

The Myb-domain protein ULTRAPETALA1 INTERACTING FACTOR 1 controls floral meristem activities in *Arabidopsis*

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ABSTRACT

Higher plants continuously and iteratively produce new above-ground organs in the form of leaves, stems and flowers. These organs arise from shoot apical meristems whose homeostasis depends on coordination between self-renewal of stem cells and their differentiation into organ founder cells. This coordination is stringently controlled by the central transcription factor WUSCHEL (WUS), which is both necessary and sufficient for stem cell specification in *Arabidopsis thaliana*. ULTRAPETALA1 (ULT1) was previously identified as a plant-specific, negative regulator of WUS expression. However, molecular mechanisms underlying this regulation remain unknown. ULT1 protein contains a SAND putative DNA-binding domain and a B-box, previously proposed as a protein interaction domain in eukaryotes. Here, we characterise a novel partner of ULT1, named ULT1 INTERACTING FACTOR 1 (UIF1), which contains a Myb domain and an EAR motif. UIF1 and ULT1 function in the same pathway for regulation of organ number in the flower. Moreover, UIF1 displays DNA-binding activity and specifically binds to WUS regulatory elements. We thus provide genetic and molecular evidence that UIF1 and ULT1 work together in floral meristem homeostasis, probably by direct repression of WUS expression.

KEY WORDS: *Arabidopsis thaliana*, Stem cell, Flower, WUSCHEL, Myb transcription factor, Repressor

INTRODUCTION

Plants differ from the majority of animals by their ability to produce new organs throughout their life cycle. Continuous organogenesis is allowed by the maintenance of stem cell reservoirs sustained through continuous cell divisions in dynamic structures called meristems (Carles and Fletcher, 2003; Holt et al., 2014; Gaillochet et al., 2015). The shoot apical meristem (SAM) is located at the growing apex and produces cells that will be incorporated into new structures such as lateral organs. Cell divisions constantly replenish the meristem in its central zone (CZ), thus providing a cell reservoir for the adjacent peripheral zone (PZ). PZ cells further differentiate into lateral organ primordia on the meristem flanks (Irish and Sussex, 1992; Leyser and Furner,

1992). Proper, continuous growth of the plant thus requires long-term function of the SAM, itself depending on a coordinated balance between cell proliferation and differentiation (Laux, 2003; Ha et al., 2010).

In *Arabidopsis thaliana*, SAM homeostasis is governed by a feedback loop signalling network involving the CLAVATA (CLV) and WUSCHEL (WUS) factors (Ha et al., 2010). The WUS homeobox transcription factor is both necessary and sufficient for stem cell specification (Laux et al., 1996; Mayer et al., 1998; Schoof et al., 2000; Lenhard et al., 2002; Gallois et al., 2004; Leibfried et al., 2005). In mutants with defective WUS function, the activity of the SAM stops at early stages of development, correlating with a lack of stem cells. WUS is expressed in a small central meristematic zone called the organizing centre (OC). The WUS protein, after being synthesised in cells of the OC, migrates in the adjacent cells of the CZ where it promotes stem-cell identity and both directly and indirectly regulates transcription of the CLV genes (Busch et al., 2010; Yadav et al., 2011; Schuster et al., 2014). In turn, the CLV3 signalling peptide restricts WUS expression to the OC (Mayer et al., 1998; Brand et al., 2000), via the CLV1-CLV2-CRN receptor protein complexes and the RPK2 receptor-like kinase (Fletcher et al., 1999; Rojo et al., 2002; Lenhard and Laux, 2003; Müller et al., 2008; Ogawa et al., 2008; Wang and Fiers, 2010; Kinoshita et al., 2010). As a consequence, *clv* loss-of-function mutants produce a widely enlarged SAM and more lateral organs, owing to extreme expansion of the WUS expression domain (Clark et al., 1993; Schoof et al., 2000). Thus, the WUS-CLV negative feedback loop is crucial for the maintenance of meristem size and function.

During the vegetative stage, the SAM produces leaves and axillary meristems that reiterate SAM development, whereas during the reproductive stage, SAM and axillary meristems produce flower meristems (FMs), which develop into flowers (Alvarez-Buylla et al., 2010). In *A. thaliana*, flowers are made of four whorls, or concentric rings, each carrying a fixed number of organs with distinct identities: four sepals, four petals, six stamens and two fused carpels. Stem cell activity at the FM determines the number of organs carried by each whorl and is regulated by the same WUS-CLV feedback loop, as flowers of *clv* mutants produce more organs per whorl, whereas *wus* hypomorphic mutants produce fewer floral organs than do wild-type (WT) plants (Laux et al., 1996; Schoof et al., 2000). Moreover and in contrast to the SAM, FM growth is determinate as organ production ceases once the carpels are initiated in the innermost whorl of the flower (Prunet et al., 2009). FM termination relies on the timely expression of the flower homeotic gene *AGAMOUS* (*AG*), which encodes a MADS domain-containing protein that both directly and indirectly represses WUS expression in the centre of the FM, leading to extinction of stem cell activity at stage 6 (Lenhard et al., 2001; Lohmann et al., 2001; Sun et al., 2009, 2014; Liu et al., 2011).

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The *A. thaliana* ULTRAPETALA1 (ULT1) factor is a key negative regulator of stem cell activity in the shoot apical and floral meristems (Fletcher, 2001; Carles et al., 2004, 2005). In particular, *ult1* loss-of-function mutants produce more flowers and supernumerary floral organs of each type. In these mutants, the inflorescence and flower meristem sizes are enlarged as a result of the expansion of the *WUS* expression domain (Carles et al., 2004, 2005). Thus, ULT1 maintains shoot and floral meristem function by restricting *WUS* expression to the OC. Moreover, FM termination is delayed in *ult1* mutants (Fletcher, 2001; Prunet et al., 2008), leading to the production of additional organs inside the innermost whorl, such as stamens and carpelloid structures. This phenotype correlates with a delay in *AG* induction, which, in turn, delays *WUS* extinction in the centre of the FM (Fletcher, 2001; Carles et al., 2004). Thus, ULT1 acts as a negative regulator of *WUS* expression in the FM, and this function is required for both meristem homeostasis and termination (Carles et al., 2004). The *ULT1* gene encodes a plant-specific protein composed of a SAND (Sp100, AIRE-1, NucP41/75 and DEAF-1) putative DNA-interacting domain and a B-box, which is likely to be involved in protein-protein interaction (Carles et al., 2005). It has been proposed that ULT1 directly activates *AG* at the centre of the FM, via interaction with the chromatin activator ARABIDOPSIS HOMOLOG OF TRITHORAX 1 (ATX1) (Alvarez-Venegas et al., 2003; Ding et al., 2011), leading to a reduction in trimethyl marks at lysine 27 of histone 3 (H3K27me3, repressive chromatin mark) at the target locus (Carles and Fletcher, 2009, 2010; Engelhorn et al., 2014a). Thus, ULT1 acts as a positive regulator of *AG* that contributes to the extinction of *WUS* expression in stage 8 flowers.

However, several questions remain open concerning the precise molecular mechanism(s) through which ULT1 regulates flower meristem activity. How does ULT1 repress *WUS* for SAM and FM homeostasis at early stages of flower development? Could ULT1 directly function in *WUS* repression? How does ULT1 interact with DNA and what defines its target gene specificity? In order to elucidate the answers to these questions, we searched for novel ULT1 interacting partners. Here, we identify and characterise ULT1 INTERACTING FACTOR 1 (UIF1), a Myb domain-containing transcription factor. We found that loss of function in *UIF1* causes phenotypes similar to *ult1* mutant phenotypes, such as production of supernumerary floral organs. We show that *UIF1* and *ULT1* function in the same pathway for the regulation of organ number in the flower. Moreover, UIF1 displays DNA-binding activity and specifically binds to *WUS* regulatory elements. Because UIF1 protein displays a transcriptional repressor activity, we propose that UIF1 and ULT1 work together in FM homeostasis, via direct regulation of *WUS* expression.

RESULTS

The Myb domain-containing protein UIF1 interacts with ULT1

To identify novel interactors of ULT1, we performed a yeast two-hybrid (Y2H) screen, using an *A. thaliana* inflorescence and flower cDNA library and the full-length ULT1 open reading frame (ORF) as bait. Upon selection, we isolated a clone corresponding to *At4g37180.1* as annotated in the tair database (<https://www.arabidopsis.org/servlets/TairObject?id=126622&type=locus>), which we named *ULT1 INTERACTING FACTOR 1 (UIF1)* (Fig. 1A). A targeted binary Y2H test using the full-length ULT1 and UIF1 proteins confirmed the interaction (Fig. 1B; Fig. S1A). The UIF1-ULT1 interaction was next validated *in planta* by a bimolecular fluorescence complementation (BiFC) assay, yielding an interaction signal in the nucleus of transformed cells (Fig. 1C;

Fig. S1B). The 1804-bp long *UIF1* gene (*At4g37180*) encodes a 356-aa long Myb domain-containing protein of unreported function, belonging to the GARP G2-like subfamily of transcription factors (Fig. 2A,B). The Myb-like putative DNA-binding domain of UIF1 (aa 209–268) contains a SHLQKYR motif at its C-terminus (Fig. 2B); this motif is found in other plant proteins (*AtCCA1*; *AtLHY*; *LeMYB1*; *ZmMRP-1*; *MybSt1*) and reported as a specific DNA-binding domain (Wang et al., 1997; Schaffer et al., 1998; Rose et al., 1999; Gómez et al., 2002; Baranowskij et al., 1994). UIF1 protein also contains two distinct EAR-like motifs (LxLxL, with L indicating leucine and x indicating any aa) at its N- and C-terminal ends, as well as a predicted nuclear localisation signal (NLS) (Hiratsu et al., 2002; Ohta et al., 2001) (Fig. 2B). We found six homologues for UIF1 in *A. thaliana* (Fig. S2A,B), which share 86–91% similarity over their Myb domain and 43–63% similarity over the full-length protein. Another globular domain rich in hydrophobic amino acids (aa 45–86) such as L and isoleucine (I) shows 78–81% similarity between all homologues (Fig. S2A). We identified orthologues of UIF1 among monocots and dicots (Fig. 2B), which share strong similarity both over the hydrophobic and Myb domains (68–83% and 88–96%, respectively). Interestingly, although the N-terminal EAR motif is conserved among all UIF1 orthologues, UIF1 differs by the presence of an additional LDLEL EAR-like motif at its C-terminus end (Fig. 2B). Phylogenetic reconstructions generated with *UIF1* orthologues in eudicots suggest that, unlike *At1g13300* and *At3g25790* on one hand, and *At1g68670* and *At1g25550* on the other hand, the *UIF1* gene does not result from duplication in the Brassicaceae lineage (Fig. S2C).

In order to map the regions necessary for ULT1-UIF1 interaction, we tested truncated versions of ULT1 and UIF1 by Y2H and BiFC (Fig. 1A,C). We showed that in the absence of the B-box domain (Carles et al., 2005), ULT1 (ULT1 1–193) does not interact with UIF1 in Y2H (Fig. 1B) or in BiFC (Fig. 1C). Furthermore, the ULT1 B-box domain alone (aa 116–237) could interact with UIF1 and restore yeast growth on selective medium (Fig. 1A,B). Similarly, we showed that deletion of the N-terminal domain of UIF1 (UIF1 93–364), which contains the (L/I)-rich motif, resulted in the loss of interaction with ULT1 (Fig. 1A,C). Taken together, these results suggest that UIF1 can interact with the ULT1 protein and that ULT1 B-box and UIF1 N-terminus are required for this interaction.

UIF1 and ULT1 display overlapping expression patterns in inflorescences

To investigate the functional significance of UIF1-ULT1 interaction, we analysed the temporal and spatial expression pattern of *UIF1* in *A. thaliana* WT tissues. Quantitative real-time RT-PCR (RT-qPCR) analysis showed that *UIF1* is expressed in all tested tissues: roots, 6-day-old (do) seedlings, inflorescences, closed flowers and open flowers, with highest levels in 6-do seedlings and inflorescences (Fig. 2C). This profile is similar to that of *ULT1* and was confirmed by microarray data grouped in the public eFP Browser database (<http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi>; Fig. S3A). In particular, *ULT1* and *UIF1* expression patterns overlap in vegetative shoot apex, floral buds and floral organs especially in stamens and carpels (in stage 12–15 flowers). At the cellular level, RNA *in situ* hybridisation experiments on inflorescence and flower tissue sections (Fig. 2D–F; Fig. S3B,C) showed that *UIF1* is expressed throughout the inflorescence meristem (Fig. 2D) and in floral primordia from stage 2 onwards, being restricted later to stamens and to the adaxial side of carpel primordia (Fig. 2E). In mature flowers, *UIF1* transcripts were also found in ovules (Fig. 2F). This pattern largely overlaps with that of

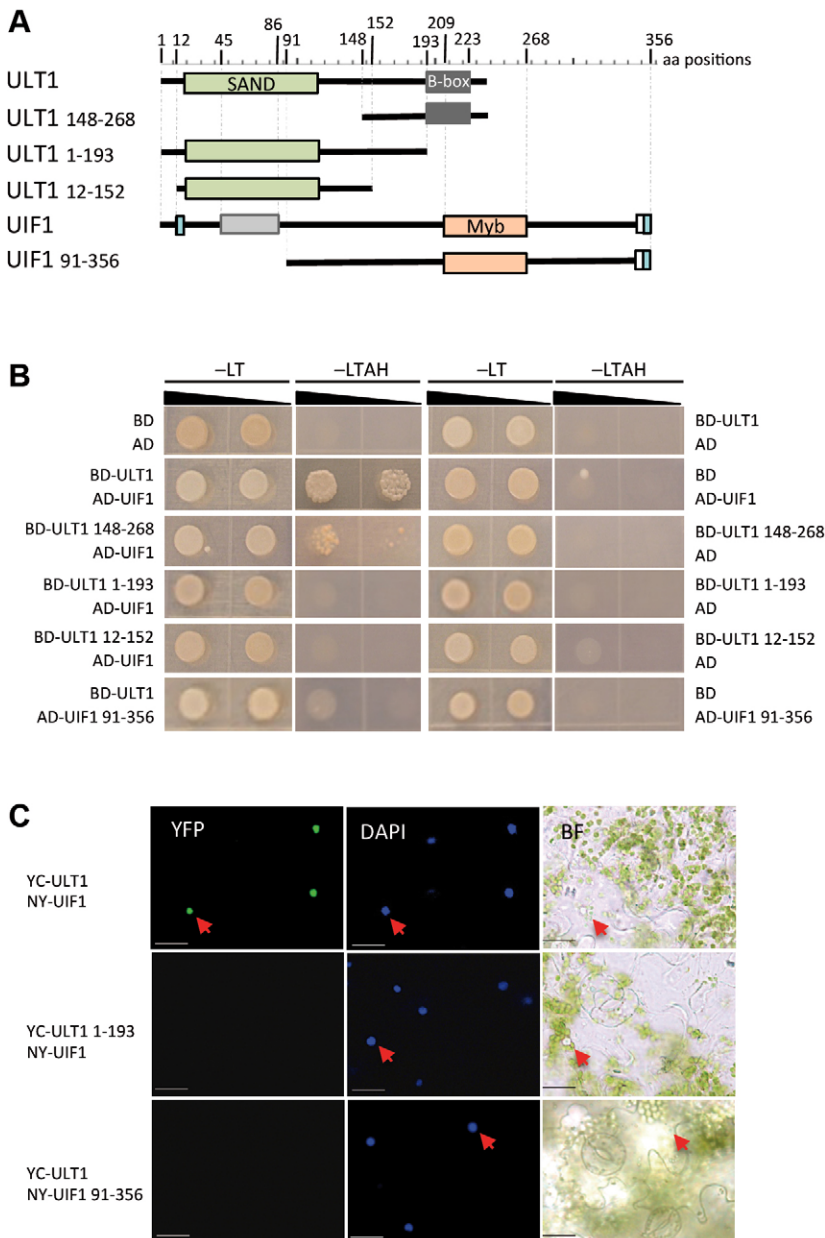


Fig. 1. ULT1 interacts with the UIF1 Myb domain-containing protein *in vivo*. (A) Diagram of ULT1 and UIF1 full length or truncated versions used in interaction studies. ULT1: SAND domain (green box), B-box like domain (dark grey box). UIF1: hydrophobic domain (light grey box), Myb domain (orange box), predicted NLS (white box), EAR-like motifs (blue boxes). (B) ULT1 interacts with UIF1 in Y2H assays. The truncated version of ULT1 carrying the B-box domain (ULT1 148-268) shows some interaction with UIF1, whereas the truncated versions lacking the B-box domain (ULT1 1-193 and ULT1 12-152) do not show any interaction. The truncated version of UIF1 lacking the N-terminus portion (UIF1 91-356) does not show interaction with ULT1. Yeast strains were dotted on –LT or –LTAH medium (on which only protein interaction allows growth). ULT1-ULT1 interaction: positive control; empty vectors: negative controls. BD, DNA-binding domain, AD, activation domain. (C) ULT1 and UIF1 interact in the nucleus of tobacco cells in BiFC experiments. The truncated ULT1 1-193 protein does not show interaction with UIF1 and the truncated UIF1 91-356 does not show interaction with ULT1. This indicates that the C-terminus of ULT1 containing the B-box domain and the N-terminus of UIF1 are required for interaction between the two proteins. From left to right, green channel (YFP filter), blue channel (DAPI filter) and bright field (BF). Red arrows indicate cell nuclei. Scale bars: 2 μ m.

ULT1 transcripts (Carles et al., 2005). Finally, at the subcellular level, we found that the GFP-UIF1 fusion protein localises in the nucleus and in cytosolic foci in the cell (Fig. 2G), a pattern very similar to that reported for the ULT1 protein (Carles et al., 2005).

Altogether, similarities in expression patterns and protein subcellular localisation further support a molecular interaction for ULT1 and UIF1 and a binomial function in inflorescences.

UIF1 and ULT1 have overlapping functions during flower morphogenesis

To assess whether *UIF1* functions in the same developmental processes as *ULT1*, we analysed two independent *A. thaliana* T-DNA insertion lines (Fig. 3A). The *uif1-1* allele contains a T-DNA insertion 132 bp upstream of the stop codon, creating an early stop codon 72 bp after the insertion (Fig. 3A). In the *uif1-3* allele, the T-DNA is located in the promoter region, 255 bp upstream of the start codon (Fig. 3A). RT-PCR analyses on inflorescences showed that for both homozygous mutants, no cDNA could be detected for

the full-length *UIF1* transcript (Fig. 3B). In the *uif1-1* line, the transcript detected upstream of the T-DNA insertion site would result in the synthesis of a truncated UIF1 protein lacking the predicted NLS domain and the C-terminus EAR-like motif (Fig. 3A). Whether the truncated transcript detected in *uif1-1* translates into the production of a truncated protein or no protein at all remains to be addressed. We found that both *uif1* mutant alleles produce flowers with more sepals and more petals than the WT control (Fig. 3C,D,G,H,K,L,O,P; Table 1), a phenotype reminiscent of that observed in *ult1* mutants (Fletcher, 2001; Carles et al., 2005). In addition, flowers of both *uif1* mutants display loss of organ boundary and identity (Table 2), producing fused sepals (Fig. 3G) as well as petaloid stamens (Fig. 3I,M,Q; Fig. S4A,B) and branching stamens fused along the filament (Fig. 3I,J,N; Fig. S4C). Altogether, these observations suggest that *UIF1* regulates floral morphogenesis and controls organ number similarly to *ULT1*. Moreover, *UIF1* regulates additional features in the flower, such as cell fate and organ identity.

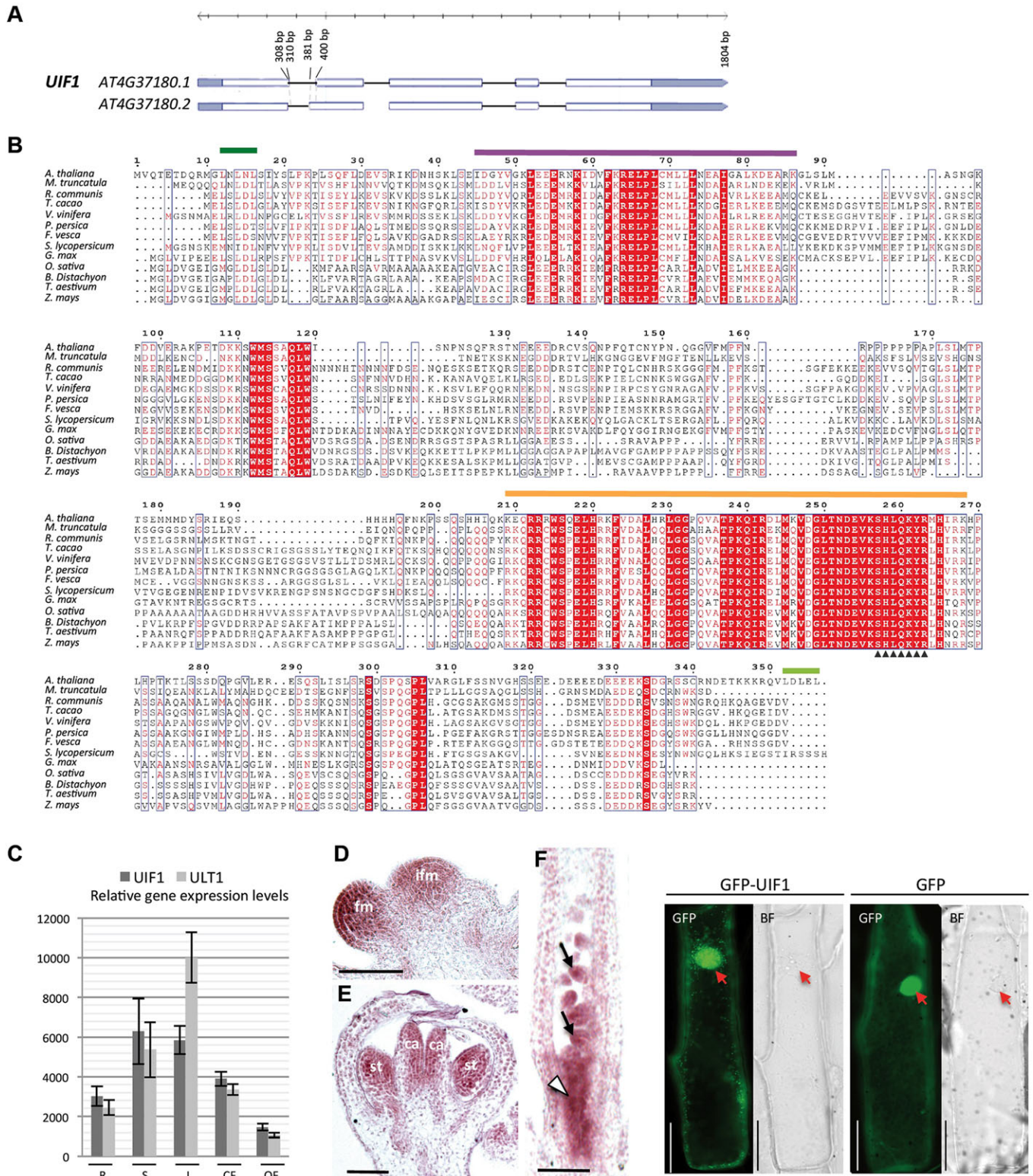


Fig. 2. See next page for legend.

ULT1 and UIF1 function in the same pathway to control flower organ number

Whereas *ult1* mutant flowers display supernumerary organs of each type (Fletcher, 2001; Carles et al., 2005), *uif1* mutants consistently produce supernumerary sepals and petals but to a lesser extent than *ult1* mutants (Fig. 4; Table 3). To test whether *ULT1* and *UIF1* are

involved in a same regulatory pathway to control organ number, *uif1-3* was crossed to the *ult1-3* null mutant. There was no significant difference between *uif1 ult1* and *ult1* mutant phenotypes for additional sepals and petals (Fig. 4; Table 3), supporting the hypothesis that *UIF1* and *ULT1* function in the same pathway for regulating organ numbers in the perianth. However, modified

Fig. 2. Molecular characterization of the *UIF1* gene. (A) *UIF1* gene structure and splicing variants. Blue boxes, untranslated regions (5', 3'-UTR); open boxes, coding regions; black lines, introns. *AT4G37180.1* (most abundant splicing variant in TAIR databases and amplified from cDNA libraries) has a first intron 21 bp longer than *AT4G37180.2*, resulting in the production of a 7-aa shorter protein (aa 75–81). (B) Alignment of predicted amino acid sequences of *UIF1* (*AT4G37180.1*) and 12 selected orthologous proteins in dicots and monocots [*Medicago truncatula* XP_003611799.1 (68%/88% similarity with *UIF1* hydrophobic domain/Myb domain, respectively), *Ricinus communis* XP_002522328.1 (83%/95%), *Theobroma cacao* XP_007047162.1 (75%/93%), *Vitis vinifera* XP_002264629.2 (80%/91%), *Prunus persica* XP_007204391.1 (80%/93%), *Fragaria vesca* XP_004288067.1 (76%/93%), *Solanum lycopersicum* XP_004233639.1 (71%/96%), *Glycine max* XP_003528384.1 (73%/83%), *Oryza sativa* Os03g0764600 (72%/90%), *Brachypodium distachyon* XP_003558989.1 (75%/91%), *Triticum aestivum* AEV91172.1 (72%/91%) and *Zea mays* NP_001146647.1 (76%/90%)]. Conserved amino acids are shaded in red, similar amino acids boxed in blue (>50% identity) and non-conserved amino acids in black. Dark green line, conserved EAR-like motif at the N-terminus; light green line, non-conserved EAR-like motif at the C-terminus; purple line, hydrophobic domain; orange line, Myb domain, with the SHLQKYR motif marked with triangles. (C) RT-qPCR analysis of *UIF1* (dark grey bars) and *ULT1* (light grey bars) gene expression in *A. thaliana* tissues (Ler): roots (R), 6-day-old seedlings (S), inflorescence apices (I), closed flowers (CF) and opened flowers (OF). Values were normalised against the *EF1 α* gene. Error bars indicate s.d. for three biological replicates. (D–F) Analysis of *UIF1* expression pattern in *A. thaliana* reproductive tissues (Ler), by RNA *in situ* hybridisation (*UIF1* antisense probe). Longitudinal sections through an inflorescence meristem (ifm) with adjacent floral meristems (fm) (D), a stage 7–8 flower (st, stamen primordia; ca, carpel primordia) (E) and the pistil of a maturing flower, showing *UIF1* transcripts at the base of the gynoecium (arrowhead) and in the ovules (black arrows) (F). (G) Subcellular localisation of the GFP-*UIF1* fusion protein in onion epidermis cells. GFP-*UIF1* is detected in the nucleus (red arrows) and cytosolic foci. GFP control is homogeneously spread in the cytosol and nucleus. BF, bright field. Scale bars: 50 μ m.

organs, such as branched stamens, were observed in flowers of *uif1 ult1* and *uif1* mutants but never in *ult1-3*, supporting an additional role of *UIF1*, independent of *ULT1* function, in the control of flower organ identity.

***UIF1* possesses DNA-binding sites in *WUS* promoter regions**

To check whether the Myb-containing *UIF1* protein is able to bind DNA on specific target sequences, a protein binding microarray (PBM) experiment (Berger and Bulyk, 2009; Godoy et al., 2011; Franco-Zorrilla et al., 2014) was performed. Analysis of DNA fragments bound by MBP-*UIF1* recombinant protein yielded a series of motifs recognised with high affinity (E-score >0.45), resembling DNA sequences recognised by other transcription factors belonging to the same GARP G2-like subfamily (Franco-Zorrilla et al., 2014; Weirauch et al., 2014) (Fig. 5A). Position weight matrices of the three top-scoring motifs were used to scan regulatory sequences in putative target genes of *UIF1* using Morpheus (<http://biodev.cea.fr/morpheus>), an algorithm that predicts binding sites in given sequences by computing affinity scores related to the used matrix (Fig. 5A). Because *uif1* mutants phenocopy *ult1* mutants, we assessed whether *WUS* could be a direct target of *UIF1* (Carles et al., 2005). Hence, binding sites for *UIF1* were searched and found in the promoter of *WUS*. The top two sites (Fig. S5; Fig. 5A) lie in specific regulatory regions as reported in the *WUS* promoter scanning study of Bäurle and Laux (2005). The *WUS-1* site (position –1182) is located in proximity to the quantitative element required for enhanced expression levels in the meristem, and the *WUS-2* site (position –2819) is embedded in the region for expression intensity in FM and ovule. Protein-DNA interactions were tested by an electrophoretic mobility shift assay (EMSA) using purified recombinant His-tagged GST-*UIF1* fusion

protein (Fig. 5A,B; Fig. S6). We found that *UIF1* specifically binds the GTAGATTCCT motif (*WUS-1*), as interaction is lost upon mutation of either of two single bases at positions 5 or 8 in the sequence. Similar results were obtained when using the full-length protein or the isolated Myb domain of *UIF1* fused to GST (Fig. 5B; Fig. S6). This suggests that *UIF1* might directly regulate *WUS*.

***UIF1* binds to DNA elements present in the *AG* gene and in *CUC* gene promoters**

We showed in this study that *UIF1* physically interacts with *ULT1*, previously reported to control *AG* expression. Moreover, we found that some of the *uif1* phenotypes, such as organ fusions, are reminiscent of those observed in the *cup-shaped cotyledon* (*cuc*) mutant backgrounds (Aida et al., 1997). This prompted us to seek putative *UIF1*-binding sites at the *AG* and *CUC* loci (Takada et al., 2001; Aida et al., 1997; Vroemen et al., 2003). Three *UIF1* binding sites were found in *AG* regulatory sequences (Fig. 5A). Among these, the best-scored AAGAATCTTT site, present in the large regulatory intron of *AG*, was confirmed by EMSA (Fig. 5B; Fig. S6). We found that *UIF1* specifically binds this motif, as interaction is lost upon mutation of a single base (*c7a*) in the sequence. *UIF1*-binding sites were also found in the promoters of the *CUC* genes: two in *CUC1*, three in *CUC2* and two in *CUC3* (Fig. 5A). Specific binding to motifs in the *CUC* genes was confirmed by EMSA (Fig. S6) and interaction with the *UIF1* Myb domain was strongly reduced upon a single base mutation (*a5t*). These data indicate that *UIF1* probably has a direct effect on *AG* and *CUC* gene regulation.

***UIF1* has a transcriptional repressor activity**

To test whether *UIF1* has a role in regulating transcription, we performed a dual luciferase reporter assay (DLRA) in onion epidermis cells (Fig. 5C). For this experiment, *UIF1* protein was fused to the Gal4 DNA-binding domain (G4DBD-*UIF1*), which binds to the upstream activation sequence (UAS) site located upstream of the CaMV35S minimal promoter driving the expression of firefly luciferase. The DLRA test showed that firefly luciferase expression was repressed by a mean of 2.7-fold in the presence of *UIF1* (G4DBD-*UIF1*) compared with the inert control (G4DBD-GFP), indicating that *UIF1* might function as a transcriptional repressor. Even though repression was not as efficient as for the EAR-containing protein IAA12/BODENLOS (BDL; 10.8-fold repression reduction; Szemenyei et al., 2008), we tested whether *UIF1* activity was due to EAR motifs mapped at the N-terminus (EAR^N) and C-terminus (EAR^C) of the protein (Fig. 2B). We used two modified versions of *UIF1*: a truncated version lacking EAR^C (*UIF1-dCT*) or the truncated version with additional mutations in EAR^N by substituting leucines with alanines (*UIF1-ANANA-dCT*). Although deletion of EAR^C did not significantly affect *UIF1* activity, additional mutation in EAR^N completely abolished its activity (Fig. 5C). Thus, the conserved EAR^N motif of *UIF1* is necessary for transcriptional repression.

Therefore, in *A. thaliana*, *UIF1* could be a transcriptional repressor of *WUS* during FM development. To investigate this, we performed RT-qPCR analyses in inflorescences of the two mutant alleles, *uif1-1* and *uif1-3*. In order to exclude *WUS* RNA derived from anthers, we used dissected inflorescences, harbouring FM younger than stage 5 (Fig. S7). We systematically found a significant increase in *WUS* expression in the mutants (Fig. 5D). This potential, direct negative regulation of *WUS* by *UIF1* could explain the production of supernumerary organs by *uif1* mutant flowers, in a *ULT1*-dependent manner (Figs 4, 5). Indeed, we previously showed that the *WUS* expression domain was laterally enlarged in *ult1* mutants, in

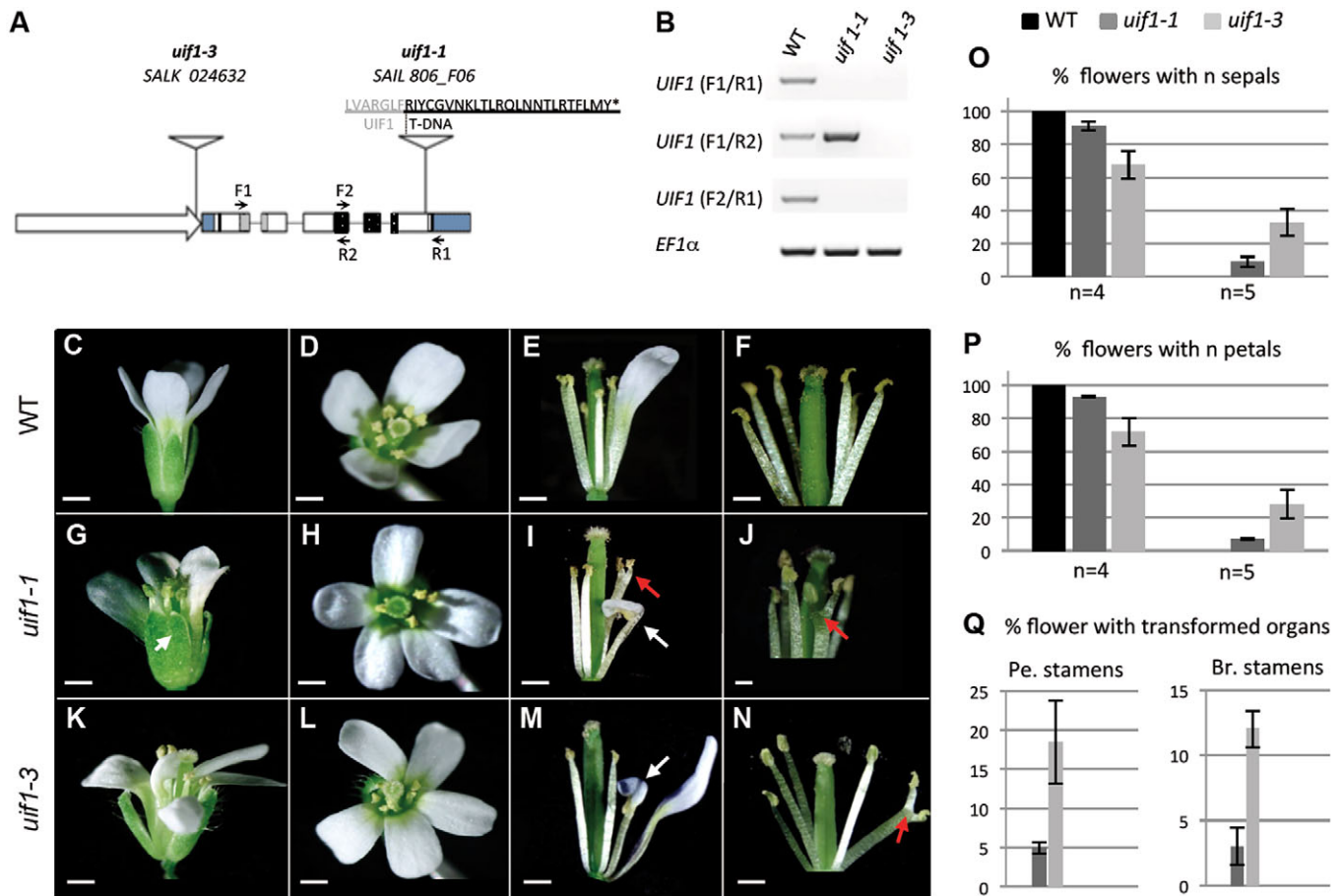


Fig. 3. *uif1-1* and *uif1-3* mutant alleles display supernumerary floral organs and loss of floral organ identity. (A) Diagram of *UIF1* genomic region and location of the T-DNA insertions in *uif1* mutant lines. Promoter (white arrow), 5'- and 3'-UTR (blue boxes), coding regions (boxes) with hydrophobic region (grey box), Myb domain (black dotted box), predicted NLS (white box) and two distinct EAR-like motifs (small black boxes close to N- and C-terminal UTR regions). The C-terminal predicted protein sequence for *uif1-1* appears on top of the diagram. Arrows indicate primers used for RT-PCR (B). (B) RT-PCR analysis of *UIF1* expression in inflorescences of mutant lines. In both *uif1-1* and *uif1-3*, cDNA corresponding to the entire *UIF1* transcript could not be detected. *EF1α*: control. (C–N) Abnormal flower development in *uif1* mutants. *uif1-1* and *uif1-3* flowers contain additional sepals (G,K) and petals (H,L), compared with WT (C,D). *uif1* flowers carry fused sepals (white arrow in G), petaloid stamens (white arrows in I,M) and branching stamens (red arrows in I,J,N). Scale bars: 1 mm. (O–Q) Organ number and transformed organs counted in *uif1* mutant flowers. (O,P) Percentage of flowers with four or five sepals (O) and petals (P) or with transformed organs (Q; Pe, petaloid stamens; Br, branching stamens) counted from 300 flowers per line. Error bars represent s.d. for three biological replicates.

a *Ler* background (Carles et al., 2005). Interestingly, and as expected in view of the relative phenotypic intensities, *uif1* mutant alleles show a weaker increase in *WUS* expression relative to the *ult1-3* null mutant in a Col-0 background (Fig. 5D).

DISCUSSION

ULT1 and UIF1 regulate homeostasis of floral meristem in a same pathway

Here, we report *in vivo* physical interaction between ULT1 and the novel Myb transcription factor UIF1 (Figs 1, 2). UIF1 was isolated from a Y2H screen against ULT1. The interaction was further confirmed in plants, where BiFC took place in the

nucleus. We show that the UIF1 N-terminus region is necessary and that the ULT1 C-terminus region containing the B-box is sufficient to mediate the interaction. This is strongly supported by reports in plants showing that the B-box domain is involved in protein-protein interactions (Datta et al., 2006, 2007; Gangappa et al., 2013). In particular, the formation of heterodimers both within and outside the B-box-containing protein family play important roles in regulating transcription and fine-tuning plant growth and development (Gangappa and Botto, 2014).

Both *ULT1* and *UIF1* genes are expressed from early stages of *A. thaliana* vegetative development and have overlapping expression patterns in inflorescence and floral meristems. However, the levels of expression of *ULT1* and *UIF1* differ in floral organs or developmental stages. Interestingly, the differences seem to be associated with the developmental status of organs, *ULT1* being expressed at higher levels in dividing tissues and *UIF1* being expressed at higher levels in differentiating tissues.

Similarly to *ult1* mutants, *uif1* mutants display supernumerary floral organs (Fig. 3). In *ult1* mutants, increase of floral organ numbers correlates with inflorescence and floral meristem enlargement (Fletcher, 2001) and with lateral enlargement of the

Table 2. Modified organs in *uif1* mutant alleles

Phenotype	Percentage of flowers exhibiting phenotype (±s.d.)		
	<i>uif1-1</i>	<i>uif1-3</i>	Ler WT
Fused sepals	5±3	13±4	0
Petaloid stamens	5±1	18±5	0
Branching stamens	4±2	12±2	0

n=300 flowers per genotype.

WUS domain of expression (Carles et al., 2005). Flowers of *ult1 uif1* double and *ult1* single mutants produce a similar number of additional sepals and petals (Fig. 4), indicating that *ULT1* and *UIF1* function in the same regulatory pathway for the production of perianth founder cells. The low penetrance of *uif1* phenotypes, together with their moderate intensity, do not allow measurement of meristem size differences, nor enlargement of the *WUS* expression domain. However, we found a slight increase in *WUS* transcript level in both *uif1* mutant alleles (Fig. 5). Together, these findings indicate that *ULT1* and *UIF1* might function together in OC size regulation.

UIF1 could provide DNA-binding specificity for ULT1 at target loci such as *WUS*

ULT1 was previously proposed to act through *WUS* to regulate meristem homeostasis (Carles et al., 2004), functioning as a negative regulator of *WUS* expression in inflorescence and floral

Table 3. Supernumerary or modified organs in *uif1 ult1* double and single mutants*

Phenotype	Percentage of flowers exhibiting phenotype (±s.d.)		
	<i>uif1-3 ult1-3</i>	<i>uif1-3</i>	<i>ult1-3</i>
Five to eight sepals	58±2	18±3	52±2
Five to eight petals	57±4	18±3	53±3
Gynoecium with three locules	28±1	0	25±4
Branching stamens	6±1	7±1	0

n=300 flowers per genotype.

*Double and single mutants were selected from the same F2 population obtained from a cross between *uif1-3* and *ult1-3* mutants.

meristems (Carles et al., 2005). Although *ULT1* protein contains a SAND domain shown to act as a DNA-binding module in animals (Bottomley et al., 2001; Surdo et al., 2003) and although *ULT1* protein shows general affinity for DNA, no evidence for *ULT1* binding at the *WUS* locus was reported thus far, nor that *ULT1* protein could recognise specific DNA motifs (Carles and Fletcher, 2009; Engelhorn et al., 2014a). In the present study, we show that *UIF1* is able to bind DNA and can recognise motifs in the promoter sequence of *WUS*. Scanning of the entire promoter using *UIF1* PBM-generated matrices led to relevant binding sites in regions previously reported as general, meristem or stem cell quantitative elements (QEs) (Fig. S5; Bäurle and Laux, 2005).

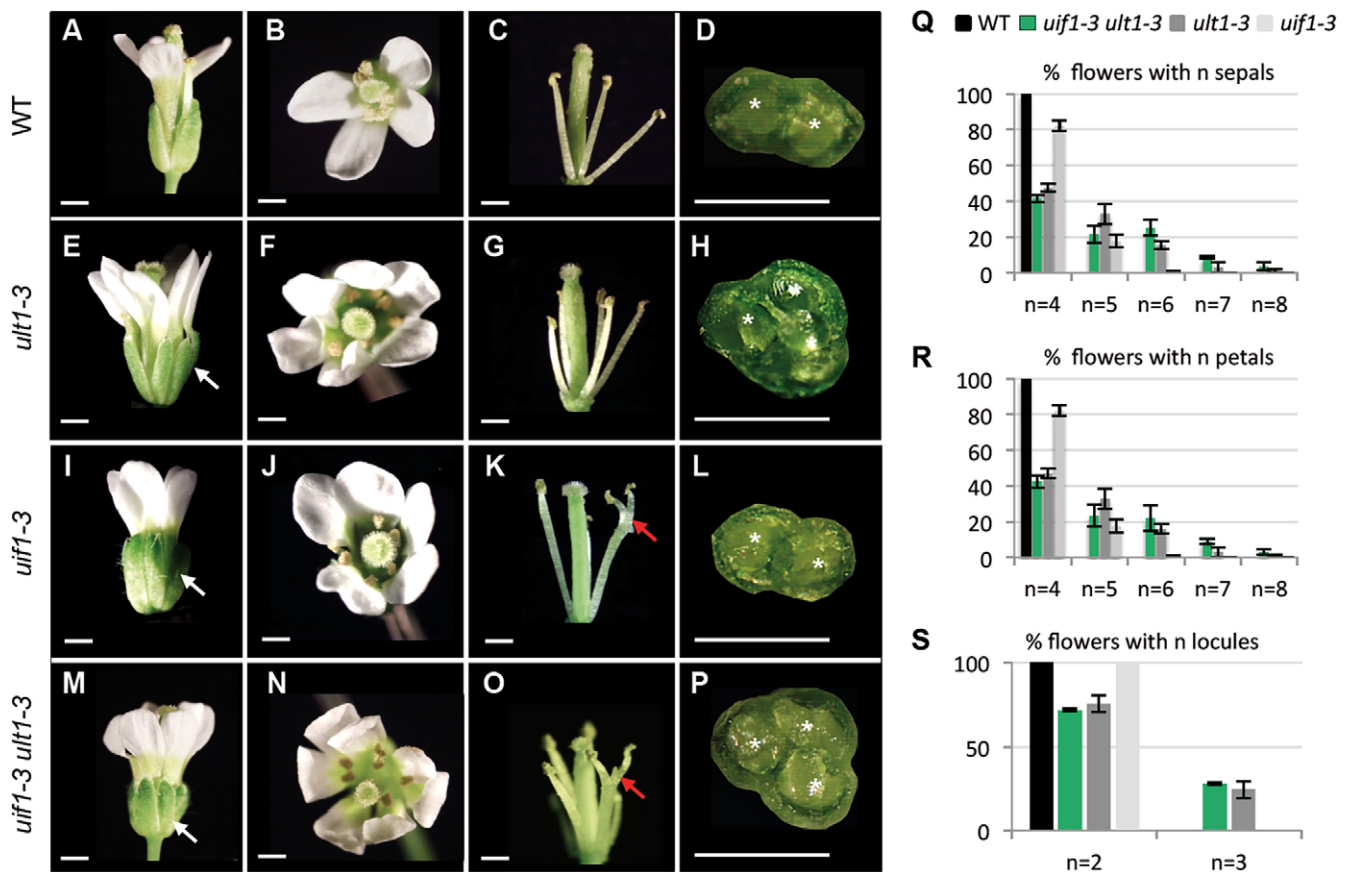


Fig. 4. *ULT1* and *UIF1* control floral organ number in the same pathway. (A–P) Phenotypes of *uif1-3 ult1-3* double and single mutants, including additional sepals (white arrows in E, I, M); pistils (P) with locules (asterisks in D, H, L, P); branching stamens (red arrows in K, O). Scale bars: 1 mm. (Q–S) Graphs showing percentage of flowers with supernumerary floral organs. (Q, R) Percentage of flowers carrying from four to eight sepals (Q) or petals (R), and two or three locules (S), counted from 300 flowers per line. Error bars represent s.d. χ^2 tests showed that each of the *uif1 ult1*, *uif1* and *ult1* populations was homogeneous (α -level=0.05) and that there was no significant difference between *uif1 ult1* and *ult1* mutants for additional floral organs (α -level=0.02 for sepal or petal number; 0.001 for locule number).

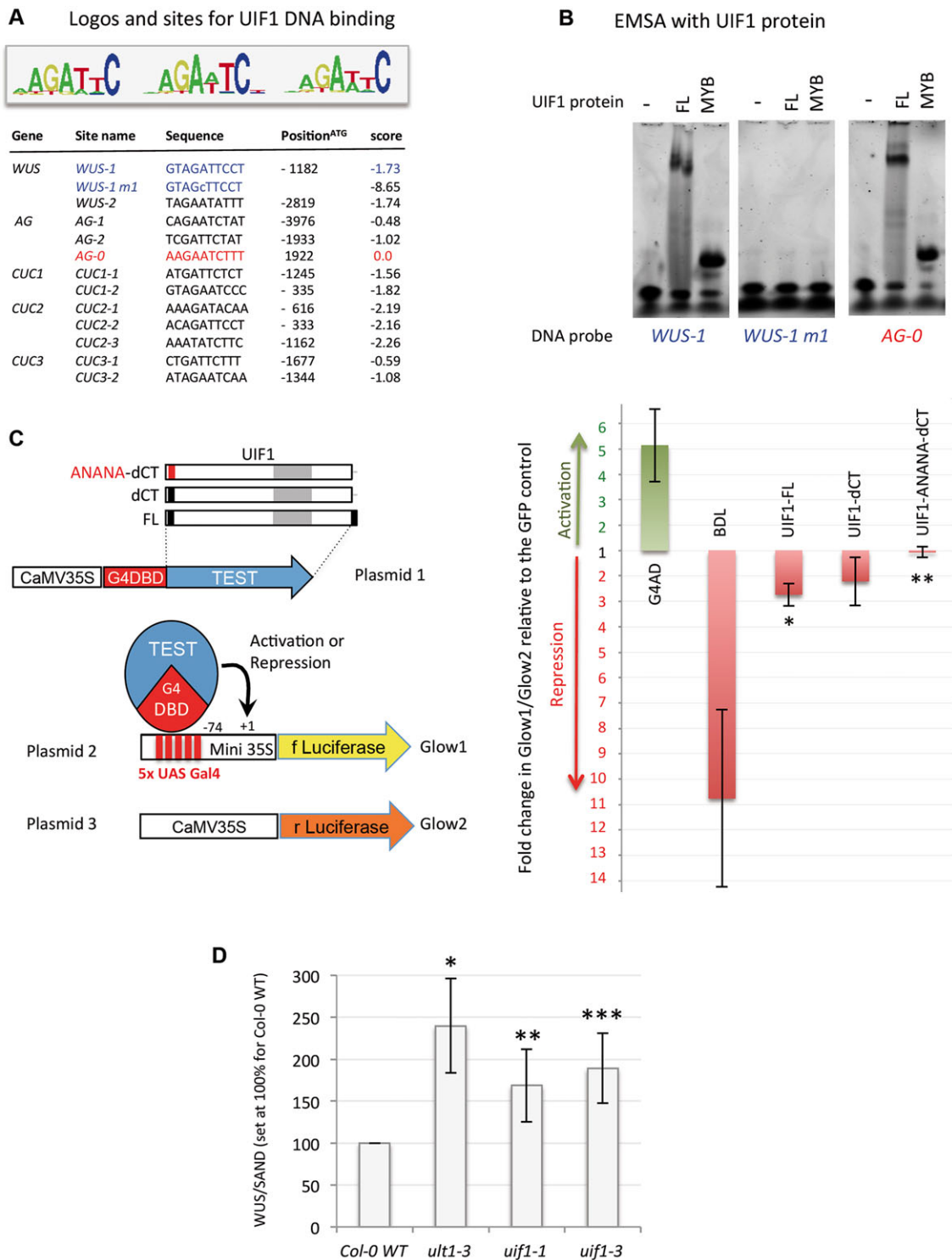


Fig. 5. UIF1 binds to DNA motifs present in *WUS*, *AG* and *CUC* genes and has transcriptional repressor activity. (A) Logos for PBM-deduced UIF1-binding motifs, and sequences of binding sites identified in *WUS*, *CUC1-3* and *AG* genes. (B) Sequences in blue and red in A (best-scored UIF1 binding sites in *WUS* and in *AG*, or mutated version) were tested by EMSA, using the full-length UIF1 protein (6His-GST-UIF1, FL) or the Myb domain (6His-UIF1-Myb, MYB). –, sample without protein. (C) Dual luciferase reporter assay using UIF1 and controls. Left: Schematic of the constructs and the three-plasmid system. Plasmid 1: UIF1 variants fused to the Gal4 DNA-binding domain (G4DBD) specific to the Gal4 5xUAS cloned upstream of the -74 bp *CaMV35S* (mini35S) and controlling expression of the firefly luciferase gene (fLuciferase, Plasmid 2). Plasmid 3: internal transfection control using the *Renilla* luciferase gene (rLuciferase) under the control of full length *CaMV35S* promoter (*CaMV35S*). Right: Graph representing transcriptional activity expressed as (Glow1/Glow2) normalised to the basal activity of G4DBD-GFP set at 1. G4AD, Gal4 activating domain used as activation control; BDL, BODENLOS/IAA12 used as repression control. *P*-values calculated by *t*-test: $*P=2.59 \times 10^{-3}$, significant repressive effect of UIF1-FL compared with GFP; $**P=0.13 \times 10^{-3}$, significant loss of repressive effect for UIF1-ANANA-dCT compared with UIF1-FL. The difference between UIF1-dCT and UIF1-FL repressive effects is not significant ($P=0.13$). (D) RT-qPCR analysis of *WUS* gene expression in dissected inflorescences (with FM younger than stage 5) of Col-0 WT, *ult1-3*, *uif1-1* and *uif1-3* mutant alleles. *WUS* expression levels were normalised against the *SAND* gene. Error bars indicate s.d. *P*-values (*t*-test) for differences with Col-0 WT: $*P=1.32 \times 10^{-3}$, $**P=4.60 \times 10^{-3}$, $***P=1.91 \times 10^{-3}$.

We showed by EMSA that UIF1 specifically binds to a site proximal to the meristem QE and that a single base mutation abolishes the binding (Fig. 5; Fig. S6). Because UIF1 protein possesses a repressor activity (Fig. 5), a reasonable hypothesis is that UIF1 binds to *WUS* and directly contributes to repress its transcription. This hypothesis is supported by the increased *WUS* expression in the inflorescences of both *uif1* mutant alleles. Moreover, UIF1 might provide DNA-binding specificity and contribute to ULT1 recruitment at the *WUS* locus for further repression via chromatin modifications. The respective mutant phenotypes support this scenario, as *ult1* defects are stronger than those of *uif1*.

Several recent studies have shown that binding of some transcriptional factors to their targets may occur prior to recruitment of the chromatin-modifying machinery, defining them as pioneers for transcriptional activation of target genes (Smale, 2010; Magnani et al., 2011; Zaret and Carroll, 2011; Iwafuchi-Doi and Zaret, 2014). In particular in plants, a pioneer function was proposed for the MADS domain-containing proteins functioning in transcriptional activation of flower morphogenesis genes (Pajoro et al., 2014). It is very likely that, via early binding to target loci, these pioneer factors could function to increase accessibility of the locus, by recruiting chromatin remodellers and modifiers (Smaczniak et al., 2012). UIF1 could be another of these TFs that recruits chromatin regulators to their targets for further regulation of chromatin accessibility. Of relevance to the important function of ULT1 in *AG* temporal activation at the centre of the FM, it cannot be excluded that UIF1 could be required to bring ULT1 to *AG* cis-regulatory elements in the proximal promoter and large intron region (Carles and Fletcher, 2009; 2010). Indeed, we found that UIF1 can specifically bind to *AG* cis-regulatory sequences located in both the promoter and the large intron, regions that we previously reported as bound by ULT1 in chromatin immunoprecipitation experiments (Carles and Fletcher, 2009).

How can we reconcile this hypothesis with a potential transcriptional repressor function, as shown by DLRA experiments? UIF1 might sit on the *AG* locus at early stages of FM development, preventing its expression, and upon an unknown signal around stage 3, bind to ULT1 protein, leading to further recruitment of chromatin factors, such as ATX1, for enrolment of the RNA PolII complex and transcriptional initiation. An alternative hypothesis is that UIF1 could carry a dual function on transcriptional regulation, depending on its interactors, acting either as repressor or as activator, as already reported for several transcription factors in *A. thaliana*, such as WUS, AP2 and FIL (Ikeda et al., 2009; Yant et al., 2010; Bonaccorso et al., 2012).

UIF1 functions in maintaining flower organ identity or spatial boundaries

The *uif1* mutants display organ fusions at their sepals and stamens. Furthermore, stamens of these mutants partially lose their identity and tend to become petaloid. Such phenotypes were observed in *uif1* and *uif1 ult1* plants but never in *ult1* mutants. This supports additional roles of *UIF1* in the control of floral organ identity and patterning that are independent of *ULT1* function. Interestingly, the organ fusion phenotypes are reminiscent of those observed in *cuc* mutants (Aida et al., 1997; Takada et al., 2001). *CUC* genes encode NAC domain transcription factors that specify boundaries between meristematic and primordia cells, as well as lateral organ separation (Aida et al., 1997; Takada et al., 2001; Vroemen et al., 2003; Breuil-Boyer et al., 2004; Cheng et al., 2012). Thus, mutations in these genes result in sepal fusions and stamens branching with variable

strengths ranging from partial to complete fusion depending on genetic backgrounds, such as single or multiple mutants in pairwise combination (Aida et al., 1997; Takada et al., 2001; Vroemen et al., 2003). The fact that *CUC* promoters contain UIF1-binding sites therefore suggests that UIF1 directly influences *CUC* gene expression (Fig. 5; Fig. S6). However, further investigations would be required to address this hypothesis.

The fact that *AG* also contains UIF1-binding sites might explain the loss of stamen identities and the development of petaloid stamens observed in *uif1* mutants. Indeed, a decrease of *AG* expression in the developing third whorl would promote an expression of A-class genes in this domain and therefore the development of petals in place of stamens. The decrease of *AG* expression is probably very mild, thus explaining the low rate of partial homeotic transformations observed in *uif1* backgrounds. However, we cannot exclude the possibility that this phenotype also results from boundary defects between whorls 2 and 3. Such hypotheses would suggest again that UIF1 may be either an activator or a repressor of transcription, promoting here activation of *AG* expression but repressing *WUS* expression in other domains.

MATERIALS AND METHODS

Plant material and growth conditions

The *A. thaliana* Columbia-0 (Col-0) and Landsberg *erecta* (*Ler*) ecotypes were used as the WT strains for the *uif1* and *ult1* alleles, respectively. Tobacco (*Nicotiana benthamiana*) plants and onion epidermis were used for transient experiments. The *uif1-1* (SAIL_806_F06) and *uif1-3* (SALK_024632) T-DNA insertional mutant lines were backcrossed two times to Col-0 before analysis. Although a third mutant allele initially named *uif1-2* (SALK-073584) putatively contains an insertion in the *UIF1* gene, we could not confirm the presence of a T-DNA at the locus in this line. Double mutant plants are in a mixed *Ler* (from *ult1-3*)/Col (*uif1* alleles) background. As controls, we used single mutants in a mixed background obtained in the F2 generation from the same cross. *A. thaliana* plants were cultivated under long-day conditions (16 h light/8 h dark) at 18°C/16°C degrees. Tobacco plants were cultivated under long-day conditions (16 h light/8 h dark) at 22°C degrees. DNA material and cloning procedures are detailed in supplementary Materials and Methods.

Analysis and alignment of protein sequences

Sequences of hydrophobic and Myb conserved domains were submitted to GlobPlot (<http://globplot.embl.de/cgiDict.py>) for prediction of structured domains and to BlastP (<http://blast.ncbi.nlm.nih.gov/>) for calculation of percentages of similarity. Sequences were aligned using the MultAlin program (Corpet, 1988) and designed under ESPript3.0 (Robert and Gouet, 2014).

Yeast two-hybrid (Y2H) screen

For binary interaction tests, ULT1 and UIF1 full-length and truncated versions were expressed as Gal4 DNA-BD (BD: binding domain) or Gal4 AD (AD: activation domain) fusion proteins. *ULT1* and *UIF1*-transformed yeast strains were mated and the resulting diploids were selected for the presence of both plasmids with medium lacking Leu and Trp (–LT). The selected strains were then dotted on –LT or –LTAH medium (lacking Leu, Trp, His and Ade) on which only the diploids presenting protein interactions can grow. For more details on the Y2H screen and protein extraction for western blot on mated Y2H diploids, see supplementary Materials and Methods.

Bimolecular fluorescent complementation (BiFC) and subcellular localisation

For BiFC tests in tobacco leaf epidermis cells, constructs were transfected by agro-infiltration into leaves of 4-week-old *N. benthamiana* (Hamilton et al., 2002; Walter et al., 2004). When OD_{600 nm}=0.5 was reached, *Agrobacterium* cultures were pelleted and resuspended in 10 mM MgCl₂ and then treated with 100 μM acetosyringone for 2 h. Tobacco leaves were infiltrated with *Agrobacterium* solutions for co-transformation allowing

expression of proteins fused to the N-terminal or C-terminal part of the split YFP (NY or YC). Cultures were grown separately and mixed at equal density before co-infiltration. For subcellular localisation, the pE2S-UIF1 constructs were transformed into onion epidermis cells using the Biolistic PDS-1000/He particle delivery system (Bio-Rad) (Sanford et al., 1993). The corresponding GFP-UIF fusion construct is suitable to address subcellular localisation of UIF1 protein as GFP-UIF can complement *uif1* mutants, indicating that the GFP does not affect UIF1 function. Tissues were observed 3 days after infiltration (tobacco) or 24–36 h after bombardment (onion), by epifluorescence microscopy under an AxioScope A1 (Carl Zeiss), using a 46 YFP filter (EX BP 500/20, BS FT 515, EM BP 535/30), a 49 DAPI filter (EX G 365, BS FT 395, EM BP 445/50) or a 38 Endow GFP filter (EX BP 470/40, BS FT 495, EM BP 525/50). Images were acquired using an AxioCam MRc camera equipped with the ZEN lite Module Hardware.

Dual luciferase reporter assay (DLRA)

Micro-projectiles were prepared with 50 ng of pRLC (*Renilla* luciferase construct), 400 ng of pBB168 (firefly luciferase construct; Blanvillain et al., 2011) and 15 µg of G4DBD-UIF1, G4DBD-UIF1-dCT or G4DBD-UIF1-ANANA-dCT. Onion epidermal cells were transfected as described above and kept in the dark for 20 h at 21°C, before being ground in liquid nitrogen. Proteins were extracted in 1 ml of PBLuc buffer [200 mM NaPO₄, pH 7, 4 mM EDTA, 2 mM dithiothreitol, 5% glycerol, 10 mg/l bovine serum albumin and EDTA-free protease inhibitor cocktail Complete (Roche)] and assayed (5–20 µl) using the Dual-Luciferase Reporter Assay System (Promega). Luciferase activities (mean±s.d.) were determined from seven independent biological replicates for each tested combination and normalised to the G4-DBD-GFP control set at 1.

Gene expression analyses

Total RNA extraction and RT-PCR experiments were performed as previously described (Engelhorn et al., 2014b), with 35 cycles of PCR (except for *EF1α*, 25 cycles). RT-qPCR experiments were performed in a 10 µl final volume, using 5 µl of SYBR Green Mix buffer (Bio-Rad) and 0.4 µM primers. The Bio-Rad CFX 384 real time system was used with the following program: 98°C, 2 min; 39 cycles (98°C, 5 s; 60°C, 5 s). Relative quantification (RQ) values were calculated using the 2^{−ΔΔCT} method (Livak and Schmittgen, 2001). ΔCt values were calculated using the *EF1α* or *SAND* genes as endogenous controls. Mean values±s.d. were calculated from three biological replicates (each quantified in three technical replicates). Sequences of *UIF1* and *WUS* primers are listed in Table S1, and those for *ULT1*, *EF1α* (At5g60390) and *SAND* (At2g28390) were already described (Carles and Fletcher, 2009; Czechowski et al., 2005).

RNA *in situ* hybridisation was performed as previously described (Carles and Fletcher, 2009). Inflorescences were harvested 4 weeks after bolting. *UIF1* sense and antisense probes were generated by T7 RNA polymerase activity from a 1-kb insert (PCR product amplified from UIF1 cDNA, using the ISH_UIF_F1/ISH_UIF_R1 primers; Table S1), cloned into the Zero Blunt PCR vector (Invitrogen).

Statistical assessment of floral organ identity and number

Counting of floral organs was carried out on the ten first flowers of first ten bolting plants for each genotype. We used the χ^2 test (Plackett, 1983) to verify that each population is homogeneous and to assess whether percentage values of additional organs counted between *uif1ult1* and *ult1* mutant lines are significantly different.

Production and purification of recombinant proteins

UIF1 protein (fused to the 6His-MBP tag for PBM experiments, or to the 6His-GST for EMSA experiments) or the UIF1 Myb domain (fused to 6His tag for EMSA experiments) were expressed in *Escherichia coli* strain Rosetta2 (DE3) (Novagen Merck Millipore), from the pETH447, pETH380 or pETH455 vector, respectively. After induction by 0.4 mM isopropyl β-D-1-thiogalactopyranoside, cells were grown overnight at 17°C. Prior to EMSA experiments, recombinant proteins were purified via their 6His tag. Pellets of 0.5 l of culture were sonicated in 50 ml lysis buffer A [500 mM

NaCl, 20 mM Tris-HCl pH 8, 1 mM Tris (2-carboxyethyl)phosphine hydrochloride], containing one protease inhibitor cocktail tablet Complete EDTA-free (Roche) and centrifuged for 30 min at 13,000 g. Clear supernatants were loaded on 1 ml Ni-Sepharose resin (GE Healthcare). Resin was washed with 20 volumes of buffer A containing 30 mM imidazole and eluted with buffer A containing 350 mM imidazole. The fractions containing recombinant proteins were pooled and dialysed overnight against buffer A.

Determination of UIF1 DNA-binding specificity by protein binding microarrays (PBM)

A pellet corresponding to 25 ml of an induced *E. coli* culture was stored at −80°C and resuspended in 1 ml 1× binding buffer prior to the DNA-binding assay. Synthesis of double-stranded microarray, protein incubation and immunological detection of DNA-protein complexes were performed as described (Godoy et al., 2011). We used the nPBM11 design containing 167,773 different oligonucleotide probes (Franco-Zorrilla et al., 2014) synthesised in an Agilent's SurePrint G3 4×180k format (Agilent Technologies). DNA microarray was scanned in a DNA Microarray Scanner at 2 µm resolution and quantified with Feature Extraction 9.0 software (Agilent Technologies). Normalisation of probe intensities and calculation of E-scores of all the possible 8-mers were carried out with the PBM Analysis Suite (Berger and Bulyk, 2009). Perl scripts were modified to adapt them to nPBM11 microarray dimensions and to input files generated by Feature Extraction software. Position weight matrices corresponding to top three motifs recognised with high affinity (UIF1_1ary, E-score=0.4832; UIF1_2ary, E-score=0.4814; and UIF1_3ary, E-score=0.4804) were obtained from analysis of all DNA fragments bound by the UIF1 using PBMs. These matrices were used for prediction of UIF1 binding sites in candidate targets with the Morpheus program (<http://biodev.cea.fr/morpheus>; Moyroud et al., 2011), using a cut-off score of −3. A score was assigned to each binding site found in candidate target genes (*WUS*, *AG*, *CUC1-3*), and only those with a score ranging from −2 to 0 (best score value) were considered for further EMSA analyses. [The best-scored AG-0 sequence is found at the 5.23×10^{−6} frequency in genome-wide regulatory regions (from a scan through the TAIR10-3 kb upstream database).] For more details of the generation of these scores, see supplementary Materials and Methods.

Electrophoretic mobility shift assays (EMSA)

For EMSA, dsDNA probes were prepared by incubating complementary single-stranded oligonucleotides (Eurofins Genomics) in annealing buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl and 1 mM EDTA). dsDNA (4 pmol) with a protruding G were fluorescently labelled with Cy5-dCTP (8 pmol) (GE Healthcare) using 1 unit of Klenow fragment polymerase (Ozyme) in 1×Klenow buffer for 1 h at 37°C. Binding reactions were performed in 20 µl binding buffer (50 mM NaCl, 10 mM Tris-HCl pH 7.5, 1% glycerol, 0.5 mM EDTA, 1 mM MgCl₂, 0.05 mM ZnCl₂ and 1% NP-40) supplemented with 28 ng/µl fish sperm DNA (Roche) and 10 nM double-stranded DNA probe. Binding reactions were loaded onto native 6% polyacrylamide gels 0.5× TBE (45 mM Tris, 45 mM boric acid and 1 mM EDTA, pH 8) and electrophoresed at 90 V for 75 min at 4°C. Gels were scanned on a Typhoon 9400 scanner [excitation light 649 nm, emission filter 670 nm band-pass filter (670 BP 30); Molecular Dynamics].

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Competing interests

The authors declare no competing or financial interests.

Author contributions

C.C.C. and C.T. designed the project and approaches; F.M., E.T., R.B., I.L.-V., J.M.F.-Z., C.T. and C.C.C. performed experiments and data analysis, with the

support of R.D., F.P. and P.M.; C.C.C. wrote the manuscript with the help of C.T. and F.M. and contribution from E.T.

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Data availability

Data are available from the Tair database (<https://www.arabidopsis.org/servlets/TairObject?id=126622&type=locus>) and the eFP Browser database (<http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi>).

Supplementary information

Supplementary information available online at <http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.127365/-/DC1>

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