STAGE	Days post injury	Case	Sex	Age at surgery (years)	Type of injury	Pathology	Lobe	Immunohistochemical & Immunofluorescent studies				
								Nestin& PD or GFAP (IF)	Mcm2& Olig2 (IF)	Mcm2 & PD (DAB/ VIP)	Aq4, GS or Cx43 (DAB)	Cx43& Nestin, PD or GFAP (IF)
ACUTE	3	E1	M	49	SUP	LEAT (DNT)	Fr	√	√		√	√
	6	E2±	F	23	SUP	NON LESIONAL	Fr	√	√		√	
	8	E3±	F	18	SUP	FCDIIB	Fr-P	√	√		√	√
	8	E4±	F	30	SUP+ DEEP	NON LESIONAL	T	√		√	√	√
	8	E5	M	32	SUP	FCD IIB	P	√		√		
	9	E6	M	39	SUP	NON LESIONAL	Fr	√	√		√	
SUBACUTE	10	E7	F	23	SUP	NON LESIONAL	T	√	√	√		
	10	E8	F	24	SUP	FCD IIB	T	√	√	√		
	10	E9	F	34	DEEP	FCDIIB	Fr			√	√	
	11	E10±	M	40	SUP	FCD IIB	Fr	√	√	√	√	√
	12	E11	M	27	SUP	OLD TBI	Fr	√				
	12	E12	M	30	SUP	NON LESIONAL	Fr	√	√		√	
	13	E13	M	29	SUP	FCDIIB	Fr	√	√	√	√	√
INTERMEDIAETE	28	E14	F	59	DEEP	NON LESIONAL	Fr	√	√			
	30	E15	F	22	DEEP	HS	T	√	√		√	
	30	E16	F	31	SUP	FCDIIB	Fr				√	√
	52	E17	M	25	DEEP	NON LESIONAL	O	√	√	√		
	70	E18	M	35	DEEP	HS	T	√	√	√	√	√
CHRONIC	107	E19	F	44	SUP	NON LESIONAL	T	√				
	175	E20	F	60	DEEP	LEAT (MENINGIOANGIOMATOSIS)	T	√				
	186	E21±	F	23	DEEP	NON LESIONAL	Fr	√				
	209	E22	M	52	DEEP	FCDIIB*	Fr	√	√	√		
	209	E23±	M	47	DEEP	NON LESIONAL	Fr	√			√	
	209	E24	M	25	DEEP	FCD IIB	T			√		
	211	E25	M	32	DEEP	NON LESIONAL	Fr	√				

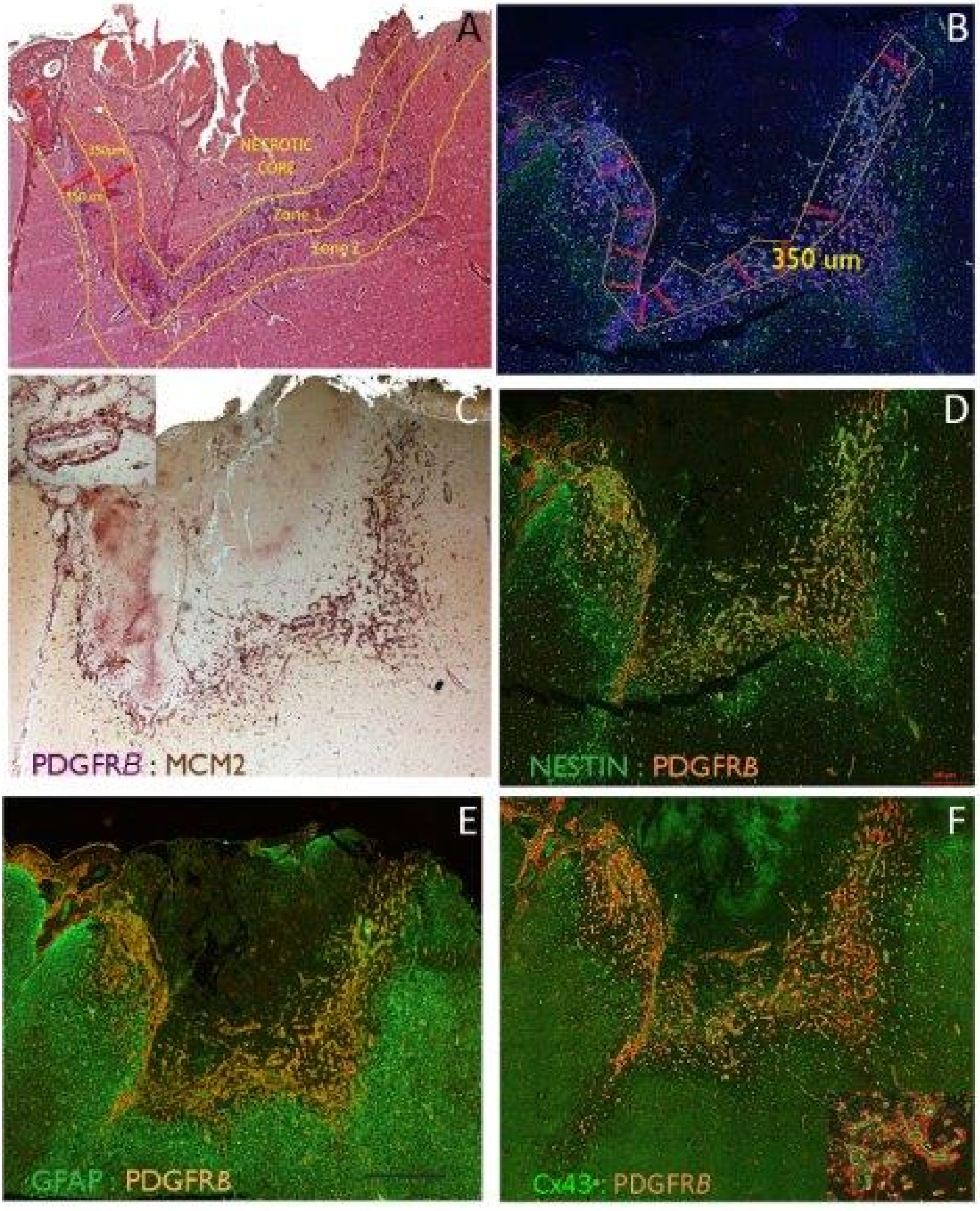
	232	E26	F	53	DEEP	LEAT (DNT)	Fr	√	√			
	264	E27	M	21	DEEP	FCDIIB	Fr	√			√	√
	301	E28	M	34	DEEP	NON LESIONAL	T	√	√	√		
	329	E29	M	49	DEEP	HS	T	√	√			
	385	E30	M	55	DEEP	NON LESIONAL	T	√			√	
	417	E31	M	87	DEEP	FCDIIID	Fr	√			√	√
	461	E32	M	49	DEEP	LEAT	T	√			√	
In vitro studies	n/a	EC1	M	49	n/a	HS	T	EdU A and Nestin immunocytochemistry				
	n/a	EC2	F	20	n/a	FCDIIB	Fr					

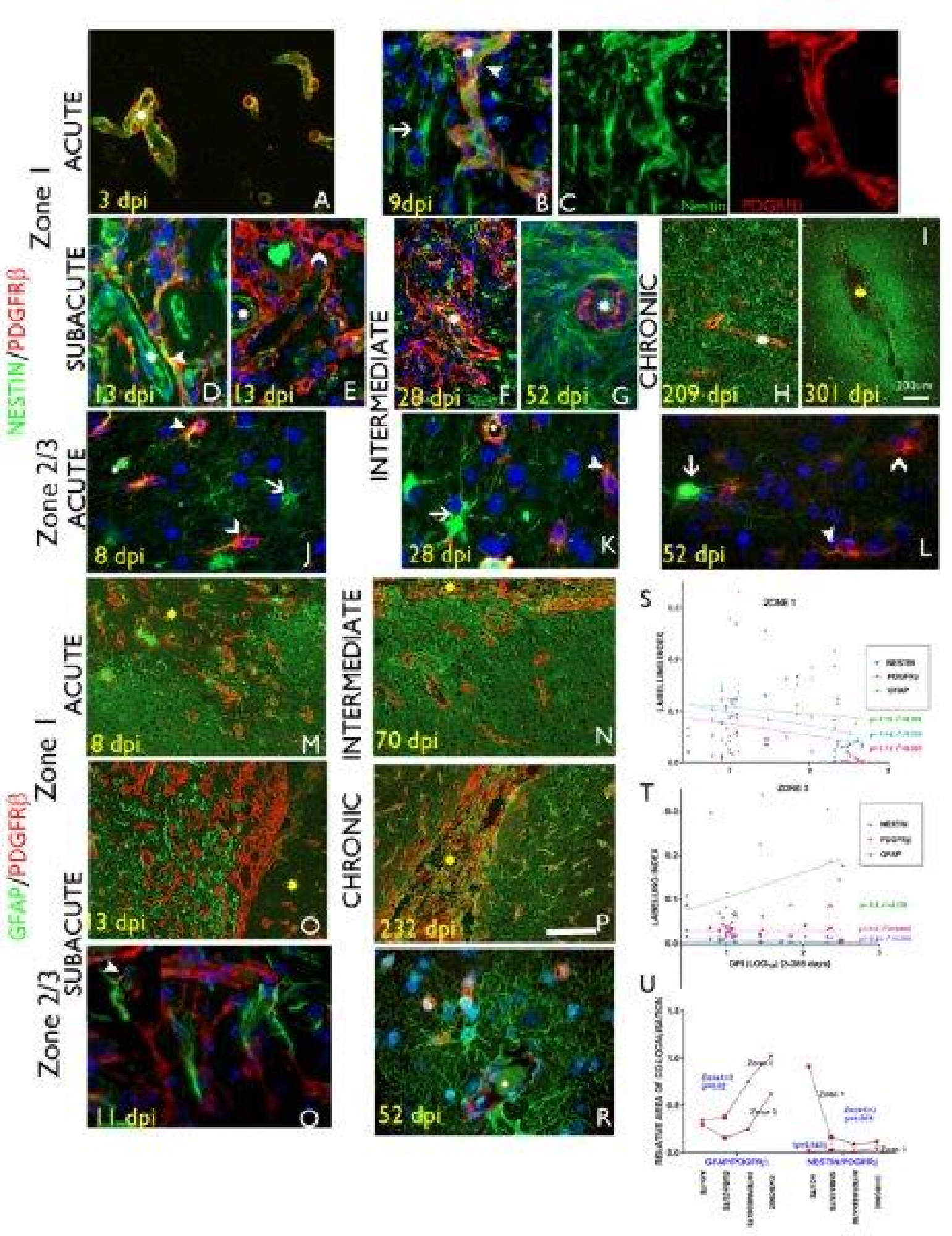
Table 1 Clinical details of cases and marker combinations for immunohistochemical studies. Under Pathology, non-lesional refers to cases with no remarkable pathology. Aq4, aquaporin 4; Cx43, connexin 43; DAB, diaminobenzidine; DNT, Dysembryoplastic neuroepithelial tumour; FCDIIB, Frontal Cortical Dysplasia type IIB; F, female; Fr, frontal lobe; GS, glutamine synthetase, HS, hippocampal sclerosis; IF, immunofluorescent studies; M, male; Mcm2, mini chromosomal maintenance 2; O, occipital lobe; P, parietal lobe; PD, platelet derived growth factor receptor beta (PDGFRβ); SMA, smooth muscle actin; T, temporal lobe. *The ICR injury was within the lesion in this case. In one case (E4) injuries in both the superficial and deep cortex were analysed separately. ±PDGFRβ and SMA immunofluorescence was performed on these cases.

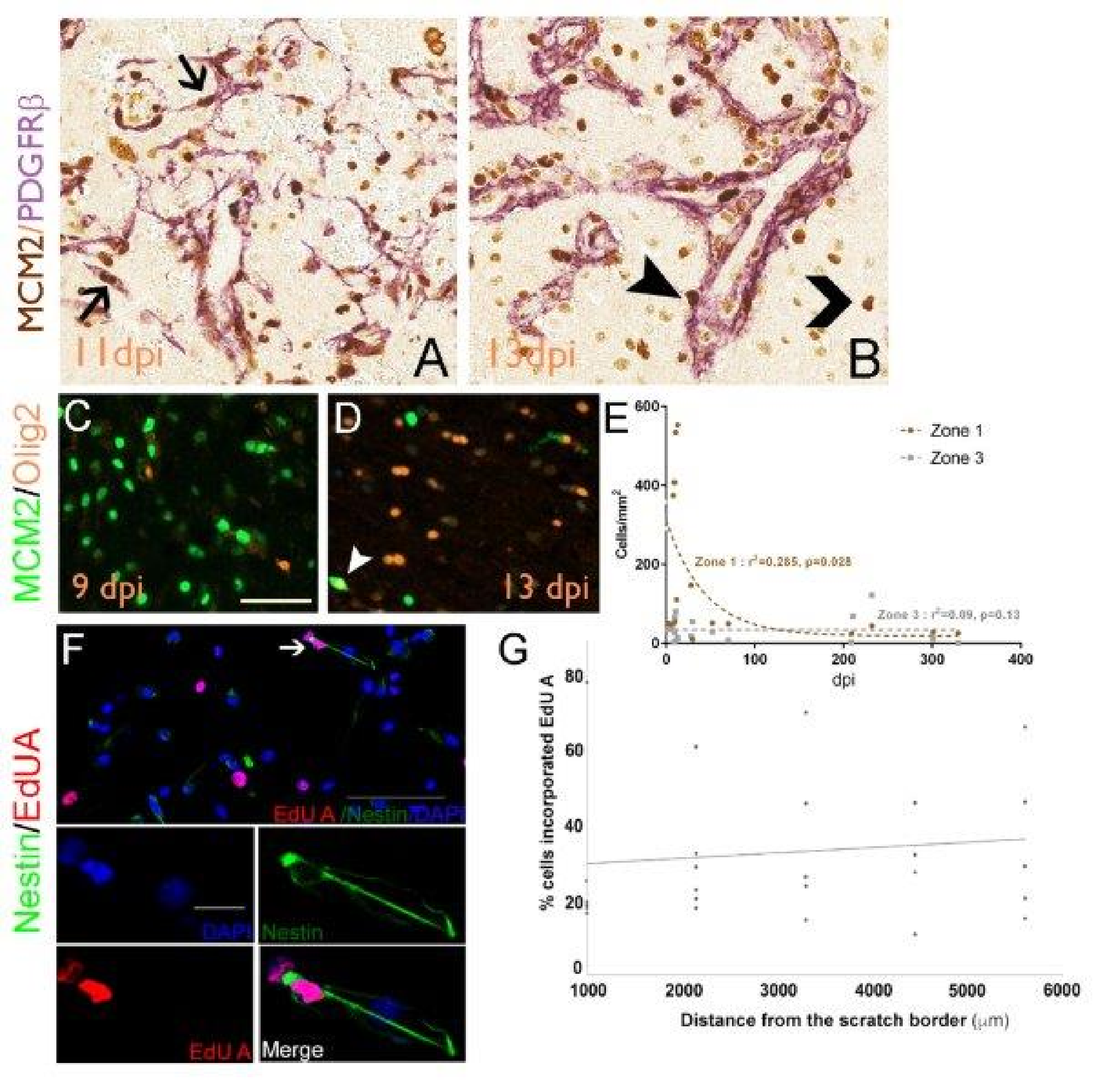
Antibodies Clone, Code	**Immunogen or target epitope**	**Labelled cell or protein type in the brain**	**Antibody supplier,** antibodies dilution, incubation conditions
Anti-Nestin 10C2, AB22035	150 aa recombinant fragment from human nestin conjugated to GST	Immature progenitors, glia, endothelial cells	EMD; 1:1000, ov
Anti-PDGFRβ Y92, AB32570	Synthetic peptide within human PDGFRβ aa 1050 to the C-terminus	Marker for Pericytes and some NG2 cells	Abcam Plc.; 1:1000, ov
Anti-GFAP Z0334	GFAP isolated from cow spinal cord	Intermediate filament in mature Astrocytic cells	DAKO; 1:1500, ov
Anti-SMA	N-terminal synthetic decapeptide of alpha-smooth muscle actin	Smooth muscle cells, myofibroblasts and myoepithelial cells	DAKO: 1:500 ov
Anti-Aq4 A5971	Recombinant fusion protein to residues 249-323 of rat AQP4 fused to GST	Aquaporin 4 Functional marker for mature astrocytes	Sigma; 1:500; ov
Anti-Cx43 CX-1B1, 13-8300	Synthetic peptide to a cytoplasmic sequence located near the C-terminus of rat Cx43	Connexin 43 Gap junction/hemi-channel Marker for Astrocytes	Thermo Fisher; 1:500; ov
Anti-GS GS-6, MAB302	purified from sheep brain.	Glutamine synthetase Marker for functional Astrocytes	EMD; 1:500; ov
Anti-MCM2 46/BM28, 610700	Human BM28 aa. 725-888	Mini-chromosome maintenance protein 2 Cells licensed for replication	BD; 1:900; ov
Anti-Olig2 AB9610	Recombinant mouse Olig-2	Nuclear based basic helix-loop-helix (bHLH) transcription factor Oligodendrocyte lineage, NG2-expressing glial progenitors, postmitotic oligodendrocytes	EMD; 1:250; ov

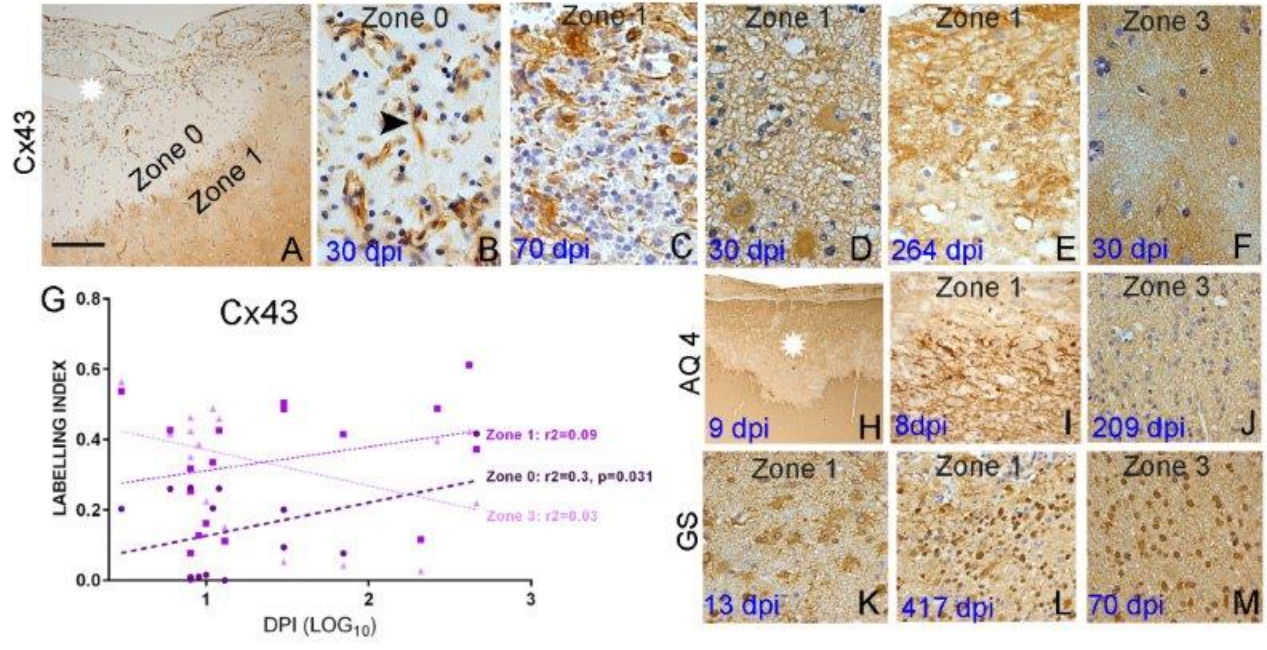
Table 2: Markers for chromogenic and immunofluorescence studies. All sections were pretreated in antigen retrieval buffer (H-3301, Vector Labs, UK), except for anti-Olig2, which require pretreatment in sodium citrate buffer, pH6, prior to primary antibody incubations. Cx43= connexin 43, GFAP, glial fibrillary acidic protein; GS, glutamine synthetase; ov, overnight at 4ºC; MCM2, mini chromosomal

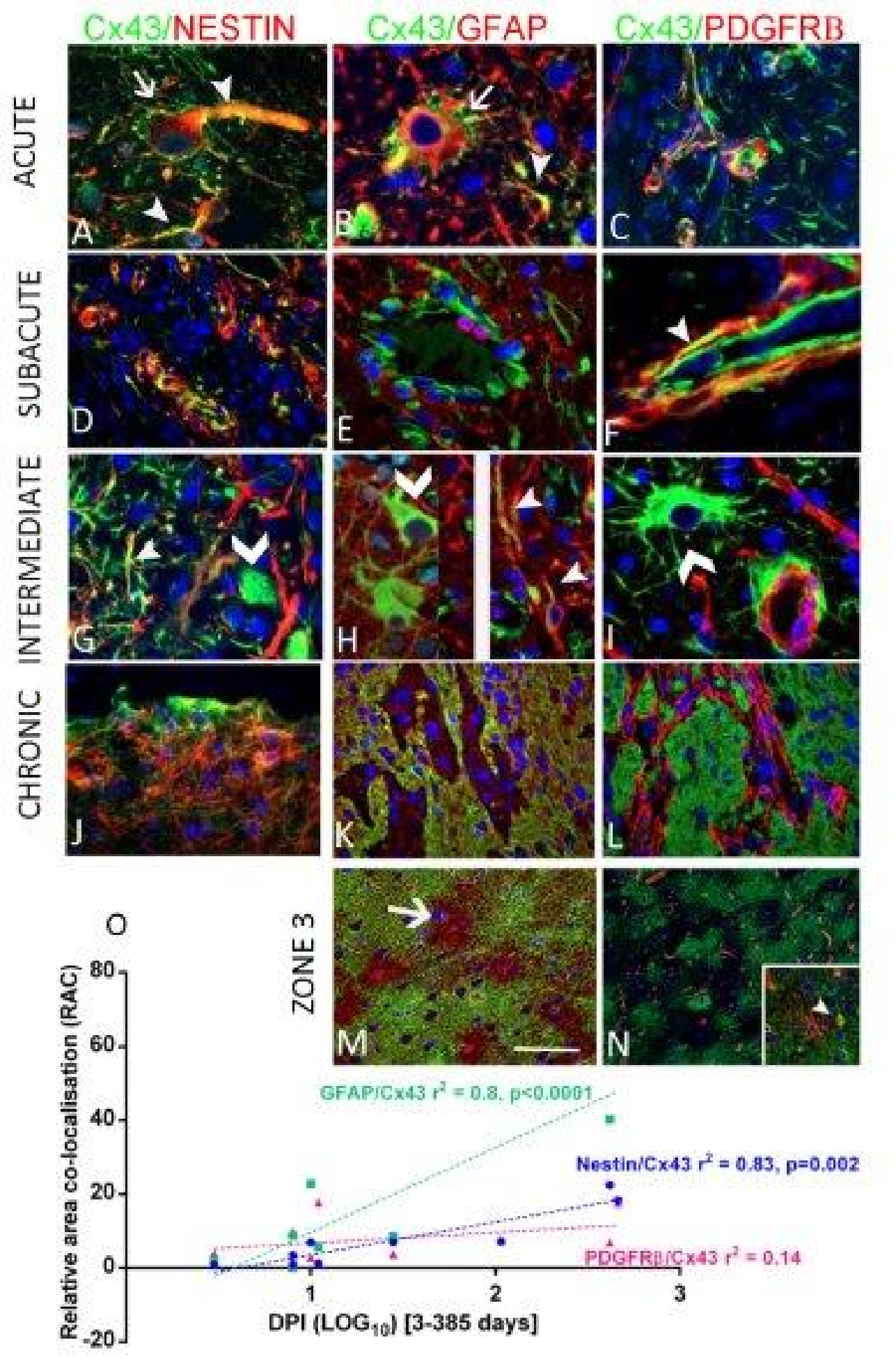
maintenance 2; PDGFRβ, platelet derived growth factors receptor beta. Suppliers: Abcam plc., Cambridge, UK; BD Transduction Lab., Oxford, UK; DAKO, Cambridgeshire, UK; EMD Millipore, Watford, UK; Sigma Aldrich Company Ltd, Dorset, UK; Thermo Fisher Scientific, Hemel Hempstead, UK.

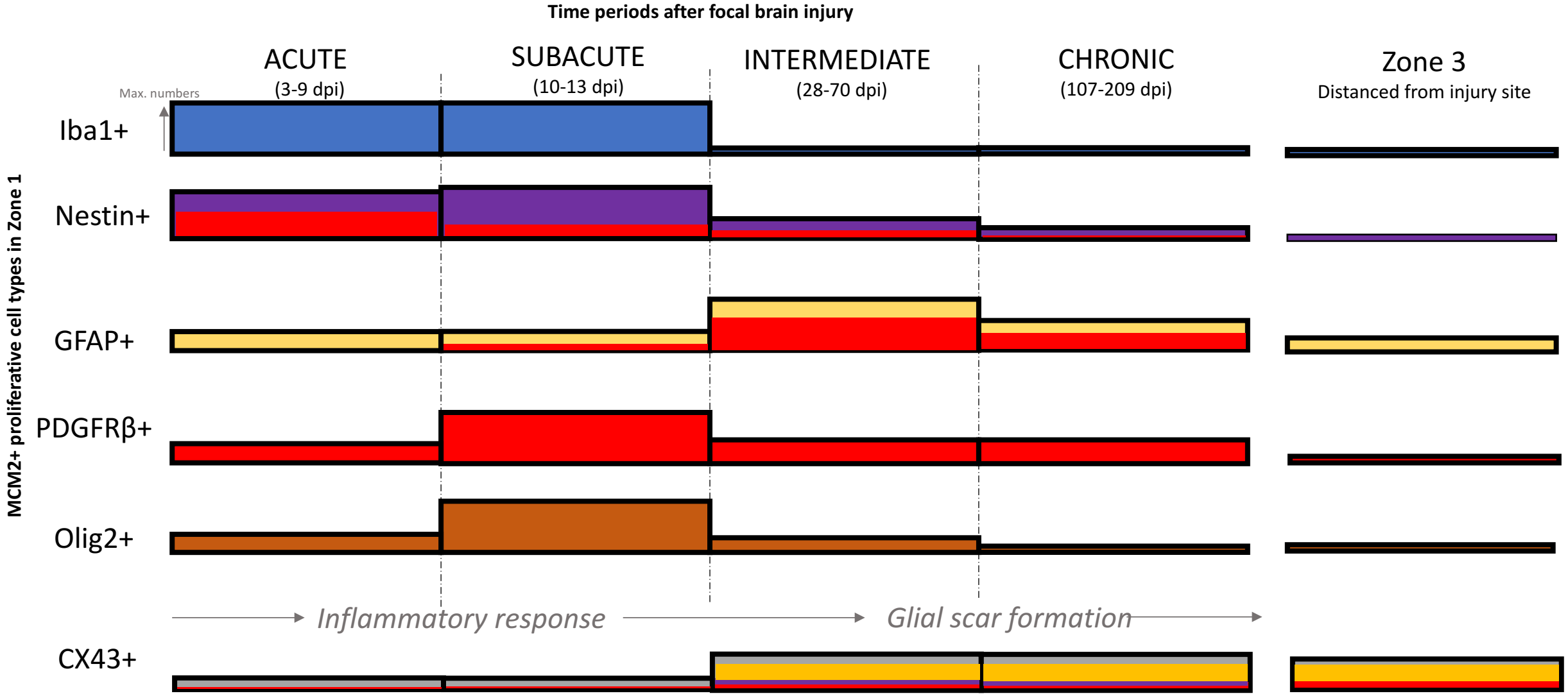

A
NECROTIC CORE
Zone 1
Zone 2
B
350 um
C
PDGFRB : MCM2
D
NESTIN : PDGFRB
E
GFAP : PDGFRB
F
Cx43* : PDGFRB

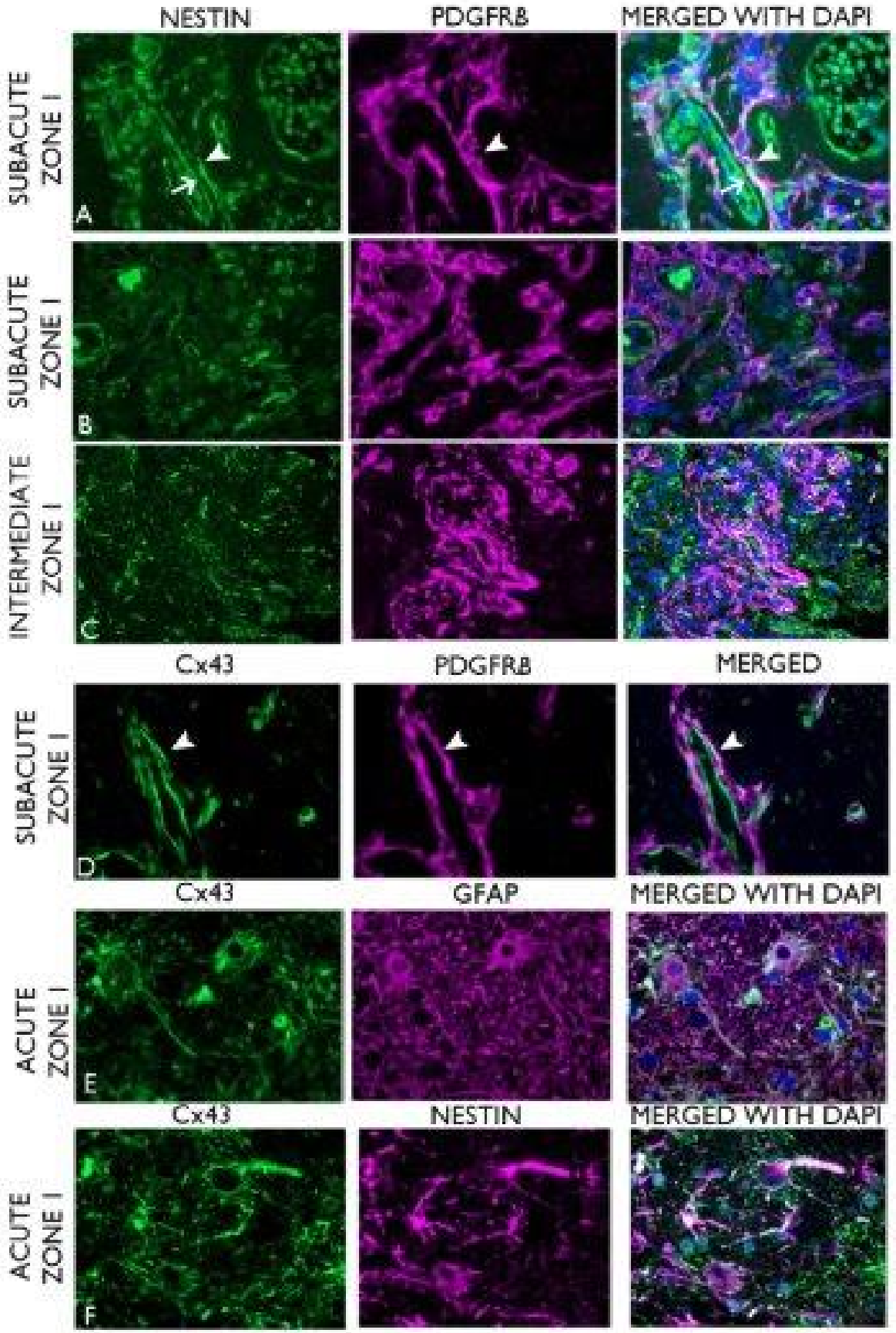

NESTIN/PDGFRβ
GFAP/PDGFRβ
Zone I
Zone 2/3
ACUTE
SUBACUTE
INTERMEDIATE
CHRONIC
3 dpi
A
9dpi
B
C
Nestin
PDGFRβ
13 dpi
D
13 dpi
E
28 dpi
F
52 dpi
G
209 dpi
H
301 dpi
I
200µm
8 dpi
J
28 dpi
K
52 dpi
L
8 dpi
M
70 dpi
N
13 dpi
O
232 dpi
P
11 dpi
Q
52 dpi
R
S
T
U
ZONE 1
ZONE 3
NESTIN
PDGFRβ
GFAP
LABELLING INDEX
DPI (LOG10) [1-365 days]
RELATIVE AREA OF COLOCALISATION
Zone 1
Zone 2
Zone 3
GFAP/PDGFRβ
NESTIN/PDGFRβ
ACUTE
SUBACUTE
INTERMEDIATE
CHRONIC


MCM2/PDGFRβ
11dpi
A
13dpi
B
MCM2/Olig2
C
9 dpi
D
13 dpi
E
Zone 1
Zone 3
Cells/mm²
Zone 1 : r²=0.285, p=0.028
Zone 3 : r²=0.09, p=0.13
dpi
Nestin/EdUA
F
EdU A /Nestin/DAPI
DAPI
Nestin
EdU A
Merge
G
% cells incorporated EdU A
Distance from the scratch border (μm)


Cx43
Zone 0
Zone 1
A
Zone 0
30 dpi
B
Zone 1
70 dpi
C
Zone 1
30 dpi
D
Zone 1
264 dpi
E
Zone 3
30 dpi
F
G
Cx43
LABELLING INDEX
0.8
0.6
0.4
0.2
0.0
1
2
3
DPI (LOG10)
Zone 1: r2=0.09
Zone 0: r2=0.3, p=0.031
Zone 3: r2=0.03
AQ 4
9 dpi
H
Zone 1
8dpi
I
Zone 3
209 dpi
J
GS
Zone 1
13 dpi
K
Zone 1
417 dpi
L
Zone 3
70 dpi
M


Cx43/NESTIN
Cx43/GFAP
Cx43/PDGFRB
ACUTE
SUBACUTE
INTERMEDIATE
CHRONIC
A
B
C
D
E
F
G
H
I
J
K
L
M
N
ZONE 3
O
Relative area co-localisation (RAC)
80
60
40
20
0
-20
1
2
3
DPI (LOG10) [3-385 days]
GFAP/Cx43 r2 = 0.8, p<0.0001
Nestin/Cx43 r2 = 0.83, p=0.002
PDGFRβ/Cx43 r2 = 0.14


Time periods after focal brain injury
ACUTE
(3-9 dpi)
SUBACUTE
(10-13 dpi)
INTERMEDIATE
(28-70 dpi)
CHRONIC
(107-209 dpi)
Zone 3
Distanced from injury site
Max. numbers
MCM2+ proliferative cell types in Zone 1
Iba1+
Nestin+
GFAP+
PDGFRβ+
Olig2+
Inflammatory response
Glial scar formation
CX43+


NESTIN
PDGFRß
MERGED WITH DAPI
SUBACUTE ZONE I
A
SUBACUTE ZONE I
B
INTERMEDIATE ZONE I
C
Cx43
PDGFRß
MERGED
SUBACUTE ZONE I
D
Cx43
GFAP
MERGED WITH DAPI
ACUTE ZONE I
E
Cx43
NESTIN
MERGED WITH DAPI
ACUTE ZONE I
F

Supplementary Methods

Single labelling immunohistochemistry (CX43, Aq4, GS)

Fixed brain sections were deparaffinized in xylene and rehydrated in alcohol. Endogenous peroxidase was blocked by immersing the sections in 0.9% hydrogen peroxide for 15 minutes. The sections were then microwaved at 800W for 12 minutes in antigen retrieval buffer (H3301; Vector Laboratories Ltd., Peterborough, UK) and allowed to cool for 20 minutes. Blocking solution consisting of 2.5% normal horse serum (Vector Laboratories, Peterborough, UK) was applied for 20 minutes. Sections were then incubated overnight at 4°C with the first set of diluted primary antibodies (Table 2). On the following day, species specific HRP-conjugated secondary antibodies were applied for 30 mins before chromogenic detection. Immunolabelled sections were counterstained in haematoxylin and processed through ascending concentrations of alcohol and xylene before coverslipped.

Double labelling methods

(Nestin/PDGFRβ, PDGFRβ/GFAP, CX43/PDGFRβ, CX43/GFAP and CX43/Nestin, olig2/MCM2, PDGFRβ/SMA)

Immunolabelled sections were underwent pre-treatment and blocking procedures as described above. Primary antibodies solution consisted of the following markers (Table 2): MCM2, PDGFRβ, GFAP, nestin, CX43, and/or olig2. On the following day, species specific HRP-conjugated secondary antibodies were applied for 30 minutes at room temperature, and fluorescein-labelled antibody in tyramide signal amplification buffer (TSA; Perkin Elmer, Buckinghamshire, UK) was applied for eight minutes. Following a ten minute incubation in 0.9% hydrogen peroxidase, the second set of primary antibodies was added overnight at 4°C. On the following day, species specific HRP-conjugated antibodies were applied for 30 minutes at room temperature, and then Cy3-labelled antibodies in TSA buffer was applied for eight minutes. Sections were coverslipped using Vectashield mounting media with DAPI (Vector Laboratories Ltd., Peterborough, UK). In negative controls, one or both primary antibodies were omitted.

Chromogenic double labelling was performed using markers, PDGFRβ and MCM2. The protocol for double labelling was similar as described above except that DAB and VIP were

used instead of fluorescein and Cy3-conjugated antibodies. Labelled sections were counterstained in haematoxylin and processed through ascending concentrations of alcohol and xylene before coverslipped.

In vitro scratch assay

Cells were cultured from a gram of fresh tissue sampled from the grey and white matter of the temporal pole of two surgical patients with MTLE and ILAE Type 1 HS (Cases EC1-2). Dissociated cells were cultured in supplemented MACS Neuro Medium (30,000 cells/mL) for four weeks using a protocol established in our previous study (Liu *et al.*, 2018). Prior to the mechanical scratch assay, a confluent monolayer of cells was grown on Fluorodish (World Precision Instruments, Florida, USA). A vertical scratch was made in the middle of the culture using a 100 µm pipette tip. 10µM of 5-ethynyl-2'-deoxyuridine (EdU A; Thermo Fisher Scientific, Massachusetts, USA), a modified thymidine analogue (Nakayama *et al.*, 2010) was added to the culture medium 24 hours after mechanical injury, for thirty minutes at 37ºC, to identify cells that were actively undergoing DNA synthesis. After incubation with EdU A solution, cells were fixed using 4% paraformaldehyde (Santa Cruz Biotechnology, Heidelberg, Germany), and double labelled immunocytochemistry was performed using reagents supplied in Click-iT EdU Imaging kit (Thermo Fisher Scientific, USA), and primary antibodies against nestin. First, EdU A was labelled with Alexa-564 conjugated antibodies following manufacture's instruction (Thermo Fisher Scientific, UK). Anti-nestin antibody diluted in PBS with 10% normal horse serum was applied at 1:1000 overnight at 4ºC. The following day, species-specific Alexa-488 conjugated secondary antibodies were applied for ninety minutes at room temperature, and Hoechst solution was applied for thirty minutes. Labelled preparations were visualised, and images were acquired using confocal microscopy (LSM700; Zeiss, Germany). Five serial images spanning 7000 µm along the horizontal axis were acquired at three points along the vertical axis of the scratch (top, middle, bottom). Images were imported into the image analysis software, Definiens Tissue Studio 3.6 and Developer X64 (Definiens AG; Munich, Germany) for automated quantification. Final results were expressed as the percentage of cells that have incorporated EdU A and expressed nestin at various distance along the x-axis from the scratch border. Statistical analysis was not undertaken due to the limited number of samples available for *in vitro* studies.