ALS- A Non-Cell Autonomous Disease

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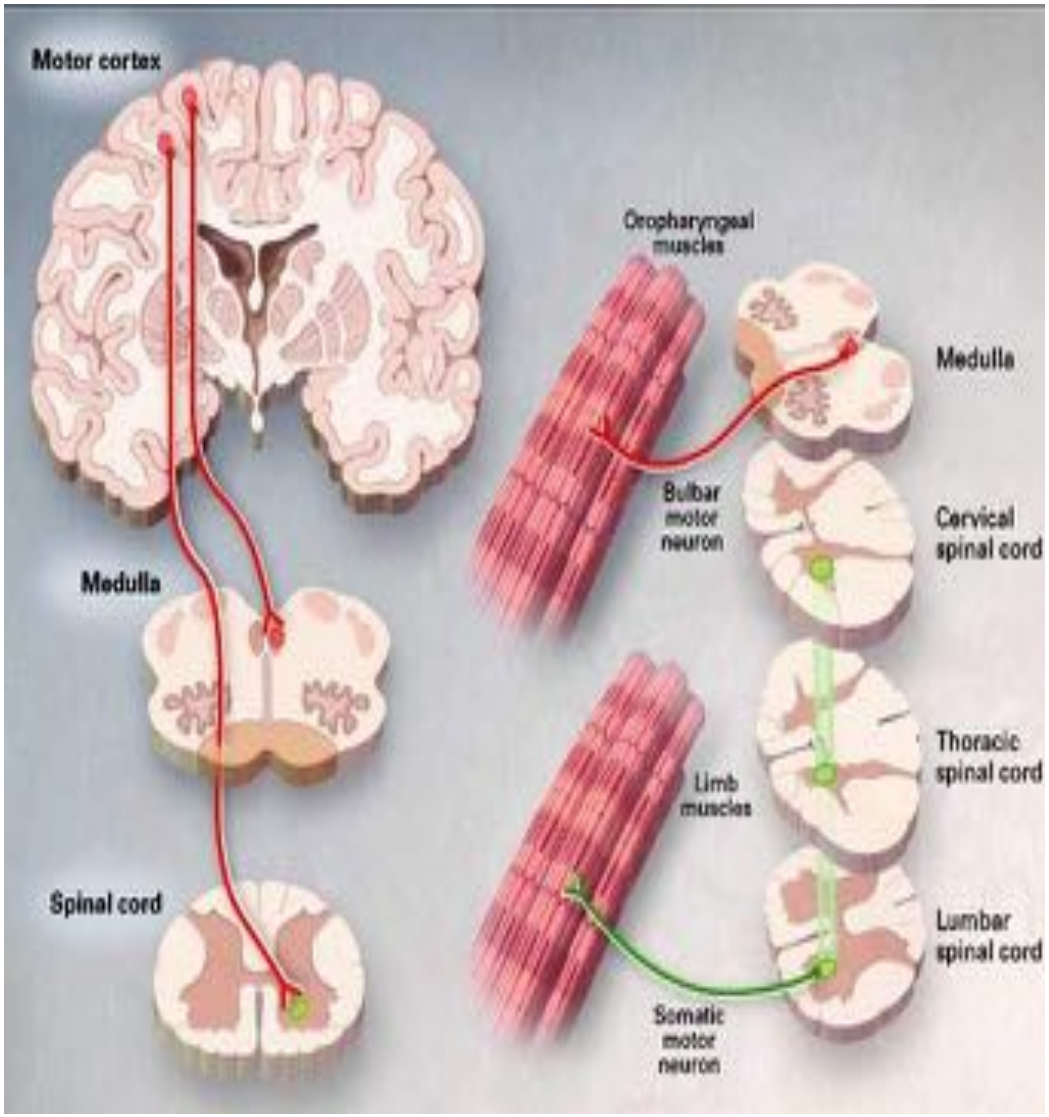
The great cell biologists of the 19th century, including Rudolph Virchow, the German physician widely known as the father of pathology, and the French physiologist Claude Bernard established the pivotal idea that individual cells function autonomously, while being part of the whole organism. Since then, many pathological conditions including all major neurodegenerative diseases have traditionally been considered mechanistically cell autonomous, meaning that damage within a selective population of affected neurons alone suffices to produce disease.

Cell Autonomous: A genetic trait in multicellular organisms in which only genotypically mutant cells exhibit the mutant phenotype.

Conversely, a **nonautonomous** trait is one in which genotypically mutant cells cause other cells (regardless of their genotype) to exhibit a mutant phenotype.

Amyotrophic Lateral Sclerosis



→ Adult onset neurodegenrative disease

→ appr. 20'000 individuals in USA

→ Degeneration of Motor Neurons

→ Paralysis and ultimately death

→ ~ 90% sporadic, 10% familial cases

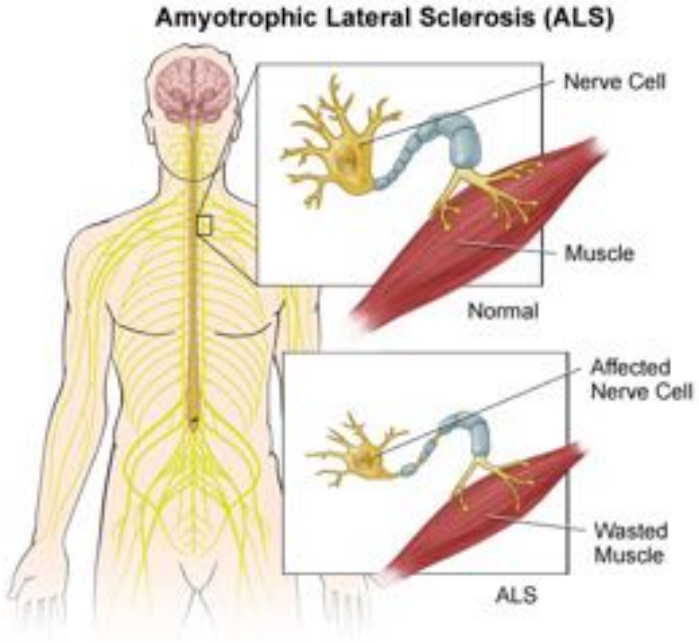
→ Various disease causing genes identified (SOD1, TDP-43, FUS, C9ORF72)

→ Vast majority of cases: cause unknown

Disease course remarkably similar → common mechanims?

Amyotrophic Lateral Sclerosis

- Adult onset, neurodegenerative disease
- Progressive & fatal loss of motor neurons in spinal cord and brain
- Amyotrophic: atrophy of muscle fibers
  Lateral sclerosis: hardening of anterior & lateral corticospinal tracts
- 5600 patients diagnosed per year in U.S.
  Life time risk 1:400
- Mean age of onset: 55-65 years
  Life expectancy: 2-5 years post-onset

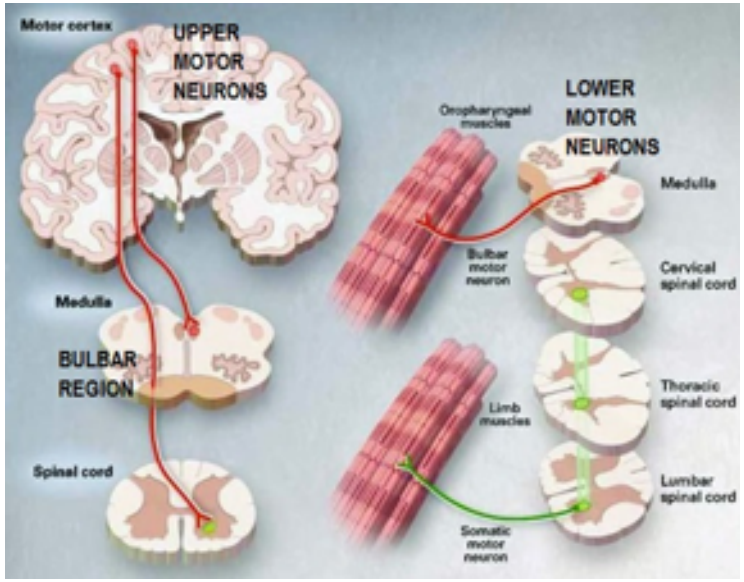


Clinical features of ALS

- Syndrome of motor neuron disorders
  - Primary ALS
  - Mimic disorders

- Clinical presentation
  - Asymmetric pattern of limb muscle weakness
  - Dysarthria and dysphagia
  - Respiratory weakness

- Diagnosis
  - Involvement of both upper and lower motor neuron degeneration
  - Exclusion of mimic disorders



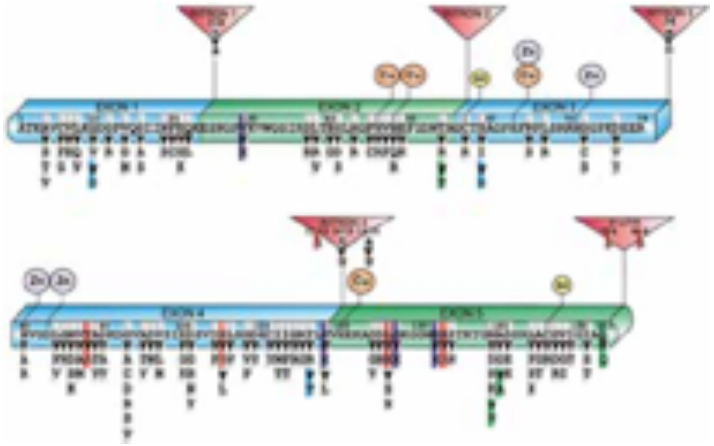
- Disease management
  - **NO CURE** for the disease
  - Symptomatic treatment and palliative care
  - Riluzole, the only FDA approved drug

Etiology of ALS

- Sporadic ALS (SALS)
  - 90% of ALS cases
  - No known mutation associated
- Familial ALS (FALS)
  - 10% of ALS cases
  - Inherited as an autosomal dominant trait
- Multiple genes associated with FALS
  - Superoxide Dismutase 1 (SOD1) (~20%)
  - TAR DNA binding protein (TDP-43) (1-5%)
  - Fused in Sarcoma (FUS) (1-5%)
  - C9ORF72 (25-40%)
- Unifying model of ALS pathogenesis remains elusive

Superoxide Dismutase 1 (SOD1)

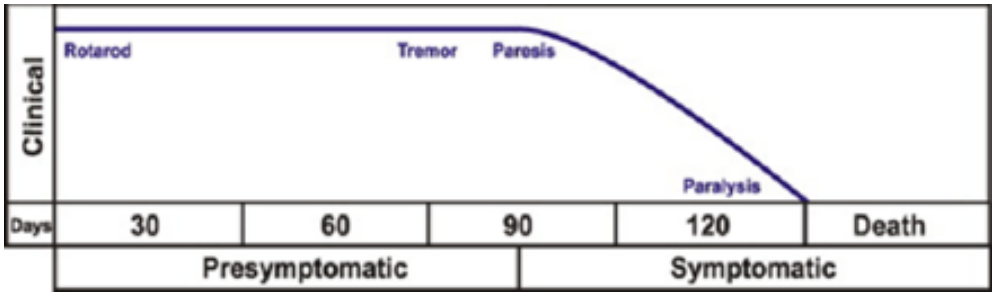
- Destruction of superoxide radicals in body
- First gene to be linked with FALS
- Over 150 mutation identified
- Toxic gain of function
- The most extensively studied
- No consensus on the mechanism of pathogenesis

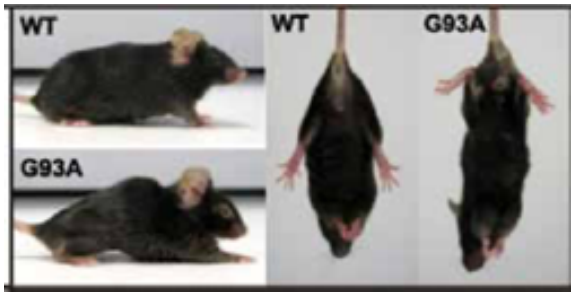


- Involvement of misfolded wild-type SOD1 in SALS pathogenesis

Turner *et al,* 2008

- **SOD1^{G93A} mice**
  - Human mutant SOD1 gene fragment with G93A mutation
  - Highly progressive
  - Disease onset: ~90d;
  - Endstage: ~130d



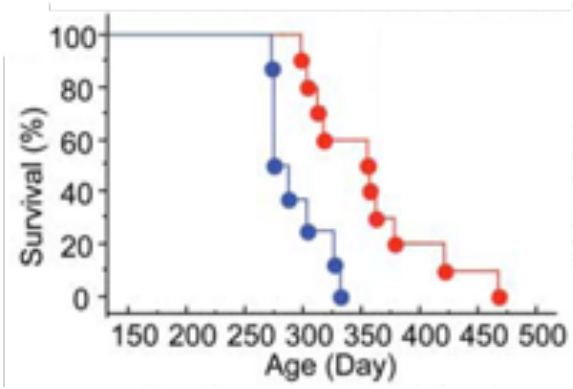


- **LoxSOD1^{G37R} mice**
  - Floxed human mutant SOD1 gene with G37R mutation
  - Slow progressing
  - Disease onset: ~197d
  - Endstage: ~390d

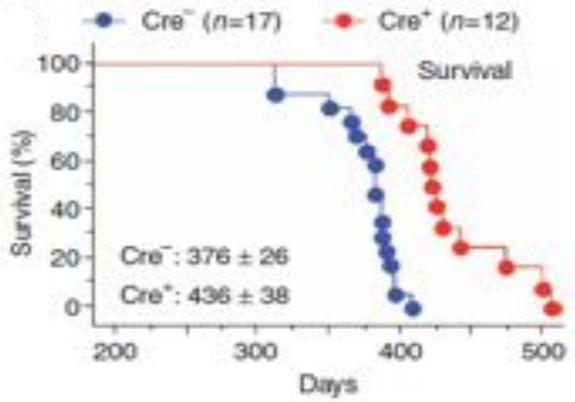
Turner *et al,* 2008

Transgenic reduction of mutant SOD1 improves survival in ALS mice

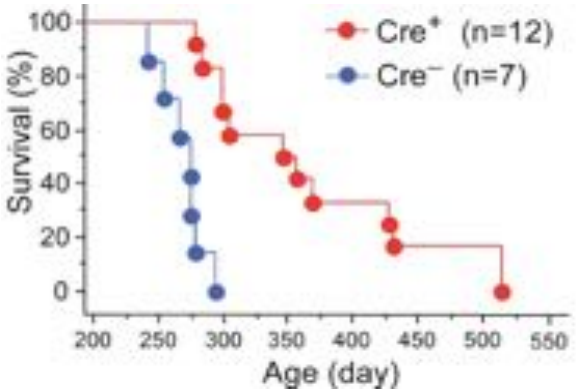
Islet 1-Cre



GFAP-Cre



Iba1-Cre

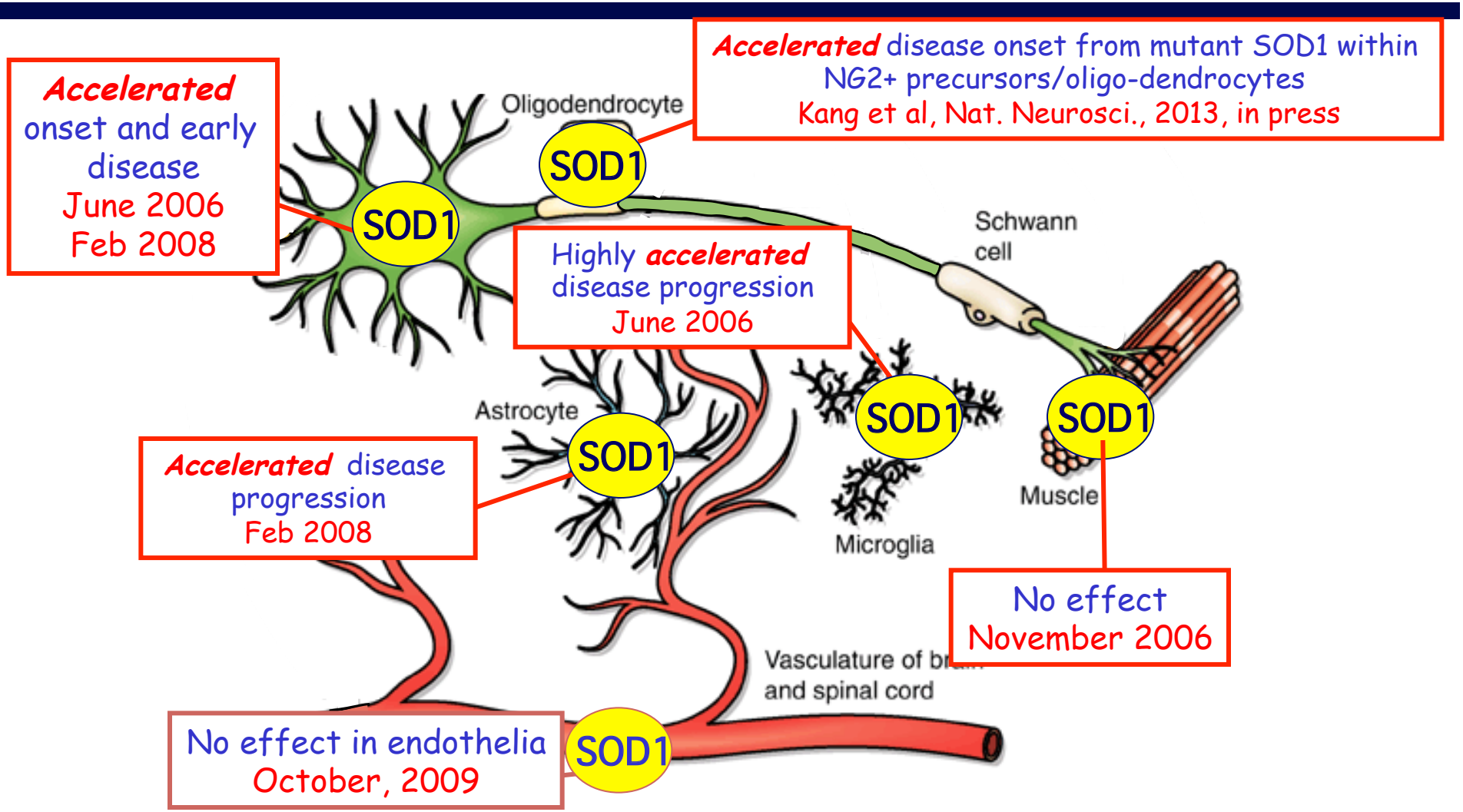


Boillee *at al*, 2006; Yamanaka *et al* 2008

Historical Survival Data in ALS mouse models

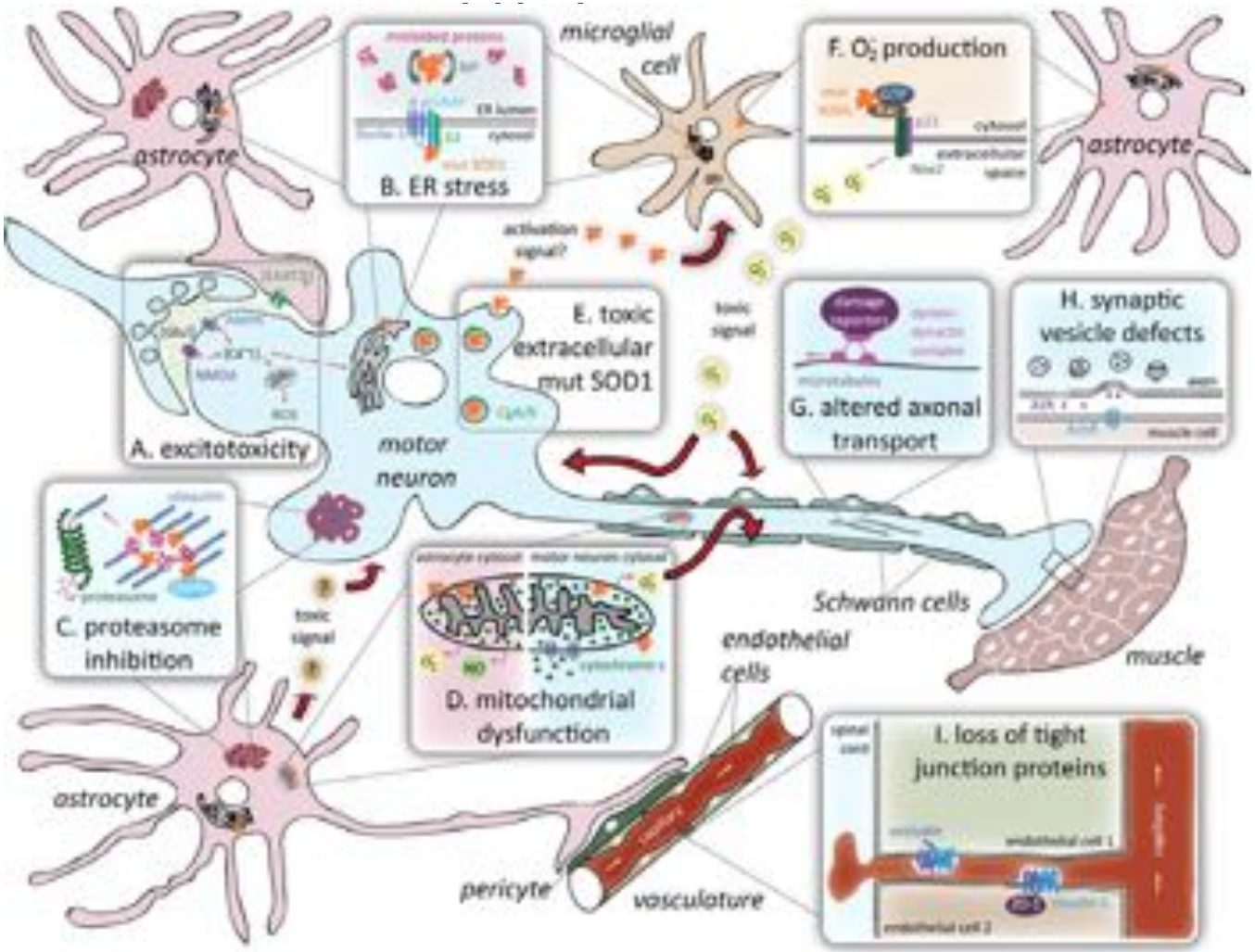
Author	Target	Model	Median Survival	Median Survival Difference	
Beers/Appel	PU.1.-/-	G93A	125-132	10-14	
			143		
Boillee/DC	Islet1 cre	G37R	Isl1Cre–, 293.5 +/- 8.7	64	21%
			Isl1Cre+, 357.5 +/- 17.2		
Boillee/DC	CD11b cre	G37R	cre- 275	75	27%
			cre+ 350		
Yamanaka/DC	GFAP cre	G37R	376+/- 26	60	16%
			436+/-38		
Zhu/Przedborski	Minocycline	G93A	125.6 +/- 3.4 days	11	
			136.8 +/- 1.2 days		
Petri	FasL KO	G93A	Control 160d+/-9	10	
			KO 170+/-17days		
Gurney	Riluzole	G93A	Ctrl 127+/- 5.7	13	
			Treated 140 +/-4.3		
BK	IGF-1	G93A	123		
			160	37	
BK	GDNF	G93A	134	12	

Cellular contributors to SOD1 mutant-derived toxicity in ALS



Boillée et al Science, 2006; Miller et al, PNAS, 2006; Lobsiger et al, PNAS, 2009
*Yamanaka *, Boillée* et al, Nat. Neurosci., 2008; Zhong et al, JCI, 2009*

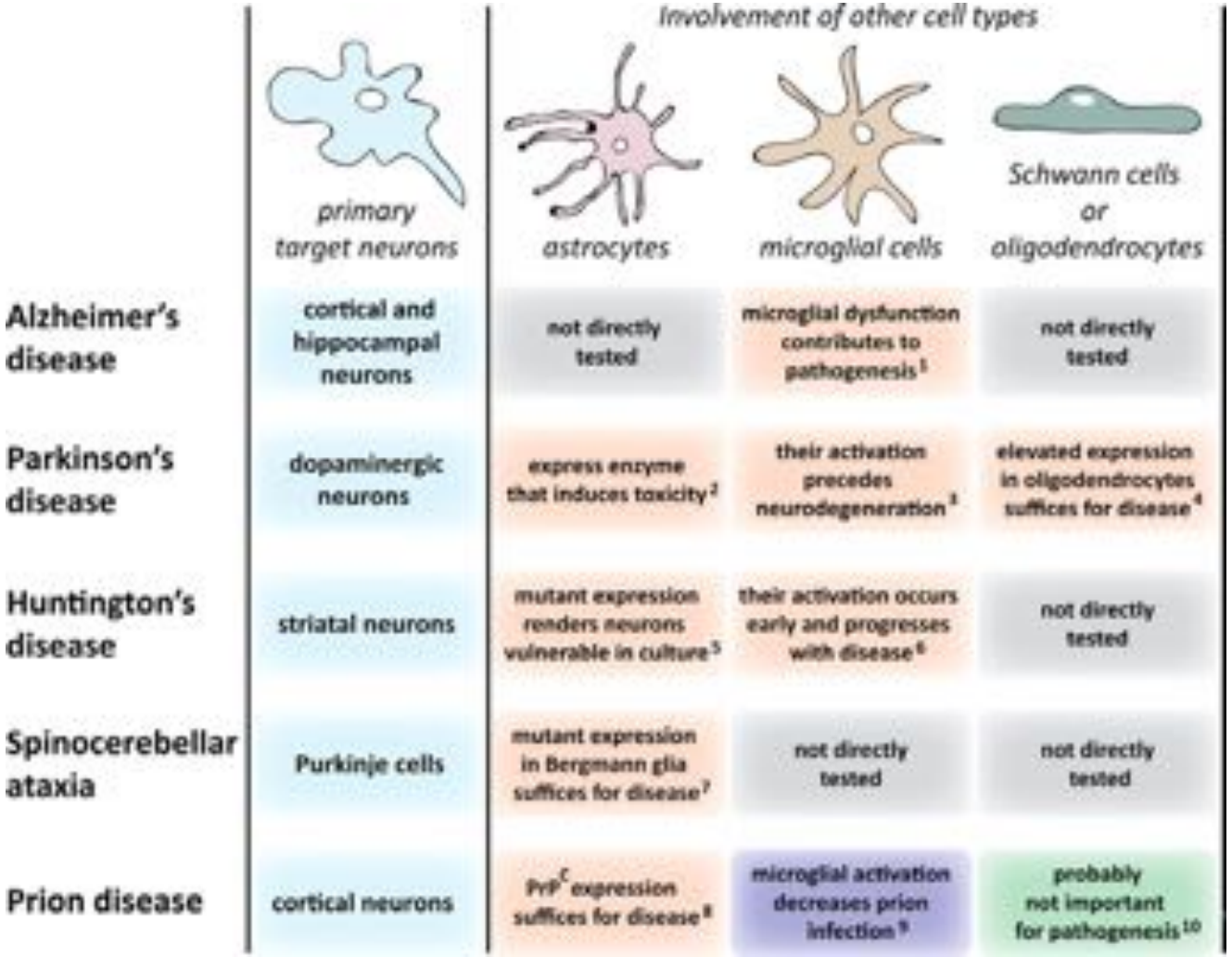
Proposed mechanisms of toxicity in SOD1-mediated ALS. (A) Excitotoxicity is the hyperactivation of motor neurons resulting from failure to rapidly remove neurotransmitter glutamate from synapses due to deficiency in the glutamate transporter EAAT2 in the



Ilieva H et al. J Cell Biol 2009;187:761-772

JCB

Non–cell autonomous pathogenesis in neurodegenerative diseases.



	primary target neurons	Involvement of other cell types: astrocytes	Involvement of other cell types: microglial cells	Involvement of other cell types: Schwann cells or oligodendrocytes
Alzheimer's disease	cortical and hippocampal neurons	not directly tested	microglial dysfunction contributes to pathogenesis[1]	not directly tested
Parkinson's disease	dopaminergic neurons	express enzyme that induces toxicity[2]	their activation precedes neurodegeneration[3]	elevated expression in oligodendrocytes suffices for disease[4]
Huntington's disease	striatal neurons	mutant expression renders neurons vulnerable in culture[5]	their activation occurs early and progresses with disease[6]	not directly tested
Spinocerebellar ataxia	Purkinje cells	mutant expression in Bergmann glia suffices for disease[7]	not directly tested	not directly tested
Prion disease	cortical neurons	PrP^{C} expression suffices for disease[8]	microglial activation decreases prion infection[9]	probably not important for pathogenesis[10]

Ilieva H et al. J Cell Biol 2009;187:761-772

JCB

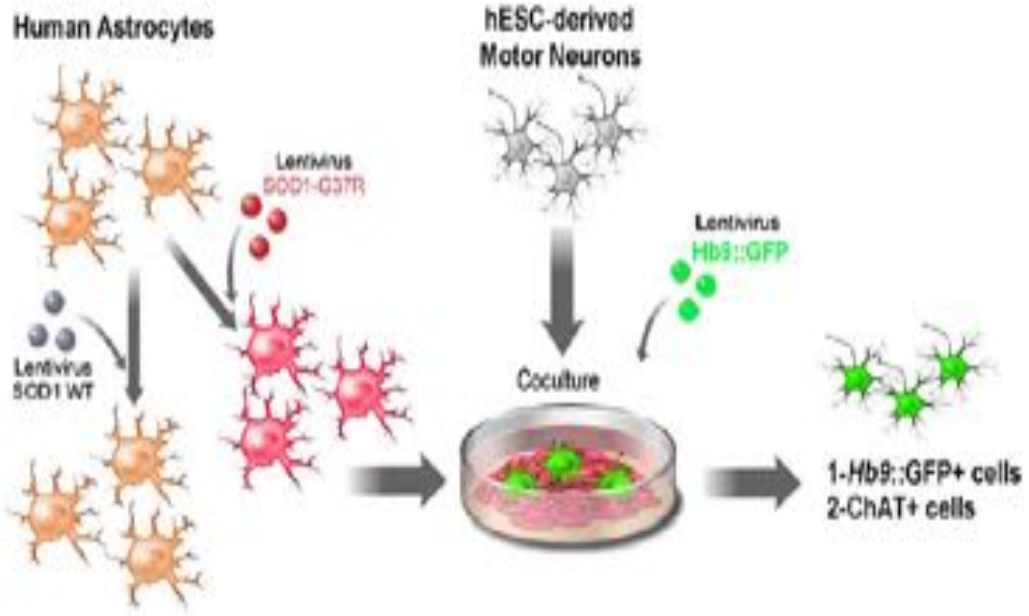
Non-cell autonomous component of ALS

Cell Stem Cell
Article

Cell PRESS

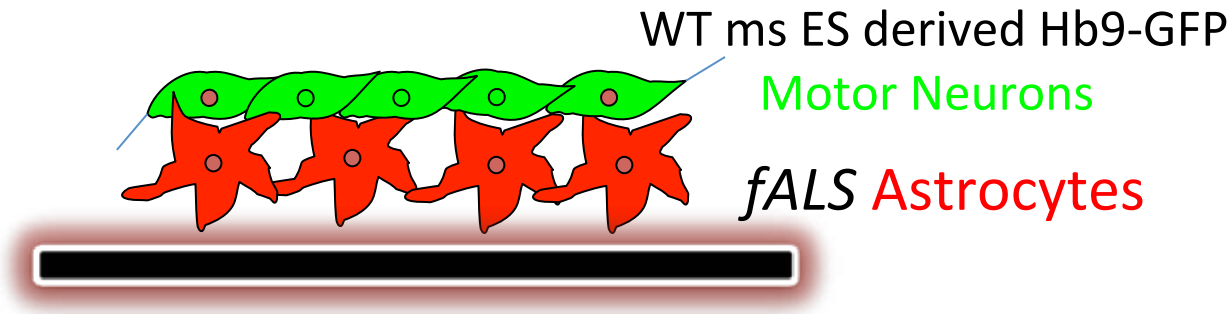
Non-Cell-Autonomous Effect of Human SOD1^{G37R} Astrocytes on Motor Neurons Derived from Human Embryonic Stem Cells

Marta C.N. Marchetto,[1] Alysson R. Muotri,[1] Yangling Mu,[1] Alan M. Smith,[2] Gabriela G. Cezar,[2] and Fred H. Gage[1,*]
[1]Laboratory of Genetics, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA
[2]Department of Animal Sciences, University of Wisconsin-Madison, Madison, WI 53706, USA



→ **Motor neuron death and disease progression is influenced by other cell types**

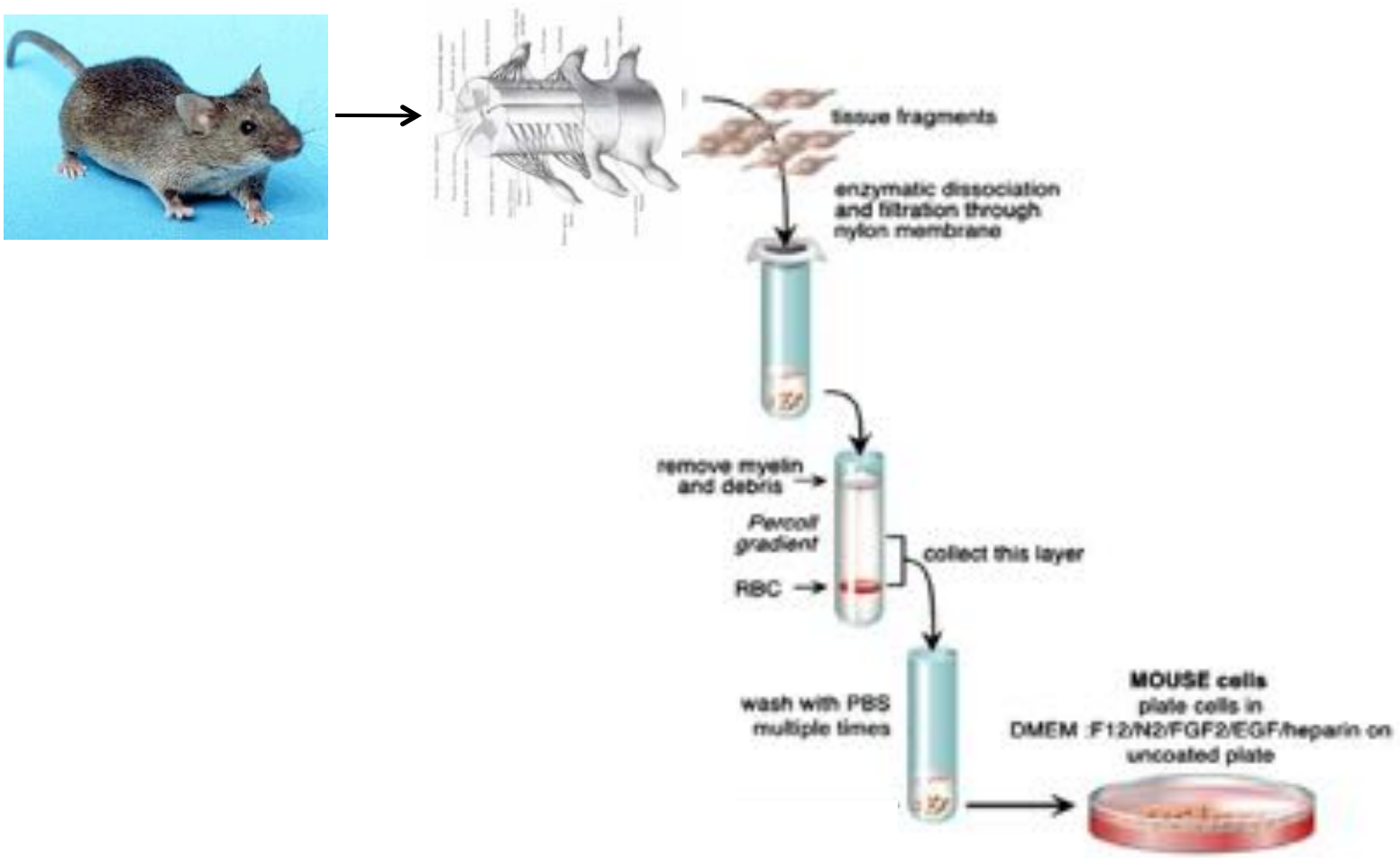
Co-culture based models of *fALS* demonstrates Astrocytes Convey Toxicity to Motor Neurons



Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons.
Nagai M, Re DB, Nagata T, Chalazonitis A, Jessell TM, Wichterle H, Przedborski S.
Nat Neurosci. 2007 May;10(5):615-22.

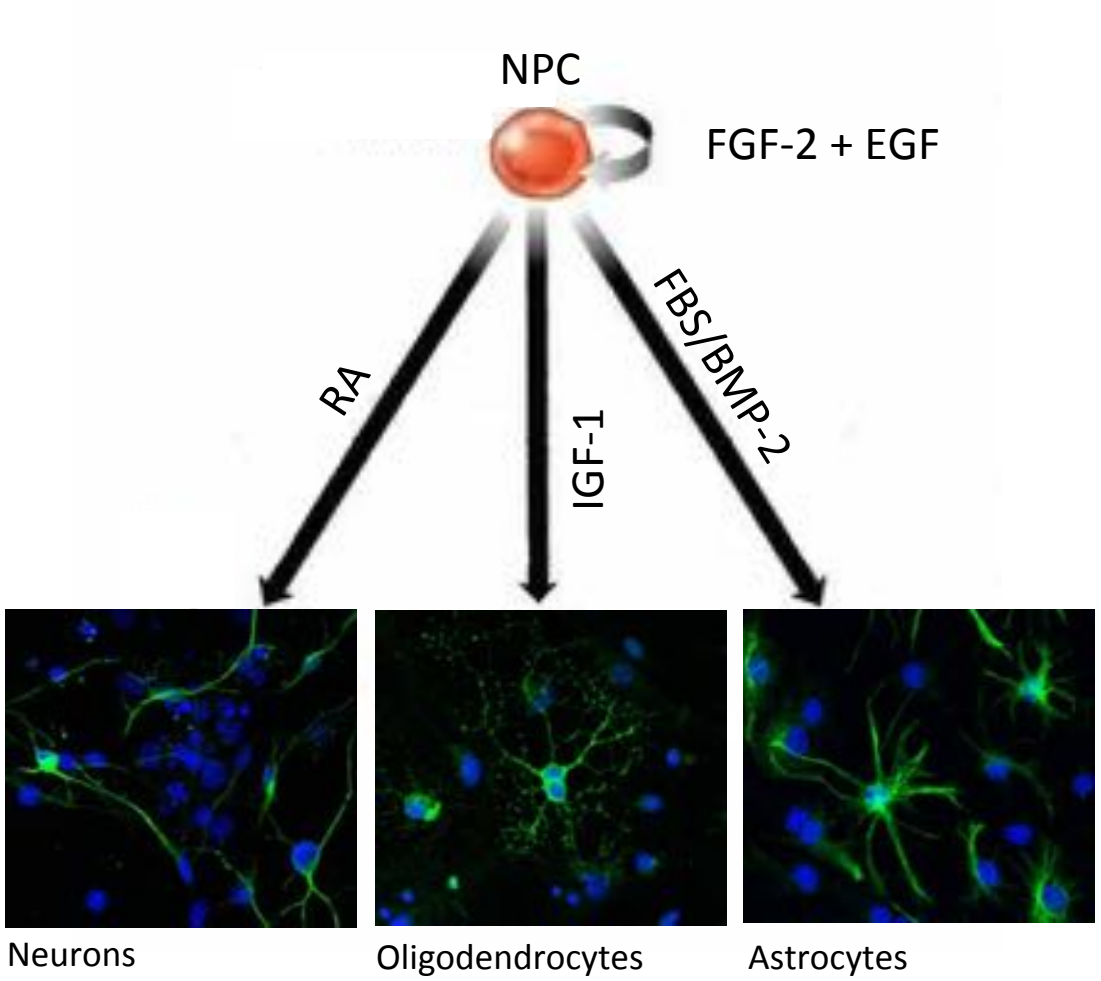
Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model.
Di Giorgio FP, Carrasco MA, Siao MC, Maniatis T, Eggan K.
Nat Neurosci. 2007 May;10(5):608-14.

Neural Progenitor Cells Can Be Isolated and Cultured from Mouse, Rodent and Human Spinal Cord

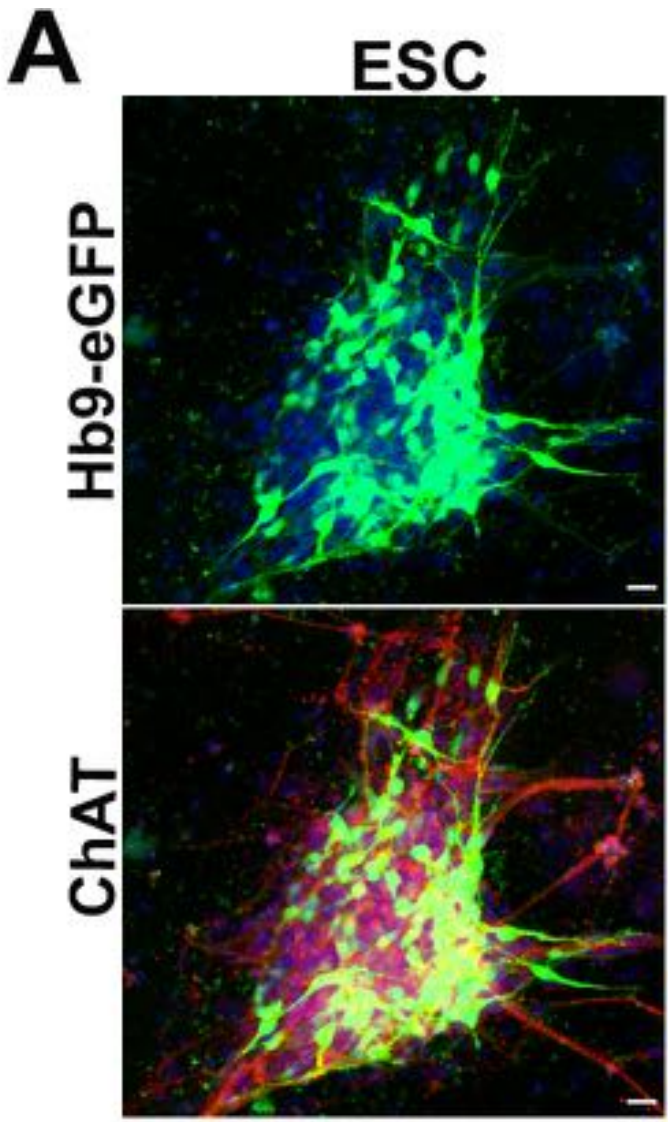


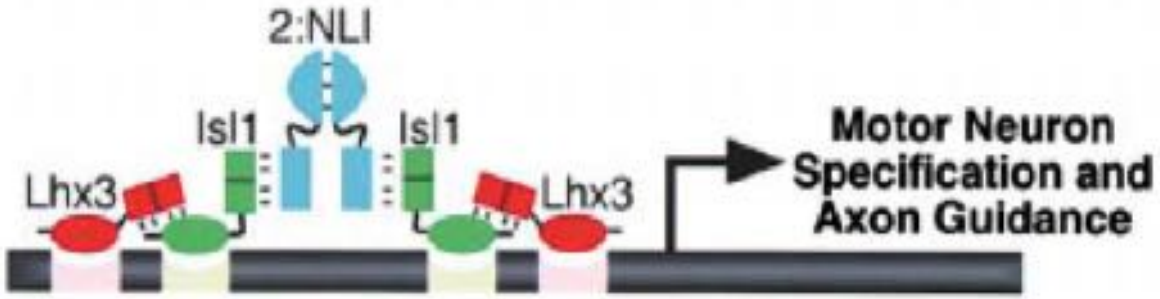
Modified from Ray el al., MCN, 2006

NPCs retain their tripotency *in vitro*

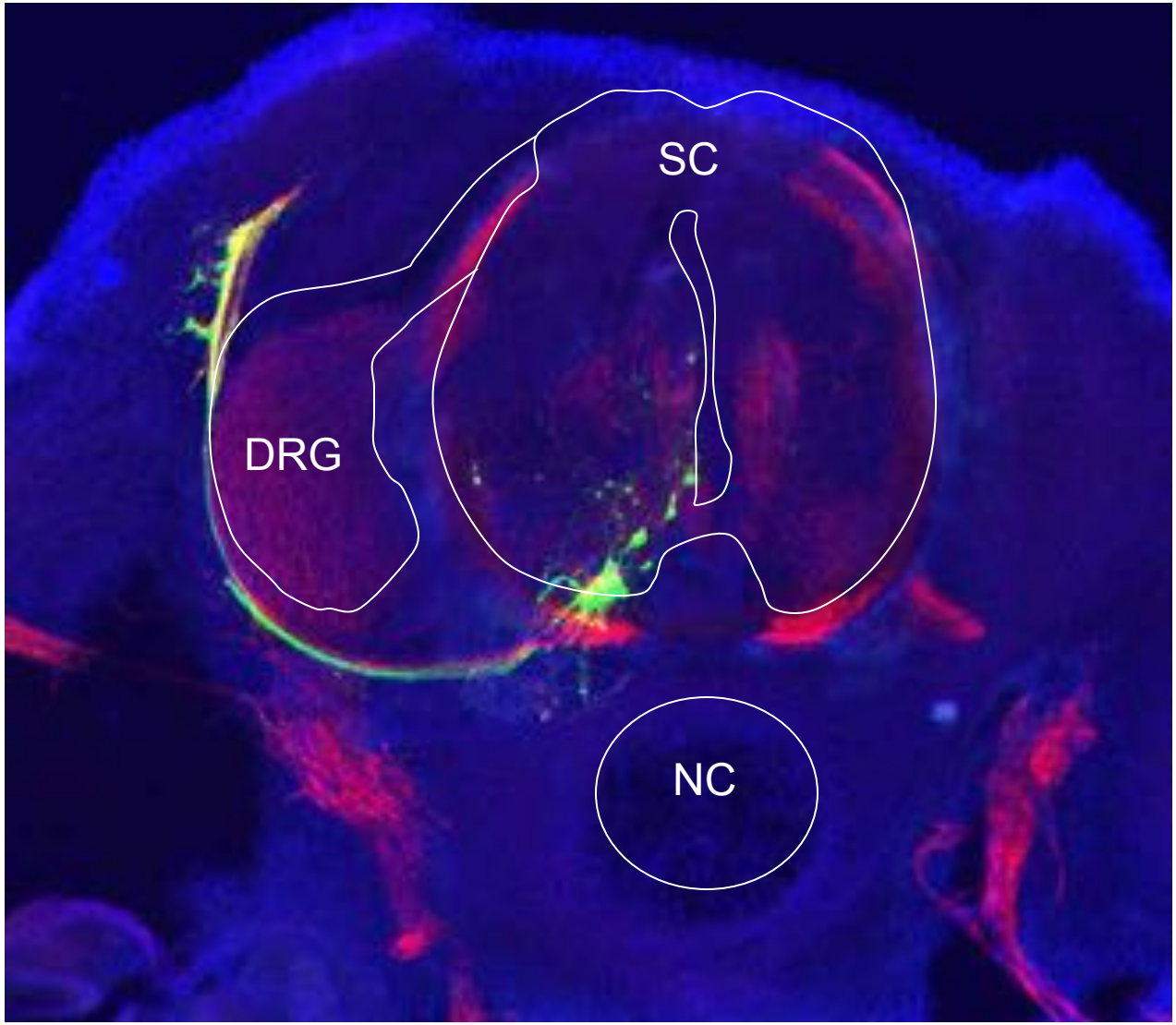


Embryonic Stem Cells are Differentiated towards Motor Neurons using RA/SHH





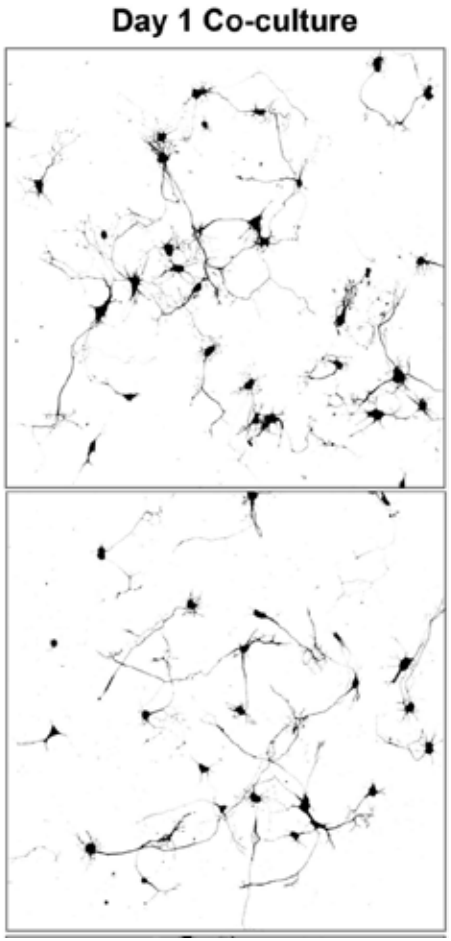
Embryonic Stem Cells are Differentiated towards Motor Neurons using RA/SHH and Can Be Studied in vivo of developing Chick Embryo



G93A SOD1 Mouse NPC-derived astrocytes convey toxicity to embryonic derived motor neurons in an *in vitro* co-culture model

Non-ALS Astrocytes

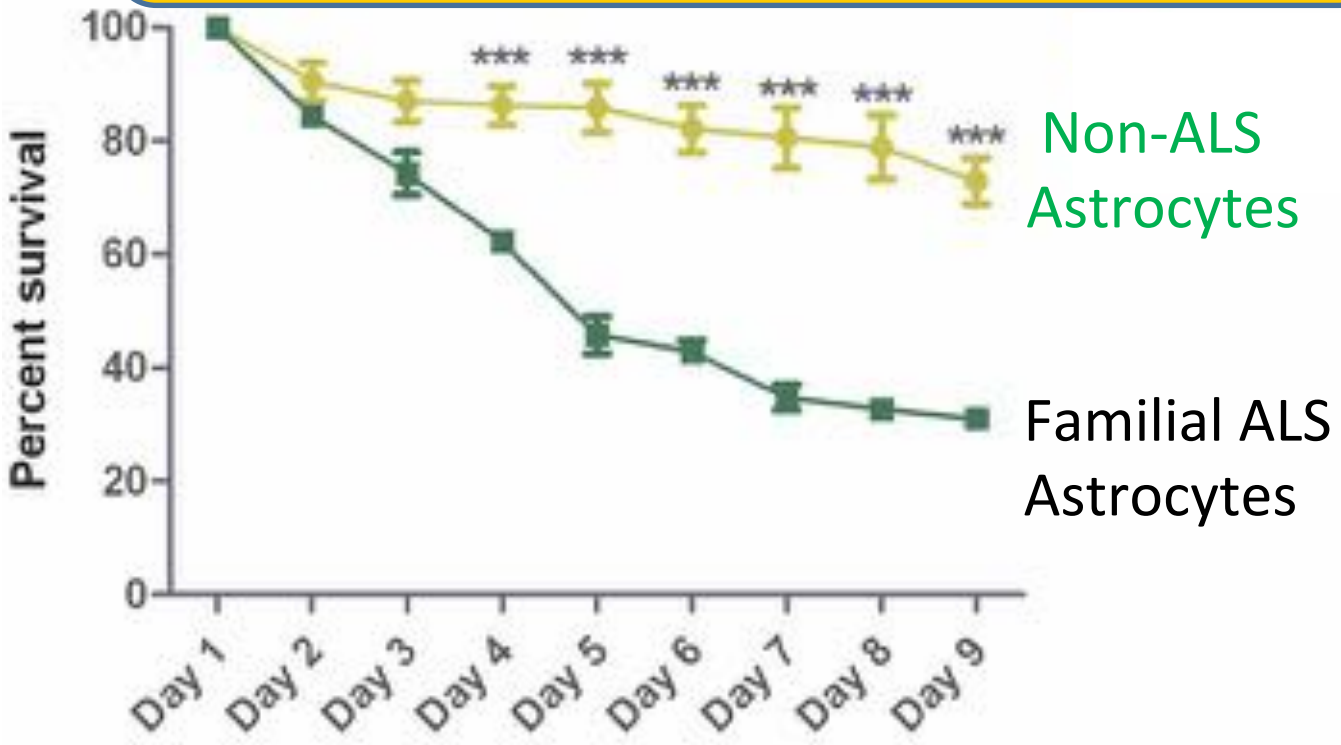
Familial ALS Astrocytes



24 Hours → 72 Hours → 120 Hours

G93A SOD1 Mouse NPC-derived astrocytes convey toxicity to embryonic derived motor neurons in an *in vitro* co-culture model

Robust, Highly Repeatable Co-culture Model
where Familial ALS astrocytes kill Motor Neurons



But, These Models Only Recapitulate familial SOD1 cases…

What about the other (sporadic) cases? iPS technology/Other Methods

Nature. 2001 May 3;411(6833):42-3.

Cell culture. Progenitor cells from human brain after death.

Palmer TD, Schwartz PH, Taupin P, Kaspar B, Stein SA, Gage FH.
The Salk Institute, Laboratory of Genetics, 10010 North Torrey Pines Road, La Jolla, California 92122, USA.

brain: Life after death

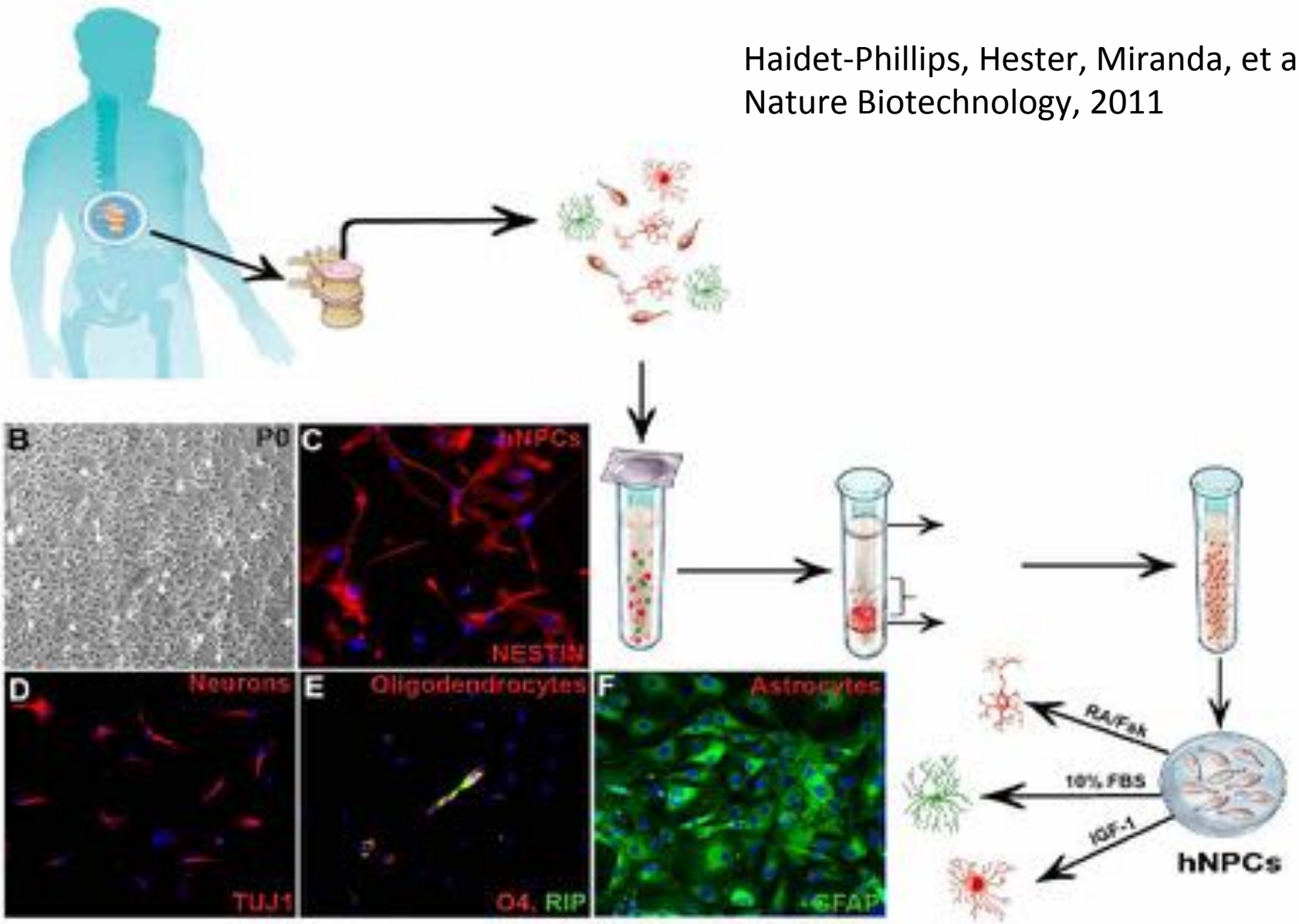
New research raises the hope that cells, like organs, could one day be taken from the dead and given to the living

Fred Gage, of the Salk Institute, and colleagues have cultured neural progenitor cells — which can go on to divide into nerve cells — from cadaver brains[1]. This could offer another, less ethically fraught alternative to fetal cells as a source of human replacement tissues.
The full medical potential of this finding is still unclear. The post-mortem cells do not divide as much as those from fetal tissue, for example, and the various types of cells they can form remain to be discovered.
"These results confirm that the adult human brain contains cells that can continue to divide and differentiate," says Gage

Palmer, T. D. et al. Progenitor cells from human brain after death. *Nature* 411, 42-43 (2001).

Strategy of deriving **human post-mortem ALS NPCs**

Haidet-Phillips, Hester, Miranda, et al
Nature Biotechnology, 2011

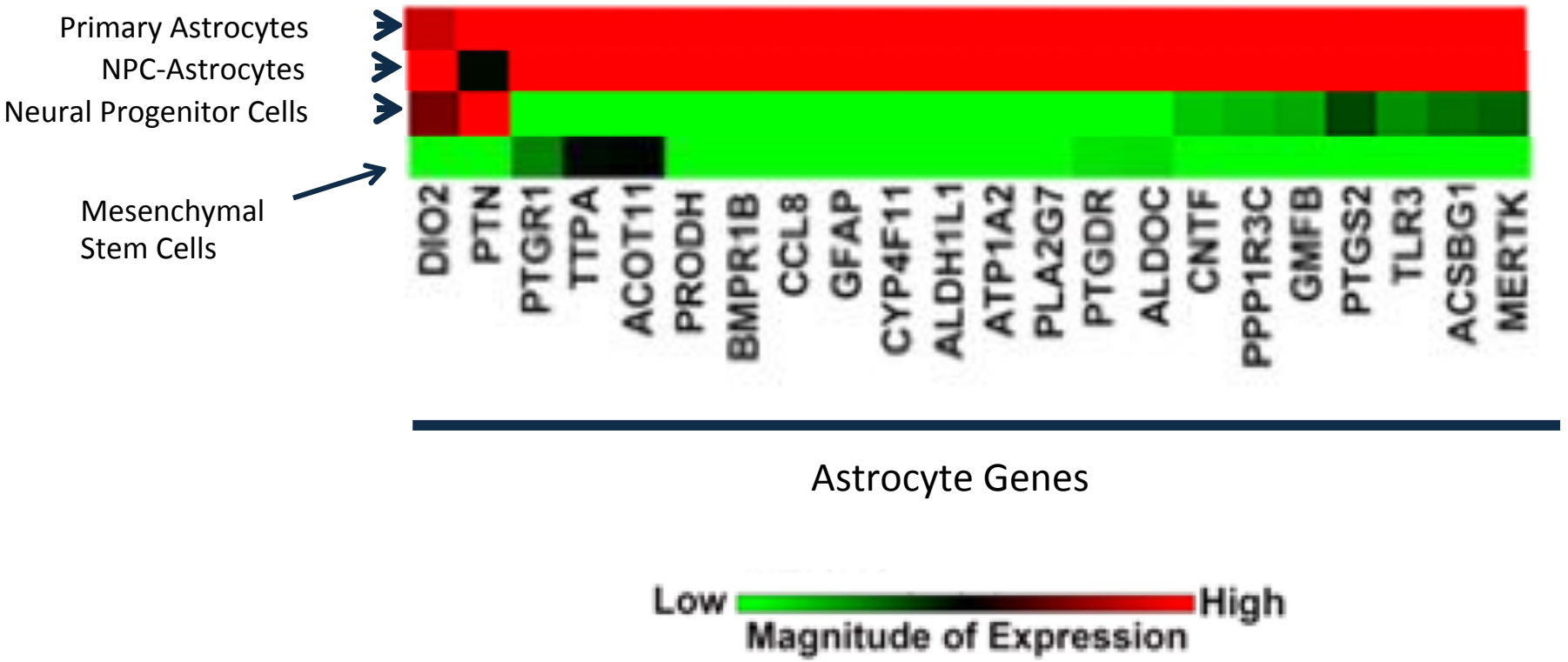


Characterizing Astrocyte "like" cells demonstrates Astrocyte like phenotypes

"Ben Barres Laboratory Astrocyte Array Analysis"

DO WE HAVE AN ASTROCYTE CELL IN CULTURE?

WE CERTAINLY HAVE AN ASTROCYTE "LIKE" CELL

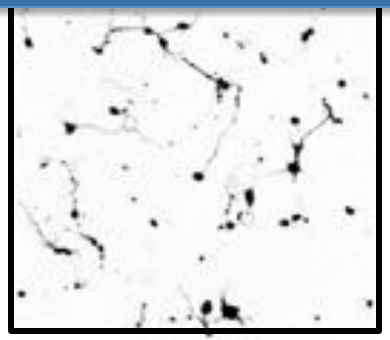


Co-Culture Evaluation of Motor Neurons with Non-ALS-, fALS- and sALS-Astrocytes

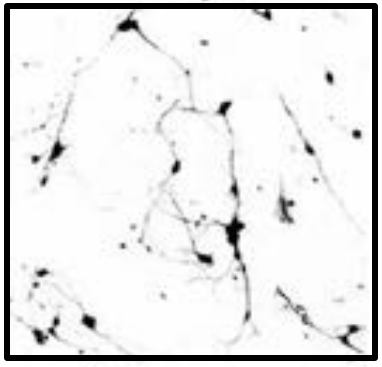
2011, 2012, 2013

Mounting Evidence in the field that
SOD1 gets misfolded in both fALS (SOD1 mutations)
and sALS and may be a much bigger target

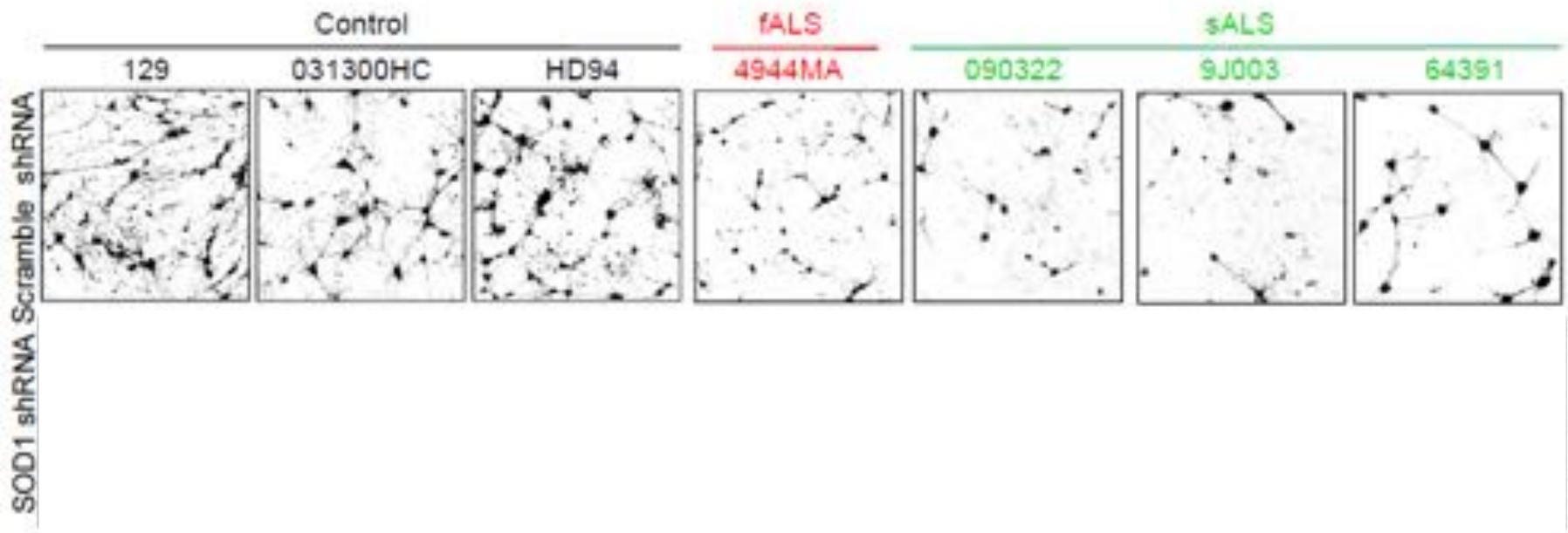
Familial ALS



Sporadic ALS



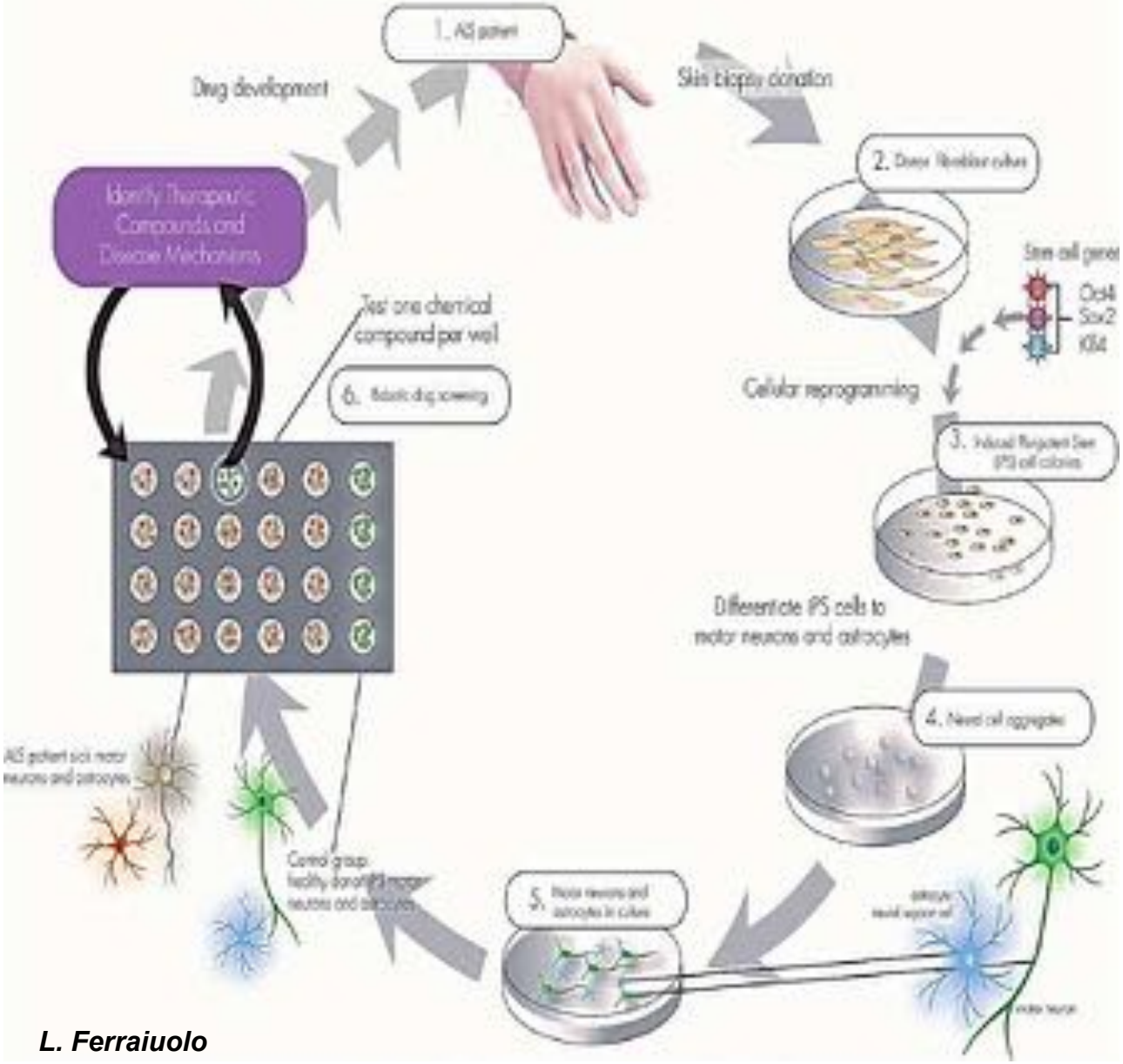
Astrocytes from familial and sporadic ALS patients
are toxic to motor neurons… in a SOD-1 dependent manner



2012 Critique

"Kaspar and colleagues work is interesting, but the cells (NPCS) they isolated were from hypoxic and Inflamed spinal cords from the sickest of patients (death), so they aren't really studying true ALS…..

Use skin fibroblasts for personalized drug screenings to find therapeutics and unravel disease mechanisms



L. Ferraiuolo

Reprogramming of terminally differentiated cells by overexpression of transcription factors

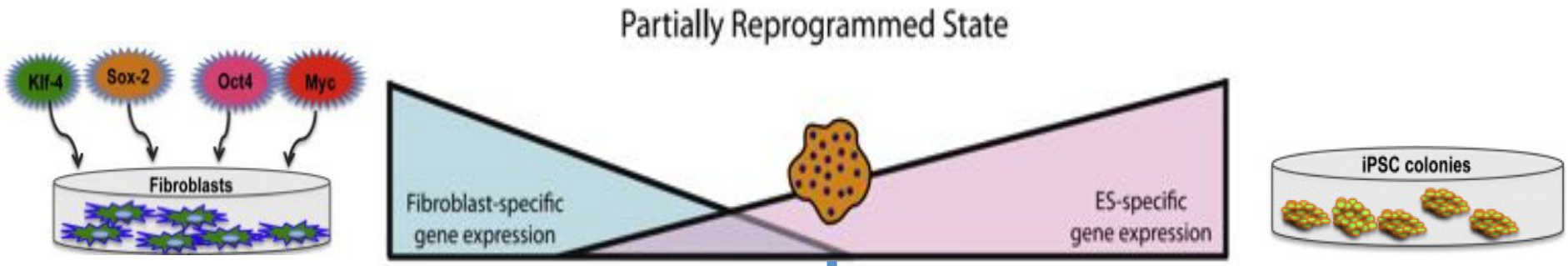


Figure modified from Nsair et. al, Advanced Drug Delivery Rev, 2011 and Sridharan et al, Cell, 2009

Unstable intermediate

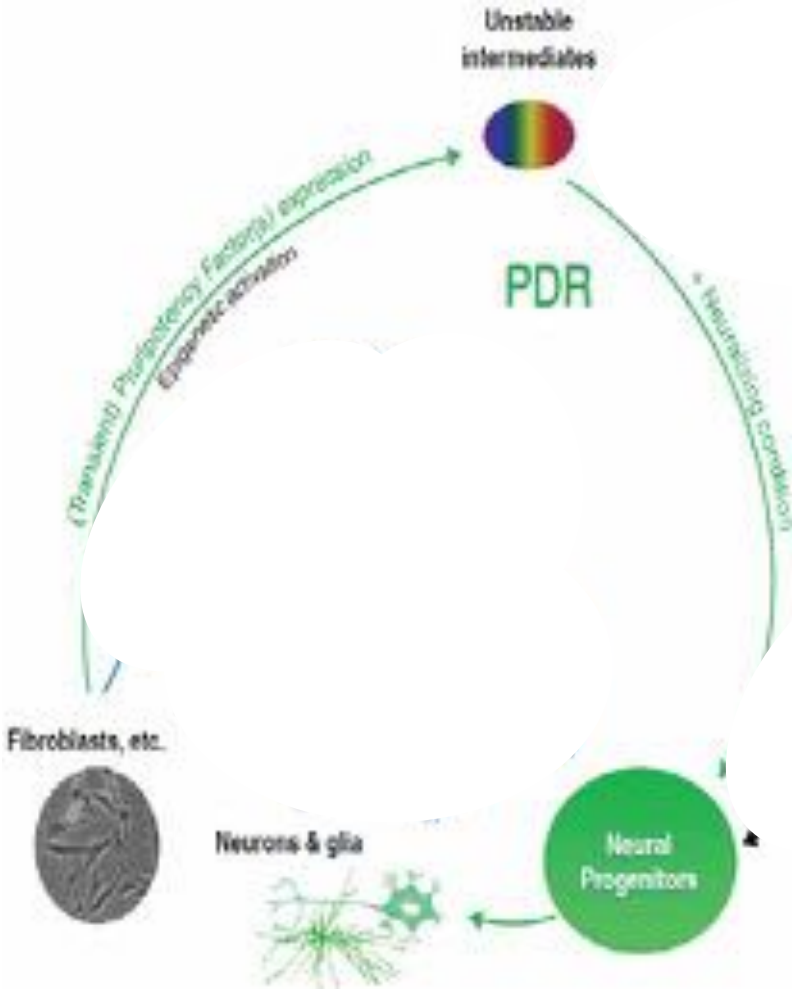
Direct reprogramming of mouse fibroblasts to neural progenitors

Janghwan Kim[a,b], Jem A. Efe[a], Saiyong Zhu[a], Maria Talantova[c], Xu Yuan[a], Shufen Wang[d,e], Stuart A. Lipton[c], Kang Zhang[d,e], and Sheng Ding[a,f,1]

Direct conversion of mouse fibroblasts to self-renewing tripotent neural precursor cells

Ernesto Lujan[a,b], Soham Chanda[a,c], Henrik Ahlenius[a,d], Thomas C. Südhof[c,e,1], and Marius Wernig[a,d,1]

Advantages of direct conversion compared to classical reprogramming



→ Direct conversion is much faster

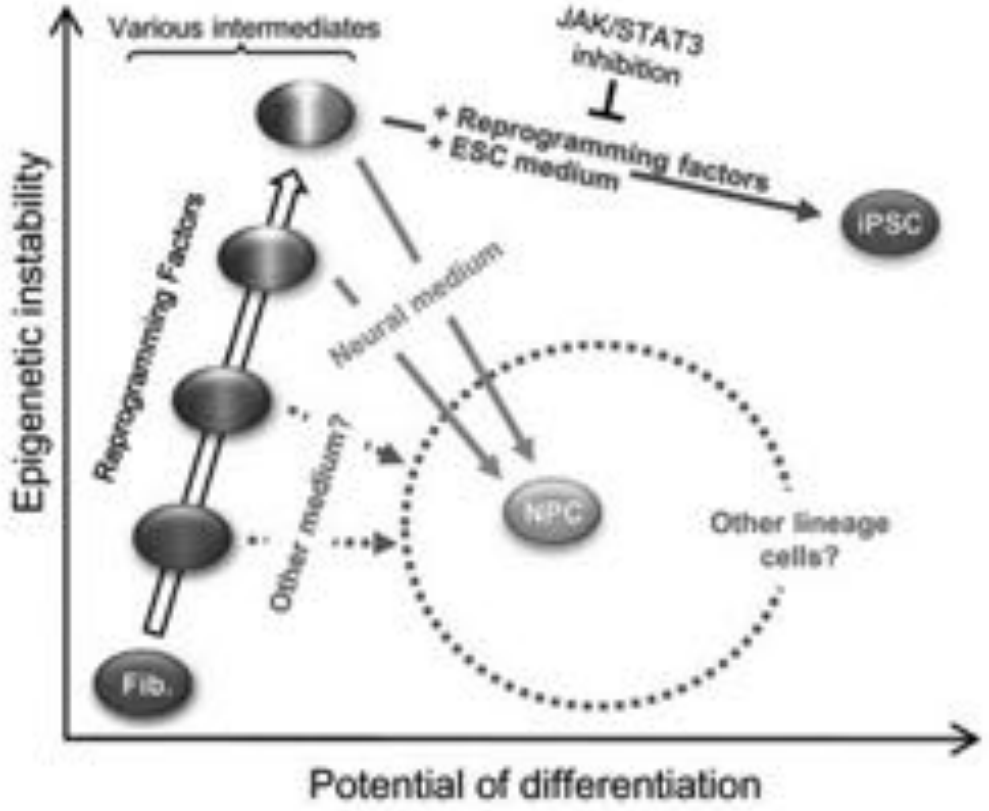
→ No clonal selection – no clonal variation

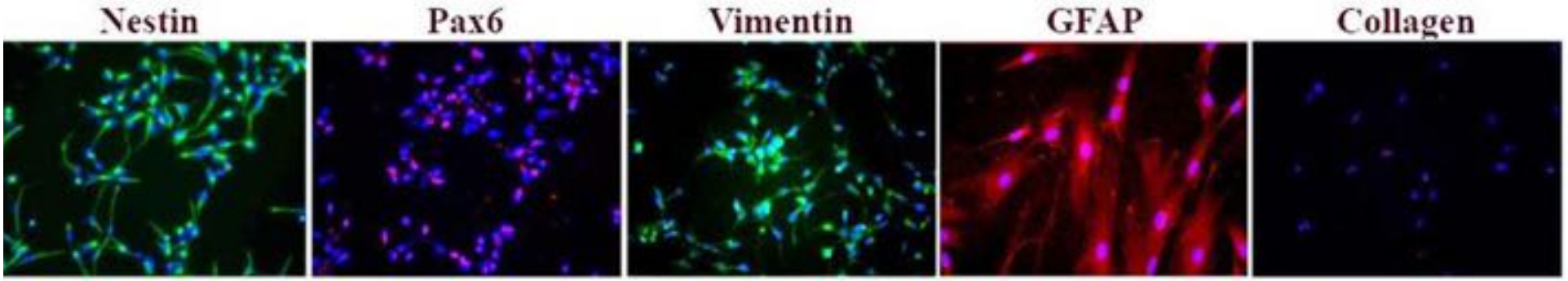
→ Maitenance much less time consuming

→ Straight forward production of neurons, astrocytes and oligodendrocytes (iPS need to be differentiated first into NPCs)

→ Still able to generate several disease relevant cell types in parallel

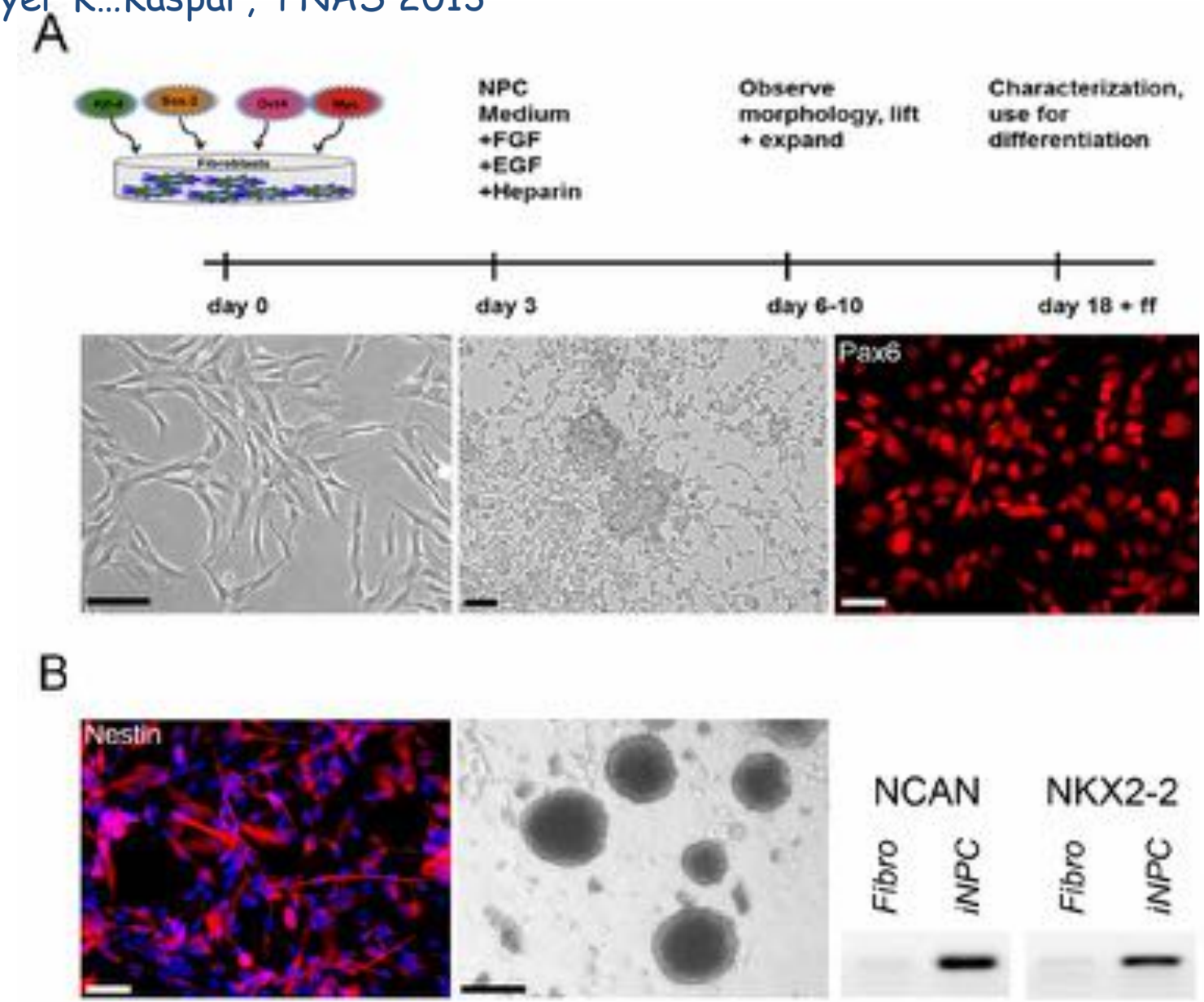
Direct Reprogramming of Fibroblasts to NPCs
New method to develop astrocytes and other cell types from live patients



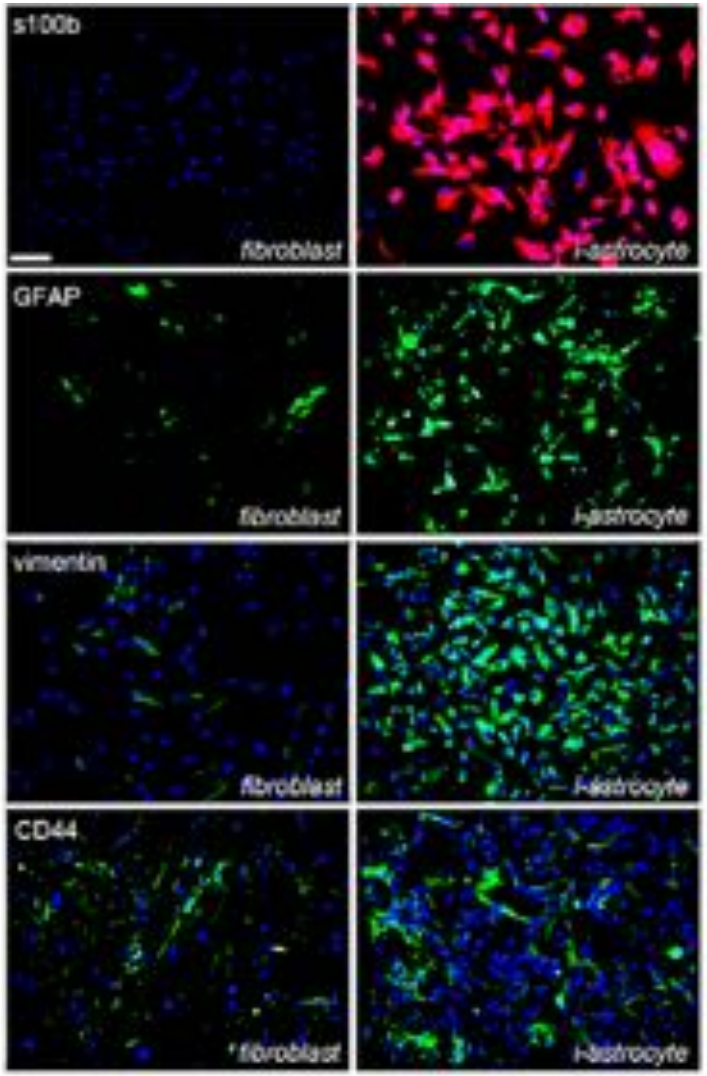


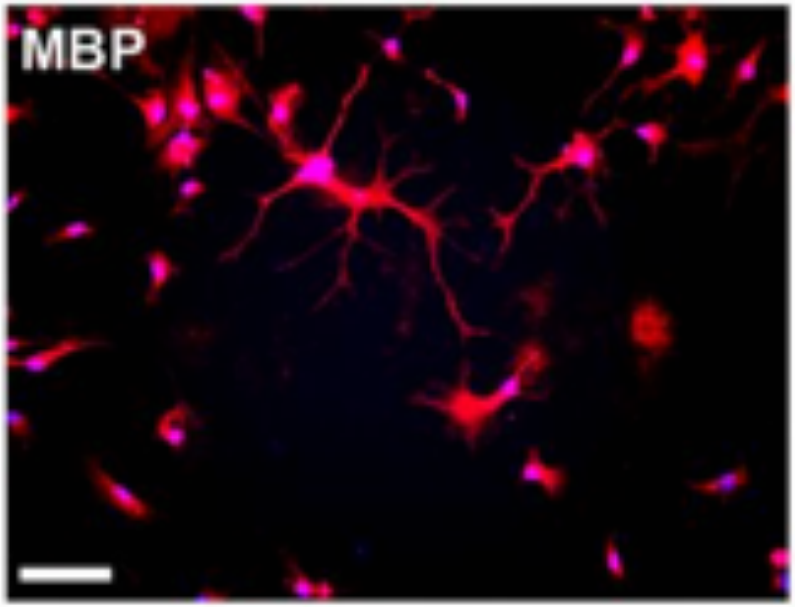
Induced NPCs from ALS patients

Meyer K…Kaspar, PNAS 2013

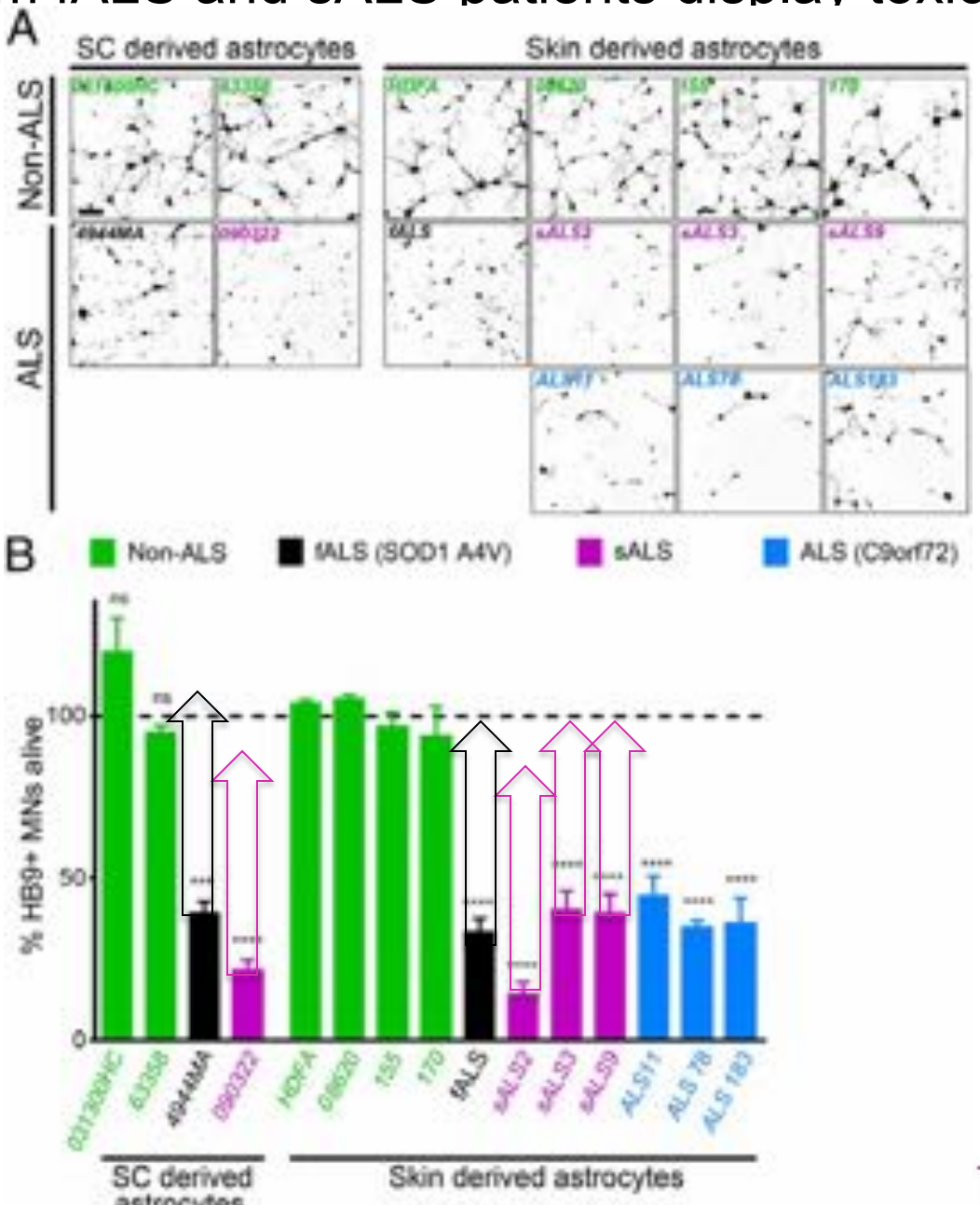


iNPCs can differentiate towards i-astrocytes and i-oligos





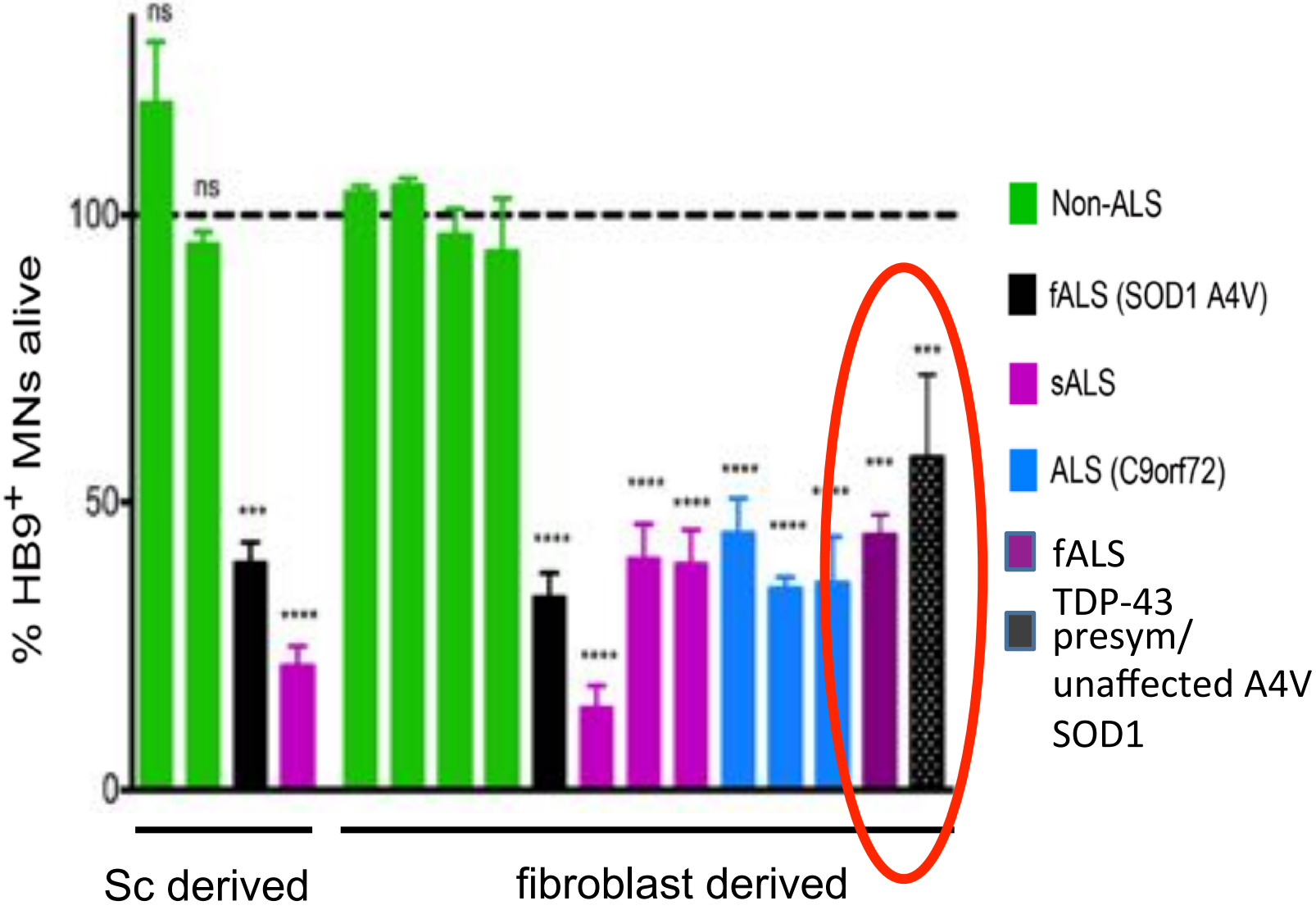
I-astrocytes from fALS and sALS patients display toxicity toward MNs.



Meyer K et al. PNAS 2014;111:829-832 PNAS

I-astrocytes from ALS patients are consistently toxic to motor neurons

EVEN in PRE-SYMPTOMATIC A4V SOD1

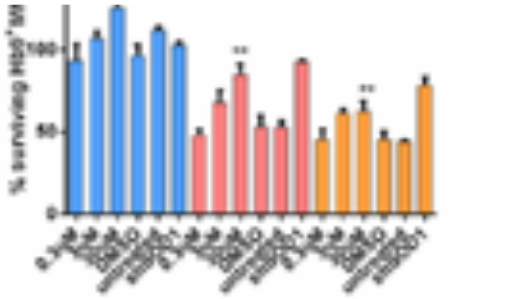


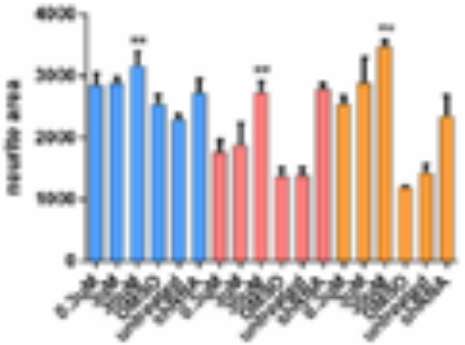
Developing High Content Screens

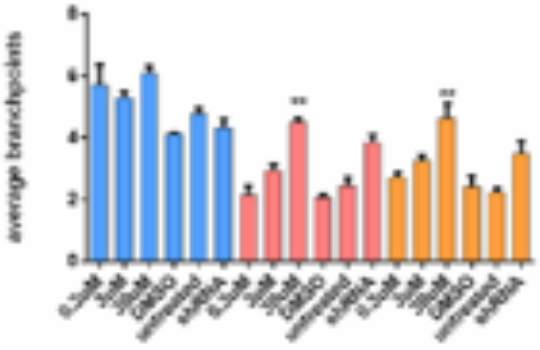
Effectively Established
384 format screening for HCA

Highly Reliable, Reproducible

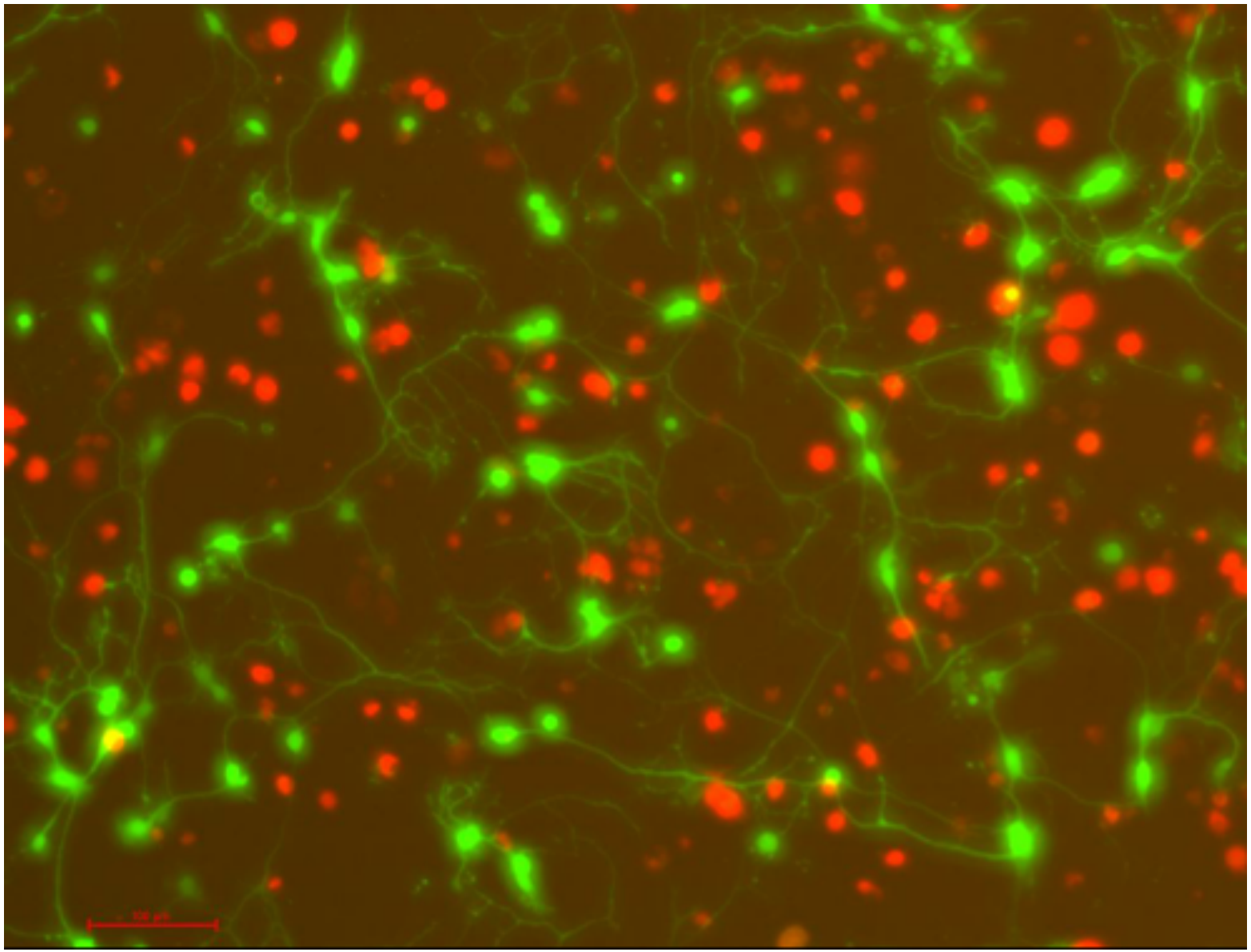
Already screening Compounds



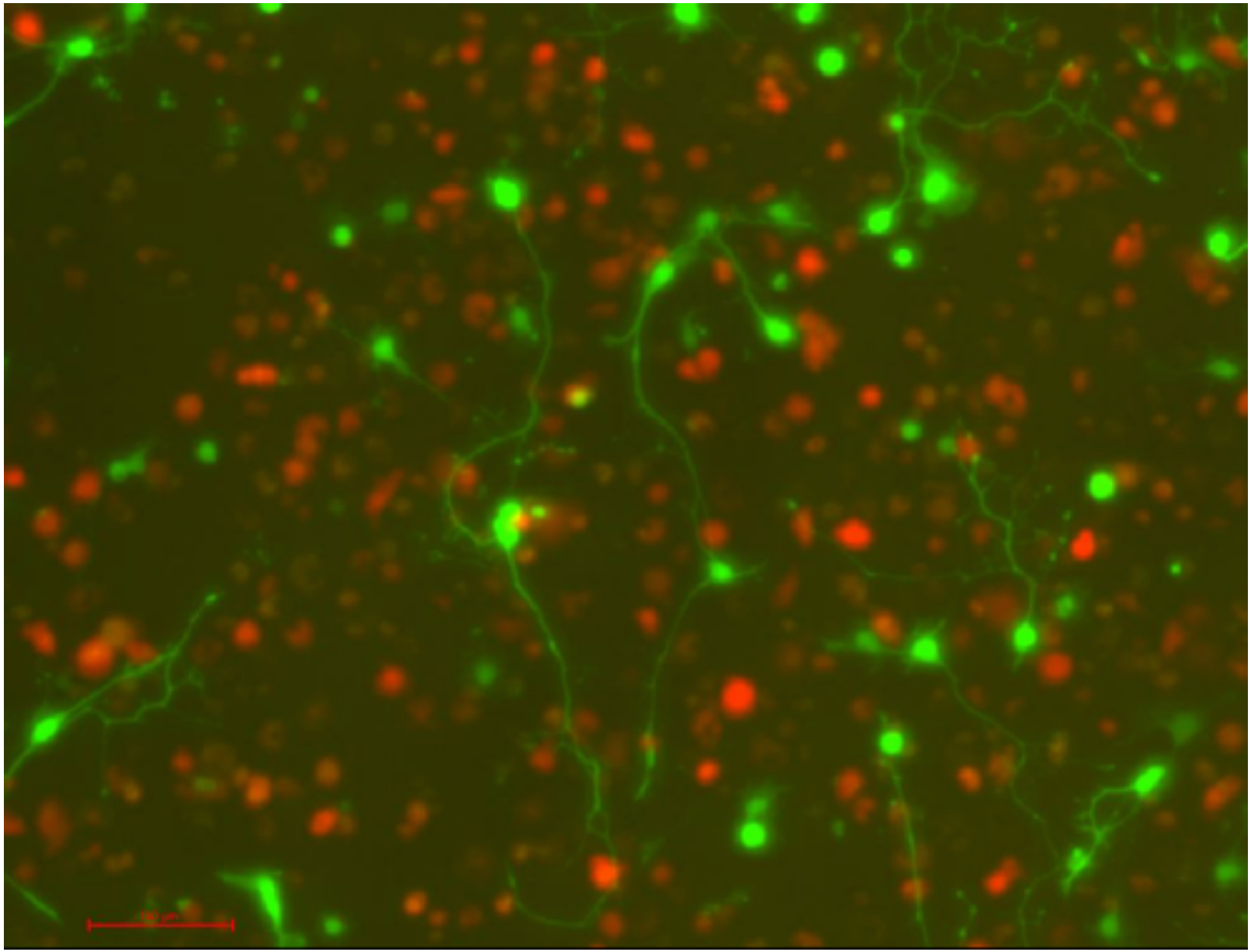




Normal WT Microglia co-culture with Motor Neurons



ALS Microglia Induce Motor Neuron Death



Role for NF-kB in ALS Microglia

Neuron
Article

Microglia Induce Motor Neuron Death via the Classical NF-κB Pathway in Amyotrophic Lateral Sclerosis

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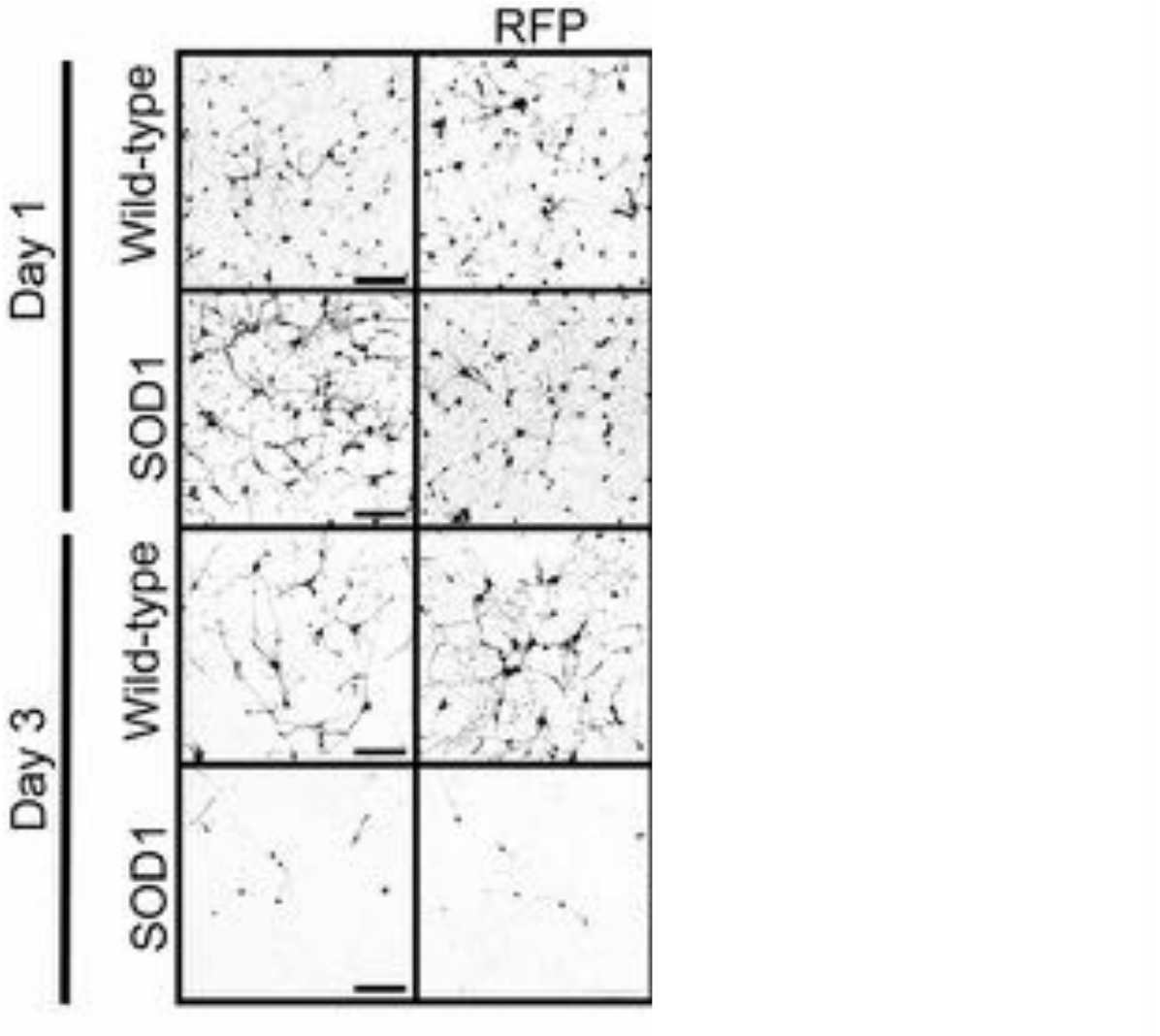
http://dx.doi.org/10.1016/j.neuron.2014.01.013

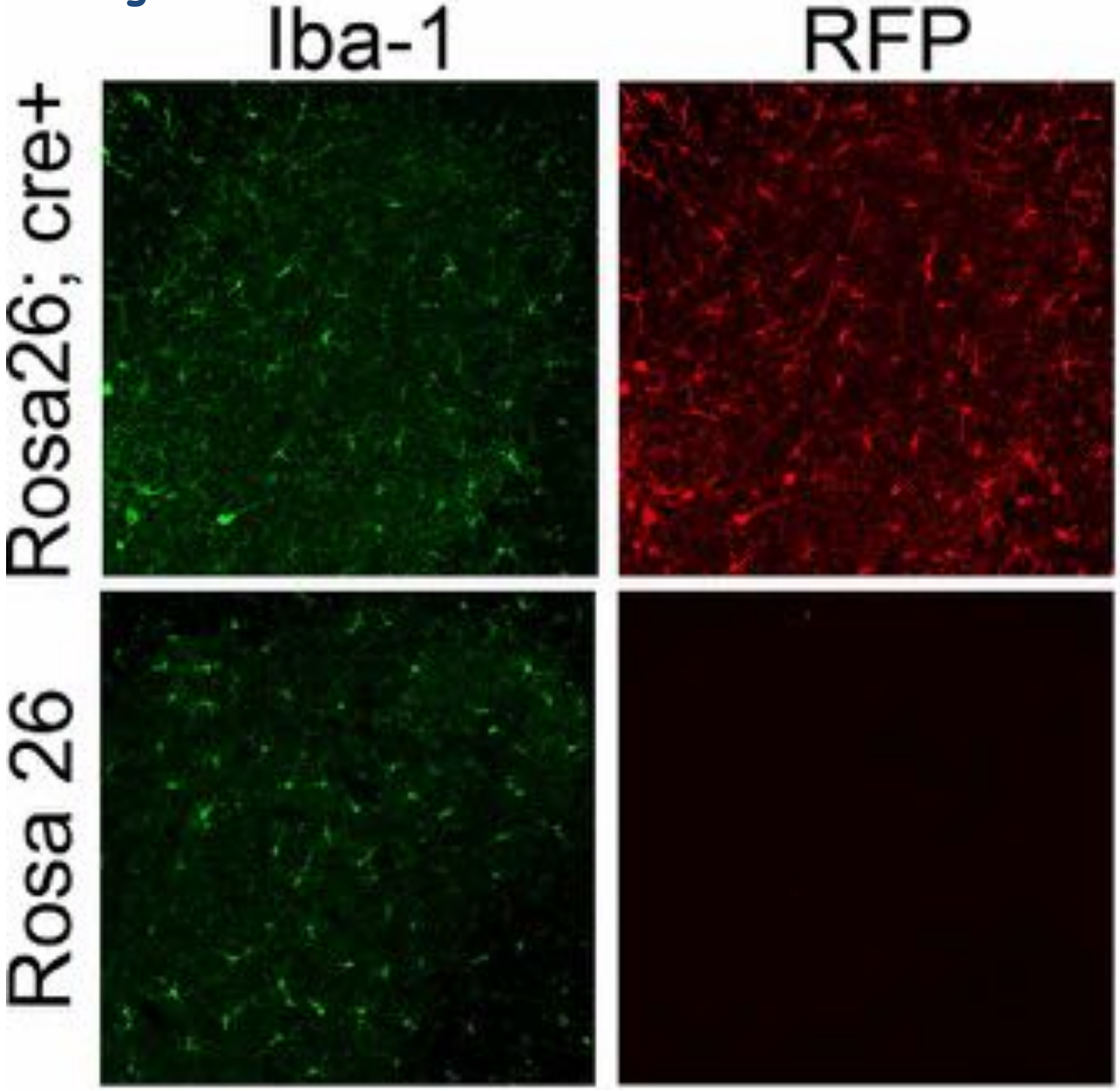
Microglia from G93A SOD1 mice kill Motor Neurons

And killing is suppressed by NfKB inhibition

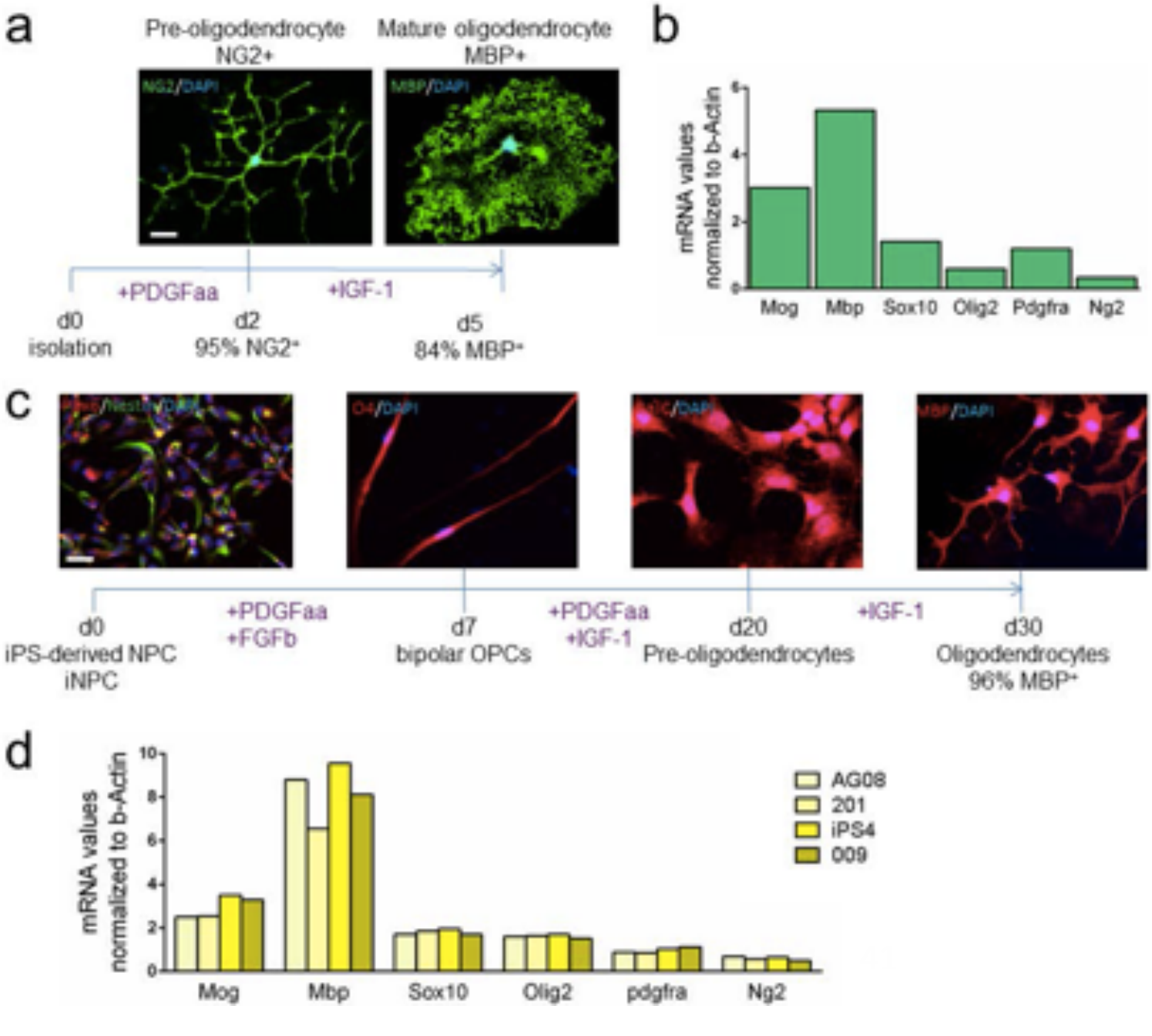


Depleting NfKB signaling in microglia extends survival in G93A SOD1 mice

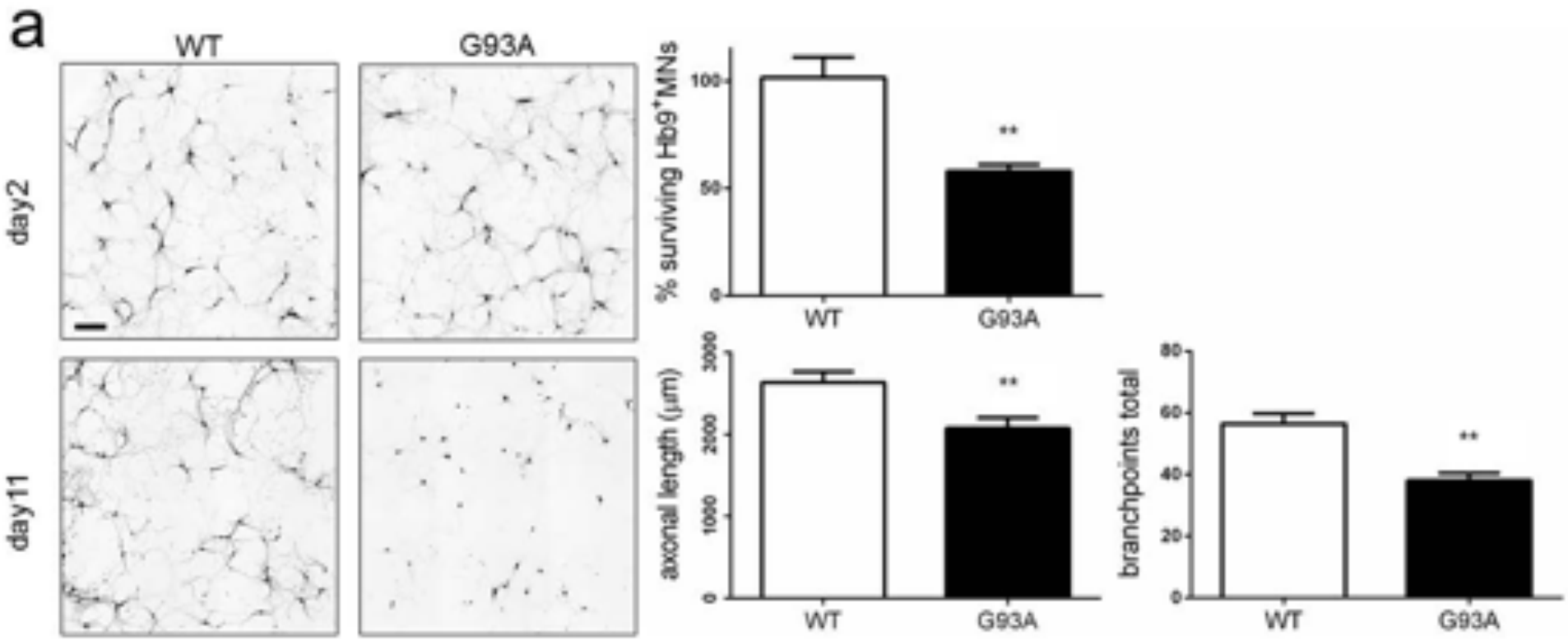
Using the CSF1R-CRE mouse x IkkB floxed x G93A SOD1



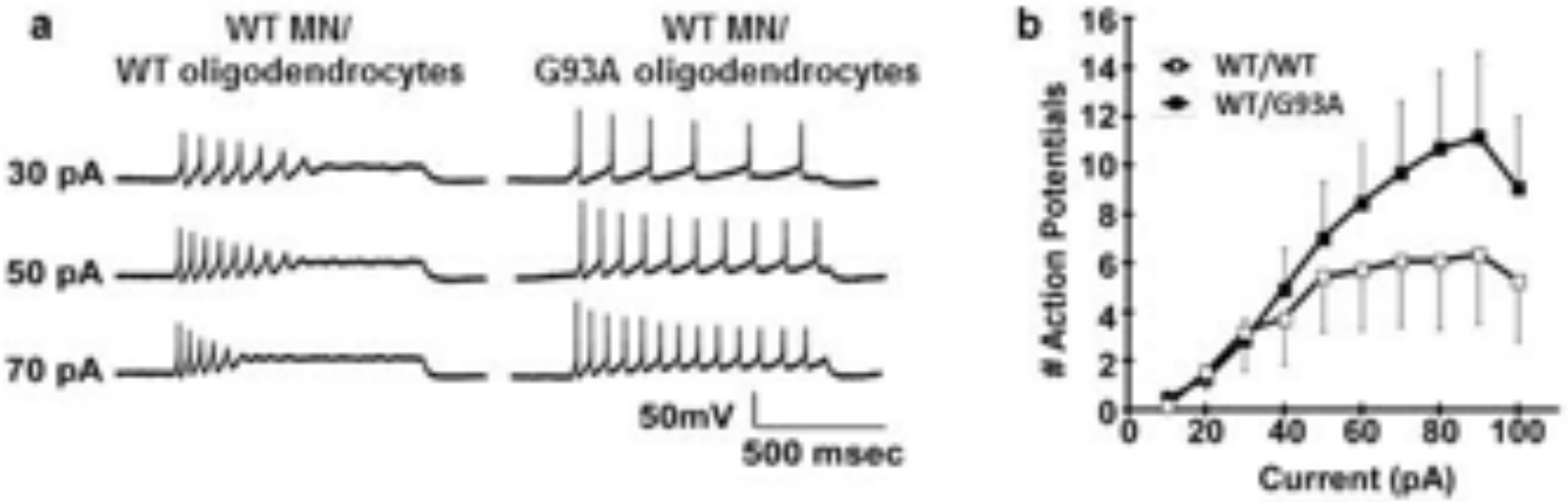
Developing Oligodendrocyte Cultures for Studying ALS



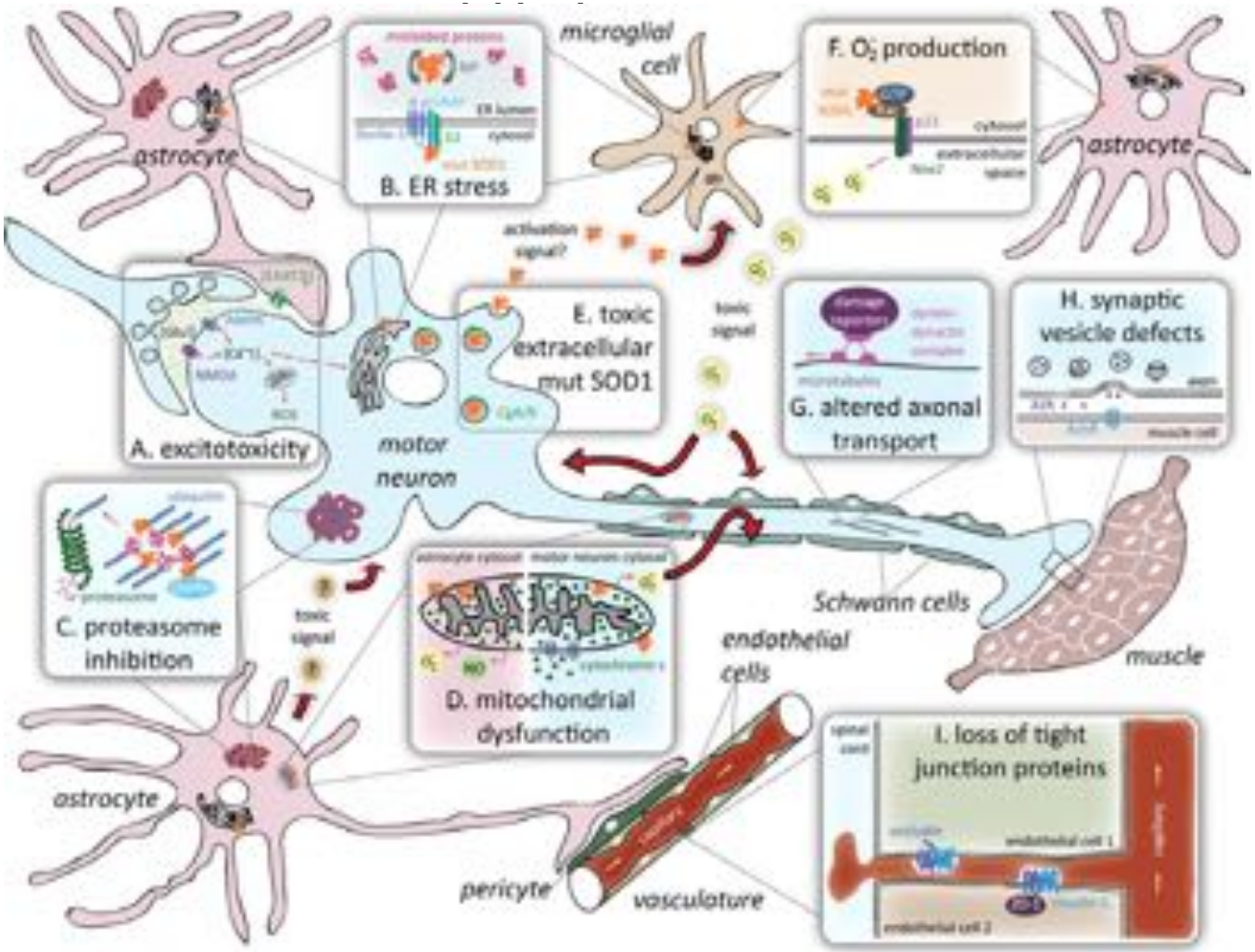
Oligodendrocytes from G93A SOD1 mice kill Motor Neurons



ALS Oligodendrocytes induce Hyperexcitability in Motor Neurons



Proposed mechanisms of toxicity in SOD1-mediated ALS. (A) Excitotoxicity is the hyperactivation of motor neurons resulting from failure to rapidly remove neurotransmitter glutamate from synapses due to deficiency in the glutamate transporter EAAT2 in the



Ilieva H et al. J Cell Biol 2009;187:761-772

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