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An essential role for *Arabidopsis* Trs33 in cell growth and organization in plant apical meristems

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Key Message

Trafficking protein particle (TRAPP) complexes subunit gene *AtTrs33* plays an important role in keeping apical meristematic activity and dominance in *Arabidopsis*.

Abstract

TRAPP complexes, composed of multimeric subunits, are guanine nucleotide-exchange factors for certain Rab GTPases and are believed to be involved in the regulation of membrane trafficking, but the cases in *Arabidopsis* are largely unknown. Trs33, recently proposed to be a component of TRAPP IV, is non-essential in yeast cells. A single copy of *Trs33* gene, *AtTrs33*, was identified in *Arabidopsis*. GUS activity assay indicated that *AtTrs33* was ubiquitously expressed. Based on a T-DNA insertion line, we found that loss-of-function of *AtTrs33* is lethal for apical growth. Knock-down or knock-in of *AtTrs33* affects apical meristematic growth and fertility, which indicates that AtTrs33 plays an important role in keeping apical meristematic activity and dominance in *Arabidopsis*. Analysis of auxin responses and PIN1/2 localization indicates that impaired apical meristematic activity and dominance was caused by altered auxin responses through non-polarized PIN1 localization. The present study reported that AtTrs33 plays an essential role in *Arabidopsis* cell growth and organization, which is different with its homologue in yeast. These findings provide new insights into the functional divergence of TRAPP subunits.

Keywords: *Arabidopsis*, Trs33, TRAPP, Apical Meristem, Auxin, PIN1/2, Subcellular localization

Introduction

In plants, the growth of both shoots and roots occurs in meristem, which consists of a population of undifferentiated pluripotent stem cells (Mizukami and Fischer 2000). These cells continuously differentiate and grow to generate new tissues and organs (Vernoux, et al. 2010). In meristems, the cell differentiation and growth are controlled by precise and complex regulatory mechanisms (Vernoux, et al. 2010). As an important phytohormone, auxin is required for initiation and outgrowth of new organs from meristems (Jiang and Feldman 2005). And its distribution is not homogeneously in meristem cells, for instance, auxin flows toward columella cells to establish local maxima for growth in root tips (Petersson, et al. 2009). The auxin flow and distribution is controlled by a series of auxin transporters (Benjamins, et al. 2005; Galweiler, et al. 1998; Kleine-Vehn, et al. 2006; Krecek, et al. 2009; Okada, et al. 1991; Petrasek and Friml 2009; Swarup, et al. 2008; Vandenbussche, et al. 2010).

Many auxin transporters are asymmetrically localized within cells and their polarity determines the directionality of intercellular auxin flow (Krecek, et al. 2009). For example, in roots, the PIN1 protein, an auxin efflux transporter, is localized to the basal part of the plasma membrane of stele cells and this polarity plays an important role in downward flow of auxin to the root tip for establishing local auxin maxima (Krecek, et al. 2009; Steinmann, et al. 1999). PIN2, another auxin efflux is mainly localized to the apical part of the plasma membrane of epidermal root cells for auxin flow back to the shoots from the root auxin maxima (Abas, et al. 2006). Polar localization PIN1 and PIN2 is dynamic. PIN proteins constitutively undergo clathrin-dependent endocytosis and ARF-GEF-dependent recycling (Kleine-Vehn, et al. 2008). It is believed that both PIN1 and PIN2 are first targeted to the plasma membrane without apparent asymmetry, they then attain their polarity by subsequent endocytosis and recycling (Dhonukshe, et al. 2008). The molecular control of endocytosis and recycling of PIN1 and PIN2 is still not very clear. When *Arabidopsis* roots were treated with latrunculin B, an actin inhibitor, causes PIN2 accumulation but not PIN1 (Rahman, et al. 2007). Subsequently, a chemical screen using endosidin1 identified that PIN2, rather than PIN1, through interactive pathways to involve in endosomal compartment (Robert, et al. 2008). TRAPP (trafficking protein particle) is a conserved modular complex that acts as a Ypt/Rab guanine-nucleotide exchange factor (GEF). Currently, four TRAPP complexes (I, II, III and IV) are known in yeast and they regulate two different intracellular trafficking pathways – secretion and autophagy (Kim, et al. 2016; Lipatova, et al. 2016; Tan, et al. 2013), but only TRAPP I and II has be confirmed in plants (Ravikumar, et al. 2017; Zhuang, et al. 2018). In plants, the polar transport of PIN2, but not PIN1, to the plasma membrane is influenced in plant cells missing TRAPP II (Qi, et al. 2011; Qi and Zheng 2011).

TRAPP II is a modified version of TRAPP I, a multi-subunit complex involved in protein trafficking around the Golgi apparatus (Barrowman, et al. 2010). In yeast, TRAPP I consists of six subunits (2 copies of Bet3 and one copy each of Bet5, Trs20, Trs23 and Trs31), which is believed to function at the ER-Golgi interface. TRAPP I serves as a tethering factor for COPII vesicles and can also act as a GEF for Ypt1 (Sacher, et al. 2001). TRAPP II consists of TRAPP I and three additional subunits (Trs120, Trs130 and Trs65) (Yamasaki, et al. 2009). In yeast, TRAPP II acts at the post-Golgi trafficking pathway and can serve as a GEF for Ypt31/2 (Morozova, et al. 2006). TRAPP III is believed to act in autophagy (Tan, et al. 2013; Zhuang, et al. 2018). TRAPP IV, an addition of Trs33 to

TRAPP I, is also proposed to be play role in autophagy [19]. Interestingly, in yeast, Trs33 is not essential for cell survival (Lipatova, et al. 2016). The mammalian TRAPP complexes showed different function and organizations with yeast (Barrowman, et al. 2010; Choi, et al. 2011), e.g. there is no homolog of Trs65 in mammalian cells (Cox, et al. 2007). TRAPP II in mammalian cells is also believed to act at pre-Golgi trafficking that can act as a GEF for Rab1 (Cai, et al. 2008).

The function and organization of TRAPP complexes have not been studied extensively in plants. In *Arabidopsis*, except for Trs65, single copies of all other eight TRAPP components exist (Cox, et al. 2007; Latijnhouwers, et al. 2005; Thellmann, et al. 2010). During cytokinesis, AtTrs120 and AtTrs130 through defining the identity of the membrane compartment to regulate cell plate assembly (Jaber, et al. 2010; Thellmann, et al. 2010). They are also involved in the post-Golgi trafficking pathway through Rab-A to establish the polarity of PIN2 and AUX1 (Qi, et al. 2011; Qi and Zheng 2011). Recently, we reported that a subunit of TRAPP, Bet5, plays an essential role in exine pattern formation and apical meristem organization (Zhang, et al. 2018). In this study, we extended our study on the function of Trs33. We show here that, unlike yeast Trs33 that is non-essential for yeast cell's survival (Tokarev, et al. 2009), *Arabidopsis* Trs33 (hereafter it is termed AtTrs33) plays an essential role in cell development in plants. The function of AtTrs33 is required for proper cell organization and auxin transport in root tips. In transgenic plant expressing AtTrs33-RNAi, polar localization of both PIN1 and PIN2 is affected.

Results

AtTrs33 is ubiquitously expressed and is essential for plant cells to develop

To explore the potential function of *AtTrs33*, we firstly analyzed its expression profile across different tissues. As shown in Figure 1A, *AtTrs33* was expressed in all tissues examined. Co-expression analysis using ATTED-II indicates that several *AtTrs33* co-expressed genes were involved in secretary pathway, such as *Trs20*, *RabA1B*, and *DUF1068* (Figure 1B). Noticeably, *Trs20* and *RabA1b* were significantly co-expressed with *Trs33* in various developmental tissues with correlation coefficient (*r*) 0.45 and 0.63, respectively (Figure 1C). GUS activity assay on transgenic *Arabidopsis* expressing *pAtTrs33::GUS* confirmed that *AtTrs33* was ubiquitously expressed (Figure 1D-H).

To study the biological function of AtTrs33, we isolated a T-DNA insertional mutant (Salk_109244), which carries a T-DNA insertion in the junction area of the third exon and the third intron. However, we failed to obtain homozygous *attrs33* mutant, but heterozygous and wild type plants from seeds harvested from plants heterozygous for the T-DNA insertion. A genetic segregation analysis revealed that, of 150 seedlings grew from seeds in individual siliques of a heterozygous *attrs33* plant, none of them were homozygous, 67 were heterozygous, 83 were wild type for the T-DNA insertion in *AtTrs33*. There was no abolished embryo in individual siliques (n=5) examined. These results indicate that the T-DNA insertion in *AtTrs33* affected the male transmission that a fundamental process in male gametophyte development is impaired.

AtTrs33-YFP is localized largely to the Golgi apparatus

To further confirm that the lack of homozygous *attrs33* from seeds produced by heterozygous *attrs33* plants is due to the T-DNA insertion in *AtTrs33* and also to examine the subcellular localization of AtTrs33, heterozygous *attrs33* mutant plants were transformed with AtTrs33-YFP. A total of 61 T1 generation plants were obtained based on the basta resistance. Three plants homozygous for *attrs33* were identified among the 61 transformants. We found that the vegetative growth of all three recovered homozygous *attrs33* mutant plants expressing AtTrs33-YFP were compatible with that of wild type plants, showing no obvious developmental defects (Figure 2A and B). However, we noted that in homozygous *attrs33* plant recovered, the fertility is not fully rescued (Figure 2B). This result confirmed that the lack of homozygous *attrs33*in seeds produced by heterozygous *attrs33* is due to the disruption of the *AtTrs33* gene that affects the pollen development.

To explore the cellular basis of the AtTrs33 function in plant cell development, the subcellular localization of AtTrs33 was examined in *attrs33* mutant plants expressing AtTrs33-YFP. In addition to faint cytosolic signal, punctate structures were marked by AtTrs33-YFP in the root epidermal cells based on confocal microscopy observation (Figure 2C). To identify the subcellular nature of AtTrs33-YFP marked punctate structures, AtTrs33-YFP were transiently expressed in tobacco lower epidermal cells. Again AtTrs33-YFP labeled numerous punctate structures in the faint cytosolic background (Figure 2I). Co-expression of AtTrs33-YFP and ST-GFP, a well characterized Golgi marker (Boevink, et al. 1998) showed that the punctate labeled by AtTrs33-YFP were largely co-localized with ST-GFP (Figure 2G - I, arrows), indicating that AtTrs33-YFP are largely associated with Golgi bodies.

Perturbation of the expression of *AtTrs33* results in a defect in shoot and root growth as well as a reduced fertility

Because we were unable to obtain homozygous *attrs33* mutant plant due to the essential role the *AtTrs33* gene plays in male transmission, we generated transcriptional knockdown lines using the RNA interference (RNAi) to assess the function of AtTrs33 in cell development in detail. In the T1 generation of *Arabidopsis* seedlings expressing AtTrs33 RNAi, we noted that roughly 50% them were small in size (Figure 3A). Unlike a single dominant primary shoot bud in the wild type (Figure 3B, see also Figure 4B), two buds were often seen in the shoot apices of these AtTrs33-RNAi lines (Figure 3B, Figure 4B), indicating that they were defective in shoot apical growth. Semi-quantitative RT-PCR analysis indicated that these seedlings with the described phenotypes were transcriptionally knocked-down in *AtTrs33* (Figure 3K and L). In the adult growth stage, these AtTrs33-RNAi lines were dwarfed and were either sterile or semi-sterile (compare Figure 3D-G to 3H). We also noticed that several AtTrs33-RNAi transgenic plants were shoot apical necrotic (Figure 3I and J). This phenotype is reminiscent of transgenic plants expressing dominant negative RAB-D2a(N121I) or *rab-D1 rab-D2b rab-D2c* triple mutants (Pinheiro, et al. 2009).

In our analysis of the T2 generation of AtTrs33-RNAi line #2, we found that there was no difference in the seed germination in DAG1 (Days After Germination) between wild type and AtTrs33-RNAi line #2 (Figure 4A). After germination, however, the growth of both shoots and roots was largely inhibited (Figure 4A). A close examination confirmed that transgenic plants expressing Attrs33-RNAi produced multi-buds (Figure 4B). The emergence of the first true leaf was also delayed 4-6 days compared to that in wild type seedlings. Similar to the defect in the shoot apical meristem, the growth of primary roots was also retarded in the AtTrs33-RNAi line (Figure 4A, from DAG3), which often resulted in a premature death of the primary root (Figure 3I and K), Interestingly, ectopic initiation of lateral roots, even in hypocotyls was often observed in the AtTrs33-RNAi line (Figure 4A DAG 8, Figure 3I).

In addition to transcriptionally knock-down of *AtTrs33* by RNAi, we also noted that over-expression of *AtTrs33* also led to developmental defects similar to that observed in AtTrs33-RNAi lines. As indicated in Figure 5, plants over-expressing AtTrs33-YFP were semi-sterile and some produced multi-inflorescences (Figure 5A and C). Furthermore, we also noticed that some transgenic plants expressing AtTrs33-YFP were shoot apical necrotic (Figure 5D and E). To investigate if shoot apical necrosis was correlated to the extent of transcriptional perturbation, we examined the transcription of *AtTrs33* of an AtTrs33 over-expressing line with shoot apical necrosis (#58) and another line (#9) without shoot apical necrosis. Comparing to wild type *Arabidopsis*, although *AtTrs33* was over-expressed in both lines (Figure 4F-4G), the over-expression of *AtTrs33* in the #58 line with shoot apical necrosis was stronger than that in #9 line with no shoot apical necrosis (Figure 4F-4G), indicating that the shoot apical necrosis is correlated the degree by which the expression of *AtTrs33* is perturbed.

Auxin response is altered in the Trs33-RNAi line

In distal root tip, auxin repress the expression of *WOX5* via ARF10/16 (Ding and Friml 2010). To explore if the defect of root growth in the AtTrs33-RNAi line is related with auxin, we therefore analyzed the auxin response through the expression of GFP under the control of DR5 promoter – an auxin responsive monitor (Liu, et al. 1994; Ulmasov, et al. 1995). As shown in Figure 6, the distribution of auxin was affected in root tip of the AtTrs33-RNAi line. In wild type, the auxin responsive peak was observed in multiple layer cells in front of the quiescent center (QC) (Figure 6A-C). However, the auxin was accumulated in a wider region in the stele cells in root tips of AtTrs33-RNAi line (Figure 6D-F). In addition, the cell arrangement was disordered in root tips of AtTrs33-RNAi line (Figure 6E). Within 5 minutes stained by propidium iodide (PI), the dye signal was observed in intracellular structures of stele cells in AtTrs33-RNAi line (Figure 6E), while it was still in the cell surface in wile type (Figure 6B).

Polar localization of PIN1 and PIN2 is disrupted in the AtTrs33-RNAi line

In plants, active auxin flow that is necessary for establishing the proper auxin distribution requires polar localization of many different auxin transporter proteins on the plasma membrane of cells. In *Arabidopsis* root tips, PIN1 and PIN2 were mainly localized in the plasma membrane of root stele cells and root epidermal cells, respectively (Grieneisen, et al. 2007). And their polarized localization plays important role in distinct intracellular transport and recycling (Grieneisen, et al. 2007; Qi, et al. 2011). To examine if the disordered auxin distribution in the AtTrs33-RNAi line is related with PIN1 and PIN2, we detected their polar localization in root tip cells. As shown in Figure 7B and F, the polar localization of PIN1 was completely disrupted in the stele cells in the AtTrs33-RNAi line and the signal was observed in the cytoplasm. Similarly, the polar localization of PIN2 was also slightly disrupted (Figure 7D and H). Although the PIN2 signal was still observed on apical region of the plasma membrane (Figure 7D), it was also detected on plasma membrane domains or punctate structures (Figure 7D and H). Moreover, the cell organization of the epidermis and cortex was also affected in the AtTrs33-RNAi line.

Discussion

Essential role of AtTrs33 in *Arabidopsis* cell development

In yeast, Trs33 is non-essential for yeast cells to survive (Lipatova, et al. 2016). Here we revealed in *Arabidopsis*, Trs33 plays an essential role in plant cell development. During our characterization of AtTrs33, we first noted that plants with a T-DNA insertion in one copy of the *AtTrs33* gene fail to produce plants homozygous for the T-DNA insertion. It seems that in plants heterozygous for the T-DNA insertion, female transmission is fine, but male transmission is impaired. When the expression of *AtTrs33* is perturbed by RNAi or over-expression, plants are also either sterile or semi-sterile. Although perturb the expression of *AtTrs33* didn't affect plant viability, the cell differentiation and organization in primary root tips were affected. This indicates that AtTrs33 plays a important role in cell differentiation and organization.

In yeast cells, Trs33 has been identified as one of seven subunits of TRAPP I (Sacher, et al. 2001) but a recent study has suggested that Trs33 is a component of TRAPP IV [19]. Although Trs33 is non-essential for yeast cells to survive, it is synthetic lethal with Trs65, whose N-terminus shares sequence similarity with Trs33 (Tokarev, et al. 2009).

Noticeably, Trs65 is missing in the *Arabidopsis* genome (Cox, et al. 2007). Although in mammalian cells there are two isoforms of Trs33 (TRAPPC6A and TRAPPC6B) (Gwynn, et al. 2006), one of them may serve as Trs65 (Tokarev, et al. 2009), *Arabidopsis* has a single copy of Trs33. This may explain why in yeast trs33 is non-essential while it is in *Arabidopsis*. However, AtTrs33-YFP largely resides in Golgi. Thus, it is also possible that in *Arabidopsis*, unlike the role of trs33 in yeast autophagy, AtTrs33 plays an essential role in trafficking around Golgi. In this regard, it is interesting to that the *rab-d1/d2b/d2c*, a triple mutant with defective in *Arabidopsis* RAB-D1 and RAB-D2a-c, *Arabidopsis* homologs of Rab1 in ER-Golgi trafficking as well as plants expressing dominant-negative RAB-D1(NI) or RAB-D2a(NI) also exhibit necrosis in the apical region of stems (Pinheiro, et al. 2009), similar to what is observed in plants with perturbed expression of AtTrs33.

Role of AtTrs33 in the organization and cell growth in root tips

Although a disruption of *AtTrs33* by a T-DNA insertion can lead a severe problem in male transmission therefore we are unable to obtain homozygous *attrs33* mutant plants, we found that some transgenic plants with transcriptional perturbation of *AtTrs33* can be generated. These plants with perturbed *AtTrs33* expression are associated with a growth defect in both shoot and root meristems. During root development, a proper auxin gradient is important for maintenance and growth of root meristem cells (Grieneisen, et al. 2007). When the expression of *AtTrs33* was perturbed, the local auxin distribution was affected. This might be the reason of cell files mis-organization and mis-identification in root tip of the AtTrs33-RNAi line.

In plants, distribution of auxin is largely under the control of transporters-facilitated auxin transport from cell to cell (Vernoux, et al. 2010). For example, PIN1 is strongly expressed in stele cells and localized to the basal part of the plasma membrane of these cells for downward auxin transport to the root tips, while PIN2 is localized to the apical part of the plasma membrane of epidermal cells for the return of auxin from the local maximum point in the distal cells below the QC. The polar localization of PIN1 and PIN2 is affected in the AtTrs33-RNAi line. This might be the reason of mis-established local auxin maximum in root tips. When the polar localization of PIN1 and PIN2 were disrupted, auxin cannot be properly transported and then it will be accumulated in other cell types.

Finally, we would like to mention that in the AtTrs33-RNAi line, it seems that the basal plasma membrane localization of PIN1 is lost in stele cells, while the polarity of PIN2 is impaired to a lesser extent in epidermis. Because AtTrs33-RNAi is expressed under the control of 35S, which is not very active in epidermis (Battraw and Hall 1990), so it is possible that the penetration of RNAi is different between epidermal and stele cells. It has been reported that the polar distribution of PIN1 and PIN2 may be mediated by different pathways (Jaillais, et al. 2007; Qi, et al. 2011; Robert, et al. 2008; Teh and Moore 2007). Whether the molecules related with PIN1 and PIN2 transport are differentially affected by AtTrs33 need to be further study.

Conclusions

In the present study, we reported AtTrs33 is essential in cell growth and organization in *Arabidopsis*. *AtTrs33* is ubiquitously expressed in different tissues and its loss-of-function mutant is lethal. Perturbation of *AtTrs33* transcription leads to apical meristematic growth defects and reduced fertility. The impaired apical meristematic activity was caused by altered auxin response. These findings provide new insights into the functional divergence of TRAPP subunits between yeast and plants.

Materials and Methods

Sequence cloning and analysis

For promoter analysis, a 1011 bp UTR5 region of *AtTrs33* was amplified and cloned into pDONR222.1 (Invitrogen). The sequencing confirmed promoter sequence was sub-cloned into the vector pMDC164 (pAtTrs33::GUS) for the GUS assay. For overexpression, the coding sequence of *AtTrs33* without stop codon was cloned into pDONR222.1 (Invitrogen) and the corrected coding sequence was sub-cloned into pEarleyGate101 (ABRC stock DB3-683) to create 35S::AtTrs33-YFP. For RNAi, the full length of *AtTrs33* was sub-cloned into pH7GWIWG2(I) to create 35S::AtTrs33-RNAi.

Plant materials and growth conditions

The T-DNA insertional line (Salk_109244) was provided by the *Arabidopsis* Biological Resource Center (http://www.arabidopsis.org). AtTrs33-YFP and AtTrs33-RNAi transgenic *Arabidopsis* lines were obtained according to *Agrobacterium*-mediated floral dip method (Clough and Bent 1998). Markers DR5-GFP, PIN1-GFP and PIN2-GFP were introduced into the AtTrs33-RNAi line #2 through crossing. For seeds germination, all seeds were sterilized in 80% ethanol for 10 minutes and were germinated on AT medium (Somerville and Ogren 1982) with corresponding antibiotics. Plants on medium or soil were grown under long-day condition (16/8 h light/dark) at 22-24°C.

Subcellular localization

Co-expression of AtTrs33-YFP and Golgi marker ST-GFP in *Nicotiana benthamiana* lower leaf epidermal cells was performed as described by Zhang et al. (Zhang, et al. 2013). The final OD600 for *Agrobacterium* cultures was 0.03 for both ST-GFP and AtTrs33-YFP. Acquisition of fluorescent images was performed on an inverted Zeiss LSM510(meta) laser scanning microscope with a C-apochromat40x/1.2W numerical aperture oil-immersion lens. More detail is required. For GFP, YFP, PI, what laser and filters used, for GFP/YFP and GFP/PI, what laser and filters used. Zeiss LSM software (Carl Zeiss, Göttingen, Germany), Photoshop (Adobe, San Jose, CA, USA), NIH ImageJ (http://rsbweb.nih.gov/ij/) were used for post-acquisition image processing.

Abbreviations

DAG: days after germination; ER: endoplasmic reticulum; GEF: guanine-nucleotide exchange factor; GFP: green fluorescent protein; PI: propidium iodide; RNAi: RNA interference; TRAPP: trafficking protein particle; YFP: yellow fluorescent proteins

Declarations

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Authors' contribution

HZ and ML designed the study. JZ, JC and WW performed the experiments, JZ and JC analyzed the data and wrote the manuscript. LW, SZ, JL, BL and XQ contributed to the data collection and data analysis. JZ, JC, HZ and ML revised the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

Abas L, Benjamins R, Malenica N, Paciorek T, Wisniewska J, Moulinier-Anzola JC, Sieberer T, Friml J, Luschnig C (2006) Intracellular trafficking and proteolysis of the Arabidopsis auxin-efflux facilitator PIN2 are involved in root gravitropism. Nature cell biology 8:249-256

Barrowman J, Bhandari D, Reinisch K, Ferro-Novick S (2010) TRAPP complexes in membrane traffic: convergence through a common Rab. Nature reviews 11:759-763

Battraw MJ, Hall TC (1990) Histochemical analysis of CaMV 35S promoter-β-glucuronidase gene expression in transgenic rice plants. Plant molecular biology 15:527-538

Benjamins R, Malenica N, Luschnig C (2005) Regulating the regulator: the control of auxin transport. Bioessays 27:1246-1255

Boevink P, Oparka K, Santa Cruz S, Martin B, Betteridge A, Hawes C (1998) Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network. Plant J 15:441-447

Cai Y, Chin HF, Lazarova D, Menon S, Fu C, Cai H, Sclafani A, Rodgers DW, De La Cruz EM, Ferro-Novick S, Reinisch KM (2008) The structural basis for activation of the Rab Ypt1p by the TRAPP membrane-tethering complexes. Cell 133:1202-1213

Choi C, Davey M, Schluter C, Pandher P, Fang Y, Foster LJ, Conibear E (2011) Organization and assembly of the TRAPPII complex. Traffic (Copenhagen, Denmark) 12:715-725

Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J 16:735-743

Cox R, Chen SH, Yoo E, Segev N (2007) Conservation of the TRAPPII-specific subunits of a Ypt/Rab exchanger complex. BMC evolutionary biology 7:12

Dhonukshe P, Tanaka H, Goh T, Ebine K, Mahonen AP, Prasad K, Blilou I, Geldner N, Xu J, Uemura T, Chory J, Ueda T, Nakano A, Scheres B, Friml J (2008) Generation of cell polarity in plants links endocytosis, auxin distribution and cell fate decisions. Nature 456:962-966

Ding Z, Friml J (2010) Auxin regulates distal stem cell differentiation in Arabidopsis roots. Proceedings of the National Academy of Sciences of the United States of America 107:12046-12051

Galweiler L, Guan C, Muller A, Wisman E, Mendgen K, Yephremov A, Palme K (1998) Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. Science (New York, NY 282:2226-2230

Grieneisen VA, Xu J, Maree AF, Hogeweg P, Scheres B (2007) Auxin transport is sufficient to generate a maximum and gradient guiding root growth. Nature 449:1008-1013

Gwynn B, Smith RS, Rowe LB, Taylor BA, Peters LL (2006) A mouse TRAPP-related protein is involved in pigmentation. Genomics 88:196-203

Jaber E, Thiele K, Kindzierski V, Loderer C, Rybak K, Jurgens G, Mayer U, Sollner R, Wanner G, Assaad FF (2010) A putative TRAPPII tethering factor is required for cell plate assembly during cytokinesis in Arabidopsis. The New phytologist 187:751-763

Jaillais Y, Santambrogio M, Rozier F, Fobis-Loisy I, Miege C, Gaude T (2007) The retromer protein VPS29 links cell polarity and organ initiation in plants. Cell 130:1057-1070

Jiang K, Feldman LJ (2005) Regulation of root apical meristem development. Annual review of cell and developmental biology 21:485-509

Kim JJ, Lipatova Z, Segev N (2016) TRAPP Complexes in Secretion and Autophagy. Front Cell Dev Biol 4:20

Kleine-Vehn J, Dhonukshe P, Sauer M, Brewer PB, Wisniewska J, Paciorek T, Benkova E, Friml J (2008) ARF GEF-dependent transcytosis and polar delivery of PIN auxin carriers in Arabidopsis. Curr Biol 18:526-531

Kleine-Vehn J, Dhonukshe P, Swarup R, Bennett M, Friml J (2006) Subcellular trafficking of the Arabidopsis auxin influx carrier AUX1 uses a novel pathway distinct from PIN1. Plant Cell 18:3171-3181

Krecek P, Skupa P, Libus J, Naramoto S, Tejos R, Friml J, Zazimalova E (2009) The PIN-FORMED (PIN) protein family of auxin transporters. Genome biology 10:249

Latijnhouwers M, Hawes C, Carvalho C (2005) Holding it all together? Candidate proteins for the plant Golgi matrix. Current opinion in plant biology 8:632-639

Lipatova Z, Majumdar U, Segev N (2016) Trs33-Containing TRAPP IV: A Novel Autophagy-Specific Ypt1 GEF. Genetics 204:1117-1128

Liu ZB, Ulmasov T, Shi X, Hagen G, Guilfoyle TJ (1994) Soybean GH3 promoter contains multiple auxin-inducible elements. Plant Cell 6:645-657

Mizukami Y, Fischer RL (2000) Plant organ size control: AINTEGUMENTA regulates growth and cell numbers during organogenesis. Proceedings of the National Academy of Sciences of the United States of America 97:942-947

Morozova N, Liang Y, Tokarev AA, Chen SH, Cox R, Andrejic J, Lipatova Z, Sciorra VA, Emr SD, Segev N (2006) TRAPPII subunits are required for the specificity switch of a Ypt-Rab GEF. Nature cell biology 8:1263-1269

Okada K, Ueda J, Komaki MK, Bell CJ, Shimura Y (1991) Requirement of the Auxin Polar Transport System in Early Stages of Arabidopsis Floral Bud Formation. Plant Cell 3:677-684

Petersson SV, Johansson AI, Kowalczyk M, Makoveychuk A, Wang JY, Moritz T, Grebe M, Benfey PN, Sandberg G, Ljung K (2009) An auxin gradient and maximum in the Arabidopsis root apex shown by high-resolution cell-specific analysis of IAA distribution and synthesis. Plant Cell 21:1659-1668

Petrasek J, Friml J (2009) Auxin transport routes in plant development. Development (Cambridge, England) 136:2675-2688

Pinheiro H, Samalova M, Geldner N, Chory J, Martinez A, Moore I (2009) Genetic evidence that the higher plant Rab-D1 and Rab-D2 GTPases exhibit distinct but overlapping interactions in the early secretory pathway. J Cell Sci 122:3749-3758

Qi X, Kaneda M, Chen J, Geitmann A, Zheng H (2011) A specific role for Arabidopsis TRAPPII in post-Golgi trafficking that is crucial for cytokinesis and cell polarity. The Plant Journal 68:234-248

Qi X, Zheng H (2011) Arabidopsis TRAPPII is functionally linked to Rab-A, but not Rab-D in polar protein trafficking in trans-Golgi network. Plant Signal Behav 6:

Rahman A, Bannigan A, Sulaman W, Pechter P, Blancaflor EB, Baskin TI (2007) Auxin, actin and growth of the Arabidopsis thaliana primary root. Plant J 50:514-528

Ravikumar R, Steiner A, Assaad FF (2017) Multisubunit tethering complexes in higher plants. Current opinion in plant biology 40:97-105

Robert S, Chary SN, Drakakaki G, Li S, Yang Z, Raikhel NV, Hicks GR (2008) Endosidin1 defines a compartment involved in endocytosis of the brassinosteroid receptor BRI1 and the auxin transporters PIN2 and AUX1. Proceedings of the National Academy of Sciences of the United States of America 105:8464-8469

Sacher M, Barrowman J, Wang W, Horecka J, Zhang Y, Pypaert M, Ferro-Novick S (2001) TRAPP I implicated in the specificity of tethering in ER-to-Golgi transport. Mol Cell 7:433-442

Somerville CR, Ogren WL (1982) Isolation of photorespiratory mutants of Arabidopsis. In Methods in Chloroplast Molecular Biology, R.B. Hallick and N.H. Chua, eds (New York: Elsevier). pp. 129-139

Steinmann T, Geldner N, Grebe M, Mangold S, Jackson CL, Paris S, Galweiler L, Palme K, Jurgens G (1999) Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF GEF. Science (New York, NY 286:316-318

Swarup K, Benkova E, Swarup R, Casimiro I, Peret B, Yang Y, Parry G, Nielsen E, De Smet I, Vanneste S, Levesque MP, Carrier D, James N, Calvo V, Ljung K, Kramer E, Roberts R, Graham N, Marillonnet S, Patel K, Jones JD, Taylor CG, Schachtman DP, May S, Sandberg G, Benfey P, Friml J, Kerr I, Beeckman T, Laplaze L, Bennett MJ (2008) The auxin influx carrier LAX3 promotes lateral root emergence. Nature cell biology 10:946-954

Tan D, Cai Y, Wang J, Zhang J, Menon S, Chou HT, Ferro-Novick S, Reinisch KM, Walz T (2013) The EM structure of the TRAPPIII complex leads to the identification of a requirement for COPII vesicles on the macroautophagy pathway. Proceedings of the National Academy of Sciences of the United States of America 110:19432-19437

Teh OK, Moore I (2007) An ARF-GEF acting at the Golgi and in selective endocytosis in polarized plant cells. Nature 448:493-496

Thellmann M, Rybak K, Thiele K, Wanner G, Assaad FF (2010) Tethering factors required for cytokinesis in Arabidopsis. Plant physiology 154:720-732

Tokarev AA, Taussig D, Sundaram G, Lipatova Z, Liang Y, Mulholland JW, Segev N (2009) TRAPP II complex assembly requires Trs33 or Trs65. Traffic (Copenhagen, Denmark) 10:1831-1844

Ulmasov T, Liu ZB, Hagen G, Guilfoyle TJ (1995) Composite structure of auxin response elements. Plant Cell 7:1611-1623

Vandenbussche F, Petrasek J, Zadnikova P, Hoyerova K, Pesek B, Raz V, Swarup R, Bennett M, Zazimalova E, Benkova E, Van Der Straeten D (2010) The auxin influx carriers AUX1 and LAX3 are involved in auxin-ethylene interactions during apical hook development in Arabidopsis thaliana seedlings. Development (Cambridge, England) 137:597-606

Vernoux T, Besnard F, Traas J (2010) Auxin at the shoot apical meristem. Cold Spring Harbor perspectives in biology 2:a001487

Yamasaki A, Menon S, Yu S, Barrowman J, Meerloo T, Oorschot V, Klumperman J, Satoh A, Ferro-Novick S (2009) mTrs130 is a component of a mammalian TRAPPII complex, a Rab1 GEF that binds to COPI-coated vesicles. Molecular biology of the cell 20:4205-4215

Zhang J, Chen J, Wang LJ, Zhao ST, Li JB, Liu BB, Li HY, Qi XY, Zheng HQ, Lu MZ (2018) AtBET5 is essential for exine pattern formation and apical meristem organization in Arabidopsis. Plant Sci 274:231-241

Zhang J, Li J, Liu B, Zhang L, Chen J, Lu M (2013) Genome-wide analysis of the Populus Hsp90 gene family reveals differential expression patterns, localization, and heat stress responses. BMC genomics 14:532

Zhuang X, Chung KP, Luo M, Jiang L (2018) Autophagosome Biogenesis and the Endoplasmic Reticulum: A Plant Perspective. Trends Plant Sci 23:677-692

Figure Legends

Figure 1. Expression pattern and GUS assay of *AtTrs33* in *Arabidopsis*.

(A) Microarray results from the public database showing ubiquitous expression pattern in various tissues.

(B) Co-expression network of *AtTrs33*.

(C) Correlation of expression pattern for Trs33 with Trs20 and RabA1B.

(D-H) Gus staining of pAtTrs33::GUS seedling, hypocotyl, root tip, and flower organs.

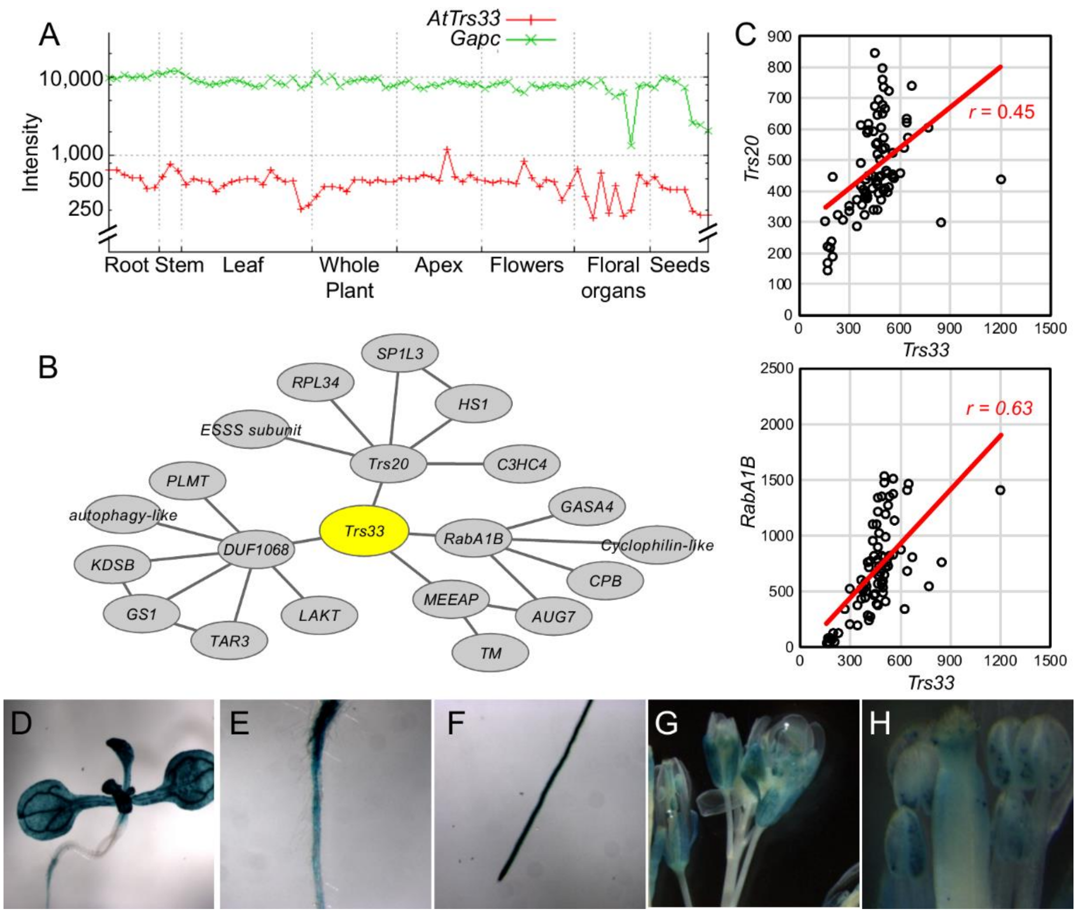

Figure 2. Molecular complementation on *trs33* mutant plants and subcellular localization of AtTrs33-YFP.

(A) Seven days seedlings of wild type Col-0 (left) and rescued *trs33* mutant plants (right).

(B) Inflorescence of wild type Col-0 (left) and rescued *trs33* mutant plants (right).

(C) AtTrs33-YFP marked punctate structures in rescued *trs33* mutant plants.

(D-F) Confocal images of ST-GFP in wild type Col-0 showing signal from GFP channel (D), YFP channel (E) and merged picture (F).

(G) Scatter plot of co-localization between ST-GFP and AtTrs33-YFP.

(H-J) Confocal images of ST-GFP in rescued *trs33* mutant plants showing ST-GFP (H), AtTrs33-YFP (I) and merged picture (J). Note ST-GFP and AtTrs33-YFP showing co-localization in (J). Arrows indicating single Golgi bodies. Scale bar is 1 cm in (A), 10 cm in (B), 5 μm in (C) and in (I) for (D-I).

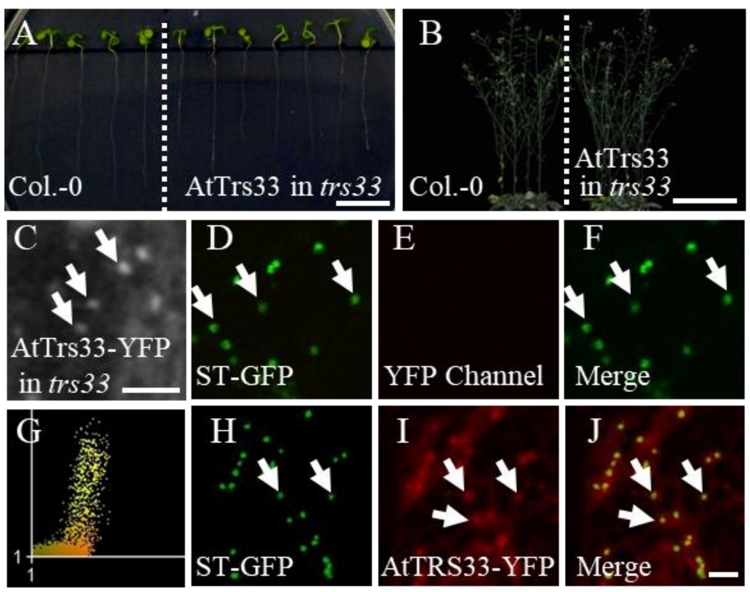

Figure 3. Perturbation of *AtTrs33* transcription by RNA interference causes defects of apical meristematic growth and a range of fertile defects.

(A) wild type Col-0 plants (left) and AtTrs33-RNAi lines showing dwarfness and multi-meristems phenotype (right). Arrow head indicating transgenic plants with multi-meristems.

(B-C) Magnified view of plants showing single bud in wild type plant (B) and multi-buds in AtTrs33 RNAi plants (C). Arrows pointing at the bud(s).

(D-G) Inflorescences of AtTrs33 RNAi plants showing a range of fertile defects from sterile (D) to fertile (G). Note the multi-inflorescences in (D-G).

(H) An inflorescence of wild type *Arabidopsis* showing normal siliques.

(I) An AtTrs33-RNAi plant showing multi-inflorescences and shoot apical necrosis.

(J) Magnified view of the shoot apical necrosis in (I).

(K-L) (B) Gel staining (K) and Quantification (L) of semi-quantitative PCR analysis of wild type and AtTrs33 RNAi lines showing interfered transcription. Scale bar is 1 cm in (A), (B) and (I), 5 cm in (H) for (D-H), 0.5 cm in (J).

Figure 4. Perturbation of AtTrs33 transcription by RNA interference causes multi-buds but not seed germination.

(A) Images of wild type and AtTrs33 RNAi lines collected at 1, 3, 5 and 8 days after germination (DAG). Note the similar germination on DAG1, and the growth defects on DAG3, DAG5 and DAG8.

(B-C) Close view of shoot apices showing single bud in wild type (B) and multi-buds in AtTrs33 RNAi plants (C).

(D-E) Cartoons showing single bud in wild type (D) and multi-buds in Trs33-RNAi line (E) located in the apical region.

(F-G) Close view of hypocotyls and roots showing normal wild type (F) and arrested primary root in AtTrs33 RNAi plants (G). Note the lateral root like organs formed in hypocotyl of AtTrs33 RNAi plants (G).

(H) Magnified view of arrested primary root in AtTrs33 RNAi plants.

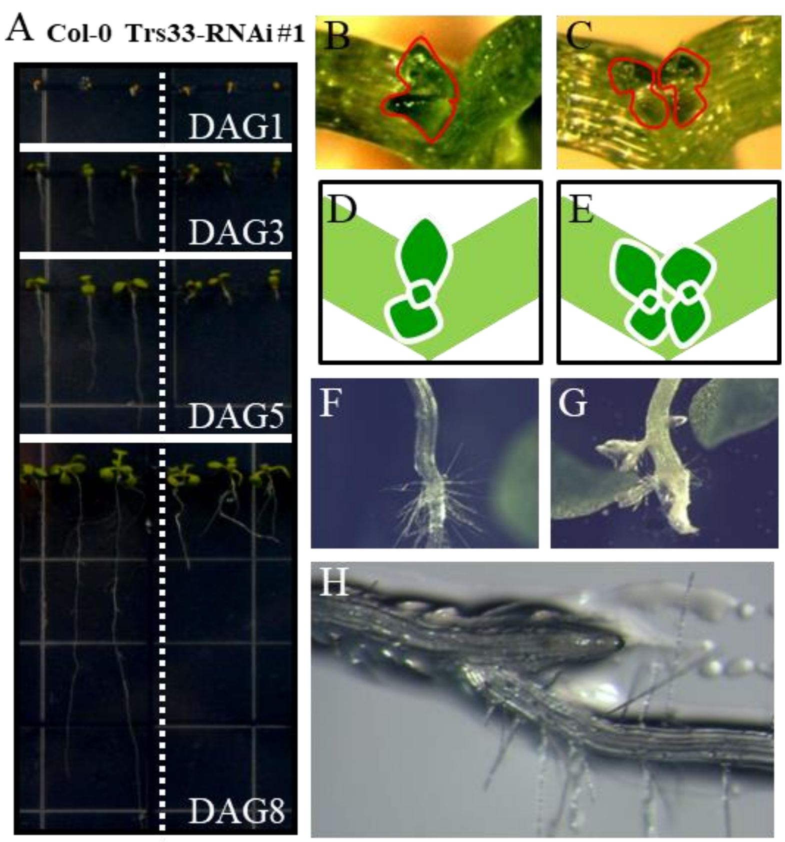

Figure 5. Perturbation of *AtTrs33* transcription by over-expression causes fertile defects, apical meristematic growth defects and shoot apical necrosis.

(A) AtTrs33-YFP over-expression lines showing a range of fertile defects. Arrow heads indicating abolished siliques and arrows pointing at multi-inflorescences.

(B) A wild type *Arabidopsis* plant showing normal inflorescence and siliques.

(C-D) Two individual plants of AtTrs33 over-expression lines with (D) or without (C) shoot apical necrosis phenotype.

(E) Magnified view of shoot apical necrosis in (D).

(F-G) Gel staining (F) and quantification (G) of semi-quantitative PCR analysis of wild type and AtTrs33 over-expression lines showing increased transcription.

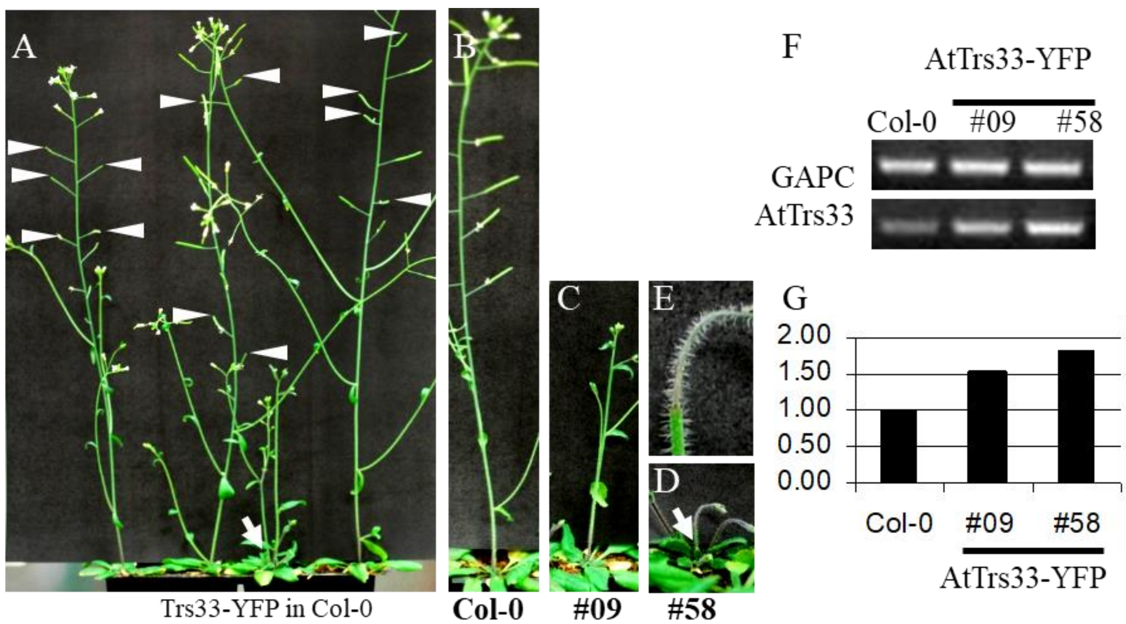

Figure 6. Auxin responses in root apical meristem are altered in AtTrs33 RNAi lines and the permeability of meristematic cells is largely increased.

(A-C) Confocal images of wild type Col-0 root tips showing DR5::GFP (A) and PI staining (B). (C) is the merged image of (A) and (B).

(D-F) Confocal images of AtTrs33 RNAi plant root tips showing altered auxin responses (D) and PI staining (E). (F) is the merged image of (D) and (E). Note the nuclei of AtTrs33 RNAi plant root meristematic cells were stained (E) at the time when wild type root meristematic cells were not (B) indicating the increased permeability of AtTrs33 RNAi plant root meristematic cells. Scale bar in (F) is 50 μm for (A-F).

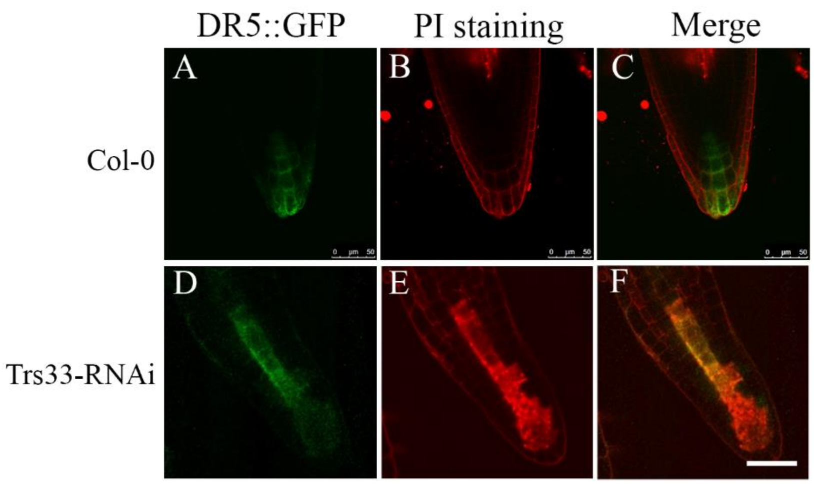

Figure 7. Polarity of PIN1 and PIN2 is changed at different extent in AtTrs33 RNAi lines. (A-D) Confocal images of PIN1/2-GFP in root tips of wild type Col-0 (A, C) and AtTrs33 RNAi plants (B, D) showing the fluoresce marked center (A-B) and peripheral cells (C-D). (E-H) Magnified view of (A-D) showing polarized localization of PIN1/2-GFP in wild type Col-0 (E, G) and changed polarity in AtTrs33 RNAi plants (F, H). Note the polarity of PIN1 is totally lost (F) and polarity of PIN2 is partially lost (H) in AtTrs33 RNAi plants. Scale bar is 50 μm in (D) for (A-D), 5 μm in (H) for (E-H).