Supplementary file 1. REAGENTS AND SOLUTIONS

Reagents and solutions protocol 1

a) Medication for anesthetic induction
   i. Ketamine 50 mg/dl (35 mg/kg) – total volume calculation: mg/50mg
   ii. Xylazine 2% (5 mg/kg) – total volume calculation: mg/20 mg
   iii. Atropine: 1.0 - 3.0 mg/kg

b) Medication for anesthetic maintenance
   i. Put 4 ml of ketamine and 3 ml of xylacine in 100 ml of FS. Connect FS with serum equipment and connect to a high-precision flow regulator, adjust it to 30 – 40 ml/h (necessary dose for an animal of 4-5 kg)
   ii. For rescue anesthesia: syringe with propofol 10 mg/kg e.v. (never > 15 – 20 mg/kg)

Reagents and solutions for protocol 2

a) Tissue digestion solution:
   i. 975 µL MEM (prewarmed).
   ii. 20 µL Papain.
   iii. Incubate 10 min at 37 ºC.
   iv. Add 10 µL DNase I.
   v. Sterile filtrate with a syringe filter.

b) Ovomucoid solution:
   i. 975 µL DMEM (prewarmed).
   ii. 10 µL DNase I.
   iii. 5 µL BSA (10 % in PBS).
   iv. 10 µL Trypsin-Inhibitor (prewarmed).
   v. Sterile filtrate with syringe filter.

c) Freezing medium:
   i. 70 % B27.
   ii. 20 % FBS.
   iii. 10 % DMSO.

d) Poly-HEMA coating:
   i. Materials:
      - Poly (2-hydroxyethyl methacrylate).
      - 96 % (v/v) ethanol.
      - H2O, deionized and sterile.
   ii. Poly-HEMA solution:
- Pour 39.5 mL of 96 % (v/v) ethanol and 500 µL of \( \text{dH}_2\text{O} \) into a 50 mL conical tube and mix them.

- After mixing, add 1.2 g of poly-HEMA into the conical tube and dissolve it using a plate rotator o.n. at RT.

- Store this poly-HEMA stock solution at 4 °C for up to 2 months.

iii. Coating of dishes and plates with Poly-HEMA solution:
- Apply the appropriate volume of the poly-HEMA solution (Table S1) to each dish or well in the tissue culture hood.
- Spread the poly-HEMA solution over the entire surface of each dish or well.
- Leave the dish or plate o.n. without lid to allow the poly-HEMA solution to completely evaporate.
- The poly-HEMA–coated dishes can be used for 3 months after coating when stored at RT in the dark.

**Table S1. Volume of poly-HEMA solution for dish or well coating.**

| Dish size          | Volume of poly-HEMA solution |
|--------------------|------------------------------|
| 90 mm (diameter)   | 3.2 mL                       |
| 60 mm (diameter)   | 1.3 mL                       |
| 35 mm (diameter)   | 500 µL                       |
| 12 well            | 200 µL                       |
| 24 well            | 100 µL                       |
| 48 well            | 70 µL                        |
| 96 well            | 25 µL                        |

**Reagents and solutions for protocol 3**

a) Laminin solution: Add \( \text{dH}_2\text{O} \) to the Laminin solution to reach a final concentration of 1 mg/mL. The amount of water added needs to be determined for each new lot. Store Laminin at -20 °C.

b) PDL solution: Dilute 50 mg PDL in 500 mL \( \text{dH}_2\text{O} \), aliquot and store at -20 °C.

c) First antibody solution immunocytostaining of neurospheres:
   - i. 1st antibody (dilution as indicated in Table S2).
   - ii. 10 % goat serum.
   - iii. PBS or PBST for intracellular epitopes.

d) Second antibody solution immunocytostaining of neurospheres:
   - i. 2nd antibody (dilution as indicated in Table S2).
   - ii. 1 % Hoechst (stock: 0.2 mg/mL).
   - iii. 2 % goat serum.
   - iv. PBS.
Table S2. Antibody solutions

| 1st antibody | Species | Type | Antigen | Dilution | Incubation |
|--------------|---------|------|---------|----------|------------|
| O4           | Oligodendrocytes | Mouse | IgM     | 1:200    | o.n., 4 °C |
| β(III)-Tubulin | Neurons | Rabbit | IgG    | 1:200    | 1h, 37 °C  |
| GFAP         | Astrocytes | Rabbit | IgG    | 1:200    | o.n., 4 °C |

| 2nd antibody | Species | Type | 1st antibody binding | Dilution | Incubation |
|--------------|---------|------|----------------------|----------|------------|
| anti-Mouse IgM, Alexa Fluor 488 | Goat | IgM | O4 | 1:200 | 30 min, 37 °C |
| anti-Rabbit IgG, Alexa Fluor 488 | Goat | IgG | β(III)-Tubulin | 1:100 | 30 min, 37 °C |
| anti-Rabbit IgG, Alexa Fluor 546 | Goat | IgG | GFAP | 1:200 | 30 min, 37 °C |

*Abbreviations: o.n.: overnight.*

e) PDL-laminin coating of 8-Chambered Cell Culture Slides:

i. Thaw PDL solution at 37 °C and add 250 µL PDL solution to chambers of an 8-chamber slide.

ii. Incubate for 1 h at 37 °C.

iii. Thaw Laminin solution at 4 °C and dilute it 1:100 in dH2O.

iv. Remove PDL solution.

v. Wash chambers/wells with dH2O.

vi. Add laminin dilution to chambers/wells.

vii. Incubate for 1 h at 37 °C.

viii. Wash chambers with dH2O and sterile PBS.

ix. Coated slides can be stored at 4 °C for up to one week.

Table S3. Volume of PDL and Laminin solution for dish or well coating.

| Dish size        | PDL and Laminin | H2O washing and PBS storage |
|------------------|-----------------|-----------------------------|
| 8-chamber-slide  | 250 µL          | 500 µL                      |
| 96 well          | 50 µL           | 100 µL                      |
| 48 well          | 150 µL          | 300 µL                      |
| 24 well          | 300 µL          | 500 µL                      |
| 12 well          | 500 µL          | 1 mL                        |
| 6 well           | 1 mL            | 2 mL                        |

f) Controls for the neurosphere differentiation and migration assays:

i. Background control: only N2 media without spheres.

ii. Solvent control: N2 media with respective solvent with spheres.

iii. Positive controls:

- Lysis control: N2 media with respective solvent with spheres.

- Oligodendrocyte differentiation: 100 ng/mL BMP7.

- Neuronal differentiation: 10 ng/mL EGF.
- Migration: 10 µM PP2.

g) Controls for cell titer blue (CTB) assay:
   i. Lysis control: culture medium with cells. Add DMSO to a final concentration of 10% 30 min before addition of CTB-reagent.
   ii. Background control: culture medium without cells.

h) Controls for proliferation assay:
   i. Solvent control: B27 media with respective solvent.
   ii. Positive control: B27 media without growth factors (B27 w/o).
   iii. If combined with CTB Assay prepare lysis control and background control.
   - Background control: only B27 media.
   - Lysis control: Solvent control with spheres.

**Reagents and solutions for protocol 5**

a) Sucrose 30 %: 30 g sucrose in 100 mL phosphate buffer 0.1 M.

**Reagents and solutions for protocol 6**

a) Solution A+B in proportion 1:1 (should be prepared 24 h prior to use, and has 1 month of viability). Prepare 5 mL of solution/1 cm³ of tissue (15 mL per brain, approximately).

b) Cresyl violet: 0.1 g/100 mL dH₂O + 2 drops of acetic acid. Prepare 24 h before use, viability 1 year.

**Reagents and solutions for protocols 7, 8, 9 and 14.**

a) IHC blocking solution:
   i. 10 % fetal bovine serum (10 mL/100 mL).
   ii. 0.2 M glycin (0.15 g/100 mL).
   iii. 0.2 M gelatin (0.2 g/100 mL).
   iv. PBST 0.3 %.

b) PBST 0.3 %: 2.4 mL Triton + 797.6 mL PBS.

c) Oligodendrocyte immunocytochemistry solutions:
   i. Primary antibody (Mouse IgM anti-O4) solution: 1 µL antibody + 49 µL PBST/cut.
   ii. Secondary antibody (Alexa Fluor 488 goat anti-mouse IgM) solution: 0.5 µL Hoechst + 49,375 µL PBST + 0.125 µL antibody/cut.

d) Perineuronal nets immunocytochemistry solutions:
   i. Primary antibody solution: 20 µL antibody + 190 µL blocking solution + 190 µL PBST 0.3 %/slide.