# Studying Ageing in the Lab

## Investigating Metabolic Changes in a Cellular Model of Ageing

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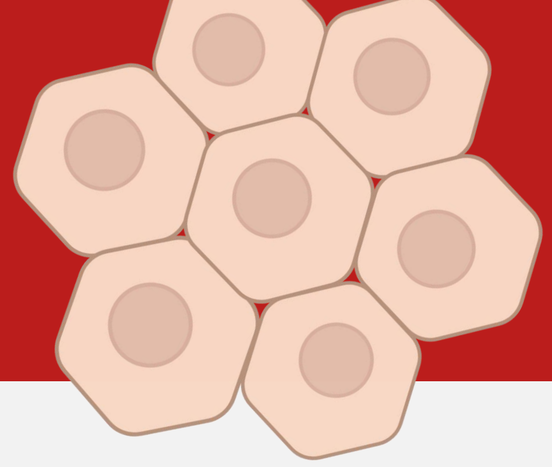


## Research objective and cell types

Because of today's technology and medicine, the elderly population is the biggest it's ever been, which is taking a toll on the provision of healthcare services and the individuals' quality of life. The incidence of metabolic syndromes such as diabetes increases with age.

Investigating age-related changes in cultured cells is the most efficient and least ethically problematic way to research ageing. In this study we compared two U2OS cell lines, one being wildtype (WT) that has its original genome, to one that has the gene for the stress granule protein G3BP1/2 "knocked out". The goal was to see how the KO of this gene may affect metabolic properties with age.

## Lab induced ageing

In figure 1 we see the process of cells becoming old. At day 5 and 7 the wildtype cells were more resistant than KO, in terms of staying more intact. KO line is more sensitive and therefore had fewer cells, especially on day 5.

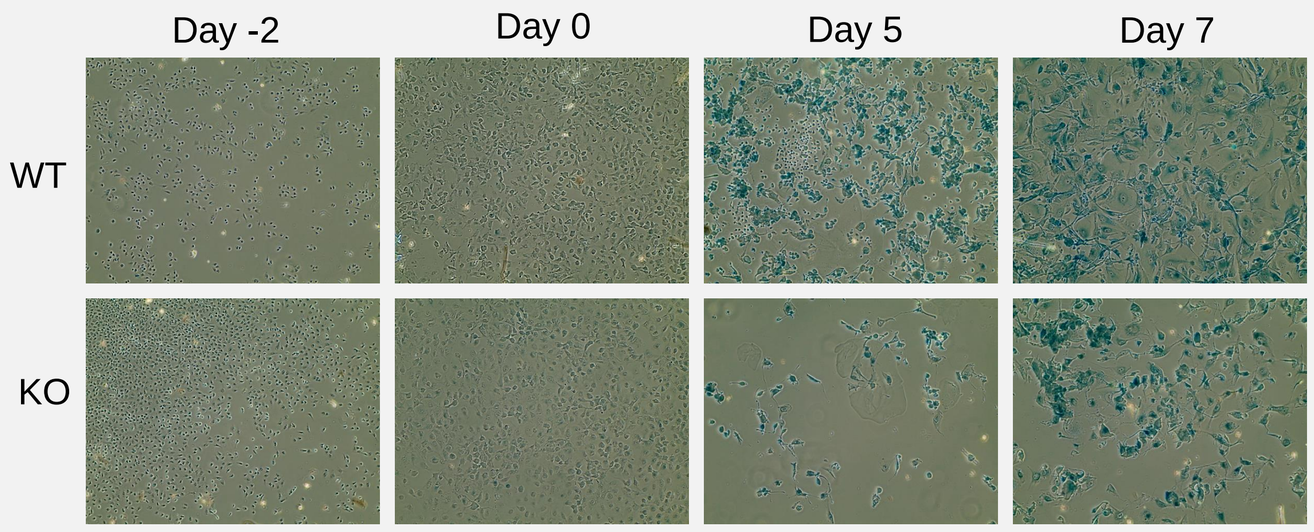
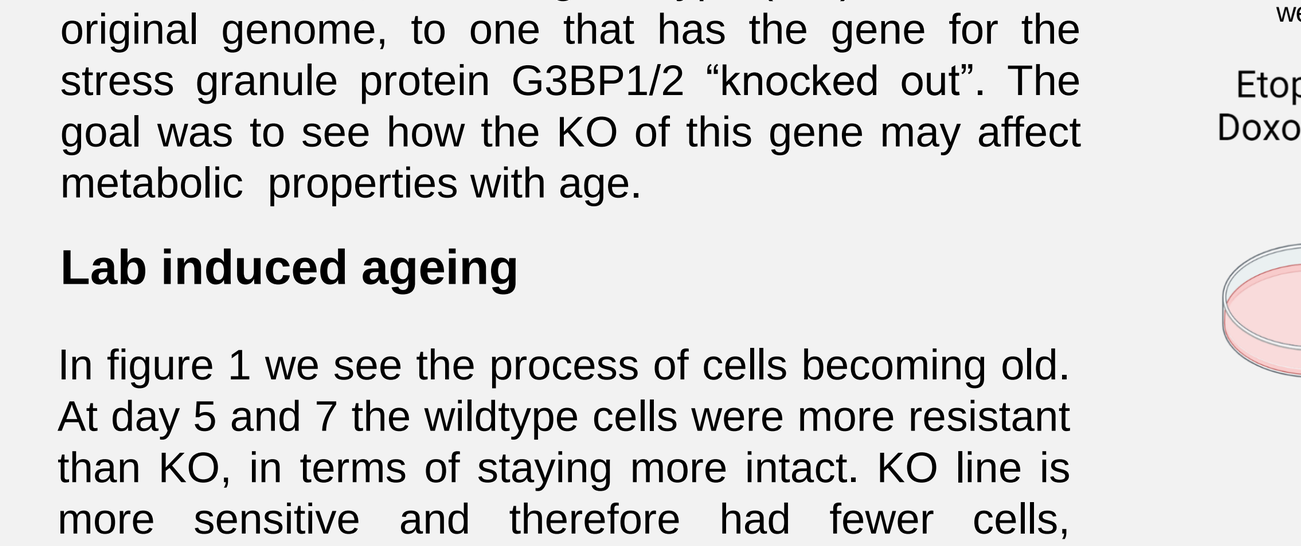



**Figure 1**: The U2OS WT and KO cell cultures were treated with 2 µm etoposide and incubated for 2 days from -2 to 0. The media was then exchanged to not contain etoposide, then samples were fixed and stained for SA-ß-Gal (makes the cells blue) activity at indicated days.

## Workflow




## Polar Metabolites

Results found in figure 2 show us that old cells had more excess NADH compared to young cells. There is more excess NADH in both young and old KO cells than there is in WT.

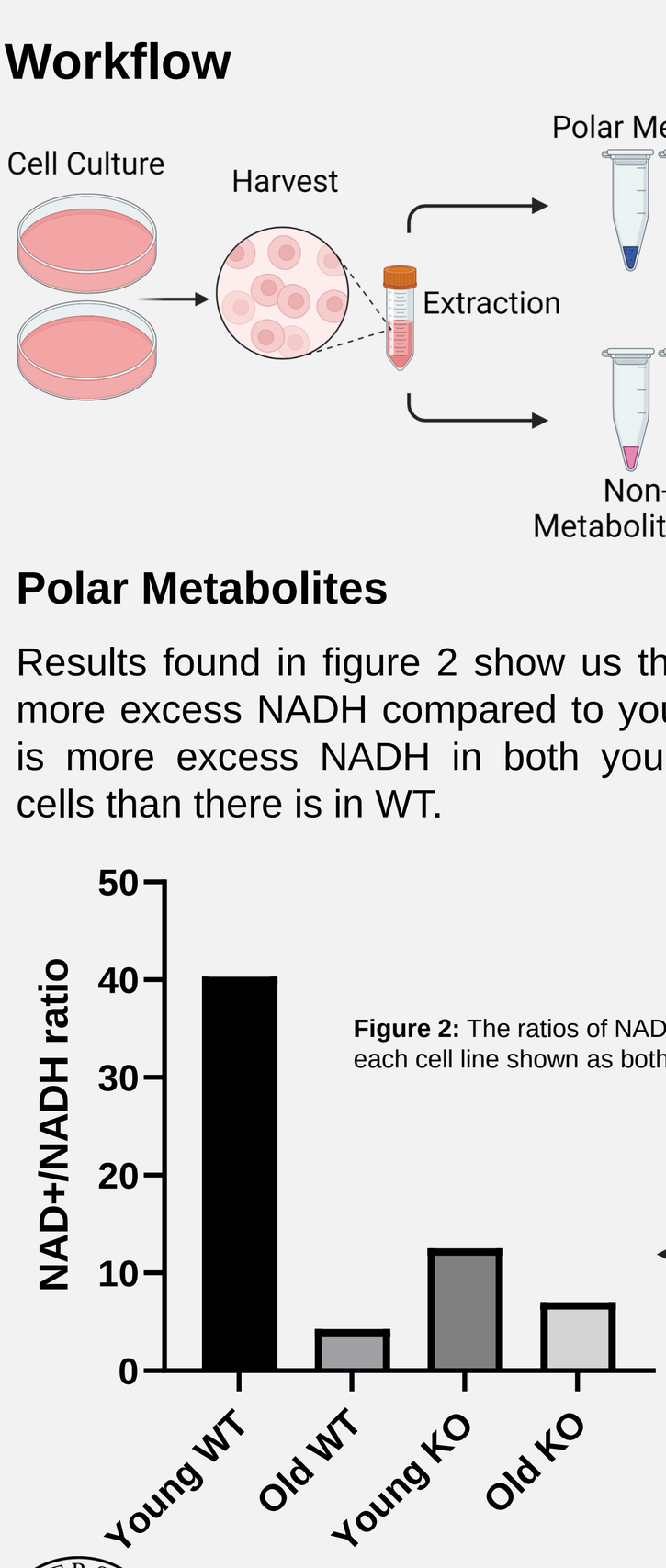
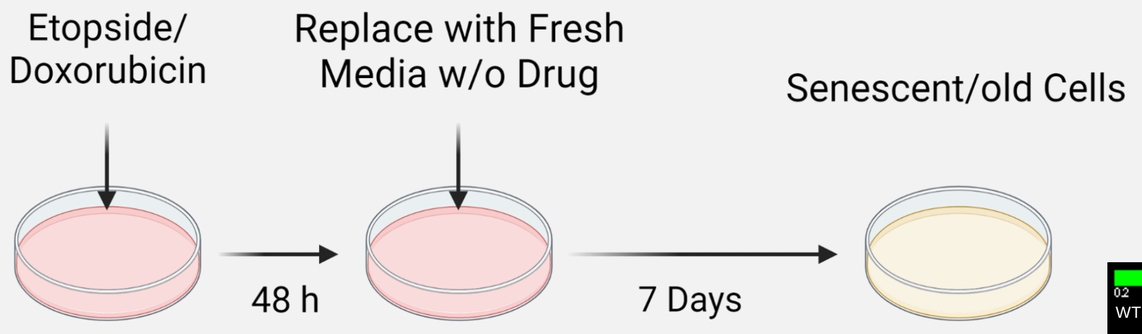



**Figure 2:** The ratios of NAD+ to NADH, for each cell line shown as both young and old.




## Non-Polar Metabolites (Lipids)

Raw data was processed and analysed using Lipid Data Analyzer 2 (Hartler et al., 2011) and the lipid profiles of cells were compared. These data shows individual lipid types and the properties of these lipids. Where there is green to red the amount of the lipid has increased from WT to KO, and decreased if it is from red to green.

WT and KO cells show significant differences in the lipid groups PC (cell membranes), and Cer/SM which may contribute to the development of diabetes.

## Where does this lead to?

The cells that have become old show very major changes in polar metabolite contents. NAD+/NADH ratio in KO cell line may be coherent with more reactive oxygen species in cells, which may be the reason why we saw more cells die in the KO induced ageing project, since reactive oxygen species are damaging to cells.

This may suggest that the protein G3BP1/2 may have a protective role against reactive oxygen species in cells. The lipid data for WT and KO are similar to published evidence of changes in diabetes, so G3BP1/2 & stress granules may have a role in lipid metabolism.

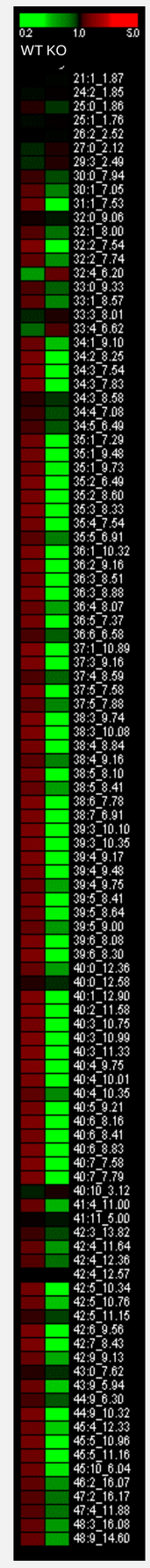



**Figure 3**:List of lipids where each color block is a specific lipid value. WT Compared to KO.

## REFERENCES

Hartler, J., Trötzmüller, M., Chitraju, C., Spener, F., Köfeler, H. C., & Thallinger, G. G. (2011). Lipid data analyzer: Unattended identification and quantitation of lipids in LC-MS data. *Bioinformatics*, *27*(4), 572–577. https://doi.org/10.1093/BIOINFORMATICS/BTQ699

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