Results

Healthy cortical neurons can be differentiated in 384-well plates for at least 5 weeks

Scheme A depicts the protocol used for the amplification (first 20 days) and detection of expression of NPCs. After neuronal induction and expression, NPCs are frozen. After thawing NPCs are adapted in 384-well plate using diluted matrigel. Numbers represent days in culture (in brackets the number of days after thawing). B, cortical identity (CTIP2) and early maturation markers (Tuj1) are expressed since day 7 of culture. Typical synaptic markers as synapsin (C) and VlakG (D) are present at 18 and 30 days respectively. MAP2/TBR1 staining shows complex connectivity network of glutamatergic cortical neurons after 30 days of differentiation (E). Cells maintain in culture for 30 days show similar metabolic activity as cells cultured for 22 days (measured by quantifying ATE CelldexG2), indicating no alteration in cell viability (n=4 independent experiments). G, no significant difference was observed in cell viability in the two experimental groups in the study after 35 days in culture. Scale bars: 10nm in B, 20nm in C, D, and 40nm in E. D, vitro days in n=5 independent experiments. One-way ANOVA was significant, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 (Dunnett’s multiple comparison test versus non seeded control).

Tau aggregation model using human cortical neurons

A shown baseline values of the assay defined by the counts obtained in all negative control used (i.e. non transduced cells, EGFP transduced cells, cells transduced with F011 but not seeded with K18 and cells only seeded with K18). In B, HTT/Tau10 AlphaLISA confirming overexpression of Tau in non-transduced cells. Optional sampling time was defined at 35 days as HTTA10/HTT10GO signal gives the highest value in the shortest time after reaching a plateau (C). The levels of total Tau do not increase after 28 days of differentiation (D). Tau aggregates were detected with HTTA10/HTT10GO (E) and ATE/ATF (F) in a dose dependent manner. The signal-to-base ratio value obtained from 384-well assay plates after 35 days provide a Z’ factor of 0.932 (G). A shorter version of the protocol also provides significant values for Tau aggregation as soon as 22 days post-seeding (HI). In these conditions: K18 cells seeded with F011-mutant fibrils; EGFP transduced cells overexpressing Tau (EGFP/Tau); control cells with EGFP and seeded with K18; F011-EGFP+K18, cells overexpressing F011 and EGFP; F011+K18; cells overexpressing F011, and seeded with K18 fibrils. n=3 biological replicates in A and D; n=6 technical replicates in E. One-way ANOVA *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 (Dunnett’s multiple comparison test versus non seeded control).

Quantification of Tau aggregates in human neurons

Future directions

• Test tau aggregation capacity of neurons differentiated from iPSCs derived from patients carrying mutations associated to tauopathies (e.g. MAPT p.391V-I3 G-A, and others).
• Optimize protocol in order to accelerate maturation and to improve electrophysiological properties of the neurons.
• Test alternative scaffolds (e.g. chemically defined) aiming to reduce potential sources of variability and costs.

REFERENCES