Increasing the neurological-disease toolbox: human iPSC-derived microglia-like cells

Technical Journal Club

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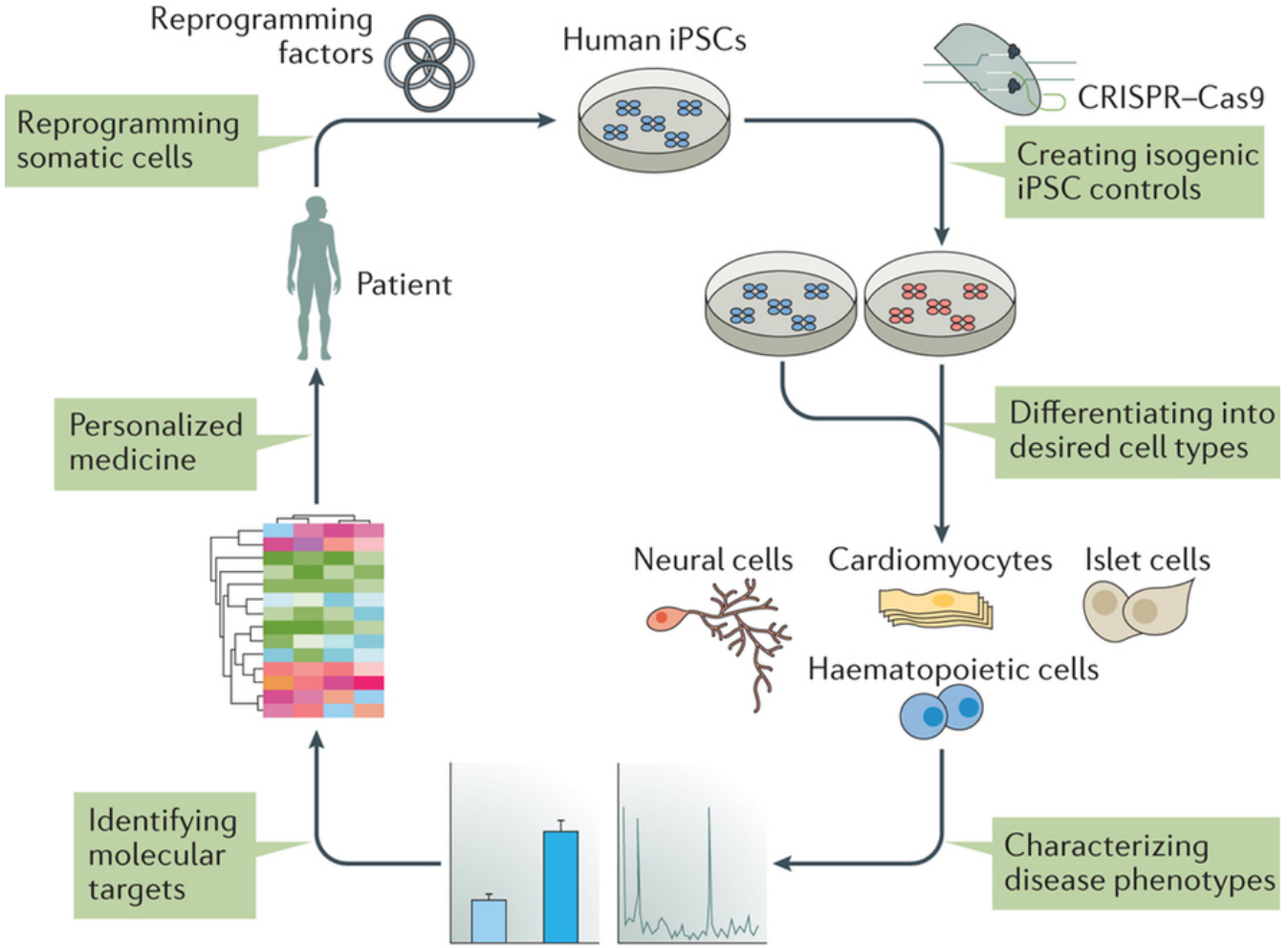
Induced pluripotent stem cells (iPSC)

- First described in 2006
- Reprogrammed mouse somatic cells by using a transcription factor cocktail of four transcription factors (OCT4, SOX2, KLF4 and MYC)
- Gene expression profile and developmental potential similar to embryonic stem cells (ESCs)
- In 2007, generation of human iPSCs (from fibroblasts)

human iPSC-based disease modelling



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human iPSC-based disease modelling

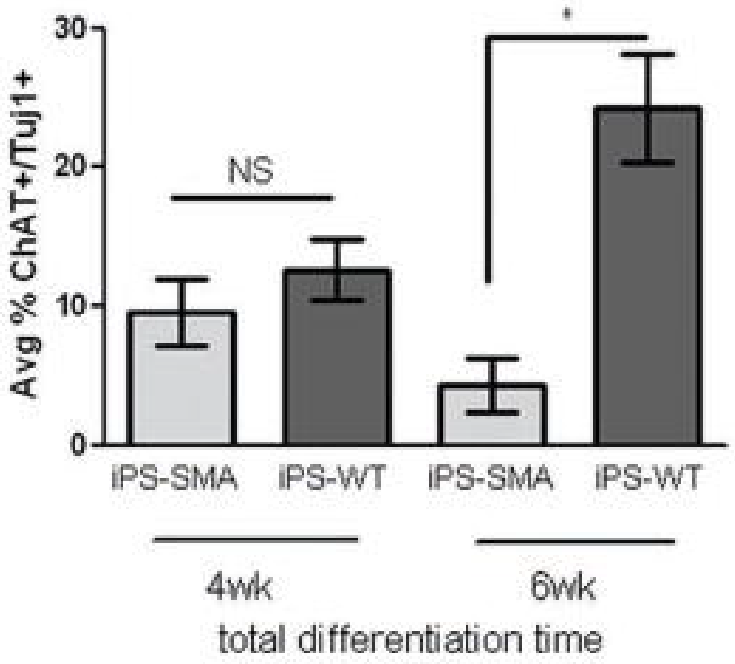
Advantages to conventional disease models

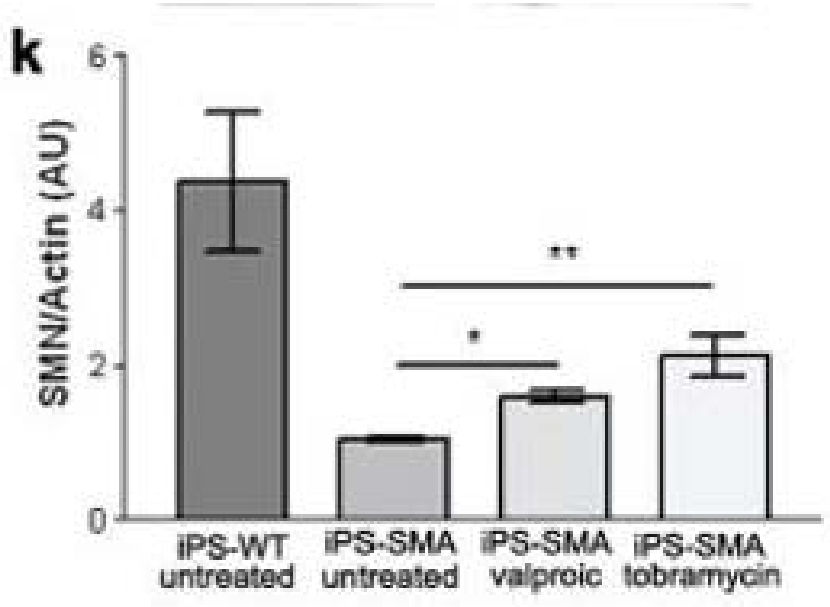
- No substantial species difference (compared to *animal models* of human disease)
- Easily accessible cells (compared to *primary cells*), which can provide large quantities of disease relevant cells and previously inaccessible cell types
- Can be derived from the relevant patients themselves (precision medicine)
- No ethical concerns (compared to *ESCs*)
- The use of gene editing technologies enables the generation of genetically matched, isogenic iPSCs as control

hiPSC-based disease modelling: example

Spinal muscular atrophy (SMA) patient derived iPSC

- Reduced survival of SMA patient iPSC derived motor neurons
- Amount of protective SMN1 could be increased by Valproic acid and tobramycin (hiPSCs as potential drug screening platform)





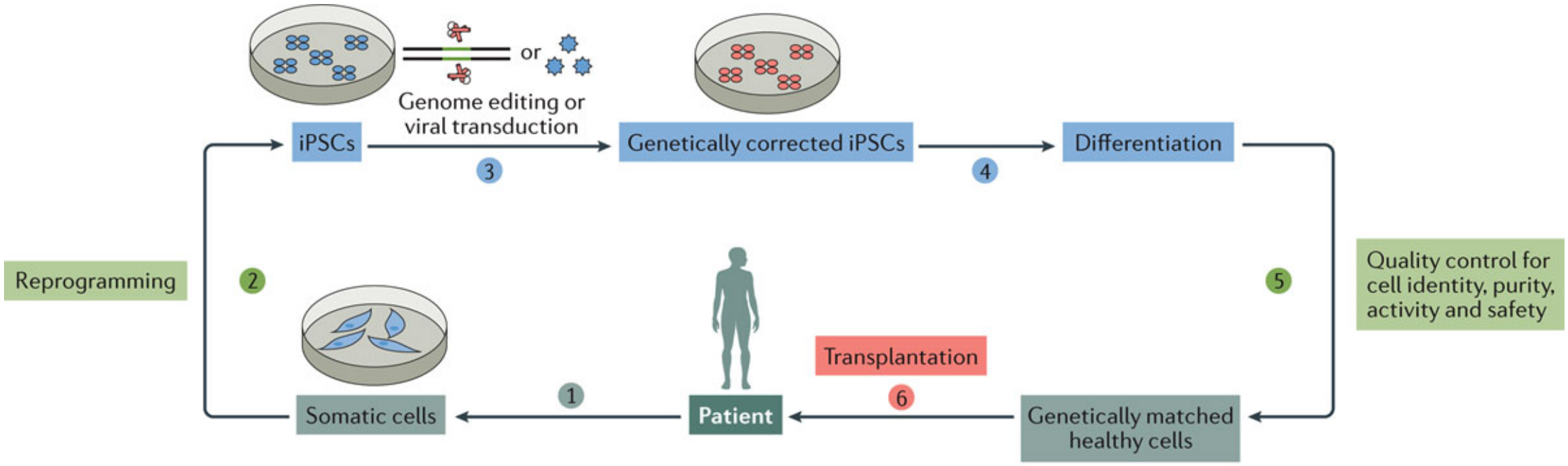
Ebert et al., Nature, 2009

hiPSC-based disease modelling

- Good way to study monogenetic diseases in the relevant cell types
- More complex for sporadic diseases
  - Development of more advanced hiPSC-based models such as Co-cultures and 3D organoids to study interaction between different cell types

Clinical application of hiPSC products

Cell therapy



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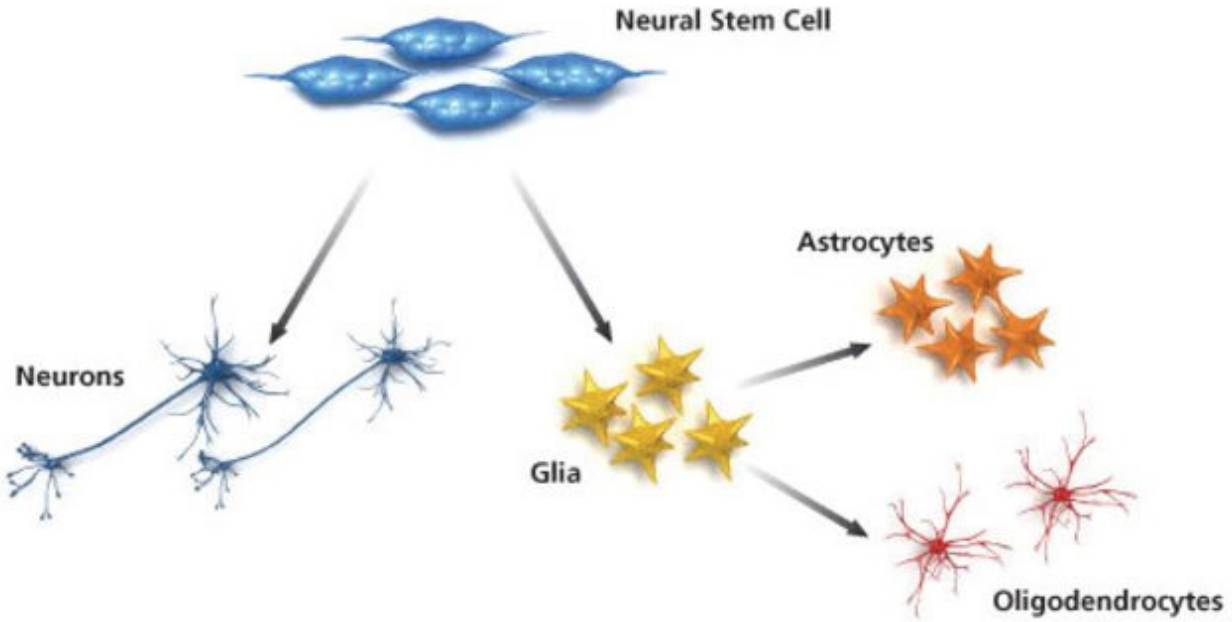
iPSCs and the human CNS

Protocols for the generation of neural progenitor cells and their derivatives from iPSCs are already available and part of extensive research



iPSCs and the human CNS

Idiopathic Parkinson's disease patient-derived induced pluripotent stem cells function as midbrain dopaminergic neurons in rodent brains. (Kikuchi et al., J Neurosci Res., 2017)

Internalized Tau Oligomers Cause Neurodegeneration by Inducing Accumulation of Pathogenic Tau in Human Neurons Derived from Induced Pluripotent Stem Cells. (Usenovic et al., J Neurosci., 2015)

iPSCs and the human CNS: Microglia

- Resident macrophages in the brain parenchmya
- Responsible for removal of dead cells and debris but also for sculpting and maintaining the brain`s neural network
- Do not share the same origin as neurons, oligodendrocytes and astrocytes

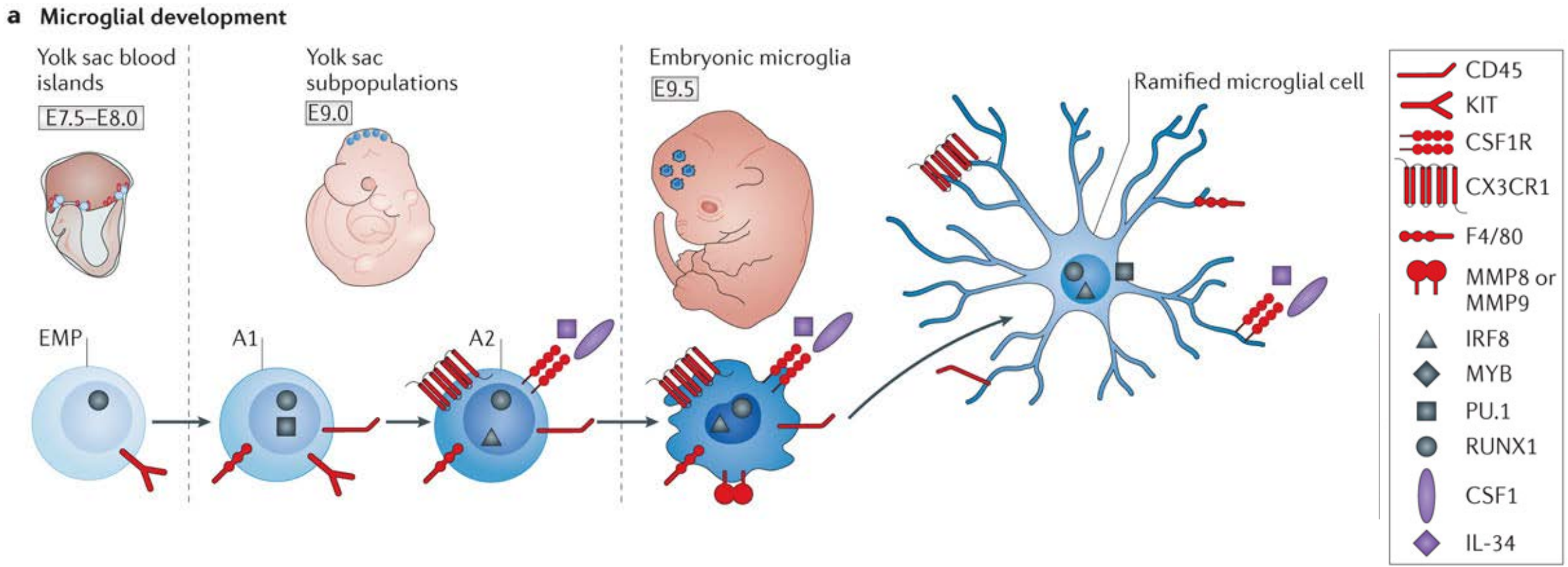
Misfunction could underlie neurological diseases

Efficient derivation of microglia-like cells from human pluripotent stem cells

Julien Muffat, Yun Li, Bingbing Yuan, Maisam Mitalipova, Attya Omer, Sean Corcoran, Grisilda Bakiasi, Li-Huei Tsai, Patrick Aubourg, Richard M Ransohoff & Rudolf Jaenisch

Muffat et al. (2016) established a protocol for the production of microglia-like cells from human (h) embryonic stem (ES) and induced pluripotent stem (iPS) cells.

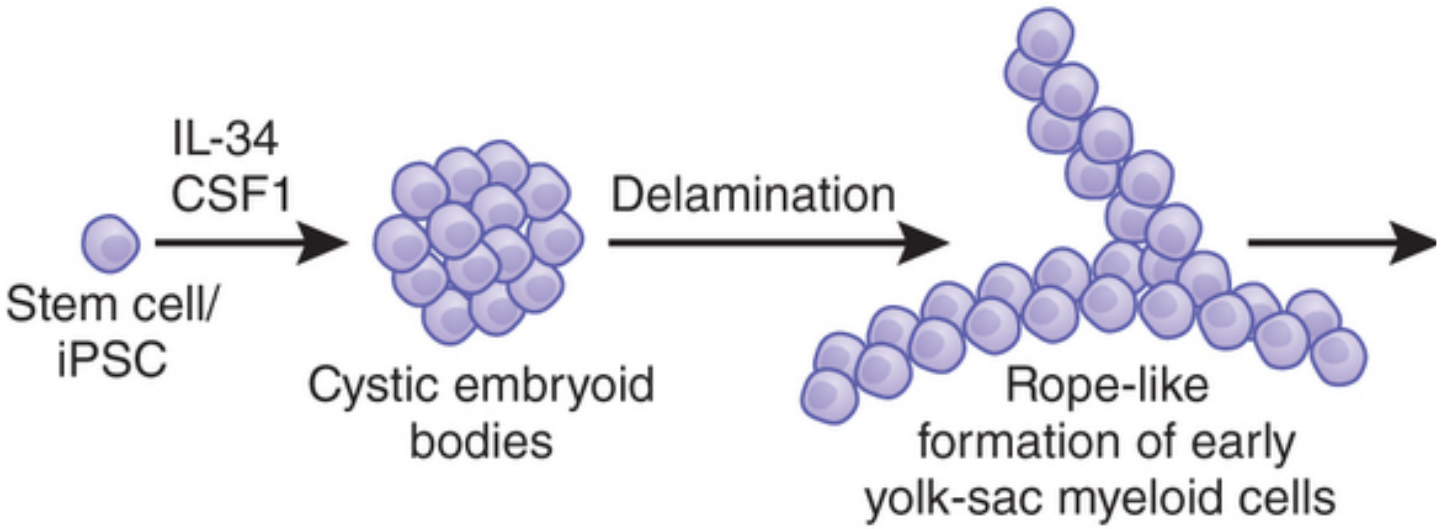
Origin of microglia



Adapted from: Prinz and Piller, Nature Reviews, 2014

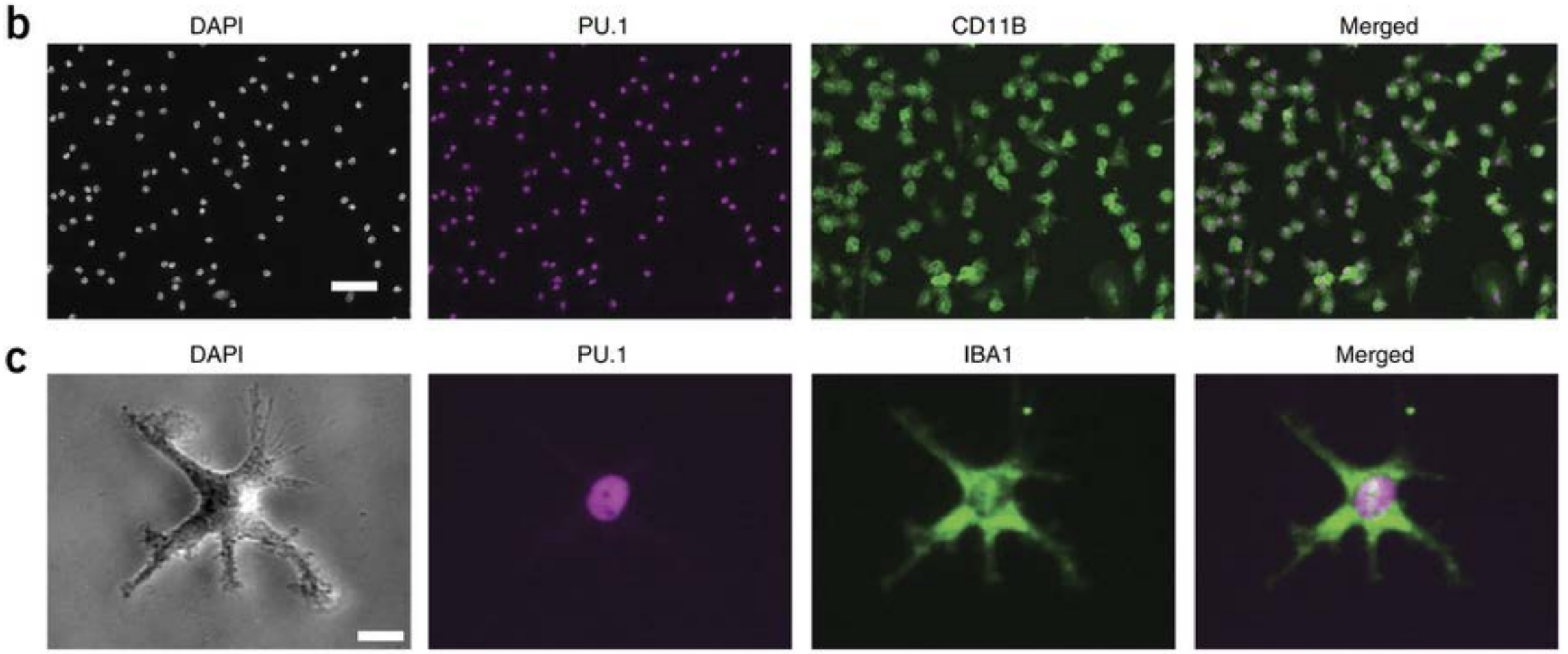
Generation of microglia-like cells from hiPSC

Procedure



Hammond and Stevens, Nature Medicine, 2016

Generation of microglia-like cells from hiPSC



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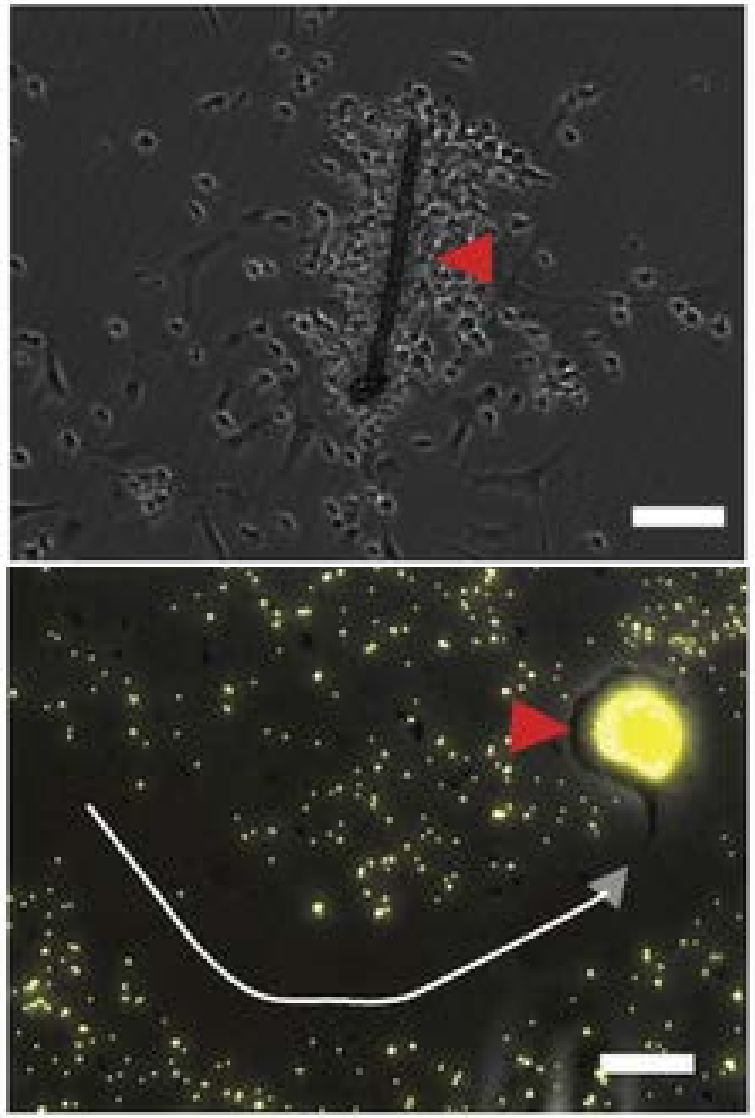

Generation of microglia-like cells from hiPSC

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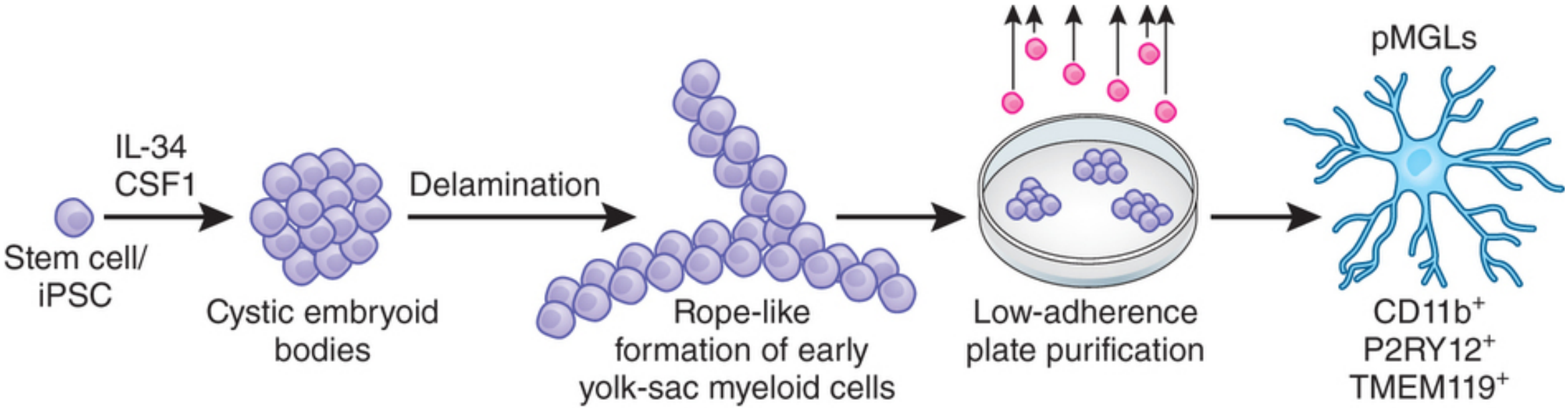
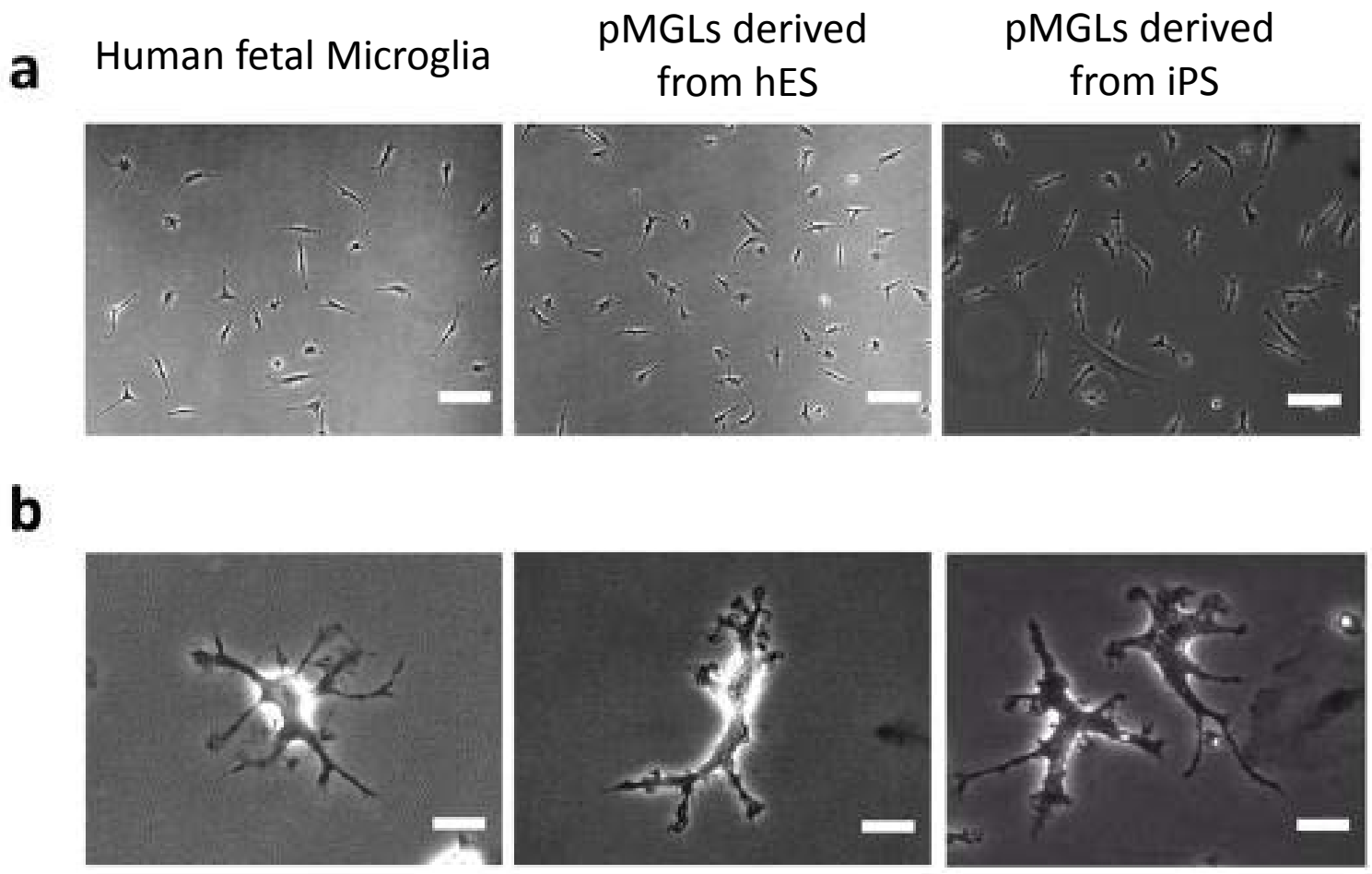


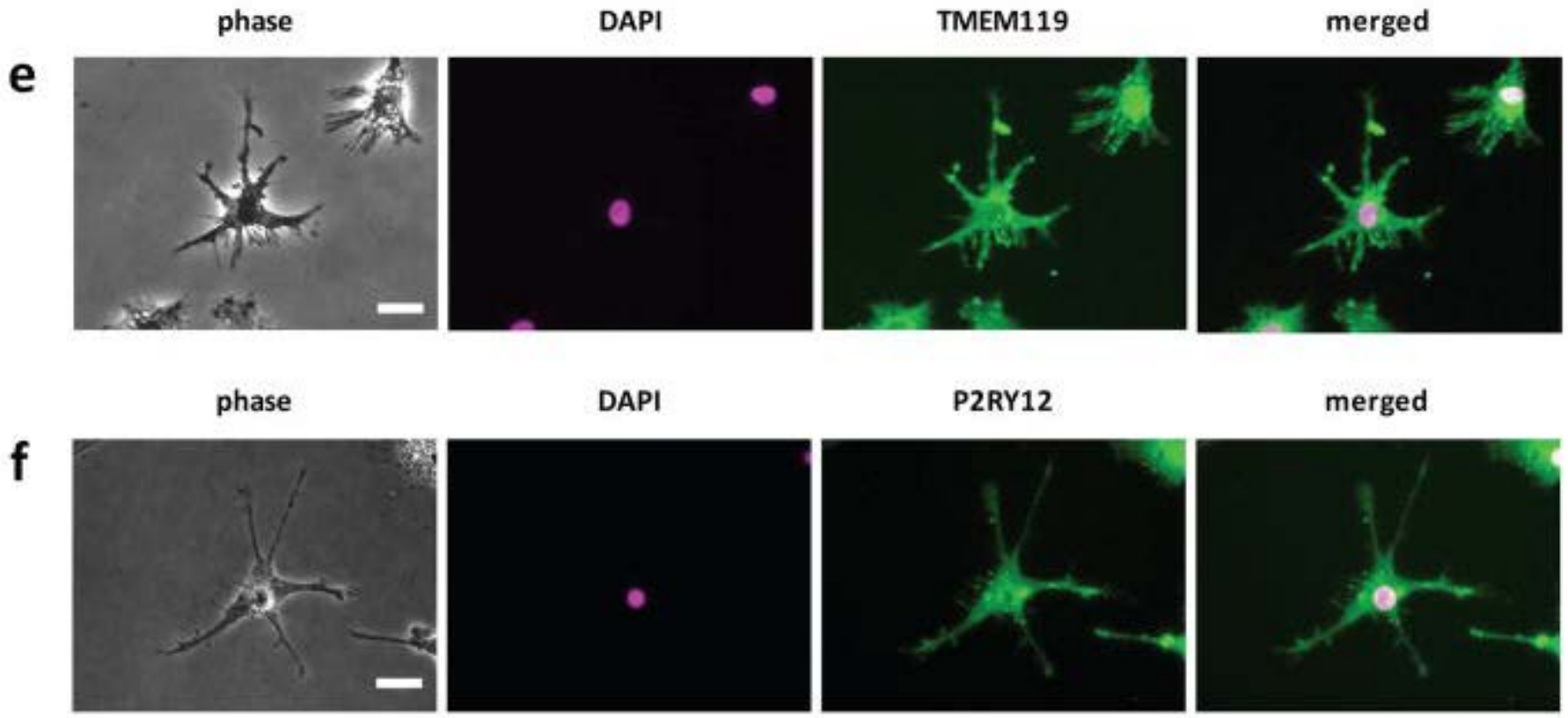
Hammond and Stevens, Nature Medicine, 2016

Phenotypic and functional characterization of pMGLs

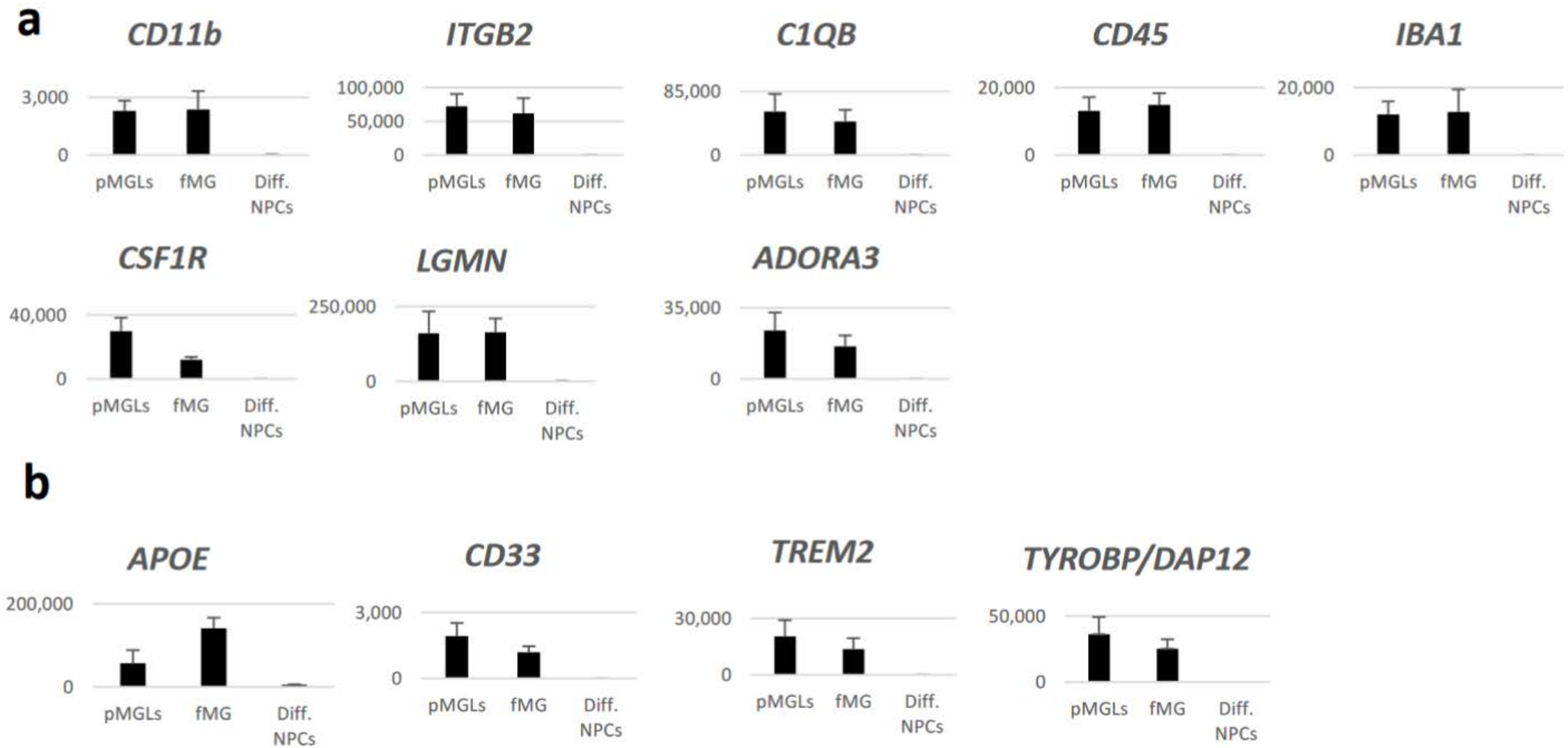


pMGL = pluripotent stem cell derived microglia-like cell

Phenotypic and functional characterization of pMGLs



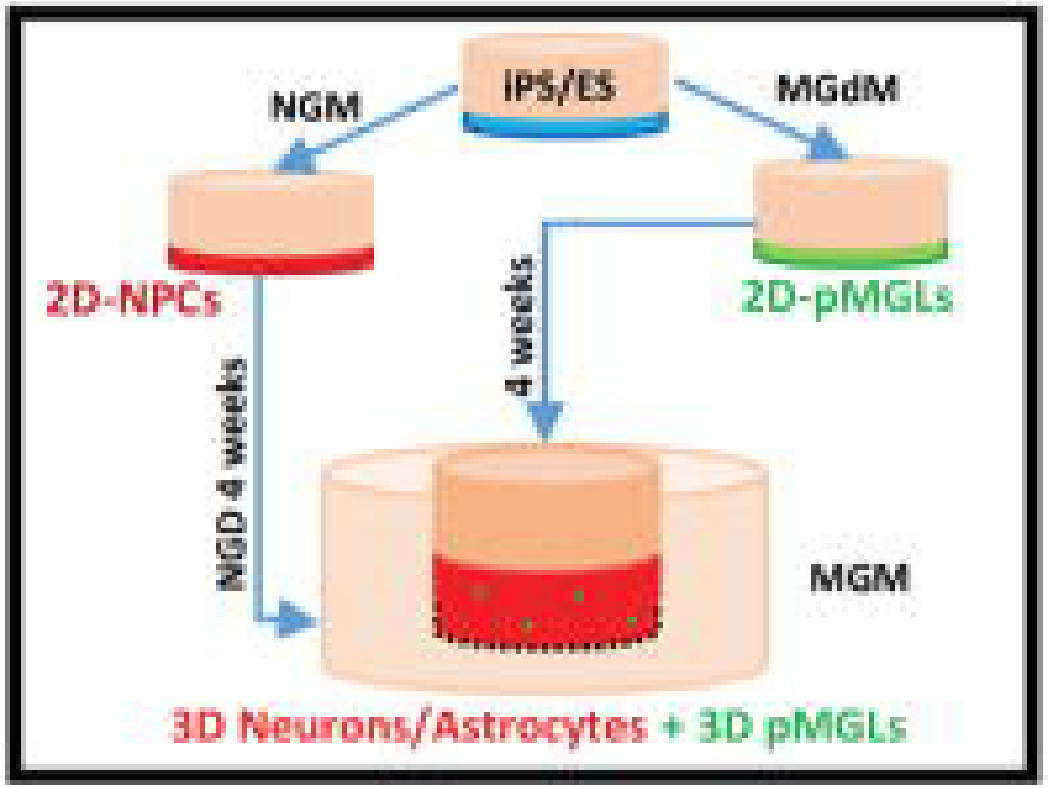
Phenotypic and functional characterization of pMGLs



pMGLs in a 3D neuroglia cell culture

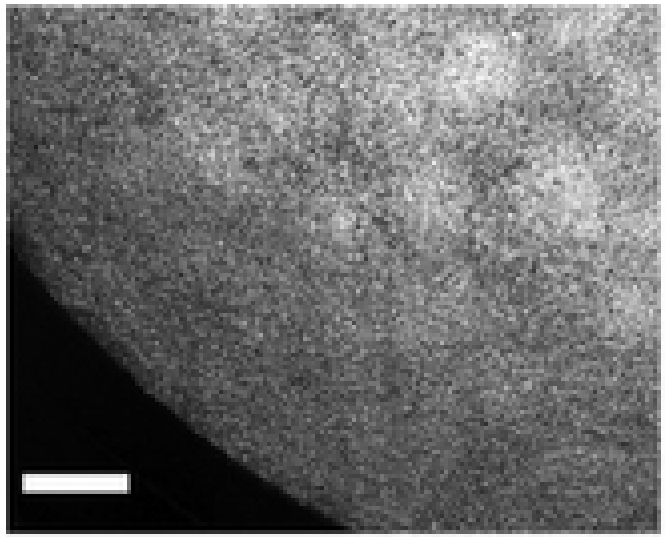
Embedding and maturation of pMGLs in a 3D organotypic neuroglial environment.

pMGLs were transduced with a GFP lentivirus and reaggregated with pre-differentiated neural cultures.

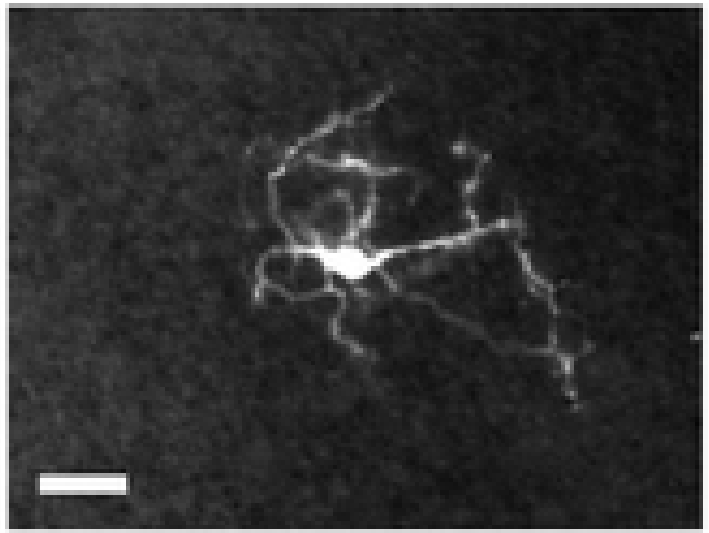


pMGLs in a 3D neuroglia cell culture

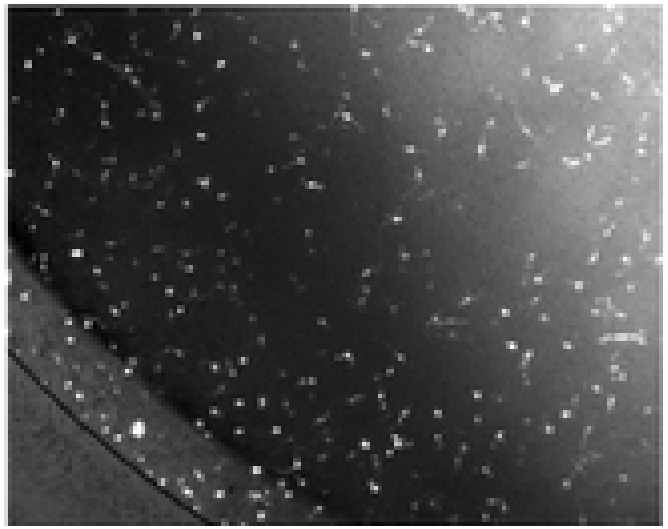
3D culture in transwell Phase contrast



Representative image of a GFP-positive pMGL

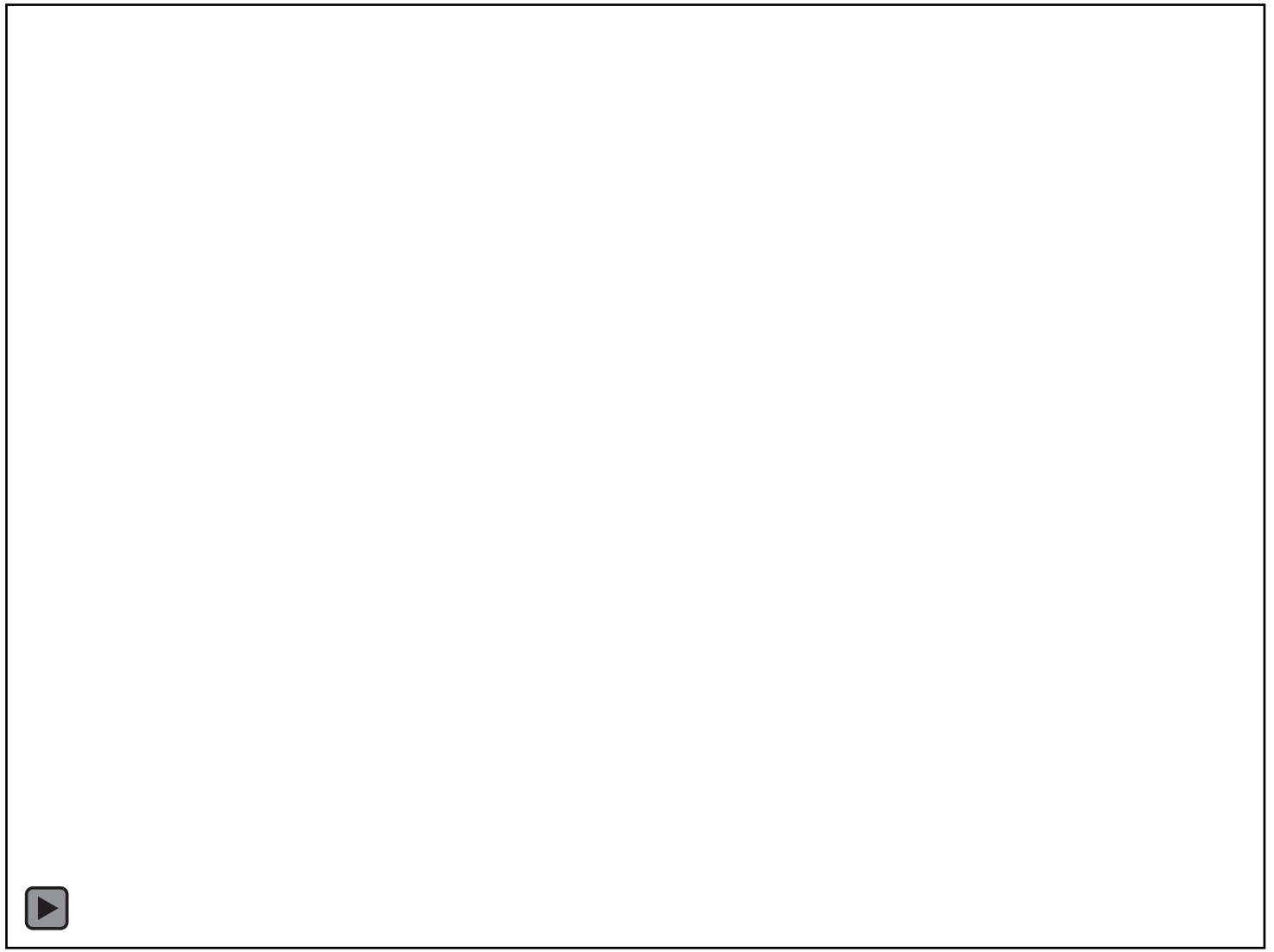


3D culture in transwell GFP labeled pMGLs



GFP-positive pMGL: Extending and retracting protrusions

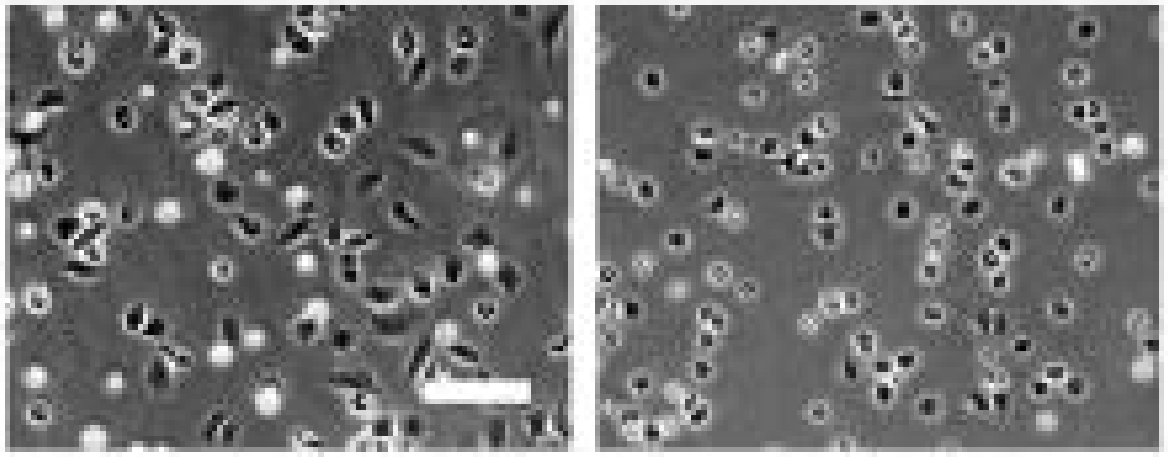
pMGLs in a 3D neuroglia cell culture

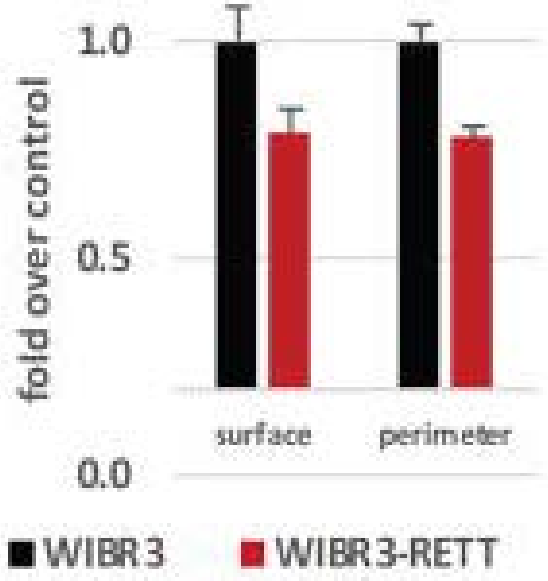


Diseasae modelling using pMGLs: Rett Syndrome

Wild type pMGLs

MECP2 mutated pMGLs





Summary 1st Paper

- Establishment of a robust protocol that allows for the derivation of microglia-like cells from human pluripotent stem cells
- Phenotype and gene expression profile of pMGLs resemble primary fetal and adult human Microglia
- pMGLs react on cellular damage in a 3D culture system
- Can be used for disesase modelling as it was shown for MECP2 mutation

NeuroResource

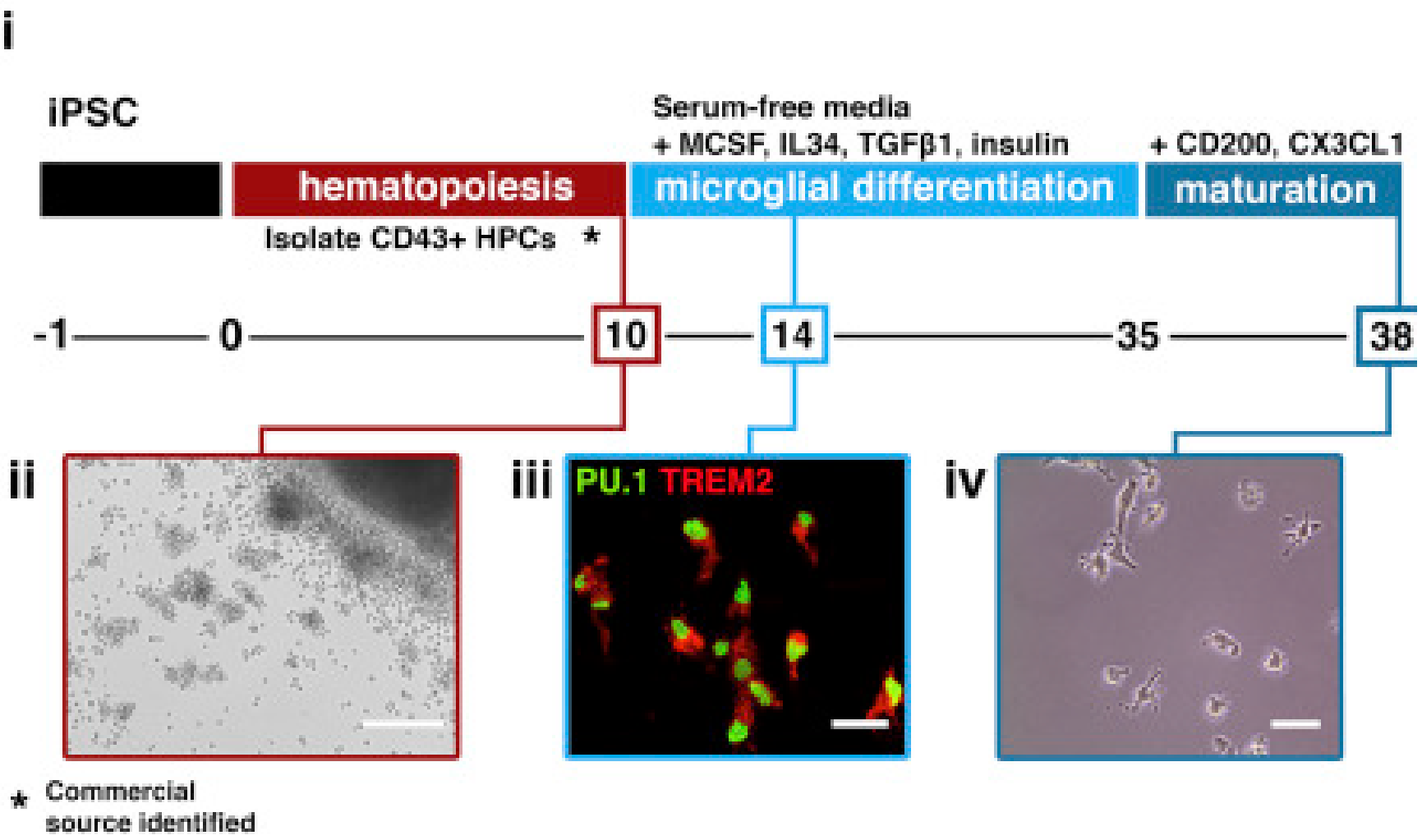
iPSC-Derived Human Microglia-like Cells to Study Neurological Diseases

Edsel M. Abud[1, 2, 3], Ricardo N. Ramirez[4], Eric S. Martinez[1, 2, 3], Luke M. Healy[5], Cecilia H.H. Nguyen[1, 2, 3], Sean A. Newman[2], Andriy V. Yeromin[6], Vanessa M. Scarfone[2], Samuel E. Marsh[2, 3], Cristhian Fimbres[3],

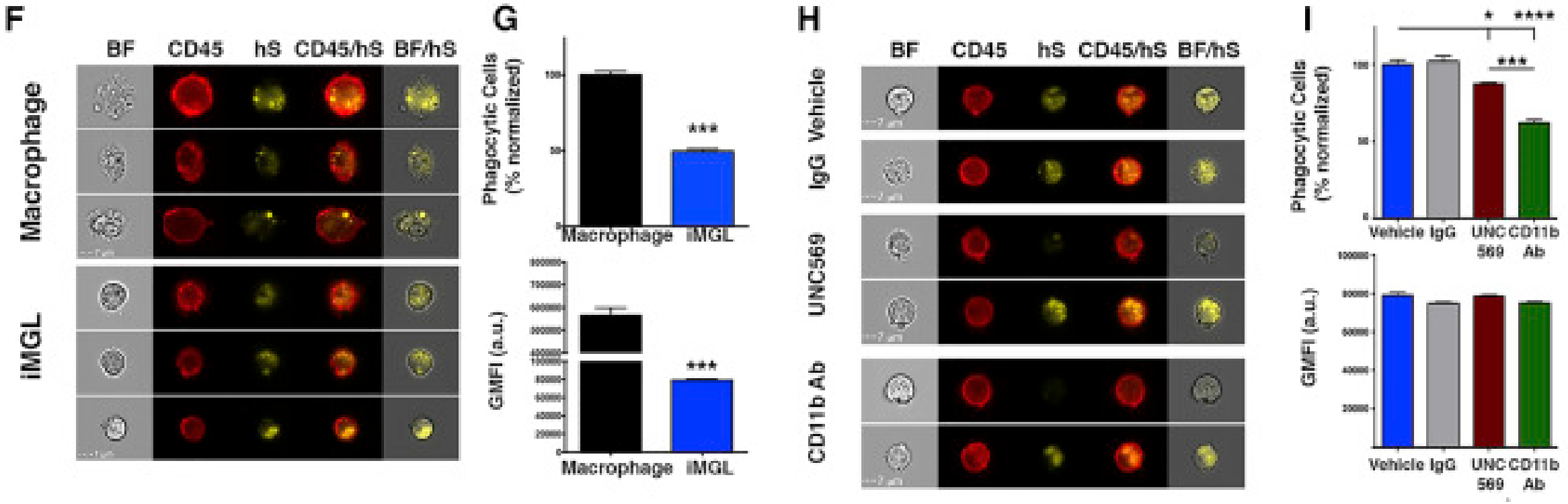
Show more

Abud et al. (2017) established a different protocol for the production of microglia-like cells from hiPSC and transplanted them into human brain organoids and transgenic mice

Differentiation of hiPSC derived microglia-like cells



Physiological function: Phagocytosis assay

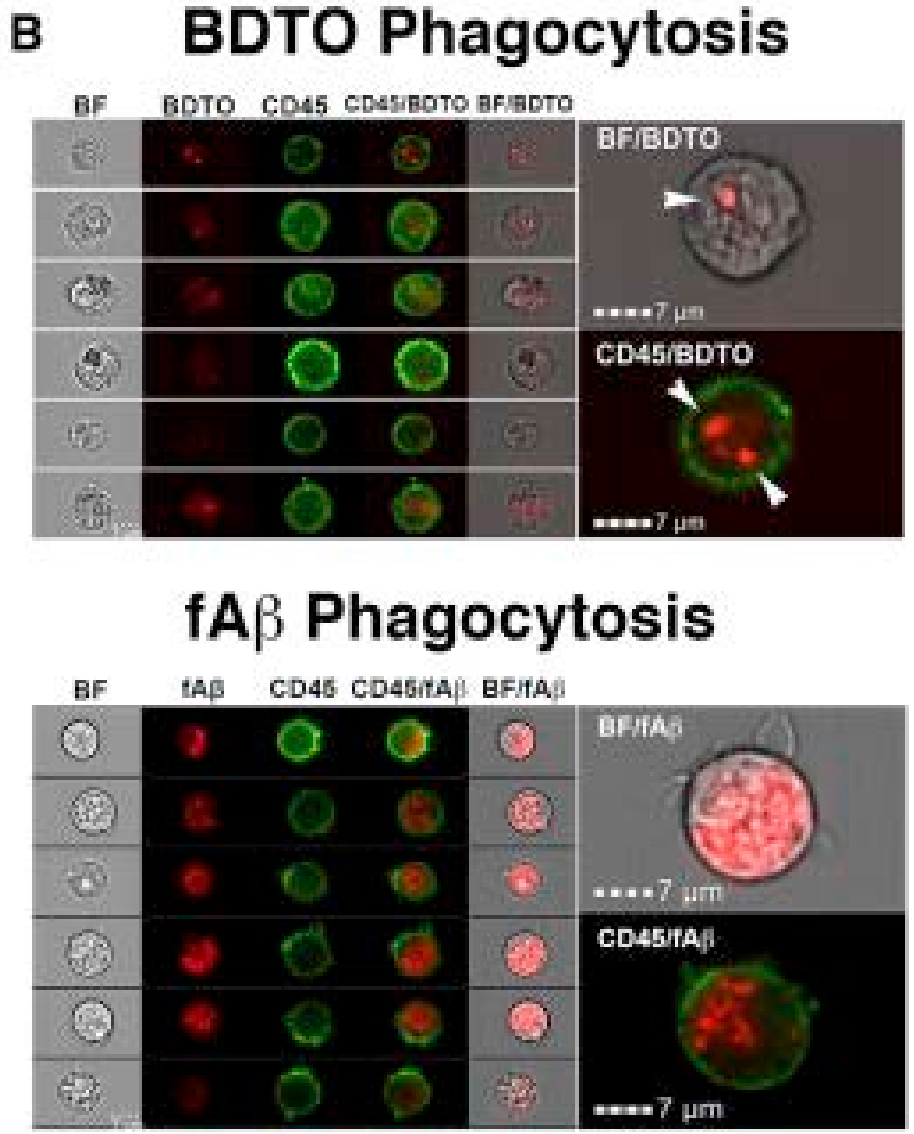


iMGL = induced pluripotent stem cell derived microglia-like cells

Phagocytosis assay: Tau and Aβ fibrils

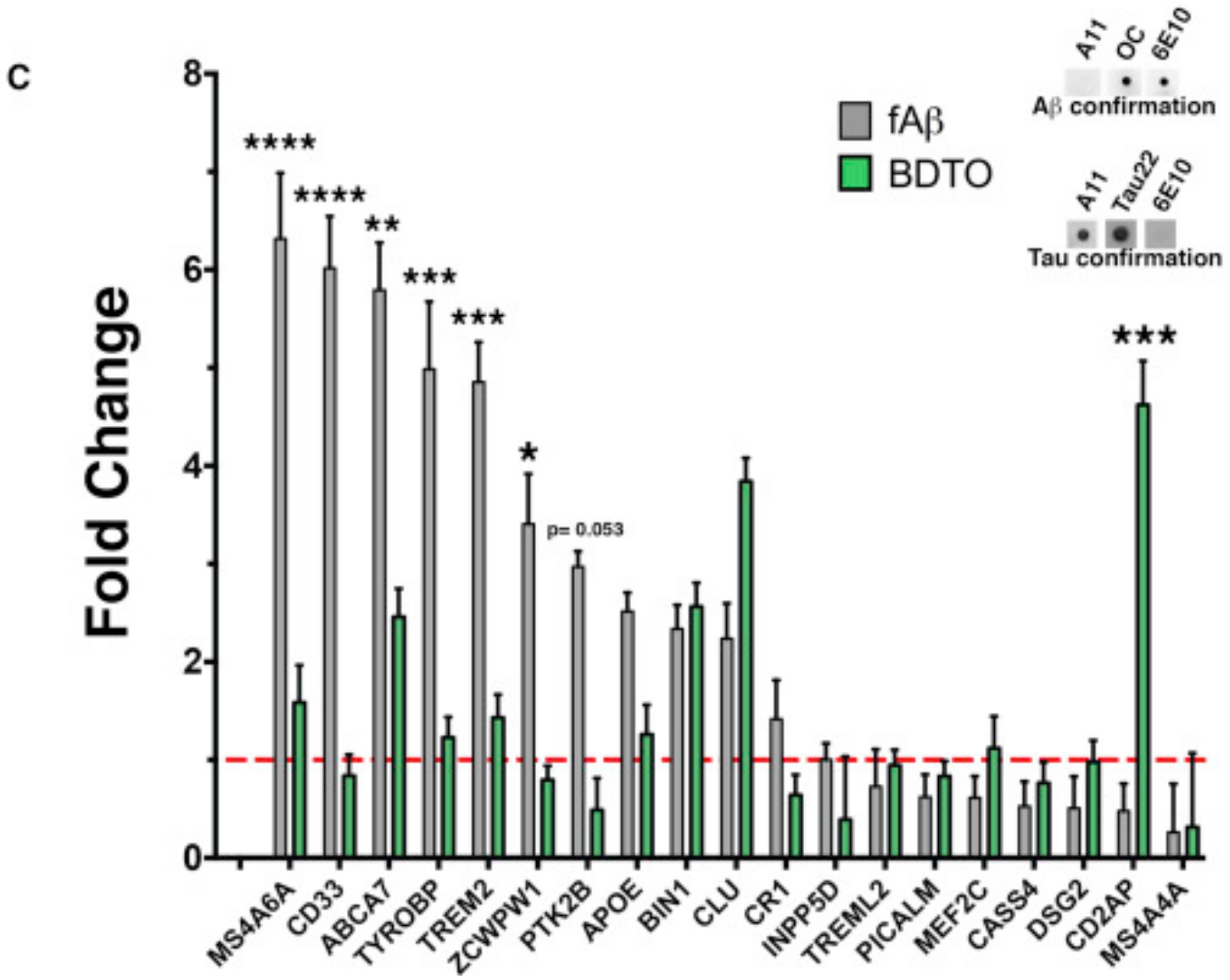
BDTO = brain-derived tau oligomers

iMGLs can internalize pHrodo dye BDTO and fluorescent labeled fAβ

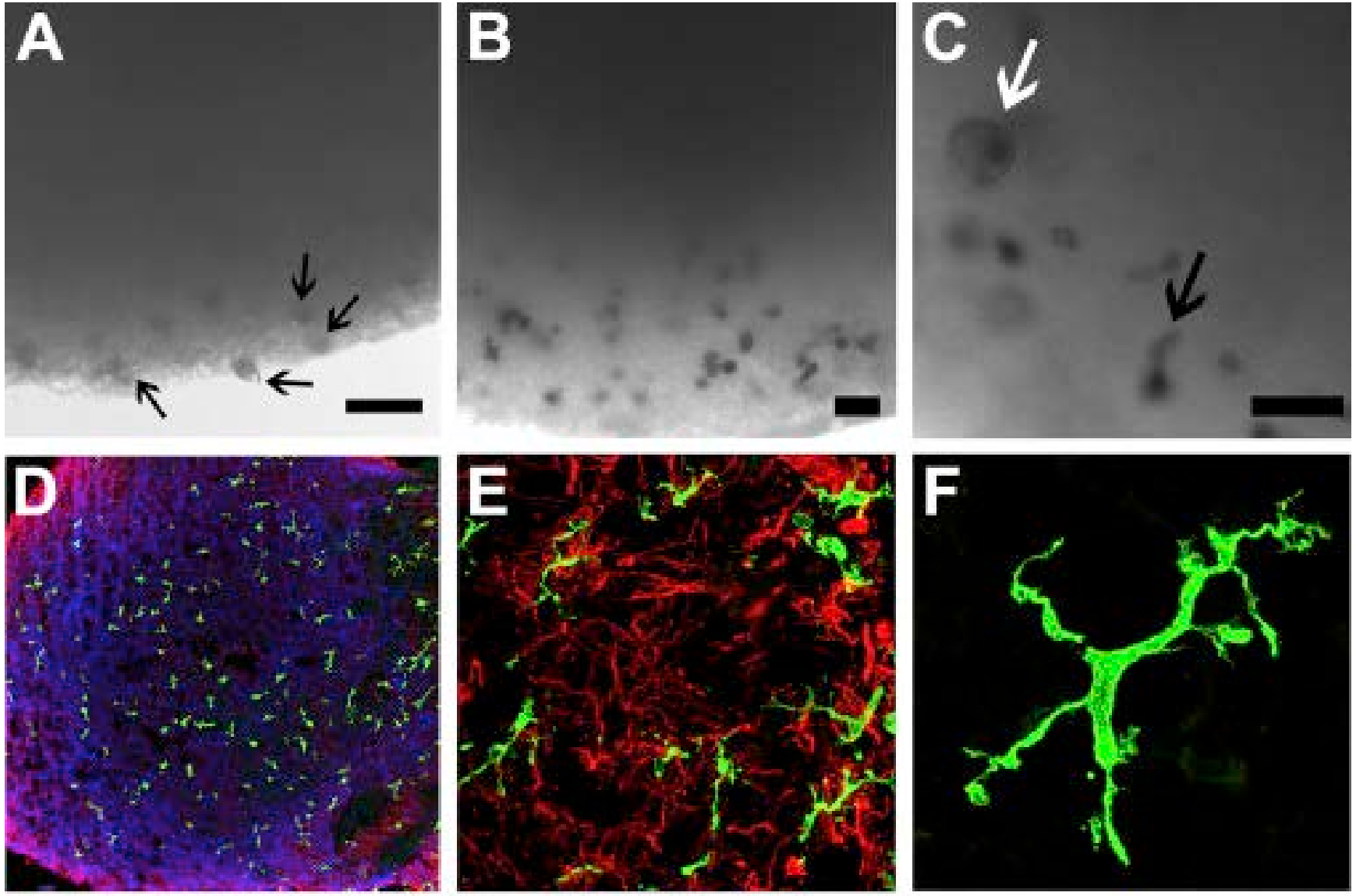


Phagocytosis assay: Tau and Aβ fibrils

mRNA expression changes of 19 GWAS genes associated with Alzheimers Disease upon exposure iMGLs to Tau and Aβ fibrils



iMGLs in a 3D brain organoid (BORG)



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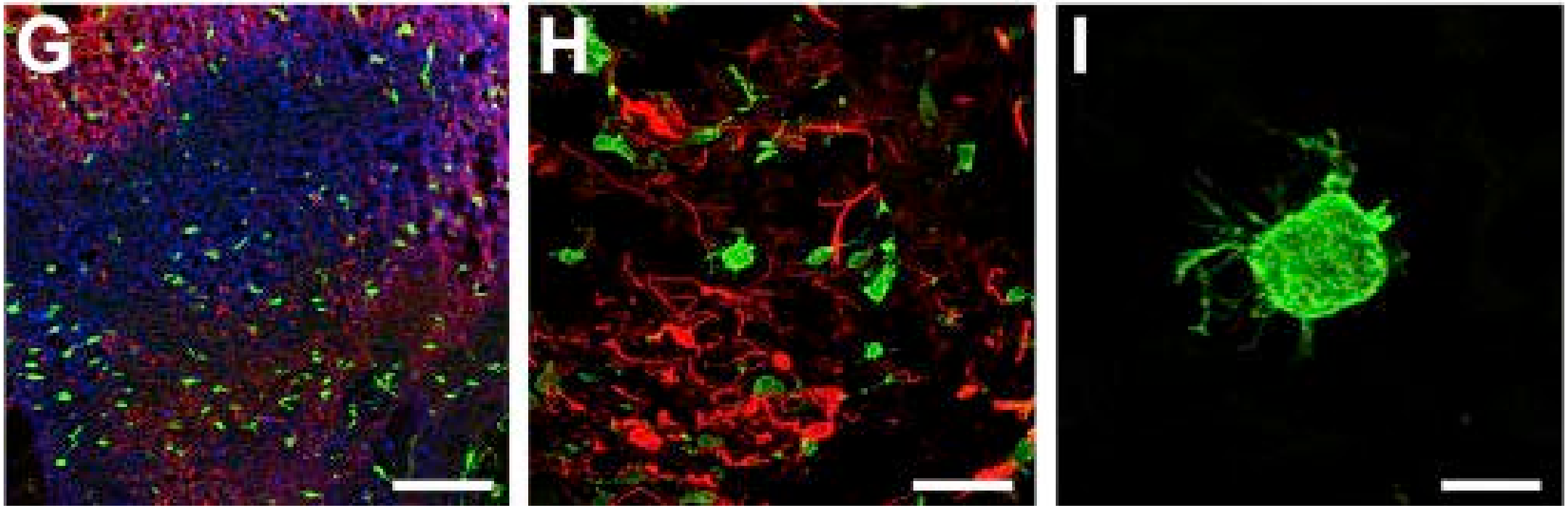
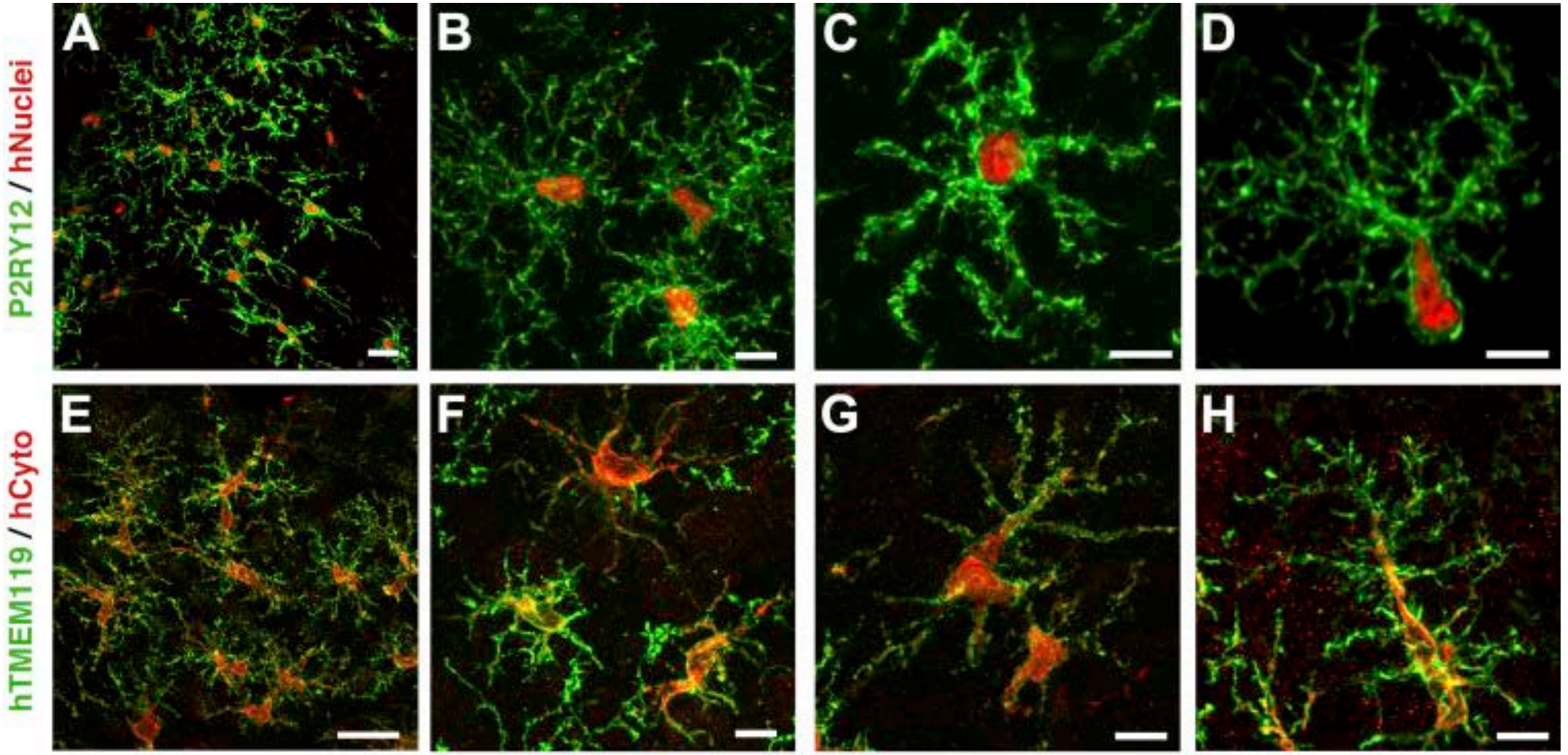


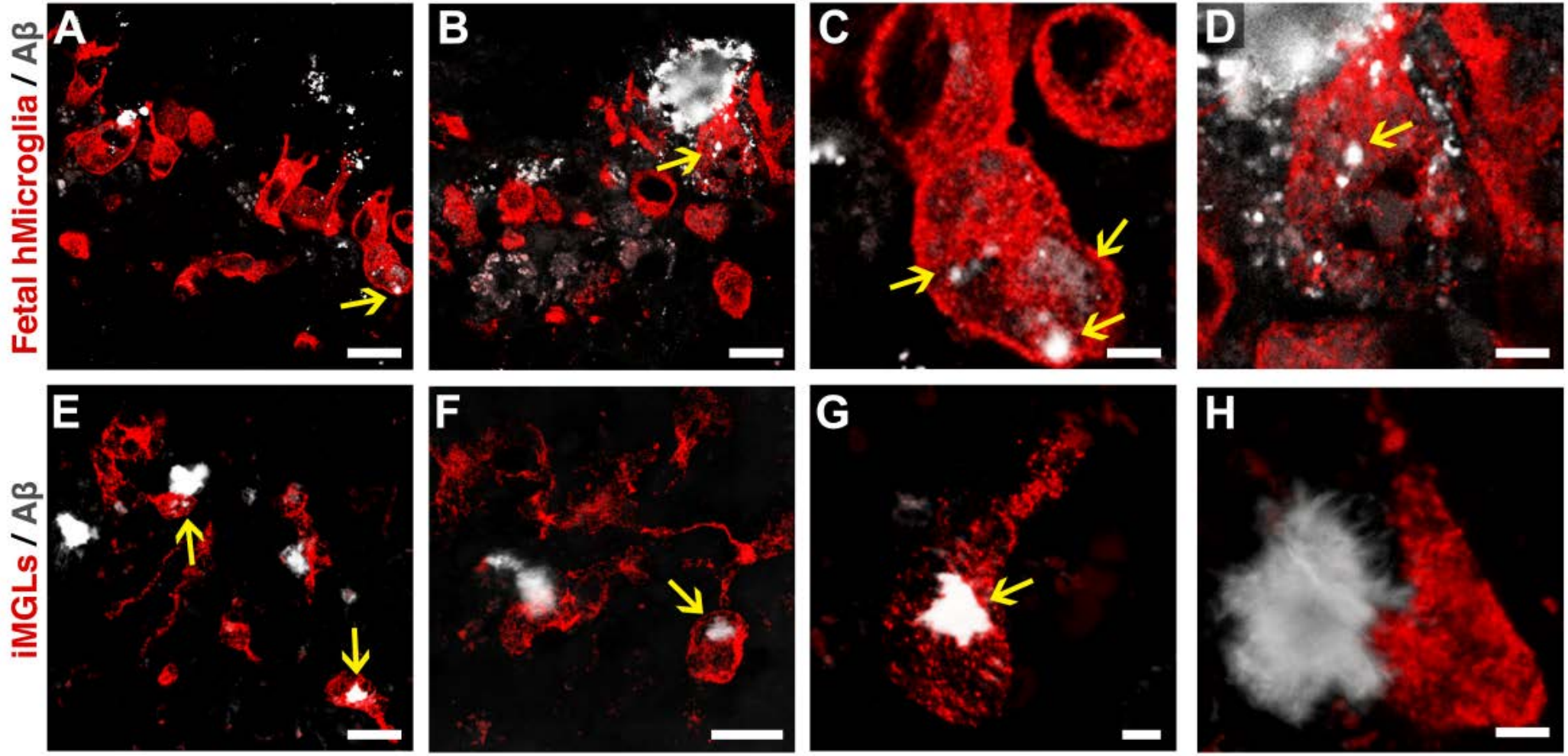
iMGLs in a CNS environment *in vivo*

Two months after transplantation

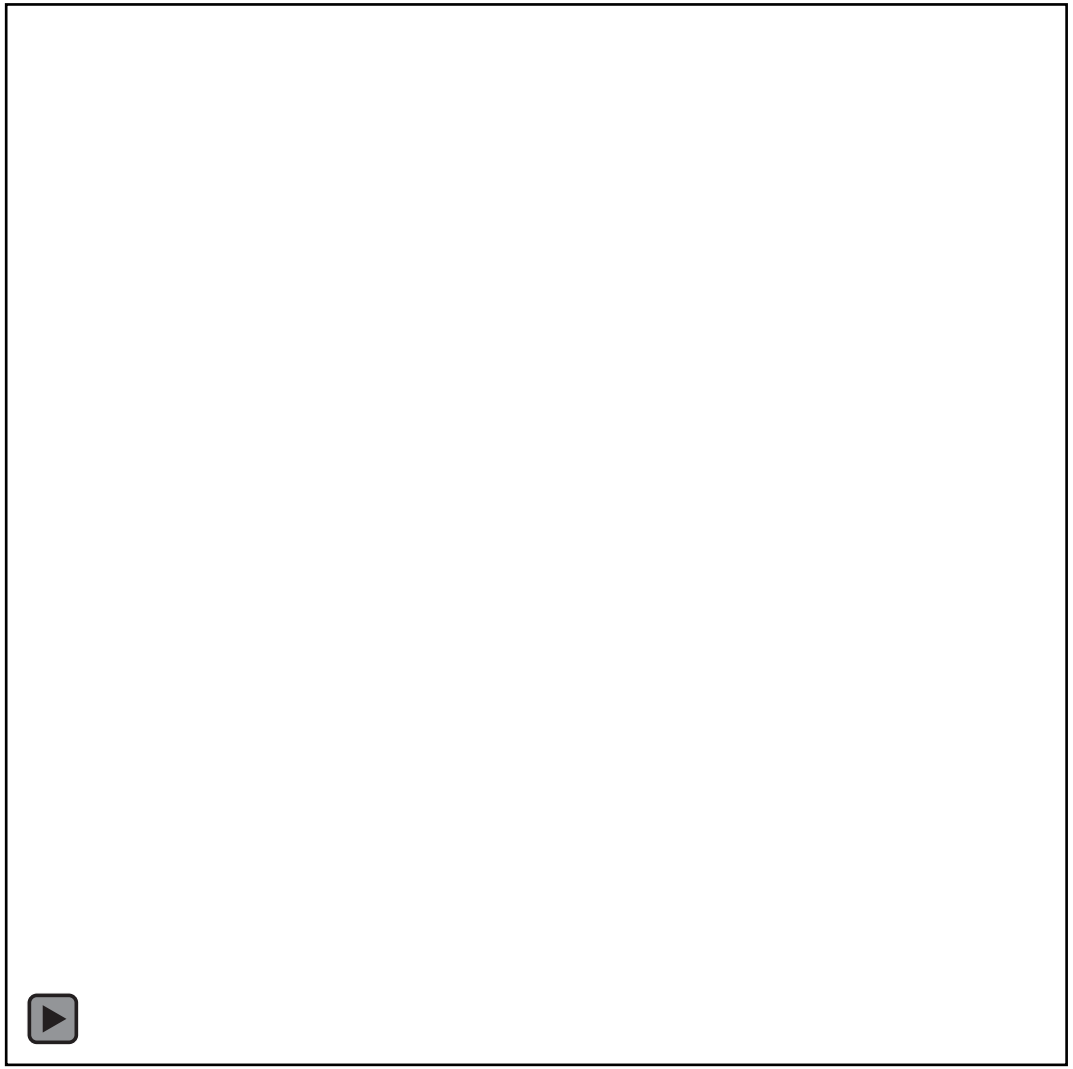


iMGLs in a CNS environment *in vivo*

Fetal microglia and iMGLs in hippocampus of AD mice



iMGLs in a CNS environment *in vivo*

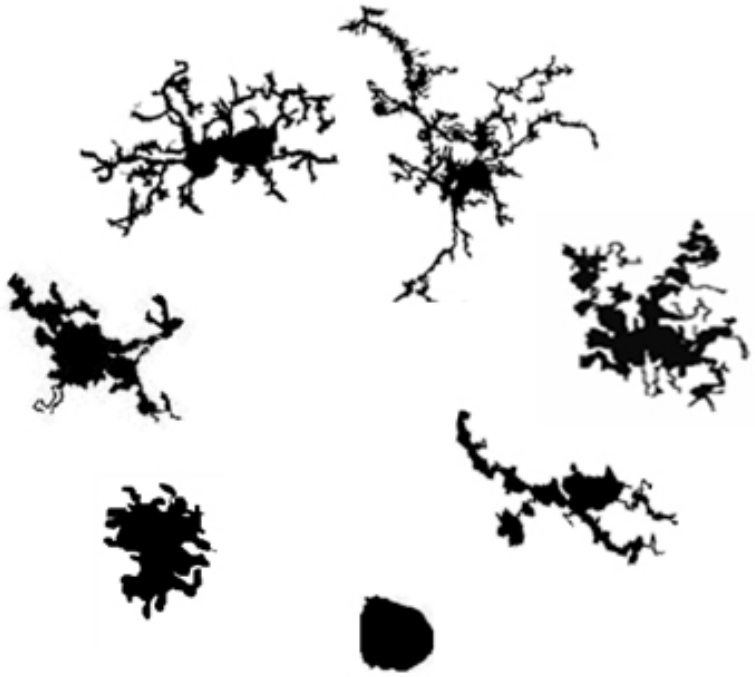


Summary 2nd Paper

- Also: Establishment of a robust protocol that allows for derivation of microglia-like cells from human pluripotent stem cells
- iMGLs responded well in a phagocytosis assay, implying normal physiological functionality
- iMGLs can intergrate within an *in vitro* 3D CNS environment, mature, ramify and respond to injury
- iMGLs as cell therapy: transplantation in AD mice

Conclusion

- iMGLs/pMGLs seem to resemble natural microglia in the characteristics shown
- Increase of the neurological disease toolbox, iPSC derived microglia might be useful for:
  - Disease modelling
  - Cell therapy



Thank you for your attention!