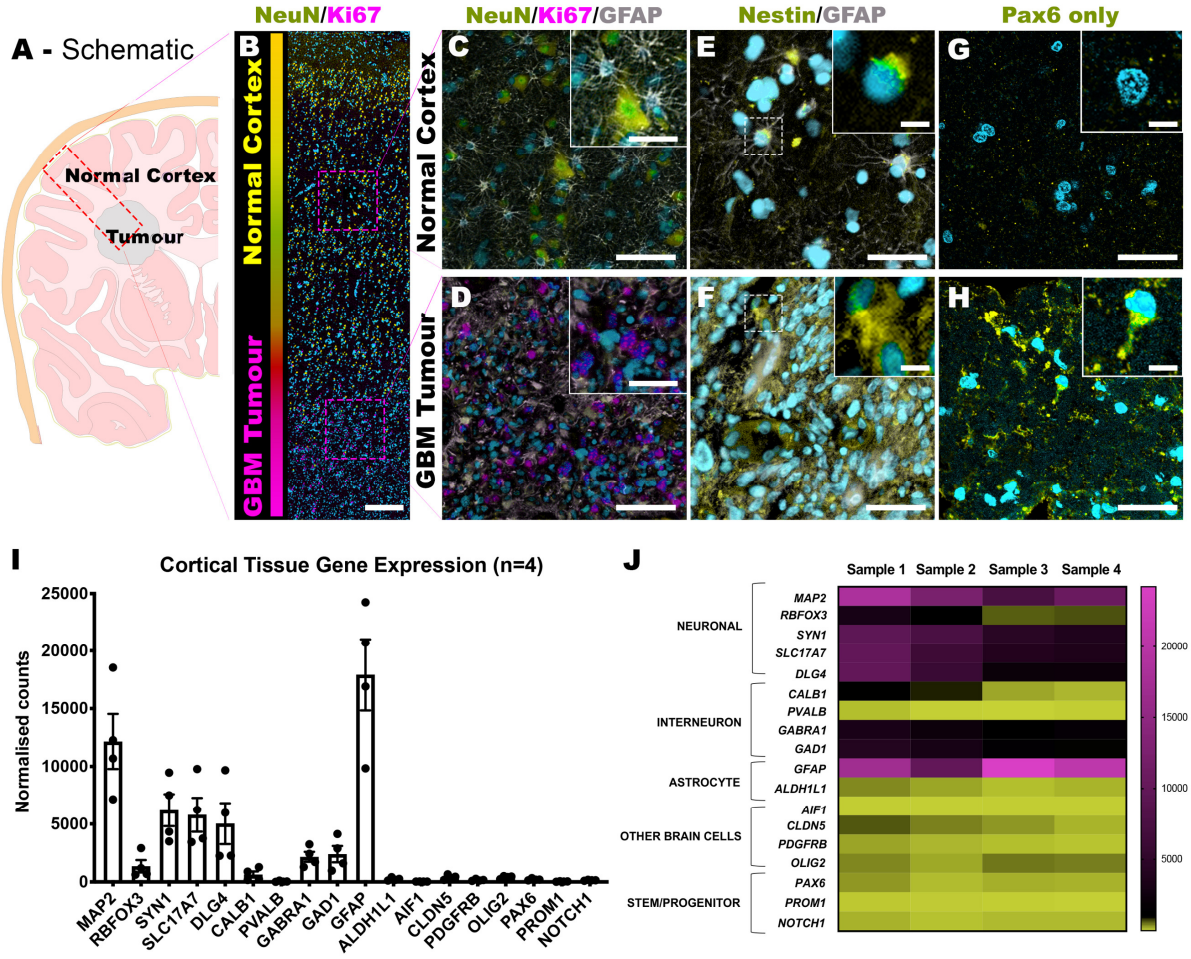
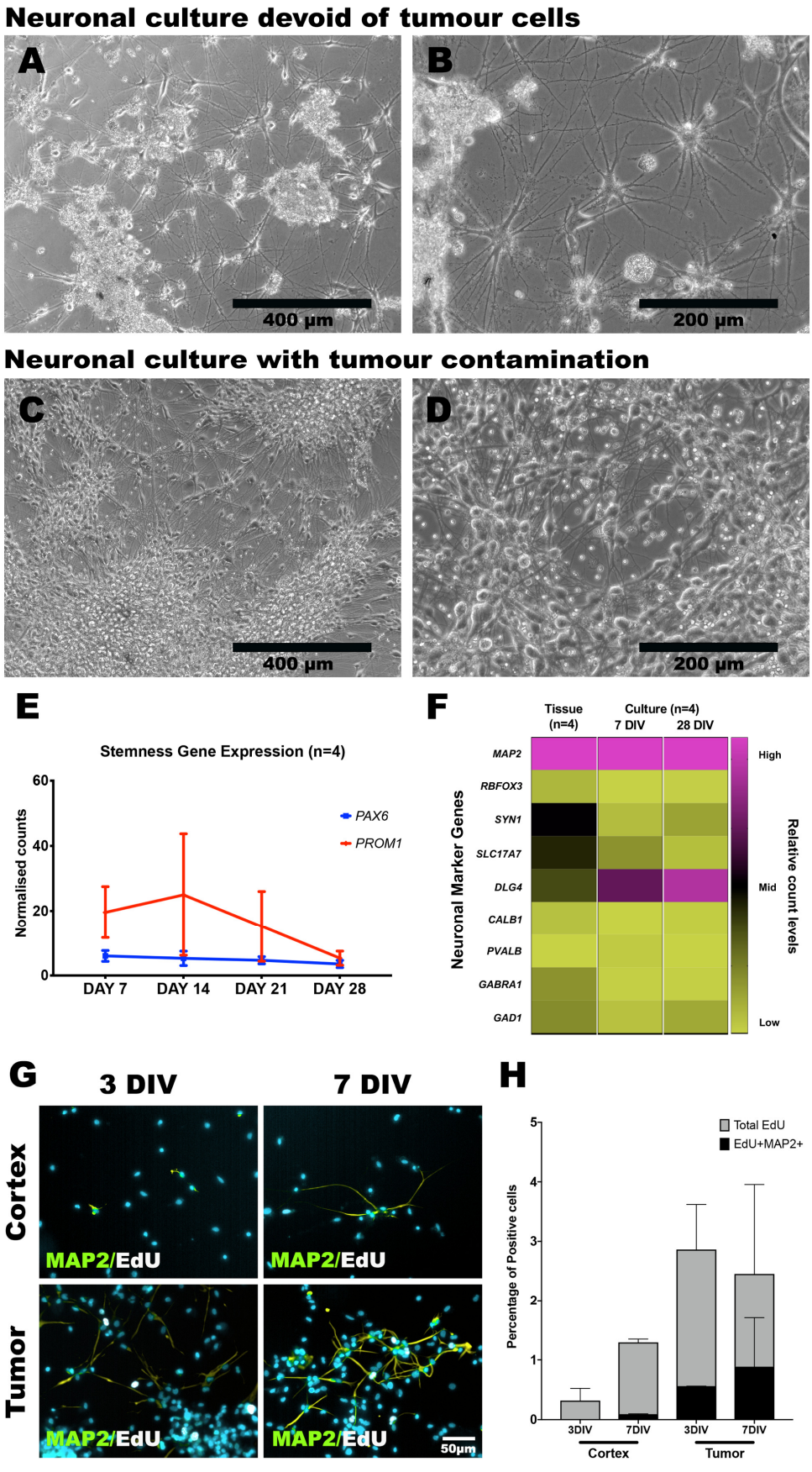
Supplementary Information

Supplementary Figure 1.



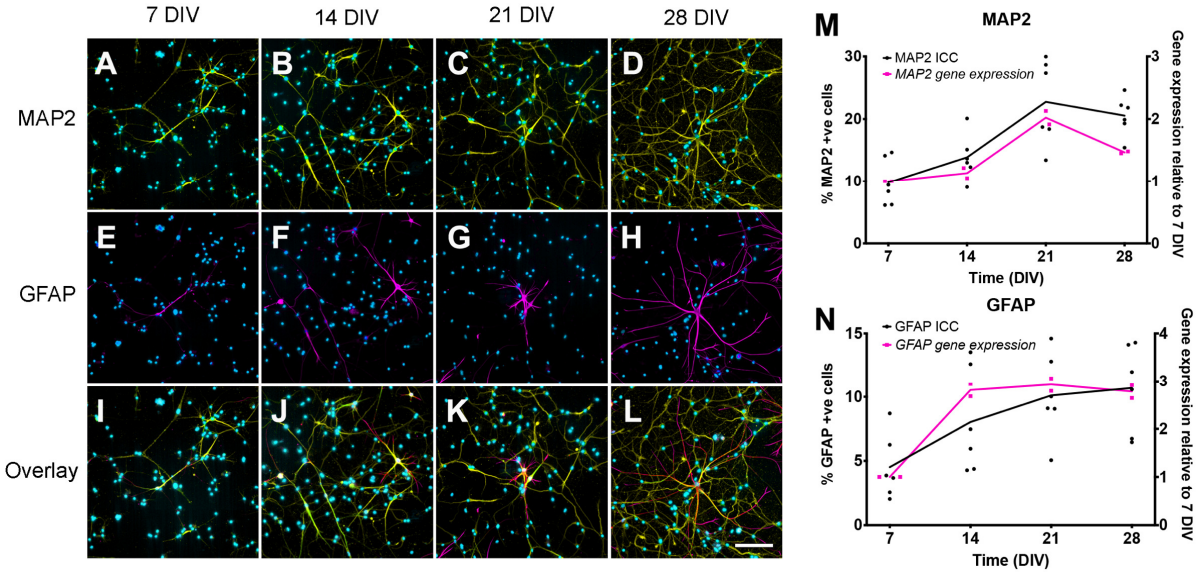
The isolation of neuronal cultures from non-neoplastic cortical tissues excised during tumour surgeries. A schematic illustrating the source of the resected cortical and tumour tissue (A). IHC analysis of the cortical tissue for the neuronal marker, NeuN (yellow) and the proliferating cell marker, ki67 (magenta; B). The outer cortical region was devoid of any proliferating ki67$^+$ cells (B and C), while the tumour region had large numbers of ki67$^+$ proliferating (B and D) cells. The cortical region also contained normal brain cells expressing neuronal marker NeuN (B and C), astrocyte marker GFAP (grey; C) and lacked progenitor marker Nestin (E) and Pax6 (G), while the tumour region contained significant numbers of ki67$^+$, GFAP$^+$, Nestin$^+$, Pax6$^+$ tumour cells (D, F, and H). I-J: Nanostring nCounter® analysis of four surgically resected cortical tissues used for neuronal culture. The average gene counts for the four cases are shown as a bar graph (I), while the expression levels of individual cases are shown as a heat map (J). Scale: B = 200 µm, C-D = 100 µm, E-H = 50 µm, insets: C-D = 50 µm, E-H = 10 µm.

Supplementary Figure 2.



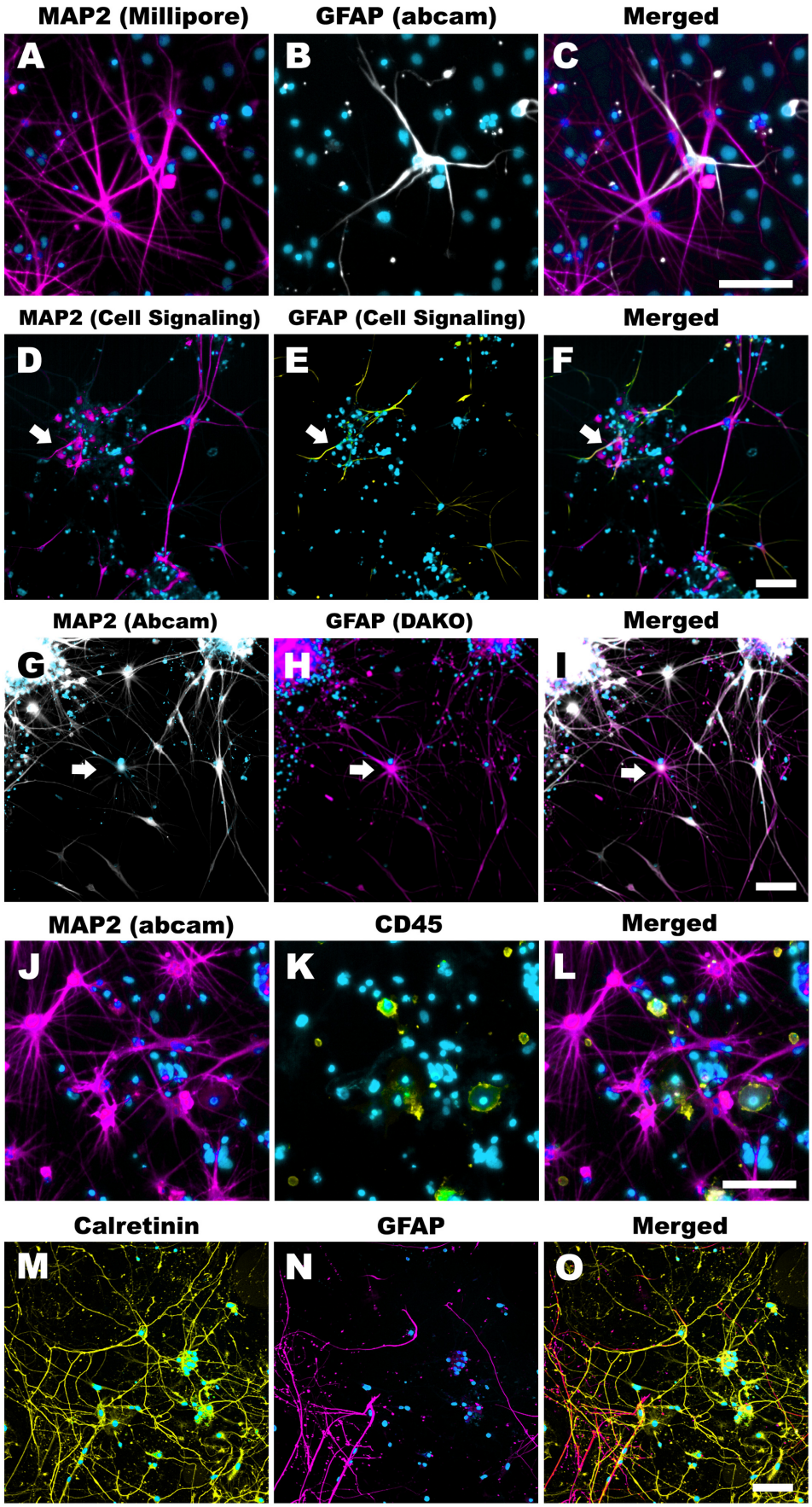
The neuronal cultures are devoid of any detectable tumour cell contamination. Photomicrographs of neuronal cultures showing a high density of neurite-possessing cells in the absence (A-B) or presence of tumour cell contamination (C-D). Gene expression analysis of cultures devoid of tumour cell contamination using Nanostring® also showed low counts of stem cell and neural progenitor cell marker genes that were not regulated throughout our culture period (E), and the pattern of neuronal marker gene expression was similar in cultured cells to those of tissue (F). Panels G-H shows the relatively low levels of EdU^+ proliferating cells in the cortical cultures compared to the patient-matched tumour cultures, as well as the near absence of EdU^+MAP2^+ cells in the cortical cultures.

Supplementary Figure 3.



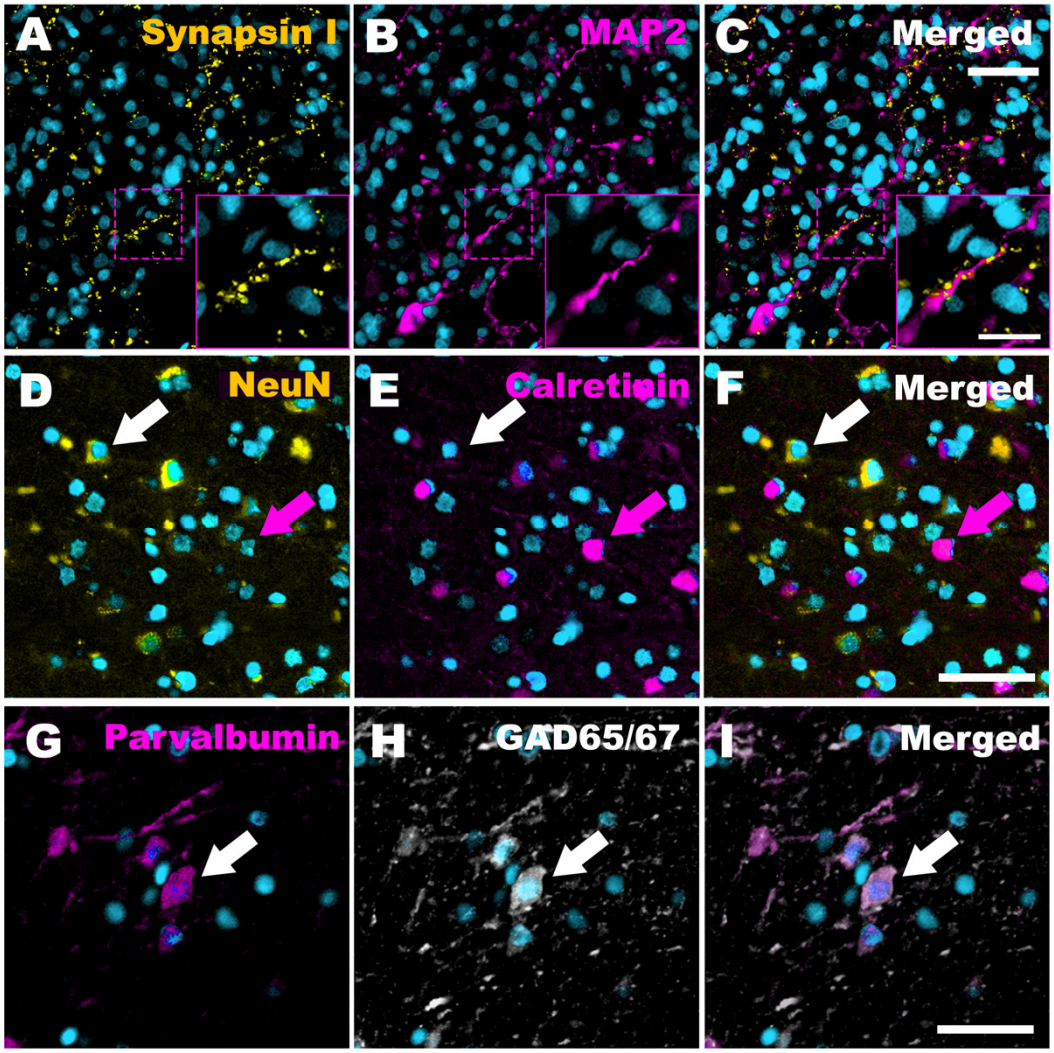
Cells isolated from the adult human brain re-establish their neuronal and astrocytic phenotypes *in vitro*. A-L: ICC images of MAP2 (A-D) and GFAP (E-H)-positive cells and their merged images (I-L) over the 28-day culture period. M-N: Image quantification of $MAP2^{+}$ (M) and $GFAP^{+}$ (N) cells in six cultures (n=6) and the gene expression changes of *MAP2* and *GFAP* in two of these cases (n=2). Scale bar:100 µm.

Supplementary Figure 4.



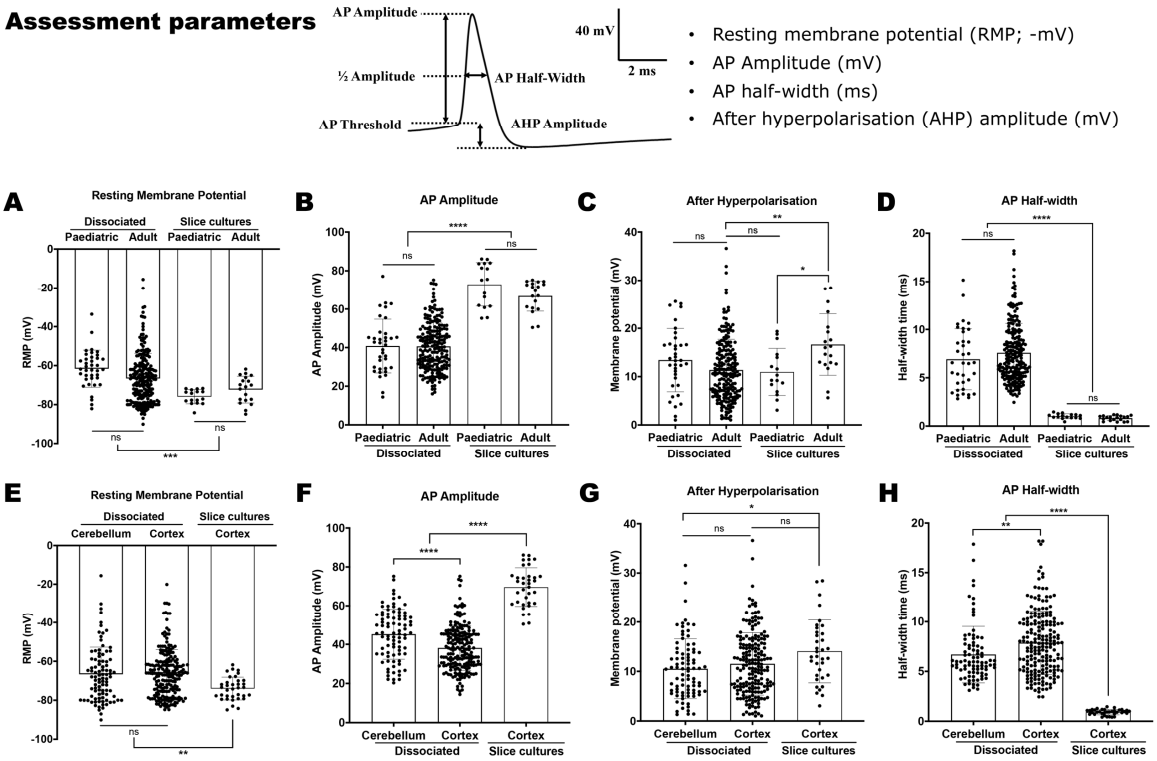
Cells isolated from the adult human brain contain neurons, astrocyte and microglia. Photomicrographs illustrating neuronal cultures ($MAP2^+$) that contain both $GFAP^+$ astrocytes and $CD45^+$ microglia. Several different combination MAP2 and GFAP antibodies were trialled and all showed MAP2 only and GFAP only cells, but also those co-expressing MAP2 and GFAP (white arrows). The cultures also contained $CD45^+$ microglia that did not co-localise with any of the neuronal markers tested. The interneuron marker, Calretinin, also labelled a large number of neurons. All images were taken from cultures at 28 DIV. Scale: 100 µm.

Supplementary Figure 5.



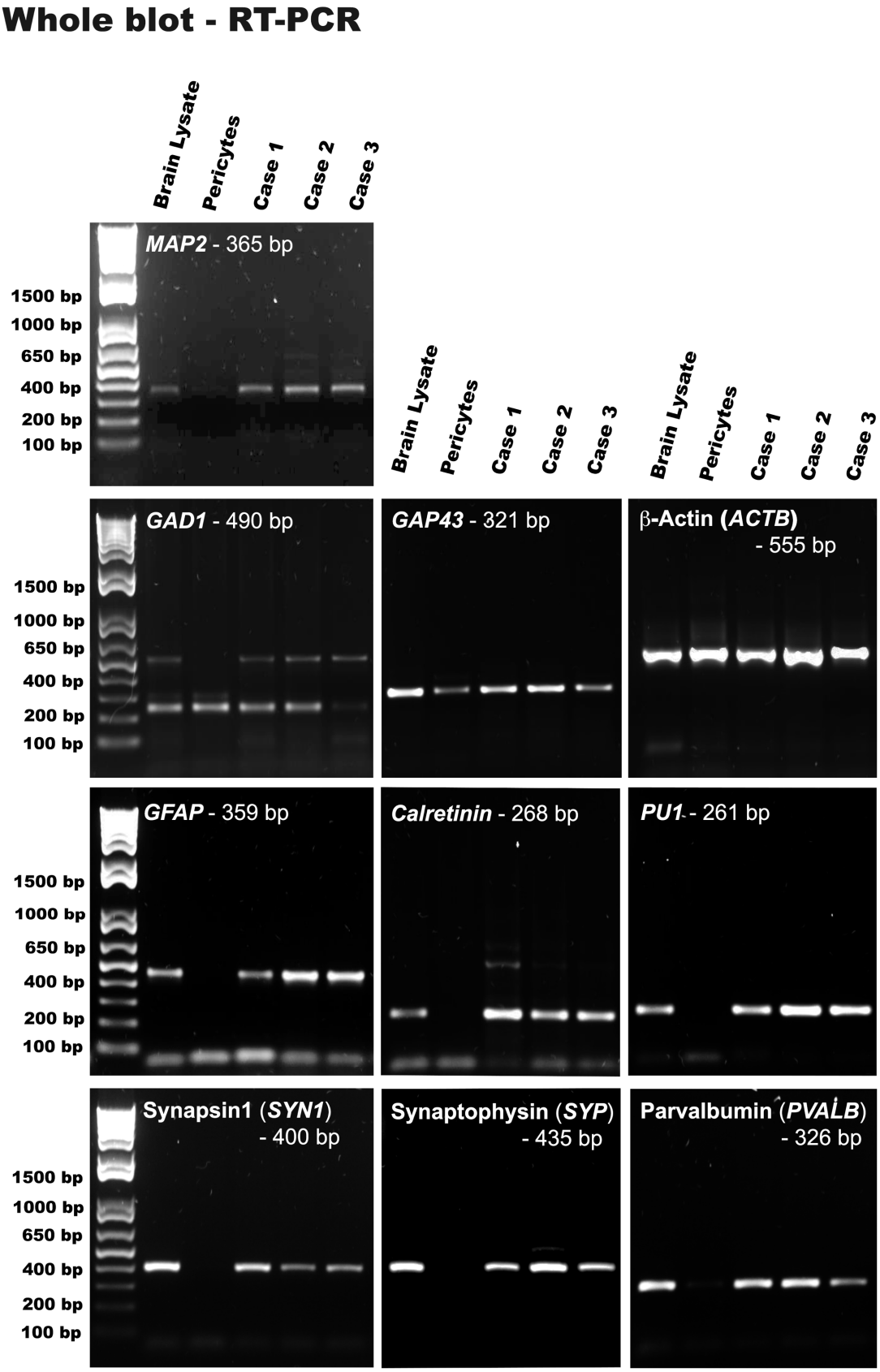
Cortical slice tissue contained the same neuronal markers expressed in the neuronal cultures. FFPE tissue from the same specimen as were cultured, were probed for the markers detected in neurons *in vitro*. The presynaptic marker, synapsin I, exhibited punctate staining onto MAP2^{+} processes (A-C). Another neuronal marker, NeuN and an interneuron marker calretinin were both present in the cortical specimens (D-F). Parvalbumin (G) and GAD65/67 (H) were also present and frequently co-localized onto a single cell (I). IHC studies of neuronal markers in the corresponding cortical tissue was conducted in 15 of the 37 specimens received and the representative case is shown in Figure S3. Scale bar = 100 µm, inset = 10 µm.

Supplementary Figure 6.



A comparison of the electrophysiological properties of dissociated primary human neurons with human brain slice cultures. The recorded dissociated primary neurons were categorised by age (A-D) and brain region (E-H), and assessed for RMP, AP amplitude, after-hyperpolarisation amplitude, and AP half-width, as shown in the **'Assessment Parameters'** illustration. These properties were also compared to equivalent neurons recorded from brain slice cultures. **Panels A-D** compare the neurons recorded from the dissociated cultures and the cortical slice cultures from paediatric and adult cases. **Panels E-H** compare the neurons cultured from dissociated cerebellar and neocortical tissues, as well as neurons recorded from neocortex-derived slice cultures. For data with equal variance, a One-way ANOVA analysis with Tukey's multiple comparison test was employed, and where variance were different, logarithmically transformed data was analysed using a general linear mixed model. ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, and **** = $p < 0.0001$.

Supplementary Figure 7.



Full sized blots for the RT-PCR experiments conducted for Figure 2K on whole-brain, pericyte (negative control) and three representative neuronal cultures.

Supplementary Tables

Supplementary Table 1. Neurosurgical specimens used for the neuronal cultures. Different regions of the human cortex were obtained as part of the required neurosurgical procedure for the neurological disorders listed. 51 specimens were obtained from 49 patients with an average age of 42 ± 3.5 years.

Underlying pathology	Cortical region	Number of specimens
Primary brain tumours – high grade (WHO grades III-IV)	Frontal lobe	6
	Temporal lobe	5
GBM, anaplastic astrocytoma III	Occipital/Parietal lobe	5
Primary brain tumours – low grade (WHO grades I-II)	Frontal lobe	1
	Temporal lobe	2
	Cerebellum	3
Metastatic brain tumours	Frontal lobe	2
	Temporal lobe	1
	Cerebellum	4
Intractable epilepsy	Temporal lobe	10
	Hippocampus	2
Paediatric cases - cortical dysplasia and cortical tubers	Frontal lobe	5
	Parietal lobe	5
		n = 51

Supplementary Table 2. Antibodies used for immunolabelling

Antibody	Species	Company	Catalog #	ICC dilution	IHC dilutions
MAP2	Mouse	Millipore	MAB3418	1:500	1:500
MAP2	Rabbit	Millipore	Ab5622	1:500	
MAP2	Chicken	Abcam	Ab5392	1:500	
NeuN	Rabbit	Millipore	ABN78	1:500	1:250
Calretinin	Rabbit	Swant Laboratories	7696	1:2,000	1:2,000
GAD65/67	Rabbit	Abcam	Ab26116	1:1,000	1:1000
Parvalbumin	Guinea Pig	Synaptic Systems	195004	1:2,000	1:2000
ki67	Mouse	DAKO	M7420	-	1:250
GFAP	Chicken	Abcam	Ab4674	1:20,000	1:3,000
GFAP	Mouse	Cell Signaling	3670	1:10,000	
GFAP	Rabbit	DAKO	Z0334	1:10,000	
Synapsin I	Rabbit	Sigma Aldrich	S193-10UG	1:200	1:200
vGlut-1	Guinea Pig	Synaptic Systems	135304	1:200	-
CD45	Mouse	Abcam	Ab8216	1:500	
Nestin	Mouse	Millipore	MAB5326	1:500	1:500
PSD95	Mouse				

Supplementary Table 3. List and sequence of primers used for RT-PCR experiments

Gene name Accession number	Primer sequence (Forward/reverse)	Start BP	Stop BP	Amplicon Length
ACTB	AGCACGGCATCGTCACCAACT	215	235	555 bp
NM_001101	AGCGGAACCGCTCATTGCCA	769	750	
MAP2	TCCAGTTTCTGCGCCCA	213	229	365 bp
NM_001039538	GACAAGTCCTTCCCCTGCTC	577	558	
GAP43	GCTGTGCTGTATGAGAAGAACC	389	410	321 bp
NM_001130064	GCGGGGTGGCATAATTCAGA	709	690	
SYP	GCCAACAAGACCGAGAGTGA	188	207	435 bp
NM_003179	GTTGAGTCCCGAGGTCACAG	622	603	
SYN1	TGAAGCCGGATTTTGTGCTG	662	681	400 bp
NM_006950	ACACGCACGTCATATTTGGC	1076	1057	
PVALB	CTCTGCCCGCTCAAACAGTT	6	25	326 bp
NM_001315532	CAACCCCAATTTTGCCGTCC	331	312	
CALB2	AGATGTCCCGACTCCTGCCT	604	623	268 bp
NM_001740	ACAATCTCCAGGTCCTTGCG	871	852	
GAD1	GCAAAACCGTGAGCTGGATT	1	20	490 bp
NM_000817	TTAGTGGTATTGGGGTCCGC	490	471	
GFAP	CAGTTATCAGGAGGCGCTGG	1032	1051	359 bp
NM_001131019	CAAAGCGCCGTGTCTGAGAG	1390	1371	
PU1	AATGTCAAGGGAGGGGGCTC	57	76	261 bp
NM_001080547	GGCGTTGGTATAGATCCGTGT	317	297	

Supplementary Table 4. List and sequence of primers used for qRT-PCR experiments

Gene name Accession number	Primer sequence (Forward/reverse)	Start BP	Stop BP	Amplicon Length
ACTB	TGGTGGGCATGGGTCAGAAGGA	131	152	94 bp
NM_001101	ATGCCGTGCTCGATGGGGTACT	224	203	
MAP2	TCGCAGAGCAGGGAAGAGTGGT	849	870	80 bp
NM_001039538	AACTTGGTGGGGTGCCAGGAGT	928	907	
GFAP	TGACCGCTTTGCCAGCTACATCG	231	253	69 bp
NM_002055	TCAGCAGCCAGCGCCTTGTTT	299	279	

Supplementary Table 5. List and sequence of probes used for Nanostring® nCounter experiments

Gene name Accession number	Target sequence (Probe A and Probe B)	Position
TBP **NM_001172085.1**	GCACGAAGTGCAATGGTCTTTAGGTCAAGTTTACAACCAAGATT CACTGTCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA CGAAAGCCATGACCTCCGATCACTCTCCTCATGATTACCGCAGCA AACCGCTTGGGATTATATTCGGCGTTTCGG	588-687
RPL13A **NM_012423.2**	TCCTTGCTCCCAGCTTCCTATGTCCCAGGGCTGCCCATCCTCTTCT TTTCTTGGTGTTGAGAAGATGCTC CGAAAGCCATGACCTCCGATCACTCATTCTCCGAGTGCTTTCAAG CAACTTCGGGAGGCAGTGACTAAGACCCTT	721-820
RPS17 **NM_001021.4**	CGGAGCTTTTTGCTGGGGATAATGGCGATCTCCTCGCACACGCGC TTGTTCACAATTCTGCGGGTTAGCAGGAAGGTTAGGGAAC CGAAAGCCATGACCTCCGATCACTCCTCTCTGAATTCGCTTCATC AGATGCGTGACATAACCTGCTATCTTGTTG	207-306
RBFOX3 **NM_001082575.1**	ACCCAAAACCCTTGGAGCCCCGCTCGTTAAAAATGATCTCCACG TCTAAACTGTTGAGATTATTGAGCTTCATCATGACCAGAAG CGAAAGCCATGACCTCCGATCACTCTTCTCCCGGGCTCGGTCAG CATCTGAGCTAGTTTCAAAAGTTACAA	577-676
MAP2 **NM_031845.2**	ACAGATTTGTAACAGTGTGTTGGAACCTCGGAATCCCAGCATAC AGAGTACAAAGACGCCTATCTTCCAGTTTGATCGGGAAACT CGAAAGCCATGACCTCCGATCACTCGCCTTTCTATGGTAACAGGC TCTAGAATTATCAGAAGAAAGAAACCCCCC	5171-5270
SYN1 **NM_006950.3**	AGAGAATCCACCATTGGCATGGGCCACAAGGTTGAGATCAGAGA ATTCGGCGAACCTAACTCCTCGCTACATTCCTATTGTTTTC CGAAAGCCATGACCTCCGATCACTCTTCAGAGACCGCACGACCT TCACCCCATTCCGAAGAACTTCCATATCCAC	566-665
DLG4 **NM_001365.3**	GTTTATACTGAGCGATGATCGTGACCGTCTGACCCGCATTCTTCA GGGCACCAATTTGGTTTTACTCCCCTCGATTATGCGGAGT CGAAAGCCATGACCTCCGATCACTCCCGAAGGTCGTGGATCTTG GCCTCGAATCGGCTGTACTCTTCTG	2461-2560
CALB1 **NM_004929.2**	CCCCAGCACAGAGAATAAGAGCAAGATCCGTTCGGTACAGCTTC CCCTTTCGGGTTATATCTATCAT CGAAAGCCATGACCTCCGATCACTCTAGATACAGTGTATCACTAG CAAGTGGTTGCGGCCACCAACTCTAGTTATTTACTTGACACCCT	911-1010
PVALB **NM_002854.2**	CGAAGGAGTCGGTAGCGCTAAAGGCTCCCACCGCCTTCTTGATG TCAACAGCCACTTTTTTTCCAAATTTTGCAAGAGCC CGAAAGCCATGACCTCCGATCACTCATCCGCACTCTTTTTCTTCA GGCCGACCATTTGGAAGAACTTTTTGTGGT	77-176
SLC17A7 **NM_020309.2**	GGCTAGGACCAGGAAGGAGATGGCCACGCCCTTGGCACCGTGT GGACGGCAACTCAGAGATAACGCATAT CGAAAGCCATGACCTCCGATCACTCTGGTTCACGTTGAACCCAG AGATGGCGAAGCCGCTGAAGCCCAC	1331-1430
GAD1 **NM_000817.2**	TTGAAGGCACTCACAAGGCGACTCTTCTCTTCCAGGCTGTTGGT CCTTTGCCTGGAGTTTATGTATTGCCAACGAGTTTGTCTTT CGAAAGCCATGACCTCCGATCACTCCCCGGTCGCTGTTTTCACA GGAAAGCAGGTTCTTGGAGGATTGCCTCTCC	576-675
GABRA1 **NM_000806.5**	GCTCAGAAGGAGGATCCAGGCCCAAAGACAGTCAGACAGACCA GATAAGGTTGTTATTGTGGAGGATGTTACTACA CGAAAGCCATGACCTCCGATCACTCTTAAGTTCATCTTGTAATGA CGGCTGTCCATAGCTTCTTCCAGTCAGTGT	476-575
GFAP **NM_002055.4**	CTCAATCTTCCTCTCCAGATCCAGACGGGCCAGGGCTGTTGAGAT TATTGAGCTTCATCATGACCAGAAG CGAAAGCCATGACCTCCGATCACTCCGTGGATCTTCCTCAAGAA CCGGATCTCCTCCTCCAGCGA	590-689
ALDH1L1 **NM_012190.2**	TCCTTTTGCACGCCACCGGGAGTACTTGAATACCGGCACTCCATC AAAGACGCCTATCTTCCAGTTTGATCGGGAAACT CGAAAGCCATGACCTCCGATCACTCCCCCAAAGCCTGGTATTTTG CCACCACATCAGGCAAAGCCTG	330-429

OLIG2 **NM_005806.2**	ATTGGATATGACCATCAGCGCTTCTGATACCGAACGCCGGCTTCC AACTACGAACCTAACTCCTCGCTACATTCCTATTGTTTTC CGAAAGCCATGACCTCCGATCACTCGGAGGAACGGCCACAGTTC TAAGAGGGTGTGGATTGACCCAGATATTGAG	1691-1790
PROM1 **NM_006017.1**	CTTGATGGATGCACCAAGCACAGAGGGTCATTGAGAGATGACCG CAGGCTCCAATTTGGTTTTACTCCCCTCGATTATGCGGAGT CGAAAGCCATGACCTCCGATCACTCGGTTGCTATTCAGCTGGCTT AGAGACAATCTGATGCTGTTGCAGGTTTCA	926-1025
NOTCH1 **NM_017617.3**	TATTTCAGATGCAAATTAATCCGCGTGCGGAAGGTGAGCCAGCTT TGCCTCTTTCGGGTTATATCTATCATTTACTTGACACCCT CGAAAGCCATGACCTCCGATCACTCCCATCTAAAACACATGGCA ACATCTAACCCATATGCTTTCACTTGTTTCC	8212-8311
PAX6 **NM_000280.3**	CTCAAACTCTTTCTCCAGGGCCTCAATTTGCTCTTGGGTAAAGGA TGTTCCAACAGCCACTTTTTTTCCAAATTTTGCAAGAGCC CGAAAGCCATGACCTCCGATCACTCATTTTGGCTGCTAGTCTTTC TCGGGCAAACACATCTGGATAATGGGTTCT	1174-1273
PDGFRB **NM_002609.3**	CTGGTGCAGGCTCCTGAAGGCTCAGGAGAACAGAGGGATGCAC CGTGTGGACGGCAACTCAGAGATAACGCATAT CGAAAGCCATGACCTCCGATCACTCGTCCCAGAGTGGGTAACAG CTGAGTAGAAGGACAGGCAGGA	266-365
CLDN5 **NM_001130861.1**	GGAAACTTCATTCCGTCTGTTAAGGGCAGGGCCGGGCTAGCCTG GAGTTTATGTATTGCCAACGAGTTTGTCTTT CGAAAGCCATGACCTCCGATCACTCCAGTCTGACACCCGCTCTG CCTATGGAAACAGCGCCGCGCACAGAAAA	1924-2023
AIF1 **NM_032955.1**	CTCCCCGGAGCCACTGGACACCTCTCCAATTAATTTCTTTAGCTC TAGGTCAGATAAGGTTGTTATTGTGGAGGATGTTACTACA CGAAAGCCATGACCTCCGATCACTCGCAGATCTCTTGCCCAGCAT CATCCTGAGAAAGTCAGGGTAGCTGAACGT	186-285

Supplementary Table 6: Table summarizing individual patient details for all the cases included in this study.

Cases (n = 49)	Age	Gender	Cortical region (n = 51)	Underlying Pathology
Tumour	60	Male	Parietal lobe	GBM
Tumour	52	Female	Parietal lobe	Anaplastic Astrocytoma III
Tumour	52	Male	Cerebellum	Metastatic tumour
Tumour	70	Male	Frontal lobe	GBM
Tumour	30	Female	Temporal lobe	Anaplastic Astrocytoma III
Tumour	57	Male	Temporal lobe	GBM
Tumour	57	Female	Parietal lobe	GBM
Tumour	55	Female	Temporal lobe	GBM
Tumour	62	Male	Temporal lobe	Anaplastic Astrocytoma III
Tumour	49	Female	Frontal lobe	Metastatic tumour
Tumour	75	Female	Temporal lobe	Diffuse Astrocytoma I-II
Tumour	58	Male	Cerebellum	Hemangioblastoma
Tumour	28	Female	Frontal lobe	Meningioma I-II
Tumour	23	Male	Temporal lobe	Metastatic tumour
Tumour	83	Male	Parietal lobe	GBM
Tumour	81	Male	Frontal lobe	GBM
Tumour	78	Female	Frontal lobe	GBM
Tumour	75	Male	Frontal lobe	GBM
Tumour	76	Male	Temporal lobe	GBM
Tumour	79	Male	Cerebellum	Hemangioblastoma
Tumour	46	Male	Cerebellum	Metastatic tumour
Tumour	24	Male	Frontal lobe	GBM
Tumour	20	Male	Cerebellum	Metastatic tumour
Tumour	64	Male	Frontal lobe	Metastatic tumour
Tumour	66	Male	Cerebellum	Meningioma
Tumour	66	Male	Occipital lobe	GBM
Tumour	58	Female	Cerebellum	Metastatic tumour
Tumour	52	Female	Frontal lobe	GBM
Epilepsy	52	Female	Temporal lobe Hippocampus	Intractable Epilepsy
Epilepsy	42	Female	Temporal lobe	Intractable Epilepsy
Epilepsy	30	Male	Temporal lobe	Intractable Epilepsy
Epilepsy	51	Female	Temporal lobe	Intractable Epilepsy
Epilepsy	40	Male	Temporal lobe	Intractable Epilepsy
Epilepsy	23	Male	Temporal lobe	Intractable Epilepsy
Epilepsy	35	Female	Temporal lobe	Intractable Epilepsy
Epilepsy	29	Female	Temporal lobe	Intractable Epilepsy

Epilepsy	36	Male	Temporal lobe	Intractable Epilepsy
Epilepsy	43	Male	Temporal lobe	Intractable Epilepsy
Paediatric	14	Female	Parietal lobe	Cortical dysplasia
Paediatric	7	Male	Frontal lobe	Cortical tuber
Paediatric	12	Female	Frontal lobe	Cortical dysplasia
Paediatric	5	Male	Parietal lobe	Cortical tuber
Paediatric	3	Male	Parietal lobe	Cortical dysplasia
Paediatric	9	Male	Parietal lobe	Cortical dysplasia
Paediatric	3	Female	Parietal lobe	Cortical tuber
Paediatric	10	Female	Frontal lobe	Intractable Epilepsy
Paediatric	3	Female	Frontal lobe	Cortical tuber
Paediatric	15	Male	Temporal lobe	Benign tumour
Paediatric	1	Male	Frontal lobe	Cortical dysplasia
Paediatric	4	Male	Parietal lobe	Cortical tuber

Supplementary Table 7: A summary of the number of cases used for each experimental paradigm.

Experimental methods	**Cases studied (total = 49)**	**Percentage**
IHC Analysis	21/49	43%
ICC Analysis	22/49	45%
RNA Analysis	14/49	29%
Electrophysiological Analysis	24/49	49%

Supplementary Table 8: The percentage of patch-clamp recorded cells from adult specimens showing active membrane properties (neuron or astrocyte-like traces) from each specimen type. Temporal lobe = Non-tumour epilepsy surgery, Peri-tumoural neocortex, Cerebellum = non-tumoural cerebellar cortex obtained in gaining access to brain stem complications.

Region	**AP Firing**		**Non-AP firing**
	Multi	Single	(Astrocyte-like)
Temporal lobe (n=175)	6 (4%)	90 (51%)	**79 (45%)**
Peritumoral Neocortex (n=60)	2 (3%)	30 (50%)	**28 (47%)**
Cerebellum (n=143)	36 (25%)	58 (41%)	**49 (34%)**
Total cells (n=378)	**44 (12%)**	**178 (47%)**	**156 (41%)**

Supplementary Table 9: The resting membrane potential (RMP) of recorded cells from each specimen type (same as above - adult cases only).

Region/ Underlying Pathology	**AP Firing (RMP ± SEM)**	**Non-AP firing (RMP ± SEM)**
TL – Epilepsy (n=175)	-66.9 ± 1.2 (n=96)	-75.5 ± 0.9 (n=79)
Neocortex – Tumour (n=60)	-62.1 ± 3.1 (n=32)	-74.3 ± 2.2 (n=28)
Cerebellum – Tumour (n=143)	-66.8 ± 1.4 (n=94)	-76.4 ± 1.1 (n=49)
Total cells (n=378)	-66.8 ± 0.9 (n=222)	-75.6 ± 0.7 (n=156)

Supplementary Table 10: Basal and active electrophysiological properties between paediatric (<16 years of age) and adult case (≥16 years of age)-derived neurons (exhibiting at least a single AP). For the average age and RMP, a two-tailed student t-test was conducted. For the percentage that exhibited multiple AP and synaptic activity, the difference in population proportions test was used.

Age groups	**Adult (n=222)**	**Paediatric (n=38)**
Average age	52.3 ± 3.1	7.5 ± 1.5 ($p < 0.001$)
RMP ± SEM	-66.6 ± 0.9	-61.6 ± 1.5 (ns)
% Multiple AP	44/222 = 19.8%	4/38 = 10.5% (ns)
% Synaptic activity	15/222 = 6.9%	4/38 = 10.5% (ns)
Total neurons (n=260)		