Neural Regeneration of Brain Injury in Animal Models via Cellular Therapy

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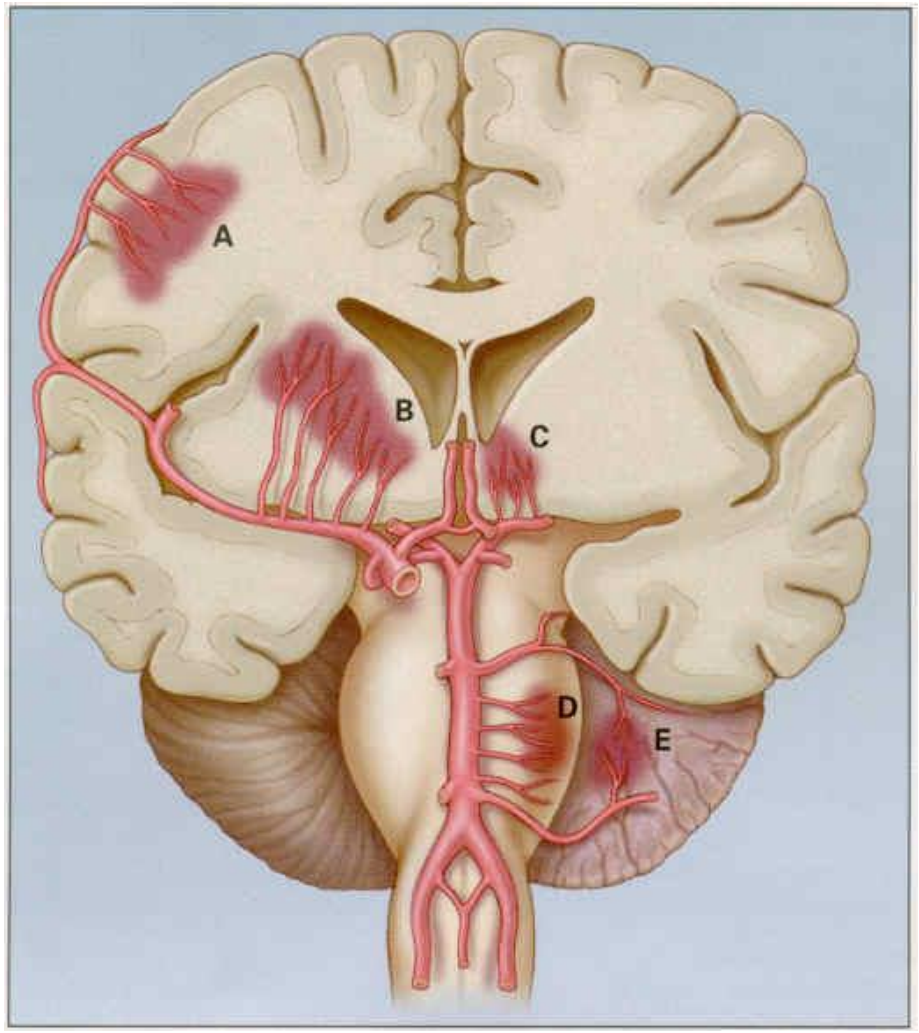
- Chen SJ, Tsai JC, Lin CY, Chang CK, Tseng TH, **Chien CL***. Brain-derived neurotrophic factor-transfected and nontransfected 3T3 fibroblasts enhance migratory neuroblasts and functional restoration in mice with intracerebral hemorrhage . ***J Neuropathol Exp Neurol***. 2012; 71:1123-1136.

- Lee WD, Wang KC, Tsai, YF, Chou PC, Tsai LK, **Chien CL*.** Subarachnoid hemorrhage promotes proliferation, differentiation, and migration of neural stem cells via BDNF upregulation. ***PLoS One*** 2016; 11(11):e0165460.

- Li YC, Chen SJ and **Chien CL***. Erythropoietin produced by genetic-modified NIH/3T3 fibroblasts enhances the survival of degenerating neurons. ***Brain Behav***. 2015; 5(8) (Cover)

- Chou PC, Tsai LK, **Chien CL***. Erythropoietin Produced by Genetic-modified NIH/3T3 Fibroblasts Facilitates Recovery in a Rat Stroke Model. 2018 (*In Preparation*)

Neurogenesis and Functional Recovery after Intracerebral Transplantation of Brain-Derived Neurotrophic Factor (BDNF) Transfected 3T3 Fibroblasts in Cerebral Hemorrhage Mice



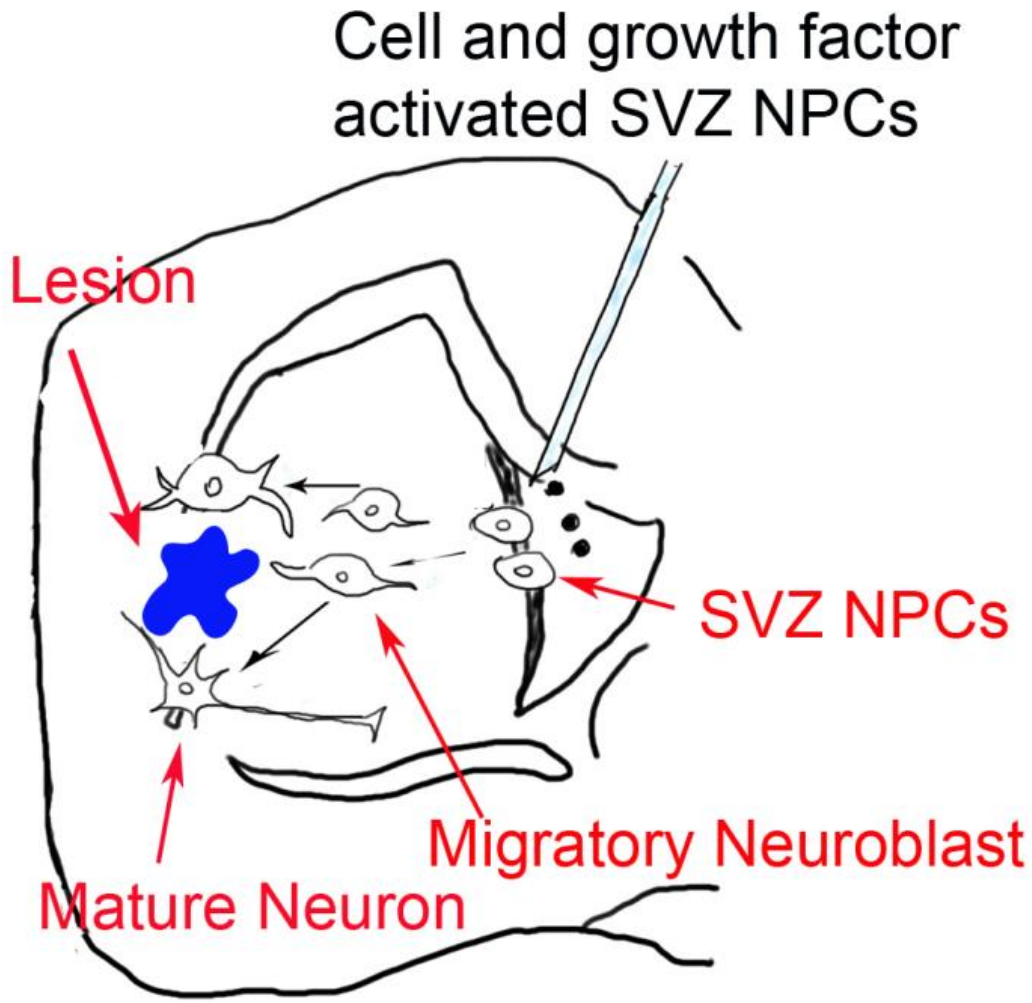
Intracerebral Hemorrhage

- Devastating form of stroke
- Surgical hematoma evacuation
- Stem cell based therapies
  - Exogenous cell transplantation
  - Endogenous neural precursor cells (NPCs) activation

(Mayer, 2003 Stroke)

Endogenous Stem Cell Activation and Repair Process

Stem cell niches: Subventricular zone (SVZ)

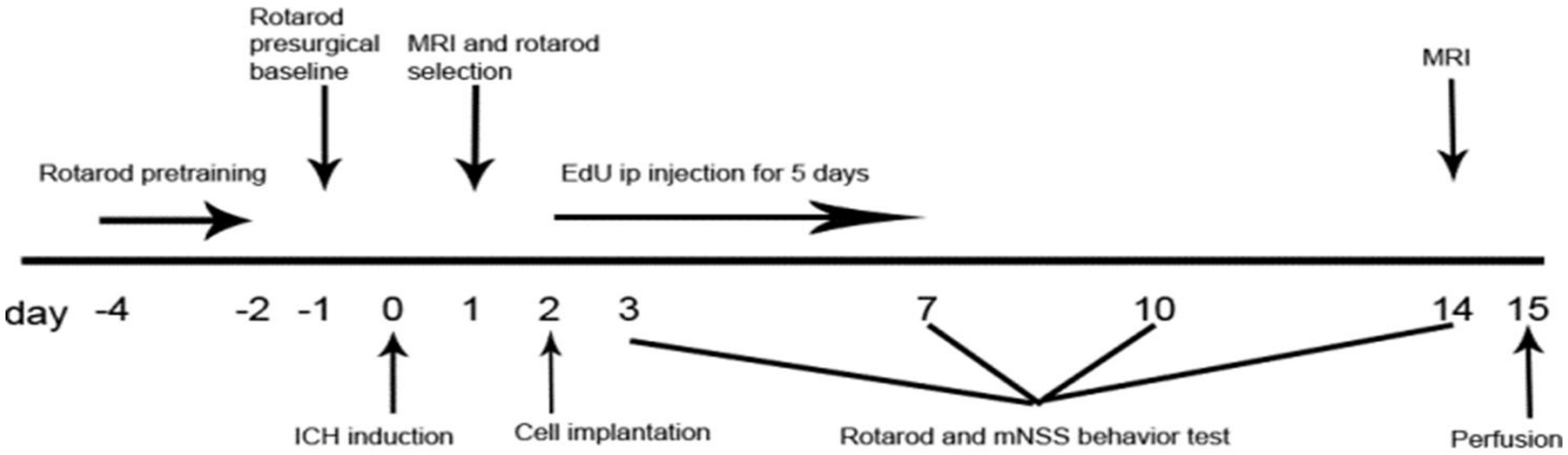


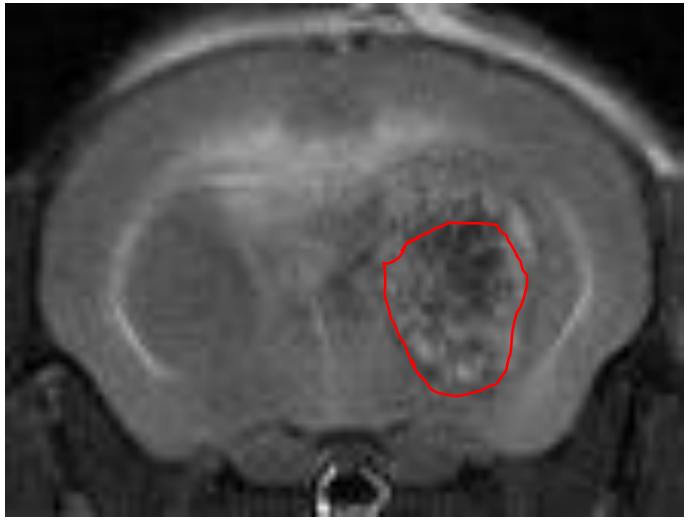
Brain–Derived Neurotrophic Factor (BDNF)

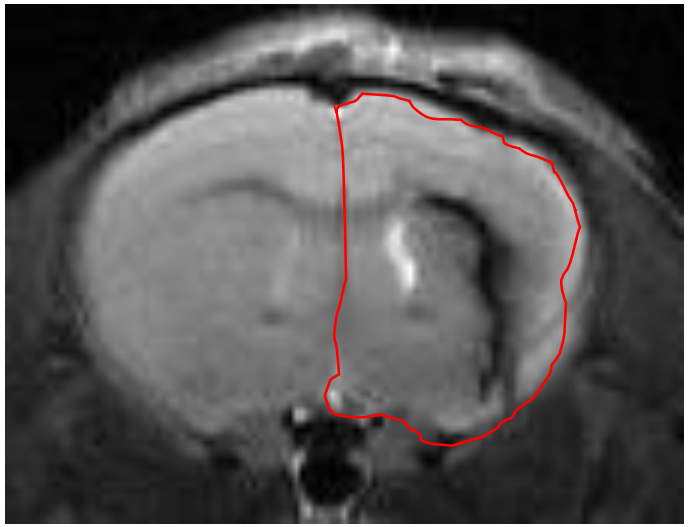
- Proliferation
- Migration
- Maturation
- Function

Aim of Study

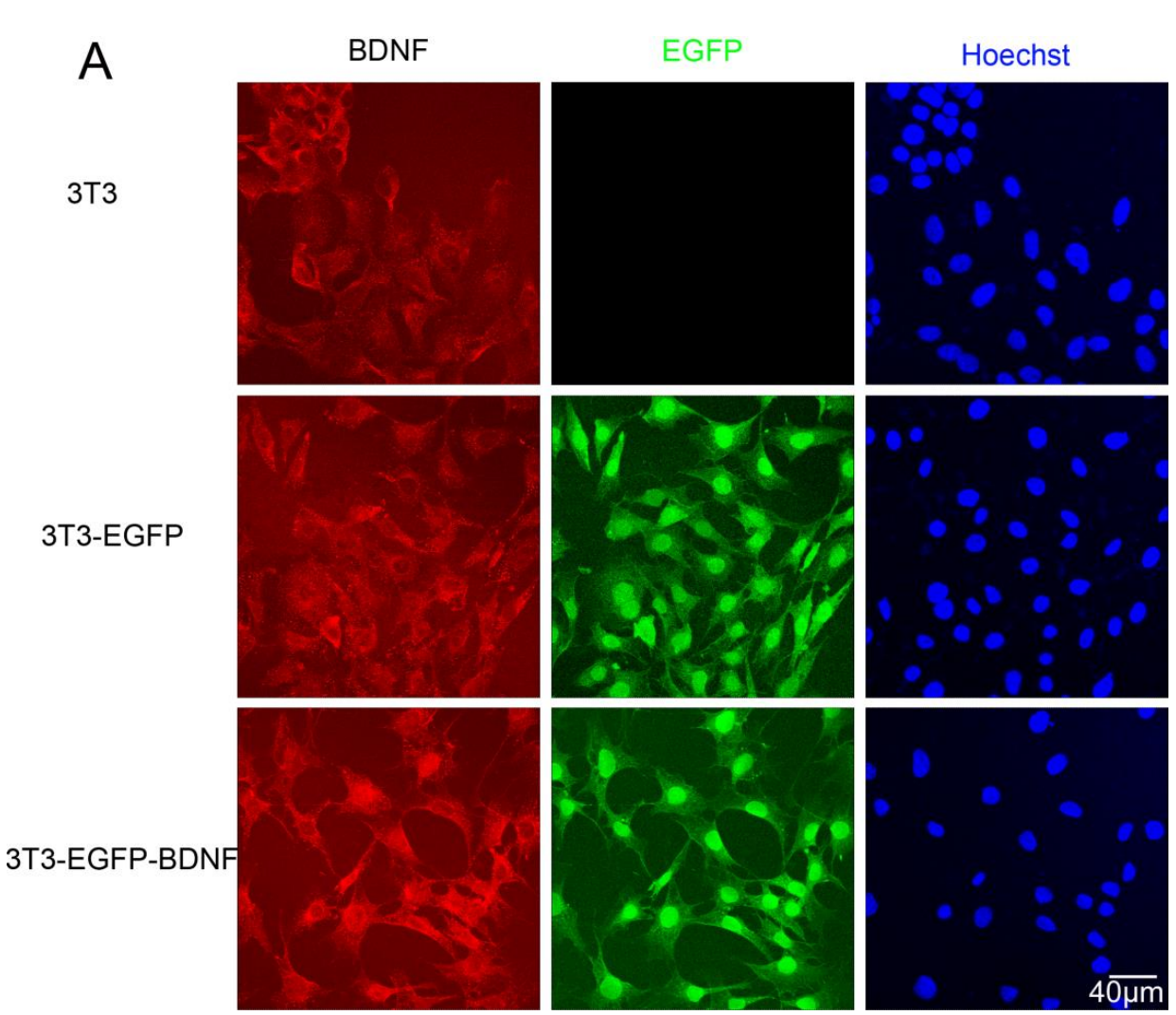
- 3T3 fibroblasts cell line expresses BDNF
- Implantation into the ICH mouse brain
- Neurogenesis and functional recovery
  - Doublecortin (DCX) immunostaining
  - 5-ethynyl-2'- deoxyuridine (EdU) staining
  - Magnetic resonance imaging (MRI)
  - Behavioral testing

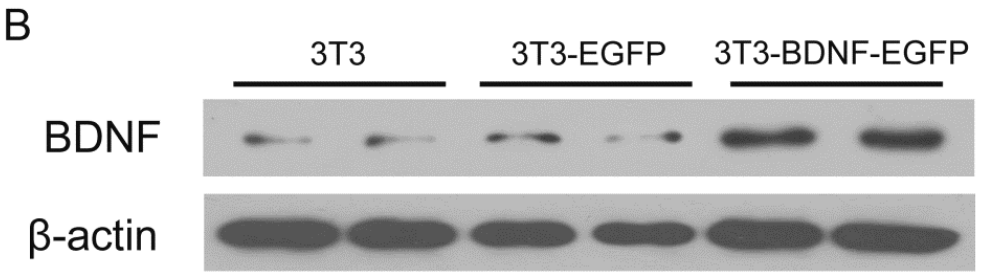


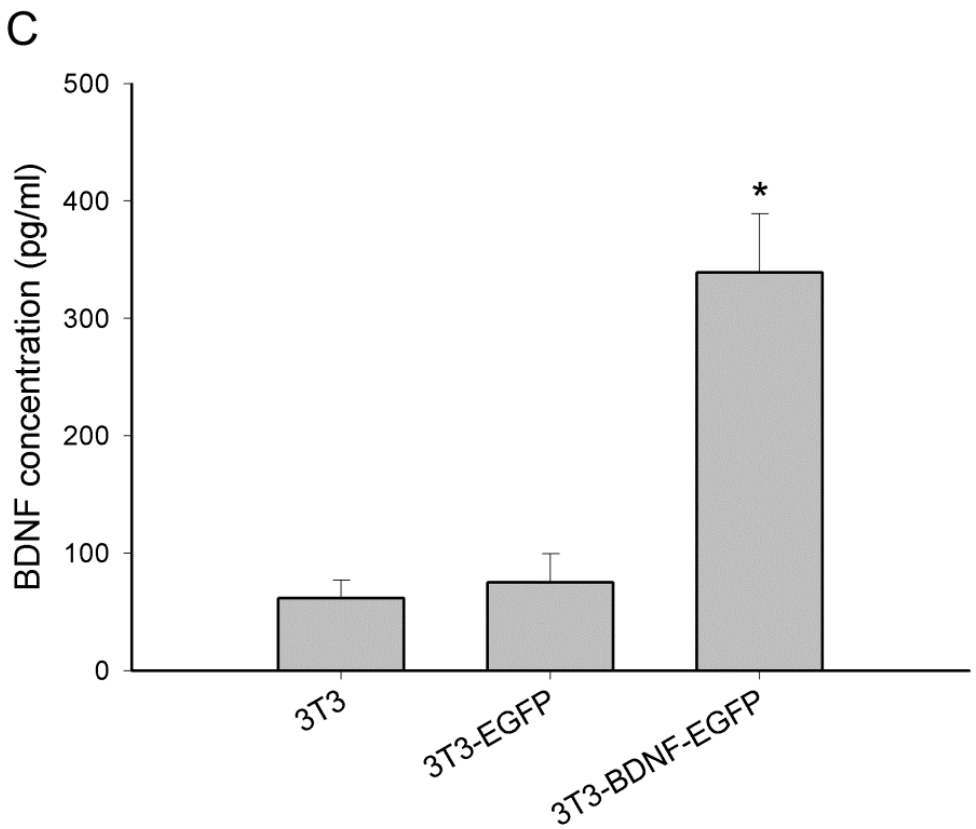


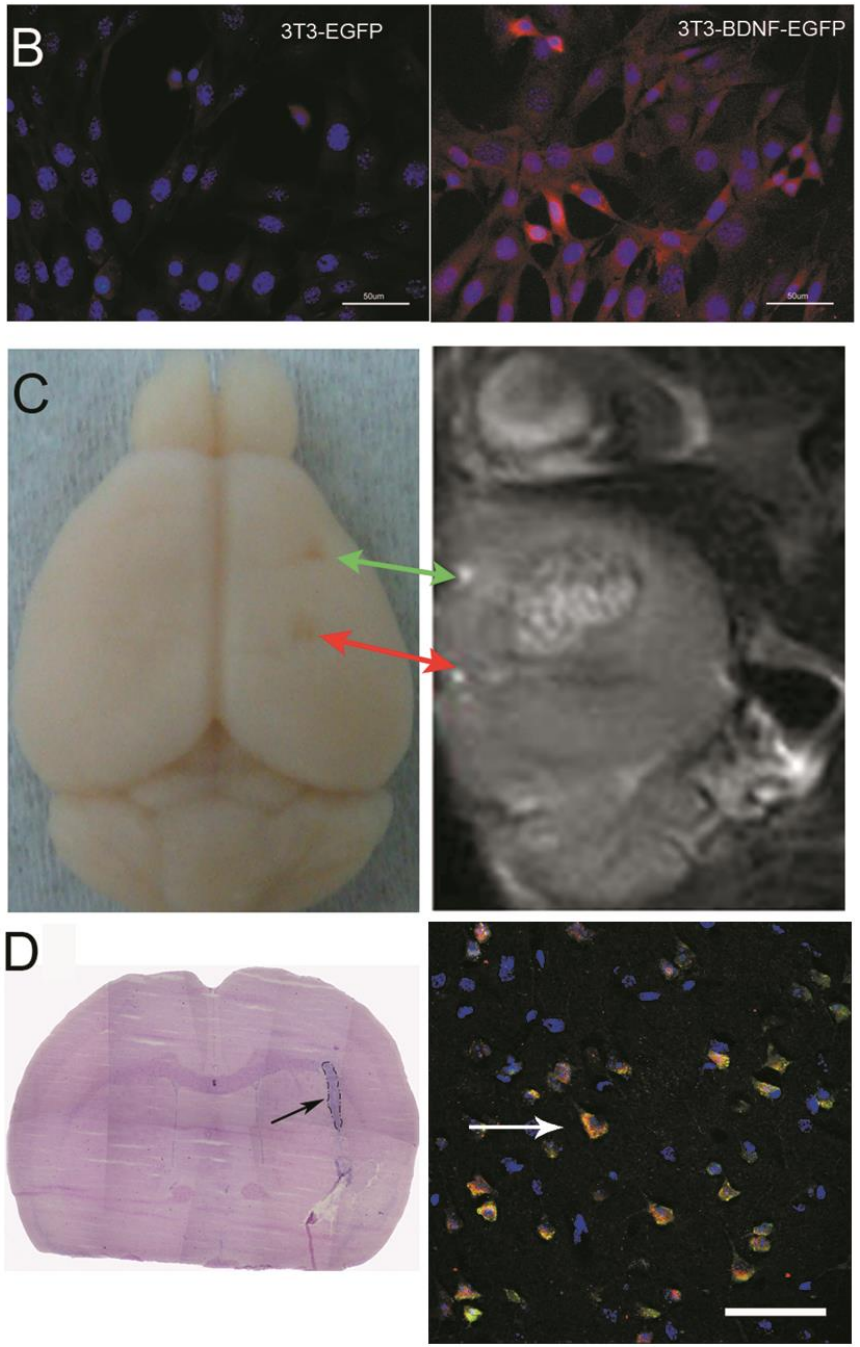


BDNF in 3T3, 3T3-EGFP, and 3T3-BDNF-EGFP Cells









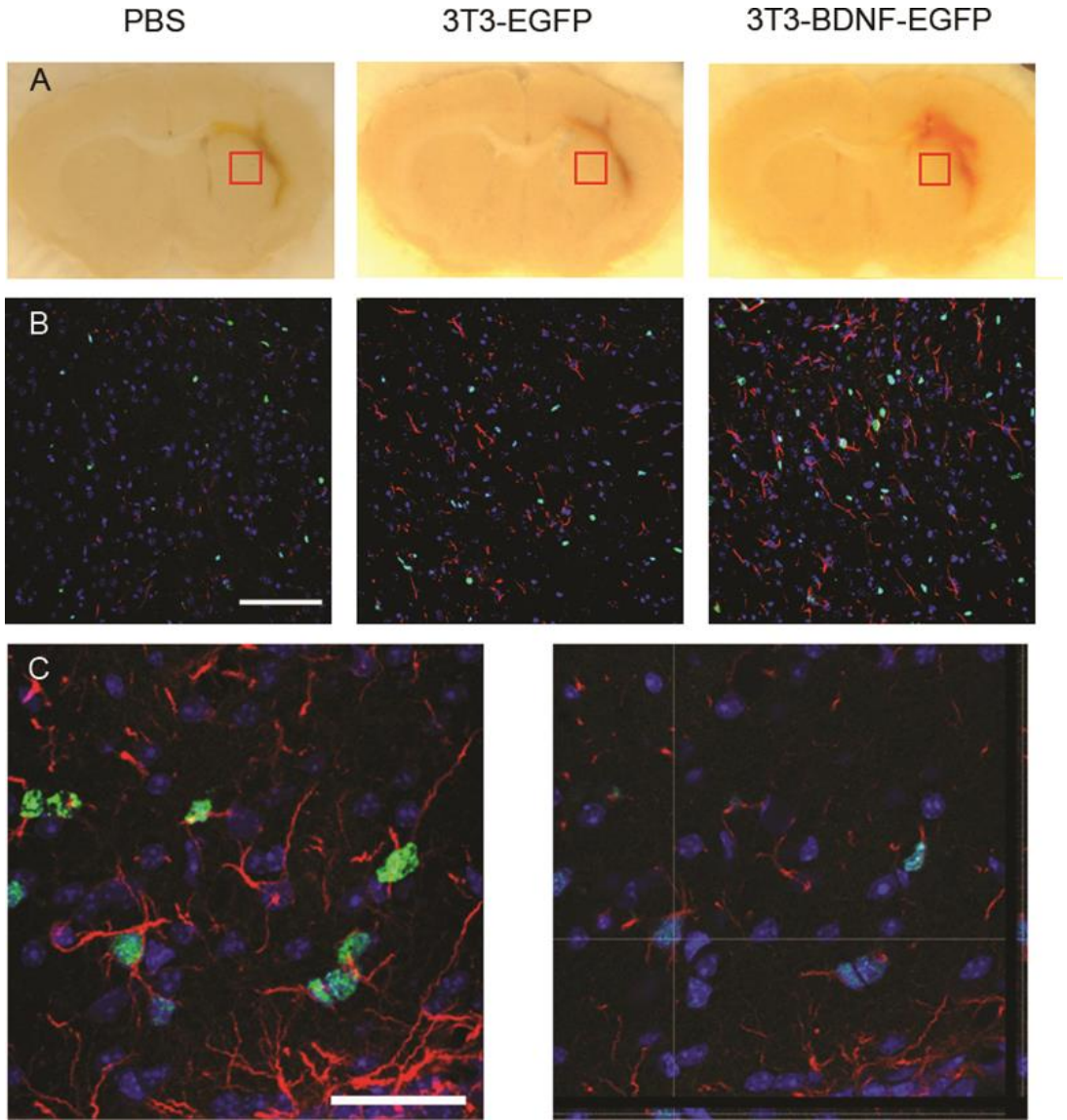
Transplanted 3T3-EGFP-BDNF Cells Produce BDNF in ICH Mice Brains

C: collagenase injection and hematoma area;

Cell implantation

D: red: BDNF, green: EFGP

Both Cell Treatment Groups Had More GFAP/EdU Positive Cells Than PBS Control Group Outside SVZ



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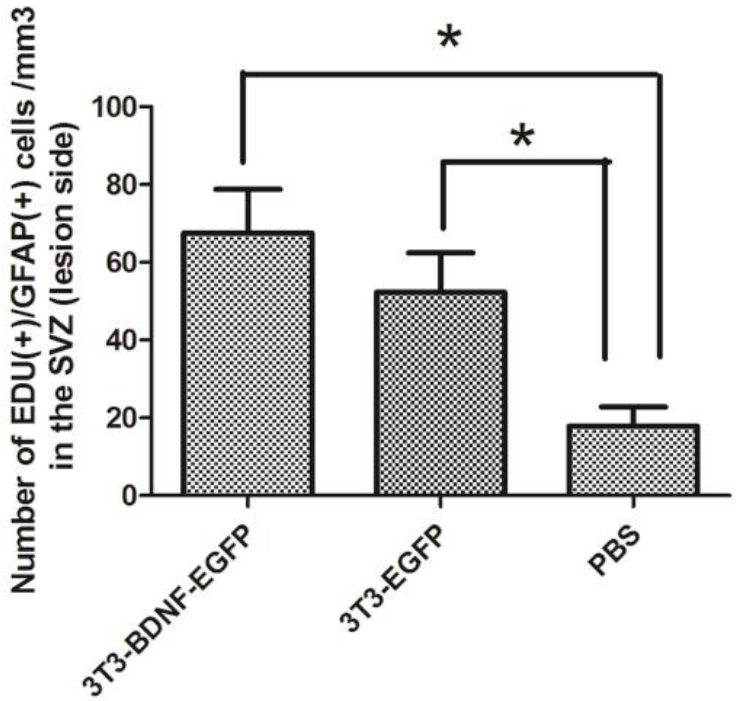
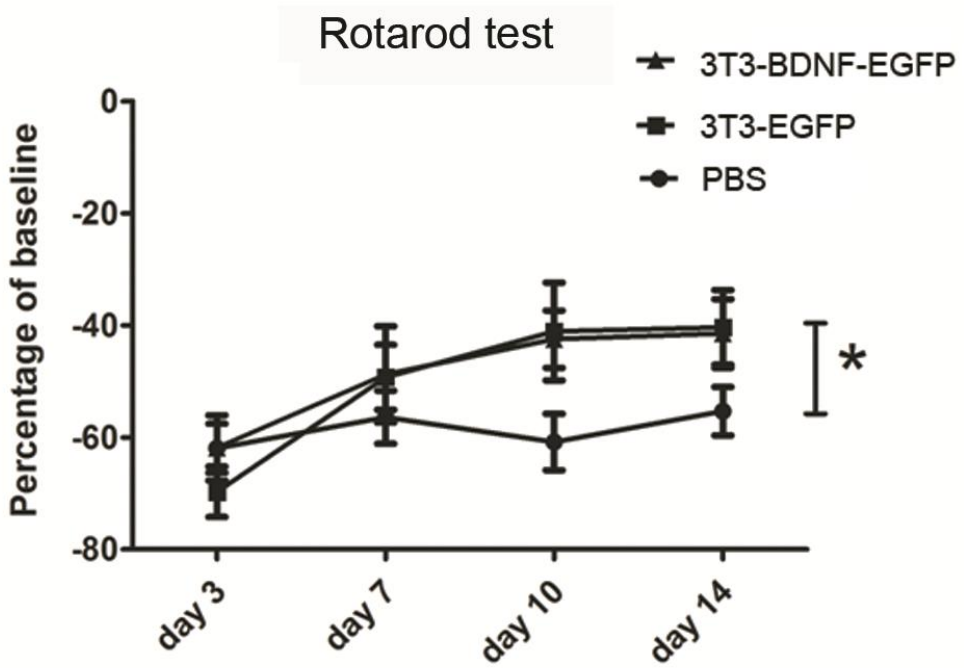
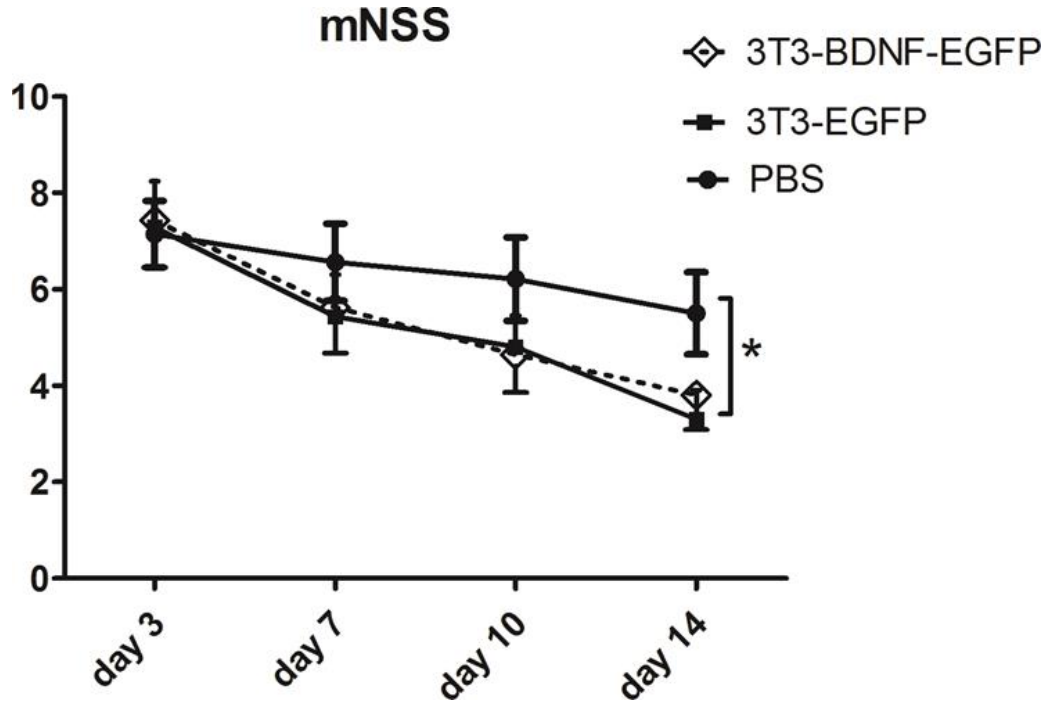


Cell Treatment Promoted Functional Recovery





Rotarod test:

Pre-trained mice with 3 trials per day for 3 days

Pre-surgical baseline data: -1

Modified Neurologic Severity Score for Mice

0-10; locomotors function score

Summary

- Growth factors provided both 3T3- BDNF-EGFP and 3T3-EGFP fibroblasts, could induce the functional recovery of ICH mice.
- The effect of BDNF may increase the number of migratory neuroblasts as well as prevent the tissue loss in area of ICH.
- Genetically manipulated fibroblasts with BDNF might have the potential therapeutic effect on the neuroregeneration after brain injury.

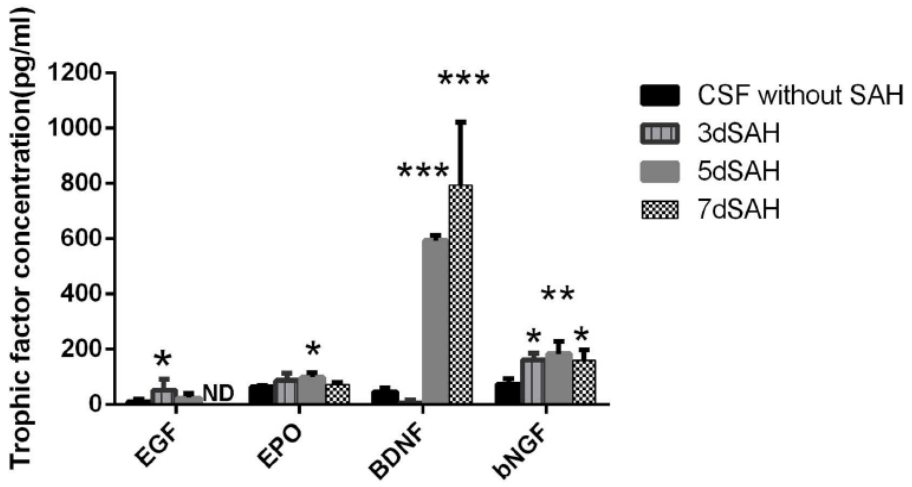
Chen SJ, Tsai JC, Lin CY, Chang CK, Tseng TH, **Chien CL***. ***J Neuropathol Exp Neurol***. 2012; 71:1123-1136.

RESEARCH ARTICLE

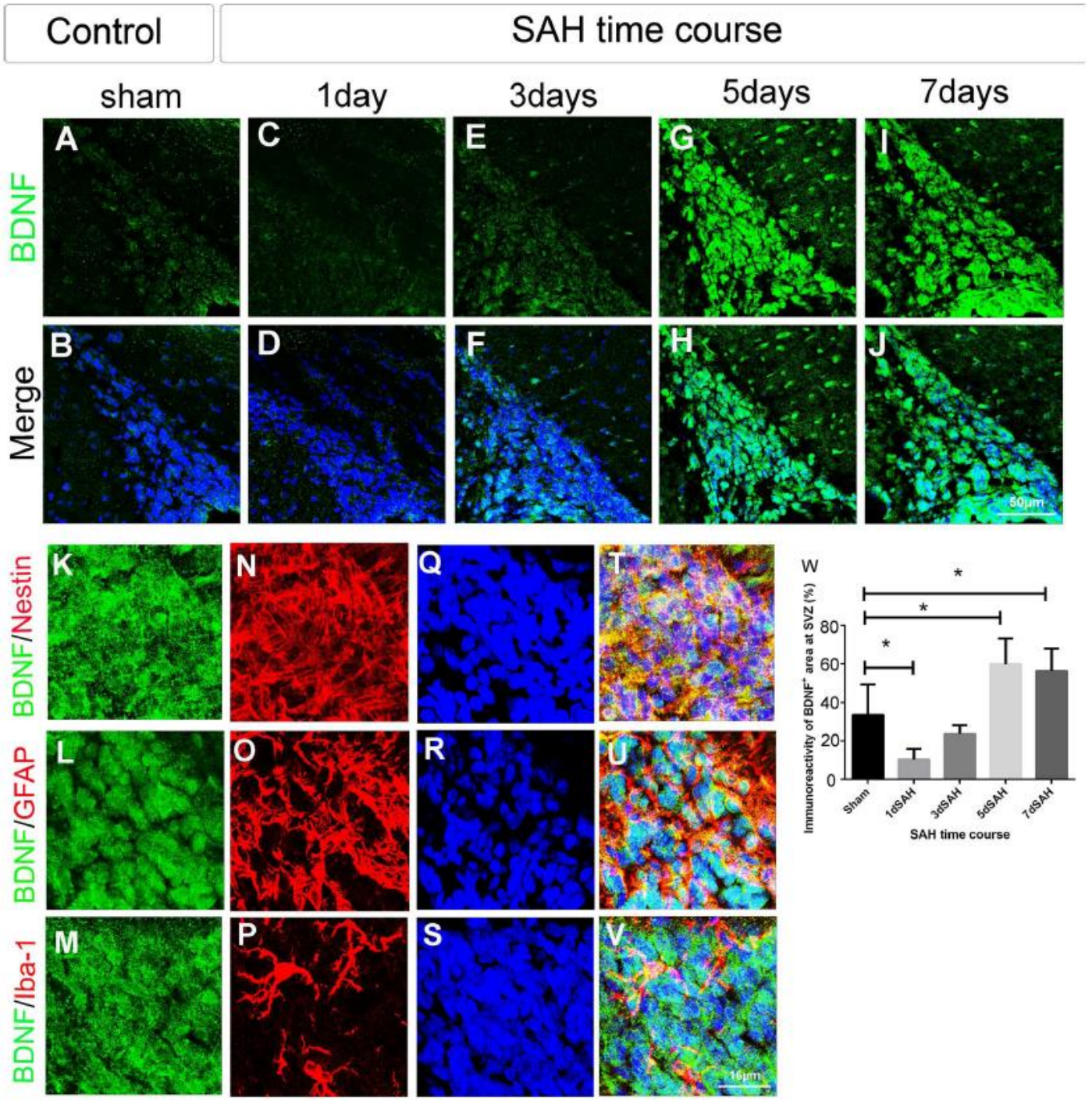
Subarachnoid Hemorrhage Promotes Proliferation, Differentiation, and Migration of Neural Stem Cells via BDNF Upregulation

Wen-Di Lee[1], Kuo-Chuan Wang[2], Yi-Fen Tsai[2], Pin-Chun Chou[1], Li-Kai Tsai[3]*, Chung-Liang Chien[1]*

PLOS ONE | DOI:10.1371/journal.pone.0165460 November 10, 2016



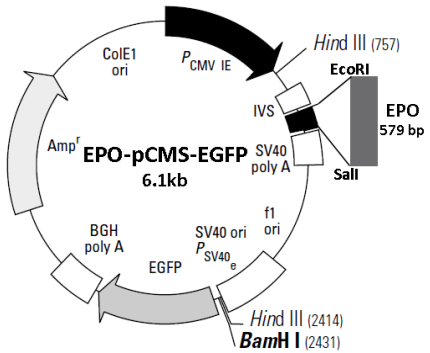
SAH triggers the expression of BDNF, which promotes the proliferation, differentiation, and migration of NSCs in the SVZ after SAH.

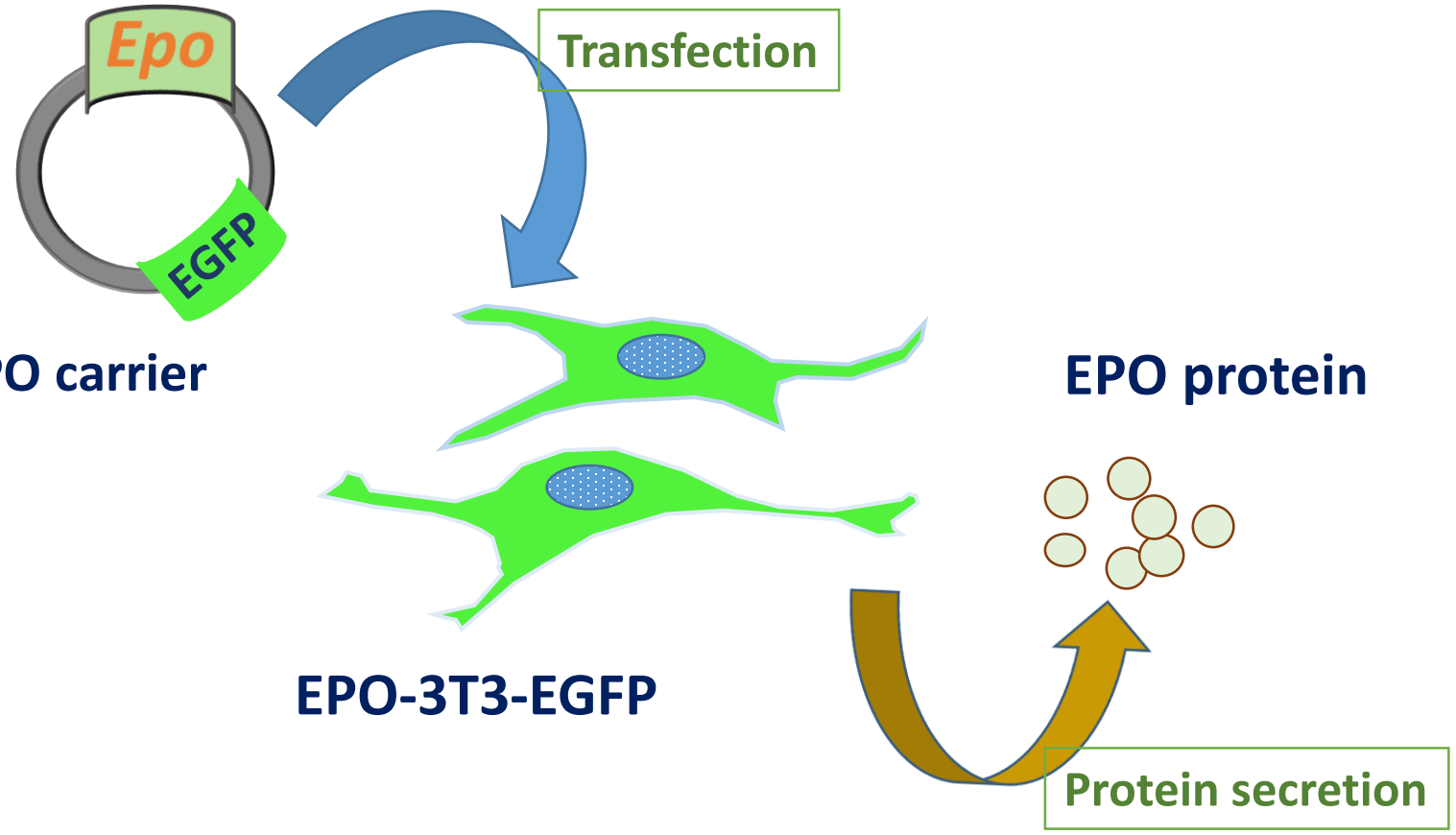


Neuroprotection effect of Erythropoitein (EPO)-overexpressing NIH/3T3 cell line

Li YC, Chen SJ and **Chien CL***.
Erythropoietin produced by genetic-modified NIH/3T3 fibroblasts enhances the survival of degenerating neurons.
Brain Behav. 2015; 5(8) **(Cover story)**

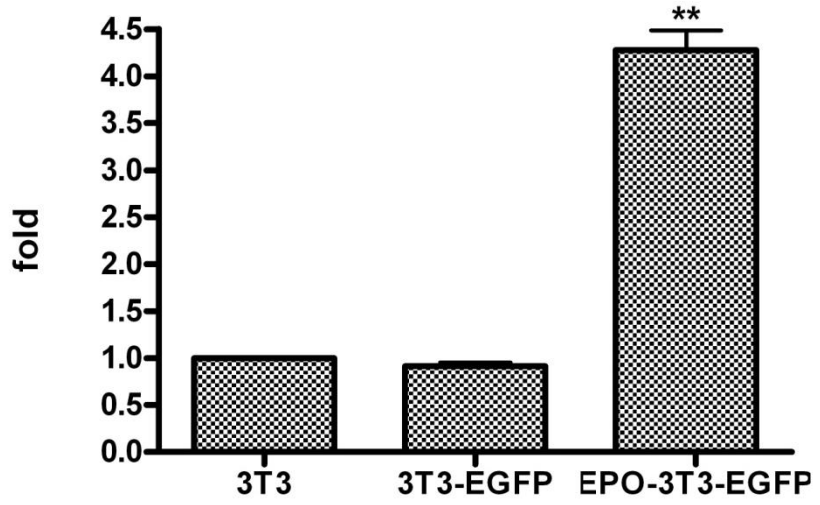
(1) Establishment of EPO-overexpressing NIH/3T3 stable cell line (EPO-3T3-EGFP)



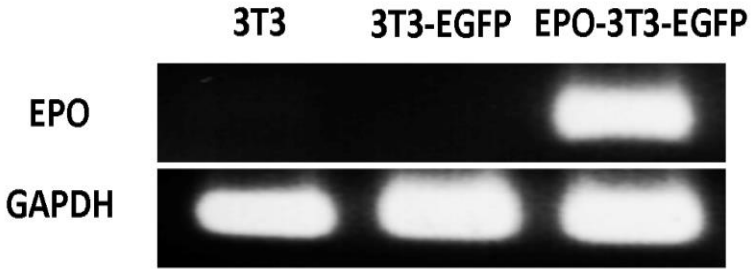


Quantitative real-time PCR analysis for EPO mRNA expression in EPO-3T3-EGFP, 3T3-EGFP and 3T3 cells

Relative quantity of mouse EPO mRNA expression

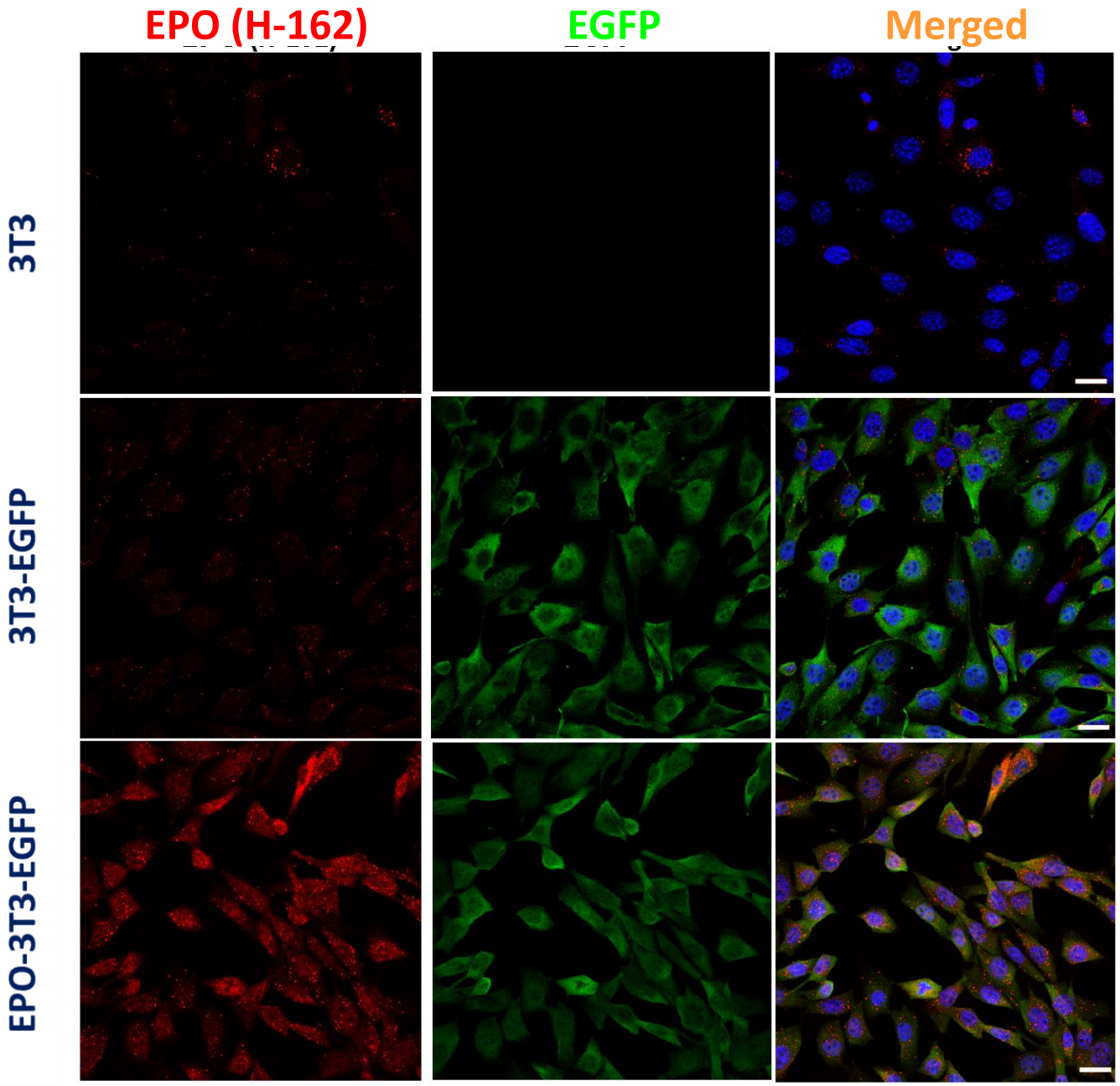


Gel Electrophoresis of cDNA

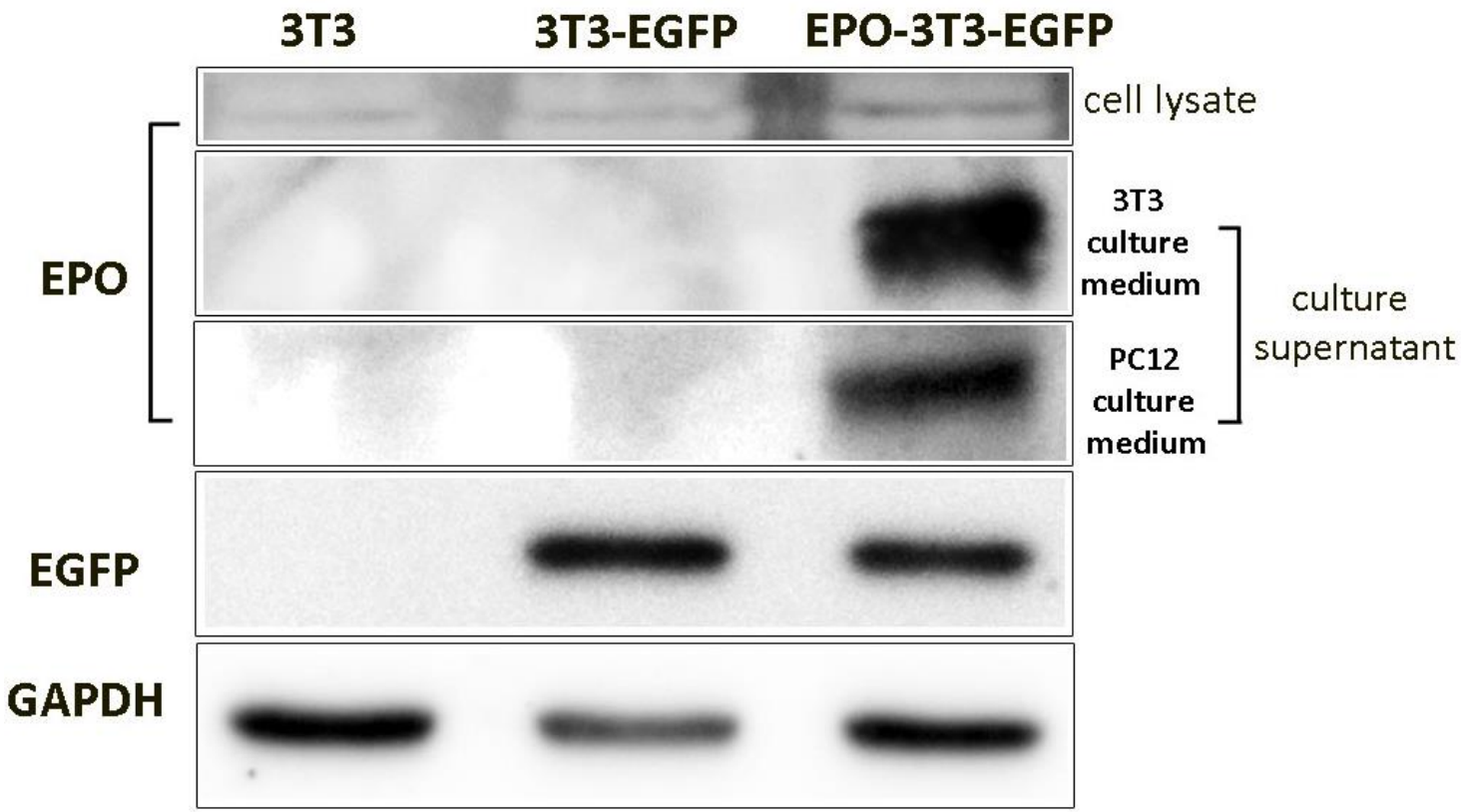


Transfected EPO gene was highly-transcribed in EPO-3T3-EGFP cells, and mRNA expression level was 4.27-fold higher than in 3T3 cells.

Immunocytochemistry for EPO in stable cell lines

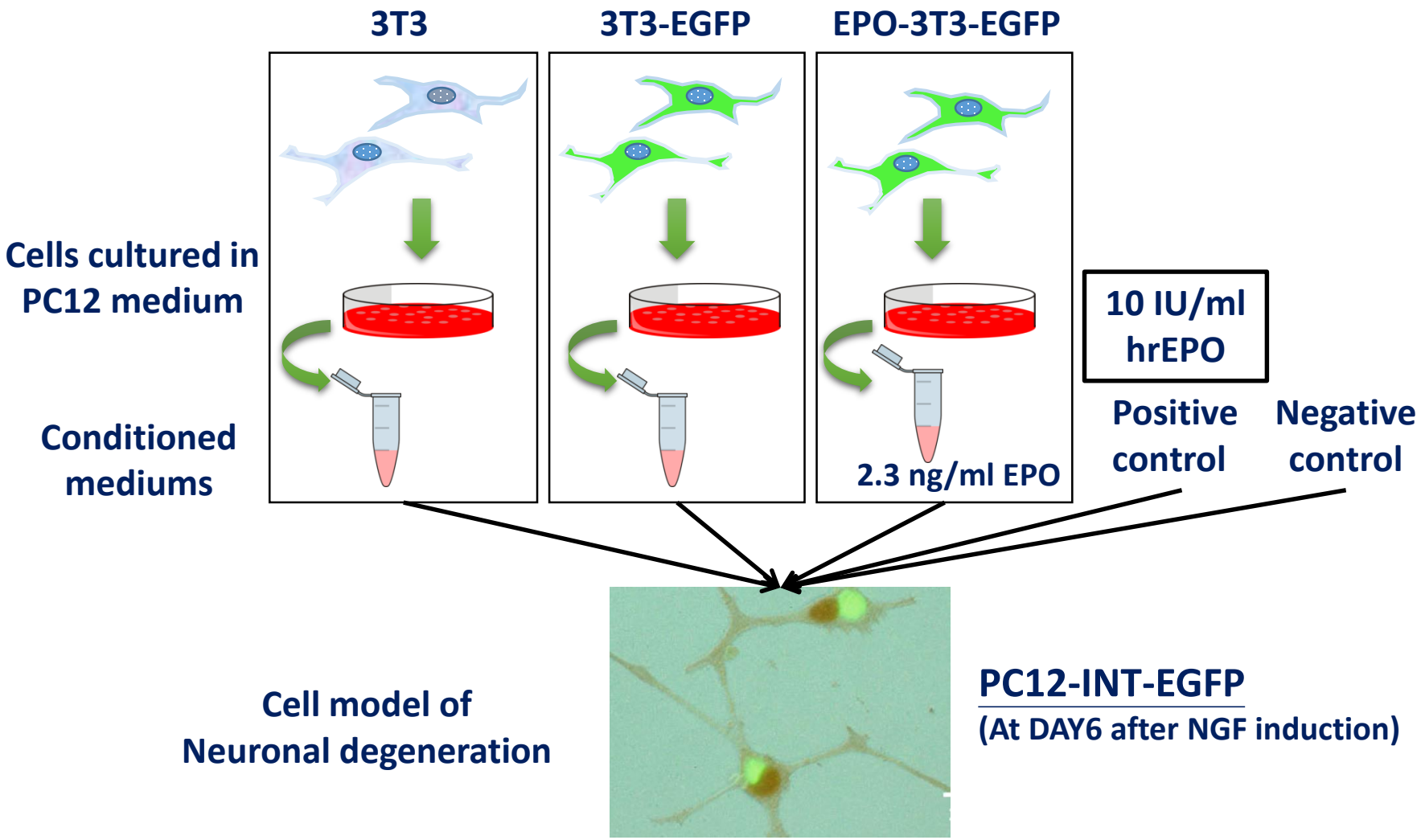


Western blot analysis for EPO in both cell lysates and culture supernatants



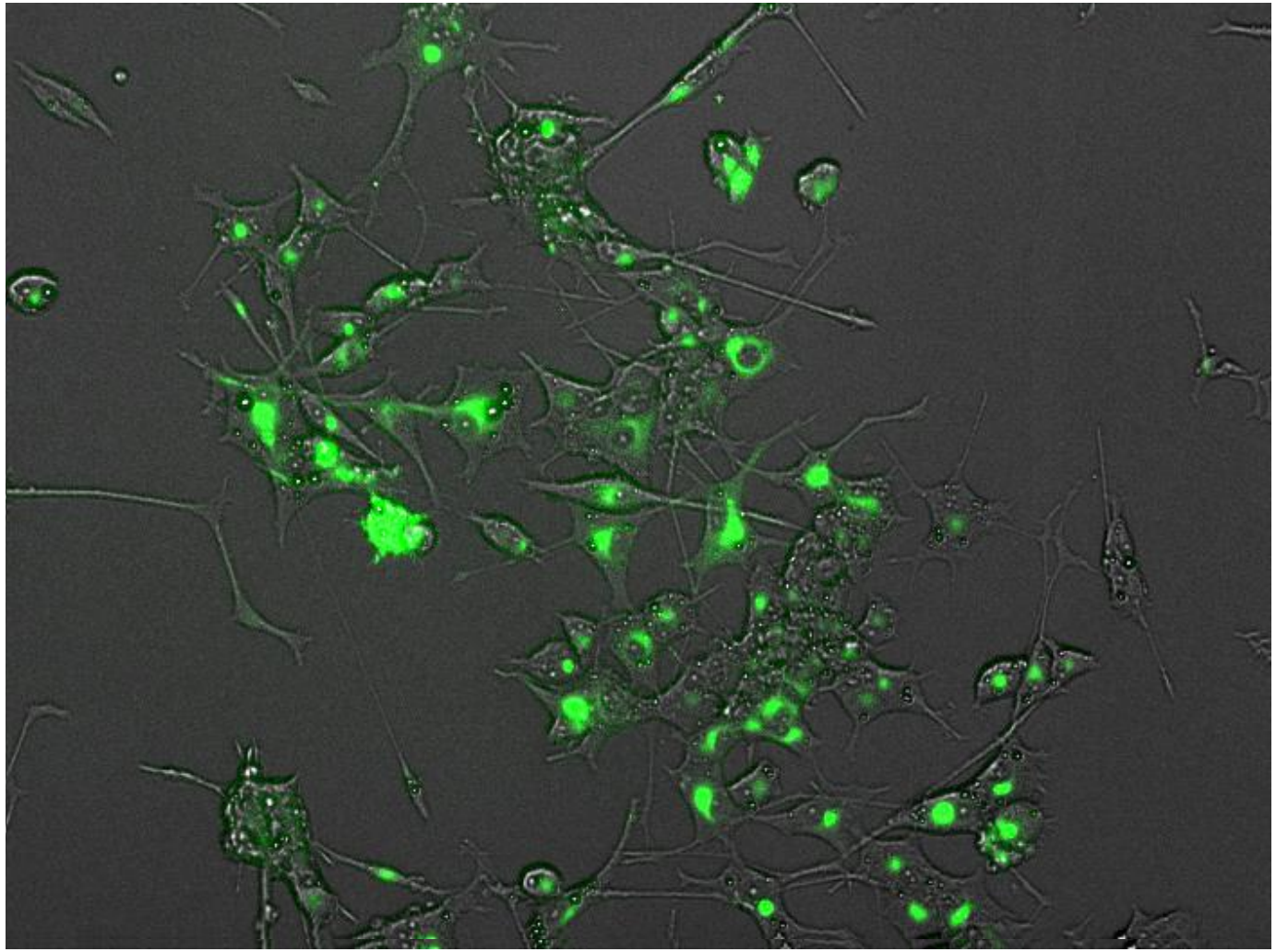
Large amount of secreted EPO in culture supernatants was detected in the EPO-3T3-EGFP stable cell line.

(2) Functional Assays for EPO-3T3-EGFP cells —— Verification of Neuro-protection ability

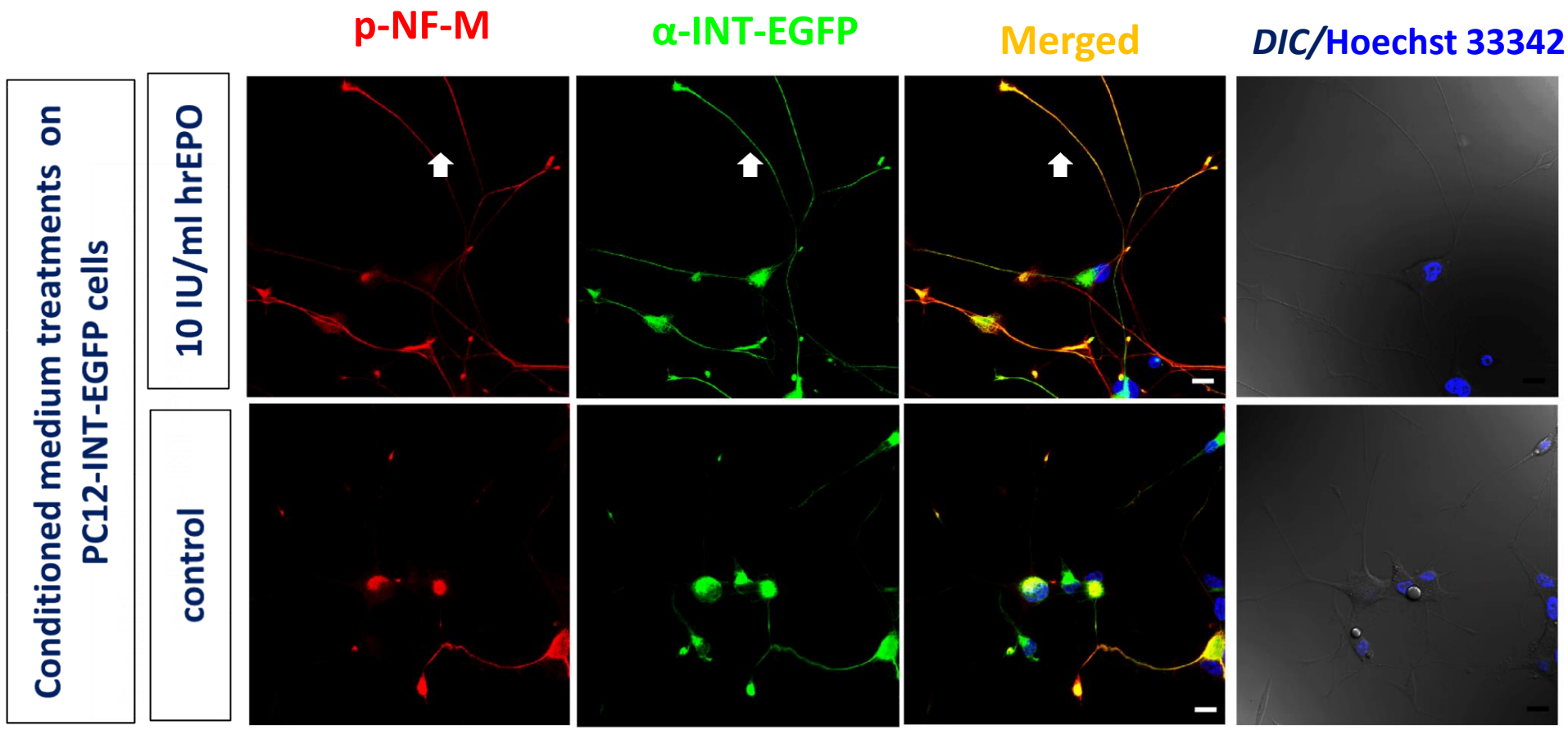


Live cell imaging recorded for 48 hours after conditioned medium treatments on PC12-INT-EGFP cells

EPO-3T3-EGFP conditioned medium treatment



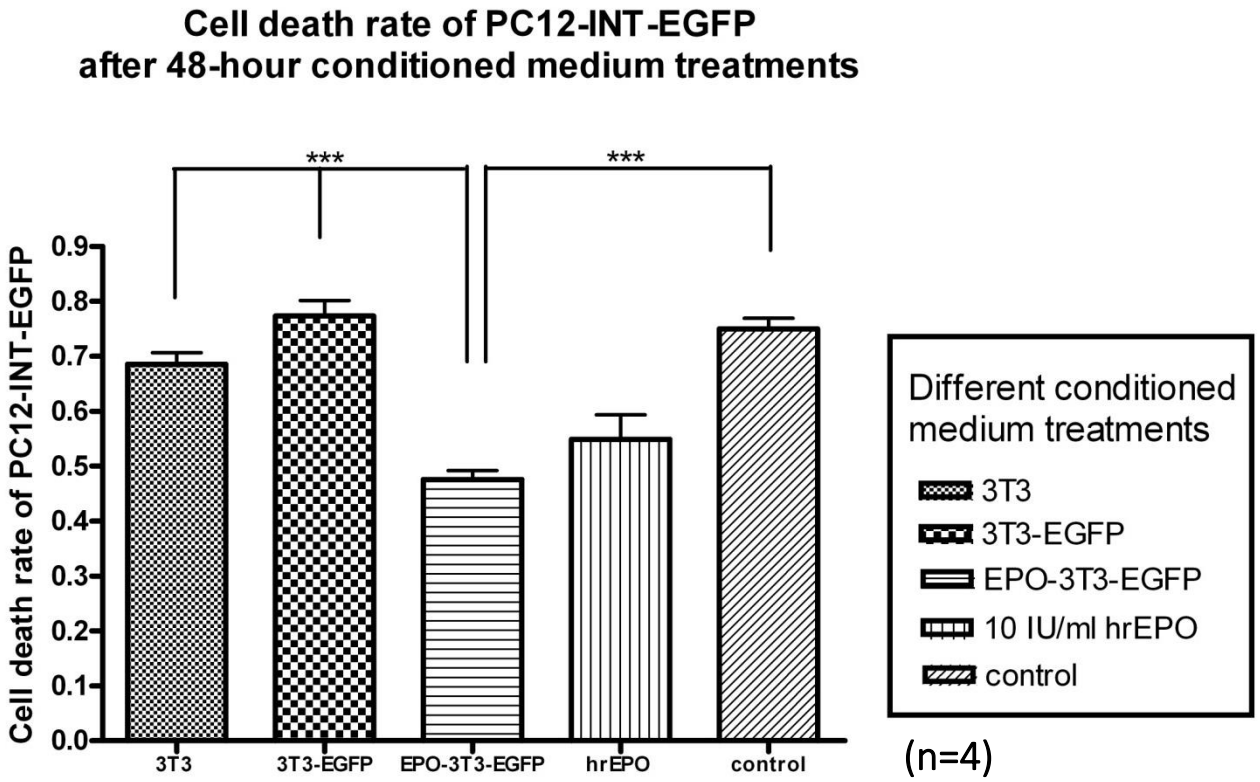
Rearrangement and disaggregation of NFs were found in PC12-INT-EGFP cells after treatments with EPO-3T3-EGGP conditioned medium and 10 IU/ml hrEPO.



Cell viability assay

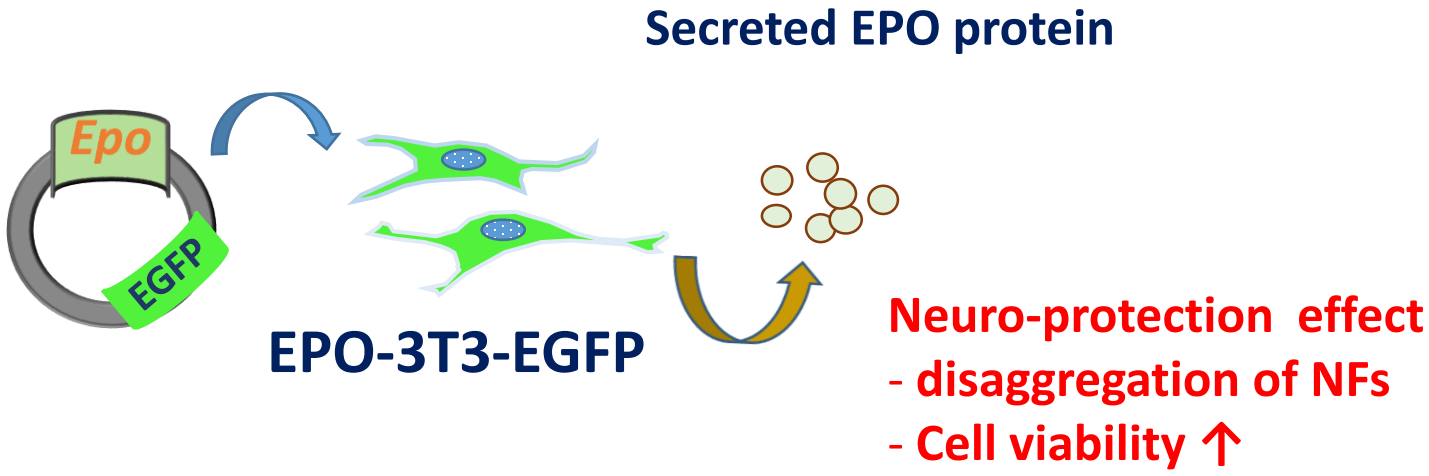
Culture cell PI/Hoechst 33342 co-stained on cell nucleus

- **PI (+), Hoechst 33342(+) ➔ dead cells**
- **PI (-), Hoechst33342 (+) ➔ live cells**



Cell death rate of PC12-INT-EGFP was significantly reduced after 48-hour treatment with EPO-3T3-EGFP conditioned medium

Summary



The neuro-protection effect of EPO-overexpressing 3T3 cell line might provide a potential material for cell therapy in neurological diseases via secreting EPO on a *short-term, high-dose* and *regional* basis.

Erythropoietin Produced by Genetic-modified NIH/3T3 Fibroblasts Facilitates Recovery in a Rat Stroke Model

Chou PC, Tsai LK, **Chien CL***

Use cell-based therapy to achieve a short-term, high-dose and *"regional"* therapeutic concept for ischemic stroke

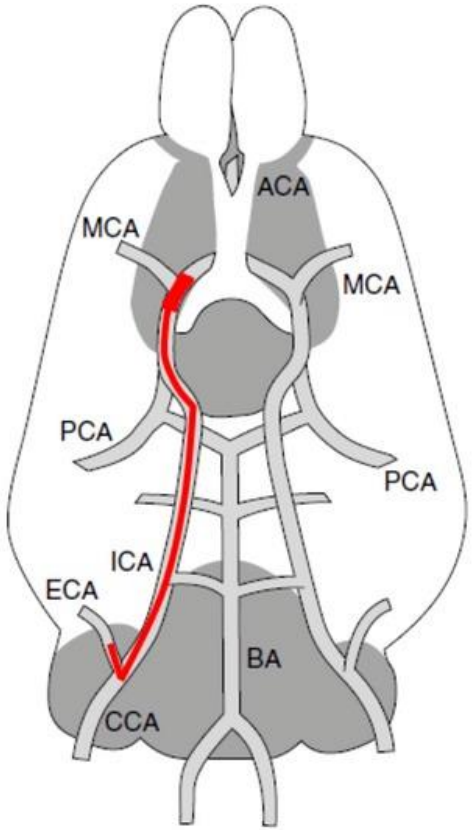
(1) To establish a cell therapy protocol for ischemic stroke
(2) To verify its possible neuroprotection and neurogenesis ability in rat stroke model

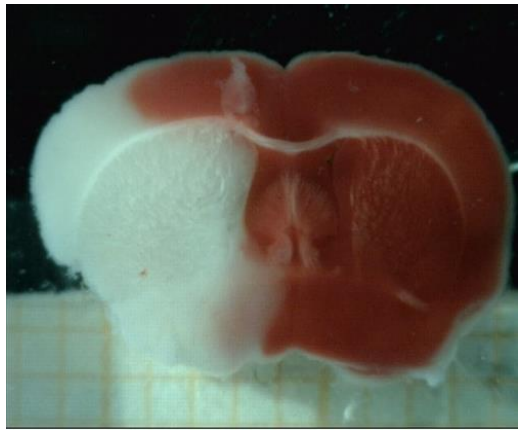
Middle Cerebral Artery Occlusion (MCAO)

- **MCAO produces primary ischemic cell death in striatum and overlying frontal, parietal, temporal, and portions of occipital cortex**

- **Damage to widespread and functionally diverse brain regions**

- **Complex motor, sensory, autonomic, and cognitive deficits and confound study of the specific circuits involved in recovery of these functions after stroke**

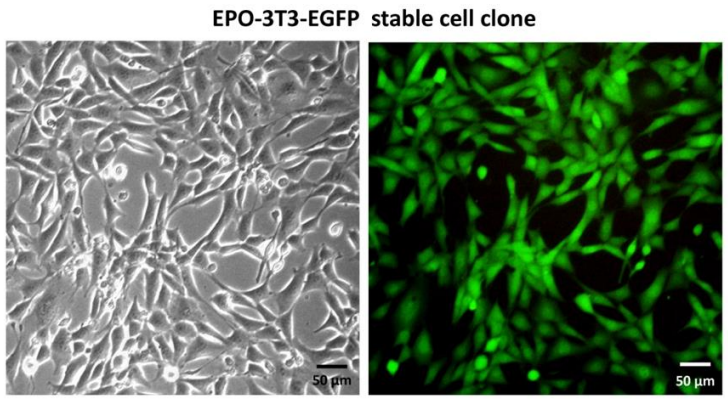




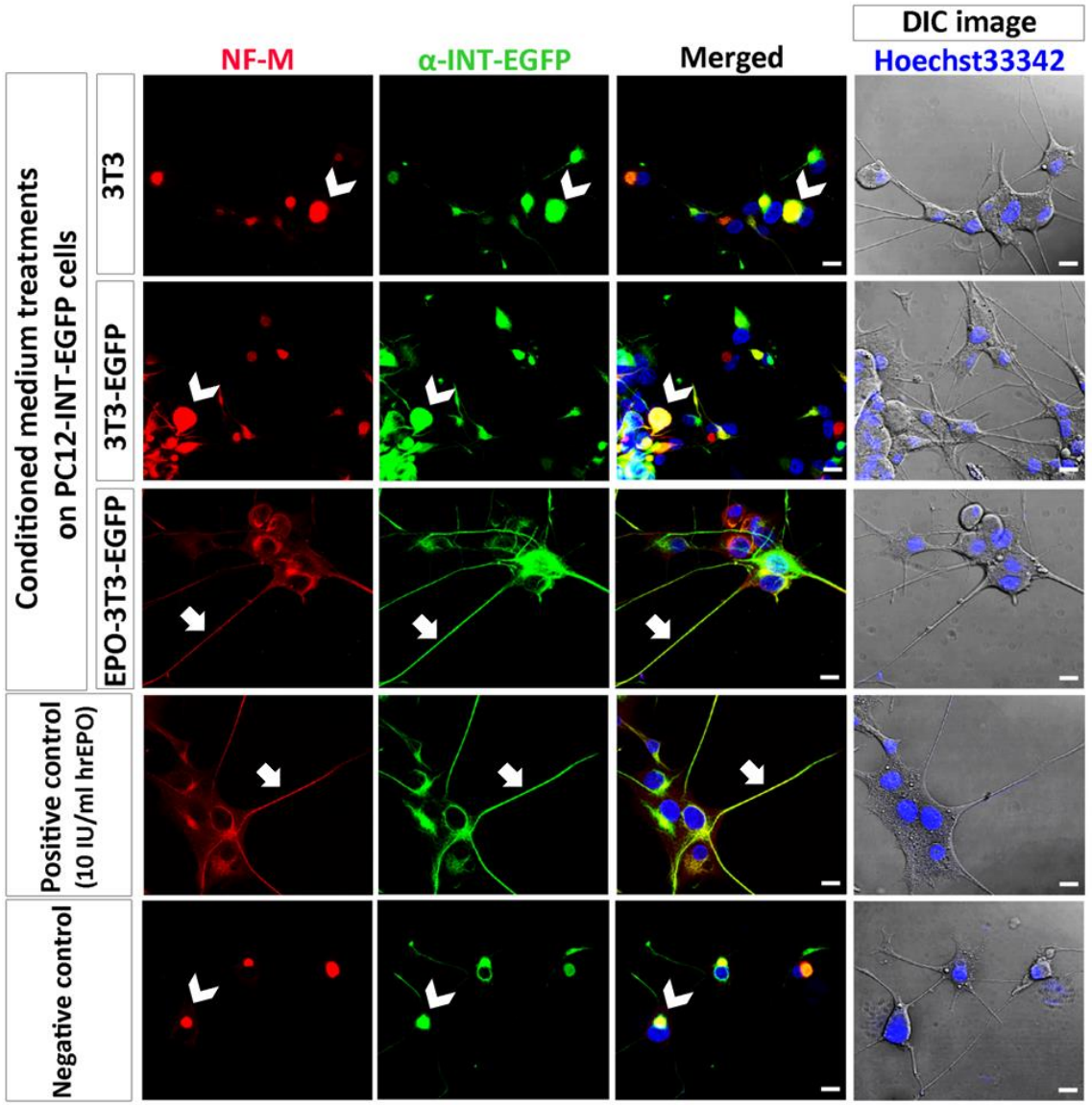
Carmichae et al., 2005

EPO-3T3-EGFP cells

- It is proven that *In vitro* experiment can increase the survival rate of PC12-INT-EGPT neuronal cells.



Cell lines	Number of hour(s) of EPO secretion (mean ± SD pg/mL, n = 3)		
	24 h	48 h	72 h
3T3	–[1]	–[1]	18.2 ± 31.5
3T3–EGFP	–[1]	18.2 ± 31.5	34.4 ± 29.9
EPO-3T3-EGFP	4428.6[2] ± 156.3	11874.6[2] ± 724.1	23888.8[2] ± 737.8



Materials & Methods

(1) Establishment of MCAO stroke rat model and use EPO-overexpressed NIH/3T3 stable cell line as cell therapy (EPO-3T3-EGFP)

Middle cerebral artery occlusion induction

↓

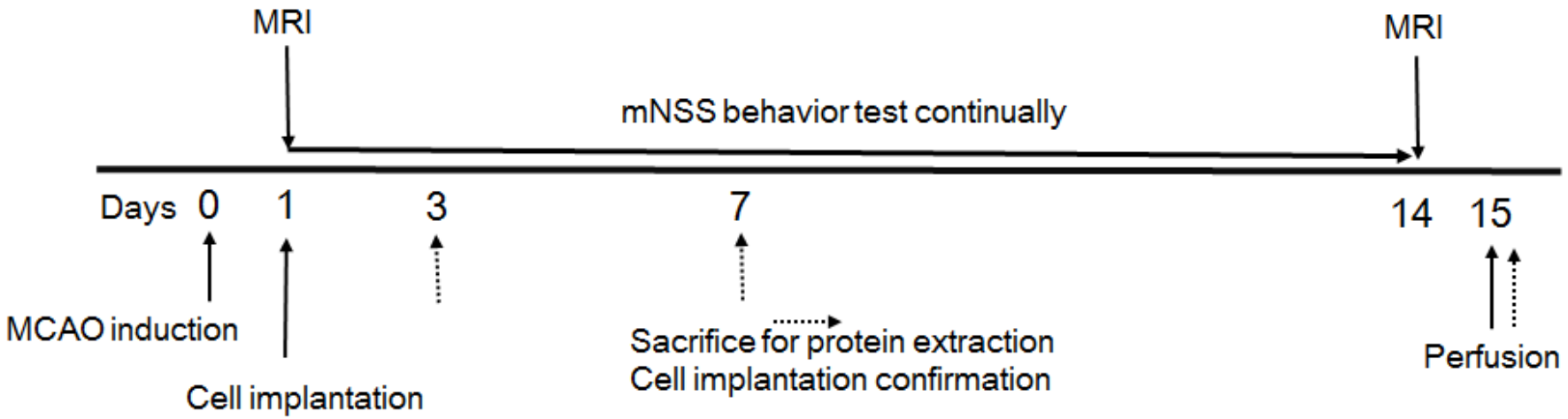
Cell therapy in stereotaxic procedure

↓

Neurological assessment

- ✓ Neurological score assessment
- ✓ MRI image: T2 image, Diffuse Weighted image

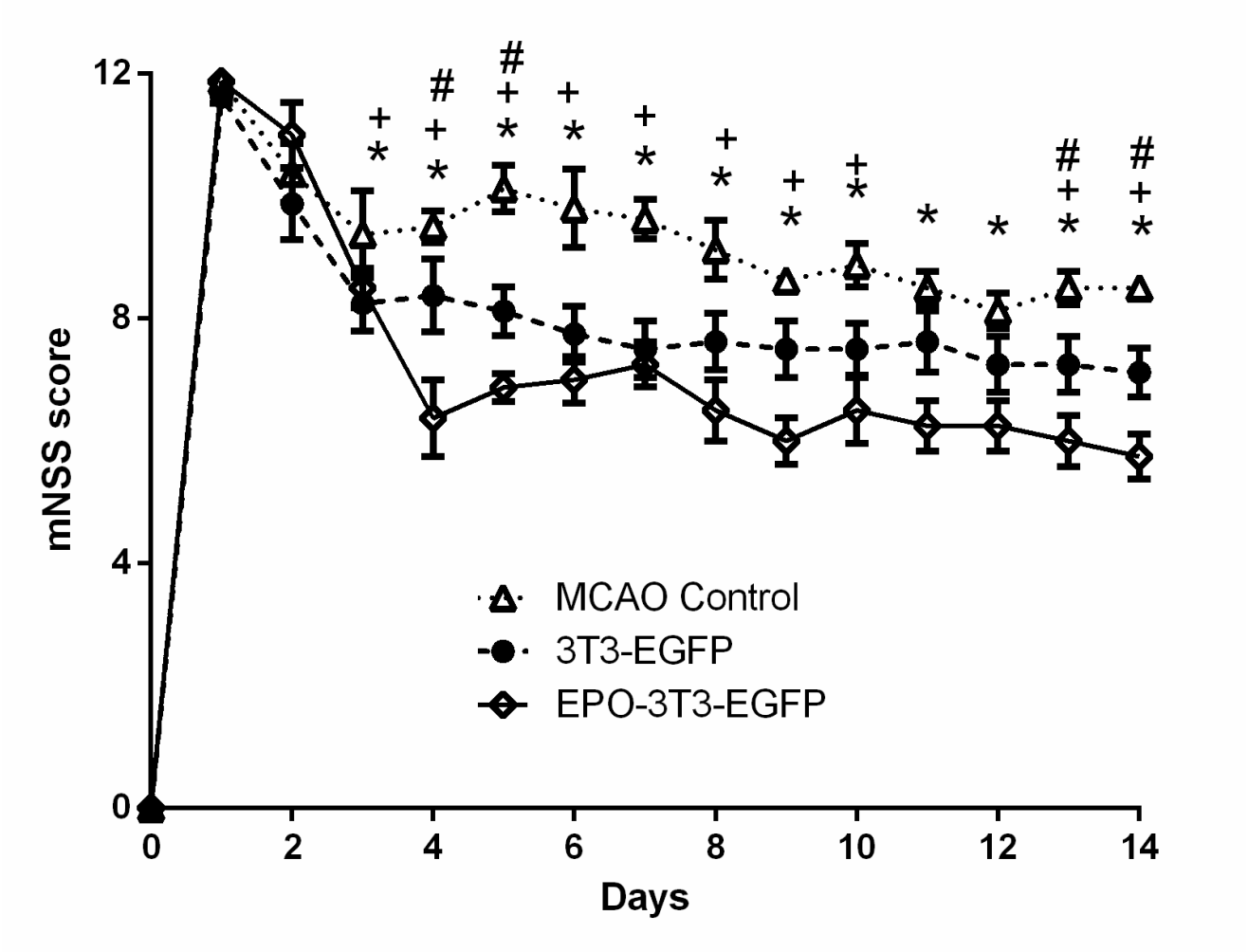
Experimental time table of the MCAO model



SD rat
Age of 7-8 weeks.
225-250g in weight

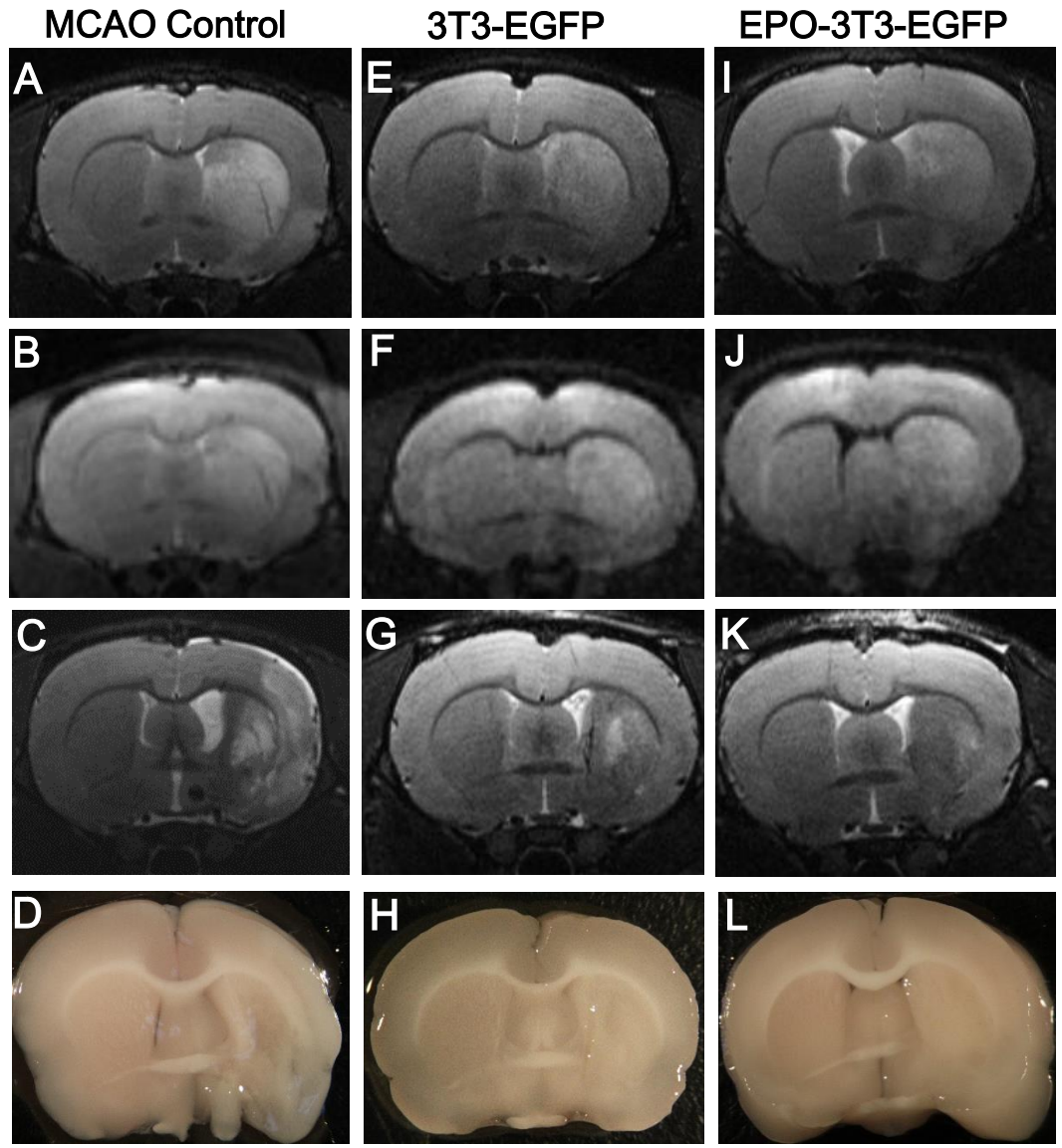
MRI: D1 and 14
Protein extraction: Day 3, 7 and 15.
Immunohistochemistry: Day 15 after perfusion

Modified Neurological Score assessment

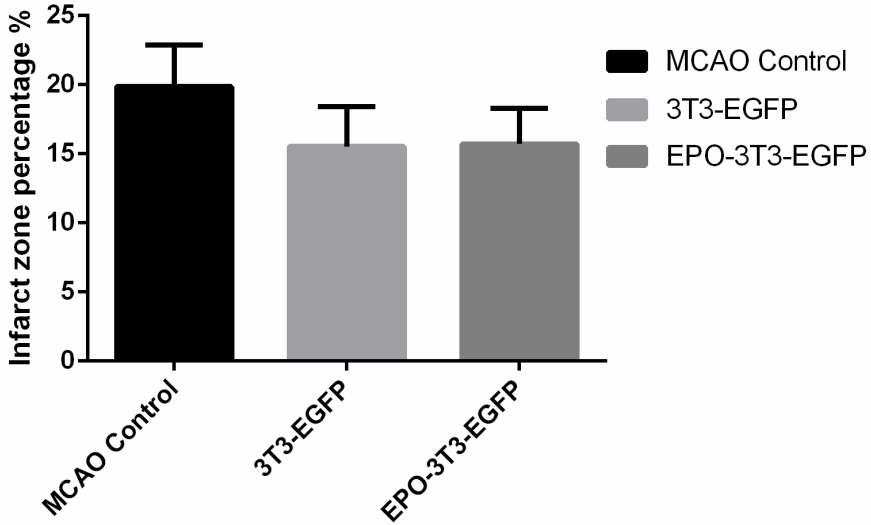


Behavior improvement was observed in the EPO treated groups.

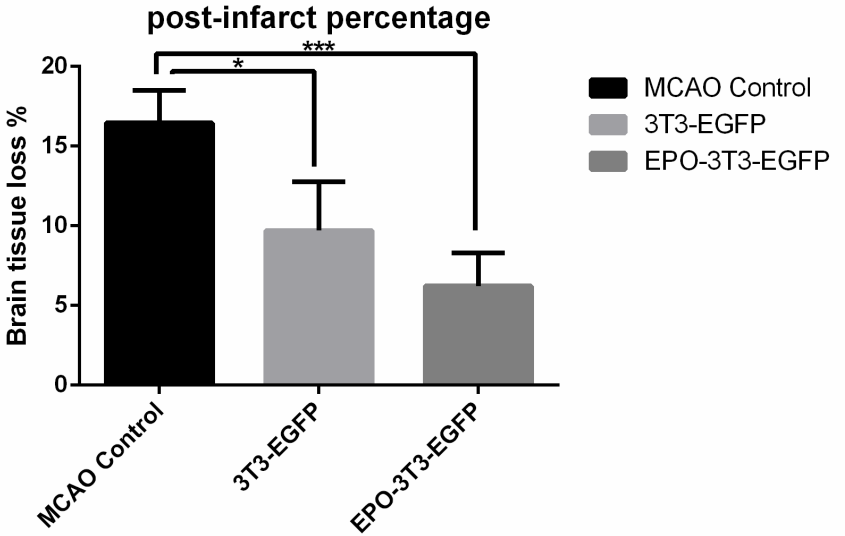
MRI analysis



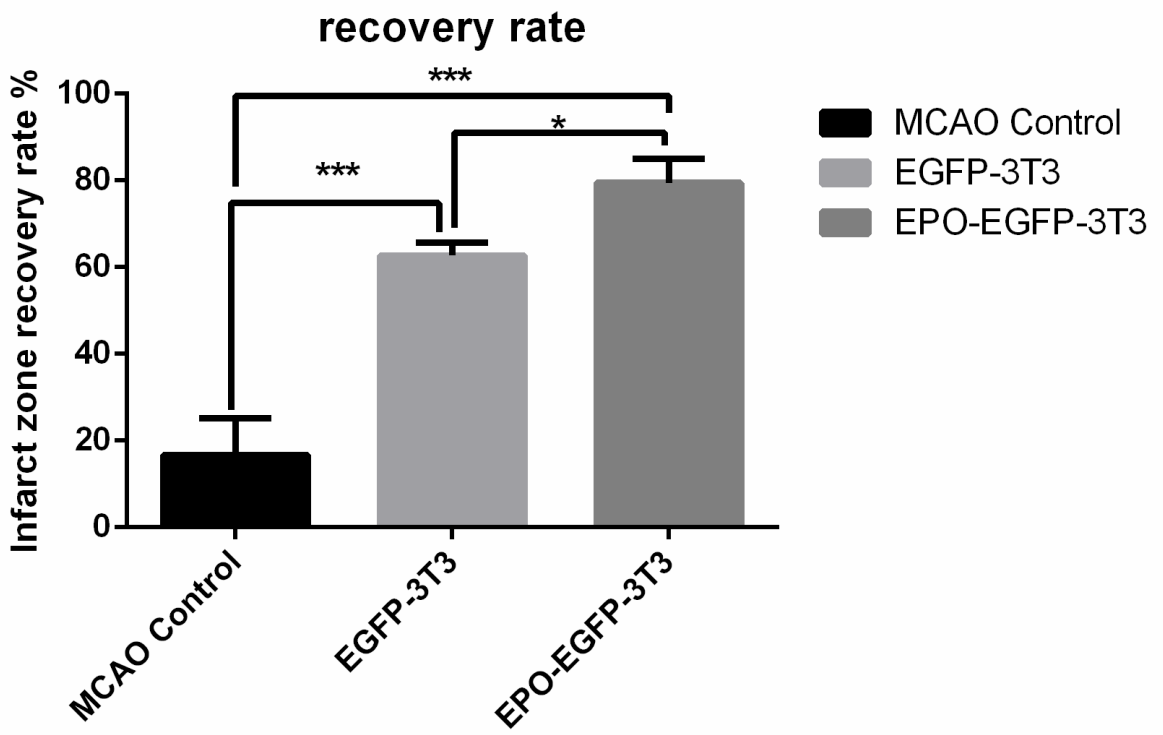
Before treatment



After treatment

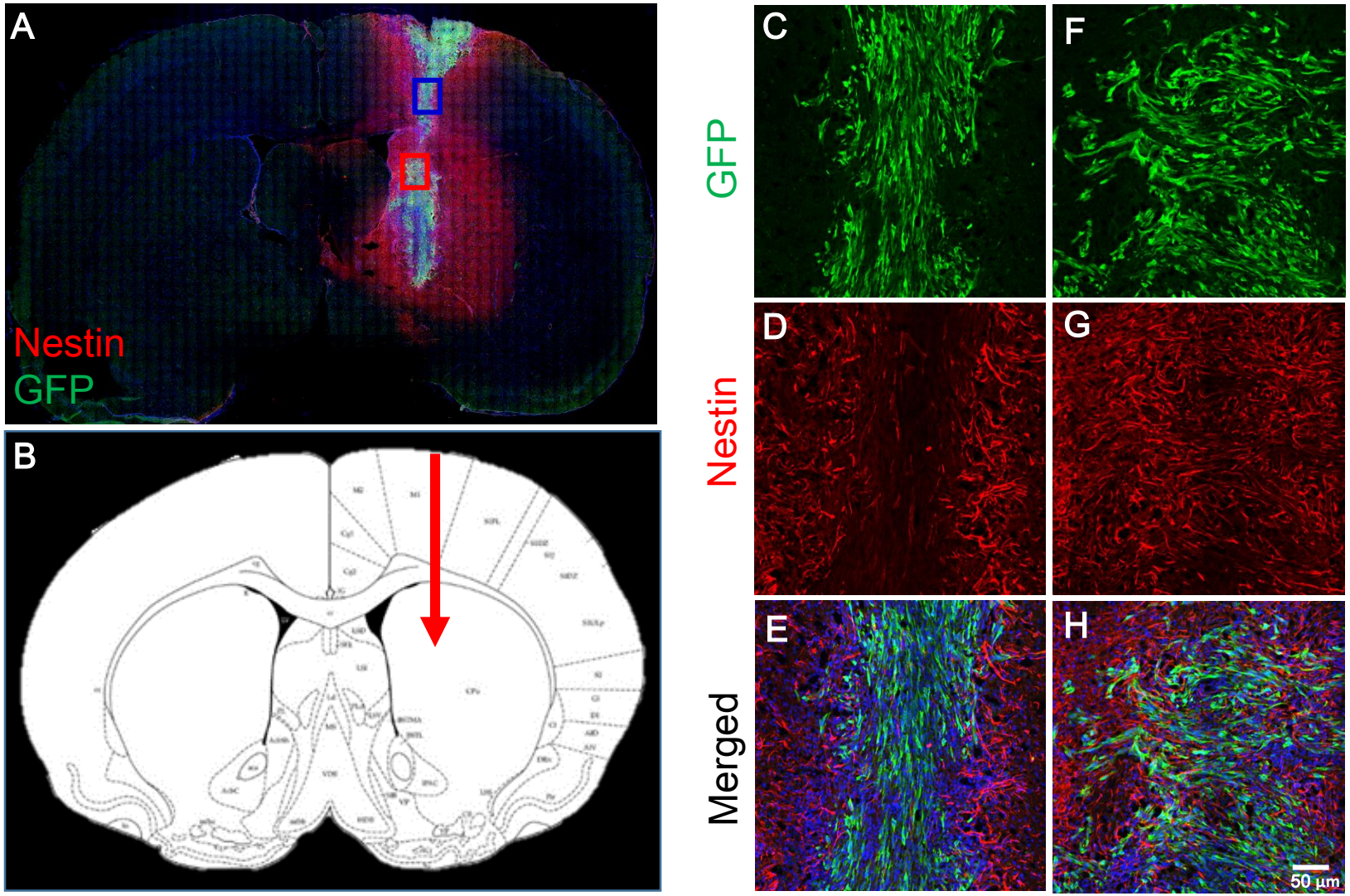


MRI analysis

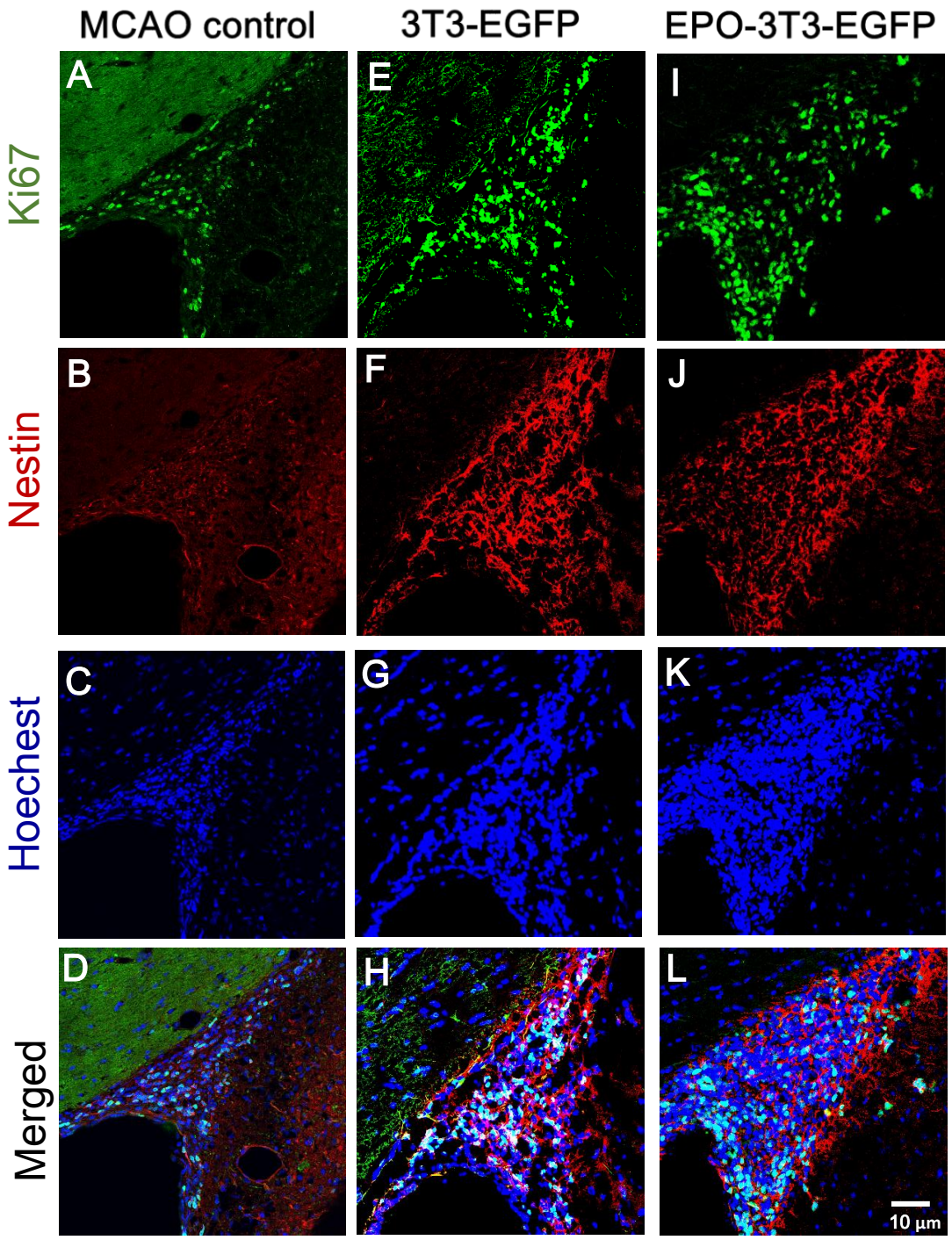


Implantation of modified cells can have the therapeutic effect to reduce the infarct area percentage and to increase functional recovery.

GFP-positive EPO-3T3-EGFP cells were identified after stereotaxic implantation for 7 days

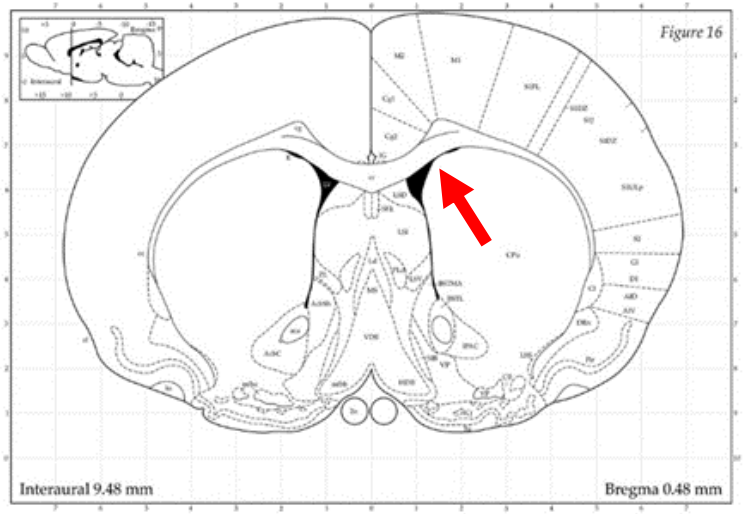


Nestin immuno-positive cells could be found around the implanted area.

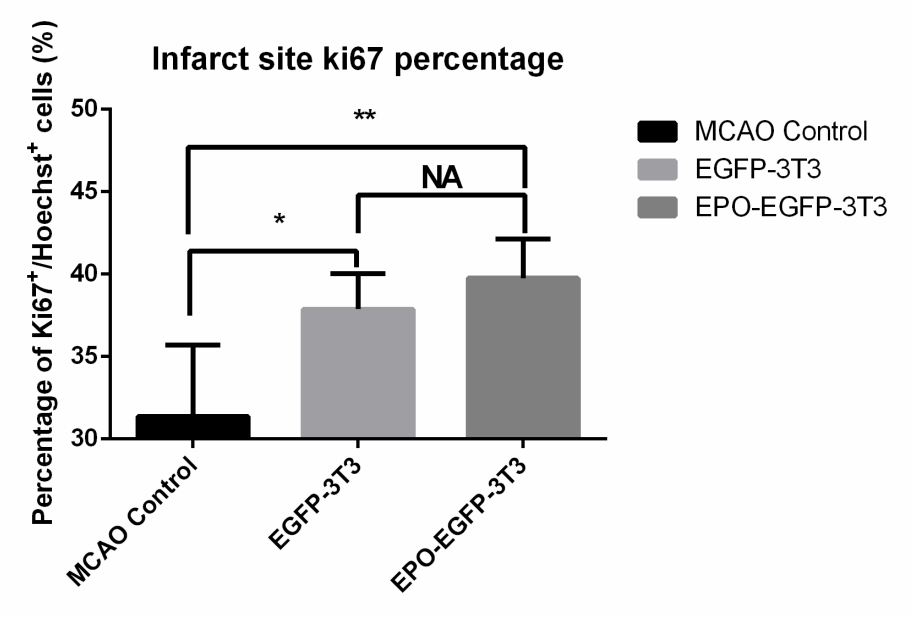


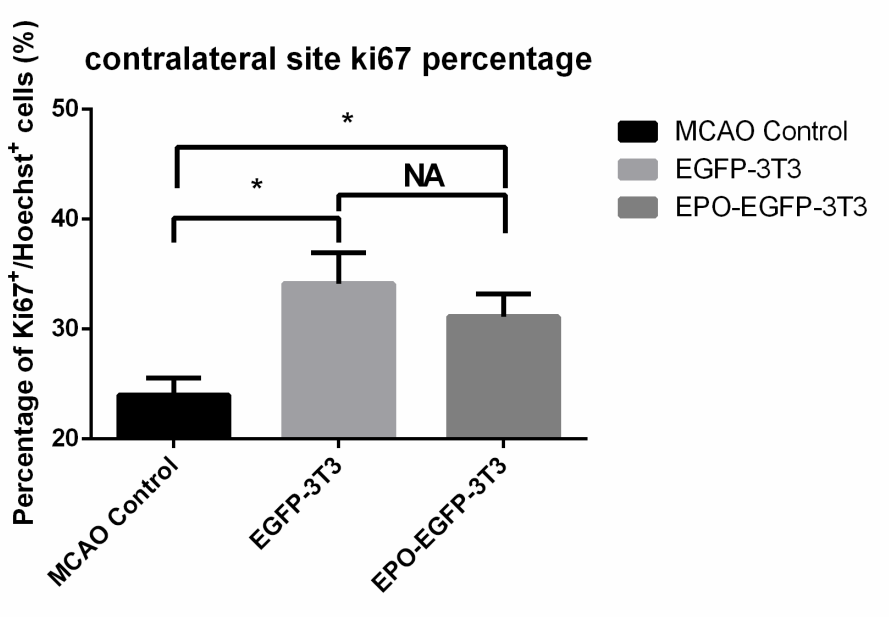
Nestin immuno-positive cells could be found co-localized with proliferation cells in SVZ – infarct site

Ki67: Cell proliferation.
Nestin: Neuralstem cell marker.

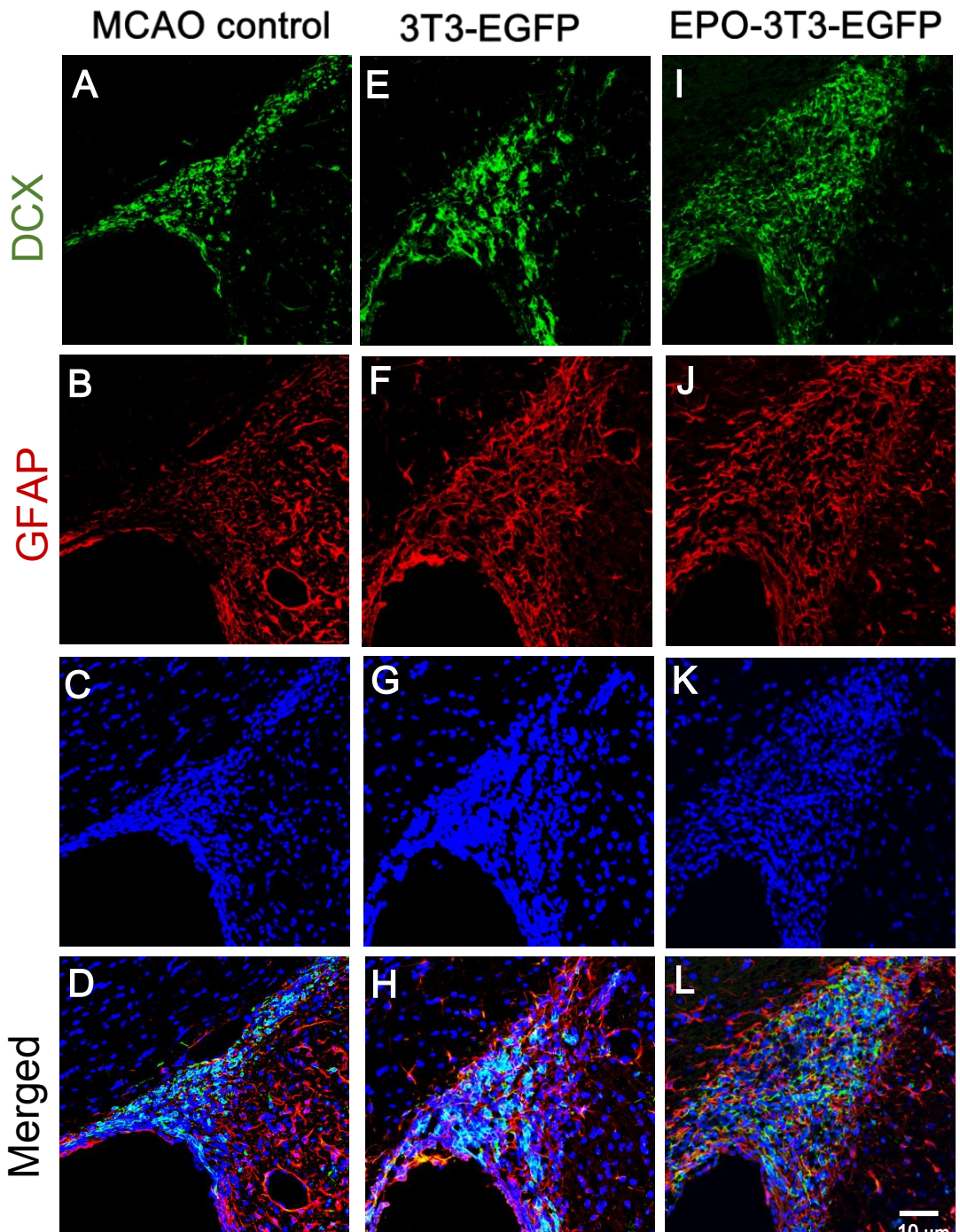


Cell proliferation in SVZ



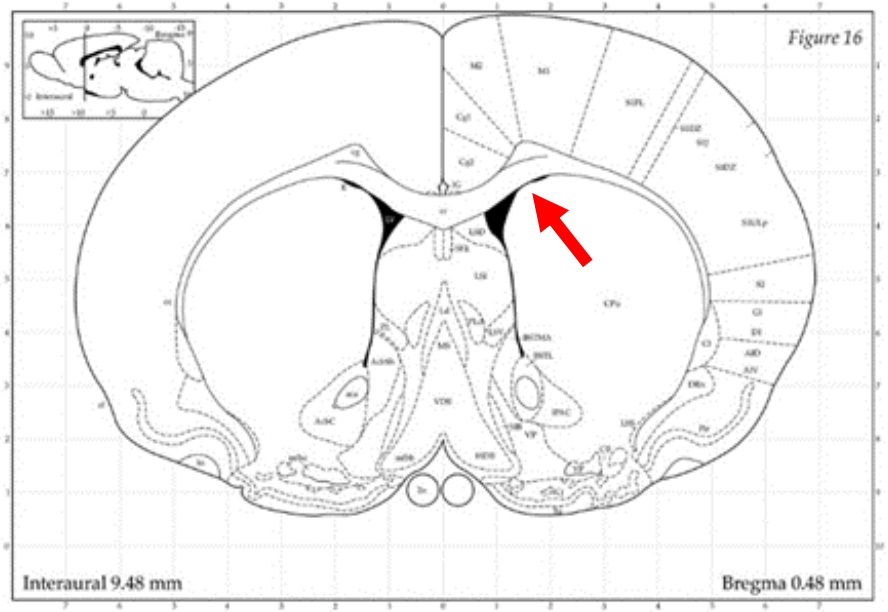


Cell proliferation was increased in SVZ after cell therapy.

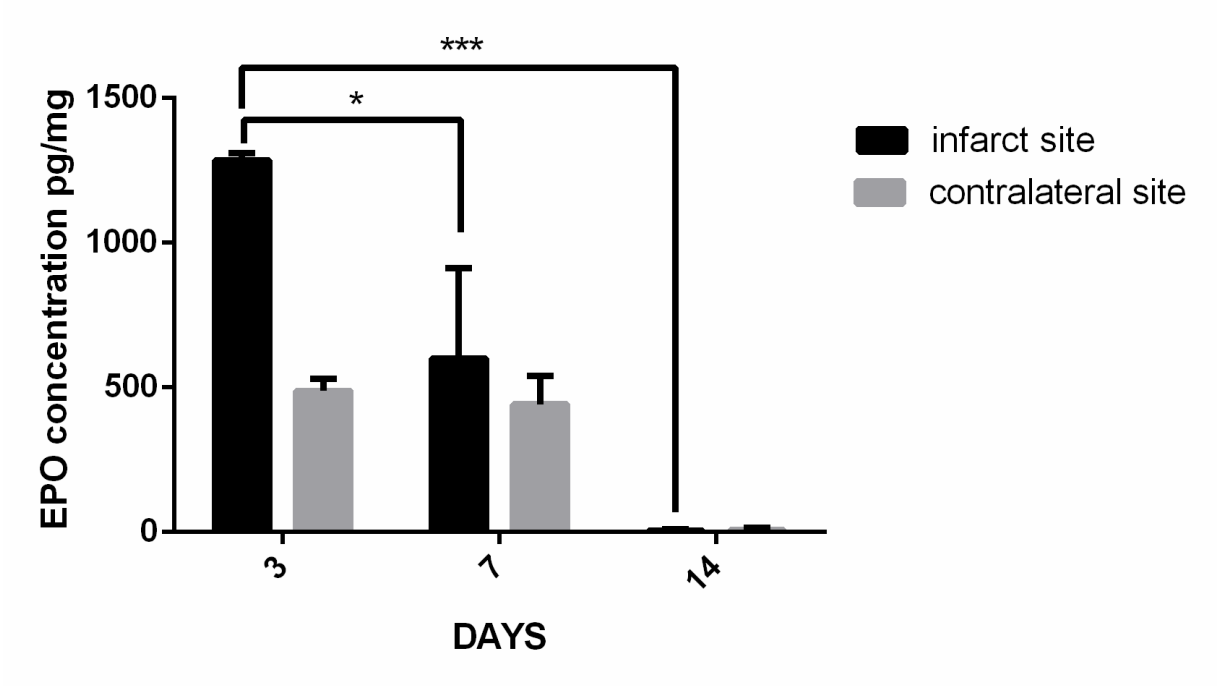


Young neurons and reactive astrocytes in SVZ – infarct site

Doublecortin (DCX) : early neuron, possible neural migration.
GFAP: reactive astrocyte.



Quantitative analysis by ELISA for EPO after EPO-3T3-EGFP injection



Abundant amount of EPO secretion by EPO-3T3-EGFP cells in the early phase of cell implantation therapy.

Neuroprotection effect of neurotrophin over-expressed fibroblasts

➢ Behavior improvement was discovered after implantation with EPO-3T3-EGFP cells. The MRI also shows a positive result after both cell therapy.

- EPO treatment can provide neuroprotection and decrease neuronal apoptosis in early phase of injury.

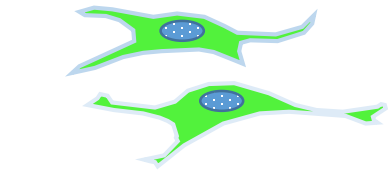
—Siren et al. (2001)

- Transfected and non-transfected BDNF 3T3 fibroblasts enhance migratory neuroblasts and functional restoration in mice with ICH.

—Chen et al. (2012)

■ EPO-3T3-EGFP cells could be a good therapeutic agent for stroke therapy or other neurological diseases in the future.

Conclusion

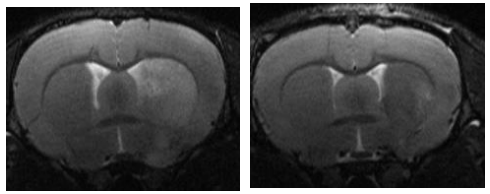


EPO-3T3-EGFP

Inject to the infarcted area

Possible Neuroprotection effects
-Neurogenesis ↑

MRI image



Rat with infarcted stroke

Functional recovery

Both EPO-overexpressing NIH/3T3 and NIH/3T3 cells treatments could facilitate neurogenesis and contribute to the functional recovery of injury brain.

Acknowledgement