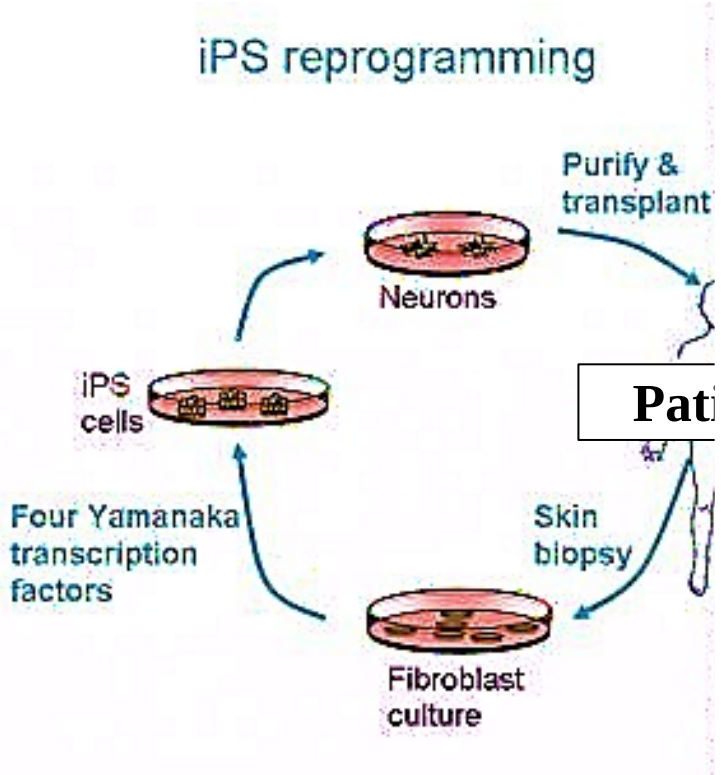
In vivo transdifferentiation- Melting scars and making neurons

Vijay Chandrasekar

Journal club presentation

25.3.14

Generation of patient derived/specific cells for transplantation/regenerative medicine



Graf, 2011

Making neurons in live animals- *In vivo* transdifferentiation

PNAS

Generation of induced neurons via direct conversion in vivo

Olof Torper[a,b], Ulrich Pfisterer[a,b,1], Daniel A. Wolf[a,b,1], Maria Pereira[a,b], Shong Lau[a,b], Johan Jakobsson[a,b], Anders Björklund[a,2], Shane Grealish[a,b], and Malin Parmar[a,b,2]

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Contributed by Anders Bjorklund, March 2, 2013 (sent for review January 27, 2013)

Cellular reprogramming is a new and rapidly emerging field in which organs such as the pancreas and heart (17, 18), the method is yet

Cell Stem Cell

Article

Cell PRESS

In Vivo Direct Reprogramming of Reactive Glial Cells into Functional Neurons after Brain Injury and in an Alzheimer's Disease Model

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PENNSTATE

Technology Available for Licensing
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The Pennsylvania State University
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Non-Confidential Description - PSU No. 3961
"Direct Conversion of Reactive Astrocytes into Functional Neurons to Treat Brain Injury and Neurological Disorders"

Keywords/Field of Invention:

Brain injury, spinal cord injury, stroke, neurological disorders, Alzheimer's disease, Parkinson disease, amyotrophic lateral sclerosis (ALS), neurons, astrocytes, glial cells, trans-differentiation, reprogramming

Inventors:

Gong Chen, Ziyuan Guo

Background

Alzheimer's disease is well-known for amyloid plaques and tau tangles, but reactive astrocytes are another hallmark of Alzheimer's disease brain. Reactive astrocytes are activated by neural injury and neurological disorders to initially serve as a defense system to protect the surrounding healthy brain tissue. After injury or disease, reactive astrocytes often over proliferate and eventually form glial scar tissue to prevent brain functional recovery. Although reactive astrocytes have been found widely associated with neural injury and neurological disorders, so far there is no method available to effectively reverse the glial scar and restore neuronal functions.

Invention Description

The subject invention represents a completely novel approach to convert reactive astrocytes into functional neurons in *in vivo* brain for internal neural regeneration and brain repair. The Penn State researchers completed *in vitro* (human) and *in vivo* (mouse) experiments demonstrating the trans-differentiation of astroglial cells into functional neurons that were immunopositive for neuronal markers such as doublecortin (DCX), Tuj1, MAP2, and neuronal nuclei (NeuN). Electrophysiological analyses demonstrated that cultured human astrocyte-converted neurons were fully functional, capable of firing action potentials and releasing neurotransmitters such as glutamate and GABA. Mouse brain slice recordings demonstrated that the *in vivo* reactive astrocyte-converted neurons after brain injury or Alzheimer's disease were also functional, firing action potentials and showing synaptic responses. These results suggest that the astrocyte conversion technology may potentially restore lost neuronal functions after brain injury or Alzheimer's disease.

Status of Invention

In vitro and *in vivo* experiments have been performed on both human and mouse astrocyte conversion into functional neurons. The researchers continue to further develop their initial discovery with governmental research support. Future research will leverage collaborations with academic and industrial laboratories to build upon and target promising clinical applications of this technology.

Commercial Applications

This innovative *in vivo* trans-differentiation technology may be the foundation for a novel therapeutic strategy that has broad commercial potential as clinical treatments for brain injury, spinal cord injury, stroke, Alzheimer's disease, Parkinson disease, amyotrophic lateral sclerosis (ALS), and other glia-related diseases.

Contact: **Matthew D. Smith**
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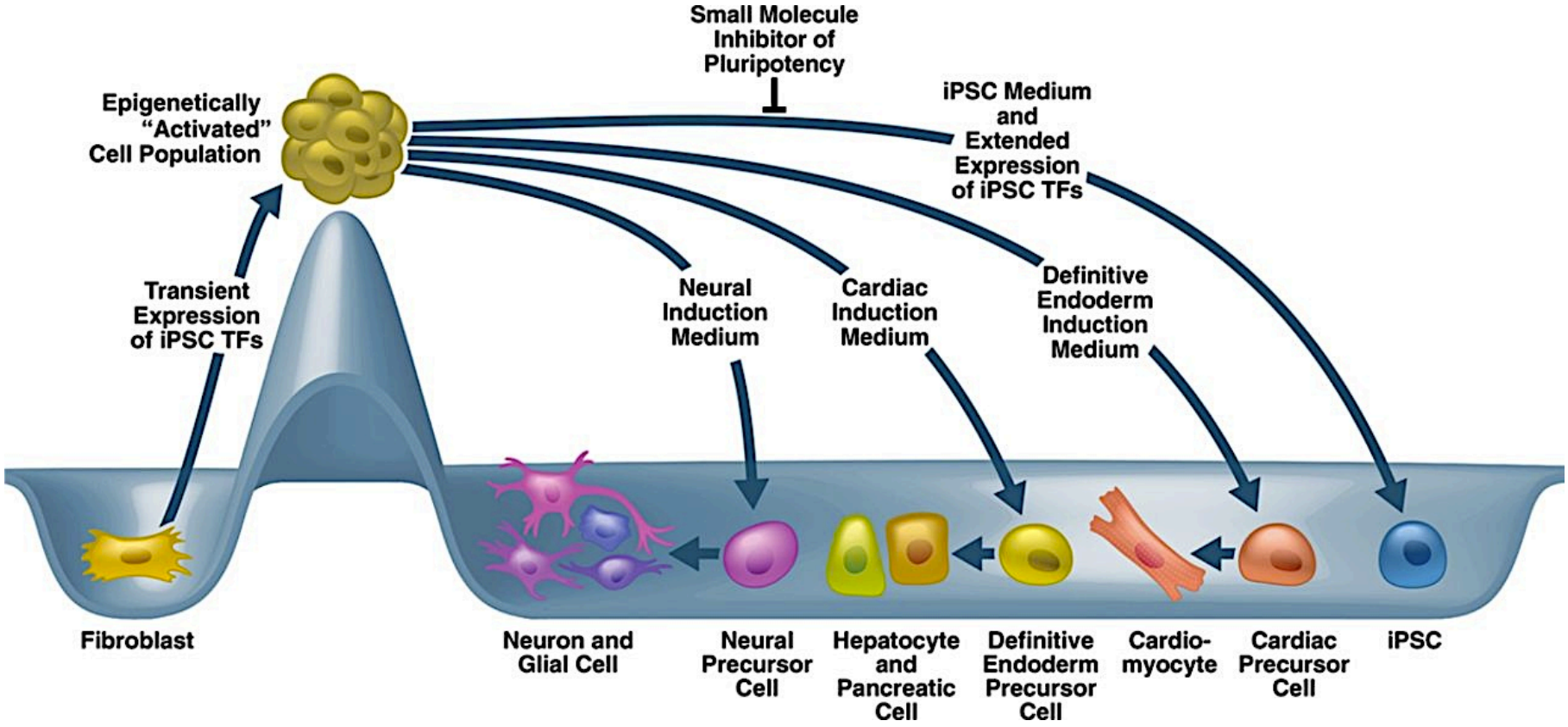
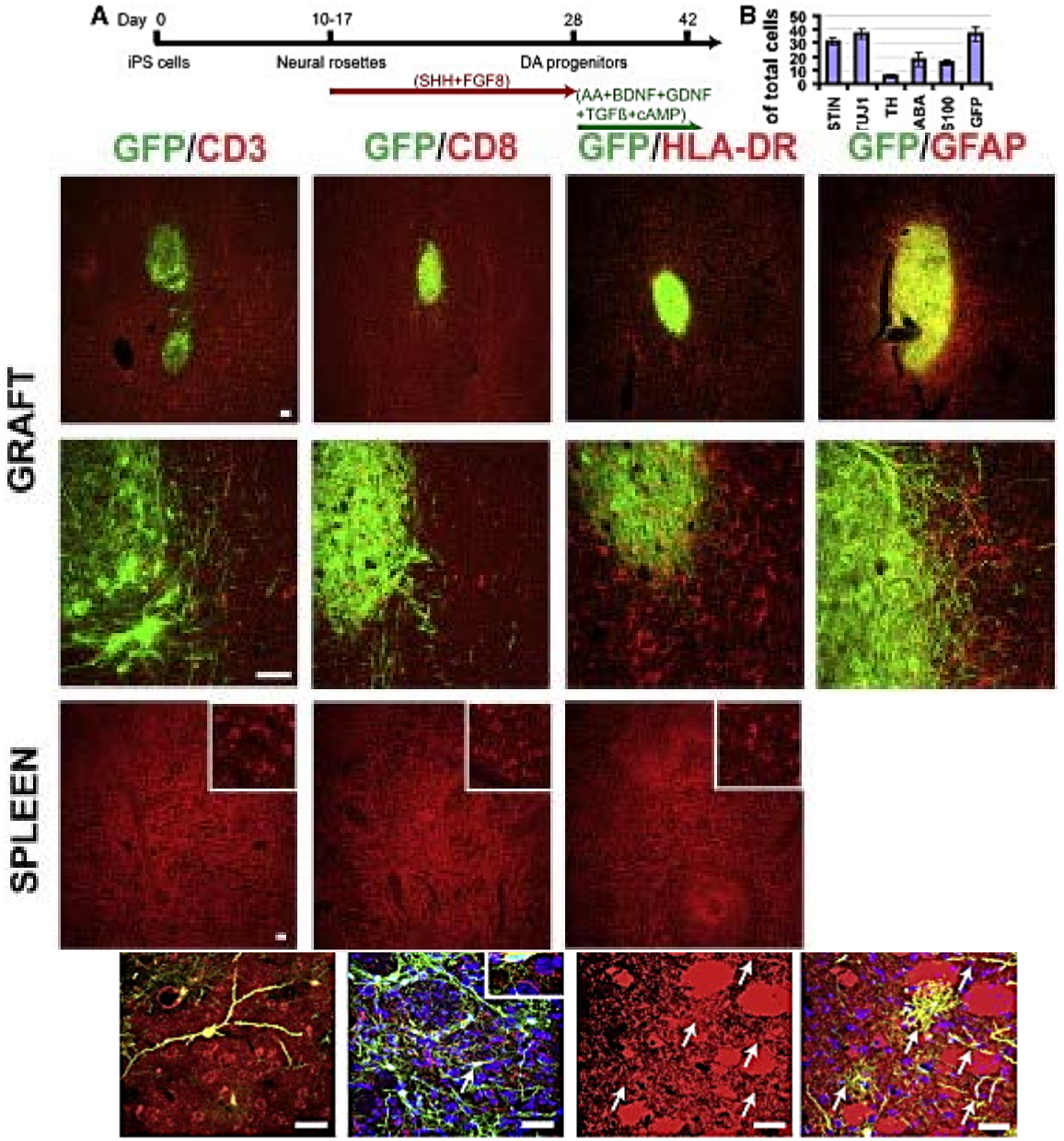
A simplified and conceptual paradigm of induced pluripotent stem cell (iPSC) transcription factor (TF)–based transdifferentiation.

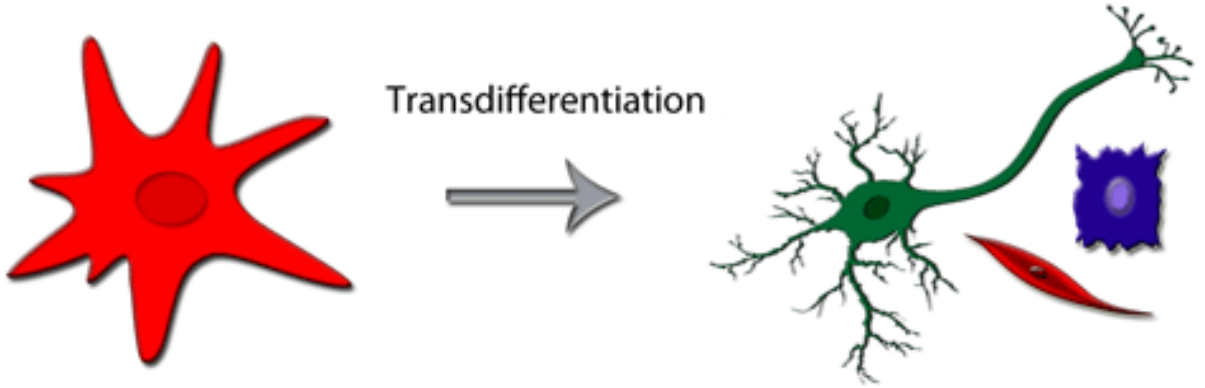


Ma T et al. *Circulation Research*. 2013;112:562-574

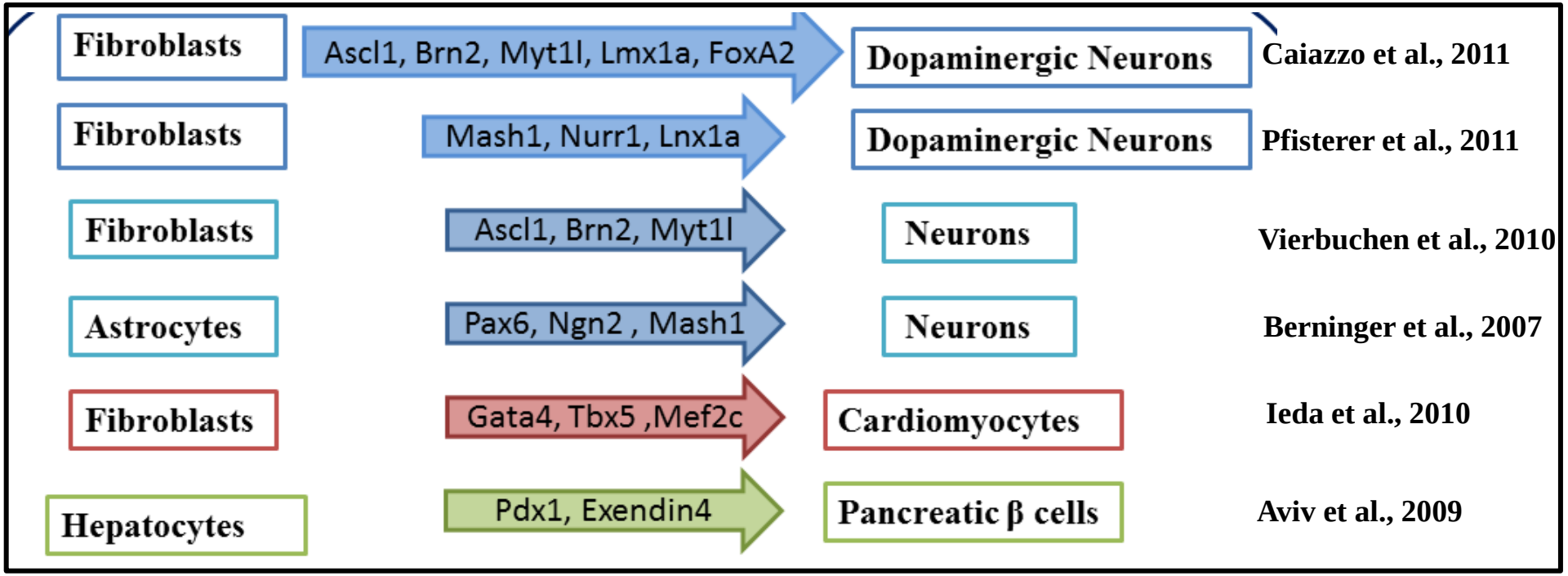
Induced Pluripotent Stem Cell-Derived Neural Cells Survive and Mature in the Nonhuman Primate Brain

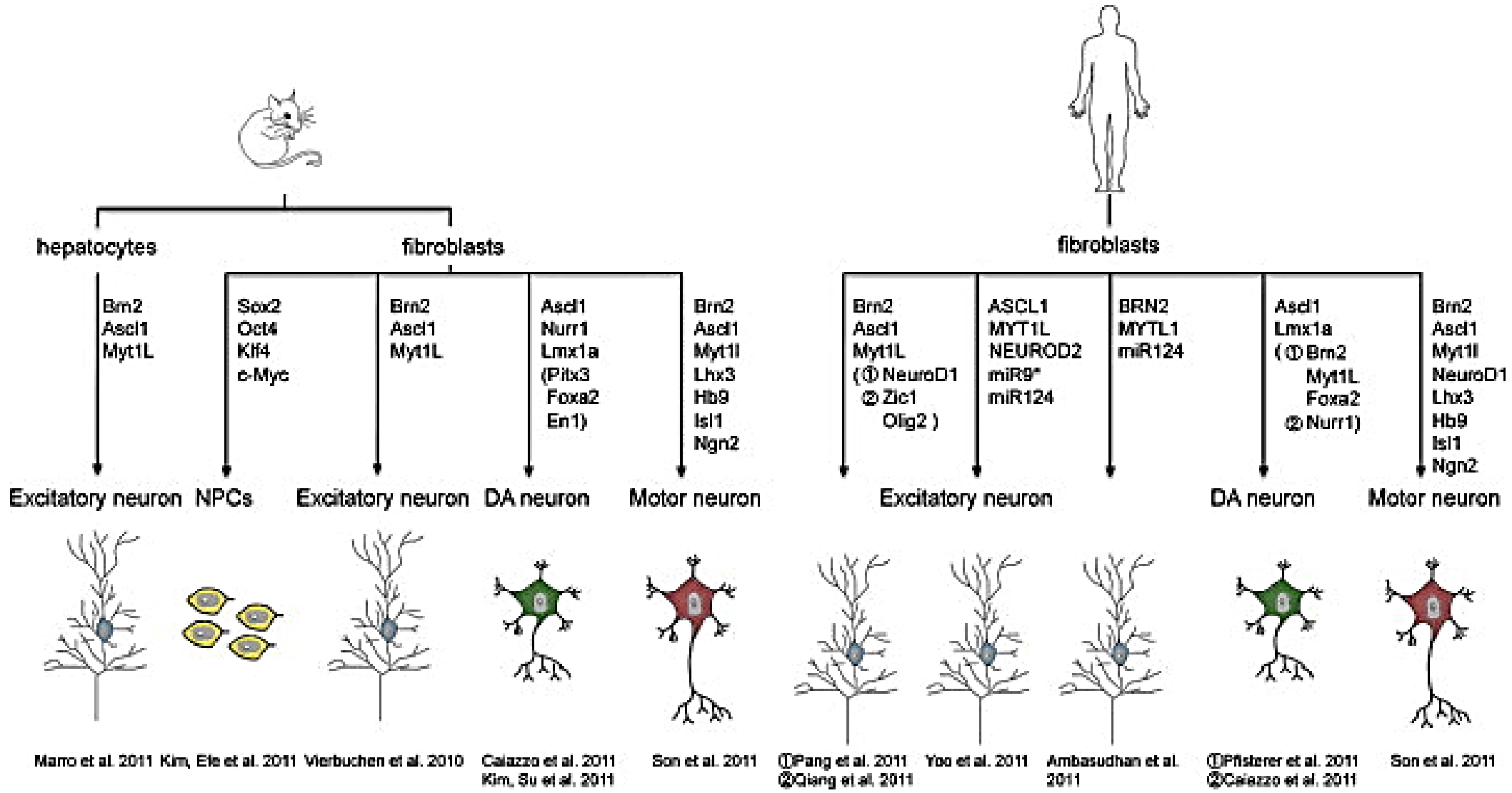


Emborg et al., 2013


Transdifferentiation

Different types of cellular transdifferentiation factors used for directing cell fate switch



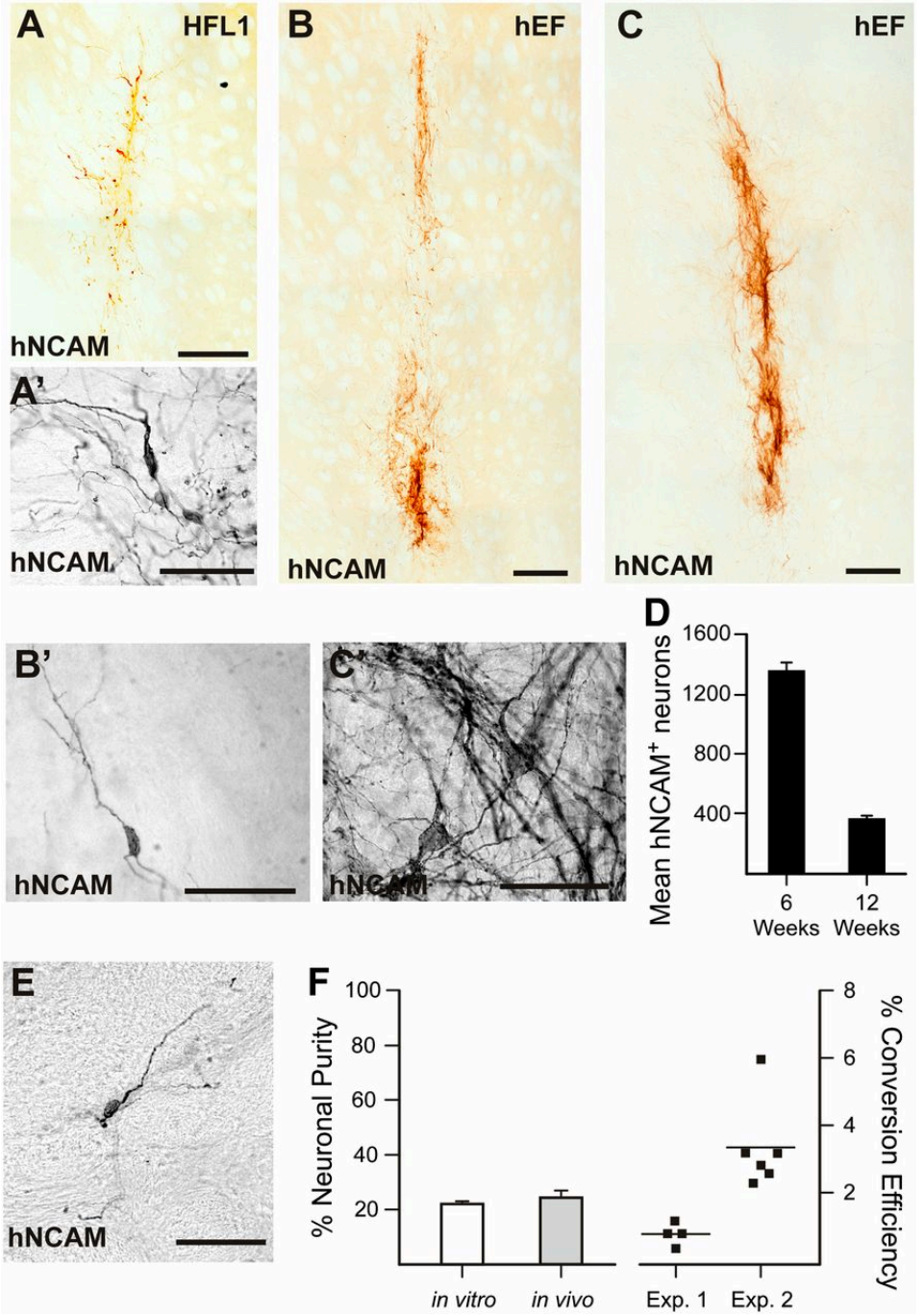


Yang et al., 2011

Ascl1, Brn2, and Myt1l

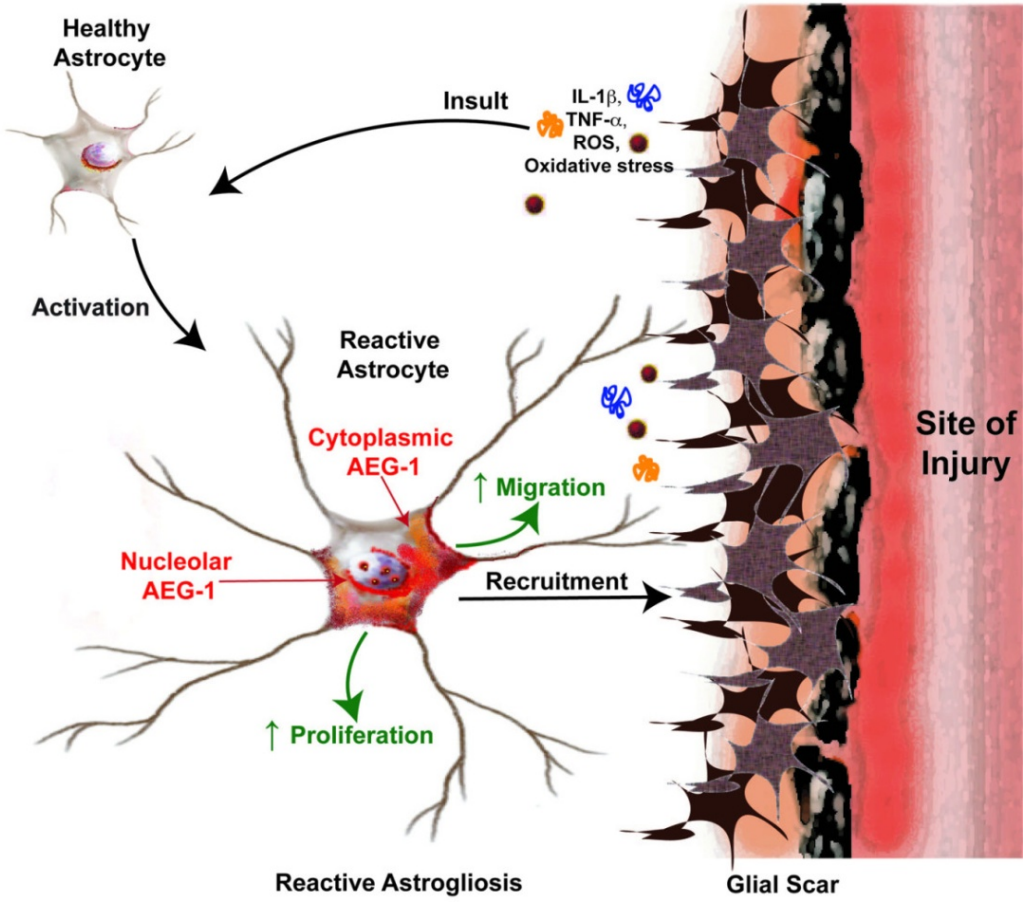
En1, **Foxa2**, Gli1, **Lmx1a**, Lmx1b, Msx1, Nurr1, Otx2, Pax2, and Pax5

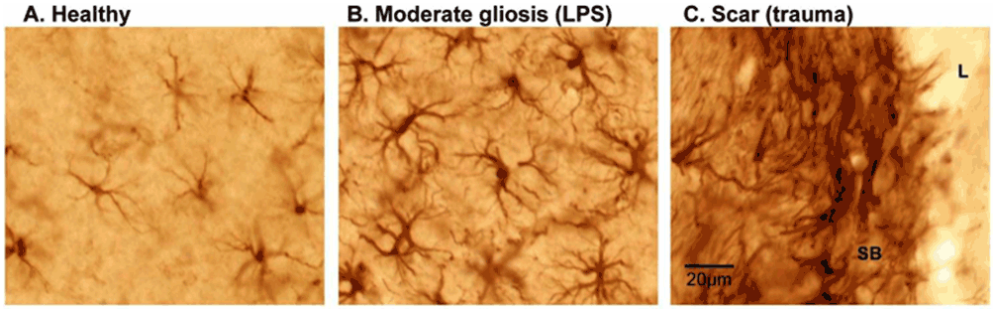
Long-term survival and stability of iN cells generated from transplanted fibroblasts via conversion *in vivo*



Torper et al. 2013

Reactive astrogliosis





Different types of reactive astrocytes in mouse cerebral cortex

Stroke,

Spinal cord injury,

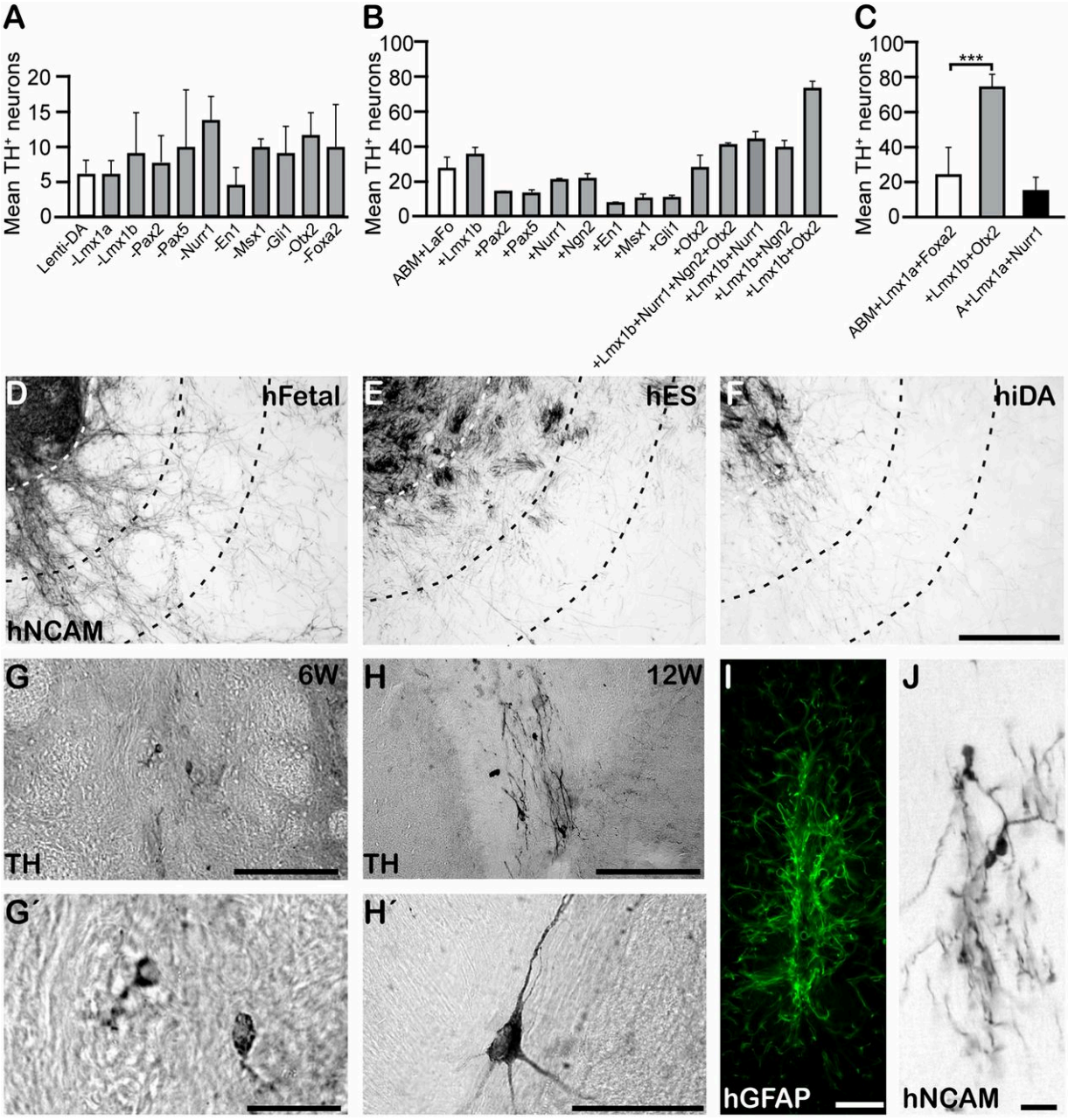
Glioma,

Neurodegenerative disorders such as Alzheimer's disease (AD)

Sharma et al., 2012

Verkhratsky et al., 2012

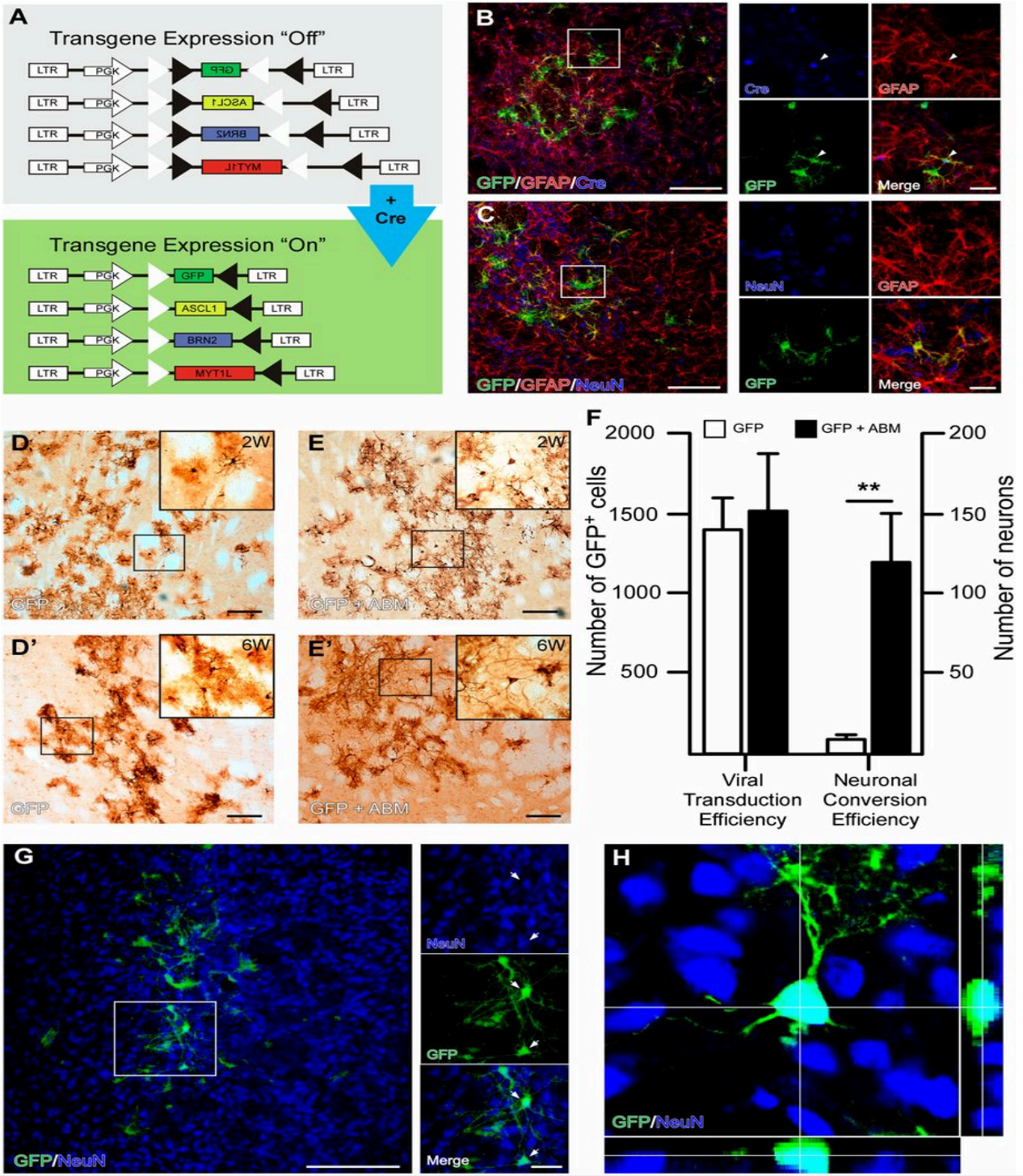
Dopaminergic fate determinants, innervation, and in vivo conversion of human astrocytes.



Torper et al. 2013

PNAS

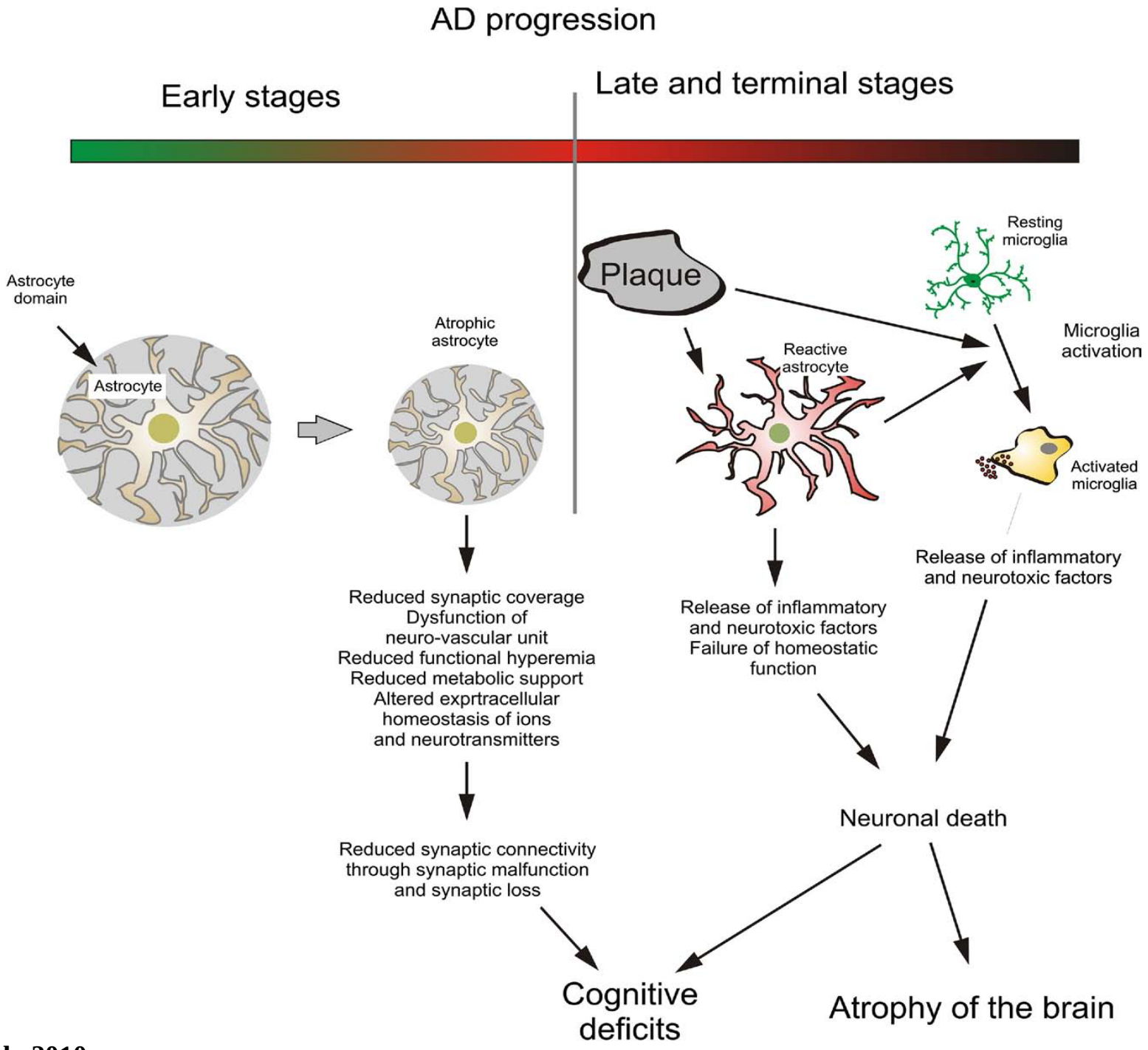
Neural conversion of striatal astrocytes in situ



Torper et al. 2013

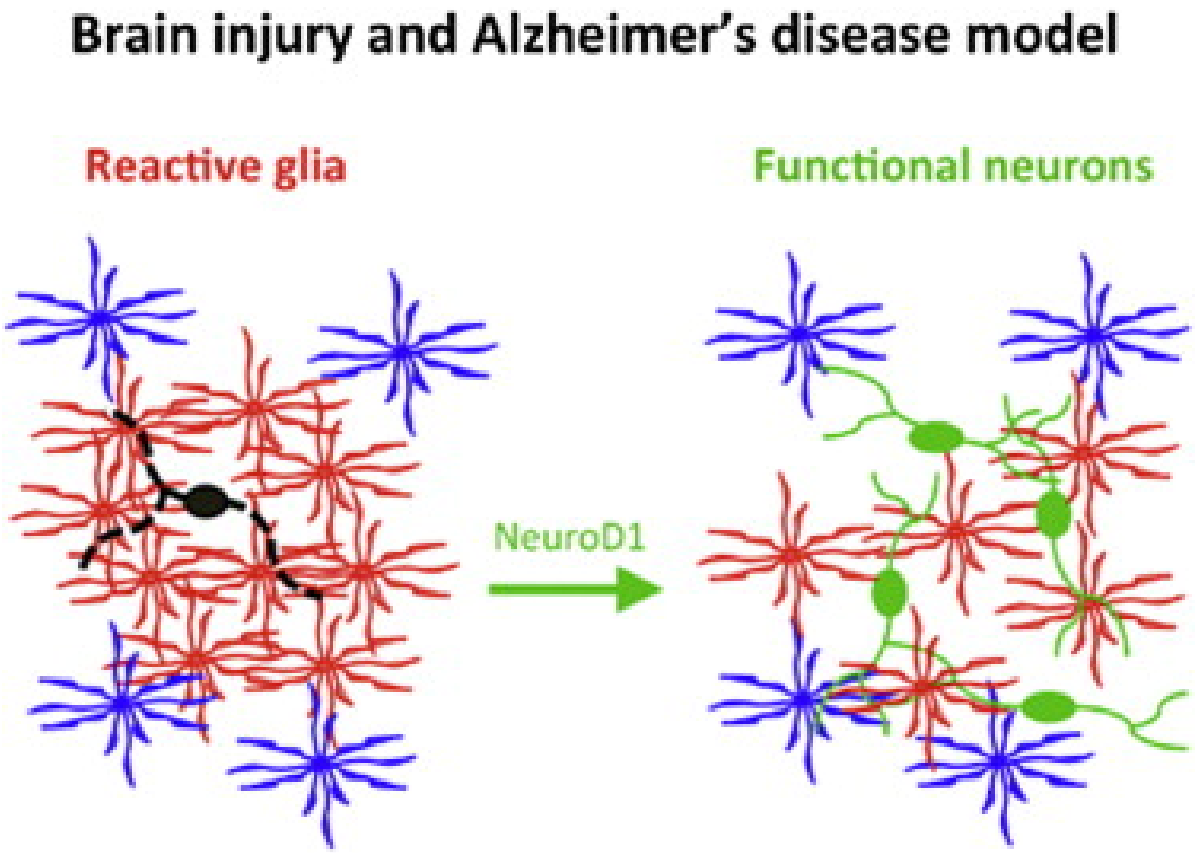
PNAS

Astroglial hypothesis of Alzheimer's disease (AD)



Verkhratsky et al., 2010

In Vivo Direct Reprogramming of Reactive Glial Cells into Functional Neurons after Brain Injury and in an Alzheimer's Disease Model



The pathological modification of astrocytes in the demented brains were initially observed by Alois Alzheimer in 1910 who had found glial cells abundantly populating neuritic plaques.

Guo et al., 2014

NeuroD1 increases reprogramming efficiency in primary human fetal fibroblasts

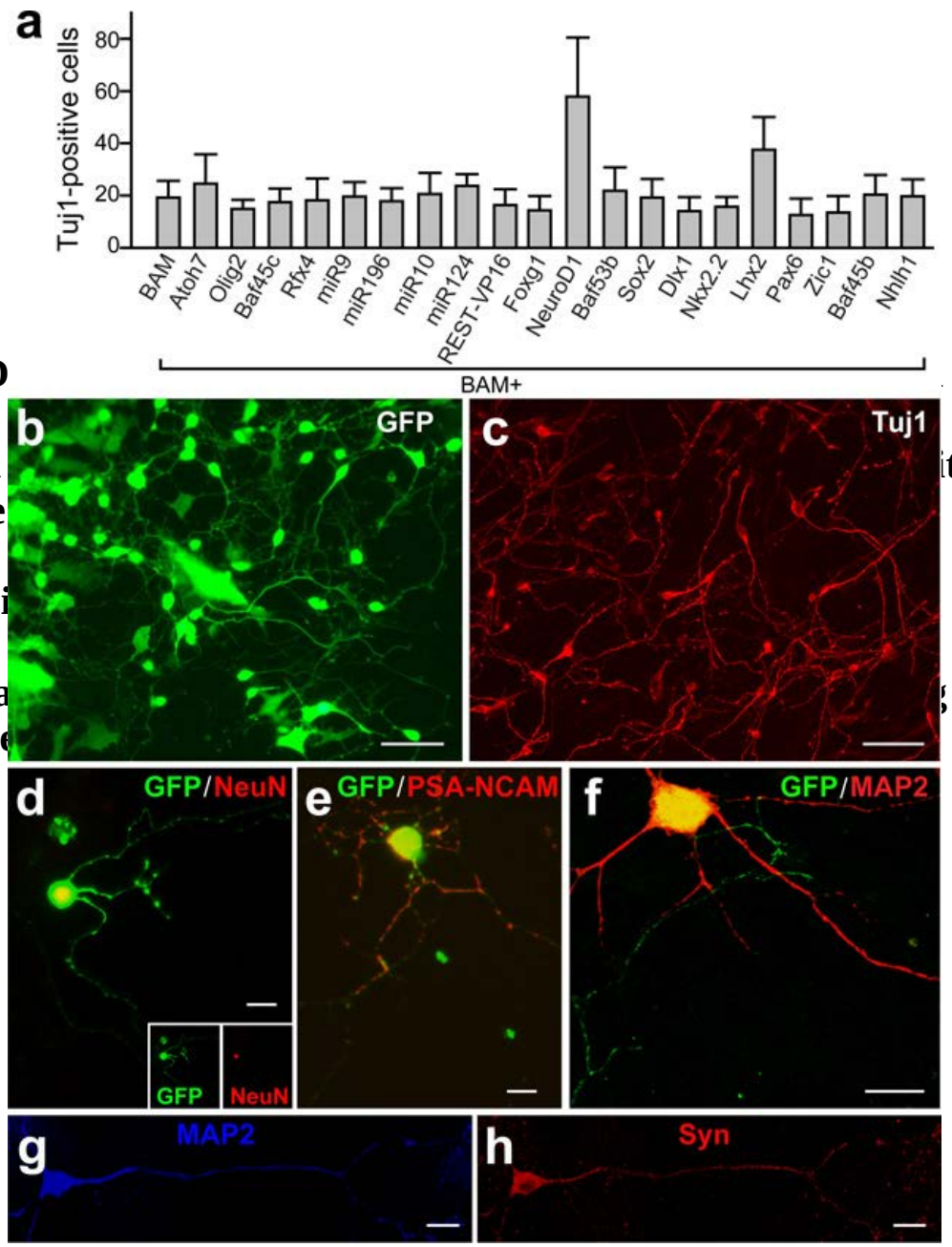
NeuroD1

Member of the NeuroD **factors**

Promote the formation **ther the cerebellum or the DG cell layer of the**

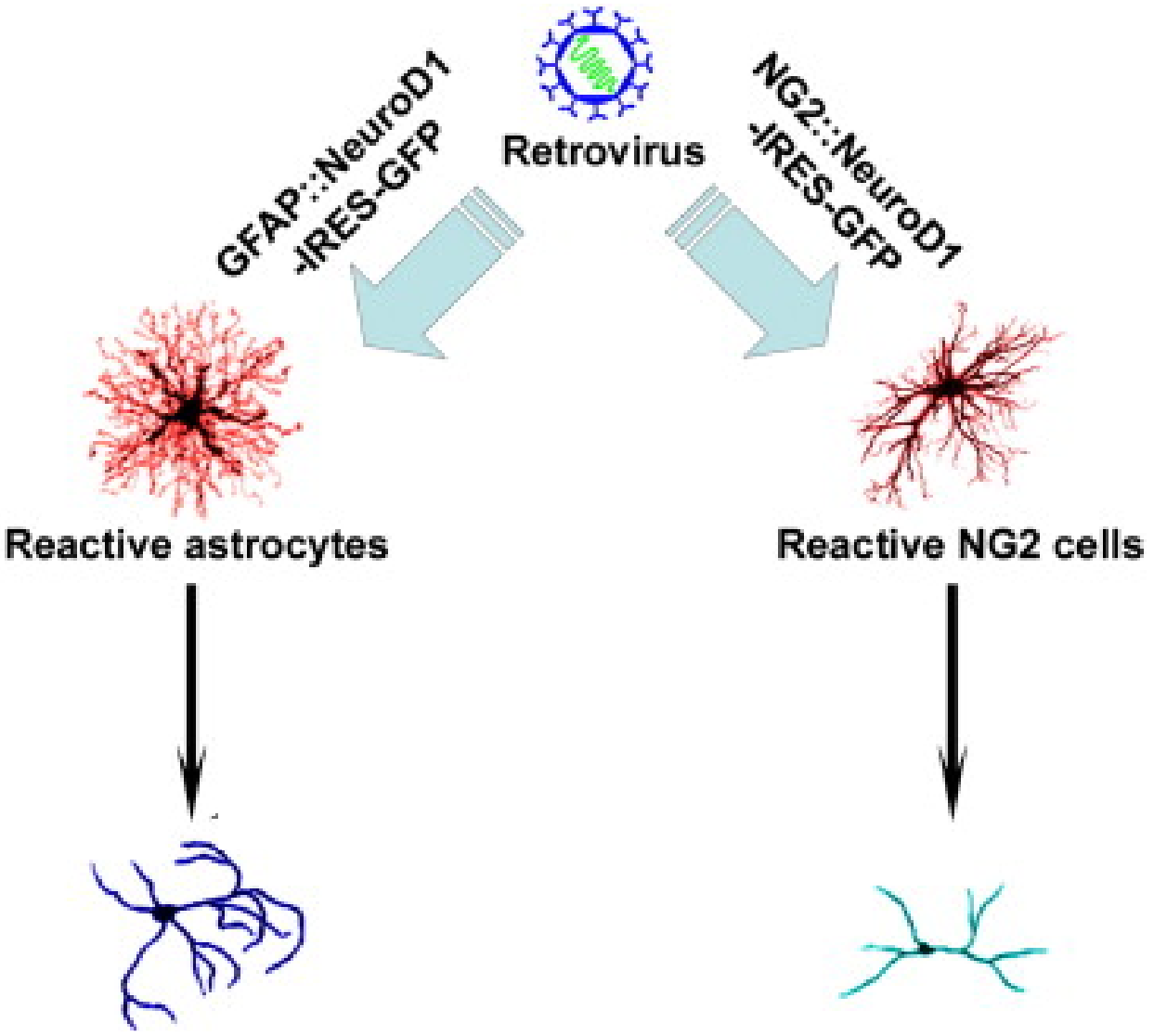
Dendrite morphogenesi

Associates with chroma **key transcriptional regulators of neurogene**

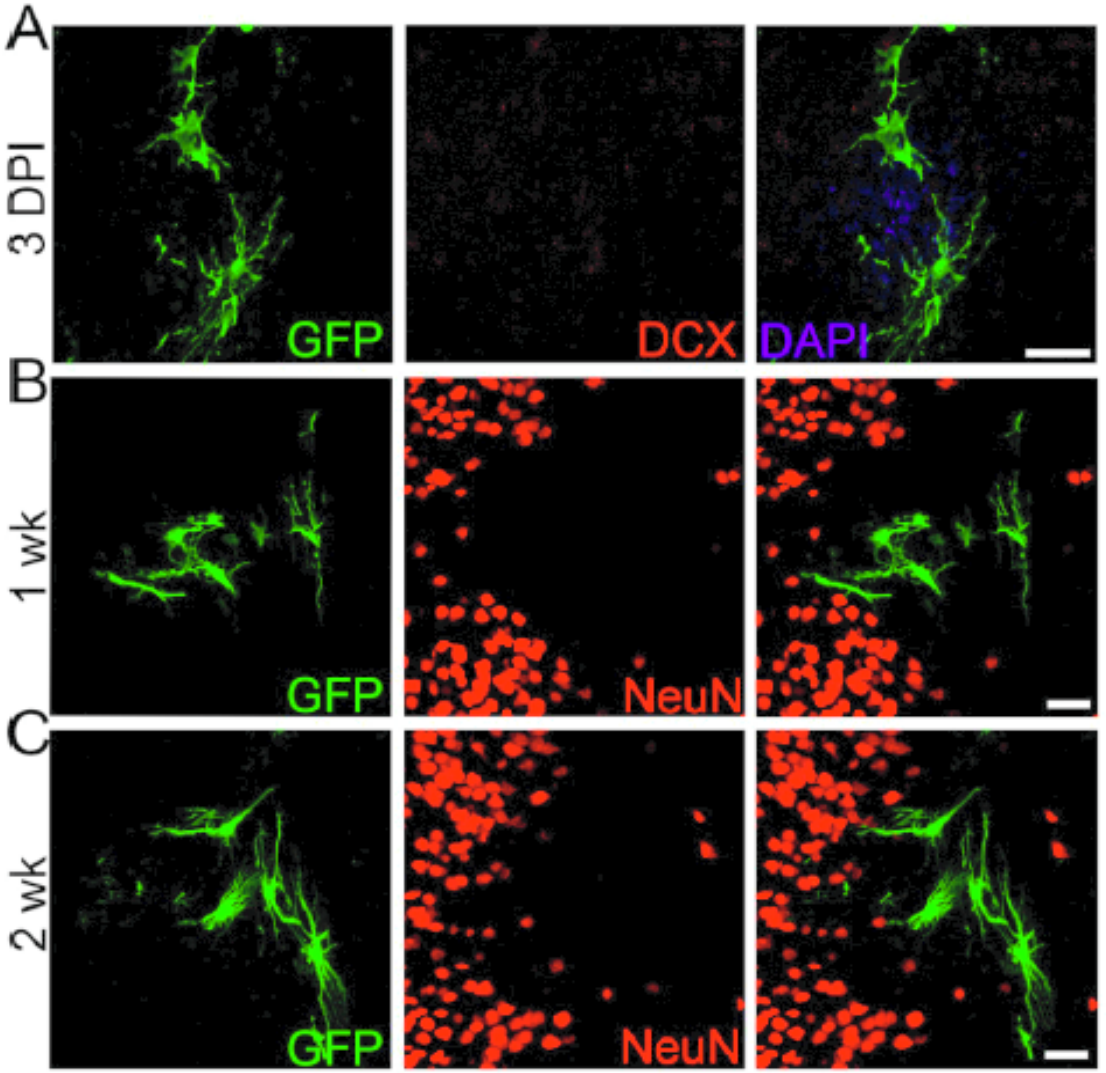


Pang et al., 2011

Direct Conversion of Reactive Glial Cells to Active Neurons via NeuroD1 Expression

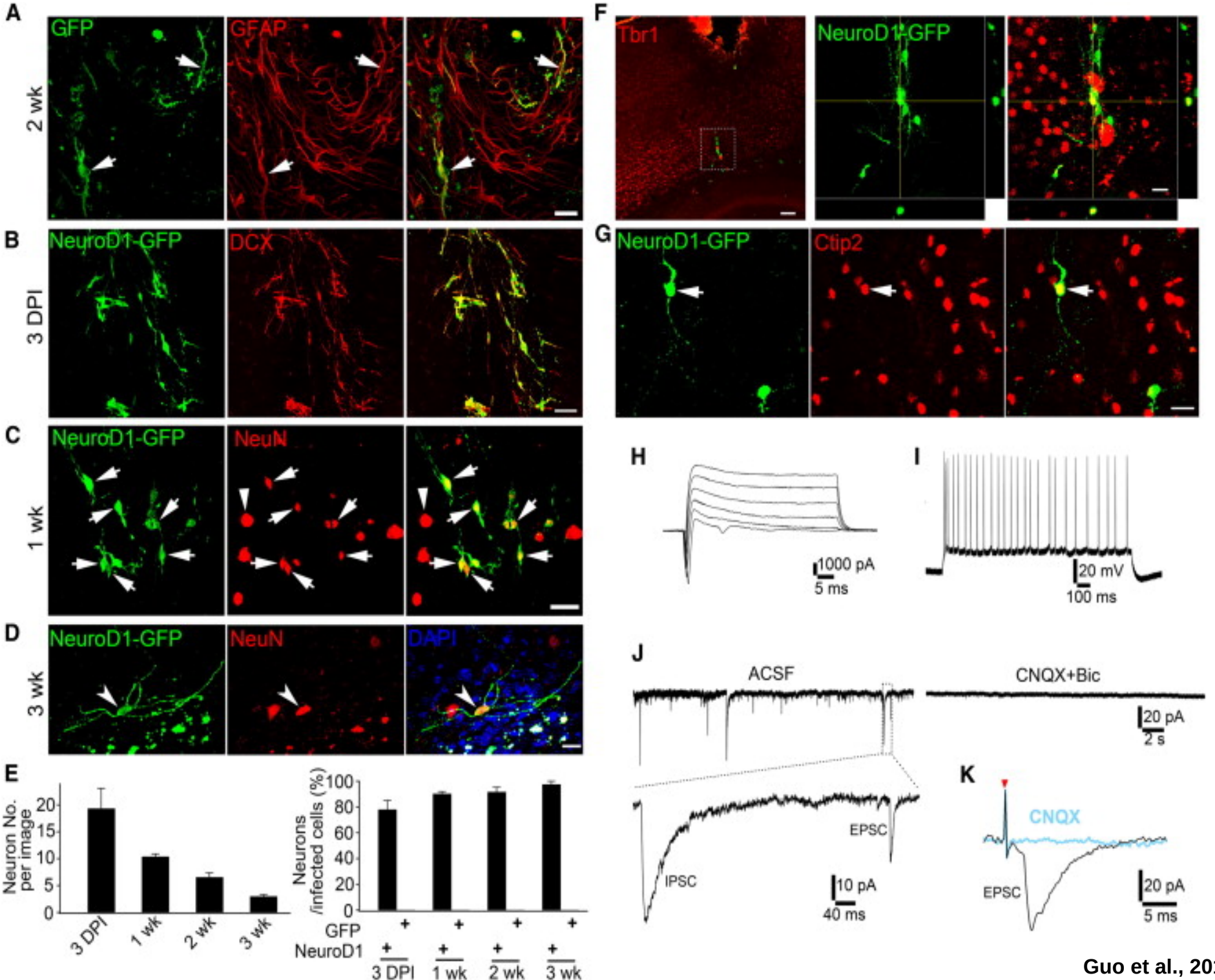


Only non-neuronal cells are infected by control retrovirus expressing GFP



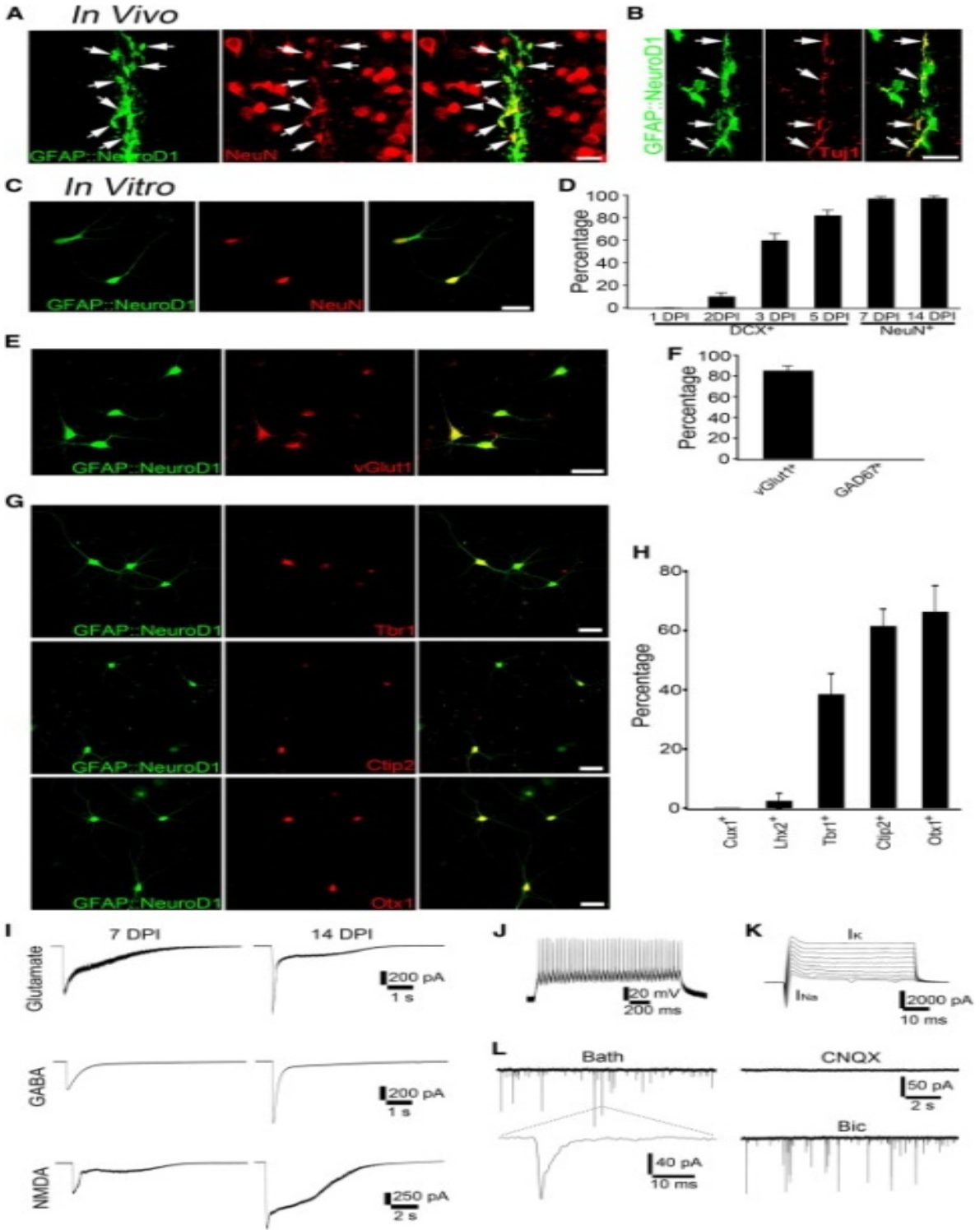
Guo et al., 2014

In Vivo Conversion of Reactive Glial Cells into Functional Neurons after Brain Injury



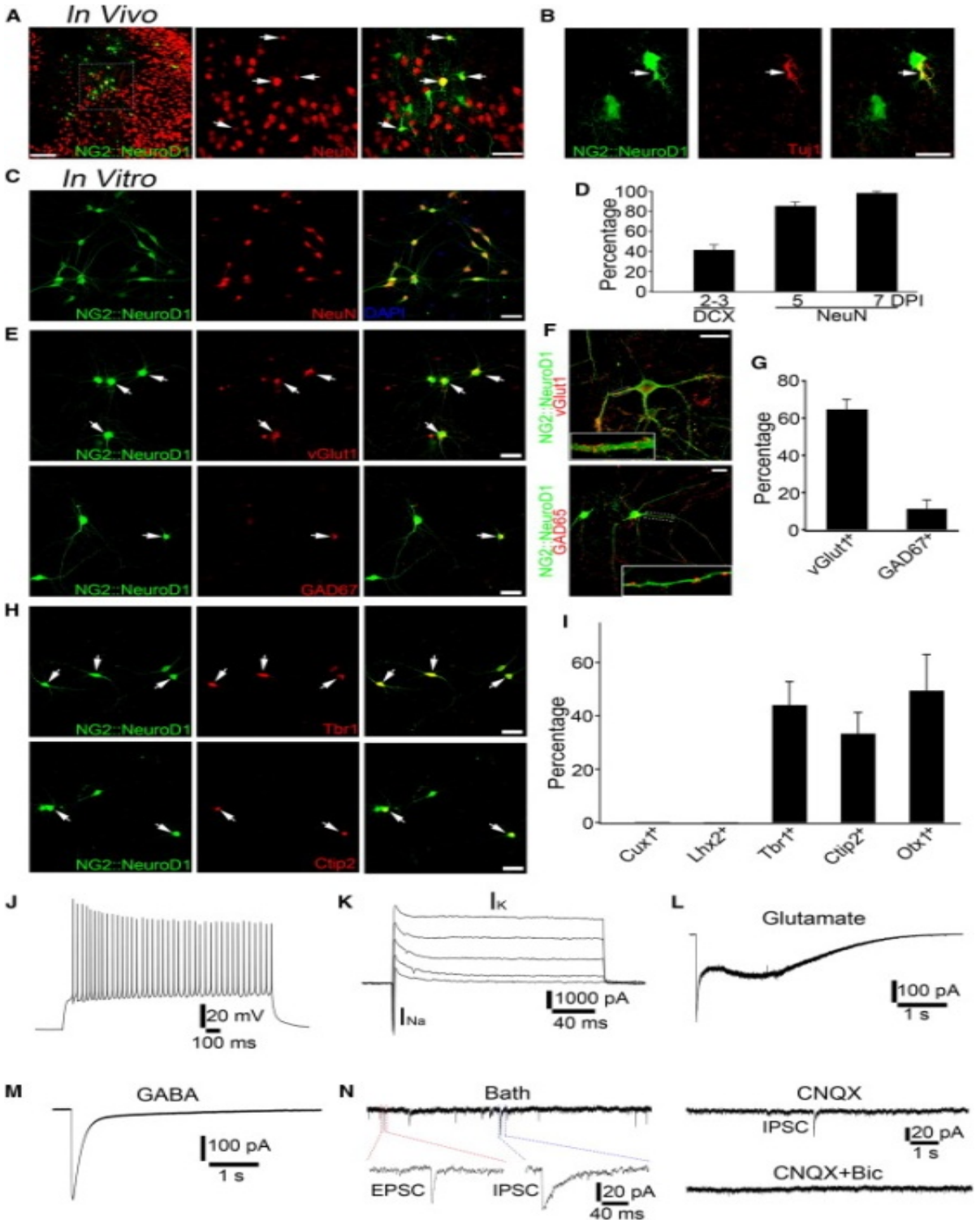
Guo et al., 2014

NeuroD1 Converts Astrocytes into Glutamatergic Neurons



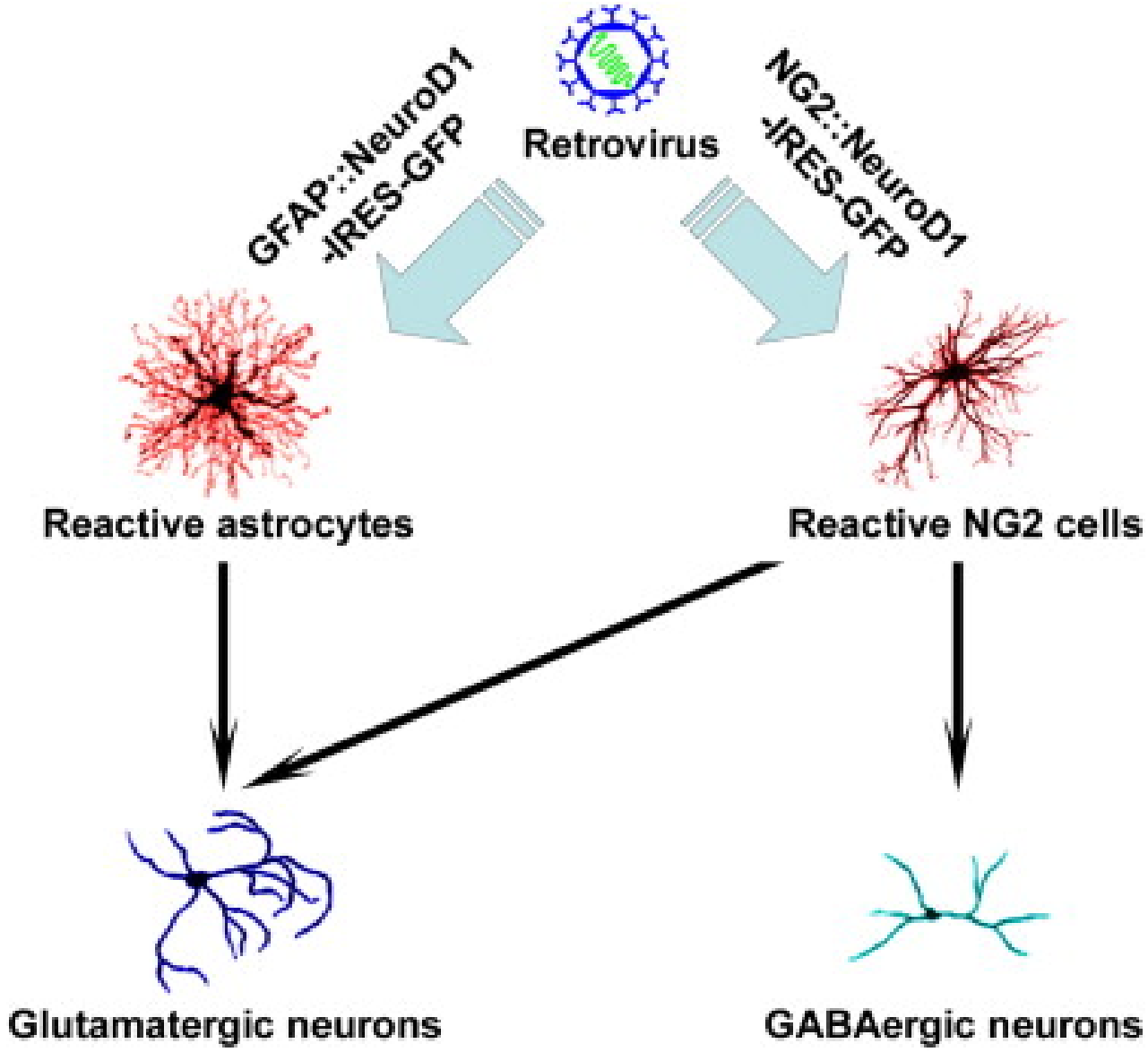
Guo et al., 2014

NeuroD1 Converts NG2 Cells into Glutamatergic and GABAergic Neurons

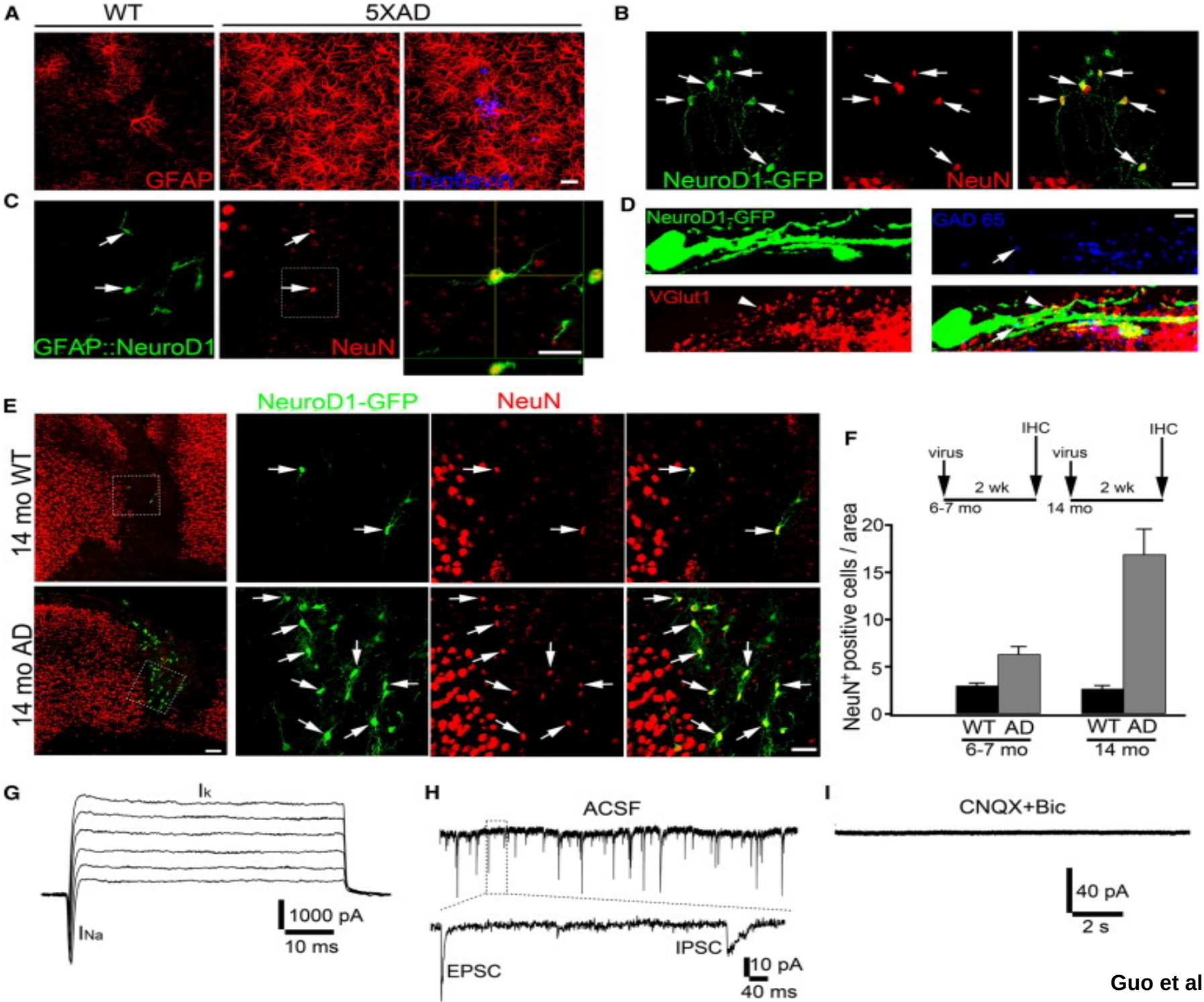


Guo et al., 2014

Direct Conversion of Reactive Glial Cells to Active Neurons via NeuroD1 Expression

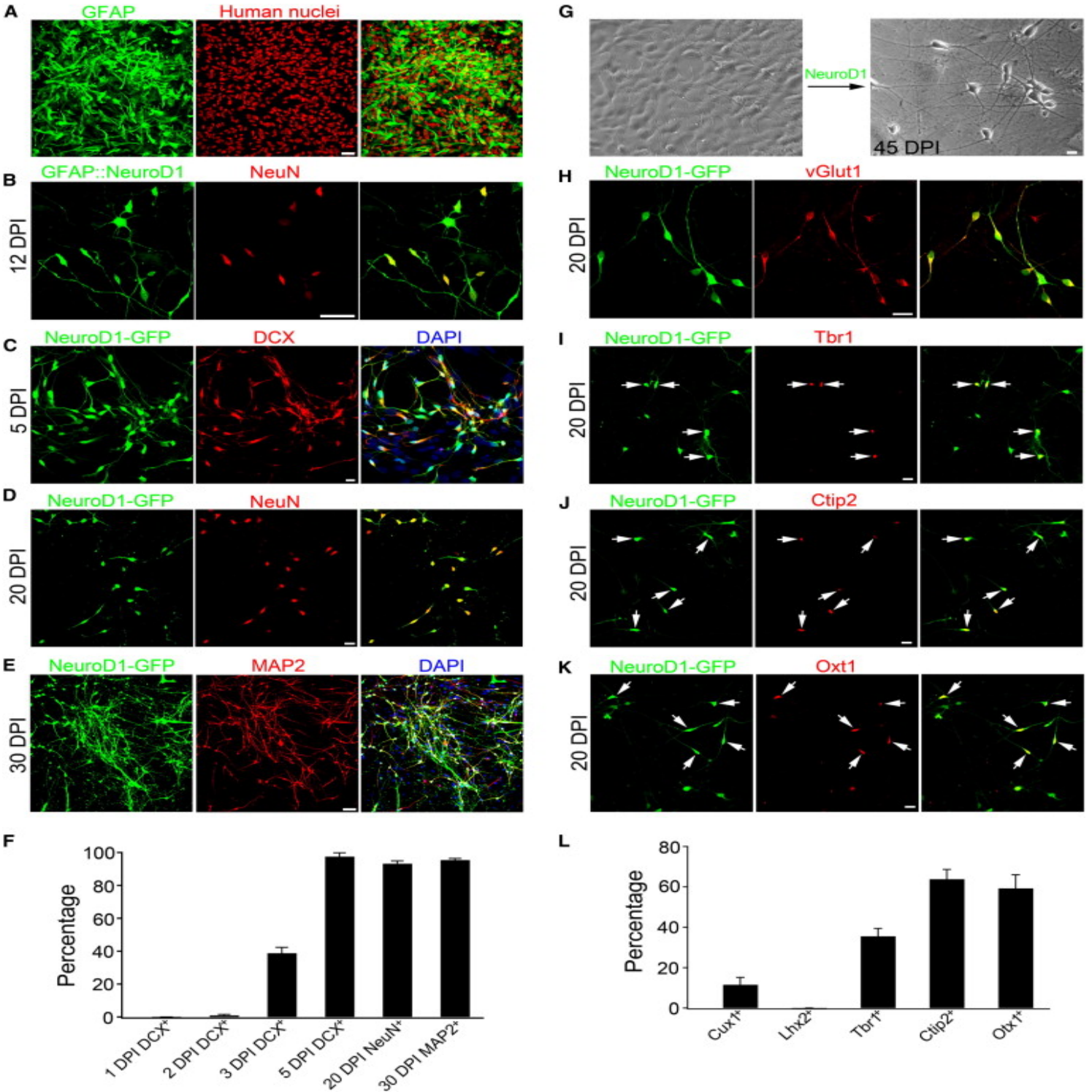


NeuroD1 Converts Reactive Glial Cells into Functional Neurons in AD Mouse Brain In Vivo



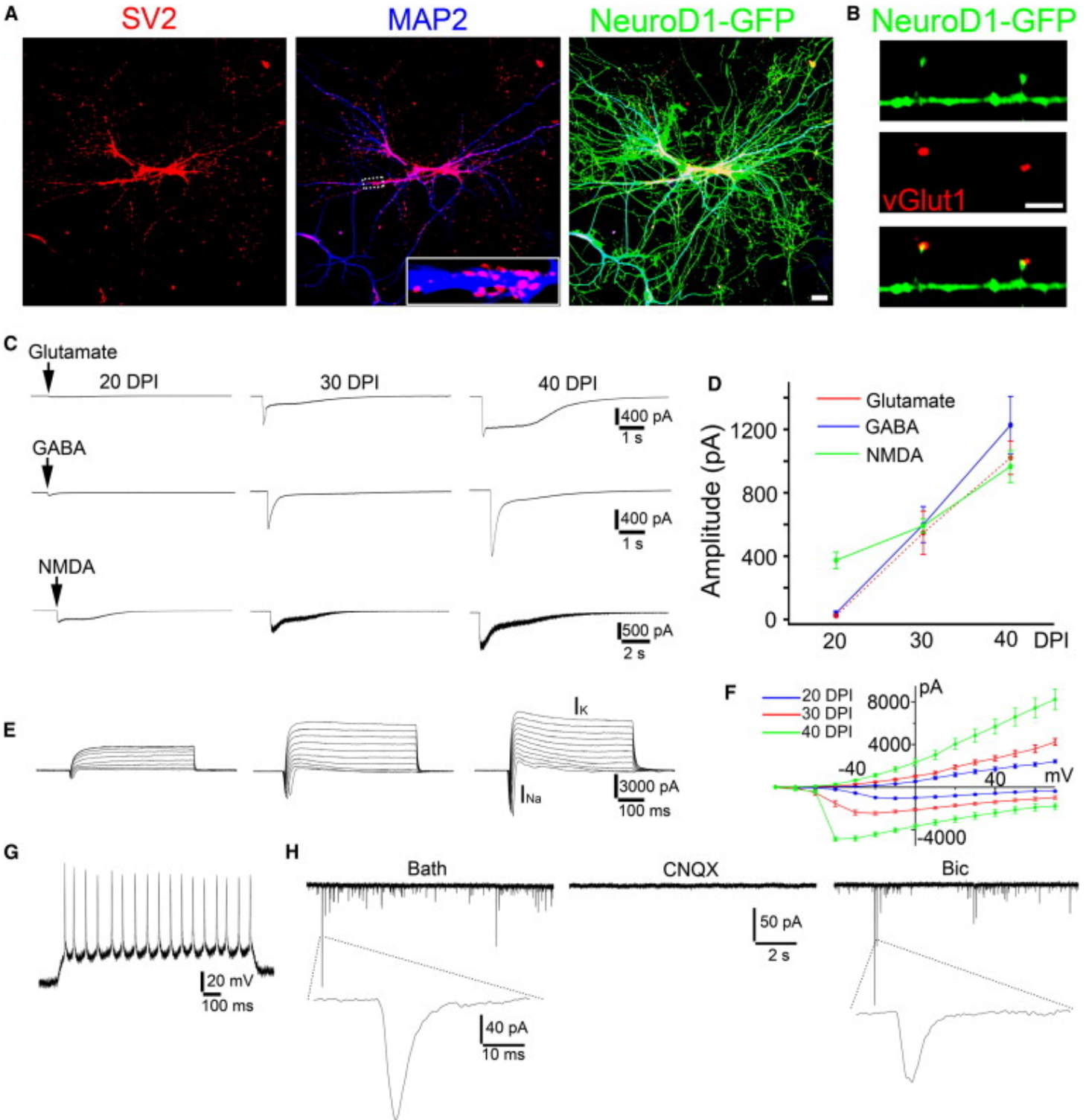
Guo et al., 2014

Conversion of Cultured Human Astrocytes into Functional Neurons



Guo et al., 2014

Functional Characterization of Human Astrocyte-Converted Neurons



Guo et al., 2014

Conclusion

Using a single factor (NeuroD1) for transdifferentiation of astrocytes and NG2 cells into neurons *in vivo*

Direct convertion reactive glia into neurons in injured or diseased brain tissue

Possibility of melting the inhibitory gliotic tissues for therapeutic gains

Efficiency is higher in older animals even in AD models

Use of retrovirus restricts the conversion to reactive proliferating astrocytes without affecting quiescent glia

Astrocytes – glutamatergic

NG2 cells - glutamatergic & GABAergic neurons

Functional neurons with deep cortical neuronal subtype

Issues that must be addressed before moving forward with potential clinical applications

A more efficient and safer method for introducing genetic material into patients' cells

Demonstrate integration of converted neurons into appropriate neural circuits and whether this contributes to functional improvement.

Elucidating the mechanisms of cell-type-specific conversion into neurons with distinct phenotypes is required

Astrocyte activation limits plaque growth and attenuates plaque-related dystrophic neurites. These activities may require intimate contact between astrocyte and plaque.

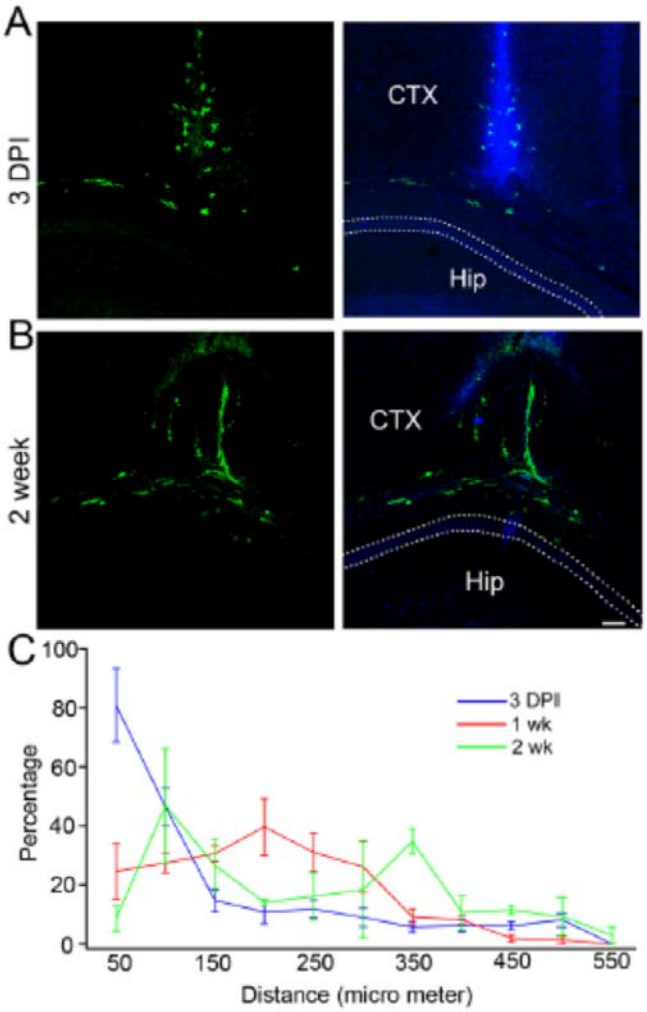
Gfap and Vim gene deletion resulted in a marked increase in dystrophic neurites

The transdifferentiated cells still carry gene or predisposition to whatever disease being treated

Thank you for your attention

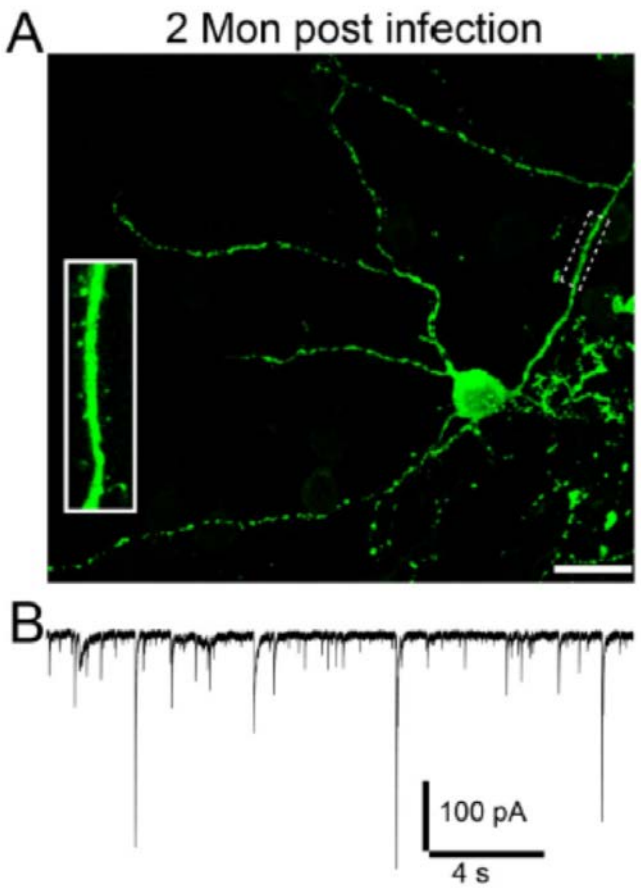
Suppl. Figure 2. Migration of NeuroD1-converted neurons after retroviral injection into mouse cortex, related to Figure 1.

(A) NeuroD1-infected cells were mostly clustered around the injection sites at 3 DPI, except a few spreading in the cingulate cortex region. (B) Two weeks after viral injection, NeuroD1-infected cells migrated away from the center of injury sites and spread more broadly in the deep layer. (C) Plot of the percentage of NeuroD1-infected cells versus the distance measured from the center of injury sites. The number of NeuroD1-infected cells was 263 for 3 dpi, 214 for 1 week, and 124 for 2 weeks. n = 3 animals for each group. Scale bar, 100 μm.



Guo et al., 2014

Suppl. Fig. 3. Long-term survival of NeuroD1-converted neurons in mouse brain *in vivo*, related to Figure 1.



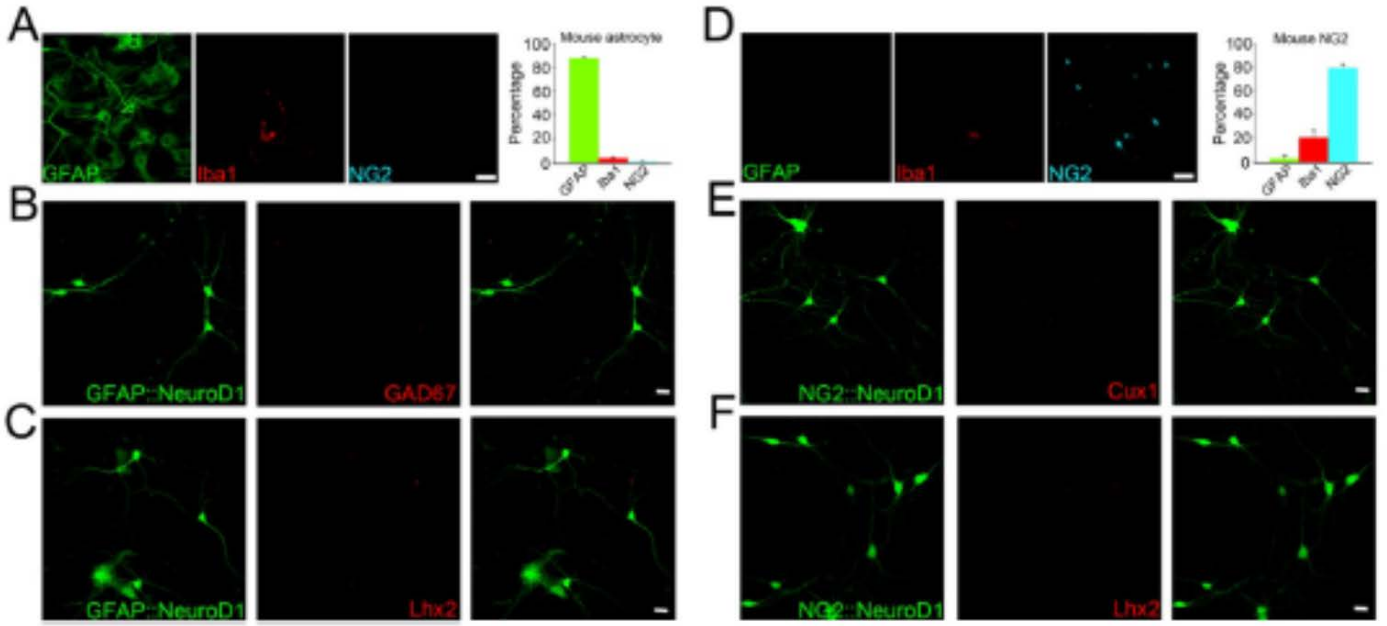
(A) A NeuroD1-converted neuron observed 2 months after viral injection. The converted neuron showed clear dendritic spines (arrows). Scale bar, 20 μm.

(B) Cortical slice recording revealed large spontaneous synaptic events from a 2-month old NeuroD1-converted neuron.

Suppl. Fig. 4. Characterization of NeuroD1-converted neurons in cultured mouse astrocytes or NG2 cells, related to Figure 2 and 3.

(A) Our cultured mouse astrocytes were mostly immunopositive for astrocytic marker GFAP (87.8 ± 1.4%), with a few positive for Iba1 but rarely NG2.

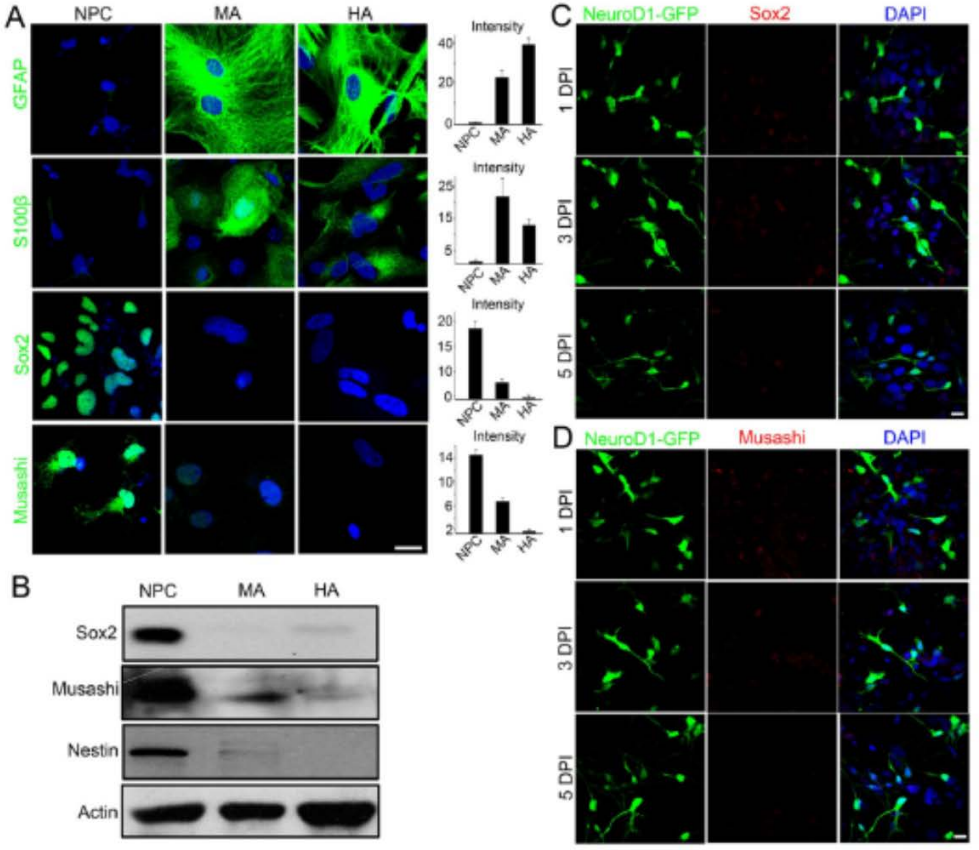
(B) GFAP::NeuroD1-GFP retrovirus-infected cells (green) were immunonegative for GAD67. (C) GFAP::NeuroD1-GFP retrovirus-infected cells were immunonegative for cortical superficial layer marker Lhx2. (D) The majority of cells in our NG2 culture were immunopositive for NG2 (~80%) with ~20% positive for microglia marker Iba1. (E-F) NG2::NeuroD1 retrovirus-infected cells were immunonegative for cortical superficial layer marker Cux1 (E) and Lhx2 (F). Scale bars, 40 μm for A and D; 20 μm for B, C, E and F.



Guo et al., 2014

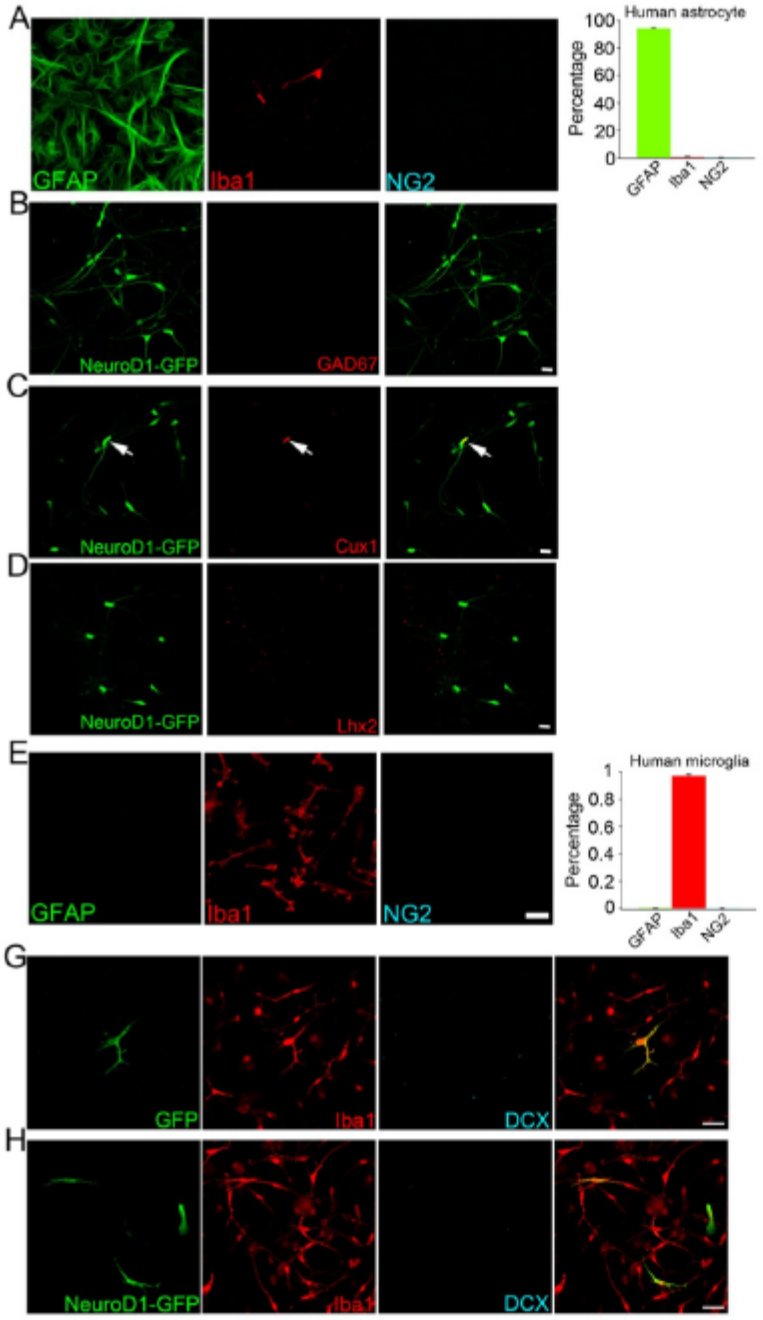
Suppl. Figure 5. No intermediate neuroprogenitor stage during human astrocyte-neuron conversion, related to Figure 5.

(A) Characterization of human astrocytes by comparing to human neuroprogenitor cells (NPC) or mouse astrocytes in primary culture. Human and mouse astrocytes were immunopositive for GFAP and S100β but negative for neural stem cell marker Sox2 or Musashi. (B) Western blot confirmed that our cultured human astrocytes were different from human NPCs. (C-D) NeuroD1-infected cells (green) did not show any increase in the expression of neural stem cell marker Sox2 (C) or Musashi (D) over 1, 3 and 5 days post infection. Scale bar, 20 μm; n = 3 cultures.



Guo et al., 2014

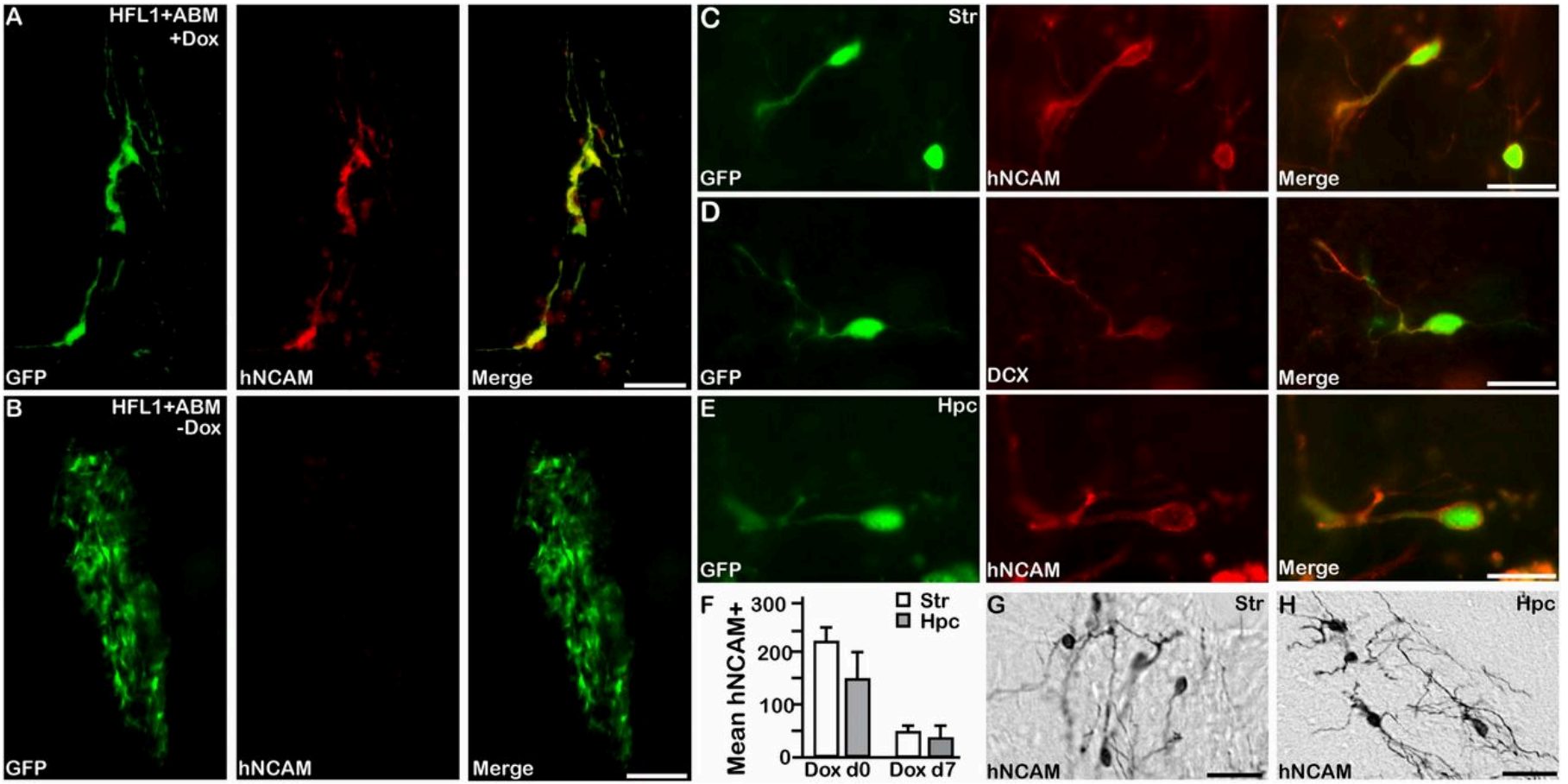
Suppl. Fig. 6. Human astrocytes can be converted into neurons but microglia cannot be converted, related to Figure 5.



(A) The majority of cells in our human astrocyte cultures were immunopositive for astrocytic marker GFAP (93.7 ± 1%). (B) NeuroD1-converted neurons from human astrocytes were immunonegative for GAD67. (C-D) Human astrocyte-converted neurons were largely negative for cortical superficial layer marker Cux1 (C) and Lhx2 (D). (E) The majority of cells in our human microglia culture were positive for Iba1 (97.1 ± 1.1%). (G-H) Human microglia not converted into neurons by NeuroD1 (20 DPI, DCX negative). n = 3-5 cultures. Scale bars, 40 μm for (A) and (E-H); 20 μm for (B-D).

Guo et al., 2014

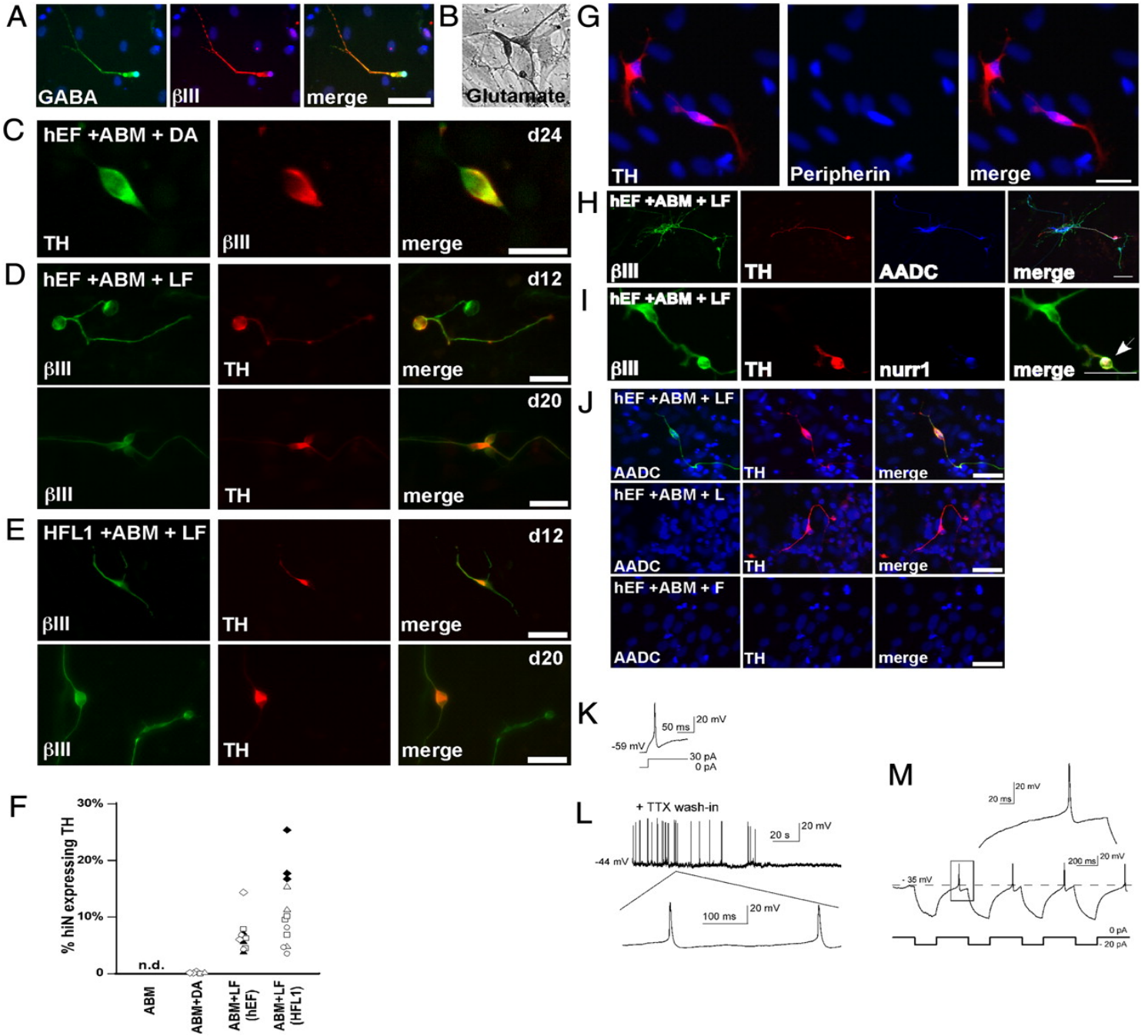
Direct neural conversion from human fibroblasts takes place *in vivo*



Torper et al. 2013

PNAS

Direct conversion of human fibroblasts to dopaminergic neurons



Pfisterer et al. 2011

PNAS