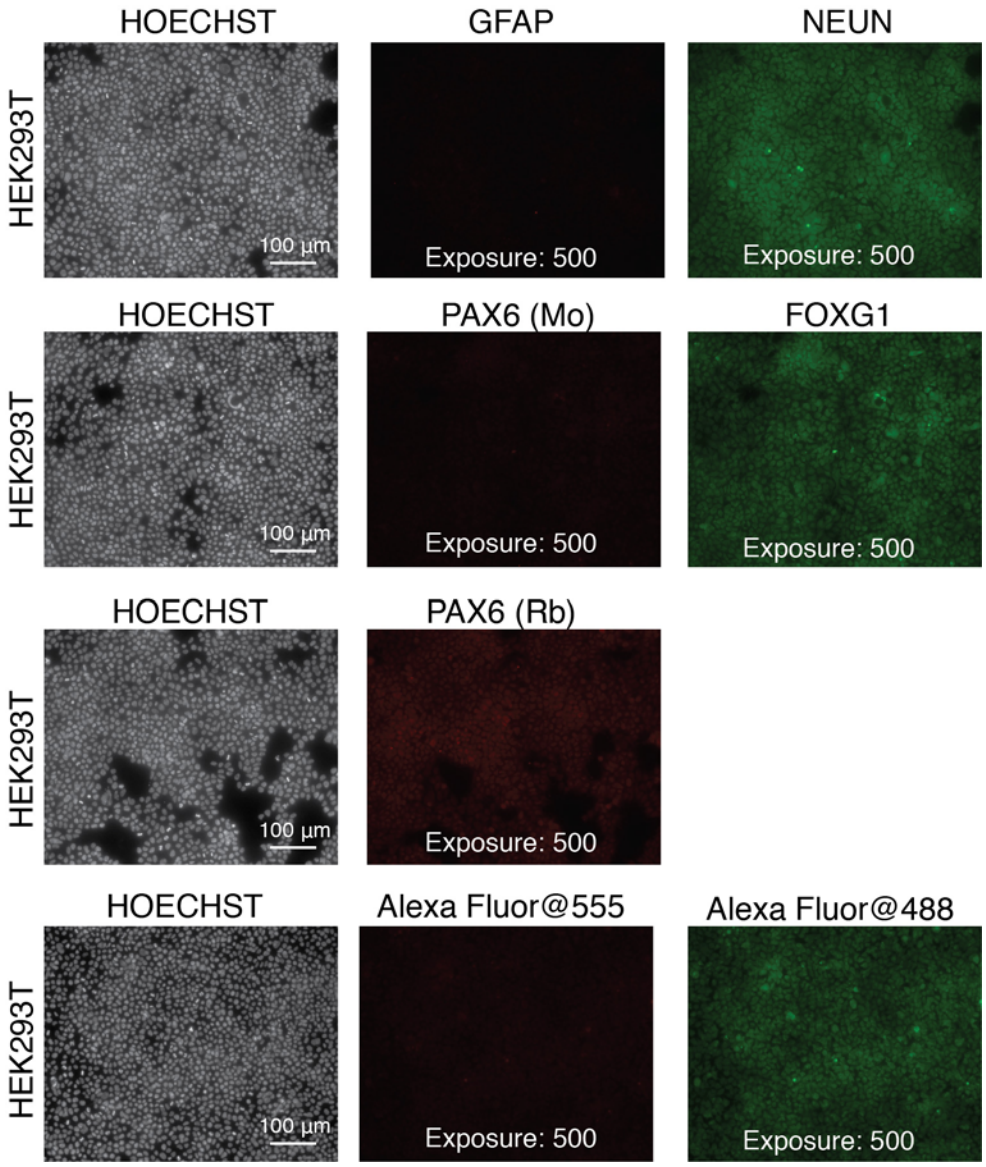
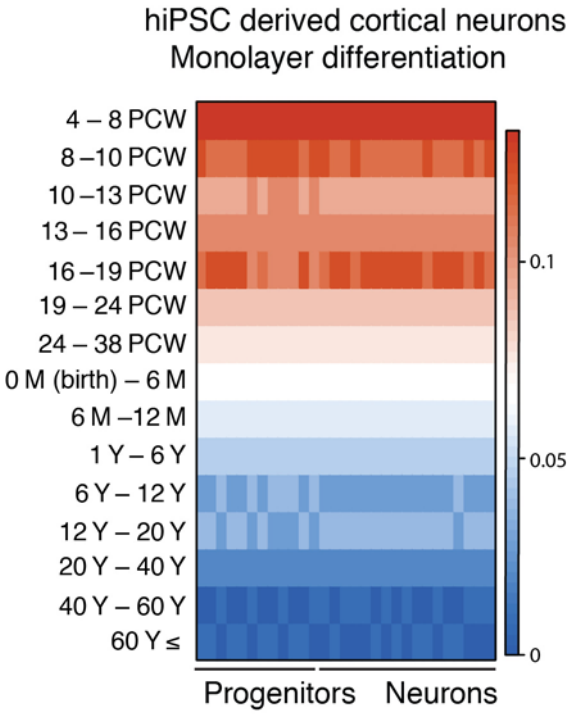


Supplementary Figure 1

Antibody specificity.

Panel showing the specificity of the antibodies against NEUN, GFAP, FOXG1, PAX6 (Rb), PAX6 (Mo) in negative cells (HEK293T). The last row shows background immunostaining for secondary-only conditions. All images were collected at a 500 ms exposure.

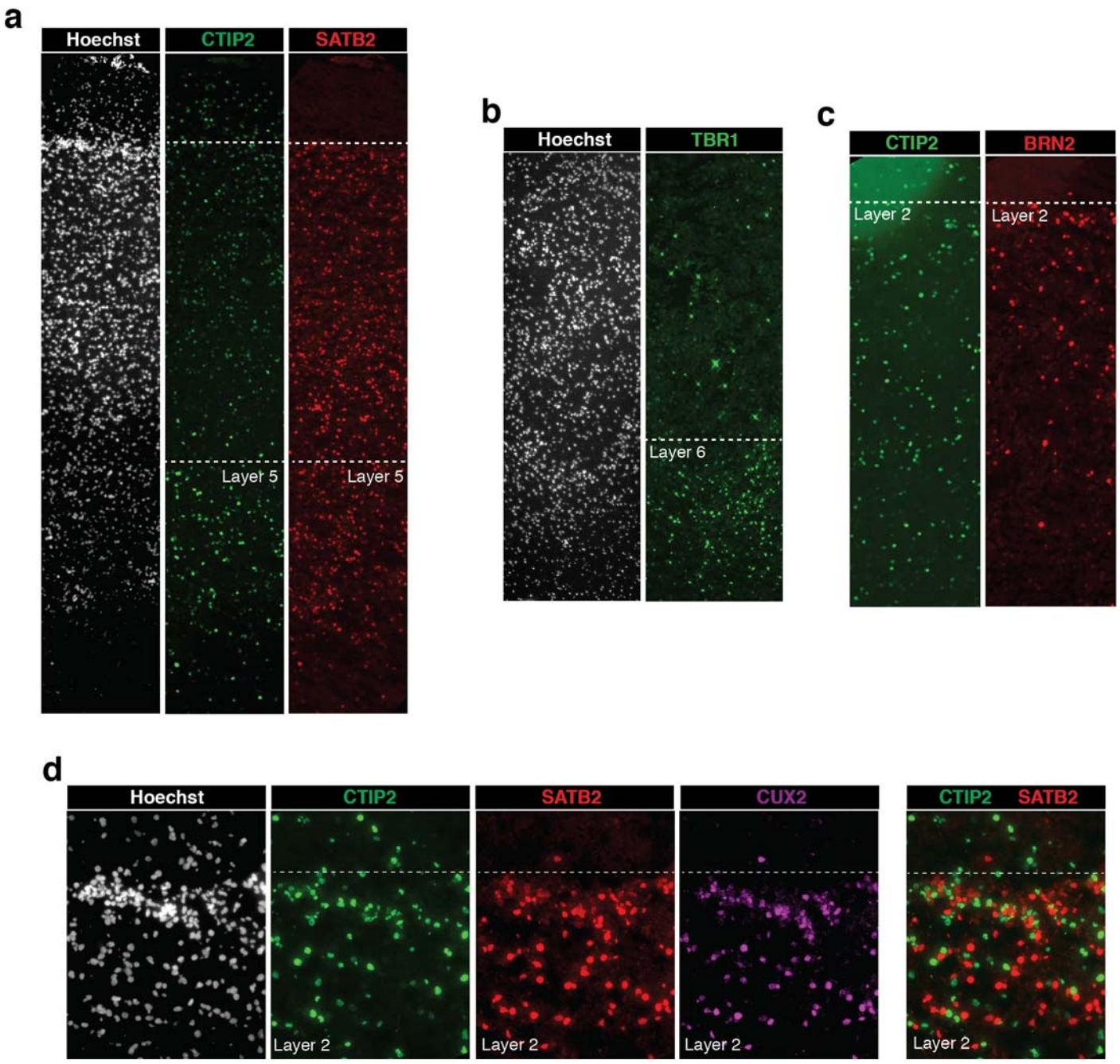


Supplementary Figure 2

Transcriptional analyses and mapping of neuronal cultures derived from hiPSCs using a monolayer approach.

The machine learning algorithm CoNTExT, which matches transcriptomes to human brain development, was used to predict the *in vivo* temporal identity of neural progenitors and neurons differentiated from hiPSC using a monolayer approach (adapted from Fig. 7 in Stein et. al., 2014). In contrast to the hCSs in **Fig. 1e** that reach up to fetal stage 6, these cultures map to earlier stages of brain development.

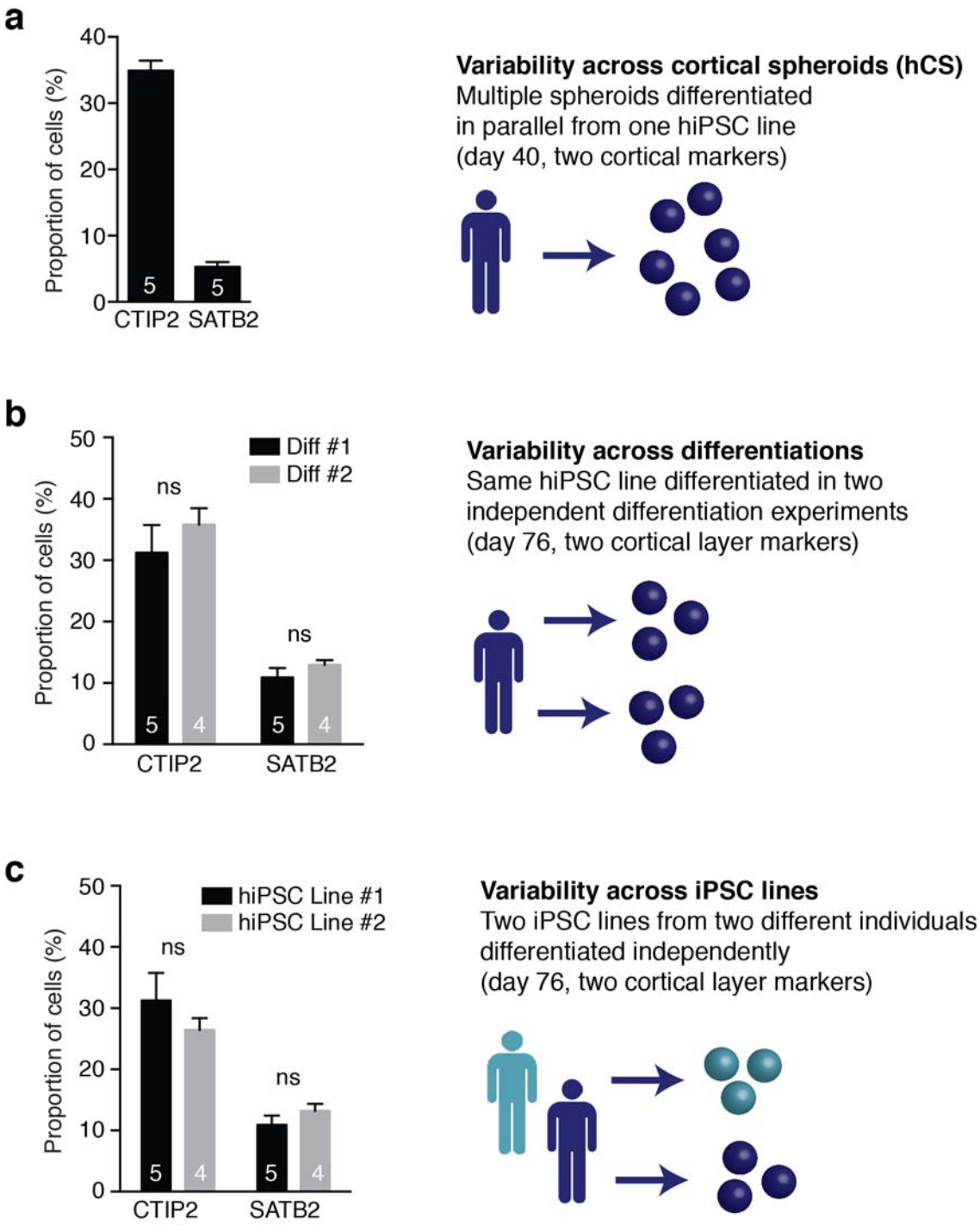
Stein, J.L., Torre-Ubieta, L., Tian, Y., Parikshak, N.P., Hernandez, I.A., Marchetto, M.C., Baker, D.K., Lu, D., Hinman, C.R., Lowe, J.K., Wexler, E.M., Muotri, A.R., Gage, F.H., Kosik, K.S., and Geschwind, D.H. A Quantitative Framework to Evaluate Modeling of Cortical Development by Neural Stem Cells *Neuron* **83**, 69-86 (2014).



Supplementary Figure 3

Validation of layer specific antibodies in the human fetal cortex at PCW36.

(**a**) CTIP2 and SATB2. (**b**) TBR1. (**c**) CTIP2 and BRN2. (**d**) CTIP2, SATB2 and CUX2.

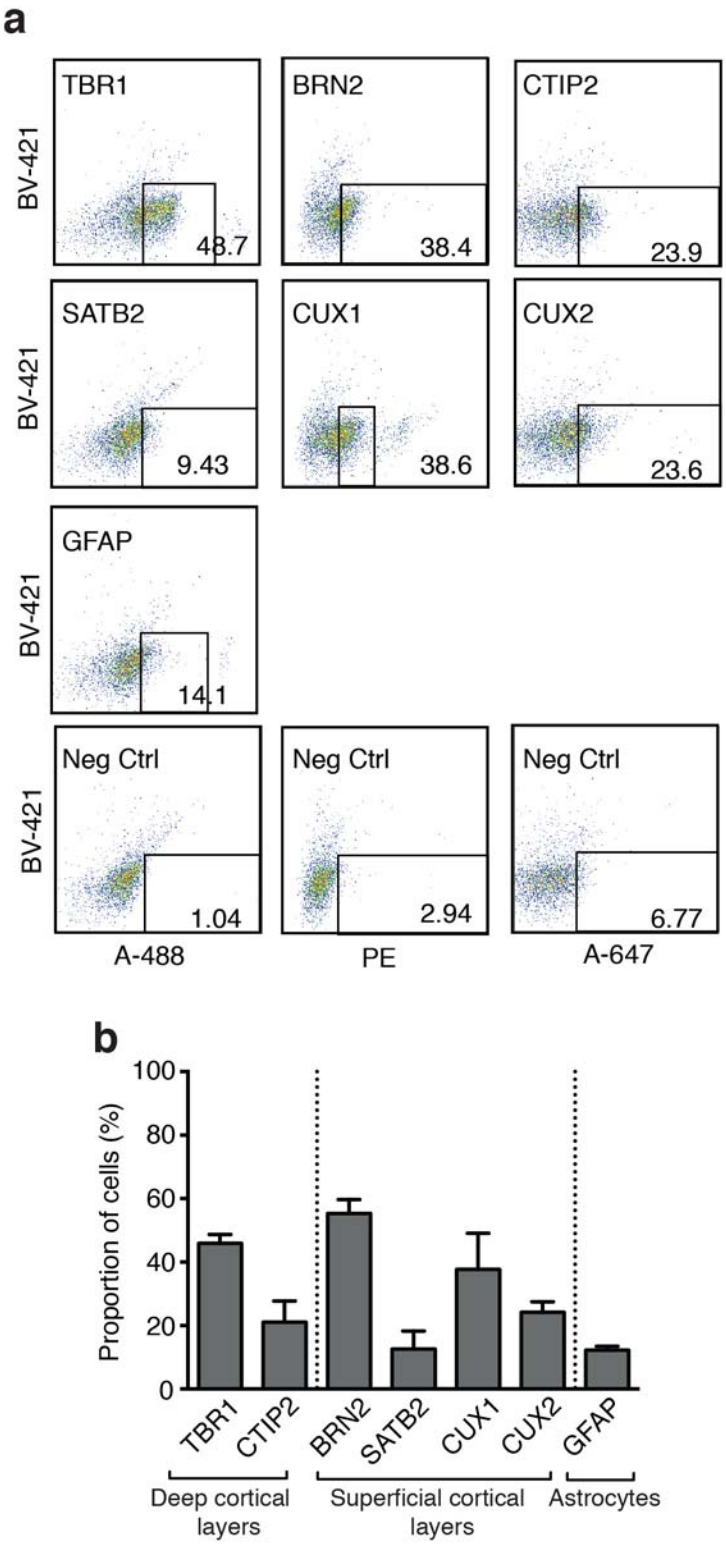


Supplementary Figure 4

Variability in the generation of hCSs.

(**a**) Proportion of neurons (mean ± s.e.m.) expressing CTIP2 and SATB2 at day 40 of differentiation. Multiple spheroids differentiated at the same time from one hiPSC line. Standard deviation is 2.9% for CTIP2 and 1.5% for SATB2. (**b**) Proportion of neurons (mean ± s.e.m.) expressing CTIP2 and SATB2 at day 76 of differentiation. The same hiPSC line was differentiated in two different experiments at two different times (multiple hCS per differentiation). Two-way ANOVA, $F_{1,14} = 0.1940$, $P = 0.66$ for hiPSC lines; multiple comparison test $P > 0.05$. (**c**) Proportion of neurons (mean ± s.e.m.) expressing CTIP2 and SATB2 at day 76 of differentiation. Two hiPSC lines derived from two individuals were differentiated at two different times (multiple hCS per differentiation). Two-way ANOVA, $F_{1,14} = 1.257$,

$P = 0.28$; multiple comparison test $P > 0.05$.

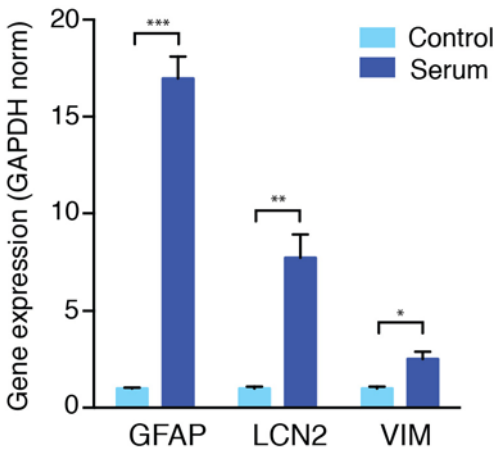


Supplementary Figure 5

Flow cytometry analysis of hCSs.

(**a**) Example of scatter plots for each of the antibodies used (first three rows) and the secondary only control conditions (fourth row). The

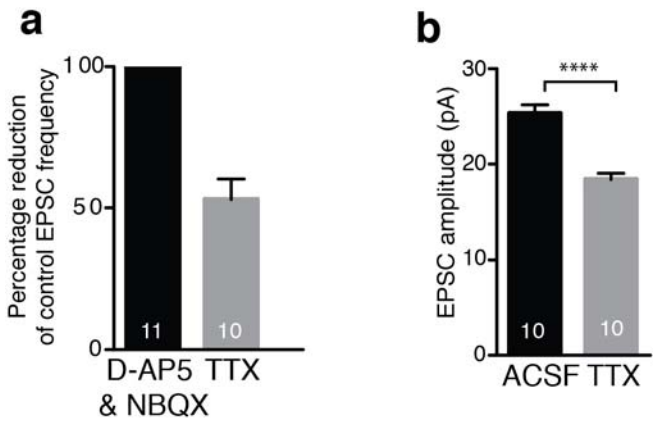
marker of interest is presented on the x-axis and the threshold gate is based on the negative control samples (cells stained with secondary antibodies alone). The y-axis represents a “dump channel”, a BV-421 fluorescent channel in which the cells were not stained with any fluorophores. Any positive signals on this BV-421 channel represent highly auto-flourescent cells or false positives and were excluded from the actual positive gates. (**b**) Quantification of the proportion of cells expressing various markers at day 76 of *in vitro* differentiation as assessed by flow cytometry.



Supplementary Figure 6

Expression of activation markers in hCSs before and after exposure to serum.

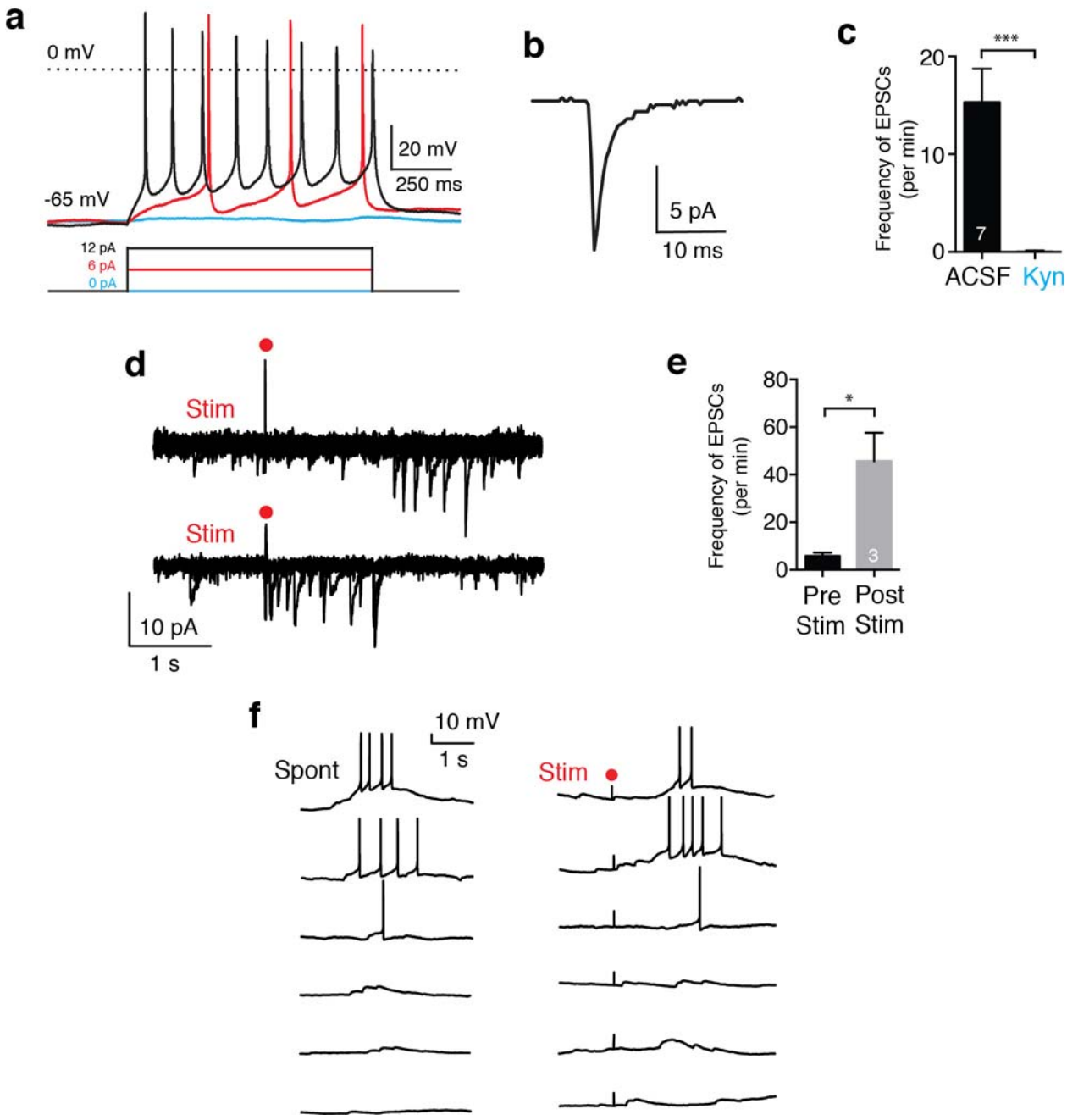
hCSs plated in monolayer were cultured in Neurobasal–B27 media with our without 20% serum (FBS). After 5 days, cells were harvested and the expression of genes associated with astrocyte activation (*GFAP*, *VIM*, *LCN2*) was measured by qPCR (t-tests with multiple comparison corrections using the Holm-Sidak method; $n = 3$ for each gene, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).



Supplementary Figure 7

Electrophysiology (hCSs plated in monolayer).

(**a**) Pharmacology of synaptic currents in neurons derived in hCS and plated in monolayer (at –70 mV). The frequency of EPSCs was abolished by NBQX (25 μM) and D-AP5 (50 μM) (paired t-test, $n = 11$ cells, $P = 0.001$), and was significantly reduced by 1 μM TTX (Wilcoxon signed-rank test, $n = 10$, $P = 0.002$). (**b**) TTX significantly reduced the amplitude of the EPSCs ($P < 0.0001$, paired t-test, versus ACSF, $n = 10$ cells).



Supplementary Figure 8

Electrophysiology (hCS slice recordings).

(**a**) Representative trace of a whole-cell current-clamp recording in an acute hCS slice preparation. Current injections (6 or 12 pA steps from –65 mV) produce sustained action potential generation. (**b**) Representative averaged trace of 53 sEPSCs in an individual hCS neuron under control conditions. (**c**) EPSCs were blocked by bath application of kynurenic acid in sliced hCSs (t-test, n = 6 cells; P = 0.0008). (**d**) Examples of voltage clamp recordings in two different hCSs showing EPSCs after electrical stimulation in an acute hCS slice preparation. The electrical stimulation artifact is designated by a red dot. (**e**) EPSC frequency 1s prior compared to 2s after electrical stimulation (t-test, n = 3 cells; P = 0.02) (**f**) Left: Representative traces of spontaneous action potentials (top three traces) and compound EPSPs (bottom three traces). Right: Representative examples of stimulus-evoked action potentials (top three traces) and compound EPSPs (bottom three traces). The electrical stimulation artifact is designated by a red dot.

ID	geneSymbol	Pval_3D Differentiation	LogFC_3D differentiation	PVal_Monolayer	LogFC_Monolayer	PVal_Fetal brain stage 1vs6	LogFC_Fetal Brain stage 1vs6
ILMN_1757081	SYN2	0.003424706	**1.881921746**	0.0304413	**0.210026365**	0.001335288	**2.620524794**
ILMN_1697189	PNCK	0.000143979	**1.815778106**	0.555391046	**0.182521156**	3.02E-07	**1.625465688**
ILMN_1670028	LPIN2	0.004990967	**1.119344082**	0.006821088	**-0.229568008**	1.91E-06	**1.113754791**
ILMN_1793241	SRD5A1	0.002626577	**1.086058965**	0.409975222	**-0.062709191**	1.88E-06	**1.517676958**
ILMN_1737089	CAPN5	0.001248015	**1.05778912**	0.031150992	**-0.284724084**	0.000482143	**1.304110264**
ILMN_1807925	GNG2	0.004873057	**1.050276872**	0.102894382	**0.294578531**	0.001175852	**1.371872185**
ILMN_1785191	TMEM14A	0.003724355	**0.991126255**	0.233787557	**0.156082104**	0.000995251	**1.455169924**
ILMN_2382505	SLC22A18	0.004825291	**0.877010208**	0.398491901	**0.106208354**	0.000817936	**0.460535107**
ILMN_1796377	C14orf37	0.000509971	**0.683039584**	0.033394192	**-0.22834774**	0.000296737	**0.855433125**
ILMN_1784749	GAS6	0.004835575	**0.651637059**	0.022776043	**0.471901789**	0.003565578	**0.565754696**
ILMN_1760922	GIT2	0.000315509	**0.617981876**	0.047505514	**-0.140457366**	0.000969808	**0.575931197**
ILMN_1790508	KCNA5	0.003460485	**0.563013651**	0.379275592	**0.09213923**	0.001931649	**0.531163348**
ILMN_1765001	NAT6	0.001044298	**0.555362121**	0.641629398	**-0.053333022**	3.70E-06	**0.711310414**
ILMN_1718771	CCDC24	0.003710791	**0.525383583**	0.170979899	**-0.195470229**	2.65E-05	**0.631296431**
ILMN_1739154	LSAMP	0.000123167	**0.468119467**	0.015264725	**-0.275352916**	3.27E-05	**1.322345313**
ILMN_2068747	OAT	0.004578799	**0.357445904**	0.929403499	**0.011031975**	0.000116989	**1.785603441**
ILMN_2400292	MAPK9	0.002369625	**0.28095182**	0.191370897	**-0.138637347**	7.79E-06	**1.193286368**
ILMN_1765257	CINP	0.00225714	**0.237842065**	0.932087722	**0.00615476**	0.002833284	**0.798138954**
ILMN_1807181	BACH1	0.00200797	**-0.129290077**	0.640773062	**0.022118782**	0.002212872	**-1.132606613**
ILMN_1742324	C1orf9	0.003879886	**-0.135825586**	0.13227243	**0.114800925**	0.000514025	**-0.723654442**
ILMN_1765159	ELMOD2	0.004146402	**-0.201928175**	0.007400399	**-0.349594829**	6.79E-07	**-1.62699728**
ILMN_1788108	TXNDC5	0.00109754	**-0.276815944**	0.049946634	**-0.204415835**	0.000397204	**-1.469944971**
ILMN_1708991	CCNY	0.000351113	**-0.299031527**	0.227821509	**0.123722519**	0.002648834	**-1.066553841**
ILMN_1799151	PPIL4	0.002490308	**-0.312236325**	0.025125958	**0.163142738**	0.001804393	**-0.426464972**
ILMN_1732566	TMTC3	0.00230638	**-0.324629165**	0.509554801	**-0.055333185**	2.82E-05	**-1.691651241**
ILMN_1756426	HDLBP	0.002642431	**-0.327575314**	0.952481193	**0.005378636**	0.00015071	**-0.918755733**
ILMN_1800412	BMP1	0.003354788	**-0.346502634**	0.238535484	**-0.24056278**	0.000268553	**-0.845675052**
ILMN_1810838	MTDH	0.004340294	**-0.38089403**	0.031137704	**0.170290345**	0.002357856	**-0.996125379**
ILMN_2397230	USP16	0.002052714	**-0.406833872**	0.94106668	**0.007647768**	0.004993946	**-0.55025428**
ILMN_2121437	NCL	0.000869961	**-0.425418628**	0.464460059	**-0.033866017**	0.000352175	**-0.851792463**
ILMN_1802089	SYMPK	0.002521406	**-0.452772726**	0.053825502	**-0.214526906**	0.004230595	**-0.532086967**
ILMN_2272074	TROVE2	0.002640189	**-0.482892591**	0.680443891	**-0.032372809**	0.000944189	**-0.970625226**
ILMN_1756767	EIF5B	0.00208903	**-0.495771964**	0.242185851	**-0.065101096**	0.00025386	**-0.827028816**
ILMN_1655645	AK2	0.002948532	**-0.512423517**	0.034920947	**-0.237593256**	0.001873008	**-1.18084599**
ILMN_2187746	EMX2	0.00173927	**-0.534775208**	0.033708115	**-0.644027316**	1.25E-06	**-2.60421998**
ILMN_1702447	IGF2BP2	0.000108119	**-0.546974679**	0.013721424	**-0.191567982**	0.000270715	**-1.558051782**
ILMN_1775937	DDB1	0.003972661	**-0.547349757**	0.248217984	**-0.096223451**	0.001219706	**-0.788770152**
ILMN_2371470	C1orf124	0.003393493	**-0.551812456**	0.373315322	**0.100462246**	7.06E-05	**-1.272116461**
ILMN_1710524	PARD3	0.000569884	**-0.555551283**	0.362972562	**-0.062788506**	3.78E-08	**-3.04530645**
ILMN_1753370	ABTB2	0.000736674	**-0.55801137**	0.342672724	**-0.156416433**	0.000504521	**-1.480342968**
ILMN_1690610	RALY	0.000661482	**-0.605694333**	0.323511904	**-0.086687316**	0.001574002	**-0.723216115**
ILMN_1763264	MRPL2	0.002184551	**-0.615348966**	0.510097281	**-0.070324547**	0.004386454	**-1.074103921**
ILMN_1765082	RBM10	0.001371822	**-0.61834817**	0.884345068	**-0.014521573**	6.08E-05	**-0.434892365**
ILMN_1811636	IFT57	0.000721043	**-0.639675966**	0.684680696	**0.051596462**	0.003519234	**-1.184788022**
ILMN_1694479	WDR18	0.004693785	**-0.649728595**	0.076095352	**-0.148458447**	0.003318257	**-0.845087287**
ILMN_2182750	DDX1	0.002594261	**-0.711498523**	0.679669508	**-0.03218584**	0.001082238	**-0.612468137**
ILMN_1741477	SMAD4	0.00354598	**-0.722083851**	0.030760355	**-0.216060088**	0.001460366	**-0.885455559**
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ILMN_1801905	ATG4D	0.002947623	**-0.760585955**	0.242684773	**0.086204435**	0.000170197	**-0.919884563**
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ILMN_1753164	IPO8	0.004119667	**-0.802039697**	0.007196824	**-0.260392915**	4.69E-05	**-1.007647634**
ILMN_2357577	PRKAA1	0.002187919	**-0.806148905**	0.175585736	**0.060067811**	7.18E-07	**-0.738753132**
ILMN_2145518	TMEM126B	0.004746557	**-0.809039951**	0.044478778	**-0.12917793**	0.000706488	**-0.821445859**
ILMN_2415529	CDK5RAP2	0.004887835	**-0.828807642**	0.074097164	**-0.188927136**	0.000351681	**-1.34988014**
ILMN_1717094	ZNF618	3.98E-05	**-0.83993616**	0.030173245	**0.180769107**	5.48E-05	**-2.55454591**
ILMN_2310253	TARBP2	0.004455577	**-0.846331994**	0.076094921	**-0.169617357**	0.003056874	**-0.860719624**
ILMN_1784523	ATP6V1G1	0.000523621	**-0.865234719**	0.233130618	**-0.073309931**	0.00015614	**-1.145152437**
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ILMN_1703524	SRP68	4.95E-05	**-1.007259746**	0.279322914	**-0.064827119**	0.000103175	**-1.039852328**
ILMN_1670899	FBN2	0.004060499	**-1.051706113**	0.005102894	**-0.547641308**	0.000564429	**-3.039533308**
ILMN_1689123	CCNK	0.000797288	**-1.057310813**	0.725039475	**0.018290134**	0.002938479	**-1.330262973**
ILMN_1758674	TMEM93	0.001952414	**-1.076383023**	0.344811525	**-0.086556598**	0.003719292	**-0.709370876**
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ILMN_2403889	PRMT5	0.00417783	**-1.171855103**	0.123915479	**-0.114107397**	0.000660678	**-1.051880568**
ILMN_1773716	MRPL9	0.004915387	**-1.191691509**	0.123009924	**-0.119770965**	0.001294591	**-0.82590083**
ILMN_1792435	STAG1	0.000527408	**-1.253028008**	0.015895085	**-0.227897439**	0.0003661	**-0.753663802**
ILMN_1761479	ZC3HC1	0.00114319	**-1.271257963**	0.008271959	**-0.321528413**	0.002066879	**-0.549346769**
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ILMN_1734317	DPF2	0.000913596	**-1.370036149**	0.027885122	**-0.219960548**	8.48E-06	**-1.502233588**