

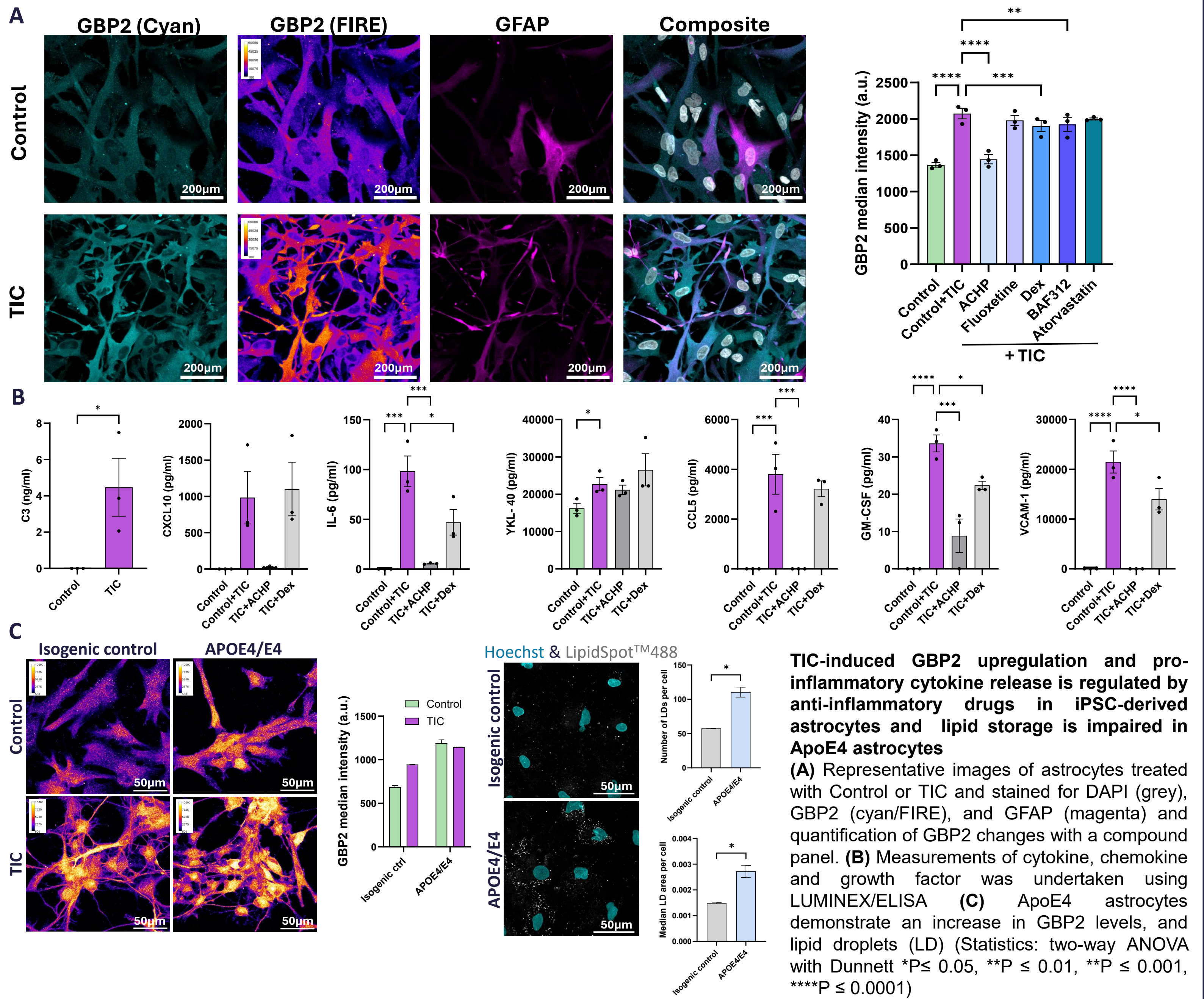
Development of a Human Reactive Astrocyte Drug Discovery Platform to Evaluate Neuroinflammation

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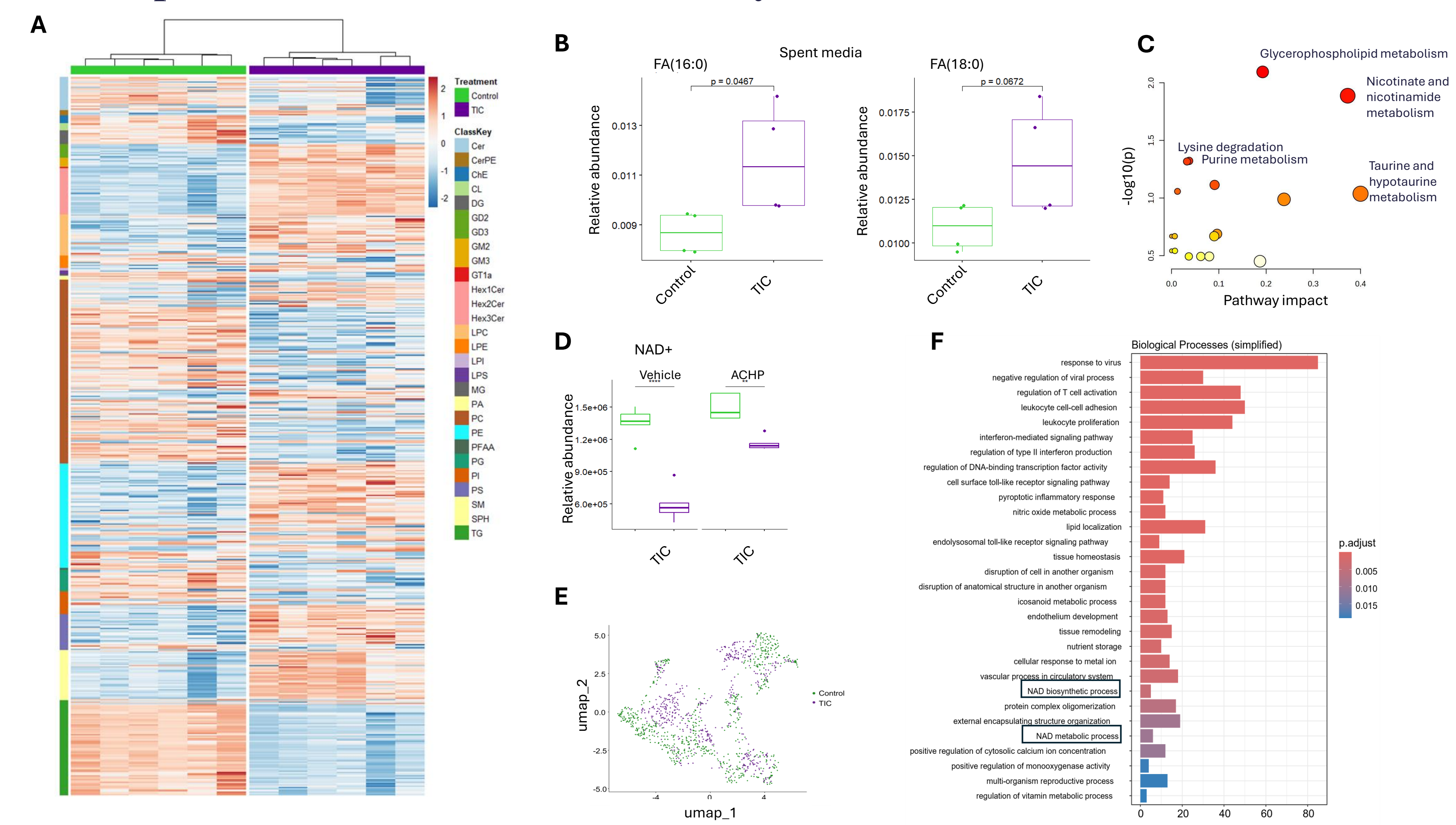
Introduction

Astrocytes are major glial cells essential for CNS homeostasis. After injury or in disease, they become reactive, altering their morphology and molecular profile to adopt distinct phenotypes that respond to pathology. These reactive astrocytes contribute to neuroinflammation in neurodegenerative disorders by releasing neuro- and immune-modulatory factors. An in vitro model using TNF- α , IL-1 α , and C1q ("TIC") induces a neurotoxic reactive state.¹ TIC-stimulated astrocytes change their molecular profile, increasing expression of inflammatory genes such as guanylate-binding protein 2 (GBP2), secrete growth factors, chemokines, pro-inflammatory cytokines, and neurotoxic lipids.² Here we show how multimodal approaches can be used to characterise astrocytes for drug discovery applications.

High-content imaging and functional profiling of human iPSC-derived astrocyte activation and drug response



LC-MS and scRNA-seq identify changes in the lipidome, metabolome and transcriptome of human reactive astrocytes



Multi-omics analysis revealed perturbation to lipid and NAD+ metabolism in TIC stimulated hiPSC-derived astrocytes (A) Heatmap shows data of 528 significantly perturbed ($q < 0.05$, TIC vs Control) confidently annotated lipids measured in either positive or negative ionisation mode. (B) Long-chain fatty acids (FAs), palmitic (left) and stearic acid (right), are upregulated in the spent media of TIC stimulated astrocytes (C) MetaboAnalyst pathway enrichment analysis against KEGG database of metabolic pathways for annotated compounds with $p < 0.05$ Control vs Control+TIC revealed changes in several pathways including NAD+ metabolism (D) N=3.(E) UMAP plots of single-cell RNA-seq data from Control and TIC stimulated astrocytes, shown by cluster (top) and by treatment type (bottom). N=1. (F) Bar graphs represents significantly (adjusted p-value < 0.05) enriched biological processes (GO terms), which includes NAD metabolic pathways (highlighted), with darker red indicating greater significance. N=1.

Conclusion

Here we show characterisation of a human iPSC-derived model of reactive astrogliosis for drug discovery. TIC stimulation increases pro-inflammatory cytokines, chemokines, growth factors, and induces morphological changes including GBP2 upregulation. Mass spectrometry and single-cell RNA-seq reveal disrupted lipid and NAD+ metabolism in TIC-treated astrocytes. Together, these tools provide a platform to study reactive astrocytes, their role in neuroinflammation, and to support development of therapeutics targeting astrogliosis in neurodegenerative diseases.

References ¹ Liddelow, S. A., et al (2017). Nature, 541(7638), 481–487. ² Guttenplan, K. A., et al (2021). Nature, 599(7883), 102–107.