BioSentinel: optimizing growth conditions for improved yeast cell viability after long-term desiccation

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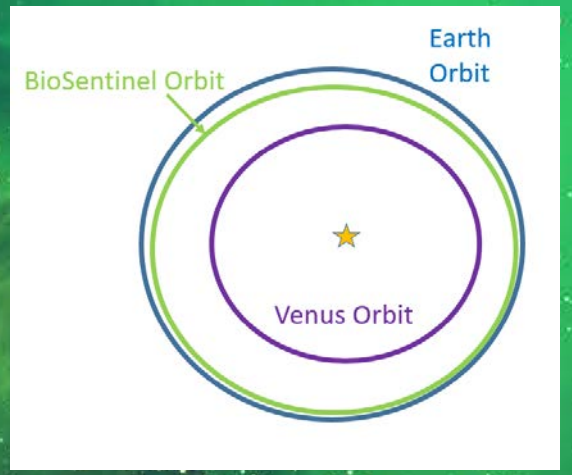
BioSentinel Overview

- Study the effects of deep space radiation on yeast, *Saccharomyces cerevisiae*
  - One type of damage generated from ionizing radiation in deep space double strand breaks (DSBs)
  - Yeast is suitable as a biological model
    - Eukaryotic, Robust analog for human cells
    - Tolerant to desiccation
      - Long duration mission: 12 months
      - Storage (pre-launch): ~6 to 9 months
    - DNA damage repair pathway is similar to that of humans
- Heliocentric orbit
- 1 of 13 secondary payloads on SLS EM1, December 2019
  - 1st biological experiment in deep space in 46 years
- ISS mission in LEO for lower radiation levels, microgravity control for deep space experiment

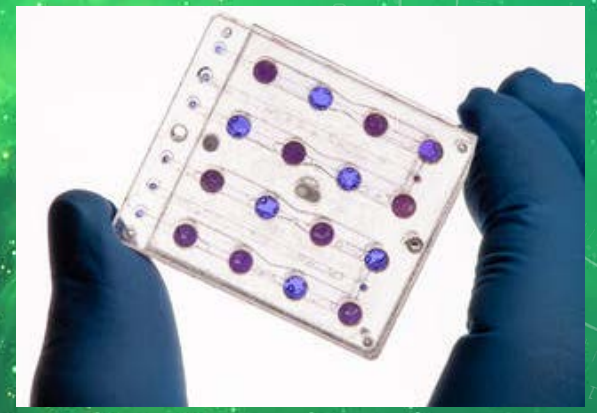


https://www.nasa.gov/mission_pages/station/main/index.html

https://www.nasa.gov/feature/around-the-moon-with-nasa-s-first-launch-of-sls-with-orion

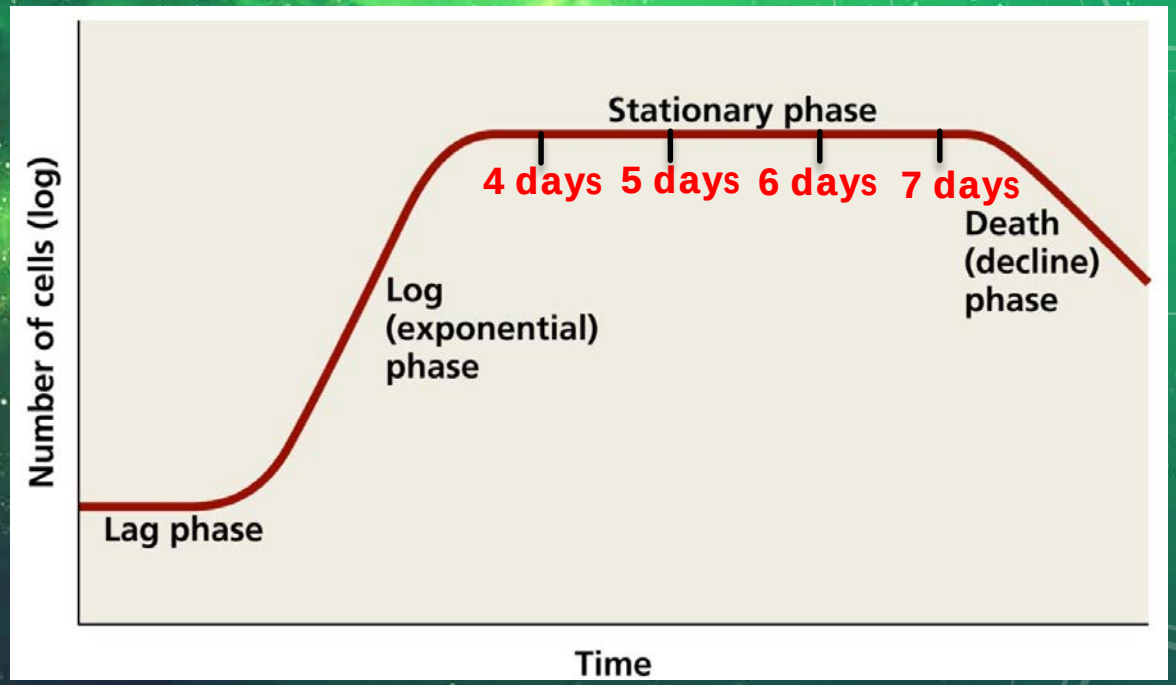
YEAST CELLS PREPARATION FOR SPACE

- Grown in liquid medium for 7 days
- Loaded and air dried into the wells of microfluidic card on Earth
- Mix of growth medium and metabolic indicator dye pumped into wells at time points to rehydrate the cells while in orbit
- 3-color LED detection system: measure changes in growth and metabolism resulting from ionizing radiation exposure
- Wild type control strain and a *rad51* mutant strain that is defective for DNA damage repair (more sensitive)



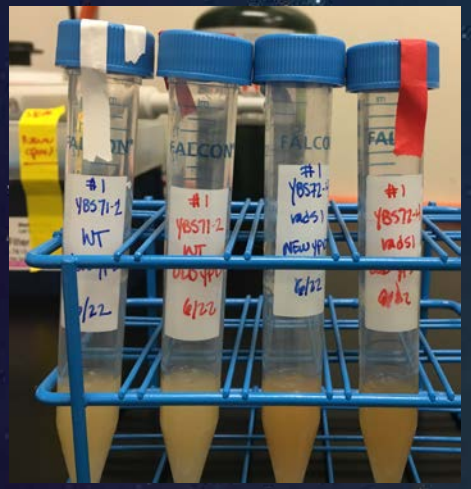
GOAL OF THE PROJECT

- Determine the growth period that improves desiccation tolerance but allows for retention of uniform radiation sensitivity
- An extended time in stationary phase: yeast are more tolerant to desiccation
- Excessive exposure in stationary phase
  - nitrogen starvation
  - loss of viability
  - heterogeneous cell population due to sporulation
    - Tetrads with 4 spores are formed
    - Indirect measure of stress
    - Spores are more tolerant to radiation



https://www.researchgate.net/figure/Typical-yeast-growth-curve-Saccharomyces-cerevisiae-grown-in-YPD-media-at-30C-for-12_fig1_235352009

YEAST DESICCATION PROCEDURE



Grow cells in liquid medium for a varying number of days (4-7 days) at room temperature

Plate some cells and grow on a YPD agar plate for 3 days at 30°C

- Provides undesiccated control which is a baseline for cell viability

Load a 96-well plate with cells with Trehalose drying buffer and left to air dry at 23°C

YPD = Yeast Extract Peptone Dextrose

Trehalose Drying Buffer = sugar to help protect cells when drying

Did this procedure for both *rad51* and wild type

YEAST REHYDRATING PROCEDURE

Place SC in the wells with dried cells, Wait 30 minutes

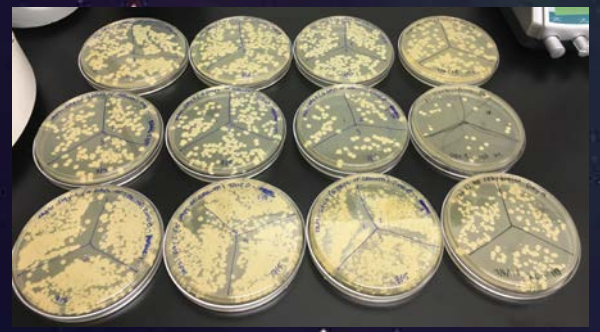
Mix and Plate on YPD agar plates

Wait 3 days at 30^{o}C

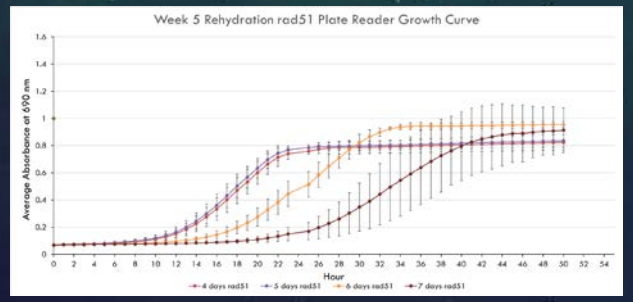
Place SC in the wells with dried cells, Wait 30 minutes

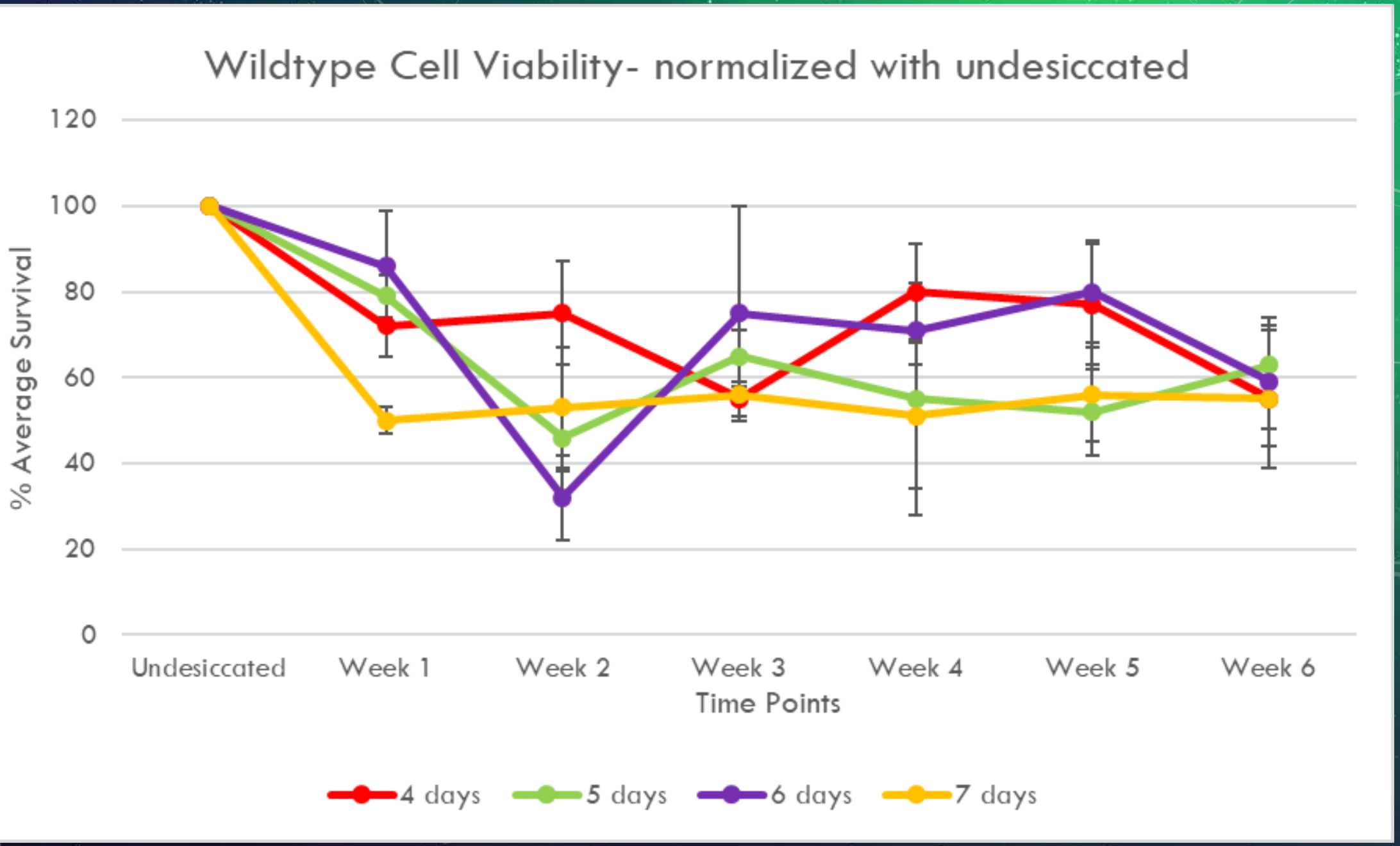
Mix and Place the mixture into another 96 well plate

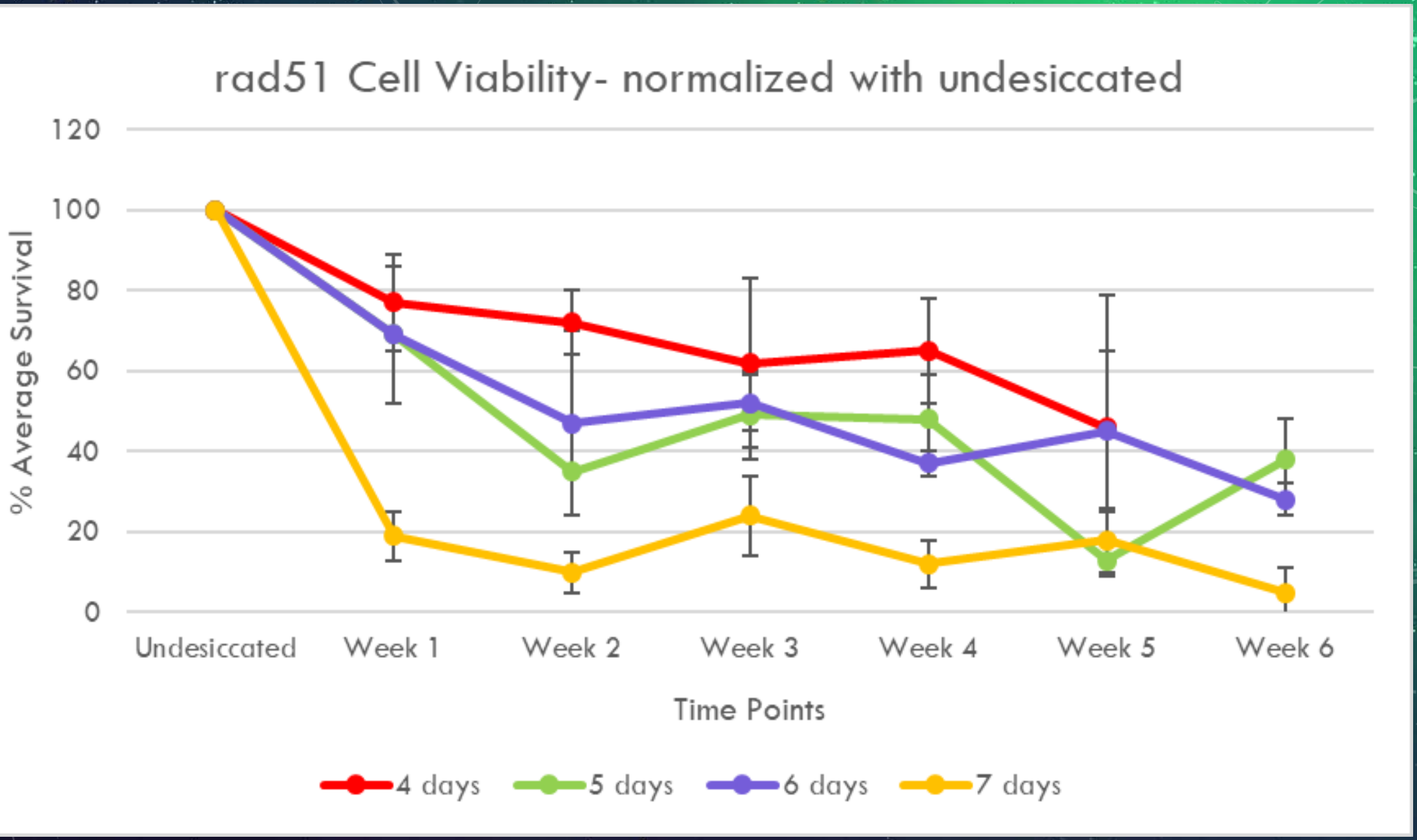
Start 24 hr plate reading, Observe growth curve at 690 nm absorbance which observes turbidity



SC = Synthetic Complete




Wildtype Cell Viability- normalized with undesiccated
120
100
80
60
40
20
0
% Average Survival
Undesiccated
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Time Points
4 days
5 days
6 days
7 days


rad51 Cell Viability- normalized with undesiccated
120
100
80
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% Average Survival
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Week 3
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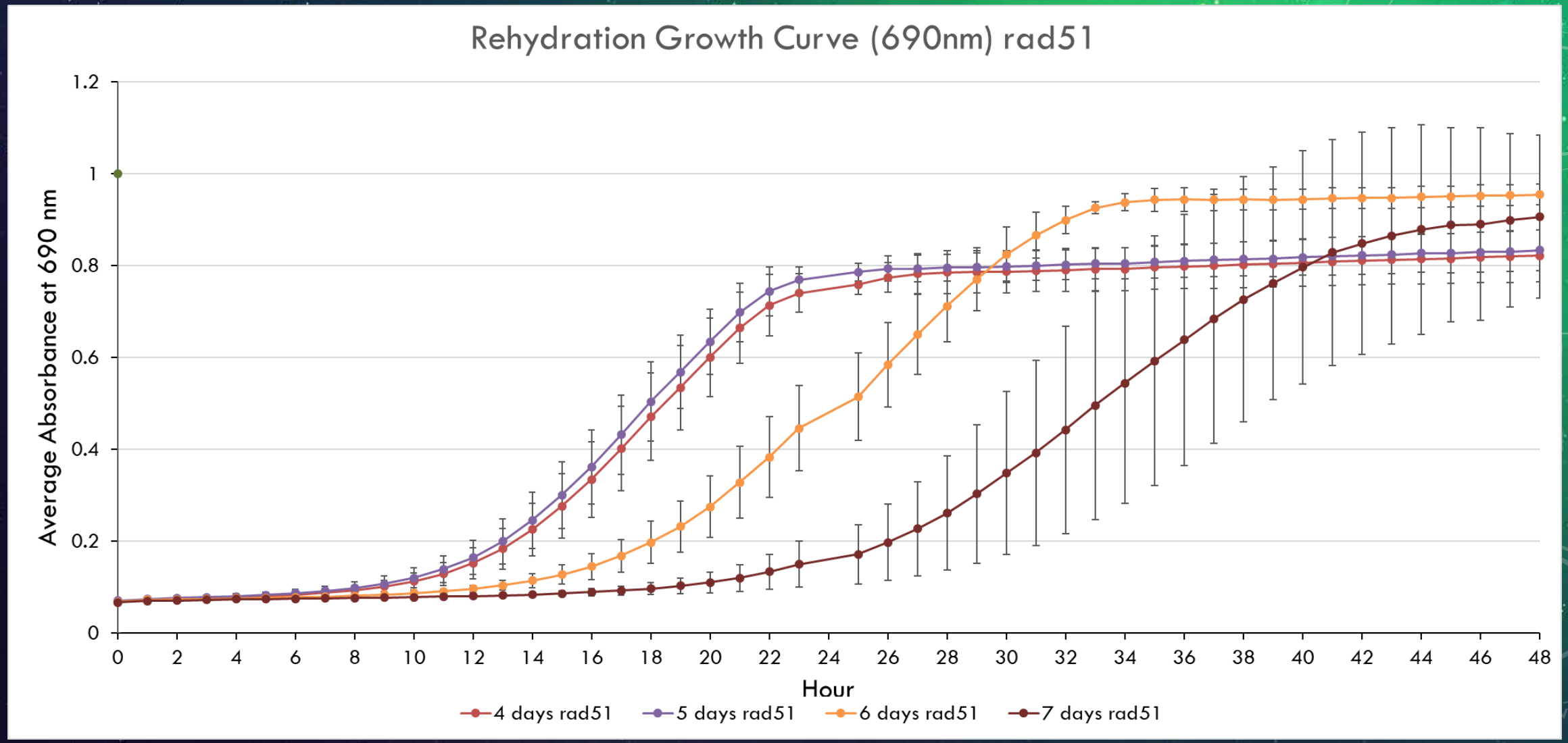
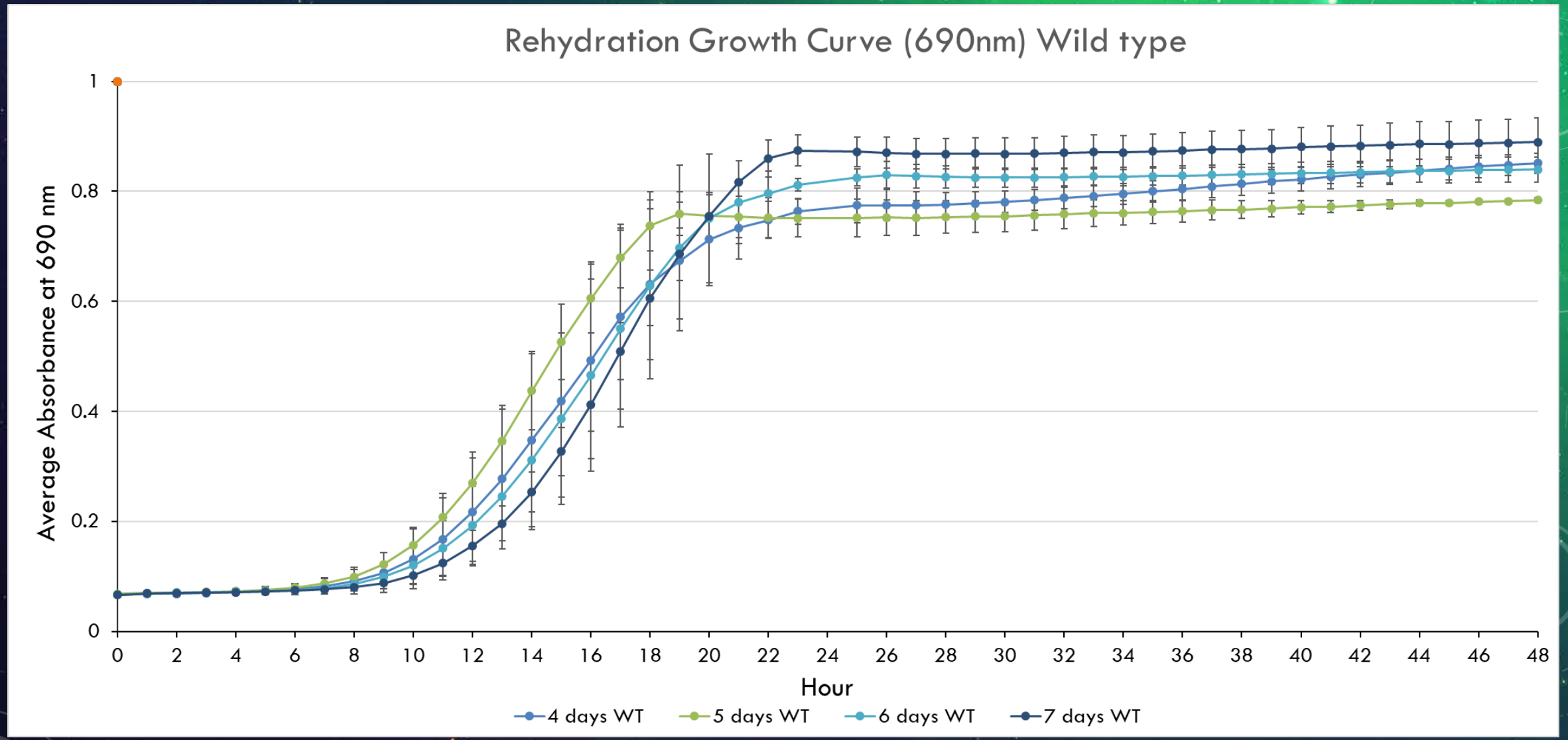
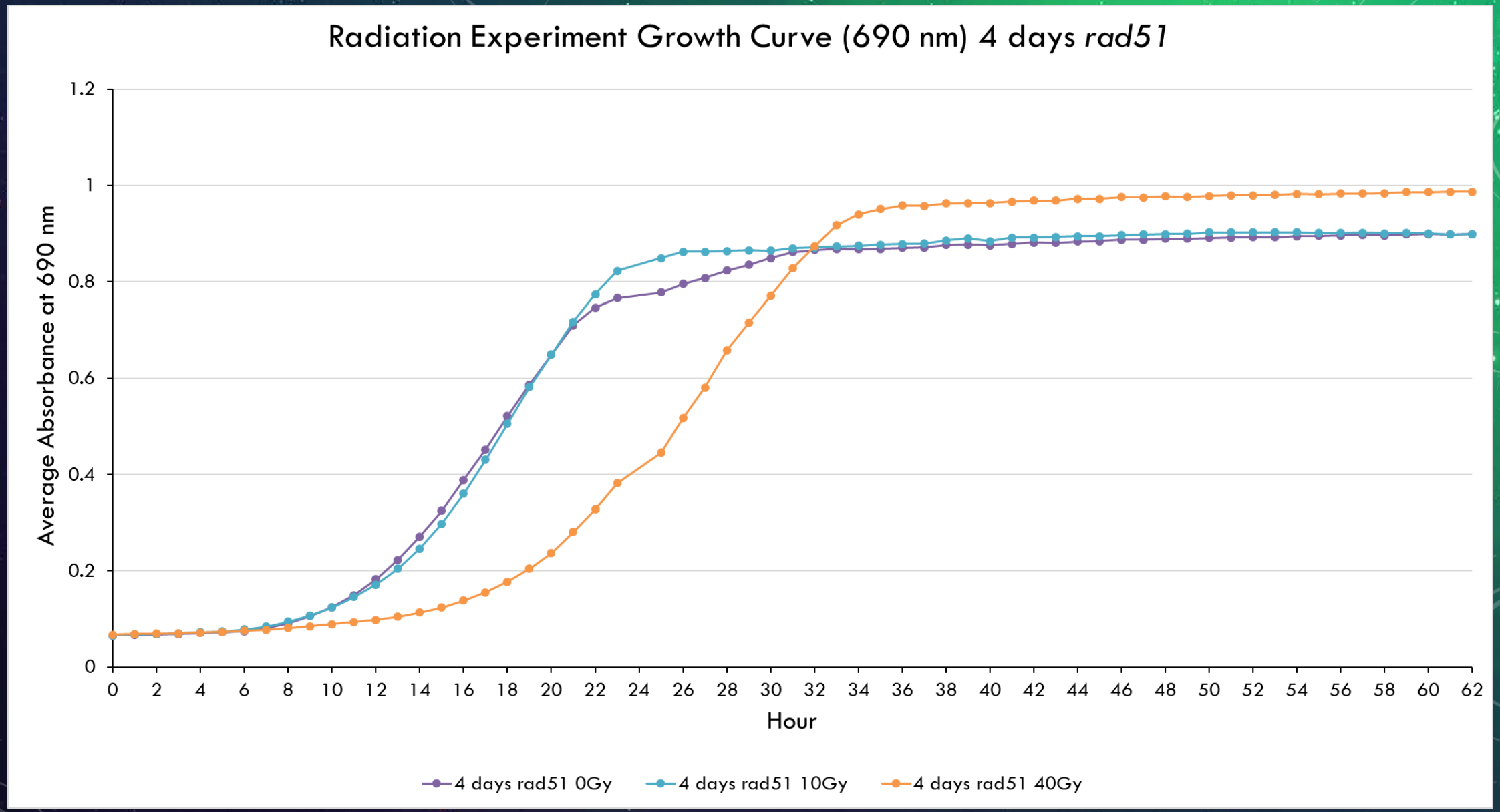


Week 5 Time Point of Rehydration Experiment

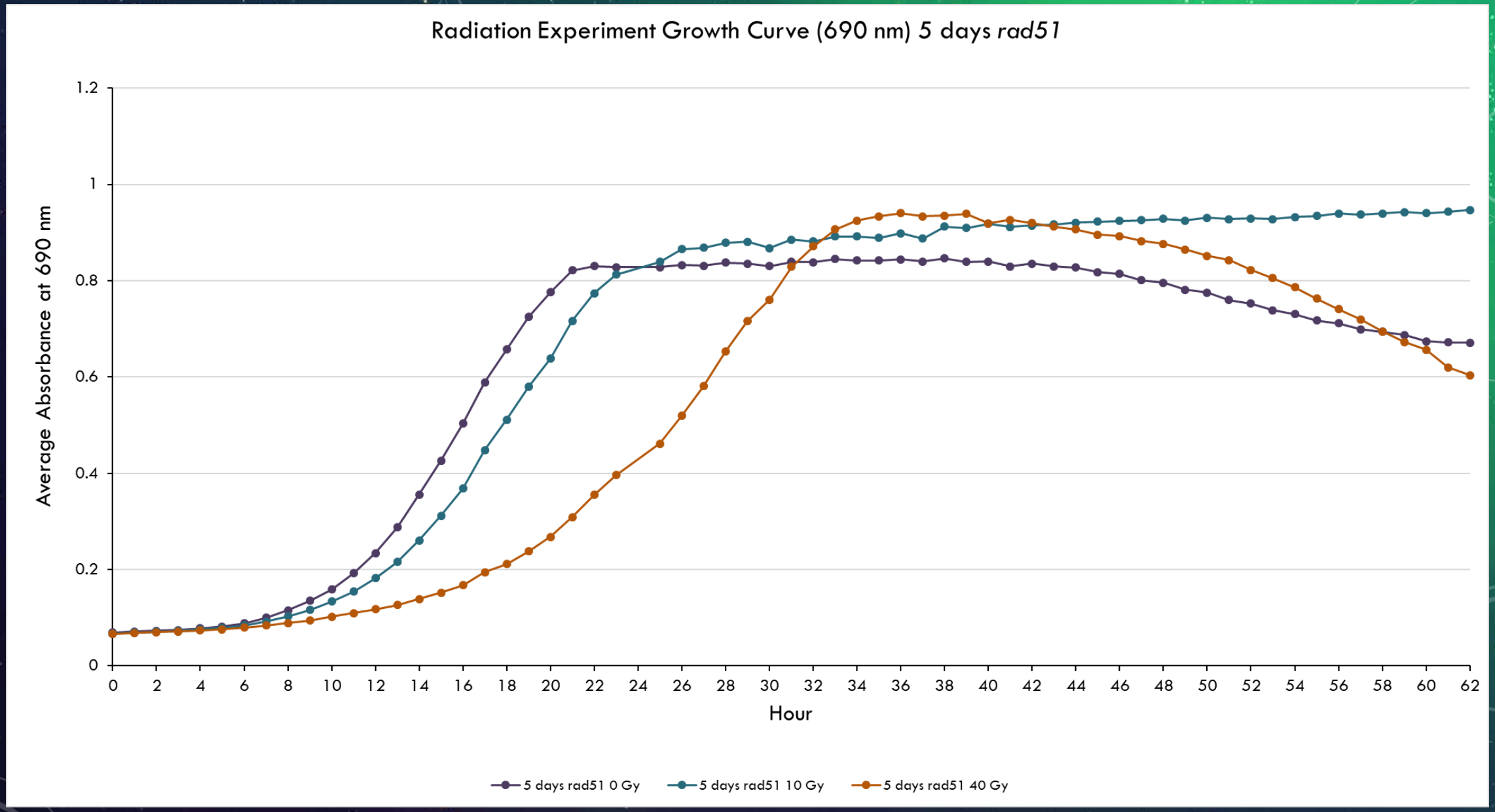


Week 5 Time Point of Rehydration Experiment



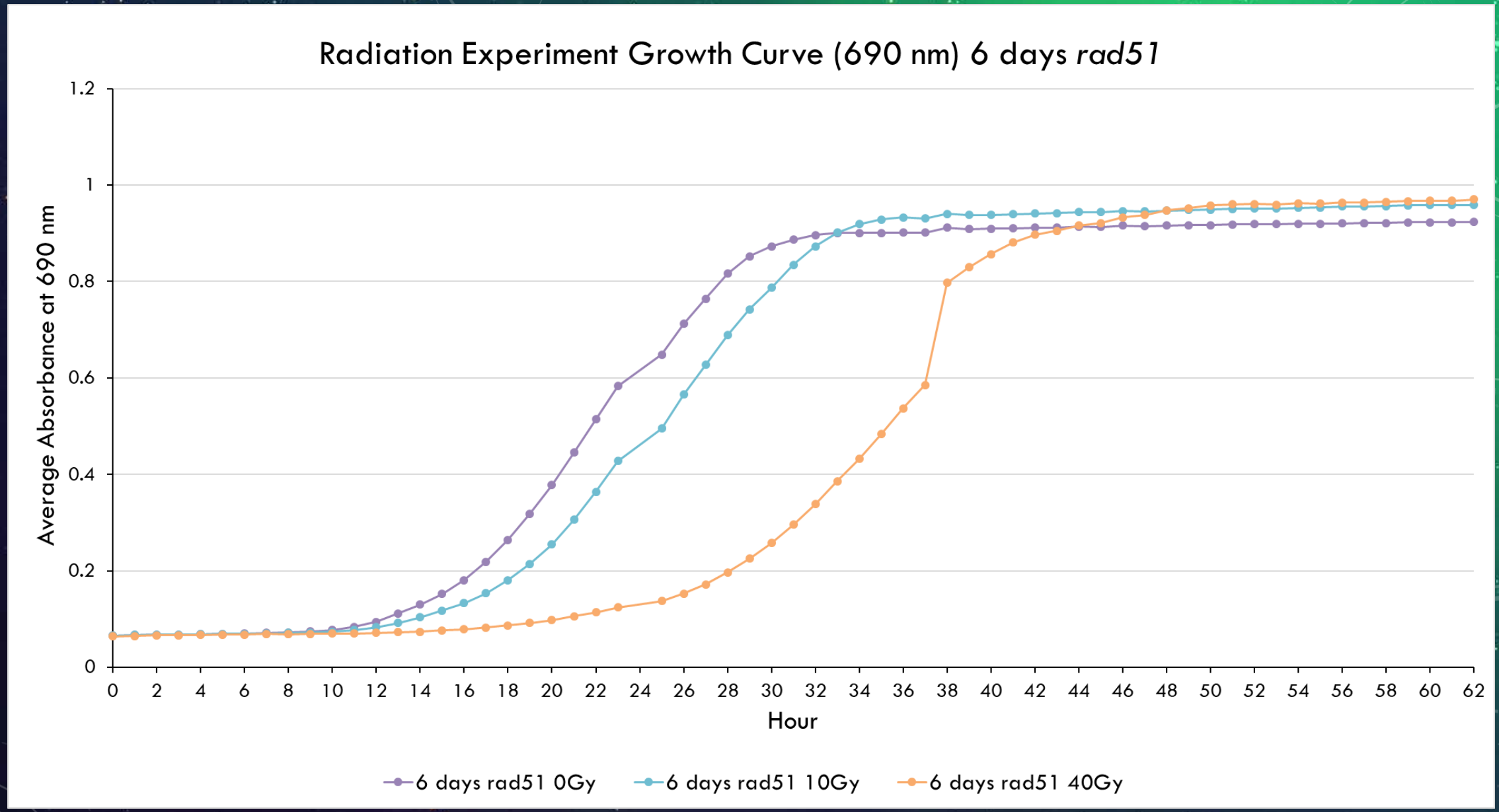
Gamma Radiation Experiment

4 days of growth *rad51*



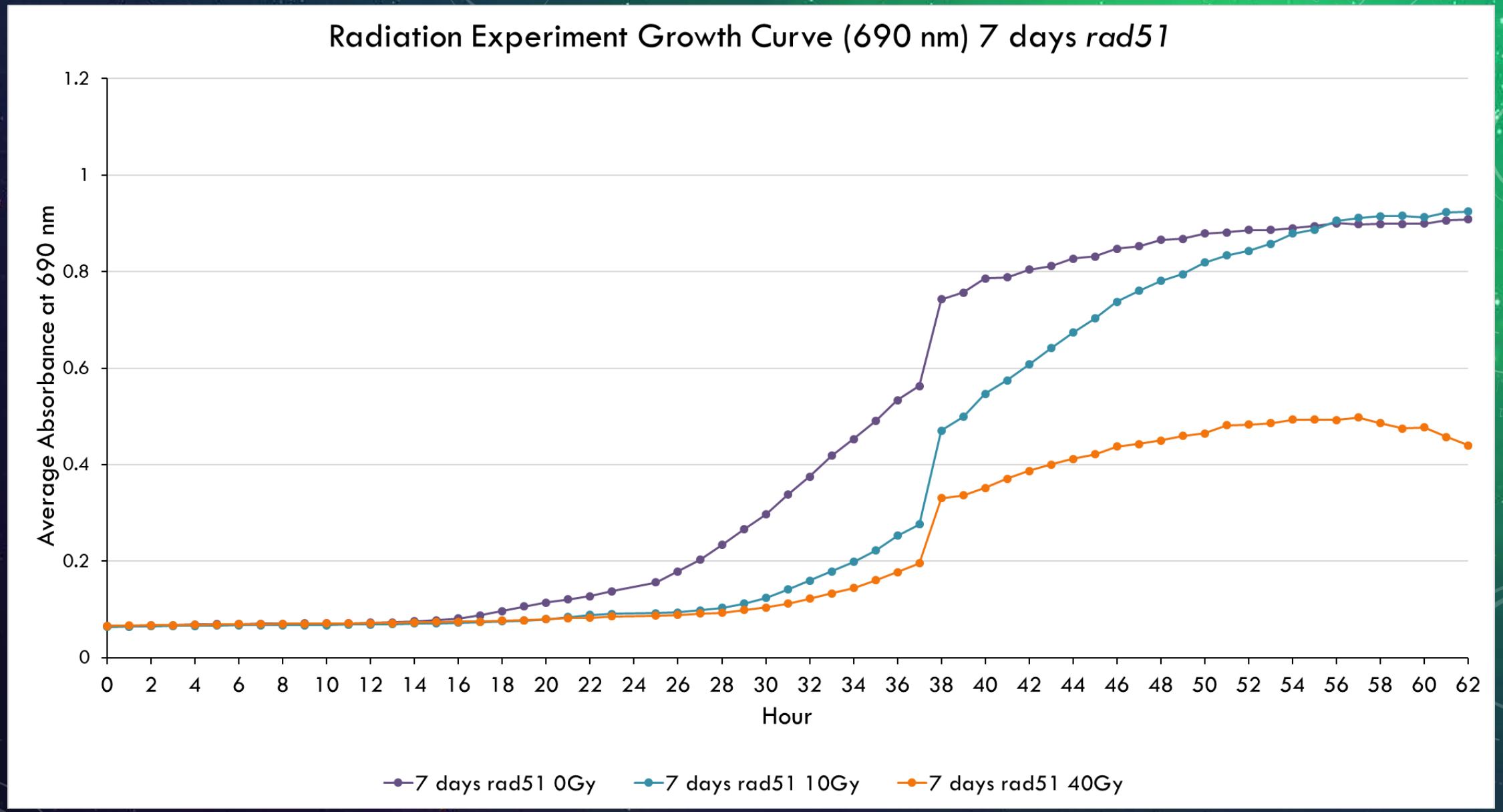
Gamma Radiation Experiment

6 days of growth *rad51*



Gamma Radiation Experiment

6 days of growth *rad51*



Gamma Radiation Experiment

7 days of growth *rad51*

CONCLUSION

- Currently 4, 5 and 6 days of growth is a better option than 7 days of growth
- Over time this may change
- Continue to take data points in the next few months
- The experiment may be repeated to confirm results

ACKNOWLEDGMENTS

- Sergio R. Santa Maria
- Sofia Tieze
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- Tristan Caro
- Aimee Johnson
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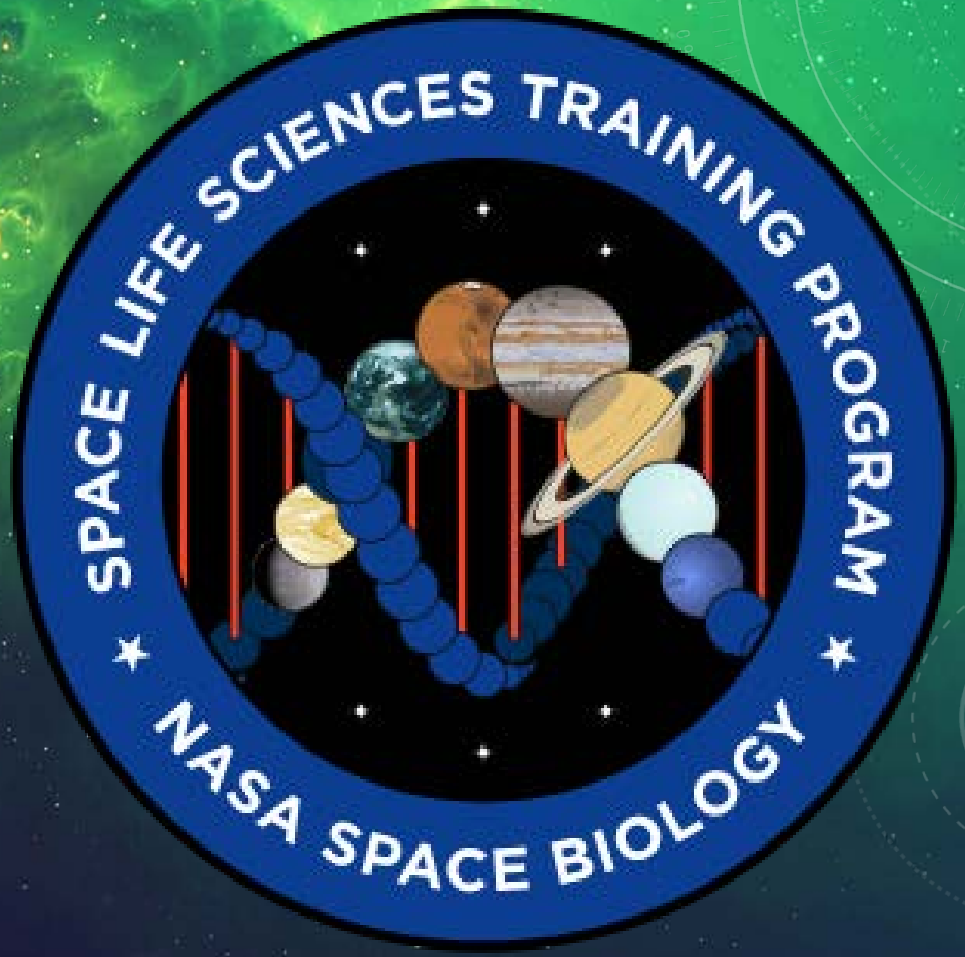
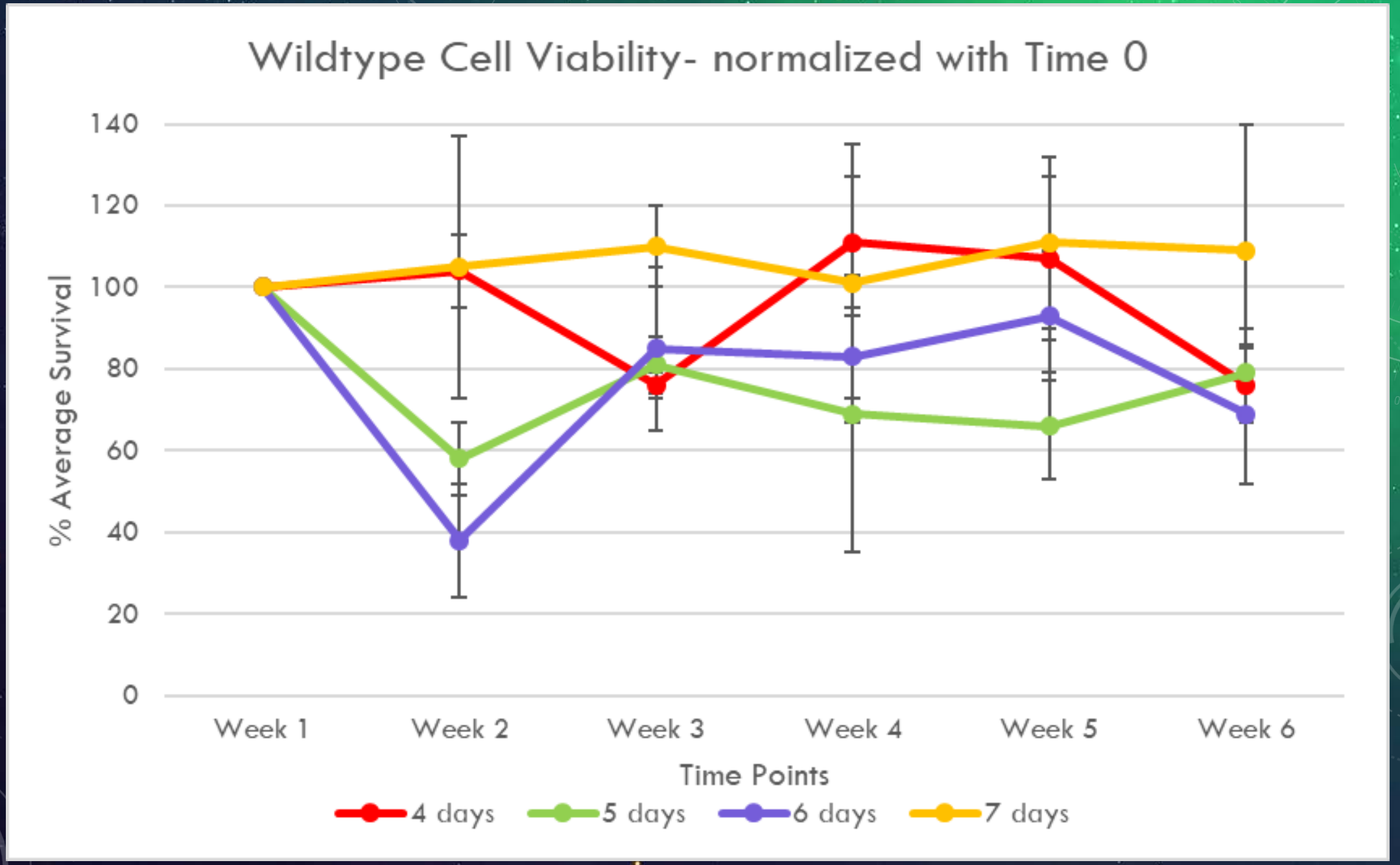
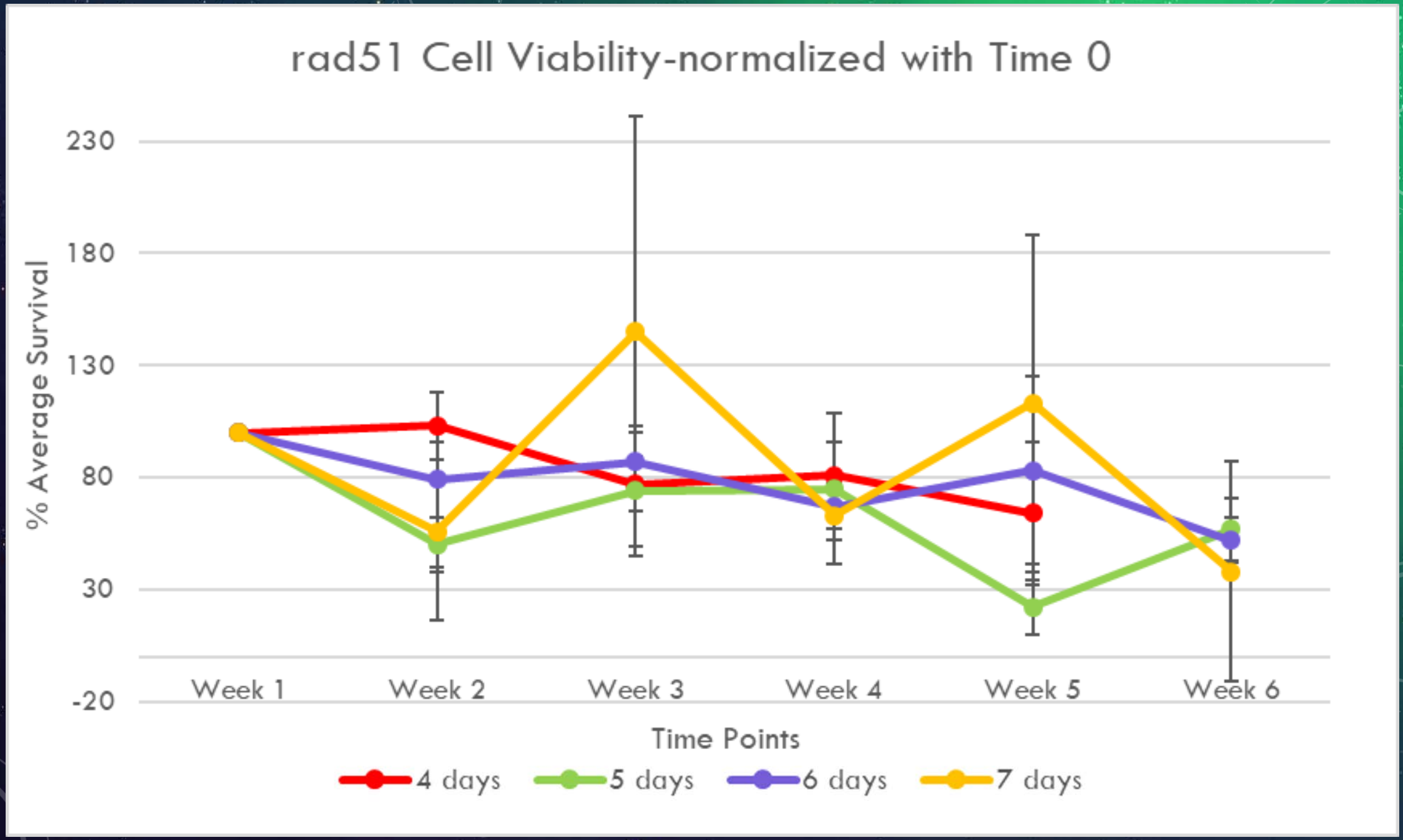
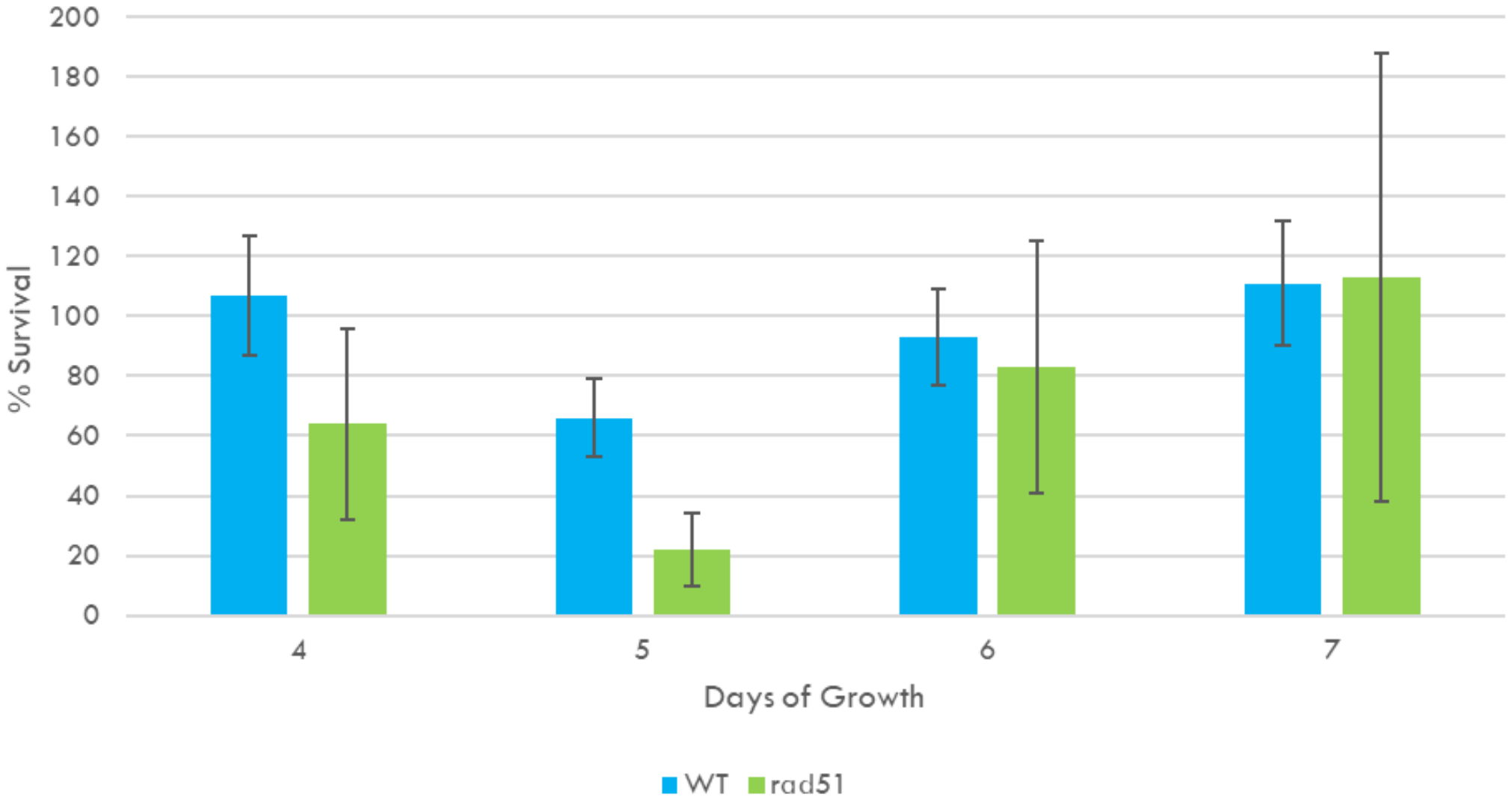
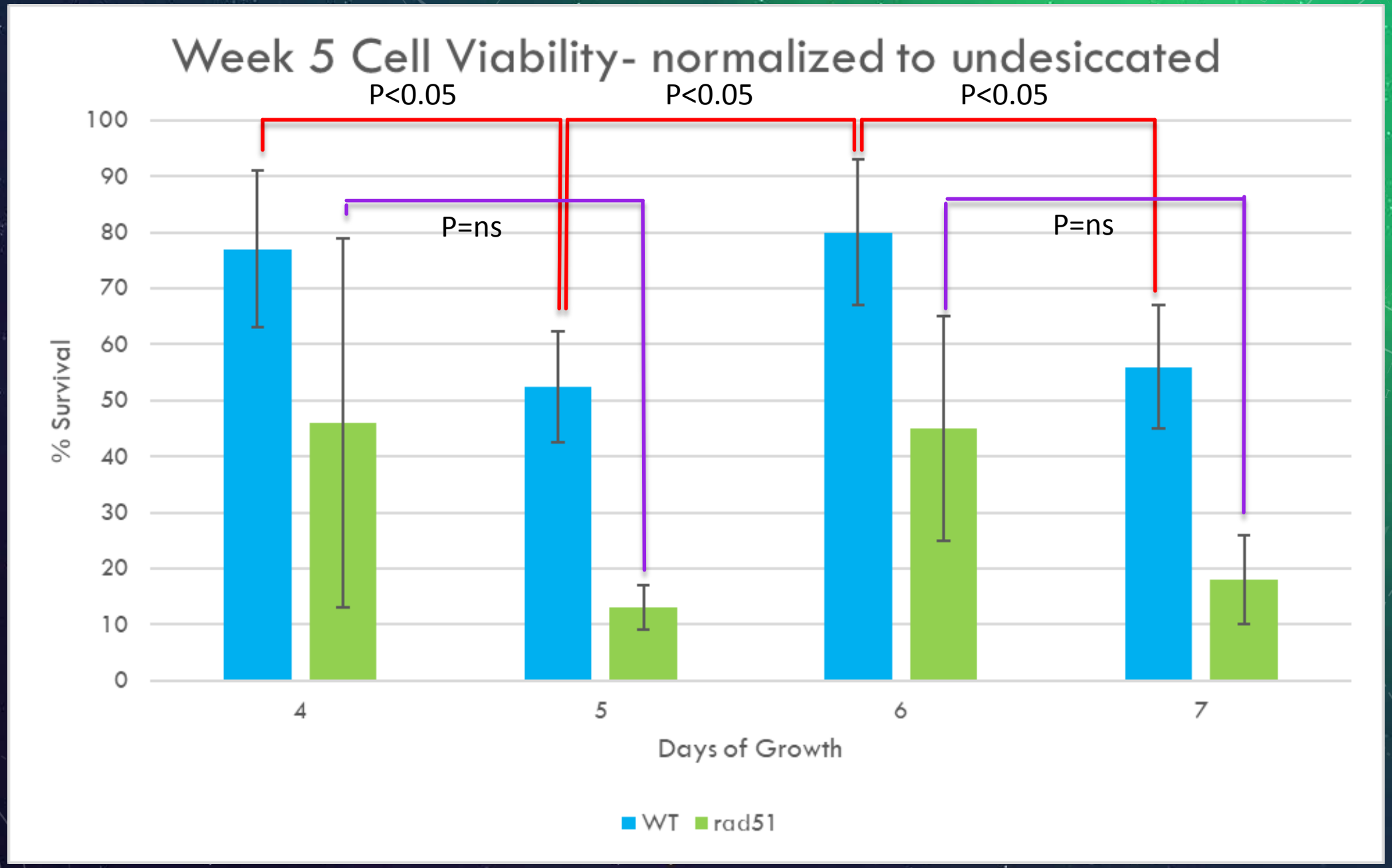


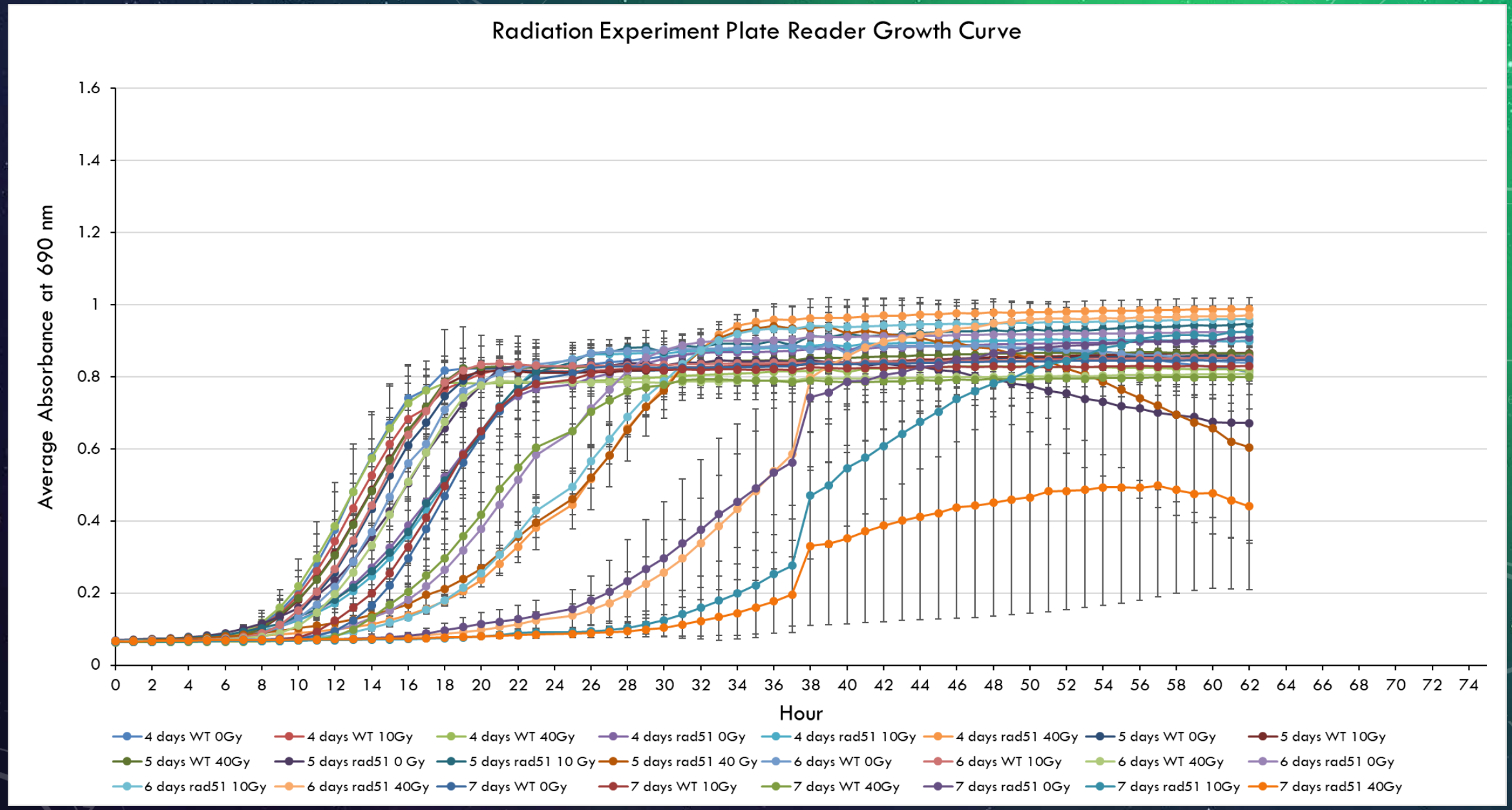

Wildtype Cell Viability- normalized with Time 0
% Average Survival
140
120
100
80
60
40
20
0
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Time Points
4 days
5 days
6 days
7 days


rad51 Cell Viability-normalized with Time 0
230
180
130
80
30
-20
% Average Survival
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Time Points
4 days
5 days
6 days
7 days

Week 5 Cell Viability- normalized to Time 0




Week 5 Cell Viability- normalized to undesiccated
P<0.05
P<0.05
P<0.05
P=ns
P=ns
100
90
80
70
60
50
40
30
20
10
0
% Survival
4
5
6
7
Days of Growth
WT
rad51


Radiation Experiment Plate Reader Growth Curve
Average Absorbance at 690 nm
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60 62 64 66 68 70 72 74
Hour
4 days WT 0Gy
4 days WT 10Gy
4 days WT 40Gy
4 days rad51 0Gy
4 days rad51 10Gy
4 days rad51 40Gy
5 days WT 0Gy
5 days WT 10Gy
5 days WT 40Gy
5 days rad51 0 Gy
5 days rad51 10 Gy
5 days rad51 40 Gy
6 days WT 0Gy
6 days WT 10Gy
6 days WT 40Gy
6 days rad51 0Gy
6 days rad51 10Gy
6 days rad51 40Gy
7 days WT 0Gy
7 days WT 10Gy
7 days WT 40Gy
7 days rad51 0Gy
7 days rad51 10Gy
7 days rad51 40Gy