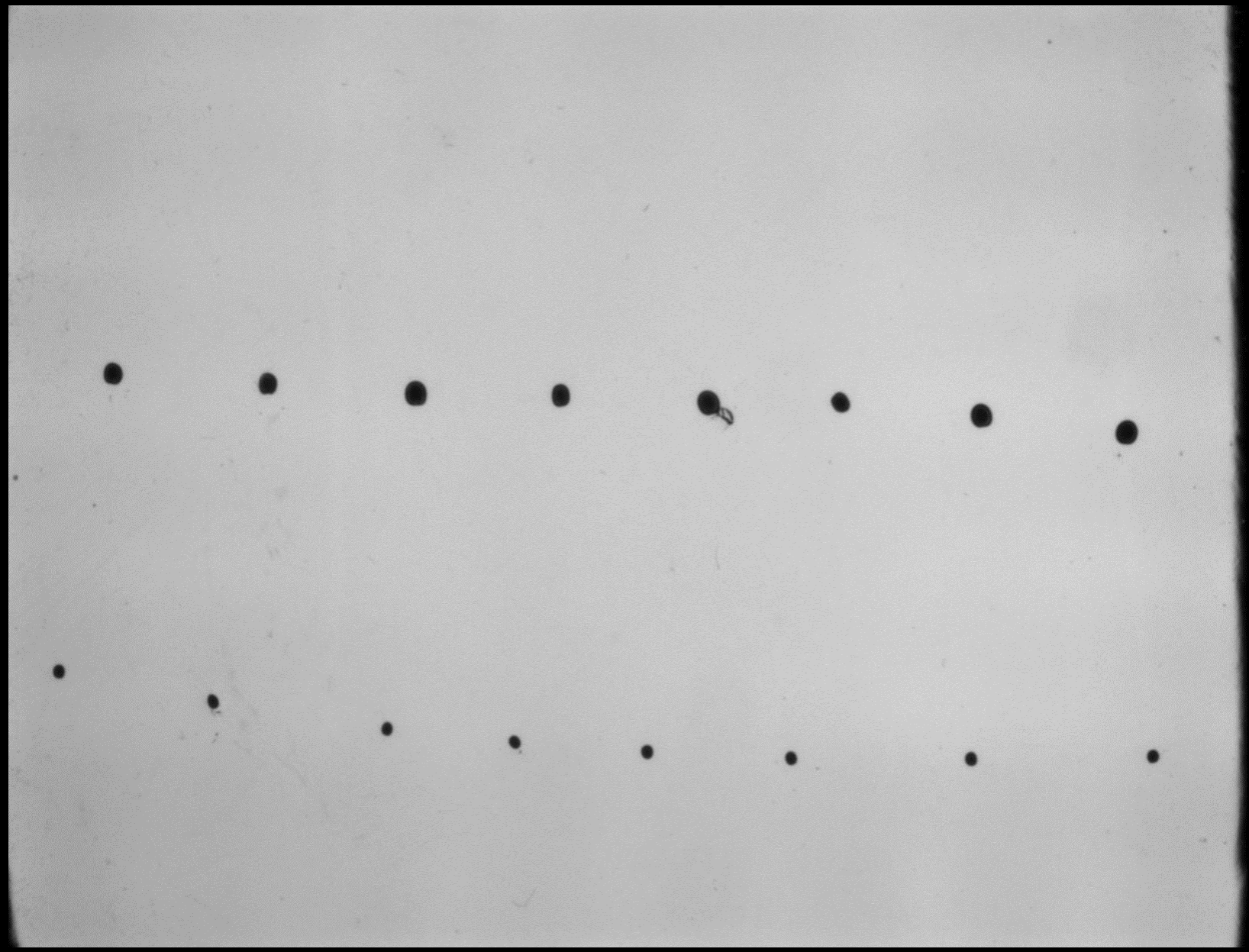
Germination: Cardamine vs Arabidopsis

Cardamine Hirsuta (OX)

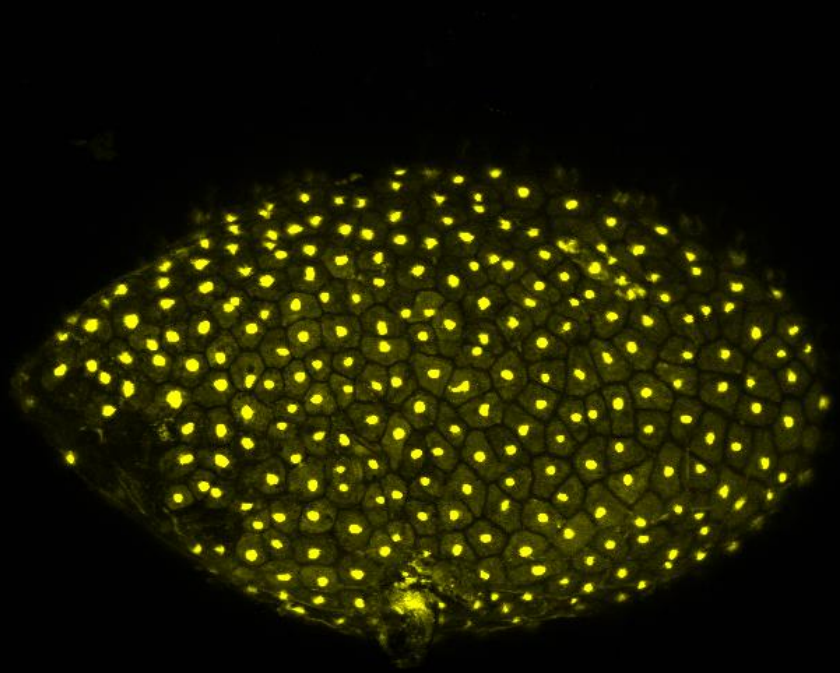
Arabidopsis Thaliana (WS)



BIOSENSORS OPTOGENETICS

Biosensors

Biosensors (‘biological sensors’) are biological tools that monitor a process or detect a given molecule. The sensor component is usually a protein that undergoes a conformational change in response to the molecule it detects.



- High selectivity
- Definite quantification
- High resolution

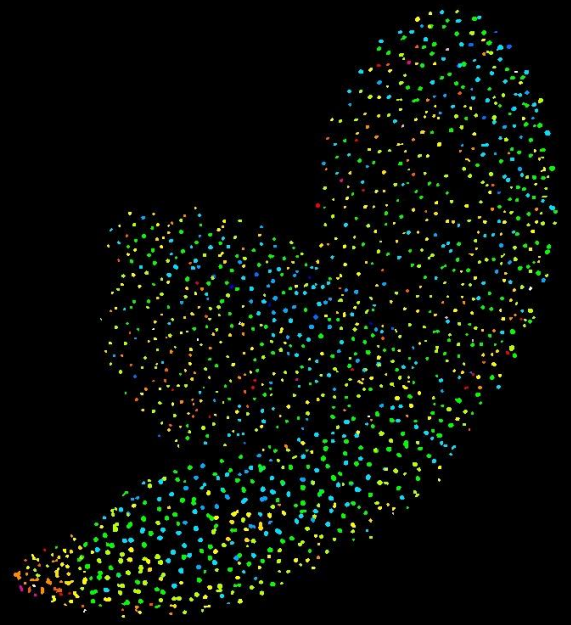
Spatio-temporal patterns of ion and metabolite levels in living cells are important for understanding signal transduction and metabolite flow. The approaches of imaging that use genetically encoded sensors are ideal for detecting such molecular dynamics, which are otherwise difficult to obtain.

Breakthrough in In Vivo Compatible Sensors: Fluorescent Proteins

Origin

Derived from Cnidaria organisms like jellyfish and corals (e.g., Aequorea species).

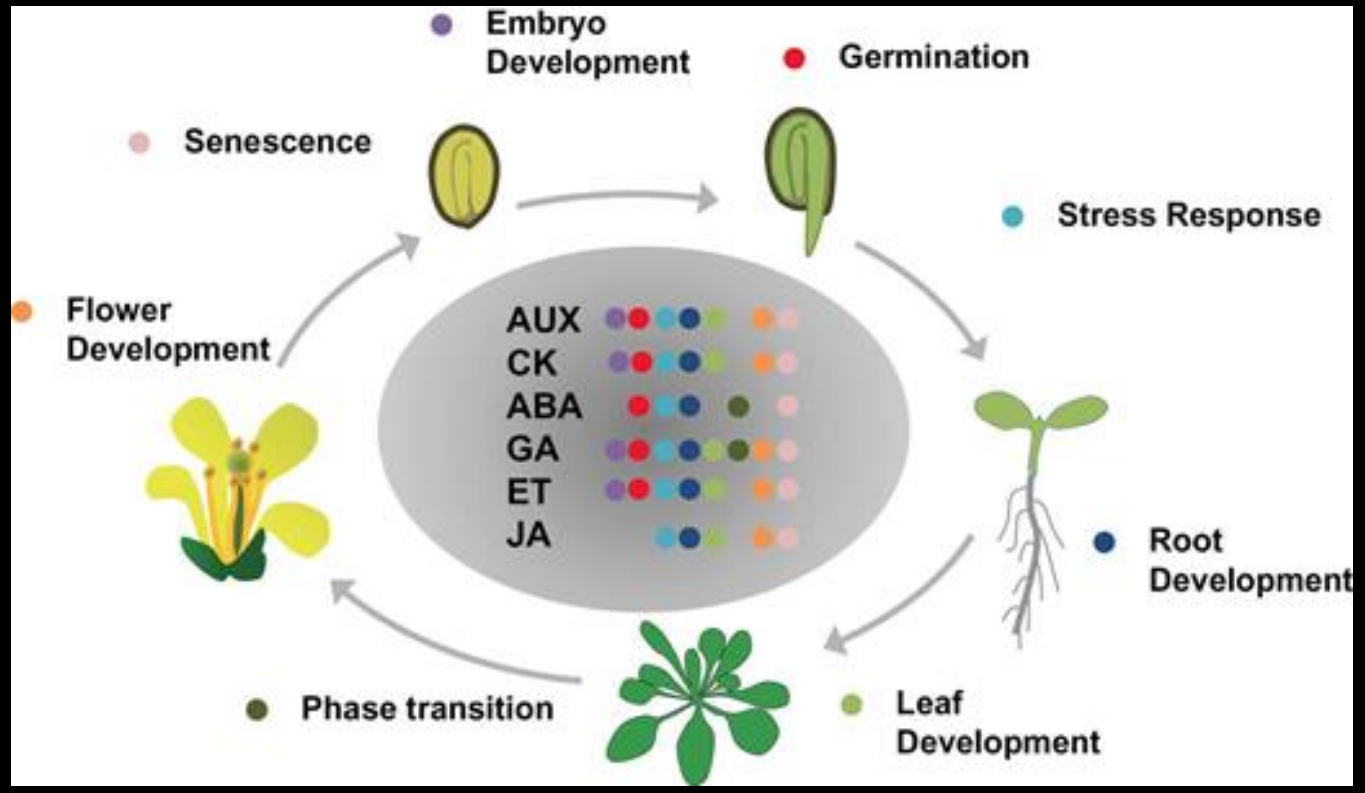
Fluorescent Proteins (FPs) are highly beneficial due to their ability to report cellular events. It has been demonstrated that two FPs acting as a pair of FRET donors and acceptors can function as reporters of biochemical events with a resolution beyond the limit of optical microscopy.



• FLORECENCE REPORTERS OR BIOSENSORS TYPES

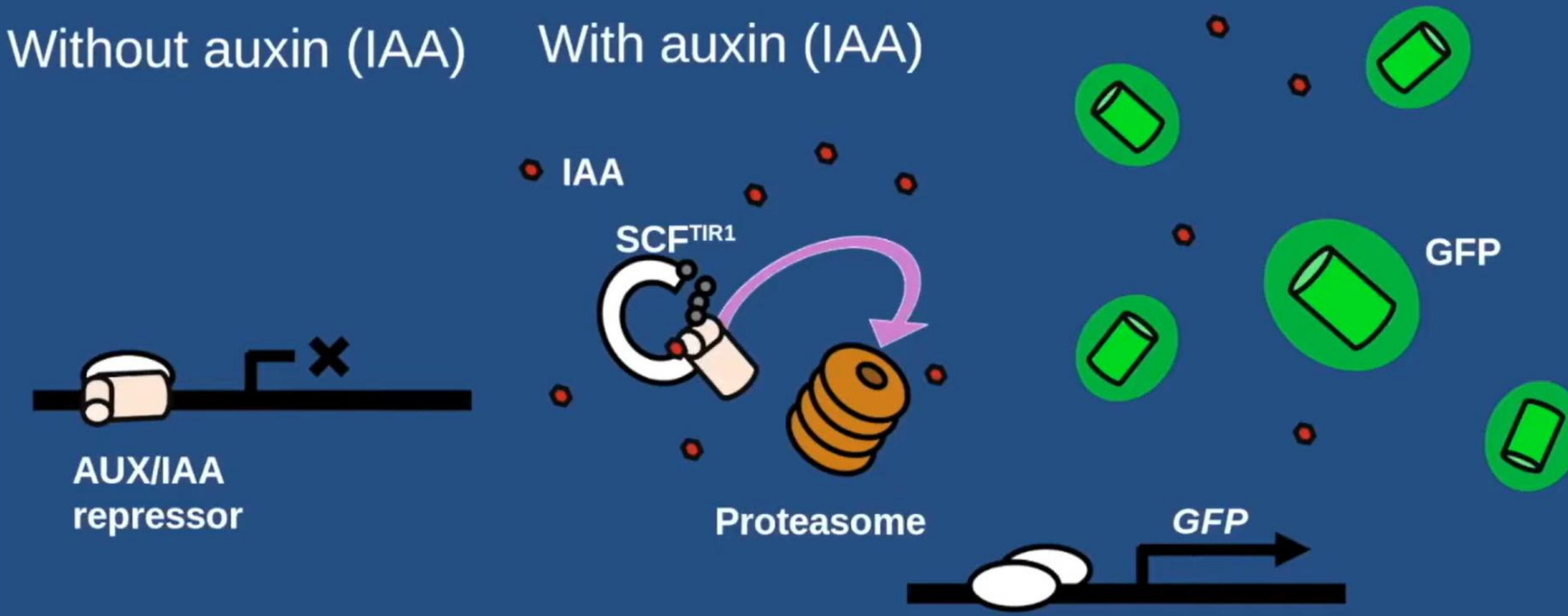
Intensiometri or Ratiometric

- Transcriptional reporters
- Degron reporters
- Direct intrinsic biosensors
- Direct extrinsic biosensors

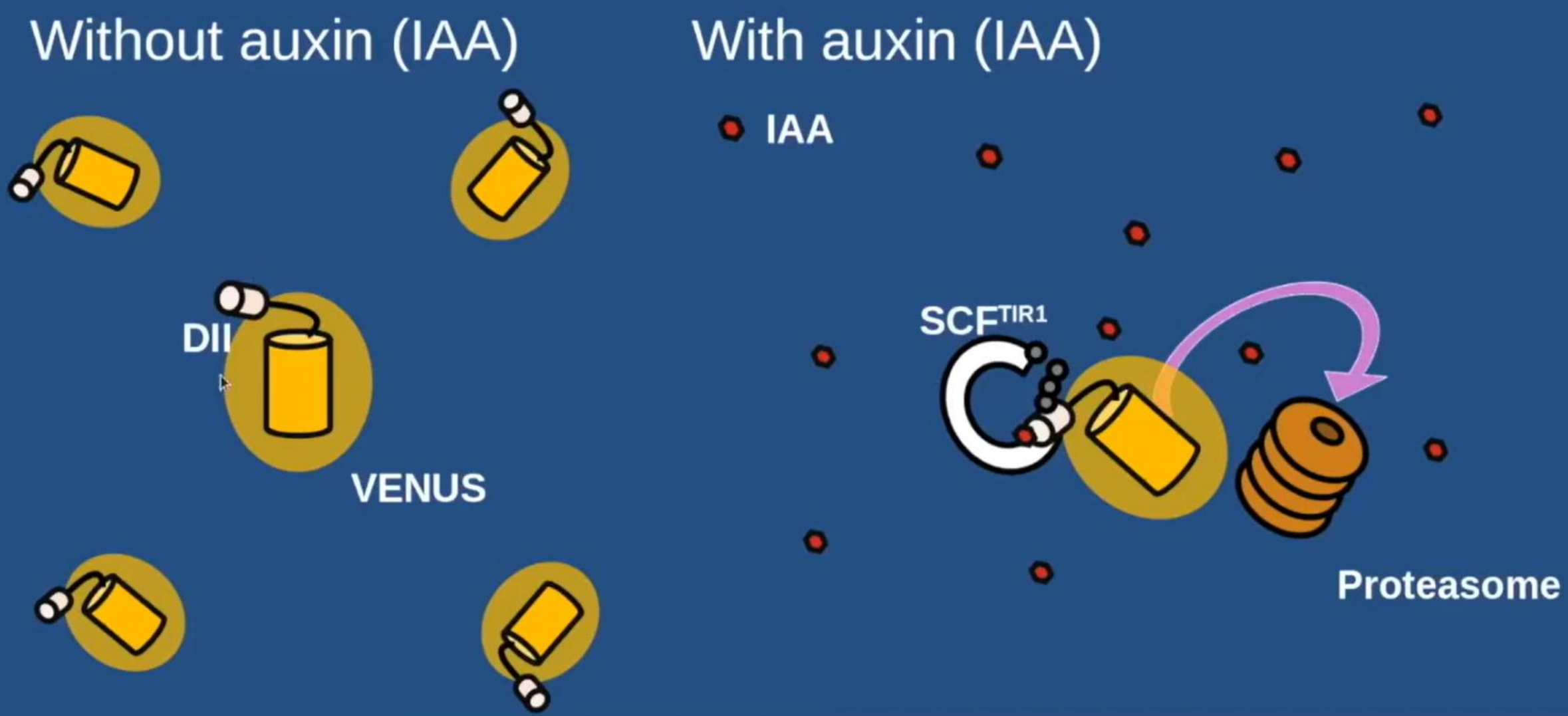


(Curaba *et al.*, 2014)

Transcriptional reporter e.g. DR5:GFP



Degron reporters e.g. DII-VENUS



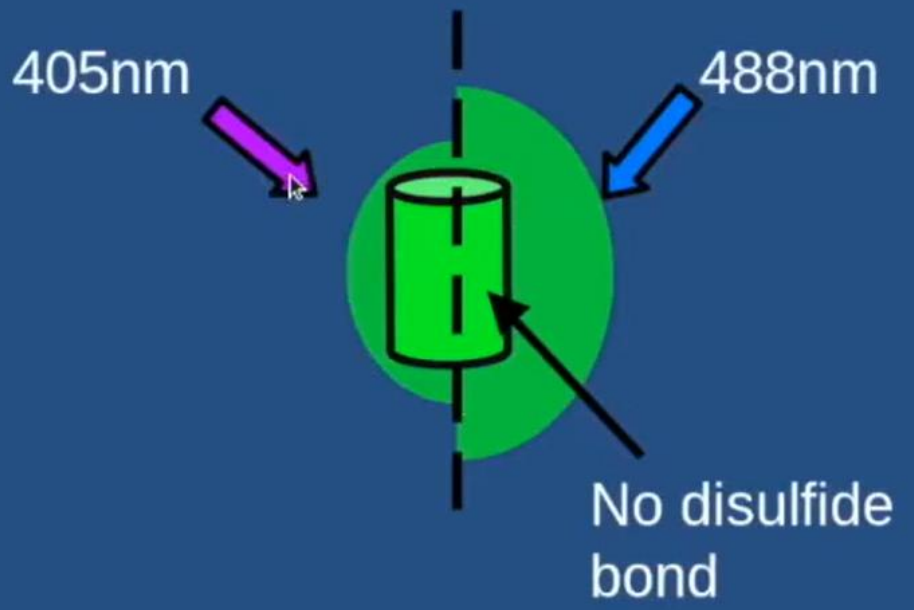
Direct biosensor benefits

- Fast responce to analyte
- Little to no requirement for endogeneous machinery
- Reversibility
- Often ratiometric

Direct intrinsic biosensors e.g. roGFP

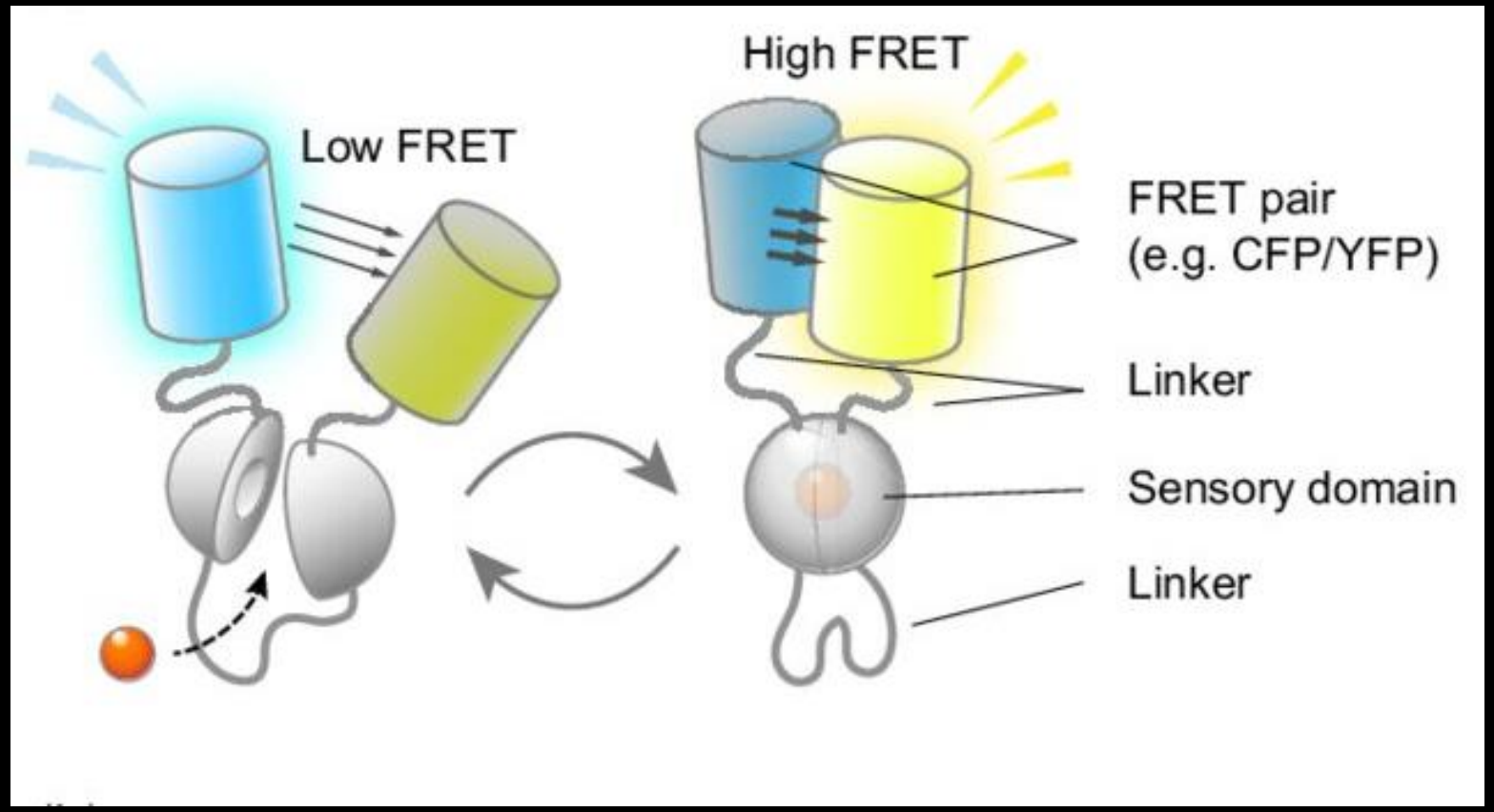
Oxidising environment

Reducing environment



Direct extrinsic Biosensor e.g. nlsGPS2

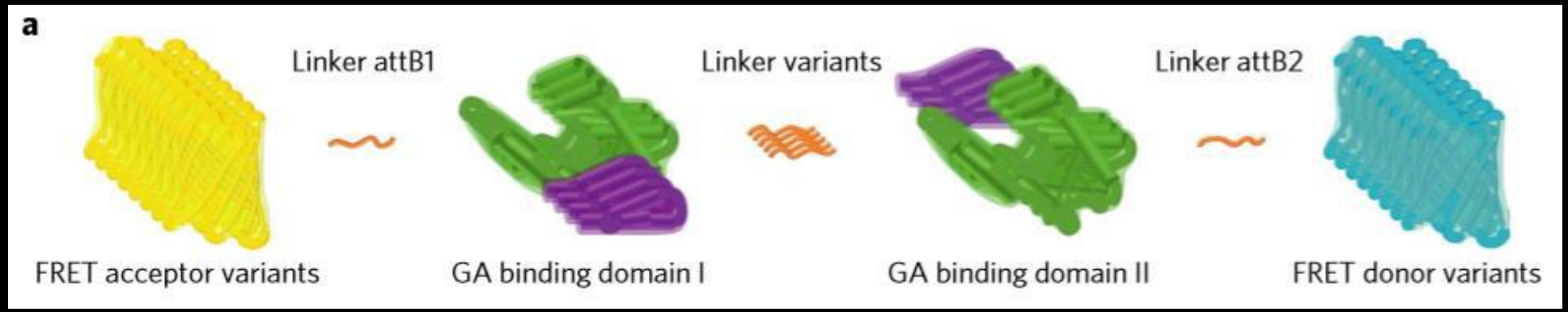
Sensory Domain: Detects changes, triggering FPs' proximity shift.
Linkers: Connect FPs to the sensory domain, allowing movement.
Low FRET: FPs are distant; low energy transfer indicates no event.
High FRET: FPs are close; high energy transfer signals an occurring event.



Gateway Cloning Strategy

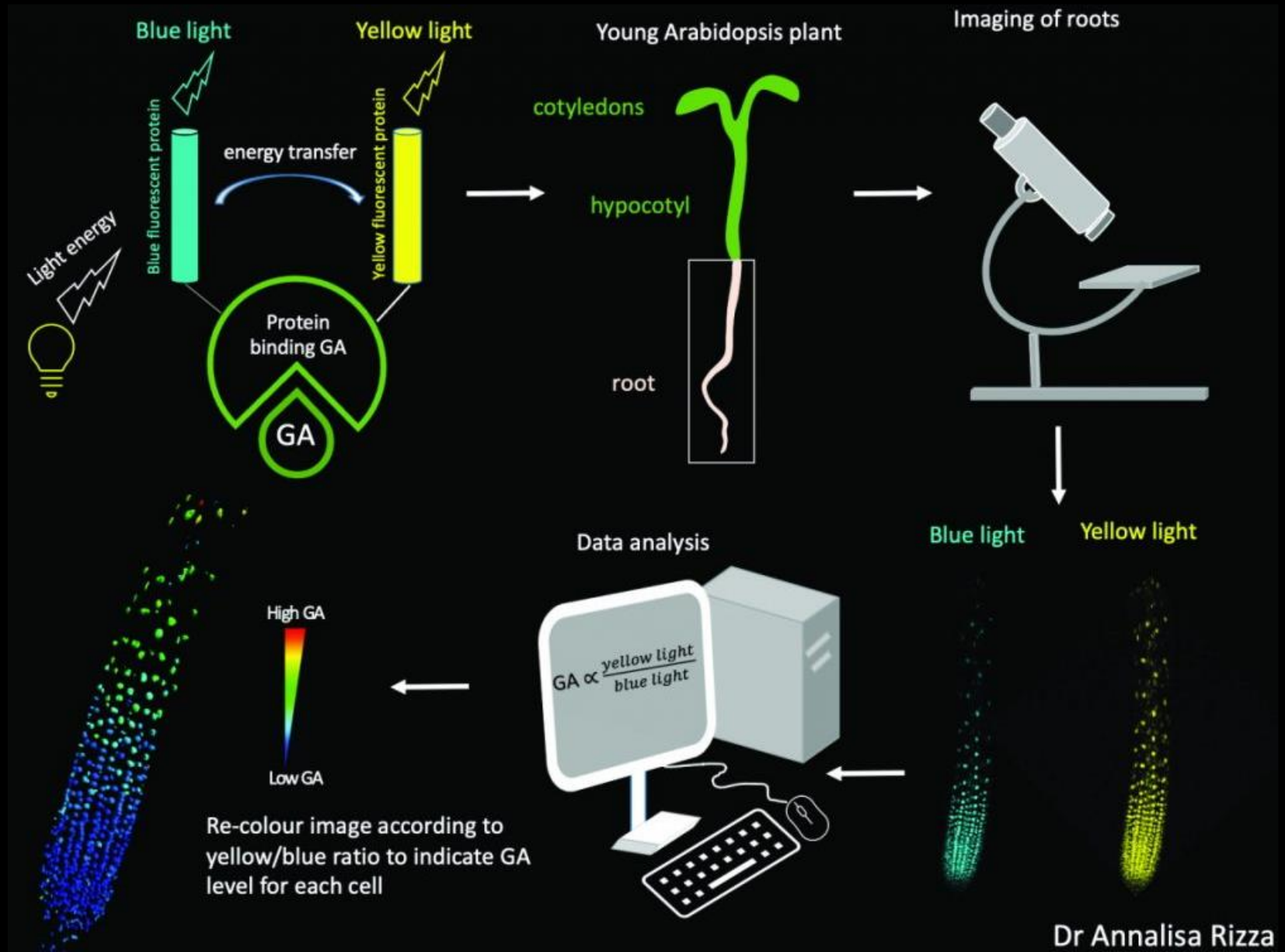
Entry clones: clones encoding potential GA-binding domains

Destination Vectors: vectors carrying genes coding for FRET pair variants



Potential binding domains (GA binding domain I and II): GA receptors, (GID1 A, B or C), linked through a linker of 12 amino acids (L12) at the N- or C-terminus of a truncated DELLA repressor, i.e. GAI or RGA. These potential candidates were recombined withdestination vectorsexpressing the FRET variants of YFP and CFP.

How does the nlsGPS-2 Biosensor work?

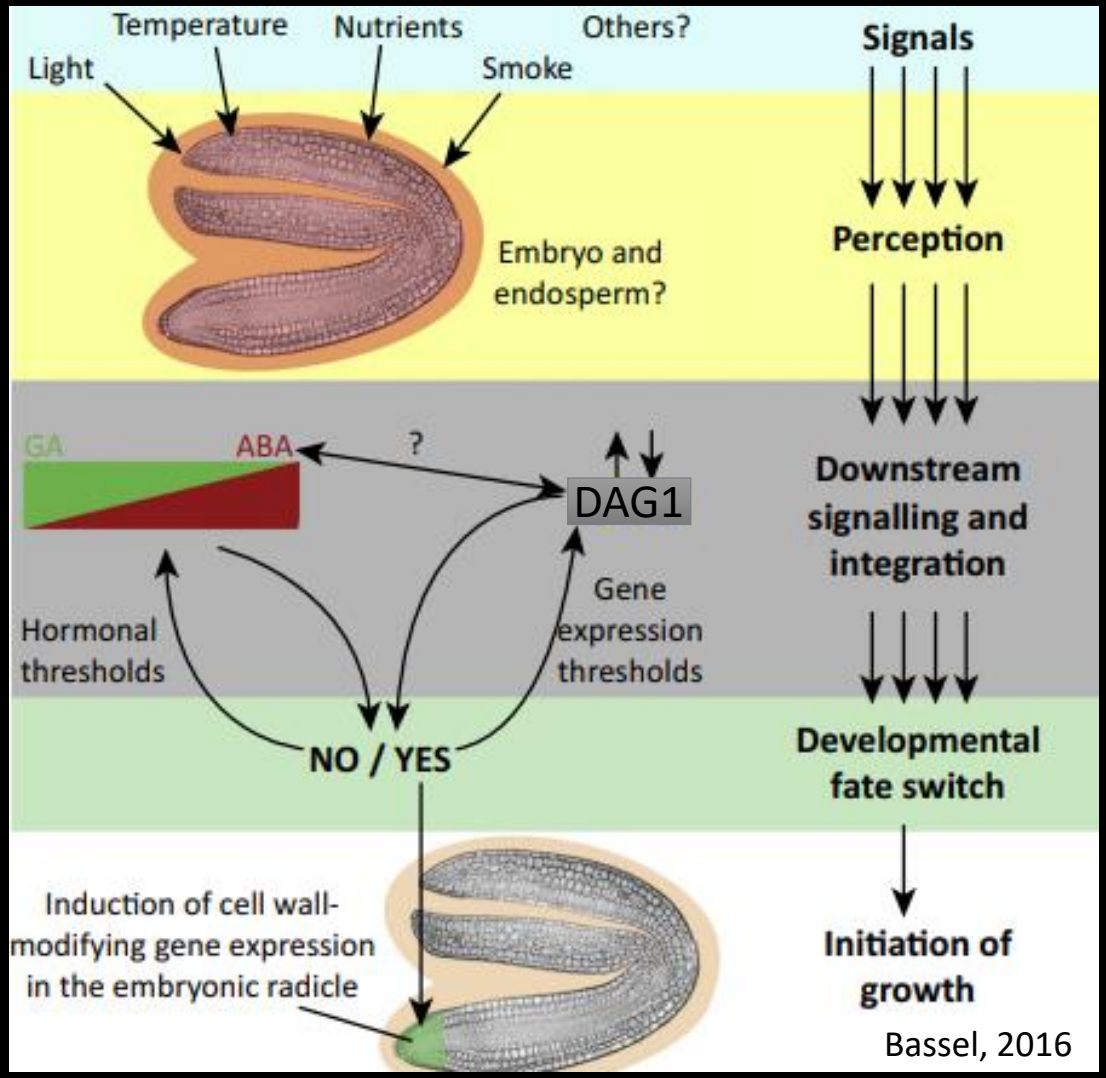


Why nlsGPS-2 ?

“The hormone behind seed germination”

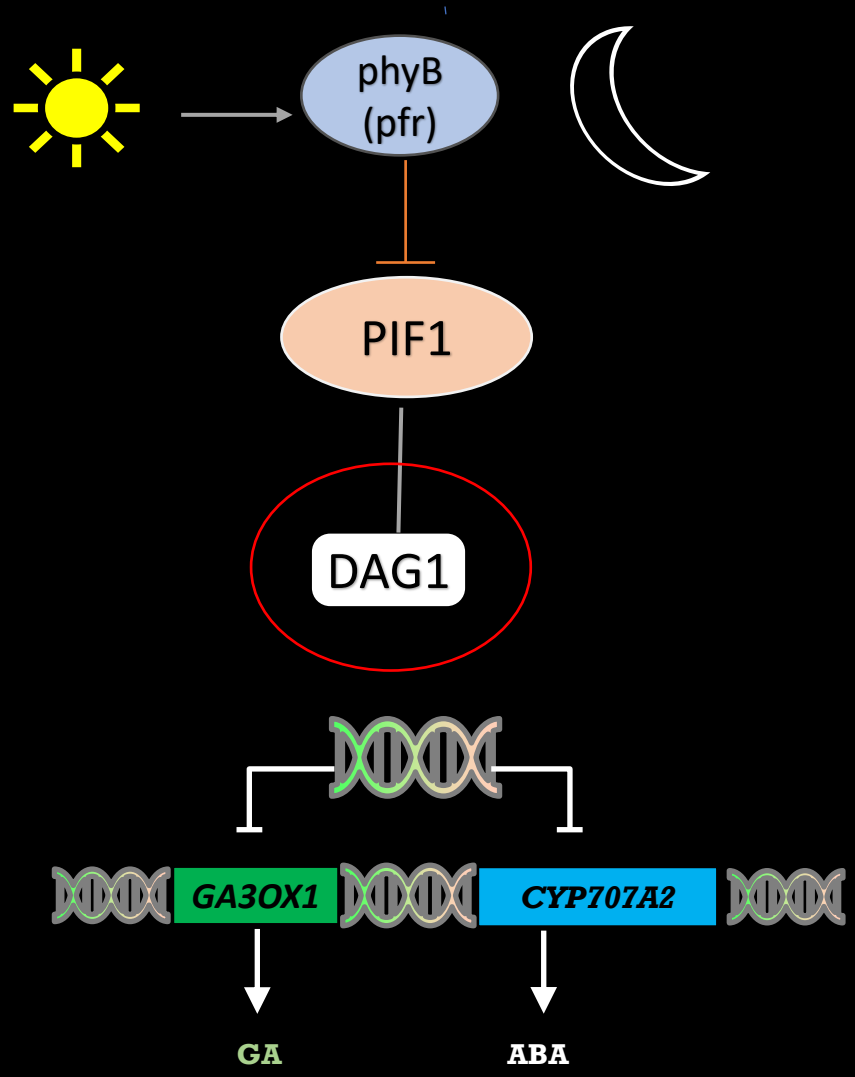
Plants coordinate their growth through hormones – chemical messengers that instruct cells to do certain activities

Let's investigate how one particular plant hormone, gibberellin, affects cells’ behaviour, and what this means for the crops of the future ?

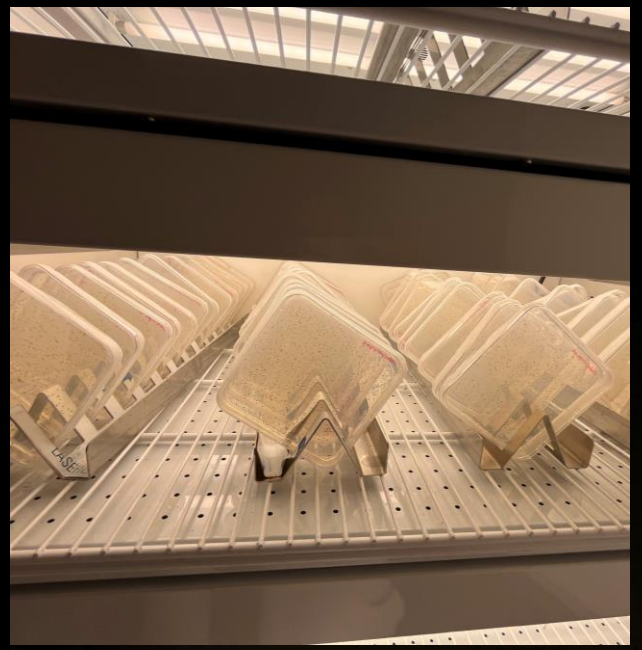


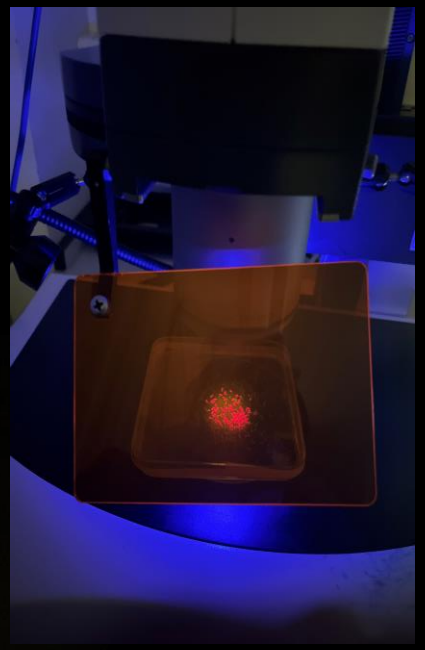
From the top down, multiple signals from the environment are perceived, initiating the process of downstream signalling and signal integration using both hormonal [abscisic acid/gibberellins (ABA/GA) balance] and gene expression (DAG1) thresholds. This integration process collectively acts upon a final irreversible developmental fate switch that, when flipped, initiates the process of embryo growth and growth promoting gene expression principally within the embryo radicle

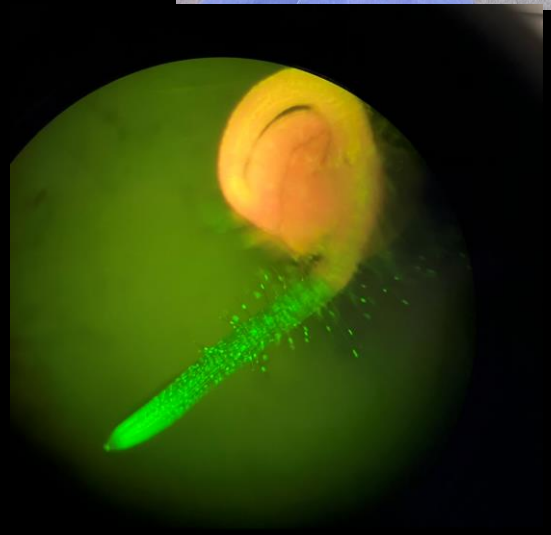
THE ROLE OF AtDAG1 IN THE LIGHT SIGNALING PATHWAY



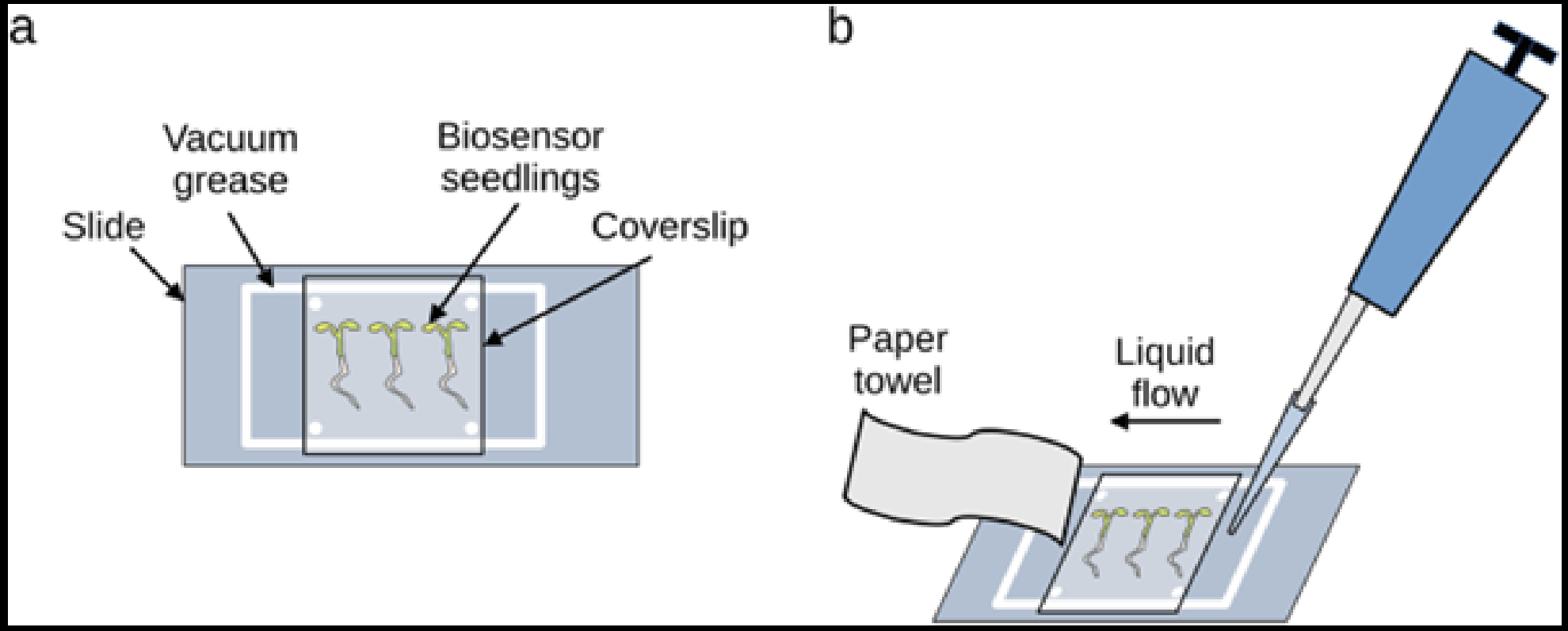
WS and *Atdag1* transformation with nlsGPS2 Biosensor







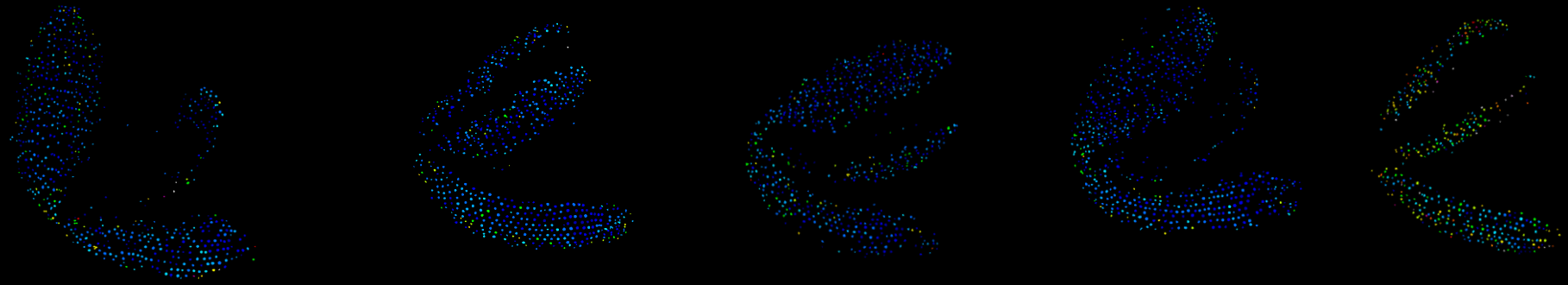
Sample preparation for confocal imaging



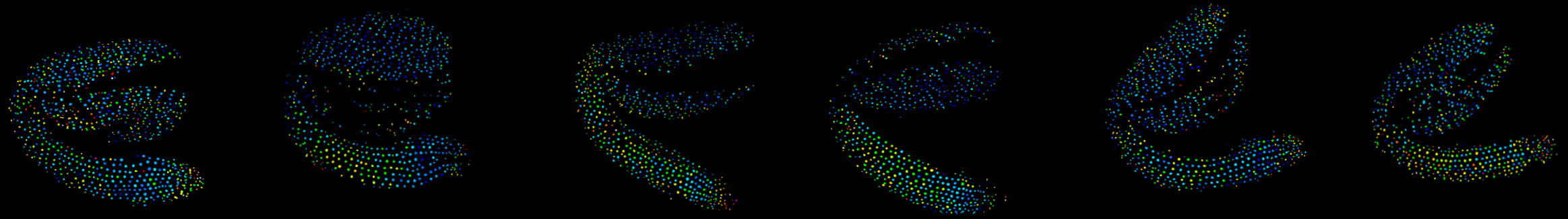
These panels show a schematic representation of the sample preparation for a steady-state experiment

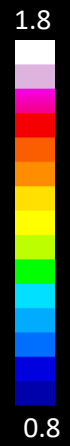
DAG1 mutation leads to increased GA emission ratio in *Arabidopsis*

WS nlsGPS1 – 3 days Stratification and 24 Hours Light

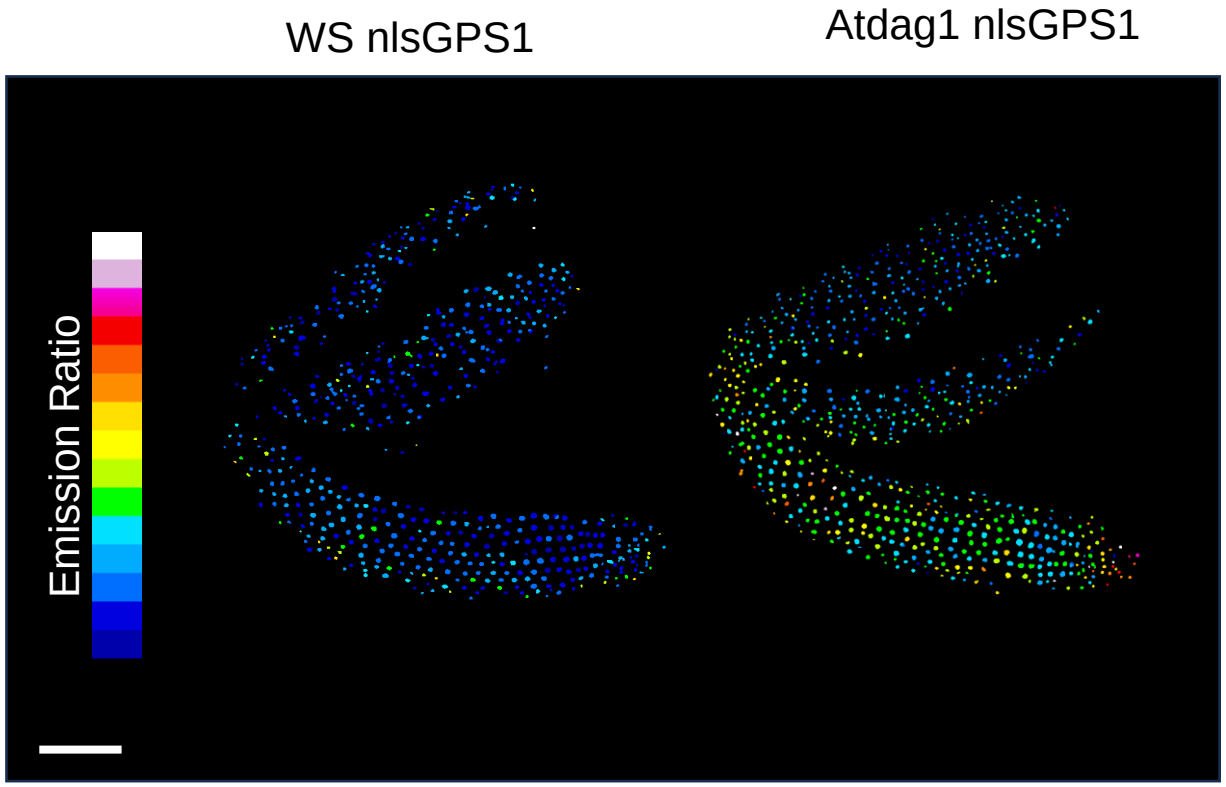


Atdag1 nlsGPS1 – 3 days Stratification and 24 Hours Light

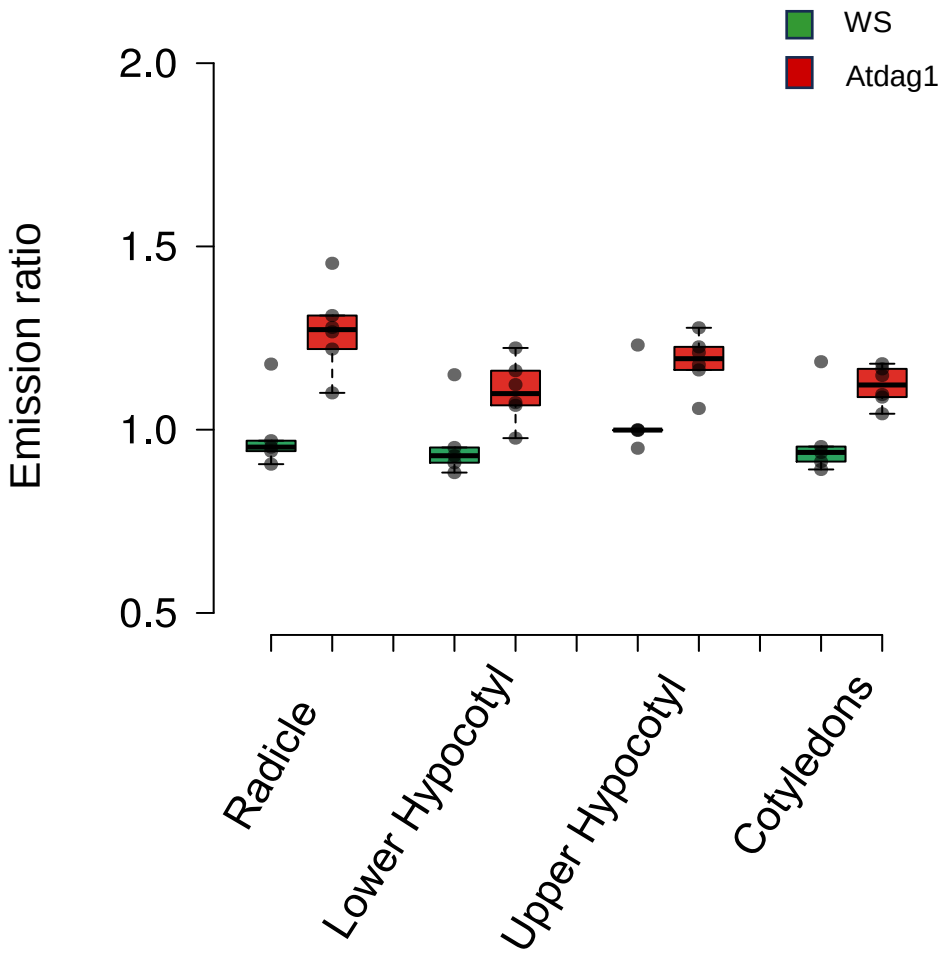




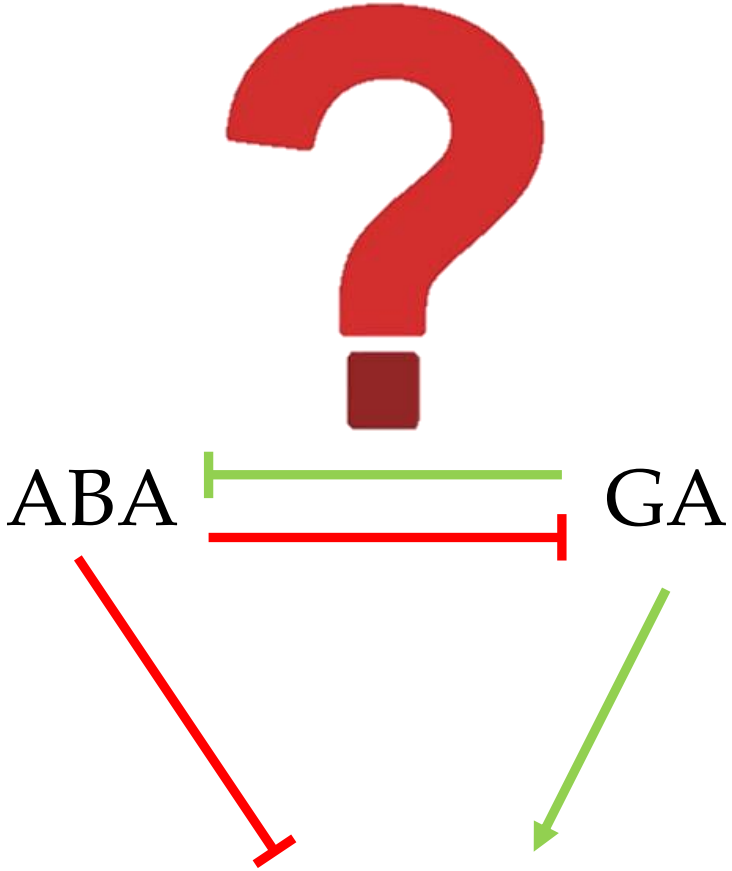
A



B

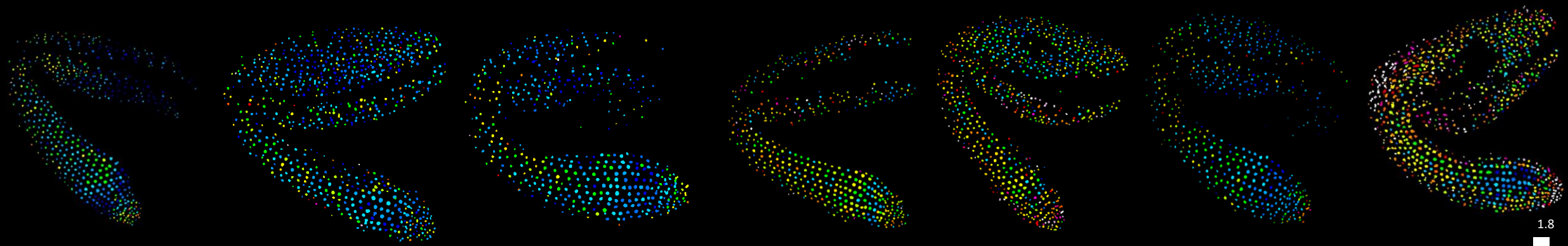


In the DAG1 mutant, where DAG1 repression is absent or reduced, we noticed higher GA levels all the different parts of embryo compared to the wild type, which correlates with increased germination rates.

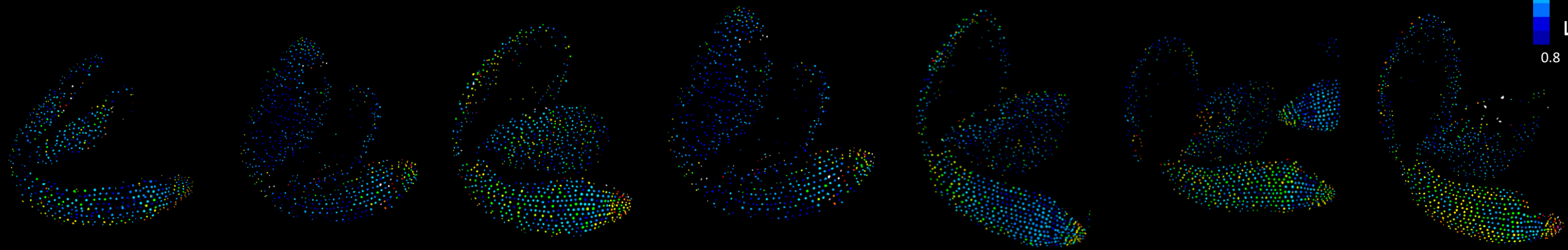


Interplay between gibberellins (GAs) and abscisic acid (ABA) is crucial for regulating plant processes, including seed germination. While GAs typically promote germination, ABA generally inhibits it. However, recent research has revealed a complex crosstalk between these two hormones, suggesting that their effects are not mutually exclusive.

Col-0 GPS1 Embryos 3 days Stratification, 24h Light

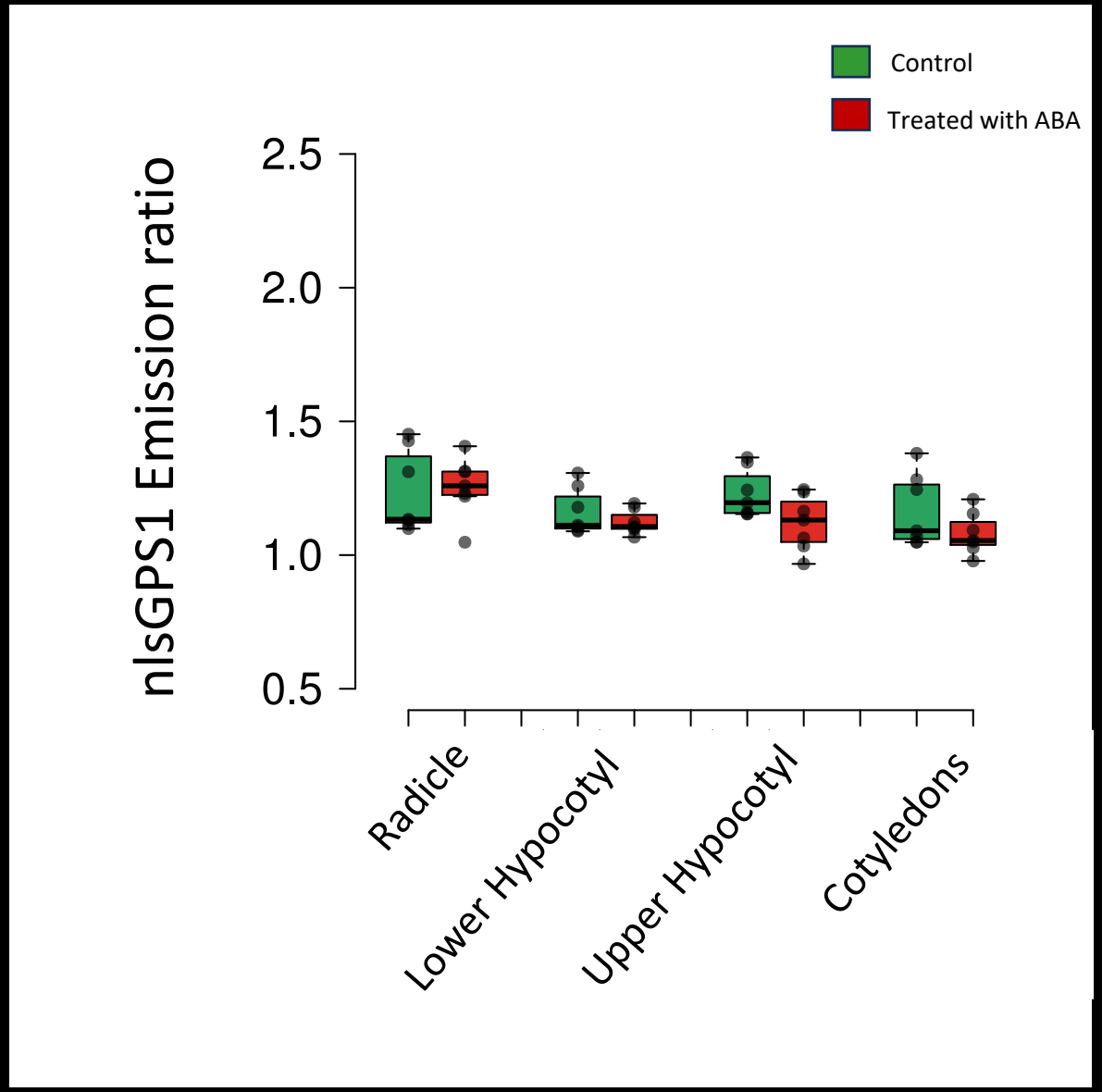


Col-0 GPS1 Embryos 3 days Stratification, 24h Light + ABA (2 µM)



Col- 0 GPS1 emission ratio in different parts of embryo before and after treatment with ABA

By treating seeds with 10 micromolar GA, I observed a slight reduction in ABA levels. This finding aligns with the established role of GAs in inducing the degradation of ABA, thereby creating a favorable environment for germination.

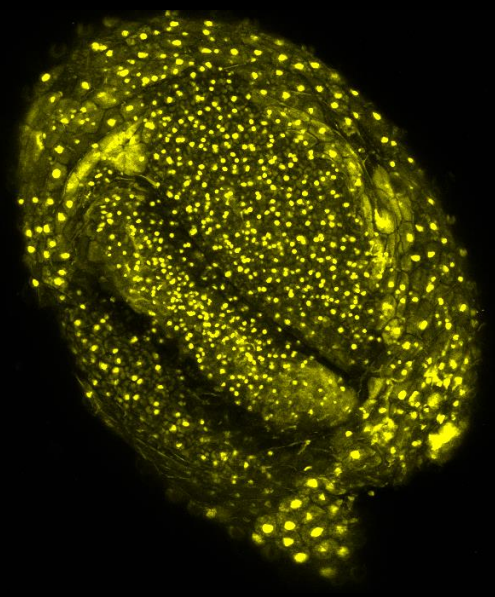


Exploring Cellular Signals in Endosperm: Understanding True Signals

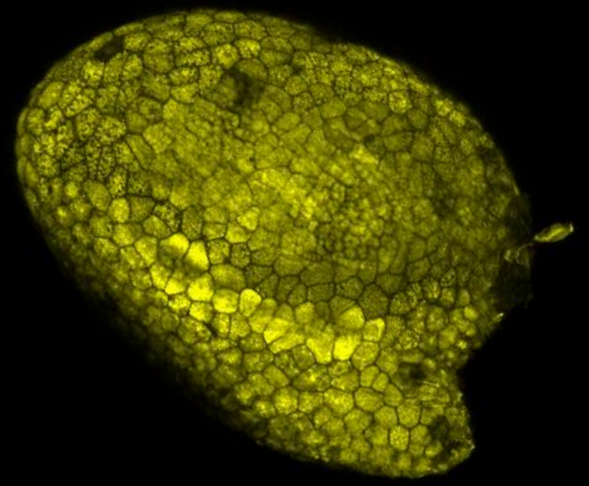
Analyzed signals in endosperm of Col-0 seeds with sensor.
Compared against endosperm of seed without sensor.

Findings:

- Observed potential signals in the endosperm of seeds transformed with Biosensor
- No signals detected in seeds without sensor, suggesting observed signal in first sample likely due to true signal.

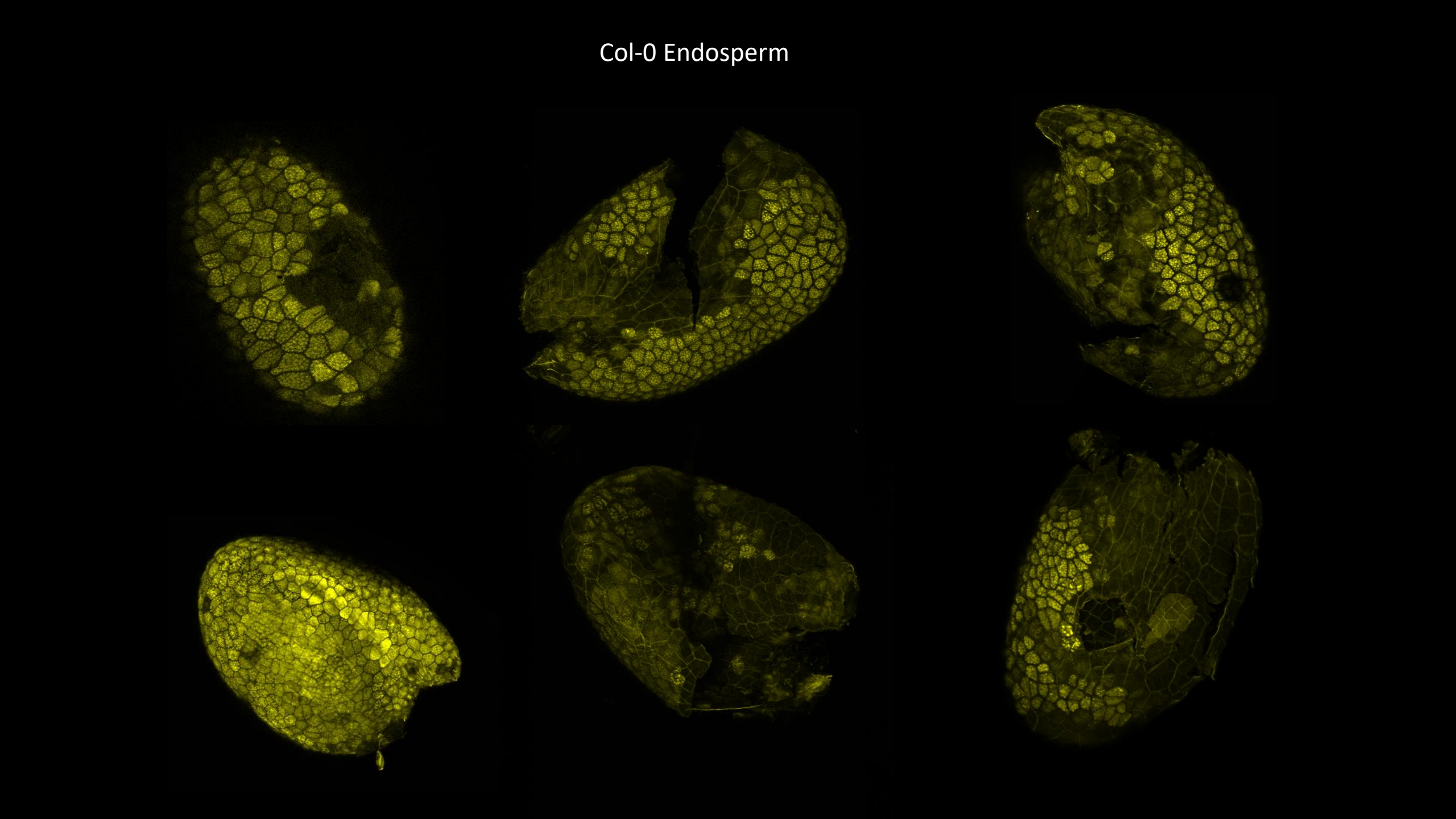


Endosperm with biosensor



Col-0 Endosperm without biosensor

Col-0 Endosperm



FINDINGS AND APPLICATIONS

With a biosensor, you can see something nobody has ever seen before. For example, Jones group found gradients of GA within growing roots and shoots and now we are looking at this gradient in seeds, to understand why these gradients are important for the plant.

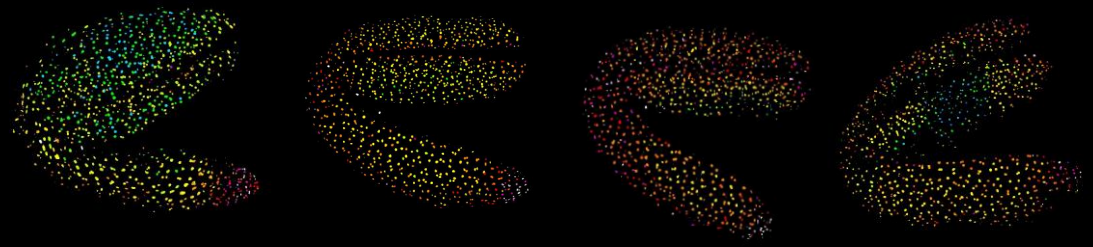
Modifications to GA levels in plants have already led to huge benefits for society. In the 1950s and 60s, concerns about possible mass starvations led to scientists finding new techniques to boost agricultural productivity – an effort known as the Green Revolution. One key way they achieved this was through breeding crops to be shorter, so they would put more of their energy towards growing edible grains that were less likely to be damaged by wind. This process involved artificially selecting plants that either made less GA or responded abnormally to GA, so the hormone did not lead to the normal growth effect.

GA manipulations are already wildly successful in agriculture, but there are sometimes unwanted side-effects. For instance, one study saw scientists genetically modify rice plants to express lower GA, but found that although modified plants were shorter, they also did not develop rice grains correctly, since GA is linked to both shoot elongation and reproductive development.

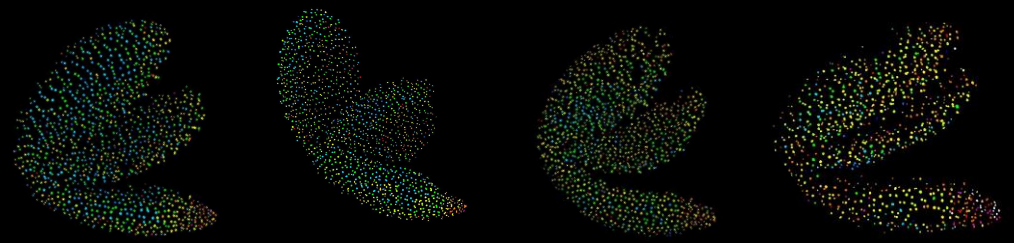
We need a deeper understanding of how GA works so we can finetune these modifications to work for us.

Col- 0 ABACUS 400n emission ratio in different parts of embryo

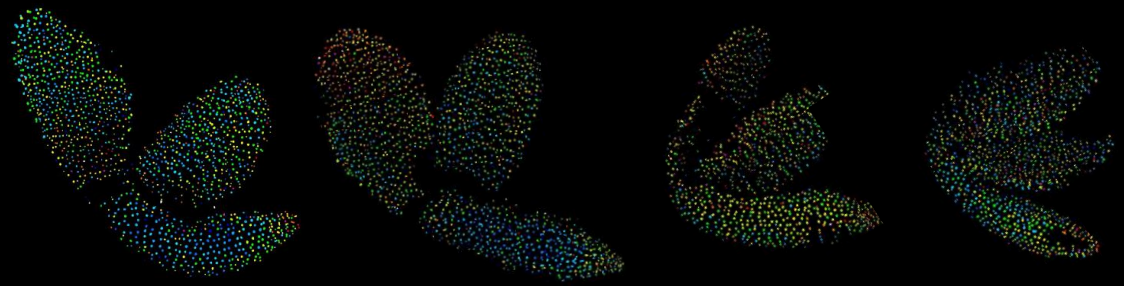
Dry seeds



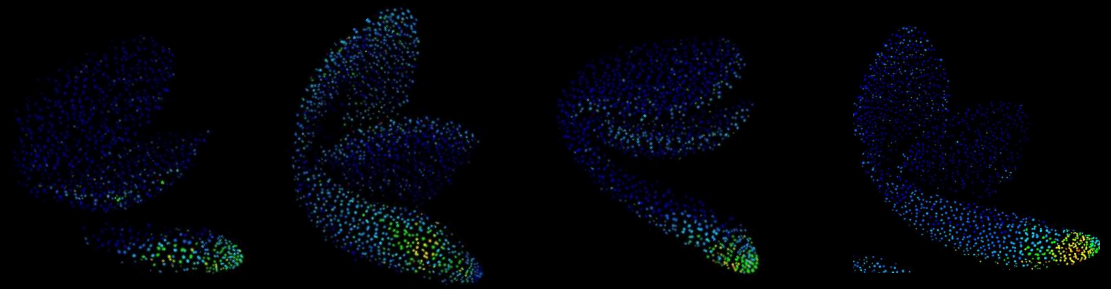
Overnight stratification



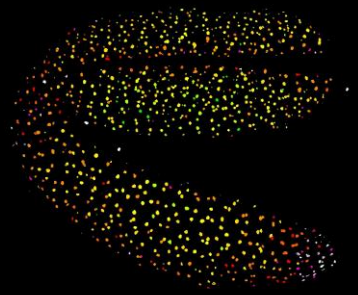
3 Days Stratification

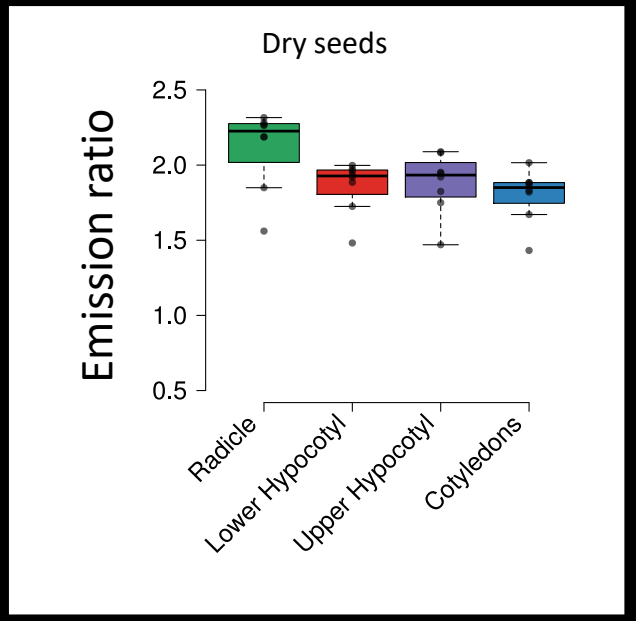


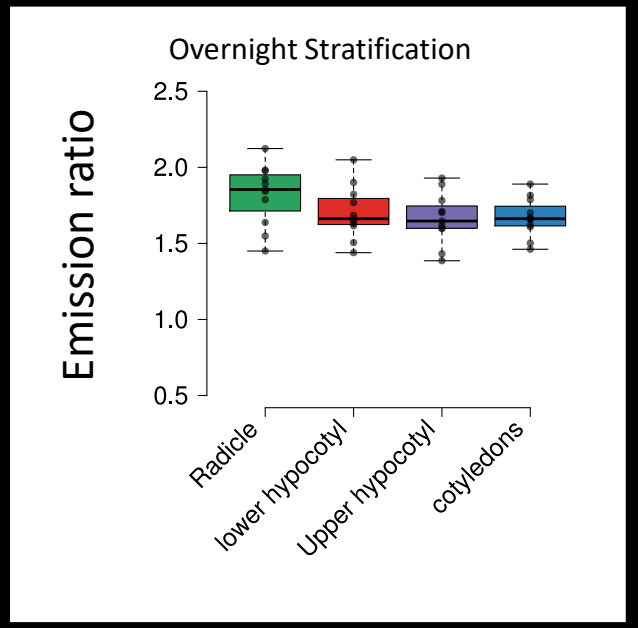
3 Days Stratification – 24h Light

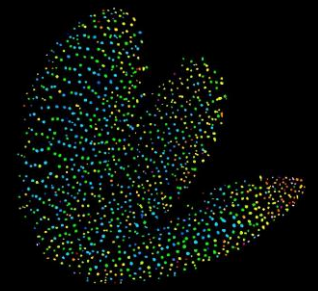


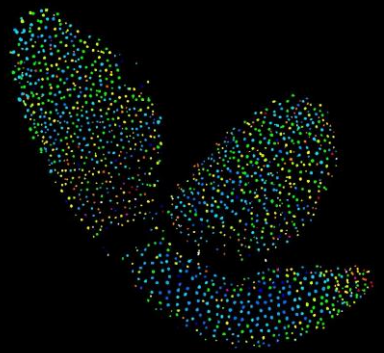
Col- 0 ABACUS 400n emission ratio in different parts of embryo

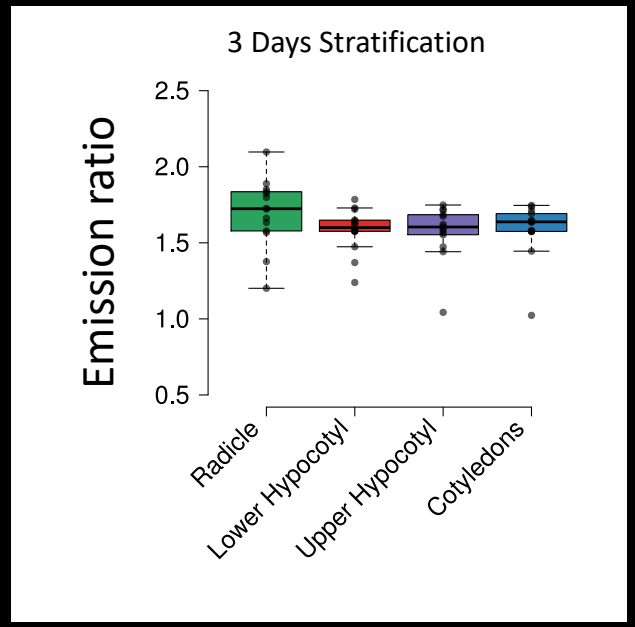


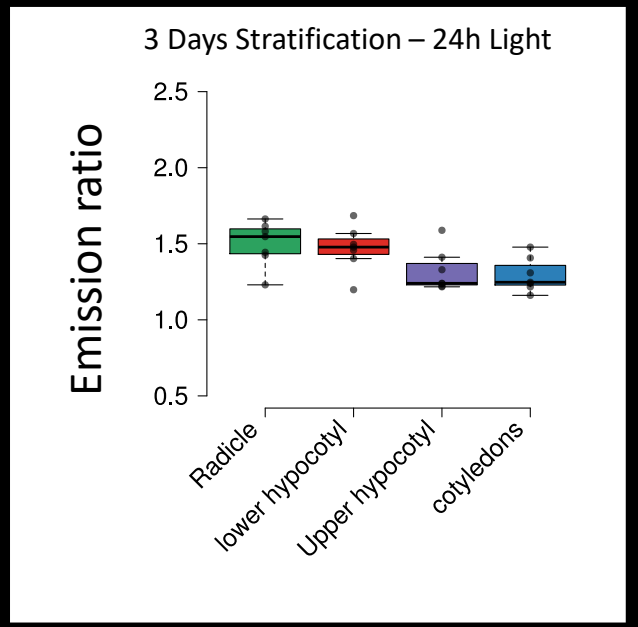


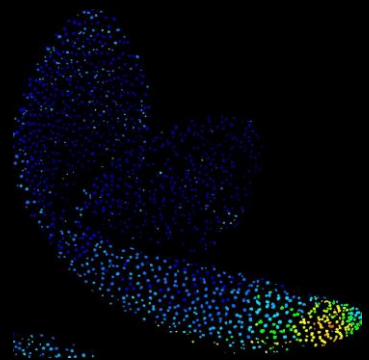


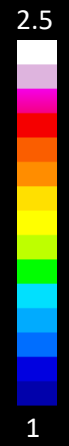




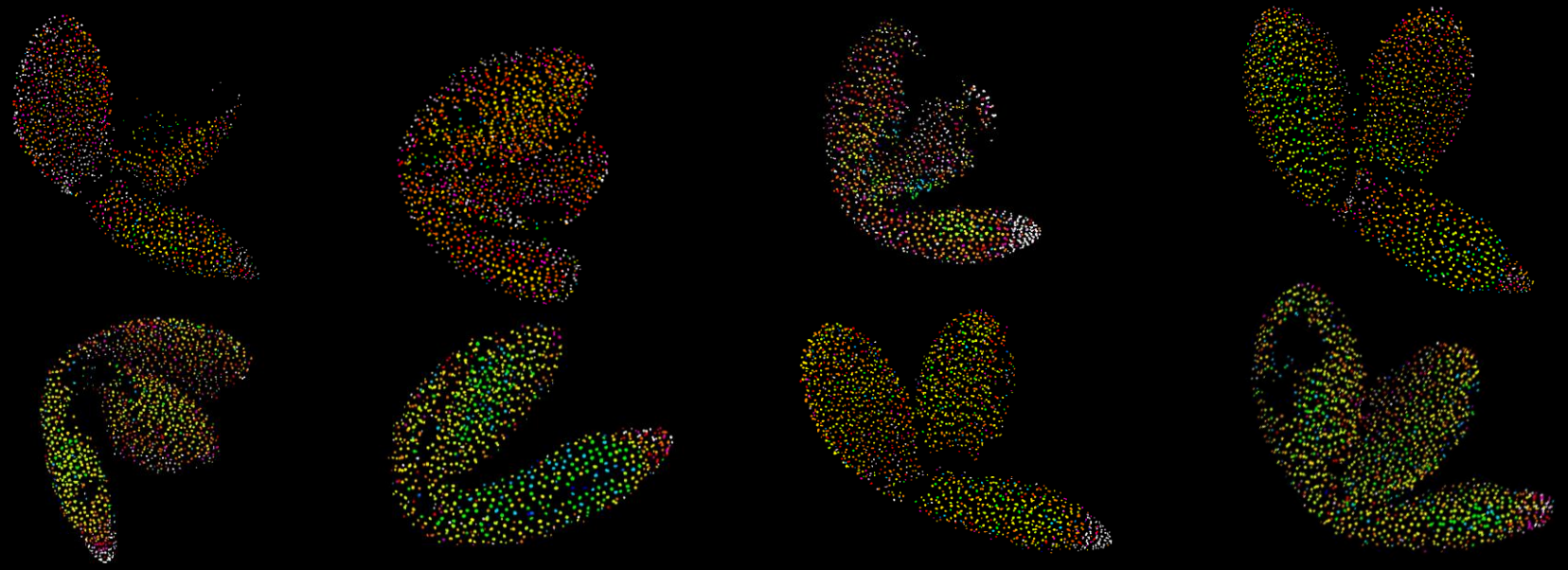


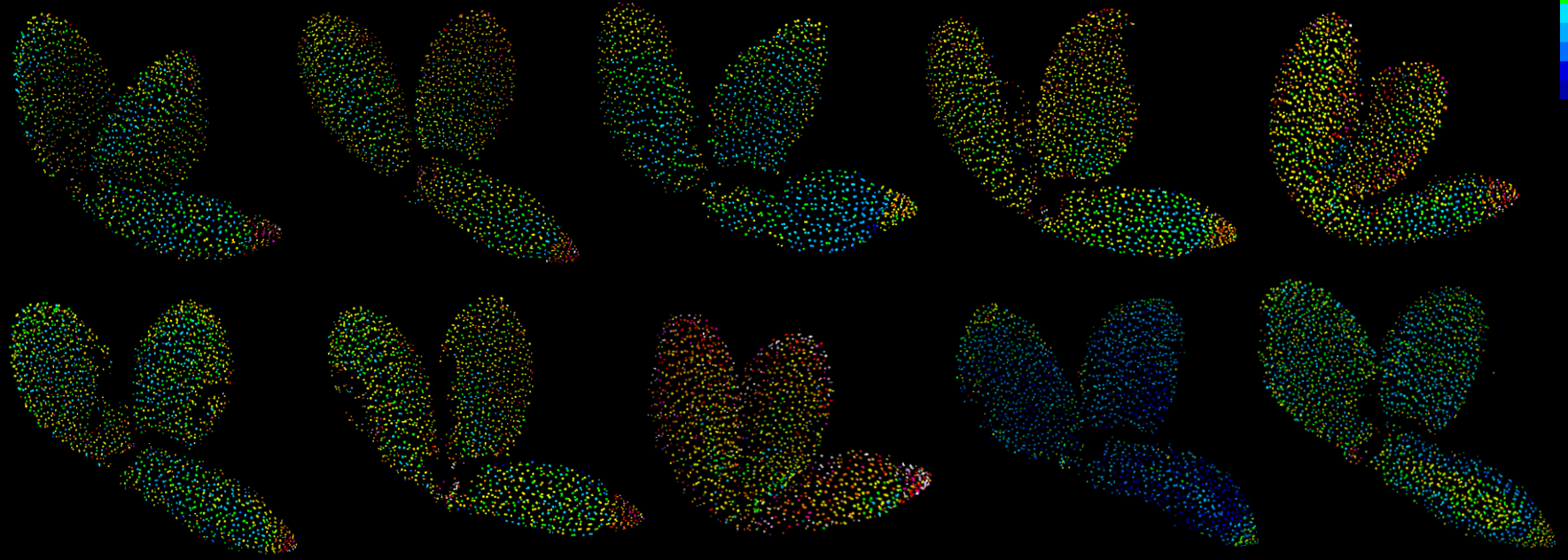




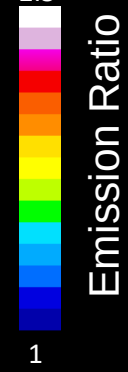


Col-0 ABACUS 400n Embryos
Overnight Stratification

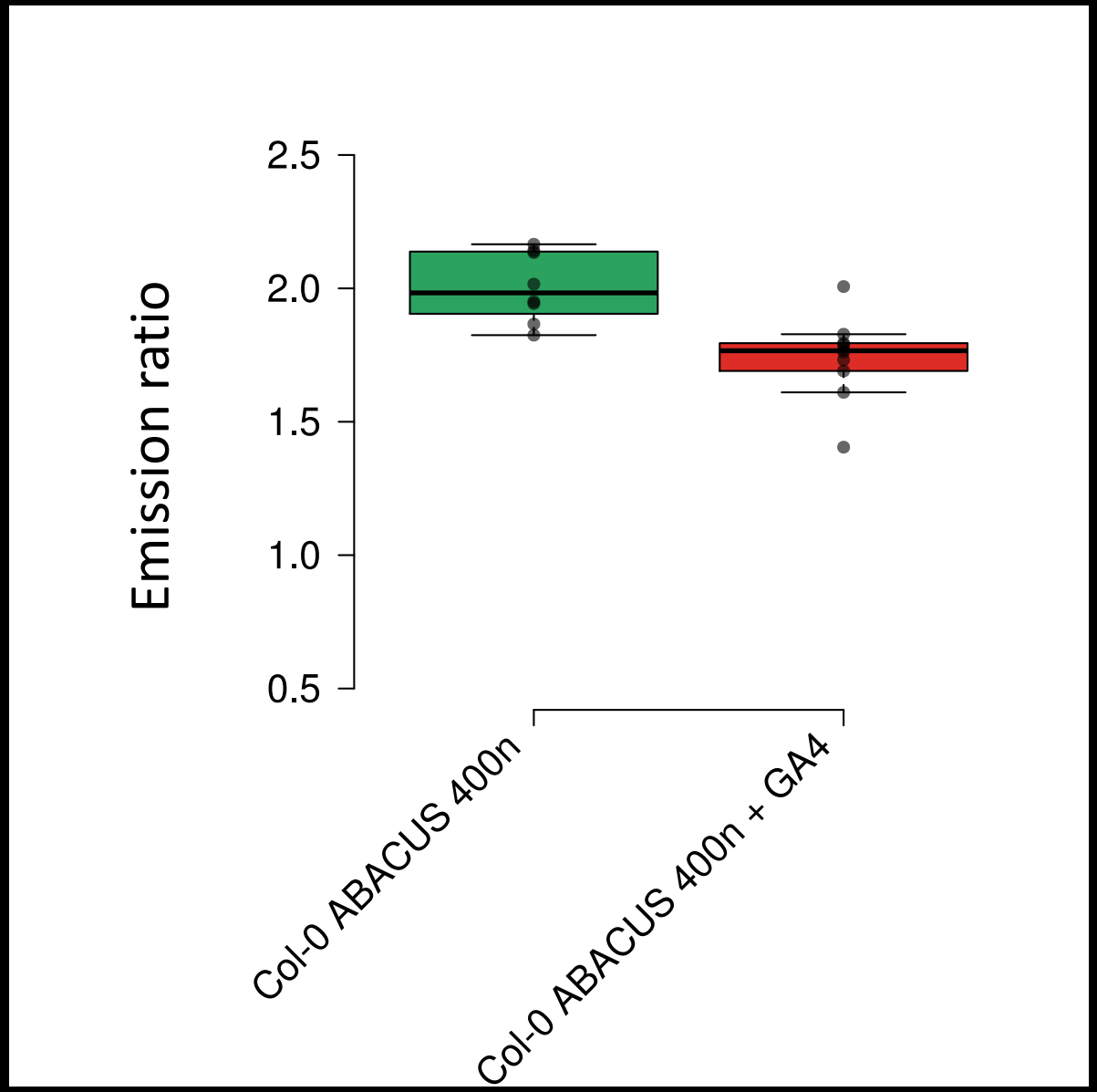


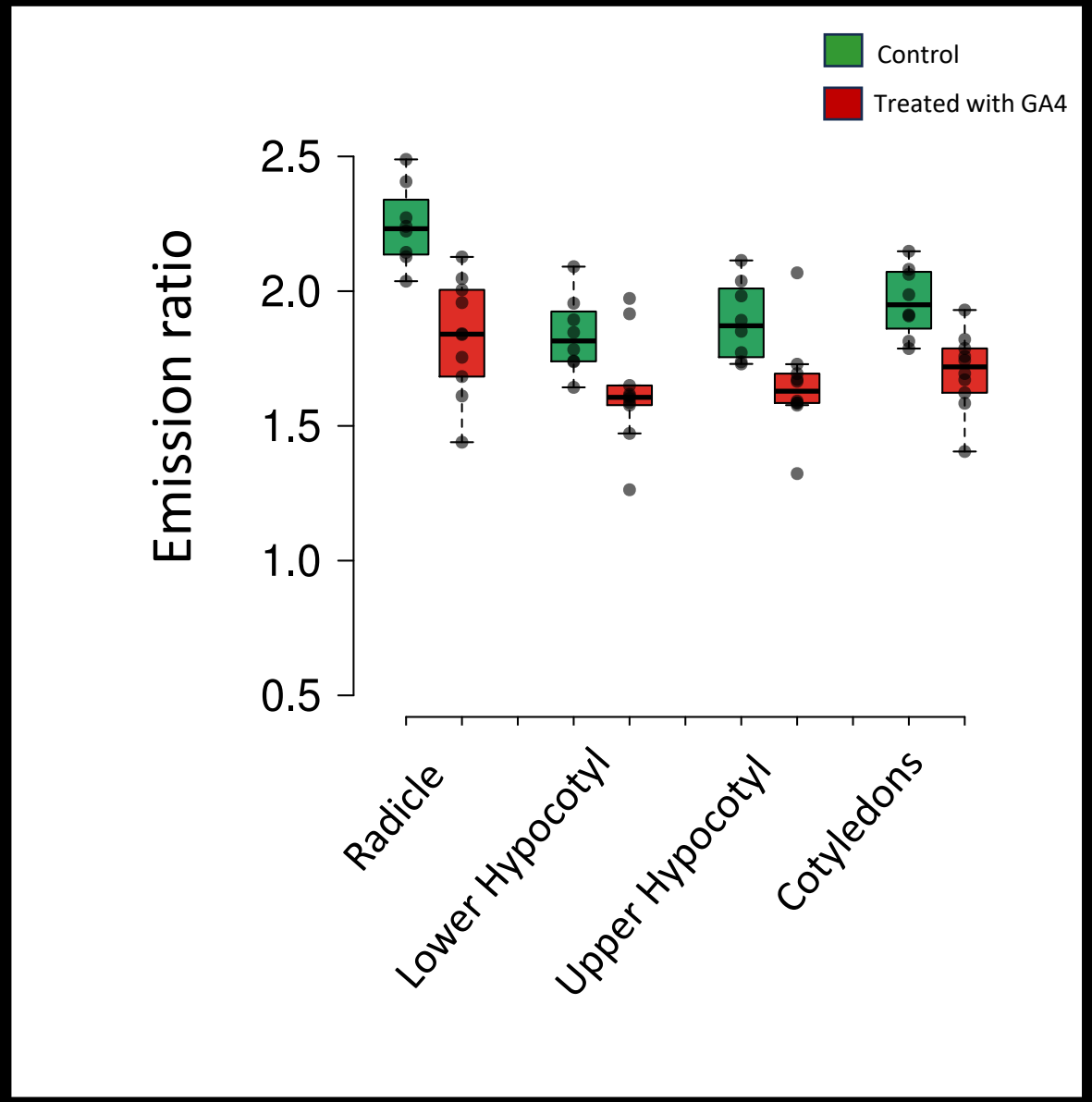


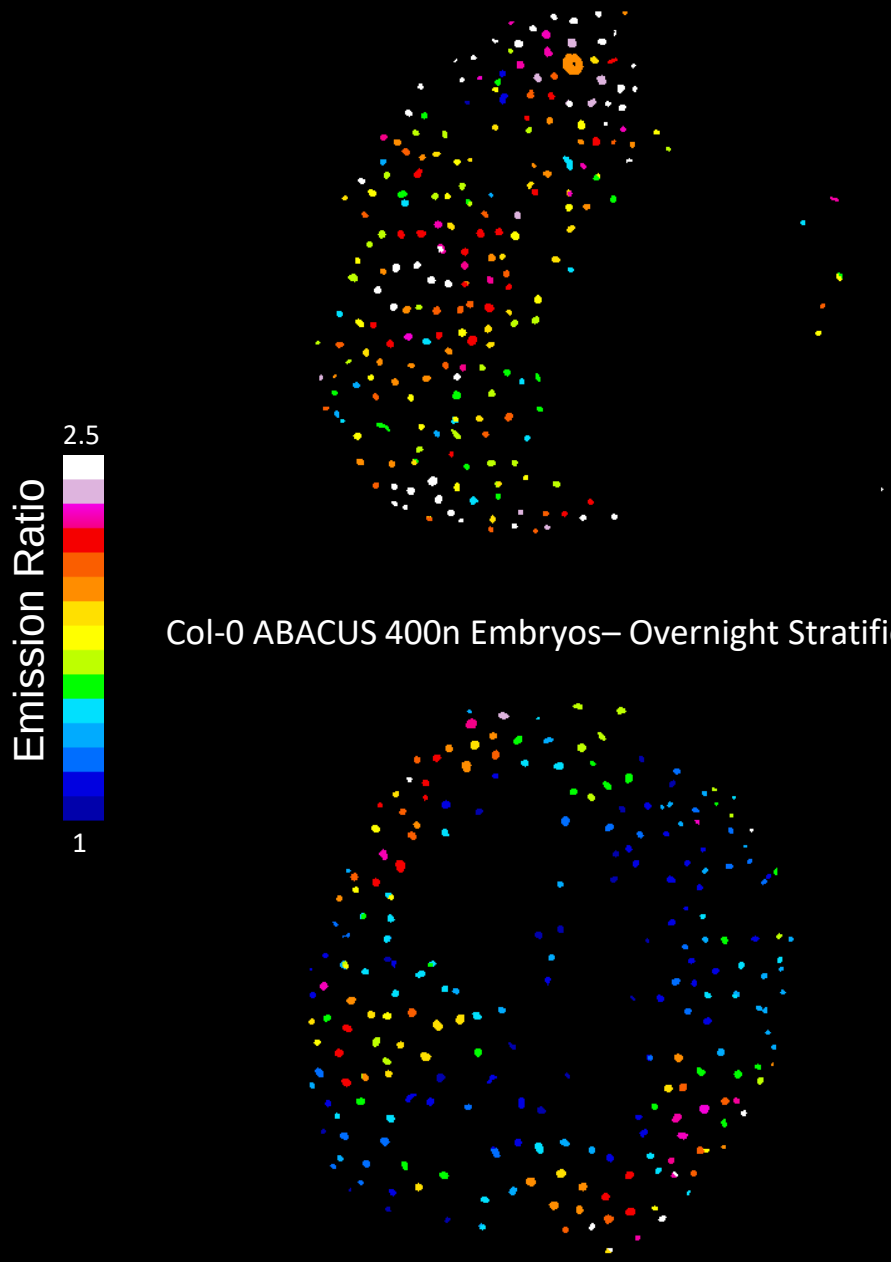
Col-0 ABACUS 400n Embryos
Overnight Stratification
+ GA4 (10 µM)



Col- 0 ABACUS 400n emission ratio in different parts of embryo before and after treatment







Col-0 ABACUS 400n Embryos– Overnight Stratification

Col-0 ABACUS 400n Embryos– Overnight Stratification + GA4 (10 µM)