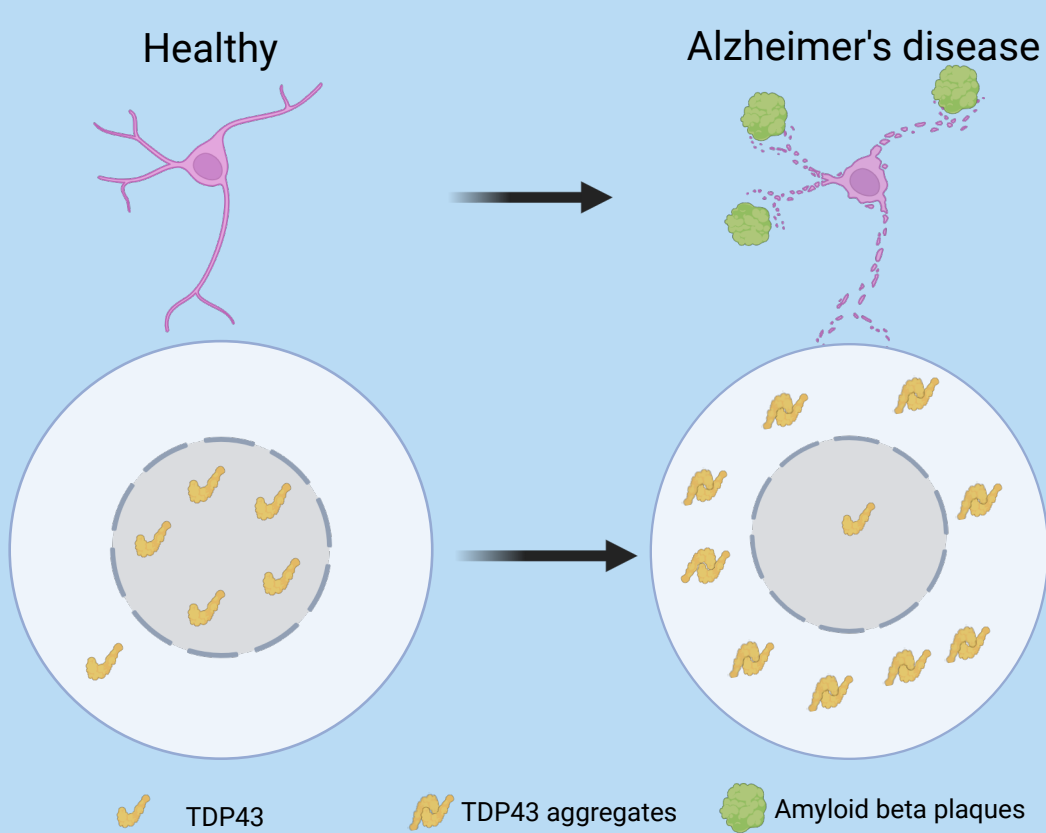


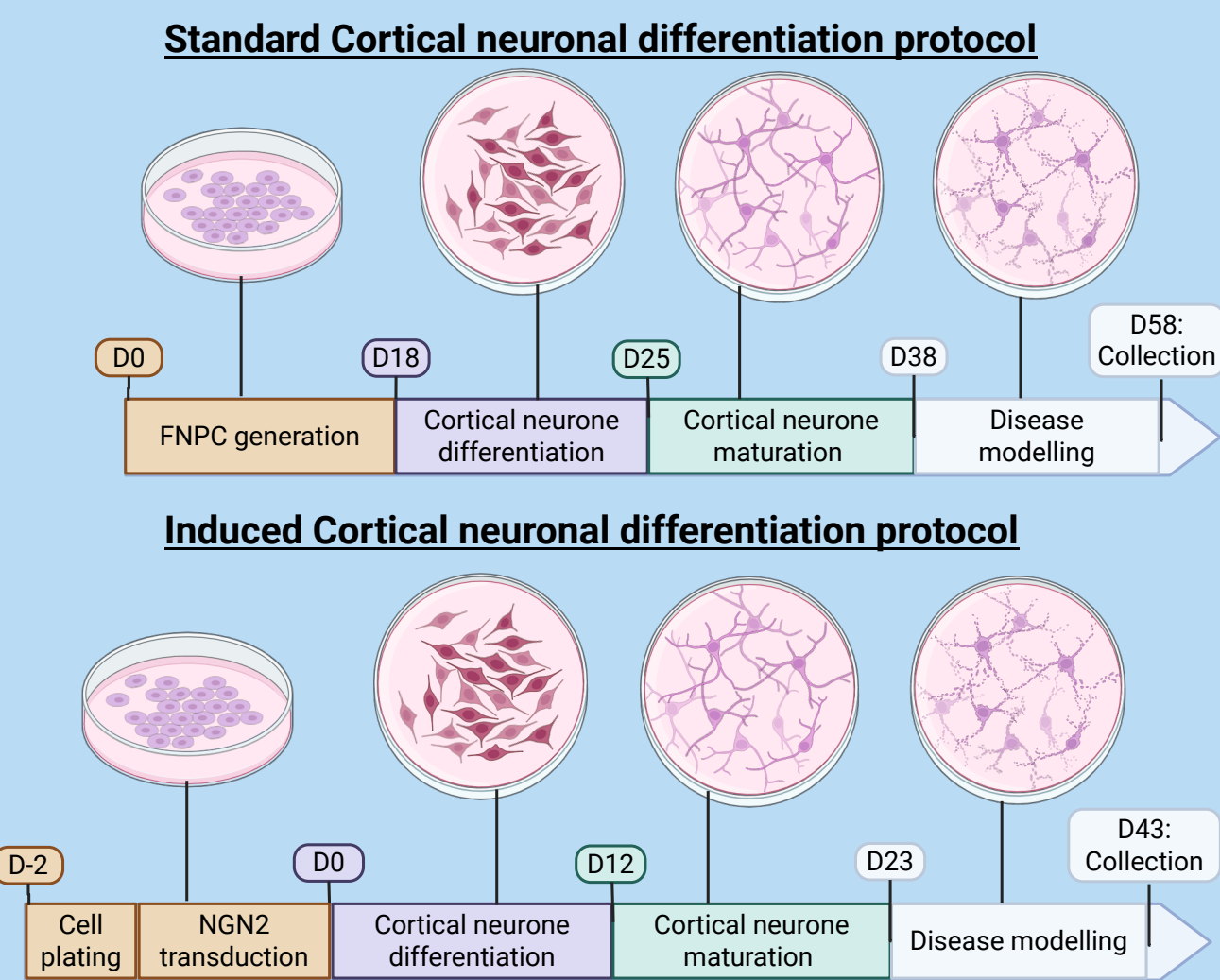
Background

- TDP43** is an RNA binding protein with several various roles including **Transcription, Translation, RNA processing**. TDP43 plays a key role in neurodegeneration as seen in **ALS**, it is typically found in the nucleus, but **mislocalises** to the cytoplasm where it aggregates. These inclusions interfere with cellular machinery triggering apoptotic pathways¹.
- Alzheimer's disease** being one of the leading causes of death with 55 million people affected worldwide in 2020². Alzheimer's disease can be identified by the aggregation of proteins such as **amyloid beta** and **Hyperphosphorylated Tau**.
- Almost **80% of severe cases of Alzheimer's Disease show TDP43 proteinopathies** suggesting that TDP43 may play a role in the characteristic neurodegeneration seen in Alzheimer's Disease³.



- Our goal:** Is there a **synergistic increase in neurodegeneration** when TDP43 is mislocalised in an Alzheimer's disease model?

Methods



IPSC derived cortical neurone differentiation

- Standard differentiation** via dual SMAD inhibition using small molecules⁴
- Induced differentiation** via expression of **Transcription factors** biased towards neural lineages ie **NGN2**.⁵

Our TDP43-mislocalisation model²:

- Makes use of nanobodies expressing the Nuclear export signal (NES).
- These **nanobodies** are specific to **GFP**.
- We've created a **cell line of iPSCs** where TDP43 is tagged with GFP.
- Expression of our nanobodies results in **TDP43 mislocalisation**.

APOE4 expression

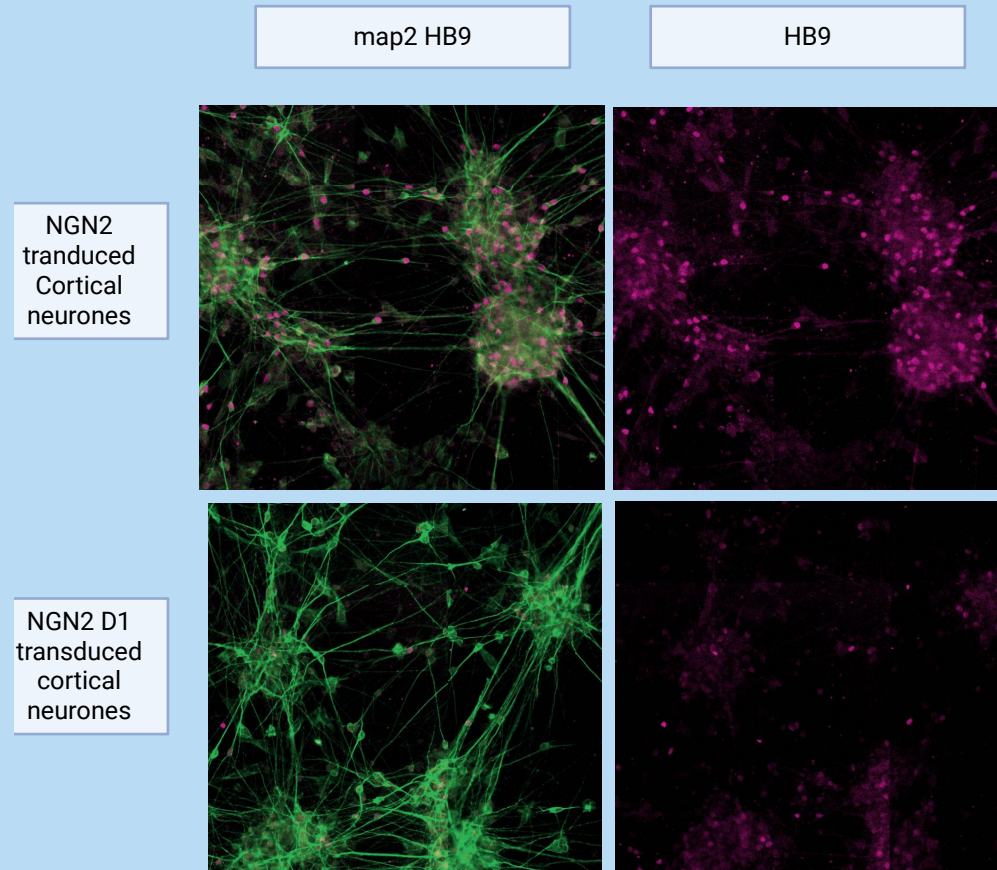
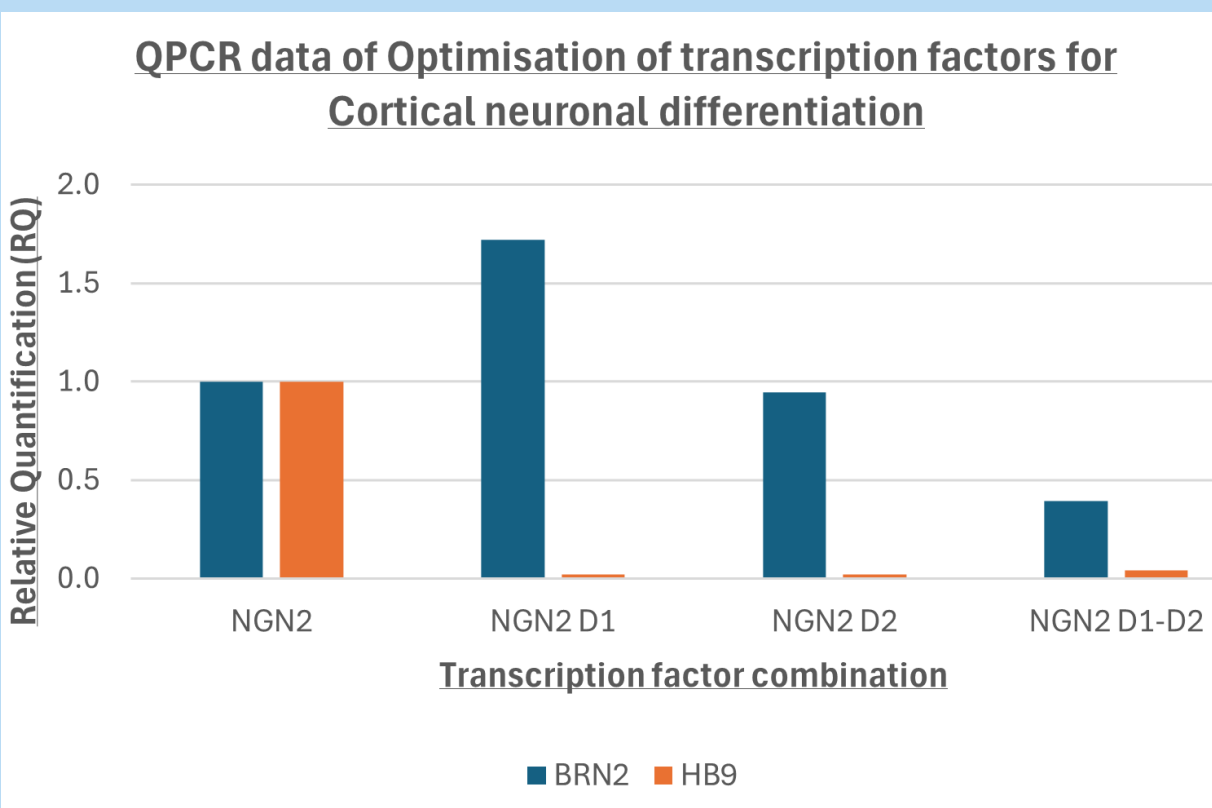
- We've generated lentiviruses capable of overexpressing with **APOE2, 3 or 4**.

Disease modelling

- Mature **IPSC-derived cortical neurones** will be transduced AAVs and LVs for the NESM nanobody and APOE4 insert respectively.

Optimisation of Cortical neurone differentiation

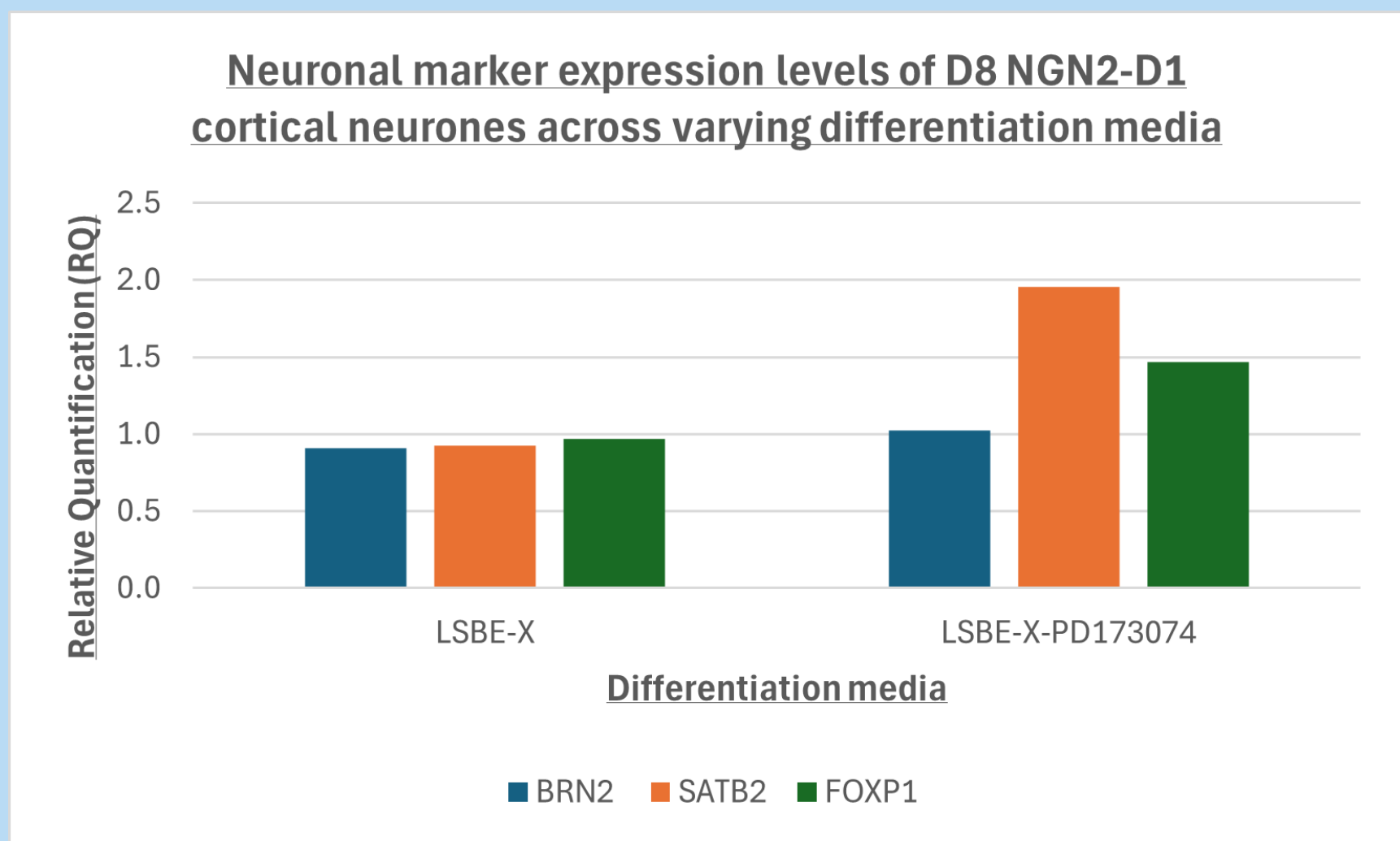
As cortical neurones are the primary affected cell type in **Alzheimer's disease** my first objective was the **optimisation** of a cortical neuronal **population** with the **highest purity possible**.



Bar graph showing expression levels of **cortical layer 2/3 marker, BRN2**, and **motor neuronal marker, HB9**, across cells transduced with various transcription factors.

These results show that we have almost **entirely eliminated** the motor neuronal population by transducing with **unique combination of transcription factors**. Thus giving us a pure cortical neuronal population.

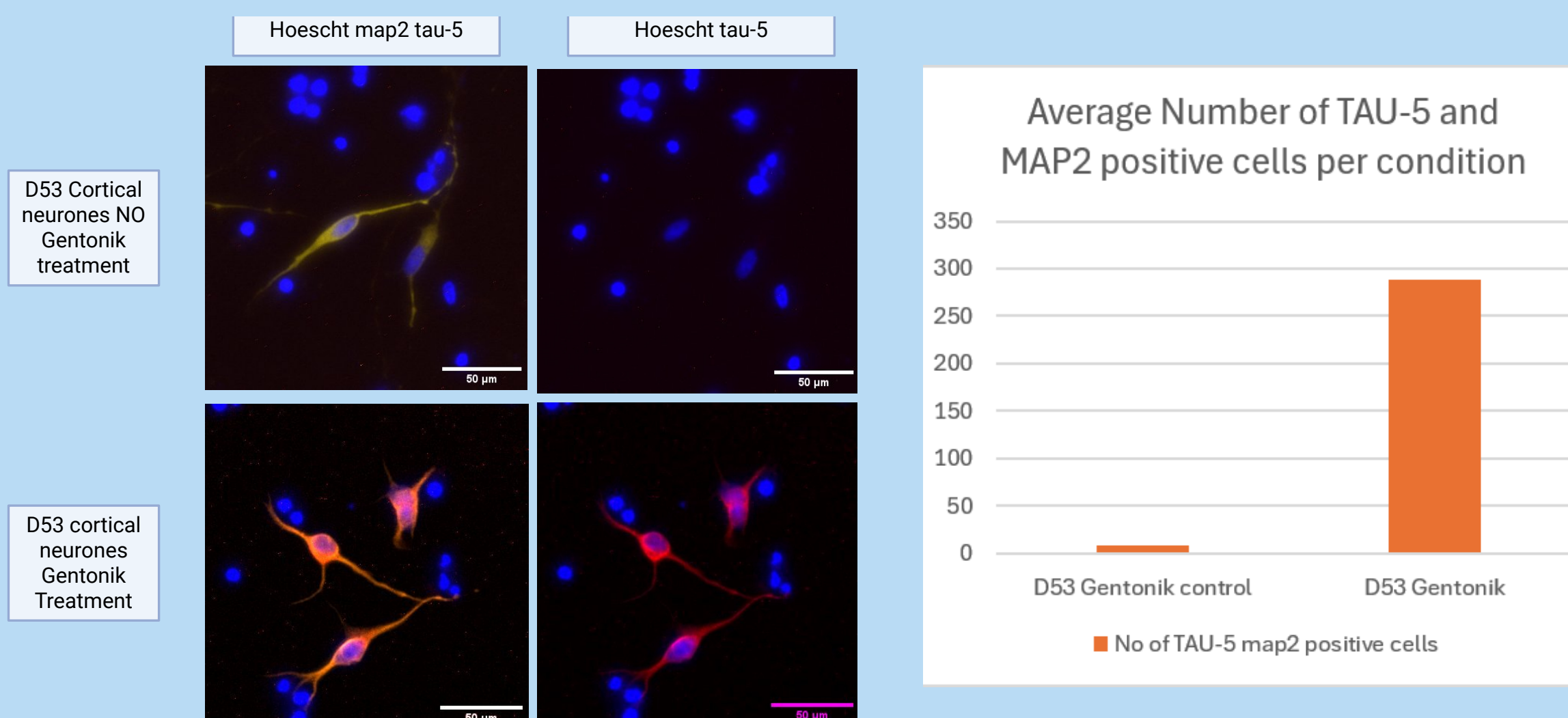
Generation of two distinct neuronal populations: Upper and Lower Cortical Neuronal layers



Bar graph demonstrating expression levels of cortical layer 2/3 marker **BRN2**, cortical layer 4/5 marker **SATB2**, and Medium spiny neuronal marker **FOXP1**, across cells incubated with various differentiation media.

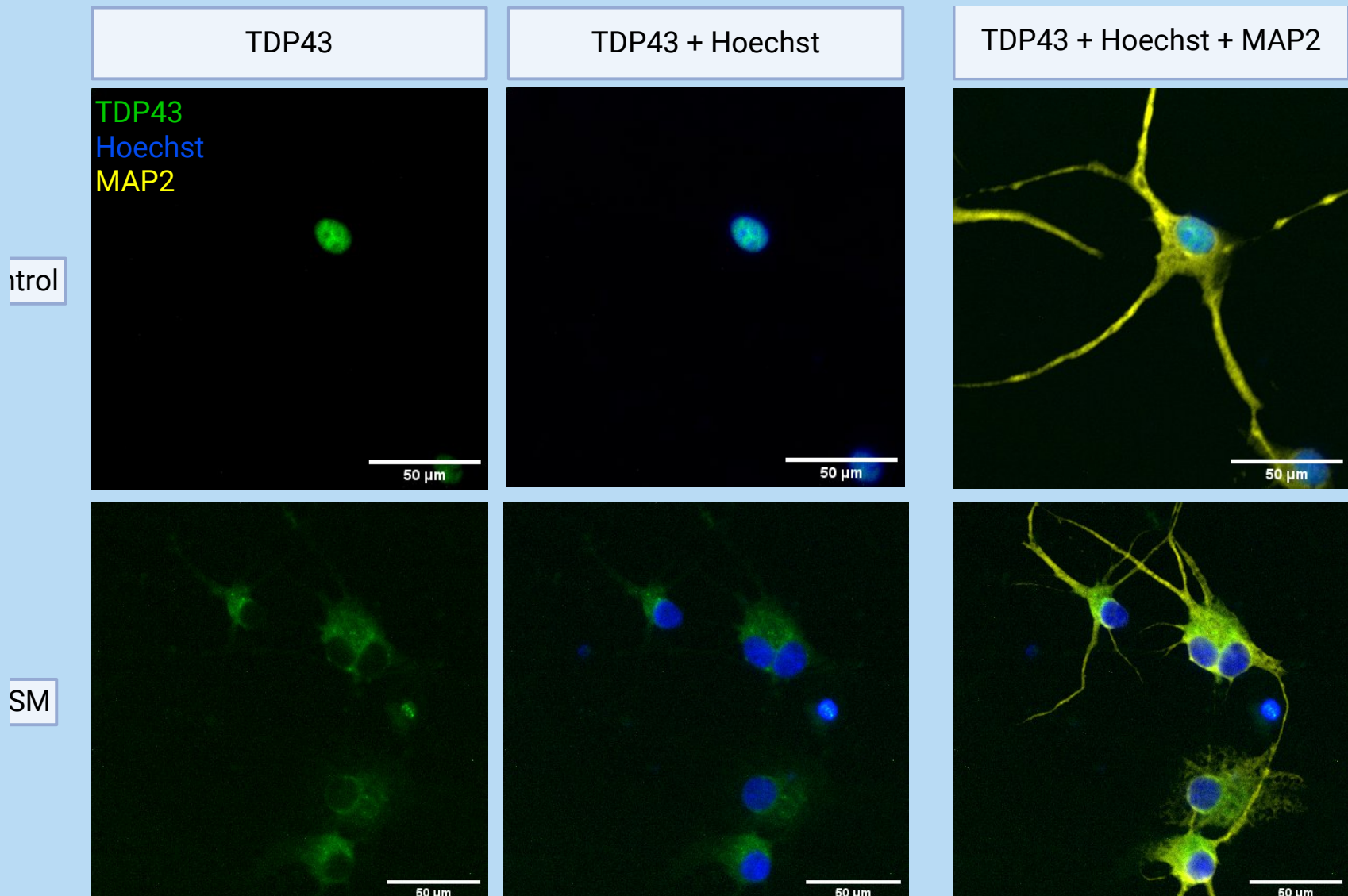
We successfully produced **two populations of cortical neurones**. This allows us to investigate how our disease modelling changes across **various neuronal populations**. The increased abundance of FOXP1 and SATB2 this population is reminiscent of **lower cortical layers** (LSBE-x-pd173074) Where as the equal abundance of all cortical markers is more reminiscent of **Upper cortical layers** (LSBE-X).

Establishment of Tau-positive Cortical Neurones

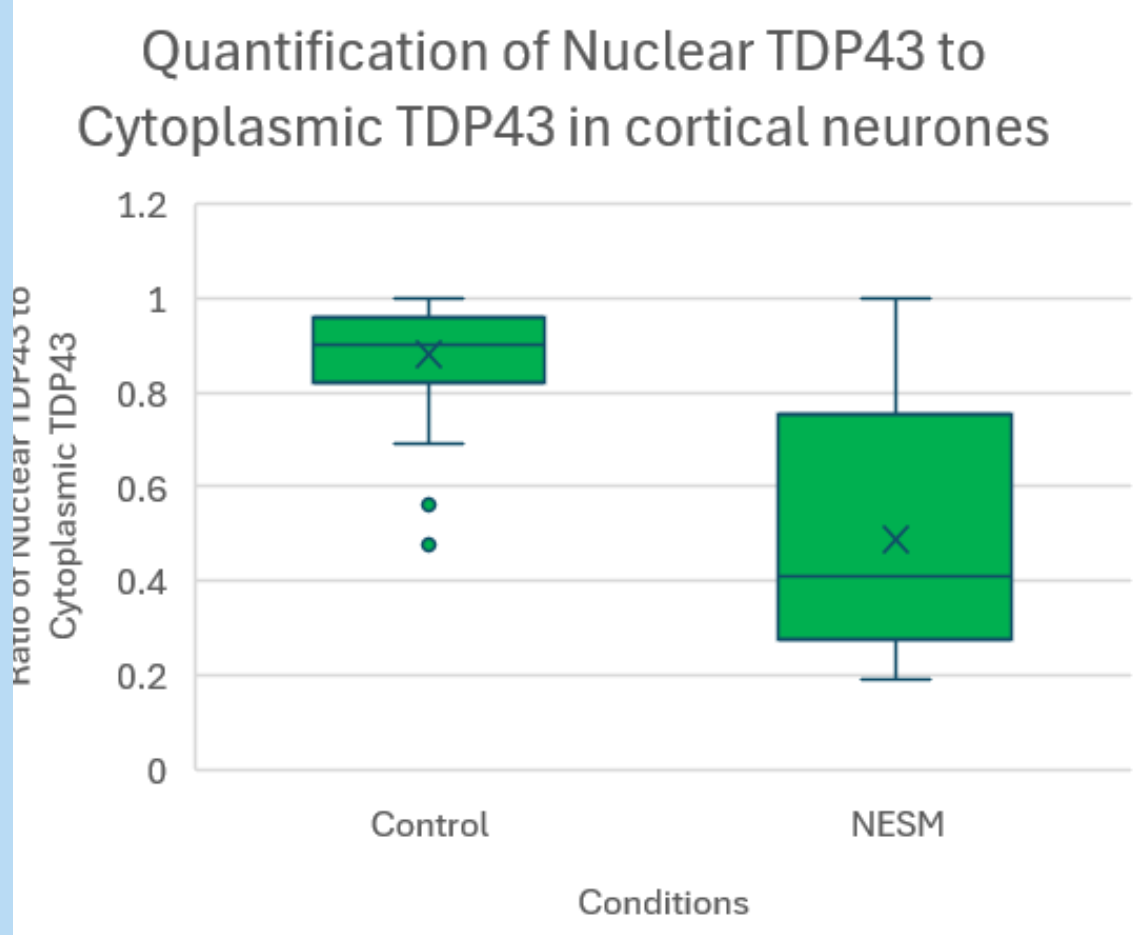


Tau is a **vital** protein in Alzheimer's disease, and potentially **mediates** its associated **neurodegeneration**, as such we decided to include **GENTonik treatment** to our protocol to ensure **Tau expression in our neurones** as seen in the above figures.

Modelling TDP43 mislocalisation in Cortical Neurones



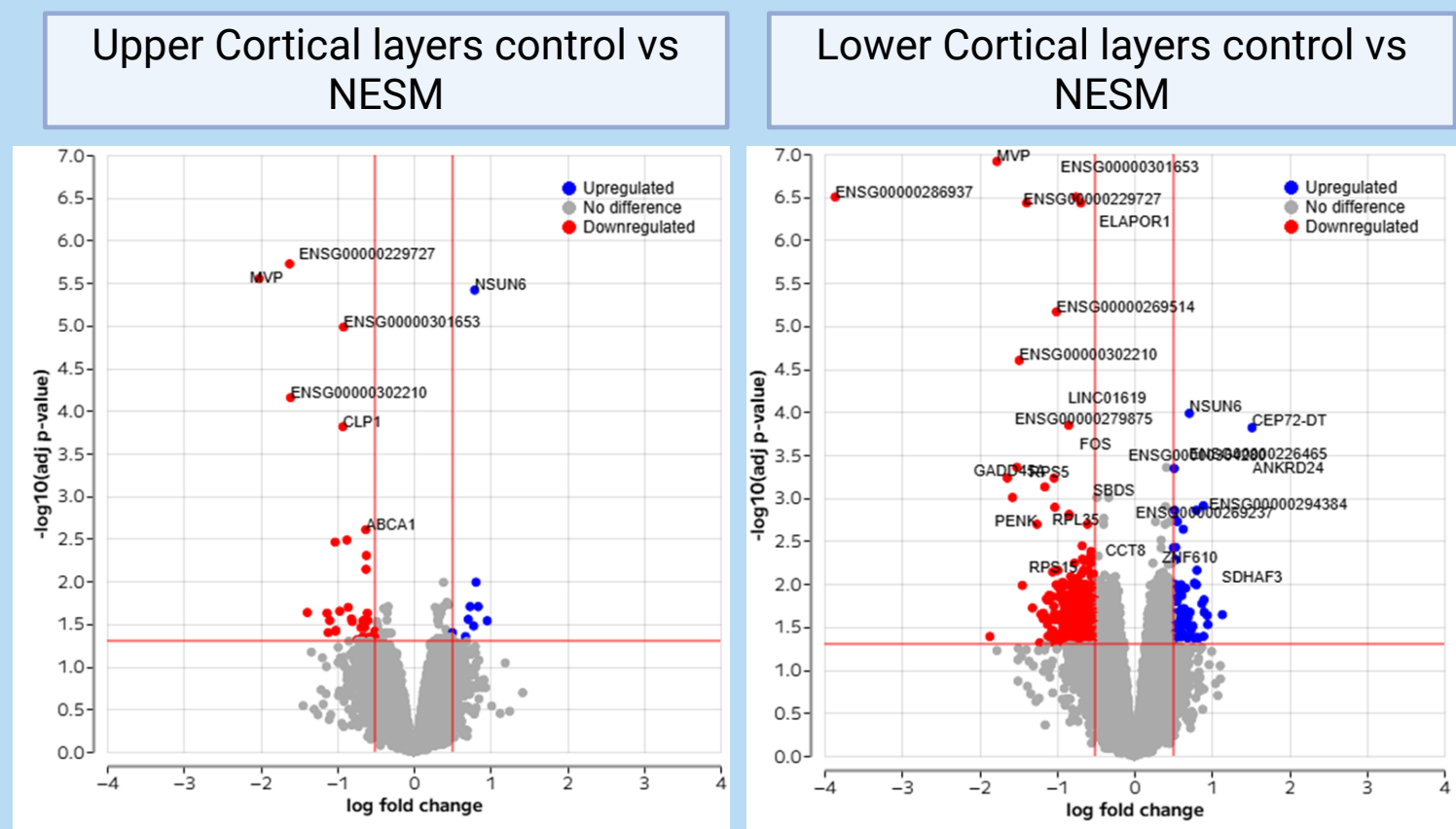
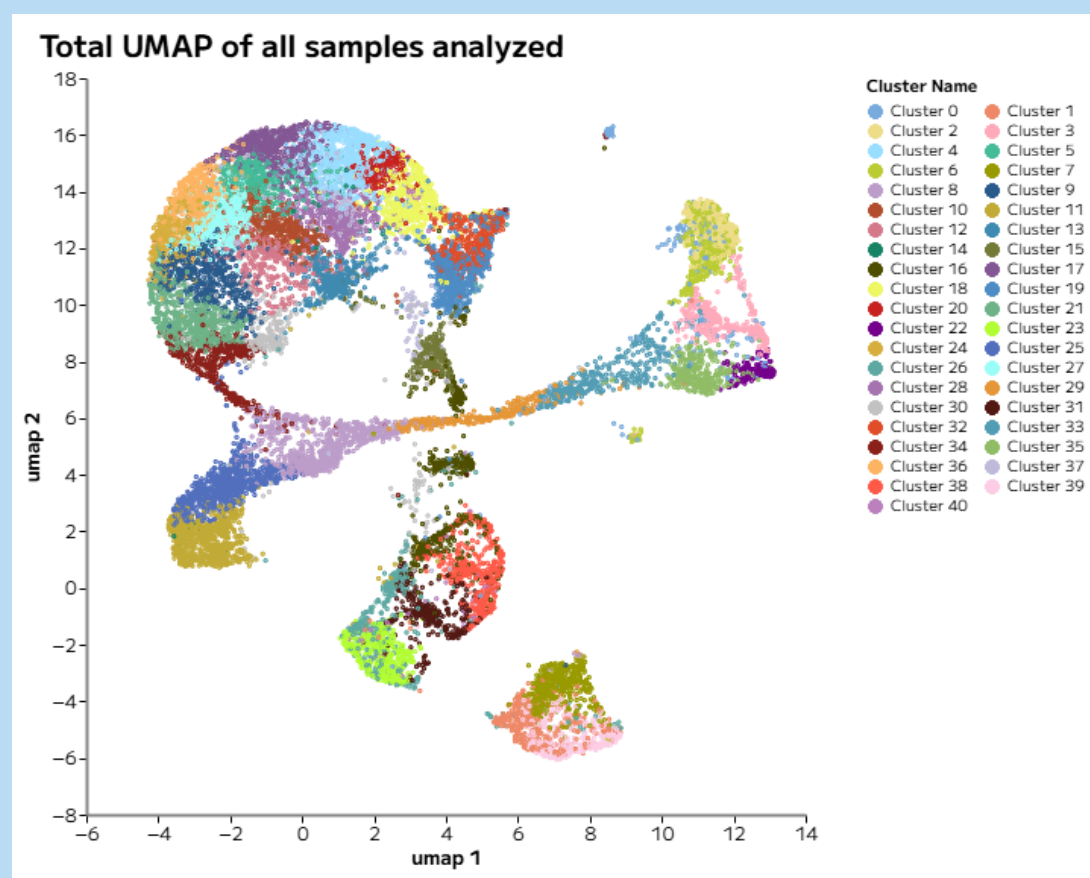
immunostain images of **TDP43 (Green)** neuronal nuclei stained with **Hoechst (Blue)**, and **MAP2 (Yellow)** in cells transduced with either a virus encoding a **control nanobody** or a virus encoding our **NESM nanobody**. Magnification is 20x.



Bar graph quantification of the ratio of TDP43 nuclear signal compared to cytoplasmic signal.

This data plainly demonstrates our ability to **mislocalise TDP43 in cortical neurones** clearly and robustly. With this established we have successfully generated the classic phenotype seen in **TDP43 proteinopathies**. As such our **next step** is to **investigate** the effects of this phenotype on **gene expression**.

Cortical layers are differentially affected by TDP43 mislocalisation



UMAP and Volcano plots resulting from **Single cell RNA sequencing** to identify genes affected by TDP43 mislocalisation. We successfully identified that the **lower cortical neuronal layers** are substantially more affected by TDP43 mislocalisation than **Upper cortical layers**.

Conclusion

Based on the current results achieved it is clear that **TDP43 mislocalisation can be achieved in cortical neurones** and in this event the cells undergo **widespread changes to their gene expression**. This is in line with current literature that shows that this phenotype results in **gain and loss of function of TDP43** triggering cascading loss of function of numerous proteins essential to healthy cell function in cortical neurones.

The next step of this project is now to analyse the effect of solely **APOE isoforms on gene expression**, as well as identify genes affected by **both pathways**. This in turn will highlight **candidate genes** which may play a key role in neurodegeneration and as such open up new pathways for **clinical and biomedical research**.

Further experiments to be conducted to provide supporting evidence of this include:

- Quantification of rate of neurodegeneration via quantification of **cleaved caspase 3 using Western Blotting**
- Identification of candidate genes via single cell RNA sequencing.
- Knockout and/or Knock-in** of candidate genes to examine their effect on **neurodegeneration** in our model.
- Quantification of Alzheimer's disease hallmark proteins ie **Phosphorylated Tau**, in our **APOE4-TDP43 model** via **western blotting**

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