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Oriane Rabesandratana, Antoine Chaffiol, Amélie Slembrouck-Brec, Sacha Reichman, Céline Nanteau, Giuliana Gagliardi, Jens Duebel, Olivier Goureau, Gael Orioux

### ► To cite this version:

Oriane Rabesandratana, Antoine Chaffiol, Amélie Slembrouck-Brec, Sacha Reichman, Céline Nanteau, et al.. Promoting the functional maturation of human iPS cell-derived retinal ganglion cells with a 3D/2D stepwise differentiation protocol. Association for Research in Vision and Ophthalmology\_ARVO\_annual congress 2017, May 2017, Baltimore, United States. hal-03495768

**HAL Id: hal-03495768**

**<https://hal.science/hal-03495768v1>**

Submitted on 20 Dec 2021

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# Promoting the functional maturation of human iPSC cell-derived retinal ganglion cells with a 3D/2D stepwise differentiation protocol

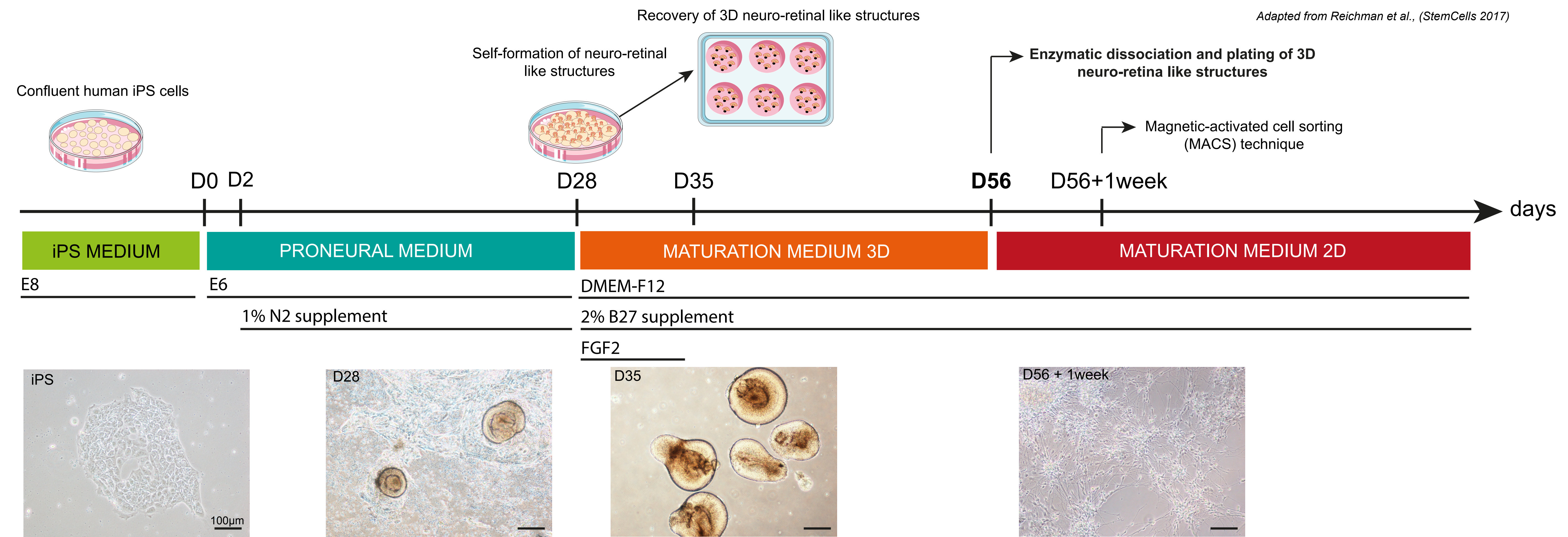
O. Rabesandratana, A. Chaffiol, A. Slembrouck, S. Reichman, C. Nanteau, G. Gagliardi, J. Duebel, O. Goureau, G. Orieux

Institut de la Vision, Sorbonnes Universités, UPMC - Univ Paris 06, INSERM U968, CNRS UMR 7210, Paris, France

Optic neuropathies have currently no effective treatment to stop the progressive loss of retinal ganglion cells (RGCs) and the degeneration of the optic nerve. Stem cell-based therapy using human induced pluripotent stem cells (hiPSCs) is one of the most promising strategies to replace lost RGCs.

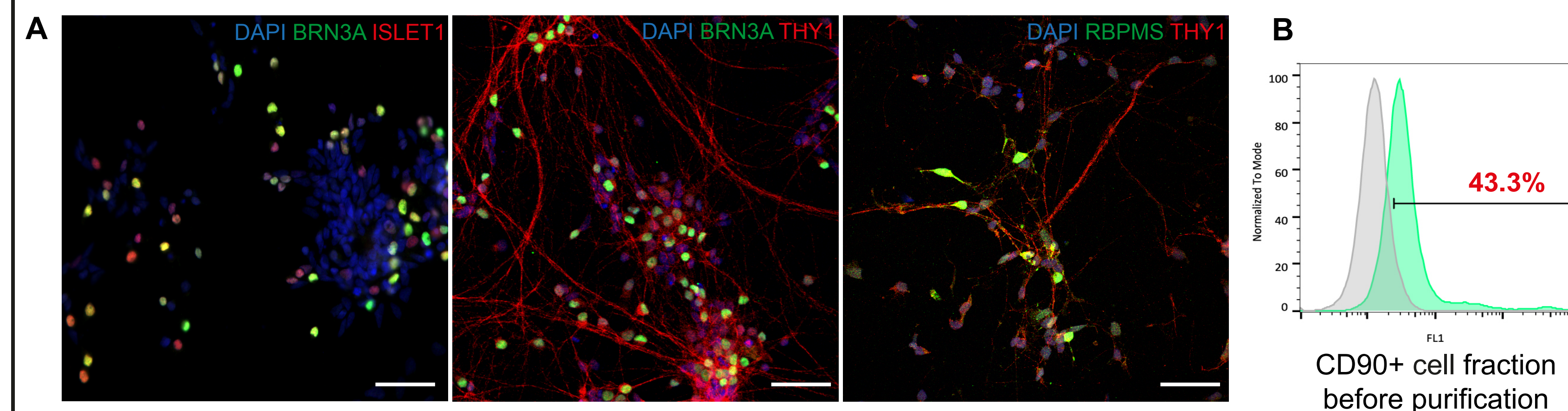
The present work displays the optimized generation and characterization of hiPSC-derived RGCs, based on our previous retinal differentiation protocol using adherent hiPSCs (Reichman et al., *StemCells* 2017).

Here, a new differentiation protocol was developed, based on two major steps in order to promote the generation of functional hiPSC-derived RGC: firstly the isolation and the culture of 3D neuro-retinal structures and secondly the culture on adherent coating of dissociated neuro-retinal cells prior to magnetic cell sorting based on CD90/Thy1 expression.



## In vitro characterization of human iPSC-RGCs

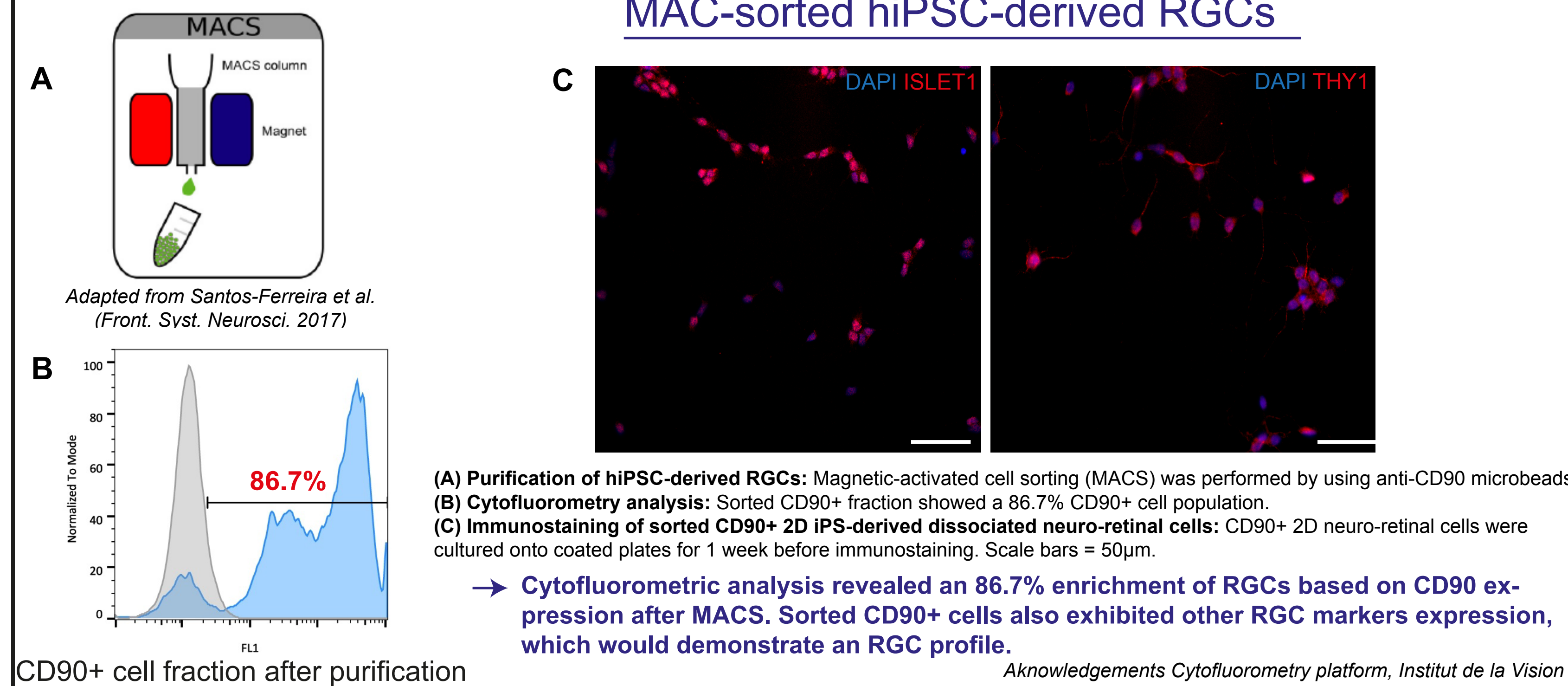
### Unsorted hiPSC-derived RGCs



(A) Immunostaining of 2D iPSC-derived dissociated neuro-retinal cells: 3D neuro-retinal structures were dissociated at D56 and cultured onto poly-D-lysine/laminin coated plates for 1-2 weeks before immunostaining. Scale bars = 50µm.  
(B) Before MACS, the cell population consists of 43.3 % CD90(Thy1)-positive cells.

→ The culture of dissociated neuro-retinal organoids on coated plates promotes neurite growth from cells expressing RGC specific markers.

### MAC-sorted hiPSC-derived RGCs



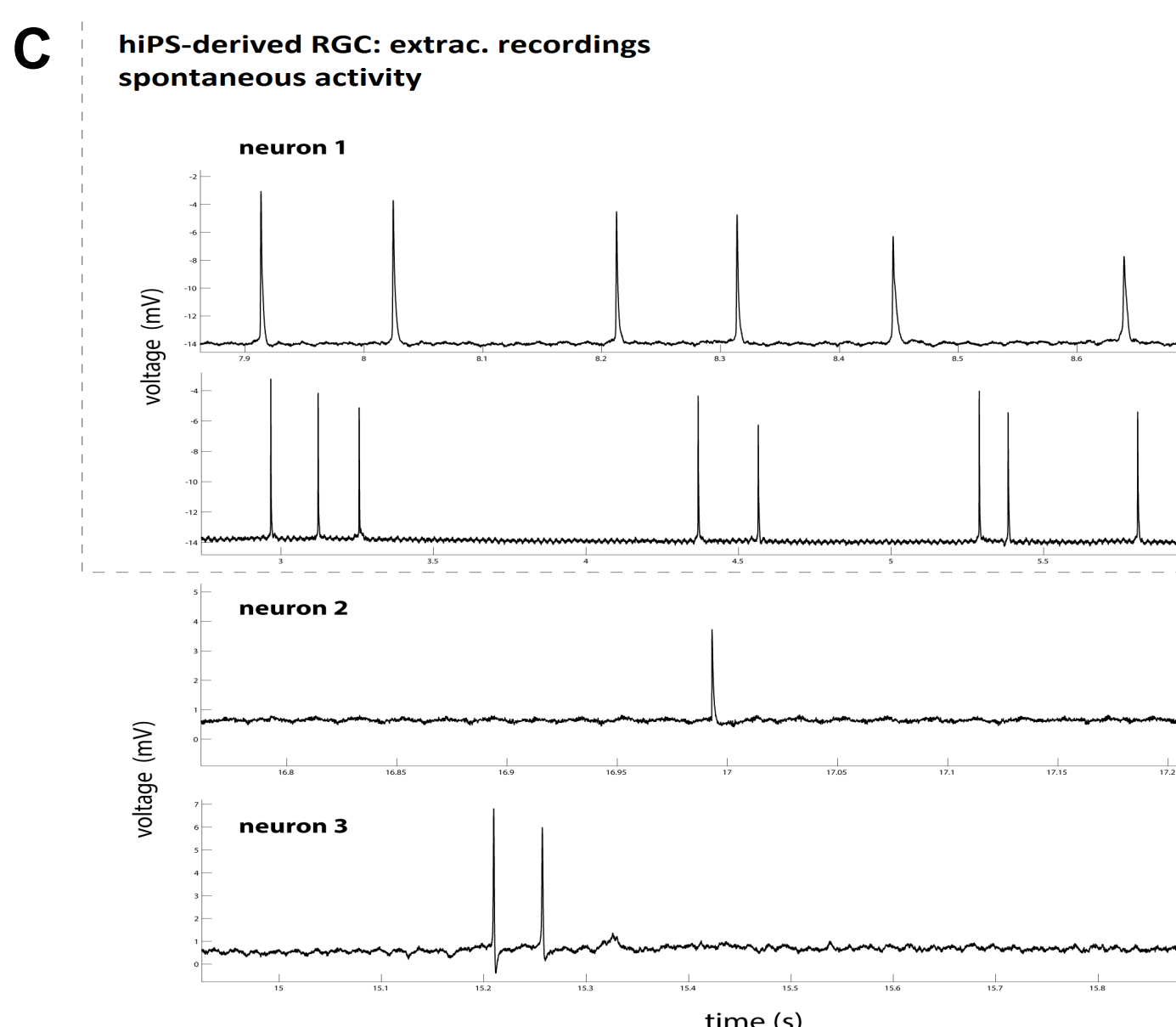
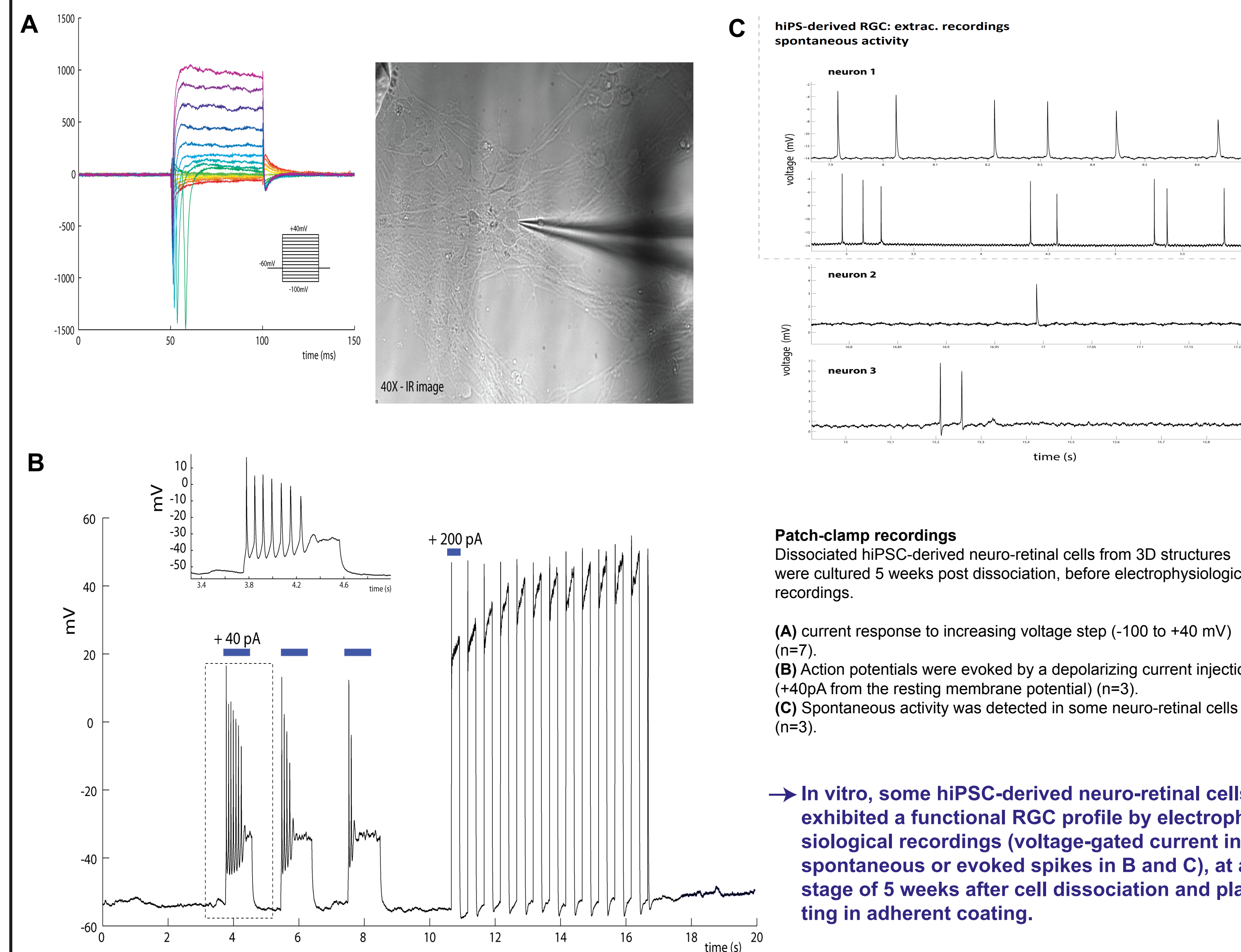
(A) Purification of hiPSC-derived RGCs: Magnetic-activated cell sorting (MACS) was performed by using anti-CD90 microbeads.  
(B) Cytofluorometry analysis: Sorted CD90+ fraction showed a 86.7% CD90+ cell population.  
(C) Immunostaining of sorted CD90+ 2D iPSC-derived dissociated neuro-retinal cells: CD90+ 2D neuro-retinal cells were cultured onto coated plates for 1 week before immunostaining. Scale bars = 50µm.

→ Cytofluorometric analysis revealed an 86.7% enrichment of RGCs based on CD90 expression after MACS. Sorted CD90+ cells also exhibited other RGC markers expression, which would demonstrate an RGC profile.

Acknowledgements Cytofluorometry platform, Institut de la Vision

## Functional characterization of human iPSC-RGCs

### Electrophysiological recordings of 5 weeks post-dissociation



Patch-clamp recordings  
Dissociated hiPSC-derived neuro-retinal cells from 3D structures were cultured 5 weeks post dissociation, before electrophysiological recordings.

(A) current response to increasing voltage step (-100 to +40 mV) (n=7).  
(B) Action potentials were evoked by a depolarizing current injection (+40pA from the resting membrane potential) (n=3).  
(C) Spontaneous activity was detected in some neuro-retinal cells (n=3).

→ In vitro, some hiPSC-derived neuro-retinal cells exhibited a functional RGC profile by electrophysiological recordings (voltage-gated current in A; spontaneous or evoked spikes in B and C), at a stage of 5 weeks after cell dissociation and plating in adherent coating.

## Discussion

Our results support the conclusion that the differentiation and the isolation of hiPSCs-derived RGC is facilitated by the sequential two step culture (3D/2D). The culture of hiPSC-derived neuro-retinal cells on coated plates stimulate the growth of Thy1-positive neurites from RGC allowing the purification of this cell population, a required step for future transplantation studies and contribute to the functional maturation of RGC as demonstrated by the electrophysiological investigations.

### In vivo characterization of human iPSC-RGCs

