

Supplementary Figures and Tables

Spatio-temporal dynamics enhance cellular diversity, neuronal function and further maturation of human cerebral organoids

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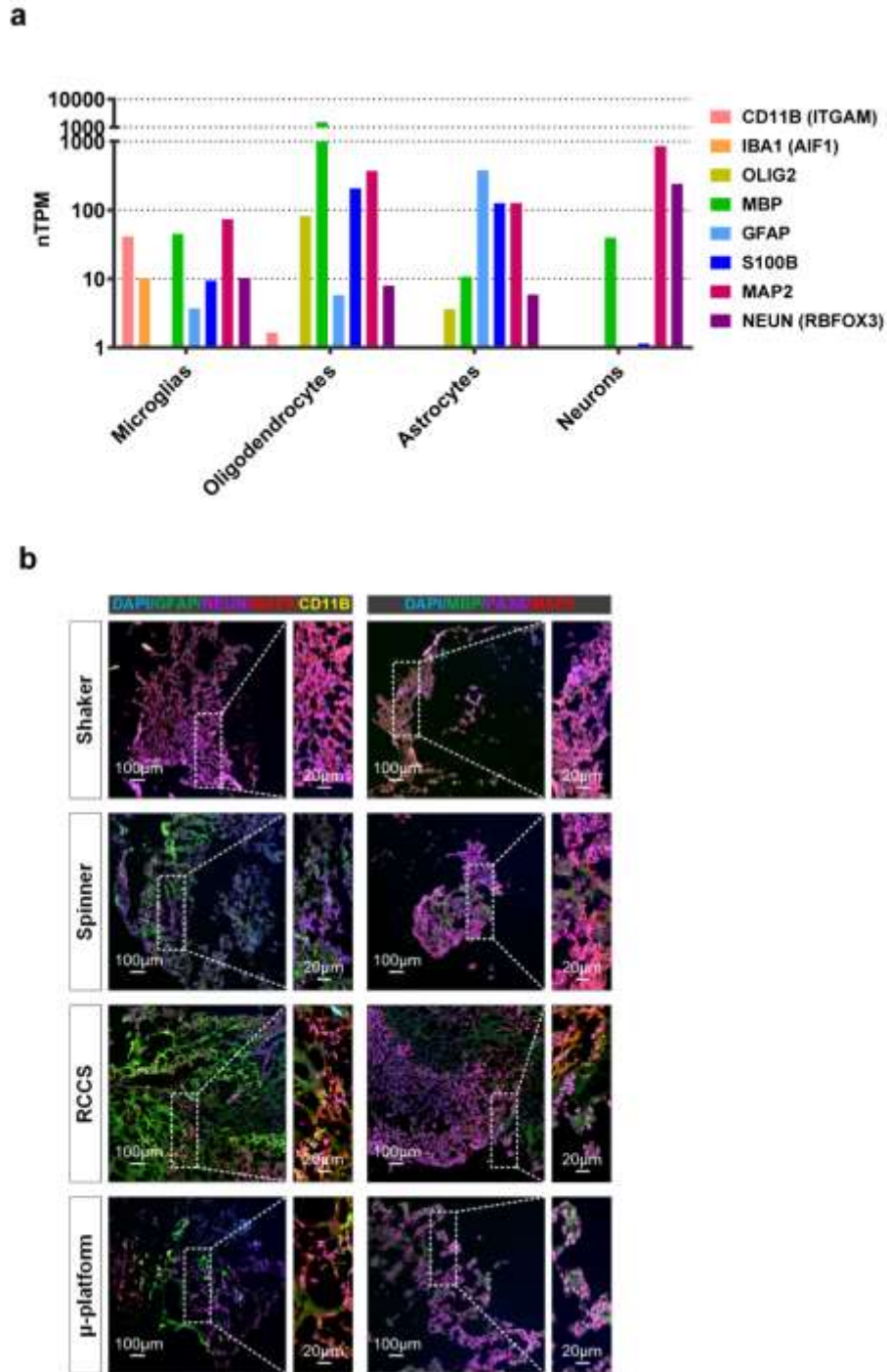
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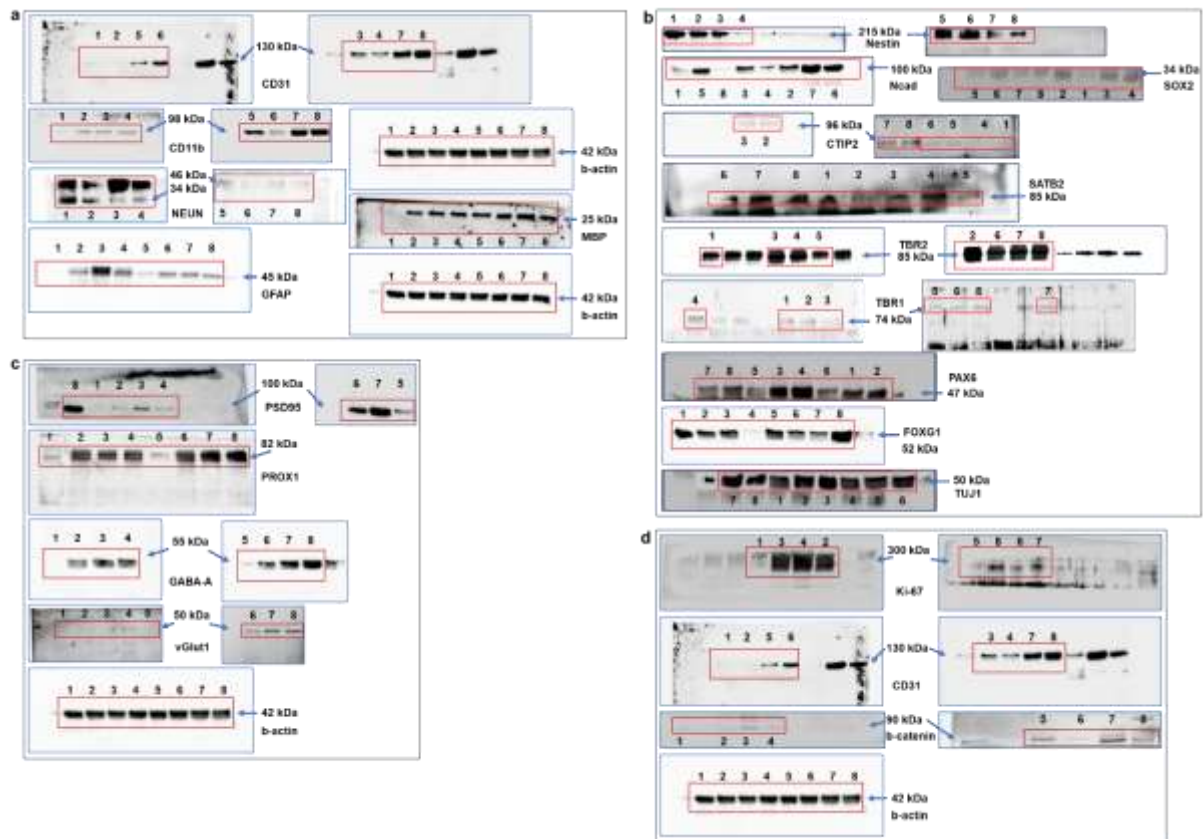
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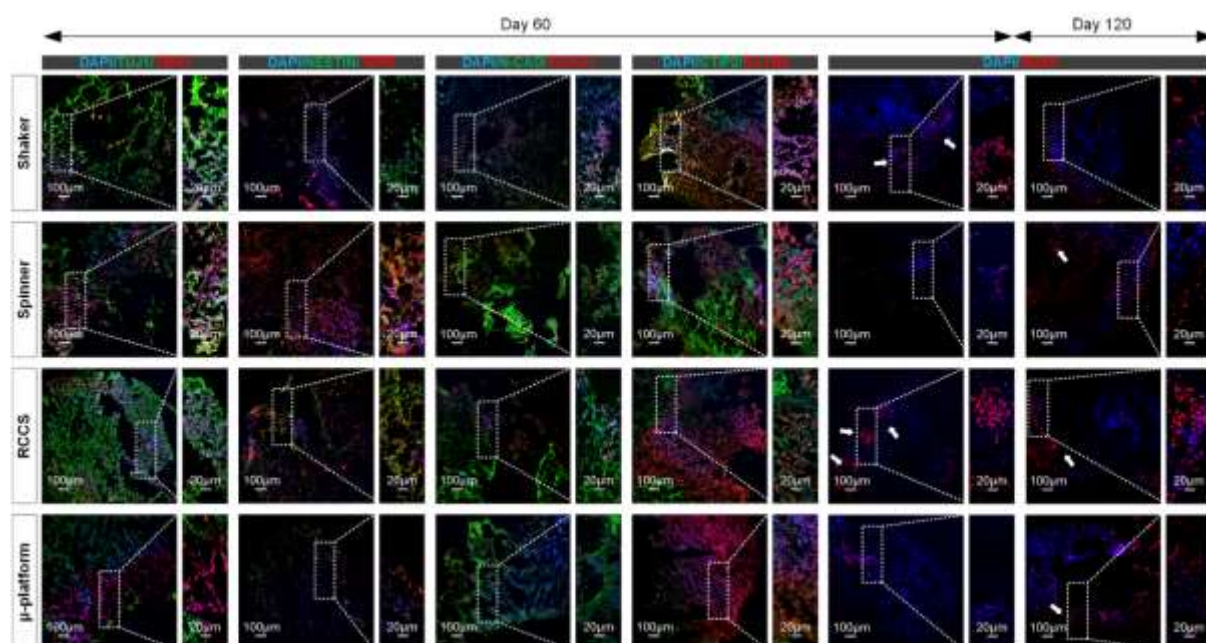
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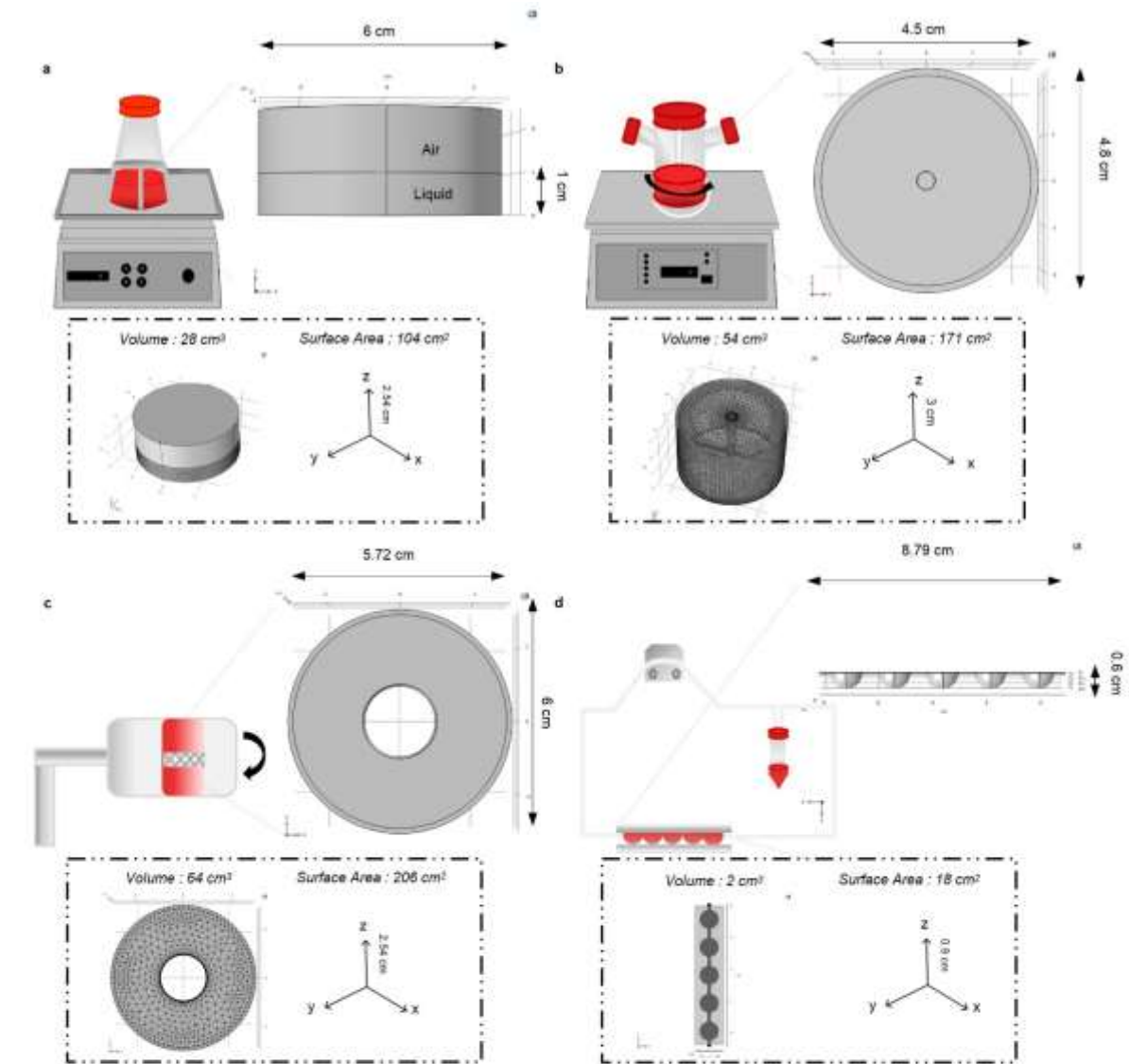
Supp. Fig. 1 a The normalized RNA expression levels of specific neuronal markers in different cell types, nTPM: normalized transcripts per million (*Human Protein Atlas V21.0* [proteinatlas.org](https://www.proteinatlas.org/); Karlsson et al., 2021) **b** Immunofluorescence staining of specific neuronal/glial cell markers (NEUN, MAP2, PAX6, GFAP, CD11B, MBP) on day 60 of organoids matured in dynamic systems (scale bars=100 μ m for 10x and 20 μ m for 25x magnification images, independent replicates=3, Zeiss LSM 880).



Supp. Fig. 2 Original western blot images. **a** Neuronal proteins, **b** specific maturation proteins, **c** advanced maturation proteins and **d** survival proteins of cerebral organoids matured in shaker (1,5), spinner (2,6), RCCS (3,7) and μ -platform (4,8) at day 60 and 120, respectively (independent replicates=2).



Supp. Fig. 3 Immunofluorescence staining of cerebral organoid maturation markers (TUJ1/TBR1, NESTIN/TBR2, N-CAD/FOXG1, CTIP2/SATB2 and SOX2) on days 60 and 120 of organoids matured in dynamic systems, white arrows indicate neural rosette-like structures (scale bars=100 µm for 10x and 20µm for 25x magnification images, independent replicates=3, Zeiss LSM 880).



CFD Module/Physics type	Equations
Laminar Flow, Navier-Stokes Equation	$\rho(\mathbf{u} \cdot \nabla) \mathbf{u} = \nabla[-p\mathbf{I} + \mu(\nabla \mathbf{u} + (\nabla \mathbf{u})^T)] + \mathbf{F}$ $\rho \nabla \cdot \mathbf{u} = 0$
Turbulent Flow, k-ε	$\rho(\mathbf{u} \cdot \nabla) \mathbf{u} = \nabla[-p\mathbf{I} + \mathbf{K}] + \mathbf{F} + \rho \mathbf{g}$ $\rho \nabla \cdot \mathbf{u} = 0$ $\mathbf{K} = (\mu + \mu_T)(\nabla \mathbf{u} + (\nabla \mathbf{u})^T)$ $\rho(\mathbf{u} \cdot \nabla) \mathbf{k} = \nabla \cdot \left[\left(\rho k + \frac{\mu_T}{\sigma_k} \right) \nabla k \right] + P_k - \rho \epsilon$ $\rho(\mathbf{u} \cdot \nabla) \epsilon = \nabla \cdot \left[\left(\rho \epsilon + \frac{\mu_T}{\sigma_\epsilon} \right) \nabla \epsilon \right] + c_{1\epsilon} \frac{\mu_T}{k} P_k - c_{2\epsilon} \rho \frac{k^2}{\epsilon}, \quad c = \epsilon \rho$ $\mu_T = \rho C_\mu \frac{k^2}{\epsilon}$ $P_k = \mu_T (\nabla \mathbf{u} : (\nabla \mathbf{u} + (\nabla \mathbf{u})^T))$
Shear Stress Equations	$\tau_{\text{lam}} = \mu \gamma \quad \gamma = \frac{dU}{dx}$ $\tau_{\text{turb}} = (\mu + \mu_T) \gamma \quad \gamma = \frac{dU}{dx}$
Boundary Condition	Values
Constant flow rate for μ-platform (m³/s)	1.67 ⁻³ , 7.33 ⁻³ , 1.33 ⁻³
Constant pressure (Pa)	0
No slip condition	
Flow properties	
Material	Water
Dynamic Viscosity (Pa.s)	0.0007
Density(kg m ⁻³)	993.37

Supp. Fig. 4 All system illustrations for 4 dynamic fluid systems, geometries and volume/surface area information used in simulations; **a** shaker, **b** spinner, **c** RCCS and **d** μ -platform. **e** Equations and variables used for CFD.

Supp. Table 1 Log2 transformation data of qRT-PCR analysis (** *p* value < 0.05)

	Day 30 +					Day 60 +					Day 120 +				
	Static	Spinner	Shaker	RCCS	μ -platform	Static	Spinner	Shaker	RCCS	μ -platform	Static	Spinner	Shaker	RCCS	μ -platform
CDH2	1.54**	2.71**	3.43**	0.82**	2.49**	3.42**	4.70**	2.34**	7.67**	2.84**	6.46**	7.16**	6.34**	6.54**	-2.00**
CTIP2	4.44**	4.14**	4.42**	6.92**	1.36**	-0.56**	2.06**	7.58**	3.65**	0.96**	0.03**	-0.43**	1.03**	4.97**	10.64**
FOXP1	-0.42**	2.65**	2.43**	-0.23**	2.27**	0.96**	-0.03	2.89**	2.24**	-1.60**	-2.12**	1.64**	0.72**	2.18**	4.73**
KROX20	1.02**	1.50**	2.30**	1.70**	2.05**	1.73**	3.57**	4.23**	5.64**	0.06**	2.05**	2.10**	0.82**	1.79**	5.42**
PAX6	-0.60**	1.43**	0.38**	-0.86**	2.30**	1.45**	2.70**	3.18**	0.14**	0.04**	0.88**	2.04**	1.57**	-0.25**	5.11**
PROX1	-0.10**	2.51**	1.25**	-2.94**	3.12**	0.43**	-1.15**	0.64**	-1.22**	-2.32**	-2.74**	6.71**	7.74**	7.86**	4.07**
PSD95	0.91**	1.63**	1.72**	0.25**	-0.60**	2.69**	-0.81**	1.60**	2.82**	0.82**	-0.22**	-0.03	-0.67**	0.94**	3.15**
SATB2	0.01	-0.09**	-0.81**	-1.60**	-1.47**	0.67**	0.07**	0.30**	1.75**	1.29**	-0.60**	-0.29**	3.58**	5.08**	5.49**
SOX1	-0.60**	0.14**	0.00	-1.00**	-1.43**	0.19**	-0.15**	0.15**	-0.43**	-2.56**	0.40**	2.46**	1.69**	0.59**	-0.94**
SOX2	0.48**	1.02**	1.06**	0.07**	0.67**	1.00**	1.80**	1.90**	1.49**	-5.06**	2.00**	4.48**	2.94**	1.29**	-0.64**
TBR1	0.79**	2.49**	1.79**	-5.06**	-6.64**	0.71**	-5.64**	-3.06**	-3.18**	-4.06**	-4.06**	-6.64**	-0.74**	0.94**	2.10**
TBR2	-2.25**	0.57**	-5.06**	-4.64**	-4.06**	-0.36**	-3.47**	-8.41**	-6.64**	-4.32**	-2.94**	-2.12**	-3.47**	-2.74**	1.51**
TTR	6.95**	7.10**	6.90**	8.99**	9.51**	6.94**	8.46**	7.89**	8.99**	-2.84**	8.34**	11.00**	8.86**	9.27**	0.06**
TUJ1	-0.36**	1.52**	1.04**	2.51**	2.74**	1.74**	1.69**	2.18**	4.12**	4.43**	-1.56**	-0.45**	-2.06**	-3.32**	-0.18**
CD11B	-1.43**	1.20**	3.20**	-1.36**	2.15**	-0.62**	1.08**	0.29**	1.63**	-5.64**	-5.06**	-5.06**	-4.64**	-1.64**	8.72**
GFAP	0.55**	1.51**	2.19**	1.14**	1.18**	2.40**	1.76**	0.59**	3.22**	-3.47**	-0.10**	0.26**	0.42**	0.05**	1.56**
IBA1	-0.60**	1.15**	0.65**	0.25**	-2.84**	1.76**	-0.47**	-2.06**	0.98**	3.72**	-2.40**	7.96**	3.80**	7.21**	4.00**
MAP2	0.56**	1.81**	1.90**	0.44**	0.90**	2.36**	4.50**	5.60**	4.75**	2.32**	5.68**	6.70**	6.17**	6.11**	1.93**
MBP	0.48**	0.64**	1.02**	1.55**	2.29**	0.02**	0.92**	2.10**	4.02**	-4.64**	-3.32**	-3.84**	-3.64**	1.29**	2.46**
NESTIN	-0.03**	0.21**	1.45**	1.94**	0.07**	1.20**	3.25**	6.77**	6.33**	-5.06**	2.39**	3.23**	1.71**	1.94**	2.92**
NEUN	4.06**	1.33**	0.52**	3.42**	-0.12**	0.67**	6.01**	6.19**	5.60**	4.32**	0.64**	1.29**	0.74**	2.51**	1.93**
OLIG2	0.16**	-1.15**	5.20**	-0.67**	3.16**	0.55**	0.01	1.53**	5.18**	-3.32**	2.37**	0.82**	3.75**	2.35**	5.09**
S100B	0.41**	1.49**	0.40**	-2.40**	-3.06**	2.31**	-1.15**	-2.25**	3.08**	-1.84**	4.25**	2.26**	1.03**	0.92**	4.53**
CD31	1.86**	1.32**	2.16**	-2.54**	-26.23**	-53.08**	-2.78**	-3.78**	-1.10**	-1.78**	2.87**	5.60**	11.69**	14.82**	46.53**
Ki-67	-1.65**	2.16**	-1.38**	2.25**	-1.95**	-3.75**	6.03**	8.44**	10.02**	11.64**	1.66**	6.25**	2.12**	11.10**	25.52**
b-catenin	1.14**	-2.35**	-1.27**	-1.42**	-1.16**	1.36**	2.51**	1.8**	2.59**	2.03**	-6.09**	10.72**	8.28**	13.12**	13.01**

Supp. Table 2 Antibody list for immunofluorescence and western blot analysis

Antibody	Host	Supplier/cat. no	IF dilution	WB dilution	FACS dilution	Region	Cellular localization
SOX2	Rabbit	Abcam, ab97959	1:200	1:1000	-	Radia glia/NSCs	Nucleus
PAX6	Rabbit	Abcam, ab195045	1:350	1:1000	-	Apical/Radia glia/NSCs	Nucleus
TUJ1	Mouse	Abcam, ab78078	1:1000	1:1000	-	Cortical neurons	Cytoplasm, cytoskeleton
N-CADHERIN	Mouse	Abcam, ab98952	1:500	1:1000	-	Apical epithelial	Cell membrane
FOXP1	Rabbit	Abcam, ab18259	1:200	1:1000	-	Forebrain	Nucleus
TBR1	Rabbit	Abcam, ab31940	1:200	1:1000	-	Preplate/Deep layer neurons	Nucleus
TBR2	Rabbit	Abcam, ab23345	1:500	1:1000	-	Intermediate progenitors	Nucleus

PROX1	Rabbit	Abcam, ab101851	1:750	1:1000	-	Hippocampus	Nucleus
CTIP2	Mouse	Abcam, ab233713	1:200	1:1000	-	Early born/Deep layer cortical neurons	Nucleus
SATB2	Rabbit	Abcam, ab34735	1:500	1:1000	-	Later born/Surface layer neurons	Nucleus matrix
PSD95	Mouse	Abcam, ab13552	1:200	1:1000	-	Postsynaptic marker	Cell membrane, junction, synapse
MAP2	Chk	Abcam, ab5392	1:1000	1:1000	-	Mature neurons	Cytoplasm, cytoskeleton
NEUN	Rabbit	Abcam, ab177487	1:200	1:1000	-	Neural marker	Nucleus, cytoplasm
CD45	Rabbit	Abcam, ab10558	-	-	1:1000	Mesodermal cell	Membrane
CD11B	Rat	Abcam, ab8878	1:500		1:1000	Mature microglia	Membrane
CD11B/C	Rabbit	Bioss, bs-1014R		1:1000	-	Mature microglia	Membrane
GFAP	Mouse	Abcam, ab10062	1:200	1:1000	1:1000	Mature astrocytes	Cytoplasm, intermediate filaments
MBP	Mouse	Abcam, ab62631	1:1500	1:2500	-	Mature oligodendrocytes	Myelin membrane. cytoplasmic side of myelin
NESTIN	Mouse	SantaCruz, sc-23927	1:500	1:1000	-	CNC progenitor cells	Intermediate filament
VGLUT1	Rabbit	Thermo, 48-2400	1:500	1:250	-	Glutamatergic neurons	Vesicular membranes
GABA-A	Rabbit	Affinity, Ab6207	1:250	1:500	-	GABAergic interneurons	Cytoplasm, cell membrane
Ki-67	Mouse	Cell signaling, 9449	1:500	1:500	-	Proliferating cells	Nucleus
B-catenin	Mouse	SantaCruz, sc-7963	1:250	1:500	-	Cell junction, adhesion	Cell membrane Cytoskeleton
CD31	Rabbit	SantaCruz, sc-376764	1:200	1:1000	-	Endothelial cells	Cell membrane
Beta-Actin	Mouse	Thermo, MA1140	1:1000	1:2500	-	A non-muscle cytoskeletal protein	Cytoskeleton
Alexa Fluor®647 conjugated Anti-Rabbit IgG H&L	Goat	Abcam, ab150079	1:1000	-	1:1000	-	-
Alexa Fluor®594 conjugated Anti-Chicken IgY H&L	Goat	Abcam, ab150176	1:1000	-	-	-	-

Alexa Fluor®488 conjugated Anti-Mouse IgG H&L	Goat	Abcam, ab150113	1:1000	-	1:1000	-	-
Alexa Fluor®555 conjugated Anti-Rat IgG H&L	Goat	Abcam, ab150158	1:1000	-	-	-	-
Alexa Fluor®488 conjugated Anti-Rat IgG H&L	Goat	Abcam, ab150157	-	-	1:1000	-	-
HRP conjugated Anti-Rabbit IgG(H+L)	Goat	ProteinTech, SA00001-2	-	1:1000	-	-	-
HRP conjugated Anti-Mouse IgG(H+L)	Goat	ProteinTech, SA00001-1	-	1:1000	-	-	-

Supp. Table 3 Primer list for qRT-PCR analysis

Gene	5'forward 3'	5' reverse 3'
<i>ACTB</i>	AGAGCTACGAGCTGCCTGAC	CGTGGATGCCACAGGACT
<i>GAPDH</i>	CGTAGCTCAGGCCTCAAGAC	GCTGCGGGCTCAATTTATAG
<i>HPRT1</i>	GACCAGTCAACAGGGGACAT	GTGTCAATTATATCTTCCACAATCAAG
<i>KLF4</i>	TTCACACTGTCTTCCCGATG	CAGTTGGGAACCTTGACCATGA
<i>C-MYC</i>	AAGAGGCGAACACACAACG	AAAAGCTCCGTTTTAGCTCGT
<i>OCT4</i>	GCTTCGGATTTTCGTCTTCTC	CTTAGCCAGGTCCGAGGAT
<i>SOX2</i>	TTGCTGCCTCTTTAAGACTAGGA	TAAGCCTGGGGCTCAAACCT
<i>TUJ1 (TUBB3)</i>	GACCGGACGGTGAGTCAG	CACGAGGGAAGAAGTTTTCG
<i>PAX6</i>	GCAGGAGGAAGTGTTTTGCT	ATGTTGCTGGGTTGGTGTG
<i>PROX1</i>	GAGGGTGGGAAAGGGGTTTT	TCAAACGGCACTGAGCTTGT
<i>FOXG1</i>	GCTTAAACGAAAATGACCCAGT	CCTAGGGCCAATAGCTCACA
<i>CDH2 (Ncad)</i>	CTCCATGTGCCGGATAGC	CGATTTACACCAGAAGCCTCTAC
<i>KROX20</i>	TCCAAAACGGCTTTTCTGAC	CGGTCATCATTTGCTCCTC
<i>SOX1</i>	TCTTTTGGGTTGGTTTGTTAATT	AATATAACTCCGCCGTCTGAAG
<i>PSD95</i>	GACGGCCTACTTTACTCACAGC	CTCCAGGATTGGAGTTGAGC
<i>TBR1</i>	TAGCAGCGCTAACATCAGCA	CCGAGCCCTAAGCCTTAAA
<i>CTIP2</i>	GCTTTCCACCTACCAGACCC	ATCACGGATGAGTGAGGGTG
<i>TTR</i>	CCGGTGAATCCAAGTGTCCT	AGATGCCAAGTGCCCTCCAG
<i>SATB2</i>	GTCTTCTCGGCTCTTGGTGT	GTGTCTTCTTCTGGTGCGGA
<i>MAP2</i>	ATGACCCCTCATCCAAAG	CATGTGGCCAGACTCAACAC
<i>TBR2 (EOMES)</i>	GAGTCGGCAGGTGGGTAG	TCTTCCGAGGGGAAGGTAA
<i>NES</i>	ACCTGTGCCAGCCTTTCTTA	GCCAAGGTAGGGGTACGG
<i>NEUN (RBFOX3)</i>	CCCAACAGAAAGGGCTGAC	CTGGGCTTCCTTCGTCCT

<i>IBA1</i>	CTCCAGCTTGGAGGAAAAGC	TGGAGGGCAGATCCTCATCA
<i>CD11B</i>	AGGACTCTGCCCAGACCAC	GTCGGGGATACTTCGCTGT
<i>GFAP</i>	CTTGCTTCAGCGGTCAGG	TGCATGAGGCAGGTGGTA
<i>S100B</i>	TGTAGACCCTAACCCGGAGG	TGCATGGATGAGGAACGCAT
<i>OLIG2</i>	TCGCATCCAGATTTTCGGGT	TCCATGGCGATGTTGAGGTC
<i>MBP</i>	CCCTGCACCTGTCAGTCC	GGCAGCTTTCTCACATACCG
<i>CD31 (PECAM1)</i>	TGAGTGGTGGGCTCAGATTG	TGAGTCTAGGTCGGGGAGTG
<i>Ki-67</i>	CTGACCCTGATGAGAGTGAGGGA	ACTCTGTAGGGTCGAGCAGG
<i>b-catenin</i>	TGTTAAATTCTTGGCTATTACGACA	CCACCACTAGCCAGTATGATGA