CLAVATA Signaling Ensures Reproductive Development in Plants across Thermal Environments

Graphical Abstract

Highlights

- CLAVATA (CLV) signaling mediates continuous flower outgrowth in Arabidopsis thaliana
- CLAVATA2/CORYNE function alongside CIK-family coreceptors
- CLV signaling promotes auxin-mediated floral primordia growth in colder temperatures
- High temperatures bypass CLV signaling, upregulating auxin through ELF3

Authors

Daniel S. Jones, Amala John, Kylie R. VanDerMolen, Zachary L. Nimchuk

Correspondence

zackn@email.unc.edu

In Brief

Jones et al. demonstrate that plants use overlapping pathways to ensure robust reproduction across diverse temperatures. In colder environments, CLAVATA peptide signaling promotes flower development by stimulating auxin-dependent growth, but at higher temperatures, auxin is upregulated in an ELF3-dependent process, bypassing the CLAVATA pathway.
CLAVATA Signaling Ensures Reproductive Development in Plants across Thermal Environments

Daniel S. Jones,1 Amala John,1 Kylie R. VanDerMolen,1 and Zachary L. Nimchuk1,2,3,*

1Department of Biology, University of North Carolina at Chapel Hill, 250 Bell Tower Drive, Chapel Hill, NC 27599, USA
2Curriculum in Genetics and Molecular Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
3Lead Contact
*Correspondence: zackn@email.unc.edu
https://doi.org/10.1016/j.cub.2020.10.008

SUMMARY

The ability to thrive in diverse environments requires that species maintain development and reproduction despite dynamic conditions. Many developmental processes are stabilized through robust signaling pathways that cooperatively ensure proper development.1 During reproduction, plants like Arabidopsis thaliana continuously generate flowers on growing indeterminate inflorescences.2 Flower primordia initiation and outgrowth depends on the hormone auxin and is robust across diverse environments.3–6 Here, we show that reproductive development under different thermal conditions requires the integration of multiple pathways regulating auxin-dependent flower production. In colder/ambient temperatures, the receptor complex CLAVATA2/CORYNE (CLV2/CRN) is necessary for continuous flower outgrowth during inflorescence development. CLV2/CRN signaling is independent of CLAVATA1 (CLV1)-related receptor signaling but involves the CLAVATA3 INSENSITIVE RECEPTOR KINASE (CIK) family co-receptors, with higher order cik mutant combinations phenocopying clv2/crn flower outgrowth defects. Developing crn inflorescences display reduced auxin signaling, and restoration of auxin biosynthesis is sufficient to restore flower outgrowth in colder and ambient temperatures. In contrast, at higher temperatures, both clv2/crn signaling and heat-induced auxin biosynthesis via YUCCA family genes are synergistically required to maintain flower development. Our work reveals a novel mechanism integrating peptide hormone and auxin signaling in the regulation of flower development across diverse thermal environments.

RESULTS AND DISCUSSION

Plants continually develop new organs throughout their life and do so across varied environmental conditions.1 This indeterminate growth requires balanced cell proliferation and differentiation in stem cell niches, called meristems, at growing apices.5,9 During the reproductive phase of Arabidopsis thaliana, flower primordia are continuously produced from inflorescence meristems (IM) dependent on the hormone auxin.3–6 Primordia then proliferate, forming flowers from secondary floral meristems.10,11 Cell recruitment into flower primordia is balanced by proliferation in the IM center. The conserved CLAVATA3 (CLV3) peptide signaling pathway dampens stem cell proliferation in shoot and floral meristems.12,13 CLV3 signals through a suite of receptors that repress the expression of WUSCHEL (WUS) in the center of the IM.8,14 WUS encodes a homeobox transcription factor that positively regulates stem cell proliferation.15 Among these receptors are the atypical receptor pair CLAVATA2/CORYNE (CLV2/CRN), a leucine rich repeat (LRR) receptor-like protein and a transmembrane pseudokinase, respectively.16–19 CLV2/CRN negatively regulate IM stem cell proliferation independent of other CLV3 receptors.17,20 Here, we define a new role for CLV2/CRN in promoting auxin-dependent flower primordia outgrowth and show that signaling through this receptor complex contributes to an environmental buffering mechanism that ensures reproductive developmental stability.

The CLV2/CRN Receptor Complex Promotes Flower Primordia Outgrowth and Development

After the production of 1–5 normal flowers in crn-null mutants (crn-10; in the Col-0 ecotype), we noticed a novel phenotype in which flower primordia initiate but fail to develop further and inflorescence internode elongation stalls (Figures 1A and 1B; clv2/crn double mutants; in Col-0) displayed a similar phenotype to clv2/crn-10 (Figures 1B and S1A; Video S1). clv2/crn mutants (rlp10-1; in Col-0) displayed a similar phenotype to crn (Figure 1C), which was previously observed in clv2 in a survey of mutants in receptor-like protein genes, but not characterized.21 To quantify primordia termination, we classified the first 30 attempts to make flowers along the primary inflorescence as normal (complete flowers; formation of all four flower organs),22 terminated flower primordia (no flower organs develop), or terminated flowers (some flower organs develop, but no gynoecium). crn and clv2 single mutants displayed equivalent defects in flower production (Figure 1E), also observed in clv2/crn double mutants, consistent with the documented co-function of CLV2/CRN (Figures 1B–1E). clv2/crn flower outgrowth
defects, and floral meristem size (measured in carpels made per flower), were complemented by expressing fusion proteins from their native promoters (Figures S1B, S1C, S1I, and S1J). Using standardized flower primordia staging, we found that crn primordia outgrowth deviated from WT (wild type) at flower primordia stage three (FP3), with little proliferation occurring afterward (Figures 1F and 1G; staging as in Reddy et al.23 or stage 2 using Smyth et al.22 stages). In crn, terminated primordia fail to develop floral organs but occasionally produce bract-like structures, likely due to de-repression of cryptic bract outgrowth (Figure 1H).24

Consistent with a role in flower primordia development, CRN expression was detected as early as incipient primordia (before primordia outgrowth) and remained throughout primordia formation (Figure 1I). Both CRN (CRN-GFP) and CLV2 (CLV2-Citrine) fusion proteins, expressed by native promoters, confirmed this expression pattern (Figures S1B and S1C). Supporting previous in situ results,15 WUS expression did not overlap with CRN spatially or temporally during primordia specification and outgrowth, when crn inflorescence phenotypes diverge from WT (Figures 1I and 1J). The WUS domain was expanded in crn IMs compared to WT, but ectopic WUS expression was not detected in terminated crn primordia (Figures 1J and 1K).17 Additionally, other mutants known to have expanded WUS IM expression domains do not display clv2/crn primordia outgrowth defects (see below).

CLV2/CRN functions have been studied extensively in the Landsberg-erecta (Ler) background.16,17,25 We found no flower primordia outgrowth defects in null clv2-1 mutants in Ler, explaining why this phenotype has not been described in this ecotype (Figures S1B and S1C). Supporting previous in situ results,15 WUS expression did not overlap with CRN spatially or temporally during primordia specification and outgrowth, when crn inflorescence phenotypes diverge from WT (Figures 1I and 1J). The WUS domain was expanded in crn IMs compared to WT, but ectopic WUS expression was not detected in terminated crn primordia (Figures 1J and 1K).17 Additionally, other mutants known to have expanded WUS IM expression domains do not display clv2/crn primordia outgrowth defects (see below).

Figure 1. Flower Primordia Outgrowth Is Disrupted in clv2/crn
(A–D) Inflorescences of Col-0, crn (crn-10), clv2 (rlp10-1), and crn clv2 double mutants. (A–D') Close up shows flower primordia termination in crn, clv2, and crn clv2.

(E) Quantification of flower termination, classifying the first 3D attempts to make a flower as normal (gray), terminated primordia (blue), or terminated flowers (yellow) in Col-0 (n = 28), clv2 (n = 29), and crn clv2 (n = 25).

(F and G) 3D reconstruction of inflorescence meristems of (F) Col-0 (n = 4) and (G) crn (n = 6). Axial view of the (F' and G') third (FP3) and (F" and G") fourth (FP4) flower primordia (labeled 3 and 4 in F and G) reveals developmental differences. FP3 and FP4 were determined by identifying the 3rd and 4th earliest detectable primordium along the IM, respectively. Staging is similar to Reddy et al.23

(H) Side view of a young inflorescence meristem of crn during the termination phase (n = 5).

(I–K) Expression patterns of YPET-N7 reporter lines in the IM with XY view of L5 layer (I–K) and axial view (Z axis) of same IM stack (I"–K") shown for each (l) CRNpro in Col-0 (n = 6), (J) WUSpro in Col-0 (n = 6), and (K) WUSpro in crn (n = 6). Tissue was stained with propidium iodide (PI) (magenta).

Statistical groupings based on significant differences found using Kruskal-Wallis and Dunn’s multiple comparison test correction (E): significance defined as p-value < 0.05.

See also Figures S1 and S4 and Video S1.
outgrowth defects segregated in a digenic semi-dominant manner in the F2 population (Figure S1M), indicating that dominant modifiers in Col-0 underlie ecotype differences in clv2/cm flower outgrowth. Additionally, ecotypic differences were not attributable to the erecta (er) allele in Ler plants, as clv2 er double mutants in the Col-0 background had equivalent flower outgrowth defects to clv2 (Figures S1N–S1P). Double mutants between CLV2 and the floral identity gene LEAFY (LFY) (clv2 Ify) displayed primordia outgrowth defects and Ify-like floral organ conversions (Figures S1Q and S1R), indicating that CLV2/CRN promote primordia formation independent of LFY-floral meristem specification. Collectively, these data show that CLV2/CRN signaling represents a novel ecotype-dependent process regulating primordia outgrowth following reproductive transition.

**CLAVATA2/CRN-Mediated Flower Outgrowth Requires CIK Co-receptors**

The CLV2/CRN receptor complex lacks signaling capacity alone, as CRN is a transmembrane pseudokinase, suggesting CLV2/CRN require associated functional kinase(s) to signal. CLAVATA candidates with active kinase domains include the CLAVATA3 INSENSITIVE RECEPTOR KINASE1/2/3/4 (CIK1/2/3/4) family co-receptors, CLV1, and the CLV1-related BARELY ANY MERISTEM1/2/3 receptors (BAM1/2/3), which all regulate IM stem cell proliferation. 

CLV1/BAM signals independent of CLV2/CRN in shoot and floral stem cell control. Consistent with this, we found negligible amounts of flower primordia termination in clv1, bam1/2, or bam1/2/3-null mutants (in Col-0) and cm was additive in each higher order mutant combination (cm clv1, cm bam1/2, and cm bam1/2/3; Figures S2A–S2J). CIK1/2/3/4 are LRR-II-receptor-like-kinase subfamily co-receptors with overlapping functions with CLAVATA primary receptors, several of which physically interact with CRN. In a previous report, we noticed a cm-like phenotype in specific ciik mutant combinations. To confirm this observation, we generated higher order CRISPR-null alleles of CIK1/2/4 in Col-0 (ciik1-3, ciik2-3, and ciik4-3; Figure S2K). In contrast to clv1/bam mutants, ciik1/2/4 displayed flower primordia termination equivalent to cm (Figures 2A–2C). Additionally, ciik1/2/4 had enlarged floral meristems quantitatively similar to cm (Figure 2D). The protein phosphatase POLTERGEIST (POL), a downstream component of CLAVATA signaling, suppresses clv2/cm meristem size defects. POL restored flower outgrowth and internode elongation defects in cm (cm pol; Figures 2E–2H). Collectively, these data demonstrate that CLV2/CRN signal alongside CIK1/2/4 co-receptors to promote flower outgrowth through a POL-dependent pathway.

CLAVATA receptors respond to CLV3/EMBRYO-SURROUNDING REGION (CLE) peptide ligand(s), and there are 32 CLEs in Arabidopsis. CLV3 and a suite of redundant CLE peptides signal via CLV1 to repress IM stem cell proliferation parallel to CLV2/CRN. Additional CLE peptides regulate flower outgrowth through CLV2/CRN/CIK (Figures S2L and S2M). Consistent with this, cm clv3 double mutants are additive with a clear disruption in flower primordia outgrowth and an enlarged disc-like IM (Figure S2N). The enlarged shoot and fasciated stem of cm clv3 made quantification of terminated primordia difficult; however, these data support previous work suggesting that CLV2/CRN can act independently of CLV3.

**Temperature and CLV2/CRN Modulate Auxin-Dependent Flower Primordia Outgrowth**

Many developmental programs are robust, ensuring optimal morphology/function across varied conditions. Populations of *A. thaliana* can be found throughout the Northern Hemisphere, thriving in diverse environments. Natural variation in traits like flowering time and freezing tolerance are influenced by and/or directly correlated with adaptations to local conditions. While investigating clv2/cm, we observed remarkable quantitative variability in flower primordia termination at different temperatures. Flower outgrowth defects in clv2/cm were suppressed when grown at higher temperatures (31°C) compared to colder/
ambient temperatures (16°C/24°C; Figures 3A–3G). Previous work noted shoot defects in crn-1 mutants (Ler background) at high temperatures; however, we did not observe this under our conditions (Figures 3H and 3I). Thermomorphogenic pathways regulate high-temperature seedling growth by enhancing auxin biosynthesis. At higher temperatures, PHYTOCHROME INTERACTING FACTOR (PIF) family transcriptional regulators activate YUCCA (YUC) genes, which encode rate-limiting enzymes in auxin biosynthesis. Under colder/ambient temperatures, thermomorphogenesis is negatively regulated by the transcriptional repressor EARLY FLOWERING 3 (ELF3). As such, elf3 seedlings display constitutive thermomorphogenic responses and higher auxin production across temperatures. To test whether the thermomorphogenesis pathway was sufficient to suppress clv2/crn flower outgrowth defects, we generated cm elf3 double mutants and grew them at colder/ambient temperatures. Consistent with high-temperature-mediated suppression of crn, flower primordia formation was suppressed in cm elf3 at colder temperatures (Figures 3J–3L). In contrast to the suppression of cm primordia outgrowth, elf3 slightly enhanced carpel numbers compared to cm (Figure 3M). This finding supports that CLV2/CRN-mediated outgrowth and CLV2/CRN-mediated meristem size regulation are separable, with primordia outgrowth being highly sensitive to thermal conditions. Our data show that CLV2/CRN/CIK signaling is critical for continuous flower production at colder/ambient temperatures but can be bypassed by thermomorphogenic responses to higher temperatures. As such, although Arabidopsis flower development is robust under various environmental conditions, distinct mechanisms maintain this stability across different temperatures.

To define the mechanisms underlying cm primordia termination, we used RNA sequencing (RNA-seq) to identify differentially expressed genes (DEGs) in terminating cm IMs compared to WT. Using a strict cutoff (p < 0.001), we found 460 DEGs between cm and WT IMs, with 236 upregulated and 224 downregulated in cm (Figure 4A; Table S1). Enriched Gene Ontology (GO) terms among DEGs included meristem maintenance, flower development, and auxin function (Figure 4B; Table S2). The first two GO term groups are consistent with CRN’s role in meristem maintenance and flower development, documented in this study (Tables S1 and S2). The overrepresentation of auxin-associated genes in cm IM DEGs (Table S1) is complementary to the thermomorphogenic suppression of cm’s primordia termination (Figure 3), suggesting CLV2/CRN regulate auxin function during early flower primordia outgrowth. Therefore, we asked whether clv2/crn were defective in auxin outputs at lower temperatures and whether auxin biosynthesis was required for the high-temperature suppression of clv2/crn. We visualized the auxin signaling reporter DR5::GFP (where GFP positively correlates with increased auxin signaling output) and the auxin perception reporter DII-Venus (where Venus negatively correlates with increased auxin perception) in terminating cm IMs (Figures 3C, 3D, and S3A–S3C). Consistently, DII::Venus accumulated in the L1 layer of termined cm IMs during termination, specifically in the L1 layer of incipient primordia (Figures 4C, 4D, and S3A–S3C). Consistently, DII::Venus accumulated in the L1 layer of terminated cm IMs, a pattern never observed in WT (Figures 4E, 4F, and S3D). DR5::GFP was restored to WT levels during cm’s recovery phase (Figures S3E and S3F). During flower development, the PIN-FORMED1 (PIN1) auxin efflux transporter concentrates auxin to the IM periphery, creating local maxima that trigger flower primordia initiation and subsequent outgrowth. PIN1 reporter levels (PIN1-GFP) were decreased in terminating cm IMs (Figures S3G and S3H), consistent with PIN1 expression from our RNA-seq DEG data (Table S1). PIN1-GFP levels increased during cm’s recovery phase, but not to WT levels (Figure S3I). These data demonstrate an overall reduction in auxin signaling/perception within the IM
and developing primordia of crn during the termination phase. This decrease is transient and corresponds with flower outgrowth defects, indicating that CLV2/CRN positively regulate auxin-dependent flower primordia outgrowth in the IM.

Several auxin biosynthetic genes had decreased expression in crn compared to WT, including YUC genes that regulate flower development (Figure S3J; Table S3). To test whether low auxin levels contribute to crn’s flower outgrowth defects, we expressed YUC1 in developing primordia of crn using the AINTEGUMENTA promoter (ANTp::YUC1)6,49 and grew plants in colder (16–18°C) and ambient (22–24°C) temperatures. At 22°C–24°C, 9/13 T1 plants suppressed crn although only 2/18 plants partially suppressed crn at 16°C–18°C (Figures 4G and 4H). This demonstrates that, at ambient temperatures (where crn terminates), ectopic YUC1 can suppress flower outgrowth defects; however, the degree of suppression correlates with temperature. Higher order mutant combinations in IM-expressed YUC1/2/4/6 severely impair floral and vasculature development; however, yuc1/4 double mutants produce more typical inflorescences with identifiable flowers.48 We generated clv2 yuc1/4 triple mutants to reduce YUC-dependent auxin in clv2 and test whether high-temperature suppression of clv2/crn flower termination was dependent on YUC-mediated auxin biosynthesis. At 16°C–18°C, clv2 yuc1/4 triple mutants displayed rates of flower primordia termination comparable to clv2 (Figure 4I). As such, CLV2/CRN promote auxin-mediated primordia outgrowth independent of YUC1/4 in colder temperatures. At 28°C–31°C, some clv2 yuc1/4 triple-mutant plants displayed clv2 flower primordia termination, indicating that, at high temperatures, YUC1/4 contribute to heat-induced suppression (Figure 4J). Surprisingly though, the majority (~60%) of clv2 yuc1/4 plants had a synergistic response to high temperatures, resulting in pin-like inflorescences completely lacking...
flower primordia (Figure 4J). These data suggest that high-temperature suppression of clv2 is dependent on YUC1/4-mediated auxin biosynthesis and that, under high temperatures, CLV2/CRN and YUC1/4 are synergistically required to maintain flower primordia initiation and outgrowth.

Ensuring robust development and reproduction across environments is a challenge all organisms face. Here, we demonstrate that robust flower production in diverse thermal environments is achieved through the synergistic deployment of CLV2/CRN signaling and ELF3-regulated auxin production via the thermomorphogenesis pathway in Arabidopsis. The relative contribution of each to flower development varies across thermal clines, with CLV2/CRN signaling being critical at colder/ambient temperatures and synergistic with heat-induced auxin production at higher temperatures (Figure S4). Arabidopsis seedlings respond to high temperatures by promoting auxin-dependent hypocotyl elongation, a process negatively regulated by ELF3.38,42 Our work demonstrates that high temperatures and ELF3 also regulate auxin-dependent primordia production. Interestingly, clv2 yuc1/4 primordia outgrowth defects were strongly enhanced in warmer conditions. Higher temperatures also enhance penetrance of seedling defects in loss-of-function mutants in the TRANSPORT INHIBITOR RESPONSE1 (TIR)-family auxin receptors.50 As such, heat might have an unappreciated negative impact on auxin function, with thermomorphogenesis-induced auxin playing a protective role rather than simply directing growth. How CLV2/CRN stimulate auxin-dependent flower initiation is unknown. Ectopic YUC1 expression or heat-induced auxin production is sufficient to restore primordia outgrowth to clv2/crn. This suggests that CLV2/CRN are not critically required for TIR-dependent auxin perception, ARF5/MONOPEROS-dependent transcriptional activity, or the TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (TAA1)-mediated conversion of tryptophan to indole-3-pyruvate (IPA) step upstream of YUCCA in auxin biosynthesis.5,51–53 clv2/crn defects manifest early in inflorescence development and are transient. Recovery of primordia production is not linked to flower meristem identity or seed/fruit-derived auxin production (clv2 lfy plants). This suggests that the transition from vegetative to reproductive meristem fate may be sensitized to CLV2/CRN signaling. Nevertheless, clv2 yuc1/4 plants reveal that CLV2/CRN signaling is required at later steps in inflorescence development as well. Heat stress is known to damage crops in ways that negatively impact yield, including the loss of flower production.54,55 As climate change increases global temperatures, it will be necessary to mitigate heat impacts on crop yield. If the environmental buffering capacity of CLV2/CRN signaling is conserved in crop species, perhaps it could be deployed to help improve plant responses to climate change.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
  - Lead Contact
  - Materials Availability
  - Data and Code Availability
  - EXPERIMENTAL MODEL AND SUBJECT DETAILS
    - Plant growth conditions
  - METHOD DETAILS
    - Plant materials
    - CRISPR mutagenesis of CIK1/2/4 and CLV2
    - Columbia-0 x Landsberg-erecta hybrid clv2 population
    - Generation of binary vectors and transgenic lines
    - Photography and time-lapse imaging
    - Confocal microscopy
    - RNA Sequencing and Data Analysis
  - QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2020.10.008.

ACKNOWLEDGMENTS

We thank Yunde Zhao, the ABRC stock center, Paul Tarr, Joe Kieber, and Elena Shpak for sharing seeds, vectors, and reporter lines. We thank Tony D. Perdue, director of the UNC Biology Microscopy Core, for assistance with imaging. We thank Jamie Winshell and James Garzoni for lab and plant growth facility support. We thank UNC’s High-Throughput Sequencing Facility for sequencing services. We thank members of the Nimchuk lab for critical feedback on this project. This research was supported by a NIGMS-MIRA award from National Institutes of Health (R35GM119614) and the National Science Foundation (NSF) Plant Genome Research Program (PGRP) (IOS-1546837) to Z.L.N. D.S.J. is supported by an NSF Postdoctoral Research Fellowship in Biology through the PGRP (NSF no. 1906389).

AUTHOR CONTRIBUTIONS

D.S.J. designed/perform experiments, analyzed data, acquired funding for support, and wrote the manuscript. A.J. designed/perform experiments and analyzed data. K.R.V. performed Col-0 × Ler population experiments. Z.L.N. conceptualized the project and experiments, analyzed data, acquired funding, and wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: April 10, 2020
Revised: September 3, 2020
Accepted: October 5, 2020
Published: November 5, 2020

REFERENCES

1. Hallgrimsson, B., Green, R.M., Katz, D.C., Fish, J.L., Bernier, F.P., Roseman, C.C., Young, N.M., Cheverud, J.M., and Marcucio, R.S. (2019). The developmental-genetics of canalization. Semin. Cell Dev. Biol. 88, 67–79.
2. Bradley, D., Ratcliffe, O., Vincent, C., Carpenter, R., and Coen, E. (1997). Inflorescence commitment and architecture in Arabidopsis. Science 275, 80–83.
3. Reinhardt, D., Mandel, T., and Kuhlemeier, C. (2000). Auxin regulates the initiation and radial position of plant lateral organs. Plant Cell 12, 507–518.
4. Benková, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertová, D., Jürgens, G., and Friml, J. (2003). Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell 115, 591–602.

Current Biology 31, 220–227, January 11, 2021 225
function analysis with the PANTHER classification system (v.14.0). Nat. Protoc. 14, 703–721.
46. Sabatini, S., Beis, D., Wolkenfelt, H., Murfett, J., Guilfoyle, T., Malamy, J., Benfey, P., Leyser, O., Bechtold, N., Weisbeek, P., and Scheres, B. (1999). An auxin-dependent distal organizer of pattern and polarity in the Arabidopsis root. Cell 99, 463–472.
47. Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T., Offringa, R., and Jürgens, G. (2003). Efflux-dependent auxin gradients establish the apical-basal axis of Arabidopsis. Nature 426, 147–153.
48. Cheng, Y., Dai, X., and Zhao, Y. (2006). Auxin biosynthesis by the YUCCA flavin monoxygenases controls the formation of floral organs and vascular tissues in Arabidopsis. Genes Dev. 20, 1790–1799.
49. Krier, Z. (2009). AINTEGUMENTA and AINTEGUMENTA-LIKE6 act redundantly to regulate Arabidopsis floral growth and patterning. Plant Physiol. 150, 1916–1920.
50. Pnigge, M.J., Pitrat, M., Kadakia, N., Zhang, Y., Greenham, K., Szutz, W., Panidey, B.K., Bhosale, R.A., Bennett, M.J., Busch, W., and Estelle, M. (2020). Genetic analysis of the Arabidopsis TIR1/AFB auxin receptors reveals both overlapping and specialized functions. eLife 9, e54740.
51. Mashiguchi, K., Tanaka, K., Sakai, T., Sugawara, S., Kawaiha, H., Natsuno, M., Hanada, A., Yaeno, T., Shirasu, K., Yao, et al. (2011). The main auxin biosynthesis pathway in Arabidopsis. Proc. Natl. Acad. Sci. USA 108, 18512–18517.
52. Won, C., Shen, X., Mashiguchi, K., Zheng, Z., Dai, X., Cheng, Y., Kasahara, H., Kamiya, Y., Chory, J., and Zhao, Y. (2011). Conversion of tryptophan to indole-3-acetic acid by TRYPTOPHAN AMINOTRANSFERASES OF Arabidopsis and YUCCAs in Arabidopsis. Proc. Natl. Acad. Sci. USA 108, 18518–18523.
53. Lavy, M., and Estelle, M. (2016). Mechanisms of auxin signaling. Development 143, 3226–3229.
54. Björkman, T., and Pearson, K.J. (1998). High temperature arrest of inflorescence development in broccoli (Brassica oleracea var. italic a L.). J. Exp. Bot. 49, 101–106.
55. Anderson, R., Bayer, P.E., and Edwards, D. (2020). Climate change and the need for agricultural adaptation. Curr. Opin. Plant Biol. 56, 197–202.
56. Torii, K.U., Mitsukawa, N., Oosumi, T., Matsuura, Y., Yokoyama, R., Whitter, R.F., and Komeda, Y. (1996). The Arabidopsis ERECTA gene encodes a putative receptor kinase protein with extracellular leucine-rich repeats. Plant Cell 8, 735–746.
57. Kinoshita, A., Betsuyaku, S., Osakabe, Y., Muzzo, S., Nagawa, S., Stahl, Y., Simon, R., Yamaguchi-Shinozaki, K., Fukuda, H., and Sawa, S. (2010). RPK2 is an essential receptor-like kinase that transmits the CLV3 signal in Arabidopsis. Development 137, 3911–3920.
58. Yu, L.P., Miller, A.K., and Clark, S.E. (2003). POLTERGEIST encodes a protein phosphatase 2C that regulates CLAVATA pathways controlling stem cell identity at Arabidopsis shoot meristem and flower meristems. Curr. Biol. 13, 178–188.
59. Hicks, K.A., Millar, A.J., Carré, I.A., Somers, D.E., Straume, M., Meeks-Wagner, D.R., and Kay, S.A. (1996). Conditional circadian dysfunction of the Arabidopsis flowering time 3 mutant. Science 274, 790–792.
60. Hazak, O., Brandt, B., Cattaneo, P., Santiago, J., Rodriguez-Villalon, A., Hothorn, M., and Hardtke, C.S. (2017). Perception of root-active CLE peptides requires CORYNE function in the phloem vasculature. EMBO Rep. 18, 1387–1381.
61. Bilou, I., Xu, J., Wildwater, M., Willemsen, V., Paponov, I., Friml, J., Heidstra, R., Aida, M., Palme, K., and Scheres, B. (2009). The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis. Nature 439, 39–44.
62. Zhang, W., To, J.P., Cheng, C.Y., Schaller, G.E., and Kieber, J.J. (2011). Type-A response regulators are required for proper root apical meristem function through post-transcriptional regulation of PIN auxin efflux carriers. Plant J. 68, 1–10.
63. Vernoux, T., Brunoud, G., Farcoat, E., Morin, V., Van den Dalee, H., Legrand, J., Oliva, M., Das, P., Lamie, A., Wells, D., et al. (2011). The auxin signaling network translates dynamic input into robust patterning at the shoot apex. Mol. Syst. Biol. 7, 508.
64. Wisniewska, J., Xu, J., Seifertova, D., Brewer, P.B., Ruzicka, K., Bilou, I., Rouqué, D., Benková, E., Scheres, B., and Friml, J. (2006). Polar PIN localization directs auxin flow in plants. Science 312, 883.
65. Hendgans, C., Nimchuk, Z.L., and Kieber, J.J. (2017). indCAPS: a tool for designing screening primers for CRISPR/Cas9 mutagenesis events. eLife 6, e018406.
66. Pertea, M., Kim, D., Pertea, G.M., Leek, J.T., and Salzberg, S.L. (2016). Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. Nat. Protoc. 11, 1650–1667.
67. Liao, Y., Smyth, G.K., and Shi, W. (2013). The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. Nucleic Acids Res. 41, e108.
68. Risso, D., Schwartz, K., Sherlock, G., and Dudoit, S. (2011). GC-content normalization for RNA-seq data. BMC Bioinformatics 12, 480.
69. Risso, D., Ngai, J., Speed, T.P., and Dudoit, S. (2014). Normalization of RNA-seq data using factor analysis of control genes or samples. Nat. Biotechnol. 32, 896–902.
70. Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139–140.
71. Bray, N.L., Pimentel, H., Melsted, P., and Pachter, L. (2016). Near-optimal probabilistic RNA-seq quantification. Nat. Biotechnol. 34, 525–527.
72. Pimentel, H., Bray, N.L., Puente, S., Melsted, P., and Pachter, L. (2017). Differential analysis of RNA-seq incorporating quantification uncertainty. Nat. Methods 14, 687–690.
73. Thomas, P.D., Campbell, M.J., Kejarwal, A., Mi, H., Kilarik, B., Davenport, R., Diemer, K., Muruganujan, A., and Narechania, A. (2003). PANTHER: a library of protein families and subfamilies indexed by function. Genome Res. 13, 2129–2141.
74. Petersen, B.A., Haak, D.C., Nishimura, M.T., Teixeira, P.J., James, S.R., Dangl, J.L., and Nimchuk, Z.L. (2016). Genome-wide assessment of efficiency and specificity in CRISPR/Cas9 mediated multiple site targeting in Arabidopsis. PLoS ONE 11, e0162169.
75. Clough, S.J., and Bent, A.F. (1998). Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. 16, 735–743.
76. Ishihara, H., Sugimoto, K., Tarr, P.T., Tenman, H., Kadokura, S., Inui, Y., Sakamoto, T., Sasaki, T., Aida, M., Suzuki, T., et al. (2019). Primed histone demethylation regulates shoot regenerative competency. Nat. Commun. 10, 1786.
77. Clough, S.J., and Bent, A.F. (1998). Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. 16, 735–743.
78. Prunet, N., Jack, T.P., and Meyerowitz, E.M. (2016). Live confocal imaging of Arabidopsis flower buds. Dev. Biol. 419, 114–120.
79. Nimchuk, Z.L., and Perdue, T.D. (2017). Live imaging of shoot meristems on an inverted confocal microscope using an objective lens inverter attachment. Front. Plant Sci. 8, 773.
80. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., et al. (2012). Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682.
## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial and Virus Strains** | | |
| *E. coli* strain DH5α | Widely distributed | N/A |
| *E. coli* strain DB3.1 | Widely distributed | N/A |
| *Agrobacterium tumefaciens* strain GV3101 | Widely distributed | N/A |
| **Chemicals, Peptides, and Recombinant Proteins** | | |
| Propidium Iodide (PI) | Thermo Fisher | Cat#P1304MP |
| Murashige and Skoog (MS) Basal Salt | Sigma-Aldrich | Cat#M0529 |
| Micronutrient Solution | Sigma-Aldrich | Cat#M6752 |
| Methyl Salicylate | Sigma-Aldrich | Cat#K240020 |
| **Critical Commercial Assays** | | |
| Gateway LR Clonase II Enzyme mix | Thermo Fisher | Cat#11791020 |
| Gateway BP Clonase II Enzyme mix | Thermo Fisher | Cat#11789020 |
| pENTR/D-TOPO Cloning Kit | NEB | Cat#E1601S |
| Golden Gate Assembly Mix | NEB | Cat#E1601S |
| EZNA Plant RNA Kit | Omega Bio-tek | SKU:R6827-01 |
| RNase-free DNase | Omega Bio-tek | SKU:E1091-02 |
| Stranded mRNA-seq Kit | Kapa Biosystems | Cat#07962169001 |
| **Deposited Data** | | |
| Raw RNaseq data | This paper | SRA BioProject: PRJNA661065 |
| Code to analyze RNaseq data | This paper | https://github.com/NimchukLab |
| *Arabidopsis thaliana* TAIR10.1 reference genome | The Arabidopsis Information Resource (TAIR) | SRA BioSample: SAMN03081427 |
| *Arabidopsis thaliana* TAIR10 Transcriptome | EnsemblPlants | https://plants.ensembl.org/Arabidopsis_thaliana/Info/Index |

### Experimental Models: Organisms/Strains

| Arabidopsis thaliana: Col-0 ecotype | N/A | N/A |
| Arabidopsis thaliana: crn-10 | 20 | N/A |
| Arabidopsis: rlp10-1 | 20 | GABI_686A09 |
| Arabidopsis: er-105 | 56 | N/A |
| Arabidopsis: ify-1 | 27 | ABRC CS6228 |
| Arabidopsis: cv3-9 | 30 | N/A |
| Arabidopsis: dodeca-cle | 13 | N/A |
| Arabidopsis: cv1-101 | 67 | N/A |
| Arabidopsis: bam1-4 | 30 | N/A |
| Arabidopsis: bam2-4 | 30 | N/A |
| Arabidopsis: bam3-2 | 30 | N/A |
| Arabidopsis: pol-6 | 58 | N/A |
| Arabidopsis: elf3-1 | 59 | ABRC CS3787 |
| Arabidopsis: yuc1 | 48 | SALK_106293 |
| Arabidopsis: yuc4 | 48 | SM_3_16128 |
| Arabidopsis thaliana: Landsberg erecta (Ler) ecotype | N/A | N/A |
| Arabidopsis: crn-1 (Ler) | 17 | ABRC CS9853 |
| Arabidopsis: cv2-1 (Ler) | 16 | N/A |
| Arabidopsis: crn CRNpro::CRN-GFP | 20 | N/A |
| Arabidopsis: cv2 CLV2pro::CLV2-CITRINE | 60 | N/A |

(Continued on next page)
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Arabidopsis: Col-0 WUSp:YN7 | gift from Paul Tarr - Caltech | N/A |
| Arabidopsis: Col-0 DR5-GFP | | N/A |
| Arabidopsis: Col-0 DII-Venus | | N/A |
| Arabidopsis: Col-0 PIN1pro::PIN1-GFP | | N/A |
| Arabidopsis: crn WUSp:YN7 | This study | N/A |
| Arabidopsis: cik1-3 | This study | N/A |
| Arabidopsis: cik2-3 | This study | N/A |
| Arabidopsis: cik4-3 | This study | N/A |
| Arabidopsis: clv2-10 (Ler) | This study | N/A |
| Arabidopsis: clv2-11 (Ler) | This study | N/A |

**Oligonucleotides**

| Oligonucleotide | Sequence |
|-----------------|----------|
| cik1 Ball dCAPS F | CTGGTTTTTGTATGGTTCTTTGATATCTCTTCTGC TACACCTTCCT |
| cik1 Ball dCAPS R | GATAAAATTCAAGAAAAAAATTGAGATTTCCTCC |
| cik2 Ball dCAPS F | CATCTTTGTTTTGTTTTTTCCCCCTCCAGAG |
| cik2 Ball dCAPS R | CAAACAAAATAATCTTACACTTGTCGAAG |
| cik4 Ball dCAPS F | AACAAGATGAAAGATGAGAAAGAGGCCTT |
| cik4 Ball dCAPS R | CTCACAGAGAAACCACACACTTC |
| ANT Pro F | AINTEGUMENTA promoter for pANTpro:GTW |
| ANT Pro R | AINTEGUMENTA promoter for pANTpro:GTW |
| YUC1 F | YUCCA1 CDS for entry vector |
| YUC1 R | YUCCA1 CDS for entry vector |

**Recombinant DNA**

| Recombinant DNA | Source |
|-----------------|--------|
| Plasmid ANTp:YUC1 | This study | N/A |
| Plasmid CRNp:YN7 | This study | N/A |
| Plasmid pENTR-GG | This study | N/A |

**Software and Algorithms**

| Software | Source |
|----------|--------|
| Fiji/ImageJ v.2.0.0-rc-69/1.52u | National Institutes of Health [https://imagej.net/Fiji](https://imagej.net/Fiji) |
| Zen Microscope Imaging Software | Zeiss [https://www.zeiss.com/corporate/int/home.html](https://www.zeiss.com/corporate/int/home.html) |
| NIS-Elements Imaging Software | Nikon [https://www.microscope.healthcare.nikon.com/products/software](https://www.microscope.healthcare.nikon.com/products/software) |
| GIMP GNU image manipulation program 2.10.4 | GIMP [https://www.gimp.org/](https://www.gimp.org/) |
| Lapse-it iOS 12.3.1 | Apple app store [http://www.lapseit.com/](http://www.lapseit.com/) |
| Prism v.8.3.2 | GraphPad [https://www.graphpad.com/](https://www.graphpad.com/) |
| indCAPS | [http://indcaps.kieber.cloudapps.unc.edu/](http://indcaps.kieber.cloudapps.unc.edu/) |
| HISAT2 2.2.0 | [https://github.com/DaehwanKimLab/hisat2](https://github.com/DaehwanKimLab/hisat2) |
| Subread 1.5.1 | [https://github.com/DeskGen/subread](https://github.com/DeskGen/subread) |
| EDASEq 2.22.0 | [https://github.com/drisso/EDASEq](https://github.com/drisso/EDASEq) |
| RUVseq 1.22.0 | [https://github.com/drisso/RUVSeq](https://github.com/drisso/RUVSeq) |
| EdgeR 3.33.0 | [https://gist.github.com/jdblischak/11384914](https://gist.github.com/jdblischak/11384914) |
| Kallisto 0.44.0 | [https://github.com/pachterlab/kallisto](https://github.com/pachterlab/kallisto) |
| Sleuth 0.33.0 | [https://github.com/pachterlab/sleuth](https://github.com/pachterlab/sleuth) |
| GO term analysis Panther | [http://geneontology.org/](http://geneontology.org/) |

**Other**

| Other | Source |
|-------|--------|
| Small glass-bottom Petri dish | MatTek Corporation Cat#P35G-1.5-10-C |
RESOURCE AVAILABILITY

Lead Contact
Information and resource/reagent requests should be directed to and will be fulfilled by the Lead Contact, Zachary Nimchuk (zackn@email.unc.edu).

Materials Availability
Plasmids and Arabidopsis lines made during this study are freely available to academic researchers through the Lead Contact.

Data and Code Availability
Raw RNaseq data described in this study has been deposited into the NCBI Short Read Archive (SRA) database under the BioProject PRJNA661065. Code used to analyze gene expression data can be found on the Nimchuk Lab GitHub page (https://github.com/NimchukLab). All other source data obtained throughout the course of this work have not been deposited to any public repository but are available upon request from the Lead Contact.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Arabidopsis thaliana accession Columbia (Col-0) was used as our primary model system throughout this work. Some phenotypic comparisons were also made with the accession Landsberg-erecta (Ler), as noted.

Plant growth conditions
Seeds were sterilized and plated on half-strength MS (Murashige-Skoog) media buffered with MES, pH 5.7. Plates were stratified in the dark at 4°C for 2 days and then moved to constant light in a custom-built grow room with environmental control (temperature maintained between 21-25°C), or in a Percival growth chamber (AR-75L3) when growing at a specified temperature. After 7-10 days, seedlings were transplanted to soil (Metro-Mix 360/sand/perlite supplemented with Marathon pesticide and Peter's 20:20:20 [N:P:K] at recommended levels) and then placed back into the chamber they were germinated in and grown until flowering for phenotypic analysis.

METHOD DETAILS

Plant materials
Mutant alleles used in this study are all in the Col-0 background, unless otherwise noted, and information for each is as follows: cm (cm-10),20 cm-1 in Ler,17 clv2 in Col-0 (clv2-10-1),31 clv2-1 in Ler,18 erecta (er-105),26 ify (ify-1; ABRC CS6228),27 clv1 (clv1-101),57 bam1 (bam1-4),30 bam2 (bam2-4),30 bam3 (bam3-2),30 pol (pol-6),30 clv3 (clv3-9),30 dodeca-cle (clv3-9; CRISPR alleles of: cle9, cle10, cle11, cle12, cle13, cle18, cle19, cle20, cle21, cle22, cle45),13 elf3 (elf3-1; ABRC CS3787),59 yuc1 (SALK_106293),10 and yuc4 (SM_3_16128).18 Information for previously published transgenic complementation and reporter lines used are as follows: CRNpro::CRN-GFP in cm,20 CLV2pro::CLV2-CITRINE in clv2,62 WUSpro::Ypet-N7 in Col-0 (gift from Paul Tarr – Caltech), DR5pro::GFP in Col-0,61,62 DII-Venus in Col-0,53 PIN1pro::PIN1-GFP in Col-0.53 The DR5pro::GFP, DII-Venus, and PIN1pro::PIN1-GFP lines were all crossed into cm (cm-10) for analysis of auxin signaling levels in cm shoots compared to WT.

CRISPR mutagenesis of CIK1/2/4 and CLV2
The pCUT vector system was used to simultaneously create cik1, cik2 and cik4 mutations in the Col-0 background (cik1-3, cik2-3, cik4-3), making the cik1/2/4 higher order mutant.74 Similarly, the pCUT system was used to make multiple unique mutations in clv2 in the Ler background (clv2-10, clv2-11). A pENTR-D/TOPO entry vector was modified for golden gate cloning by TOPO cloning in Pmel and Bsal cut sites (pENTR-GG). pENTR-GG was used to clone tandem cassettes (cik1/2/4) or a single cassette (clv2) of the U6 promoter, 20 bp guide sequence, and the gRNA scaffold. These guide constructs were then gateway cloned into pCUT4GW vector that expresses Cas9 from the UBQUITIN10 promoter. Hygromycin resistant plants were selected in the T1 generation and sequenced to detect editing of the target genes; CIK1 (AT1G60800), CIK2 (AT2G23950), and CIK4 (AT5G45780) or CLV2 (AT1G65380). To make stable cik1/2/4, T2 seed from editing cik1/2/4 lines were grown for 2 weeks under standard conditions, heat shocked for 12-24 hours at 35°C, and screened for stable edits using caps primers designed by the indcaps webtool (http://indcaps.kieber.cloudapps.unc.edu).65 Cas9 was segregated out of plants that had stable homozygous mutations in CIK1/2/4. To make stable clv2 alleles in Ler, T2 seed, collected from single branches of T1 plants that had the clv2 carpel phenotype, were grown in a Percival growth chamber at 16°C (see above), screened for stable mutations while segregating Cas9 out prior to phenotypic analysis.

Columbia-0 X Landsberg-erecta hybrid clv2 population
Null clv2 lines in the Col-0 (clv2-10) and Ler (clv2-1) backgrounds were crossed to generate a hybrid population with fixed clv2 mutations. Segregation of flower termination traits was assessed in the F2 generation. Phenotypic ratios were compared to expected values of a single causative locus (1:2:1) and digenic semi-dominant modifiers (7:6:3) using Chi-square analysis (Microsoft Excel v.16.40) to determine the underlying genetic complexity of the ecotypic variability of clv2 termination.
Generation of binary vectors and transgenic lines

New transgenic lines were generated using floral-dip transformation of binary vectors into specified backgrounds.76 pWUSpro::Ypet-N7 (gift from Paul Tarr – Caltech; cloning methods as in27 but with Ypet-N7) was transformed directly into crn and the transgene was selected on ½ MS plates with Kanamycin. Four independent lines were selected for downstream analysis with the WUS expression domain being equivalent across all lines imaged (see imaging methods for data acquisition details). For pCRNpro::Ypet-N7, a pENTR-D Ypet-N7 (2xYpet-N7 fusion) entry vector was recombined by LR reaction into the pCRNpro::Gateway binary vector in the pMOA33 backbone. For pANTpro::YUC1, the 4 kb S’ANT promoter was amplified from genomic DNA, and cloned up stream of a gateway::OCS terminator cassette in the pCR2.1 shuttle vector, and sequence verified. The resulting ANTPro::Gateway::OCS cassette was then mobilized as a NotI fragment in to the pMOA33 binary vector backbone as previously described to create pANTpro::GTW.75 The YUC1 CDS was amplified from Arabidopsis Col-0 cDNA and cloned into the pENTR-D topo vector and sequence verified. This vector was then recombined in an LR reaction into pANTpro::GTW to create the pANTpro::YUC1.

Photography and time-lapse imaging

Unless specified differently below, young inflorescences were staged at similar developmental time points and photographed using a Canon EOS Rebel T5 equipped with a Tokina 100mm f/2.8 AT-X M100 AF Pro D macro lens. Images were edited for brightness and contrast using Gimp v2.10.4 (https://www.gimp.org/). Young inflorescences from Col-0, crn, clv2, crn clv2, clk1/2/4, yuc1/4, and clv2 yuc1/4 (Figures 1A’–1D’, 2B, 4E, 4F, 4H, and 4I) were imaged using a Zeiss Stemi 2000-C stereo microscope equipped with a Zeiss AxioCam 105-color digital camera and acquired using Zeiss ZEN software. Time-lapse imaging was done using the Lapse-it (http://www.lapseit.com/) app on an iPhone 6 operating iOS 12.3.1. Imaging began with ~3-week-old Col-0 (WT) and crn plants growing in our custom-built grow room. A single image was taken every 30 minutes over the course of 10-12 days. The final movie was compiled at 30 frames per sec and exported into iMovie where it was cropped to show only two plants for comparison of early flowering phenotypes.

Confocal microscopy

Live imaging of inflorescence meristems (IMs) was performed as previously reported.25,78 We used either an inverted Zeiss 710 (for: propidium iodide (PI) stained Crn-0, crn, CRNpro::Ypet-N7, WUSpro::Ypet-N7, DR5pro::GFP, DII-Venus, and PINIpro::PIN1-GFP) or a Zeiss 880 (for CRNpro::CRN-GFP and CLV2pro::CLV2-CITRINE) confocal laser scanning microscope equipped with an inverter (setup described in78). Young IMs were dissected immediately following floral transition in order to analyze expression patterns and reporter levels at the same developmental stage as clv2/crn flower primordia termination. When analyzing reporter status in recovered IMs of crn, shoots were dissected at a later time point after flower buds were visibly developing again. All IMs were briefly (~5 mins for WT and ~15 mins for crn) stained with PI (final concentration of 50 μg/mL for WT shoots and 150 μg/mL for crn) on ice and placed into a Petri dish with 2% agarose (w/v) and immersed in cold water, ensuring no bubbles formed around the IM.78 IMs were imaged using a W Plan-APoCHROMAT 40X (NA = 1.0) water dipping objective. Laser excitation and detected emission ranges were as follows: PI only – laser 561nm diode, PI channel 600-750nm; Ypet/Venus markers with PI – 514nm argon laser, Ypet channel 520-581nm, PI channel 655-758nm; GFP markers (on Zeiss 710) with PI – 488nm argon laser, GFP channel (GaAsP detector) 490-550nm, PI channel (GaAsP detector) 520-581nm, PI channel 655-758nm; GFP markers (on Zeiss 880) with PI – 488nm argon laser, GFP channel (GaAsP detector) 490-550nm, PI channel (GaAsP detector) 565-610nm; Citrine on Zeiss 880 with PI – 514nm argon laser, Citrine channel (GaAsP detector) 519-550nm, PI channel (GaAsP detector) 565-610nm. Whole IMs were imaged as a z stack series with a step size optimized for three-dimensional reconstruction of data. All images comparing reporter levels in different backgrounds were obtained with identical specifications: DR5pro::GFP, DII-Venus, and PINIpro::PIN1-GFP in both WT and crn shoots. Live inflorescence micrographs were all post-processed using ZEN (Zeiss) for three-dimensional reconstructions of IMs and Fiji/ImageJ v.2.0.0-rc-69/1.52u (National Institutes of Health)80 for single scan images as well as axial views of IMs. Channels corresponding to PI staining in crn IMs were almost always gamma corrected (0.8) as penetrance of this fluorescent dye in enlarged crn shoots was sometimes limited. DR5pro::GFP fluorescence quantification comparing WT and crn IMs was done as follows using Fiji/ImageJ v.2.0.0-rc-69/1.52u. Z stacks were rendered as maximum intensity projections of the GFP channel only using data from the entire IM. A region of interest (ROI) was drawn around each of the first 3 developing primordia (Identified as the 3 early primordia with the highest GFP intensities). GFP levels were quantified for each ROI, normalized to the final area of each ROI, and then averaged together to obtain a single value for WT and crn IMs. DII-Venus quantification was done using Fiji/ImageJ v.2.0.0-rc-69/1.52u. Due to rapid photobleaching of the Venus fluorescent reporter, single scan images were taken in the L1 layer of WT and crn IMs prior to z stack scans and used for direct comparison. The percentage of L1 cells with the Venus reporter were determined across all imaged IMs, with WT IMs never having reporter in this layer.

Whole shoot reconstruction of crn during termination (Figure 1H) was done using fixed and cleared tissue, imaging structural auto-fluorescence (as in17). Young crn IM were fixed in FAA (2% formaldehyde, 5% acetic acid, 60% ethanol (w/v)) at 4°C overnight and then dehydrated in a graded ethanol series (70%, 80%, 95% and 100%) for 30 minutes each at room temperature. Tissue was then cleared overnight in methyl salicylate (catalog no. M6752; Sigma Aldrich) and placed in a small glass-bottom Petri dish (catalog no. P35G-1.5-10-C; MatTek Corporation) and imaged on a Zeiss 710 CLSM using a Plan-APoCHROMAT 10X (NA = 0.45). Autofluorescence was detected using a 488nm argon laser for excitation and combining two channels for emission detection; Channel 1 ~ 504-597nm and channel 2 ~ 629-731nm. Data was gathered as a z stack and three-dimensional reconstruction was done in Nikon NIS-Elements as a shaded render of both channels combined in grayscale.
RNA Sequencing and Data Analysis
Total RNA was isolated using the EZNA Plant RNA kit (Omega Bio-tek) from 45-50 inflorescence meristems for three biological replicates of both Col-0 and crn (crn-10) plants. RNA was treated with RNase-free DNase (Omega Bio-tek). Approximately 1.5 ug RNA was used as input material for library preparation, using the Stranded mRNA-Seq kit (Kapa Biosystems) at the High-throughput Sequencing Facility at UNC Chapel hill. 50bp paired-end reads were generated on the NovaSeq 6000 sequencer (illimina) with a read depth of 23-35 million reads per biological replicate. Trimmed raw data was aligned to the A. thaliana genome (TAIR10.1) using HISAT2 version 2.2.0 and reads were counted using Subread version 1.5.1. Subsequent analysis was performed on RStudio with reads normalized using EDASeq version 2.22.0 and RUVseq version 1.22.0 (upper quartile normalization) and differentially expressed genes were identified with a p value < 0.001 using EdgeR version 3.33.0. These top 460 DEGs were used for GO term analysis from Panther. To obtain the average TPM counts of auxin biosynthetic genes, reads were pseudoaligned to the Arabidopsis transcriptome (TAIR10 from https://plants.ensembl.org) using Kallisto version 0.44.0 and quantified with Sleuth version 0.33.0.

Quantification and Statistical Analysis
Quantitative data from all experiments was compiled and analyzed in GraphPad Prism v.8.3.2. We performed at least two biological replicates for each experiment ensuring consistent results (sample sizes indicated in figure legends). For comparisons of flower termination across conditions, genotypes and/or transgenic lines (Figures 1E, 2C, 2G, 3G, 3L, 4G, 4H, S1I, S2C, S2D, S2I, and S2J), only % flower primordia termination (as defined in the paper) was compared across samples using a non-parametric Kruskal-Wallis and a Dunn’s multiple comparison test correction where significance was defined as p value < 0.05. For comparisons of carpel number across genotypes (Figures 2D, 2H, 3M, and S1J), 10 consecutive flowers on the primary inflorescence were counted, starting after the recovery phase in crn, clv2, and cik1/2/4 while starting at the 11th flower in genotypes that had no flower primordia termination or partially suppressed flower primordia termination (Col-0, Ler, clv2-1, cm-1, pol, cm pol, elf3, and cm elf3). Carpil number was compared statistically using a non-parametric Kruskal-Wallis and a Dunn’s multiple comparison test correction where significance was defined as p value < 0.05. For comparison of DR5pro::GFP levels, WT and crn IM values were compared statistically using an unpaired t test, where the p value = 0.0007. Sample size (n) for all analyses can be found with each figure in the legend and refers to the number of individual plants analyzed.