

# SCF<sup>FBS1</sup> Regulates Root Quiescent Center Cell Division via Protein Degradation of APC/C<sup>CCS52A2</sup>

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**Homeostatic regulation of meristematic stem cells accomplished by maintaining a balance between stem cell self-renewal and differentiation is critical for proper plant growth and development. The quiescent center (QC) regulates root apical meristem homeostasis by maintaining stem cell fate during plant root development. Cell cycle checkpoints, such as anaphase promoting complex/cyclosome/cell cycle switch 52 A2 (APC/C<sup>CCS52A2</sup>), strictly control the low proliferation rate of QC cells. Although APC/C<sup>CCS52A2</sup> plays a critical role in maintaining QC cell division, the molecular mechanism that regulates its activity remains largely unknown. Here, we identified SCF<sup>FBS1</sup>, a ubiquitin E3 ligase, as a key regulator of QC cell division through the direct proteolysis of CCS52A2. FBS1 activity is positively associated with QC cell division and CCS52A2 proteolysis. FBS1 overexpression or *ccs52a2-1* knockout consistently resulted in abnormal root development, characterized by root growth inhibition and low mitotic activity in the meristematic zone. Loss-of-function mutation of *FBS1*, on the other hand, resulted in low QC cell division, extremely low *WOX5* expression, and rapid root growth. The 26S proteasome-mediated degradation of CCS52A2 was facilitated by its direct interaction with FBS1. The FBS1 genetically interacted with APC/C<sup>CCS52A2</sup>-ERF115-PSKR1 signaling module for QC division. Thus, our findings establish SCF<sup>FBS1</sup>-mediated CCS52A2 proteolysis as the molecular mechanism for controlling QC cell division in plants.**

**Keywords:** CCS52A2, E3 ubiquitin ligase, FBS1, quiescent center, root apical meristem

## INTRODUCTION

Plants, as sessile organisms, must cope with a variety of environmental stresses to properly adapt to terrestrial ecosystems. The postembryonic regulation of plant growth and development from the meristematic stem cell tissues is strongly influenced by environmental cues. The quiescent center (QC), a central organizing center that regulates the homeostasis of the root stem cell niche (SCN), ensures proper development over extended periods of time (Aichinger et al., 2012; Hong et al., 2017; Rahni et al., 2016; Scheres et al., 2002). Under normal growth conditions, the QC cells exhibit a lower proliferation rate and mitotic activity than the surrounding SCN. However, stress-induced meristem cell damage increases QC cell division, replacing the damaged cells with new stem cells (Cruz-Ramirez et al., 2013; Scheres, 2007; Timilsina et al., 2019). This indicates that the low mitotic activity and proliferation of QC cells are required for replenishing the pluripotent stem cell pools under fluctuating environmental conditions (Heyman et al., 2013; 2014). Consistently, stress-related plant hormones, such as jasmonic acid (JA), ethylene, and brassinosteroid (BR), as well as mechanical wounding and DNA damage facilitate QC cell division and tissue regeneration (Campos et al., 2016; Chen et al., 2004; Hou et al.,

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2010; Nemhauser et al., 2006; Zhou et al., 2019).

Recent studies have identified several key molecular pathways that control QC cell homeostasis and its implications for SCN homeostasis (Heyman et al., 2013; 2014). CELL CYCLE SWITCH 52 A2 (CCS52A2), a highly conserved activator subunit of the anaphase promoting complex/cyclosome (APC/C), was identified as an essential factor controlling QC cell division. APC/C is an E3 ubiquitin ligase that inhibits QC cell division through proteasomal degradation of cell cycle control proteins recruited by CCS52A2 (Vanstraelen et al., 2009). ETHYLENE RESPONSE FACTOR 115 (ERF115) and A-type cyclin CYCA3;4 have been identified as proteolytic targets of APC/C<sup>CCS52A2</sup> for regulating QC homeostasis and formative cell division control, respectively (Heyman et al., 2013; Willems et al., 2020). BR-induced *ERF115* expression promotes QC cell division through the direct transcriptional regulation of *PHYTOSULFOKINES 5 (PSK5)* and activation of signaling pathways downstream of PSKR RECEPTOR (PSKR) (Hartmann et al., 2013; Heyman et al., 2013). The APC/C<sup>CCS52A2</sup>-ERF115-mediated regulation of QC homeostasis is importantly linked to environmental stress adaptation by replenishing damaged stem cells (Cheng et al., 2013; Heyman et al., 2013; Hong et al., 2017; Zhou et al., 2019). This suggests that the modulation of APC/C<sup>CCS52A2</sup>-mediated QC cell division is likely crucial for plant adaptation to environmental changes. Although external stresses and plant signaling pathways are intimately linked with root meristem organization, it has yet to be determined which factor primarily connects APC/C<sup>CCS52A2</sup> to environmental stress cues for QC maintenance and SCN replenishment.

In this study, we reveal that F-BOX STRESS INDUCED 1 (FBS1), a stress-inducible F-box protein, regulates QC cell division and root apical meristem (RAM) organization through the previously established APC/C<sup>CCS52A2</sup>-ERF115-PSKR1 signaling pathways. *FBS1* overexpression lines displayed enhanced QC cell division, phenocopying the *ccs52a2-1* mutant, whereas *fbs1-1* knockout plants displayed minimal QC division and increased stem cell proliferation in the meristematic zone. Direct physical interaction between FBS1 and CCS52A2 facilitated 26S proteasome-mediated CCS52A2 protein degradation. These findings suggest that SCF<sup>FBS1</sup> governs root development by fine-tuning the homeostatic regulation of RAM via APC/C<sup>CCS52A2</sup>-mediated QC maintenance.

## MATERIALS AND METHODS

### Plant materials and growth condition

*Arabidopsis thaliana* Col-0 was grown in a growth chamber at 22°C-23°C with a 16-h light/8-h dark cycle on half-strength Gamborg B5 plates containing 0.5% MES-KOH, pH 5.7, and 0.75% agarose. T-DNA insertion mutant of *fbs1-1* (WISDCDSLOX485-488N17: CS858174) was obtained from ABRC. *ccs52a2-1*, *pskr1-3*, and *pWOX5:GFP* lines were provided by Prof. Lieven De Veylder group. All transgenic and double mutant plants were generated by the floral dipping method using *Agrobacterium tumefaciens* (GV3101) and genetic crossing, respectively. To generate FBS1 overexpression lines, genomic fragments of *FBS1* (AT1G61340) were cloned into 2X HA contained *pCB302ES* binary vector (Ryu et al.,

2007; 2010). For a *pFBS1-gFBS1-HA* line, the coding region of *gFBS1* with 4 kb promoter sequence was cloned into 2X HA contained *pBI121* or GUS contained *pCAMBIA1303* binary vector. All primer lists used in this study were presented in Supplementary Table S1.

### Immunoprecipitation

The plasmid DNA 35S:CCS52A2-FLAG (AT4G11920) was transformed into protoplasts with or without 35:FBS1-HA and then incubated at 20°C-23°C for 5 h under light conditions. After incubation, transformed samples were treated with MG132 and then incubated additionally for 3 h. To perform the immunoprecipitation (IP), proteins were extracted from transformed protoplasts with IP buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM EDTA, 1% TritonX-100, 1 mM DTT, 1× protease inhibitor). The extracts were centrifuged at 18,000 × *g* for 10 min at 4°C to remove cellular debris (input). Anti-FLAG magnetic bead (10 μl) was added to protein extracts followed by 5 h incubation at 4°C. After incubation with anti-FLAG, beads were washed five times to remove the non-specific binding proteins using wash buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% TritonX-100). The FLAG magnetic beads were eluted by boiling with protein sample buffer (60 mM Tris-Cl, pH 6.8, 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol, 0.1% Coomassie bromophenol blue).

### Pull-down assay

To purify the GST (glutathione-S-transferase) and GST-FBS1 protein, proteins were expressed with bacterial protein expression vector in *Escherichia coli* BL21-RIL strain which was transformed with construct *pGEX5* vector or *pGEX5-FBS1* in 400 ml LB culture, until OD<sub>600</sub> reached 0.4-0.6, 1 mM IPTG (Duchefa Biochemie, Netherlands) was added to cells and incubated at 37°C for 4 h additionally. The cells were centrifuged at 3,000 × *g* at 4°C for 10 min and resuspended with lysis buffer (100 mM Tris-HCl, 150 mM NaCl, 1 mM DTT, 1% Triton X-100, and protease inhibitor cocktail [Roche, Switzerland]) and performed sonication on ice. After sonication, the lysate was divided into a soluble fraction and pellet fraction by centrifugation at 15,000 × *g* at 4°C for 15 min. GST and GST-FBS1 were purified with Pierce™ Glutathione Agarose beads (GE Healthcare, USA) following the manufacturer's instructions. The 35S:CCS52A2-HA transformed protoplasts and incubated for 5 h under light conditions were collected by centrifugation at 1,000 × *g* for 1 min. Protein was extracted with extraction buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM EDTA, 1% TritonX-100, 1 mM DTT, 1× protease inhibitor) and centrifuged at 18,000 × *g* for 10 min, to remove the cellular debris. The protoplast extracted supernatant was incubated with purified GST or GST-FBS1 for 1 h. After incubation, beads were washed five times with wash buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% TritonX-100) to remove the nonspecific binding. After binding, GST and GST-FBS1 samples were eluted from beads by boiling with sample buffer (60 mM Tris-Cl, pH 6.8, 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol, 0.1% Coomassie bromophenol blue).

### Protoplast transient expression and Western blotting assay

The plasmid DNA *p35S:CCS52A2-FLAG* was transformed into protoplasts with or without *p35S:FBS1-HA* and then incubated at 20°C–23°C for 5 h under light conditions. After that, samples were treated with cycloheximide (CHX; Sigma, USA) with or without the MG132 (Sigma) and incubated for 3 h additionally. Before the treatment of the CHX and MG132, some samples were harvested (0 h samples) using centrifugation at 1,000 × *g* for 1 min. After 3 h of additional incubation, all samples were harvested and proteins were extracted from the harvested protoplast by boiling with protein sample buffer (60 mM Tris-Cl, pH 6.8, 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol, 0.1% Coomassie bromophenol blue).

The prepared protein samples were separated by SDS-PAGE using 10% acrylamide gel and transferred onto a polyvinylidene fluoride (PVDF) membrane. The membrane containing protein samples was incubated with 1× PBST (Phosphate-buffered saline with 0.1% (v/v) Tween 20) solution containing 6% (w/v) skim milk for 30 min. The membrane was incubated with an anti-HA-HRP antibody (1:1,000 dilution; Roche) and anti-FLAG-HRP (1:1,000 dilution; Sigma) at room temperature (RT) for 1 h 30 min. After incubation, membranes were washed three times with 1× PBST, the chemiluminescence images were developed using ECL reagents (Sigma) and ChemiDoc™ XRS+ imaging system (Bio-Rad Laboratories, USA).

### Histological analysis and Laser-scanning confocal microscopy

Histochemical staining which is modified pseudo-Schiff propidium iodide (mPS-PI) to observe the root membrane and structure. Whole seedlings were immersed in fixation solution (50% (v/v) methanol, 5% (v/v) glacial acetic acid) for over 12 h to fix the roots. After fixation, rinse briefly with water and incubated with 1% periodic acid (80% (v/v) ethanol, 1% (w/v) periodic acid) at RT for 40 min, and then rinse with water. After that, samples were incubated in Schiff reagent (100 mM sodium metabisulphite, 0.15 N HCl, 100 µg/ml PI) with propidium iodide (PI). For clearing, samples were incubated in chloral hydrate solution (15% (v/v) glycerol, 1 g/ml chloral hydrate) for 1 or 2 days at RT in dark and closed conditions. Before imaging, remove the excess chloral hydrate solution and drop the Hoyer's mounting solution (12% (w/v) gum arabic, 0.8 mg/ml chloral hydrate, 8% (v/v) glycerol). *pWOX5:GFP*, *pWOX5:GFP/35S-gFBS1* and *pWOX5:GFP/fbs1-1* plants were incubated in a 1 g/ml PI (Sigma) dissolved in water for observing GFP fluorescence and stained the cell walls using the PI staining solution for 1 to 5 min. All plant samples were observed using a laser beam at two different wavelengths, a laser 488 nm and a 495–514 nm band-pass filter for observing the GFP, and a laser 561 nm and a 581–652 nm band-pass filter for observing the PI. To observe the *FBS1* expression pattern in the root, *pFBS1-GUS* seedlings were grown on half-strength Gamborg B5 plates for 7 days and transferred to 150 mM NaCl plate for 1 h. After treatment of NaCl, plants were stained with GUS-staining buffer (100 mM Tris-HCl [pH 7.0], 2 mM ferricyanide, and 1 mM

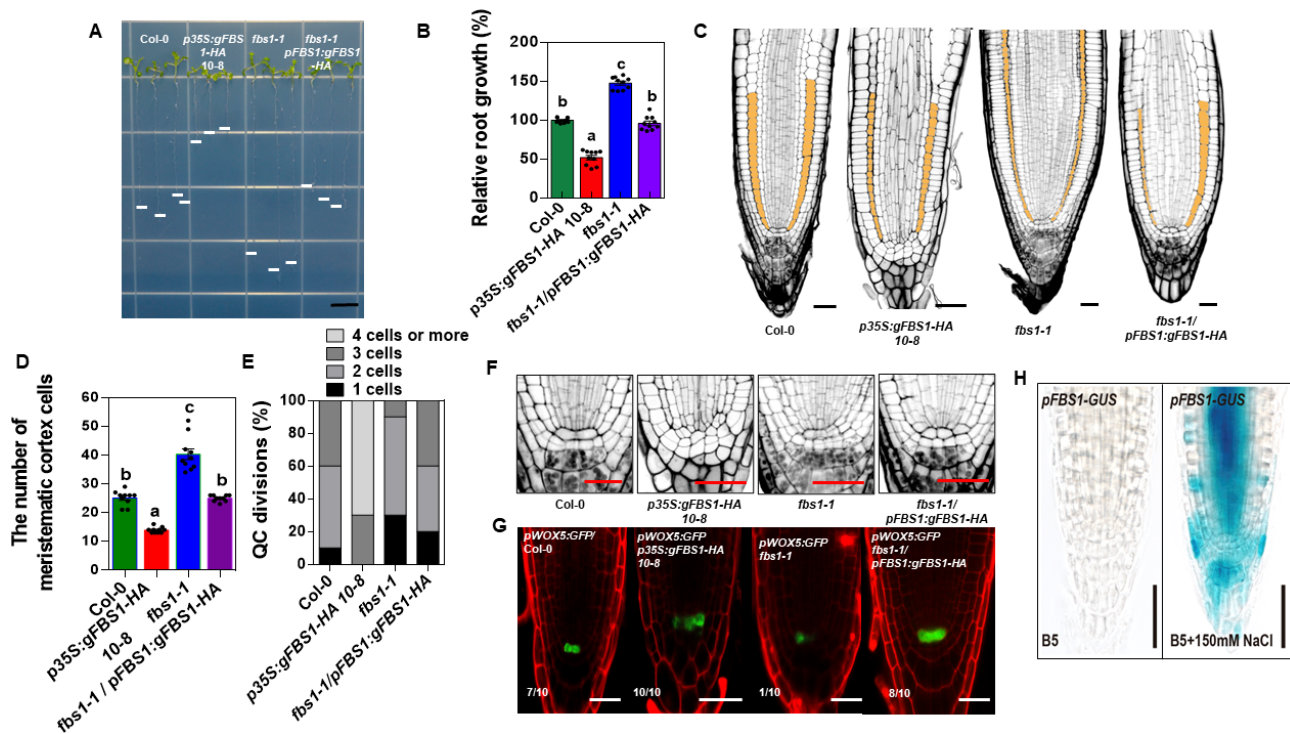
X-Gluc [5-bromo-4-chloro-3-indolyl-β-d-glucuronidase]) for 12 h at 37°C.

## RESULTS

Fine-tuning protein turnover via ubiquitin-mediated proteolysis is one of the most critical processes for the proper execution of cell signaling pathways and developmental process in eukaryotic organisms. FBS1, a member of the SCK1-CUL1-F-box (SCF)-type E3 ubiquitin ligase family, has been identified as a stress- or stress-related hormone-inducible protein (Gonzalez et al., 2017; Maldonado-Calderon et al., 2012); however, little is known about its biological role in plant growth and stress adaptation. To investigate the function of *FBS1* in plant growth and development, we initially generated transgenic lines ectopically expressing *FBS1*. Interestingly, root growth was significantly reduced in three independent *FBS1* overexpression (*p35S:FBS1*) plants compared with the wild type (WT; control). By contrast, *fbs1-1* knockout mutant plants showed greater root elongation than the WT. The root growth phenotype of *fbs1-1* plants was completely restored by expressing *FBS1* under the control of its own promoter, and root growth inhibition correlated well with the *FBS1* protein level (Figs. 1A and 1B, Supplementary Figs. S1A–S1E). To precisely compare the differences in root growth between *35S:FBS1* and *fbs1-1* plants, we stained the roots with PI and performed histological analysis by confocal laser scanning microscopy. The cortex cell division in the meristematic zone was correlated with *FBS1*-mediated root growth phenotype; compared with the WT, the *p35S:FBS1* plants showed the reduced division of meristematic cortex cells, whereas *fbs1-1* plants displayed enhanced cortex cell division, which could be restored by *FBS1* expression (Figs. 1C and D, Supplementary Figs. S1F–S1H).

We also found a link between meristematic cell division and QC cell division in *FBS1* overexpression, *fbs1-1* mutant, and *fbs1-1/pFBS1:FBS1* complementation plants. Compared with the WT, the *35S:FBS1* plants showed more frequent QC cell division and greater *pWOX5:GFP* expression, whereas *fbs1-1* plants maintained QC homeostasis and showed almost no *pWOX5:GFP* expression (except weak GFP signal in 1 out of 10 samples), which could be fully restored to WT levels by *FBS1* complementation (Figs. 1E–1G). Interestingly, these *pWOX5:GFP* expression patterns in the Col-0, *fbs1-1*, and *p35S:FBS1-HA* backgrounds were persistently maintained at days 5, 8, and 12 along with a similar root growth phenotype (Supplementary Fig. S2). Moreover, the stress-induced specific expression pattern in the RAM of *pFBS1-GUS* further validated these findings. Exogenous NaCl treatment stimulated *FBS1* expression in the QC, SCN, and vascular stele of the root, as previously reported (Maldonado-Calderon et al., 2012; Fig. 1H). These results indicate that SCF<sup>FBS1</sup> participates in root growth and development by controlling QC cell division.

Next, we investigated how SCF<sup>FBS1</sup> regulates QC cell division. *CCS52A2* is a reasonable SCF<sup>FBS1</sup>-target candidate as it plays critical roles in maintaining QC homeostasis and stress response. Consistent with this notion, the *35S:FBS1* transgenic and *ccs52a2-1* knockout mutant plants showed similar



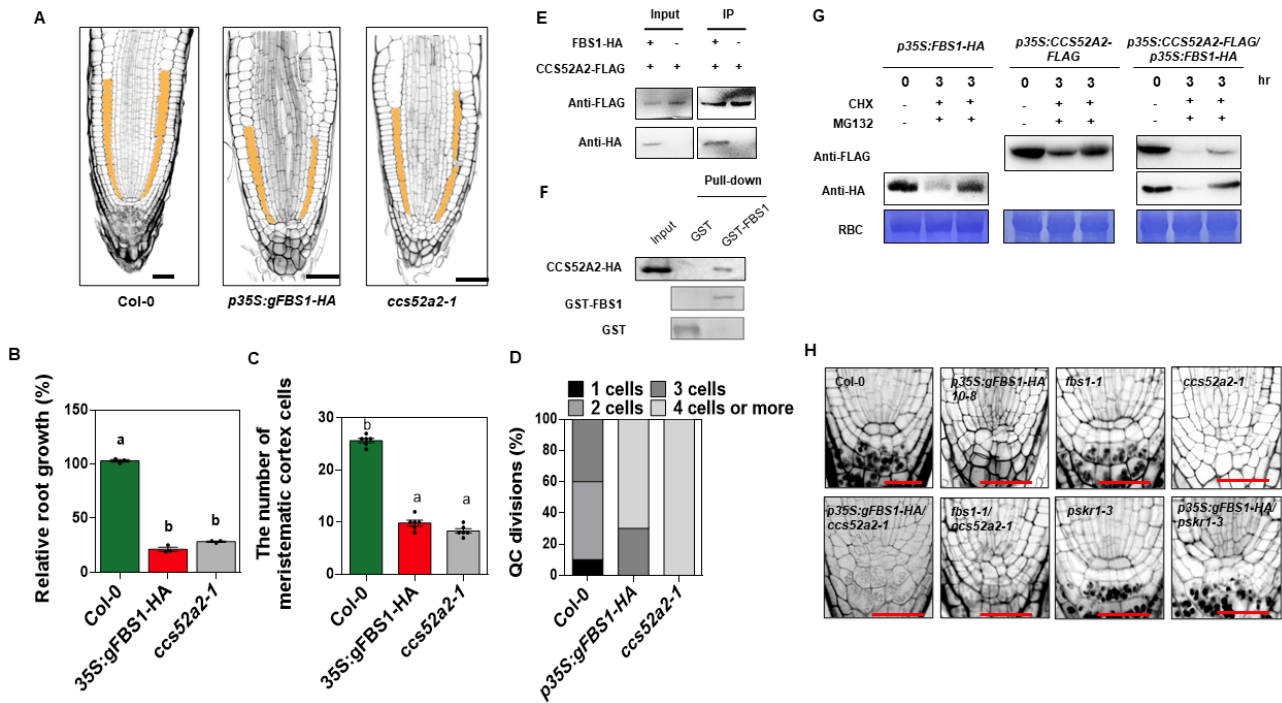
**Fig. 1. FBS1 plays a crucial role in root growth and development via the regulation of QC division.** (A) Root growth phenotype of 5-day-old plants. Scale bar = 1 cm. (B) Relative root growth rate was measured ( $n = 20$ ;  $P < 0.05$ ; one-way ANOVA with Tukey's multiple range test). (C) mPS-PI staining in 5-day-old root of Col-0, p35S:gFBS1-HA, *fbs1-1*, and *fbs1-1*/pFBS1:gFBS1-HA plants. Orange color indicates the cortex cells of proximal meristem. Scale bars = 100  $\mu$ m. (D) The cell number in meristematic cortex cells ( $n = 10$ ;  $P < 0.05$ ; one-way ANOVA with Tukey's multiple range test). (E) Quantification of QC cell divisions ( $n = 50$ ). (F) Cell organization phenotypes of stem cell niche and QC. Scale bars = 100  $\mu$ m. (G) Confocal images showing QC with expression of pWOX5:GFP in 5-day-old roots. Numbers represent GFP expressed samples out of 10 tests. Scale bars = 100  $\mu$ m. (H) FBS1 is rapidly induced in the SCN and vascular stele in roots by salt stress. Expression of pFBS1:GUS in the root of 7-day-old plants with or without 150 mM NaCl. Scale bars = 100  $\mu$ m.

root growth phenotypes, with robust QC proliferation and low meristematic cell division (Figs. 2A-2D). Furthermore, results of the co-IP assay indicated that FBS1 directly interacts with CCS52A2 (Fig. 2E); this finding was confirmed by a pull-down assay conducted using GST-tagged FBS1 and CCS52A2 (Fig. 2F). To learn more about how SCF<sup>FBS1</sup> regulates CCS52A2, we conducted a protein degradation assay using a protoplast transient expression system. The FBS1 protein was rapidly degraded in the presence of cycloheximide, consistent with a previous study; however, FBS1 proteolysis was inhibited upon treatment with MG132, a 26S proteasome inhibitor (Fig. 2G, left). The level of CCS52A2 was regulated by the 26S proteasome-mediated protein degradation process in a similar manner (Figs. 2G [middle] and 2H). However, in the co-expression of FBS1, the rate of CCS52A2 degradation increased, which was partially inhibited by MG132 (Fig. 2G [right], Supplementary Fig. S3). These findings suggest that SCF<sup>FBS1</sup> controls QC homeostasis by regulating CCS52A2 abundance. To confirm the genetic interaction of FBS1 with CCS52A2-ERF115-PSKR1 signaling pathway, we next investigated the QC cell division phenotypes in 35S-FBS1/ccs52a2-1 or pskr1-3 double mutants, as well as *fbs1-1*/ccs52a2-1 double mutants. The QC proliferation of 35S-FBS1 plants was almost completely recovered by *pskr1-3*, but *ccs52a2-1* was

not repaired by either 35S-FBS1 or *fbs1-1* (Fig. 2H, Supplementary Fig. S4). These findings imply that SCF<sup>FBS1</sup> controls QC homeostasis through regulating CCS52A2 protein turnover and acting as epistatic to the CCS52A2-ERF115-PSKR1 signaling pathway. Moreover, given that environmental stress signals play critical roles in the homeostatic regulation of the QC and SCN, the stress-inducible SCF<sup>FBS1</sup> would serve as a key mediator of stress signaling-controlled plant meristem tissue organization.

## DISCUSSION

Over the past few decades, considerable research has been conducted to understand the mechanisms controlling QC cell division necessary for proper plant root growth and development. The maintenance of QC homeostasis has been identified as a critical mechanism that regulates root organization by preserving the stem cell fate and pool (Heyman et al., 2013; 2014; Vanstraelen et al., 2009). Recent studies show that stress-related plant hormones and chilling-, wounding-, and DNA breakage-induced stem cell damage can increase the QC cell division rate, allowing for the replenishment of damaged stem cells surrounding the QC. Notably, the modulation of ERF115 transcriptional activity by JA, ERF109, and



**Fig. 2. SCF<sup>FBS1</sup> regulates QC quiescence through CCS52A2 protein degradation.** (A) mPS-PI staining in 5-day-old roots of Col-0, *p35S:gFBS1-HA*, and *ccs52a2-1*. Scale bars = 100  $\mu$ m. Orange color indicates the cortex cells of proximal meristem. (B) Relative root growth (n = 10), (C) the number of meristematic cortex cells (n = 10), and (D) QC divisions were measured (n = 50).  $P < 0.05$ ; one-way ANOVA with Tukey's multiple range test. (E) Protein-protein interaction between FBS1 and CCS52A2 by a co-IP assay. FBS1-HA and CCS52A2-FLAG were co-expressed in protoplasts. IP with anti-FLAG antibody-tagged magnetic beads and detection with anti-HA antibody. (F) Pull-down assay shows the direct interaction between FBS1 and CCS52A2. CCS52A2-HA was expressed in protoplasts and pull-downed with 1  $\mu$ g of GST or GST-FBS1 proteins. Anti-HA antibody was used for the CCS52A2-HA protein detection. (G) FBS1 promotes 26S proteasome-mediated CCS52A2 proteolysis. *p35S:FBS1-HA*, *p35S:CCS52A2-FLAG* with or without *p35S:FBS1-HA* were transformed into protoplasts and incubated 5 hrs. The transfected protoplasts were further incubated with or without MG132 for 3hr in the presence of cycloheximide. The protein expression levels were determined with anti-HA and anti-FLAG antibodies. Rubisco large subunit proteins were used as an internal loading control. (H) QC division phenotypes of Col-0, *p35S:gFBS1-HA*, *fbs1-1*, *ccs52a2-1*, *p35S:gFBS1-HA/ccs52a2-1*, *fbs1-1/ccs52a2-1*, *pskr1-3*, and *p35S:gFBS1-HA/pskr1-3* plants with mPS-PI staining. Scale bars = 100  $\mu$ m.

APC/C<sup>CCS52A2</sup> in the QC and SCN is a major mechanism for the proper regeneration of damaged stem cells and root tissues. (Aichinger et al., 2012; Chen et al., 2011; Cruz-Ramirez et al., 2013; Gonzalez-Garcia et al., 2011; Heyman et al., 2013; Hong et al., 2017; Zhang et al., 2013). Local auxin maxima and BR signaling activation in the SCN are also critically linked to the root regeneration network (Blilou et al., 2005; Gonzalez-Garcia et al., 2011; Grieneisen et al., 2007; Sabatini et al., 1999; Zhou et al., 2019). These findings suggest that stress-induced tissue damage can directly stimulate tissue regeneration by increasing QC cell division, thus contributing to developmental plasticity in response to environmental changes. Although several previous key findings indicate that QC homeostasis is closely linked to environmental stresses via the APC/C<sup>CCS52A2</sup>-ERF115-PSKR1 signaling module (Heyman et al., 2013; Hong et al., 2017; Zhou et al., 2019), the factor that primarily controls APC/C<sup>CCS52A2</sup> for the maintenance of QC cell division and homeostasis remains unknown. In this study, we report that SCF<sup>FBS1</sup> acts as a core signaling regulator of QC homeostasis by directly regulating the CCS52A2 protein turnover. Furthermore, the stress- and stress-related

hormone-inducible nature of *FBS1* expression leads to the assumption that *FBS1* is closely involved in the stress-mediated modulation of plant developmental plasticity. *FBS1* also plays important roles in the formation of JA- and stress signaling-mediated transcriptional networks (Gagne et al., 2002; Maldonado-Calderon et al., 2012). The recent finding that JA plays a crucial role in the plant defense response and post-wounding tissue regeneration through ERF115 activity is a major breakthrough (Zhou et al., 2019). Together, these data suggest that proper *FBS1* activity is required for regulating the JA-mediated tradeoff between plant defense and tissue repair. However, how the stress-inducible *FBS1* and its role in post-translational control of a wide range of proteolytic fates of unidentified targets are integrated into the regulation of plant developmental plasticity remains unclear. This understanding will provide insights into how plants have successfully learned to cope with the unpredictable environmental fluctuations and overcome the limitations imposed by their sessile lifestyle.

One of the most intriguing discoveries of this study is the contrasting primary root growth rate and meristem cell

mitotic activity phenotypes of *35S:FBS1* and *fsb1-1* plants. In *35S:FBS1* overexpression plants, a larger QC region with enhanced *pWOX5* activity and many QC cell divisions resulted in decreased root growth and low mitotic activity of meristematic cells. Conversely, the *fsb1-1* knockout mutant showed almost no *pWOX5-GFP* expression but enhanced primary root growth and high meristematic cortical cell division rate (Supplementary Fig. S2). These phenotypes imply that increased signaling activity for the QC proliferation could antagonistically decrease cell proliferation in the SCN and meristematic zone during primary root growth. Any environmental stress can reduce root growth and development, and several stress responses contribute directly to not only breaking the homeostatic state of the QC but also activating *FBS1* expression. This notion is further supported by the inhibitory effects of stress-related hormones, such as JA and ethylene, on root growth and QC cell division. Together, these data suggest that meristem tissue organization and homeostasis may be regulated by an-as-yet unidentified antagonistic regulatory machinery or non-cell autonomous signaling pathway via a feedback inhibitory loop that controls the cell cycle in the SCN and its surrounding QC. This precise regulation could serve as a general mechanism for maintaining a balance between the development and environmental adaptation of plants under stress conditions. Additional in-depth studies are needed to understand how stress-induced *FBS1* expression modulation and other post-translational modifications affect the balance between stem cell division and differentiation.

Note: Supplementary information is available on the Molecules and Cells website ([www.molcells.org](http://www.molcells.org)).

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## AUTHOR CONTRIBUTIONS

H.R. designed the experiments and supervised this study. K.R.G. and H.K. carried out all experiments. K.R.G. and H.R. analyzed all data and wrote the manuscript.

## CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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