nuclear Targets Modified by the SUMO Ligase SI1 during Heat Stress. The Plant cell. 2018; 30(3):1077–99. https://doi.org/10.1105/tpc.17.00995 PMID: 29568388

41. Crozet P, Margalha L, Butowt R, Fernandes N, Elias CA, Orosa B, et al. SUMOylation represses SnRK1 signaling in Arabidopsis. The Plant journal: for cell and molecular biology. 2016; 85(1):120–33. Epub 2015/12/15. https://doi.org/10.101111/tpc.13096 PMID: 26602259; PubMed Central PMCID: PMCPMC4817235.

42. Zheng Y, Schumaker KS, Guo Y. Sumoylation of transcription factor MYB30 by the small ubiquitin-like modifier E3 ligase SI1 mediates abscisic acid response in Arabidopsis thaliana. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109(31):12822–7. https://doi.org/10.1073/pnas.1202630109 PMID: 22814374; PubMed Central PMCID: PMC3411956.

43. Miura K, Lee J, Jin JB, Yoo CY, Miura T, Hasegawa PM. Sumoylation of ABI5 by the Arabidopsis SUMO E3 ligase SI1 negatively regulates abscisic acid signaling. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106(13):5418–23. Epub 2009/03/12. https://doi.org/10.1073/pnas.0811088106 PMID: 19276109; PubMed Central PMCID: PMC2664011.

44. Lee J, Nam J, Park HC, Na G, Miura K, Jin JB, et al. Salicylic acid-mediated innate immunity in Arabidopsis is regulated by SI1 SUMO E3 ligase. The Plant journal: for cell and molecular biology. 2007; 49(1):79–90. https://doi.org/10.1111/j.1365-313X.2006.02947.x PMID: 17163880.

45. Miura K, Lee J, Gong Q, Ma S, Jin JB, Yoo CY, et al. SI1-mediated sumoylation functions in secondary cell wall formation: MPMI. 2017; 30(4):334–42. Epub 2017/04/15. https://doi.org/10.1094/MPMI-02-17-0041-R PMID: 28409353.
51. Knuesel M, Cheung HT, Hamady M, Barthel KK, Liu X. A method of mapping protein sumoylation sites by mass spectrometry using a modified small ubiquitin-like modifier 1 (SUMO-1) and a computational program. Molecular & cellular proteomics: MCP. 2005; 4(10):1626–36. Epub 2005/07/16. https://doi.org/10.1074/mcp.T500011-MCP200 PMID: 16020427.

52. Taylor-Teeple M, Lin L, de Lucas M, Turco G, Toal TW, Gaudinier A, et al. An Arabidopsis gene regulatory network for secondary cell wall synthesis. Nature. 2015; 517(7536):571–5. https://doi.org/10.1038/nature14099 PMID: 25533953; PubMed Central PMCID: PMCPMC4333722.

53. Rao X, Dixon RA. Current Models for Transcriptional Regulation of Secondary Cell Wall Biosynthesis in Grasses. Frontiers in plant science. 2018; 9:399. https://doi.org/10.3389/fpls.2018.00399 PMID: 29670638; PubMed Central PMCID: PMCPMC5893761.

54. Huang C, Zhang R, Gui J, Zhong Y, Li L. The Receptor-Like Kinase AtVRLK1 Regulates Secondary Cell Wall Thickening. Plant physiology. 2018; 177(2):671–83. https://doi.org/10.1104/pp.17.01279 PMID: 29678858; PubMed Central PMCID: PMCPMC6001334.

55. Kong Y, Xu P, Jing X, Chen L, Li L, Li X. Decipher the ancestry of the plant-specific LBD gene family. BMC Genomics. 2017; 18(Suppl 1):951. https://doi.org/10.1186/s12864-016-3264-3 PMID: 28198677; PubMed Central PMCID: PMCPMC5310275.

56. Song D, Shen J, Li L. Characterization of cellulose synthase complexes in Populus xylem differentiation. The New phytologist. 2010; 187(3):777–90. Epub 2010/06/16. https://doi.org/10.1111/j.1469-8137.2010.03315.x PMID: 20546138.

57. Zhang D, Xu Z, Cao S, Chen K, Li S, Liu X, et al. An Uncanonical CCCH-Tandem Zinc-Finger Protein Represses Secondary Wall Synthesis and Controls Mechanical Strength in Rice. Molecular plant. 2018; 11(1):163–74. https://doi.org/10.1101/j.molp.2017.11.004 PMID: 29175437.

58. Foster CE, Martin TM, Pauly M. Comprehensive compositional analysis of plant cell walls (lignocellulosic biomass) part II: carbohydrates. Journal of visualized experiments: JoVE. 2010; (37). Epub 2010/03/17. https://doi.org/10.3791/1837 PMID: 20228730; PubMed Central PMCID: PMCPMC3145335.
68. Yu L, Chen H, Sun J, Li L. PtrKOR1 is required for secondary cell wall cellulose biosynthesis in Populus. Tree physiology. 2014; 34(11):1289–300. Epub 2014/04/15. https://doi.org/10.1093/treephys/tpu020 PMID: 24728296.

69. Foster CE, Martin TM, Pauly M. Comprehensive compositional analysis of plant cell walls (Lignocellulosic biomass) part I: lignin. Journal of visualized experiments: JoVE. 2010;(37). Epub 2010/03/13. https://doi.org/10.3791/1745 PMID: 20224547; PubMed Central PMCID: PMCPMC3144576.

70. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25(4):402–8. https://doi.org/10.1006/meth.2001.1262 PMID: 11846609.

71. Yu L, Sun J, Li L. PtrCel9A6, an endo-1,4-beta-glucanase, is required for cell wall formation during xylem differentiation in populus. Molecular plant. 2013; 6(6):1904–17. Epub 2013/06/19. https://doi.org/10.1093/mp/sst104 PMID: 23770836.

72. McVety S, Li L, Thiffault I, Gordon PH, Macnamara E, Wong N, et al. The value of multi-modal gene screening in HNPCC in Quebec: three mutations in mismatch repair genes that would have not been correctly identified by genomic DNA sequencing alone. Familial cancer. 2006; 5(1):21–8. Epub 2006/03/11. https://doi.org/10.1007/s10689-006-2572-6 PMID: 16528605.