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Adult Neural Stem Cells

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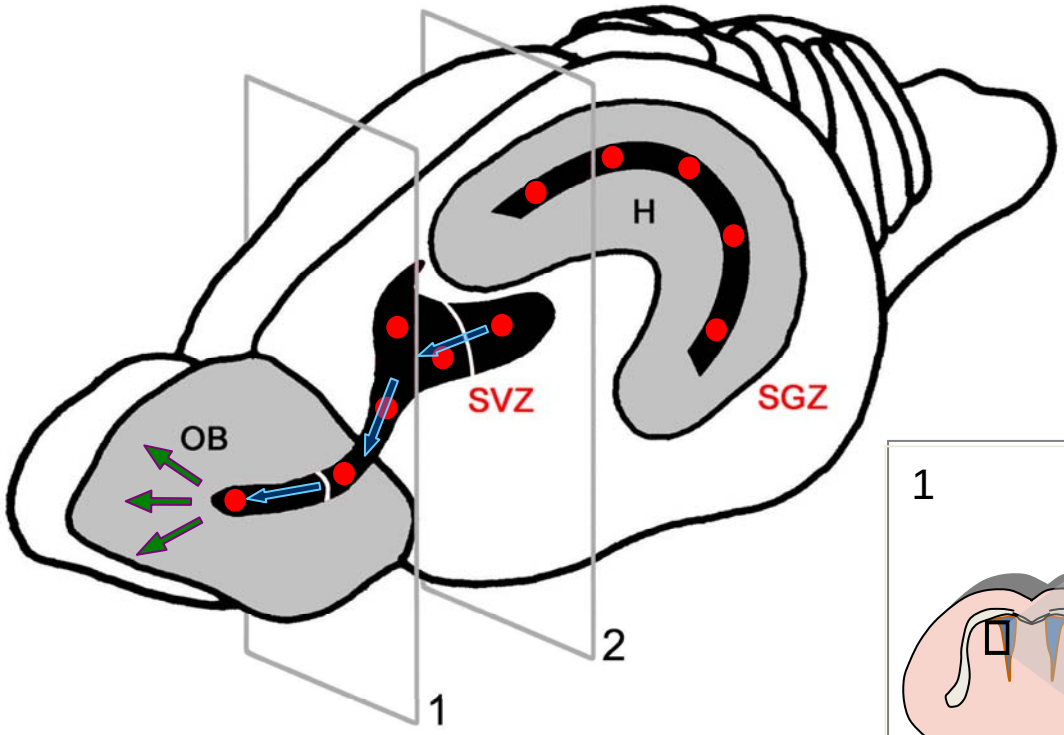
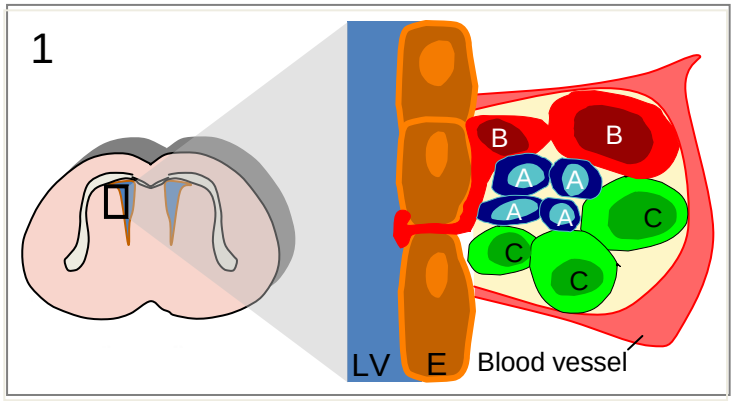
SGZ = sub-granular zone
SVZ = sub-ventricular zone
OB = olfactory bulb

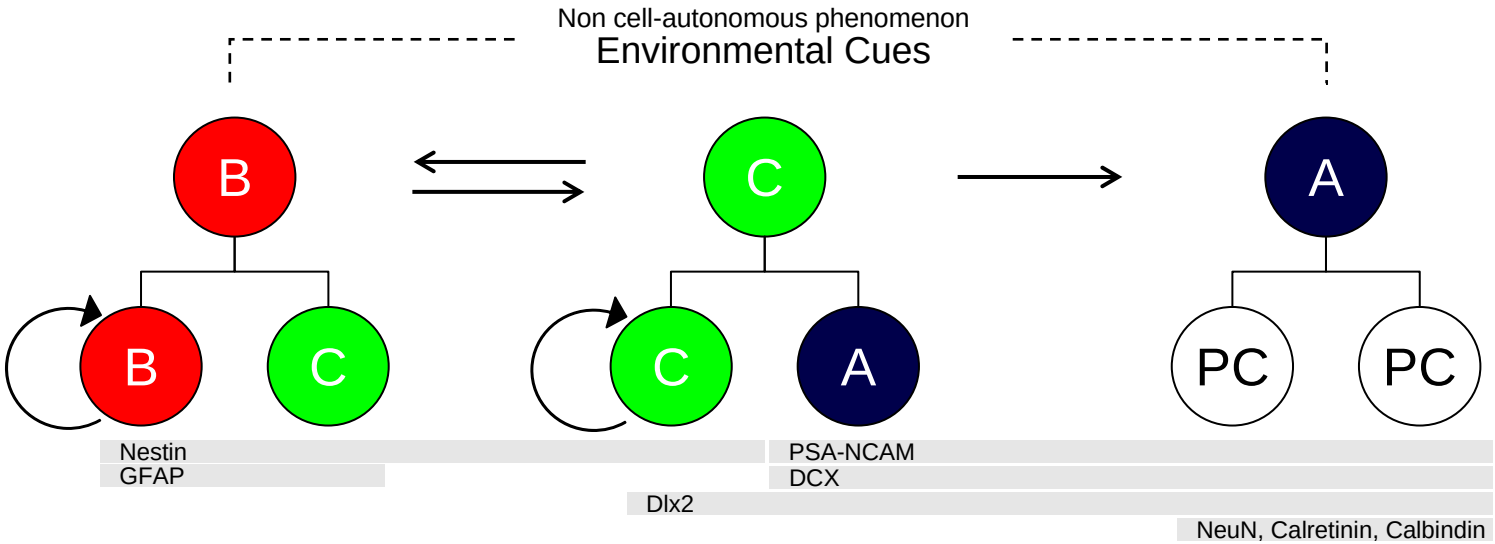




Tangential migration
Radial migration
(2 weeks)

SVZ stem cell compartment (niche)
B = SVZ astrocyte; C = Transit amplifying cell; A = Neuroblast

Adult (somatic) NPCs: the nomenclature



SVZ astrocyte
(type-1 cell)
Self-renewing
Proliferative (long cell-cycle)
Multipotent (all neuroectodermal lineages)

Transit amplifying cell
(type-2a type-2b type-3 cells)
Limited self-renewal
Proliferative (short cell-cycle)
Multipotent (at least two different lineages)

Neuroblast
(post-mitotic immature neurons)
Unipotent: one specific cell lineage (neuronal, glial, astroglial, oligodendroglial)

Neural Stem Cells

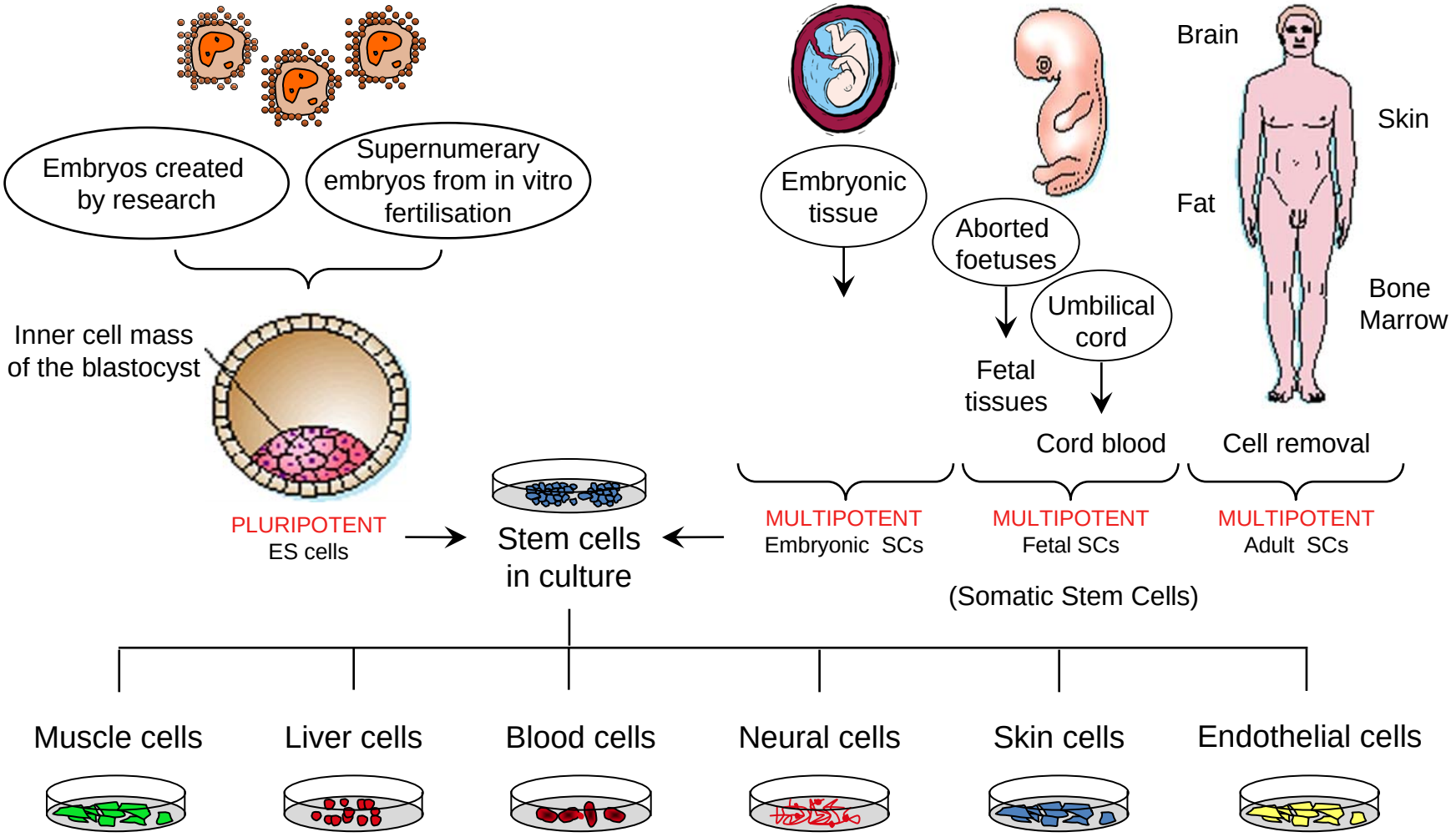
Multipotent Progenitors

Lineage-specific Precursors

Precursors or Progenitors

Adult Neural Precursor Cells (aNPCs)

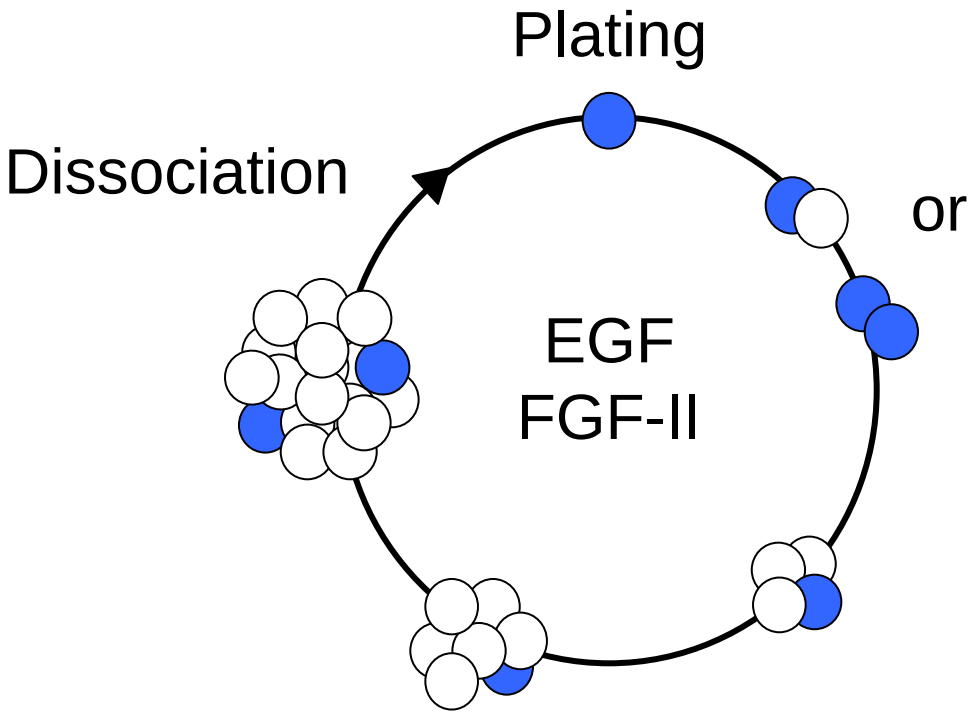
Nat. Rev. Neurosci. 2006



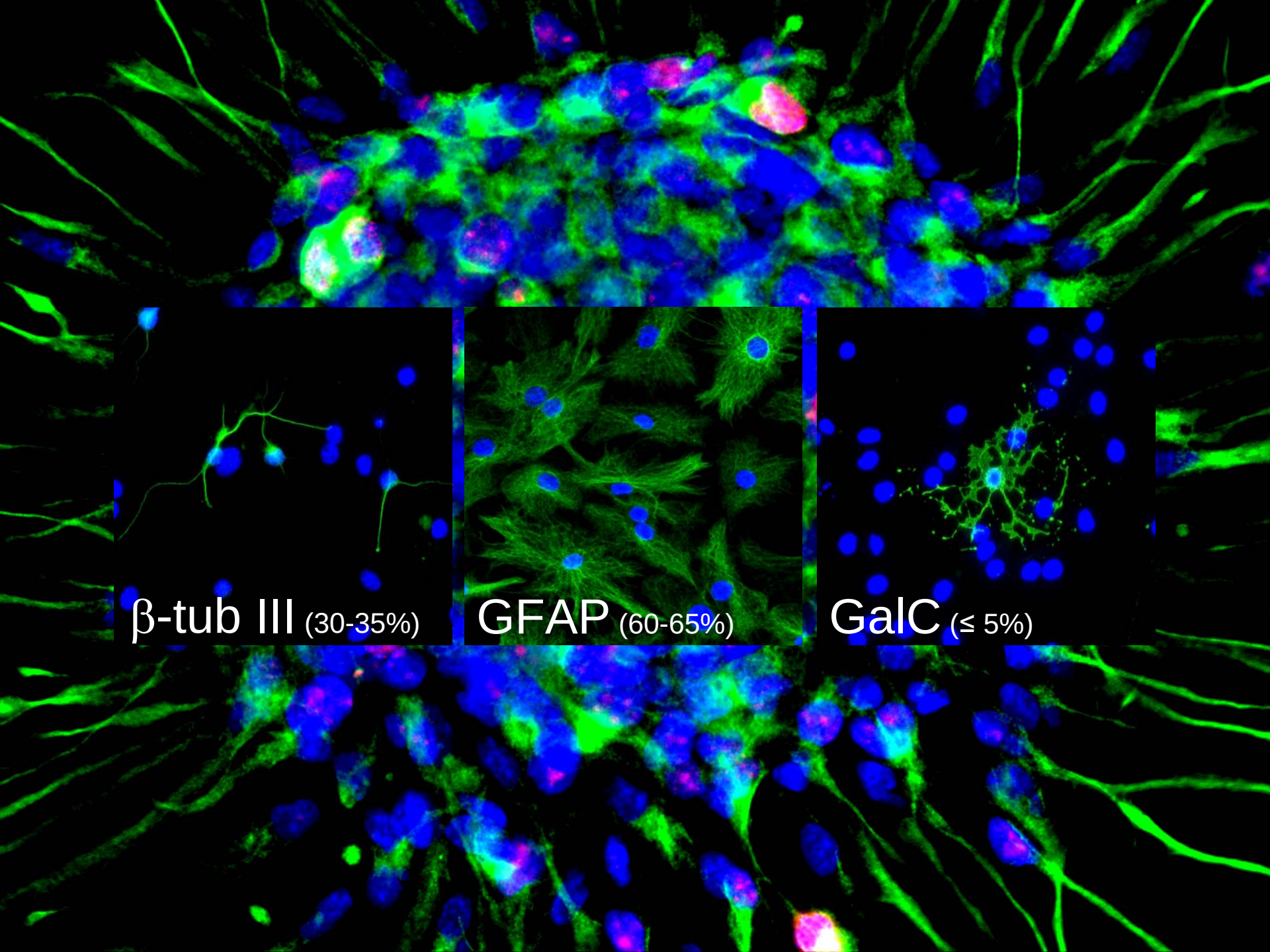
Embryonic stem (ES) cells: intrinsic tumorigenic potential, undetermined differentiation potential.
Somatic (fetal and adult) stem cells: limited growth potential

Neurosci. Lett. 2009

In vitro epigenetic aNPCs propagation



Neurosphere-forming cell (stem cell)


β-tub III (30-35%)
GFAP (60-65%)
GalC (≤ 5%)

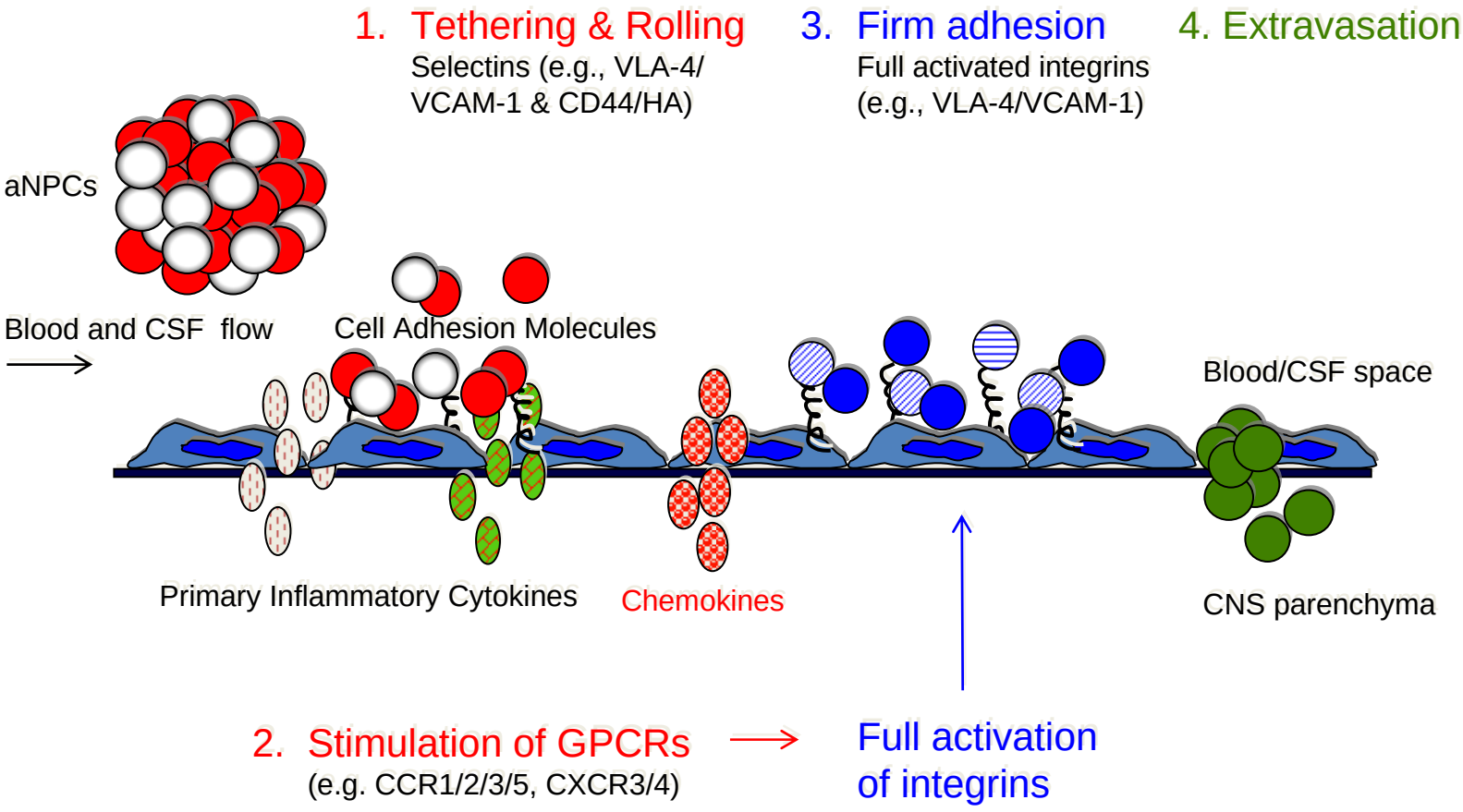
Cell administration route

aNPCs: routes of delivery

- **Intraparencymal administration**
  - Single site
  - Multiple sites
- **Intrathecal administration**
  - Lumbar
  - Cisternal
  - Intraventricular
- **Systemic administration**
  - Intravenous
  - Intra-arterial

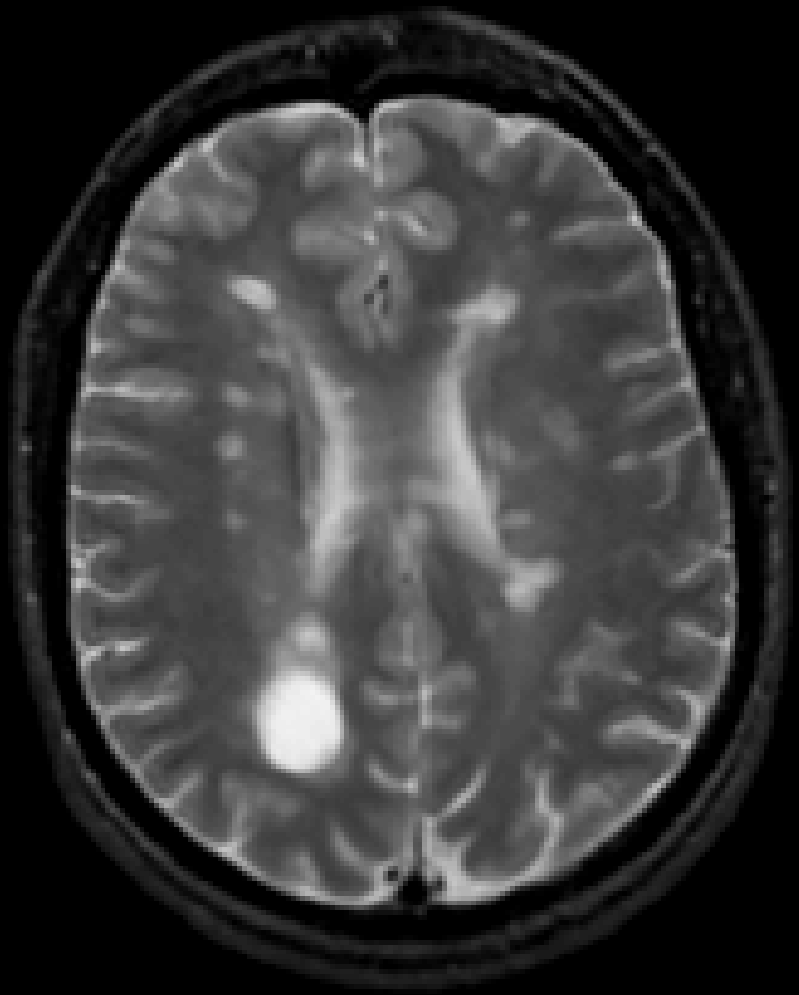
- **Focal disorders:**
  - Parkinson’s Disease
  - Huntington’s Disease
  - Spinal cord Injury
  - Ischemic Stroke
  - Brain Tumours
- **Multifocal disorders:**
  - Multiple Sclerosis
  - Alzheimer’s Disease
  - Amyotrophic Lateral Sclerosis
  - Lysosomal Storage Disorders
  - Dysmyelinating Disorders

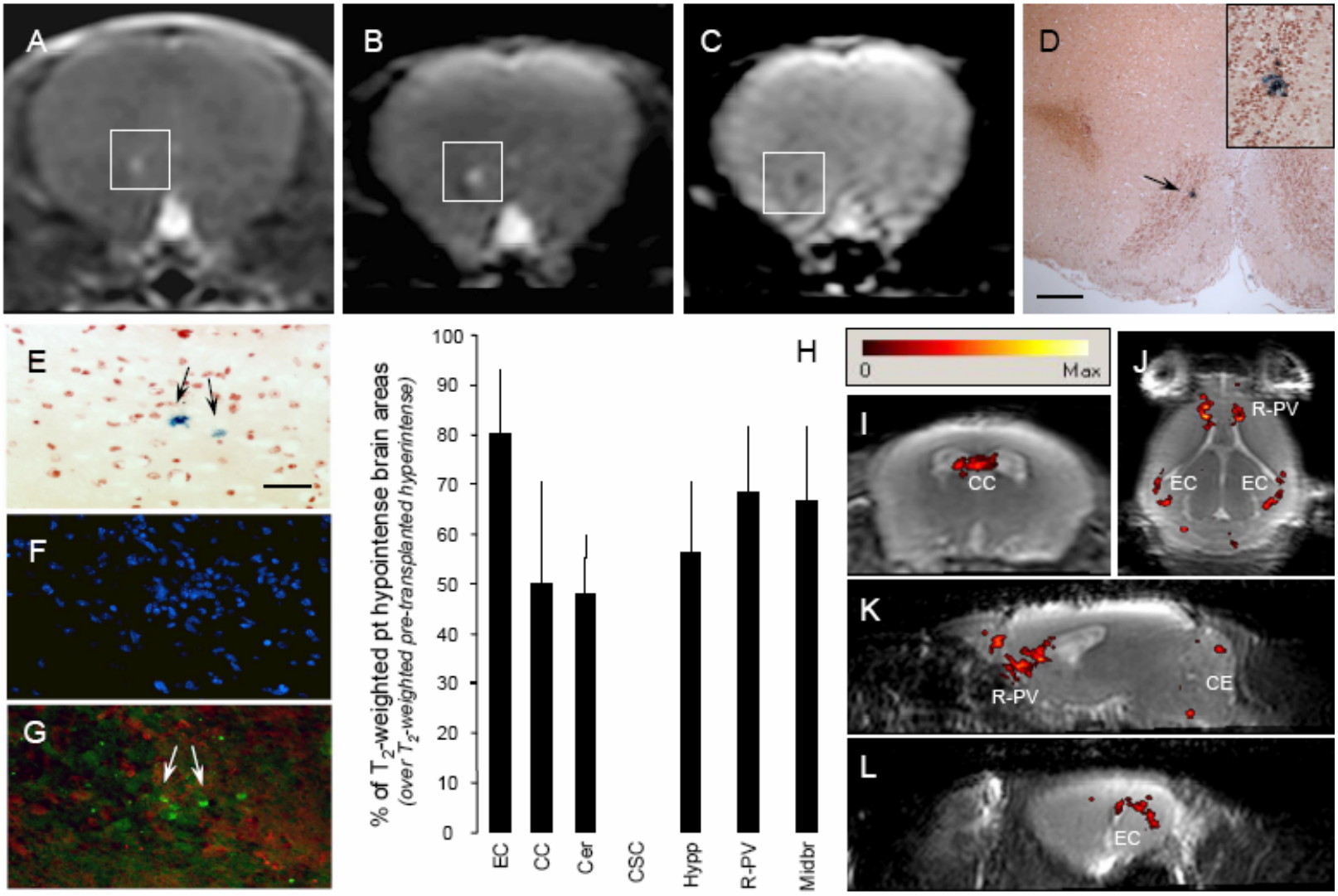
Neural stem cell pathotropism



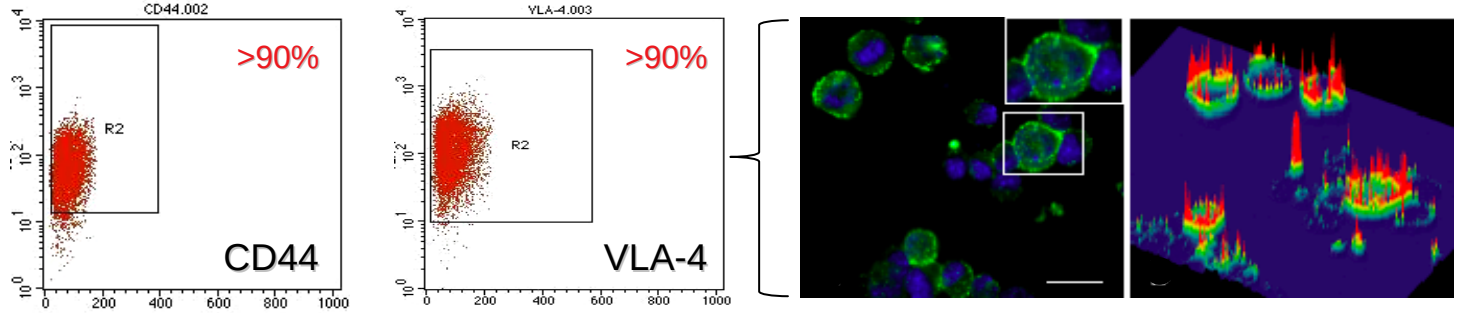
Nat Rev Neurosci, 2006

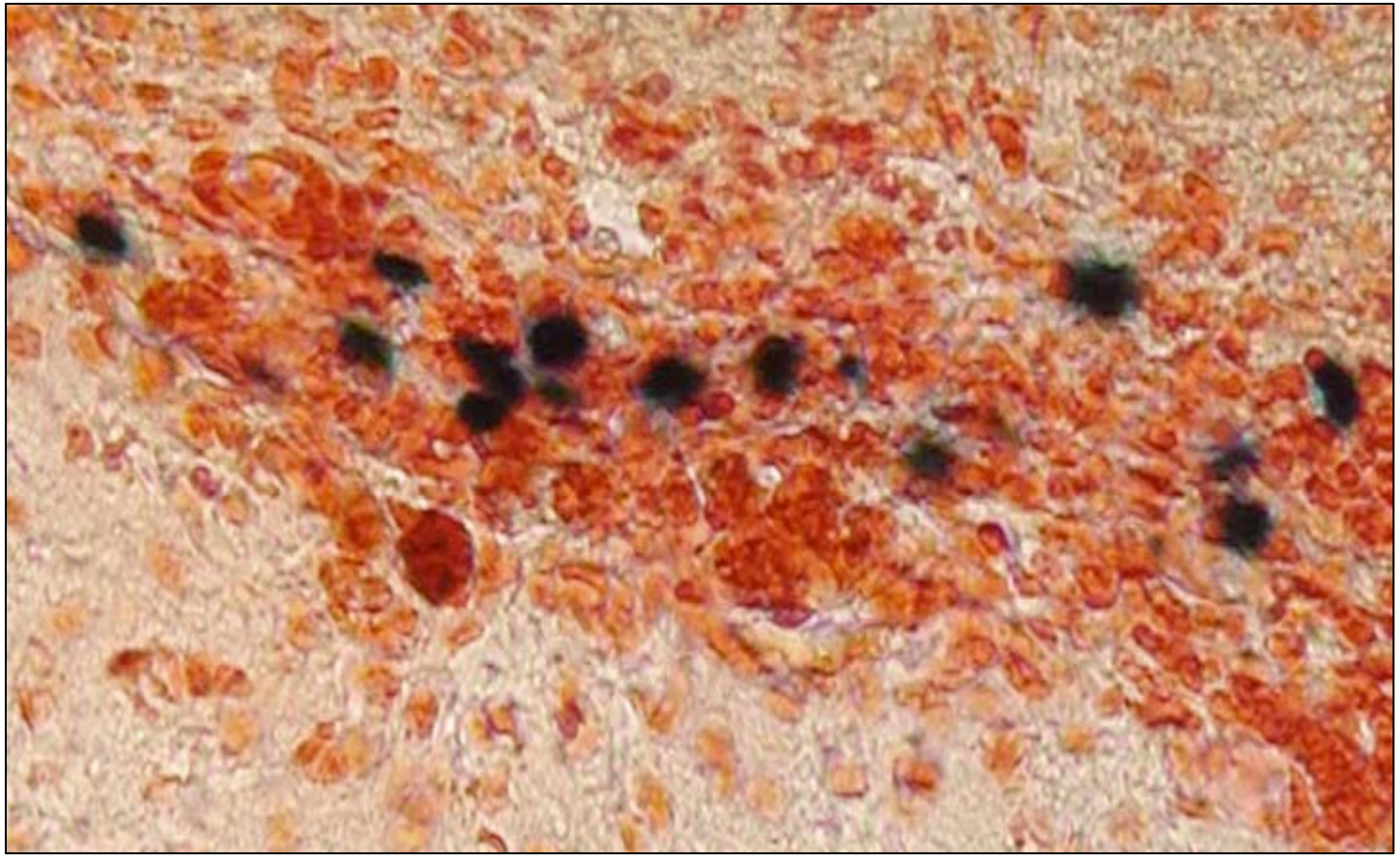
Multiple Sclerosis



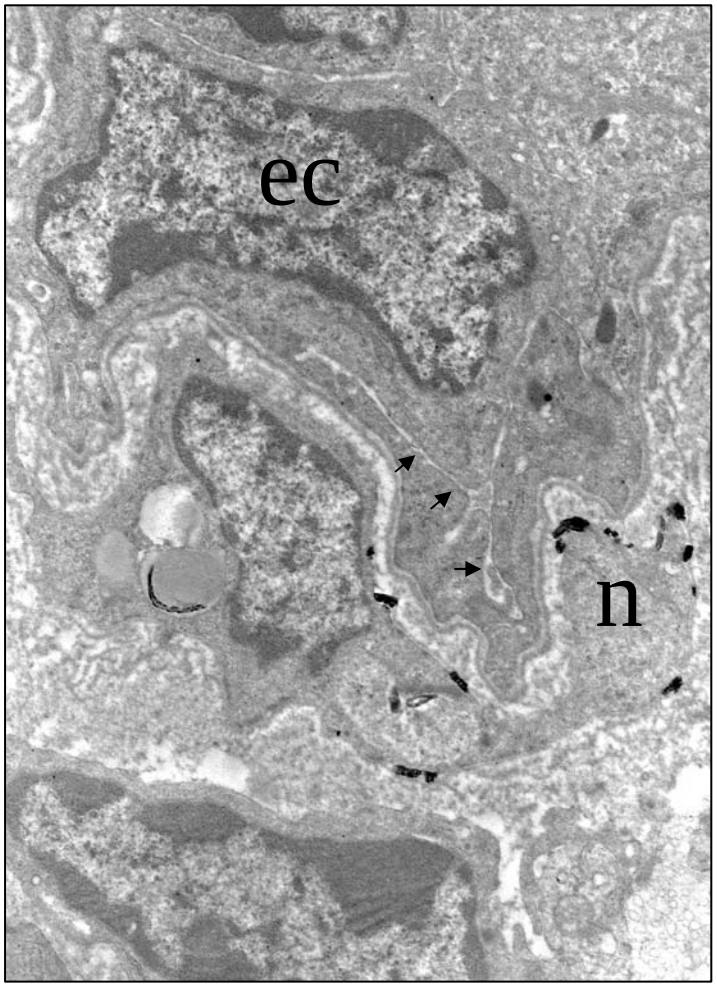


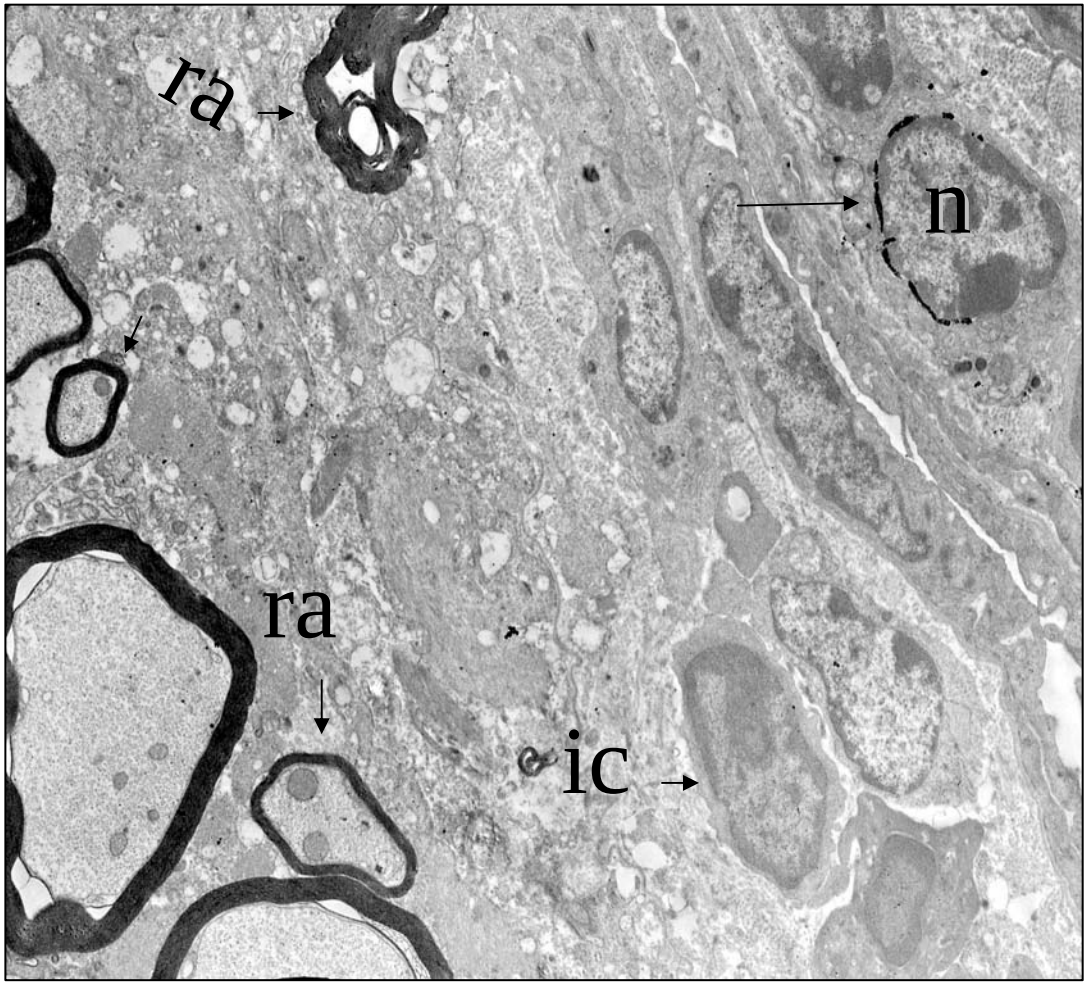
Stem Cells, 2007

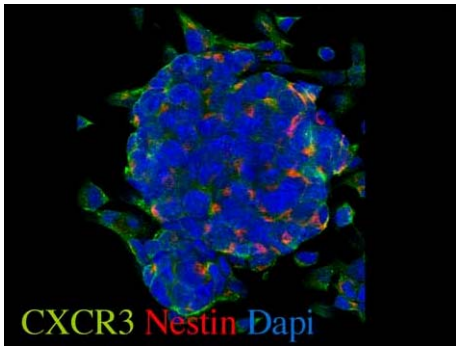


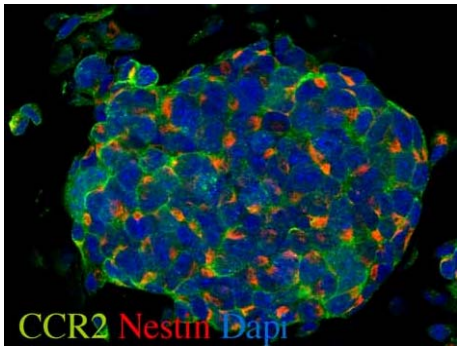


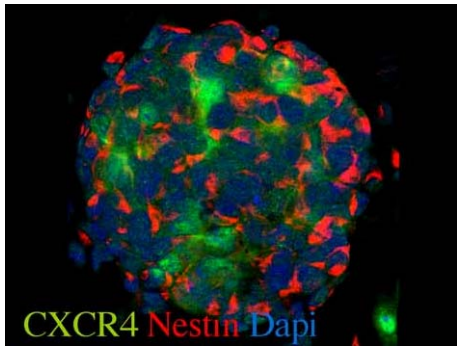
Nature, 2003

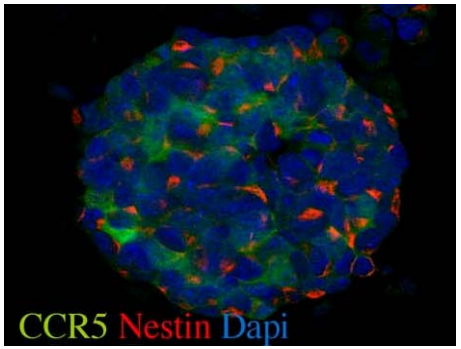

ec
n


ra
n
ra
ic

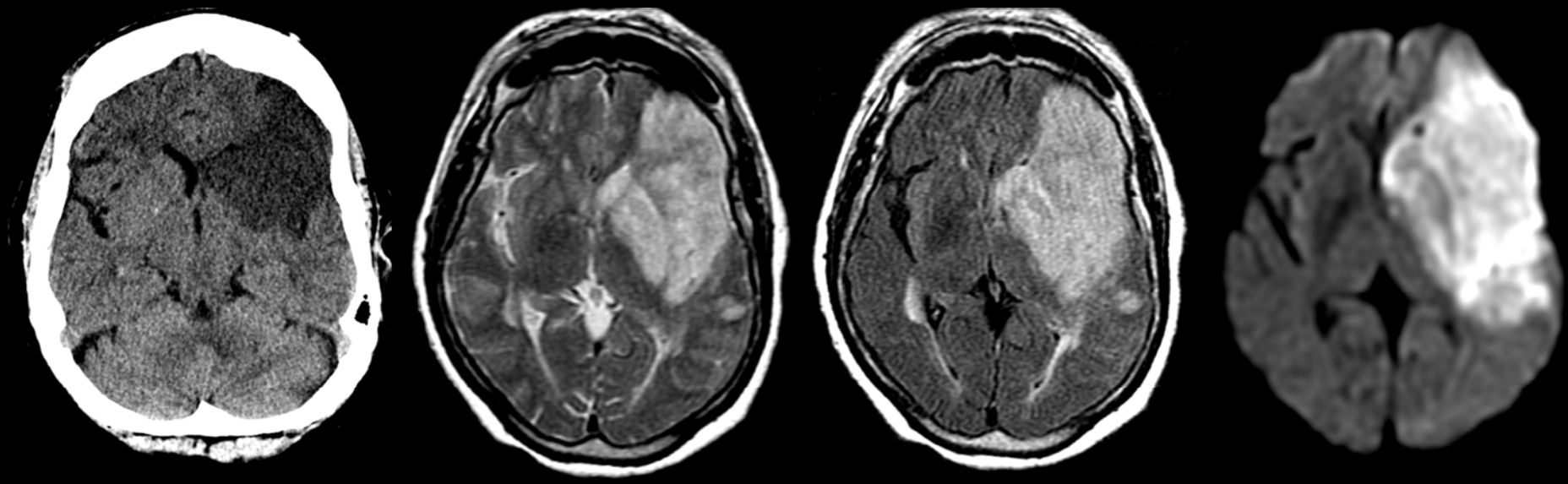

CXCR3 Nestin Dapi

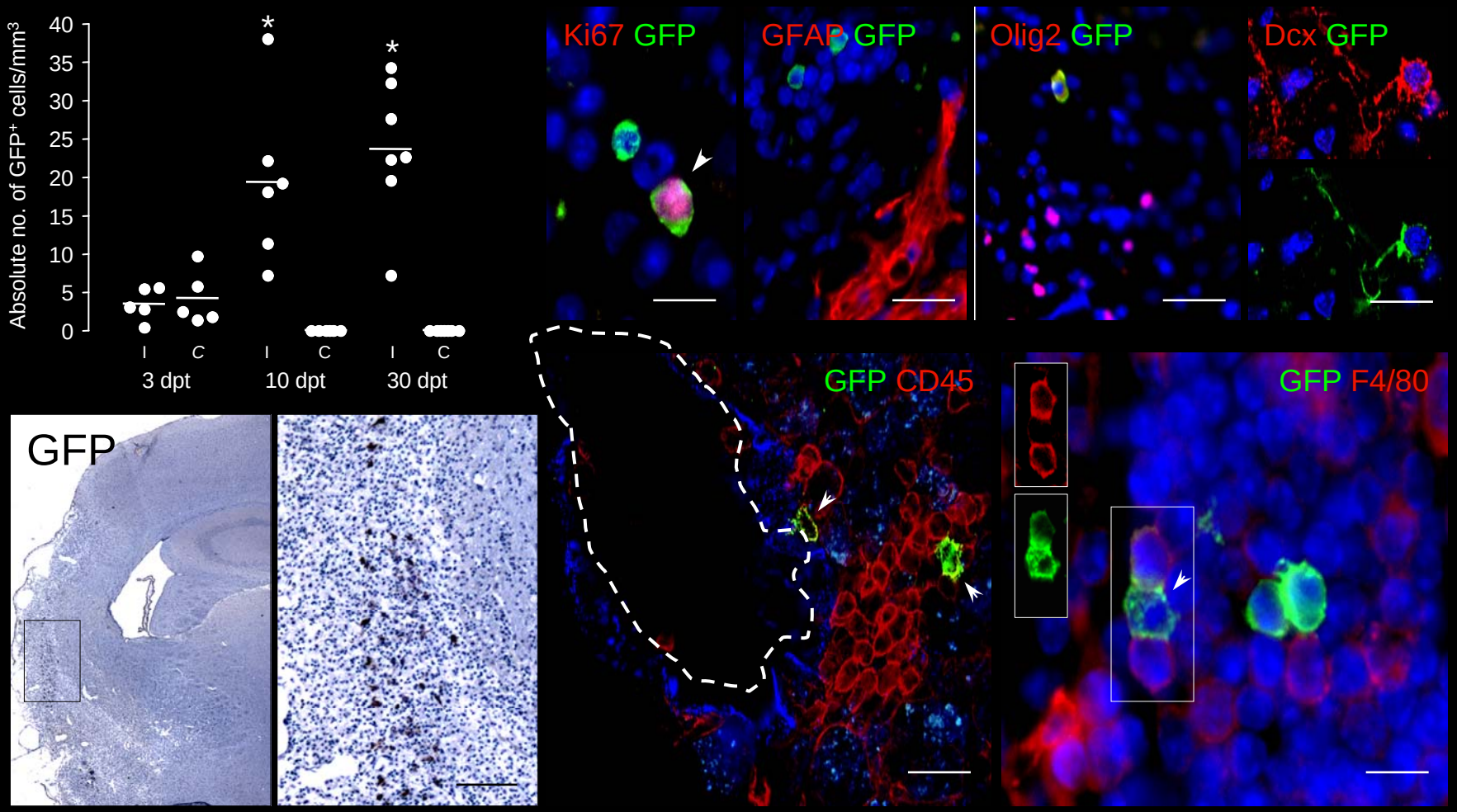

CCR2 Nestin Dapi

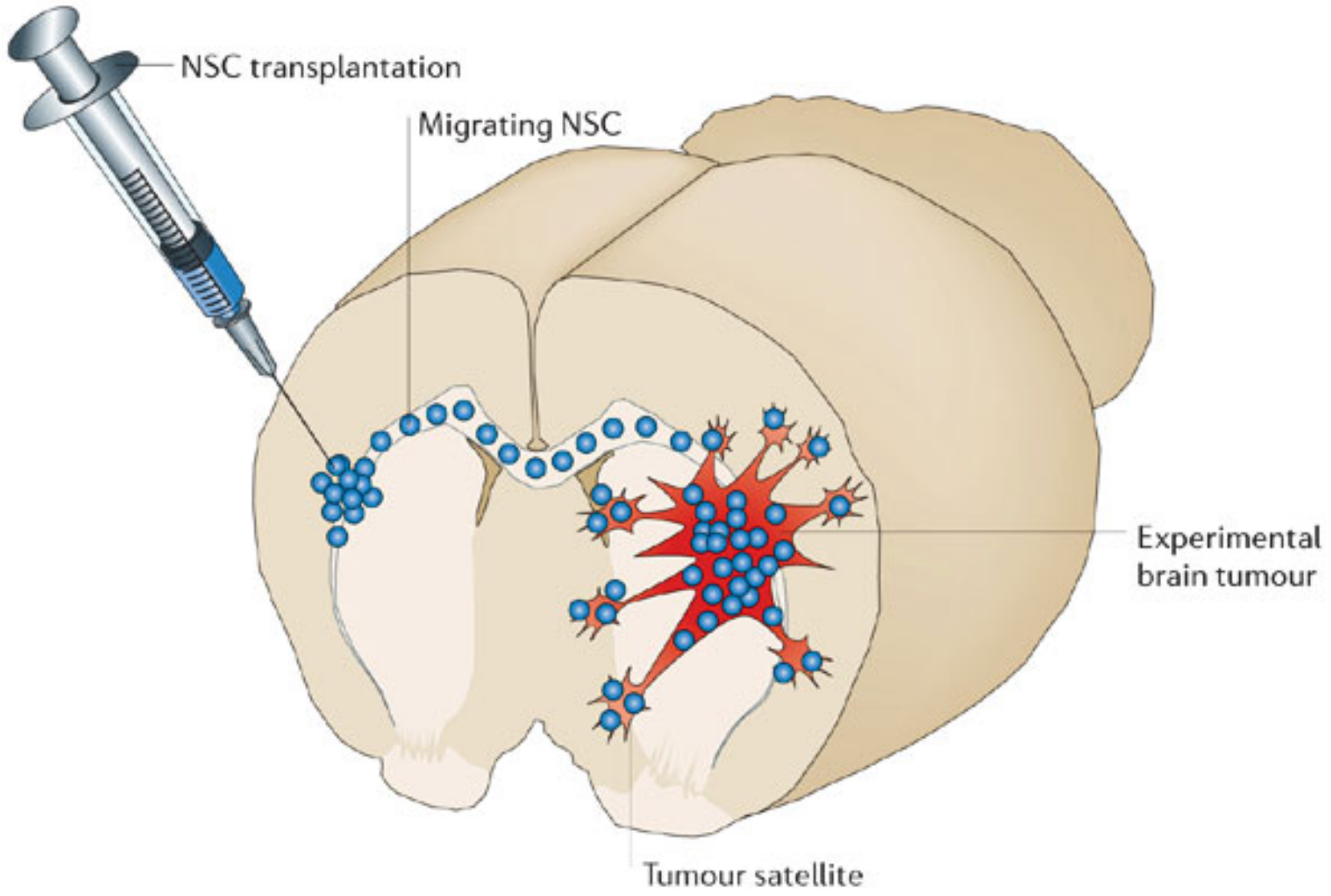

CXCR4 Nestin Dapi


CCR5 Nestin Dapi

Ischemic Stroke




Absolute no. of GFP+ cells/mm3
40
35
30
25
20
15
10
5
0
I
C
I
C
I
C
3 dpt
10 dpt
30 dpt
*
*
Ki67 GFP
GFAP GFP
Olig2 GFP
Dcx GFP
GFP
GFP CD45
GFP F4/80



Nat. Rev. Neurosci. 2006;7:75–84

aNPC therapeutic plasticity

aNPC Therapeutic Plasticity

- Replacement of damaged cells (exogenous repair):
  - Functional integration of differentiated cells
- Bystander activity (endogenous repair):
  - Immunemodulation:
  - Inhibition of T cell proliferation
  - Fostering of pro-inflammatory (Th1) cell apoptosis
  - Inhibition of dendritic cell (DC) antigen presentation capacity
  - Neurotrophic support:
  - Inhibition of scar formation
  - Inhibition of neural cell apoptosis

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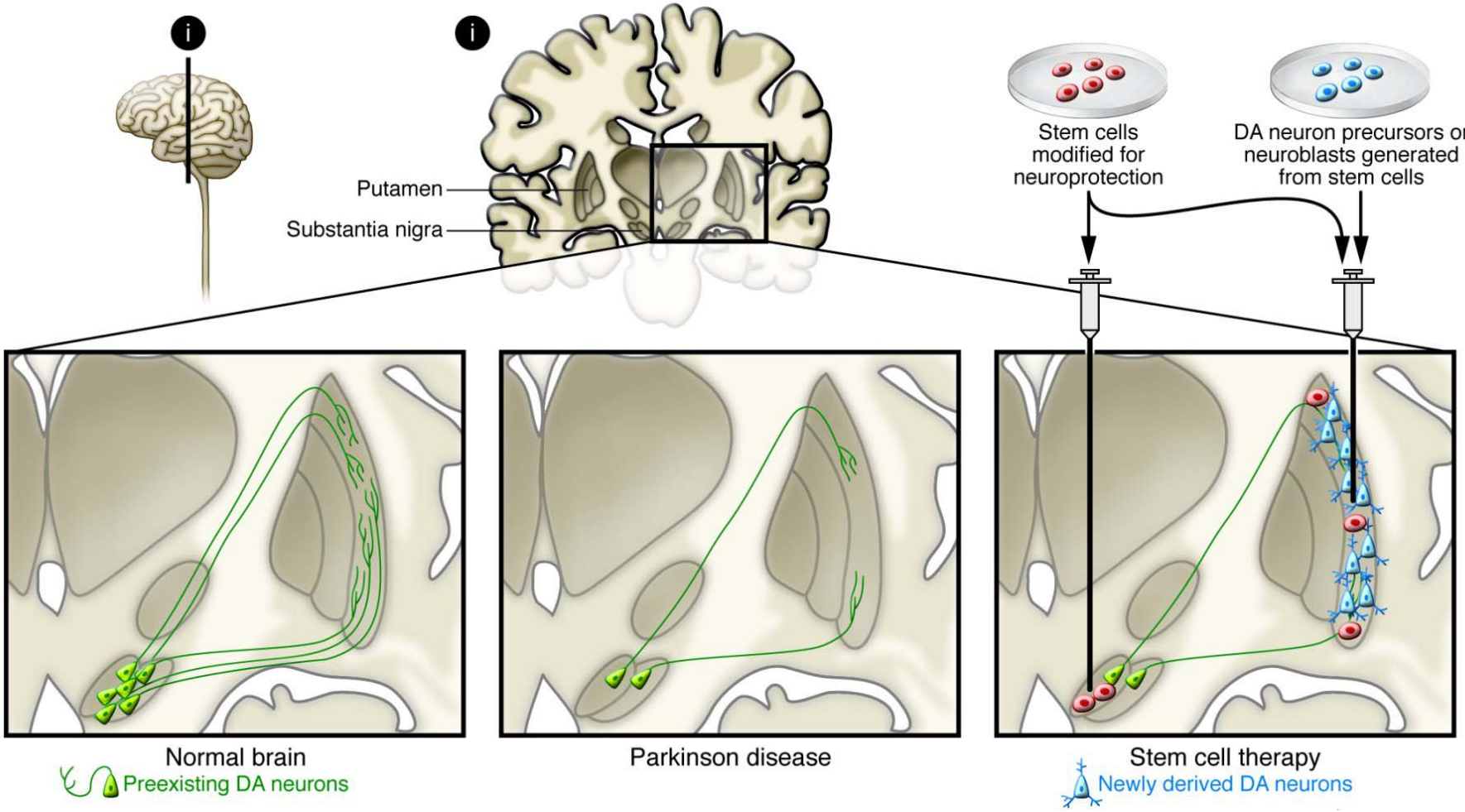
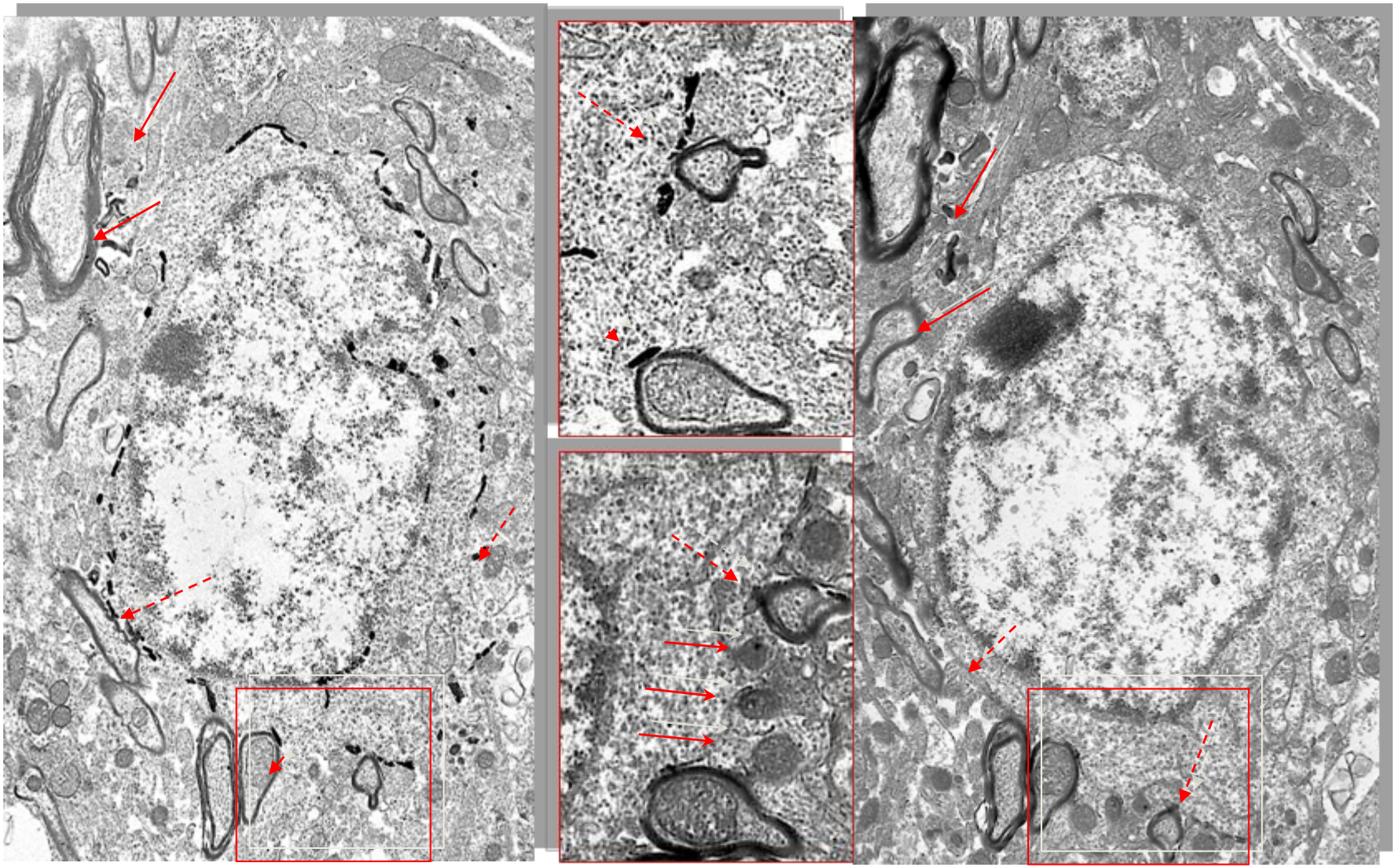


J Clin Invest. 2010;120:29–40



Nature 2003

aNPC Therapeutic Plasticity

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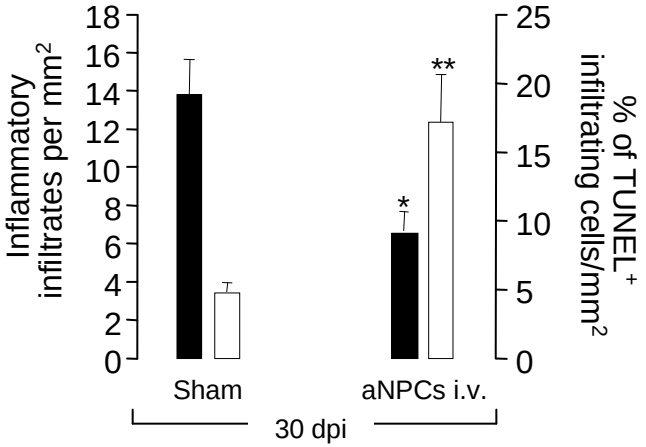
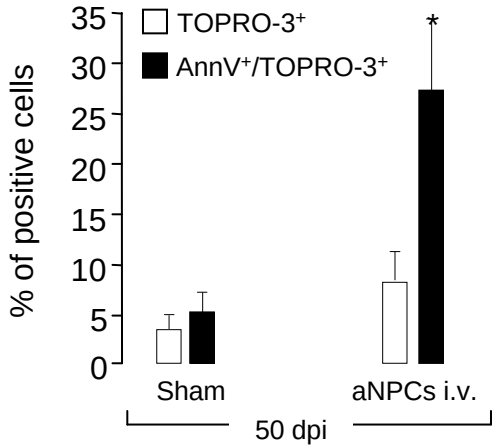
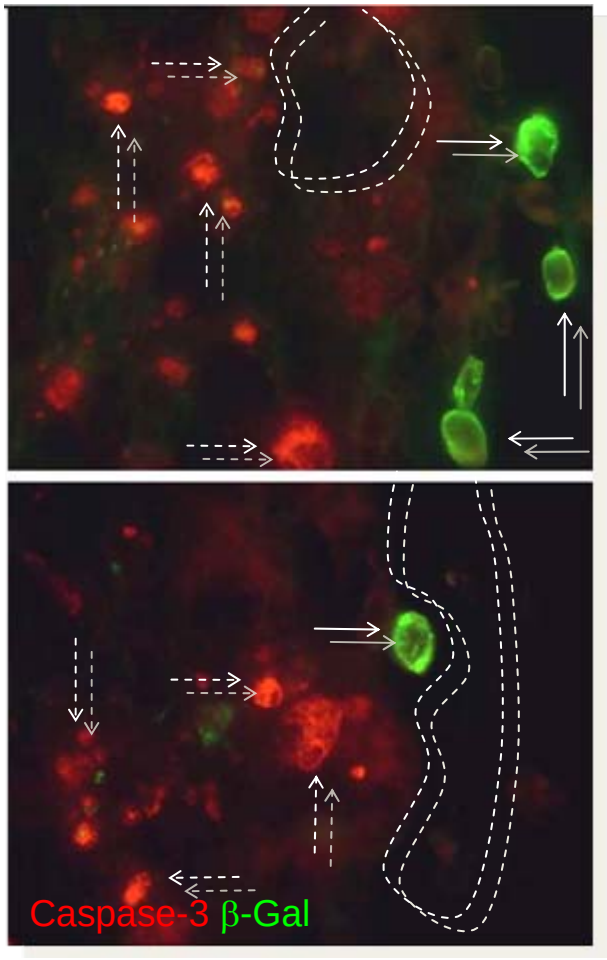
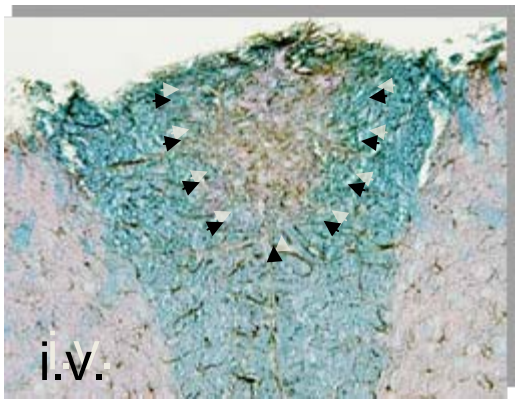
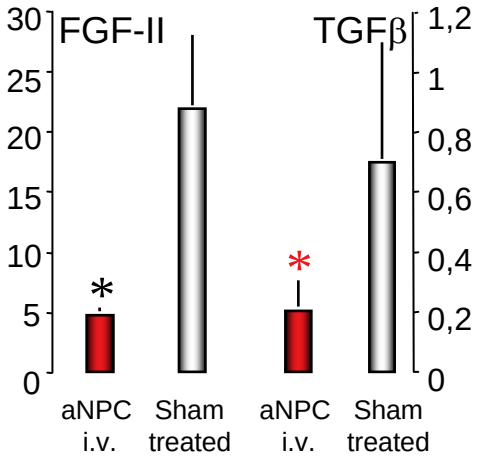

Inflammatory infiltrates per mm²
% of TUNEL⁺ infiltrating cells/mm²
18
16
14
12
10
8
6
4
2
0
25
20
15
10
5
0
*
**
Sham
aNPCs i.v.
30 dpi

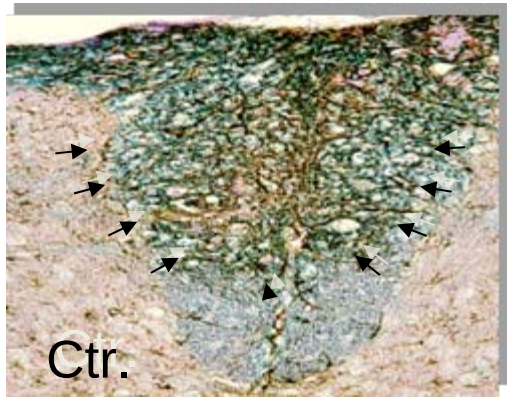

% of positive cells
TOPRO-3⁺
AnnV⁺/TOPRO-3⁺
35
30
25
20
15
10
5
0
*
Sham
aNPCs i.v.
50 dpi

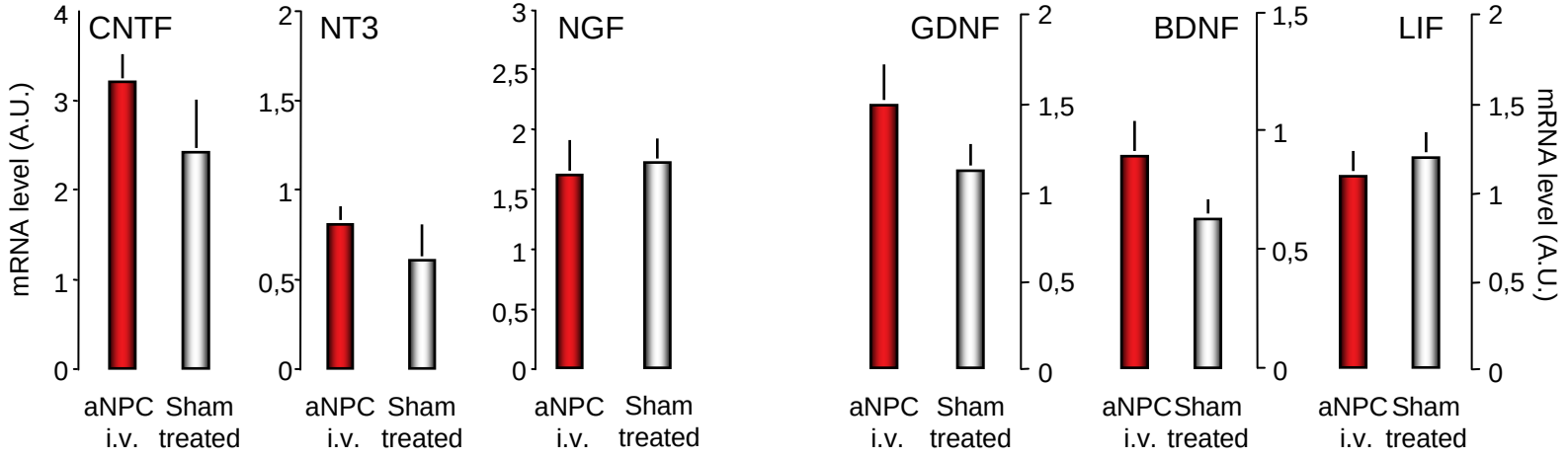

Caspase-3 β-Gal

Nature, 2005

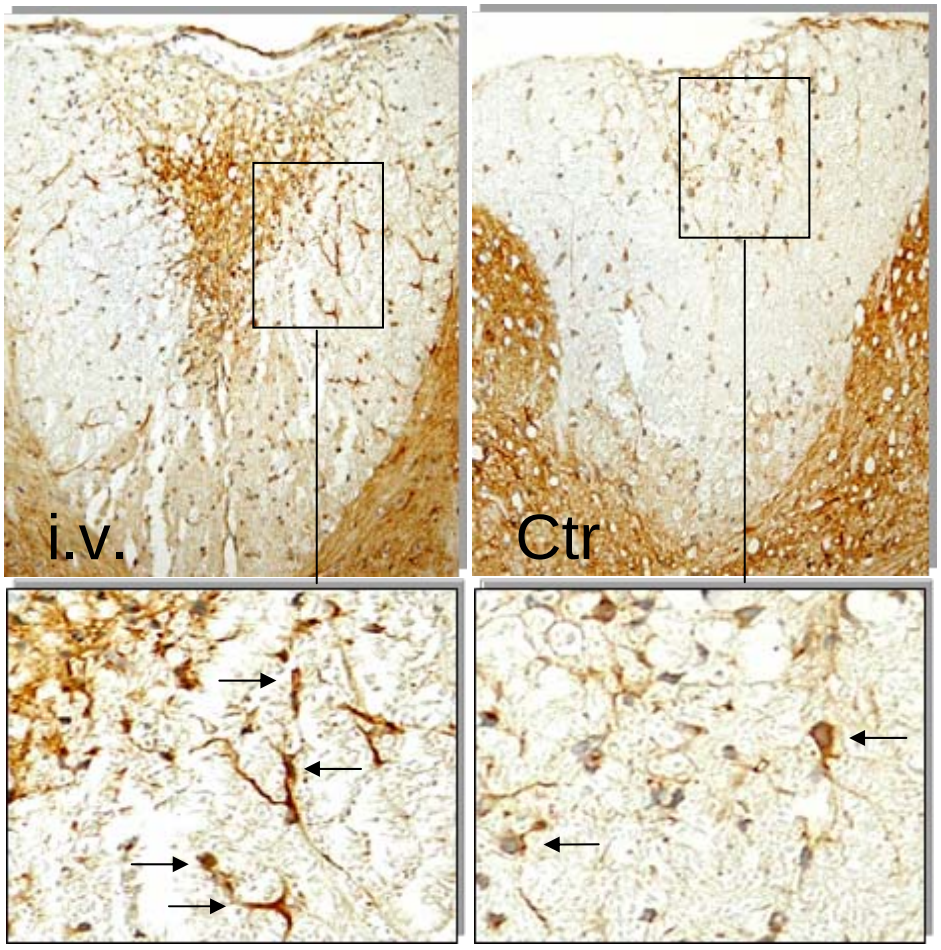

i.v.

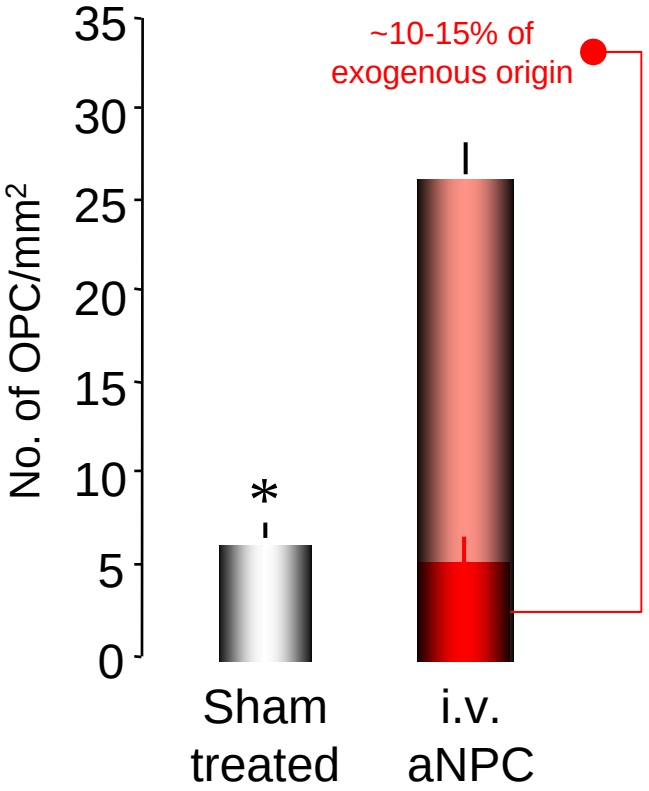

FGF-II
TGFβ
30
25
20
15
10
5
0
1,2
1
0,8
0,6
0,4
0,2
0
*
*
aNPC i.v.
Sham treated
aNPC i.v.
Sham treated


Ctr.

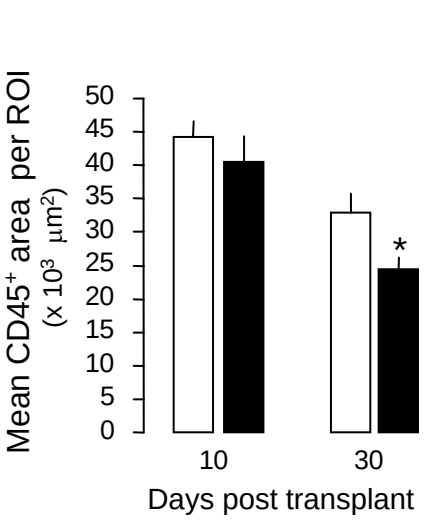

mRNA level (A.U.)
CNTF
NT3
NGF
GDNF
BDNF
LIF
aNPC i.v.
Sham treated

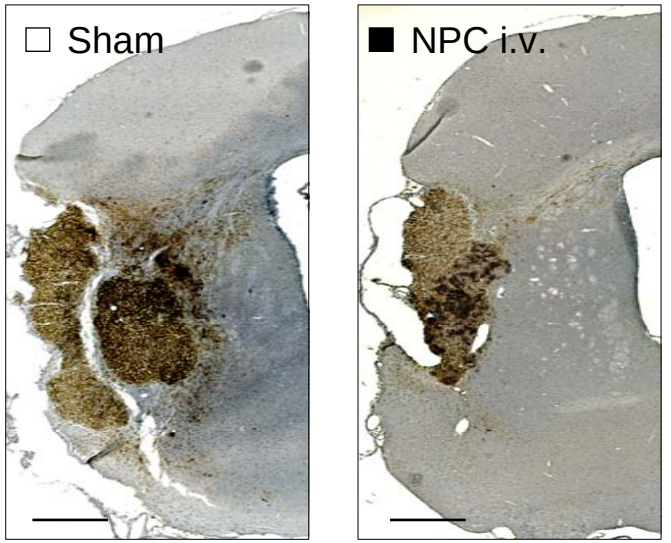
Nature, 2003

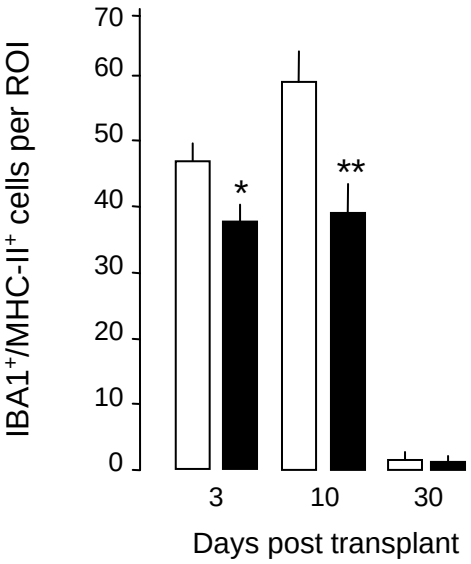

i.v.
Ctr

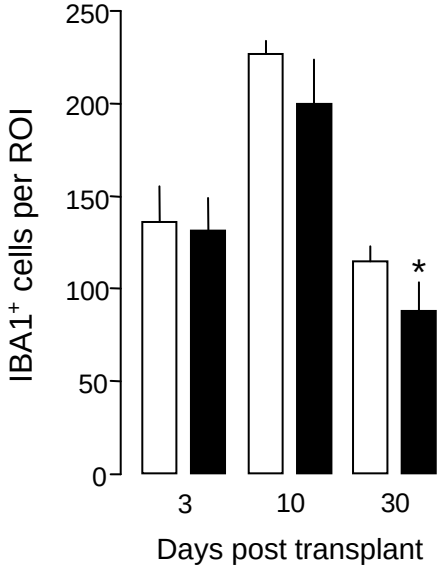

No. of OPC/mm2
35
30
25
20
15
10
5
0
*
~10-15% of
exogenous origin
Sham
treated
i.v.
aNPC

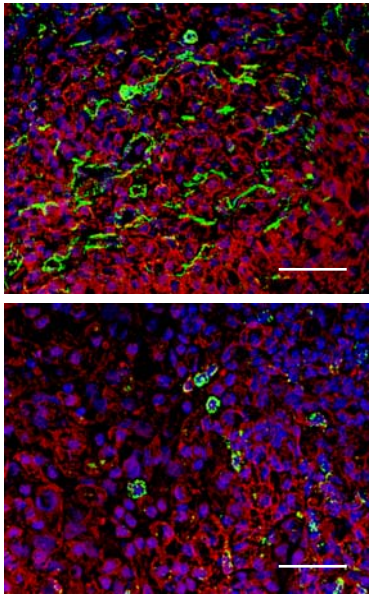
Nature, 2003


Mean CD45+ area per ROI
(x 10³ µm²)
50
45
40
35
30
25
20
15
10
5
0
*
10
30
Days post transplant

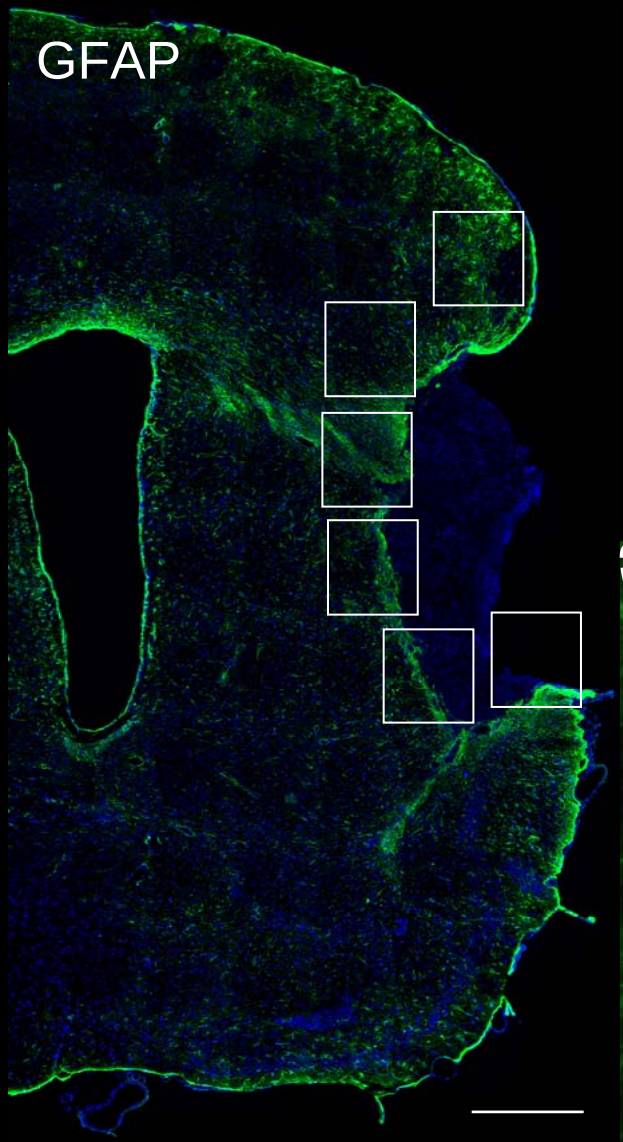

Sham
NPC i.v.

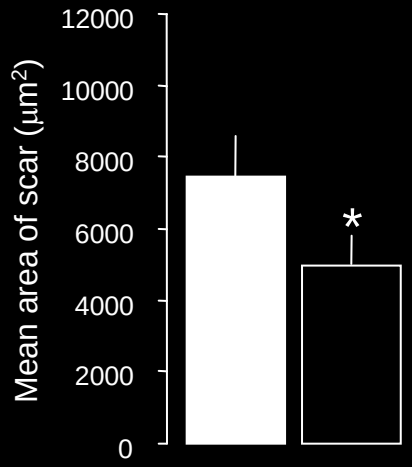

IBA1+/MHC-II+ cells per ROI
70
60
50
40
30
20
10
0
*
**
3
10
30
Days post transplant

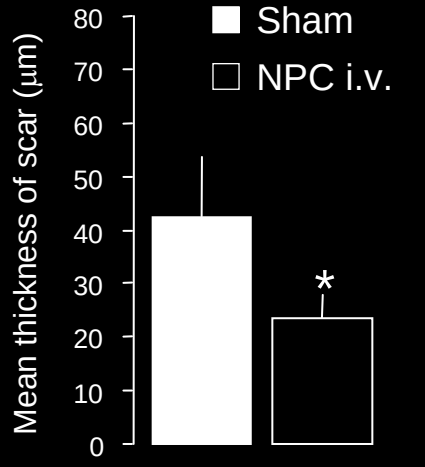

IBA1+ cells per ROI
250
200
150
100
50
0
*
3
10
30
Days post transplant

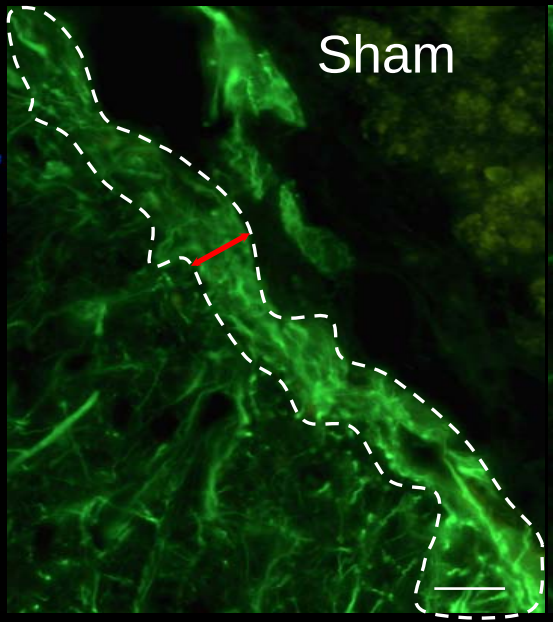


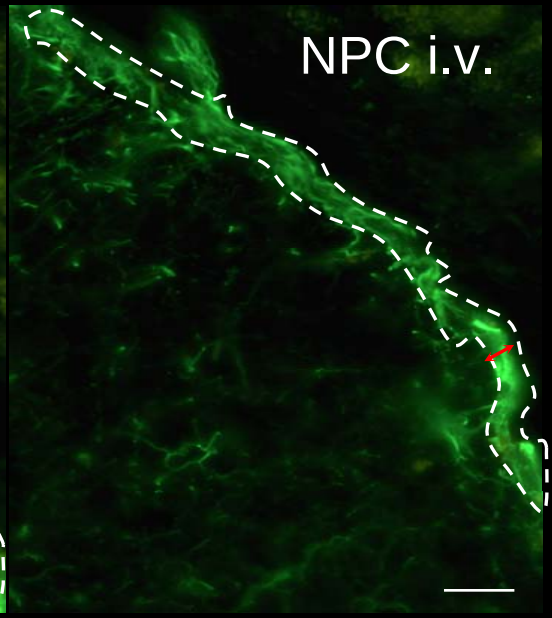
Brain, 2009


GFAP

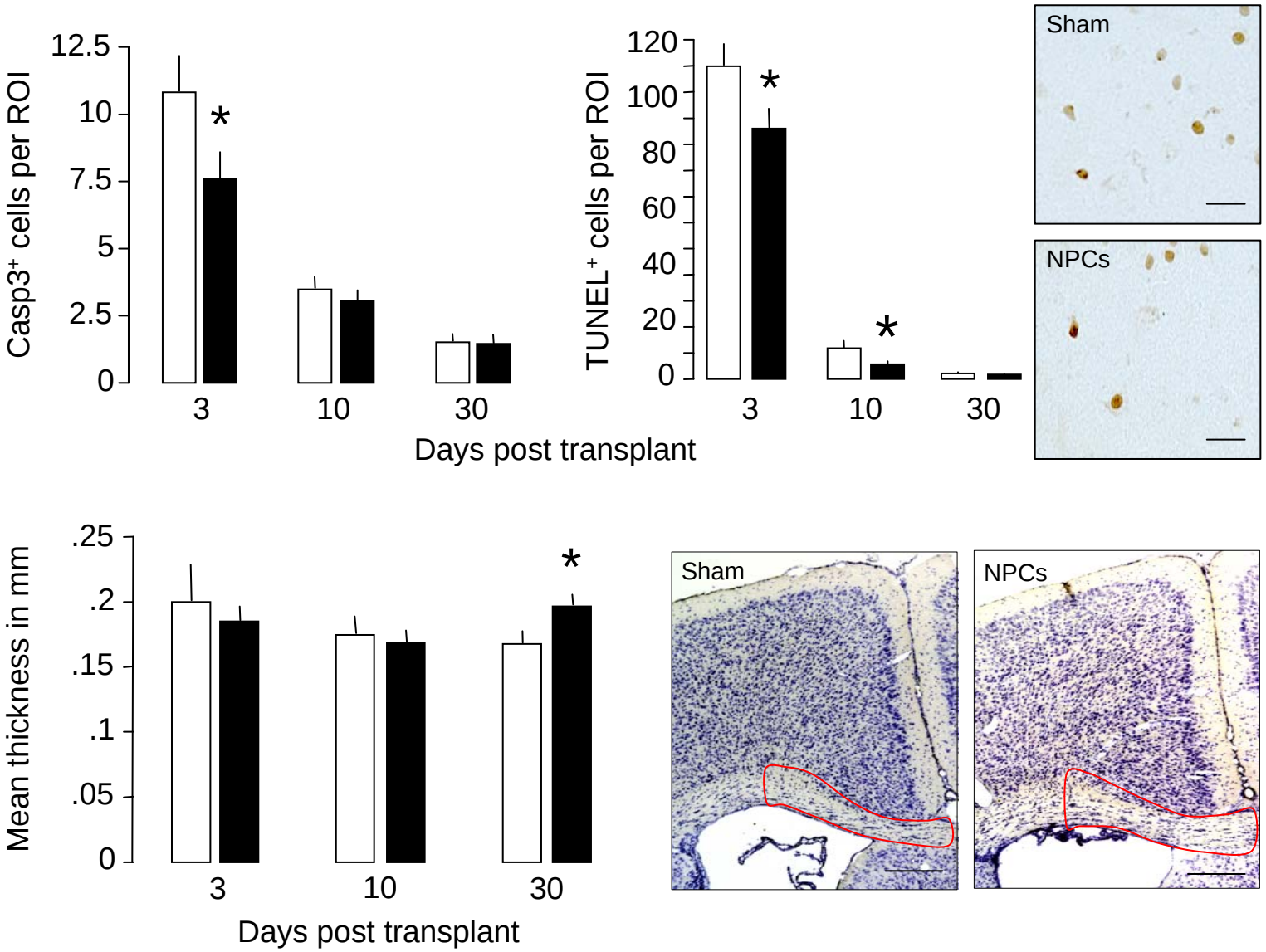

Mean area of scar (μm²)
12000
10000
8000
6000
4000
2000
0
*

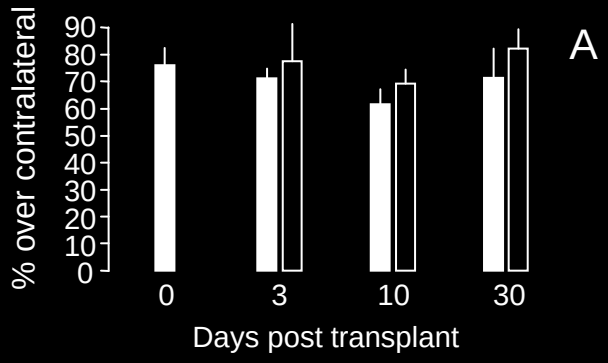

Mean thickness of scar (μm)
80
70
60
50
40
30
20
10
0
*
Sham
NPC i.v.

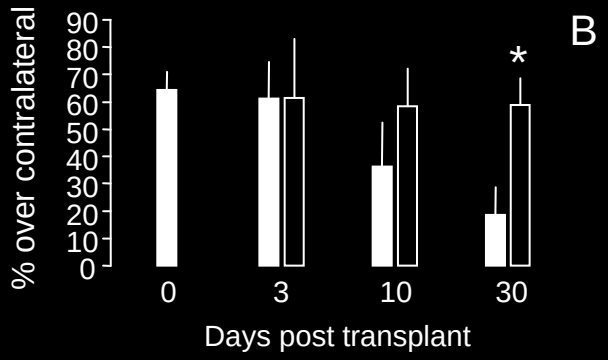

Sham

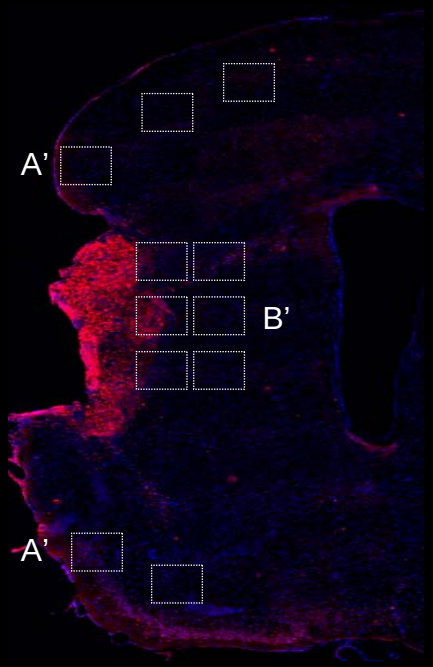

NPC i.v.

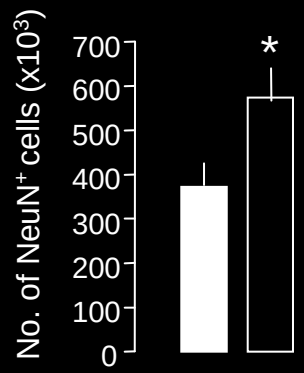
Brain, 2009

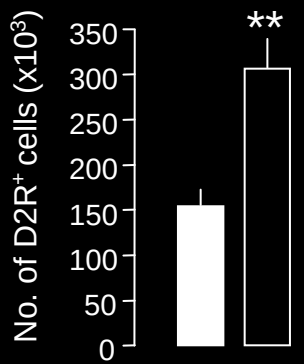

Casp3+ cells per ROI
12.5
10
7.5
5
2.5
0
*
3
10
30
TUNEL+ cells per ROI
120
100
80
60
40
20
0
*
*
3
10
30
Days post transplant
Sham
NPCs
Mean thickness in mm
.25
.2
.15
.1
.05
0
*
3
10
30
Days post transplant
Sham
NPCs

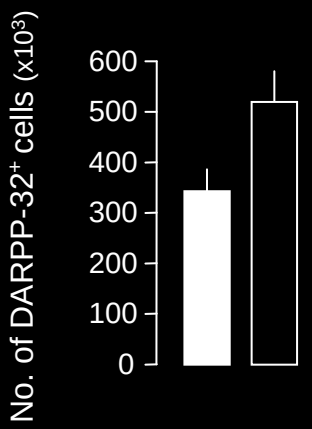

% over contralateral
90
80
70
60
50
40
30
20
10
0
0
3
10
30
Days post transplant
A

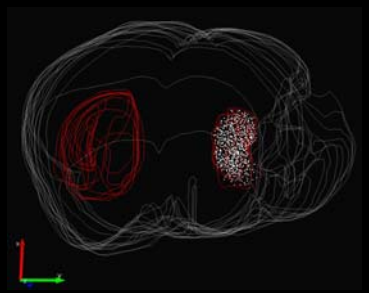

% over contralateral
90
80
70
60
50
40
30
20
10
0
*
0
3
10
30
Days post transplant
B

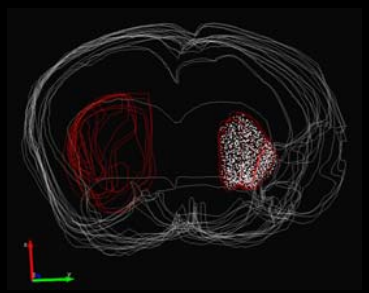

A'
B'
A'

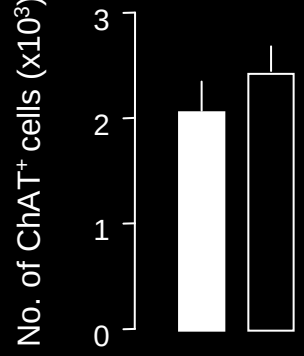

No. of NeuN+ cells (x10³)
700
600
500
400
300
200
100
0
*


No. of D2R+ cells (x10³)
350
300
250
200
150
100
50
0
**


No. of DARPP-32+ cells (x10³)
600
500
400
300
200
100
0



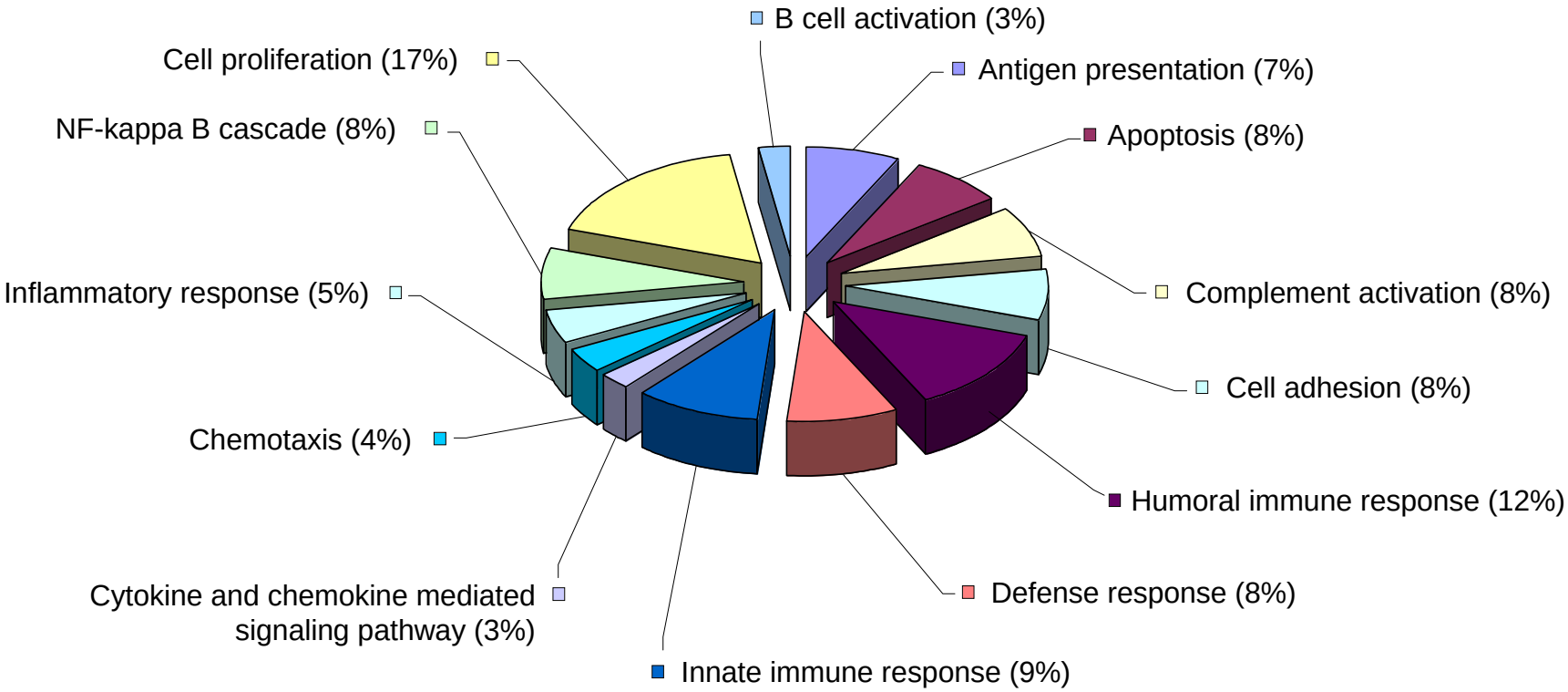



No. of ChAT+ cells (x10³)
3
2
1
0

Brain, 2009

Is aNPC therapeutic plasticity a scalable system?

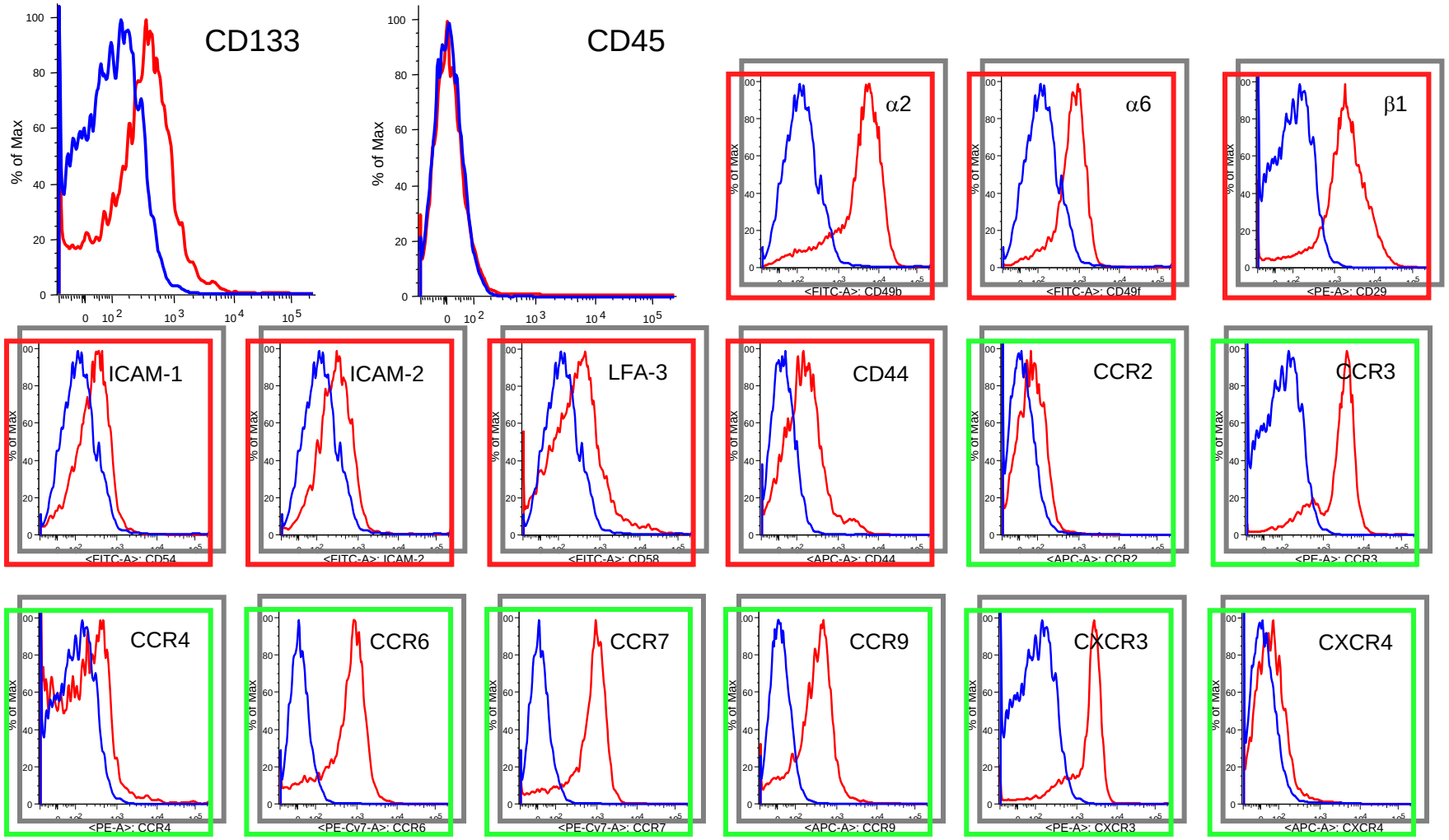
The Immune Signature of Human aNPCs



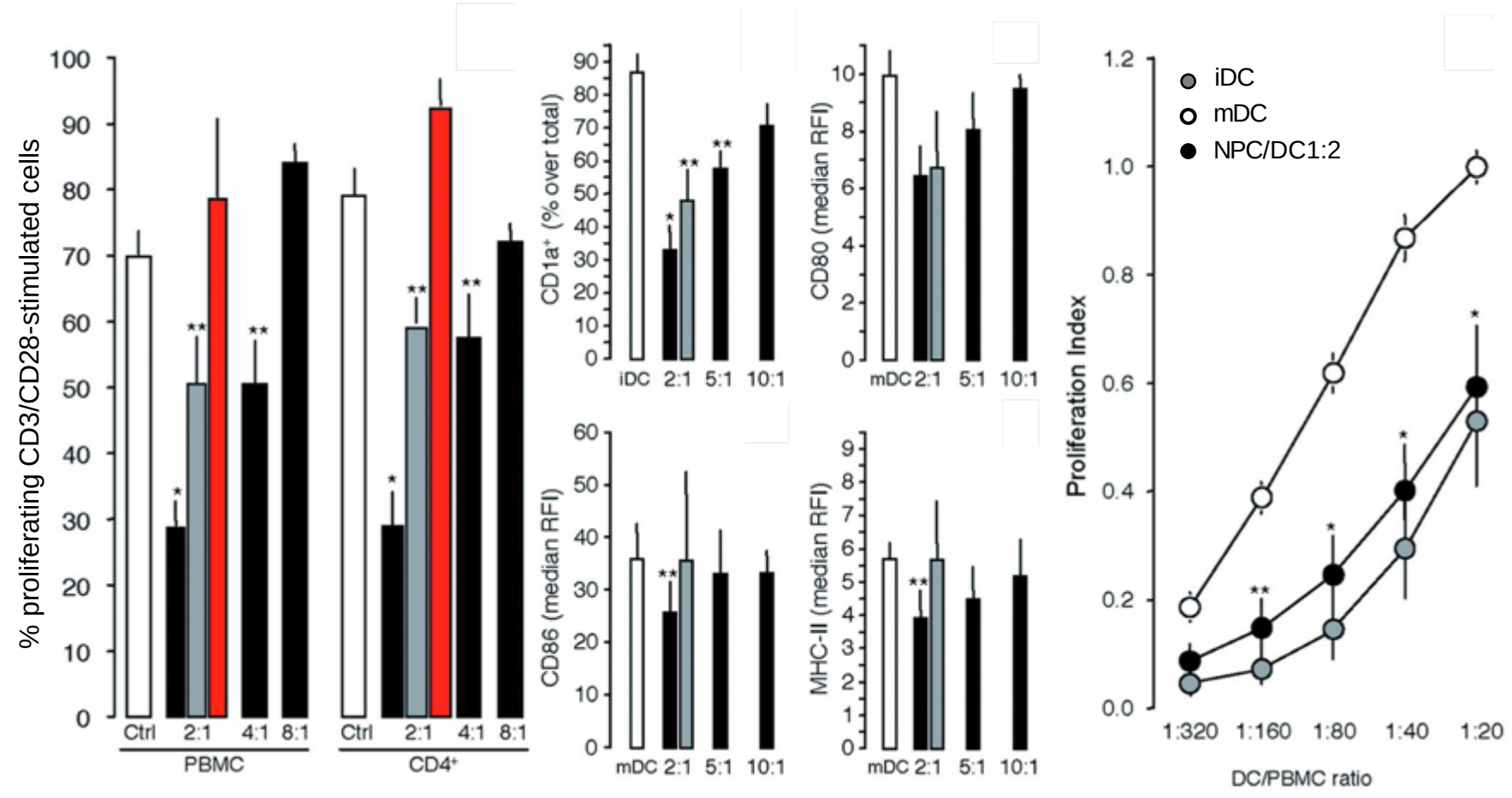
HGU133A Affymetrix® chip (GO:0006955)

22.215 probe set →637 immune response genes →117 expressed genes (18,3%)

Neurosci Lett, 2009



Ann Neurol, 2009

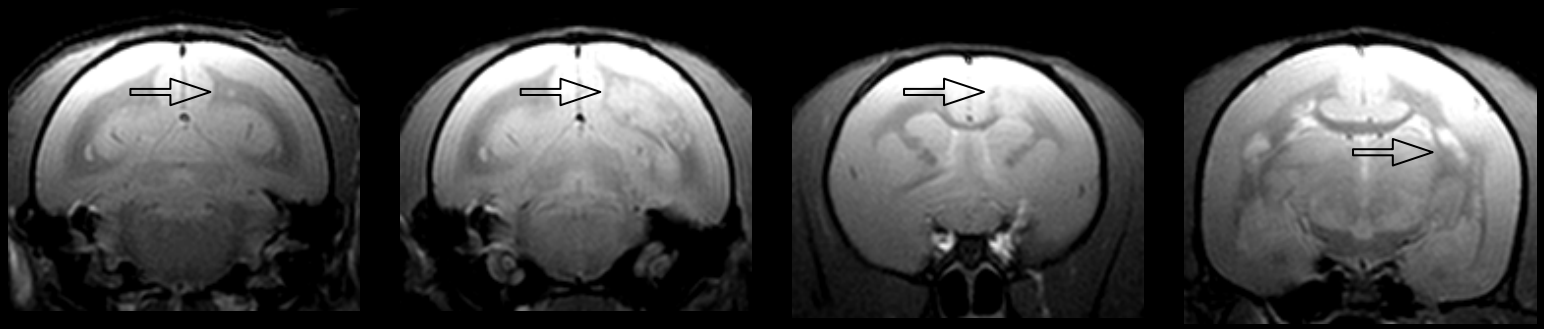


PFA-fixed NPCs

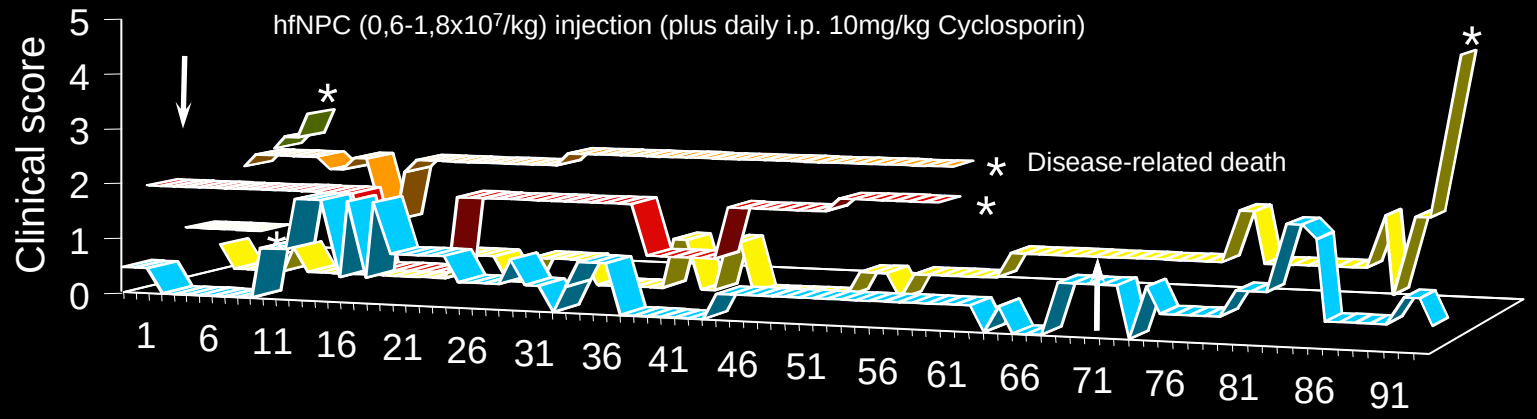
iDC/mDC = immature/mature CD14+ myeloid PC-derived dendritic cells

Ann Neurol, 2009

EAE in Callithrix jacchus

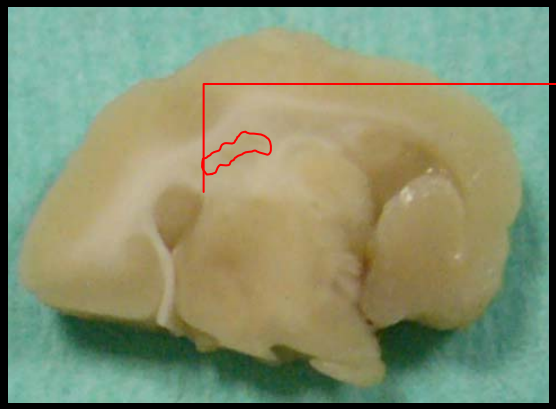


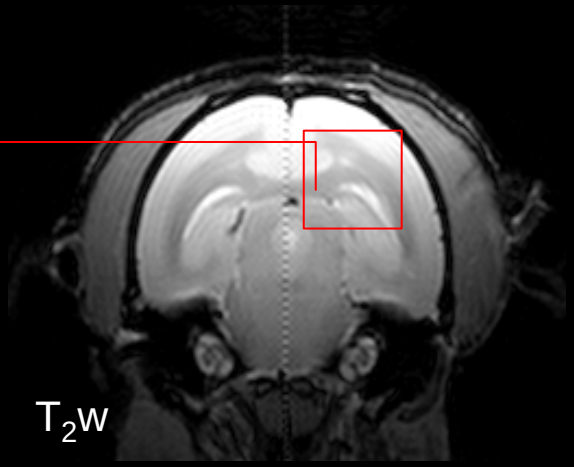
MRI T2w lesion map

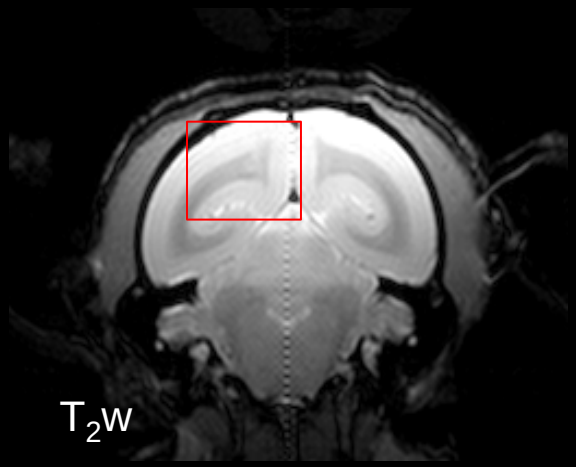


(20-150 days post-immunization with hMOG$_{1-125}$)

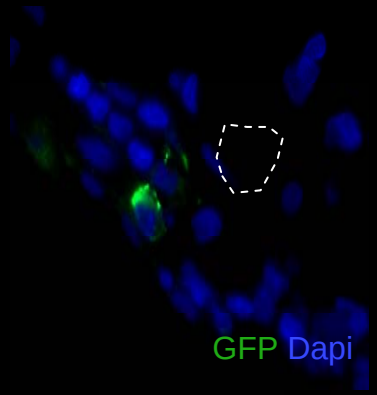
aNPCs are derived from the telencephalon and diencephalon of 10.0 - 10.5 post-conception week human foetuses

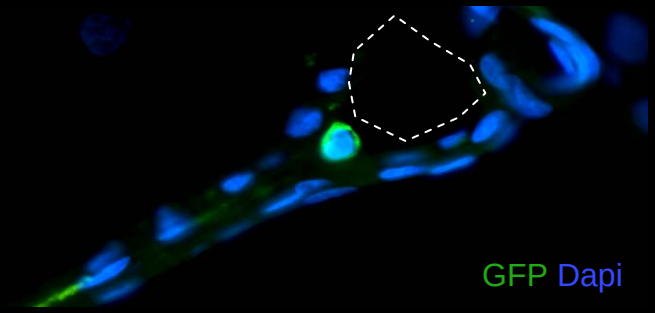


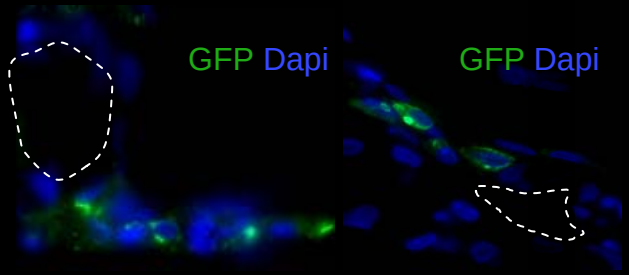

T_2W

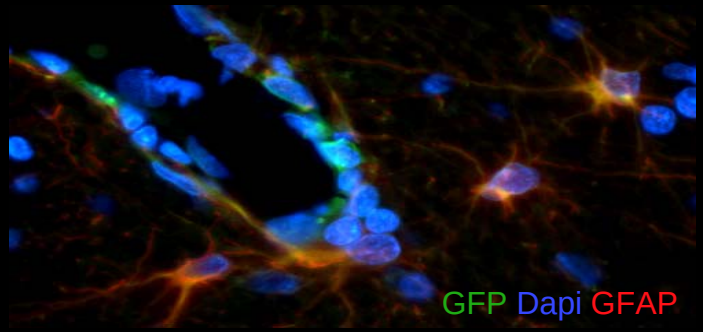

T_2W

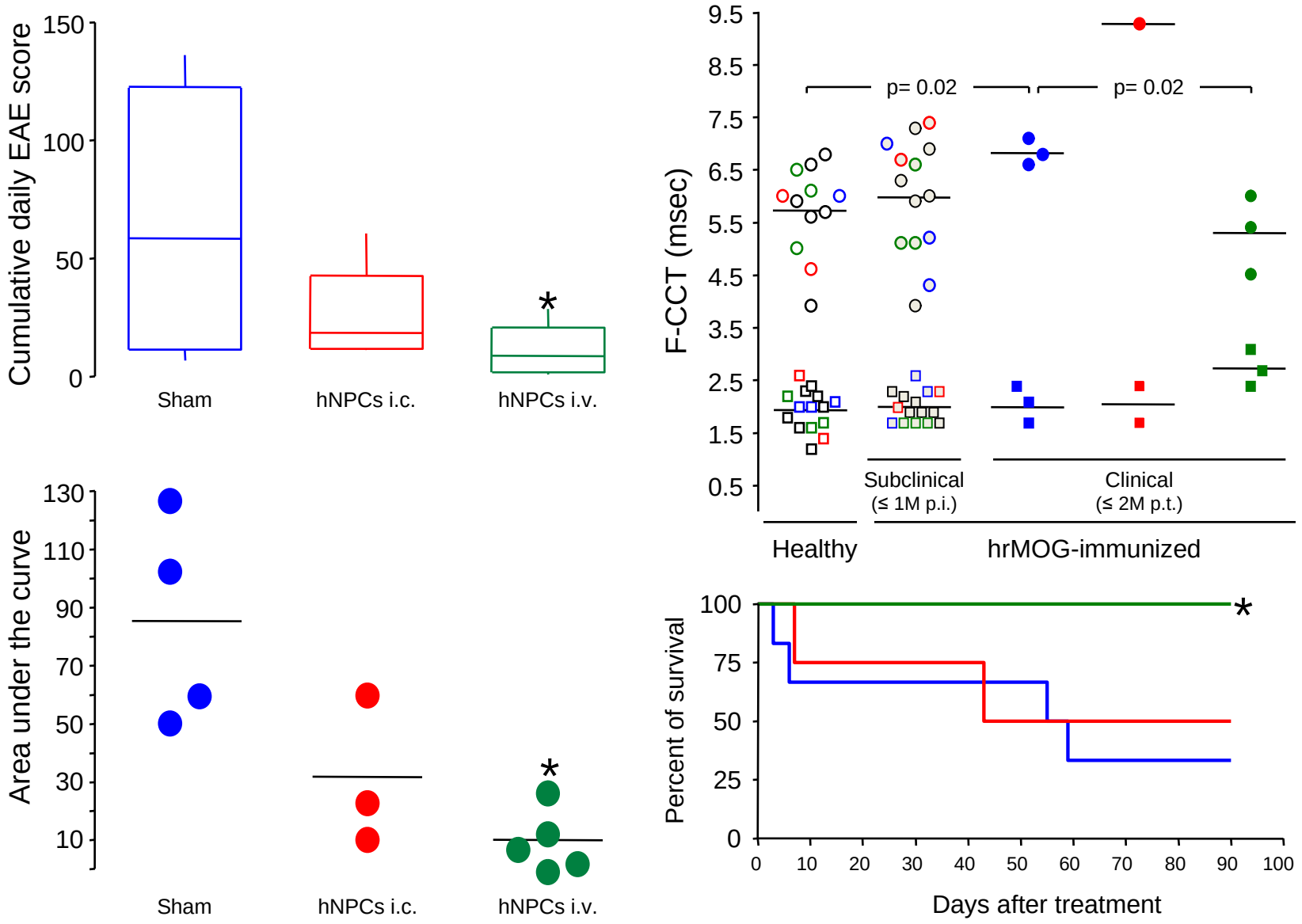

FISH-Y


GFP Dapi

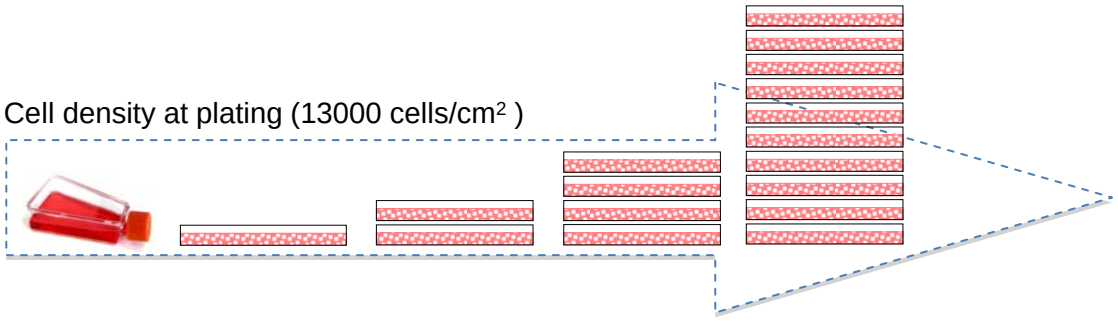

GFP Dapi


GFP Dapi
GFP Dapi

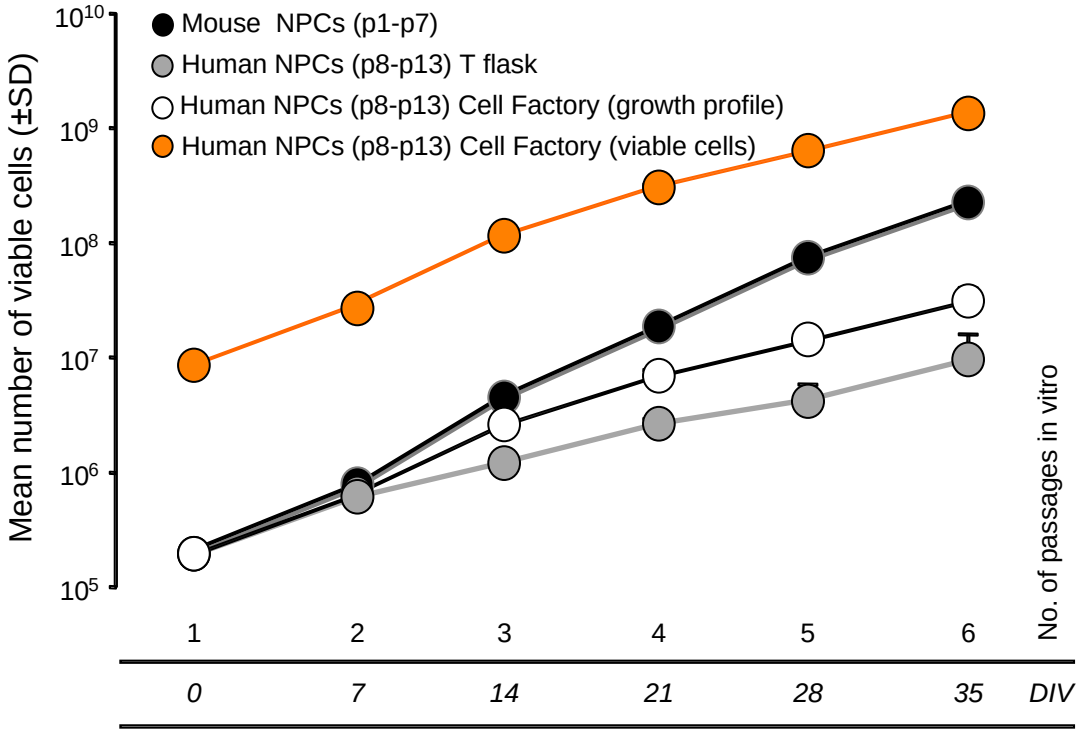

GFP Dapi GFAP

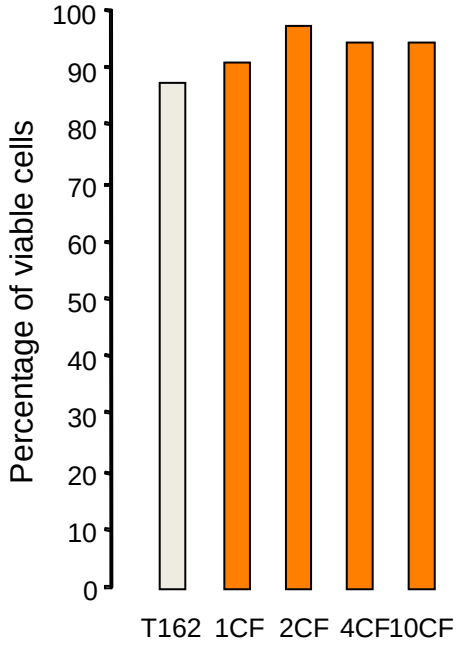


Ann Neurol, 2009

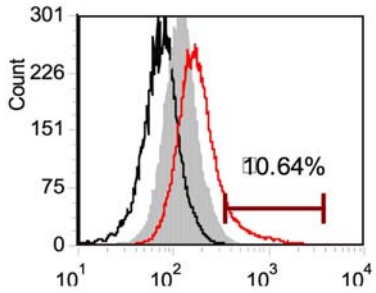


Growth area (632 cm^2)
1, 2, 10 and 40 tray versions for easy scale-up

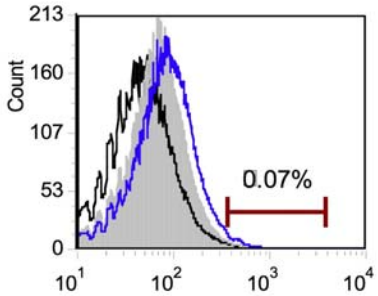




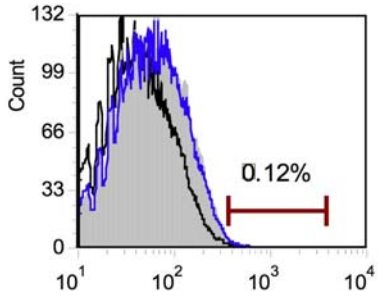
p53



HT-29 colon adenocarcinoma cells

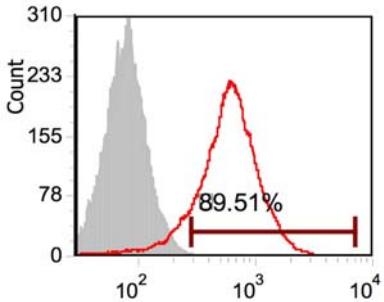


#050411 hNPCs (p15)

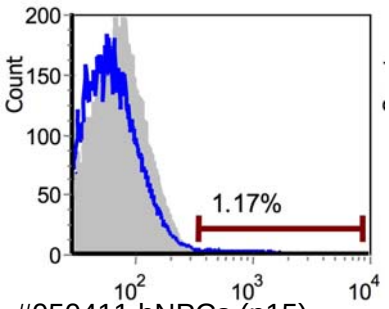


#050411 hNPCs (p30)

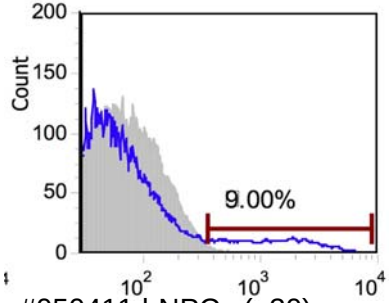
p16



HeLa cervical carcinoma cells



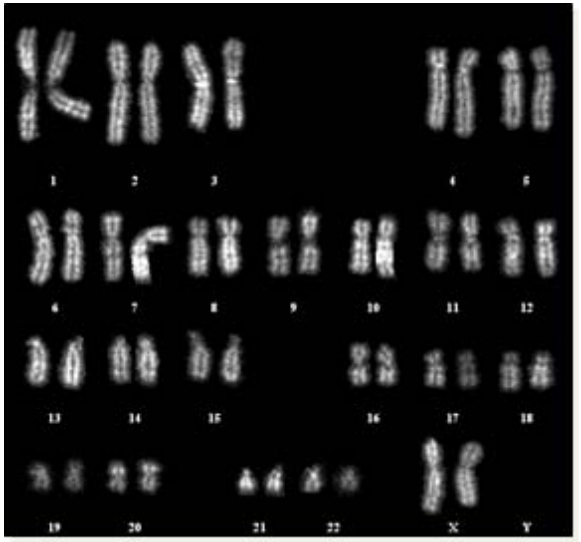
#050411 hNPCs (p15)



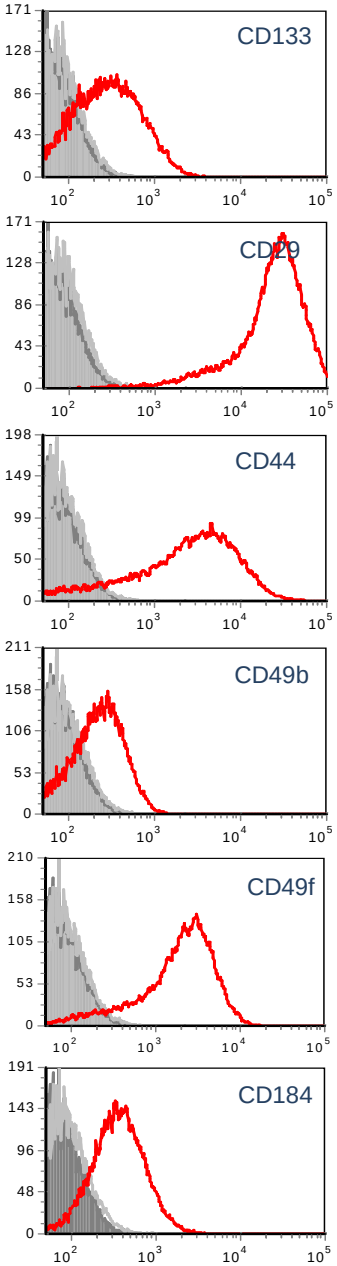
#050411 hNPCs (p30)

#050411 hNPCs (p10)

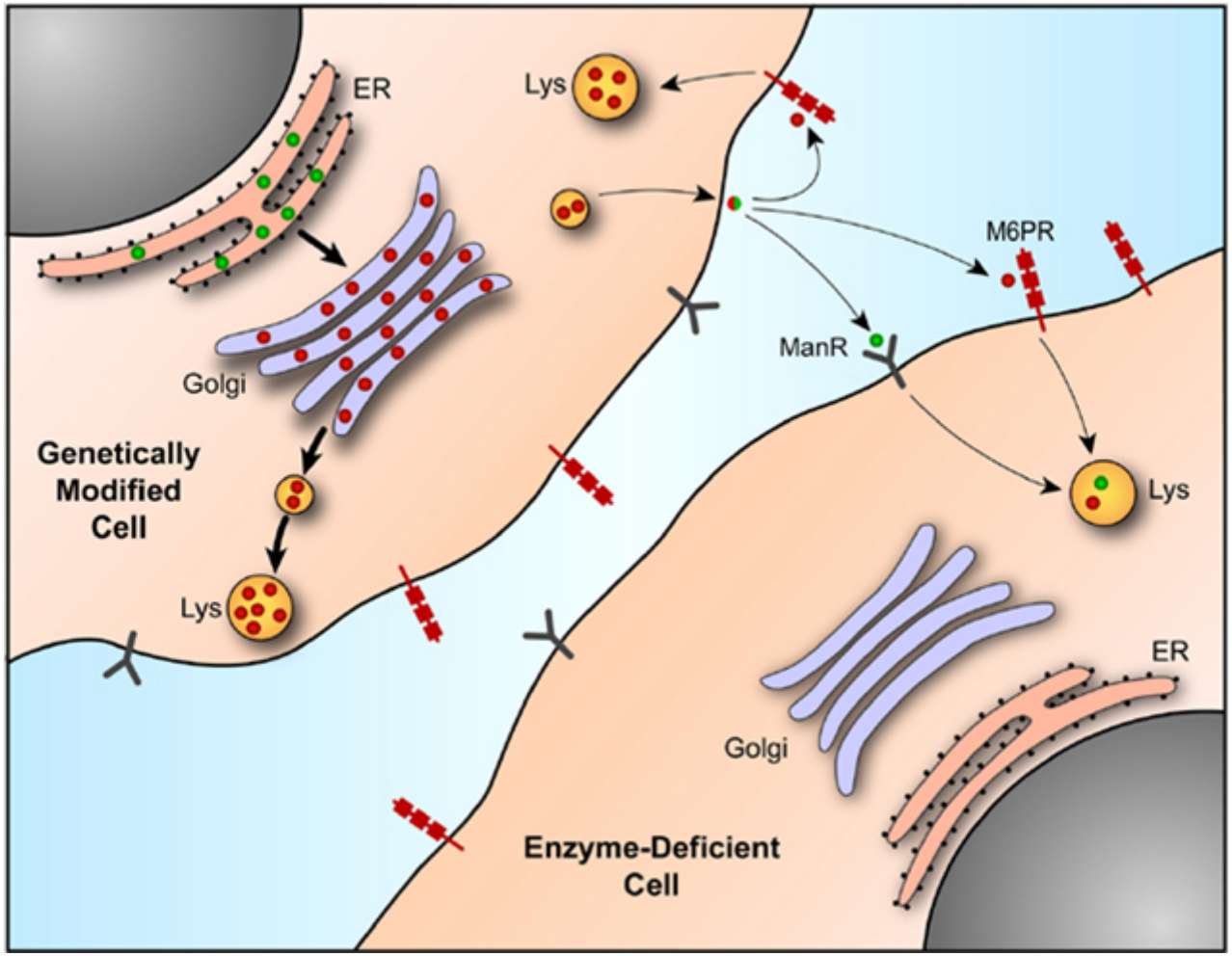
#050411 hNPCs (p18)



Immune Marker	T flask p8	T flask p15	Cell factory p12
CD34	+	-	-
CD45	-	-	-
CD133	-	+	-/+
CD18 (integrin β2 chain)		-	-
CD29 (integrin β1 chain)	++	++	++
CD44	+	++	++
CD49a (integrin α1 chain)	-/+	-	-
CD49b (integrin α2 chain)	++	+	+
CD49d (integrin α4 chain)	-	-	-
CD49f (integrin α6 chain)	+	++	++
CD192 (CCR2)	+	-	-
CD193 (CCR3)	+	-	-
CD194 (CCR4)	+	-	-
CD195 (CCR5)	+	-	-
CD197 (CCR7)	++	-	-
CD183 (CXCR3)	++	-	-
CD184 (CXCR4)	-/+	+	+
Integrin β7 chain	+	-	-
HLA ABC	+	+	+
HLA DR	-	-	-



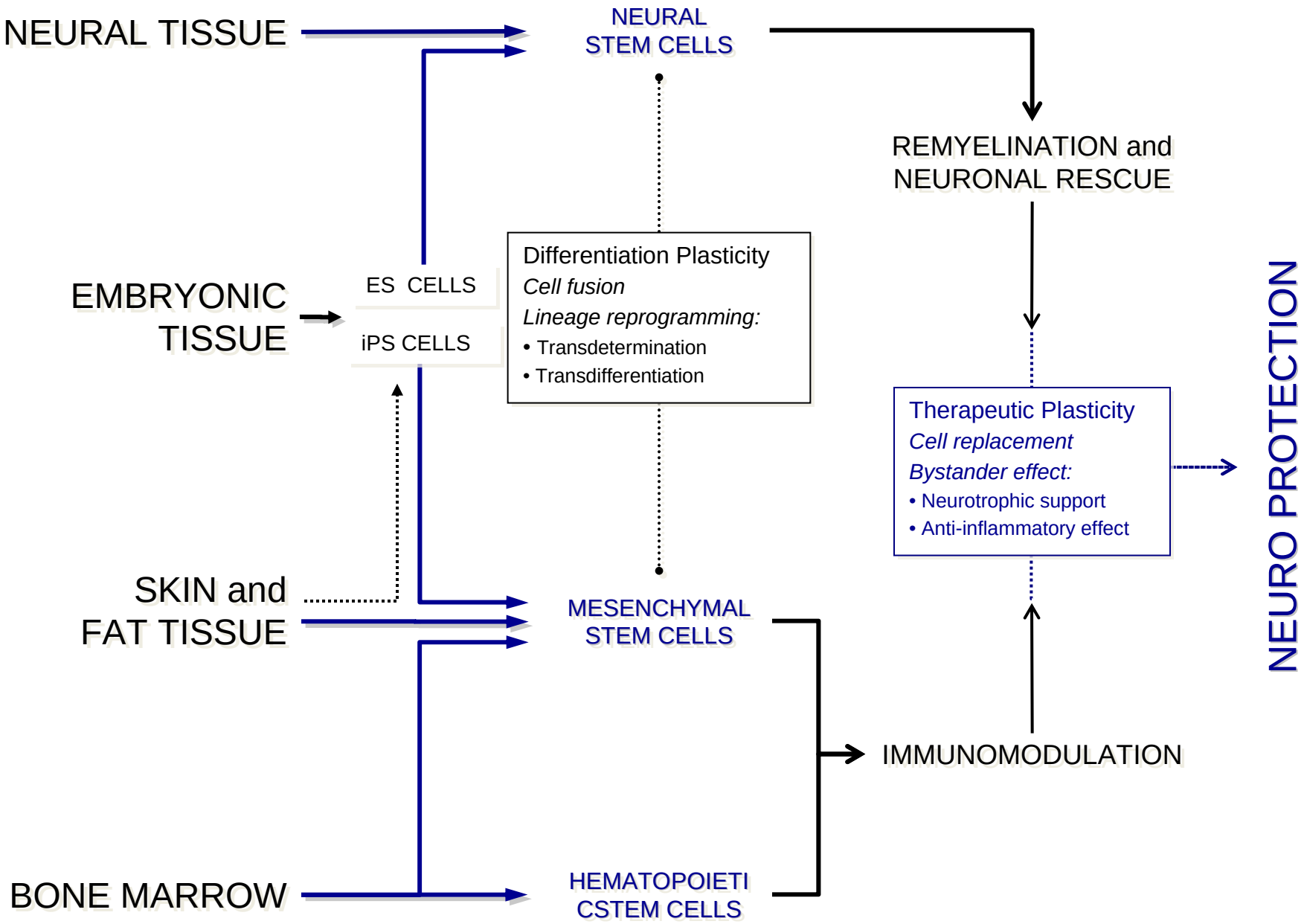
aNPCs: Enzyme cross-correction



Molecular Therapy 2006;**13:839–849**

aNPC Therapeutic Plasticity

- Neural stem cells do not therapeutically work exclusively throughout cell replacement.
- Neural stem cells do also promote CNS repair also via intrinsic *neuroprotective* bystander capacities – which may explain the CNS repair capabilities of other sources of somatic stem cells with very low capabilities of neural (trans) differentiation (e.g. mesenchymal stem cells) – via the release, at the site of tissue damage, of :
  - immunomodulatory substances, neurotrophic growth factors, stem cell regulators, etc.
- Somatic neural stem cells are *therapeutically plastic* since they are capable to adapt their fate and function(s) to specific environmental needs occurring as a result of different pathological conditions.



Nat Rev Neurol, 2010

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