# Self-promoted and self-terminated PIN2-mediated auxin transport coordinates pavement cell morphogenesis in *Arabidopsis* cotyledons

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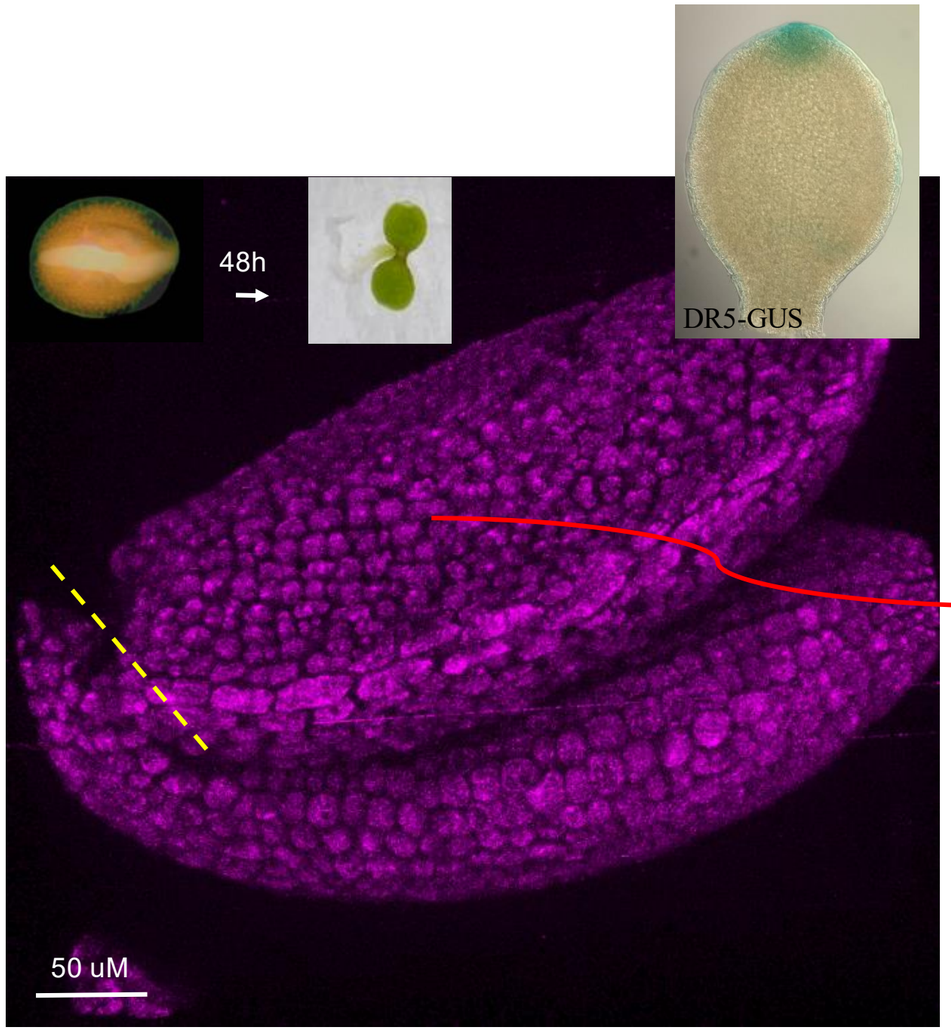
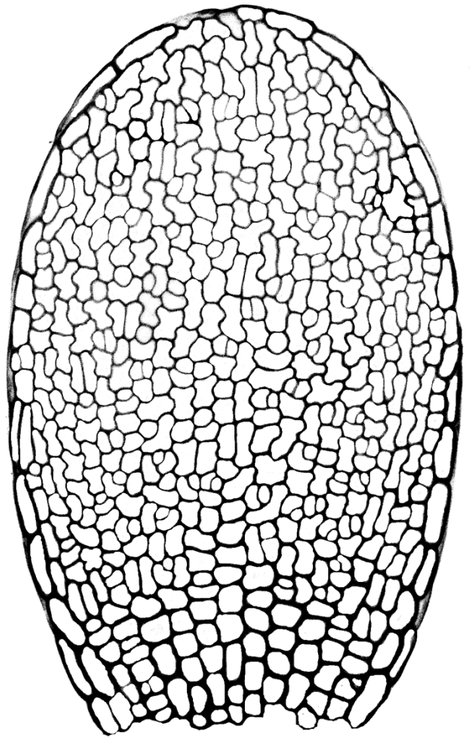
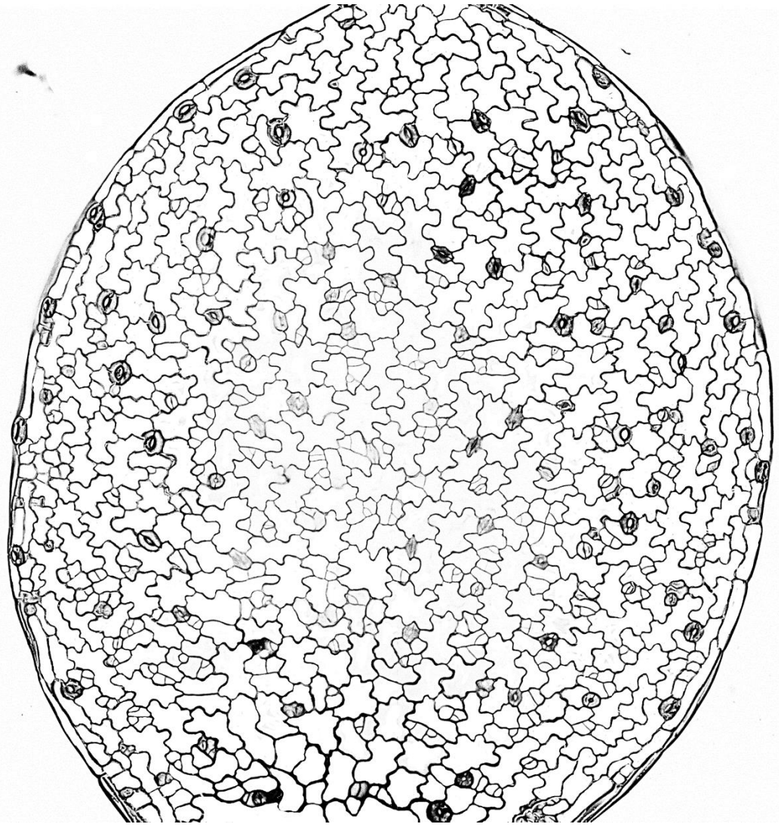
Auxin meeting 2022

# Arabidopsis cotyledon expansion is a good model for understanding auxin-driven growth coordination

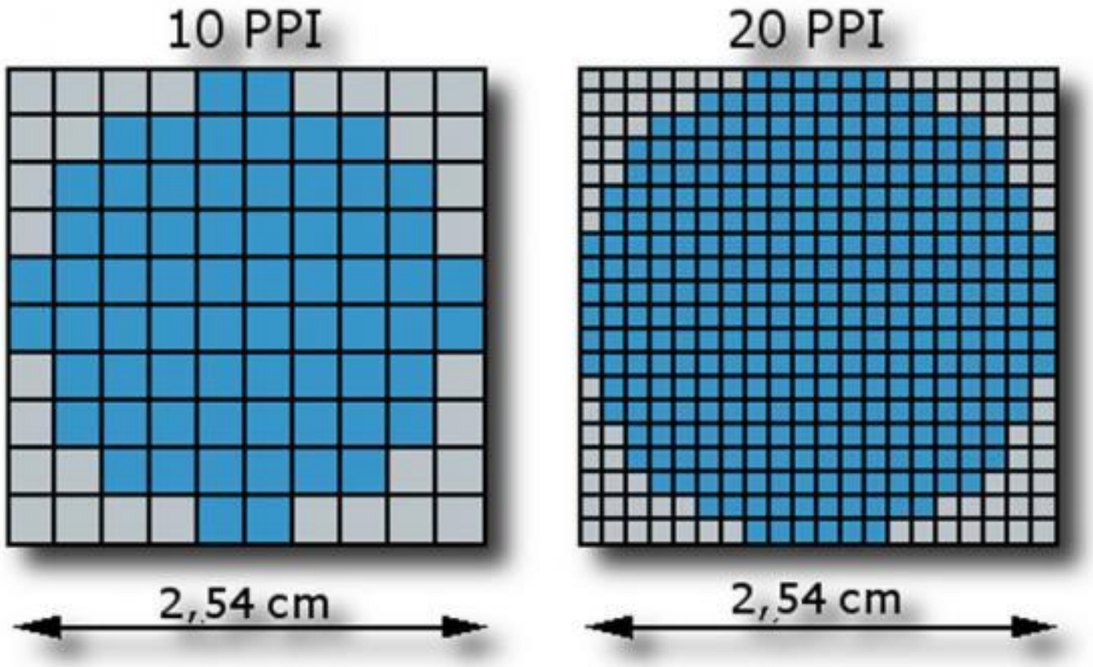


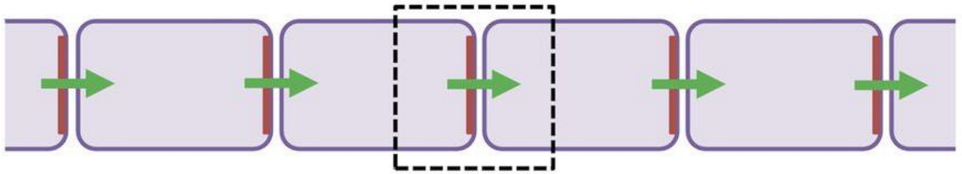


# How is the auxin maxima at the tip formed?

# Compare the shape

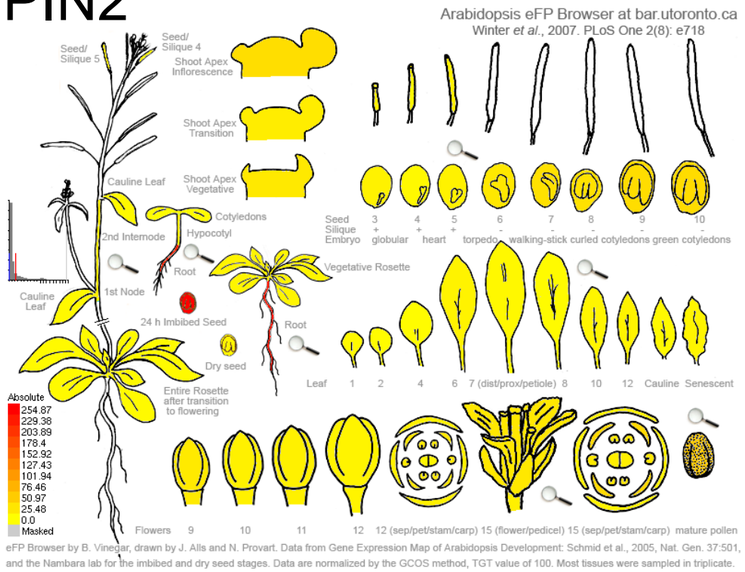


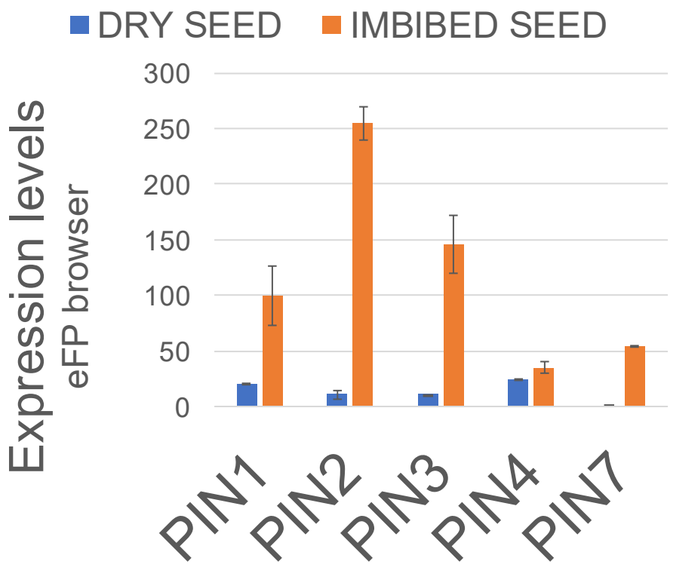
# PIN-based auxin transport



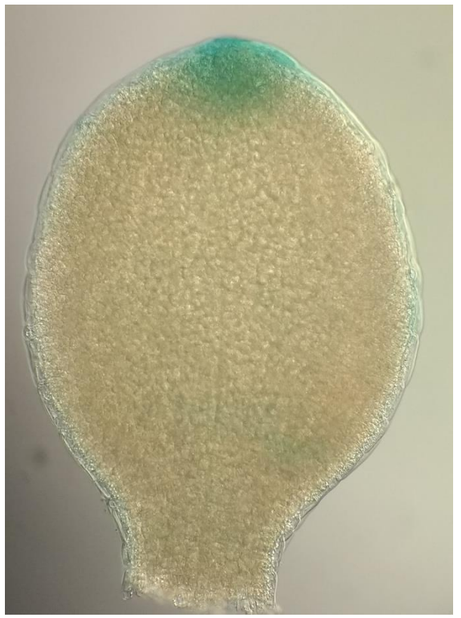
Friml and Jones. 2010. Plant Phys
Adamowski and Friml 2015. The Plant Cell

PIN2



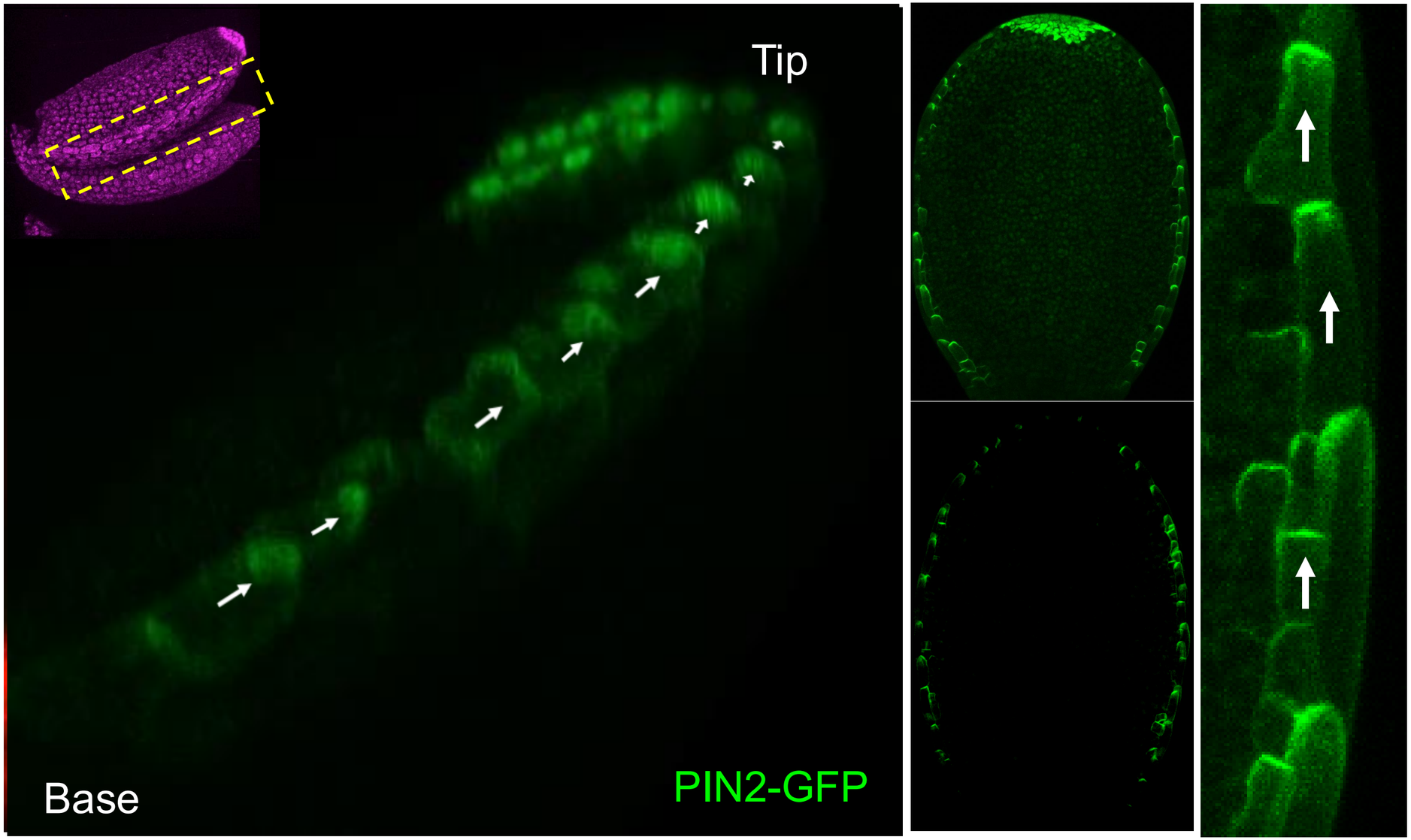




DR5-GUS



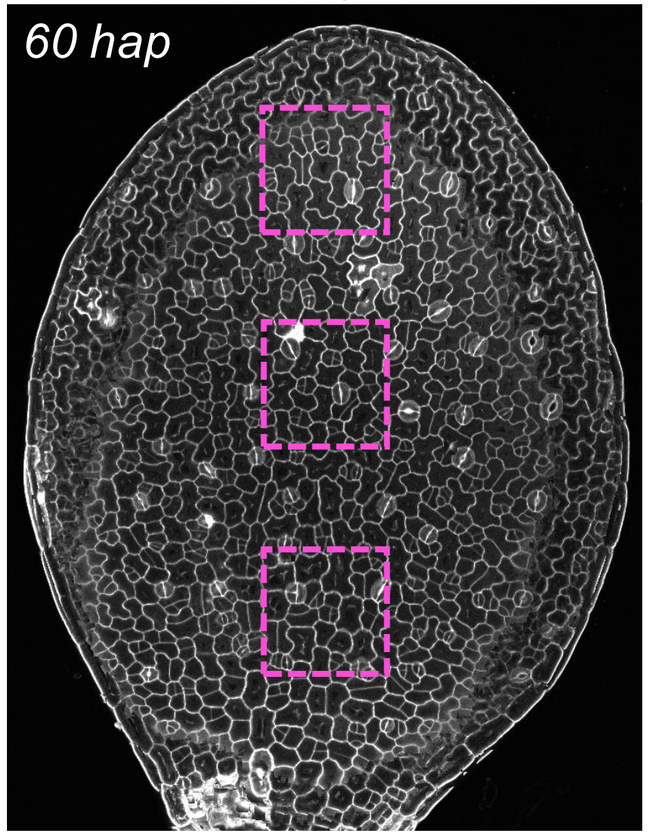
*eir1-1/pin2*
DR5-GUS

# PIN2 is expressed in margin cells (MCs)

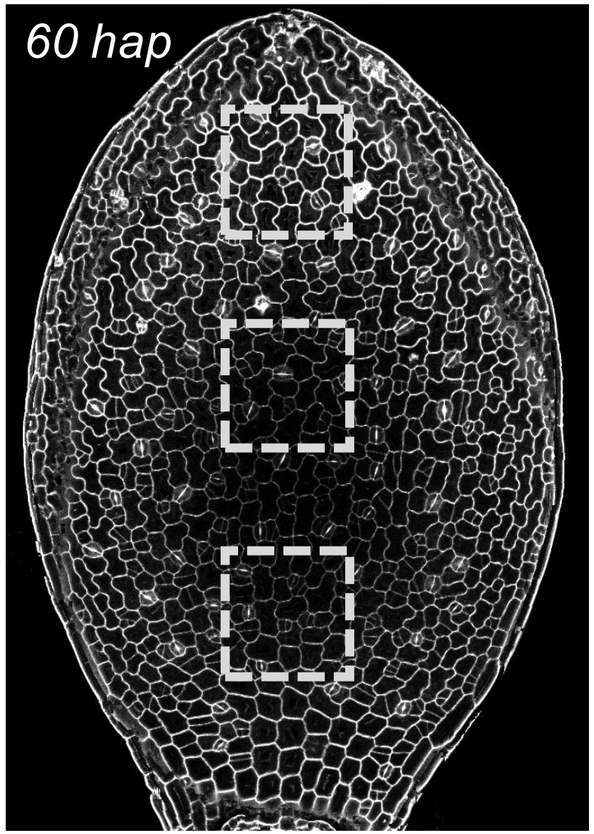


# Interdigitation phenotype of *eir1-1* / pin2 mutant

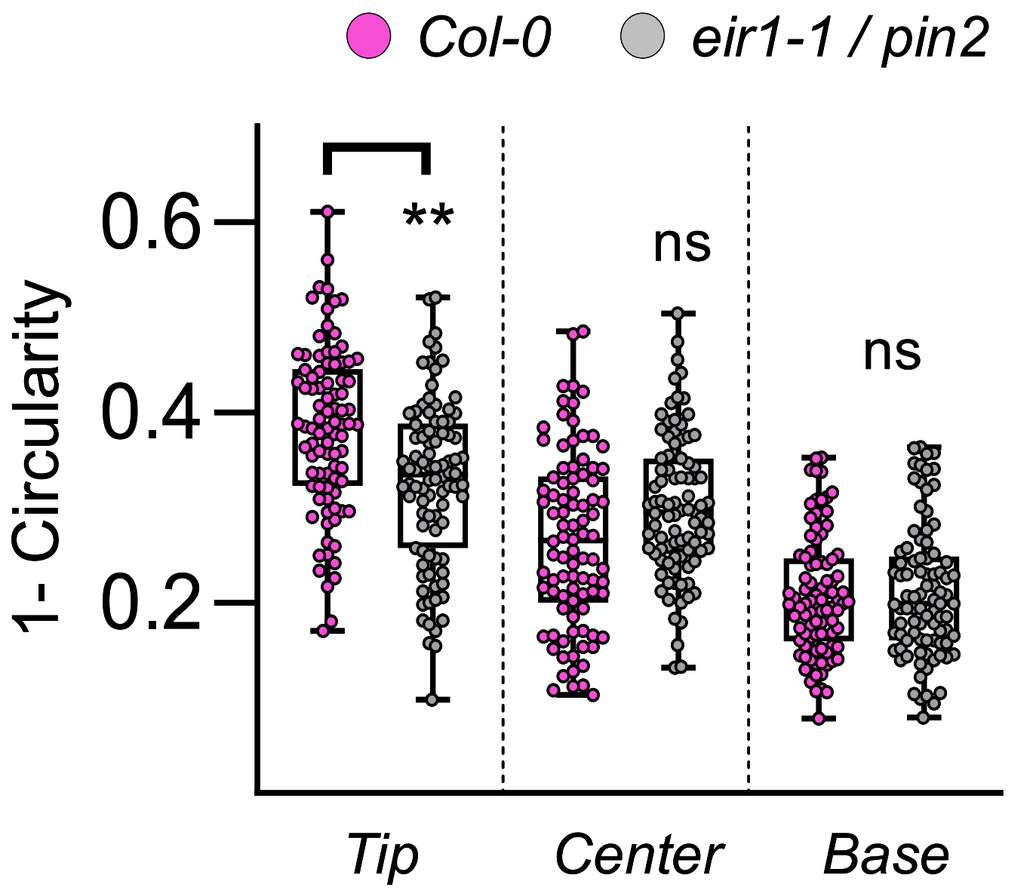
Wild type



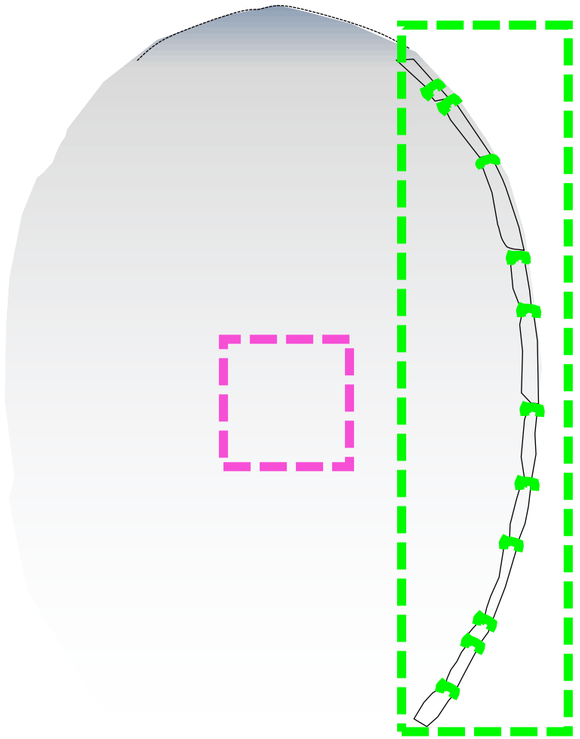

*eir1-1 / pin2*




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

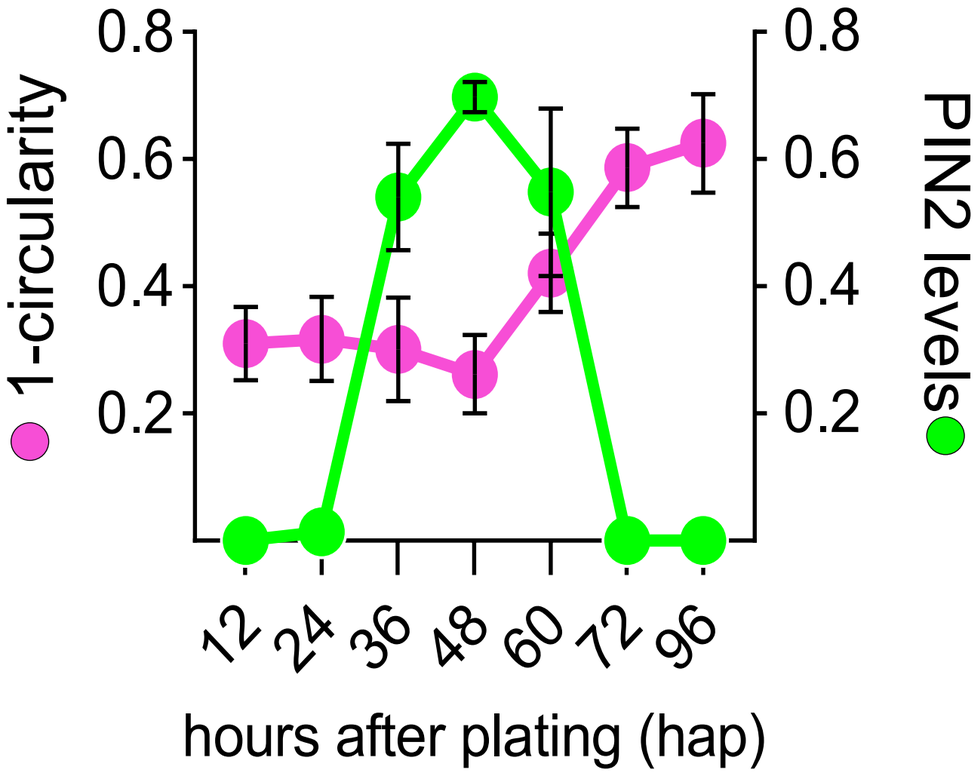


# PIN2 precedes the start of interdigitation



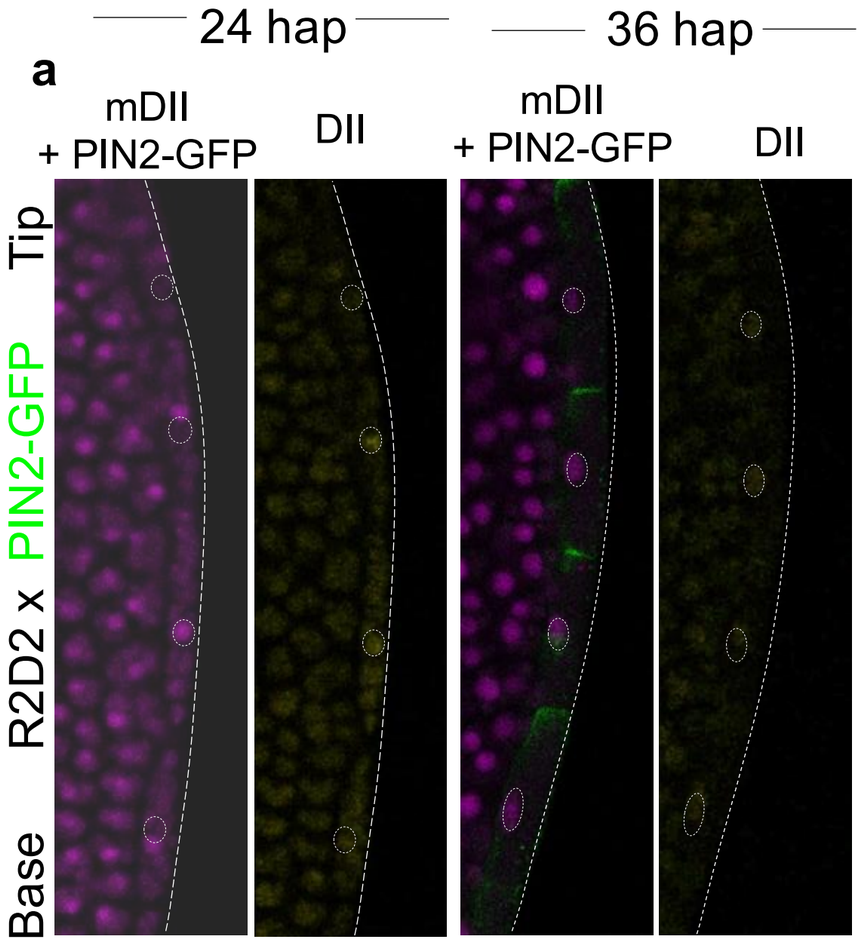
$$\text{PIN2-GFP levels} = \frac{\text{MCs PIN2+}}{\text{Total MCs}}$$

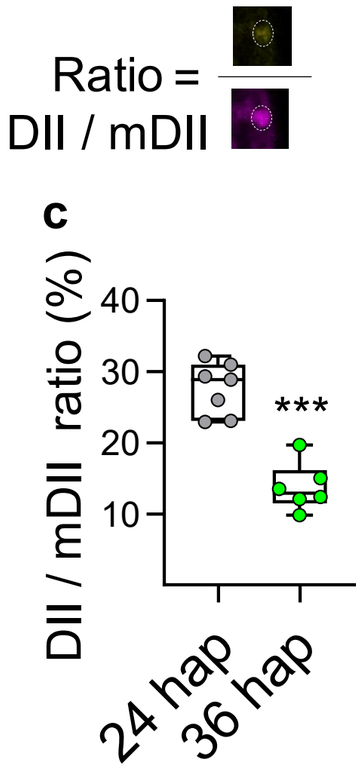
$$\text{Circularity} = \frac{4 \cdot \pi \cdot [\text{Area}]}{[\text{Perimeter}]^2}$$

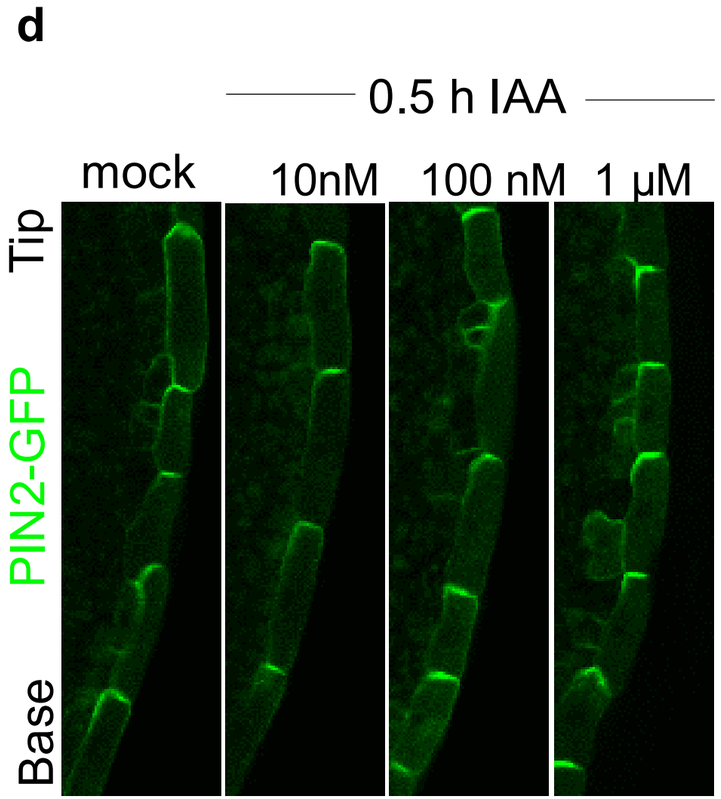


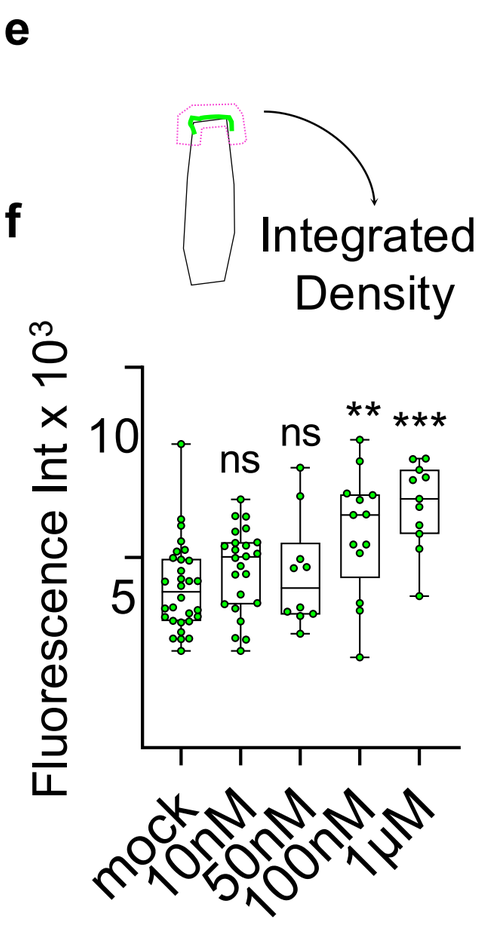

This system is transient BUT is it transporting auxin (functional)?

# PIN2 along MCs is fully functional



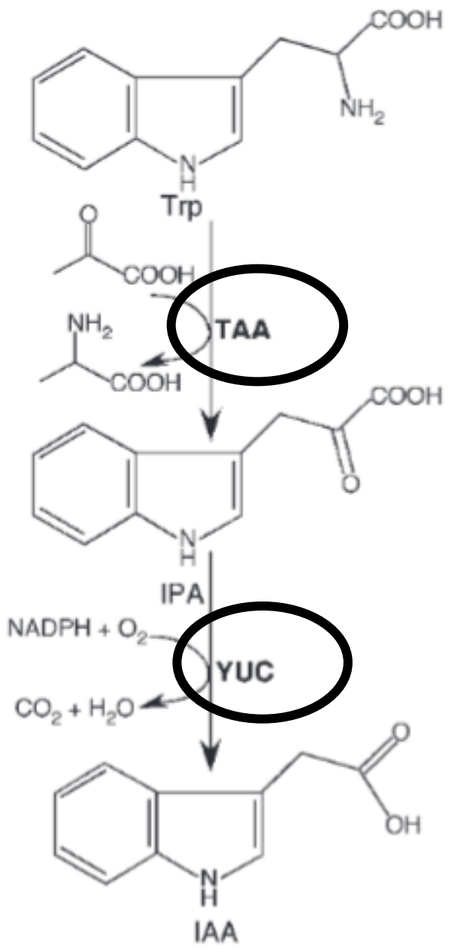




PIN2 is transporting auxin BUT is transient…how?

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

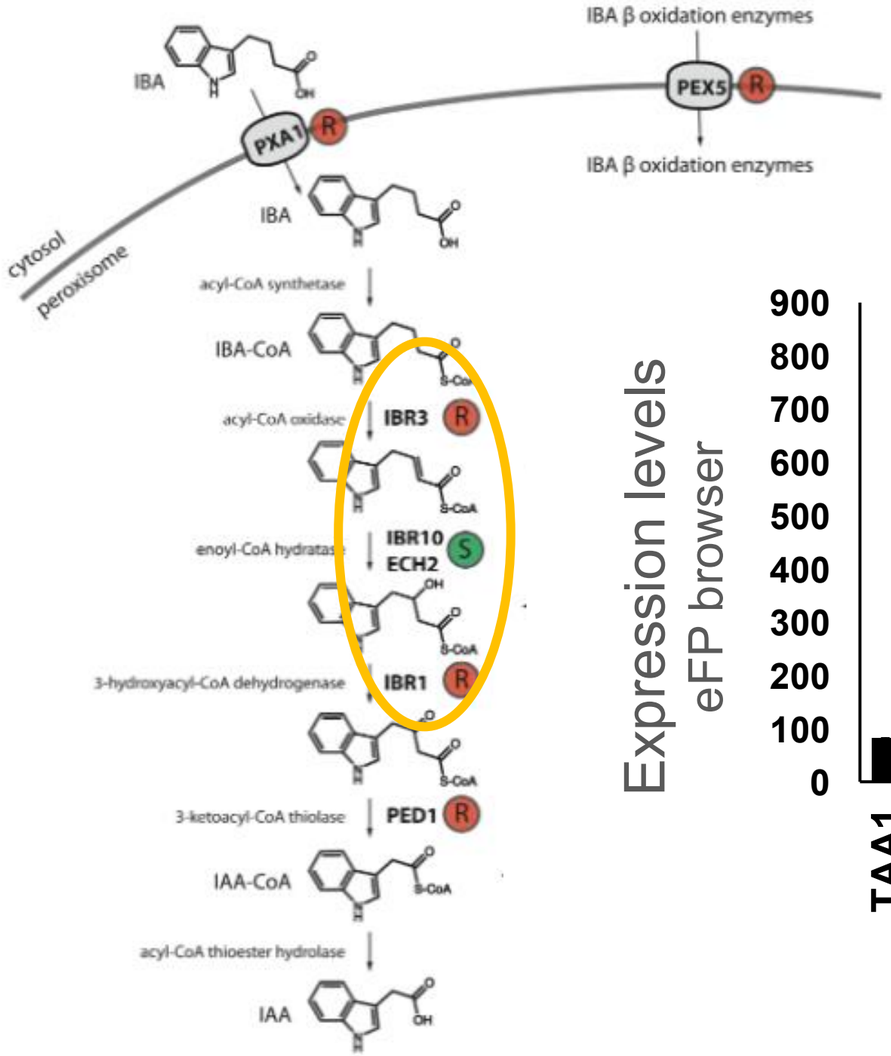
# What is the initiating signal?

## IPA pathway

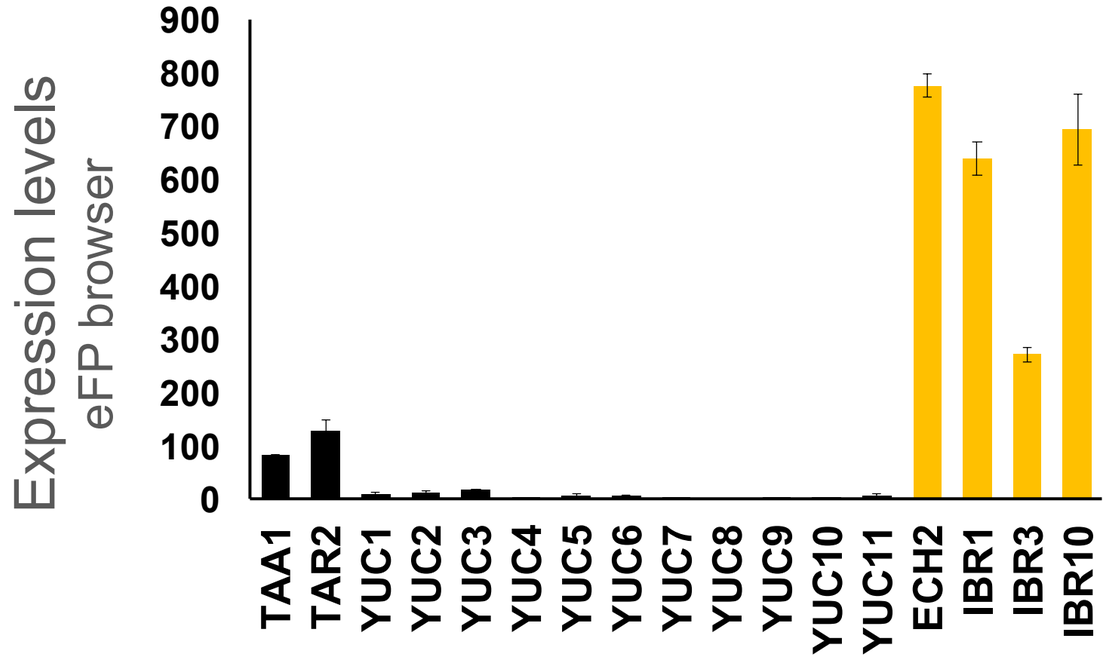



Zhao et al 2012, Molecular Plant

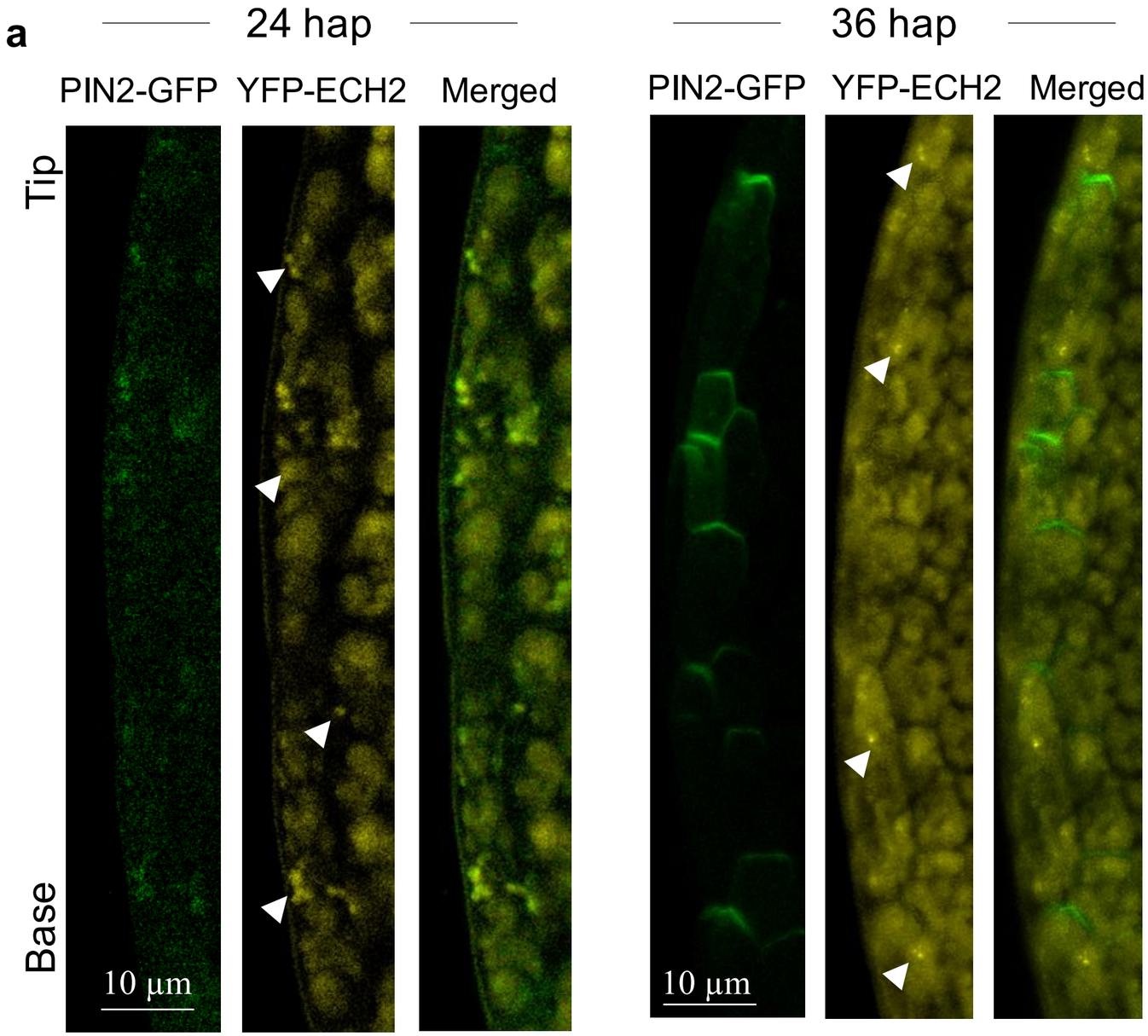
## IBA-to-IAA conversion

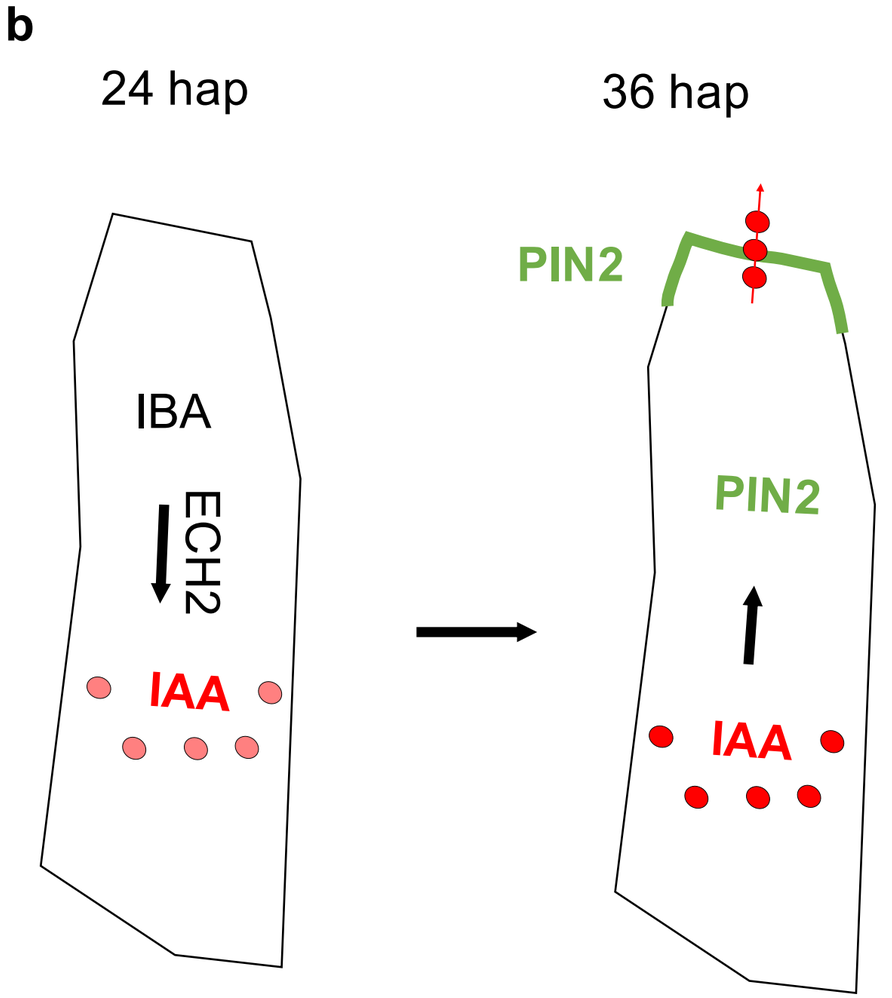



De Rybel et al 2011, Nature Chem Biol

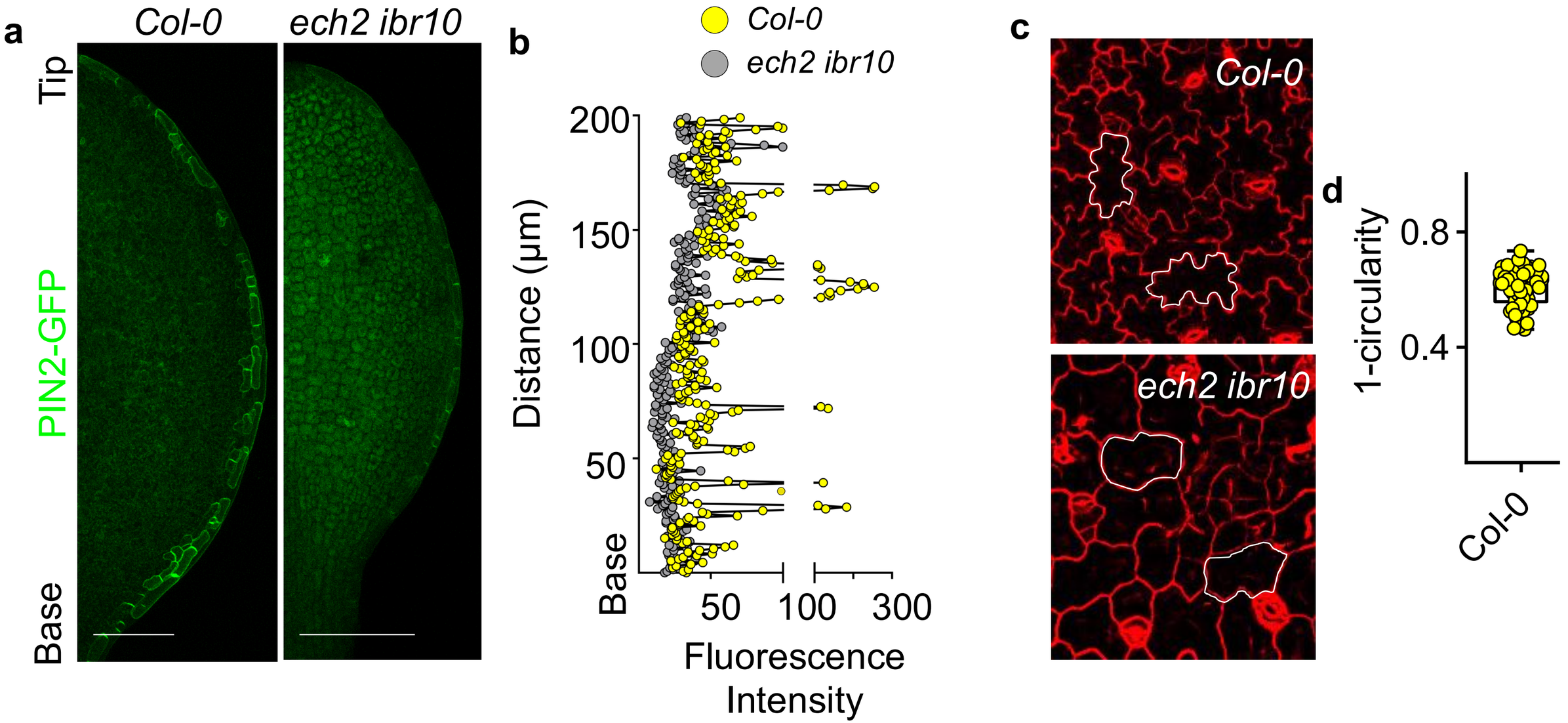


# IBA-derived auxin precedes PIN2 levels



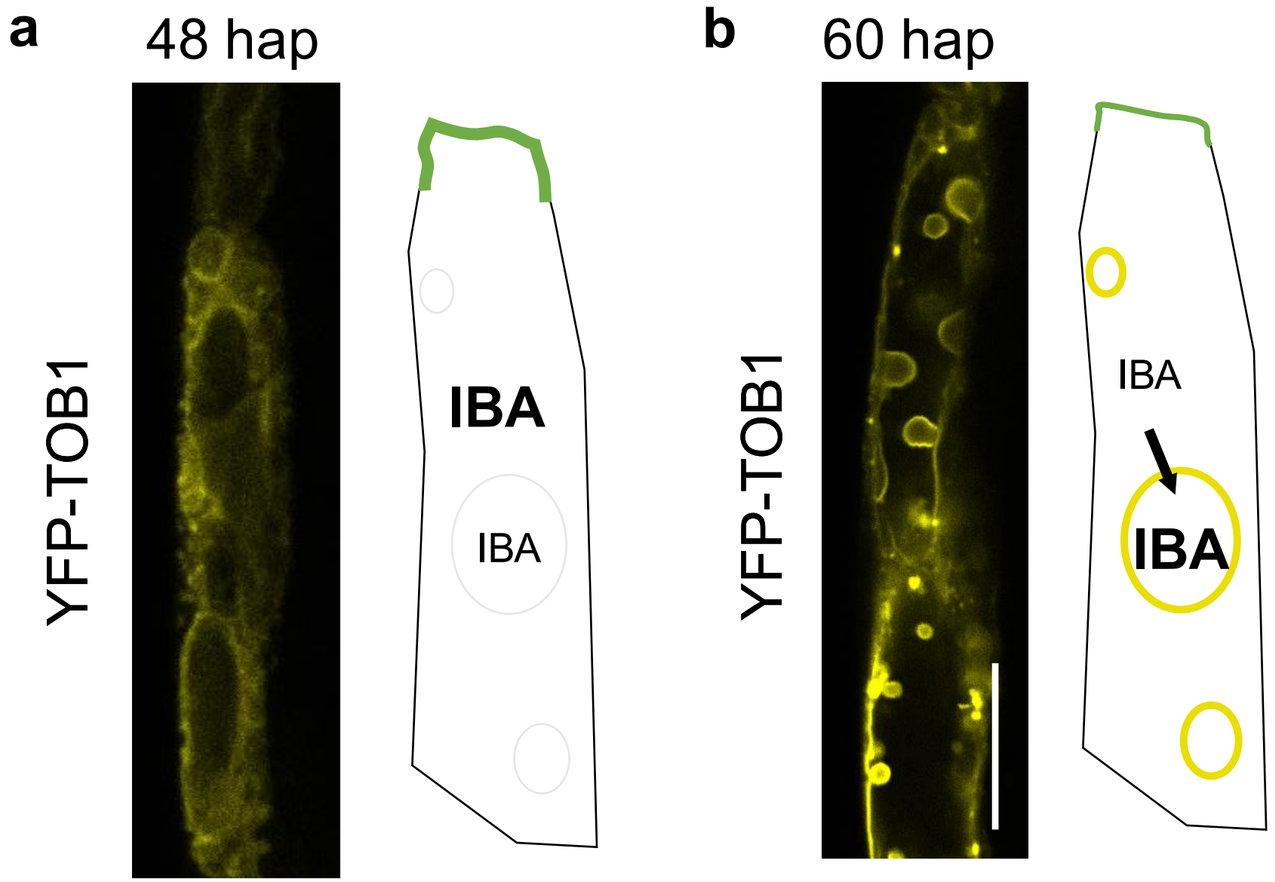



# IBA-derived auxin regulates PIN2 levels at MCs



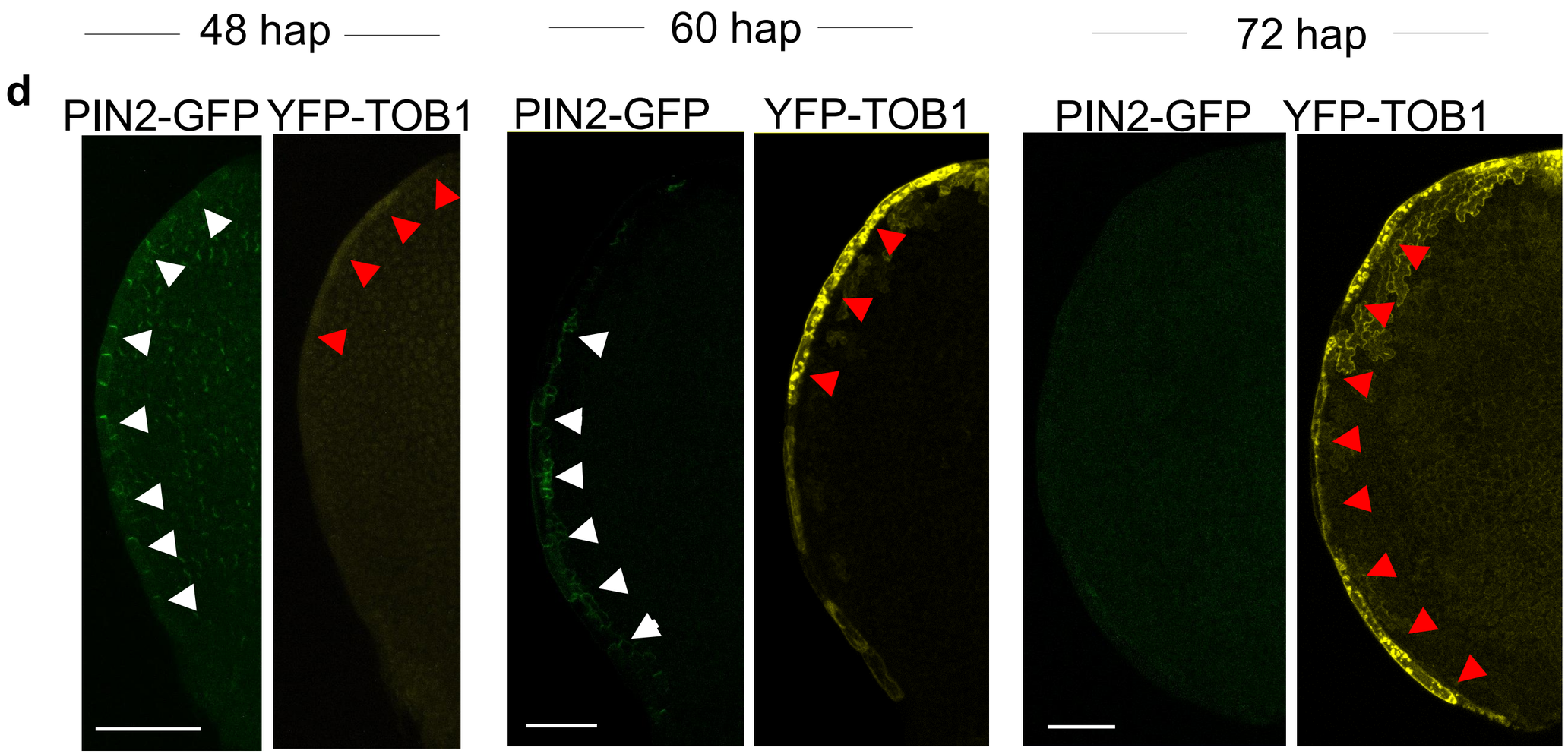

The initiating is signal IBA-derived auxin  BUT what is the terminating signal?

# Vacuole-localized TOB1 correlates with reduced PIN2 levels

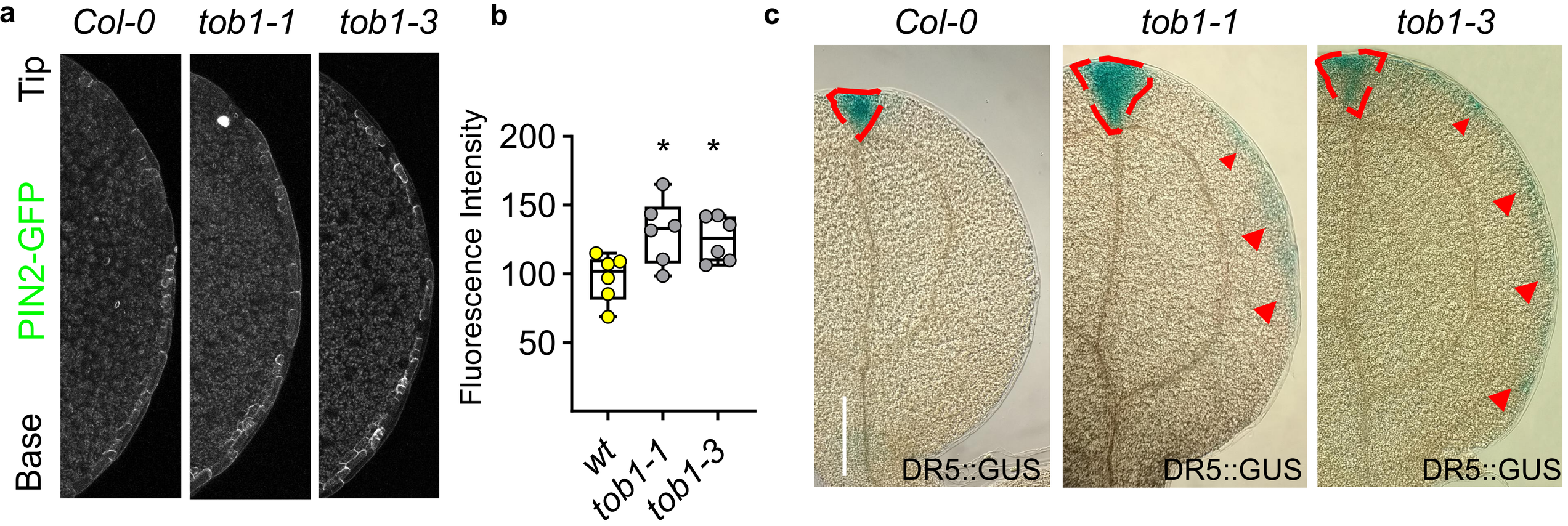



The hypothesis might be correct …

# Vacuole-localized TOB1 correlates with reduced PIN2 levels

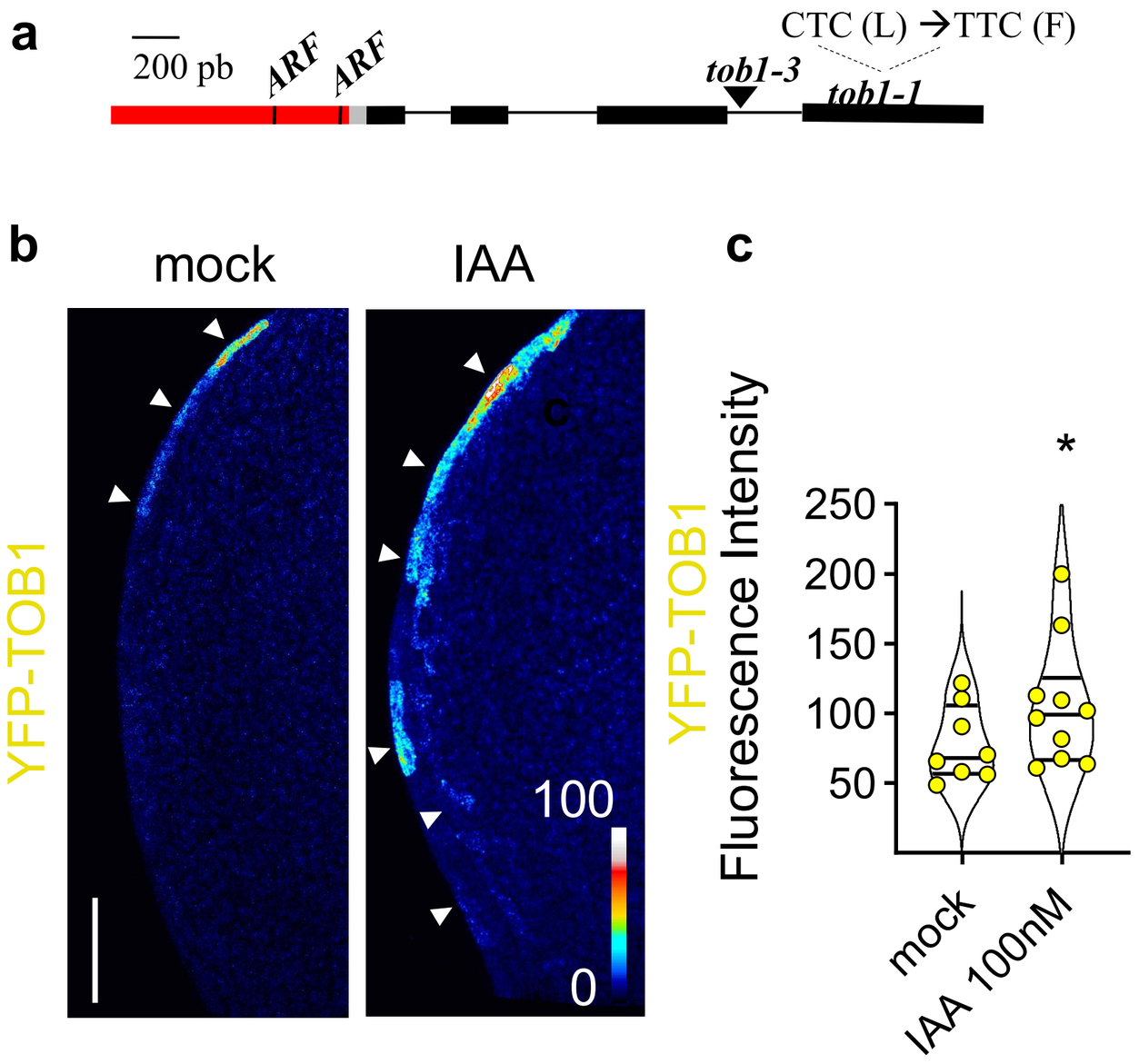


# TOB1 regulates PIN2 levels in margin cells

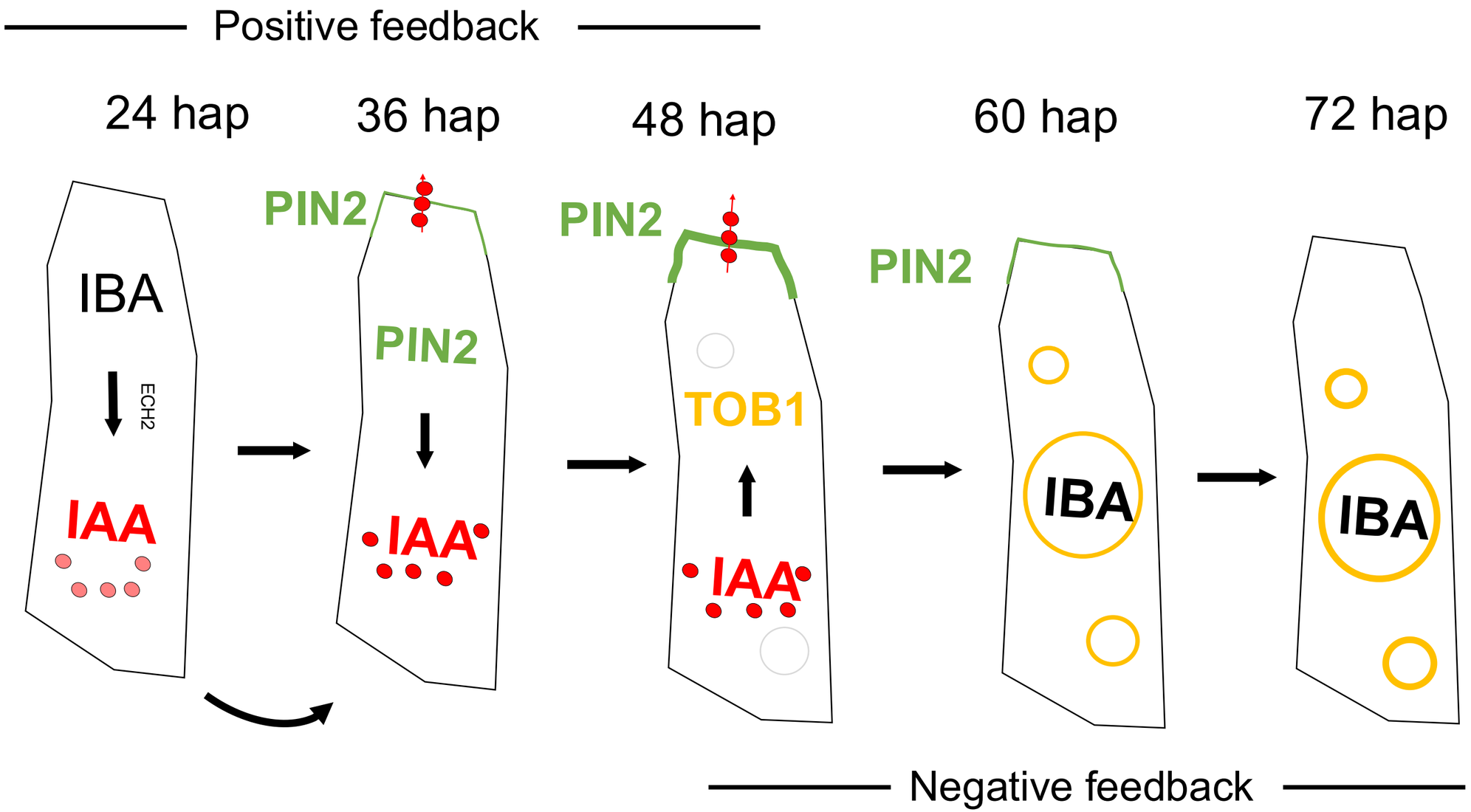



TOB1-mediated IBA internalization into vacuoles is the terminating signal

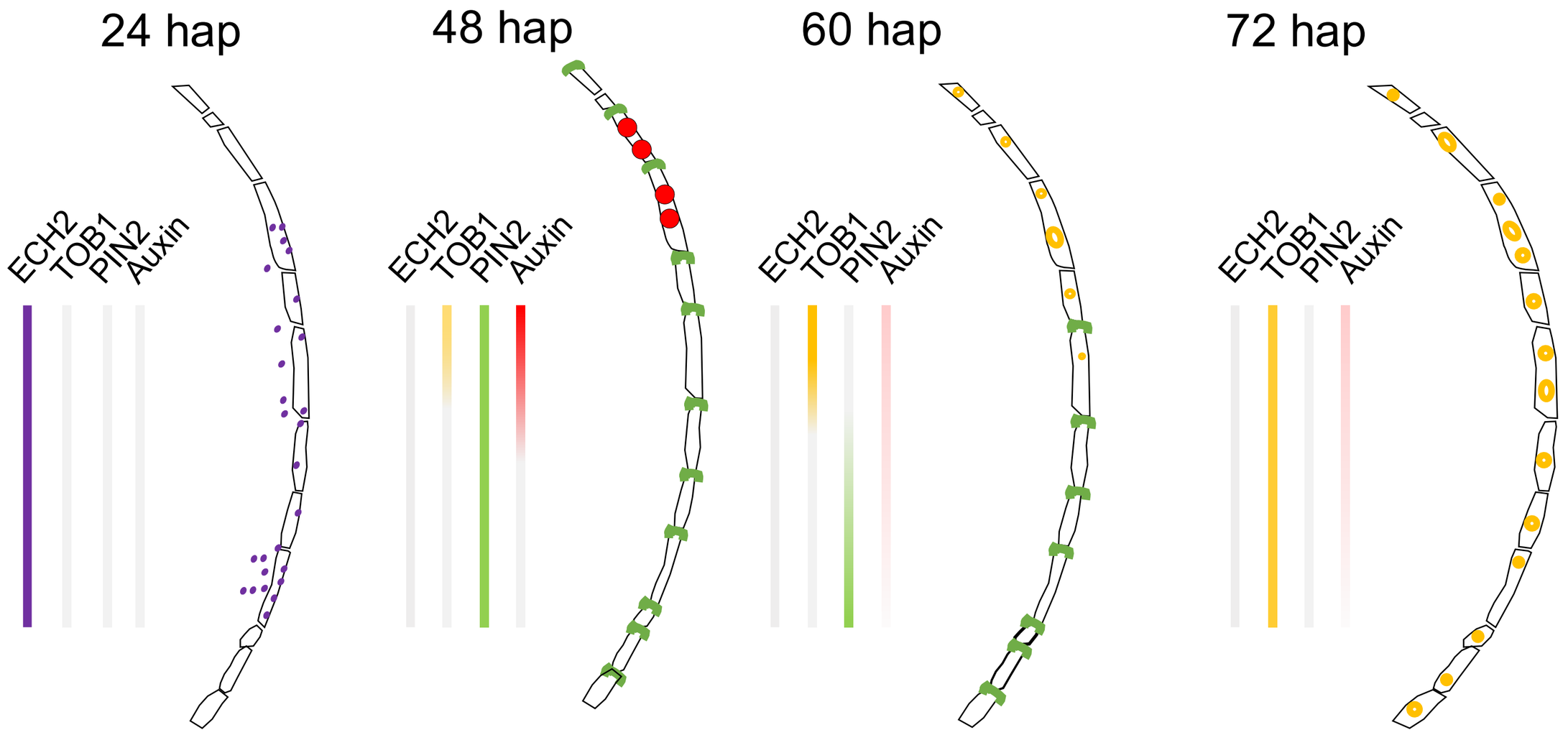
# Auxin regulates TOB1 levels



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



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



# Acknowledgments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Ken-Ichiro Hayashi



UC RIVERSIDE UNIVERSITY OF CALIFORNIA

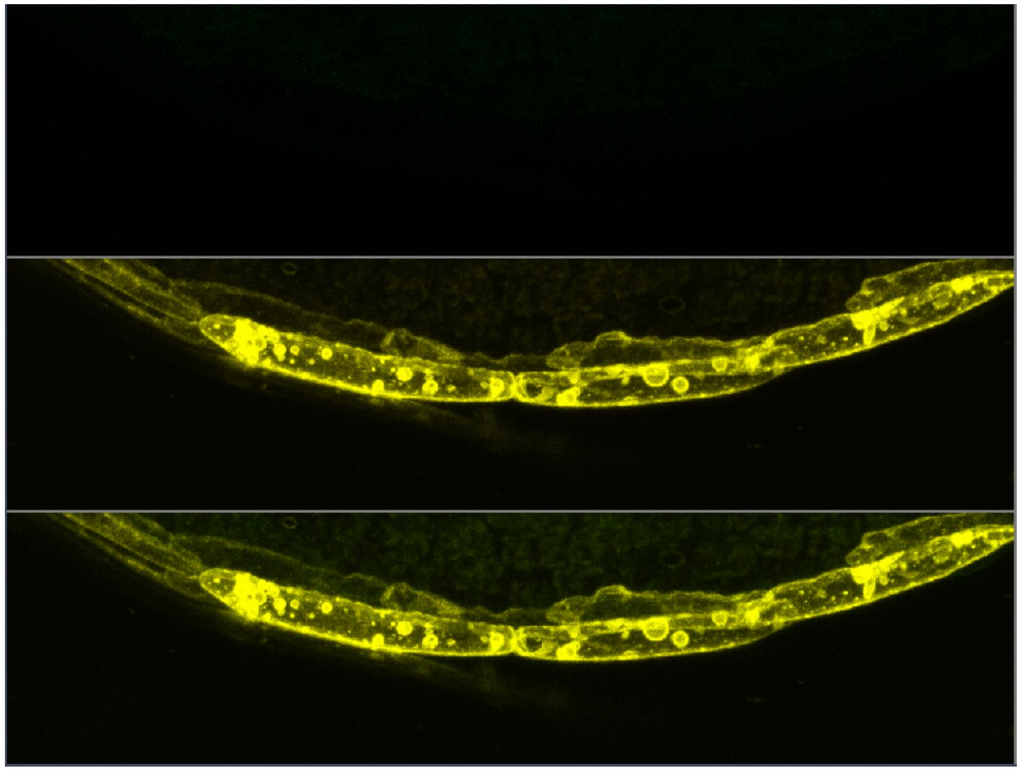
FAFU-
UCR
HBMC

NSF

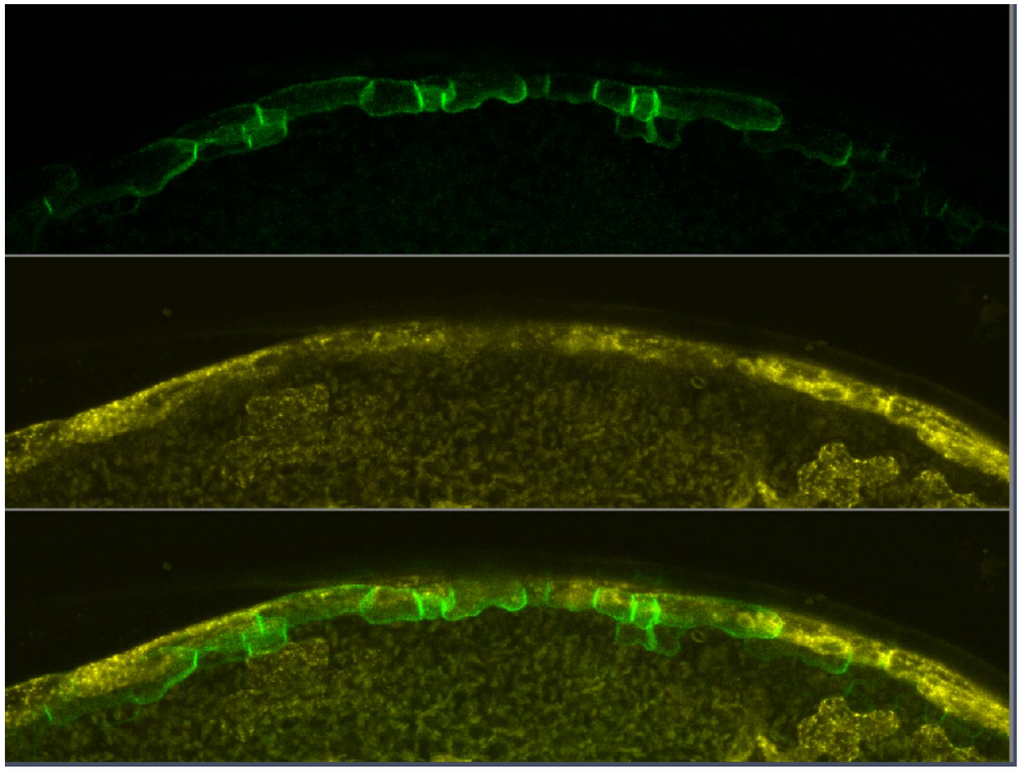
NIH

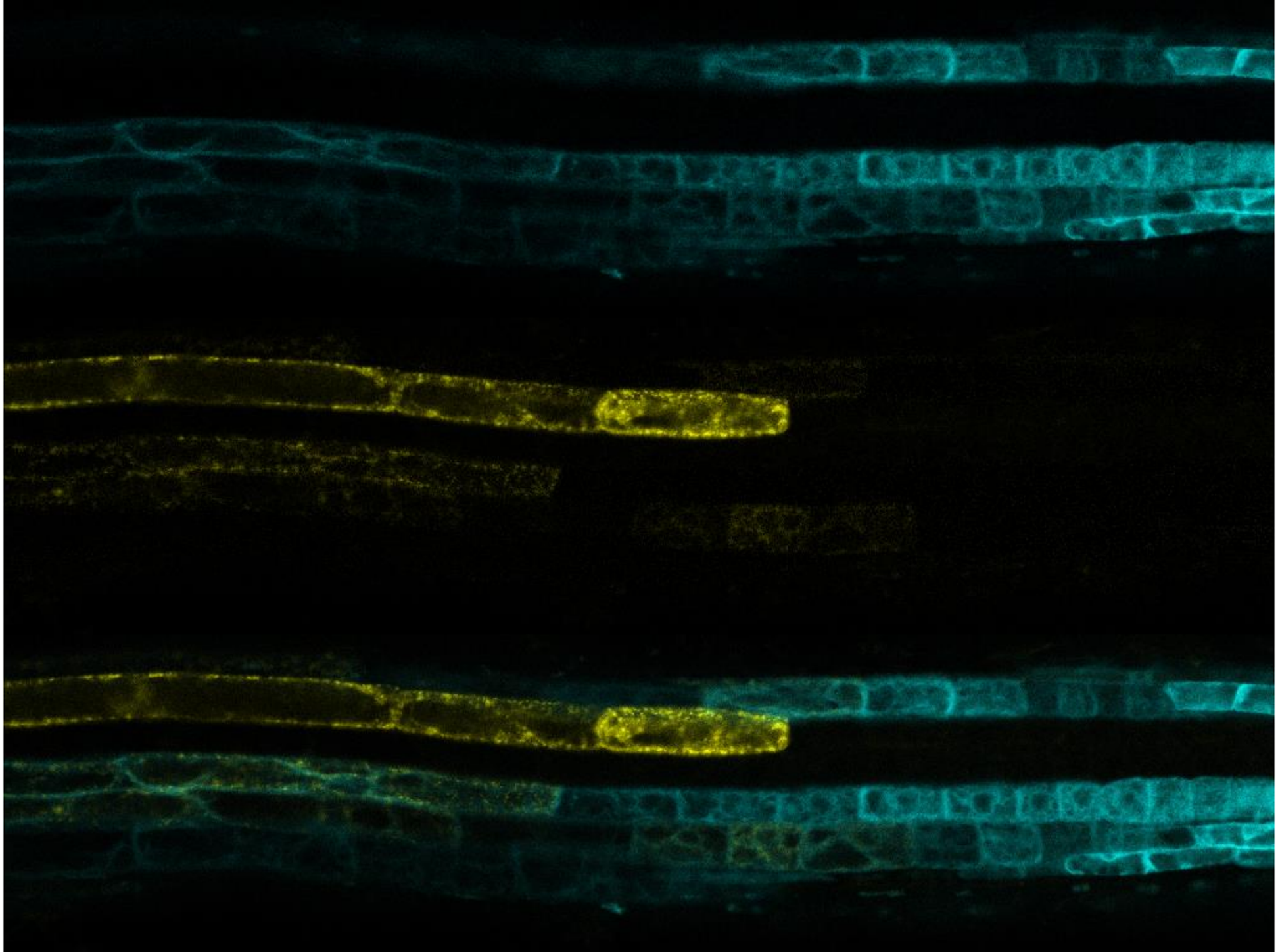
The END

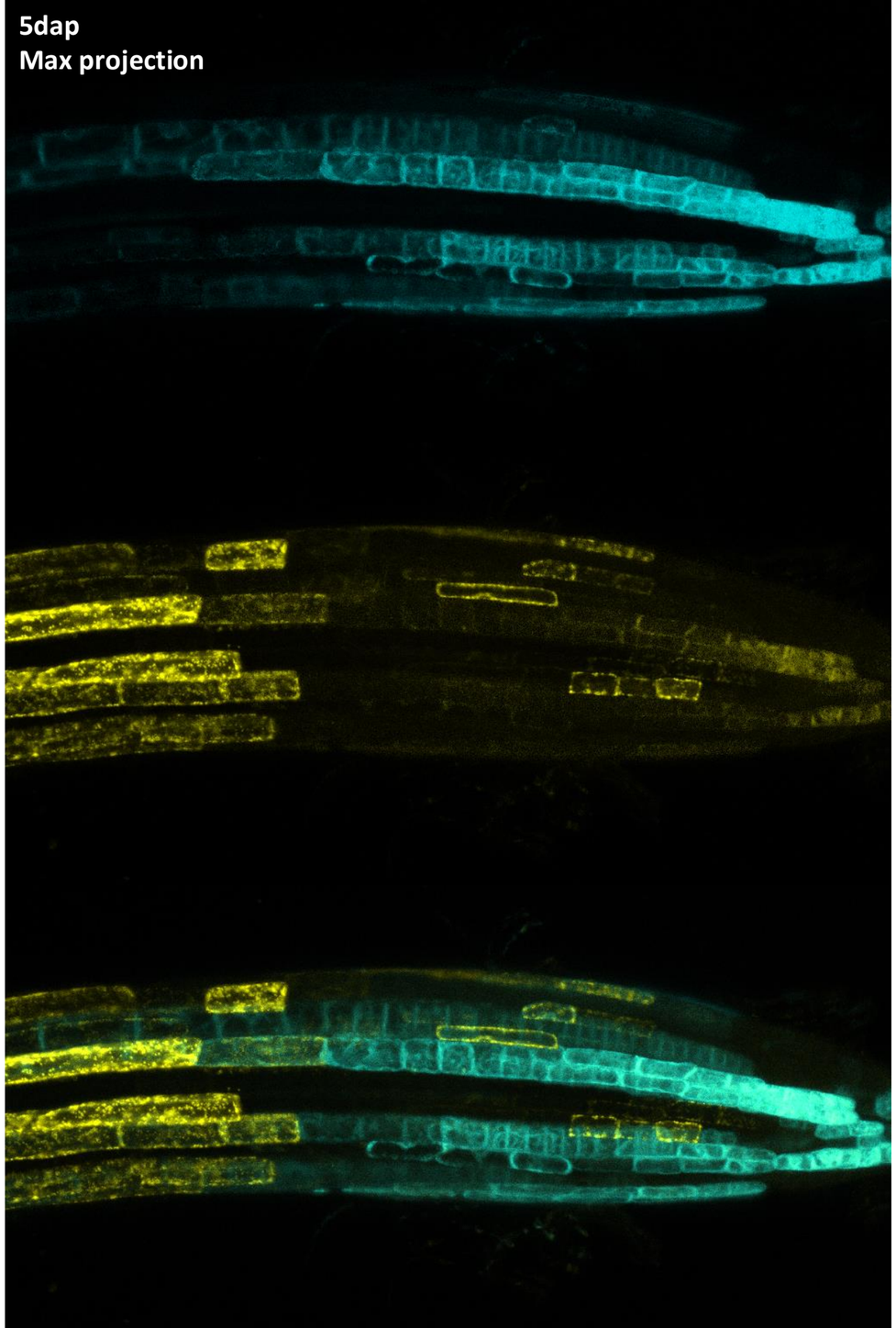
TOB1 vacuole → no PIN2



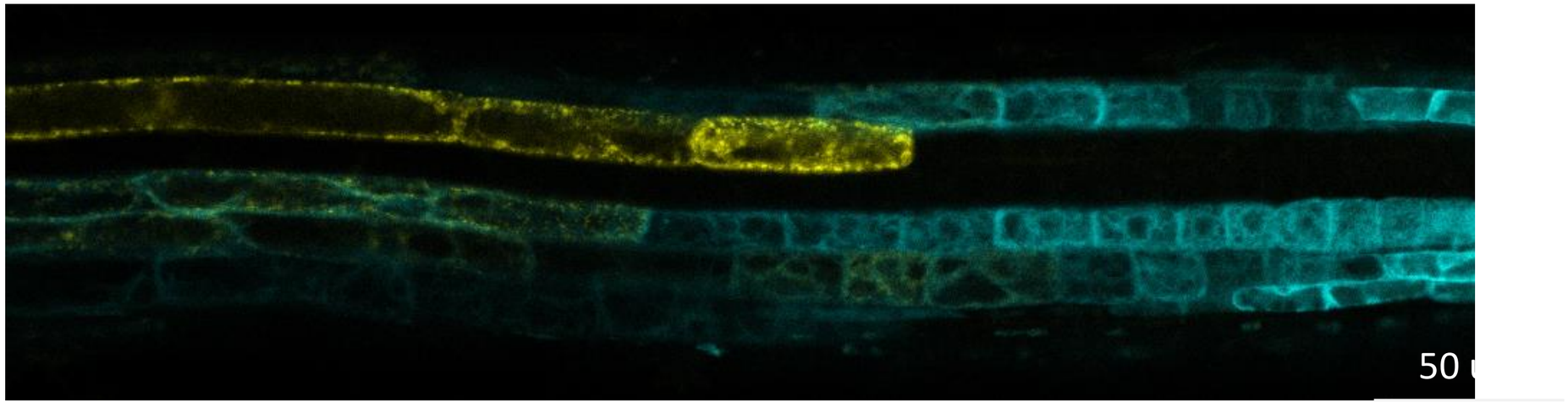
TOB1 reticular → PIN2



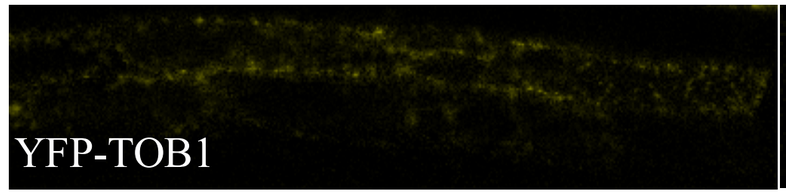


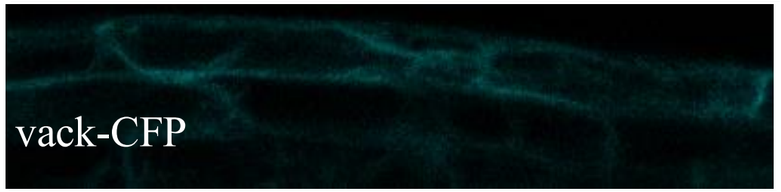

5dap
Max projection

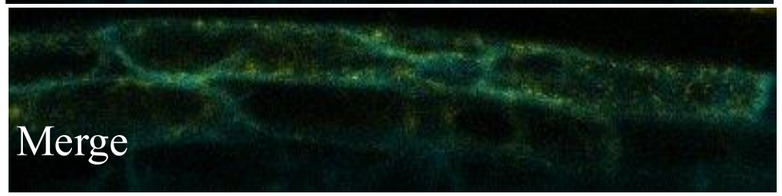
Extract of Montage of Image 76-Linear Unmixing-02



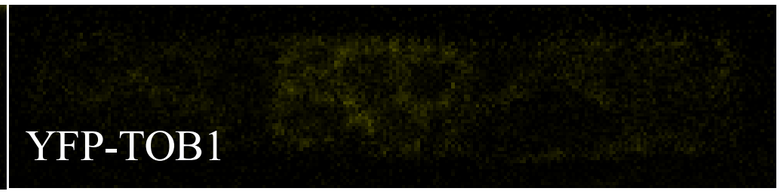

Non-vacuole localization

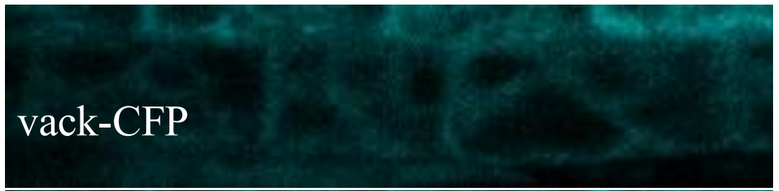


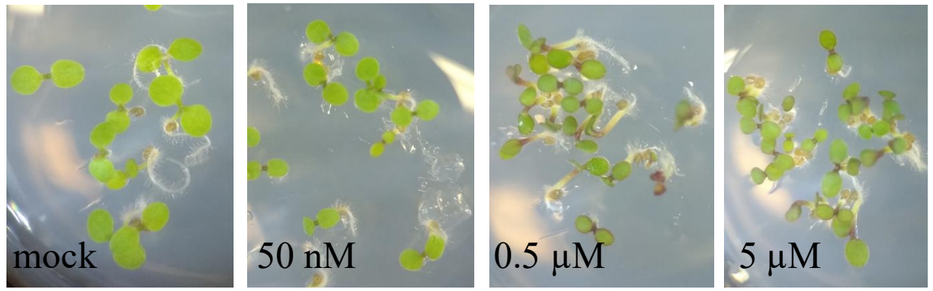

Vacuole localization

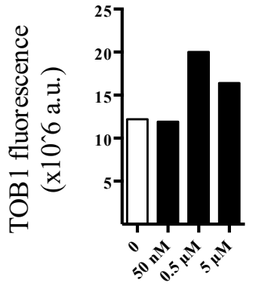


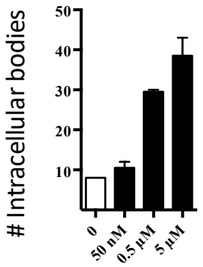




exp1

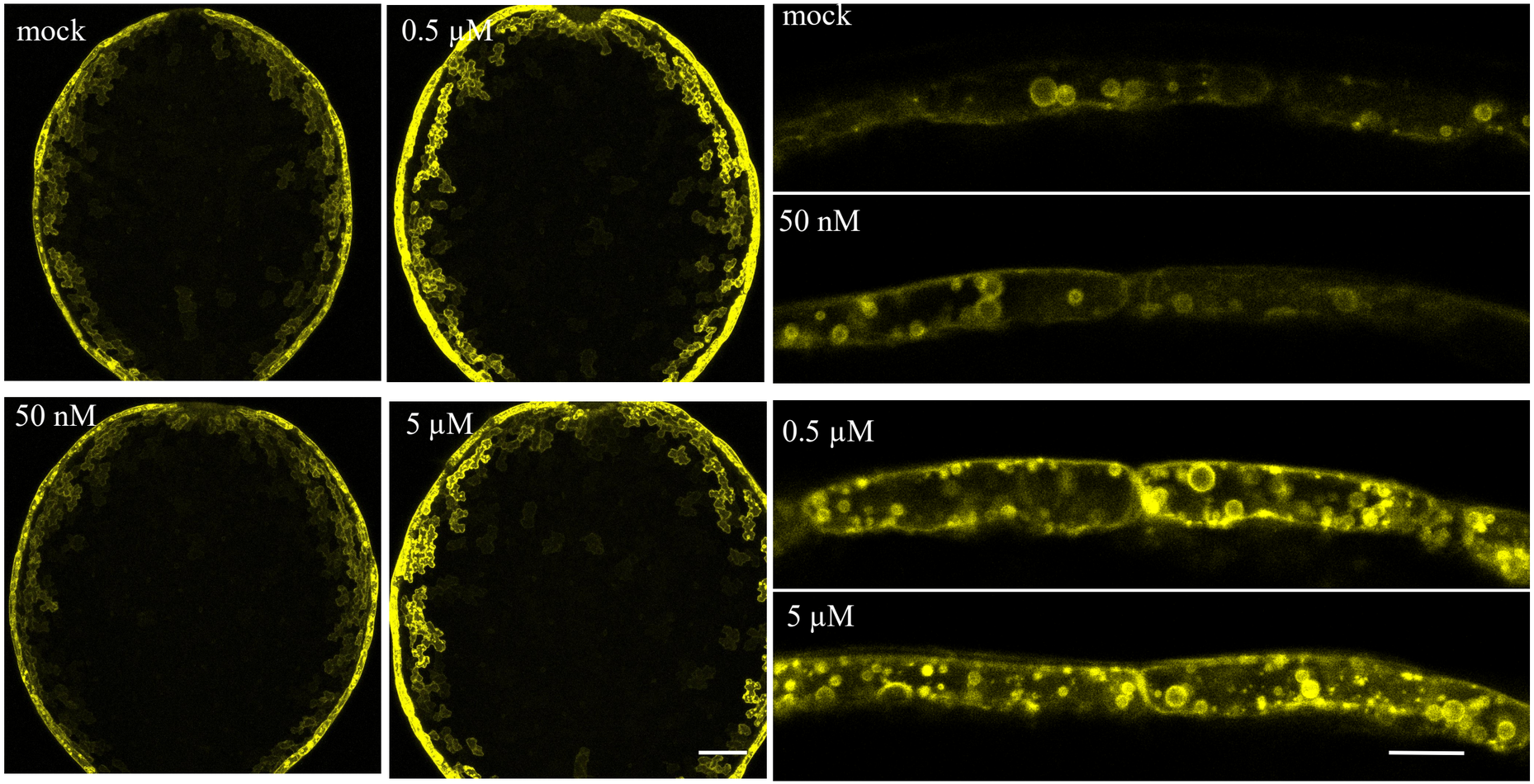
# CK induces TOB1 accumulation



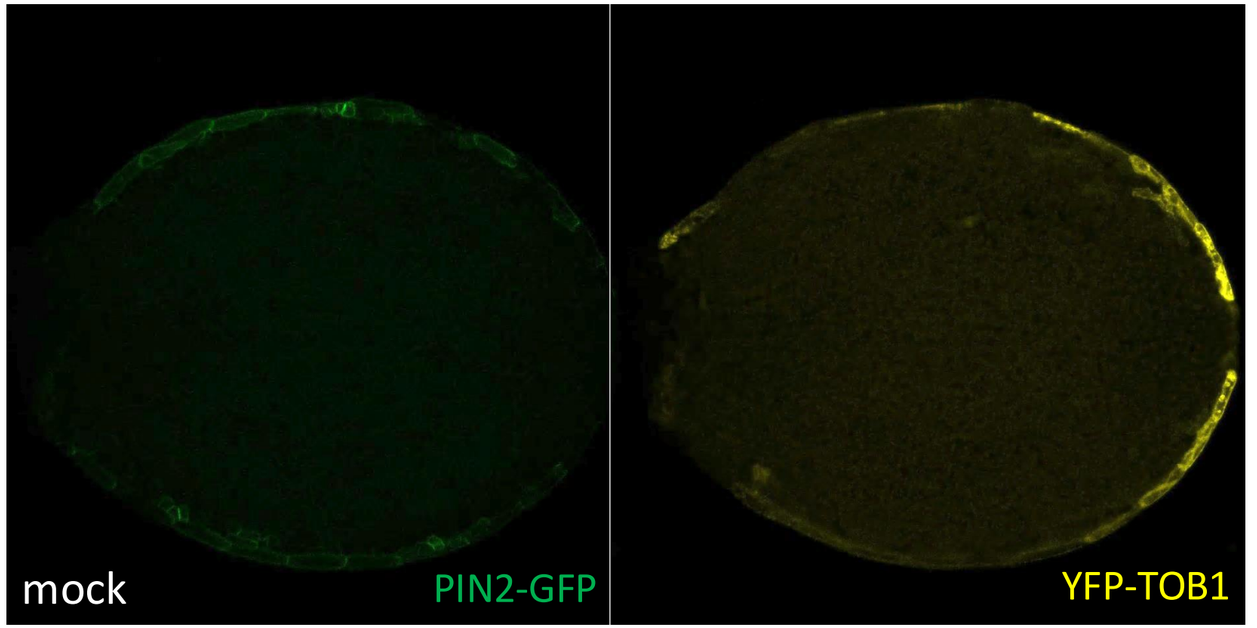




Cotyledon size

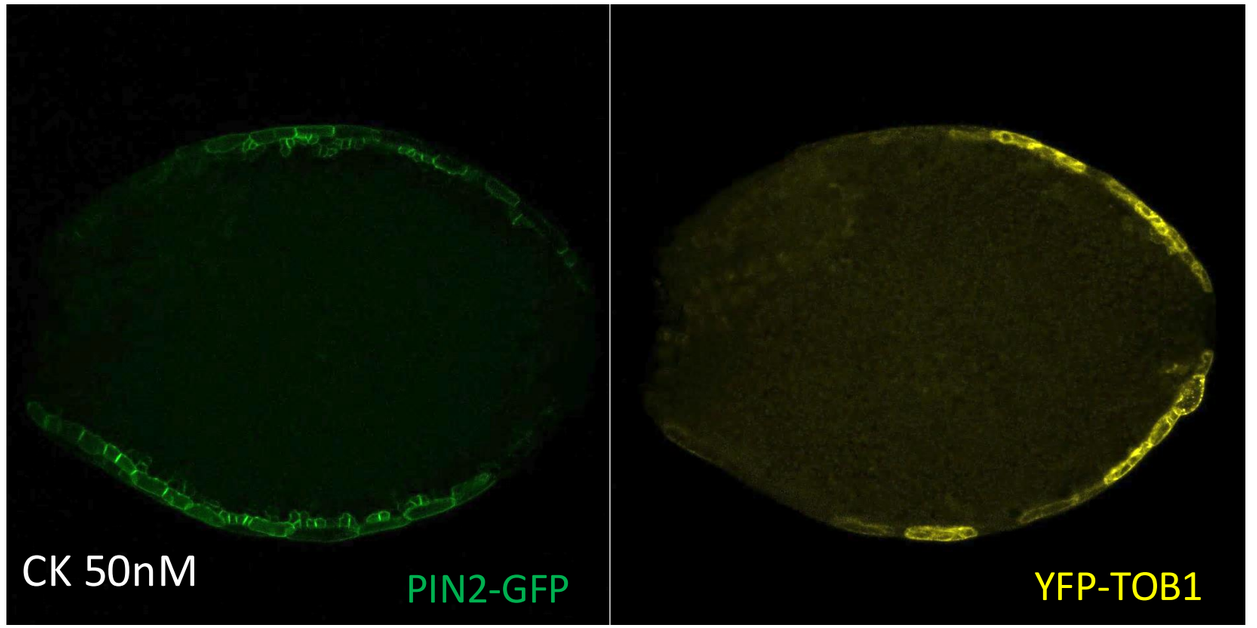



bar = 100 µM (whole coty) , bar= 20 µM (zoom)

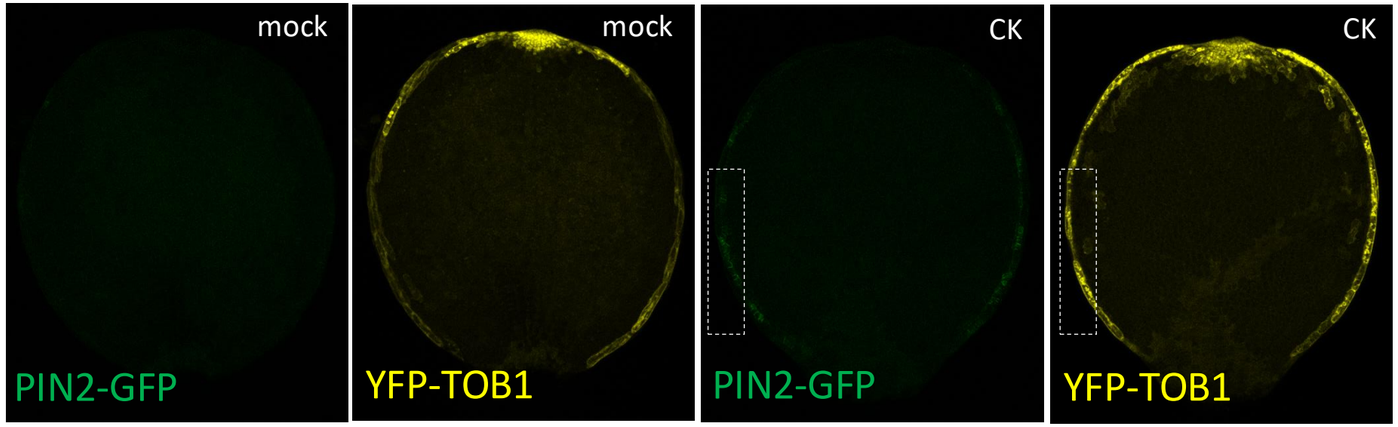
24 hai seeds → 24h mock

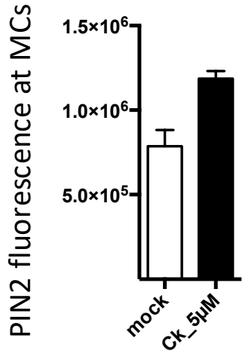


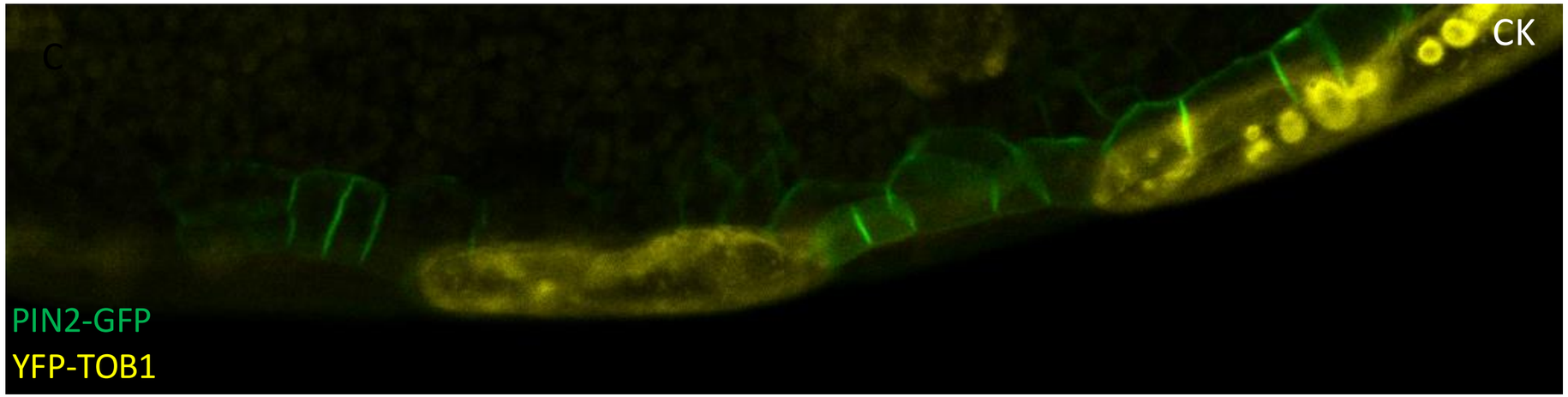

24 hai seeds → 24h CK 50nM



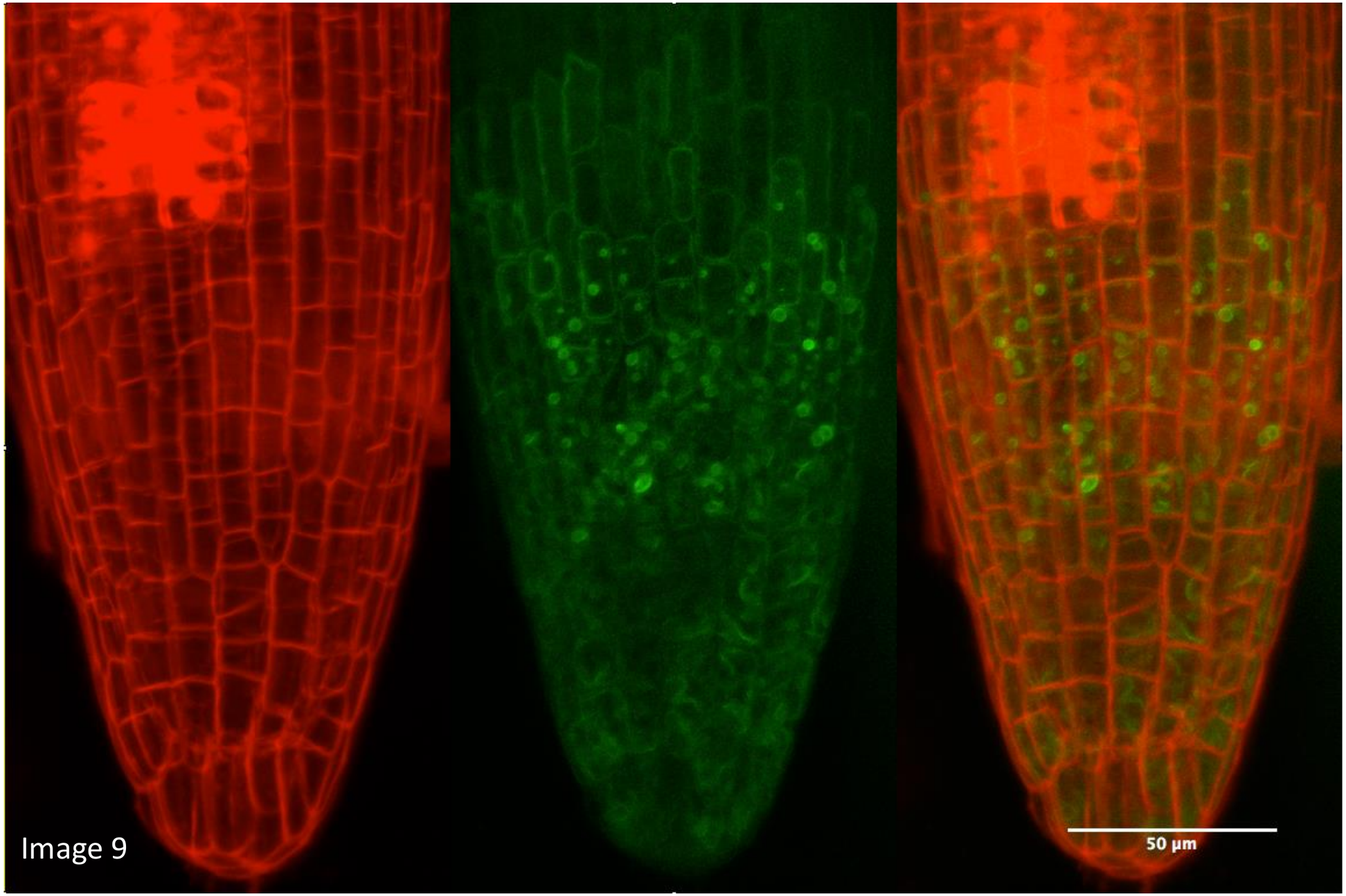
# TOB1 and PIN2 interplay in response to citokinin



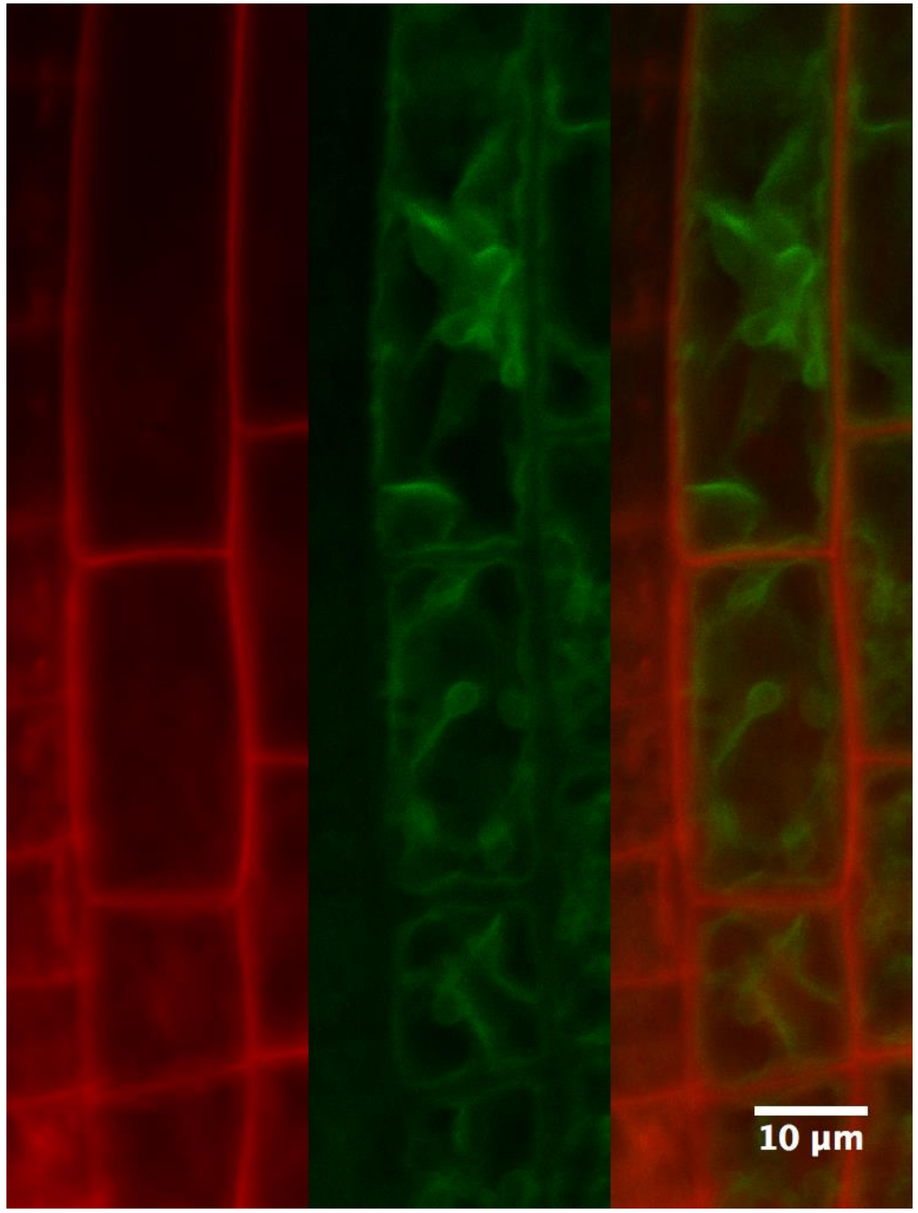



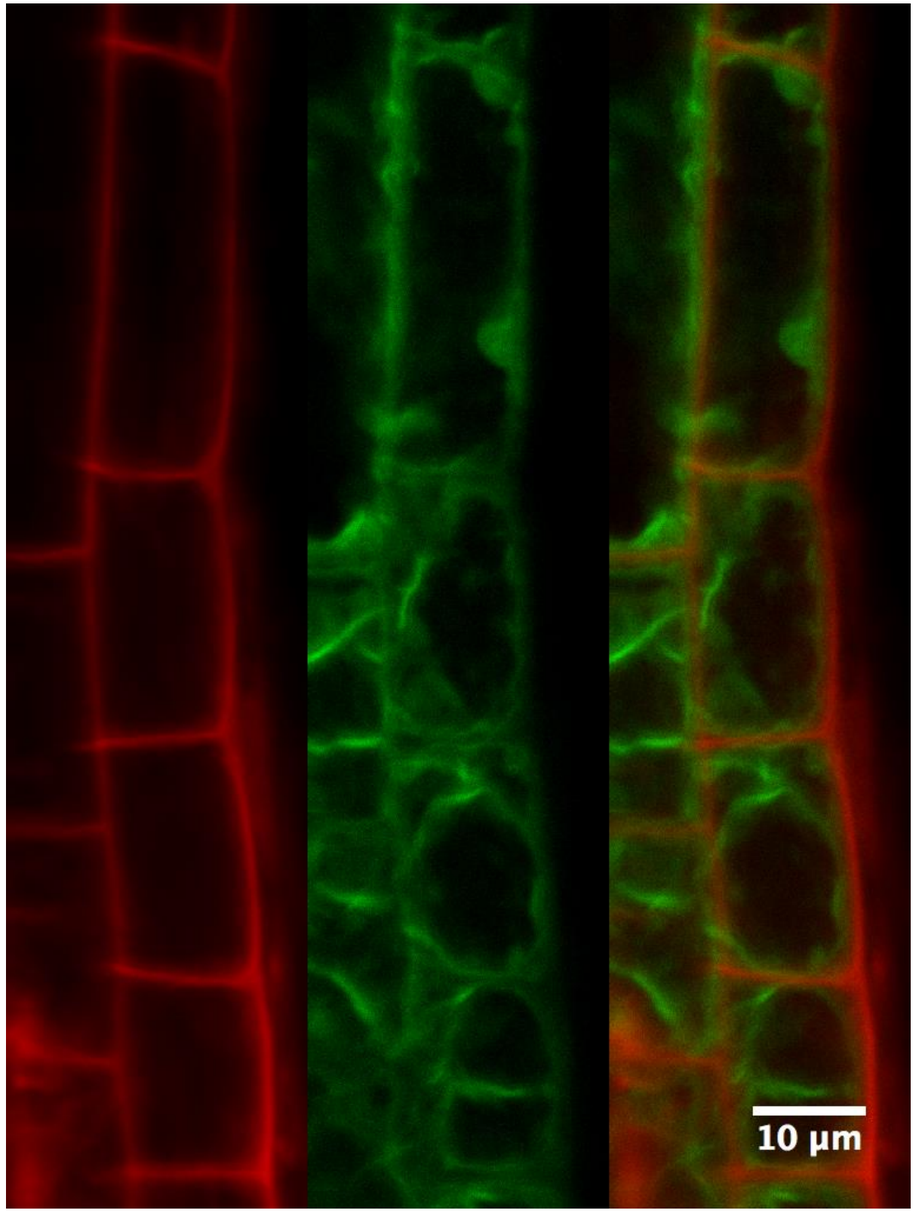



35S-YFP TOB1 / PI (100µM)

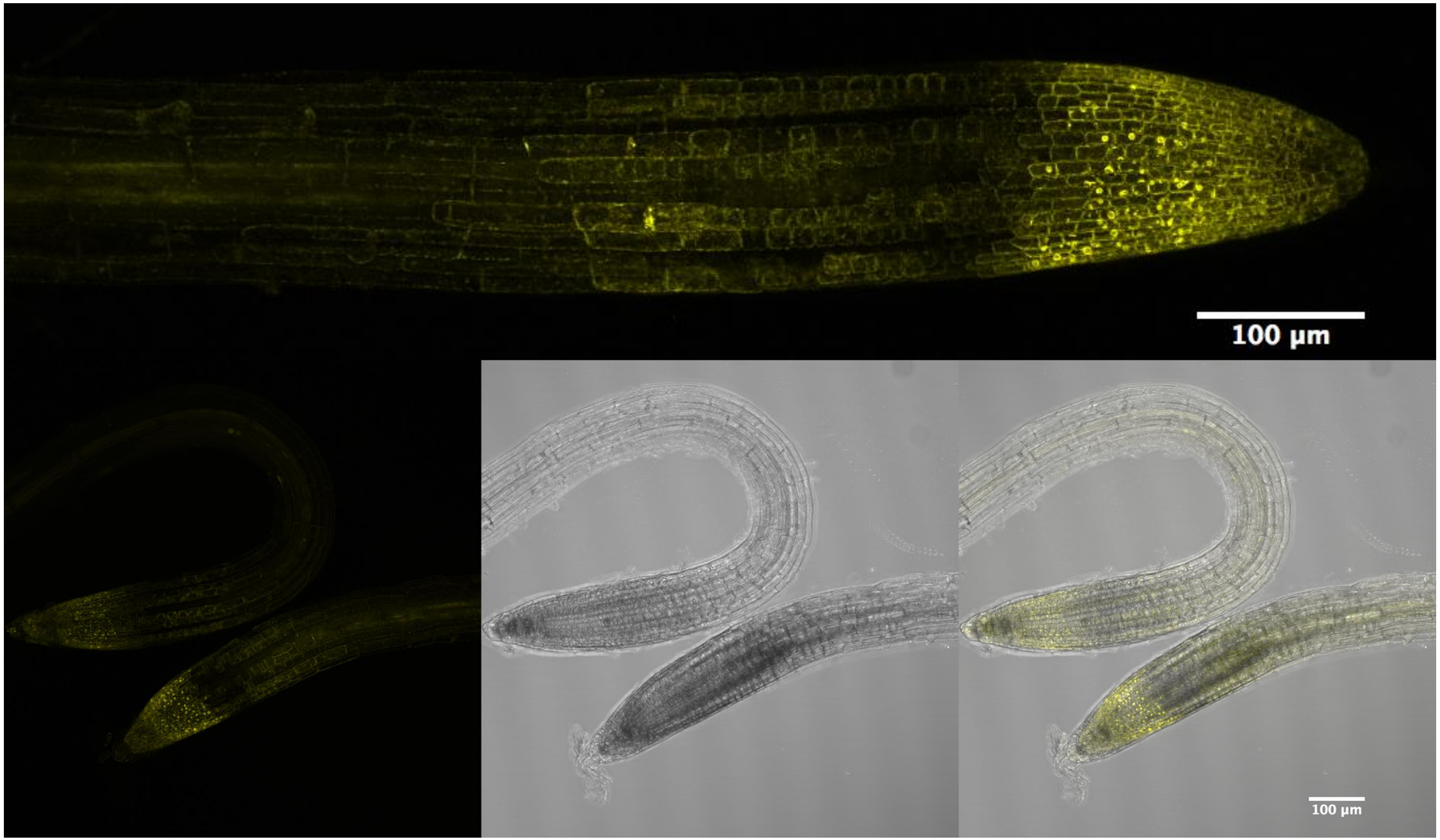


35S-YFP TOB1 / PI (100µM) _ transition zone



35S-YFP-TOB1 signal in roots (see below).




<u>Need to check the following:</u> I can find plants of different size when analyzing seeds from tube Hom T2-A

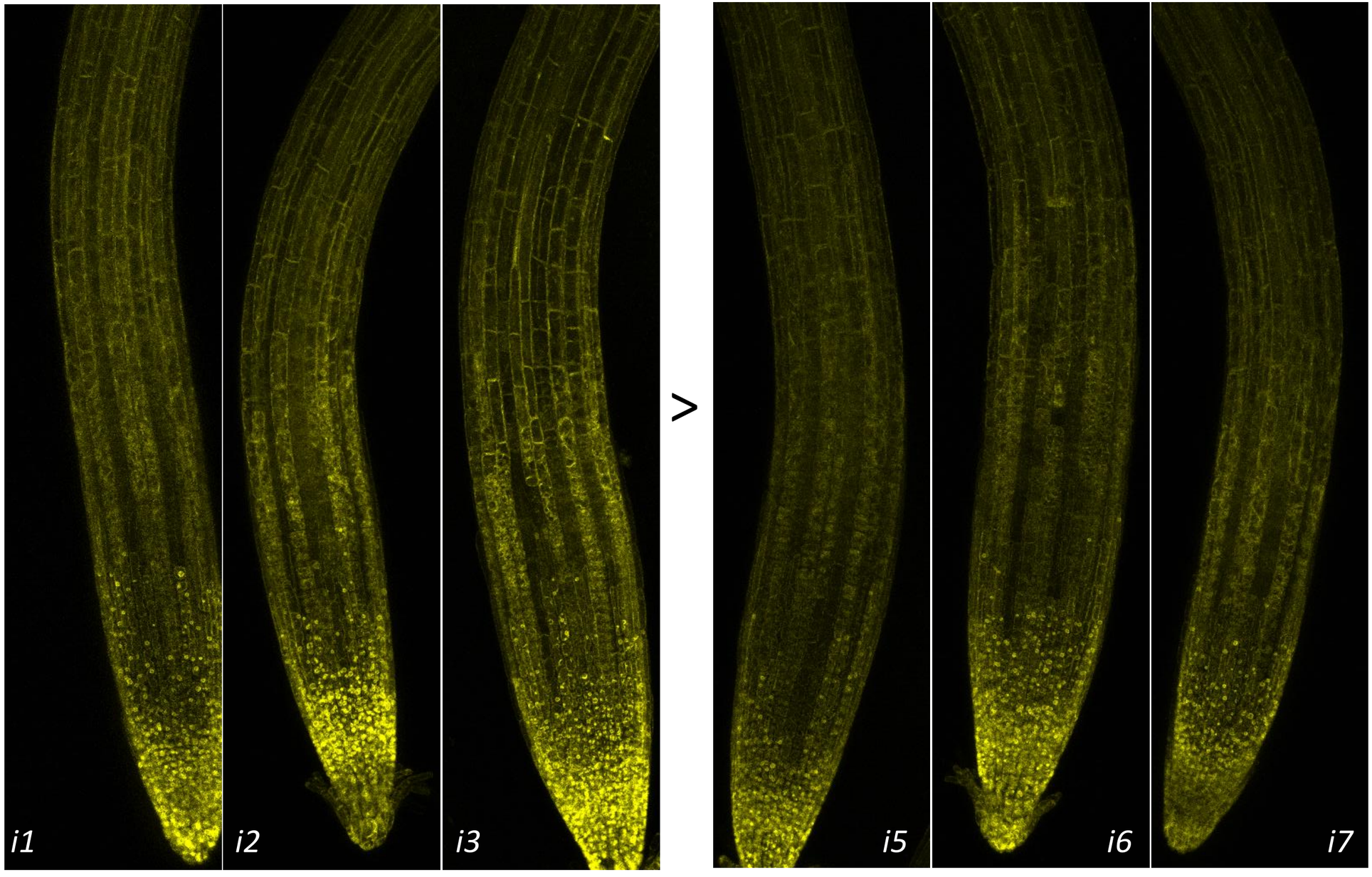
Smaller plants → showed higher fluorescence (on epi) and big (huge) PCs. It might be useful to **image those small plants (compare to the big plants) in the confocal.**

**2018-11-09_35S_YFP_TOB1**

Images corroborated that 35S::YFP-TOB1 T2 A* showed stronger signal than A

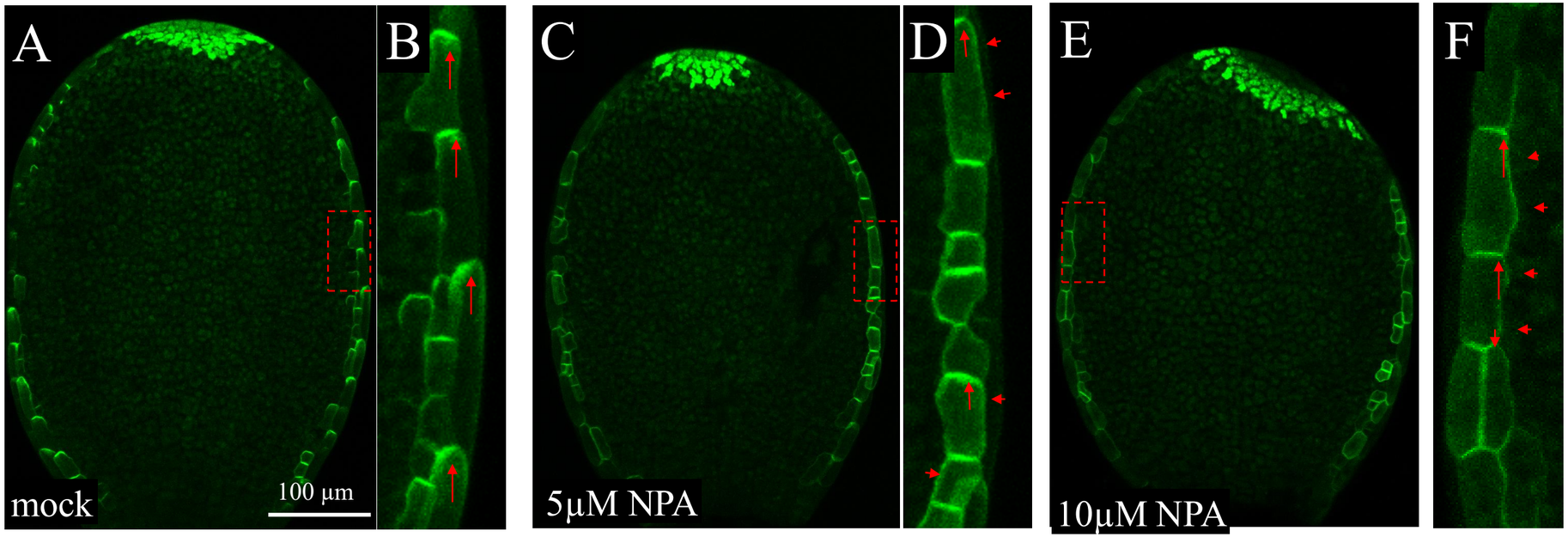
Line A*

Line A

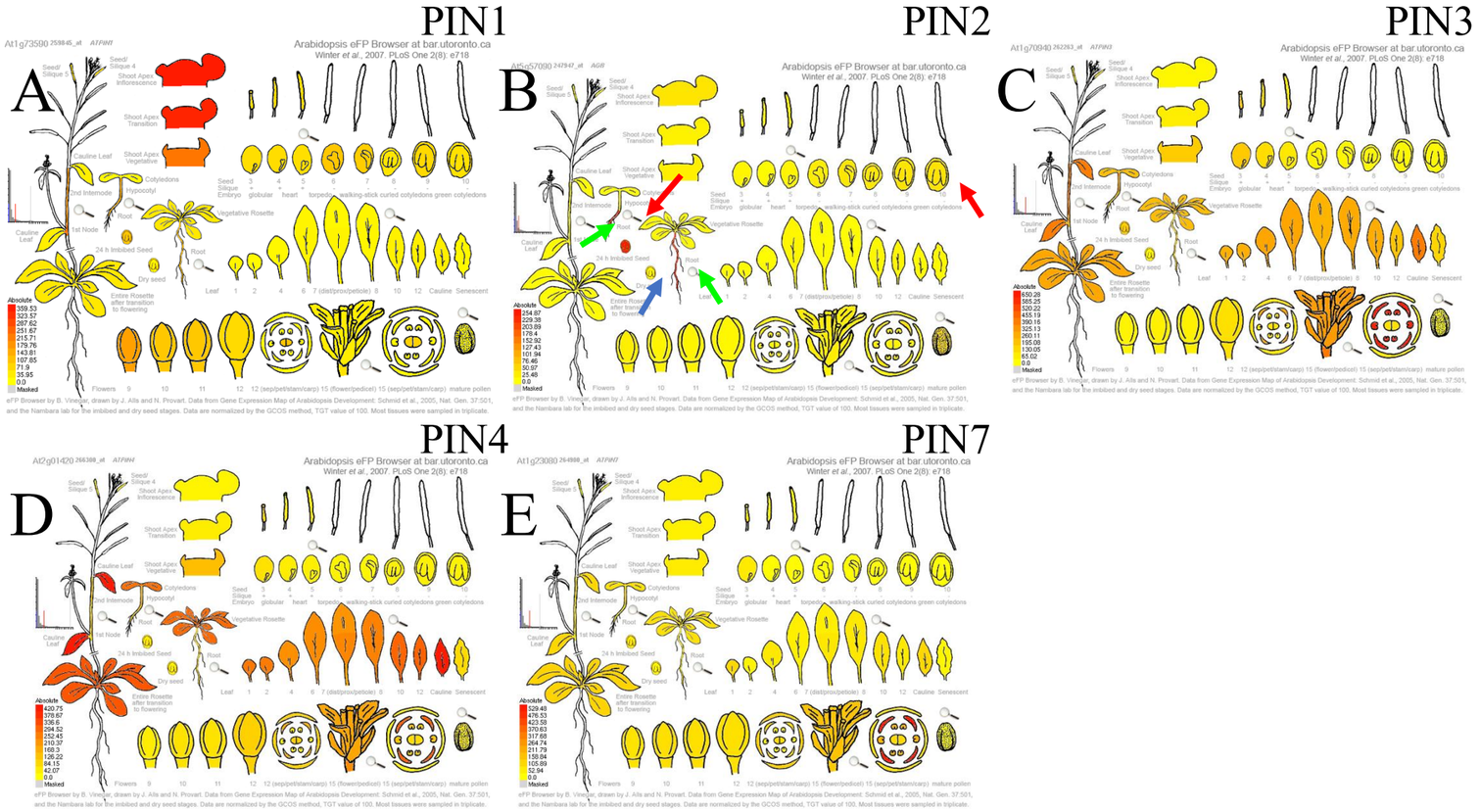


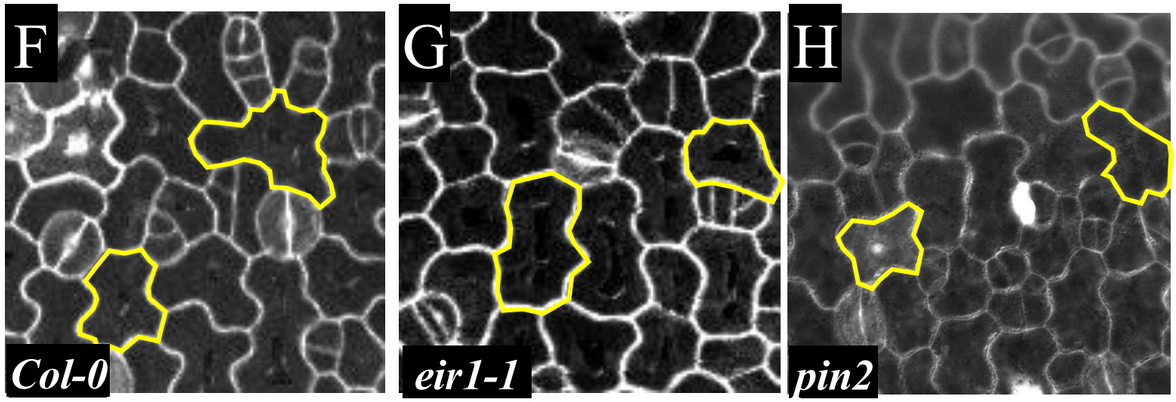
**Interrupting auxin transport with NPA, disrupted both PIN2 polar localization and PC interdigitation.**

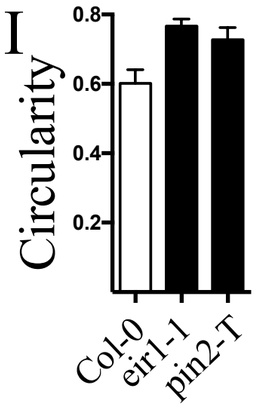
24 hap → NPA → 48 hap



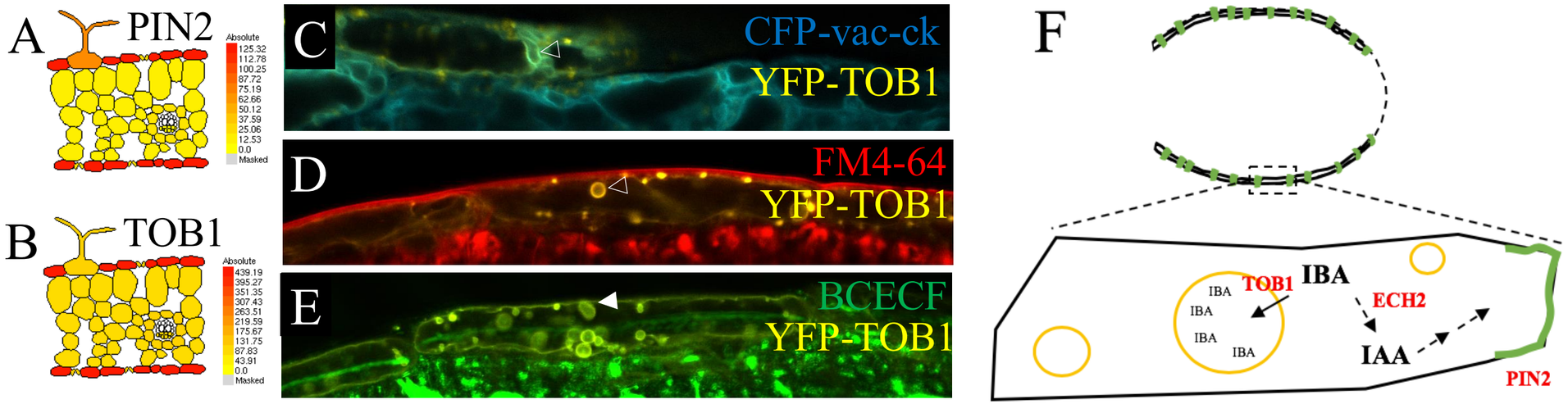
# PIN2 is expressed in imbibed seeds and its absence generates reduced PC morphogenesis



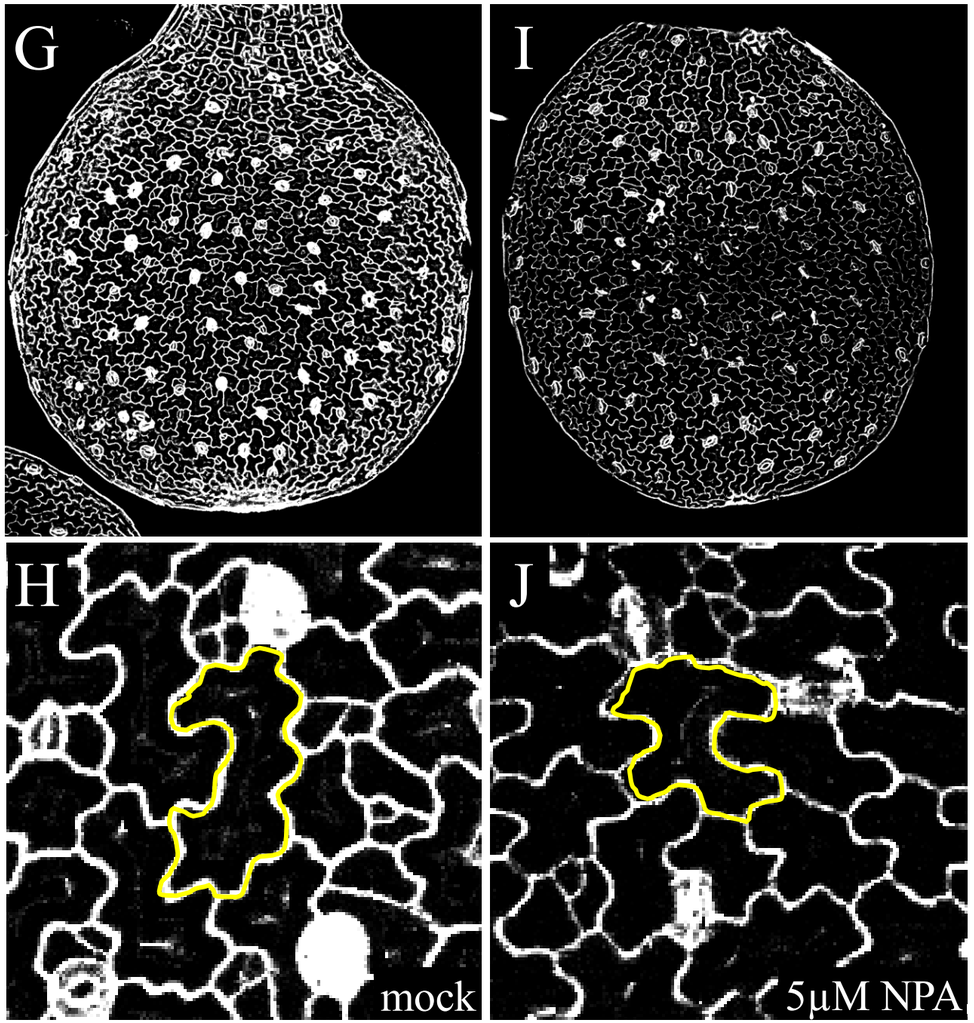



# TOB1-mediated vacuole internalization of IBA. Terminating signal?

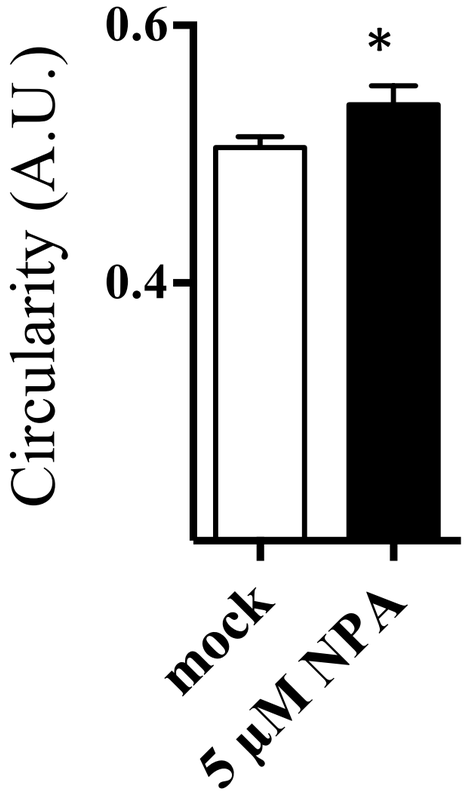


**Interrupting auxin transport with NPA, disrupted both PIN2 polar localization and PC interdigitation.**

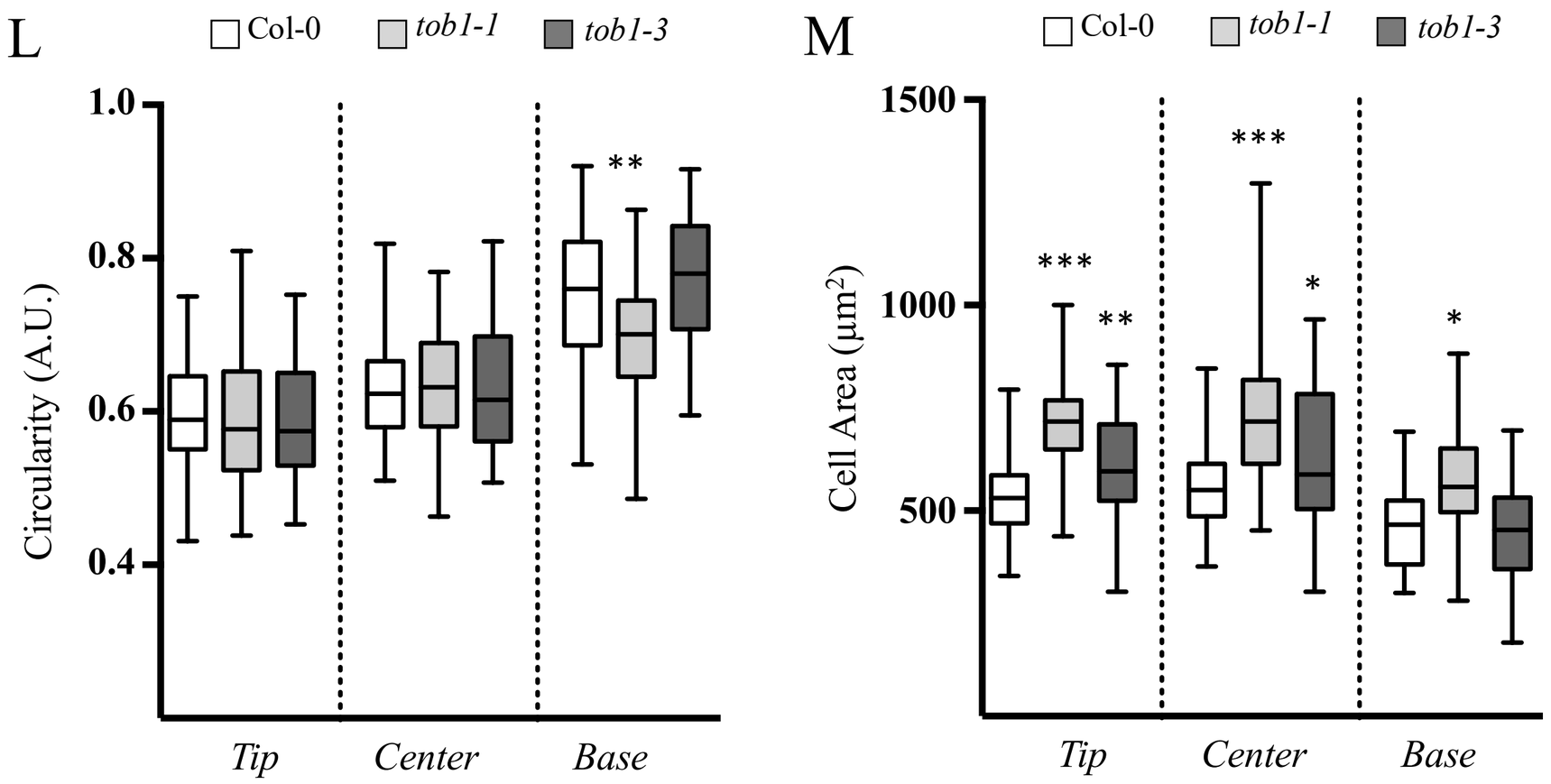
24 hap → NPA → 72 hap



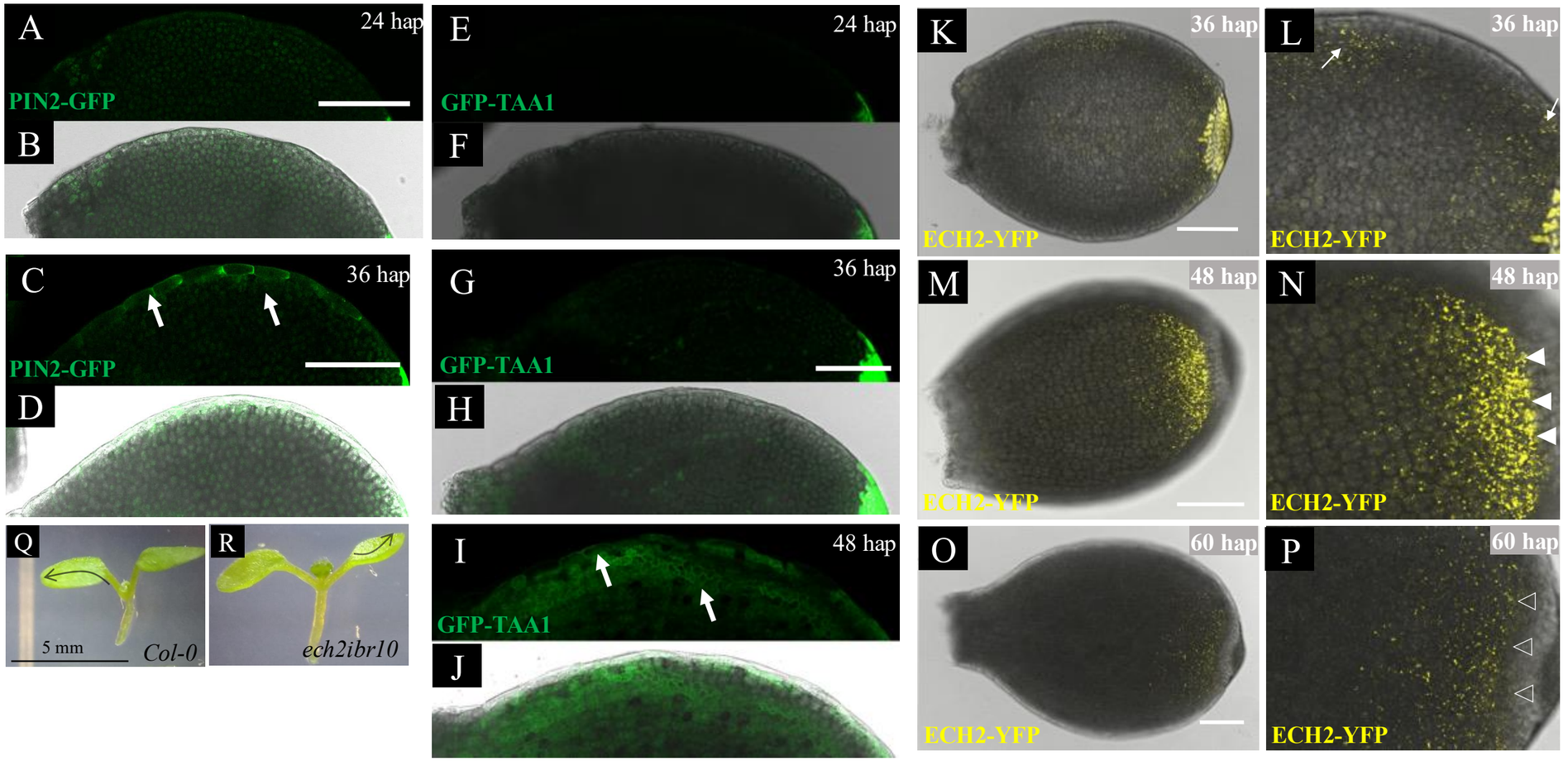

K

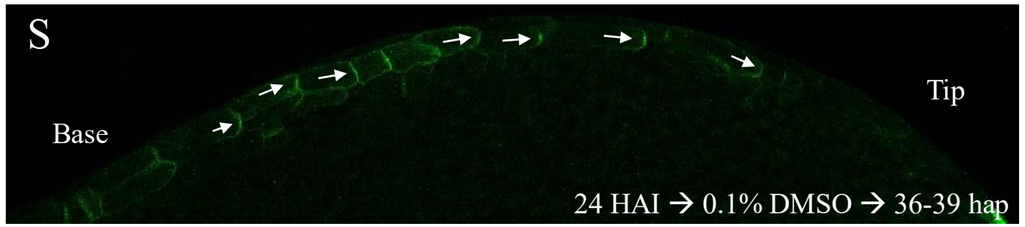


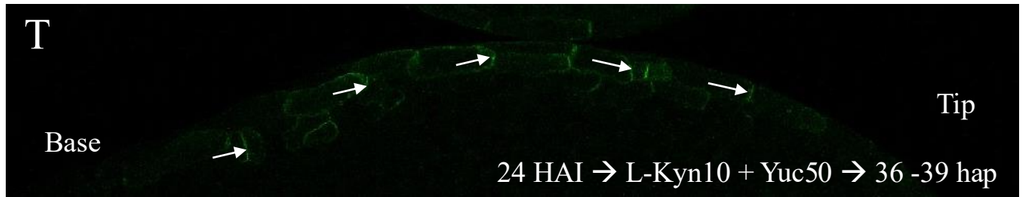
# TOB1 regulates cotyledon size also by increasing cell size in early stages

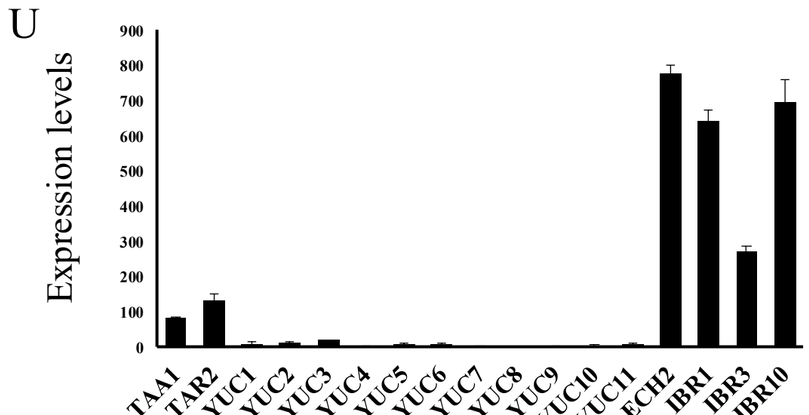


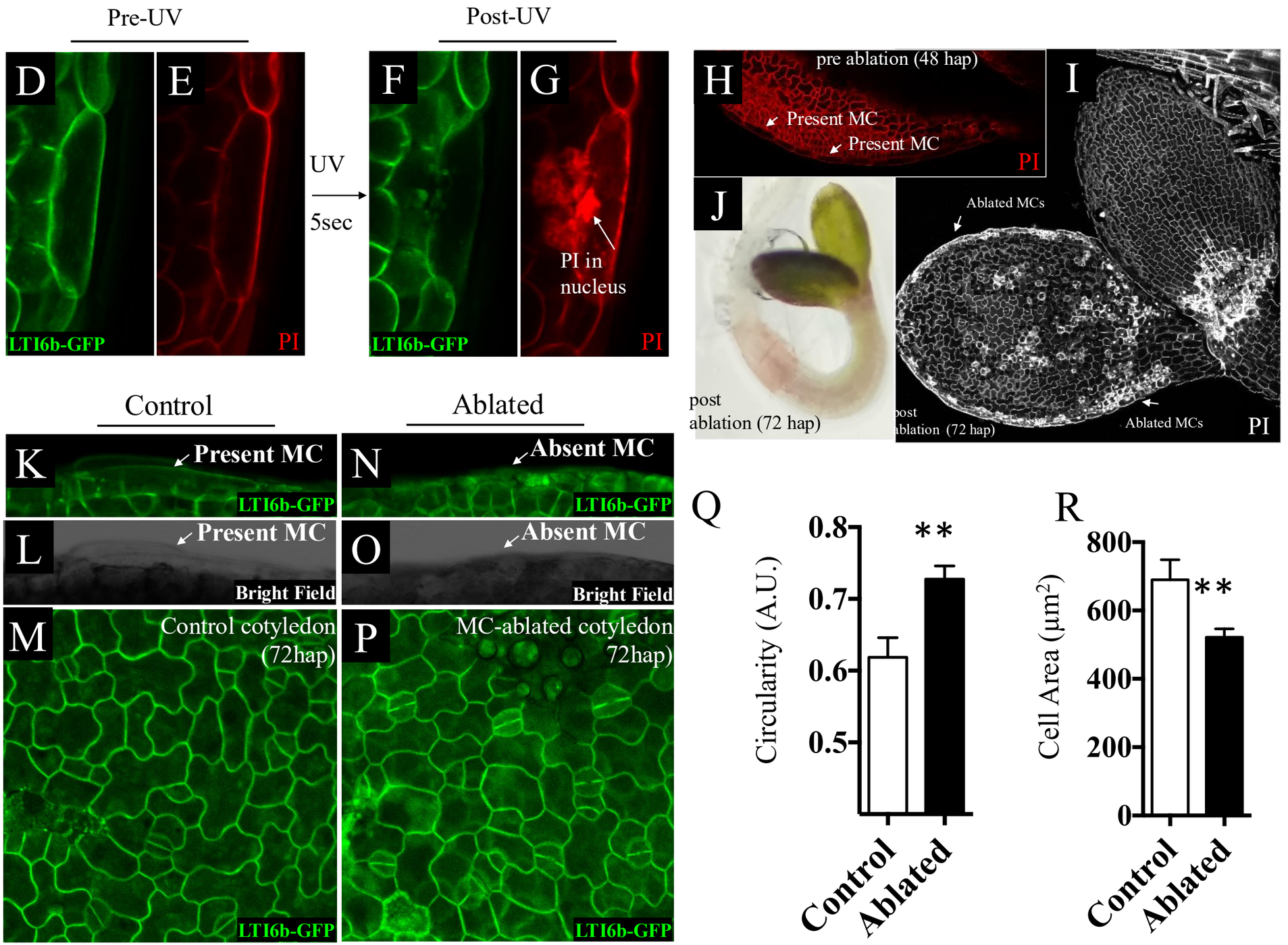
**PIN2-mediates polar auxin transport in *Arabidospis* cotyledon margin cells (MCs) is initially organized by IBA-to-IAA conversion and not but IPA *de novo* auxin synthesis pathway.**



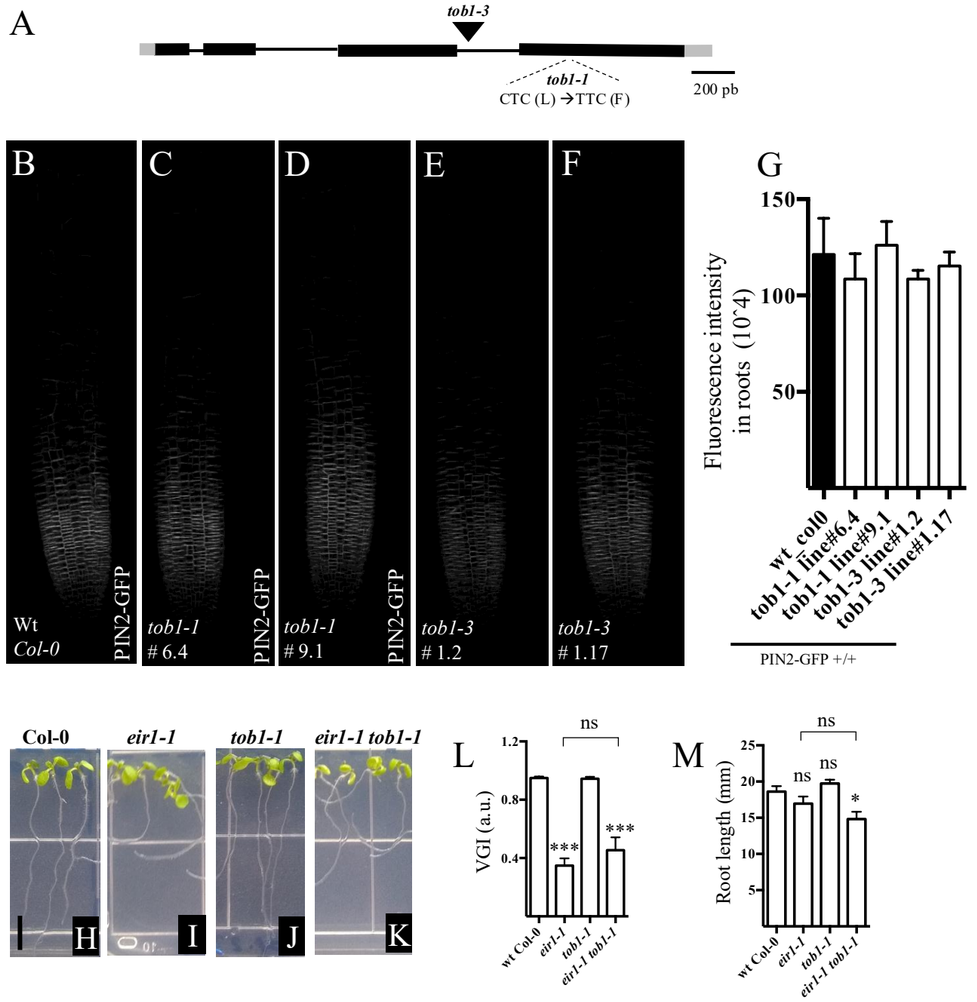




Pre-UV
Post-UV
D
E
F
G
UV
5sec
LTI6b-GFP
PI
LTI6b-GFP
PI
PI in nucleus
H
pre ablation (48 hap)
Present MC
Present MC
PI
I
J
post ablation (72 hap)
Ablated MCs
post ablation (72 hap)
Ablated MCs
PI
Control
Ablated
K
Present MC
LTI6b-GFP
N
Absent MC
LTI6b-GFP
L
Present MC
Bright Field
O
Absent MC
Bright Field
M
Control cotyledon (72hap)
LTI6b-GFP
P
MC-ablated cotyledon 72hap)
LTI6b-GFP
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.



# FAC sorting of IQD22pro:GUS-mCit leaf protoplasts can enrich for palisade mesophyll cells

17 day old seedlings

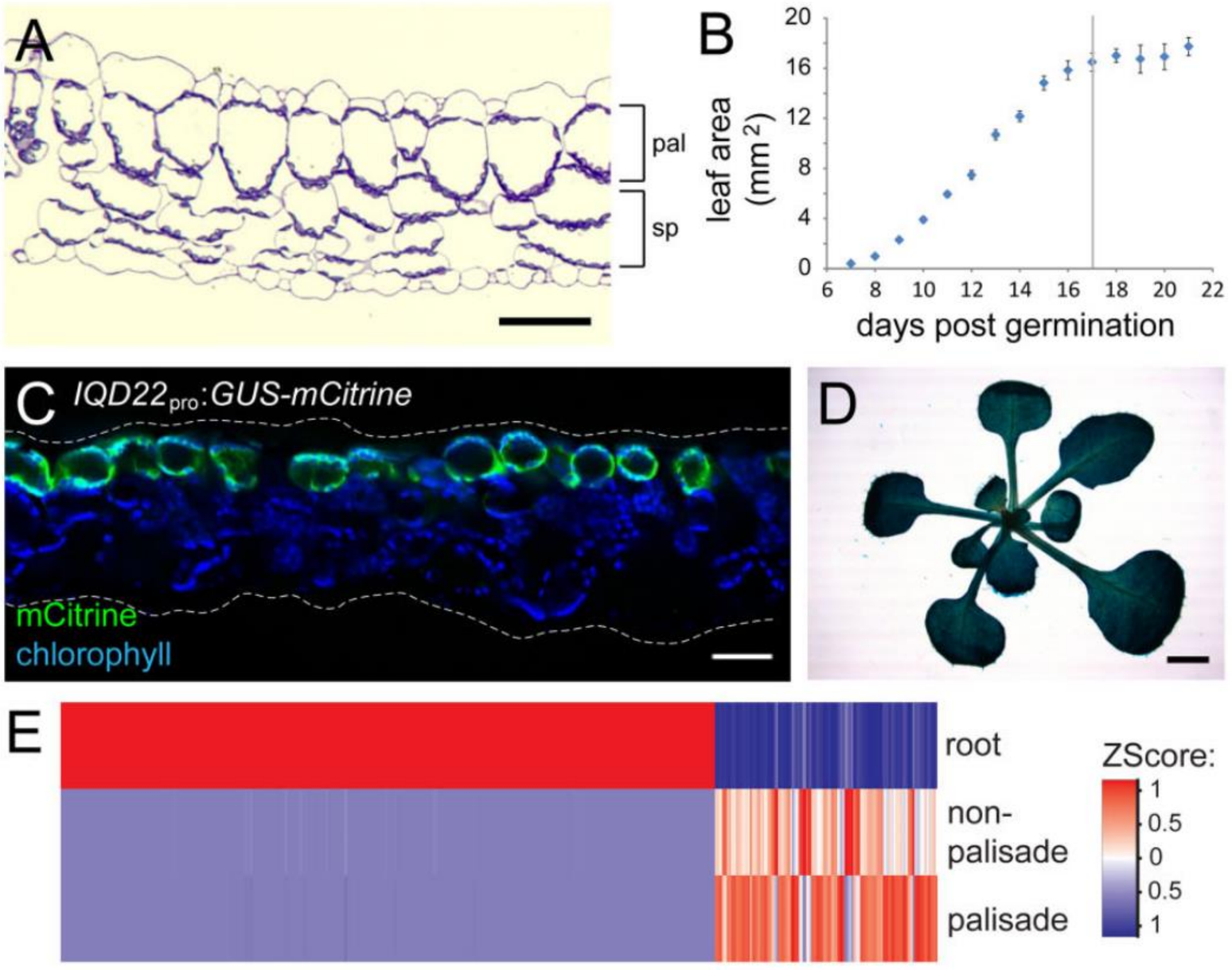


Fig. 1. Anatomical and morphological characteristics of the mature Arabidopsis thaliana leaf