Application of stem cells in brain diseases from the perspective of preclinical trials

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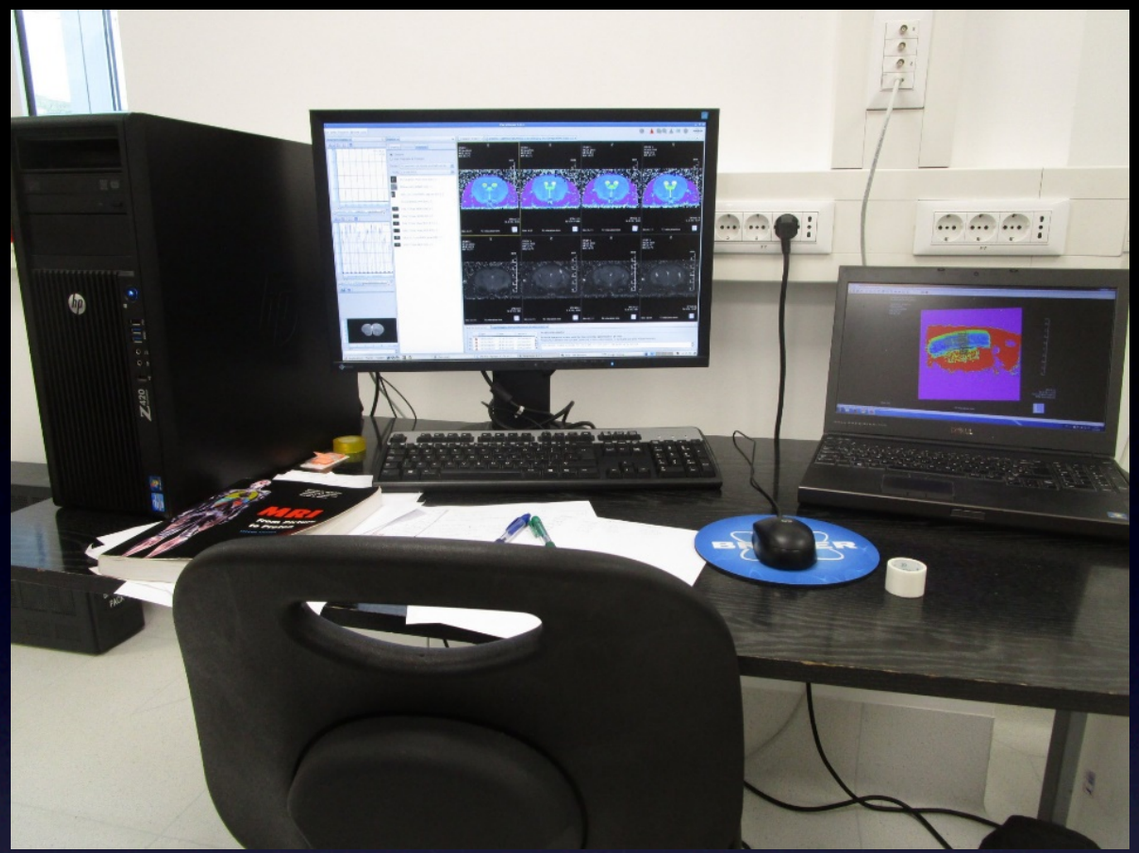
HRVATSKI INSTITUT ZA ISTRAŽIVANJE MOZGA

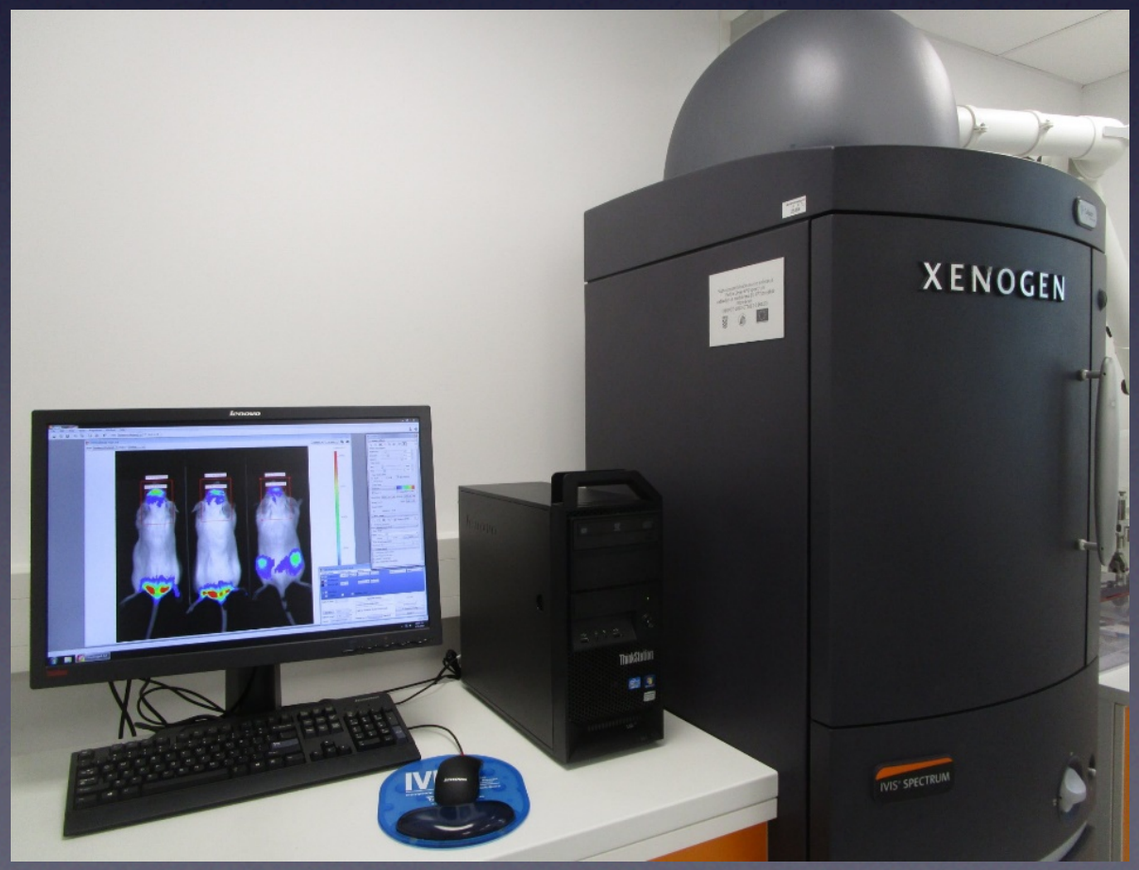
CROATIAN INSTITUTE FOR BRAIN RESEARCH

Human brain bank


BRUKER

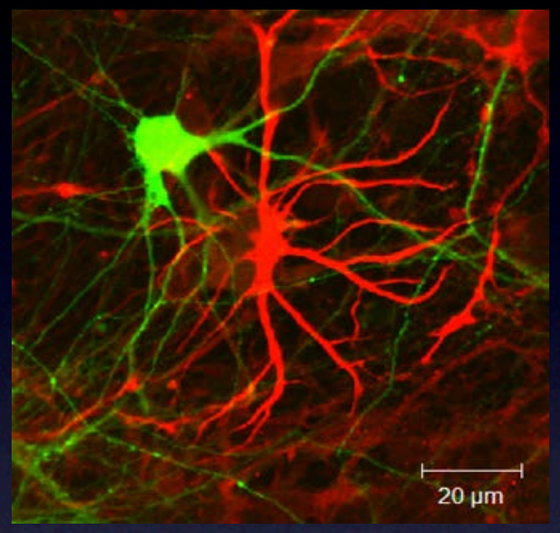

MRI

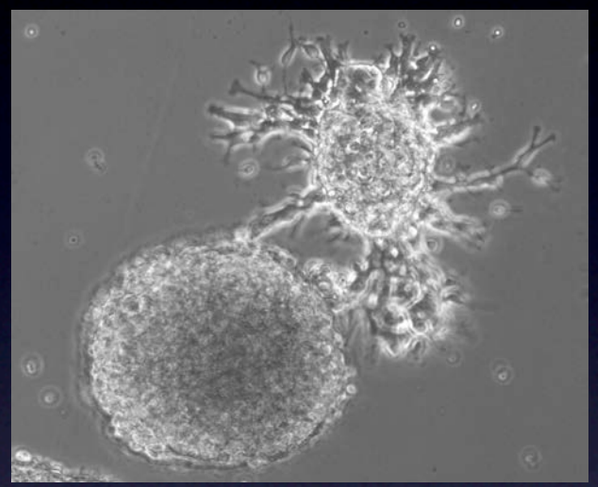

BRUKER

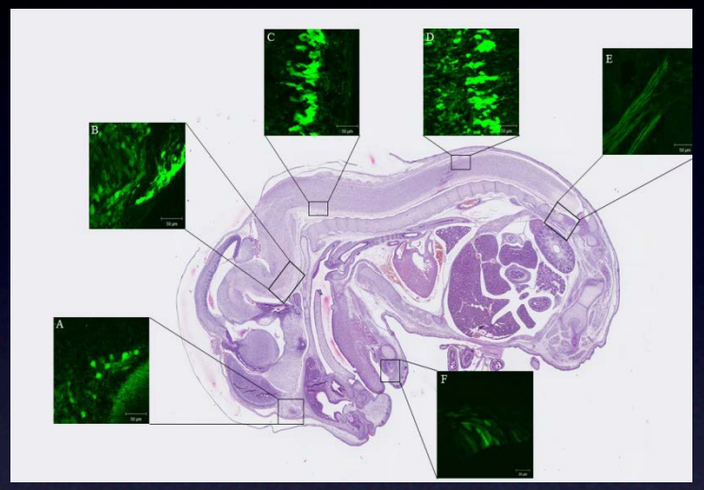

XENOGEN
IVIS SPECTRUM

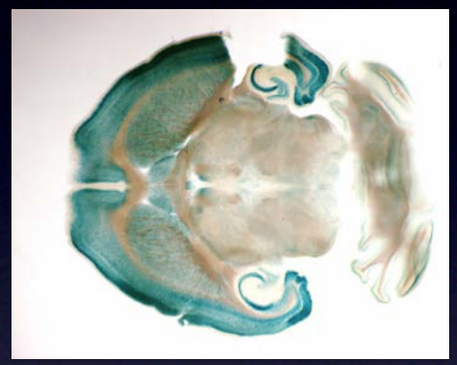
Stereotaxic transplantation of NSC

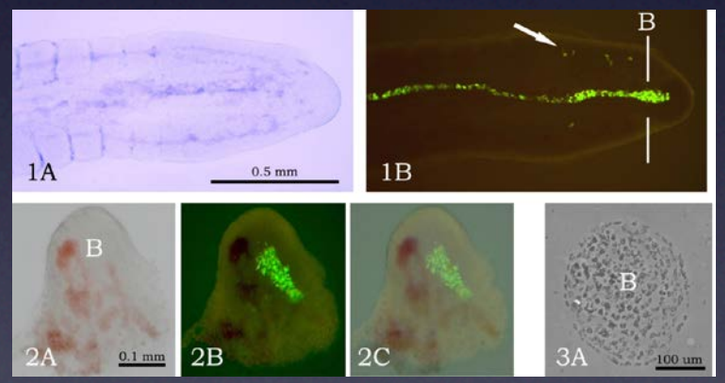

EVOS FL Auto
Nabavljeno uz pomoć Europske unije iz projekta:
"Mladi Mozak"
FONDOVI

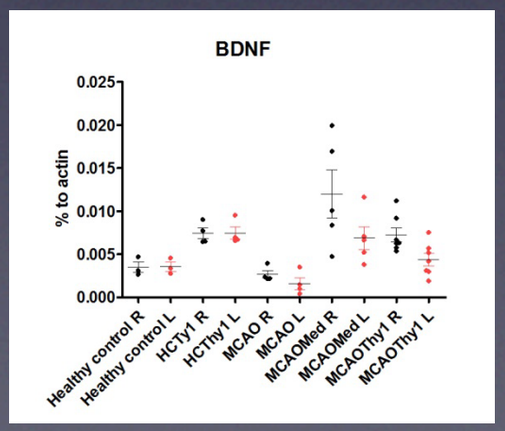

20 µm

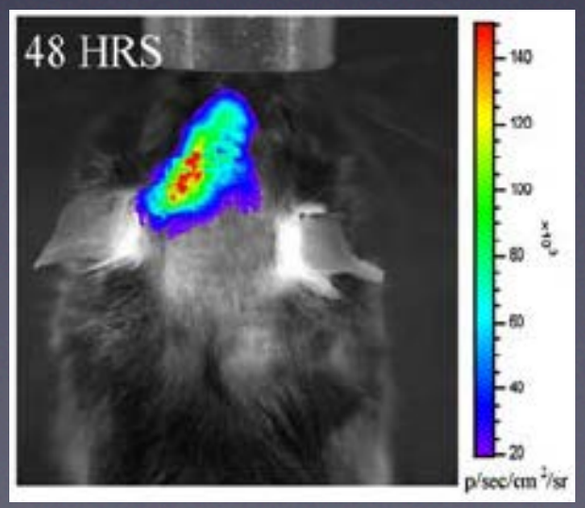


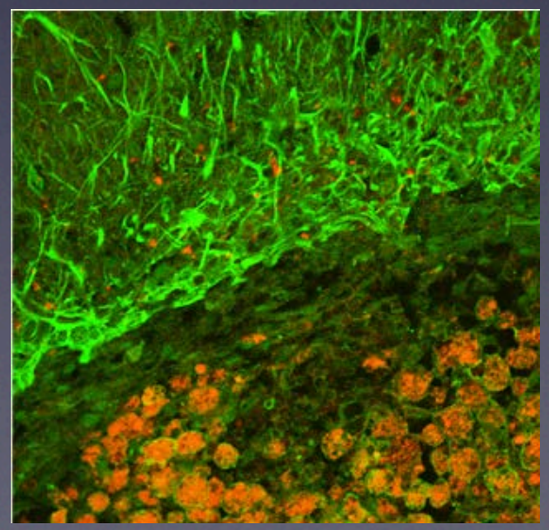

A
B
C
D
E
F




1A
0.5 mm
1B
B
2A
0.1 mm
2B
2C
3A
100 um
B
B


BDNF
% to actin
0.025
0.020
0.015
0.010
0.005
0.000
Healthy control R
Healthy control L
HCTy1 R
HCThy1 L
MCAO R
MCAO L
MCAOMed R
MCAOMed L
MCAOThy1 R
MCAOThy1 L


48 HRS
140
120
100
80
60
40
20
p/sec/cm²/sr



When did we start to use „stem cells”?

1963. McCulloch i Till

1968. The first bone marrow transplantation

1978. SCs in the umbilical cord

1980s In vitro cultivation

The first brain transplants

1989. The first transplants in the human brain

1990s SC lines

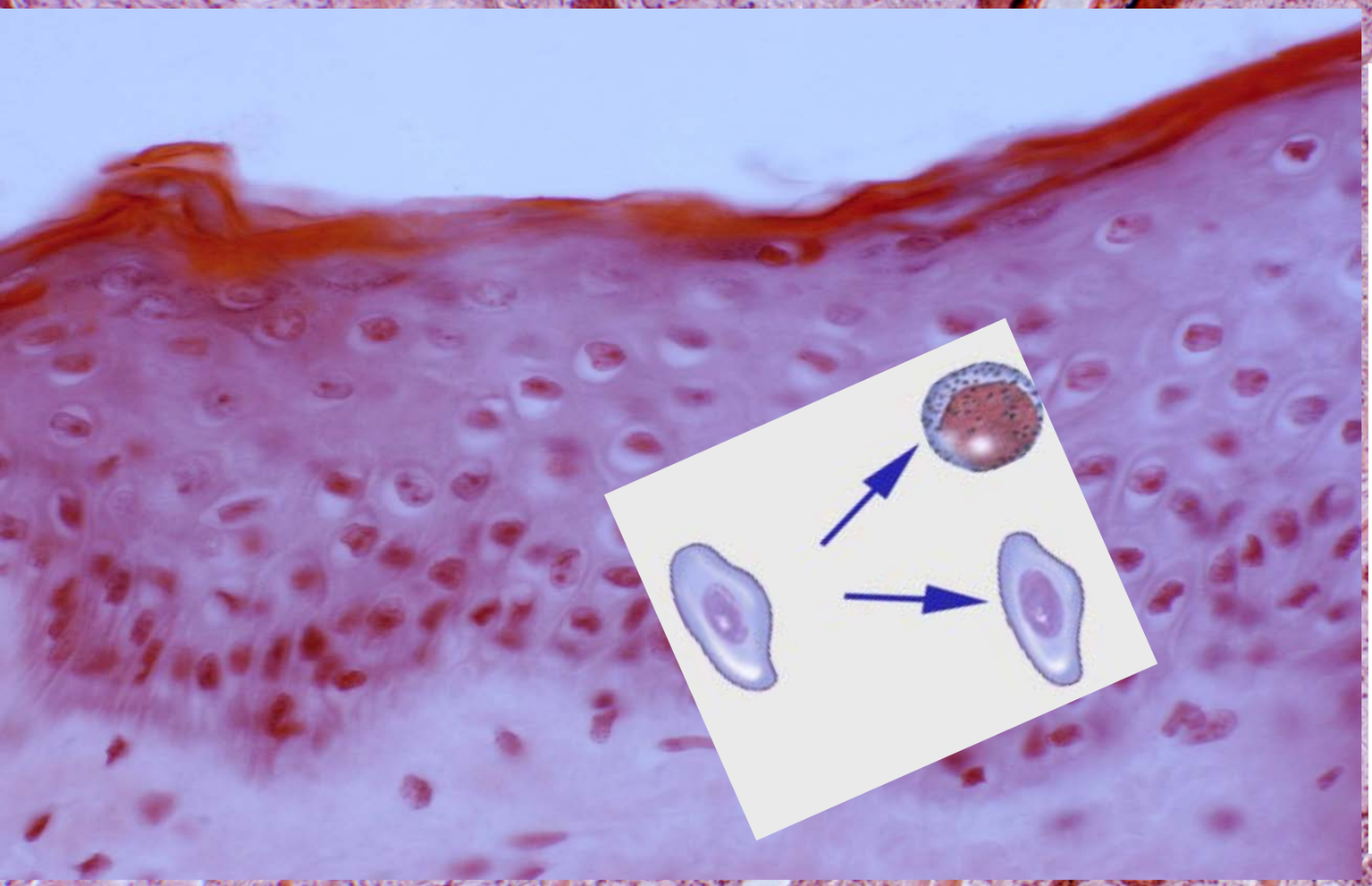
1995. Dolly the sheep

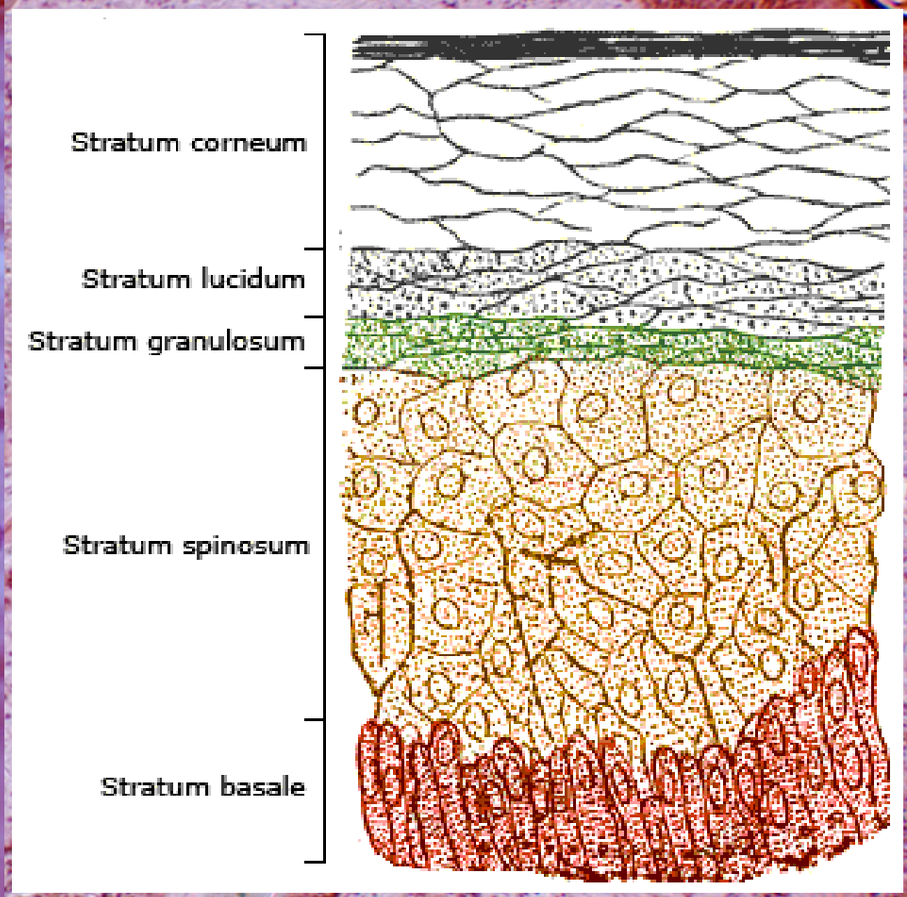
1998. Human SCs

2006. iPSCs

2007. Nobel prize Capecchi, Evans, Smithies *"for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"*.

2012. Nobel prize iPSCs Gurdon, Yamanaka




Stratum corneum
Stratum lucidum
Stratum granulosum
Stratum spinosum
Stratum basale

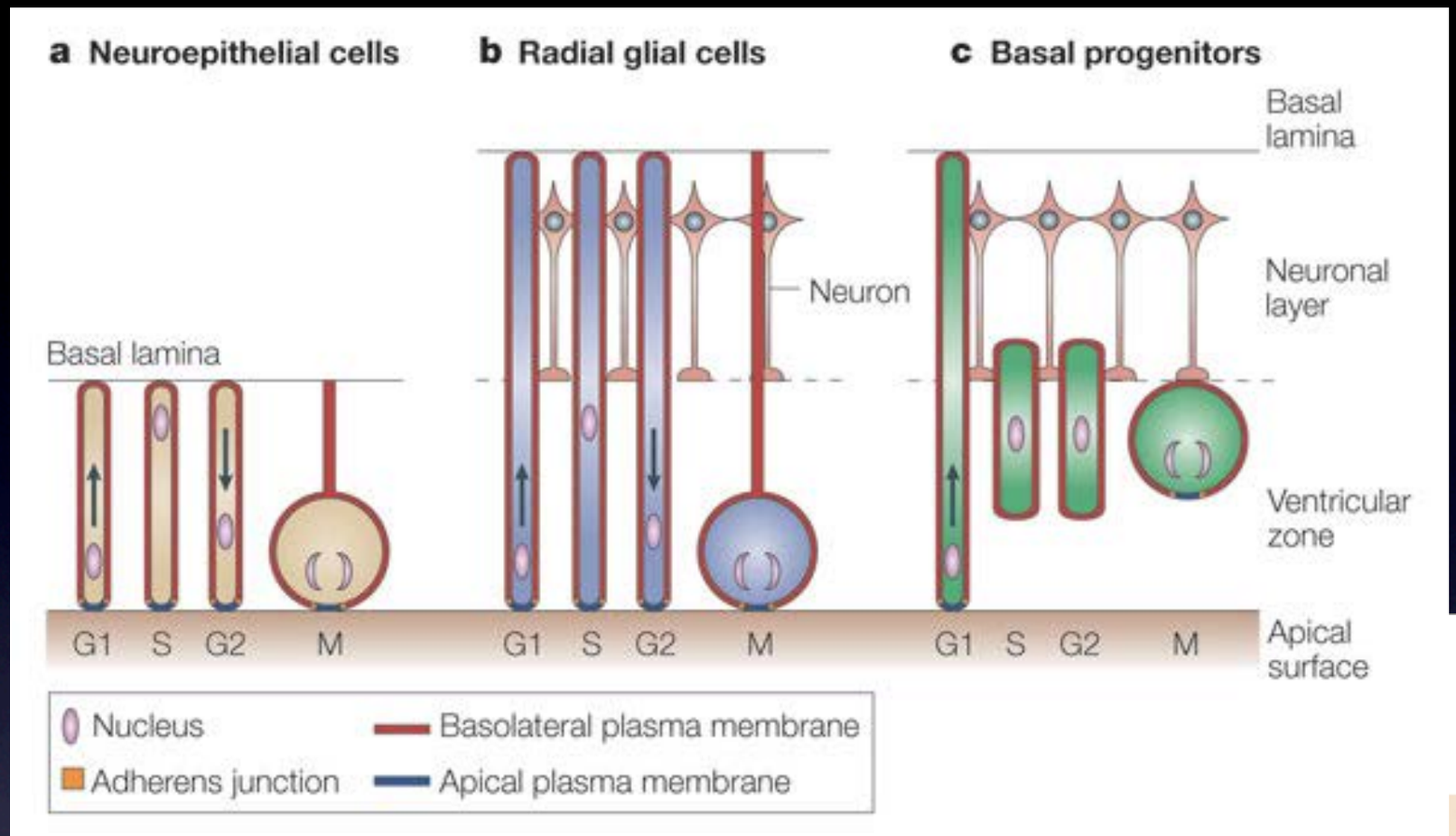
1) General classification of stem cells

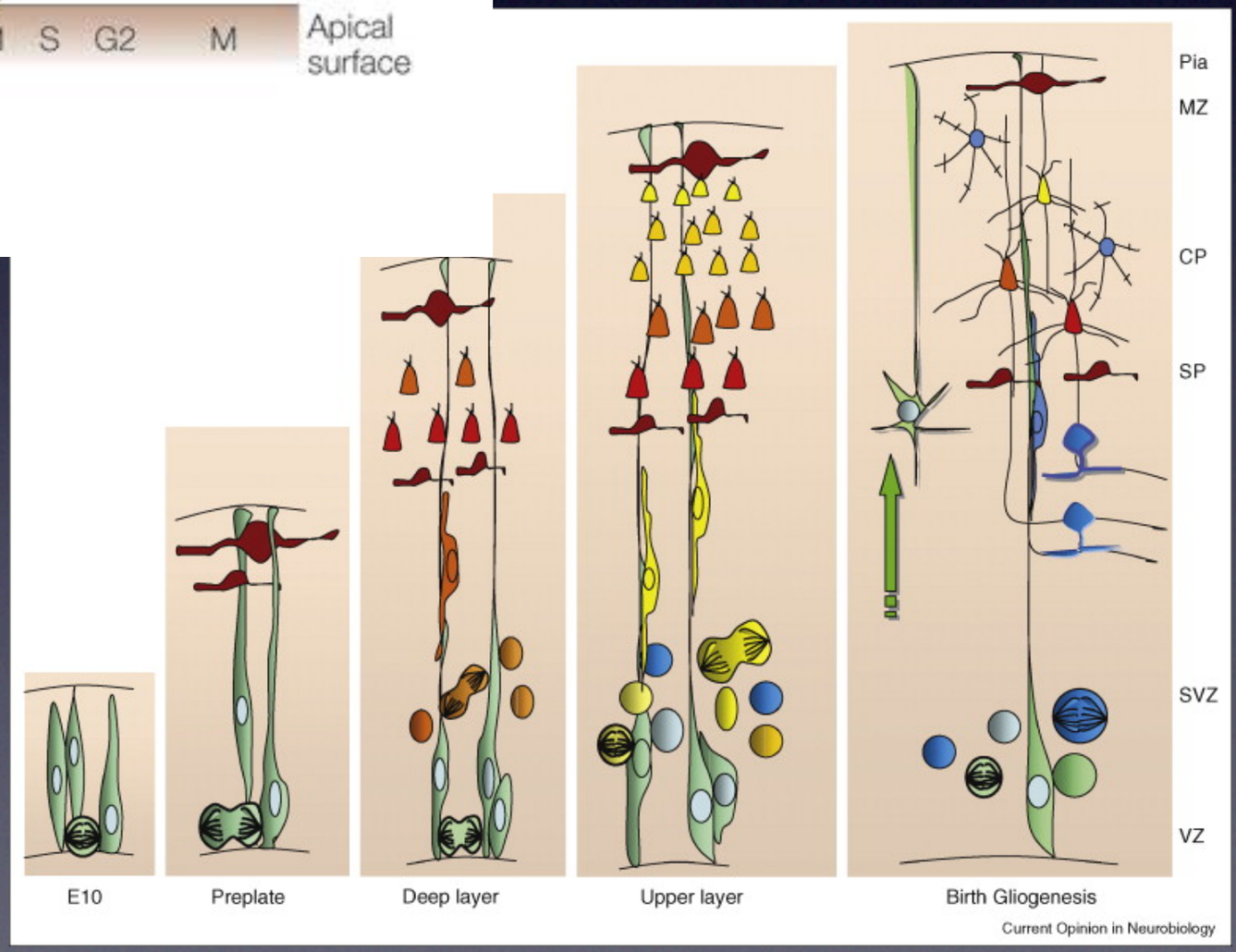
2) Introduction to regenerative neurology

3) Animal models

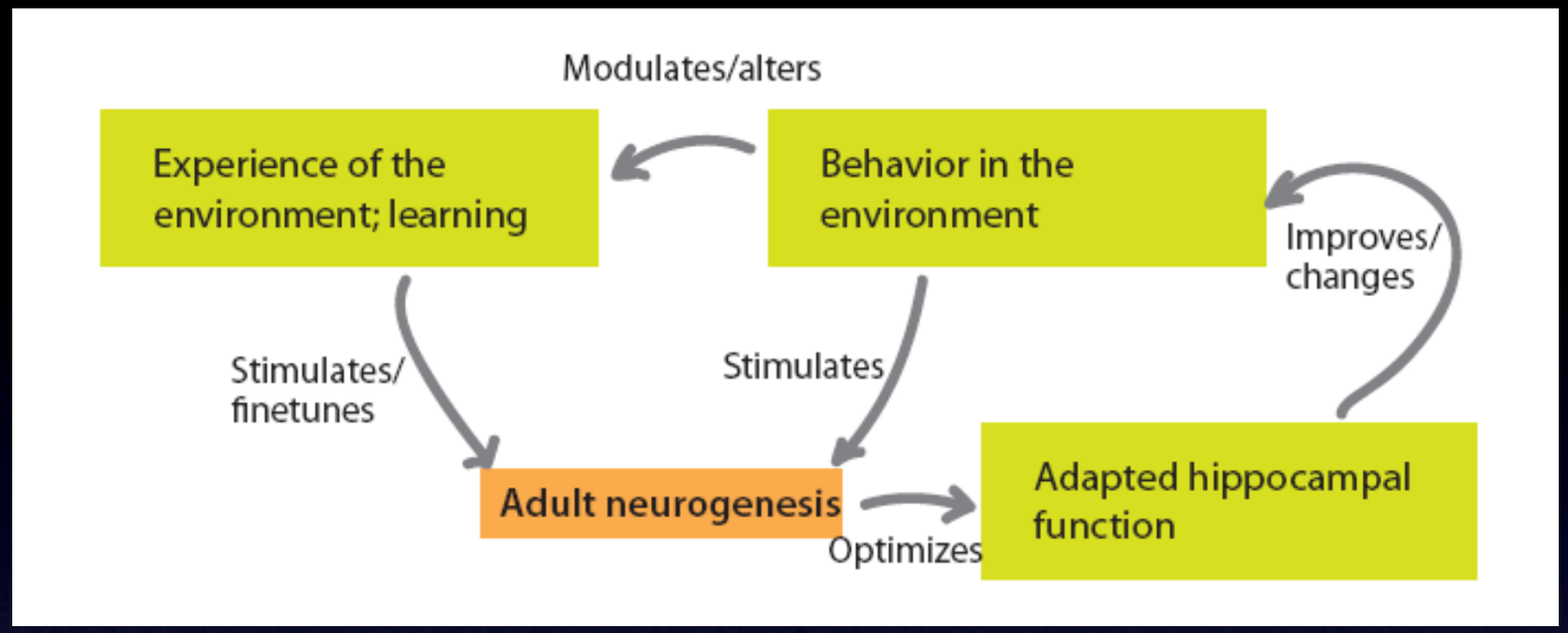
4) Cell tracing

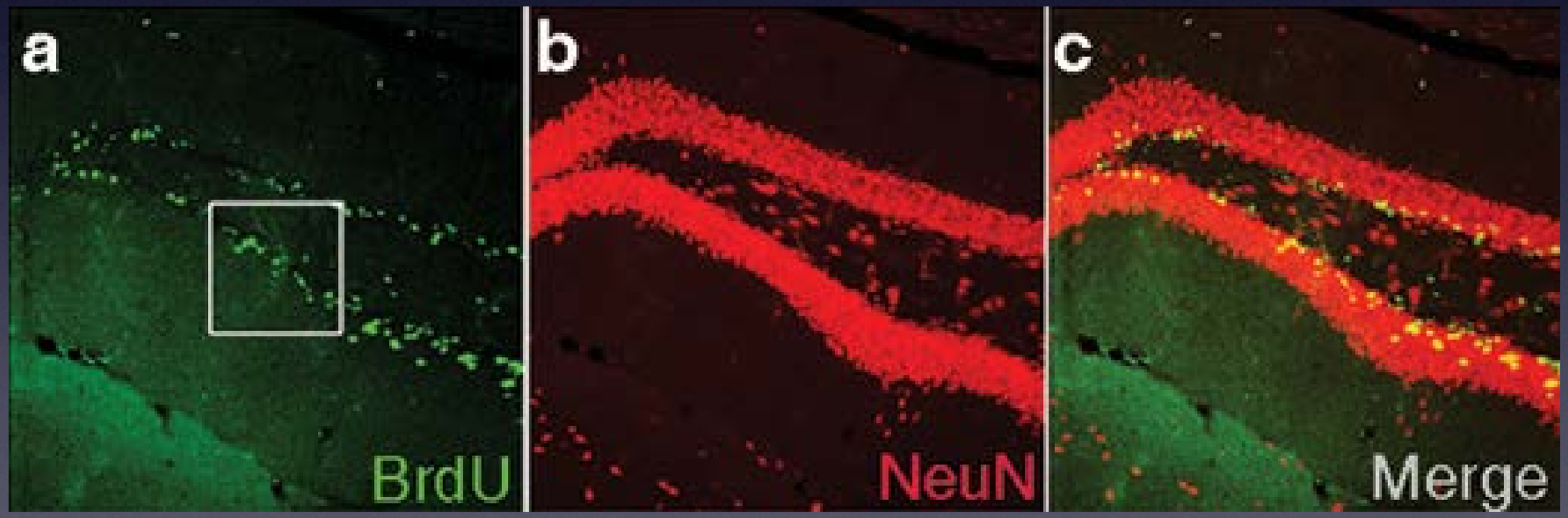
1. Embryonic SC





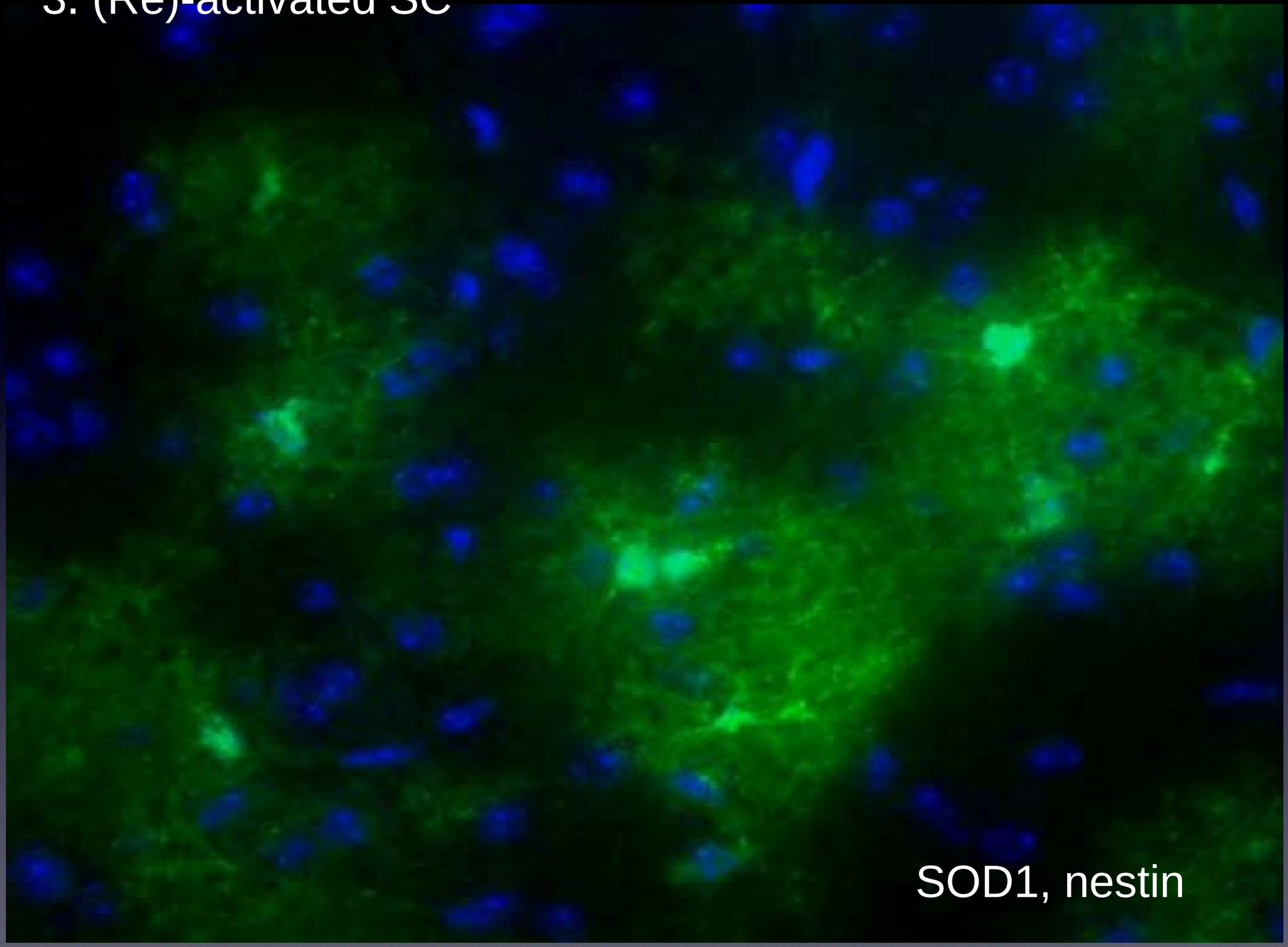
2. Adult SC



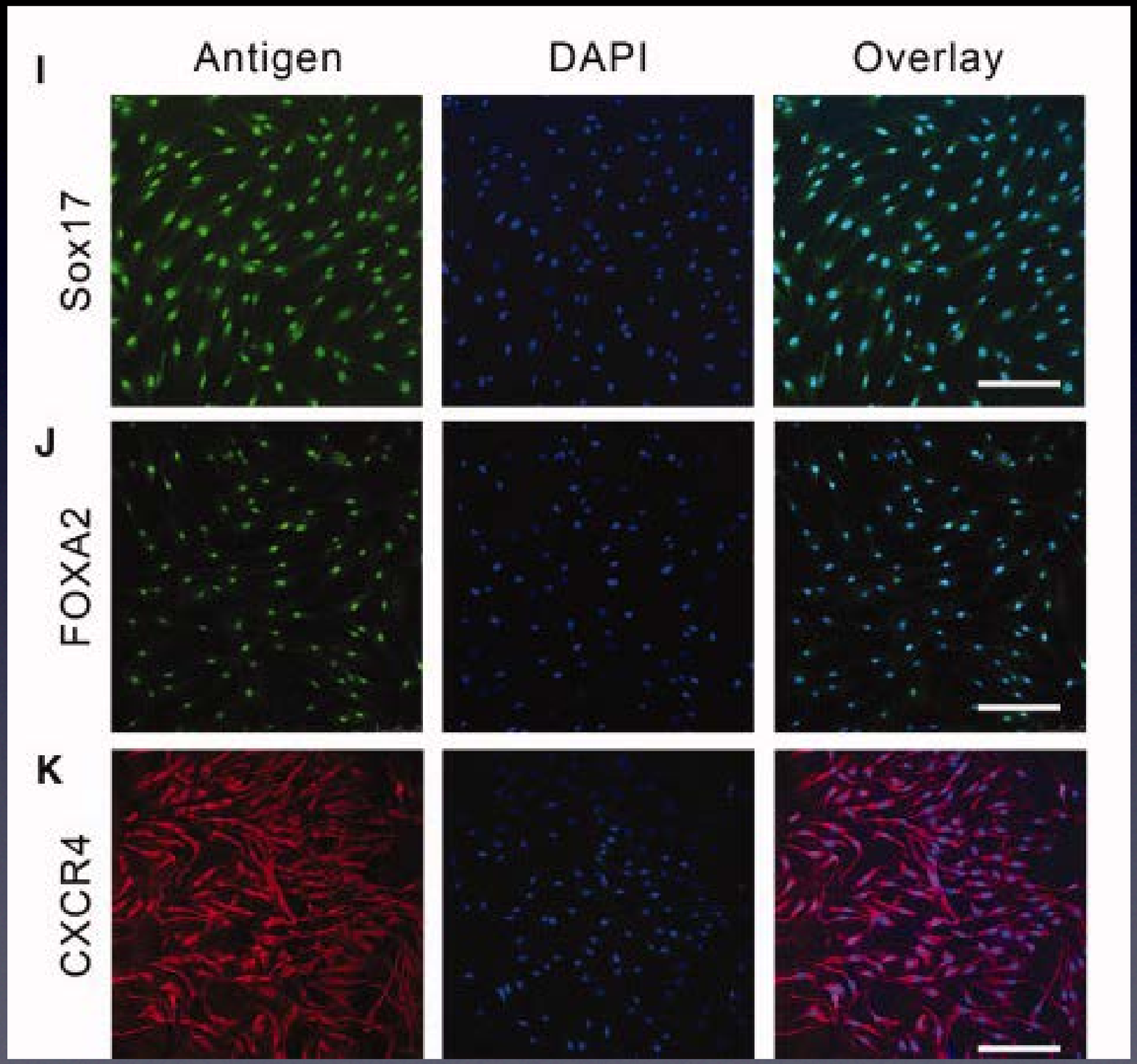


Thuret et al 2009.

3. (Re)-activated SC



Induced pluripotent stem cells



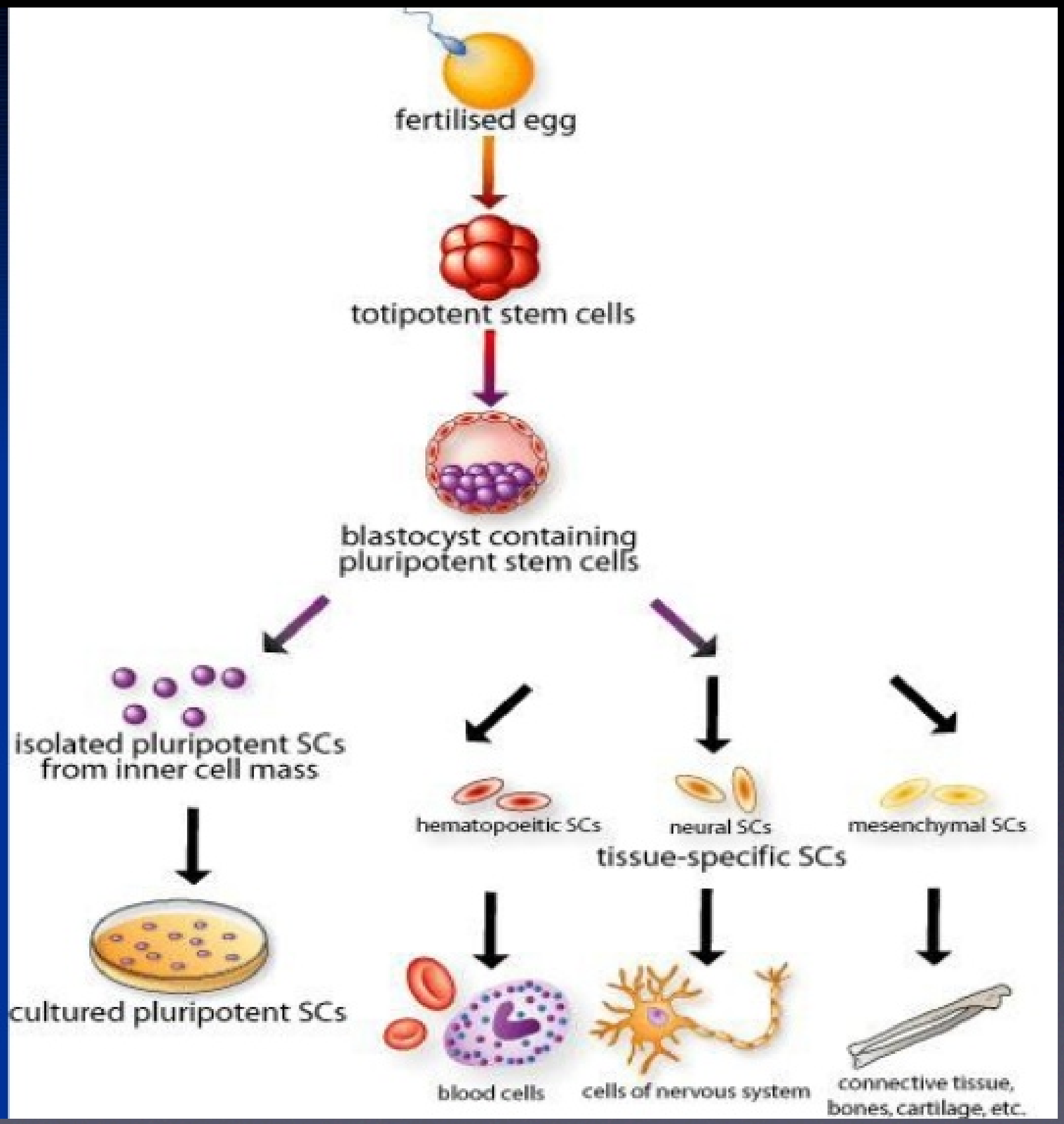
Embryonic Stem cells

Totipotent

Pluripotent

Multipotent

Unipotent



European Neuropsychopharmacology (2011) 21, 718–779

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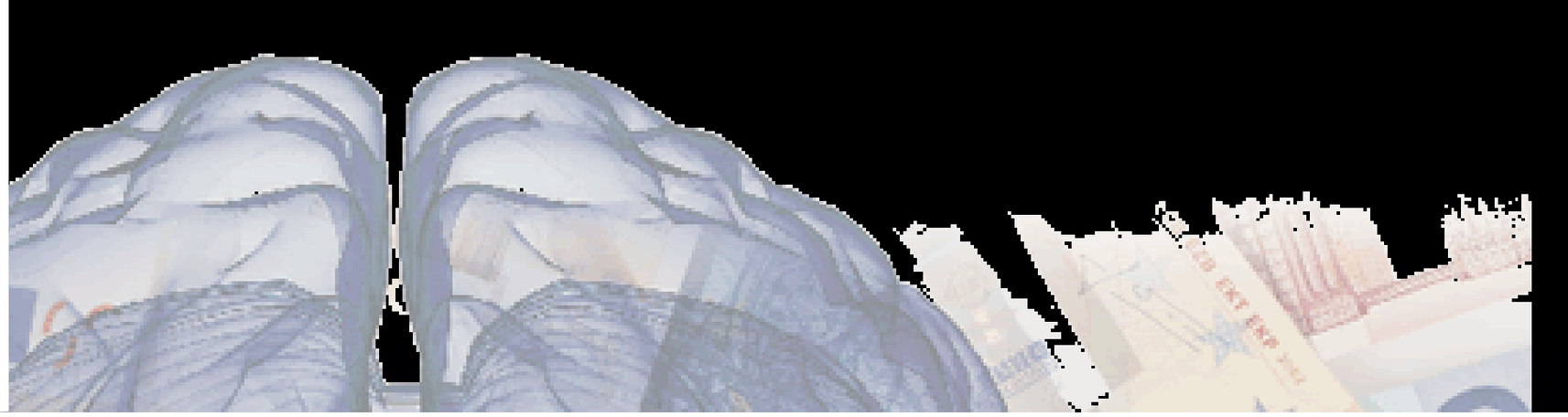
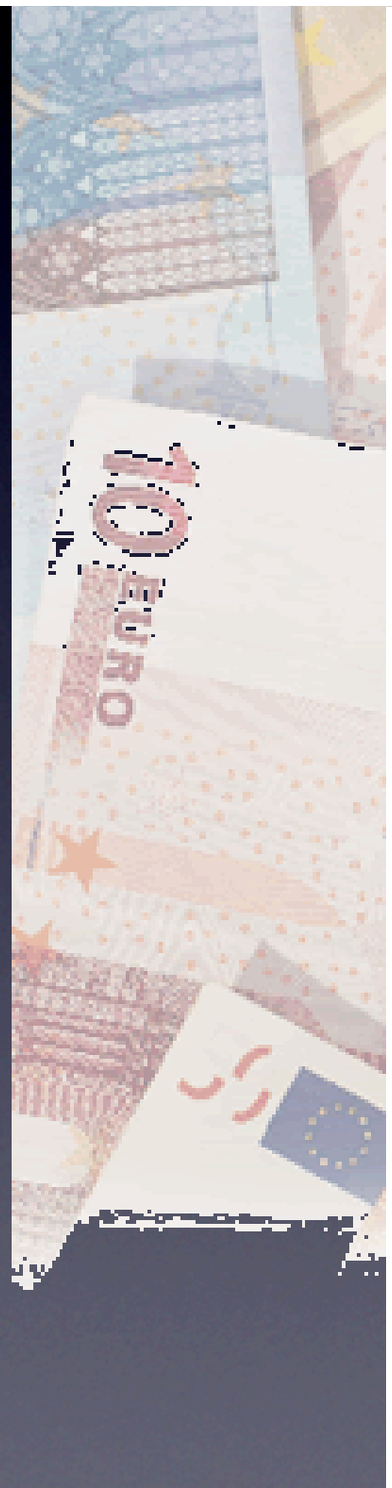
www.elsevier.com/locate/euroneuro

Cost of disorders of the brain in Europe 2010

Anders Gustavsson [a], Mikael Svensson [b], Frank Jacobi [c], Christer Allgulander [d], Jordi Alonso [e], Ettore Beghi [f], Richard Dodel [g], Mattias Ekman [a], Carlo Faravelli [h], Laura Fratiglioni [i], Brenda Gannon [j], David Hilton Jones [k], Poul Jennum [l], Albena Jordanova [m,n,o], Linus Jönsson [a], Korinna Karampampa [a], Martin Knapp [p,q], Gisela Kobelt [r,s], Tobias Kurth [t], Roselind Lieb [u], Mattias Linde [v,w], Christina Ljungcrantz [a], Andreas Maercker [x], Beatrice Melin [y], Massimo Moscarelli [z,aa], Amir Musayev [a], Fiona Norwood [ab], Martin Preisig [ac], Maura Pugliatti [ad], Juergen Rehm [ae,af], Luis Salvador-Carulla [ag,ah], Brigitte Schlehofer [ai], Roland Simon [aj], Hans-Christoph Steinhausen [ak,al,am], Lars Jacob Stovner [an], Jean-Michel Vallat [ao], Peter Van den Bergh [ap], Jim van Os [aq,ar], Pieter Vos [as], Weili Xu [t], Hans-Ulrich Wittchen [c], Bengt Jönsson [at], Jes Olesen [au,*] on behalf of the CDBE2010 study group [1]





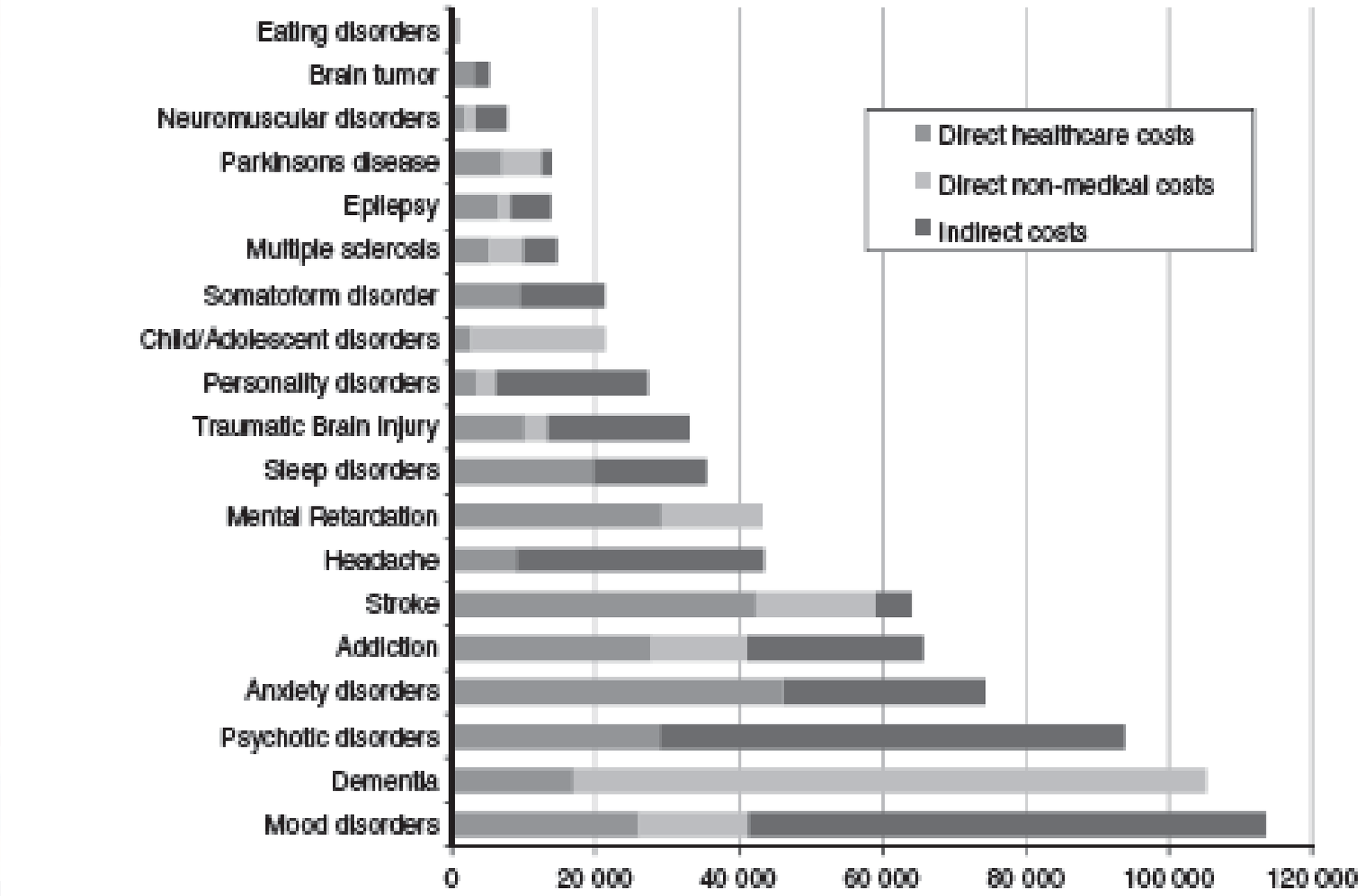
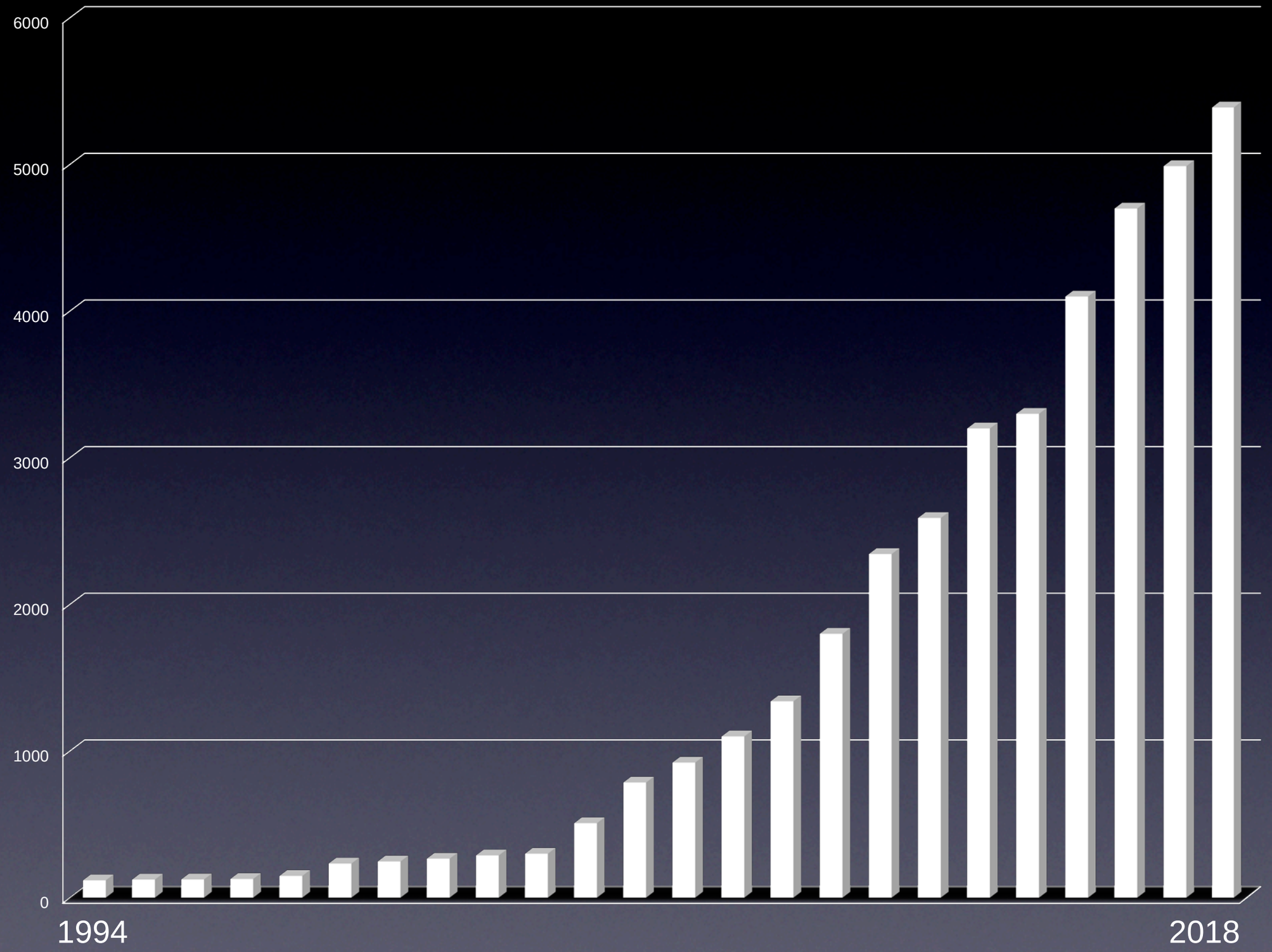
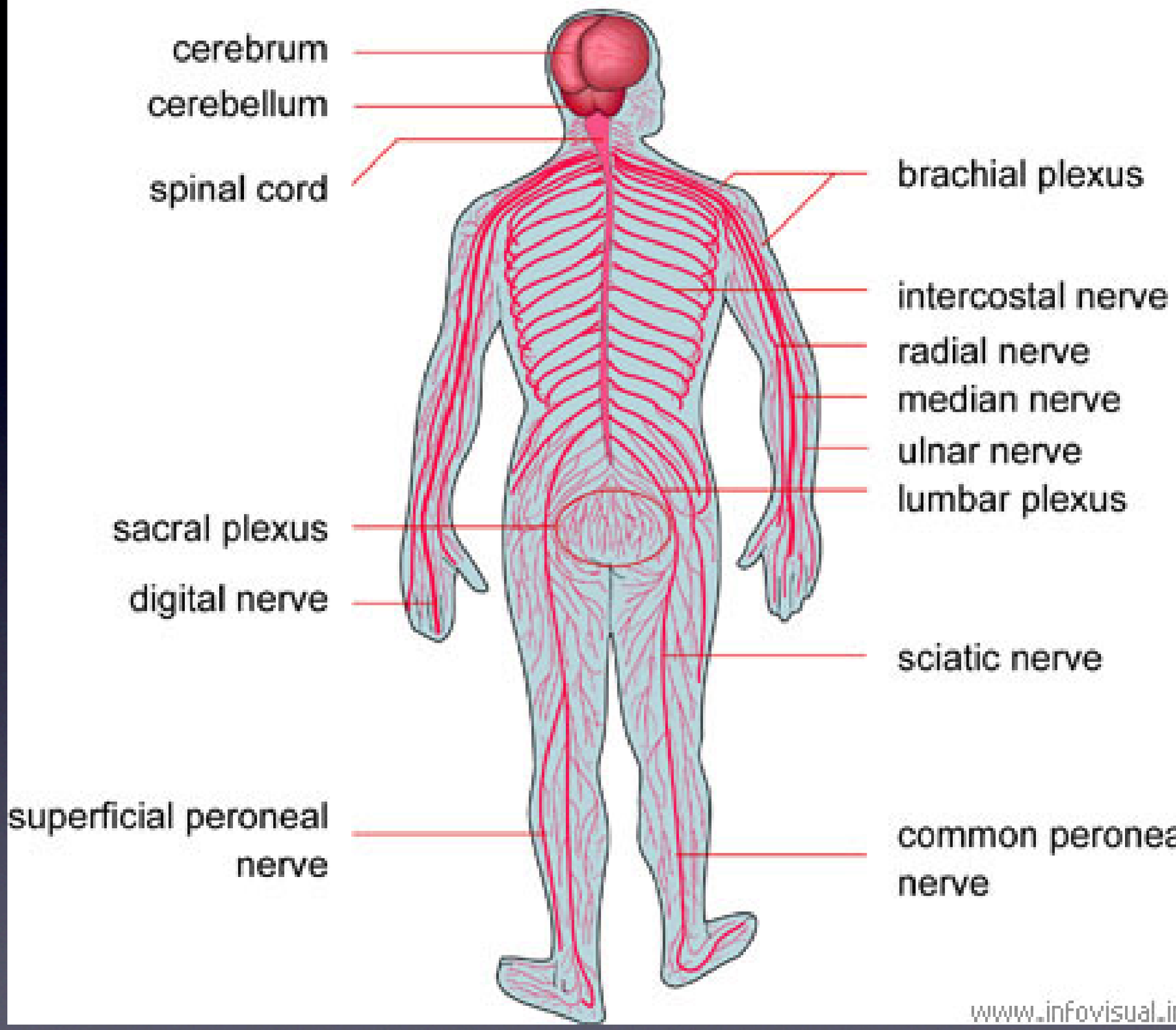


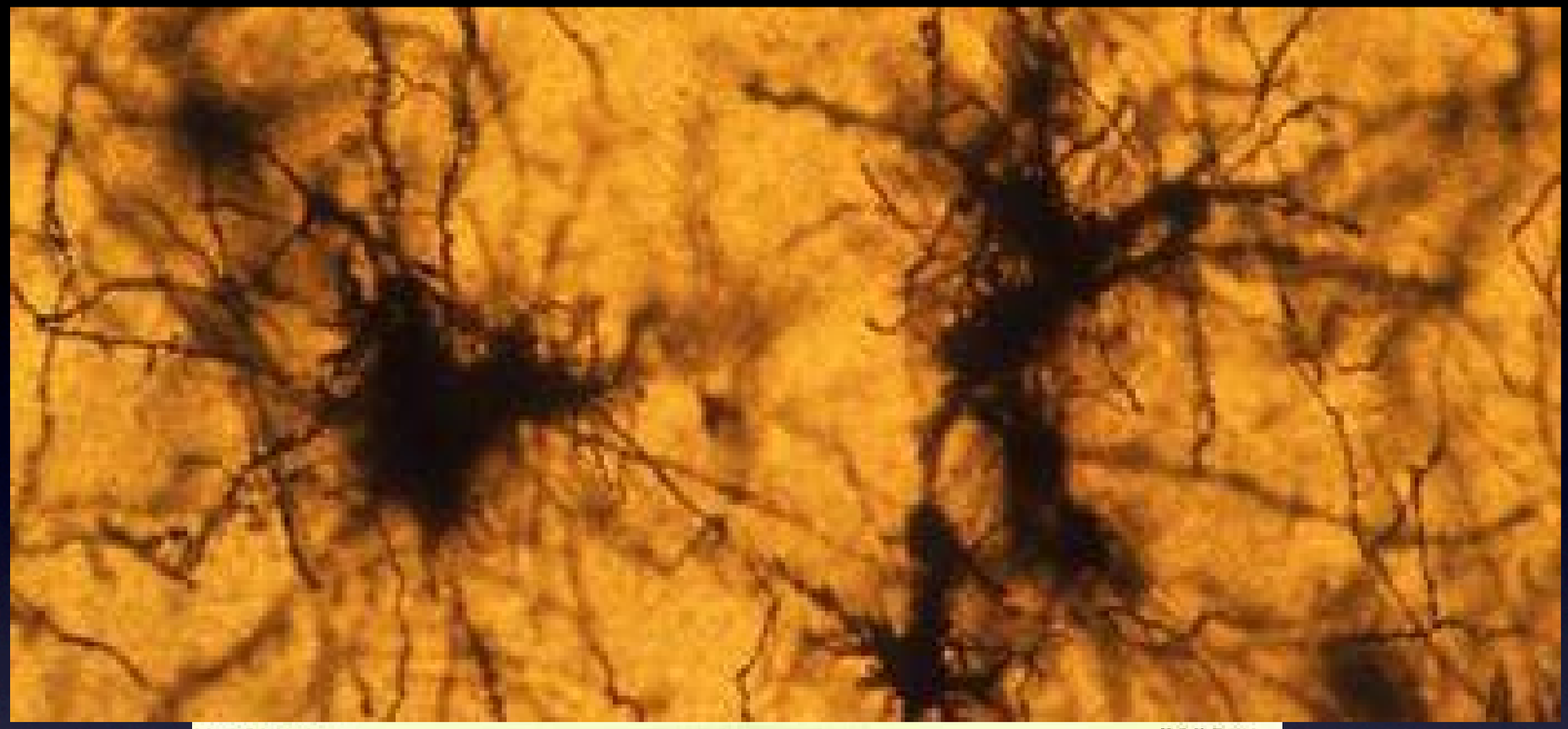
Figure 3 Total cost by disorder and type of cost (€PPP million, 2010), all disorders.

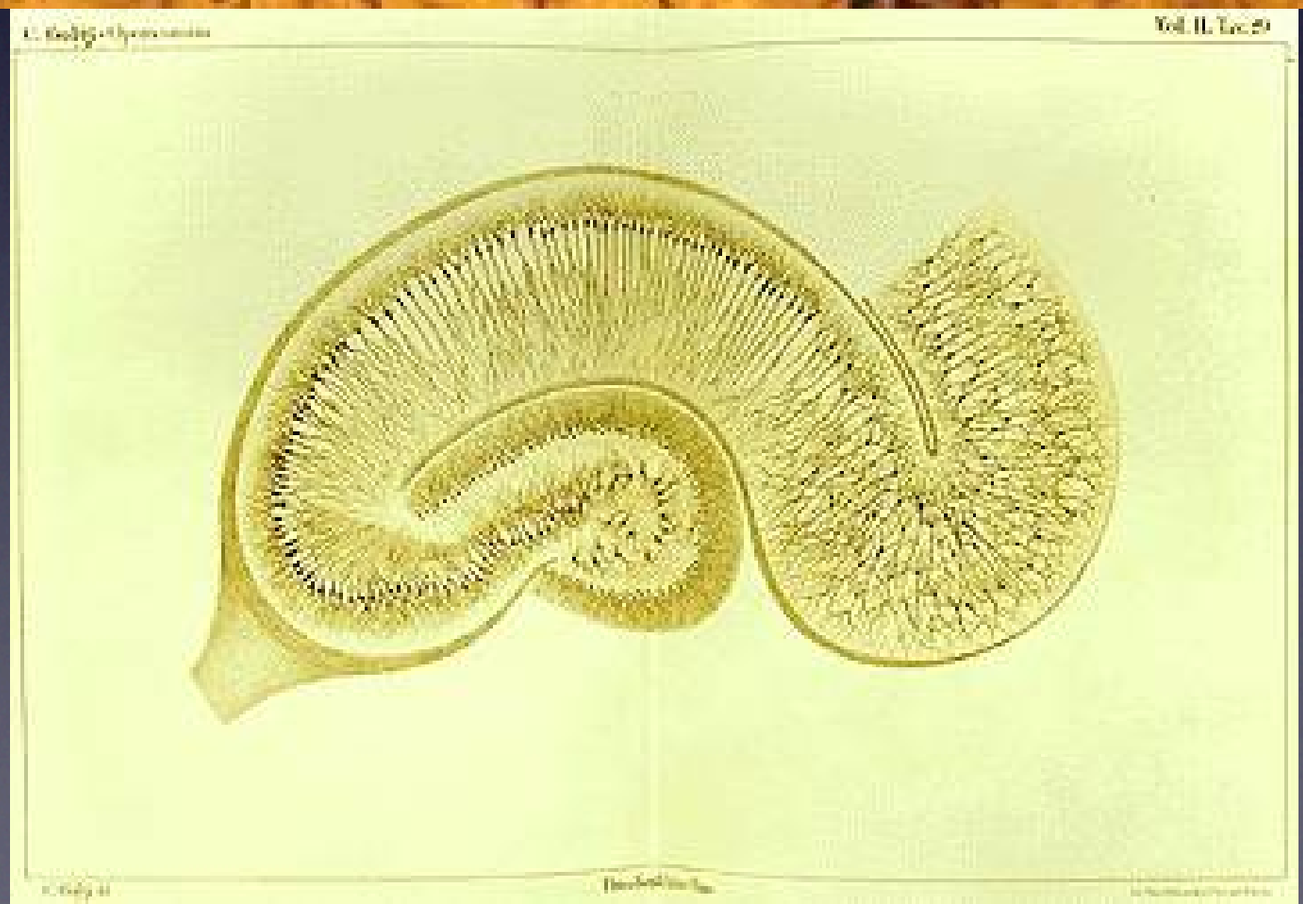

6000
5000
4000
3000
2000
1000
0
1994
2018

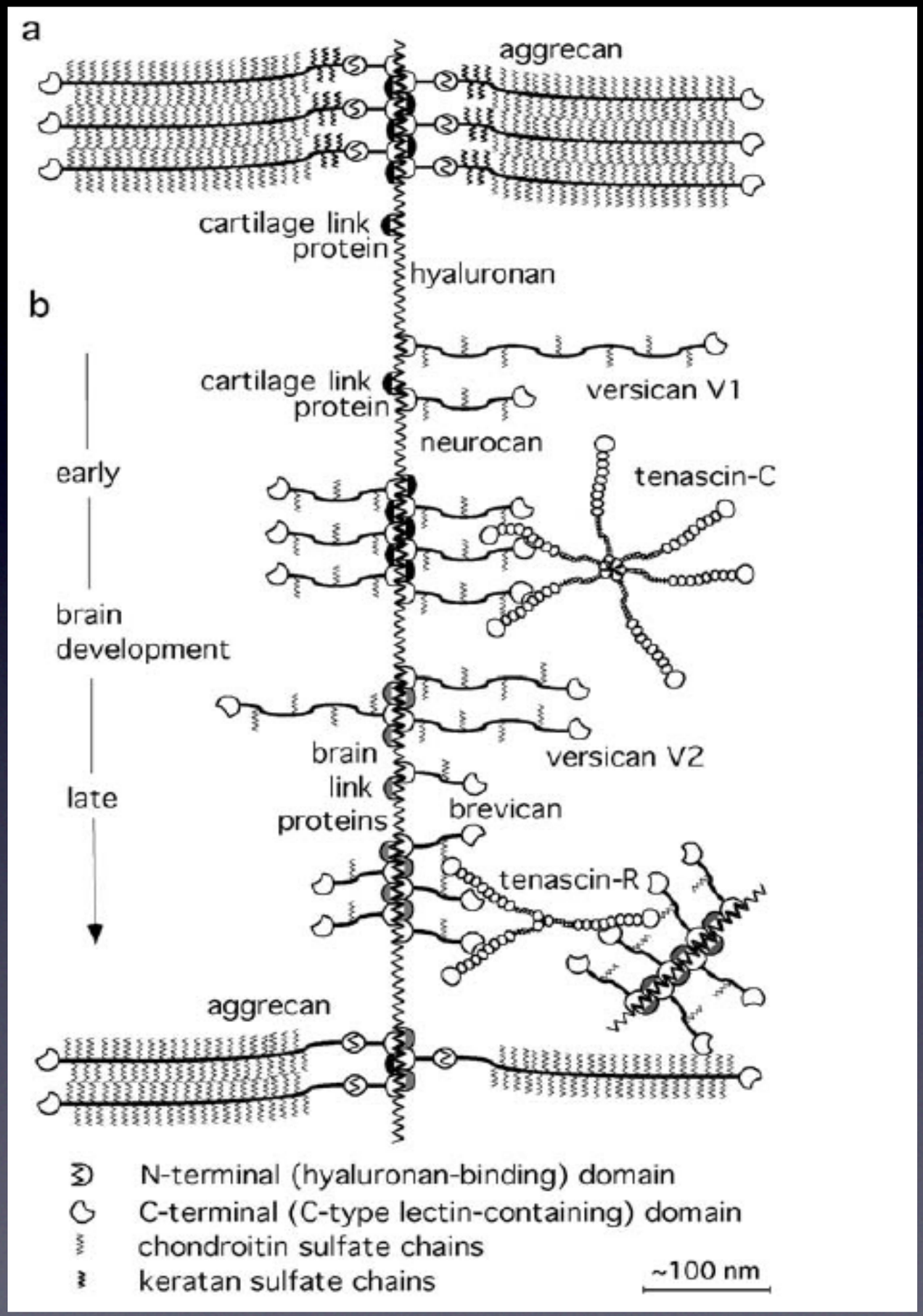
NERVOUS SYSTEM



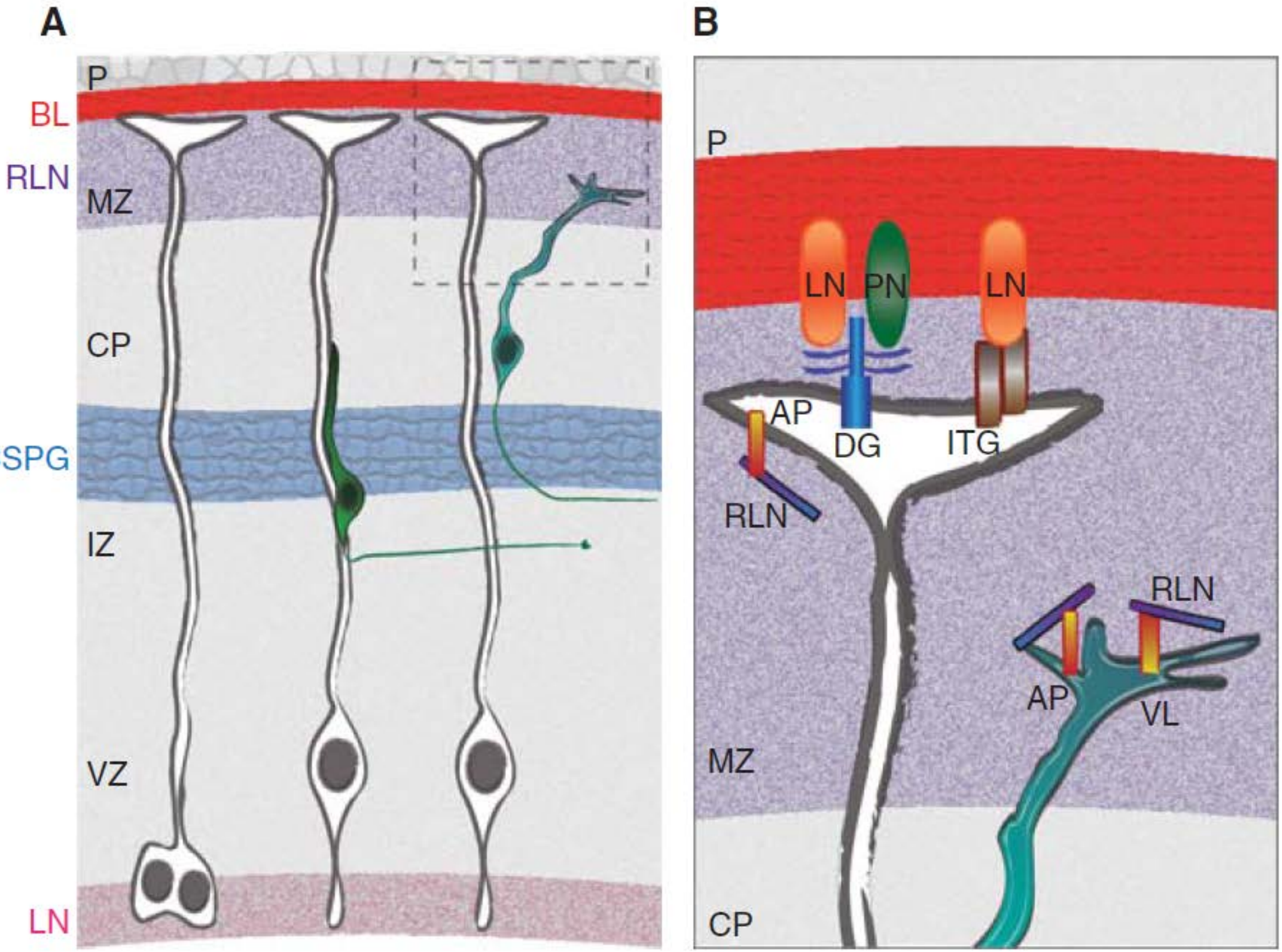
Golgi staining 1873



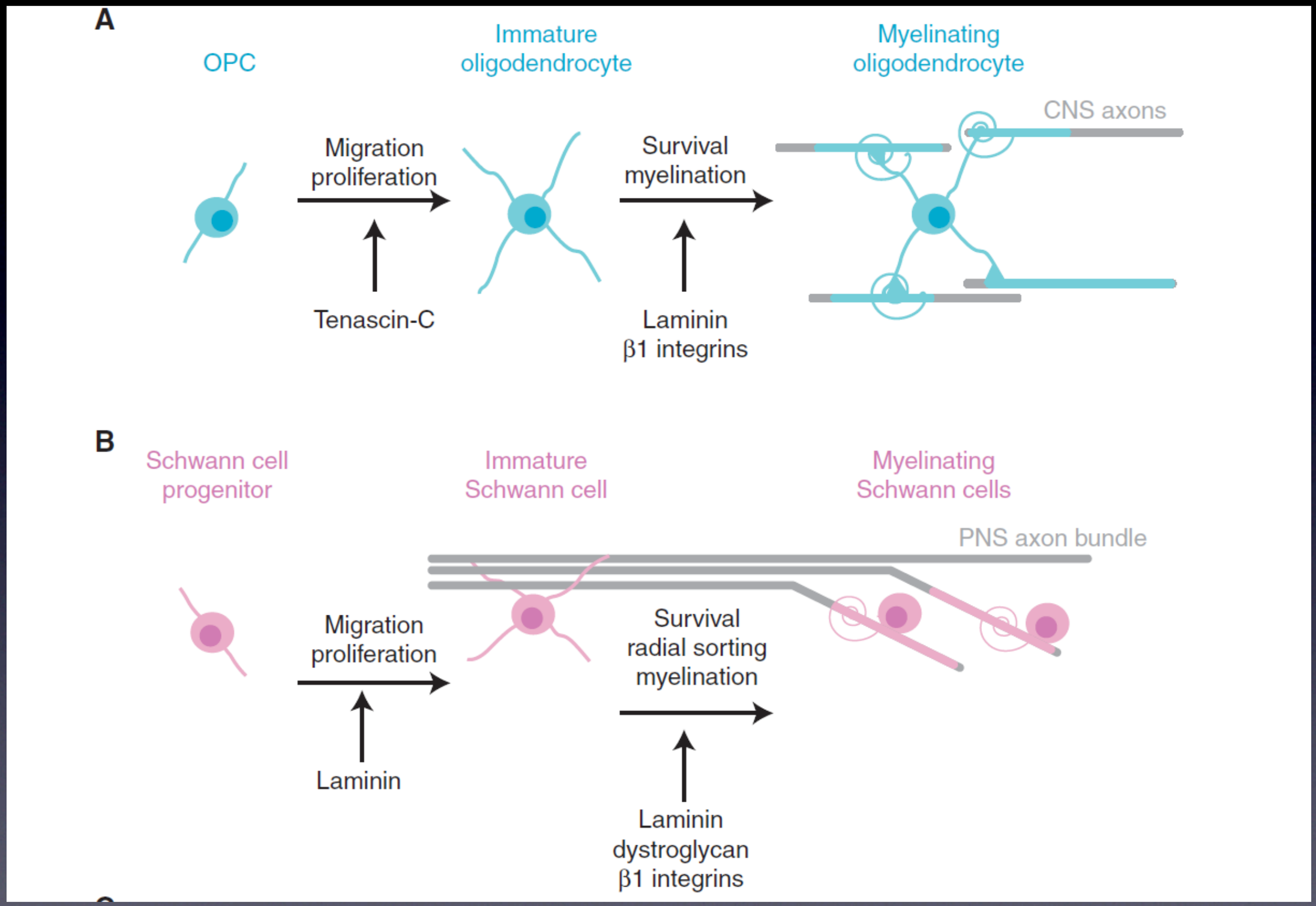


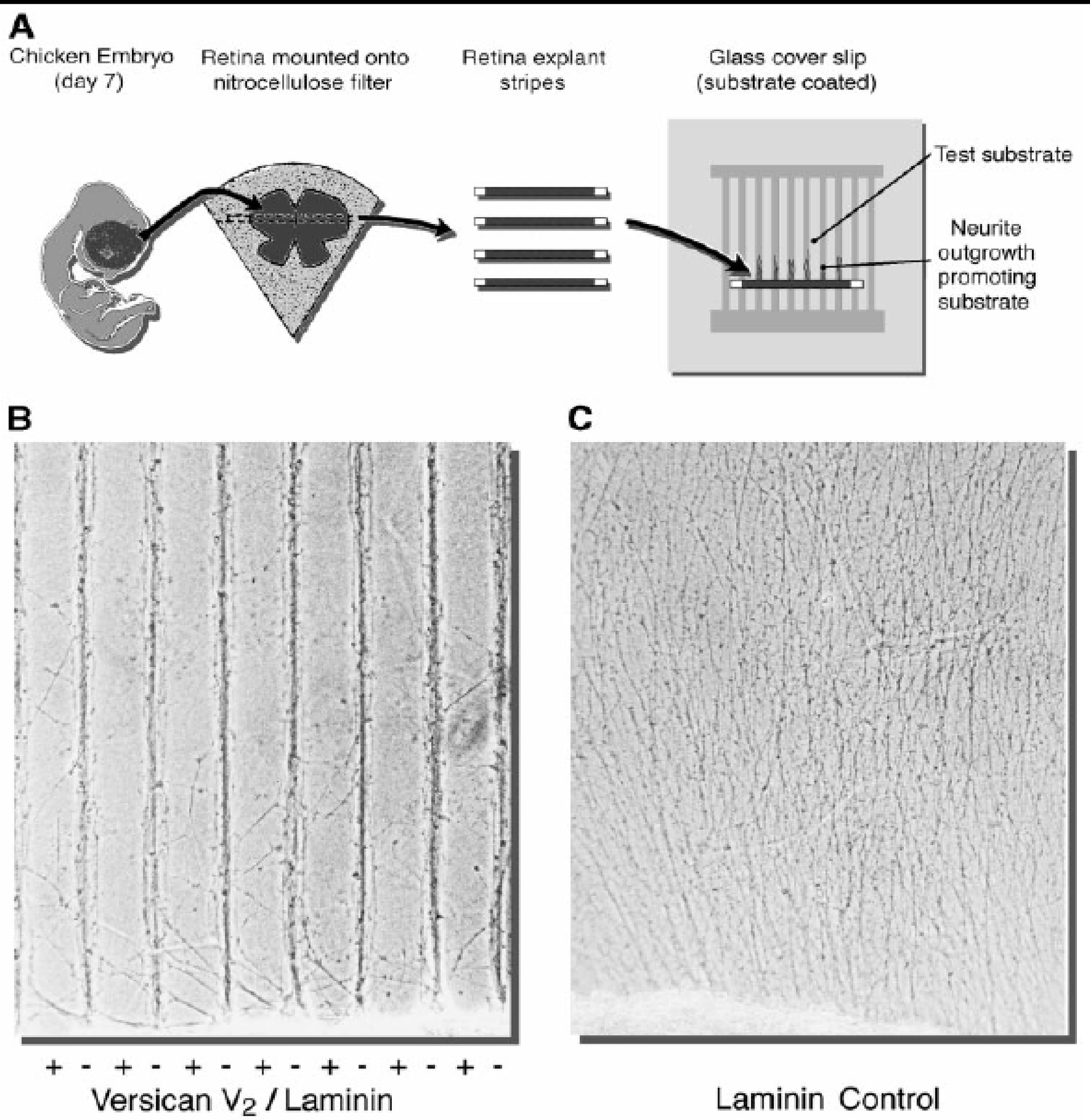

a
aggrecan
cartilage link protein
hyaluronan
b
cartilage link protein
versican V1
neurocan
tenascin-C
early
brain development
late
brain link proteins
versican V2
brevican
tenascin-R
aggrecan
N-terminal (hyaluronan-binding) domain
C-terminal (C-type lectin-containing) domain
chondroitin sulfate chains
keratan sulfate chains
~100 nm

During development

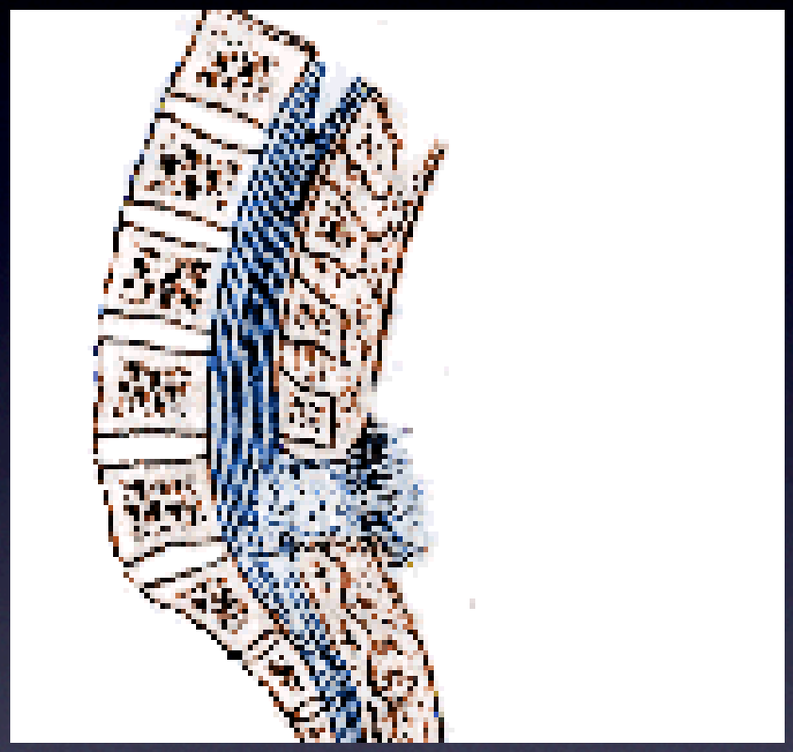


During myelination

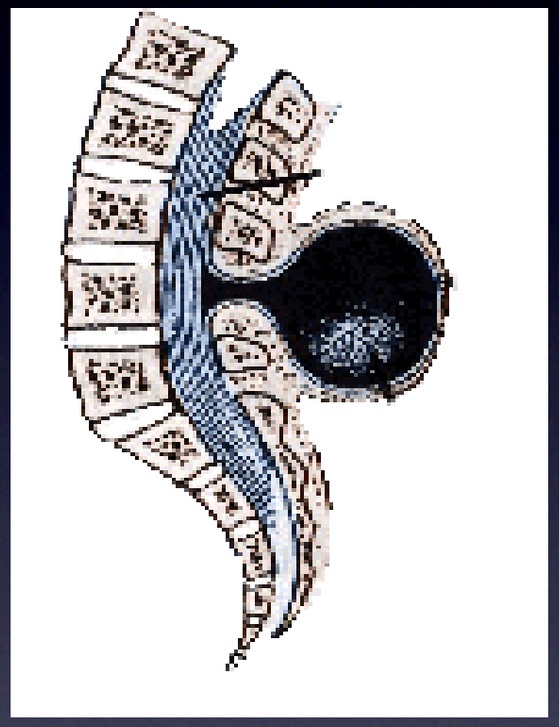



A
Chicken Embryo
(day 7)
Retina mounted onto
nitrocellulose filter
Retina explant
stripes
Glass cover slip
(substrate coated)
Test substrate
Neurite
outgrowth
promoting
substrate
B
+ - + - + - + - + - + - + -
Versican V2 / Laminin
C
Laminin Control

Spina bifida



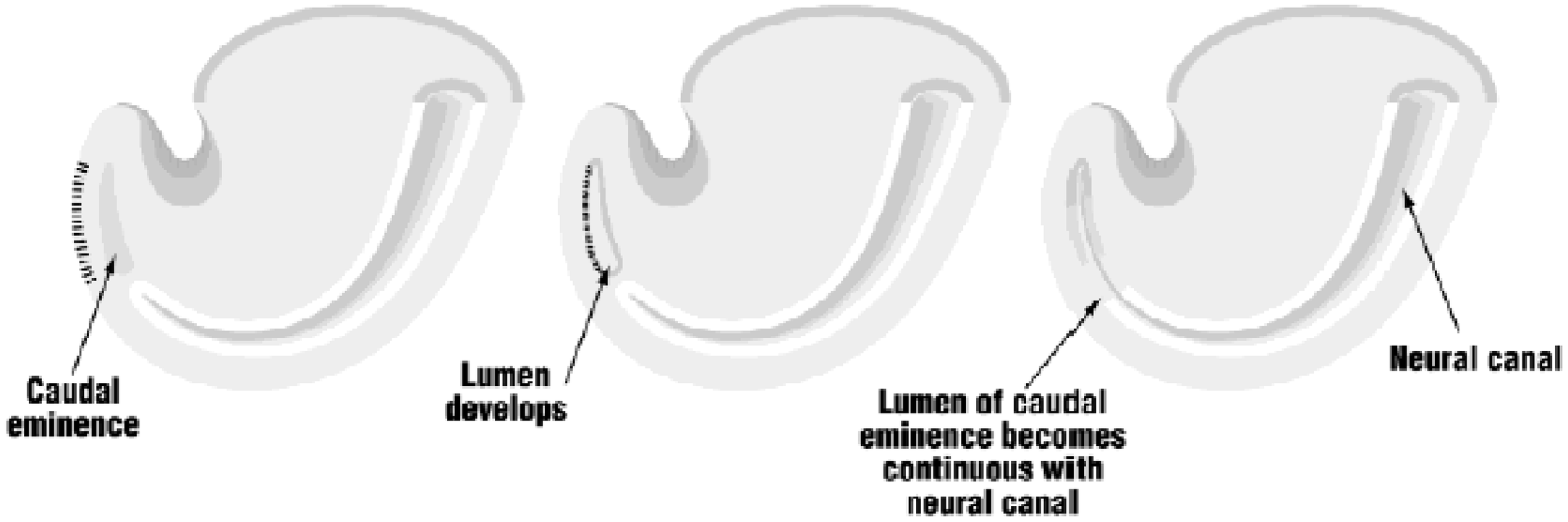
a) occulta

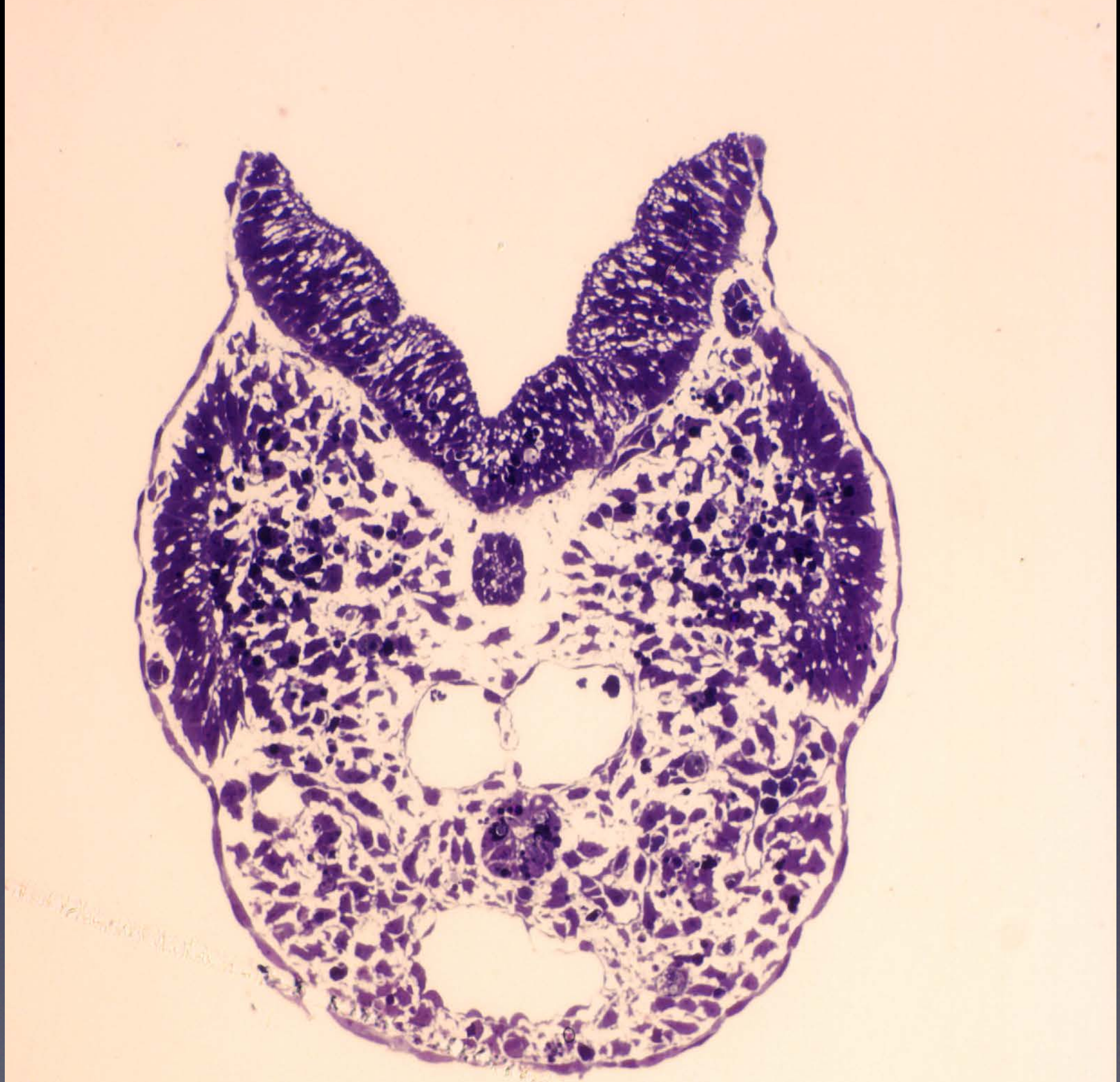


b) meningocela

c) myelomeningocela


1
2


20 Days
40 Days
Caudal eminence
Lumen develops
Lumen of caudal eminence becomes continuous with neural canal
Neural canal



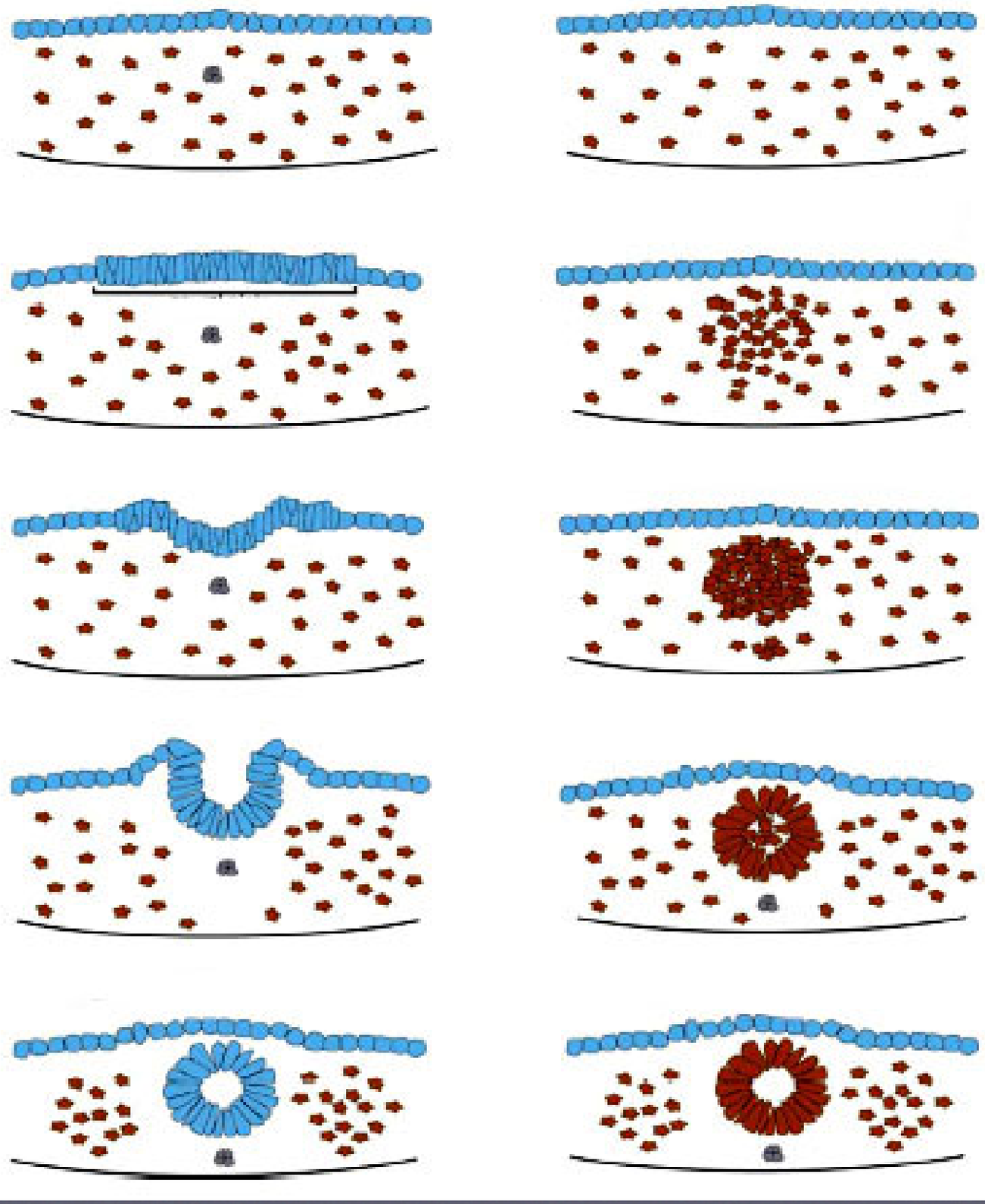
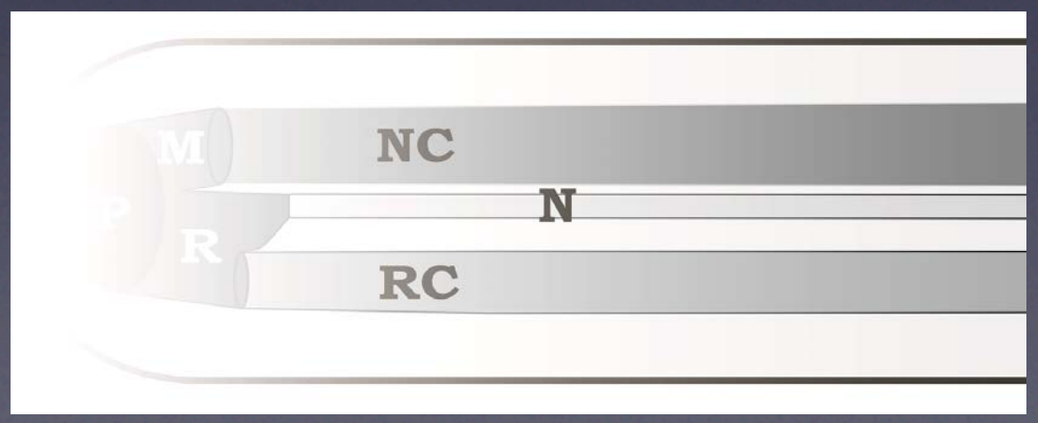
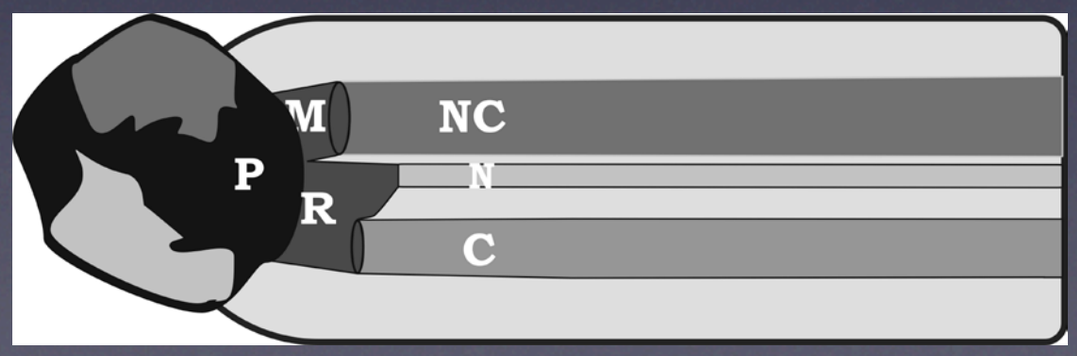


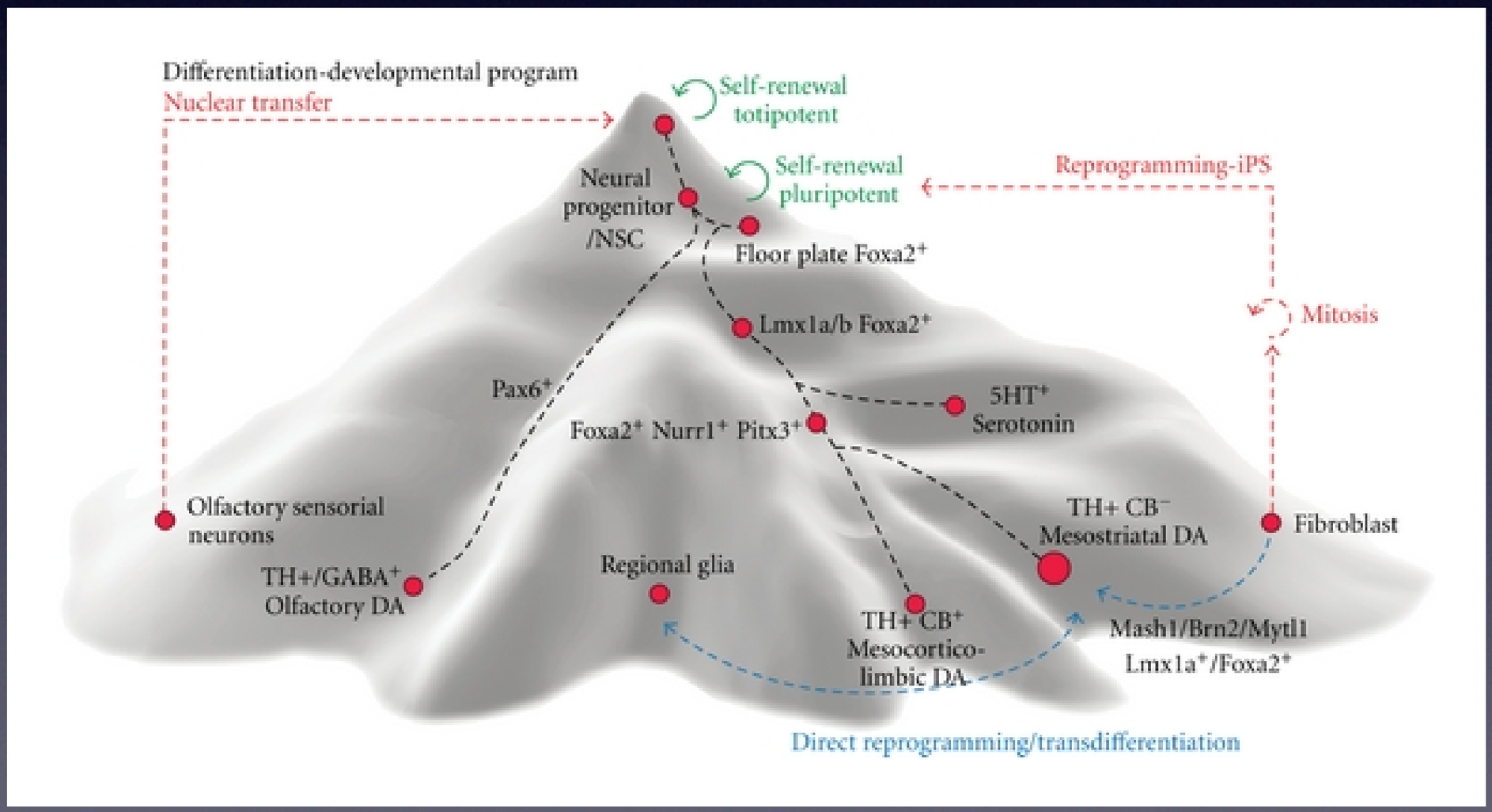

Photo by Essay


M
NC
P
N
R
RC

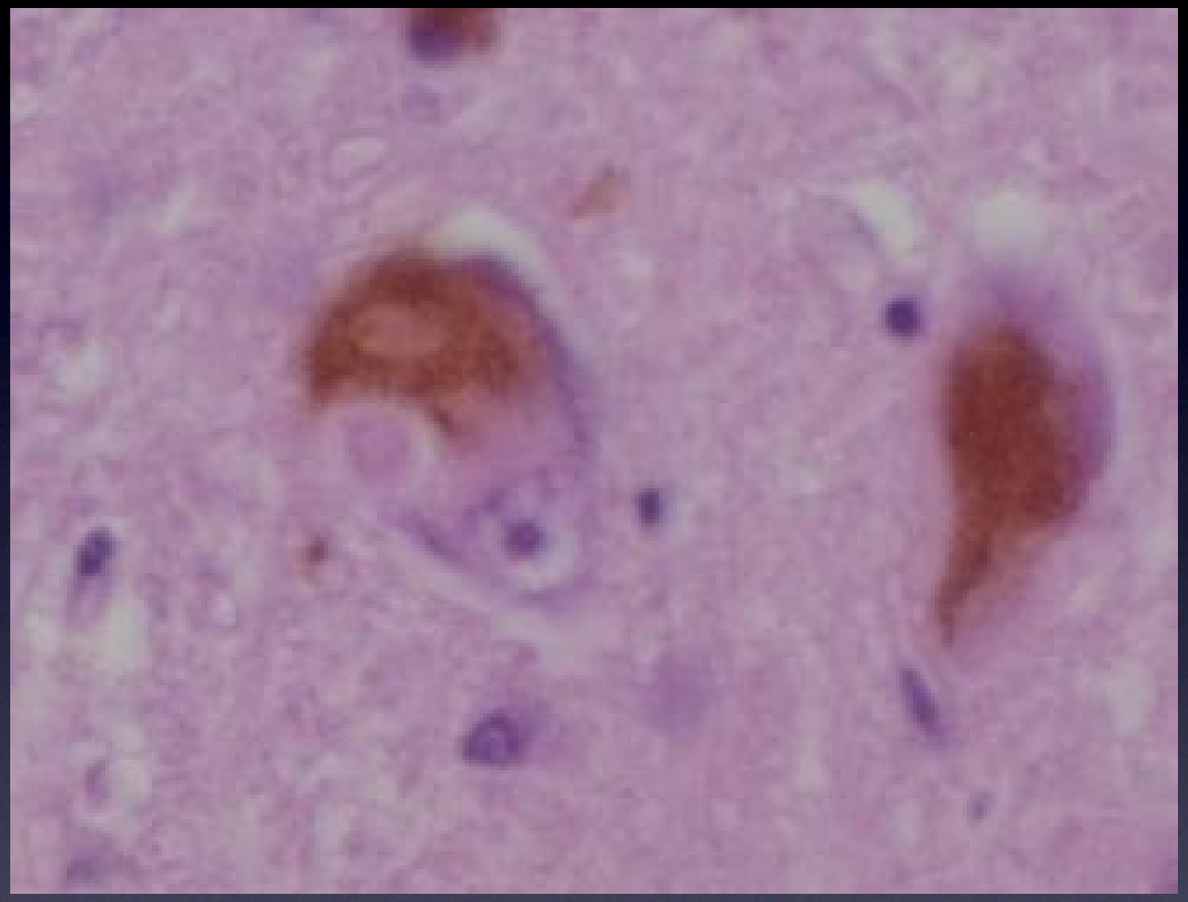

M
NC
P
N
R
C

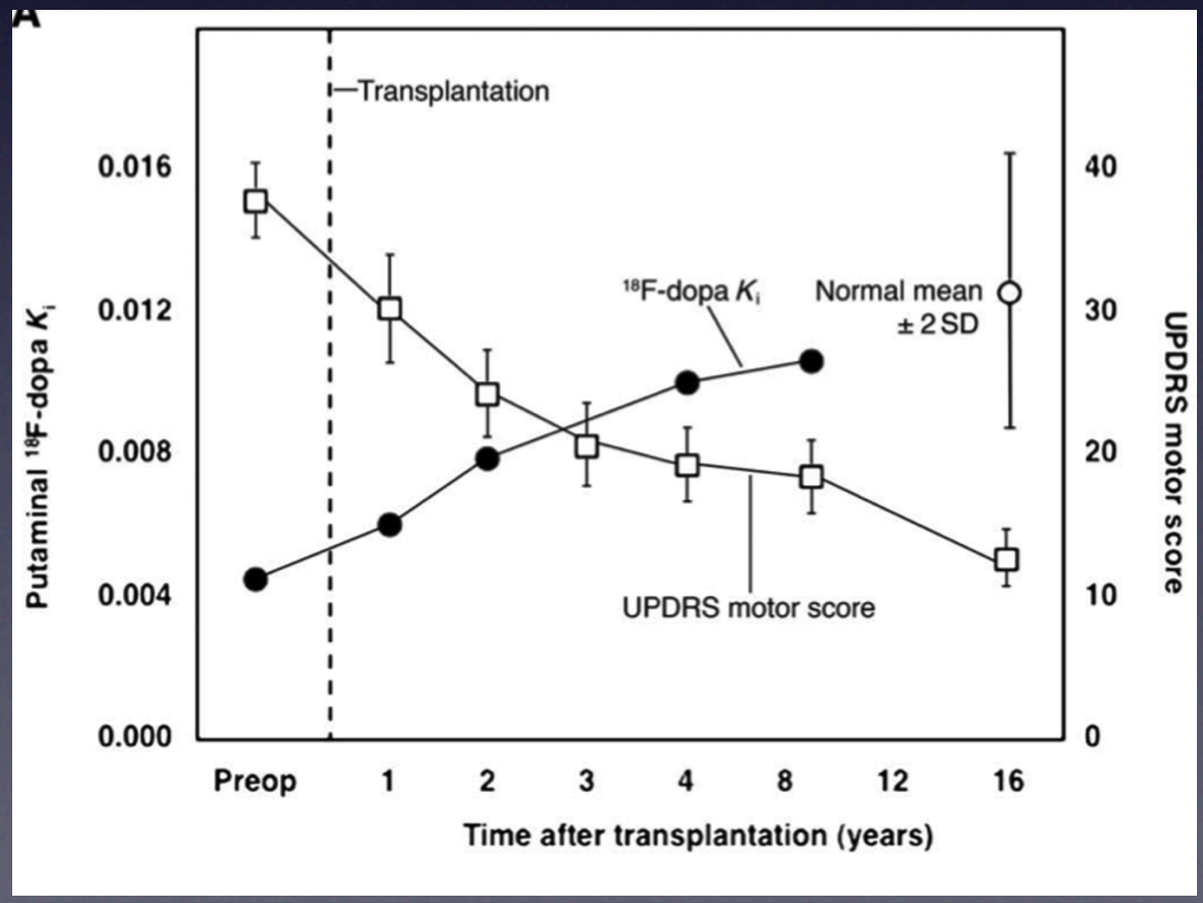
MCCULLOCH EA, TILL JE. PROLIFERATION OF HEMOPOIETIC COLONY-FORMING CELLS TRANSPLANTED INTO IRRADIATED MICE
Radiat Res. 1964 Jun;22:383-97.

McCulloch EA, Till JE. **Perspectives on the properties of stem cells.**
Nat Med. 2005 Oct;11(10):1026-8.



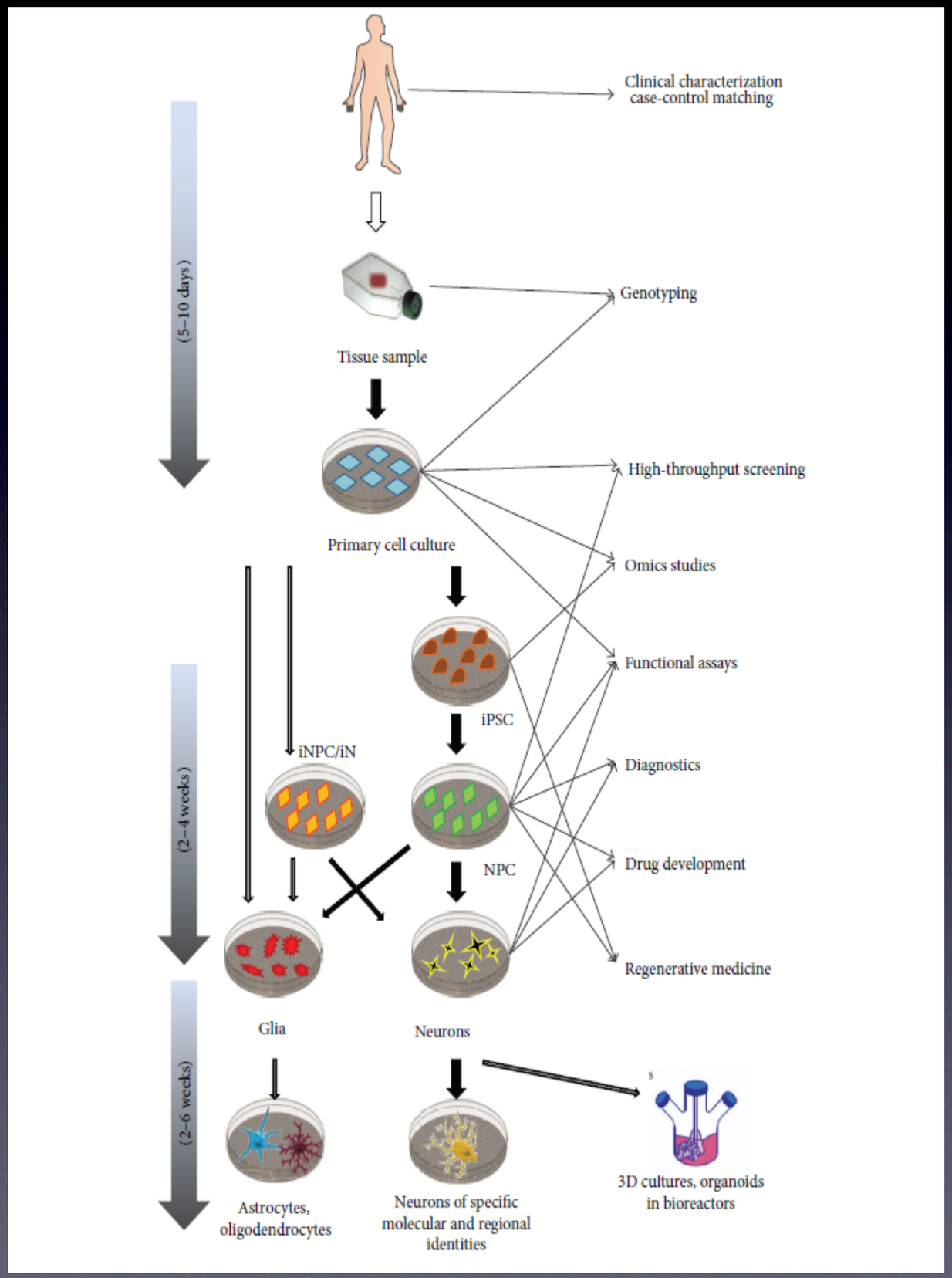
Björklund A, Lindvall O, Brundin P **Characterization of Lewy body pathology in 12- and 16-year-old intrastriatal mesencephalic grafts surviving in a patient with Parkinson's disease.** Mov Disord. 2010 Jun 15;25(8):1091-6.





What do you expect from the stem cells?

- Cell replacement
- Influence on cell survival
- Disease modeling



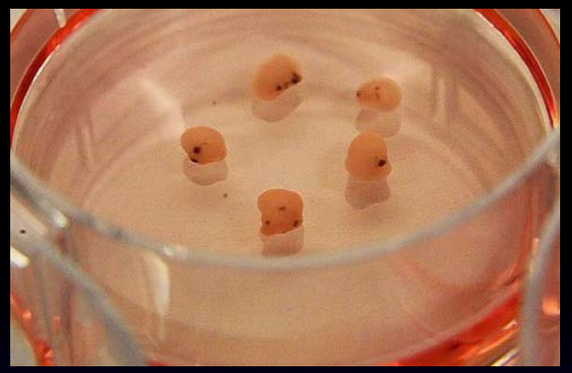
K. J. Brennand, A. Simone, J. Jou et al., “Modelling schizophrenia using human induced pluripotent stem cells,” *Nature*, vol. 473, no. 7346, pp. 221–225, 2011.

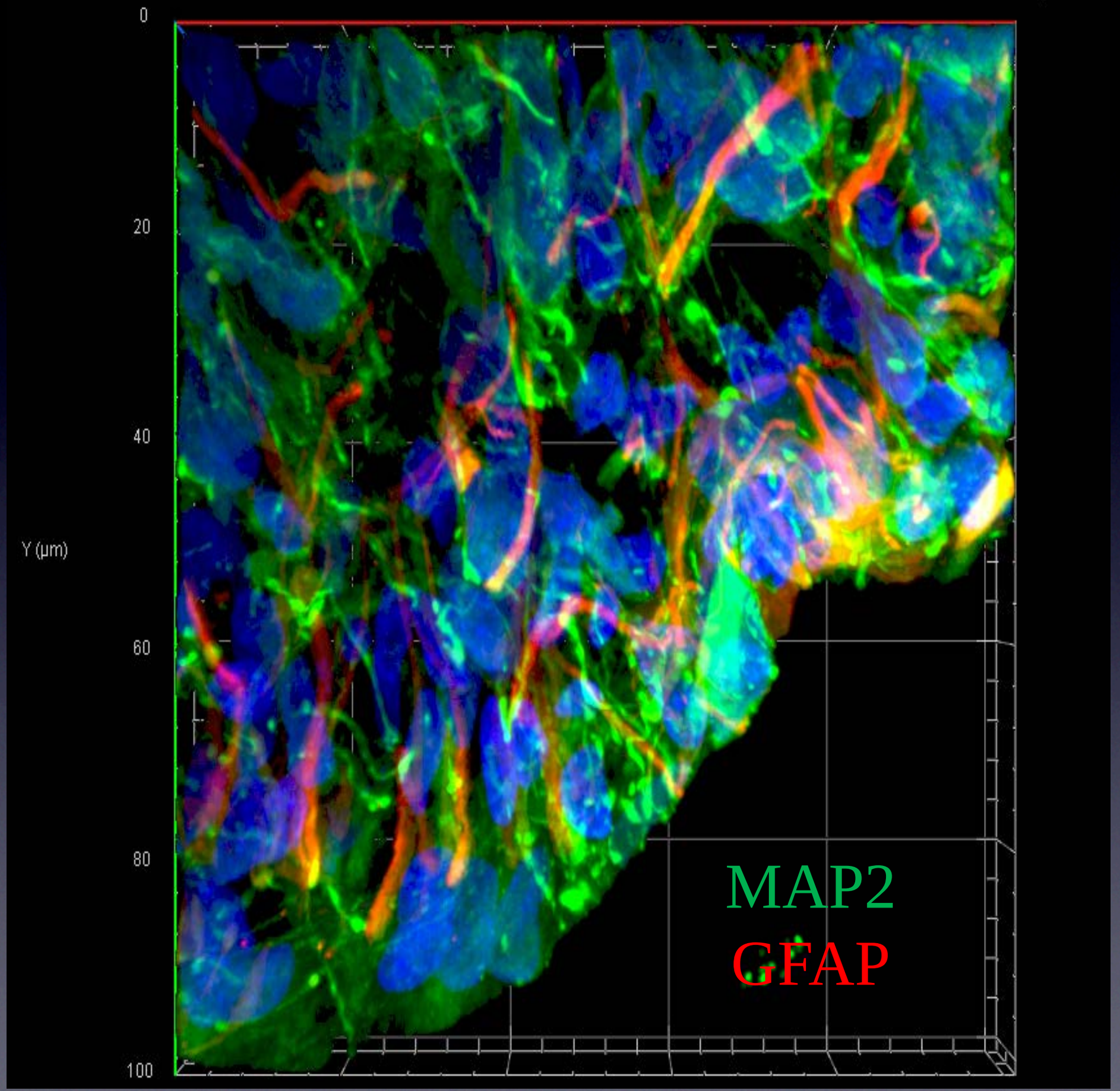
Altered WNT signaling rescued by antipsichotic treatment

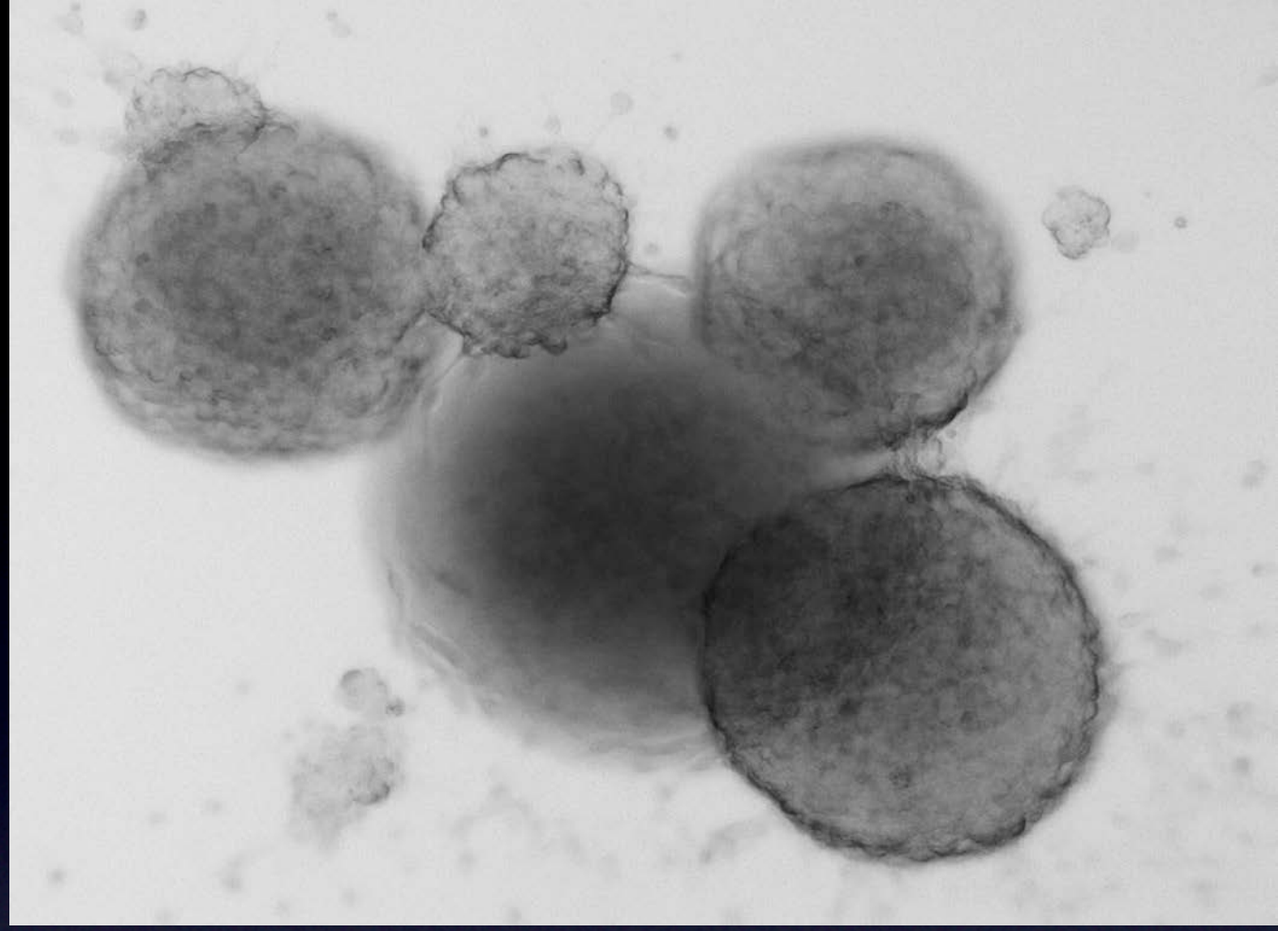
J. L. Wang, S. M. Shamah, A. X. Sun, I. D. Waldman, S. J. Haggarty, and R. H. Perlis, “Label-free, live optical imaging of reprogrammed bipolar disorder patient-derived cells reveals a functional correlate of lithium responsiveness,” Translational Psychiatry, vol. 4, article e428, 2014.

iNCs derived from lithium responders and lithium-nonresponders demonstrated different cell adhesion characteristics.

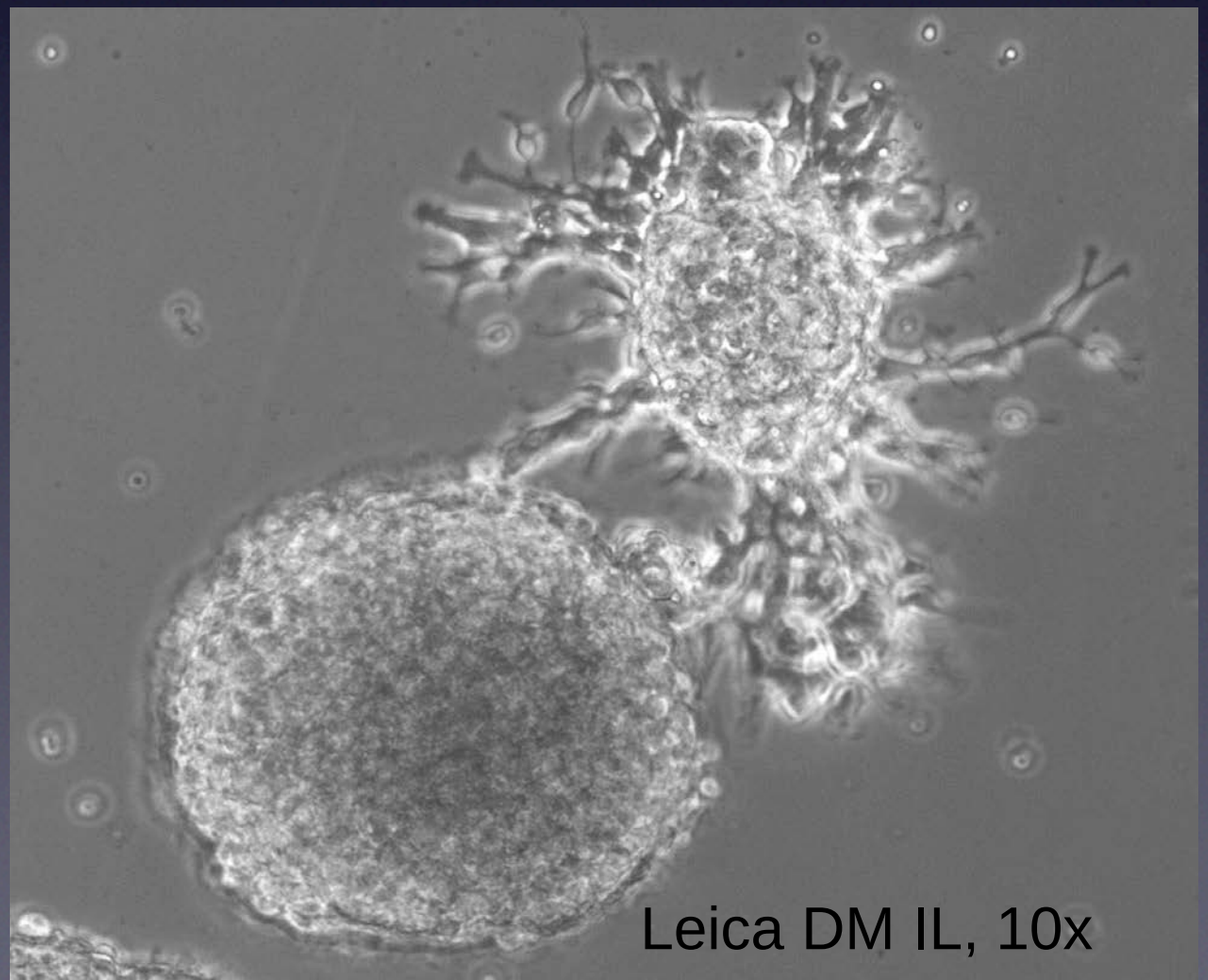
Organoids



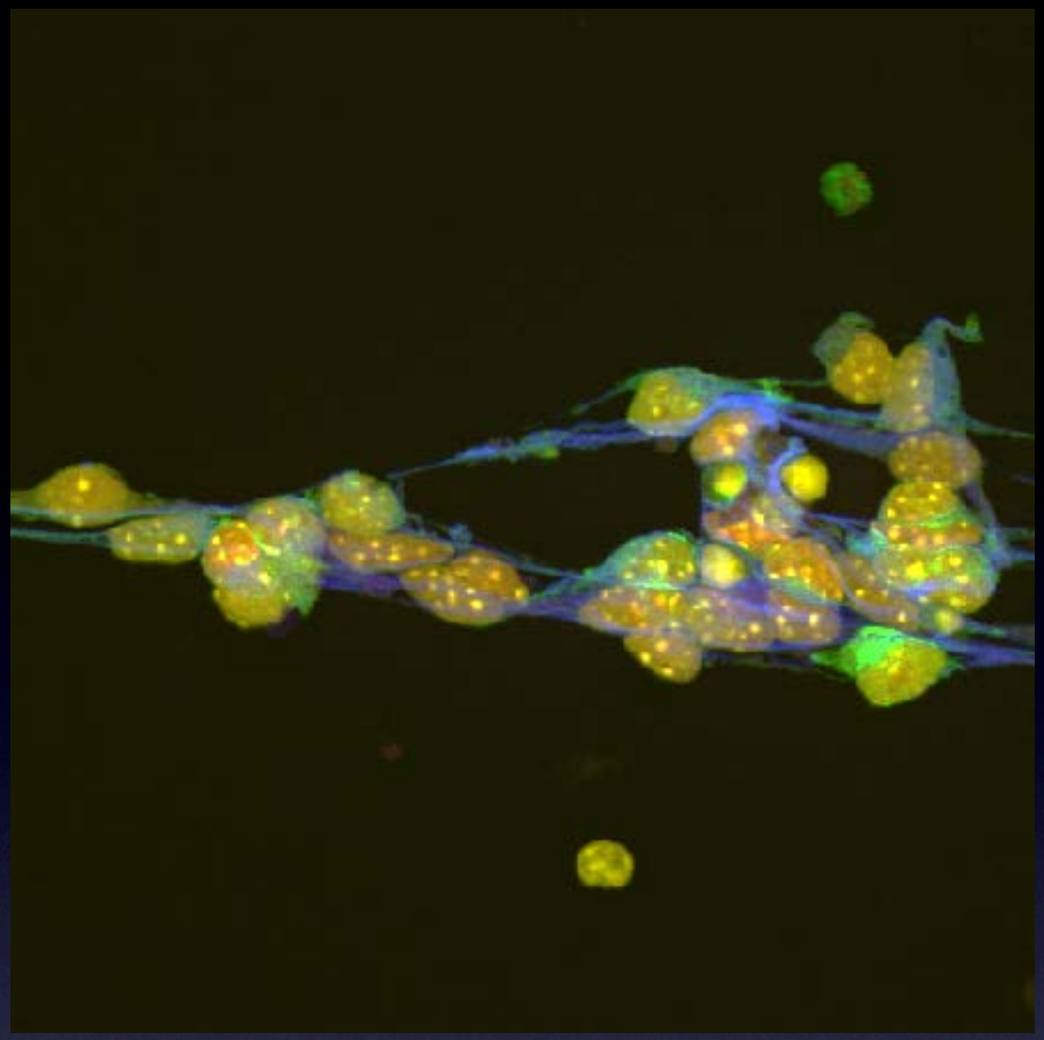


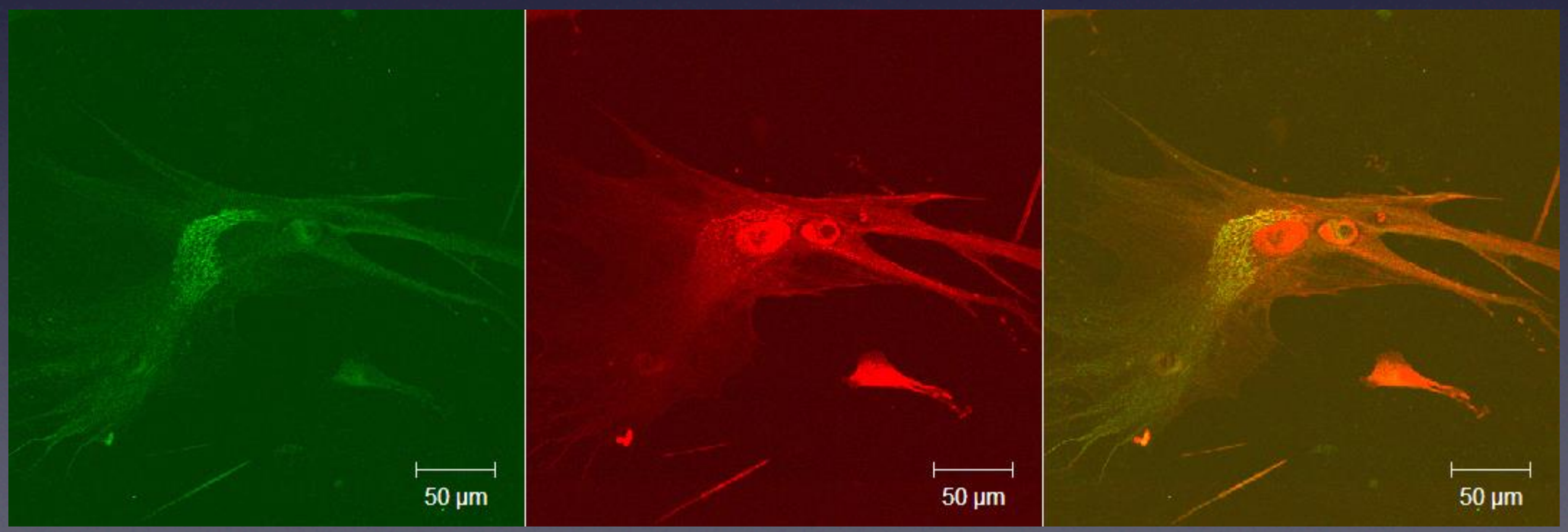


N2 (Invitrogen, 100x)
B27 (Invitrogen, 50x)
DMEM/F12 (Gibco, 31331-093)
EGF (Peprotech): 20 ng/ml
bFGF (Peprotech): 10ng/ml
Pen/Strep



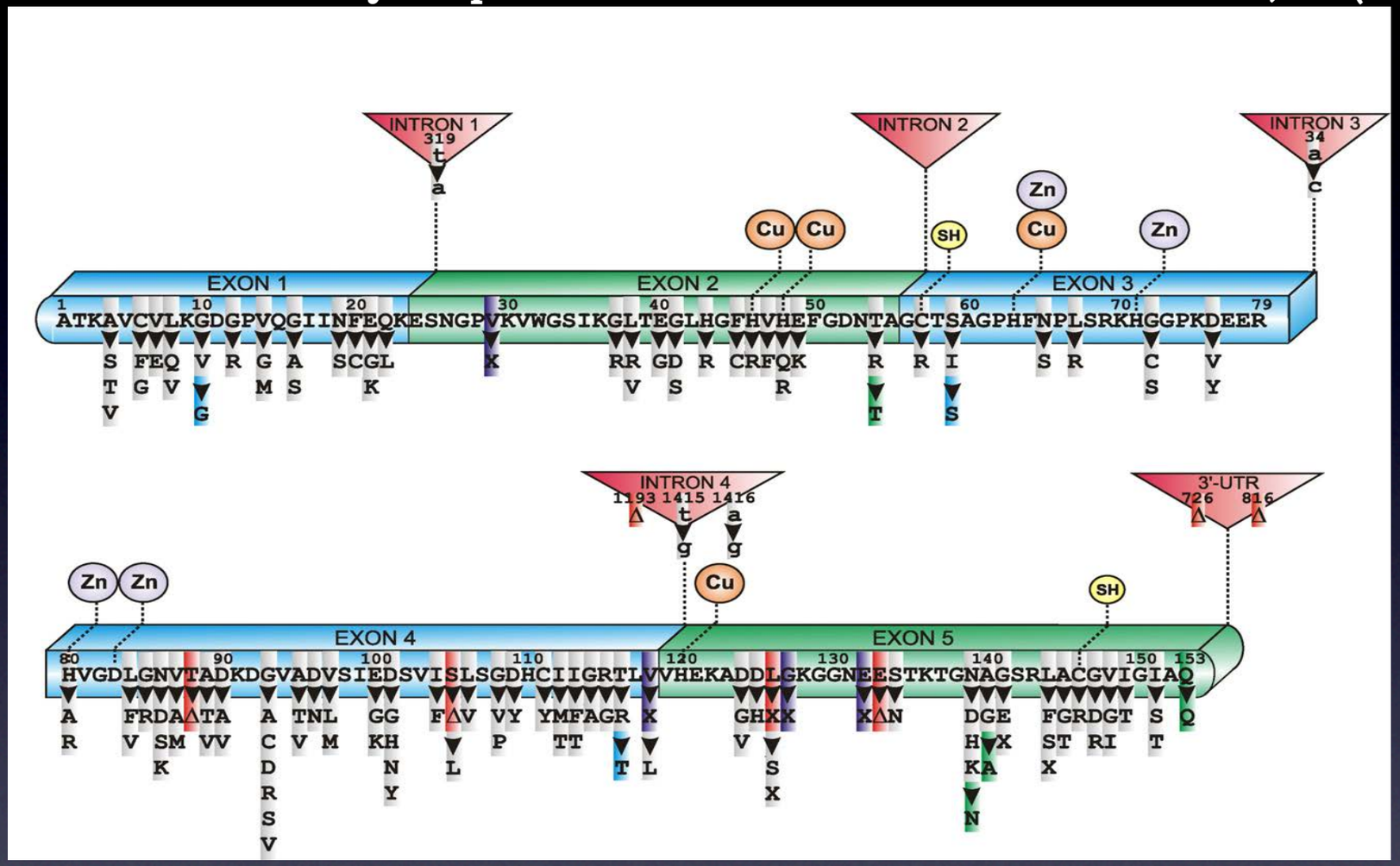
Leica DM IL, 10x

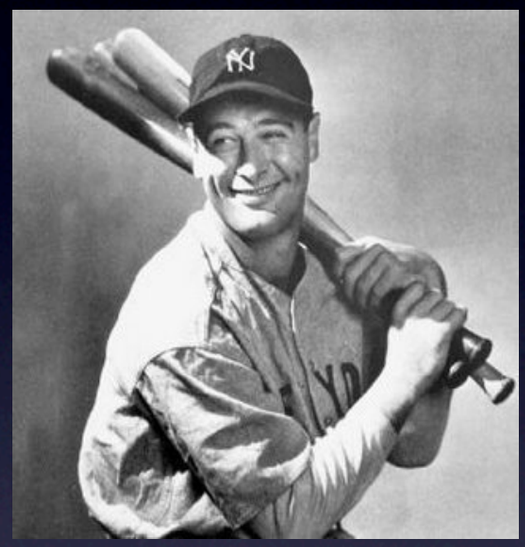



50 µm
50 µm
50 µm

SOD1 (Cu/Zn-SOD1)

Rosen DR et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature. 1993 Mar 4;362(6415):59-62





The SOD-catalysed dismutation of superoxide :

$$M(n+1)+ - SOD + O2- \rightarrow Mn+ - SOD + O2$$
$$Mn+ - SOD + O2- + 2H+ \rightarrow M(n+1)+ - SOD + H2O2.$$

BRAIN RESEARCH 1301 (2009) 152–162

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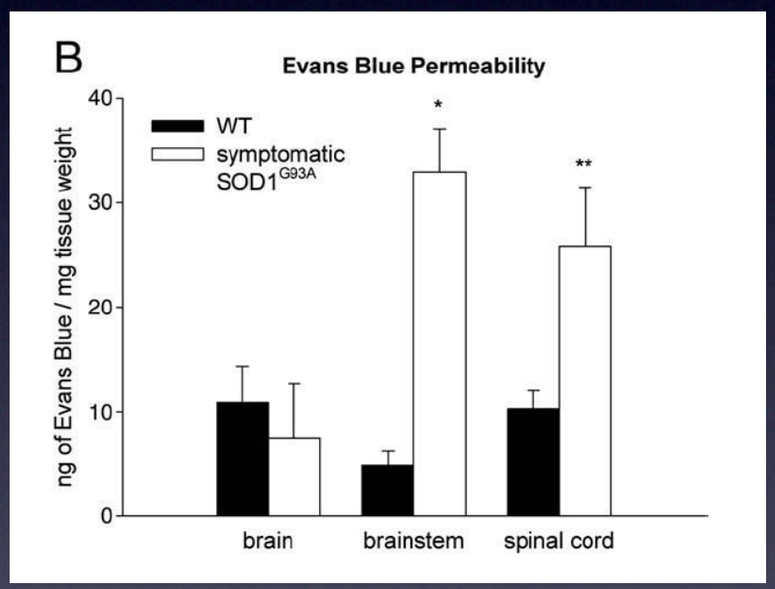
BRAIN RESEARCH

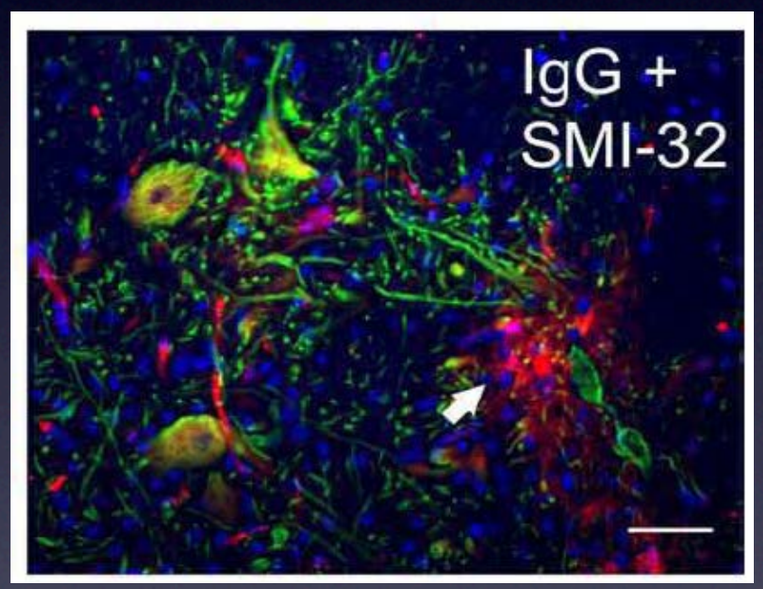
Research Report

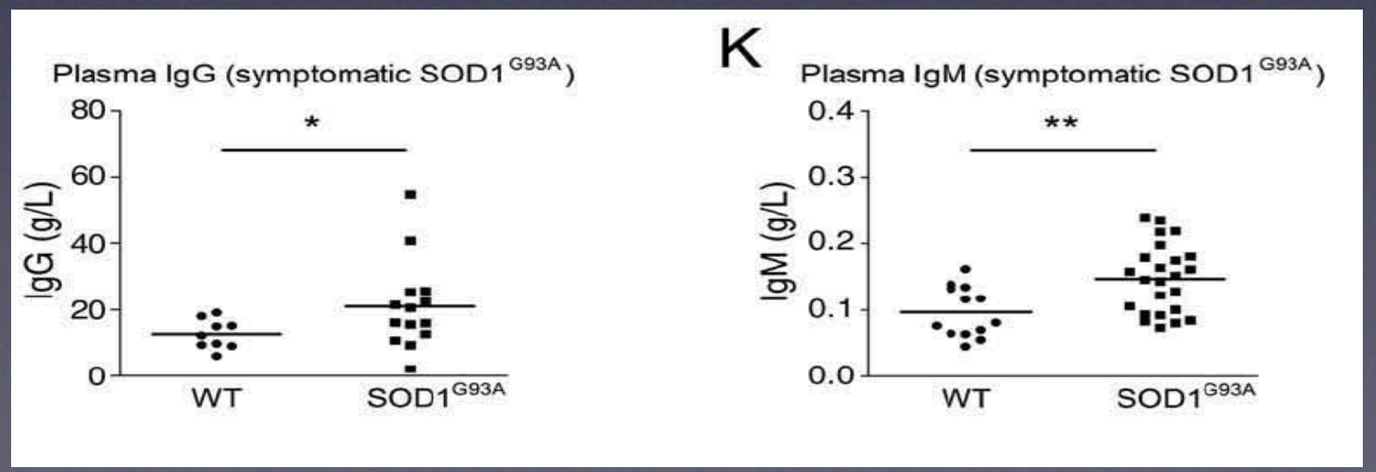
Impaired blood–brain and blood–spinal cord barriers in mutant SOD1-linked ALS rat

Charles Nicaise[a], *Dinko Mitrecic*[a], *Pieter Demetter*[b], *Robert De Decker*[a], *Michèle Authelet*[a], *Alain Boom*[a], *Roland Pochet*[a,*]

[a]*Laboratory of Histology, Neuroanatomy and Neuropathology, Université Libre de Bruxelles, Brussels, Belgium*
[b]*Department of Pathology, Erasme Hospital, Brussels, Belgium*



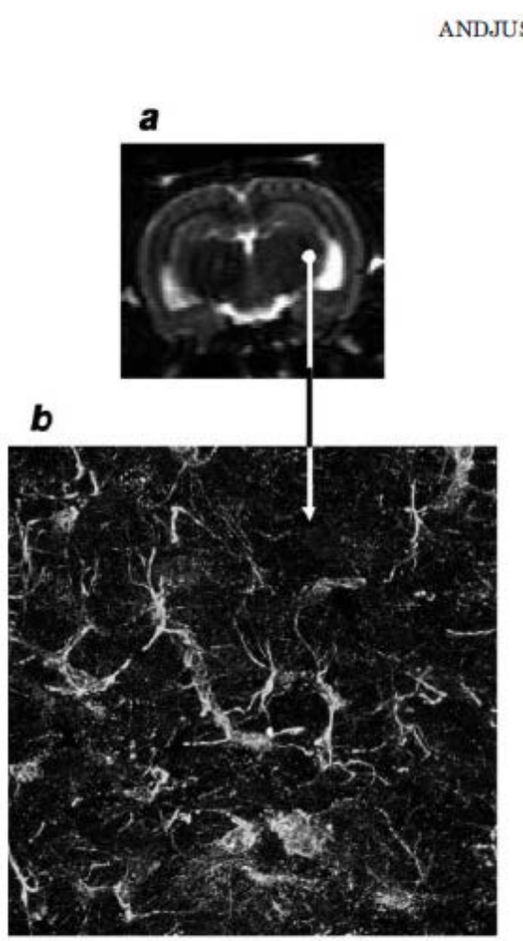


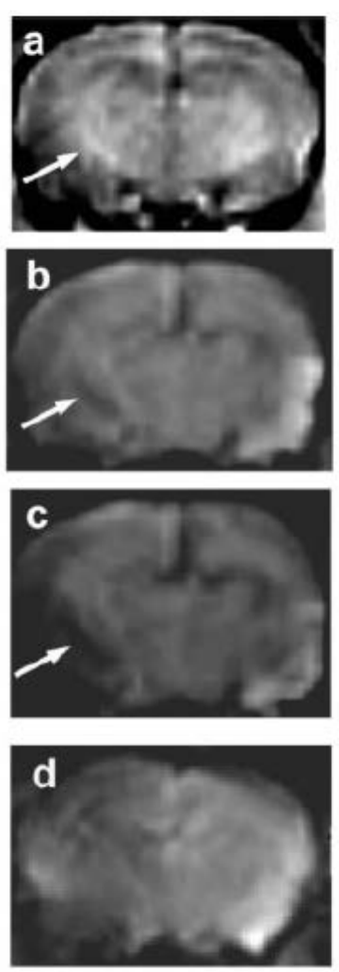


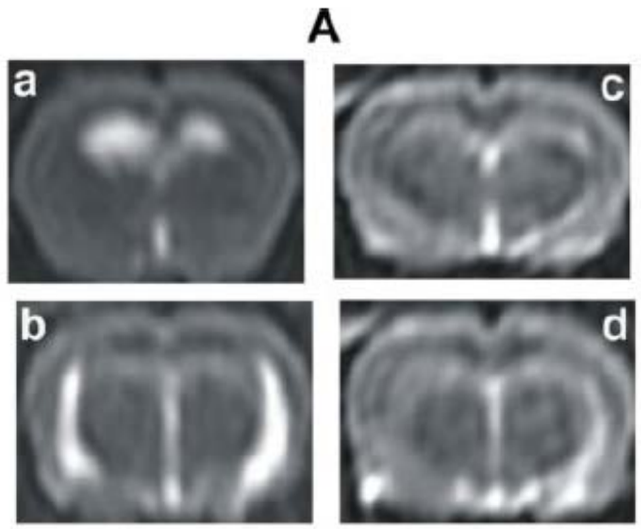
THE ANATOMICAL RECORD 292:1882–1892 (2009)

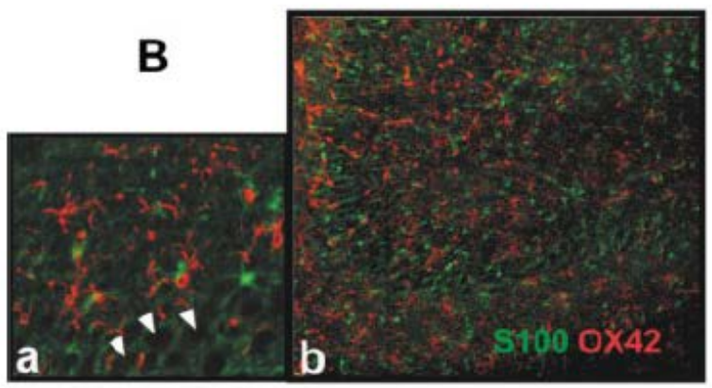
In Vivo Morphological Changes in Animal Models of Amyotrophic Lateral Sclerosis and Alzheimer's-Like Disease: MRI Approach

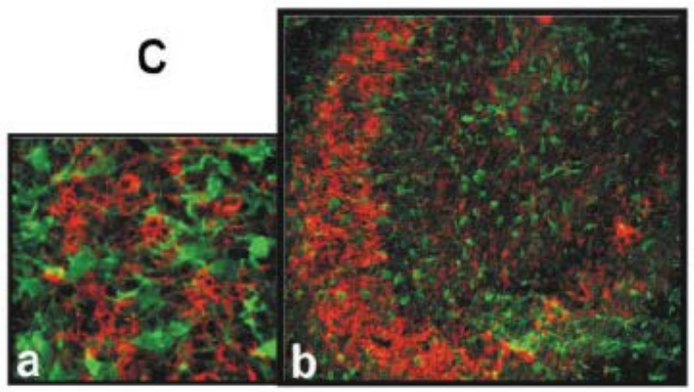
PAVLE R. ANDJUS,[1*] DANIJELA BATAVELJIĆ,[1] GREETJE VANHOUTTE,[2] DINKO MITRECIC,[3] FABRIZIO PIZZOLANTE,[4] NEVENA DJOGO,[1] CHARLES NICAISE,[3] FABRICE GANKAM KENGNE,[3] CARLO GANGITANO,[4] FABRIZIO MICHETTI,[4] ANNEMIE VAN DER LINDEN,[2] ROLAND POCHET,[3] AND GORAN BAČIĆ[5]







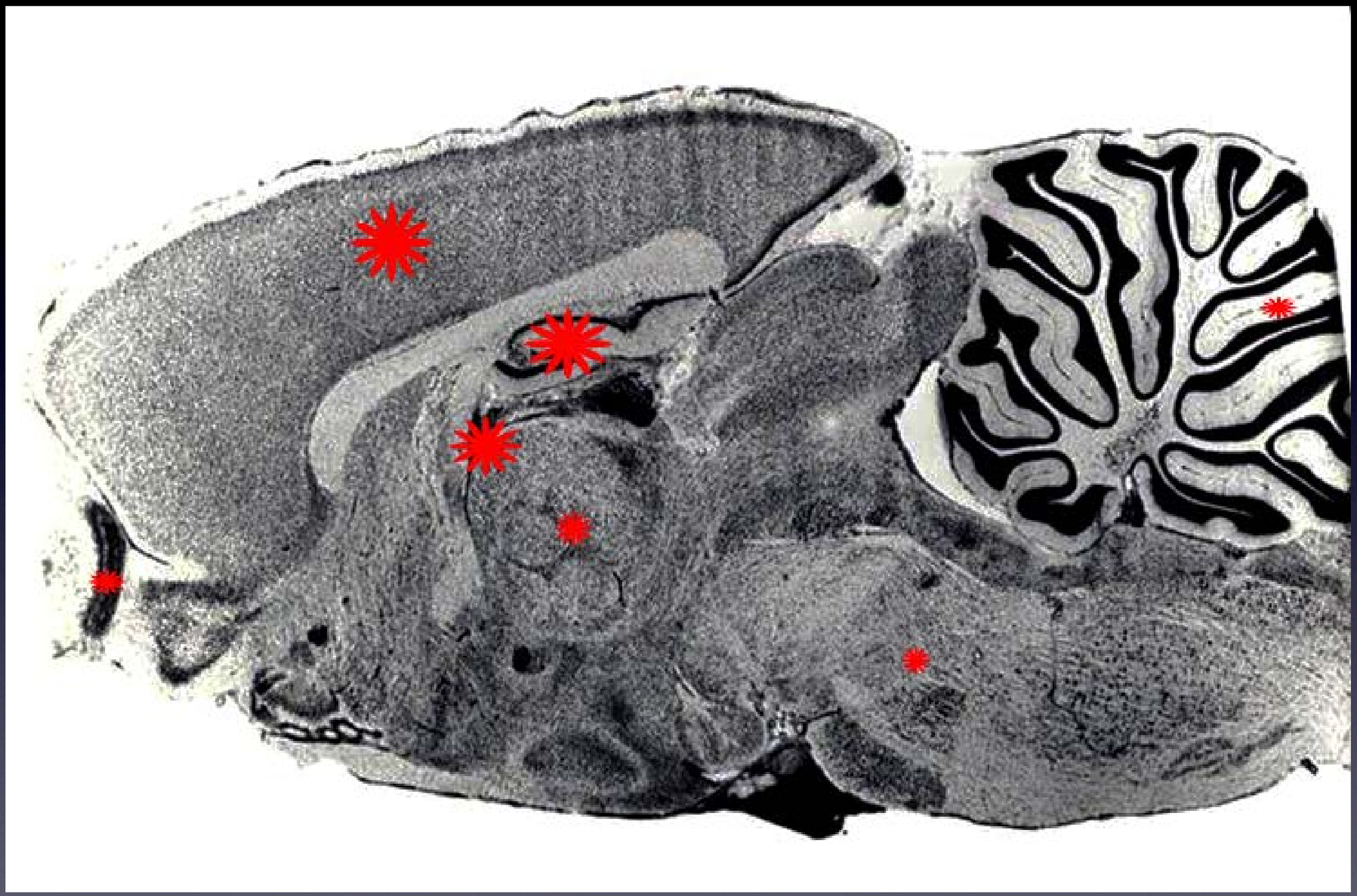




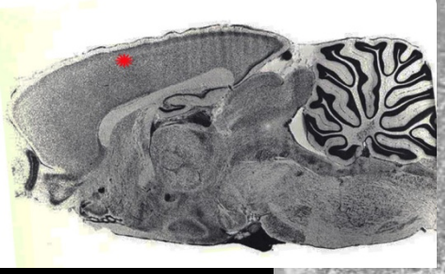
Cell tracing principles:

1. Endogeneous marking

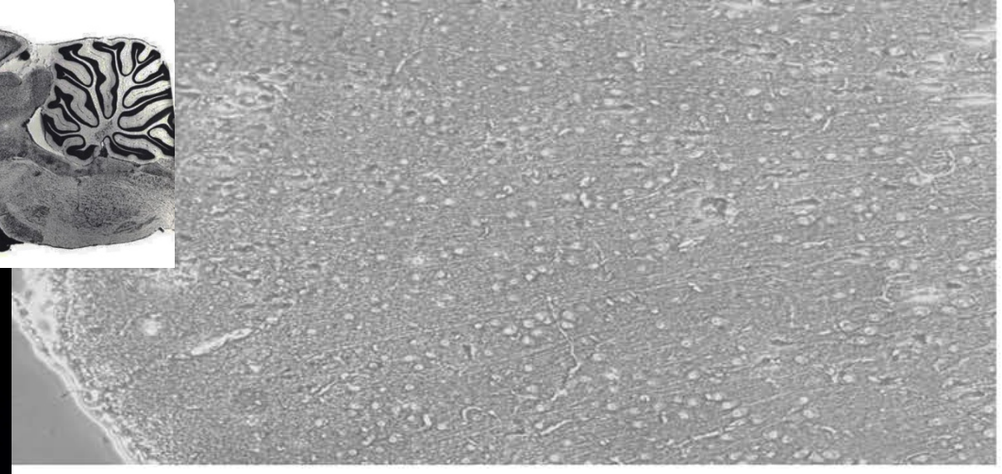
2. Exogeneous marking

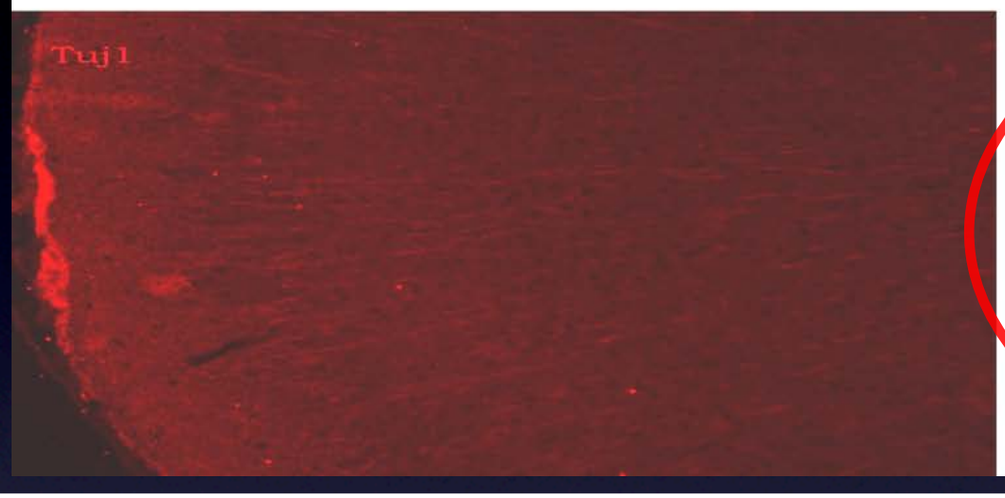


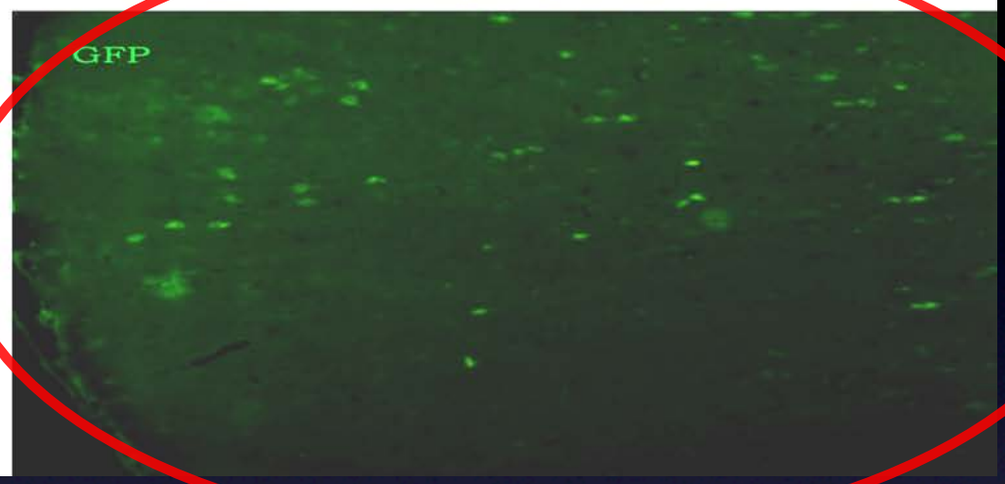
Mitrecic et al, Cell Transp, 2010



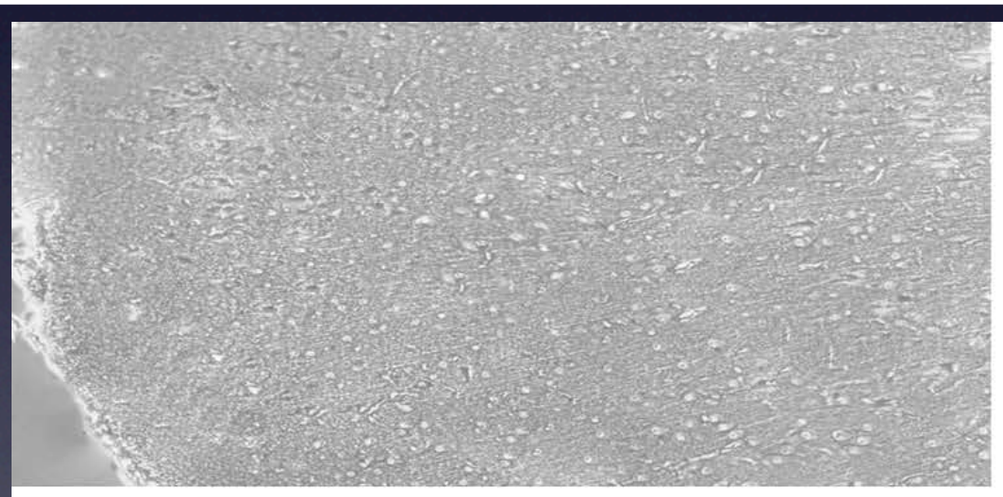
SOD1

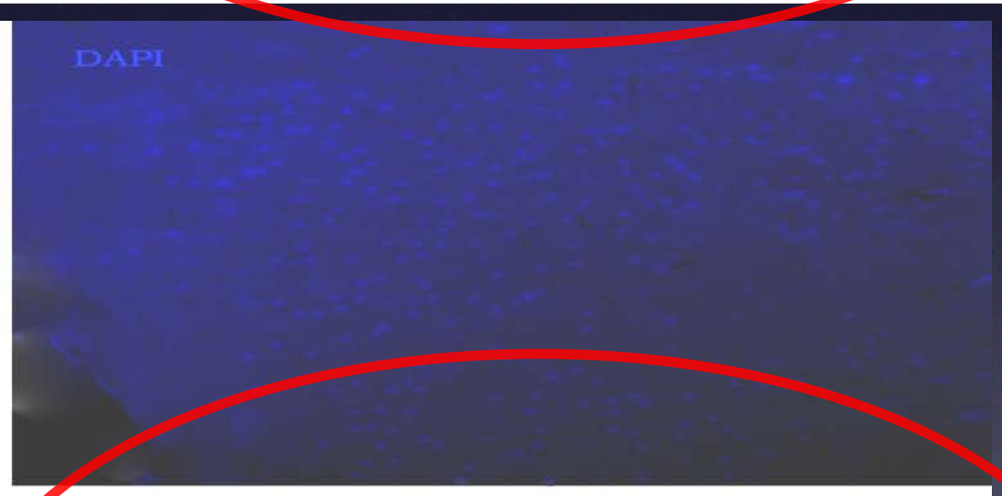


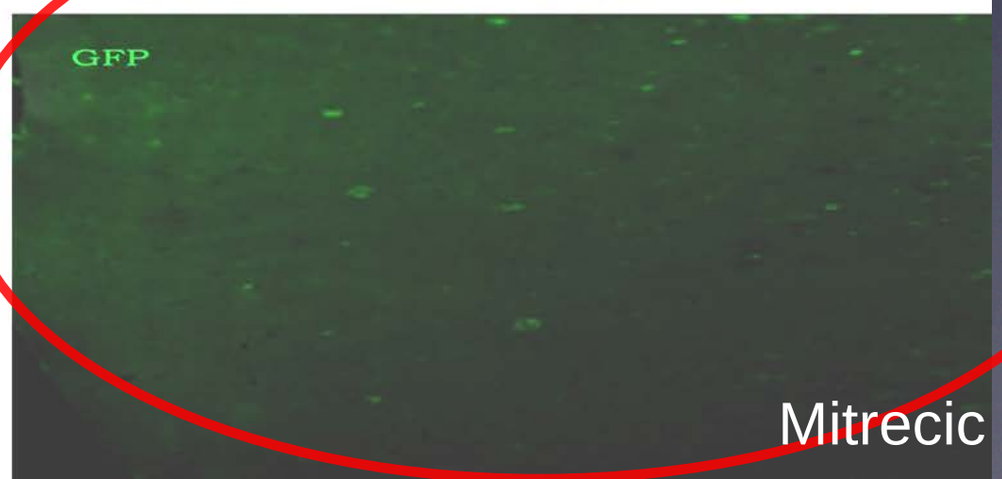




Wt

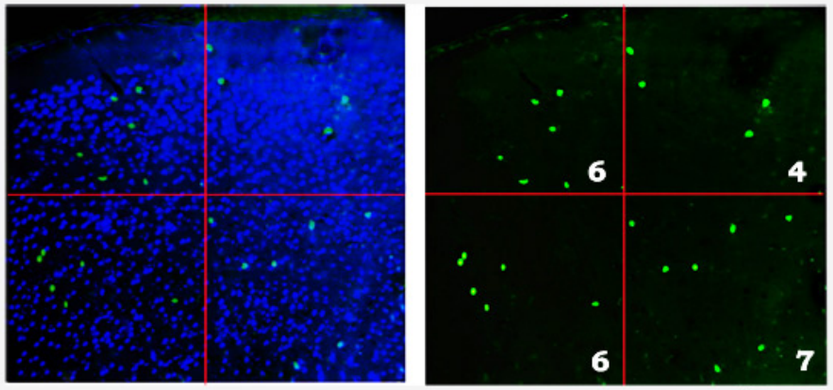






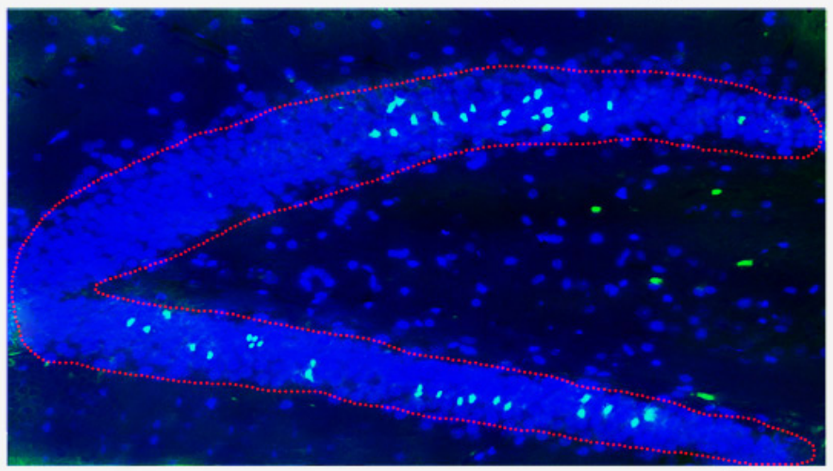
Mitrecic et al, 2010

GFP marked NSC in the brain cortex



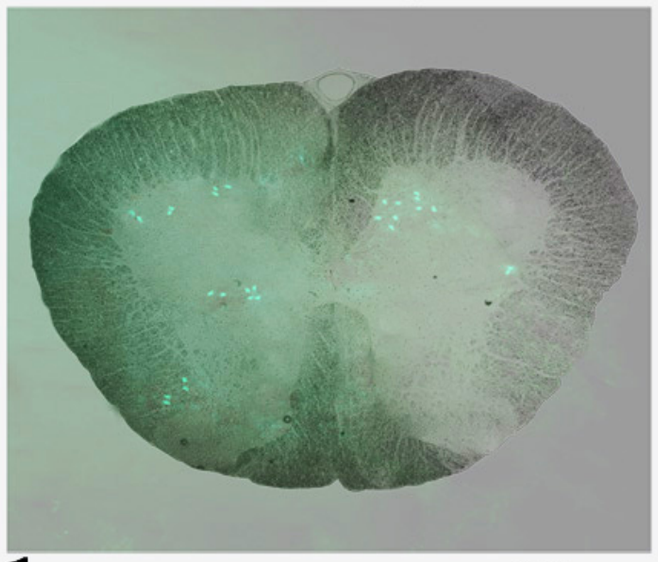
area: 1.00 mm^2,
total number of GFP cells: 23,
cells/mm : 23

GFP marked NSC in the dental gyrus of hippocampus



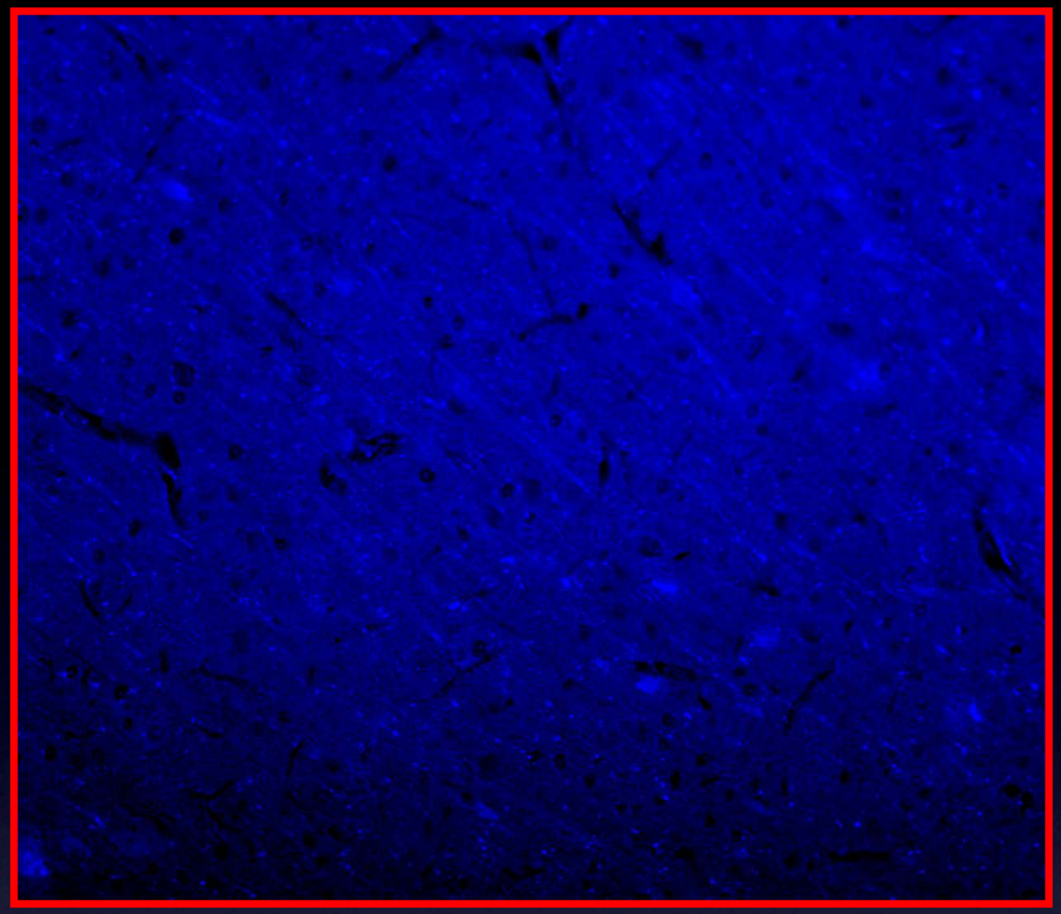
area: 0.22 mm^2,
total number of GFP cells: 42,
cells/mm^2: 189

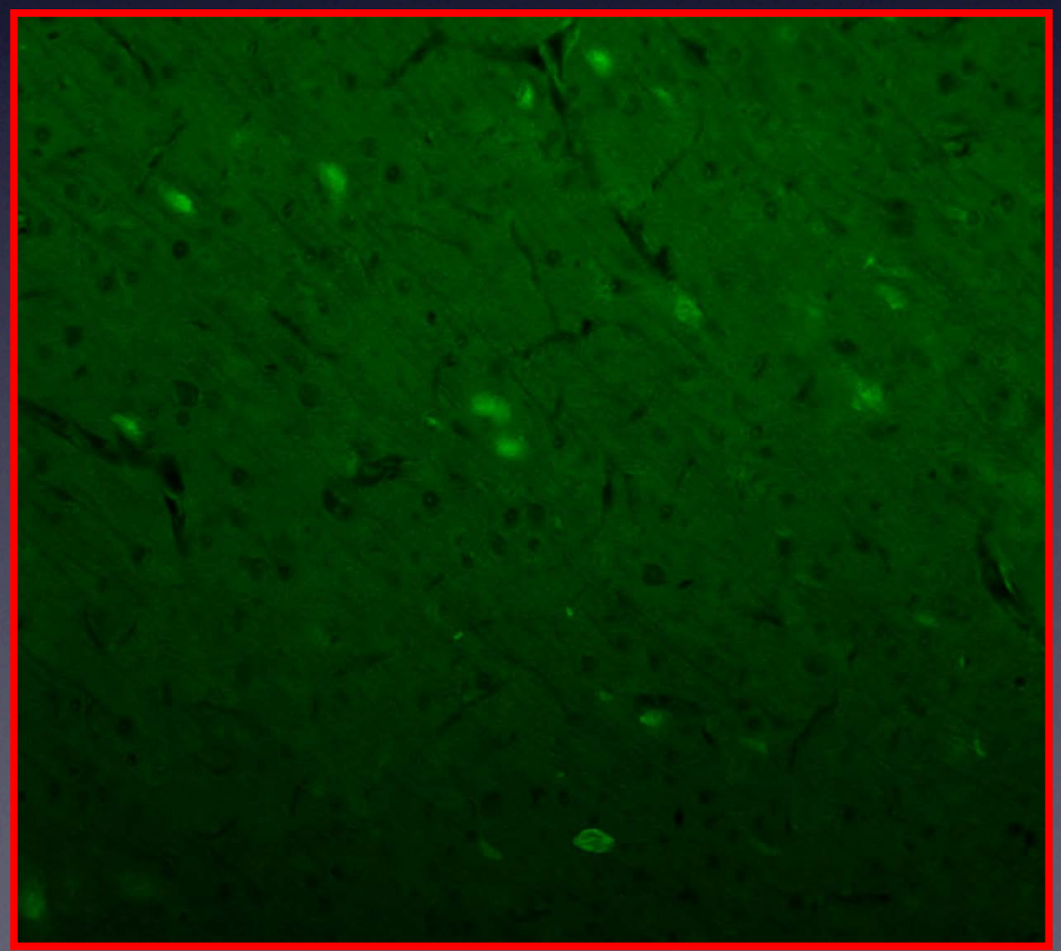
GFP marked NSC in the spinal cord

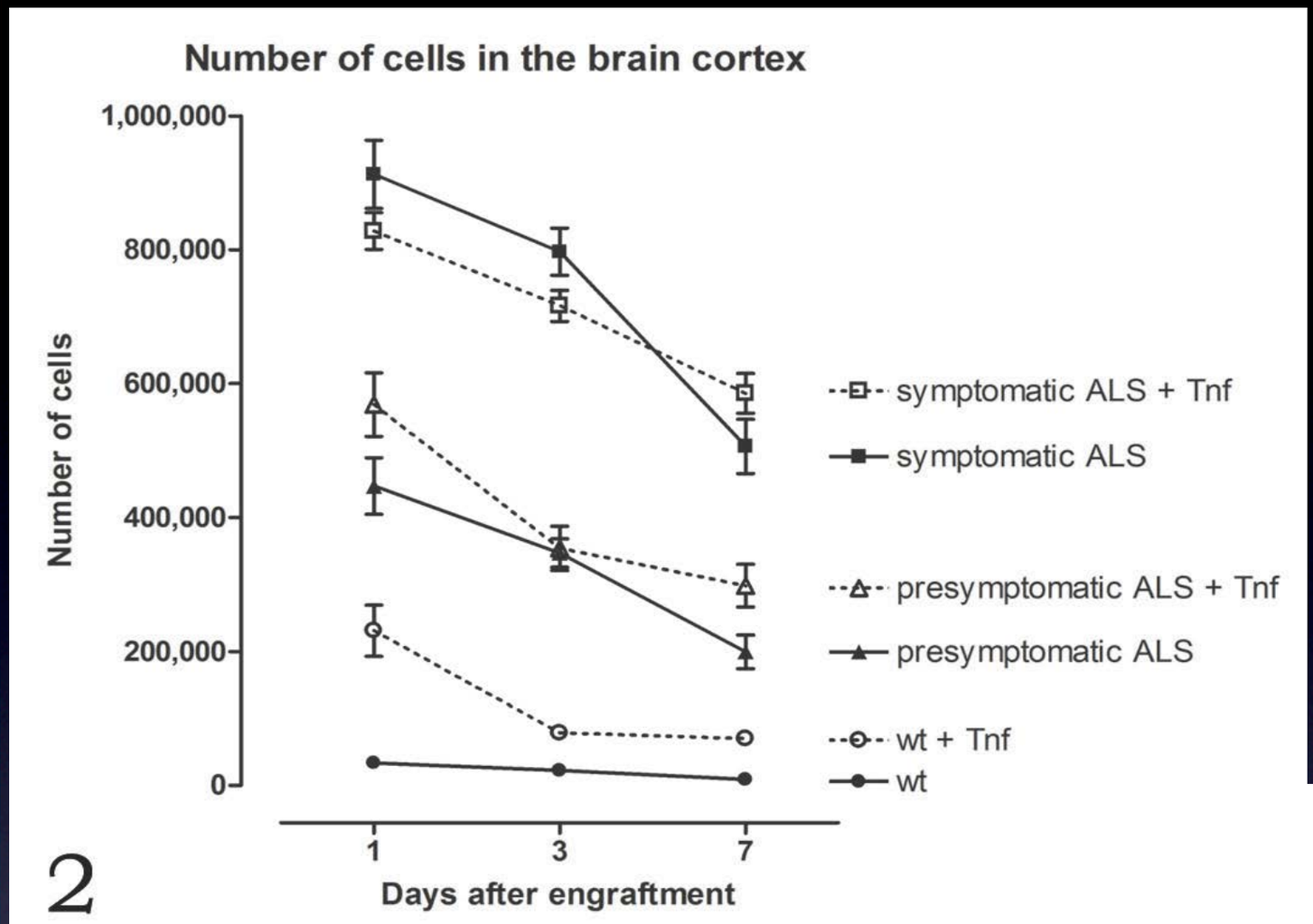


area: 4.87 mm^2,
total number of GFP cells: 28,
cells/mm^2: 6

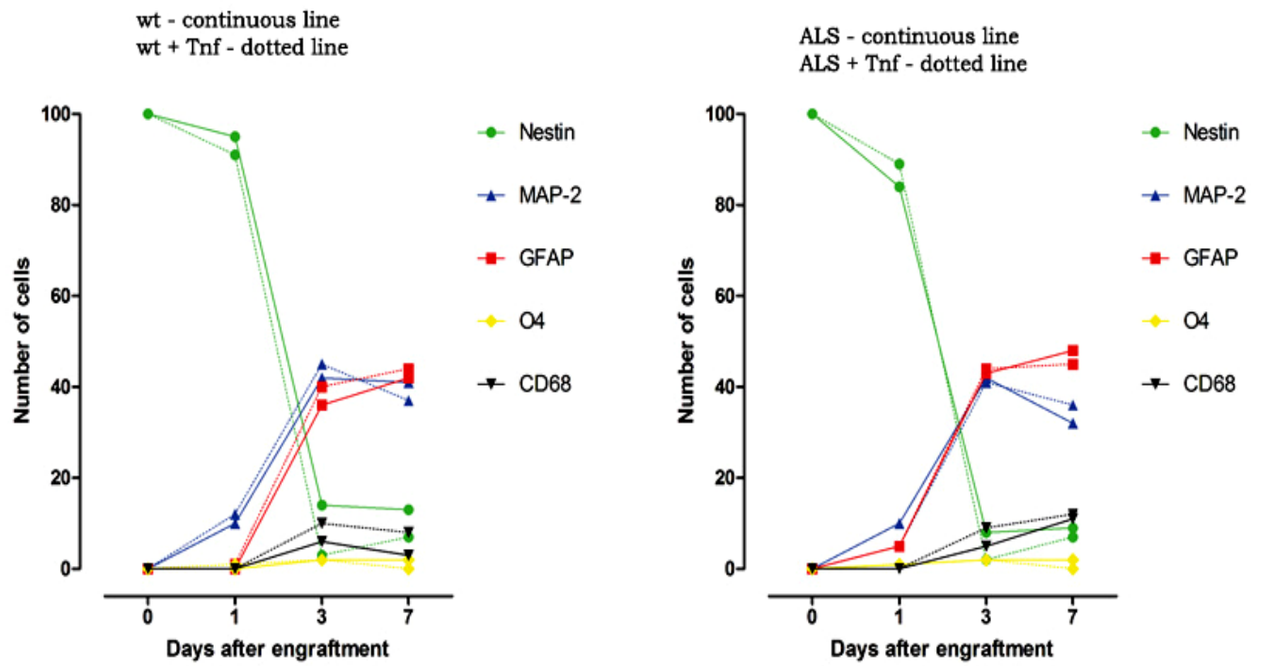
1



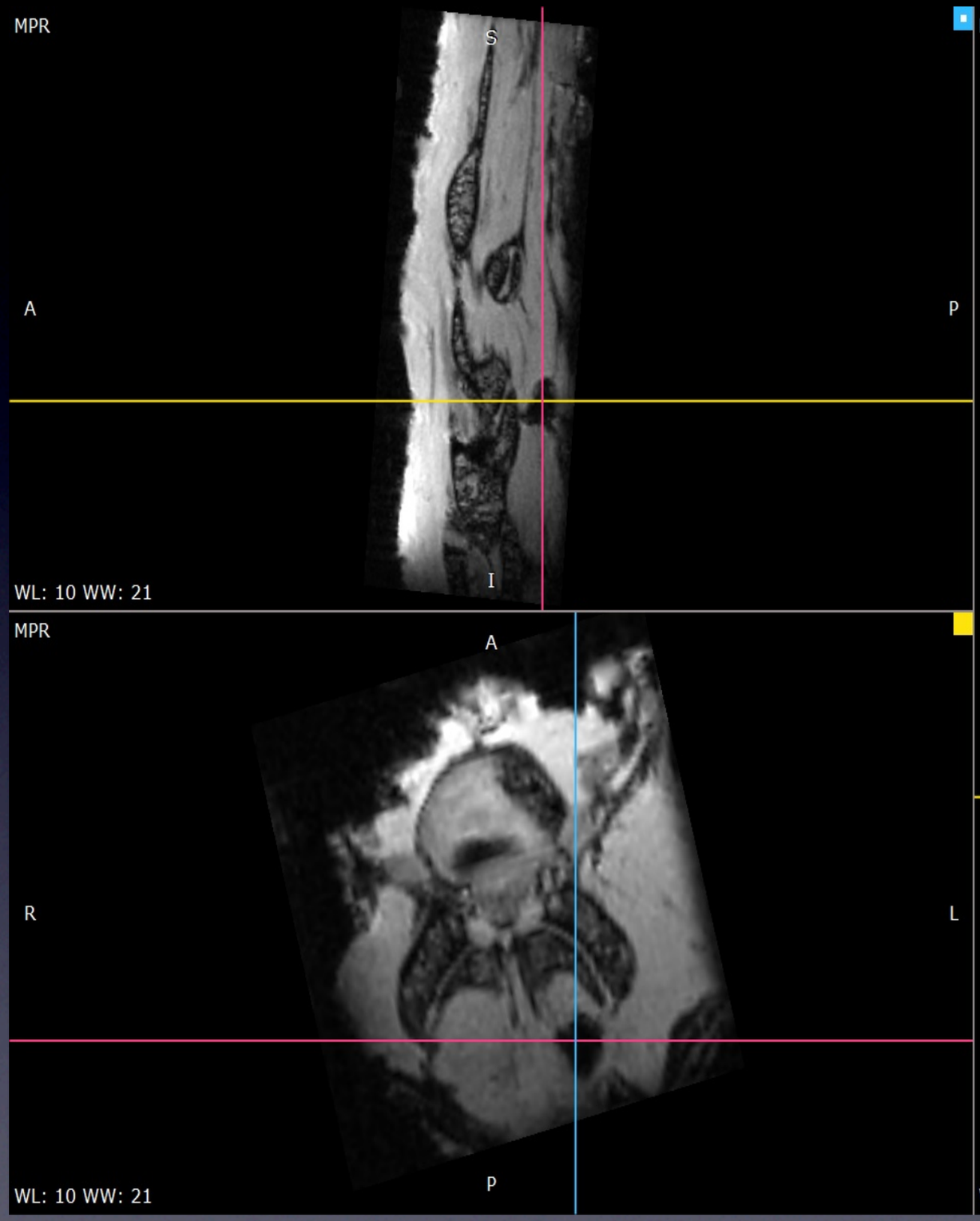



Number of cells in the brain cortex
1,000,000
800,000
600,000
400,000
200,000
0
Number of cells
1
3
7
Days after engraftment
symptomatic ALS + Tnf
symptomatic ALS
presymptomatic ALS + Tnf
presymptomatic ALS
wt + Tnf
wt

2

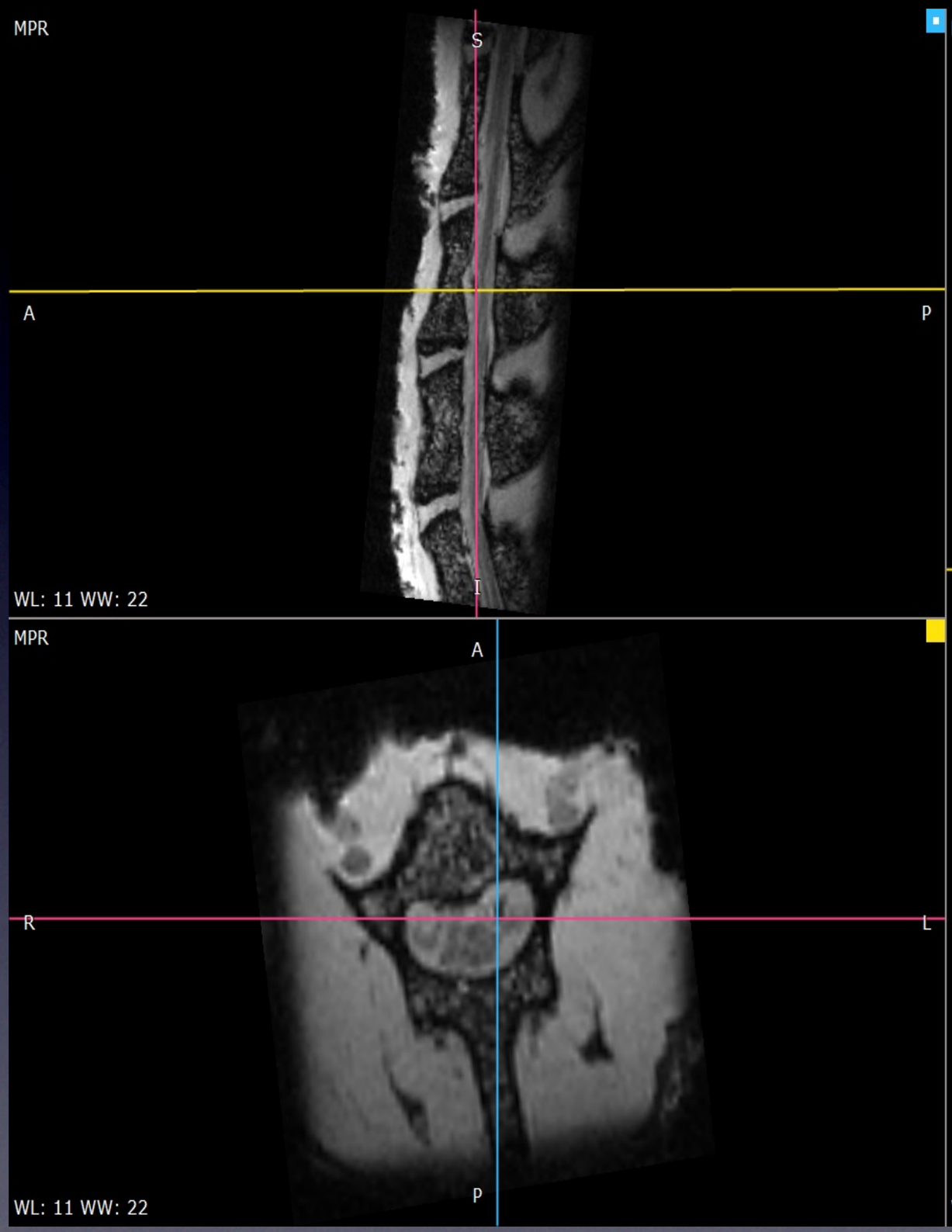

wt - continuous line
wt + Tnf - dotted line
ALS - continuous line
ALS + Tnf - dotted line
100
80
60
40
20
0
Number of cells
0
1
3
7
Days after engraftment
Nestin
MAP-2
GFAP
O4
CD68

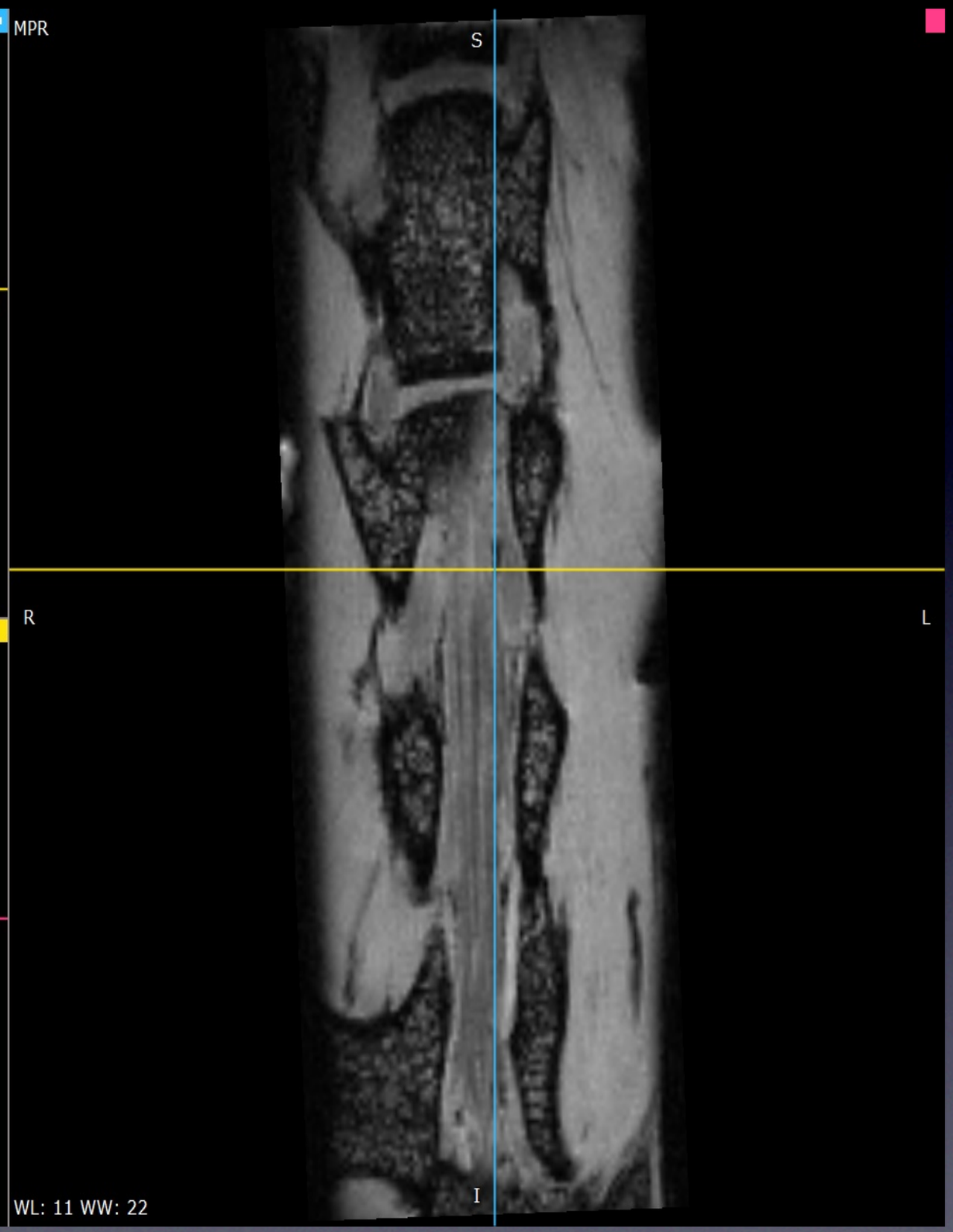
4


MPR
S
A
P
I
WL: 10 WW: 21

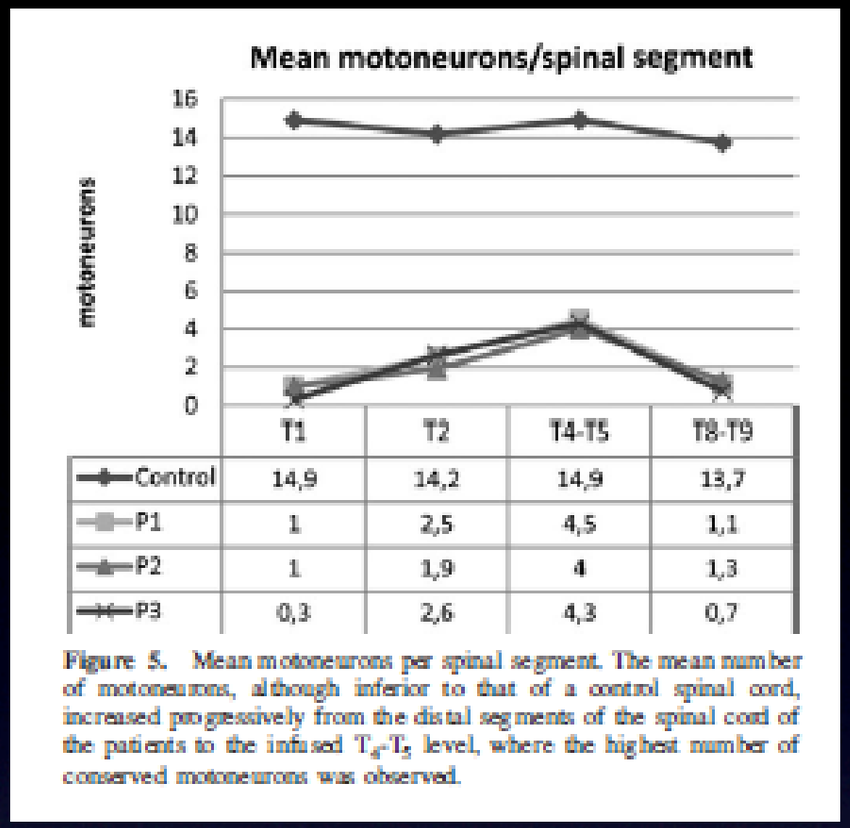

MPR
S
R
L
I
WL: 10 WW: 21

MPR
A
R
L
P
WL: 10 WW: 21


MPR
S
A
P
I
WL: 11 WW: 22
MPR
A
R
L
P
WL: 11 WW: 22


MPR
S
R
L
WL: 11 WW: 22
I

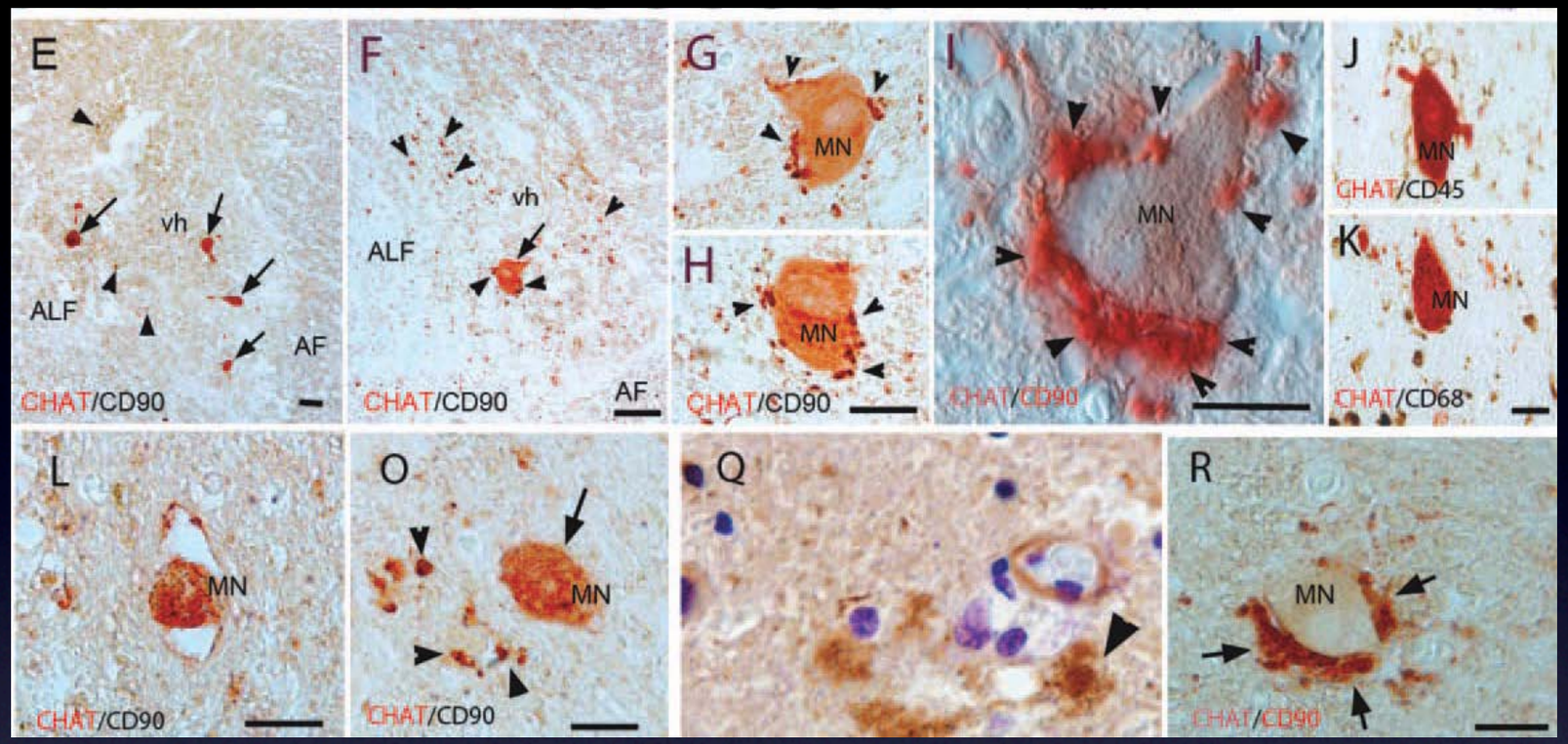
NSC vs MSC

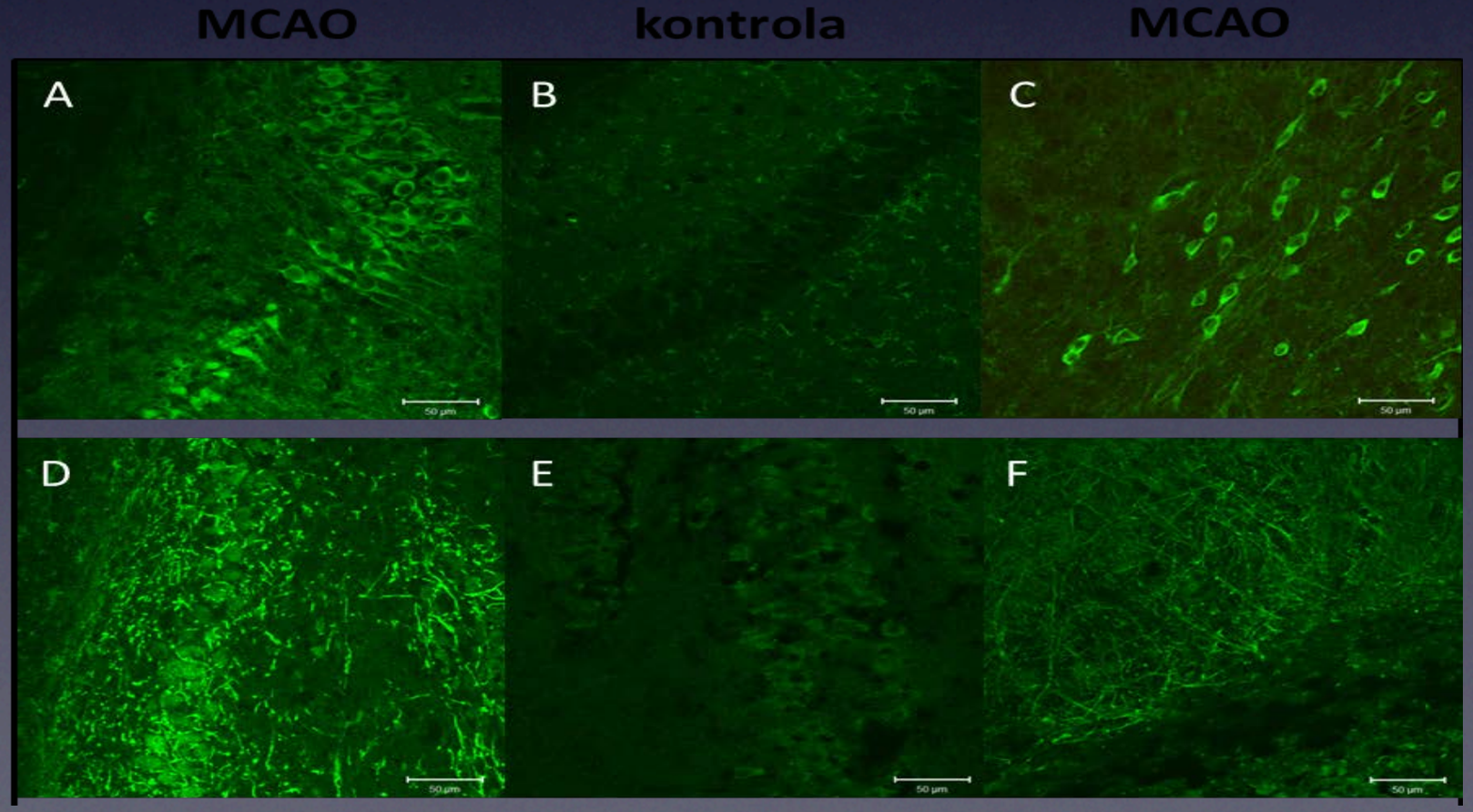


	T1	T2	T4-T5	T8-T9
Control	14,9	14,2	14,9	13,7
P1	1	2,5	4,5	1,1
P2	1	1,9	4	1,3
P3	0,3	2,6	4,3	0,7

Figure 5. Mean motoneurons per spinal segment. The mean number of motoneurons, although inferior to that of a control spinal cord, increased progressively from the distal segments of the spinal cord of the patients to the infused T_4-T_5 level, where the highest number of conserved motoneurons was observed.

Percentage of „protected motoneurons” in treated segments: 65±9%





BRAIN RESEARCH 1597 (2015) 65–76

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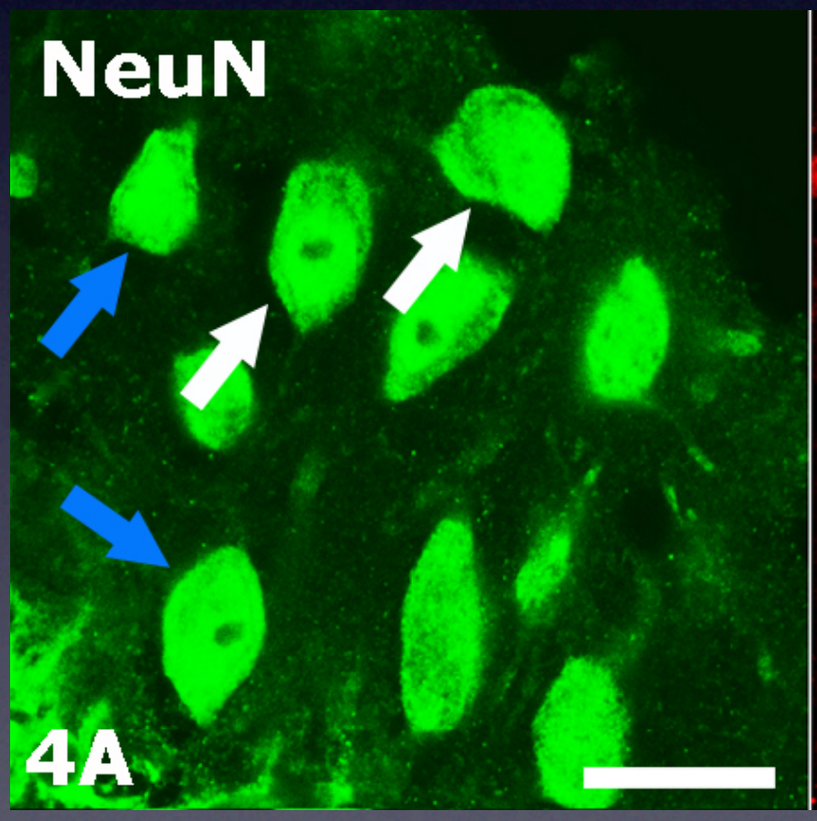
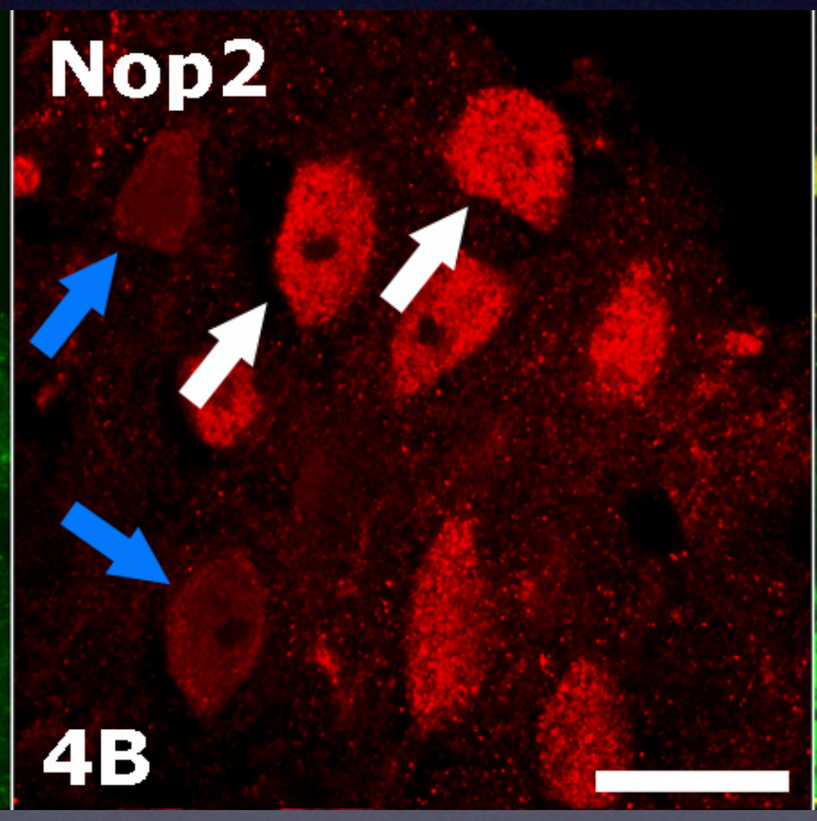
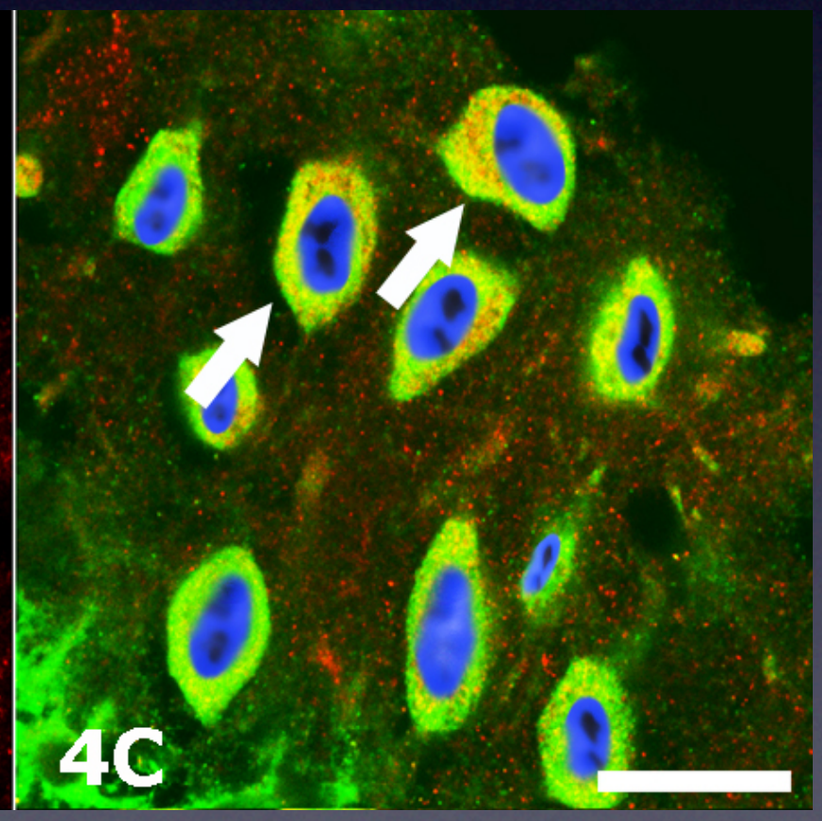
Research Report

Nop2 is expressed during proliferation of neural stem cells and in adult mouse and human brain

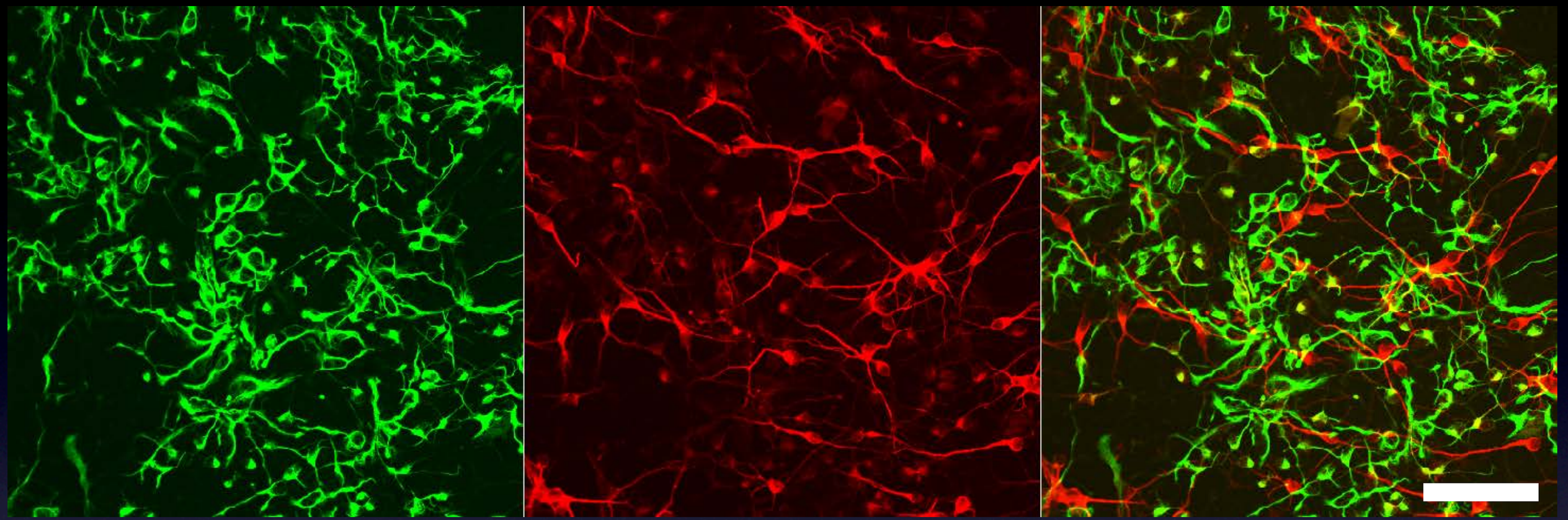
Nina Kosi[a], Ivan Alić[a,b], Matea Kolačević[a], Nina Vrsaljko[a], Nataša Jovanov Milošević[c], Margarita Sobol[d], Anatoly Philimonenko[d], Pavel Hozák[d], Srećko Gajović[a,e], Roland Pochet[a,e,f], Dinko Mitrečić[a,e,*]



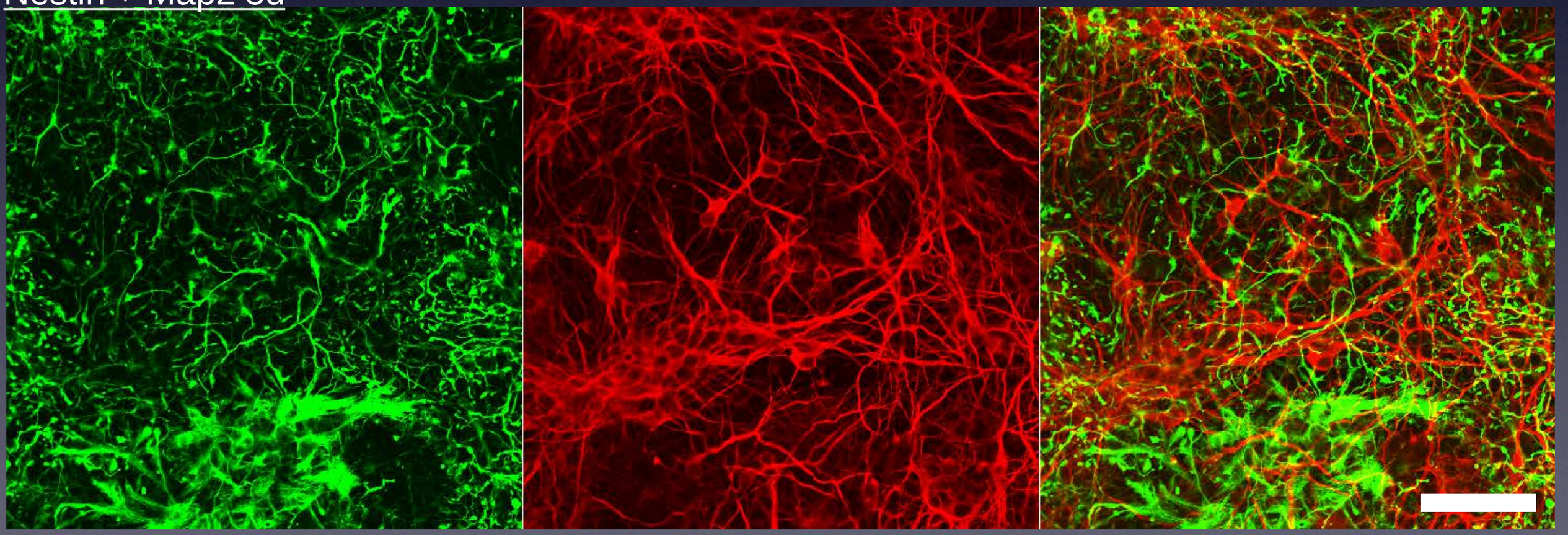


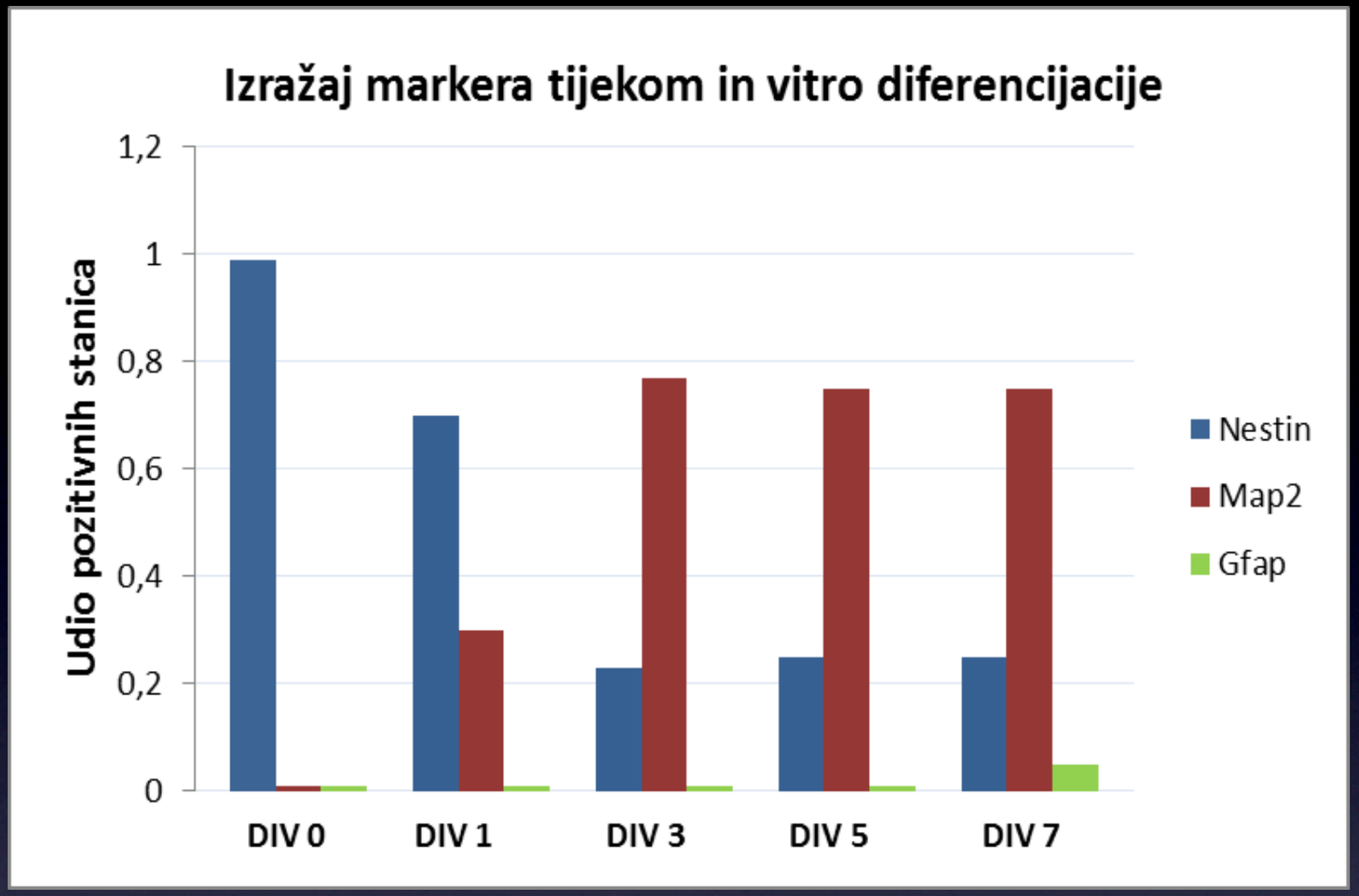


Nestin + Map2 1d



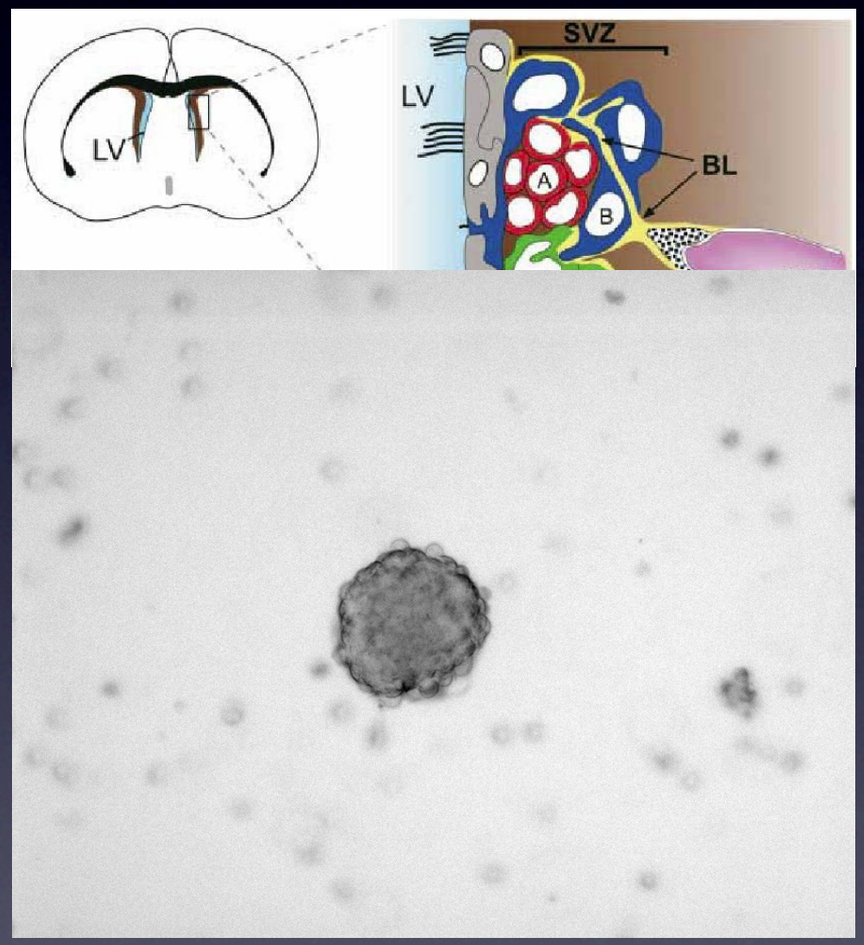
Nestin + Map2 5d





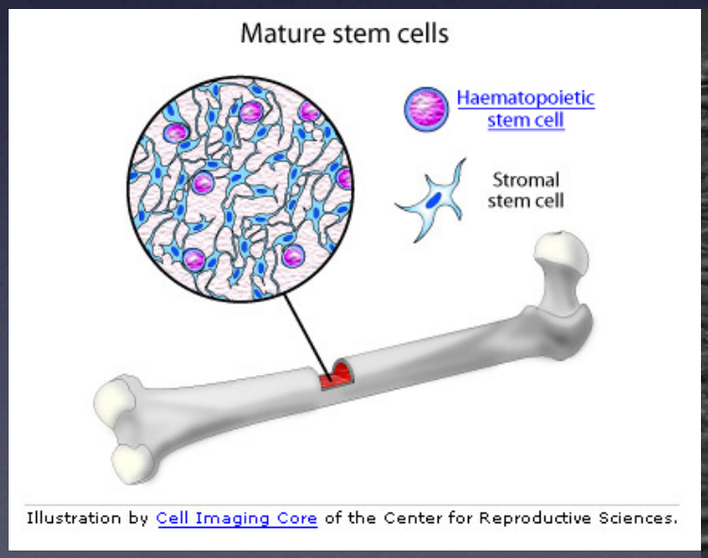
	0d	1d	3d	5d	7d
Nestin	99%	70%	23%	25%	25%
Map2	1%	30%	77%	75%	75%
Gfap	1%	1%	1%	1%	5%

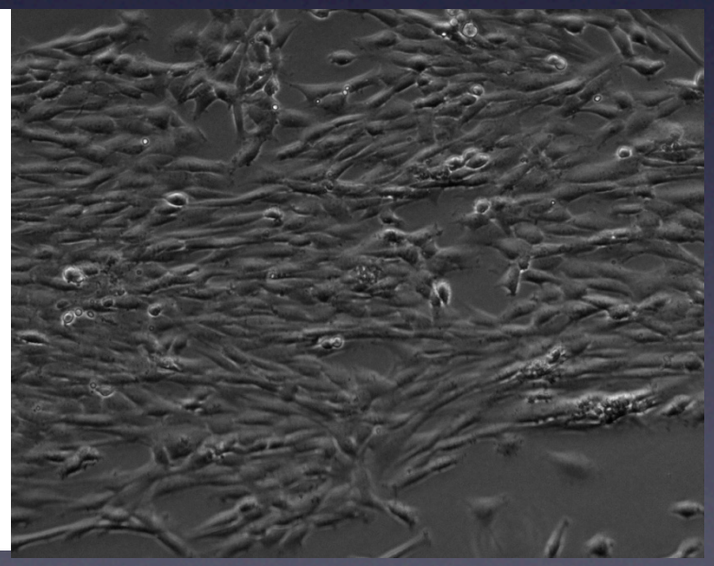
Neural stem cells (NSC)



Mitrecic et al., Cell Transplant, 2010.

Mesenchymal stem cells (MSC)



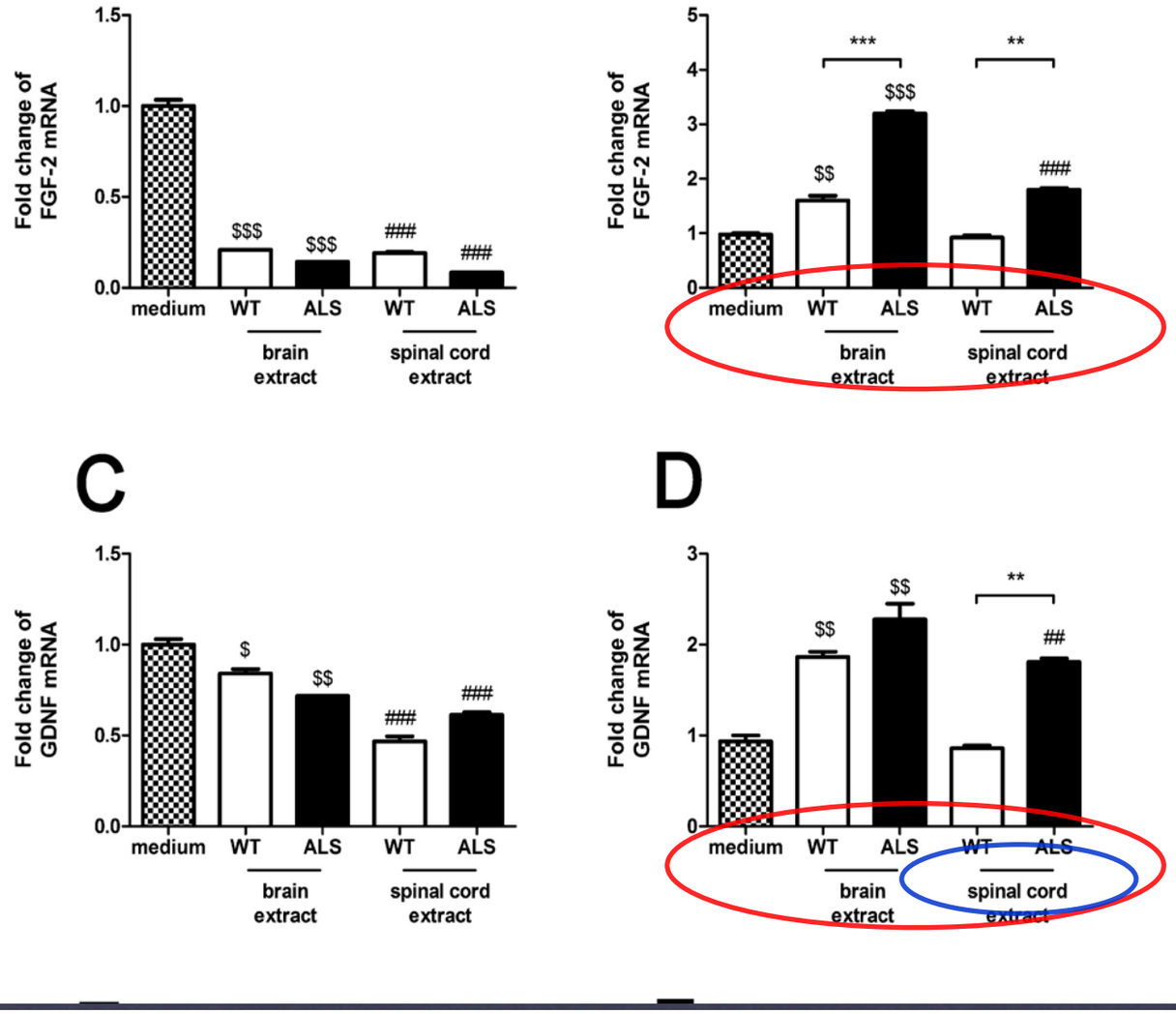


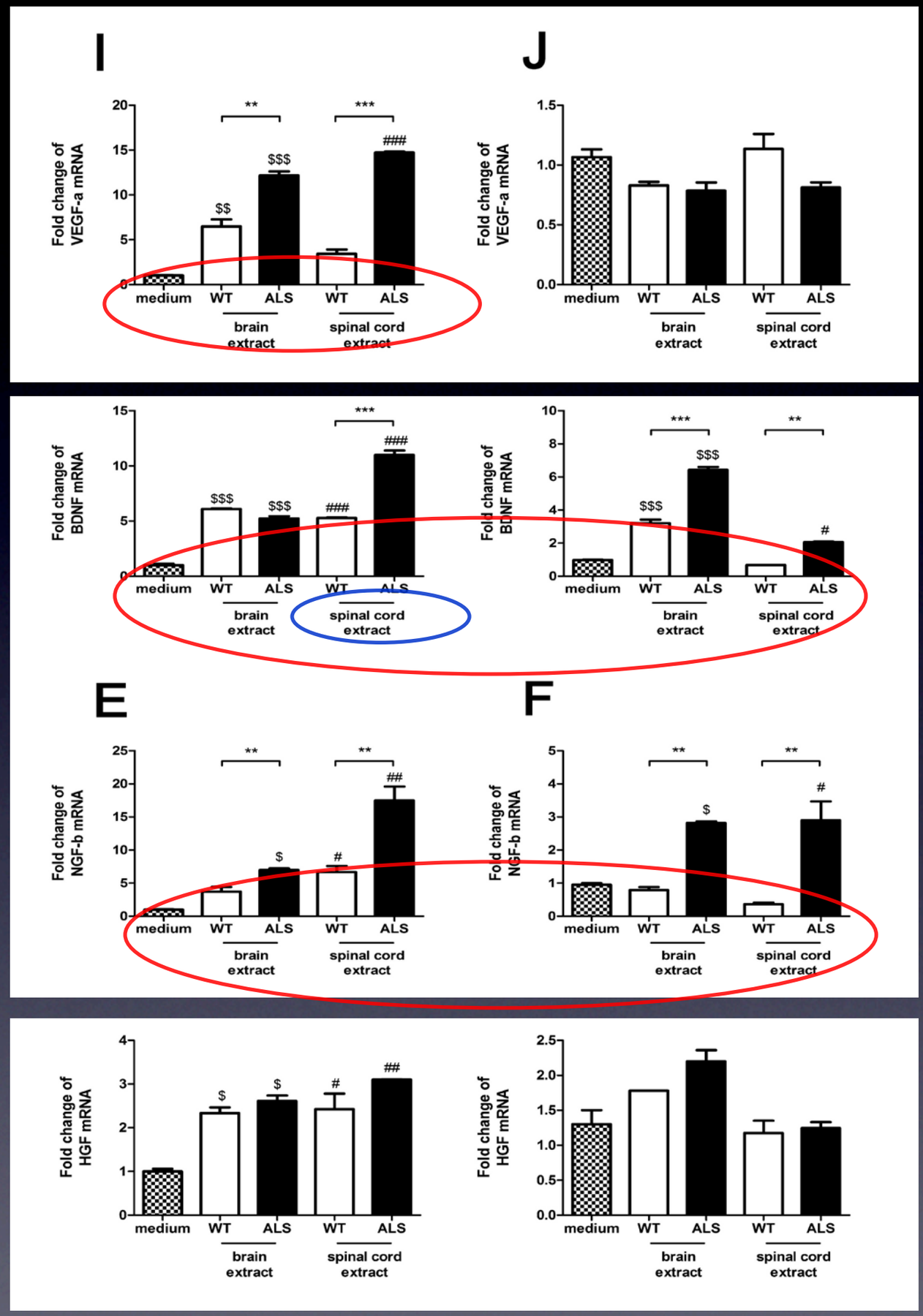
Based on E. Hermans protocol, UCL

Stimulation of MSC and NSC with brain and spinal cord extracts for 16 hours

Mesenchymal Stem Cells

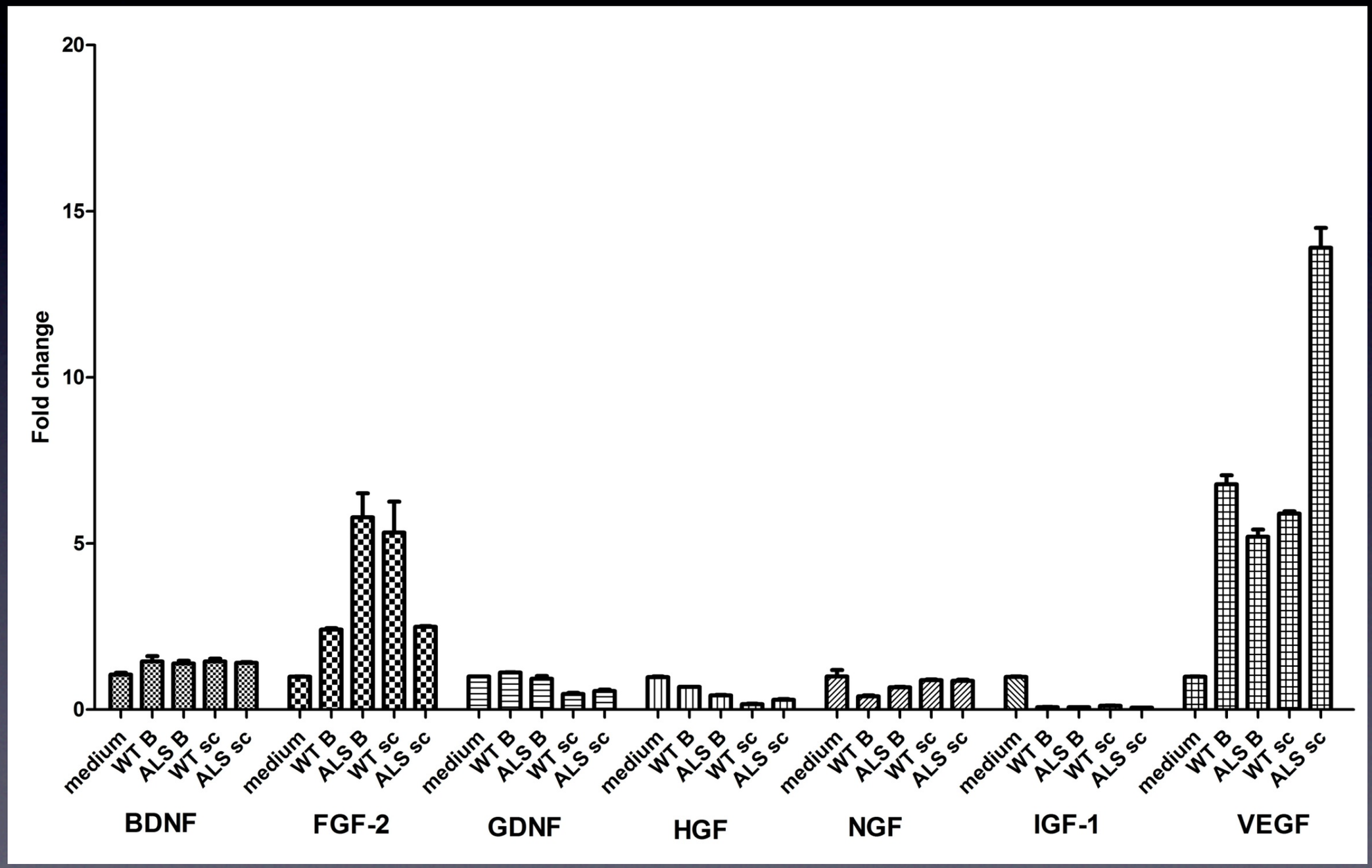
Neural Stem Cells





Nicaise, Mitrecic and Pochet:: Neuropathology and Applied Neurobiology, 2010.

Do we need stem cells?



B6.Cg-Tg(Thy1-YFP)16Jrs/J

(YFP -16)

- ✓ transgenic mouse strain
- ✓ *Thy 1* gene promoter
- ✓ YFP

Neuron, Vol. 28, 41–51, October, 2000, Copyright ©2000 by Cell Press

Neurotechnique

Imaging Neuronal Subsets in Transgenic Mice Expressing Multiple Spectral Variants of GFP

Guoping Feng,* Rebecca H. Mellor,† Michael Bernstein,* Cynthia Keller-Peck,* Quyen T. Nguyen,* Mia Wallace,* Jeanne M. Nerbonne,† Jeff W. Lichtman,* and Joshua R. Sanes*‡
*Department of Anatomy and Neurobiology
†Department of Molecular Biology and Pharmacology
Washington University School of Medicine
St. Louis, Missouri 63110

variants with altered spectral properties and improved translational efficiency, thermostability, and quantum yield. As a result of these favorable properties, GFP and its variants have been used to follow molecules and cells in at least a dozen species, ranging from slime molds to ferrets (reviewed in Chalfie and Kain, 1998; Tsien, 1998; Conn, 1999).

The first report on GFP expression in heterologous cells illustrated its use as a vital stain for neurons (Chalfie et al., 1994). Since that time, neuroscience has been one of the greatest beneficiaries of GFP technology, and GFP has been used to facilitate the study of neuronal

Neuroscience Letters 634 (2016) 32–41

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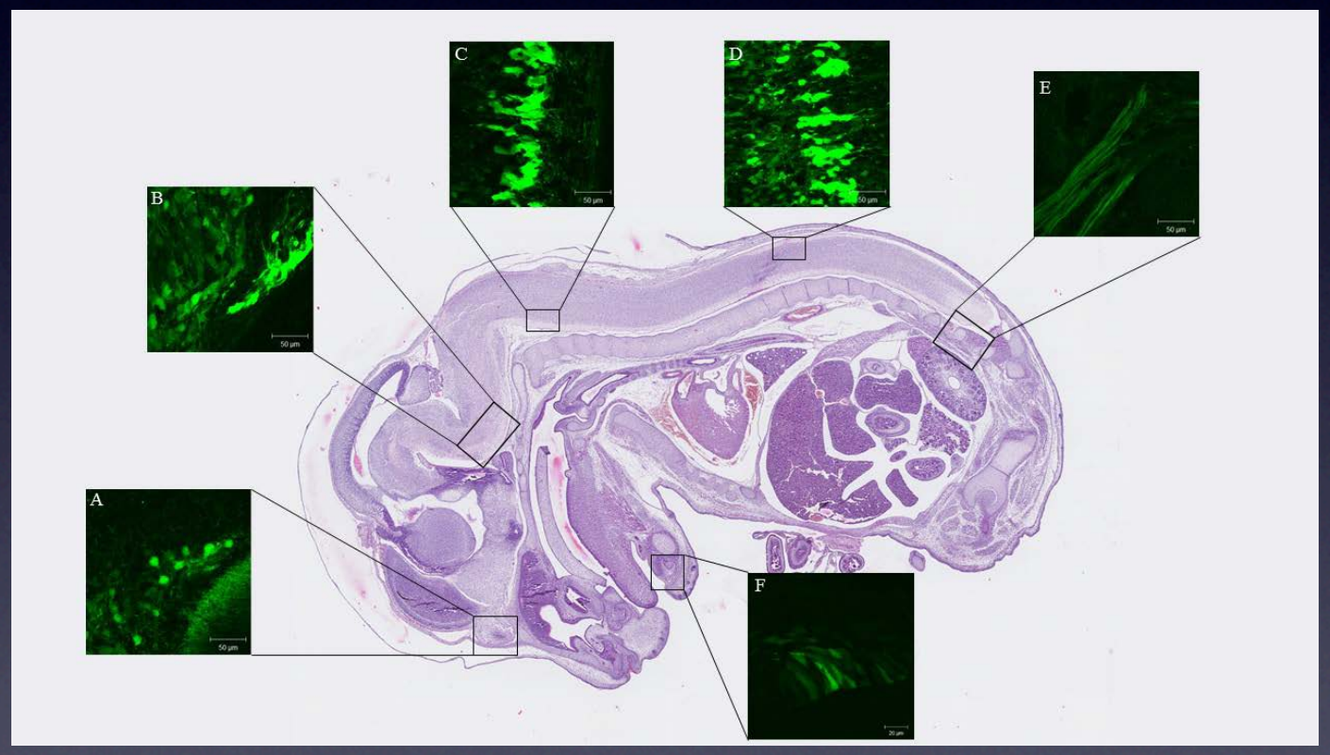
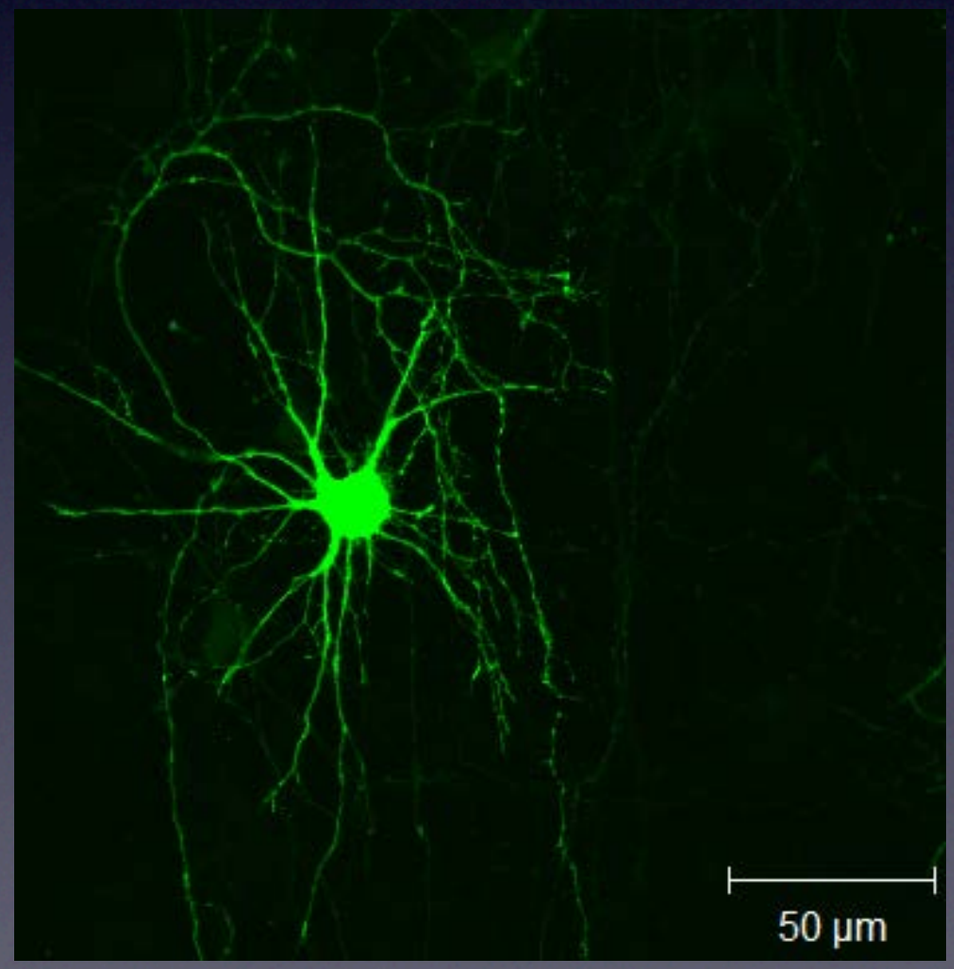
Research article

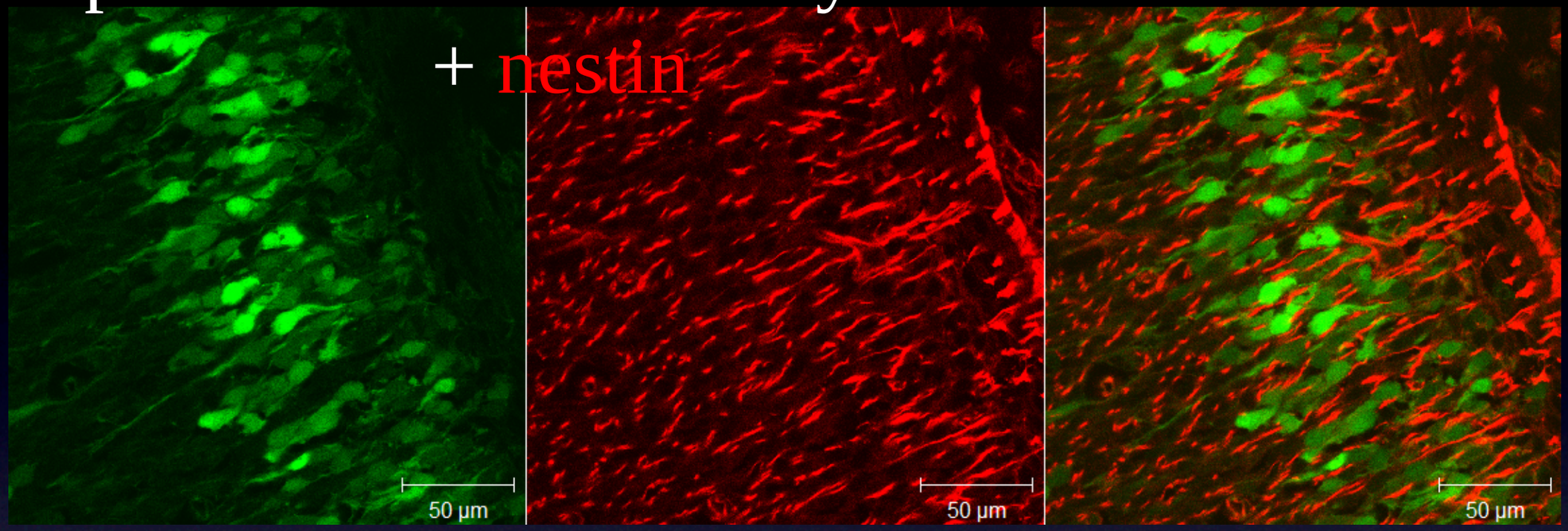
Neural stem cells from mouse strain Thy1 YFP-16 are a valuable tool to monitor and evaluate neuronal differentiation and morphology

Ivan Alić[a,b], Nina Kosi[a], Katarina Kapuralin[c], Dunja Gorup[a], Srećko Gajović[a], Roland Pochet[d], Dinko Mitrečić[a,*]

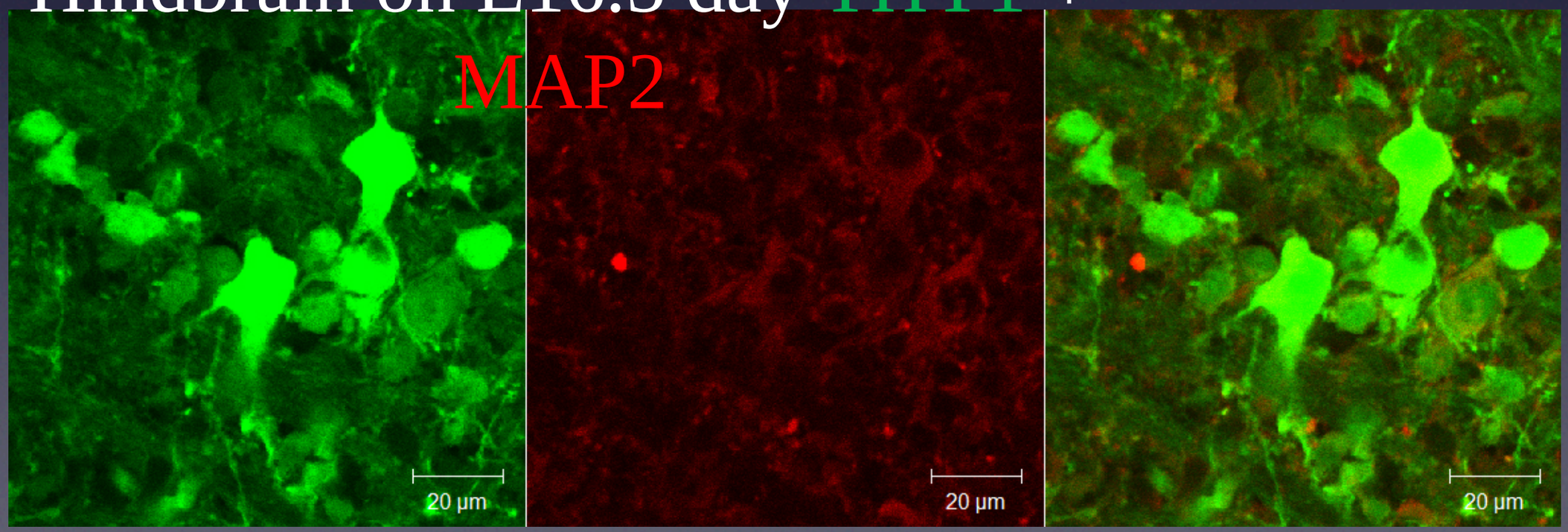




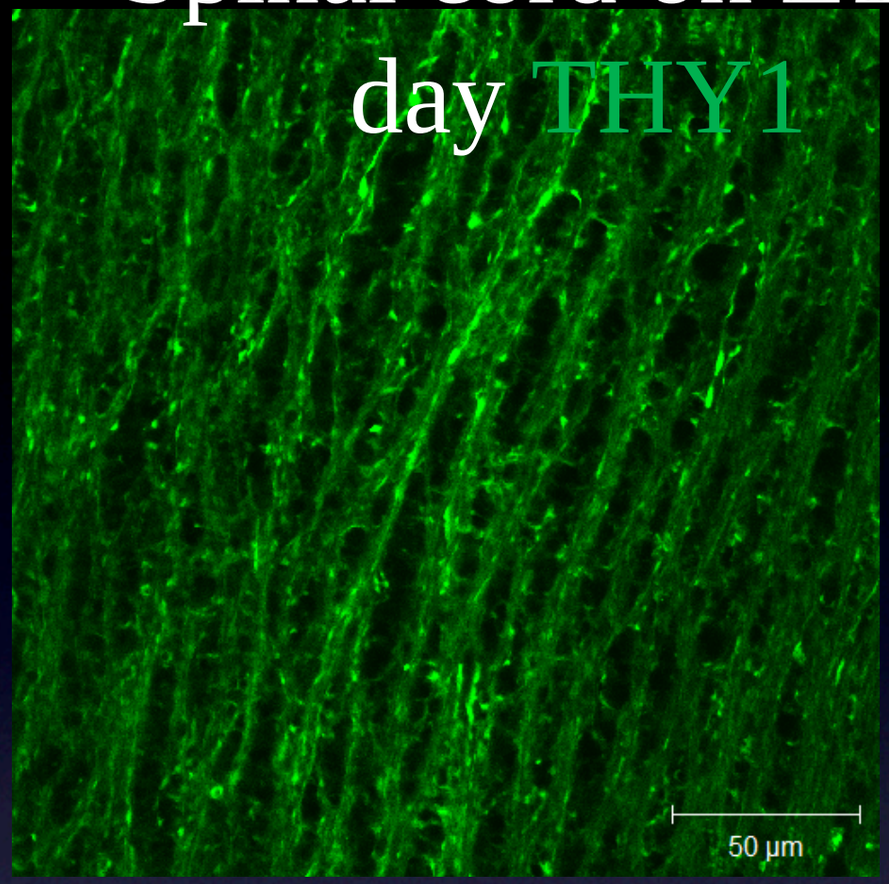
Spinal cord on E12.5 day THY1 + nestin



Hindbrain on E16.5 day THY1 + MAP2

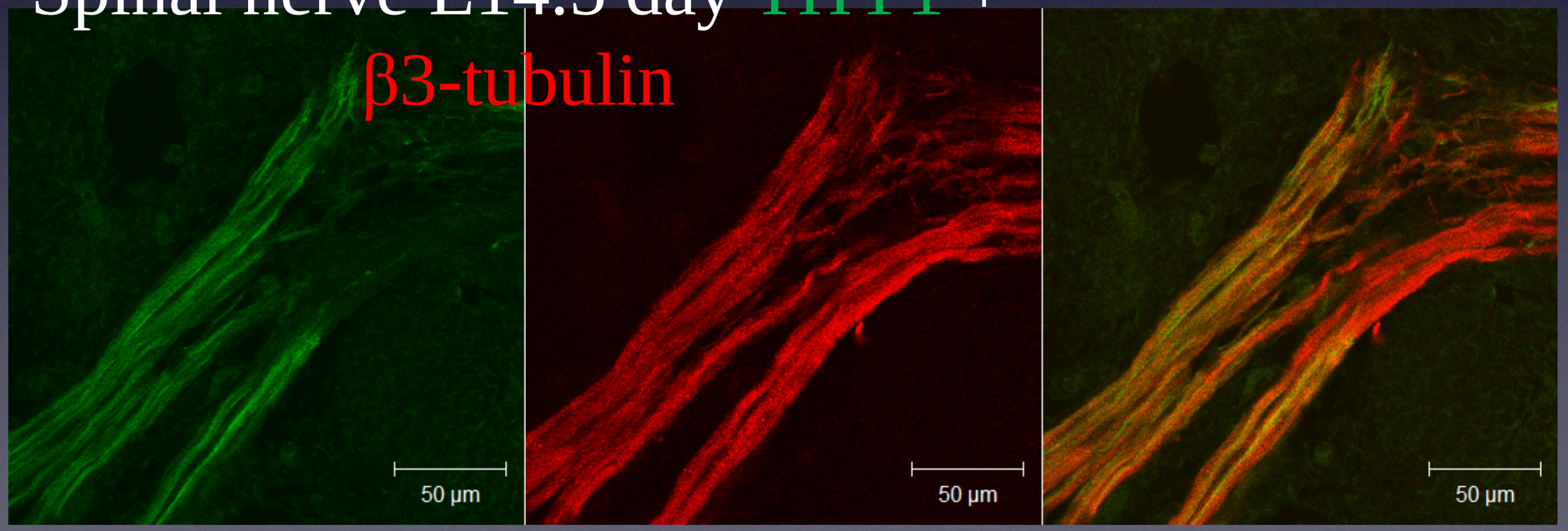


Spinal cord on E15.5 day THY1

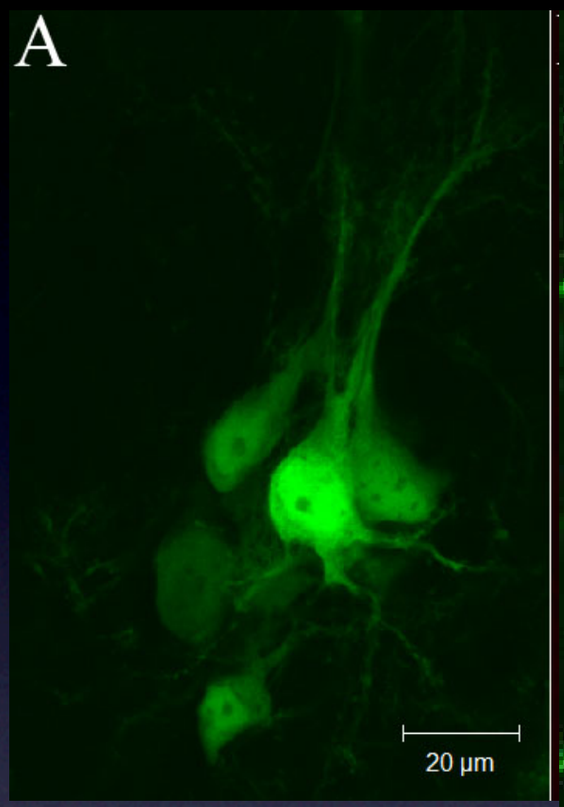


Retina in adult mouse THY1

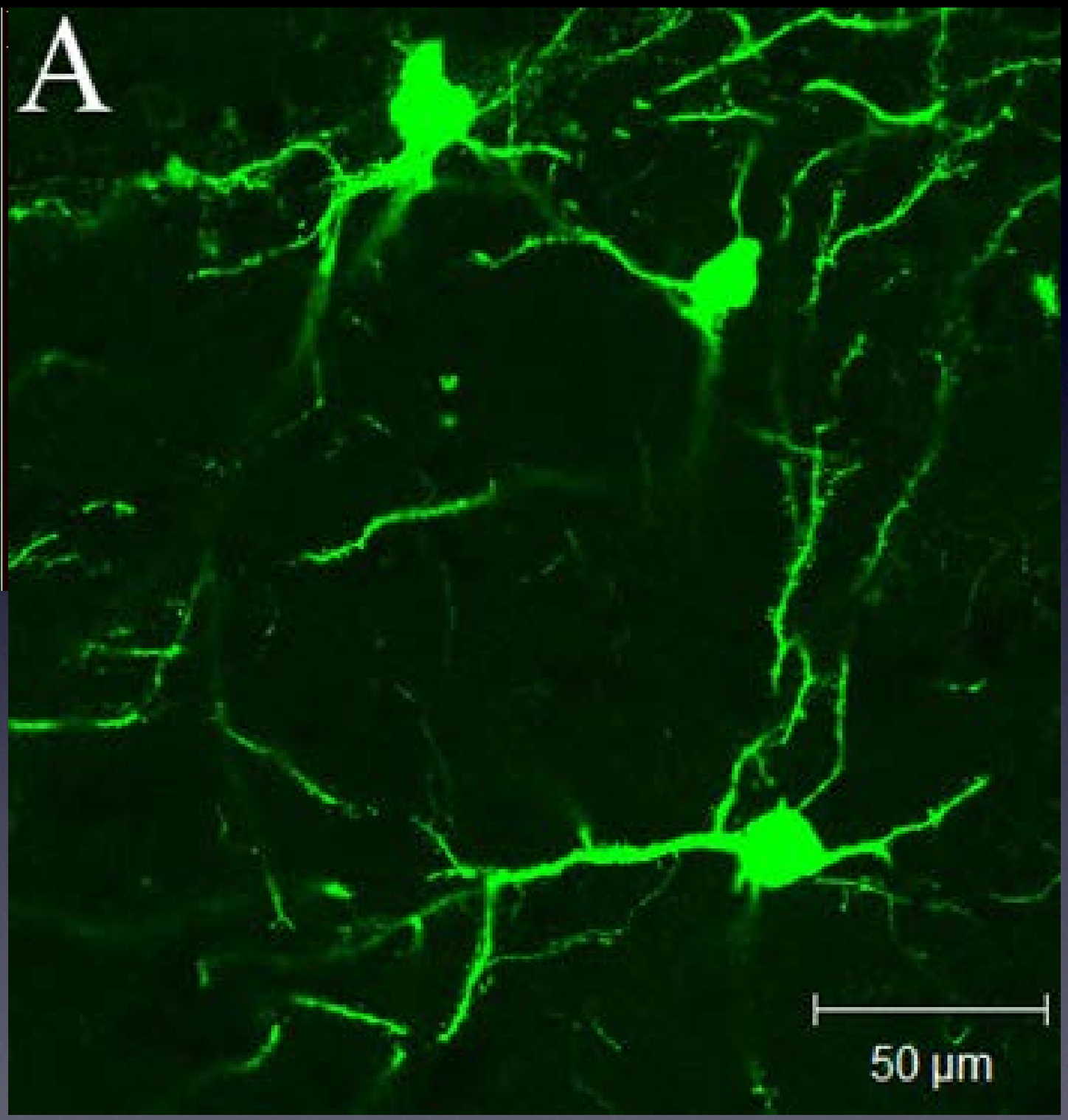
Spinal nerve E14.5 day THY1 + β3-tubulin



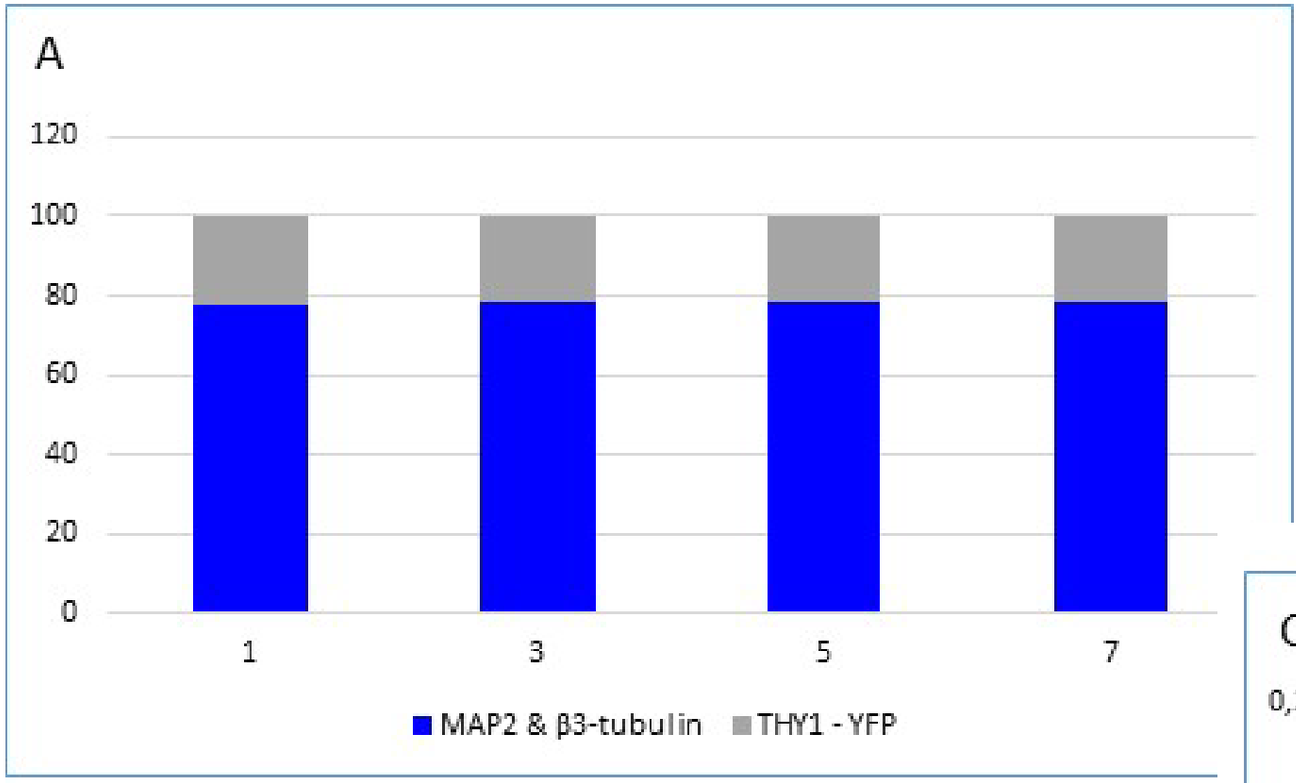
8 weeks
THY1 +
PKH26



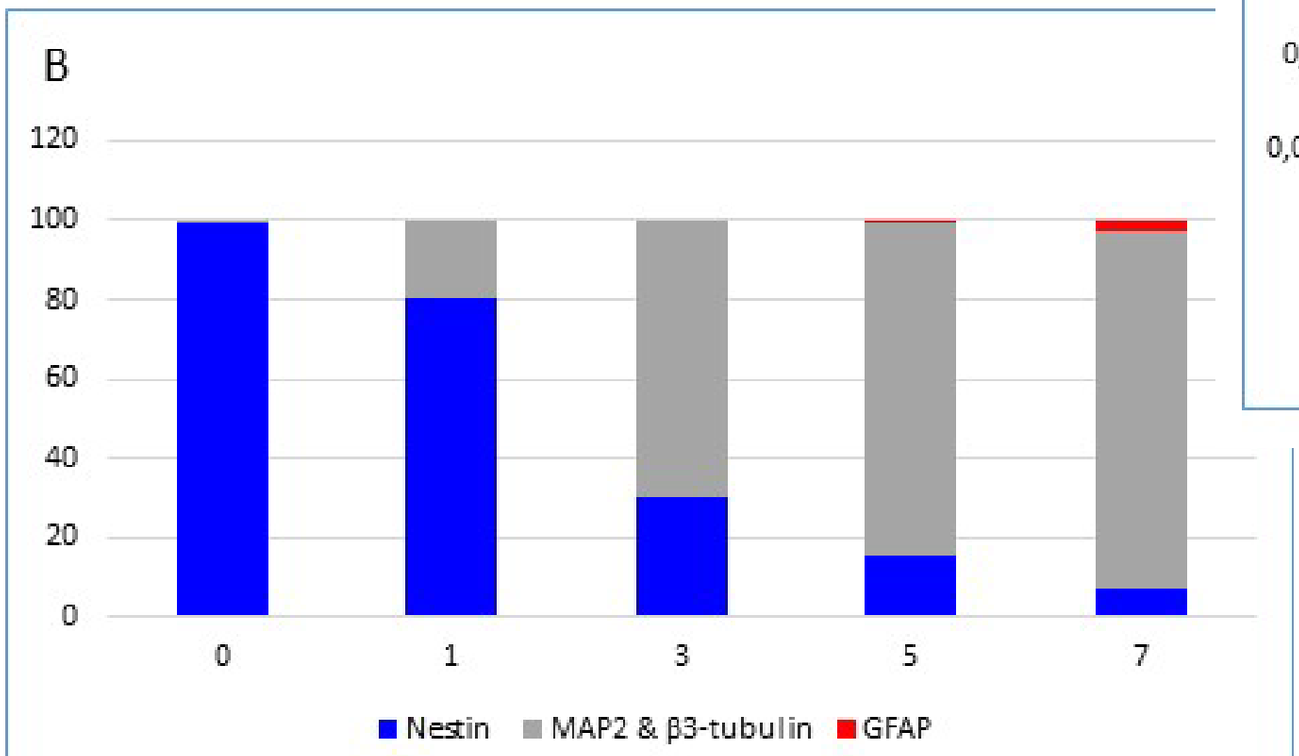
14 weeks
THY1



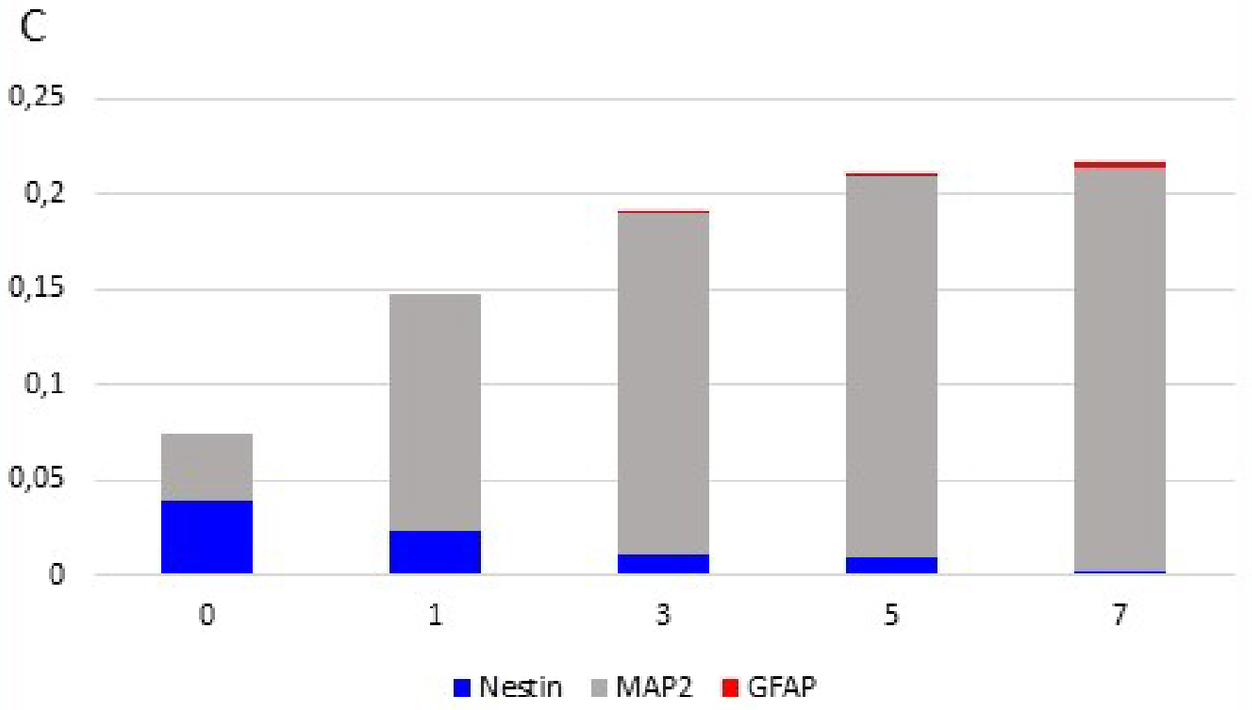
A

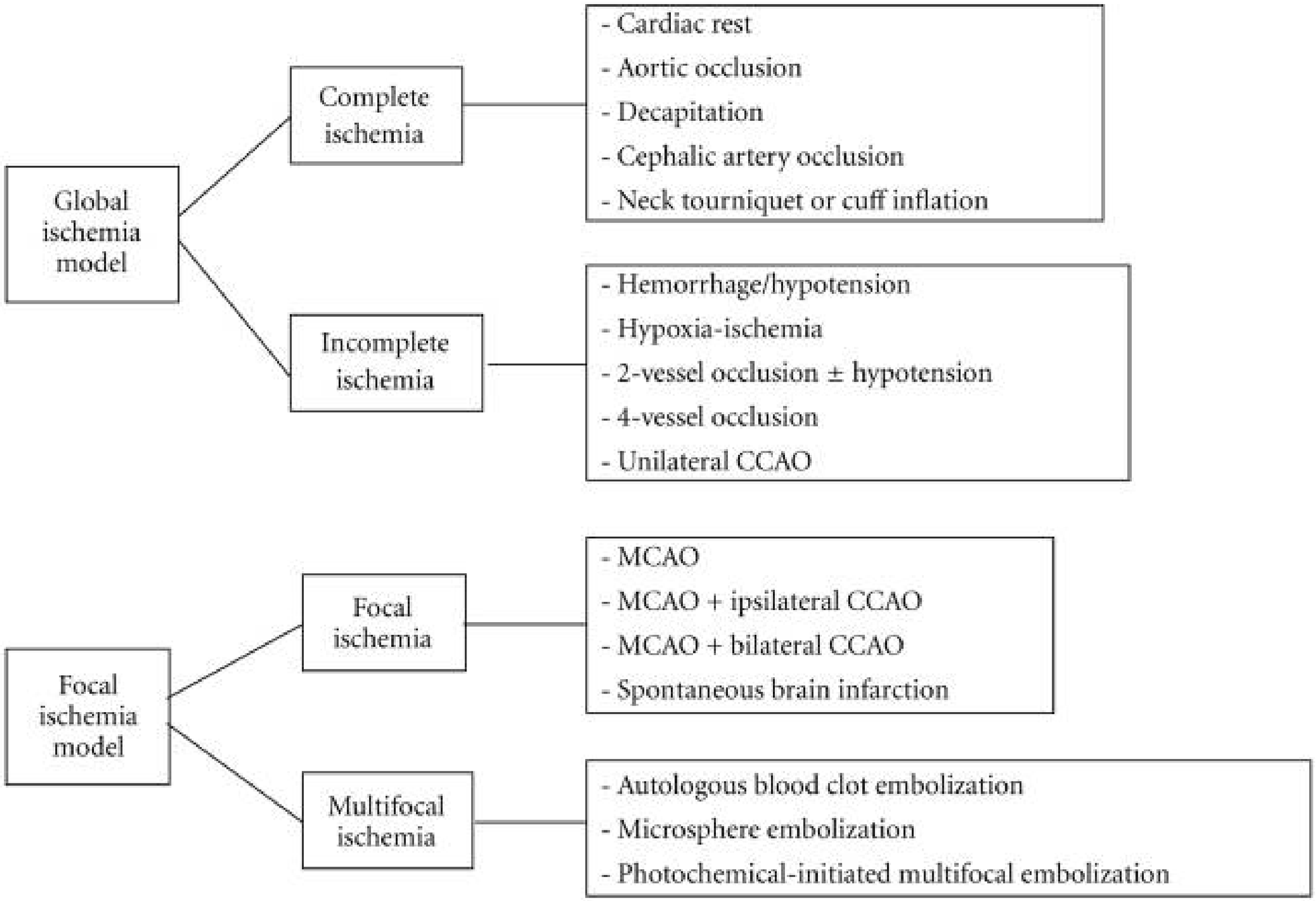

120
100
80
60
40
20
0
1
3
5
7
MAP2 & β3-tubulin
THY1 - YFP

B


120
100
80
60
40
20
0
0
1
3
5
7
Nestin
MAP2 & β3-tubulin
GFAP

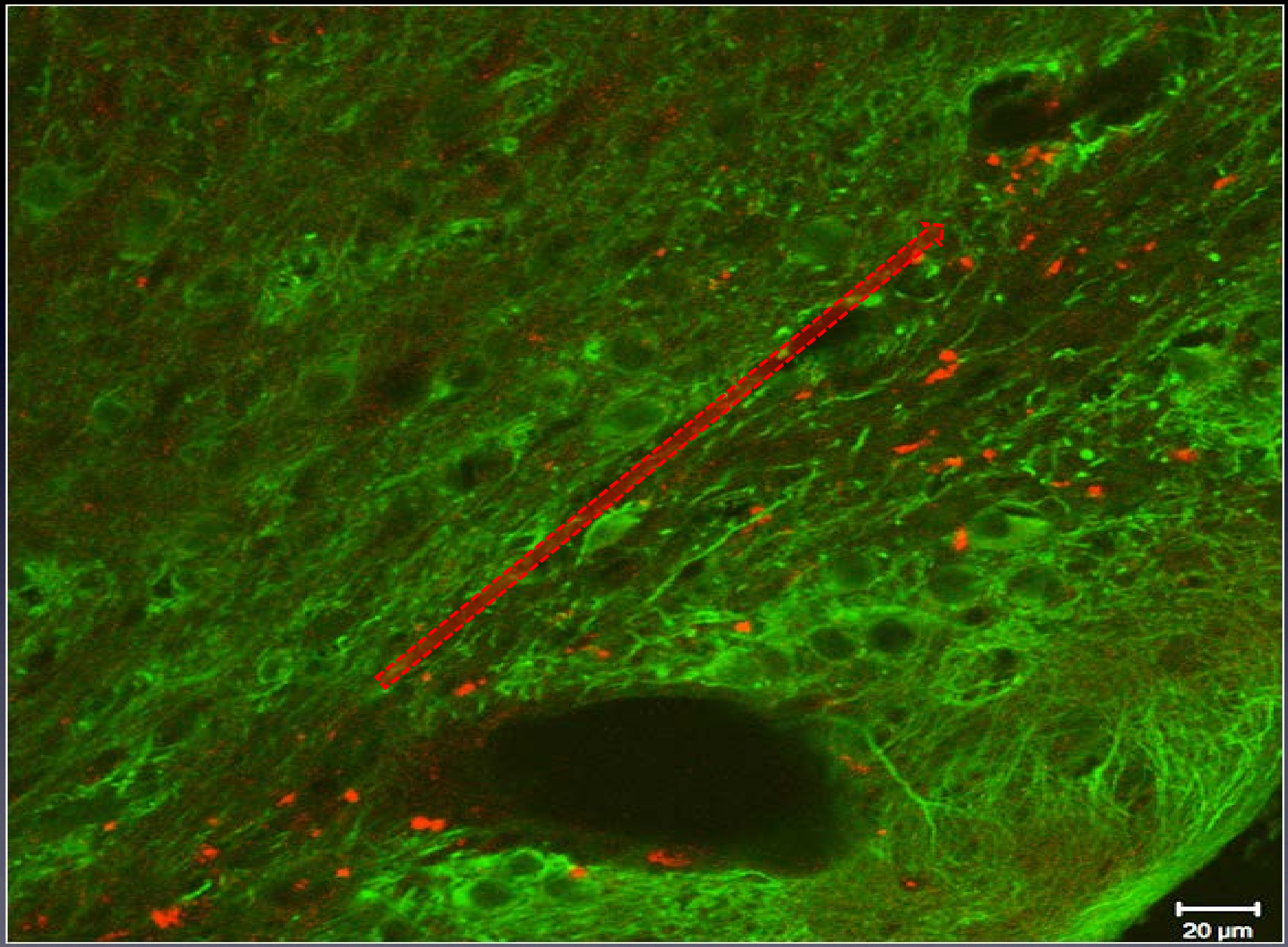
C


0,25
0,2
0,15
0,1
0,05
0
0
1
3
5
7
Nestin
MAP2
GFAP

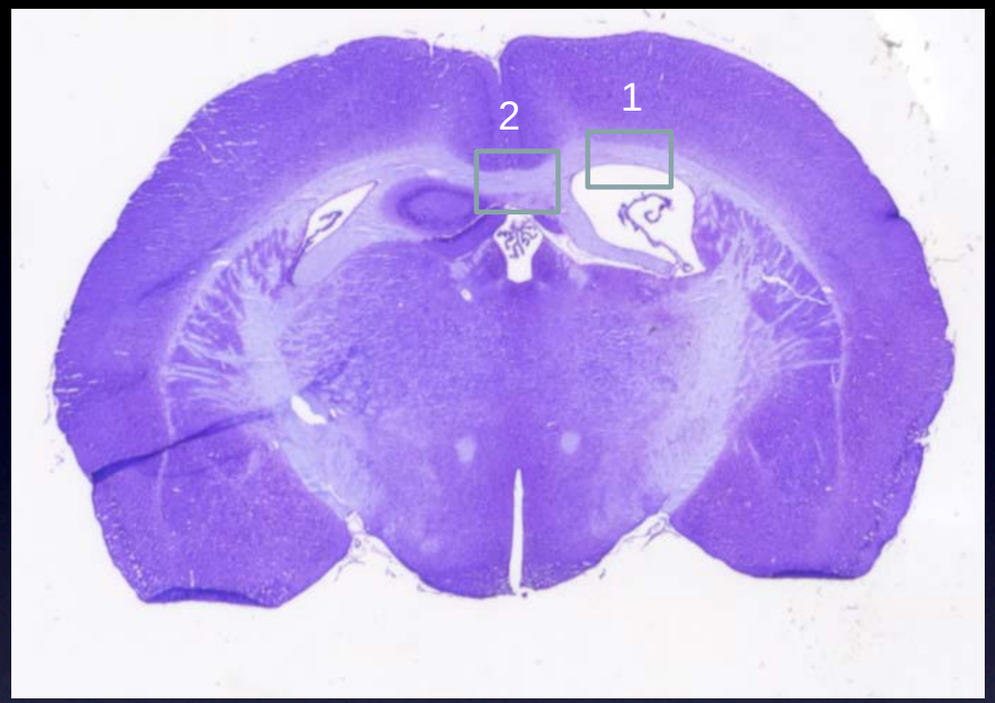


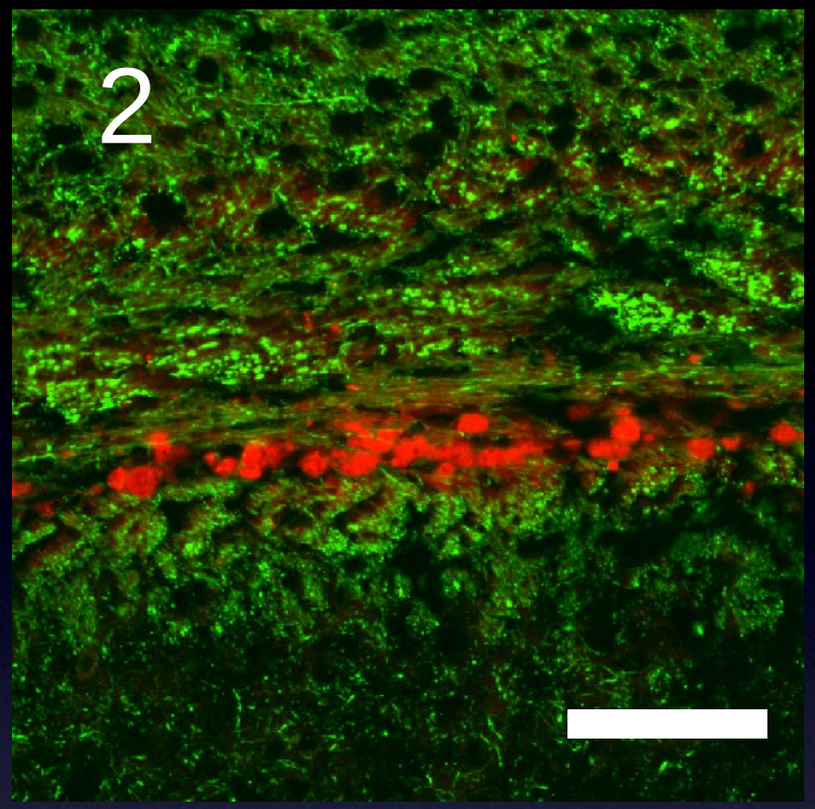
Liu et al., Journal of Biomedicine and Biotechnology, 2011.

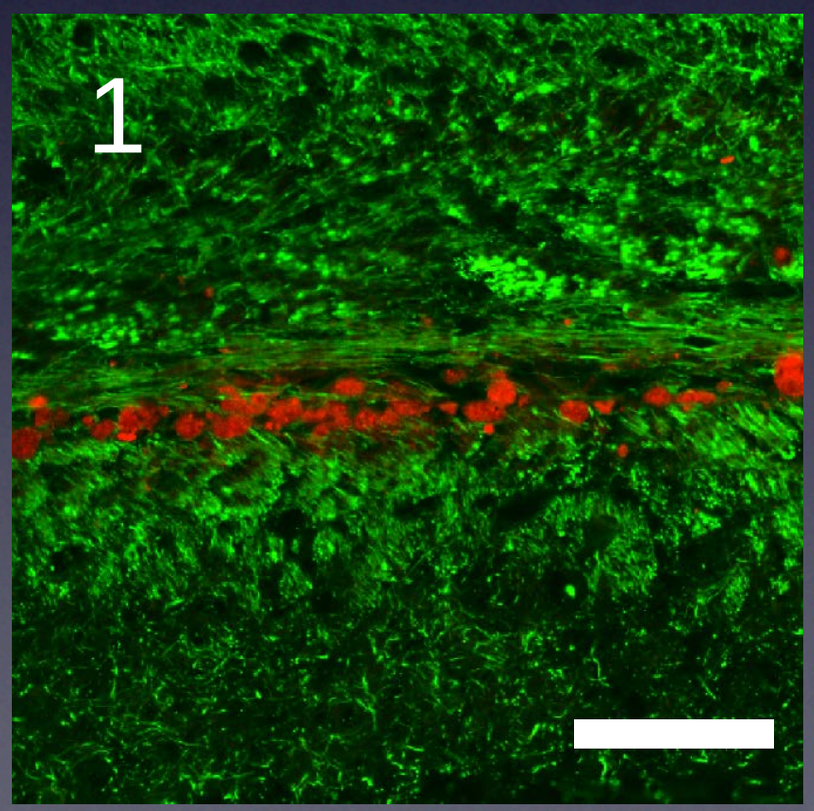
1. Migration (PKH + Thy1)



Migration using corpus callosum

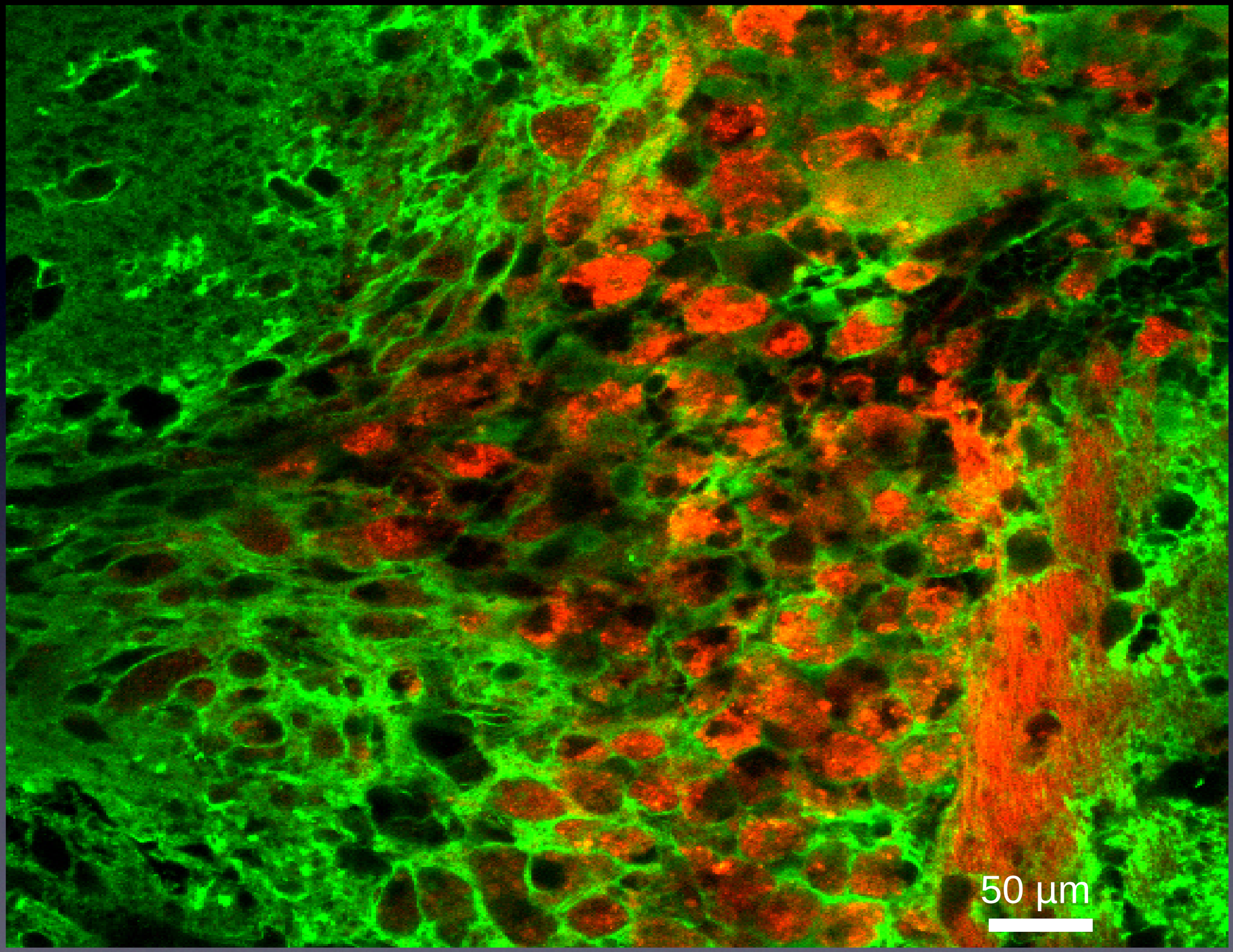




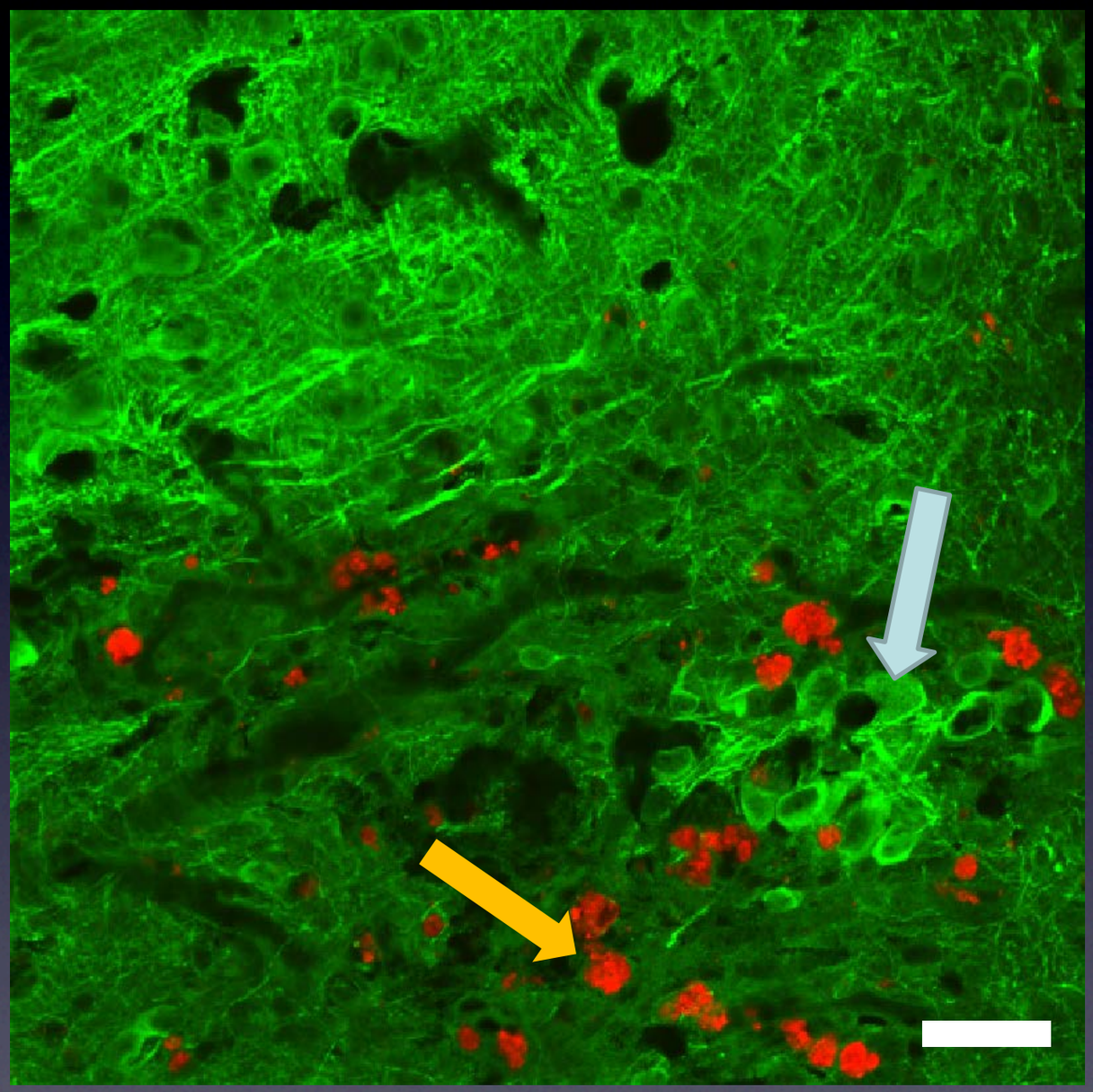


SMI32 + PKH26

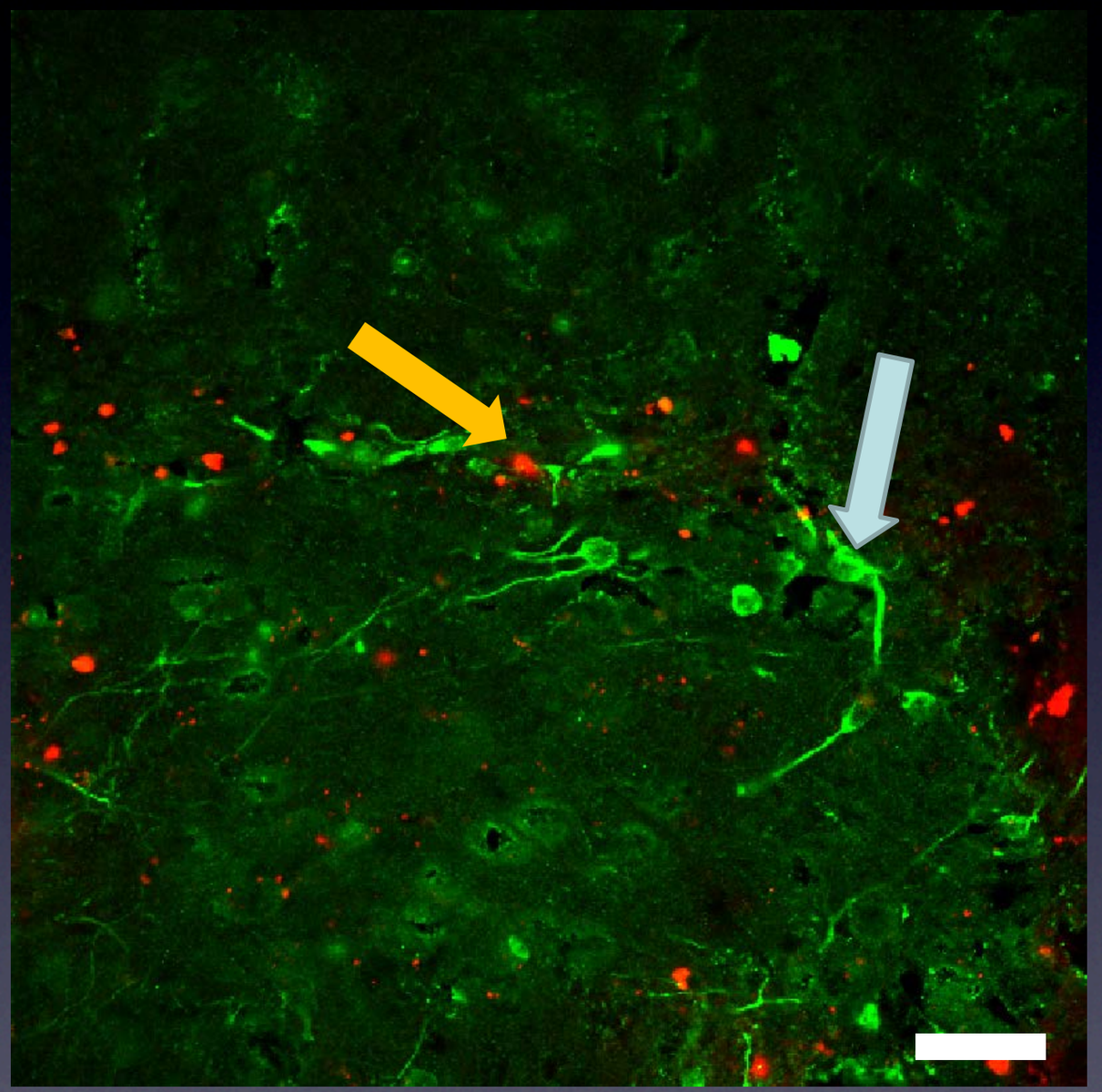
Non - Migration



At the site of injection, wt animal

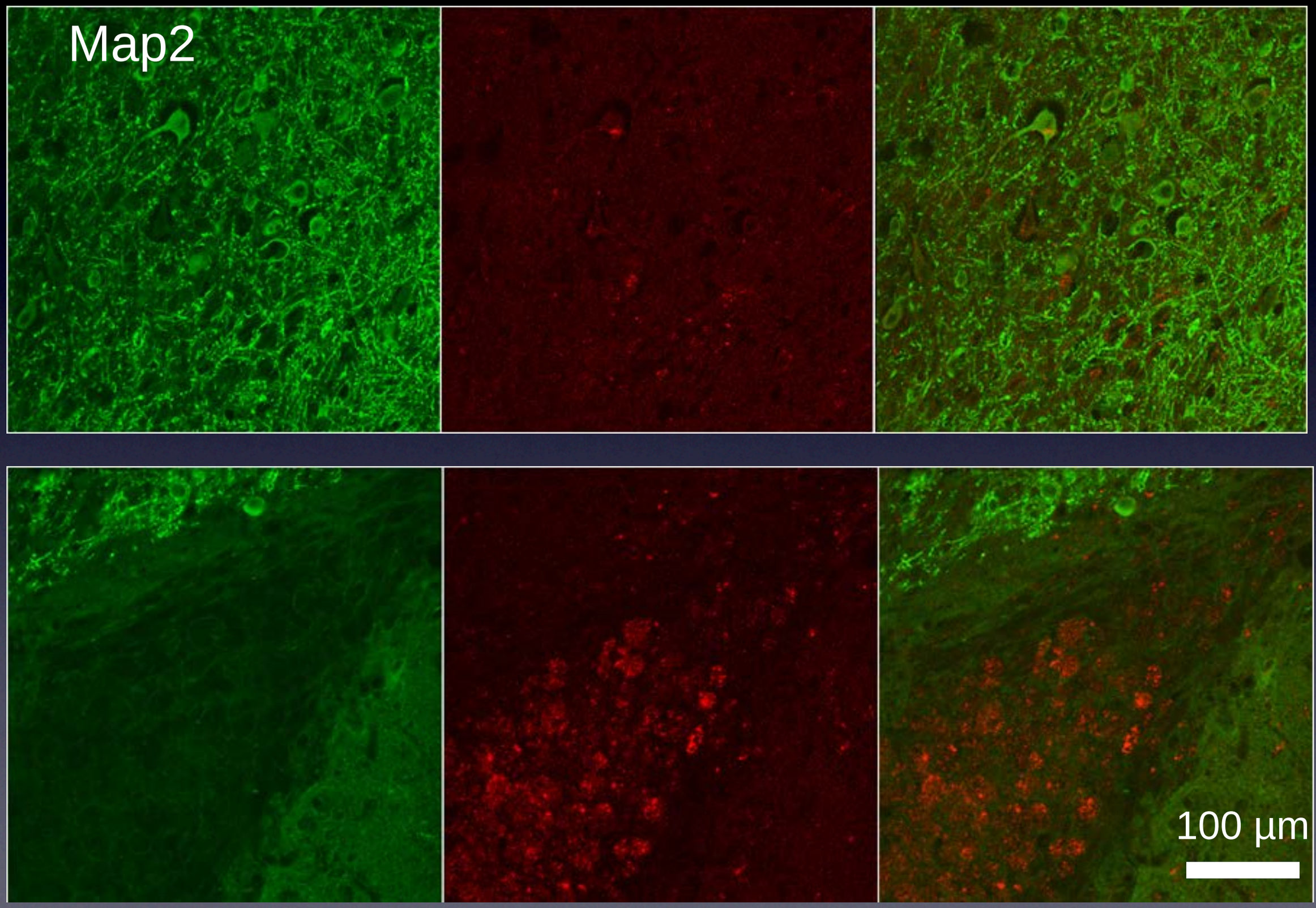


Map2 + PKH26

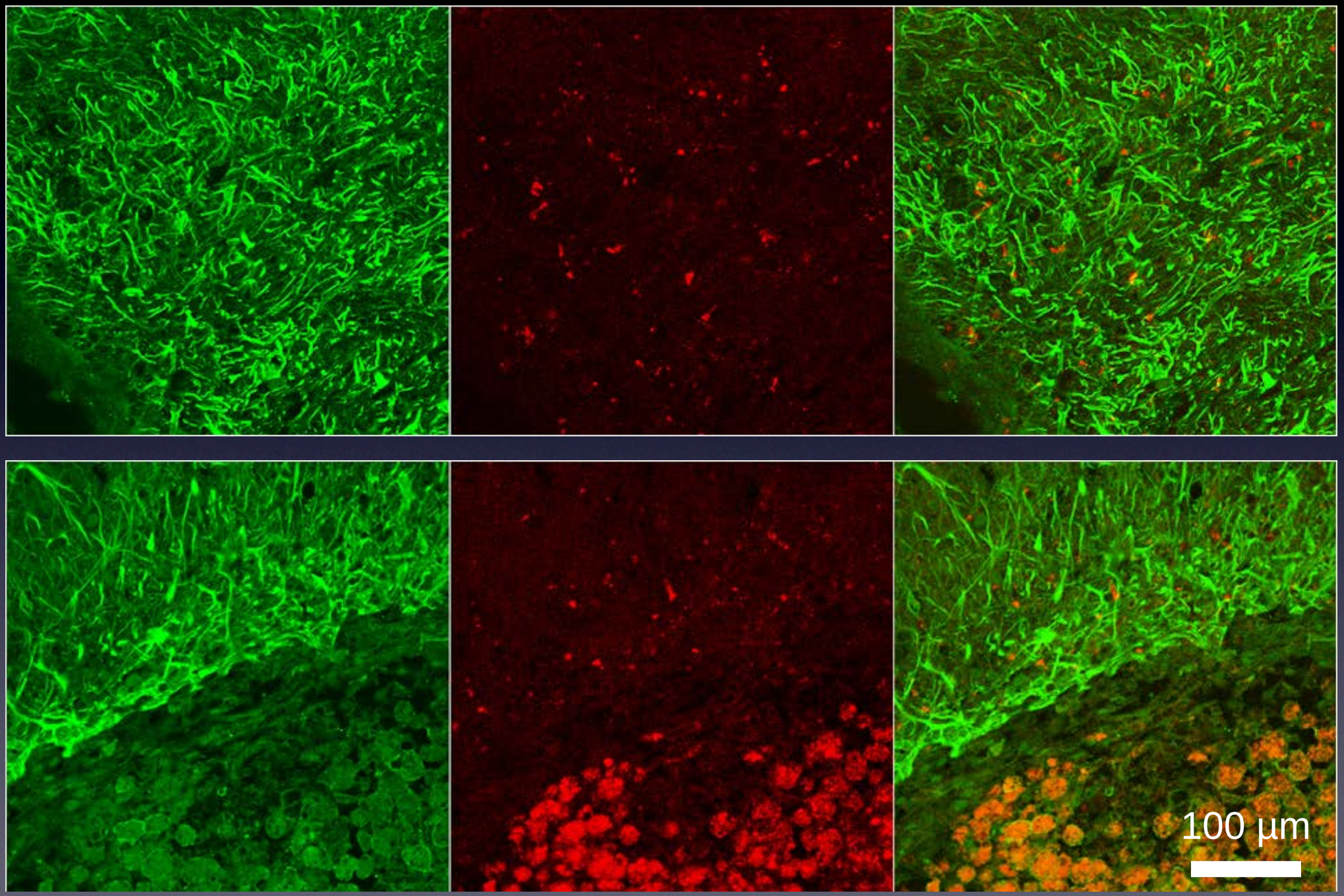


Dcx + PKH26

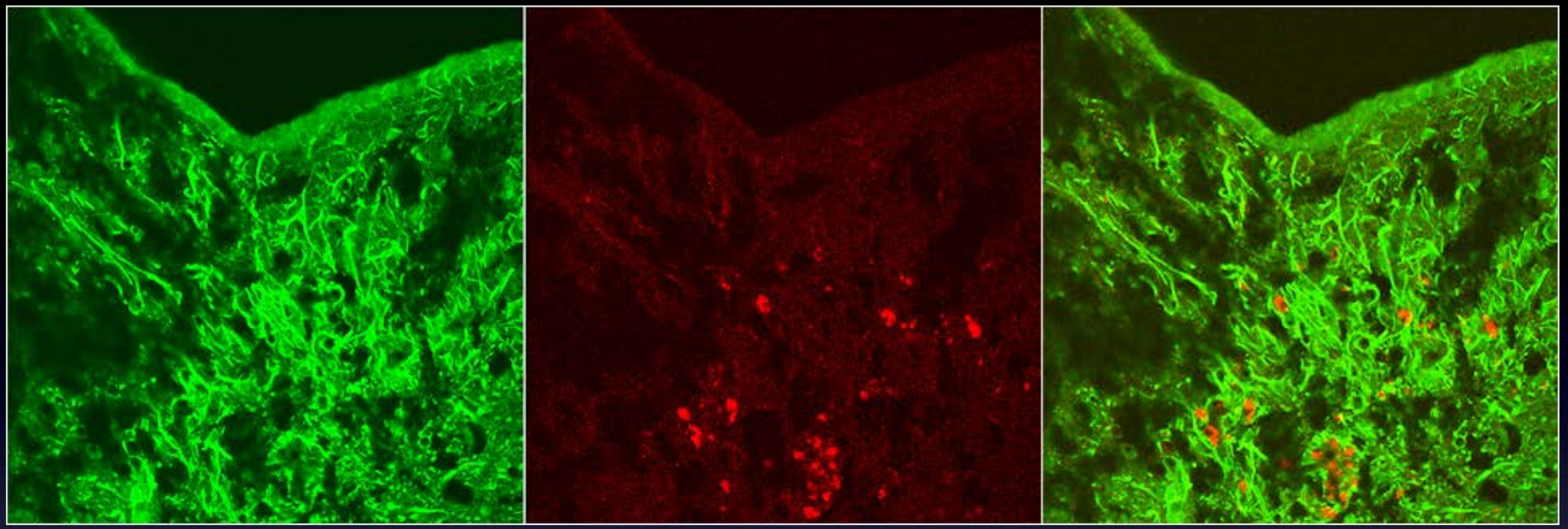
2. Accumulation



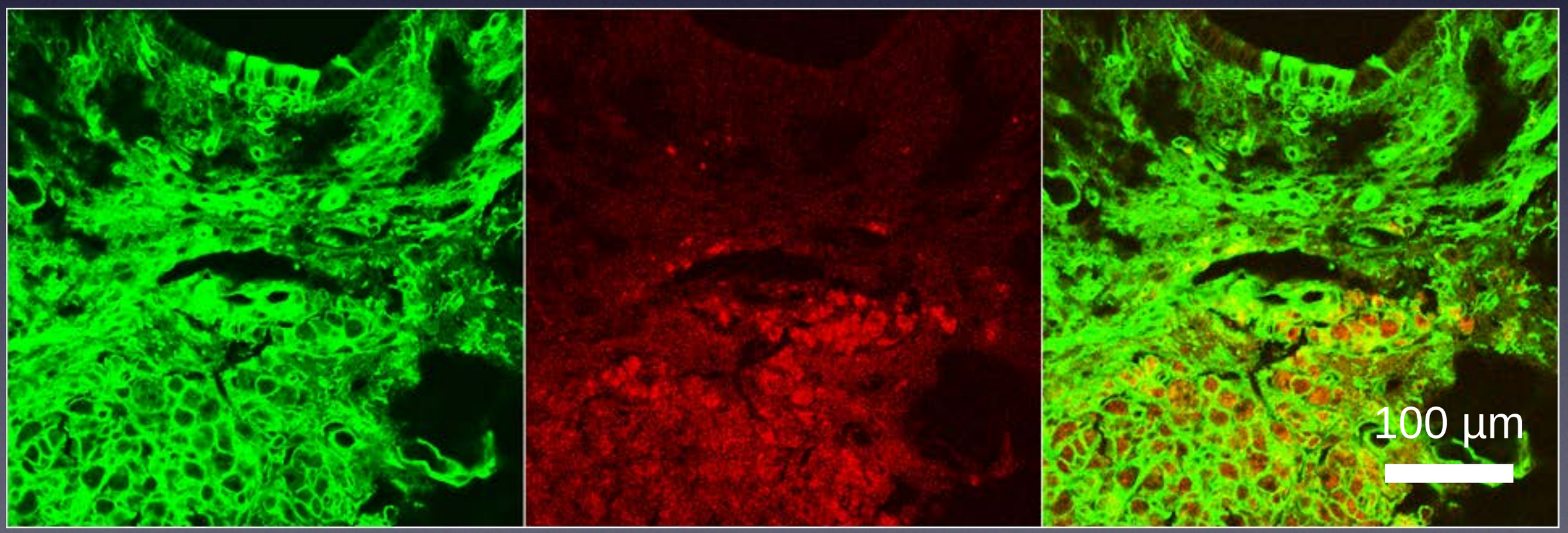
Gfap



Gfap



Nestin



Experience from more than 150 transplanted animals

1. In corticostriatal border
   (MCAO – the fastest directed migration vs.
   WT – slow bi-directional migration, cell loss)

2. hippocampus (the most efficient cell survival and differentiation)

3. ventricules (silent keepers of cells)

The hypothesis:

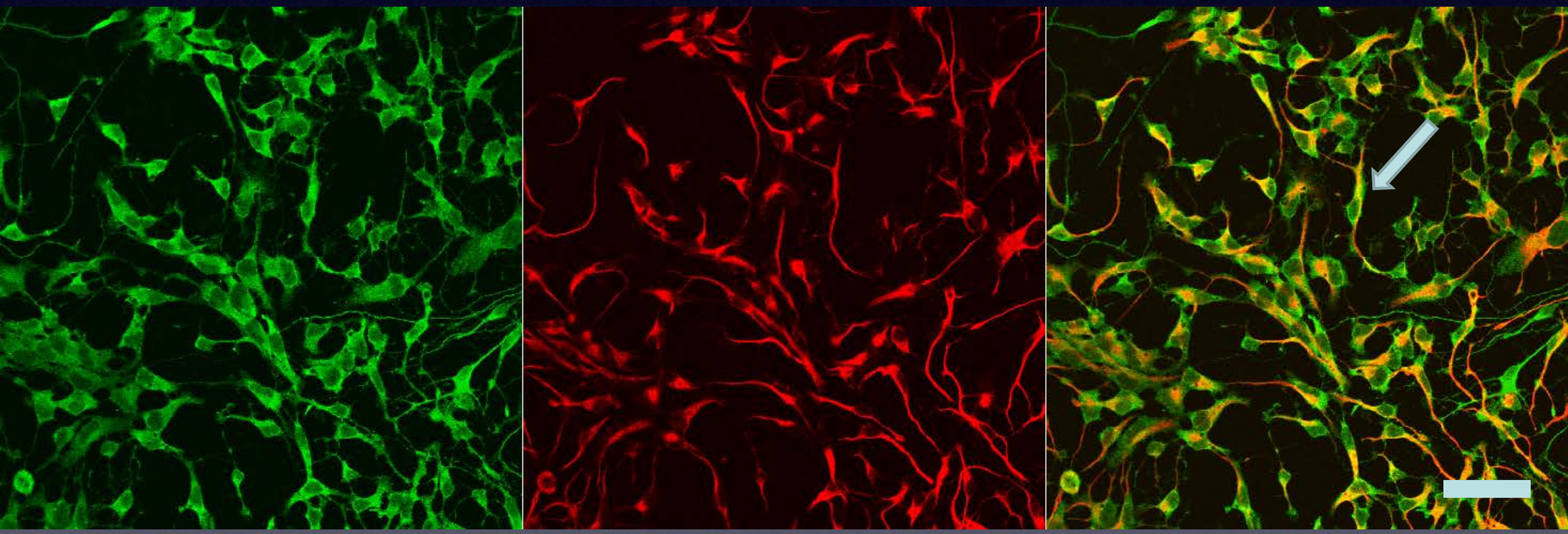
Stroke-associated inflammation **negatively** influences survival of transplanted cells faced with a hostile environment

AND

it **promotes differentiation and synaptogenesis** in order to speed up integration of cells

Neuroligin

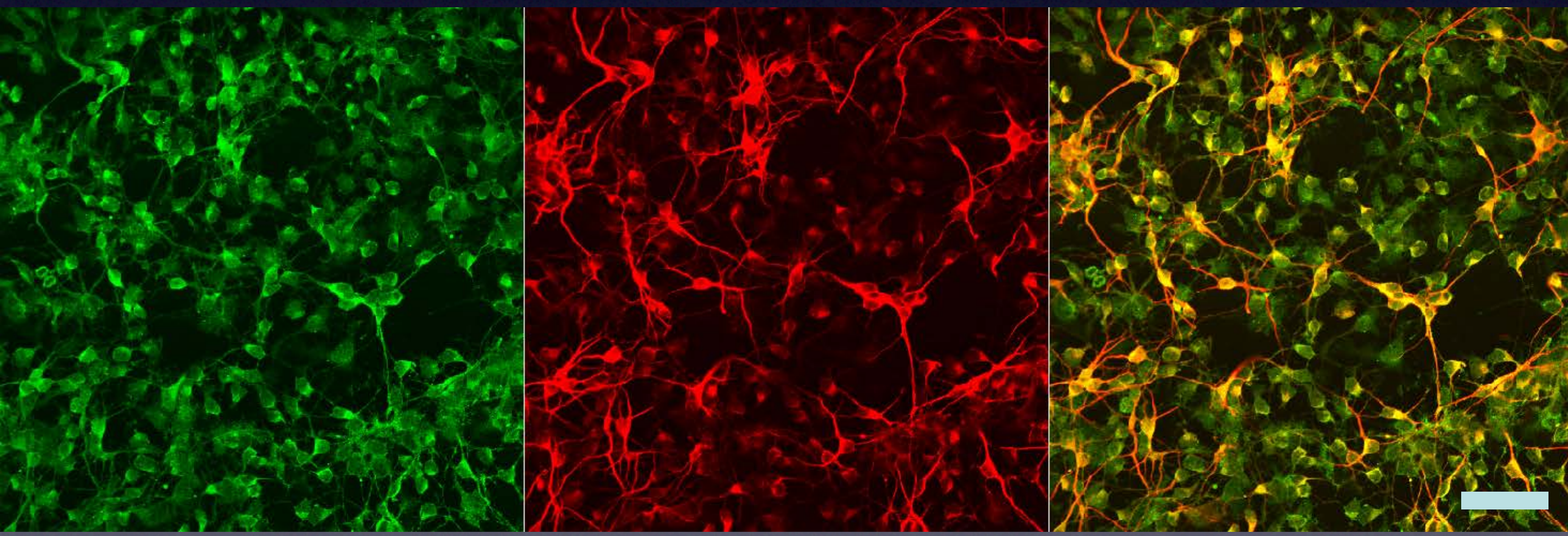
Neuroligin + Nestin 0d



Nlg Nestin

Nlg follows the increase of Map2

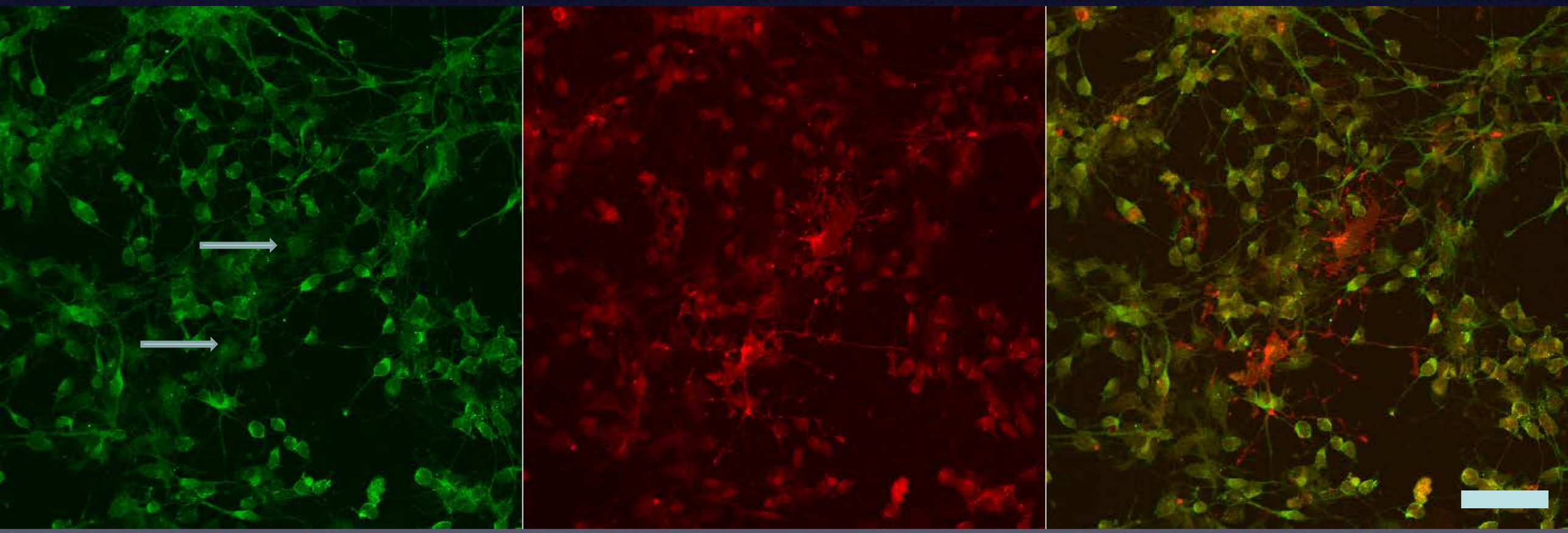
Neuroligin i Map2 1d



Nlg Map2

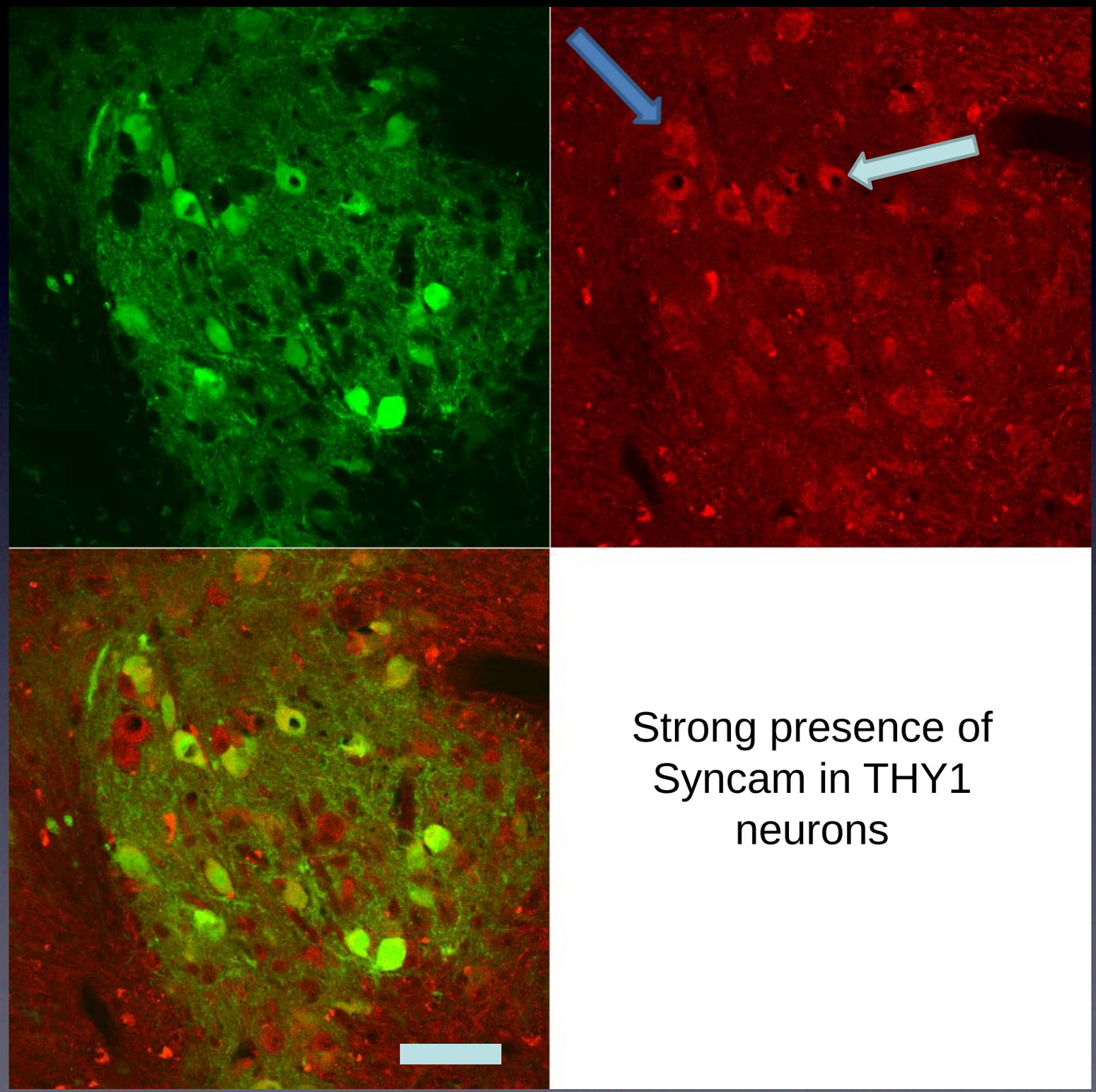
Nlg is present only in immature Gfap cells

Neuroligin + Gfap 1d



Nlg Gfap

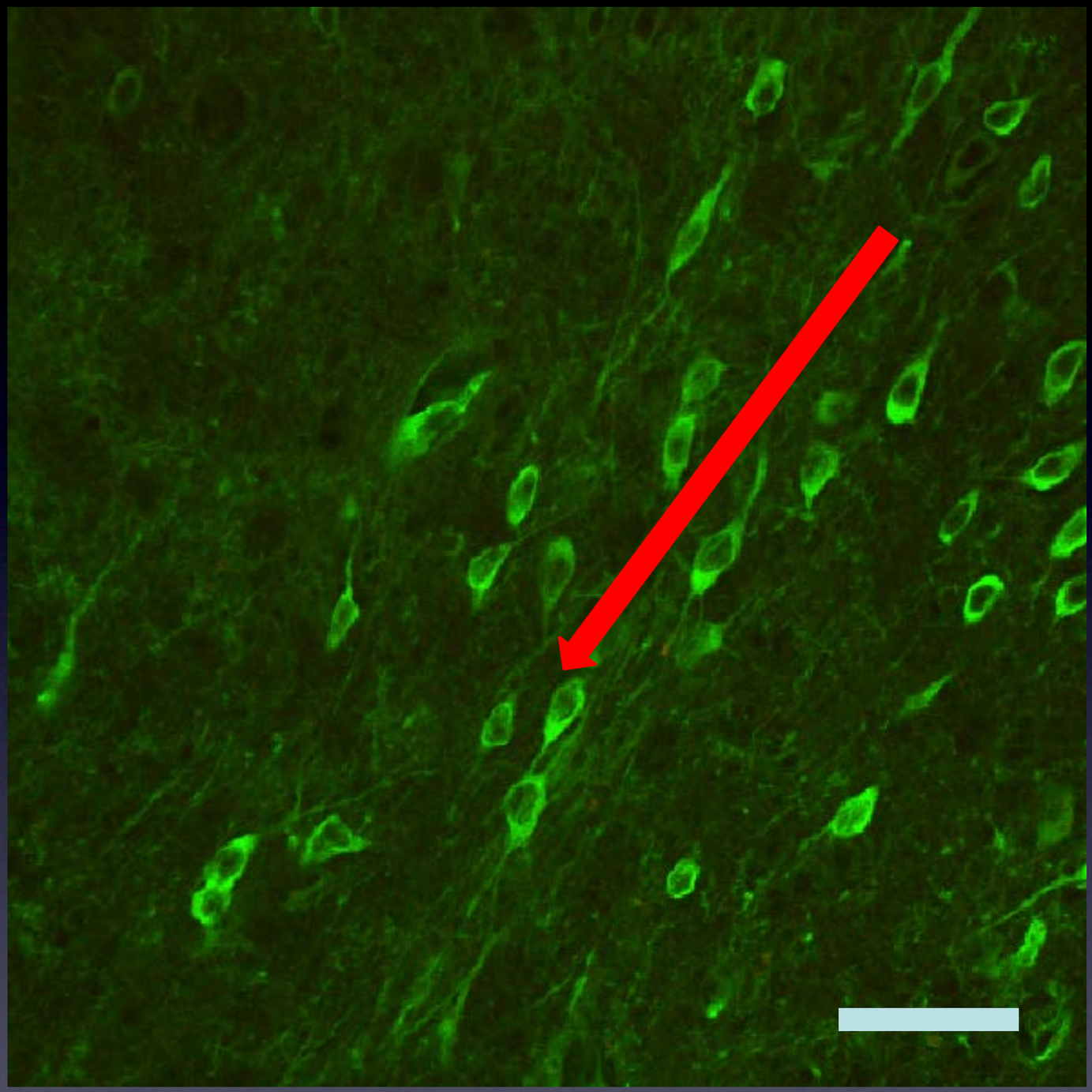
Expression of synaptic markers in the transplant (4w, MCAO vs. WT)



Strong presence of Syncam in THY1 neurons

Syncam

SVZ



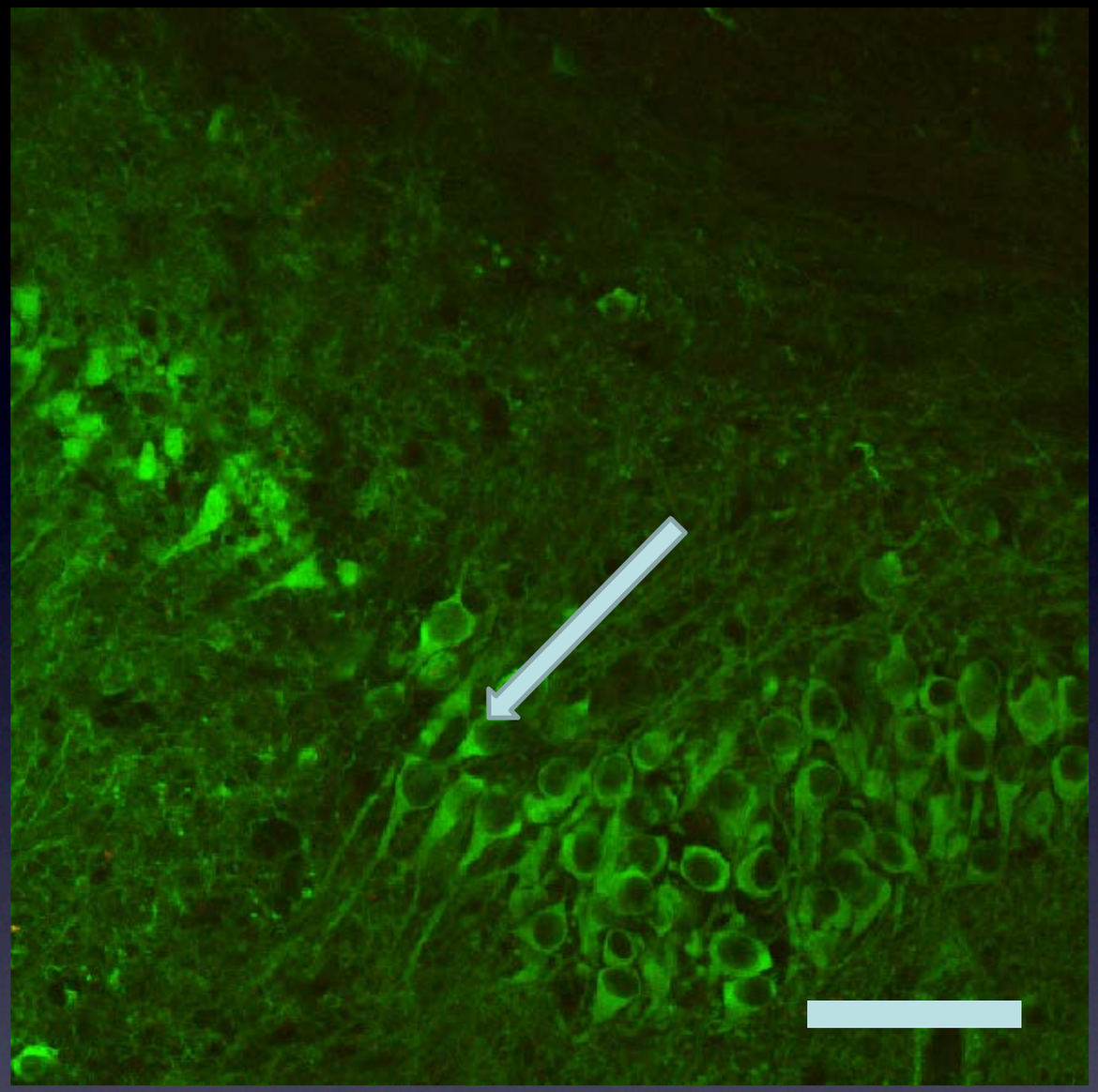
INJURY

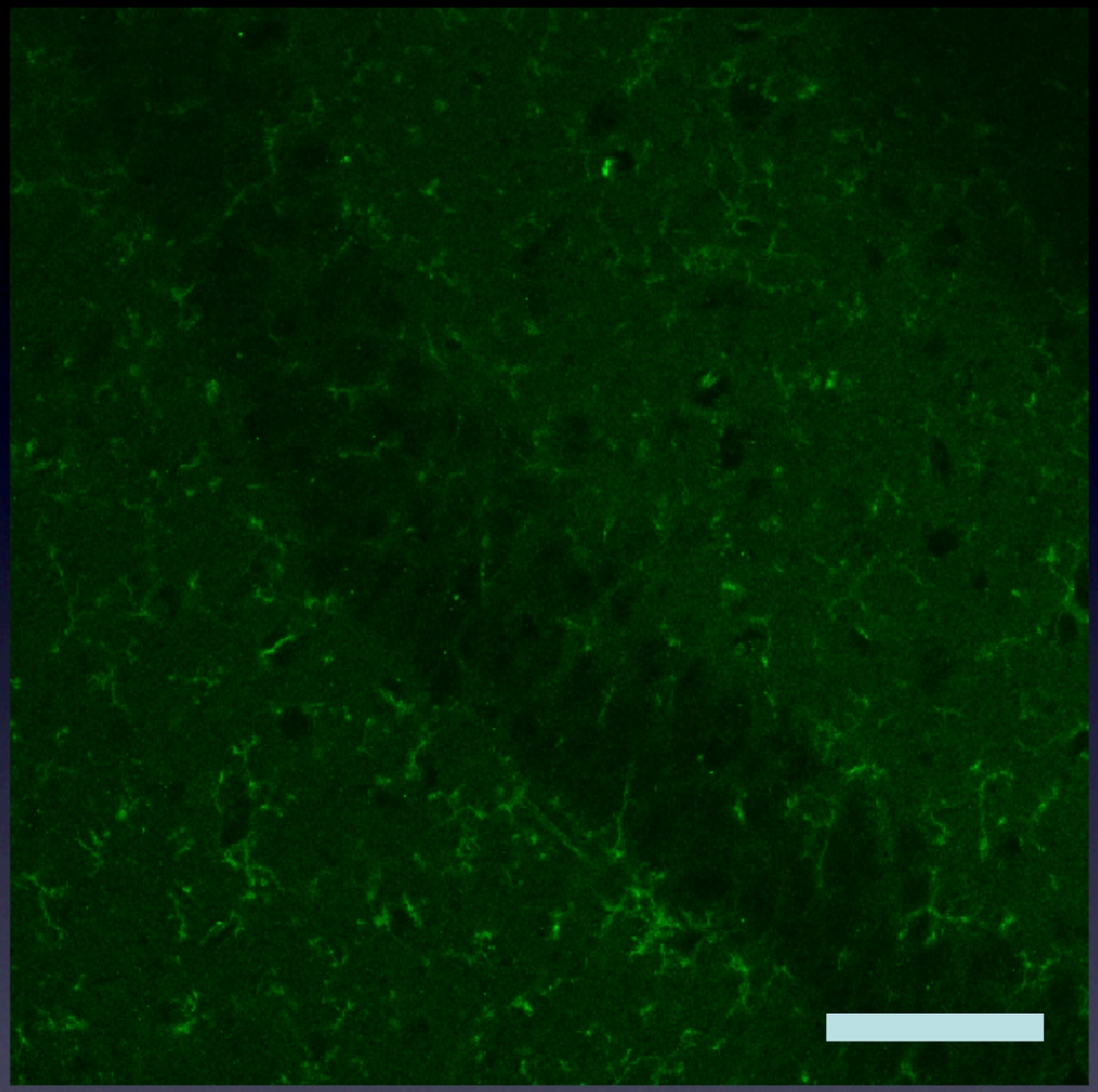
A great majority of endogeneous progenitors during migration strongly express Nlgn

What do you expect from the stem cells?

- Cell replacement
- Influence on cell survival
- Disease modeling
- Activation of endogeneous stem cells

Nlgn

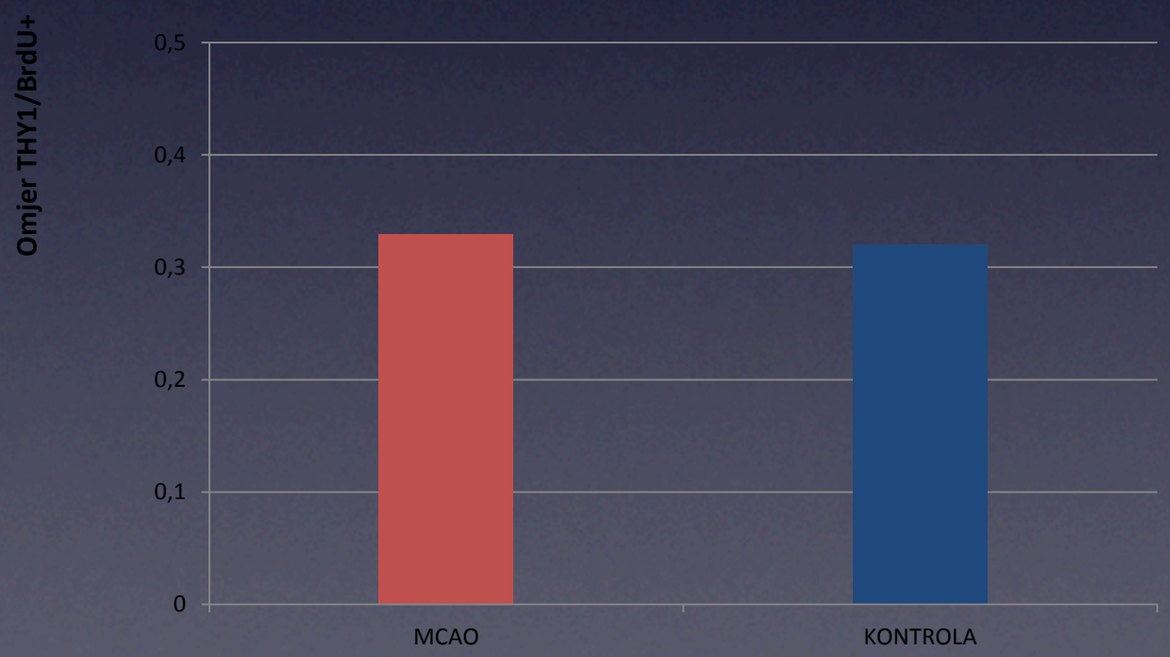




Hippocampus: MCAO vs WT

How does ischemia affects differentiation of NSCs?

Not significantly!

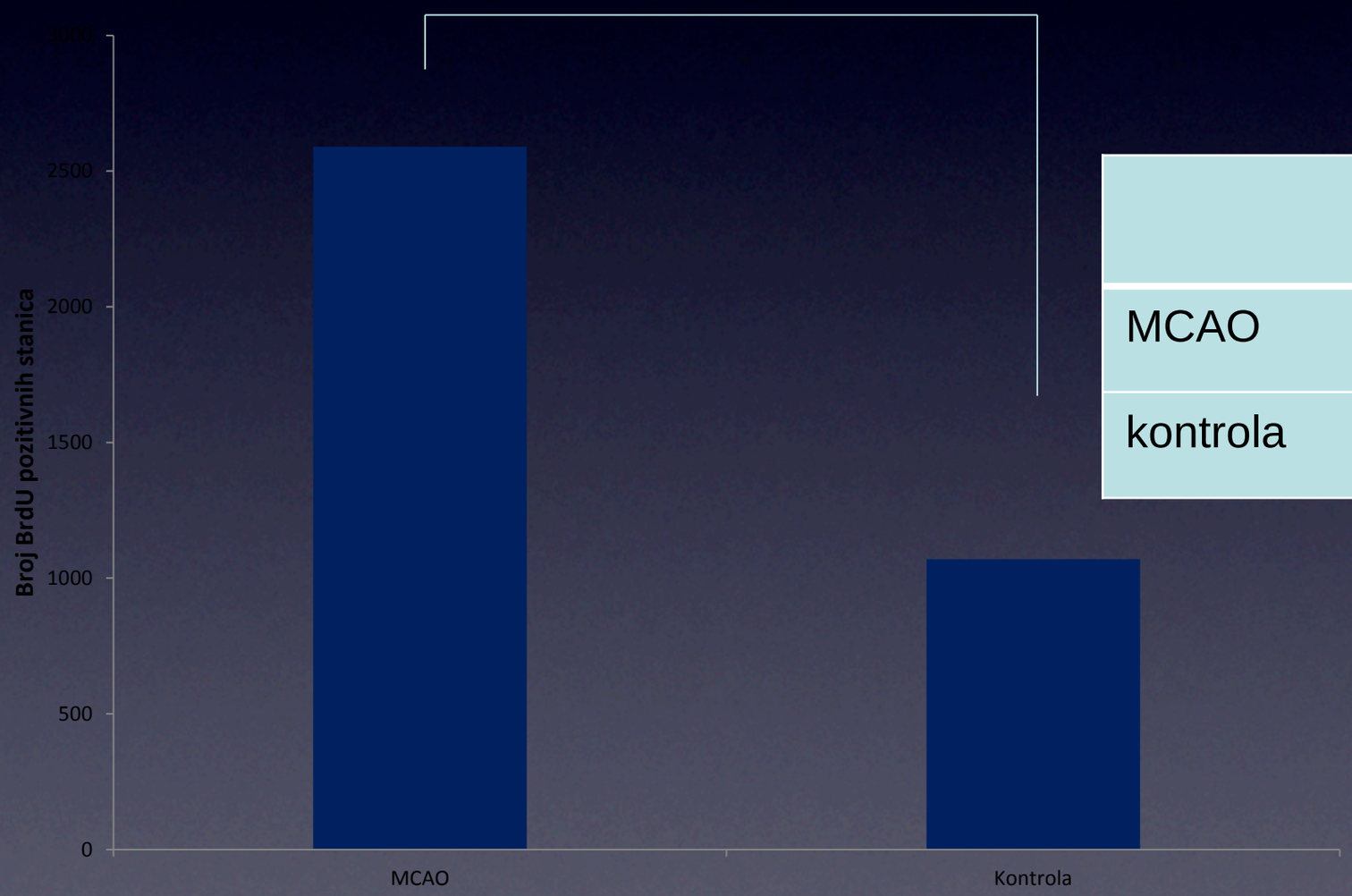


	THY1/BrdU+
MCAO	0,33
kontrola	0,32

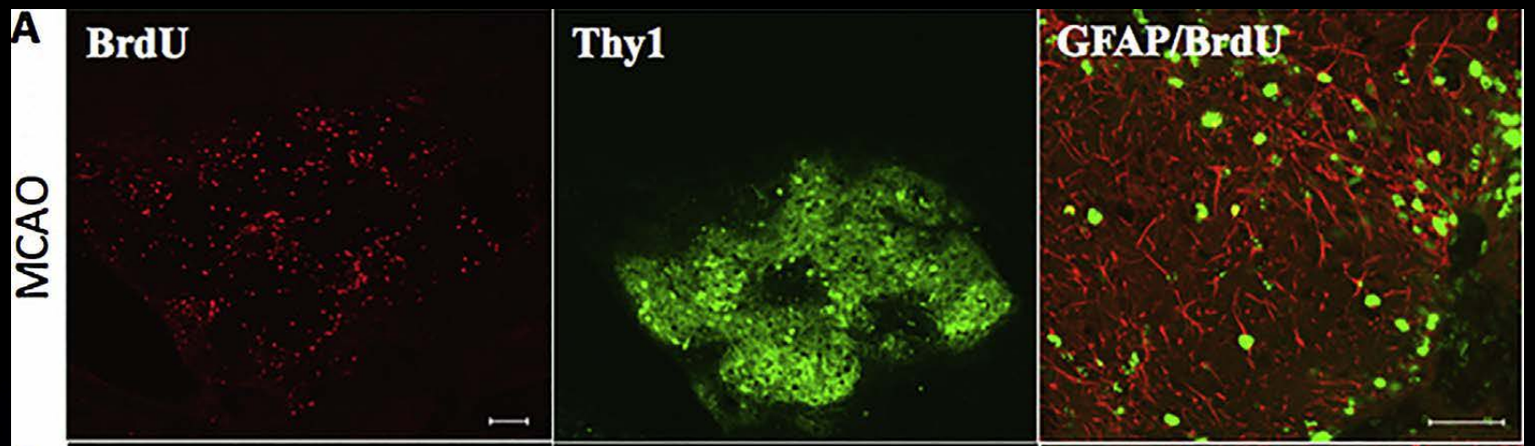
How does ischemia affects survival of NSCs?

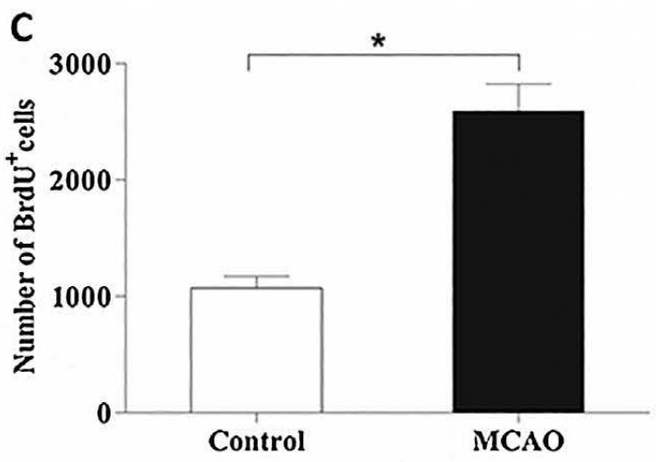
Surprisingly – it supports survival

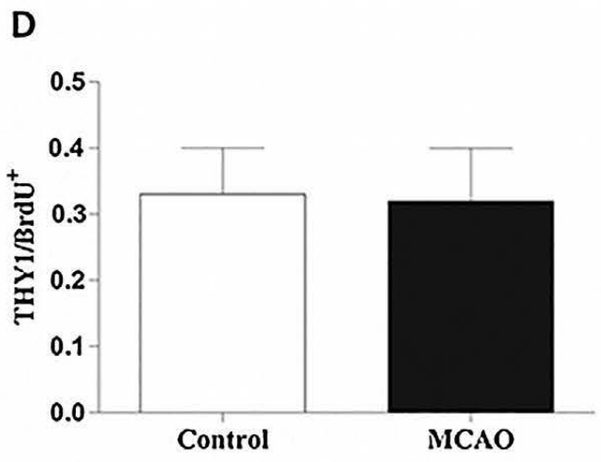
*

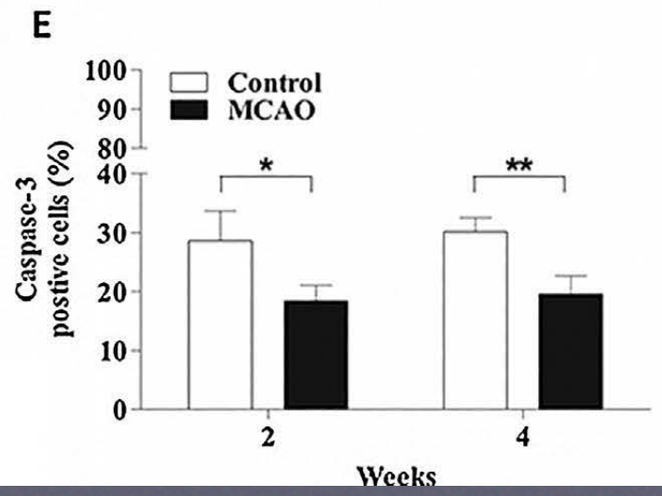


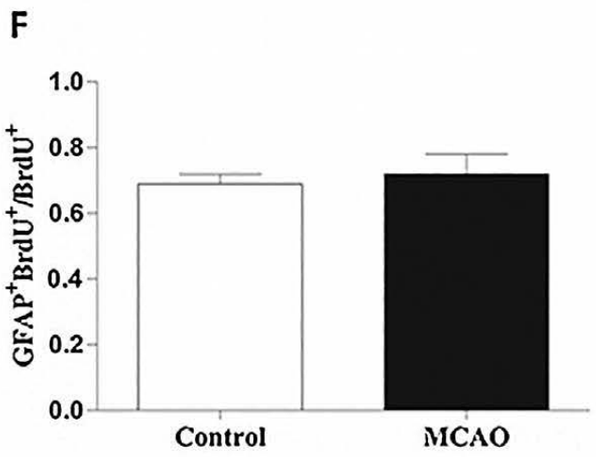
	Average number of cells in the unit
MCAO	2590
kontrola	1070



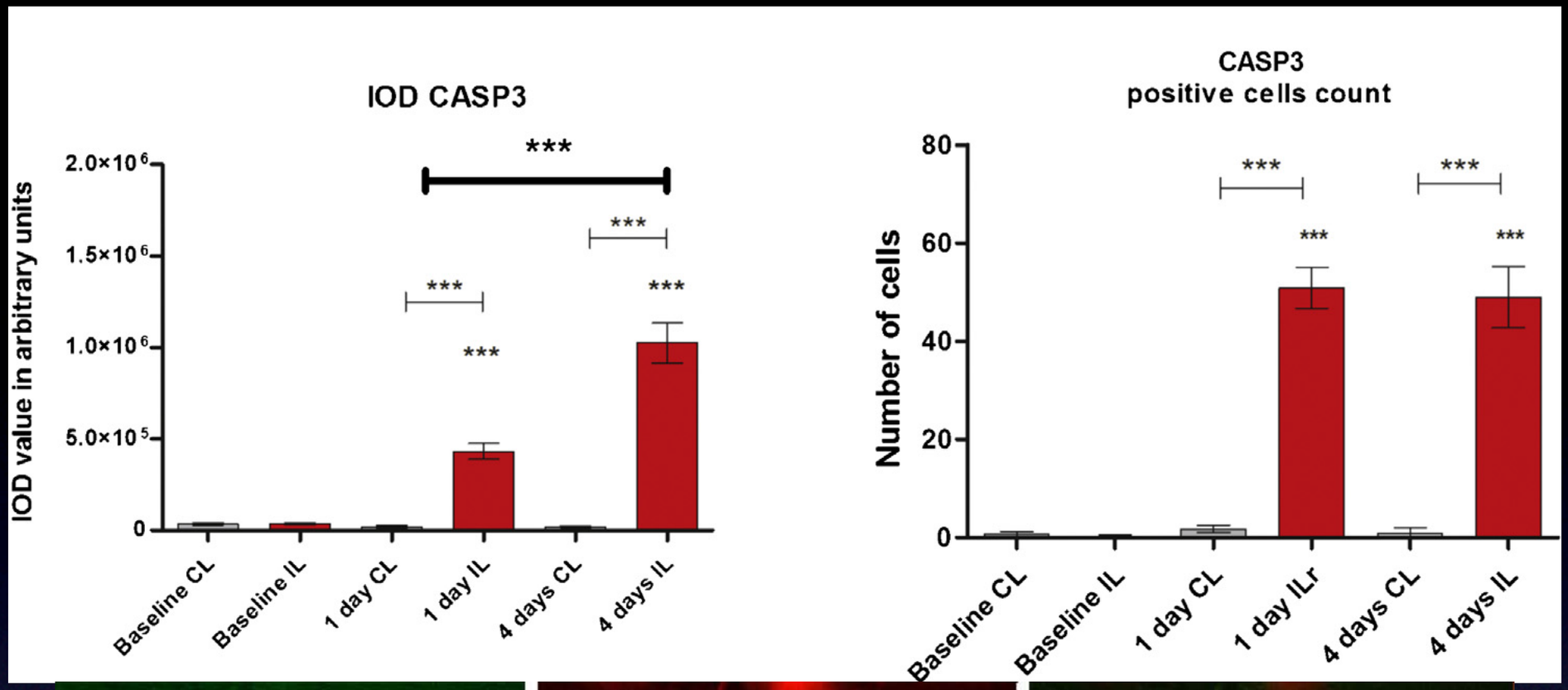


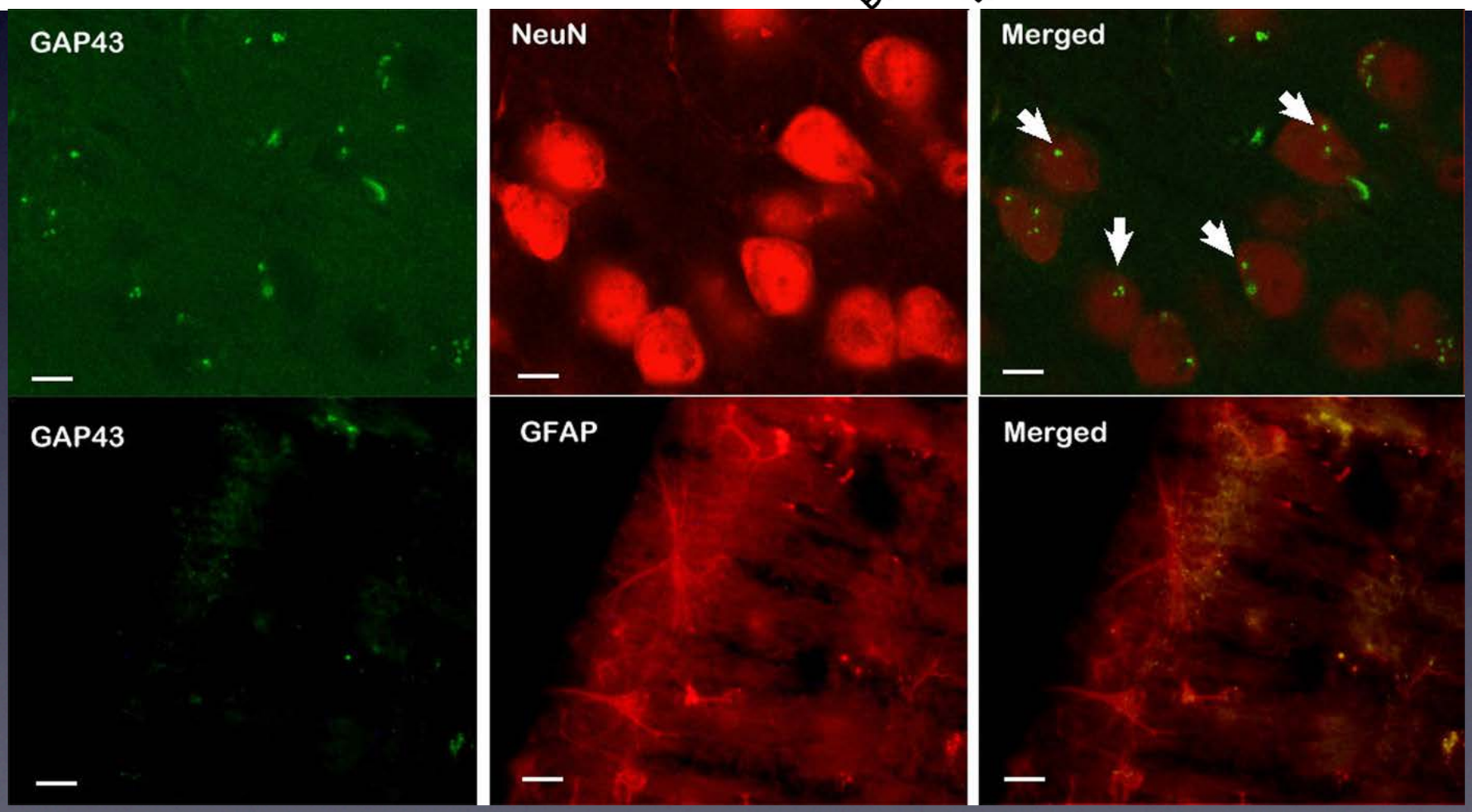






Kosi et al, Neurosci Lett. 2018





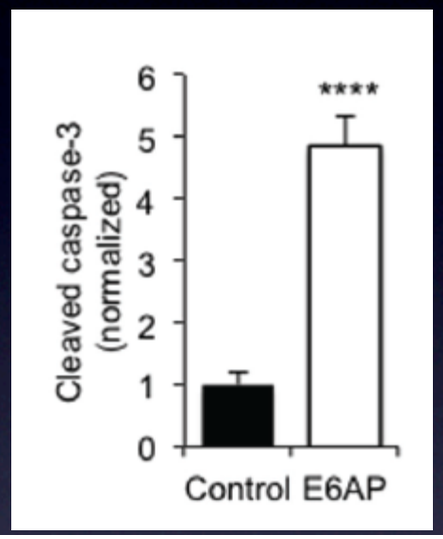
Gorup et al, Neurosci Lett, 2015

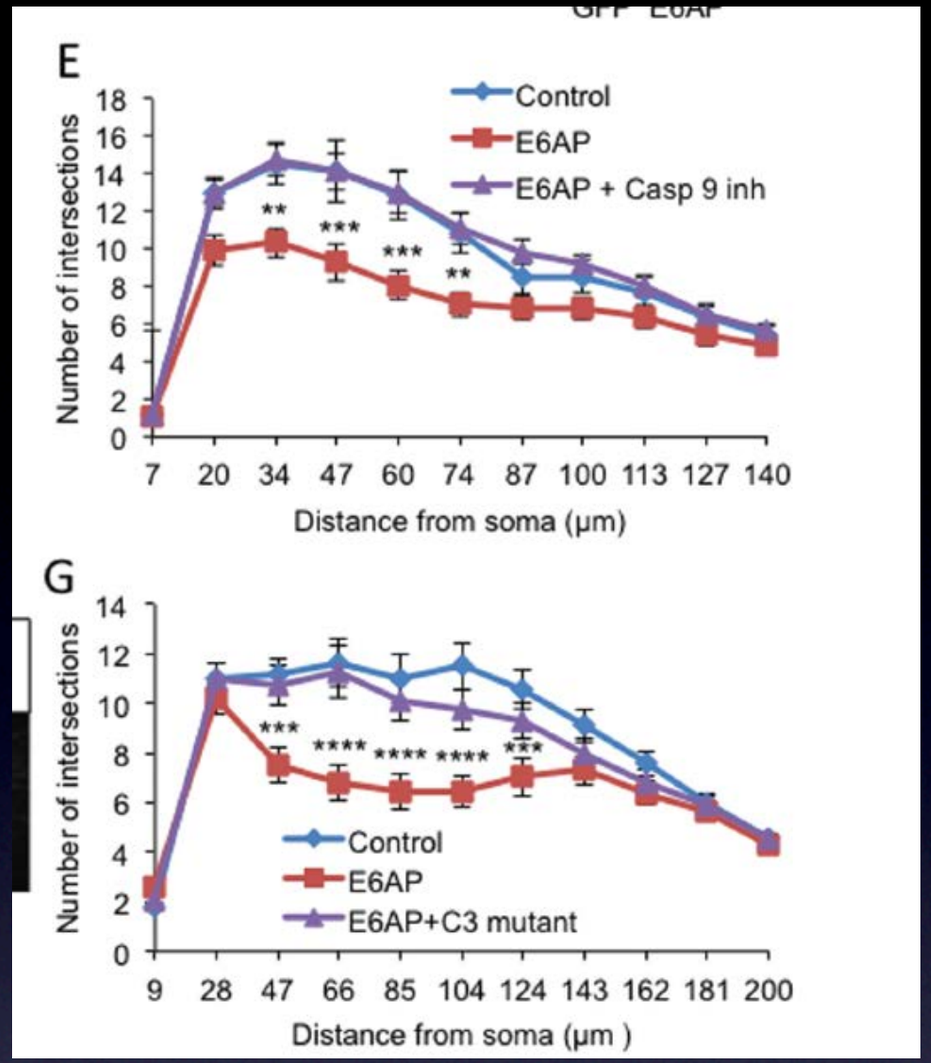
JNeurosci
THE JOURNAL OF NEUROSCIENCE

Research Articles: Neurobiology of Disease

The autism protein Ube3A/E6AP remodels neuronal dendritic arborization via caspase-dependent microtubule destabilization

Natasha Khatri[1,2], James P. Gilbert[1], Yuda Huo[1], Roozhin Sharaflari[1], Michael Nee[1], Hui Qiao[1] and Heng-Ye Man[1,2]





Neuroscience Letters 666 (2018) 111–119

Contents lists available at ScienceDirect

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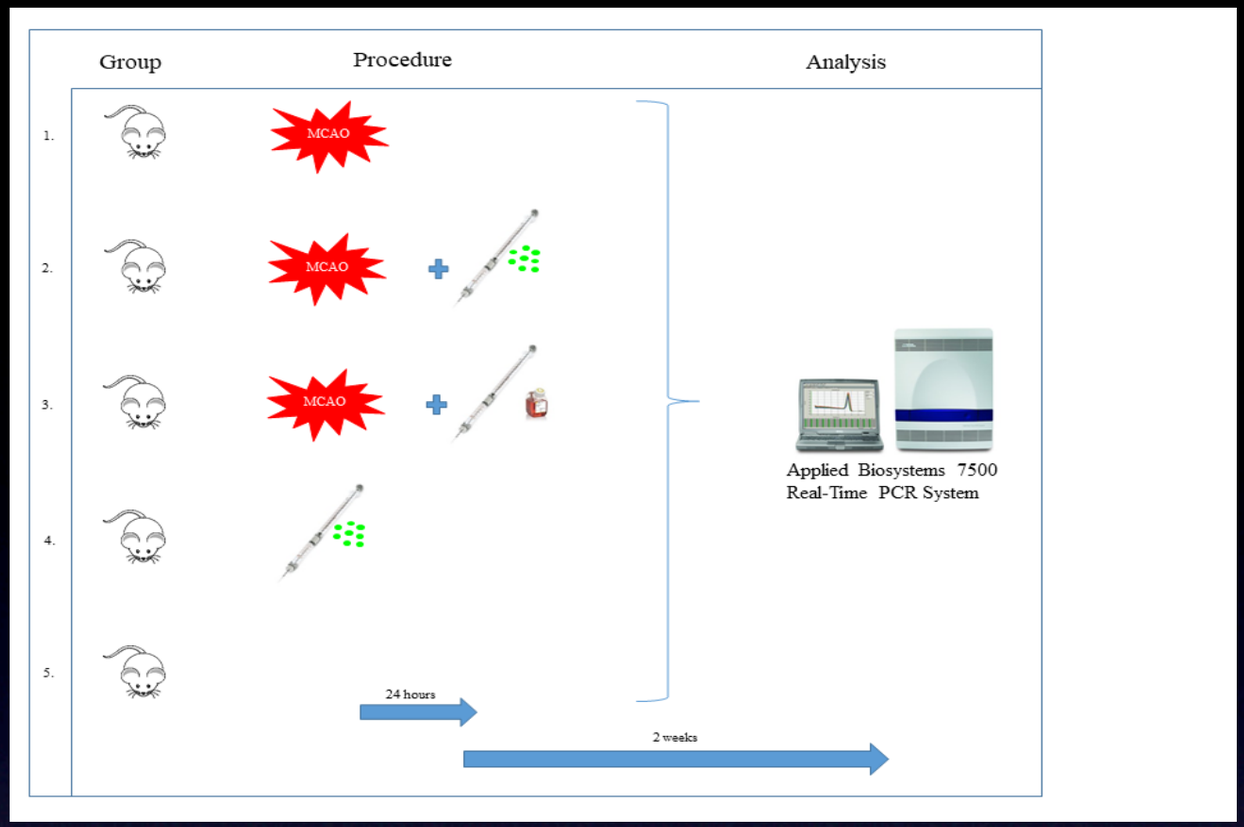
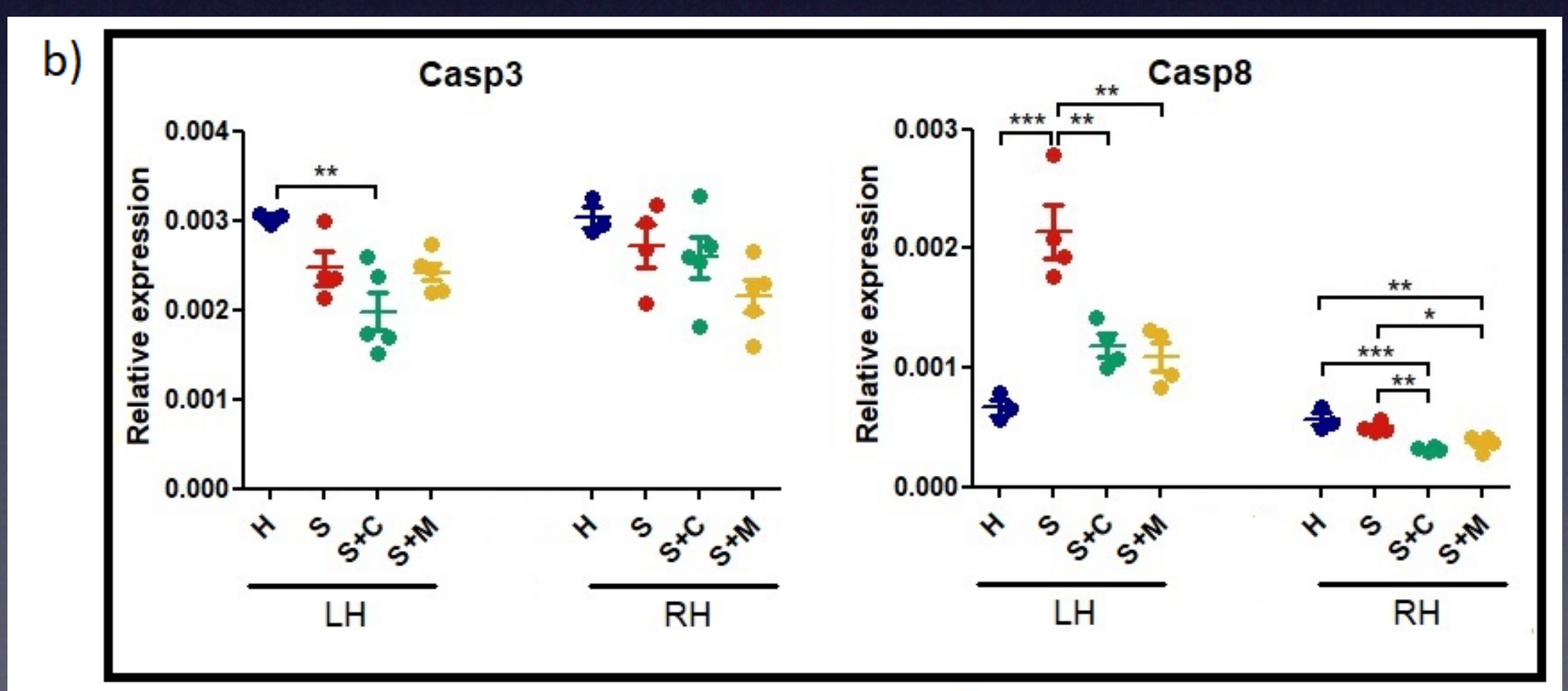
Research article

Stroke promotes survival of nearby transplanted neural stem cells by decreasing their activation of caspase 3 while not affecting their differentiation

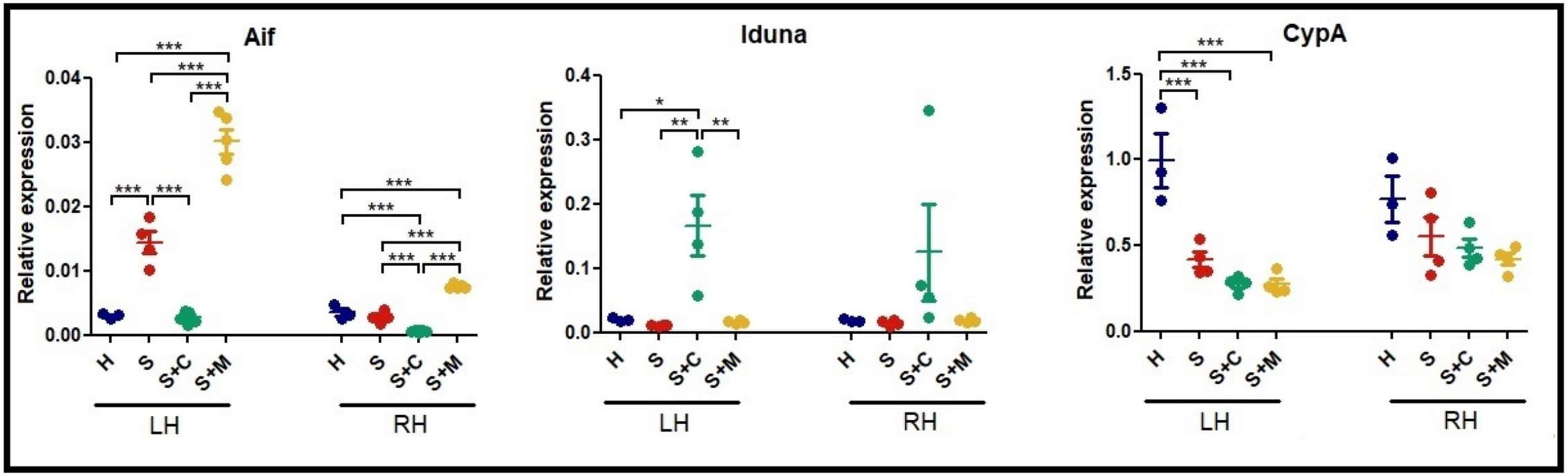
Nina Kosi[a], Ivan Alić[a,b], Iva Salamon[a], Dinko Mitrečić[a,*]

[a] Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia
[b] Faculty of Veterinary Medicine, University of Zagreb, 10 000 Zagreb, Croatia and Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

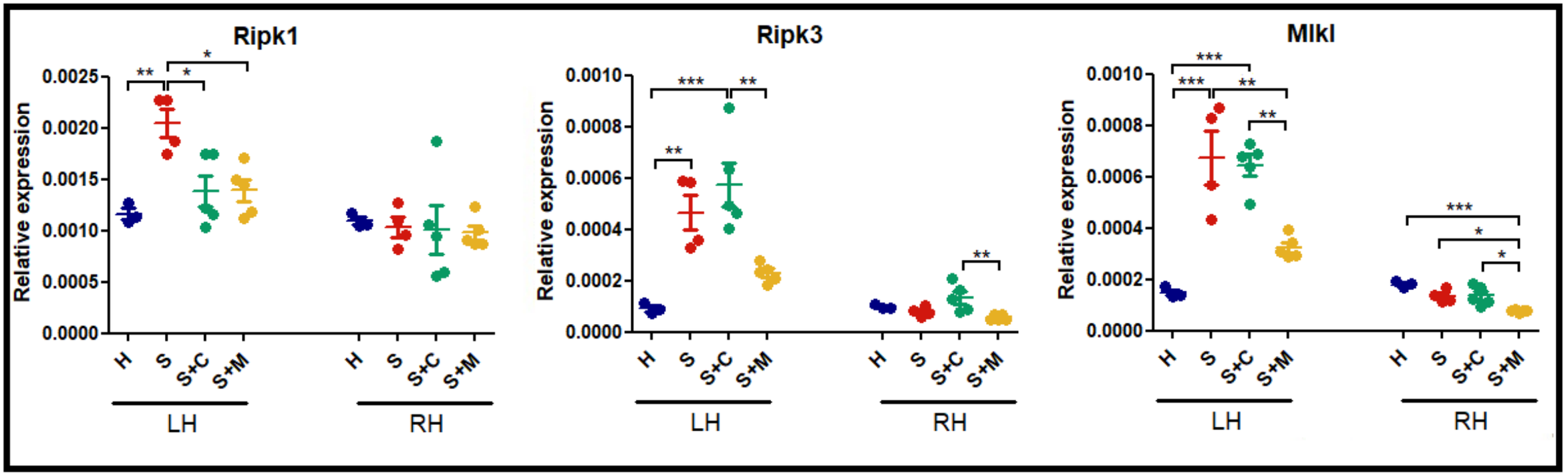

Group
Procedure
Analysis
1.
2.
3.
4.
5.
MCAO
MCAO
MCAO
24 hours
2 weeks
Applied Biosystems 7500
Real-Time PCR System


b)
Casp3
Relative expression
0.004
0.003
0.002
0.001
0.000
**
H
S
S+C
S+M
LH
RH
Casp8
***
**
**
*
H
S
S+C
S+M
LH
RH

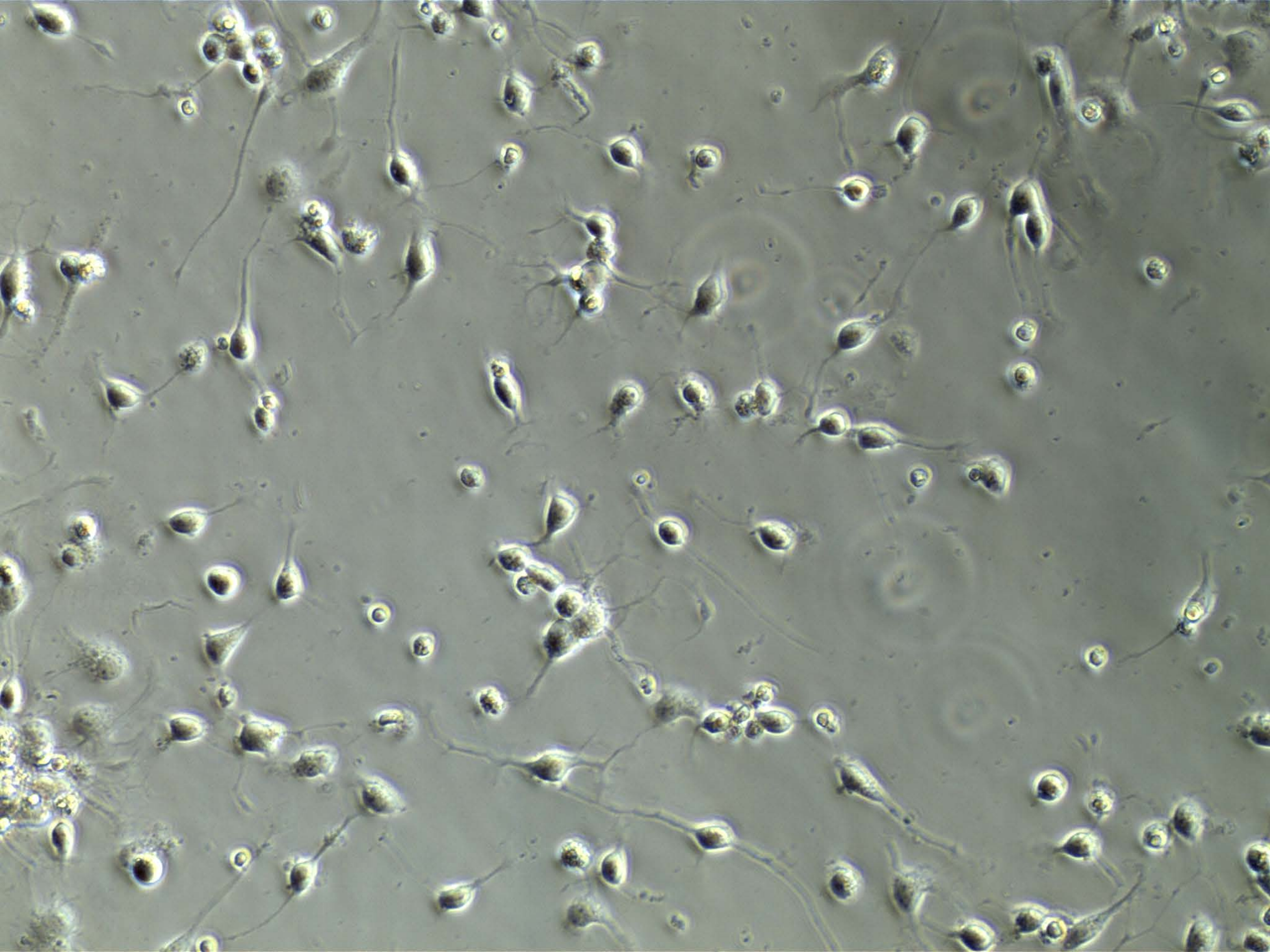
a)


Aif
Iduna
CypA
Relative expression
H
S
S+C
S+M
LH
RH

b)


Ripk1
Ripk3
Mlkl
Relative expression
H
S
S+C
S+M
LH
RH


EVOS FL Auto
Nabavljeno uz pomoć Europske unije iz projekta:
"Mladi Mozak"
FONDOVI



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CROATIAN INSTITUTE FOR BRAIN RESEARCH

Laboratory for Stem Cells

Laboratorij za matične stanice