Successful replacement of animal models:

Stem cell based models systems

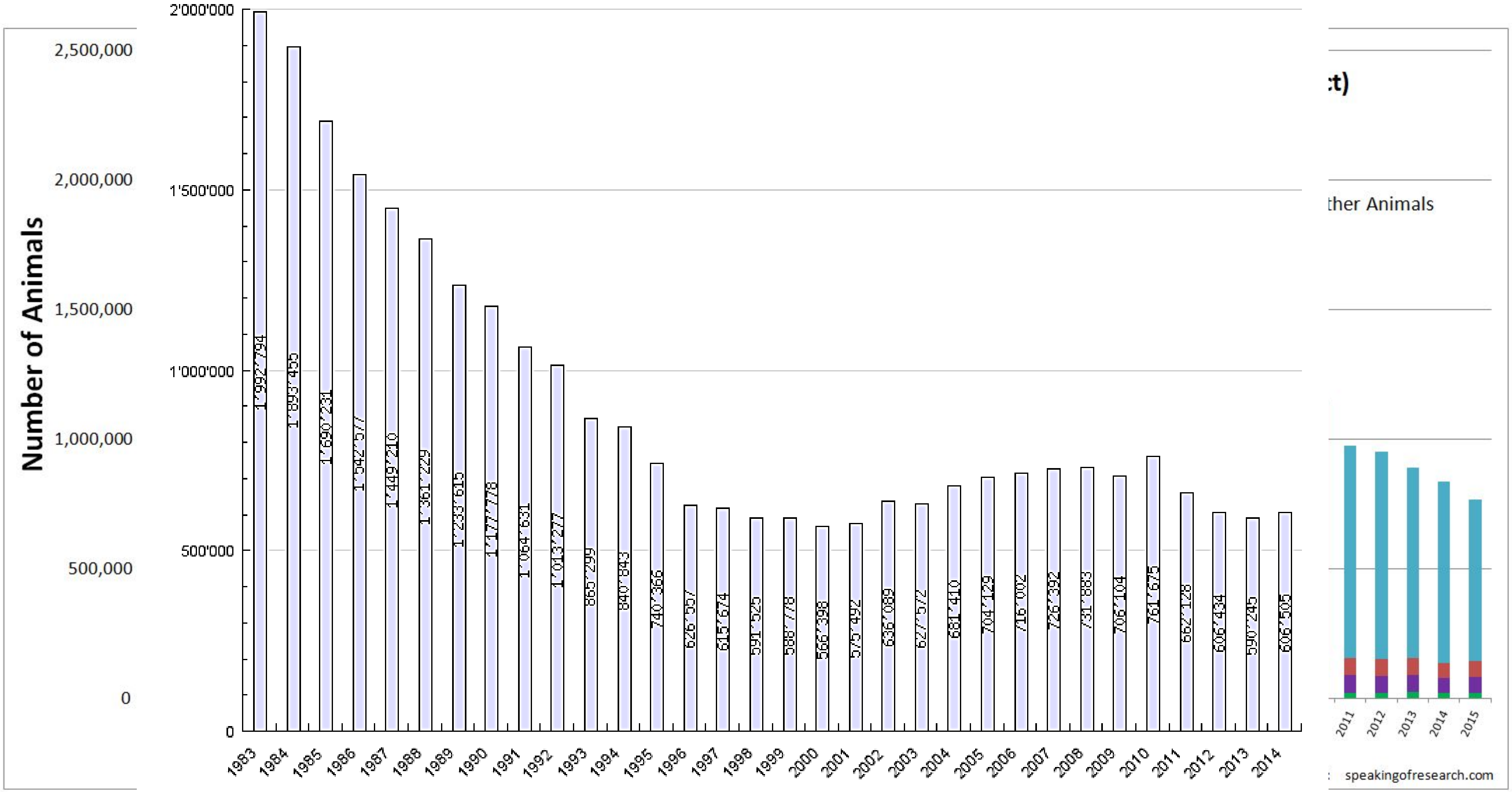
Study, Screen, Substitute

Journal club
5.7.2016

Vijay Chandrasekar
Aguzzi lab

Covered Animal use in US 1973-2015

Animal use in Schweiz 1983-2014



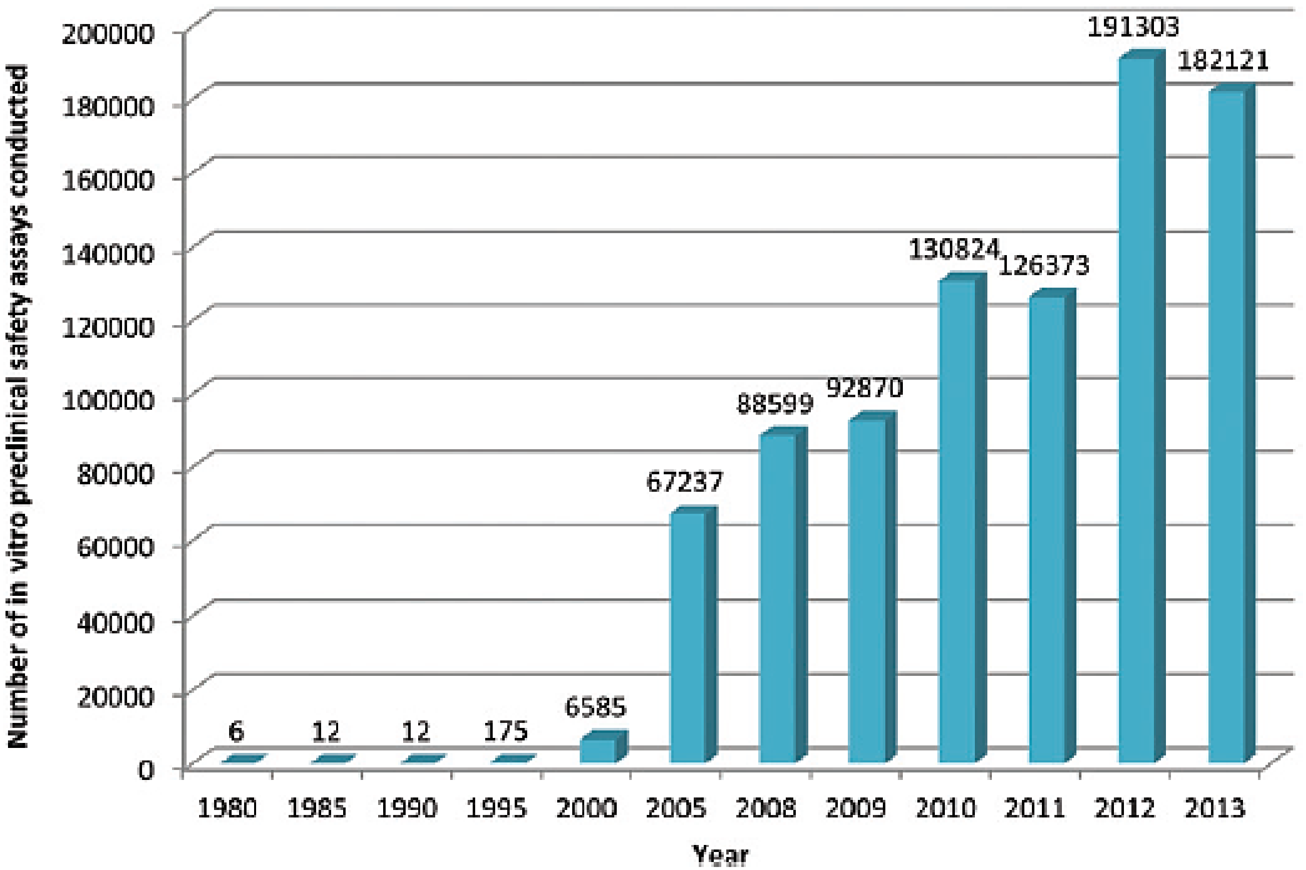
Tierversuche 2014 in der Schweiz

(Swiss National Statistics on animal research)
http://tv-statistik.ch/de/statistik/index.php

data from US AWA(1966) Reports, 1973-2015

3Rs :Reduction, Replacement and Refinement

Alternative assays simply can't model complex biological systems yet



UK's pharmaceutical industry umbrella body, the ABPI

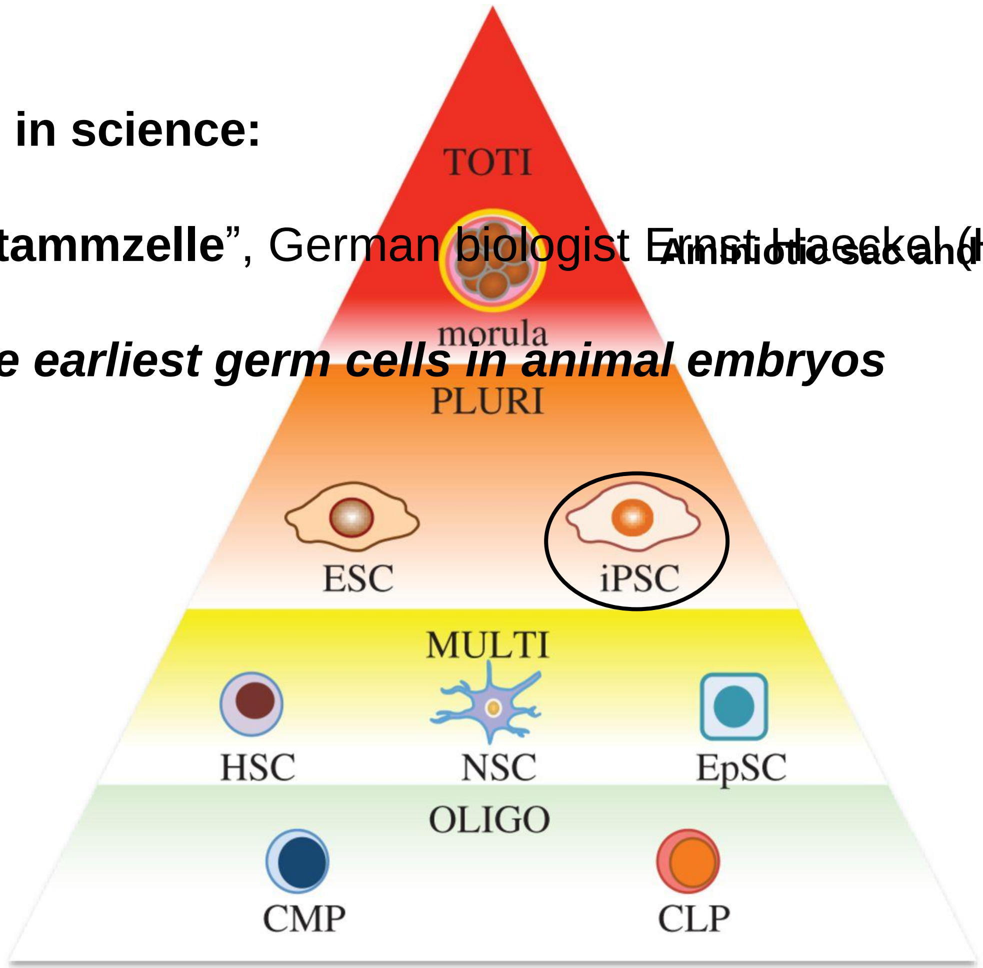
Types of Stem cells

Stem cells in science:

Origin : “**Stammzelle**”, German biologist Ernst Haeckel (Haeckel, 1868)

Amniotic sac and placenta

identify the earliest germ cells in animal embryos

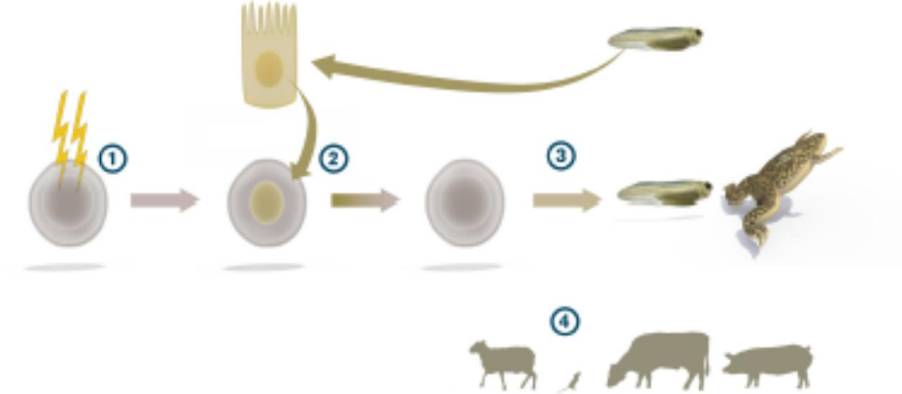


1981: First *in vitro* mouse stem cell line

Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells.

Martin, 1981 (PNAS)

John B. Gurdon (1962)

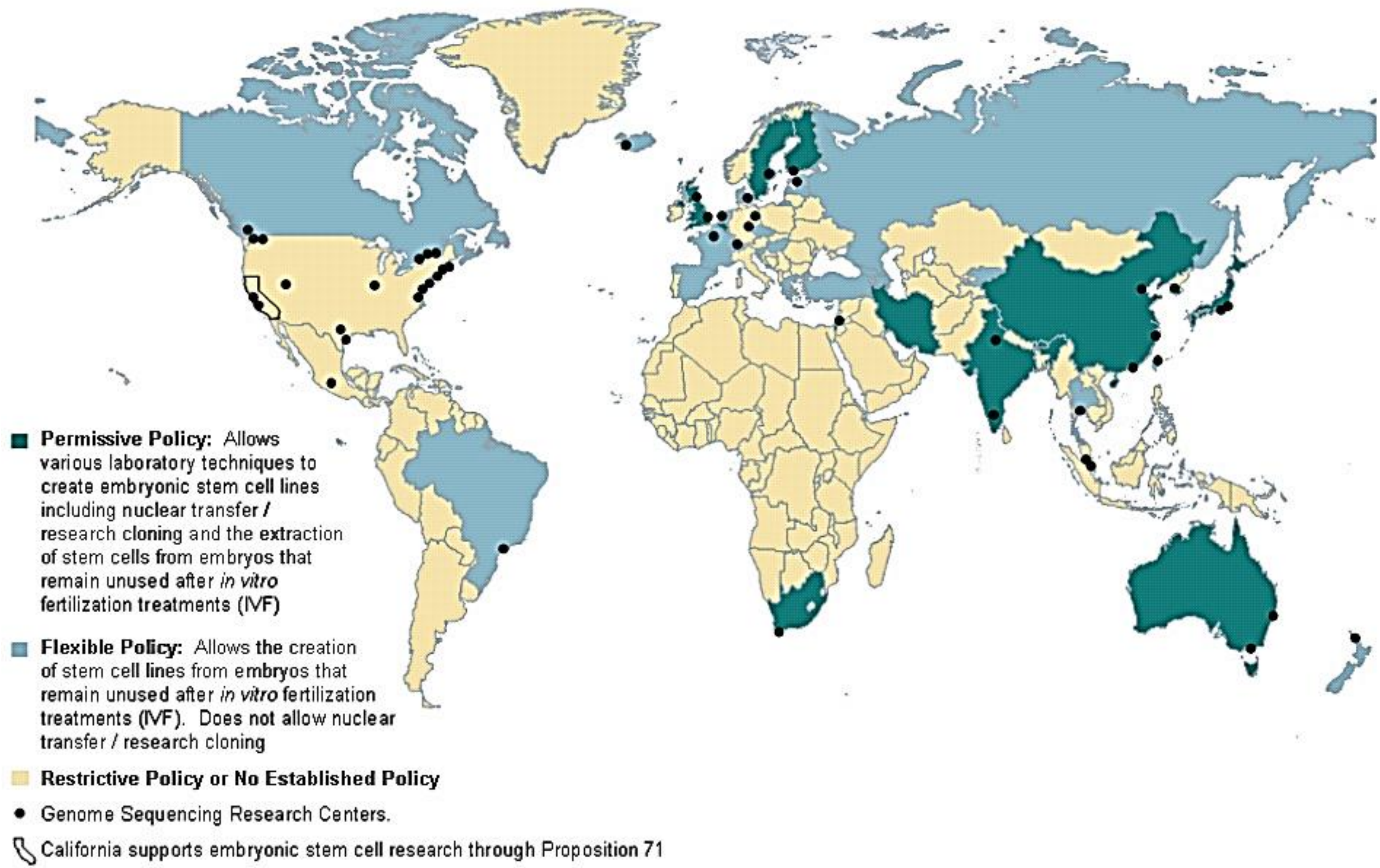


Dolly(1996) had developed prematurely arthritis & lung disease

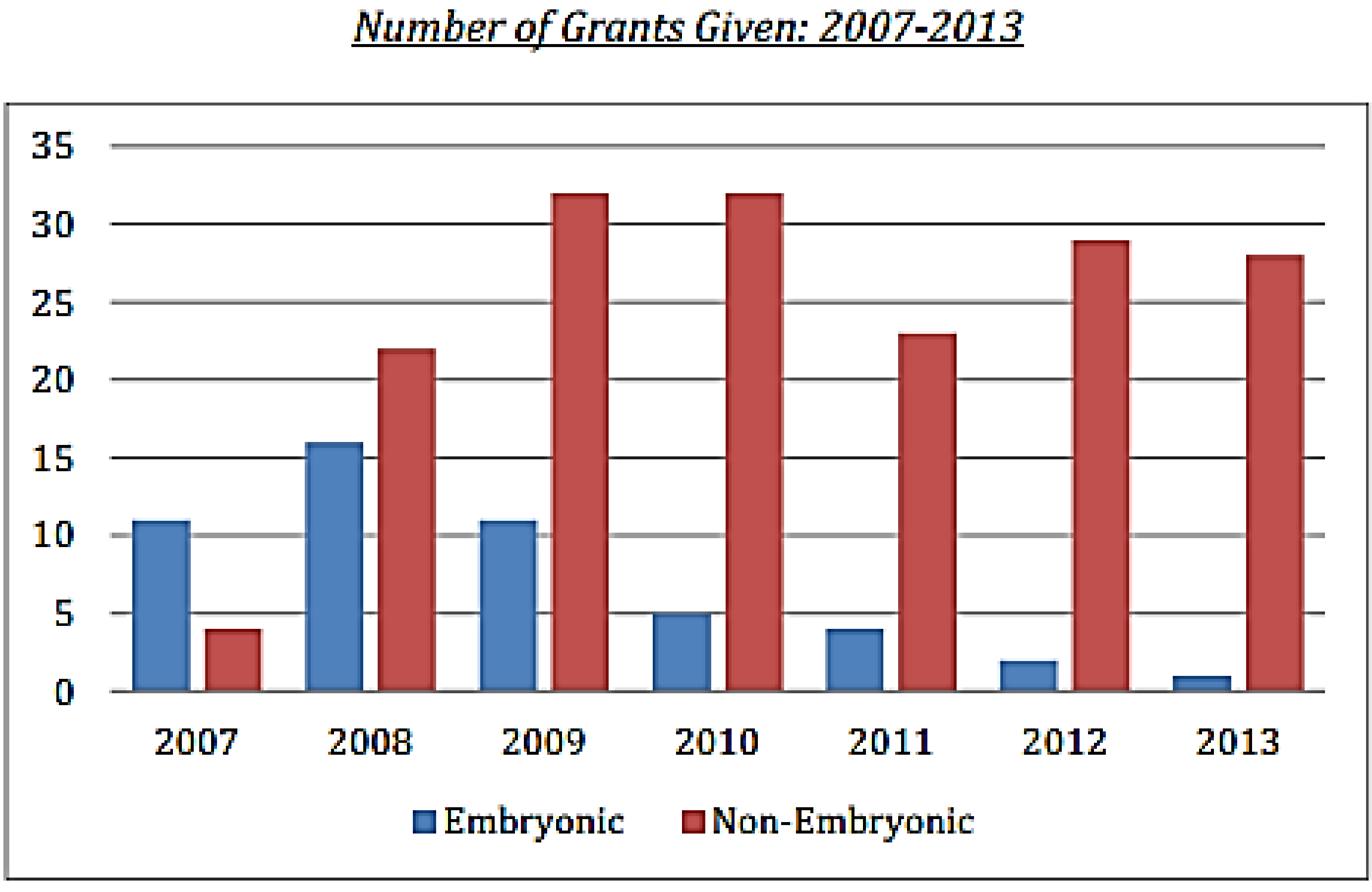
Cloned mammals, including Dolly, have shorter telomeres than other animals of the same age

The 2012 Nobel Prize in Physiology or Medicine - Press Release". Nobelprize.org. Nobel Media

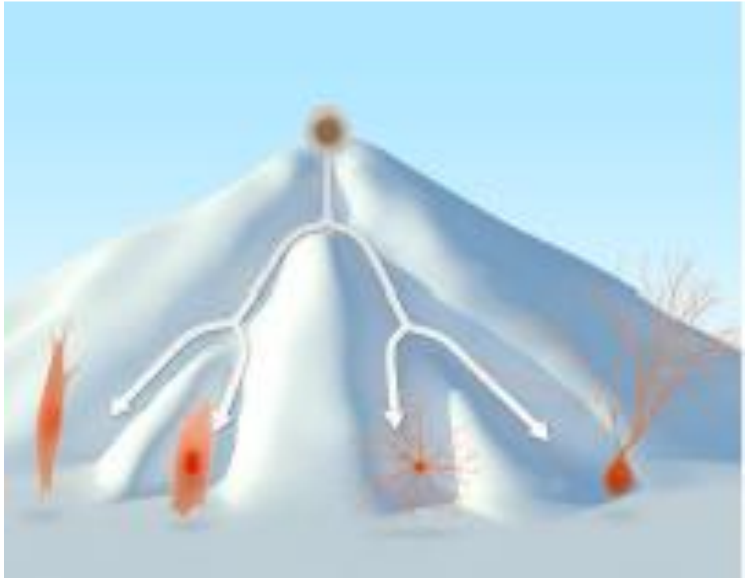
World Stem Cell Policy



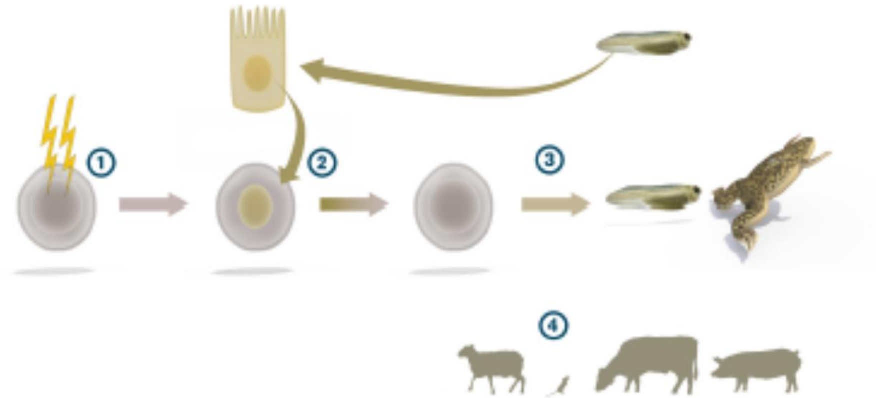
Source: William Hoffman, MBBNet, University of Minnesota. For more detail, see: **http://www.mbbnet.umn.edu/scmap.html**
Published by the Monitor Company Group, Cambridge, Massachusetts, USA

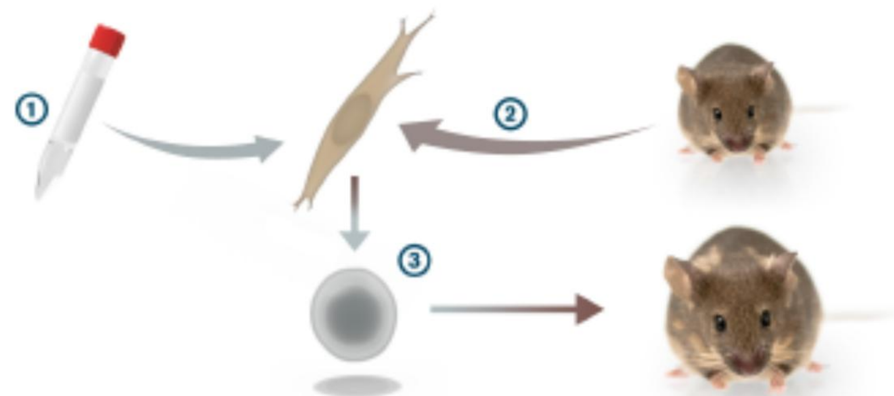


The Maryland Stem Cell Research Commission



John B. Gurdon (1962)





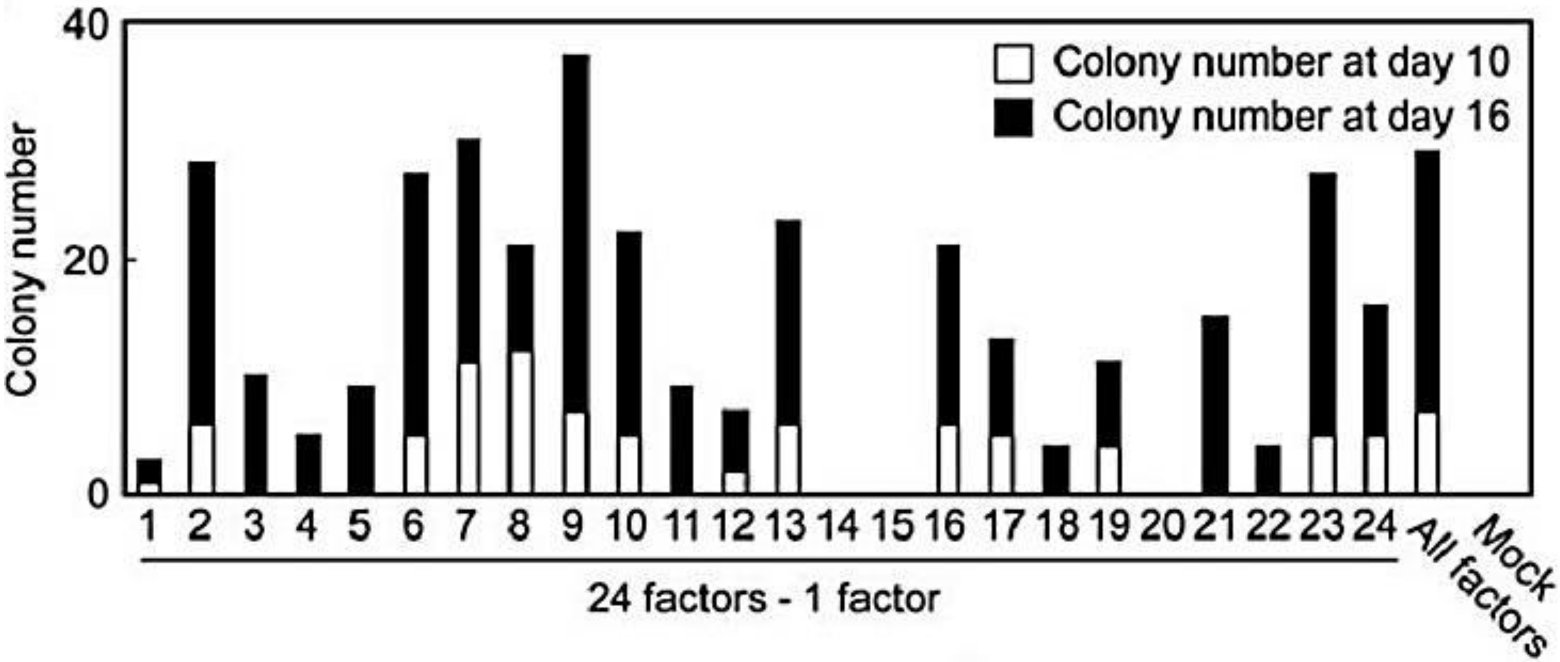
Shinya Yamanaka (2006)

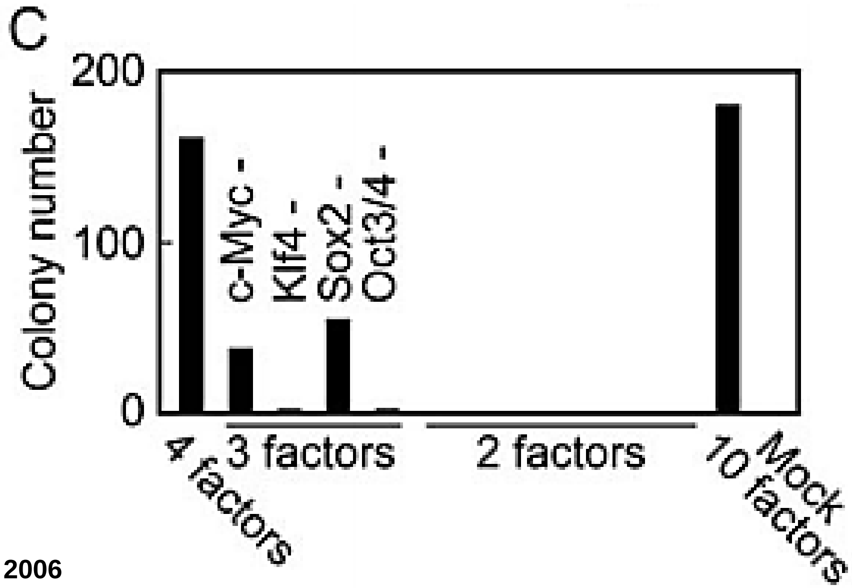
Cell

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024

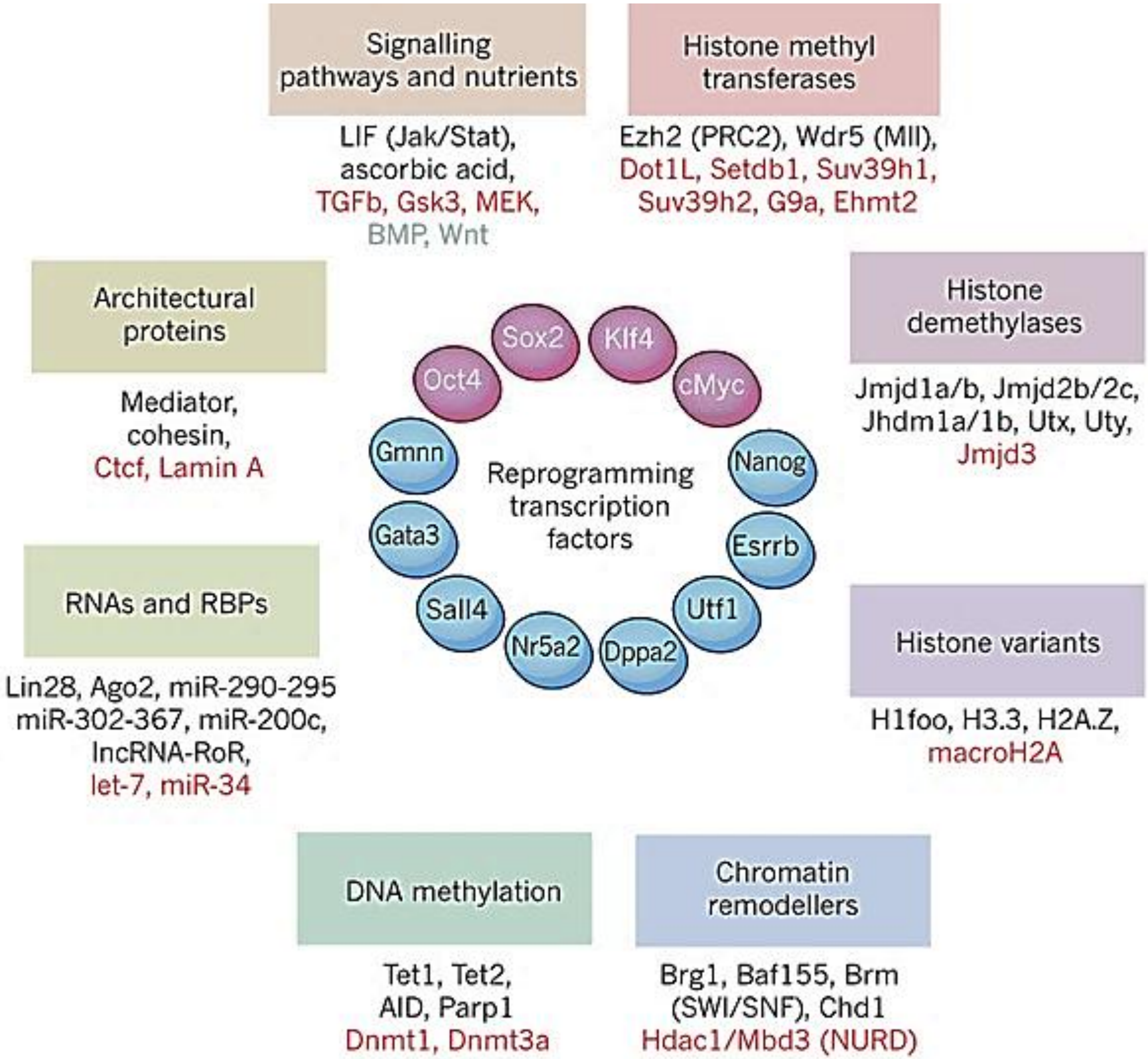
Retroviral transduction of fibroblasts to generate iPSC colonies





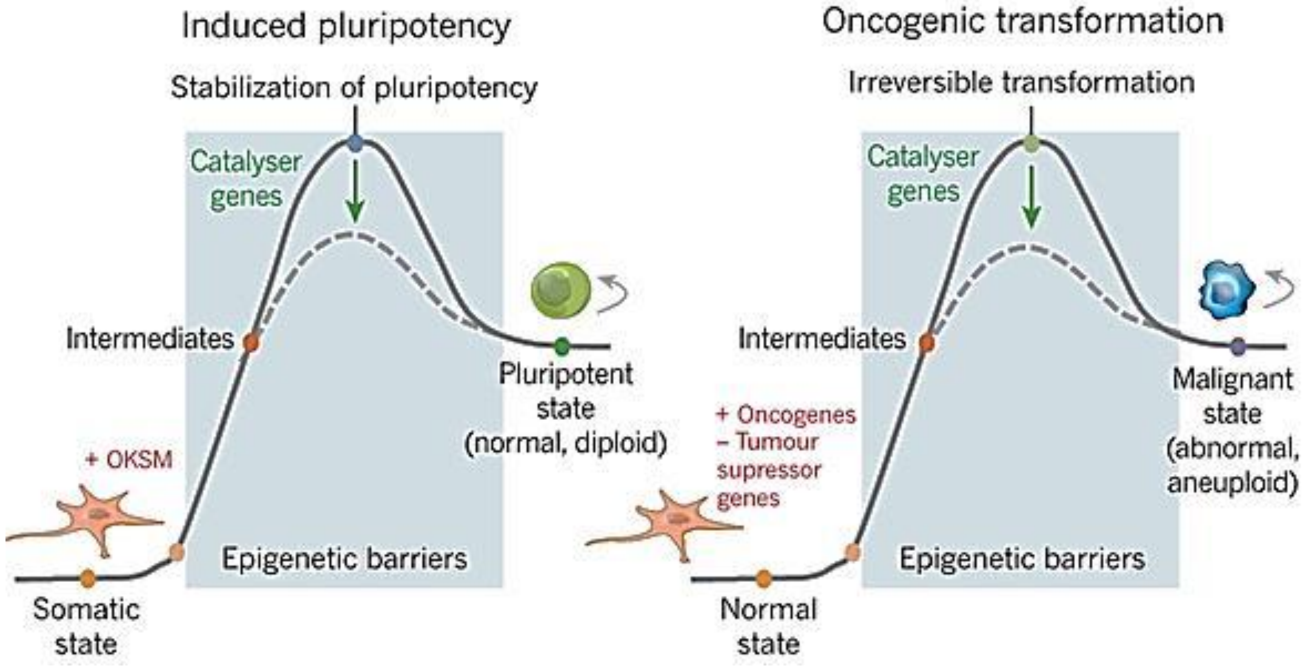
Kazutoshi Takahashi & Shinya Yamanaka, 2006

Gene involved in reprogramming and stemness



Apostolou & Konrad Hochedlinger, 2013

Stem cell Vs Cancer cell

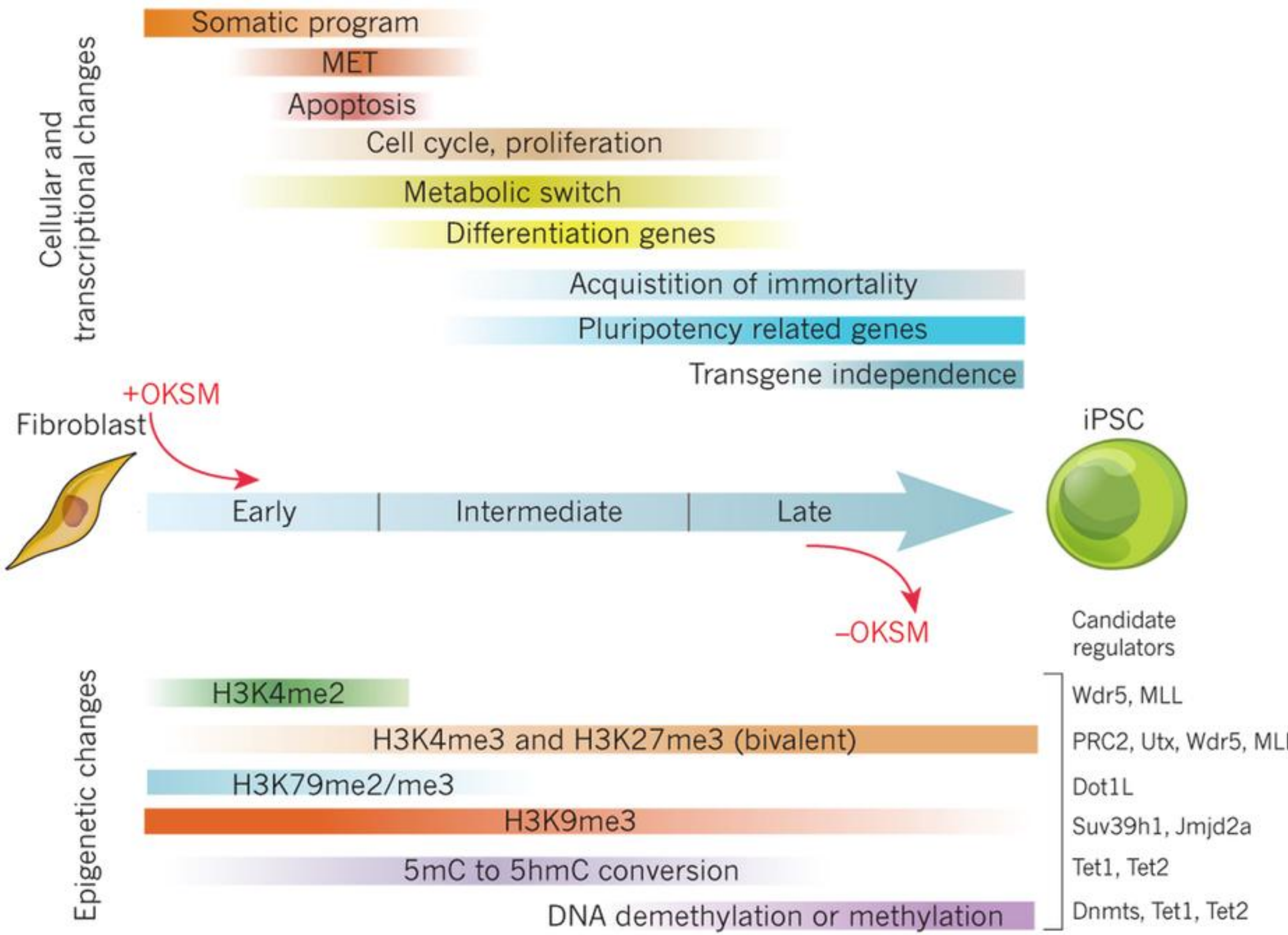


Some epigenetic regulators and histone modifications have been shown to have opposite roles during reprogramming and malignancy.

Loss of Tet2 causes myeloid transformation in mice (Moran-Crusio, K. et al. 2011), consistent with a tumour suppressor function, whereas depletion of Tet2 protein abrogates reprogramming (Doege, C. A. et al., 2012).

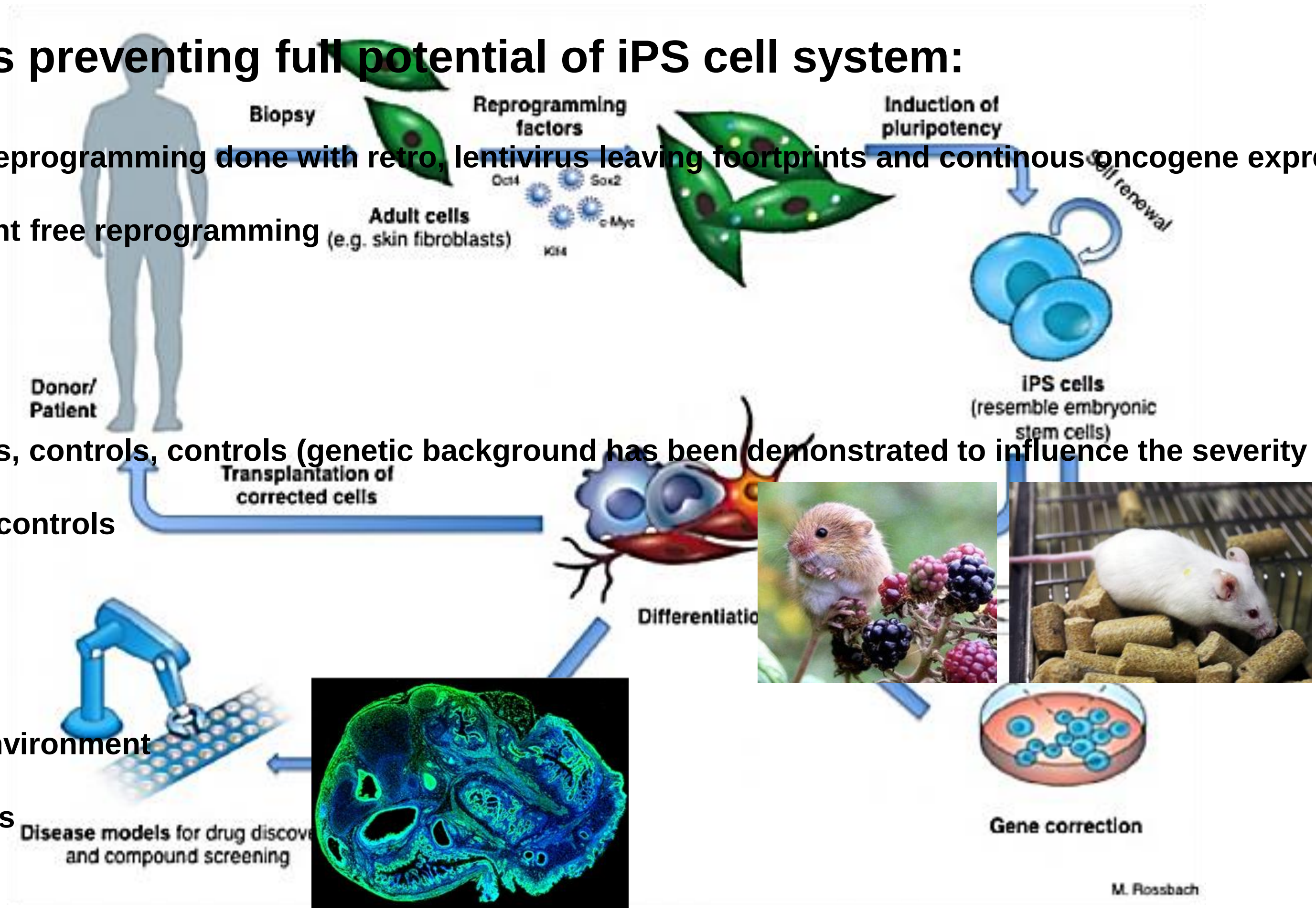
H3K79 methyltransferase Dot1L promotes leukaemia formation induced by MLL–AF9 translocations, although it prevents iPSC formation

Chromatin dynamics during cellular reprogramming



Apostolou & Konrad Hochedlinger, 2013

Potential of iPS cells technology.



Pit falls preventing full potential of iPS cell system:

1. Early reprogramming done with retro, lentivirus leaving footprints and continous oncogene expression

- Footprint free reprogramming

2. Controls, controls, controls (genetic background has been demonstrated to influence the severity of disease)

-Isogenic controls

3. Microenvironment

-Organoids

Eli et al., Cerebral organoid derived from ALS patient stem cells.
Image from USC Stem Cell.

4. Well refined cell lineage derivation procedures and acceleration of physiological ageing

- Small molecules and stress

http://www.eurostemcell.org/factsheet/ips-cells-and-reprogramming-turn-any-cell-body-stem-cell

Study:

Disease modelling

Cell Stem Cell
Article

Global Transcriptional and Translational Repression in Human-Embryonic-Stem-Cell-Derived Rett Syndrome Neurons

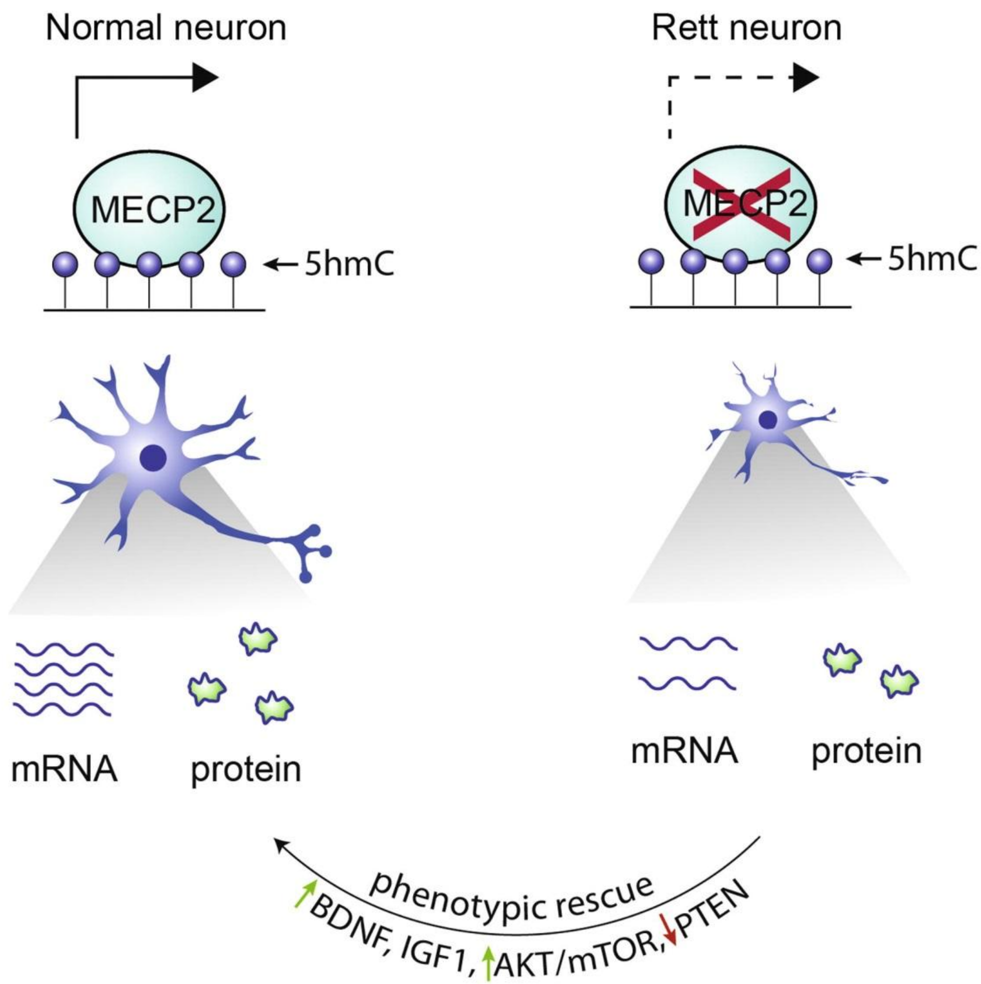
Yun Li,[1] Haoyi Wang,[1] Julien Muffat,[1] Albert W. Cheng,[1,2] David A. Orlando,[1] Jakob Lovén,[1] Show-ming Kwok,[3] Danielle A. Feldman,[3] Helen S. Bateup,[4] Qing Gao,[1] Dirk Hockemeyer,[1] Maisam Mitalipova,[1] Caroline A. Lewis,[5] Matthew G. Vander Heiden,[5] Mriganka Sur,[3] Richard A. Young,[1,6] and Rudolf Jaenisch[1,6,*]
[1]The Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA
[2]Department of Computational and Systems Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[3]The Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[4]Howard Hughes Medical Institute, Department of Neurobiology, Harvard Medical School, Boston, MA 02115, USA
[5]Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[6]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
*Correspondence: jaenisch@wi.mit.edu
http://dx.doi.org/10.1016/j.stem.2013.09.001

Rett syndrome (RTT)- monogenic X-linked neurodevelopmental disease

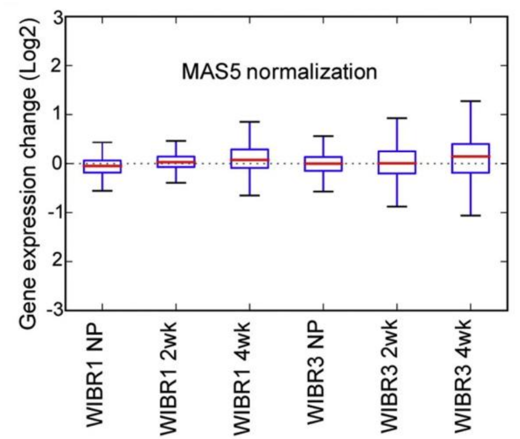
is a rare genetic postnatal neurological disorder of the grey matter of the brain that almost exclusively affects females

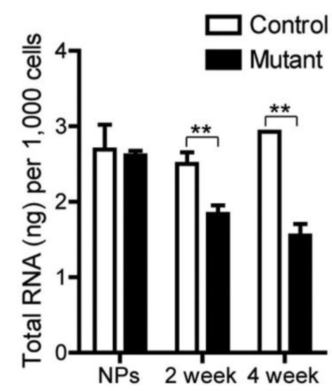
Huda Zoghbi (1999) Rett syndrome is caused by mutations in the gene MECP2

Figure summary

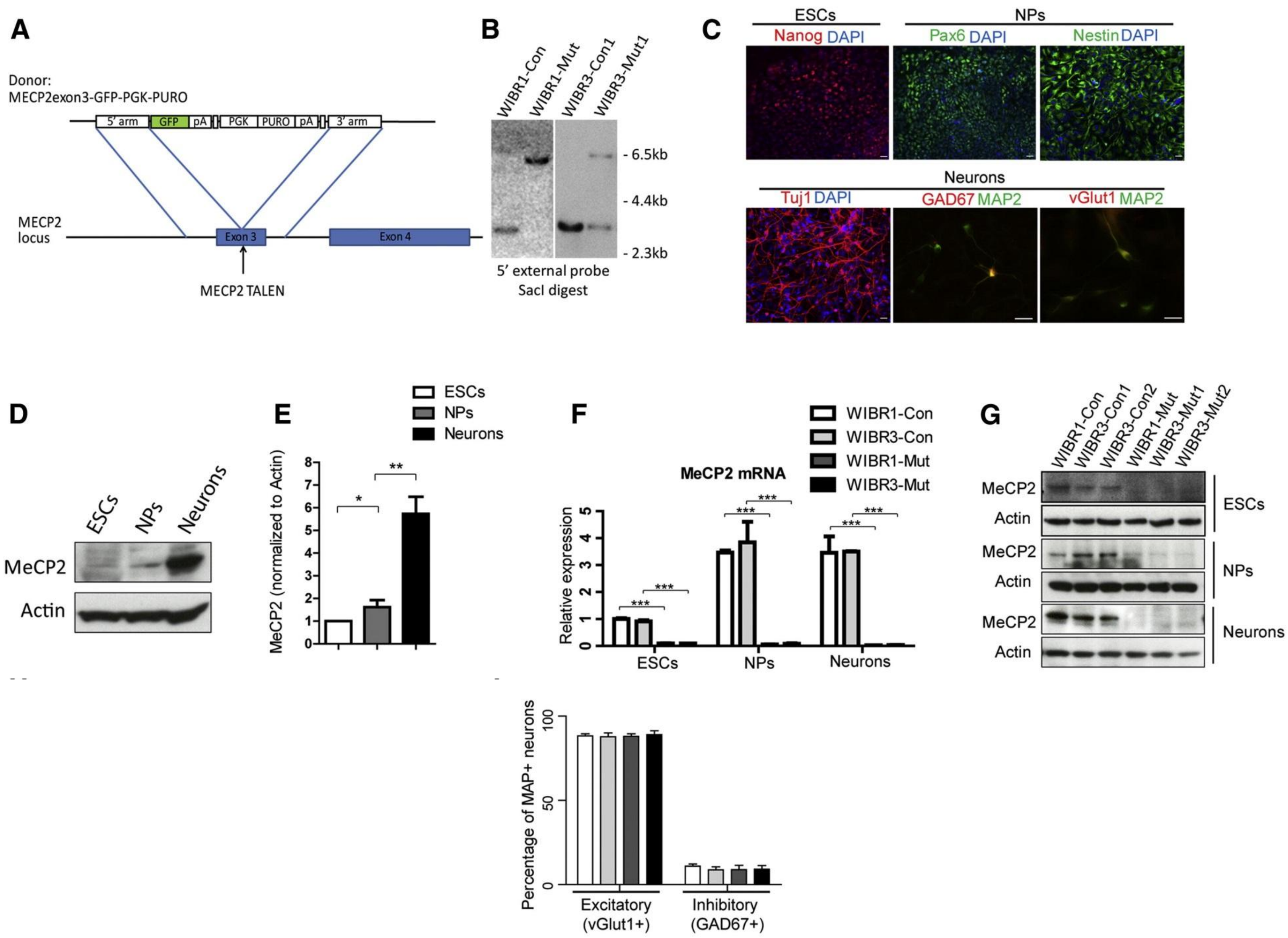


Conventional normalizing individual gene expression to total RNA (MAS5 for Affymetrix)

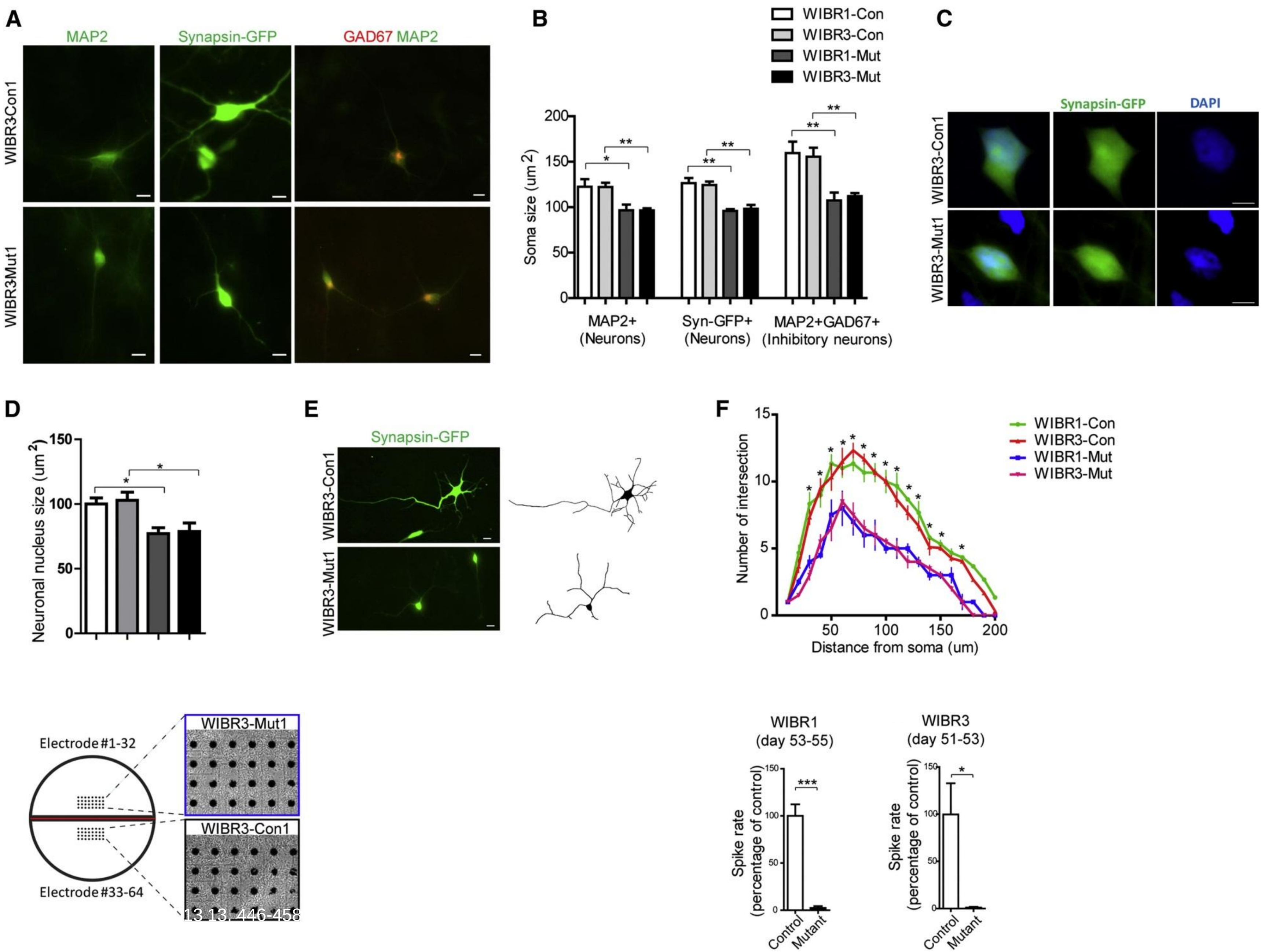




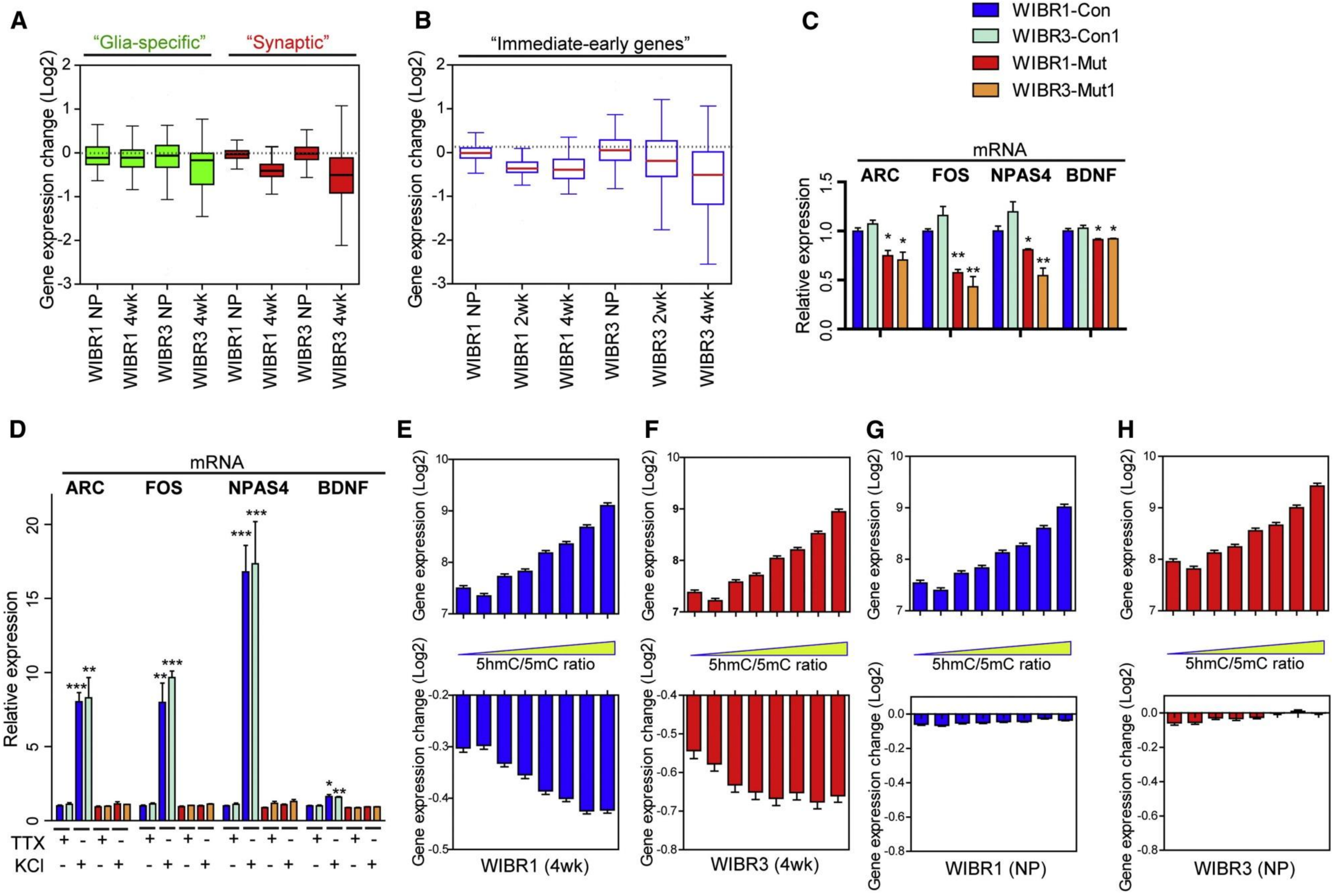
Generation of Isogenic Pairs of Control and MECP2 Mutant Human ESCs, NPs, and Neurons



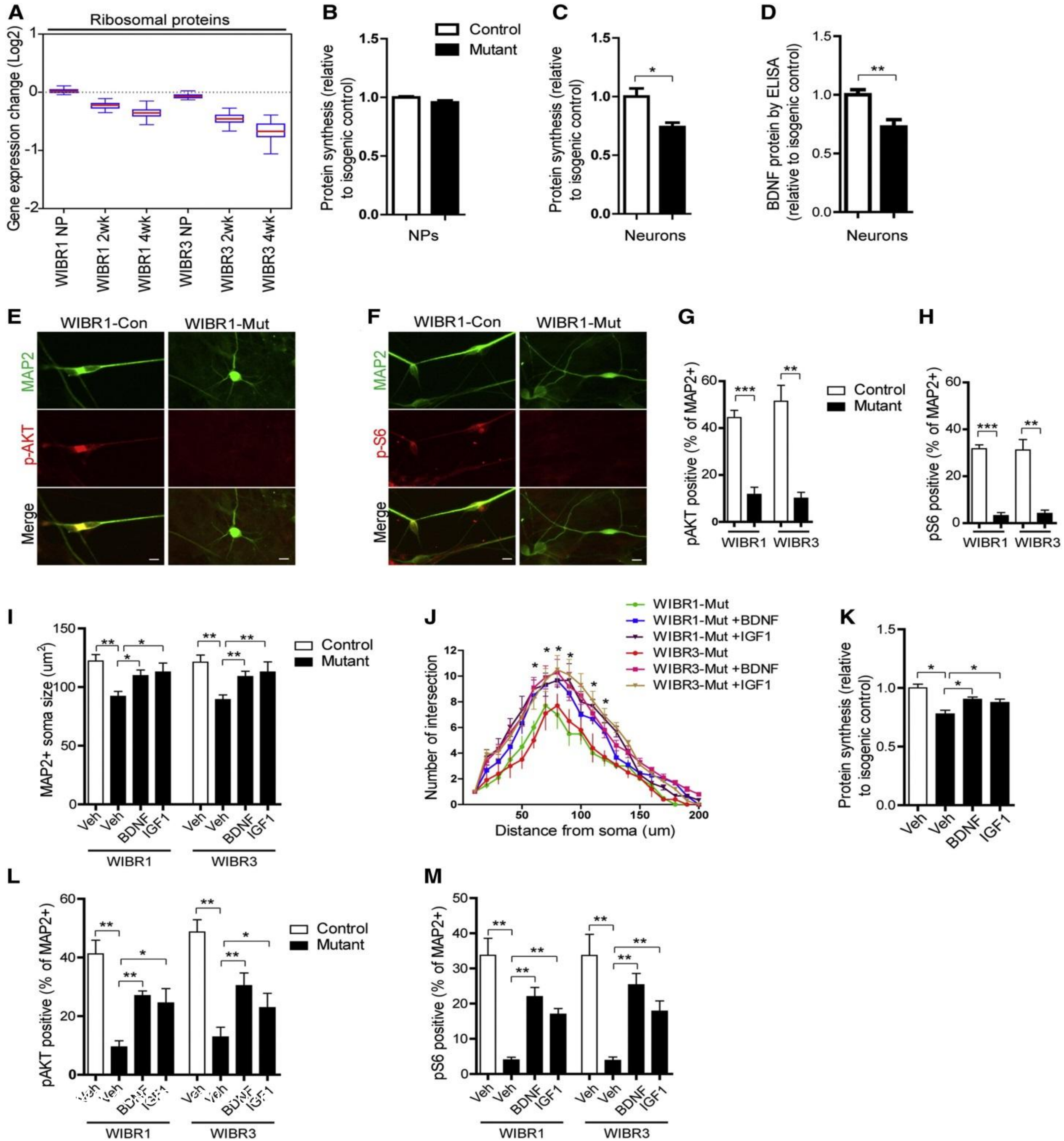
MECP2 Mutant Human Neurons Show Morphological and Electrophysiological Defects



Preferential Reduction of Active Transcription in MECP2 Mutant Neurons

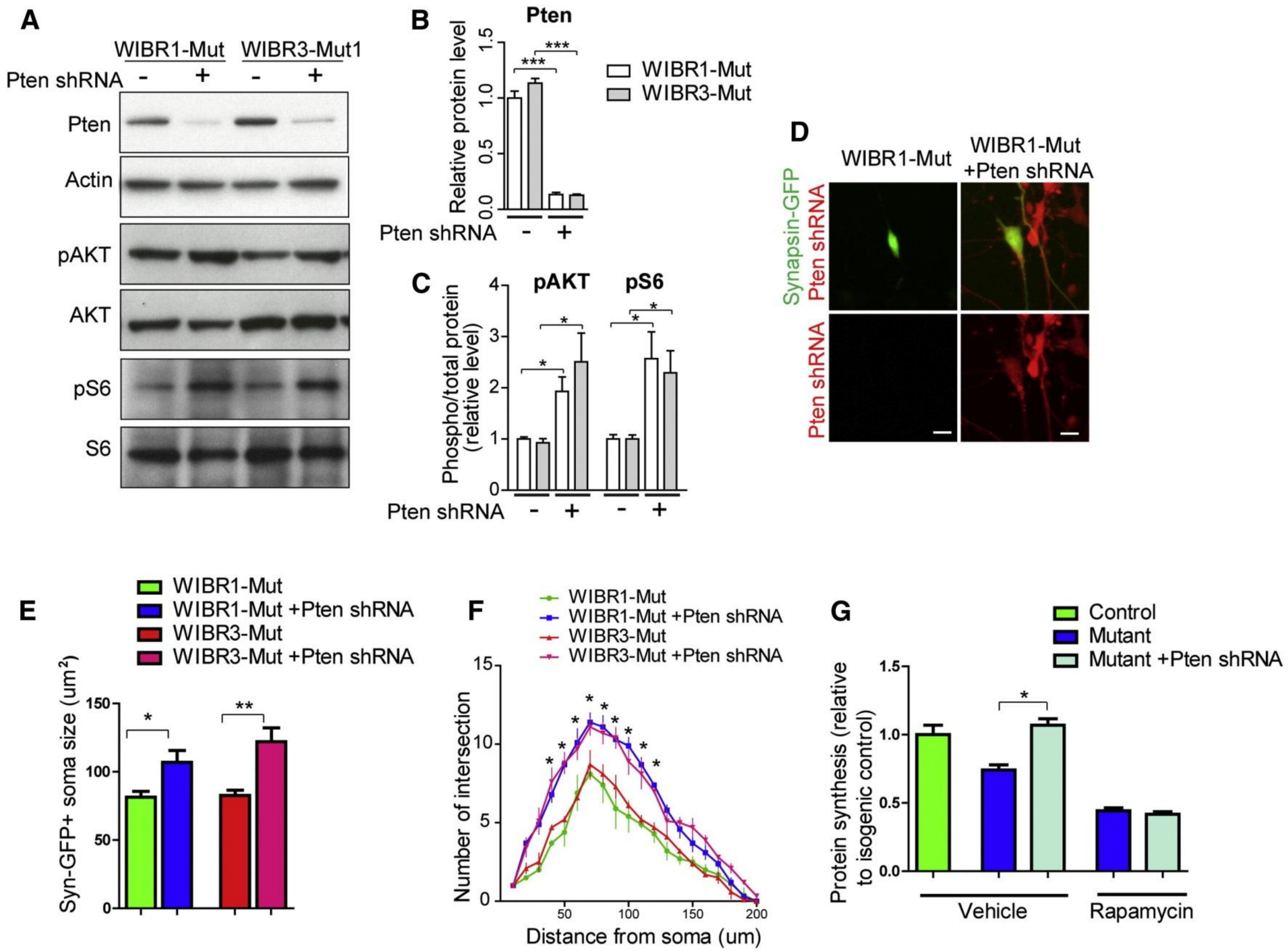


Reduced Protein Synthesis and AKT/mTOR Activity in MECP2 Mutant Neurons Were Rescued by BDNF and IGF1 Treatment

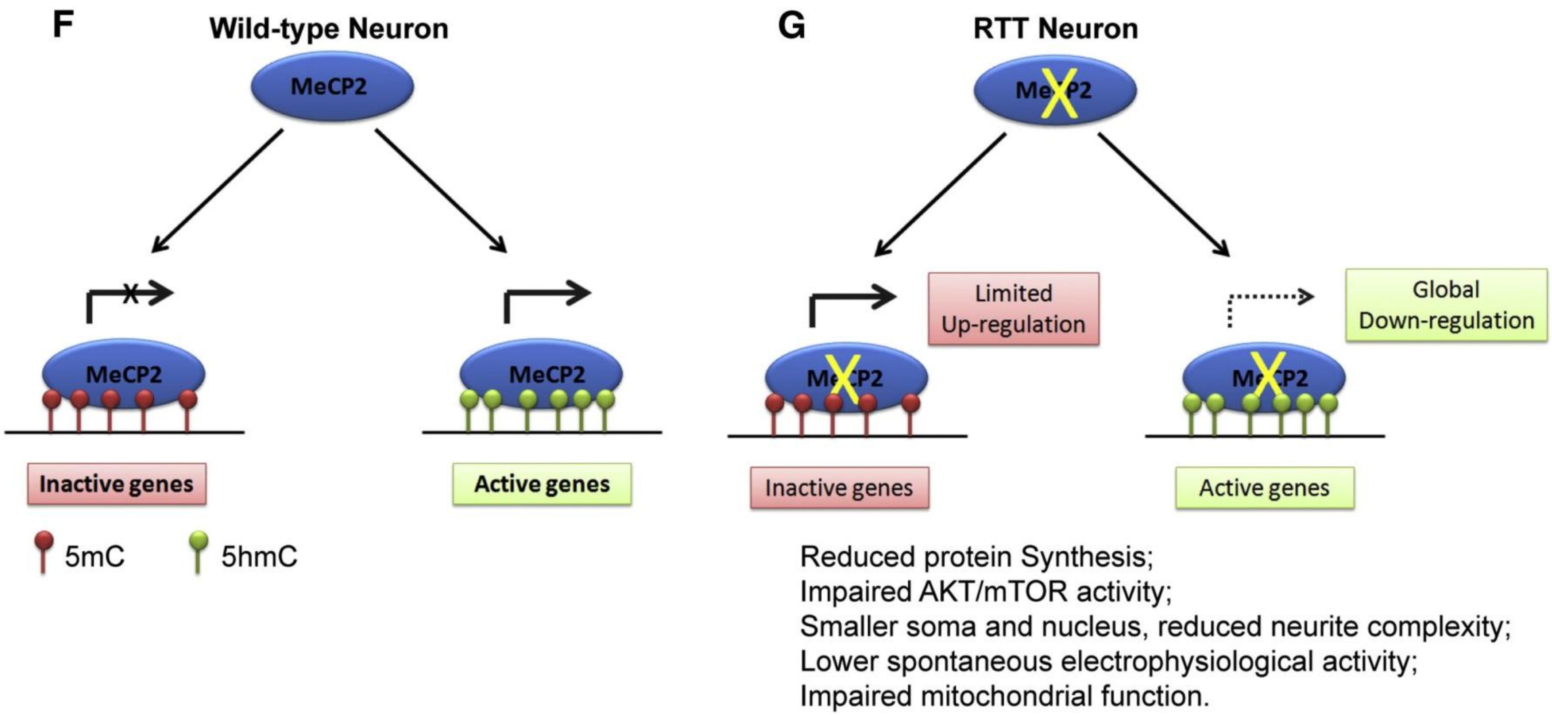


PTEN Knockdown Rescues Protein Synthesis and Morphological Defects in MECP2 Mutant Neurons

PI3K activation



A model of MECP2-regulated gene expression in human neurons



Cell

Volume 165, Issue 5, 19 May 2016, Pages 1238–1254

Resource

Brain-Region-Specific Organoids Using Mini-bioreactors for Modeling ZIKV Exposure

Xuyu Qian[1, 2, 18], Ha Nam Nguyen[1, 3, 4, 18], Mingxi M. Song[1, 9], Christopher Hadiono[1, 10], Sarah C. Ogden[11], Christy Hammack[11], Bing Yao[12], Gregory R. Hamersky[5], Fadi Jacob[1], Chun Zhong[1, 4], Ki-jun Yoon[1, 4], William Jeang[1, 14], Li Lin[12], Yujing Li[12], Jai Thakor[1], Daniel A. Berg[1], Ce Zhang[1, 4], Eunchai Kang[1, 4], Michael Chickering[1], David Nauen[1, 6], Cheng-Ying Ho[15, 16], Zhexing Wen[1, 4], Kimberly M. Christian[1, 4], Pei-Yong Shi[17], Brady J. Maher[5, 7], Hao Wu[13], Peng Jin[12], Hengli Tang[11], Hongjun Song[1, 3, 4, 8], ,

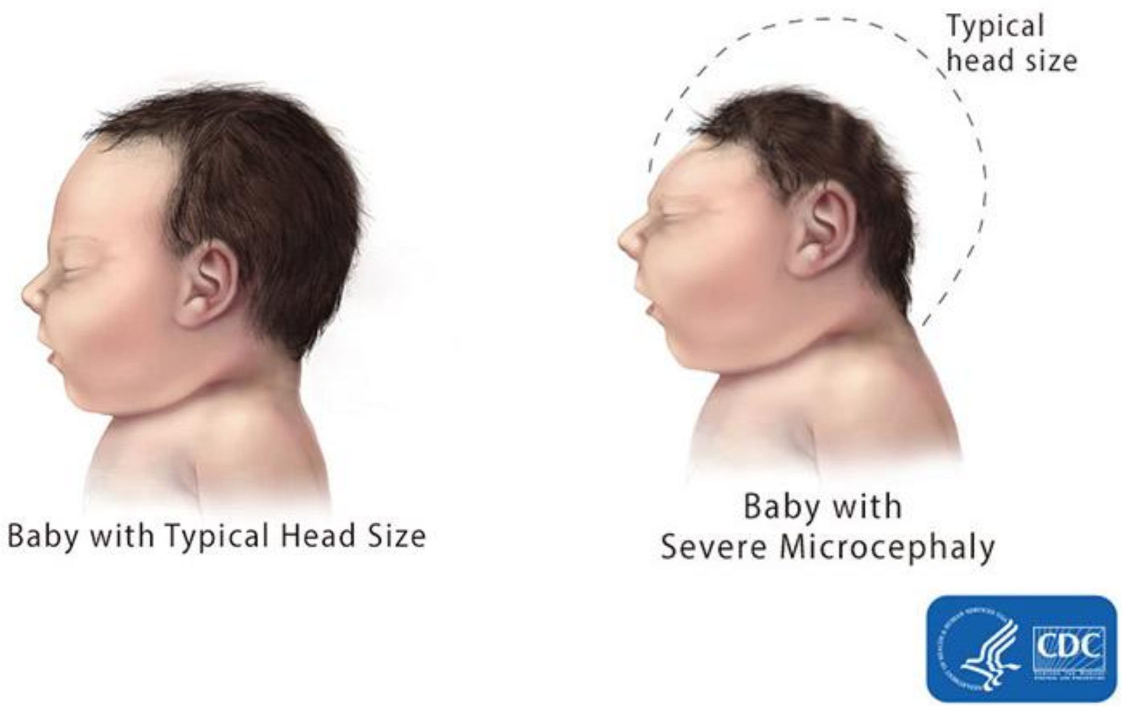
⊞ **Show more**

doi:10.1016/j.cell.2016.04.032

Zika Virus Disease:

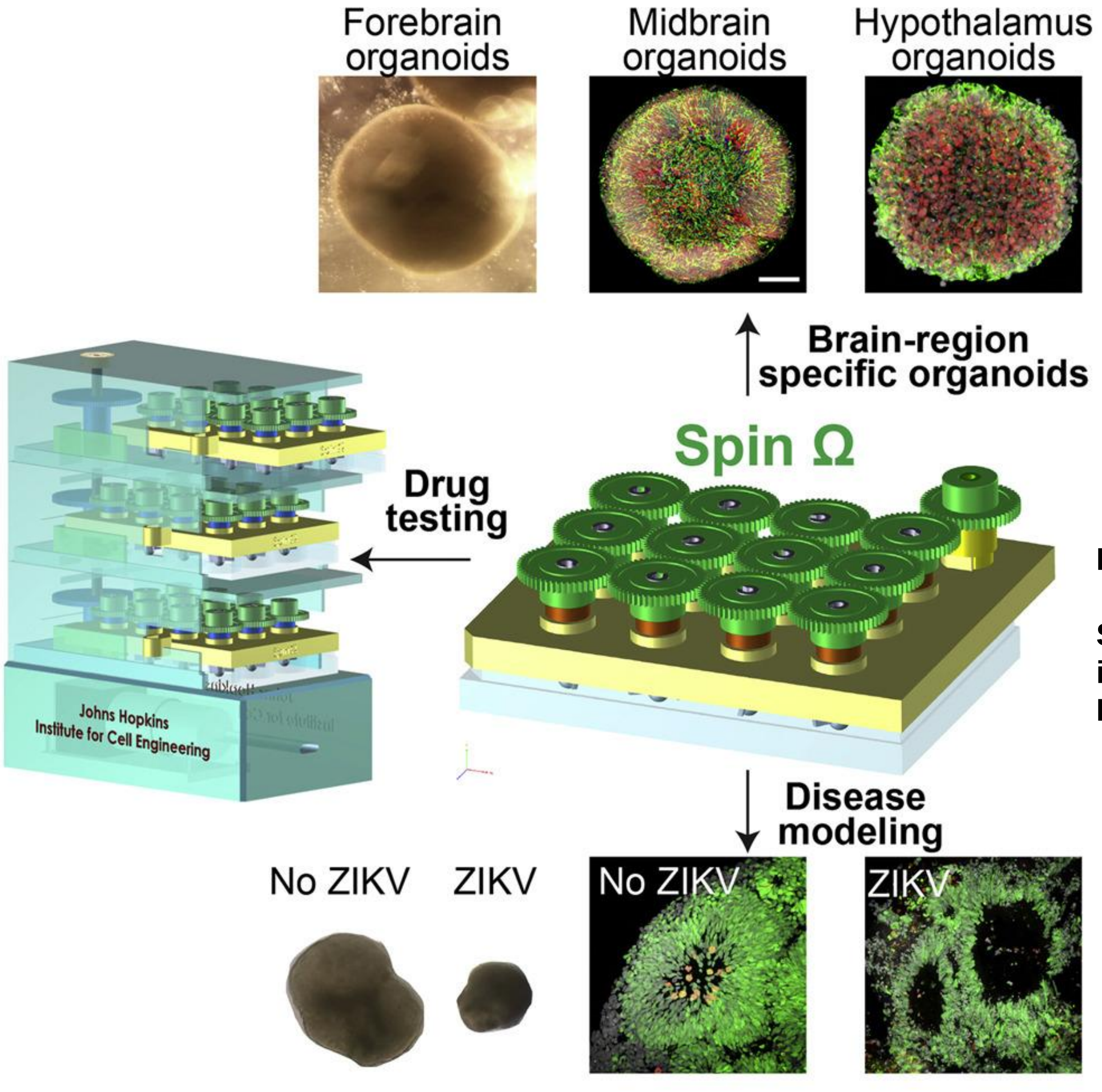
Flavivirus

Aedes species mosquito



February 1, 2016, the World Health Organization (WHO) declared Zika virus a Public Health Emergency of International Concern (PHEIC)

Figure summary



Modelling human organogensis

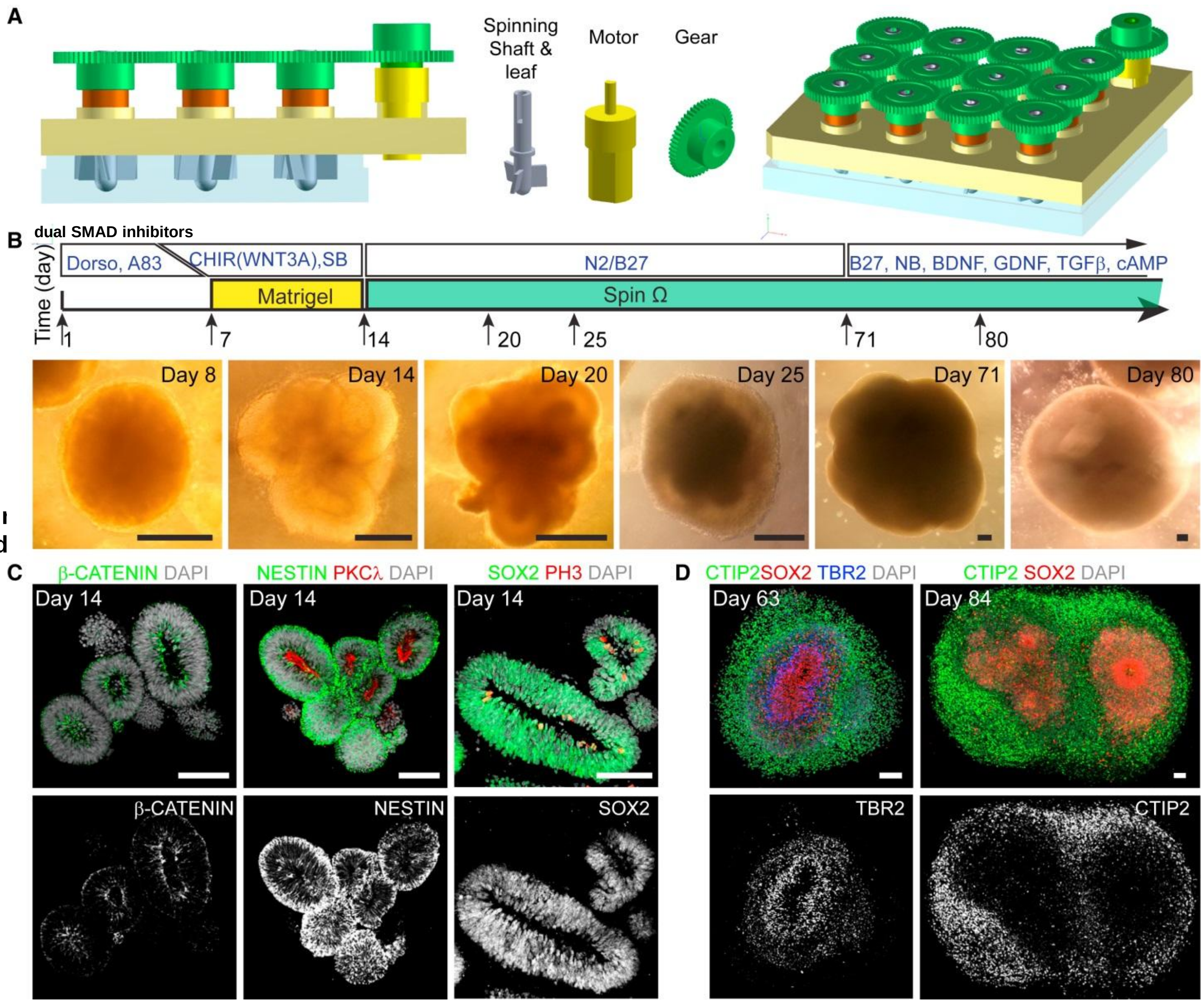
Finding:

Suppressed proliferation,
increased cell death,
Macroscopic features resembling microcephaly

Cell 2016 165, 1238-1254DOI: (10.1016/j.cell.2016.04.032)

12-well version SpinΩ bioreactor

(an early NASA-designed rotating wall vessel bioreactor to simulate microgravity)



Above the cover, spinning shafts are attached to a set of 13 interconnecting gears, driven by a single electric motor

2 ml of media per well

Forebrain organoid

Neuroepithelium

NPCs: NESTIN+SOX2+

Adherent junction markers: (β-CATENIN and PKCλ)

Proliferation marker: phospho-histone H3 (PH3)

Multi-layer stratified structures

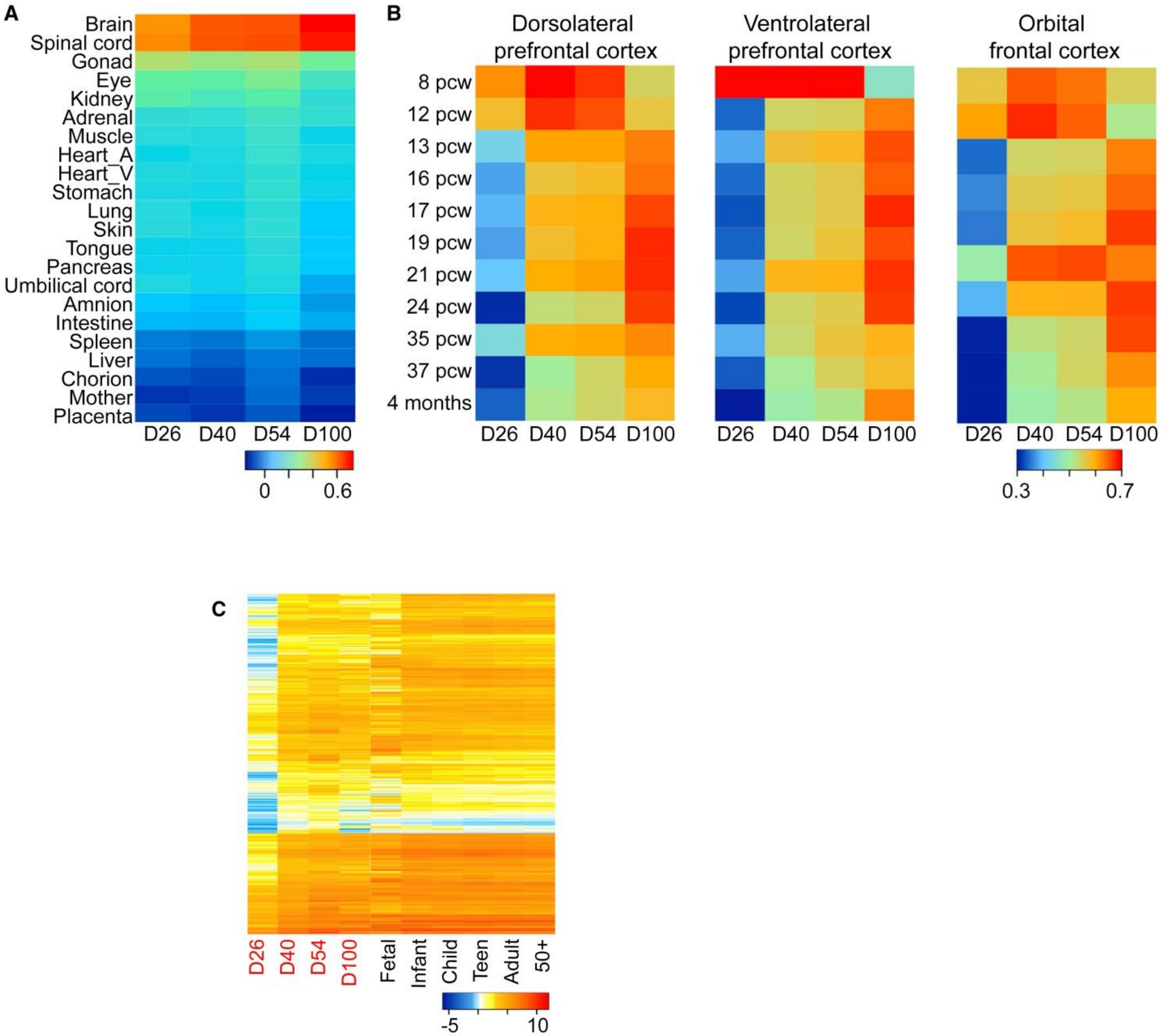
Intermediate progenitor cells: TBR2⁺

Neuron: CTIP2

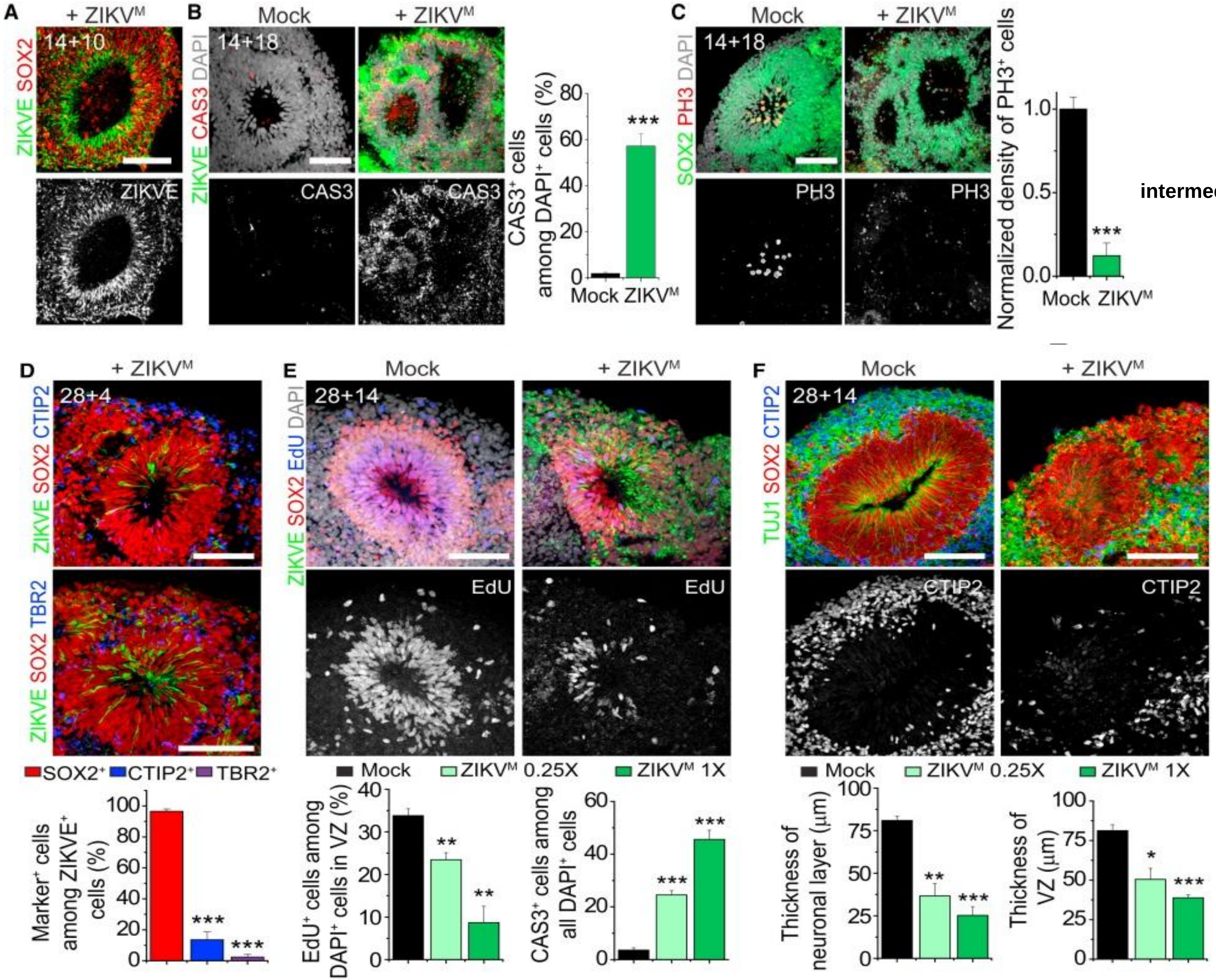
Cell 2016 165, 1238-1254DOI: (10.1016/j.cell.2016.04.032)

Correlation of Global Transcriptomes between Forebrain Organoids and Fetal Human Brain Development

Heatmap of Pearson's correlation analysis of RNA-seq datasets



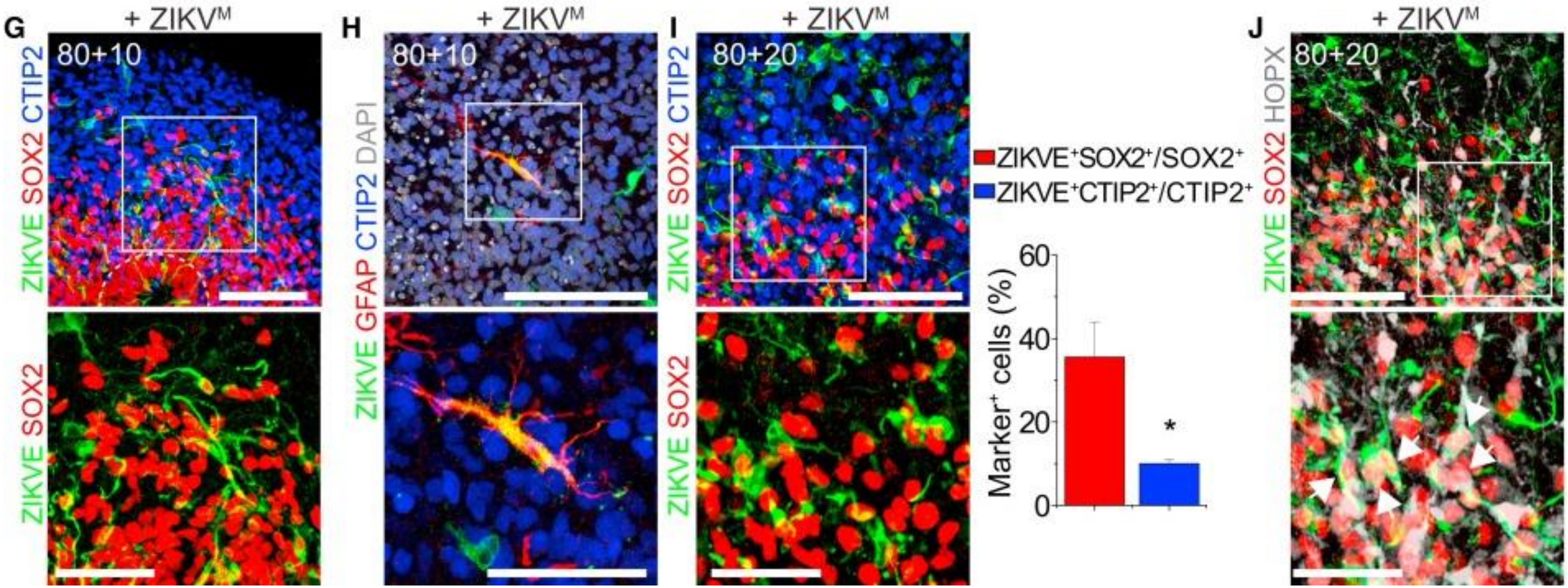
Modeling Impact of ZIKV Exposure Using Forebrain Organoids



intermediate progenitor cells

The infection appeared less robust in D80 compared to that of earlier stages of organoids

outer radial glia cells: HOPX, GFAP



Key findings:

A miniaturized spinning bioreactor for cost-effective culturing of organoids

ZIKV, upon access to the fetal brain, targets NPCs, decreases neuronal cell-layer volume and causes microcephalic-like deficits in cortical development.

Both African and Asian ZIKV infect neural progenitors in organoids

Screen:

Drug target screening

RESEARCH ARTICLE

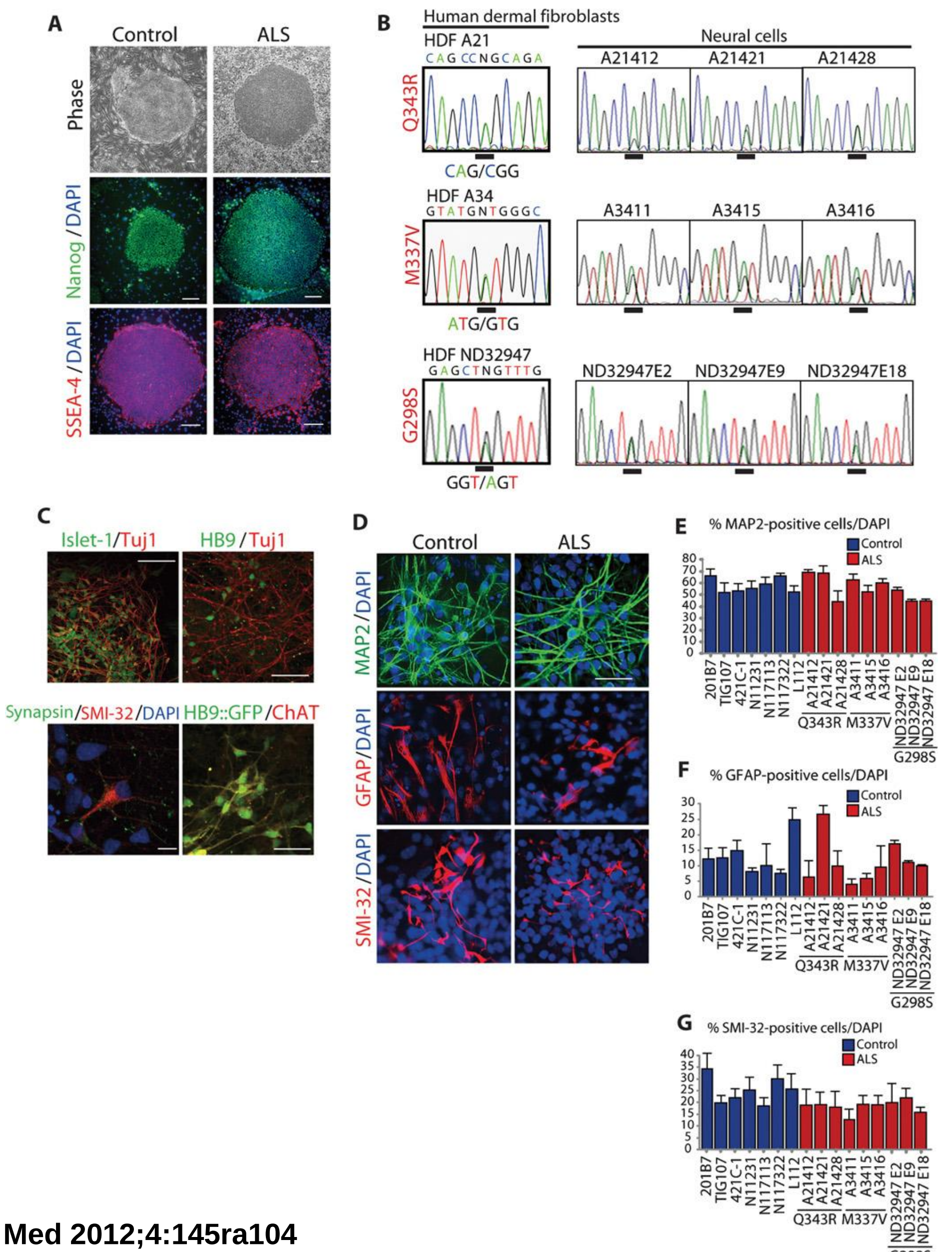
STEM CELLS

Drug Screening for ALS Using Patient-Specific Induced Pluripotent Stem Cells

Naohiro Egawa,[1,2*] Shiho Kitaoka,[1,2*] Kayoko Tsukita,[1,2] Motoko Naitoh,[3] Kazutoshi Takahashi,[1] Takuya Yamamoto,[1,4] Fumihiko Adachi,[1] Takayuki Kondo,[1,5] Keisuke Okita,[1] Isao Asaka,[1] Takashi Aoi,[1] Akira Watanabe,[1,4] Yasuhiro Yamada,[1,4] Asuka Morizane,[1,6] Jun Takahashi,[1,6] Takashi Ayaki,[5] Hidefumi Ito,[5] Katsuhiro Yoshikawa,[3] Satoko Yamawaki,[3] Shigehiko Suzuki,[3] Dai Watanabe,[7] Hiroyuki Hioki,[8] Takeshi Kaneko,[8] Kouki Makioka,[9] Koichi Okamoto,[9] Hiroshi Takuma,[10] Akira Tamaoka,[10] Kazuko Hasegawa,[11] Takashi Nonaka,[12] Masato Hasegawa,[12] Akihiro Kawata,[13] Minoru Yoshida,[14] Tatsutoshi Nakahata,[1] Ryosuke Takahashi,[5] Maria C. N. Marchetto,[15] Fred H. Gage,[15] Shinya Yamanaka,[1,4,16] Haruhisa Inoue[1,2,16†]

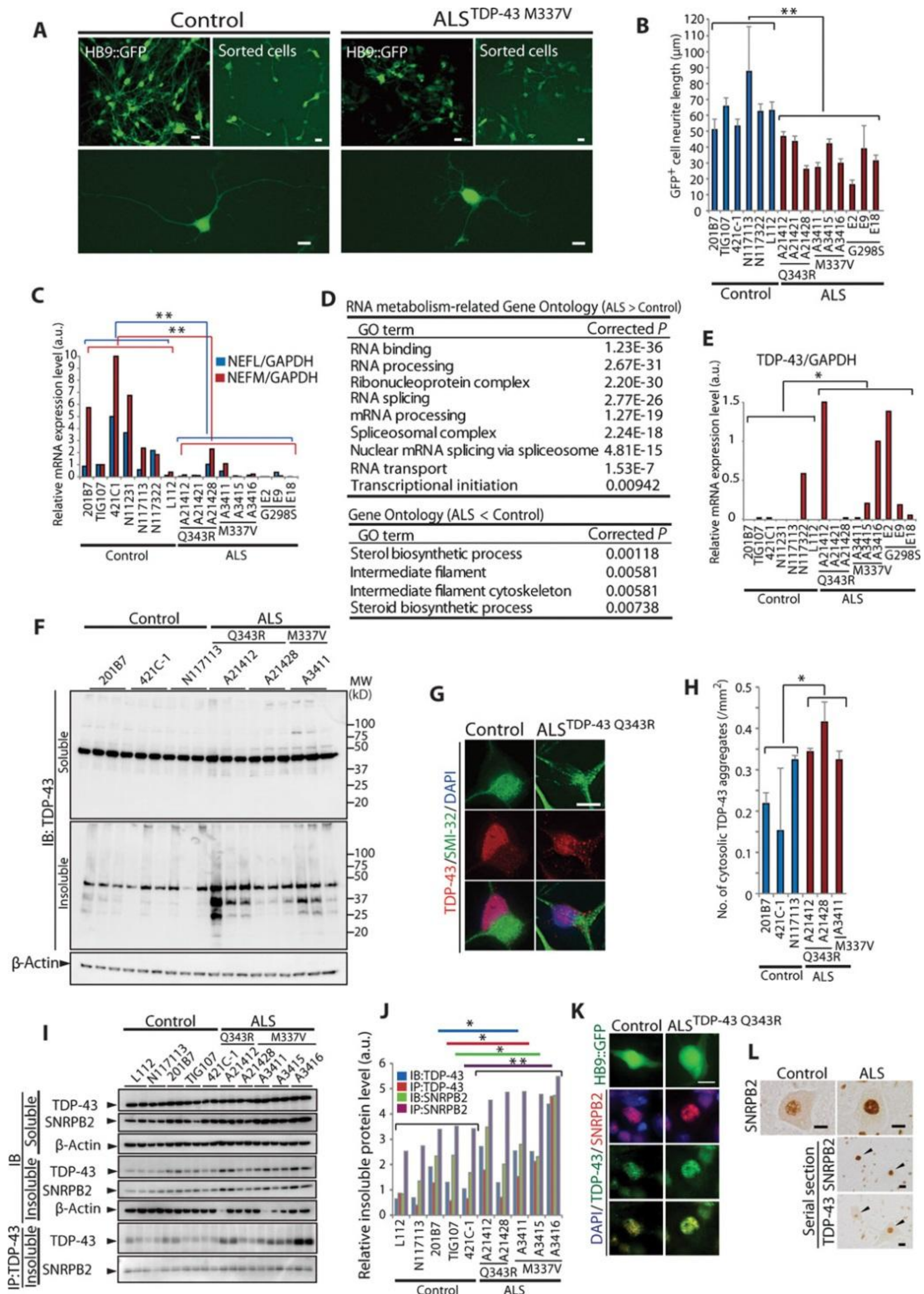
Naohiro Egawa et al., Sci Transl Med 2012;4:145ra104

Generation of ALS patient–specific iPSCs and iPSC-derived motor neurons.



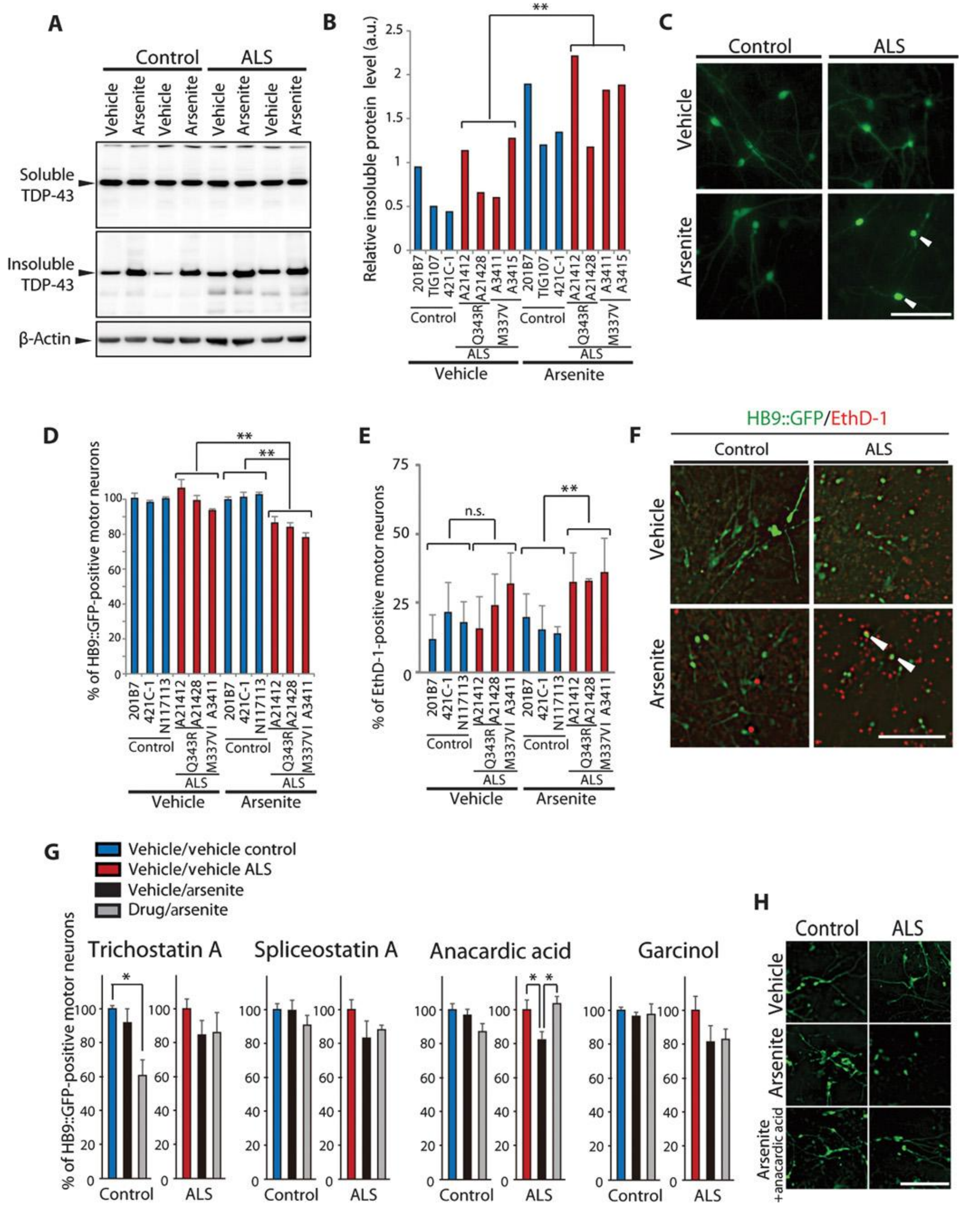
Naohiro Egawa et al., Sci Transl Med 2012;4:145ra104

Phenotypes of ALS iPSC–derived motor neurons.



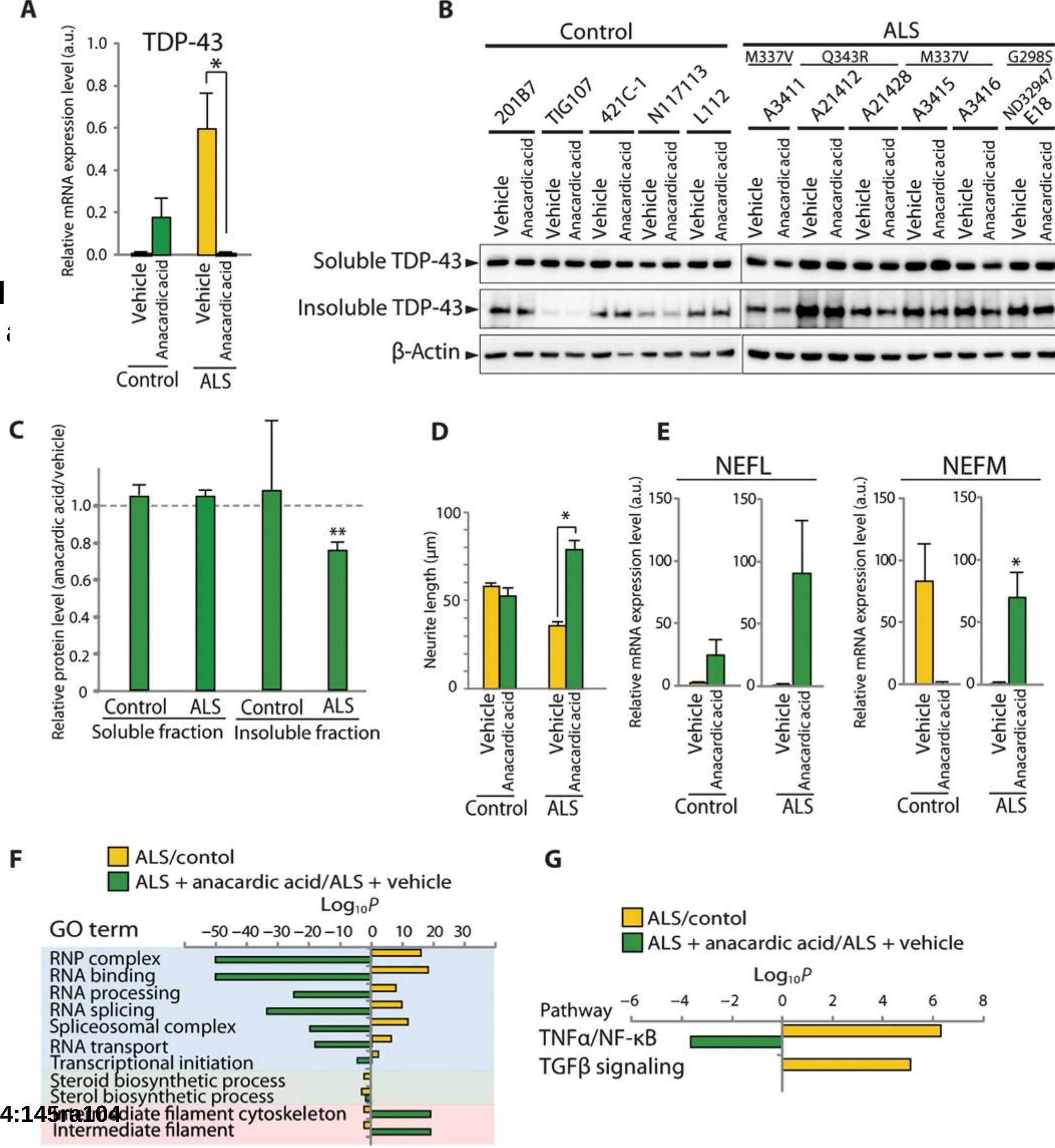
Naohiro Egawa et al., Sci Transl Med 2012;4:145ra104

Arsenite-induced death of ALS and control iPSC–derived motor neurons.



Naohiro Egawa et al., Sci Transl Med 2012;4:145ra104

Anacardic acid–induced phenotypic changes in ALS and control motor neurons.



Naohiro Egawa et al., Sci Transl Med 2012;4:145ra104

Substitute:

Transplantation and tissue replacement

Grafted tissues provide a necessary substrate for the formation of new learning (rather than simply being necessary for the execution of the response) (Brasted et al., 1999).

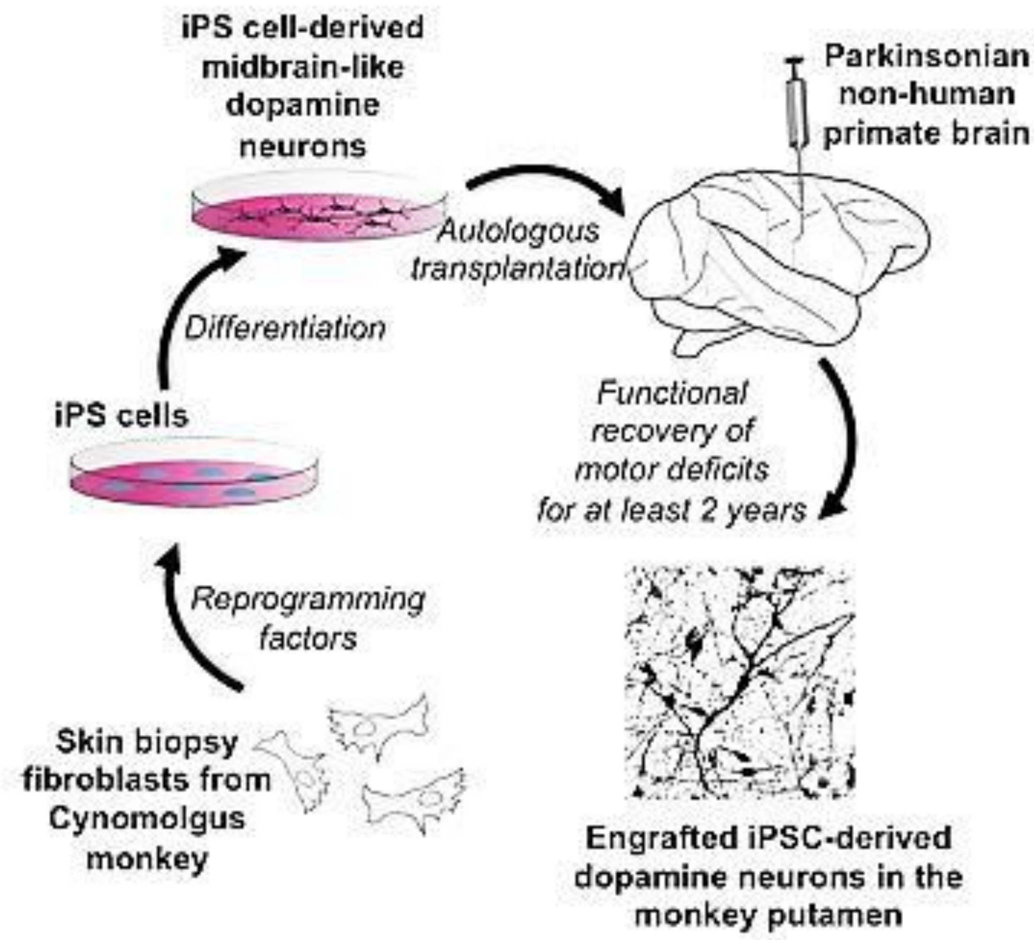
The patient requires to restore the lifetime of acquired motor skills and habits lost to the disease process (Döbrössy and Dunnett, 2001).

Cell Stem Cell
Brief Report

Successful Function of Autologous iPSC-Derived Dopamine Neurons following Transplantation in a Non-Human Primate Model of Parkinson's Disease

Penelope J. Hallett,[1] Michela Deleidi,[1] Arnar Astradsson,[1] Gaynor A. Smith,[1] Oliver Cooper,[1] Teresia M. Osborn,[1] Maria Sundberg,[1] Michele A. Moore,[1,2] Eduardo Perez-Torres,[1] Anna-Liisa Brownell,[1,3] James M. Schumacher,[1] Roger D. Spealman,[1,2] and Ole Isacson[1,4,*]
[1]Neuroregeneration Research Institute, McLean Hospital and Harvard Medical School, Belmont, MA 02478, USA
[2]New England Primate Research Center, Harvard Medical School, Southborough, MA 01772, USA
[3]MGH/HST Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129, USA
[4]Harvard Stem Cell Institute, Cambridge, MA 02138, USA
*Correspondence: isacson@hms.harvard.edu
http://dx.doi.org/10.1016/j.stem.2015.01.018

Figure summary



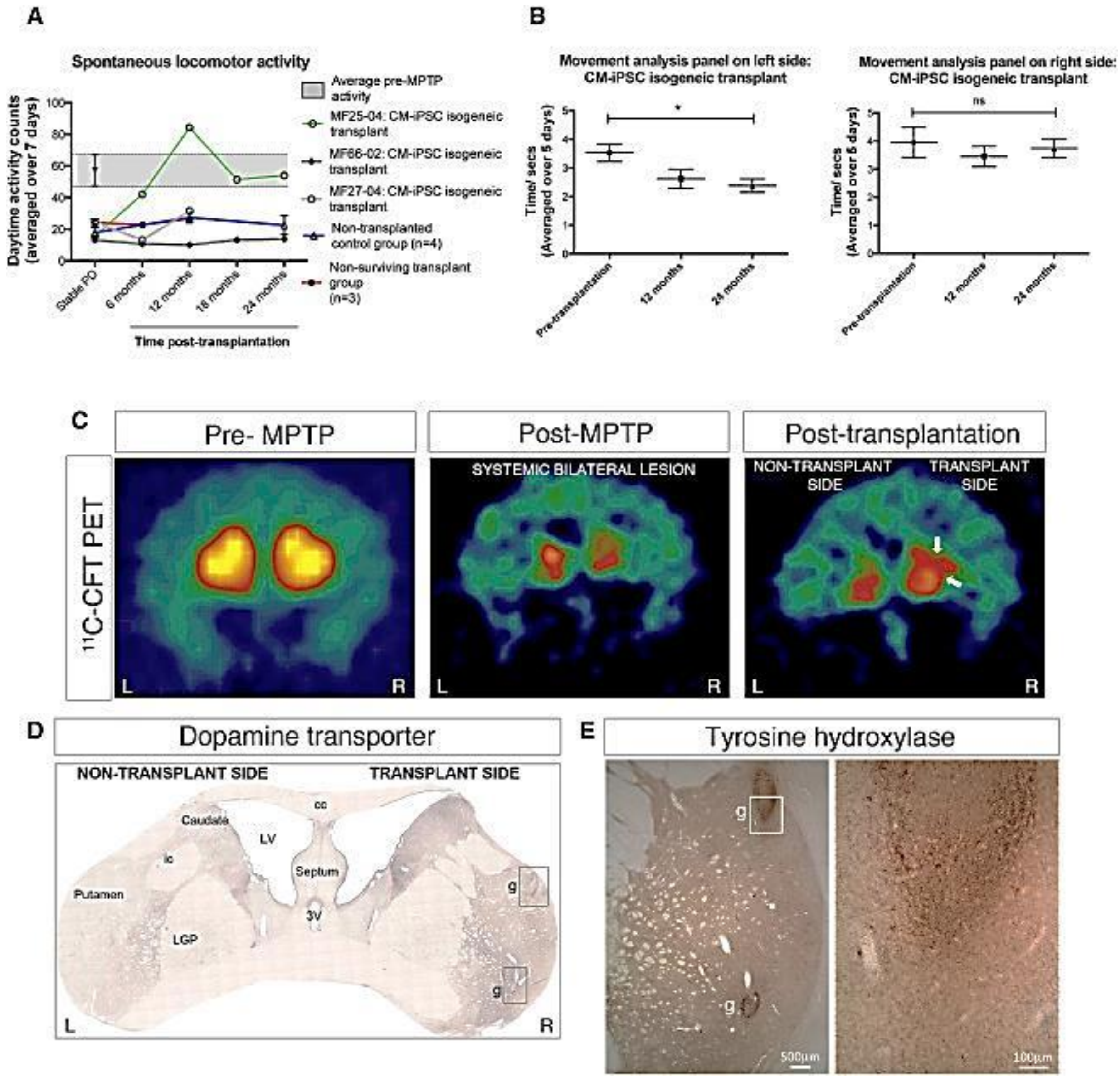
1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP),

Stable bilateral parkinsonian syndrome, including tremor, rigidity, bradykinesia, hypokinesia,

Hallett et al.,

Successful Function of Autologous iPSC-Derived Dopamine Neurons following Transplantation in a Non-Human Primate Model of Parkinson’s Disease

Contralateral functional Improvement of PD Motor Symptoms, Increased Dopamine Reuptake, and Reinnervation of the Transplanted Putamen after Autologous Transplantation of CM iPSC-Derived Dopamine Neurons

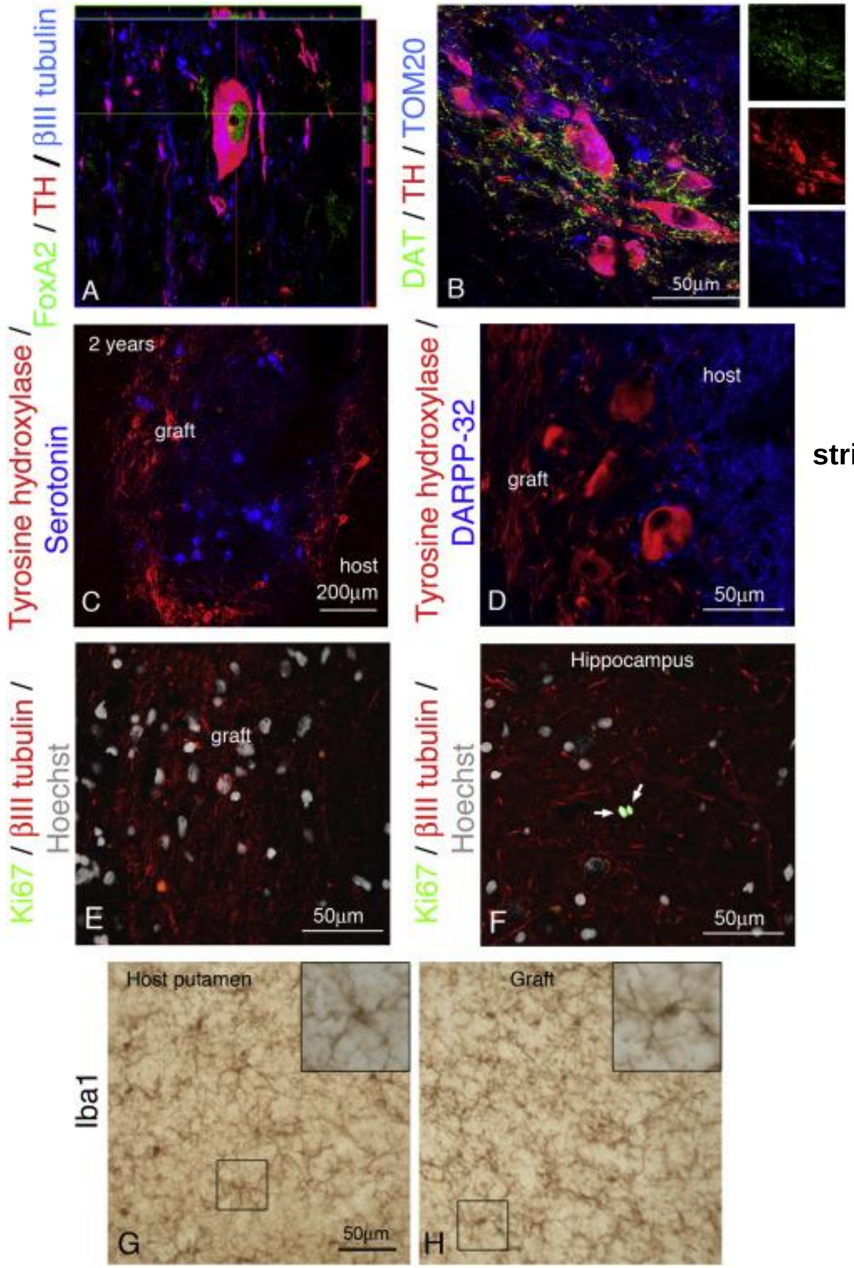


functional motor improvement and increased motor activity, without a need for immunosuppression

Phenotypes of Engrafted CM iPSC-Derived Neurons at 2 Years after Autologous Transplantation

midbrain-like dopamine neurons

striatal medium spiny GABAergic neurons



OPEN ACCESS

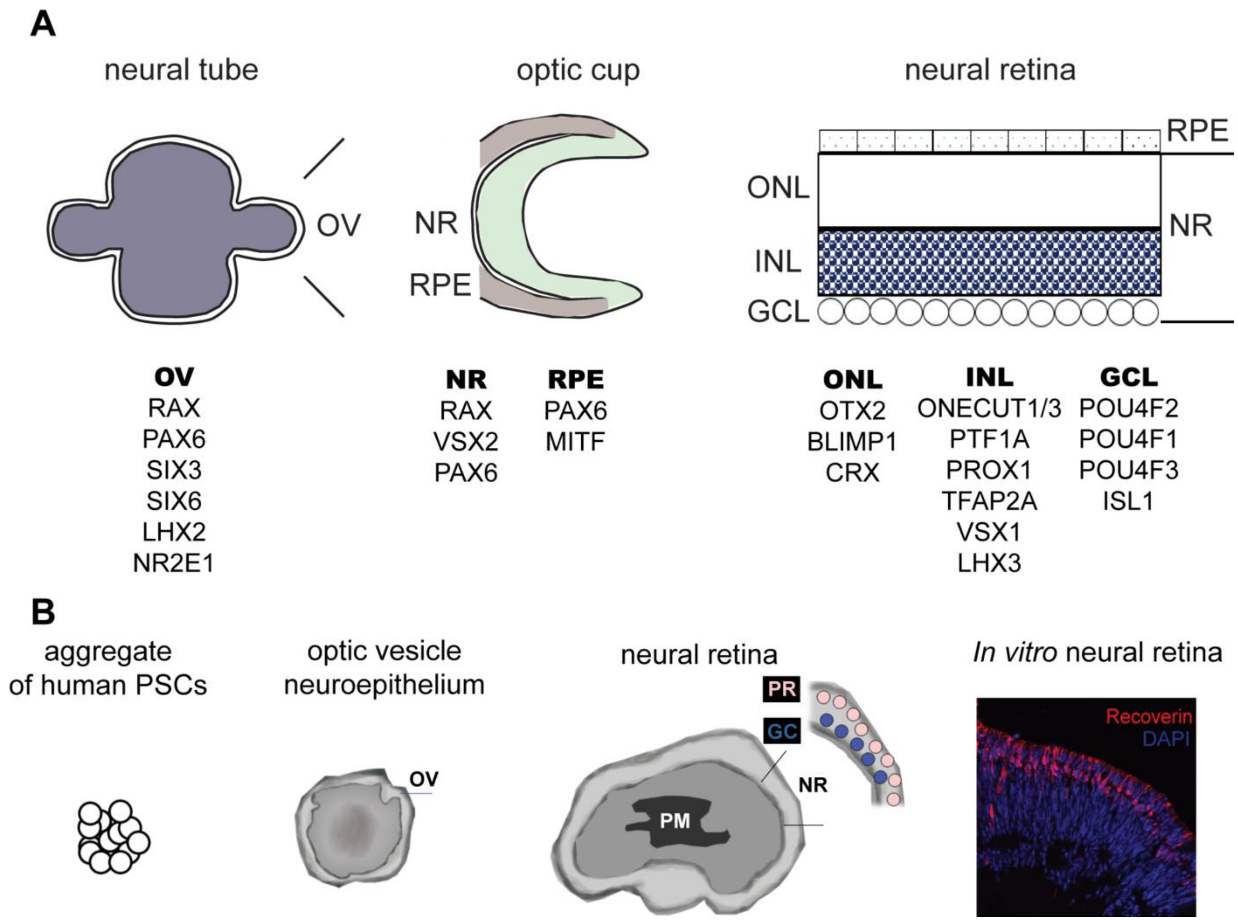
Articles | April 2016

Treatment Paradigms for Retinal and Macular Diseases Using 3-D Retina Cultures Derived From Human Reporter Pluripotent Stem Cell Lines

Rossukon Kaewkhaw; Manju Swaroop; Kohei Homma; Jutaro Nakamura; Matthew Brooks; Koray Dogan Kaya; Vijender Chaitankar; Sam Michael; Gregory Tawa; Jizhong Zou; Mahendra Rao; Wei Zheng; Tiziana Cogliati; Anand Swaroop

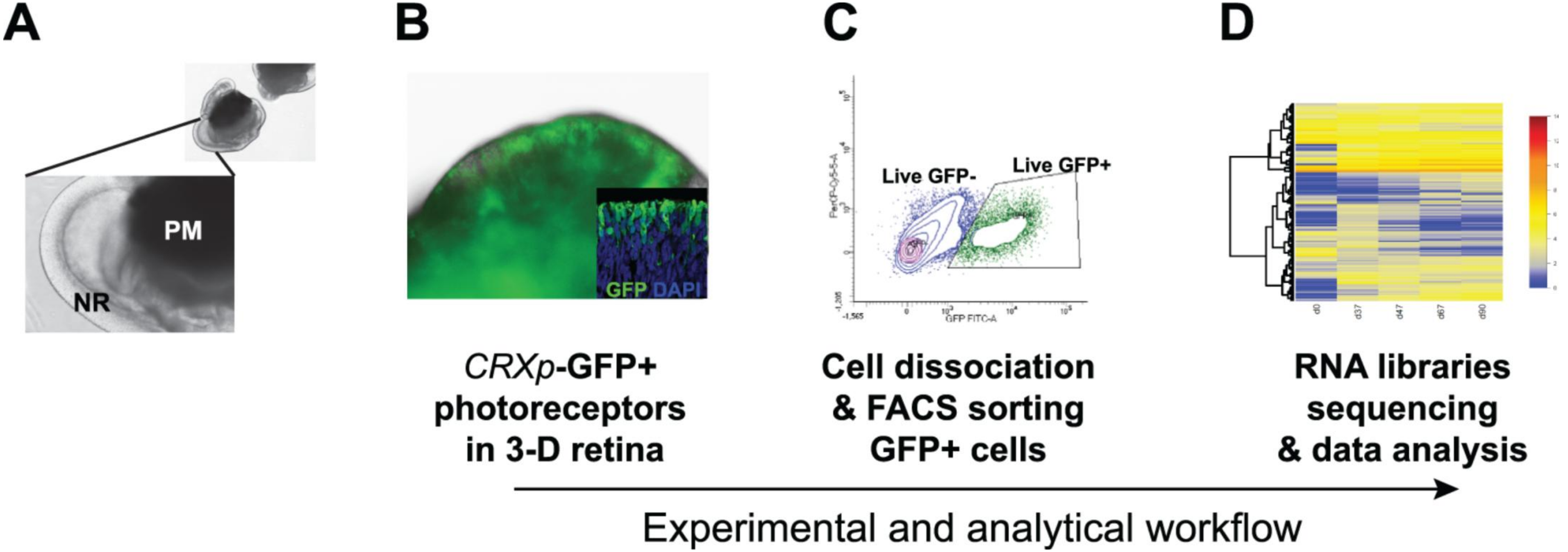
+ Author Affiliations & Notes

Investigative Ophthalmology & Visual Science April 2016, Vol.57, ORSFl1-ORSFl11. doi:10.1167/iovs.15-17639



Development of neural retina and retinal pigment epithelium cells. (A) Neural retina (NR) and retinal pigment epithelium (RPE) cells develop from the optic vesicle (OV) that emerges from the neural tube. Invagination of the optic vesicle, placing the prospective NR alongside the prospective RPE, gives rise to the optic cup. Key transcription factors that regulate each developmental stage and determine the fate of cell types in the retinal layers are listed. (B) Three-dimensional retina in vitro can be generated by floating aggregates of PSCs, which form optic vesicles where NR and pigment mass (PM) develop. ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer; PR, photoreceptor; GC, ganglion cell.

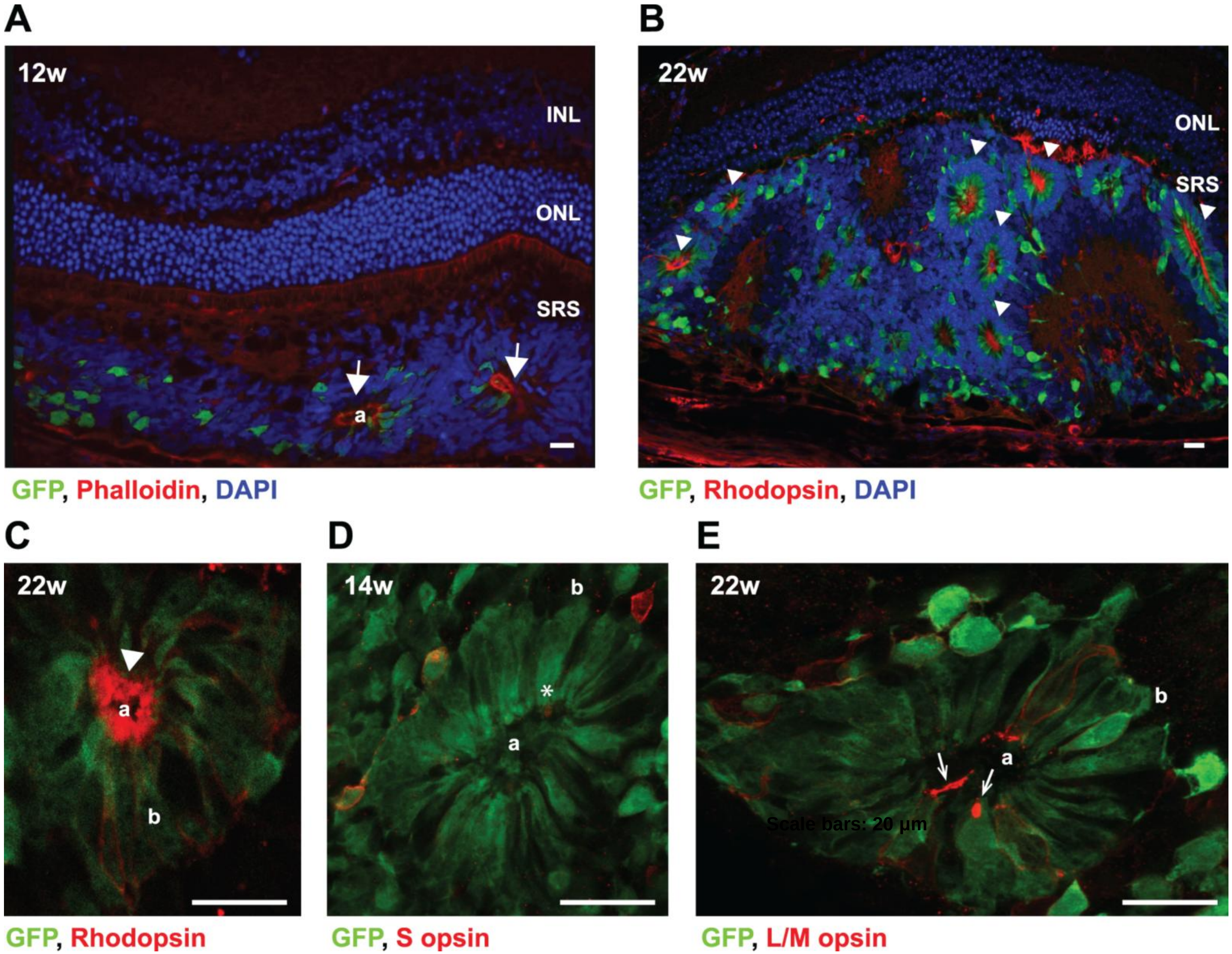
Three-dimensional retina derived from the CRXp-GFP H9 hESC reporter line



In vivo differentiation of CRXp-GFP H9 hESC-derived retinal cells from 3-D cultures

CRX+/GFP+ cells formed polarized "rosette" structures with the apical side facing inward based on phalloidin staining

cells were injected in the subretinal space of recipient adult C57BL/6 mice



A PROMISING FUTURE:

First human transplantation of tissue derived from induced pluripotent stem cells

Kobe City Medical Center General Hospital implanted a 1.3 by 3.0 millimetre sheet of RPE cells into an eye 70 year old Japanese woman who suffers from age-related macular degeneration

Masayo Takahashi (RIKEN Center for Developmental Biology (CDB)), had developed and tested the epithelium sheets

Two-hour procedure, four days after a health-ministry committee gave clearance for the human trial

Monitoring the cells whether they prevent further destruction of the retina while avoiding potential side effects, such as bringing about an immune reaction or inducing cancerous growth.

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Thank you for your attention