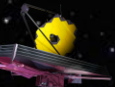


# Investigating the Relationship between the Cell Wall Integrity Pathway and Unfolded Protein Response in *Arabidopsis thaliana*

Ryan B., Brooke H.,  
Margaret H., and Fatima K.

321 – Space Farmers!

OSD-321





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# Meet the Space Farmers



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# Overview of Presentation

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Introduction

What is the mission?

02

Preliminary Data

What is the data + How  
did we access it?

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Hypothesis and  
Aims

Why do we think this is  
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Why does this matter?

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01

# The Introduction

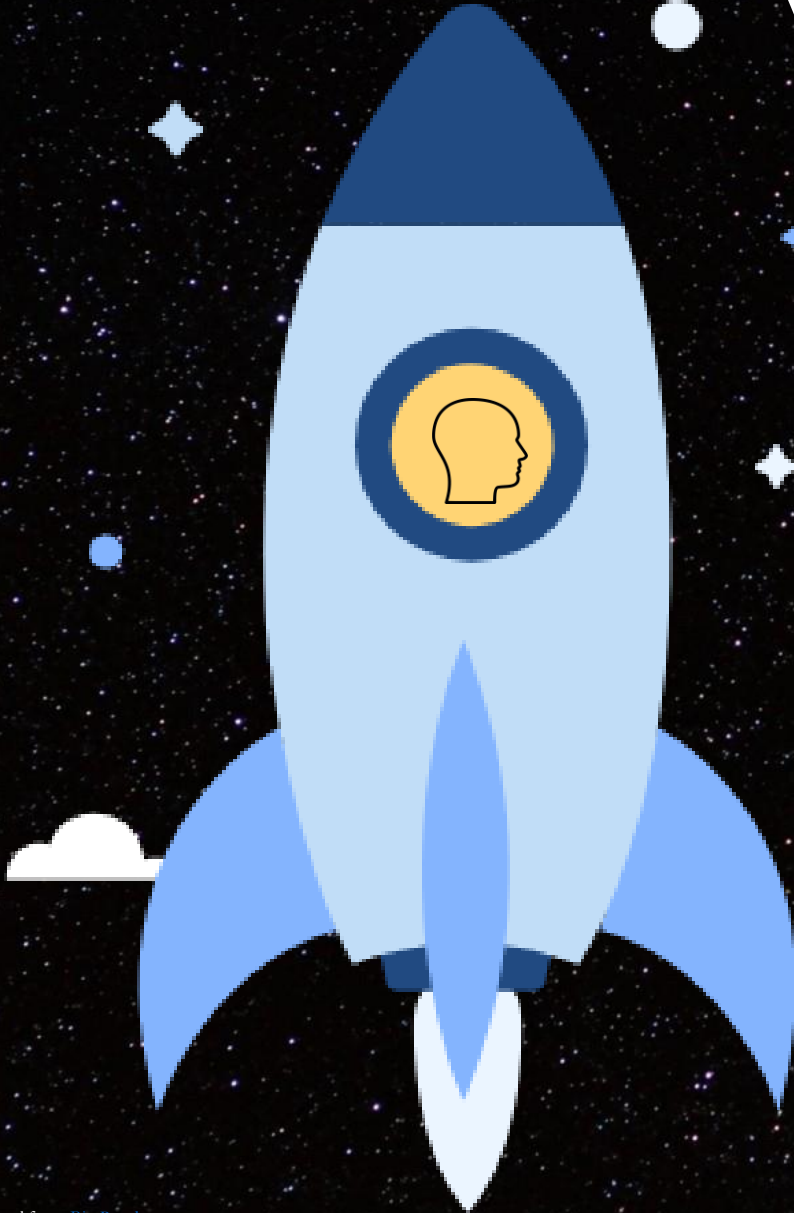
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What is the mission?



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# Why *Arabidopsis*?





## "Relevance of Unfolded Protein Response to Spaceflight-Induced Transcriptional Reprogramming in *Arabidopsis*"

### *Arabidopsis thaliana*

#### Dataset

#### Goals

- Understand how plants grow in orbit
- Study the new pathways induced in spaceflight caused by the removal of the UPR regulators

#### Conditions

**Control:** Wild Type (WT) (5 Petri dishes)

**Experimental:** KO bZIP28 (4 Pd), bZIP60 (4 Pd), bZIP28/bZIP60 (5 Pd), atire1 (4 Pd)

**Growing Period:** 14 days (on KSC ISS Environmental Simulator & on ISS)

**Sample Size:** 70-80 seeds per petri dish replicate

**Hardware:** BRIC (Biological Research In Canisters)



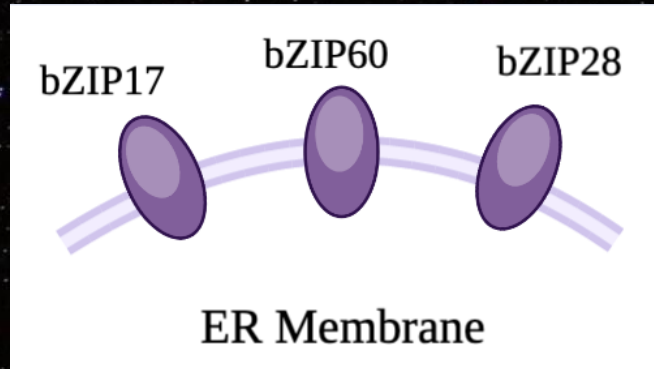


# What are bZIPs?

"ER-Anchored Transcription Factors bZIP17 and bZIP28 Regulate Root Elongation"

Kim JS, Yamaguchi-Shinozaki K, Shinozaki K et al.

Three bZIP transcription factors regulate the UPR in *Arabidopsis thaliana*:



The bZIPs remain inactive and anchored to the ER under nonstress circumstances until evoked

## GLDS-321

bZIP28

In UPR; transducer in ER  
Stress signaling pathway

bZIP60

In UPR; Abiotic stress  
response & plant immunity

atire1

In UPR; Senses unfolded  
proteins in the ER



---

# Key Concepts Used Today



**Cell Wall:** a rigid layer of polysaccharides lying outside the plasma membrane of the cells of plants, fungi, and bacteria. It helps keep out pathogens from entering the cell.



**Endoplasmic Reticulum (ER):** the transportation system of eukaryotic cells generally responsible for protein production



**Unfolded Protein Response (UPR):** a cellular stress response linked to the Endoplasmic Reticulum.

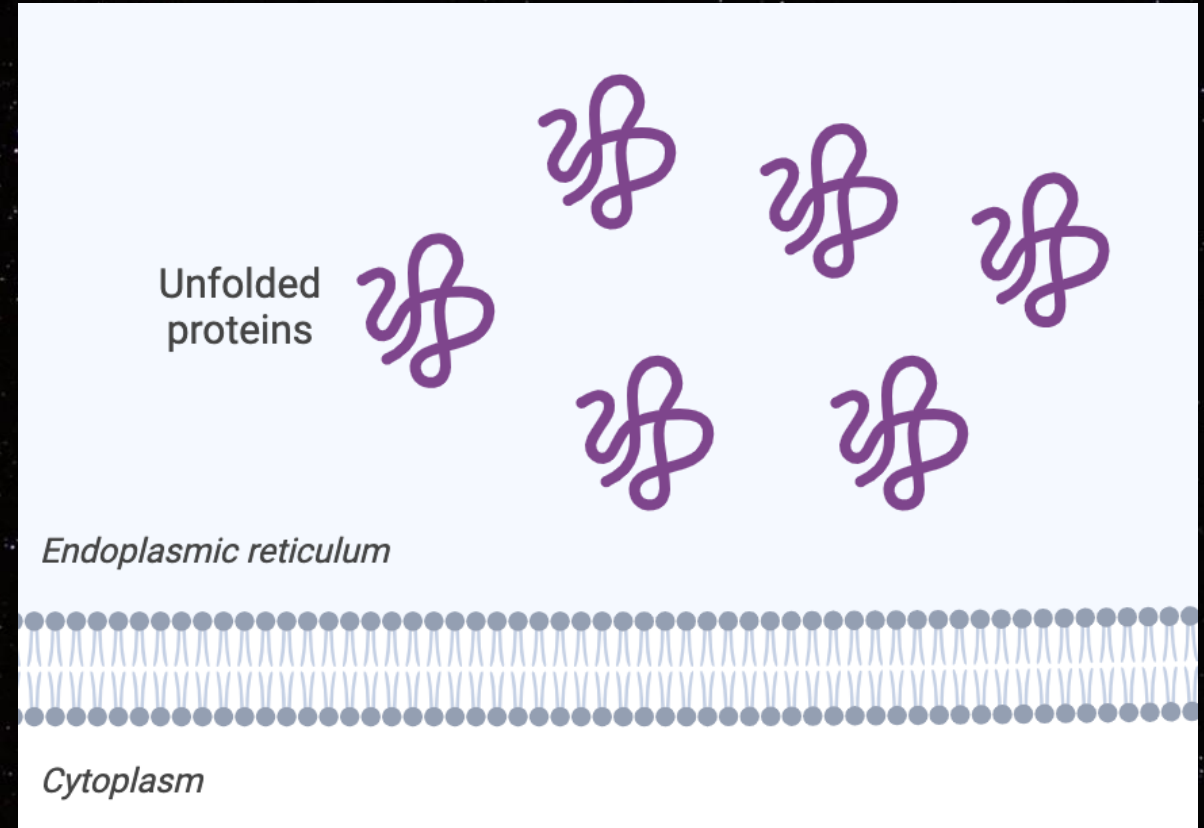


**Cell Wall Integrity Pathway (CWI):** the central signaling cascade required for the adaptation to a wide spectrum of cell wall perturbing conditions, including heat, oxidative stress and antifungals.



# What is Unfolded Protein Response?

- ✿ Key mediator of stress
- ✿ Located in the ER
- ✿ Occurs when there are too many unfolded proteins
- ✿ Cell death if UPR doesn't work





02

# Preliminary Data

---

What is the data? + How  
did we access it?



# Overview: Methods

WT & bZIP28 (both GC & FLT)

DESeq2

Data Normalization

P-value < 0.05

Log(FC) > 1.0

Volcano  
Plot

Differentially  
Expressed  
Genes

ShinyGo

Lollipop Plot  
Fold Enrichment

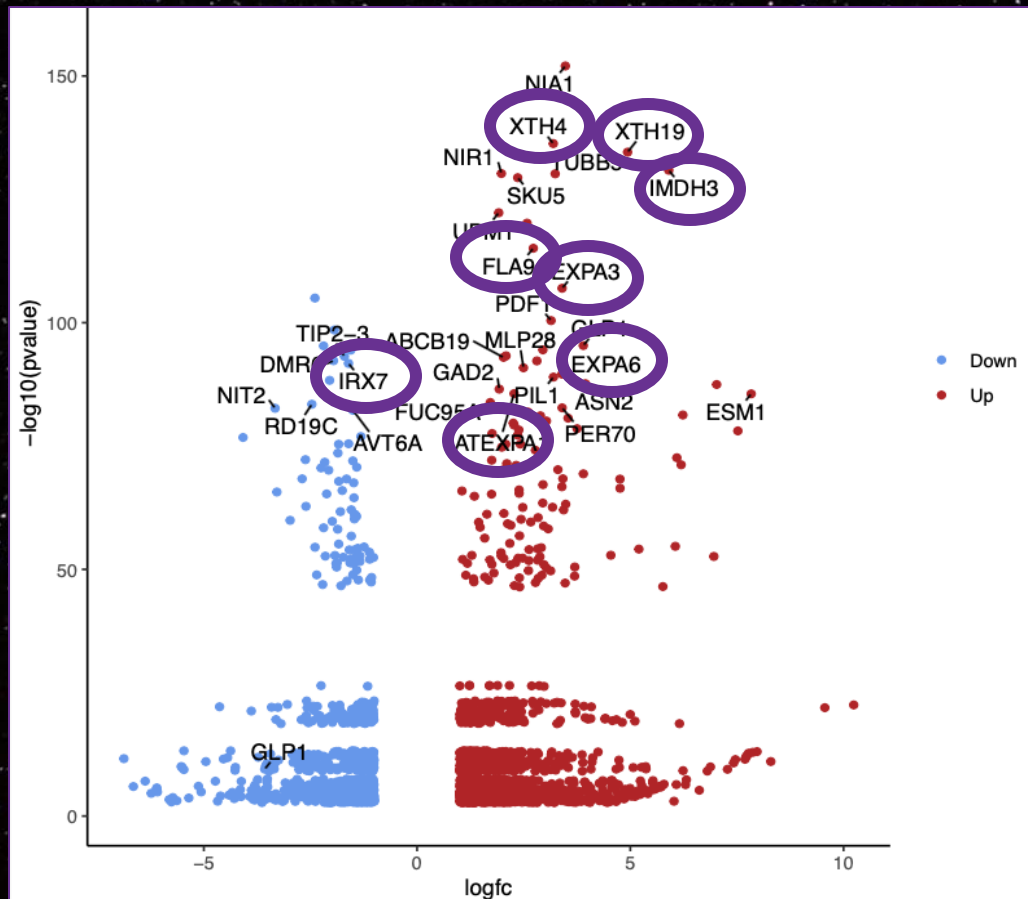
Interacti  
Venn

Compares &  
Contrasts Genes

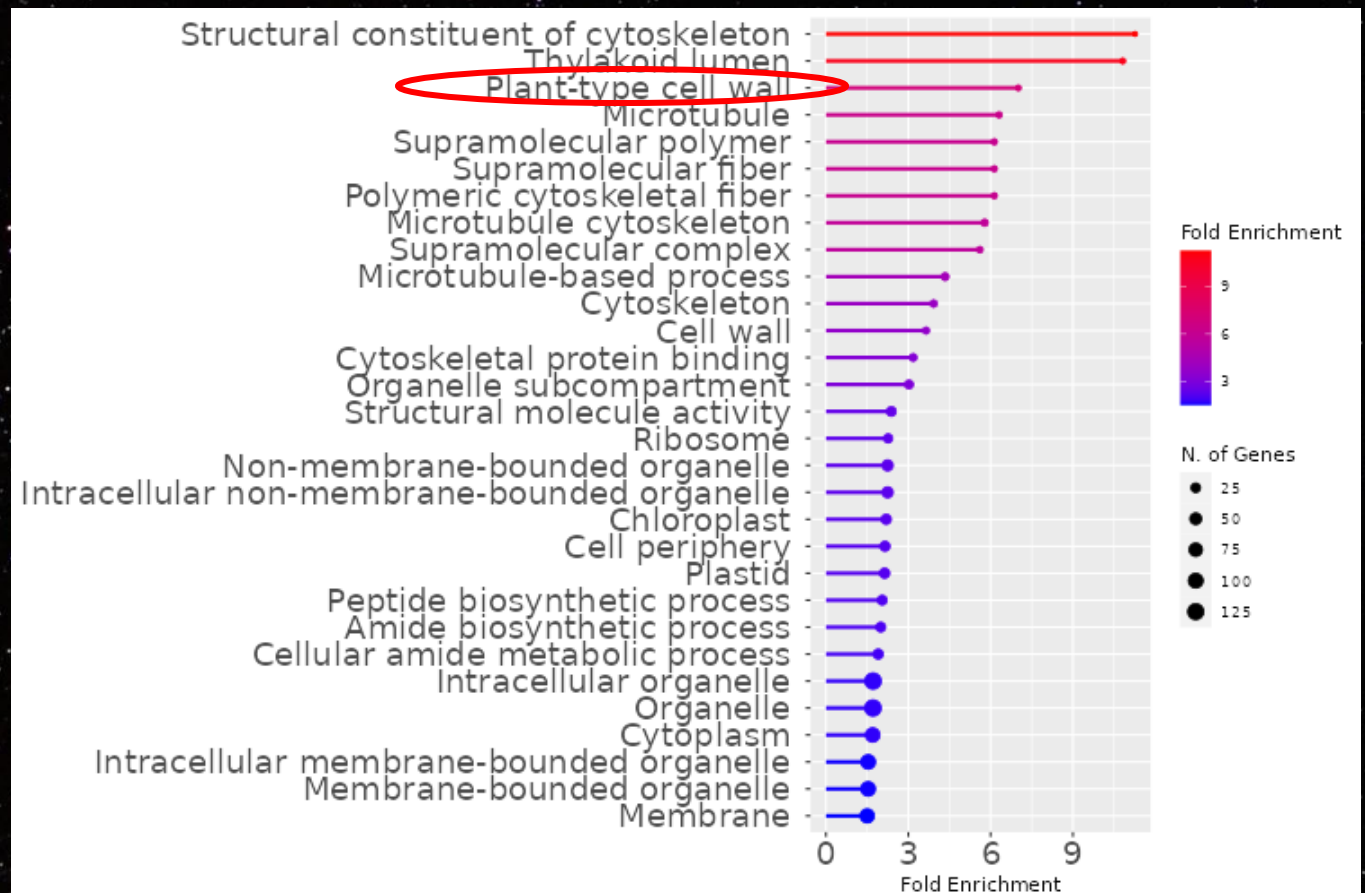


# bZIP28: Pathway Analysis

(Involved in UPR; transducer in ER Stress signaling pathway)



Volcano Plot

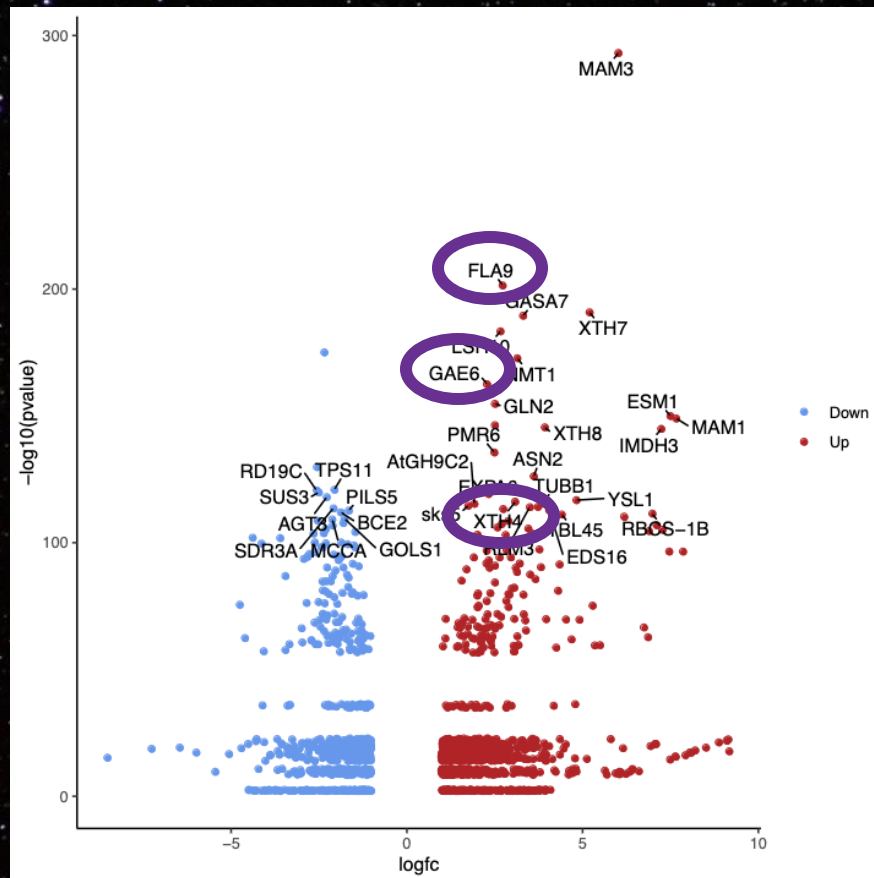


Lollipop Plot

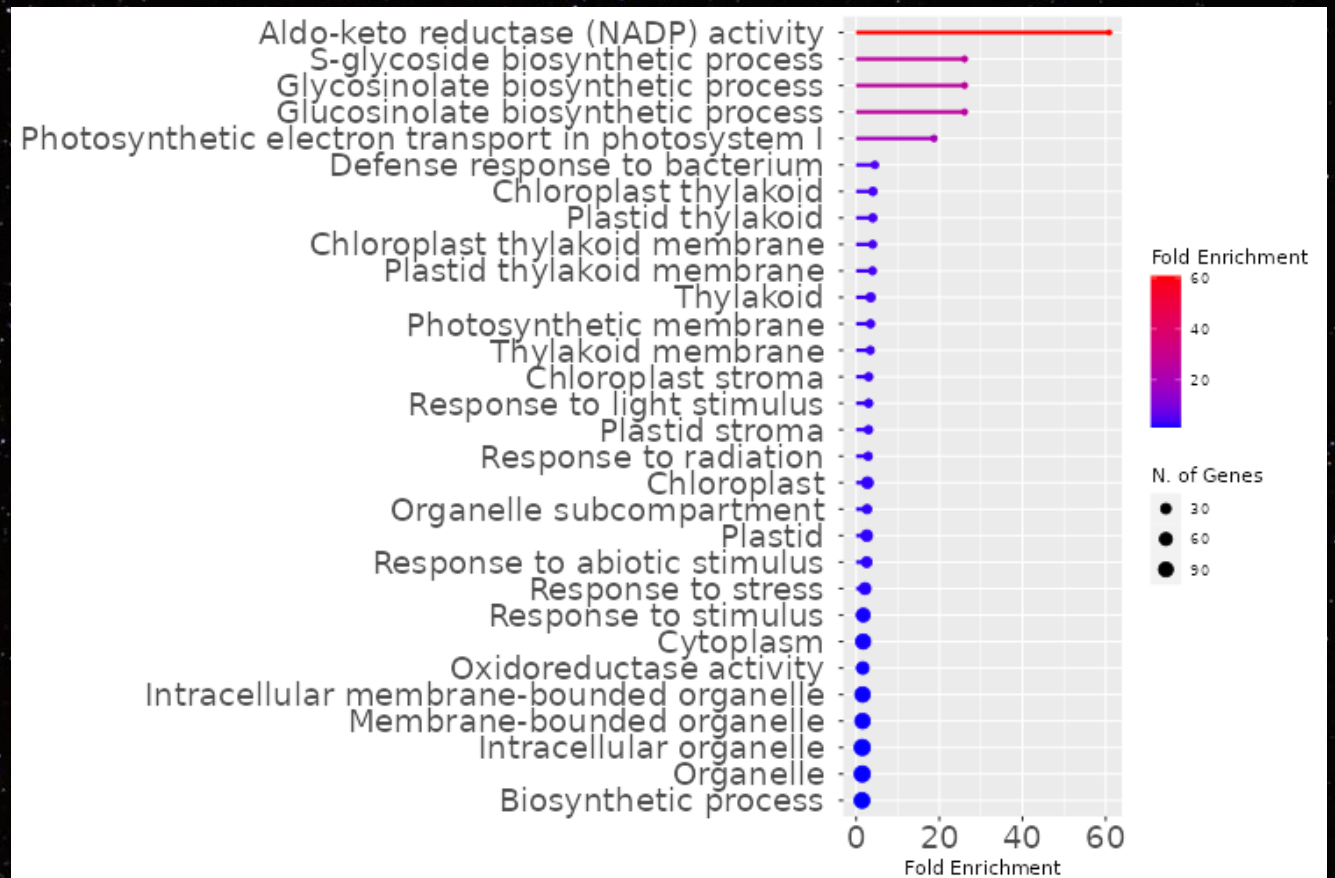


# WT: Pathway Analysis

(Wild Type *Arabidopsis thaliana*)



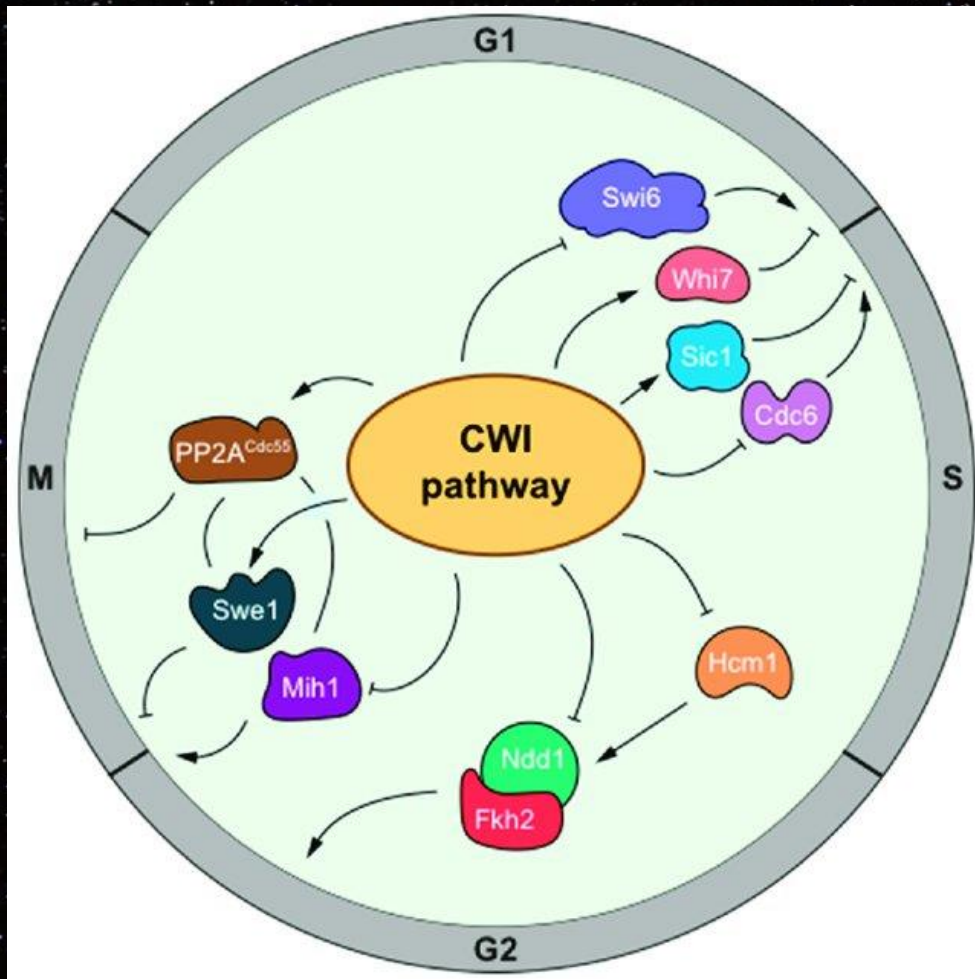
Volcano Plot



Lollipop Plot



# What is Cell Wall Integrity Pathway?



- ✿ Responds to stress factors
- ✿ Regulates cell wall biosynthesis
- ✿ Repairs damage to cell wall
- ✿ Confers cell shape



# How are CWI & UPR activated?

"The unfolded protein response is induced by the cell wall integrity mitogen-activated protein kinase signaling cascade and is required for cell wall integrity in *Saccharomyces cerevisiae*"

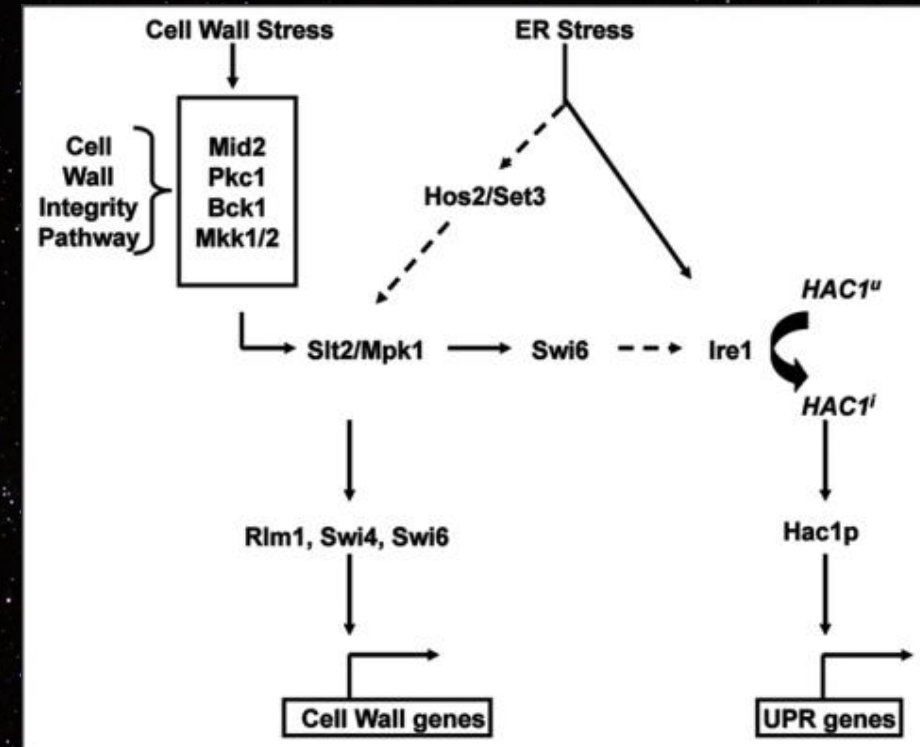
Krysan et al.

Cell wall  
stress

CWI  
Pathway  
activated

UPR  
activated

Cell wall  
integrity  
maintained





# How are CWI & UPR activated? (Cont.)

"The cell wall and endoplasmic reticulum stress responses are coordinately regulated in *Saccharomyces cerevisiae*"

Krysan et al.



UPR and CWI work together to protect cells against harsh environments in yeast cells



Unsure if CWI and UPR are linearly linked



Yeast (fungi) and *Arabidopsis* (plant) have similar cell wall structures

## **Food for thought!**

If Yeast (Fungi) and *Arabidopsis* are similar in cell wall structure, could CWI pathway be similar?



# Commonalities in Yeast & *Arabidopsis thaliana*

Cell wall integrity signaling in plants: “To grow or not to grow that's the question”

Voxeur A, Höfte H. et al

## Fungi

- Cell Wall Integrity (CWI) pathways monitor cell architecture
- Stress responses compensate for changes in the CW

## Plant

- Evolved signaling modules to maintain mechanical homeostasis during cell wall growth
- Extensive damage in cell wall structure triggers stress response

Plants and fungi have synonymous pathways that enable the maintenance of cell wall integrity



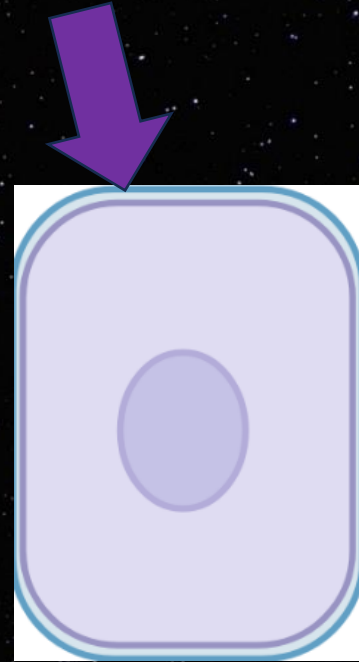
# Gene of Interest

Adj P-value:  $1.717e-89$

**IRX7**

Log Fold Change:  
-1.61

Secondary cell  
wall biogenesis  
genes are **down-  
regulated** in  
bZIP28



- ✿ Issues with biogenesis can cause cell wall defects (Ramírez et al.)
- ✿ IRX7 is only differentially expressed in bZIP28

\*This graphic is not representative of where the IRX7 gene is located\*



# What we know

Which Can

UPR regulator  
bZIP28 have  
been removed

Interrupt Cell  
Wall Integrity  
pathway; vital  
cell process

Decrease in efficiency  
of CWI  
pathway; interference  
with the biosynthesis &  
repair of the cell wall

Which May Impact



# 03

## Hypothesis

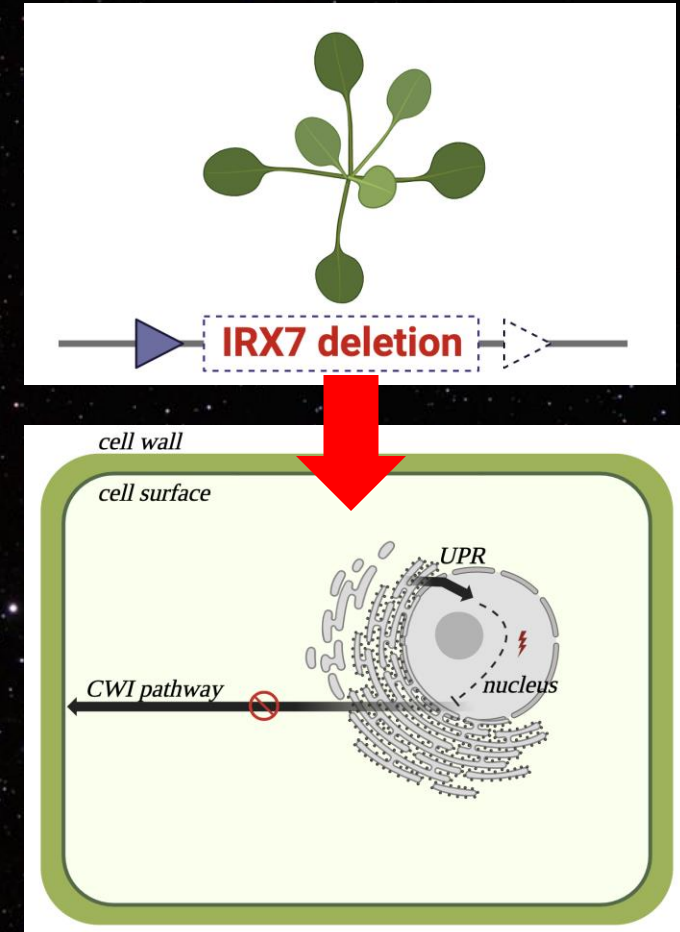
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Why do we think this is  
occurring?



# Hypothesis

We hypothesize that **IRX7**, which oversees the secondary cell wall biogenesis, affects the communication between the UPR & CWI pathways, leading the cell wall integrity to be compromised.





04

# Experimental Design

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What is our mission?



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# Aims

#1

To investigate the effect of over-expressing & silencing IRX7 on the CWI and UPR pathways

#2

To determine how cell-wall integrity affects the morphology of *Arabidopsis*



# Overview on Experimental Design

## *Arabidopsis:*

- 2,100 seedlings total
- 70-80 per petri dish replicate
- 5 plates per experimental group
- Wild Type *Arabidopsis*

## **Duration:**

- 14 days

## **BRIC Hardware:**

- ISS
- ISS-environment simulator
- WT, IRX7

## **Analysis:**

- DeepLabCut
- RNA seq
- Galaxy
- Atomic Force Microscope





# Our Design

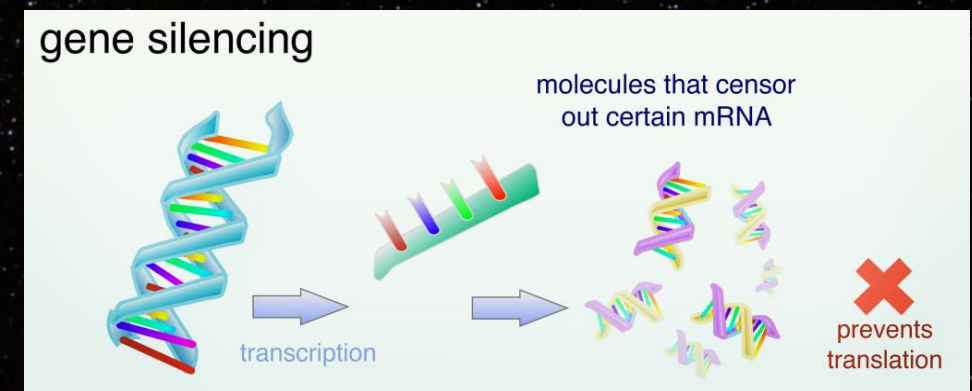
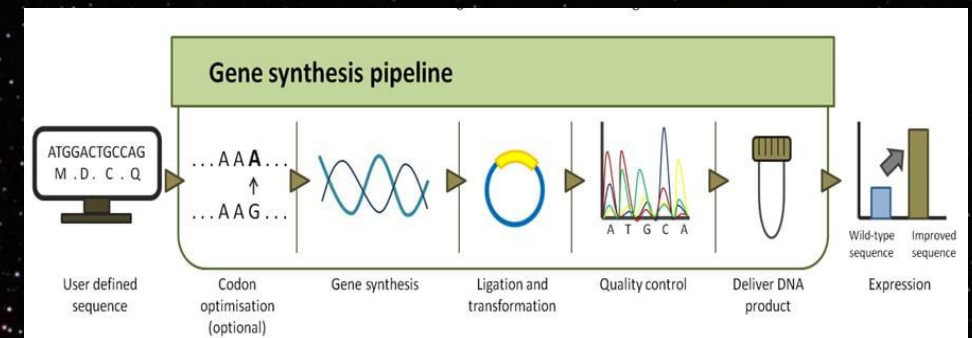
Group	Qualities	Methods
<b>Control Group:</b> 70 <i>Arabidopsis</i> seedlings per plate replicate 14 days exposure	<b>Ground:</b> ISS simulator; <b>Wild Type <i>Arabidopsis thaliana</i></b> ; conditions standardized; ISS-environment Simulator at KSC  <b>Space:</b> ISS; <b>Wild Type <i>Arabidopsis thaliana</i></b> ; conditions standardized; BRIC	Use DeepLabCut; Paired end RNA seq  Atomic force microscope to analyze the cell wall
<b>Experimental Group 1:</b> 70 <i>Arabidopsis</i> seedlings per plate replicate 14 days exposure	<b>Ground:</b> ISS simulator; <b>silenced IRX7</b> ; conditioned standardized; ISS-environment Simulator at KSC  <b>Space:</b> ISS; <b>silenced IRX7</b> ; conditions standardized; BRIC	siRNA – silence IRX7  Use DeepLabCut; Paired end RNA seq  Atomic force microscope to analyze the cell wall
<b>Experimental Group 2:</b> 70 <i>Arabidopsis</i> seedlings per plate replicate 14 days exposure	<b>Ground:</b> ISS simulator; <b>over-expressed IRX7</b> ; conditioned standardized; ISS-environment Simulator at KSC  <b>Space:</b> ISS; <b>over-expressed IRX7</b> ; conditions standardized; BRIC	Artificial gene synthesis – over-express IRX7  Use DeepLabCut; Paired end RNA seq  Atomic force microscope to analyze the cell wall



# Aim 1

To investigate the effect of IRX7 on the CWI and UPR pathways

Group:	Control & Experimental Groups 1&2 Silencing IRX7 via siRNA in WT Over-expressing via artificial gene synthesis IRX7 in WT
Data Analysis:	Measuring change in UPR gene expression (ie., atire1, bZIP28) Assess CWI & associated gene expression
Tools:	<ul style="list-style-type: none"> <li>- RNA Seq</li> <li>- UseGalaxy Analysis Platform</li> <li>- Atomic force microscope</li> </ul>



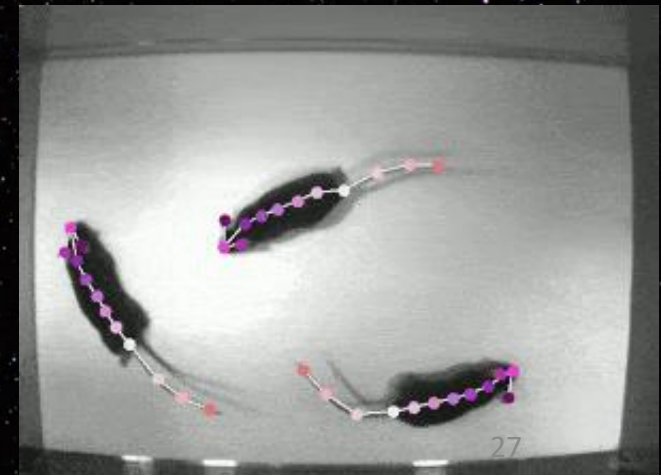


## Aims 2

To determine how cell wall integrity affects the morphology of the plant following the silencing of IRX7

### DeepLabCut – Machine Learning

- Pose estimation software that utilizes machine learning
- Used in many research projects to study animal movement in unique conditions
- Study the morphology of the plants while growing on the ISS
- Camera somewhere in the BRIC to capture movement images





# Aims

#1

To investigate the effect of silencing IRX7 on the CWI and UPR pathways



# Expected Results

When silencing IRX7, UPR will be downregulated. The CWI will be overall downregulated and may lead to cell wall defects/damage.

When over-expressing IRX7, UPR and CWI will be upregulated, allowing for the cell wall to become more resilient to stressors.

#2

To determine how cell-wall integrity affects the morphology of *Arabidopsis*



IRX7 experimental groups will have issues in plant morphology & growth. It could grow at odd angles caused by the lack of cell wall repair and synthesis/overexpression of gene.



05

# The Significance

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Why should you care?



# Significance – Cell Wall Health

## Cell Wall Defects

- Misfolded proteins can cause Cell Wall Defects

### Meaning

- Dwarfed plant stature
- Changed plant morphology
- Reduced seed production



Reduced feasibility of growing fresh veggies long term



Possibly less nutrients and edible biomass available for crew members

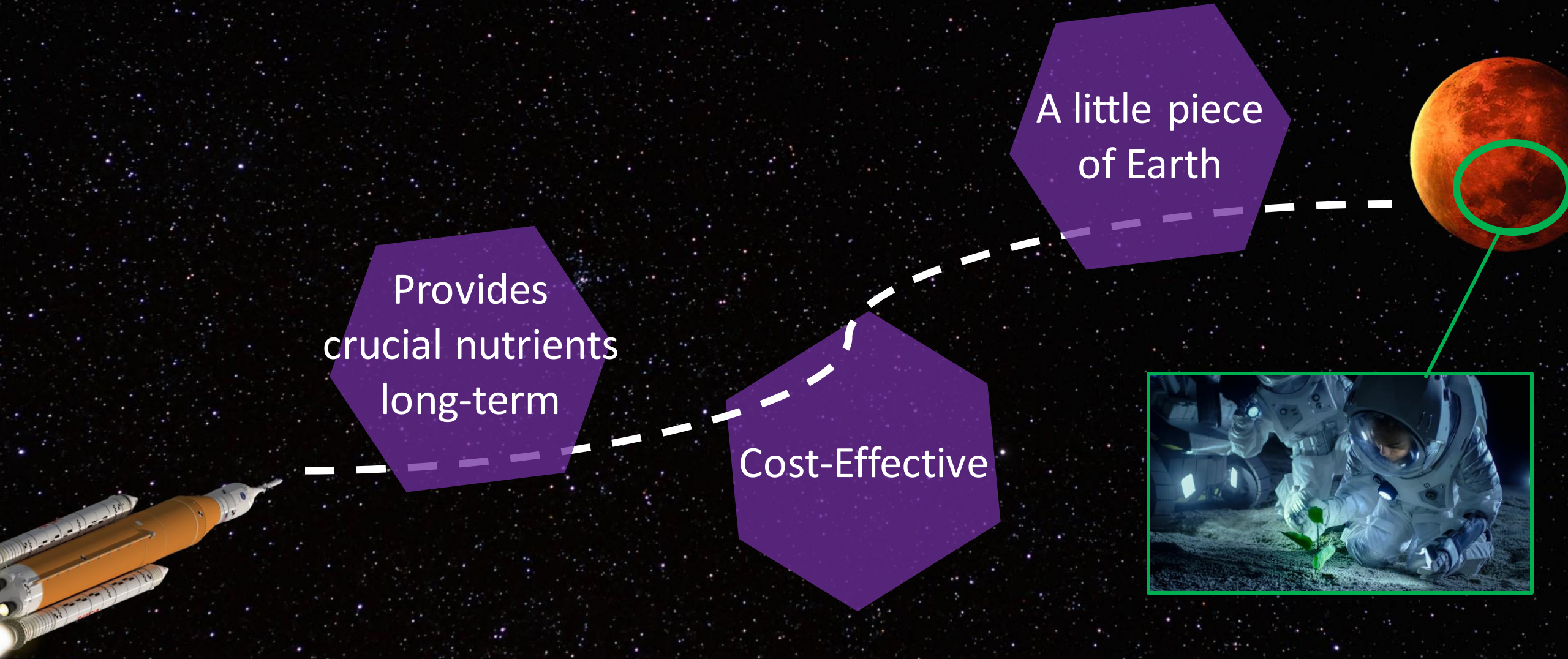


Inefficient use of funds & insufficient for large-scale growth

**Understanding the relationship between CWI & UPR will help prevent Cell Wall defects!**



# Significance – Long-Term Space Missions





# Significance – Psychological & Physical Impact

Reminds  
Astronauts  
of Home

Astronaut  
Megan McArthur



"I loved the smell of the chili peppers  
when I was taking care of them."

Strong Flavor  
(ie., chili, mizuna)

Astronaut  
Jessica Meir

Vit. C, B1  
No nutrient  
degradation



Brings  
"Green"  
to the ISS



# Significance – Economic Impact

Cell-Wall defects will  
reduce the  
**cost-effectiveness** of  
growing plants in space

**Insight into  
mitigating  
env't stressors**

**Cost-effective  
Large-Scale  
plant growth**

**Sustainable  
plant cultivation  
in space**





# Significance - Robust Plants in a Changing Climate

Spaceflight



Extreme Environments



Plants built to withstand spaceflight could lead to insight on how to create plants able to survive in extreme environments caused by climate change



06

# Acknowledgements

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Citations and Questions



# Thank You!

## Thank You To Our Program Directors:

Dr. Elizabeth Blaber

Mrs. Jennifer Claudio

Mrs. Kimberly Cadmes

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Dr. Richard Barker

Dr. Lauren Sanders

Dr. Gbolaga Oluwasegun

Ms. Emma Canaday

All our families & friends

**AND THANK YOU TO OUR LISTENERS**





# Questions?

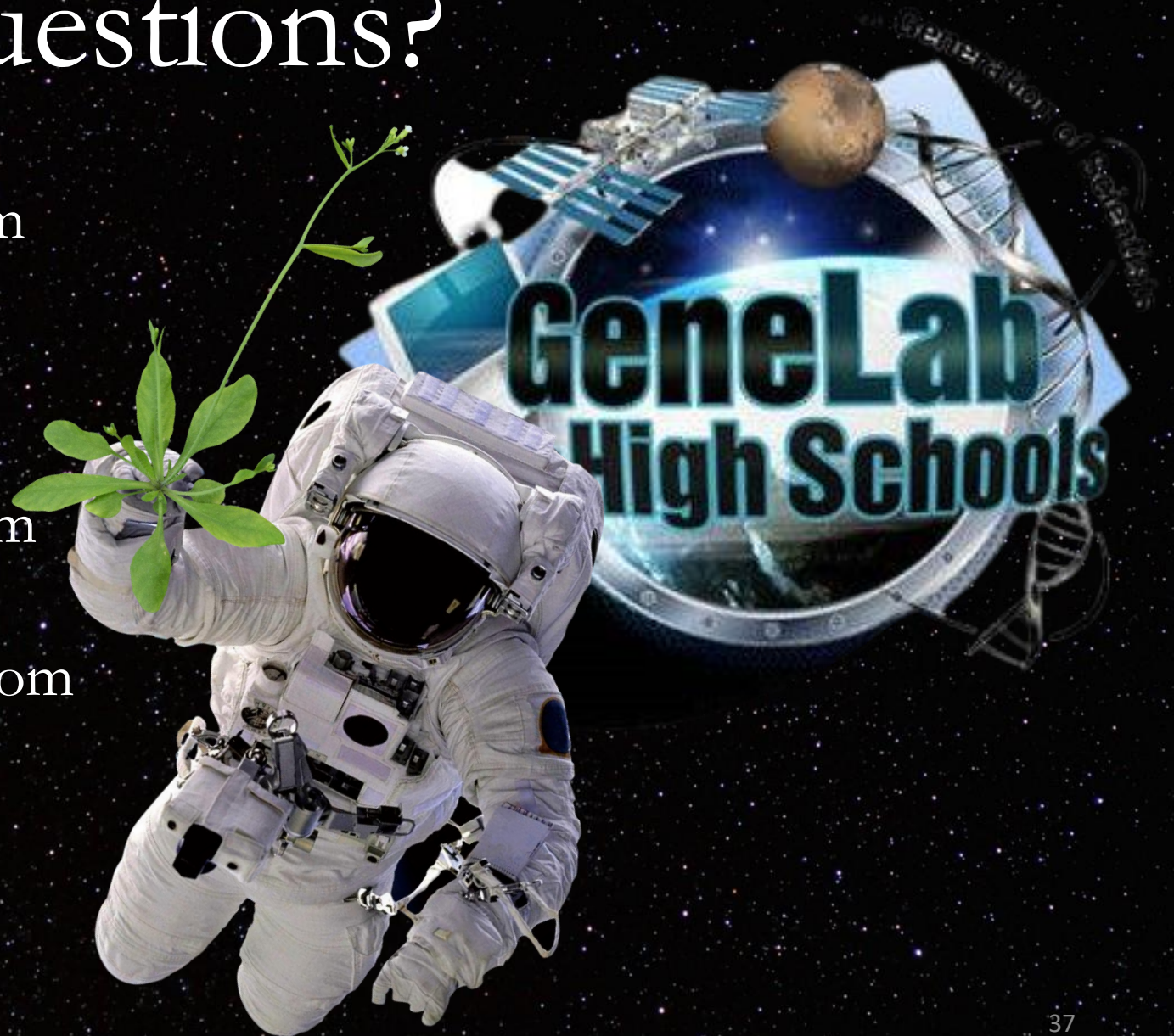
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