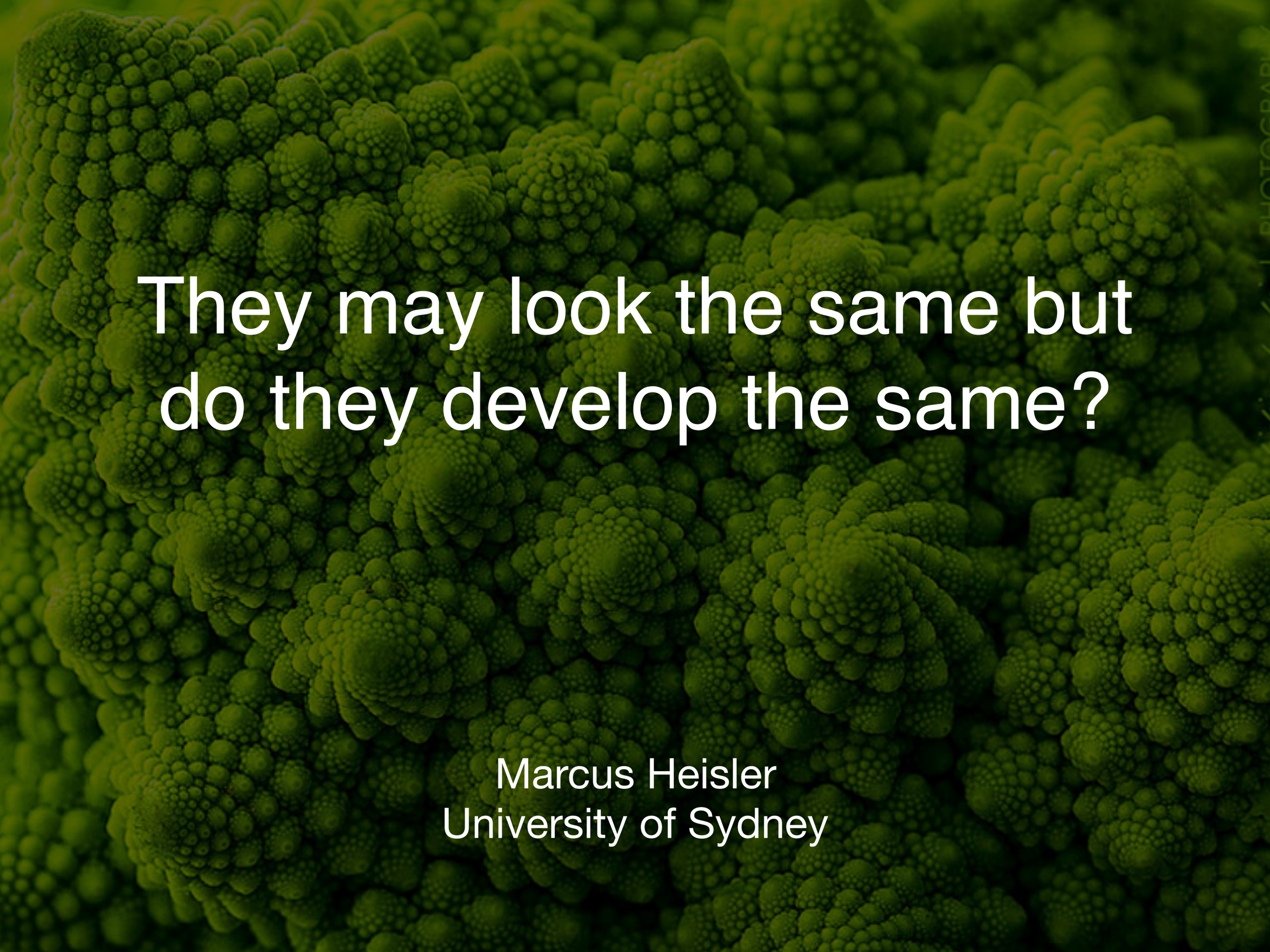
They may look the same but
do they develop the same?
Marcus Heisler
University of Sydney

Acknowledgments

Heisler Lab

Xiulian Yu
Neha Bhatia
Carolyn Ohno
Monica Pia Caggiano
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Jonsson lab
Sainsbury lab
Cambridge

Meyerowitz lab
Caltech

EMBO excellence in life sciences
EMBL
Australian Government
Australian Research Council
THE UNIVERSITY OF SYDNEY
erc
European Research Council
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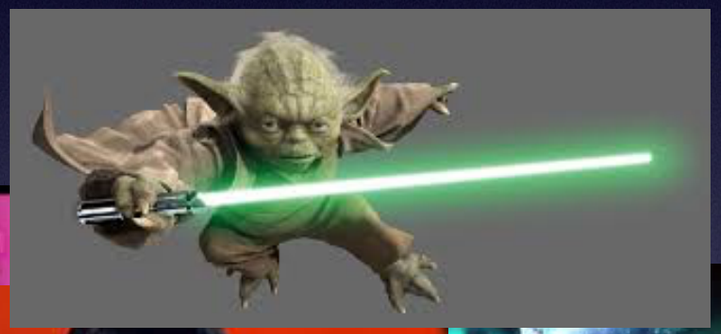
They may look the same but do they develop the same?

They may look the same but do they develop the same?

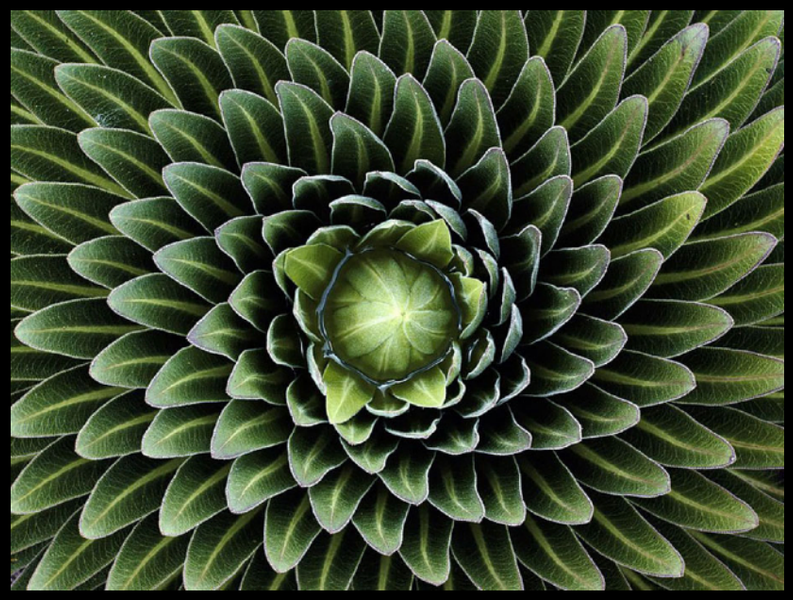


They may look the same but do they develop the same?

- Might certain developmental mechanisms be of such general utility that they appear repeatedly in evolution (need to exclude homology)?

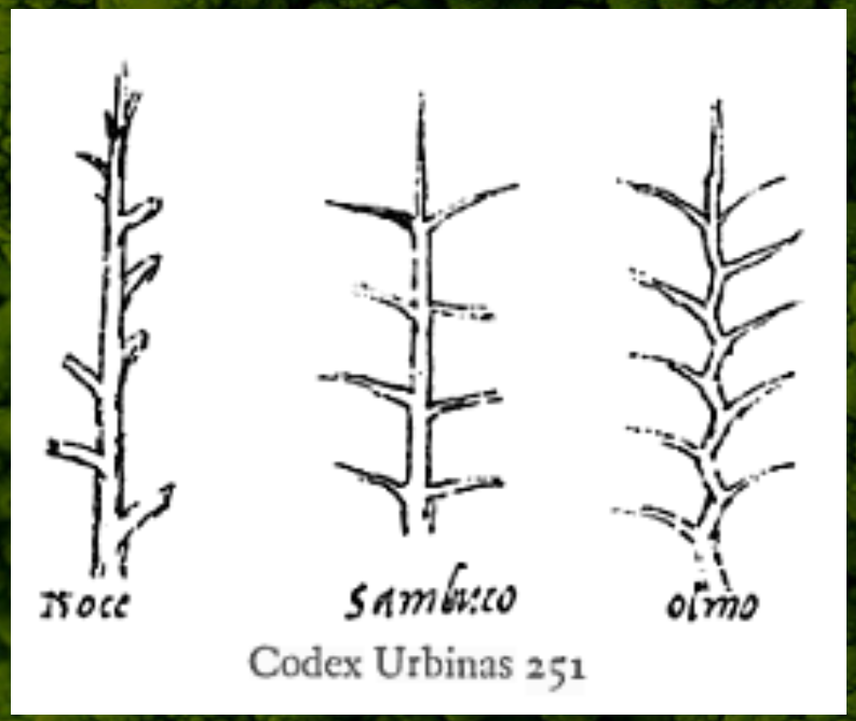
- From these instances can we extract general principles - perhaps at a higher level than morphogens and mechanics?

Common features of plant lateral organs - periodicity and a flattened shape



Plant organ positioning (phyllotaxis) - *an old question*

Leonardo Da Vinci c.1500



Codex Urbinas 251

Elm Elderberry Walnut

Plant organ positioning (phyllotaxis) - *an old question*

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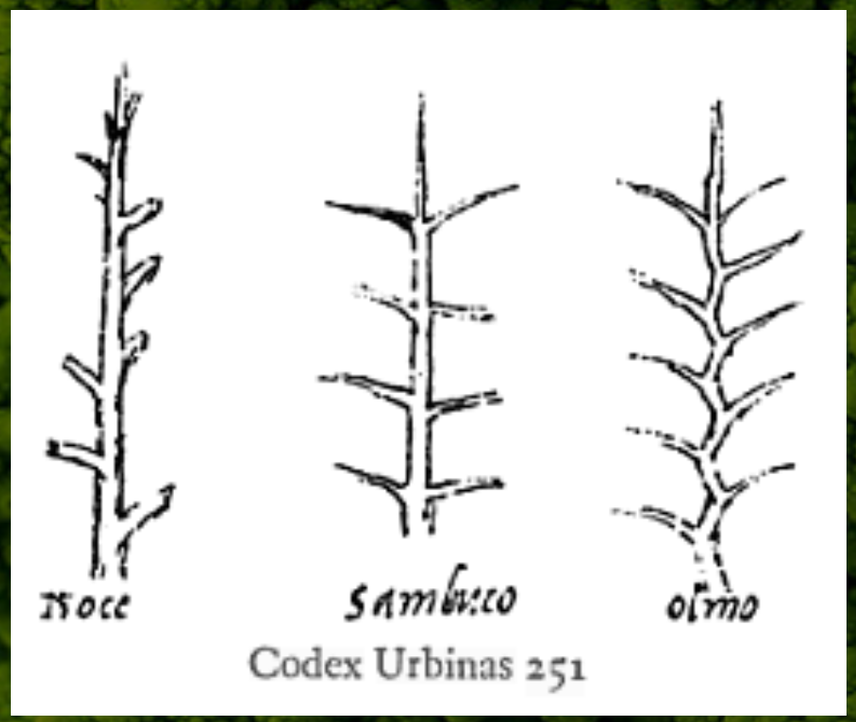
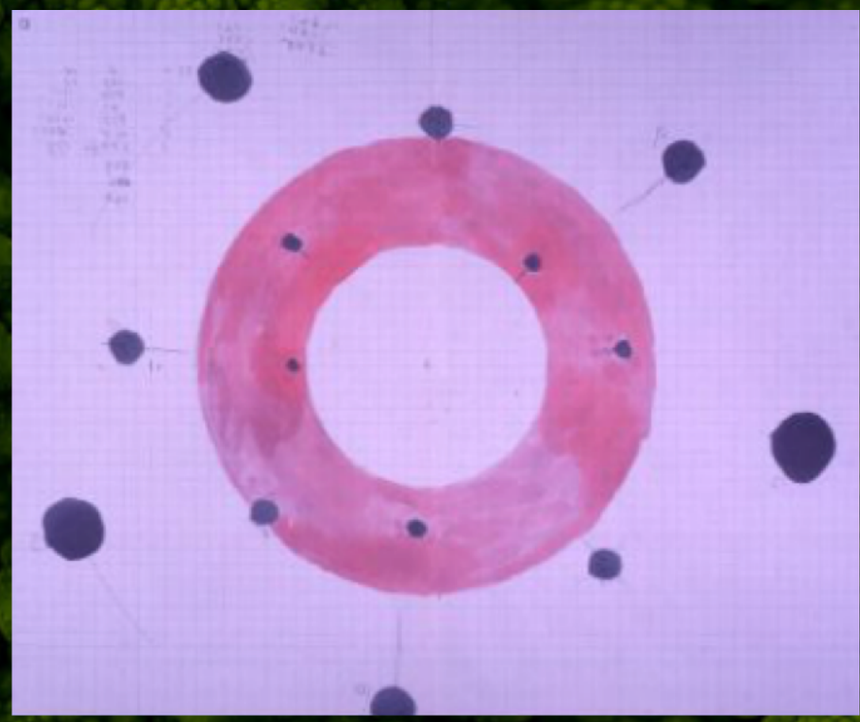


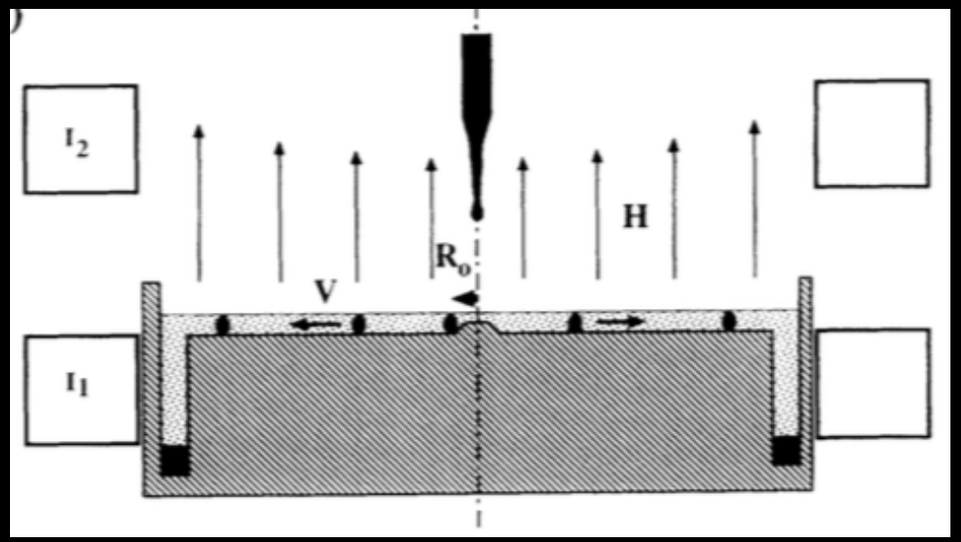
Elm Elderberry Walnut

Alan M. Turing c.1950



Turing applied his diffusion-reaction mechanism to create phyllotactic patterns

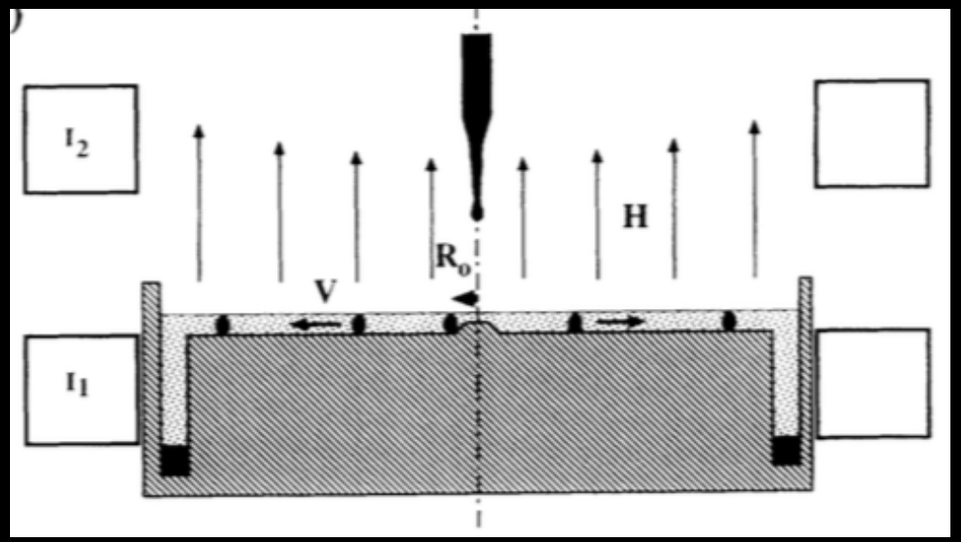
A physical demonstration….



Douady and Couder (1992)

EMBL

A physical demonstration….

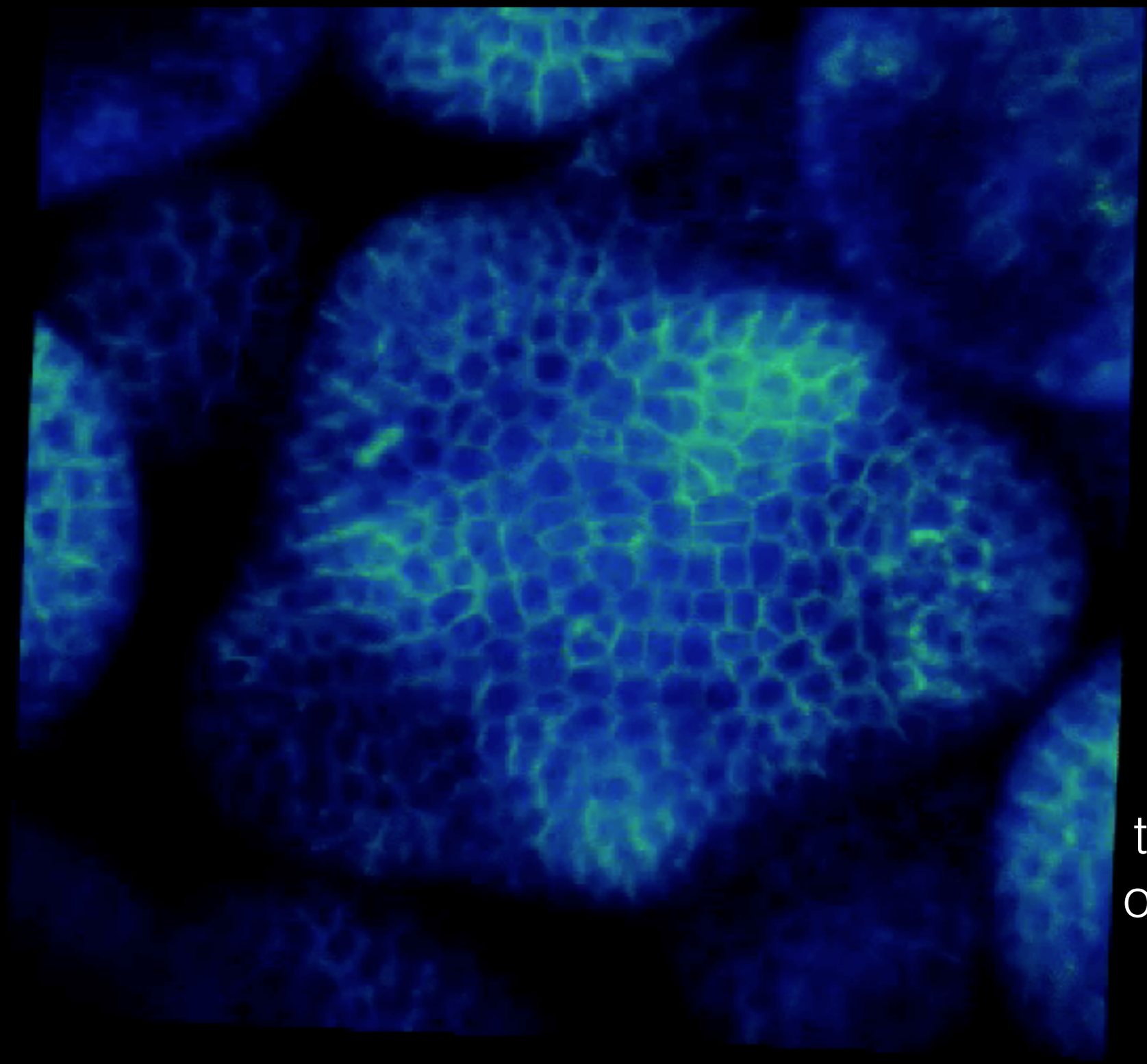


Douady and Couder (1992)

EMBL

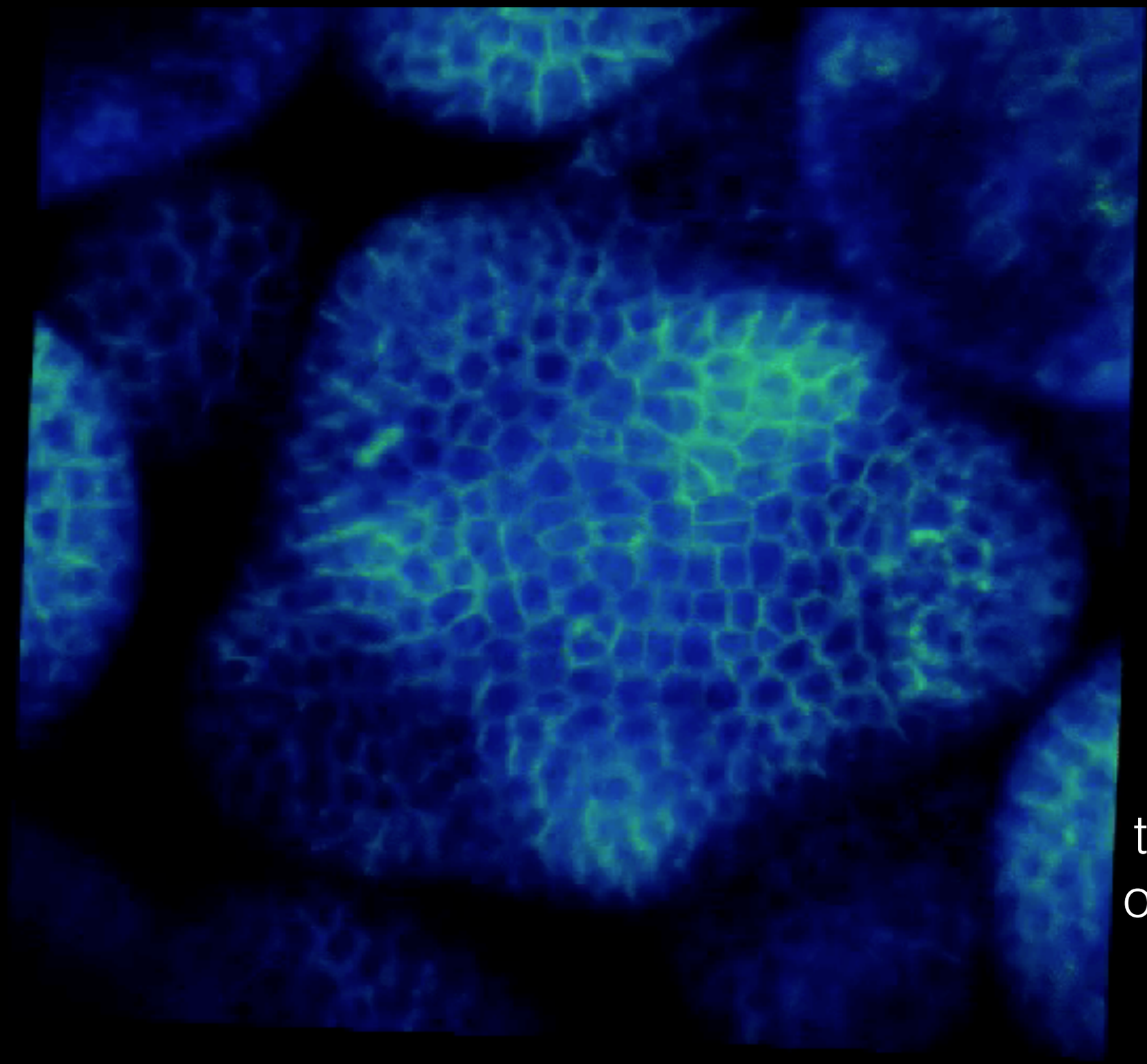
The Arabidopsis shoot meristem

Organogenesis at the shoot meristem



time lapse
over 40 hrs

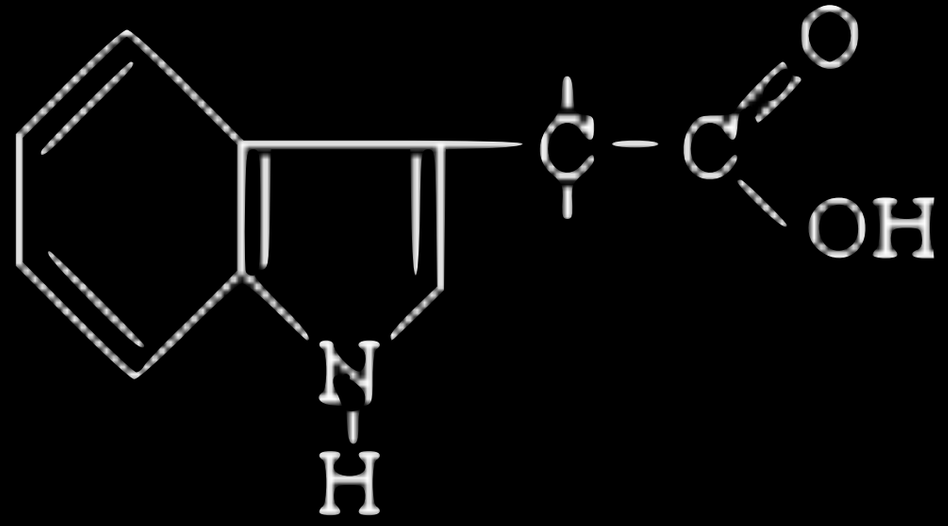
Organogenesis at the shoot meristem



time lapse
over 40 hrs

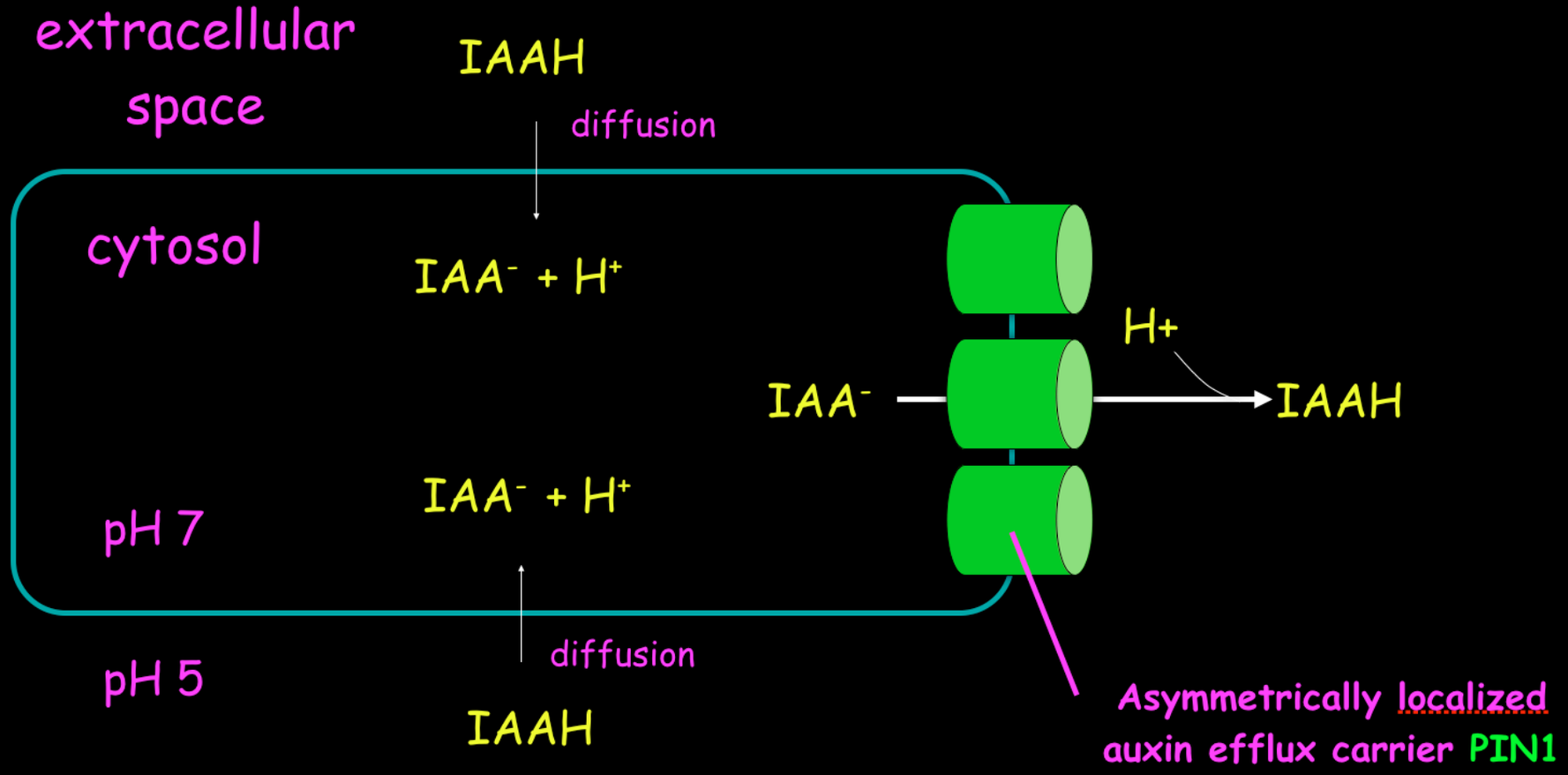
How does the plant actually create such a spacing?

Auxin - Master regulator of plant development



Indole-3-acetic acid (IAA)

Auxin is directionally transported from cell to cell via PIN1



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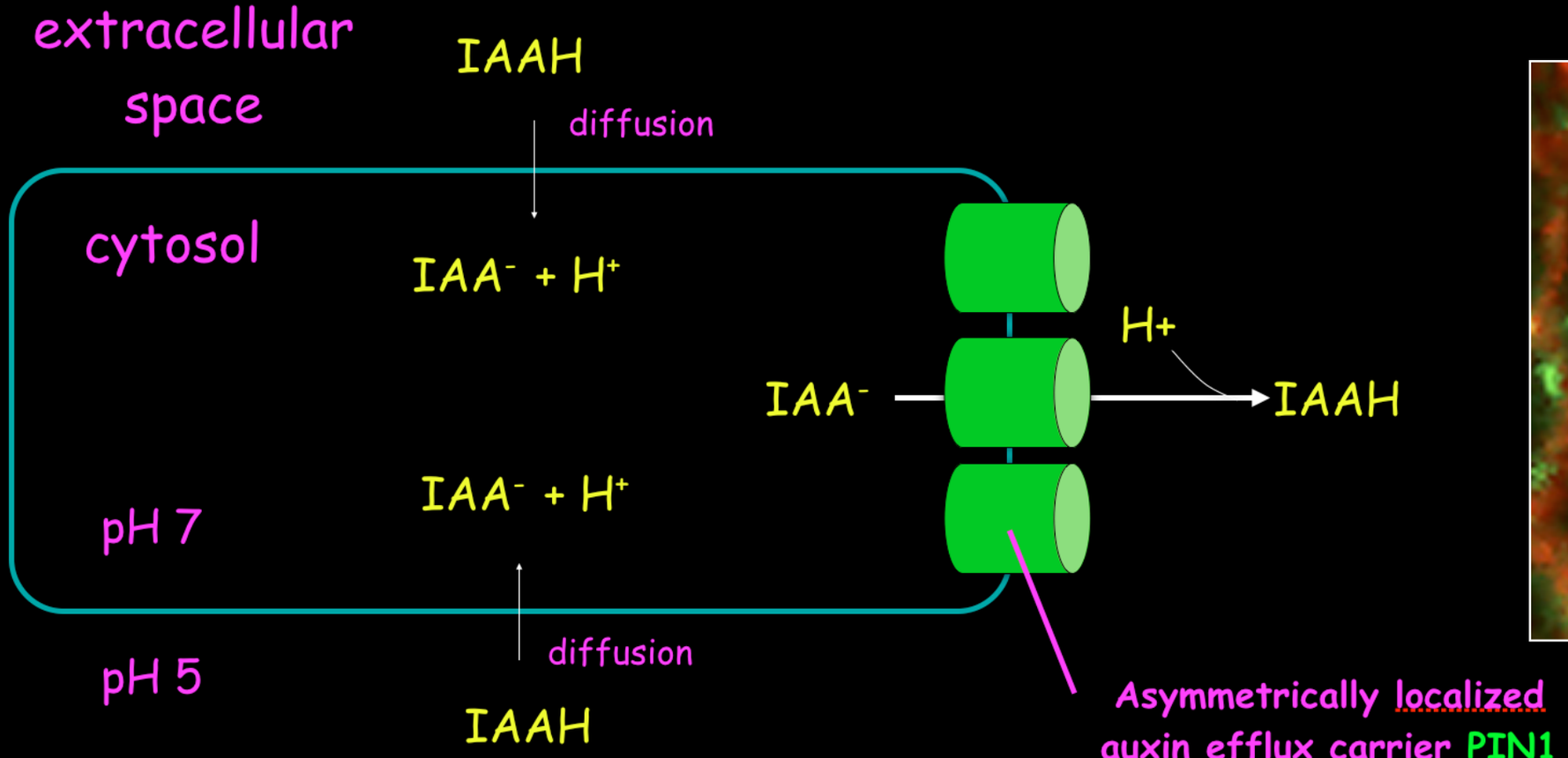
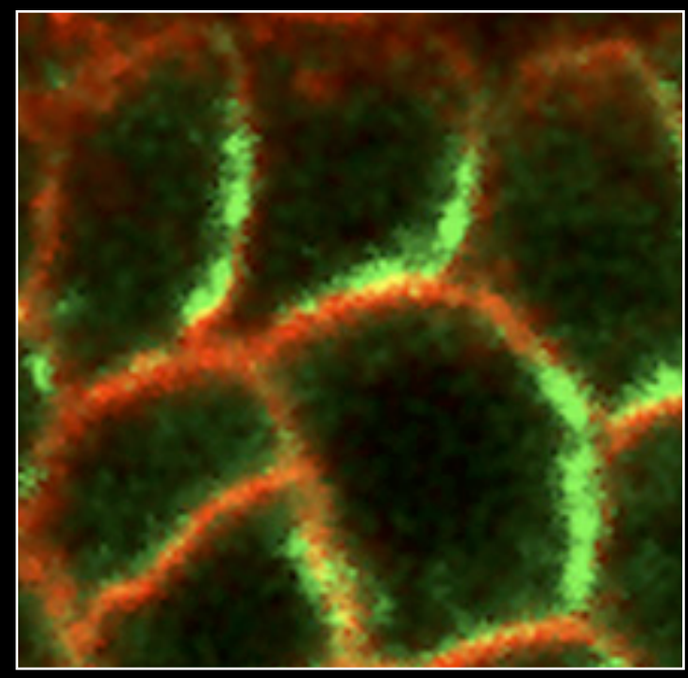
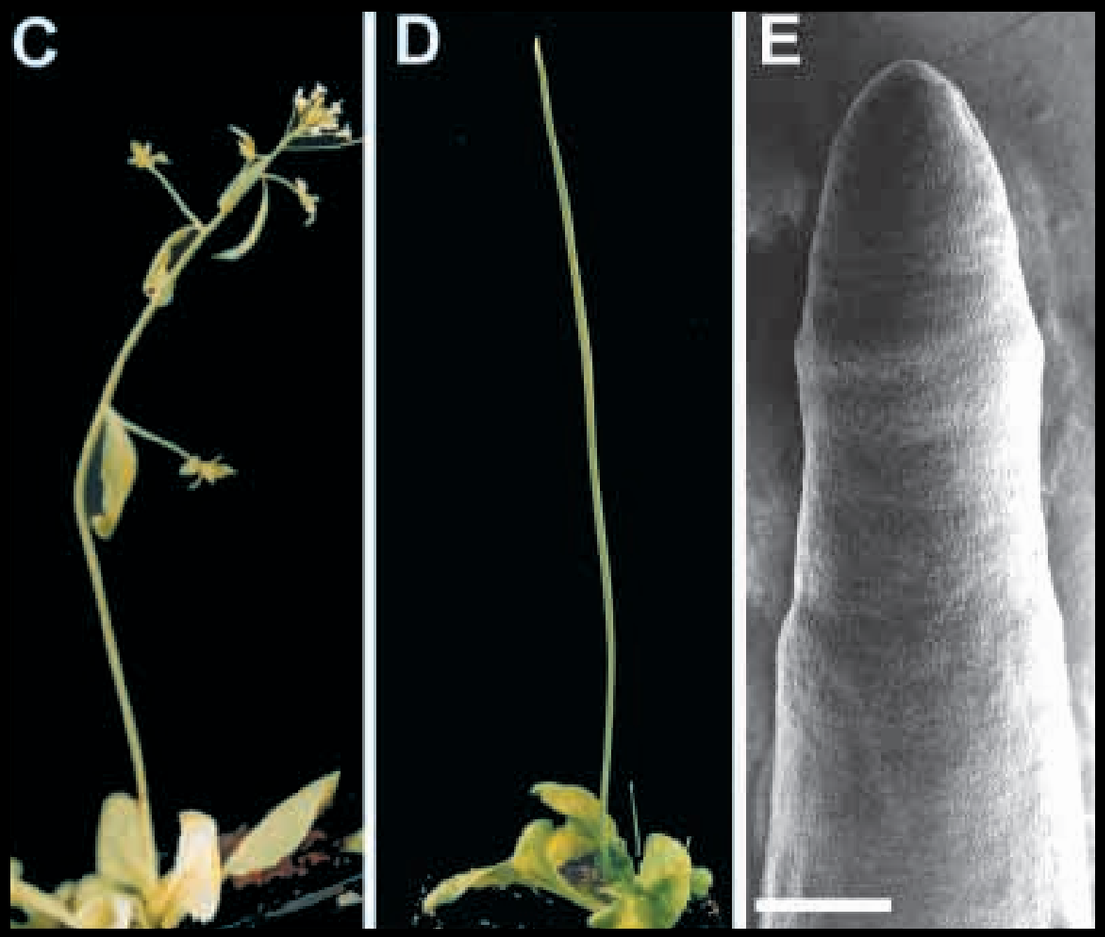




FM 4-64
PIN1-GFP

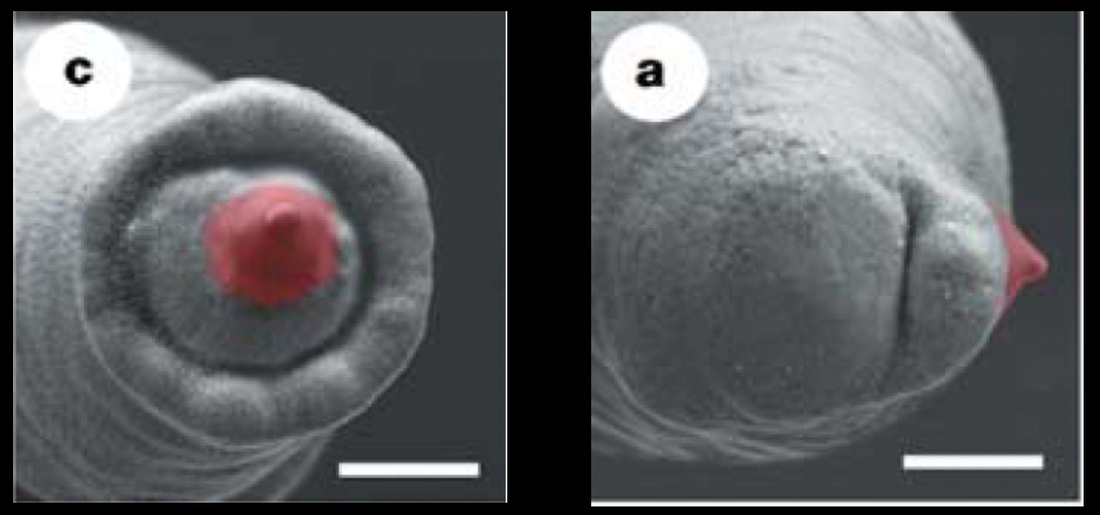
Auxin transport is required for organ formation



Wild type apex

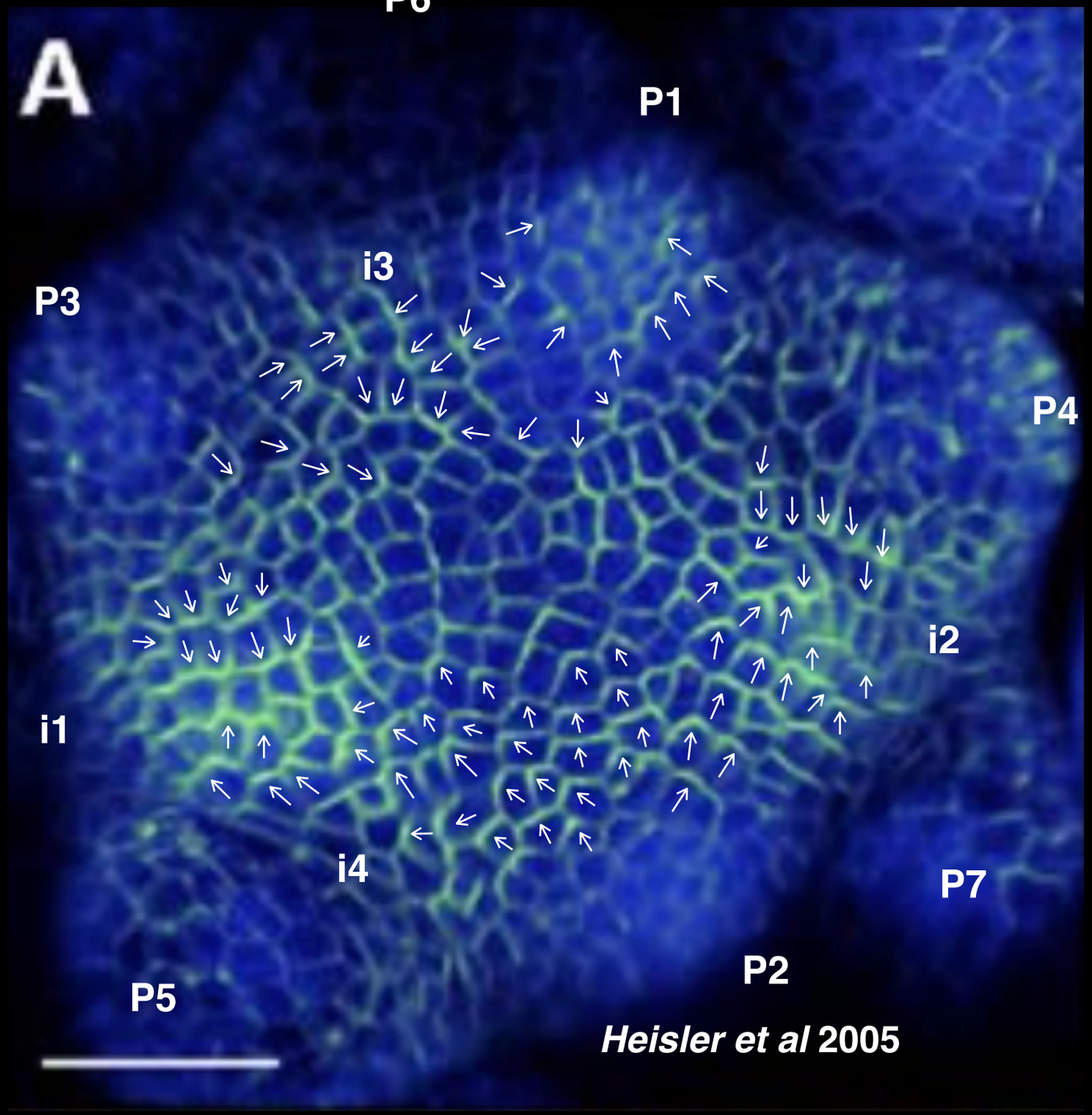
pin1 mutants fail to form primordia

Vernoux et al., 2000.



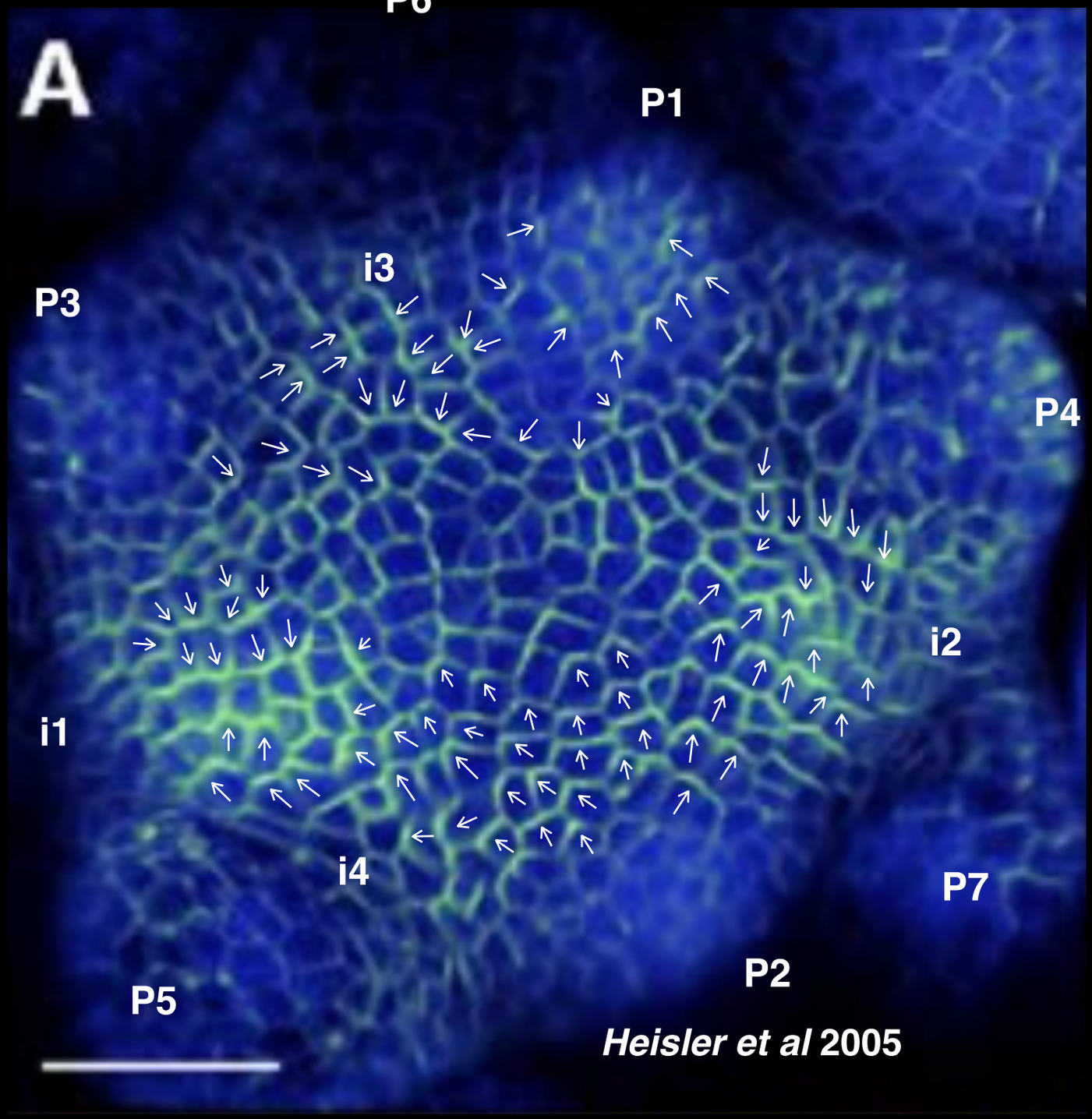
Auxin application can rescue primordium formation Reinhardt et al 2000, 2004

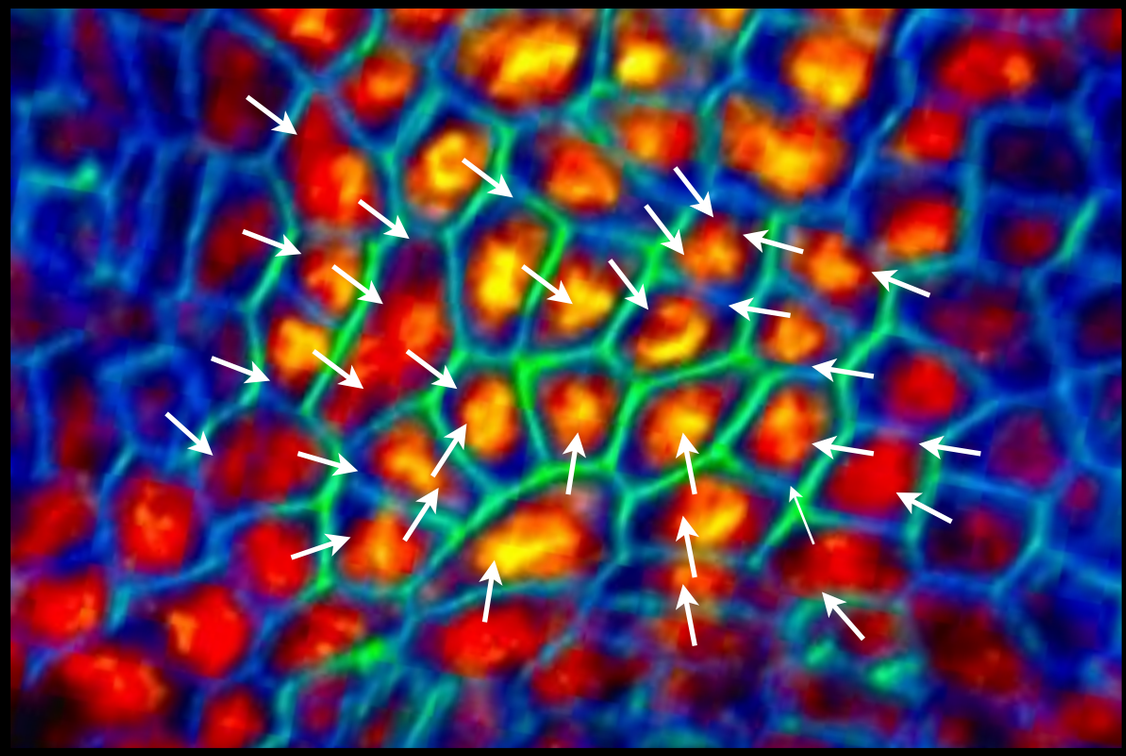
PIN1 polarities form convergence patterns that concentrate auxin locally



Heisler et al 2005

PIN1 polarities form convergence patterns that concentrate auxin locally

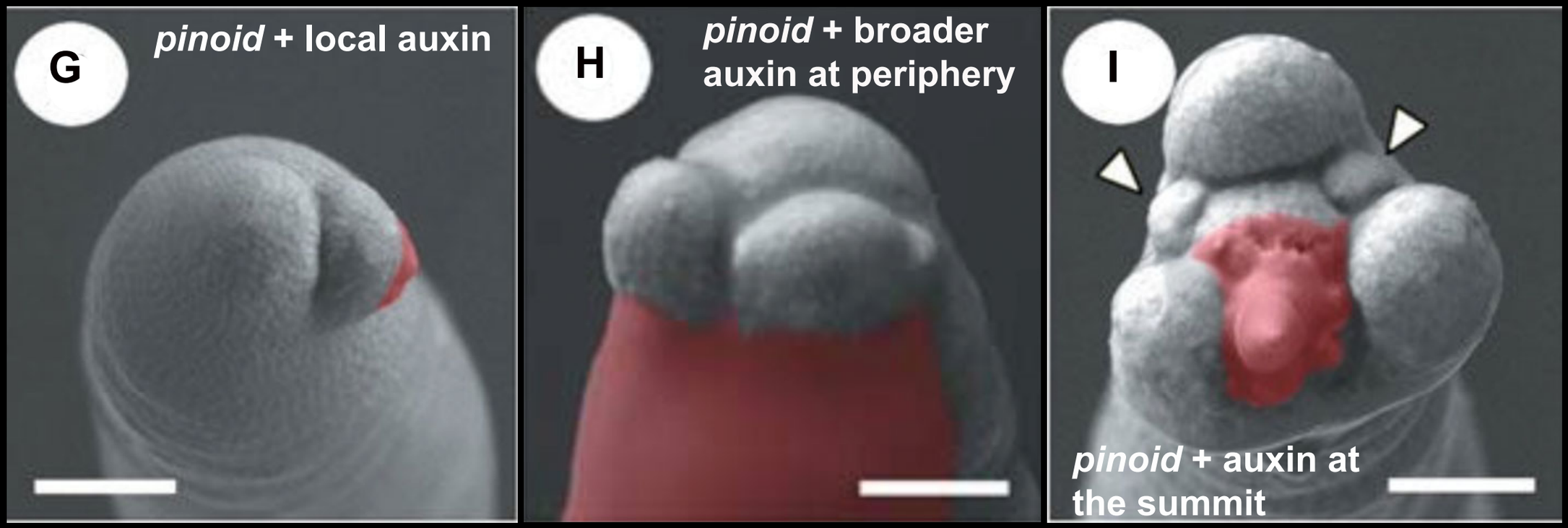




R2D2 auxin sensor

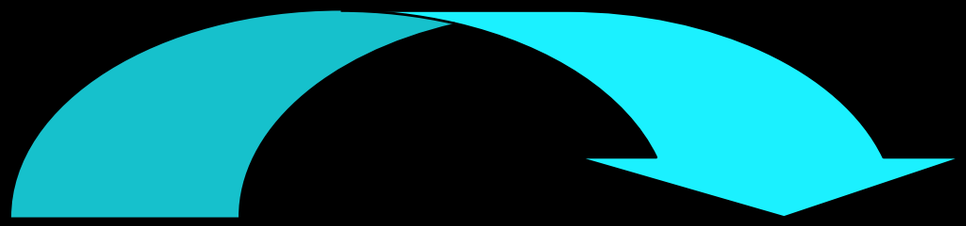
How are cell polarities patterned?

Evidence of self-organisation



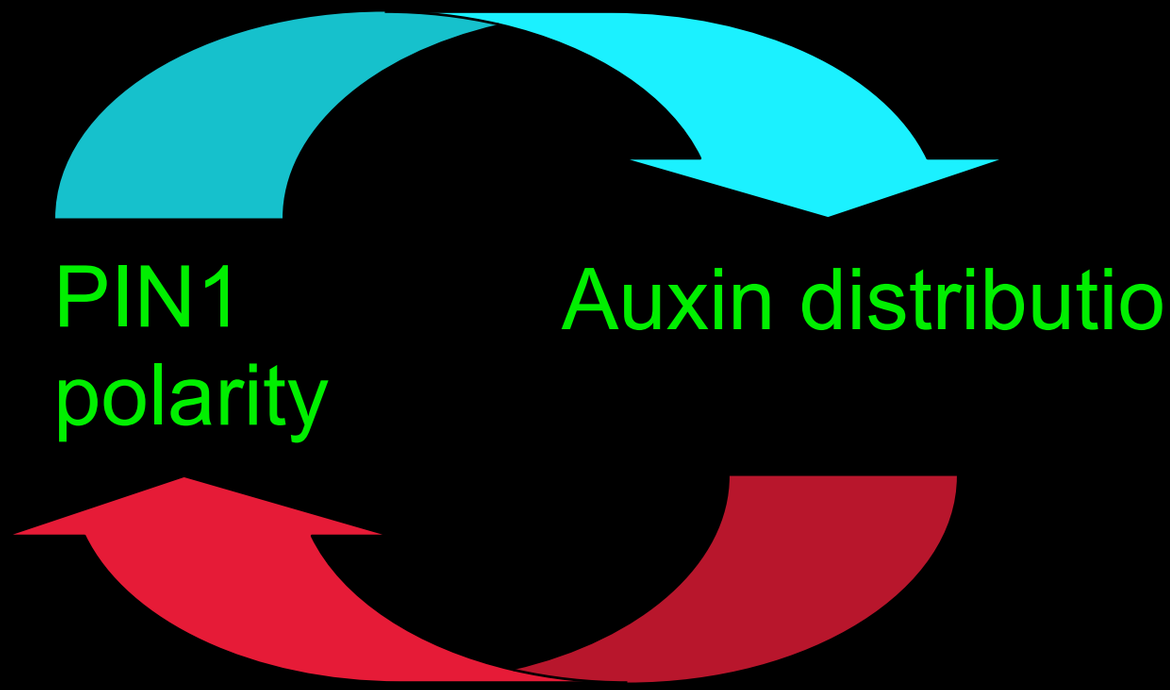
A periodic spacing is generated no matter the initial distribution of auxin

Reinhardt et al (2003) *Nature*



PIN1 polarity

Auxin distribution



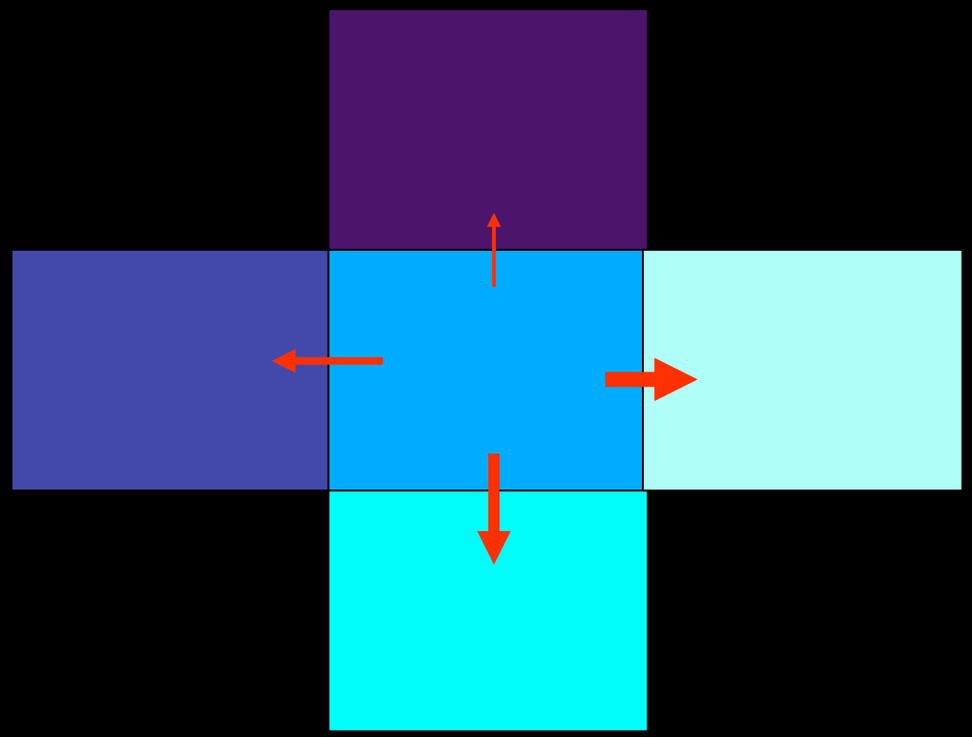
Could auxin act as a polarity cue?

If so, how?

Feedback between PIN1 and auxin can generate periodic patterns

Jonsson and Heisler et al., 2006, *PNAS*

Feedback between PIN1 and auxin can generate periodic patterns

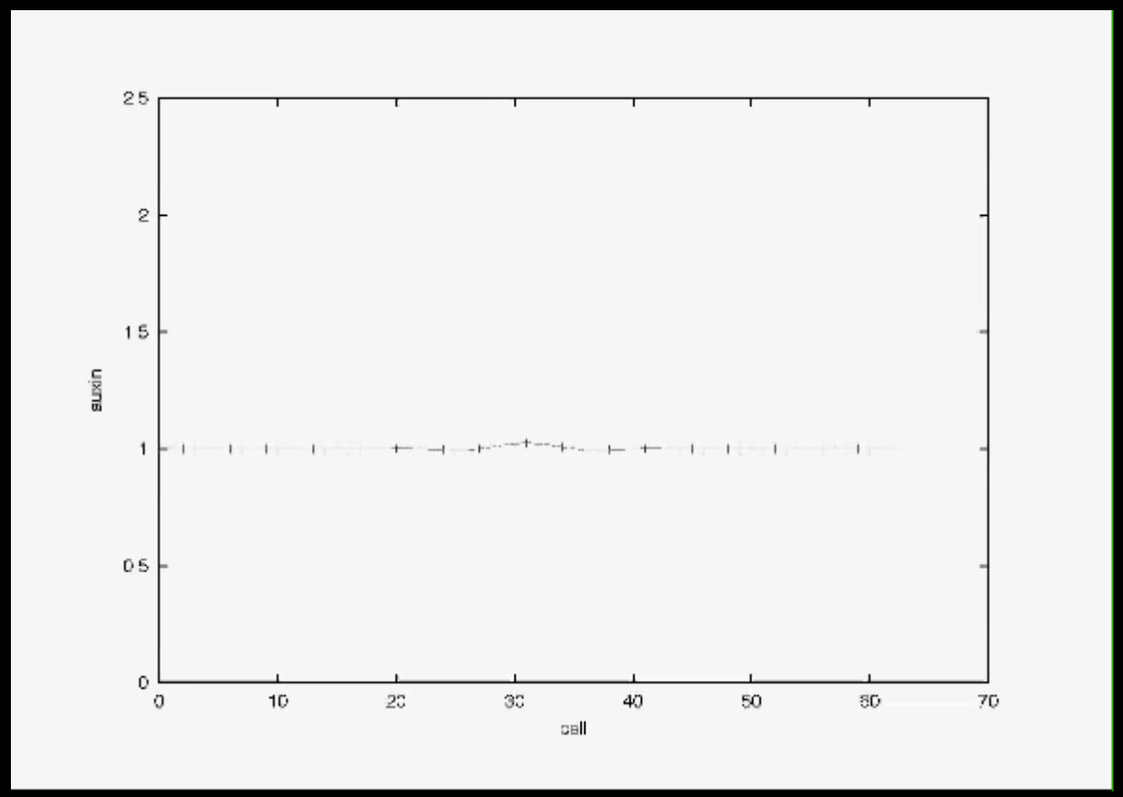


$$P_{ij} = P_i \frac{a_j}{\sum_k^{N_i} a_k} \propto P_i a_j$$

Jonsson and Heisler et al., 2006, *PNAS*

Feedback between PIN1 and auxin can generate periodic patterns

auxin conc.



cells

Jonsson and Heisler et al., 2006, *PNAS*

Feedback between PIN1 and auxin can generate periodic patterns

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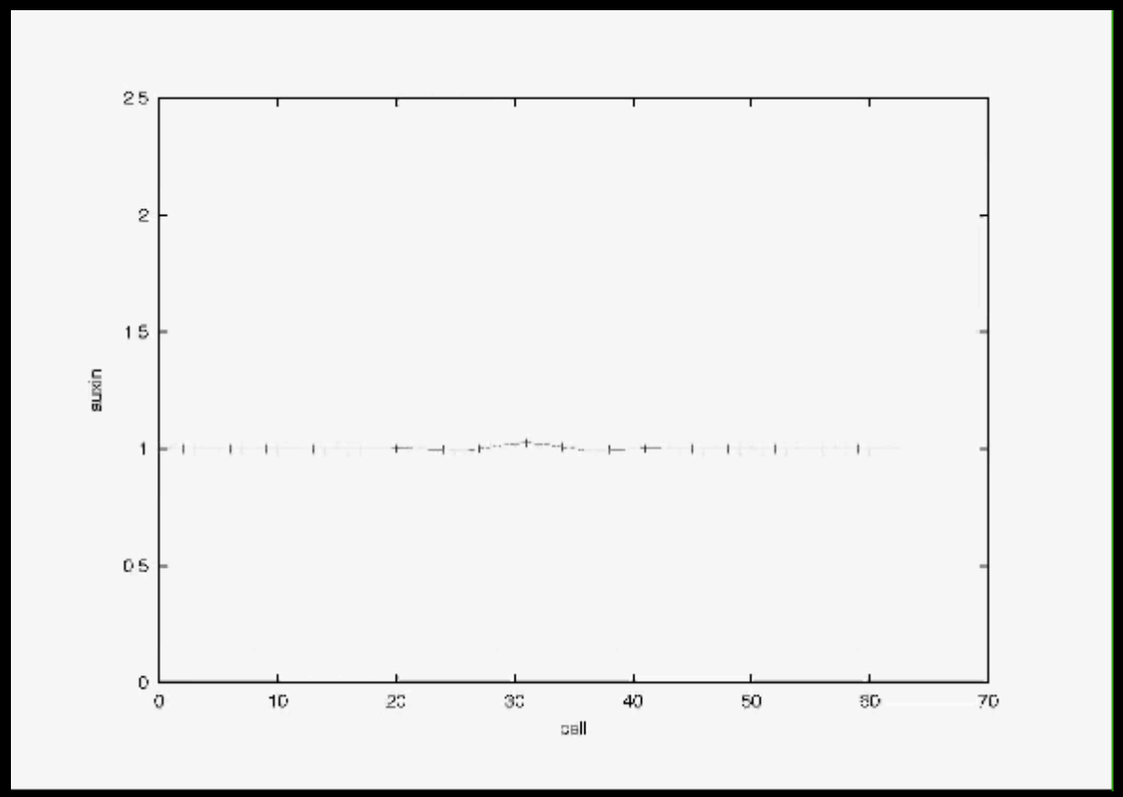

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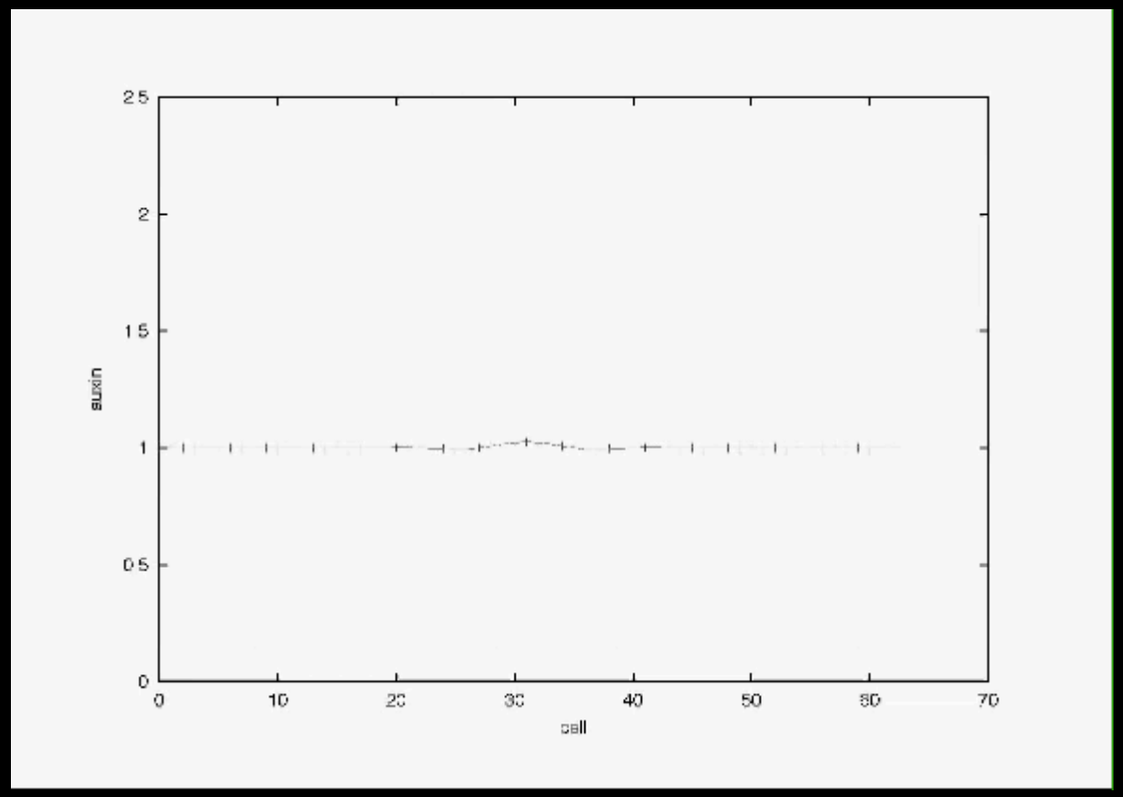
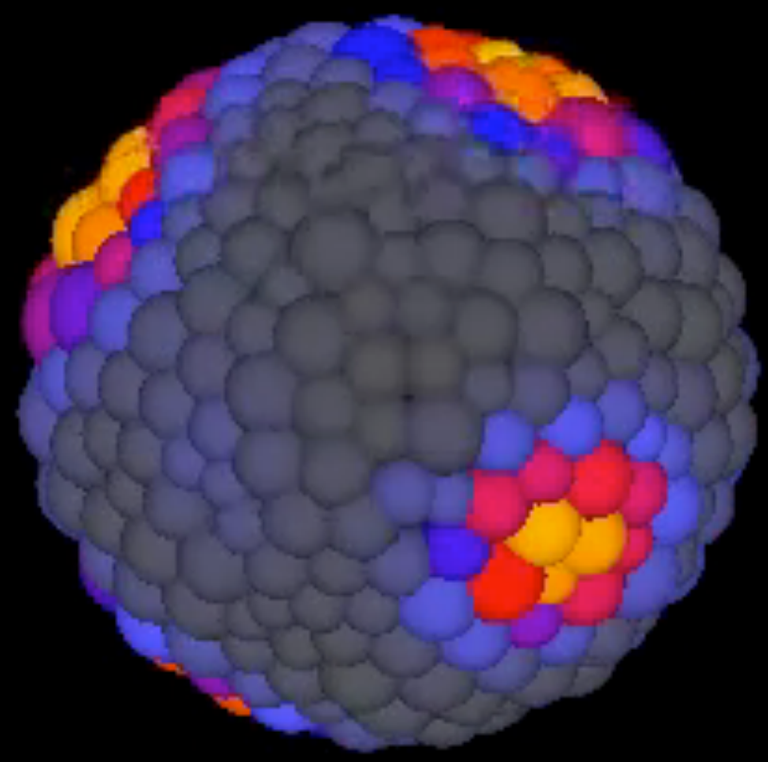


cells



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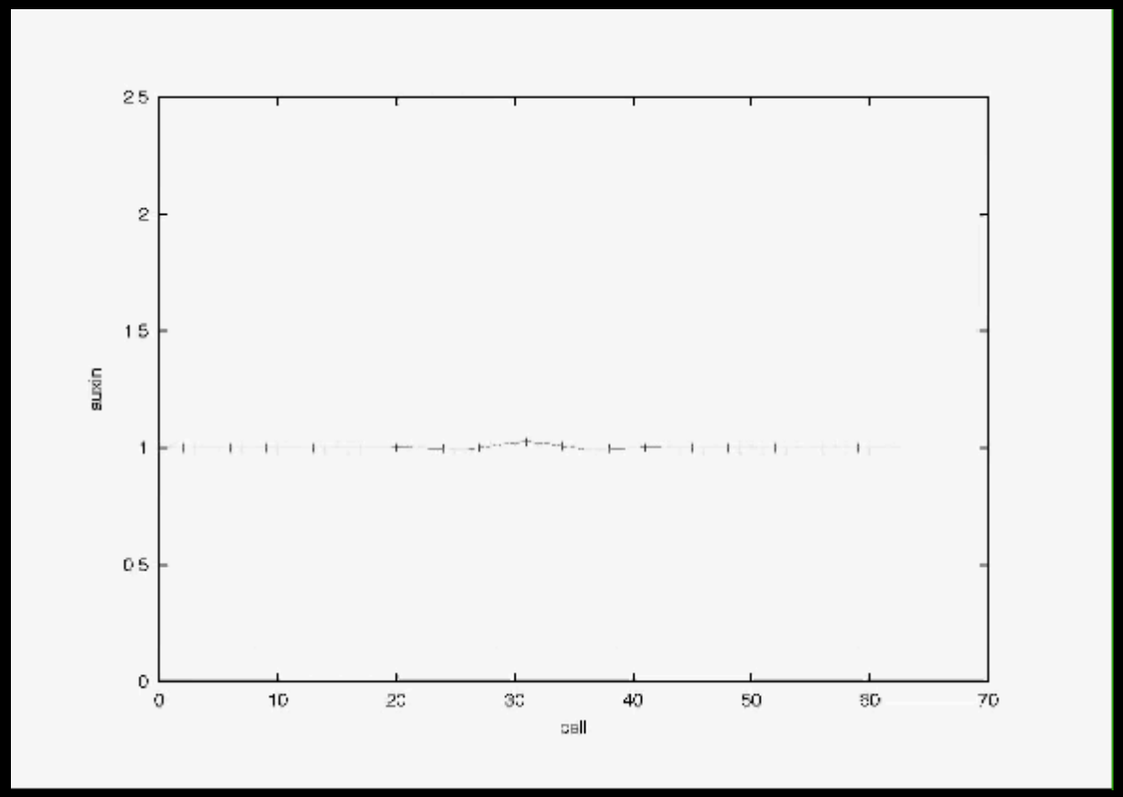

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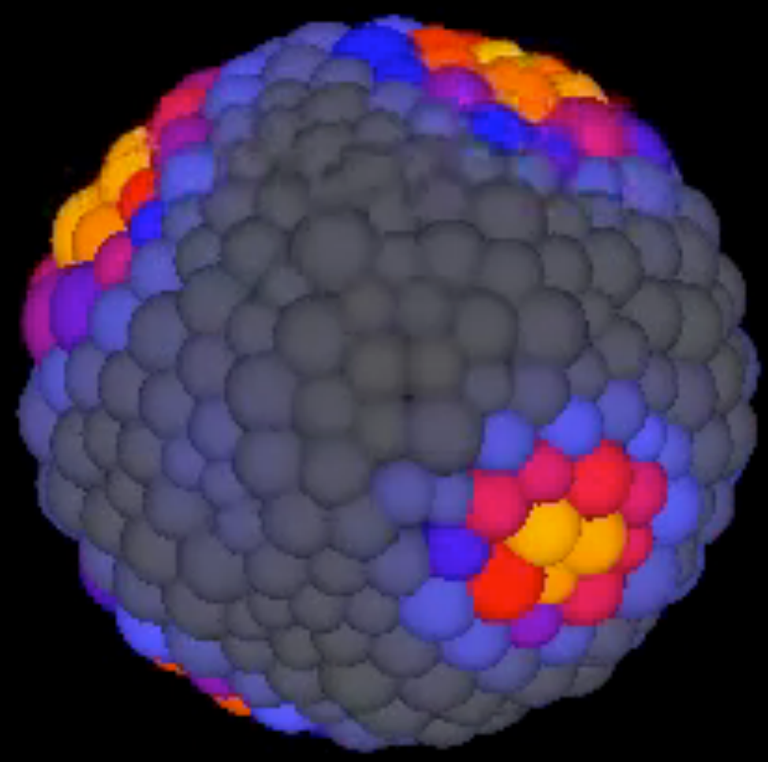
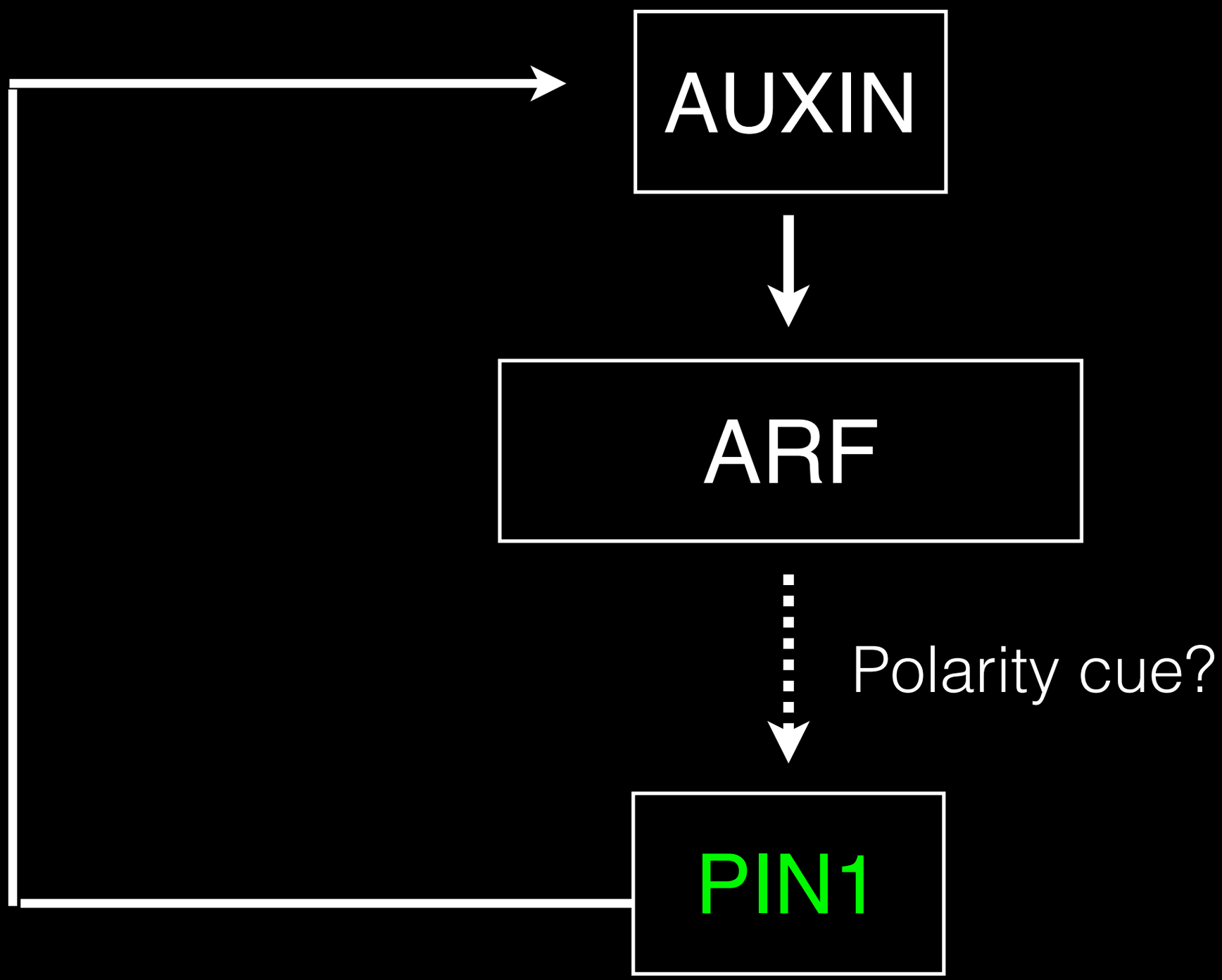
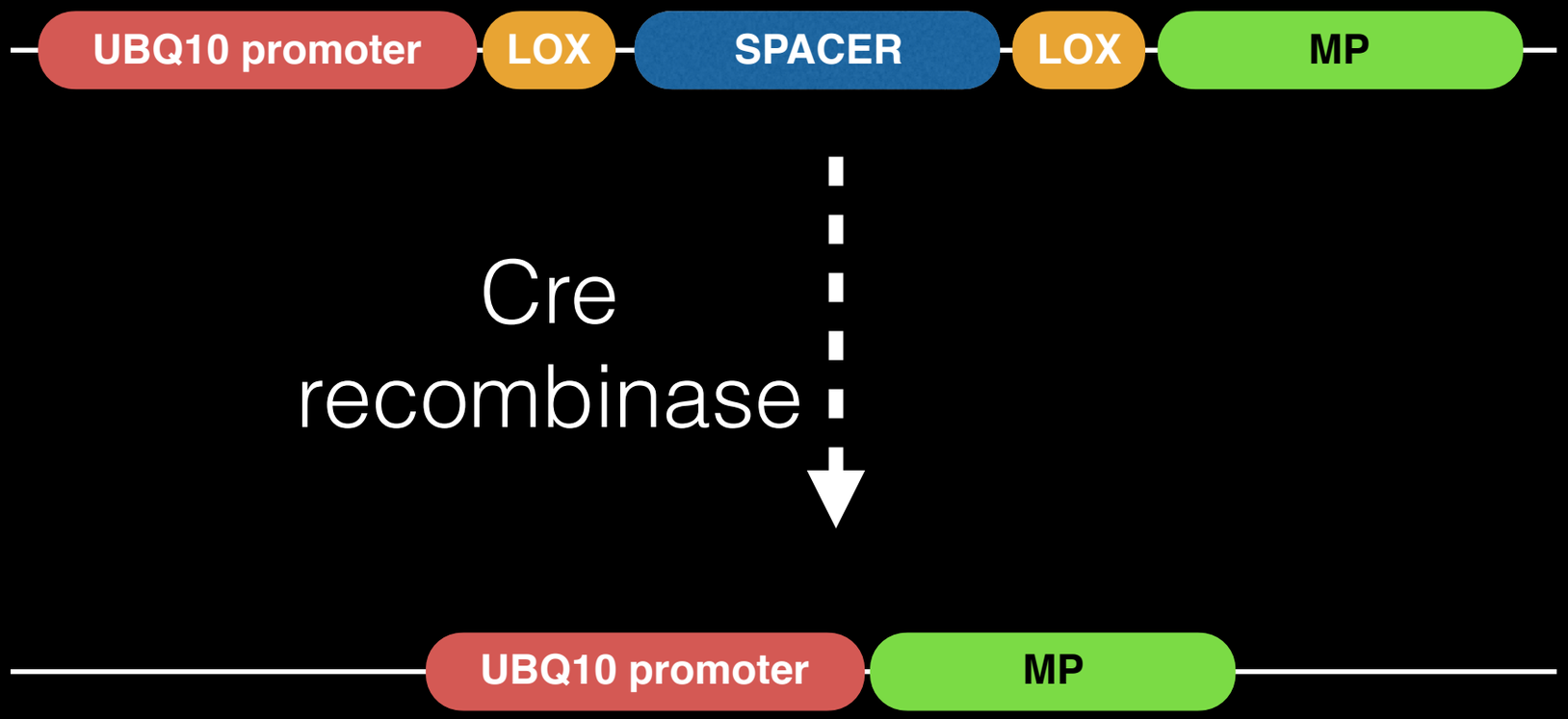


Jonsson and Heisler et al., 2006, *PNAS*

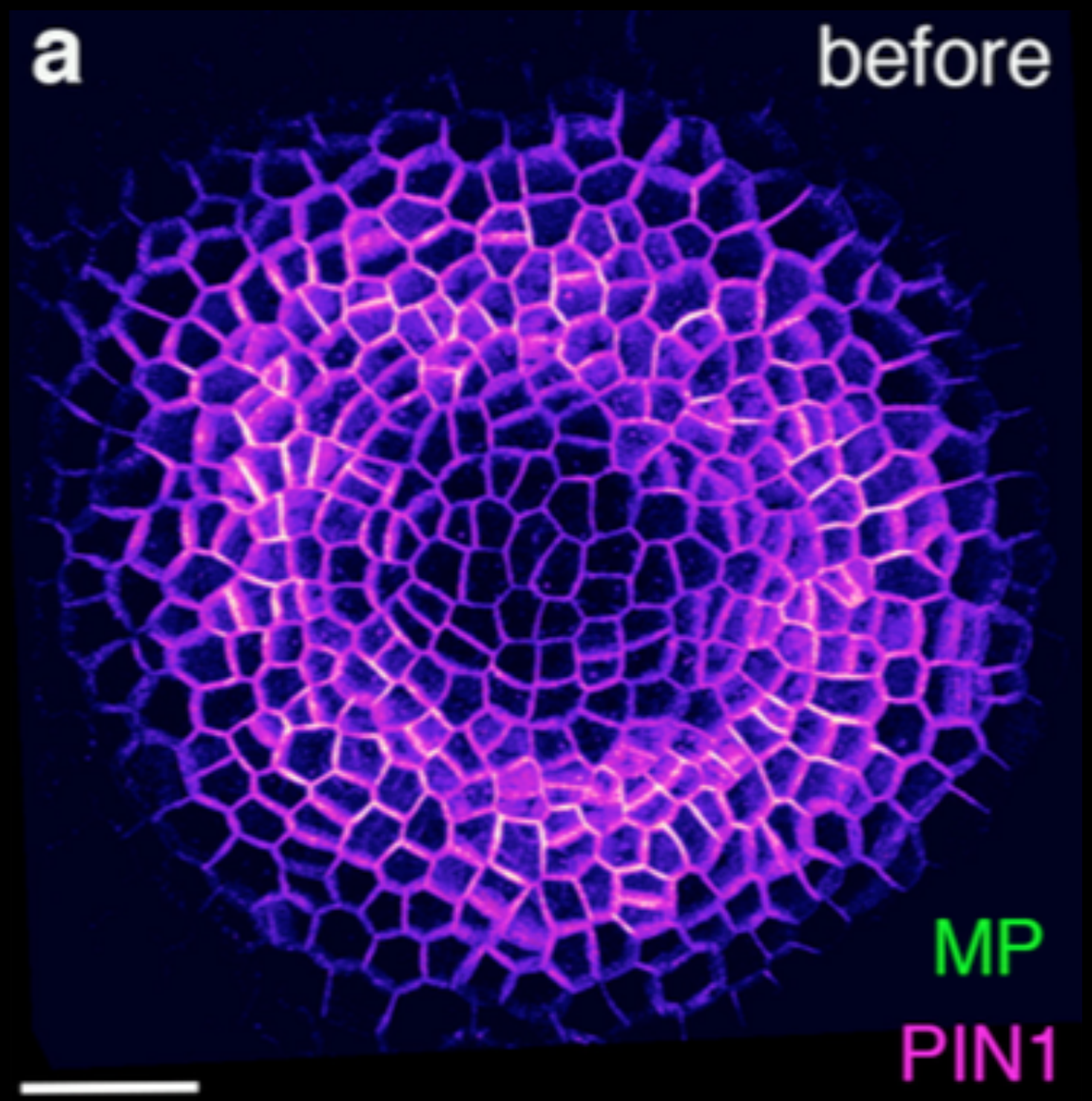
A candidate pathway for the "up-the-gradient" model



Using Cre-Lox to create cell-cell differences in MP activity

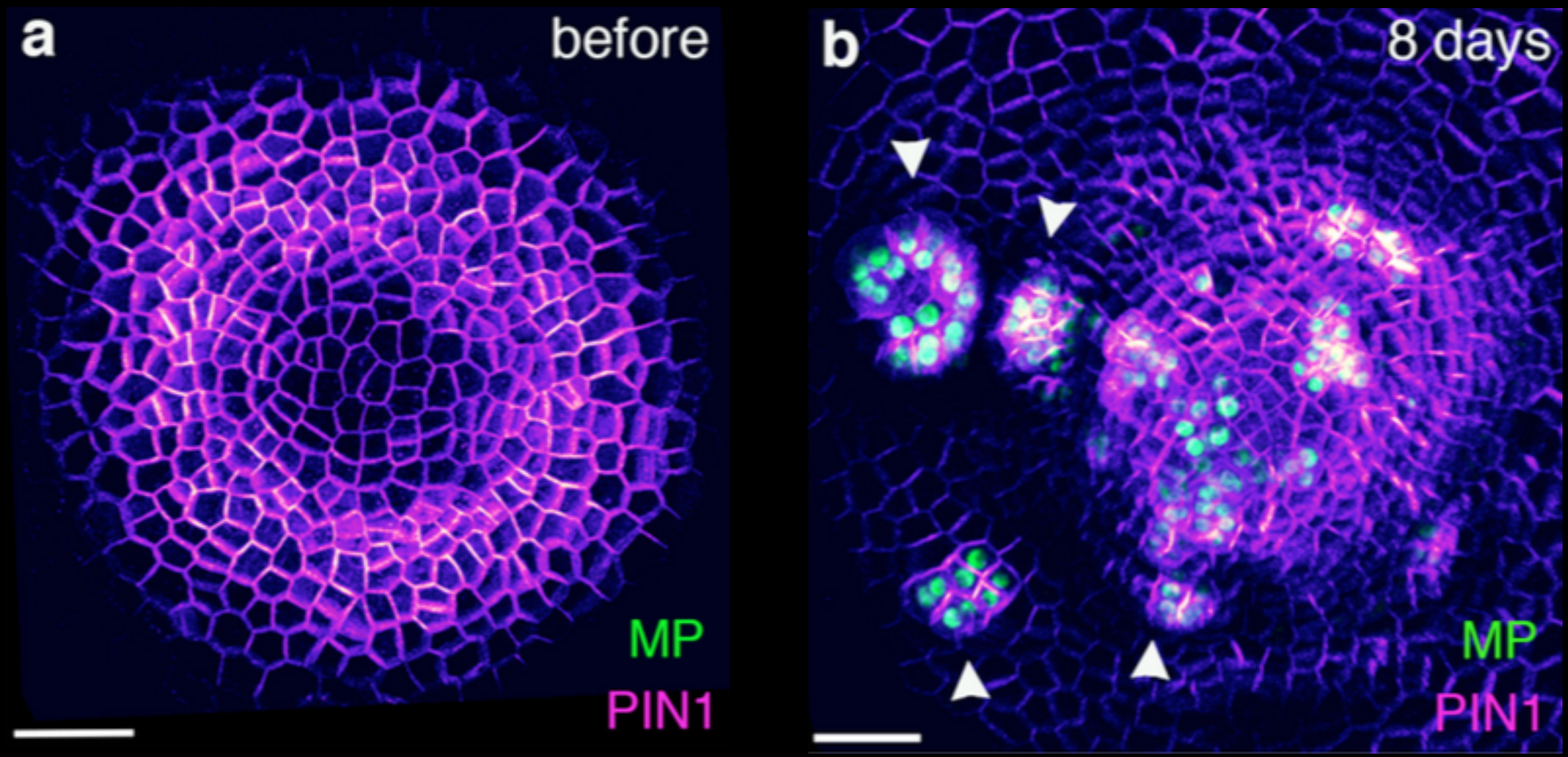


MP clones mark PIN1 convergence points from where organ initiate



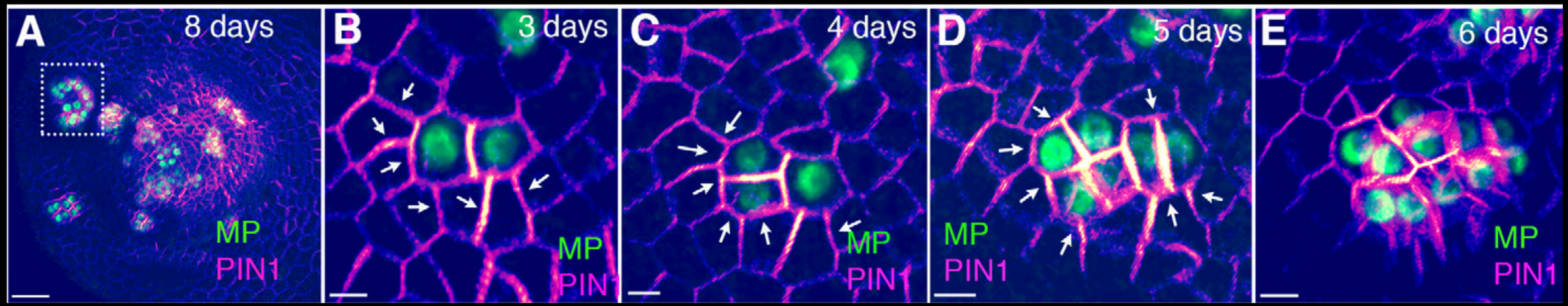
Low High

MP clones mark PIN1 convergence points from where organ initiate



ARF5 clones orient cell polarity non-cell autonomously

PIN1



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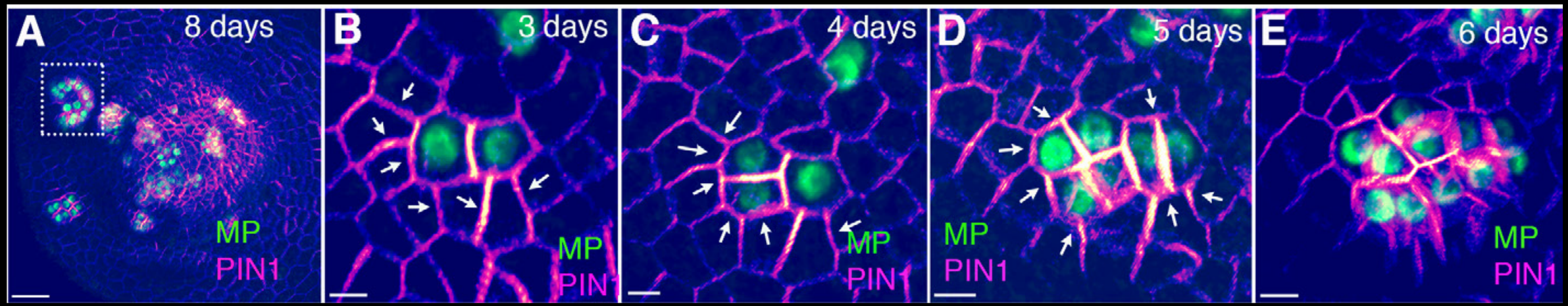

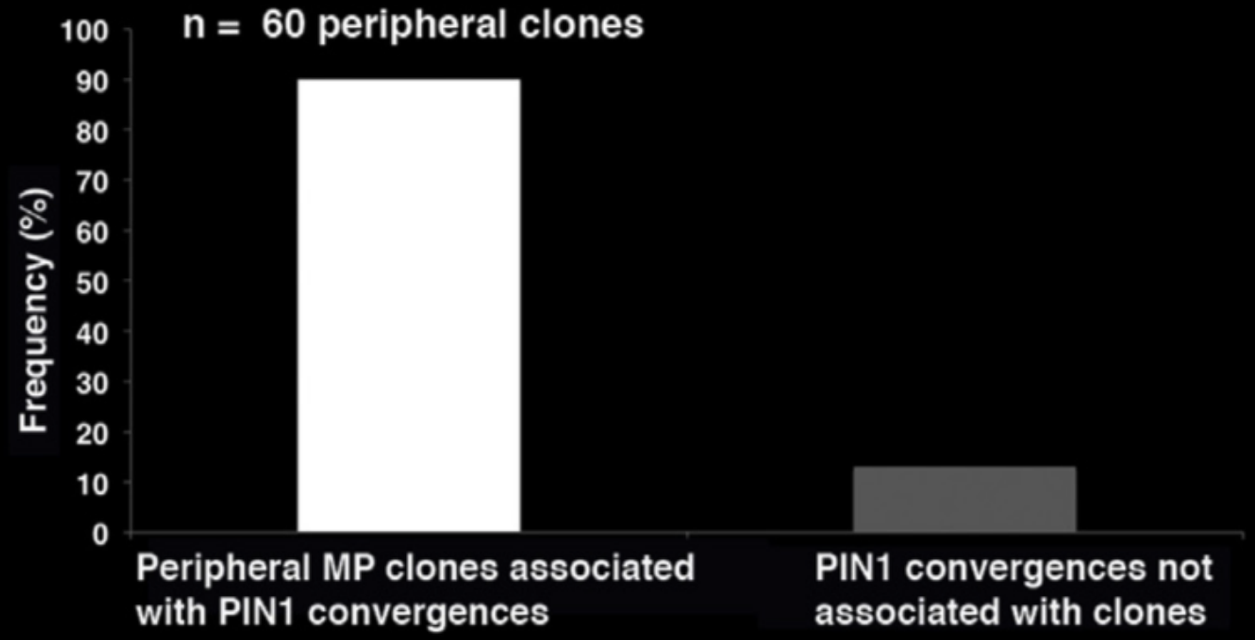

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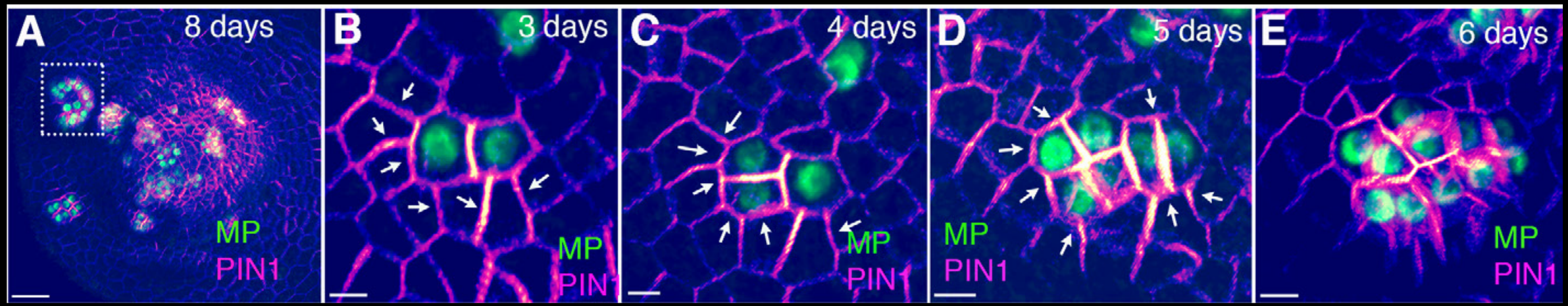

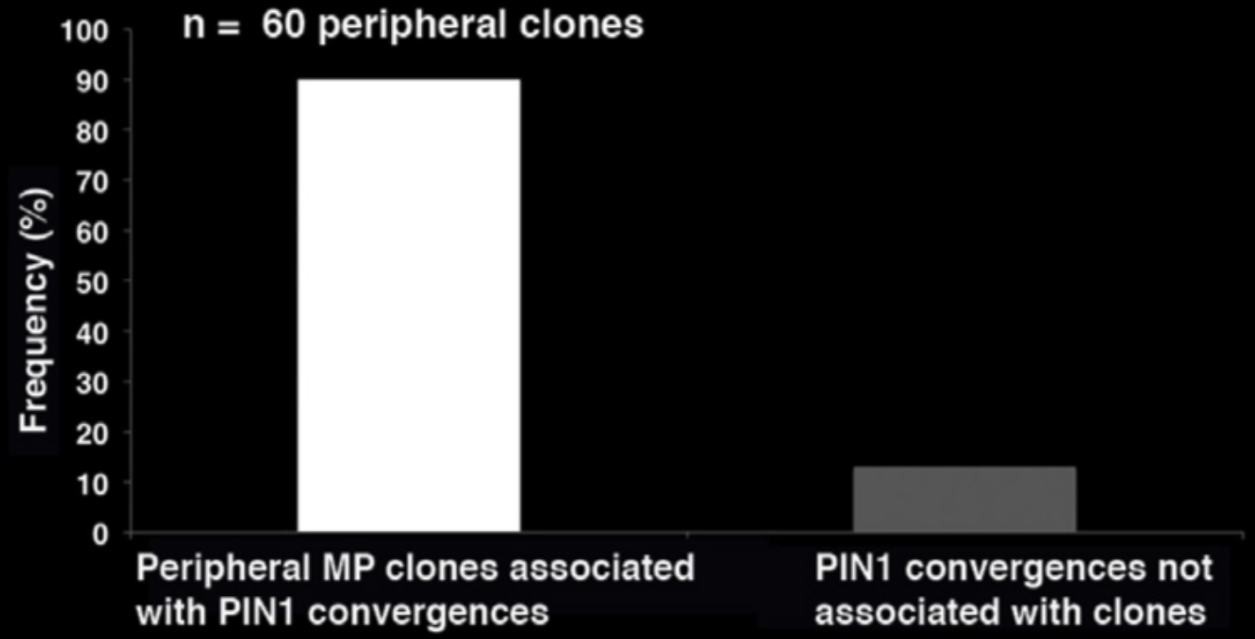

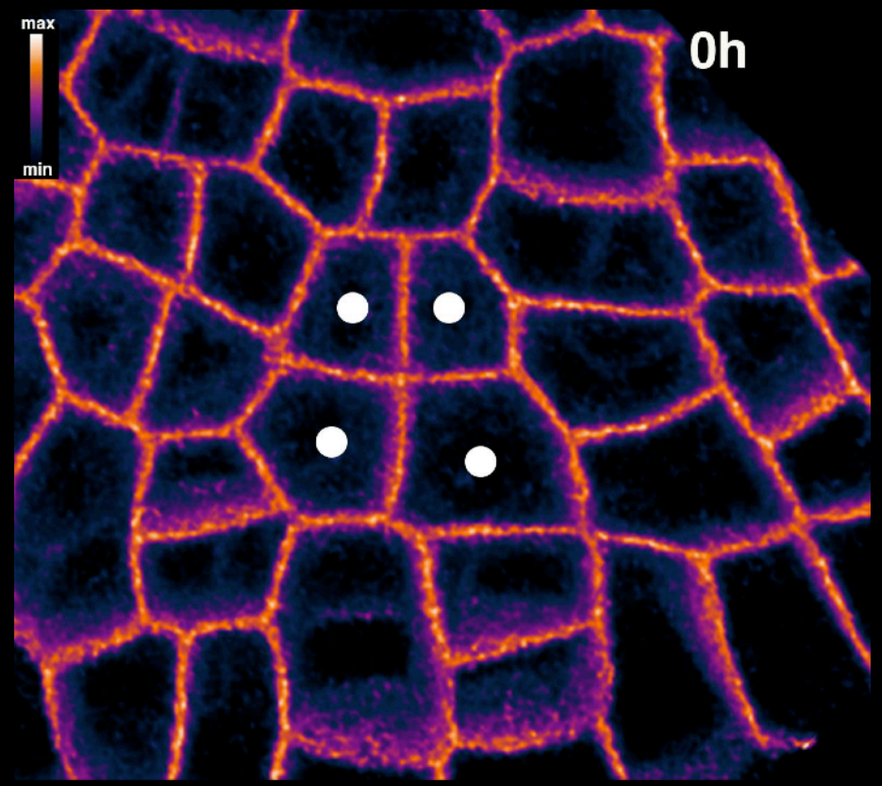

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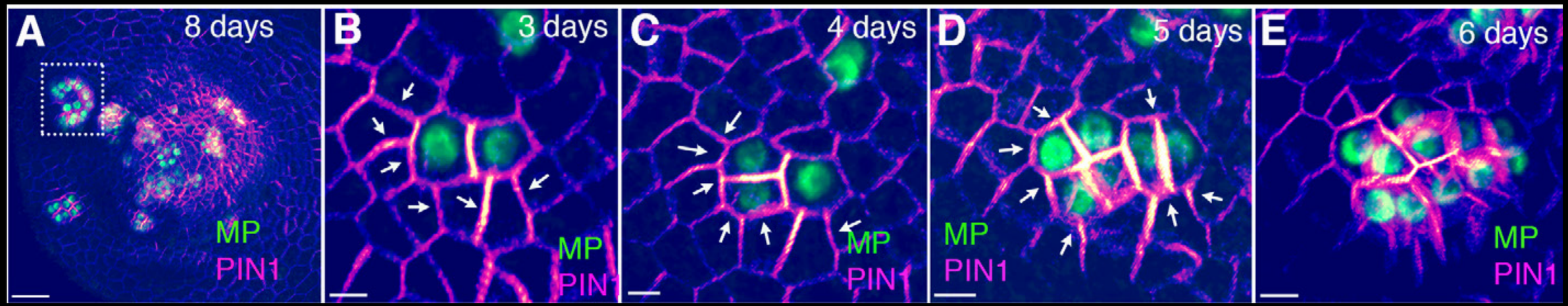
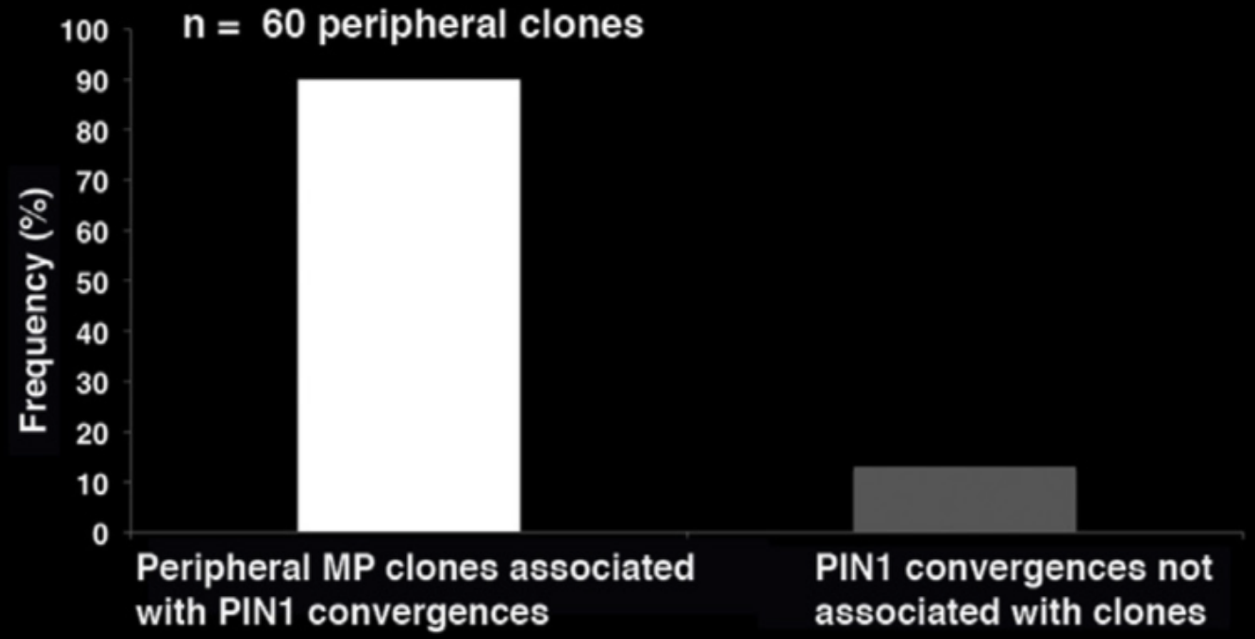
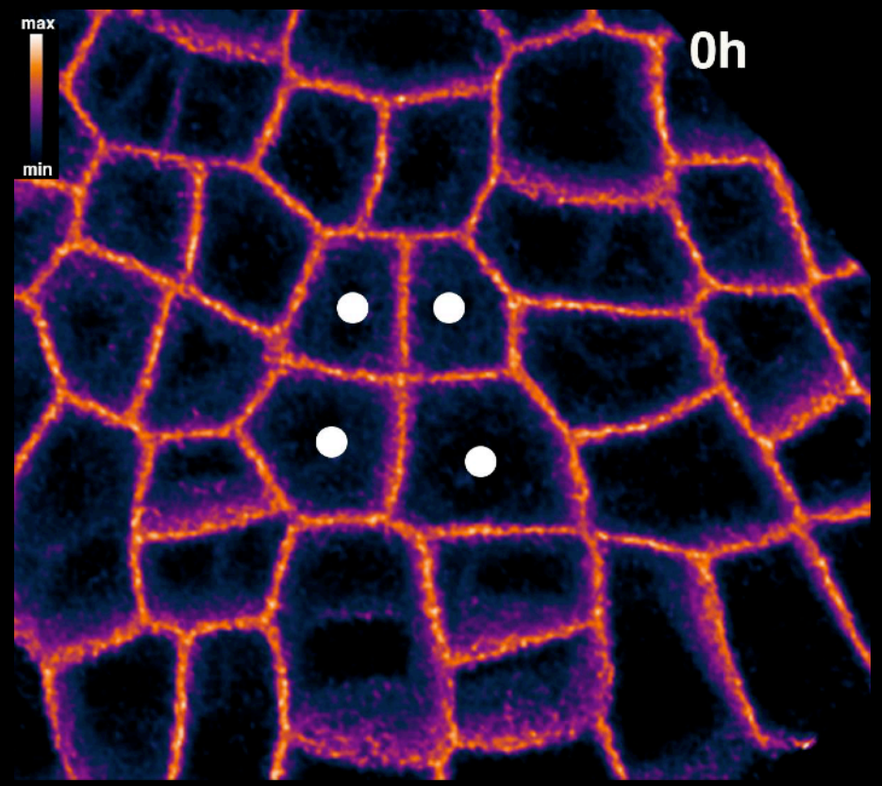
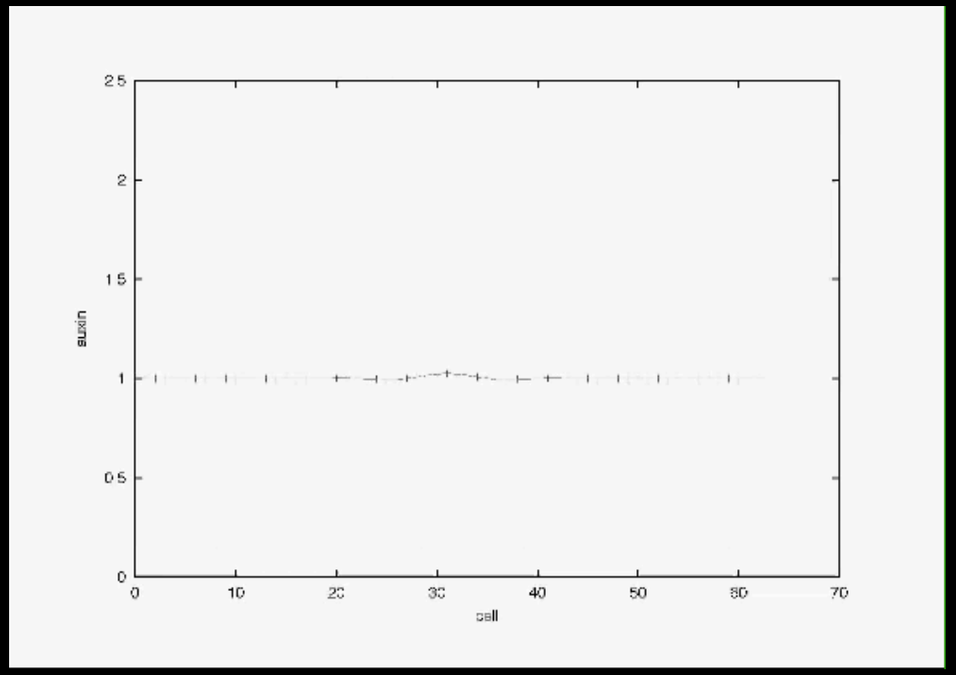
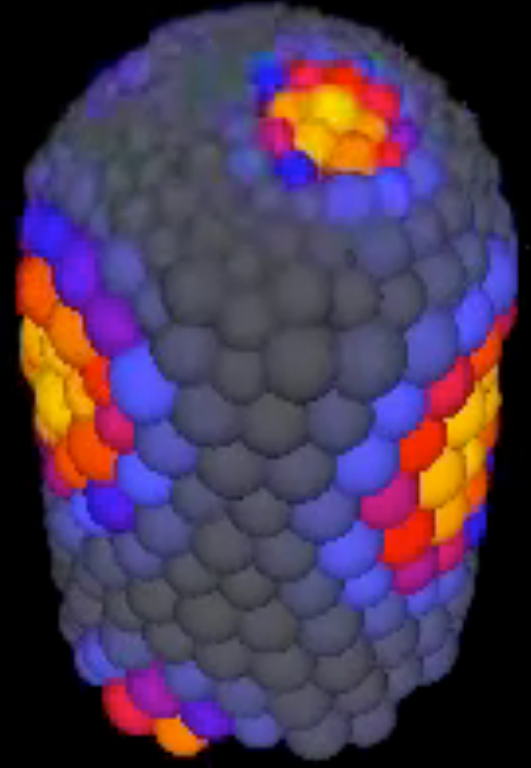






Is epidermal MP expression sufficient to generate periodic patterns?





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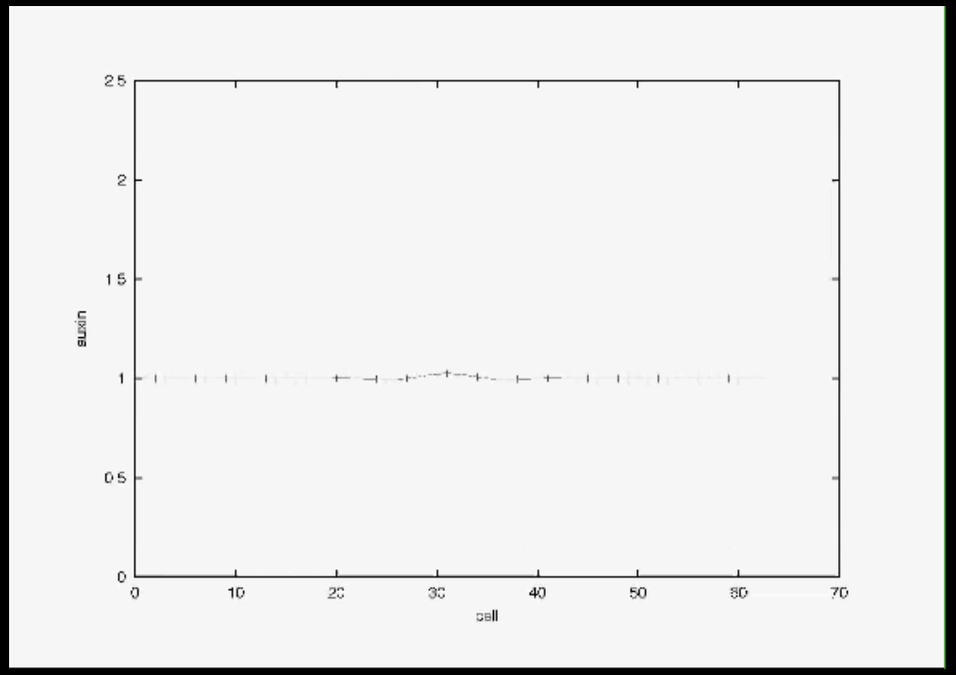

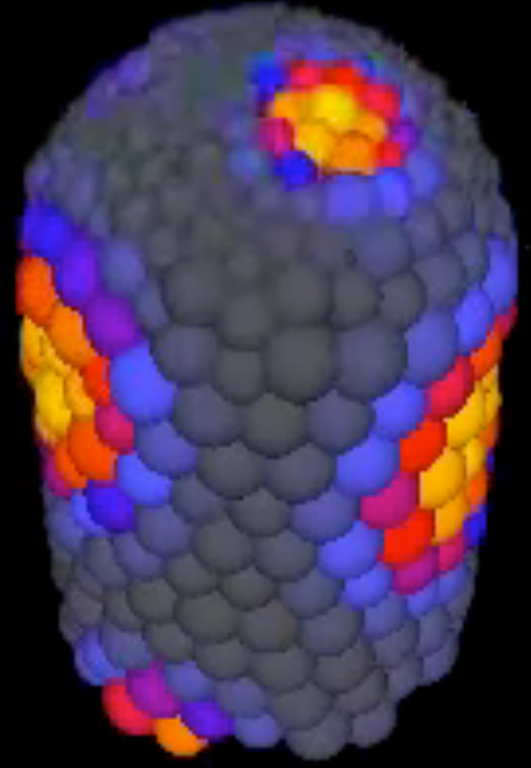

Restriction of MP activity to the epidermis resulted in two continuous spirals of organ tissue

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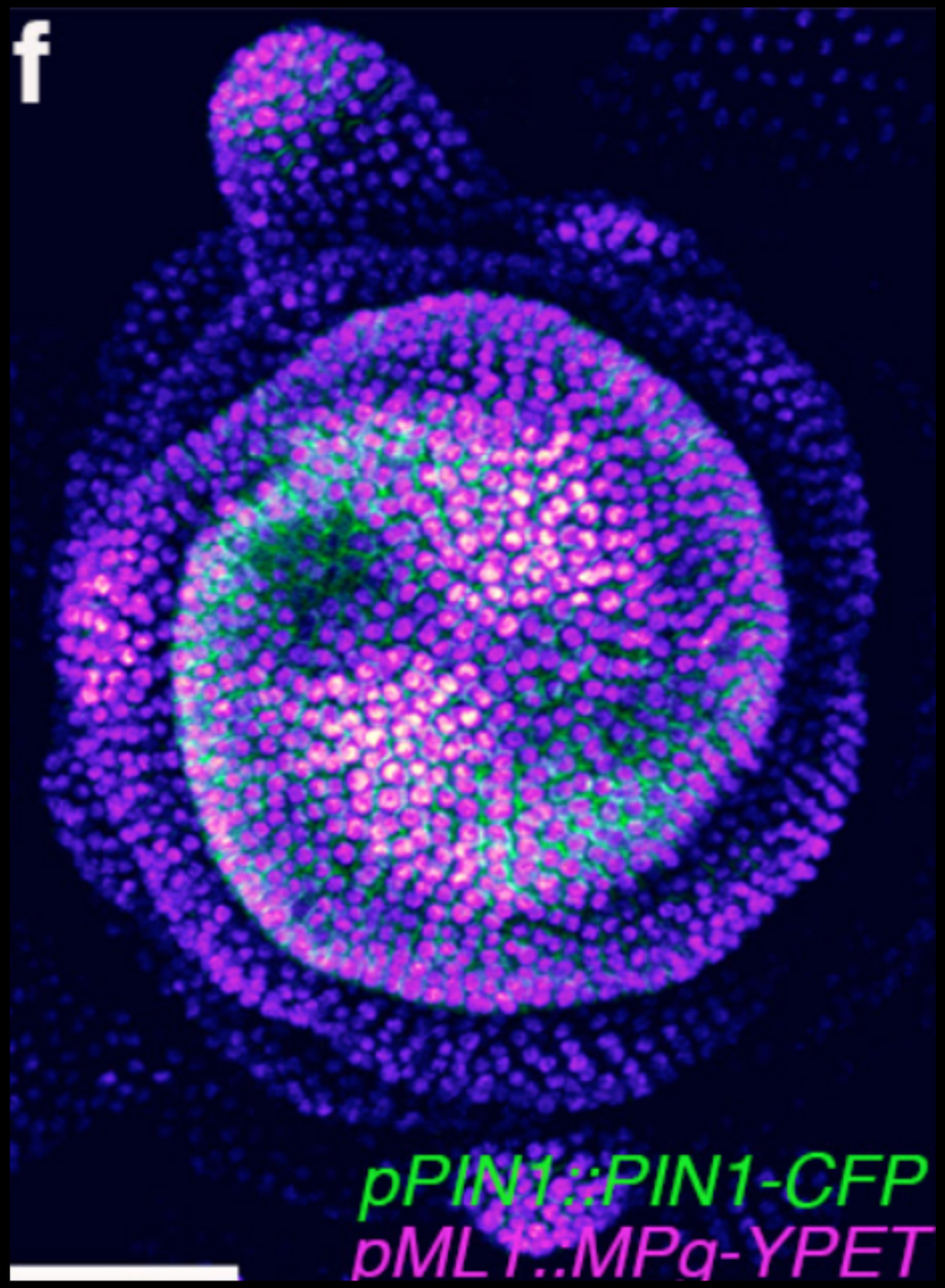
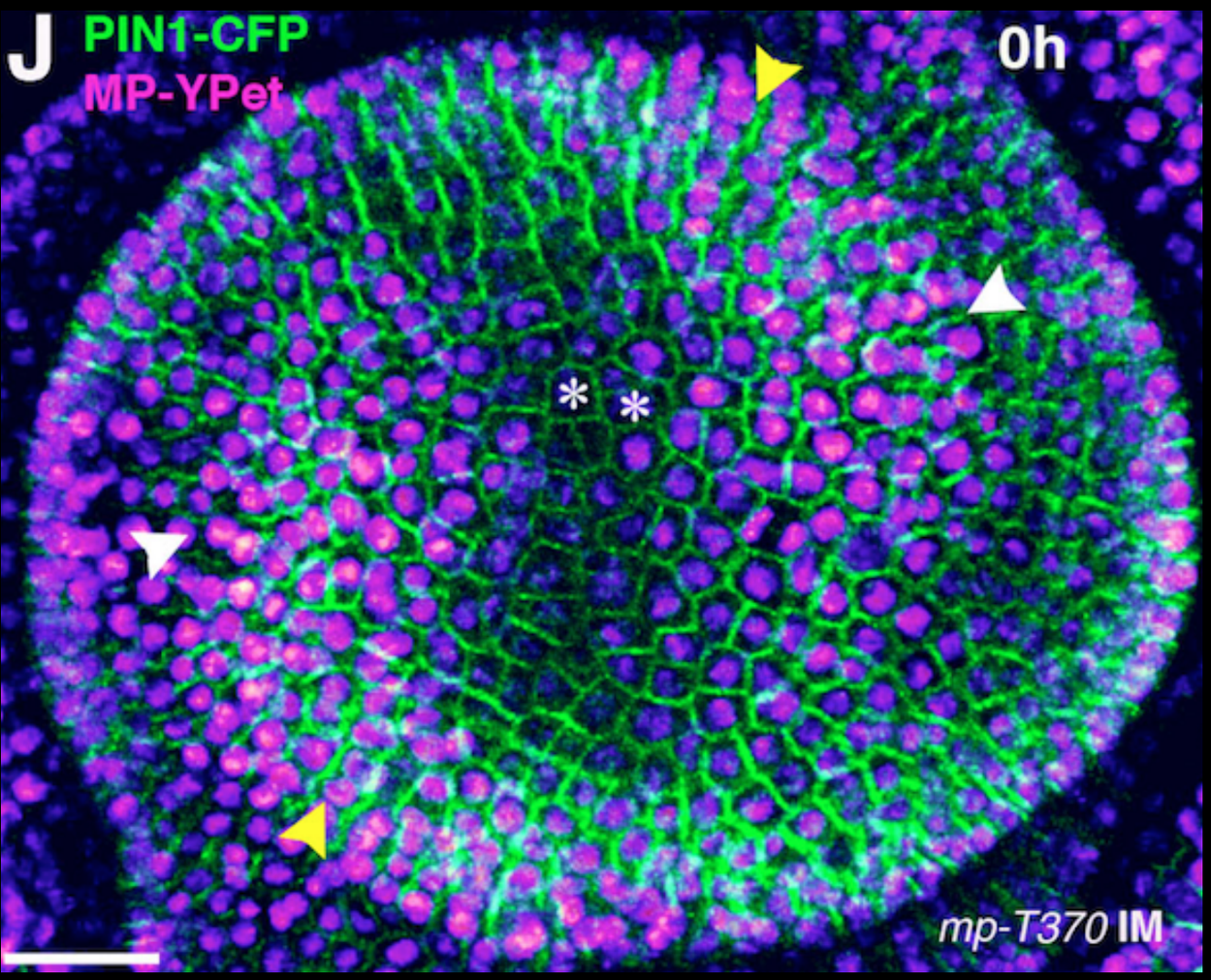


Lack of Sub epidermal MP activity results in the formation of mobile auxin maxima



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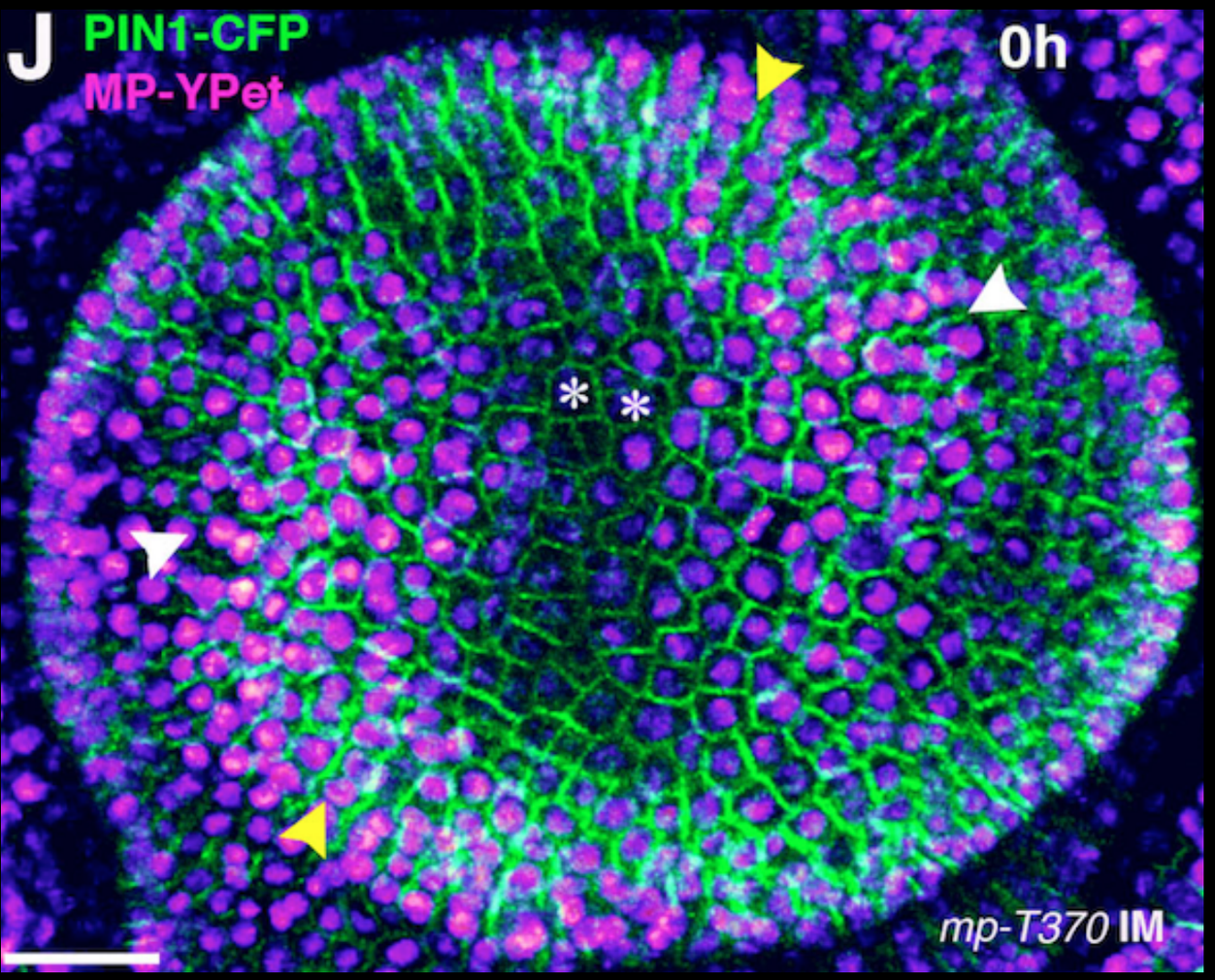
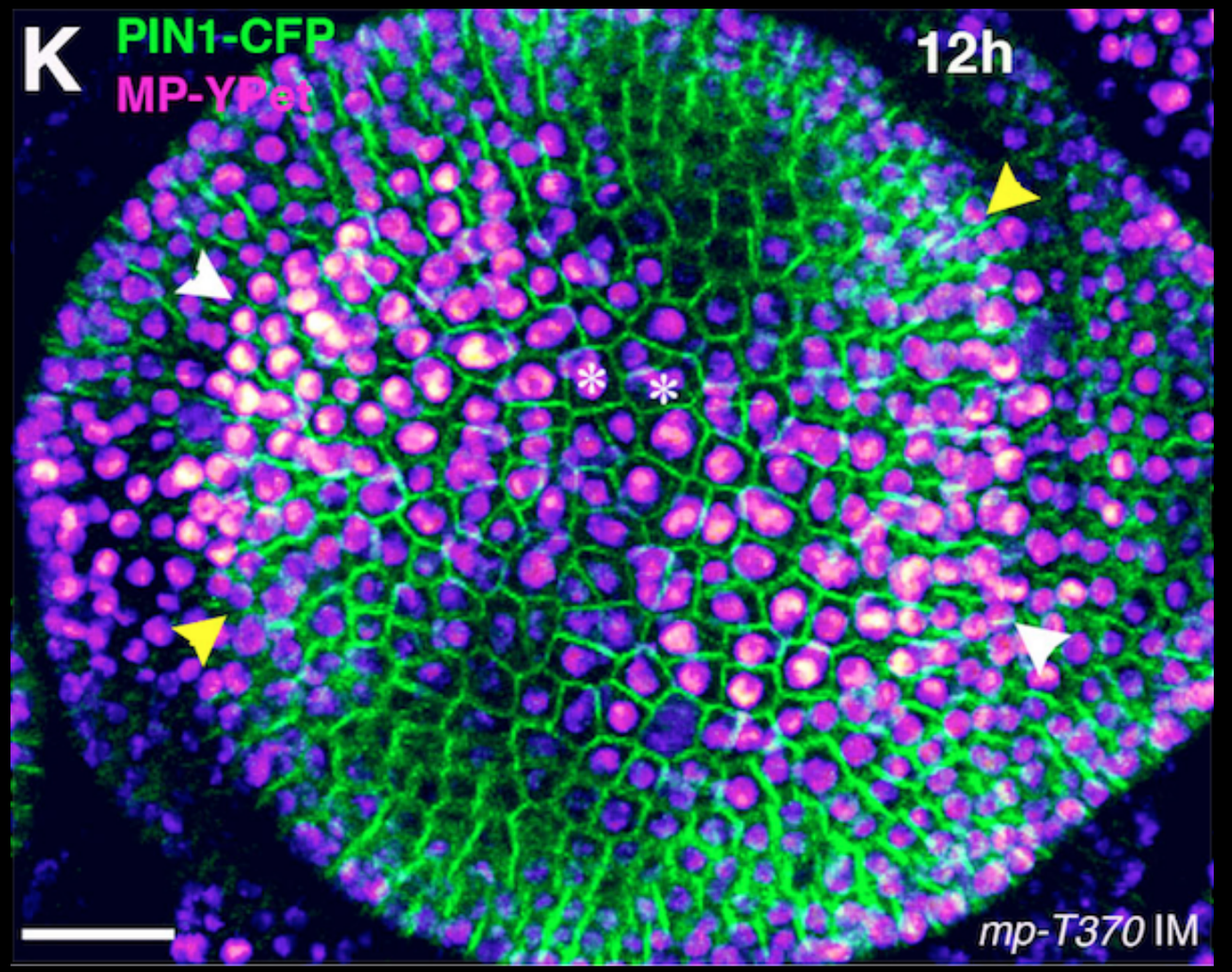




Summary

- Plant phyllotaxis is an example of a periodic patterning mechanism that is self-organising
- So far such mechanisms have been classified into three classes including
    - 1) Turing reaction diffusion (e.g. digit spacing in vertebrates)
    - 2) Cell-cell interactions (fish stripes, lateral inhibition)
    - 3) Mechanical instabilities, e.g. buckling (villi), cell-substrate interactions (hair follicle spacing)
- Plant system represents a new class that relies on feedback between signalling molecule and its directional cell-to-cell transport

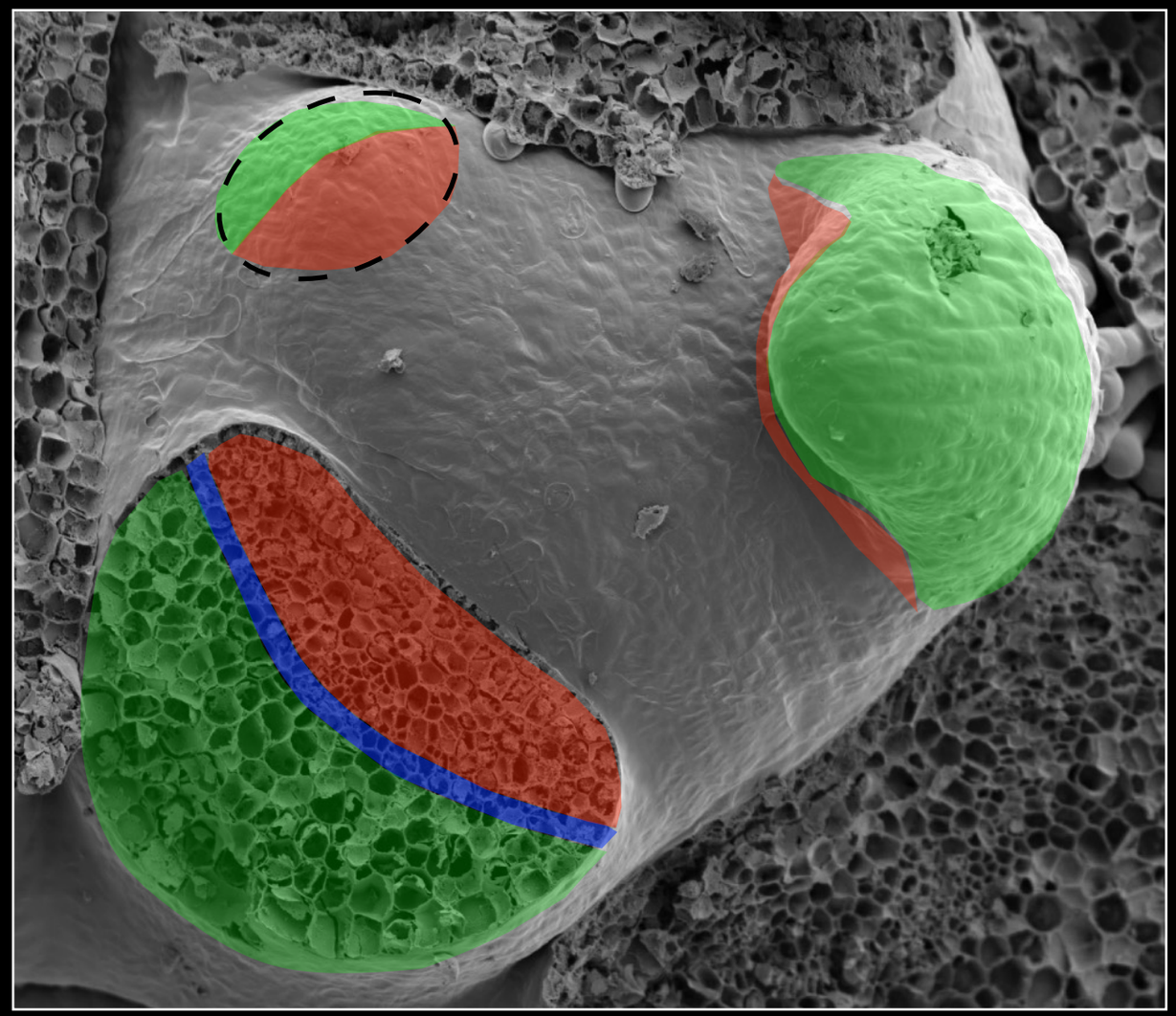
The role of cell type boundaries in regulating organogenesis

Dorsoventral polarity

Dorsoventral polarity

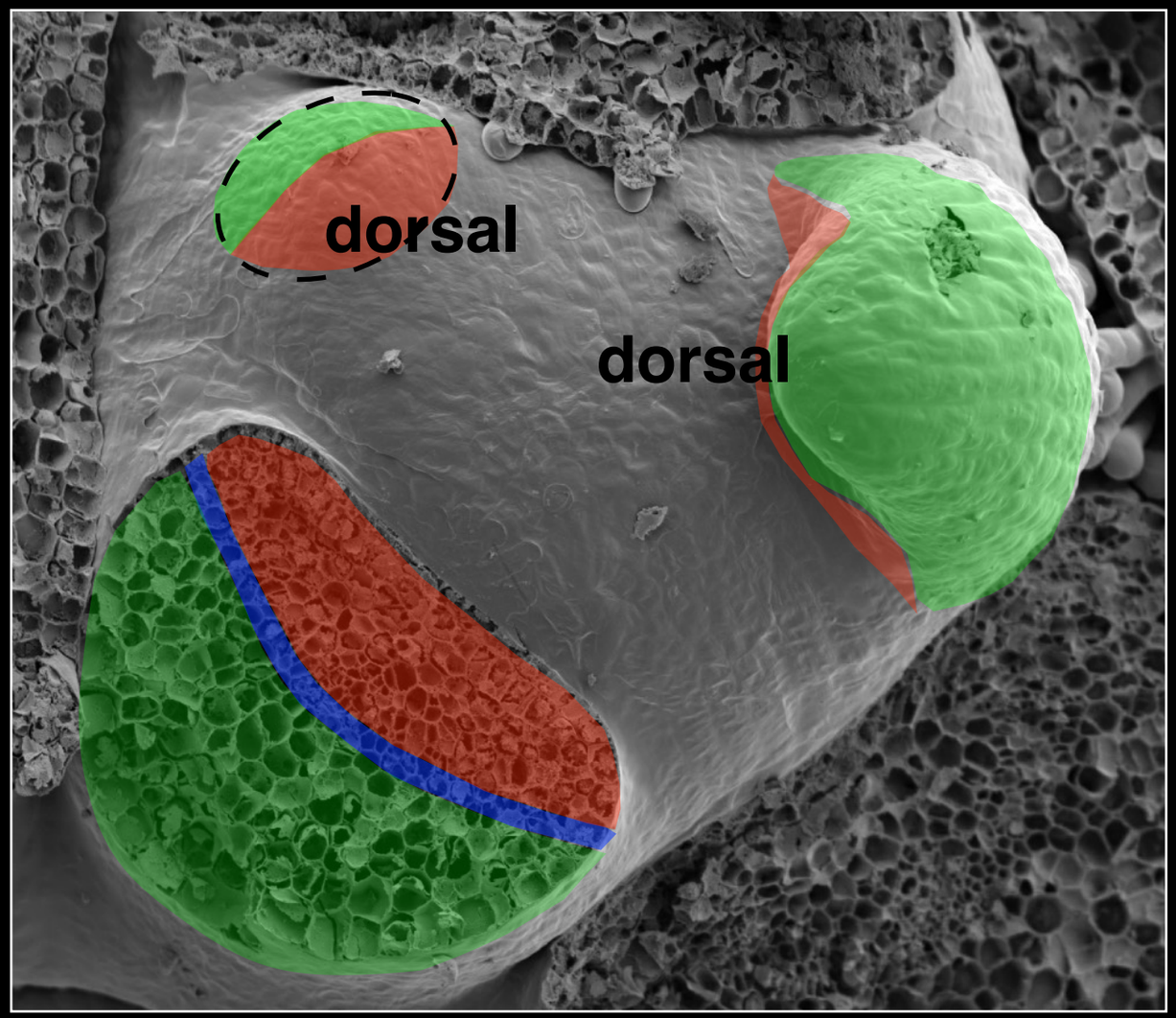
Plant organs have distinct dorsal and ventral cell types

Plant organs have distinct dorsal and ventral cell types



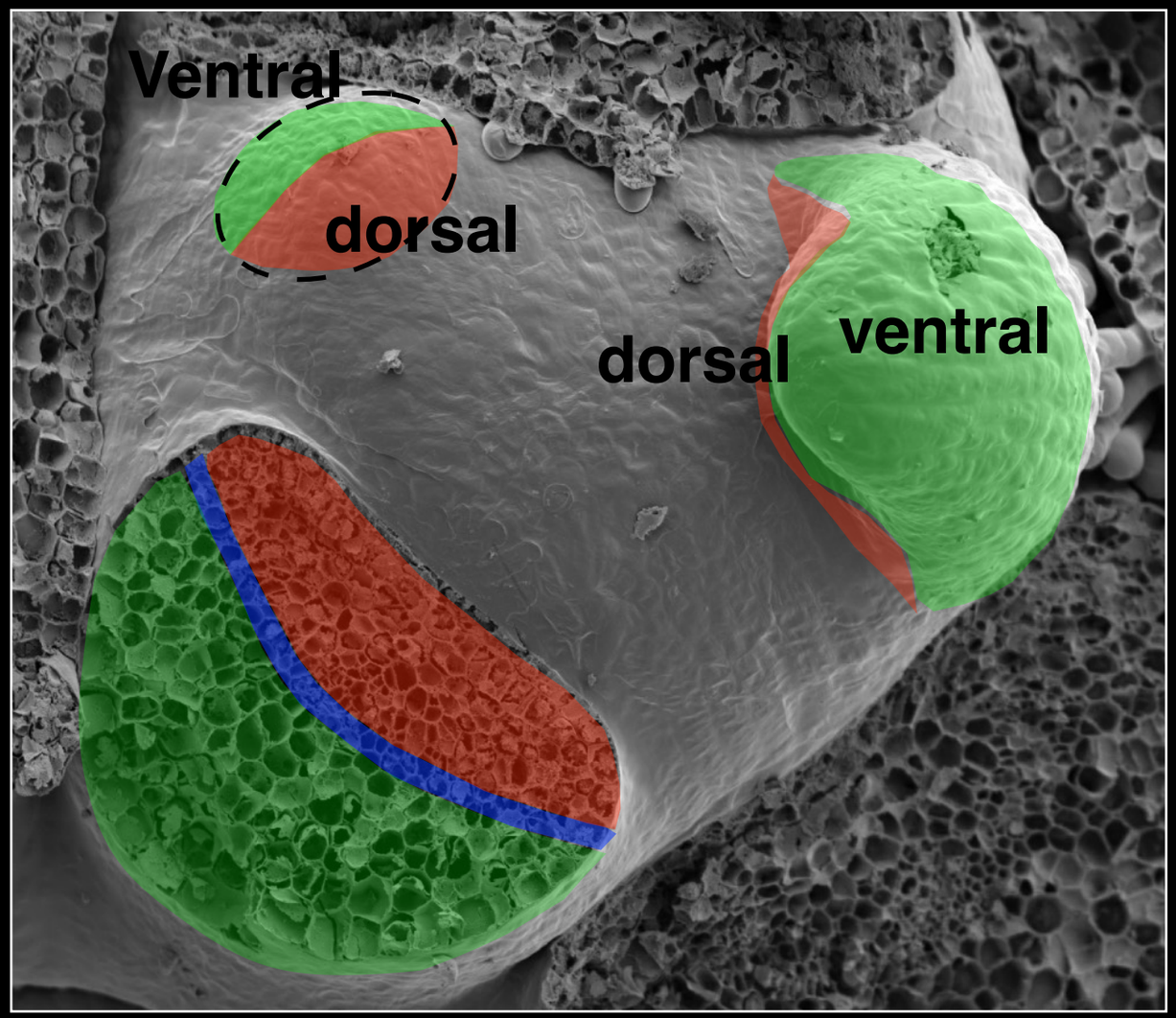
Adapted from Eshed *et al*. 2001

Plant organs have distinct dorsal and ventral cell types



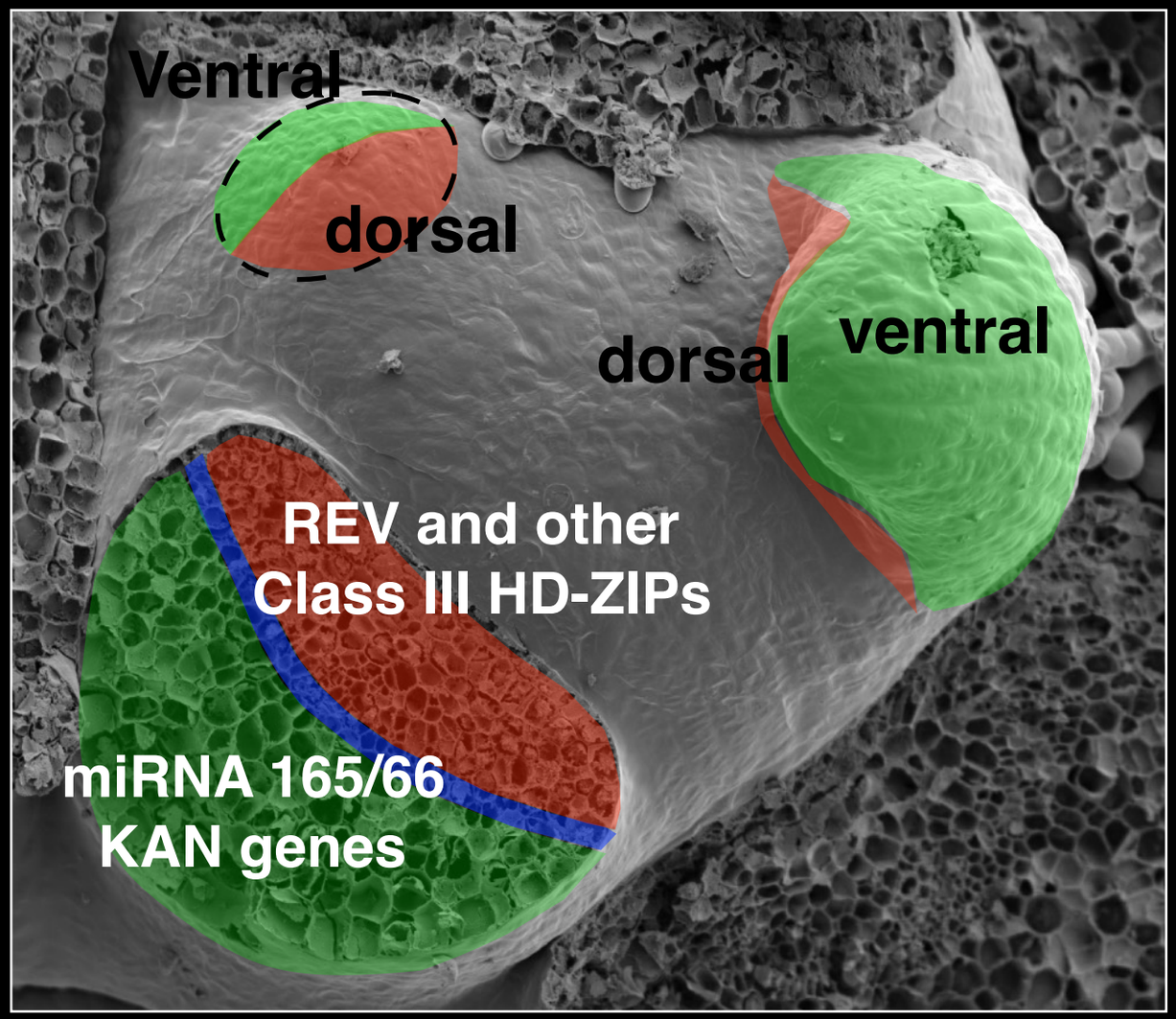
Adapted from Eshed *et al*. 2001

Plant organs have distinct dorsal and ventral cell types



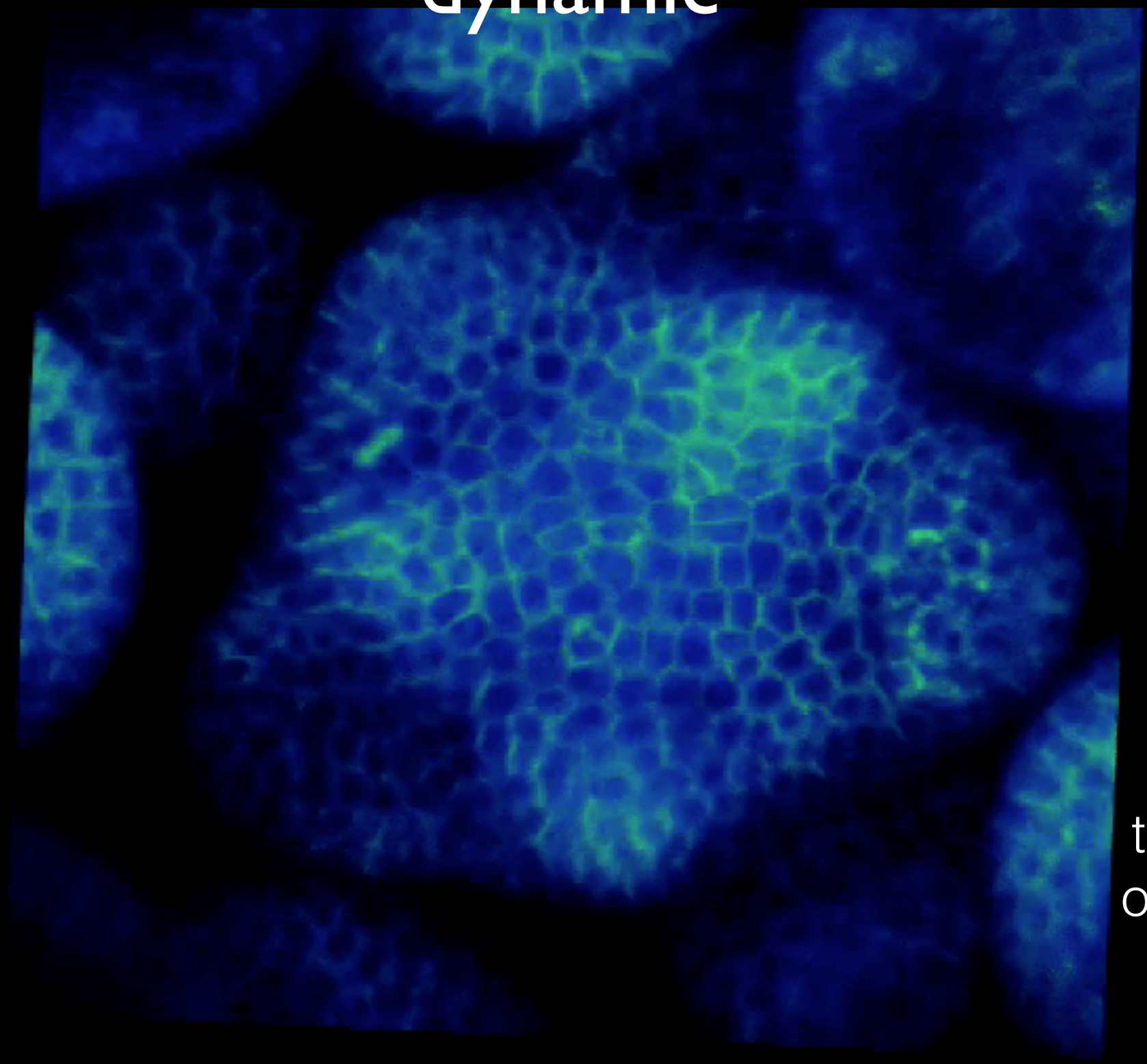
Adapted from Eshed *et al*. 2001

Plant organs have distinct dorsal and ventral cell types



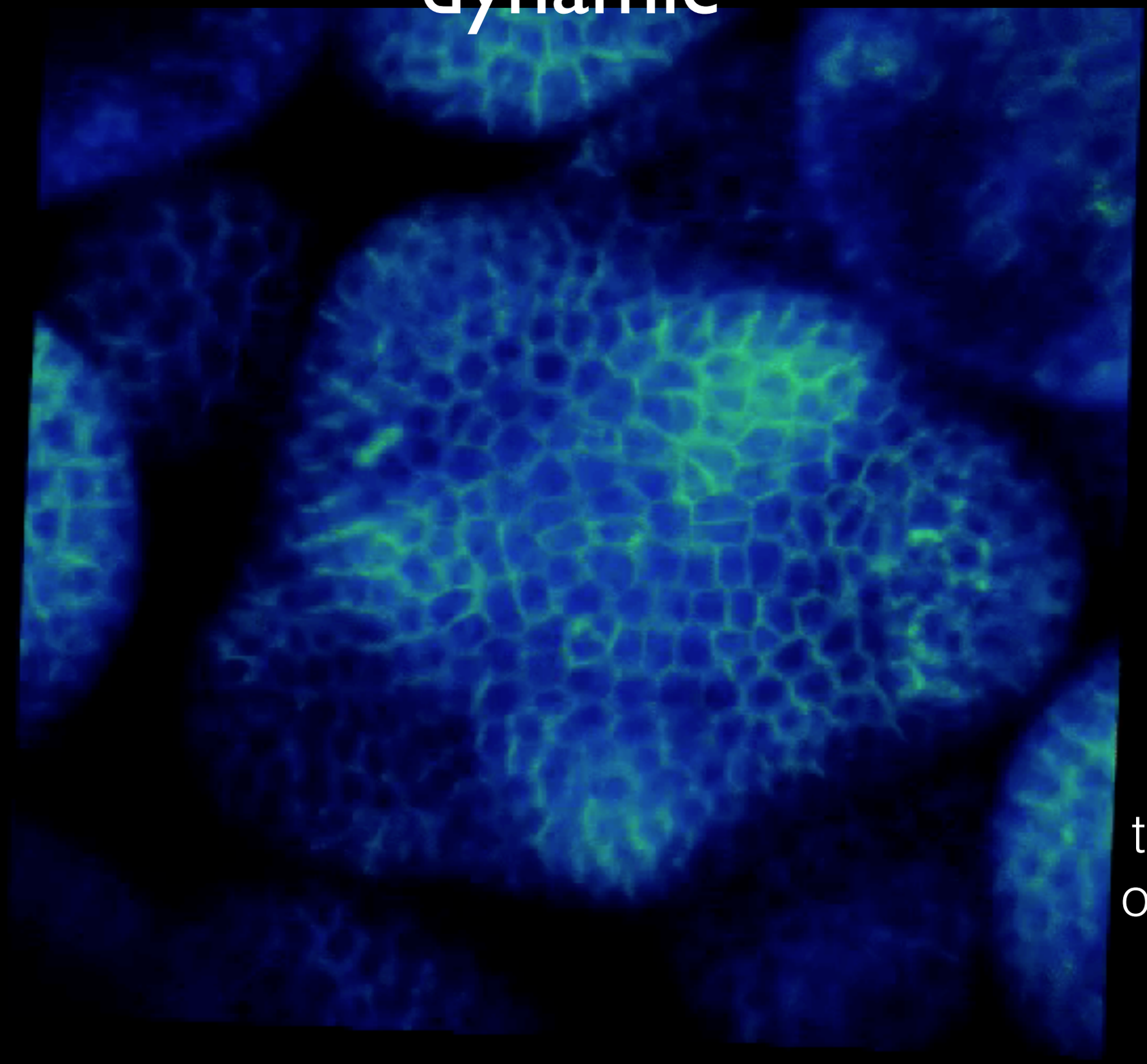
Adapted from Eshed *et al*. 2001

Cell positions and organ positions are dynamic



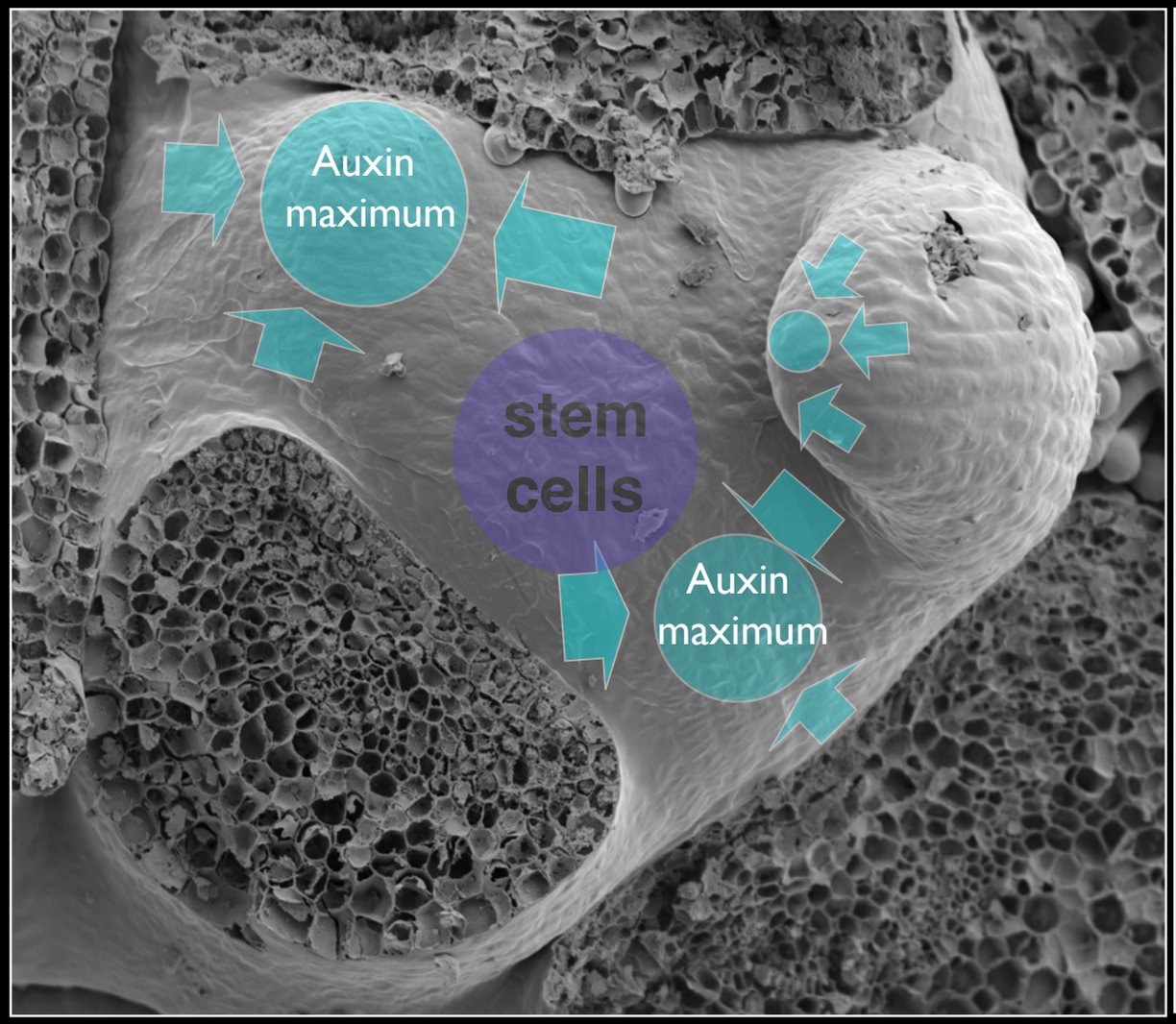
time lapse
over 40 hrs

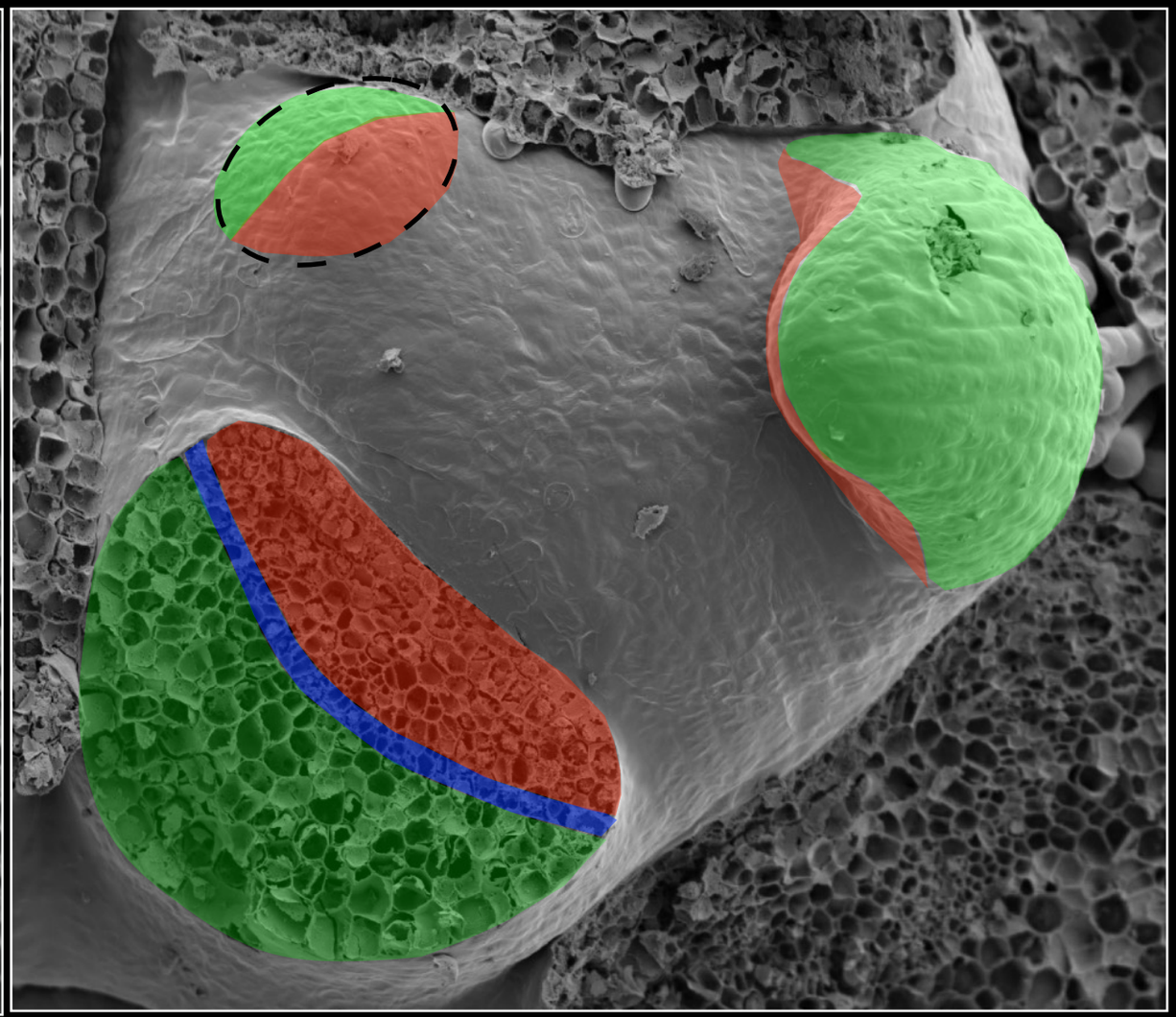
Cell positions and organ positions are dynamic



time lapse
over 40 hrs

How are dorsal and ventral cell types first specified?

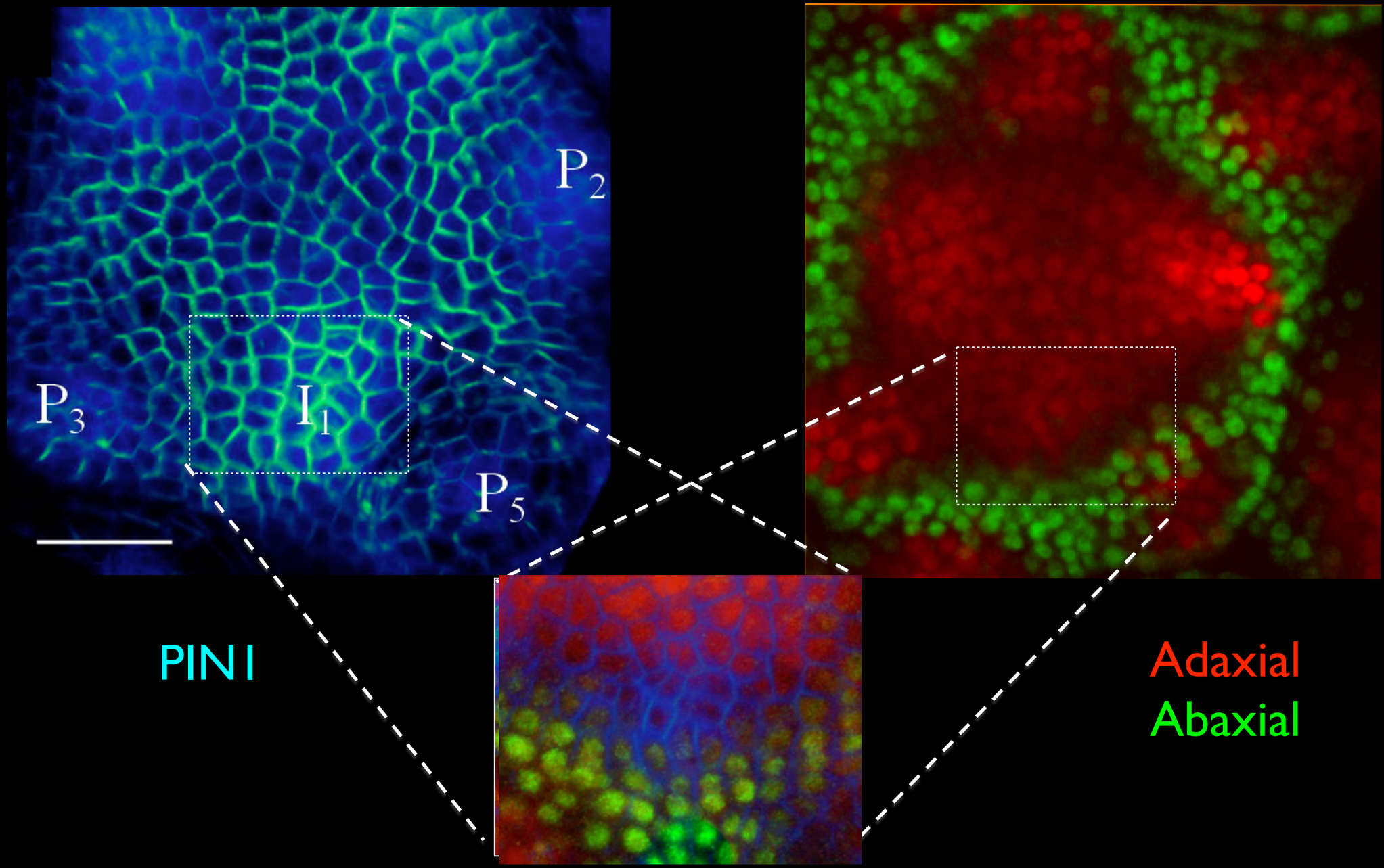




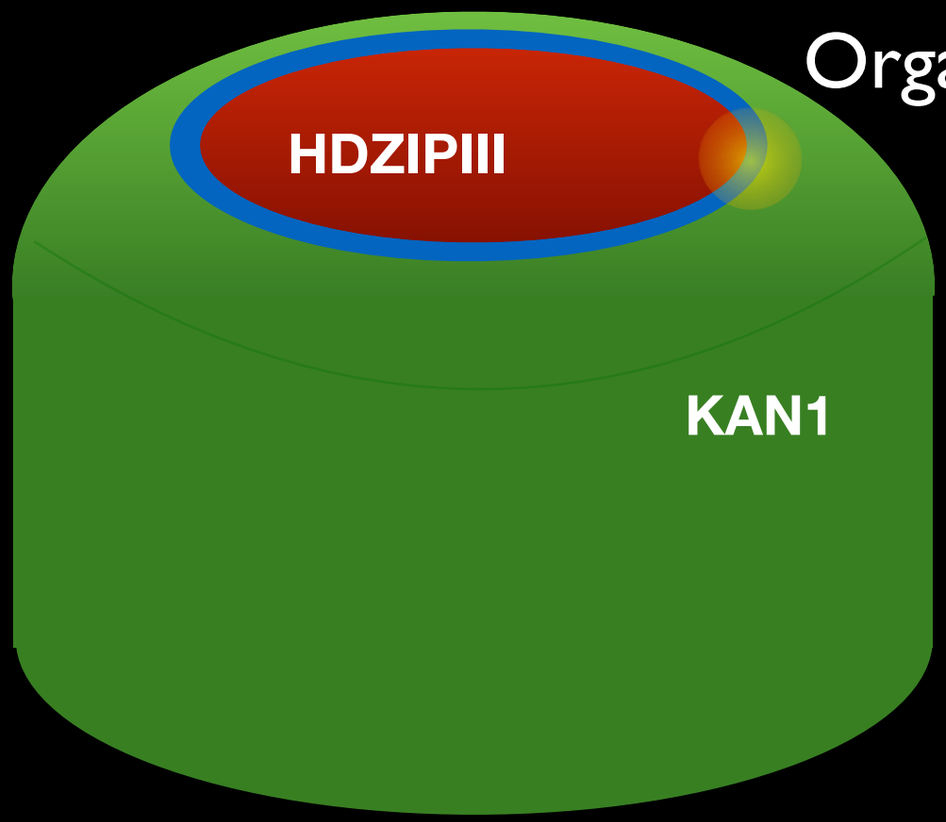
Adapted from Eshed *et al*. 2001

EMBL

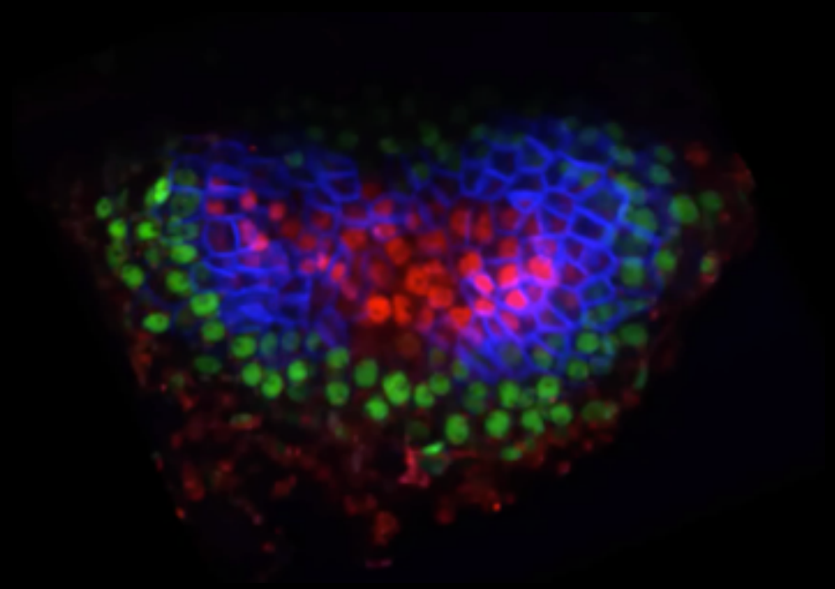
Organ positioning occurs on the boundary between "dorsal" and "ventral" transcription factors



The meristem is pre-patterned with HD-ZIPIII and KAN gene expression

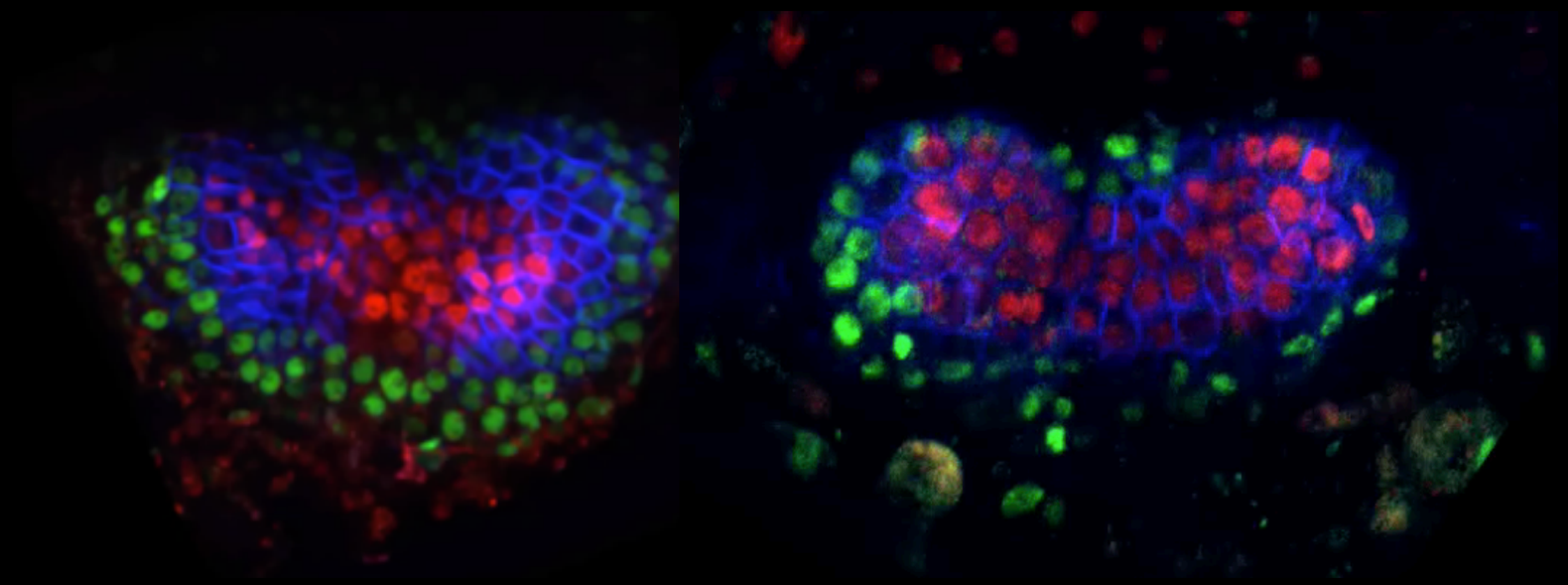


Dorsal and ventral gene expression is propagated into developing organs



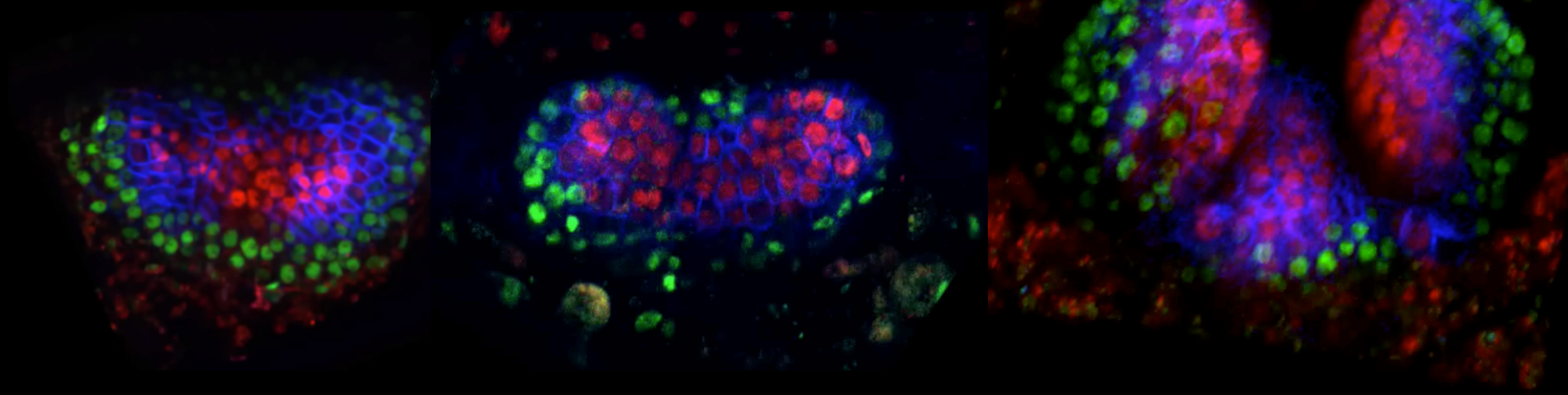
REVOLUTA PIN1
KANADI1

Dorsal and ventral gene expression is propagated into developing organs



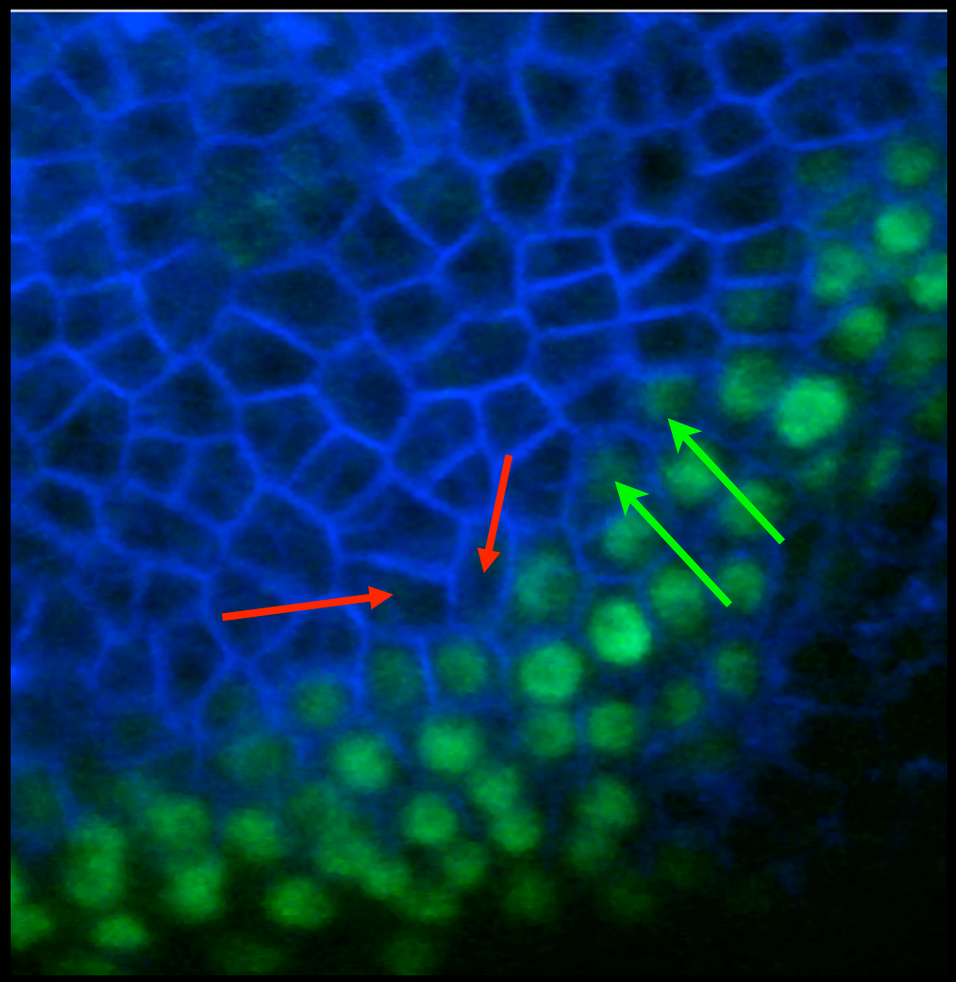
REVOLUTA PIN1
KANADI1

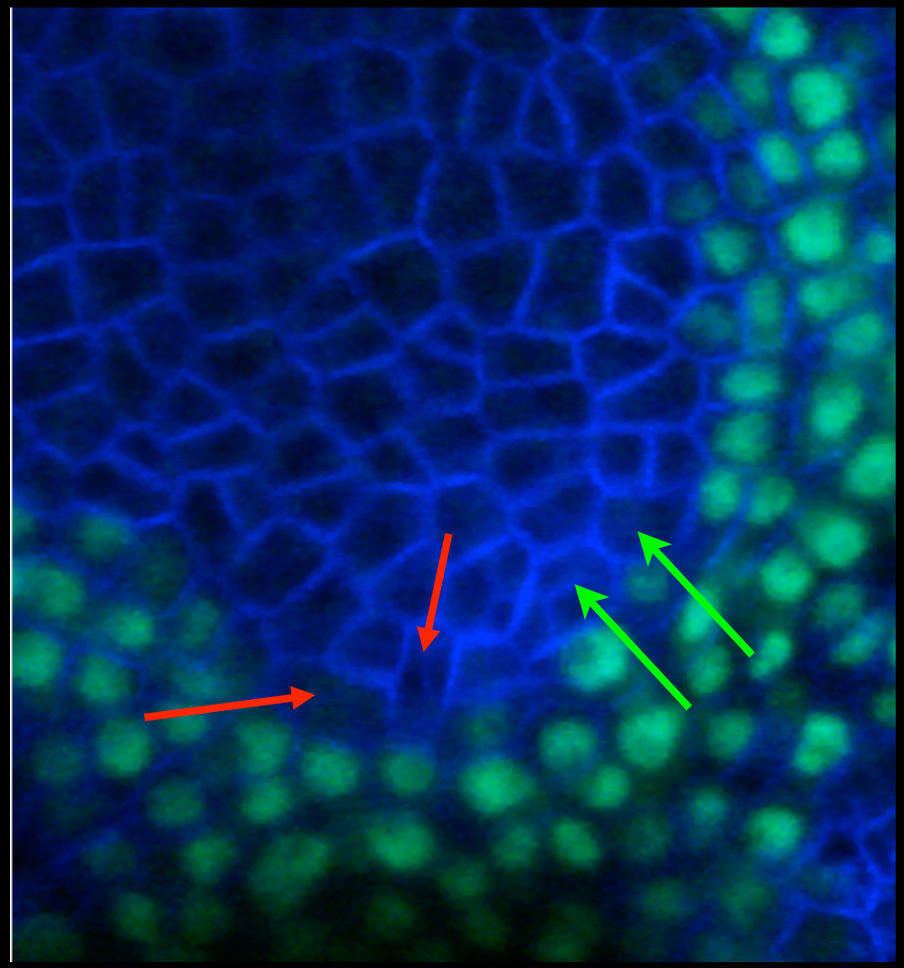
Dorsal and ventral gene expression is propagated into developing organs

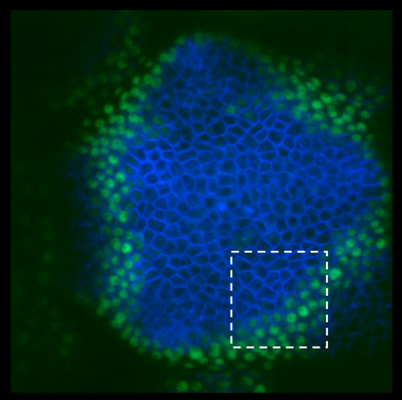


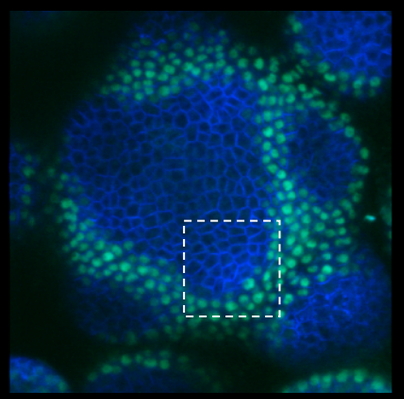
REVOLUTA PIN1
KANADI1

Boundary position is relatively stable during organ inception

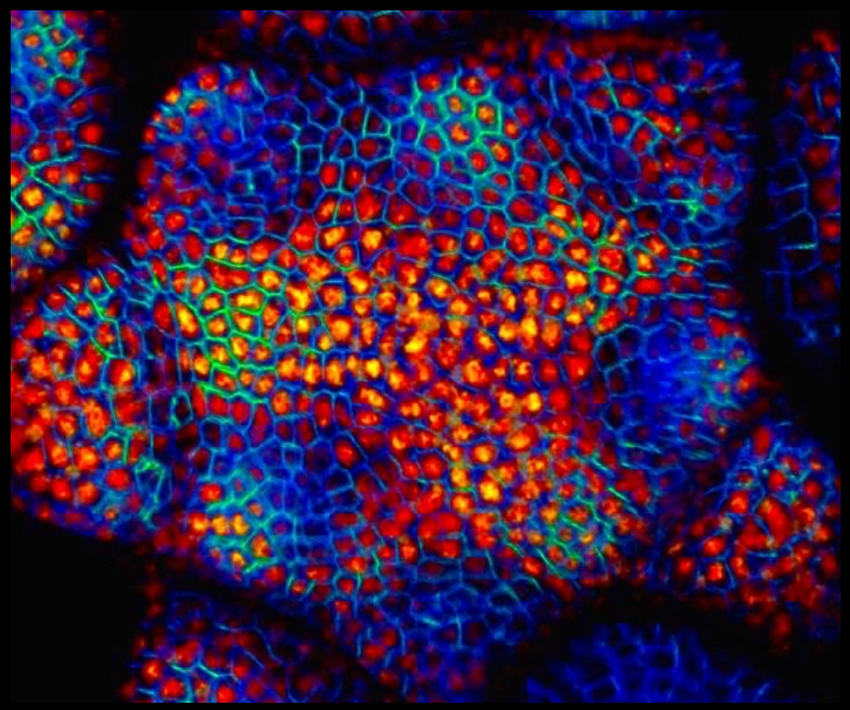






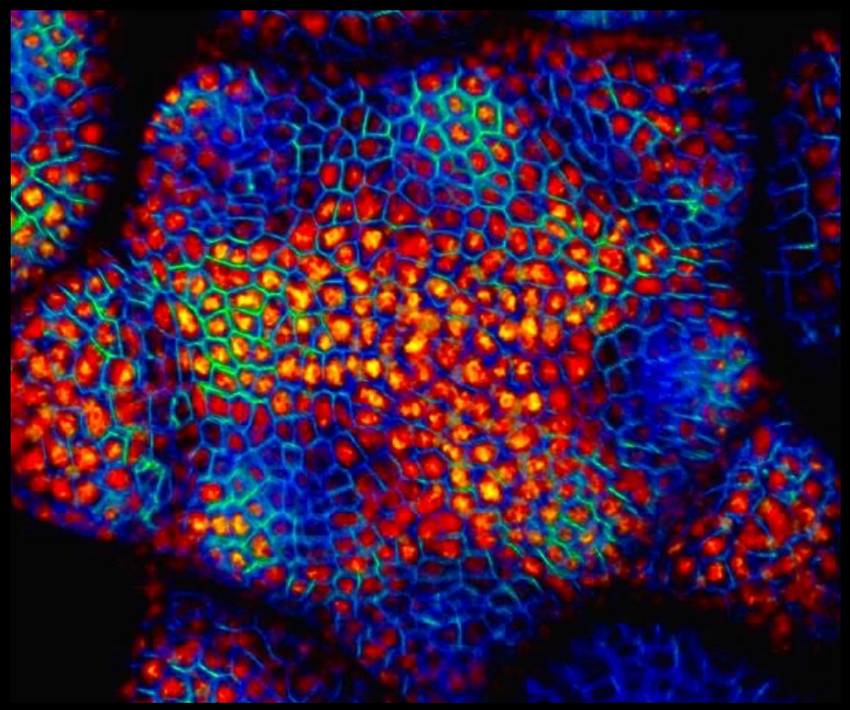


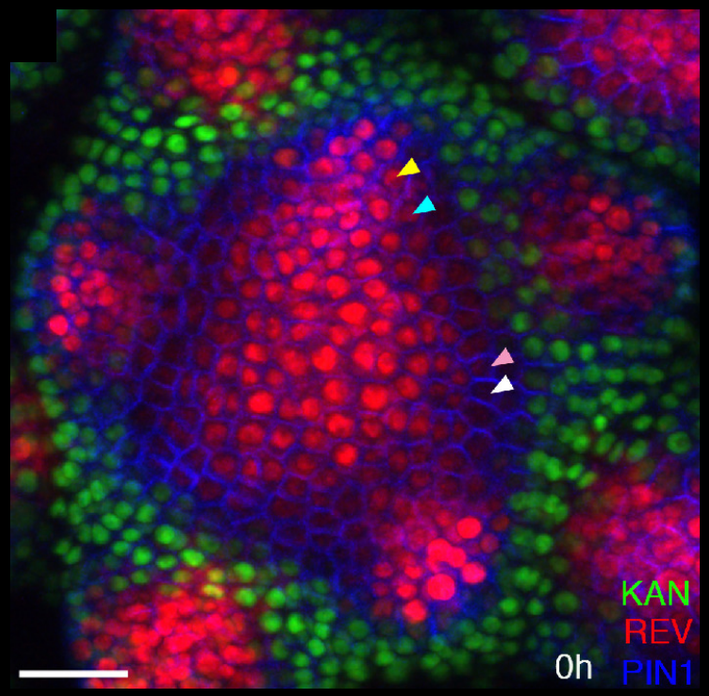
The establishment adaxial/abaxial boundaries is itself indirectly patterned by auxin



Auxin levels according to R2D2
PIN1

The establishment adaxial/abaxial boundaries is itself indirectly patterned by auxin

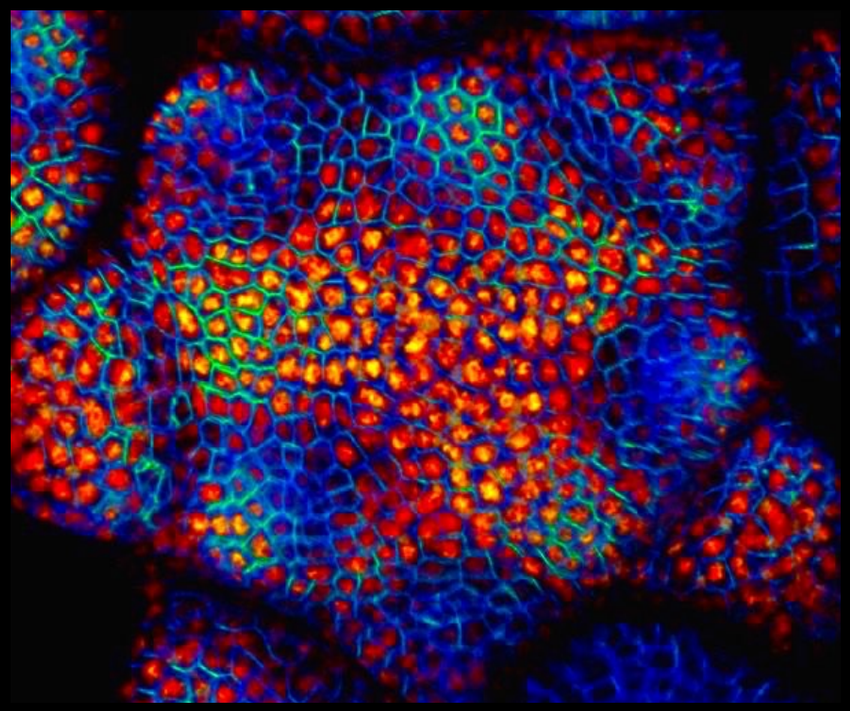


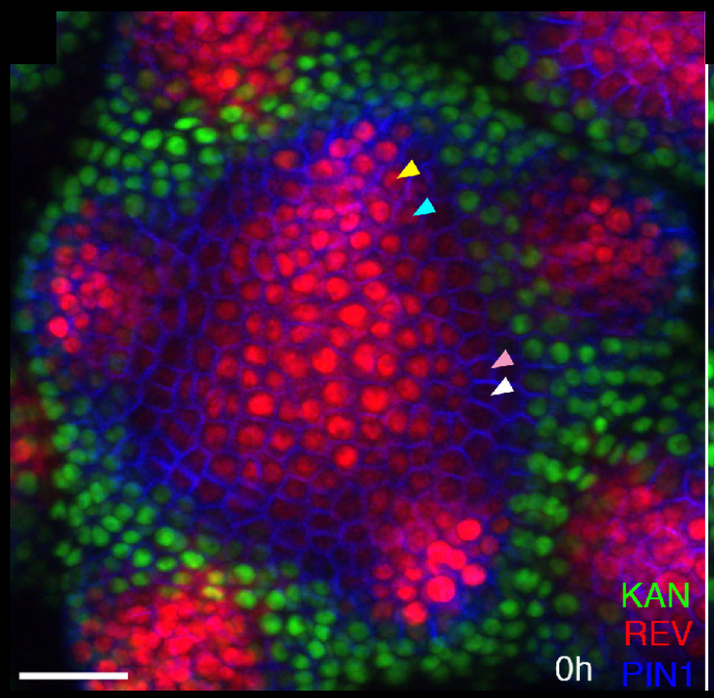


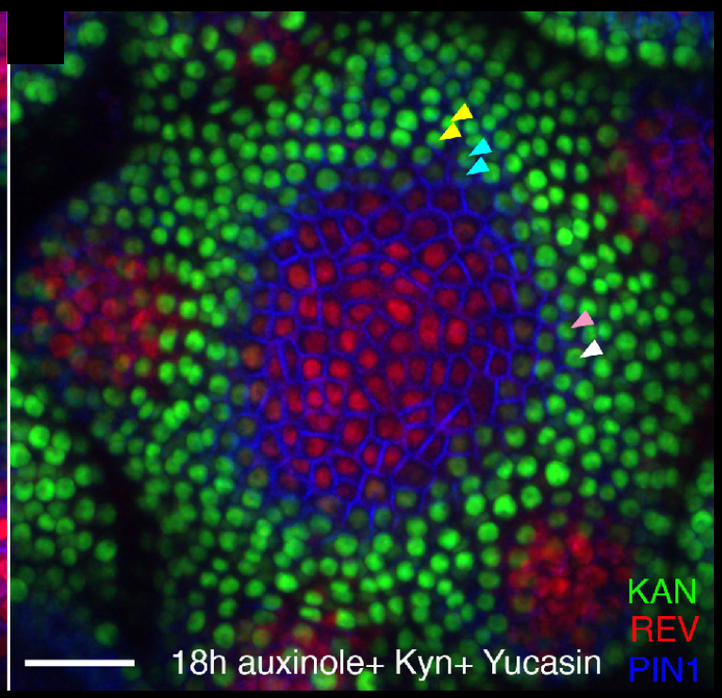
Auxin levels according to R2D2

PIN1

The establishment adaxial/abaxial boundaries is itself indirectly patterned by auxin



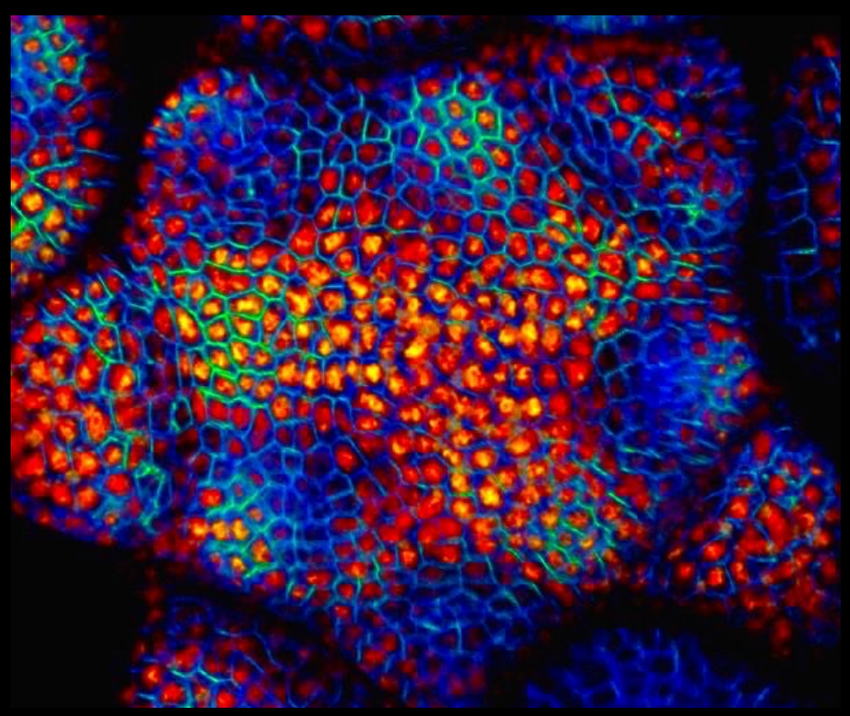


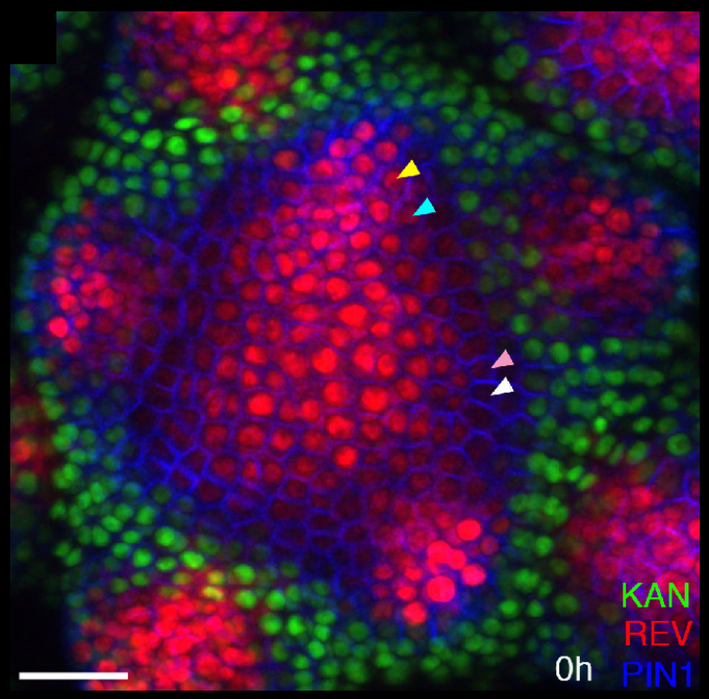


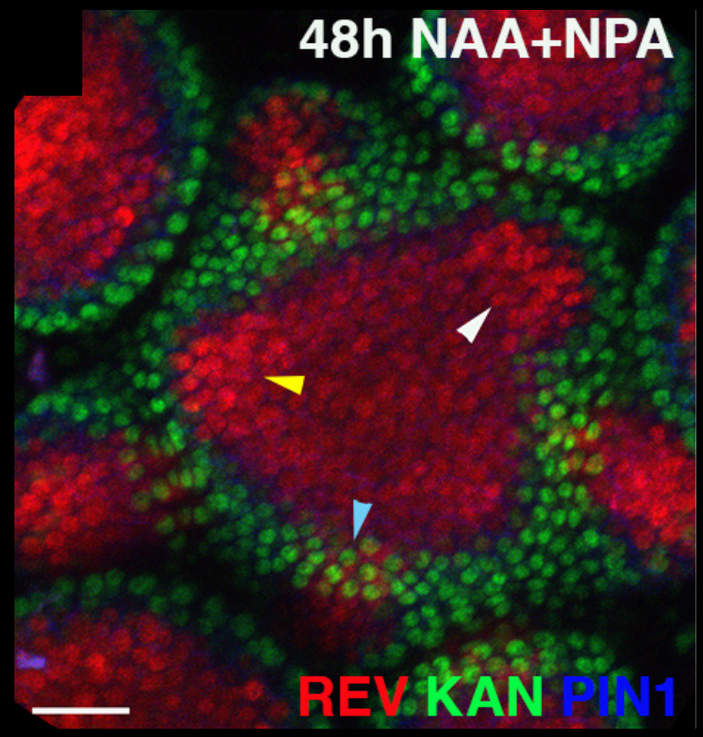
Auxin levels according to R2D2

PIN1

The establishment adaxial/abaxial boundaries is itself indirectly patterned by auxin

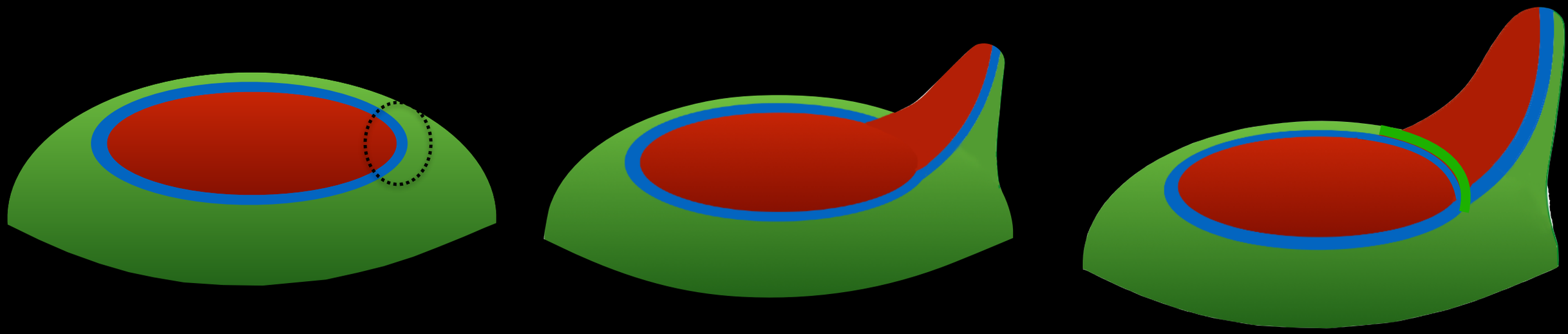




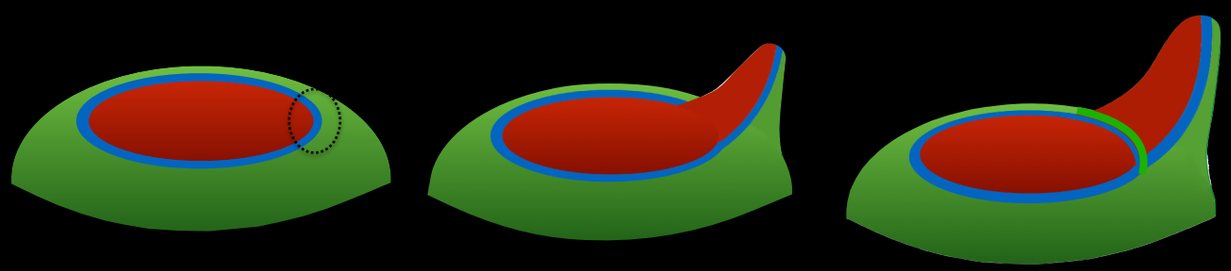


Auxin levels according to R2D2
PIN1

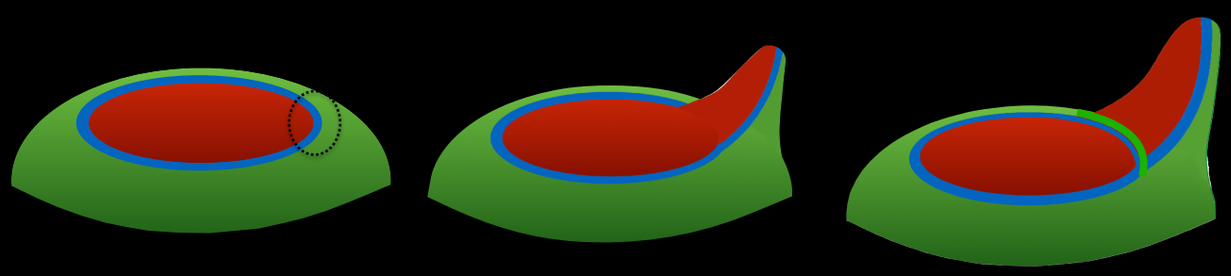
Leaves arise on a pre-existing cell-type boundary - auxin stabilises cell identities

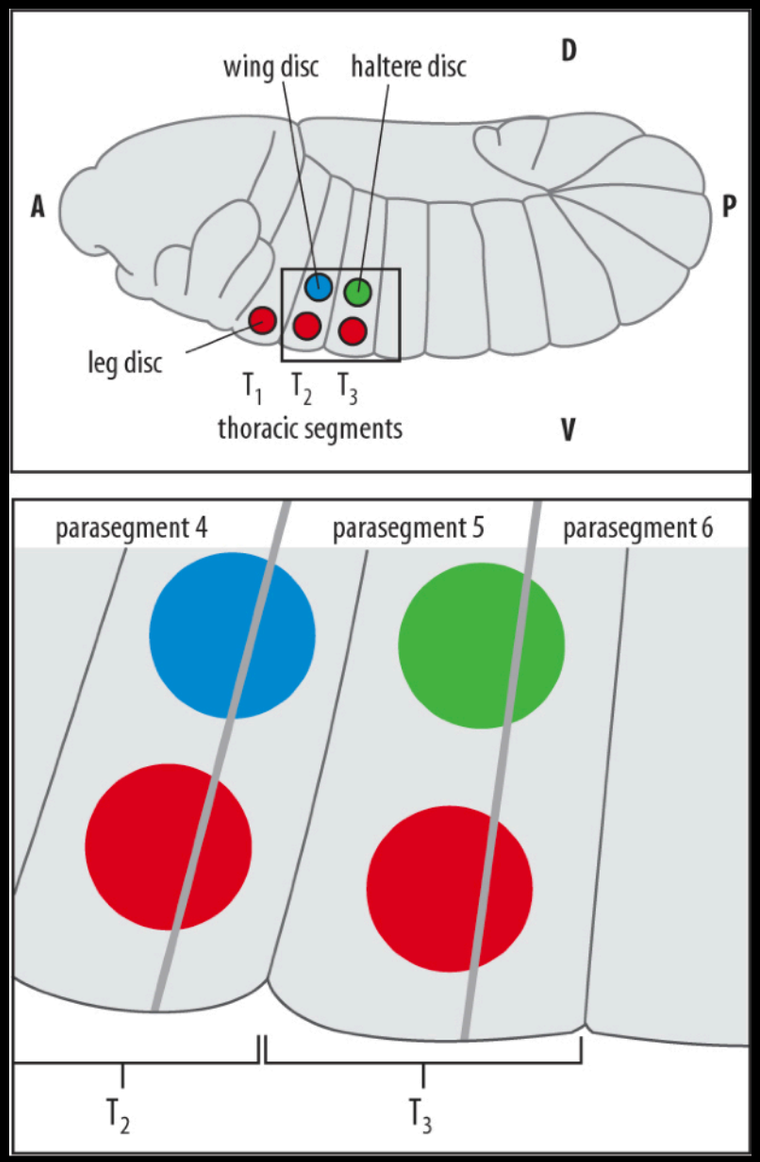


Leaves arise on a pre-existing cell-type boundary - auxin stabilises cell identities

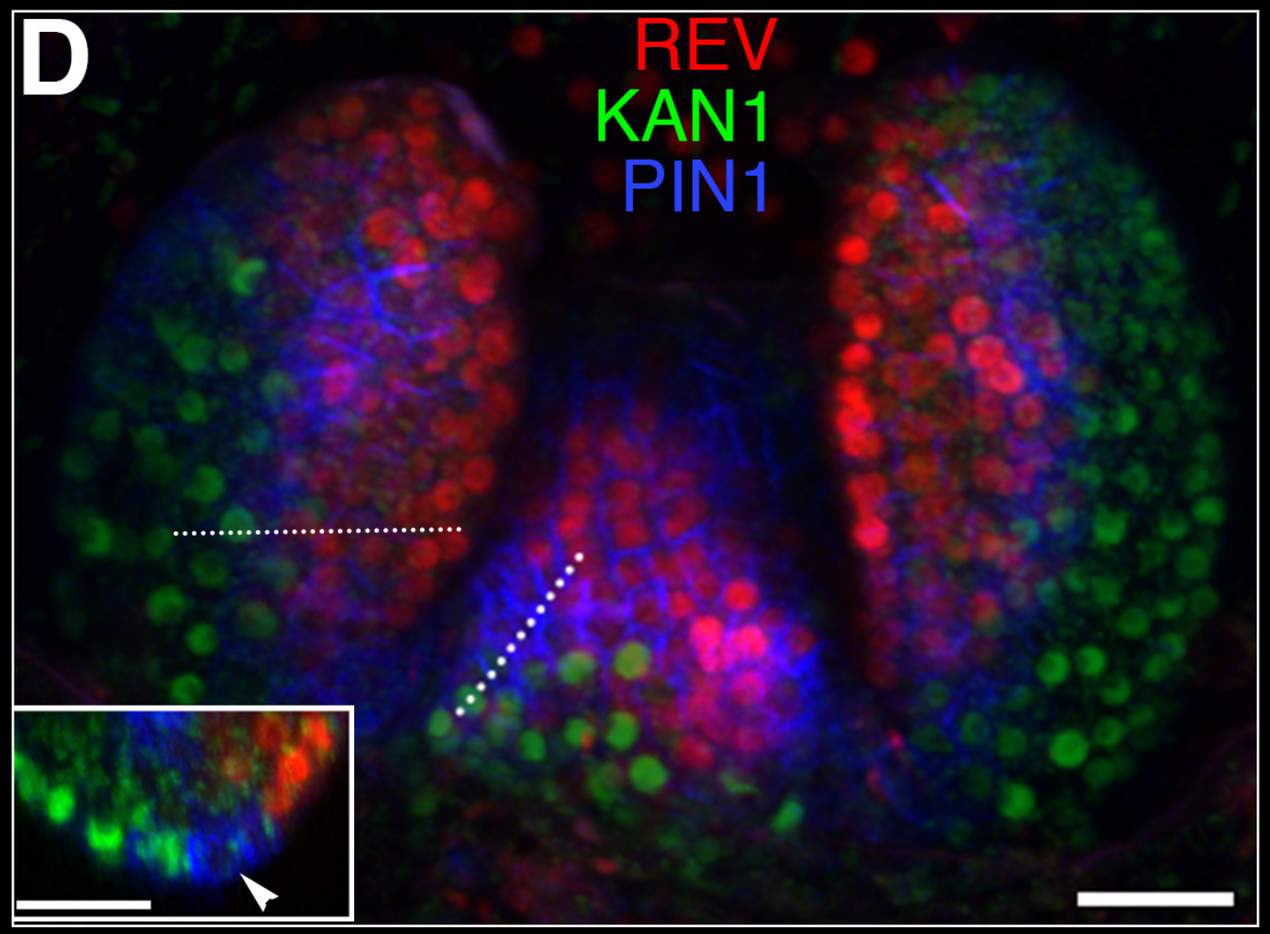


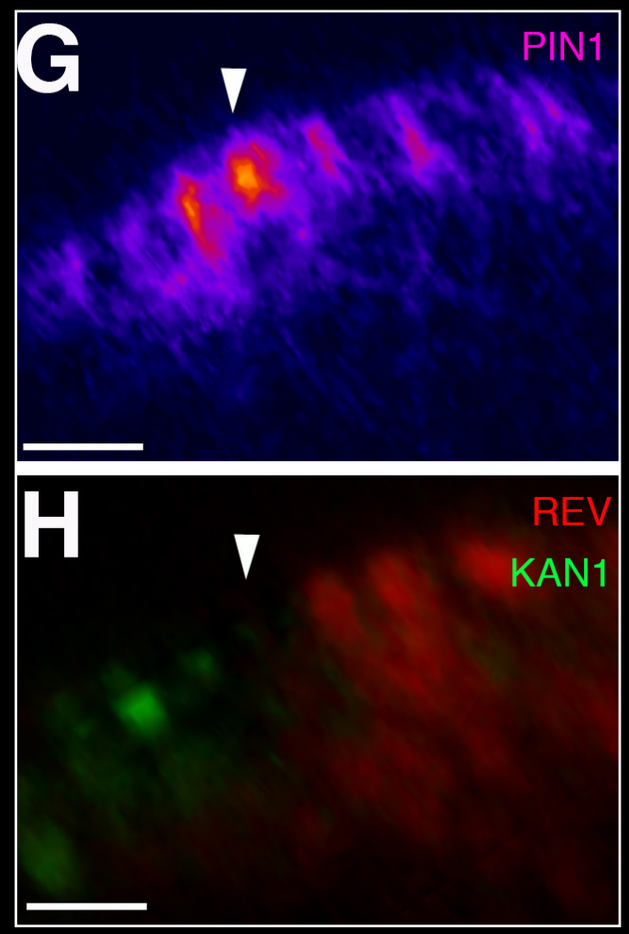
Leaves arise on a pre-existing cell-type boundary - auxin stabilises cell identities



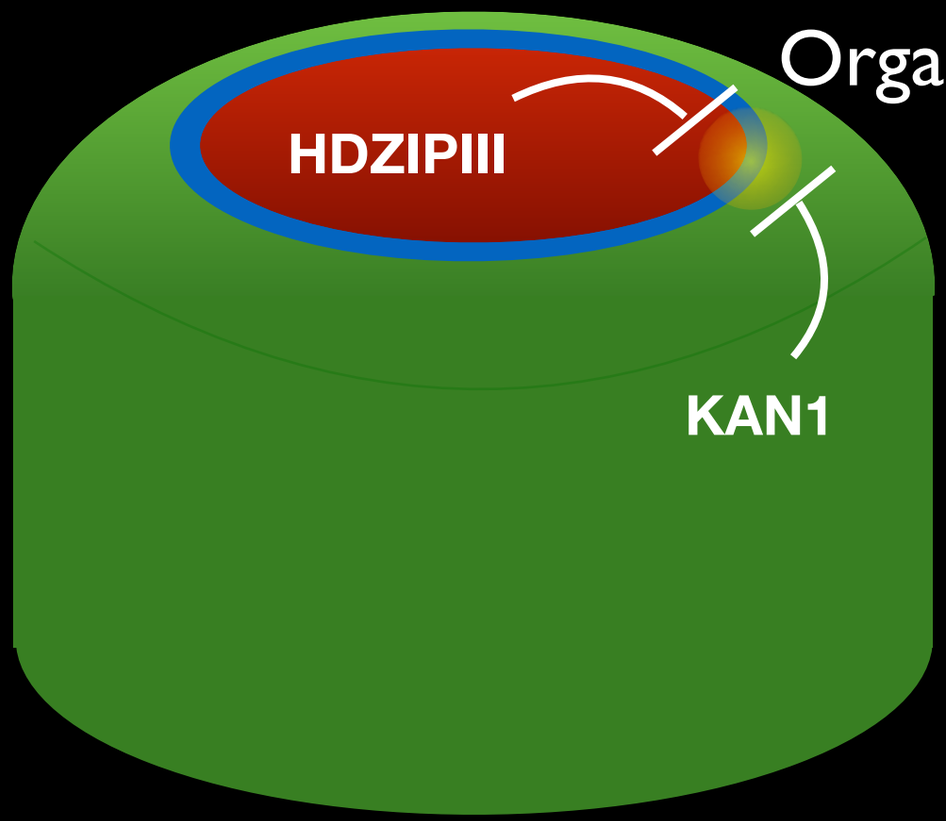


The boundary corresponds to a “gap” between HD-ZIPIII and KAN expression



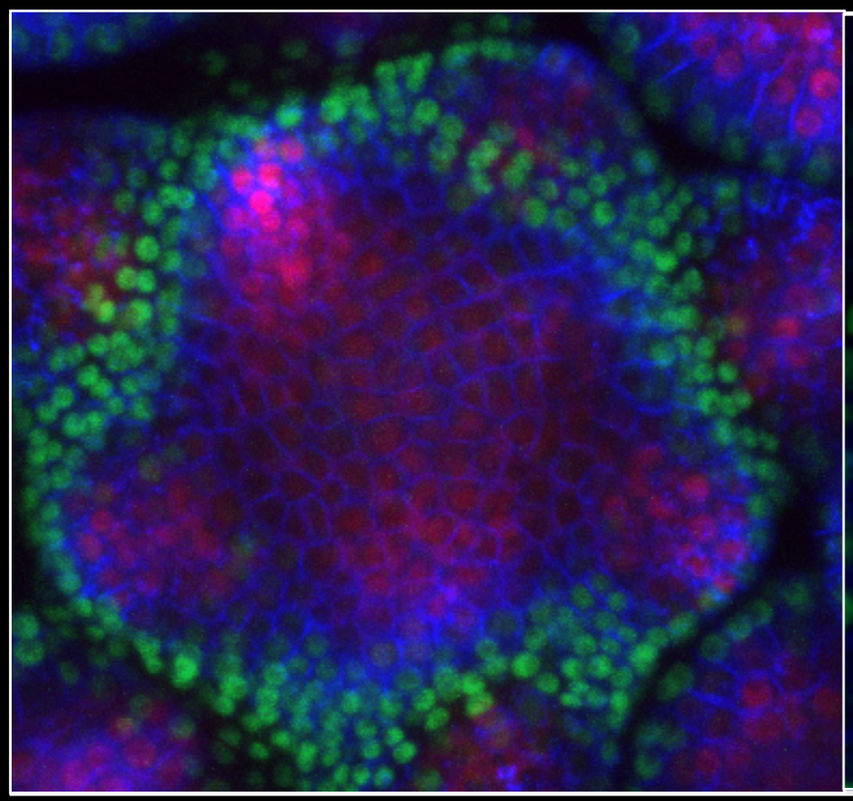


Do HD-ZIPIII and KAN genes repress organogenesis where they are expressed?



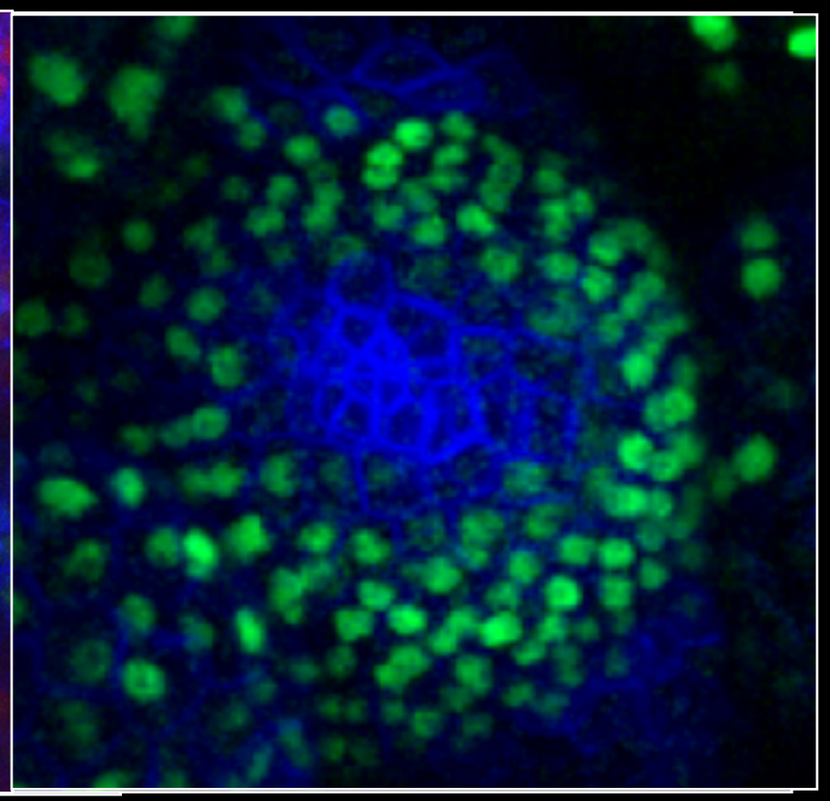
Loss of dorsal or ventral gene expression leads to ectopic organ formation

WT



REV PINI KANI

Loss of REV



pUBQ > miR165

Loss of KAN

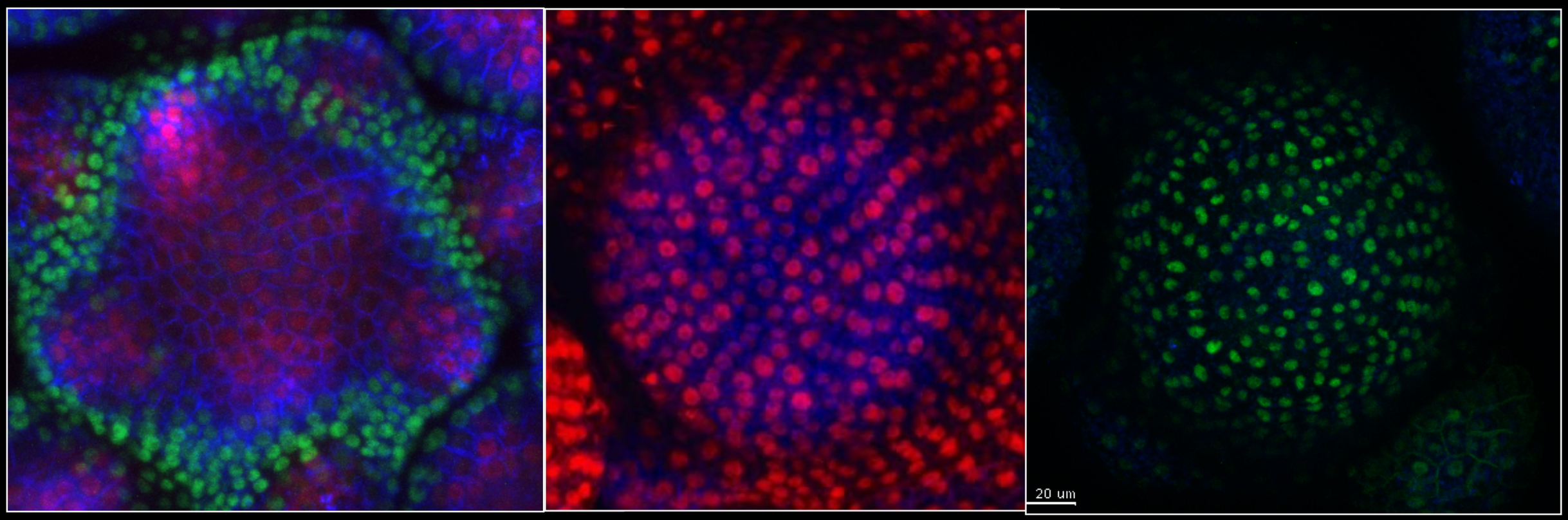
kan1kan2kan4

Ectopic dorsal or ventral gene expression represses organ formation

WT

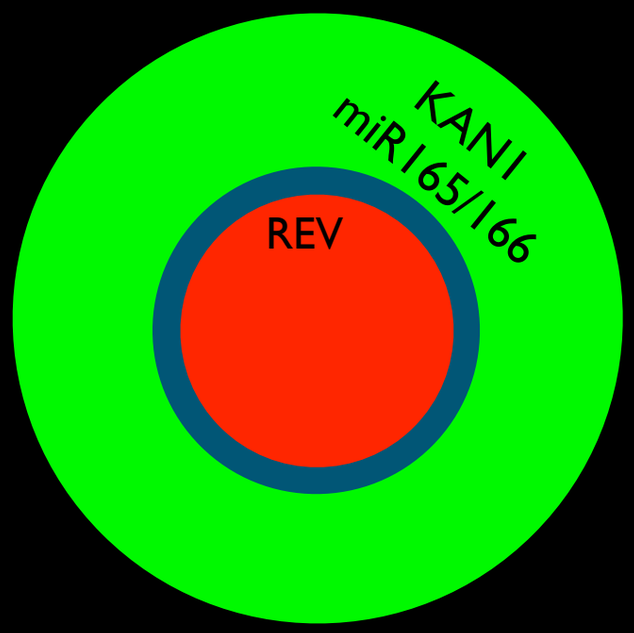
pML1 > REV

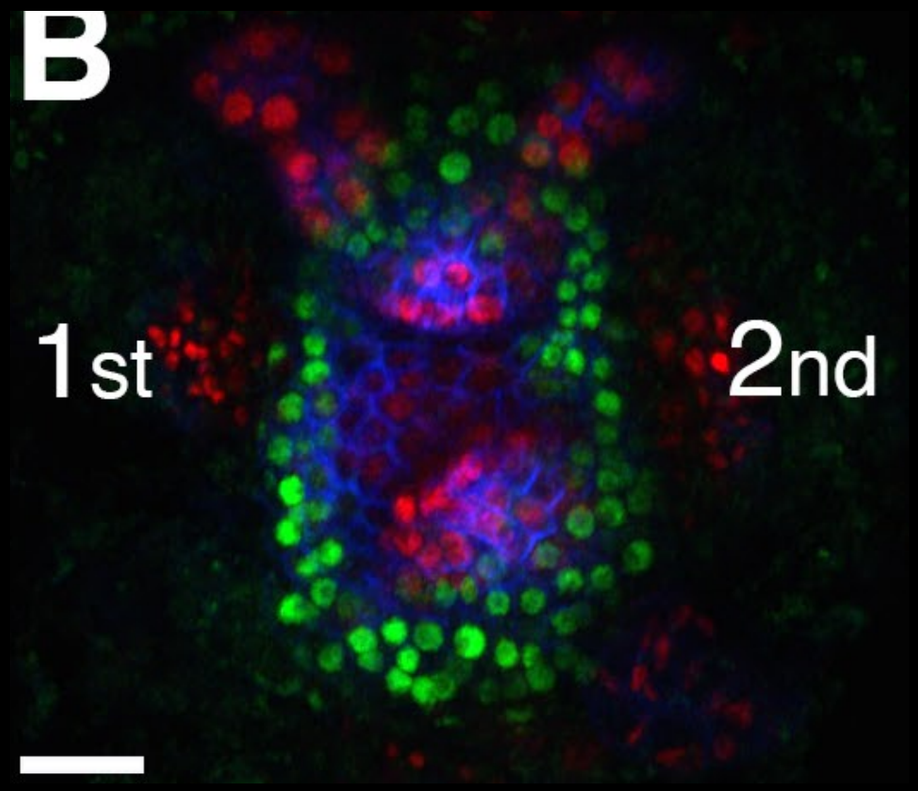
pML1 > KAN1



REV PINI KANI

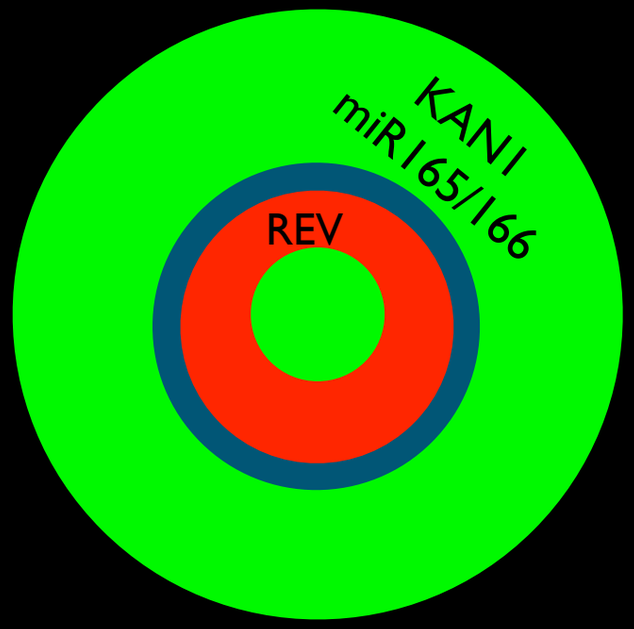
DV patterning in the meristem also influences organ morphogenesis

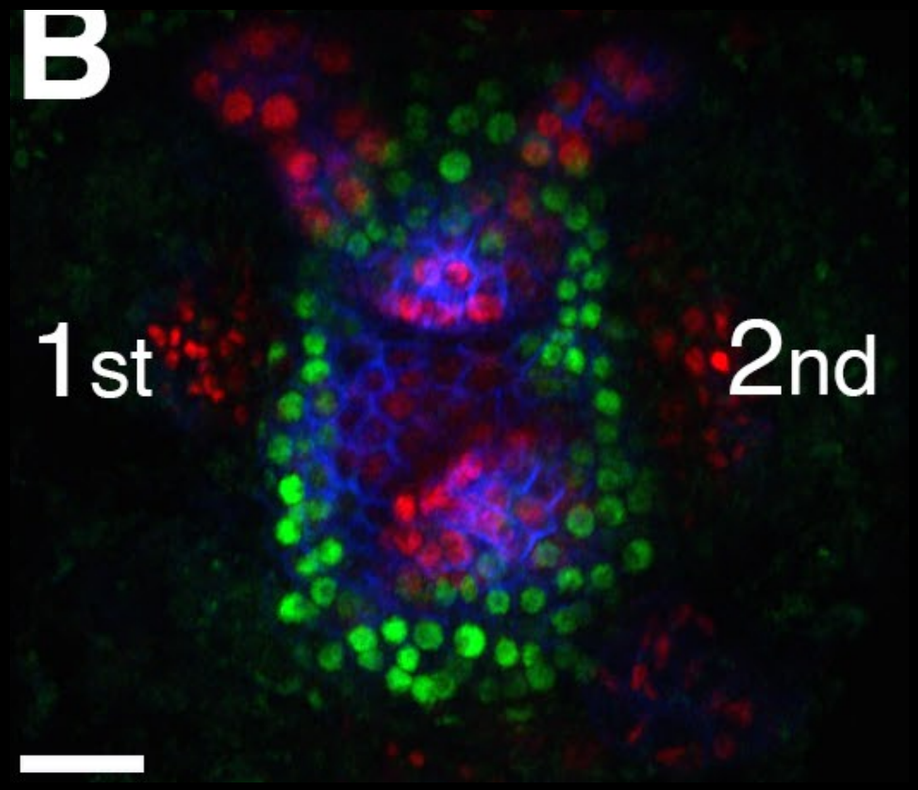


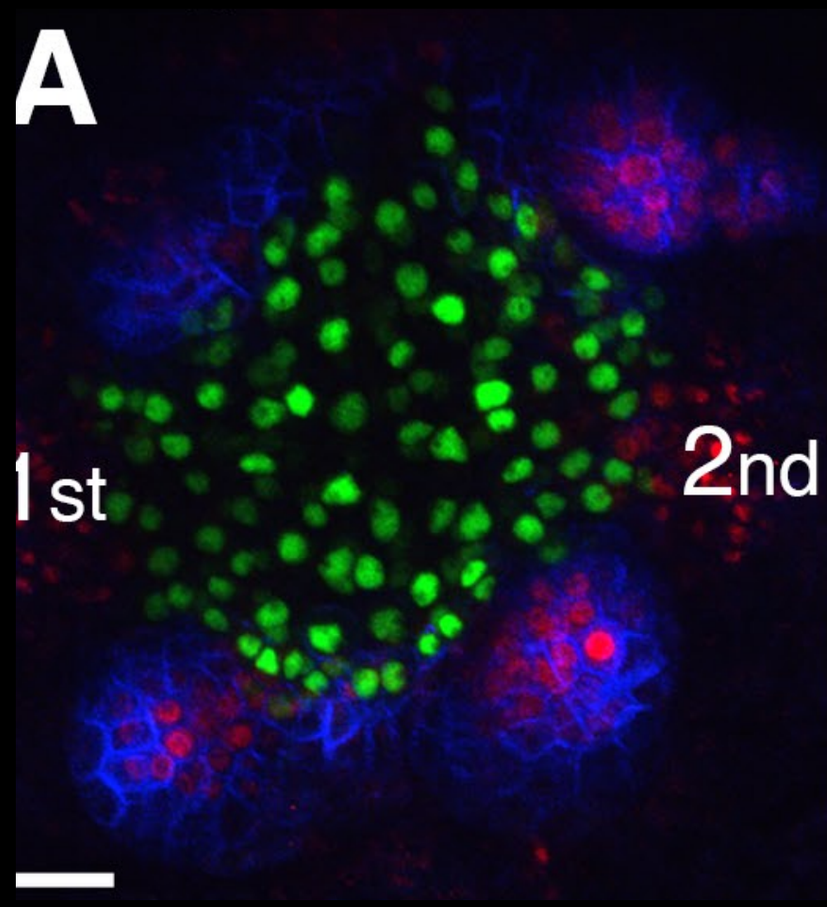


REV KAN PIN1

DV patterning in the meristem also influences organ morphogenesis



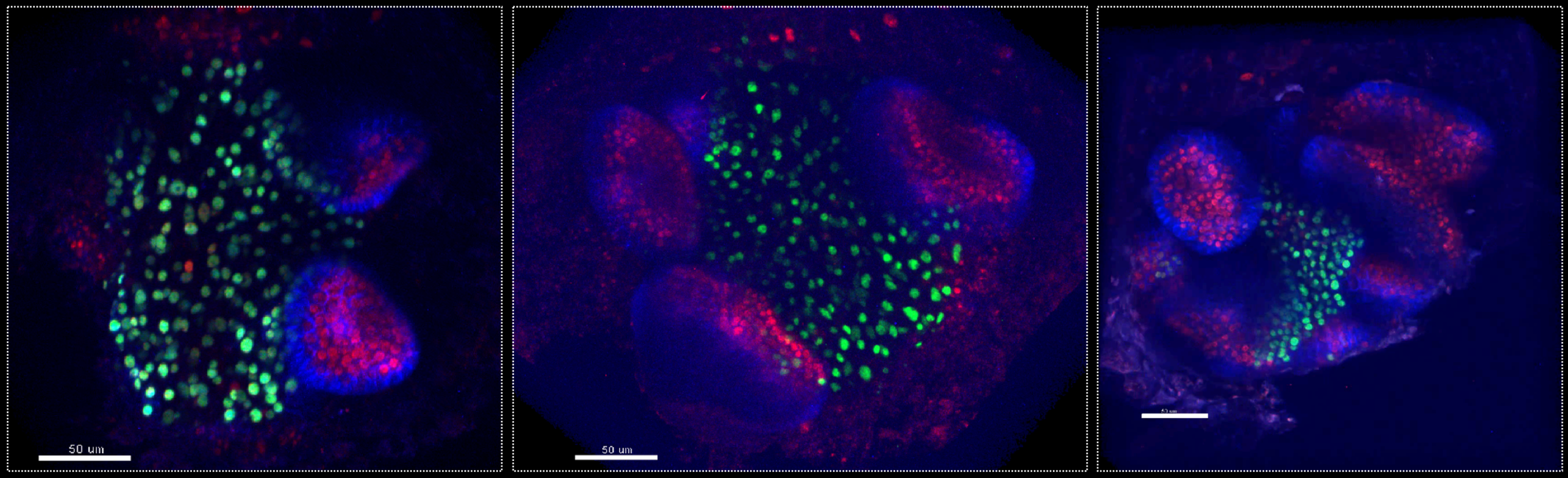




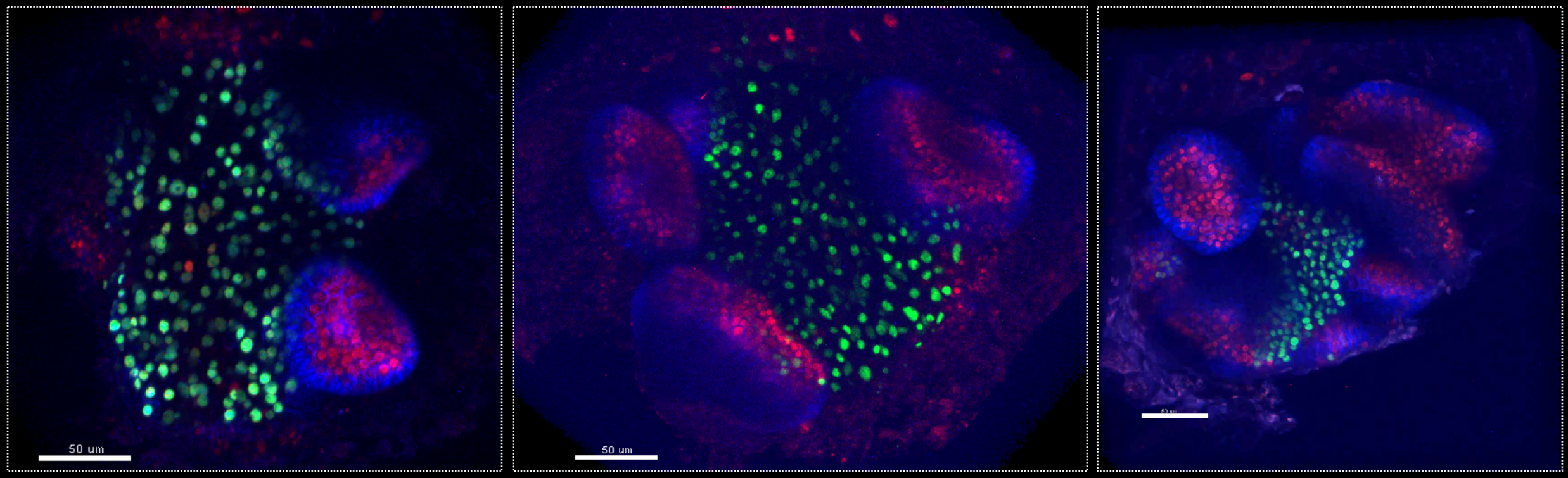
CLV3>>KAN1-2GFP

REV KAN PIN1

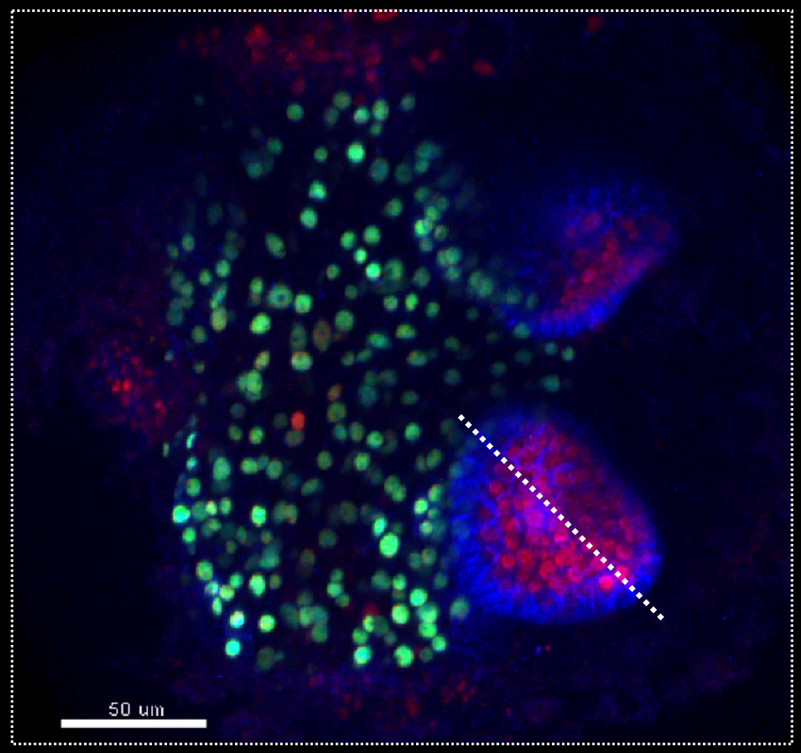
Boundary configuration in SAM determines organ morphogenesis

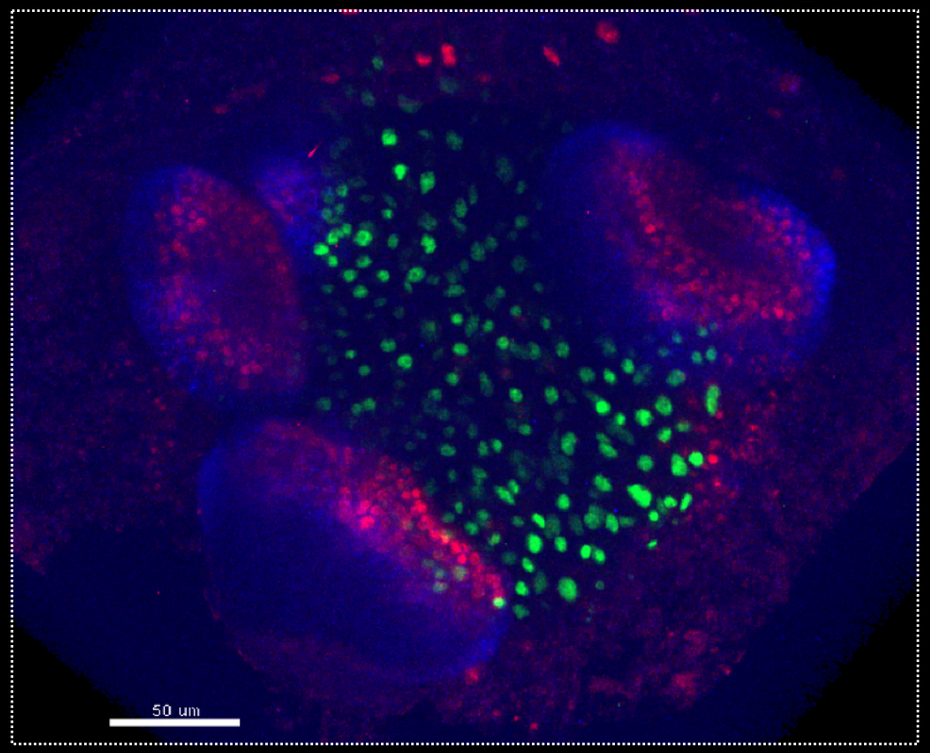


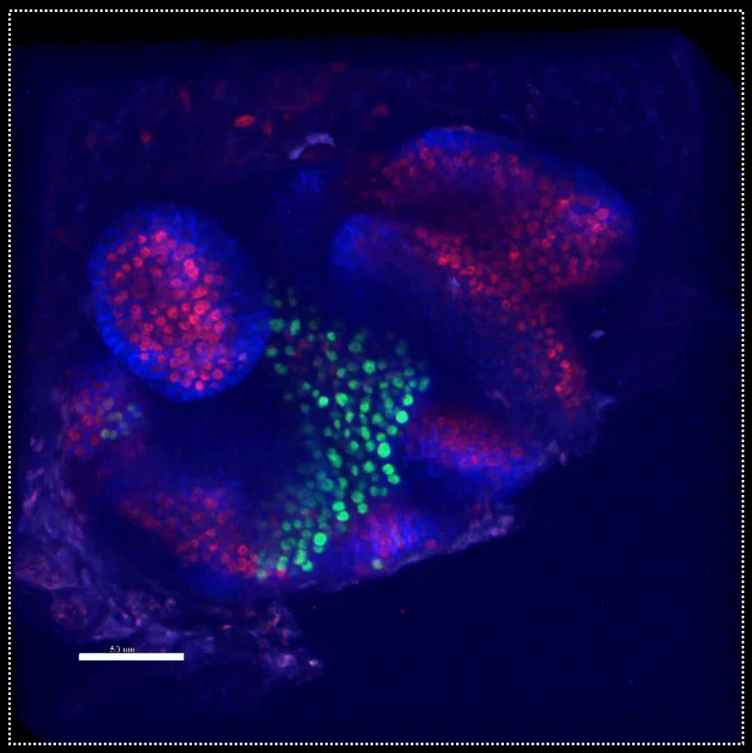
Boundary configuration in SAM determines organ morphogenesis

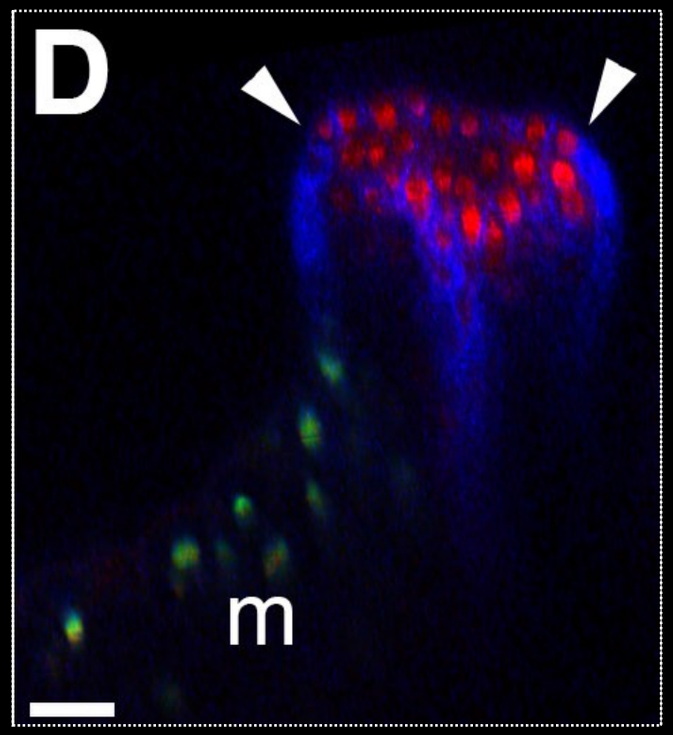


Boundary configuration in SAM determines organ morphogenesis

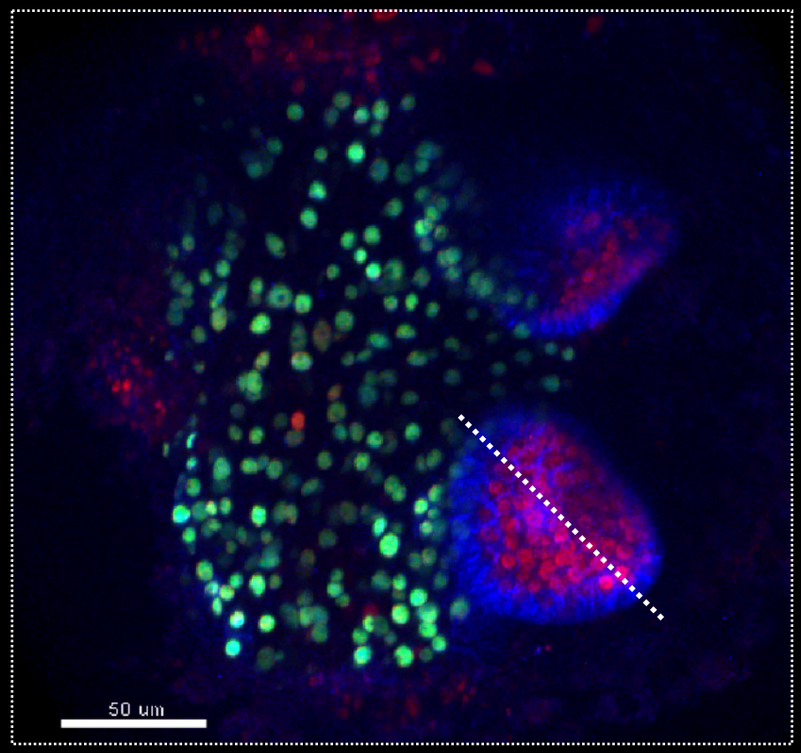


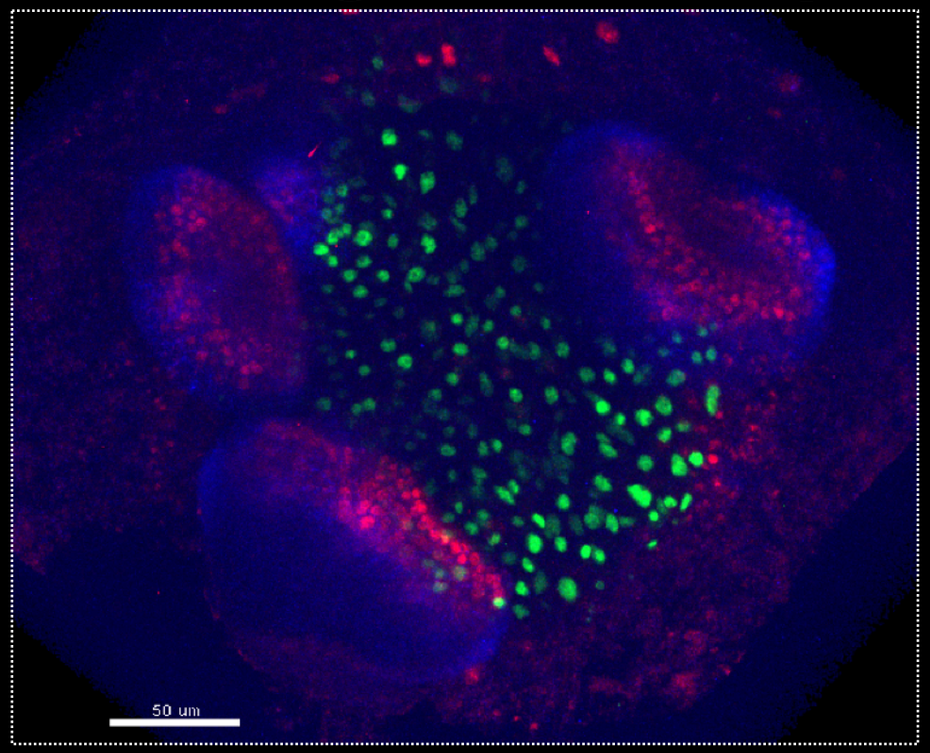


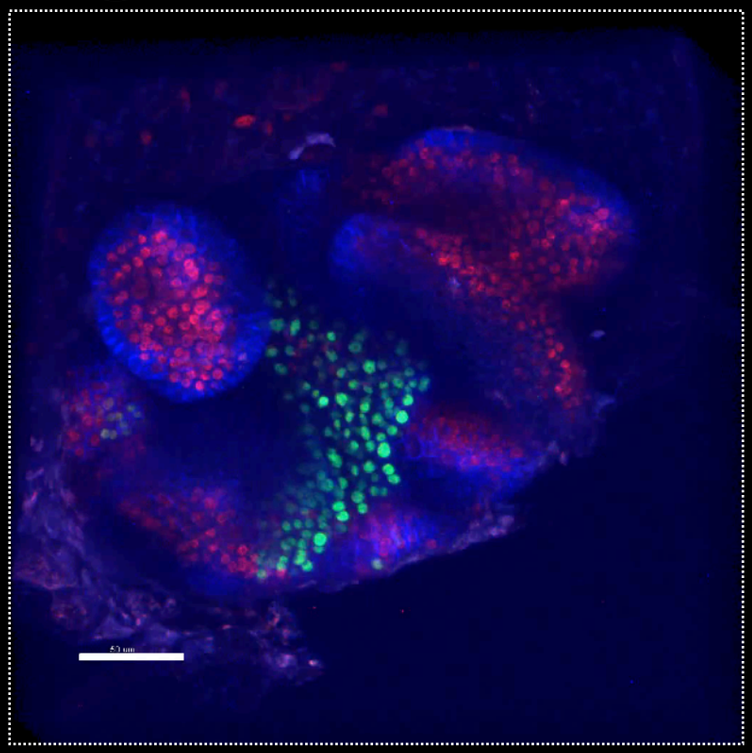


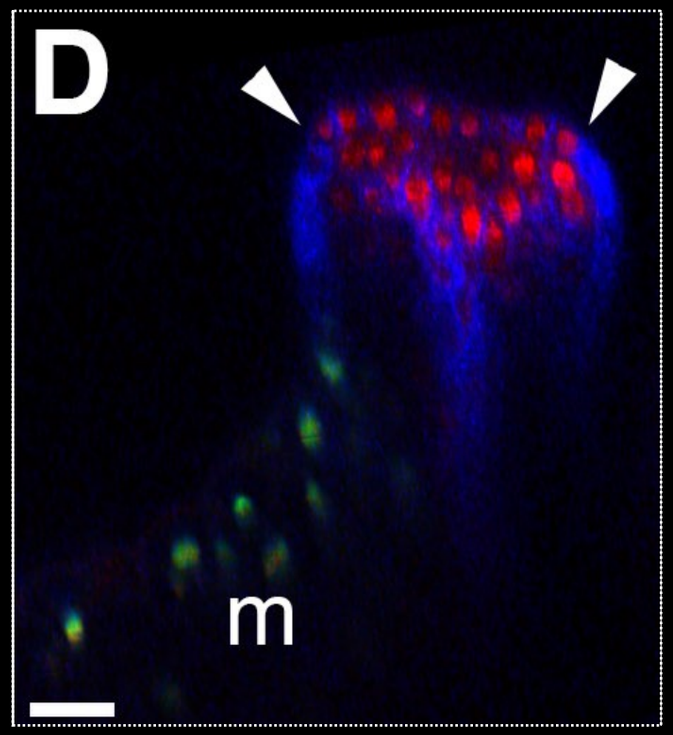


Boundary configuration in SAM determines organ morphogenesis

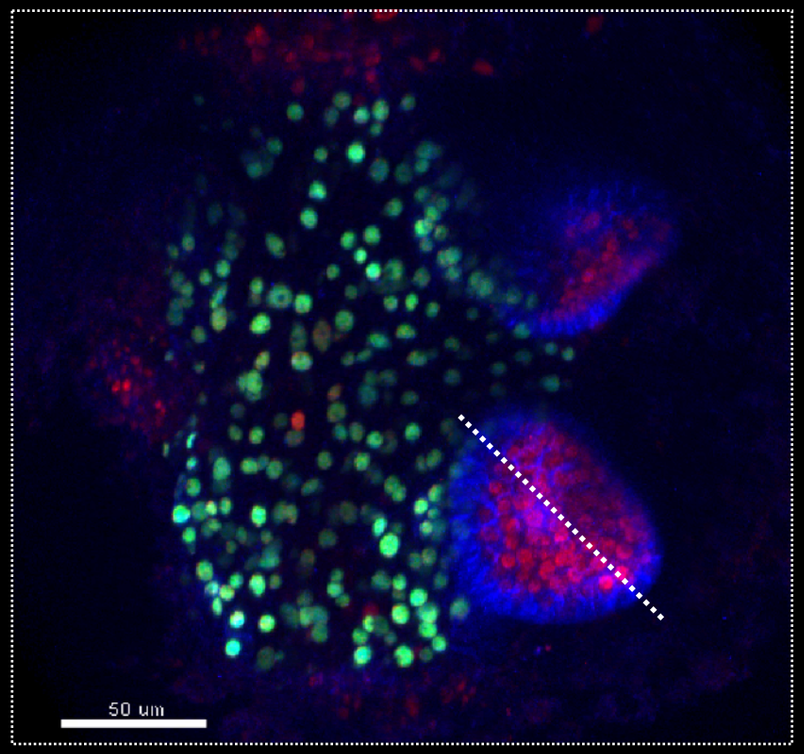


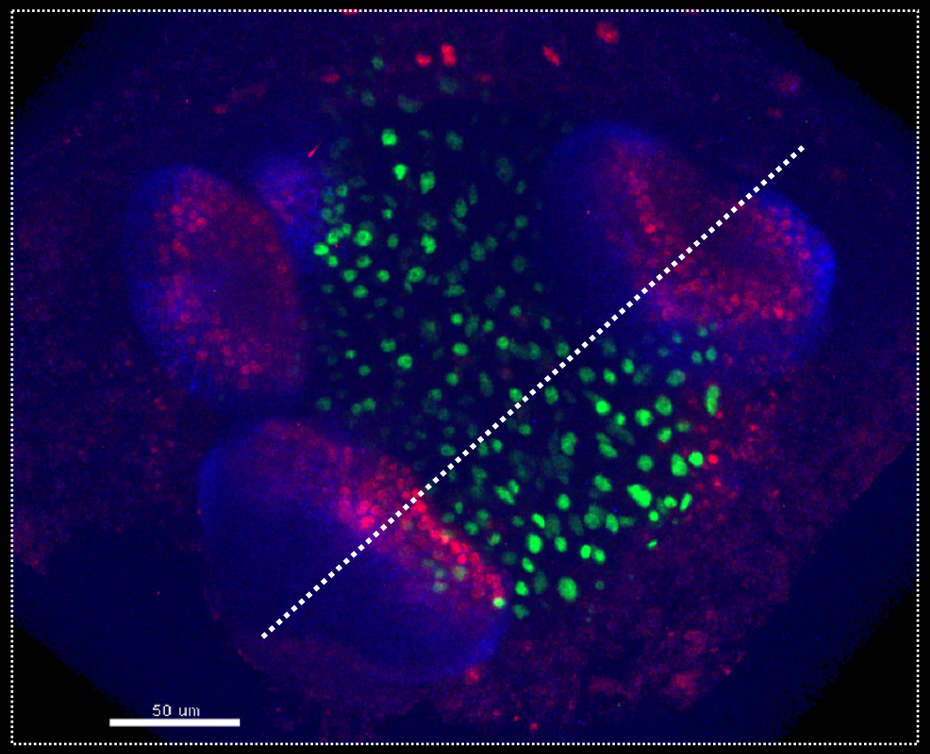


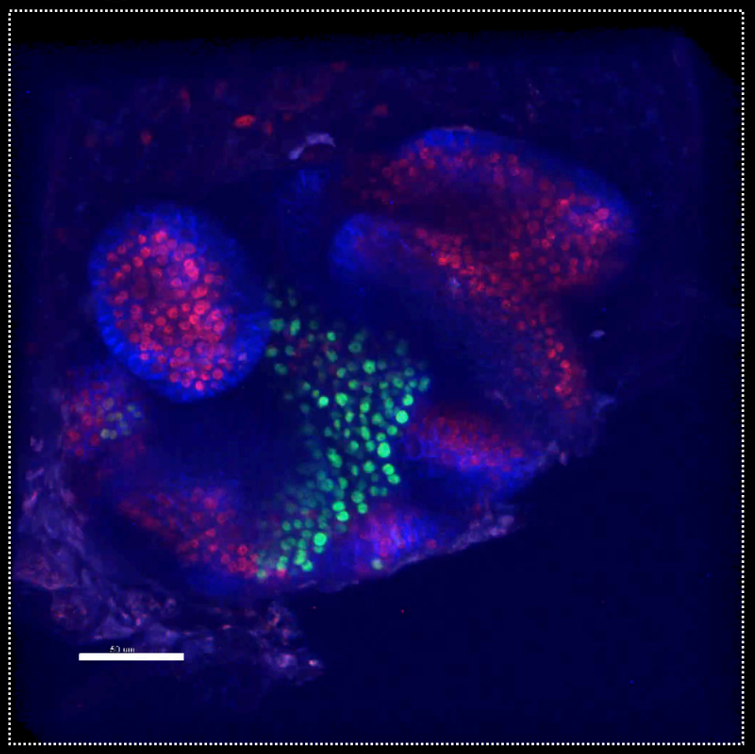


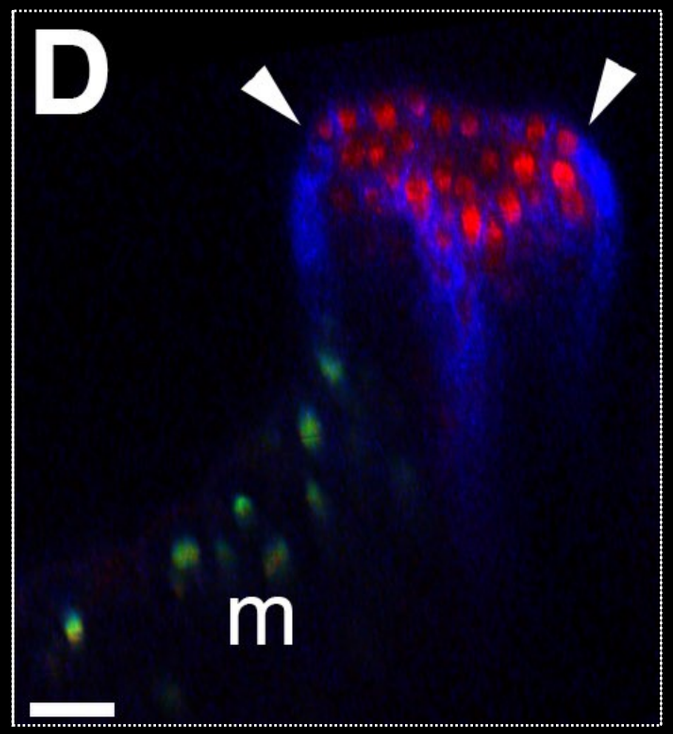


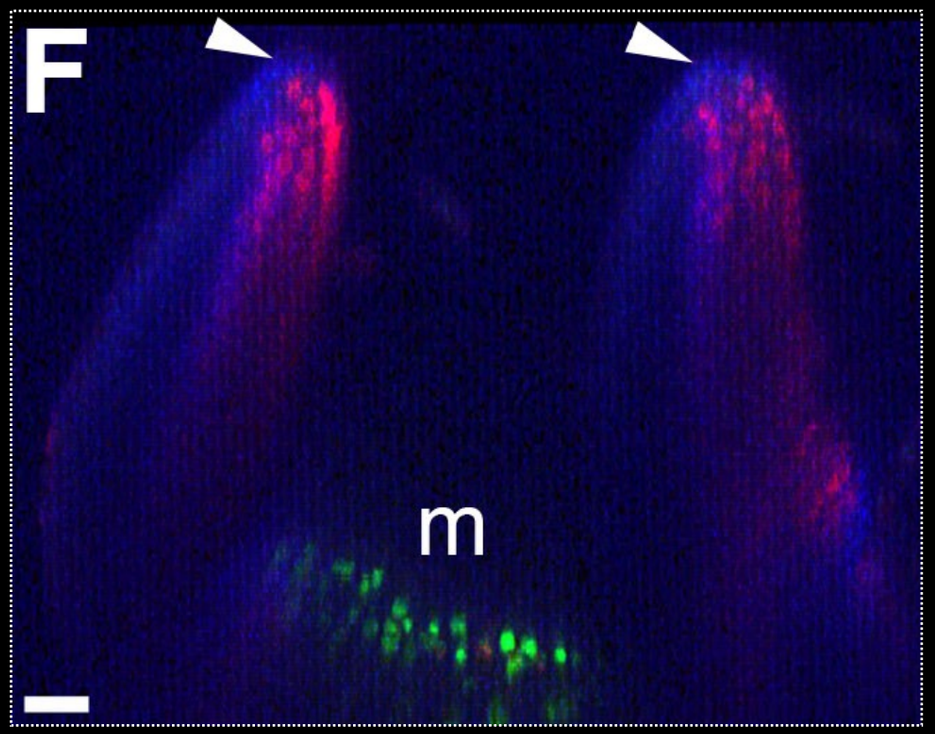
Boundary configuration in SAM determines organ morphogenesis



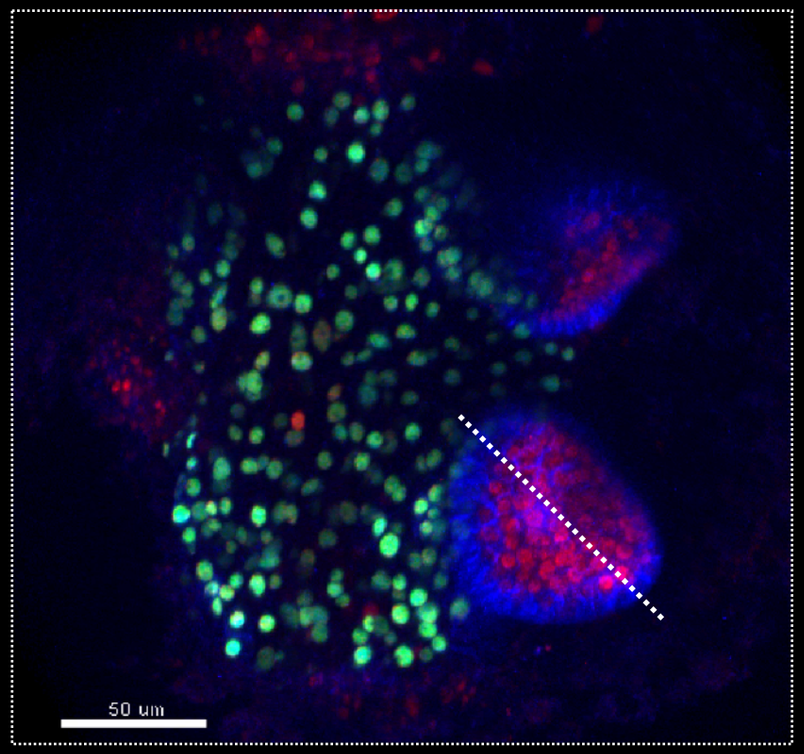


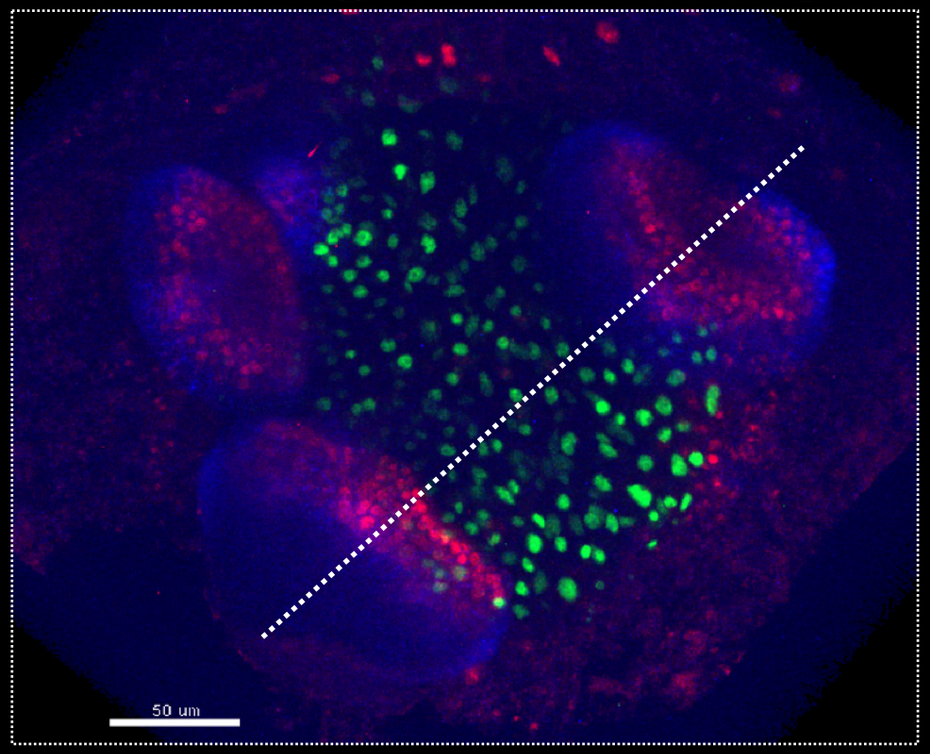


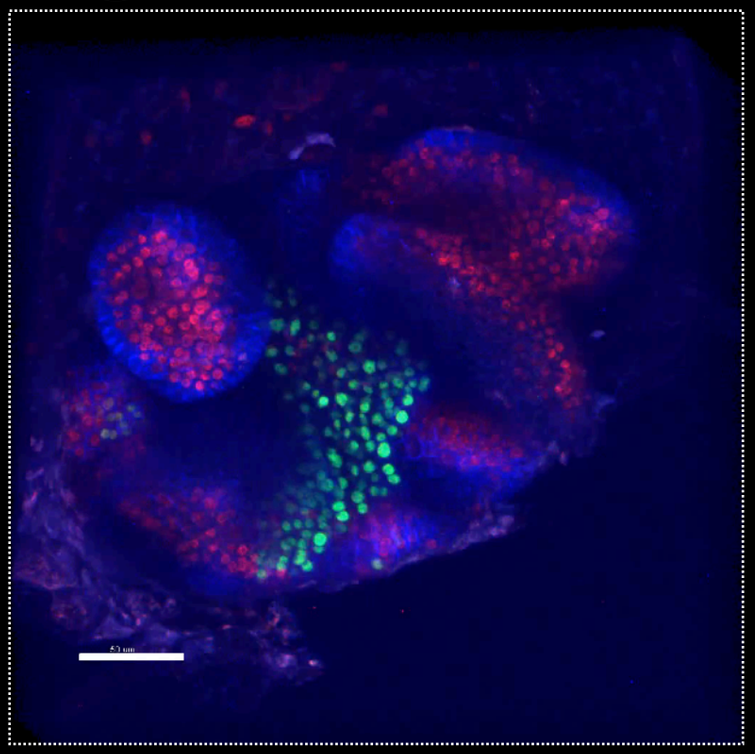


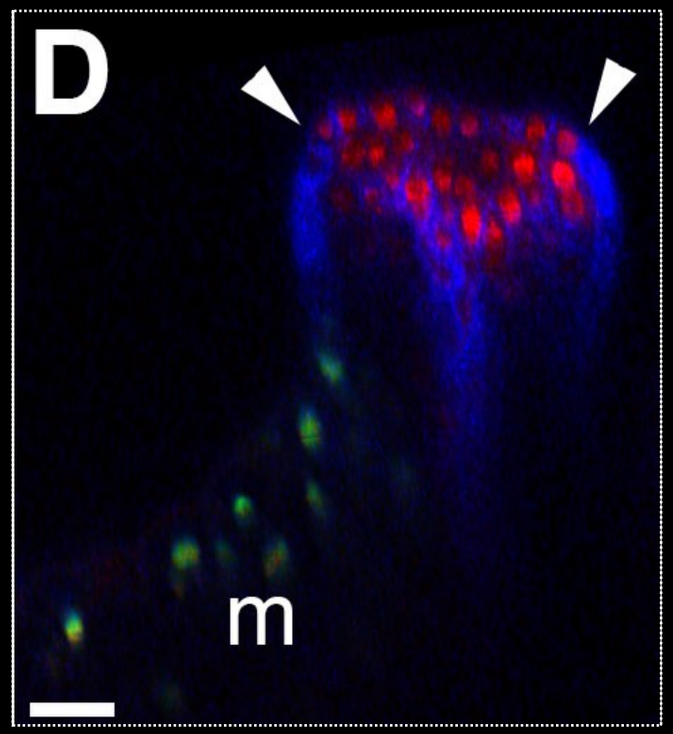


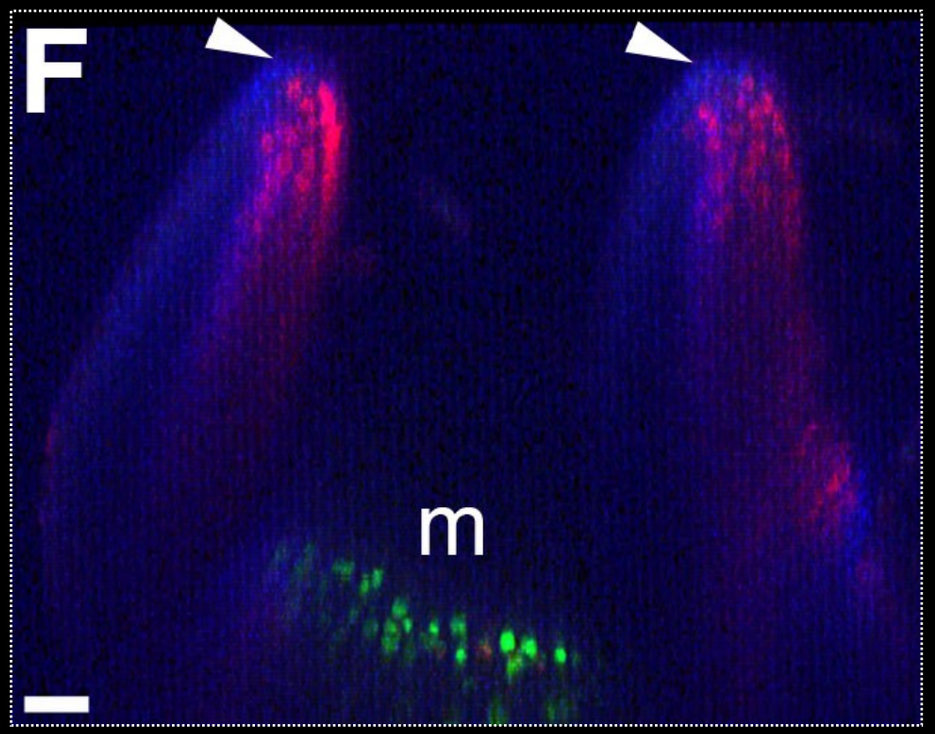
Boundary configuration in SAM determines organ morphogenesis



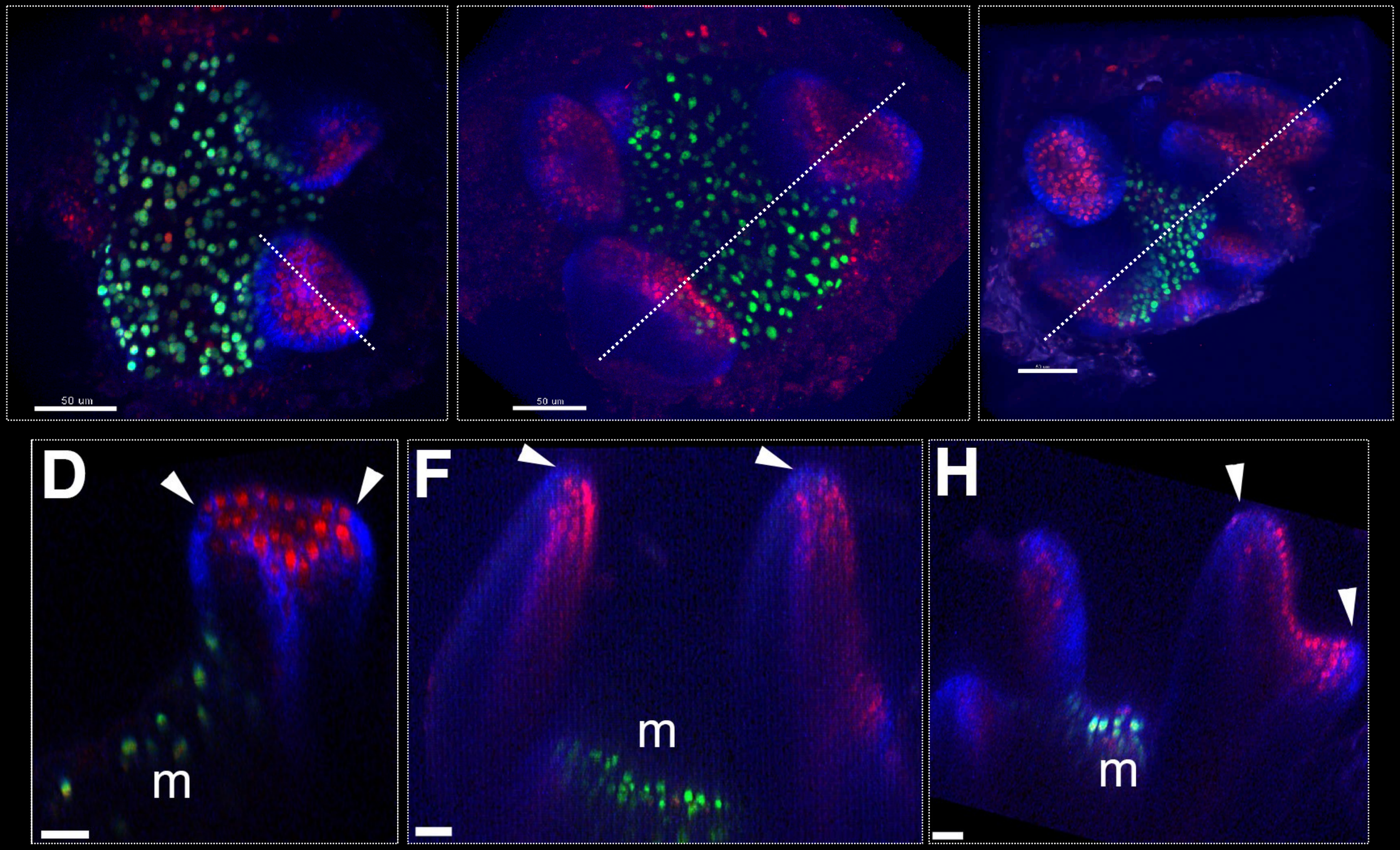








Boundary configuration in SAM determines organ morphogenesis



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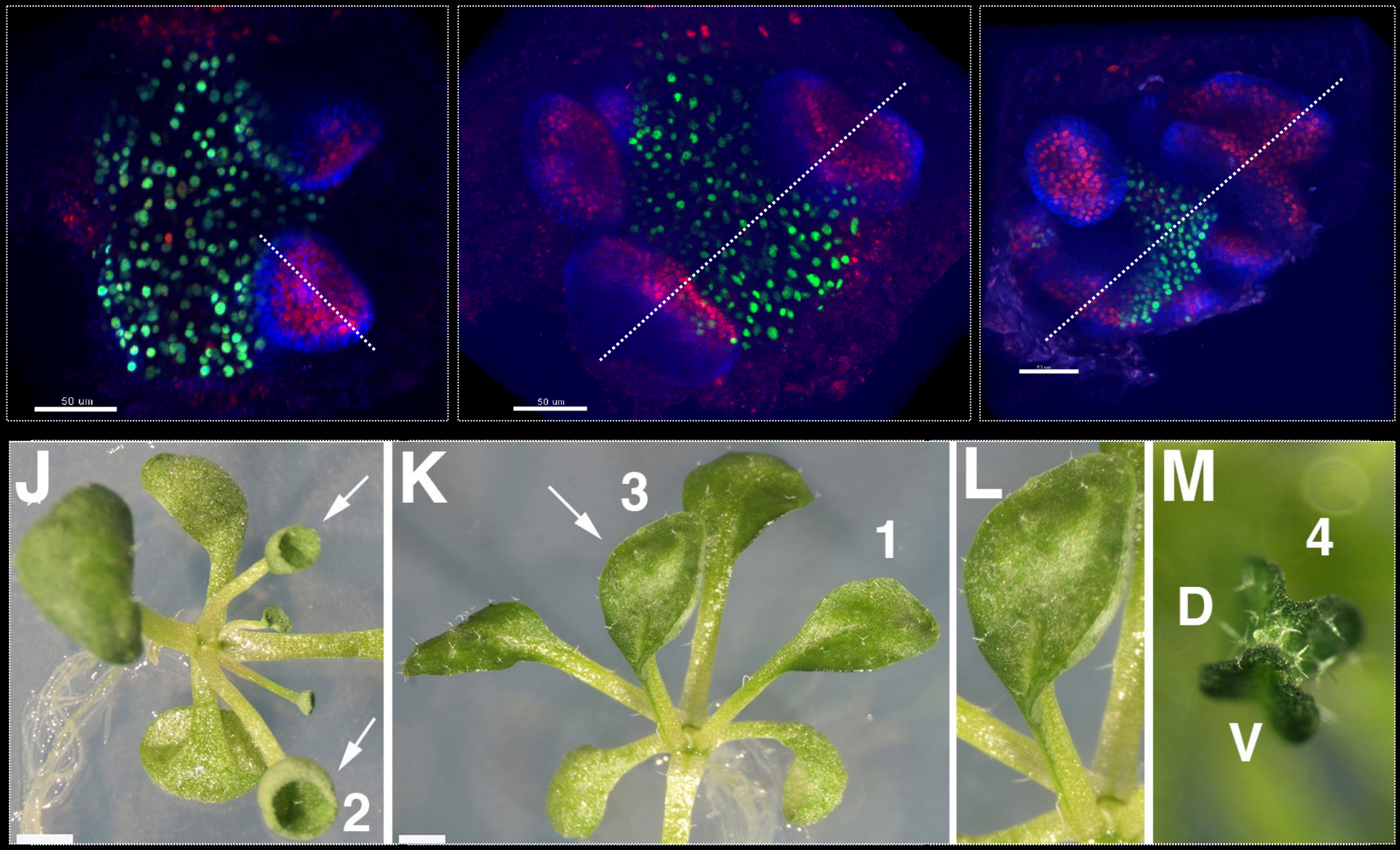
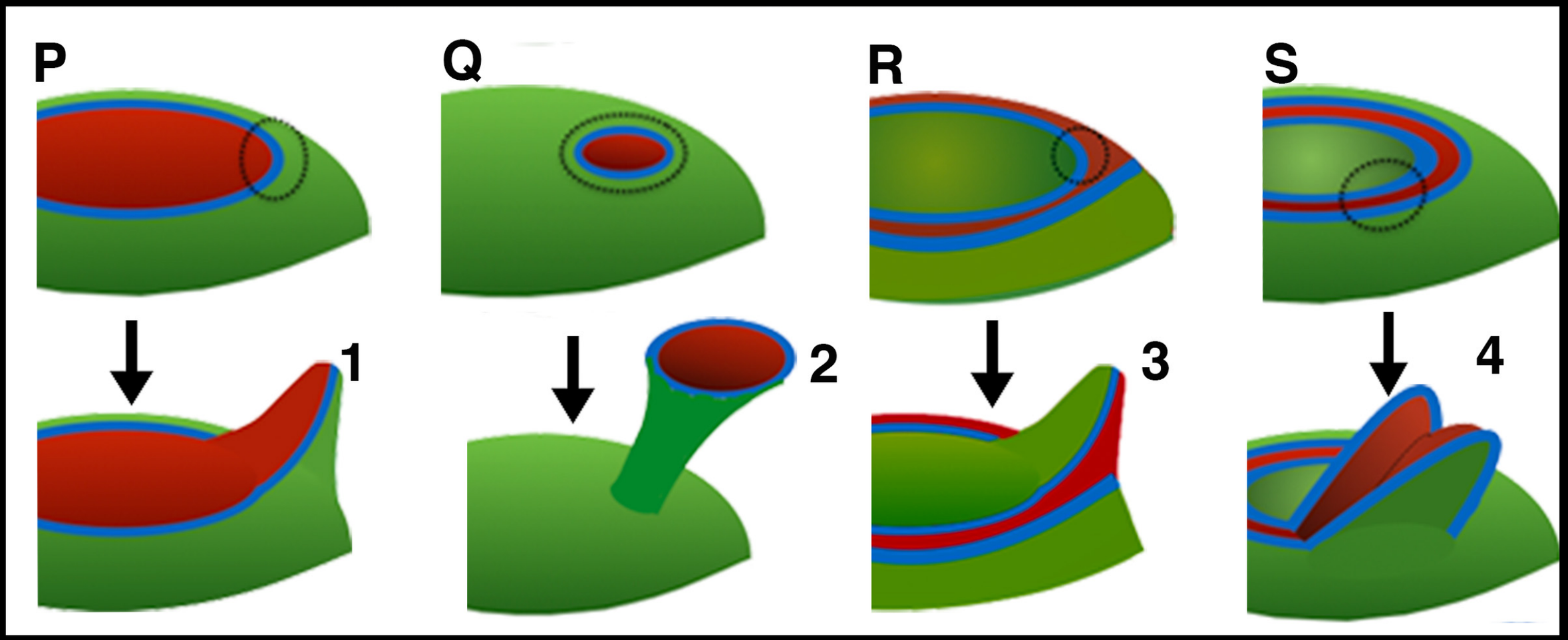


Proposed relationship between boundary configuration in founder cells and leaf shape



Summary

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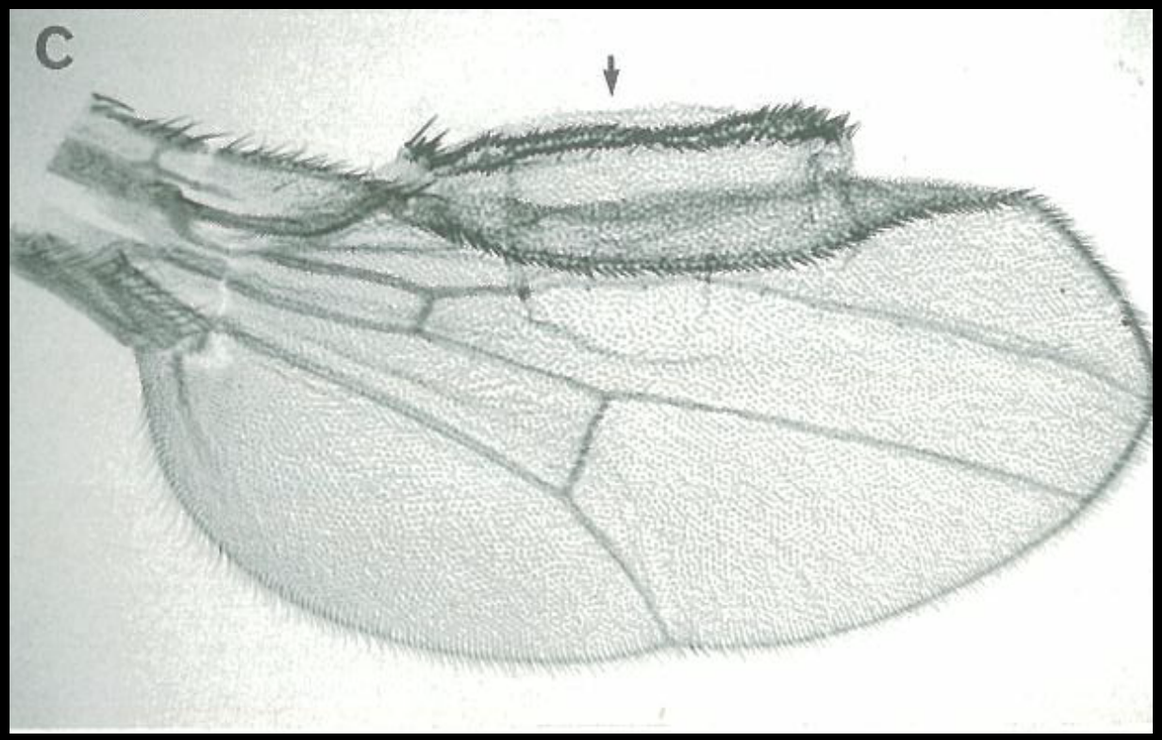
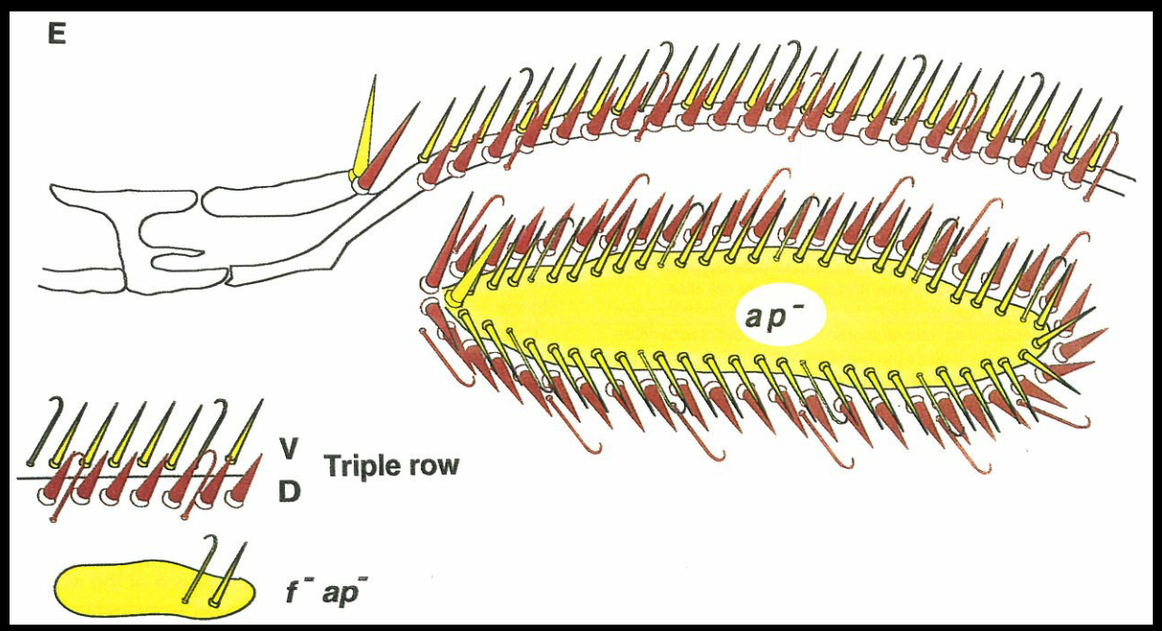
Boundary regulated development in the fly wing



(Diaz-Beniumea and Cohen. 1993)



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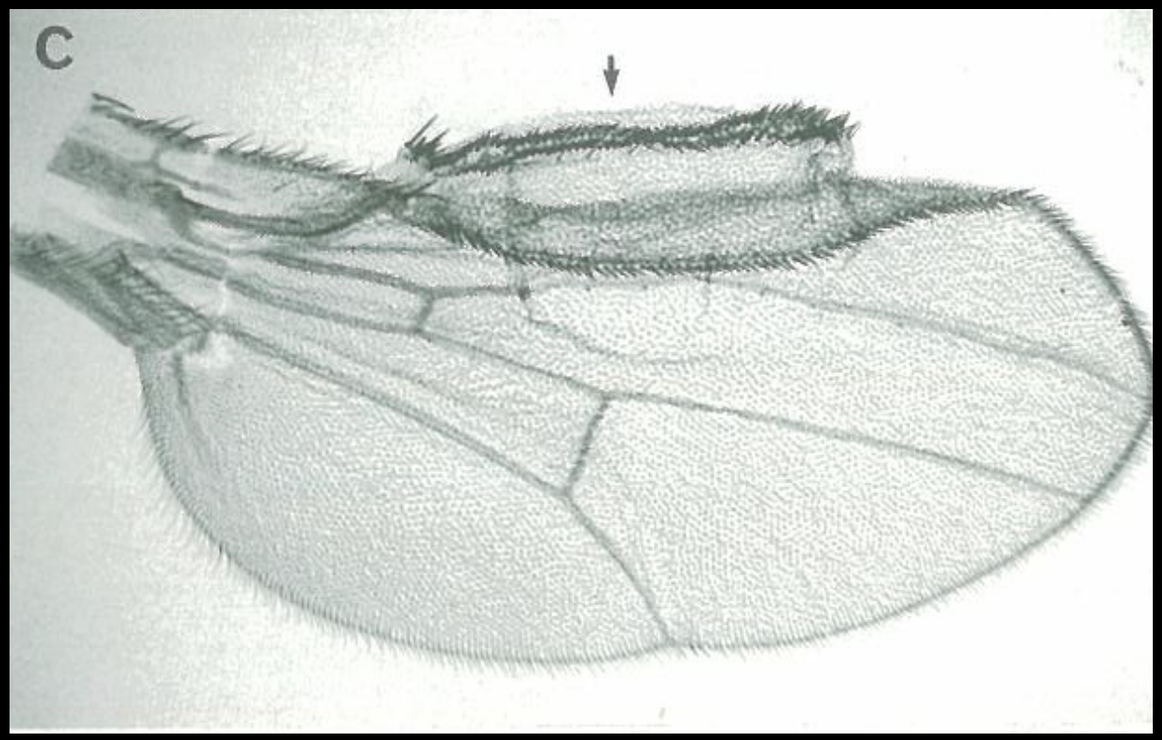

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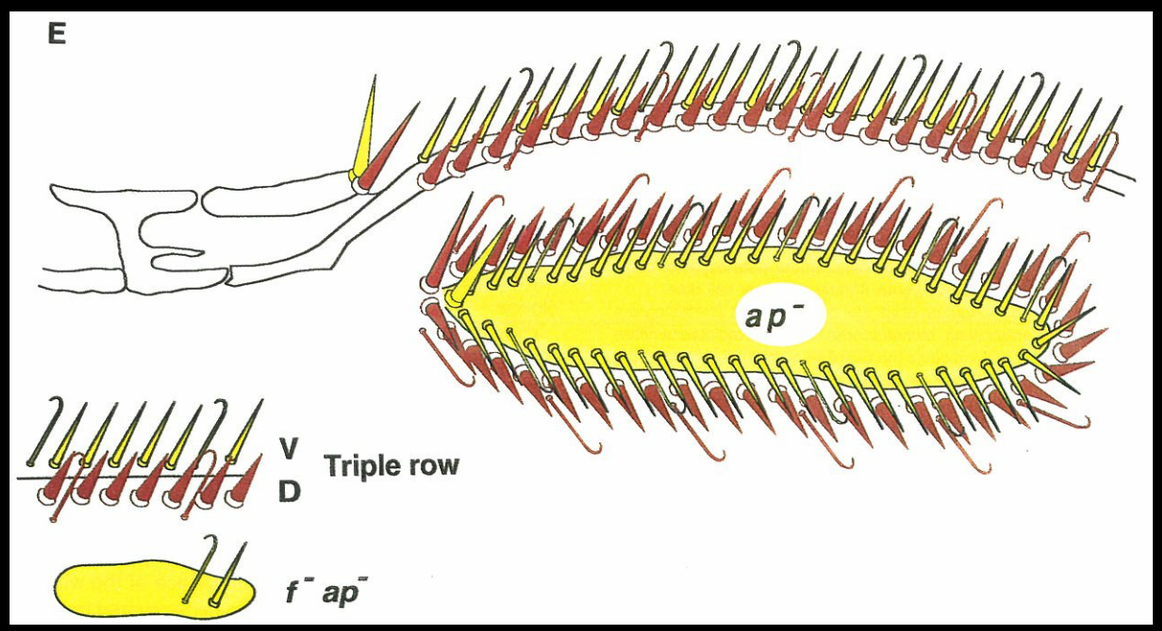

(Tanaka et al., 1997)

Transplantation of dorsal tissue into ventral domain leads to ectopic limb bud (AER)

Boundary organizers in animals and plants - similar mechanisms?

Meinhardt (1983)

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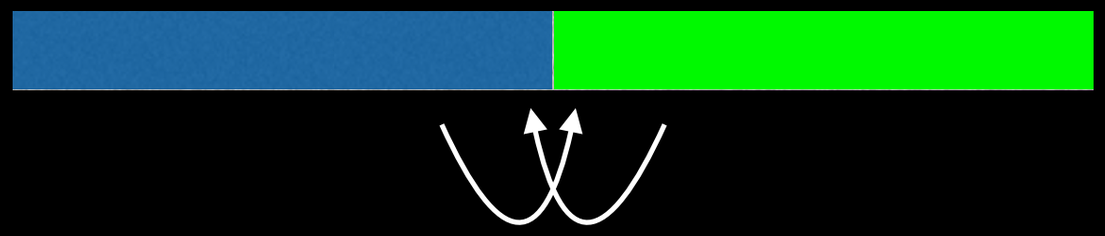

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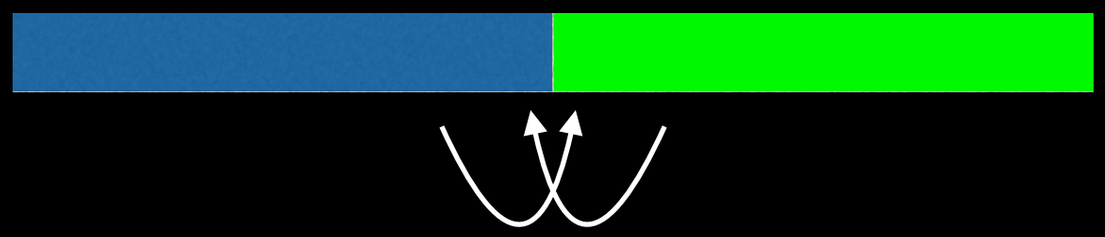

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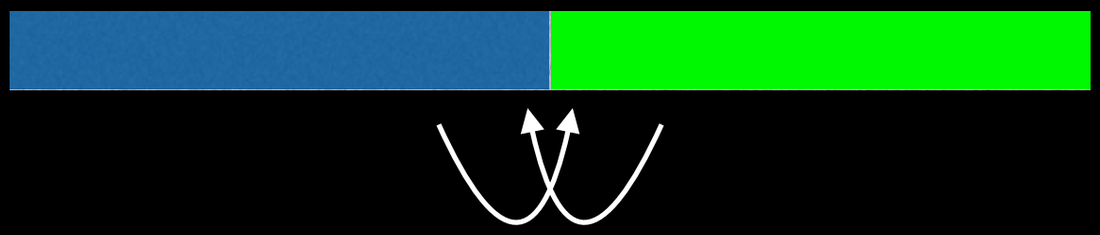
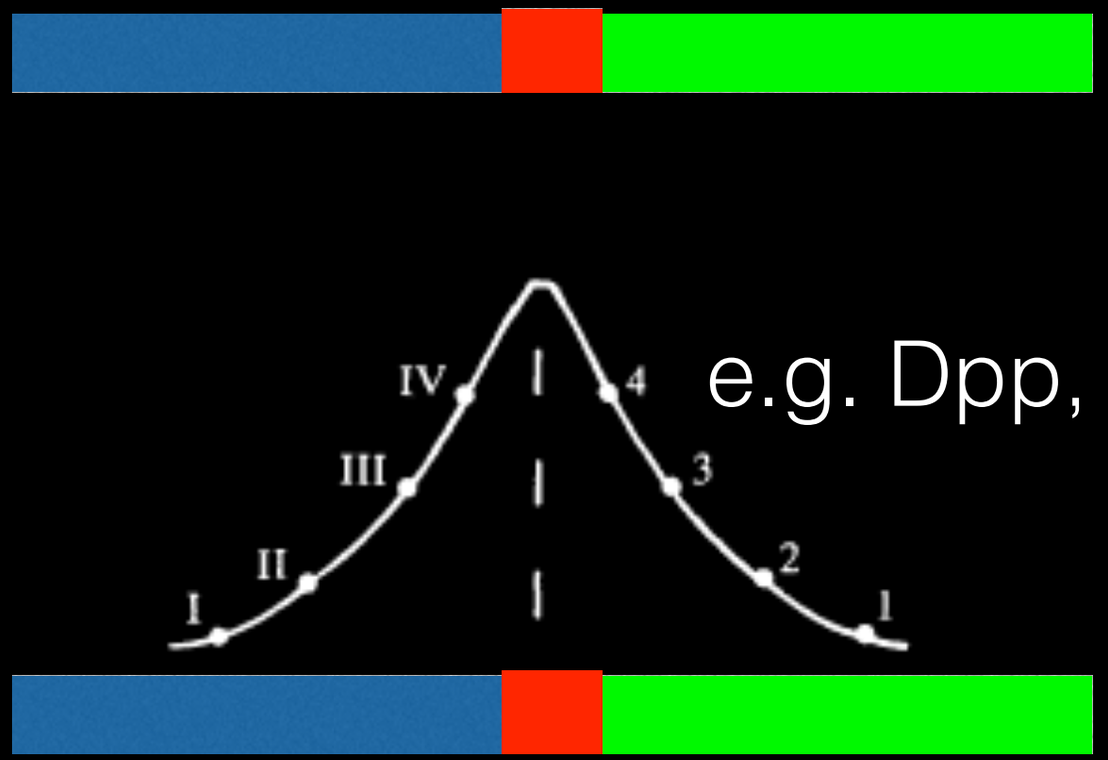
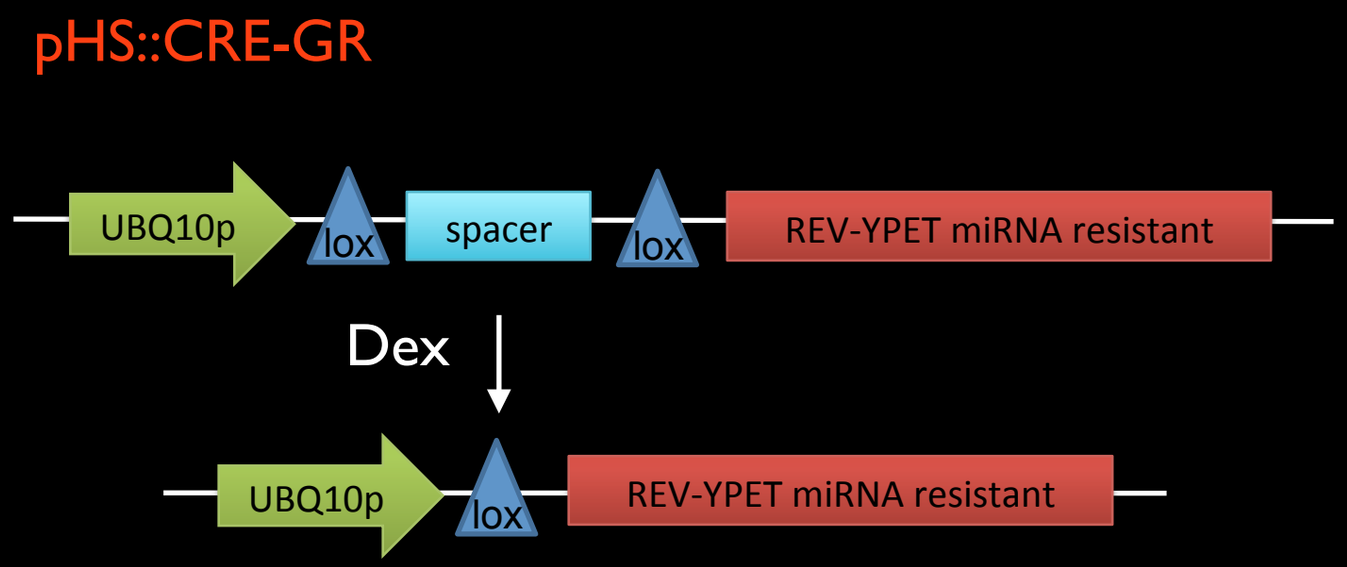


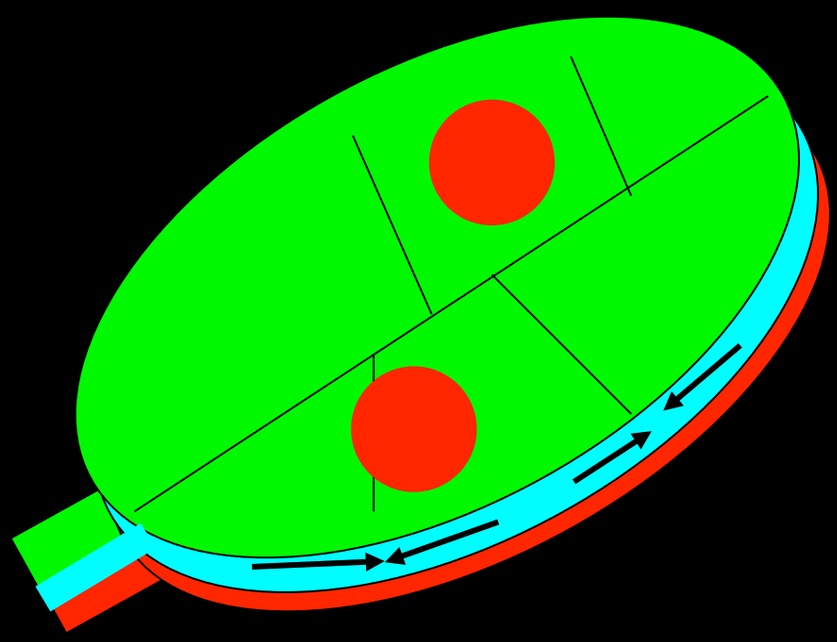


e.g. Dpp, Wg

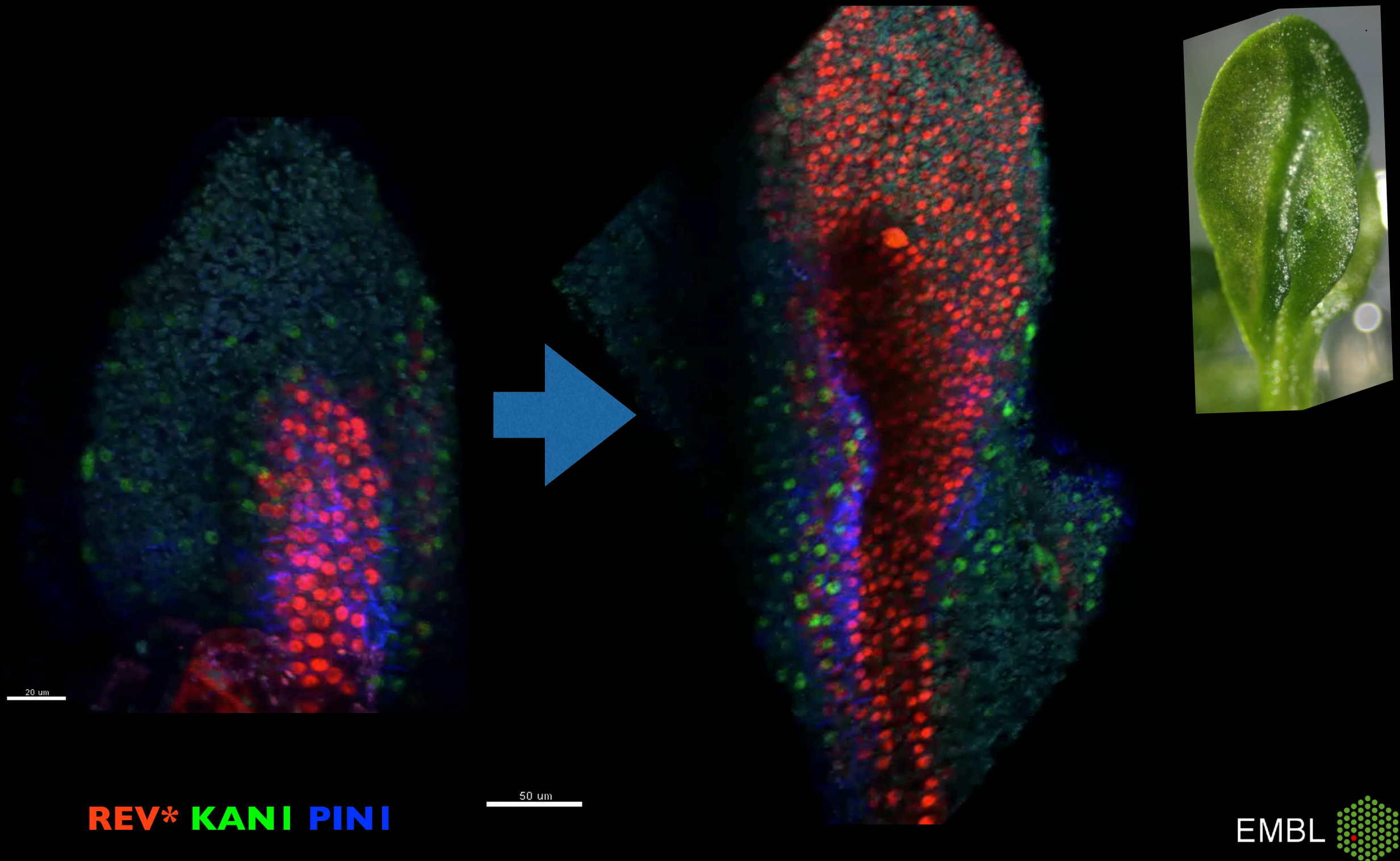
Meinhardt (1983)

Can juxtaposition of dorsal and ventral gene expression induce ectopic leaf tissue?





Juxtaposition of DV gene expression provokes ectopic leaf lamina



EMBL

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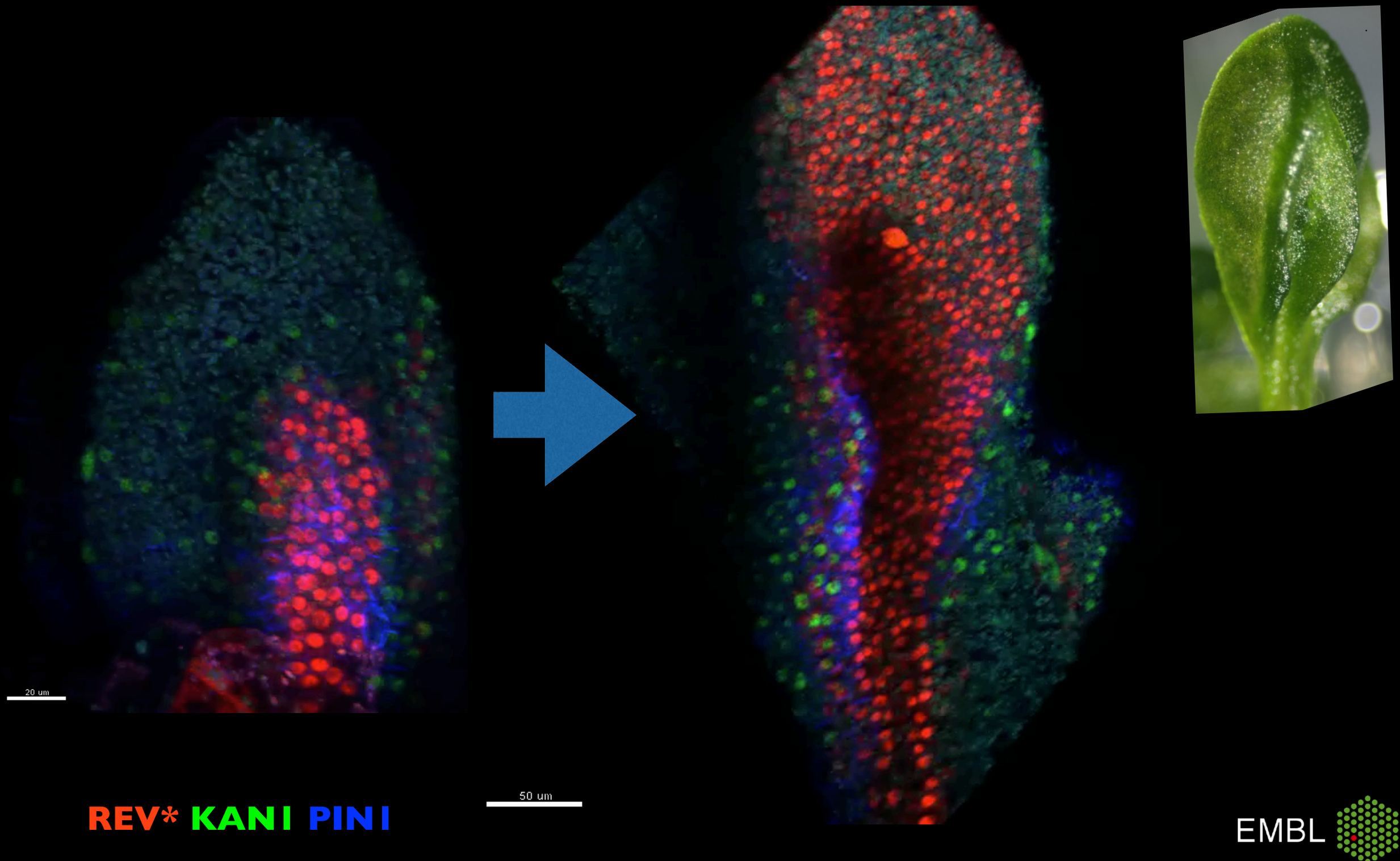
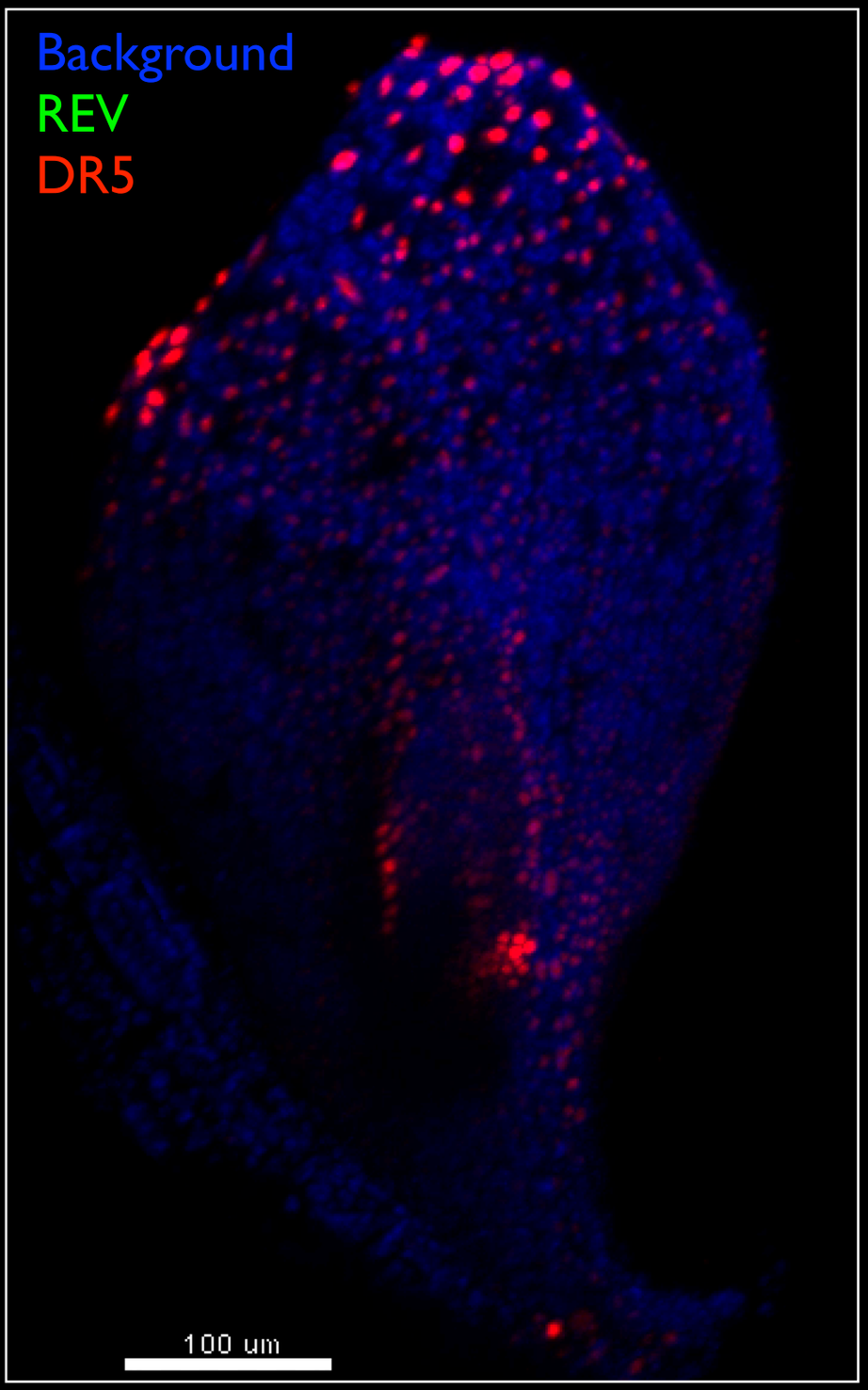
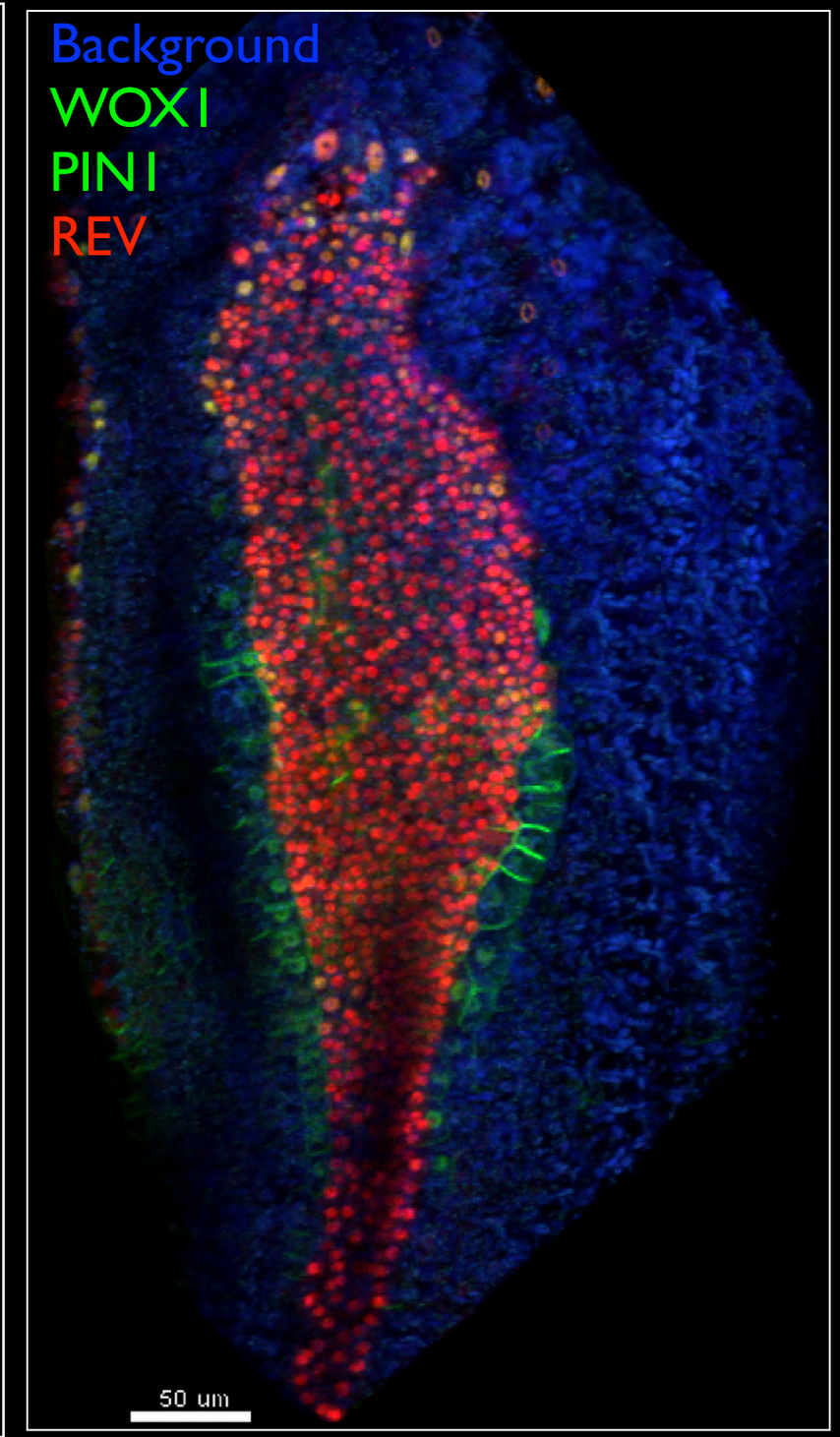
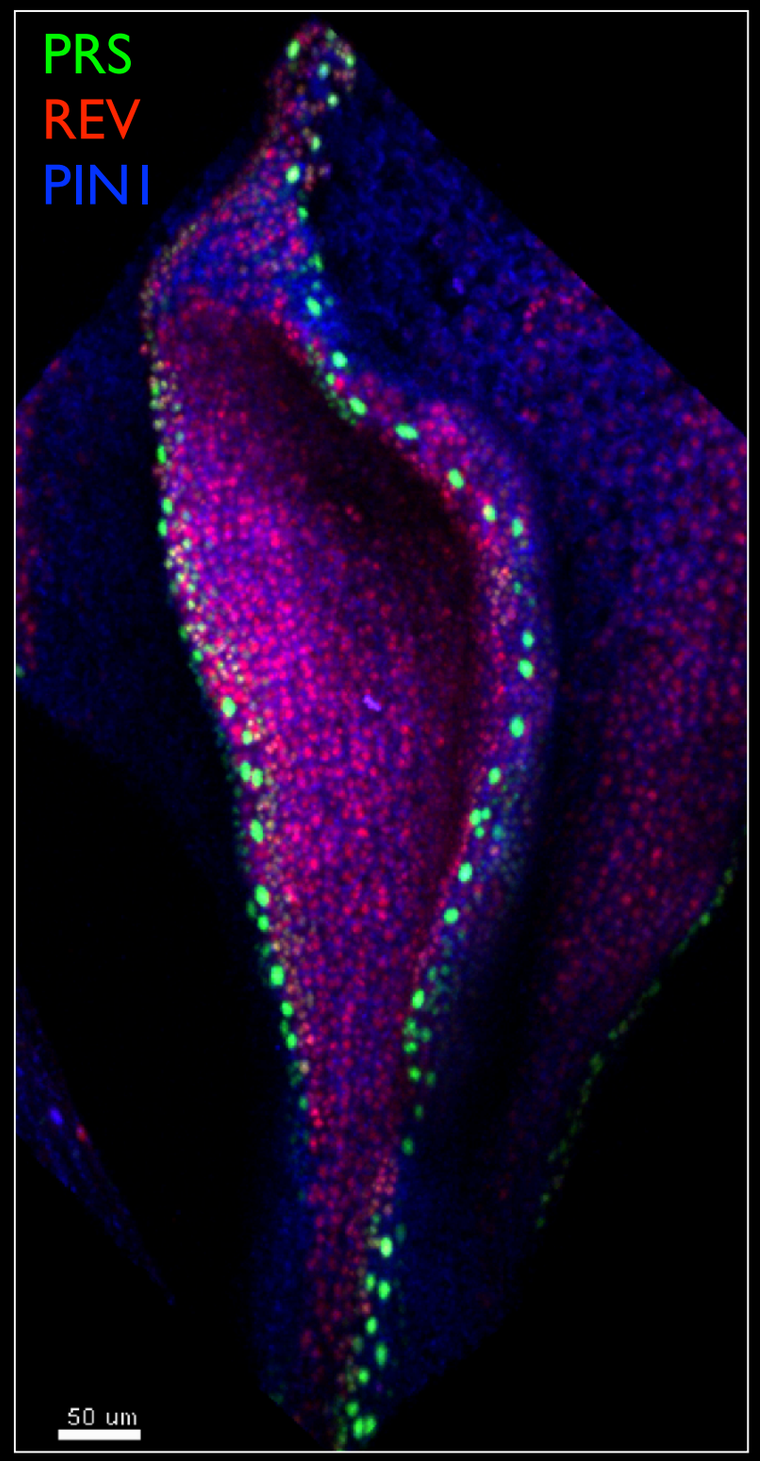


EMBL

Short range signals from REV cells can promote boundary associated gene expression







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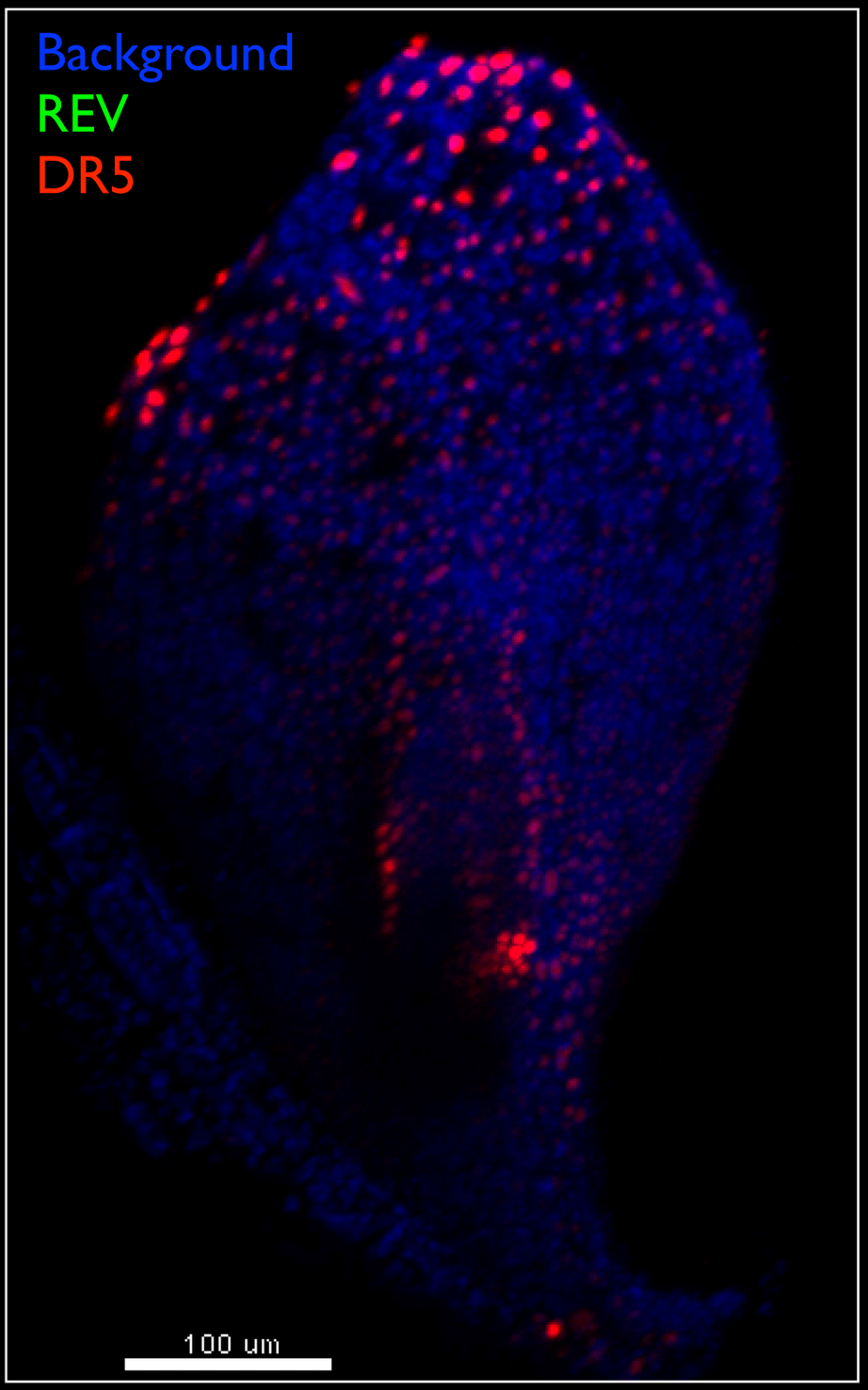

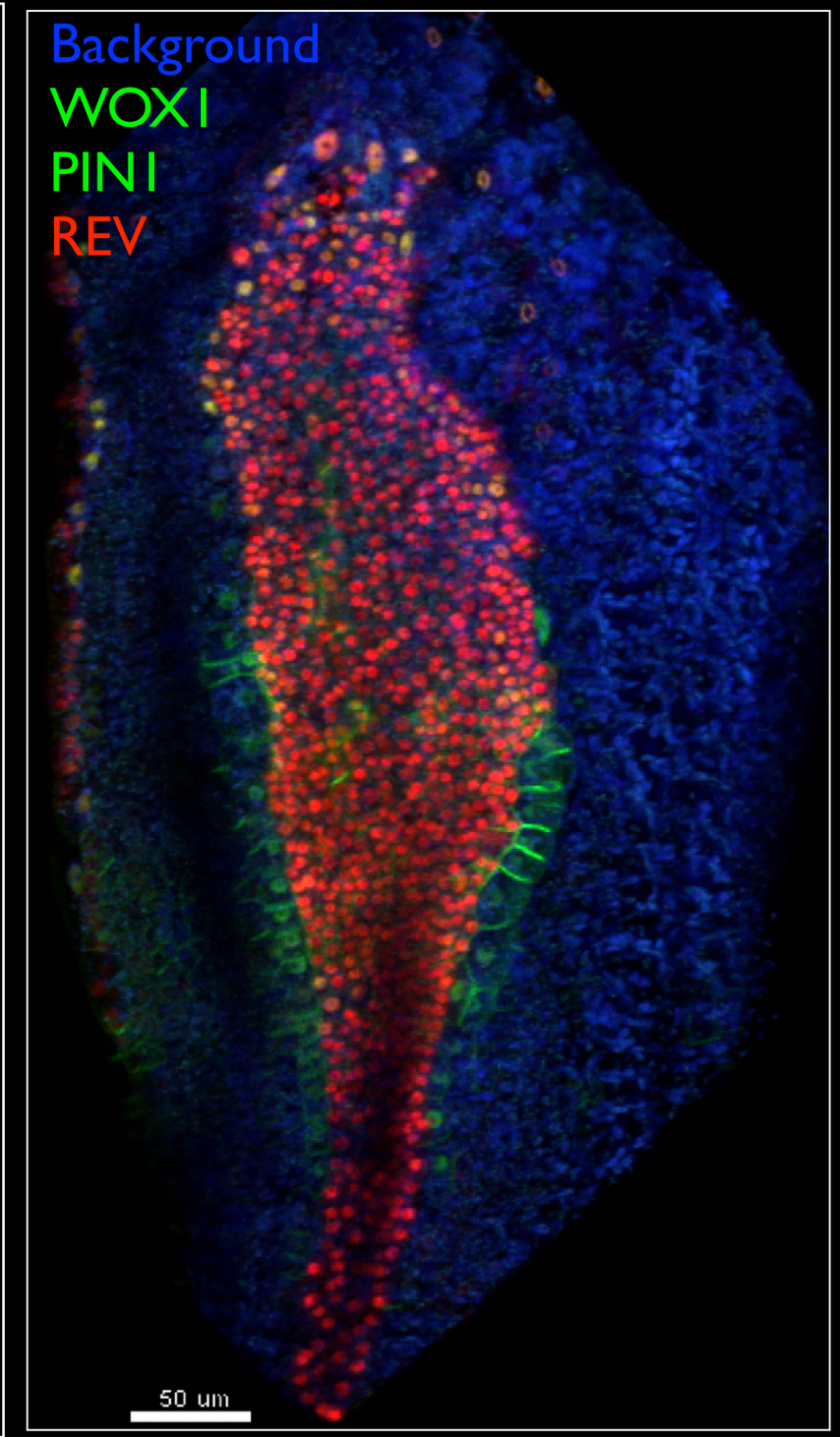

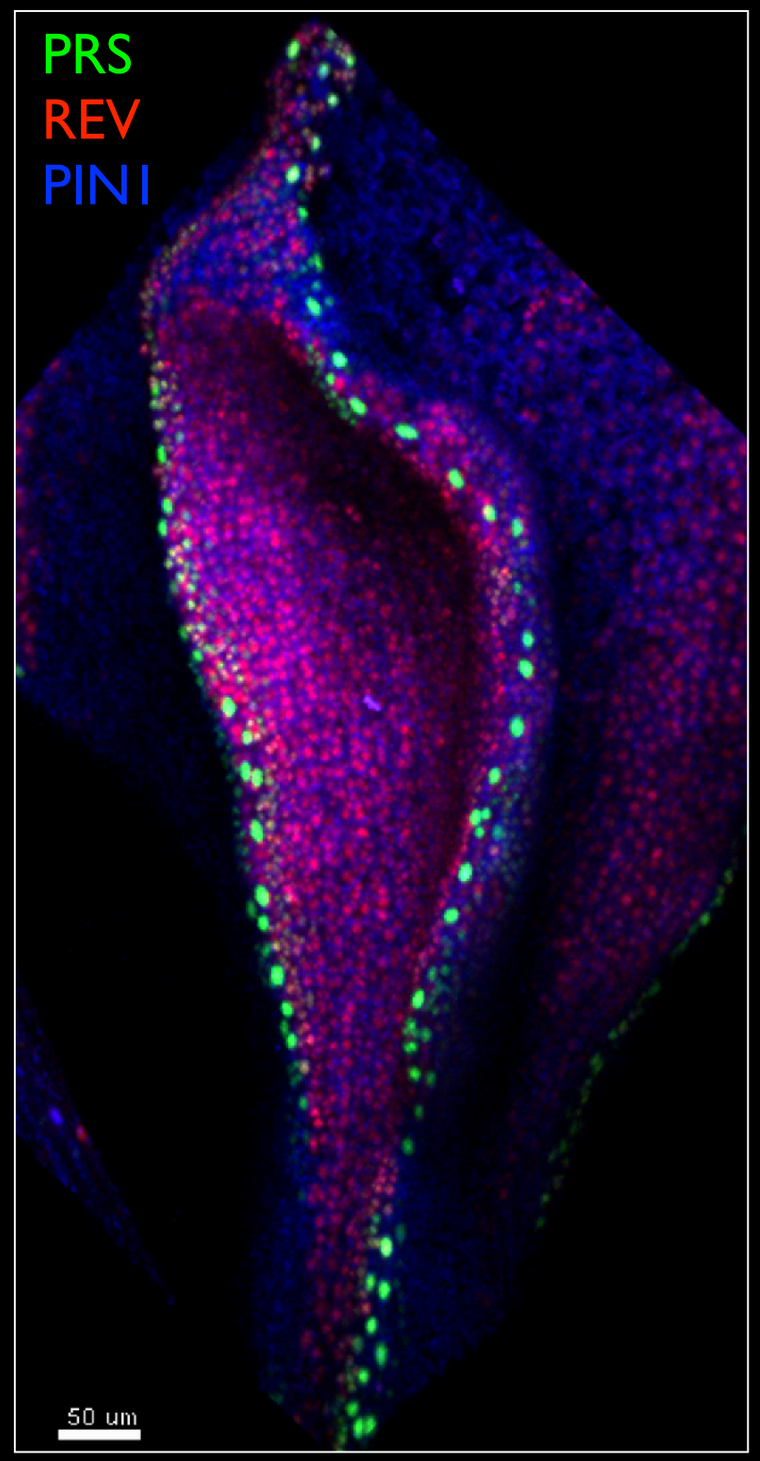

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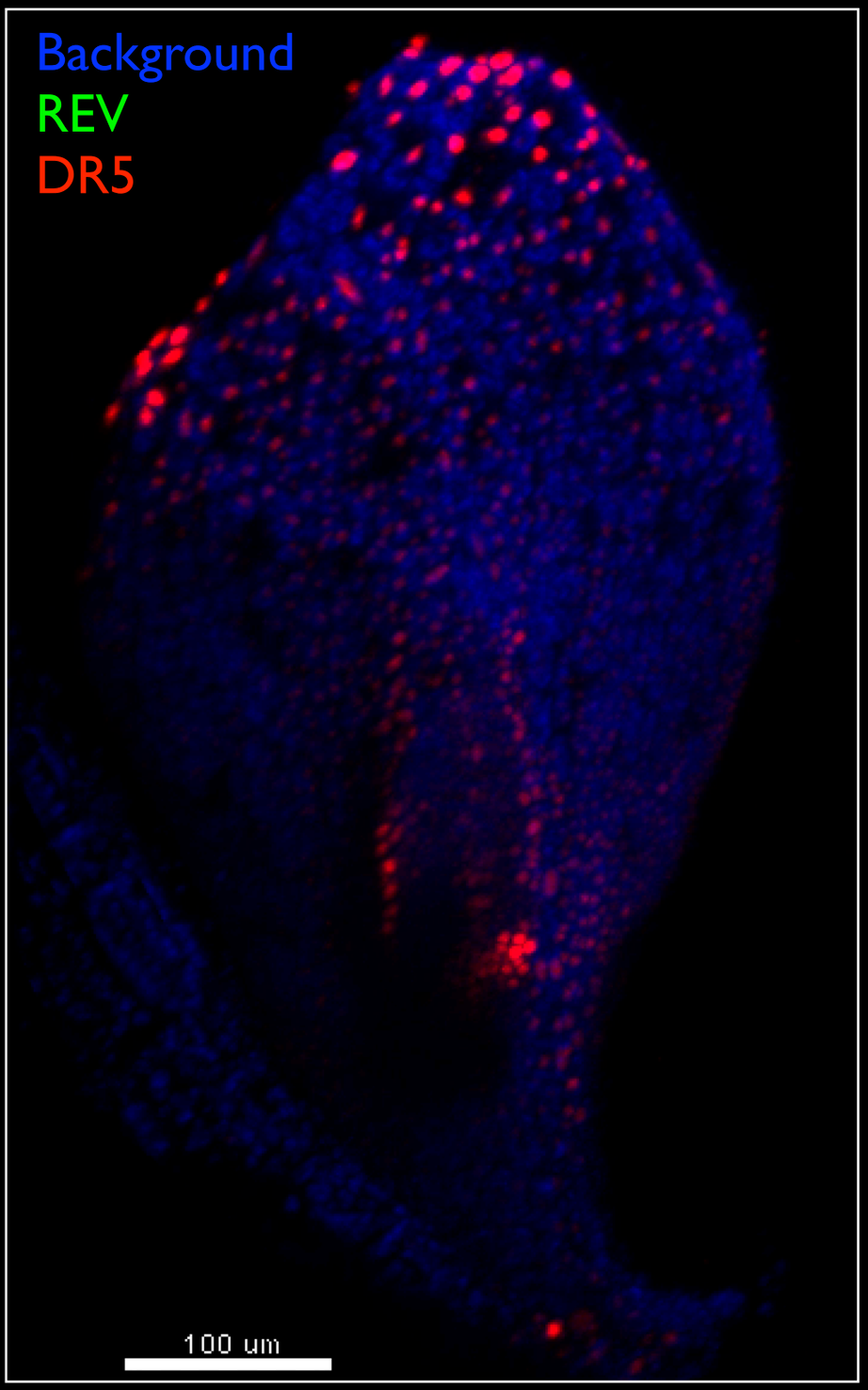

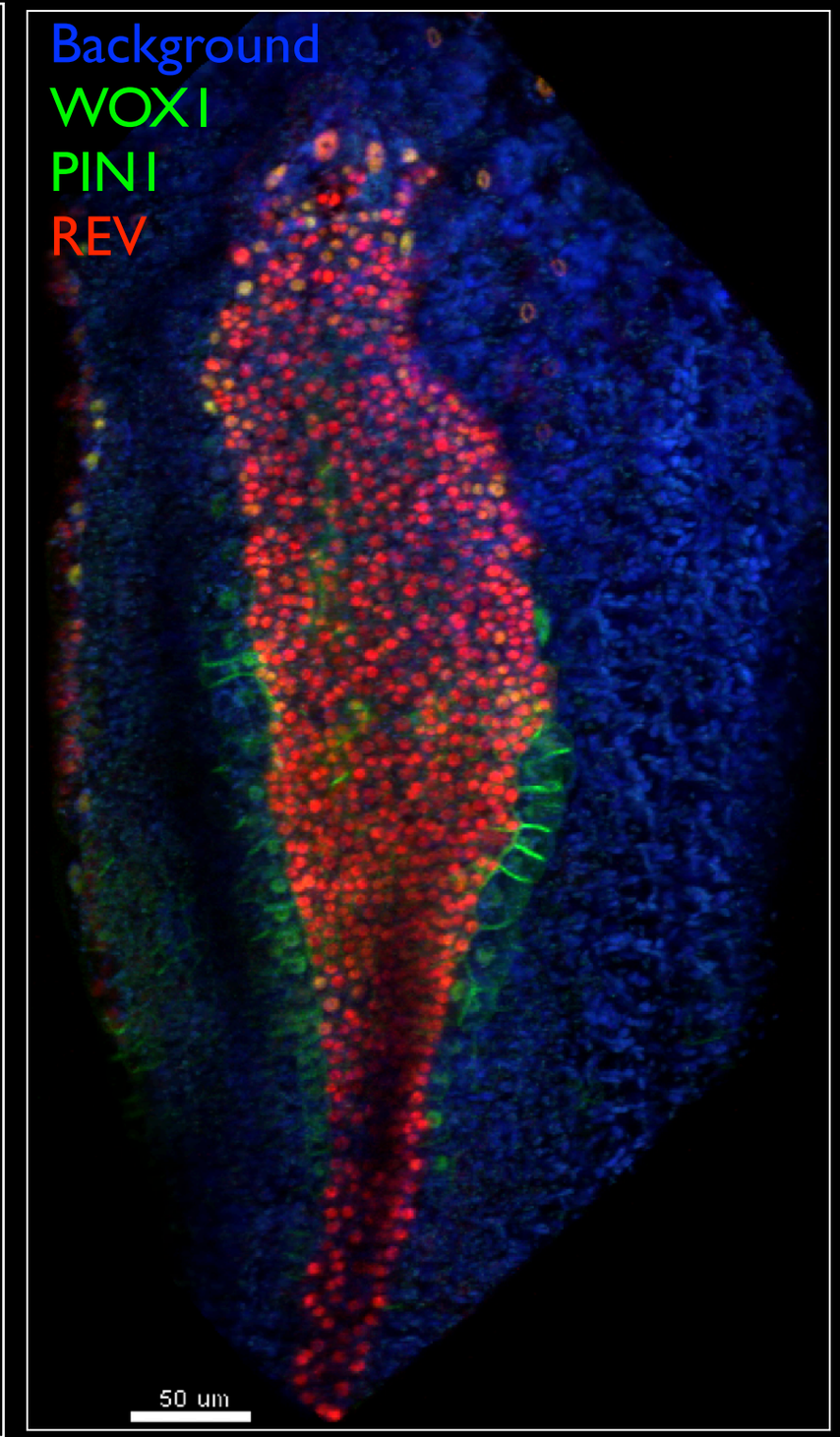

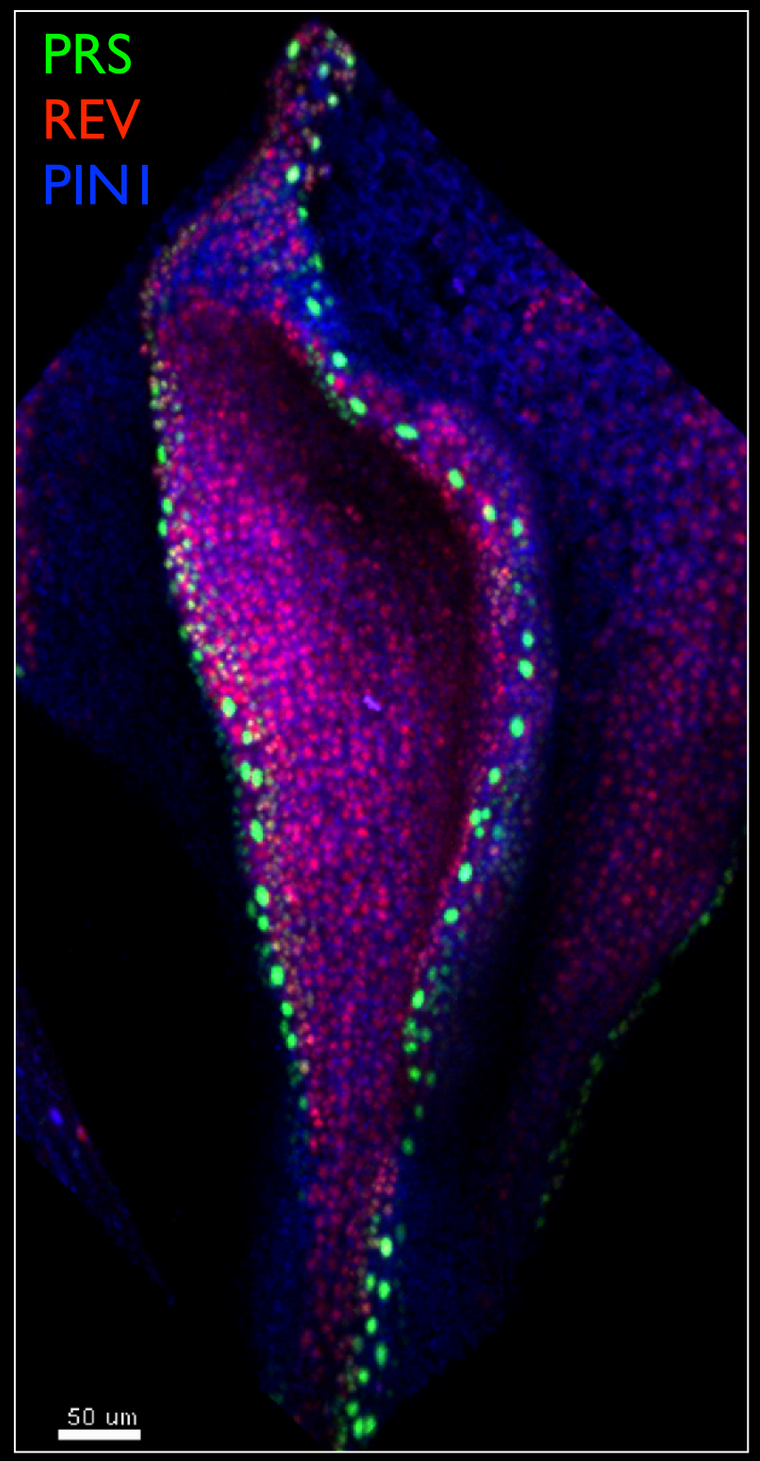

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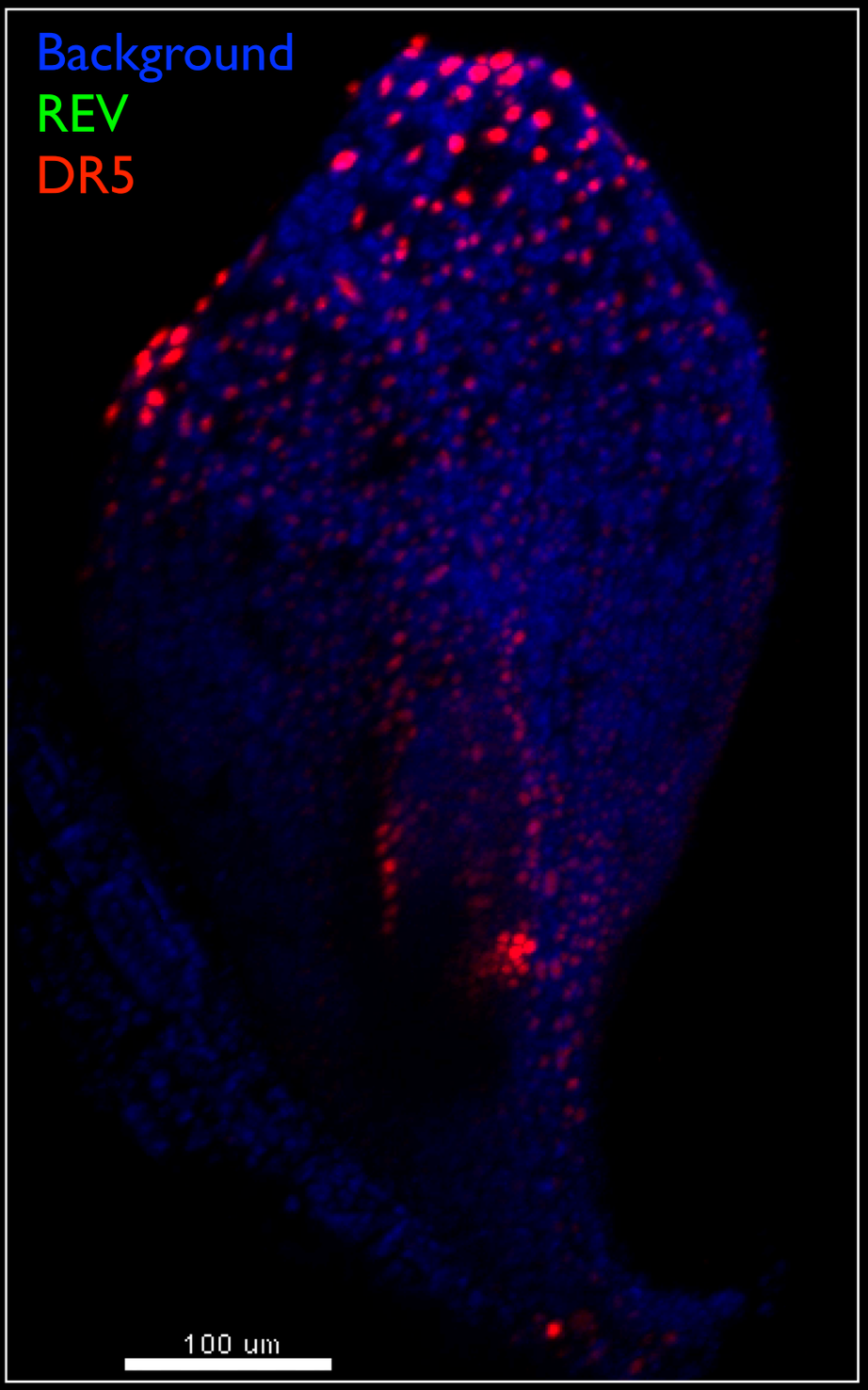
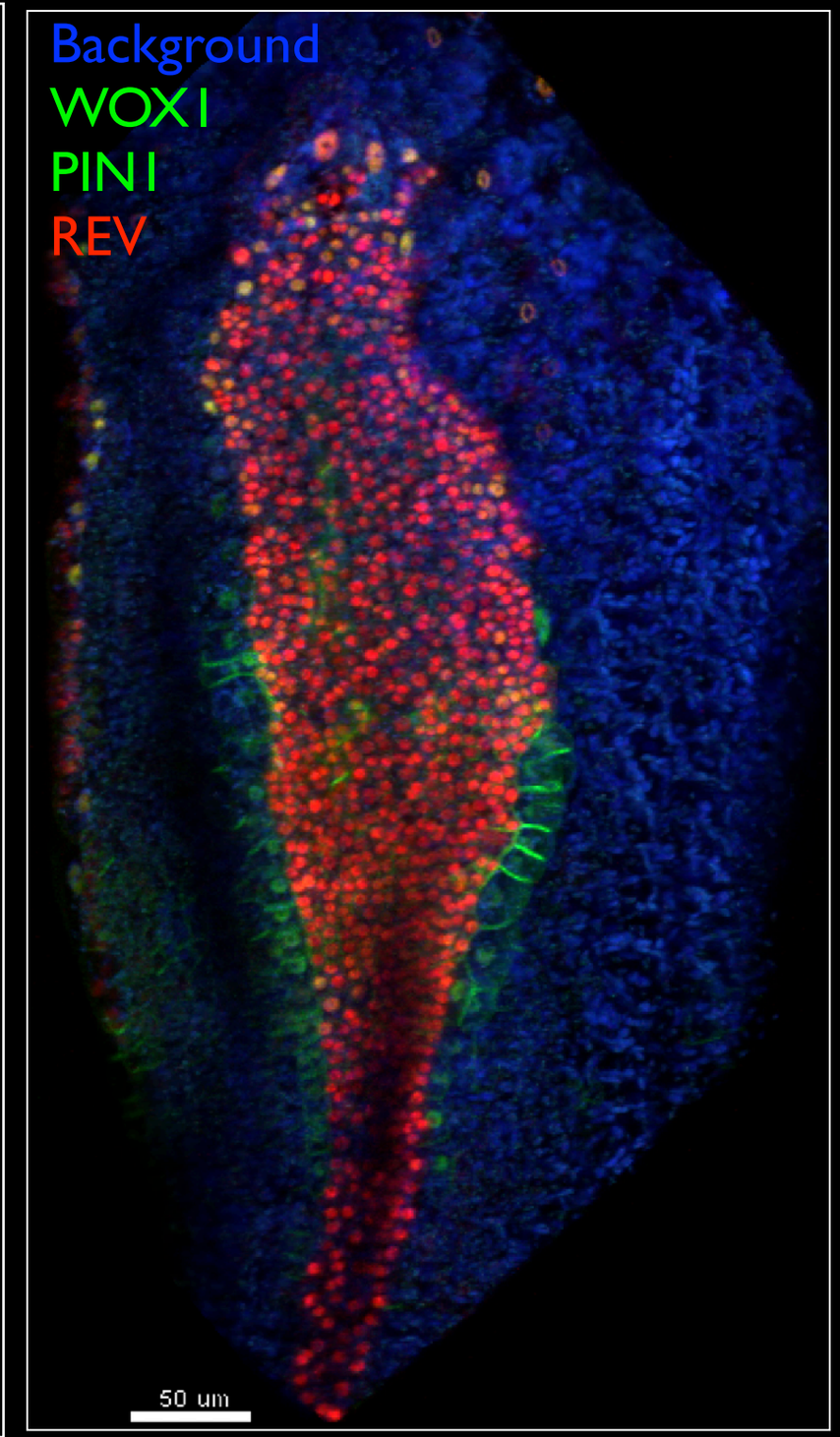
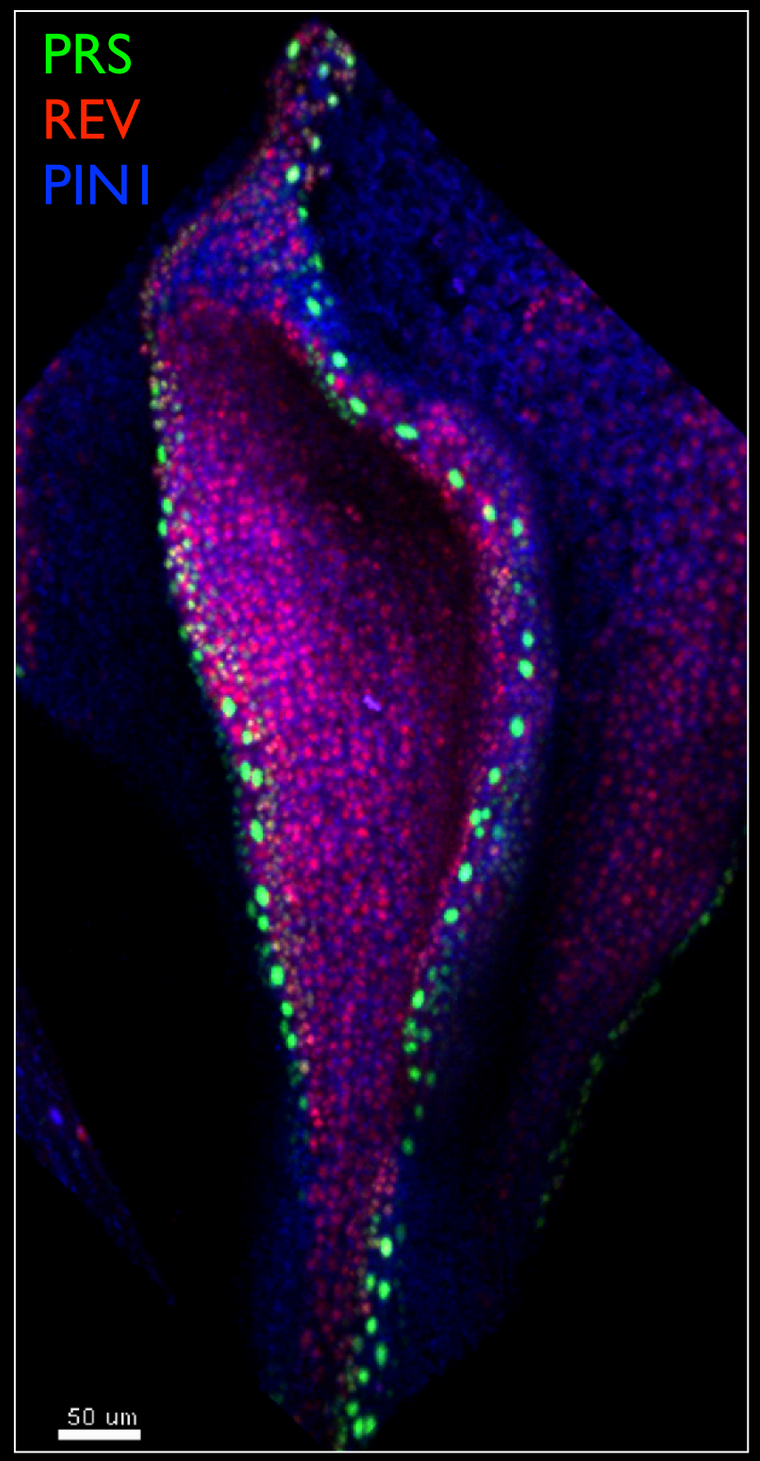
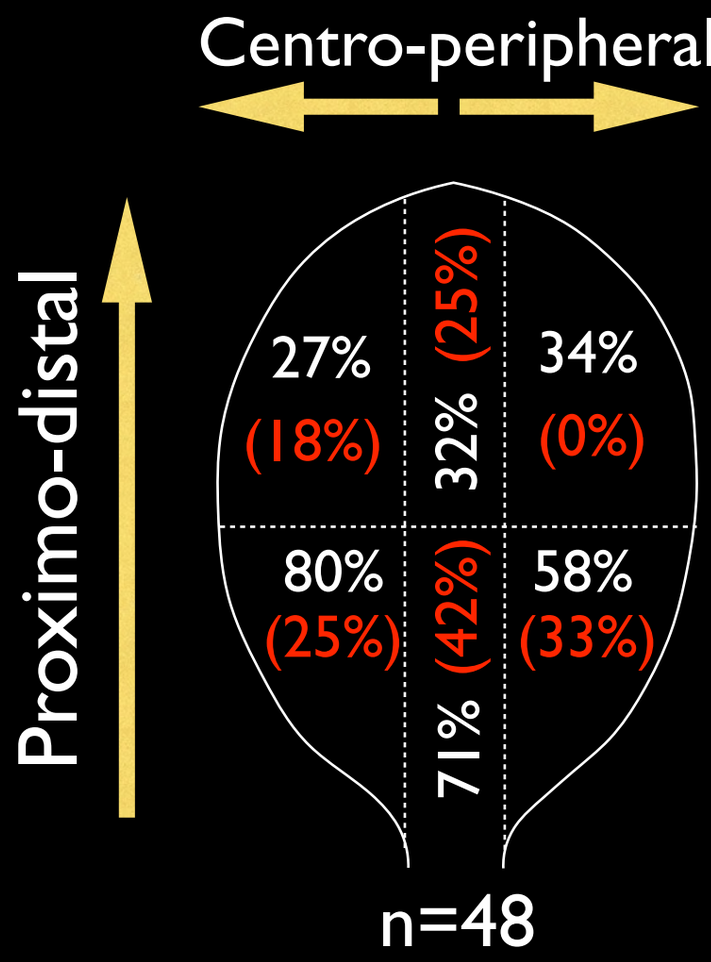






REV triggered outgrowth is variable

Ventral (bottom)

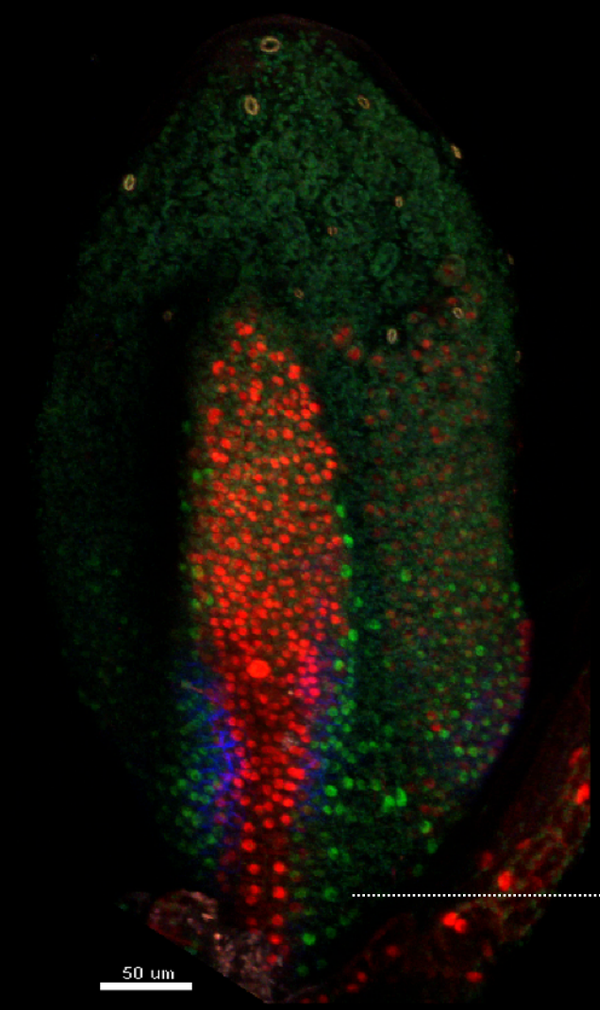


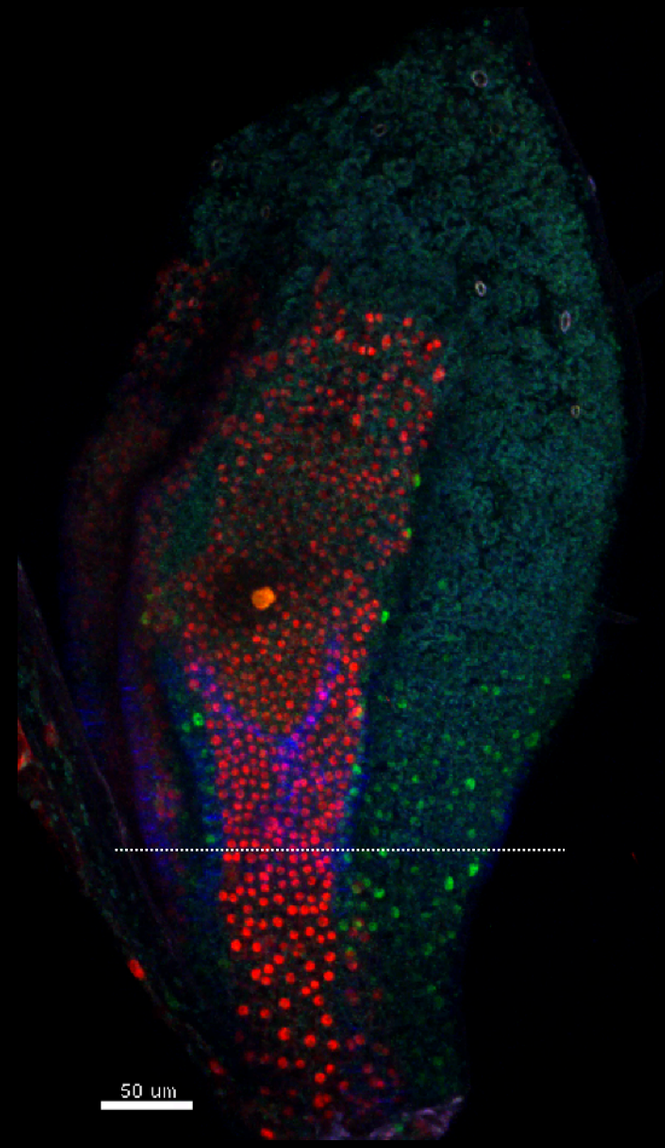
Frequency of sectors associated with "bump-like" growth

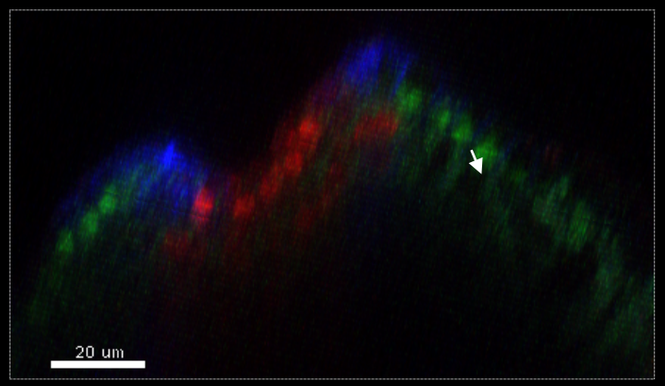
Frequency of sectors associated with lamina-like growth

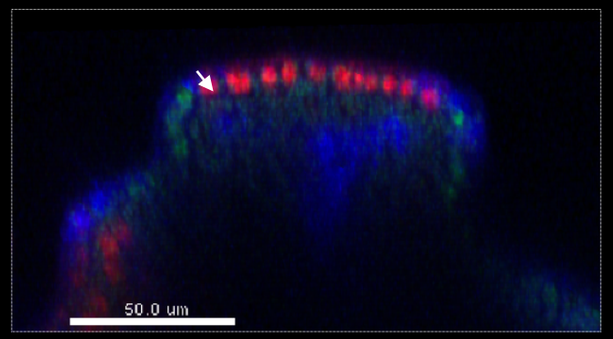
Laminar VS bump

REV
KAN1
PIN1









Laminar VS bump

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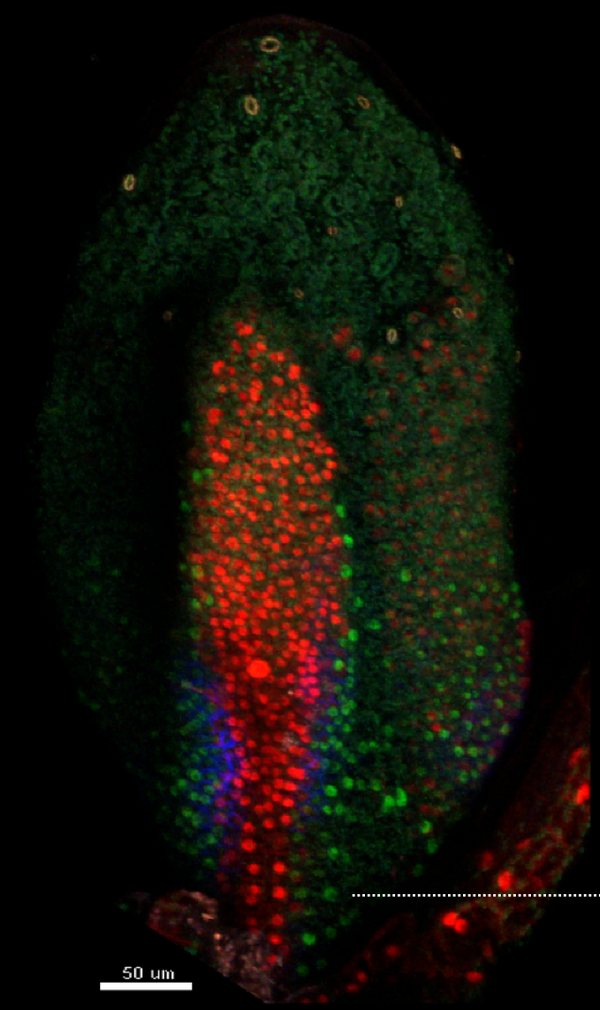

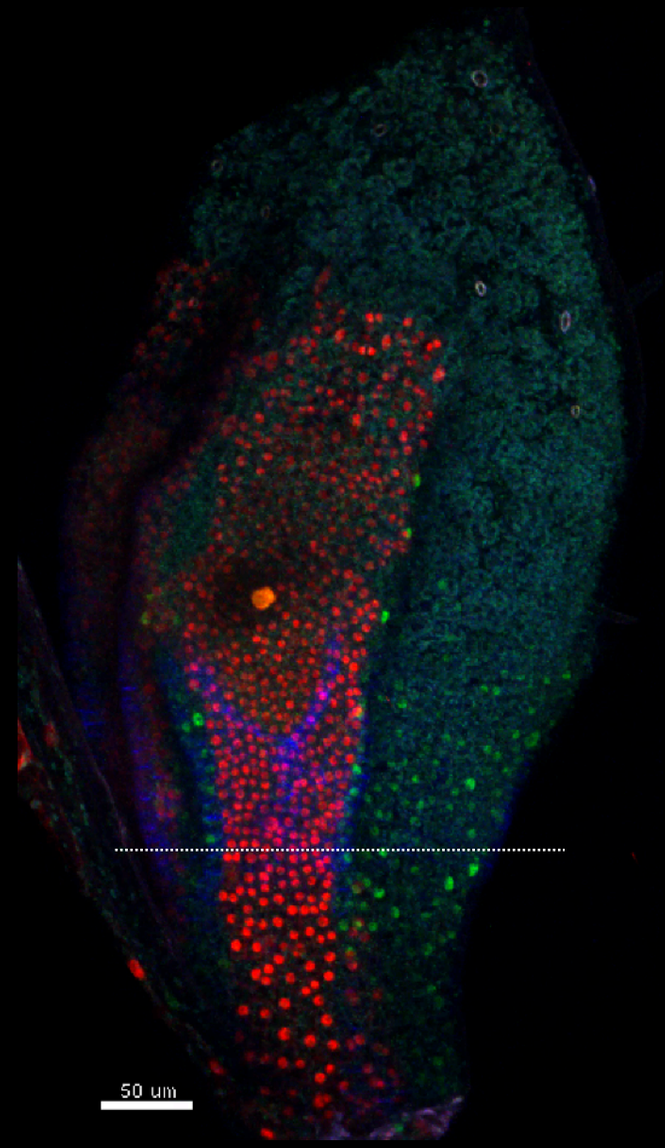

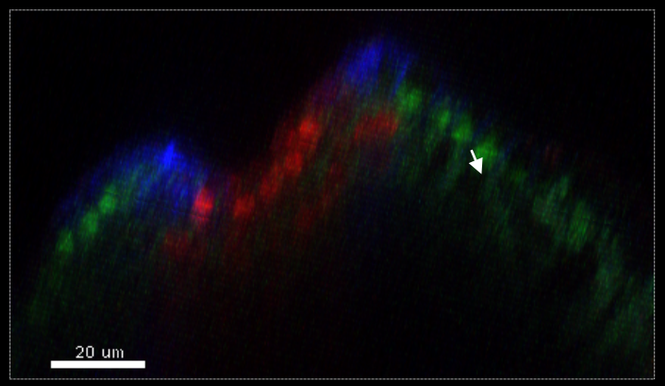

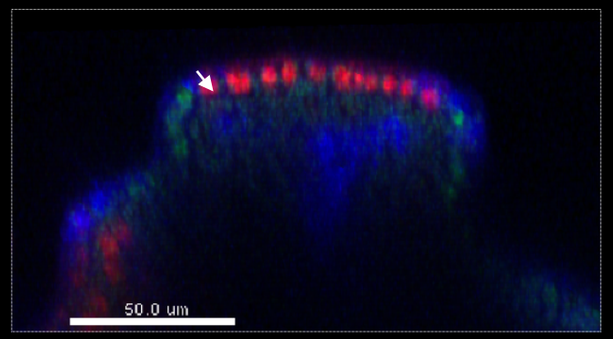

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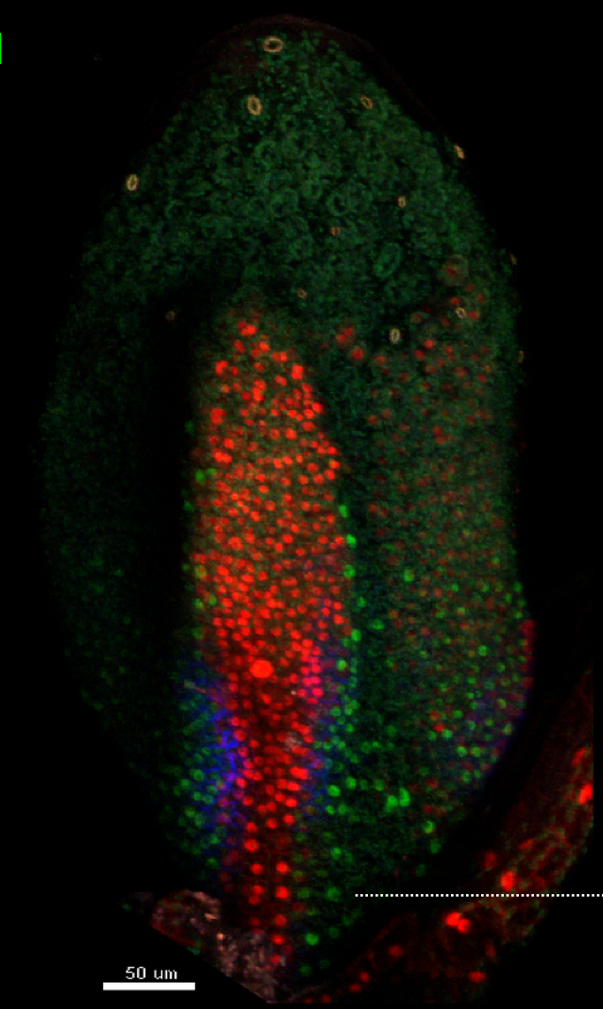
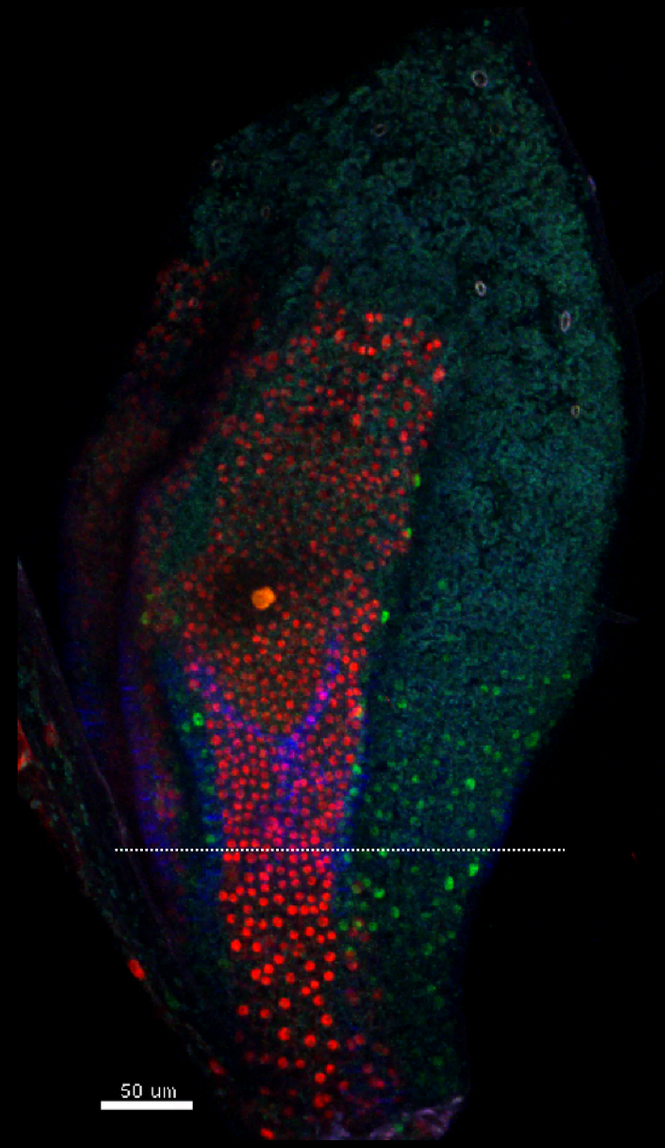
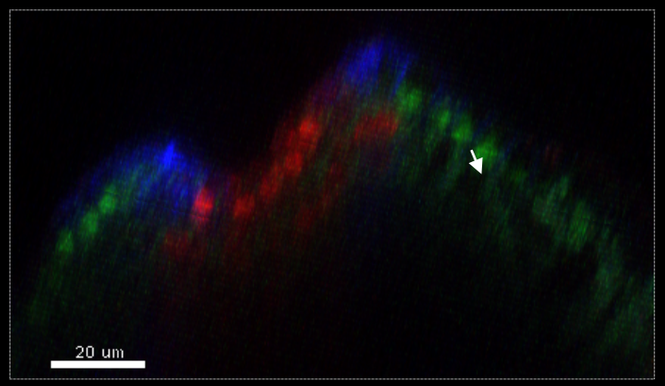
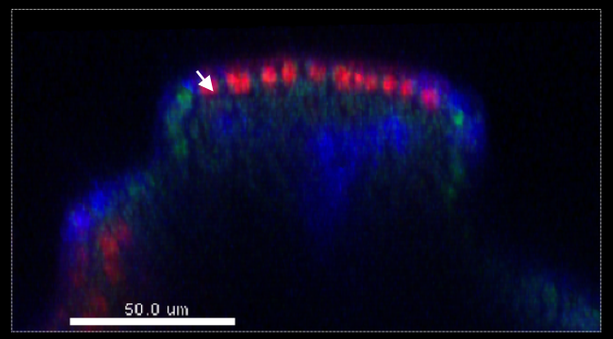
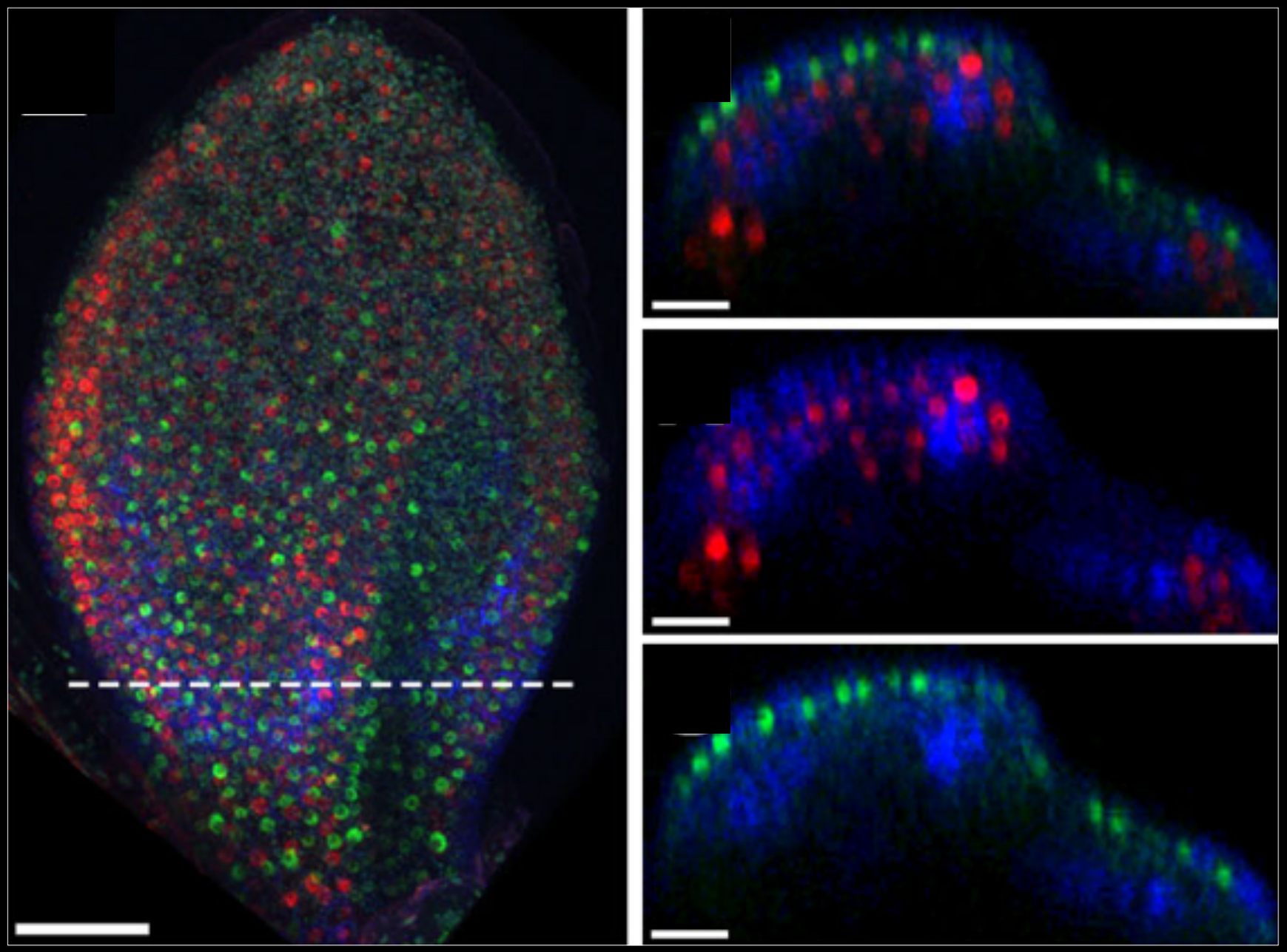




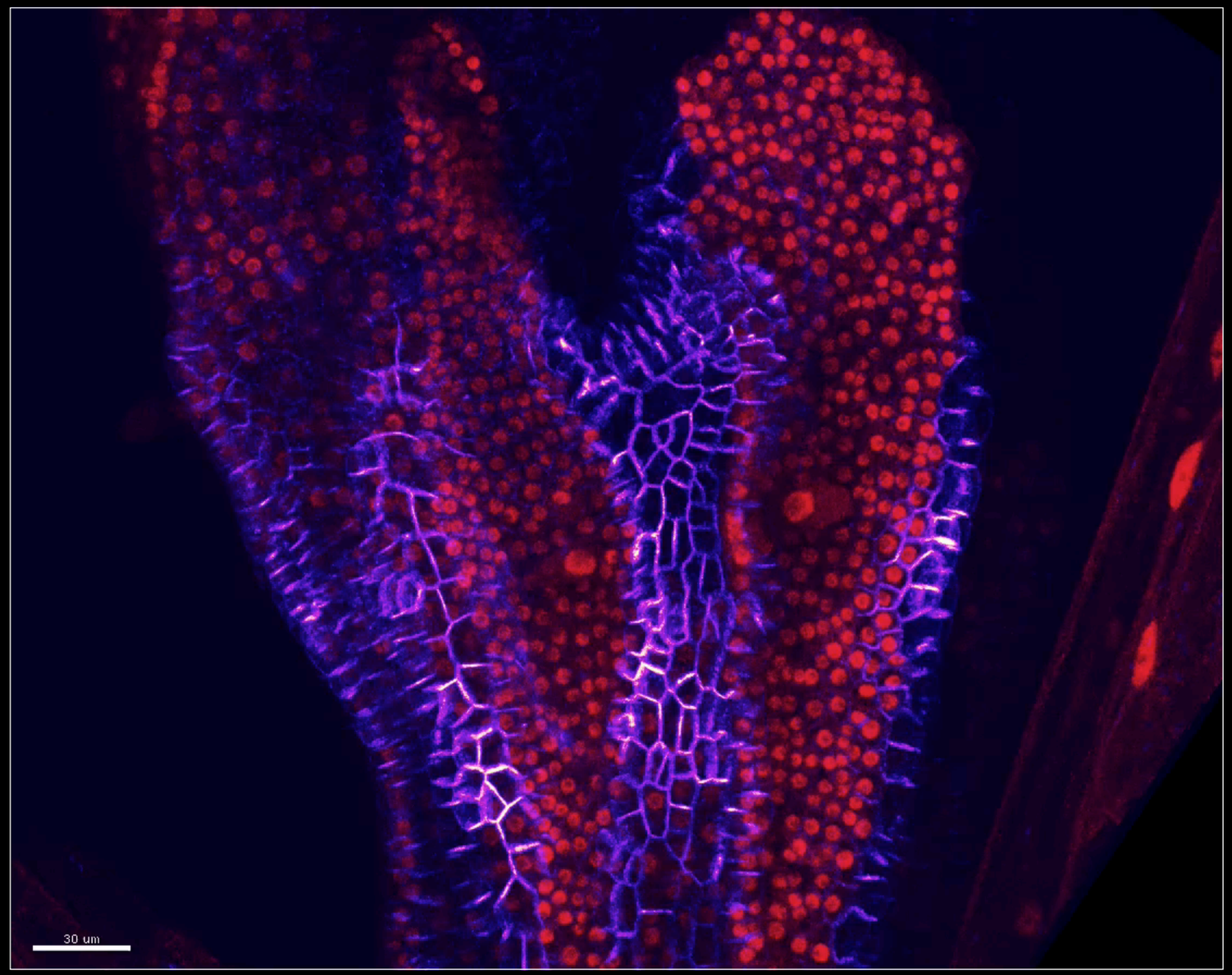


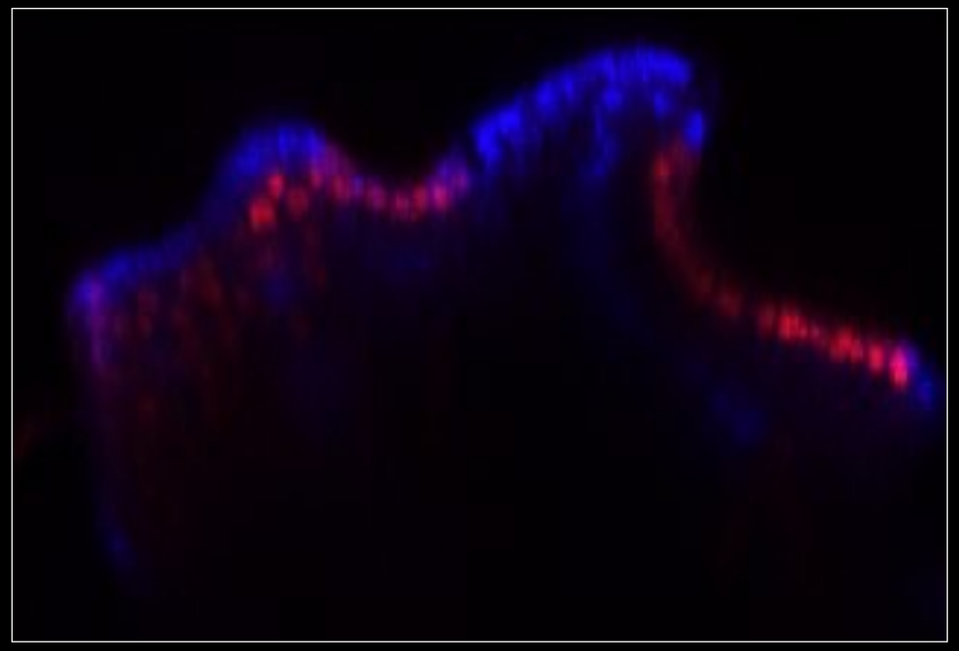


Non-cell autonomous up-regulation of PIN1 and growth does not absolutely require absence of KAN1



Two adjacent REV clones also promote outgrowth





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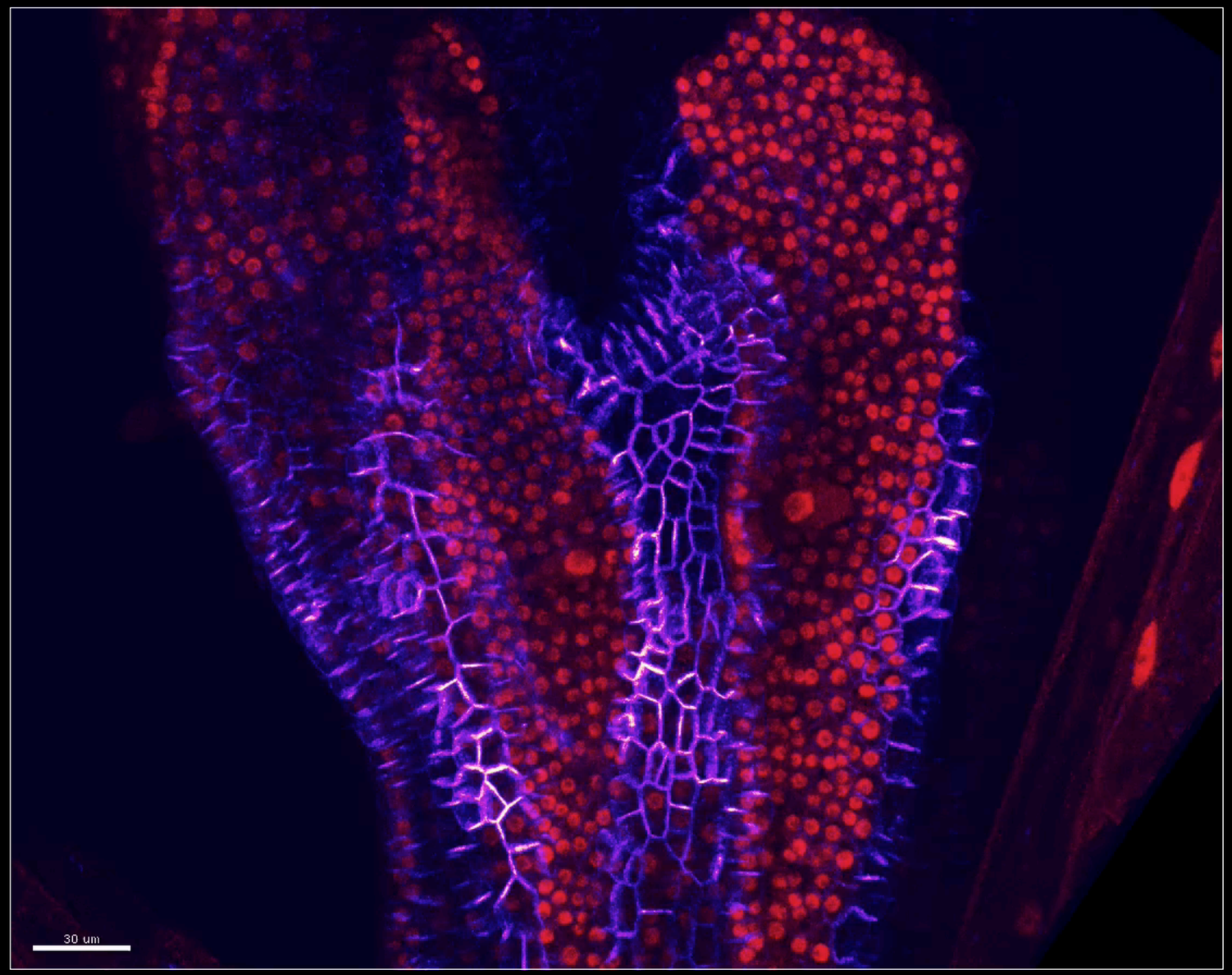
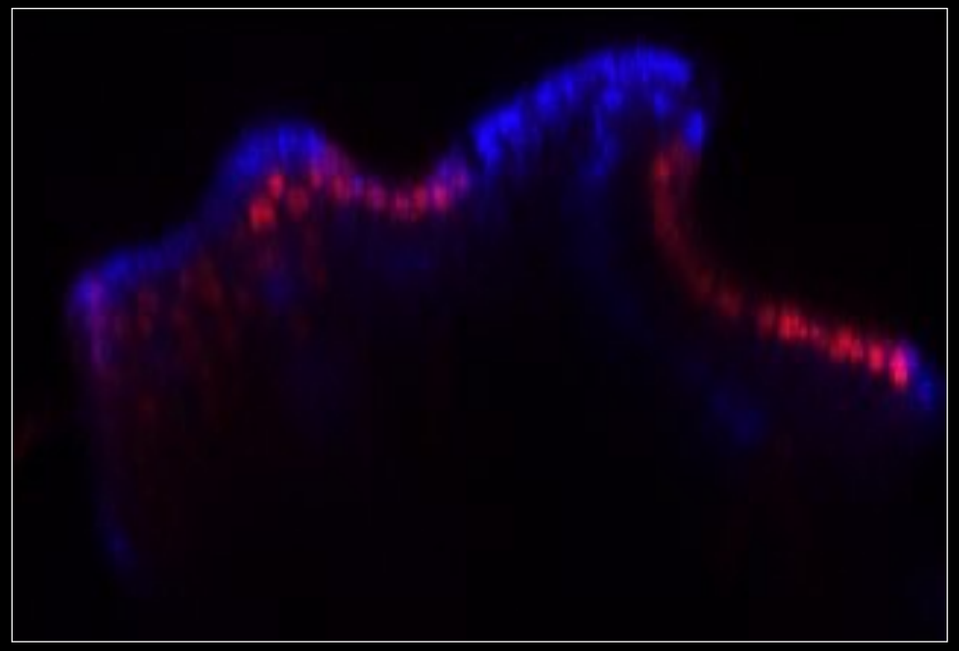
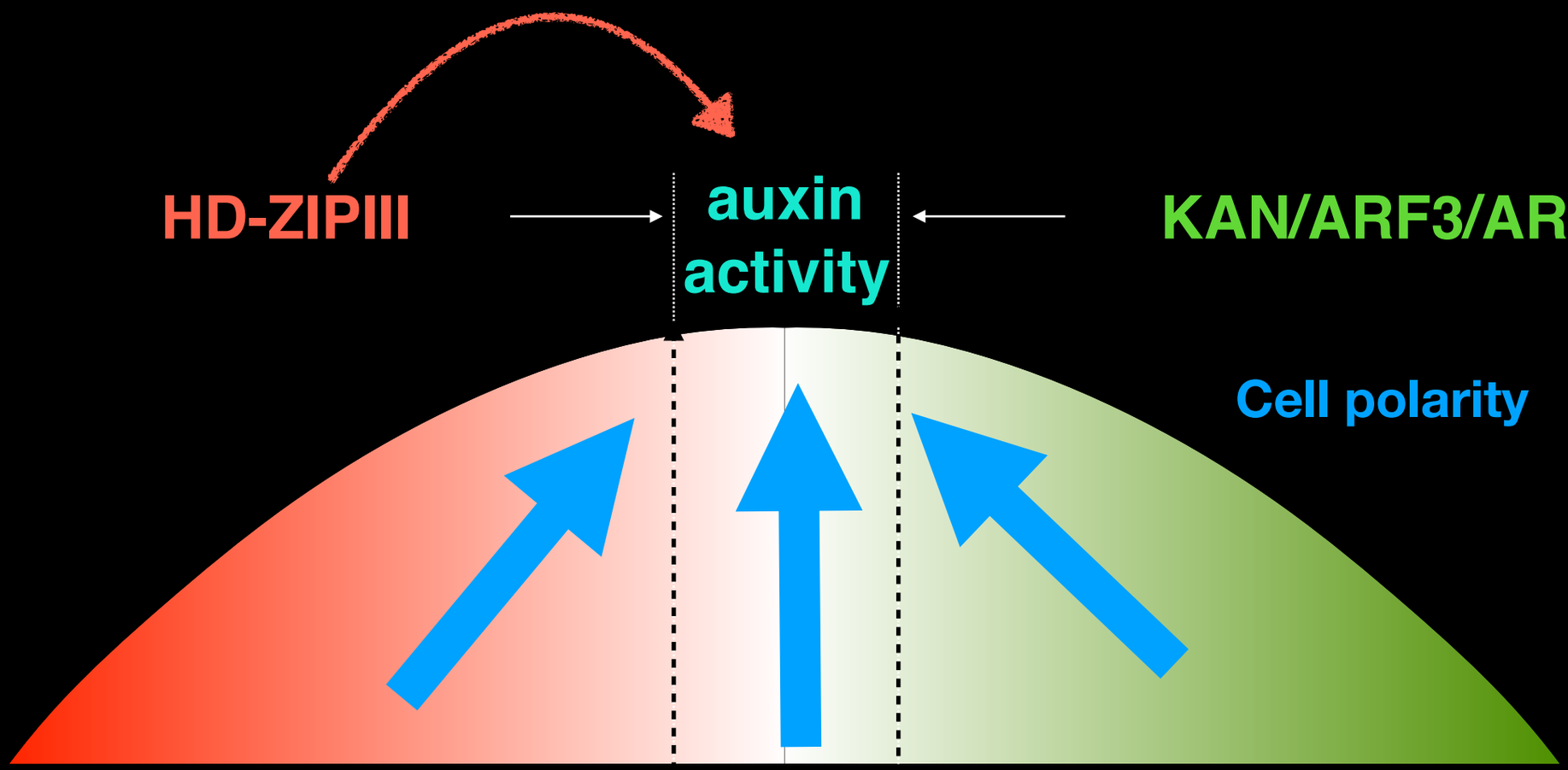


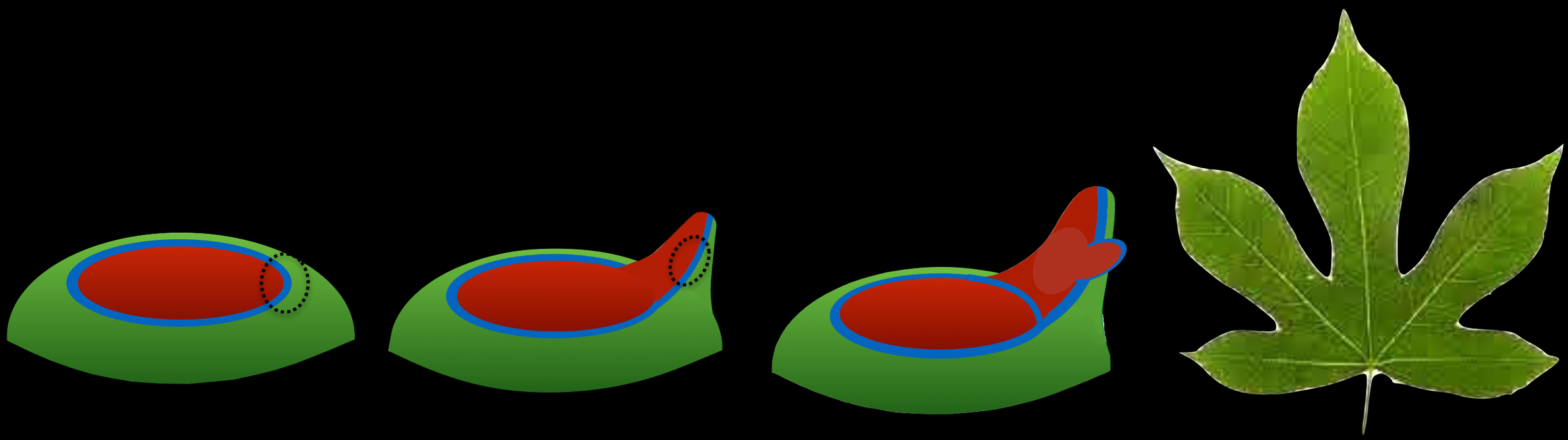


Summary



Similarities but also differences compared to boundary-localised organisers in animals

Combining periodic patterning with boundary oriented growth creates diversity



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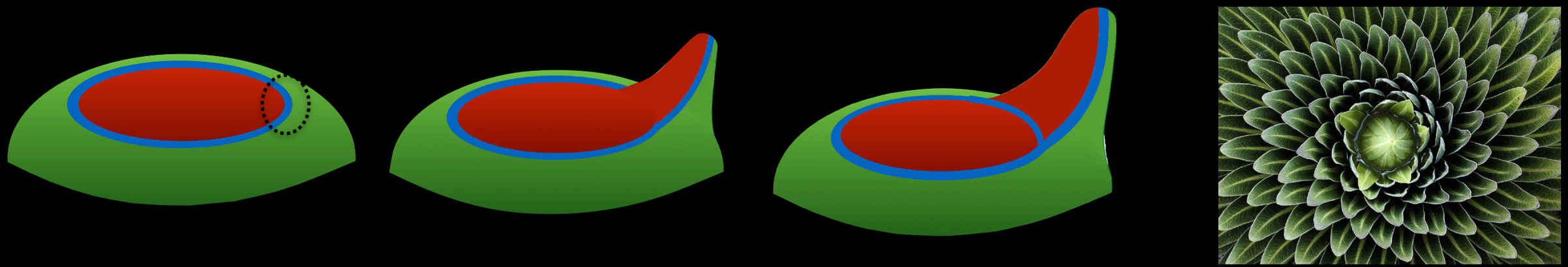
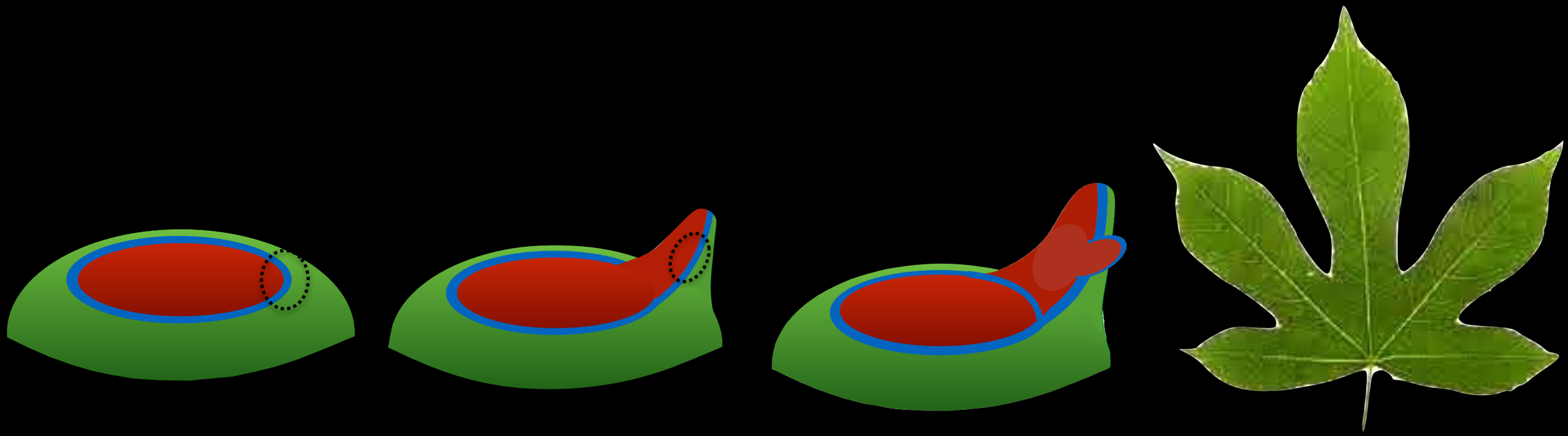
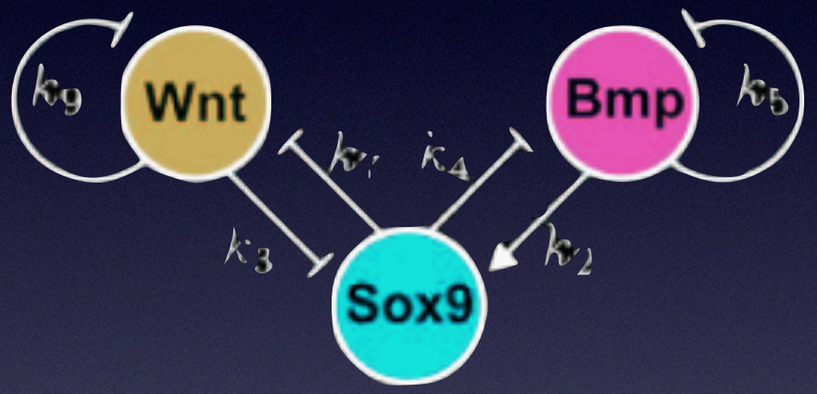
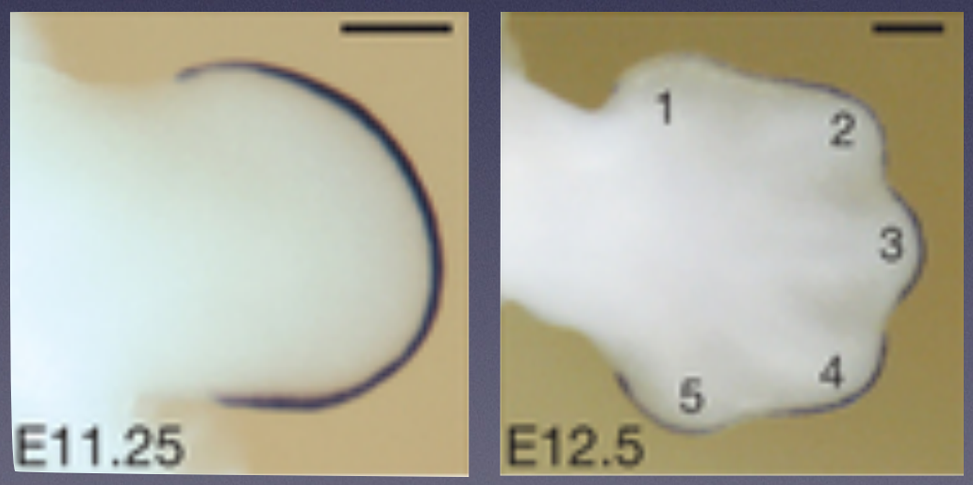




Self-organizing periodic system operates to position digits

- Constrained to 2D plane





Lopez-Ríos et al. (2014)

Raspopovic et al. (2014)

EMBL

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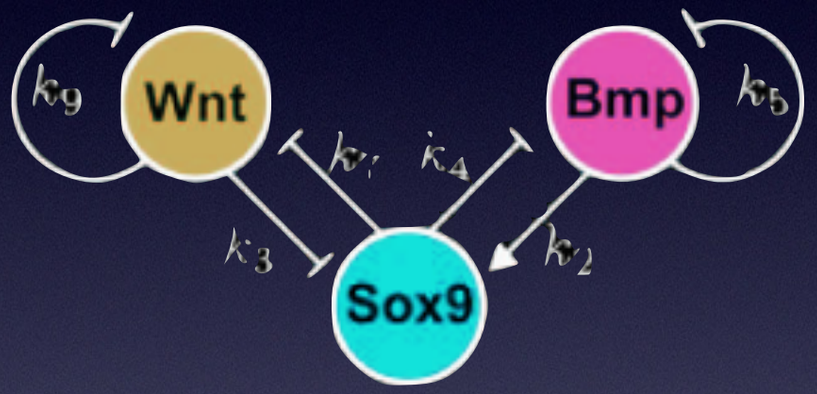

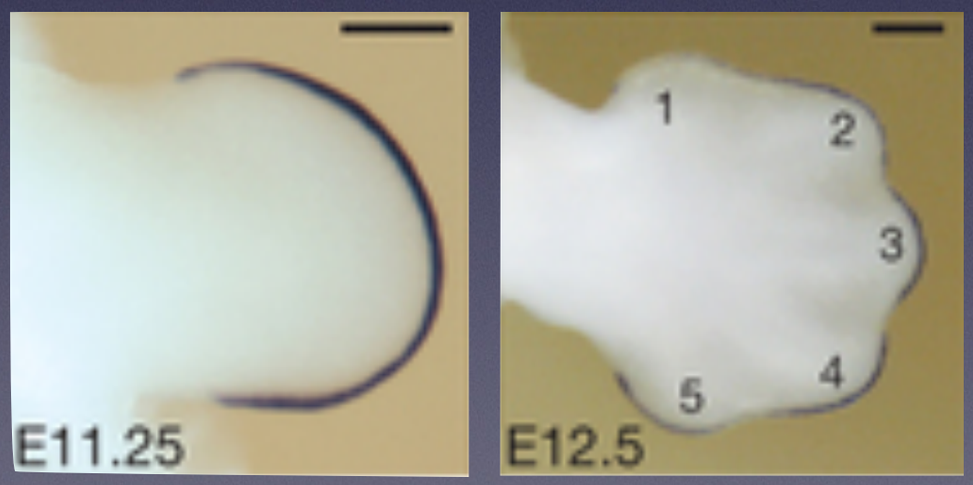

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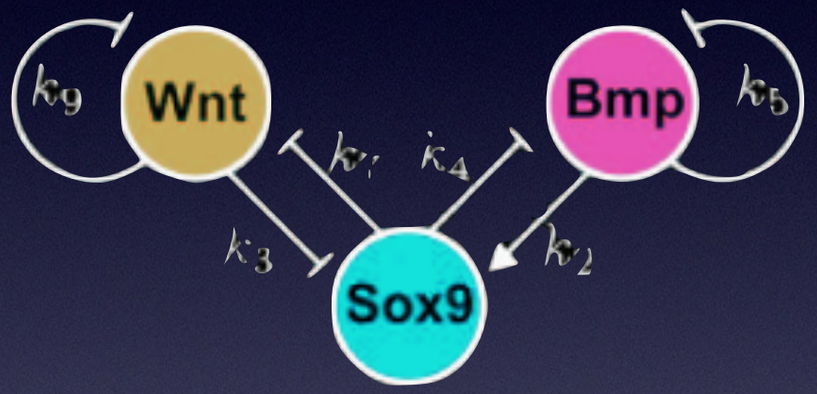
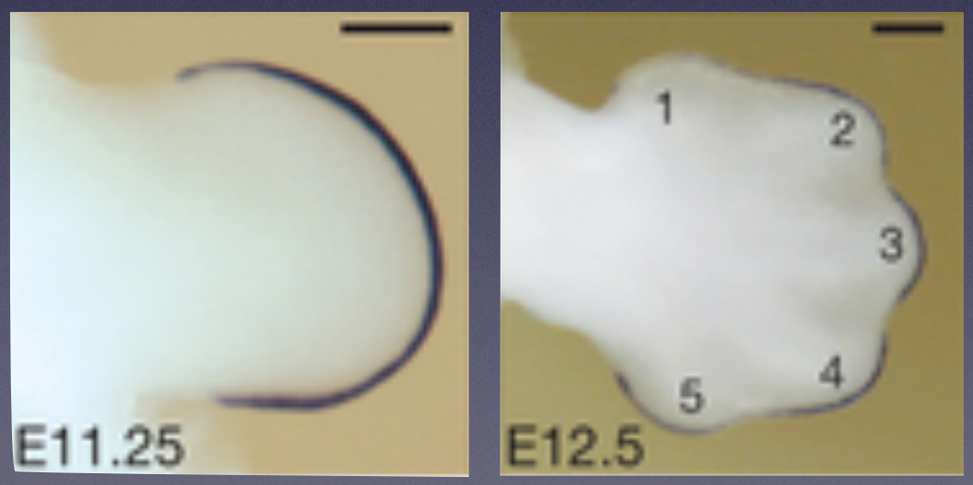
Self-organizing periodic system operates to position digits

- Constrained to 2D plane



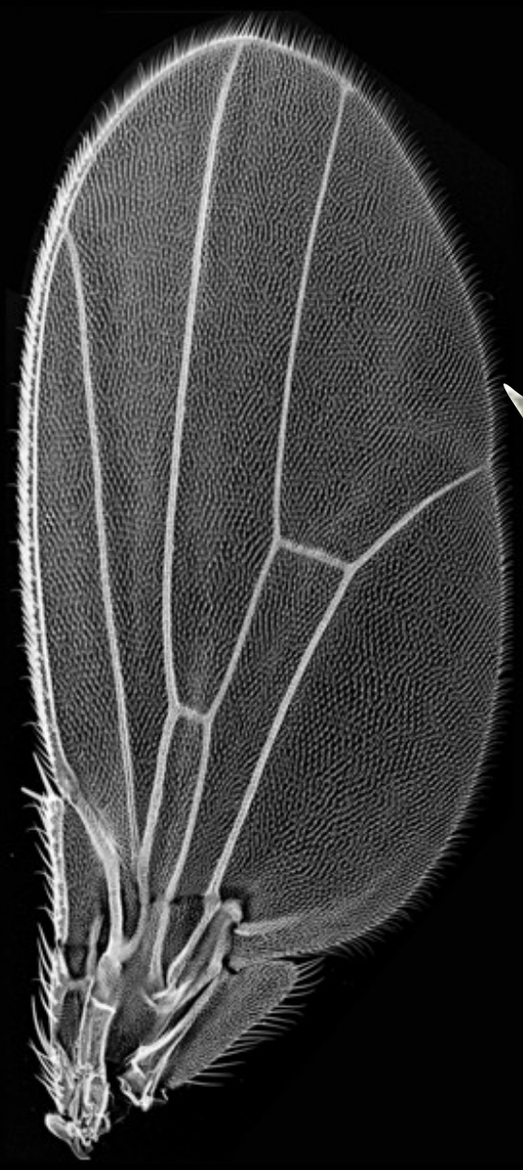


Lopez-Ríos et al. (2014)

EMBL

Common themes?

Common themes?



Common themes?

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Pierre Le Gars

Jonsson lab
Sainsbury lab
Cambridge

EMBO excellence in life sciences

EMBL

Australian Government
Australian Research Council

THE UNIVERSITY OF SYDNEY

erc
European Research Council
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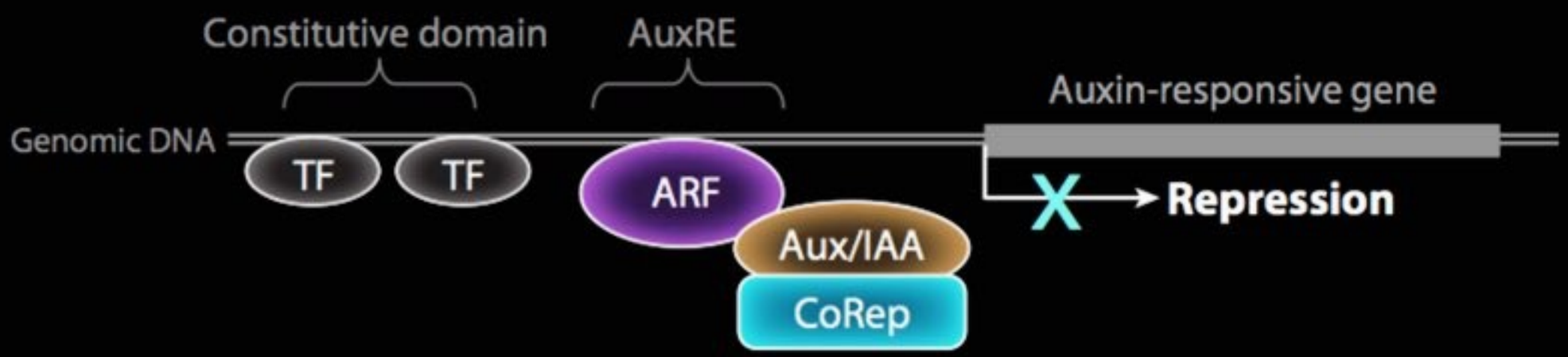
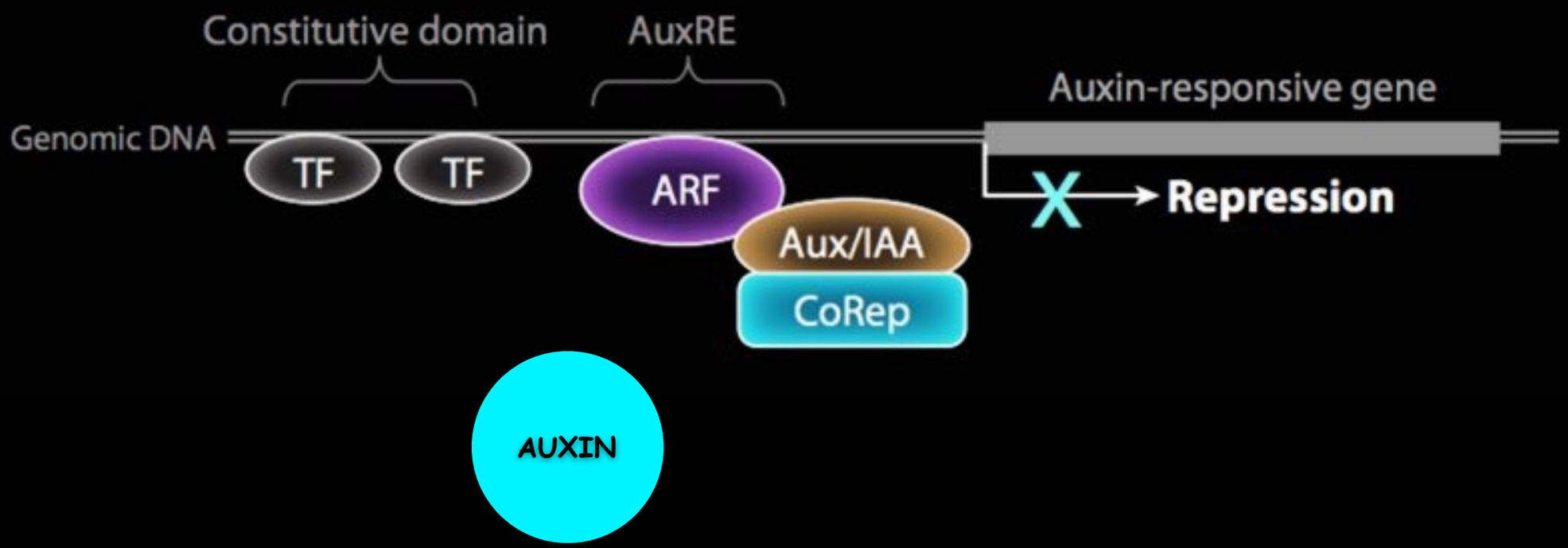
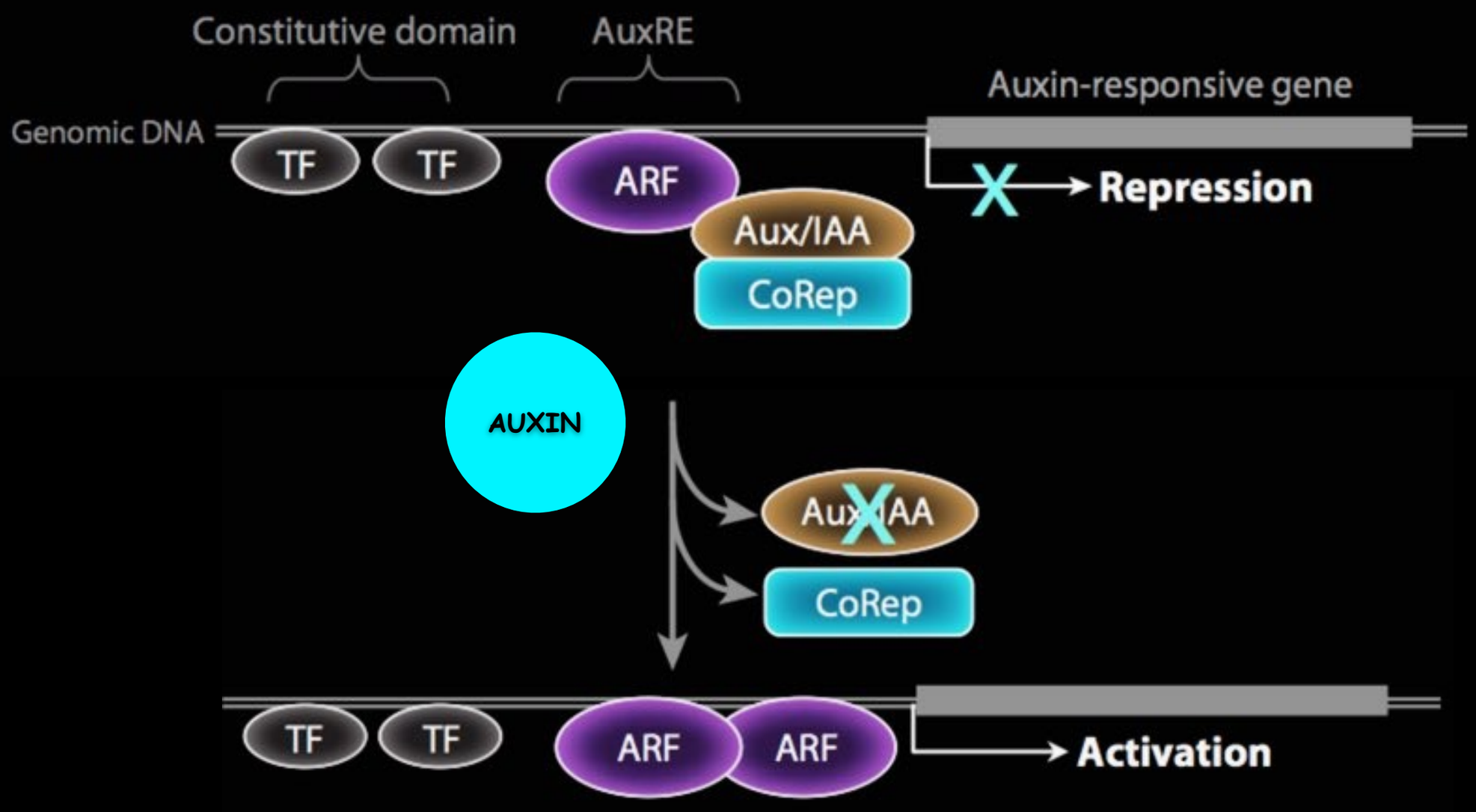
Auxin mediated transcription



Auxin mediated transcription

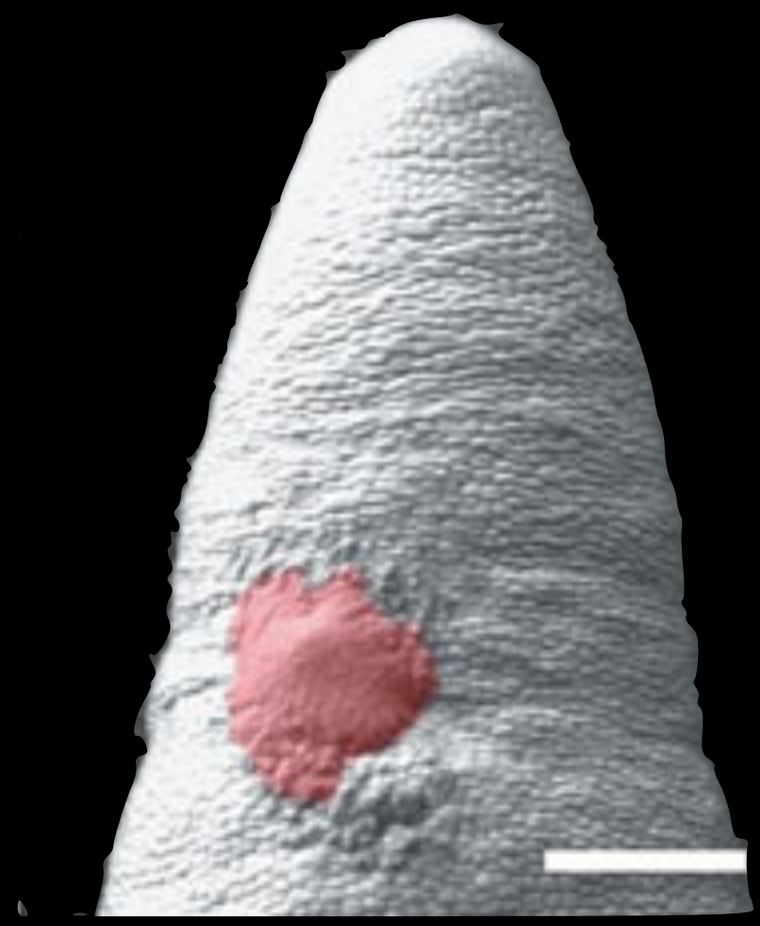


Auxin mediated transcription



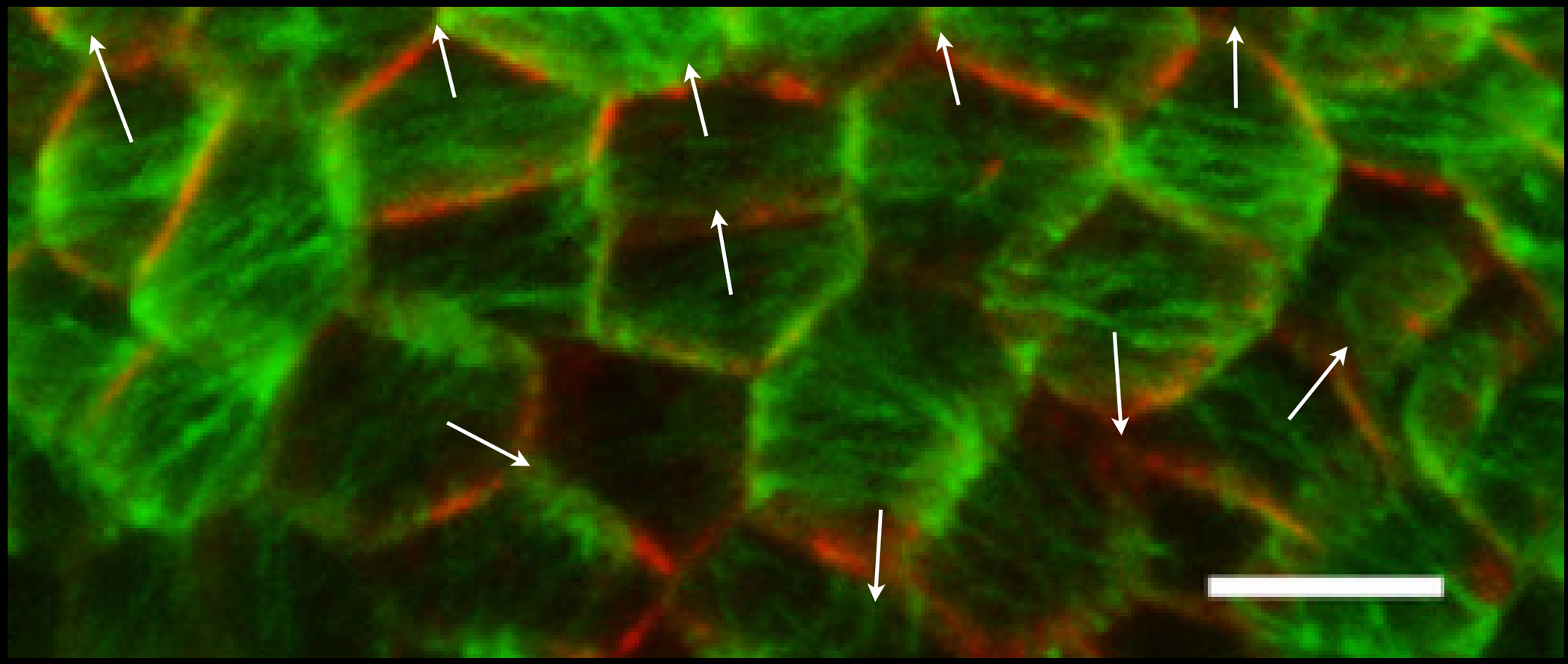
mp (arf5) mutants do not form organs in response to auxin

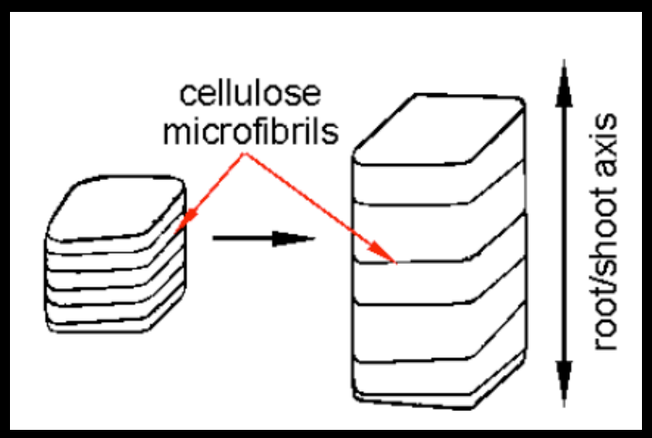
pin1 treated with auxin



mp treated with auxin

PIN1 polarities correlate with microtubule array orientations

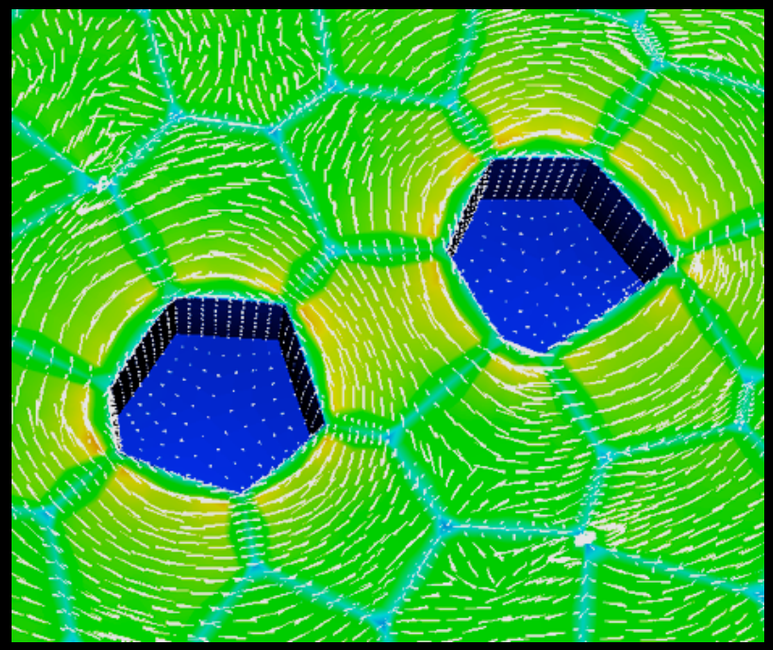


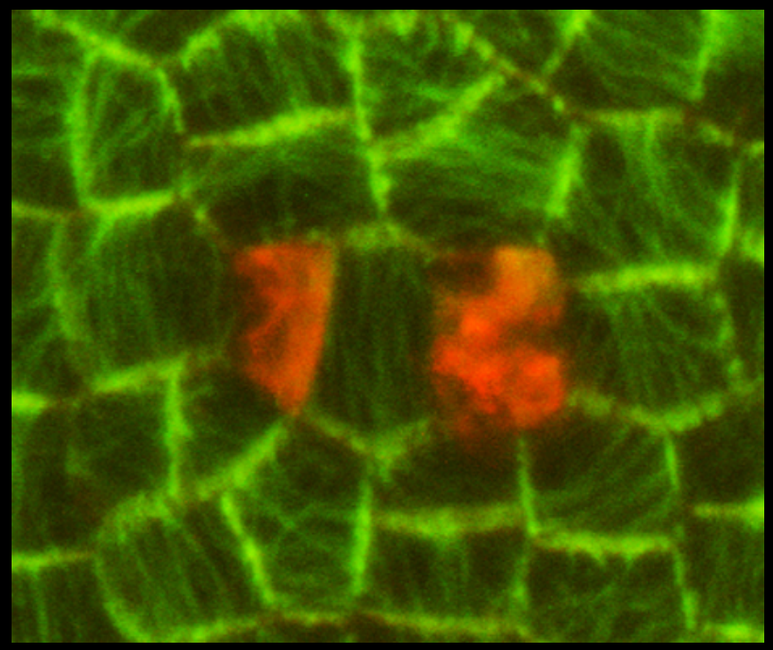


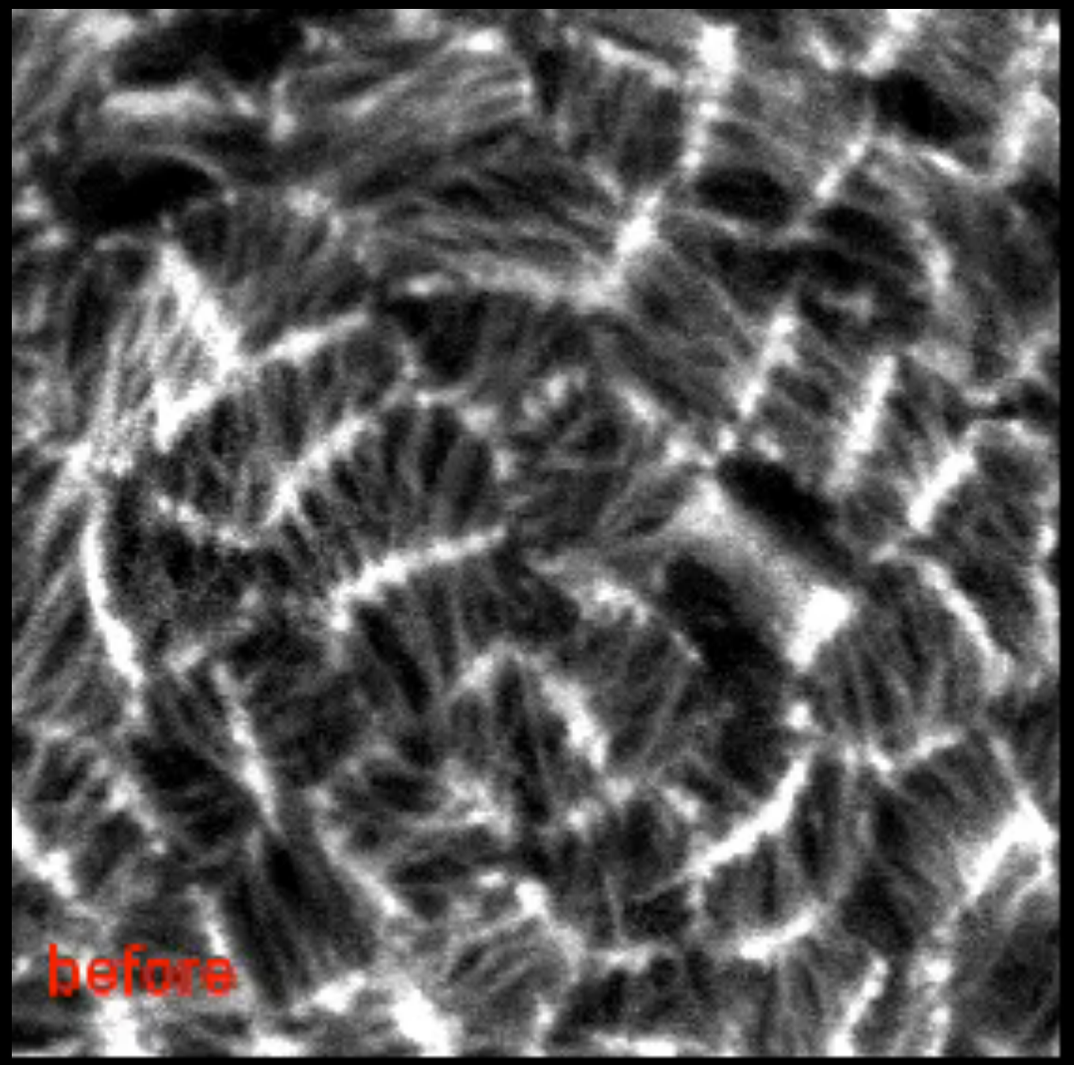
PIN1
MICROTUBULES

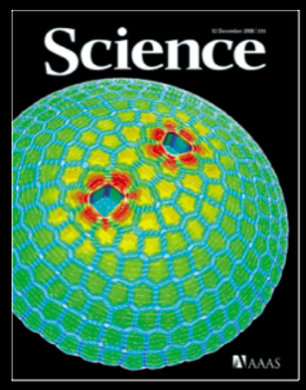
Heisler et al,2010

Mechanical stresses orient microtubules

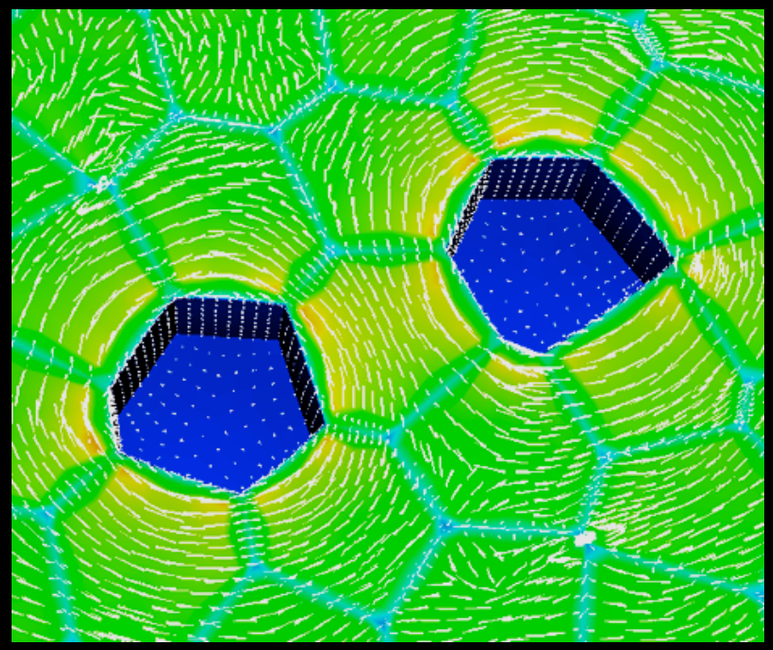


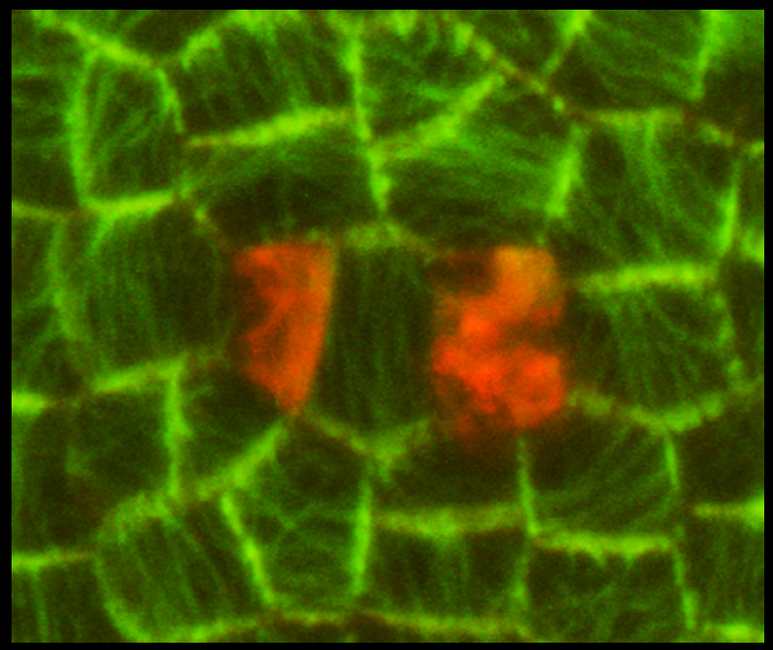


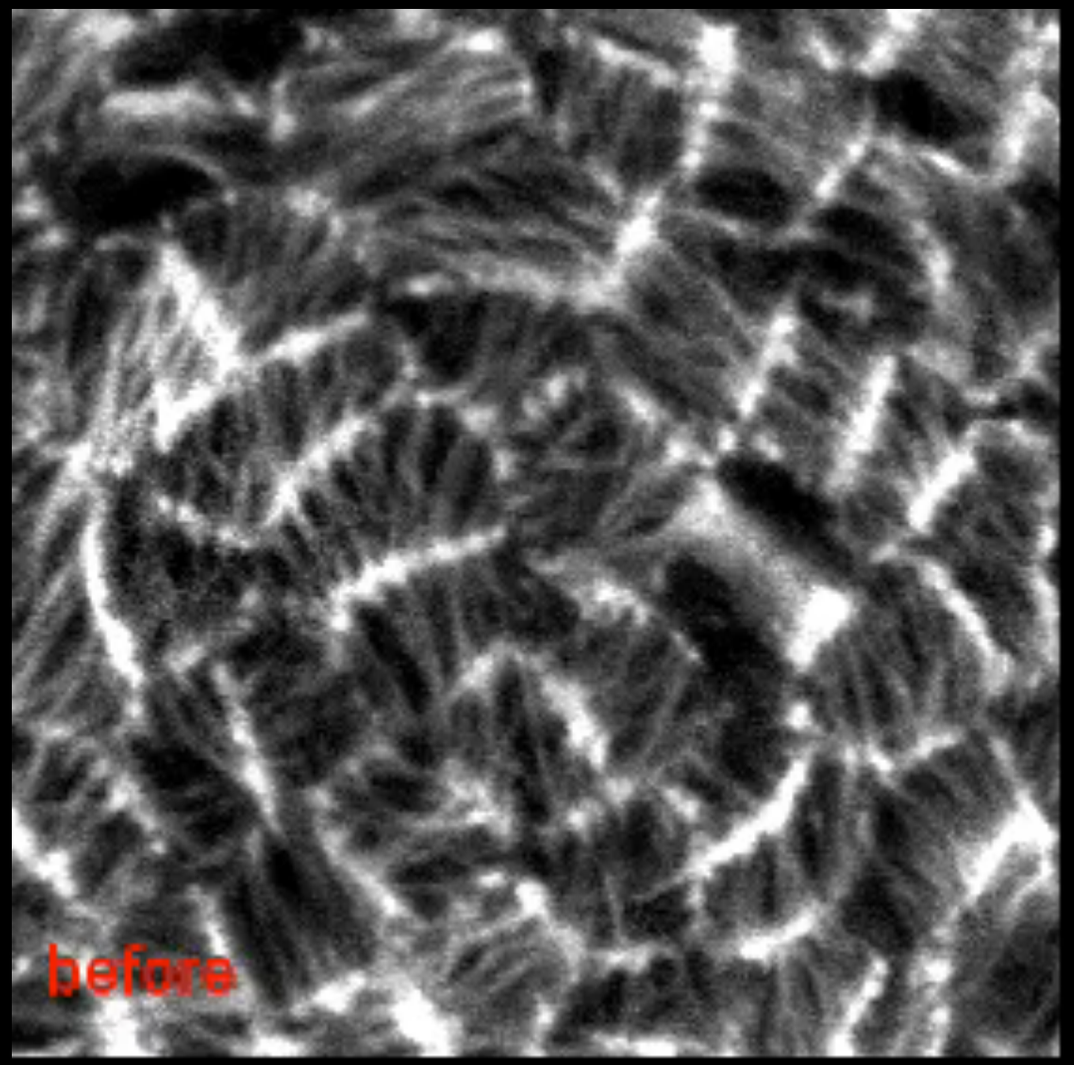


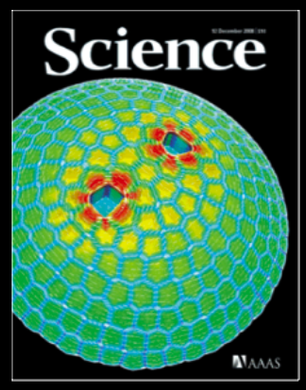


Mechanical stresses orient microtubules

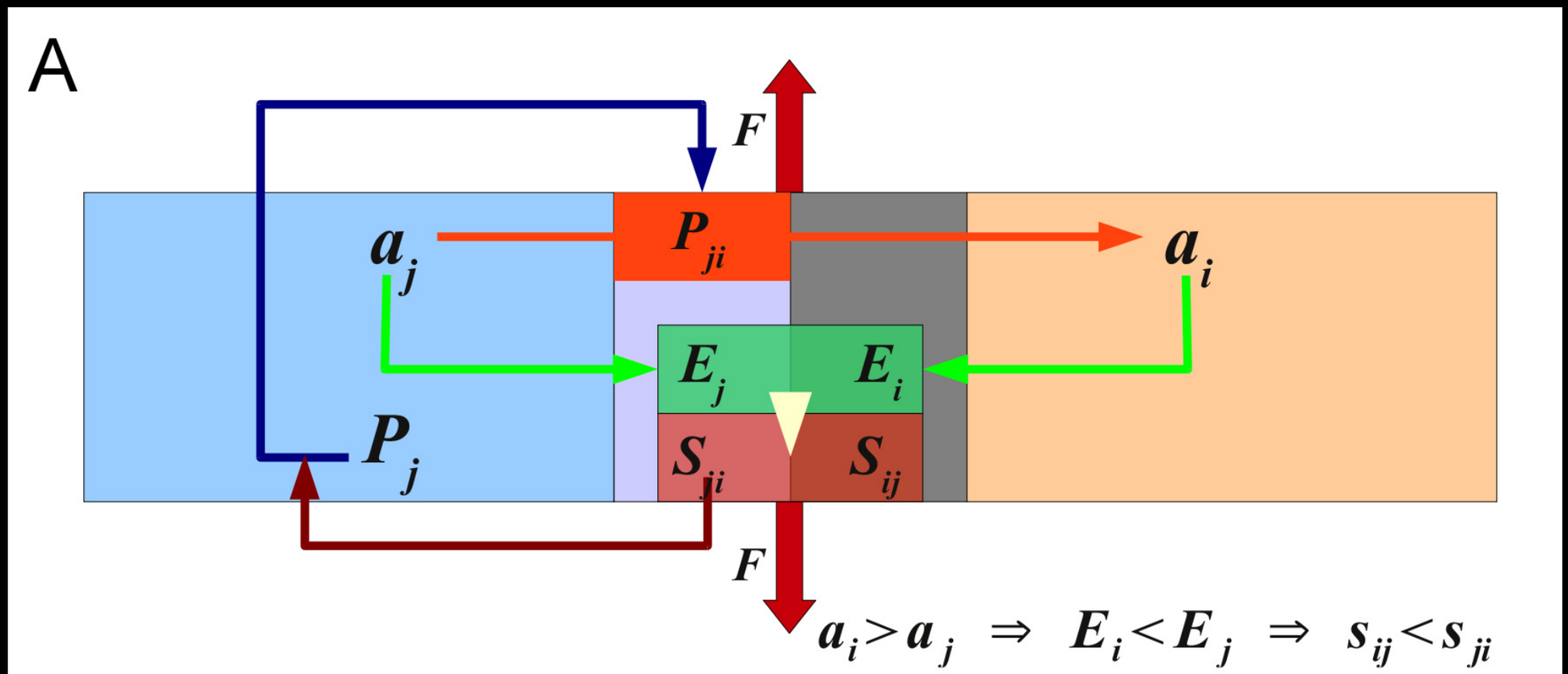






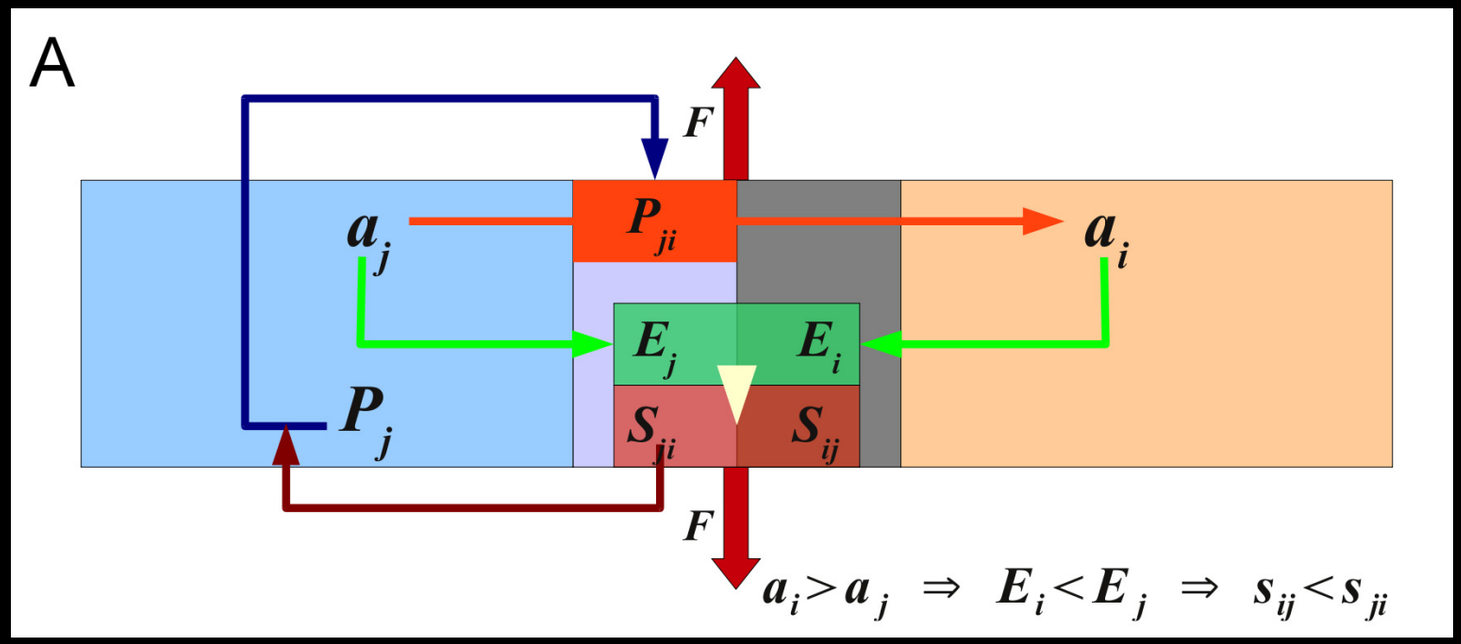


Auxin may polarise cells via mechanics



Heisler, Hamant and Krupinski et al (2010)

Auxin - cell polarity feedback probably involves mechanics



Heisler, Hamant and Krupinski et al (2010)

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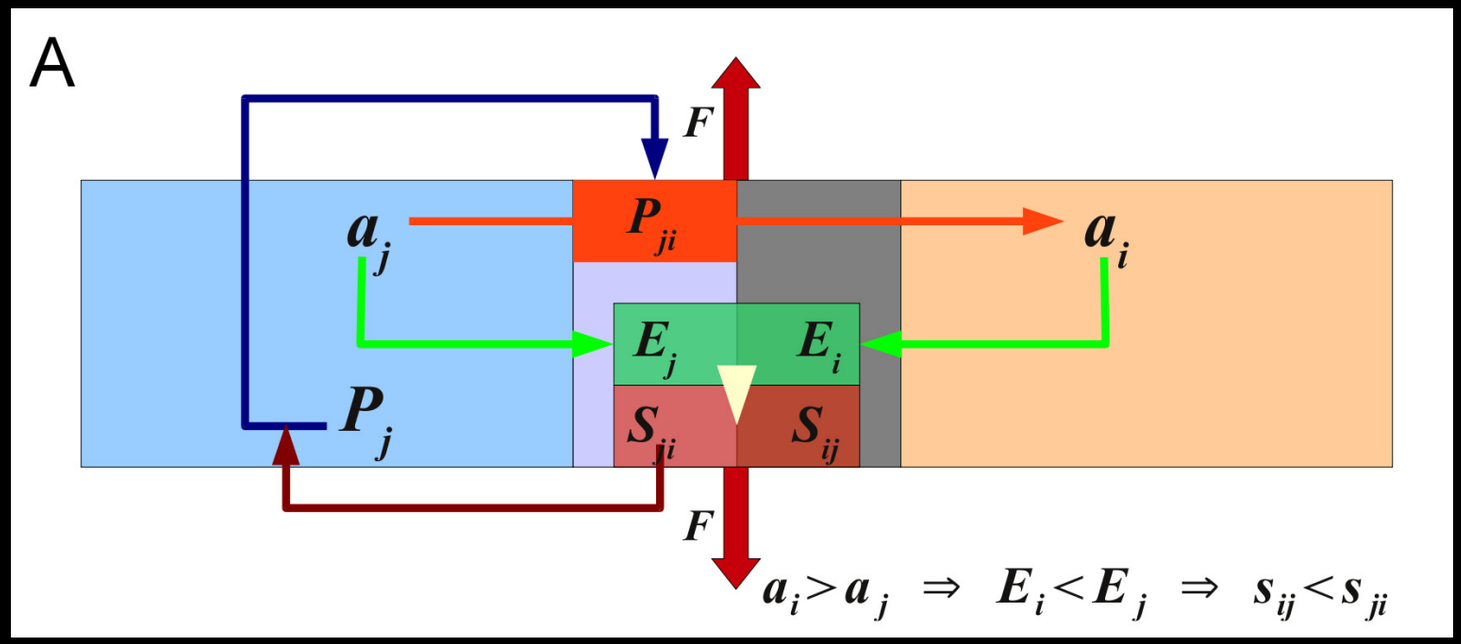
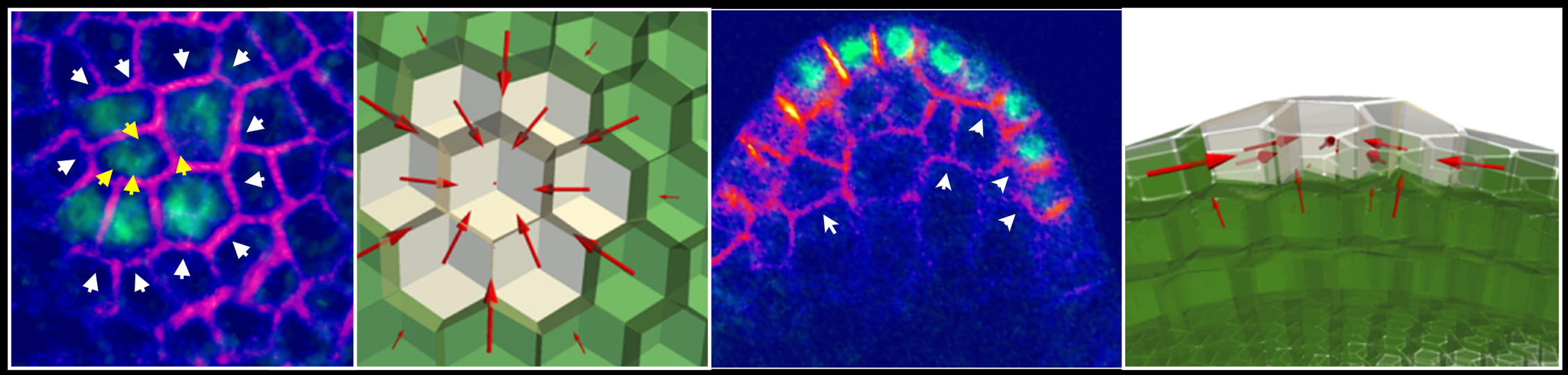
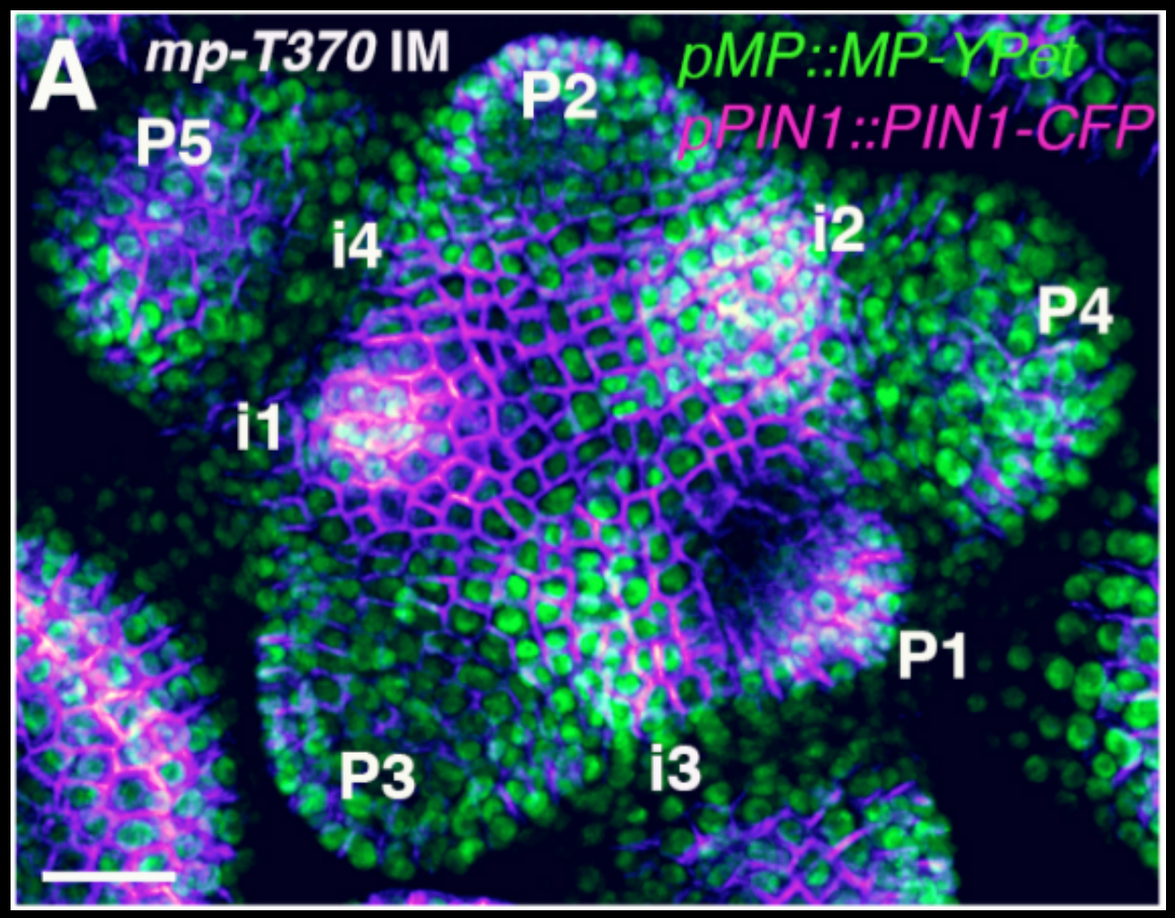
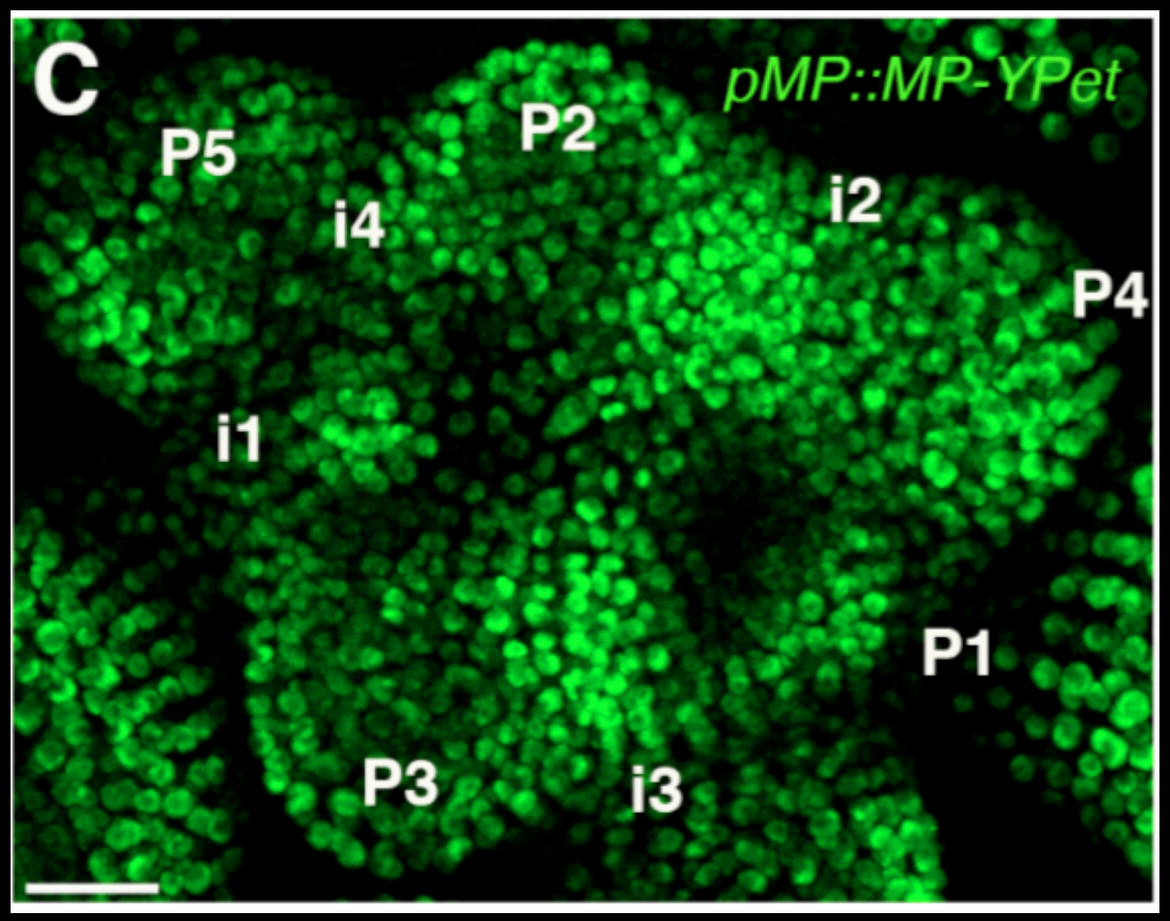


Heisler, Hamant and Krupinski et al (2010)



PIN1 polarity patterns follow MP expression





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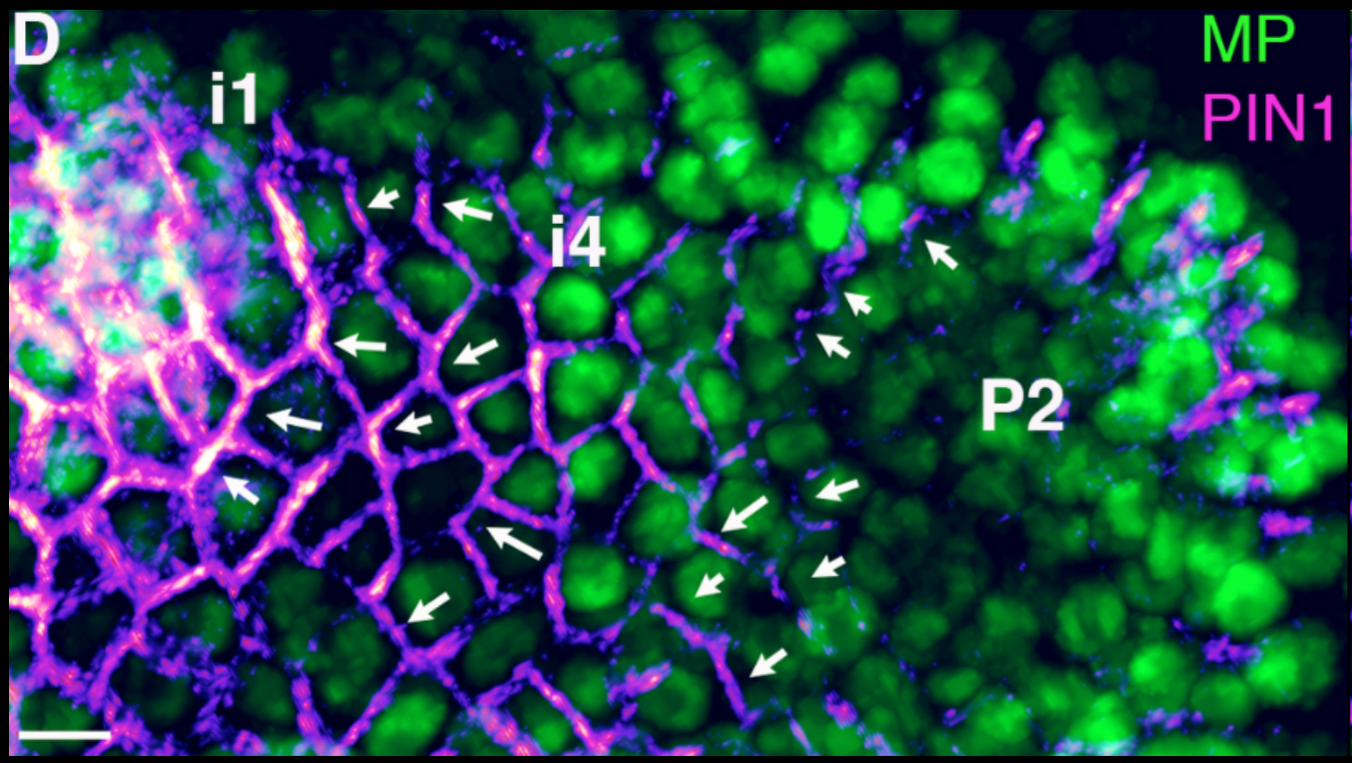

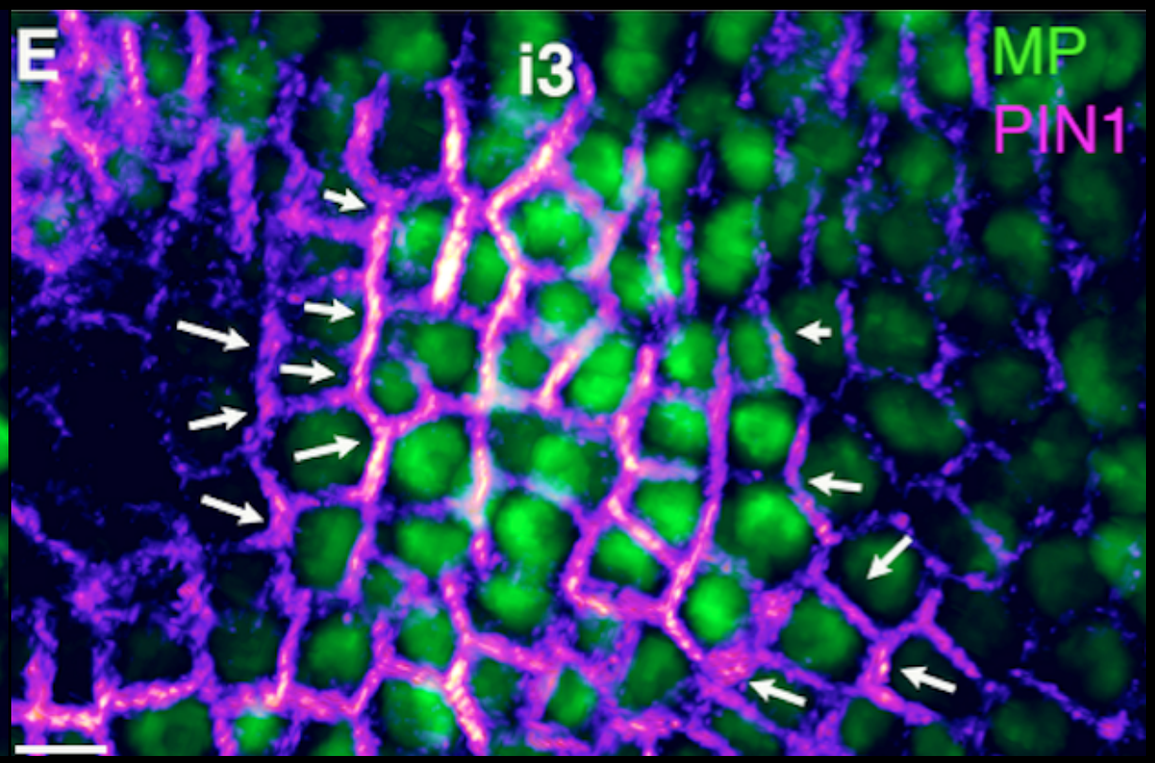

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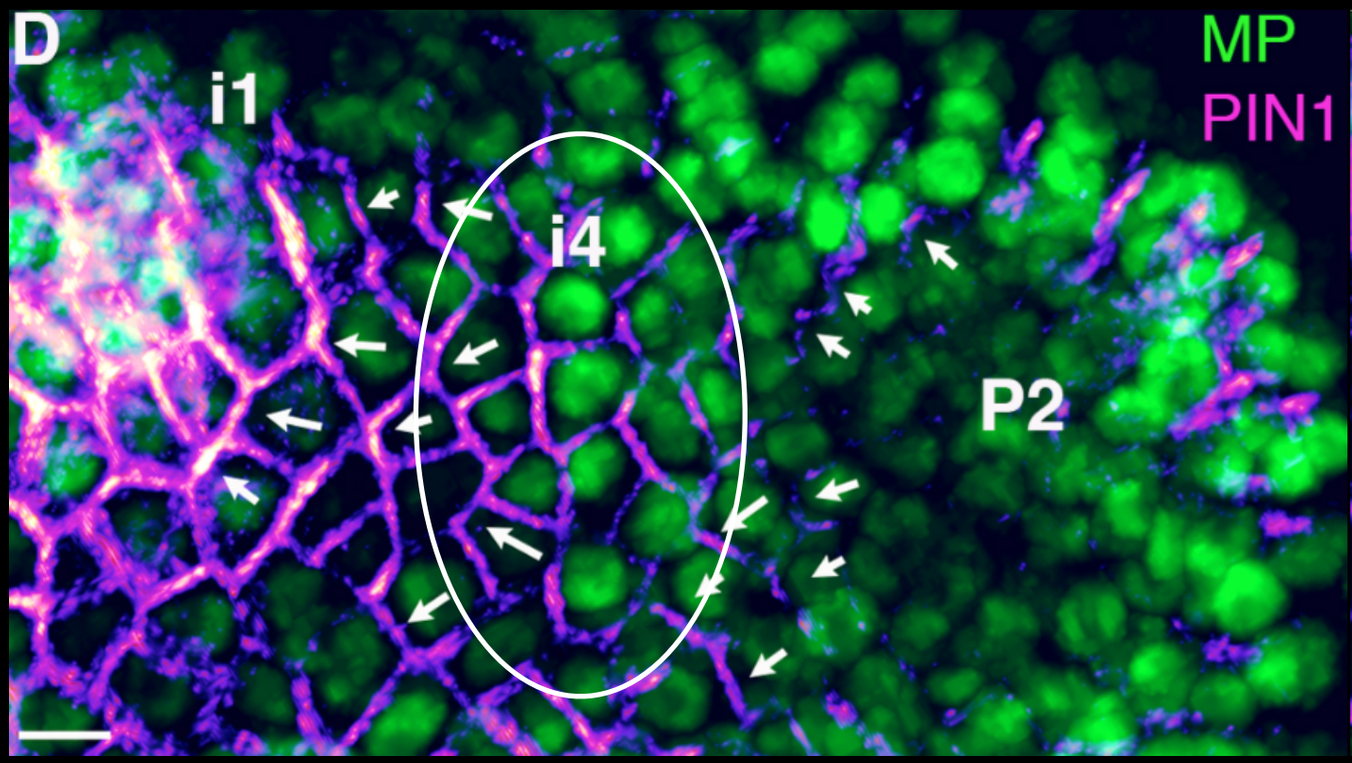
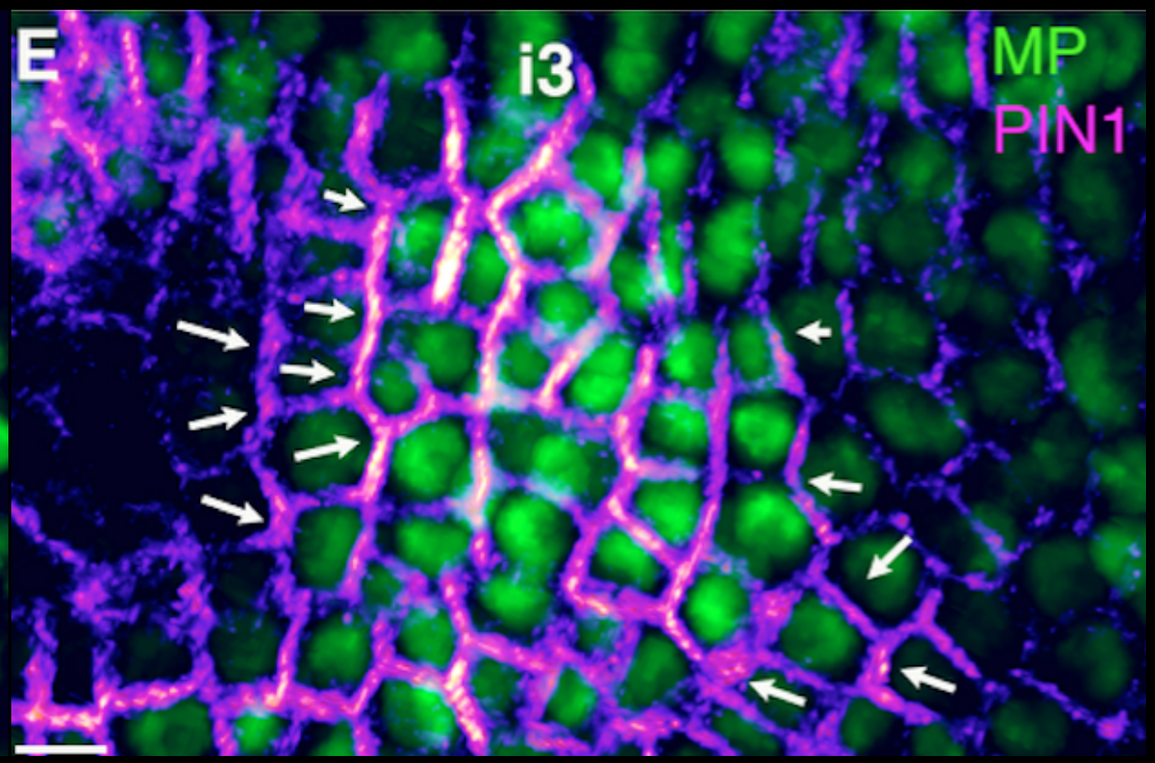
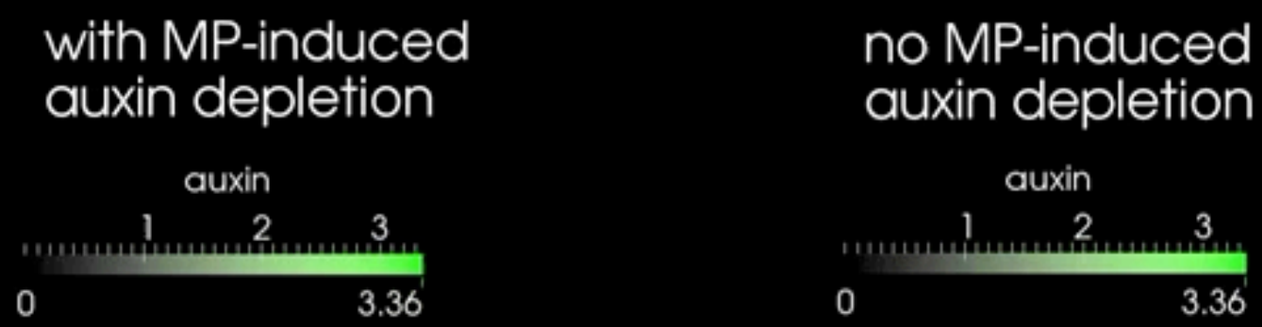




Phenotype can be recapitulated by models if auxin levels are too high



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