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Title

The F-box protein UFO controls flower development by redirecting the master transcription factor LEAFY to novel cis-elements

Authors

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Abstract

In angiosperms, flower development requires the combined action of the transcription factor (TF) LEAFY (LFY), and the ubiquitin ligase adaptor F-box protein, UNUSUAL FLORAL ORGANS (UFO), but the molecular mechanism underlying this synergy has remained unknown. Here, we show in transient assays and stable transgenic plants that the connection to ubiquitination pathways suggested by the UFO F-box domain is mostly dispensable. Based on biochemical and genome-wide studies, we establish that UFO instead acts by forming an active transcriptional complex with LFY at newly discovered regulatory elements. Structural characterization of the LFY-UFO-DNA complex by cryo-electron microscopy further demonstrates that UFO performs this function by directly interacting with both LFY and DNA. Finally, we propose that this complex might have a deep evolutionary origin, largely predating flowering plants. This work reveals a novel mechanism of an F-box protein directly modulating the DNA-binding specificity of a master TF.

Main text

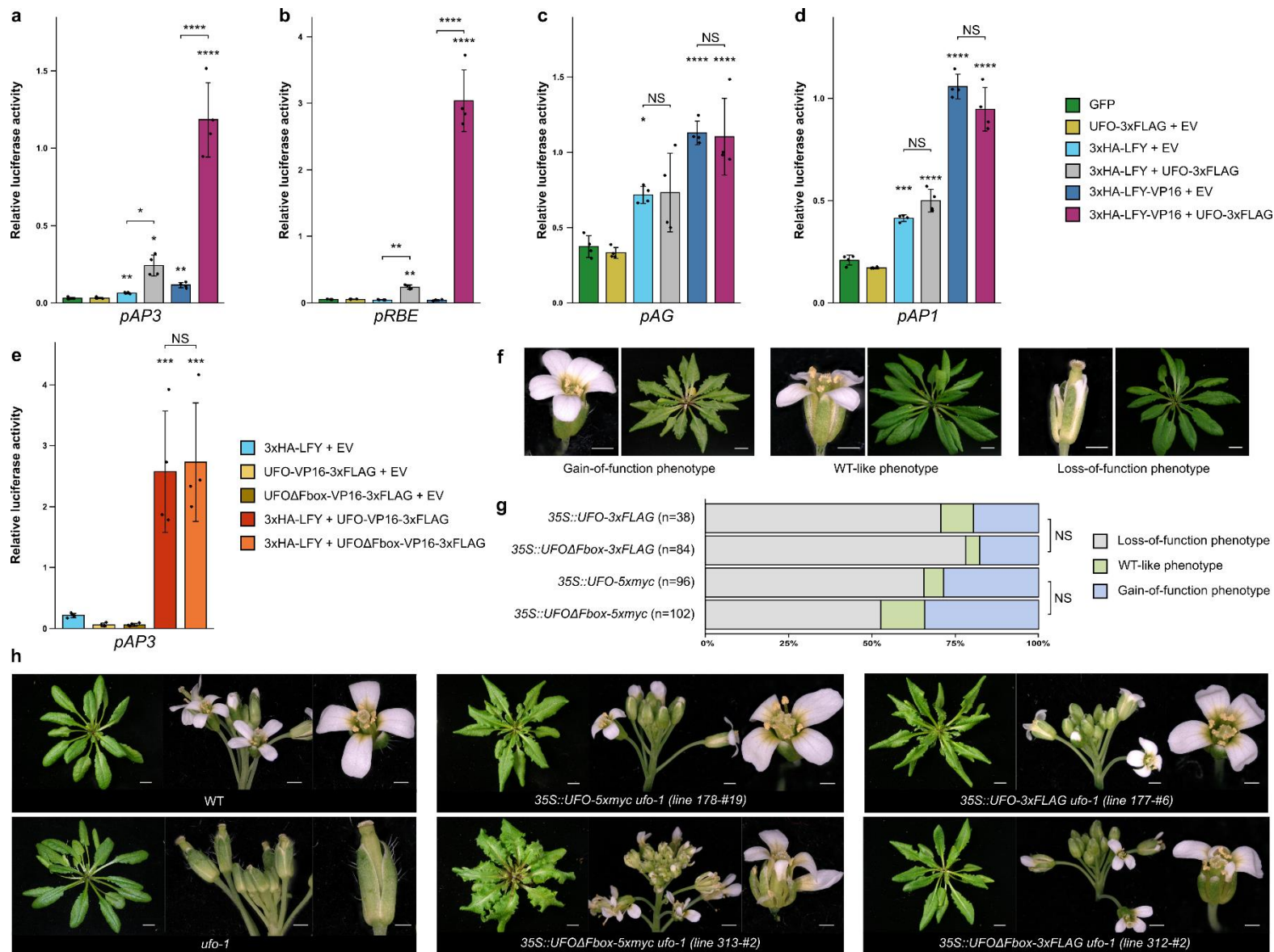
The formation of flowers is key to the reproductive success of angiosperms. Flowers are made of four types of organs (sepals, petals, stamens and carpels) arranged in concentric whorls. The patterning of flower meristems requires the localized induction of the ABCE floral homeotic genes that determine specific floral organ identities. In *Arabidopsis thaliana*, this developmental step is largely controlled by the master transcription factor (TF) LEAFY (LFY) that activates the ABCE genes^{1,2}. LFY directly activates the A class gene *APETALA1* (*API*) uniformly in the early flower meristem^{3,4}, while activations of B and C genes are local and require the activity of cofactors. For instance, LFY regulates the C class gene *AGAMOUS* (*AG*)

in conjunction with the TF WUSCHEL to specify third (stamen) and fourth whorl (carpel) identities⁵. The activation of the B class gene *APETALA3* (*AP3*), necessary to specify the identity of the second (petal) and third whorls of the flower, requires the combined activity of LFY and the spatially-delineated cofactor UNUSUAL FLORAL ORGANS (*UFO*)⁶⁻⁸. In Arabidopsis, the main function of LFY and *UFO* is to activate *AP3*⁹ but in numerous species (such as rice, wheat, tomato or petunia), their joint role goes well beyond *B* genes activation and is key to floral meristem and inflorescence development¹⁰⁻¹³.

At the molecular level, little is known on the nature of LFY-*UFO* synergy. Unlike most floral regulators, *UFO* does not encode for a TF but for an F-box protein, one of the first to be described in plants¹⁴⁻¹⁶. *UFO* is part of a SKP1-Cullin1-F-box (SCF) E3 ubiquitin ligase complex through the interaction of its F-box domain with ARABIDOPSIS SKP1-LIKE (ASK) proteins^{15,17}. In addition, its predicted C-terminal Kelch-type β -propeller domain physically interacts with LFY DNA Binding Domain (DBD)¹⁸. As the control of TF activity through proteolytic and non-proteolytic ubiquitination is a well-described mechanism¹⁹, it was suggested that LFY is targeted for ubiquitination and possibly degradation by the SCF^{*UFO*} complex. Other data showed that adding a repression or an activation domain to *UFO* changes its activity and that *UFO* is recruited at the *AP3* promoter in a LFY-dependent manner, rather suggesting a more direct role of *UFO* in gene regulation^{18,20}. However, direct evidence explaining how *UFO* regulates a specific subset of LFY targets was still missing and the molecular mechanism underlying LFY-*UFO* synergistic action remained elusive.

Here, we show that *UFO* connection to the SCF complex is largely dispensable for its activity and that an important role of *UFO* is to form a transcriptional complex with LFY at genomic sites devoid of canonical high-affinity LFY binding sites (LFYBS). Our study presents a unique mechanism by which an F-box protein acts as an integral part of a transcriptional complex.

78 Results



79

80 **Fig. 1. UFO action is largely independent on its F-box domain.** a-e, Promoter activation in
 81 Arabidopsis protoplasts, with indicated effectors (right) and promoters (below each graph). EV
 82 = Empty Vector. Data are mean \pm SD (n = 4 biological replicates). One-way ANOVA with
 83 Tukey's multiple comparisons test (c,d) or Welch's ANOVA with Games-Howell post-hoc test
 84 (a,b,e). Stars represent a significant statistical difference compared to GFP (a-d) or to 3xHA-
 85 LFY+EV (e), non-significant (NS) otherwise. Other comparisons are indicated with brackets.
 86 (NS: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ and ****: $p < 0.0001$). f, Representative
 87 pictures of the different phenotypic classes obtained in the T1 population of indicated transgenic

plants (bars, 1 mm for flowers and 1 cm for rosettes). **g**, Distribution of T1 plants in phenotypic classes as described in (f). The distribution of *35S::UFO* and *35S::UFOΔFbox* lines within phenotypic classes is not significantly different (χ^2 tests, NS: $p > 0.05$). n = number of independent lines. **h**, *ufo-1* complementation assay by the *35S::UFO* and *35S::UFOΔFbox* transgenes. Rosette (scale bar, 1 cm), inflorescence (scale bar, 1 mm) and flower (scale bar, 0.5 mm) are shown.

UFO F-box domain is partially dispensable for its floral role. A Dual Luciferase Reporter Assay (DLRA) in Arabidopsis protoplasts was used to study floral promoter activation by LFY and UFO. We used promoter versions known to allow full complementation of mutants or able to recapitulate a WT expression pattern (see Methods). We found that the *AP3* promoter (*pAP3*) was more strongly activated when LFY (or LFY-VP16, a fusion of LFY with the VP16 activation domain) was co-expressed with UFO (or UFO-VP16) than by either effector alone (Fig 1a,e). Similar results were obtained with the promoter of *RABBIT EARS* (*RBE*), another UFO target (Fig. 1b)²². We also analyzed the promoters of *APETALA1* (*pAPI*) and *AGAMOUS* (*pAG*), two LFY targets regulated independently of UFO^{3,4,21} and that are required for organ identity of the first and second (*API*) or third and fourth (*AG*) floral whorls. We found that their activation by LFY and LFY-VP16 were insensitive to UFO (Fig. 1c,d). Thus, the protoplast assay accurately reproduced several floral promoter activation patterns.

We next investigated the involvement of a SCF^{UFO}-dependent ubiquitination pathway in *pAP3* activation by LFY-UFO. We found that, when co-expressed with LFY, N-terminally truncated UFO versions lacking the F-box domain (*UFOΔFbox* and *UFOΔFbox-VP16*) activated *pAP3* similarly to the full-length (FL) UFO (Fig. 1e). Thus, the connection of UFO to an SCF complex appears dispensable for the *pAP3* activation in transient protoplast assays. The previously reported inactivity of UFO with an internal deletion of its F-box likely reflects the poor folding

of this protein variant rather than the functional importance of the F-box domain (Extended Data Fig. 1a-c)²⁰.

We also constitutively expressed tagged versions of UFO and UFO Δ Fbox in Arabidopsis. Irrespective of the presence of the F-box, plants displaying a detectable UFO or UFO Δ Fbox expression (Extended Data Fig. 1d) showed a typical UFO gain-of-function phenotype (Fig. 1f,g). In addition, both UFO versions complemented the strong *ufo-1* mutant and induced gain-of-function phenotypes (Fig. 1h and Extended Data Fig. 1e,f)⁸. Still, minor defects (such as some missing or misshapen petals and disorganized flowers) were specifically observed in the absence of the F-box, suggesting that this conserved domain might be important for a subset of UFO functions (Fig. 1h and Extended Data Fig. 1g). Overall, UFO and UFO Δ Fbox have a very similar activity, showing that the role of the F-box domain is largely dispensable and that a ubiquitination-independent mechanism determines the LFY-UFO synergy.



138

CFC. LFY-UFO-specific peaks used to build mLUBS and dLUBS motifs in (e) are triangle-shaped. e, Logos for mLUBS, dLUBS and LFY binding site. The LFY logo was generated using the 600 peaks with the strongest LFY ampDAP-seq signal. f, EMSA with mLUBS and dLUBS highest score sequence DNA probes. Drawings represent the different complexes with LFY (blue) and ASK1-UFO (red) on DNA.

LFY and UFO form a transcriptional complex on a new DNA motif. Protoplast assays established that *AP3* and *RBE* promoter sequences contain the information that dictates their specific activation by LFY-UFO. Several regulatory regions driving *AP3* regulation in early floral meristem have been identified, including the Distal and the Proximal Early Elements (DEE and PEE; Fig. 2a)^{23,24}. The DEE contains a predicted canonical LFY Binding Site (LFYBS) but in protoplasts, like in plants²⁴, this site is not sufficient to explain *pAP3* activation (Extended Data Fig. 2). By systematically testing *AP3* promoter variants in the transient assay, we identified a 20-bp DNA element around the PEE important for LFY-UFO-dependent activation but devoid of canonical LFYBS (Fig. 2b and Extended Data Fig. 3a-c). We investigated the possibility that LFY and UFO form a complex on this DNA element using electrophoretic mobility shift assay (EMSA). For this, we mixed either recombinant LFY DNA Binding Domain (DBD, the LFY domain interacting with UFO)¹⁸ or *in vitro*-produced FL LFY, with a reconstituted ASK1-UFO complex. None of the proteins bound the DNA probe alone, but a shift was observed when LFY-DBD or FL LFY were mixed with ASK1-UFO (Fig. 2c). Thus, a presumptive ASK1-UFO-LFY complex was formed on a *pAP3* DNA element (hereafter named LFY-UFO Binding Site 0 or LUBS0) that each partner did not bind on its own. We did note that UFO had a weak affinity for DNA as ASK1-UFO shifted the DNA probe when performing EMSA with low competitor DNA concentrations (Extended Data Fig. 3d). Mutating

164 LUBS0 on various bases provided evidence that the formation of the complex is sequence-
 165 specific and suggested a bipartite DNA motif (Extended Data Fig. 3f).

166 To identify all genome regions possibly targeted by the ASK1-UFO-LFY complex, we
 167 performed ampDAP-seq (amplified DNA Affinity Purification sequencing) with a reconstituted
 168 ASK1-UFO-LFY complex (Extended Data Fig. 4a,b). We identified numerous genomic regions
 169 where LFY binding was strongly enhanced by the presence of ASK1-UFO. For each bound
 170 region, we computed the ratio (named Coverage Fold Change or CFC) between the coverage
 171 of peaks in the presence or absence of ASK1-UFO (Fig. 2d). Searches for enriched DNA motifs
 172 in the 600 regions with the highest CFC (> 4.7) identified two bipartite motifs made of a 6-bp
 173 RRNRCA (N=A/C/G/T, R=A/G) sequence, 4 bases of variable sequence and either a
 174 monomeric or a dimeric site resembling canonical LFYBS but with more variability (Fig. 2e).
 175 Consistent with the presence of a sequence resembling LFYBS, we found that *pAP3* activation
 176 in protoplasts required the LFY amino-acid residues involved in binding to canonical LFYBS
 177 (Extended Data Fig. 4c,d).

178 We named the identified motifs mLUBS and dLUBS for monomeric and dimeric LFY-UFO
 179 Binding Sites, respectively (Fig. 2e). Since it is observed specifically with ASK1-UFO, the
 180 RRNRCA element will be called UFO Recruiting Motif (URM). dLUBS and to a lesser extent
 181 mLUBS Position Weight Matrices (PWM) outperformed LFY canonical PWM showing they
 182 reliably predicted binding of ASK1-UFO-LFY (Extended Data Fig. 4e). The LFYBS present
 183 within the LUBS of high CFC regions tended to have a lower predicted affinity than those
 184 present in regions bound by LFY alone (Extended Data Fig. 4f), explaining why LFY binding
 185 to those sequences occurs only with UFO and the URM. Remarkably, we also identified the
 186 URM *de novo* from published LFY ChIP-seq data (Extended Data Fig. 4g)²⁵. Moreover, we
 187 found that the LFY-ChIP-seq performed in inflorescences²⁵ correlates better with the ASK1-
 188 UFO-LFY ampDAP-seq than with the LFY ampDAP-seq (Spearman rank correlation 0.481 vs

0.338 for the first 1000 ChIP-seq peaks), strongly suggesting that many regions are bound *in vivo* by UFO (see examples of such regions in Extended Data Fig. 7b,c).

AmpDAP-seq findings were validated by EMSA with DNA probes corresponding to optimal mLUBS and dLUBS motifs (Fig. 2f and Extended Data Fig. 4h). We observed a complex of slower mobility with dLUBS as compared to mLUBS, consistent with the presence of two LFY molecules on dLUBS. ASK1-UFO also supershifted LFY bound to canonical LFYBS from *pAP1* and *pAP3* DEE (Extended Data Fig. 4i), sometimes (but not systematically) increasing apparent LFY binding.

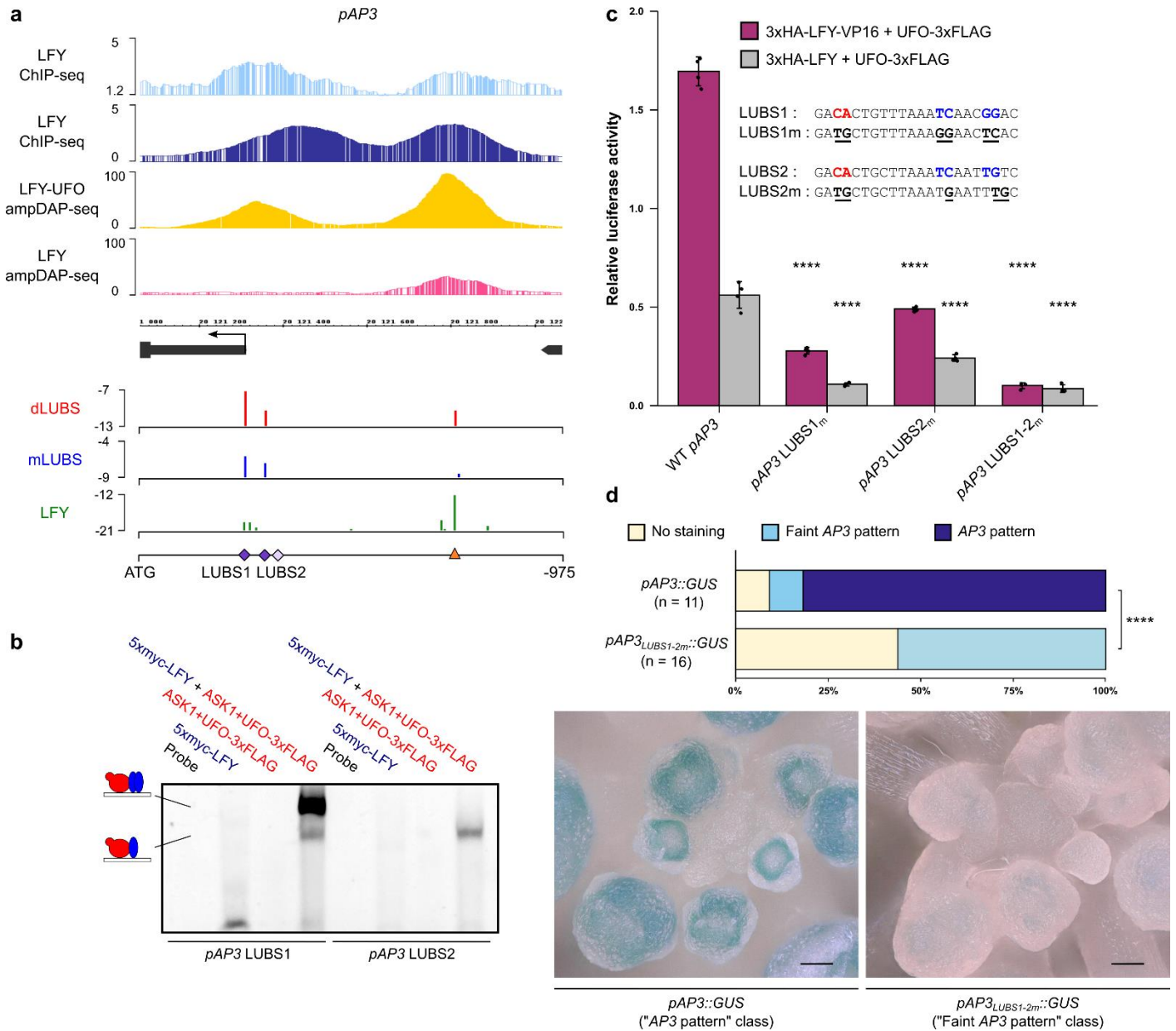


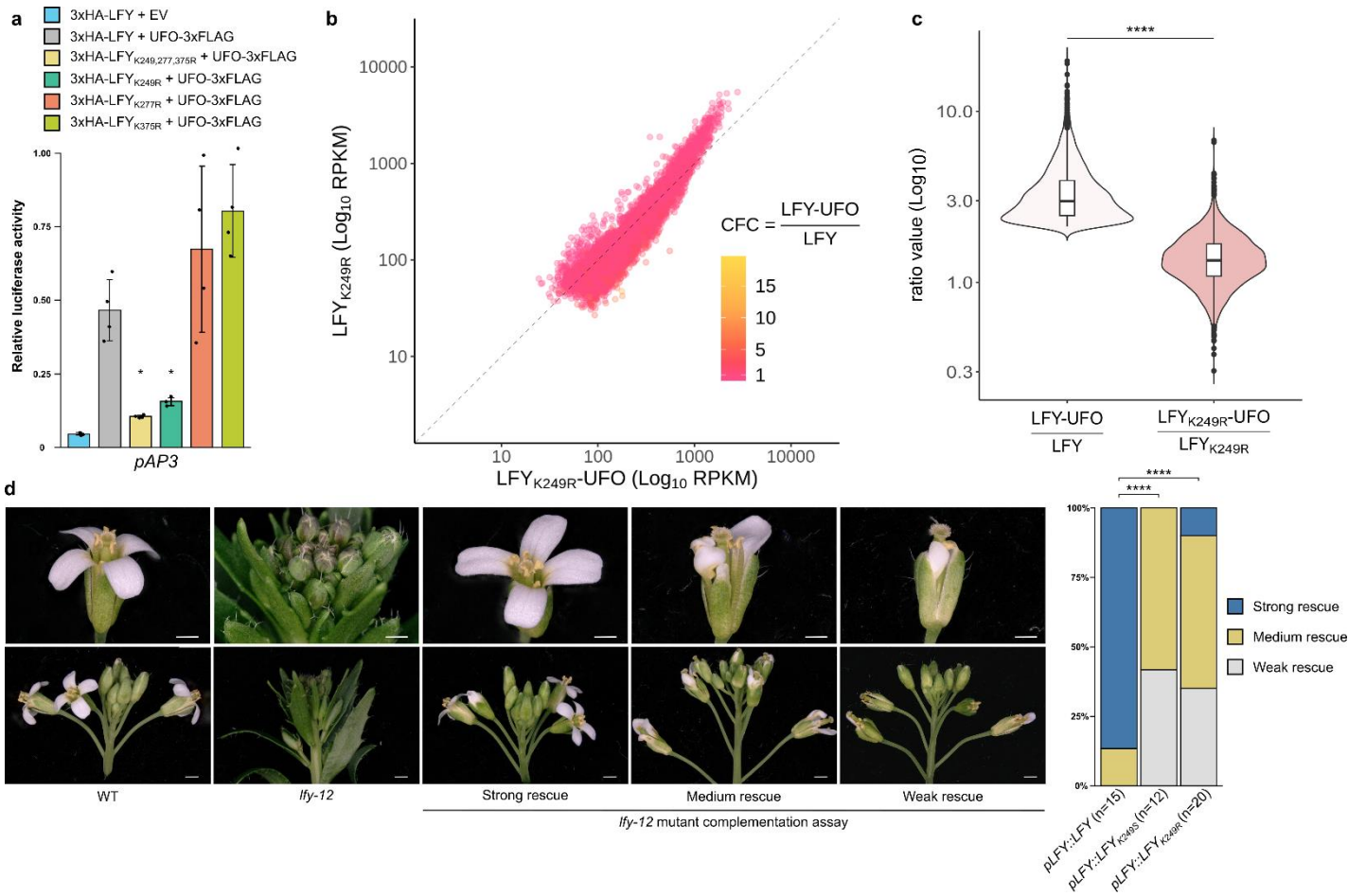
Fig. 3. Functional validation of LUBS. **a**, Integrated Genome Browser (IGB) view of *pAP3* showing LFY ChIP-seq in inflorescences (light blue)²⁵ or seedlings (dark blue)²⁶, LFY-UFO ampDAP-seq (yellow) and LFY ampDAP-seq (pink)²⁷, y-axis indicates read number range (top). Identification of LUBS in *pAP3* (bottom). Predicted binding sites using dLUBS and mLUBS models and LFY PWM, y-axis represents score values. LUBS1 and LUBS2 are indicated with purple squares, canonical LFYBS as an orange triangle. LUBS0 (light purple square) is not visible because of its low score. **b**, EMSA with *pAP3* LUBS probes. Drawings

represent the different complexes involving LFY (blue) and ASK1-UFO (red) on DNA. **c**, *pAP3* activation in Arabidopsis protoplasts. Effect of mutations (underlined) in URM (red) and LFYBS (blue) bases of *pAP3* LUBS were assayed. Data are mean \pm SD (n = 4 biological replicates). One-way ANOVA performed with data from the same effector and with Games-Howell post-hoc test. Stars represent a statistical difference compared to WT promoter (****: p < 0.0001). **d**, *In vivo* analysis of *pAP3::GUS* fusions. Percentage of transgenic lines with an *AP3* pattern, a faint *AP3* pattern or absence of staining (top). Pattern distributions are different between the two constructs (χ^2 test, ****: p < 0.0001). n = number of independent lines. Representative pictures of plants with an *AP3* pattern (bottom left) and a faint *AP3* pattern (bottom right, scale bar, 50 μ m). Note the staining in the ring corresponding to 2nd and 3rd whorl primordia in the left picture.

LUBS are functional regulatory elements. Examination of *pAP3* genomic region in ASK1-UFO-LFY ampDAP-seq revealed a peak that is absent in the experiment performed with LFY alone (Fig. 3a). This peak is roughly located on the PEE and is consistent with LFY ChIP-seq peaks^{25,26}. We searched for LUBS under this peak and, to our surprise, we identified several sites predicted to be better than LUBS0 (Fig. 3a). In EMSA, the two highest score sites, LUBS1 and LUBS2, were specifically bound by LFY in the presence of ASK1-UFO (Fig. 3b and Extended Data Fig. 5a). EMSAs performed with a LFY mutant version affected in its ability to dimerize further confirmed the stoichiometry of LFY-UFO complexes on LUBS1 and LUBS2 (Extended Data Fig. 5b). A similar binding was also observed when combining LFY and UFO Δ Fbox (Extended Data Fig. 5c,d), consistent with the F-box being facultative for LFY-UFO transcriptional activity (Fig. 1). In the protoplast assay, mutating LUBS1 or LUBS2 (or both) significantly reduced *pAP3* activation (Fig. 3c) with a stronger effect of the LUBS1

mutation. Specifically mutating the URM of *pAP3* LUBS1 and LUBS2, that abolished LFY-UFO binding on individual sites in EMSA (Extended Data Fig. 5e), also reduced *pAP3* activation albeit less effectively than mutating the whole LUBS (Extended Data Fig. 5f). Finally, the previously described *pAP3::GUS* staining pattern in the second and third whorls of Arabidopsis early floral meristems was severely reduced when LUBS1 and LUBS2 were mutated, demonstrating the importance of these sites *in vivo* (Fig. 3d and Extended Data Fig. 5g). Similarly, the *RBE* promoter contains an ASK1-UFO-LFY ampDAP-seq peak that is absent with LFY alone (Extended Data Fig. 6a), and the functional importance of the single LUBS identified under this peak was confirmed using EMSA, transient assay in protoplasts and stable reporter constructs in plants (Extended Data Fig. 6b-e).

In addition to *AP3* and *RBE*, LFY and UFO together likely regulate many other genes in Arabidopsis. To identify such potential LFY-UFO targets, we established a list of genes bound (in ampDAP and ChIP) and regulated by LFY-UFO (Extended Data Fig. 7a). This procedure identified the other B gene *PISTILLATA*, previously proposed as a LFY-UFO target but through an unknown regulatory element that the LUBS model precisely localized (Extended Data Fig. 7b). We also found floral regulators such as *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 5* and *FD* as well as novel candidates likely regulated by LFY and UFO (Extended Data Fig. 7a,c).



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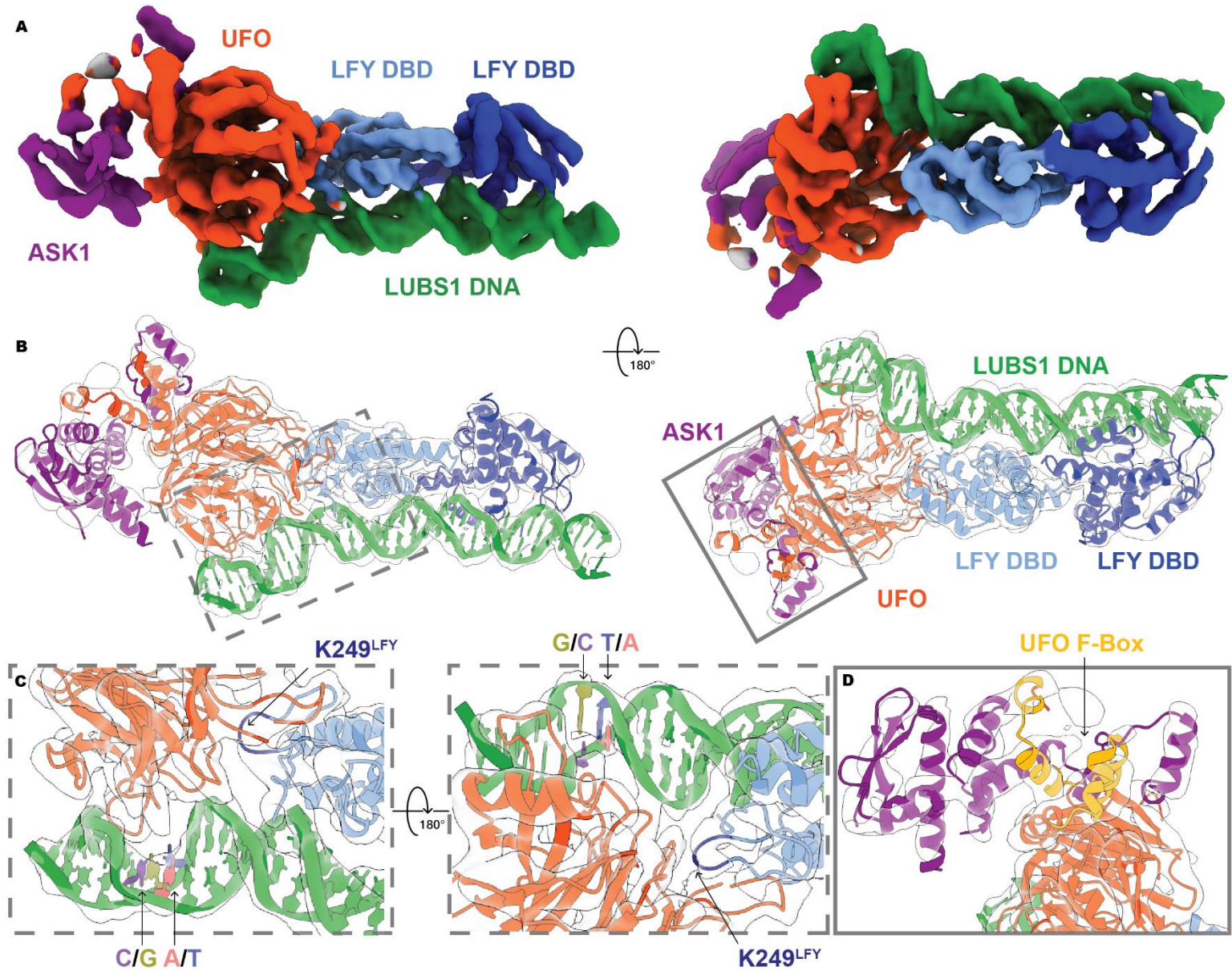
Fig. 4. The LFY K249R mutation disrupts the LFY-UFO synergy. **a**, *pAP3* activation in Arabidopsis protoplasts. Data are mean \pm SD (n = 4 biological replicates). Welch's ANOVA with Games-Howell post-hoc test. Stars indicate a statistical difference compared to 3xHA-LFY+UFO-3xFLAG. (NS: $p > 0.05$, *: $p < 0.05$). **b**, Comparison of peak coverage in LFY_{K249R}-UFO (x-axis) and LFY_{K249R} (y-axis) ampDAP-seq experiments, colored by peak coverage ratio (CFC) as in Fig. 2d. Note that, in contrast to Fig. 2d, the LFY-UFO-specific regions are mostly absent. **c**, Distribution of coverage ratios for LFY and LFY_{K249R} for LFY-UFO-specific regions (20% highest CFC; n = 3843 genomic regions). Wilcoxon's rank sum test (***: $p < 0.0001$). Median (solid line), interquartile range (box edges), $\pm 1.5 \times$ interquartile range (whiskers) and outliers (black dots) are shown. **d**, *lfy-12* mutant complementation assay. Pictures of WT, *lfy-12* mutant and of representative plants of the different phenotypic complementation classes

(left, scale bar 1 mm for top pictures and 1 cm for bottom pictures). Distribution of the different lines within phenotypic complementation classes (right). Plants complemented with LFY_{K249R} and LFY_{K249S} show different complementation patterns compared to plants complemented with LFY (χ^2 tests, ****: $p < 0.0001$). n = number of independent lines.

The LFY K249R mutation specifically affects UFO-dependent LFY functions. In Arabidopsis, LFY performs UFO-dependent and independent functions³, and we wondered whether they could be uncoupled by introducing specific mutations in LFY. As we were initially looking for LFY ubiquitination mutants, we mutated exposed lysines of LFY-DBD into arginines, and tested the effect of such mutations on LFY-UFO-dependent *pAP3* activation in protoplasts. We found one mutation (LFY K249R; Extended Data Fig. 8a) that strongly reduced *pAP3* activation by LFY-UFO (Fig. 4a) or LFY-VP16-UFO (Extended Data Fig. 8b) without affecting the UFO-independent *pAG* activation (Extended Data Fig. 8c) or the LFY-UFO interaction (Extended Data Fig. 8d). AmpDAP-seq experiments showed that the LFY K249R mutation specifically impaired the binding of LFY-UFO but not that of LFY alone (Fig. 4b,c and Extended Data Fig. 8e-i), revealing that K249 plays a key role in LFY-UFO interaction with the LUBS DNA.

The importance of LFY K249 for UFO-dependent LFY functions was also confirmed using complementation assay of the Arabidopsis *lfy-12* null mutant²⁸. *lfy-12* plants expressing LFY_{K249R} or LFY_{K249S} under the control of *LFY* promoter developed flowers with normal sepals and carpels but with defective third and more importantly second whorl organs, resulting in flowers similar to those observed in weak *ufo* mutants (Fig. 4d). When expressed under the constitutive 35S promoter, LFY_{K249R} triggered ectopic flower formation and early flowering

299 like WT LFY (Extended Data Fig. 8j), consistent with these LFY functions being independent
300 of UFO and thus not affected by the K249R mutation²⁹.



301

302 **Fig. 5. Structural characterization of the ASK1-UFO-LFY-DNA complex.** **a**, Cryo-EM
 303 density map of the ASK1-UFO-LFY-DBD-LUBS1 complex under two angles, colored with
 304 regard to the underlying macromolecule (green: LUBS1 DNA; pale and dark blue: LFY-DBD;
 305 red: UFO; purple: ASK1). **b**, The same views of the cryo-EM density map in transparent gray
 306 with fitted structures of LFY-DBD dimer, UFO, ASK1 and LUBS1 DNA. Same colors as in
 307 **(a)**. The frames roughly indicate the regions shown in **(c)** and **(d)**. **c**, Zoom on the UFO-DNA
 308 contact region (left) and on the LFY-UFO interface (right). Only the high-information CA of

the URM and its complement is highlighted by filled coloring the rings for each base (red for A, blue for T, pale green for G and purple for C). The LFY-DBD loop containing the K249 residue is highlighted in dark blue. **d**, Zoom on the ASK1-UFO interface, with the UFO F-box highlighted in gold.

Structural characterization of the ASK1-UFO-LFY-DNA complex. In order to understand how the LFY-UFO complex recognizes its cognate DNA binding site and how the K249 mutation impedes this interaction, we purified the ASK1-UFO-LFY-DBD-LUBS1 complex and we structurally characterized it using cryo-electron microscopy (Fig. 5a and Extended Data Fig. 9a-d). A structure at a 4.27 Å resolution was obtained (Extended Data Fig. 9g-i) into which were fit the AlphaFold2 predicted structure for UFO and ASK1, and the LFY-DBD dimer/DNA crystallographic structure³⁰ (PDB, 2VY1; Fig. 5b and Extended Data Fig. 9e,f). Due to the modest resolution, specific interacting amino acids could not be unambiguously identified. However, the major protein-protein and protein-DNA interaction surfaces were clearly identifiable.

The structure revealed that UFO directly contacts the DNA in the major groove around the URM (Fig. 5c). This binding likely involves basic residues present on loops projecting from the UFO Kelch-type β -propeller and results in a bend of roughly 30 degrees in the DNA double helix (Extended Data Fig. 9f). The structure also shows an interface between UFO and one LFY-DBD monomer (Fig. 5c). The LFY-DBD loop containing the K249 residue lies in this interface and likely interacts with one of the DNA-binding loops of UFO, consistent with the key role of LFY K249 in the ternary complex formation. As expected, ASK1 interacts with UFO F-box domain¹⁵ (Fig. 5D).

333 These data show how a β -propeller protein is able to modify the specificity of a TF, and offer a
334 structural explanation on how LFY and UFO synergistically recognize a novel DNA element
335 via direct interactions by both proteins with the DNA.

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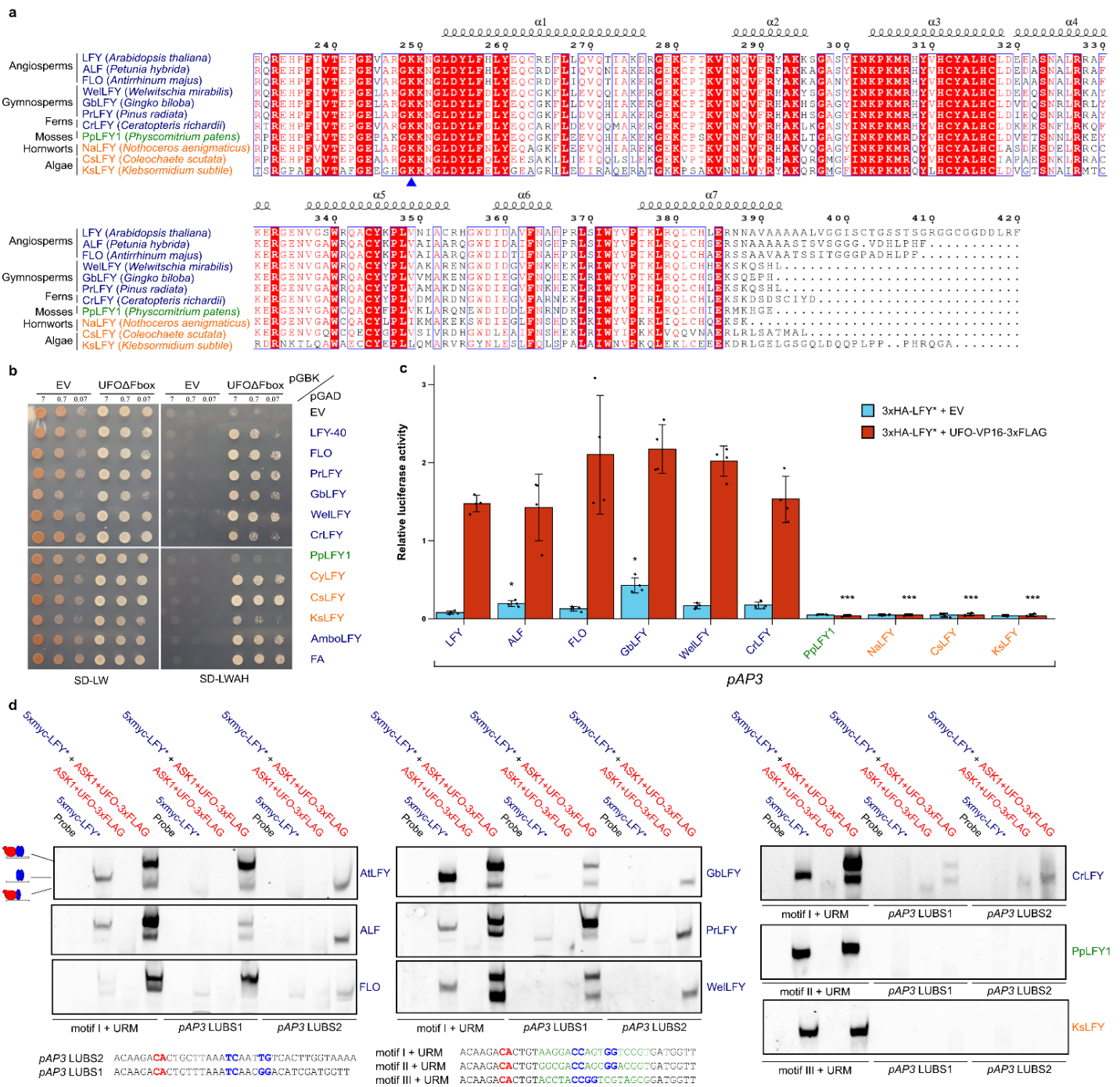


Fig. 6. LFY-UFO interaction is conserved beyond angiosperm species. a, Alignment of LFY DBDs. Amino acid numbering and secondary structure annotation are based on LFY from *A. thaliana*. LFY K249 residue is indicated with a blue triangle. DNA binding specificities are color-coded, type I (blue), II (green) and III (orange). FLO = FLORICAULA; ALF = ABERRANT LEAF AND FLOWER. **b**, Interaction between LFY orthologs and AtUFOΔFbox in Y2H. LFY orthologs are described in (a) except CyLFY (*Cylindrocystis sp.*), AmboLFY

(*Amborella trichopoda*) and FA (FALSIFLORA; *Solanum lycopersicum*). See Extended Data Fig. 4d for legends. **c**, *pAP3* activation measured by DLRA in Arabidopsis protoplasts. EV = Empty Vector. 3xHA-LFY* refers to the different LFY orthologs indicated under the x-axis. Data represent averages of independent biological replicates and are presented as mean \pm SD, each dot representing one biological replicate (n = 4). Welch's ANOVA with Games-Howell post-hoc test. One-way ANOVA was performed with data from the same effector (described in the legend), and stars represent a statistical difference compared to AtLFY, NS otherwise. (NS: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). **d**, EMSA with indicated DNA probes (bottom). URM and LFYBS bases are depicted in red and blue, respectively. *pAP3* LUBS1 sequence was modified to insert the perfect sequence of motif I, II or III³¹ (depicted in green): these DNA probes were used as positive controls for binding of LFYs alone and LFY-UFO complex formation. 5xmyc-LFY* refers to the different LFY orthologs indicated next to each EMSA and described in (a).

The LFY-UFO complex might have a deep evolutionary origin

As genetic and physical LFY-UFO interactions have been described in diverse angiosperms, we wondered whether the mechanism unraveled for Arabidopsis proteins could also apply to LFY from other species, including non-angiosperm ones. We selected LFY orthologous proteins from several species and with different DNA binding specificities (Fig. 6a). Indeed, through evolution, LFY specificity evolved with three major DNA binding specificities³¹. Type I specificity is the one described in Arabidopsis and valid for other angiosperms, gymnosperms, ferns and the moss *Marchantia polymorpha*, with two half-sites separated by a 3-bp spacer (Fig. 2e). LFY from the moss *Physcomitrium patens* have a type II specificity with specific half-sites (different from type I half-sites) also separated by a 3-bp spacer. Finally, type III specificity is found for LFY from algae and corresponds to a type II motif without the spacer. Because

functional UFO homologs have not been identified outside angiosperms, we used Arabidopsis UFO (AtUFO) in all the following experiments.

We tested the interaction of various LFY orthologs with AtUFO in Y2H (Fig. 6b), in DLRA in protoplasts with Arabidopsis *pAP3* (Fig. 6c) and in EMSA (Fig. 6d). In Y2H, all LFYs except LFY from *P. patens* (Type II) interact with AtUFO (Fig. 6b). However only Type I LFY from angiosperms, gymnosperms and ferns form a complex on *pAP3* LUBS and activate *pAP3* in the protoplast assay (Fig. 6c,d). These results suggest that the ability of LFY and UFO to act together by forming a complex is ancient, largely predating the origin of angiosperms. We obtained no evidence that type II and III LFY (from moss and algae) could form a complex with AtUFO on LUBS1 and LUBS2. A detailed and more trustworthy history of the LFY-UFO interaction will await further analyses, notably with the identification of UFO orthologs from non-angiosperm genomes.

Discussion

LFY was long known to interact with UFO to control flower and inflorescence development in numerous angiosperm species. However, the molecular nature of their synergistic action had remained unknown. As UFO encodes an F-box protein taking part in an SCF complex^{17,32,33}, it was thought to target proteins for a SCF^{UFO}-dependent ubiquitination and possible degradation. LFY was an obvious target candidate but clear evidence of LFY ubiquitination was missing^{12,18}. The results we present here suggest that the F-box domain, required for ubiquitination, is dispensable for most UFO-dependent LFY activity. Nevertheless, the high conservation level of UFO F-box sequence in angiosperms, together with slight differences in UFO activity when the F-box is deleted suggest that this domain might still be needed for some elusive facets of UFO function. UFO may work redundantly with other F-box proteins in ubiquitination

pathways like with the F-box protein HAWAIIAN SKIRT identified in a genetic screen as an enhancer of *ufo* mutant phenotype³⁴. It is thus possible that UFO acts as a moonlighting protein³⁵ with functions in both transcription and ubiquitination, and these two activities could be related or independent.

The molecular mechanism we discovered here is consistent with most published data on *AP3* and *PI* regulation^{18,23,36,37}. However, a detailed understanding of the expression pattern of *AP3* and *RBE* will require further work on other *cis* and *trans*- elements. Why *AP3* is not transcribed in floral stage 0-1 despite the expression of LFY and UFO is unclear²⁰. It could be because SUPPRESSOR OF OVEREXPRESSION OF CO 1 (SOC1), AGAMOUS-LIKE 24 (AGL24) and SHORT VEGETATIVE PHASE (SVP) act as early *AP3* repressors as *AP3* mRNA is detected in the floral anlage in a *soc1 svp agl24* mutant^{38,39}. Another explanation could be that *AP3* expression requires the *SEPALLATA3* activator⁴⁰. Why *pAP3* is not activated by LFY (or LFY-VP16) alone through the canonical LFYBS is also an open question.

Our work unraveled an unsuspected function unrelated to ubiquitination for UFO: it forms a transcriptional complex with LFY at regulatory sites that are different from the canonical sites bound by a LFY homodimer. UFO was previously proposed to act in transcription, but in the absence of direct evidence that a LFY-UFO complex forms on novel binding sites, it was difficult to understand how UFO controls only a subset of LFY targets. These novel regulatory sites (mLUBS and dLUBS) are made of a low-affinity or half LFYBS (poorly or not bound by LFY alone) and a motif located at a fixed distance from it and responsible for UFO recruitment. The formation of such a sequence-specific complex is explained at the structural level by the capacity of UFO to interact with both LFY and DNA. The poor ability of UFO to bind DNA alone explains its complete dependence on LFY to perform its transcriptional functions *in planta*^{6,20}. Thus, depending on *cis*-elements present in regulatory regions, LFY either binds

DNA as a homodimer or requires UFO to form a ternary complex. Mutation of the LFY K249 residue allows uncoupling these two types of binding by specifically disrupting the formation of the LFY-UFO-DNA complex. The position of this residue in the 3D structure at the interface between LFY, UFO and DNA is consistent with the key role of this residue in the complex formation. It is possible that replacing K249 with a bulkier R residue displaces the UFO loops involved in DNA binding without affecting the LFY-UFO interaction. Obtaining a higher-resolution structure will help to understand precisely the interactions occurring in this complex. Although it might be a common regulatory mechanism, only few cases where non-TF proteins modify TF DNA binding specificity have been described so far (for example Met4 and Met28 modifying the binding of TF Cbf1 in yeast⁴¹, or the herpes simplex virus transcriptional activator VP16 changing specificity of the Oct-1/HCF-1 complex⁴²). None of these examples involves an F-box protein or a Kelch-type β -propeller protein and neither of them has been characterized at the structural level. TF DNA binding specificity modification by non-TF proteins offers additional possibilities for a combinatorial control of gene expression and explains how a master regulator such as LFY accesses novel *cis*-elements to perform different functions in distinct territories.

Since LFY and UFO play key roles together in numerous plants species (including ornamental, crops and model plants), our findings expand the molecular understanding of flower and inflorescence development in a large variety of angiosperms. Because the LFY-UFO synergy is observed with LFY orthologs from gymnosperms and ferns as well, we speculate that this complex largely predated the origin of flowers and could have been coopted for flower development from a yet unknown ancestral role.

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Author contributions

FP and PR designed the project. PR performed the plant experiments helped by GT. PR and ET performed the biochemical experiments helped by HC for evolutionary analyses. LT performed the bioinformatics analyses helped by JL and RBM. EZ and GS performed cryo-EM experiments and MN, EZ, CZ and GS analyzed the data. PR and LT assembled the figures. PR and FP wrote the paper with contributions from LT and CZ.

Competing interests

We declare no competing interests.

Methods

Arabidopsis growth. All mutants and transgenic lines are in the *A. thaliana* Columbia-0 accession. Seeds were sown on soil, stratified 3 days at 4 °C, and then grown at 22°C under long-day conditions (16 h light). Transgenic plants were obtained with *Agrobacterium tumefaciens* C58C1 pMP90 using the floral dip method. Transformants were identified using GFP or Basta selection.

Arabidopsis cell suspension culture. *Arabidopsis thaliana* (ecotype Columbia-0) cells in suspension cultures were grown under continuous light (90 μmol of photons $\text{m}^{-2} \text{s}^{-1}$) at 21°C with shaking at 135 rpm in Murashige and Skoog (MS) medium supplemented with 30 g/L sucrose and 2 mg/L 2,4-dichlorophenoxyacetic acid (2,4D), pH 5.5. Suspension cells were subcultured every week with a 5-fold dilution. Suspension cells at 4 or 5 days following subculture were used for protoplast preparation.

Cloning. DNA fragments were amplified by PCR with Phusion high fidelity polymerase (NEB). Plasmids were all obtained by Gibson Assembly (GA) with either PCR-amplified or restriction enzyme-digested backbone vectors. We used the 420 aa LFY version. For site-directed mutagenesis, primers containing the desired mutations were used for GA mutagenesis. Plasmids were obtained using DH5 α bacteria and were all verified by Sanger sequencing. A list of plasmids and cloning procedures is provided in Supplementary Data 1. Oligonucleotide sequences are listed in Supplementary Data 2.

Yeast-two-hybrid. Coding sequences were cloned in pGADT7-AD or pGBKT7 vectors (Clontech) by GA. Y187 and AH109 yeast strains (Clontech) were transformed with pGADT7-

AD or pGBKT7 vectors and selected on plates lacking Leucine (SD-L) or Tryptophan (SD - W), respectively (MP Biomedicals). After mating, yeasts were restreaked on plates lacking Leucine and Tryptophan (SD -L-W) for 2 days. Yeasts were then resuspended in sterile water and OD_{600nm} was adjusted to indicated values for all constructions; two ten-fold dilutions were performed, and 6 µL drops were done on SD -L-W or SD -L-W-A-H (lacking leucine, tryptophan, histidine and adenine) plates. Yeasts were grown at 28°C and pictures were taken at indicated times.

Dual Luciferase Reporter Assay (DLRA) in Arabidopsis protoplasts. Effector plasmids with a 3xHA tag were obtained by cloning indicated genes in the modified pRT104 vector containing a 3xHA N-terminal tag (pRT104-3xHA)⁴³. The pRT104 empty plasmid was reengineered to insert a 3xFLAG C-terminal tag. For reporter plasmids, indicated promoter fragments were cloned upstream a Firefly Luciferase gene in pBB174⁴⁴. We used a 975-bp *pAP3* fragment and a 2-kb *pRBE* promoter fragment upstream of the ATG, known to induce a WT pattern in plant^{23,45}. *pAG* corresponds to *AG* second intron fused to a minimal 35S promoter, known to induce a WT pattern in plant²¹. For *pAPI*, we used a 600-bp fragment upstream of the ATG. This version is sufficient to give a WT pattern in plant⁴⁶, and the use of longer promoter versions induced a very high background noise in protoplasts. The pRLC reference plasmid contains Renilla Luciferase sequence under the control of the 35S promoter. Plasmids were obtained in large amounts using NucleoBond Xtra Maxi Plus kit (Macherey-Nagel). Protoplasts were prepared from Arabidopsis Col-0 cell suspension and transformed following the procedure described by Iwata et al.⁴⁷. Cell wall was digested using Onuzuka R-10 cellulase and macerozyme R-10 (Yakult Pharmaceutical). Digested cells were passed through two layers of Miracloth to remove debris, and protoplast concentration was adjusted to 2-5x10⁵ cells/mL. Protoplasts were then PEG-mediated transformed using 10 µg of indicated effector and reporter

plasmids and 2 µg of reference plasmid. After 17 h of incubation at RT, protoplasts were lysed. Firefly (F-LUC) and Renilla Luciferase (R-LUC) activities were measured using Dual Luciferase Reporter Assay System (Promega) and a TECAN Spark 10M 96-well plate reader. F-LUC/R-LUC luminescence ratios were calculated with background-corrected values. Four biological replicates were done for each plasmid combination. All DLRA data were analyzed using R Studio software and are presented as mean ± SD. All statistical methods are indicated within the figure legends. One-way ANOVA was used to analyze experimental data with more than two experimental groups. Welch's ANOVA was performed when the homogeneity of variance assumption was not met. Two-tailed unpaired Student's t-test was used for other data analyses.

Electrophoretic Mobility Shift Assay (EMSA). DNA probes used in EMSA are listed in Supplementary Data 2. Complementary oligos were annealed overnight in annealing buffer (10 mM Tris pH 7.5, 150 mM NaCl and 1 mM EDTA). 4 pmol of double-stranded DNA was then fluorescently labeled with 1 unit of Klenow fragment polymerase (NEB) and 8 pmol Cy5-dCTP (Cytiva) in Klenow buffer during 1 h at 37°C. Enzymatic reaction was stopped with a 10-min incubation at 65°C.

Proteins used in EMSA were obtained by different methods (bacteria, insect cells or TnT). Recombinant proteins (6xHis-LFY-DBD, UFOΔFbox-3xFLAG) and recombinant complexes (ASK1-UFO, ASK1-UFO-3xFLAG) concentration was adjusted to 500 nM for all reactions. All the 5xmyc-tagged proteins were obtained in vitro by TnT. 50 µL TnT reactions were done by mixing for 2 h at 25°C 5 µg of pTNT-5xmyc plasmid containing the gene of interest with TnT SP6 High-Yield Wheat Germ Protein Expression System (Promega). For EMSA with TnT-produced proteins, 5 µL of TnT reaction was used. Recombinant protein buffer or TnT mix was used as control when comparing reactions with multiple proteins.

All binding reactions were performed in 20 μ L binding buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1% glycerol, 0.25 mM EDTA, 2 mM MgCl₂, 0.01% Tween-20 and 3 mM TCEP) with 10 nM labelled probe. Reactions were supplemented with 140 ng/ μ L fish sperm DNA (Sigma-Aldrich) for EMSAs performed with in vitro-produced LFY, and 200 ng/ μ L for EMSAs performed with recombinant 6xHis-LFY-DBD. Binding reactions were incubated for 20 min on ice and then loaded on a 6 % native polyacrylamide gel. Gels were electrophoresed at 90 V for 75 min at 4°C and revealed with an Amersham ImageQuant 800 imager (Cytiva). Uncropped gels are shown in Source data.

Recombinant protein production and purification from bacteria. 6xHis-LFY-DBD was produced in E.Coli Rosetta2 (DE3) cells (Novagen) and purified as previously described ³⁰. ASK1 was cloned into the pETM-11 expression vector ⁴⁸, and the resulting plasmid was transformed into E.Coli BL21 cells (Novagen). Bacteria were grown in LB medium supplemented with kanamycin and chloramphenicol at 37°C up to an OD_{600nm} of 0.6. Cells were then shifted to 18°C and 0.4 mM isopropyl b-D-1-thiogalactopyranoside (IPTG) was added. After an overnight incubation, cells were sonicated in UFO buffer (25 mM Tris pH8, 150 mM NaCl, 1 mM TCEP) supplemented with one EDTA-free Pierce Protease Inhibitor Tablets (ThermoFisher). Lysed cells were then centrifuged for 30 min at 15000 rpm. Supernatant was mixed with Ni Sepharose High Performance resin (Cytiva) previously equilibrated with UFO buffer (25 mM Tris pH 8, 150 mM NaCl, 1 mM TCEP). Resin was then washed with UFO buffer containing 20 and 40 mM imidazole. Bound proteins were eluted with UFO buffer containing 300 mM imidazole and dialyzed overnight at 4 °C against UFO buffer without imidazole.

Recombinant protein production and purification from insect cells. The different tagged versions of ASK1, LFY and UFO were cloned in acceptor and donor plasmids (pACEBac1, pIDK and pIDS respectively; Geneva Biotech). Final acceptor plasmids containing the combination of desired coding sequences were obtained with Cre recombinase (NEB). DH10EmBacY competent cells containing the baculovirus genomic DNA (bacmid) were transformed with final acceptor plasmids. Blue-white selection was used to identify colonies with a recombinant bacmid with acceptor plasmid inserted. Bacmid was then isolated from bacteria and mixed with X-tremeGENE HP DNA Transfection Reagent (Roche) to transfect Sf21 insect cells. 96 h after transfection, supernatant containing the recombinant baculovirus (V0) was collected and used to infect fresh Sf21 cells. When infected cells reached DPA (Day Post Arrest), V1 virus was collected. For large expression, Sf21 cells were infected with either V1 virus or frozen baculovirus-infected cells. The pellet of a 0.75 L culture was sonicated in 50 mL of UFO buffer supplemented with one EDTA-free Pierce Protease Inhibitor Tablets (ThermoFisher). Sonicated cells were centrifuged for 1.5 h at 30 000 rpm, 4 °C. Supernatant was then incubated for 1 h at 4°C with Ni Sepharose High Performance resin (Cytiva) previously equilibrated with UFO buffer. Beads were transferred into a column, and washed with 20 column volumes of UFO buffer, then UFO buffer + 50 mM imidazole. Proteins were eluted with UFO buffer containing 300 mM imidazole. Elution was dialyzed overnight at 4 °C against UFO buffer. TEV protease was added to cleave tags (0.01% w/w). When ASK1 was limiting compared to UFO, recombinant 6xHis-ASK1 from bacteria was added. The following day, elution was repassed on Dextrin Sepharose High Performance (Cytiva) and Ni Sepharose High Performance resins (Cytiva) to remove tags and contaminants. For ASK1-UFO, ASK1-UFO-3xFLAG or UFO Δ Fbox-3xFLAG, proteins were concentrated with a 30 kDa Amicon Ultra Centrifugal filter (Millipore) and further purified by Size Exclusion Chromatography (SEC). For ASK1-UFO-LFY-DBD complex purification, contaminant DNA was removed by

passing proteins on Q Sepharose High Performance resin (Cytiva) pre-equilibrated with UFO buffer. Increasing salt concentrations allowed obtaining DNA-free proteins. Indicated annealed HPLC-purified oligos (Supplementary Data 2) were then added and incubated with proteins on ice for 20 min. Proteins were concentrated with a 30 kDa Amicon Ultra Centrifugal filter (Millipore) and further purified by SEC.

Size Exclusion Chromatography (SEC) and Size Exclusion Chromatography coupled to Multi-Angle Laser Light Scattering (SEC-MALLS). SEC was performed with a Superdex 200 Increase 10/300 GL column (Cytiva) equilibrated with UFO buffer. Unaggregated proteins of interest were frozen in liquid nitrogen and stored at -80°C. SEC-MALLS was performed with a Superdex 200 Increase 10/300 GL column (Cytiva) equilibrated with UFO buffer. For each run, 50 µL containing 1 mg/mL of complex was injected. Separations were performed at RT with a flow rate of 0.5 mL/min. Elutions were monitored by using a Dawn Heleos II for MALLS measurement (Wyatt Technology) and an Optilab T-rEX refractometer for refractive index measurements (Wyatt Technology). Molecular mass calculations were performed using the ASTRA software with a refractive index increment (dn/dc) of 0.185 mL/g.

ampDAP-seq. pTnT-5xmyc-LFY²⁷ was used to produce 5xmyc-LFY in vitro using TnT SP6 High-Yield Wheat Germ Protein Expression System (Promega). We used the ampDAP-seq libraries described in Lai et al.²⁷. ampDAP-seq experiments were performed in triplicates (LFY-UFO) or in duplicates (LFY_{K249R} and LFY_{K249R}-UFO).

A 50 µL TnT reaction producing 5xmyc-LFY was mixed with an excess of recombinant ASK1-UFO-3xFLAG (2 µg) and 20 µL of Pierce Anti-c-Myc Magnetic Beads (ThermoScientific). DAP buffer (20 mM Tris pH 8, 150 mM NaCl, 1 mM TCEP, 0.005% NP40) was added to reach 200 µL. Mix was incubated for 1 h at 4°C on a rotating wheel. Beads were then immobilized

and washed 3 times with 100 μ L DAP buffer, moved to a new tube and washed once again. ampDAP-seq input libraries (50 ng) were then added, and protein-DNA mixes were incubated for 1.5 h at 4°C on a rotating wheel. Beads were immobilized and washed 5 times with 100 μ L DAP buffer, moved to a new tube and washed 2 more times. Finally, beads were mixed with 30 μ L of elution buffer (10 mM Tris pH 8.5) and heated for 10 min at 90°C. IP-ed DNA fragments contained in the elution were amplified by PCR according to published protocol⁴⁹ with Illumina TruSeq primers. Remaining beads were mixed with 20 μ L of 1X SDS-PAGE Protein Sample Buffer and WB were performed to check the presence of tagged proteins. PCR products were purified using AMPure XP magnetic beads (Beckman Coulter) following manufacturer's instructions. Library molar concentrations were determined by qPCR using NEBNext Library Quant Kit for Illumina (NEB). Libraries were then pooled with equal molarity. Sequencing was done on Illumina HiSeq (Genewiz) with specification of paired-end sequencing of 150 cycles.

GUS staining. The different promoter versions were cloned upstream *GUS* gene in the pRB14 backbone vector⁴⁶. Transformants were selected with GFP seed fluorescence. The number of independent lines analyzed for each construct is indicated in each figure. GUS staining was performed on the apex of primary inflorescences of T2 plants. Tissues were placed in ice-cold 90% acetone for 20 min at RT, and then rinsed in GUS buffer without X-Gluc (0.2% Triton X-100, 50 mM NaPO₄ pH 7.2, 2 mM potassium ferrocyanide, 2 mM potassium ferricyanide). Tissues were transferred in GUS buffer containing 2 mM X-Gluc substrate (X-Gluc DIRECT) and placed under vacuum for 5 min. Samples were then incubated overnight at 37°C unless specified in the legend. Finally, tissues were washed with different ethanol solutions (35%, 50%, and 70%) and pictures were taken with a Keyence VHX-5000 microscope with a VH-

Z100R objective. χ^2 tests were used to test for independency between constructs and staining classes.

***In planta* overexpression and mutant complementation assay.** Tagged versions of UFO and UFO Δ Fbox were cloned under the control of the 35S promoter in pEGAD⁵⁰. Transformants were selected with Basta treatment. Overexpressing lines with a strong gain-of-function phenotype were crossed to the strong *ufo-1* mutant. Basta-resistant F2 plants were individually genotyped to select *ufo-1* $-/-$ homozygous plants. For this, a fragment was amplified by PCR with oligos oGT1085 and oPR578 (Supplementary Data 2) and digested with DpnII enzyme (NEB). Based on digestion profile, *ufo-1* $-/-$ plants were kept and analyzed once they reached flowering.

Mutated versions of LFY were cloned in pETH29³⁰ or pCA26⁵¹ to express LFY cDNA under the control of its endogenous promoter or the 35S promoter, respectively. For *lfy-12* complementation assay, heterozygous *lfy-12/+* plants were transformed. Transformants were selected with GFP fluorescence and genotyped with a previously described protocol⁴⁶ to select *lfy-12* $-/-$ plants. Complementation assay was performed with T2 plants and was based on the analysis of the first 10 flowers from the primary inflorescence. Pictures were taken with a Keyence VHX-5000 microscope with a VH-Z20R objective. χ^2 tests were used to test for independency between constructs and complementation classes.

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Western Blot. For Western Blots on plant total protein extracts, indicated tissues were crushed in 2X SDS-PAGE Protein Sample Buffer (100 mM Tris pH 6.8, 20% glycerol, 2% SDS, 0.005% Bromophenol blue, and 0.8% w/v dithiothreitol) at a 1:2 w:v ratio and boiled for 5 min. Samples

were then loaded on a 12% acrylamide SDS-PAGE gel. For all WB, transfer was performed with iBlot2 Dry Blotting System (Invitrogen) using default parameters. Membranes were blocked for 1 h at RT with 5% milk TBST and then incubated overnight at 4 °C with 5% milk TBST solution containing HRP-conjugated antibody (1:1000 for anti-FLAG (Sigma-Aldrich; Cat# A8592) and 1:5000 for anti-myc (Invitrogen; Cat# R951-25)). Revelation was performed with Clarity Western ECL substrate (Bio-Rad). Pictures were taken with a ChemiDoc MP Imaging System (BioRad). Uncropped gels are shown in Source Data.

Cryo-EM sample preparation, data collection and data processing. An aliquot of the SEC-purified ASK1-UFO-LFY-LUBS1 complex was thawed on ice (see Supplementary Data 2 for LUBS1 DNA sequence). Subsequently, 3.5 µl of the complex at 1 mg/mL were deposited onto glow-discharged (25 mA, 30 s) C-flat Au grid R 1.2/1.3 300 mesh (Electron Microscopy Sciences), blotted for 5.5 s with force 0, at 20°C and 100% humidity using a Mark IV Vitrobot (FEI, Thermo Fisher Scientific) and plunge-frozen in liquid ethane for specimen vitrification. A dataset of about 1'000 movies of 40 frames was acquired on a 200 kV Glacios (Thermo Fisher Scientific) electron microscope (Supplementary Data 3) at a nominal magnification of 36'000 with a physical pixel size of 1.145 Å.

The raw movies, acquired with SerialEM on a Gatan K2 Summit camera (Supplementary Data 3), were imported to Cryosparc live⁵² for motion correction and CTF estimation. The dose-weighted micrographs were used for particle picking with crYOLO 1.7.6 and the general model for low-pass filtered images⁵³. Particle coordinates were imported to Cryosparc, where all subsequent steps were performed. After manual inspection, a subset of 761 micrographs was selected based on CTF fit resolution, total and per frame motion, average defocus and relative ice thickness. A raw particle stack of 282'567 images was extracted at 256x256 pixels² box size, binned twice and subjected into 2D classification to remove false positive picks. 207'392

particles from the selected class averages were re-extracted, re-centered at full size and submitted for a second round of 2D classification. All class averages showing clear protein features were selected and the resulting 147'849 particles were used for ab initio reconstruction with 3 classes and subsequent heterogeneous refinement of the resulting volumes. Of those 3 classes, 2 looked like a protein-DNA complex with the most apparent difference being the presence or not of an extra electron density at one edge of the DNA helix. The last class had no recognizable features and was used as a decoy to remove "junk" particles. Each subset and volume of the 2 first classes was refined separately with Non-Uniform refinement⁵⁴ resulting into 2 distinct reconstructions of about 4.2 Å resolution, where the DNA model, the crystal structure of LFY-DBD and the AlphaFold2 models of UFO and ASK1 could be unambiguously fitted into the electron density. The second of these classes could fit a LFY-DBD dimer, while in the first class there was density only for the LFY-DBD molecule that directly interacts with UFO (Extended Data Fig. 9d). The unsharpened maps of each reconstruction were used for post-processing with DeepEMhancer⁵⁵. Figures were prepared with Chimera⁵⁶ or ChimeraX⁵⁷.

Cryo-EM model building. Ideal B-form DNA was generated in Coot⁵⁸ and then manually built into the electron density. The resulting model was further refined using phenix.real_space_refine⁵⁹. A single monomer of LFY-DBD was manually placed in the electron density, followed by fitting in ChimeraX⁵⁷. The biological LFY-DBD dimer was then downloaded from the RCSB PDB (2VY1)³⁰ and used as a guide to place the second LFY monomer, followed by fitting to density in ChimeraX. Alphafold models⁶⁰ of ASK1 (uniprot ID: Q39255) and UFO (uniprot ID: Q39090) were both downloaded from the EBI, preprocessed to remove low confidence regions in phenix.process_predicted_model⁶¹, then placed manually and then fit to density in ChimeraX.

Bioinformatic analyses.

Read mapping and peak calling. Reads processing and peak calling of LFY, LFY-UFO, LFY_{K249R} and LFY_{K249R}-UFO ampDAP-seq data were performed as previously published⁶². Briefly, the quality of sequencing data was analyzed with fastQC v0.11.7 and adapters were removed with NGmerge v0.2_dev⁶³. Bowtie2 v2.3.4.1 was used for mapping to the TAIR10 *A. thaliana* reference genome⁶⁴. Reads mapped to a single location and with maximum two mismatches were retained. Duplicates were removed with the samtools dedup program v1.8. Bound regions (i.e. peaks) were identified with MACS2 v2.2.7.1, using input DNA from Lai et al. as control²⁷. Consensus peaks were selected with MSPC v4.0.0⁶⁵ by retaining peaks called in all replicates, and resizing them by ± 200 bp around the peak maximum for further analysis.

Analyses of ampDAP-seq experiments. To compare binding in different experiments, peaks were merged according to a previously published procedure⁶². Bound peaks were considered as common if they overlapped by at least 80%, while the remaining non-overlapping portion of either peak was $< 50\%$. Peaks that did not overlap by at least 50% were considered as new peaks. The same procedure was used to assess experimental reproducibility (comparisons between replicates of the same experiment), where peaks were normalized by the number of reads mapped in library (RPKM).

As the fraction of reads mapped in peaks is much lower for LFY than LFY-UFO ampDAP-seq ($\sim 25\%$ vs $\sim 40\%$, respectively), normalizing reads count by all reads mapped along the genome would introduce a bias and estimate the LFY relative coverage (RPKM) towards lower values compared to LFY-UFO. In addition to this consideration, experimental proof from EMSAs suggests that UFO does not strongly affect binding intensity of the complex at canonical LFYBS (which represent most peaks). Hence, reads count at each peak was normalized by the total number of reads mapped within all LFY and LFY-UFO merged peaks. Then, the mean

normalized coverage from each experiment, divided by the peak size, was computed for each peak. The same strategy was applied when comparing LFY_{K249R} and LFY_{K249R}-UFO (Fig. 4b), LFY_{K249R} and LFY (Extended Data Fig. 8h) and LFY, LFY-UFO, LFY_{K249R} and LFY_{K249R}-UFO (Fig. 4c). The Coverage Fold Change (CFC) was computed on merged peaks as the ratio between mean normalized peak coverage in LFY-UFO and LFY (Fig. 2d) or mean normalized coverage in LFY_{K249R}-UFO and LFY_{K249R} (Fig. 4b).

Motif search in bound regions. Merged peaks of LFY and LFY-UFO datasets were sorted based on decreasing CFC value. The top 600 peaks (i.e. highest CFC values) were used for a motif search using MEME-ChIP v4.12.0 using options -nmeme 600 -meme-maxsize 600*1000 -meme-nmotifs 1 -dreme-m 0 -noecho and the JASPAR 2018 core plants non-redundant database⁶⁶. For dLUBS, we used options -meme-minw 20 -meme-maxw 30, while for mLUBS we used -meme-minw 16 -meme-maxw 19. To retrieve the LFY motif in Fig. 2e the 600 LFY ampDAP-seq peaks with strongest coverage were fed to MEME-ChIP with options -nmeme 600 -meme-nmotifs 1 --meme-minw 19 -meme-maxw 19 -pal.

Receiver Operating Characteristics (ROC) analysis. From the dataset of merged peak set (peaks found in LFY or in LFY-UFO experiments or in both), peaks were sorted based on decreased CFC value, the top 20% peaks were selected, and among these, the first 600 used for motif determination were excluded to avoid overfitting, for a total of 3243 final peaks. A negative set of the same size was created using a previously published method, which allows searching for sequences from the *A. thaliana* genome (TAIR10 reference) with the same GC content and genomic origin as the positive set⁶⁷. Both sets were scanned with dLUBS and mLUBS PWMs as well as with the LFY PWM with dependencies as published previously⁶⁸

766 using an in-house script available on our GitHub page. The ROC plot was then created with the
767 R 'plotROC' package v2.2.1.

768

769 *LFY in dLUBS within LFY-UFO-specific regions vs LFY in LFY-specific regions.* To assess
770 whether the scores of LFYBS within dLUBS were comparable to the scores of canonical
771 LFYBS, we used the peaks from the comparison of LFY vs LFY-UFO ampDAP-seq and
772 resized them (+/-50 bp around the peak maximum). We used the dLUBS matrix to scan the
773 resized sequences and retained the best site per sequence. We then retrieved sequences
774 corresponding to the dLUBS site and computed the score of the LFYBS present in dLUBS
775 using the LFY PWM⁶⁸. The values obtained in the 20% most LFY-UFO-specific sequences
776 (20% highest CFC) is shown in the boxplot. The 20% lowest CFC peaks were scanned with the
777 LFY PWM to generate the box-plot in Extended Data Fig. 4f.

778

779 *Microarray data analysis.* Microarray data were retrieved from AtGenExpress⁶⁹ for
780 inflorescence tissue in the *ufo* (ATGE_52A-C) vs Col-0 background (ATGE_29A-C). The
781 'gcrma' R package was used to adjust probe intensities and convert them to expression
782 measures, and then the 'limma' package was used to fit the model and smooth standard errors.
783 A Benjamini-Hochberg correction was applied to p-values and fold change (FC) was computed
784 as the ratio between expression in WT versus the *ufo* mutant. Only genes with $|\log_2(\text{FC})| > 0.5$
785 and adjusted p-value < 0.05 were considered as significantly differentially expressed.

786

787 *ChIP-seq datasets and analysis of ChIP-seq vs ampDAP-seq.* We collected the raw data of all
788 available LFY ChIP-seq datasets: GSE141704⁷⁰, GSE96806²⁵, GSE64245²⁶, GSE24568⁶⁸.
789 Mapping and peakcalling analysis were performed with the same procedure as ampDAP-seq,
790 except that peaks were resized to 600 bp around the peak maximum, and the -q option of

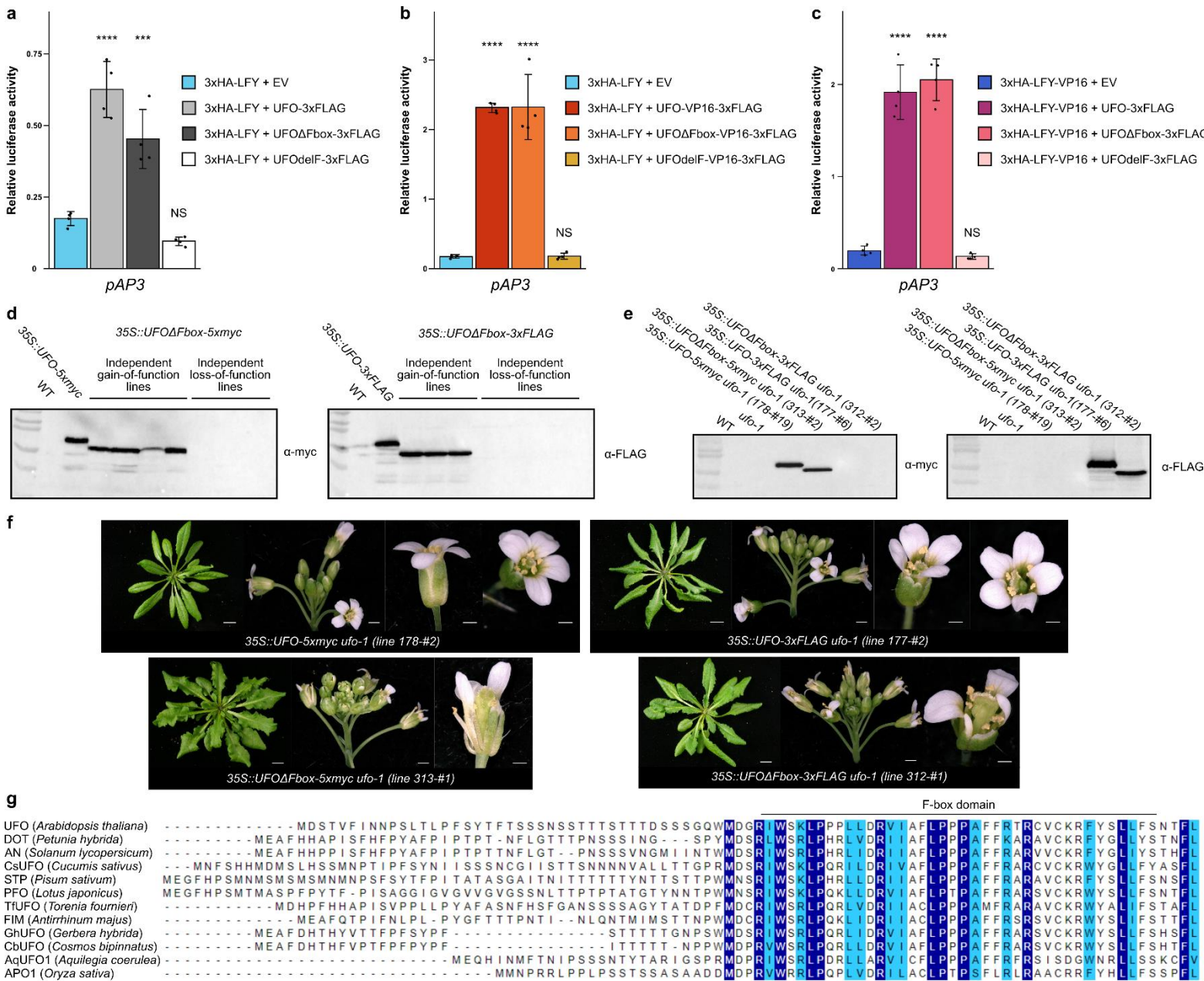
MACS2 was set to 0.1. Coverage of the resulting peaks was calculated as the average of normalized read coverage for each replicate. Peaks from the four datasets were merged through a four-way comparison following the same procedure used for ampDAP-seq. Bedtools intersect (v2.30.0) was used with options -wa -f 0.8 -F 0.8 -e to find the peaks common to the merged ChIP-seq peaks and the 20% most LFY-UFO-specific genomic regions (highest CFC value from ampDAP-seq). Peaks were assigned to genes by extending gene regions 3 kb upstream of the TSS and 1 kb downstream of the TTS and using bedtools intersect (options -f 0.8 -F 0.8 -e) to identify genes in the vicinity of peaks. The bound genes obtained were crossed with the list of differentially expressed genes in *ufo* inflorescences.

Identification of the URM from published LFY ChIP-seq data. To test whether the URM could be identified *de novo* (Extended Data Fig. 4g), we collected the 298 regions bound by LFY ChIP-seq data of inflorescence tissue²⁵ for which the binding intensity was twice greater in vivo relative to in vitro (LFY ampDAP-seq). We resized these regions +/- 55 bp around the ChIP-seq peak maximum. The corresponding sequences were searched with the LFY PWM⁶⁸ to identify all LFYBS with a PWM score > -23. Assuming that a recruiting motif should be at a fixed distance from the LFYBS, we created 140 batches, corresponding to sequences with size ranging from 4 to 10 bp, distant from 1 to 20 bp at both sides of the canonical LFYBS. Each of the 140 batches of sequences was used as input with MEME-ChIP for motif discovery with the motif size constrained to the length of the sequences in a given batch.

Data and code availability

ampDAP-seq data have been deposited at GEO and are publicly available as of the date of publication (GSE204793; reviewer link: mlufkwogxjurvcj). All original code has been

816 deposited at github (https://github.com/Bioinfo-LPCV-RDF/LFYUFO_project) and is publicly
817 available as of the date of publication. The cryo-EM structure determined in this study is
818 deposited in the EM data bank under the reference number EMD-15145. The .pdb file of the
819 model is available in Supplementary information. Any additional information required to
820 reanalyze the data reported in this paper is available from the lead contact upon request.
821



823

824 **Extended Data Fig. 1. UFO has SCF-dependent and independent functions. a-c, pAP3**

825 activation measured by DLRA in Arabidopsis protoplasts. EV = Empty Vector (pRT104-

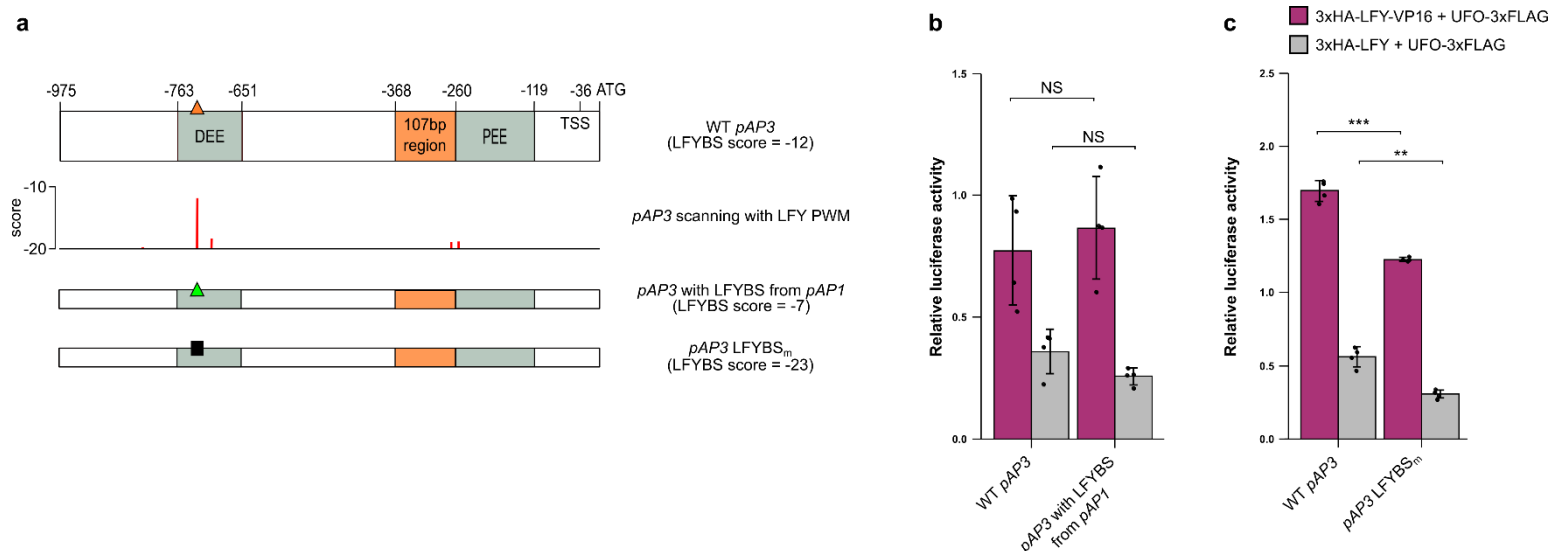
826 3xHA). UFOΔFbox corresponds to a deletion of the whole N-terminal part comprising the F-

827 box domain (aa. 1-90), while UFOdelF corresponds to a previously-described internal deletion

828 in the F-box domain (aa. 50-62)²⁰. Data represent averages of independent biological replicates

829 and are presented as mean ± SD, each dot representing one biological replicate (n = 4). One-

way ANOVA with Tukey's multiple comparisons test. Stars above bars represent a significant statistical difference compared to 3xHA-LFY + EV or 3xHA-LFY-VP16 + EV negative controls (NS: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ and ****: $p < 0.0001$). **d**, Western Blot on protein extracts from independent T1 plants from different phenotypic classes described in Fig. 1g (one independent line per lane). *35S::UFO-5xmyc* (line 178-#19) and *35S::UFO-3xFLAG* (line 177-#6) plants were used as positive controls. Total proteins were extracted from rosette leaves. Note the difference of molecular weight between UFO and $\text{UFO}\Delta\text{Fbox}$. Loss-of-function defects are likely due to silencing of both transgene-encoded $\text{UFO}\Delta\text{Fbox}$ and endogenous UFO. **e**, Western Blot on protein extracts from F2 plants described in Fig. 1h. Total proteins were extracted from rosette leaves. **f**, *ufo-1* complementation assay with other *35S::UFO* and *35S::UFO\Delta Fbox* lines. Rosette leaves (right, scale bar, 1 cm), inflorescence (middle, scale bar 1 mm) and flower (right, scale bar, 0.5 mm) phenotypes are shown. Primary inflorescences were removed to observe rosette phenotype. For each construct, at least 5 plants were analyzed per line. As in Risseuw et al, our *35S::UFO* lines displayed relatively milder phenotypes than the *35S::UFO* phenotypes reported by Lee et al.^{6,20}. Note that the *35S::UFO-5xmyc* 178-#2 line did not display the serrated leaves phenotype. **g**, Sequence alignment of UFO N-terminal region. The F-box domain is represented⁷¹. In selected species, presented proteins were identified as UFO homologs and their role was confirmed genetically^{7,11,12,16,72-79}.



850

851 **Extended Data Fig. 2. *pAP3* DEE LFYBS is not required for LFY-UFO-dependent *pAP3***

852 **activation. a**, Schematic representation of *pAP3*. Top row represents WT *pAP3* with regulatory

853 regions and *cis*-elements. Orange triangle represents LFYBS. The second row represents the

854 scores for the best LFYBS obtained by scanning WT *pAP3* sequence with LFY PWM⁶⁸ (the

855 best binding sites correspond to the less negative score values). Other rows represent the

856 different *pAP3* versions used in (b) and (c). LFYBS mutation corresponds to the previously

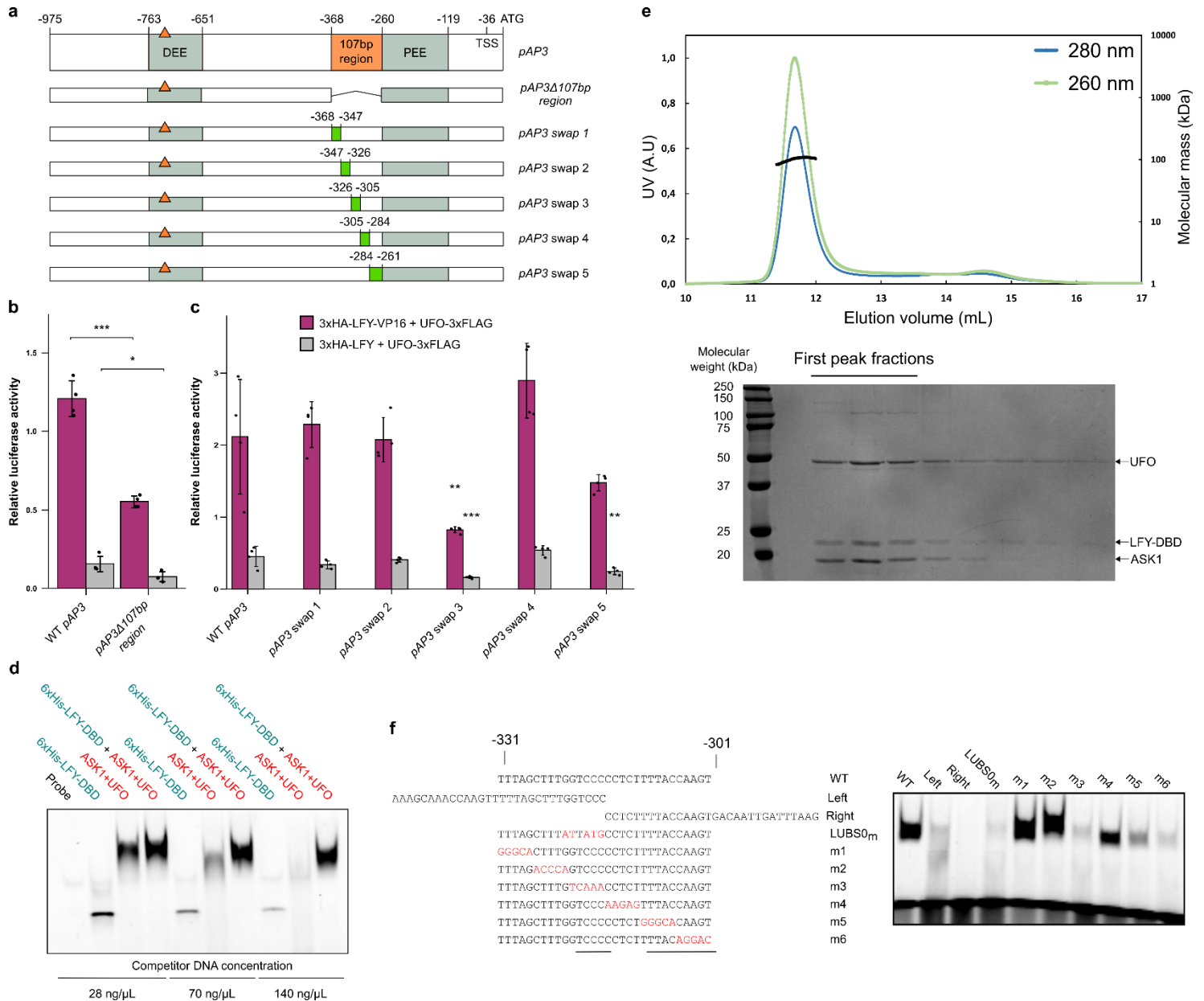
857 described *site1m-site2m* mutation²⁴. **b,c**, *pAP3* activation with promoter versions described in

858 (a) and indicated effectors. For bar charts, data represent averages of independent biological

859 replicates and are presented as mean \pm SD, each dot representing one biological replicate (n =

860 4). Unpaired t-tests (b,c). (NS: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

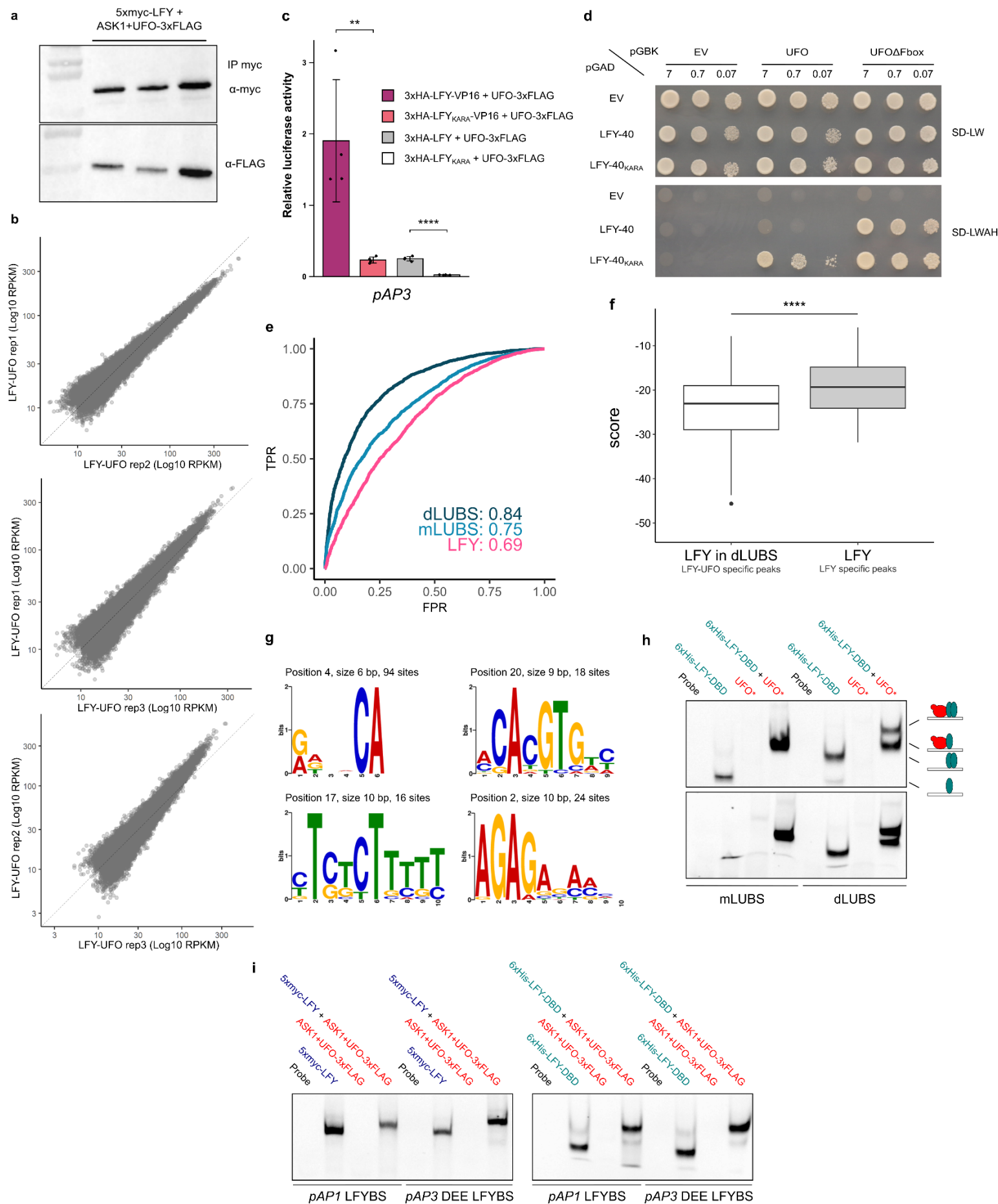
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Extended Data Fig. 3. Analysis of *pAP3* activation by LFY-UFO. **a**, Description of *pAP3*. Top line represents WT *pAP3* with regulatory regions and *cis*-elements. Coordinates are relative to *AP3* start codon. TSS: Transcription Start Site. Orange triangle represents LFYBS. Other rows show the promoter versions used in **(b)** and **(c)**. Green rectangles in swapped versions correspond to the same random sequence. **b,c**, *pAP3* LFY-UFO response element mapping with *pAP3* versions described in **(a)** by DLRA in Arabidopsis protoplasts. Data represent averages of independent biological replicates and are presented as mean \pm SD, each dot

870 representing one biological replicate ($n = 4$). One-way ANOVA with Tukey's multiple
871 comparisons test (c). One-way ANOVA was performed with data from the same effector, and
872 stars represent a statistical difference compared to WT *pAP3*. Unpaired t-test (b). (NS: $p >$
873 0.05 , *: $p < 0.05$, **: $p < 0.01$; ***: $p < 0.001$). d, EMSA with ASK1-UFO, LFY-DBD and
874 LUBS0 DNA probe. Different competitor DNA concentrations were tested as indicated. e,
875 Molecular mass determination for ASK1-UFO-LFY-DBD in complex with LUBS0 DNA by
876 SEC-MALLS (top). Elution profiles correspond to absorbance at 280 nm and 260 nm (left
877 ordinate axis, A.U: Arbitrary Unit). The black line shows the molecular mass distribution (right
878 ordinate axis). A mass of 102 ± 3.3 kDa was found for this ASK1-UFO-LFY-DBD-LUBS0
879 complex, consistent with one copy of each protein per DNA molecule (theoretical mass of 108
880 kDa). Coomassie-stained SDS-PAGE gel of the different SEC-MALLS fractions (bottom).
881 Each lane corresponds to a 0.5 mL fraction. Molecular weights of the protein standards are
882 indicated (BioRad Precision Plus). Faint bands above UFO likely correspond to contaminants.
883 f, EMSA with ASK1-UFO, LFY-DBD and indicated DNA probes. Sequences with coordinates
884 relative to *AP3* start codon (left). Red letters indicate mutated bases. Bars under sequences
885 represent the regions required for ASK1-UFO-LFY-DBD binding. EMSA with described DNA
886 probes (right). Each DNA probe was mixed with the same ASK1-UFO-LFY-DBD protein mix.
887 Note that the LUBS0 mutation also reduced *pAP3* activation in protoplasts (Fig. 2b).

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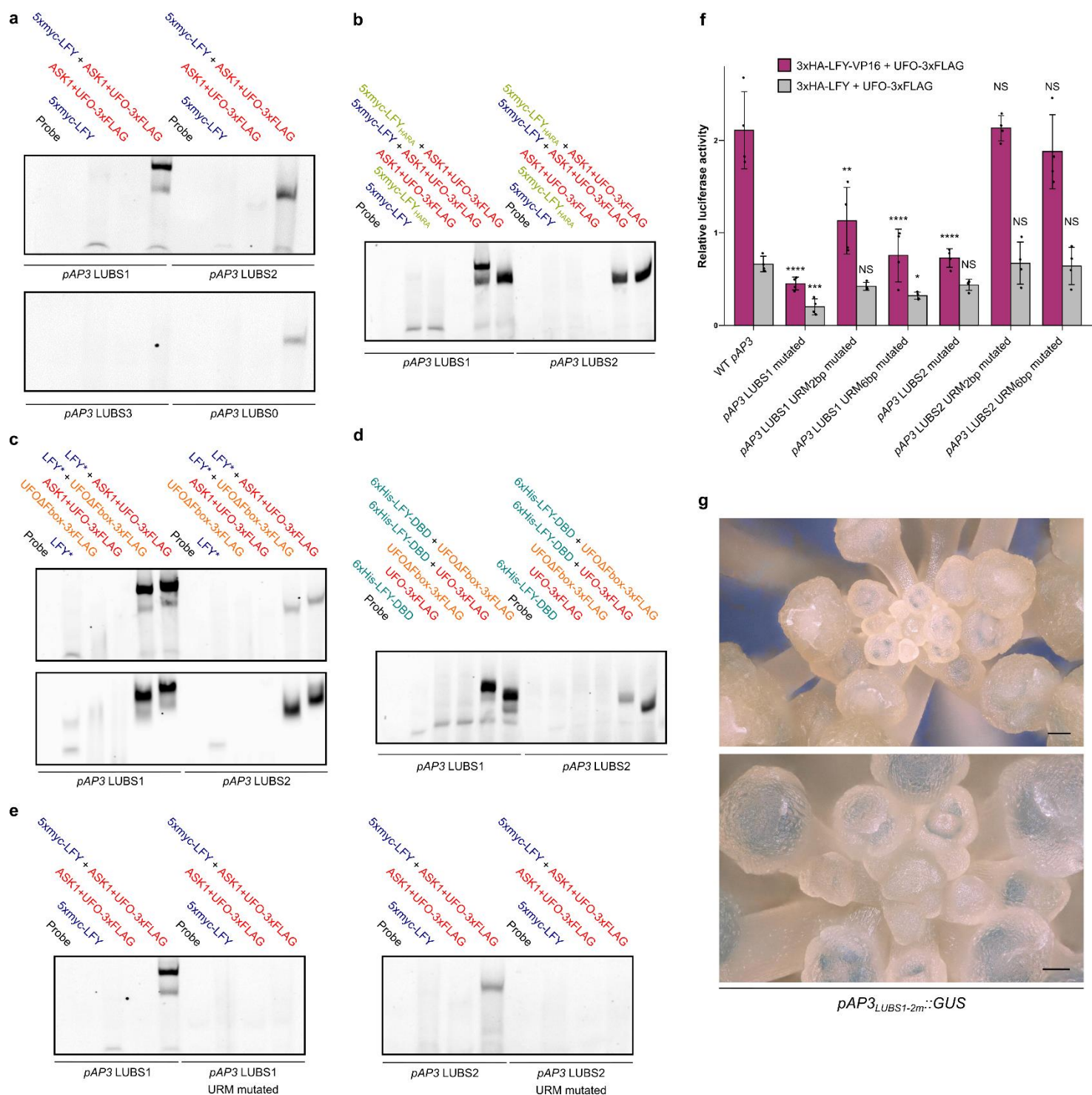


Extended Data Fig. 4. Genome-wide analysis of LFY-UFO binding. **a**, Western Blot after DNA elution during ampDAP-seq experiment. After DNA elution, 20 μ L of 1X SDS-PAGE Protein Sample Buffer was added to the remaining beads to run WB. Each lane represents one replicate. **b**, Assessment of experimental reproducibility of ampDAP-seq experiment through the comparison of replicates datasets 2 by 2. **c**, Effect of the LFY KARA mutation (K303A-R233A)⁵¹ on *pAP3* activation in Arabidopsis protoplasts. Data represent averages of independent biological replicates and are presented as mean \pm SD, each dot representing one biological replicate (n = 4). Unpaired t-tests (**: p < 0.01; ****: p < 0.0001). **d**, The LFY KARA mutation (K303A-R233A) does not disrupt LFY-UFO interaction in Yeast-Two-Hybrid (Y2H). EV = Empty Vector. LFY-40 is a LFY version lacking the first 40 aa and better tolerated by yeast cells. Values correspond to the different dilutions (OD = 7, 0.7 and 0.07). Top picture corresponds to the non-selective plate lacking Leucine and Tryptophan (SD -L-W), and bottom picture to the selective plate lacking Leucine, Tryptophan, Histidine and Adenine (SD -L-W-A-H). Pictures were taken at day + 4. **e**, Receiver operating characteristics (ROC) curves for mLUBS, dLUBS and LFY using the top 20% high-CFC LFY-UFO-specific peaks. Area under the curve (AUC) values are shown. TPR: True Positive Rate, FPR: False Positive Rate. **f**, Score distribution of LFY PWM with dependencies⁶⁸ within dLUBS (best site on 20% most LFY-UFO-specific genomic regions, high CFC, n = 3843 genomic regions) and in canonical LFYBS (best site on 20% most LFY-specific genomic regions, low CFC, n = 3843 genomic regions). Best sites were selected within ± 25 bp around the peak maximum. Wilcoxon rank sum test (****: p < 0.0001). Median (solid line), interquartile range (box edges), $\pm 1.5 \times$ interquartile range (whiskers) and outliers (black dot) are shown. **g**, *De novo* identification of URM from LFY ChIP-seq data²⁵. Motifs identified at a fixed distance from LFY canonical binding sites in 298 regions harboring high LFY ChIP-seq to LFY ampDAP-seq coverage ratio. The text above each motif gives the motif's start position relative to the canonical LFYBS, its length and the

915 number of sites used to build the motif. **h**, EMSA with mLUBS and dLUBS highest score
916 sequences. 6xHis-LFY-DBD is recombinant. UFO* refers to either recombinant ASK1-UFO-
917 3xFLAG complex (top gel) or *in vitro* produced UFO-3xFLAG (bottom gel). Drawings
918 represent the different types of complexes involving LFY-DBD (pale blue) and ASK1-UFO
919 (red) on DNA. LFY-DBD binds as a monomer as previously reported³⁰. The fact that *in vitro*
920 produced UFO-3xFLAG shifts DNA in the presence of LFY indicates that ASK1 is not required
921 for the UFO-LFY-DNA complex formation *in vitro*. **i**, EMSA with DNA probes corresponding
922 to *pAP1* and *pAP3* DEE LFYBS and indicated proteins. Note that probes used here have the
923 same length as those used to study LUBS.

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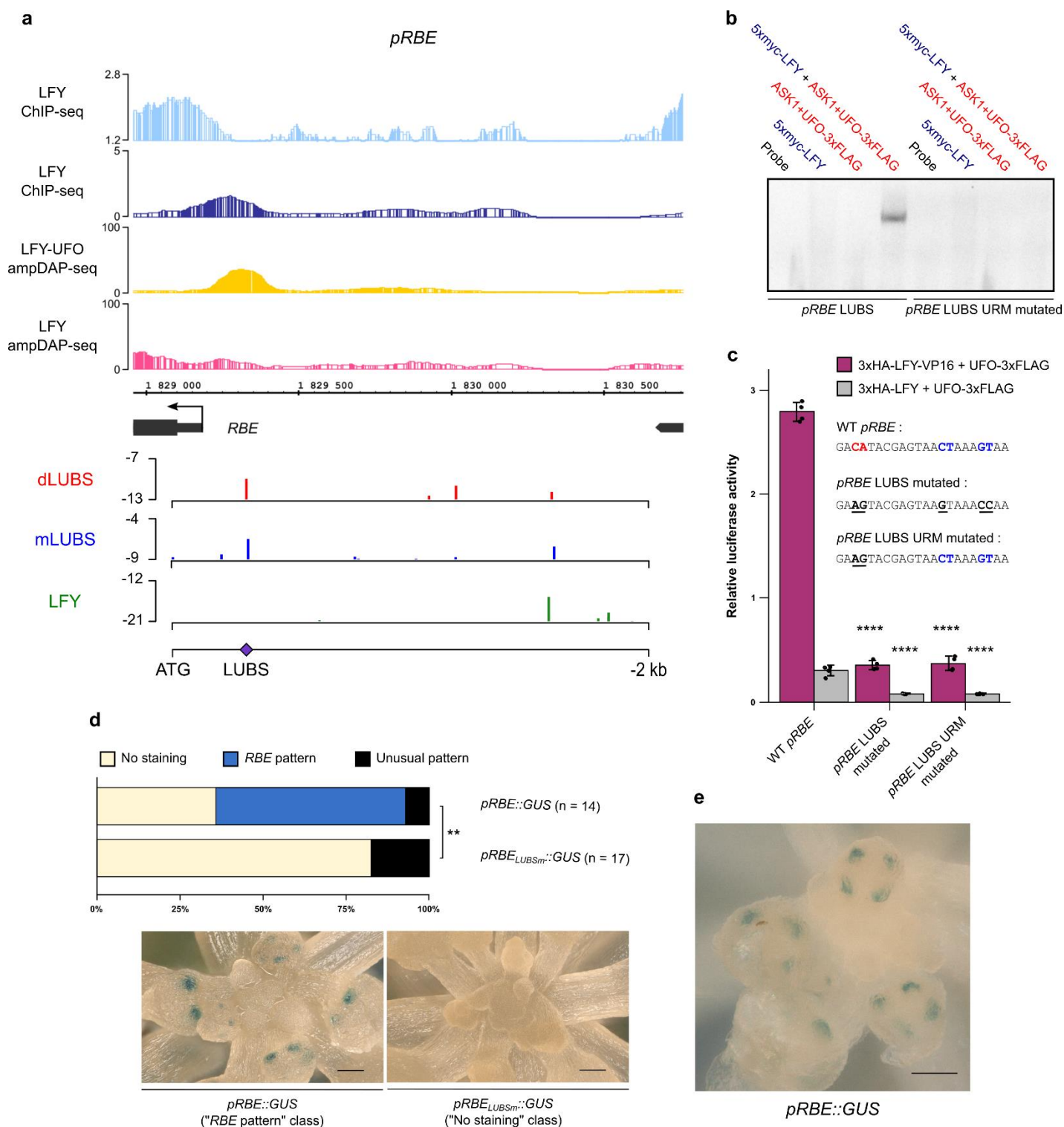
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926

927 **Extended Data Fig. 5. *pAP3* LUBS are required for LFY-UFO-dependent activation. a,**
 928 **EMSA with indicated probes and proteins. LUBS3 is the third highest-score *pAP3* LUBS.**
 929 **Because LUBS0 is bound with a lower affinity by LFY-UFO compared to LUBS1 and LUBS2,**
 930 **we then focused on LUBS1 and LUBS2. b, EMSA with *pAP3* LUBS1 and LUBS2 DNA probes**

and indicated proteins. LFY_{H383A-R386A} (LFY_{HARA}) is a LFY mutated version affected in its ability to dimerize^{30,51}. Note the absence of the complex with a slower mobility on LUBS1 with LFY_{HARA}. **c**, EMSA with *pAP3* LUBS1 and LUBS2 DNA probes and indicated proteins. LFY* refers to either *in vitro*-produced 5xmyc-LFY (top) or recombinant 6xHis-LFY-DBD (bottom). Note the difference of complex size between UFO and UFOΔFbox. **d**, Same as in (c) except that UFO-3xFLAG and UFOΔFbox-3xFLAG were produced *in vitro*. Note that *in vitro* produced UFO-3xFLAG and UFOΔFbox-3xFLAG behave similarly as recombinant UFO versions. **e**, EMSA with indicated proteins and DNA probes corresponding to *pAP3* LUBS1 (left) and LUBS2 (right), WT or with URM mutated. **f**, Promoter activation measured by DLRA in Arabidopsis protoplasts with indicated effectors. Different promoter versions were tested as indicated under x-axis. Either 2 bp (high-informative CA) or 6 bp (whole URM) of *pAP3* LUBS1 and LUBS2 URM were mutated. Data represent averages of independent biological replicates and are presented as mean ± SD, each dot representing one biological replicate (n = 4). One-way ANOVA with Tukey's multiple comparisons tests. One-way ANOVA were performed with data from the same effector and stars represent a statistical difference compared to WT *pAP3* promoter. (NS: p > 0.05, *: p < 0.05, **: p < 0.01, ***: p < 0.001 and ****: p < 0.0001). **g**, *In vivo* analysis of *pAP3_{LUBS1-2m}::GUS* fusions. Same as in Fig. 3d, except that staining incubation time was increased to 17 h (4h incubation in Fig. 3d). Representative pictures are shown (top scale bar, 100 μm, bottom scale bar, 50 μm). The faint *AP3* pattern suggests that other LUBS (such as LUBS0) may take over but less efficiently. Note that with this staining incubation time, all plants expressing *pAP3::GUS* showed a highly saturated staining.



954

955 **Extended Data Fig. 6. *pRBE* LUBS is required for LFY-UFO-dependent activation.** **a**, IGB

956 view of *pRBE* showing LFY ChIP-seq in inflorescences (light blue)²⁵ or seedlings (dark blue)²⁶,

957 LFY-UFO ampDAP-seq (yellow), LFY ampDAP-seq (pink)²⁷, numbers indicate read number

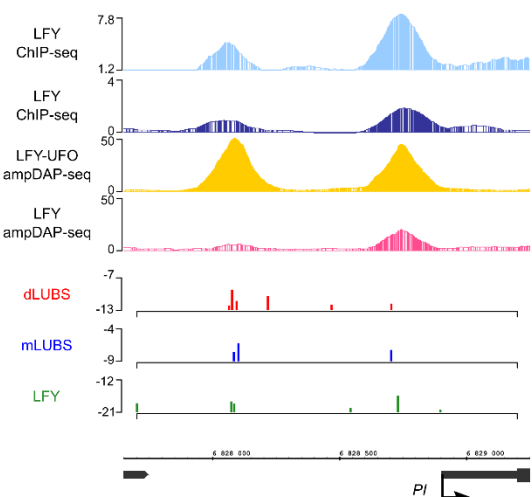
958 range (top). Identification of LUBS in *pRBE* (bottom). Predicted binding sites using dLUBS

and mLUBS models from Fig. 2e and LFY PWM with dependencies⁶⁸, y-axis represents score values (bottom). The best binding sites correspond to the less negative score values. Studied LUBS is indicated with a purple square. **b**, EMSA with probes corresponding to *pRBE* LUBS, WT or with URM mutated. **c**, *pRBE* activation in Arabidopsis protoplasts. Effect of mutations (underlined) in URM (red) and in LFYBS (blue) bases of *pRBE* LUBS were assayed. Data represent averages of independent biological replicates and are presented as mean \pm SD, each dot representing one biological replicate (n = 4). One-way ANOVA with Tukey's multiple comparisons test. One-way ANOVA were performed with data from the same effector, and stars represent a statistical difference compared to WT promoters (****: p < 0.0001). **d**, *In vivo* analysis of *pRBE::GUS* fusions. The percentage of transgenic lines with *RBE* pattern, unusual pattern or absence of staining was scored (top; χ^2 test, **: p < 0.01). n = number of independent lines. Unusual pattern refers to staining in unexpected tissues, each pattern seen in a single line. Representative pictures of plants with no staining (bottom left) and a *RBE* pattern (bottom right) are shown (scale bar, 50 μ m). **e**, *In vivo* analysis of *pRBE::GUS* fusions. Same as in (**d**), with another view showing staining in the four petal primordia (scale bar, 50 μ m).

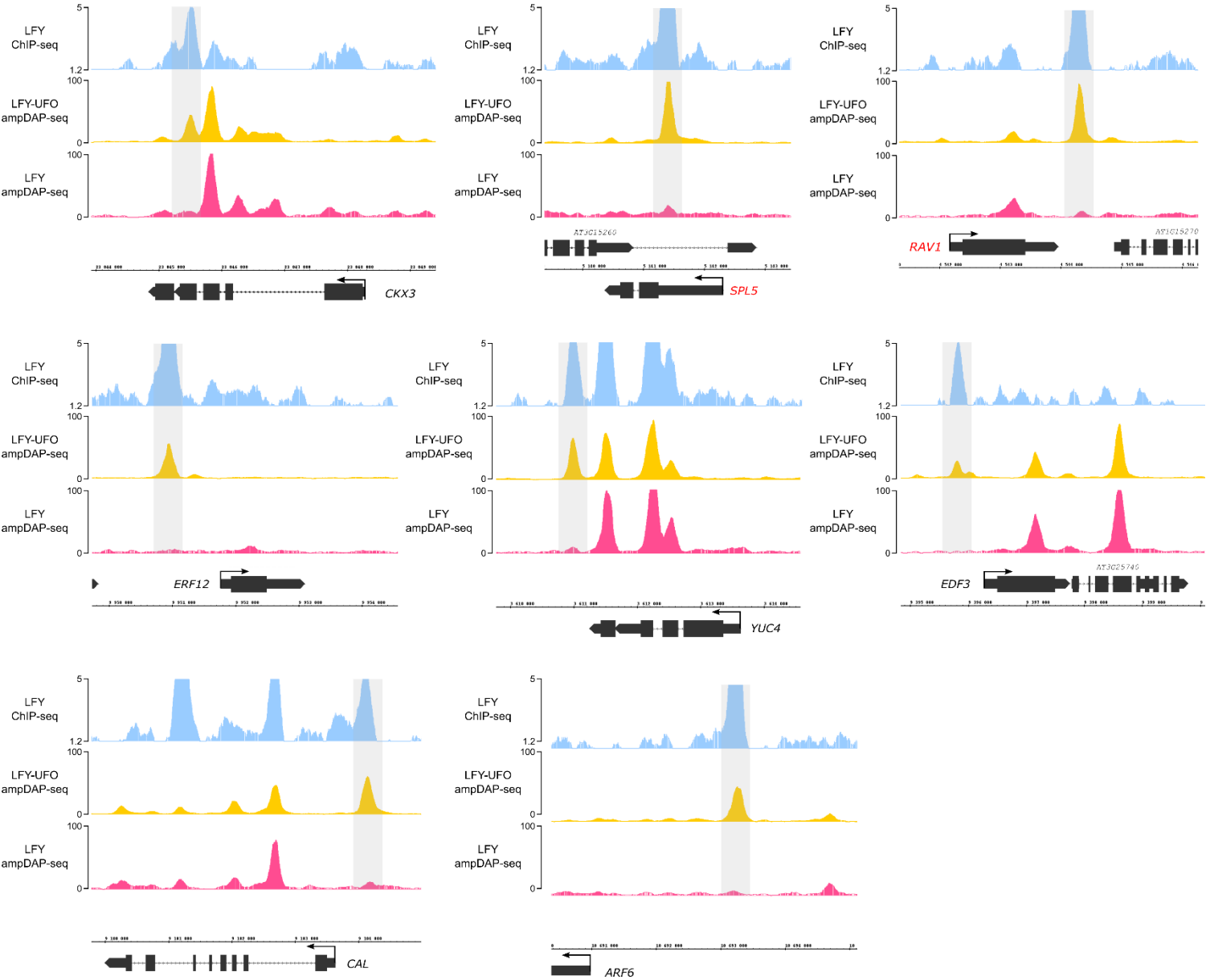
a

Gene	Name	Description	Up/Down regulated	LFY ChIP-Seq set
AT1G13260	RAV1	related to ABI3/VP1 1	down	A
AT1G24530	NA	Transducin/WD40 repeat-like superfamily protein	down	B
AT1G34110	NA	Leucine-rich receptor-like protein kinase family protein	up	C; B
AT1G54010	NA	GDSL-like Lipase/Acylhydrolase superfamily protein	down	A
AT1G73805	NA	Calmodulin binding protein-like	down	C; B; A
AT1G74440	NA	Protein of unknown function (DUF962)	down	A
AT1G75450	ATCKX5	cytokinin oxidase 5	up	C; B; D
AT1G76110	NA	HMG (high mobility group) box protein with ARID/BRIGHT DNA-binding domain	up	A
AT1G80840	ATWRKY40	WRKY DNA-binding protein 40	up	C; B; A
AT3G15270	SPL5	squamosa promoter binding protein-like 5	up	A
AT3G20810	NA	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	up	A
AT3G28180	ATCSLC04	Cellulose-synthase-like C4	down	B
AT3G54340	AP3	K-box region and MADS-box transcription factor family protein	up	B
AT3G55560	AGF2	AT-hook protein of GA feedback 2	up	C; B; A
AT4G00730	ANL2	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein	up	A
AT4G02380	AILEA5	senescence-associated gene 21	down	B
AT4G34160	CYCD3	CYCLIN D3	up	A
AT4G35900	FD	Basic-leucine zipper (bZIP) transcription factor family protein	down	C; B; A
AT5G02540	NA	NAD(P)-binding Rossmann-fold superfamily protein	up	B; D
AT5G20240	PI	K-box region and MADS-box transcription factor family protein	up	A

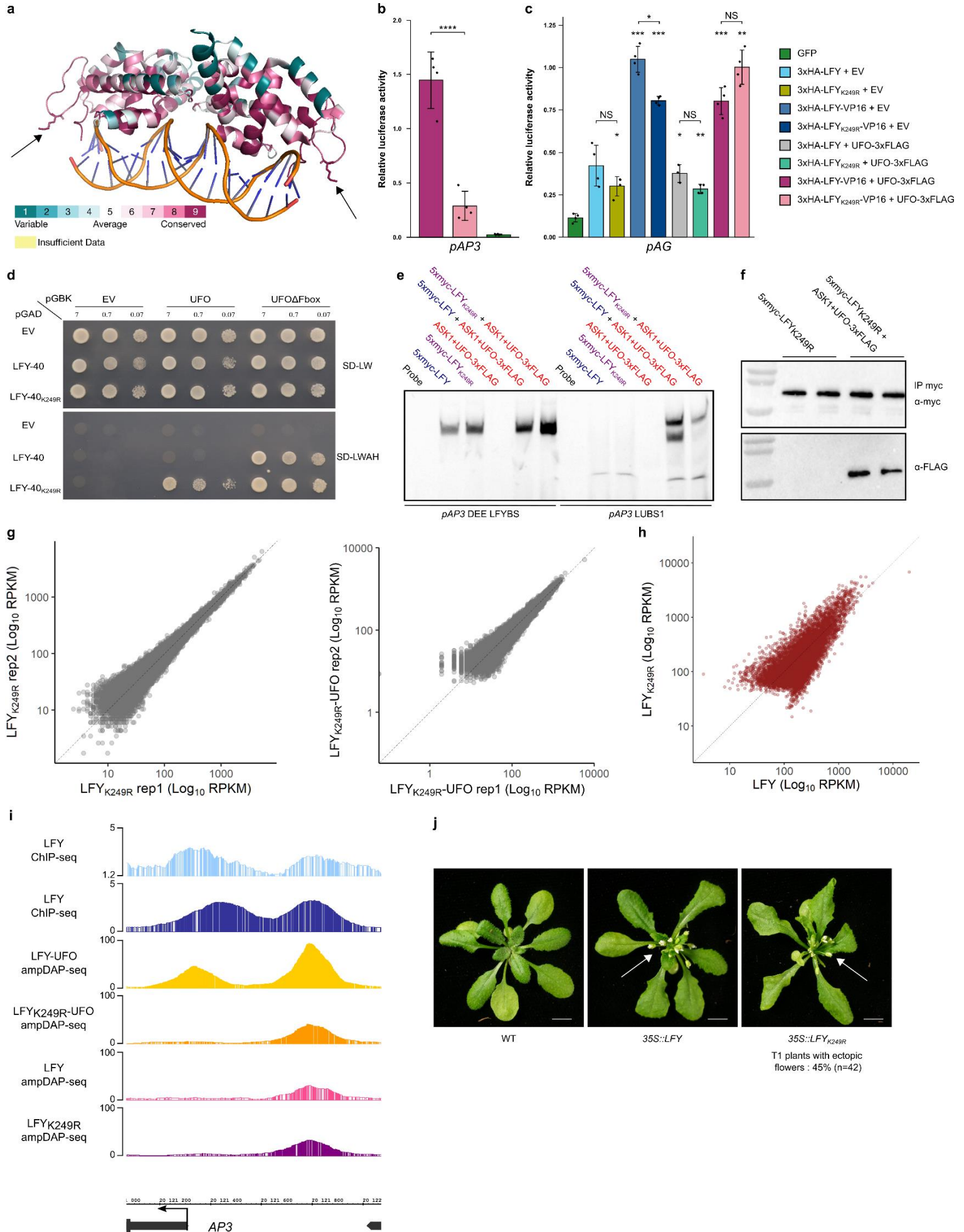
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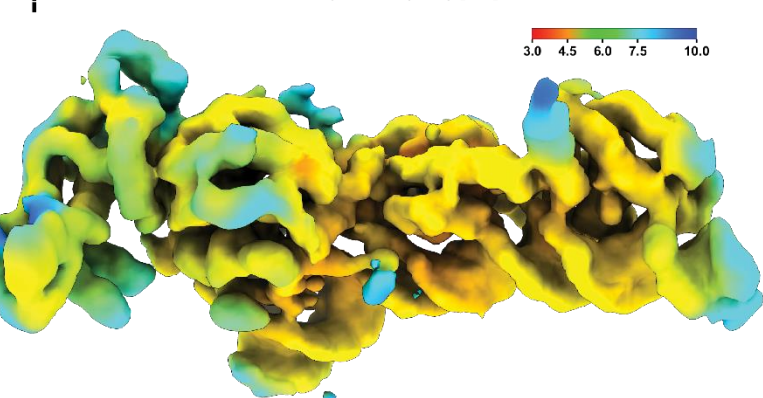
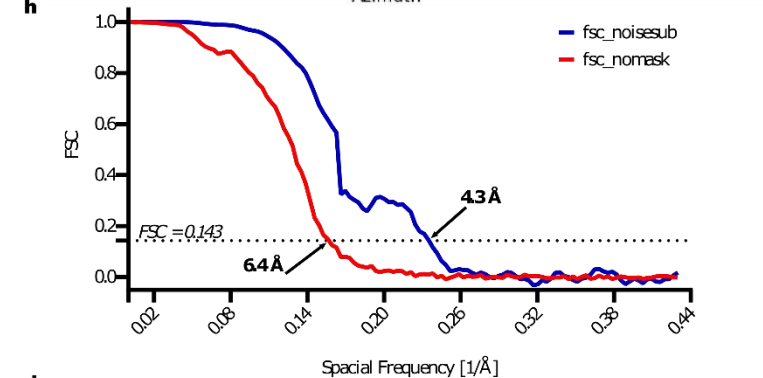
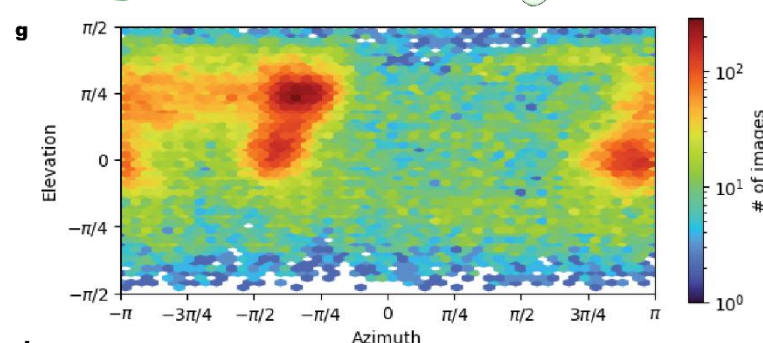
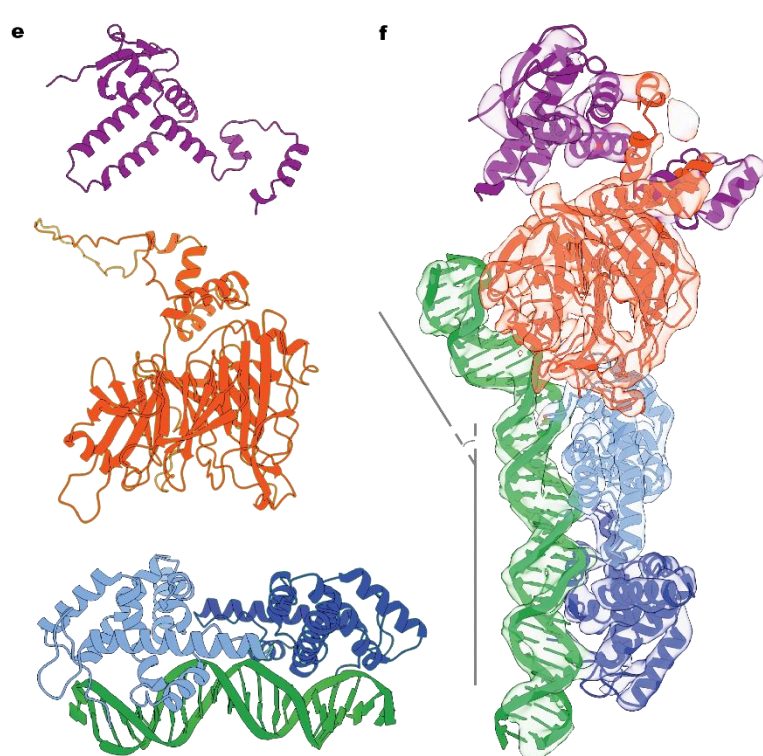
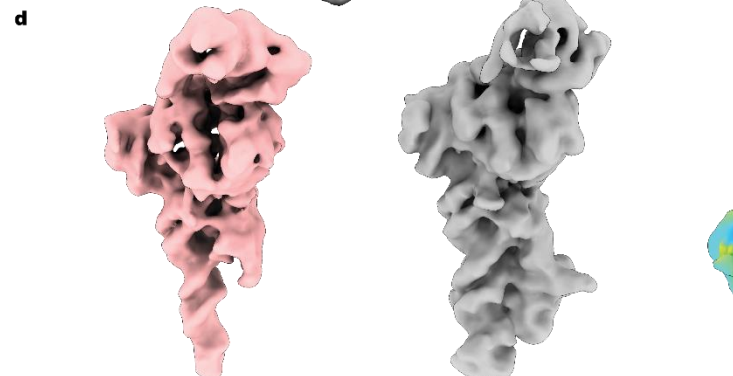
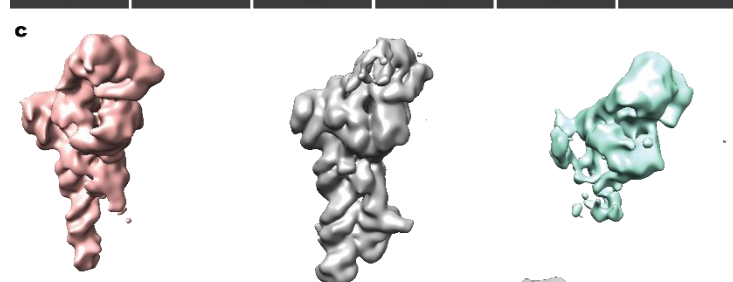
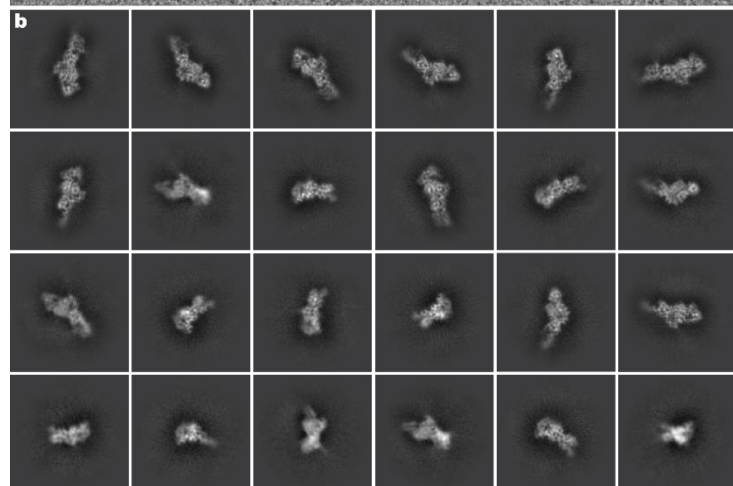
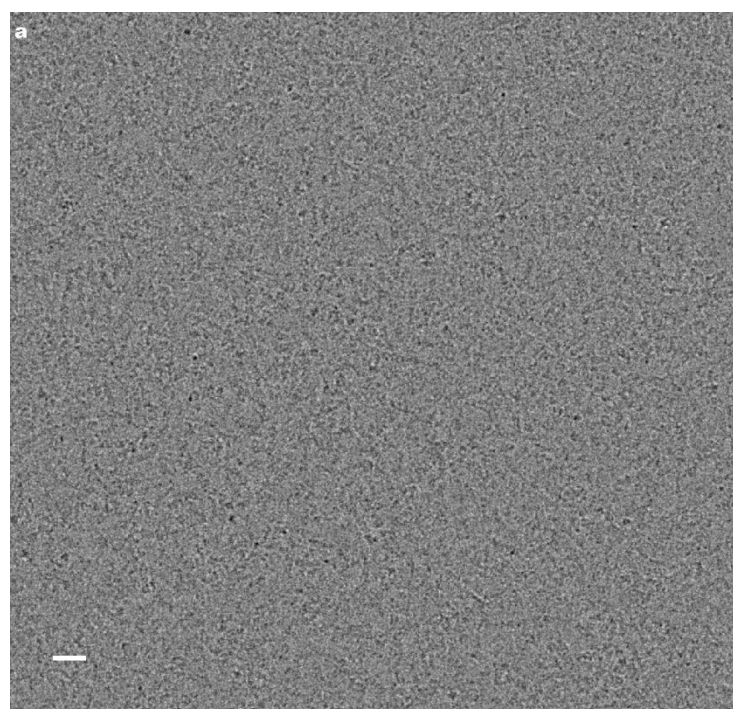
976 **Extended Data Fig. 7. LFY and UFO likely regulate other genes in Arabidopsis. a,** List of
 977 candidate LFY-UFO target genes selected as i) present in regions specifically bound by LFY-
 978 UFO in ampDAP-seq (high CFC) ii) bound *in vivo* in LFY ChIP-seq experiments (A²⁵; B²⁶;
 979 C⁶⁸; D⁷⁰) and iii) deregulated in *ufo* inflorescences⁶⁹. **b,** IGB view of *PISTILLATA* promoter
 980 region showing LFY ChIP-seq in inflorescences (light blue)²⁵ or seedlings (dark blue)²⁶, LFY-
 981 UFO ampDAP-seq (yellow), LFY ampDAP-seq (pink)²⁷, numbers indicate read number range
 982 (top). Predicted binding sites using the dLUBS, mLUBS models from Fig. 2e and LFY PWM
 983 with dependencies⁶⁸, y-axis represents score values (bottom). **c,** IGB view of selected genes
 984 showing LFY ChIP-seq in inflorescences (light blue)²⁵, LFY-UFO ampDAP-seq (yellow), LFY
 985 ampDAP-seq (pink)²⁷, numbers indicate read number range. Genes in red are deregulated in
 986 *ufo* inflorescences⁶⁹. ChIP-seq peaks better explained by LFY-UFO than by LFY alone are
 987 shaded in grey.
 988



Extended Data Fig. 8. The LFY K249 is essential for LFY-UFO-LUBS complex formation.

a, Structure of LFY-DBD³⁰. Residues were colored by conservation using Consurf with default parameters⁸⁰. K249 residues on each LFY monomer are represented as sticks and indicated with arrows. Note that the K249-containing loop is highly conserved. **b,c**, Promoter activation measured by DLRA in Arabidopsis protoplasts with indicated effectors (right). EV = Empty Vector (pRT104-3xHA). Tested promoters are indicated below each graph. Note that for 3xHA-LFY+UFO-3xFLAG on *pAG* only $n = 3$ biological replicates are shown. Data represent averages of independent biological replicates and are presented as mean \pm SD, each dot representing one biological replicate ($n = 4$ unless specified). One-way ANOVA with Tukey's multiple comparisons tests (**b**) or Welch's ANOVA with Games-Howell post-hoc test (**c**). In (**c**), stars above bars represent a statistical difference compared to GFP. Other comparisons are indicated with brackets. (NS: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ and ****: $p < 0.0001$). **d**, Effect of the LFY_{K249R} mutation on LFY-UFO interaction in Y2H. EV = Empty Vector. LFY-40 is a LFY version lacking the first 40 aa and better tolerated by yeast cells. Values correspond to the different dilutions (OD = 7, 0.7 and 0.07). Top picture corresponds to the non-selective plate lacking Leucine and Tryptophan (SD -L-W), and bottom picture corresponds to the selective plate lacking Leucine, Tryptophan, Histidine and Adenine (SD -L-W-A-H). Pictures were taken at day + 4. **e**, EMSA with DNA probes corresponding to *pAP3* DEE LFYBS and *pAP3* LUBS1 and indicated proteins. *pAP3* DEE LFYBS DNA probe was used as a control for binding on canonical LFYBS. **f**, WB after DNA elution during ampDAP-seq experiment. After DNA elution, 20 μ L of 1X SDS-PAGE Protein Sample Buffer was added to the remaining beads to run WB. Each lane represents one replicate. **g**, Reproducibility of ampDAP-seq experiments with LFY_{K249R} (left) and LFY_{K249R}-UFO (right) through the comparison of replicates datasets 2 by 2. **h**, Comparison of peak coverage in LFY_{K249R} (y-axis, this study) and LFY (x-axis)²⁷ ampDAP-seq experiments. **i**, Integrated Genome Browser (IGB)

1015 view of *pAP3* showing LFY ChIP-seq in inflorescences (light blue)²⁵ or seedlings (dark blue)²⁶,
1016 LFY-UFO ampDAP-seq (yellow; this study), LFY ampDAP-seq (pink)²⁷ and LFY_{K249R}
1017 ampDAP-seq (purple; this study). Numbers indicate read number range. **j**, Pictures of WT and
1018 representative transgenic plants expressing *35S::LFY* or *35S::LFY_{K249R}* (scale bar, 1 cm). The
1019 white arrows indicate ectopic rosette flowers. *35S::LFY* was obtained previously²⁶. 42 T1 plants
1020 expressing *35S::LFY_{K249R}* were analyzed; the percentage of plants with a LFY overexpressing
1021 phenotype is comparable to the one obtained with *35S::LFY*²⁶.
1022



Extended Data Fig. 9. UFO binds DNA and LFY DBD. **a**, A representative micrograph of the ASK1-UFO-LFY-DNA complex in vitreous ice (scale bar, 20 nm). **b**, Selected 2D class averages of the particles submitted to *ab initio* reconstruction and heterogeneous refinement for 3D classification. **c**, Intermediate reconstructions of the 3D classes after heterogeneous refinement. **d**, Final reconstructions of ASK1-UFO-LFY-DNA complexes (involving either a LFY-DBD monomer (pink) or a LFY-DBD dimer (gray)) after Non-Uniform refinement. **e**, Unprocessed AlphaFold2 model for ASK1 (top, purple; uniprot ID, Q39255), UFO (middle, red; uniprot ID, Q39090) and the LFY-DBD dimer/DNA crystallographic structure (bottom, pale and dark blue for the LFY-DBD dimer and green for the DNA; PDB, 2VY1). **f**, Cryo-EM density map color-coded by fitted molecule. Note the kink on DNA induced by the presence of UFO. **g**, Heat map of the angular distribution of particle projections contributing for the final reconstruction of the complete ASK1-UFO-LFY-DNA complex (with a LFY-DBD dimer). **h**, Gold-standard Fourier shell correlation (FSC) curves. The dotted line represents the 0.143 FSC threshold, which indicates a nominal resolution of 6.4 Å for the unmasked (red) and 4.3 Å for the masked (blue) reconstruction. **i**, View of the post-processed map of the complete ASK1-UFO-LFY-DNA complex, colored according to the local resolution.

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