

THE AMENDED CELL SIGNALLING OF *IN VIVO*-MIMETIC 3D STRUCTURES

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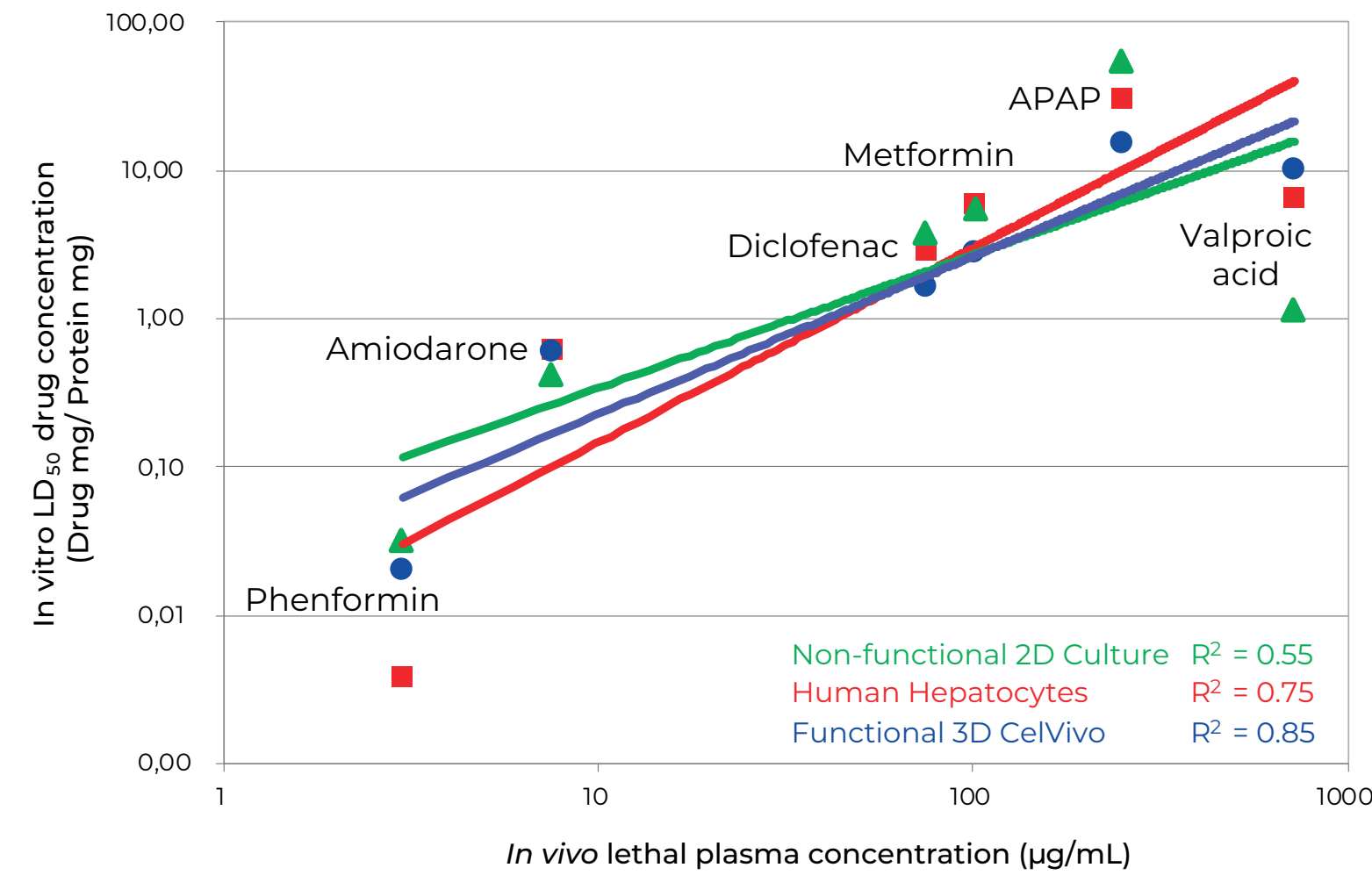
INTRODUCTION

We have developed a 3D model which mimics *in vivo* functionality

Using a clinostat we grow the hepatocellular cell line (HepG2/C3A) into functionally mature liver-mimetic spheroids. They are large (~450µm radius) and can be maintained for >300 days.

They are more predictive of *in vivo* toxicology than primary human hepatocytes. Cell lines grown in 2D cultures are essentially not predictive.

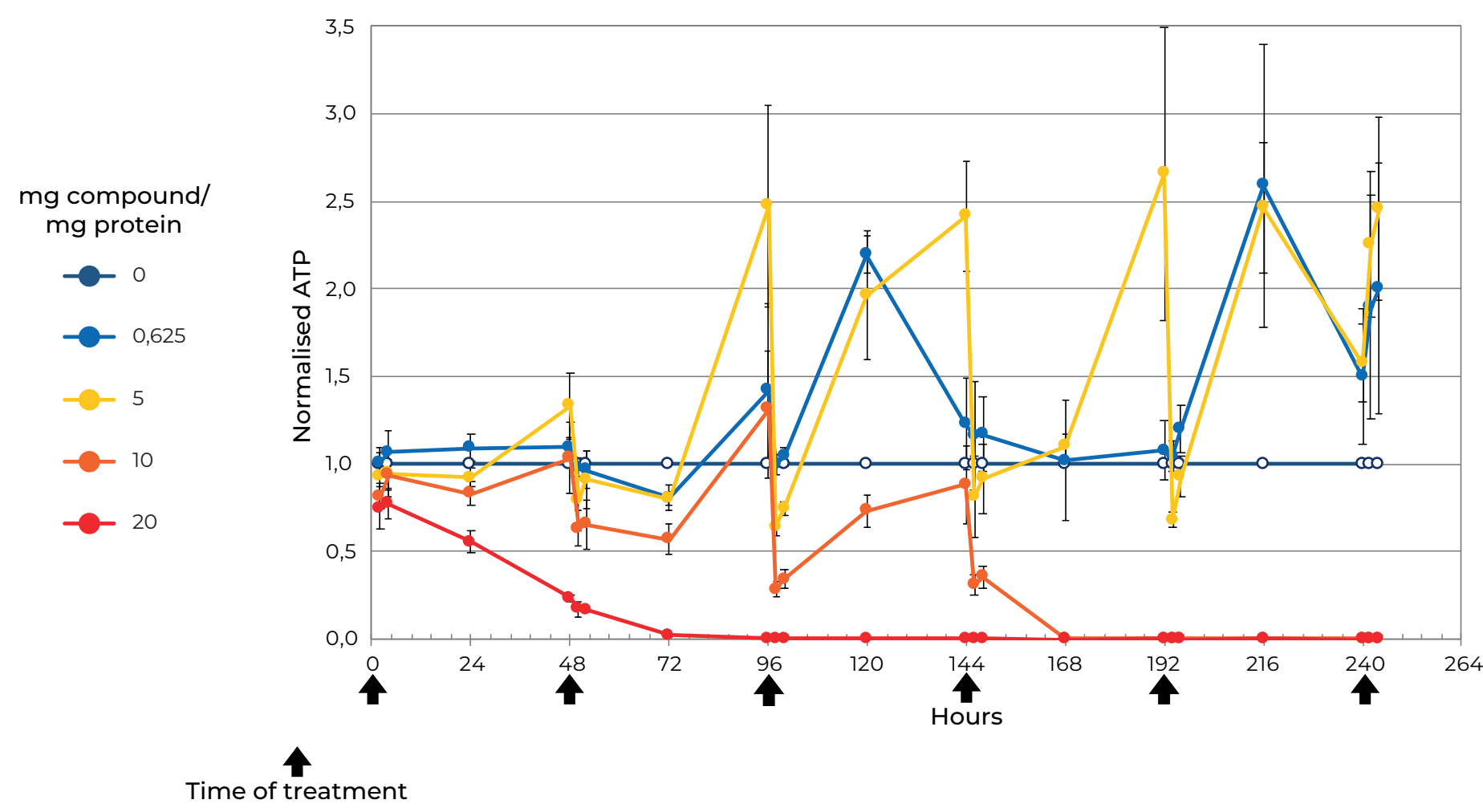
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Treatment of 21 day old C3A spheroids with APAP

This clinostat model can also be used for repeated dose testing. A single high doses of APAP (20 mg / mg cellular protein) kill the spheroids (acutely lethal). Half of that dose can kill the spheroids if given repetitively (chronically lethal). Half the dose again produces a response after which the spheroids recover. This can be repeated many times. Lower doses again produce the same response and recovery result.

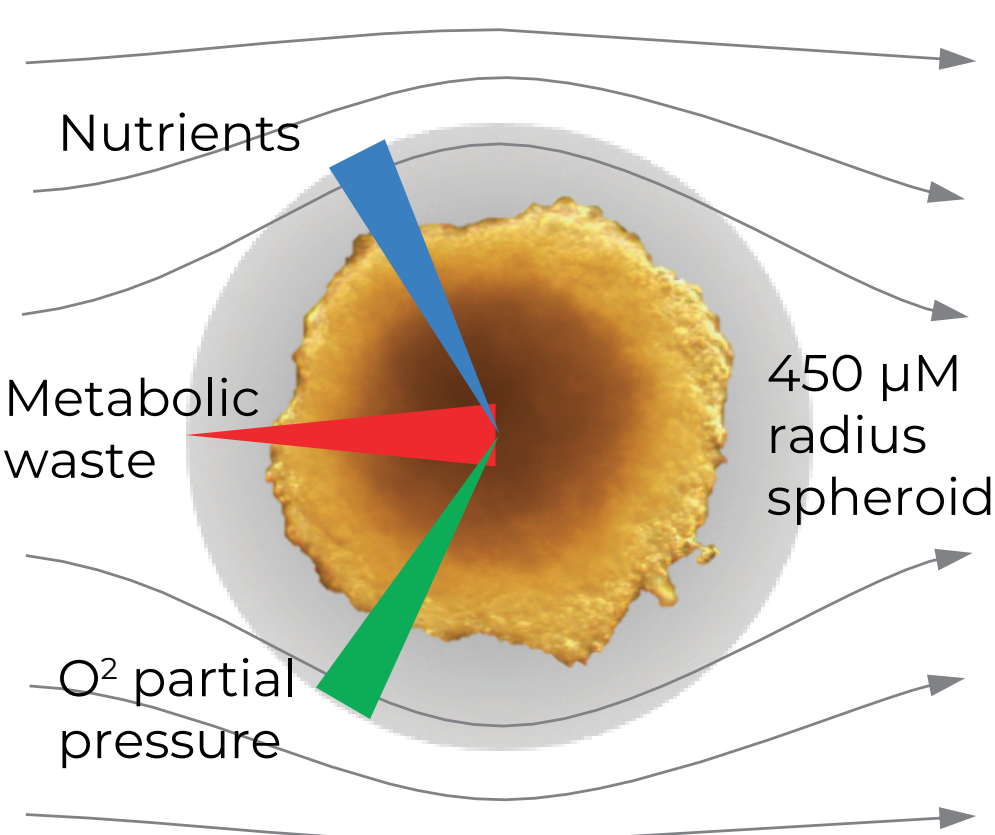
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The factors that drive recovery of the physiological phenotype are hypoxia and nutrient shortage

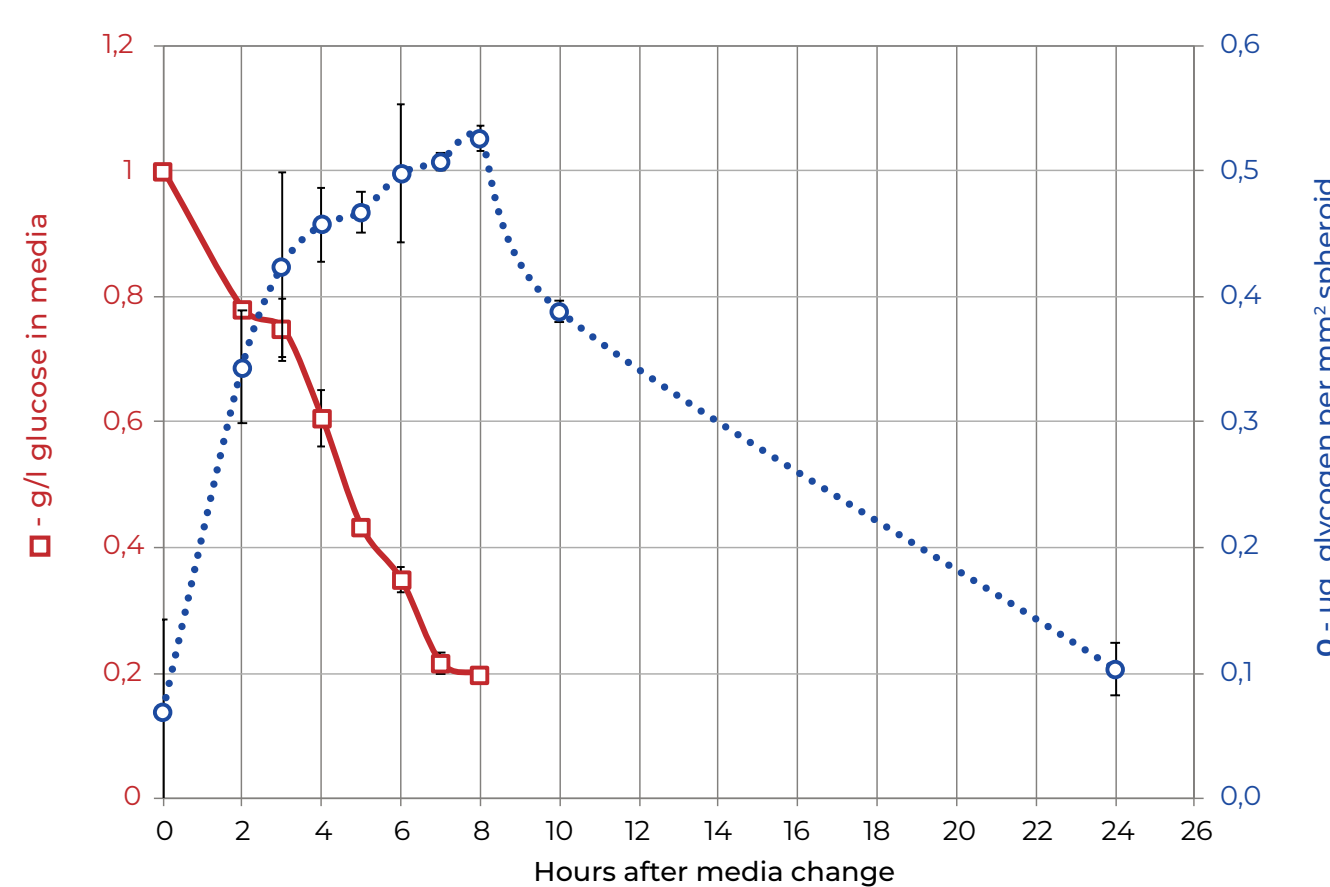
The 3D structure results in an oxygen gradient into the spheroid (as is seen in tissues *in vivo*).

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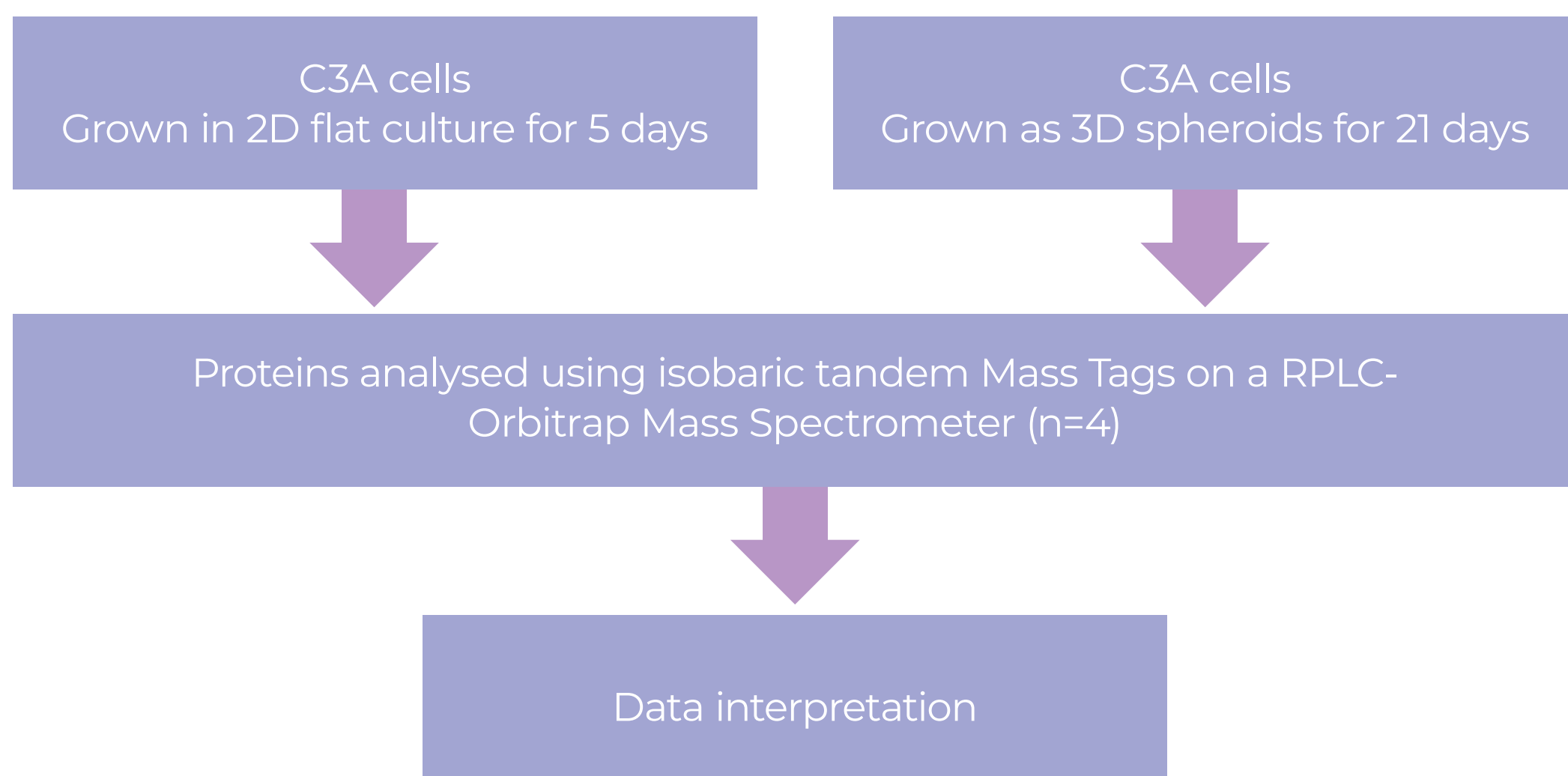
The spheroids rapidly consume all the glucose in the media. This means that they quickly become nutrient depleted.

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In order to determine why cells grown in 2D and 3D are so different, we analysed cells grown in 2D and 3D by mass spectrometry.

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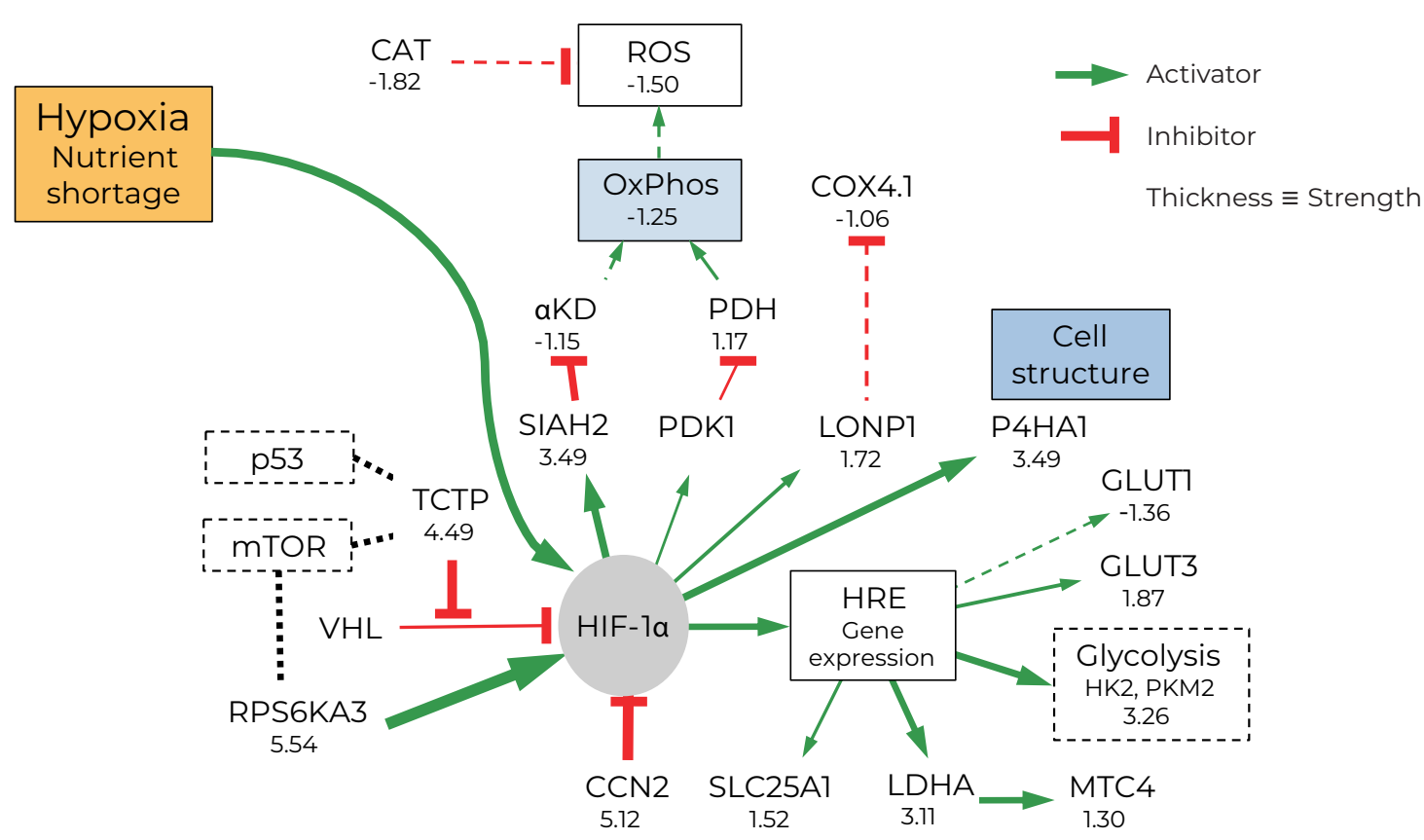


RESULTS

Hypoxia and nutrient shortage

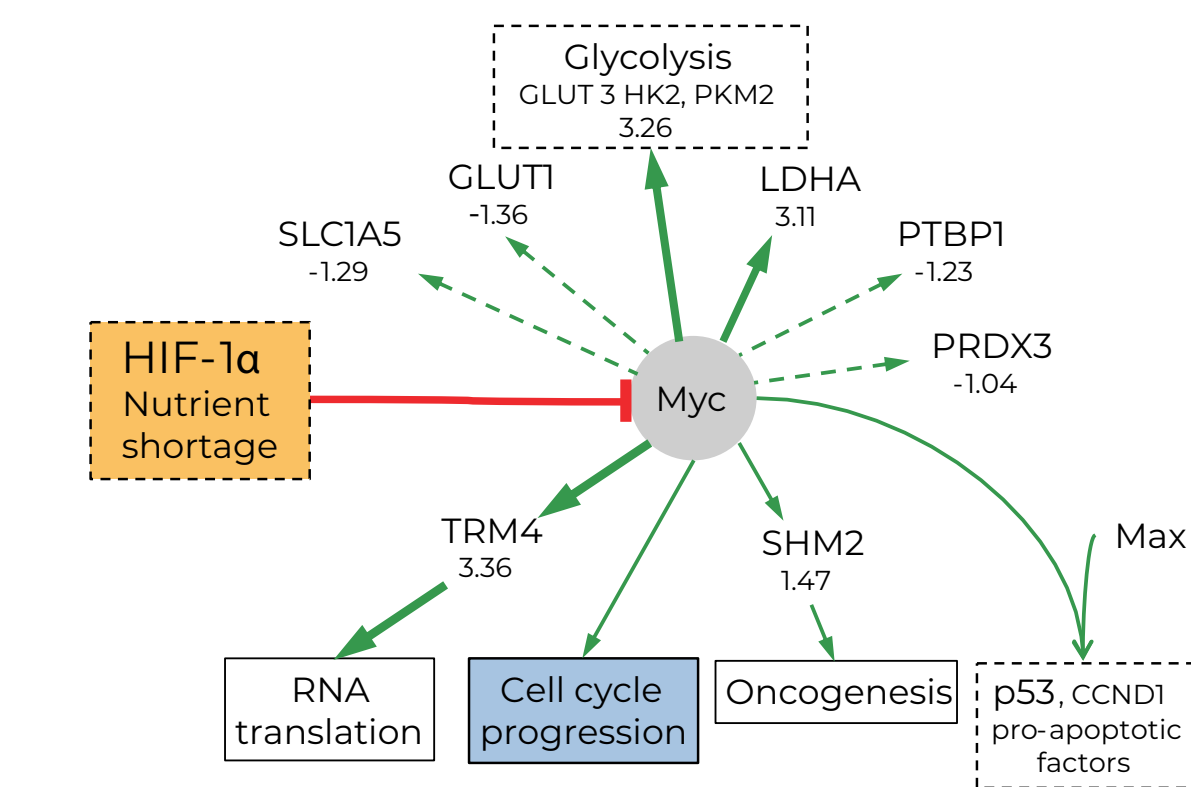
Hypoxia and nutrient shortage appear to be the primary driver of changes in various signalling pathways leading to alterations of the cell structure and a slight decrease in oxidative phosphorylation.

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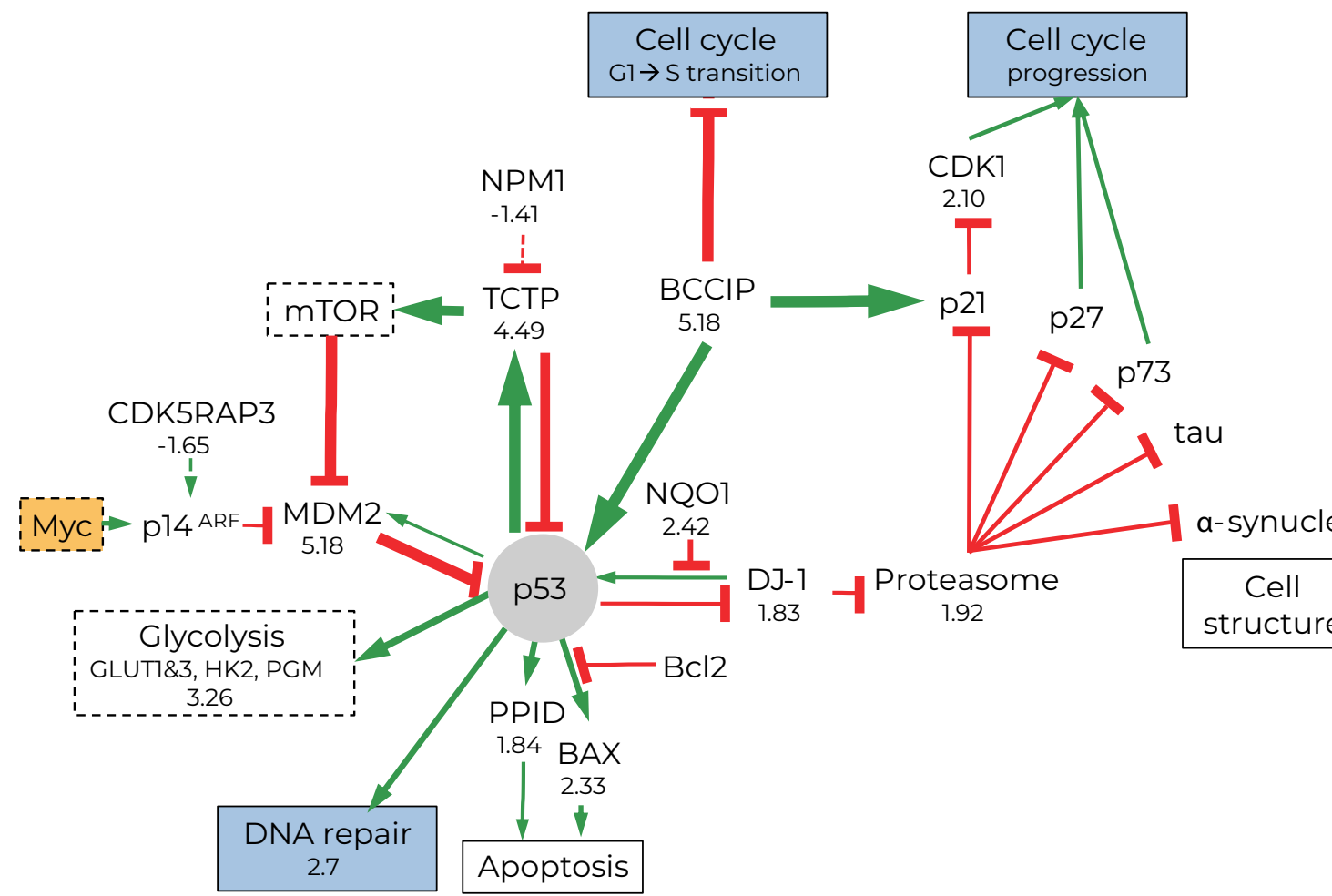
Hypoxia and nutrient shortage also affect Myc

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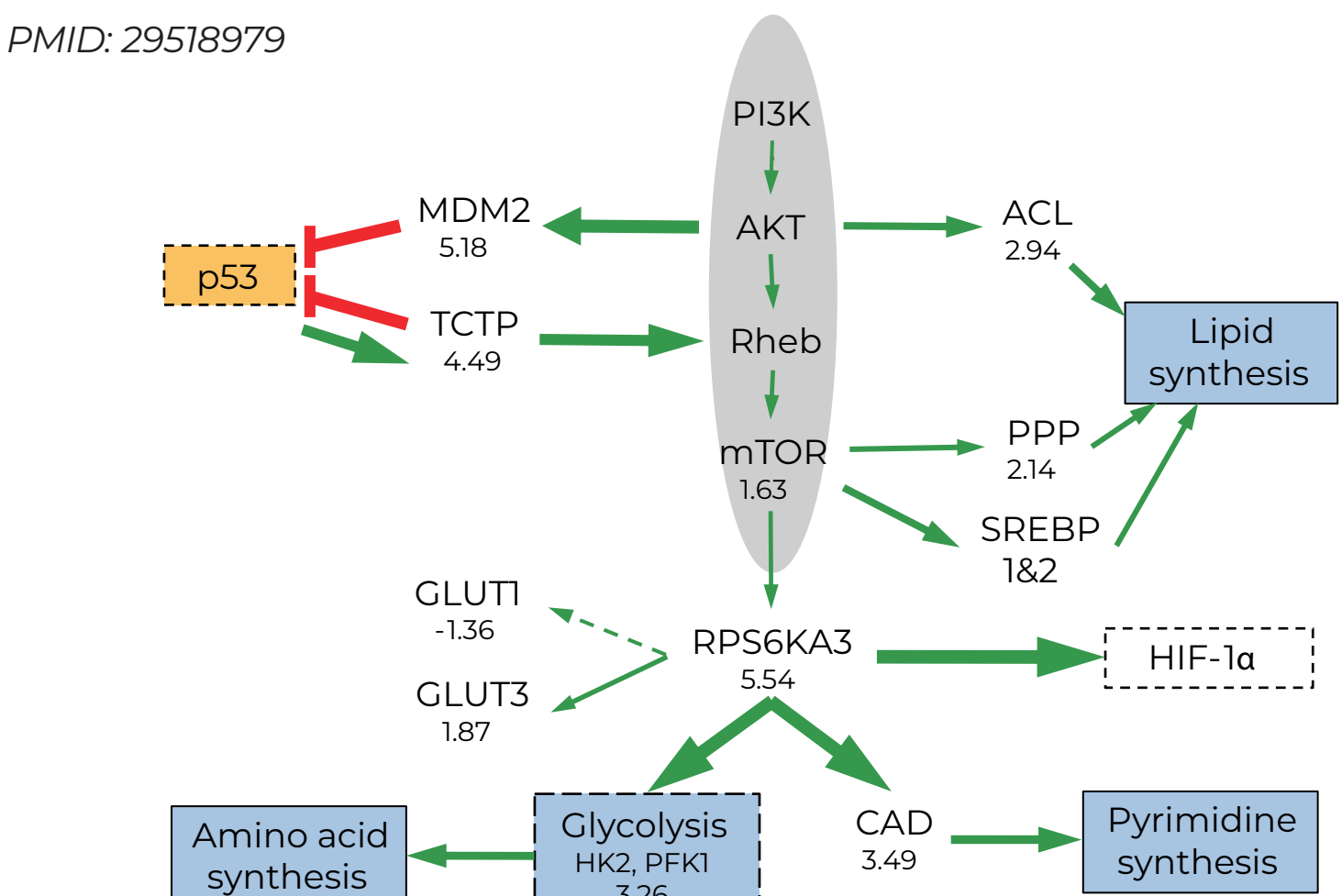
Myc affects the p53 pathway leading to changes in DNA organisation and transcription

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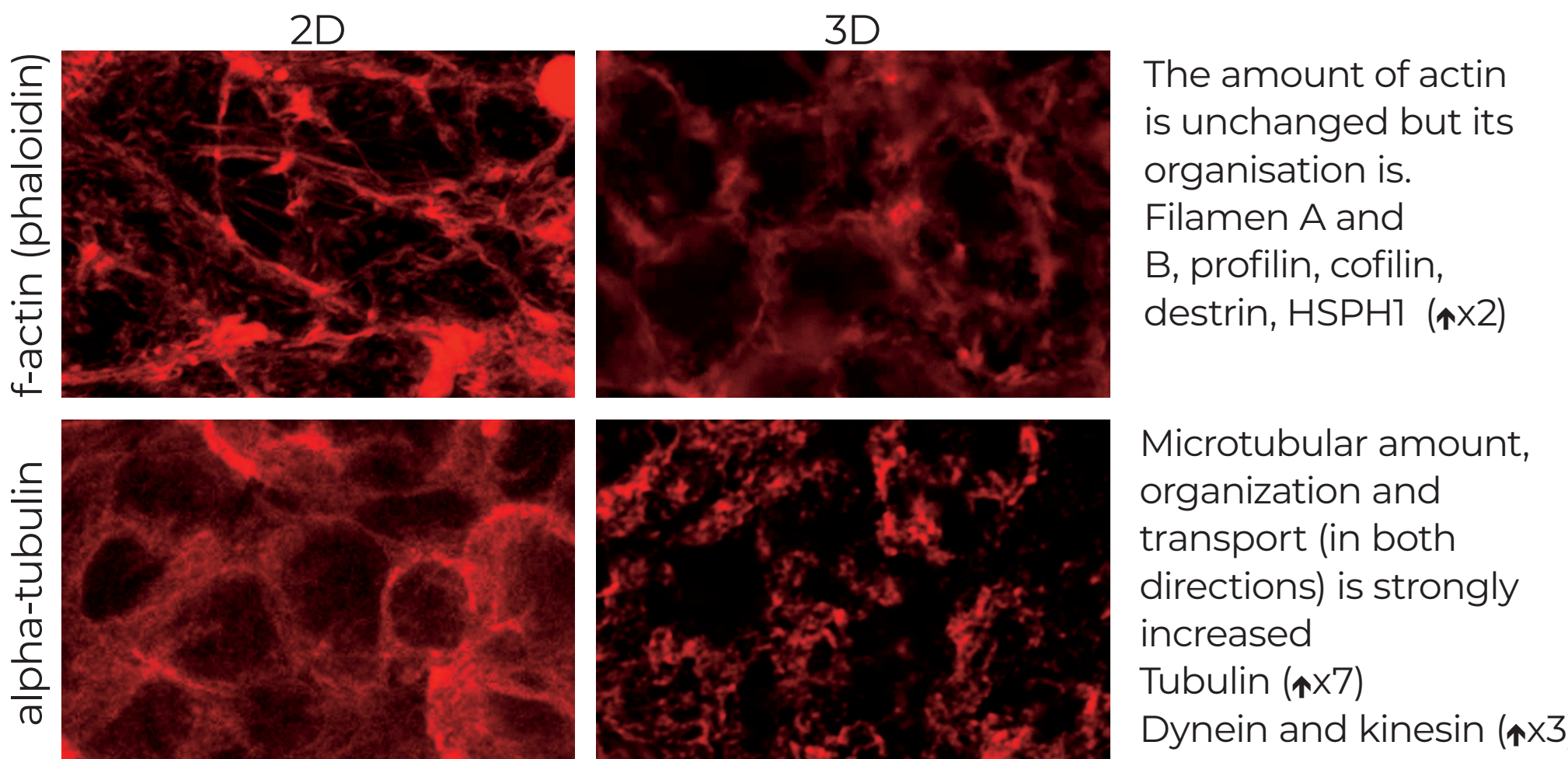
P53 and TCTP interact with the mTOR pathway increasing anabolic processes

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Significant changes in cell structure

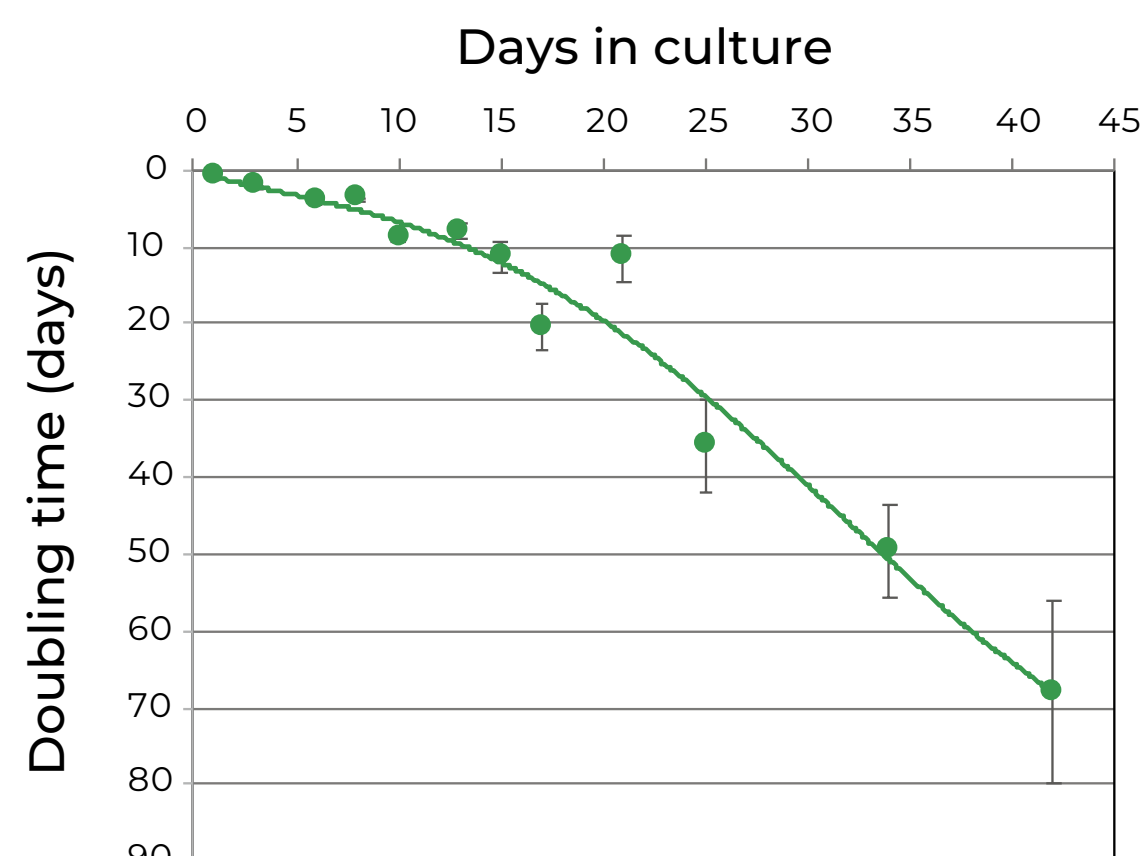
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Cell cycle progression slows significantly

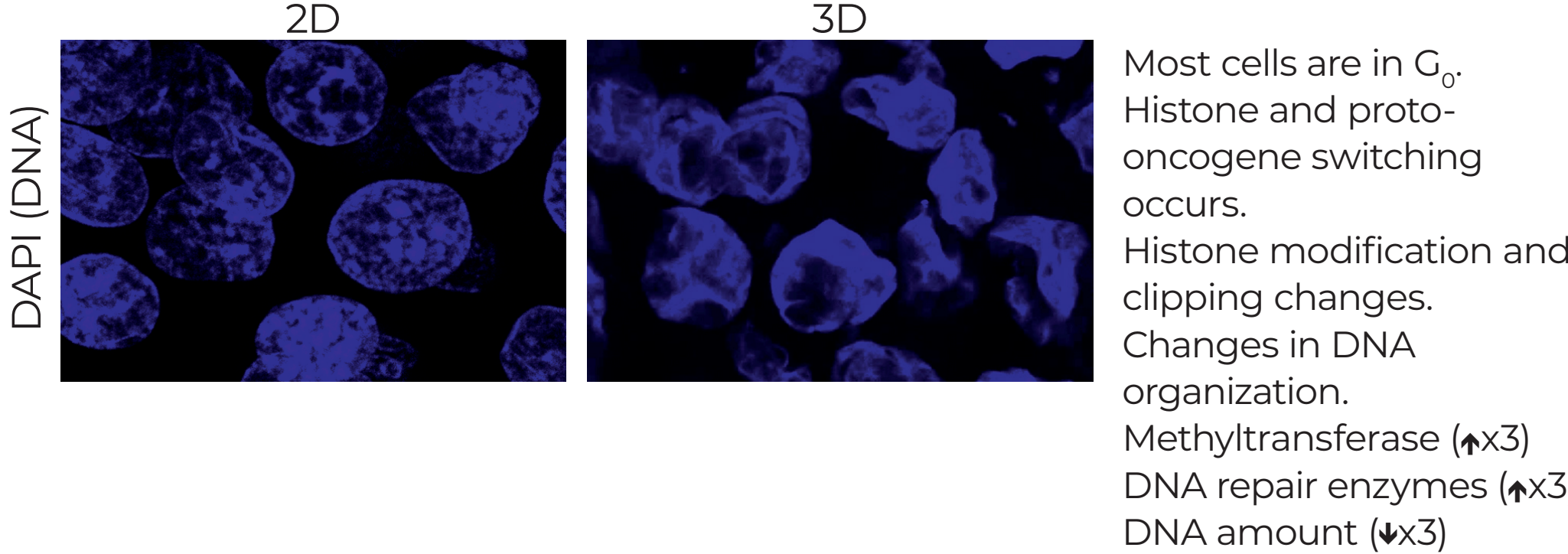
The growth rate of cells in the 3D clinostat spheroids decreases from a doubling time of one day to 70 days after 42 days. For comparison, human liver hepatocytes are replaced every 200 – 300 days.

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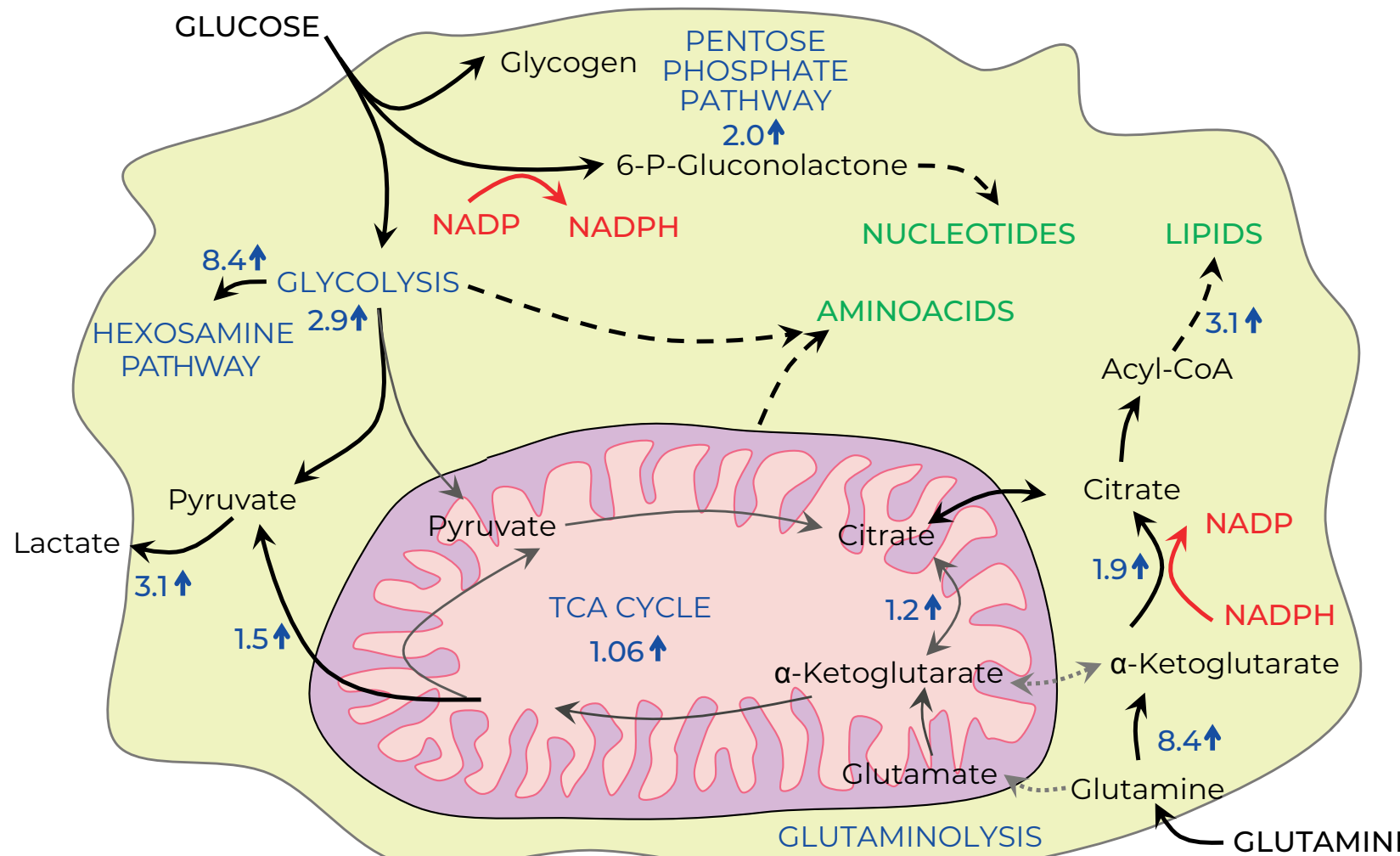
DNA repair is increased despite that there is less DNA per cell

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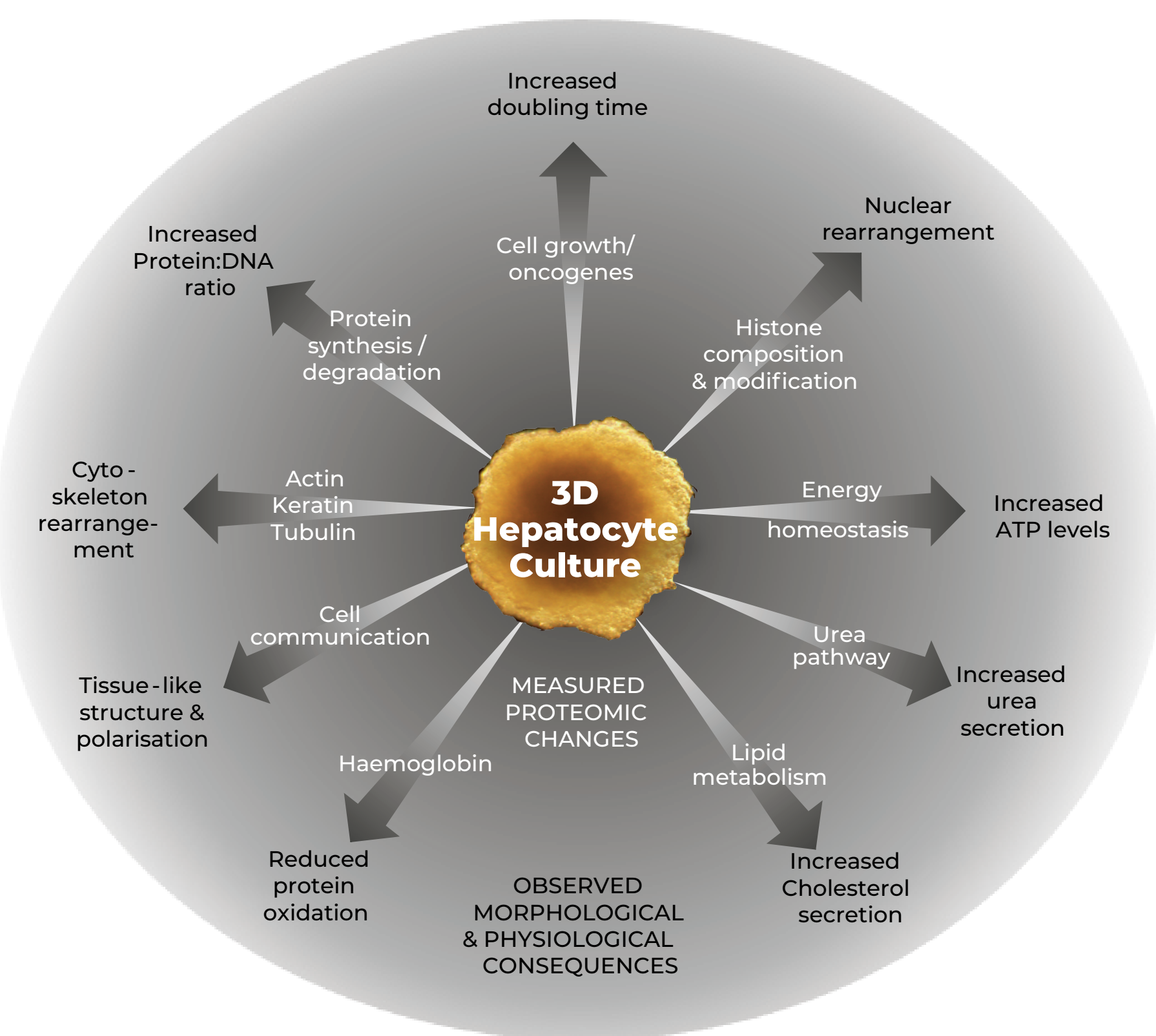
Changes in signalling pathways drive metabolic reprogramming from oxidative phosphorylation towards aerobic glycolysis

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CONCLUSION

For 3D spheroids to be able to mimic human tissues, virtually every cellular process needs to be changed



The importance of various actors in signalling pathways *in vivo* is clearly very different to that seen *in vitro* in 2D cultures.

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