Single cell dynamics of a plant stem cell niche

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Project overview

1. **Pipeline design for analysis of confocal data**

2. **Single cell *in vivo* dynamics of aerial stem cell niche in *A. thaliana***

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Goal: Form a cohesive picture of stem cell regulation over time

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1. **Pipeline design for analysis of confocal data**

2. **Single cell *in vivo* dynamics of aerial stem cell niche in *A. thaliana***

Goal: Form a cohesive picture of stem cell regulation over time

1. **The experimental conditions perturb the system**

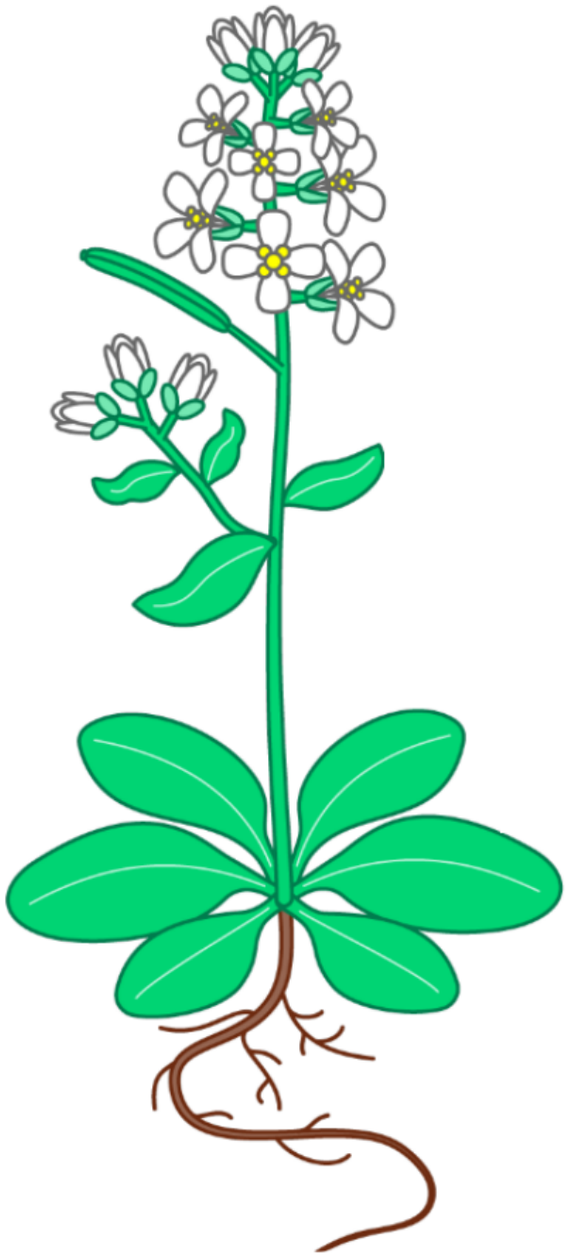
2. **Stem cell niche robust despite perturbations**

3. **Longevity is centrally regulated**

4. **Overthrowing the textbook: Where is the stem cell niche really?**

The SAM drives aerial development

A. thaliana



- **Development through stem cells in root (RAM) and shoot (SAM)**

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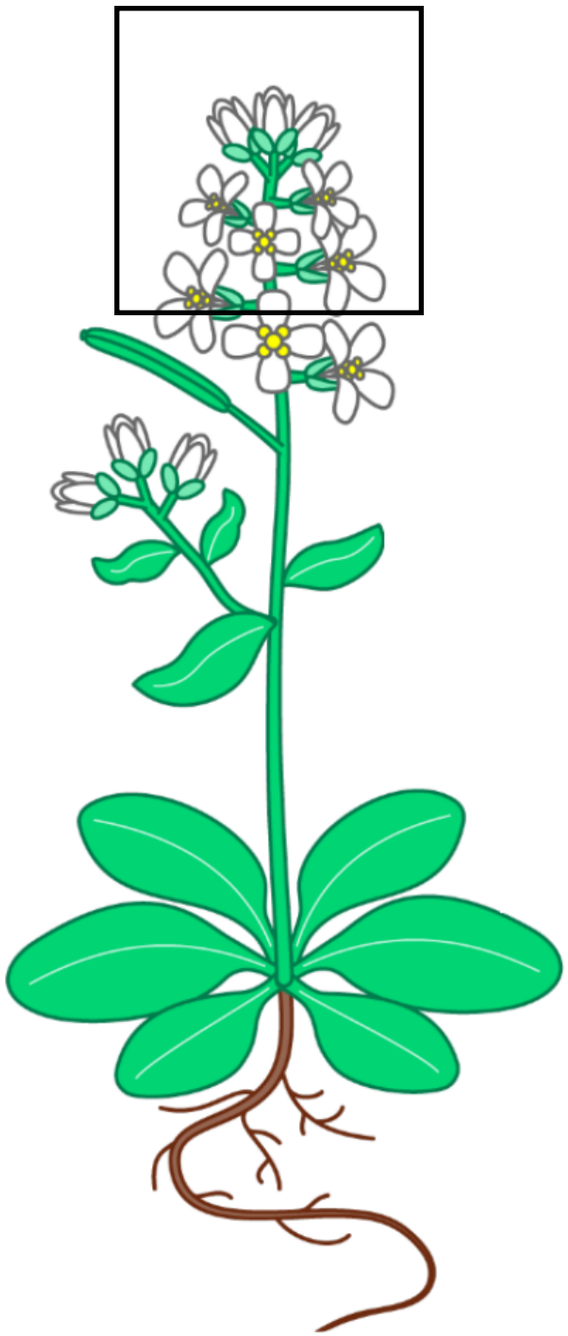

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- **Stem cells located at apex**

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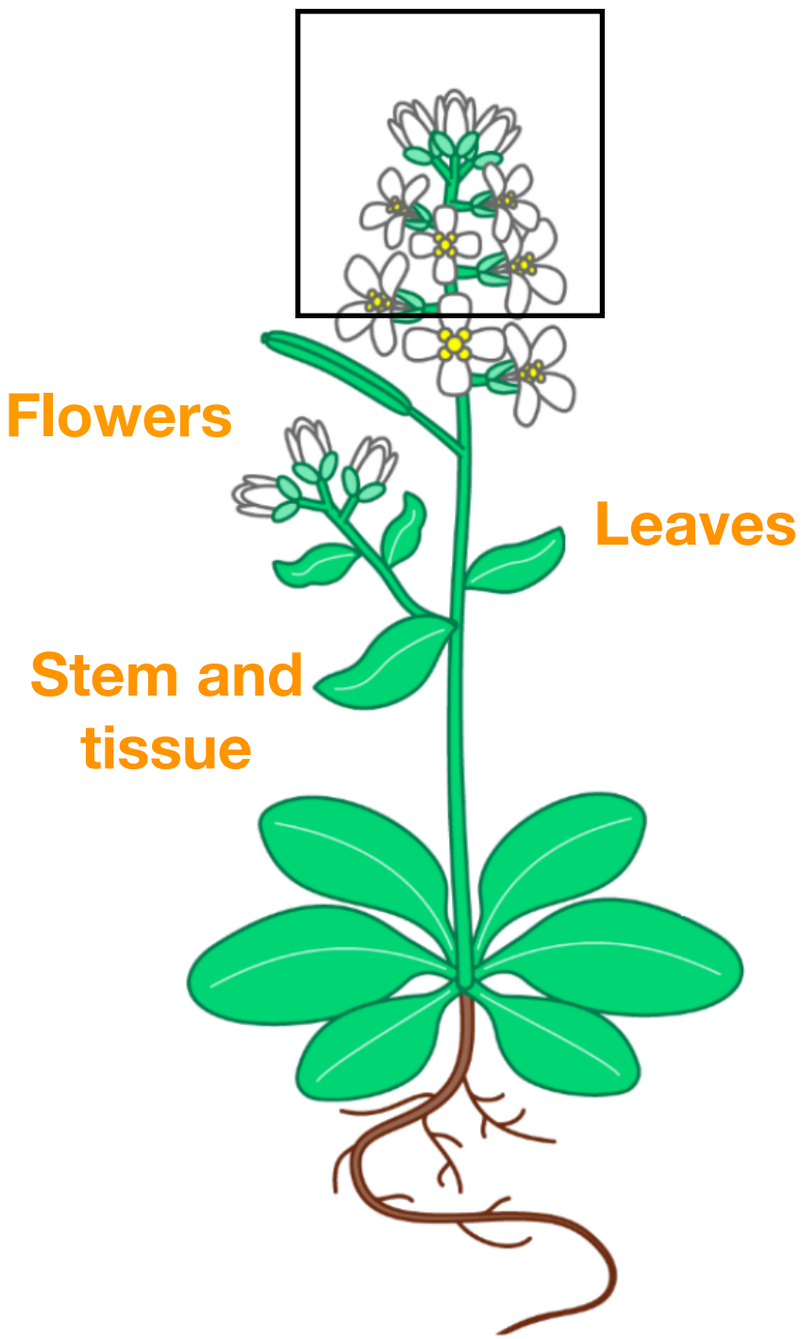

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- **Responsible for formation of organs**

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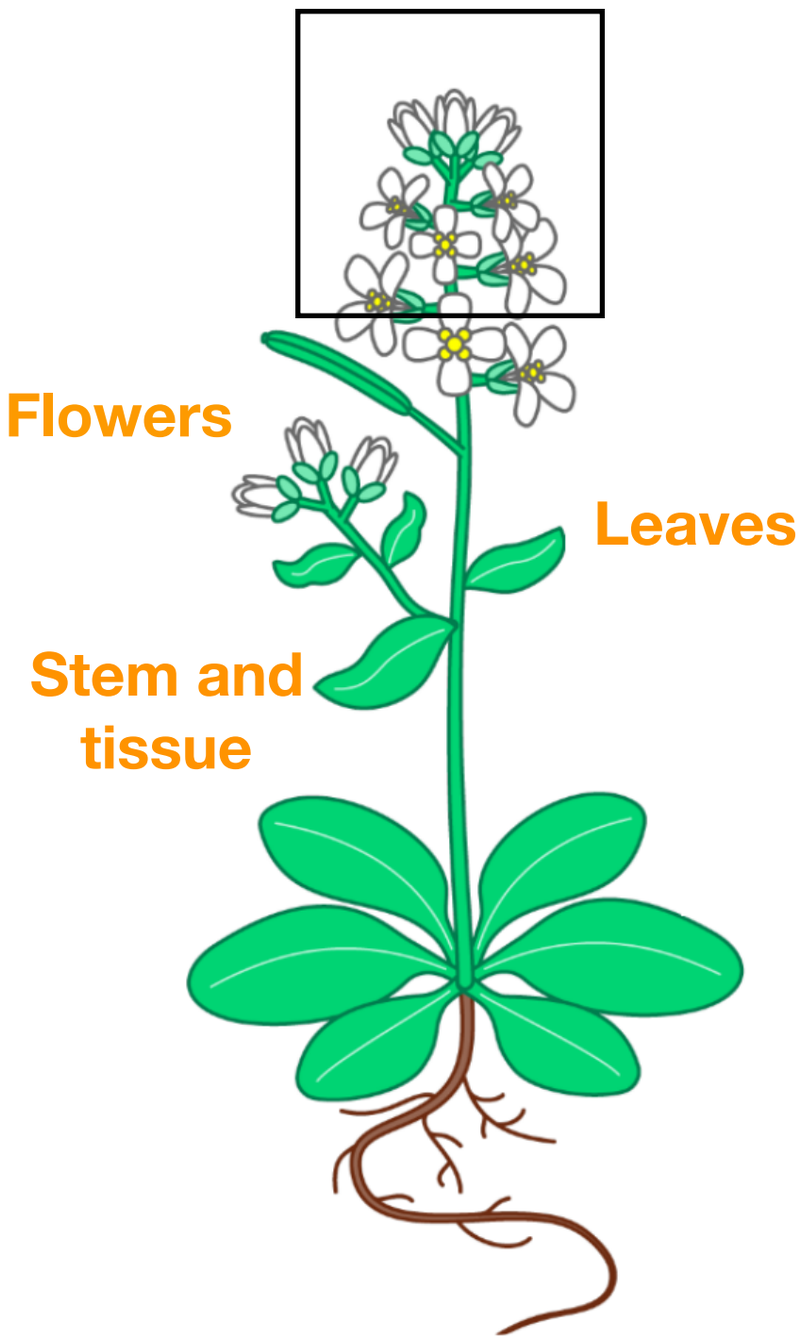
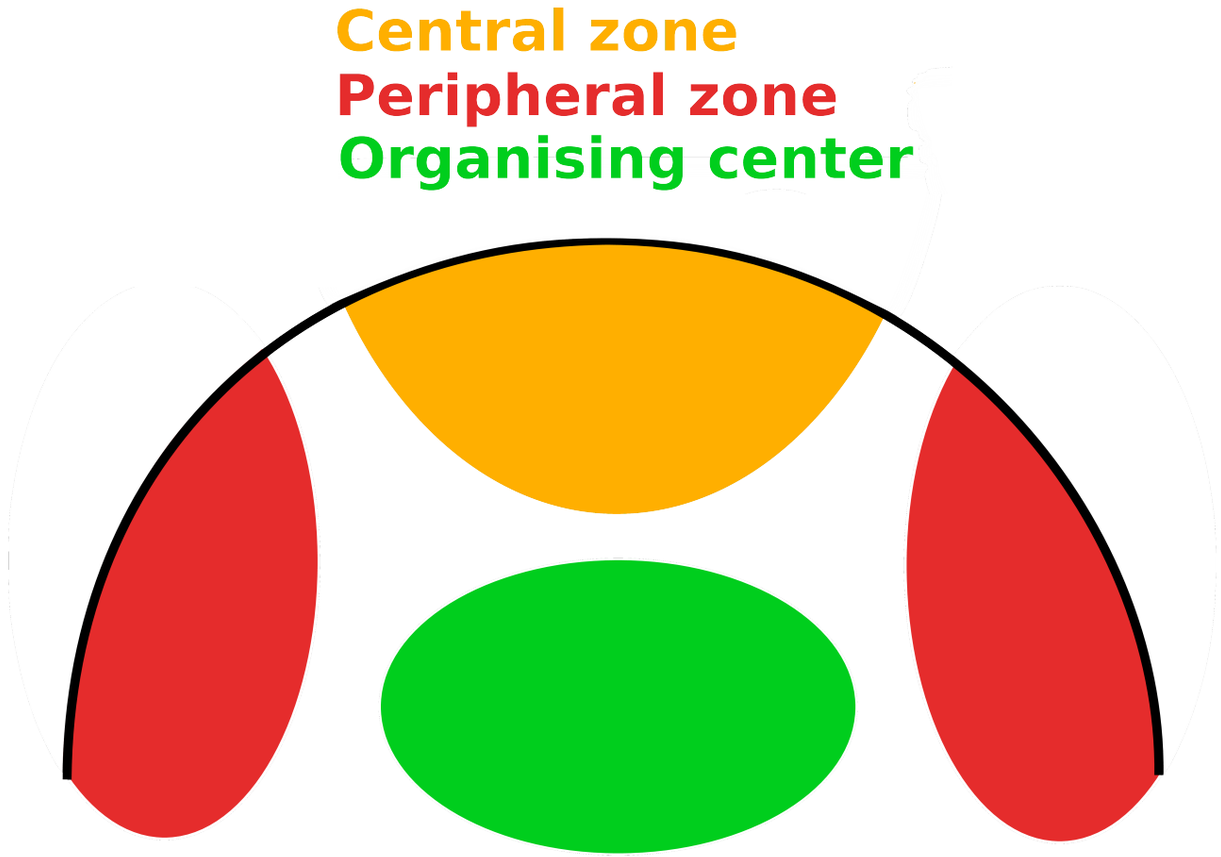


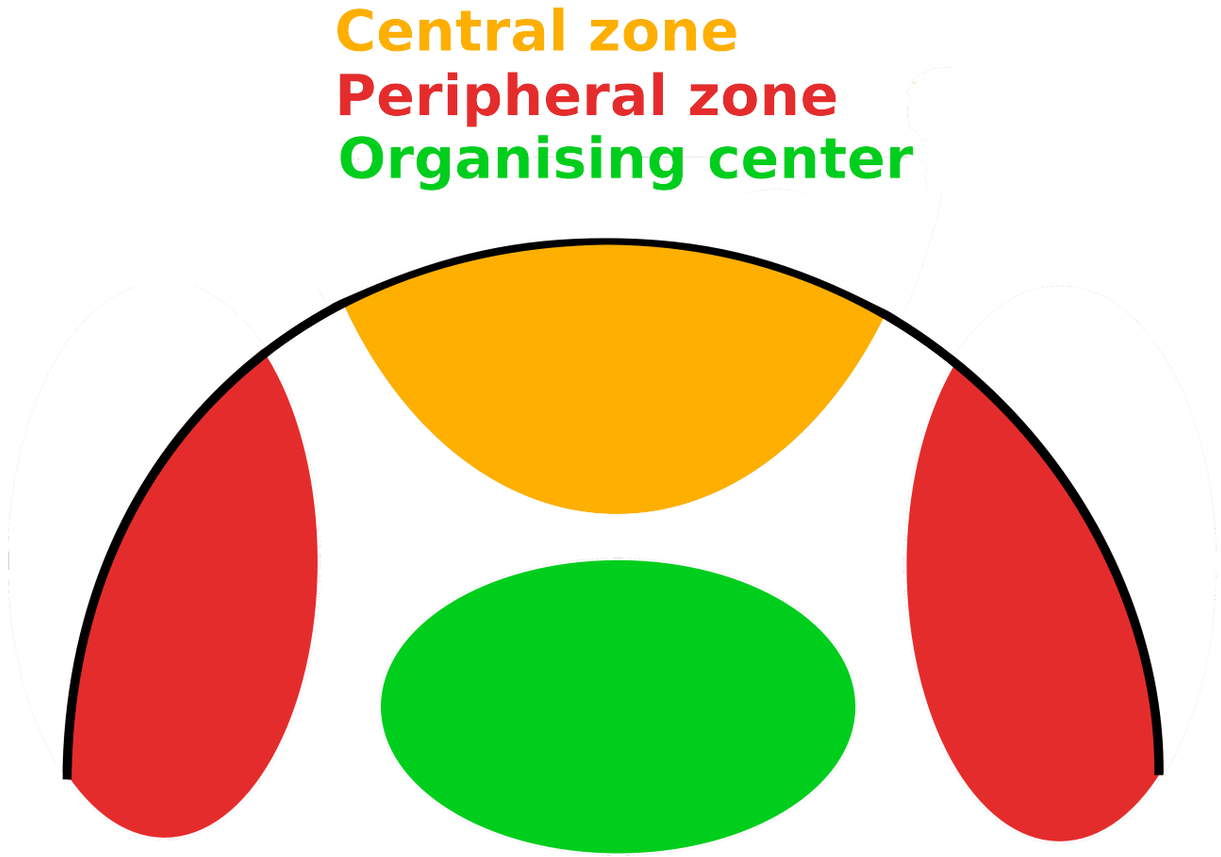
- **Development through stem cells in root (RAM) and shoot (SAM)**
- **Stem cells located at apex**
- **Responsible for formation of organs**
- **80 % of food supply derives from SAMs**

Composition of the SAM



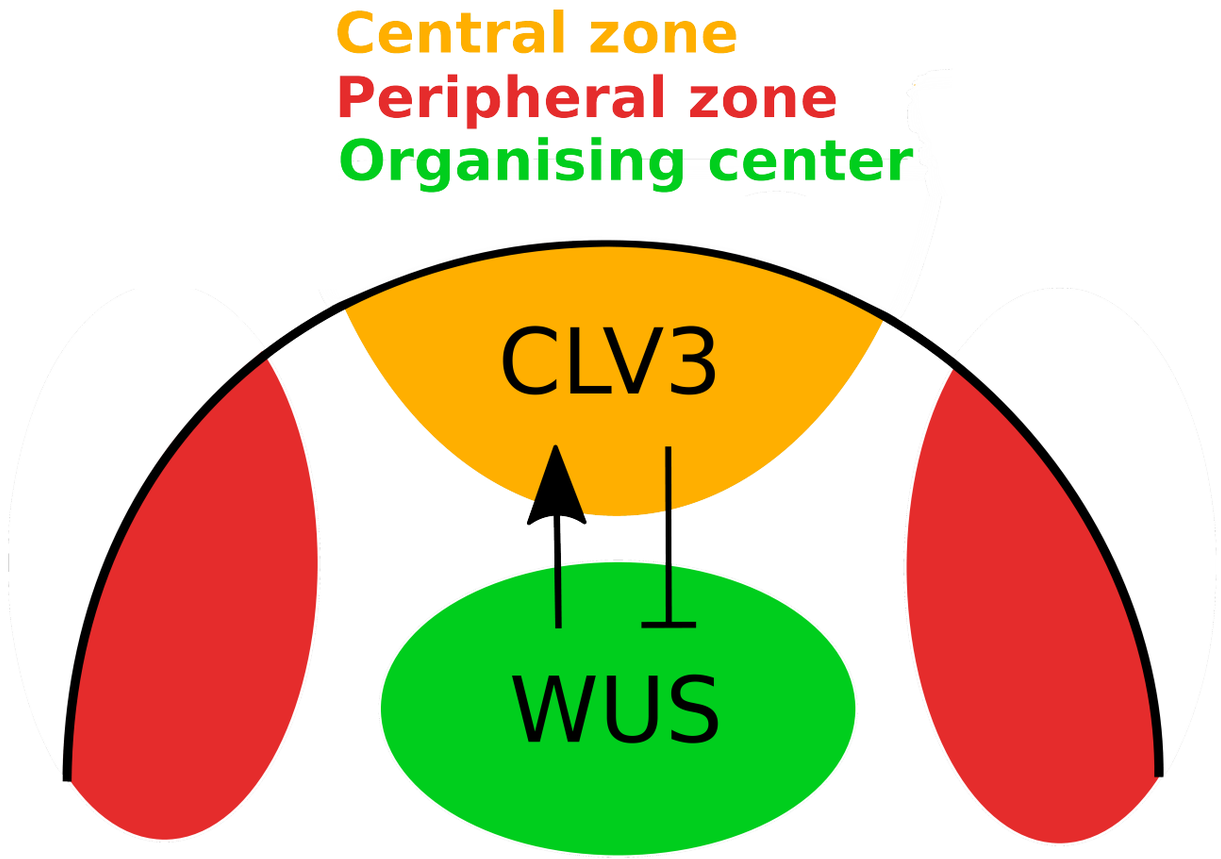
Composition of the SAM

- **Stem cells located at apex**



Composition of the SAM

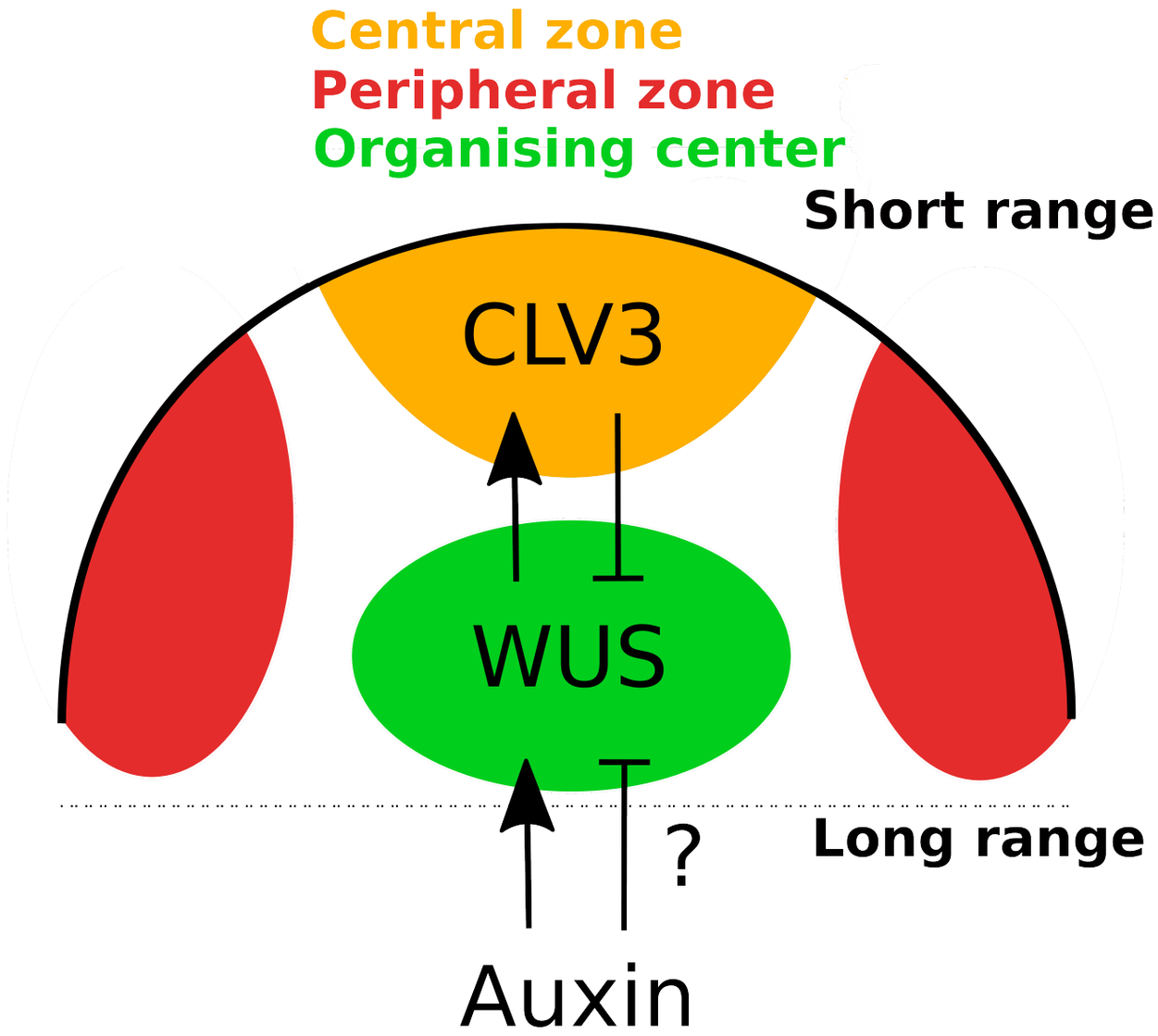
- Stem cells located at apex
- **CLV3-WUS specify pluripotency**



Composition of the SAM

- Stem cell population located apex
- CLV3-WUS specify pluripotency
- **Hormonal regulation happens**

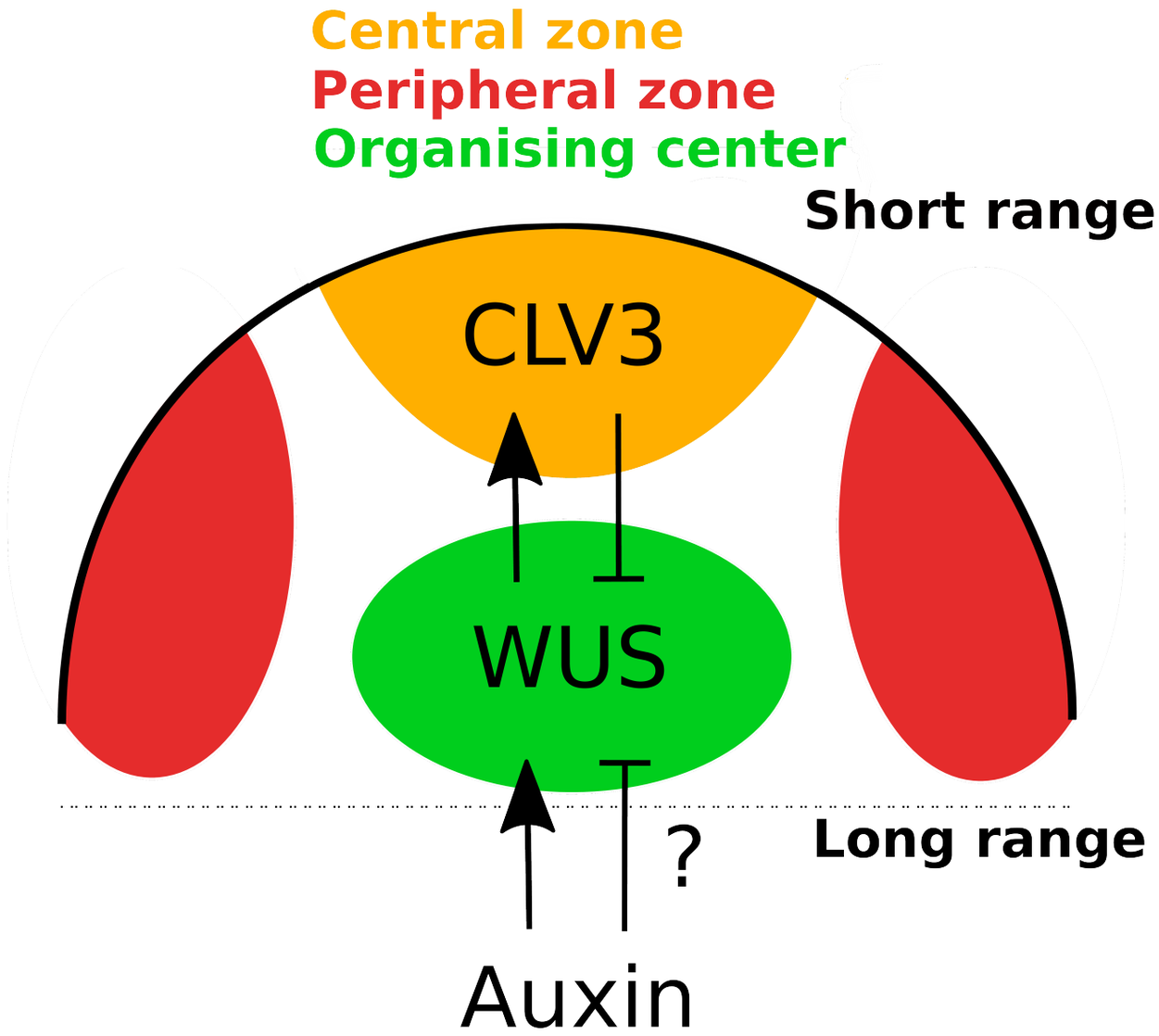
  directly and indirectly



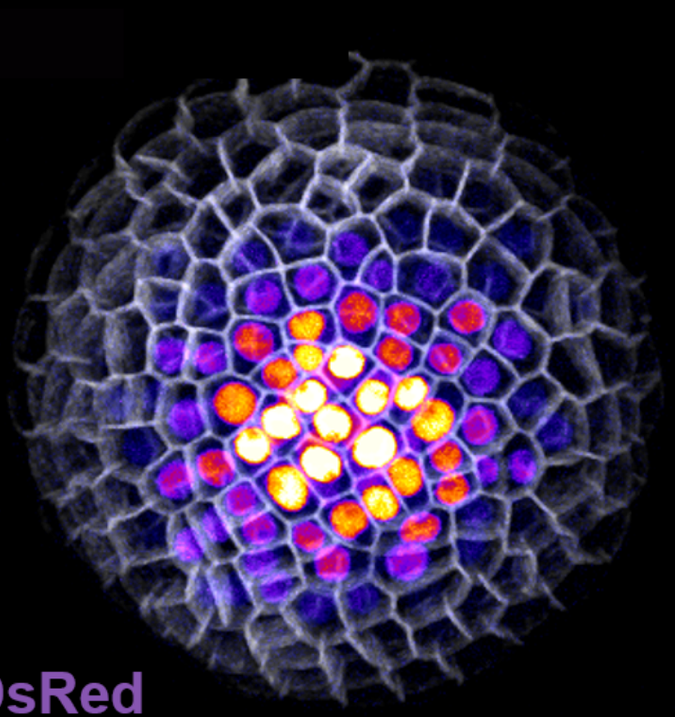
Composition of the SAM

- Stem cell population located apex
- CLV3-WUS specify pluripotency
- Hormonal regulation directly and indirectly

- **Exact size and regulation not well understood**



Data: Time-series of 3D confocal images



pCLV3::DsRed
pUBQ::acyl-YFP

Sample every 4 hours

0 h → 84 h

NPA
(auxin transport inhibitor)

- Quantify **membrane** and **nuclear** signal for six plants
- **Track over time** using membranes
- NPA induces **small**, **naked** meristems

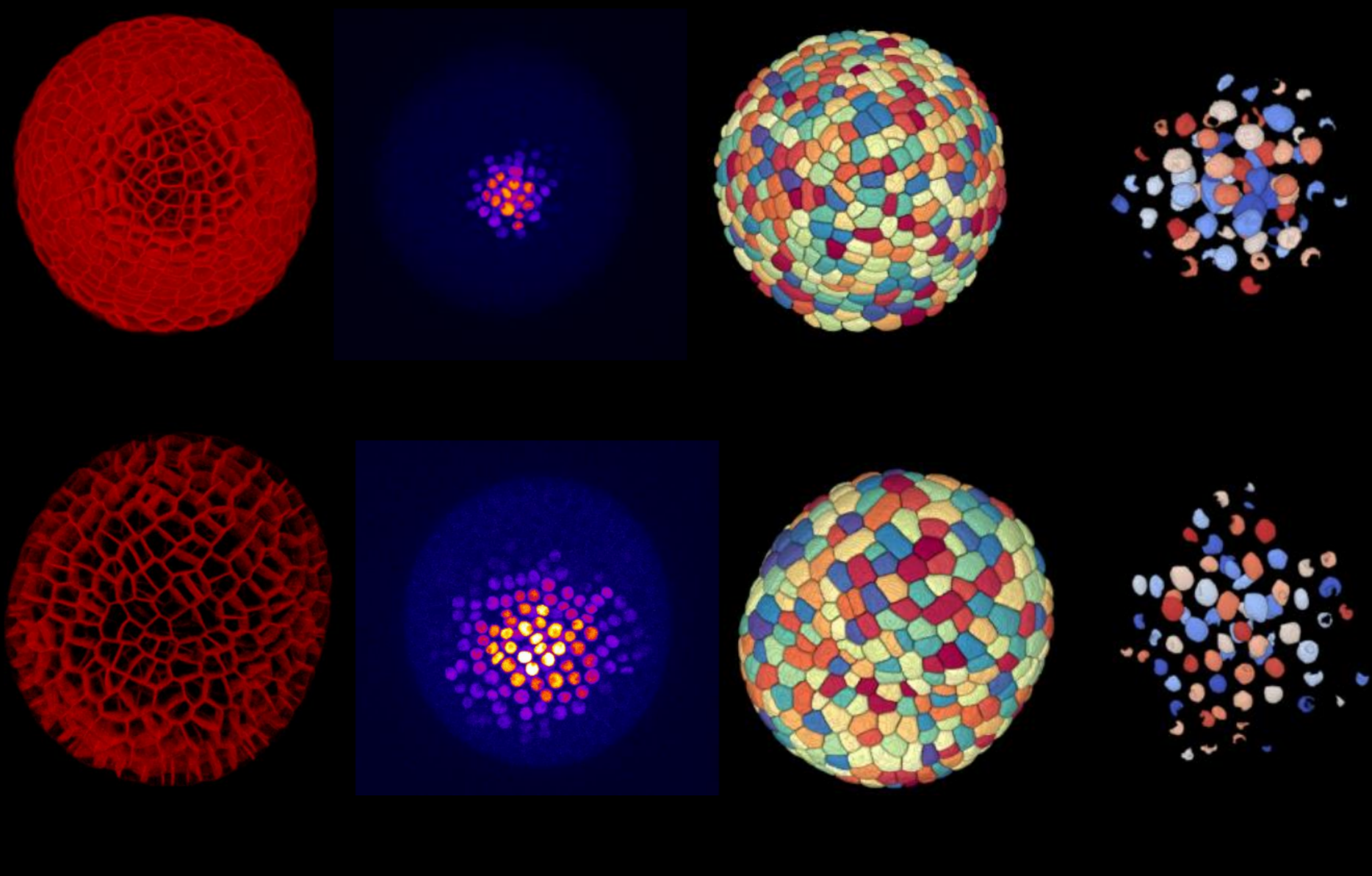
Data: Time-series of 3D confocal images

Raw data

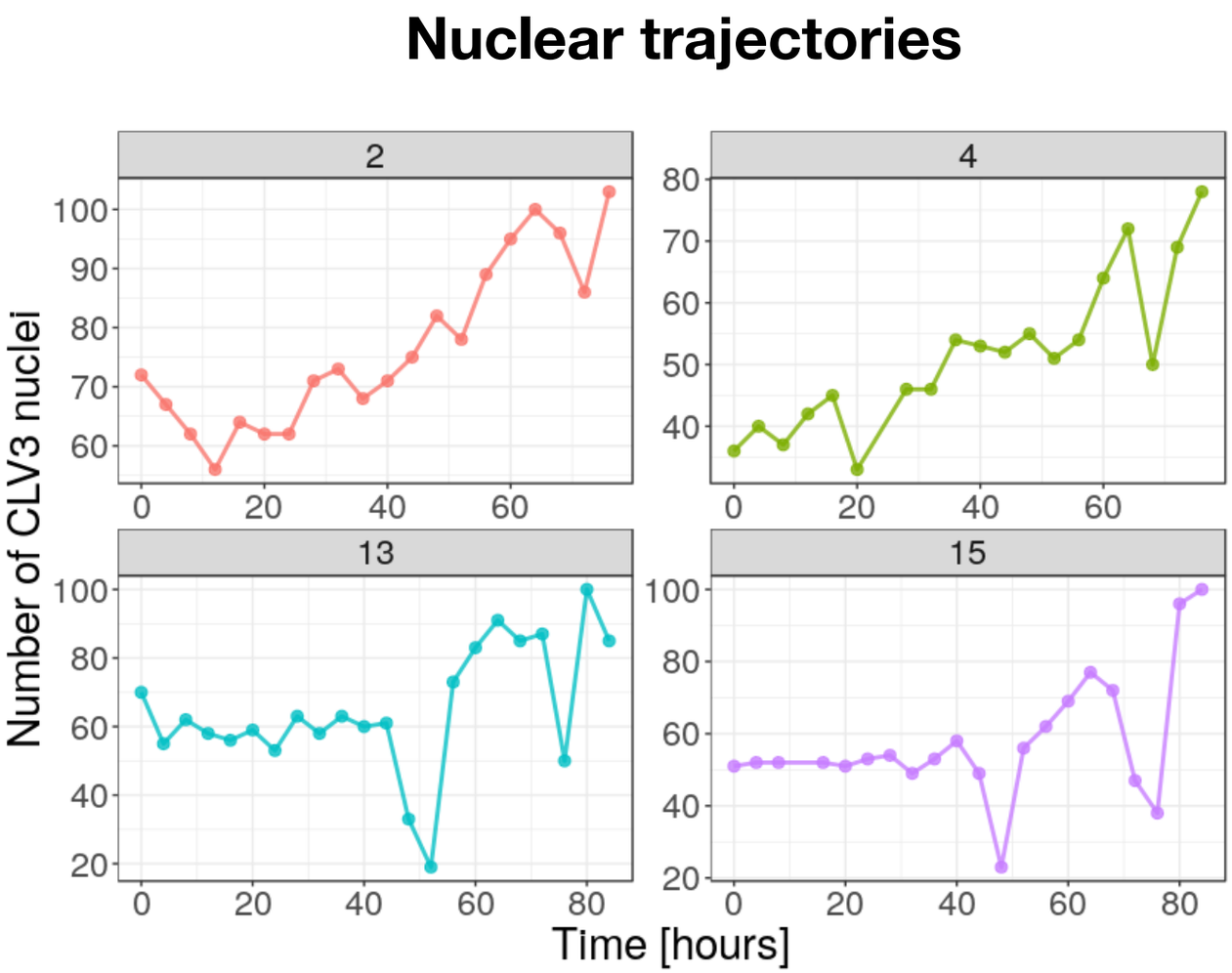
Segmentation

t = 0h

t = 84h



1: NPA dilution gives feedback response



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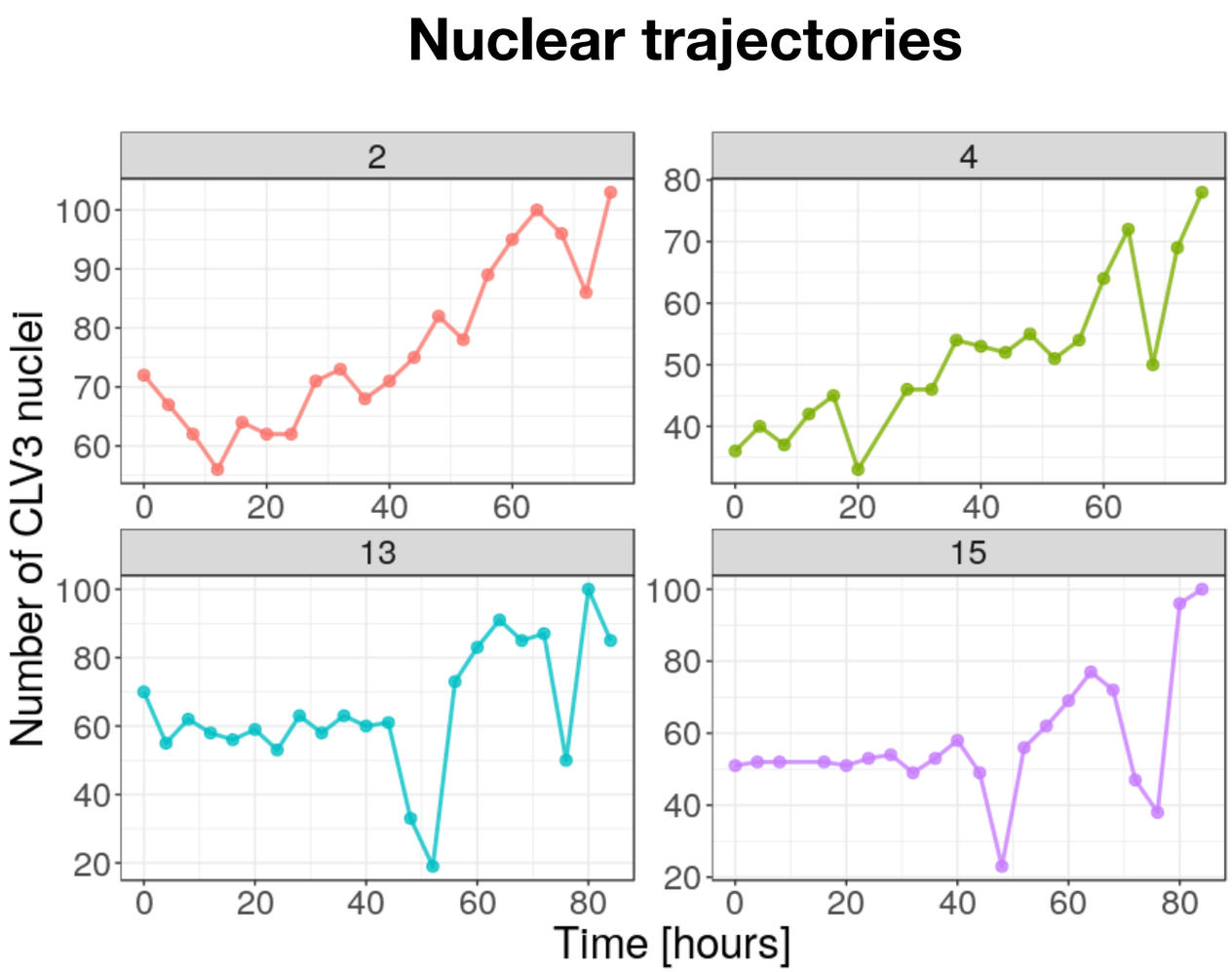

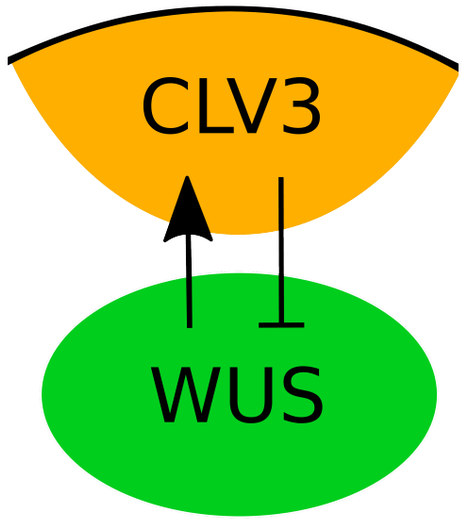

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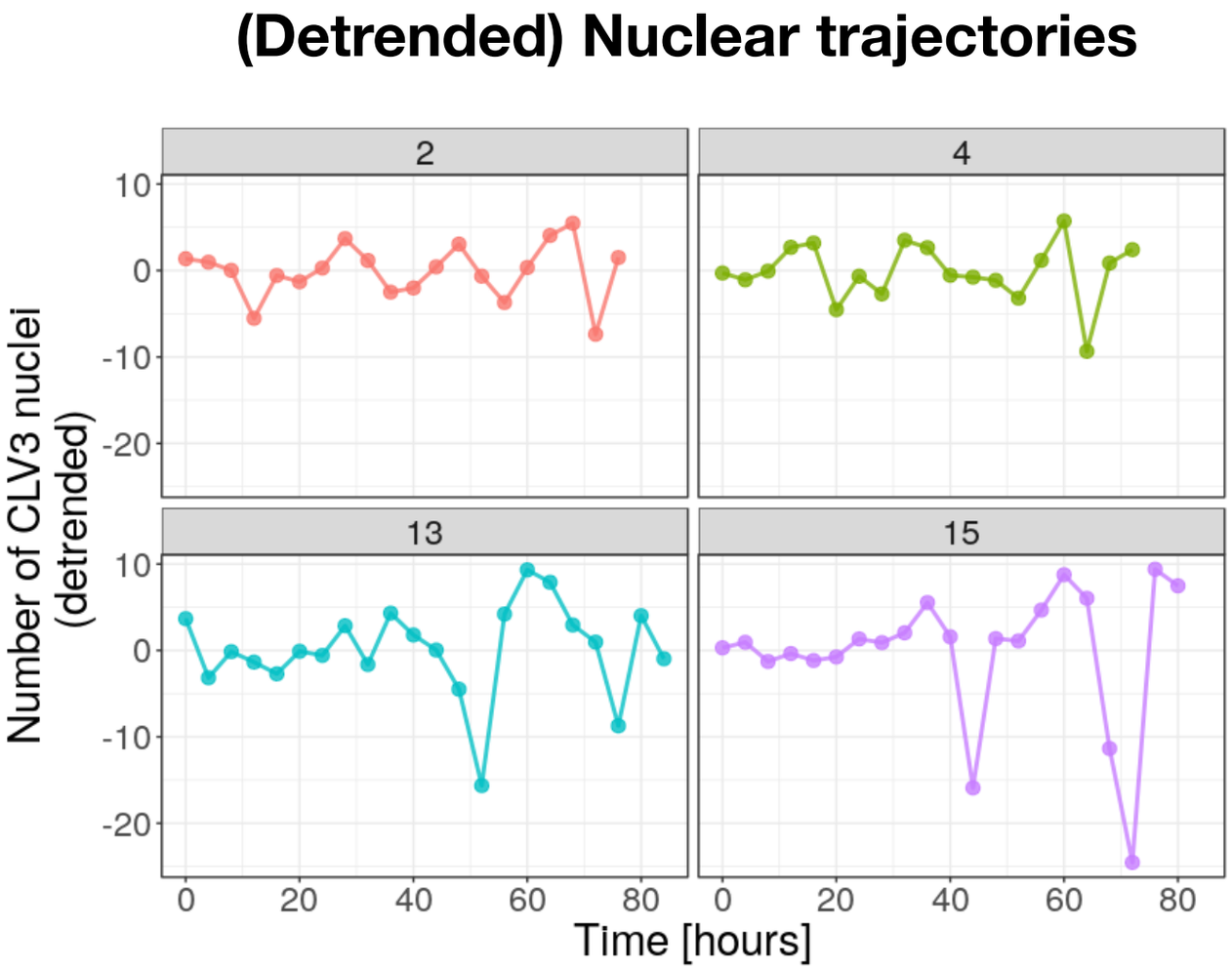

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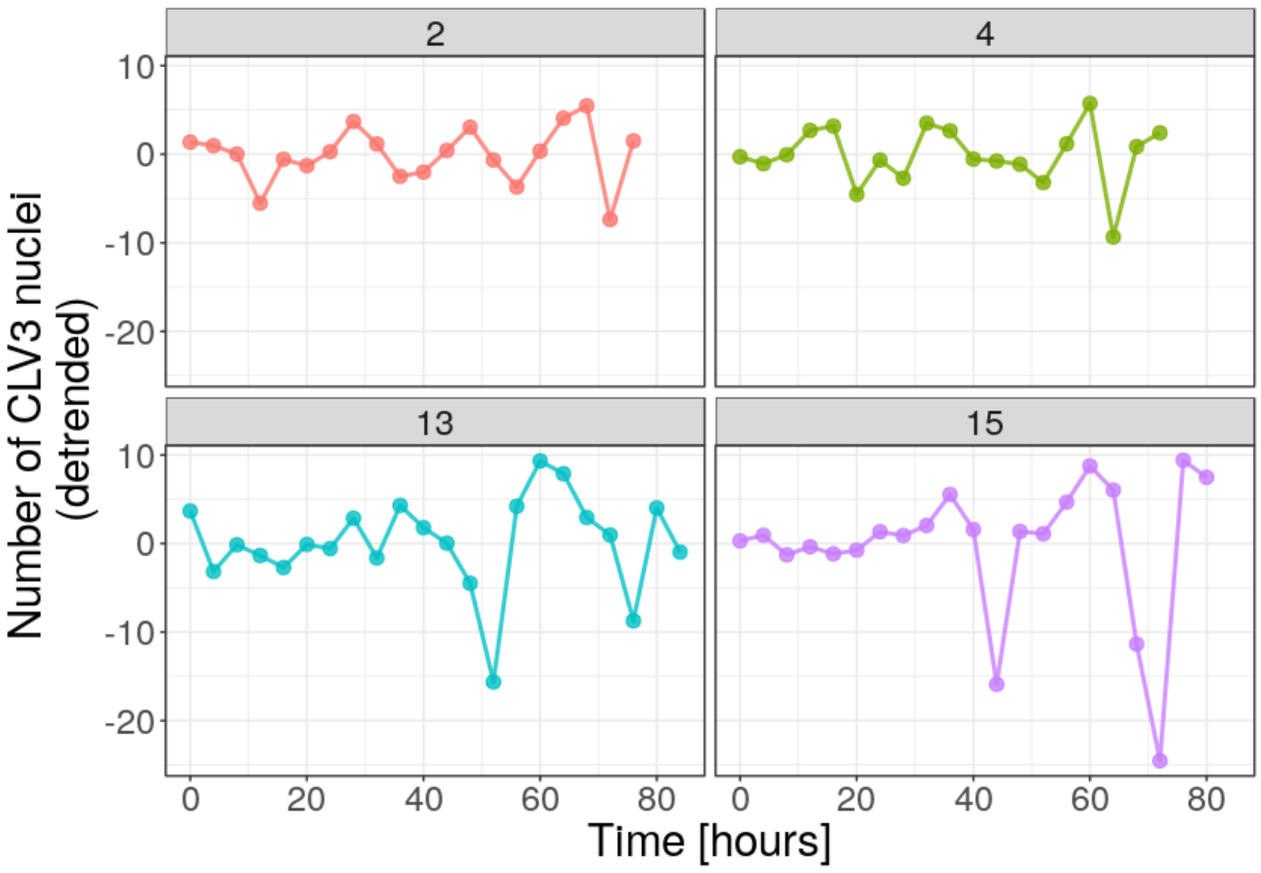
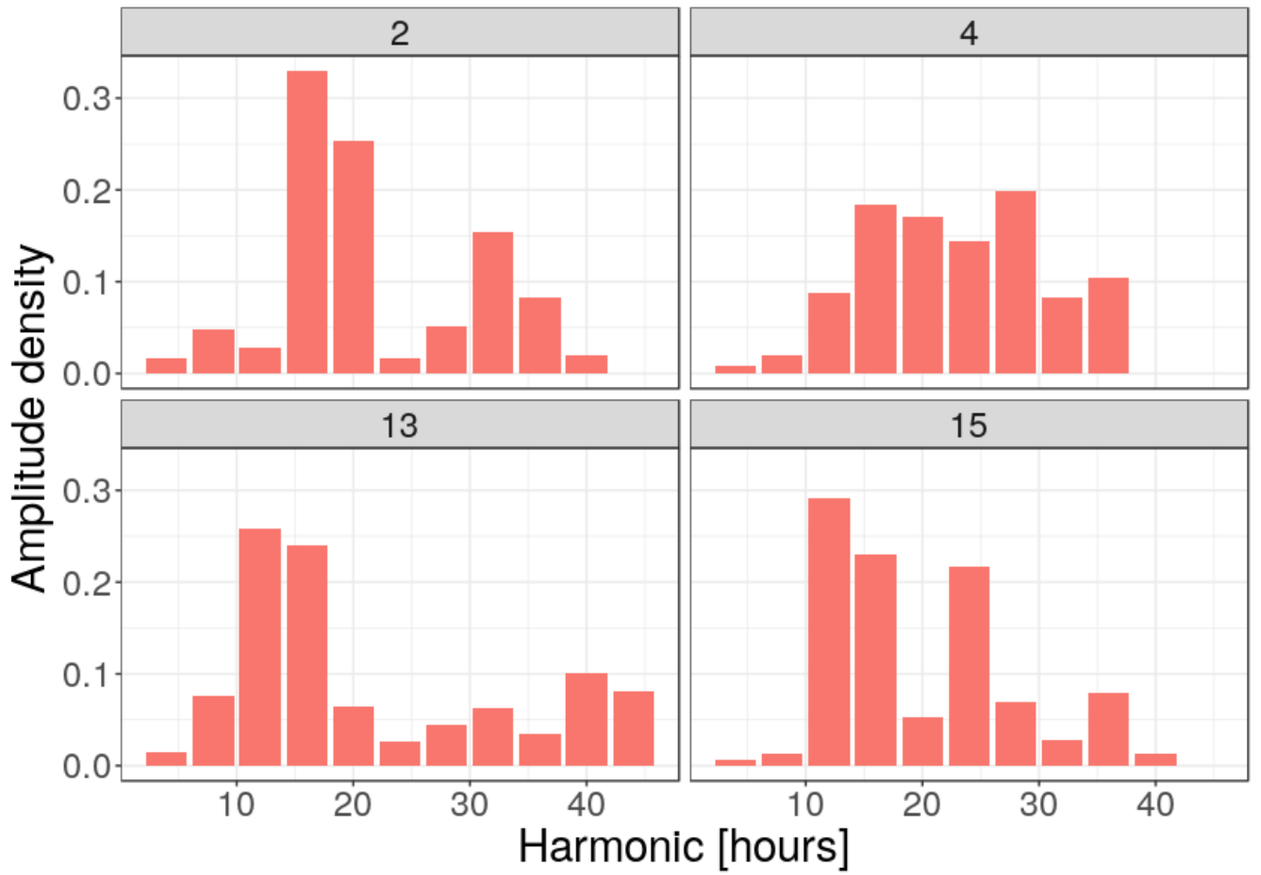
(Detrended) Nuclear trajectories



Fourier decomposition



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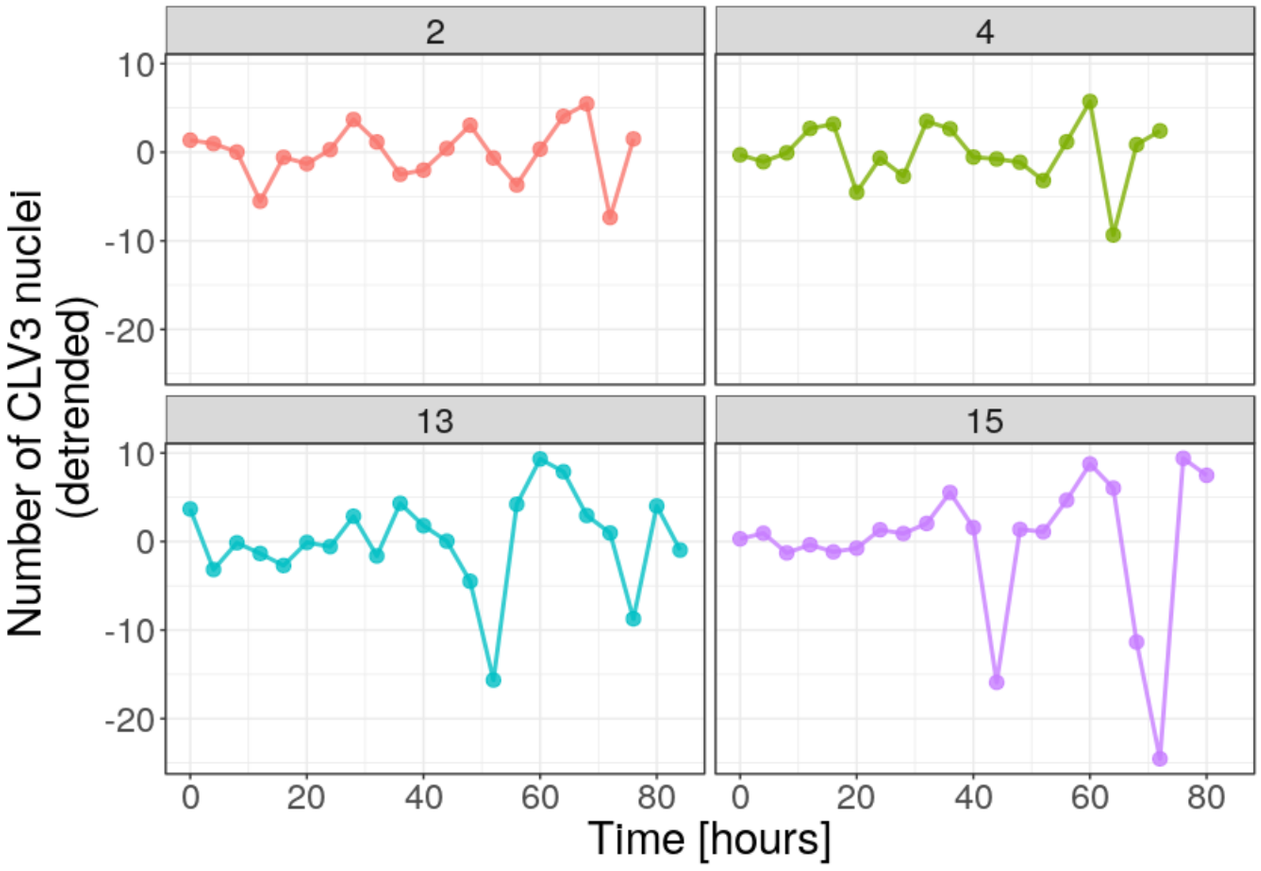

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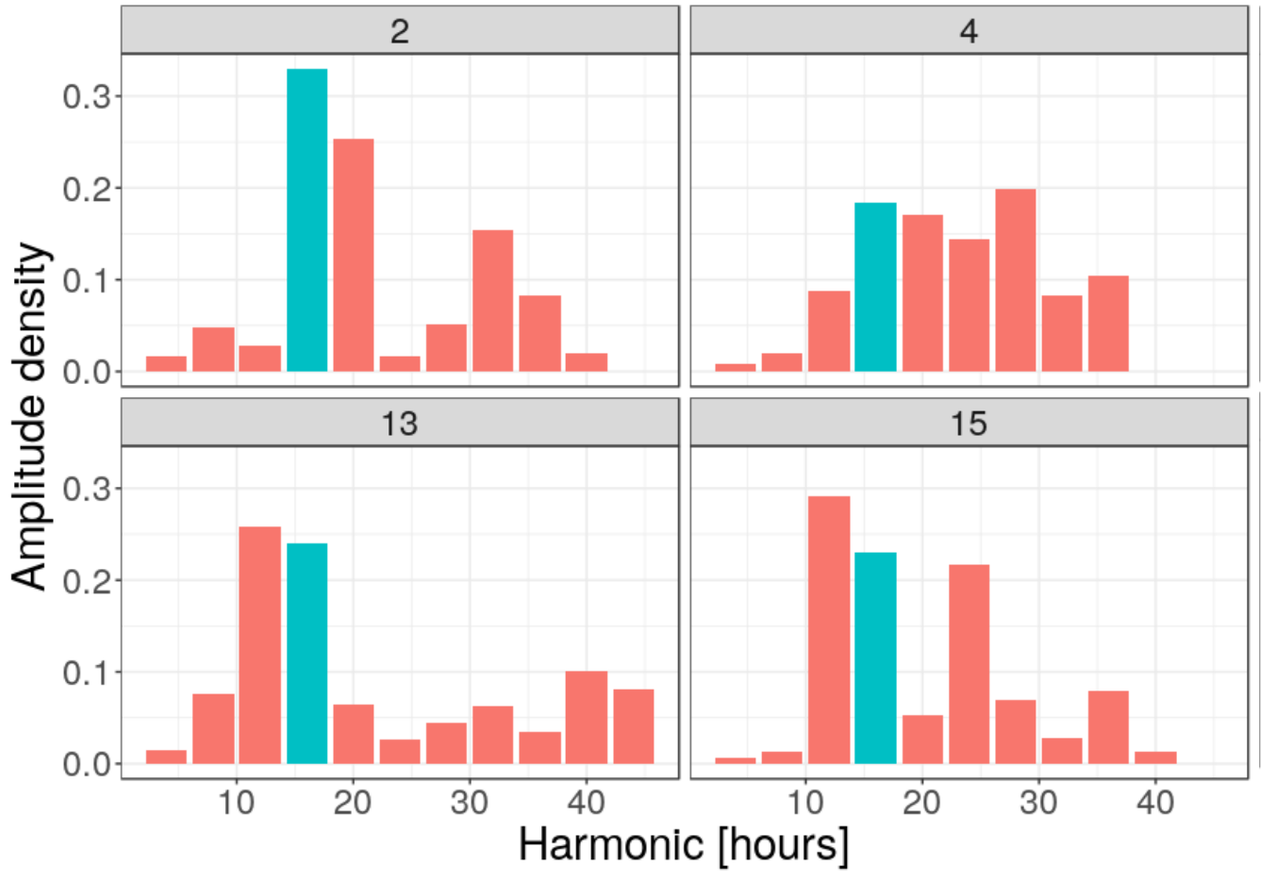

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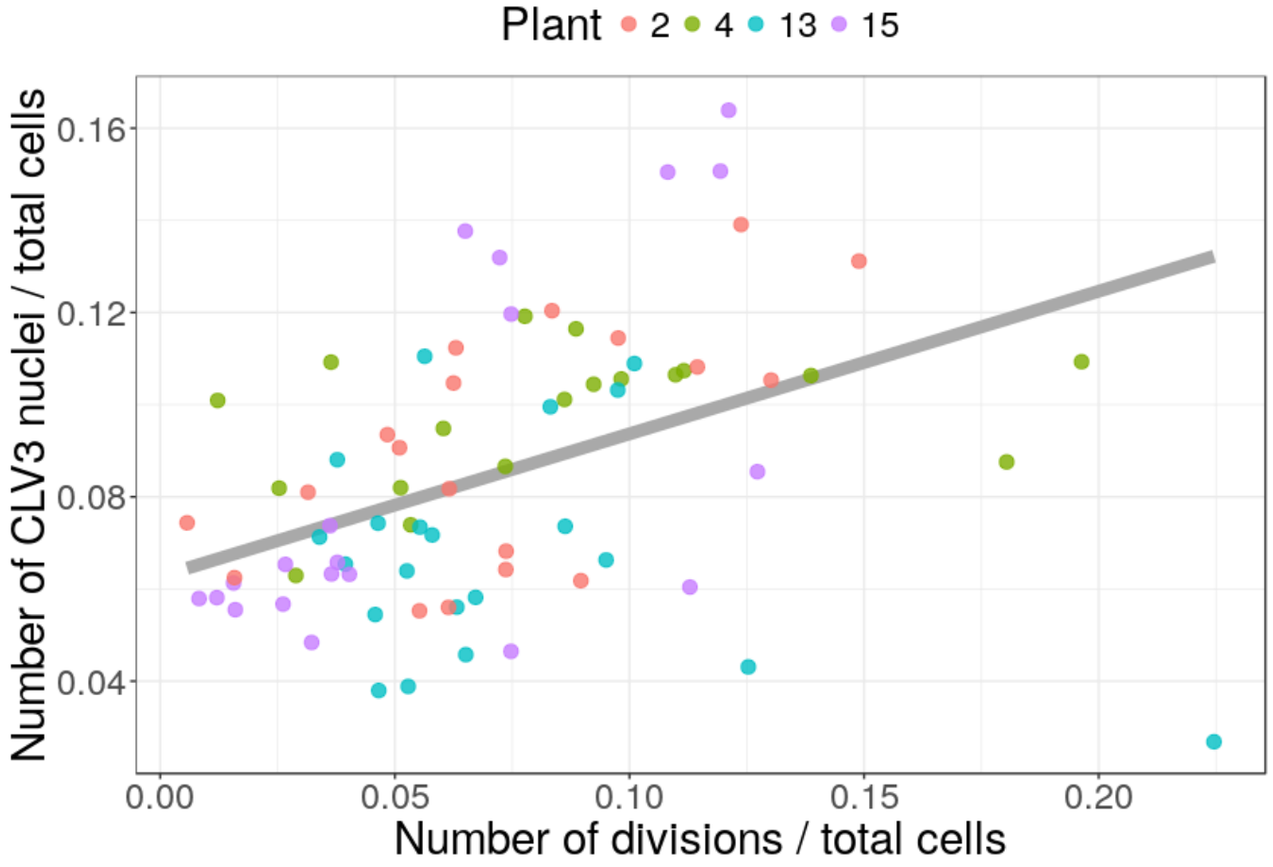
Divisions increase with #CLV3 nuclei



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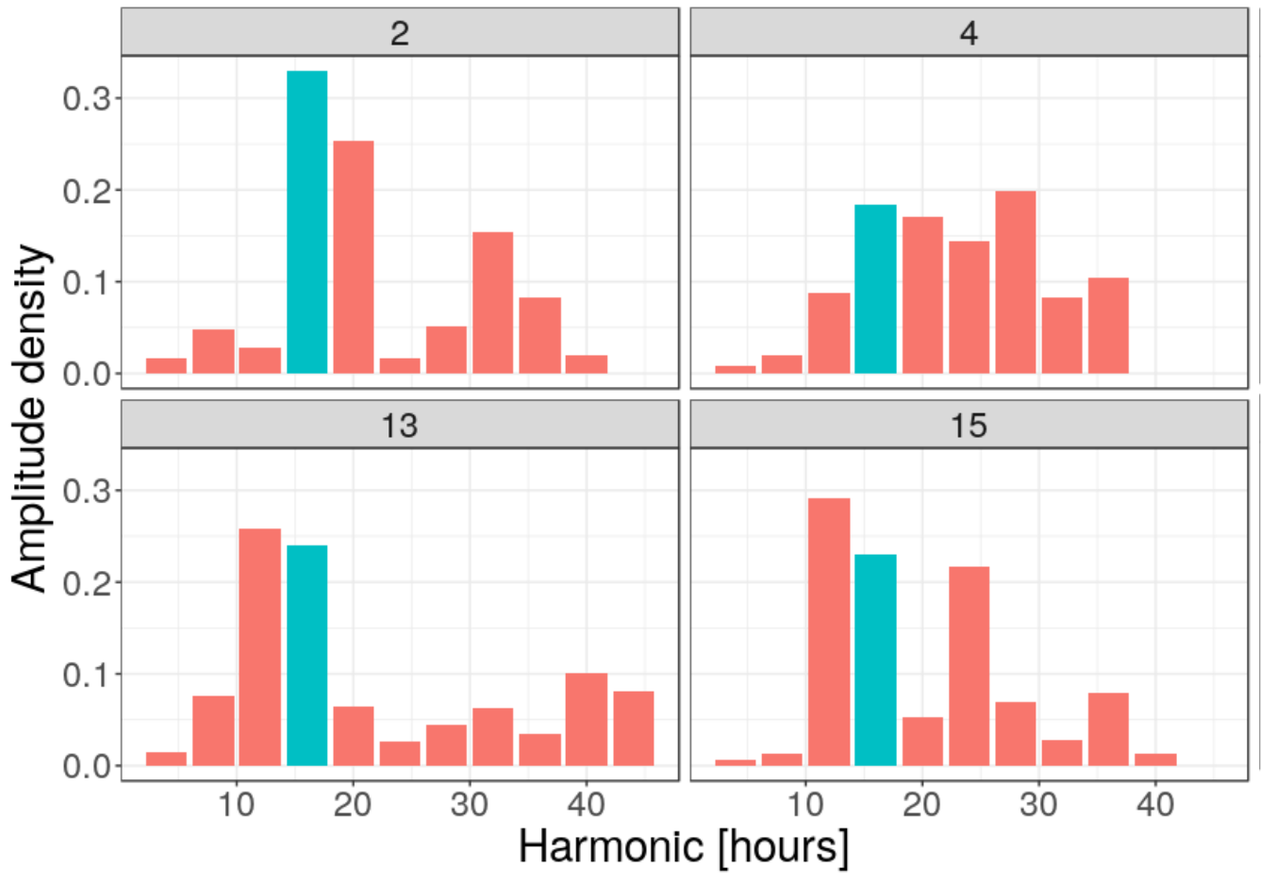

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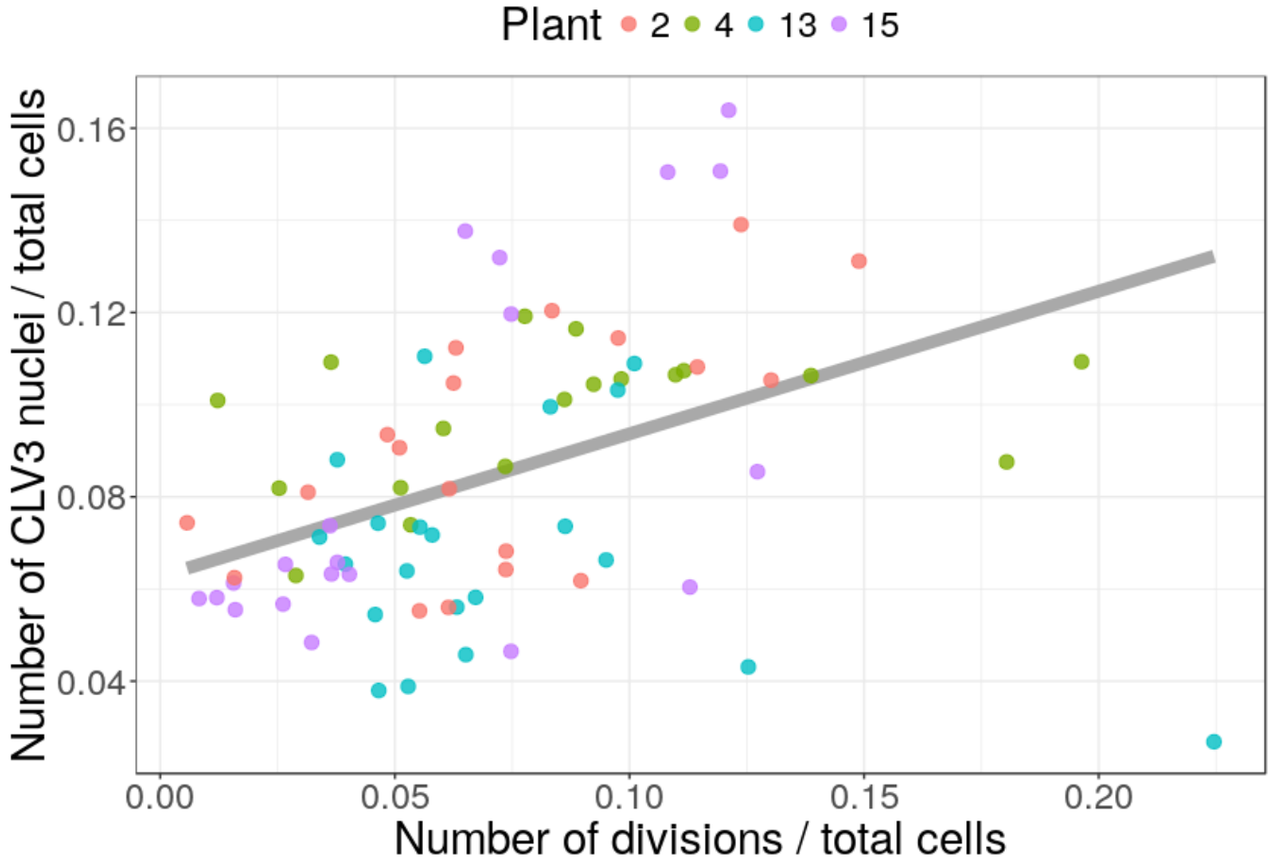

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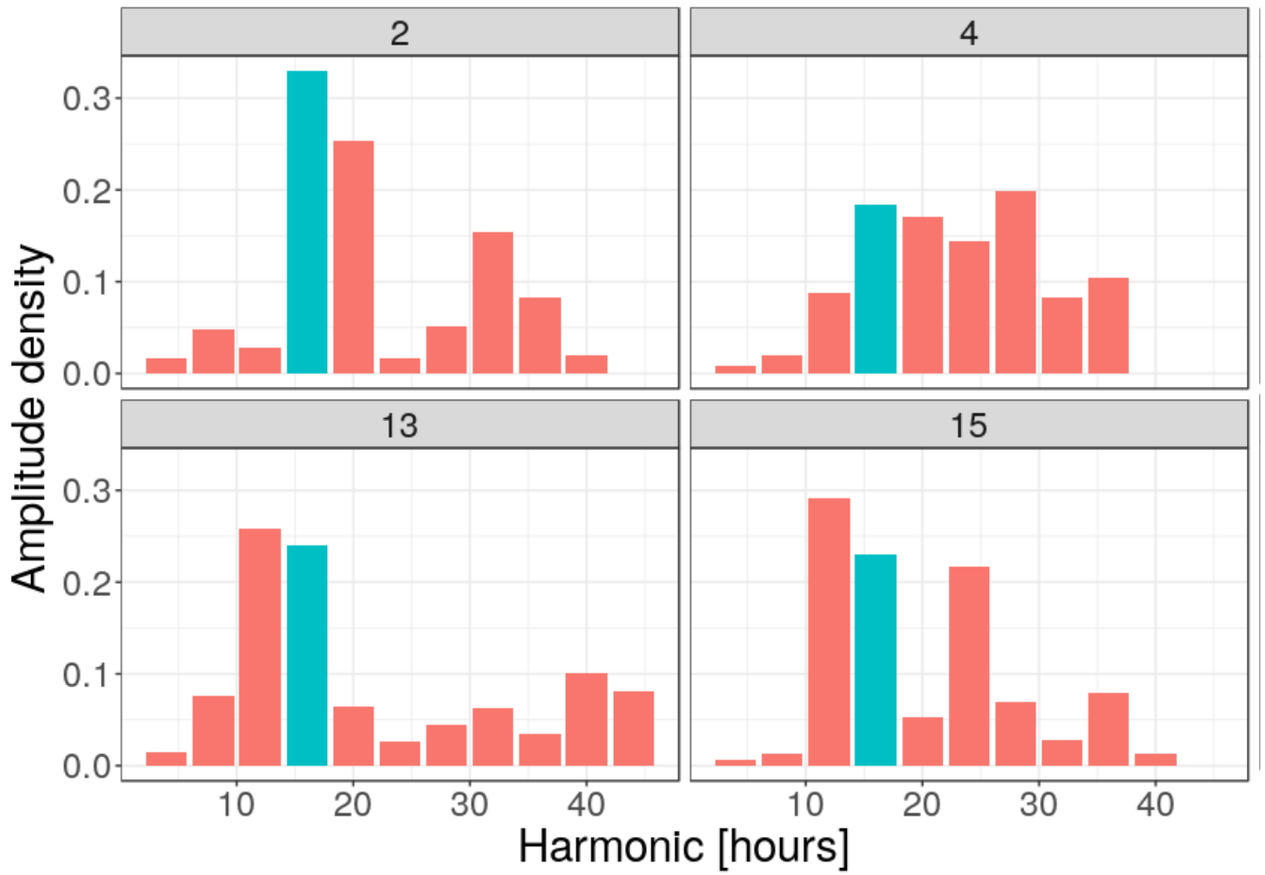
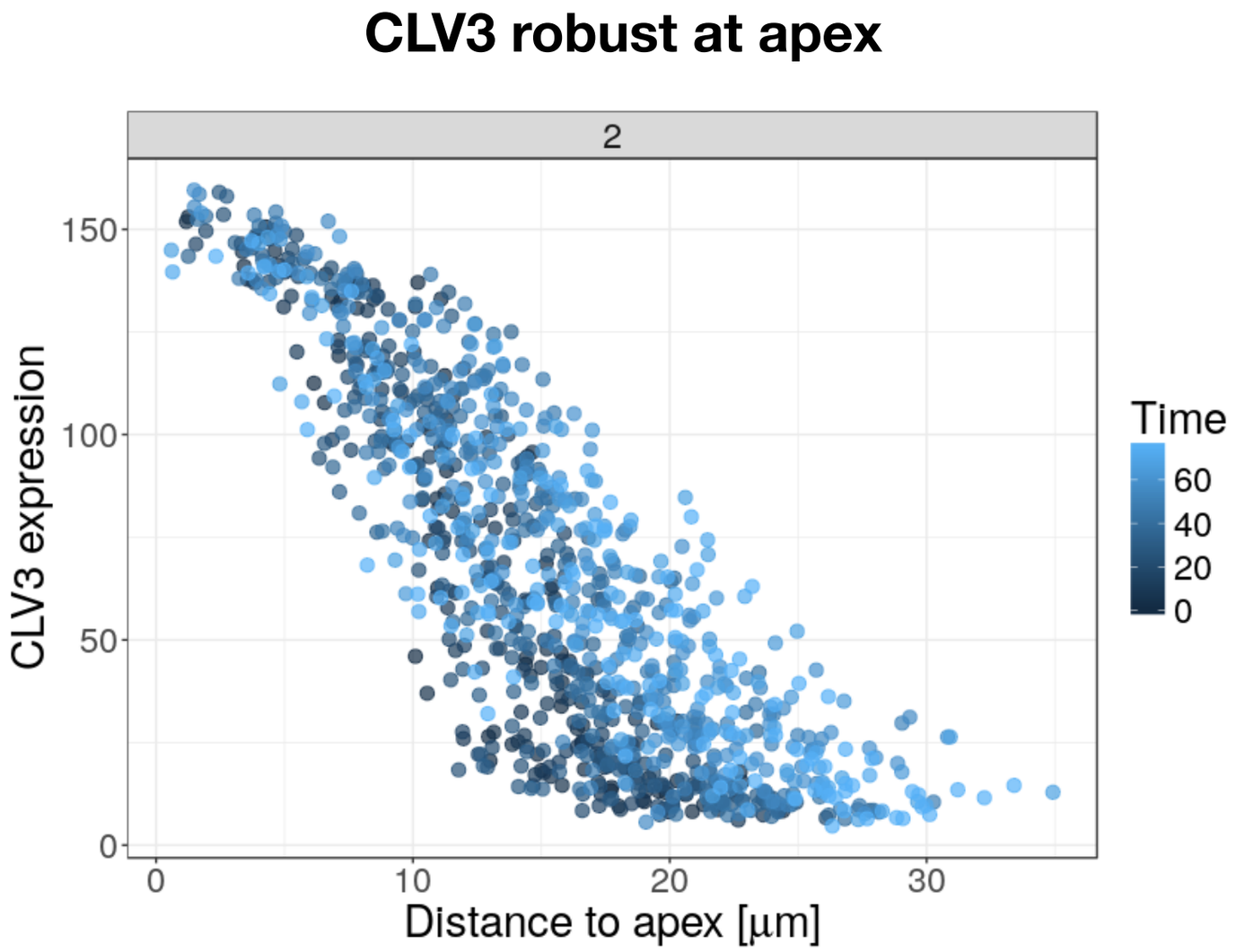


Globally: Not stable!

2: Robust regulation of apical cells

- **Low-high-low variability pattern for CLV3**



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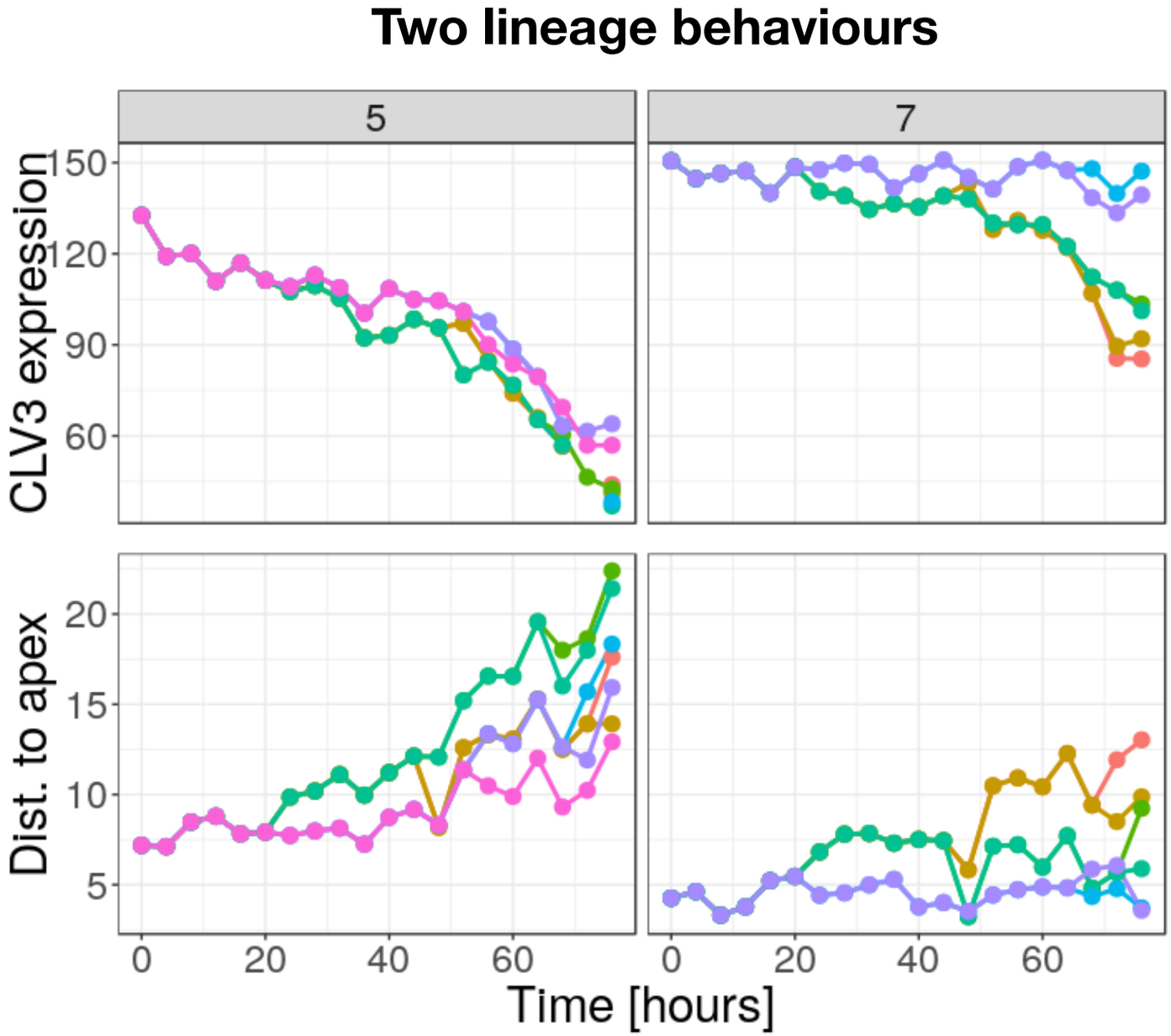

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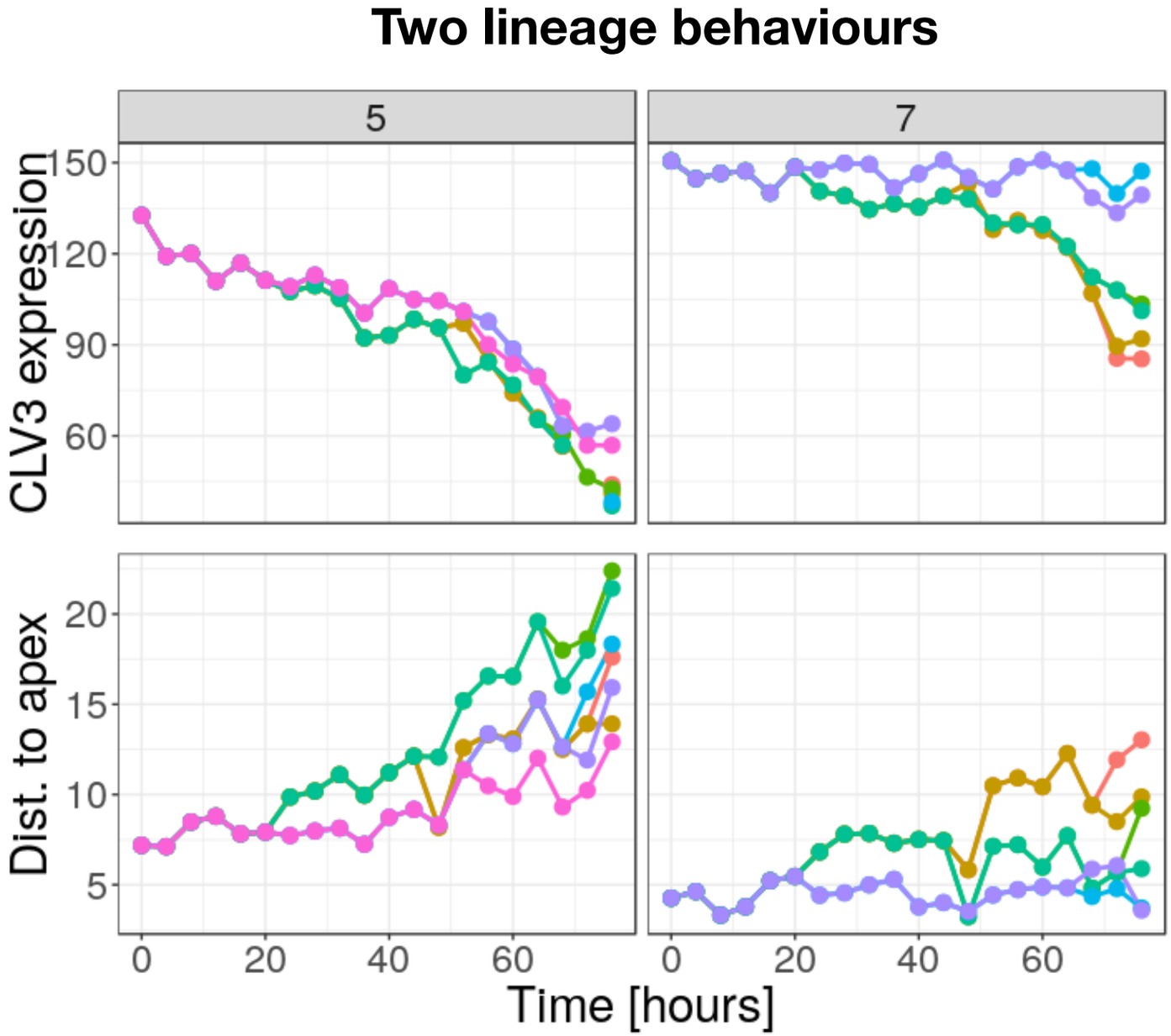
- **Q: Possible regulation of robustness?**



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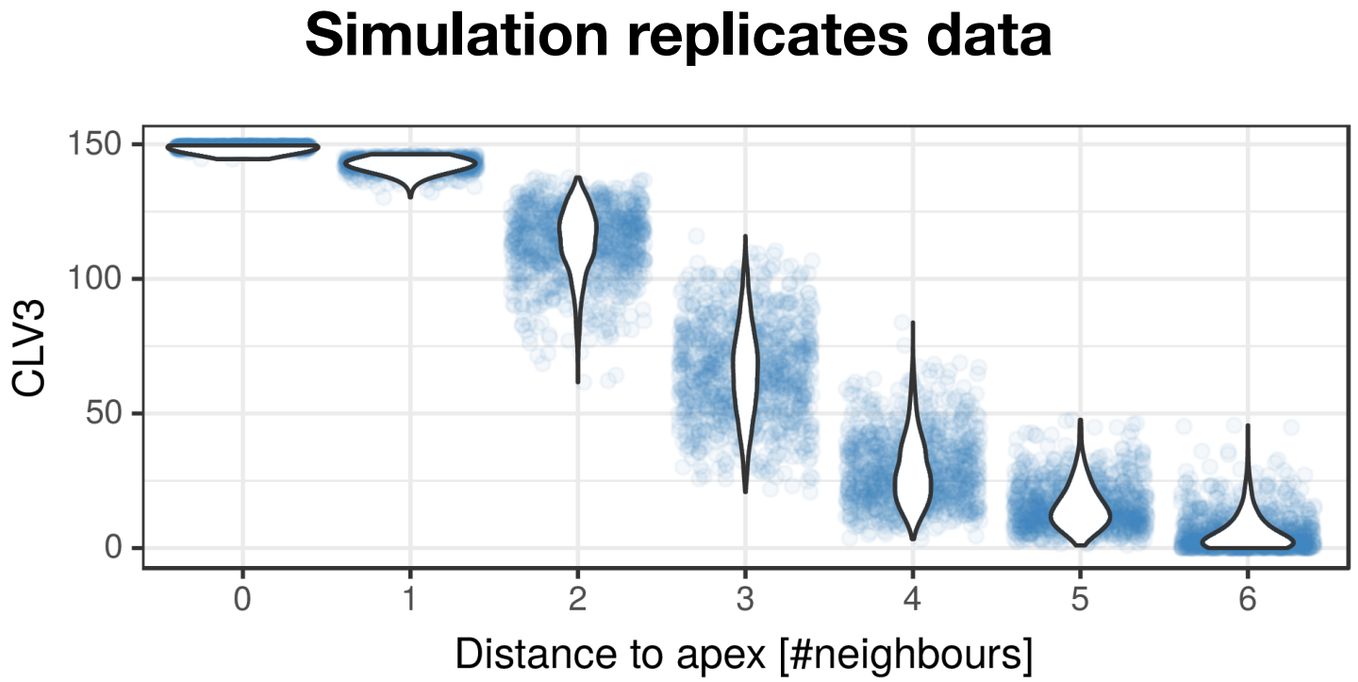
- **A: Enzymatic CLV3 activation sufficient**

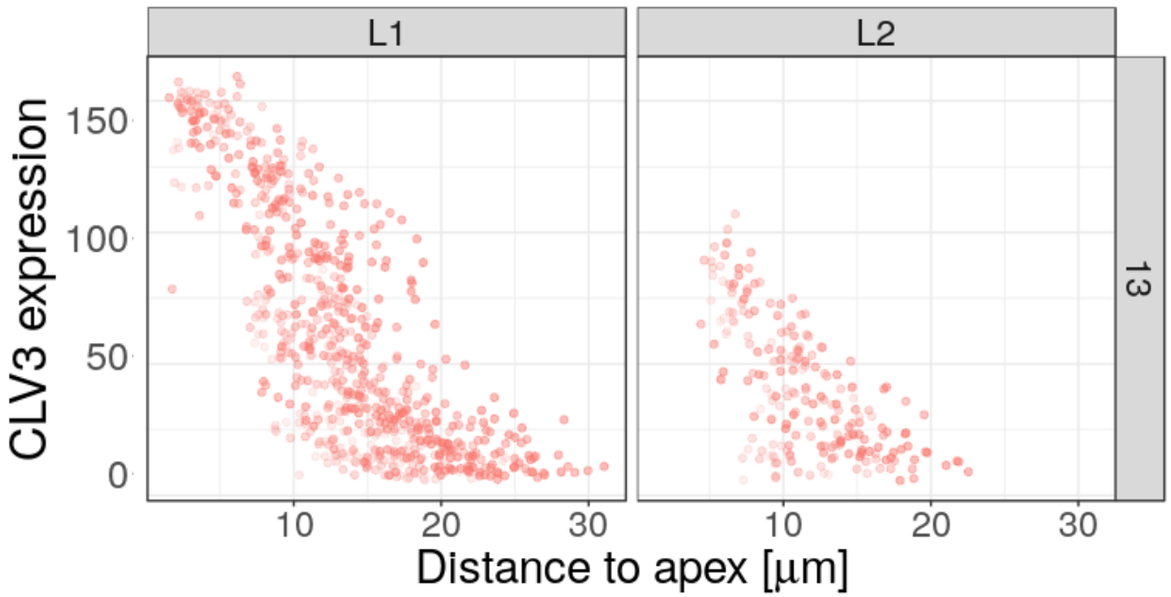


$$\dot{CLV3} \sim v_{max} \frac{[WUS]^{n_1}}{[K_1]^{n_1} + [WUS]^{n_1}}$$

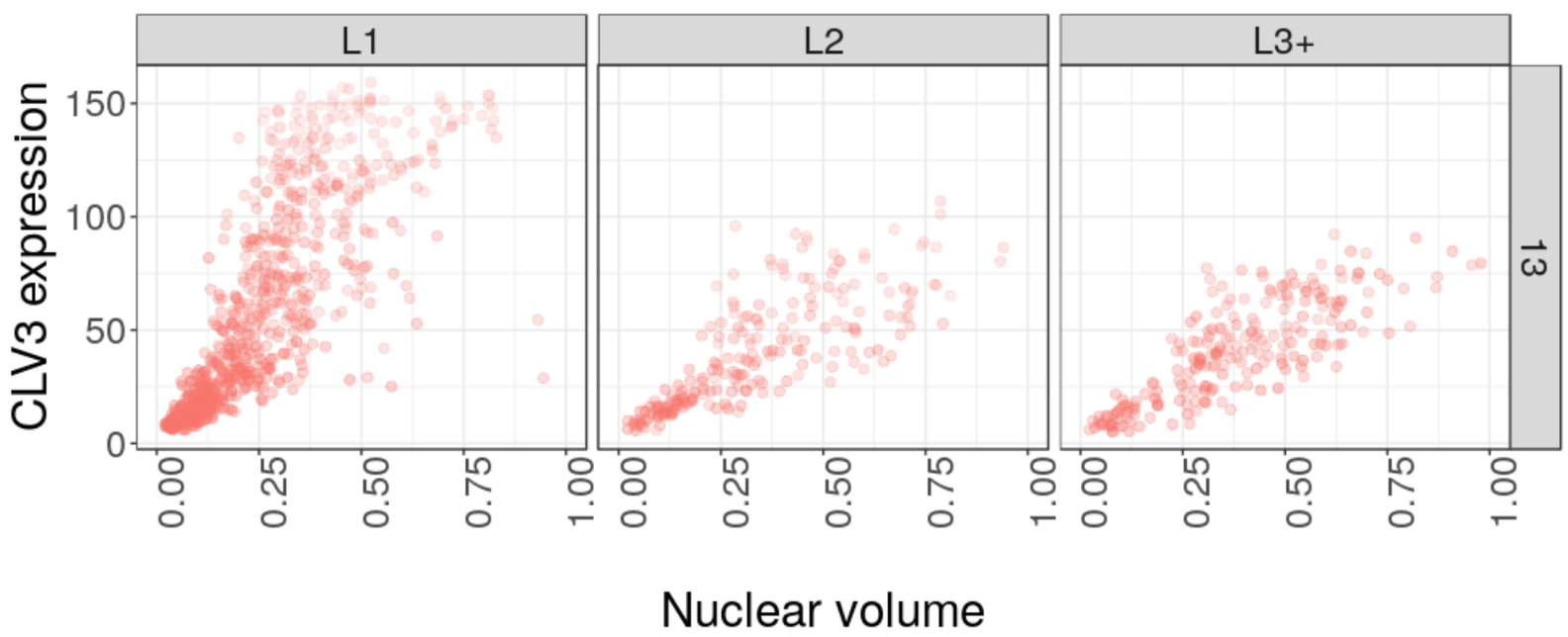
2: Robust regulation of apical cells

- **Expression-distance-volume relationship hints at epidermal regulation**

Distributions change between layers



Sigmoidal **Linear** **Linear**



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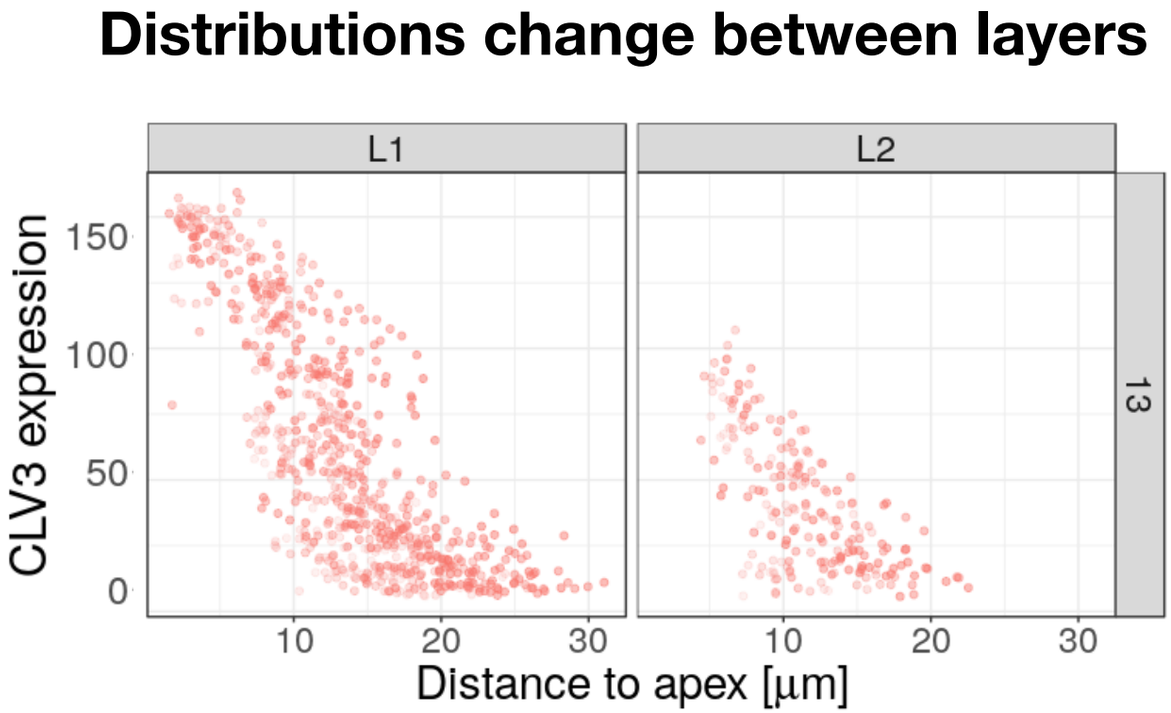
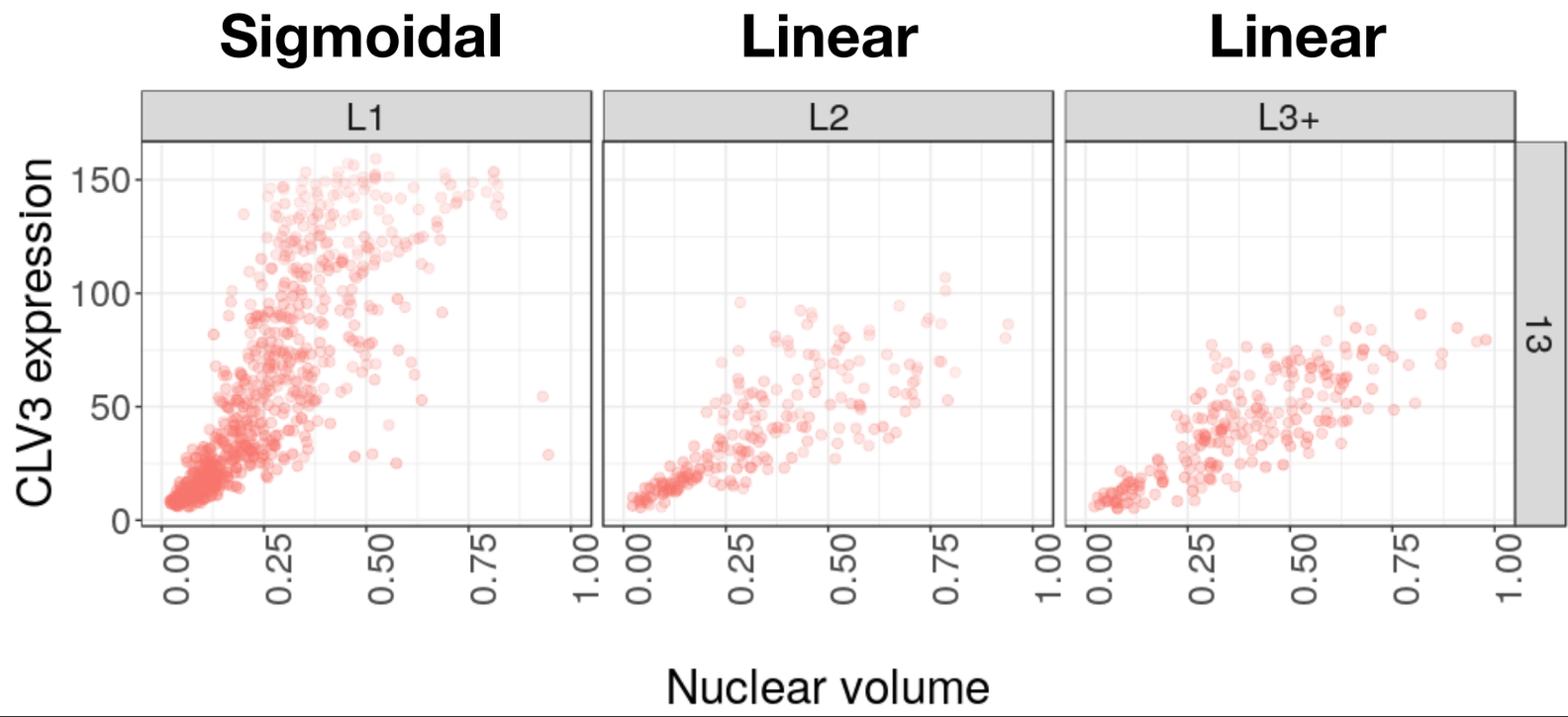
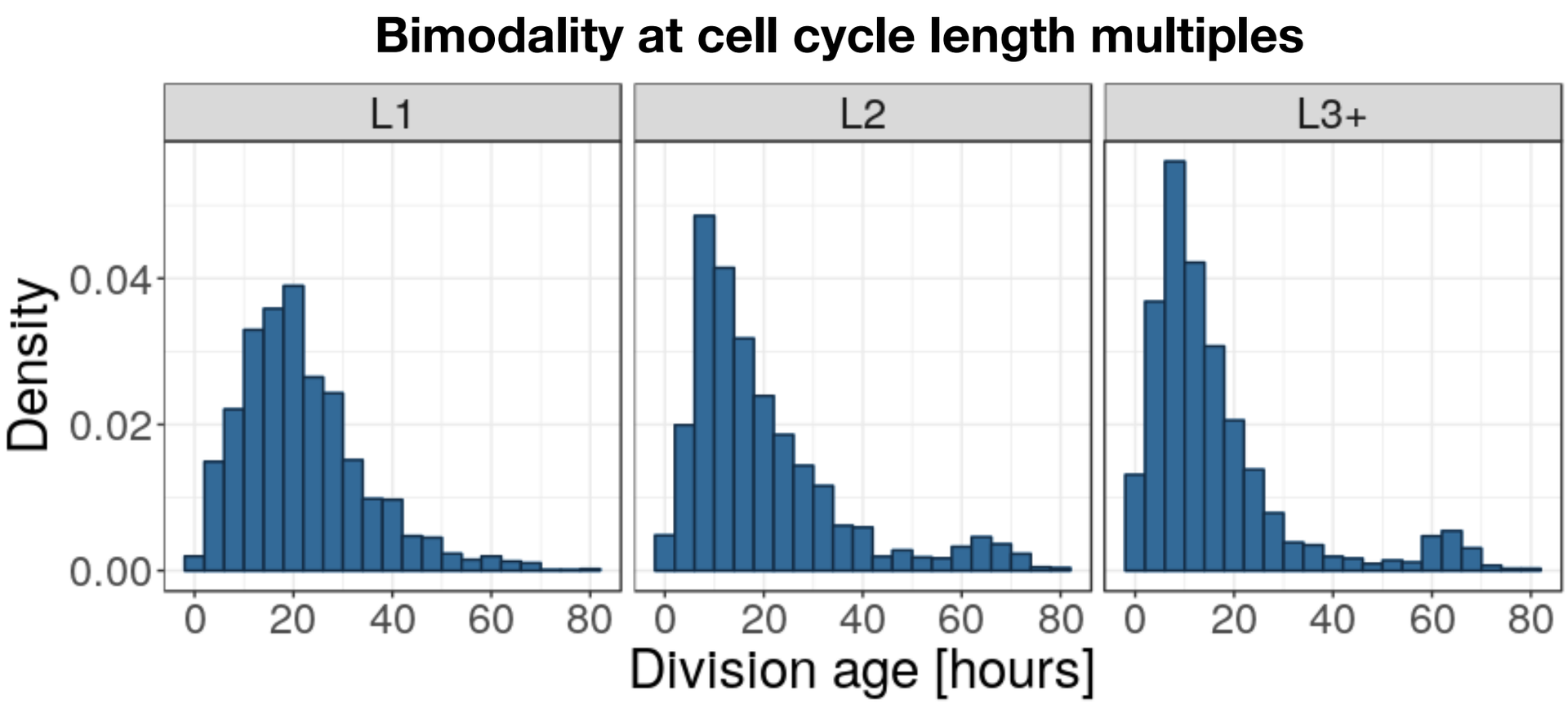


- **Model shows L1 activation plausible**

$$\dot{CLV3} \sim v_{max} \frac{[WUS]^{n_1}}{[K_1]^{n_1} + [WUS]^{n_1}} \frac{[L1]^{n_2}}{[K_2]^{n_2} + [L1]^{n_1}}$$



3: Is higher age induced in center?



- **Clusters in 3x typical division time**

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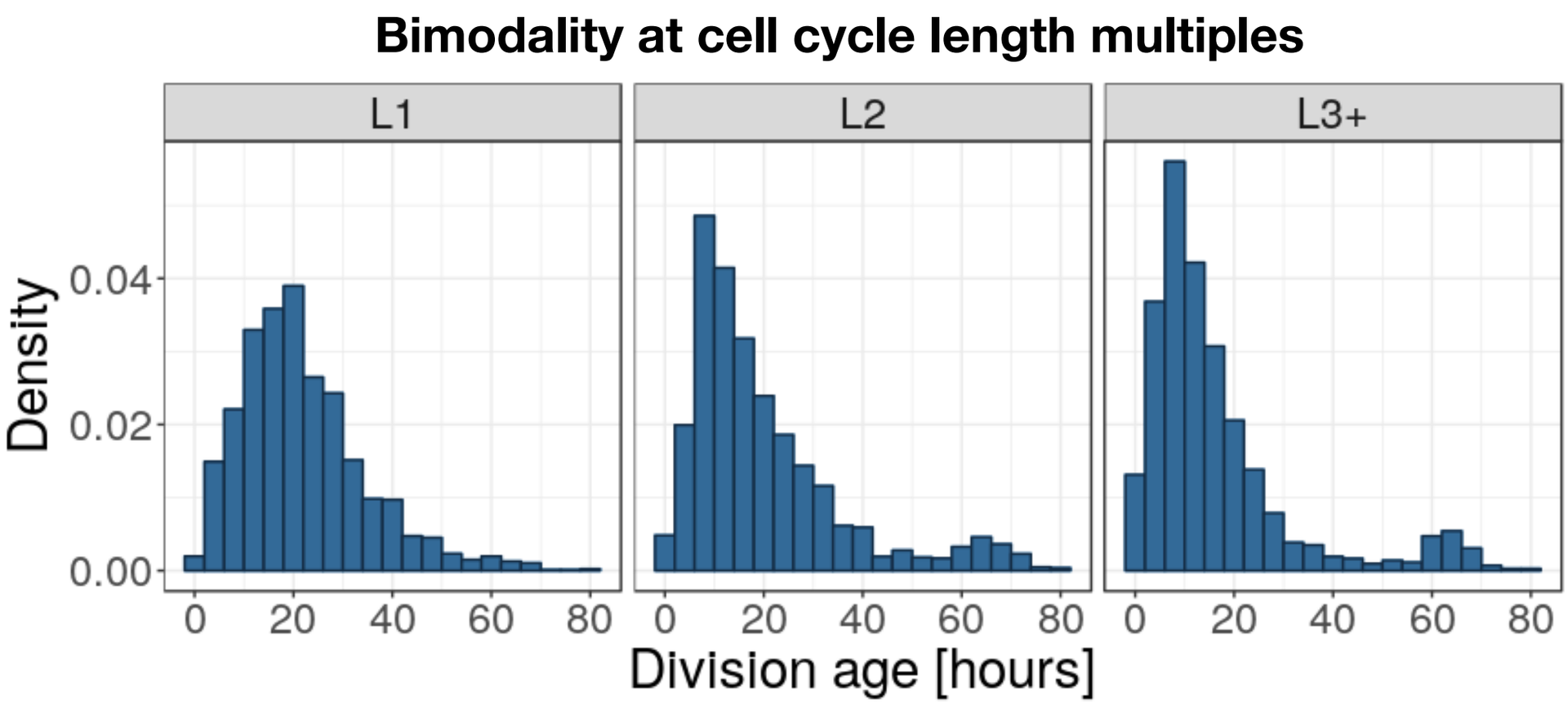
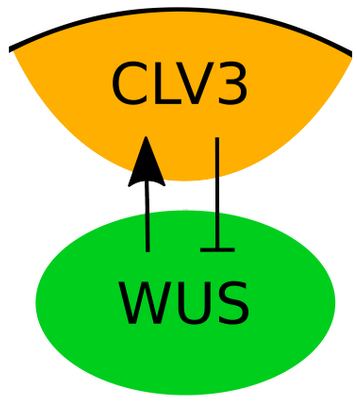


- Clusters in 3x typical division time

- **Possible domain effect in organising center**



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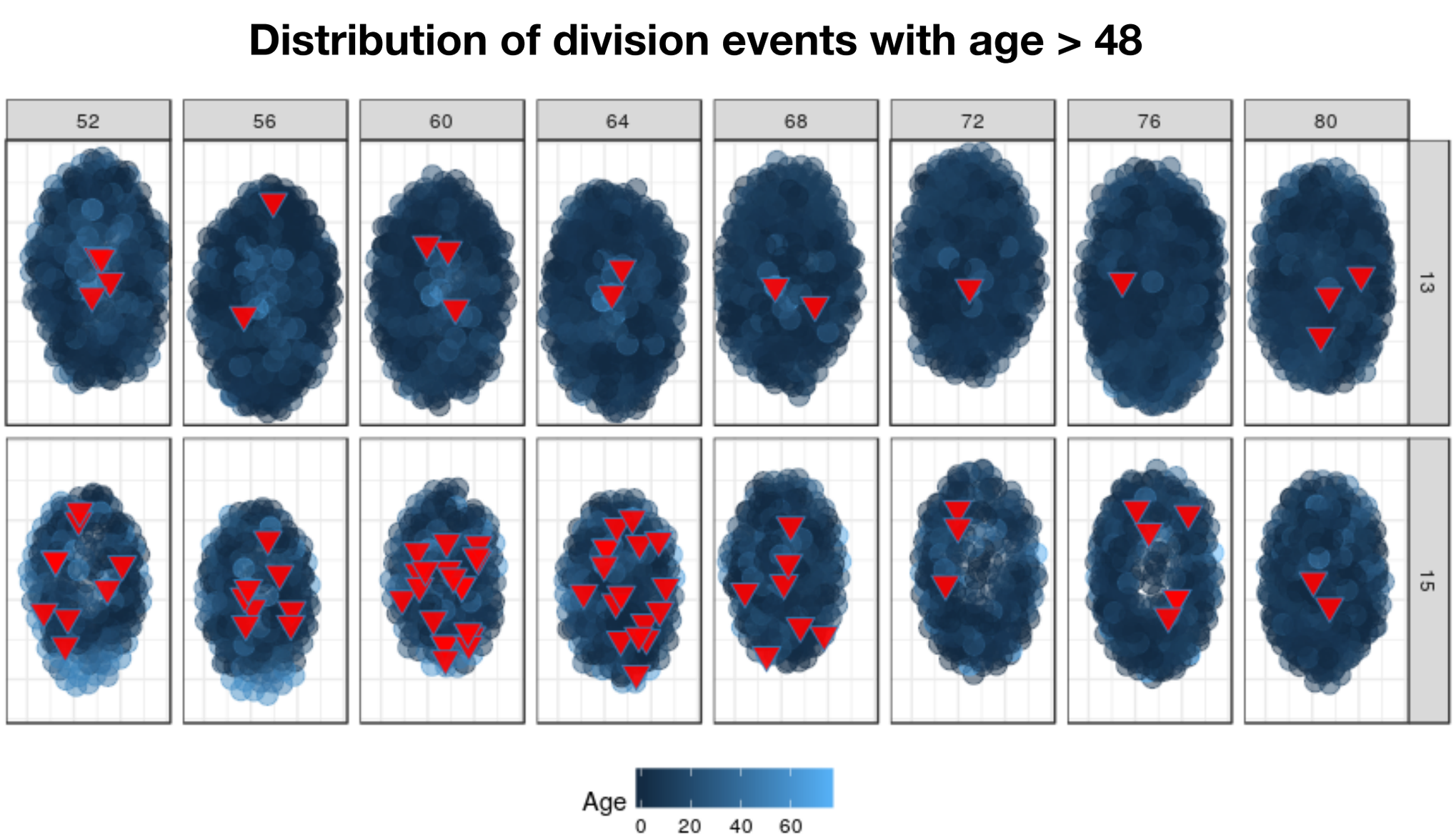
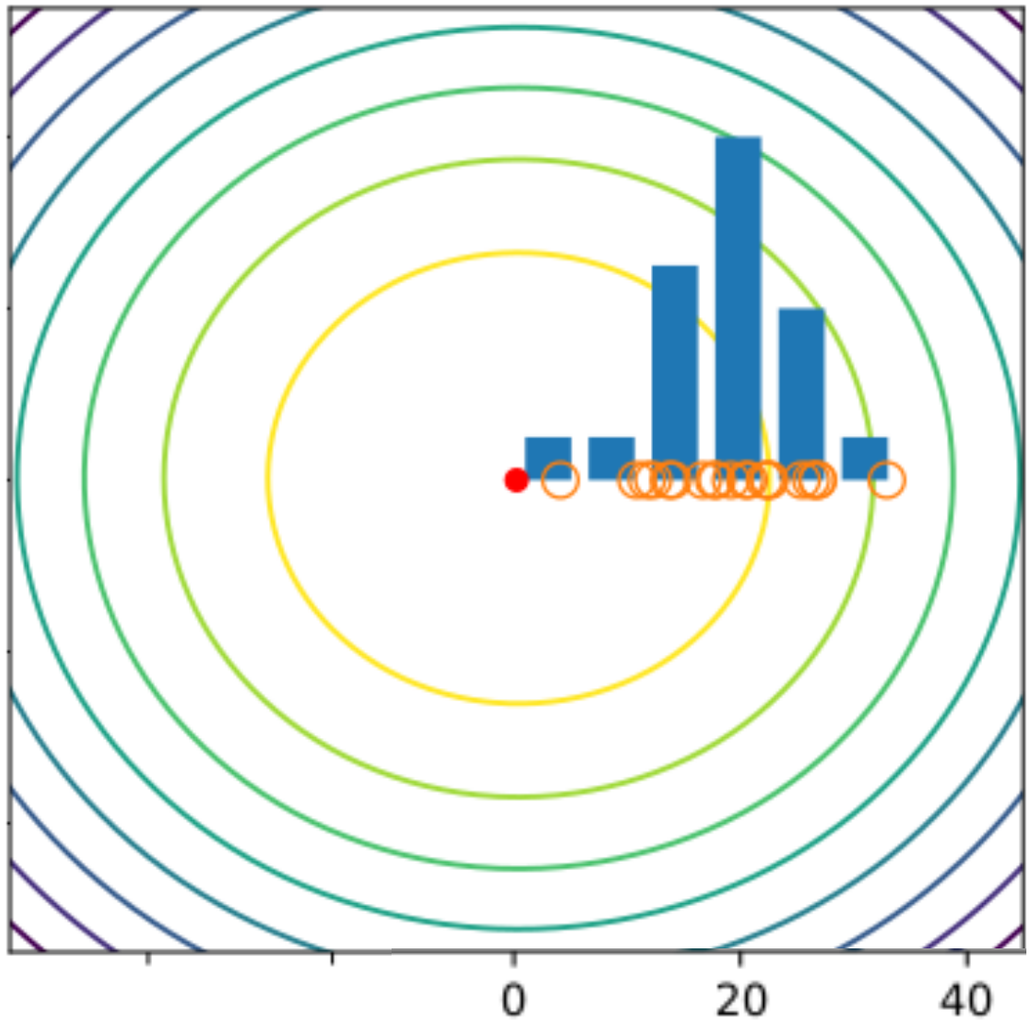


4. Geometric apex ≠ chemical apex

- **Paraboloid fit to raw meristem image**

- **CLV3 peak distribution shifted ~3 cells from geometric apex**

Paraboloid top-view



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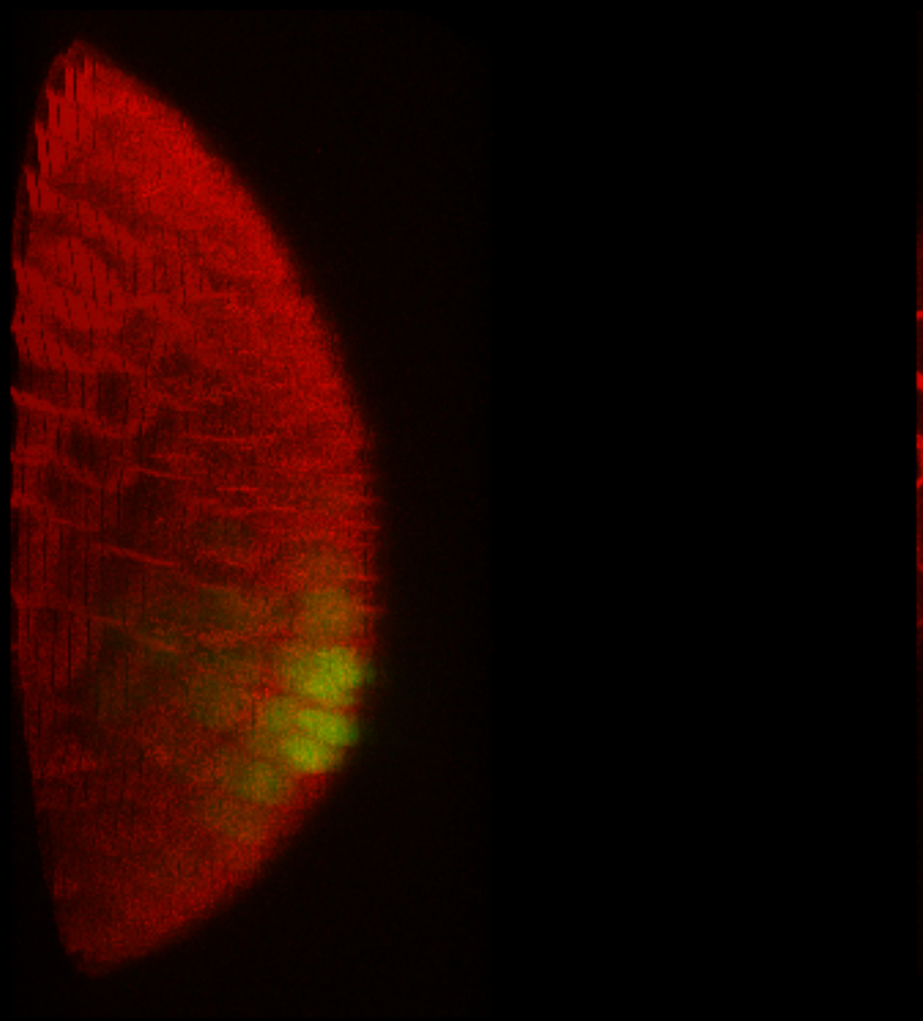
Side view



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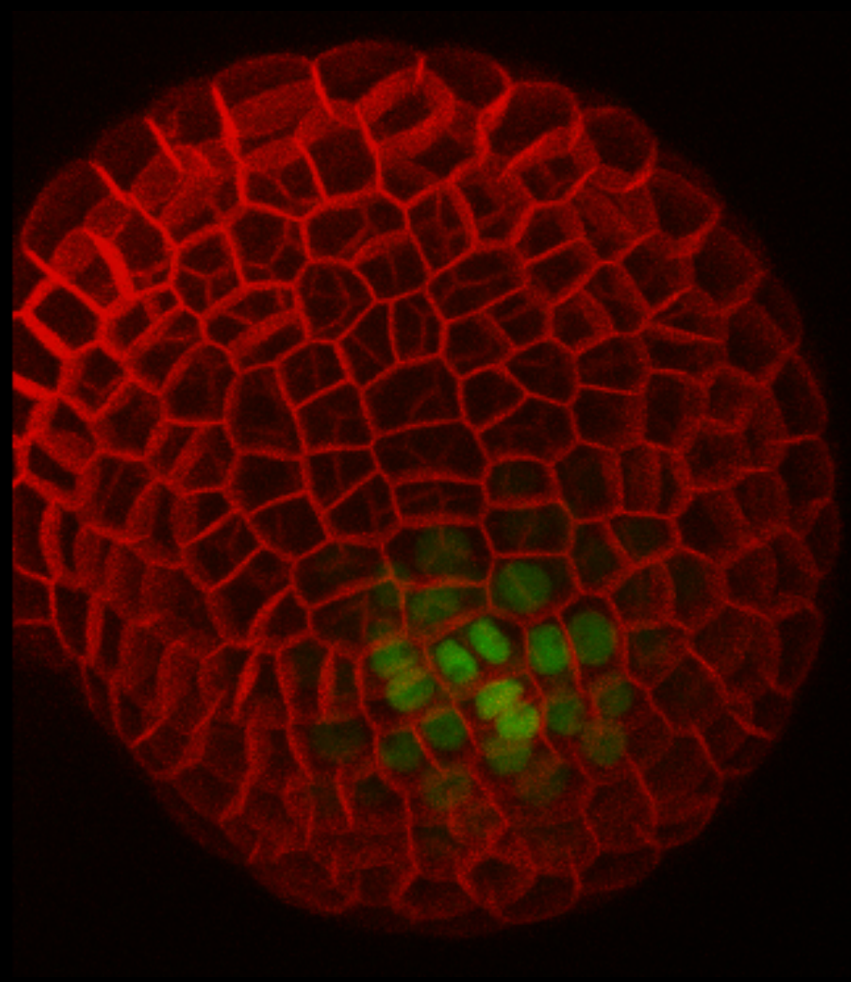

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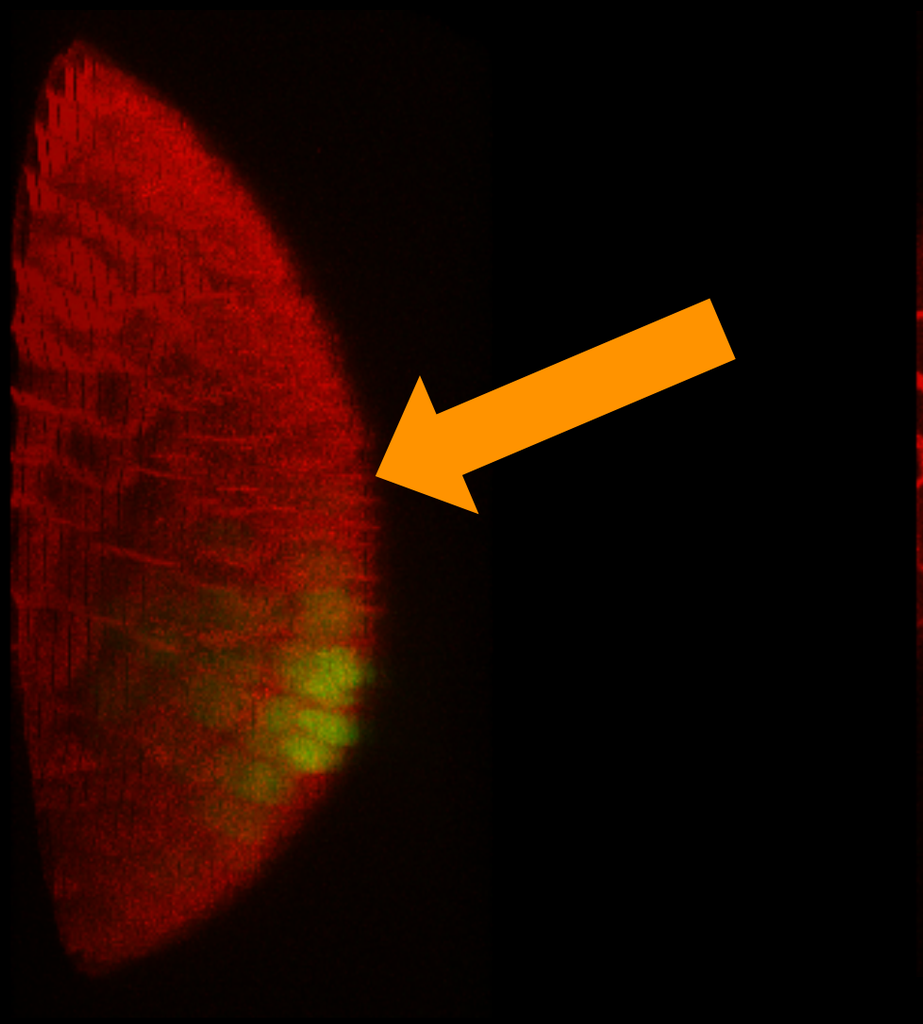

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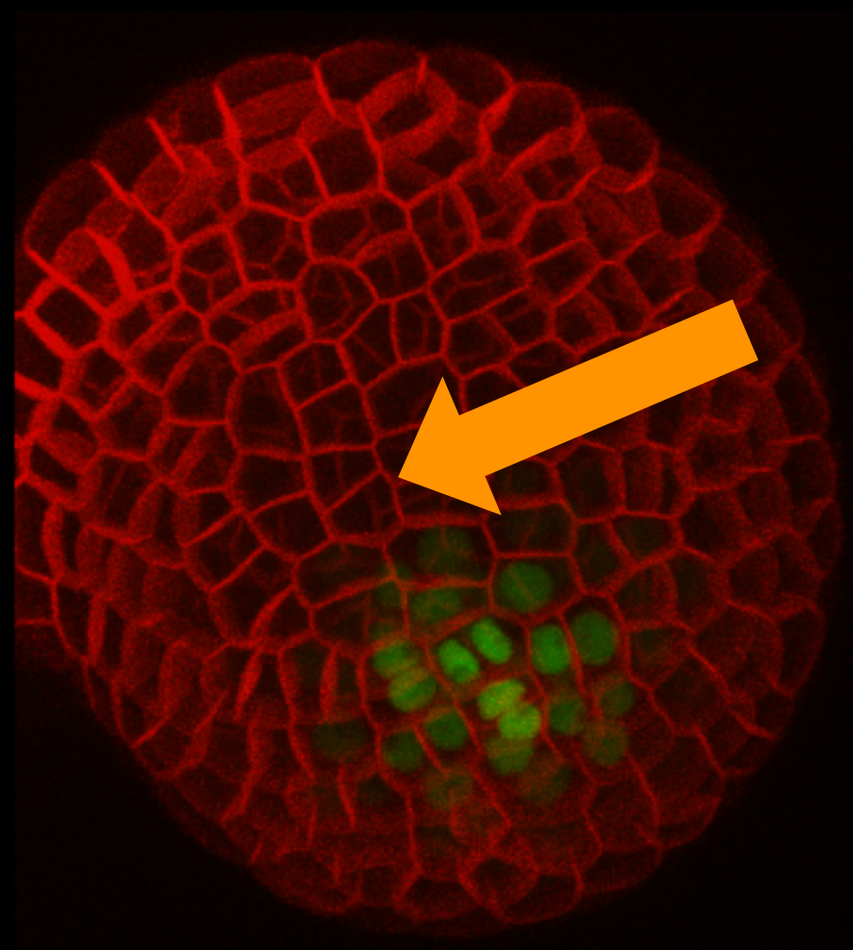

Conclusions

In vivo **quantification at single cell level**

Robustness

Possible regulation

Longevity

Growth / phyllotaxis