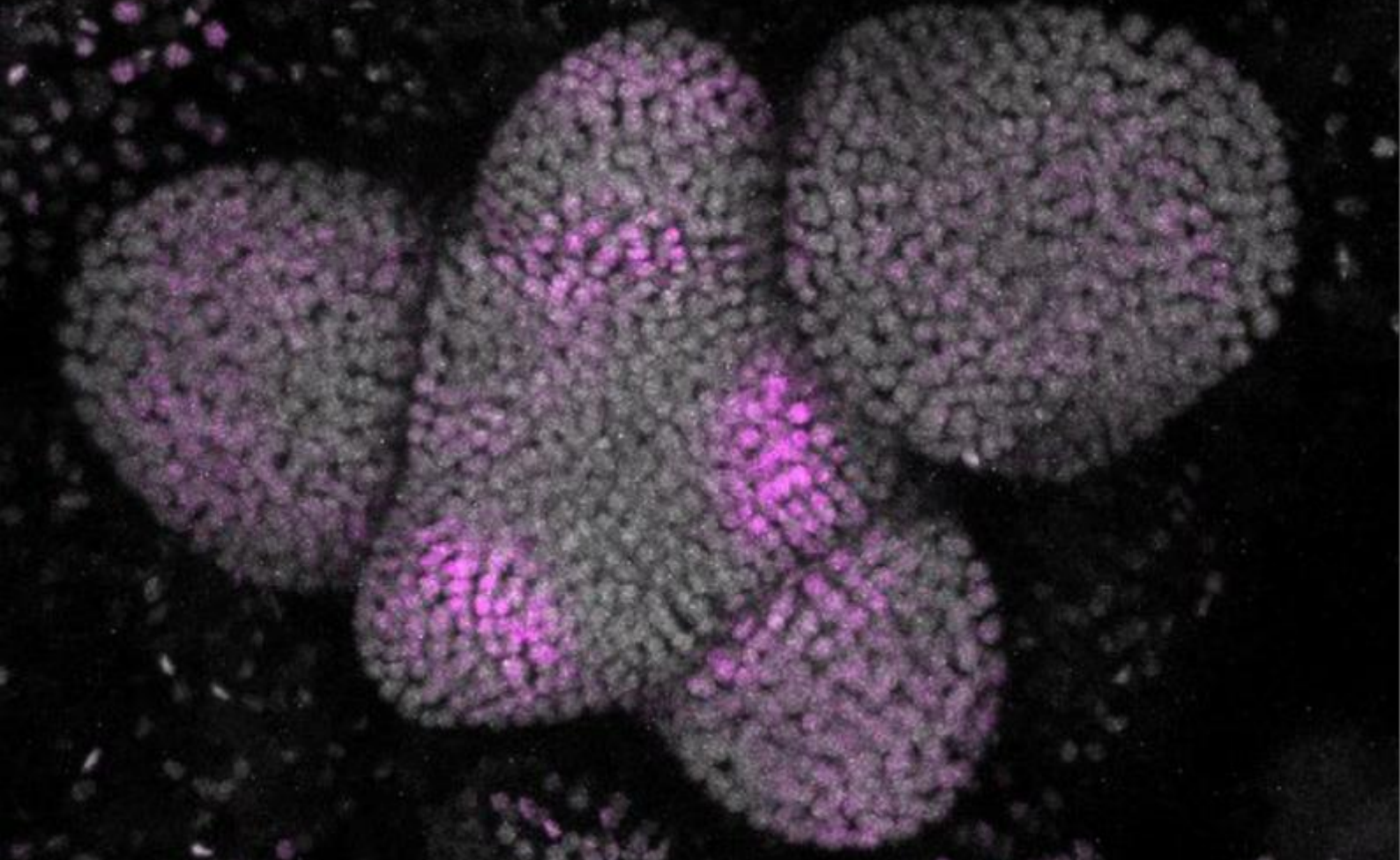
UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Study of hormone signaling and the mode of action of FRUITFULL during proliferative arrest in *Arabidopsis thaliana*

PhD Thesis

Irene González Cuadra



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Study of hormone signaling and the mode of action of FRUITFULL during proliferative arrest in *Arabidopsis thaliana*

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ABSTRACT

The regulation of both the initiation and termination of the flowering period is essential to ensure reproductive success. In monocarpic plants, such as *Arabidopsis thaliana* and many crop species (e.g., legumes or solanaceae spp.), this regulation is particularly important since they undergo a single reproductive cycle in their lifetime. The reproductive phase initiates with the floral transition, during which the shoot apical meristem (SAM) starts producing flowers, and ends with proliferative arrest (PA), marked by the cessation of all inflorescence meristem (IM) activity (IM arrest) and floral bud development (floral arrest). While the start of reproductive development has been deeply studied, the mechanisms controlling its termination remain poorly understood, despite the critical role of PA in optimizing the size and viability of the offspring before plant death.

In recent years, substantial research has been conducted to understand the environmental, hormonal, genetic, and other signaling factors involved in PA. It is known that fruit and seed signals play a key role in promoting PA. At the genetic level, PA is controlled by the *FRUITFULL-APETALA2* (*FUL-AP2*) pathway. It has been proposed that FUL accumulates in the inflorescence along the flowering period and negatively regulates *AP2*. This repression of *AP2* leads to the downregulation of *WUSCHEL* (*WUS*), a key regulator of stem cell maintenance, ultimately resulting in IM arrest. Additionally, hormones are crucial in PA control. Cytokinin (CK) response needs to be repressed in the SAM for its arrest as well as for floral arrest. Furthermore, FUL represses CK-related pathways to promote this process. Two modes of action of FUL can be distinguished during PA. First, FUL, together with additional unknown factors, contribute to the repression of the CK-related events (decline phase). Then, FUL completely blocks these CK-related events (shutdown phase). On the other hand, abscisic acid (ABA) promotes floral arrest at the end of flowering, and auxin has been proposed to act as an intermediate factor in fruits/seeds derived signals that promote floral arrest.

In this work, we aim to extend the current knowledge about the molecular mechanisms and specific factors that regulate PA. In particular, we have

visualized with high spatio-temporal resolution changes in auxin biosynthesis, transport and response and have analyzed the effect of auxin content modifications in the SAM on PA. Our results suggest that PA is tightly regulated by local auxin biosynthesis as well as by auxin transport and response. These auxin-related processes must be repressed in the SAM for its arrest. Furthermore, auxin and CK pathways must be simultaneously and coordinately repressed in the SAM during PA.

Moreover, we have characterized the spatial pattern of FUL within the SAM at advanced stages of the flowering period. Our findings suggest that increased nuclear localization of FUL during the decline and shutdown phases may be necessary for its function and thus, for PA control. Furthermore, significant gene expression changes predominantly occur during these two phases. Our data also indicate that FUL controls meristem activity and PA, both independently or through AP2, by positively regulating ABA-related genes, while repressing CK-, auxin- and jasmonic acid (JA)-related genes. Notably, JA content and JA signaling decrease in the apex during PA, suggesting that JA may act as a negative regulator of this process.

Together, these findings provide a comprehensive view of the molecular mechanisms and hormonal interactions underlying PA, highlighting the central role of FUL in orchestrating this process.

RESUMEN

La regulación tanto del inicio como de la finalización del periodo de floración es esencial para garantizar el éxito reproductivo. En plantas monocárpicas, como *Arabidopsis thaliana* y otras especies cultivadas, como leguminosas o solanáceas, esta regulación es particularmente importante ya que solo experimentan un único ciclo reproductivo a lo largo de su vida. La fase reproductiva se inicia con la transición floral, cuando el meristemo apical del tallo (SAM) comienza a producir flores, y finaliza con la parada proliferative (PA), marcada por el cese de la actividad de todos los meristemos inflorescentes (IM) y el desarrollo de las yemas florales (parada floral). Mientras que el inicio del desarrollo reproductivo ha sido ampliamente estudiado, los mecanismos que controlan su terminación siguen siendo poco conocidos, a pesar de su papel crítico en la optimización del tamaño y la viabilidad de la descendencia antes de la muerte de la planta.

En los últimos años, se ha avanzado considerablemente en la compresión de factores ambientales, hormonales, genéticos y de señalización implicados en el PA. Se sabe que la producción de frutos y semillas juegan un papel clave en el PA. A nivel genético, el PA está controlado por la ruta *FRUITFULL-APETALA2* (*FUL-AP2*). Se ha propuesto que FUL se acumula en la inflorescencia a lo largo del periodo de floración regulando negativamente a *AP2*, lo que promueve la represión de *WUSCHEL* (*WUS*), un regulador clave en el mantenimiento de las células madre, dando lugar a la parada del IM. Además, las hormonas son cruciales en el control del PA. La respuesta de las citoquininas (CKs) necesita ser reprimida en el SAM para su detención como para la parada floral. A su vez, FUL reprime las rutas relacionadas con las CK, promoviendo el PA. Durante el PA, pueden distinguirse dos modos de acción de FUL. Primero, FUL, junto con factores adicionales, contribuye a la represión de los eventos relacionados con las CK (fase de declive). Luego, FUL bloquea completamente estos eventos (fase de parada) Por otro lado, el ácido abscísico (ABA) promueve la parada floral al final de la floración y se ha propuesto que las auxinas actúen como un

factor intermediario de las señales derivada de los frutos y semillas que promueve la parada floral.

En este trabajo pretendemos ampliar el conocimiento actual sobre los mecanismos moleculares que regulan el PA. En particular, hemos visualizado con alta resolución espacio-temporal los cambios en la biosíntesis, transporte y respuesta de las auxinas y hemos analizado el efecto de modificaciones en el contenido de las auxinas en el SAM sobre el PA. Nuestros resultados sugieren que el PA está estrechamente regulado por la biosíntesis local de auxinas así como por su transporte y respuesta. Estos procesos relacionados con las auxinas tienen que reprimirse en el SAM para su parada. Además, las rutas relacionadas con auxinas y CKs deben reprimirse simultánea y coordinadamente en el SAM durante el PA.

Por otro lado, hemos caracterizado el patrón espacial de FUL en el SAM en fases avanzadas del periodo de floración. Nuestros resultados sugieren que un incremento en la localización nuclear de FUL durante las fases de declive y parada podría ser necesaria para su función, y con ello el control del PA. Además, cambios significativos en la expresión génica ocurre predominantemente durante estas dos fases. Nuestros datos indican que FUL controla la actividad del meristemo y el PA, de manera independiente o a través de AP2, regulando positivamente genes relacionados con el ABA, mientras que reprime genes relacionados con CKs, auxinas y el ácido jasmónico (JA). En particular, la señalización y el contenido de JA disminuyen durante el PA, lo que sugiere que el JA puede actuar como un regulador negativo de este proceso.

En conjunto, estos resultados proporcionan una visión completa de los mecanismos moleculares y las interacciones hormonales que subyacen al PA, destacando el papel central de FUL en la regulación de este proceso.

RESUM

La regulació tant de l'inici com de la finalització del període de floració és essencial per a garantir l'èxit reproductiu. En plantes monocàrpiques, com *Arabidopsis thaliana* i altres espècies cultivades, com ara lleguminoses o solanàcies, esta regulació és especialment important, ja que només experimenten un únic cicle reproductiu al llarg de la seua vida. La fase reproductiva s'inicia amb la transició floral, quan el meristem apical de la tija (SAM) comença a produir flors, i finalitza amb la parada proliferativa (PA), caracteritzada pel cessament de l'activitat de tots els meristemes inflorescents (IM) i el desenvolupament de les gemmes florals (parada floral). Mentres que l'inici del desenvolupament reproductiu ha sigut àmpliament estudiat, els mecanismes que controlen la seua terminació continuen sent poc coneguts, malgrat el seu paper crític en l'optimització de la grandària i la viabilitat de la descendència abans de la mort de la planta.

En els darrers anys, s'ha avançat considerablement en la compressió dels factors ambientals, hormonals, genètics i de senyalització implicats en el PA. Se sap que la producció de fruits i llavors juga un paper clau en el PA. A nivell genètic, el PA està controlat per la ruta *FRUITFULL-APETALA2* (*FUL-AP2*). S'ha proposat que FUL s'acumula en la inflorescència al llarg del període de floració regulant negativament *AP2*, cosa que promou la repressió de *WUSCHEL* (*WUS*), un regulador fonamental en el manteniment de les cèl·lules mare, donant lloc a la parada de l'IM. A més, les hormones són crucials en el control del PA. La resposta de les citoquinines (CKs) necessita ser reprimida en el SAM tant per a la seua parada com per a la parada floral. Per altra banda, FUL reprimeix les rutes relacionades amb les CK per tal de promoure aquest procés. Durant el PA es poden distingir dos modes d'acció de FUL. En primer lloc, FUL, juntament amb altres factors encara desconeguts, contribueix a la repressió dels esdeveniments relacionats amb les CK (fase de declivi). Posteriorment, FUL bloqueja completament aquestos esdeveniments (fase de parada). D'altra banda, l'àcid abscísic (ABA) promou la parada floral al final de la floració, i s'ha proposat que les auxines actuen com a factors intermediaris en les senyals derivades dels fruits i llavors que promouen aquesta parada floral.

Amb este treball pretenem ampliar el coneixement actual sobre els mecanismes moleculars que regulen el PA. En particular, hem visualitzat amb alta resolució espai-temporal els canvis en la biosíntesi, transport i resposta de les auxines i hem analitzat l'efecte de modificacions en el contingut de les auxines en el SAM sobre el PA. Els nostres resultats suggerixen que el PA està estretament regulat per la biosíntesi local d'auxines, així com pel seu transport i resposta. Aquests processos relacionats amb les auxines han de ser reprimits en el SAM perquè aquest entre en parada. A més, les rutes relacionades amb auxines i CK han de ser reprimides simultàniament i de manera coordinada en el SAM durant el PA.

D'altra banda, hem caracteritzat el patró espacial de FUL en el SAM en fases avançades del període de floració, Els nostres resultats suggereixen que un augment en la localització nuclear de FUL durant les fases de declivi i parada pot ser necessari per a la seua funció i, per tant, per al control del PA. A més, els canvis significatius en l'expressió gènica es produeixen predominantment durant aquestes dues fases. Les nostres dades indiquen que FUL controla l'activitat meristemàtica i el PA, de manera independent o a través d'*AP2*, regulant positivament gens relacionats amb el ABA, mentres que reprimix gens relacionats amb CKs, auxines i l'àcid jasmònic (JA). En particular, la senyalització i el contingut de JA disminueixen durant el PA, cosa que suggereix que el JA pot actuar com un regulador negatiu d'este procés.

En conjunt, estos resultats proporcionen una visió completa dels mecanismes moleculars i les interaccions hormonals que subjauen al PA, destacant el paper central de FUL en la regulació d'este procés.

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ABBREVIATIONS

%: Percentage

µg: Micrograms

µL: Microliter

µm: Micrometres

µM: Micromolar

A. tumefaciens: Agrobacterium Tumefaciens

ABA: Abscisic acid

ABI: ABA INSENSITIVE

AGL: AGAMOUS-LIKE

AHG: ABA-HYPERSENSITIVE GERMINATION

AOS: ALLENE OXIDE SYNTHASE

AP: APETALA

Arabidopsis: *Arabidopsis thaliana*

ARF: AUXIN RESPONSE FACTOR

ARR: ARABIDOPSIS RESPONSE REGULATOR

Aux/IAA: Auxin/INDOLE-3-ACETIC ACID INDUCIBLE

BAP: N6-benzylaminopurine

BASTA: Glufosinate ammonium

bHLH: Basic Helix-Loop-Helix

BRC1: BRANCHED1

CDK: CYCLIN-DEPENDENT KINASE

ChIP: Chromatin Immunoprecipitation

CK(s): Cytokinin(s)

CKX: CYTOKININ OXIDASE

CLV: CLAVATA

COI: CORONATINE INSENSITIVE

Col-0: Columbia ecotype

CRF: CYTOKININ RESPONSE FACTOR

C-termius: Carboxy-terminus

CYC: CYCLIN

CYP: CYTOCHROME P450

dab: Day after bolting

dad: Day after defruiting

DAO: DIOXYGENASE FOR AUXIN OXIDATION

DEG(s): Differentially expressed gene(s)

dot: Day of treatment

Dex: Dexamethasone

DRN: DORNROSCHEN

E. coli: Escherichia Coli

et al.: And others

FC: Fold change

FUL: FRUITFULL

GA(s) Gibberellin(s)

GA20ox: GIBBERELLIN 20-OXIDASE

GA2ox: GIBBERELLIN 2-OXIDASE

GFP: Green Fluorescent Protein

GID: GIBBERELLIC ACID INSENSITIVE DWARF

GO Term: Gene Ontology Term

HAI2: HIGHLY ABA-INDUCED PP2C GENE

HB: HOMEOBOX-PROTEIN

IM: Inflorescence meristem

JA: Jasmonic acid

JAM: JA-ASSOCIATED MYC2-LIKE

JAR1: JASMONATE RESISTANT1

JAZ: JASMONATE ZIM-DOMAIN

JMT: JA CARBOXYL METHYLTRANSFERASE

JOX: JASMONATE-INDUCED OXYGENASE2

JR1: JACALIN-LECTIN LIKE 1

kb: Kilobases

KMD: KISS ME DEADLY

L*er*: Landsberg ecotype

LOX: LIPOXYGENASES

M: Molar

MeJA: Methyl jasmonate

mg: Milligrams

miR172: MicroRNA172

miRNA: MicroRNA

ML: Meristem layer

mL: Millilitres

mM: Millimolar

MP: MONOPTEROS

MPK: MITOGEN-ACTIVATED PROTEIN KINASE

MS: Murashige and Skoog

NAC: NAM/ATAF/CUC

NCED: 9-CIS-EPOXICAROTENOIR DIOXIGENASE

nm: Nanometres

ºC: Degree Celsius

OC: Organizing center

OPR: 12-OXOPHYTODIENOATE-REDUCTASE

PA: Proliferative arrest

PIN: PIN-FORMED

P-value: P-adjusted value

PZ: Peripheral zone

RAM: Root apical meristem

RGL: RGA-LIKE

RNA: Ribonucleic acid

RNase: Ribonuclease

RNA-seq: RNA sequencing

RP: RIBOSOMAL PROTEIN

RZ: Rib zone

SAG; SENESCENCE-ASSOCIATED GENE

SAM: SHOOT APICAL MERISTEM

SAUR: SMALL AUXIN UPREGULATED

SMZ: SCHLAFMÜTZE

SNZ: SCHNARCHZAPFEN

SOC: SUPPRESSOR OF OVEREXPRESSION OF CONSTANT

SPL: SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE

STM SHOOT MERISTEMLESS

T-DNA: Transfer DNA

TEM: TEMPRANILLO

TF(s): Transcription Factor(s)

TOE: TARGET OF EAT

TPMs: Transcripts Per Kilobase Million

TAA1: TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS

TAR: TRYPTOPHAN AMINOTRANSFERASE RELATED

TCSn: Two-Component signaling Sensor new

v/v: Volume per volume

VENUS Variant of YFP

vs.: versus

VSP: VEGETATIVE STORAGE PROTEIN

wab: Week after bolting

wad: Week after defruiting

wot: Week of treatment

wt: Wild-type

WUS: WUSCHEL

YFP: Yellow Fluorescent Protein

YPet: Variant of YFP

YUC: YUCCA

General introduction

GENERAL INTRODUCTION

Reproductive strategies in Angiosperms: monocarpic plants

Throughout evolutionary history, both plants and animals have developed different life strategies to adapt to their environment and ensure an optimal number of descendants, allowing the survival of the species.

Angiosperms, or flowering plants, have evolved two strategies to ensure reproductive success: semelparity and iteroparity. These strategies are based on the number of flowering events plants undergo in their lifetime. Iteroparous plants, also known as polycarpic, reproduce multiple times during their lifetime. In contrast, semelparous plants, or monocarpic, complete a single reproductive cycle before the senescence and death of the whole plant (Bleeckerl *et al*., 1997; Amasino *et al*., 2009; Albani & Coupland 2010).

When environmental conditions are favourable, plants transition to reproductive development and meristems begin to produce flowers. However, the flowering period must be terminated to promote offspring viability and regulate optimal progeny size, an important ecological trait, thereby ensuring reproductive success.

The initiation of reproductive development, or floral transition, has been extensively studied, yielding a wealth of information regarding the inputs and regulatory pathways involved in this process (Kinoshita *et al*., 2020; Maple *et al*., 2024). In contrast, the end of flowering remains less well understood. Only in recent years, various studies have provided insights into the environmental, genetic, hormonal and other signaling factors that regulate this process (Wuest *et al*., 2016; Balanzà *et al*., 2018; González-Suárez *et al*., 2020; Martínez-Fernández *et al*., 2020; Ware *et al*., 2020; Merelo *et al*., 2022; Walker *et al*., 2023; Sánchez-Gerschon *et al*., 2024).

Studying reproductive development in monocarpic plants is especially interesting as this group includes many important crops, such as cereals, legumes and solanaceous. Thus, understanding the molecular mechanisms that regulate this process would allow the adaptation of production to environmental conditions and

improve crop yield. Furthermore, the model organism *Arabidopsis thaliana* (Arabidopsis) is also monocarpic, which aids in understanding the fundamental aspects of this process. Its simple structure, short life cycle (6-8 weeks) with high seed production, small genome size, and well-established protocols for gene editing techniques are some of the characteristics that make Arabidopsis an excellent model organism (Koornneef and Meinke, 2010; Yaschenko *et al*., 2025).

Proliferative arrest in Arabidopsis

Plants can maintain indeterminate growth throughout their life cycles. This process of continuous organogenesis depends on the maintenance of the stem cells in the meristems. During embryogenesis, plants establish the shoot apical meristem (SAM) and the root apical meristem (RAM), which are responsible for the development of most of the aboveground and underground organs, respectively.

In Arabidopsis, the reproductive SAM, also known as inflorescence meristem (IM), displays indeterminate growth and produces flowers continuously (**Figure I.1A**) until the end of the flowering period. Flowering termination is also known as proliferative arrest (PA) and is characterized by the cessation of all IM activity before the plant death (Hensel *et al*., 1994). PA in Arabidopsis encompasses two processes: meristem and floral arrest. Flower production rate decreases at 4-5 wab, leading to a reduction in the rate of fruit accumulation (**Figure I.1D**). The decrease in flower and fruit production correlates with a gradual reduction in cell size and number, and thus, SAM growth (**Figures I.E**) (Wang *et al*., 2020; Merelo *et al*., 2022). Hence, at 4 wab, SAM activity would be compromised, and no new primordia are initiated (meristem arrest). Lastly, the unopened floral buds produced before meristem arrest (stage 9 or below) block their development (floral arrest). These two events result in the characteristic PA phenotype, a cluster of non-developing buds at the apex of the plant (**Figure I.1B**) (Merelo *et al*., 2022; Walker *et al*., 2023).

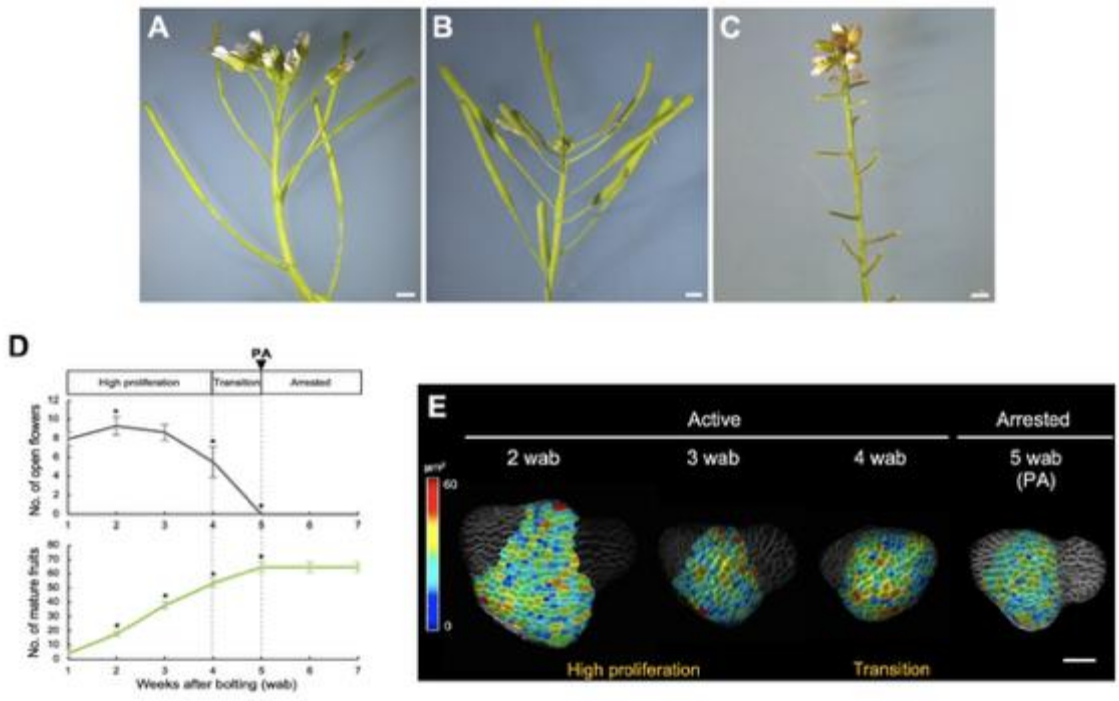

Figure I. 1. End of the flowering period. (A-C) Active apices 2 weeks after bolting (wab) (A), arrested apices 4 wab (B) and reactivated apices 1 week after defruiting (dad) (C) of Arabidopsis thaliana plants. (D) The number of flowers and fruits in the primary stem decreases at 4-5 wab until the conspicuous PA (5 wab), when no more flower buds are produced. (E) Cell size, cell number and SAM size decrease at 3-5 wab in comparison with 2 wab. The decrease in these parameters correlates with the reduction in flower and fruit production. Scale bars, 1 mm (A-C) and 20 µm (E). Adapted from Merelo *et al*. (2022).

Fruit and seed production is an important factor controlling PA. Several works have proposed the existence of a communication system between fruits and IMs in several species, including Arabidopsis (Murneek, 1926; Lockhart & Gottschall, 1961; Hensel *et al*., 1994). In male-sterile mutant plants (*ms1-1*) or plants where fruits have been continuously removed, PA is delayed, and the IM differentiates into a terminal flower. Moreover, in plants that have already arrested, defruiting is able to reactivate IM activity (**Figure I.1C**), suggesting that meristem arrest is a reversible state (Hensel *et al*., 1994; Wuest *et al*., 2016). In addition, it has been observed that mutants with reduced fertility (less than 50% of seeds per fruit) increase in proliferative capacity. Thereby, the authors proposed that a threshold of more than 30% of seeds per fruit is necessary to induce PA (Hensel *et al*.,

1994). These findings suggest the presence of mobile signals derived from the seeds/fruits that may mediate PA. These signals would need to reach a critical level to induce PA. However, later research proposed that in addition to the accumulation of certain levels of fruit-derived signals, the plant needs to acquire the ability to respond to these signals (Ware *et al*., 2020). Furthermore, a small number of fruits proximal to the apex (six-eight) are sufficient to trigger PA, only when the inflorescence becomes competent to arrest (Ware *et al*., 2020). The nature of these signals was not initially elucidated. Previous authors proposed that fruit-derived signals may be phytohormones, either the established ones (Noodén & Penney, 2001) or an unidentified "death hormone" (Engvild, 1989; Wilson, 1997). Hensel *et al*. (1994) observed that different hormone-related mutants (auxin, gibberellins, abscisic acid or ethylene-related mutants) do not show significant PA alterations. Recently, auxin and cytokinins (CKs) have been suggested as some of the seed/fruit-derived signals responsible for PA (Ware *et al*., 2020; Walker *et al*., 2023).

Shoot apical meristem: structure, regulation and maintenance

As introduced before, PA involves the cessation of IM activity (Hensel *et al*., 1994; Wuest *et al*., 2016; Balanzà *et al*, 2018; Martínez-Fernández *et al*., 2020; Ware *et al*., 2020; Merelo *et al*., 2022). Understanding the structure, organization, and function of the SAM is therefore essential to elucidate the molecular mechanisms controlling PA.

The SAM is a highly organized structure divided into the outer tunica, which consists of two cell layers (L1 and L2) and the inner corpus (L3). The cells in the tunica divide perpendicularly to the surface of the meristem, whereas the corpus cells divide in all directions. During development, the L1 contributes to the epidermis of the shoot organs, such as leaves and flowers, the L2 produces the mesodermal cells and the germ cells of ovules and pollen, and the L3 is responsible of the development of the vascular tissues of the stem and most internal tissues of flowers and leaves (**Figure I.2A**) (Satina *et al*., 1940; Poethig, 1987; Fletcher, 2002; Carles & Fletcher, 2003).

Additionally, the SAM can also be divided into different functional domains: the central zone (CZ), the organizing center (OC), the rib zone (RZ) and the peripheral zone (PZ). The CZ is composed of stem cells with low mitotic activity. The OC is located below the CZ and at the top of the RZ and maintains the stem cell population. The PZ and the RZ contain rapidly dividing cells that differentiate and integrate into the lateral organs and the stem core, respectively (**Figure I.2B**) (Mayer *et al*., 1998; Steeves & Sussex, 1989; Carles & Fletcher, 2003). Although there are no clear boundaries between the domains, several studies reveal different gene expression patterns within each zone (Yadav *et al*., 2009; 2014). For instance, cells from the CZ are marked by the expression of *CLAVATA3* (*CLV3*) gene (Fletcher *et al*., 1999), whereas OC cells are defined by the expression of *WUSCHEL* (*WUS*) gene (**Figure I.2B**) (Mayer *et al*., 1998).

The maintenance of the stem cell niche in the shoot apex is essential for providing new cells that allow continuous organ formation and plant growth, while simultaneously replenishing its reservoir. This maintenance is controlled by different genetic pathways.

WUS encodes a WOX family homeodomain transcription factor that specifies stem cell identity and promotes stem cell proliferation and renewal (Mayer *et al*., 1998). WUS acts in a negative feedback loop together with CLV3. WUS protein, synthesized in the OC, migrates to the CZ where it induces *CLV3* expression. *CLV3* encodes a small peptide that, together with the receptors CLV1 and CLV2, represses *WUS* expression and restricts stem cell proliferation in the CZ (Fletcher *et al*., 1999; Brand *et al*., 2000). This negative feedback loop ensures SAM homeostasis by maintaining a stable number of stem cells (**Figures I.2B and I.3**) (Schoof *et al*., 2000; Ha *et al*., 2010; Yadav *et al*., 2011; Fuchs & Lohmann, 2020; Wang *et al*., 2023).

Together with the WUS-CLV loop, a pathway mediated by the class I KNOTTED family homeodomain transcription factor STM is also involved in SAM maintenance. *STM* is expressed throughout most of the SAM, except for young primordia, where it is downregulated, and is required to maintain stem cells and prevent their differentiation (Long *et al*., 1996; Heisler *et al*., 2005; Landrein *et al*., 2015). STM directly activates *CLV3* and interacts with WUS to enhance WUS binding to the *CLV3* promoter (Lenhard *et al*., 2002; Su *et al*., 2020).

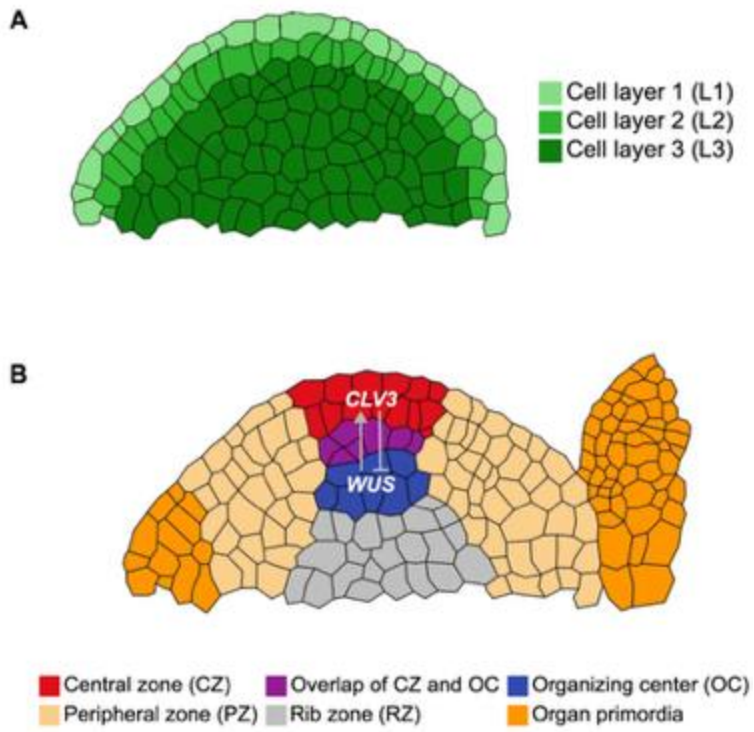

Figure I. 2. Schematic representation of the SAM of *Arabidopsis thaliana*. (A) Schematic representation of distinct cell layers (L) in the SAM. L1 and L2 group cells divide anticlinally, whereas cells in L3 divide in all directions. (B) Schematic representation of SAM functional domains. Adapted from Fuchs & Lohmann (2020).

Phytohormones are essential for maintaining stem cell homeostasis and SAM organization. Cytokinins are key in sustaining cell proliferation in the SAM. Plants that contain mutations in CK receptors display smaller SAMs (Riefler *et al*., 2006), and those with mutations in CK biosynthesis genes, such as *ISOPENTENYL TRANSFERASE* (*IPT*), *LONELY GUY* (*LOG*) and *CYTOCHROME P450 MONOOXYGENASE 735A* (*CYP735A*), display early SAM termination and smaller inflorescence meristems that produce fewer organs (Kurakawa *et al*., 2007; Landrein *et al*., 2018). In contrast, plants with mutations in *CYTOKININ OXIDASE 3* (*CKX3*) and *CKX5*, whose products participate in the degradation of CKs, show larger inflorescence meristems (Bartrina *et al*., 2011).

CK signaling is mediated by two classes of ARABIDOPSIS RESPONSE REGULATORS (ARRs) transcription factors. The type-B ARRs activate the

transcription of CK-responsive genes, whereas type-A ARRs are negative regulators of CK signaling (Kieber & Schaller, 2018).

A positive feedback loop between CK signaling and *WUS* expression contributes to stem cell homeostasis. WUS activates CK signaling by repressing *type-A ARRs* (Leibfried *et al*., 2005). In addition, type A-ARRs ARR7 and ARR15 are required for *CLV3* expression (Zhao *et al*., 2010). On the other hand, type B-ARRs promote *WUS* expression through both *CLV*-dependent and *CLV*-independent pathways (**Figure I.3**) (Gordon *et al*., 2009; Meng *et al*., 2017; Xie *et al*., 2018). Furthermore, STM increases CK levels in the SAM through the activation of *IPT7*, which encodes an enzyme involved in the first step of CK biosynthesis (Jasinski *et al*., 2005; Yanai *et al*., 2005).

Besides CKs, auxin has also been connected to SAM homeostasis. Auxin biosynthesis-related genes, such as *YUCCA 1* (*YUC1*), *YUC2*, *YUC4*, *YUC6*, *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1* (*TAA1*) and *TRYPTOPHAN AMINOTRANSFERASE RELATED 2* (*TAR2*) are expressed in different domains in the SAM, playing a role in maintaining auxin responses necessary for its growth and development (Cheng *et al*., 2006; Stepanova *et al*., 2008; Zhao, 2014; Galvan-Ampudia *et al*., 2020; Yadav *et al*., 2023).

Cell differentiation and organ formation require the coordinated regulation of auxin transport and signaling. Convergences of PINFORMED1 (PIN1), an auxin efflux carrier, determine auxin accumulation in specific sites of the PZ to promote organ primordia initiation (Reinhardt *et al*., 2000; Heisler *et al*., 2005; Vermoux *et al*., 2011). Although auxin accumulates mainly in the PZ, a minimal auxin response in the center of the SAM is required to maintain the stem cell niche. WUS coordinates auxin signaling in the stem cell niche via the reduction of transcription of several genes involved in auxin transport, perception, signaling and response, restricting auxin activity in stem cells while, at the same time, maintaining it at basal levels (Ma *et al*., 2019; Galvan-Ampudia *et al*., 2020). This regulation occurs downstream of the AUXIN RESPONSE FACTOR (ARF) ARF5/MONOPTEROS (MP) (Ma *et al*., 2019). ARF5/MP protein is primarily located in the PZ, but is also present in the CZ, where WUS reduces its expression without completely suppressing it (Zhao *et al*., 2010; Ma *et al*., 2019). Conversely, in the CZ, ARF5/MP directly represses

DORNROSCHEN/ ENHANCER OF SHOOT REGENERATION 1 (*DRN/ESR1*), which encodes a transcription factor expressed in the center of the meristem that positively regulates *CLV3* expression (Luo *et al*., 2018). Additionally, ARF5/MP inhibits the expression of two *type-A ARRs* (*ARR7* and *ARR15*) in the CZ, inducing CK signalling and, in turn, *WUS* expression (Zhao *et al*., 2010). This interplay reveals a crosstalk between CK and auxin that ensures a balance between stem cell proliferation and differentiation and, thus, SAM homeostasis and function (**Figure I.3**).

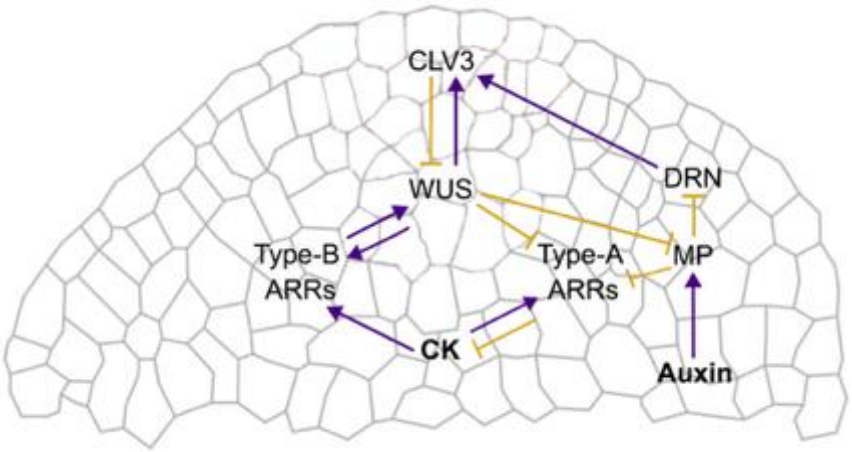

Figure I. 3. CK and auxin signaling and the gene regulatory network involved in SAM maintenance. The type-A and type-B ARRs, involved in CK signaling, regulate *WUS* expression. In turn, WUS regulates the expression of *type-A* and *type-B ARRs*. Moreover, WUS represses *MP*, a gene related to auxin response. On the other hand, MP represses *DRN* and *type-A ARRs*, ensuring *WUS* expression by repressing *CLV3* and promoting CK signaling.

Genetic control of proliferative arrest

The first genetic factors potentially involved in the control of PA were identified through transcriptomic comparisons between SAMs of growing and arrested plants and SAMs that were reactivated by defruiting (Wuest *et al*., 2016). The transcriptomic profiles of growing and arrested meristems exhibit significant differences, whereas reactivated meristems closely resemble growing meristems, suggesting that the transcriptional stage of arrested meristems is rapidly reverted upon defruiting. Arrested meristems are characterized by low

mitotic activity and reduced CK responsiveness, while stress-, abscisic acid (ABA)- and senescence-related genes are upregulated (Wuest *et al*., 2016; Martínez-Fernández *et al*., 2020). Notably, bud dormancy has been related to ABA accumulation (Yao & Finlayson, 2015; González-Grandío *et al*., 2017), reduced CK levels (González-Grandío & Cubas 2014; Roman *et al*., 2016) and low mitotic activity (González-Grandío & Cubas 2014). This resemblance suggests that IM arrest would represent a dormant-like stage at the end of flowering.

Balanzà *et al*. (2018) provided the first model of a genetic pathway controlling PA. This study showed that PA is under the control of age-dependent factors that act in parallel to the seed/fruit-derived factors. As the plant ages, the expression of *FRUITFULL* (*FUL*) and *microRNA172* (*miR172*) increases in the IM. These factors directly repress the expression of *APETALA2* (*AP2*) and *AP2-like* genes and, in turn, *WUS* expression, resulting in IM arrest (**Figure I.6**).

FUL encodes a MADS-domain transcription factor involved in fruit development, meristem identity and floral transition (Gu *et al*., 1998; Ferrándiz *et al*., 2000). In *ful* mutant plants, meristem activity never arrests, and flower and fruit production continue indefinitely (**Figure I.4**).

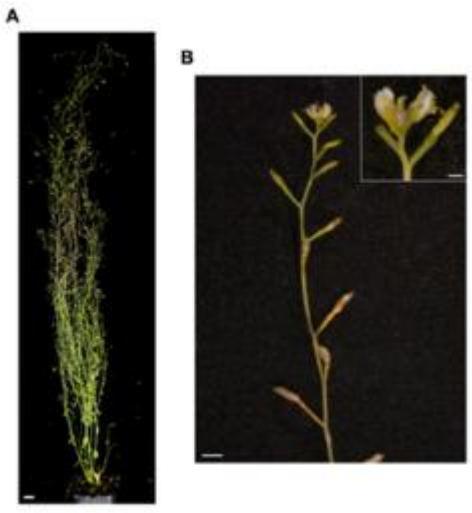

Figure I. 4. *ful* mutant plants at advanced stages of the flowering period. (A) *ful* plant 6 wab. (B) The upper part of the main stem of *ful* plant 11 wab. The shoot apex is shown in the magnified image (from Merelo *et al*. 2022). Scale bars represent 1 cm (A) and 1 mm (B).

On the other hand, AP2 is a transcription factor of the euAP2 lineage (*TARGET OF EAT1* [*TOE1*], *TOE2*, *TOE3*, *SCHLAFMÜTZE* [*SMZ*], its paralog *SCHNARCHZAPFEN* [*SNZ*], and *AP2*) characterized by being targets of the miR172 (Aukerman & Sakai, 2003). AP2 is involved in regulating the stem cell niche, floral organ development and floral transition (Würschum *et al*., 2006; Yant *et al*., 2010). During PA, AP2 plays an antagonistic role to FUL in the regulation of this process. The *ap2-170* mutant, in which the miR172 binding site on *AP2* is mutated, shows a delay in PA (Balanzà *et al*., 2018). Interestingly, *ap2* null mutation does not fully rescue the PA phenotype of *ful*, suggesting that PA control by FUL occurs not exclusively through AP2 (Balanzà *et al*., 2018). Additionally, the induction of *AP2* is able to reactivate SAM activity (Balanzà *et al*., 2018; Martínez-Fernández *et al*., 2020). Transcriptomic analyses revealed that AP2 is involved in PA control by regulating genes related to hormones (i.e., CKs and ABA) and environmental factors (i.e., light and temperature) (Martínez-Fernández *et al*., 2020). Three homeodomain leucine zipper (HD-ZIP) transcription factors, HOMEOBOX PROTEIN21 (HB21), HB40 and HB53 have been identified as putative AP2 targets. These transcription factors are related to ABA response and bud dormancy (González-Grandío *et al*., 2017) and are directly repressed by AP2 (Martínez-Fernández *et al*., 2020). *HB21/40/53* expression is upregulated shortly before PA and further increases in arrested inflorescence apices, while in mutants where PA is delayed (*ap2-170*) or never happens (*ful*), their expression is not detected until the arrest of the plants or never detected, respectively. The triple mutants *hb21 hb40 hb53* do not show differences in IM arrest but exhibit delayed floral arrest, suggesting that their role is more closely associated with this response. Hence, the decline in AP2 levels at the end of flowering would lead to the accumulation of HB21/40/53 in the inflorescence apex, which would induce ABA accumulation, and thus, floral arrest at the end of flowering (Sánchez-Gerschon *et al*., 2024).

As previously mentioned, the *WUS-CLV3* feedback loop is essential to maintain SAM activity. WUS was not detected in arrested meristems (Balanzà *et al*., 2018; Goetz *et al*., 2021; Merelo *et al*., 2022), and when arrested meristems are reactivated by defruiting, WUS levels are rapidly restored in the SAM (Merelo *et al*., 2022). Moreover, in *ful* mutants, WUS levels are maintained throughout the

flowering period, in comparison with wild-type plants (Merelo *et al*., 2022). However, FUL does not regulate *WUS* directly (Balanzà *et al*., 2018). Since AP2 positively regulates *WUS* expression and FUL is a repressor of *AP2*, FUL has been proposed to regulate *WUS* through *AP2* at the end of the flowering period (**Figures I.5A and I.6**) (Balanzà *et al*., 2018; Martínez-Fernández *et al*., 2020; Merelo *et al*., 2022).

Hormonal control of proliferative arrest

In the last few years, different hormone signaling pathways have been linked to the control of PA. As we introduced before, CKs are essential for maintaining cell proliferation in the SAM. Several studies have shown that CK-related pathways need to be repressed locally in the SAM for its arrest (Martínez-Fernández *et al*., 2020; Merelo *et al*., 2022). In particular, PA involves a coordinated and gradual repression of CK signaling and CK-dependent processes, such as CYCB1;2-promoted cell division, WUS-mediated SAM maintenance and SAM growth (**Figure I.5**). Moreover, the application of CKs to both active and arrested apices revealed that CKs are sufficient to prevent and revert PA, respectively. The early repression of CK-related pathways, which causes a decrease in SAM activity and flower production, led to propose a model that distinguishes two different phases at the end of flowering. First, a gradual repression of the SAM activity regulators and flower production is observed (decline phase; 3 wab). At this time point, no new primordia would be generated. Subsequently, a complete block of the SAM activity regulators (shutdown phase; 4 wab) and the conspicuous PA takes place, where a cluster of unopened buds remains at the apex of the plant (**Figure I.5**) (Merelo *et al*., 2022). A recent study has proposed that CKs, besides repressing IM arrest, negatively regulate floral arrest (Walker *et al*., 2023). Mutant plants in genes encoding the CK receptors ARABIDOPSIS HISTIDINE KINASE2 (AHK2) and AHK3, which have increased CK sensitivity (Bartrina *et al*., 2017), display alterations in IM arrest. Gain-of-function mutations in *AHK2* cause alterations in both floral and IM arrest, whereas those in *AHK3* primarily affect floral arrest. According to this, the authors proposed that the redistribution of CK between inflorescence and fruits controls the timing of both IM and floral arrest. Inflorescences and fruits would act as sinks for CK. As new inflorescences and

fruits are formed during the flowering period, thereby increasing CK sinks, CK levels within the inflorescences become progressively diluted. This would contribute to a reduction in IM activity, ultimately resulting in IM arrest, followed by floral arrest (Walker *et al*., 2023).

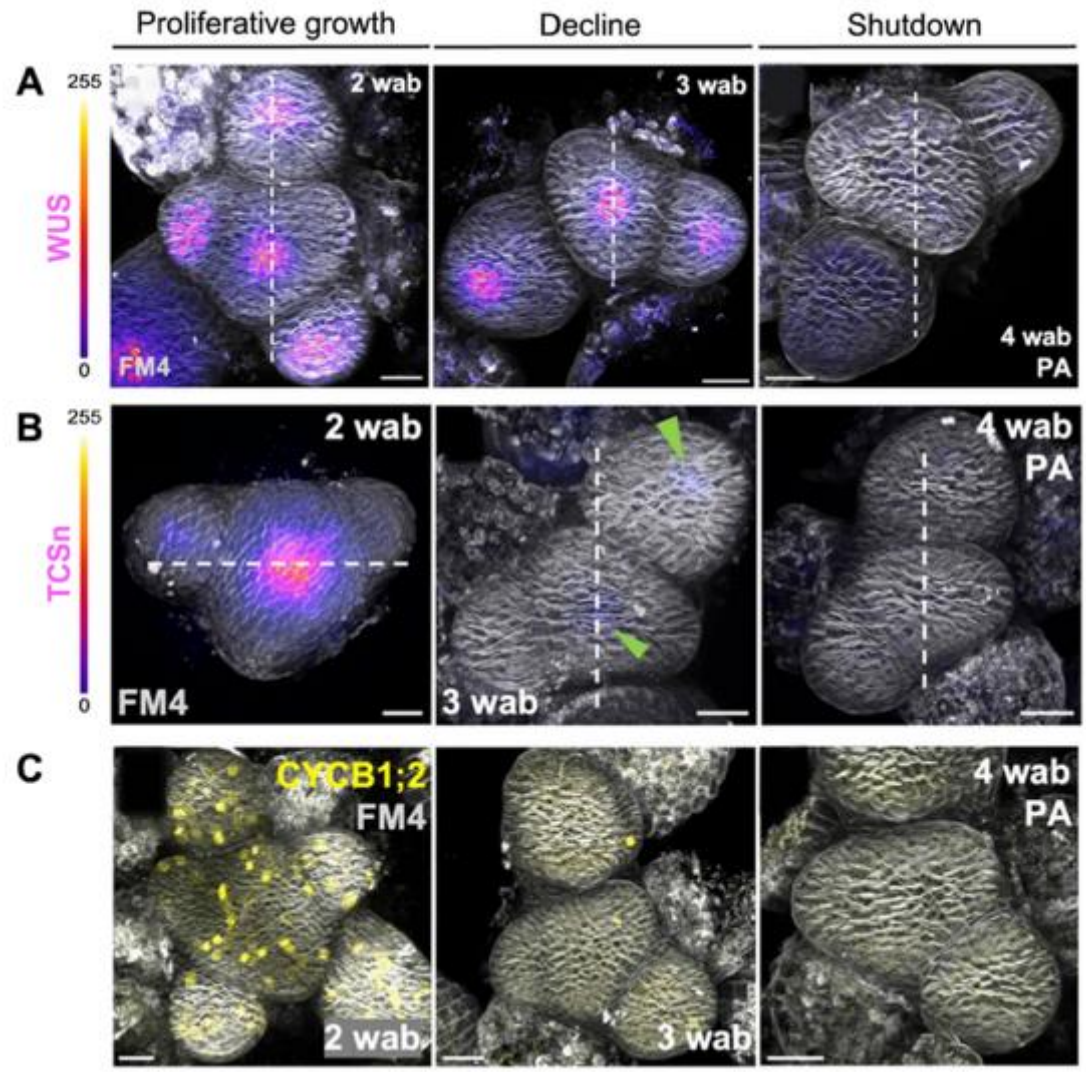

Figure I. 5. CK-related factors are gradually repressed during PA. (A-C) WUS levels (A), CK signaling (TCSn) (Zürcher *et al*., 2013) (B) and cell division (CYCB1;2-GFP) (Merelo *et al*., 2022) (C) are high during proliferative growth. These factors decrease during the decline phase and are completely blocked at the shutdown phase. Adapted from Merelo *et al*. (2022).

This role of CK during PA has also been related to the FUL-AP2 pathway. Since CK-related events are continuously active in the SAM of *ful* mutants, FUL would repress CK-related pathways to promote PA. In addition, the mode of action of

FUL would be biphasic: first, FUL, together with additional unknown factors, gradually represses the CK-related pathways (decline phase) and, later, FUL completely blocks these events (shutdown phase) (Merelo *et al*., 2022). Furthermore, AP2 may directly repress the negative regulators of CK signaling KISS ME DEADLY2 (KMD2) and KMD4. Thus, at the end of flowering, the repression of CK-related pathways, either through direct FUL activity or through the increase in KMD proteins resulting from the decline of AP2 activity, would trigger PA (**Figure I.6**) (Martínez-Fernández *et al*., 2020).

On the other hand, auxin has been proposed as a negative regulator of PA. Auxin is produced at high levels in fruits and seeds (Gustafson, 1939; Matilla, 2020), and early works proposed that signals derived from the fruits or seeds (named as the death hormone) trigger PA (Murneek, 1926; Lockhart & Gottschall, 1961; Hensel *et al*., 1994). The application of auxin to sterile fruits or to pedicels after fruit removal induces PA, whereas mutants with reduced auxin transport delay IM arrest (Ware *et al*., 2020). Furthermore, auxin transport decreases at the apical region of the stem below the shoot apex, where the last fruits develop. However, auxin response increased in the apical region. The authors proposed that these changes in auxin response would repress auxin transport in the apical region at the conspicuous PA (Goetz *et al*., 2021). However, this would be in conflict with the positive feedback between auxin response and transport previously reported (Bhatia *et al*., 2016). These works have led these authors to propose that auxin exported from the developing fruits proximal to the inflorescence triggers PA by disrupting polar auxin transport in the apical region of the stem: first, inflorescences would canalize auxin transport coming from the fruits; later, when the inflorescences reach a critical age and acquire the competence to arrest, a high amount of auxin would be exported from the last six to eight fruits and the apex would no longer be able to canalize this transport, promoting the inflorescence arrest. Meanwhile, if plants are sterile or fruits are removed, the auxin export from fruits is reduced, allowing the apex to canalize the auxin transport and continue flowering (Ware *et al*., 2020; Goetz *et al*., 2021).

Nevertheless, the follow-up study of these same authors that described the distinction between IM and floral arrest (Walker *et al*., 2023) seems to contradict this model for the proposed role of auxin exported from the fruits in IM arrest.

According to their new data, IM arrest occurs at an earlier developmental stage, precluding the possibility that it is induced by auxin exported from the late-developing fruits proximal to the inflorescence. These late-developing fruits continue their development even after IM arrest has occurred, suggesting that the auxin-mediated mechanism is more closely associated with floral arrest than with IM arrest (Walker *et al*., 2023). However, although auxin plays an important role in PA (López-Martín *et al*., 2025), more work is needed to define the molecular mechanisms under its mode of action in PA.

Another important hormone associated with PA is ABA. Transcriptomic profiles of arrested meristems revealed that ABA-related genes are upregulated in the SAM at the moment of the conspicuous PA and that their expression levels are reduced in reactivated meristems (Wuest *et al*., 2016). Most of these ABA-related genes are also repressed after *AP2* induction. Among these putative AP2 targets, there are genes related to ABA biosynthesis (*ABA DEFICIENT1* [*ABA1*], *NINE-CIS-EPOXYCAROTENOID DIOXYGENASE3* [*NCED3*]), perception (*PYR1-LIKE7* [*PYL7*]), signaling (*SNF1-RELATED PROTEIN KINASE 2-3* [*SNRK2.3*], *ABA INSENSITIVE2* [*ABI2*]) and response (*RESPONSIVE TO DESICCATION20* [*RD20*], *HB21*, *HB53*) (Martínez-Fernández *et al*., 2020). ABA levels are also increased in arrested inflorescences. The triple mutants *hb21 hb40 hb53* accumulate lower ABA levels than wild-type plants, suggesting that *HB* genes would promote ABA biosynthesis through *NCED3* and *4 genes (*Sánchez-Gerschon *et al*., 2024). Moreover, the application of ABA on the inflorescences causes their arrest, while treatments with an ABA antagonist (ABA-az) delay floral arrest *(*Sánchez-Gerschon *et al*., 2024). These studies suggest that ABA accumulation in the apex could mediate IM and floral arrest (**Figure I.6**).

Finally, jasmonic acid (JA), a hormone involved in various developmental processes such as floral organ development (Song *et al*., 2011; Reeves *et al*., 2012), floral transition (Zhai *et al*., 2015; Zhao *et al*., 2022) and leaf senescence (Qi *et al*., 2015; Zhang *et al*., 2020), may be also involved in the control of PA. Mutations in the JA biosynthesis genes *13-LIPOXYGENASE3* (*LOX3*) and *LOX4* or in the JA co-receptor *CORONATINE INSENSITIVE1* (*coi1-37*) cause higher flower production and a delayed PA (Caldelari *et al*., 2011; Kim *et al*., 2013).

However, these mutations cause male sterility, making unclear whether the effects on PA are due to the absence of seed production or to JA itself.

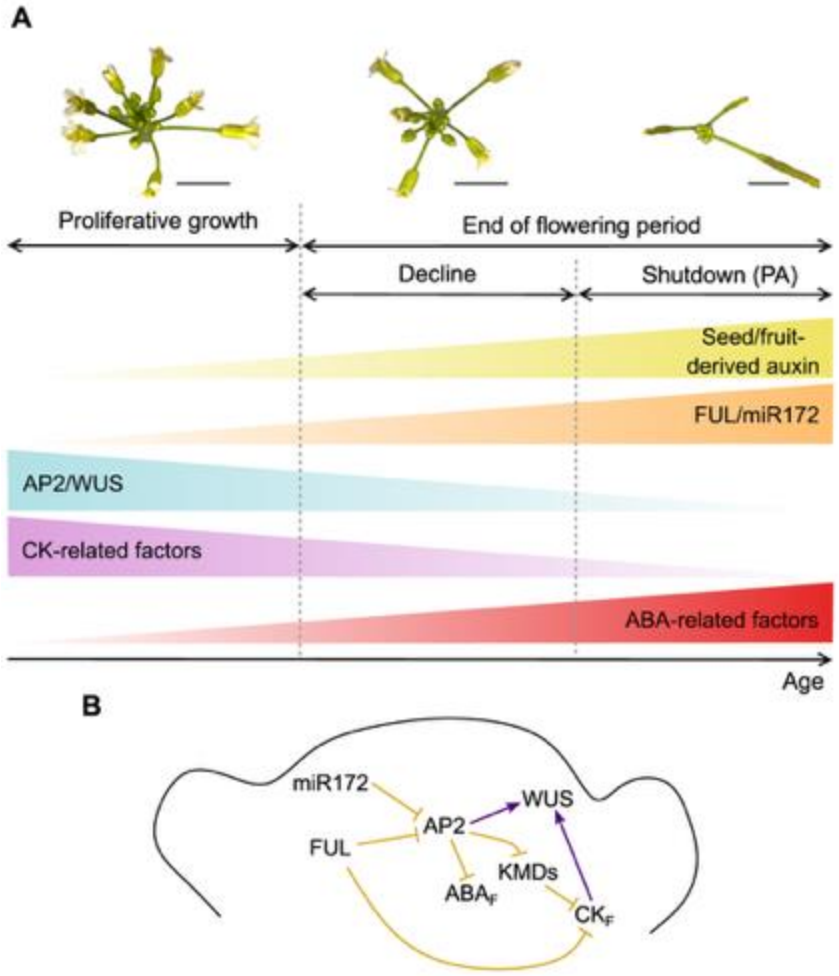

Figure I. 6. Hormonal and genetic factors involved in the control of PA. (A) Endogenous clues such as age or the production of seeds/fruits are major players in the control of PA. In inflorescences at the proliferative growth stage, CK-related factors and the transcription factors AP2 and WUS maintain the activity of the meristem, while FUL or miR172 are present at low levels. At the end of the flowering phase, CK-related factors, AP2 and WUS levels decrease, whereas FUL and miR172 accumulate (decline phase). Later, at the shutdown phase, CK-related factors, *AP2* and *WUS* expression are completely blocked in the SAM. Additionally, ABA-related factors increase at the end of flowering, inducing a dormant-like stage in the meristem. Finally, auxin exported from fruits increases with the age of the plants and promotes floral arrest. (B) CK-related factors (CK_F) and *AP2* and *WUS* expression may be repressed, in part, by the negative regulation of FUL. Moreover, the repression of AP2 may lead to an increase of ABA-related factors (ABA_F). These factors, together with the negative effect of fruit-derived auxin, trigger PA.

These recent advances have provided valuable insights into the regulation of the end of the reproductive phase. Nonetheless, significant gaps in this knowledge persist. In this context, we tackle two main questions. The first explores how auxin signaling impacts meristem dynamics, particularly in the context of PA. As mentioned above, several works have assigned a role for auxin in PA control. However, the molecular bases underlying the auxin mode of action in this process remain unclear. Our second question seeks to elucidate how and what factors FUL regulates during PA. FUL is a key component promoting meristem arrest, but its precise mode of action and expression pattern within the SAM during this process remain poorly understood. FUL promotes meristem arrest, in part, by repressing *AP2-like* genes and CK-related processes. Furthermore, PA control by FUL may not occur exclusively through AP2 (Balanzà *et al*., 2018; Martínez-Fernández *et al*., 2020; Merelo *et al*., 2022). Merelo *et al*. (2022) proposed that FUL initially functions as a mild repressor and later as a strong repressor during PA. However, this biphasic role has only been described in the regulation of CK-related pathways, leaving its regulation of other factors during PA largely unexplored.

Therefore, this thesis aims to address this gap in knowledge by exploring new ideas and hypotheses to enhance our understanding of the process. Addressing this challenge requires an integrative approach that combines genetic and transcriptomic assays, as well as live imaging confocal microscopy analyses of the SAM, enabling a precise characterization of the molecular, cellular and morphological changes in the meristem associated with PA.

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Objetives

OBJECTIVES

The general objective of this thesis is to advance the understanding of the molecular mechanisms that control proliferative arrest at the end of flowering. In order to identify novel factors implicated in the control of this process, elucidate how these factors are coordinated and integrated within the spatio-temporal context, and generate a comprehensive map of the molecular events that trigger proliferative arrest in *Arabidopsis thaliana*, the following specific objectives are proposed:

1. To study auxin dynamics within the shoot apical meristem during proliferative arrest and how it is integrated into the spatio-temporal framework of PA control.

2. To elucidate the interaction between auxin and cytokinins in this process and to evaluate the regulation of auxin-related pathways by FRUITFULL during the flowering period.

3. To study the mode of action of FRUITFULL through advanced stages of the flowering period by characterizing its distribution within the SAM and identifying potential molecular pathways downstream of FRUITFULL involved in PA regulation.

4. To investigate the role of jasmonic acid in the regulation of PA and its connection to FRUITFULL at the end of the flowering period.

Local regulation of auxin-related pathways in the shoot apical meristem plays a major role during proliferative arrest

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INTRODUCTION

To ensure reproductive success, plants regulate the timing of both the start and the end of the flowering period. In monocarpic plants, such as *Arabidopsis thaliana* (Arabidopsis), the end of flowering is determined by the proliferative arrest process (PA) (Hensel *et al*., 1994; Balanzà *et al*., 2018). In Arabidopsis, PA is characterized by the cessation of all inflorescence meristem (IM) activity (IM arrest) and floral bud development (floral arrest). Thus, no new primordia are initiated after IM arrest, and the unopened floral buds at this point do not continue their development. These two processes, IM and floral arrest, produce a cluster of unopened buds at the end of flowering (Merelo *et al*., 2022; Walker *et al*., 2023). Despite their importance, the molecular events underlying the regulation of PA have remained largely unexplored. Only recently, several studies — mainly in Arabidopsis, but also in other monocarpic species such as pea and tomato — have provided information regarding the genetic, signaling and environmental factors involved in this regulation (Murneek, 1926; Wuest *et al*., 2016; Balanzà *et al*., 2018; Martínez-Fernández *et al*., 2020; Merelo *et al*., 2022; Walker *et al*., 2023; Balanzà *et al*., 2023; Burillo *et al*., 2024; Marínez-Fernández *et al*., 2024; Sánchez-Gerschon *et al*., 2024; López-Martín *et al*., 2025).

Cytokinins (CKs) are crucial in the control of PA. CK response and CK-related events (cell division, shoot apical meristem (SAM) growth and *WUSCHEL* [*WUS*]-dependent SAM maintenance) need to be repressed locally in the SAM at the end of flowering (Martínez-Fernández *et al*., 2020; Merelo *et al*. 2022). A recent work has also proposed that CKs negatively regulate floral arrest and that the distribution of CKs between fruits and inflorescences controls the timing of IM and floral arrest (Walker *et al*, 2023). Furthermore, PA is influenced by the age of the inflorescence, mainly through the action of FRUITFULL (FUL). FUL accumulates in the SAM to promote this process and is essential for it, as *ful* mutants do not undergo PA. First, FUL, together with unknown factors, appears to contribute to the gradual repression of CK-related events (decline phase) and, later, FUL would completely inhibit these events (shutdown phase) (Merelo *et al*., 2022). By contrast, AP2, a target of FUL negative regulation in this age-dependent pathway, regulates CK response by repressing negative regulators of CK signaling

(Martínez-Fernández *et al*., 2020). Abscisic acid (ABA), on the other hand, plays a positive role in floral arrest. ABA accumulates in the inflorescence apex, and ABA-related genes are induced in arrested meristems downstream of AP2 (Wuest *et al*., 2016; Martínez-Fernández *et al*., 2020; Sánchez-Gerschon *et al*., 2024).

Together with genetic and hormonal control, the production of fruits and seeds is an important factor controlling PA (Murneek, 1926; Lockhart & Gottschall, 1961; Hensel *et al*., 1994). In sterile plants, or in those from which fruits are continuously removed, PA is delayed. In addition, fruit removal from arrested plants reactivates meristem activity. These results suggest the presence of a mobile signal produced by fruits/seeds that triggers PA (Hensel *et al*., 1994). Recently, some studies have proposed that auxin exported from developing fruits proximal to the inflorescence could be this mobile signal. Auxin accumulation in the apical region of the stem would disrupt auxin transport, leading to PA once the inflorescences acquire the competence to arrest (González-Suárez *et al*., 2020; Ware *et al*., 2020; Goetz *et al*., 2021). These authors later proposed that auxin promotes floral arrest rather than IM arrest, as older fruits — which are responsible for the cessation of flower opening — continue developing after IM arrest (González-Suárez *et al*., 2020; Walker *et al*., 2023).

However, while these works convincingly assign a role for auxin in PA regulation, more work is needed to determine the molecular bases underlying the auxin mode of action and how auxin dynamics within the SAM is integrated into the spatio-temporal framework of PA control.

In this work, we show that repression of auxin biosynthesis, transport and response locally in the SAM may promote PA. We also observe a decrease and shutdown of auxin-related reporter expression during PA, correlating with described changes in CK pathways (Merelo *et al*., 2022). These findings suggest that auxin and CK pathways may be coordinated in regulating PA. Additionally, analysis of auxin markers in *ful* mutants, which fail to arrest, and after *FUL* induction in the SAM, suggests that FUL may promote meristem arrest by locally repressing auxin pathways. Overall, our study provides new insights into auxin dynamics in the SAM at the end of flowering and its integration into the temporal framework of IM arrest.

RESULTS

Repression of auxin-related pathways in the SAM correlates with the PA

Previous studies have proposed that auxin export from fruits that are proximal to the apex could promote PA by interrupting auxin transport in the apical region of the stem, mainly affecting floral arrest rather than IM arrest (González-Suárez *et al*., 2020; Ware *et al*., 2020; Goetz *et al*., 2021; Walker *et al*., 2023). However, these works do not provide any insight into auxin dynamics within the SAM during PA. Auxin is essential to promote organ initiation and growth in the SAM, and auxin transport and polar flow, as well as auxin response, are tightly and locally regulated in the SAM to ensure such processes (Vernoux *et al*., 2000; Reinhardt *et al*., 2003; Heisler *et al*., 2005; Bhatia *et al*., 2016). PA implies the cessation of new flower primordia initiation and development, and previous studies have shown that local regulation of fundamental cellular and molecular events for meristem activity is necessary to trigger IM arrest (Merelo *et al*., 2022). Therefore, to test whether PA, and especially IM arrest, depends on changes in auxin-related pathways locally in the SAM, we monitored with high spatio-temporal resolution the expression of *pPIN1:PIN1-GFP* (an auxin transport reporter based on the *PIN-FORMED 1* gene; PIN1-GFP) (Benková *et al*., 2003) and R2D2 (an auxin ratiometric signaling reporter) (Liao *et al*., 2015) in the SAM close to and during PA. Strong PIN1 convergences and auxin signaling correlate with the incipient primordia, where auxin peaks are created promoting organ initiation and differentiation (Vernoux *et al*., 2000; Reinhardt *et al*., 2003; Heisler *et al*., 2005; Ma *et al*., 2019). Therefore, these specific reporters can provide a readout of auxin-dependent changes in organ initiation during PA. Quantification of flower and fruit production during the flowering period allowed us to distinguish the two different phases before the PA, as previously described (Merelo *et al*., 2022): a high proliferation phase (0-3 weeks after bolting; wab), where a high number of open flowers and fertile fruits were produced in the primary apex, and a decline phase (3-4 wab), characterized by a decay in the flower production rate and fruit production until the moment of conspicuous PA (4 wab; shutdown), when no more flower buds were produced (**Figure S1.1**). Based on this kinetics, we visualized

these reporters in the SAM at 2, 3, and 4 wab as well as in apices reactivated by defruiting (1 day after defruiting, dad; 5 wab/1 week after defruiting, wad) (Hensel *et al*., 1994; Balanzà *et al*., 2018; Merelo *et al*., 2022). In highly active apices (2 wab), PIN1-GFP and R2D2 expression were patterned as previously reported (Vernoux *et al*., 2000; Reinhardt *et al*., 2003; Heisler *et al*., 2005; Ma *et al*., 2019) (**Figures 1.1A and 1.1F**). In apices at 3 wab, a marked decline of expression for these auxin markers was observed in the SAM (**Figures 1.1B and 1.1G**), correlating with the onset of the decline phase of PA (Merelo *et al*., 2022). The signal of these reporters continued to decline in the following days (**Figure S1.2**). This signal decline correlated with the decay in the flower production rate (3-4 wab; **Figure S1.1**). At the conspicuous PA (4 wab; cluster of arrested buds; shutdown phase) (Merelo *et al*., 2022), PIN1-GFP and R2D2 signal were almost undetectable in the SAM (**Figures 1.1C and 1.1H**). On the other side, the signal of these auxin reporters was reestablished quickly in the SAM after reactivation by defruiting (1 dad) and maintained longer (1 wad) at levels like in prearrested meristems (**Figures 1.1D, 1.1E, 1.1I and 1.1J**). Besides auxin transport and response, we monitored *pTAA1:GFP-TAA1* (an auxin biosynthesis reporter based on the *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS* gene; GFP-TAA1) (Stepanova *et al*., 2008; He *et al*., 2011) to test whether changes in local auxin biosynthesis might also correlate with the onset and advance of IM arrest, in addition to the proposed effect of the fruit-derived auxin (González-Suárez *et al*., 2020; Ware *et al*., 2020; Goetz *et al*., 2021; Walker *et al*., 2023). In fact, the expression of auxin biosynthesis genes is spatially restricted to specific domains in the SAM (Cheng *et al*., 2006; Pinon *et al*., 2013; Yadav *et al*., 2023), suggesting that this is also a tightly regulated process in active SAMs, and it could be also locally regulated during PA leading to IM arrest. GFP-TAA1 expression was detected in the epidermal cell layer (L1) of the central region of the SAM and some primordia (stages ~P_3-P_n) 2 wab (**Figures 1.1K and 1.1P**). Interestingly, a decay and shutdown of GFP-TAA1 signal were also observed 3 and 4 wab, respectively, as well as a recovery of signal in reactivated SAMs 1 dad and 1 wad (**Figures 1.1L-1.1O and 1.1Q-1.1T**).

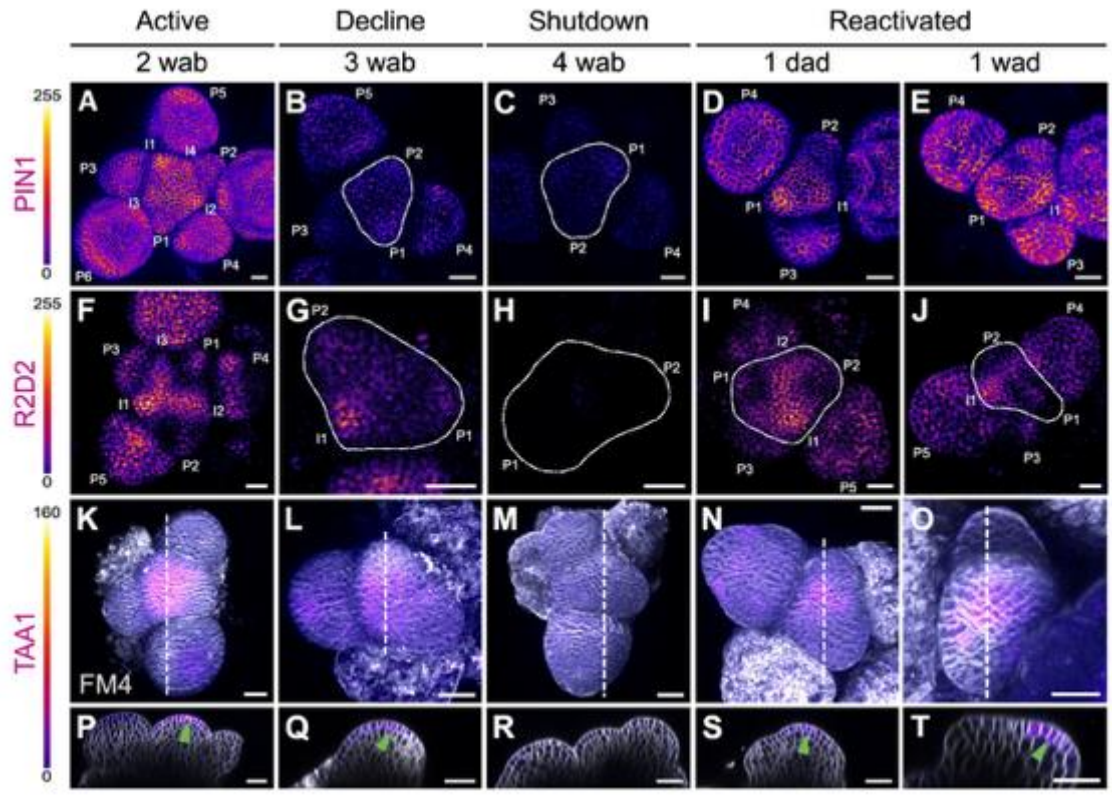

Figure 1. 1. Auxin biosynthesis, transport and signaling are repressed during PA. (A-E) *pPIN1:PIN1-GFP* expression (magenta; signal intensity calibration bar) in the shoot apex 2 (A), 3 (B) and 4 weeks after bolting (wab) (C) and in reactivated apices (D and E; 1 day after defruiting [dad] and 1 week after defruiting [wad], respectively). (F-J) Confocal projections of the shoot apex showing R2D2 expression (magenta; mDII/DII ratio intensity distribution) 2 (F), 3 (G) and 4 wab (H) and 1 dad (I) and 1wad (J). The white dashed line outlines young primordia (~In-P2) and meristems. Pn, flower primordia that have grown out from the meristem; In, incipient primordia. Both Pn and In are numbered in order of appearance from youngest (P1 or I4) to oldest (P6 or I1). (K-O) Expression of *pTAA1:GFP-TAA1* in the shoot apex (magenta; signal intensity calibration bar) 2 (K), 3 (L) and 4 wab (M) and 1 dad (N) and 1 wad (O). (P-T) Corresponding longitudinal sections of the shoot apex along the dashed lines in (K)-(O). Green arrowheads point to GFP-TAA1 signal in the L1 of the center of the SAM. Cell membranes were highlighted using FM4-64 staining (gray). Scale bars represent 20 µm. A total of 6 to 10 apices were visualized for each reporter and time point.

Additionally, we quantified IAA levels in active (2 wab), arrested (4 wab) and reactivated (1 wad) apices. IAA levels decreased significantly 4 wab with respect to early stages (2 wab). After reactivation by defruiting (1wad), IAA levels were restored in the apex (**Figure S1.3**). These results suggest that repression of auxin-related factors at different levels (biosynthesis, transport and signaling),

and probably a decrease in local auxin content, promote IM arrest. Moreover, the temporal correlation of changes in GFP-TAA1 expression with the repression of PIN1 and R2D2 reporters in the SAM suggests that changes in the levels of local auxin might be necessary to repress auxin-mediated organ formation during PA, and that the potential effect from the fruit-derived auxin on PA might be additive, indirect or rely on sites different to the SAM such as the apical region of the stem or buds/flowers (Ware *et al*., 2020; Goetz *et al*., 2021). In fact, the fast recovery of auxin reporters and IAA levels within the SAM after fruit removal suggests that fruit or seed signals, such as signals dependent on fruit-derived auxin (González-Suárez *et al*., Ware *et al*., 2020; Goetz *et al*., 2021), may also regulate arrest via auxin-related pathways locally in the SAM. The changes observed in auxin pathways follow the same timing as the CK-related factors involved in this process. The repression of auxin pathways starts at 3 wab and is gradual and prior to the observation of the arrested inflorescence (4 wab), as we observed in the context of CK-related events (Merelo *et al*., 2022). CK and auxin act together in the maintenance of SAM activity by promoting stem cell proliferation and differentiation, respectively (Reinhardt *et al*., 2000; Heisler *et al*., 2005; Gordon *et al*., 2009; Shi & Vernoux, 2019). PA may imply a simultaneous and coordinated repression of pathways regulated by these two closely related hormones, and therefore of these two coupled processes, proliferation and differentiation. Therefore, besides testing the importance of the local auxin biosynthesis in the control of IM arrest, we have performed different assays to elucidate whether these hormones may interact in this process as well.

PA is affected by modifications in auxin biosynthesis and catabolism within the SAM

Our results showed that auxin-related factors are repressed locally in the SAM during PA (**Figure 1.1**). Repression of auxin biosynthesis, transport and signaling reporters, which provide a readout of auxin levels, suggests that low auxin content in the SAM may lead to PA. To assess the significance of auxin on the regulation of meristem arrest, we modified auxin content in the SAM by inducing auxin biosynthesis or catabolism genes (*TAA1* and *DIOXYGENASE FOR AUXIN OXIDATION 1* [*DAO1*], respectively) in the L1 and analyzed whether PA is

affected. For this aim, we used the two-component GR-LhG4 system (Craft *et al*., 2005) driven by the *MERISTEM LAYER 1* (*ML1*) promoter (*pML1:GR-LhG4_6xOp:TAA1*, *pML1:GR-LhG4_6xOp:DAO1*; *pML1>TAA1*, *pML1>DAO1*) (Sessions *et al*., 1999). Continuous *TAA1* induction by dexamethasone (Dex) treatment of active apices from ~2 wab delayed PA for about four days and led to a higher total number of fruits in comparison with mock-treated apices (control), which arrested after 2 weeks of treatment (wot) (4 wab) (**Figures 1.2A-1.2C**). We also compared the expression of the auxin reporters PIN1-GFP and R2D2, which respond to changes in auxin levels (Heisler *et al*., 2005; Adamowski & Friml 2015; Bhatia *et al*., 2019; Yadav *et al*., 2023), in Dex-treated and control SAMs. PIN1-GFP and R2D2 expression was almost undetectable in control-arrested SAMs after 2 wot, whereas SAMs of Dex-treated apices still showed signal for both auxin reporters (**Figures 1.2D-1.2G**). On the other hand, *TAA1* induction in arrested SAMs (4 wab) caused their reactivation. Reactivated apices showed new buds and flowers after 1 wot (5 wab), while control apices stayed arrested (**Figures 1.2H-1.2K**). Moreover, PIN1-GFP and R2D2 expression was restored in the SAM after 1 day of treatment (dot) (**Figures 1.2M and 1.2Q**), indicating a rapid reactivation of auxin pathways and primordia formation, and was maintained after 1 wot (**Figures 1.2O and 1.2S**). By contrast, the expression of these auxin-related markers was very low in control SAMs after 1 dot and 1 wot (**Figures 1.2L, 1.2N, 1.2P and 1.2R**). These results suggest that local auxin synthesis mediated by TAA1 is sufficient to maintain longer or to reactivate SAM activity.

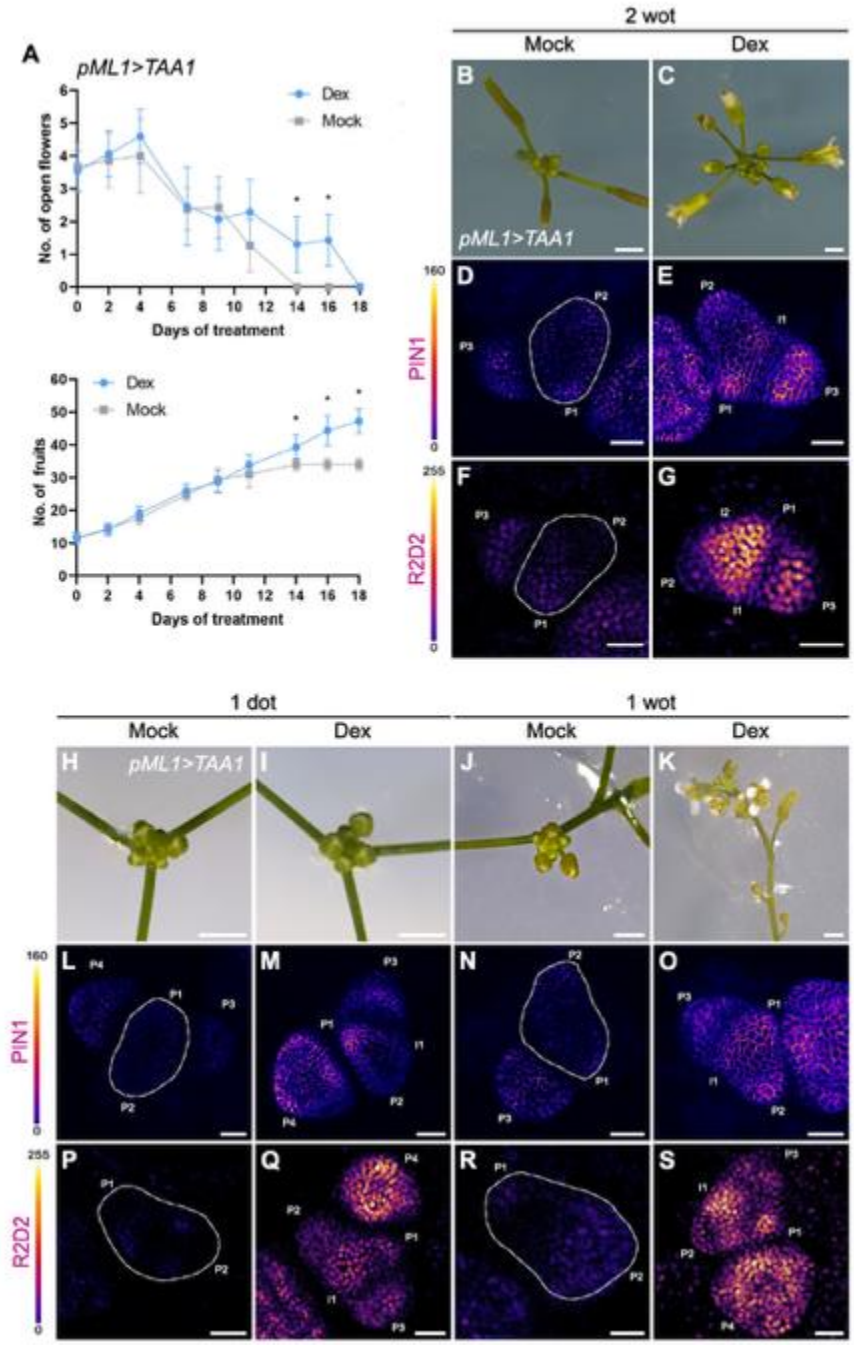

Figure 1. 2. PA is affected by modifications in auxin biosynthesis within the SAM. (A) Quantification of number of open flowers (stages 12-15) in the primary apex (upper) and total number of fertile fruits (stages 16-20) in the primary stem (lower) along the mock and dexamethasone (Dex) (10 µM) treatment in *pML1:GR-LhG4_6xOp:TAA1* transgenic lines. Apices were treated every day from 2 wab. Data are represented as mean ± SD of 15 biological replicates. Asterisks indicate significant differences ($p<0.005$) according to two-tailed Student's test comparing each treatment. (B and C) Apices after 2 weeks of mock (B) and Dex treatment (wot) (C) (or 4 wab). (D-G) *pPIN1:PIN1-GFP* expression (magenta; signal intensity calibration bar) (D and E) and R2D2 expression (magenta; mDII/DII ratio intensity distribution) (F and G) in the shoot apex after 2 weeks of mock (D and F) and Dex treatment (E and G) of apices of

pML1>TAA1 plants. Apices were treated every day from 2 wab. (H-K) Apices of *pML1>TAA1* plants that were in PA after 1 day (H and I) and 1 week (J and K) of mock (H and J) and Dex treatment (I and K). Apices were treated every day from 4 wab (PA). (L-S) Expression of *pPIN1:PIN1-GFP* (magenta; signal intensity calibration bar) (L-O) and R2D2 (magenta; mDII/DII ratio intensity distribution) (P-S) in *pML1>TAA1* apices after 1 day of mock (L and P) and Dex treatment (M and Q) and 1 week of mock (N and R) and Dex treatment (O and S). The white dashed line outlines young primordia (~In-P2) and meristems. Scale bars represent 1 mm (B, C, and H-K) and 20 µm (D-G and L-S). Between 8 and 10 apices were visualized for each reporter, treatment, and time point.

Continuous DAO1-mediated auxin catabolism (*pML1>DAO1*) in active SAMs from ~2 wab caused a quick decay of the flower production rate (2 dot), and Dex-treated apices were arrested after 1 wot, while control apices still produced new buds and flowers (**Figures 1.3A-1.3C**). Moreover, *pML1>DAO1* induction caused changes in PIN1-GFP and R2D2 expression similar to those observed in arrested apices (**Figures 1.1C, 1.1H, 1.3 and S1.4**). After 1 wot, their signal was almost undetectable in Dex-treated SAMs in comparison with control SAMs, where signal levels were still high (**Figures 1.3D-1.3G**). We also analyzed flower and fruit production in the loss-of-function auxin biosynthesis mutant *taa1-1*. In line with our previous results, *taa1-1* mutant plants displayed an earlier decrease of the flower production rate (from 2 wab) in comparison with wild-type plants (from 3 wab) and arrested one week before wild-type plants (**Figure S1.5A**). Moreover, the *taa1-1* mutant produced a lower total number of fruits than control plants (**Figure S1.5B**).

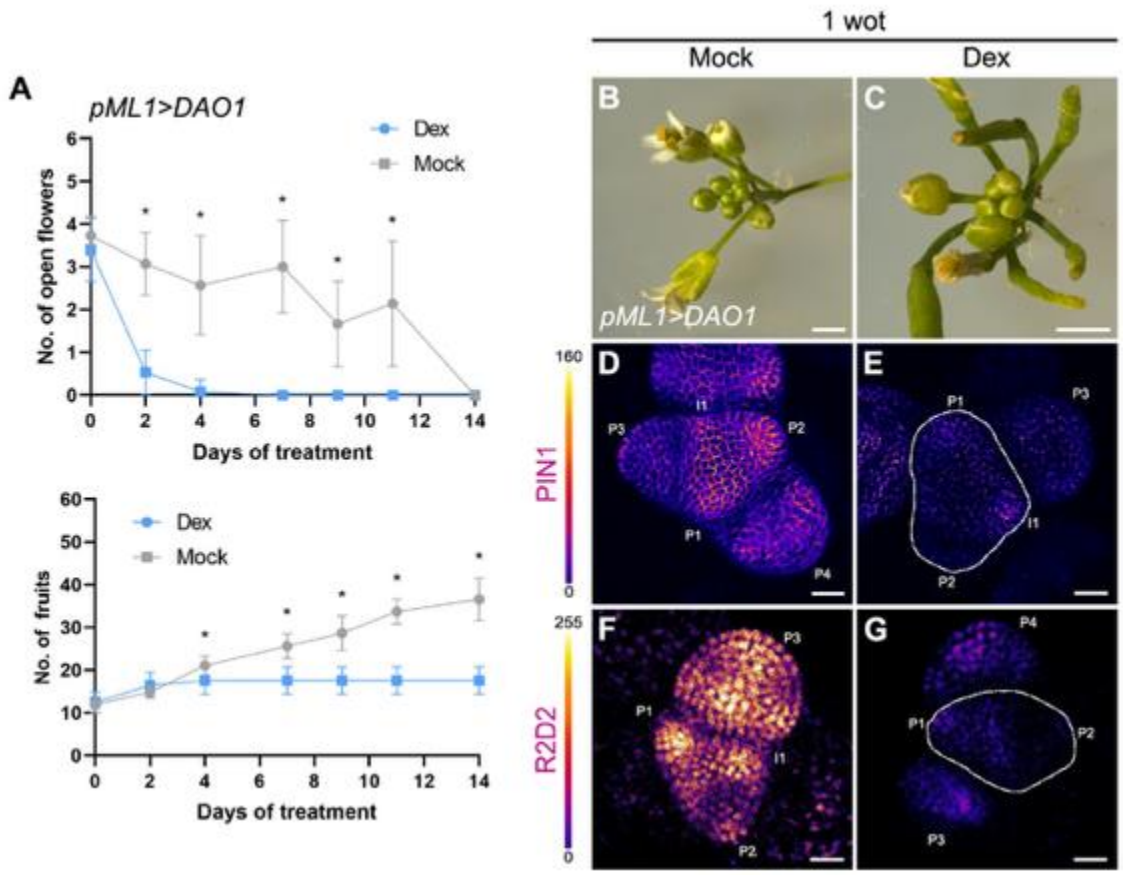

Figure 1. 3. Auxin catabolism within the SAM affects PA. (A) Quantification of number of flowers at stages 12-15 in the primary apex (upper) and total number of fertile fruits (stages 16-20) in the primary stem (lower) along the mock and Dex treatment in *pML1:GR-LhG4_6xOp:DAO1* transgenic lines. Apices were treated every day from 2 wab. Data are represented as mean ± SD of 15 biological replicates. Asterisks indicate significant differences ($p<0.005$) according to two-tailed Student's test comparing each treatment. (B and C) Apices after 1 week of mock (B) and Dex treatment (wot) (C) (or 3 wab). Apices were treated every day from 2 wab. (D-G) Expression of *pPIN1:PIN1-GFP* (magenta; signal intensity calibration bar) (D and E) and R2D2 (magenta; mDII/DII ratio intensity distribution) (F and G) after 1 week of mock (D and F) and Dex treatment (E and G) in the shoot apex of *pML1>DAO1* plants. The white dashed line outlines young primordia (~In-P2) and meristems. Scale bars represent 1 mm (B and C) and 20 µm (D-G). Between 7 and 9 apices were visualized for each reporter and treatment.

Altogether, these data suggest that auxin and auxin-related pathways negatively regulate PA and that PA entails not only stem cell proliferation arrest (Merelo *et al.*, 2022), but also the cessation of auxin-dependent primordia initiation. In addition, the effect of local modifications of auxin content on auxin transport and response and, consequently, on meristem arrest, in the presence of the fruits

(auxin source) (González-Suárez *et al*., 2020; Ware *et al*., 2020; Goetz *et al*., 2021; Walker *et al*., 2023), suggests that additional auxin-dependent mechanisms regulate PA within the SAM apart from the systemic control.

The effect of alterations in auxin levels on PA correlates with changes in CK response

Repression of auxin and CK factors in the SAM was temporally correlated during PA. To further investigate the relationship between auxin and CK, we tested whether CK pathways could depend on changes in auxin levels in the SAM. To this aim, we monitored the CK signaling reporter *TCSn:GFP-ER* (Two-Component signaling Sensor new; TCSn) (Zürcher *et al*., 2013; Liu & Müller, 2017) in the SAM after continuous *TAA1* induction, TAA1-mediated reactivation and continuous *DAO1* induction (**Figure 1.4**). In the SAM, after continuous *TAA1* induction (2 wot) or TAA1-mediated reactivation (1 dot and 1 wot), auxin promoted CK signaling in the boundaries between the SAM and primordia (**Figures 1.4B, 1.4D, 1.4F, 1.4H, 1.4J and 1.4L**; white arrowheads), where it has been proposed that CK and CK-dependent cell divisions trigger boundary formation while new primordia are developing (Merelo *et al*., 2022). TCSn expression was also detected in the organizing center (OC) after these *TAA1* induction assays, but in a few cells (**Figures 1.4B, 1.4D, 1.4F, 1.4H, 1.4J and 1.4L**; green asterisks). No TCSn signal was detected in the boundaries or OC of corresponding arrested control SAMs (**Figures 1.4A, 1.4C, 1.4E, 1.4G, 1.4I and 1.4K**). These results indicate that CK pathways may be downstream of auxin at these domains (boundaries and OC), but that auxin would promote CK-related events in the boundaries and therefore differentiation and organ formation in a higher extent than stem cell proliferation.

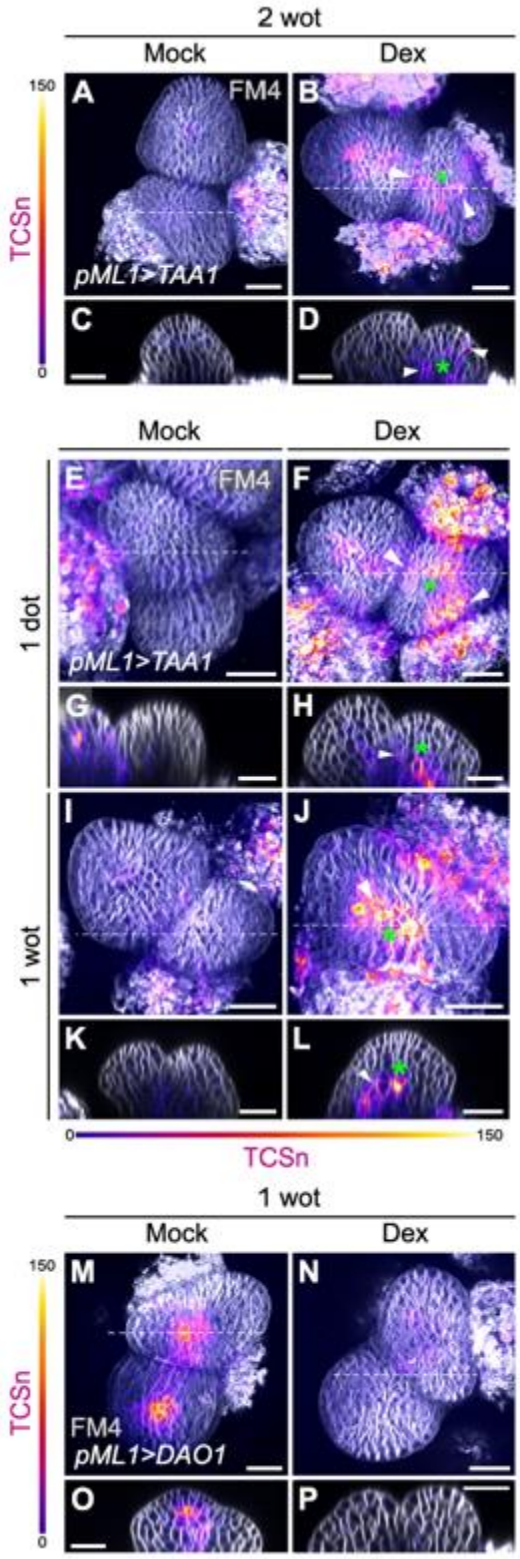

Figure 1. 4. Modifications in auxin levels in the SAM correlate with changes in CK response during PA. (A and B) Confocal projections of the shoot apex showing *TCSn:GFP-ER* expression (magenta; signal intensity calibration bar) after 2 weeks of mock (A) and Dex treatment (wot) (B) of *pML1>TAA1* plants (or 4 wab). Apices were treated every day from 2 wab. (C and D) Corresponding longitudinal sections of the shoot apex along the dashed lines in (A)

and (B). (E-L) *TCSn:GFP-ER* expression (magenta; signal intensity calibration bar) after 1 day (E-H) and 1 week (I-L) of mock (E, G, I and K) and Dex treatment (F, H, J and L) of arrested *pML1>TAA1* apices. Apices were treated every day from 4 wab (PA). Confocal projections of the shoot apex are shown in (E), (F), (I) and (J) and the corresponding longitudinal sections along the dashed lines are shown in (G), (H), (K) and (L). (M and N) Expression of *TCSn:GFP-ER* (magenta; signal intensity calibration bar) after 1 week of mock (M) and Dex (N) treatment in apices of *pML1>DAO1* plants. Apices were treated every day from 2 wab. (O and P) Corresponding longitudinal sections of the shoot apex along the dashed lines in (M) and (N). Cell membranes were highlighted using FM4-64 staining (gray). White arrowheads point to TCSn signal in the boundaries between the SAM and primordia. Green asterisks mark TCSn signal in the organizing center. Scale bars represent 20 µm. A total of 7 to 13 apices were visualized for each treatment and time point.

On the other hand, auxin catabolism through *pML1>DAO1* induction caused CK signaling repression at early stages (1 and 3 dot; **Figure S1.6**) and a complete TCSn expression shutdown after 1 wot either in the boundaries or the OC (**Figures 1.4M-1.4P**). Previous studies have shown that auxin signaling pathways are repressed in shoot apical stem cells to limit differentiation to the peripheral zone, but that low levels of auxin signaling are necessary at the same time to allow stem cell proliferation (Luo *et al*., 2018; Shi *et al*., 2018; Ma *et al*., 2019), which is in line with the induction of TCSn expression in the OC after *TAA1* induction or its repression after *DAO1* induction. Moreover, these studies would also explain that *pML1>TAA1* induction assays had a weaker effect on SAM size and PA delay than CK treatments (Merelo *et al*., 2022). Taken together, our results suggest that auxin and auxin-related factors are necessary to maintain SAMs active along the flowering period and that auxin and CK signaling pathways are closely regulated and interconnected during PA.

CK-mediated alterations in PA are linked to changes in auxin biosynthesis and response

To confirm that local repression of auxin pathways within the SAM leads to meristem arrest and that auxin and CK pathways are tightly co-regulated during PA, we tested the response of auxin-related reporters to CK treatment in the SAM, previously shown to alter meristem arrest (Merelo *et al*., 2022). We treated

with CKs (100 µM N6-benzylaminopurine; BAP) and mock active apices from 2 wab and arrested meristems (4 wab) to prevent and revert meristem arrest, respectively (Merelo *et al*., 2022). Then, we analyzed auxin dynamics in the SAM. The temporal expression pattern of auxin reporters tightly correlated with the previously reported TCSn pattern (Merelo *et al*., 2022). Thus, GFP-TAA1 and R2D2 signal was maintained at high levels in active SAMs treated with BAP after 2 wot (4 wab) and 3 wot (5 wab) (**Figures 1.5B, 1.5D, 1.5F, 1.5H, 1.5J and 1.5L**) and in BAP-reactivated SAMs after 1 dot and 1 wot (5 wab) (**Figures 1.5N, 1.5P, 1.5R, 1.5T, 1.5V and 1.5X**), while the corresponding control SAMs, which were arrested at these time points, displayed very low GFP-TAA1 and R2D2 signal (**Figures 1.5A, 1.5C, 1.5E, 1.5G, 1.5I, 1.5K, 1.5M, 1.5O, 1.5Q, 1.5S, 1.5U and 1.5W**). These results indicate that local CK treatment promotes auxin pathways in the SAM, which correlates with the maintenance or the restoration of SAM activity by these assays (**Figure S1.7**). Our results show again that local treatments in the SAM can bypass the effect of auxin exported from the fruits, suggesting that an additional regulation of PA mediated by auxin pathways may act locally in the SAM. Finally, these experiments suggest that auxin pathways may act downstream of CKs. Together with the regulation of CK response by auxin shown before (**Figure 1.4**), our results point to a close coordination of these two hormones, not only in active SAMs along the flowering period, but also during PA.

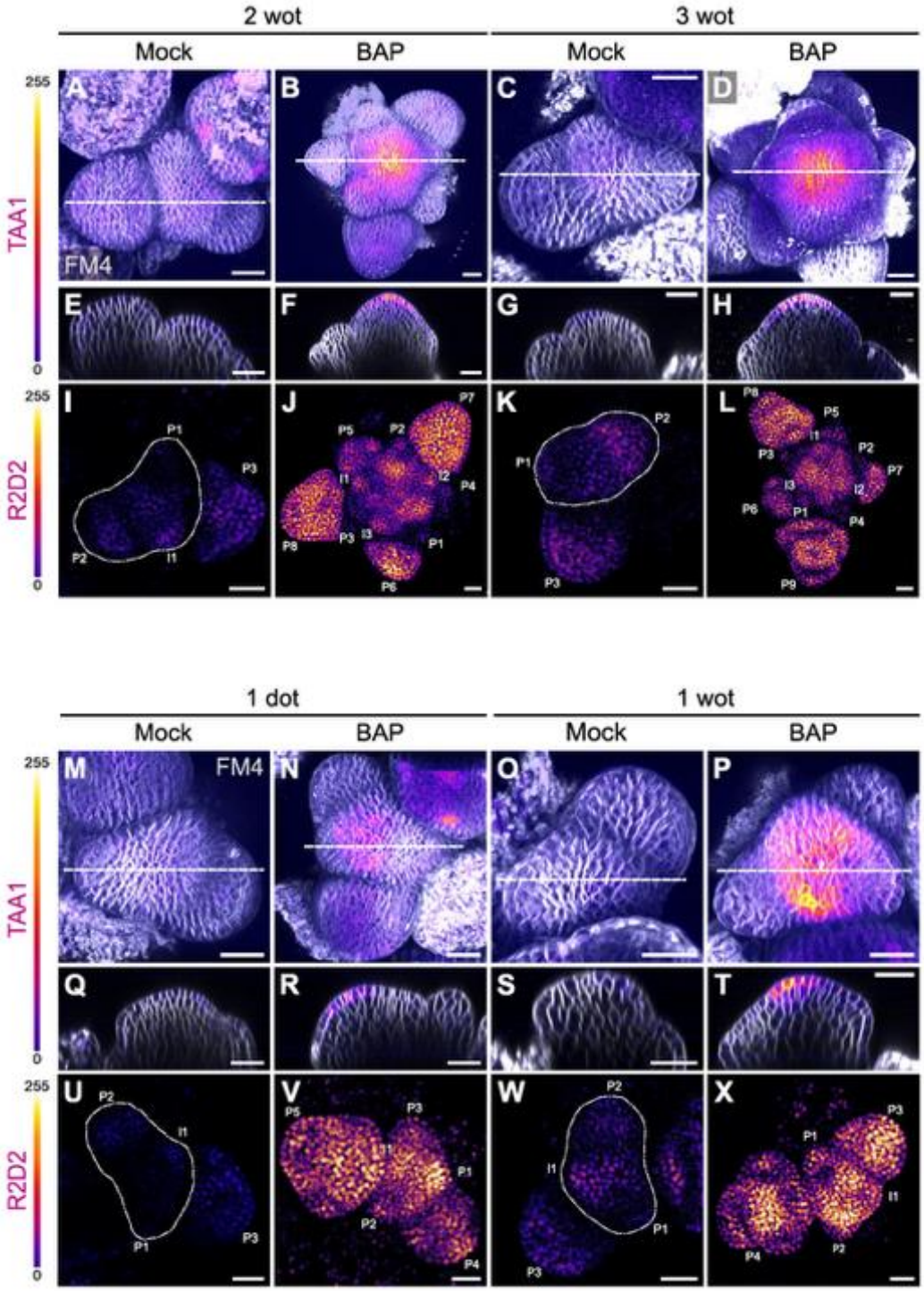

Figure 1. 5. Auxin biosynthesis and response are maintained longer or recovered after CK treatments. (A-D) *pTAA1:GFP-TAA1* expression (magenta; signal intensity calibration bar) in the shoot apex after 2 (A and B) and 3 (C and D) weeks of mock (A and C) and N6-benzylaminopurine (BAP) (100 mM) treatment (wot) (B and D) (or 4 and 5 wab). Apices were treated every 3 days from 2 wab. (E-H) Corresponding longitudinal sections of the shoot apex along the dashed lines in (A)-(D). Cell membranes were highlighted using FM4-64 staining (gray). (I-L) Confocal projections of the shoot apex showing R2D2 expression (magenta; mDII/DII ratio intensity distribution) after 2 (I and J) and 3 (K and L) weeks of mock (I and K) and BAP treatment

(J and L). (M-P) *pTAA1:GFP-TAA1* expression (magenta; signal intensity calibration bar) in the shoot apex after 1 day (M and N) and 1 week (O and P) of mock (M and O) and BAP treatment (N and P). Apices were treated every 3 days from 4 wab (PA). (Q-T) Corresponding longitudinal sections of the shoot apex along the dashed lines in (M)-(P). Cell membranes were highlighted using FM4-64 staining (gray). (U-X) Confocal projections showing R2D2 expression (magenta; mDII/DII ratio intensity distribution) in the shoot apex after 1 day (U and V) and 1 week (W and X) of mock (U and W) and BAP (V and X) treatment. The white dashed line outlines young primordia (~In-P2) and meristems. Scale bars represent 20 µm. Between 5 and 8 apices were visualized for each reporter, treatment, and time point.

Auxin-related pathways may be regulated by FRUITFULL in the SAM during PA

Based on the correlation between auxin and CKs in the control of PA and on the previous reported link between FRUITFULL and CKs (Merelo *et al*., 2022), we analyzed the behavior of auxin reporters in the SAM of *ful-2* mutant plants, which do not undergo PA (**Figure S1.8**). We also used this genetic background to evaluate the significance of auxin pathways in the regulation of the process and whether FUL may promote meristem arrest by repressing auxin factors. We observed that PIN1-GFP, R2D2 and GFP-TAA1 signal in *ful-2* apices was detected and patterned along the flowering period as in active wild-type apices (**Figures 1.1 and 1.6A-1.6R**). PIN1 convergences and R2D2 signal accumulation in the young primordia (~In-P2) of *ful-2* SAMs were clearly detectable at time points equivalent to PA in wild-type plants (4-5 wab) (Merelo *et al*., 2022), where PIN1 and R2D2 signal was almost undetectable (**Figures 1.1C, 1.1H, 1.6C, 1.6D, 1.6I, 1.6J**; white arrowheads). Therefore, the maintenance of the expression level and the spatial pattern of these auxin reporters would explain the continuous organ initiation and differentiation in the apex of *ful-2* mutants (**Figure S1.8**). The number of PIN1 and R2D2-marked primordia decreased 4 and 5 wab, correlating with the decline in flower production in *ful-2* mutants (decline phase; **Figures 1.6C, 1.6D, 1.6I, 1.6J and S1.8A**). The low number of primordia formed is maintained 6 and 7 wab, matching with the low flower production rate at these time points in *ful-2* mutant plants (low proliferation phase; **Figures 1.6E, 1.6F, 1.6K, 1.6L and S1.8A**). Importantly, TAA1 was expressed in

the SAM of *ful-2* plants 4 and 5 wab, while was almost absent in wild-type SAMs 4 wab (arrested) (**Figures 1.1M, 1.1R, 1.6O, 1.6P**; green arrowheads).

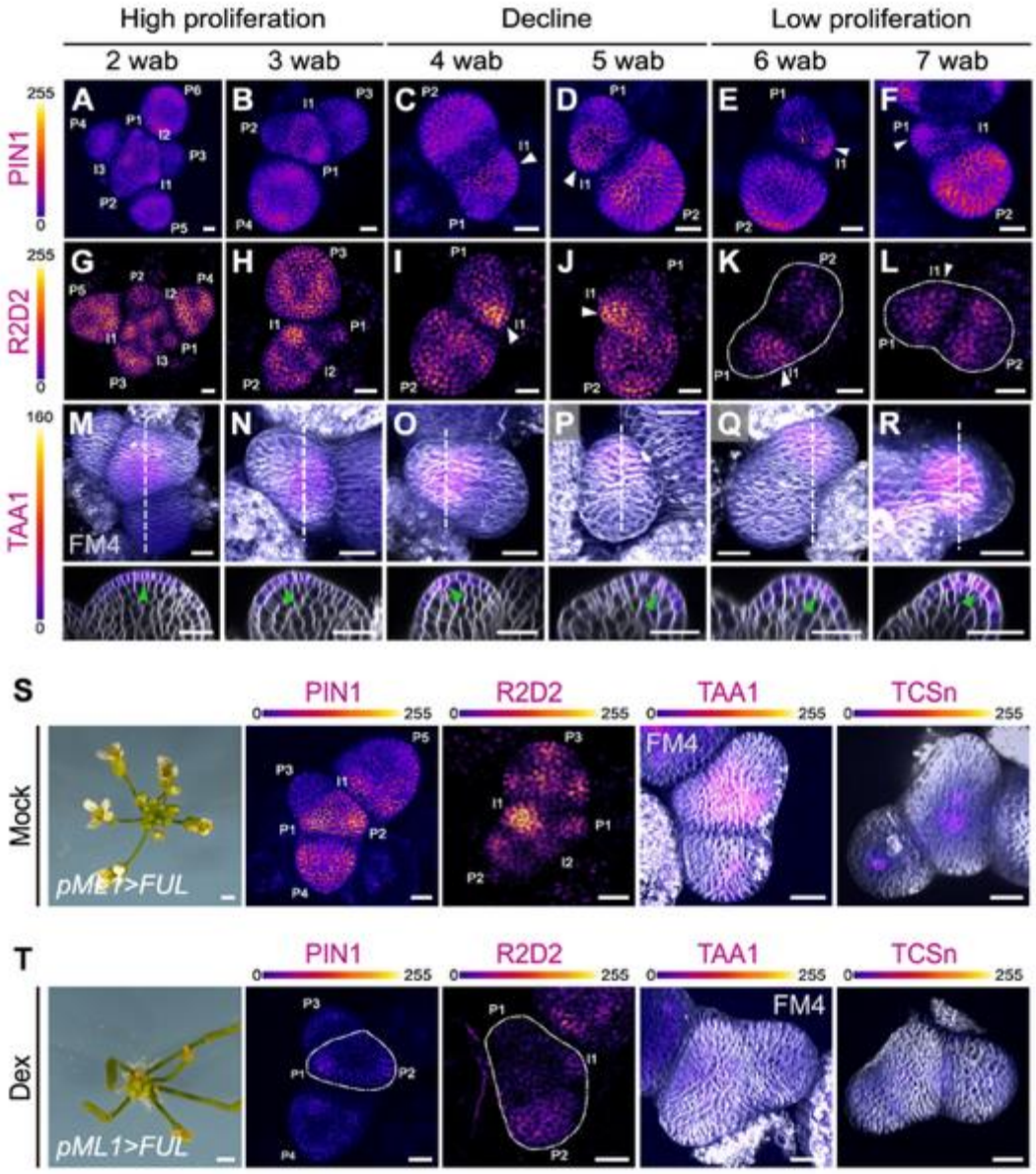

Figure 1. 6. FUL represses auxin-related pathways during PA. (A-L) Expression of *pPIN1:PIN1-GFP* (magenta; signal intensity calibration bar) (A-F) and R2D2 (magenta; mDII/DII ratio intensity distribution) (G-L) in *ful-2* apices 2 (A and G) 3 (B and H), 4 (C and I), 5 (D and J), 6 (E and K) and 7 wab (F and L). White arrowheads point to *pPIN1:PIN1-GFP* and R2D2 signal in the young primordia (~In-P1). (M-R) Expression of *pTAA1:GFP-TAA1* in *ful-2* apices 2 (M) 3 (N), 4 (O), 5 (P), 6 (Q) and 7 wab (R). Corresponding longitudinal sections of the shoot apex along the dashed lines are shown in the lower panels. Green arrowheads point to *pTAA1:GFP-TAA1* expression in the L1. The high proliferation, decline and low proliferation phases are established

based on the changes in the flower production rate of *ful-2* mutant plants. See also Figure S1.8. (S and T) Apices and confocal projections of the shoot apex showing expression of *pPIN1:PIN1-GFP* (magenta; signal intensity calibration bar), R2D2 (magenta; mDII/DII ratio intensity distribution), *pTAA1:GFP-TAA1* (magenta; signal intensity calibration bar) and *TCSn:GFP-ER* (magenta; signal intensity calibration bar) after 1 week of mock (S) and Dex treatment (T) of *pML1>FUL* plants. Apices were treated every day from 2 wab. Cell membranes were highlighted using FM4-64 staining (gray). The white dashed line outlines young primordia (~In-P2) and meristems. Scale bars represent 20 µm (A-R, S and T, last four panels) and 1mm (S and T, first panel). A total of 5 to 13 apices were visualized for each reporter, treatment, or time point.

Furthermore, IAA levels in *ful-2* apices were maintained along the flowering period (**Figure S1.9**). On the other hand, local auxin catabolism in the SAM through *pML1>DAO1* induction in *ful-2* mutant apices 2 wab led to PA. Dex-treated apices displayed a decay of the flower production rate after 2 dot and did not show new open flowers after 1 wot in comparison with mock-treated apices, that contained open flowers (**Figure S1.10A, S1.10B and S1.10E**). In addition, when we increased FUL protein levels locally in the SAM by making use of the same inducible system mentioned above (*pML1:GR-LhG4_6xOp:FUL*; *pML1>FUL*), PA occurred one week after the first Dex treatment either in wild-type or *ful-2* plants, while control plants still produced new flowers in both backgrounds (**Figures 1.6S, 1.6T, S1.10C, S1.10D, S1.10F and S1.10G**). Then, we monitored the effect of *FUL* induction on PIN1, R2D2 and TAA1 reporters in wild-type apices and observed a general decline of signal to undetectable levels in Dex-treated SAMs after 1 wot (**Figure 1.6T**), while in control SAMs signal was still high (**Figure 1.6S**). Taken together, these data suggest that FUL may repress auxin biosynthesis, transport and signaling, and consequently organ initiation, in the SAM leading to PA. In this line, previous chromatin immunoprecipitation sequencing (ChIP-seq) data (Bemer *et al*., 2017; van Mourik *et al*., 2023) indicated that FUL directly represses the expression of an auxin inducible gene (*SAUR10*) and binds auxin biosynthesis (*YUCCA1* [*YUC1*], *YUC2* and *YUC6*), transport (*PIN3*, *PIN4*, *PIN7* and *ABCB19*) and response (*AUXIN RESPONSE FACTOR 2* [*ARF2*], *ARF3/ETTIN* [*ETT*] and *ARF5/MONOPTEROS* [*MP*]) genes. Among these FUL direct targets, we identified in a transcriptomic analysis (GSE29917; unpublished dataset), comparing *ful-1* and wild-type apices at 2 and 4 wab, that *YUC2*, *PIN7*, *ABCB19*, *ARF2* and *ARF3* showed significant

differences in expression (**Table S1.1**). As indicated by the monitorization of PIN1, R2D2 and TAA1 markers in *ful-2* mutants and after *pML1>FUL* induction (**Figure 1.6**), FUL would repress the expression of *YUC2*, *PIN7*, *ABCB19* and *ARF3* at 4 wab (shutdown phase), whereas it would promote the expression of *ARF2*, a potential repressor of the maintenance of SAM cells (Roodbarkelari *et al*., 2015). Moreover, FUL may act as an integrator of the auxin or auxin-dependent signal coming from the fruits because these mutants have fruits and viable seeds, but still auxin pathways and the SAM are active 4-7 wab independently on the systemic source of auxin. Our study does not resolve whether fruit-derived auxin, or a signal dependent on fruit auxin, additionally regulates auxin pathways in the SAM. However, taken together, our results point towards a local regulation within the SAM of auxin-related events that could be mediated directly or indirectly by FUL. Finally, a parallel decrease of TCSn signal was also observed after one week of *pML1>FUL* induction, whereas control SAMs showed higher TCSn signal (**Figures 1.6S and 1.6T**). Our previous work (Merelo *et al*., 2022) showed that FUL may repress CK-related pathways. This previous observation, together with the current data, suggests that both CK and auxin pathways may be tightly regulated by FUL in the SAM.

DISCUSSION

In the last few years, several hormones have been related to the regulation of PA at different levels: by controlling IM arrest (Wuest *et al*., 2016; Martínez-Fernández *et al*., 2020; Merelo *et al*., 2022) and/or floral arrest (Walker *et al*., 2023; Sánchez-Gerschon *et al*., 2024), or through a systemic control from fruits/seeds (González-Suárez *et al*., 2020; Ware *et al*., 2020).

One of the novelties of our current work lies on the evidence of a local role of auxin within the SAM during PA. We have characterized with detailed spatio-temporal resolution the changes in auxin pathways within the SAM at advanced stages of the flowering period and have shown that either auxin biosynthesis, transport or signaling must be repressed in the meristem for PA to initiate and progress. These changes perfectly fit with the previous model that defined two

phases at the end of the flowering period (Merelo *et al*., 2022): (i) decline in SAM activity regulators (i.e., CK-related factors) and flower production, and (ii) shutdown of SAM activity regulators and conspicuous PA (cluster of arrested buds). Thus, a strong decrease of expression of auxin markers correlates with the onset of the first phase (decline; 3 wab). The reduction in the local auxin biosynthesis mediated by TAA1, the almost absence of PIN1 convergences, and the low auxin signaling indicate that probably, at this first phase, no new primordia would be initiated, and then SAM activity would be highly compromised. This correlates with the decline in CK-related events at this stage (Merelo *et al*., 2022). Furthermore, our current data show that the shutdown of *TAA1*, *PIN1* and R2D2 expression in the SAM at the conspicuous PA (4 wab) perfectly matches with the complete inhibition of CK pathways at this phase (Merelo *et al*., 2022). Walker *et al*. (2023) suggested that the fruit-derived auxin would be only involved in floral arrest at the end of the process. However, changes in IM arrest because of modifications of auxin content locally in the SAM, together with the early PA observed in auxin biosynthesis mutants, suggest that local auxin biosynthesis is an alternative mechanism controlling PA. Therefore, our study extends previous knowledge by proposing a local auxin-mediated regulation in the SAM during PA, highlighting the role of local auxin biosynthesis. Interestingly, the induction of local auxin biosynthesis mediated by TAA1 is able to maintain longer SAM activity and reactivate arrested SAMs in the presence of fruits. The similar reactivation achieved by defruiting or by the induction of auxin biosynthesis in the SAM supports the importance of the local auxin-mediated regulation. Different studies have proposed that local auxin biosynthesis is key in maintaining auxin maxima and hence meristem activity (Chen *et al*., 2014; Brumos *et al*., 2018; Yadav *et al*., 2023). Based on these studies and our observations, we propose that fruit-derived auxin is not sufficient to regulate inflorescence activity during PA. Moreover, our data indicate that a simultaneous and coordinated repression of auxin and CK-related pathways is needed for IM arrest, what would lead to the coupled cessation of stem cell differentiation and proliferation processes during PA (**Figure 1.7**). These results, along with the reciprocal effects observed in auxin and CK markers following modifications in CK and auxin content (**Figures 1.4 and 1.5**), respectively, strongly suggest a positive interaction between these

hormones in the control of PA. Previous studies have already put forward an interaction between these two hormones in the SAM, although in highly active SAMs. Besides its main role in stem cell differentiation and organ formation at the peripheral zone (Vernoux *et al*., 2000; Reinhardt *et al*., 2003; Heisler *et al*., 2005), auxin participates in stem cell proliferation in the center of the SAM by positively regulating the CK signaling cascade (Zhao *et al*., 2010; Luo *et al*., 2018; Ma *et al*., 2019). Auxin promotes the expression of *MP/ARF5* (Bhatia *et al*., 2016; Krogan *et al*., 2016), which directly represses the transcription of *DORNROSCHEN* (*DRN*), a positive regulator of *CLAVATA3* (*CLV3*) expression (Luo *et al*., 2018). CLV3 is a peptide that controls the levels of stem cell proliferation through the repression of *WUS*, which promotes CK signaling (Leibfried *et al*., 2005; Gordon *et al*., 2009; Meng *et al*., 2017). Additionally, MP directly represses the expression of CK signaling negative regulators (*type-A ARABIDOPSIS RESPONSE REGULATORs*) (Zhao *et al*., 2010). Hence, during PA, the decrease in auxin content in the center of the SAM, because of *TAA1* repression, together with low auxin signaling may lead to a decline in *MP* expression, which would ultimately cause CK signaling repression. On the other hand, local CK treatment of active apices maintained the expression of auxin markers in the SAM along the extended reproductive phase and reactivated auxin pathways and SAM activity in arrested apices. In active SAMs, it has been described that although WUS mainly restricts auxin responses to avoid stem cell differentiation in the center of the SAM, it also maintains low auxin signaling levels, which are required for stem cell maintenance (Ma *et al*., 2019). CKs promote *WUS* expression (Meng *et al*., 2017) and we previously showed that a decrease in CK signaling correlated with a reduction in WUS levels during PA (Merelo *et al*., 2022). Based on these studies and our data, we hypothesize that the decline in auxin pathways during PA may be also due to the decline in CK signaling and then in WUS levels. In addition to this positive and mutual regulation of auxin and CK pathways in the center of the SAM, our results also showed this correlation at the PZ (flower primordia and meristem-primordia boundaries) (**Figure 1.7**). Therefore, our study expands the observation of a positive interaction between these two hormones to another developmental process in the SAM (i.e., PA), but also to different SAM regions. How this auxin-CK interaction occurs at the molecular level in all these SAM domains constitutes an additional

point to be further studied in the context of PA, but also specifically in the primordia and boundaries during organ development in active SAMs. The analysis of auxin markers in *ful-2* mutants and after *FUL* induction suggests that FUL may promote PA by repression of auxin pathways locally in the SAM (**Figure 1.7**). Moreover, because these pathways are active and IAA levels are maintained in *ful-2* apices in the presence of fruits/seeds, FUL may act as an integrator of the fruit/seed systemic signals in the regulation of auxin-related pathways in the SAM during PA. Our previous work showed a transient decrease of CK markers in *ful* SAMs (3-5 wab) similar to wild-type SAMs from 3 wab to the PA, although milder, and the absence of a complete shutdown at the time points equivalent to the PA (4-5 wab) (Merelo *et al*., 2022). Based on this, we proposed that FUL, together with additional factors or the seed signals, would act as a mild repressor of CK-related events during the decline phase and as a strong repressor in the shutdown phase of PA. Differently from CK markers, auxin reporters maintained similar signal levels along the flowering period in *ful-2* SAMs (2-7 wab) in comparison with wild-type plants (2-4 wab). This could indicate that the regulation of these auxin pathways locally in the SAM strongly depends on FUL activity during the two PA phases rather than on fruit/seed signals or additional factors.

Importantly, our work shows that local auxin biosynthesis, transport and response are fundamental in IM arrest control and that FUL regulates these pathways to promote PA. In addition, auxin and CK pathways are coupled in the SAM during PA. Our results suggest that the repression of both hormone pathways and the disruption of the balance between them leads to meristem arrest (**Figure 1.7**). But still there are interesting aspects to unveil that will need additional investigation in the future. For instance, it remains unclear how FUL integrates specific systemic signals in the SAM upstream of auxin- and CK-related regulatory events, and whether FUL directly modulates auxin and CK factors, as it has been shown in distinct physiological contexts (Bemer *et al*., 2017; van Mourik *et al*., 2023), and which specific factors. Moreover, it is not known whether the regulation of these pathways occurs exclusively through the FUL-AP2 module (Balanzà *et al*., 2018; Martínez-Fernández *et al*., 2020), or what potential additional factors confer the different behavior between auxin and CK pathways. Among these additional factors, reactive oxygen species may function upstream

of CK pathways, as they have been previously proposed as regulators of *WUS* expression during PA (Wang *et al*., 2020, 2022). Finally, besides the biological importance of this process in plants, the control of PA entails agronomic interest in monocarpic crop species. Thus, a comprehensive understanding of the molecular mechanisms controlling this process could facilitate the development of biotechnological and agronomical strategies. Based on our current study, we propose that auxin pathways could be potential targets for future biotechnological programs aimed at controlling PA, not only to enhance crop yield but also to adapt production to the environmental conditions.

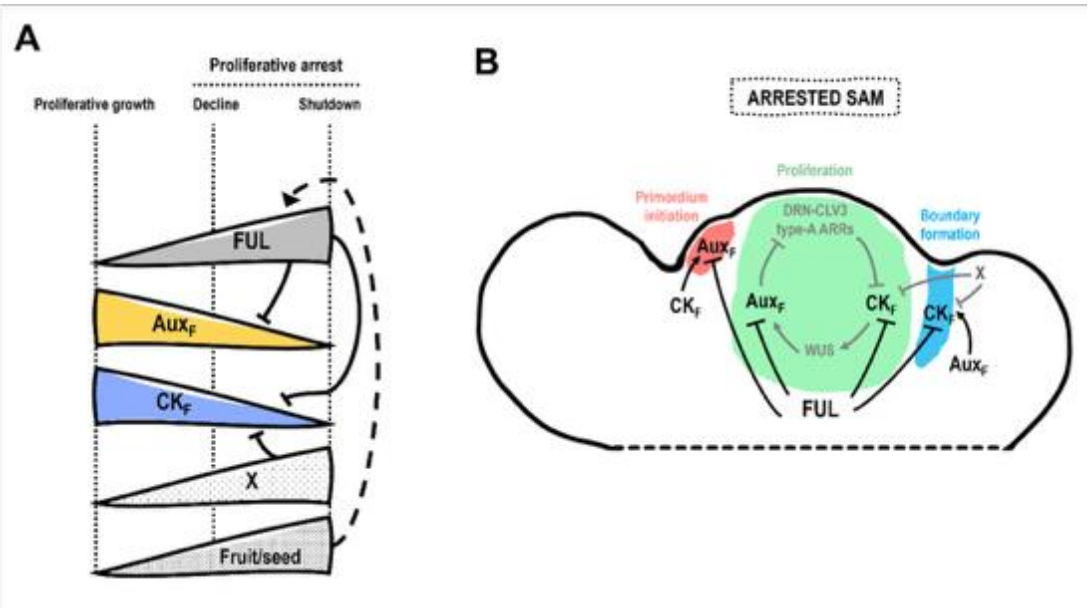

Figure 1. 7. Auxin-related factors regulate meristem arrest, and their expression pattern correlates with the temporal framework of CK-dependent changes promoting PA. (A) Repression of auxin-related factors (Aux_F) such as TAA1, involved in auxin biosynthesis (Stepanova *et al*., 2008; He *et al*., 2011), PIN1, which controls auxin transport (Benková *et al*., 2003), and auxin signaling (R2D2) (Liao *et al*., 2015), triggers meristem arrest during the PA process. The repression of Aux_F in the SAM correlates with the temporal framework previously described (Merelo *et al*., 2022) that differentiates two phases during PA based on changes in CK-related factors (CK_F; CK response, CYCB1;2-dependent mitosis, WUS and SAM size). During the first phase (decline; 3 wab), the levels of Aux_F and CK_F decrease, and during the second phase (shutdown; 4 wab; conspicuous PA), these factors are completely blocked. Both Aux_F and CK_F are regulated by FUL, whose relative contribution, first as a mild repressor and later as a strong repressor, leads to the differentiation of these two phases during PA. FUL

may contribute, probably together with fruit/seed signals, by integrating such signals within the SAM. Unlike CK-related pathways, that could be regulated by other factors (X), auxin-related events would be mainly controlled by FUL in the SAM. (B) Aux_F mainly promote stem cell differentiation and organ initiation in the periphery of proliferative SAMs (Vernoux *et al*., 2000; Reinhardt *et al*., 2003; Heisler *et al*., 2005), but also stem cell proliferation in the center, although in a less extent (Zhao *et al*., 2010; Luo *et al*., 2018; Ma *et al*., 2019). Our results suggest that SAM activity arrest during PA entails a coordinated negative regulation of these two processes (differentiation and proliferation) via the potential simultaneous repression of Aux_F and CK_F, which are interconnected at the same time in different domains of the SAM (center, primordia and meristem-primordia boundaries). As previously shown in active SAMs, this interconnection during PA could involve MP, DRN, CLV3, type-A ARRs or WUS, that are part or act downstream of the Aux_F and CK_F described in our studies.

MATERIAL AND METHODS

Plant material

All *Arabidopsis thaliana* plants used in this work were ecotype Columbia-0 (Col-0) and Landsberg *erecta* (L*er;* Table S1.1). The mutant and reporter lines used in this study have been previously described: *ful-2* (Ferrándiz *et al*., 2000), *ful-1* (Table S1.1) (Gu *et al*., 1998), *taa1-1*, *pTAA1:GFP-TAA1* (Stepanova *et al*., 2008; He *et al*., 2011)*, pPIN1:PIN1-GFP* (Benková *et al*., 2003)*,* R2D2 (Liao *et al*., 2015) and *TCSn:GFP-ER* (Zürcher *et al*., 2013). *pTAA1:GFP-TAA1, pPIN1:PIN1-GFP* and R2D2 lines were crossed to *ful-2* and the assays were carried out with F3 homozygous plants.

Plants were grown on soil (a mixture of sphagnum:perlite:vermiculite, 2:1:1) at 21ºC under LD conditions (16 h light/8 h dark) and illuminated by cool-white fluorescent lamps (150 µE m^{-2} s^{-1}). Before germination, seeds were stratified on soil at 4 ºC during 3 days in dark conditions. Plants were watered with a dilution of the Hoagland's nutrient solution 1.

Construction of transgenes and plant transformation

For dexamethasone-inducible expression, the p6xOp/GR-LhG4 two component system was used (Craft *et al*., 2005). To generate the constructs *pML1:LhG4-*

GR_6xOp:TAA1 (*pML1>TAA1*), *pML1:LhG4-GR_6xOp:DAO1* (*pML1>DAO1*) and *pML1:LhG4-GR_6xOp:FUL* (*pML1>FUL*), the coding sequence of *TAA1, DAO1* and *FUL* was amplified and cloned into the PCR8 vector (pCR8/GW/TOPO TA Cloning Kit; Invitrogen) using the next primers: 5'-ATGGTGAAACTGGAGAACTCG-3' (TAA1-F; forward), 5'-CTAAAGGTCAATGCTTTTAATGAGC-3' (TAA1-R; reverse), 5'-ATGGGGGAACTAAACGGAGTC-3' (DAO1-F; forward), 5'-TCATTTATCTAGTCCTGCATGGG-3' (DAO1-R; reverse), 5'-ATGGGAAGAGGTAGGGTTCAGC-3' (FUL-F; forward), 5'-CTACTCGTTCGTAGTGGTAGGACG-3' (FUL-R; reverse). Then, the coding sequences were cloned into the destination pOpOn2.1 binary vector (Moore *et al*., 2006) by LR recombination (Invitrogen). A 3.4 kb promoter region of the *ML1* gene (*AT4G21750*) (Sessions *et al*., 1999) was previously introduced into the binary vector by In-Fusion cloning (Takara) using the next primers: 5'-AGCTTATCAAAGAAAAAACAAGAAC-3' (pML1-F; forward) and 5'-CACACCCGGTGGATTCAG-3' (pML1-R; reverse). We decided to use the *ML1* promoter rather than a constitutive promoter to avoid extreme or pleiotropic effects and to promote alterations mainly in the meristem. Also, because auxin is a mobile molecule that can diffuse from the L1 and most of the auxin activity on organ initiation begins in the L1 (Reinhardt *et al*., 2000; Kierzkowski *et al*., 2013; Bhatia *et al*., 2016; Galvan-Ampudia *et al*., 2020; Mellor *et al*., 2020), this system ensures proper auxin function in the SAM. Arabidopsis plants were transformed with *Agrobacterium tumefaciens* C58 using the floral dip method (Clough & Bent, 1998). Homozygous T3 transgenic lines carrying a single transgene insertion were selected on Murashige and Skoog (MS) (Duchefa-Biochemie) plates containing kanamycin (Duchefa-Biochemie).

Flower and fruit number quantification

Total number of fertile fruits (stages 16-20) in the primary stem and flowers in stages 12-15 present at each time point in the primary apex were quantified. For wild-type, *ful-2* and auxin biosynthesis mutant plants quantification was performed every week from 0 to 7 wab. We considered 0 wab the time when the cluster of flower buds becomes visible after floral transition. Quantifications were

performed in at least 16 plants of each genotype. For BAP and dexamethasone (Dex) treatments, quantification was carried out every 2 days from 2 to 4 wab or the moment of PA in at least 15 plants of each treatment.

Reactivation, chemical and hormonal treatments

For defruiting-mediated reactivation assays, we removed the fruits in the main stem as well as all the rosette-leaf and cauline-leaf branches. For each reactivation assay, 20 plants of each genotype were used.

For the Dex induction experiments in the SAM, a 10 µL drop of 10 µM Dex solution (Sigma; stock solution was prepared in ethanol) containing 0.03% [v/v] Tween-20 (Sigma-Aldrich) was applied to the shoot apices. Mock solution (ethanol and 0.03% [v/v] Tween-20) was used to treat control apices. Active apices of plants from 2 wab (experiments to delay or promote PA) or arrested apices (4 wab) (experiments of reactivation) were treated everyday with Dex or mock solution. For live imaging assays, the lines treated with Dex/mock that we used were GFP-TAA1 containing the *pML1>FUL* construct and PIN1-GFP, R2D2 and TCSn containing the *pML1>TAA1*, *pML1>DAO1* or *pML1>FUL* constructs. Quantification of flowers and fruits in the primary apex of Dex and mock-treated plants (*pML1>TAA1* in Col-O background and *pML1>DAO1* and *pML1>FUL* in Col-0 and *ful-2* background) was carried out as described above.

The treatments with CK (100 µM N6-benzylaminopurine, BAP; Duchefa-Biochemie) were performed as described in Merelo *et al*. (2022). GFP-TAA1 and R2D2 lines treated with BAP/mock were used for live imaging assays.

Quantification of IAA

Apices of Col-0 and *ful-2* plants were collected 2 and 4 wab and 1 wad or 6 wab. Flowers and older buds were carefully removed with clean tweezers. Three biological replicates containing 20-25 apices were harvested and analyzed. Plant material (about 50 mg) was resuspended in 80% (v/v) methanol and 1% (v/v) acetic acid including [$^{2}H_{5}$]indole-3-acetic acid [D-IAA] internal standards (OlChemIm) and mixed by shaking during 1 h at 4ºC. IAA levels were quantified as described in Seo *et al*. (2011).

Confocal microscopy and image analysis

Live imaging analyses in Figures 1.1 and 1.6A-1.6R and R2D2 imaging experiments were performed on a Zeiss LSM780 confocal microscope (Zeiss, Germany) using a water-dipping 40X objective. For the rest of the experiments, live imaging analyses were performed on a Stellaris 8 FALCON confocal microscope (Leica, Germany) using a water-dipping 25X objective. Dissection of shoot apices, preparation for imaging and FM4-64 staining (Invitrogen) were previously described in Merelo *et al*. (2022). In the Zeiss LSM780 confocal microscope, GFP was imaged using an argon laser with an excitation wavelength of 488 nm together with 499-527 nm collection. FM4-64 was excited with the argon laser (488 nm) and collected at 666-759 nm. Venus was imaged using an argon laser (excitation wavelength of 514 nm) while tdTomato was imaged using a DPSS 561-10 laser (excitation wavelength of 561 nm). In the Stellaris 8 FALCON confocal microscope, GFP and FM4-64 were imaged using a White Light Laser (WLL, Supercon) emitting at 488 nm together with the corresponding collection settings mentioned above. To image GFP/FM4-64 and Venus/tdTomato combinations, we used sequential scanning in line-scan mode. For all the samples in each experiment, GFP, Venus and tdTomato gain were set up equally. We used a resolution of 12-bit depth, a Z step of 0.8 µm and a line average of 2 for Z stack acquisition. For the analysis of the confocal stacks, we used ImageJ (FIJI, http://fiji.sc/) (Schindelin *et al*., 2012), that allowed to obtain maximum intensity projection images, longitudinal section images, and the fluorescence intensity scale (signal heat-map). Brightness was modified equally for all the samples in every assay to properly visualize GFP-TAA1 (Figures 1.1 and 1.6M-1.6R), PIN1-GFP (Figures 1.2, 1.3, 1.6S and 1.6T) and TCSn (Figure 1.4). Ratio-metric calculations for the R2D2 auxin sensor were performed using ImageJ as described in Bhatia *et al*. (2019).

Statistical analyses

All statistical analyses were performed using graphPad Prism 9 software (https://www.graphpad.com). Significance of data was determined by two-tailed Student's t-test.

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SUPPLEMENTAL INFORMATION

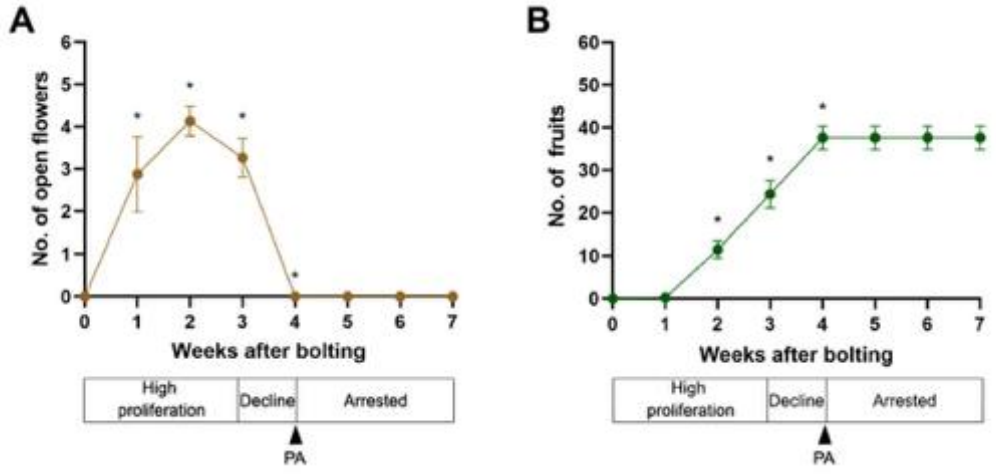

Figure S1. 1. Flower and fruit production during the flowering period in Col-0 wild-type plants. (A) Number of flowers at stages 12-15 in the primary apex of wild-type plants from 1 to 7 weeks after bolting (wab). (B) Total number of fertile fruits (stages 16-20) in the primary stem in wt plants from 1 to 7 wab. Data are represented as mean ± SD of 16 biological replicates. Asterisks indicate significant differences ($p < 0.005$) according to two-tailed Student's test comparing each time point to the previous one.

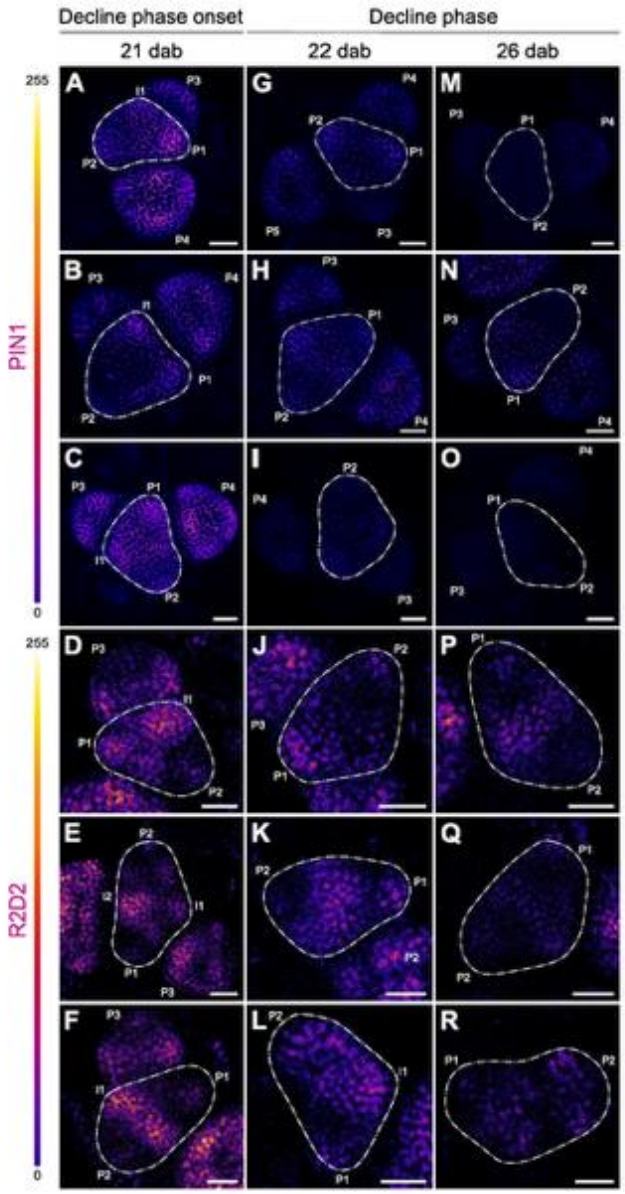

Figure S1. 2. Changes in PIN-GFP and R2D2 signal during the decline phase of PA. (A-R) Confocal projections of three different shoot apices showing the expression of *pPIN1:PIN1-GFP* (magenta; signal intensity calibration bar) (A, B, C, G, H, I, M, N and O) and R2D2 (magenta; mDII/DII ratio intensity distribution) (D, E, F, J, K, L, P, Q and R) at 21 (3 wab or the onset of the decline phase of PA) (A-F), 22 (G-L) and 26 days after bolting (dab) (M-R). The signal decreased markedly at 3 wab compared to 2 wab and continued to decline in the following days until the shutdown phase (4 wab; cluster of arrested buds) (see also Figure 1). Pn, flower primordia that have grown out from the meristem; In, incipient primordia. Both Pn and In are numbered in order of appearance from youngest (P1 or I1) to oldest (P4). The white dashed line outlines young primordia (~In-P2) and meristems. Scale bars represent 20 µm. A total of 6 to 10 apices were visualized for each reporter and time point.

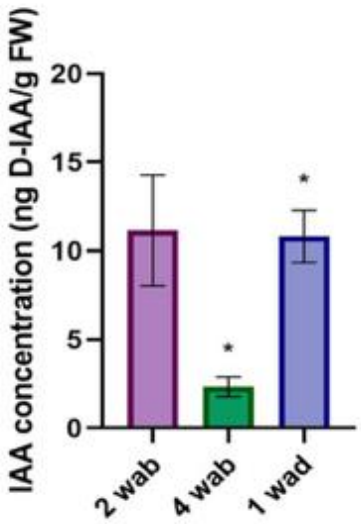

Figure S1. 3. Auxin content in the shoot apex decreased during PA. IAA levels in active (2 wab), arrested (4 wab) and reactivated (1 wad) apices. Data are represented as mean ± SD of 3 biological replicates. Asterisks indicate significant differences ($p < 0.05$) according to two-tailed Student's test comparing each time point to the previous one. FW, fresh weight.

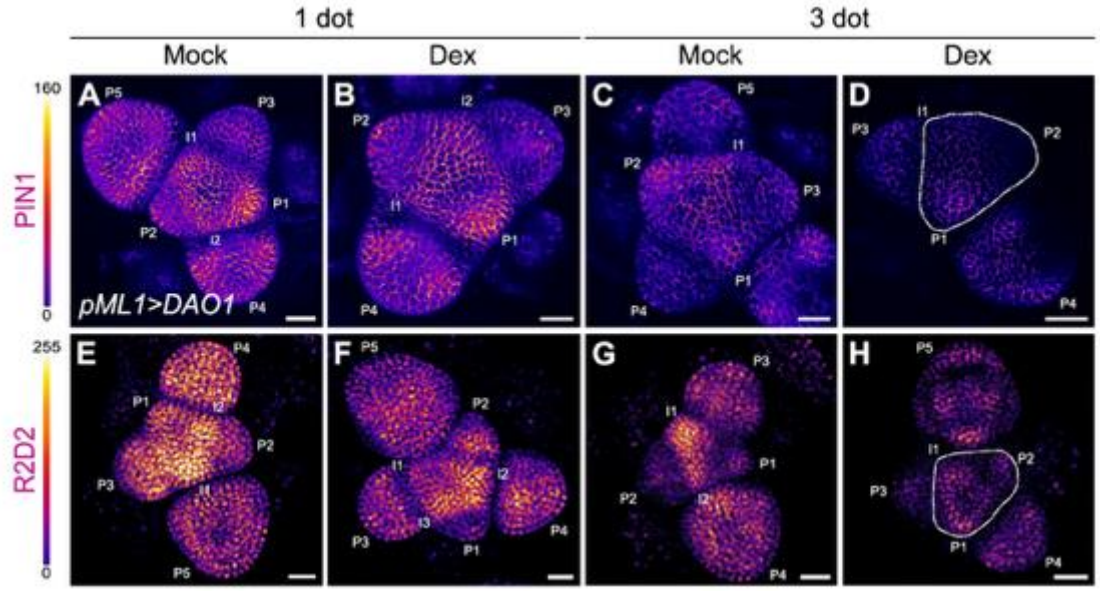

Figure S1. 4. Changes in PIN1-GFP and R2D2 expression during short time points after pML1>DAO1 induction. (A-D) *pPIN1:PIN1-GFP* expression (magenta; signal intensity calibration bar) in the shoot apex after 1 and 3 days of mock (A and C, respectively) and dexamethasone (Dex) (10 μM) treatment (dot) (B and D, respectively). (E-H) R2D2 expression (magenta; mDII/DII ratio intensity distribution) in the shoot apex after 1 and 3 days of mock (E and G, respectively) and Dex treatment (F and H, respectively). Apices were treated every day from 2 wab. Pn, flower primordia that have grown out from the meristem; In, incipient primordia. Both Pn and In are numbered in order of appearance from youngest (P1 or I3) to oldest (P5 or I1). The white dashed line outlines young primordia (~In-P2) and meristems. Scale bars represent 20 μm. Between 7 and 9 apices were visualized for each reporter and treatment.

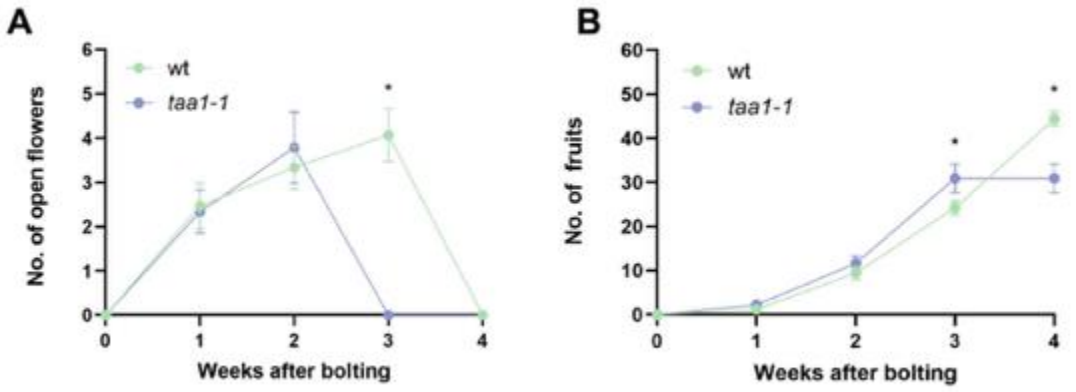

Figure S1. 5. Fruit and flower production in loss-of-function auxin biosynthesis mutants. (A) Number of flowers at stages 12-15 in the primary apex of wild-type and *taa1-1* mutant plants 1 to 4 weeks after bolting. (B) Total number of fertile fruits (stages 16-20) in the primary stem in wt and *taa1-1* mutant plants 1 to 4 weeks after bolting. Data are represented as mean ± SD of 16 biological replicates. Asterisks indicate significant differences ($p<0.005$) according to two-tailed Student's test comparing *taa1-1* and wt plants.

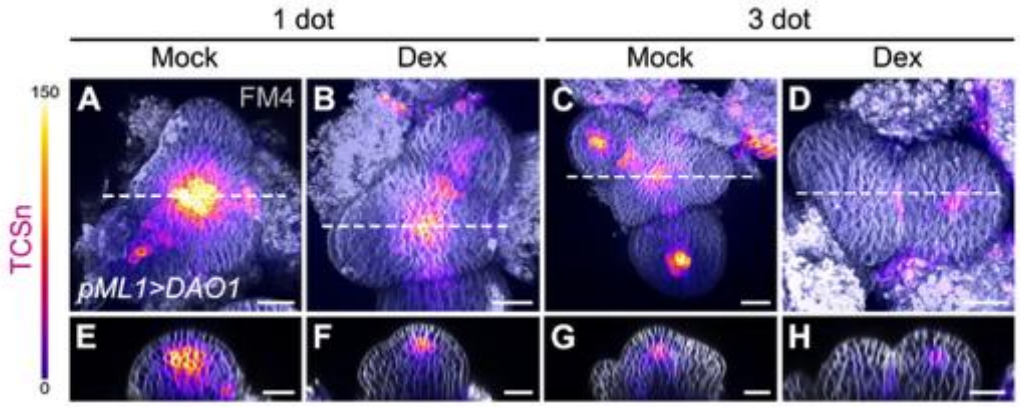

Figure S1. 6. TCSn expression at early stages after *pML1>DAO1* induction. (A-D) Confocal projections of shoot apices showing *TCSn:GFP-ER* expression (magenta; signal intensity calibration bar) after 1 and 3 days of mock (A and C, respectively) and Dex treatment (dot) (B and D, respectively). Apices were treated every day from 2 wab. (E-H) Corresponding longitudinal sections of the apices along the dashed lines in (A)-(D). Cell membranes were highlighted using FM4-64 staining (gray). Scale bars represent 20 µm. Between 7 and 9 apices were visualized for each reporter and treatment.

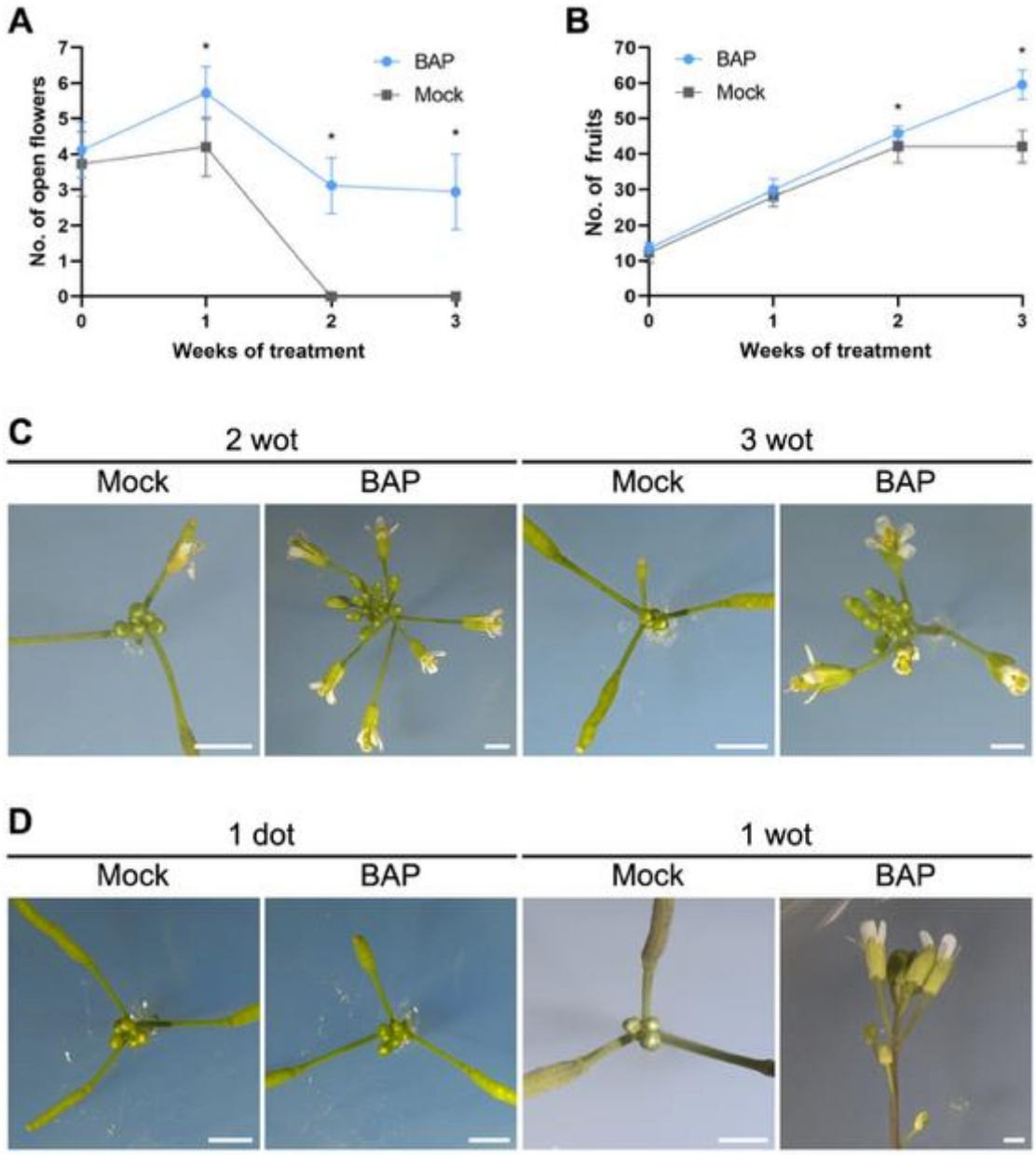

Figure S1. 7. CKs treatments prevent and revert PA. (A and B) Quantification of number of open flowers (stage 12-15) in the primary apex (A) and total number of fertile fruits (stages 16-20) in the primary stem (B) along the mock and BAP treatment. Data are represented as mean ± SD of 16 biological replicates. Asterisks indicate significant differences ($p<0.005$) according to two-tailed Student's test comparing each treatment. (C) Apices after 2 and 3 weeks of mock and N6-benzylaminopurine (BAP) (100mM) treatment (wot) (or 4 and 5 wab). (D) Apices after 1 day and 1 week of mock and BAP treatment. Apices were treated every 3 days from 2 (A-C) or 4 wab (PA) (D). Scale bars represent 1 mm.

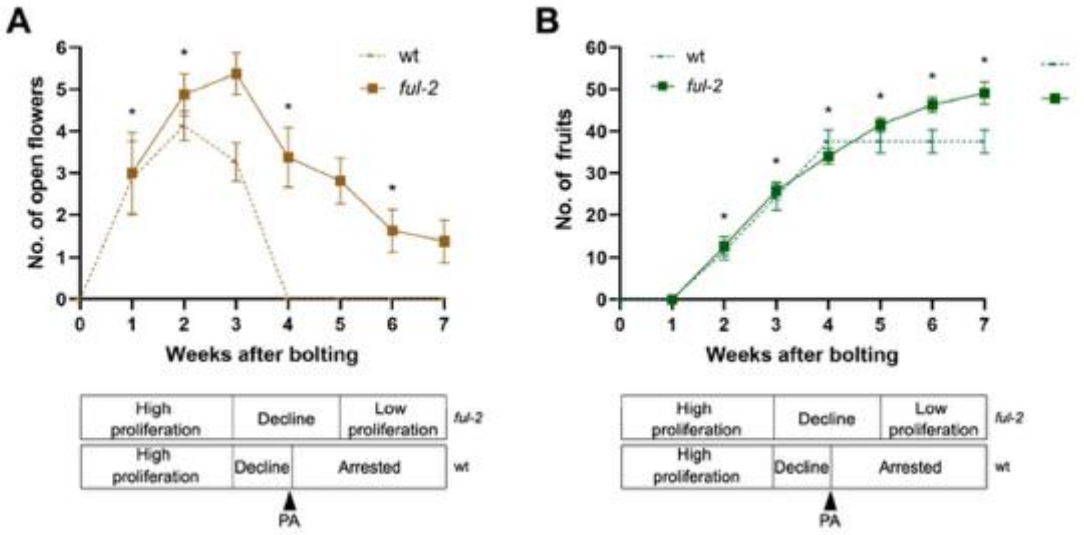

Figure S1. 8. Quantification of flower and fruit production in *ful-2* mutant plants. (A) Number of flowers at stages 12-15 in the primary apex of *ful-2* mutant plants 1 to 7 weeks after bolting. (B) Total number of fertile fruits (stages 16-20) in the primary stem in *ful-2* mutant plants 1 to 7 weeks after bolting. Data are represented as mean ± SD of 16 biological replicates. Asterisks indicate significant differences ($p < 0.005$) according to two-tailed Student's test comparing each time point to the previous one of *ful-2* mutant plants. Wild-type data are also shown (dashed line).

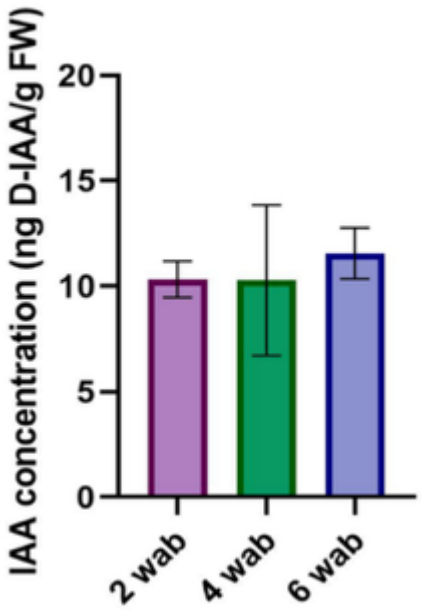

Figure S1. 9. IAA levels are maintained in apices of *ful-2* mutant plants. IAA levels in apices 2, 4 and 6 wab. Data are represented as mean ± SD of 3 biological replicates. No significant differences ($p < 0.05$) according to two-tailed Student's test comparing each time point to the previous one. FW, fresh weight.

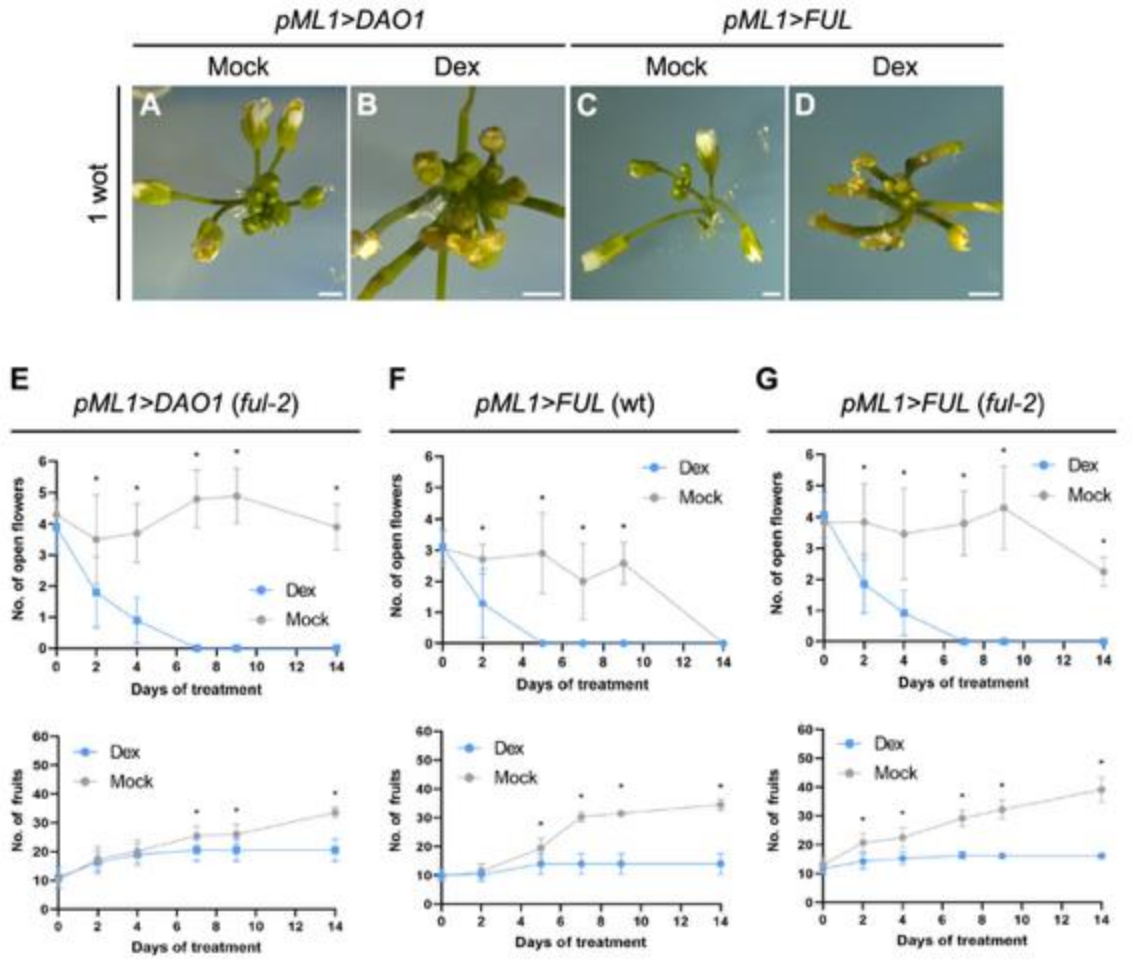

Figure S1. 10. *ful-2* mutants arrest after auxin inactivation or *FUL* induction. (A and B) Apices of *ful-2* mutant plants containing the *pML1>DAO1* construct after 1 week of mock (A) and Dex treatment (wot) (B) (or 3 wab). (C and D) Apices of *ful-2* mutant plants transformed with the *pML1>FUL* construct after 1 week of mock (C) and Dex treatment (D). Apices were treated every day from 2 wab. Scale bars represent 1 mm. (E-G) Quantification of number of open flowers (stages 12-15) in the primary apex (upper) and total number of fertile fruits (stages 16-20) in the primary stem (lower) along the mock and Dex treatment in *pML1>DAO1* (E) or *pML1>FUL* transgenic lines (F and G). Apices were treated every day from 2 wab. Data are represented as mean ± SD of 15 biological replicates. Asterisks indicate significant differences ($p < 0.005$) according to two-tailed Student's test comparing each treatment.

Table S1. 1. Auxin-related genes that show significant differences in a transcriptomic analysis of wt and *ful-1* apices (Ler). The FC and the P-value are indicated for each differentially expressed gene comparing wt and *ful-1* apices at 2 and 4 wab. Four biological replicates (4 pools of 22 shoot apices) were used for each genotype and time point. Blue colour indicates negative FC (genes promoted by FUL) and yellow colour represents positive FC (genes repressed by FUL). FC, log_2 fold change; P-value, P-adjusted value; –, no differential gene expression.

SYMBOL	TAIR_ID	2 wab		4 wab	
		FC	P-value	FC	P-value
YUC2	AT4G13260	-	-	2,47	9,95E-07
PIN7	AT1G23080	-	-	1,03	3,71E-21
ABCB19	AT3G28860	-	-	1,35	1,52E-25
ARF2	AT5G62000	-	-	-1,31	3,64E-63
ARF3	AT2G33860	-	-	1,39	3,96E-20

Transcriptional control of proliferative arrest by FRUITFULL in Arabidopsis: A role for jasmonic acid-related factors

INTRODUCTION

In *Arabidopsis thaliana* (Arabidopsis), the end of the flowering period, also known as proliferative arrest (PA) (Hensel *et al*., 1994), ensures reproductive success, optimizing nutrient allocation for seed and fruit production before plant senescence. This process involves two distinct events: first, the cessation of inflorescence meristem (IM) activity and primordia initiation (IM arrest), and second, the developmental arrest of the unopened floral buds already formed at the moment of IM arrest (floral arrest). Consequently, inflorescences that have reached PA are characterized by a cluster of non-developing buds at the apex of the plant (Merelo *et al*., 2022; Walker *et al*., 2023). Despite the relevance of regulating the cessation of the flowering phase, the PA process is still largely uncharacterized. Recently, there has been an upturn of studies focused on this process, highlighting its complexity as a developmental event controlled by a combination of hormonal, genetic, environmental, and other signaling factors (González-Suárez *et al*., 2020; Balanzà *et al*., 2023).

Different works suggested that fruits/seeds communicate with the inflorescence through a hormonal signal, which would promote PA (Murneek, 1926; Lockhart & Gottschall, 1961; Engvild, 1989; Hensel *et al*., 1994; Wuest *et al*., 2016). It has been proposed that, during late stages of the flowering period, seed/fruit-derived auxin would accumulate at high levels in the apical region of the stem disrupting auxin transport and promoting floral arrest (González-Suárez *et al*., 2020; Ware *et al*., 2020; Goetz *et al*., 2021; Walker *et al*., 2023). However, our recent work has provided more detailed insights into the relevance and dynamics of auxin signaling in the control of PA, suggesting that local repression of auxin-related pathways in the shoot apical meristem (SAM) promotes IM arrest and highlighting the importance of local auxin synthesis over fruit-derived auxin export (González-Cuadra *et al*., 2025). In addition to auxin, abscisic acid (ABA) is also involved in PA regulation. ABA-related genes are induced in arrested meristems and ABA accumulates in the arrested inflorescence apex, pointing to ABA as a positive regulator of PA (Wuest *et al*., 2016; Martínez-Fernández *et al*., 2020). In particular, a recent work shows that ABA would predominantly promote floral arrest at the end of flowering through the activation of ABA synthesis in the

unpollinated floral bud petioles (Sánchez-Gerschon *et al*., 2024). Cytokinin (CK) response, on the other hand, is repressed locally in the SAM, causing its arrest (Martínez-Fernández *et al*., 2020; Merelo *et al*., 2022). The repression of CK response is associated with a consequent decrease in cell division rate, the expression of the key stem cell maintenance factor *WUSCHEL* (*WUS*), and SAM size (Merelo *et al*., 2022). A recent study proposes that CKs would also participate in the negative regulation of floral arrest. Moreover, the distribution of CKs between fruits and inflorescences would control the timing of IM and floral arrest (Walker *et al*., 2023). Other hormones, like jasmonic acid (JA), have also been suggested as potential regulators of PA, as JA biosynthesis and signaling mutants display alterations in flower production and PA. However, these mutations also cause sterility (Caldelari *et al*., 2011; Kim *et al*., 2013), raising the possibility that their effects on PA result from defects in seed production rather than a direct role of JA in meristem activity. Therefore, the contribution of JA to the control of PA still needs further investigation.

At the genetic level, PA is controlled by the age-dependent pathway *FRUITFULL-APETALA2* (*FUL-AP2*). *FUL* encodes a MADS-box transcription factor that participates in several developmental processes such as fruit development, meristem identity, and floral transition (Gu *et al*., 1998; Ferrándiz *et al*., 2000). FUL is also a major factor controlling PA, since *fruitfull* (*ful*) mutant plants do not show IM activity arrest and instead produce flowers and fruits indefinitely (Balanzà *et al*., 2018; Merelo *et al*., 2022). Throughout the development of Arabidopsis, FUL is expressed in different tissues (Gu *et al*., 1998; Urbanus *et al*., 2009; Bemer *et al*., 2017; van Mourik *et al*., 2023), but its expression pattern within the SAM during PA has not been described in detail. It has been previously shown that FUL may accumulate in the IM along the flowering period to repress the expression of *AP2* and *AP2-like* genes (*SCHNARCHZAPFEN* [*SNZ*], *TARGET OF EARLY ACTIVATION TAGGED 1* [*TOE1*], and *TOE3*) (Balanzà *et al*., 2018). The decrease in the expression of *AP2* and *AP2-like* genes would lead to the downregulation of *WUS*, resulting in IM arrest. Moreover, FUL represses other CK-related events in the IM, such as cell division and SAM growth, to promote PA. Two modes of action of FUL can be distinguished during PA in relation to CK. First, FUL, together with additional unknown factors, contributes

to the repression of the CK-related events (decline phase). Then, FUL completely blocks these CK-related events (shutdown phase) (Merelo *et al*., 2022). In addition, FUL represses auxin-related pathways locally in the meristem to promote PA (González-Cuadra *et al*., 2025).

Analyses that compare the transcriptome of inflorescence meristems at different developmental stages or in different genetic backgrounds have uncovered potential genetic and signaling pathways involved in PA regulation, such as CK and ABA responses, and factors that respond to environmental signals or trigger senescence (Wuest *et al*., 2016; Martínez-Fernández *et al*., 2020; Sánchez-Gerschon *et al*., 2024). However, little is known about how these pathways and factors are related to FUL activity at the end of flowering or whether additional factors would be involved in PA under the control of FUL. In this work, we have characterized with high spatio-temporal resolution the expression pattern of FUL within the SAM. Nuclear accumulation of FUL during the decline and shutdown phases reveals that changes in its cellular localization may be implicated in the control of PA. Furthermore, we have performed a transcriptomic study comparing apices of *ful* and wild-type plants during advanced stages of the flowering period and PA. Our results indicate that FUL may control meristem activity and PA by directly repressing CK- and auxin-related pathways, while positively regulating ABA-related genes. In addition to processes and pathways previously linked to PA, our transcriptomic data provide new insights into different factors involved in PA regulation in a FUL-dependent manner. Notably, JA signaling and JA content decrease during PA, suggesting that JA may act as a negative regulator of this process. Finally, the analysis of JA signaling distribution in the SAM of *ful* mutants confirms the transcriptomic data, suggesting that FUL represses JA response to promote PA.

RESULTS

FUL accumulates in the nucleus during PA

As introduced before, FUL is involved in the age-dependent genetic pathway that controls PA. FUL promotes this process, at least in part, through the repression

of *AP2* and *AP2-like* genes and, consequently, *WUS* expression (Balanzà *et al*., 2018). It has been proposed that FUL accumulates gradually in the inflorescence meristem during the reproductive cycle (Balanzà *et al*., 2018), but the expression pattern of FUL within the SAM throughout this phase has not been studied in detail. To address this point, we generated a reporter line of the FUL protein and studied its expression pattern through different stages of the flowering period. For this aim, we used a recombineering-based tagging system using JAtY clones (Brumos *et al*., 2020). We fused *3xYPet* to the C-terminus of the *FUL* genomic sequence, encompassing 10 Kb upstream and 5 Kb downstream of *FUL* (*pFUL:FUL-3xYPet*, FUL-3xYPet; **Figure S2.1A**). This translational reporter was transformed into *ful-2* mutant plants, which do not arrest, to test its functionality. FUL-3xYPet rescued the PA phenotype of the mutant. Thus, the reporter line arrested 4 wab as the wild-type (wt) control plants. The flower production rate and total number of fruits along the flowering period until PA were similar to control plants as well. Moreover, the fruit *ful-2* mutant phenotype was rescued almost completely (**Figures S2.1B-S2.1I**).

We then analyzed with high spatio-temporal resolution the distribution of FUL-3xYPet in the SAM at advanced stages of the flowering period, considering the different phases of PA and the flower production kinetics previously reported (Merelo *et al*., 2022; González-Cuadra *et al*., 2025). Hence, we visualized the FUL reporter in SAMs 2 (highly active SAM), 3 (decline phase) and 4 weeks after bolting (wab) (shutdown phase), and in the SAM of plants reactivated by defruiting (1 day after defruiting, dad; and 1 week after defruiting, wad) (Hensel *et al*., 1994; Balanzà *et al*., 2018; Merelo *et al*., 2022). FUL-3xYPet signal was similar along these time points, but we observed changes in its subcellular localization. In active apices (2 wab), the signal was located in the nucleus and the cytoplasm (**Figures 2.1A and 2.1F**). In the SAM of less active (3 wab) and arrested apices (4 wab), FUL-3xYPet signal was predominantly nuclear (**Figures 2.1B, 2.1C, 2.1G and 2.1H**). In addition, the signal of FUL-3xYPet was reestablished into the cytoplasm 1 dad, while remaining detectable in the nucleus, and this distribution was maintained longer (1 wad) (**Figures 2.1D, 2.1E, 2.1I and 2.1J**). These results suggest that FUL activity may be modulated by its subcellular localization and, thus, PA control. The observed changes in FUL localization

follow the timing of the previous model of PA regulation (Merelo *et al*., 2022; González-Cuadra *et al*., 2025). The higher accumulation of FUL in the nucleus at the onset of PA (decline phase; 3 wab) and at the conspicuous PA (shutdown phase; 4 wab) correlated with the gradual repression and complete blocking of the CK and auxin-related factors previously involved in this process (Merelo *et al*., 2022; González-Cuadra *et al*., 2025). Furthermore, the early recovery of FUL cytoplasmic localization after defruiting suggests that fruit/seed-derived signals may regulate arrest via this change in the cellular localization of FUL within the SAM.

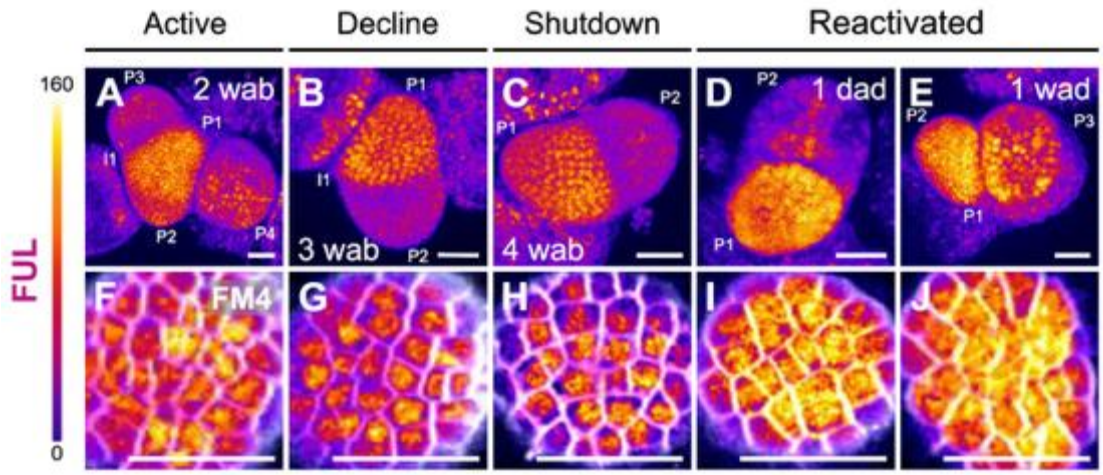

Figure 2. 1. Changes in FUL localization correlate with PA. (A-J) Expression of *pFUL:FUL-3xYPet* (FUL-3xYPet; magenta; signal intensity calibration bar) in apices 2 (A and F), 3 (B and G; decline) and 4 wab (C and H; shutdown) and in reactivated apices at 1 day (D and I) and 1 week after defruiting (dad and wad, respectively) (E and J). Confocal projections of the shoot apices are shown in (A)-(E). Magnified transversal sections of the apices in (A)-(E) are shown in (F)-(J). FM4-64 (white) was used to visualize the cell membrane in (F)-(J). In, incipient primordia; Pn, flower primordia that have grown out from the meristem. The numbering of both Pn and In corresponds to the sequence of appearance, from the youngest (P1 or I1) to the oldest (P3). Scale bars, 20 µm.

FUL controls ABA, auxin and cytokinin pathways during PA

Our previous studies (Balanzà *et al*., 2018; Merelo *et al*., 2022; González-Cuadra *et al*., 2025) have proposed *AP2* and *AP2-like*, CK- and auxin-related genes as FUL targets in the context of PA control. To identify other potential genes regulated by FUL and then to delve deeper into its mode of action, we have

performed a transcriptomic analysis comparing apices of wt and *ful-1* (*ful*) plants at different time points. Based on the flower and fruit production kinetics (**Figure S2.2**), the FUL-3xYPet expression pattern (**Figure 2.1**) and previous results (Merelo *et al*., 2022; González-Cuadra *et al*., 2025), we collected wt apices at 2, 3 and 4 wab (PA) and 1 wad, and *ful* apices at the equivalent time points (2, 3, 4 and 6 wab). Four independent biological replicates were analyzed per time point and genotype (**Figure S2.2**), and the transcriptome of each genotype was compared at each time point. Transcripts with a $\log_2$ fold change (FC) ≤ -1 and ≥ 1 and a P-adjusted value (P-value) ≤ 0.05 were considered as differentially expressed genes (DEGs) and selected for further analysis. We identified 632 DEGs in the comparison 2 wab *ful* vs. wt, 457 DEGs 3 wab *ful* vs. wt, 7605 DEGs 4 wab *ful* vs. wt and 1107 DEGs 6 wab (*ful*) vs. 1 wad (wt) (**Figure 2.2A and Tables S1A-S1D, respectively**). Based on the early (3 wab, decline phase) and late regulation of PA (4 wab, shutdown phase) by FUL (Merelo *et al*., 2022; González-Cuadra *et al*., 2025), and on the increased nuclear localization of FUL at 3 and 4 wab (**Figure 2.1**), we hypothesized that the DEGs at these time points could participate in the regulation of PA and, potentially, downstream of FUL activity. Thus, we focused on the DEGs between *ful* and wt apices at 4 wab exclusively (Group I; shutdown), 3 wab exclusively (Group II; decline) and at 3 and 4 wab (Group III; decline and shutdown) (**Figure 2.2A and Table S2A-S2C**, respectively). Most of the DEGs were grouped in Group I (6515 DEGs; **Figure 2.2A and Table S2A**), suggesting a major transcriptional shift, potentially associated with FUL activity, at the shutdown phase (4 wab). However, it is worth noting that the comparison between *ful* and wt apices at 4 wab represents a comparison between arrested and active apices, respectively. Then, some of DEGs may not be associated with FUL activity, but rather with the physiological state of the meristem. Furthermore, a markedly high number of DEGs was also detected at 3 wab, and at 3 and 4 wab (101 and 181 DEGs; Group II and III, respectively; **Figure 2.2A and Tables S2B and S2C**), indicating that 3 wab would be a critical regulatory point of cellular processes as well and influenced, at least in part, by FUL. We performed a Gene Ontology (GO) analysis using the ShinyGO platform (Ge *et al*., 2020) and selected the enriched terms in the Biological Process category. The categories or set of genes described below were selected because of their high representation during PA, their reported link

to PA or their particular biological interest. Among these categories, response to abscisic acid (168 genes), auxin (76 genes), ethylene (69 genes), jasmonic acid (49 genes), gibberellin (35 genes) and cytokinin (28 genes) were highlighted. Cell cycle (248 genes), shoot system development (169 genes) and aging (56 genes) categories were also overrepresented (**Figures 2.2B-2.2E and Table S3**).

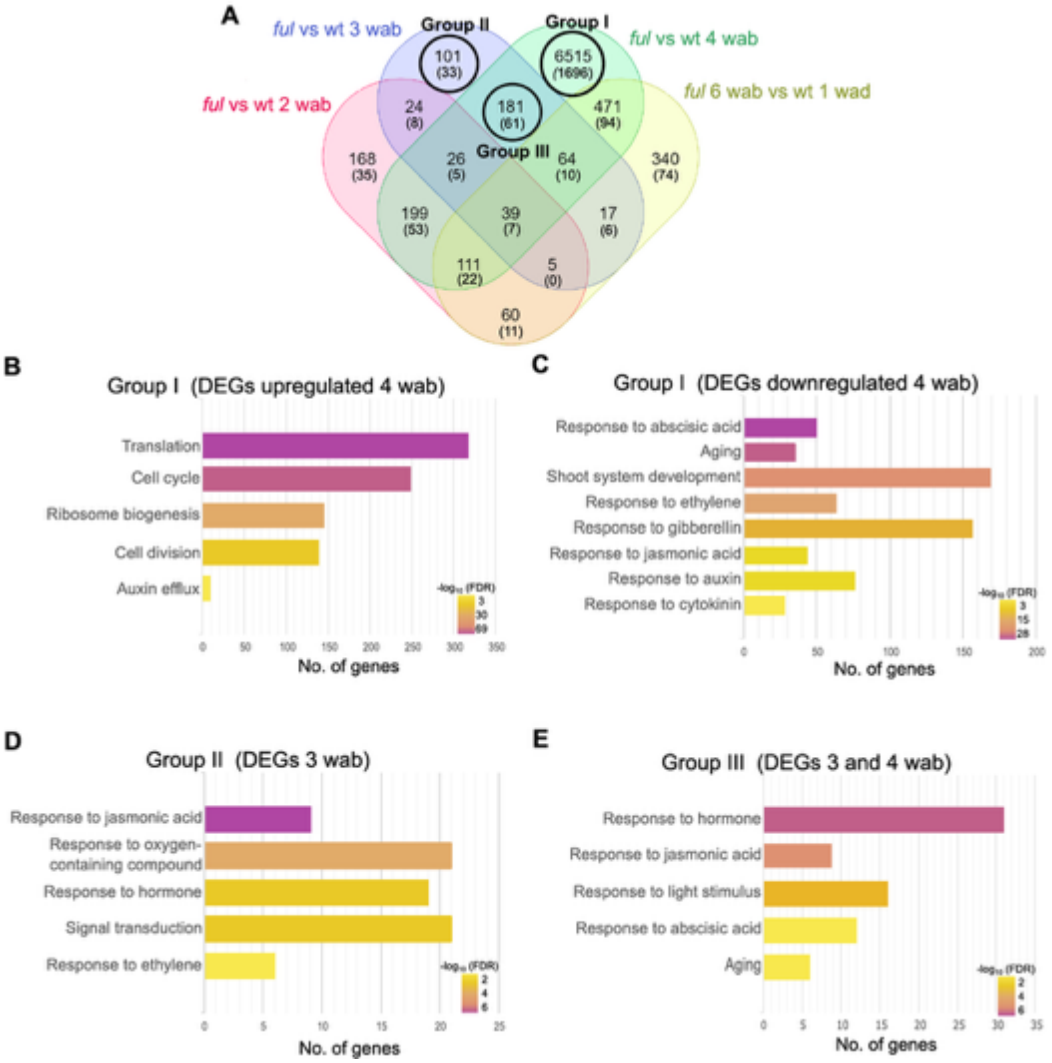

Figure 2. 2 Differential gene expression between *ful* and wt apices and functional enrichment analysis showing overrepresented GO biological process categories. (A) Venn diagram showing the number of DEGs in *ful* vs. wt apices 2, 3, 4, 6 wab or 1 wad. Numbers in parentheses indicate the DEGs that are potential direct targets of FUL (van Mourik *et al*., 2023). (B-E) The top five-eight most relevant GO terms, selected because of the enrichment degree, biological interest or previous relationship with PA, in Group I (B and C; upregulated and downregulated categories enriched 4 wab, respectively), Group II (D; categories enriched 3 wab) and Group III (E; categories enriched 3 and 4 wab). Significantly enriched GO terms were

identified using ShinyGO v0.75. The colour code shows the significance degree (FDR-adjusted P-value) of selected GO terms depending on the intensity, from yellow (lowest enrichment degree) to purple (highest enrichment degree). See Table S3 for full details.

Furthermore, these categories have been highlighted because some of the genes that they grouped may be direct targets of FUL. We identified these potential direct targets of FUL by comparing our transcriptomic data with previous chromatin immunoprecipitation sequencing (ChIP-seq) data obtained from proliferative inflorescence meristems expressing pFUL:FUL-GFP (**Figure 2.2A and Table S2**) (van Mourik *et al*., 2023). Indeed, since the comparison of *ful* and wt apices at 4 wab could be related to the different physiological state of the meristem, we compared these datasets to restrict our initial transcriptomic dataset to genes with a higher potential to be dependent on FUL in the control of PA. Moreover, to assess whether FUL may regulate this set of genes independently of AP2, the other major regulator of the age-genetic pathway controlling PA (Balanzà *et al*., 2018), we compared the DEGs potentially regulated by FUL with the DEGs responding to the induction of *AP2* (**Table S2**) (Martínez-Fernández *et al*., 2020).

Within this set of potential FUL targets, we found genes involved in ABA biosynthesis, signaling and response that may be promoted by FUL at 3 and/or 4 wab (**Tables 2.1, S2 and S3**). Martínez-Fernández *et al*. (2020) established a direct relationship between AP2 and ABA-related genes, suggesting that AP2 maintains meristem activity by repressing ABA response. A high number of potential FUL targets were previously described as regulated by AP2 (**Tables 2.1 and S2**) (Martínez-Fernández *et al*., 2020), suggesting that the regulation of ABA pathways could occur through the FUL-AP2 module. Contrary to AP2, FUL would promote ABA-related genes, as could be expected if FUL activity on them was downstream of AP2. However, and somehow surprisingly, many of the DEGs common to FUL and AP2 experiments were also direct targets of FUL in proliferative meristems (van Mourik *et al*, 2023), suggesting that FUL could be co-regulating ABA pathways together with AP2 at the end of the flowering period.

Table 2. 1. Direct targets of FUL related to abscisic acid pathways and regulated at 3 and/or 4 wab. The FC and the P-value are indicated for each gene. FC of DEGs between *ful* and wt apices at 3 and 4 wab is shown in the fourth and sixth columns, respectively. FC of DEGs after *AP2* induction (*AP2* ind; mock- vs. Dex-treated apices) is shown in the last column. Blue colour indicates negative FC (DEGs promoted by FUL and/or repressed by AP2) and yellow colour represents positive FC (DEGs repressed by FUL and/or promoted by AP2). -, no differential gene expression.

BIOLOGICAL PROCESS	SYMBOL	TAIR_ID	*ful* vs. wt 3 wab		*ful* vs. wt 4 wab		*AP2* ind
			FC	P-value	FC	P-value	FC
ABA biosynthesis	NCED3	AT3G14440	-3.51	1.23E-10	-7.49	1.74E-147	-2.42
Positive regulation of ABA signaling	SNRK2.3	AT5G66880	-	-	-2.20	6.39E-71	-0.85
	ABF3	AT4G34000	-	-	-3.59	1.07E-105	-1.90
	ABI5	AT2G36270	-	-	-2.70	7.29E-73	-0.89
Negative regulation of ABA signaling	HAI2	AT1G07430	-	-	-4.37	8.01E-46	-
	AHG3	AT3G11410	-	-	-2.89	5.88E-100	-
	ABI2	AT5G57050	-	-	-4.07	3.58E-112	-0.87
ABA response	HB12	AT3G61890	-1.26	2.29E-03	-4.11	1.30E-71	-
	HB53	AT5G66700	-1.63	2.72E-04	-5.02	5.54E-121	-2.97
	RD29	AT5G52310	1.60	3.01E-02	-4.00	1.89E-46	-3.42

In our previous work (González-Cuadra *et al*., 2025), we showed that repression of auxin biosynthesis mediated by TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (TAA1), PIN-FORMED1 (PIN1)-mediated auxin transport and auxin signaling correlates with PA and that the regulation of these processes strongly depends on FUL activity. Our current results support this previous study and extend these findings by identifying additional FUL targets associated with auxin biology in PA regulation (**Tables 2.2, S2 and S3**). Specifically, FUL may directly repress *YUCCA2* (*YUC2*) and *CYTOCHROME P450-CYP79B3,* both of which play critical roles in auxin biosynthesis (Zhao *et al*., 2002; Cheng *et al*., 2006), *PIN7* and *ABCB19*, auxin transport-related genes that maintain auxin distribution patterns (Mravec *et al*., 2008; Titapiwatanakun *et al*., 2009), and *PATELLIN5* (*PATL5)*, which has been related to PIN polarity regulation (Tejos *et al*., 2018). On the other hand, FUL predominantly promoted the expression of genes encoding repressors of auxin signaling, such as *Auxin/INDOLE-3-ACETIC ACID INDUCIBLE* (*Aux/IAA*) genes, and *MITOGEN-ACTIVATED PROTEIN KINASE 1* (*MPK1*), which have been linked to the regulation of auxin signaling during cell division and expansion (Enders *et al*., 2017; Bao *et al*., 2024). Moreover, FUL may directly promote *AUXIN RESPONSE FACTOR 2* (*ARF2*), whose repression is required for maintaining shoot meristem

stem cells (Roodbarkelari *et al*., 2015). Interestingly, most of the auxin-related genes regulated by FUL were not downstream of AP2 activity (**Tables 2.2 and S2**). These results, together with our previous work (González-Cuadra *et al*., 2025), indicated that inhibition of organ initiation and growth during PA, at least in part through the repression of these auxin-related genes, may be strongly dependent on FUL rather than on the FUL-AP2 module.

Table 2. 2. Direct targets of FUL related to auxin and cytokinin pathways and regulated at 3 and/or 4 wab. The FC and the P-value are indicated for each gene. FC of DEGs between *ful* and wt apices at 3 and 4 wab is shown in the fourth and sixth columns, respectively. FC of DEGs after *AP2* induction (*AP2* ind; mock- vs. Dex-treated apices) is shown in the last column. Blue colour indicates negative FC (DEGs promoted by FUL and/or repressed by AP2) and yellow colour represents positive FC (DEGs repressed by FUL and/or promoted by AP2). -, no differential gene expression.

BIOLOGICAL PROCESS	SYMBOL	TAIR_ID	*ful* vs. wt 3 wab		*ful* vs. wt 4 wab		*AP2* ind
			FC	P-value	FC	P-value	FC
Auxin biosynthesis	CYP79B3	AT2G22330	2.21	8.33E-10	1.09	4.96E-03	-
	YUC2	AT4G13260	-	-	2.48	2.29E-06	-
Auxin transport	PIN7	AT1G23080	-	-	1.03	2.36E-20	-
	ABCB19	AT3G28860	-	-	1.35	1.24E-24	-
Negative regulation of auxin signaling	MPK1	AT1G10210	-	-	-2.29	1.12E-34	-0.97
	IAA4	AT5G43700	-	-	-2.08	3.81E-17	-
	IAA14	AT4G14550	-	-	-4.03	2.19E-26	-
Auxin response	ARF2	AT5G62000	-	-	-1.31	1.50E-61	-
	PATL5	AT4G09160	-	-	1.39	3.96E-20	-
Negative regulation of CK signaling	ARR4	AT1G10470	-	-	-1.02	2.74E-05	-
	ARR5	AT3G48100	-	-	-1.94	1.13E-05	-
	ARR7	AT1G19050	-	-	-1.49	1.59E-05	-
	KMD1	AT1G80440	-1.20	1.98E-05	-5.87	1.40E-137	-2.3
CK response	CRF1	AT4G11140	-	-	2.94	3.24E-12	-

FUL may directly promote genes encoding repressors of CK signaling, such as *type-A ARABIDOPSIS RESPONSE REGULATORs* (*ARRs*), which have been previously related to the control of SAM maintenance and activity by repressing not only CK response but also *WUS* (Shi & Vernoux, 2022), and *KISS ME DEADLY1* (*KMD1*), a repressor of CK response already linked to PA regulation (Meng *et al*., 2017; Martínez-Fernández *et al*., 2020) (**Tables 2.2, S2 and S3**). Particularly, it has been described that AP2 promotes SAM activity in part by repressing *KMD1*, *KMD2* and *KMD4*. Thus, FUL would act oppositely to AP2 by

promoting *KMD1* expression at the end of flowering. On the other hand, FUL may directly repress a positive regulator of CK response, *CYTOKININ RESPONSE FACTOR 1 (CRF1)*, that promotes SAM activity and shoot growth (Raines *et al*., 2016). Interestingly, our analysis identified additional regulators of CK response acting downstream of FUL, whose repression would probably lead to the inhibition of SAM maintenance and growth, and then to PA (**Table 2.2, S2 and S3**) (Merelo *et al*., 2022). Moreover, many of the CK-related genes regulated by FUL were not downstream of AP2 activity (**Table 2.2 and S2**) (Martínez-Fernández *et al*., 2020), suggesting that the repression of these CK-related genes may be specifically dependent on FUL rather than on the FUL-AP2 module. Altogether, our results suggest that FUL represses auxin- and CK-related pathways, while promoting ABA-related pathways to induce PA. Since FUL is a repressor of *AP2* (Balanzà *et al*., 2018), and AP2 may promote SAM activity by repressing ABA response and negative regulators of CK signaling (Martínez-Fernández *et al*., 2020), FUL may regulate CK- and ABA-related pathways through the FUL-AP2 pathway, but also directly, as suggested by the ability of FUL to bind the promoters of a subset of these genes (Martínez-Fernández *et al*., 2020; Merelo *et al*., 2022). While the repression of auxin-related genes may be strongly dependent on FUL rather than on the FUL-AP2 module.

Identification of potential FUL targets related to hormonal, shoot development, cell cycle and senescence pathways during PA

Our transcriptomic analyses also revealed DEGs associated with hormonal pathways or developmental processes that remain less explored in the context of PA. FUL may directly promote the expression of genes involved in GA biosynthesis and catabolism, such as *GA20-oxidases* and *GA2-oxidases*, respectively (Lange & Lange, 2020). In addition, FUL may directly promote the expression of the GA receptor *GA INSENSITIVE DWARF1* (*GID1*) and repress the expression of *RGA-LIKE2* (*RGL2*), a DELLA protein that negatively regulates GA response, 3 and/or 4 wab (**Tables 2.3, S2 and S3**) (Lee *et al*., 2002; Murase *et al*., 2008). However, these results do not show a clear tendency for FUL to potentially promote or repress GA pathways. GAs are known to positively regulate cell division and meristem size (Serrano-Mislata *et al*., 2017; Kinoshita

et al., 2020; Shi *et al*., 2024), and components of GA biosynthesis and degradation, such as GA20-oxidase 2 and GA2-oxidase 4, respectively, as well as DELLA proteins, have been detected in the SAM (Kinoshita *et al*., 2020; Shi *et al*., 2024). Previous studies have shown that GA catabolism pathways are downregulated after *AP2* induction (**Table 2.3 and S2**) (Martínez-Fernández *et al*., 2020). Bioactive GA levels are tightly regulated through feedback and feedforward acting on GA metabolic and signaling genes (Yamaguchi, 2008). Thus, FUL and AP2 could participate in the regulation of GA homeostasis. Taken together, these findings suggest that the regulation of GA pathways may occur not only through FUL-AP2 but also through a direct control by FUL, thereby modulating meristem activity at the end of the flowering period.

Table 2. 3. Direct targets of FUL related to gibberellin pathways and regulated at 3 and/or 4 wab. The FC and the P-value are indicated for each gene. FC of DEGs between *ful* and wt apices at 3 and 4 wab is shown in the fourth and sixth columns, respectively. FC of DEGs after *AP2* induction (*AP2* ind; mock- vs. Dex-treated apices) is shown in the last column. Blue colour indicates negative FC (DEGs promoted by FUL and/or repressed by AP2) and yellow colour represents positive FC (DEGs repressed by FUL *and/or promoted by AP2*). -, no differential gene expression.

BIOLOGICAL PROCESS	SYMBOL	TAIR_ID	*ful* vs. wt 3 wab		*ful* vs. wt 4 wab		*AP2* ind
			FC	P-value	FC	P-value	FC
GA biosynthesis	GA20OX1	AT4G25420	-1.14	3.73E-05	-	-	-
GA inactivation	GA2OX4	AT1G47990	-1.32	3.66E-03	-2.66	8.57E-18	-2.67
	GA2OX2	AT1G30040	-	-	-3.34	2.18E-77	-1.2
GA perception	GID1B	AT3G63010	-1.10	4.18E-10	-2.96	1.99E-87	-
	GID1C	AT5G27320	-	-	-2.14	7.36E-61	-
Negative regulation of GA signaling	RGL2	AT3G03450	-	-	1.49	3.87E-13	-

On the other hand, ethylene signaling and response genes may be directly activated by FUL during the shutdown phase (4 wab) (**Tables 2.4, S2 and S3**). Ethylene has been associated with negative roles in cell division and expansion (Dubois *et al*., 2018) and plays a well-established role in promoting senescence (Guo *et al*., 2021; Wang *et al*., 2021). Furthermore, FUL may directly promote the expression of several *NAC* and *WRKY* family genes, such as *ANAC002/ATAF1*, *ANAC029/NAP, ANAC046*, *ANAC087* and *WRKY22*, all of which play important roles in senescence regulation (**Tables 2.4, S2 and S3**) (Cao *et al*., 2023). A recent study proposed that senescence pathways are induced at the end of the

flowering period, leading to the death of shoot stem cells (Wang *et al*., 2020, 2023). Our results suggest that FUL may act upstream of these cellular processes, and this regulation would depend in a higher extent on FUL rather than on the FUL-AP2 pathway (**Tables 2.4 and S2**).

Table 2. 4. Direct targets of FUL related to ethylene and senescence pathways and regulated at 3 and/or 4 wab. The FC and the P-value are indicated for each gene. FC of DEGs between *ful* and wt apices at 3 and 4 wab is shown in the fourth and sixth columns, respectively. FC of DEGs after *AP2* induction (*AP2* ind; mock- vs. Dex-treated apices) is shown in the last column. Blue colour indicates negative FC (DEGs promoted by FUL and/or repressed by AP2) and yellow colour represents positive FC (DEGs repressed by FUL and/or promoted by AP2). -, no differential gene expression.

BIOLOGICAL PROCESS	SYMBOL	TAIR_ID	*ful* vs. wt 3 wab		*ful* vs. wt 4 wab		*AP2* ind
			FC	P-value	FC	P-value	FC
Ethylene signaling	EIN3	AT3G20770	-	-	-1.73	1.25E-42	-
Ethylene response	ERF11	AT1G28370	-	-	-3.06	4.14E-03	-
	ERF14	AT3G15210	-	-	-1.08	2.09E-04	-
	ESE3	AT5G25190	-	-	-2.35	3.11E-23	-
Plant organ senescence	WRKY22	AT4G01250	-	-	-1.02	2.67E-03	-
	ATAF1	AT1G01720	-	-	-1.30	2.28E-19	-
	NAC046	AT3G04060	-	-	-6.07	3.78E-52	-
	ANAC087	AT5G18270	-	-	-3.17	2.45E-35	-
	NAP	AT1G69490	-1.67	1.30E-4	-7.54	1.13E-180	-2.45

Additionally, FUL regulated JA-related genes at 3 and/or 4 wab (**Figure 2.4A and Tables S2 and S3**). It is worth noting that the potential role of JA-related factors in the regulation of PA remains less characterized (Wuest *et al*., 2016; Martínez-Fernández *et al*., 2020). Further details regarding JA-related factors and PA are discussed in later sections.

Genes encoding transcription factors involved in floral transition and meristem development may also be directly regulated by FUL, further supporting its role as a central regulator of shoot apex activity during plant aging. *TEMPRANILLO* genes (*TEM1*/*TEM2*), which encode transcriptional repressors that regulate multiple flowering pathways (Castillejo & Pelaz, 2008; Osnato *et al*., 2012; Hu *et al*., 2021), *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*), which regulates flowering time and floral meristem determinacy (Lee & Lee, 2010) and *AGAMOUS-LIKE 16* (*AGL16*), which encodes a known floral repressor (Hu *et al*.,

2014), may be directly promoted by FUL 4 wab (**Tables 2.5, S2 and S3**). Additionally, the TCP transcription factor *BRANCHED1* (*BRC1*), which promotes axillary bud dormancy (Aguilar-Martínez *et al*., 2007), was identified as a FUL target at 4 wab. Since most of these genes are crucial for the initiation of reproductive development and shoot system architecture, our results suggest that FUL may also modulate the end of flowering by regulating their expression. A previous work showed that mitotic divisions are repressed during PA and that FUL negatively regulates mitotic activity within the SAM (Merelo *et al*., 2022). Our current results support this previous study and extend these findings by identifying additional FUL targets associated with cell cycle regulation. Particularly, FUL may compromise the progression of the cell cycle by directly repressing genes encoding cyclin (CYC) and cyclin-dependent kinases (CDK), whose interactions positively regulate cell cycle progression, but also by promoting *ICK1*, a CDK inhibitor that represses cell proliferation (**Tables 2.5, S2 and S3**) (Zhou *et al*., 2003; Nakai *et al*., 2006; Shimotohno *et al*., 2025).

Table 2. 5. Direct targets of FUL related to plant growth and development pathways and regulated at 3 and/or 4 wab. The FC and the P-value are indicated for each gene. FC of DEGs between *ful* and wt apices at 3 and 4 wab is shown in the fourth and sixth columns, respectively. FC of DEGs after *AP2* induction (*AP2* ind; mock- vs. Dex-treated apices) is shown in the last column. Blue colour indicates negative FC (DEGs promoted by FUL and/or repressed by AP2) and yellow colour represents positive FC (DEGs repressed by FUL and/or promoted by AP2). -, no differential gene expression.

BIOLOGICAL PROCESS	SYMBOL	TAIR_ID	*ful* vs. wt 3 wab		*ful* vs. wt 4 wab		*AP2* ind
			FC	P-value	FC	P-value	FC
Shoot system development	BRC1	AT3G18550	-	-	-8.65	9,61E-43	-
	TEM1	AT1G25560	-	-	-1.23	8.21E-04	-
	TEM2	AT1G68840	-	-	-5.78	7.64E-53	-
	SOC1	AT2G45660	-	-	-1.55	2.65E-19	-1.32
	AGL16	AT3G57230	-	-	-1.05	1.07E-16	-
Cell cycle	CYCB2;2	AT4G35620	-	-	3,31	2,46E-25	-
	CDC6B	AT1G07270	-	-	2,67	5,94E-16	-
	CDKD1;1	AT1G73690	-	-	2,16	1,75E-17	1.93
Negative regulation of cell cycle	ICK1	AT2G23430	-	-	-3,24	7,96E-20	-
Ribosome biogenesis	RPL18	AT3G05590	-	-	1,96	2,58E-64	-
	PRPL35	AT2G24090	-	-	1,92	3,20E-82	-
	RPL27	AT5G40950	-	-	2,26	3.05E-85	-

Altogether, these results suggest that hormonal pathways, such as GA, ethylene and JA, and developmental processes, including shoot development, cell cycle progression and senescence, may be regulated by FUL at the end of the flowering period. Furthermore, since most of the genes related to these pathways or processes do not appear to be downstream of AP2 activity, their regulation may be strongly mediated by FUL activity, rather than on the FUL-AP2 module, pointing out the central role of FUL in controlling meristem activity and growth to promote PA.

Temporal expression profiles of wild-type and *ful* apices at the end of the flowering period

The analysis described above allowed to identify genes whose expression may be regulated by FUL to promote PA. However, we were also interested in understanding how the temporal dynamics of gene expression throughout the flowering period are affected by the presence or absence of FUL. Our previous studies suggest that FUL could act as the main regulator of certain pathways involved in IM arrest, such as auxin-related pathways (González-Cuadra *et al*., 2025), or together with other factors in the control of specific pathways, such as those related to CK (Merelo *et al*., 2022). To identify which potential genes might be specifically regulated by FUL or by other factors, we compared the temporal expression profiles of wt and *ful* apices. Genes were clustered based on common temporal expression patterns within genotypes (wt or *ful* apices), and along the time points previously mentioned (**Figure 2.3 and Tables S4 and S5**), using the fuzzy c-means algorithm implemented in the Mfuzz package (Kumar & Futschik, 2007). Then, within the clusters of wt apices, we focused on those showing significant expression changes at 3 and/or 4 wab (PA phases) in comparison with active or reactivated apices (2 wab and 1 wad) (**Figure 2.3A and Table S4**). Genes that showed higher or lower expression at the moment of the conspicuous PA (4 wab) were grouped in clusters Aw and Bw, respectively (**Figure 2.3A and Tables S4A and S4B**). Clusters Cw and Dw grouped genes highly expressed 3 wab (**Figure 2.3A and Table S4C**), and genes whose expression decreased from 3 wab, respectively (**Figure 2.3A and Table S4D**). Interestingly, *FUL* was upregulated 4 wab (**Figure 2.3C**). The increase of *FUL* expression at the

conspicuous PA correlates with FUL protein localization mostly in the nucleus, suggesting that its activity is key at this time point.

Next, we focused on genes that showed an opposite behaviour between wt and *ful* apices because the regulation of these genes may be dependent on FUL activity (**Figures 2.3A and 2.3B**). This involved genes whose expression increased or decreased 3 and/or 4wab in wt apices, but decreased or increased in *ful* apices, respectively (186 genes; **Tables S4 and S5**). In contrast, genes with similar temporal expression profiles in both wt and *ful* apices may be regulated by other factors (292 genes; **Tables S4 and S5**). Additionally, we found that the majority of genes clustered within the wt temporal expression profiles displaying significant expression changes during the PA phases did not show significant expression changes in *ful* apices at the end of the flowering period (2930 genes; **Tables S4 and S5**). In accordance with our previous findings (upper sections, **Tables S2 and S3**), genes related to ABA biosynthesis, signaling and response, auxin transport, JA signaling and shoot development showed significant expression changes in wt apices at 3 and/or 4 wab but not in *ful* mutants at the end of the flowering period, indicating FUL-dependent regulation (**Tables S6 and S7**).

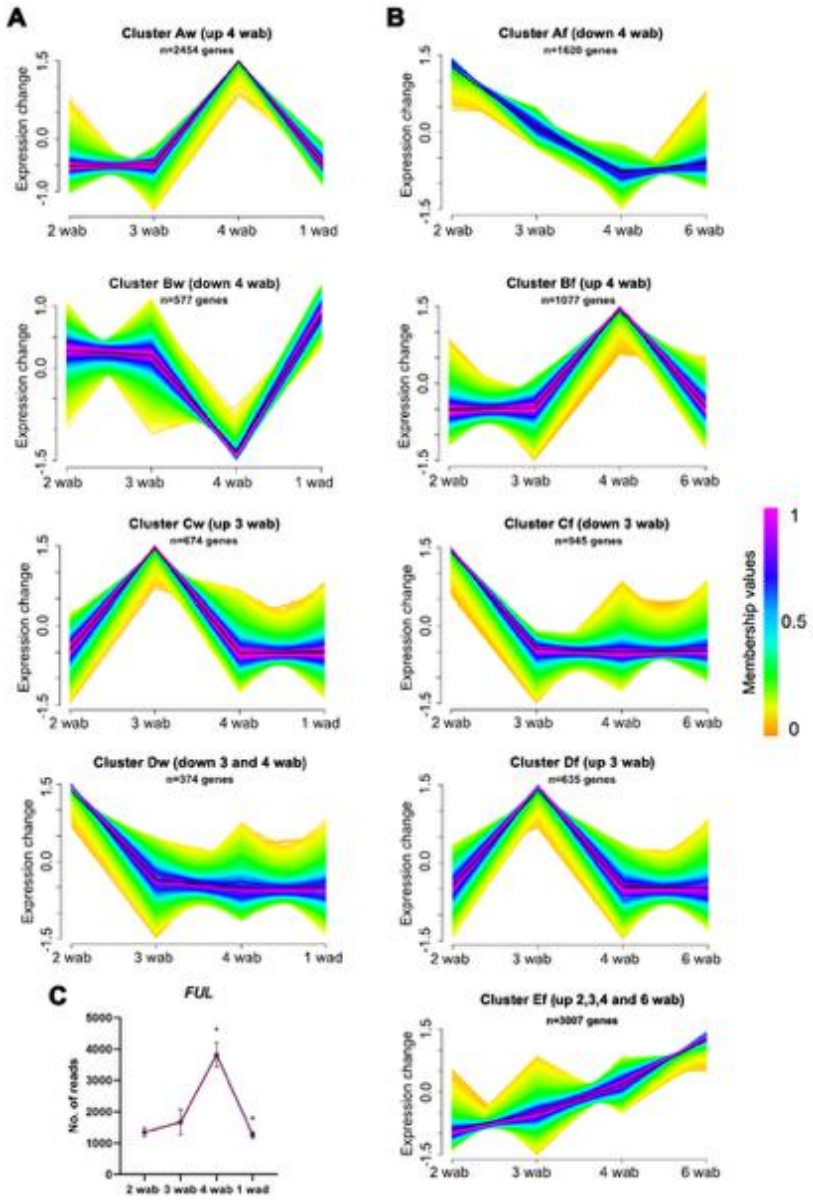

Figure 2. 3. Time course expression patterns in wt and *ful* apices. (A) Clustering of genes based on their temporal expression patterns in wt apices 2 (active), 3 (decline phase), and 4 wab (shutdown phase) and in reactivate apices (1 wad). (B) Clustering of genes based on their temporal expression patterns in *ful* apices along advanced stages of the flowering period (from 2 to 6 wab). Fuzzy c-means clustering of normalized and variance-stabilized read counts was applied, resulting in the identification of four (A) and five common expression patterns (B). Each line represents the expression pattern of an individual gene. The colour bar on the right indicates the colour-encoding of the membership values. Each colour represents the degree of belonging to a specific cluster. Core expression values for each cluster are shown in black. (C). Number of reads of *FUL* at 2, 3 and 4 wab and 1 wad in wt apices. Data are represented as mean ± SD of 4 biological replicates (4 pools of 22 shoot apices). Asterisks indicate significant differences ($p < 0.05$) according to two-tailed Student's test comparing each time point to the previous one.

Moreover, a large number of less-characterized genes showed temporal expression profiles with significant changes during the PA phases in wt apices, but not in *ful* apices, or displayed opposite behaviour between wt and *ful* apices (**Tables S4 and S5**). These results highlight a broad dataset for future investigations concerning PA control. Altogether, our results support the role of FUL as a central regulator of PA, with the transcriptional changes that drive the transition to PA being largely dependent on its activity.

FUL regulates the expression of genes related to JA pathways during PA

Our transcriptome analyses showed a prominent representation of DEGs related to JA metabolism, signaling and response, which could be directly or indirectly regulated by FUL during PA (3 and/or 4 wab; **Figure 2.4A and Tables S2-S6**). Some of them are also direct targets of FUL in active meristems, based on ChIP-seq published data (**Figure 2.4A**; striped rectangles) (van Mourik *et al*., 2023). JA regulates several processes of plant growth and development, including root growth (Raya-González *et al*., 2012; Cai *et al*., 2014), floral organ development (Song *et al*., 2011; Reeves *et al*., 2012), floral transition (Zhai *et al*., 2015; Wang *et al*., 2017) and leaf senescence (Qi *et al*., 2015; Zhuo *et al*., 2020). Moreover, two studies have proposed that JA could play a role in the control of PA (Caldelari *et al*., 2011; Kim *et al*., 2013). Mutations in the *13-LIPOXYGENASES 2 (LOX3)* and *LOX4* genes, which encode JA biosynthesis enzymes, and the *CORONATINE INSENSITIVE 1* (*COI1*), which encodes a JA co-receptor, cause alterations in PA but also in male fertility. However, since it has been previously described that sterile mutants display alterations in PA (Hensel *et al*., 1994; Wuest *et al*., 2016), it is likely that the observed PA alterations in these mutants are at least in part due to the lack of seeds rather than to defects on JA pathways. Therefore, the role of JA in the control of PA has not been well established yet, highlighting the need for further investigation in this regard.

As JA pathways are less explored in the context of PA, we decided to take into account all JA-related DEGs, regardless of whether they are potential direct targets of FUL, to elucidate their putative role at the end of the flowering period. Our transcriptomic analyses indicated that FUL promoted the expression of

several genes related to the negative regulation of JA signaling, such as *JA-ASSOCIATED MYC2-LIKE1* (*JAM1*), JASMONATE ZIM-DOMAIN1 (*JAZ1*), *JAZ2*, *JAZ3*, *JAZ6*, *JAZ7*, *JAZ9* and *JAZ11* (Chini *et al*., 2007; Nakata *et al*., 2013; Thines *et al*., 2007), at 4 wab (shutdown phase) (**Figure 2.4A**). Additionally, we detected that FUL repressed the expression of genes related to JA biosynthesis, such as *ALLENE OXIDE SYNTHASE* (*AOS*), *12-OXOPHYTODIENOATE-REDUCTASE 1 (OPR1)*, *OPR3*, *LOX2* and *LOX3* (Vick & Zimmerman, 1984; Bannenberg *et al*., 2009; Schaller & Stintzi, 2009), and *JASMONIC ACID CARBOXYL METHYLTRANSFERASE* (*JMT*), which catalyzes the formation of the active JA form methyl jasmonate (Seo *et al*., 2011), at 3 and/or 4 wab (**Figure 2.4A**). Interestingly, although FUL promoted the expression of *JASMONATE RESISTANT1* (*JAR1)*, which encodes an enzyme responsible for the formation of the biologically active jasmonoyl-isoleucine conjugate (Staswick & Tiryaki, 2004), it also promoted the expression of *JASMONATE-INDUCED OXYGENASE2* (*JOX2*) and *CYTOCHROME P450 CYP94B3*, which are involved in the inactivation of the active JA-amino acid conjugates (Kitaoka *et al*., 2011; Caarls *et al*., 2017). This suggests that FUL may fine-tune JA metabolism, ultimately reducing the levels of biologically active JA (**Figure 2.4A**). Most of these JA-related genes displayed temporal expression profiles that fit the relevant patterns in PA, and their expression changes temporally matched with the changes in FUL subcellular localization (**Figures 2.1 and 2.4B**). Furthermore, most of the JA-related genes do not appear to be regulated by AP2, suggesting that the regulation of JA pathways at the end of the flowering period may be strongly dependent on FUL activity, rather than on the FUL-AP2 module (**Figure 2.4 and Table S2**) (Martínez-Fernández *et al*., 2020). Therefore, a potential decrease in active JA content and the suppression of JA signaling mediated by FUL may participate in meristem arrest.

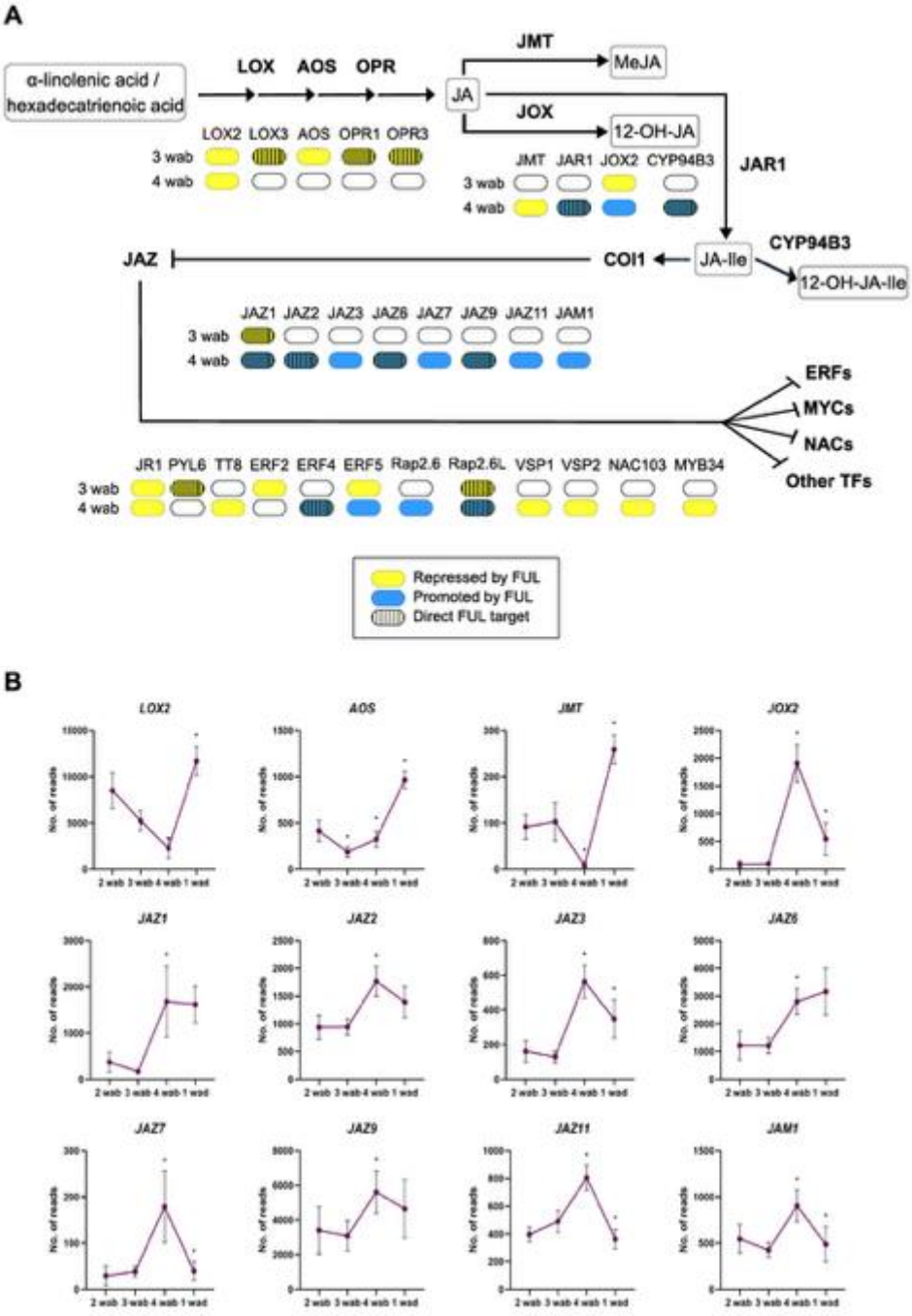

Figure 2. 4. Differentially expressed genes related to JA pathways. (A) Summary of JA-related DEGs 3 and/or 4 wab (*ful* vs. wt). Solid coloured rectangles indicate detection of differential expression of the corresponding gene. Blue and yellow colours represent downregulated DEGs (promoted by FUL) and upregulated DEGs (repressed by FUL), respectively. Striped rectangles represent potential direct targets of FUL. Enzymes are indicated in bold letters, and metabolites are shown as rectangles. (B) Number of reads of *LOX2*, *AOS*, *JMT*, *JOX2*, *JAZ1*, *JAZ2*, *JAZ3*, *JAZ6*, *JAZ7*, *JAZ9, JAZ11 and JAM1* at 2, 3 and 4 wab and 1 wad in wt apices. Data are represented as mean ± SD of 4 biological replicates (4 pools of 22 shoot apices). Asterisks indicate significant differences ($p < 0.05$) according to two-tailed Student's test comparing each time point to the previous one.

Changes in a JA signaling reporter and JA content in the apex correlate with PA

To corroborate the transcriptomic results, we characterized JA dynamics within the SAM by monitoring the negative JA signaling fluorescent reporter *35S:Jas9-N7-VENUS* (Jas9-VENUS) (Larrieu *et al*., 2015) at the end of the flowering period. This reporter is based on the JA-dependent degradation of JAZ proteins. The Jas motif of the JAZ9 protein directs the degradation of VENUS in the presence of JA, providing a negative readout of JA distribution. In highly active SAMs (2 wab), no signal of Jas9-VENUS was detected (**Figures 2.5A and 2.5F**). One week later (3 wab, decline phase), Jas9-VENUS was detected in some cells of the young primordia (around I1-P1) and the meristem-primordia boundaries (**Figures 2.5B and 2.5G**; white and green arrowheads, respectively). At the moment of the conspicuous PA (4 wab, shutdown phase), Jas9-VENUS signal was observed in many cells of primordia at different developmental stages (around P1-P3; **Figures 2.5C and 2.5H**; white arrowheads) and at their meristem-primordia boundaries (**Figures 2.5C and 2.5H**; green arrowheads). On the other hand, after reactivation of arrested apices by defruiting, Jas9-VENUS signal was reduced throughout the SAM, becoming restricted to a few cells, 1 dad and 1 wad, similarly to apices 3 wab (**Figures 2.5D, 2.5E, 2.5I and 2.5J**). Additionally, we quantified JA levels in active (2 wab), arrested (5 wab) and reactivated (1 wad) apices to test whether changes at the level of JA biosynthesis may also be linked to PA control, as the transcriptomic data suggested. JA content decreased significantly 4 wab compared to early stages (2 wab). After reactivation (1 wad), JA levels were restored and remained high (**Figure S2.3A**). Altogether, these results suggest that repression of JA response and a decrease in JA levels, which correlate with a downregulation of JA biosynthesis genes 3 and 4 wab, may promote IM arrest. We previously showed (Merelo *et al*., 2022) that, in active meristems, cell divisions are mainly located in developing primordia and at the meristem-primordia boundaries, whereas no cell divisions were observed in arrested SAMs. Interestingly, Jas9-VENUS monitoring indicated that repression of JA signaling in cells of these specific domains could promote PA. This correlation suggests that repression of JA signaling pathways may be linked to the inhibition of cell divisions and, in turn, of primordia initiation and

development during PA. In this line, previous works suggested that JA may be involved in the regulation of the cell cycle by inducing cell division during the activation of the stem cell niche in the root apical meristem (Chen *et al*., 2011; Zhou *et al*., 2019). Finally, the restoration of JA signaling in the SAM after defruiting, suggested that fruit/seed signals may impact JA response.

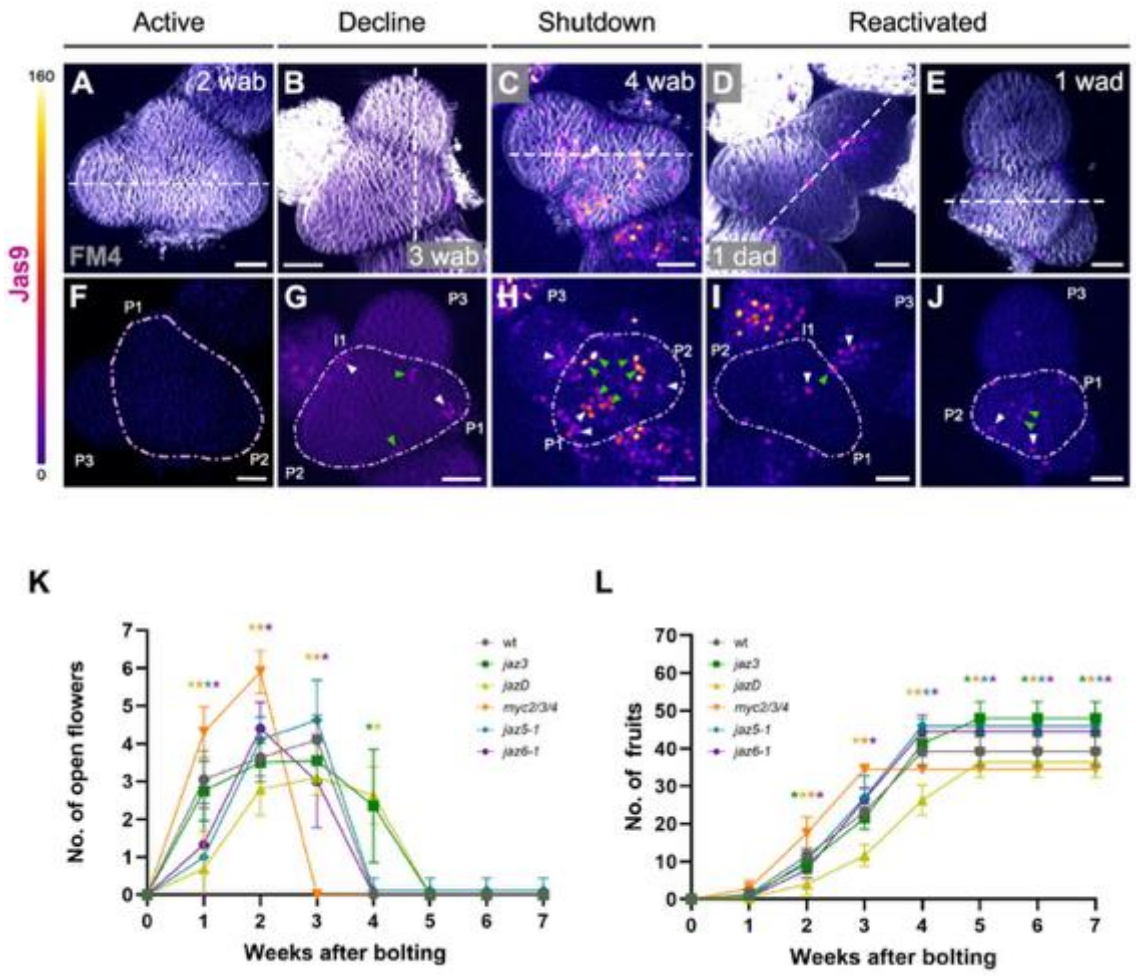

Figure 2. 5. JA signaling is repressed during PA. (A-J) Confocal projections showing *35S:Jas9-N7-VENUS* signal (Jas9-VENUS; magenta; signal intensity calibration bar) in apices 2 (A and F), 3 (B and G; decline) and 4 wab (C and H; shutdown), 1 dad (D and I) and 1 wad (E and J). FM4-64 (gray) was used to visualize the cell membrane. Confocal projections of the shoot apices combining both Jas9-VENUS and FM4-64 channels are shown in (A)-(E). The same projections with the single Jas9-VENUS channel are shown in (F)-(J). Green and white arrowheads point to Jas9-VENUS signal in the meristem-primordia boundaries and in the primordia, respectively. The pink dashed line outlines young primordia and meristems. Scale bars, 20 µm. (K) Number of flowers at stages 12-15 produced in primary apex in wild-type and

jaz3, *jaz5-1*, *jaz6-1*, *jazD* and *myc2/3/4* mutant plants from 0 to 7 wab. (L) Total number of fertile fruits (stages 16-20) in the primary stem in wt and *jaz3*, *jaz5-1*, *jaz6-1*, *jazD* and *myc2/3/4* mutant plants from 0 to 7 wab. Data are represented as mean ± SD of 16 biological replicates. Asterisks indicate significant differences ($p<0.005$) according to two-tailed Student's test comparing each mutant genotype to wt plants. Each colour of the asterisk corresponds to the colour legend.

To further investigate the role of JA in the control of PA, we quantified flower and fruit production in several mutant lines related to the JA signaling pathway. We characterized some loss-of-function mutants related to members of the JAZ protein family, which repress JA signaling and biosynthesis genes (Chini *et al*., 2007): *jaz3*, *jaz5-1*, *jaz6-1* and the decuple mutant *jazD* (containing the mutations *jaz1-jaz7*, *jaz9*, *jaz10* and *jaz13*) (Guo *et al*., 2018). We also characterized the triple mutant *myc2-1 myc3-1 myc4-1* (*myc2/3/4*), which contains mutations in the basic helix-loop-helix (bHLH) transcription factors *JASMONATE INSENSITIVE 1* (*JIN1/MYC2*), *MYC3*, and *MYC4* and displays lower JA levels than wt plants (Fernández-Calvo *et al*., 2011; Zhang *et al*., 2020). The single mutants *jaz3*, *jaz5-1* and *jaz6-1* produced a higher total number of fruits (48 ± 4.43, 46 ± 1.85 and 44 ± 4.48, respectively) with respect to wt plants (39 ± 3.93) (**Figure 2.5K**), but only *jaz3* arrested one week after wt plants (5 wab instead of 4 wab) (**Figure 2.5L**). The mutant *jazD* also arrested 1 week after wt plants (5 wab and 4 wab, respectively) but produced a lower total number of fruits (36 ± 4.01) with respect to wt plants (**Figures 2.5K and 2.5L**). This may be attributed to the lower flower production rate (**Figure 2.5K**) and the reduced relative growth rate observed in *jazD* mutant plants (Guo *et al*., 2018). On the other hand, the triple mutant *myc2/3/4* arrested one week before (3 wab) and produced a lower total number of fruits (34 ± 1.35) in comparison with wt plants (4 wab) (**Figures 2.5K and 2.5L**). These results are in line with our previous findings and suggest that JA signaling factors negatively regulate PA.

JA treatments delay PA and reactivate arrested meristems

To further investigate the relevance of JA on the control of PA, we treated active apices from 2 wab and arrested apices from 4 wab with JA (50 µM Methyl jasmonate, MeJA) or mock (control) every 2 days. Plants continuously treated

with MeJA from 2 wab displayed a delay of four days in PA and produced a higher number of total fruits in comparison with control plants, which arrested after 2 weeks of treatment (wot) (4 wab) (**Figures 2.6A-2.6C**). We also analyzed the expression of the WUS reporter line *pWUS:EGFP-WUS* (GFP-WUS) (Yadav *et al*., 2011) in MeJA-treated and control apices to check whether SAM activity correlated with the observed phenotype. GFP-WUS expression was almost undetectable in control arrested SAMs 2 wot (**Figures 2.6D and 2.6F**), whereas SAMs of MeJA-treated apices still showed GFP-WUS signal (**Figures 2.6E and 2.6G**). On the other hand, arrested apices treated with MeJA (4 wab) were reactivated and still showed open flowers after 1 wot, while control apices remained arrested (**Figures 2.6H-2.6K**). Moreover, GFP-WUS expression was restored after 1 day of treatment (dot) (**Figures 2.6M and 2.6Q**), indicating a rapid reactivation of SAM activity, and was maintained longer (1 wot; **Figures 2.6O and 2.6S**). In control apices, GFP-WUS expression was almost undetectable after 1 dot and 1 wot (**Figures 2.6L, 2.6N, 2.6P and 2.6R**). JA treatments delayed and reverted meristem arrest, demonstrating that this hormone would participate in the negative regulation of PA.

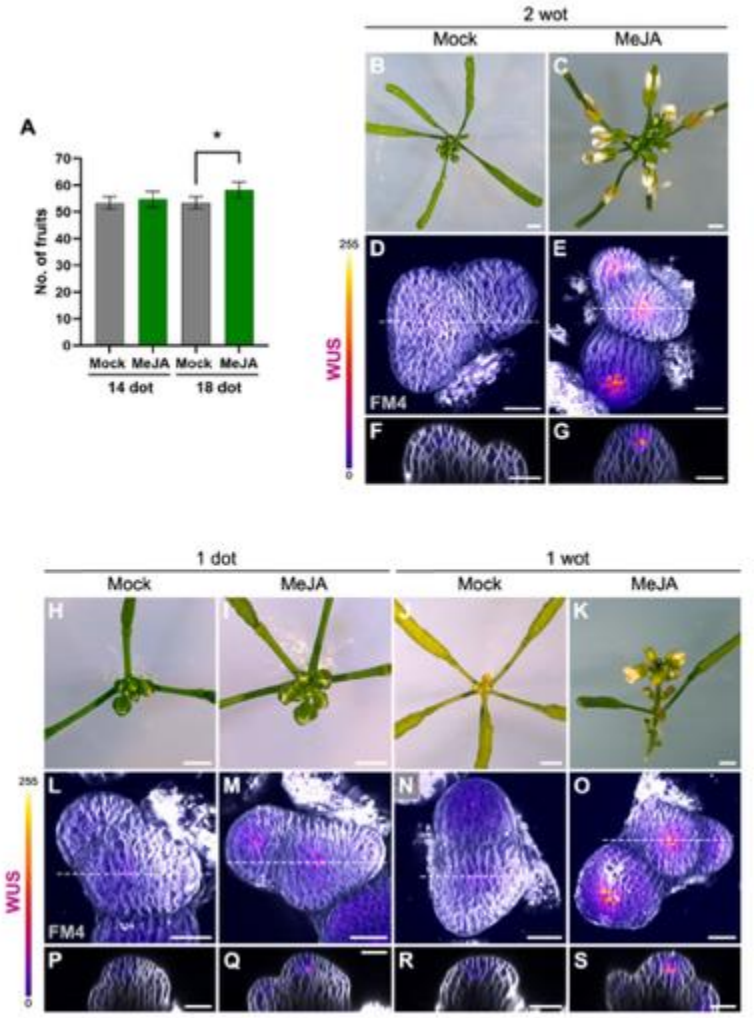

Figure 2. 6. JA delays PA and reactivates arrested apices. (A) Quantification of fertile fruits (stages 16-20) in the primary stem of methyl jasmonate (MeJA; 50 µM) and mock-treated plants after 14 and 18 days of treatment. Apices were treated every 2 days from 2 wab. Data are represented as mean ± SD of 15 biological replicates. Asterisks indicate significant differences ($p< 0.005$) according to two-tailed Student's test comparing each treatment. (B and C) Apices after 2 weeks of mock (B) and MeJA treatment (C). (D-G) *pWUS:EGFP-WUS* expression (GFP-WUS; magenta; signal intensity calibration bar) in the shoot apex after 2 weeks of mock (D and F) and MeJA treatment (E and G). Confocal projections are shown in (D) and (E), and the corresponding longitudinal sections along the dashed lines are shown in (F) and (G). (H-K) Apices after 1 day (H and I) and 1 week (J and K) of mock (H and J) and MeJA treatment (I and K). Apices were treated every 2 days from 4 wab (PA). (L-S) Expression of GFP-WUS (magenta; signal intensity calibration bar) in the shoot apex after 1 day (L, M, P and Q) and 1 week (N, O, R and S) of mock (L, N, P and R) and MeJA treatment (M, O, Q and S). Confocal projections are shown in (L)-(O), and the corresponding longitudinal sections along the dashed lines are shown in (P)-(S). FM4-64 (gray) was used to visualize the cell membrane. Scale bars, 20 µm (D-G and L-S) and 1 mm (B, C and H-K).

FUL represses JA response during PA

Our current results suggest that the expression of JA-related genes may be regulated by FUL from the decline (3 wab) to the shutdown phase (4 wab) (**Figure 2.4**). Moreover, changes in the subcellular localization of FUL 3 and 4 wab correlated with the repression of JA signaling within the SAM. To further assess whether JA signaling could be linked to FUL in the control of PA, we monitored the Jas9-VENUS reporter in the SAM of *ful-2* mutant plants. Jas9-VENUS signal was never detectable from 2 to 7 wab, indicating that JA signaling would be highly active along the flowering period in *ful-2* apices, as in active wt plants 2 wab (**Figures 2.5A and 2.5F and 2.7**). The maintenance of active JA signaling in *ful-2* SAMs, especially 3 and 4 wab, based on the complete absence of Jas9-VENUS signal, correlates with our transcriptomic data (**Figures 2.4, 2.7B, 2.7C, 2.7H and 2.7I**) and the identification of potential FUL direct targets (**Table S2**) (van Mourik *et al*., 2023). These analyses suggested that FUL may directly promote genes involved in the negative regulation of JA signaling during the decline and shutdown phase of PA. Additionally, we quantified JA levels in the shoot apices of *ful-2* mutant plants through the flowering period (2, 4 and 6 wab). JA level increased significantly with plant age (**Figure S2.3B**). High JA levels detected in advanced stages of plant development in both wt and *ful-2* apices (1 wad and 6 wab, respectively; **Figure S2.3**) are not reflected by Jas9-VENUS reporter signal. This suggests that the reporter may have limitations in sensitivity, potentially failing to detect subtle changes in JA levels. Therefore, the use of more suitable reporters, such as those based on JA biosynthesis, may be necessary to overcome this limitation. On the other hand, high JA levels 1 wad and 6 wab in wt and *ful-2* apices, respectively (**Figure S2.3**), could be associated with senescence processes at these time-points. Genes related to senescence processes, such as *SENESCENCE-ASSOCIATED GENE29* (*SAG29*)*, SAG12*, *or WRKY DNA-BINDING PROTEIN45*, are induced in our transcriptomic data at these developmental stages in wt and *ful* apices (**Tables S2, S4A, S5B** and **S5E**). SAM senescence has already been linked to PA. At the end of the flowering period, age-induced senescence programs are triggered, ending with the death of the SAM and the whole plant (Wang *et al*., 2020, 2023). Furthermore, JA has been reported to control leaf senescence (Qi *et al*., 2015; Zhuo *et al*., 2020). Therefore, in addition to the potential participation of JA-related factors in the

regulation of PA, it is possible that the increase in JA content in the apex at the end of the flowering period might be related to the onset of the senescence program.

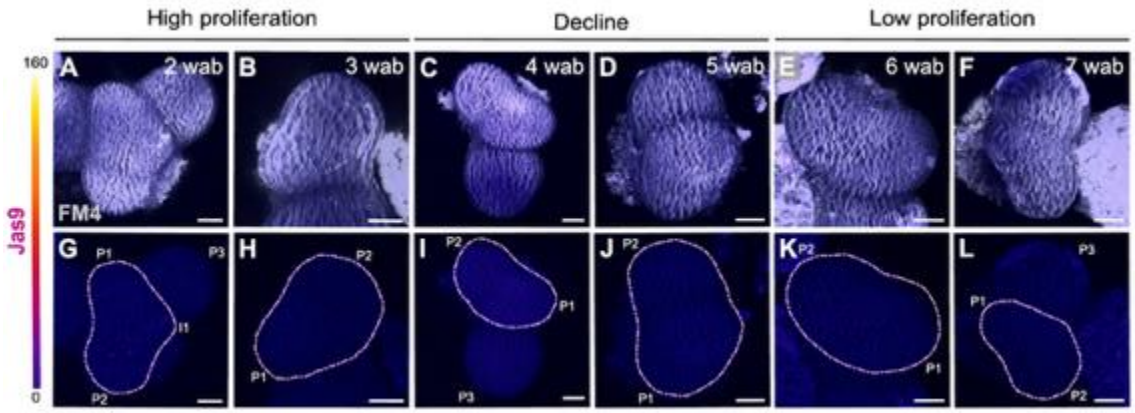

Figure 2. 7. JA signaling is active along the flowering period in *ful-2* apices. (A-L) Expression of Jas9-VENUS (magenta; signal intensity calibration bar) in *ful-2* apices 2 (A and G), 3 (B and H), 4 (C and I), 5 (D and J), 6 (E and K) and 7 wab (F and L). FM4-64 (gray) was used to visualize the cell membrane. Confocal projections of the shoot apices combining both Jas9-VENUS and FM4-64 channels are shown in (A)-(F). Projections with the single Jas9-VENUS channel are shown in (G)-(L). The pink dashed line outlines young primordia and meristems. The high proliferation, decline and low proliferation phases are established according to flower production rate of *ful-2* mutant plants, Figure S2.1. Scale bars, 20 μm.

DISCUSSION

The molecular mechanisms that control PA at the end of flowering have started to be elucidated in the last few years. IM arrest is genetically regulated by the transcription factors FUL and AP2. It has been proposed that FUL accumulates in the IM as the plant ages and represses *AP2-like* genes, triggering PA (Balanzà *et al*., 2018). Moreover, FUL would act as a main repressor of auxin-related pathways (biosynthesis, transport and response), and as a co-regulator, together with other factors, of CK-related events (i.e., CK-signaling, cell divisions and *WUS* expression) during PA (Merelo *et al*., 2022; González-Cuadra *et al*., 2025). Here, to delve deeper into the mode of action of FUL during PA, we have characterized

the distribution of FUL protein within the SAM at high spatio-temporal resolution and have performed a transcriptomic analysis comparing apices of *ful* and wild-type plants during advanced stages of the flowering period. Furthermore, the availability of published transcriptomic data of inflorescence meristems responding to *AP2* induction (Martínez-Fernández *et al*., 2020) has allowed to identify putative molecular pathways downstream of FUL that are independent of AP2 activity. Moreover, published ChIP-seq data identifying potential FUL direct targets in active IMs (van Mourik *et al*., 2023) has also enabled the identification of potential direct targets of FUL involved in PA control.

FUL protein was detected mostly in the nucleus of the stem cells at 3 and 4 wab while, in active SAMs, it was located at both cytoplasm and nucleus. Moreover, *FUL* expression increases at the conspicuous PA. Thus, the higher transcriptional regulation observed at 3 and 4 wab, as well as the biphasic regulation of PA by FUL previously proposed (Merelo *et al*., 2022; González-Cuadra *et al*., 2025), may be due, at least in part, to FUL nuclear translocation. The activity of some transcription factors is regulated by nuclear translocation through interactions with other proteins (Lu *et al*., 2021; Marathe *et al*., 2024). However, the protein interaction partners of FUL in active meristems previously published (van Mourik *et al*., 2023) do not show significant expression changes at 3 wab (decline phase), when FUL protein location starts to be predominantly nuclear (**Table S8**). Interestingly, SOC1, which can translocate to the nucleus when it is expressed with specific partners (Lee *et al*., 2008), interacts with FUL at 4 wab. FUL-SOC1 heterodimer has previously been reported to promote flower initiation (Balanzà *et al*., 2014). In the context of PA, this interaction may enhance the nuclear localization of FUL at the end of flowering, thereby ensuring its activity.

Our current study shows that FUL may act as a direct activator of genes involved in ABA biosynthesis, signaling and response in the apex. The up-regulation of ABA-related genes is consistent with recent works showing that increased ABA levels and response in the inflorescence apex promote floral arrest at the end of flowering (Wuest *et al*., 2016; Martínez-Fernández *et al*., 2020; Sánchez-Gerschon *et al*., 2024). Furthermore, ABA-responsive genes are repressed by AP2 at the end of flowering (Martínez-Fernández *et al*., 2020), suggesting that ABA regulation occurs downstream of the FUL-AP2 module. Interestingly, our

data show that BRC1, which mediates bud dormancy by ABA accumulation through the activation of *HB21*, *HB40* and *HB53* expression (Aguilar-Martínez *et al*., 2007; González-Grandío *et al*., 2017) and has not previously been reported in relation to PA (Wuest *et al*., 2016; Martínez-Fernández *et al*., 2020), is induced in arrested meristems, likely regulated by FUL at the end of flowering. Since FUL represses *AP2* to promote PA (Balanzà *et al*., 2018), it may reinforce the regulation of ABA pathways by repressing *AP2*, thereby derepressing *HB* gene expression, and by promoting *BRC1* expression, which is upstream of this same route (**Figure 2.8**).

A previous work showed that FUL would repress CK response to promote PA, acting first as a mild repressor, together with additional factors, and then as a strong repressor that completely inhibits CK response (Merelo *et al*., 2022). Here, we show that FUL may repress CK-related pathways by directly promoting negative regulators of CK signaling, such as *ARRs*, and by directly repressing positive regulators of CK signaling, such as *CRF1*, while also repressing *AP2*, which in turn would negatively regulate CK signalling factors to maintain SAM activity (Martínez-Fernández *et al*., 2020). Our results, together with previous data, indicate that CK-related factors would be regulated both through the FUL-AP2 module and also independently and directly by FUL at the end of flowering (**Figure 2.8**).

We previously proposed a local role of auxin within the SAM during PA (González-Cuadra *et al*., 2025). FUL would start to repress auxin-related pathways (TAA1-mediated biosynthesis, PIN1-related transport and response) at 3 wab (onset of the decline phase) and completely block them at 4 wab (shutdown phase, respectively). In the current work, we have identified additional auxin-related factors potentially acting downstream of FUL in the regulation of PA. Particularly, genes encoding auxin biosynthesis (*YUC* family) and transport (*PIN* family) proteins may be directly repressed by FUL during the shutdown phase. Moreover, FUL promotes the expression of several *Aux/IAA* genes, which are negative regulators of auxin response (Bao *et al*., 2024). Since auxin-related pathways do not appear to be regulated by AP2 (Martínez-Fernández *et al*., 2020), our data further support previous results suggesting that FUL is the principal repressor of auxin-related pathways in the SAM at the end of flowering

(**Figure 2.8**) (González-Cuadra *et al*., 2025). Interestingly, FUL may also regulate JA-related pathways, which have not been extensively explored in the context of PA. The repression of JA biosynthesis genes and the induction of genes that encode negative regulators of JA signaling by FUL, along with the resulting decrease in JA content and signaling (Jas9-VENUS) during PA, suggests that JA negatively regulates this process downstream of FUL. Furthermore, FUL physically interacts with the JA-responsive proteins VEGETATIVE STORAGE PROTEIN 1 (VSP1), VSP2 and JACALIN-LECTIN LIKE 1 (JR1) at 3 and/or 4 wab (**Table S8**) (Berger *et al*., 1996; van Mourik *et al*., 2023). Given that the JA pathways may be repressed in arrested meristems, FUL could interact with them and inhibit their activity. Interestingly, the decrease in JA levels and signaling in the SAM started at the decline phase and became more pronounced at the shutdown phase, perfectly correlating with the gradual repression of the CK- and auxin-related pathways (Merelo *et al*., 2022; González-Cuadra *et al*., 2025). These observations also suggest the need of a coordinated and simultaneous regulation of these three hormonal pathways to control PA. Previous studies have demonstrated interactions among these hormones. JA and auxin share some signaling components, and their pathways are linked at different levels (Tiryaki & Staswick, 2002; Pauwels *et al*., 2010; Sun *et al*., 2011; Cai *et al*., 2014). During lateral root formation, JA induces the expression of several auxin biosynthesis genes (Hentrich *et al*., 2013), and JA biosynthesis mutant seedlings exhibit a reduction in auxin content (Gupta *et al*., 2024). Moreover, auxin-responsive factors promote JA production during flower development (Nagpal *et al*., 2005). On the other hand, the interaction between JA and CK differs depending on the specific developmental process. While JA increases CK content during root development (Avalbaev *et al*., 2016; Dob *et al*., 2021), it also inhibits CK response during xylem development (Jang *et al*., 2017). In the context of PA, our results suggest that JA and CK responses undergo similar temporal repression within the SAM. Moreover, our transcriptomic analyses reveal that genes related to CK-dependent processes, such as cell divisions, show expression patterns that correlate with those of JA-related genes. Cell cycle regulators, such as *CYCs*, may be directly repressed by FUL during PA, and the repression of JA signaling matches with the inhibition of cell division in primordia and meristem-primordia boundaries. Notably, JA has been proposed to regulate cell division in the

quiescent centre of the root meristem (Chen *et al*., 2011; Zhou *et al*., 2019). Hence, this leads us to propose that the reduction of JA signaling observed during PA may contribute to the repression of auxin-related factors and CK-related events, besides the potential direct repression by FUL, to inhibit SAM activity and growth during this process (**Figure 2.8**). How JA interacts with other hormones at the molecular level in the SAM constitutes an additional point to be further studied in the context of PA.

JA treatments are able to maintain for longer SAM activity and reactivate arrested SAMs. The AP2-related transcription factors TOE1 and TOE2, which promote meristem activity through the activation of *WUS* (Balanzà *et al*., 2018) and act then as negative regulators of PA, are directly repressed by JAZs (Zhai *et al*., 2015). High levels of JA promote JAZ degradation, thereby activating JA response and relieving the repression of TOE1/2 (Chini *et al*., 2007; Thines *et al*., 2007). Our transcriptomic data reveal that the temporal expression patterns of 7 *JAZ* genes and *WUS* are opposite at the end of the flowering time. Both *JAZ* and *WUS* genes may be directly or indirectly regulated by FUL 3 and/or 4 wab. Therefore, we could hypothesize that, during PA, the decrease in JA levels together with the upregulation of *JAZ* encoding genes, would cause the repression of *TOE1* and *TOE2* and, in turn, of *WUS*. Moreover, the analysis of the JA response reporter in *ful-2* mutant plants suggests that FUL would repress JA signaling, as JA signaling is active in the SAM of *ful-2* mutants along the flowering period in comparison with wt plants. FUL promotes IM arrest by repressing *AP2-like* genes (Balanzà *et al*., 2018) and CK response factors (Merelo *et al*., 2022), both of which maintain meristem activity through *WUS* expression (Martínez-Fernández *et al*., 2020; Meng *et al*., 2017). Altogether, these results suggest that FUL would mediate the repression of *WUS* expression not only through the repression of *AP2-like* genes or CK-related events, but also through JA-related pathways, ultimately leading to meristem arrest (**Figure 2.8**).

In conclusion, our work expands previous knowledge about the mode of action of FUL during the regulation of PA and proposes JA as a hormone involved in the negative control of PA downstream of FUL activity. JA-related factors could be directly connected to other hormones and genetic factors previously implicated in the regulation of PA, such as CKs, auxin, or AP2-WUS. However, further

research is required to elucidate the molecular mechanisms underlying these potential interactions at the end of the flowering period. Thus, a comprehensive understanding of these molecular mechanisms controlling PA could facilitate the development of new biotechnological and agronomic approaches to generate crops more productive but also more resilient to environmental changes.

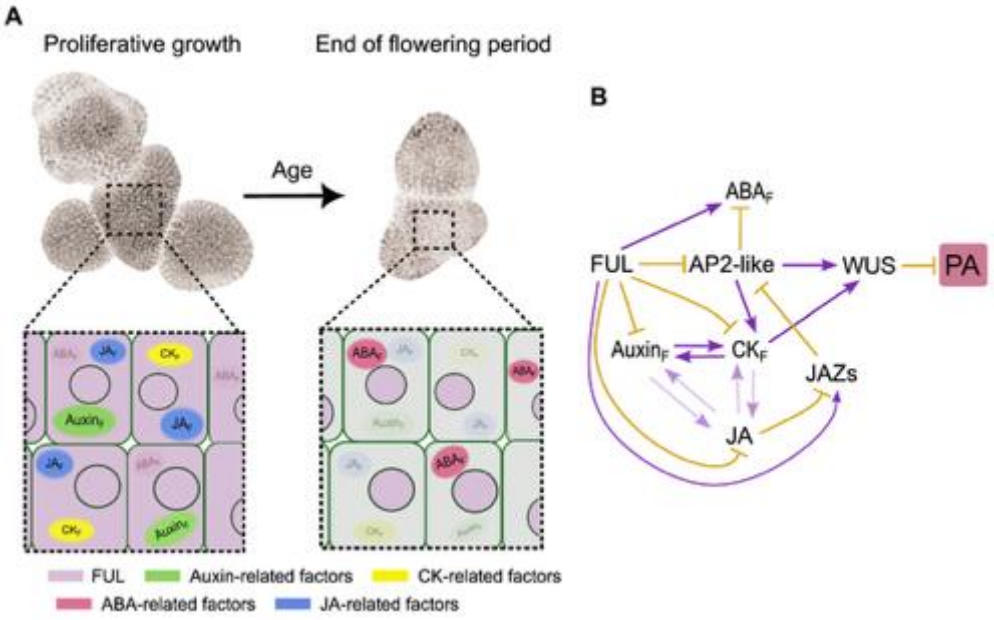

Figure 2. 8. JA-related factors negatively regulate PA. (A) Repression of JA-related factors (JA_F) promotes PA at the end of flowering. The reduction of JA levels and signaling in the SAM starts at the decline phase and becomes more pronounced at the shutdown phase, correlating with the gradual repression of CK- and auxin-related factors (CK_F and $Auxin_F$, respectively). FUL contributes as the main repressor of these factors (JA_F, CK_F, $Auxin_F$) during the shutdown phase to promote meristem arrest. Furthermore, FUL promotes ABA-related factors (ABA_F) from the decline until the shutdown phase, triggering PA. These changes fit with the subcellular localization of FUL in high proliferative (nuclear and cytoplasmic) and arrested SAMs (decline and shutdown phases; nuclear). (B) The decrease in AP2 activity, together with the FUL-mediated increase in *BRC1* expression, may promote the increase of ABA_F and, consequently, the acquisition of the dormant stage in arrested meristem. Moreover, the reduction of JA_F observed during PA may contribute to the repression of $Auxin_F$ and CK_F, besides the potential direct repression by FUL, to inhibit SAM activity and growth during PA. Furthermore, the decrease in JA levels and the accumulation of FUL in the nucleus may promote the expression of *JAZs*, which would cause the repression of *AP2-like* genes and then *WUS* repression and PA. Thus, FUL may mediate *WUS* repression by downregulating AP2-, CK-, and JA-related factors, ultimately triggering PA.

MATERIAL AND METHODS

Plant material

The *Arabidopsis thaliana* plants used in the RNA-seq and MeJA treatment assays were in ecotype Landsberg *erecta* (L*er*): *ful-1 (Gu et al., 1998)* and *pWUS:EGFP-WUS (Yadav et al., 2011)*, respectively. The following lines were in Columbia-0 (Col-0) background and have been previously described: *ful-2 (Ferrándiz et al., 2000)* and *35S:JAS9-N7-VENUS* (Larrieu *et al*., 2015). We obtained the following T-DNA insertion lines from the SALK collection (Alonso *et al*., 2003) or NASC: *jaz3* (SALK_139337C), *jaz5-1* (SALK_053775), *jaz6-1 (SAIL_1156_C06)*, *jazD* (N72544), *myc2/3/4* (N73349). *35S:JAS9-N7-VENUS* was crossed to *ful-2* and the assays were performed with F3 homozygous plants.

For all the analyses described, Arabidopsis seeds were stratified on soil at 4 ºC for 3 days under dark conditions. Plants were grown in the greenhouse under long-day conditions (16 h light, 8 h dark) at 21 ºC, with light provided by cool-white fluorescent lamps (150 µE m^{-2} s^{-1}). The growth substrate consisted of a 2:1:1 (v/v/v) mixture of sphagnum:perlite:vermiculite and a dilution of the Hoagland's nutrient solution 1 was used to water the plants.

Generation of constructs and plant transformation

pFUL:FUL-3xYPet transgene was generated through the recombineering-based tagging system, using JAtY clones with universal adaptors at the 5' and 3' ends of the recombineering cassette (Brumos *et al*., 2020). We used the pYLTAC17 vector that contains the *FUL* locus (AT5G60910) within the JAtY clone JAtY53I20 69586 (JAtY library; JIC; https://abrc.osu.edu/stocks/number/CD4-96). *FUL* was amplified and cloned into SW105 competent bacteria using the next primers: 5'-GCTTCCGGCTTGGATGTTACGTCCTACCACTACGAACGAGGGAGGTGGAG GTGGAGCT-3' (FUL-Rec-F; forward), 5'-ACATTAATTATATTATCATTATATTATAAAGAGTGAGATAGTTCTAGGCCCC AGCGGCCGCAGCAGCACC-3' (FUL-Rec-R; reverse). Then, the JAtY clone was trimmed to obtain a final genomic region of *FUL*, extending 10 Kb upstream

and 5 Kb downstream of *FUL*. For this, we used the following primers: 5'-AGTTCTAGATGAAGTCATGAAGGTTGTTTTTTATATTTACCAATGCTTAATCAGTG-3' (FUL-TRIMING-LEFT; forward), 5'-ATTAAATCAGAAAGCTGTTGTTTCCTAGCTACATGAACACAGCGAATGCTAGTCTAGCTGTTGC-3' (FUL-TRIMING-RIGHT; reverse).

3xYPet was amplified and cloned into the trimmed clone using the next primers:

5'- GCTTCCGGCTTGGATGTTACGTCCTACCACTACGAACGAG-3' (3xYPet-Rec-F; forward), 5'-ACATTAATTATATTATCATTATATTATAAAGAGTGAGATAGTTCTA-3' (3xYPet-Rec-R; reverse). The final construction was introduced in *Agrobacterium tumefaciens* C58. Arabidopsis plants were transformed using the floral dip method (Clough & Bent, 1998). Homozygous T3 transgenic lines carrying a single transgene insertion were selected on Murashige and Skoog (MS) (Duchefa-Biochemie) plates containing Basta (Duchefa-Biochemie). The optimal lines were selected after analyzing FUL-3xYPet signal under the confocal.

RNA-seq and data analysis

For the RNA-seq analysis, 22 shoot apices of L*er* and *ful-1* plants at different stages of the flowering period were collected. L*er* apices were harvested 2, 3 and 4 wab and 1 wap, and *ful-1* apices were collected 2, 3, 4 and 6 wab. Flowers and older flower buds were removed with clean tweezers and the samples were immediately frozen in liquid nitrogen. Four biological replicates were sampled for each time point and genotype. RNA was extracted with the commercial RNeasy Plant Mini Kit (QUIAGEN). RNA integrity was determined using an Agilent 2100 Bioanalyzer. RNA sequencing was performed by Novogene Company (UK), with 20M reads and 6 Gb of raw data per sample in FASTQ format. For the bioinformatic analysis, the raw reads were cleaned to remove adapters and eliminate low quality regions using the *cutadapt* software (Martin, 2010). The clean reads were mapped to the reference genome of Arabidopsis TAIR10 available at the TAIR database (Lamesch *et al*., 2012), using the *HISAT2* program (Kim *et al*., 2019). Finally, the counting of reads per gene was performed using the *htseq-count* tool (Anders *et al*., 2015). *DESeq2* package (Love *et al*.,

2014) was used to normalize read counts across samples and to identify DEGs. All the processing was carried out by the IBMCP bioinformatic service.

GO enrichment analysis was performed using the online tool ShinyGO v0.75 (Ge *et al*., 2020). Enriched biological process categories were analyzed with a FDR ≤ 0.05 against *Arabidopsis thaliana* genes TAIR10 as background. The enrichment GO charts were generated using a set of the output from the GO term biological process selected because of the biological interest or previous relationship with PA.

Gene expression clustering was carried out using the fuzzy c-means algorithm implemented in the Mfuzz package (Kumar & Futschik, 2007). Read counts were normalized and variance-stabilized to recover four and five clusters per genotype (wild-type or *ful*), respectively, using default parameters.

The RNA-seq data discussed in this chapter have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus (Edgar *et al*., 2002) and are accessible through the GEO Series accession number GSE299176.

Flower and fruit number quantification

Flowers in stages 12-15 present in the primary apex and the total number of fertile fruits (stages 16-20) in the primary stem were quantified at each time point. For wild-type, *ful,* FUL-3xYpet and JA-related mutant plants, quantifications were carried out every week from 0 to 7 wab in at least 10 plants. 0 wab is considered the time when the cluster of flower buds becomes visible after floral transition. For the MeJA treatment assay, fruit quantifications were performed every 2 days in at least 15 plants.

Reactivation and hormonal treatments

Reactivation of arrested apices by defruiting was performed by removing all the fruits in the main stem as well as the rosette-leaf and cauline-leaf branches. For every reactivation assay, 15-20 plants of each genotype were used.

Methyl jasmonate (MeJA) treatments were carried out applying a solution of 50 µM MeJA (Sigma-Aldrich; stock solution was prepared in water) and 0.03% [v/v] Tween-20 (Sigma-Aldrich) to the shoot apices by spraying. Mock solution (water and 0.03% [v/v] Tween-20) was used to treat control apices. For the assay of PA delay, active apices of 24 *pWUS:EGFP-WUS* plants were sprayed from 2 wab and every two days with MeJA or mock solution. For the MeJA-mediated reactivation assay, arrested apices (4 wab) of 24 *pWUS:EGFP-WUS* plants were sprayed every 2 days with MeJA or mock solution. Quantification of flowers and fruits in the primary apex of MeJA and mock-treated plants was carried out as described above.

Confocal microscopy and image analysis

To perform live imaging analyses, we used a Stellaris 8 FALCON confocal microscope (Leica, Germany) and a water-dipping 25X objective. Shoot apices were imaged under water on MS medium-containing boxes. All flower buds were removed with clean tweezers and a fine needle. After dissection, cell membranes were dyed by incubating in FM4-64 (30 mg/mL; Invitrogen) as described in Merelo *et al*. (2022). YPet and VENUS were imaged using a White light laser (WLL; Supercon) emitting at the wavelength of 514 nm and collected at 508-545nm. GFP was imaged using a WLL with an excitation wavelength of 488 nm together with a 499-527 nm collection. FM4-64 was excited with the WLL emitting at the wavelength of 488 nm and collected at 666-759 nm. We used sequential scanning in line-scan mode to image YPet/FM4-64, GFP/FM4-64 and VENUS/FM4-64 combinations. For Z stack acquisition, we used a resolution of 12-bit depth, a Z step of 0.8 µm and a line average of 2. YPet, GFP and VENUS gain were set up equally in all the samples of each experiment. We used ImageJ (FIJI; https://fiji.sc/) (Schindelin *et al*., 2012) to obtain maximum intensity projection images, longitudinal sections images, and the fluorescence intensity scale (signal heat-map). In every assay, brightness was modified equally for all the samples to properly visualize YPet and VENUS.

Quantification of JA

Apices of Col-0 wild-type plants were collected 2 and 4 wab, and 1 wad. Apices of *ful-2* plants were collected 2, 4 wab and 6 wab. Previously, we removed flowers and older buds with clean tweezers. Three biological replicates were harvested and analyzed. 50 mg of plant material was resuspended in a solution that contained 80% (v/v) methanol, 1% (v/v) acetic acid and an internal standard (9,10-DIHYDROJASMONIC ACID [DJA]; OlChemIm). Then, all the components were mixed by shaking during 1 h at 4ºC. Quantification of JA levels was carried out as described in Seo *et al*. (2011) (IBMCP hormone quantification service).

Statistical analyses

We used the GraphPad Prism 9 software (https://www.graphpad.com/) to perform all the statistical analyses. The two-tailed Student's t-test was used to determine the significance of the data.

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SUPPLEMENTAL INFORMATION

The Supplementary Tables of this chapter are available online in the following link:

https://docs.google.com/spreadsheets/d/1G6FWAuRV70tx0sa22clLjI0ZHcy6ymNXUgIZJjO-1yo/edit?usp=sharing

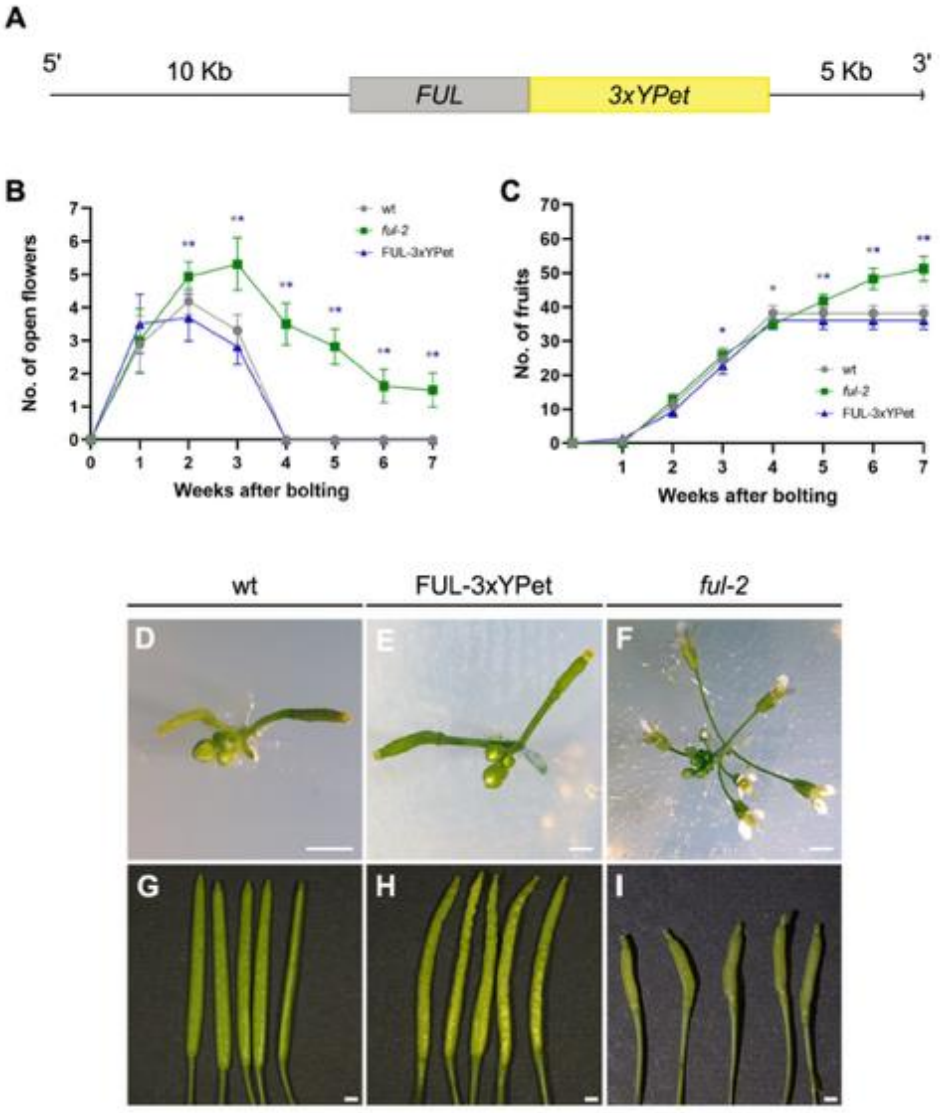

Figure S2. 1. FUL-3xYPet rescues *ful-2* phenotype. (A) Schematic representation of FUL-3xYPet construct. Gray and yellow rectangles represent *FUL* gene and *3xYPet* sequence, respectively. The final genomic region encompasses 10 Kb upstream and 5 Kb downstream of *FUL*. (B) Number of flowers at stages 12-15 in the primary apex of wild-type (wt), *pFUL:FUL-3xYPet* (FUL-3xYPet) and *ful-2* plants from 0 to 7 weeks after bolting (wab). (C), Total number of fertile fruits (stages 16-20) in the primary stem of wt, FUL-3xYPet and *ful-2* plants 0-7 wab. Data are represented as mean ± SD of 16 biological replicates. Asterisks indicate significant differences ($p<0.005$) according to two-tailed Student's test comparing *ful-2* with wt and FUL-3xYPet plants. Each colour of the asterisk corresponds to the colour legend. (D-F) Apices of wt (C), FUL-3xYPet (D) and *ful-2* (E) plants 4 wab. (G-H). Fruits of wt (G), FUL-3xYPet (H) and *ful-2* (H) plants. Scale bars, 1 mm.

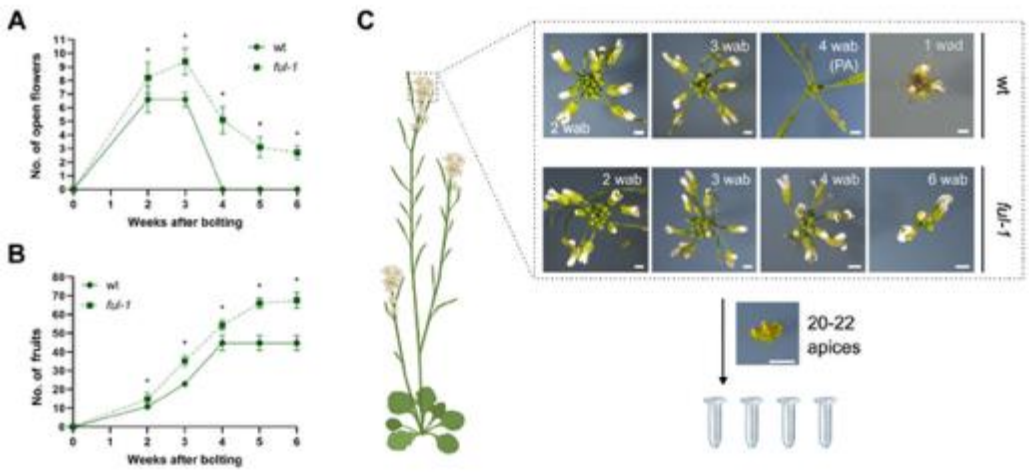

Figure S2. 2. Workflow scheme for the RNA-seq experiment. (A) Number of flowers (stages 12-15) in the primary apex of wt and *ful-1* plants from 2 to 6 wab. (B) Total number of fertile fruits (stages 16-20) in the primary stem of wt and *ful-1* plants 2-6 wab. Data are represented as mean ± SD of 10 biological replicates. Asterisks indicate significant differences ($p < 0.005$) according to two-tailed Student's test comparing each genotype. (C) Images of apices at the different time points when were sampled. Inflorescences of the main stems were harvested and dissected to eliminate older buds. Four biological replicates were sampled, each one containing 20-22 apices. Scale bars, 1 mm. Arabidopsis plant image obtained from BioRender.com.

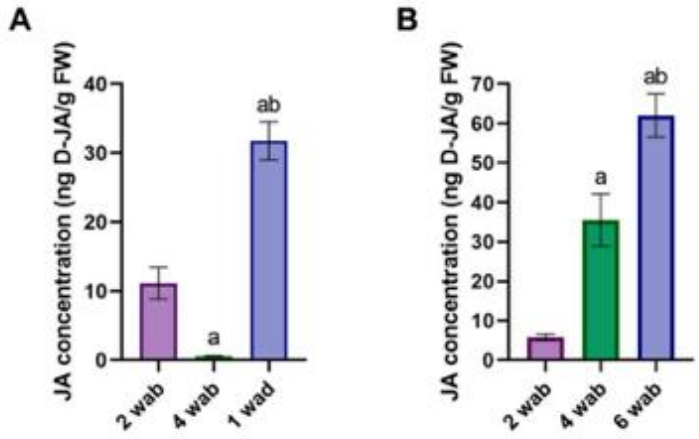

Figure S2. 3. **Changes in JA content in apices of wt and *ful-2* plants.** (A) JA levels in active (2 wab), arrested (4 wab) and reactivated apices (1 wad) of wt plants. (B) JA levels in apices 2, 4 and 6 wab of *ful-2* mutant plants. Data are represented as mean ± SD of 3 biological replicates (3 pools of 50 mg of plant material). Letters indicate significant difference $p < 0.05$: a, two-tailed Student's test versus 2 wab time point and b, two-tailed Student's test versus the previous time point.

General discussion

During the last few years, different studies have highlighted the complexity of the proliferative arrest (PA) process, identifying environmental, genetic and hormonal pathways, as well as other signaling factors involved in its regulation (González-Suárez *et al*., 2020; Balanzà *et al*., 2023). The work presented in this thesis contributes to extend this previous knowledge on PA control by shedding light on two major aspects: understanding auxin dynamics within the SAM and identifying new players that potentially act downstream of FUL at the end of the flowering period.

Auxin exported from fruits has been proposed to promote floral arrest by disrupting auxin transport in the apical region of the stem (González-Suárez *et al*., 2020; Ware *et al*., 2020; Goetz *et al*., 2021; Walker *et al*., 2023). However, auxin dynamics within the SAM during PA had not been characterized to date. The detailed spatio-temporal characterization of changes in auxin pathways in the SAM performed in the current thesis has shown that auxin biosynthesis, transport and response must be locally repressed in the SAM for PA to occur. Furthermore, local changes in auxin content within the SAM affect PA, as the induction of auxin biosynthesis delays IM arrest and reactivates arrested SAMs, whereas inducing auxin degradation suppresses SAM activity. Therefore, besides previous findings suggesting that fruit-derived auxin may indirectly promote floral arrest (González-Suárez *et al*., 2020; Ware *et al*., 2020; Walker *et al*., 2023), this thesis proposes that local control of auxin levels, transport and signaling within the SAM would function as a mechanism regulating meristem arrest.

Furthermore, local repression of auxin pathways correlates with the gradual repression of CK-related factors (CK response, CYCB1;2-dependent mitosis, *WUS* expression and SAM growth) at the end of flowering (Merelo *et al*., 2022). Modifications in auxin content influence CK signaling: auxin biosynthesis induction promotes CK signaling, while auxin degradation leads to its repression. At the same time, CK treatments promote auxin-related factors, thereby maintaining SAM activity and reverting IM arrest. Thus, the synchronized temporal regulation of both hormonal pathways, together with their reciprocal

effects upon auxin and CK content modifications, suggests a coordinated and simultaneous regulation of both hormones during PA (**Figure D.1**) (Meng *et al*., 2017; Ma *et al*., 2019; Merelo *et al*., 2022; González-Cuadra *et al*., 2025). Since CK and auxin regulate cell proliferation and differentiation (Heisler *et al*., 2005; Gordon *et al*., 2009; Zhao *et al*., 2010; Luo *et al*., 2018; Shi *et al*., 2019), the arrest of SAM activity during PA would involve the coordinated downregulation of these processes.

Moreover, both hormonal pathways are regulated by FUL. FUL would promote meristem arrest, at least in part, by repressing auxin- and CK-related factors (Merelo *et al*., 2022; González-Cuadra *et al*., 2025). In our previous study, we reported a decline in CK-related markers in *ful* meristems at 3-5 wab, as in wt meristems, but the absence of a complete shutdown comparable to that observed in wt plants at the equivalent time point to PA. Thus, we proposed that FUL, together with additional factors, would regulate CK-related factors. Initially (3 wab; decline), FUL appeared to act as a mild repressor of these factors, and later (4 wab; shutdown), as a strong repressor (Merelo *et al*., 2022). Differently from CK pathways, auxin pathways remained active throughout the flowering period in *ful* meristems. This suggests that the regulation of auxin pathways would mainly depend on FUL activity (Merelo *et al*., 2022; González-Cuadra *et al*., 2025). Transcriptomic analyses comparing apices of wt and *ful* plants at advanced stages of the flowering period, combined with FUL-related ChIP-seq data, have identified potential direct targets of FUL associated with auxin pathways. Most of these genes were not regulated by AP2, the other major regulator of PA (Martínez-Fernández *et al*., 2020). These analyses also indicated that FUL may directly repress CK-related factors. It has been described that CK response is promoted by AP2 in active SAMs through the direct repression of negative CK signaling regulators (Martínez-Fernández *et al*., 2020). Since FUL is a repressor of *AP2* (Balanzà *et al*., 2018), it may regulate CK-related pathways through direct control, as well as through the FUL-AP2 module (Martínez-Fernández *et al*., 2020; Merelo *et al*., 2022). Interestingly, the different behaviour observed between auxin and CK pathways suggests that the regulation of auxin pathways would mainly depend on FUL activity, whereas CK pathways may be regulated directly by FUL, through the FUL-AP2 module and by additional factors. *AP2* is

negatively regulated by the microRNA172 (miR172), whose expression increases in the inflorescence meristem at the end of the flowering period (Balanzà *et al*., 2018). This upregulation could contribute to the repression of CK pathways through the downregulation of *AP2*. However, further studies are required to clarify these regulatory mechanisms that control PA.

Previous studies have identified that some ABA-related genes regulated by AP2 contribute to ABA accumulation in the inflorescence apex, leading to floral arrest (Martínez-Fernández *et al*., 2020; Sánchez-Gerschon *et al*., 2024). In this thesis, we show that a high number of ABA-related genes that are regulated by AP2 and, therefore by the FUL-AP2 module, may also be direct targets of FUL in proliferative meristems (van Mourik *et al*, 2023). Taken together, the results described in this thesis suggest that FUL may contribute, at least in part, to the arrest of SAM activity by directly promoting ABA-related factors and repressing auxin and CK-related factors (**Figure D.1**). FUL antagonizes AP2 in the regulation of PA-related pathways through the FUL-AP2 module, and both factors exhibit opposite temporal expression patterns at the end of flowering (Balanzà *et al*., 2018). Since FUL and AP2 share common downstream targets in the IM, FUL could compete with AP2 for binding to these targets, thereby modulating their transcriptional regulation. However, future research will reveal the molecular basis of the antagonistic function between FUL and AP2 during PA control.

FUL-dependent repression of JA biosynthesis, metabolism and response genes, along with the resulting decrease in JA content and response in the inflorescence apex during PA, suggests that JA negatively regulates this process downstream of FUL (**Figure D.1**). These changes correlate with the gradual repression of CK- and auxin-related pathways during the two PA phases previously established (decline and shutdown) (Merelo *et al*., 2022; González-Cuadra *et al*., 2025), suggesting a coordinated regulation of these three hormonal pathways in PA control. The repression of JA signaling pathways could be linked to the inhibition of CK-dependent cell divisions and, consequently, to the suppression of primordia initiation and development during PA (Merelo *et al*., 2022). This inhibition fits with the repression of auxin pathways, which ultimately drive new organ formation (González-Cuadra *et al*., 2025). Thus, the coordinated repression of these three hormones would repress SAM activity and growth,

triggering PA (**Figure D.1**). Moreover, genes encoding negative regulators of JA signaling (*JAZs*) directly repress *AP2-like* genes (Chini *et al*., 2007; Zhai *et al*., 2015). During PA, the reduction in JA levels and the subsequent upregulation of JAZ encoding genes may contribute to the repression of *AP2-like* genes, further reinforcing the negative regulation exerted by FUL, resulting in reduced WUS protein levels (Balanzà *et al*., 2018; Martínez-Fernández *et al*., 2020). Additionally, auxin signaling and CK-related factors, which modulate the WUS-CLV3 feedback loop to maintain SAM activity, are also regulated by FUL (Meng *et al*., 2017; Ma; Luo *et al*., 2018; Ma *et al*., 2019; Merelo *et al*., 2022; González-Cuadra *et al*., 2025). Thus, FUL may contribute to the repression of *WUS* by downregulating *AP2*, CK-, auxin- and JA-related factors, leading to PA (**Figure D.1**). Additional assays beyond JA treatments and the characterization of JA-related interaction networks in the PA context will help to better understand the regulatory network contribution to PA control.

Finally, we have shown that FUL protein accumulates in the nucleus during the decline and shutdown phases. Thus, the higher transcriptomic regulation related to CK, auxin, ABA and JA pathways observed at these stages, together with the FUL's biphasic role in PA regulation (Merelo *et al*., 2022; González-Cuadra *et al*., 2025), may be partly attributed to its subcellular localization dynamics (**Figure D.1**).

In conclusion, the work described in this thesis provides new insights into the spatio-temporal regulation of PA. We have provided new evidence on auxin dynamics within the SAM at the end of flowering, showing that local auxin biosynthesis, transport and response play a key role in IM arrest. Additionally, our findings support a tight positive interaction between auxin and cytokinin in the regulation of meristem arrest. We have identified additional factors related to hormones previously linked to PA (auxin, CKs and ABA) (Wuest *et al*., 2016; Martínez-Fernández *et al*., 2020; Ware *et al*., 2020; Merelo *et al*., 2022; Walker *et al*.; 2023; Sánchez-Gerschon *et al*., 2024), as well as other hormonal pathways and developmental processes downstream of FUL (i.e., JA, GA and ethylene pathways, shoot development, cell cycle and senescence), highlighting the central role of FUL in controlling meristem activity during PA. Our results point to JA as a novel player in the control of this process. However, several interesting

aspects remain to be addressed. For instance, what are the molecular mechanisms underlying the potential interactions between JA-related factors and other hormones and genetic factors previously involved in PA control (CK, auxin, ABA and AP2-WUS)? What additional elements contribute to the differing behavior of auxin, compared to CKs, during PA? How are the new candidate regulators of PA temporally and spatially distributed within the SAM during this process? Do additional factors or fruit-derived signals regulate PA, impacting the pathways discussed in this thesis or by mainly independent mechanisms? Could FUL act as an integrator of the fruit-derived signals in PA control? What are the molecular mechanisms underlying the differing regulation of the pathways mainly regulated by FUL versus those regulated through the FUL-AP2 module? Addressing these questions in the future will be crucial to further understanding the complex regulatory network that controls PA.

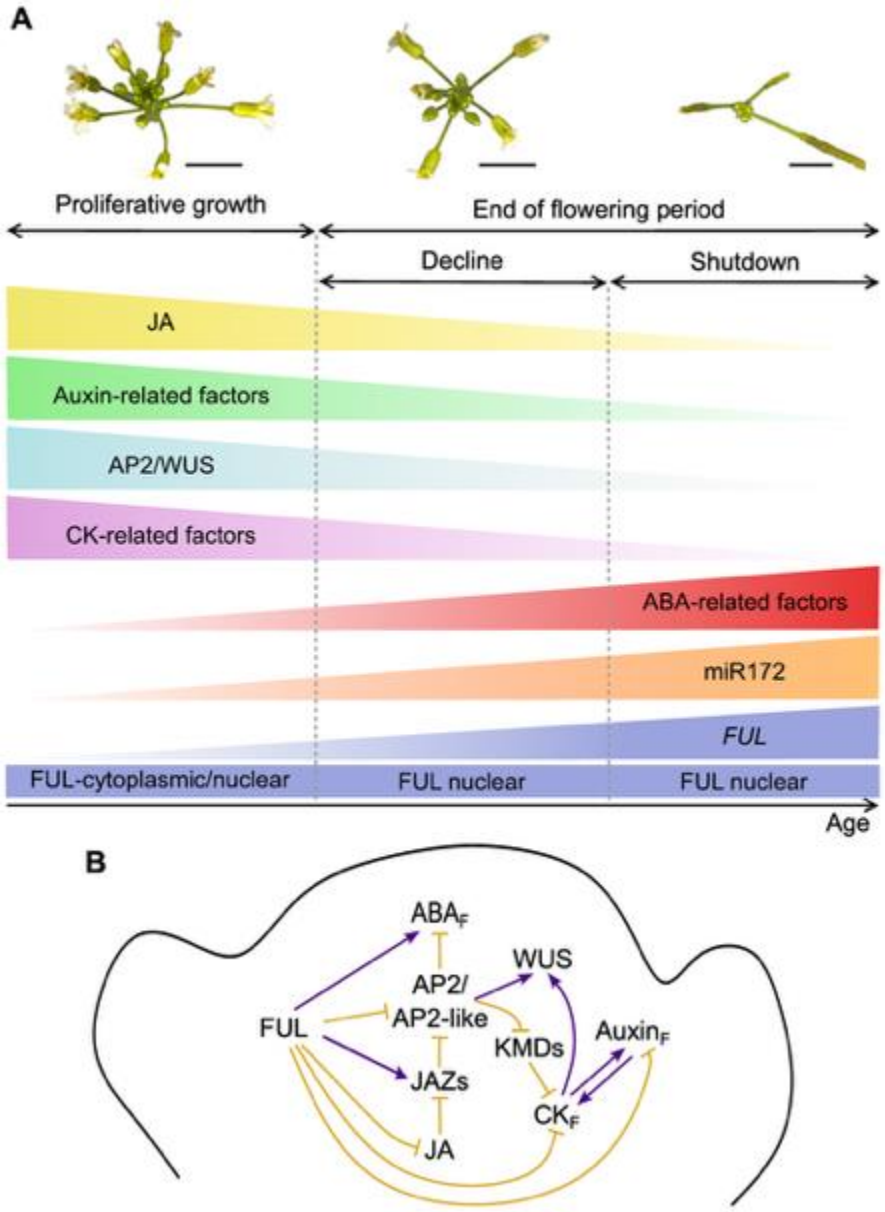

Figure D. 1. Temporal changes in genetic and hormonal factors involved in meristem arrest during PA. (A) CK-, auxin- and JA-related factors and the transcription factors AP2 and WUS maintain the activity of the meristem during the proliferative growth stage. At the end of the flowering phase, these factors are repressed (decline phase) and completely blocked (shutdown phase) in the SAM. On the other hand, ABA signaling increases and miR172 accumulates at the end of flowering. Finally, the increase in *FUL* expression at the conspicuous PA (shutdown phase), together with the accumulation of FUL in the nucleus during the decline and shutdown phases, would ensure transcriptional regulation of its target genes during PA. (B) CK- and auxin-related factors (CK_F and Aux_F, respectively) and *AP2* and *WUS* expression may be repressed, in part, by FUL. On the other hand, FUL would promote the expression of ABA-related genes (ABA_F) and negative regulators of JA signaling (*JAZs*) during PA.

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Conclusions

The work described in this thesis has allowed us to establish the following conclusions:

1. Local auxin biosynthesis, along with its transport and response, are fundamental in the control of PA. These auxin-related pathways must be locally repressed in the SAM for PA to initiate and progress.
2. Regulation of auxin and CK pathways is coupled within the SAM during PA. The simultaneous and coordinated repression of factors related to these two hormones inhibits both stem cell differentiation and proliferation processes, leading to IM arrest.
3. FUL would promote PA by repressing auxin-related pathways locally in the SAM. These pathways strongly depend on FUL activity rather than on fruit/seed-derived signals or additional factors.
4. The increase in nuclear localization of FUL during the two phases of PA (decline and shutdown) would be necessary for its function during this process. Moreover, these subcellular localization changes temporally coincide with an increased transcriptional activity in the apex, potentially mediated by FUL.
5. Auxin, JA-, GA- and ethylene-related pathways, along with developmental processes, such as shoot development, cell cycle progression and senescence, would be mainly regulated by FUL rather than by the FUL-AP2 module. However, FUL may directly regulate ABA- and CK-related pathways, as well as via *AP2* repression.
6. The repression of JA biosynthesis genes and the consequent decrease in JA content in the shoot apex, as well as the repression of JA signaling in the meristem, may promote PA.
7. The coordinated repression of JA, auxin and CK pathways at the end of the flowering period would contribute to the suppression of SAM activity.