AtF-box gene expression fine-tunes Arabidopsis thaliana root development

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Abstract: Root growth is under constant dynamic regulation for optimal response to developmental and environmental stimuli. At the posttranslational level, protein abundance is controlled by proteasomal degradation of targeted proteins. The substrate-specificity of this process is exerted by F-box proteins taking part in the SCFs (SKP1-CULLIN-F-box protein ligase) E3 ubiquitin protein ligases. In this work an Arabidopsis thaliana AtF-box, which regulates leucine homeostasis, was analyzed in the context of root development. Publicly available data sets and reporter lines revealed AtF-box expression in the primary and lateral roots. Aberrant stem cell divisions were detected in the distal stem cells (DSC) of the AtF-box knockdown lines (AtF-boxamiRNA), suggesting that AtF-box is required for the optimal cell division. Microscopic observations revealed the premature exit from cell proliferation and slower cell division activity. Conversely, in AtF-box overexpression (AtF-boxOE) lines the cell division phase was prolonged. The root growth rate was respectively reduced and enhanced in the AtF-boxamiRNA and AtF-boxOE lines compared to the control. From the results of these studies, we concluded that the AtF-box gene is important for the fine-tuning of root growth.

Keywords: Arabidopsis thaliana; cell division; F-box gene; meristem; primary root; root growth rate

Abbreviations: DAS, days after sowing; DSC, distal stem cells; PD, proliferation domain of the root meristem; QC, quiescent center; TD, transition domain of the root meristem; VisuaLRTC, Visual Lateral Root Transcriptome Compendium; XPP, xylem pole pericycle

Introduction

The well-established reference organism Arabidopsis thaliana has opened a new era in root developmental studies, especially through the identification of many mutants and genes involved. The achievements of the A. thaliana root community have led to a broader understanding of fundamental aspects of root biology, such as meristem organization, gravitropic response, root branching and the response of roots toward their environment (Santosh et al. 2015; Motte et al. 2019). Root growth is predominantly driven by cell proliferation in its meristem that holds the stem cells being regulated by interconnected signaling pathways. On the other hand, little information is available about the regulatory networks that control root development in response to environmental cues (Osment et al. 2007).

Posttranscriptional control represents an important part of the plant’s response to internal and external environmental changes. A key posttranslational control system comprises protein degradation by the 26S proteasome (Vierstra 2009). Plant F-box proteins are part of enzymatic complexes taking part in targeted ubiquitination of proteins, thereby marking them for subsequent degradation by the proteasome (Lechner et al. 2006). The principal function of the F-box proteins is to...
provide substrate specificity of the SCFs (SKP1-CULLIN-F-box protein ligase) E3 ubiquitin protein ligases in the process of protein turnover (Xu et al. 2009). Previous reports implicated a role for F-box proteins in defense response (Kim and Delaney 2002), drought and salt tolerance (Bu et al. 2014; Zhao et al. 2017), hormone signaling (Li et al. 2016; Williams et al. 2019), and plant secondary metabolism (Zhang et al. 2013; Zhang et al. 2017). Developmental processes controlled by F-box genes include control of organ growth (Gonzalez-Carranza et al. 2007), pollen development (Gusti et al. 2009), floral development (Song et al. 2012), leaf size control (Baute et al. 2017), and seed germination (Majee et al. 2018). Research on F-box proteins has been performed in different plant species, such as rice (Jain et al. 2007), maize (Jia et al. 2013), tobacco (Bu et al. 2014), Medicago truncatula (Iantcheva et al. 2015; Song et al. 2015) and soybean (Jia et al. 2017), however, most information was achieved by studies on A. thaliana. It is expected that the data from A. thaliana can be extended to crop species to optimize productivity (Boycheva et al. 2015; Iantcheva et al. 2021).

The present work is focused on A. thaliana F-box gene (AtF-box; AT1G10780) which is an ortholog of the M. truncatula F-box gene (MtF-box; MT2G007220), that was previously identified in Tnt1 retrotransposon M. truncatula mutant collection (Revalska et al. 2011). In a study performed on the legume model plant M. truncatula, Iantcheva et al. (2015) revealed the association of a gene encoding a novel F-box protein with the cell division cycle. In the same report, based on an established M. truncatula root tip synchronization system, the expression of the investigated MtF-box gene was linked to the DNA replication phase of the mitotic cycle. Moreover, the detailed analysis of pMtF-box promoter activity using reporter lines demonstrated specific expression in young leaves, petiole, sepal, and primary roots, while no expression was detected in the mature nitrogen fixing nodules (Boycheva et al. 2015). Likewise, the pMtF-box promoter was shown to be functional in heterologous systems as A. thaliana (being active in the dividing tissues of leaf, root and flower) and Lotus japonicus (showing expression during somatic embryogenesis, in the root and mature nodules). Upon heterologous expression of the MtF-box gene in A. thaliana transgenic lines, modulations were observed in root and hypocotyl growth, endoreplication levels in leaf cells (Boycheva et al. 2015). Iantcheva et al. (2021) dissected the role of MtF-box during leaf development of M. truncatula. In the same study, it was shown that leucine content was increased and decreased in the knockdown (AtF-boxRNAi and MtF-boxRNAi) and overexpression (AtF-boxOE and MtF-boxOE) lines, respectively. Based on this research, a conserved role of the F-box gene in regulation of leucine homeostasis was highlighted, and the specific effect of the F-box gene on the transition from cell division cycle to differentiation was demonstrated (Iantcheva et al. 2021).

In the present work, we extended our studies towards unraveling the role of the A. thaliana AtF-box in root development. Transgenic plants with modified AtF-box expression were analyzed in comparison to the control. First, information about the endogenous AtF-box expression in roots was collected from the publicly available data sets. In a following step, phenotypic analyses of AtF-box control and transgenic lines were performed at the microscopic level in young root apical meristems in respect to cell division effects. Moreover, root growth rates of the transgenic lines were estimated in a five-day period.

Materials and Methods

Description of online tools for data sets analyses

The eFP Browser (electronic Fluorescent Pictograph) is a tool, available at http://www.bar.utoronto.ca/ to facilitate the interpretation and analysis of microarray data and data from other large-scale data sets (Winter et al. 2007; Klepikova et al. 2016). This eFP Browser engine paints data onto pictographic representations of the experimental samples for data visualization. The ePlant (http://bar.utoronto.ca/eplant) is a visual analytic tool for exploring multiple levels of A. thaliana data through a zoomable user interface (Waese and Provart 2017). The Visual Lateral Root Transcriptome Compendium (VisuaLRTC) is a spreadsheet application which combines several publicly available data sets and can be used for any biological process although the focus is on lateral root development (Parizot et al. 2010).

Plant material and growth conditions

The A. thaliana AtF-box gene is annotated under the AT1G10780 identification number and possesses an F-box domain of approximately 34 aa. Gene family search in the PLAZA database (Vandepoele et al. 2013; Van Bel et al. 2017) revealed only one gene present in the genome of A. thaliana, as well as in M. truncatula (Fig. S1). According to the classification of F-box proteins in A. thaliana, the investigated F-box gene is categorized in group of Others/Unknown (Kuroda et al. 2002; Xu et al. 2009).
**A. thaliana** (L.) Heynh. ecotype Columbia-0 (Col-0) was used for generation of transgenic lines as described in Iantcheva et al. (2021). Transgenic plants with inactivated function of the gene encoding AtF-box-protein were constructed via method of artificial microRNAs (AtF-box<sup>amiRNA</sup>, Fig. S2A). Synthetic small RNAs (microRNAs (amiRNAs)) are 21 bp small RNAs that can be designed and operated by specifically inactivating one or several genes of interest (Schwab et al. 2006). In the database of the site listed below the sequence of the test gene, that should be inactivated, is sent (in Fasta format), and a plurality of 21 bp amiRNAs sequences are generated and are obtained in the form of an Excel file. The artificial microRNA design WMD program (http://wmd3.weigelworld.org) sends the sequences of a large number of gene attenuation suggestions, with one or two mismatches (Fig. S2A). The chosen amiRNA was subjected to additional BLAST search confirming its specificity (Fig. S2B).

The overexpression of AtF-box in transgenic lines was under the control of *Cauliflower Mosaic Virus* (CaMV) 35S promoter and C-terminal translational green fluorescent protein /GFP/ fusion to the open reading frame (p35S:AtF-box-GFP, AtF-box<sup>OE</sup>). Homozygous lines T<sub>1</sub> were planted on half-strength MS medium and used for analyses. The sterilized seeds were incubated in the dark at 4°C for 48 h and transferred to light. Plants were grown under controlled growth conditions (at 21°C, continuous light, 100 µmol m<sup>-2</sup> s<sup>-1</sup> photosynthetically active radiation, 55% humidity) on vertical plates containing ½ MS medium supplemented with 0.8% plant tissue culture agar (LAB M Ltd, Heywood, UK) and 1% sucrose.

**Root phenotypic analyses of A. thaliana transgenic plants with modified AtF-box expression**

T<sub>1</sub> homozygous AtF-box transgenic lines and *A. thaliana* control plants were examined for root phenotypes. For observation of cells in the root meristem, the root cell walls were stained with 10 µM propidium iodide (PI) for 3 min and imaged under an Axiovert100M confocal laser scanning microscope with LSM510 software package version 3.2 (Zeiss) using epifluorescent light with the appropriate filter set (excitation filter, 535-550 nm; dichroic mirror, 565 nm; barrier filter, 590 nm). GFP fluorescence at 5 and 9 days after sowing (DAS) was detected with a 500 nm to 550 nm band-pass emission filter. The microscopic observations in the proliferation domain (PD) and transition domain (TD) zones were performed at 5 DAS. These regions were defined according to Verbelen et al. (2006) and Garcia-Gómez et al. (2017). The PD zone was defined as the region of isodiametric cortex cells from the quiescent center (QC) up to the cell that was twice the length of the immediately preceding cell. The following TD zone was measured up to the cell that was more than three times the length of the first TD cell. The number of cells in the PD and TD was counted, and the respective average cell length was calculated by dividing the zone length to the cell number. Roots of seedlings at 5 DAS were scanned and root length measured with ImageJ v1.4.3 software. The length of the primary root was measured at 5 DAS and root growth rate was recorded every 24 h for further 5 days.

**Statistical analyses**

Data represent the mean ± SE and in average three independent lines per transgenic plant were analyzed with n indicated in the respective figure legend. The One-way ANOVA (Holm-Sidak) and Student (t-test) statistical tests were applied to estimate the difference between all the variants (*P* < 0.05 was considered statistically significant).

**Results and Discussion**

*AtF-box* expression is enhanced in dividing cells in root

Based on publicly available data sets, we collected information about the endogenous *AtF-box* root expression pattern (Table 1; Fig. 1). The VisuaLRTC combines data sets involved in *A. thaliana* lateral root development and links them with additional information on tissue-specific expression and cell cycle involvement, thus allowing the extraction of novel information from existing data sets in a visual and user-friendly manner (Parizot et al. 2010; Table 1). According to VisuaLRTC, *AtF-box* is expressed preferentially in the xylem pole pericycle (XPP) cells that are primed to form lateral roots (De Smet et al. 2007). In the XPP cells, local auxin accumulation triggers the initiation of lateral roots (Benková et al. 2003) through an auxin signaling cascade that leads to the first anticlinal asymmetric division of two neighboring XPP cells. This result is in accordance with experiments performed on the primary root, mapping spatiotemporal gene expression data, where *AtF-box* transcripts were detected in developing xylem (protoxylem and 2/3 metaxylem designated as S4 by Brady et al. 2007). The *A. thaliana* eFP Browser offers respective visualization of the *AtF-box* endogenous expression in primary root that is upregulated in developing xylem tissue at the root tip (Fig. 1B). As well, in 7-day-old *A.
thaliana seedlings, the heterologous MtF-box promoter activity was observed in the vasculature of primary roots and in the lateral root primordia and root tips (Boycheva et al. 2015).

De Smet et al. (2007) studied asymmetric cell division using a lateral root initiation system (LRIS; Himanen et al. 2004). Upon addition of an auxin transport inhibitor, lateral root formation was prevented which was rescued by application of the auxin NAA (naphthaleneacetic acid). The

| AtF-box | Tissue-specific expression in root | Asymmetric Division | Cell division cycle phase |
|---------|-----------------------------------|--------------------|--------------------------|
| AT1G10780 | Significantly differentially expressed | XPP-PPP | XPP-P | PPP-P | Radial Layers | 0-2 | 2-6 | 0-6 | Cell cycle phase | Max fold change |
| Fold change: | X’ | 4.21 | 1.33 | -3.16 | S4” | 1.02 | 18.19 | 18.62 | M*** | 3.73 |
| P-values | 3.4E-05 | 0.08 | 0.000129 | 0.91 | 0.00011 | 7.1E-05 |

* Parizot et al. (2010);  † Brady et al. (2007);  ‡ De Smet et al. (2007);  § Menges et al. (2003)

X - preferential expression in the Xylem Pole Pericycle (X)
S4 - protoxylem and 2/3 metaxylem
M - mitotic phase of the cell division cycle
XPP-PPP - Fold change between the Xylem Pole Pericycle and the Phloem Pole Pericycle
XPP-P - Fold change between the Xylem Pole Pericycle and the Pericycle
PPP-P - Fold change between the Phloem Pole Pericycle and the Pericycle
0-2; 2-6; 0-6 - hours after NPA-mediated inhibition of lateral root formation followed by application of the auxin NAA for lateral root initiation (LRI/NAA).

0-2 - Fold change from 0h (NPA) to 2 hours NAA treatment;
2-6 - Fold change from 2h NAA to 6h NAA treatment;
0-6 - Fold change from 0h NAA to 6h NAA treatment

P-values below 0.05 are statistically significant

Fig. 1. Root eFP browser representation of root localization of AtF-box. (A) View of the whole primary root. [Link to visual dataset](http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi?primaryGene=AT1G10780&dataSource=Root&modeInput=Absolute) (B) Magnification of the root tip. Red coloration indicates abundance of the AtF-box transcript in Xylem Pole Pericycle (XPP).
A combination of LRIS with cell sorting of the XPP allowed to zoom in on cell-specific events related to the process of asymmetric cell division that is crucial for proper lateral root initiation (De Smet et al. 2007; Parizot et al. 2010). *AtF-box* was found to be expressed between 2 to 6 hours after auxin application and its expression increased by nearly 20-fold, corresponding to cell division activation (Table 1). In support, *AtF-box* was found to be specifically expressed during mitosis in synchronized cell cultures (Table 1; Menges et al. 2003; Parizot et al. 2010). These data obtained by search in the available databases are supported by Boycheva et al. (2015) showing that the *M. truncatula* MtF-box promoter is active in proliferating cells in roots of *M. truncatula*, *L. japonicus* and *A. thaliana*. Iantcheva et al. (2021) further confirmed G2/M-specific endogenous expression of *AtF-box* in synchronized *A. thaliana* root tip cells, which correlated with expression of the G2/M-specific marker genes CYCLIN A1;1 (CYCA1;1), CYCLIN DEPENDENT KINASE B1;1 (CDKB1;1) and CYCB1;1.

*AtF-box* is required for the cellular organization in the stem cell niche of the root

To study the role of the *AtF-box* gene on *A. thaliana* root development, *AtF-boxOE* and *AtF-boxamiRNA* plants were constructed and the expression profiles of these transgenic lines were presented in our previous paper (Iantcheva et al. 2021). The selected transgenic lines were used in all the analyses in this study. In the *AtF-boxOE* lines the coding sequence of the *AtF-box* gene was in translational fusion with the GFP reporter (p35S:AtF-box-GFP) (Fig. S3). Our next step was to check the role of *AtF-box* in the root growth at the cellular level. Root growth and development in plants is supported by a group of cells called stem cells that form a stem cell niche in the root apical meristem and divide asymmetrically. These stem cells maintain a balance between cell division and differentiation and give rise to different tissues in the root, thereby ensuring its continuing growth (Bennett and Scheres 2010). At the center of the stem cell niche are 4 to 8 cells that form the QC which is responsible for the fate of the adjacent stem cells (Van den Berg et al. 1997). The QC cells are of regular shape, undifferentiated and at rest. Microscopic analyses on roots revealed that in the control and *AtF-boxOE* lines were observed 4 QC cells with regular arrangement of DSC (distal stem cells) (Fig. 3). Downregulation of *AtF-box* in the *AtF-boxamiRNA* lines resulted in QC cells and DSC that were chaotically arranged likely caused by unusual aberrant cell division pattern (Fig. 2).

*AtF-box* fine-tunes the exit from cell division to maintain the root growth rate

The root consists of roughly three longitudinal zones: the meristematic, elongation, and differentiation zones (Motte et al. 2019). In the meristematic zone (meristem), all cell types are derived from one or more stem cell precursors located at the stem cell niche. As stem cells divide, they generate tissue-specific transit-amplifying cells, which in turn divide anticlinally to produce the bulk of cells constituting the meristem. These dividing cells form the proliferation domain (PD) followed by a transition domain (TD), where cells diminish their division rate before transiting to the elongation zone, followed by final differentiation (Verbelen et al. 2006; García-Gómez et al. 2017). The observed atypical shape and cellular activity of the QC and DSC cells in lines with inactivated *AtF-box* expression correlated with a shorter primary root length at 5 DAS (Fig. 3A). Therefore, we performed a more detailed analysis in PD and TD of the root apical meristem (Fig. 3B and C). The PD was reduced in both *AtF-boxOE* and *AtF-boxamiRNA* roots. In *AtF-boxOE*, the shorter PD correlated with smaller

![Fig. 2. Effect of modified *AtF-box* level on cell division in root tips of *A. thaliana* (*AtF-boxOE*, *AtF-boxamiRNA*, and control). Magnification of the root tip cells around QC are shown. Asterisks indicate aberrant cell divisions.](image-url)
Fig. 3. Effect of modified AtF-box level on root development in *A. thaliana* plants (AtF-boxOE, AtF-boxamiRNA, and control). (A) Root length at 5 DAS. (B) Root apical meristem stained with propidium iodide and imaged by confocal microscope. Arrowheads indicate QC, cortex proliferation domain (PD; cells are highlighted with white dots) and cortex transition domain (TD; cells are highlighted with grey dots). (C) Measurements of PD and TD (length, cell size and number). In (A) and (C) data represent the mean ± SE (*n* =60 and *n* =12 with 3 independent transgenic lines, respectively). The One-way ANOVA (Holm-Sidak) statistical test is applied to estimate the difference between all the variants. Different letters denote statistically significant differences. (D) Root growth in *A. thaliana* transgenic lines with modified F-box level AtF-boxOE, AtF-boxamiRNA and control. Mean values ± SE are shown (*n* =25 with 3 independent transgenic lines). Statistical difference compared to the control was estimated by Student *t*-test for AtF-boxOE and AtF-boxamiRNA, respectively, for primary root growth after 5 DAS, i.e. after: 24 h (*P* < 0.005 and *P* < 0.05), 48 h (*P* < 0.05 and *P* < 0.001), 72 h (*P* < 0.005 and *P* < 0.001), 96 h (*P* < 0.01 and *P* > 0.05), 120 h (*P* > 0.05 and *P* < 0.05).
cell size, while in AtF-box\textsuperscript{amiRNA} this was result of lower cell number. Upon entering the root TD, the cells of the control started to expand. In comparison, the TD of AtF-box\textsuperscript{OE} and AtF-box\textsuperscript{amiRNA} roots was significantly increased in length which was due to increase in cell number rather than change in cell size compared to the control. These observations hinted that AtF-box is required for cell division in the root meristem and an optimal gene expression controls the exit towards the transition to cell elongation.

The rate of cell proliferation and subsequent elongation propels the root stem cell niche forward through the soil and defines the rate of root growth (Motte et al. 2019). To trace the root growth rate after 5 DAS, the primary root length was determined and root growth rate was recorded after 24, 48, 72, 96, and 120 h (Fig. 3D). The delay in root growth of AtF-box\textsuperscript{amiRNA} lines was maintained during the first 24 h up to 48 h and 72 h, and persisted at 120 h. At 5 DAS, the average root length of AtF-box\textsuperscript{OE} did not differ significantly from the control (Fig. 3A). The root growth of AtF-box\textsuperscript{OE} lines was comparable to the control or higher for the first 48 h and increased significantly at 72 and 96 h (Fig. 3D). Based on these data, it could be assumed that knockdown of AtF-box causes defects in the root meristem that lead to premature exit from proliferation and slower progression towards cell elongation, resulting in a reduced root growth. In opposite, AtF-box overexpression appeared to provoke prolonged cell division activity in PD and TD of the root meristem, which resulted in an enhanced root growth rate compared to wild type plants. In support, Iantcheva et al. (2021) demonstrated in synchronized roots that at the onset of the G2/M checkpoint of the cell division cycle, when cells could exit proliferation, the transcript level of G2/M marker genes were higher in the AtF-box\textsuperscript{amiRNA} line compared to the control which suggests arrest at the G2 point of the cell division progression. On the contrary, in the AtF-box\textsuperscript{OE} line the level of the G2/M markers was lower than in the control prompting to higher cell division activity that is supported by enhanced root growth rate.

**Hypothetical model about AtF-box role in root development**

In our previous study focused on the F-box gene (Iantcheva et al. 2021), we demonstrated that the abundance of the F-box gene correlated in an opposite manner with the level of the amino acid leucine in M. truncatula and A. thaliana. Leucine-related amino acids have been shown to have a negative effect on root growth by feed-back inhibition of enzyme catalyzing key step of the biosynthetic pathway (Chen et al. 2010), which is in corroborated with the shorter root and slower growth rate of AtF-box\textsuperscript{amiRNA} lines (with higher leucine content as shown by Iantcheva et al. 2021). According to Yu et al. (2013), deficiency of isoleucine also impairs root development which suggest that both, strong feed-back control and lack
of amino acids could inhibit root growth. The level of another amino acid, threonine, was found to be a limiting factor for root apical meristem maintenance in roots of *A. thaliana* (Reyes-Hernández et al. 2019). A recent report demonstrated that several amino acids, including leucine, are perceived at the root apex and repress *A. thaliana* primary root growth by inhibition of both cell division and elongation (Ravelo-Ortega et al. 2021). The authors revealed that leucine and few other amino acids diminish the auxin response around the stem cell niche and columella and antagonize auxin flux via reduced expression of the PIN auxin transporter proteins. In addition, they appointed a role for the mitogen-activated protein kinase MPK6 in coordinating the signaling of different amino acids in root cells. In agreement with these results, in our study the lack of *AtF-box*, had different impacts on cell division – at the stem cell niche asymmetric cell divisions were observed, while in the PD and TD premature proliferation exit and slower cell division progression occurred. In *AtF-boxOE* roots, we hypothesize that the increased *AtF-box* expression (with lowered leucine level) leads to respective feed-back suppression, which promoted cell division progression in the root apical meristem and enhanced root growth rate. Based on the overall data, we suggest that *AtF-box* is required to prevent cell division in the stem cell niche, while it promotes proliferation in PD (Fig. 4).

**Conclusion**

In the present study, the *AtF-box* gene was shown to be an important player in early root development. Expression analyses in published data sets and in the analyzed *AtF-boxOE* lines with AtF-box-GFP demonstrated that *AtF-box* is expressed in the primary and lateral roots. The diminished *AtF-box* expression caused defects in DSC near the QC in *AtF-box*<sup>RNAi</sup> root tips. This malfunction in the cell division pattern correlated with premature exit from proliferation and slower cell division progression in the root meristem. As an overall result of *AtF-box* inactivation, root growth was reduced. In *AtF-boxOE* lines, the root meristem cells retained their cell division activity for a prolonged time and, subsequently, root growth rate was enhanced. Since the F-box was previously shown to have conserved functions in *A. thaliana* and *M. truncatula*, the results in the present study could be applied to crops of economic importance to monitor the F-box expression and respective root growth for improved agronomic traits (yield and sustainability to stress factors).

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Fig. S1. Phylogenetic tree of F-box (MT2G007220 and AT1G10780) in PLAZA 3.0. Taxonomic abbreviations in order of appearance: Brassica rapa (bra), Thellungiella parvula (tpa), Capsella rubella (cru), Arabidopsis lyrata (aly), Arabidopsis thaliana (ath), Beta vulgaris (bvu), Vitis vinifera (vvi), Malus domestica (mdo), Prunus persica, Fragaria vesca (fve), Citrullus lanatus (cla), Cucumis melo (cme), Theobroma cacao (tca), Gossypium raimondii (gra), Carica papaya (cpa), Citrus sinensis (csi), Populus trichocarpa (ptr), Ricinus communis (rco), Manihot esculenta (mes), Eucalyptus grandis (egr), Glycine max (gma), Medicago truncatula (mtr), Solanum lycopersicum (sly), Solanum tuberosum (stu), Amborella trichopoda (atr), Oryza sativa ssp. japonica (osa), Zea mays (zma).
Fig. S2. Target of amiRNA in *AtF-box*transgenic lines. (A) *AtF-box* gene model and target position of the amiRNA sequence (in red). Asterisks point mismatches between the amiRNA fragment and the *AtF-box* gene. (B) BLAST results after search with the targeted *AtF-box* fragment by choosing options: Standard databases ‘Reference RNA sequences’; Organism ‘Arabidopsis thaliana (taxid:3702)’; Optimized for ‘Highly similar sequences’. Box in (B) highlights significant E-value.
Fig. S3. AtF-box localization in roots of AtF-boxOE plants. (A-F) Confocal microscopy images in root at 5 and 9 DAS. (A) Expression in root tip at 5 DAS. (B) Magnification of (A) indicating the QC and temporal accumulation of AtF-box in one of the QC cells. (C-F) Expression in root at 9 DAS with pointing arrows: root tip (C); localization in root cell cytoplasm at the transition domain (TD) (D); root hair at the differentiation zone (E); emerging lateral root in the differentiation zone and cytosol inclusions pointed with arrows (F). Red – propidium iodide (PI) staining, green – GFP fluorescent signal, yellow – merge of PI and GFP. Bars=100 µm.

Fig. S4. eFP Browser representation of single cell RNA-Seq data of AtF-box endogenous expression in QC cells and dividing meristem cells. Red rectangular is used for highlighting. (http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi?dataSource=Single_Cell)