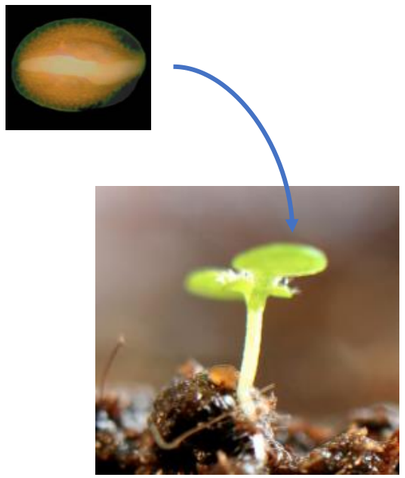
# Collaborative auxin signaling for pattern formation in embryonic leaves



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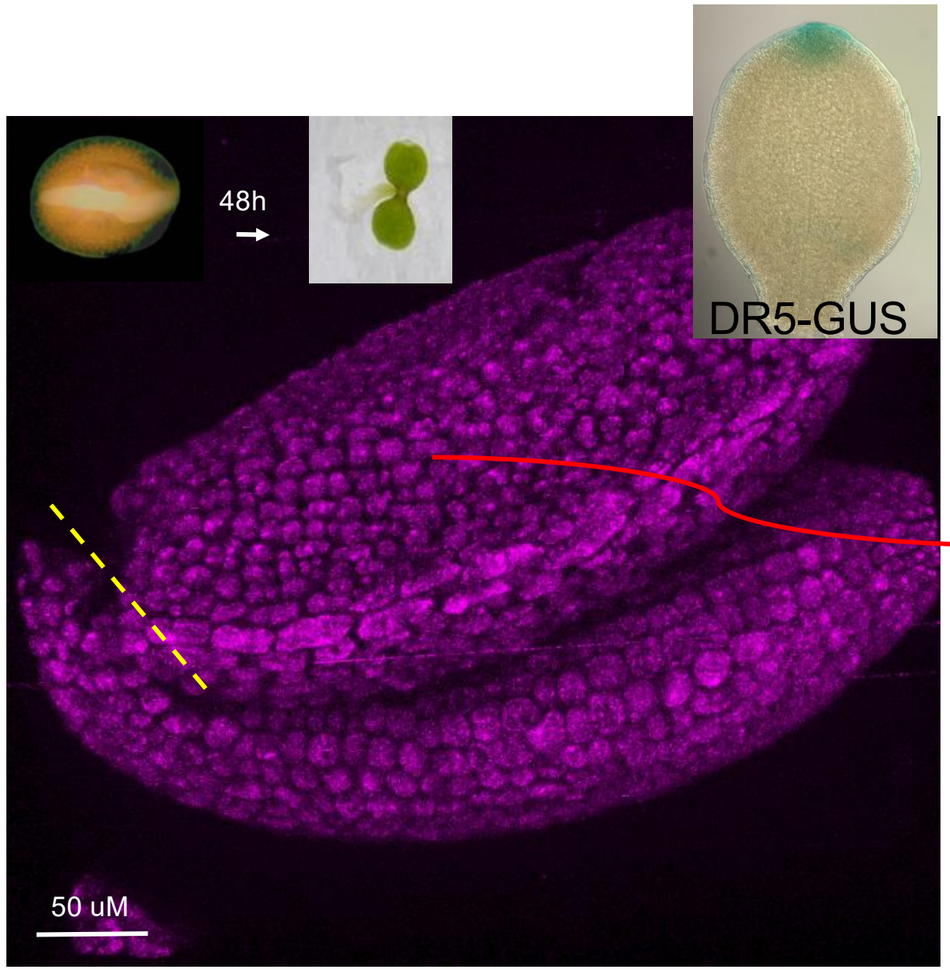
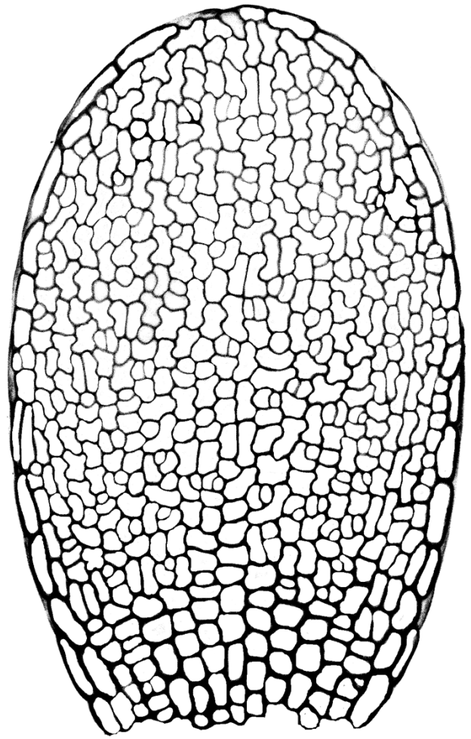
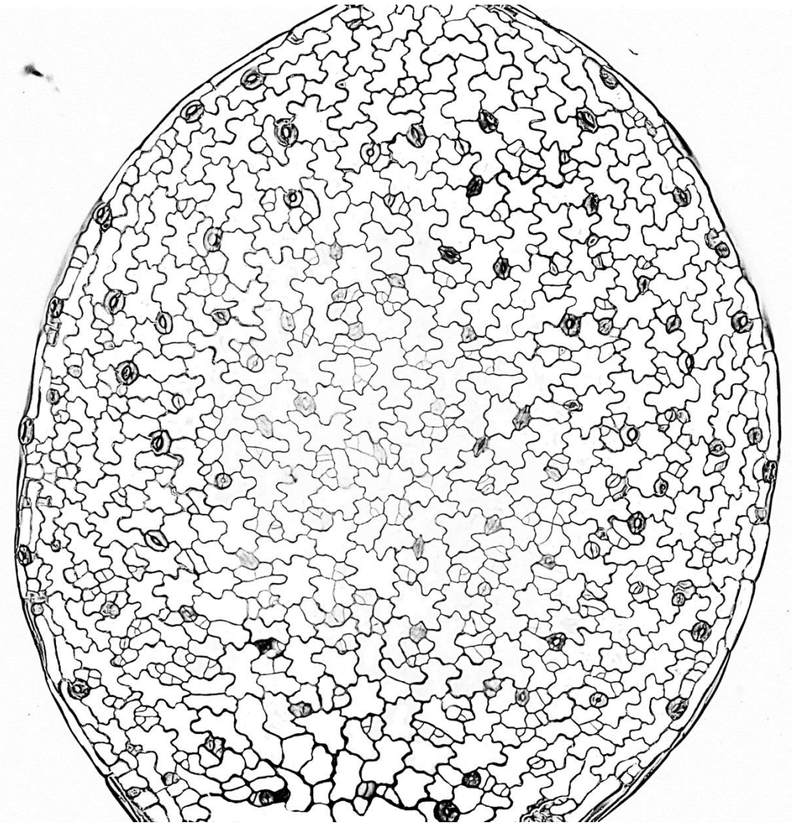
Zhenbiao Yang Lab
University of California, Riverside

Zhenbiao Yang Lab (2016-2022)
Jaimie Van Norman Lab (2022-to date)

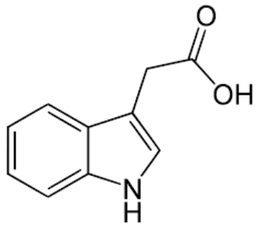
# Cotyledon expansion for understanding pattern formation

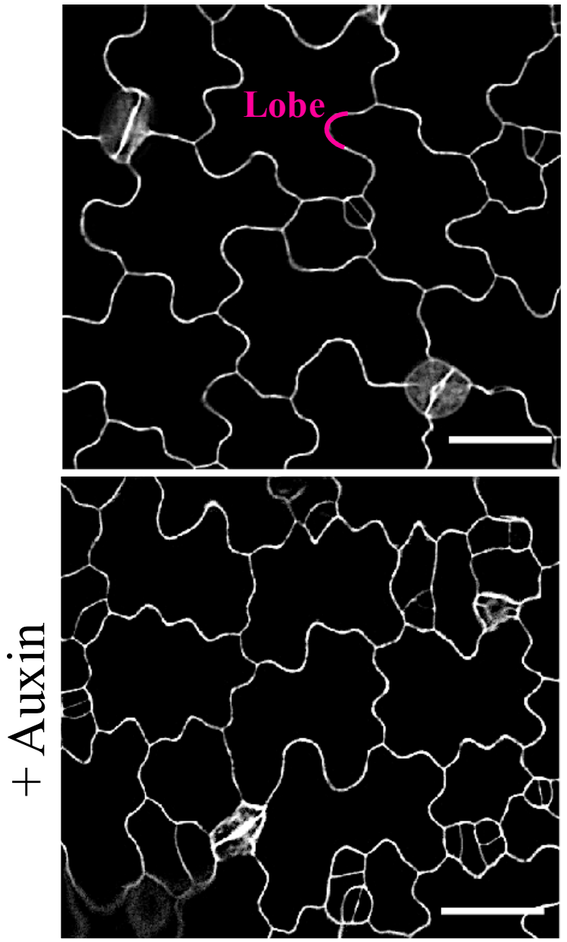


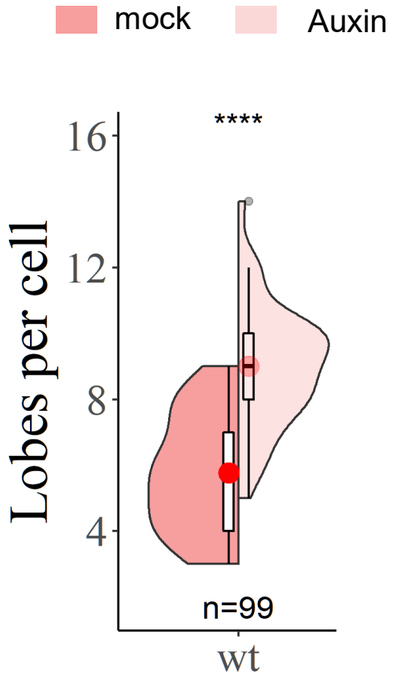



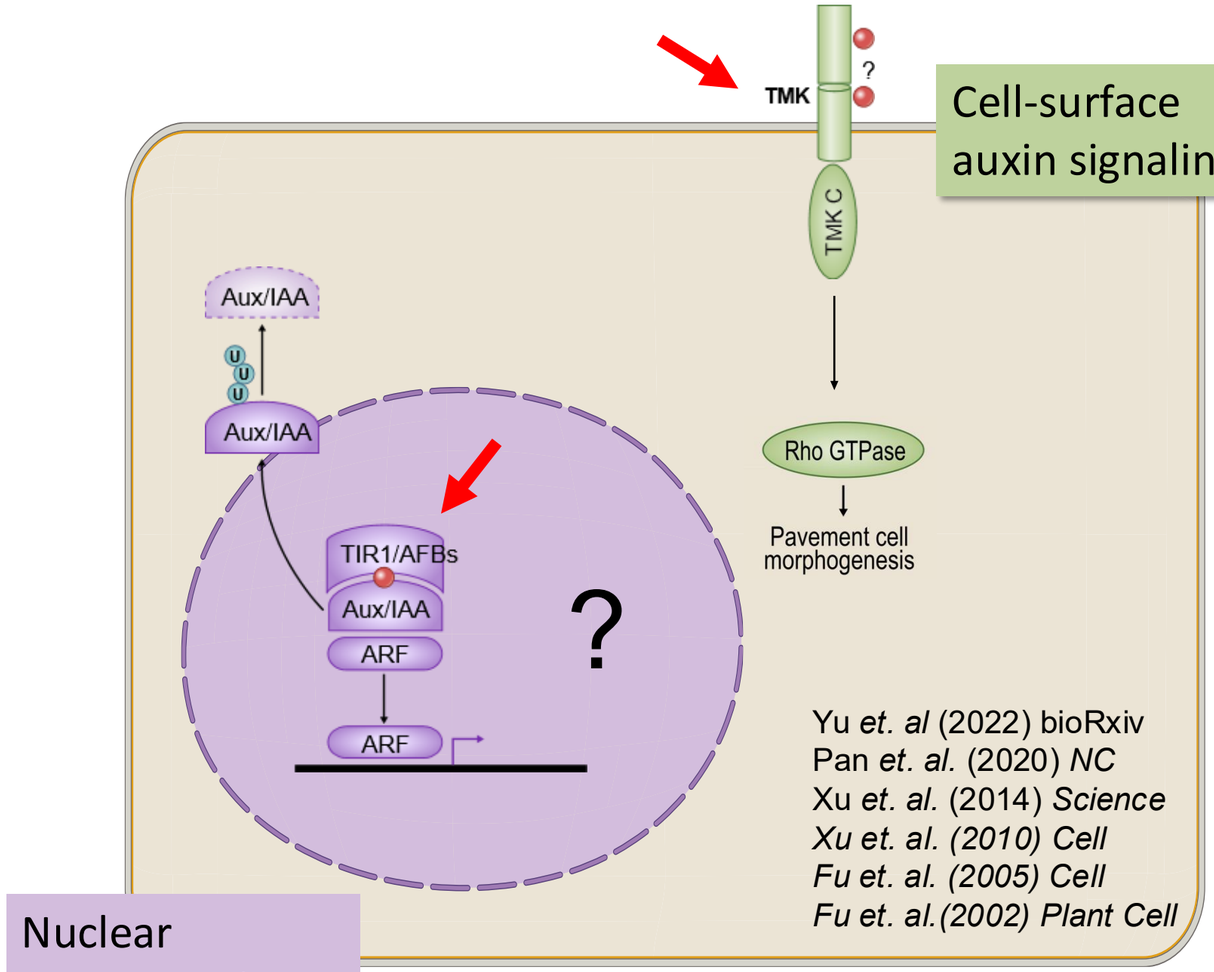


# Auxin signaling and pavement cell morphogenesis

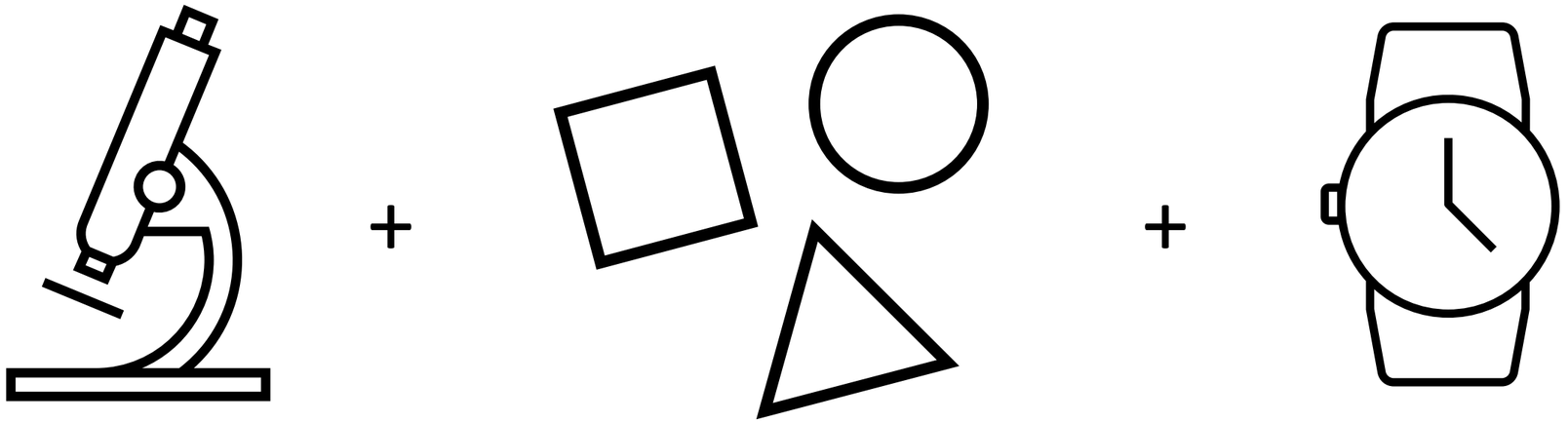


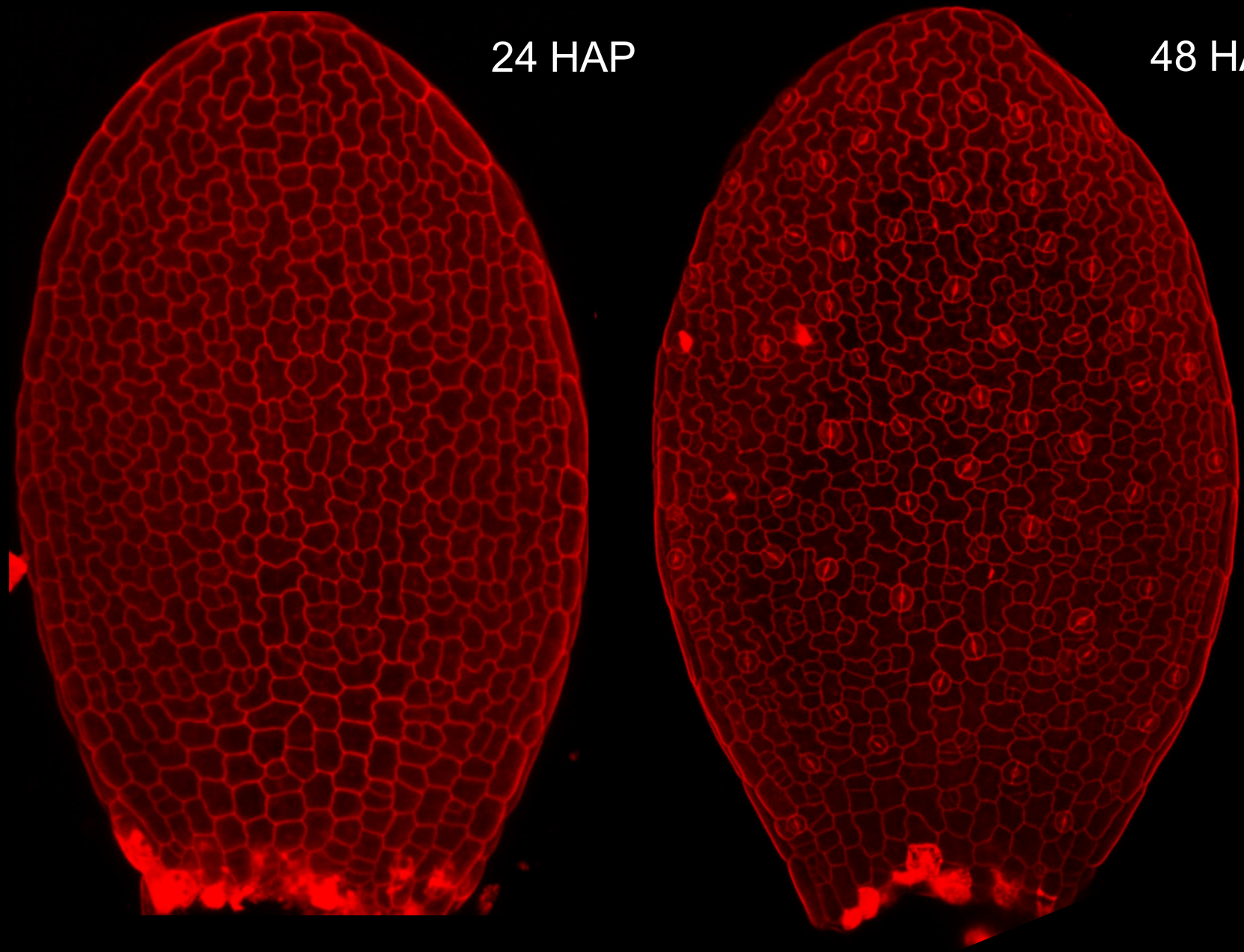




Yu *et. al* (2022) bioRxiv
Pan *et. al.* (2020) *NC*
Xu *et. al.* (2014) *Science*
*Xu et. al. (2010) Cell*
*Fu et. al. (2005) Cell*
*Fu et. al.(2002) Plant Cell*



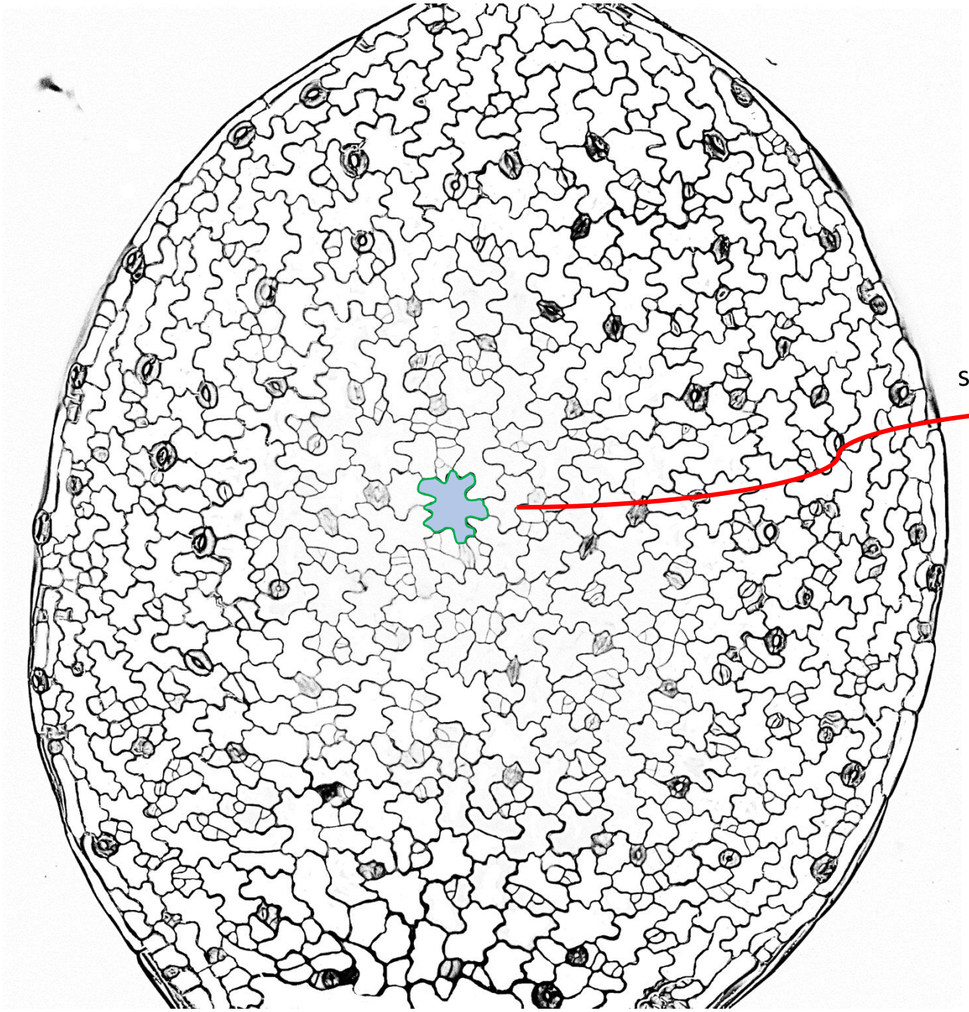

24 HAP
48 HAP

“A picture is worth a thousand words”

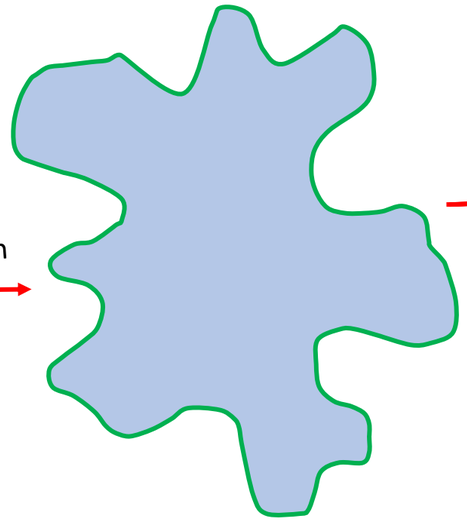
Confucius
Napoleon
Fred Barnard

but a formula is worth a thousand pictures”

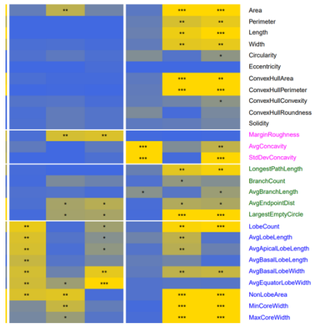
Edsger Dijkstra



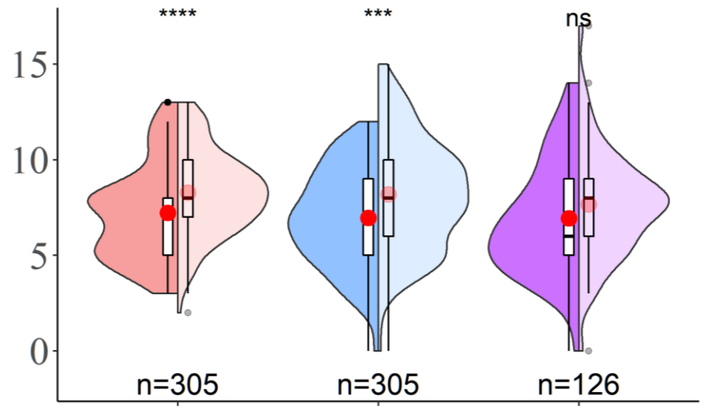
segmentation



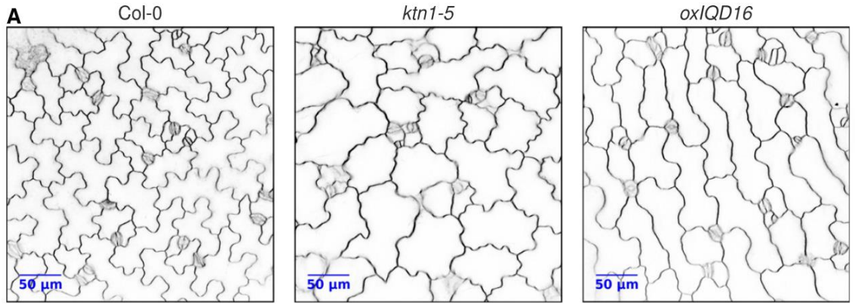
Calculate 28 descriptors

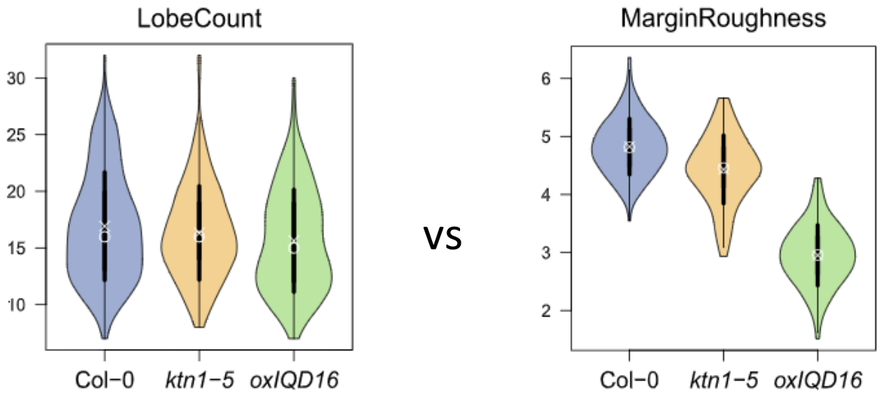



graph



# A new descriptor that accounts for early lobes

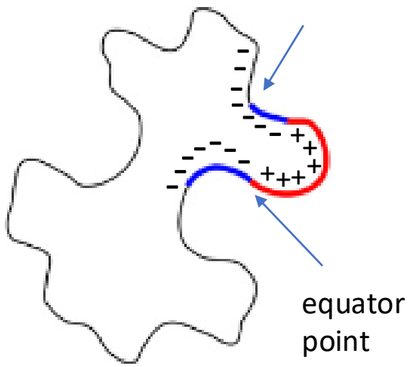



vs

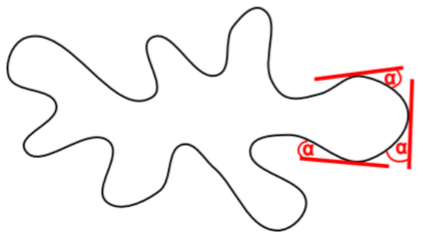



| | | Lobe Count | Margin Roughness |
|---|---|---|---|
| Early Lobe |  | undetectable | detectable |
| Late Lobe |  | detectable | detectable |



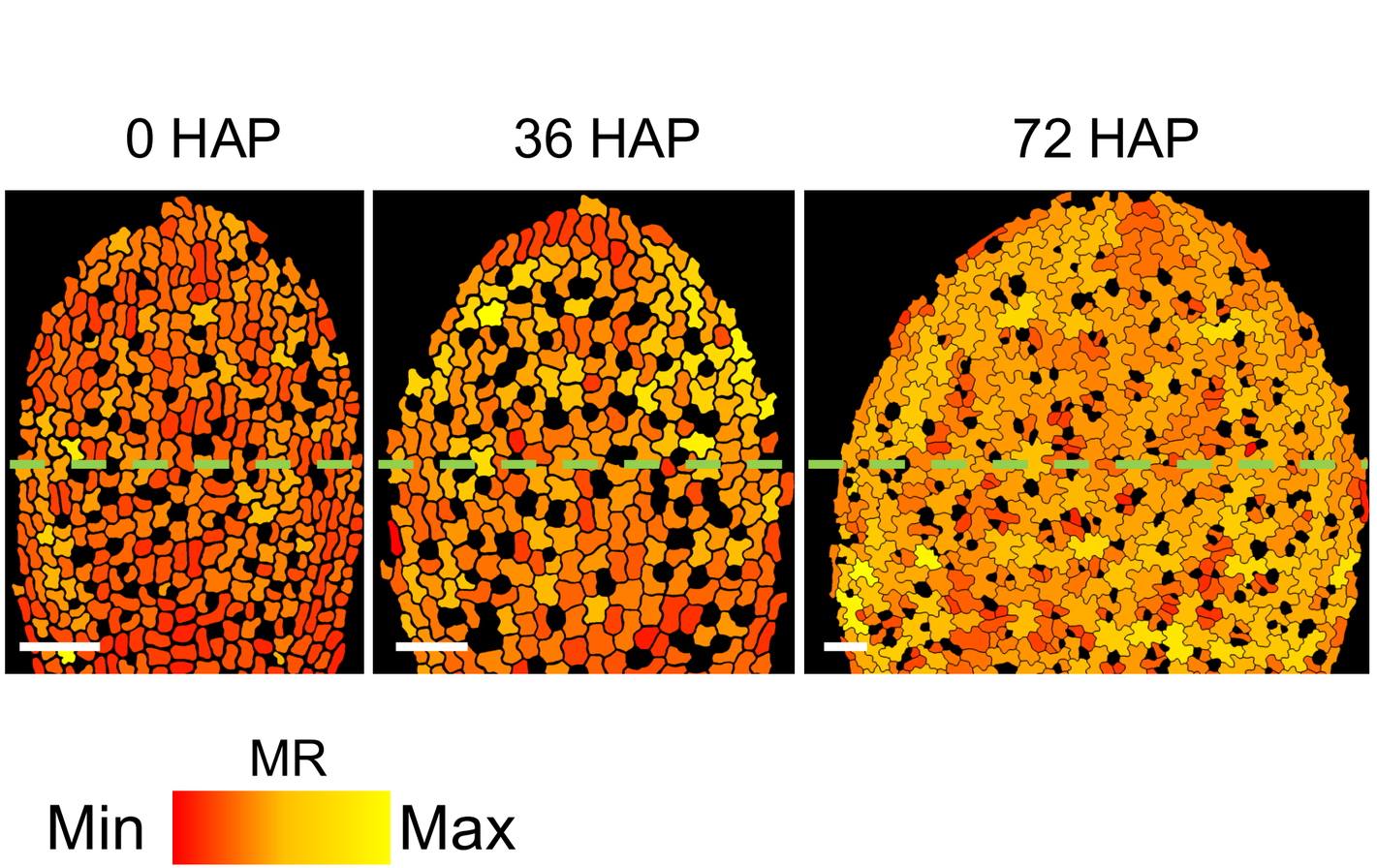

Lobes = Necks /2

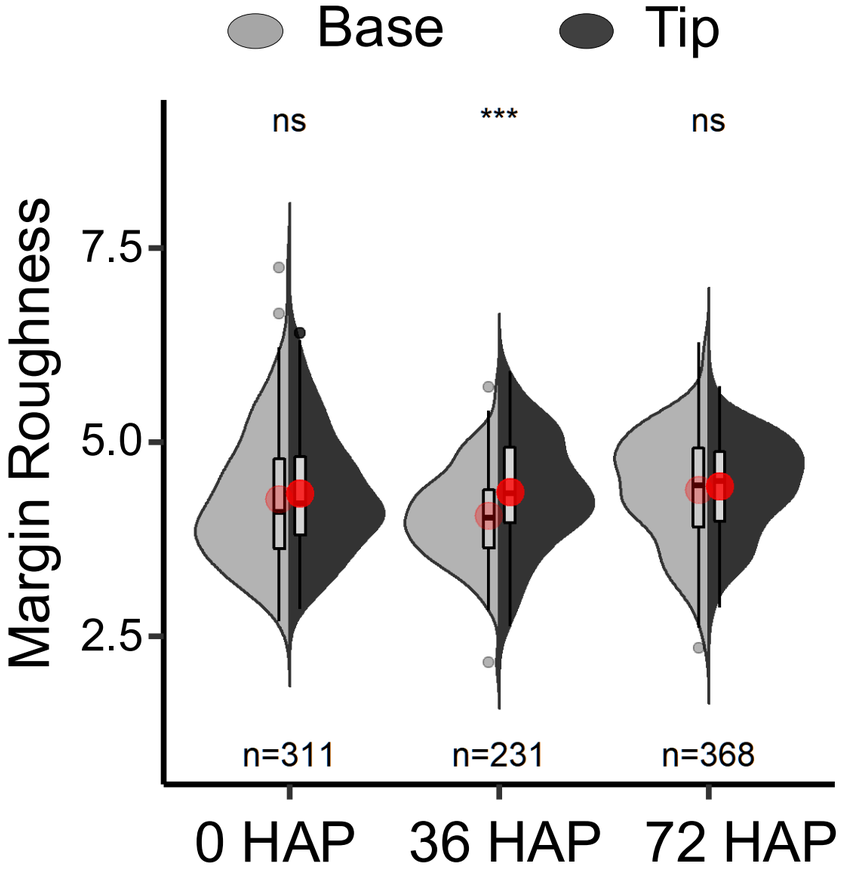
$$MR(R) = \left(\frac{1}{N}\sum_{i=1}^{N}|A_i|\right) - \frac{360}{N},$$



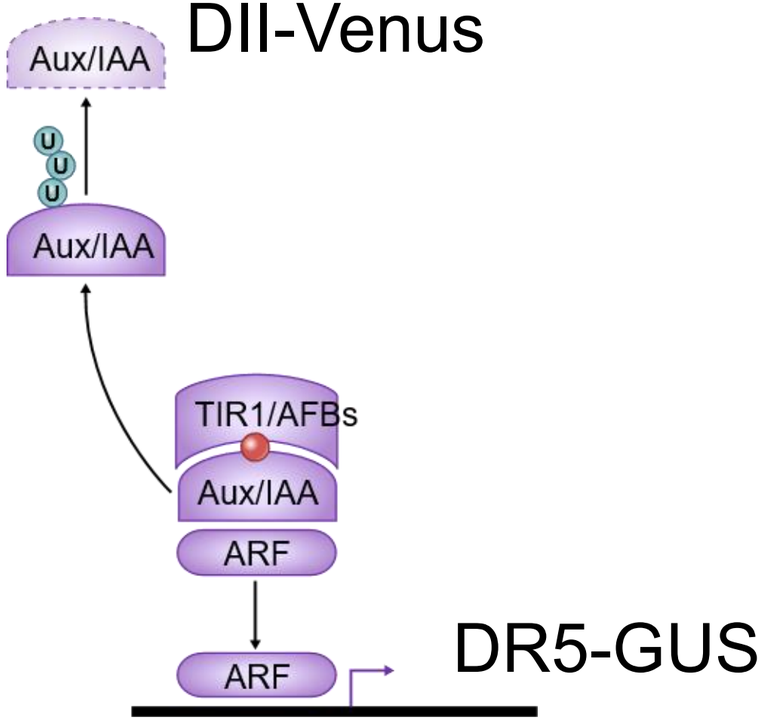

Moller et al 2017. *Plant Phys*

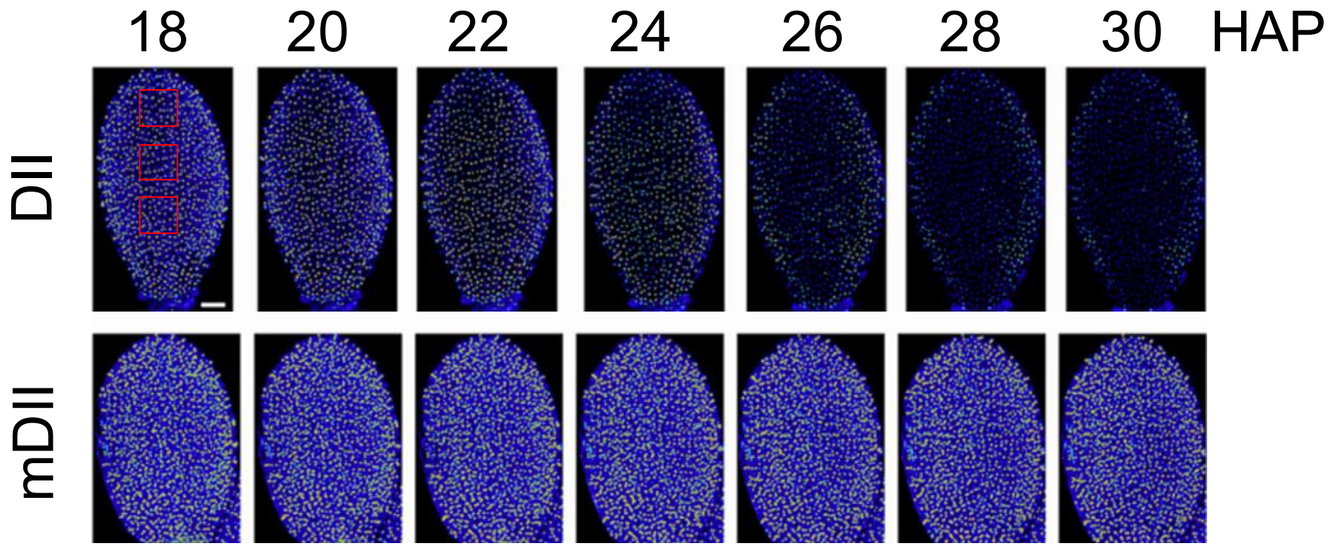
# Transient interdigitation gradient

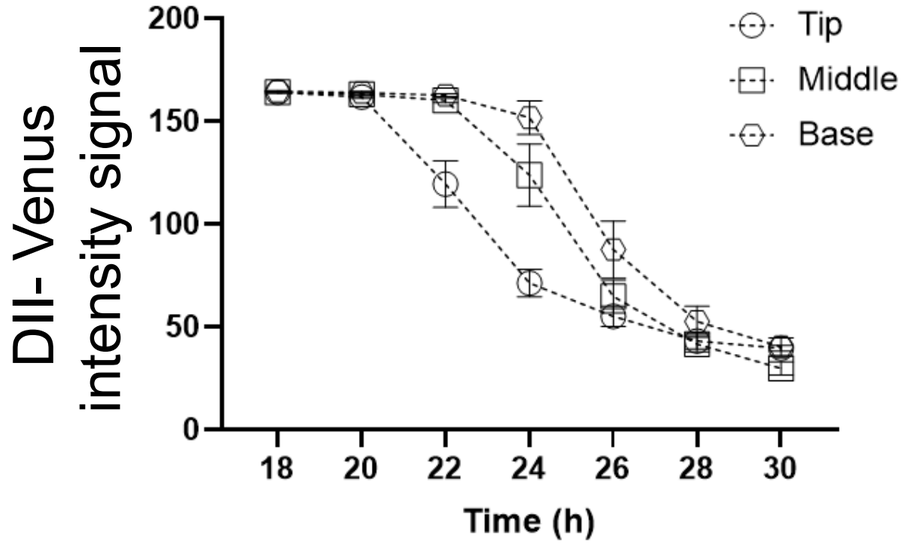


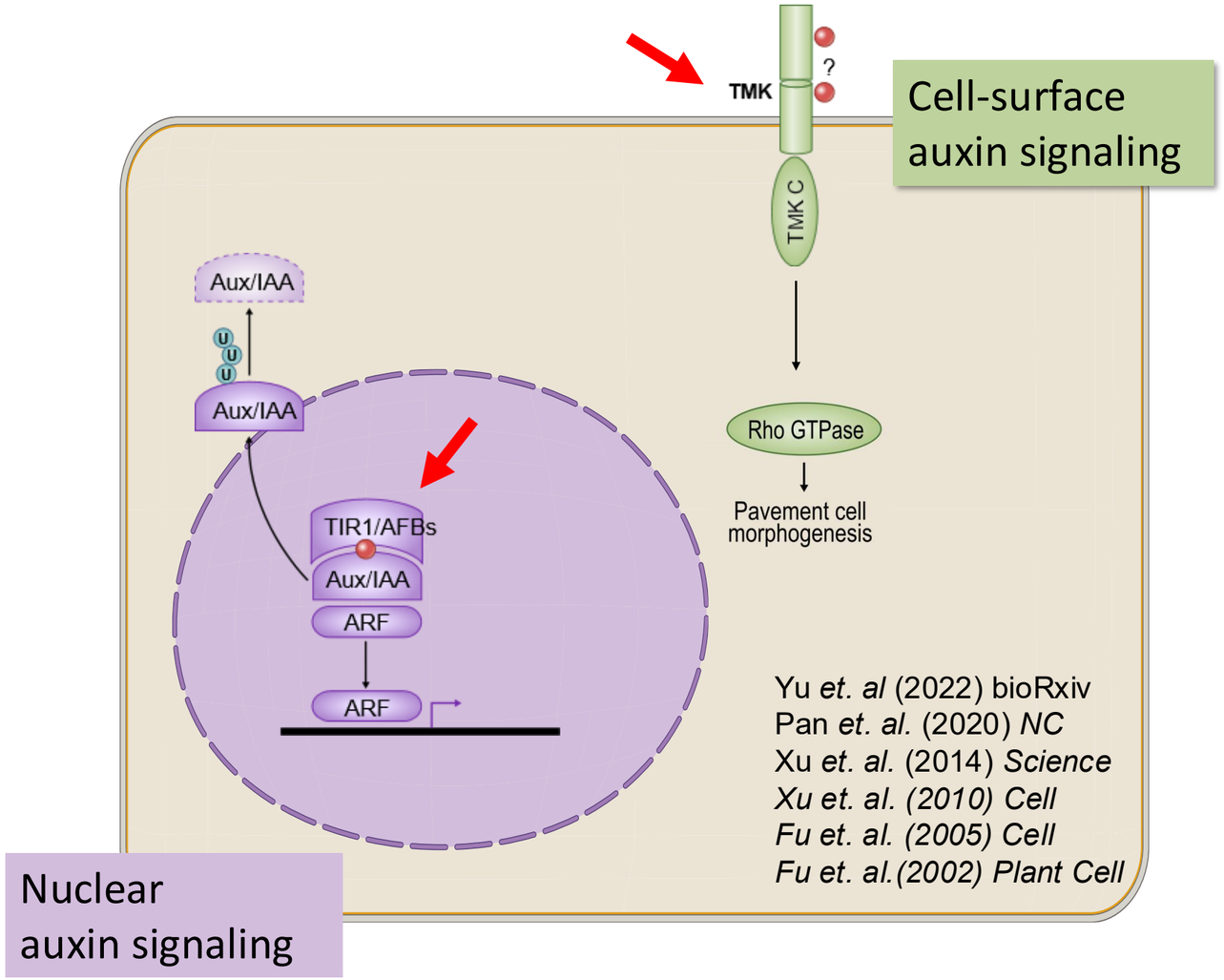



# Transient auxin wave precedes interdigitation gradient



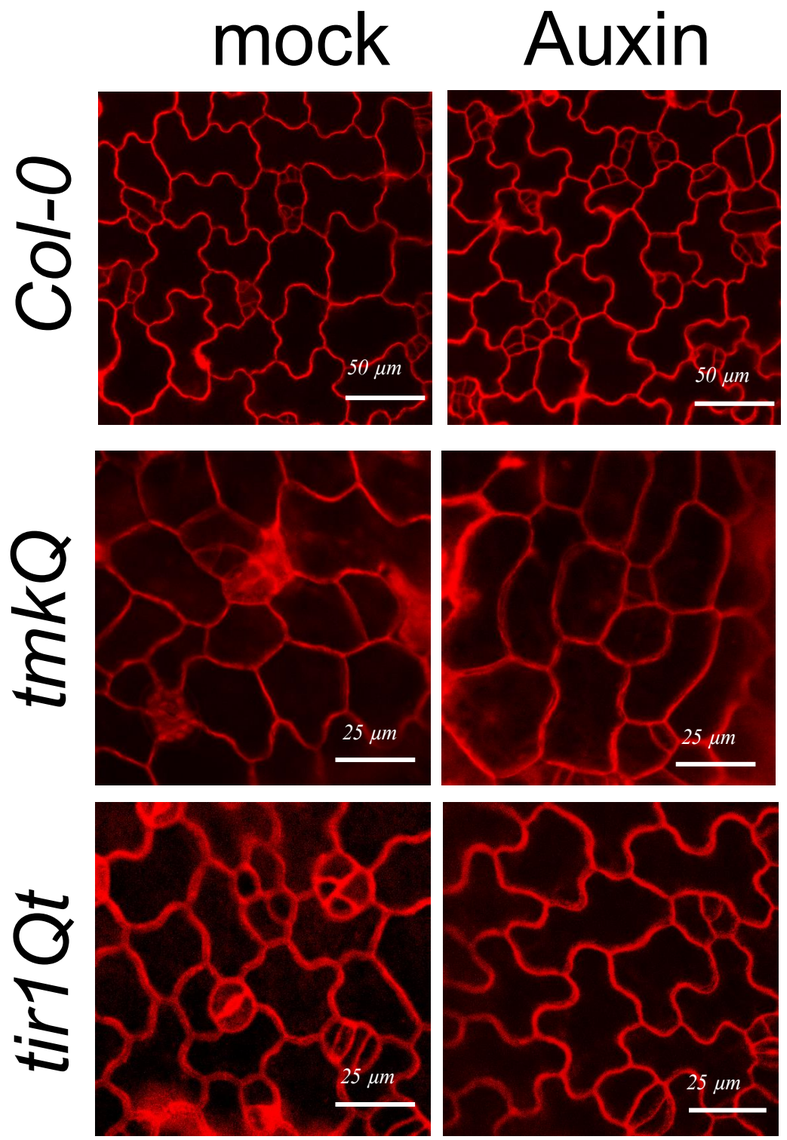



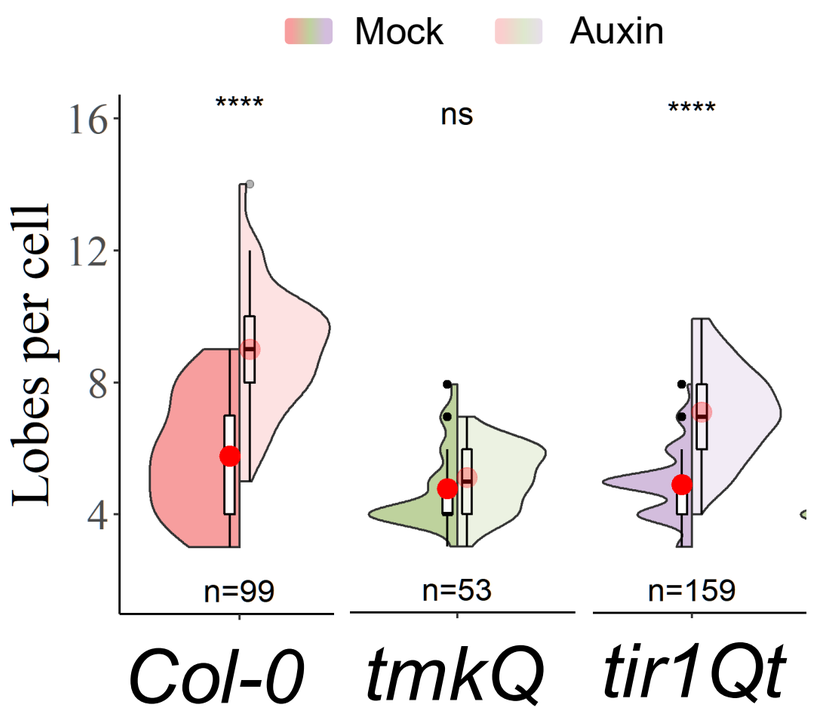



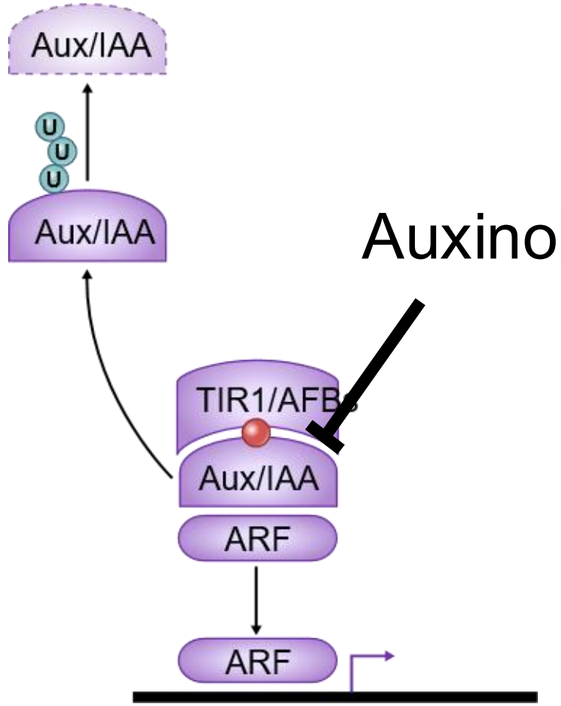



Is there a functional coordination between nuclear and surface auxin signaling ?

# Nuclear auxin signaling is involved in interdigitation



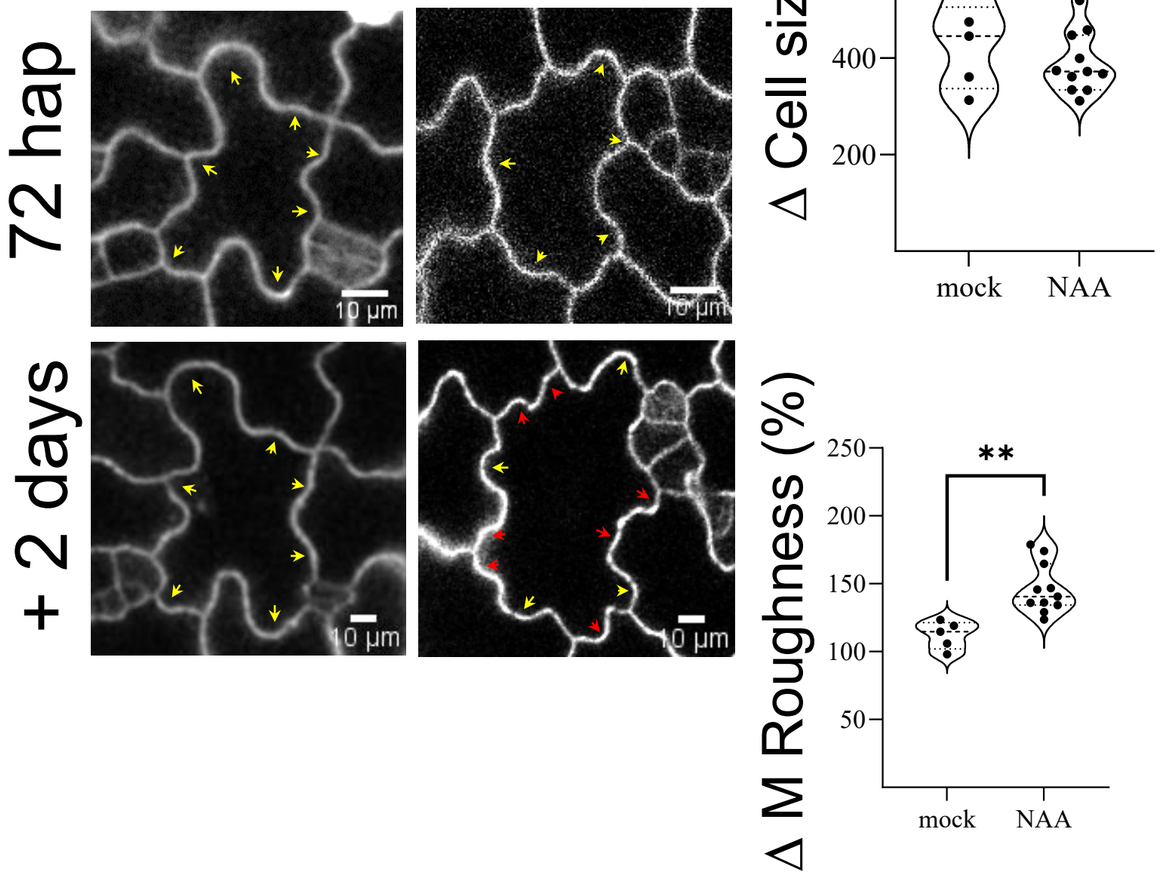




Nuclear auxin signaling acting via regulating auxin levels?

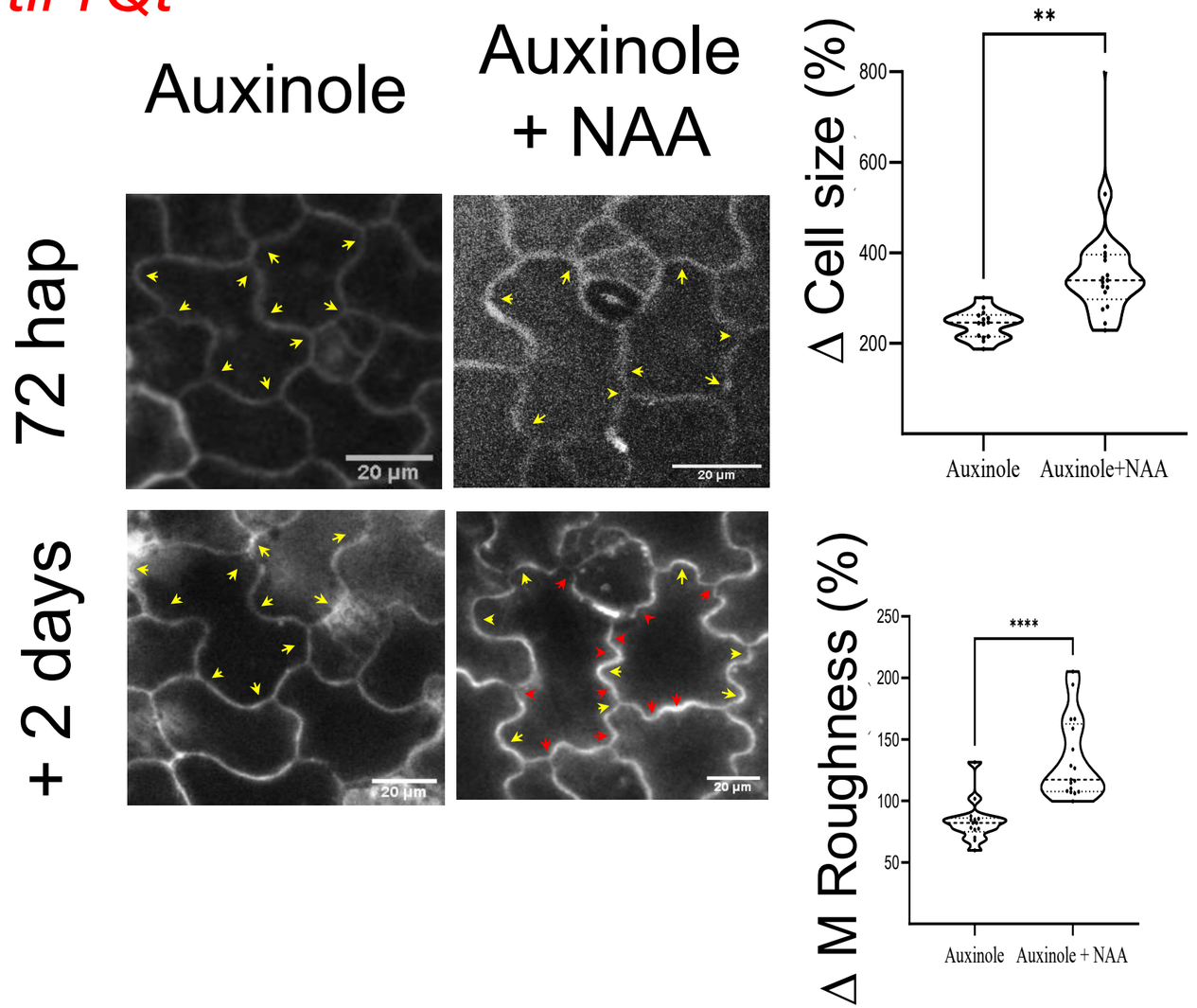
Xu et al. (2014) *Science*

# Single cell tracking corroborates nuclear auxin signaling role in interdigitation

*Col-0*



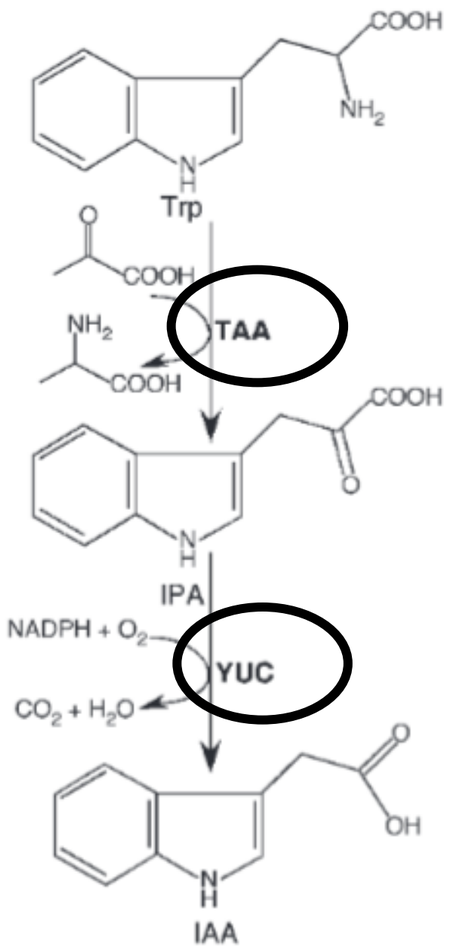

*tir1Qt*




Nuclear auxin signaling acts via regulating auxin levels

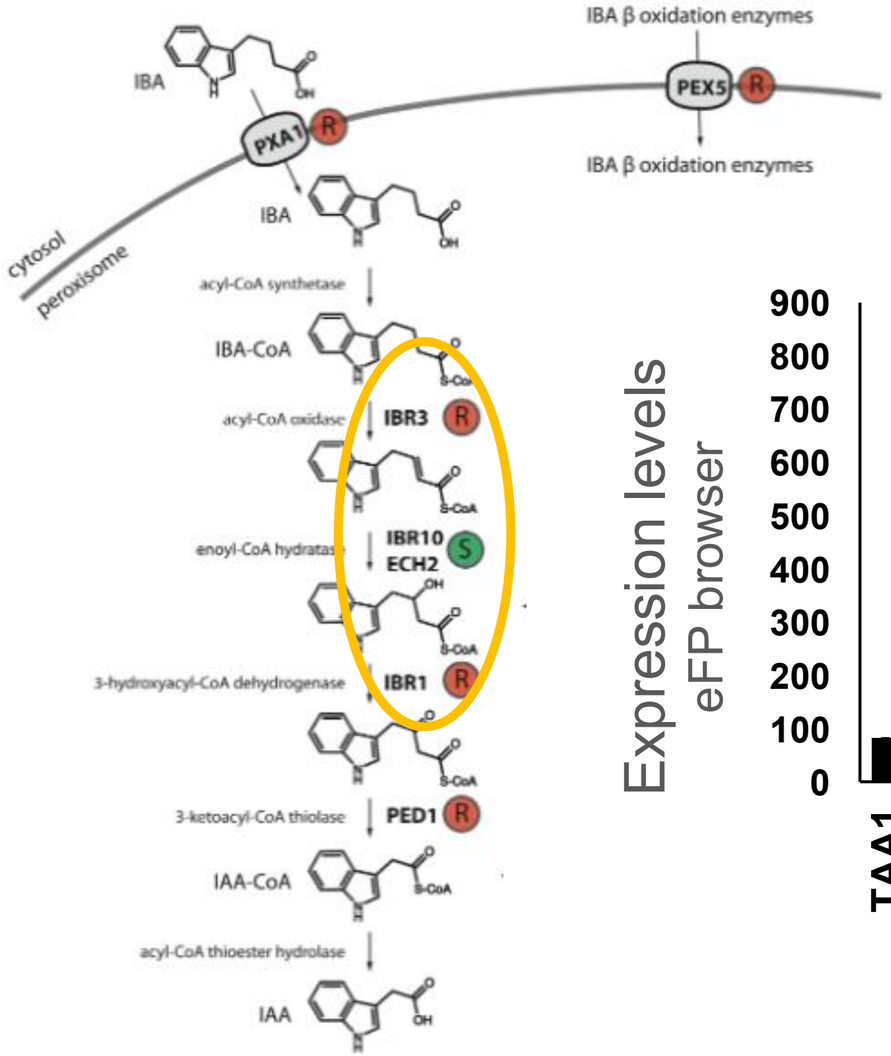
# How?

## IPA pathway

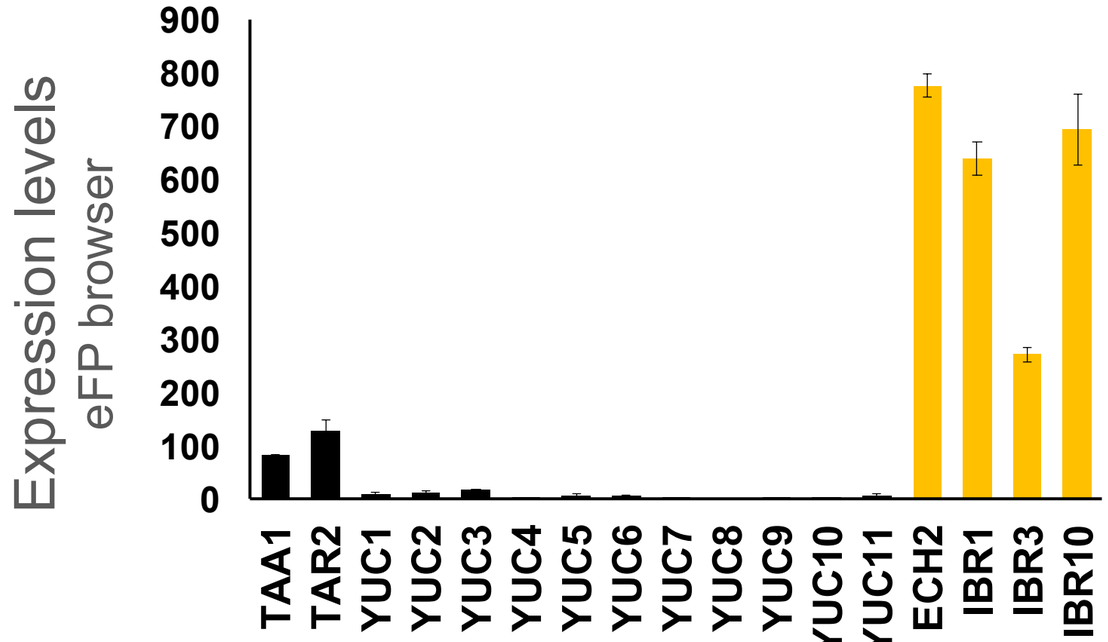



Zhao et al 2012, Molecular Plant

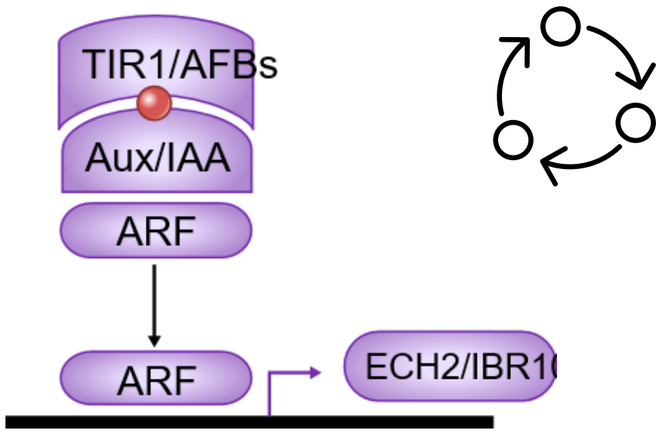
## IBA-to-IAA conversion

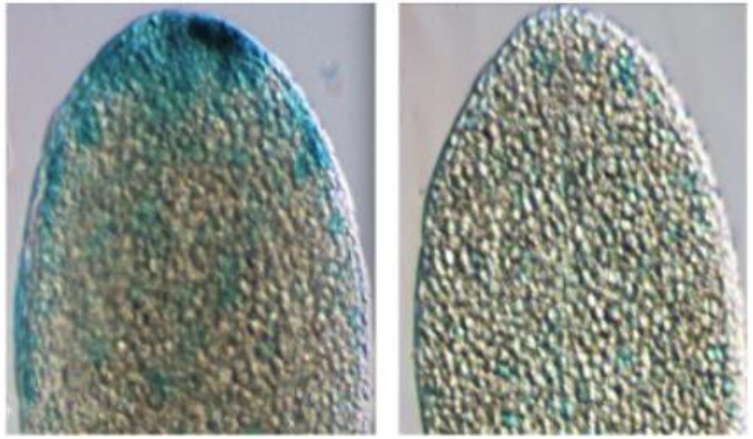


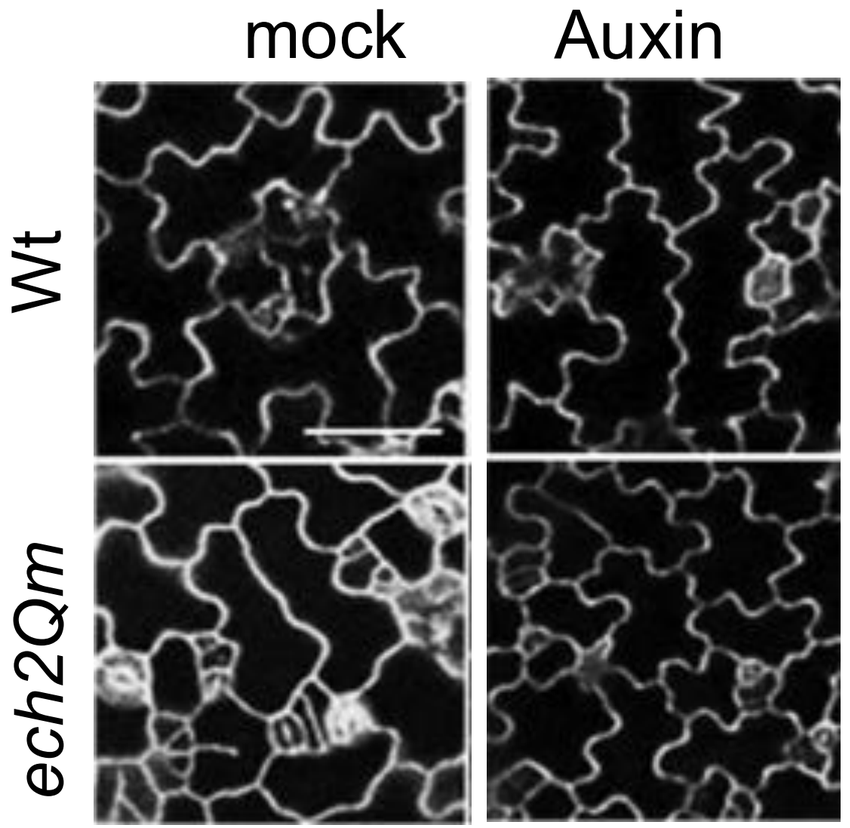

De Rybel et al 2011, Nature Chem Biol

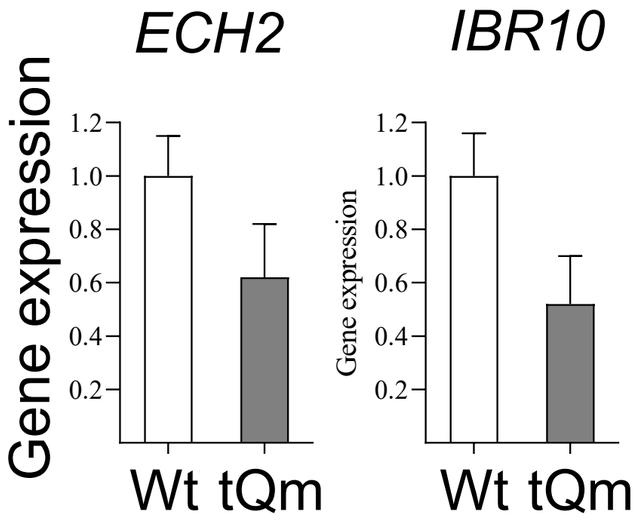


# Nuclear auxin signaling regulates auxin synthesis



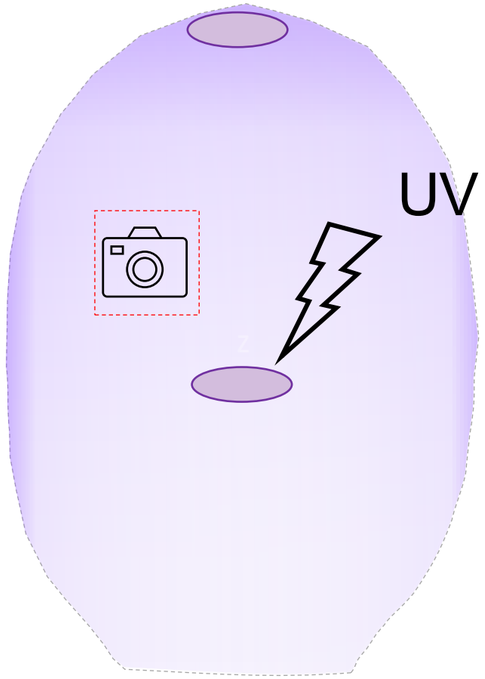



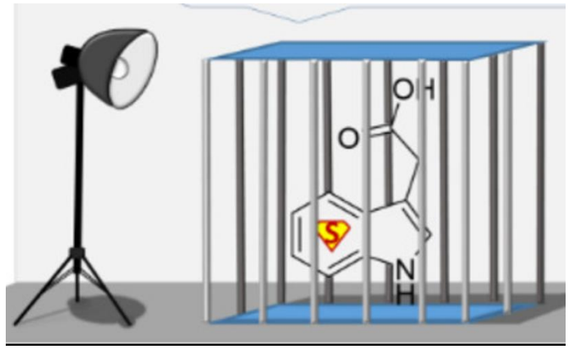



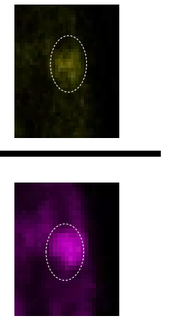

TIR1/AFB auxin signaling function to produce a local auxin maximum at the tip

# Caged auxin to produce ectopic auxin maxima

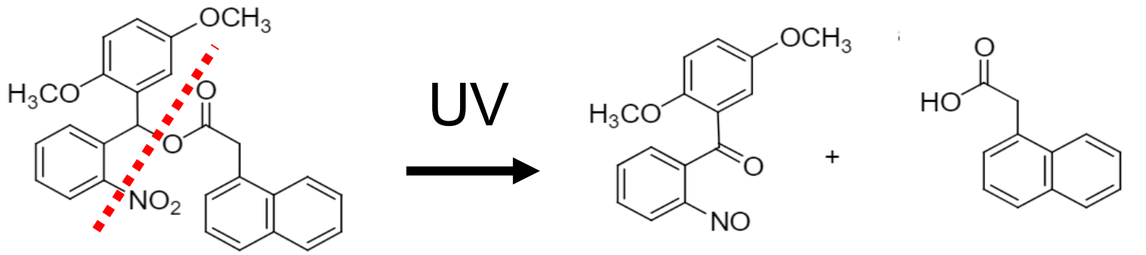


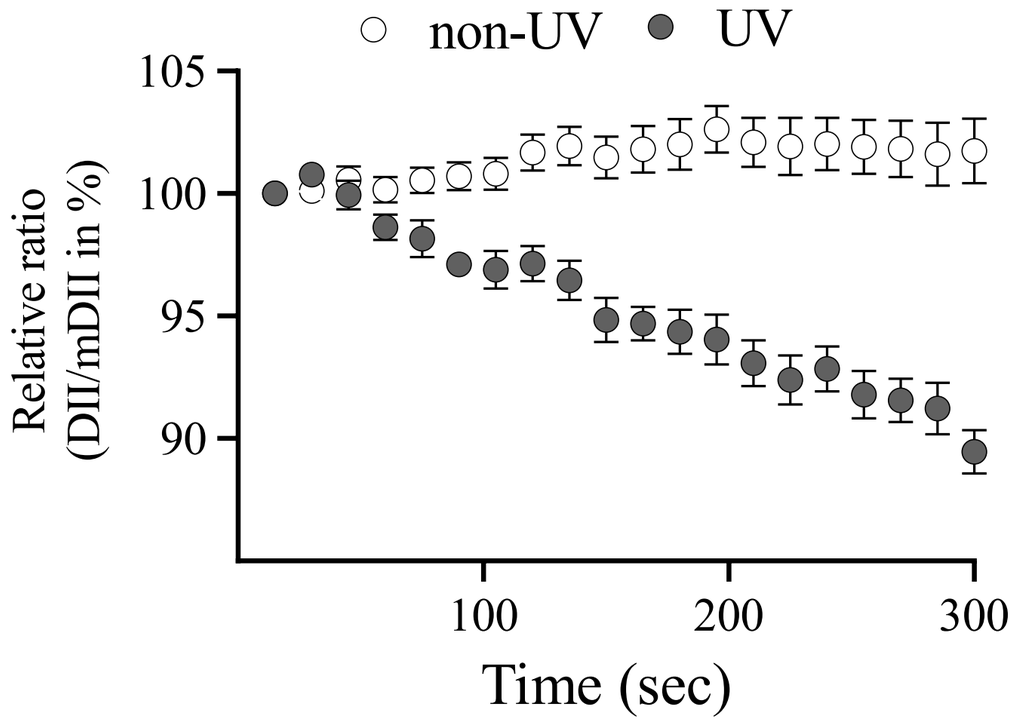
Lights, camera, auxin!

Ratio DII / mDII = 

Lower ratio = more auxin

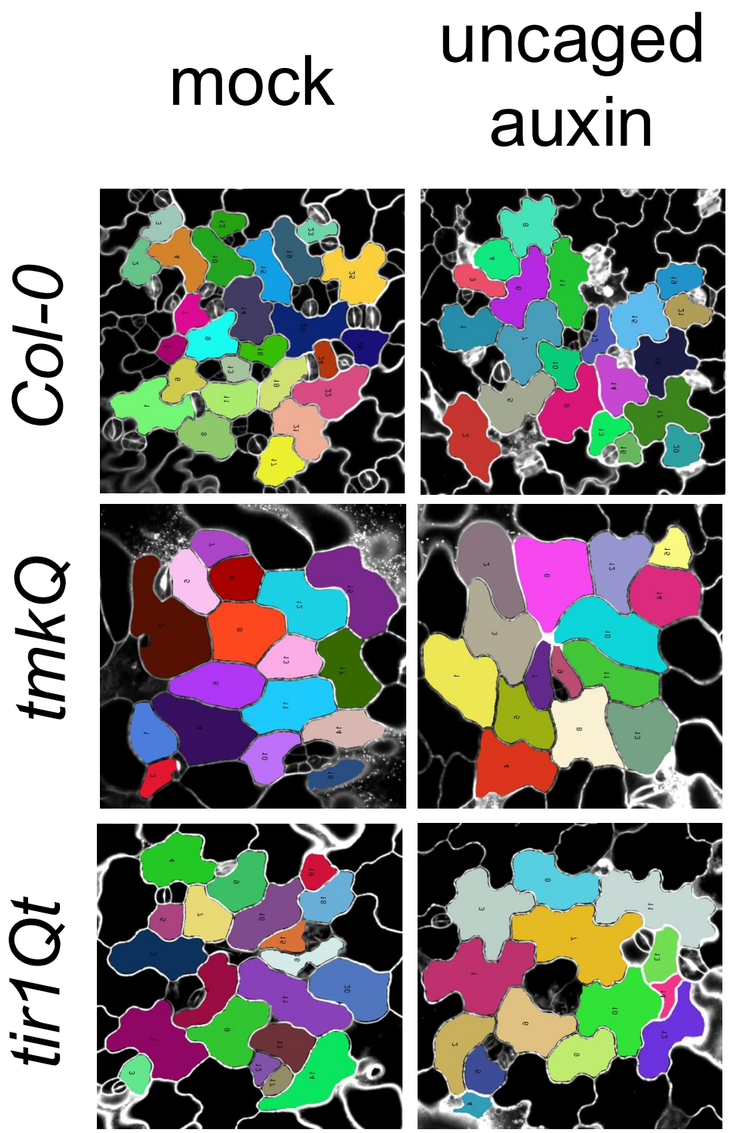


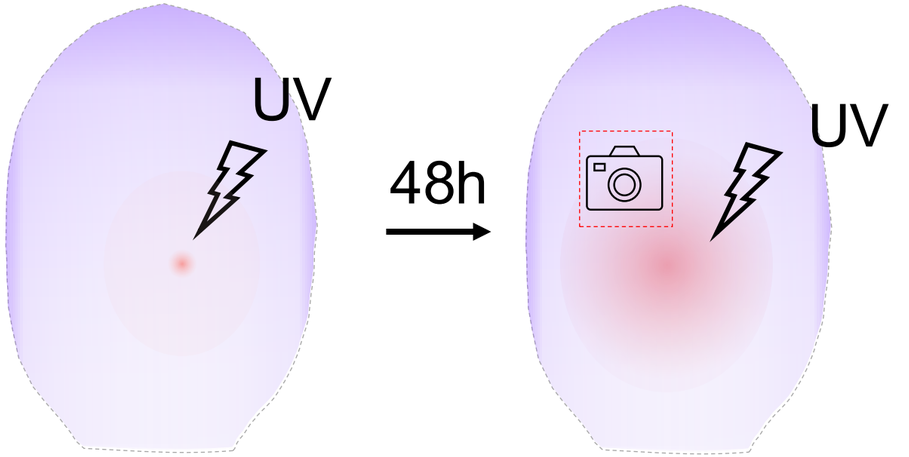

DMPNB-NAA → DMPNB + uncaged NAA

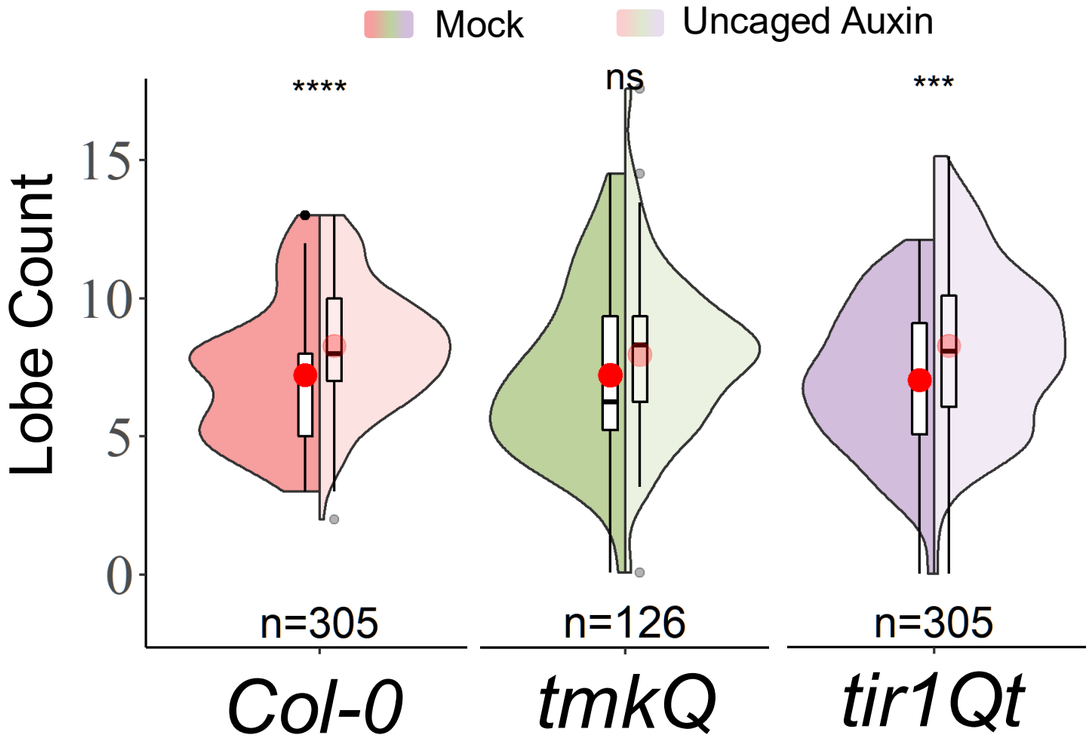



*Hayashi et al 2012, ACS chemical biology*
*Kusaka et al 2009, ChemBioChem*
*Hemelikova et al (2021) J Agric Food Chem*

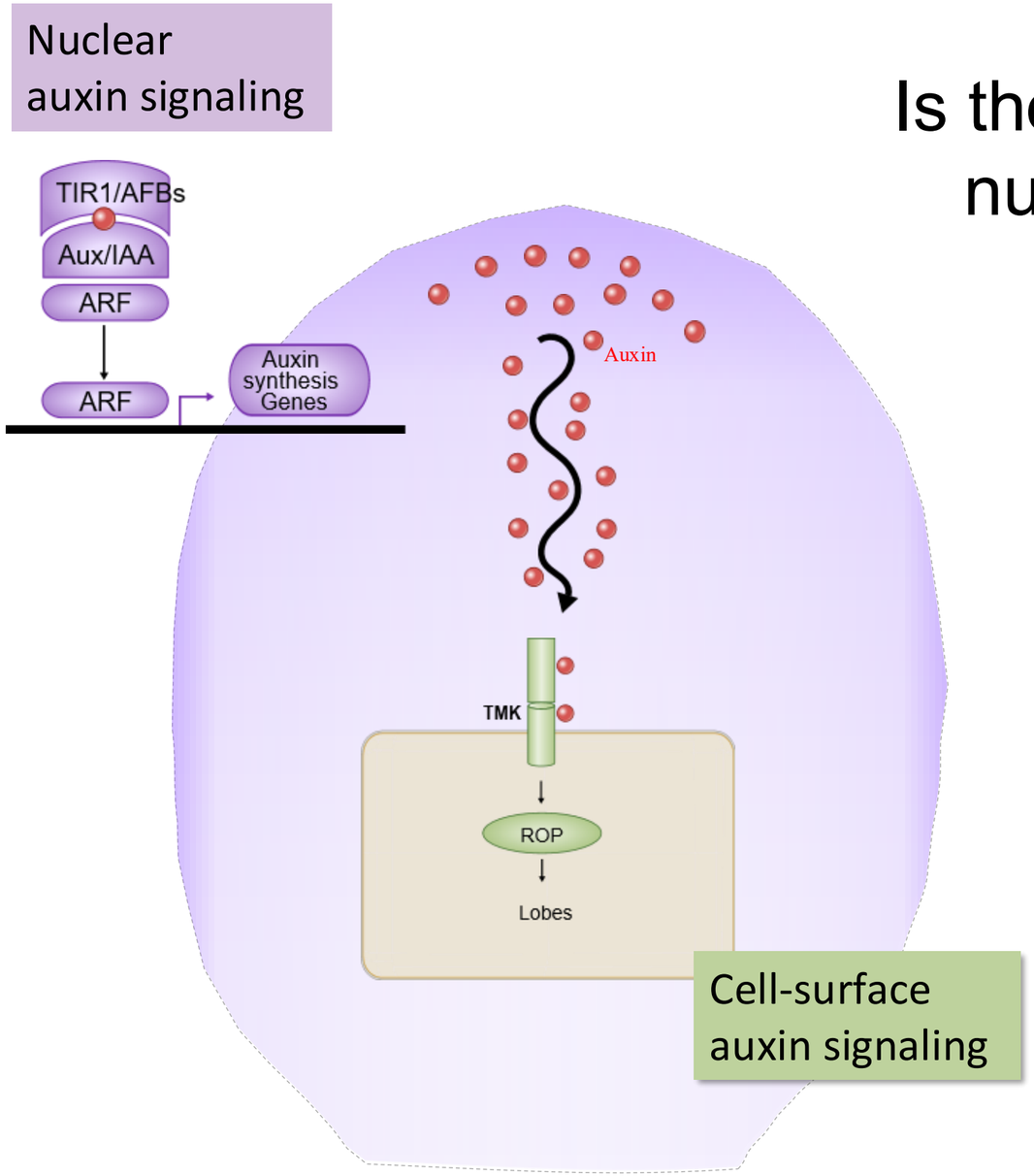
# Local auxin maxima can globally coordinate interdigitation



# Hierarchical auxin signaling




Is there a functional coordination between nuclear and surface auxin signaling ?

Yes!

- Nuclear and cell-surface signaling collaboratively work in patter formation

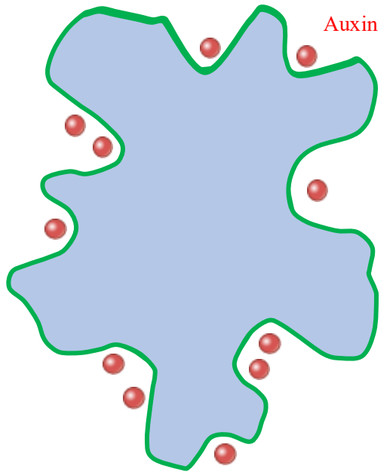
- TIR1/AFB regulates ECH2 to accumulate auxin at the tip

- TMK locally induces lobing

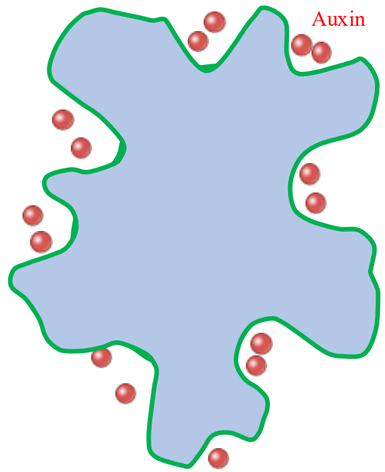
# Future of the interdigitation wave

- Continuous live-imaging

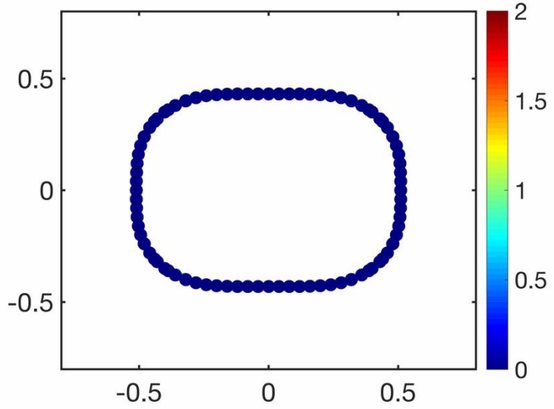
hyp1




hyp2

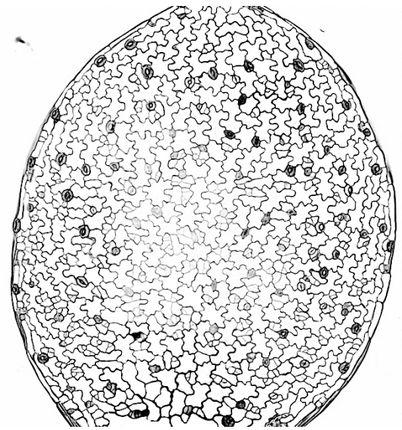



- Mathematical Modeling

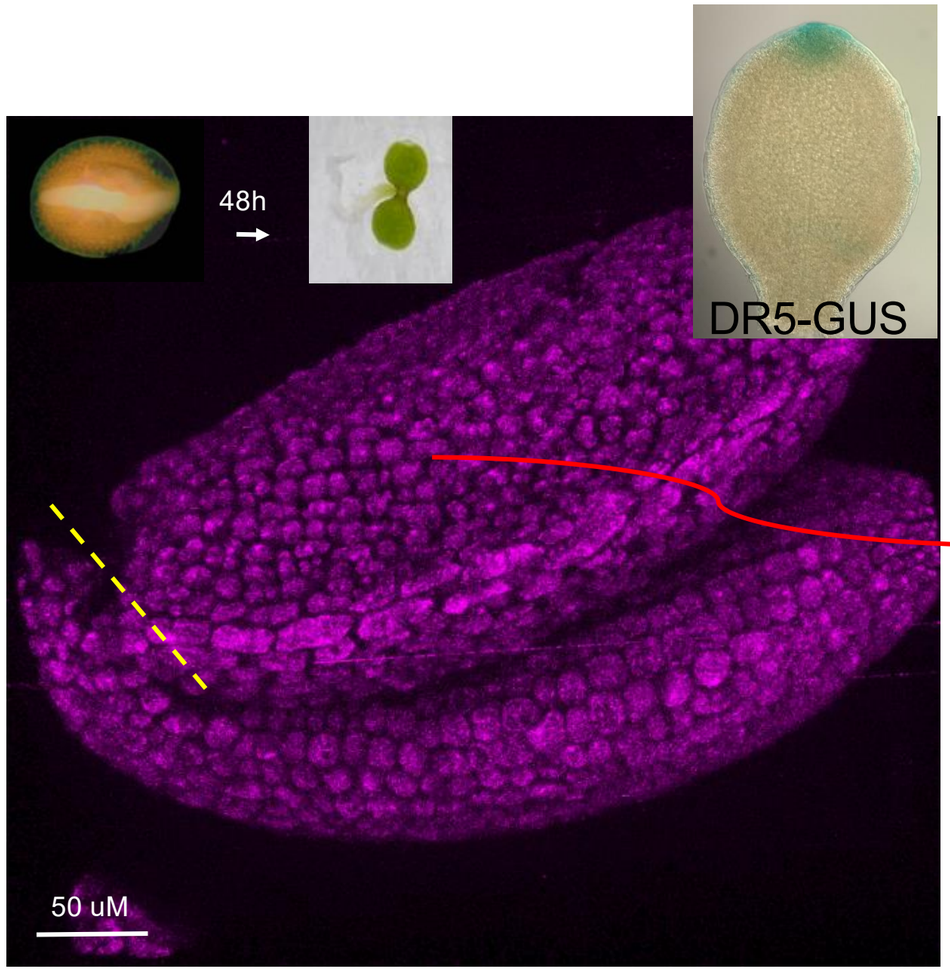


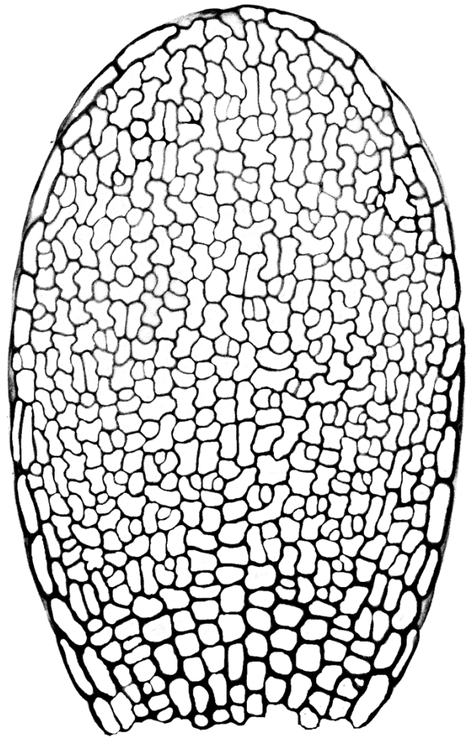

- Xue Pan, U Toronto
- Weitao Chen, UC Riverside

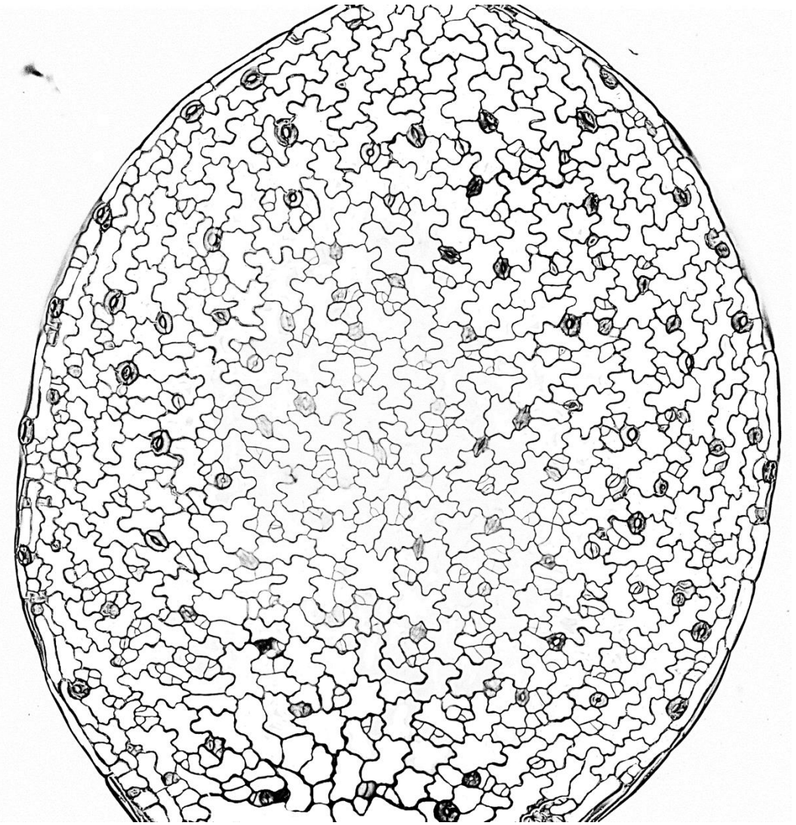
- Tissue wide modeling



# Cotyledon expansion for understanding pattern formation

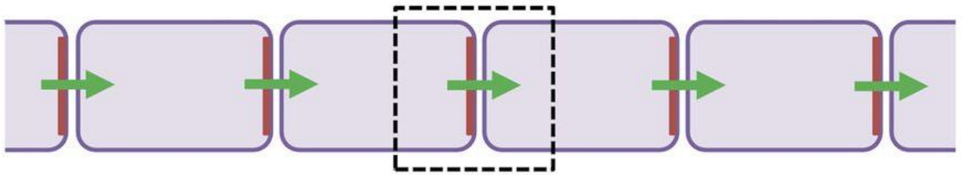




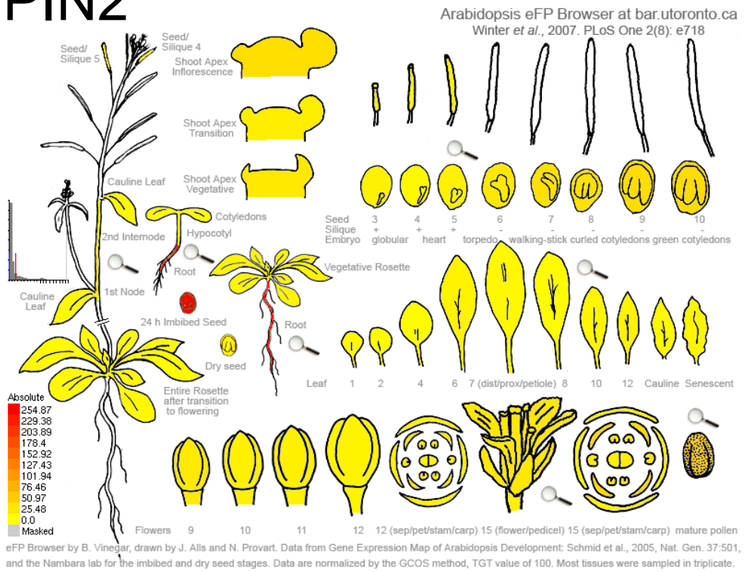
# How is the auxin maxima at the tip formed?

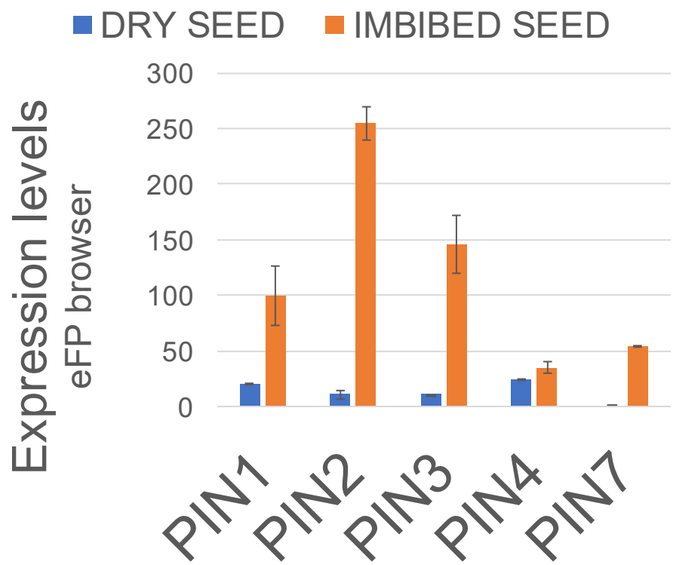
# PIN-based auxin transport



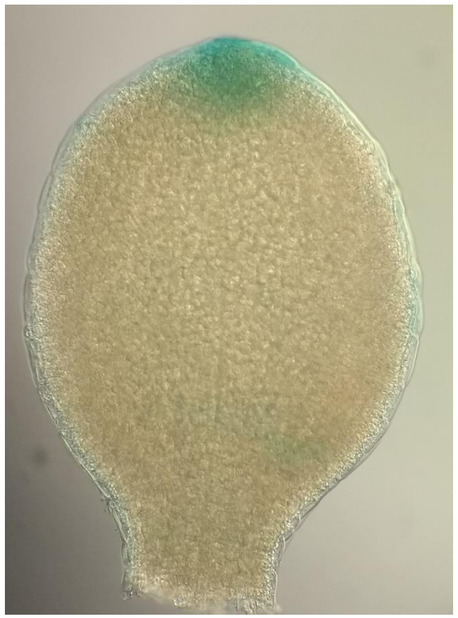
Friml and Jones. 2010. Plant Phys
Adamowski and Friml 2015. The Plant Cell

PIN2



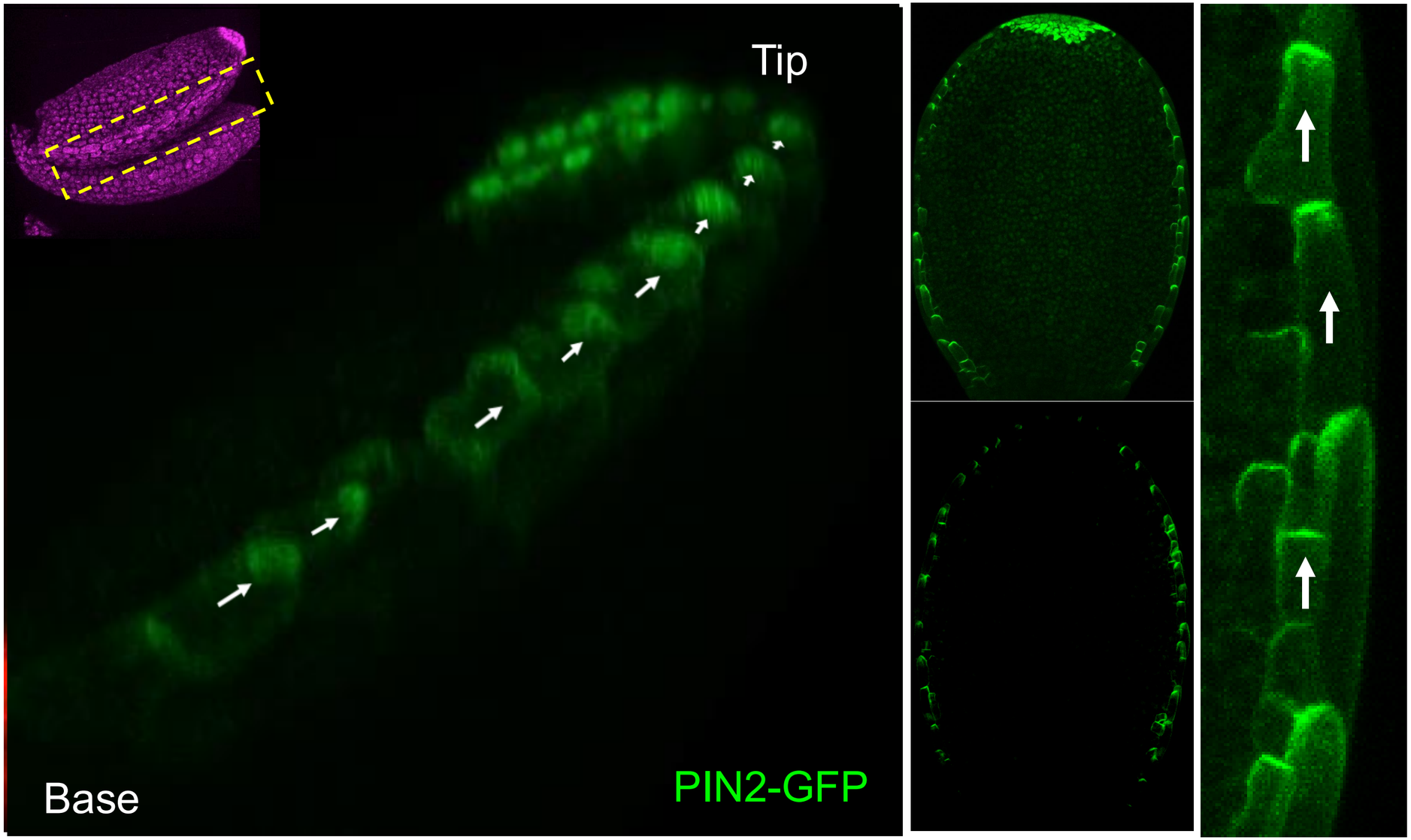




DR5-GUS



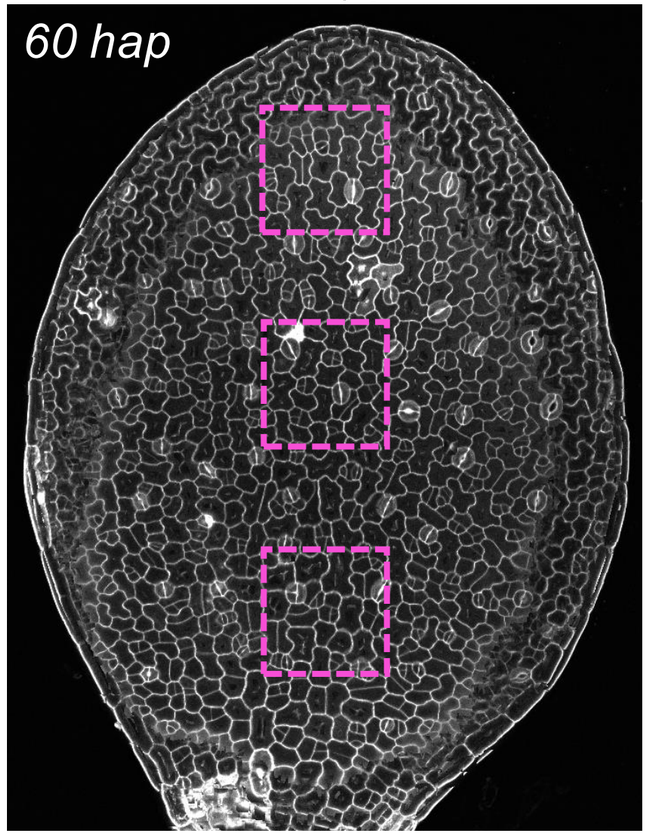
*eir1-1/pin2*
DR5-GUS

# PIN2 is expressed in margin cells (MCs)

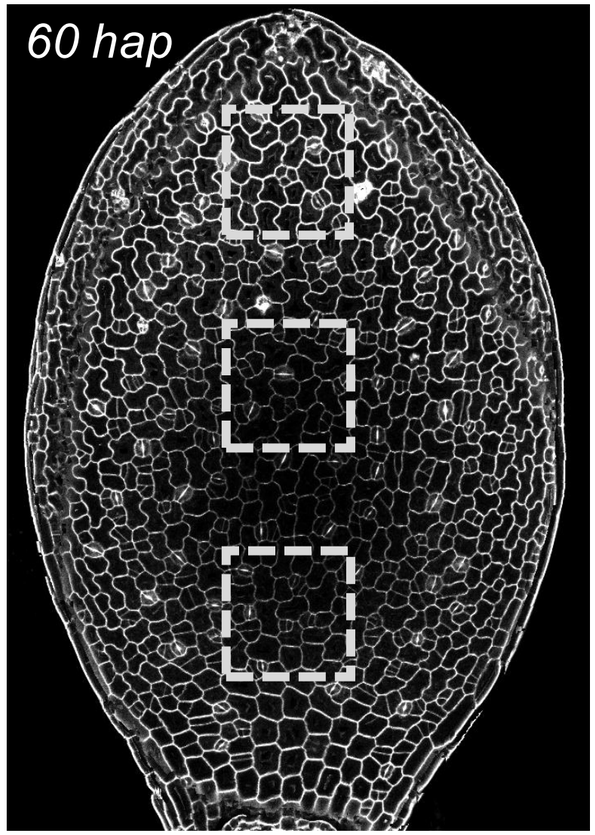


# Interdigitation phenotype of *eir1-1* / pin2 mutant

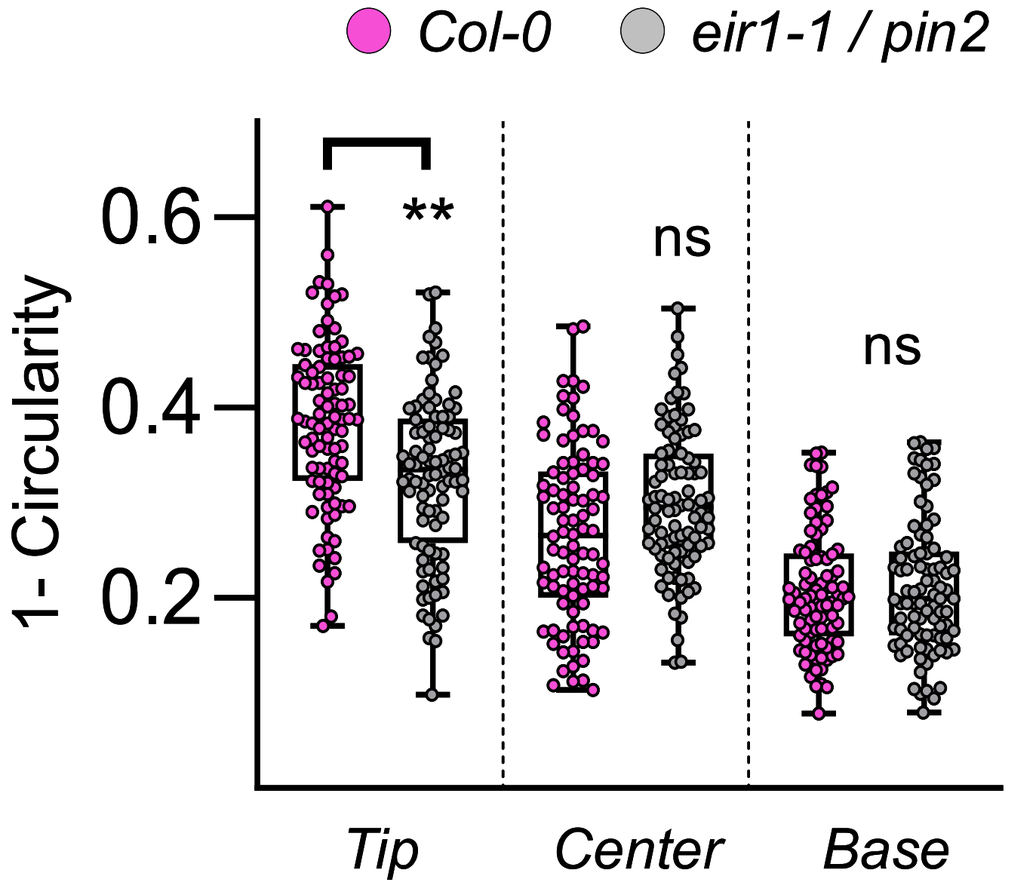
Wild type



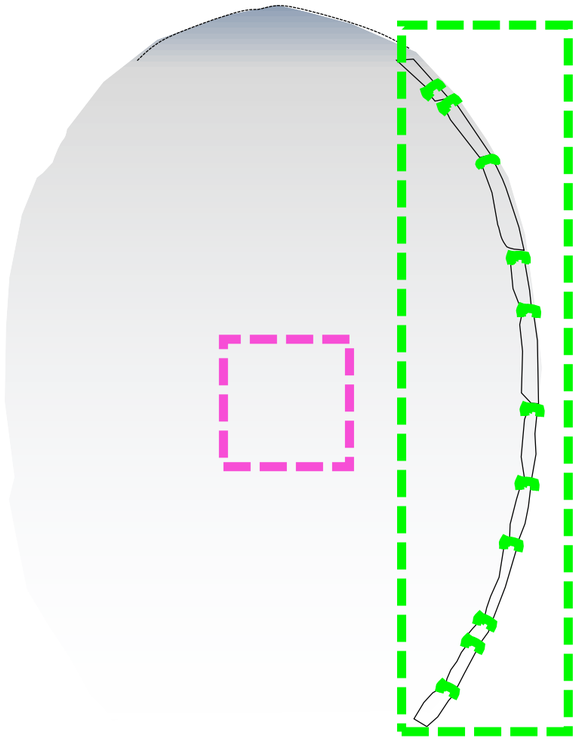

*eir1-1 / pin2*




$$\text{Circularity} = \frac{4 \cdot \pi \cdot [\text{Area}]}{[\text{Perimeter}]^2}$$

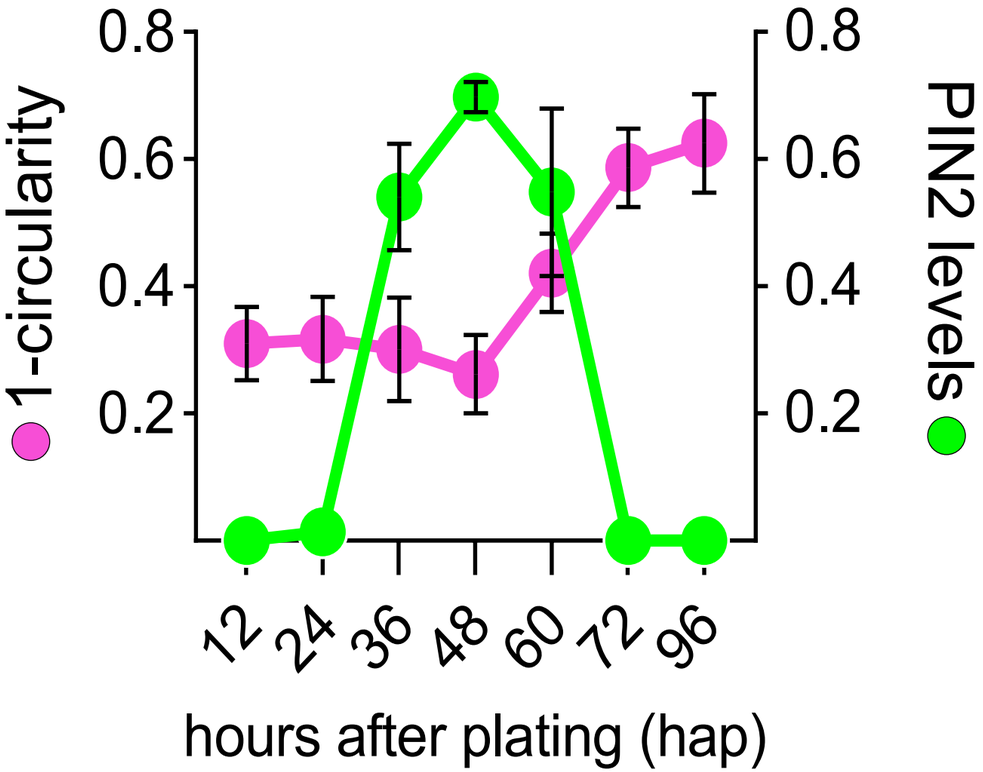


# PIN2 precedes the start of interdigitation



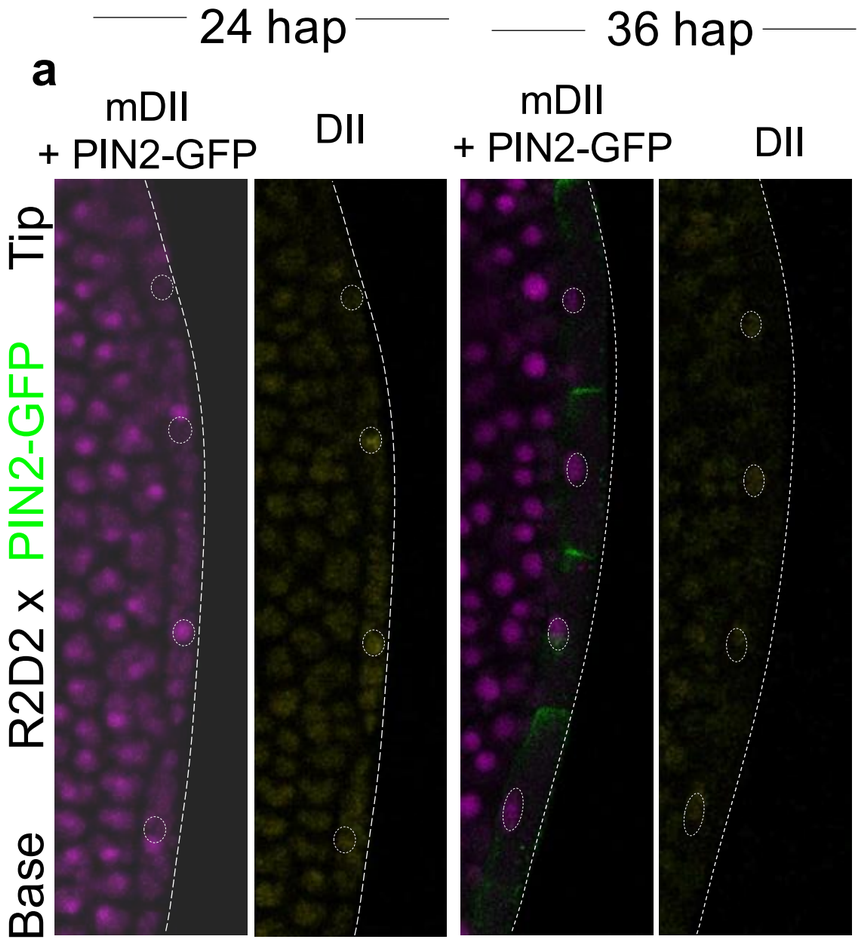
$$\text{PIN2-GFP levels} = \frac{\text{MCs PIN2+}}{\text{Total MCs}}$$

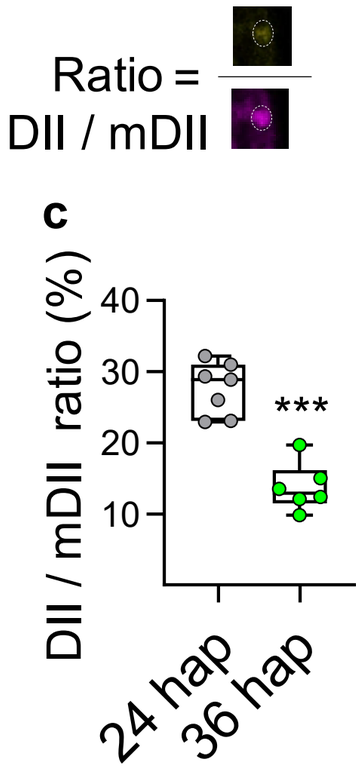
$$\text{Circularity} = \frac{4 \cdot \pi \cdot [\text{Area}]}{[\text{Perimeter}]^2}$$

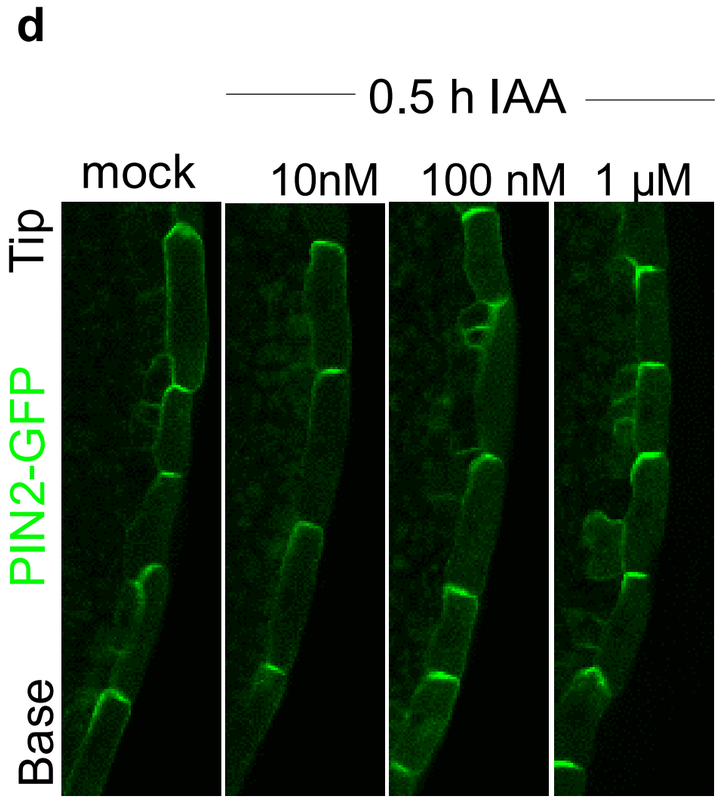


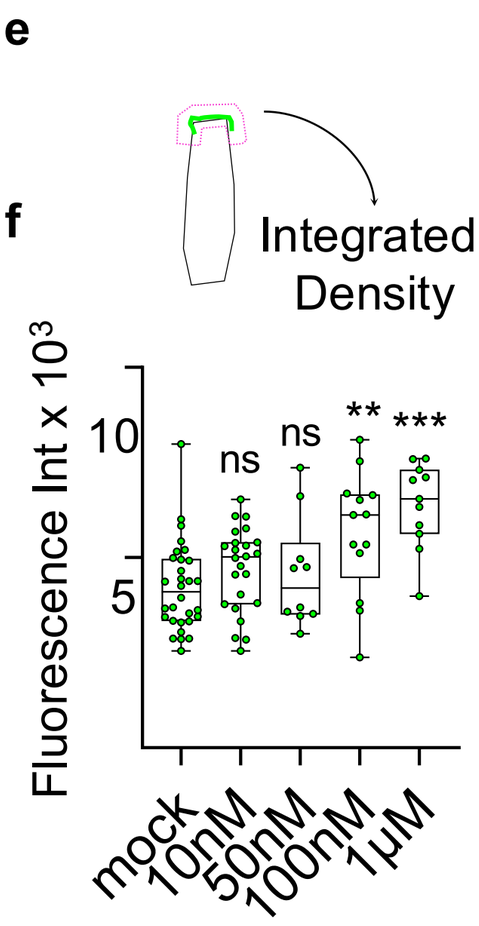

This system is transient BUT is it transporting auxin (functional)?

# PIN2 along MCs is fully functional



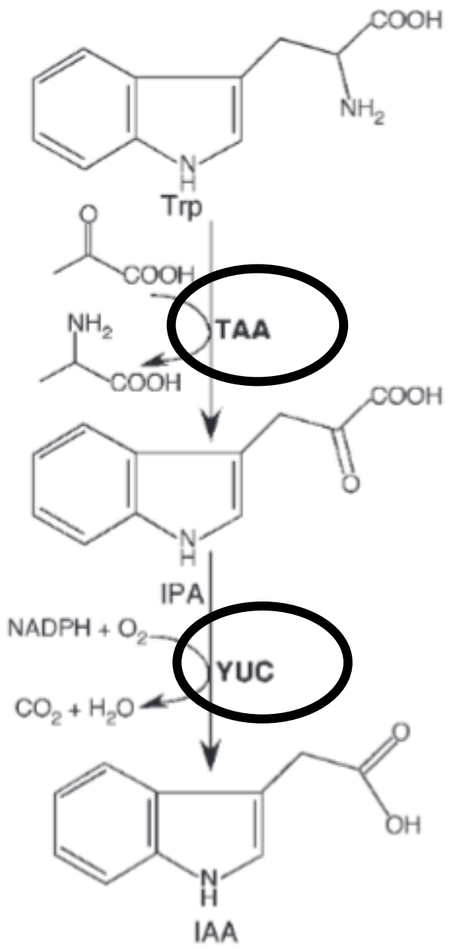




PIN2 is transporting auxin BUT is transient…how?

What are the initiating and terminating signals for the transient PIN2-based auxin transport system?

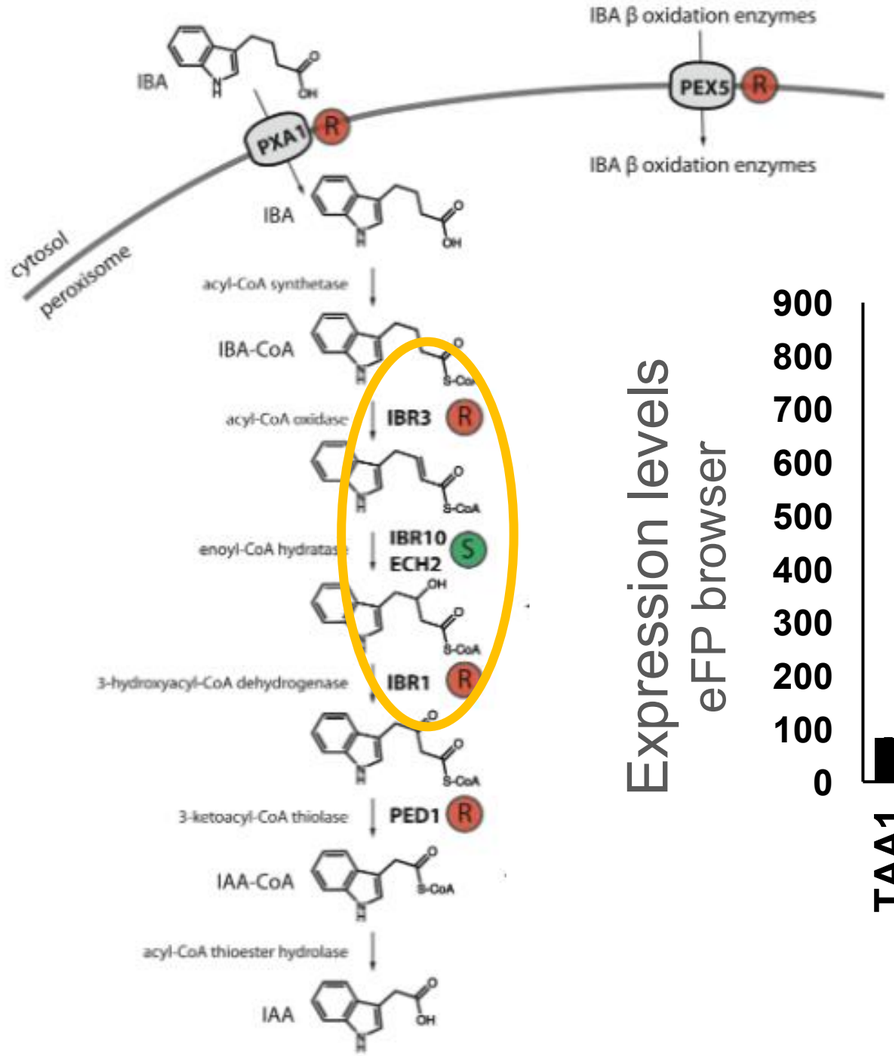
# What is the initiating signal?

## IPA pathway

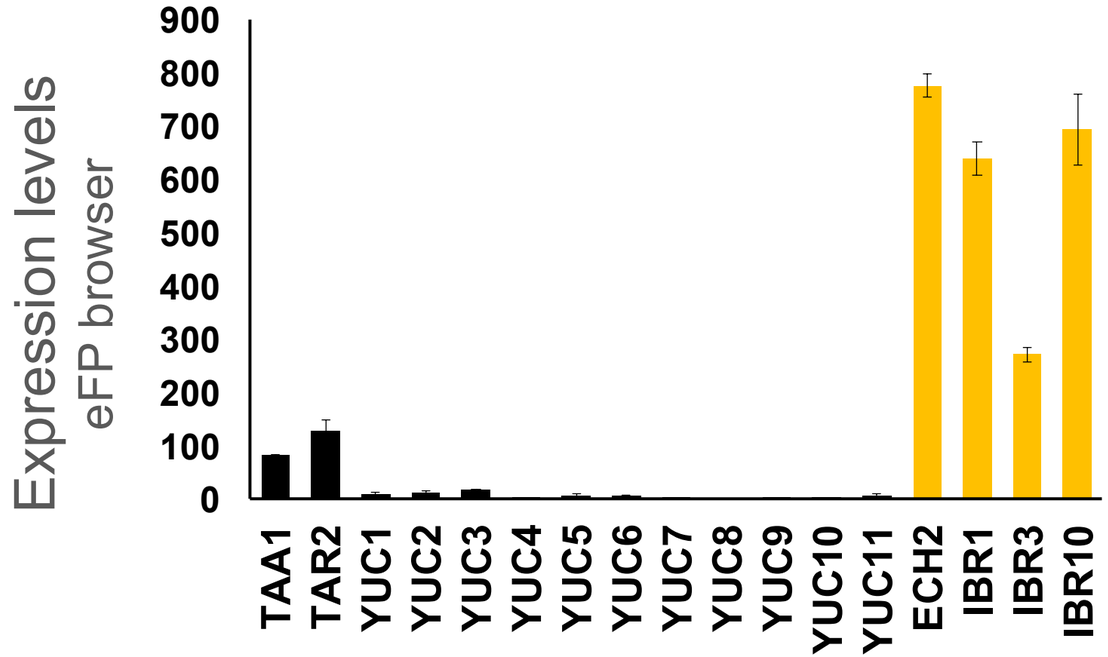



Zhao et al 2012, Molecular Plant

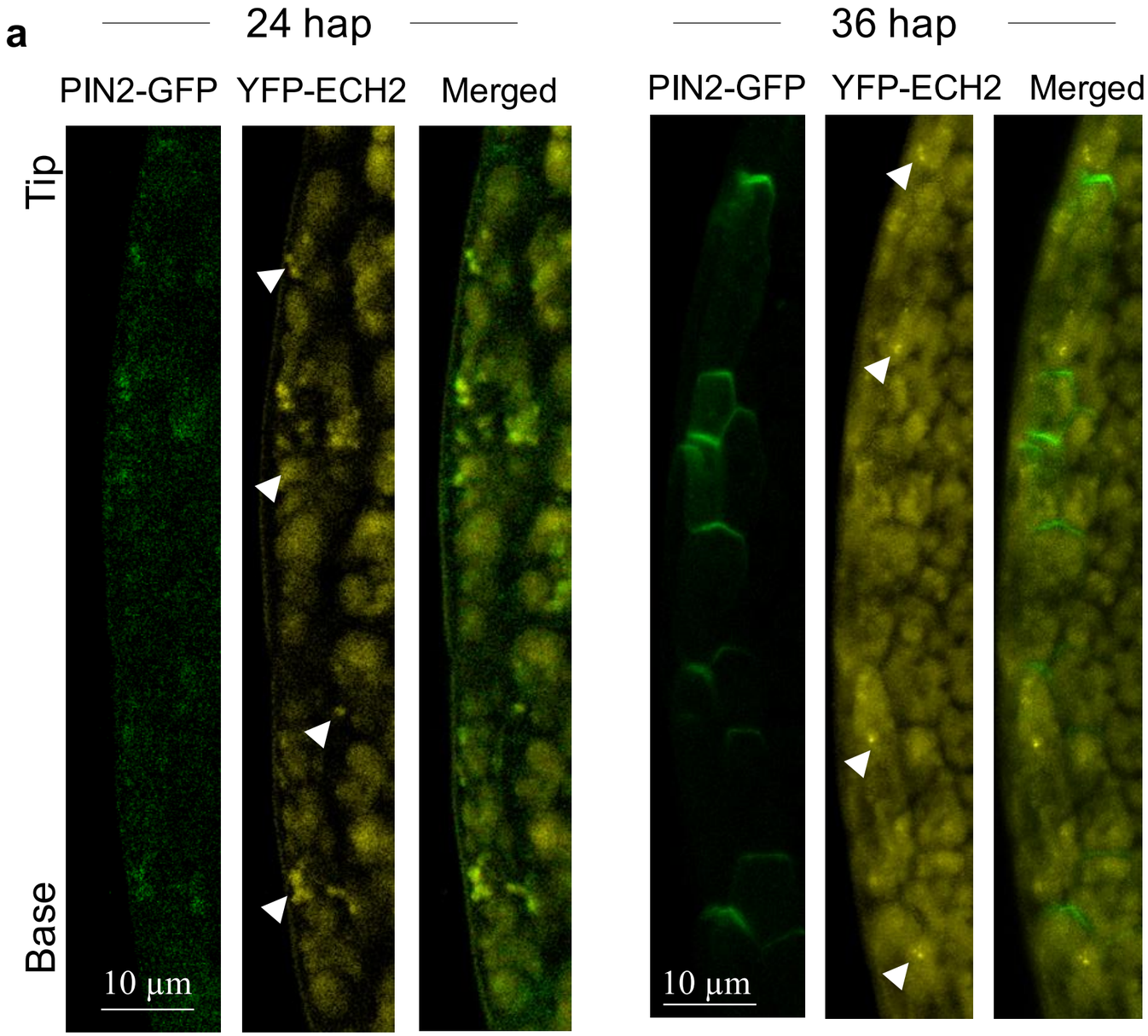
## IBA-to-IAA conversion

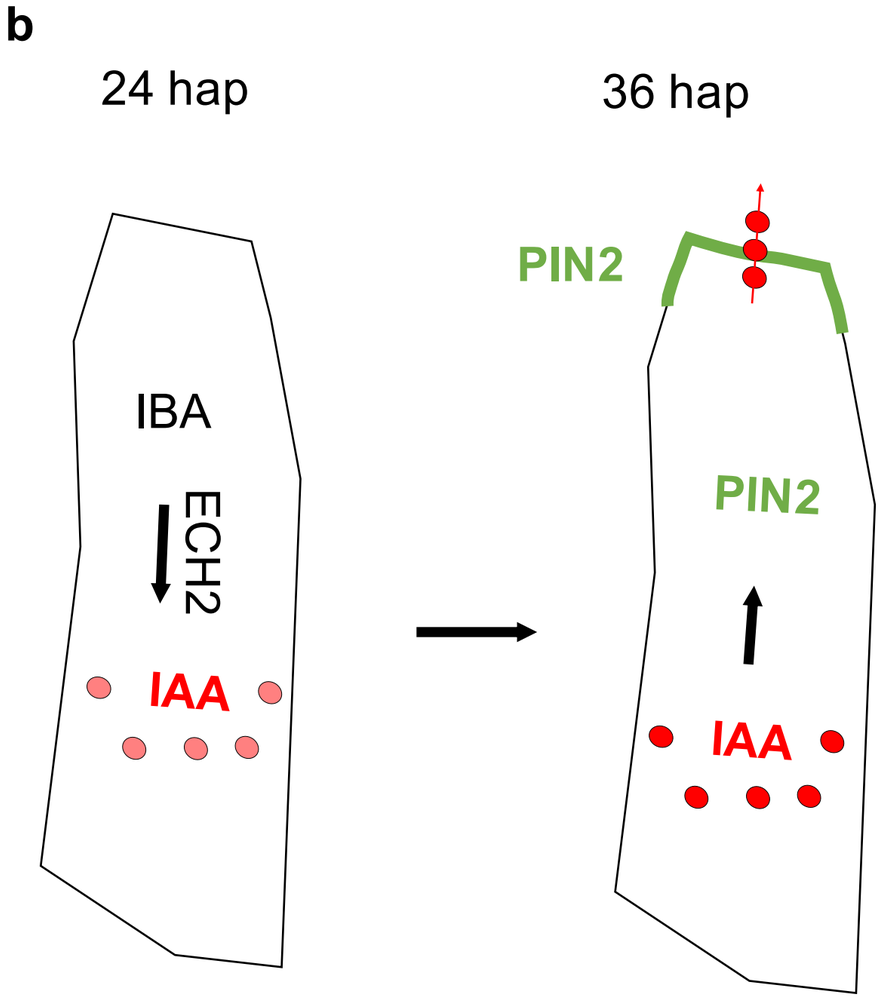



De Rybel et al 2011, Nature Chem Biol

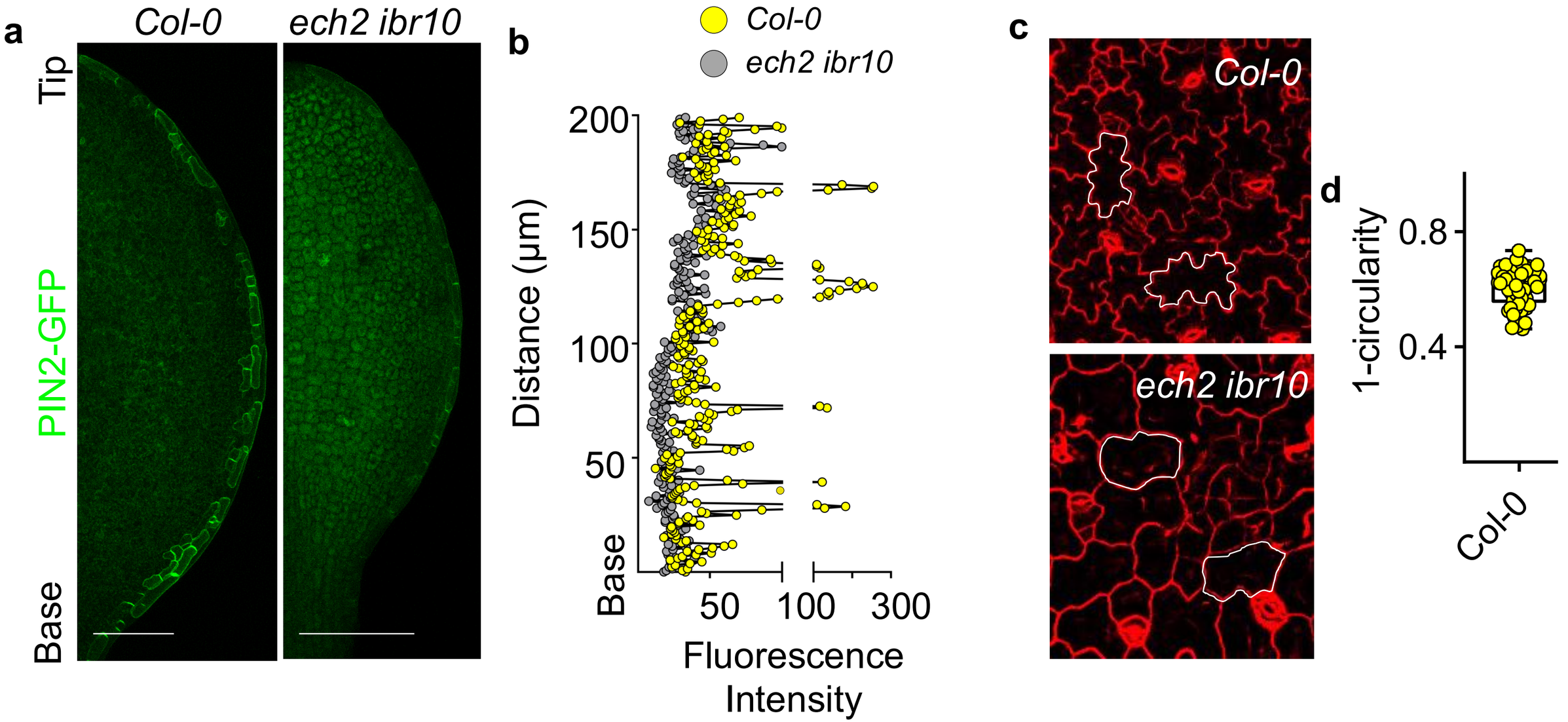


# IBA-derived auxin precedes PIN2 levels



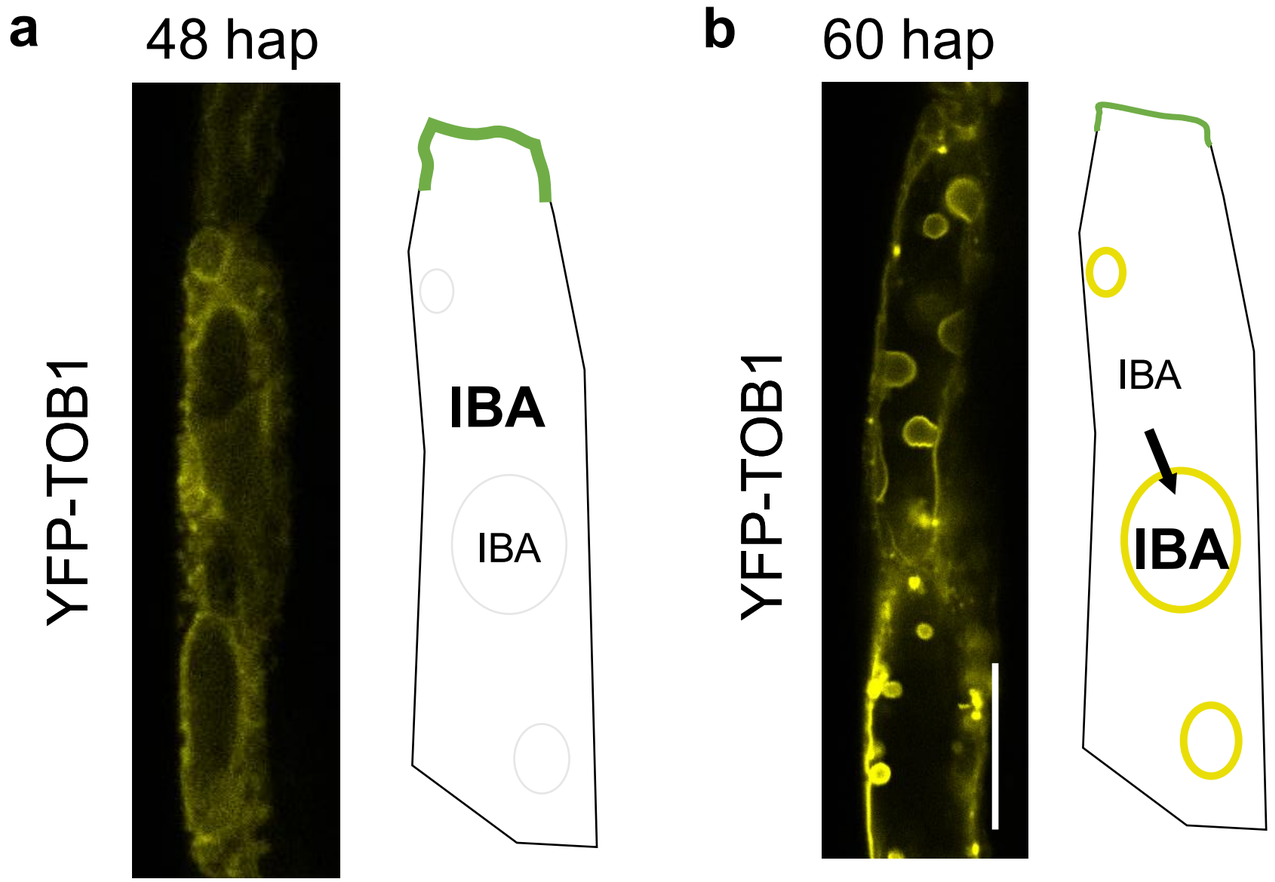



# IBA-derived auxin regulates PIN2 levels at MCs



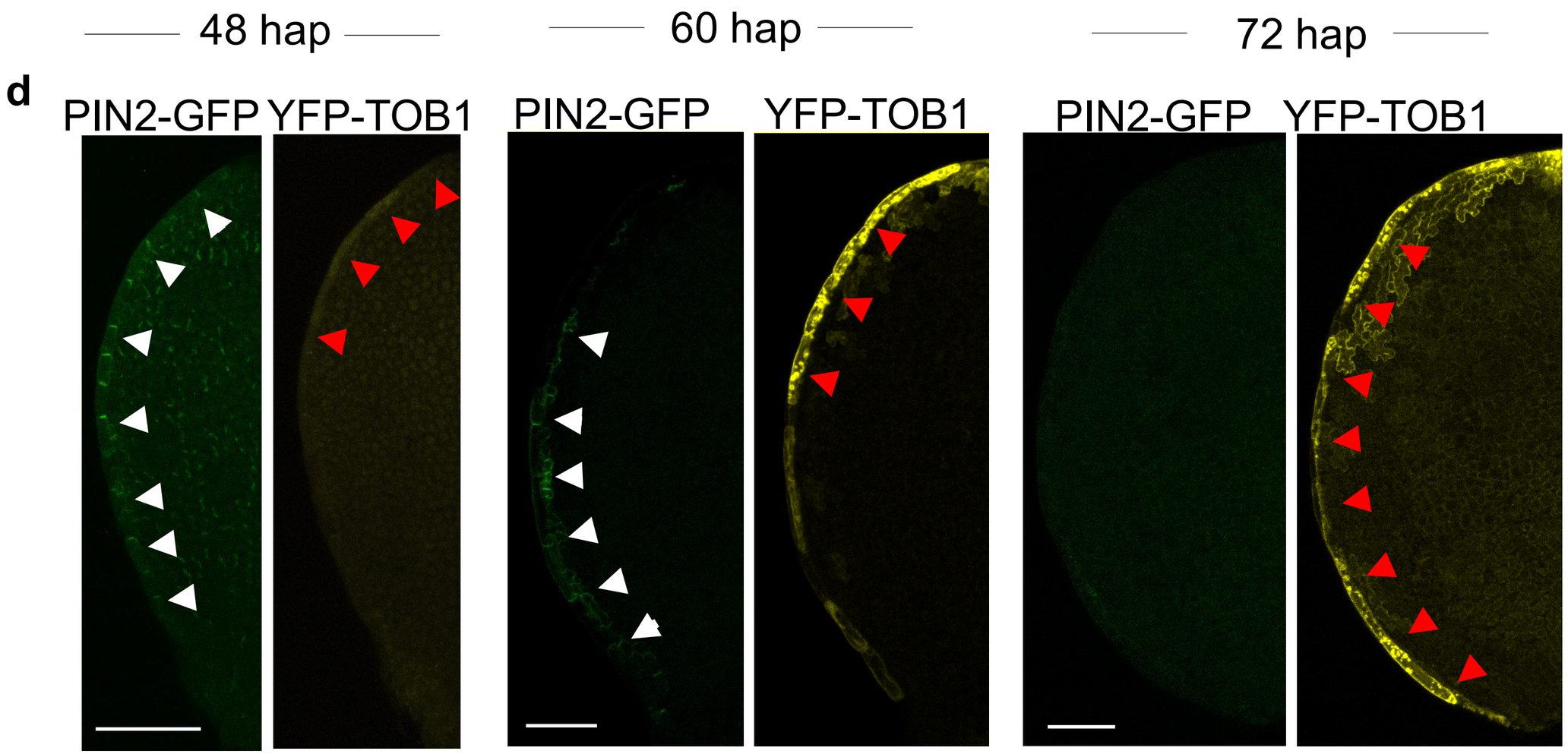

The initiating is signal IBA-derived auxin  BUT what is the terminating signal?

# Vacuole-localized TOB1 correlates with reduced PIN2 levels

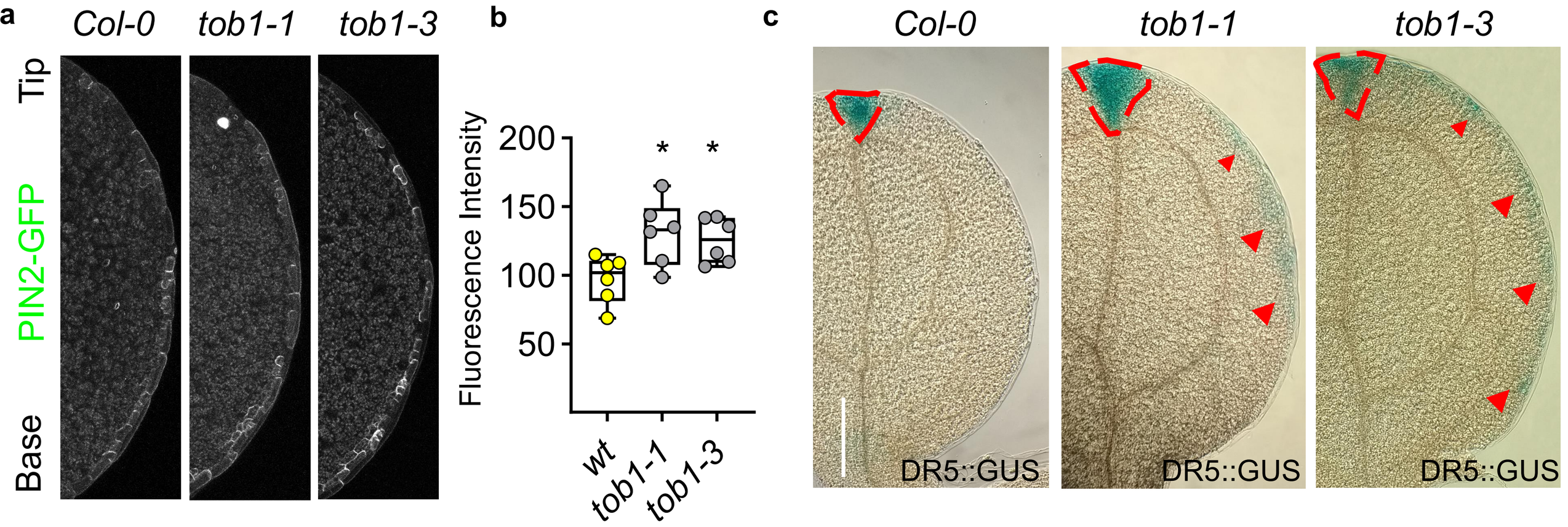



The hypothesis might be correct …

# Vacuole-localized TOB1 correlates with reduced PIN2 levels

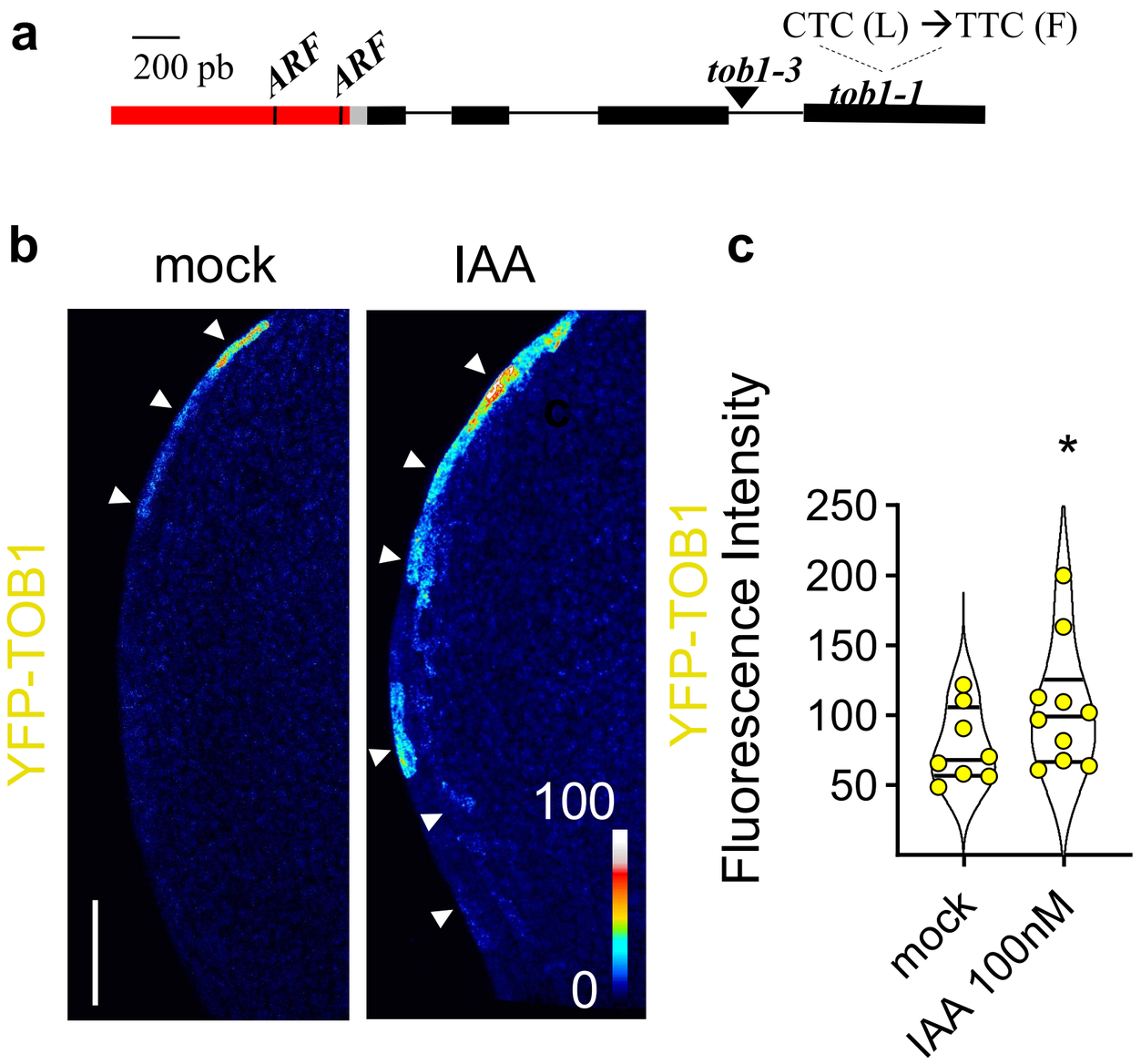


# TOB1 regulates PIN2 levels in margin cells

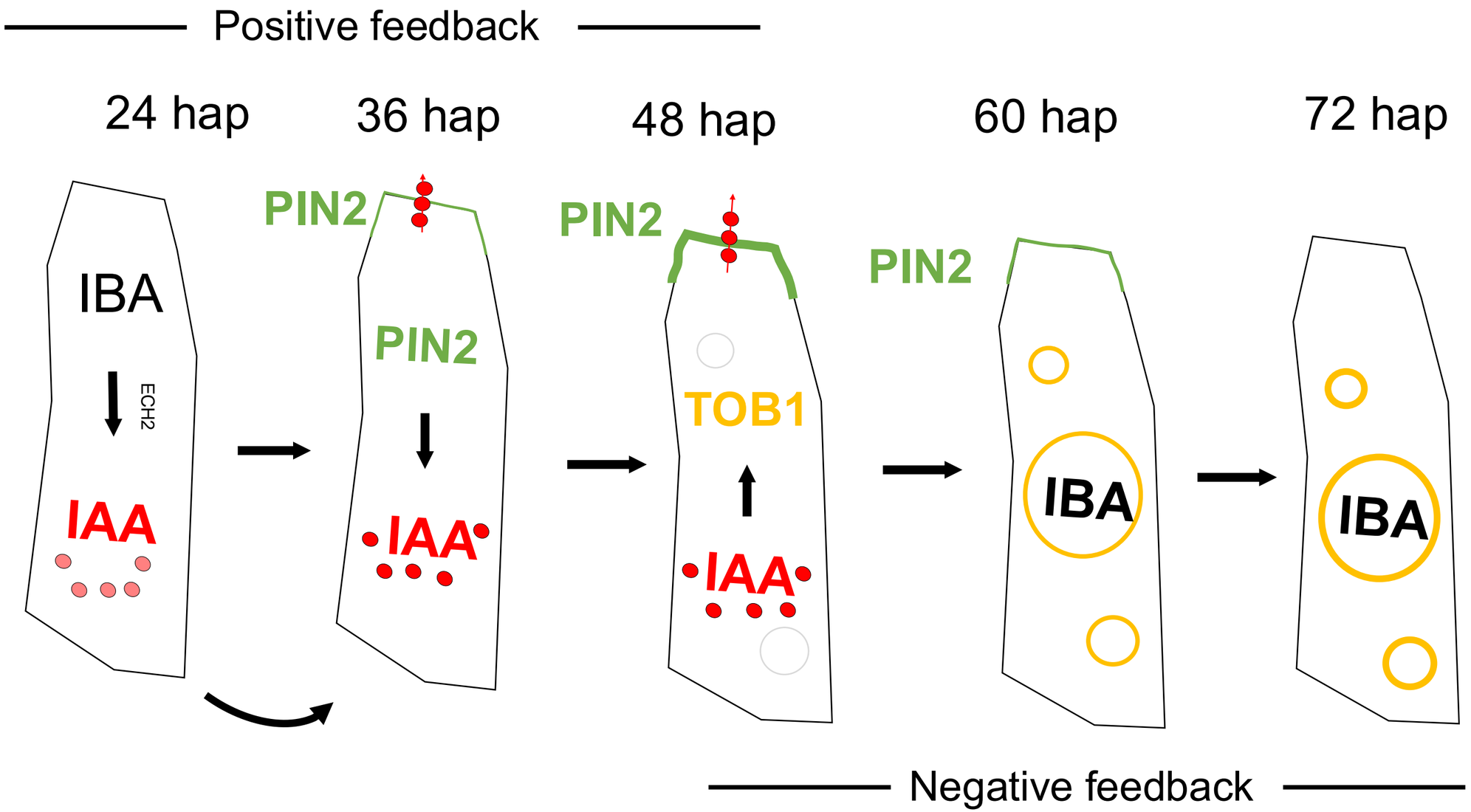



TOB1-mediated IBA internalization into vacuoles is the terminating signal

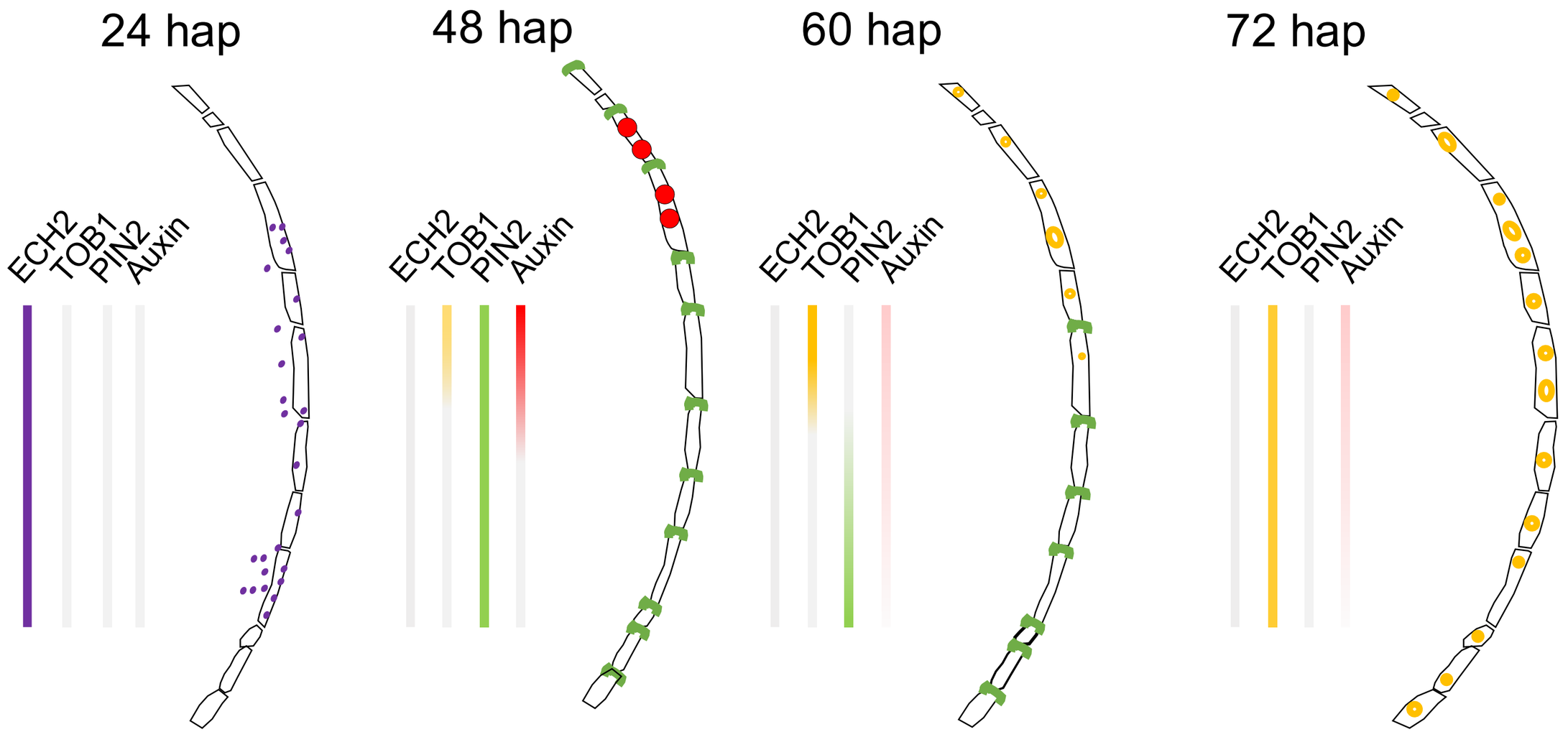
# Auxin regulates TOB1 levels



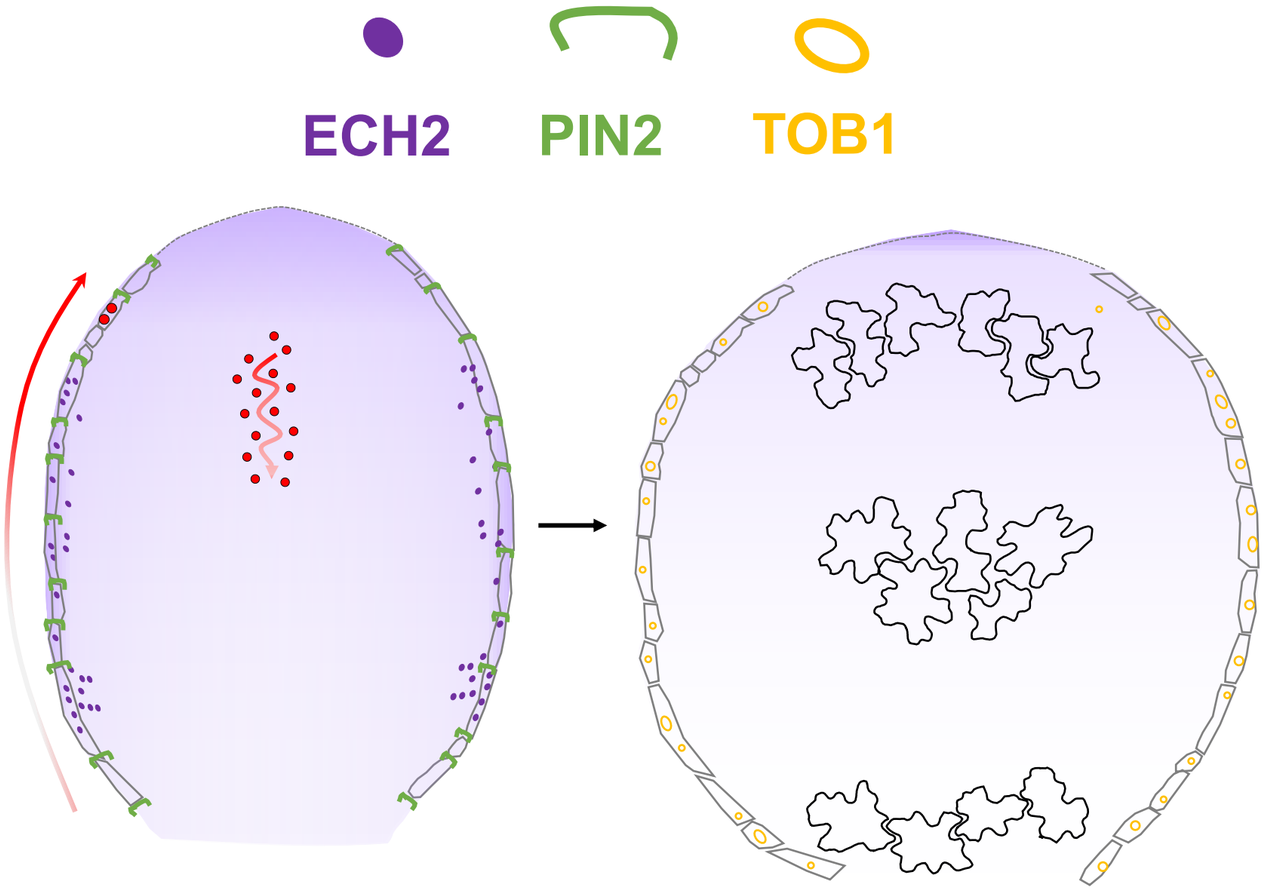
# Working model for self-organizing transient auxin flow at MCs



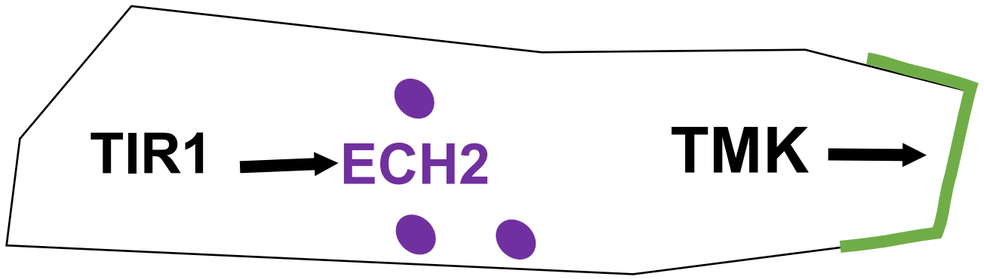
# Working model for self-organizing transient auxin flow at MCs



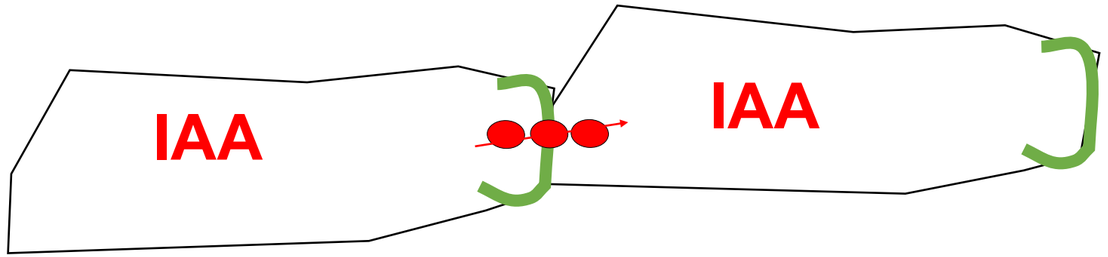
# Future of auxin flow in margin cells

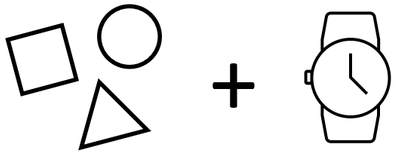


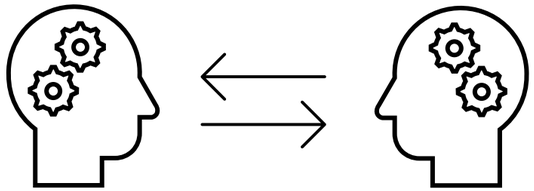

- Hierarchical auxin signaling

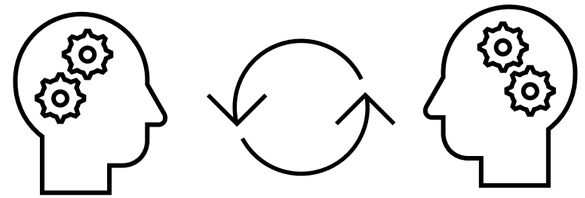


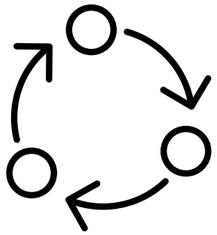

- Canalization hypothesis



Pattern formation 

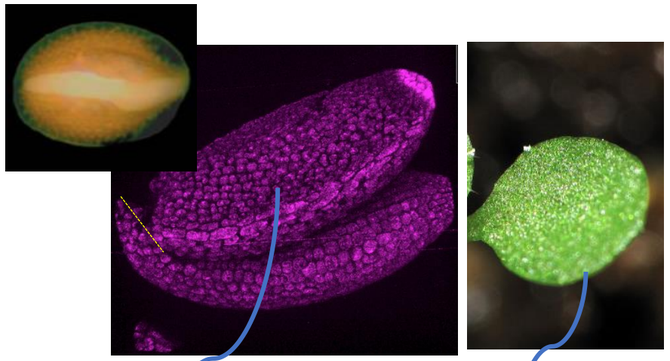


Collaborative auxin signaling 

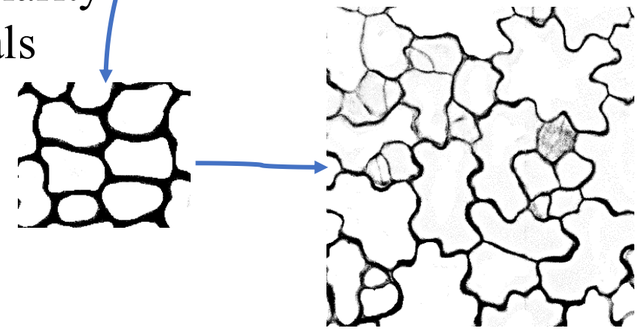
Self-organization 

# Proposal for the future research

a. Fast morphogenetic changes

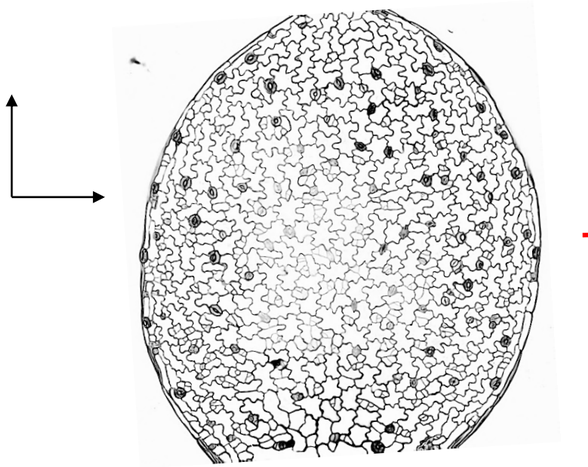


b. Polarity signals

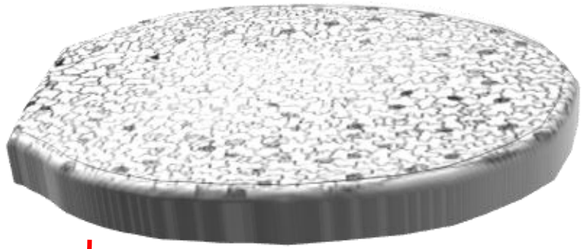


c. Flat structure

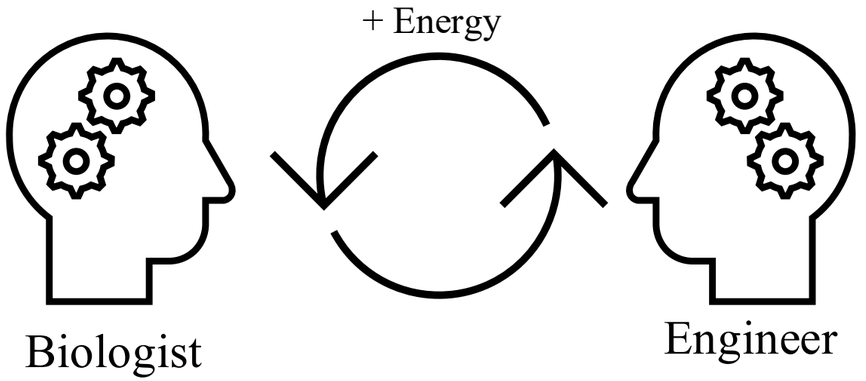
d. postdoc; coordination in 2D



e. Future; coordination in 3D



f. career-long plan



# Acknowledgments


Zhenbiao Yang

Xue Pan
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Jingzhe Guo

Jaimie Van Norman

Lucia Strader

Ken-Ichiro Hayashi



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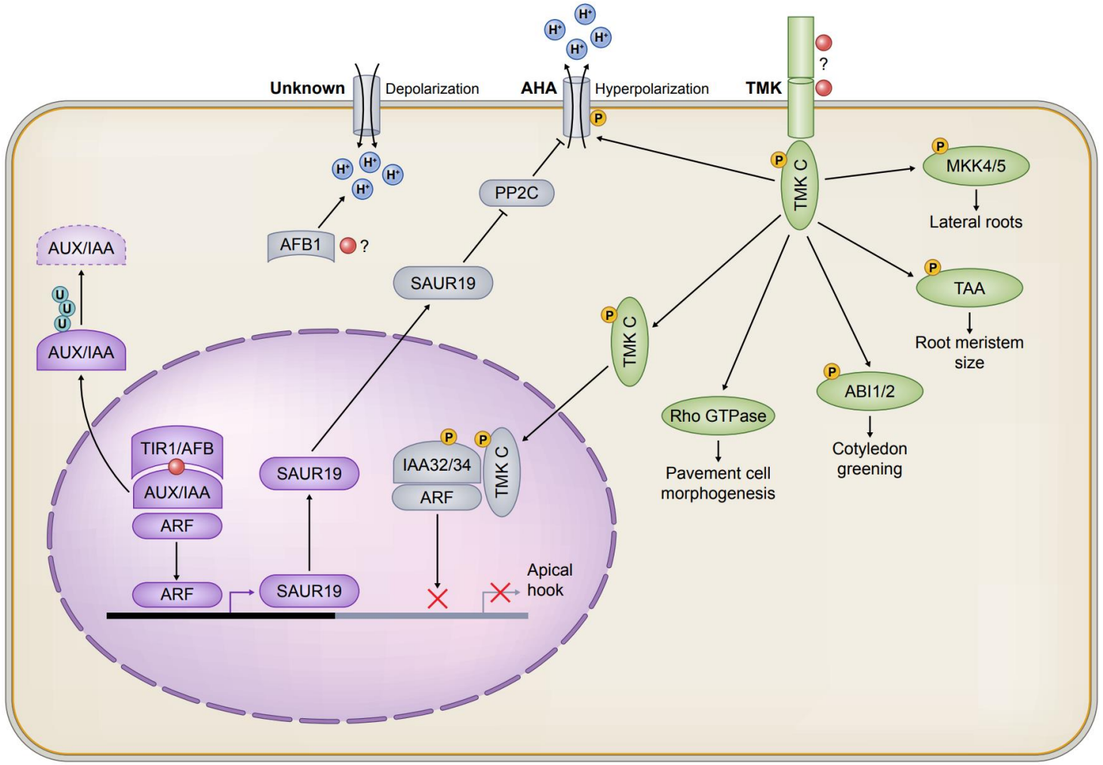


Perez-Henriquez & Yang (2022). *New Phytologist*

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Ken-Ichiro Hayashi, Okayama U


# Funding


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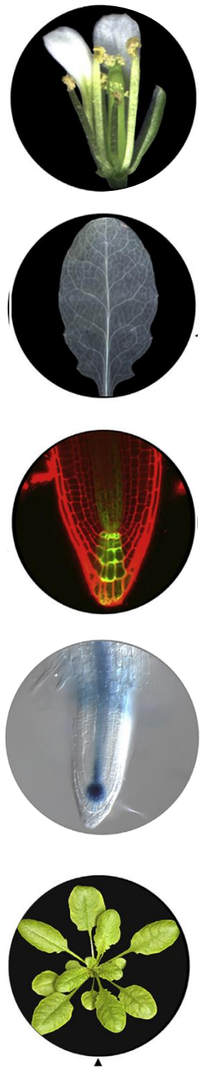
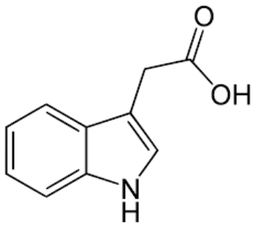
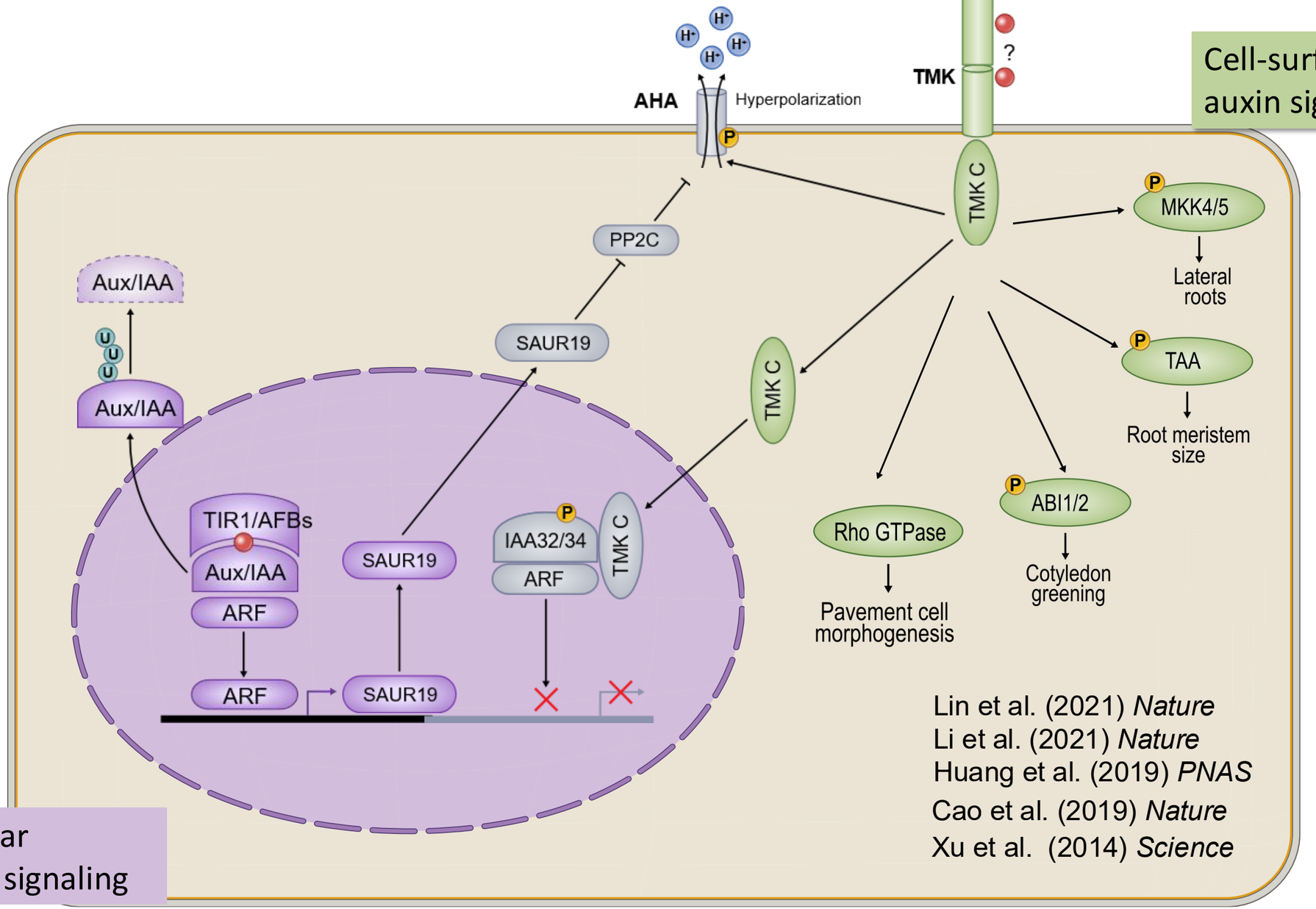
# Auxin signaling

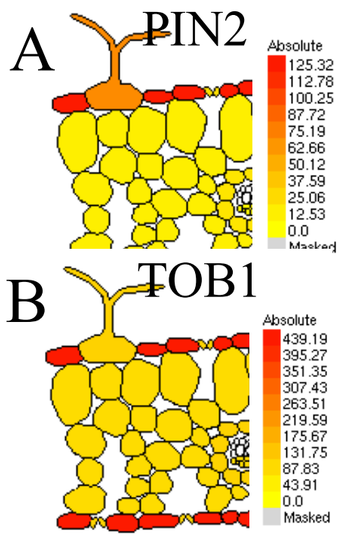


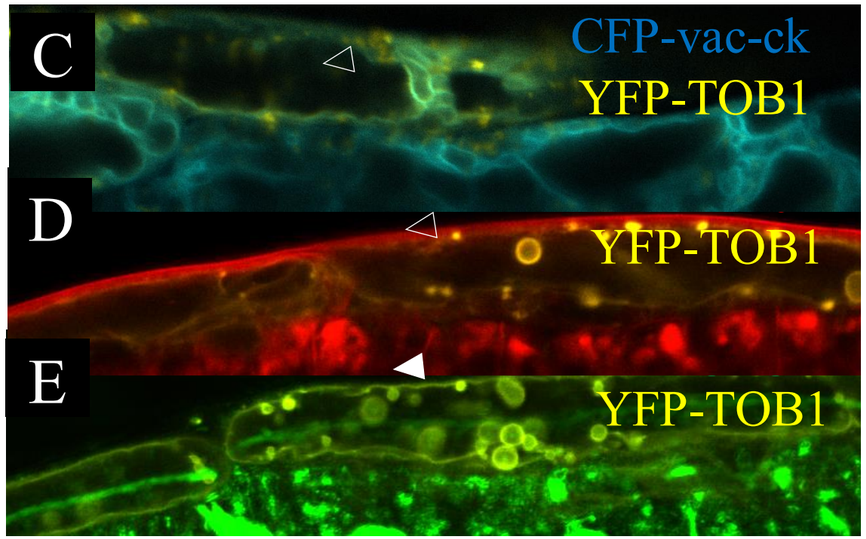


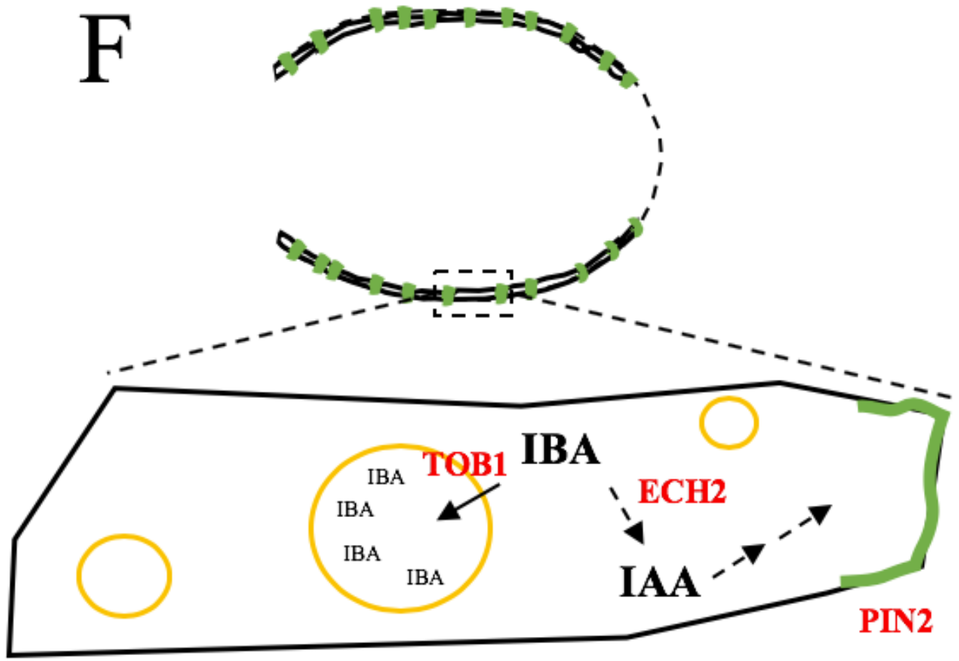




Perez-Henriquez & Yang (2022). *New Phytologist*

# TOB1 sub-cellular localization

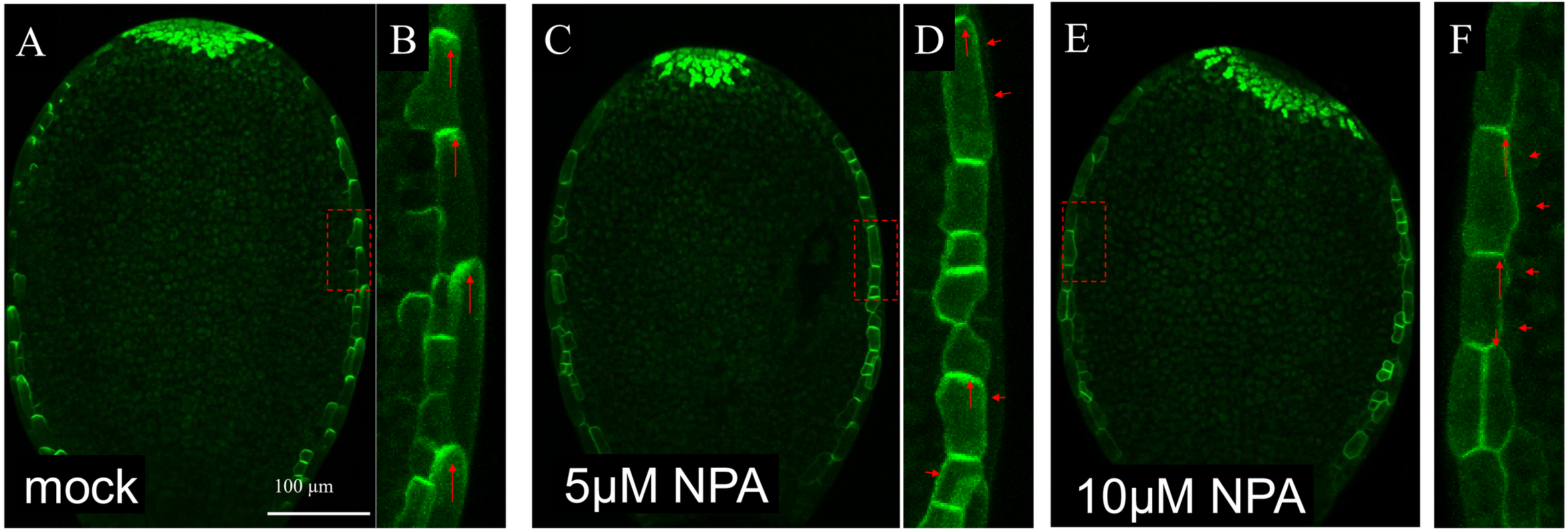


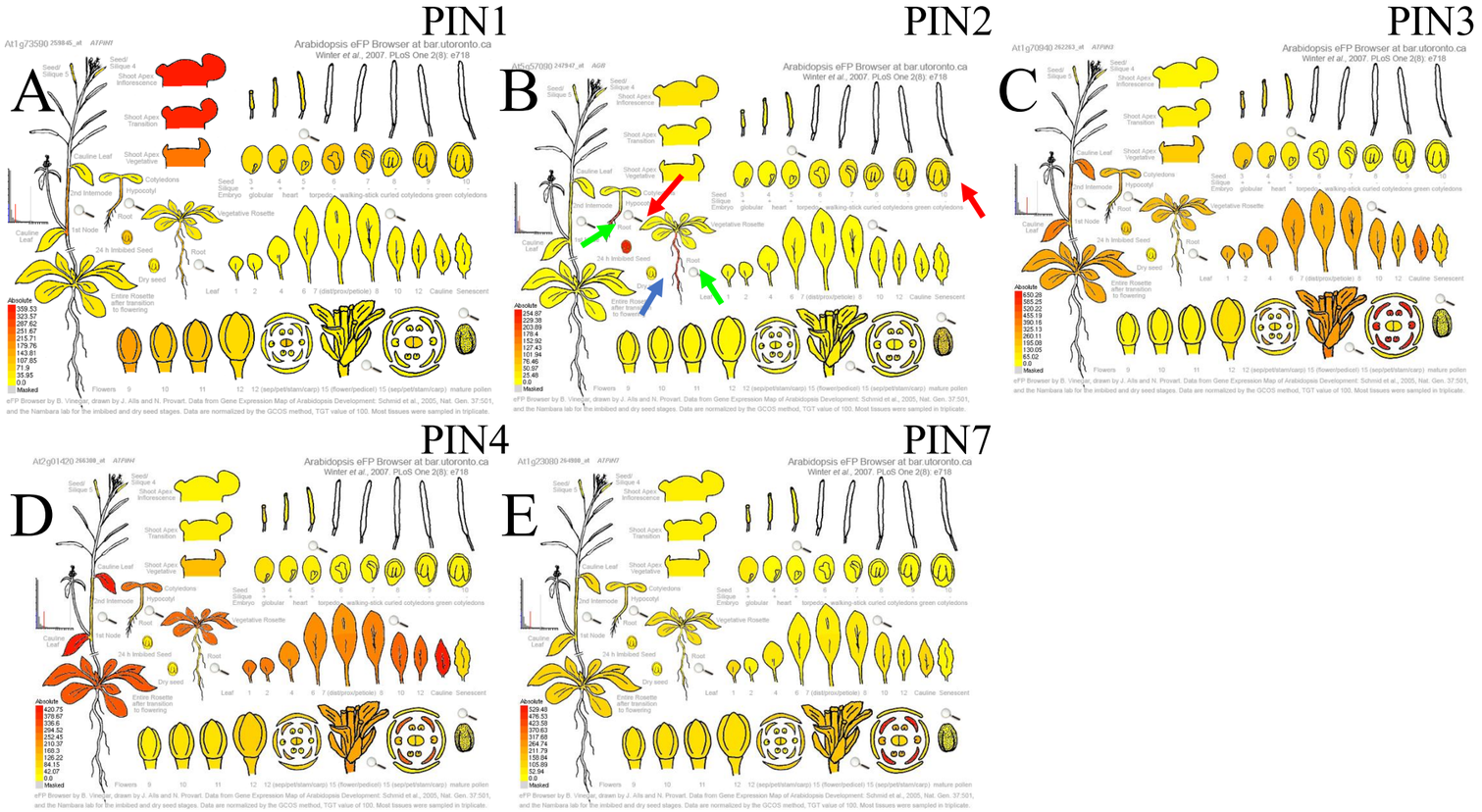



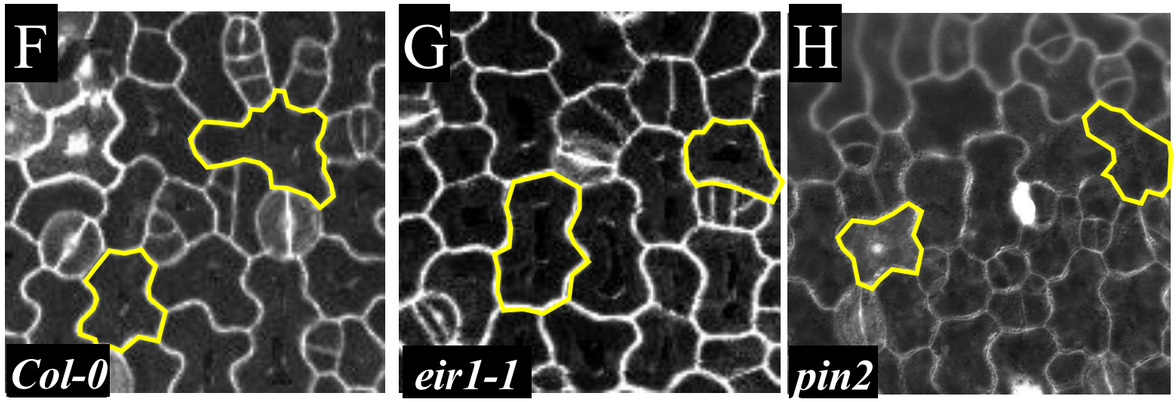
# NPA disrupts PIN2 polar localization

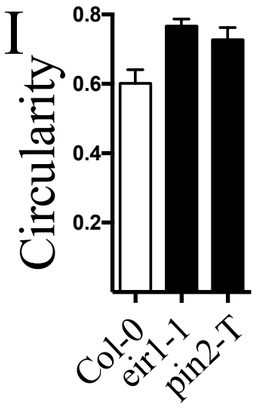
24 hap → NPA → 48 hap



**PIN2 is expressed in imbibed seeds and its absence generates reduced PC morphogenesis**

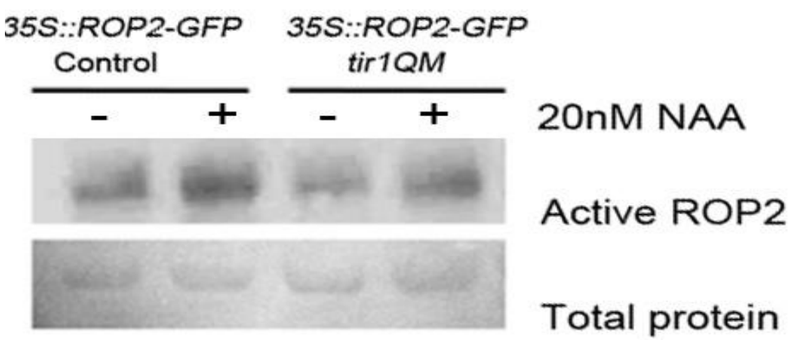


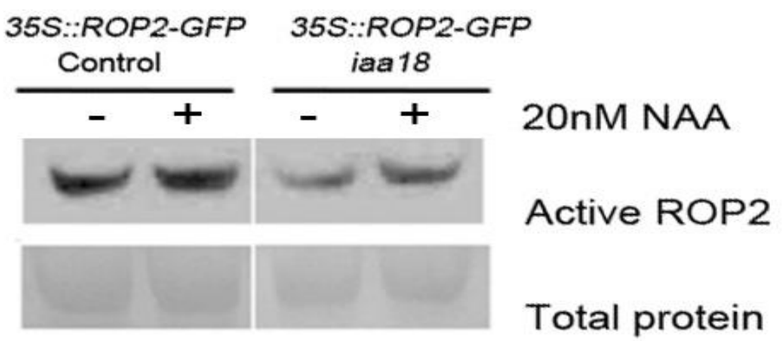



# TIR1/AFBs dependent auxin activates locally ROP signaling

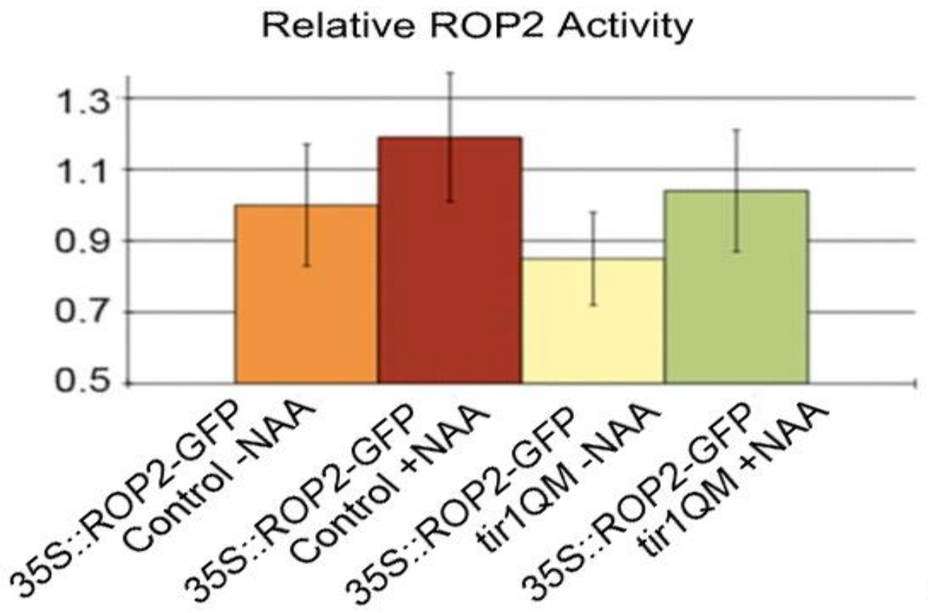
A



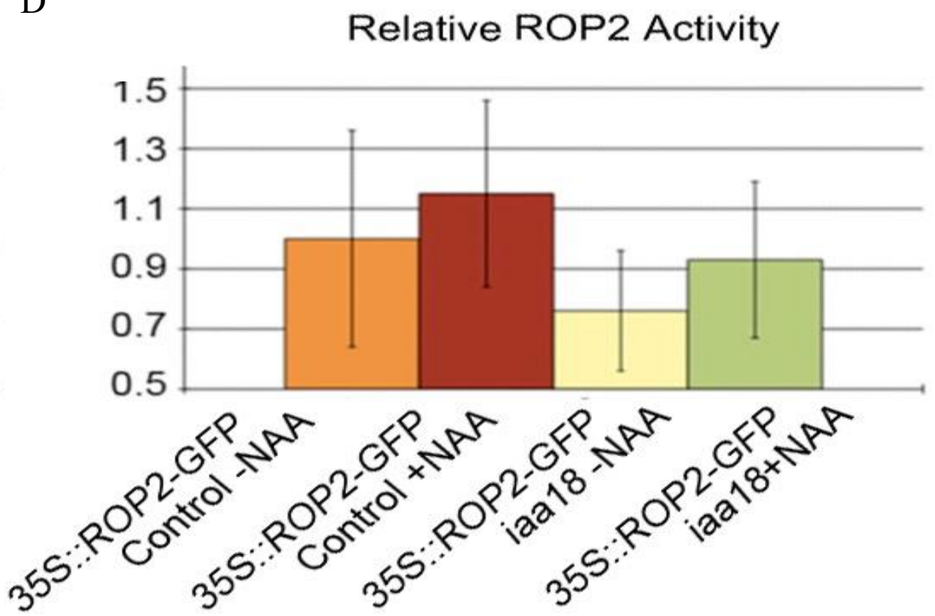

B




C

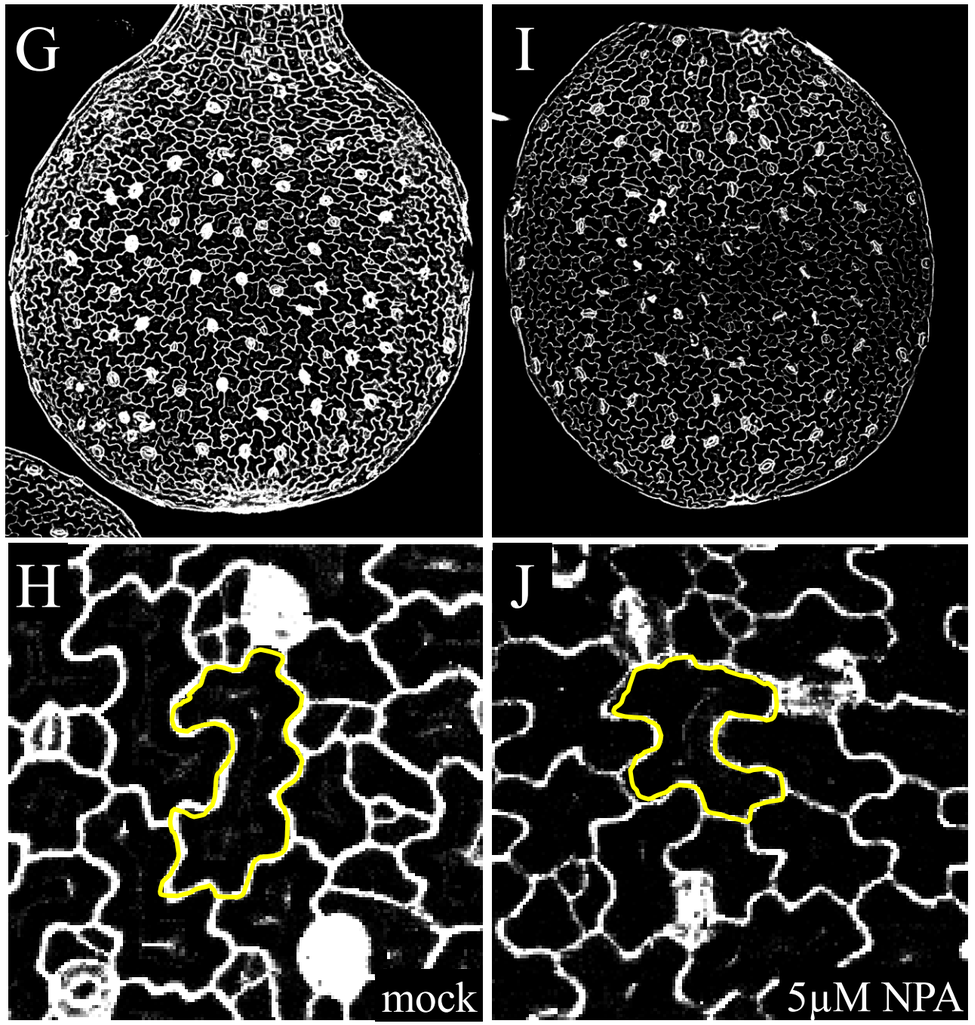


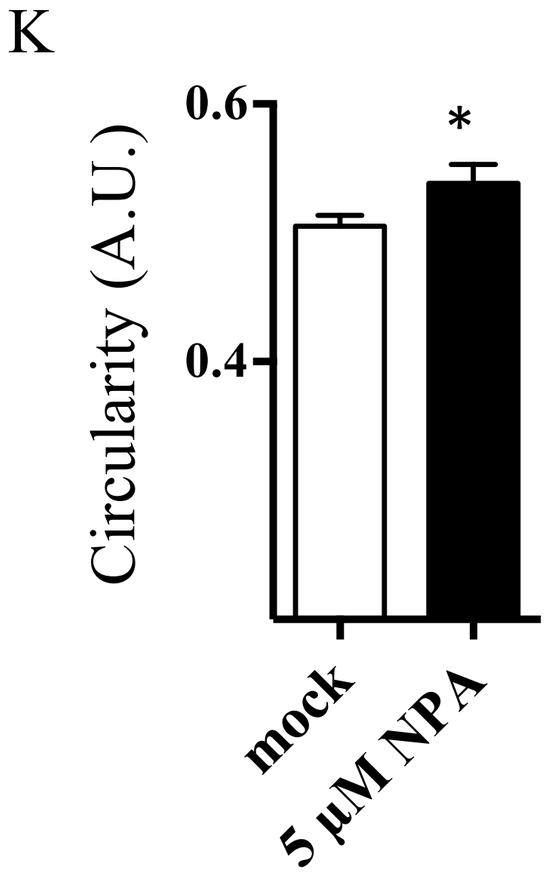

D



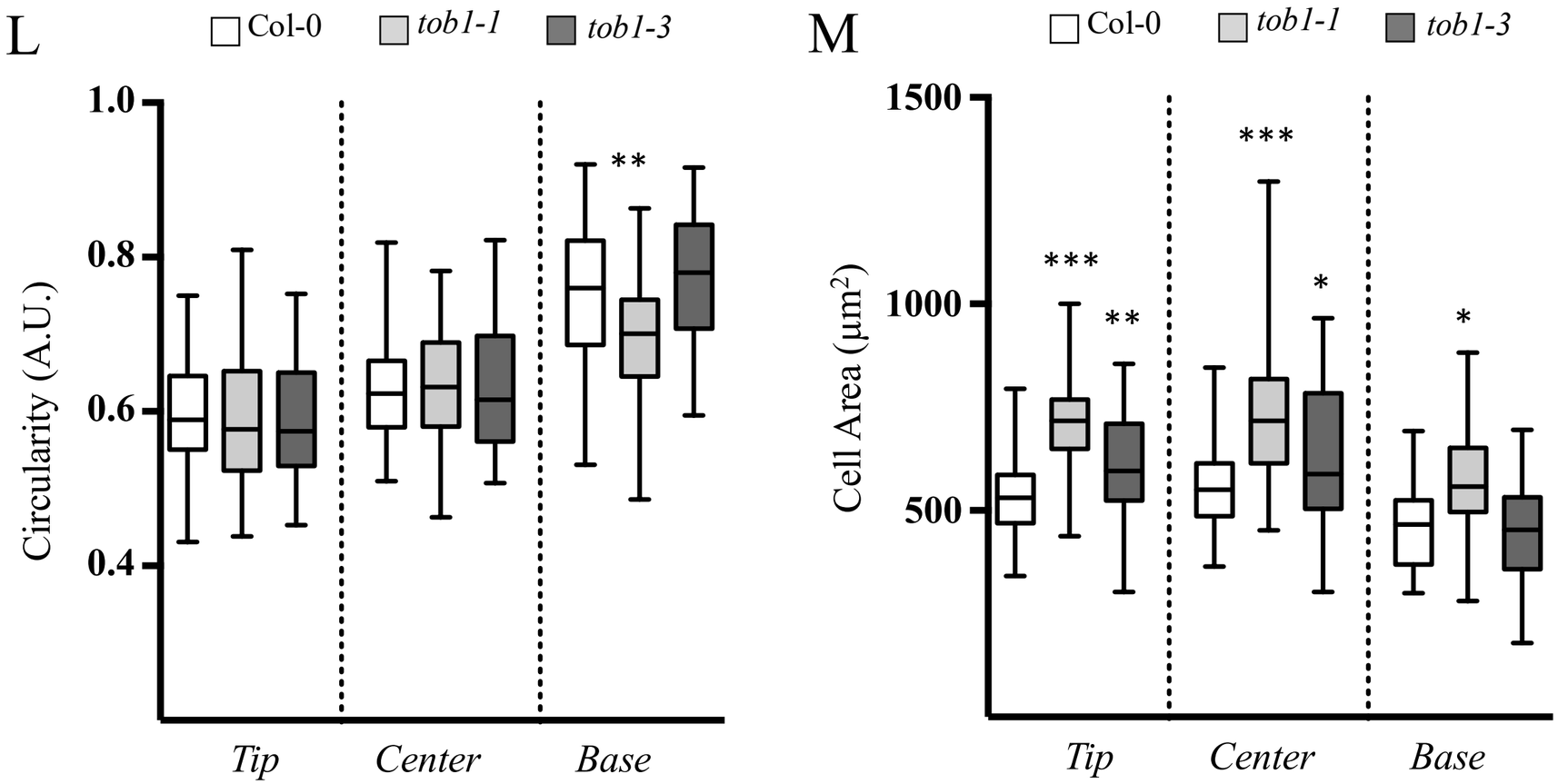
# Interrupting auxin transport with NPA, disrupted both PIN2 polar localization and PC interdigitation.

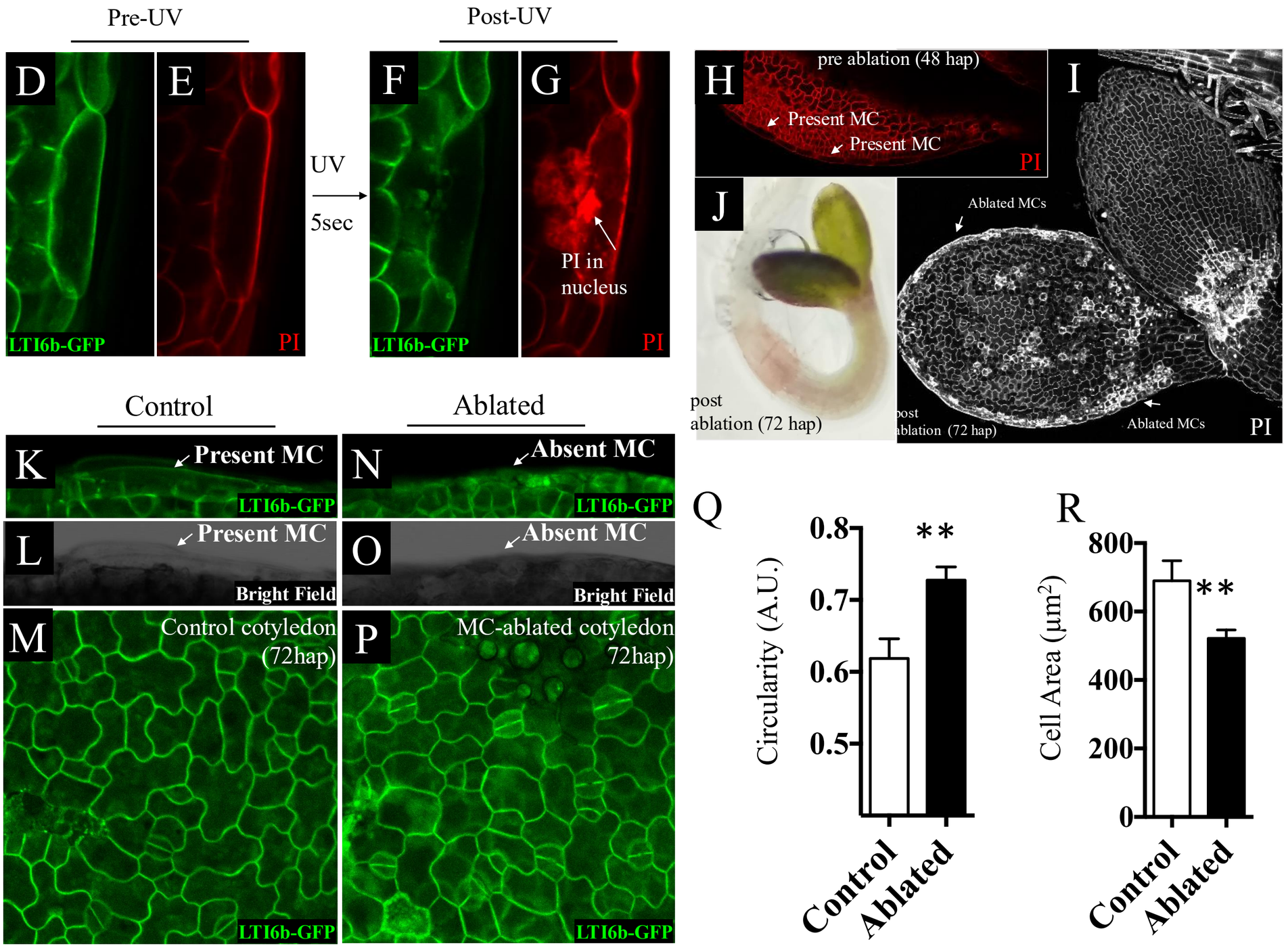
24 hap → NPA → 72 hap



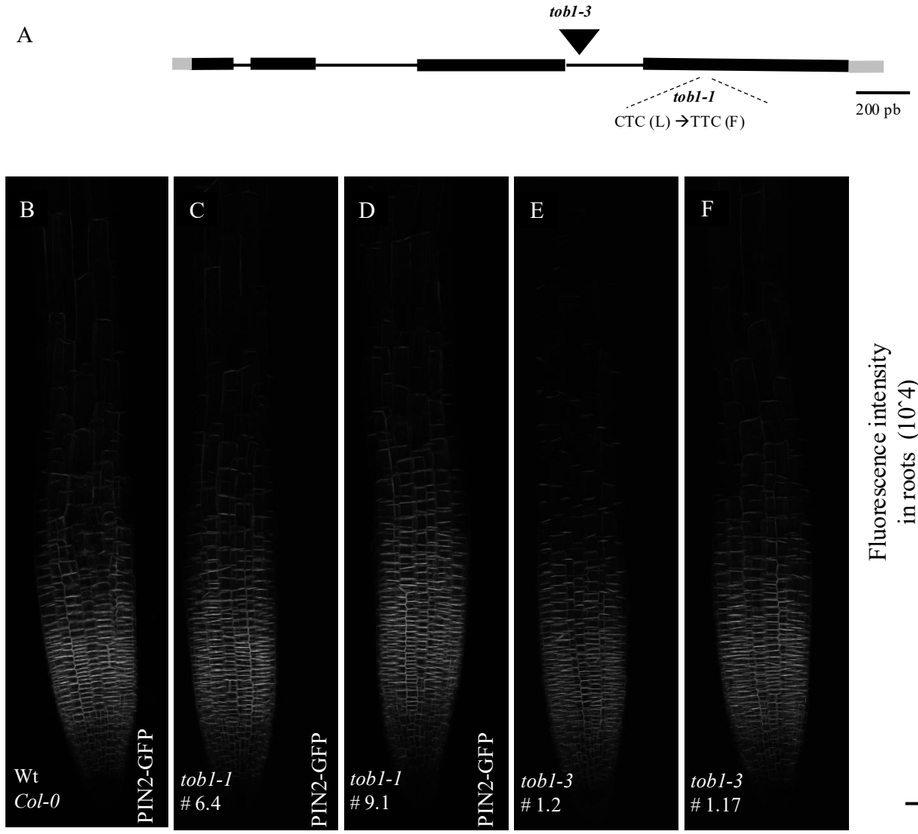



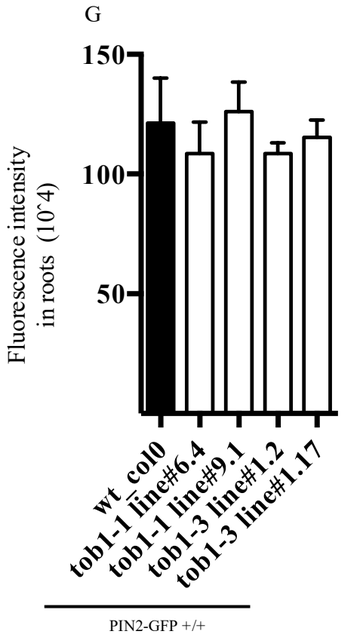
# TOB1 regulates cotyledon size also by increasing cell size in early stages

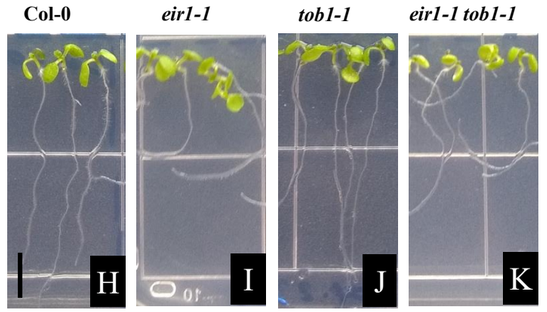


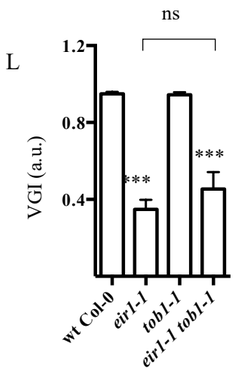

Pre-UV
Post-UV
D
E
F
G
UV
5sec
LTI6b-GFP
PI
PI in nucleus
H
pre ablation (48 hap)
Present MC
Present MC
I
J
post ablation (72 hap)
Ablated MCs
Ablated MCs
Control
Ablated
K
Present MC
N
Absent MC
L
Present MC
O
Absent MC
Bright Field
M
Control cotyledon (72hap)
P
MC-ablated cotyledon 72hap)
Q
Circularity (A.U.)
0.8
0.7
0.6
0.5
**
Control
Ablated
R
Cell Area (µm²)
800
600
400
200
0
**
Control
Ablated

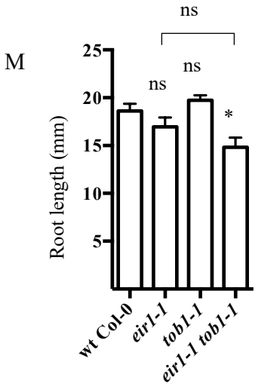
# TOB1 does not regulate PIN2 levels in *Arabidopsis* root tips.

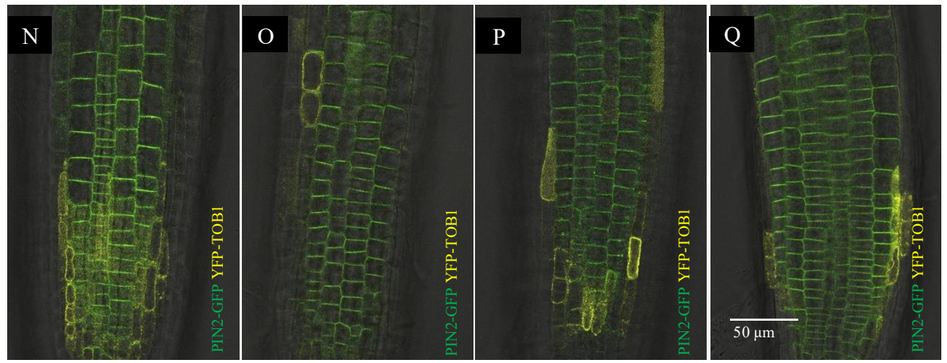


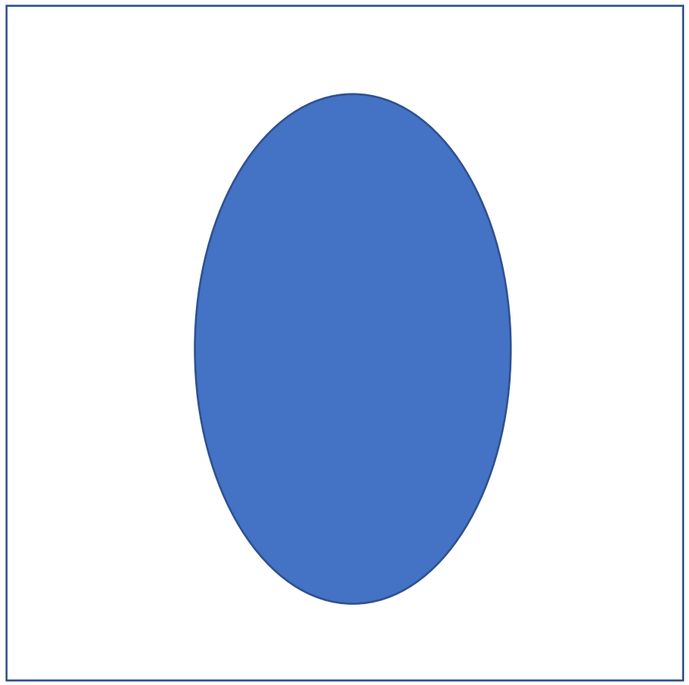



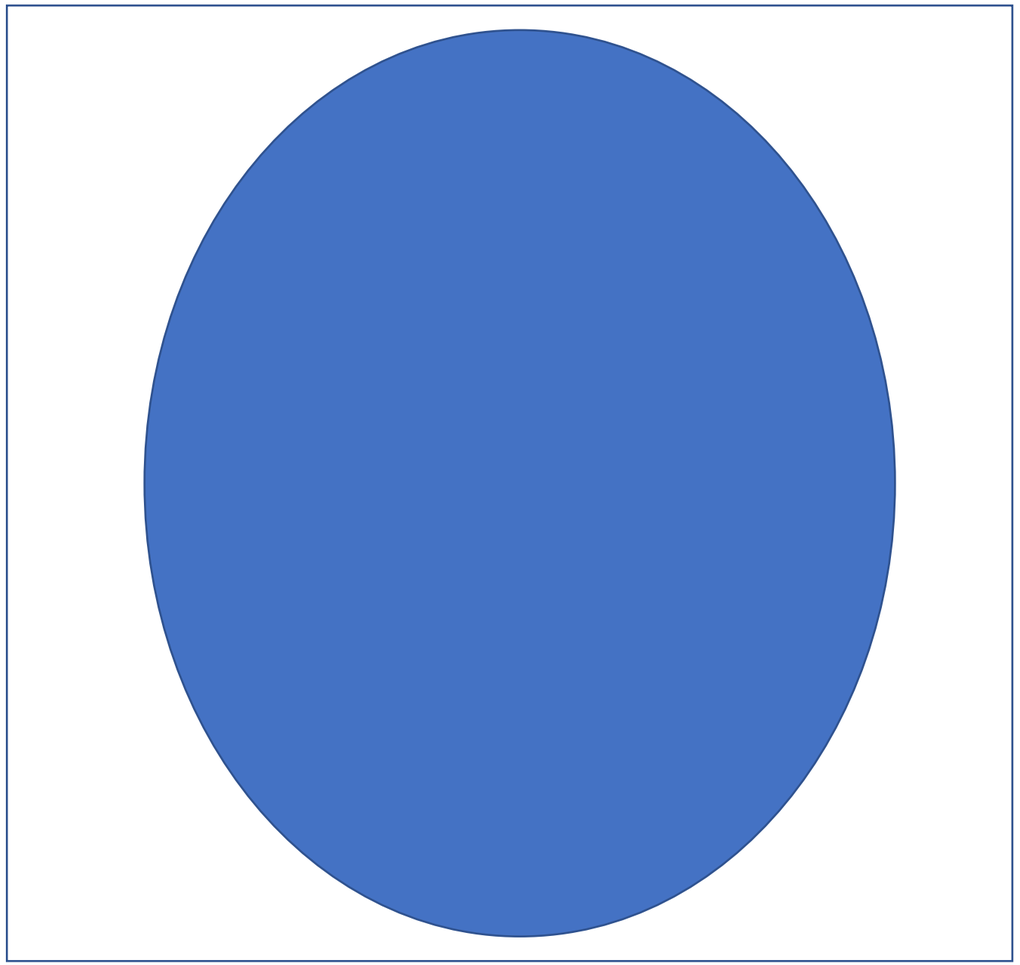



# Pixel length

300 µm x 300 µm (1024 x 1024 px)



500 µm x 500 µm (1024 x 1024 px)



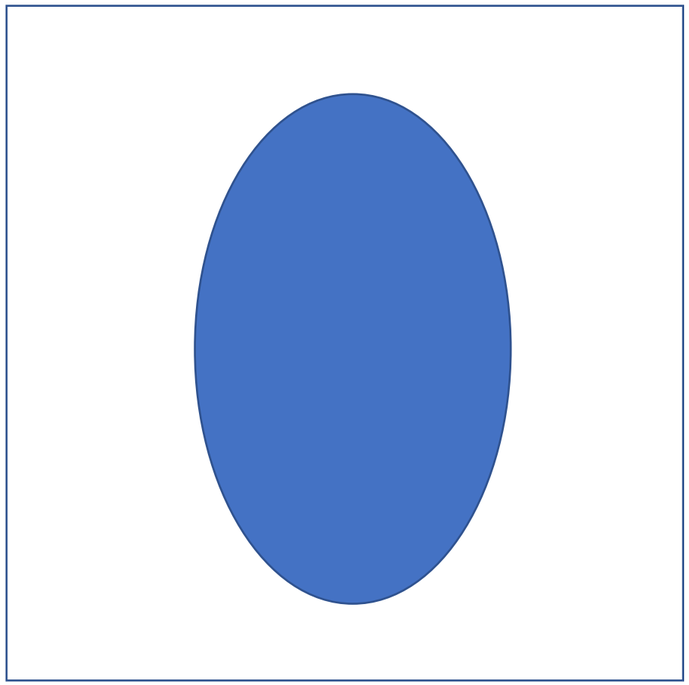
**different pixel length**

300/1024 = 0.29 µm/px

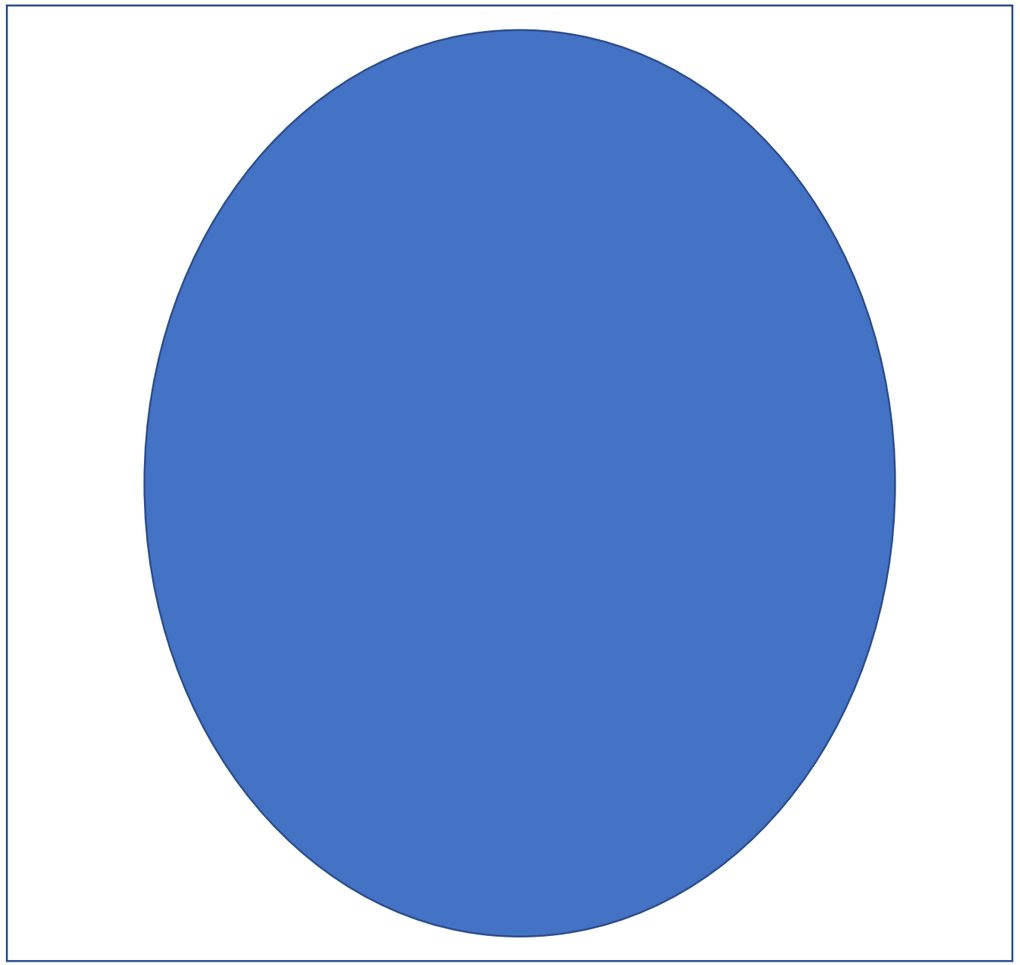
500/1024 = 0.48 µm/px

# Size matters

300 µm x 300 µm (1024 x 1024 px)



500 µm x 500 µm (**1725 x 1725 px**)

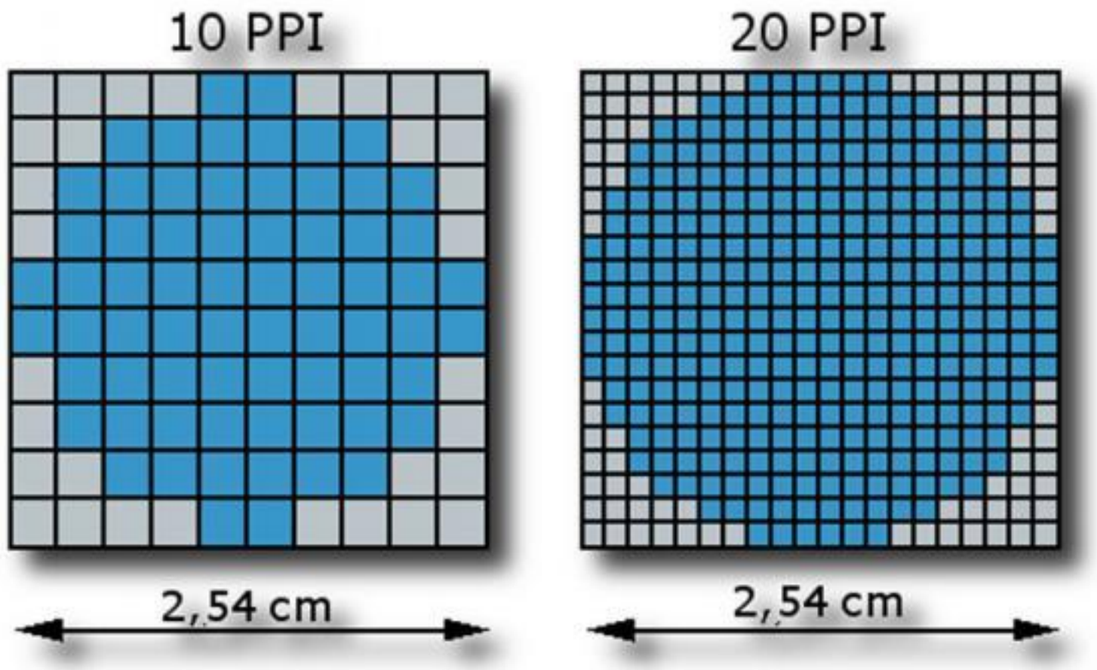


**same pixel length**

300/1024 = 0.29 µm/px

500/1725 = 0.29 µm/px

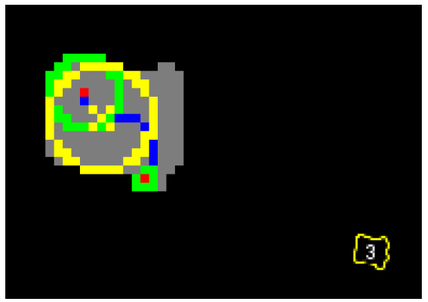
# Compare the shape



# In-house testing

512 x 512

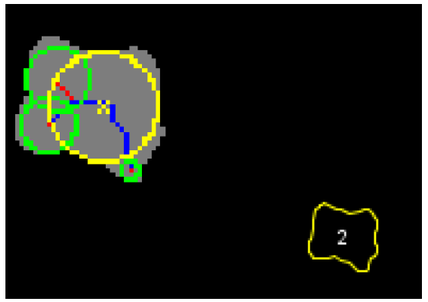
0.83 µm/px




| Parameter | Value |
|---|---|
| Area | 132.9 |
| Perimeter | 46.1 |
| MR | 7.76 |
| BranchCount | 0 |
| CH Conv | 0.940 |
| LobeCount | 2 |
| Circularity | 0.785 |

1024 x 1024

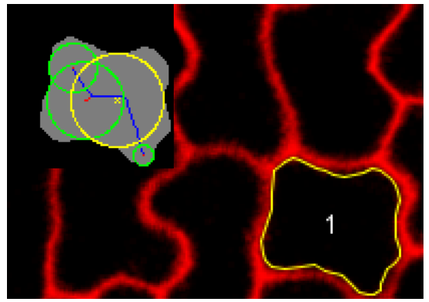
0.42 µm/px




| Parameter | Value |
|---|---|
| Area | 130.7 |
| Perimeter | 47.6 |
| MR | 7.06 |
| BranchCount | 3 |
| CH Conv | 0.926 |
| LobeCount | 4 |
| Circularity | 0.722 |

2048 x 2048

0.21 µm /px




| Parameter | Value |
|---|---|
| Area | 130.5 |
| Perimeter | 47.6 |
| MR | 4.9 |
| BranchCount | 3 |
| CH Conv | 0.927 |
| LobeCount | 5 |
| Circularity | 0.712 |