Use of human cerebral organoids to study the role of Alzheimer's disease risk factor in distinct cell types

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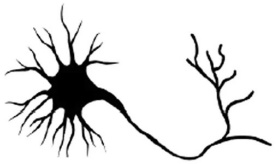
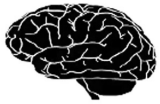
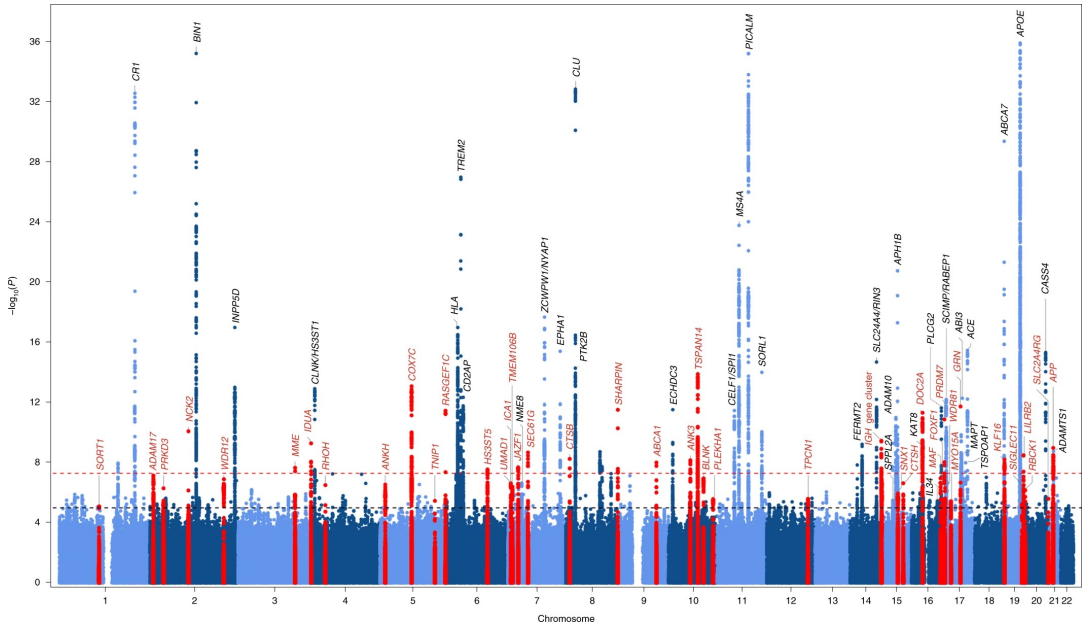
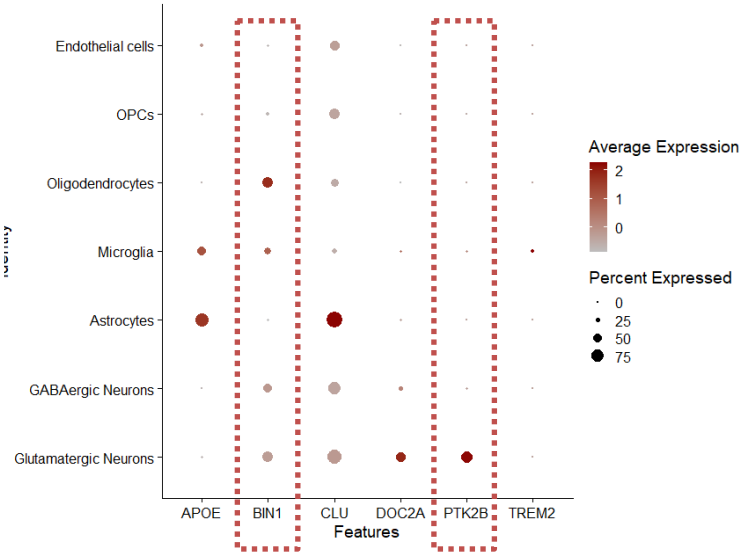
HUMANIZED AND HUMAN MODELS TO THE STUDY OF CELLULAR AND MOLECULAR MECHANISMS OF ALZHEIMER'S DISEASE (AD) PATHOLOGY

	Features	Limitations
'Humanized' animal models  Overexpression / Knock-in human gene APP, PS1 carrying familial mutations	Reproduce Aβ pathology (Plaques, oligomers) Immune reaction (microglial cells) Cognitive impairment	No neurofibrillary tangles (Unless Tau mutations) Accelerated phenotype (Low contribution of aging) No sporadic pathology
2D iPSC neurons culture  Derived from sAD/FAD patients	Features of Aβ and Tau pathology Genuine background Screening of sporadic phenotype 'case by case'	2D limitations: - No plaques or tangles, - Limited cell interactions - Altered transcriptomic Lack of immune cells
3D iPSC 'organoids'  Derived from sAD/FAD patients	Complete Aβ and Tau pathology with neuronal degeneration 3D features: - Spreading - Organized neuronal population - Complex cell interactions	Immature cells (limited vascularization) No microglial cells Rare oligodendrocytes Pre-natal brain transcriptomic profile Low synaptic activity

Gerakis & Hetz, 2019

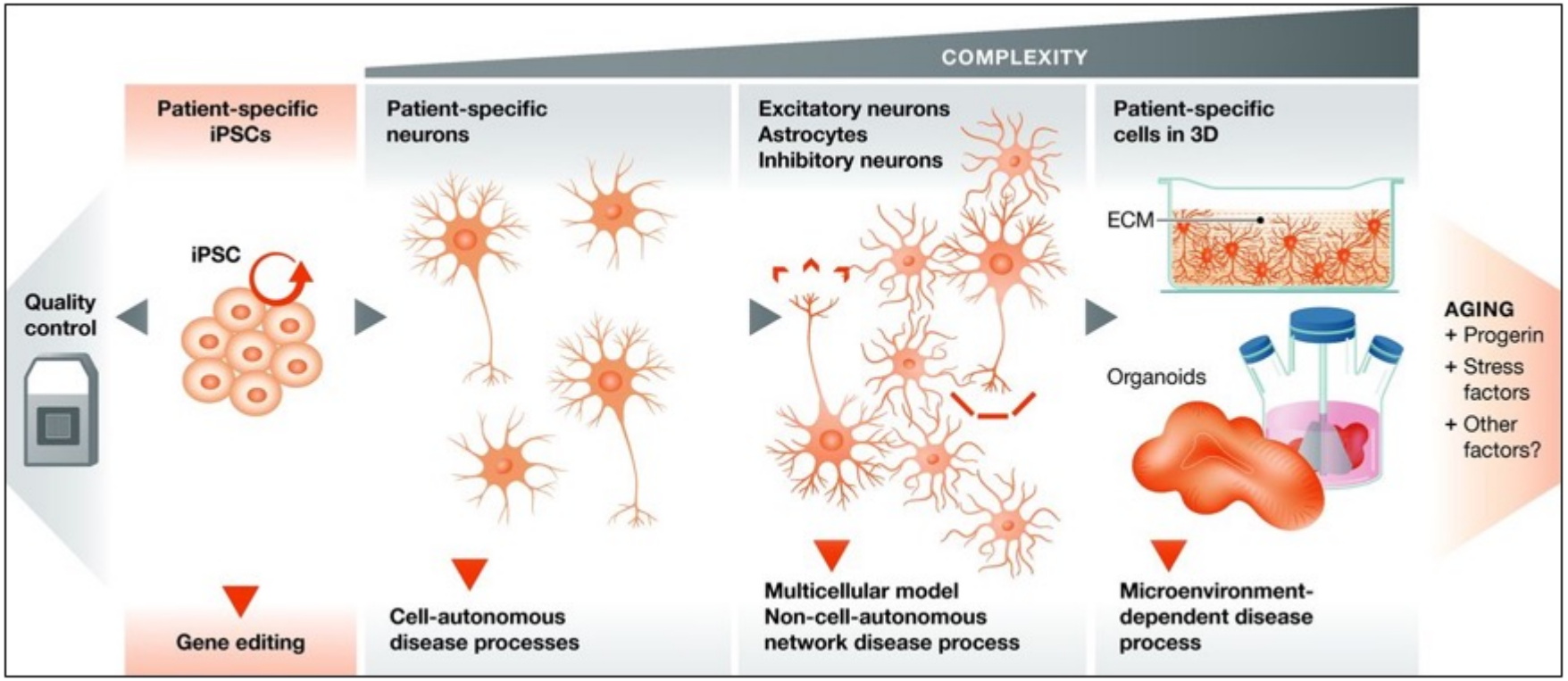
CELL TYPE SPECIFIC EXPRESSION OF AD RISK FACTORS





Bellenguez et al, 2022

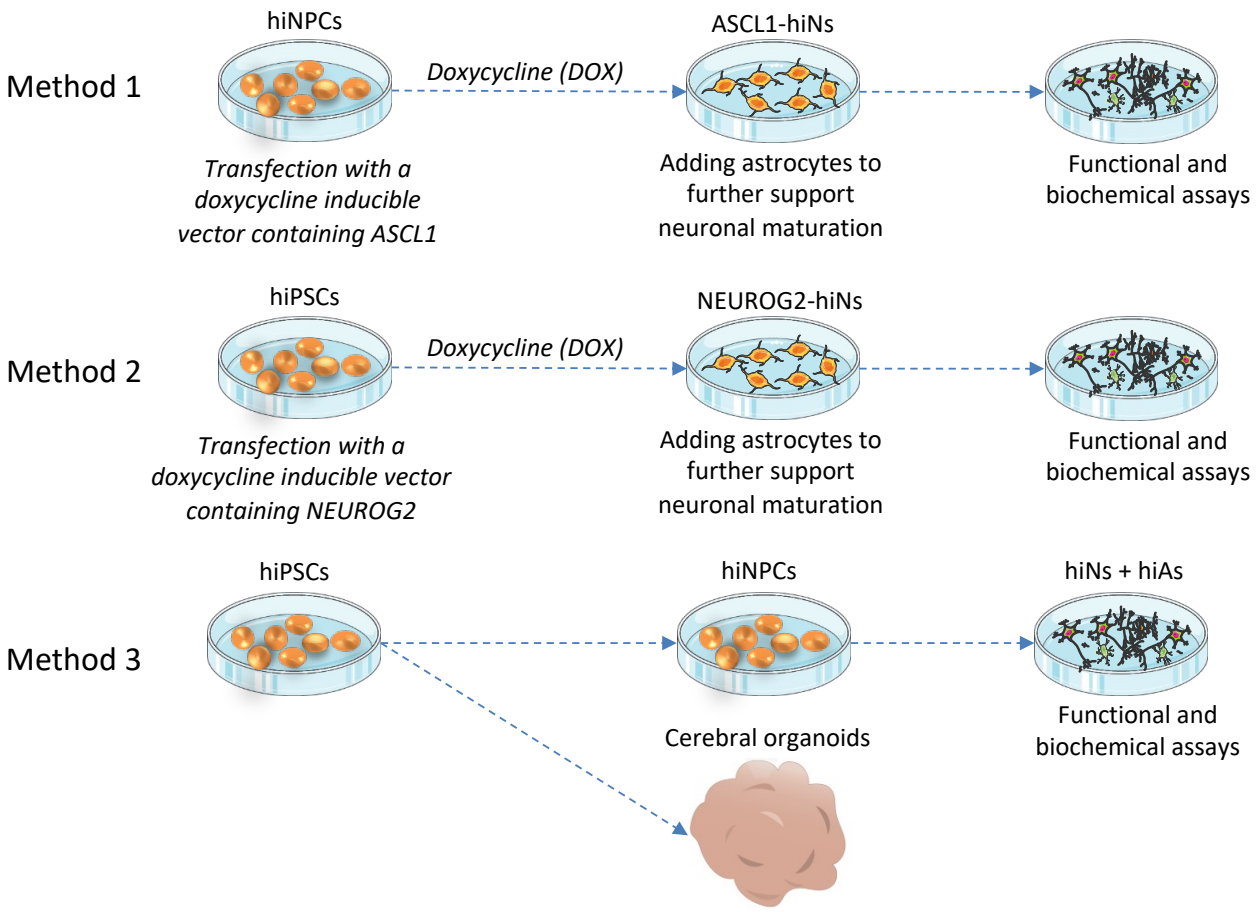
HiPSC TECHNOLOGIES ALLOW THE GENERATION AND STUDY OF MUTATED NEURONAL AND NON-NEURONAL CELLS IN A CONTROLLED MANNER

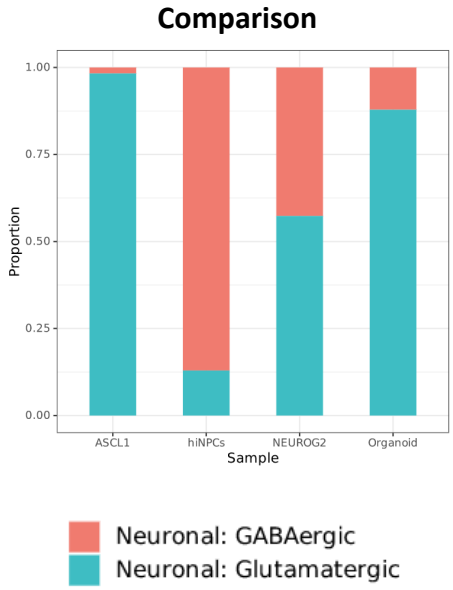


CRISPR/Cas9
Lentivirus

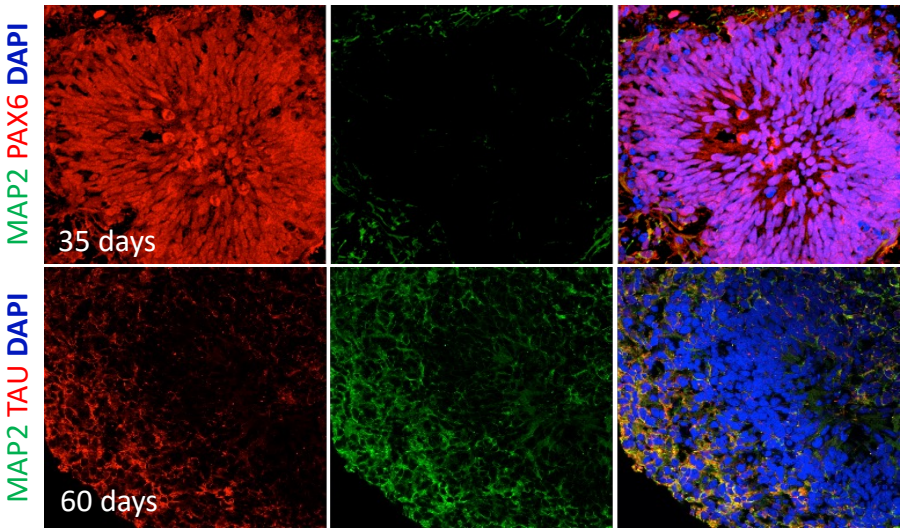
Ichida & Kiskinis, 2015

GENERATING HIGHLY PURE GLUTAMATERGIC NEURONAL POPULATIONS USING THE PRO-NEURAL FACTOR ASCL1

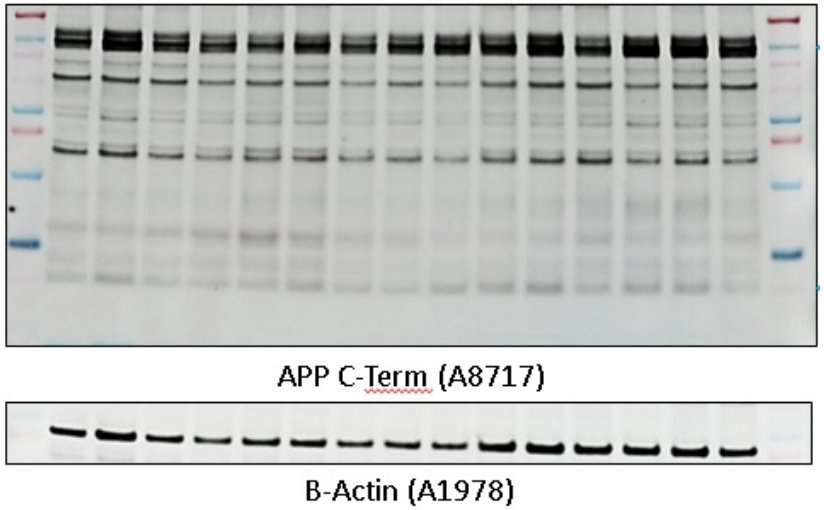




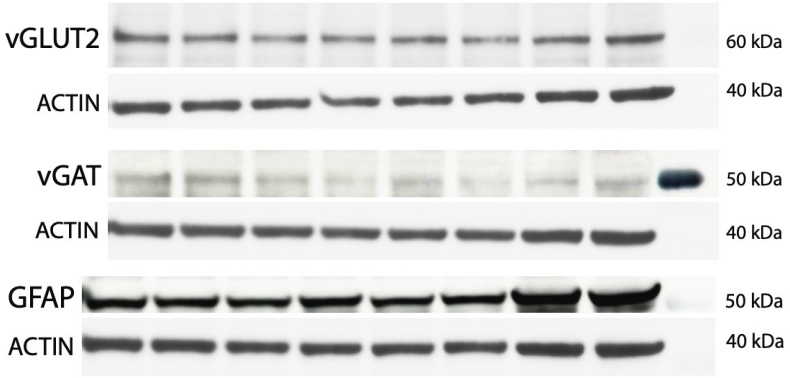
CEREBRAL ORGANOIDS EXPRESS SIMILAR LEVELS OF CELL TYPE SPECIFIC PROTEINS AND ALZHEIMER'S RELATED PROTEINS



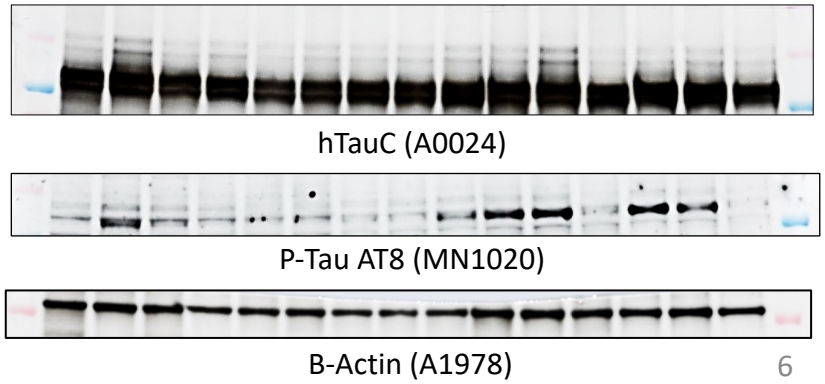
APP processing (190d)



Cell markers (190d)

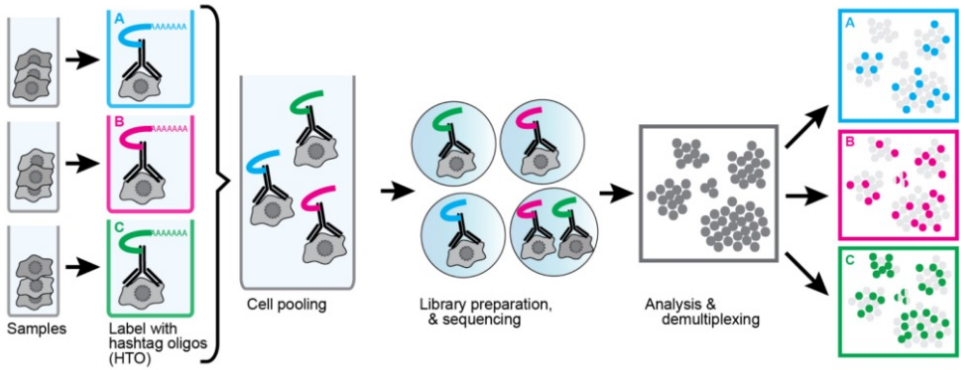


TAU phosphorylation (190d)

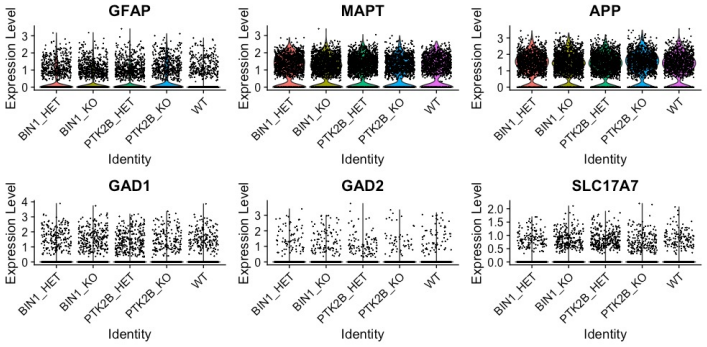


REDUCING CELLULAR HETEROGENEITY IN SNRNA-SEQ USING POOLED CDNA LIBRARIES OBTAINED FROM 190 DAYS CEREBRAL ORGANOIDS

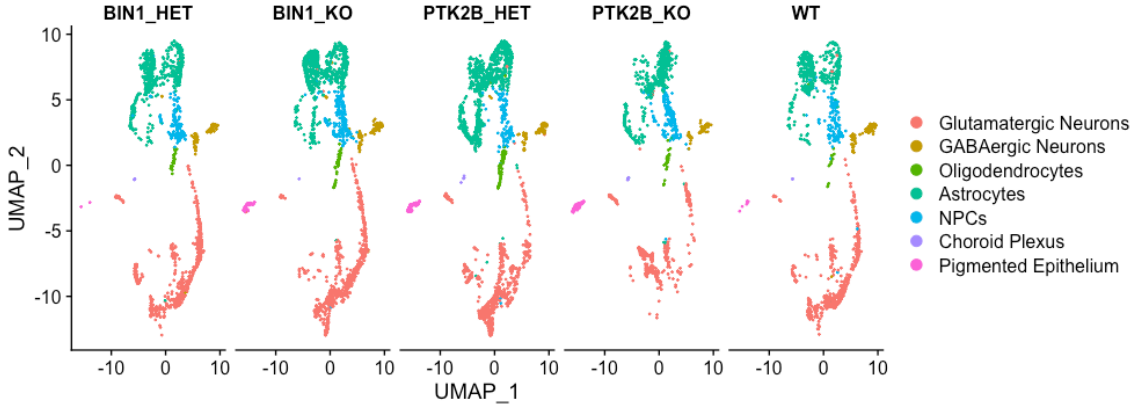
STRATEGY: Pool 5 cerebral organoids per genotype and mix libraries (CITE-Seq)



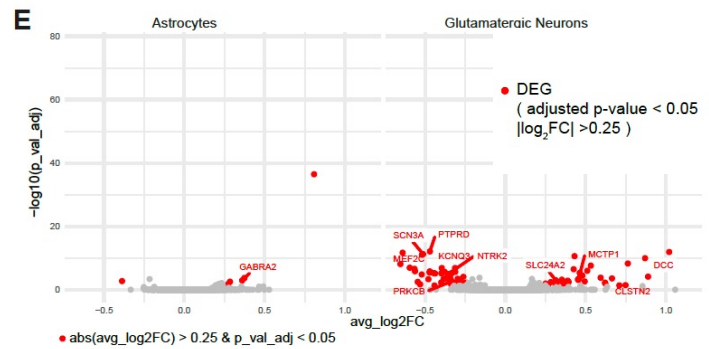
Cell type and AD markers



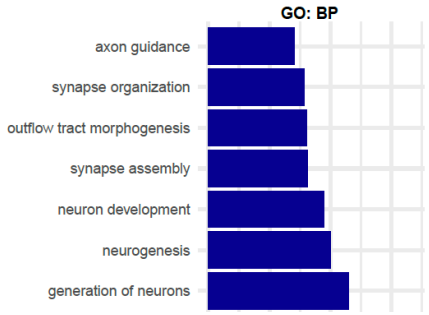
Similar cellular composition in all samples



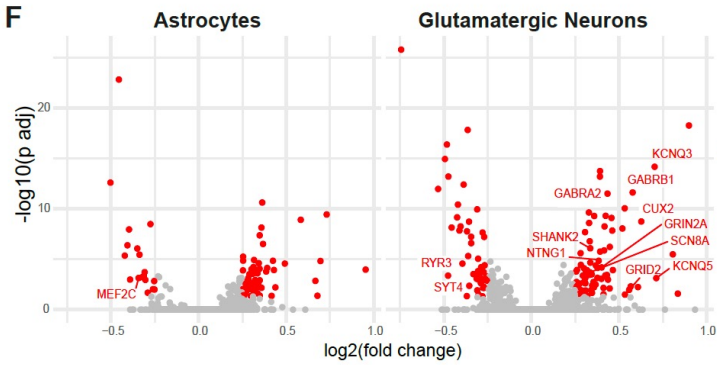
STUDYING CELL TYPE SPECIFIC TRANSCRIPTIONAL ALTERATIONS IN CEREBRAL ORGANOIDS USING SNRNASEQ (I)

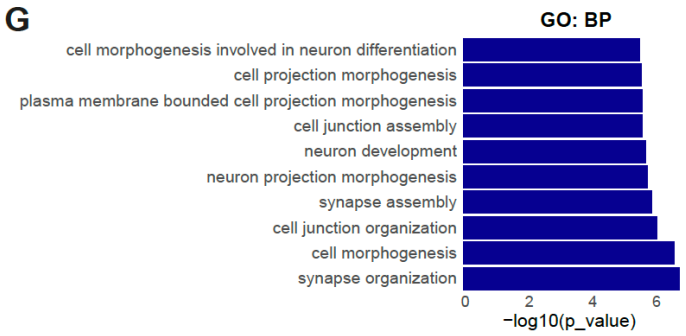


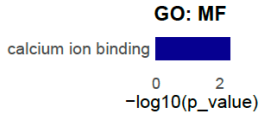
BIN1 HET vs WT



BIN1 KO vs WT

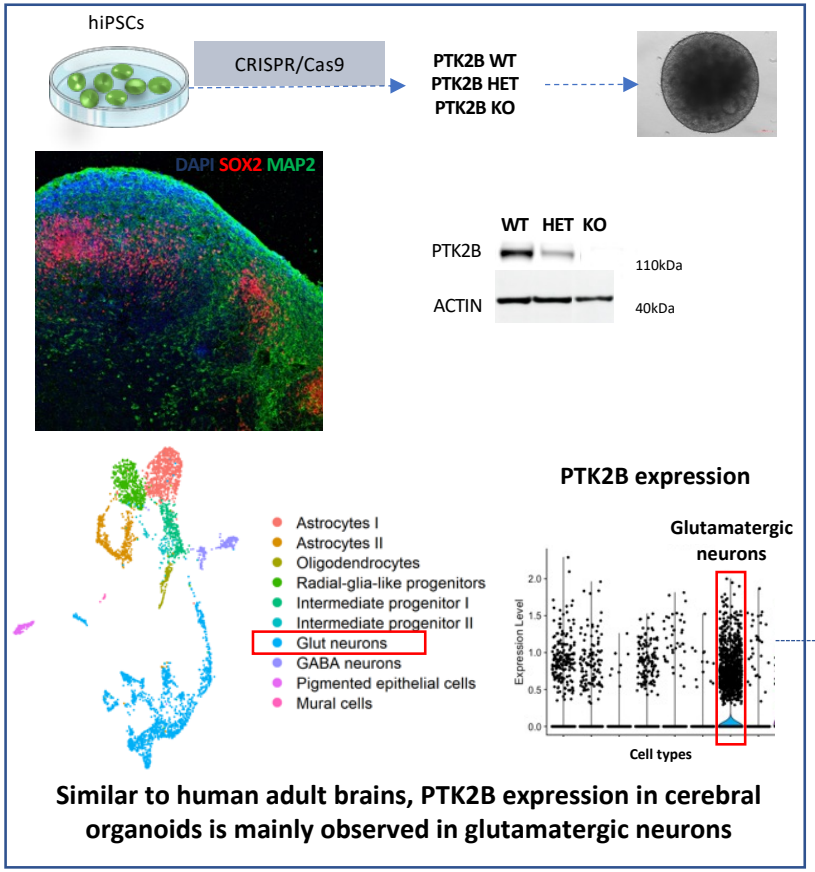


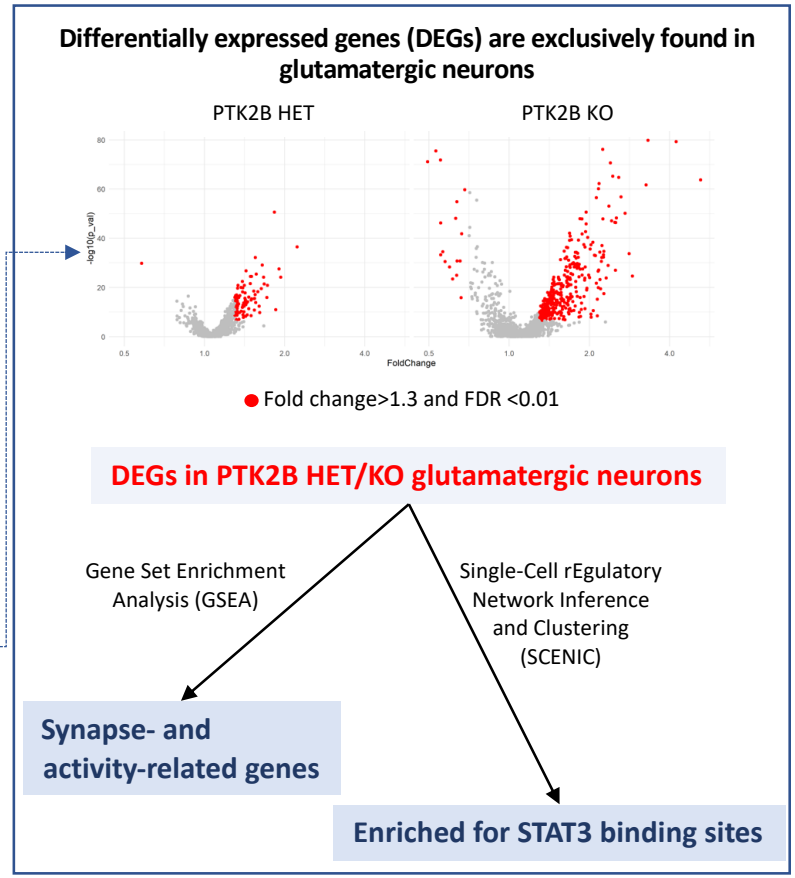




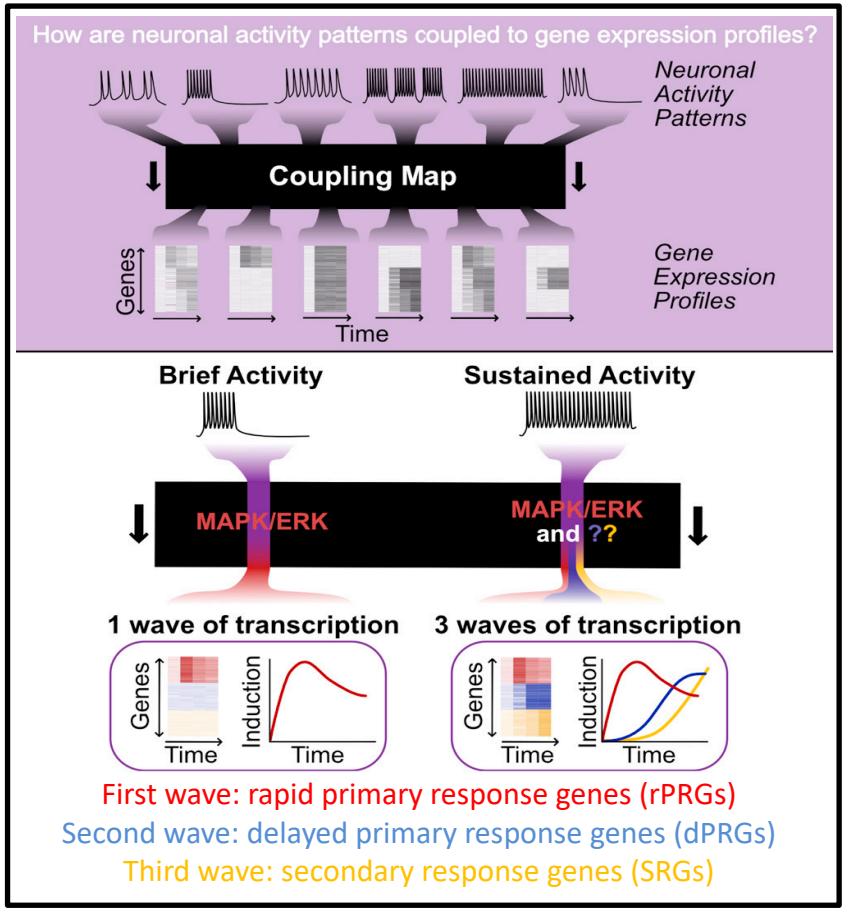
Saha et al, 2022

STUDYING CELL TYPE SPECIFIC TRANSCRIPTIONAL ALTERATIONS IN CEREBRAL ORGANOIDS USING SNRNASEQ (II)





USING ACTIVITY-RELATED GENES SIGNATURES AS A READ-OUT OF ELECTRICAL ACTIVITY IN CEREBRAL ORGANOIDS

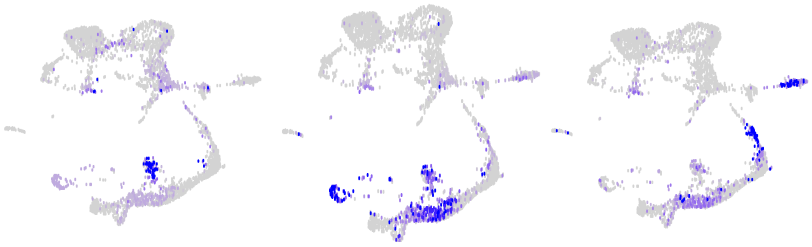


Tyssowski et al., 2018

Gene signature extraction and cell identity recognition at the single-cell level

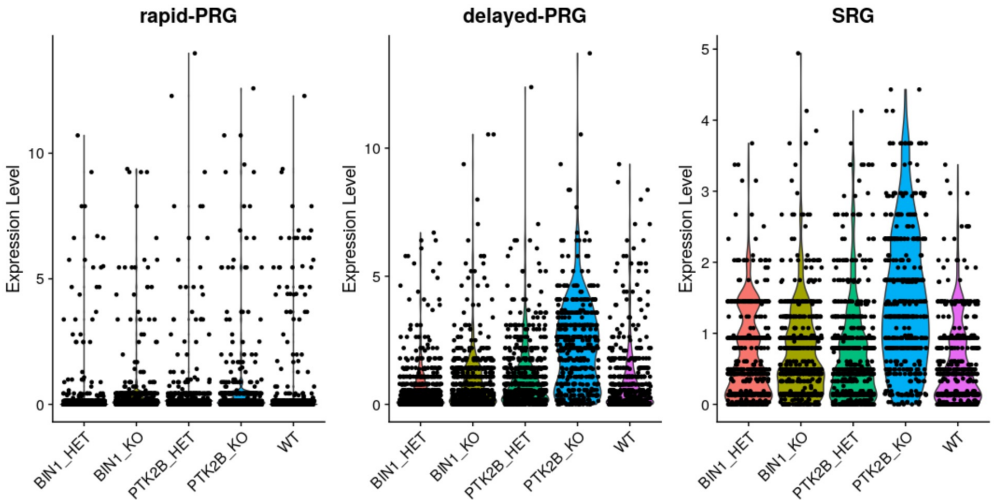
Cell ID

rPRG_ID dPRG_ID SRG_ID

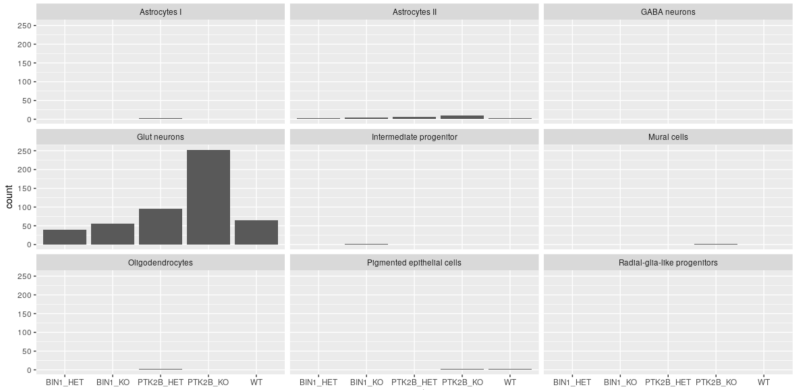


Evidence for altered electrical activity in BIN1 and PTK2B KO cerebral organoids

BIN1 and PTK2B HET and KO glutamatergic neurons express higher levels of dPRGs and SRGs

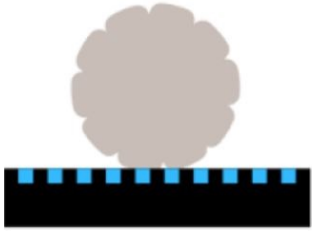


Glutamatergic neuron-specific

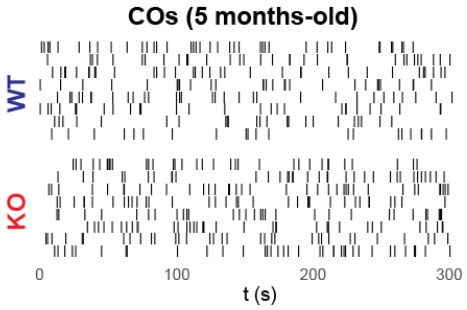


DIRECT MEASUREMENT OF ELECTRICAL ACTIVITY IN CEREBRAL ORGANOIDS USING MULTI-ELECTRODE ARRAYS

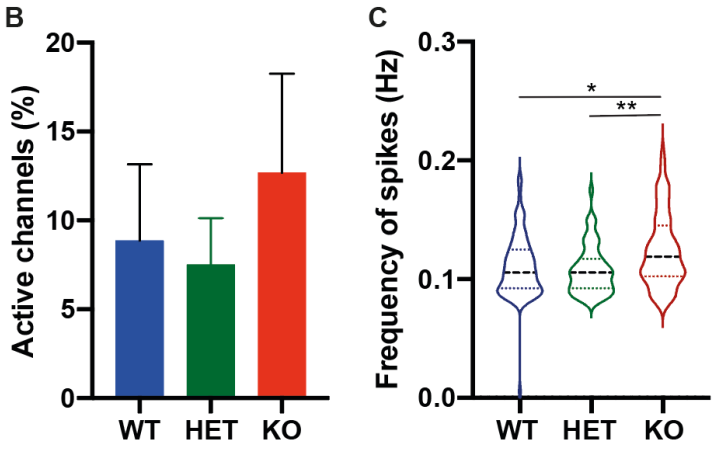
256 channels MEA



Acute recording in 5 to 6 months-old organoids



Spike detection threshold > 5.5 SD

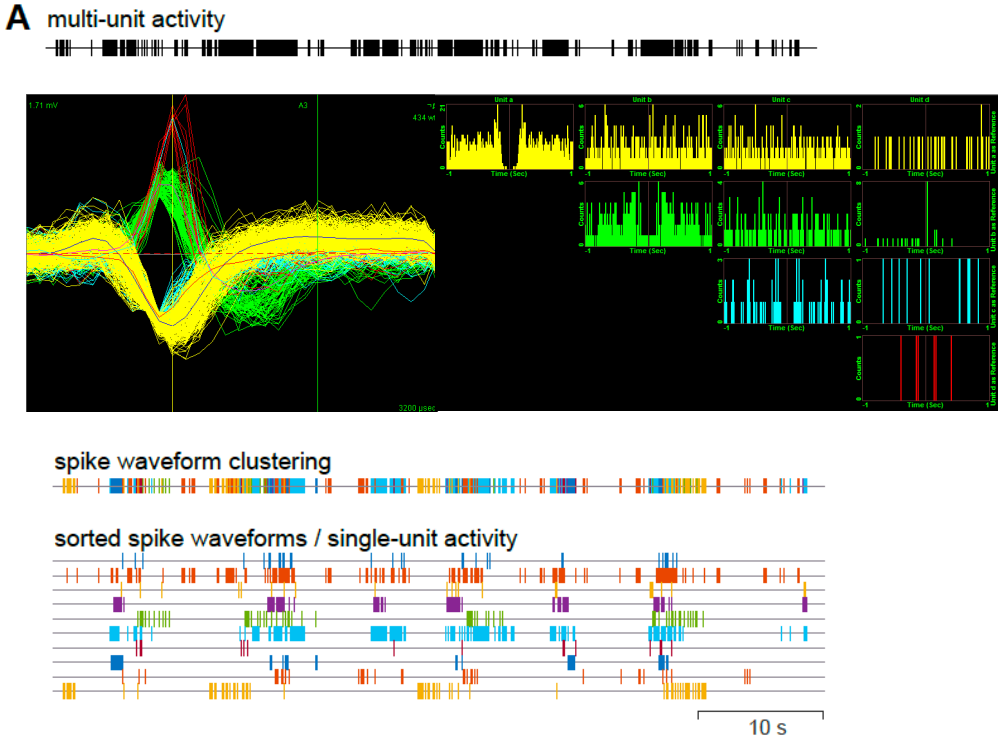


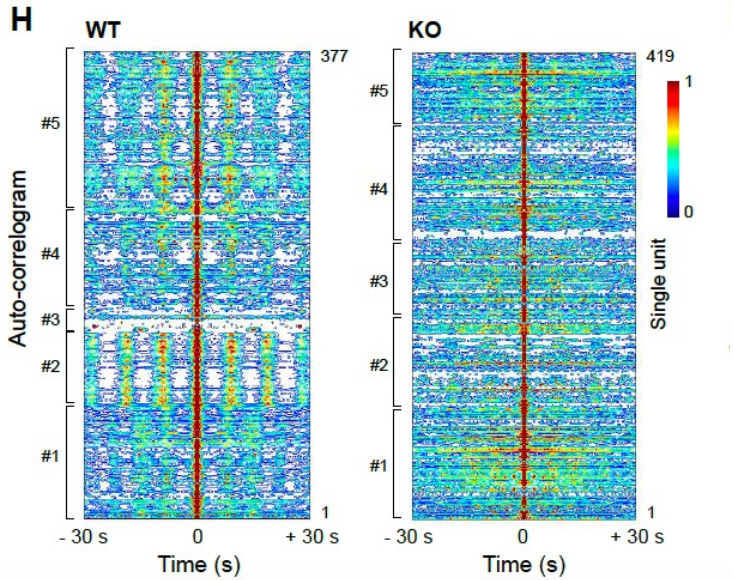
N = 4 organoids from each genotype
Dunn's multiple comparisons test

Saha et al, 2022

DIRECT MEASUREMENT OF ELECTRICAL ACTIVITY IN CEREBRAL ORGANOIDS USING MULTI-ELECTRODE ARRAYS - LIMITATIONS

Despite the high expression of ARGs in 6.5 months-old cerebral organoids, single-unit activity recorded was much lower than that observed in 2D ASCL1-hiNs





Future improvements: use of perforating electrodes

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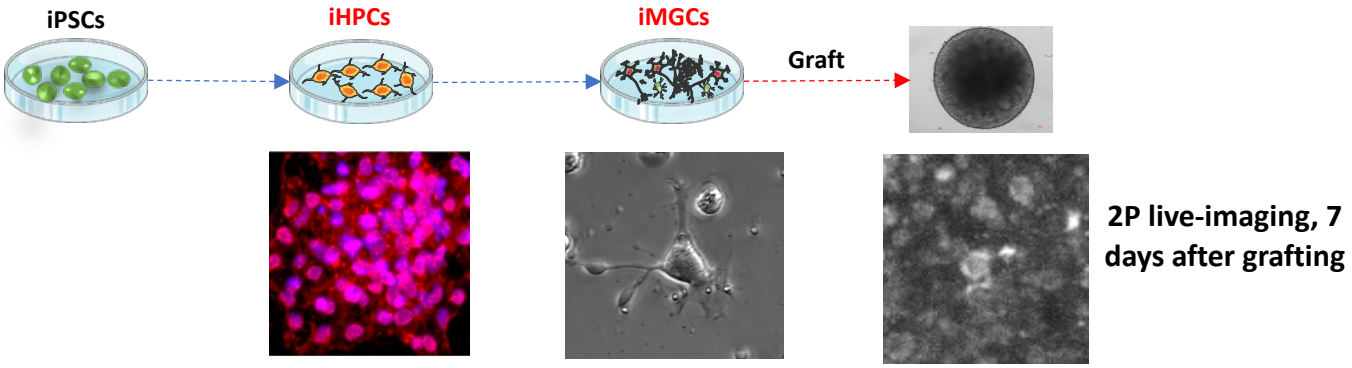
ONGOING WORK AND FUTURE IMPROVEMENTS FOR HUMAN CEREBRAL ORGANOIDS MODELS

- Co-culture of hiPSC-derived microglial cells (hiMGCs) and cerebral organoids



2P live-imaging, 7 days after grafting

- Vascularization of cerebral organoids and prolongation of culture times (up to 2 years)

- Recapitulation of blood brain barrier properties – self-assembly?

CONCLUSIONS

- Cerebral organoids generated from isogenic iPSC lines are comparable in terms of cellular composition and expression of AD-related proteins
- Human cerebral organoids are a powerful tool to unravel and study cell type specific roles of genes associated with Alzheimer's and likely other neurological diseases
- Neuronal electrical activity can be assessed in cerebral organoids by direct and indirect (molecular) methods
- Co-culture with microglial cells and vascularization are important improvements to leverage the use of cerebral organoids as ideal models in neurodegenerative diseases

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