Plant Physiology Preview. Published on November 13, 2017, as DOI:10.1104/pp.17.00823

Short Title: Spatial control of gene expression by TCPs.

Corresponding author: Carla Schommer, IBR-CONICET, Predio CCT, Ocampo y Esmeralda s/n, 2000 Rosario, Argentina; Phone: +54-3414237070-663; Email: schommer@ibr-conicet.gov.ar

Spatial control of gene expression by miR319-regulated TCP transcription factors in leaf development

Edgardo G. Bresso[1], Uciel Chorostecki[1,2], Ramiro E. Rodriguez [1,2,3], Javier F. Palatnik[1,3*] and Carla Schommer[1,3*]

[1]Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET and Universidad Nacional de Rosario, Rosario, Argentina.

[2]Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (UNR), Rosario, Argentina.

[3]Centro de Estudios Interdisciplinarios, Universidad Nacional de Rosario (UNR), Rosario, Argentina.

*To whom correspondence should be directed: Carla Schommer schommer@ibr-conicet.gov.ar

One sentence summary: microRNA319 regulated TCP transcription factors influence leaf development in distinct ways in central and marginal parts of the organ.

Author contributions: E.G.B., R.E.R. and C.S. performed the experiments. E.G.B., U.C., R.E.R., C.S. and J.P. analysed the data. C.S. and J.P. wrote the article. All authors revised and approved the manuscript.

Footnote

The work was mainly supported by grants from the Argentine Ministry of Science to C.S. (PICT2013-2763) and J.F.P. (PICT2016-0761). It was supported by an EMBO Short-Term Fellowship to E.G.B., CONICET fellowships to E.G.B. and U.C.. J.F.P. and R.E.R. are members of CONICET. C.S. is contracted by CONICET. Work performed at the Max Planck Institute was supported by SFB1101 to Detlef Weigel.

***Corresponding author email**: Correspondence should be addressed to Carla Schommer: schommer@ibr-conicet.gov.ar

Abstract

The characteristic leaf shapes we see in all plants are in good part outcome of the combined action of several transcription factor networks that translate into cell division activity during the early development of the organ. We show here that wild-type leaves have distinct transcriptomic profiles in center and marginal regions. Certain transcripts are enriched in margins, including those of *CINCINNATA* (*CIN*)-like *TCP*s, and members of the *NGATHA* (*NGA*) and *STYLISH* (*STY*) gene families. We study in detail the contribution of miR319 regulated TCP (TEOSINTE BRANCHED CYCLOIDEA, PCF1/2) transcription factors to the development of the center and marginal regions of Arabidopsis leaves. We compare in molecular analyses wildtype, a *tcp2 tcp4* mutant that has enlarged flat leaves and a *tcp2 tcp3 tcp4 tcp10* mutant with strongly crinkled leaves. The different leaf domains of the *tcp* mutants show changed expression patterns for many photosynthesis related genes, indicating delayed differentiation, especially in the marginal parts of the organ. At the same time, we found an upregulation of cyclin genes and other genes that are known to participate in cell division, specifically in the marginal regions of *tcp2 tcp3 tcp4 tcp10*. Using GUS reporter constructs we confirmed extended mitotic activity in the *tcp2 tcp3 tcp4 tcp10* leaf which persisted in small defined foci in the margins when the mitotic activity had already ceased in wild-type leaves. Our results describe the role of miR319-regulated TCP transcription factors in the coordination of activities in different leaf domains during the organs development.

Introduction

Leaves are impressive examples for the plasticity found in plant development. The size and shape of leaves vary not only between different species, but also depend considerably on plant age and the environment. Leaves are generated at the flanks of the shoot apical meristem. They first appear as rod-shaped primordia, which then expand and grow to form flat laminas through the activity of a marginal meristem (Donnelly et al., 1999). Cell divisions in the growing leaf lamina are maintained mainly by a plate meristem and occurs first throughout the organ, and then become restricted to a region in the proximal part of the organ, until ceaseing rather abruptly (Donnelly et al., 1999; Beemster et al., 2005; Kazama et al., 2010; Andriankaja et al., 2012; Powell and Lenhard, 2012; Rodriguez et al., 2014). Dispersed meristematic cells producing stomata and vascular cells continue to proliferate for a longer period of time (White, 2006; Andriankaja et al., 2012). After cell proliferation stops, cell enlargement becomes the driving force for organ growth. The coordination of cell division and cell expansion throughout the organ is a key to establish leaf size and shape. Around this time of transition from division to expansion, chloroplasts already develop and genes involved in photosynthesis are being activated and influence in turn cell proliferation (Andriankaja et al., 2012).

The *TCP* transcription factors are a plant specific family of transcription factors, named after the first identified members, *TEOSINTE BRANCHED*, *CYCLOIDEA* and *PCF1/PCF2* (Kosugi and Ohashi, 1997; Cubas et al., 1999). In Arabidopsis, 24 family members have been identified which can be further classified according to similarities in the TCP domain and biological functions (Nicolas and Cubas, 2016). Five *TCPs*, *TCP2*, *TCP3*, *TCP4*, *TCP10* and *TCP24* and their homologs in different species are regulated by the evolutionary conserved microRNA (miRNA) miR319 (Palatnik et al., 2003). High levels of miR319 down-regulate these *TCPs* and cause important changes in Arabidopsis leaf morphogenesis and the generation of crinkled leaves (Palatnik et al., 2003; Koyama et al., 2007; Efroni et al., 2008). A triple mutant in miR319-regulated TCPs also affects leaf development severely, as well as mutations in the snapdragon gene *CINCINNATA*, a *TCP4* homolog (Nath et al., 2003; Schommer et al., 2008). The effects of miR319 regulated TCPs in the generation of crinkles are enhanced by three other TCPs: TCP5, TCP13, and TCP17 (usually referred to as CIN-LIKE TCPs). These three *TCP*s lack the regulation by miR319 but are closely related to the miRNA regulated family members, based on similarities in their amino acid sequence (Efroni et al., 2008; Li, 2015). Transcriptome analysis of leaves with modified TCP levels have shown that their differentiation program is modified (Efroni et al., 2008). However, TCPs have also been shown to directly activate *MIR396b* and *CYCLIN DEPENDENT KINASE INHIBITOR1* (*ICK1*) (Schommer et al., 2014), which are known to

regulate cell proliferation (Wang et al., 2000;; Rodriguez et al., 2010;). Furthermore, miR319 regulated TCPs are involved in hormone biosynthesis and response [reviewed in (Nicolas and Cubas, 2016)].

Leaf margins can be smooth, lobed or serrated. The typical shape that is acquired varies between species and depends on plant age. How the margin is defined and which genes are involved in constructing the typical shape of a leaf is not well understood. Various studies describe genes and mutants or transgenic lines that affect margin development such as *ICK1* overexpressors*, yucca* mutants or plants with changes the *CUP SHAPED COTYLEDON* (*CUC)*/miR164 balance and *pin formed1,* which affect the degree of serration of the Arabidopsis leaf (Nikovics et al., 2006; Kawamura et al., 2010; Engelhorn et al., 2012; Steiner et al., 2012). *NGATHA* (*NGA*) and *STYLISH* (*STY*) genes initially described by their function in gynoecium development have also been found to be important in the generation of leaf margins. Both gene families promote auxin biosynthesis (Sohlberg et al., 2006; Martinez-Fernandez et al., 2014) and multiple knock outs have leaves with more serrations than wild type (Kuusk et al., 2002; Beemster et al., 2005; Alvarez et al., 2009; Trigueros et al., 2009; Ballester et al., 2015; Alvarez et al., 2016). Recent studies show that simultaneous downregulation of miR319 regulated *TCP*s and *NGA*s results in dramatic leaf phenotypes with indefinite growth at the margins (Alvarez et al., 2016).

Transcriptome studies have been carried out at different stages of leaf development and their analyses have led to the identification of genes involved in cell proliferation and differentiation in different leaves (Beemster et al., 2005; Schmid et al., 2005; Efroni et al., 2008), as well as genes involved in the transition from primary to secondary morphogenesis (Andriankaja et al., 2012). Detailed studies during the progression of leaf development have identified markers of leaf differentiation, implicating functions for the TCPs in the regulation of developmental timing, especially in the control of early differentiation events during leaf development (Efroni et al., 2008).

Interestingly, previous transcriptomic studies have been performed in whole leaves, which might underscore the events occurring in specific leaf domains. Here, we isolate a quadruple knock out *tcp2 tcp3 tcp4 tcp10*, which has crinkled margins in a similar or stronger way as the *jaw*-D mutant that overexpresses miR319. In contrast, *tcp* double mutants have larger leaves with no obvious appearance of crinkles. We performed transcriptomic studies on the margins and central parts of leaves from wildtype, *tcp2 tcp4* and *tcp2 tcp3 tcp4 tcp10* mutants. We found that the reduction of TCP activity had general effects on the transcriptome of the organ as well as we detected domain specific differences. We also found a strong

activation of cell proliferation markers in leaf margins, which had not been detected in previous transcriptomic studies using whole leaves. We found that most of the genes enriched in leaf margins are affected by downregulation of miR319-regulated *TCPs*. Still, a group of margin specific genes with an over-representation on transcription factors is acting independent of TCP control. Our data provide new insights into leaf development and the role of miRNA regulated TCPs in the coordination of organ growth.

Results

Differential effects of *tcp* double and quadruple mutants on leaf development

To study the functions of miR319-regulated *TCPs* we characterized a series of loss-of-function *tcp* insertional knock-out mutants including single, double, triple and quadruple mutants (Figure 1). The leaves and rosettes of *tcp* single mutants were slightly larger than wild type (Figure 1). In contrast to small effects on the size of the single *tcp* knockouts, we observed a significant increase of leaf size in *tcp2 tcp4* double knock outs (Figure 1). The *tcp* quadruple mutant had a different phenotype, with strongly crinkled leaves (Figure 1). While changes in the leaf curvature are seen in all quadruple knock out leaves, we observed that crinkles and small folds at the margins become more predominant starting with leaf five and onwards. We determined the area of the crinkles by flattening the leaves and considering the areas that were folded one or more times on top of each other and entered them as multiples into the calculations of the total leaf area (Figure 1). Analysis of the first leaf revealed that the complete area was smaller than wild type, if not considering folded areas (Figure 1). Yet, the *tcp* quadruple mutant had smaller leaves than the double mutant (Figure 1).

The triple mutant *tcp2 tcp4 tcp10* had an intermediate phenotype between double and quadruple mutants. The leaves already had some crinkles (Figure 1), however to a much lower extent than the quadruple *tcp* mutant. Leaves of *tcp2 tcp4 tcp10* were larger than wild type, but similar to *tcp2 tcp4,* even after considering folded regions (Figure 1).

We analyzed the first leaf at cellular level. The number of palisade parenchyma mesophyll cells increased in single and double mutants with respect to wild-type leaves (Figure 1). As the cell size remained fairly constant in these leaves (Figure 1), the increase of leaf size in *tcp* single and double mutants correlated directly with an increase in cell number. A different scenario was observed in plants with a strong decrease in TCP activity. First, the cell number did not increase steadily with the combination of *tcp* mutants, rather, the total number of cells in double, triple and quadruple *tcp* knock-outs was similar (Figure 1). We also noticed that the

cell size in the *tcp2 tcp3 tcp4 tcp10* quadruple knock-out was reduced (Figure 1), which explains its reduced in leaf area (Figure 1). Therefore, a mild decrease in TCP activity such as seen in single and double mutants caused an increase in cell number, which in turn produced larger leaves. Further loss of TCP activity triggered additional changes in leaf morphogenesis, namely a modification of organ curvature and a reduction of cell expansion.

Transcriptome analysis of leaf margins and inner lamina

Previous transcriptomic analyses have focused on whole developing leaves or leaves dissected into proximal and distal parts (Efroni et al., 2008; Li et al., 2010; Pettko-Szandtner et al., 2015; Nicolas and Cubas, 2016). Here, we decided to analyze the plant transcriptome in the margins and the center of the leaf. To do this we collected developing fifth leaves of wildtype, *tcp2 tcp4* and *tcp2 tcp3 tcp4 tcp10* mutants (Figure 2, see methods for details). The leaves were dissected with the help of a stereo microscope as shown in Figure 2 and the marginal or central areas respectively were collected. Biological triplicates representing the margins and central regions of more than 50 individual plants were subjected to transcriptome analysis by RNA sequencing (Anders et al., 2013).

In a first step, we focused on the analysis of the wild type samples. We identified 141 transcripts enriched in the margins and 237 enriched in the center samples (at least two-fold change in expression levels and FDR < 0,01) (Figure 2, Table S1), indicating that the expression profile of the leaf is not homogeneous but that margins and center parts have their specific signature.

Among the 141 genes enriched in wild-type margins we detected 13 transcription factors of the *WRKY*, *BHLH*-like, *NGA, STY* and *TCP* gene families (Table S1). Interestingly, several of those have been shown to affect leaf shape and size when misregulated (Kuusk et al., 2002; Alvarez et al., 2006; Kuusk et al., 2006; Efroni et al., 2008; Alvarez et al., 2009; Trigueros et al., 2009; Ballester et al., 2015; Lee et al., 2015).

We inspected the 141 genes that are enriched in wild-type margins also in the margins of the *tcp* mutants. We found that the margin-specific genes decreased their expression in the double and quadruple *tcp* mutant margins (Figure 2C). In contrast, we observed that the expression of 237 center enriched genes from wild-type leaves were less affected in the centers of the *tcp* mutant leaves (Figure 2C, Table S1). These results are consistent with the morphological changes observed in the margins of the plants with low TCP activity.

An analysis of the 6 different samples in the RNAseq experiment displayed in multidimensional scaling (MDS) and smear plots further visualized that the margins of the quadruple *tcp* mutant were strikingly different in their expression profiles from wildtype and the double *tcp* knock out (Figure 2, Figure S1). However, we also detected that the *tcp2 tcp4* knock out, which does not appear to have morphologically changed margins compared to wildtype, on molecular level displayed more changes in the marginal than in the central part of the leaf (Figure 2, Figure S1). Therefore, our analysis detected approximately 140 genes enriched in the borders of developing leaves and showed that miR319-regulated TCPs are necessary to assure a correct regulation of many of those.

Differential control of gene expression by TCPs in leaf domains: down-regulated genes in *tcp* mutants

Next, we inspected the genes that were down-regulated in the margins and the center of the *tcp* mutant leaves compared to wildtype (Table S2). We found that 863 genes were down-regulated in the margins of the quadruple *tcp* mutants and 254 in the center of the organ (Figure 3, Table S2). That *tcp2 tcp3 tcp4 tcp10* had more changes in the margin than in the center is consistent with mutant leaves having crinkles and changes in leaf curvature especially along the border regions. 210 genes were downregulated in both, margin and center of the *tcp2 tcp3 tcp4 tcp10* mutants (Figure 3), and most of the genes affected in *tcp2 tcp4* were a subgroup of those affected in the quadruple knockout (Table S2) demonstrating that the double mutant reflects to a certain extent a partial state of TCP activity between wild type and the *tcp2 tcp3 tcp4 tcp10* quadruple mutant.

MiR319-regulated TCPs bind a core sequence GGACCA (Schommer et al., 2008). We observed that the presence of this sequence was enriched in the promoters of the downregulated genes (defined as 1kb upstream of the transcription start) in *tcp2 tcp4* margins, and in the promoters of *tcp2 tcp3 tcp4 tcp10* center and margin samples (Figure 3, Table S3). Among the downregulated genes were validated TCP targets such as *LIPOXYGENASE2, ICK1* and the *ARABIDOPSIS RESPONSE REGULATER 16* (Table S2) (Schommer et al., 2008; Efroni et al., 2013; Schommer et al., 2014) and noteworthy also the *NGA2* gene, whose activity had been shown to be reduced in the miR319a overexpressing *jaw*-D background (Ballester et al., 2015).

Analysis of GO-term enrichment in the down-regulated genes in the center and the margins of *tcp2 tcp3 tcp4 tcp10* leaves revealed that ‘response to stimuli’ was the most

frequent category (Table S4). Most interesting, we saw that among down-regulated genes in the quadruple *tcp* mutant margins were many genes related to photosynthesis (Figure 3, Table S2). It has been recently found that many photosynthesis related genes start being expressed close to the timepoint when cell proliferation stops during leaf development (Andriankaja et al., 2012).

We then looked at the expression of photosynthesis related genes (light reactions) in the center and margin of wild-type plants, and found that many of them were stronger expressed in the margins than in the center (Figure 3). However, when we looked at the relative activity levels of these genes in the *tcp* knock outs we found an opposite pattern of expression, with a tendency of photosynthetic genes to be expressed at higher levels in the center compared to the margins (Figure 3). As expected, the effect was stronger in the quadruple than in the double *tcp* mutant, but already clear in *tcp2 tcp4* (Figure 3). Overall, these results strongly suggest that TCPs are necessary to coordinate the maturation program of the photosynthetic machinery in the margin and center of the leaf. We estimated the chlorophyll levels in chloroplasts of developing leaves of wildtype and the *tcp* quadruple knock out by analysing the fluorescence intensity with a laser scanning confocal microscope. Like this we visualized the distribution of chloroplasts and displayed the state of maturation of the leaf in terms of photosynthesis in its different areas. In agreement with other studies (Andriankaja et al., 2012) in wildtype more chlorophyll had accumulated in the distal parts of the leaf and along the margins when compared to the center of the leaf. In the quadruple knockout mutant we detected a lower level of chlorophyll fluorescence in the distal part of the leaf indicating a delayed maturation of chloroplasts (Figure 3). Furthermore, and in contrast to wildtype, less chlorophyll fluorescence was emitted from the marginal regions compared to the central part of the leaf (Figure 3). This confirmed our results towards photosynthesis related genes that we had obtained from the RNAseq experiments and showed the delayed maturation in general and especially in the marginal regions of the quadruple knock out leaves. However, we did not find an enrichment of the GGACCA motif in the promoters of the photosynthetic genes, suggesting that the effect of the TCPs is indirect or at least not caused by their direct binding.

TCPs repress cell proliferation and early leaf developmental programs in the margins

Next, we analyzed upregulated genes in the *tcp* knock outs. We found that in the center region of the *tcp2 tcp4* mutant only 56 genes increased their expression levels (Figure 4), while at the same time 279 genes were upregulated in the *tcp2 tcp3 tcp4 tcp10* blade (Figure 4). 47 of the

56 genes that were enriched in *tcp2 tcp4* out were also upregulated in *tcp2 tcp3 tcp4 tcp10* (Figure 4, Table S5), confirming that largely the genes that are changing their expression levels in the double mutant are a subgroup of those affected in the quadruple mutant.

Drastic were the changes in the margins of the *tcp2 tcp3 tcp4 tcp10* quadruple mutant compared to wildtype, as 799 genes were upregulated (Figure 4, Table S5). Again, the difference between *tcp2 tcp4* and wildtype was much less pronounced with only 123 genes being enriched (Figure 4, Table S5). An analysis for GO-term enrichment within the genes upregulated in the center region of the *tcp2 tcp3 tcp4 tcp10* mutants revealed GO-term enrichment related to stimulus (Figure 4, Table S4). We obtained a different view once we analyzed genes up-regulated in the margins. The most up regulated categories were related to cell cycle and microtubule based processes, as required during mitosis (Figure 4, Table S6). All Arabidopsis *CYCLINB* genes were upregulated in the margins of *tcp2 tcp3 tcp4 tcp10* leaves, while they were unchanged or diminished in the center (Figure 4, Table S6). This is interesting as B type cyclins control the G2 to M transition of the cell cycle (Polyn et al., 2015). We extended the analysis to a list of mitosis specific genes (Menges et al., 2005) and other genes that have already been described to be involved in early leaf development and obtained similar results, finding them to be expressed at higher levels in the *tcp2 tcp3 tcp4 tcp10* compared to wildtype margin (Figure 4, Table S6).

JAGGED was the most up-regulated gene in *tcp* quadruple knock out margins, and *GROTH REGULATING FACTOR 5* and *WUSCHEL-RELATED HOMEOBOX 5* were among the top ten (Figure 4, Table S5). An analysis of genes known to participate in leaf development revealed the upregulation of *ASYMETRIC LEAVES1*, *ASYMETRIC LEAVES2*, *AINTETGUMENTA, and WUSCHEL-RELATED HOMEOBOX 1* (Figure 4, Table S5, Table S6). Most interestingly, these genes changed in the margins of the quadruple knock outs but were largely unaffected in the central leaf regions (Figure 4, Table S6). Overall these results suggest that processes related to early leaf development and cell division remain active specifically in the margins of the *tcp2 tcp3 tcp4 tcp10* leaves when they are already shut down in the wildtype leaf or the central zones of the *tcp* mutant leaves.

Interaction between cell proliferation programs and miR319-regulated TCPs in different leaves.

Previous studies in snapdragon *cin* mutants and young leaves of miR319 overexpressors have shown a delay in the repression of B-type cyclin activity mostly in the organ margins, which has

been associated with a corresponding change in leaf curvature, although microarray studies of these leaves did not reveal an increase in mitotic activity (Nath et al., 2003; Palatnik et al., 2003). That crinkles at the leaf margins of *jaw*-D or quadruple *tcp* mutants increase in leaves that are generated later during the plants life cycle and that cell proliferation markers represent the most up-regulated group of genes in our transcriptomic experiments in leaf five prompted us to look in more detail into the interaction between cell proliferation and the miR319-*TCP* network during development of different leaves.

To study this, we overexpressed miR319a, which causes the same phenotypic effects as a *tcp2 tcp3 tcp4 tcp10* mutant (Figure 1), in the context of a *CYCLINB1;1:GUS* reporter line, which labels cells undergoing mitosis (Donnelly et al., 1999). In wild-type plants the reporter is expressed initially in the whole leaf primordia but then becomes restricted to the base of young developing leaves, until cell proliferation stops and leaves continue to grow by cell expansion (Figure 5) (Donnelly et al., 1999). In the first true leaves, we observed that upon overexpression of miR319 cell proliferation was extended to the margins (Figure 5) and lasted longer than in wildtype, in agreement with previous observations (Nath et al., 2003; Efroni et al., 2008).

However, a closer inspection of the B-type cyclin *CYCLINB1;1* behavior in the fourth leaf of Arabidopsis plants overexpressing miR319 revealed additional changes in the pattern of proliferating cells in these leaves. We found that discrete regions at the margins of the leaf harbored cells undergoing mitosis, in contrast to wild-type leaves (Figure 5, also see insets). This effect was even more pronounced in young seventh leaves (Figure 5). Therefore, Arabidopsis leaves with low TCP activity do no simply have a delay in the mitotic arrest front, but also cell proliferation continues to be activated in specific subdomains at the leaf margins. The *foci* of dividing cells persisted for extended periods, even after proliferation in wild-type leaves had completely ceased (Figure 5). Leaves produced by older plants normally have more serrations than the younger ones (Poethig, 2003). In the case of miR319 overexpressors, we also noticed that more discrete *foci* harboring proliferating cells were detected rather in later than in earlier leaves, which is consistent with a larger number of crinkles in these leaves. We propose that probably an extension of the cell proliferation, such as seen in the first leaves of Arabidopsis or snapdragon would cause a change in leaf curvature, but the formation of discrete *foci* would result in crinkles along the leaf margins as seen in leaves produced later during development of miR319 overexpressors or *tcp* mutants (Figure 5).

Identification of margin specific genes independent of TCPs

In our analysis we had identified 237 genes enriched in the center and 141 genes in the margins of wild-type leaves (Figure 2, Table S1). We performed the same analysis with the margin and center samples of the *tcp2 tcp4* and *tcp2 tcp3 tcp4 tcp10* leaves and searched for center and margin enriched genes in the mutants. We found that of the 237 genes enriched in the center of the wild-type leaves, 154 (approximately 65%) were still enriched in the center of the quadruple *tcp* mutant leaves (Table S1, Figure S2). In contrast, when we looked at the expression of the 141 margin-specific genes that we identified in wild-type leaves, we observed that only 31 continued to be enriched in the margin of quadruple *tcp* mutants (Table S1, Figure S2).

We focused then on the genes that were enriched in the margins of both wild-type and *tcp* quadruple mutant leaves, suggesting that the expression of these genes will be at least partially independent of the miR319-regulated TCPs. Interestingly, among the total of 31 genes (Table 1), we found eight transcription factors belonging to the groups of *NGA*, *STY* and *CIN*-LIKE *TCP*s. More specifically, we identified *NGA1*, *NGA3*, *STY1*, *STY2* and *SHI-RELATED SEQUENCE 4* as well as the three *CIN*-LIKE *TCP*s *TCP5*, *TCP13* and *TCP17* to be enriched in both, wild-type and mutant margins (Table 1). Interesting was also the identification of the auxin biosynthesis gene *YUCCA2* as margin enriched, as it is thought to be regulated by *NGA*s (Trigueros et al., 2009). The *STY* gene family consists of eight members, three of which we determined to be margin enriched. Noteworthy, some of these genes have already been described to play a role in margin development either of the gynoecium or the leaf. So have multiple *sty* mutants and plants with reduced *NGA* activity stronger serrated leaves than wildtype (Kuusk et al., 2002; Kuusk et al., 2006; Alvarez et al., 2009).

We suggest that at least part of the identified group of 31 genes that continued to be expressed in the margins of the *tcp* mutants likely represent pathways that are involved in margin development in a manner independent of miR319 regulated *TCP*s. This would also be in agreement with the additive or synergistic effects observed in margin development when overexpressing miR319 and simultaneously decreasing other *CIN*-like *TCP*s (Efroni et al., 2008) or *NGA* genes (Alvarez et al., 2016). Still, several of these genes quantitatively decrease in *tcp* mutants (Table 1), suggesting that complex regulatory networks might exist that relate these transcription factors. In good agreement, *NGA2* was enriched in the margins of the quadruple knockout compared to its blade region, albeit its level of expression was decreased (Table S7).

Vasculature development is impaired in the *tcp2 tcp3 tcp4 tcp10* mutant

In the analysis of the RNAseq experiments we also observed that several genes related to auxin biosynthesis or signaling were downregulated in the margins of the *tcp2 tcp3 tcp4 tcp10* mutant, including 19 *SMALL AUXIN UPREGULATED RNA* genes and eight *INDOLE-3-ACETIC ACID INDUCIBLE* genes (Table S2). Auxin is known to play an important role in the establishment of the vasculature (Scarpella et al., 2006) and observations of alterations in the vasculature organization have also been reported in studies with TCP3-SRDX constructs (Li and Zachgo, 2013). We analyzed the venation patterns in the *tcp2 tcp4* double and *tcp2 tcp3 tcp4 tcp10* quadruple mutants compared to wildtype. In the Columbia wildtype cotyledon we usually observed one midvein and secondary vein loops parting from it, generating four areoles (Figure 6). In the *tcp2 tcp4* and *tcp2 tcp3 tcp4 tcp10* mutants we observed significant occurrence of distal pegs, ectopic vein pieces distal from the closing loops which end unconnected and close to the distal margin of the cotyledon (Figure 6). We further observed an increased tendency for secondary veins ending unconnected and therefore not generating proximal areoles. However, the total average number of areoles was slightly higher in the quadruple mutant, due to an increased number of branchpoints (Figure 6). Furthermore, the quadruple mutant veins were often not straight but had a wiggly appearance (Figure 6 insets).

We also analyzed the vasculature of the first true leaf, which is more complex than that of cotyledons. We found that the numbers of branch points and areoles significantly increased in the double and quadruple mutants with respect to wildtype (Figure 6). Moreover, the vasculature per leaf area increased, and in turn decreased the average size of the areoles in double and quadruple knock outs (Figure 6). Taken together these data demonstrated that the *tcp2 tcp3 tcp4 tcp10* leaf had a more extended vascular system than wildtype, and that these differences are already seen between wildtype and *tcp2 tcp4* double mutants.

Discussion

In this study, we analyzed the effects of reduced TCP activity on different domains of the growing leaf. We employed a set of cumulative single and multiple mutants with decreasing TCP levels focusing on *tcp2 tcp4* double mutants that have large flat leaves and *tcp2 tcp3 tcp4 tcp10* quadruple mutants with strongly crinkled organs. We dissected the leaf center and marginal regions. In a first step our analyses revealed many genes that were enriched in either margins or central areas of developing wildtype Arabidopsis leaves. In subsequent analyses of

the *tcp* double and quadruple mutant samples we identified both, organ-wide and margin specific effects of the *TCP*s. Most conspicuously, cell proliferation genes were significantly upregulated only in the margins of the *tcp* mutants.

Spatial control of gene expression by TCP transcription factors

Leaf size initially increased with loss of TCP activity. However, it did not continue to increase steadily in triple and quadruple knockouts, but rather stagnated. Leaves from quadruple *tcp* mutants were smaller than those from double mutants and had mesophyll cells that were significantly smaller than those of their wildtype and double mutant counterparts. The reduced cell size is likely the consequence of a disturbed or delayed cell differentiation in the quadruple knock-out leaves, which is in agreement with other reports describing a role for TCPs in the control of differentiation (Efroni et al., 2008; Sarvepalli and Nath, 2011, 2011).

Previous work has focused on the analysis of whole leaves or transversal organ sections (Efroni et al., 2008; Li et al., 2010; Pettko-Szandtner et al., 2015), reflecting the developmental program along the proximo-distal axis of the leaf. Here, we focus on the medial to lateral axis of the organ by performing a comparative analysis of leaf margins and center regions. Using this approach, we detected large numbers of genes differentially expressed in the center or margin of the leaf. Mutations in the TCPs affected gene activity in both, the center and margin of the leaves, however, margins were much more affected even *in tcp2 tcp4* double mutants that have flat leaves, confirming a primary role of these transcription factors in the developmental control of the leaf margins. Even though *tcp* mutants have largely affected leaf margins, we detected a small group of genes that continued to be enriched in wildtype and *tcp* mutant leaf margins. Several of the margin enriched genes detected here have already been described with biological functions related to margins. They include for example eight transcription factor genes of the *CIN-like TCPs, NGA* and *STY* gene families and hormone biosynthetic genes such as *YUCCA2*. Downregulation of the CIN-like *TCP*s *TCP5*, *TCP13* and *TCP17* has been shown to synergistically enhance the effects of miR319-regulated TCPs in the control of leaf growth (Efroni et al., 2008). *NGA* and *STY* genes have been known to be important in the generation of margins, initially of the gynoecium but also in leaf development. They influence organ shape, as seen in *sty1 sty2* double knock outs, as well as quadruple *nga1 nga2 nga3 nga4* knock outs, which also have leaves that are larger and more serrated than wild type. In contrast, over-expressors of *STY1* or *NGA genes* have smooth leaf margins (Kuusk et al., 2002; Alvarez et al., 2009; Trigueros et al., 2009; Lee et al., 2015). *NGA*

genes also seem to regulate *YUCCA2* (Trigueros et al., 2009), which we identified to be margin enriched. Recently, a combined knockdown of miR319 regulated *TCP*s and *NGA* genes has been shown to have a dramatic additive effect on leaf development with undetermined growth of the margins (Alvarez et al., 2016). In this elegant study it was concluded that miR319-regulated *TCP*s and *NGA*s are working redundantly in the inhibition of meristematic activity in the leaf margins and its proximal part. It may be plausible to think that simultaneous down-regulation of the margin enriched genes identified here together with the miR319-regulated *TCP*s would also cause synergistic effects in margin development. Furthermore, it is worthwhile to note that all three group of transcription factors have not only be implied in leaf development, affecting shape and vasculature, but also in gynoecium or fruit development, suggesting that indeed they may be able to entertain close interactions (Palatnik et al., 2003; Kuusk et al., 2006; Martinez-Fernandez et al., 2014).

The origination of the vascular pattern in the leaf is closely related to the establishment of leaf shape and to the presence of auxin convergence points in the leaf margins (Scarpella et al., 2006; Scarpella et al., 2010). Therefore, disturbed margin development may go hand in hand with defects in auxin signaling or biosynthesis and may be the reason for the observed alterations in the *tcp* mutants vasculature.

A careful analysis of the group of downregulated genes in the *tcp* mutant margins compared to the center samples revealed that it contained many genes related to photosynthesis. This is in contrast to what is seen in wildtype where in general photosynthesis related genes were expressed at higher levels in the margins than in the center region of the leaf. We visualized by confocal microscopy that the distribution of chlorophyll in the leaf was as predicted by the transcriptome analysis and confirmed a delayed maturation of the photosynthetic apparatus in the *tcp2 tcp3 tcp4 tcp10* leaf, especially in the marginal regions.

Simultaneous downregulation of miR319 regulated *TCP*s and *NGA* genes results in strongly crinkled leaves with white margins indicating the lack of chlorophyll (Alvarez et al., 2016). Interestingly, we observed a change in the relative expression domain of photosynthetic genes also in *tcp2 tcp4* double mutants, suggesting that TCP activity quantitatively regulates the expression of photosynthesis related genes. The activation of photosynthesis happens upon the decision of differentiation very early in leaf development, and chloroplast differentiation is linked to the control of cell proliferation (Andriankaja et al., 2012). Therefore, a delay in the activation of photosynthesis related genes upon the reduction or lack of TCP activity aids further into the role of the *TCP*s as coordinators of cell proliferation and differentiation with specific roles in the medial-lateral axis of the leaf blade. Our results are

459 also in agreement with earlier observations which indicated that TCPs trigger the
460 differentiation of leaf cells (Efroni et al., 2008).

461 Although the *NGA* genes were identified as margin enriched genes in wild-type and in
462 *tcp2 tcp3 tcp4 tcp10* margins the expression levels of all detected *NGA* genes were reduced in
463 the double and quadruple *tcp* mutant samples compared to wildtype in both center and
464 marginal regions. These results are consistent with previous data showing that TCP2 and TCP3
465 can bind to a conserved promoter element of all *NGA* genes in yeast experiments and also to
466 the *NGA3* promoter in transient *Nicotiana benthamiana* assays and activate it (Ballester et al.,
467 2015). Overall, the results suggest that the microRNA regulated TCPs partially control *NGA*
468 expression, both in the center and margins of the leaf (our results, (Ballester et al., 2015)), and
469 that the two groups of transcription factors synergistically control margin development
470 (Alvarez et al., 2016). The margin specific *STY* genes did not show a uniform downregulation in
471 the *tcp* knock out samples which suggests that their functions in margin development might be
472 less connected to the *TCPs* than those of the *NGAs*.

473

474 **Margin-specific control of cell proliferation by miR319-regulated TCPs**

475 While previous transcriptomic analyses did not detect changes in the expression of cell
476 cycle genes upon overexpression of miR319 (Efroni et al., 2008; Schommer et al., 2008), our
477 specific analysis of young leaf margins identified a major up-regulation of genes known to
478 participate in cell proliferation in the *tcp* mutants. An analysis for GO term enrichment in the
479 *tcp2 tcp3 tcp4 tcp10* margin samples resulted in rankings with GO terms related to cell cycle
480 and microtubule based processes being the top overrepresented groups.

481 An interaction between the TCP network and the cell cycle machinery, with other
482 factors involved in the control of plant age has been suggested. Recent results point towards
483 the TCPs interacting with SQUAMOSA PROMOTER BINDING PROTEIN-LIKE transcription factors
484 (Rubio-Somoza et al., 2014), which might provide further explanations for different effects of
485 the TCPs in different leaves. Analysis of a CYCLINB1;1 reporter revealed changes in its
486 expression pattern that were dependent on the point of appearance of the leaf during the
487 plants ontogeny. Leaves of plants overexpressing miR319 and of *tcp2 tcp3 tcp4 tcp10* knock
488 outs that are produced later in plant life history have strong crinkles at their margins in
489 addition to a general change in leaf curvature. In the first pair of true leaves, we found that cell
490 proliferation was extended towards the margins and detectable for a longer period of time,
491 similar to what has been described in previous studies (Nath et al., 2003; Efroni et al., 2008).

However, in higher order leaves we observed that cell proliferation was maintained in discrete *foci* along the leaf margins, until later stages of leaf development. We propose that theses *foci* are responsible for the localized generation of extra cells that cause the conspicuous phenotype of crinkled leaves as observed in adult plants with low TCP activity due to the overexpression of miR319 or loss of function of multiple *TCP* genes.

The changes in cell proliferation observed in the margins of plants with very low TCP activity are consistent with a role of the miR319-regulated TCPs in the control of the marginal meristem of the leaf (Donnelly et al., 1999; Alvarez et al., 2016). However, despite their prominent effects on margin development the TCPs do have functions in the control of cell proliferation in the leaf blade. Leaves of the single and double knock outs that do not obviously change their morphological appearance are larger than wild-type leaves. Furthermore, the snap dragon *cincinnata* mutant has been described to show bulky growth of the intervein sections (Nath et al., 2003) which might suggest that also in the leaf blade some cells remain with mitotic activity that should have ceased to proliferate to achieve the neutral curvature of the blade.

That *TCP2*, *TCP3*, *TCP4*, and *TCP10* were not identified as margin-enriched genes, hints that their tissue-specific effects may not be directly achieved through their transcriptional regulation. Recent studies show that TCPs can interact and form protein complexes with other transcription factors. In a global analysis where dimerization abilities of all Arabidopsis TCP family members were tested by yeast two hybrid assays, it turned out that miR319 regulated TCPs have a strong tendency to hetero-dimerize with TCP5, TCP13 and TCP17 (Danisman et al., 2013). Curiously, the transcripts of these exact three members of the TCP family were identified in our analyses to be margin enriched. It might be that heterodimers between the miR319 regulated TCPs and the closely related TCP5, 13 and 17 are necessary to guarantee normal leaf development in the margins. In addition, it has been suggested that changes in leaf morphogenesis during the plant life cycle can be achieved by competition of transcription factor complexes between TCPs, members of the SQUAMOSA PROMOTER BINDING PROTEIN-LIKE and CUC transcription factor families. The function of TCPs may be to sequester CUC2 and CUC3 proteins in order to avoid functional CUC dimers to build (Rubio-Somoza et al., 2014), with CUC2 especially being expressed in the marginal leaf regions (Nikovics et al., 2006). Furthermore, TCP4 interacts with other proteins like TCP INTERACTOR CONTAINING EAR MOTIF PROTEIN 1 which then leads to the establishment of functional complexes that regulate transcription (Tao et al., 2013). TCP4 has also been shown to interact with chromatin remodeling complexes harboring BRHAMA ATPases (Efroni et al., 2013). Taken together, it

seems possible that the formation of different protein complexes may be one of the ways in which the TCPs achieve their various ways of action throughout the plants lifecycle and in different regions of the leaf.

Materials and Methods

Plant Material

Arabidopsis thaliana accession Columbia (Col-0) wildtype and mutants were grown at 23°C in long day conditions (16h light/8h dark). *tcp2*, *tcp4* and *tcp10* insertional mutants and the cyclinB reporter have been described in earlier work (Schommer et al., 2008; Rodriguez et al., 2010). The *tcp3* insertional mutant was obtained from ABRC, stock number: CS855978.

Measurements of Leaf Size

Leaves were detached and mounted between two glass plates before photographing with illumination from below. Leaf circumferences were calculated with ImageJ software and the folded areas that were identified by darker color in the image were calculated correspondingly.

Measurement of Cell Size and Number and Observation of Vasculature

To obtain paradermal views of palisade cells, leaves were fixed with FAA and cleared with chloral hydrate solution as described (Horiguchi et al., 2005). Palisade leaf cells were observed using differential interference contrast (DIC) microscopy.

β-glucoronidase assays

To visualize the activity of the reporters, transgenic plants were subjected to GUS staining, according to Donnelly et al. (Donnelly et al., 1999). Stained tissue was transferred to 70% EtOH.

RNA-Seq experiments

For this experiment seeds were surface sterilized sown on plates with MS-Agar medium and stratified for at least 2 days. To synchronize the developmental stage of the three genotypes at tissue collection, an offset of 2 days was generated before placing in the greenhouse the plates of the next plant line, starting with *tcp2 tcp3 tcp4 tcp10* and ending with Col-0. Once in the greenhouse, plates were grown at 23°C in long day conditions (16h light/8h dark) for 5 days, at which point seedlings were transplanted to individual soil pots. At emergence of the 7th leaf, the 5th leaf of each plant was harvested at Zeitgeber time (ZT) 6, dissected on a cold plate into "Center" and "Margin" samples as shown in Figure 2, and transferred to separate tubes containing 200ul of RNALater™ solution (Ambion #AM7020). Each "Center" and "Margin" sample consisted of the center and margin portions respectively of the 5th leaf of 15-20 different individual plants. This was performed by quadruplicates for each line. After tissue harvest, RNALater™ was removed by placing the contents of each tube on a piece of sterile absorbent paper, and the sample was immediately flash frozen in liquid nitrogen. Total RNA was extracted using TRIzol™ (Invitrogen #15596-018), treated with Turbo™ DNase (Ambion #AM2238), and re-purified by Phenol-Chloroform-Isoamyl Alcohol (25:24:1, v/v) extraction. Total RNA integrity was confirmed on the BioAnalyzer™ (Agilent). Barcoded libraries were constructed using the Illumina TruSeq™ RNA kit with an average of 1 μg of total RNA as starting material. Library quality was monitored on the BioAnalyzer™ and then sequenced as 100-bp single end reads using an Illumina HiSeq™ sequencer. Resulting RNA-Seq raw data was processed as described elsewhere (Anders et al., 2013) and differential expression assessed with edgeR v.3.8.6 (Robinson et al., 2010). Differentially expressed genes were selected based on a minimum fold-change of ±2 and a FDR < 0.01 (False Discovery Rate, i.e. p-value adjusted for multiple testing with the Benjamini-Hochberg procedure). GO analysis was performed with AGRIGO (Du et al., 2010; Tian et al., 2017). Functional enrichments were visualized with MAPMAN (Thimm et al., 2004).

Supplemental Information

Supplemental Figure 1
M (´minus') vs. A ('add') plots for RNAseq data.
Supplemental Figure 2
Center and Margin enriched transcripts.
Supplemental Table S1
Center and Margin enriched genes.
Supplemental Table S2
Downregulated Genes in *tcp* mutants.

Supplemental Table S3
Identification of GGACCA boxes in downregulated genes.
Supplemental Table S4
GO-term enrichment analysis in center and marginal parts of *tcp* mutants.
Supplemental Table S5
Upregulated Genes in *tcp* mutants.
Supplemental Table S6
Mitosis and early leaf development associated genes in *tcp* mutants.
Supplemental Table S7
Expression levels of *NGA* and *STY* genes.

Accession Numbers

The RNAseq data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (Edgar et al., 2002) and are accessible through GEO Series accession number GSE99854 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GSE99854).

Acknowledgements: RNAseq experiments were carried out in Detlef Weigel's lab under the supervision of N. Rubio-Somoza and with the help of C. Becker. We thank members of the RNA Biology lab for critical discussions.

Table 1. Expression levels of margin specific genes independent of TCP activity. Expression values of double and quadruple knock out centers and margins are expressed as normalized counts per million (CPM) and fold change of margin samples compared to center samples. n/d: not detected; n/s: non-significant.

		normalized counts per million			normalized counts per million			Fold-change (FC)		
		Center			Margin			Margin/Center		
Transcription Factors		WT	2KO	4KO	WT	2KO	4KO	WT	2KO	4KO
AT1G01030	NGA3	2.80±0.38	1.31±0.86	0.51±0.09	6.79±0.82	5.01±0.64	3.43±0.91	2,41	4,16	6,49
AT2G18120	SRS4	0.81±0.07	0.92±0.39	1.26±0.32	2.25±0.52	2.26±0.67	4.04±0.73	2,78	n/s	3,15
AT2G46870	NGA1	8.77±1.10	4.43±1.33	2.47±0.90	25.54±2.62	18.93±2.09	13.2±0.46	2,92	4,45	5,33
AT3G02150	TCP13	5.79±1.52	5.97±1.53	5.04±1.44	12.19±2.24	13.34±1.10	10.75±2.04	2,11	2,19	2,09
AT3G51060	STY1	0.12±0.00	0.09±0.09	n/d	1.18±0.39	1.24±0.52	0.47±0.10	8,45	11.60	39,15
AT4G36260	STY2	0.16±0.31	0.04±0.07	0.31±0.31	2.23±0.88	1.85±0.20	3.44±1.13	12,25	30,58	10,81
AT5G08070	TCP17	1.46±0.24	1.01±0.52	1.52±0.69	4.34±0.68	4.59±0.69	4.45±0.60	2,95	4.30	2,94
AT5G60970	TCP5	4.99±0.50	4.93±0.53	5.53±1.56	11.73±2.31	13.31±1.93	14.05±1.35	2,32	2,62	2,58
Hormones and Metabol.										
AT1G56710	Pectin lyase superfam.	0.08±0.16	0.04±0.07	0.13±0.19	2.69±0.75	3.01±1.99	0.92±0.33	27,44	50,85	6,17
AT1G70560	CKRC1	3.09±0.20	2.82±0.45	1.51±0.38	13.77±0.19	9.17±1.60	5.77±1.03	4,45	3,35	3,78
AT2G04160	AIR3	10.50±1.78	5.93±0.79	0.67±0.31	53.78±8.03	26.16±2.61	3.35±0.53	5,09	4,44	5,03
AT2G23170	GH3.3	0.20±0.16	0.20±0.29	1.36±0.32	1.23±0.79	0.27±0.26	4.37±0.86	5,72	n/s	3,12
AT2G43840	UGT74F1	1.42±0.41	0.97±0.35	0.54±0.03	3.56±0.22	1.54±0.43	1.59±0.44	2.50	n/s	2.90
AT3G13380	BRL3	4.74±1.56	4.48±1.40	2.58±0.82	10.09±2.46	7.36±0.97	5.26±0.15	2,13	n/s	2,02
AT4G13260	YUC2	2.35±0.44	2.21±1.23	1.28±0.44	8.06±1.22	6.71±0.96	4.81±0.31	3,38	3.20	3,79
AT5G26220	GGCT2;1	1.02±0.66	3.49±2.98	1.46±0.65	4.55±1.91	7.08±4.55	6.83±3.32	4,43	n/s	4,67
AT5G28030	DES1	2.76±0.51	1.84±0.21	0.58±0.27	15.33±4.13	11.13±0.41	5.63±1.33	5,49	5,98	9,43
AT5G59130	Subtilase fam.	15.83±0.21	18.76±4.4	12.99±3.19	34.49±3.43	39.81±3.05	30.67±3.51	2,18	2,14	2,37

Transport related

AT1G74810	BOR5	5.28±1.11	7.87±0.34	9.96±2.35	11.43±0.83	13.87±1.1	20.41±0.66	2,15	1,75	2.10
AT2G36590	PROT3	3.97±0.30	3.24±0.49	2.35±0.70	10.30±0.56	6.95±2.13	5.28±1.23	2,59	2,11	2,21
AT4G24120	YSL1	7.14±1.58	6.17±0.93	4.98±1.94	14.61±6.32	18.42±1.55	10.61±1.42	2,03	2,96	2,08
AT5G17700	MATE efflux fam.	16.59±3.74	16.27±2.94	6.13±1.85	43.7±1.04	50.25±7.07	19.62±3.36	2,64	3,14	3,25
AT5G50790	SWEET10	0.61±0.23	0.43±0.27	0.14±0.07	2.00±0.55	3.42±0.83	1.85±0.57	3,29	7,08	12,71
AT5G55930	OPT1	9.00±3.88	7.36±1.61	4.18±0.75	23.03±5.30	19.51±1.28	9.59±1.31	2,57	2,61	2,28

Others

AT1G22900	Disease resist.-respons.	0.12±0.24	0.15±0.04	0.14±0.28	6.15±0.12	5.00±0.48	1.16±0.79	44,65	32,28	7,97
AT2G47880	Glutaredoxin fam.	2.68±1.56	3.13±1.51	0.77±0.11	30.60±5.42	26.33±6.45	2.16±0.12	11,39	8,63	2,84
AT3G05730	unknown	15.44±3.12	4.55±2.44	4.39±3.06	652.26±60.06	341.85±26.96	11.08±1.97	42,17	76,86	2,52
AT3G09520	EXO70H4	3.21±0.94	3.61±1.14	3.11±0.57	7.41±1.86	6.13±1.39	7.72±1.50	2,33	n/s	2,44
AT3G16120	Dyn.light chain t1 fam.	0.97±0.12	1.18±0.44	1.64±0.50	2.66±0.76	3.79±0.76	5.71±0.75	2,76	3,26	3.40
AT3G16660	Pollen Ole e 1	1.58±0.15	0.36±0.19	0.65±0.53	69.08±5.14	36.25±3.71	3.27±0.41	43,25	93,08	5.20
AT5G37540	Euk. Asp. protease fam.	2.68±0.41	2.57±0.19	2.35±0.24	5.86±1.80	5.67±1.97	5.84±1.13	2,21	2.10	2,48

621 **Figure Legends**
622

623 **Figure 1.** Effects of decreasing TCP levels on leaf size and shape. A, Four weeks old rosettes of
624 Arabidopsis plants with decreased levels of TCP activity. B, Disassembled rosettes of indicated
625 genotypes. C, Leaf area of leaf number one and two of the indicated genotypes. Marked in orange the
626 contribution of folded regions to the total leaf area (see Materials and Methods). D, Number of cells in
627 the same leaves as in C. E, Cell area of the same leaves in C, asterisk indicating statistical significance of
628 difference according to $p<0.01$ by Student's t-test. F, Cleared and flattened leaves of the different
629 genotypes that have reduced TCP activity. Yellow arrows indicate folded regions. Violet bars used to
630 illustrate how leaf size and curvature develop with respect to the gradual reduction of TCP activity. The
631 mutants *tcp2 tcp4*, *tcp2 tcp4 tcp10* and *tcp2 tcp3 tcp4 tcp10* are labelled *tcp2-4, tcp2-4-10* and *tcp2-3-4-*
632 *10* respectively.

633
634 **Figure 2.** Transcriptome analysis of leaf domains of wild type and *tcp* mutants. A, Rosettes with
635 emerging leaf number five as used for transcriptome analysis by RNAseq experiments. B, Schematic
636 display of regions that were used as marginal and center samples to obtain RNA for the RNAseq
637 experiments. In wild type 237 center and 141 margin enriched genes were identified. C, Graphical
638 display as fold change of behaviour of genes that were determined in B to be margin or center enriched
639 in wild type samples, in *tcp2 tcp4* and *tcp2 tcp3 tcp4 tcp10* mutants. The black line represents the
640 average expression level of all margin or center enriched genes. D, Multidimensional scaling (MDS) plot
641 for count data (all genes). Distances correspond to leading log-fold-changes between each pair of RNA
642 samples.

643
644 **Figure 3**. Coordination of gene expression related to photosynthesis by miR319-regulated TCPs. A, Venn
645 diagram showing the intersections of downregulated genes in margins and centers of *tcp2 tcp4* and *tcp2*
646 *tcp3 tcp4 tcp10* compared to wildtype. B, Promoter analysis of downregulated genes in *tcp2 tcp4* and
647 *tcp2 tcp3 tcp4 tcp10* center and margin regions for overrepresentation of TCP binding motif GGACCA. C,
648 Graphical display of MapMan analysis of gene expression for photosynthesis related genes in *tcp2 tcp3*
649 *tcp4 tcp10* margins compared to wildtype. D, Graphical display of relative expression changes of
650 photosynthesis related genes between center and marginal regions in wildtype*, tcp2 tcp4* and *tcp2 tcp3*
651 *tcp4 tcp10* as determined by RNAseq experiments. The mutants *tcp2 tcp4* and *tcp2 tcp3 tcp4 tcp10* are
652 labelled *tcp2-4* and *tcp2-3-4-10* respectively. E, Chlorophyll content in developing young leaves as
653 estimated by their fluorescence under a light scanning confocal microscope. Left with close up wildtype,
654 *tcp2 tcp3 tcp4 tcp10* and close up, right. The coloured bar indicates the fluorescence intensity profile
655 used from blue (low fluorescence) to white (high fluorescence).
656

657 **Figure 4.** Up-regulation of the leaf developmental program and mitotic genes in *tcp* mutant margins. A,
658 Venn diagram showing the intersections of upregulated genes in margins and centers of *tcp2 tcp4* and
659 *tcp2 tcp3 tcp4 tcp10* compared to wildtype. B, Output of gene ontology (GO) enrichment analysis for
660 upregulated genes in *tcp2 tcp3 tcp4 tcp10* margin and center regions. C, Display of expression changes
661 of B-type cyclin genes in *tcp2 tp4* and *tcp2 tcp3 tcp4 tcp10* center and marginal leaf samples in RNAseq
662 analysis. Y-axis is log fold change compared to wildtype. D, Display of expression changes of selected
663 genes that are active in early stages of leaf development in *tcp2 tp4* and *tcp2 tcp3 tcp4 tcp10* center and

marginal leaf samples. Y-axis is log of fold change of mutant samples compared to wildtype. The mutants *tcp2 tcp4* and *tcp2 tcp3 tcp4 tcp10* are labelled *tcp2-4* and *tcp2-3-4-10* respectively.

Figure 5. Activation of discrete foci expressing CYCLINB1;1GUS upon TCP downregulation. A-L, β–glucoronidase assays. A-D,I,K young developing leaves of a CYCLINB1;1-GUS reporter line. E-H,J,L, young leaves of the same a CYCLINB1;1-GUS reporter overexpressing miR319a, resulting in crinkly leaves. A, Leaf one seven DAS. B, Leaf one nine DAS. C, Leaf one at 11 DAS. D, Leaf four, E, Leaf one of miR319 overexpressor at eight DAS. F, Leaf one of miR319 overexpressor at 10 DAS. G, Leaf one of miR319 overexpressor at 12 DAS. The dashed line delimits the domain containing cycling cells. H, Leaf four miR319 overexpressor. I, Leaf seven. J, Leaf seven of miR319 overexpressor. K,L More mature leaves of wildtype (K) and miR319a overexpressor (L). M, Model showing the effect of miR319 regulated TCPs on cell division in the developing leaf of wild-type (top) and *tcp* quadruple knock outs or miR319 overexpressing plants (bottom). Blue dots indicate proliferating cells. Scale bars: A,E,I,J 0,2mm, B,C,F,G 0,5mm, D,H,K,L 1mm.

Figure 6. Modification of the venation pattern in *tcp* mutants. A-C, Cleared cotyledons of wildtype (A), *tcp2 tcp4* (B) and, *tcp2 tcp3 tcp4 tcp10* (C). D-F, Cleared first leaves of wildtype (D), *tcp2 tcp4* (E) and *tcp2 tcp3 tcp4 tcp10* (F). G, table summarizing the scored parameters describing the venation pattern. Scale bars: 1mm.

References

Alvarez JP, Furumizu C, Efroni I, Eshed Y, Bowman JL (2016) Active suppression of a leaf meristem orchestrates determinate leaf growth. Elife 5

Alvarez JP, Goldshmidt A, Efroni I, Bowman JL, Eshed Y (2009) The NGATHA distal organ development genes are essential for style specification in Arabidopsis. Plant Cell 21: 1373-1393

Alvarez JP, Pekker I, Goldshmidt A, Blum E, Amsellem Z, Eshed Y (2006) Endogenous and synthetic microRNAs stimulate simultaneous, efficient, and localized regulation of multiple targets in diverse species. Plant Cell 18: 1134-1151

Anders S, McCarthy DJ, Chen Y, Okoniewski M, Smyth GK, Huber W, Robinson MD (2013) Count-based differential expression analysis of RNA sequencing data using R and Bioconductor. Nat Protoc 8: 1765-1786

Andriankaja M, Dhondt S, De Bodt S, Vanhaeren H, Coppens F, De Milde L, Muhlenbock P, Skirycz A, Gonzalez N, Beemster GT, Inze D (2012) Exit from proliferation during leaf development in Arabidopsis thaliana: a not-so-gradual process. Dev Cell 22: 64-78

Ballester P, Navarrete-Gomez M, Carbonero P, Onate-Sanchez L, Ferrandiz C (2015) Leaf expansion in Arabidopsis is controlled by a TCP-NGA regulatory module likely conserved in distantly related species. Physiol Plant 155: 21-32

Beemster GT, De Veylder L, Vercruysse S, West G, Rombaut D, Van Hummelen P, Galichet A, Gruissem W, Inze D, Vuylsteke M (2005) Genome-wide analysis of gene expression profiles associated with cell cycle transitions in growing organs of Arabidopsis. Plant Physiol 138: 734-743

Cubas P, Lauter N, Doebley J, Coen E (1999) The TCP domain: a motif found in proteins regulating plant growth and development. Plant J 18: 215-222

709 Danisman S, van Dijk AD, Bimbo A, van der Wal F, Hennig L, de Folter S, Angenent GC, Immink RG (2013)
710 Analysis of functional redundancies within the Arabidopsis TCP transcription factor family. J Exp
711 Bot 64: 5673-5685
712 Donnelly PM, Bonetta D, Tsukaya H, Dengler RE, Dengler NG (1999) Cell cycling and cell enlargement in
713 developing leaves of Arabidopsis. Dev Biol 215: 407-419
714 Du Z, Zhou X, Ling Y, Zhang Z, Su Z (2010) agriGO: a GO analysis toolkit for the agricultural community.
715 Nucleic Acids Res 38: W64-70
716 Edgar R, Domrachev M, Lash AE (2002) Gene Expression Omnibus: NCBI gene expression and
717 hybridization array data repository. Nucleic Acids Res 30: 207-210
718 Efroni I, Blum E, Goldshmidt A, Eshed Y (2008) A protracted and dynamic maturation schedule underlies
719 Arabidopsis leaf development. Plant Cell 20: 2293-2306
720 Efroni I, Han SK, Kim HJ, Wu MF, Steiner E, Birnbaum KD, Hong JC, Eshed Y, Wagner D (2013) Regulation
721 of leaf maturation by chromatin-mediated modulation of cytokinin responses. Dev Cell 24: 438-
722 445
723 Engelhorn J, Reimer JJ, Leuz I, Gobel U, Huettel B, Farrona S, Turck F (2012) Development-related PcG
724 target in the apex 4 controls leaf margin architecture in Arabidopsis thaliana. Development 139:
725 2566-2575
726 Horiguchi G, Kim GT, Tsukaya H (2005) The transcription factor AtGRF5 and the transcription coactivator
727 AN3 regulate cell proliferation in leaf primordia of Arabidopsis thaliana. Plant J 43: 68-78
728 Kawamura E, Horiguchi G, Tsukaya H (2010) Mechanisms of leaf tooth formation in Arabidopsis. Plant J
729 62: 429-441
730 Kazama T, Ichihashi Y, Murata S, Tsukaya H (2010) The mechanism of cell cycle arrest front progression
731 explained by a KLUH/CYP78A5-dependent mobile growth factor in developing leaves of
732 Arabidopsis thaliana. Plant Cell Physiol 51: 1046-1054
733 Kosugi S, Ohashi Y (1997) PCF1 and PCF2 specifically bind to cis elements in the rice proliferating cell
734 nuclear antigen gene. Plant Cell 9: 1607-1619
735 Koyama T, Furutani M, Tasaka M, Ohme-Takagi M (2007) TCP transcription factors control the
736 morphology of shoot lateral organs via negative regulation of the expression of boundary-
737 specific genes in Arabidopsis. Plant Cell 19: 473-484
738 Kuusk S, Sohlberg JJ, Long JA, Fridborg I, Sundberg E (2002) STY1 and STY2 promote the formation of
739 apical tissues during Arabidopsis gynoecium development. Development 129: 4707-4717
740 Kuusk S, Sohlberg JJ, Magnus Eklund D, Sundberg E (2006) Functionally redundant SHI family genes
741 regulate Arabidopsis gynoecium development in a dose-dependent manner. Plant J 47: 99-111
742 Lee BH, Kwon SH, Lee SJ, Park SK, Song JT, Lee S, Lee MM, Hwang YS, Kim JH (2015) The Arabidopsis
743 thaliana NGATHA transcription factors negatively regulate cell proliferation of lateral organs.
744 Plant Mol Biol 89: 529-538
745 Li P, Ponnala L, Gandotra N, Wang L, Si Y, Tausta SL, Kebrom TH, Provart N, Patel R, Myers CR, Reidel EJ,
746 Turgeon R, Liu P, Sun Q, Nelson T, Brutnell TP (2010) The developmental dynamics of the maize
747 leaf transcriptome. Nat Genet 42: 1060-1067
748 Li S (2015) The Arabidopsis thaliana TCP transcription factors: A broadening horizon beyond
749 development. Plant Signal Behav 10: e1044192
750 Li S, Zachgo S (2013) TCP3 interacts with R2R3-MYB proteins, promotes flavonoid biosynthesis and
751 negatively regulates the auxin response in Arabidopsis thaliana. Plant J 76: 901-913
752 Martinez-Fernandez I, Sanchis S, Marini N, Balanza V, Ballester P, Navarrete-Gomez M, Oliveira AC,
753 Colombo L, Ferrandiz C (2014) The effect of NGATHA altered activity on auxin signaling
754 pathways within the Arabidopsis gynoecium. Front Plant Sci 5: 210

755 Menges M, de Jager SM, Gruissem W, Murray JA (2005) Global analysis of the core cell cycle regulators
756 of Arabidopsis identifies novel genes, reveals multiple and highly specific profiles of expression
757 and provides a coherent model for plant cell cycle control. Plant J 41: 546-566
758 Nath U, Crawford BC, Carpenter R, Coen E (2003) Genetic control of surface curvature. Science 299:
759 1404-1407
760 Nicolas M, Cubas P (2016) TCP factors: new kids on the signaling block. Curr Opin Plant Biol 33: 33-41
761 Nikovics K, Blein T, Peaucelle A, Ishida T, Morin H, Aida M, Laufs P (2006) The balance between the
762 MIR164A and CUC2 genes controls leaf margin serration in Arabidopsis. Plant Cell 18: 2929-2945
763 Palatnik JF, Allen E, Wu X, Schommer C, Schwab R, Carrington JC, Weigel D (2003) Control of leaf
764 morphogenesis by microRNAs. Nature 425: 257-263
765 Pettko-Szandtner A, Cserhati M, Barroco RM, Hariharan S, Dudits D, Beemster GT (2015) Core cell cycle
766 regulatory genes in rice and their expression profiles across the growth zone of the leaf. J Plant
767 Res 128: 953-974
768 Poethig RS (2003) Phase change and the regulation of developmental timing in plants. Science 301: 334-
769 336
770 Polyn S, Willems A, De Veylder L (2015) Cell cycle entry, maintenance, and exit during plant
771 development. Curr Opin Plant Biol 23: 1-7
772 Powell AE, Lenhard M (2012) Control of organ size in plants. Curr Biol 22: R360-367
773 Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression
774 analysis of digital gene expression data. Bioinformatics 26: 139-140
775 Rodriguez RE, Debernardi JM, Palatnik JF (2014) Morphogenesis of simple leaves: regulation of leaf size
776 and shape. Wiley Interdiscip Rev Dev Biol 3: 41-57
777 Rodriguez RE, Mecchia MA, Debernardi JM, Schommer C, Weigel D, Palatnik JF (2010) Control of cell
778 proliferation in Arabidopsis thaliana by microRNA miR396. Development 137: 103-112
779 Rubio-Somoza I, Zhou CM, Confraria A, Martinho C, von Born P, Baena-Gonzalez E, Wang JW, Weigel D
780 (2014) Temporal control of leaf complexity by miRNA-regulated licensing of protein complexes.
781 Curr Biol 24: 2714-2719
782 Sarvepalli K, Nath U (2011) Hyper-activation of the TCP4 transcription factor in Arabidopsis thaliana
783 accelerates multiple aspects of plant maturation. Plant J 67: 595-607
784 Sarvepalli K, Nath U (2011) Interaction of TCP4-mediated growth module with phytohormones. Plant
785 Signal Behav 6: 1440-1443
786 Scarpella E, Barkoulas M, Tsiantis M (2010) Control of leaf and vein development by auxin. Cold Spring
787 Harb Perspect Biol 2: a001511
788 Scarpella E, Marcos D, Friml J, Berleth T (2006) Control of leaf vascular patterning by polar auxin
789 transport. Genes Dev 20: 1015-1027
790 Schmid M, Davison TS, Henz SR, Pape UJ, Demar M, Vingron M, Scholkopf B, Weigel D, Lohmann JU
791 (2005) A gene expression map of Arabidopsis thaliana development. Nat Genet 37: 501-506
792 Schommer C, Debernardi JM, Bresso EG, Rodriguez RE, Palatnik JF (2014) Repression of cell proliferation
793 by miR319-regulated TCP4. Mol Plant 7: 1533-1544
794 Schommer C, Palatnik JF, Aggarwal P, Chetelat A, Cubas P, Farmer EE, Nath U, Weigel D (2008) Control of
795 jasmonate biosynthesis and senescence by miR319 targets. PLoS Biol 6: e230
796 Sohlberg JJ, Myrenas M, Kuusk S, Lagercrantz U, Kowalczyk M, Sandberg G, Sundberg E (2006) STY1
797 regulates auxin homeostasis and affects apical-basal patterning of the Arabidopsis gynoecium.
798 Plant J 47: 112-123
799 Steiner E, Efroni I, Gopalraj M, Saathoff K, Tseng TS, Kieffer M, Eshed Y, Olszewski N, Weiss D (2012) The
800 Arabidopsis O-linked N-acetylglucosamine transferase SPINDLY interacts with class I TCPs to
801 facilitate cytokinin responses in leaves and flowers. Plant Cell 24: 96-108

802 Tao Q, Guo D, Wei B, Zhang F, Pang C, Jiang H, Zhang J, Wei T, Gu H, Qu LJ, Qin G (2013) The TIE1
803 transcriptional repressor links TCP transcription factors with TOPLESS/TOPLESS-RELATED
804 corepressors and modulates leaf development in Arabidopsis. Plant Cell 25: 421-437
805 Thimm O, Blasing O, Gibon Y, Nagel A, Meyer S, Kruger P, Selbig J, Muller LA, Rhee SY, Stitt M (2004)
806 MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic
807 pathways and other biological processes. Plant J 37: 914-939
808 Tian T, Liu Y, Yan H, You Q, Yi X, Du Z, Xu W, Su Z (2017) agriGO v2.0: a GO analysis toolkit for the
809 agricultural community, 2017 update. Nucleic Acids Res
810 Trigueros M, Navarrete-Gomez M, Sato S, Christensen SK, Pelaz S, Weigel D, Yanofsky MF, Ferrandiz C
811 (2009) The NGATHA genes direct style development in the Arabidopsis gynoecium. Plant Cell 21:
812 1394-1409
813 Wang H, Zhou Y, Gilmer S, Whitwill S, Fowke LC (2000) Expression of the plant cyclin-dependent kinase
814 inhibitor ICK1 affects cell division, plant growth and morphology. Plant J 24: 613-623
815 White DW (2006) PEAPOD regulates lamina size and curvature in Arabidopsis. Proc Natl Acad Sci U S A
816 103: 13238-13243

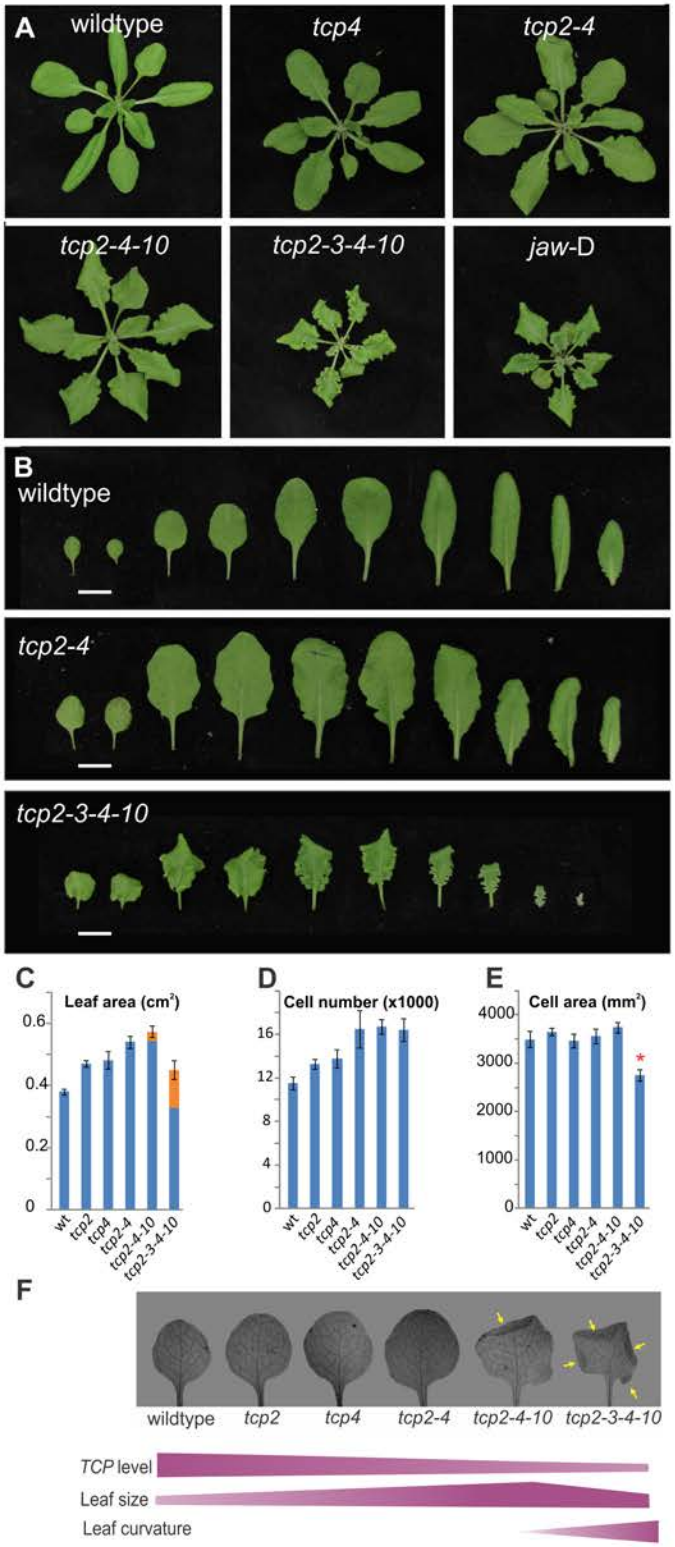


Figure 1. Effects of decreasing TCP levels on leaf size and shape. A, Four weeks old rosettes of Arabidopsis plants with decreased levels of TCP activity. B, Disassembled rosettes of indicated genotypes. C, Leaf area of leaf number one and two of the indicated genotypes. Marked in orange the contribution of folded regions to the total leaf area (see Materials and Methods). D, Number of cells in the same leaves as in C. E, Cell area of the same leaves in C, asterisk indicating statistical significance of difference according to $p<0.01$ by Student's t-test. F, Cleared and flattened leaves of the different genotypes that have reduced TCP activity. Yellow arrows indicate folded regions. Violet bars used to illustrate how leaf size and curvature develop with respect to the gradual reduction of TCP activity. The mutants *tcp2 tcp4*, *tcp2 tcp4 tcp10*, *tcp2 tcp4 tcp3 tcp10* and *tcp2-4-10* are labelled *tcp2*, *tcp4*, *tcp2-4* and *tcp2-3-4-10* respectively.

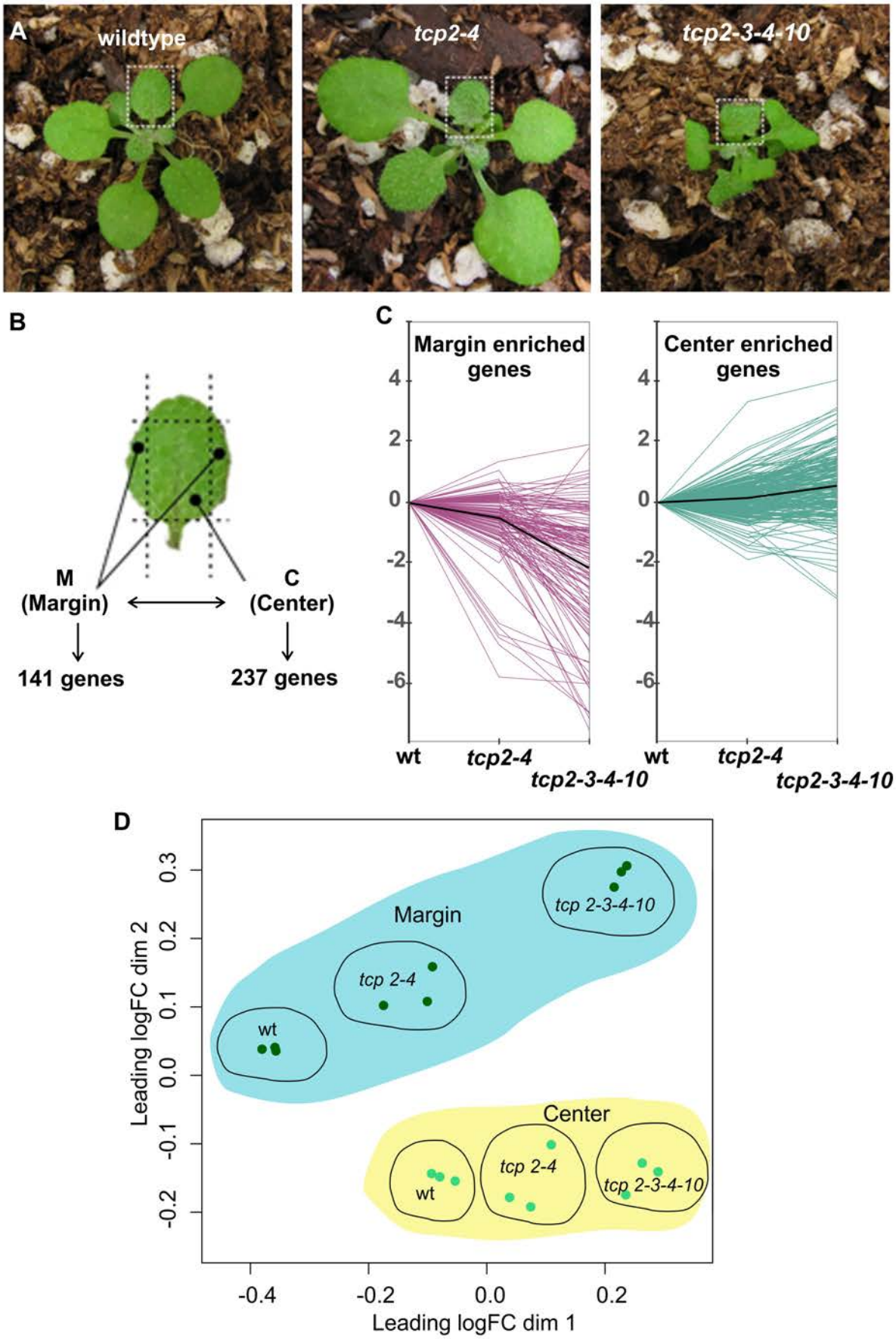
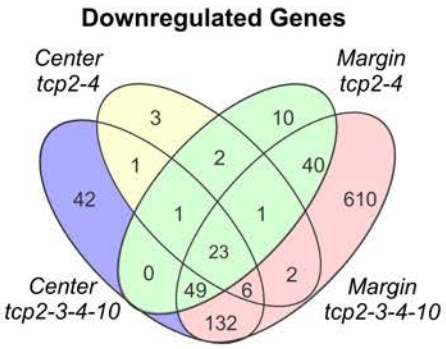


Figure 2. Transcriptome analysis of leaf domains of wild type and *tcp* mutants. A, Rosettes with emerging leaf number five as used for transcriptome analysis by RNAseq experiments. B, Schematic display of regions that were used as marginal and center samples to obtain RNA for the RNAseq experiments. In wild type 237 center and 141 margin enriched genes were identified. C, Graphical display as fold change of behaviour of genes that were determined in B to be margin or center enriched in wild type samples, in *tcp2 tcp4* and *tcp2 tcp3 tcp4 tcp10* mutants. The black line represents the average expression level of all margin or center enriched genes. D, Multidimensional scaling (MDS) plot for count data (all genes). Distances correspond to leading log-fold-changes between each pair of RNA samples.

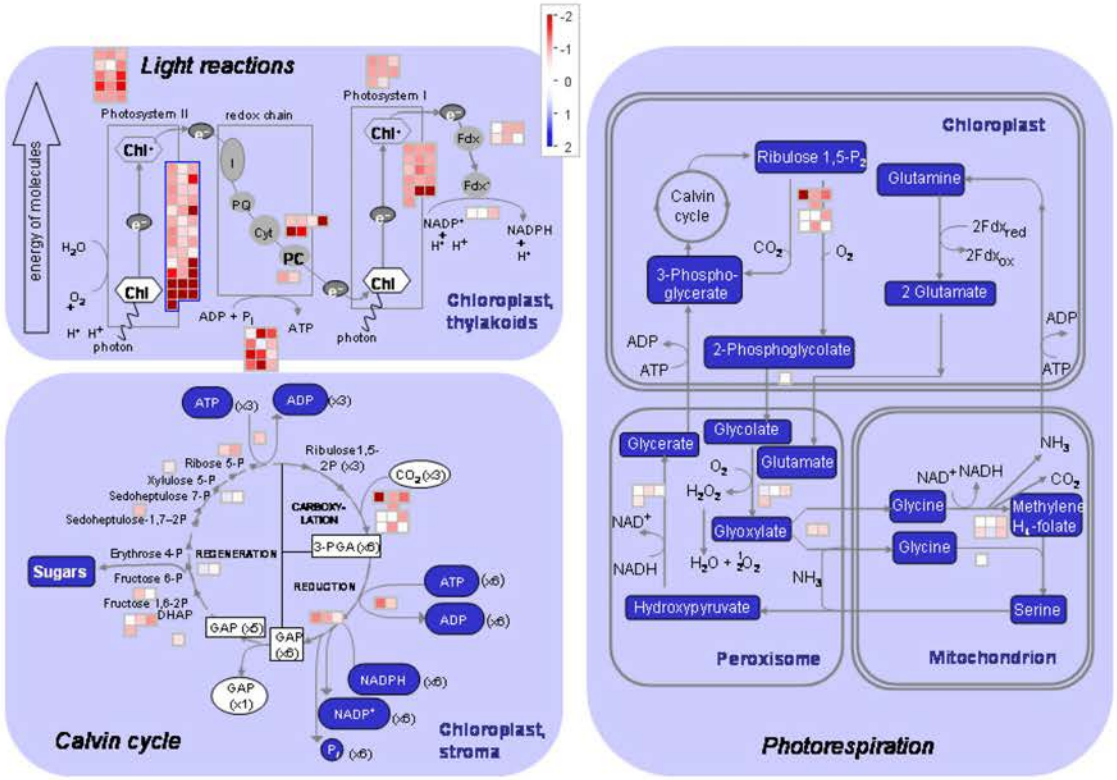
A



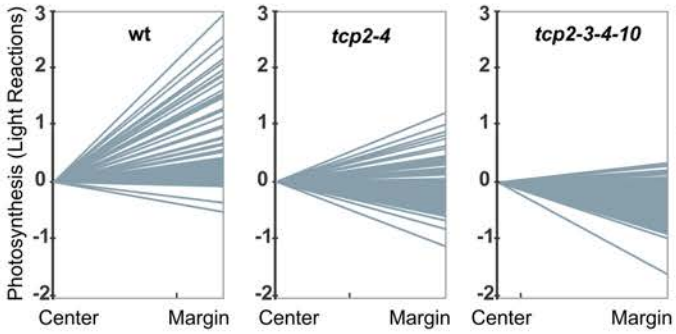
B

		total genes	GGACCA total hits	GGACCA promoters	p
tcp 2-4	margin	126	62	49	8.3E-06
tcp 2-3-4-10	center	254	135	106	4.9E-13
tcp 2-3-4-10	margin	863	368	295	1.5E-17
tcp 2-3-4-10	margin only	653	251	201	3.1E-08

C



D



E

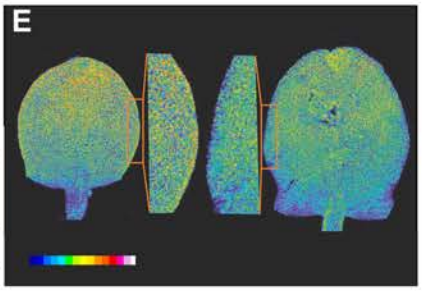


Figure 3. Coordination of gene expression related to photosynthesis by miR319-regulated TCPs. A, Venn diagram showing the intersections of downregulated genes in margins and centers of *tcp2 tcp4* and *tcp2 tcp3 tcp4 tcp10* compared to wildtype. B, Promoter analysis of downregulated genes in *tcp2 tcp4* and *tcp2 tcp3 tcp4 tcp10* center and margin regions for overrepresentation of TCP binding motif GGACCA. C, Graphical display of MapMan analysis of gene expression for photosynthesis related genes in *tcp2 tcp3 tcp4 tcp10* margins compared to wildtype. D, Graphical display of relative expression changes of photosynthesis related genes between center and marginal regions in wildtype, *tcp2 tcp4* and *tcp2 tcp3 tcp4 tcp10* as determined by RNAseq experiments. The mutants *tcp2 tcp4* and *tcp2 tcp3 tcp4 tcp10* are labelled *tcp2-4* and *tcp2-3-4-10* respectively. E, Chlorophyll content in developing young leaves as estimated by their fluorescence under a light scanning confocal microscope. Left with close up wildtype, *tcp2 tcp3 tcp4 tcp10* and close up, right. The colour bar indicates the fluorescence intensity profile used from blue (low fluorescence) to white (high fluorescence).

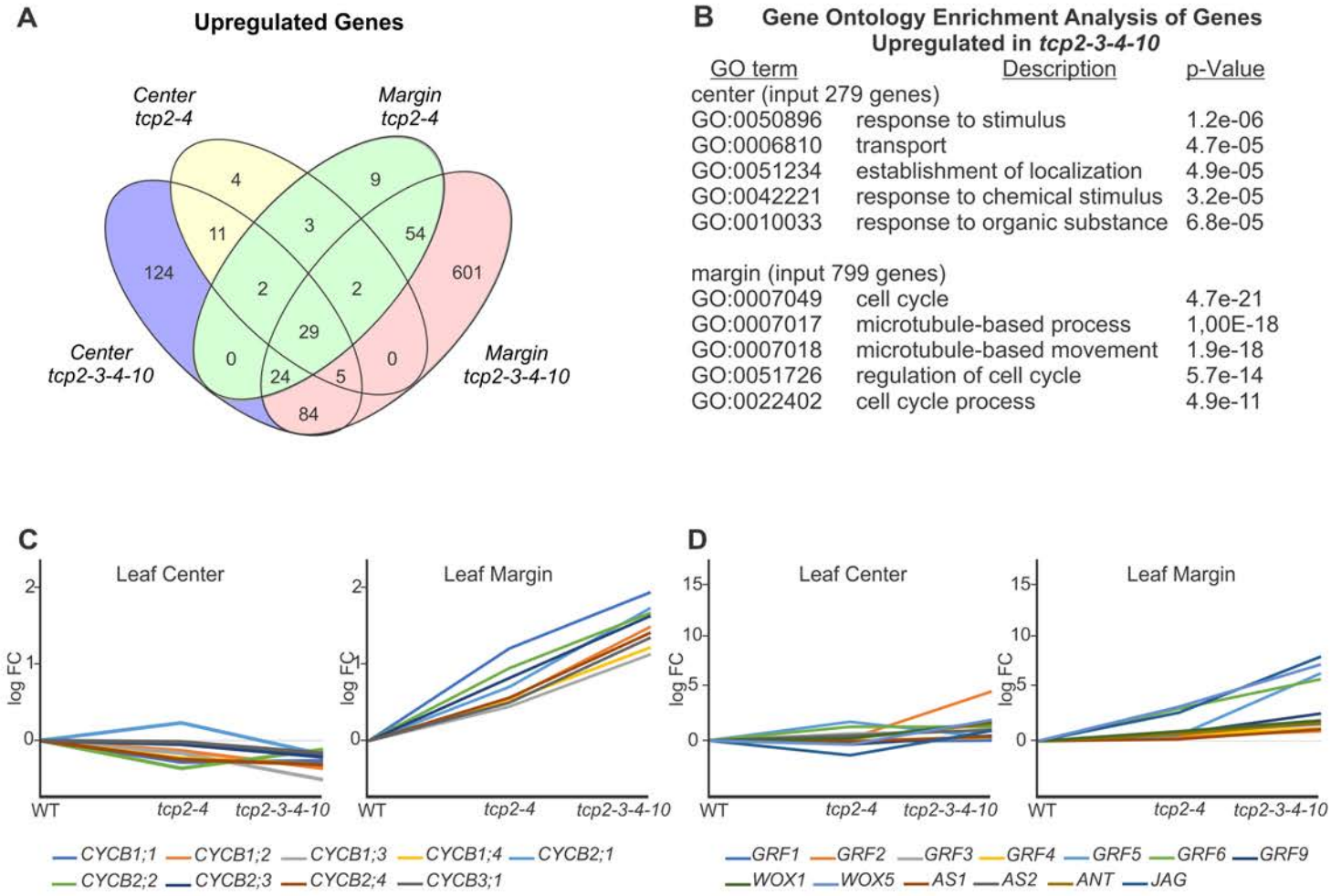


Figure 4. Up-regulation of the leaf developmental program and mitotic genes in *tcp* mutant margins. A, Venn diagram showing the intersections of upregulated genes in margins and centers of *tcp2 tcp4* and *tcp2 tcp3 tcp4 tcp10* compared to wildtype. B, Output of gene ontology (GO) enrichment analysis for upregulated genes in *tcp2 tcp3 tcp4 tcp10* margin and center regions. C, Display of expression changes of B-type cyclin genes in *tcp2 tp4* and *tcp2 tcp3 tcp4 tcp10* center and marginal leaf samples in RNAseq analysis. Y-axis is log fold change compared to wildtype. D, Display of expression changes of selected genes that are active in early stages of leaf development in *tcp2 tp4* and *tcp2 tcp3 tcp4 tcp10* center and marginal leaf samples. Y-axis is log of fold change of mutant samples compared to wildtype. The mutants *tcp2 tcp4* and *tcp2 tcp3 tcp4 tcp10* are labelled *tcp2-4* and *tcp2-3-4-10* respectively.

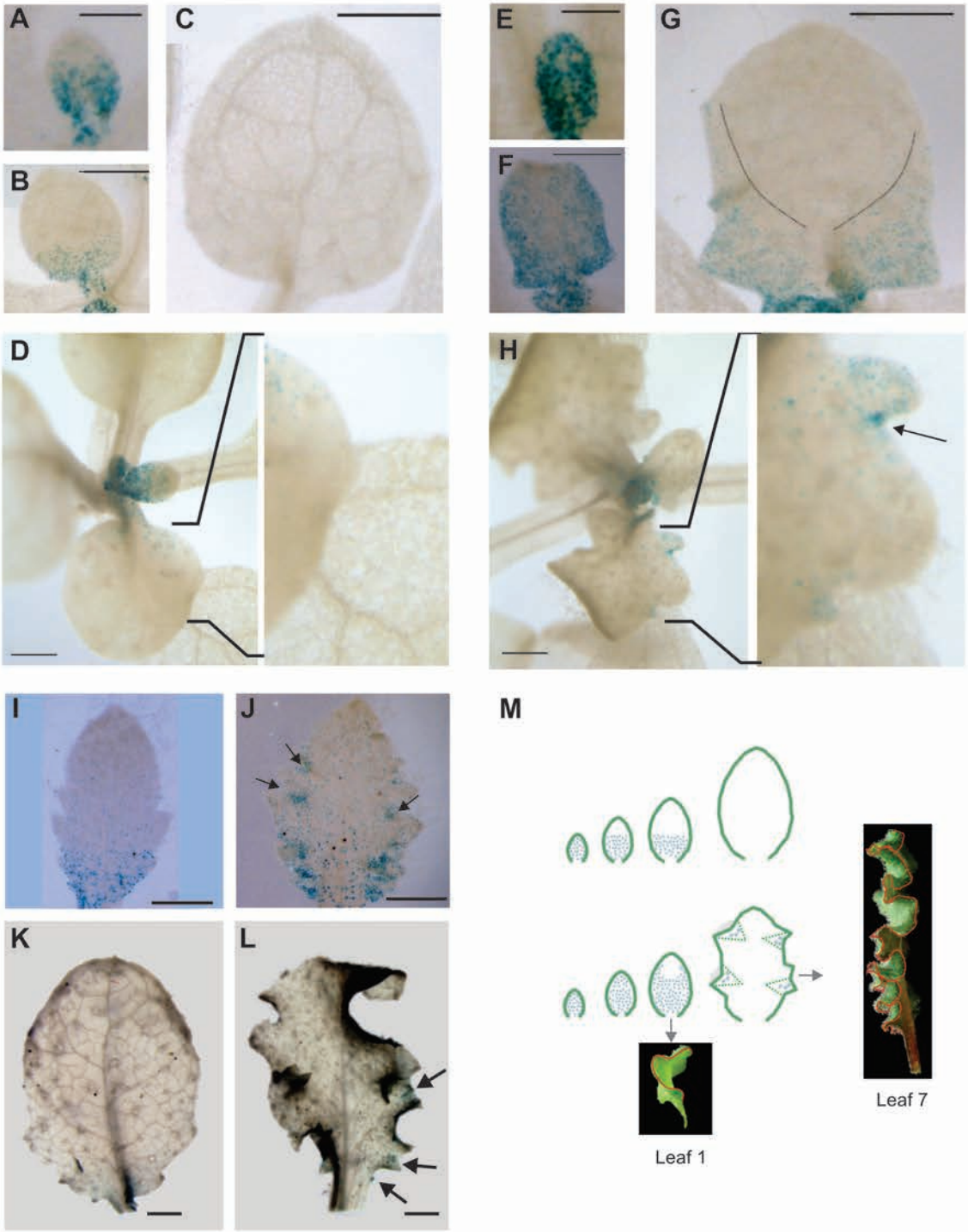
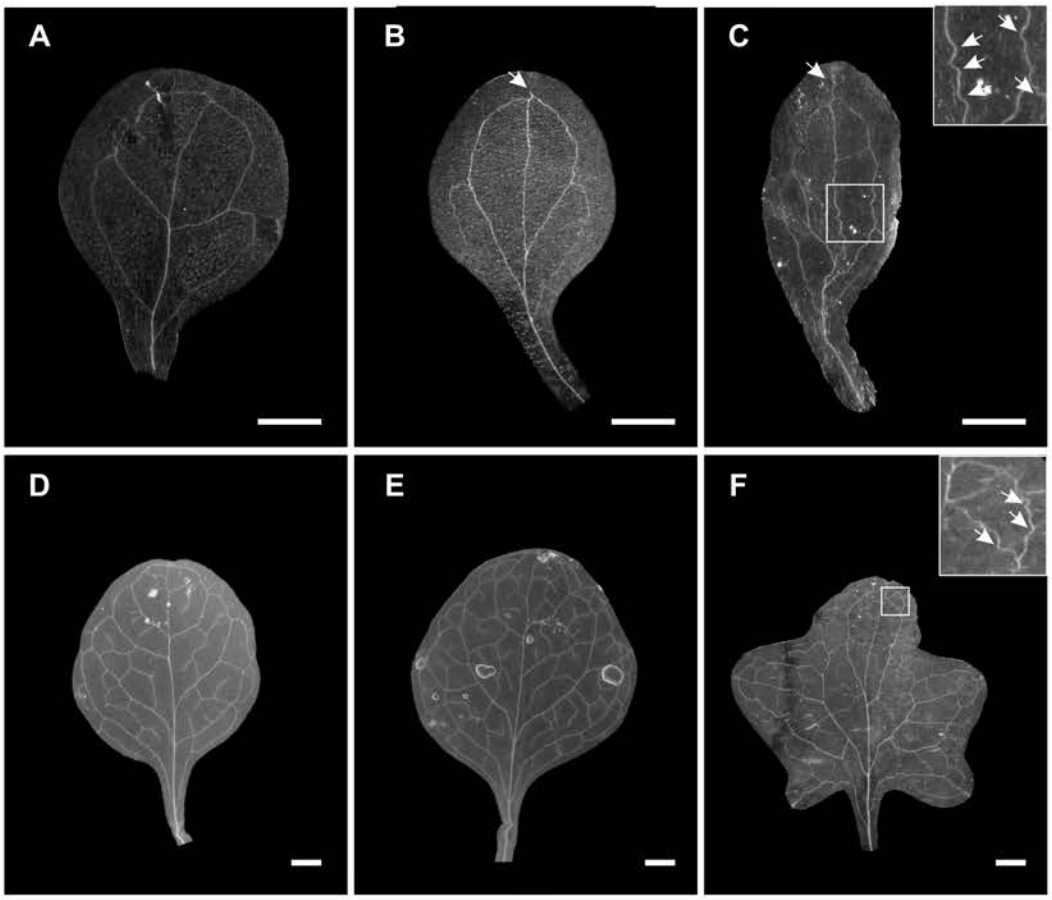


Figure 5. Activation of discrete foci expressing CYCLINB1;1GUS upon TCP downregulation. A-L, β–glucoronidase assays. A-D,I,K young developing leaves of a CYCLINB1;1-GUS reporter line. E-H,J,L, young leaves of the same a CYCLINB1;1-GUS reporter overexpressing miR319a, resulting in crinkly leaves. A, Leaf one seven DAS. B, Leaf one nine DAS. C, Leaf one at 11 DAS. D, Leaf four, E, Leaf one of miR319 overexpressor at eight DAS. F, Leaf one of miR319 overexpressor at 10 DAS. G, Leaf one of miR319 overexpressor at 12 DAS. The dashed line delimits the domain containing cycling cells. H, Leaf four miR319 overexpressor. I, Leaf seven. J, Leaf seven of miR319 overexpressor. K,L More mature leaves of wildtype (K) and miR319a overexpressor (L). M, Model showing the effect of miR319 regulated TCPs on cell division in the developing leaf of wild-type (top) and *tcp* quadruple knock outs or miR319 overexpressing plants (bottom). Blue dots indicate proliferating cells. Scale bars: A,E,I,J 0,2mm, B,C,F,G 0,5mm, D,H,K,L 1mm.



G

	Areoles	Branch Points	Secondary Veins	Distal Peg	Free Ends
cotyledons					
wildtype	3.4 ± 0.1	4.6 ± 0.5	3.3 ± 0.2	0%	30.8%
tcp2-4	2.9 ± 0.3	3.5 ± 0.3	3.1 ± 0.2	36.8%	47.4%
tcp2-3-4-10	4.2 ± 0.3*	8.0 ± 0.5*	3.5 ± 0.2	85.2%	74.%

	Areoles	Branch Points	Leaf Area (cm^2)	Areole Size (cm^2)	Total Length of Vasculature (cm)	Vasculature/Leaf Area (cm/cm^2)
leaf one						
wildtype	22.6 ± 1.1^a	57 ± 3^a	0.39 ± 0.02^a	1.75 ± 0.1^a	8.24 ± 0.31^a	21.3 ± 0.4^a
tcp2-4	43.9 ± 3.4^b	112 ± 8^b	0.58 ± 0.04^b	1.33 ± 0.1^b	13.76 ± 0.81^b	23.8 ± 0.4^a
tcp2-3-4-10	40.7 ± 4.5^b	97 ± 6^b	0.34 ± 0.02^a	0.89 ± 0.1^c	9.63 ± 0.52^a	28.7 ± 1.5^b

Figure 6. Modification of the venation pattern in *tcp* mutants. A-C, Cleared cotyledons of wildtype (A), *tcp2 tcp4* (B) and, *tcp2 tcp3 tcp4 tcp10* (C). D-F, Cleared first leaves of wildtype (D), *tcp2 tcp4* (E) and *tcp2 tcp3 tcp4 tcp10* (F). G, table summarizing the scored parameters describing the venation pattern. Scale bars: 1mm.

Parsed Citations

Alvarez JP, Furumizu C, Efroni I, Eshed Y, Bowman JL (2016) Active suppression of a leaf meristem orchestrates determinate leaf growth. Elife 5

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Alvarez JP, Goldshmidt A, Efroni I, Bowman JL, Eshed Y (2009) The NGATHA distal organ development genes are essential for style specification in Arabidopsis. Plant Cell 21: 1373-1393

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Alvarez JP, Pekker I, Goldshmidt A, Blum E, Amsellem Z, Eshed Y (2006) Endogenous and synthetic microRNAs stimulate simultaneous, efficient, and localized regulation of multiple targets in diverse species. Plant Cell 18: 1134-1151

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Anders S, McCarthy DJ, Chen Y, Okoniewski M, Smyth GK, Huber W, Robinson MD (2013) Count-based differential expression analysis of RNA sequencing data using R and Bioconductor. Nat Protoc 8: 1765-1786

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Andriankaja M, Dhondt S, De Bodt S, Vanhaeren H, Coppens F, De Milde L, Muhlenbock P, Skirycz A, Gonzalez N, Beemster GT, Inze D (2012) Exit from proliferation during leaf development in Arabidopsis thaliana: a not-so-gradual process. Dev Cell 22: 64-78

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ballester P, Navarrete-Gomez M, Carbonero P, Onate-Sanchez L, Ferrandiz C (2015) Leaf expansion in Arabidopsis is controlled by a TCP-NGA regulatory module likely conserved in distantly related species. Physiol Plant 155: 21-32

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Beemster GT, De Veylder L, Vercruysse S, West G, Rombaut D, Van Hummelen P, Galichet A, Gruissem W, Inze D, Vuylsteke M (2005) Genome-wide analysis of gene expression profiles associated with cell cycle transitions in growing organs of Arabidopsis. Plant Physiol 138: 734-743

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Cubas P, Lauter N, Doebley J, Coen E (1999) The TCP domain: a motif found in proteins regulating plant growth and development. Plant J 18: 215-222

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Danisman S, van Dijk AD, Bimbo A, van der Wal F, Hennig L, de Folter S, Angenent GC, Immink RG (2013) Analysis of functional redundancies within the Arabidopsis TCP transcription factor family. J Exp Bot 64: 5673-5685

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Donnelly PM, Bonetta D, Tsukaya H, Dengler RE, Dengler NG (1999) Cell cycling and cell enlargement in developing leaves of Arabidopsis. Dev Biol 215: 407-419

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Du Z, Zhou X, Ling Y, Zhang Z, Su Z (2010) agriGO: a GO analysis toolkit for the agricultural community. Nucleic Acids Res 38: W64-70

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Edgar R, Domrachev M, Lash AE (2002) Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 30: 207-210

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Efroni I, Blum E, Goldshmidt A, Eshed Y (2008) A protracted and dynamic maturation schedule underlies Arabidopsis leaf development. Plant Cell 20: 2293-2306
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Efroni I, Han SK, Kim HJ, Wu MF, Steiner E, Birnbaum KD, Hong JC, Eshed Y, Wagner D (2013) Regulation of leaf maturation by chromatin-mediated modulation of cytokinin responses. Dev Cell 24: 438-445
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Engelhorn J, Reimer JJ, Leuz I, Gobel U, Huettel B, Farrona S, Turck F (2012) Development-related PcG target in the apex 4 controls leaf margin architecture in Arabidopsis thaliana. Development 139: 2566-2575
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Horiguchi G, Kim GT, Tsukaya H (2005) The transcription factor AtGRF5 and the transcription coactivator AN3 regulate cell proliferation in leaf primordia of Arabidopsis thaliana. Plant J 43: 68-78
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kawamura E, Horiguchi G, Tsukaya H (2010) Mechanisms of leaf tooth formation in Arabidopsis. Plant J 62: 429-441
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kazama T, Ichihashi Y, Murata S, Tsukaya H (2010) The mechanism of cell cycle arrest front progression explained by a KLUH/CYP78A5-dependent mobile growth factor in developing leaves of Arabidopsis thaliana. Plant Cell Physiol 51: 1046-1054
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kosugi S, Ohashi Y (1997) PCF1 and PCF2 specifically bind to cis elements in the rice proliferating cell nuclear antigen gene. Plant Cell 9: 1607-1619
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Koyama T, Furutani M, Tasaka M, Ohme-Takagi M (2007) TCP transcription factors control the morphology of shoot lateral organs via negative regulation of the expression of boundary-specific genes in Arabidopsis. Plant Cell 19: 473-484
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kuusk S, Sohlberg JJ, Long JA, Fridborg I, Sundberg E (2002) STY1 and STY2 promote the formation of apical tissues during Arabidopsis gynoecium development. Development 129: 4707-4717
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kuusk S, Sohlberg JJ, Magnus Eklund D, Sundberg E (2006) Functionally redundant SHI family genes regulate Arabidopsis gynoecium development in a dose-dependent manner. Plant J 47: 99-111
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lee BH, Kwon SH, Lee SJ, Park SK, Song JT, Lee S, Lee MM, Hwang YS, Kim JH (2015) The Arabidopsis thaliana NGATHA transcription factors negatively regulate cell proliferation of lateral organs. Plant Mol Biol 89: 529-538
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li P, Ponnala L, Gandotra N, Wang L, Si Y, Tausta SL, Kebrom TH, Provart N, Patel R, Myers CR, Reidel EJ, Turgeon R, Liu P, Sun Q, Nelson T, Brutnell TP (2010) The developmental dynamics of the maize leaf transcriptome. Nat Genet 42: 1060-1067
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li S (2015) The Arabidopsis thaliana TCP transcription factors: A broadening horizon beyond development. Plant Signal Behav 10: e1044192
Pubmed: Author and Title
CrossRef: Author and Title

Google Scholar: Author Only Title Only Author and Title

Li S, Zachgo S (2013) TCP3 interacts with R2R3-MYB proteins, promotes flavonoid biosynthesis and negatively regulates the auxin response in Arabidopsis thaliana. Plant J 76: 901-913
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Martinez-Fernandez I, Sanchis S, Marini N, Balanza V, Ballester P, Navarrete-Gomez M, Oliveira AC, Colombo L, Ferrandiz C (2014) The effect of NGATHA altered activity on auxin signaling pathways within the Arabidopsis gynoecium. Front Plant Sci 5: 210
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Menges M, de Jager SM, Gruissem W, Murray JA (2005) Global analysis of the core cell cycle regulators of Arabidopsis identifies novel genes, reveals multiple and highly specific profiles of expression and provides a coherent model for plant cell cycle control. Plant J 41: 546-566
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Nath U, Crawford BC, Carpenter R, Coen E (2003) Genetic control of surface curvature. Science 299: 1404-1407
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Nicolas M, Cubas P (2016) TCP factors: new kids on the signaling block. Curr Opin Plant Biol 33: 33-41
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Nikovics K, Blein T, Peaucelle A, Ishida T, Morin H, Aida M, Laufs P (2006) The balance between the MIR164A and CUC2 genes controls leaf margin serration in Arabidopsis. Plant Cell 18: 2929-2945
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Palatnik JF, Allen E, Wu X, Schommer C, Schwab R, Carrington JC, Weigel D (2003) Control of leaf morphogenesis by microRNAs. Nature 425: 257-263
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Pettko-Szandtner A, Cserhati M, Barroco RM, Hariharan S, Dudits D, Beemster GT (2015) Core cell cycle regulatory genes in rice and their expression profiles across the growth zone of the leaf. J Plant Res 128: 953-974
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Poethig RS (2003) Phase change and the regulation of developmental timing in plants. Science 301: 334-336
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Polyn S, Willems A, De Veylder L (2015) Cell cycle entry, maintenance, and exit during plant development. Curr Opin Plant Biol 23: 1-7
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Powell AE, Lenhard M (2012) Control of organ size in plants. Curr Biol 22: R360-367
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26: 139-140
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Rodriguez RE, Debernardi JM, Palatnik JF (2014) Morphogenesis of simple leaves: regulation of leaf size and shape. Wiley Interdiscip Rev Dev Biol 3: 41-57
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Rodriguez RE, Mecchia MA, Debernardi JM, Schommer C, Weigel D, Palatnik JF (2010) Control of cell proliferation in Arabidopsis thaliana by microRNA miR396. Development 137: 103-112
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Rubio-Somoza I, Zhou CM, Confraria A, Martinho C, von Born P, Baena-Gonzalez E, Wang JW, Weigel D (2014) Temporal control of leaf complexity by miRNA-regulated licensing of protein complexes. Curr Biol 24: 2714-2719
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Sarvepalli K, Nath U (2011) Hyper-activation of the TCP4 transcription factor in Arabidopsis thaliana accelerates multiple aspects of plant maturation. Plant J 67: 595-607
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Sarvepalli K, Nath U (2011) Interaction of TCP4-mediated growth module with phytohormones. Plant Signal Behav 6: 1440-1443
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Scarpella E, Barkoulas M, Tsiantis M (2010) Control of leaf and vein development by auxin. Cold Spring Harb Perspect Biol 2: a001511
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Scarpella E, Marcos D, Friml J, Berleth T (2006) Control of leaf vascular patterning by polar auxin transport. Genes Dev 20: 1015-1027
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Schmid M, Davison TS, Henz SR, Pape UJ, Demar M, Vingron M, Scholkopf B, Weigel D, Lohmann JU (2005) A gene expression map of Arabidopsis thaliana development. Nat Genet 37: 501-506
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Schommer C, Debernardi JM, Bresso EG, Rodriguez RE, Palatnik JF (2014) Repression of cell proliferation by miR319-regulated TCP4. Mol Plant 7: 1533-1544
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Schommer C, Palatnik JF, Aggarwal P, Chetelat A, Cubas P, Farmer EE, Nath U, Weigel D (2008) Control of jasmonate biosynthesis and senescence by miR319 targets. PLoS Biol 6: e230
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Sohlberg JJ, Myrenas M, Kuusk S, Lagercrantz U, Kowalczyk M, Sandberg G, Sundberg E (2006) STY1 regulates auxin homeostasis and affects apical-basal patterning of the Arabidopsis gynoecium. Plant J 47: 112-123
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Steiner E, Efroni I, Gopalraj M, Saathoff K, Tseng TS, Kieffer M, Eshed Y, Olszewski N, Weiss D (2012) The Arabidopsis O-linked N-acetylglucosamine transferase SPINDLY interacts with class I TCPs to facilitate cytokinin responses in leaves and flowers. Plant Cell 24: 96-108
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Tao Q, Guo D, Wei B, Zhang F, Pang C, Jiang H, Zhang J, Wei T, Gu H, Qu LJ, Qin G (2013) The TIE1 transcriptional repressor links TCP transcription factors with TOPLESS/TOPLESS-RELATED corepressors and modulates leaf development in Arabidopsis. Plant Cell 25: 421-437
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Thimm O, Blasing O, Gibon Y, Nagel A, Meyer S, Kruger P, Selbig J, Muller LA, Rhee SY, Stitt M (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J 37: 914-939
Pubmed: Author and Title
CrossRef: Author and Title

Google Scholar: Author Only Title Only Author and Title

Tian T, Liu Y, Yan H, You Q, Yi X, Du Z, Xu W, Su Z (2017) agriGO v2.0: a GO analysis toolkit for the agricultural community, 2017 update. Nucleic Acids Res

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Trigueros M, Navarrete-Gomez M, Sato S, Christensen SK, Pelaz S, Weigel D, Yanofsky MF, Ferrandiz C (2009) The NGATHA genes direct style development in the Arabidopsis gynoecium. Plant Cell 21: 1394-1409

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wang H, Zhou Y, Gilmer S, Whitwill S, Fowke LC (2000) Expression of the plant cyclin-dependent kinase inhibitor ICK1 affects cell division, plant growth and morphology. Plant J 24: 613-623

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

White DW (2006) PEAPOD regulates lamina size and curvature in Arabidopsis. Proc Natl Acad Sci U S A 103: 13238-13243

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title