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We use this protocol and it's working

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Non-guided neural organoids differentiation

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ABSTRACT

This protocol describes how to perform non-guided neural organoid differentiation

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hiPSCs lines (LINC01876 KD)

1 To generate the human cerebral-like organoids we followed the protocol detailed in Johansson et al., 2022, based on the protocol published by Lancaster et al., 2013.

We used three hIPSC6-derived lines obtained by transduction and FACS sorting as described in detail in the protocol CRISPR inhibition of LINC01876 in hiPSCs and fbNPCs.
The cell lines included one control line (with a gRNA against LacZ) and two LINC01876 CRISPRi KD lines (guide 2 and guide 3).

Embroid body formation

2 8000 cells/well were plated in a 96-wells plate (Costar, Ultra Low Attachment, round bottom; REF 7007) with 250 µL of mTeSR1 (StemCell Technologies, Inc.) and RY27632 10 µM. This is considered day −5 of the

differentiation of the iPSCs-derived non-guided neural organoids.

3 On days −3 and −1 the medium was changed (150 µL and 200 µL of mTeSR1, respectively).

Neural induction

4 At day 0 the cells are fed with Neural Induction Medium (NIM; DMEM/F12 media, N2 Supplement (1:100), L-Glutamine (2mM), Penicillin/Streptomycin (1:500), Non-Essential Amino acids (1:100) and Heparin (2ug/ml).) enriched with 3% KSR.

5 At day 2, 4, and 6, the organoids were fed with NIM with no added KSR.

Embedding

6 On day 8 the organoids were embedded in 30-50 µL of Matrigel (Corning) and incubated at 37°C for 25 minutes to allow the Matrigel to solidify.

Differentiation

7 The organoids were then transferred in Corning REF 3471 6-wells plates with flat bottom containing 4ml/well of Cortical Differentiation Medium (CDM; F12 Media (-Glut) (48.5%), Neurobasal (48.5%), N2 Supplement (1:200), B27 Supplement (-Vit.A, 1:100), L-Glutamine (2mM), Penicillin/Streptomycin (1:500), Non-Essential Amino acids (1:200), Beta MercaptoEtOH (50uM) and Insulin (2.5 ug/mL)).

8 On day 10 and 12 of the differentiation, the medium was changed exchanging 3 ml/well for 3 mL of fresh CDM.

9 On day 15, 17, 19, 21 and 23, ~4 mL the medium was replaced with 4 mL of Improved Differentiation Medium + A (IDM, F12 Media (-Glut) (48.5%), Neurobasal (48.5%), N2 Supplement (1:200), B27 Supplement (+Vit.A, 1:50), L-Glutamine (2mM), Penicillin/Streptomycin (1:500), Non-Essential Amino acids (1:200), Beta MercaptoEtOH (50uM), Insulin (2.5 ug/mL) and Ascorbic Acid (400uM)).

10 From day 25, the medium was changed every 3 days with 3-4 mL of Cortical Terminal Differentiation Medium (CTDM, F12 Media (-Glut) (48.5%), Neurobasal (48.5%), N2 Supplement (1:200), B27 Supplement (+Vit.A) – (1:50) 800uL, L-Glutamine (2mM), Penicillin/Streptomycin (1:500), Non-Essential Amino acids (1:200), Beta MercaptoEtOH (50uM), Insulin (2.5 ug/mL) and Ascorbic Acid (400uM), BDNF (10ng/uL), cAMP (200uM), GDNF (10ng/uL)).

Organoids' size measurements

11 All the diameter measurements of the organoids were taken with the Measure tool from the Image J software. The chosen measuring unit was mm.