TIC TOC: Targeting Improved Cotton Through Orbital Cultivation

ISS National Laboratory

CENTER FOR THE ADVANCEMENT OF SCIENCE IN SPACE

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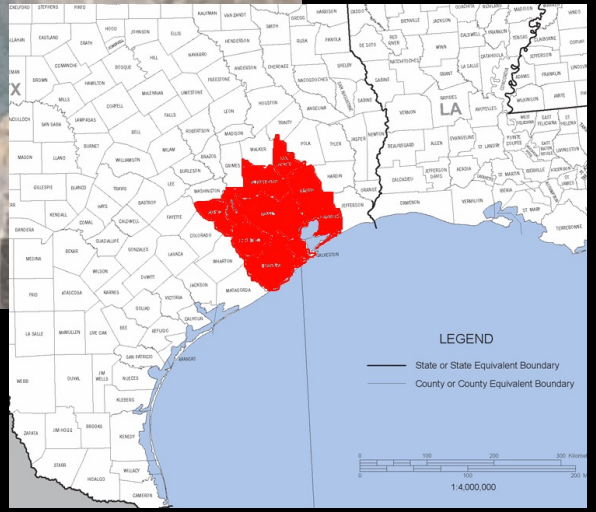
Lucas Bauer
Sarah Swanson
Simon Gilroy

& all the awesome folk at KSC
Astronauts: Shane Kimbrough, Mark Vande Hei

Cotton is a thirsty crop.
1kg of cotton uses 10,000 liters of water

Greater Houston Area

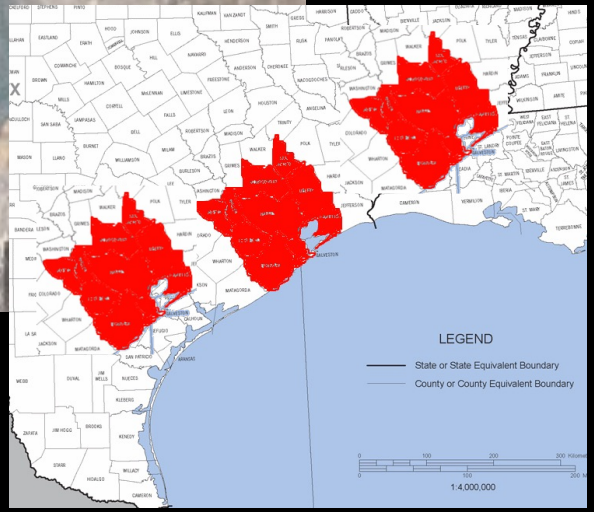


Known environmental impact

Cotton field irrigation caused the decline of the Aral sea (1993-present).

Cotton is a thirsty crop.
1kg of cotton uses 10,000 liters of water

3 x Houston = 1 Aral sea



Known environmental impact

Cotton field irrigation caused the decline of the Aral sea (1993-present).

So how can we adjust plant biology to reduce this environment impact?

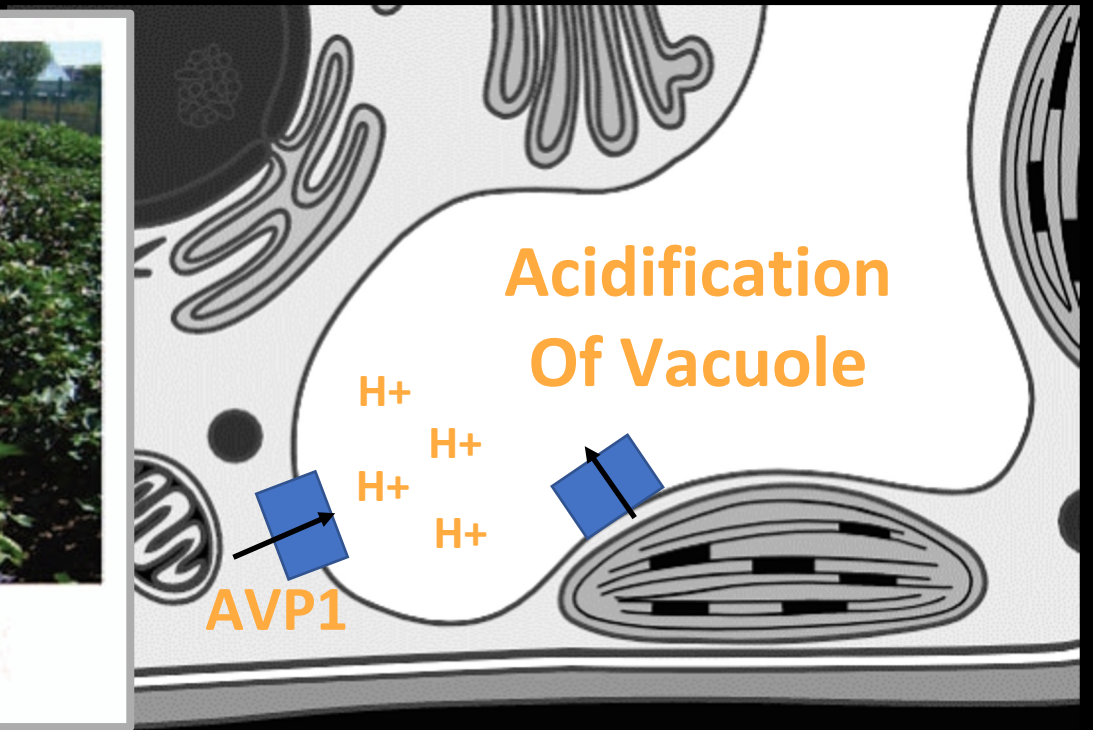
Auxin can cause proton movement.
Allowing shoots and roots to change their morphology.

GMO Cotton plant that is drought and salt resistance

Zhange *et al*., 2011

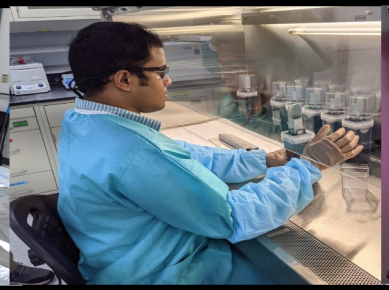
Arabidopsis Vacuolar Pyrophosphatase Over Expression
AVP1-OX -> A68 & D130

Zhange *et al*., 2011



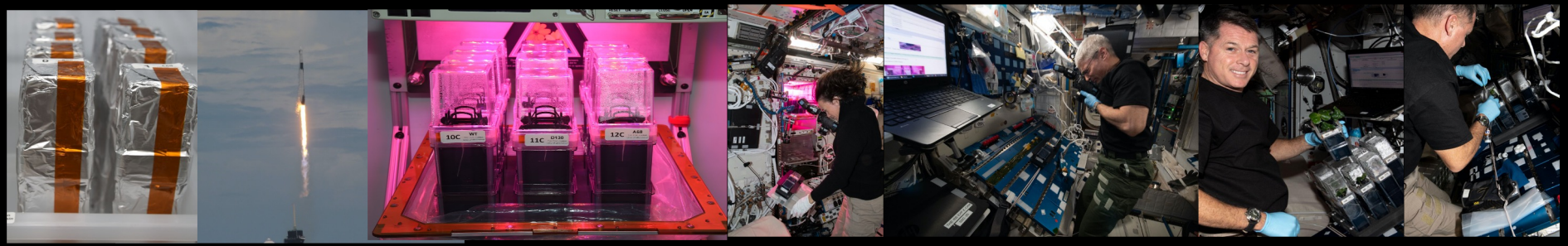
Cellwall / Apoplast

Hypothesis: AVP-over expressing plants could be resistant to spaceflight



Hypothesis: AVP-over expressing plants could be resistant to spaceflight?

WT and AVP1-ox lines (A68 & D130) grew on the ISS and photographed by astronauts.



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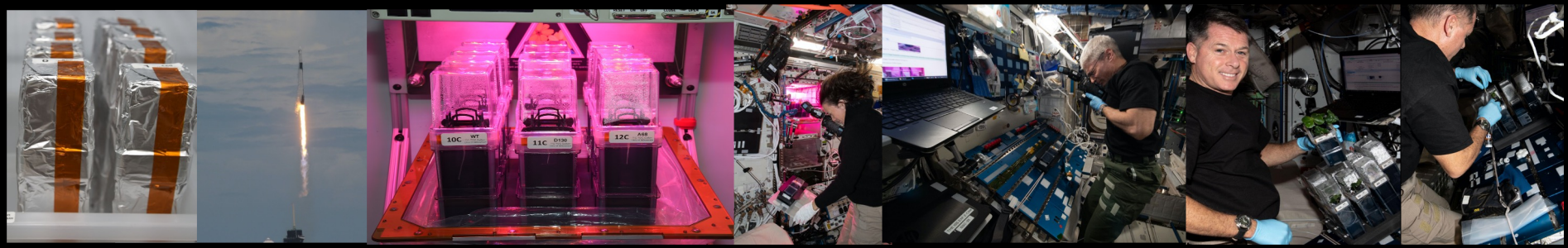
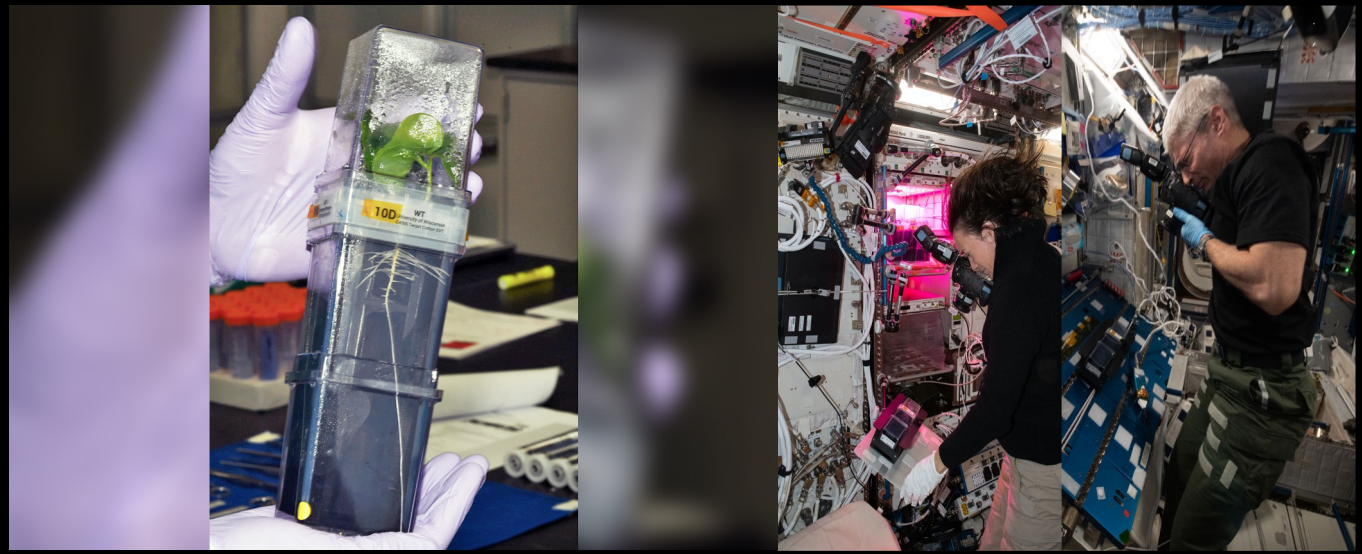


Samples were frozen and brought back to Earth for processing by Dr Arko Bakshi

Image analysis pipeline -> No spectral analysis possible

Original Image

- Rename geno_age_treatment
- RGB Color
- Tiif
- ~5-12Mb



Note: Variation in the ambient light environment

Image analysis pipeline - > Morphological normalization

Original Image

- Rename geno_age_treatment
- RGB Color
- Tiif/JPG
- ~5Mb

Adobe bridge rename

- Image raw conversion
- -100% saturation (Grey scale)
- +100% clarity (sharpens edges)
- Crop (reduce size)
- Align parallel lines

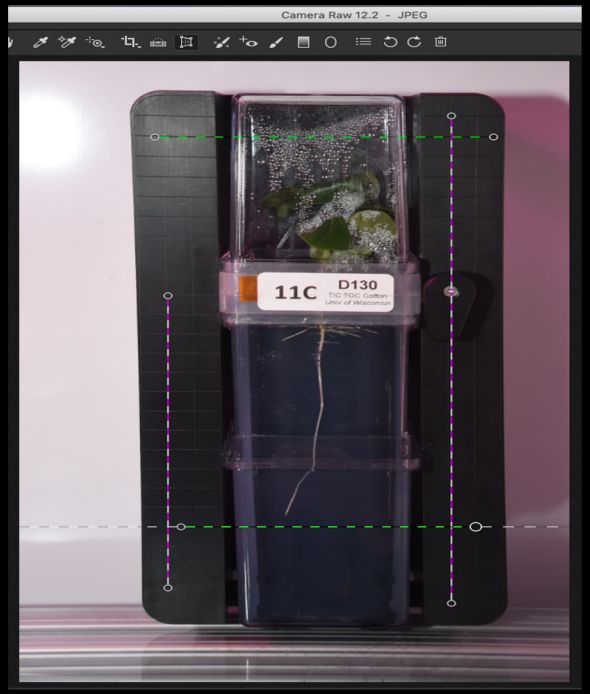


Image analysis pipeline -> Semi-automated root system detection

Original Image

- Rename geno_age_treatment
- RGB Color
- Tiif/JPG
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Adobe bridge rename

- Image raw conversion
- -100% saturation (Grey scale)
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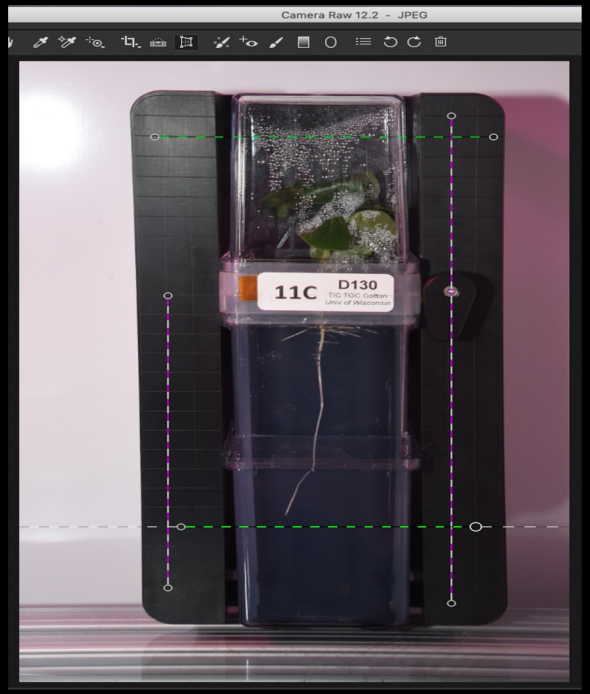
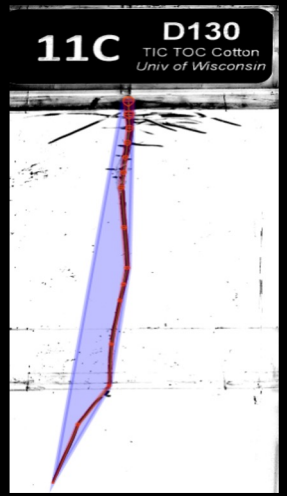
ImageJ & Smart Root Plugin

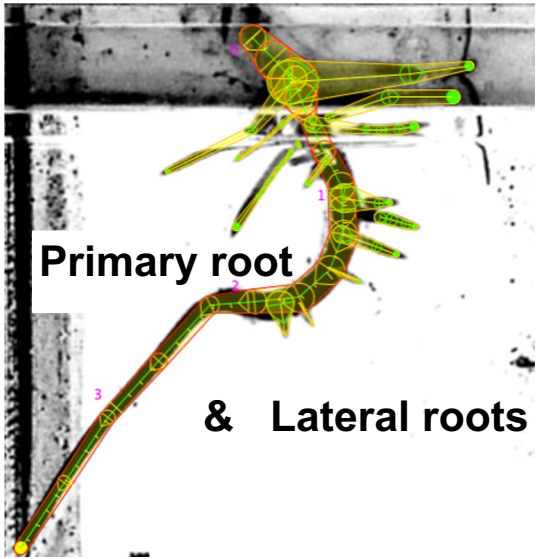
- Invert image
- User define scale conversion based on image marker
- User define root spine, lateral root autodetection.
- RSML output

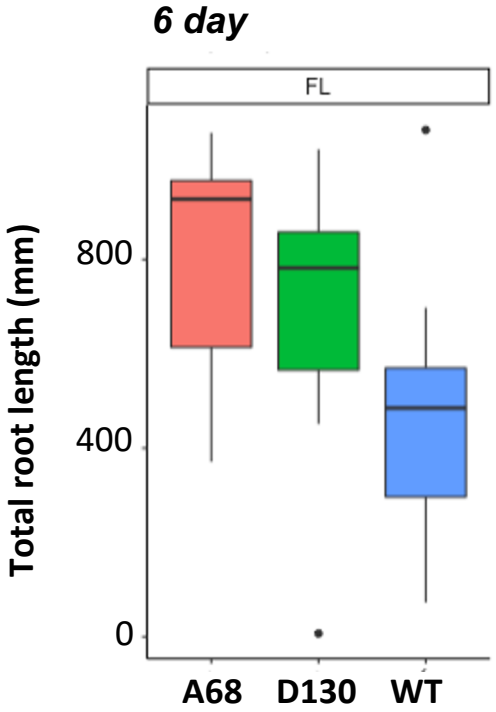




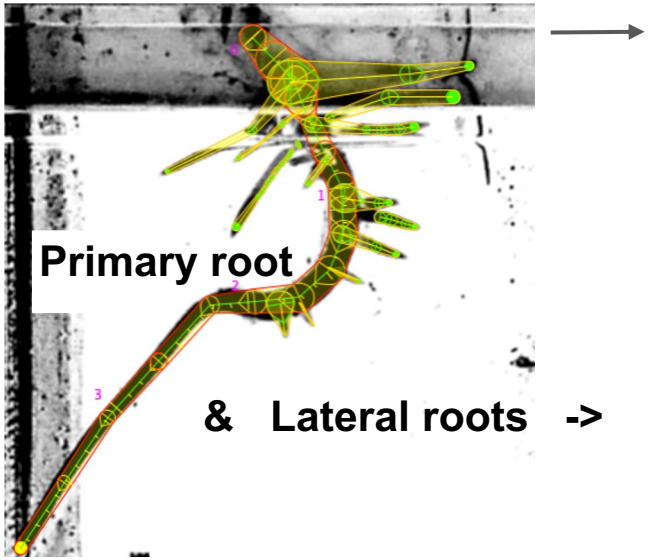
Check out Lucas's Bauer SOAPP poster - UH36

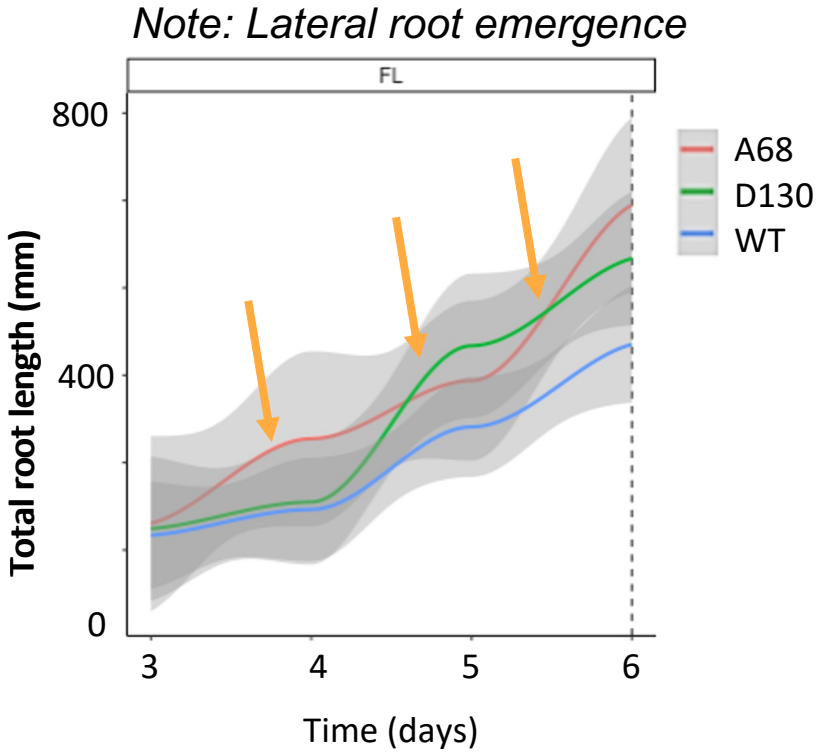
AVP-ox roots grew larger than WT during flight





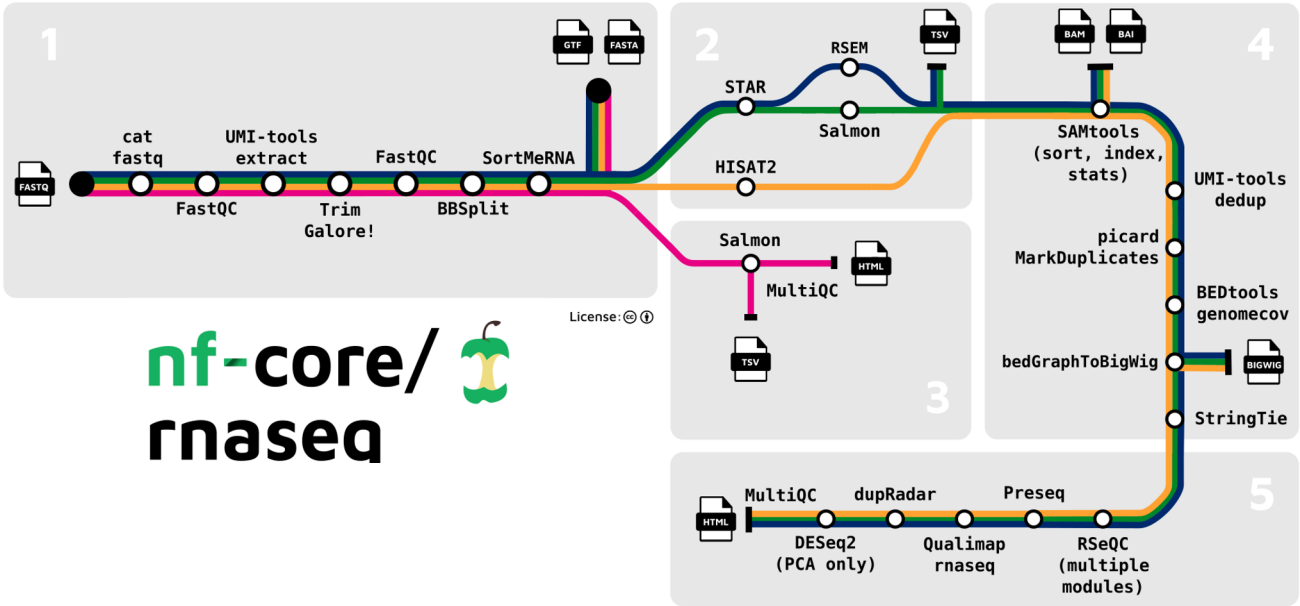
AVP-ox lateral roots emerged earlier than WT



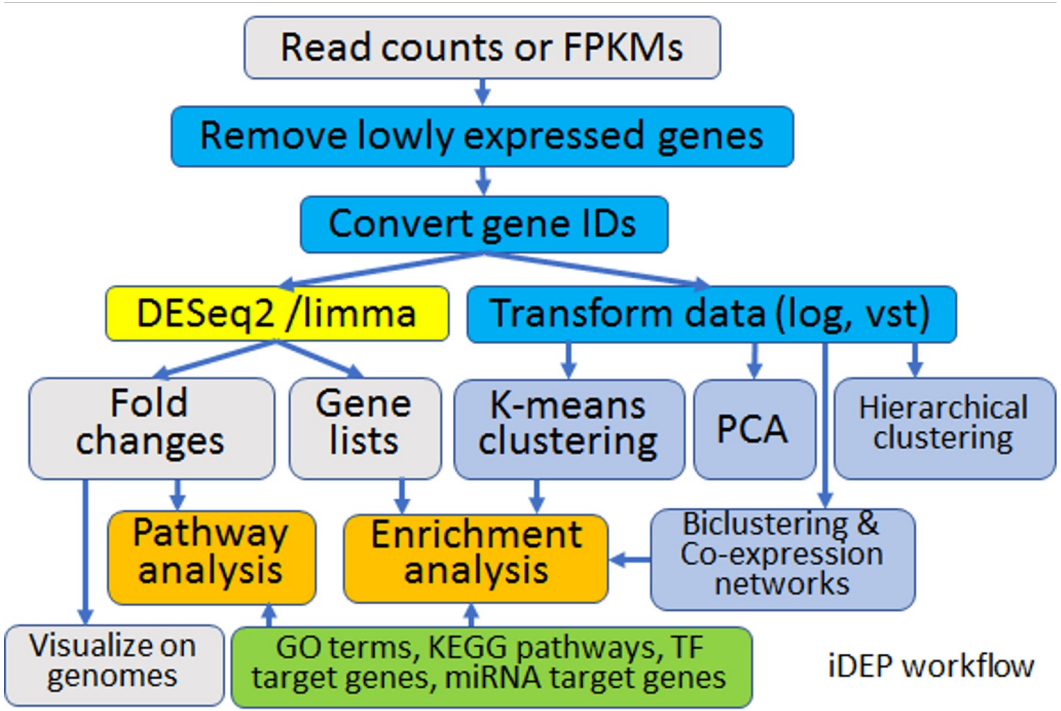


Gold line: nf-core was used HiSat2 for FASTQ alignment and transcript count performed with Dup-radar (*similar to BAM2count*).

Arko line:
RNA extraction with SPECTRUM kit Illumina NOVAseq sequencing.

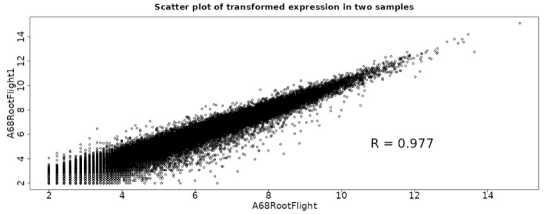


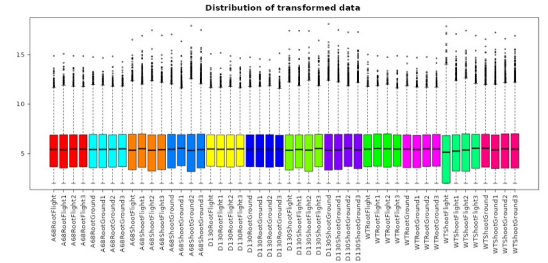
QC, normalization and differential expression calculation. Enabling ontological enrichment visualization.

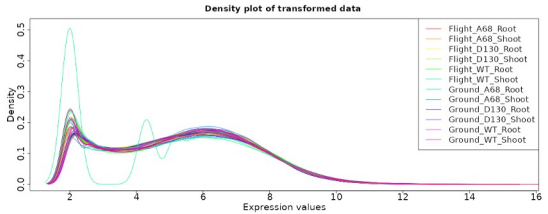


iDEP workflow

Ge *et al.*, 2022







RNAseq DEG count WT (FDR <=0.1 and logFC >=2)

Comparisons	Up	Down
WT Shoot Flight -vs- WT Shoot Ground	580	740
WT Root Flight -vs- WT Root Ground	3946	2433

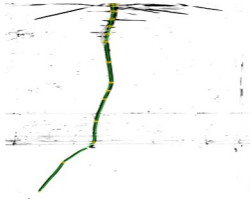
Differential Expressed Gene (DEG) Count

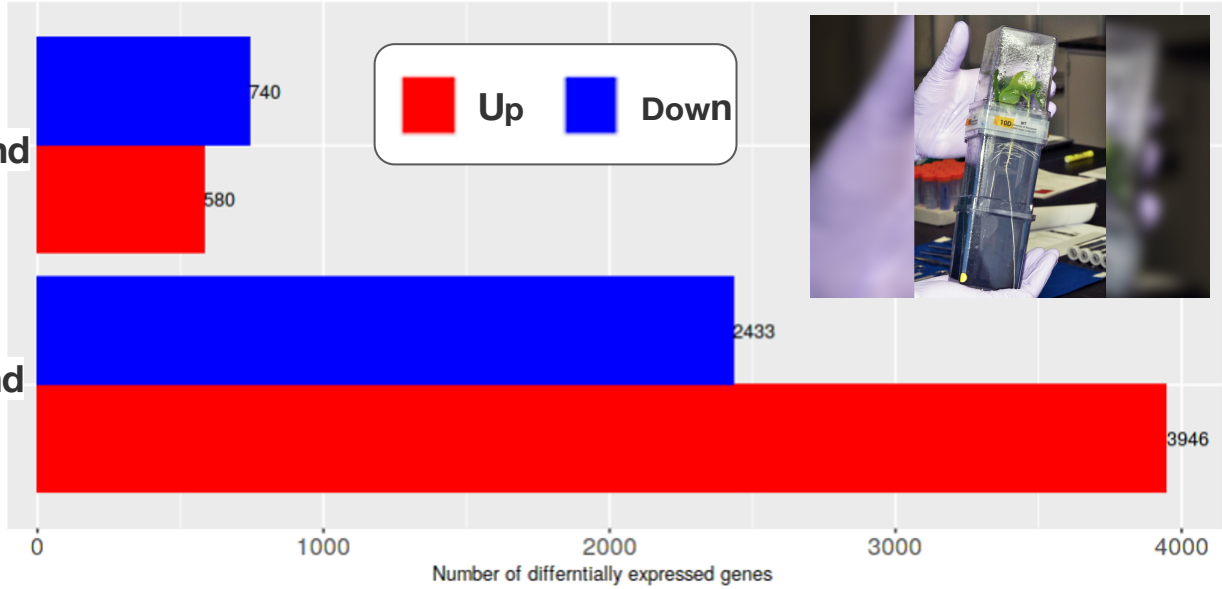
DESeq2 DEG Count Thresholds

Fold Change (FC) and FDR false discovery rate

WT Shoot Flight -vs- WT Shoot Ground

WT Root Flight -vs- WT Root Ground





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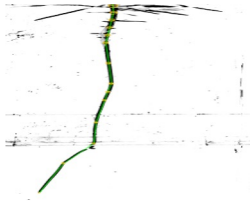
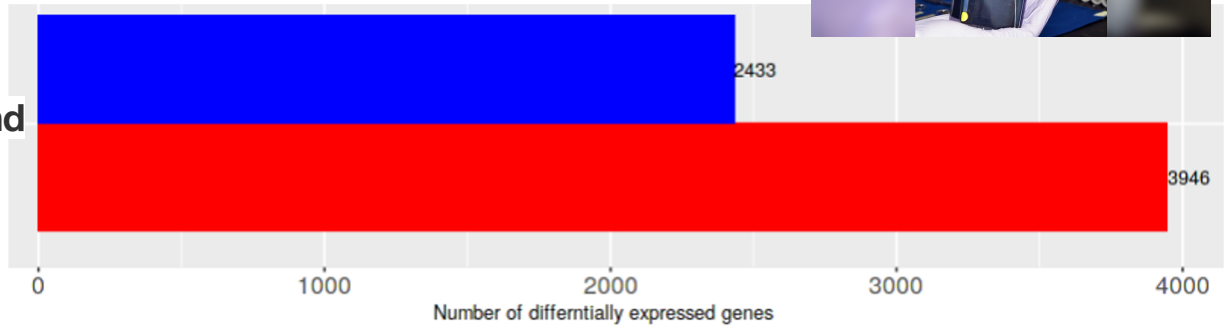
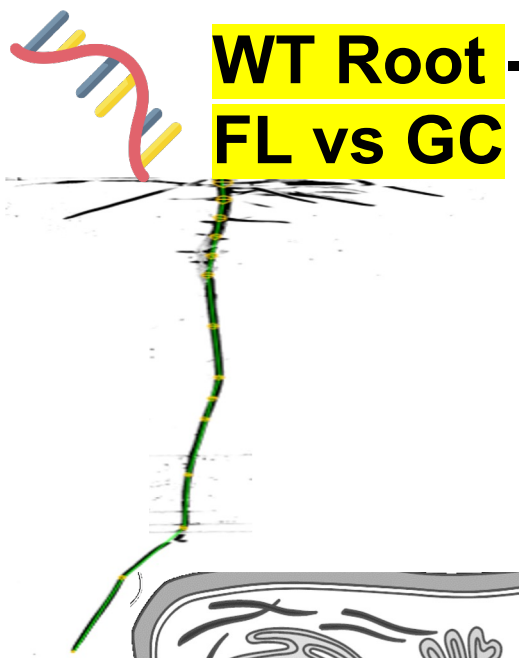
Focus on roots



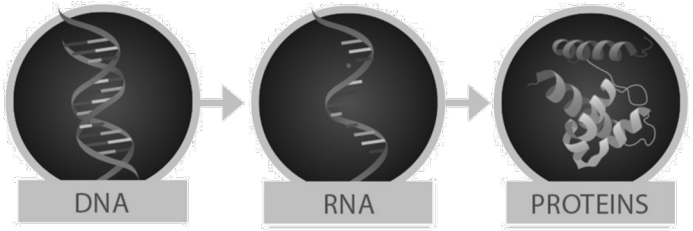


WT Root - mRNA translation suppression

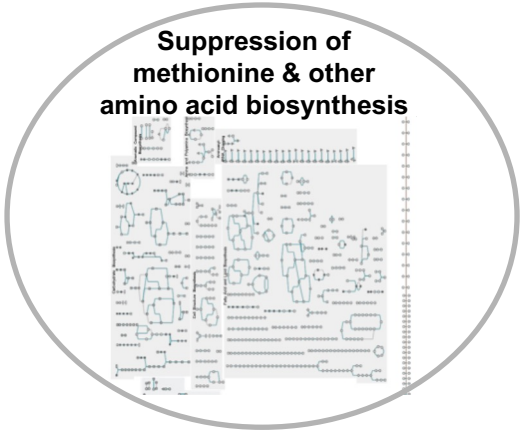
FL vs GC

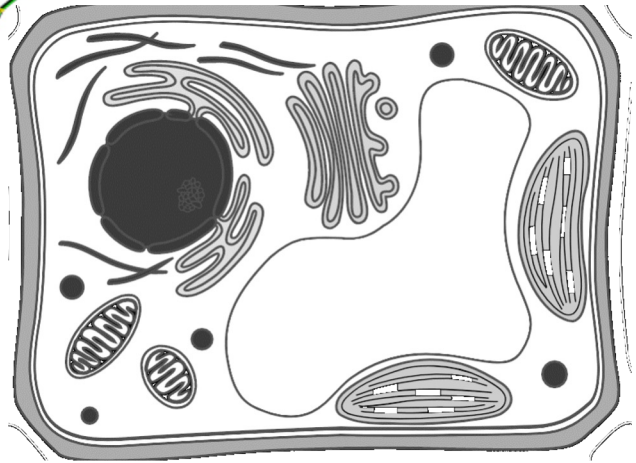


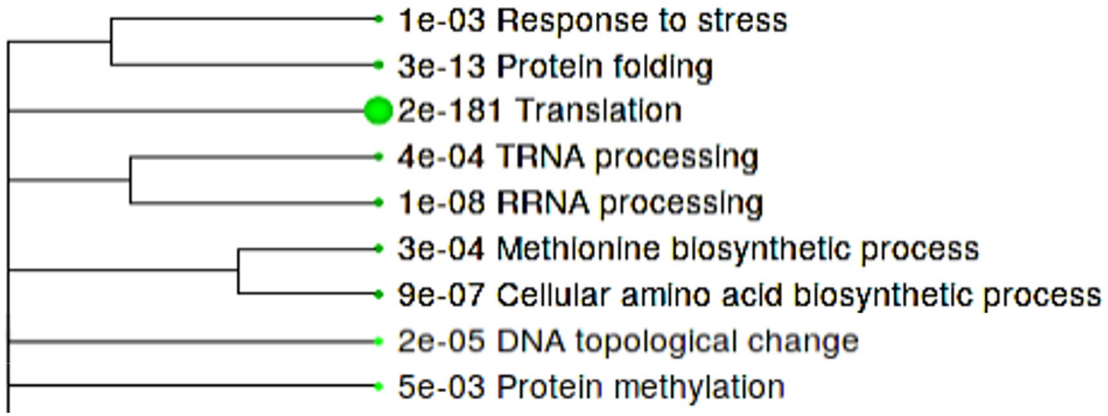
Down (N=2433)



Topology Translation Folding



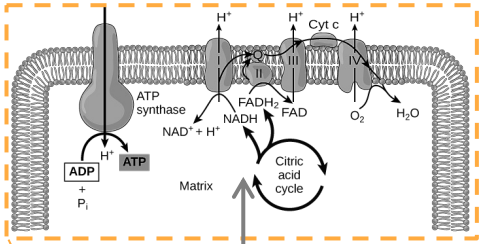




WT Root - Oxidative-Phosphorylation up-regulation

FL vs GC

Up (N=3946)

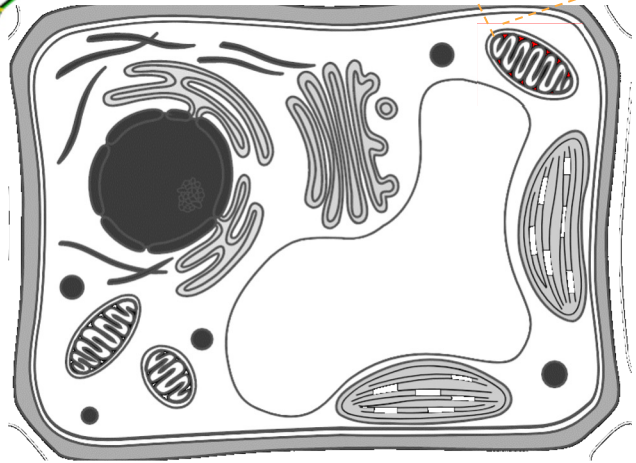


Carbohydrate : Lipid metabolism

Protein UBQ & ERAD pathway

Protein phosphorylation / dephosphorylation

Signaling / Exocytosis



1e-06 Coenzyme A metabolic process

2e-12 Oxidation-reduction process

3e-03 Glycerol-3-phosphate catabolic process

6e-03 Isoprenoid biosynthetic process

2e-05 Lipid metabolic process

3e-13 Signal transduction

8e-46 Regulation of transcription-DNA-templated

2e-04 Protein dephosphorylation

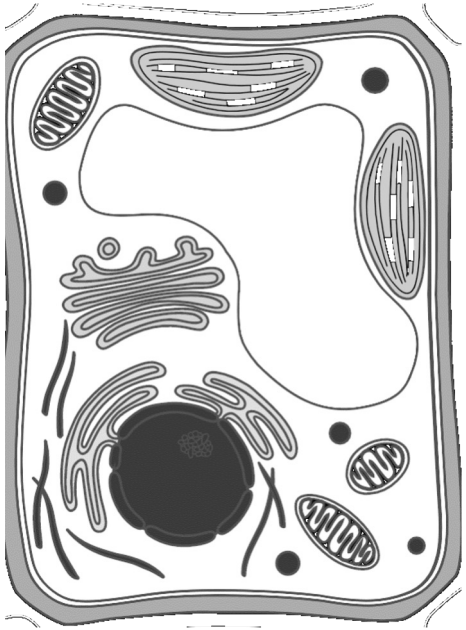
2e-04 Exocytosis

8e-04 Protein kinase C-activating G-protein coupled receptor

3e-03 Carbohydrate derivative biosynthetic process

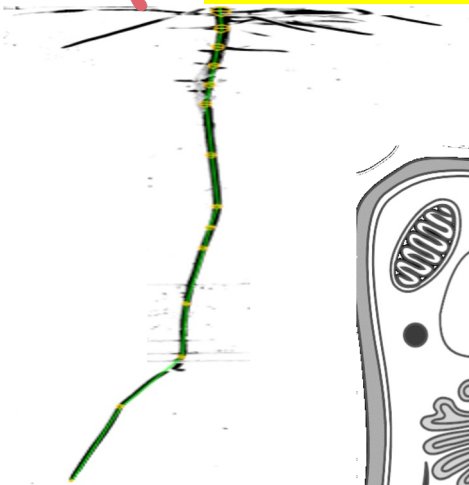
WT vs A68 – Root -> Pathways effected by AXP-1OX FL vs FL

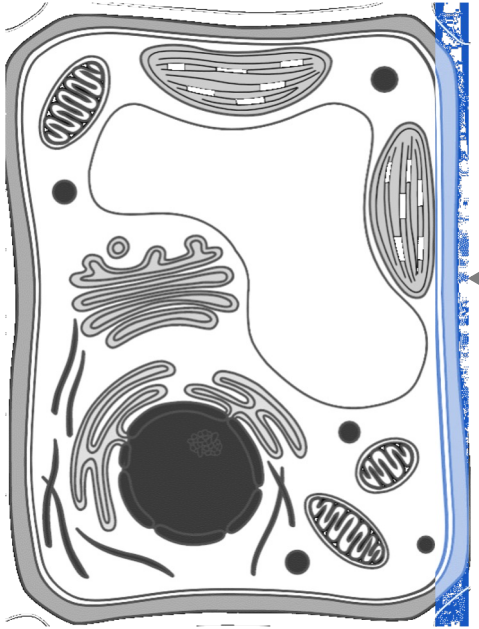
Note: No significant difference D130 vs A68 in Flight

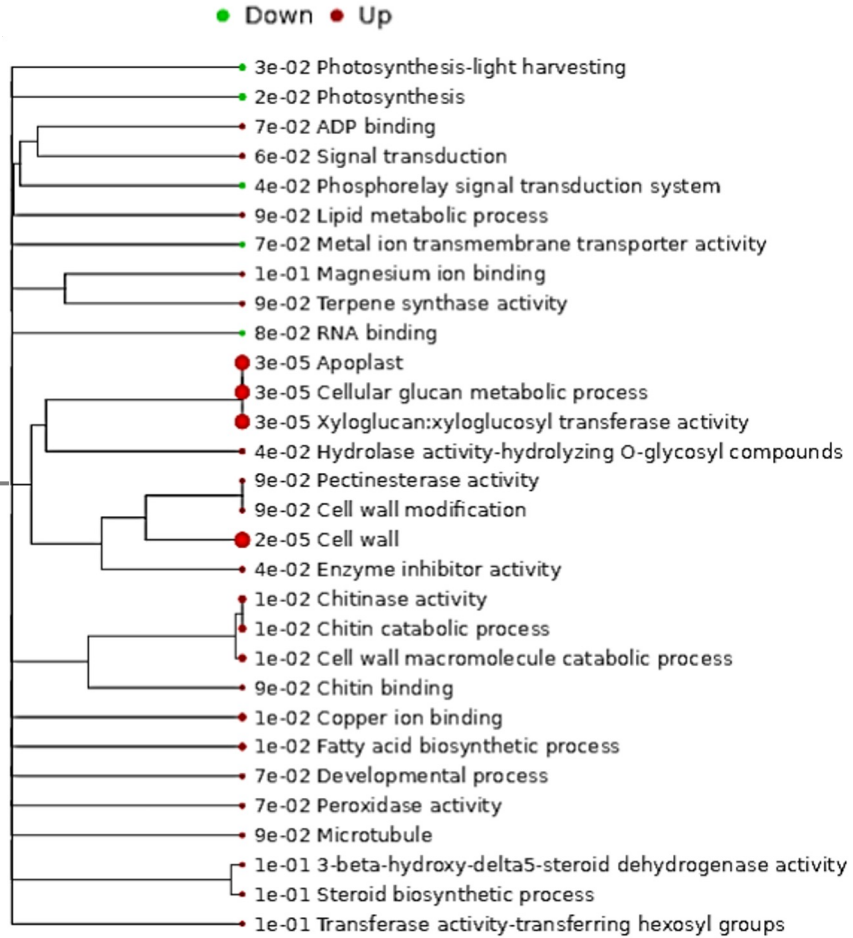


WT vs A68 – Root -> Pathways effected by AXP1-OX FL vs FL

Let's break this down…

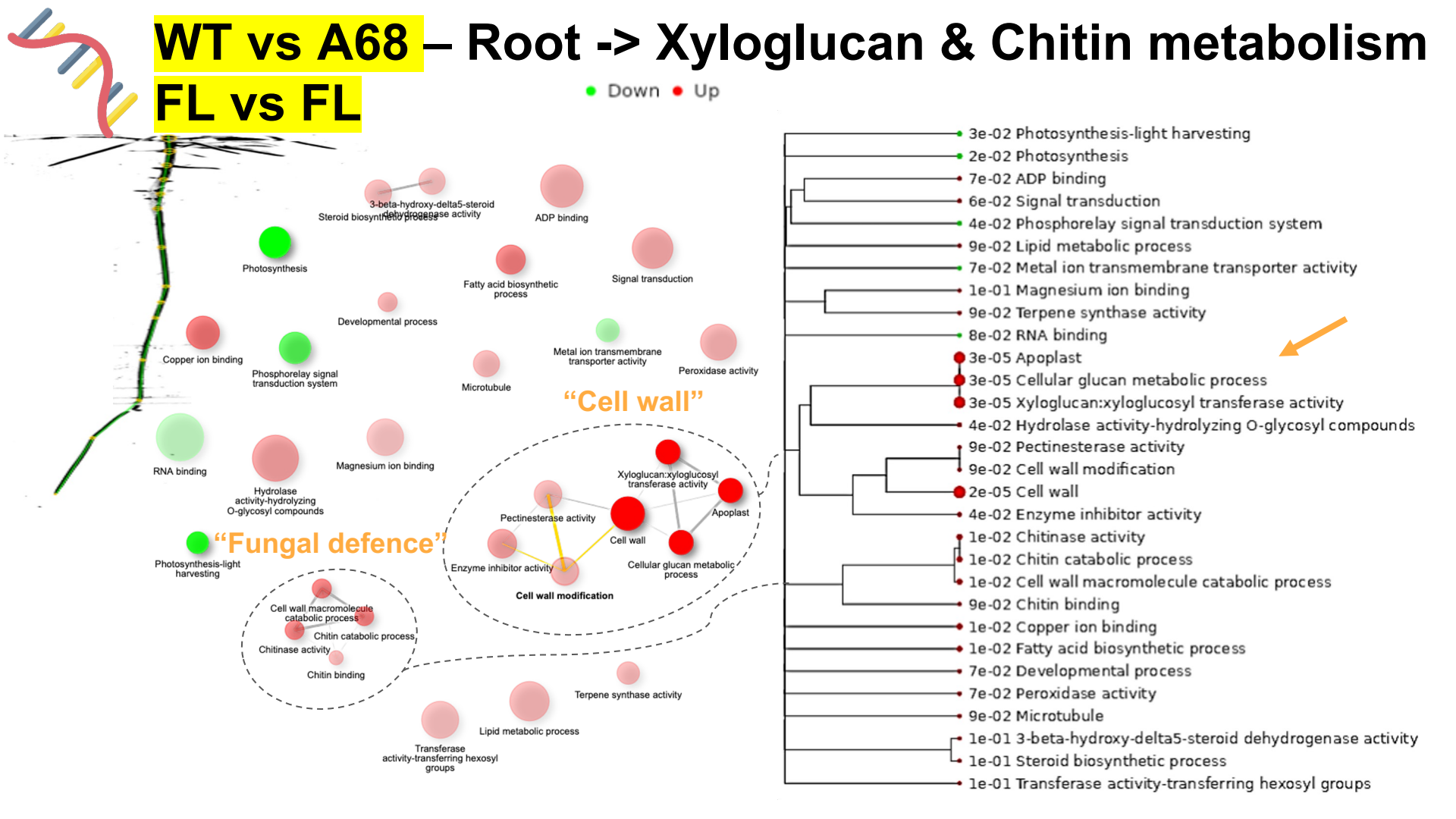




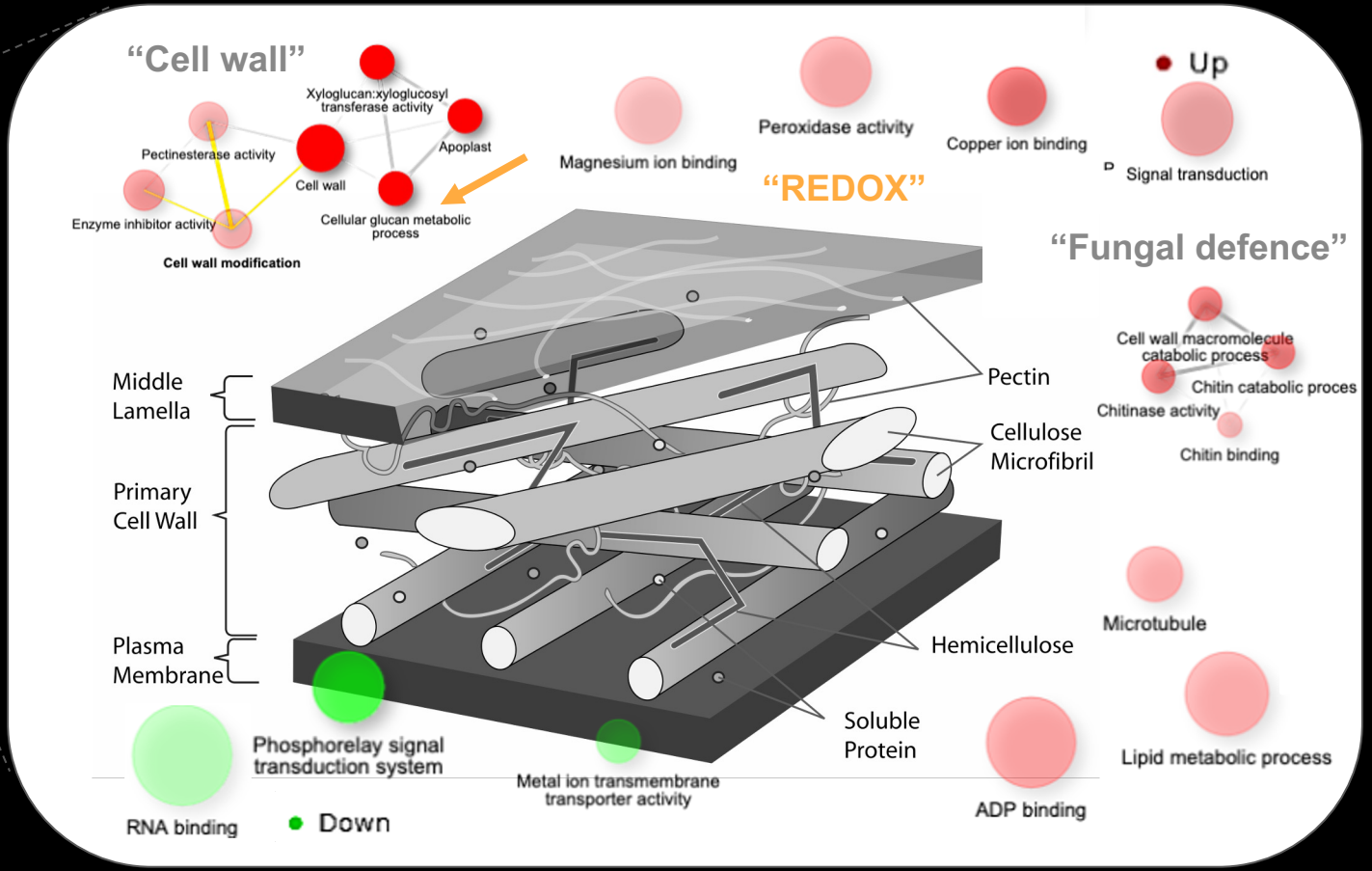


WT vs A68 – Root -> Xyloglucan & Chitin metabolism

FL vs FL



WT Root vs AVP1-Ox -> Cell wall REDOX
FL vs FL

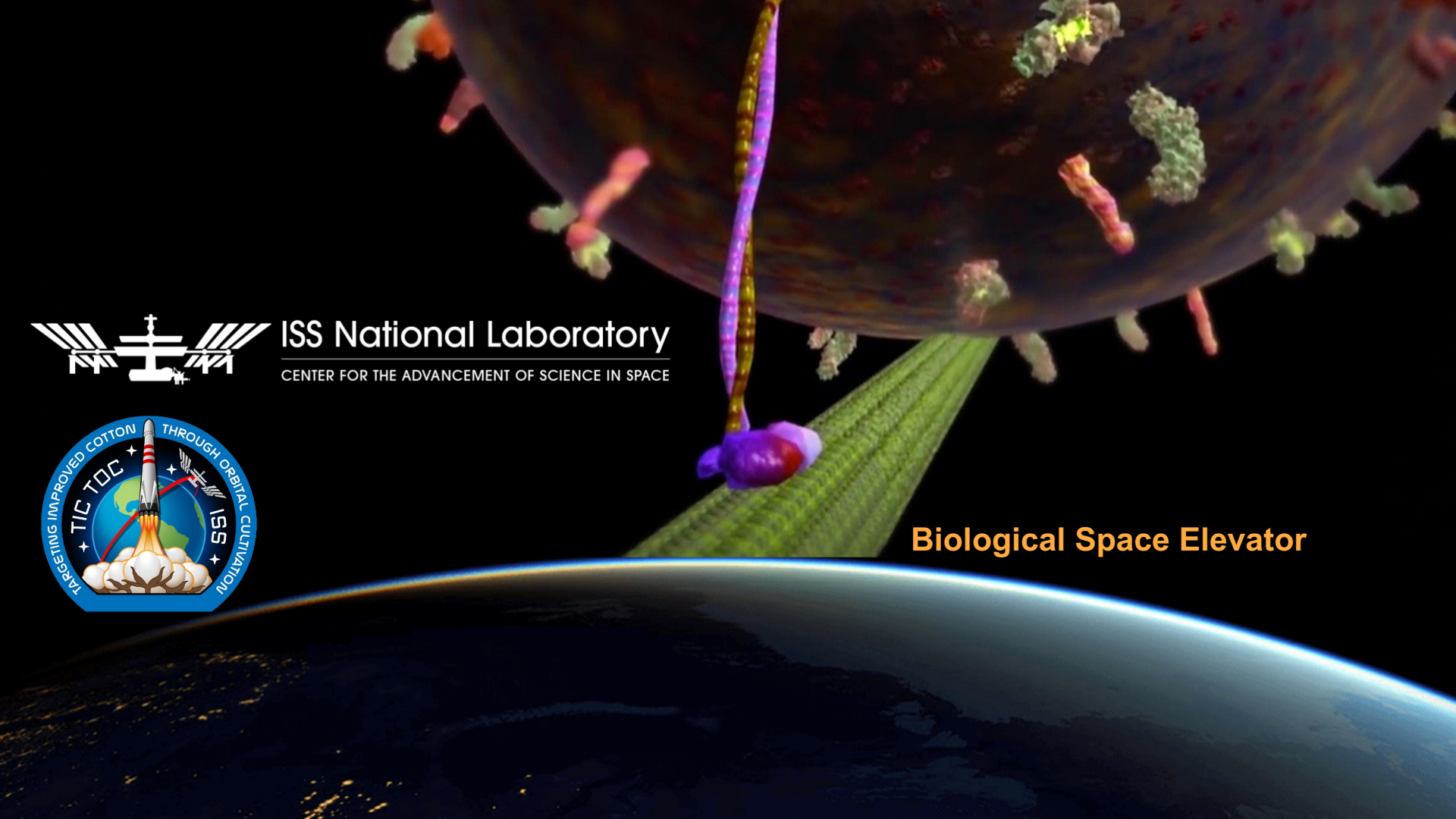


Change ion transport and phosphorelay signaling.

TICTOC Conclusion AGSR 2022

- WT Cotton RNAseq “functionally resembles WT Col-0 *Arabidopsis thaliana* response to space flight.”
- The AVP-OX lines exhibited greater growth in flight relative to WT controls.
- AVP-OX may changes vesicle trafficking system influencing cation ion transporters and cell wall modifying enzymes.

YURI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TIC TOC
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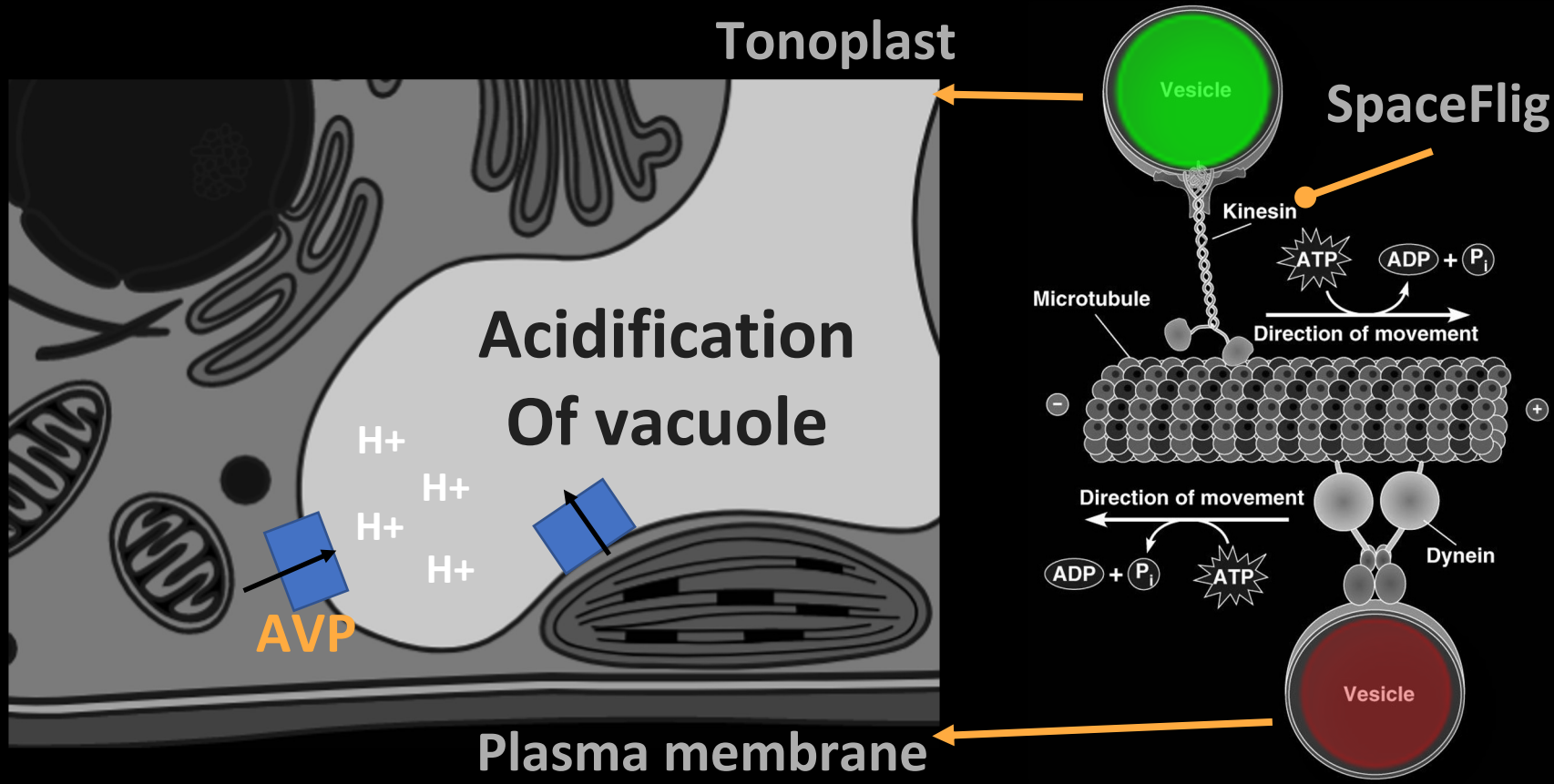
Biological Space Elevator

AVP-ox might alter the orientation of vesicle movement?



&/Or how does this change what is delivered to vacuole or apolast?