TCS1, a Microtubule-Binding Protein, Interacts with KCBP/ZWICHEL to Regulate Trichome Cell Shape in Arabidopsis thaliana

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Abstract

How cell shape is controlled is a fundamental question in developmental biology, but the genetic and molecular mechanisms that determine cell shape are largely unknown. Arabidopsis trichomes have been used as a good model system to investigate cell shape at the single-cell level. Here we describe the trichome cell shape 1 (tcs1) mutants with the reduced trichome branch number in Arabidopsis. TCS1 encodes a coiled-coil domain-containing protein. Pharmacological analyses and observations of microtubule dynamics show that TCS1 influences the stability of microtubules. Biochemical analyses and live-cell imaging indicate that TCS1 binds to microtubules and promotes the assembly of microtubules. Further results reveal that TCS1 physically associates with KCBP/ZWICHEL, a microtubule motor involved in the regulation of trichome branch number. Genetic analyses indicate that kcbp/zwi is epistatic to tcs1 with respect to trichome branch number. Thus, our findings define a novel genetic and molecular mechanism by which TCS1 interacts with KCBP to regulate trichome cell shape by influencing the stability of microtubules.

Author Summary

The particular shape of plant cells is not only crucial for their biological functions but also affects the overall shape of organs. How cell shape is controlled is a fundamental question in developmental biology, and the study of plant cell shape regulation is an interesting part of plant biology. Arabidopsis trichomes have been used as a good model system to investigate cell shape at the single-cell level. In this study, we use Arabidopsis trichomes as a model to identify the trichome cell shape 1 (tcs1) mutants with the reduced trichome branch number. TCS1 encodes a microtubule binding protein, which is required for the...
stability of microtubules. We further find that TCS1 physically interacts with a microtubule motor involved in the regulation of trichome branch number. TCS1 acts genetically with this microtubule motor to control trichome branch number. Thus, our findings provide important insights into how the microtubule cytoskeleton determines cell shape.

Introduction

The particular shape of plant cells not only relates to their functions but also influences the overall shape of organs. Arabidopsis trichomes are well established as a system for studying cell shape at the single-cell level [1–3]. Arabidopsis trichomes differentiate from single epidermal cells, which stop proliferating and begin endoreduplication cycle or endocycle. After three or four endoreduplication cycles, trichome cells have two successive branching events and morphological changes, and then form mature trichomes [1]. Trichomes on Arabidopsis leaves are regularly spaced and exhibit a distinctive shape with a stalk and three or four branches. The cytoskeletons appear to be important for establishing and maintaining the branching pattern of trichomes [4–6]. It is generally accepted that mutations in genes involved in the regulation of actin cytoskeleton often cause distorted trichomes, while the disruption of genes regulating the microtubule cytoskeleton usually influences the number of trichome branches [4,5,7–12]. However, the genetic and molecular mechanisms by which the cytoskeletons determine trichome cell shape remain largely unknown in plants.

In trichomes, microtubules, a major component of the plant cytoskeletons, not only regulate anisotropic cell expansion but also control cell branching. Several factors that regulate trichome branch number by influencing the microtubule cytoskeleton have been described in Arabidopsis. Arabidopsis TUBULIN FOLDING COFACTOR (TCF) C and TCFA have been suggested to be required for microtubule biogenesis, and their loss-of-function mutants show the reduced trichome branch number and shape as well as multiple growth defects [13,14], suggesting that the formation of new microtubules is likely to be important for the formation of new branches. KINESIN-13A has the microtubule-depolymerizing activity in vitro and in vivo, and kinesin-13a mutants produce trichomes with more branches [15]. Kinesin-like calmodulin-binding protein (KCBP/ZWICHEL) is involved in the regulation of microtubule stability and trichome morphogenesis in plants [4,16]. Trichomes on kcbp/zwichel (zwi) leaves have a short stalk and only one or two branches compared with wild-type trichomes with three or four branches [16]. KCBP-interacting Ca$^{2+}$ binding protein (KIC) represses the activity of KCBP in response to Ca$^{2+}$ and regulates trichome branching [17]. Plants overexpressing KIC produce trichomes with reduced branch number [17]. KCBP also physically interacts with ANGUSTIFOLIA (AN) in yeast cells, which is involved in the regulation of the microtubule cytoskeleton [18]. Trichomes on an leaves have one or two branches, indicating AN is required for normal trichome branching [18,19]. KCBP has been suggested to function with suppressors of zwi (SUZ) in a complex to control the number of trichome branches, but the SUZ genes remain to be cloned in Arabidopsis [20]. KCBP has also been recently reported to interact with both microtubules and F-actin to affect trichome branch initiation and elongation, respectively [21]. These studies imply that KCBP acts as an important node linking cytoskeletons with trichome cell shape.

To further understand the genetic and molecular mechanisms of cell shape control, we characterize tcs1 mutants, which form trichomes with the reduced branch number. Mutations in TCS1 influence the stability of microtubules. TCS1 encodes a coiled-coil domain-containing protein, which binds to microtubules in vitro and in vivo and promotes the assembly of microtubules. Further results reveal that TCS1 interacts physically and genetically with KCBP/ZWI
to control the number of trichome branches. Thus, our findings reveal a novel genetic and molecular mechanism of TCS1 and KCBP in trichome cell shape control.

Results

The tcs1 mutants exhibit the reduced number of trichome branches

We isolated the trichome cell shape 1 (tcs1) mutants in a screen of publicly available T-DNA mutant collections of Arabidopsis thaliana. The tcs1-1, tcs1-2 and tcs1-3 trichomes had the reduced branch number compared with wild-type trichomes (Fig 1). By contrast, the tcs1
mutants did not show any obvious defects in plant growth. Progeny of crosses of the three lines indicated that they are allelic. We further measured the number of trichome branches using the first pair of leaves. In wild-type leaves, trichomes normally had two branching points with three branches (92%), although trichomes with four branches were occasionally found (Fig 1C). By contrast, about 70% and 25% of trichomes on tcs1 leaves had two and three branches, respectively (Fig 1C). The tips of tcs1 trichome branches were sharp, as those observed in wild-type trichome branches (Fig 1B). Thus, these results show that TCS1 influences the number of trichome branches in Arabidopsis.

**Trichomes of tcs1 are hypersensitive to the microtubule-disrupting drug oryzalin and the microtubule-stabilizing drug paclitaxel**

In Arabidopsis, the reduced branch number of trichomes is often correlated with a decrease in the level of endoreduplication or the destabilization of microtubules [18,22]. We firstly investigated whether TCS1 could affect endoreduplication in trichome cells. As the nuclear size is often associated with the ploidy level, we measured the nuclear size of Col-0 and tcs1-1 trichomes. The average nuclear size of tcs1-1 trichomes was similar to that of wild-type trichomes (S1A and S1B Fig). The ploidy levels in tcs1-1 leaves were comparable with those in wild-type leaves (S1 Fig). These results suggest that TCS1 may not regulate endoreduplication. We then asked whether TCS1 could influence the microtubule cytoskeleton. The microtubule-disrupting drug oryzalin has been shown to destabilize microtubules, leading to a decrease in the number of trichome branches in Arabidopsis [23]. We therefore treated 4-day-old seedlings of Col-0 and tcs1-1 with 20 μM oryzalin for 2 hours. After a 10-day recovery on ½ MS medium, we examined the branch number of Col-0 and tcs1-1 trichomes. As shown in Fig 2A and 2B, the oryzalin treatment caused a 7.7% decrease in the average number of Col-0 trichome branches, while the oryzalin treatment resulted in an 18.9% reduction in the average number of tcs1-1 trichome branches. The microtubule-stabilizing drug paclitaxel (taxol) has been reported to stabilize microtubules [23]. We asked whether taxol could rescue the trichome branch phenotype of tcs1. Four-day-old seedlings of Col-0 and tcs1-1 were treated with 20 μM taxol for 2 hours. After a 10-day recovery on ½ MS medium, we examined the branch number of Col-0 and tcs1-1 trichomes. In our growth condition, the taxol treatment caused a 2.6% increase in the average number of Col-0 trichome branches, while the taxol treatment resulted in a 9.3% increase in the average number of tcs1-1 trichome branches (Fig 2C and 2D), suggesting that taxol partially rescues the phenotype of tcs1-1 trichome branches.

As the microtubule is crucial for hypocotyl elongation [24], we asked whether TCS1 affects hypocotyl growth. As shown in Fig 2E and 2F, the average length and width of dark-grown tcs1-1 hypocotyls was comparable with that of dark-grown Col-0 hypocotyls. We then treated dark-grown Col-0 and tcs1-1 seedlings with oryzalin and measured their hypocotyl length and width. After oryzalin treatment, hypocotyls of tcs1-1 were significantly shorter and wider than those of the wild type (Fig 2E and 2F). Epidermal cells in tcs1-1 hypocotyls were short and wide in comparison with those in wild-type hypocotyls (S2 Fig). These results show that hypocotyls of tcs1-1 are hypersensitive to oryzalin treatment than wild-type hypocotyls.

**Disruption of TCS1 influences the stability of microtubules**

As tcs1 trichomes had the reduced branch number and were hypersensitive to oryzalin and taxol, we asked whether TCS1 affects the stability of microtubules in trichome cells. We therefore crossed GFP-TUB6 transgenic plants with the tcs1-1 mutant and generated GFP-TUB6; tcs1-1 plants. Cortical microtubule arrays in tcs1-1 trichome cells were similar to those in wild-type trichome cells (Fig 3A). We then applied the microtubule-disrupting drug oryzalin to trichome cells of GFP-TUB6 and GFP-TUB6;tcs1-1 leaves. As shown in Fig 3A and 3B, cortical
Fig 2. Trichomes and hypocotyls of tcs1-1 are hypersensitive to the microtubule-disrupting drug oryzalin and the microtubule-stabilizing drug paclitaxel. (A) Trichome branch distribution of Col-0 and tcs1-1 treated with (T) or without (UT) 20 μM oryzalin for 2 hours. The branch number of Col-0 and tcs1-1 trichomes was examined after a 10-day recovery on ½ MS medium. (B) The average number of Col-0 and tcs1-1 trichome branches treated with (T) or without (UT) 20 μM oryzalin for 2 hours. The branch number of Col-0 and tcs1-1 trichomes was examined after a 10-day recovery on ½ MS medium. (C) Trichome branch distribution of Col-0 and tcs1-1.
microtubule arrays disappeared faster in tcs1-1 trichome cells than those in wild-type trichome cells. We counted the number of cortical microtubules in the trichome branch junction. Microtubules were similar in density before oryzalin treatment. However, more cortical microtubules were disrupted in tcs1-1 than in the wild type after drug treatment. These results indicate that TCS1 influences the stability of microtubules in trichomes. Similarly, we observed that microtubule arrays disappeared relatively faster in epidermal cells of tcs1-1 cotyledons than those in epidermal cells of wild-type cotyledons after oryzalin treatment (S3 Fig).

As tcs1 hypocotyls were hypersensitive to the microtubule-disrupting drug oryzalin, we investigated whether TCS1 is required for the stability of microtubules in hypocotyl cells. Cortical microtubule arrays in epidermal cells of GFP-TUB6;tcs1 hypocotyls were comparable with those of GFP-TUB6 hypocotyls (Fig 3C and 3E). We then applied the microtubule-disrupting drug oryzalin to epidermal cells of etiolated GFP-TUB6 and GFP-TUB6;tcs1-1 hypocotyls. Cortical microtubule arrays disappeared relatively faster in epidermal cells of tcs1-1 hypocotyls than those in epidermal cells of wild-type hypocotyls (Fig 3D and 3E). When oryzalin was washed off after the treatment, the recovery of cortical microtubules in epidermal cells of tcs1-1 hypocotyls was slower than that in epidermal cells of wild-type hypocotyls (Fig 3D and 3E). Taken together, these results indicate that TCS1 influences the stability of microtubules.

**TCS1 encodes a coiled-coil domain-containing protein**

The tcs1-1, tcs1-2 and tcs1-3 mutants were identified from the T-DNA insertions in the fourth exon and the sixth exon of the gene At1g19835, respectively (Fig 4A). T-DNA insertions were confirmed using T-DNA specific and flanking primers (S4A–S4C Fig). We further investigated the expression level of At1g19835 in tcs1-1, tcs1-2 and tcs1-3 mutant seedlings. As shown in S4D Fig, the full length transcript of At1g19835 was not detected in tcs1 mutants, suggesting that tcs1 mutants are loss-of-function alleles. A plasmid containing wild-type At1g19835 cDNA driven by a 35S promoter was introduced into the tcs1-2 mutant. Transgenic plant exhibited complementation of tcs1-2 phenotypes (Fig 4C and 4D). In addition, transformation of tcs1-1 with TCS1-GFP fusion protein under the control of the TCS1 promoter (pTCS1:TCS1-GFP) restored a wild-type phenotype (S4E Fig). Therefore, these results indicate that At1g19835 is the TCS1 gene.

TCS1 encodes a 982-amino-acid protein that contains four coiled-coil domains, which belongs to a family of long coiled-coil protein that consists of 7 members in Arabidopsis [25] (Fig 4B; S5 Fig). Although the family members have been named as filament-like plant proteins (AtFPP), their biochemical and biological functions are totally unknown in Arabidopsis [25]. By performing a BLAST search in the databases, we identified TCS1 homologs in *Brassica rapa*, *Gossypium raimondii*, *Sorghum bicolor*, *Zea mays*, and *Oryza sativa*, but we did not find convincing homologs from animals and yeasts (S5 Fig), suggesting that TCS1 and its homologs might have evolved to control cell morphogenesis in plants.

To determine the expression pattern of TCS1, RNA from roots, flowers, seedlings and leaves were investigated by RT-PCR analysis. TCS1 mRNA was detected in all plant organs tested (S6 Fig). Tissue-specific expression pattern of TCS1 was examined using histochemical assay of
GUS activity of transgenic plants containing the TCS1 promoter: GUS fusion (pTCS1:GUS). GUS activity was detected in cotyledons, leaves, inflorescences and developing etiolated hypocotyls (Fig 4E–4H). GUS activity was also observed in trichomes (Fig 4I), consistent with the role of TCS1 in trichome morphogenesis.

**TCS1 binds to microtubules and promotes microtubule assembly**

As tcs1 affects the stability of microtubules, we asked whether TCS1 could directly bind to the microtubules. A cosedimentation assay was used to analyze the binding of TCS1 to taxol-
Fig 4. Identification of the TCS1 gene. (A) The TCS1 gene structure. The start codon (ATG) and the stop codon (TAA) are indicated. Closed boxes indicate the coding sequences, open boxes show the 5’ and 3’ untranslated regions, and the line between boxes indicates the intron. The T-DNA insertion sites (tcs1-1, tcs1-2 and tcs1-3) in TCS1 are shown. (B) The predicted TCS1 protein contains four coiled-coil domains. aa, amino acids. (C) The average number of trichome branches in Col-0, tcs1-2, 35S::TCS1;tcs1-2#1 and 35S::TCS1;tcs1-2#6 leaves. Values are given as mean ± SE. **P<0.01 compared with Col-0 (Student’s t test). (D) Scanning electron microscope images of epidermal cells in Col-0, tcs1-2 and 35S::TCS1;tcs1-2 hypocotyls grown in ½ MS containing 0.3 μM oryzalin for 15 days in dark. Bars = 200 μm. (E-I) TCS1 expression activity was monitored by pTCS1::GUS transgene expression. Histochemical analysis of GUS activity in a cotyledon (E), a leaf (F), an inflorescence (G), a 4-day-old dark-grown seedling (H) and a trichome (I). Bars = 1 mm in (E-H) and 0.1 mm in (I).

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stabilized microtubules. TCS1 was expressed as a maltose binding protein (MBP) fusion protein (MBP-TCS1) in *E. coli*. As shown in Fig 5A and S7A Fig, MBP-TCS1 was cosedimented...
with the microtubules. The binding of TCS1 to microtubules was saturated at a stoichiometry of about 0.38 M MBP-TCS1 per mole of tubulin dimers (S7C Fig). The binding of the positive control (AUGMIN subunit 8, AUG8) to microtubules was saturated at a stoichiometry of about 0.22 M His-AUG8 per mole of tubulin dimers in our experimental conditions (S7B and S7C Fig) [26]. We then asked whether TCS1 could directly interact with tubulins. To test this, we conducted pull-down experiments. As shown in Fig 5B, MBP-TCS1 bound to tubulins, while the negative control (MBP-TCP14) did not interact with tubulins. Thus, these results indicate that TCS1 physically interacts with tubulins in vitro.

We further performed co-immunoprecipitation analyses to detect the interaction of TCS1 with tubulins in Arabidopsis. Total proteins from pTCS1:TCS1-GFP or 35S:GFP plants were isolated and incubated with GFP-Trap-A agarose beads to immunoprecipitate TCS1-GFP and GFP. The anti-GFP and anti-tubulin antibodies were used to examine immunoprecipitated proteins, respectively. As shown in Fig 5C, tubulins were found in the immunoprecipitated TCS1-GFP complex but not in the negative control (GFP), indicating that TCS1 physically associates with tubulins in Arabidopsis.

To further investigate whether TCS1 localizes to cortical microtubules, we conducted live-cell imaging using a functional TCS1-GFP fusion under the control of TCS1 promoter. As shown in Fig 5D–5F, TCS1-GFP localizes to puncta along cortical microtubules (mCherry-TUB6) in pavement cells, indicating that TCS1 binds to the microtubules. We then investigated the co-localization of TCS1-GFP and microtubules in developing trichomes. We have previously showed that it is difficult to observe the signal of mCherry labeled-microtubules in trichomes [21]. We therefore used pTCS1:TCS1-GFP and GFP-TUB6-expressing lines to compare TCS1 with microtubules. In GFP-TUB6 trichomes, transverse microtubule arrays formed rings encircling the elongating branches, without the signal at the extreme apex (Fig 5G) [21]. Similarly, we observed that TCS1-GFP was present in elongating trichome branches, but leave a TCS1-depleted zone at the extreme apex (Fig 5H). These results indicate that TCS1 and microtubules exhibit similar organization patterns in trichomes, further suggesting that TCS1 is a microtubule-binding protein.

As TCS1 directly interacts with microtubules, we asked whether TCS1 could affect microtubule assembly. We therefore added various concentrations of MBP-TCS1 (0, 0.25, 0.5 and 1 μM) and 1 μM MBP to a 20 μM tubulin solution, and tubulin polymerization was investigated by measuring turbidity. As shown in Fig 5I, the presence of MBP-TCS1 increased turbidity, indicating that MBP-TCS1 increases microtubule mass. The assembly rate of tubulins was increased in a dosage-dependent manner with the addition of MBP-TCS1. To confirm this result, we observed the assembly of rhodamine-labeled tubulins incubated with MBP and MBP-TCS1 under confocal microscopy. As shown in Fig 5J, the assembly of microtubules was detected in the presence of MBP-TCS1 rather than MBP. Taken together, these results indicate that TCS1 promotes microtubule assembly.

TCS1 physically interacts with KCBP/ZWI in vitro and in vivo

To further understand the molecular mechanism of TCS1 in the regulation of trichome branch number, we performed a yeast two-hybrid screen to identify putative TCS1-binding proteins. TCS1 was fused to the GAL4 DNA binding domain (BD) and used as a bait. In this screen, KCBP/ZWI was identified as a putative TCS1-interacting protein. KCBP/ZWI has been shown to affect microtubules and trichome branches [16], suggesting that TCS1 could interact with KCBP/ZWI to control trichome branches. We tested the interactions between TCS1 and the full length KCBP in yeast cells. As shown in Fig 6A, TCS1 interacted with KCBP in a yeast two-hybrid assay. We then investigated the interaction of TCS1 with KCBP using in vitro pull-
Fig 6. TCS1 physically and genetically interacts with KCBP to control the number of trichome branches. (A) TCS1 interacts with KCBP in yeast cells. (B) TCS1 physically interacts with KCBP in vitro. MBP-TCS1 was pulled down (PD) by GST-KCBP immobilized on Glutathione Sepharose 4B and analyzed by immunoblotting (IB) using an anti-MBP antibody. MBP was used as a negative control. (C) TCS1 interacts with KCBP in vivo. Total proteins from pTCS1::TCS1-GFP; 35S:Myc-KCBP and 35S::GFP; 35S:Myc-KCBP plants were immunoprecipitated with GFP-Trap-A (IP), and the immunoblots (IB) were probed with anti-GFP and anti-Myc antibodies, respectively. Myc-KCBP was detected in the immunoprecipitated TCS1-GFP complex. (D) Trichome branch (br) distribution of Col-0, tcs1-2, zwi-101, and zwi-101 tcs1-2 first pair of leaves at 15 days after germination (DAG). Values are given as mean ± SE. (E) The average number of Col-0, tcs1-2, zwi-101, zwi-101 tcs1-2 trichome branches treated with (T) or without (UT) 20 μM oryzalin for 2 hours. The branch number of Col-0, tcs1-2, zwi-101, zwi-101 tcs1-2 trichomes was examined after a 10-day recovery on ½ MS medium.

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down experiments. TCS1 was expressed as a maltose binding protein (MBP) fusion protein (MBP-TCS1), while KCBP was expressed as a glutathione S-transferase (GST) fusion protein (GST-KCBP). As shown in Fig 6B, MBP-TCS1 bound to GST-KCBP, while the negative control (MBP) did not bind to GST-KCBP. This result indicates that TCS1 physically interacts with KCBP in vitro.

We further performed co-immunoprecipitation analysis to investigate the association of TCS1 with KCBP in Arabidopsis. We generated 35S:Myc-KCBP transgenic plants. We crossed the pTCS1:TCS1-GFP and 35S:GFP transgenic lines with 35S:Myc-KCBP transgenic plants to generate pTCS1:TCS1-GFP;35S:Myc-KCBP and 35S:GFP;35S:Myc-KCBP plants, respectively. Total proteins were isolated and incubated with GFP-Trap-A agarose beads to immunoprecipitate TCS1-GFP and GFP. The anti-GFP and anti-Myc antibodies were used to detect immunoprecipitated proteins, respectively. Myc-KCBP was found in the immunoprecipitated TCS1-GFP complex but not in the negative control (GFP) (Fig 6C), indicating that TCS1 physically associates with KCBP in Arabidopsis.

TCS1 genetically interacts with KCBP to control the number of trichome branches

As TCS1 physically interacts with KCBP, and tcs1 mutants showed similar trichome branching phenotypes to kcbp/zwi mutants, we sought to establish genetic relationships between TCS1 and KCBP in the regulation of trichome branch number. We obtained the zwi-101 mutant (SALK_017886) harboring the T-DNA insertion in the KCBP/ZWI gene (S8 Fig). The full length mRNA of KCBP could not be detected in zwi-101, suggesting that zwi-101 is a loss-of-function allele. The zwi-101 trichomes exhibited the reduced number of branches (Fig 6D), consistent with previous results [16]. We then generated a zwi-101 tcs1-2 double mutant and investigated its trichome branch number. As shown in Fig 6D and S9 Fig, the branch number of zwi-101 tcs1-2 double mutant trichomes was comparable to that of zwi-101 single mutant trichomes, suggesting that zwi-101 is epistatic to tcs1-2 with respect to the number of trichome branches. Considering that both TCS1 and KCBP affect the stability of microtubules, we asked whether genetic interactions between TCS1 and KCBP in trichome branch number are related to microtubule stability. We therefore treated 4-day-old seedlings of Col-0, zwi-101, tcs1-2 and zwi-101 tcs1-2 with 20 μM oryzalin for 2 hours. After a 10-day recovery on ½ MS medium, the number of Col-0, zwi-101, tcs1-2 and zwi-101 tcs1-2 trichome branches was investigated. After oryzalin treatment, the number of zwi-101 tcs1-2 trichome branches was similar to that of zwi-101 trichome branches (Fig 6E). The oryzalin treatment caused a similar decrease in the average number of zwi-101 tcs1-2 and zwi-101 trichome branches. These results suggest that TCS1 acts genetically with KCBP to regulate the number of trichome branches by influencing the stability of microtubules.

KCBP was reported to physically interact with AN in yeast cells [18]. The an mutants showed the reduced branches of trichomes in leaves [18,19]. We asked whether TCS1 and AN could function in a common pathway to control trichome branches. To test this, we obtained the an-101 mutant (SALK_026489) harboring the T-DNA insertion in the AN gene (S10 Fig). The full length mRNA of AN could not be detected in an-101, suggesting that an-101 is a loss-of-function allele. The an-101 mutant trichomes mainly had one or two branches (S11 Fig), consistent with previous results [18]. We then generated the an-101 tcs1-2 double mutant and investigated its trichome branches. The number of an-101 tcs1-2 trichome branches was similar to that of an-101 trichome branches (S11 Fig), suggesting an epistatic genetic interaction. We further tested whether TCS1 could physically interact with AN. As shown in S12 Fig, TCS1 did not directly interact with AN in vitro (S12 Fig).
Discussion
A fundamental question in developmental biology is how cell shape is controlled. In plants, cell shape is crucial not only for the function of the individual cell, but also for its role in organ shape and size control. However, the genetic and molecular mechanisms that determine cell shape remain largely unknown in plants. In this study, we report that the TCS1 gene, which encodes a microtubule binding protein with long coiled-coil domains, is required for trichome cell shape in Arabidopsis. TCS1 directly binds to microtubules and promotes microtubule assembly. TCS1 physically and genetically interacts with KCBP/ZWICHEL to regulate the number of trichome branches by influencing microtubule stability. Thus, our findings reveal a novel genetic and molecular mechanism of TCS1 and KCBP in trichome cell shape control.

TCS1 regulates trichome cell shape by influencing the stability of microtubules
The tcs1 trichomes showed the reduced branch number (Fig 1), although tcs1 plants appear to be similar to wild-type plants. Trichome branching is a complicated process, which is regulated by a number of factors. In Arabidopsis, DNA replication (endoreduplication) in trichome cells influences the number of trichome branches [22,27,28]. However, mutations in TCS1 did not affect ploidy levels in leaves and nuclear size in trichome cells (S1 Fig). Thus, it is unlikely that TCS1 regulates trichome branch number by influencing DNA replication events. After endoreduplication, a cytoskeleton-dependent polarization event happens during trichome morphogenesis [23], resulting in a total of three to four branches in the mature trichome on leaves. Molecular-genetic and pharmacological studies have established that microtubules are essential for trichome branching in Arabidopsis [23,29]. Interestingly, the trichomes of tcs1 were hypersensitive to the microtubule-disrupting drug oryzalin in comparison with those of the wild type (Fig 2A and 2B). Similarly, tcs1 hypocotyls were more sensitive to oryzalin than wild-type hypocotyls (Fig 2E, 2F, and S2 Fig). By contrast, the microtubule-stabilizing drug taxol treatment partially rescued the branch number of tcs1 trichomes (Fig 2C and 2D). These results suggest that TCS1 may affect the stability of microtubules, which are crucial for trichome cell morphogenesis. Consistent with this notion, we observed that microtubules in tcs1 cells disappeared faster than those in wild-type cells when treated with oryzalin (Fig 3 and S3 Fig). Thus, these results support that mutations in TCS1 influence the stability of microtubules, resulting in the altered trichome cell shape in Arabidopsis.

TCS1 is a microtubule-binding protein and promotes microtubule assembly
The TCS1 gene encodes a coiled-coil domain-containing protein, which belongs to a family of long coiled-coil protein that consists of 7 members in Arabidopsis [25]. However, the biological functions of the TCS1 family members are totally unknown in Arabidopsis [25]. Therefore, TCS1 is a novel regulator of trichome cell shape in Arabidopsis. Sequence analyses show that TCS1 homologs are plant-specific proteins (S5 Fig), suggesting that TCS1 and its homologs might have evolved to regulate cell shape in plants. Expression of TCS1 was detected in all tested tissues (S6 Fig), although the only visible phenotype in tcs1 mutants was found in trichomes. It is possible that TCS1 might function redundantly with other proteins to influence cell growth in other tissues or cell types.

Several microtubule binding proteins have been known to influence the branch number of trichomes in Arabidopsis [30,31]. Our biochemical analyses showed that TCS1 physically interacts with microtubules in vitro and in vivo (Fig 5A–5C). Live-cell imaging assay found
that TCS1 directly bound to the microtubule in Arabidopsis cells (Fig 5D–5F). In addition, TCS1-GFP and GFP-TUB6 showed similar organization patterns in elongating trichome branches (Fig 5G and 5H). These results support that TCS1 is a microtubule binding protein. It is possible that TCS1 directly binds to microtubules during trichome development and stabilizes microtubules, thereby influencing the formation of trichome branches in Arabidopsis. Biochemical analyses showed that TCS1 promotes microtubule assembly, consistent with the function of TCS1 in stabilizing microtubules (Fig 5I and 5J). The microtubule assembly has been known to influence trichome branch number. For example, mutations in the TUBULIN FOLDING COFACTOR (TCF) C and TCFA result in the unbranched trichome phenotype [13,14]. These mutants were proposed to affect the making of assembly component α/β tubulin dimmers and possibly decrease the assembly of new microtubules. Mutations in KINESIN-13A, which promotes microtubule depolymerization, resulted in the increased number of trichome branches [15,32]. Thus, it is possible that TCS1 promotes microtubule assembly and increases the stability of microtubules, thereby influencing trichome branch number in Arabidopsis.

A possible genetic and molecular mechanism of TCS1 in regulating the number of trichome branches

KCBP, a microtubule motor, regulates cell division and trichome cell shape in Arabidopsis [16,17]. Trichomes on zwi leaves had one or two branches with blunt tips. It has been suggested that KCBP participates in the trichome morphogenesis by regulating the local reorientation and stability of microtubules [30,31]. Similarly, TCS1 regulates trichome branch number by influencing the stability of microtubules. The tcs1 mutants showed similar trichome branch number phenotype to kcbp/zwi mutants, suggesting that TCS1 could genetically interact with KCBP to control the branch number of trichomes. Consistent with this idea, our genetic analyses show that zwi is epistatic to tcs1 with respect to trichome branch number. Further results demonstrated that TCS1 physically interacted with KCBP in vitro and in vivo (Fig 6A–6C). As both TCS1 and KCBP influence the stability of microtubules, it is possible that TCS1 functions with KCBP to control trichome branch number by affecting the dynamics and stability of microtubules in Arabidopsis. Supporting this notion, zwi-101 tcs1-2 and zwi-101 trichomes showed a similar level of hypersensitivity to oryzalin (Fig 6E). A recent study have shown that KCBP interacts with both microtubules and actin cytoskeleton to regulate trichome branching and elongation in Arabidopsis [21]. The zwi trichomes had the reduced number of branches, shortened stalks and stunted branches [16]. The reduced number of branches in zwi trichomes is likely caused by defects in microtubules. By contrast, the transverse cortical F-actin cap at the trichome branch apex has been proposed to regulate polarized branch elongation and tip sharpening [21]. tcs1 mutants only affected the trichome branch number and had normal stalks and trichome branch tips (Fig 1A and 1B), suggesting that KCBP and TCS1 may have the overlapped function in the regulation of microtubule cytoskeleton rather than actin cytoskeleton.

Genetic studies suggested that KCBP may interact with multiple factors, such as SUZ1, SUZ2 and SUZ3 and might function as a complex, although the SUZ genes have not been cloned in Arabidopsis [20]. KCBP has been shown to physically interact with a plant-specific protein kinase termed KCBP-interacting protein kinase (KIPK), a calcium binding protein (KIC) and AN [17,18,33]. KIC regulates trichome morphogenesis by influencing microtubule binding and microtubule-stimulated ATPase activities of KCBP, although the genetic interactions between KCBP and KIC remain unknown [17]. AN is also required for normal trichome morphogenesis in Arabidopsis although AN has been suggested to indirectly regulate microtubules [18,19]. It has been proposed that the level of each protein in the KCBP complex is likely to be crucial for trichome morphogenesis [17]. As TCS1 physically and genetically interacted
with KCBP, it is possible that TCS1 and other members in the KCBP complex may have genetic interactions in trichome branching. Supporting this notion, we found an epistatic interaction between AN and TCS1 with respect to the number of trichome branches, although TCS1 does not physically interact with AN (S12 Fig). It will be a worthwhile challenge to build up the genetic and molecular interactions between TCS1 and other members of the KCBP complex in the future. Taken together, our findings reveal a novel genetic and molecular mechanism by which TCS1 interacts with KCBP to control trichome cell shape by influencing the stability of microtubules.

**Materials and Methods**

**Plant materials and growth conditions**

The *tcs1-1* (SAIL_403_D02), *tcs1-2* (SALK_040648), *tcs1-3* (SALK_078664), *zwi-101* (SALK_017886), and *an1-101* (SALK_026489) mutants were obtained from the Nottingham Arabidopsis Stock Centre (NASC). The T-DNA insertions were verified by PCR and sequencing using the primers described in S1 Table. Arabidopsis seeds were sterilized with 100% isopropanol for 2 min and 10% NaClO (v/v) for 10 min and then washed six times with sterile water. Arabidopsis seeds were dispersed on ½ Murashige and Skoog (MS) medium containing 0.9% agar and 1% glucose and then stored at 4°C for 3 days in the darkness. Plants were grown at 22°C under long-day conditions (a 16-h-light /8-h-dark cycle). To observe etiolated hypocotyls, we grow plants in dark at 22°C.

**Construction and plant transformation**

A PCR-based Gateway system was used to generate 35S:TCS1, pTCS1:TCS1-GFP and pTCS1:GUS constructs. The TCS1 CDS was amplified using the primers TCS1-CDS-LP and TCS1-CDS-RP (S1 Table). PCR product was subcloned into the pCR8/GW/TOPO TA cloning vector (Invitrogen) using TOPO enzyme. The TCS1 CDS was then subcloned into the Gateway binary vector pMDC32 to generate the 35S:TCS1 construct. The TCS1 genomic sequence containing a 2012-bp promoter sequence and 3298bp gene was amplified using the primers gTCS1-GFP-LP and gTCS1-GFP-RP. PCR products were firstly cloned into the pCR8/GW/TOPO TA cloning vector (Invitrogen) using TOPO enzyme. The TCS1 genomic sequence was then subcloned into the pMDC107 vector to generate the construct pTCS1:TCS1-GFP. The 2164bp promoter sequence of TCS1 was amplified using the primers TCS1pro-LP and TCS1pro-RP. PCR products were cloned into the pCR8/GW/TOPO TA cloning vector (Invitrogen) using TOPO enzyme. The TCS1 promoter was then subcloned into the pMDC164 vector to generate the transformation plasmid pTCS1:GUS. The plasmids 35S:TCS1, pTCS1:TCS1-GFP and pTCS1:GUS were transferred into *tcs1-2* or Col-0 plants using Agrobacterium GV3101, and medium with hygromycin (30μg/mL) was used to select transgenic plants.

**Morphological and cellular analysis**

Trichome branches on the first pair of Col-0 and *tcs1-1* leaves were counted at 15 days after germination (DAG). Leaves and etiolated hypocotyls of wild-type and *tcs1-1* mutants were fixed in a solution (formalin, acetic acid, ethanol and H₂O in a ratio of 1: 0.5: 4.75: 3.75) for 24 hours, dehydrated with a graded ethanol series and dried at critical point in liquid CO₂. Samples were coated with gold and then observed in an S-4160 Field Emission Scanning Electron Microscope (SEM) (Hitachi).

To determine the effect of TCS1 on cortical microtubules, the microtubule-disrupting drug oryzalin (3,5-dinitro-N4, N4-dipropylsulfanilamide; Sigma-Aldrich) was applied to trichomes
and hypocotyl epidermal cells of the wild type (Col-0) and tcs1-1 for specific times. The microtubule-stabilizing drug taxol (paclitaxel, Sigma-Aldrich) was used to treat trichomes of the wild type and tcs1-1.

To quantify the numbers of cortical microtubules in trichome and hypocotyl cells, the ImageJ software was employed. A line of fixed length (10 μm or 20 μm) perpendicular to the orientation of the most cortical microtubules was drawn, and the number of cortical microtubules across the line was counted. At least 10 cells from each treatment were used, and four lines of fixed length were drawn for each cell. The average number of cortical microtubules before and after treatments was calculated. The Student’s test was used to analyze the significance of the difference.

**GUS staining**

Samples (pTCS1:GUS) were putted into a GUS staining solution [0.1% Nonidet P-40, 1 mM 5-bromo-4-chloro-3-indolyl-b-D-glucuronic acid, 10 mM EDTA, 100 mM Na₃PO₄ buffer, and 3 mM each K₃Fe(CN)₆/K₄Fe(CN)₆] and incubated at room temperature for 6 hours. After GUS staining, 70% ethanol was used to remove chlorophyll.

**Confocal microscopy observation**

GFP fluorescence in cells of trichomes and hypocotyls was detected using a Zeiss LSM710 META confocal microscope. GFP was observed using wave lengths of 510 to 530 nm. To study the co-localization of TCS1 and microtubules, we crossed pTCS1:TCS1-GFP transgenic plants with mCherry-TUB6 expressing plants. Seeds were germinated on ½ Murashige and Skoog (MS) medium supplemented with 0.9% agar and 1% glucose. Leaves of 6-day-old mCherry-TUB6;pTCS1:TCS1-GFP seedlings were observed under a spinning disk confocal microscope equipped with lasers for GFP and mCherry (Intelligent Design).

**RNA isolation and semiquantitative RT-PCR analysis**

Leaves, stems, cotyledons and roots from 12-day-old seedlings were collected to isolate total RNAs using an RNasy Plant Mini kit (TIANGEN). Reverse transcription (RT)-PCR was performed using Superscript III reverse transcriptase (Invitrogen). ACTIN2 mRNA was an internal control. The specific primers used for RT-PCR are shown in S1 Table.

**Yeast two-hybrid assays**

The coding sequence of TCS1 was cloned into NotI and SalI sites of the bait vector pDBleu (Invitrogen) and the prey vector pEXP-AD502 (Invitrogen) to generate TCS1-BD and TCS1-AD constructs, respectively. The specific primers for TCS1-BD and TCS1-AD were TCS1-Y2H-NotI-LP and TCS1-Y2H-NotI-RP (S1 Table). The coding sequence of KCBP was cloned into NotI and SalI sites of the bait vector pDBleu (Invitrogen) and the prey vector pEXP-AD502 (Invitrogen) to generate KCBP-BD and KCBP-AD constructs. The specific primers for KCBP-BD and KCBP-AD constructs were KCBP-Y2H-SalI-LP and KCBP-Y2H-NotI-RP (S1 Table). The prey and bait plasmids were co-transformed into the yeast strain PT69-4A to investigate their interactions.

**In vitro protein-protein interaction**

The coding sequence of TCS1 was cloned into the vector pMAL-C2 to generate the MBP-TCS1 construct. The specific primers for the MBP-TCS1 construct were MBP-TCS1-LP and MBP-TCS1-RP (S1 Table). The coding sequence of KCBP was cloned into the vector pGEX-
4T-1 (Amersham-Pharmacia) to generate the GST-KCBP construct. The specific primers for the GST-KCBP constructs were GST-KCBP-LP and GST-KCBP-RP (S1 Table). The coding sequence of AN was subcloned into the vector pET-NT to generate the AN-His construct. The specific primers for the AN-His construct were AN-His-LP and AN-His-RP (S1 Table). The MBP-TCS1, GST-KCBP and AN-His plasmids were transformed and expressed in E. coli Rosetta (DE3).

To investigate protein-protein interaction, we performed the pull-down assay. Bacterial lysates containing ~15 μg of MBP-TCS1 or MBP-TCP14 fusion proteins were mixed with ~20 μg of tubulins. Bacterial lysates containing ~15 μg of MBP-TCS1 or MBP proteins were mixed with ~20 μg of AN-His fusion proteins. Amylose resin (30 μL; New England Biolabs) was added into each combined solution with gently rocking at 4°C for 1 h. Bacterial lysate containing ~20 μg of GST-KCBP was mixed with lysate containing ~15 μg of MBP-TCS1 or MBP. Glutathione Sepharose 4B (30 μL;GE Healthcare) was added into each combined solution with gently rocking at 4°C for 1 h. The TGH buffer (1× Complete Protease Inhibitor cocktail [Roche], 150 mM NaCl, 10% glycerol, 1.5 mM MgCl₂, 50 mM HEPES, pH 7.5, 1 mM phenylmethylsulfonyl fluoride, 1 mM EGTA, pH 8.0, and 1% Triton X-100) was used to wash beads for five times. The isolated proteins were then analyzed by 10% SDS-PAGE and determined by immunoblot analysis with anti-MBP, anti-tubulin, anti-GST, and anti-His antibodies (Abmart), respectively.

Assays of microtubule cosedimentation and assembly
For the microtubule cosedimentation assay, different concentrations of MBP-TCS1 were added to paclitaxel-stabilized microtubules in the PEMT buffer (1 mM MgCl₂, 1 mM EGTA, 100 mM PIPES, and 20 μM taxol, pH 6.9). After incubation at 25°C for 30 min, the samples were centrifuged at 100,000g at 25°C for 30 min to separate supernatants and pellets. They were then analyzed by 10% SDS-PAGE and determined by staining the gels with Coomassie Brilliant Blue R 250.

For the microtubule polymerization assay, different concentrations of MBP-TCS1 were added to 20 μM tubulin solution in the PEM buffer (1 mM MgCl₂, 1 mM EGTA, 1 mM GTP, and 100 mM PIPES, pH 6.9). The polymerization was investigated turbidimetrically by absorbance at 350 nm with a 0.4-cm path quartz cell at 37°C in a DU-640 spectrophotometer (Beckman Coulter, Fullerton, CA). 1 μM MBP was used as a negative control. The data was recorded from time 0 to 35 min, when the turbidity in all samples did not increase any more.

For the observation of microtubule assembly, 1μM MBP-TCS1, 20μM Rhodamine labeled tubulins and 1mM GTP were incubated at 37°C for 30 min. The microtubule polymerization was then stopped using 1% glutaraldehyde. Spinning-Disc Confocal Microscopy Imaging was performed on an Olympus IX81 inverted microscope equipped with a Yokogawa spinning-disc confocal head (Yokogawa Electric) and an Andor iXon charge-coupled device camera (Andor Technology). 1μM MBP was used as a negative control. Images were captured using Andor iQ software, version 1.1 (Andor Technology), and processed using ImageJ software.

Co-immunoprecipitation
The coding sequence of KCBP was cloned into the XmaI and SpeI sites of the pCAMBIA1300-221-Myc vector to generate the transformation plasmid 35S:Myc-KCBP. The specific primers used for 35S:Myc-KCBP construct were MYC-KCBP-XmaI-LP and MYC-KCBP-SpeI-RP (S1 Table). The 35S:Myc-KCBP plasmid was transferred into tcs1-2 plants using Agrobacterium GV3101, and hygromycin (30μg/mL)-containing medium was used to select transformants. Total proteins from pTCS1:TCS1-GFP;35S:Myc-KCBP and 35S:GFP;35S:Myc-KCBP were
isolated with the extraction buffer (1× Complete protease inhibitor cocktail, 50 mM Tris/HCl, pH 7.5, 2% Triton X-100, 1 mM EDTA, 150 mM NaCl, 20 mg/mL MG132, and 20% glycerol) and mixed with GFP-Trap-A (Chromotek) for 1 h at 4°C. Beads were washed four times with the wash buffer (1×Complete protease inhibitor cocktail, 50mMTris/HCl, pH7.5, 150mM NaCl, and 0.1% Triton X-100). The immunoprecipitates were analyzed by 10% SDS-polyacrylamide gel and determined by immunoblot analysis with anti-GFP (Abmart) and anti-Myc (Abmart) antibodies, respectively.

**Accession numbers**

Arabidopsis Genome Initiative locus identifiers for the genes mentioned in this article are as follows: AT1G19835 (TCS1), AT5G65930 (KCBP), and AT1G01510 (AN).

**Supporting Information**

**S1 Fig. TCS1 does not affect endoreduplication.** (A) The size of nuclei in wild-type Col-0 and tcs1-1 trichomes. The nuclei were stained by DAPI. (B) The average area of nuclei in Col-0 and tcs1-1 trichomes. (C) Nuclear DNA ploidy distribution of cells in Col-0 and tcs1-1 first pair of leaves measured at 13 days after germination (DAG). Values (B and C) are given as mean ± SE. Bars = 20 μm in (A).

**S2 Fig. Hypocotyl cells of tcs1-1 are hypersensitive to the microtubule-disrupting drug oryzalin.** (A) Scanning electron microscope images of Col-0 and tcs1-1 cells in the top and middle regions of etiolated hypocotyls grown in ½ MS for 15 days in dark. Bars = 200 μm. (B) Scanning electron microscope images of Col-0 and tcs1-1 cells in the top and middle regions of etiolated hypocotyls grown in ½ MS containing 0.3 μM oryzalin for 15 days in dark. Bars = 200 μm. (C) The average length of epidermal cells in the middle regions of Col-0 and tcs1-1 hypocotyls treated with oryzalin. Col-0 and tcs1-1 seedlings were grown in ½ MS containing 0, 0.25 μM and 0.3 μM oryzalin (OZ) for 15 days in dark. (D) The average width of epidermal cells in the middle regions of Col-0 and tcs1-1 hypocotyls treated with oryzalin. Col-0 and tcs1-1 seedlings were grown in ½ MS containing 0, 0.25 μM and 0.3 μM oryzalin (OZ) for 15 days in dark. Values (C and D) are given as mean ± SE. **P<0.01 compared with the wild type (Student’s t test).

**S3 Fig. Microtubules in epidermal cells of tcs1-1 cotyledons are hypersensitive to the microtubule-disrupting drug oryzalin.** Cortical microtubules in epidermal cells of GFP-TUB6 and GFP-TUB6;tcs1-1 cotyledon veins treated with 5 μM oryzalin for 10 minutes. Bars = 20 μm.

**S4 Fig. Identification of the TCS1 gene.** (A) PCR identification of the T-DNA insertion in tcs1-1 with T-DNA specific primers (LB1) and flanking primers (LP and RP). (B) PCR identification of the T-DNA insertion in tcs1-2 with T-DNA specific primers (LBa1) and flanking primers (LP and RP). (C) PCR identification of the T-DNA insertion in tcs1-3 with T-DNA specific primers (LBa1) and flanking primers (LP and RP). (D) RT-PCR analysis of TCS1 expression in Col-0, tcs1-1, tcs1-2 and tcs1-3 seedlings. RT-PCR was performed on first-strand cDNA prepared from 2-week-old seedlings. cDNA was standardized by reference to an ACTIN2 standard. (E) The average trichome branch number of Col-0, tcs1-1, pTCS1: TCS1-GFP;tcs1-1#1 and pTCS1: TCS1-GFP;tcs1-1#2 first pair of leaves at 15 days after germination (DAG). Values (E) are given as mean ± SE. **P<0.01 compared with the wild type.
S5 Fig. Phylogenetic tree of TCS1 and its homologs in different species. The phylogenetic tree was constructed using the neighbor-joining method of the MEGA6 program (http://www.megasoftware.net/mega.html). Values at nodes represent percentages of 1000 bootstrap replicates. The scale bar at the bottom represents the genetic distance.

S6 Fig. Expression of the TCS1 gene. RT-PCR analysis of TCS1 expression in roots, flowers, 10-day-old seedlings, rosette leaves and cauline leaves.

S7 Fig. Quantification of the binding affinity of TCS1 and AUG8 with microtubules. (A) MBP-TCS1 fusion protein was cosedimented with paclitaxel-stabilized microtubules (5 μM). After high-speed centrifugation, the amount of MBP-TCS1 in pellets increased when higher concentrations of MBP-TCS1 proteins were added before reaching saturation. (B) His-AUG8 fusion protein was cosedimented with paclitaxel-stabilized microtubules (5 μM). After high-speed centrifugation, the amount of His-AUG8 in pellets increased when higher concentrations of His-AUG8 proteins were added before reaching saturation. (C) Quantification of the binding affinity of TCS1 with microtubules shown in (A) compared with that of AUG8 with microtubules shown in (B). The binding of TCS1 and AUG8 to microtubules was saturated at a stoichiometry of about 0.38 M MBP-TCS1 and 0.22 M His-AUG8 per mole of tubulin dimers, respectively.

S8 Fig. Identification of the zwi-101 mutant. (A) The insertion of T-DNA in zwi-101 (SALK_017886) is shown. (B and C) PCR identification of the T-DNA insertion in zwi-101 with T-DNA specific primers (LBa1) and flanking primers (LP and RP). (D) Expression levels of KCBP in Col-0 and zwi-101 seedlings as determined by RT-PCR.

S9 Fig. zwi-101 is epistatic to tcs1-2 with respect to trichome branch number. (A) The average number of Col-0, tcs1-2, zwi-101 and zwi-101 tcs1-2 trichome branches of the first pair of leaves at 15 days after germination (DAG). (B) Scanning electron microscope images of Col-0, tcs1-2, zwi-101 and zwi-101 tcs1-2 trichome branches of first pair of leaves at 15 days after germination (DAG). Values (A) are given as mean ± SE. **P<0.01 compared with the respective controls (Student’s t test). Bars = 100 μm.

S10 Fig. Identification of the an-101 mutant. (A) The insertion of T-DNA in an-101 (SALK_026489) is shown. (B and C) PCR identification of the T-DNA insertion in an-101 with T-DNA specific primers (LBa1) and flanking primers (LP and RP). (D) Expression levels of AN in Col-0 and an-101 seedlings as determined by RT-PCR.

S11 Fig. an-101 is epistatic to tcs1-2 with respect to trichome branch number. (A) The average number of Col-0, tcs1-2, an-101 and an-101 tcs1-2 trichome branches of the first pair of leaves at 15 days after germination (DAG). (B) Scanning electron microscope images of Col-0, tcs1-2, an-101 and an-101 tcs1-2 trichome branches of the first pair of leaves at 15 days after germination (DAG). Values (A) are given as mean ± SE. Bars = 100 μm.
S12 Fig. **TCS1 does not physically interact with AN.** AN-His proteins were pulled down (PD) by MBP-TCS1 immobilized on amylose resin and analyzed by immunoblotting (IB) using an anti-His antibody. MBP was used as a negative control.

(PDF)

**S1 Table. List of primers used in this study**

(PDF)

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**Author Contributions**

Conceived and designed the experiments: LC YP YL.

Performed the experiments: LC YP JT XW.

Analyzed the data: LC YP JT ZK TM MY YL.

Contributed reagents/materials/analysis tools: ZK TM MY.

Wrote the paper: LC YL.

**References**


