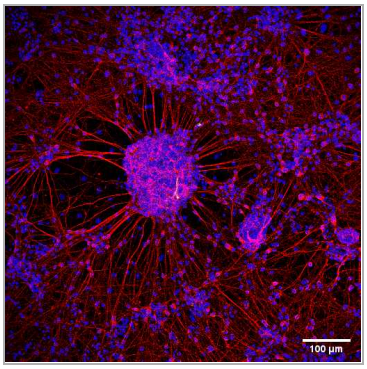

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We use this protocol and it's working

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Differentiation of mature neurons from mouse NPCs

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ABSTRACT

This is a step by step protocol used to differentiate mouse NPCs to mature neurons.

MATERIALS

·Mouse NPCs: vial of mNPCs derived from mES mettl3#6Cre from TEF
·Neurobasal (Thermo scientific, cat. no. 21103-049)
·DMEM/F12 (Thermo scientific, cat. no. 11320-033)
·N2 supplement (Gibco, cat. no. 17502-048)
·Phosphate-buffered saline (PBS; Life technologies, cat. no. 14190-169)
·EDTA 0.5M pH 8
·B27 minus vitamin A (Gibco, cat. no. 11500446)
·Non-essential amino acids (Thermo scientific, cat no.11140-035)
·2-mercaptpethanol (Thermo scientific, cat no.31350-010)
·Glutamax (life technologies, cat no. 35050-061)
·P/S (Thermo scientific, cat no. 15140-122)
·Laminin (Sigma cat no. L2020)
·Poli-L-Ornithine solution (Sigma cat no. A004-C)
·EGF (Gibco, cat no. PMG3043)
·FGF2 (aka bFGF) (Stem cell technologies, cat no. 78003)
·DAPT (Selleck,cat no. S2215)
·BDNF (Sigma,cat no. B3795)
·FBS
·DMSO

●N2B27 medium:

Component	Volume for 50ml
Neurobasal	24 mL
DMEM/F12	24 mL
P/S	0.5 mL
MEM Non-Essential Amino Acids Solution	0.5 mL

GlutaMAX™- 100X	0.5 mL
B-27 without vitamin A	0.5 mL
N2	0.25 mL
β-mercaptoethanol, 1000X. Sigma M-7522	50 uL

This medium could be stored 1 month at 4 C

●**NPC medium:**
N2B27 + EGF 10 ng/ml + FGF2 10 ng/ml
●**differentiation medium:**
N2B27 + BDNF 40 ng/ml + DAPT 10 uM

Coating

1 -Dilute Poly-L-Ornithine (PLO) 1:6 in H2O

2 -Fully coat the wells with diluted PLO (i.e. 500 ul per well in a 12WP)

3 -Leave in the incubator for minimum 2h (also overnight works)

4 -Aspirate the PLO and wash three times with sterile H2O

5 -Dilute Laminin 1:500 in sterile H2O

6 -Fully coat the wells with diluted Laminin (i.e. 500 ul per well in a 12WP)

7 -Leave in the incubator for minimum 2h (also overnight works)

Plating - Day -1

8 -count the desired number of cells: Plate ~100.000 cells on a coverslip, ~150.000 cells in a 24WP well, ~250.000 cells in a 12WP well.

9 -seed NPCs for the final differentiation in NPC medium

Day 0

10 -aspirate all NPC medium

11 -immediately add differentiation medium not directly on the bottom of the well but making it go through the walls to avoid disturbing the cells.

Day 2-14

12 -Change medium (remove half volume and add half volume of new medium) avoiding the cells' contact with air every 2-3 days. If at diff day 2-3 you notice overgrowth of glial cells, add AraC 1uM to the media in order to stop proliferation.

Cells can be kept in colture for >14 days, but they are already MAP2+ and TUJ1+ at day 7.

Tamoxifen treatment

13 -Add 2.5 uM Tamoxifen to the medium, and the corresponding volume of metOH as control. Incubate for at least 6 days.