Marcelo N. Rivolta

Centre for Stem Cell Biology.
Department of Biomedical Science
The University of Sheffield. Reino Unido

- Director de laboratorio en el Centro de Biología de Células Madre de la Universidad de Sheffield
- Dedicado al estudio pionero de las células madre humanas y sus posibles aplicaciones terapéuticas para la sordera
- Recientemente su laboratorio identificó y aisló por vez primera una población de células madres cocleares humanas (hFASCs)

FUNDACION RAMON ARECES

Unidad de Sordera y Vértigo

Marcelo N. Rivolta
Centre for Stem Cell Biology.
Department of Biomedical Science
The University of Sheffield. Reino Unido

Aplicación experimental de las células madre en el tratamiento de la sordera: estado actual y nuevas perspectivas

Experimental application of stem cells for the treatment of deafness: state of the art and new perspectives

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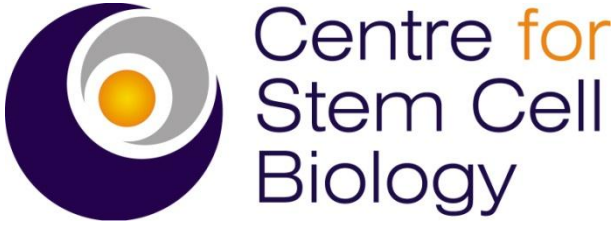
Unidad de Sordera y Vértigo

Experimental Application of Stem Cells for the Treatment of Deafness: State of the Art and New Perspectives

Marcelo N. Rivolta

Centre for Stem Cell Biology and Dept of Biomedical Sciences

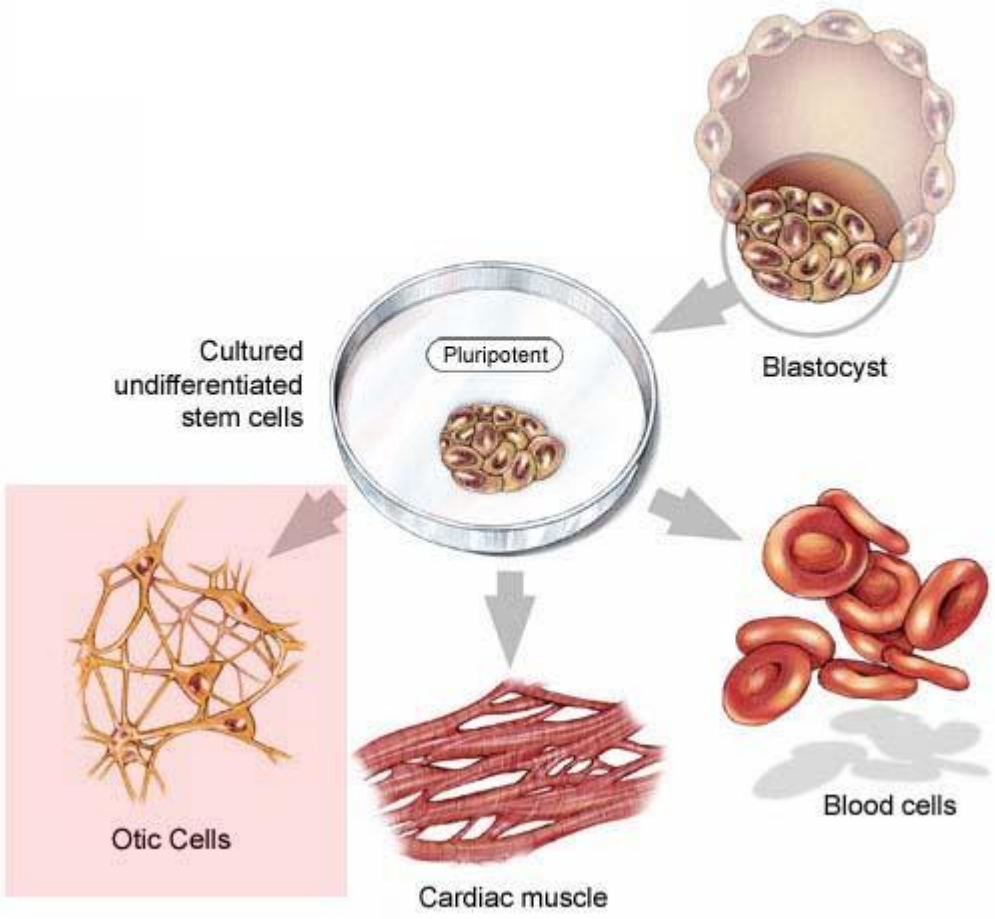


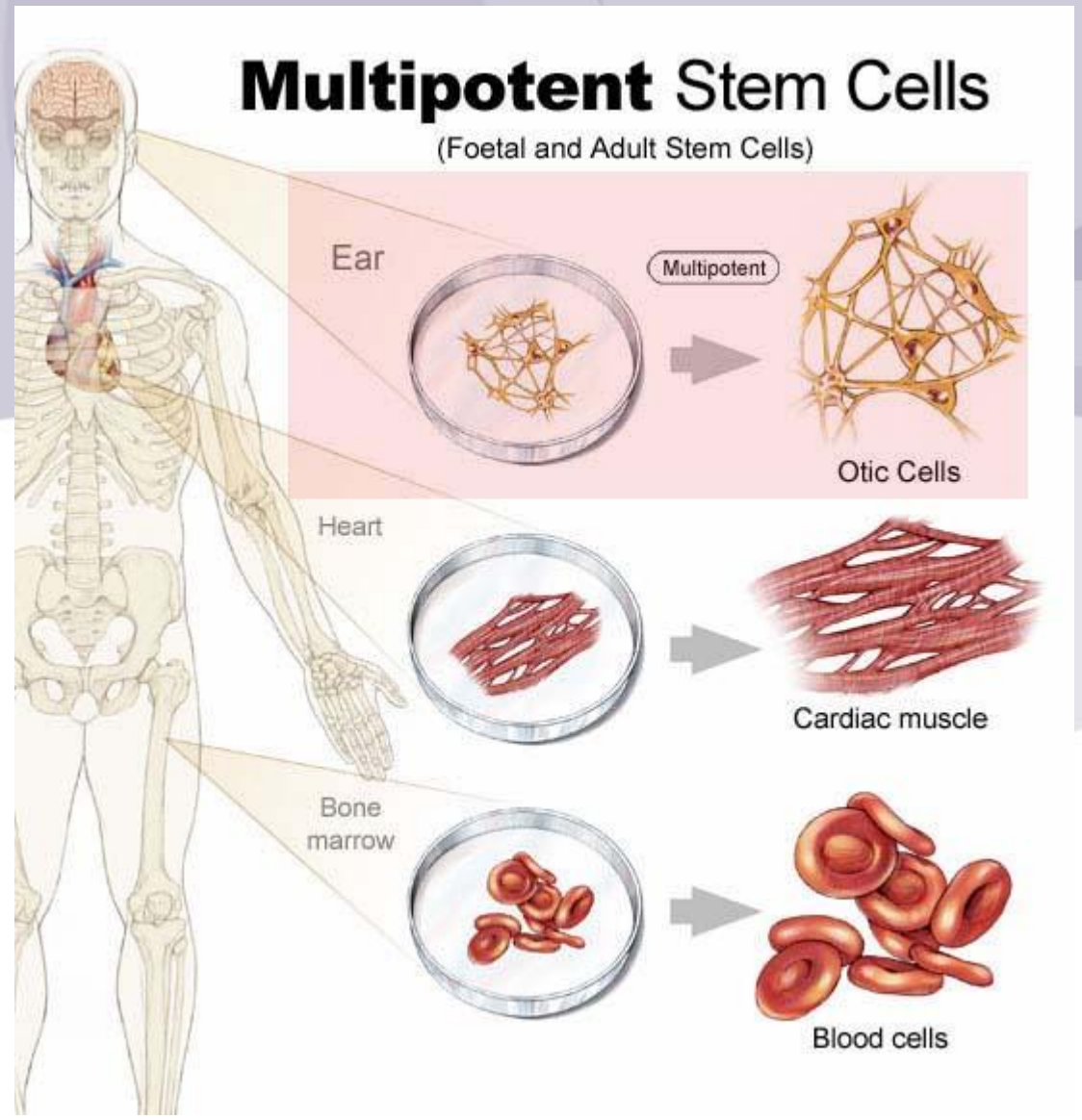
Isolation of human auditory stem cells

- The need for model systems to study human auditory biology
  - Physiology, Gene function
- In vitro systems for toxicity assays and to facilitate drug discovery
- Direct therapeutic application (cell replacement)

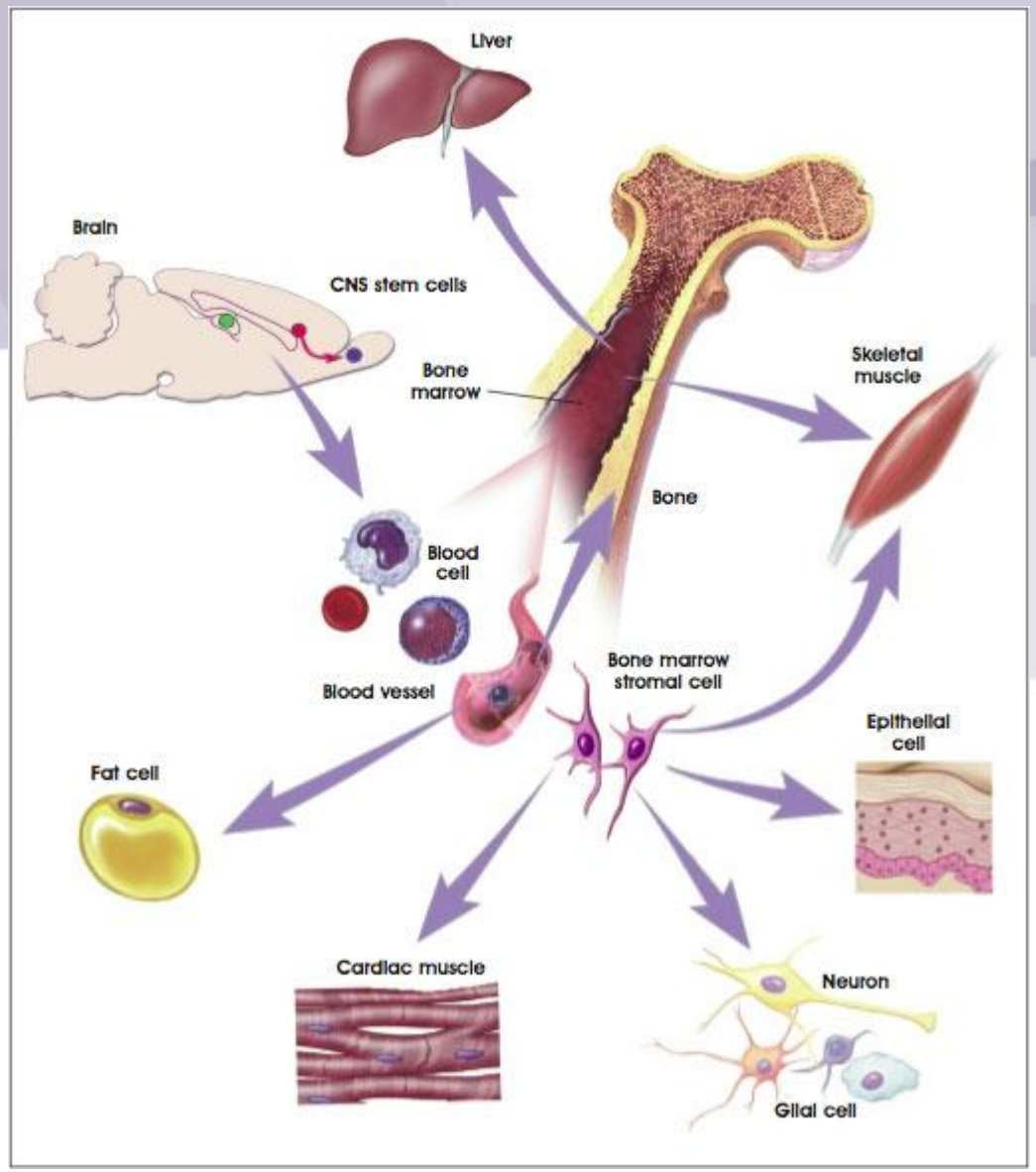
Pluripotent Stem Cells

(Embryonic Stem Cells)

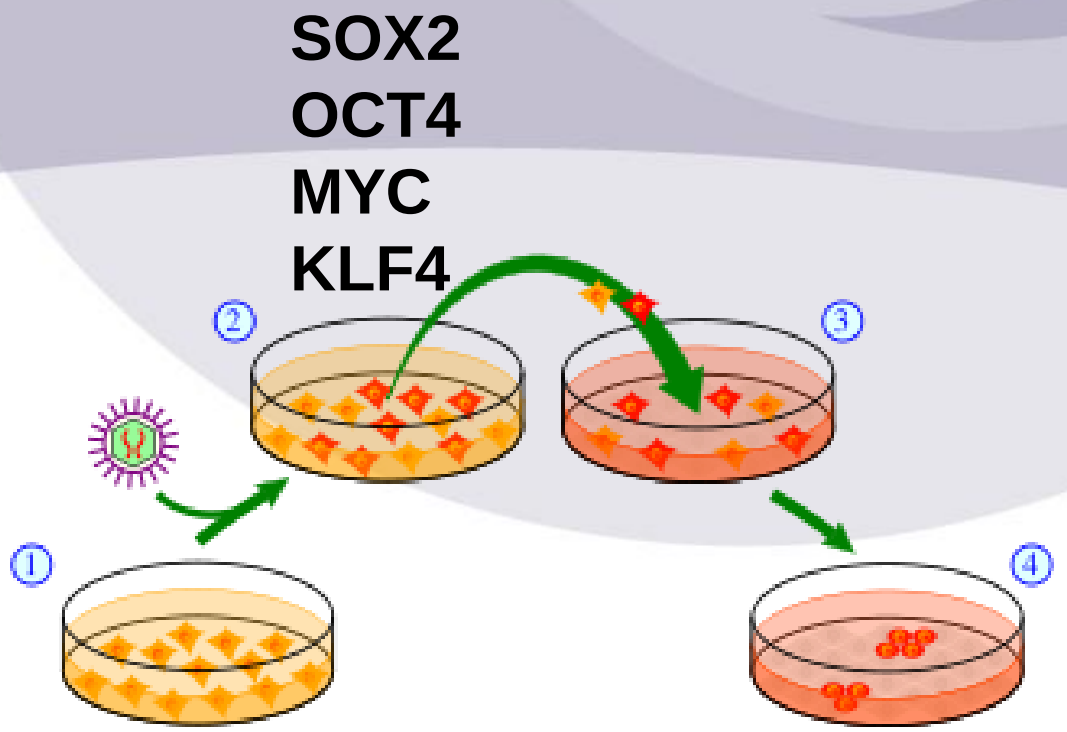



Multipotent Stem Cells
(Foetal and Adult Stem Cells)
Ear
Multipotent
Otic Cells
Heart
Cardiac muscle
Bone marrow
Blood cells

•The bone marrow is one of the adult organs with larger number of stem cells, although with a limited potential (multipotents)



Generation of iPS cells (induced pluripotency)



Skin cells

iPS cells
(similar to the embryonic stem cells)

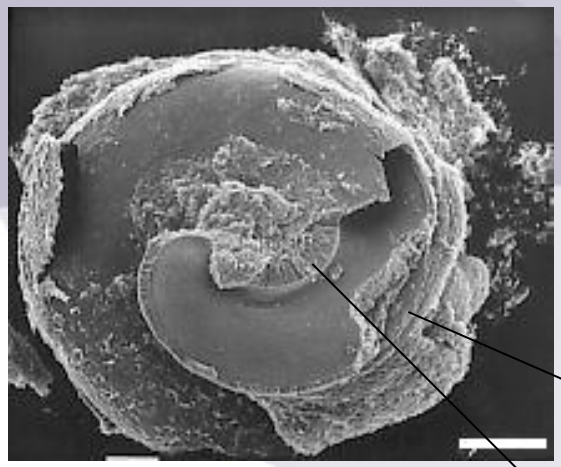
Isolation of human auditory stem cells

- Multipotent auditory stem cells from human fetal tissues (hFASCs)
  - *Wei Chen*
- Step-wise differentiation of auditory phenotypes from hES cells
  - *Wei Chen, Nop Jong, Jo Thurlow, Sarah Jacob, Leila Abbas*
- And from induced-Pluripotent stem cells (iPSCs)
  - *Ricardo Romero*
- Phenotypic conversion of adult stem cells (i.e. bone marrow MSCs)
  - *Wei Chen, Ricardo Romero, Sarah Boddy*

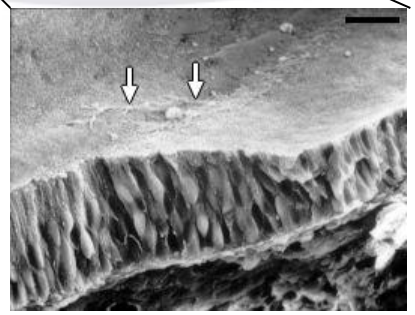
- Isolation of auditory stem cells from the fetal cochlea

Chen et al. (2007) *Hearing Res* 233:23-29
Chen et al. (2009) *Stem Cells* 27:1196-1204.

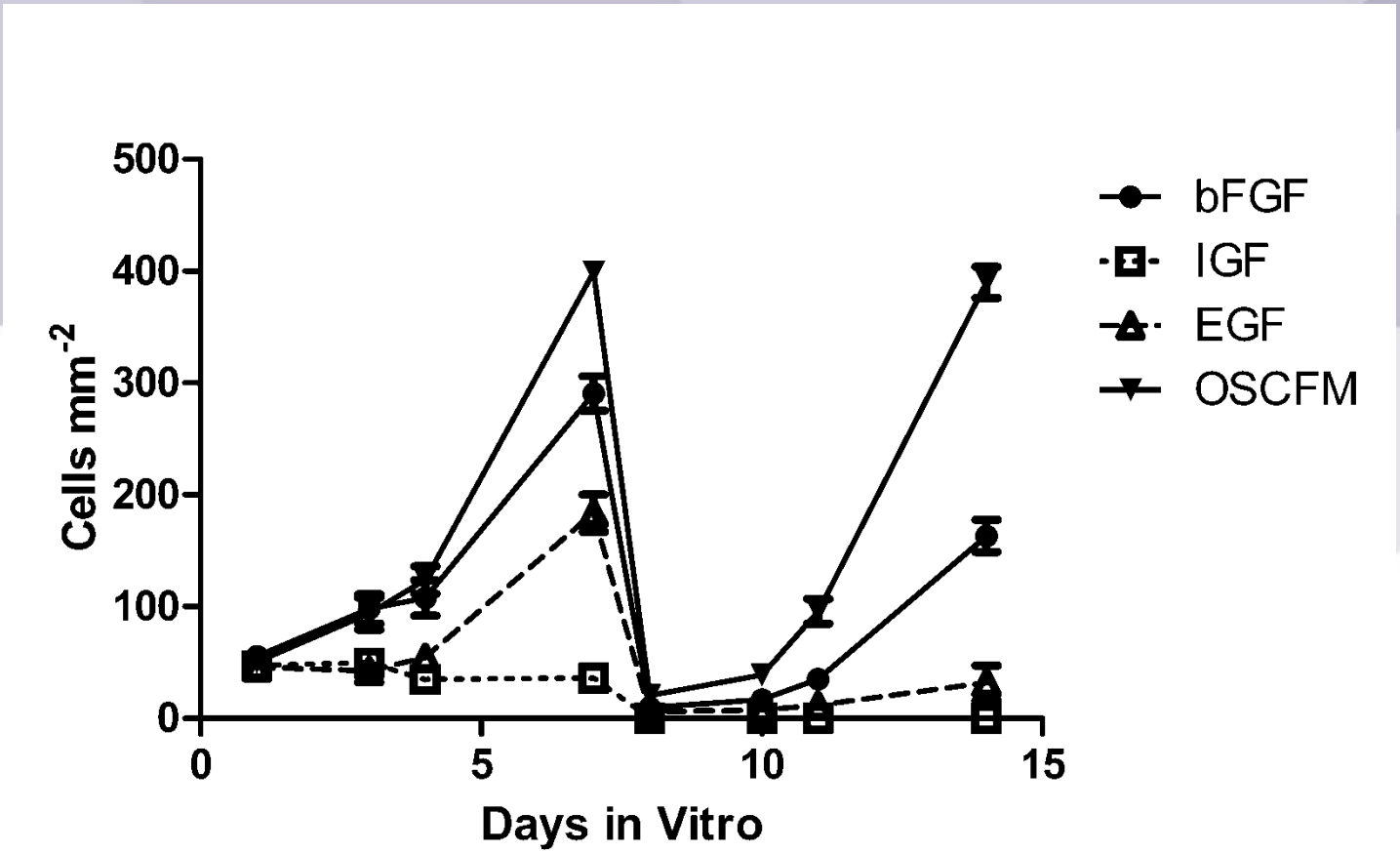
View of a 9-10 weeks old human cochlea



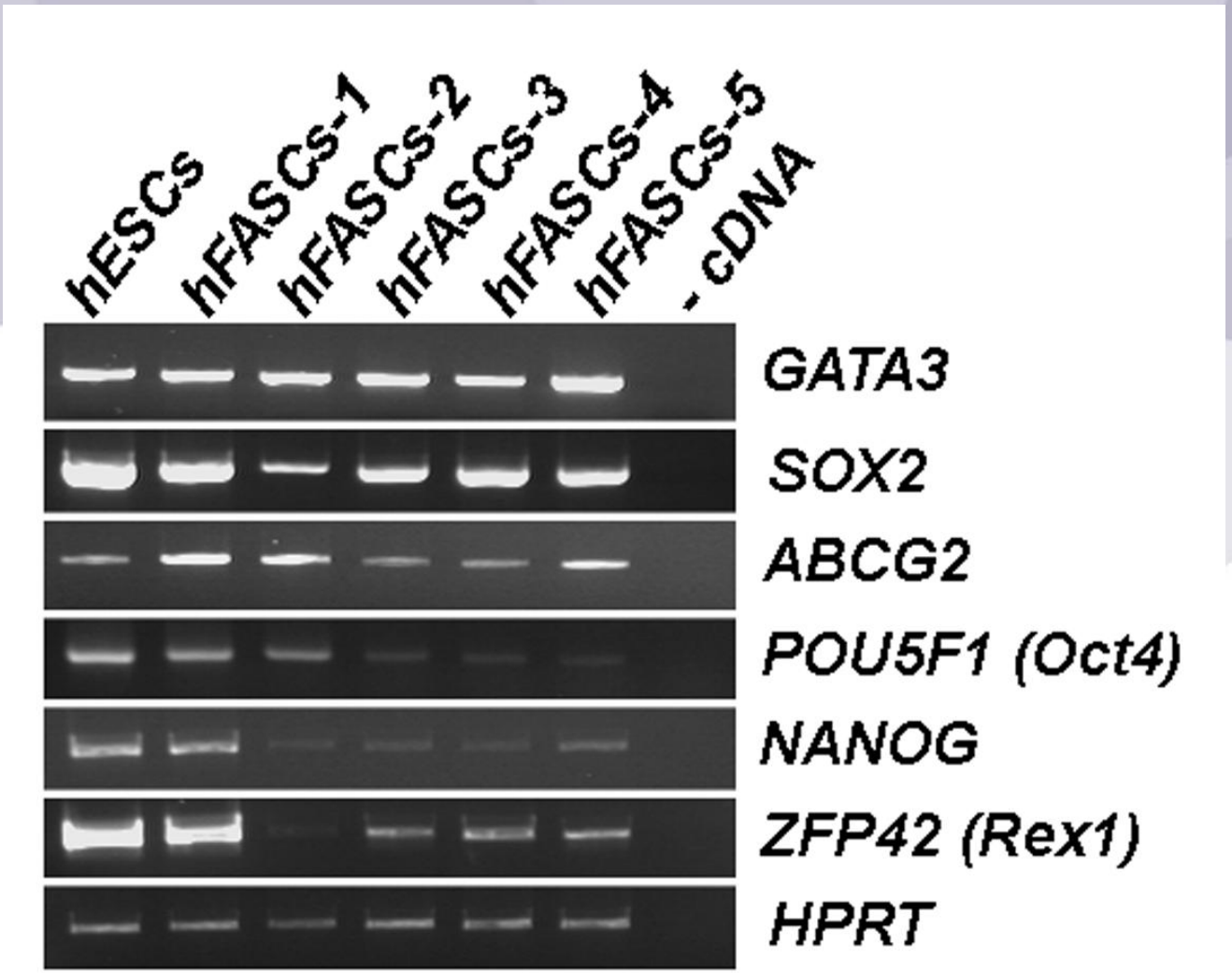
(Adapted from M. Lavigne-Rebillard, http://www.cochlea.org/)

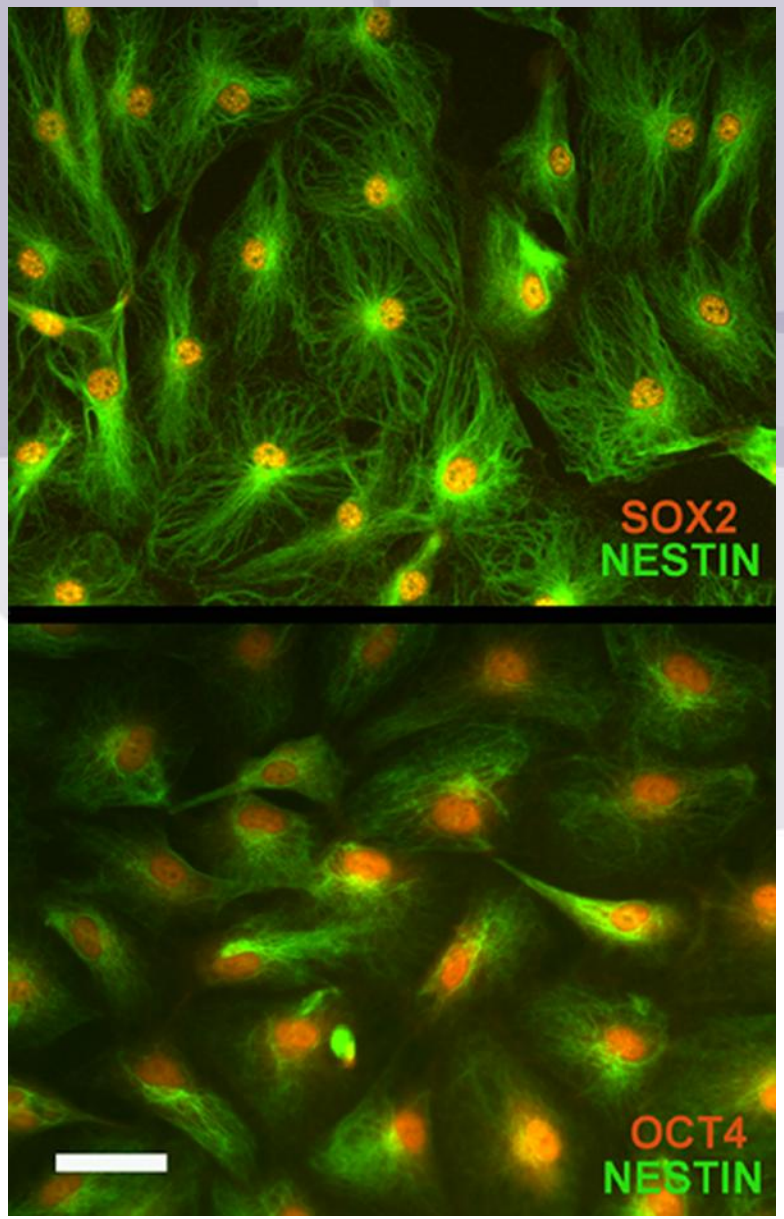


Serum-free conditions support the culture of hFASCs

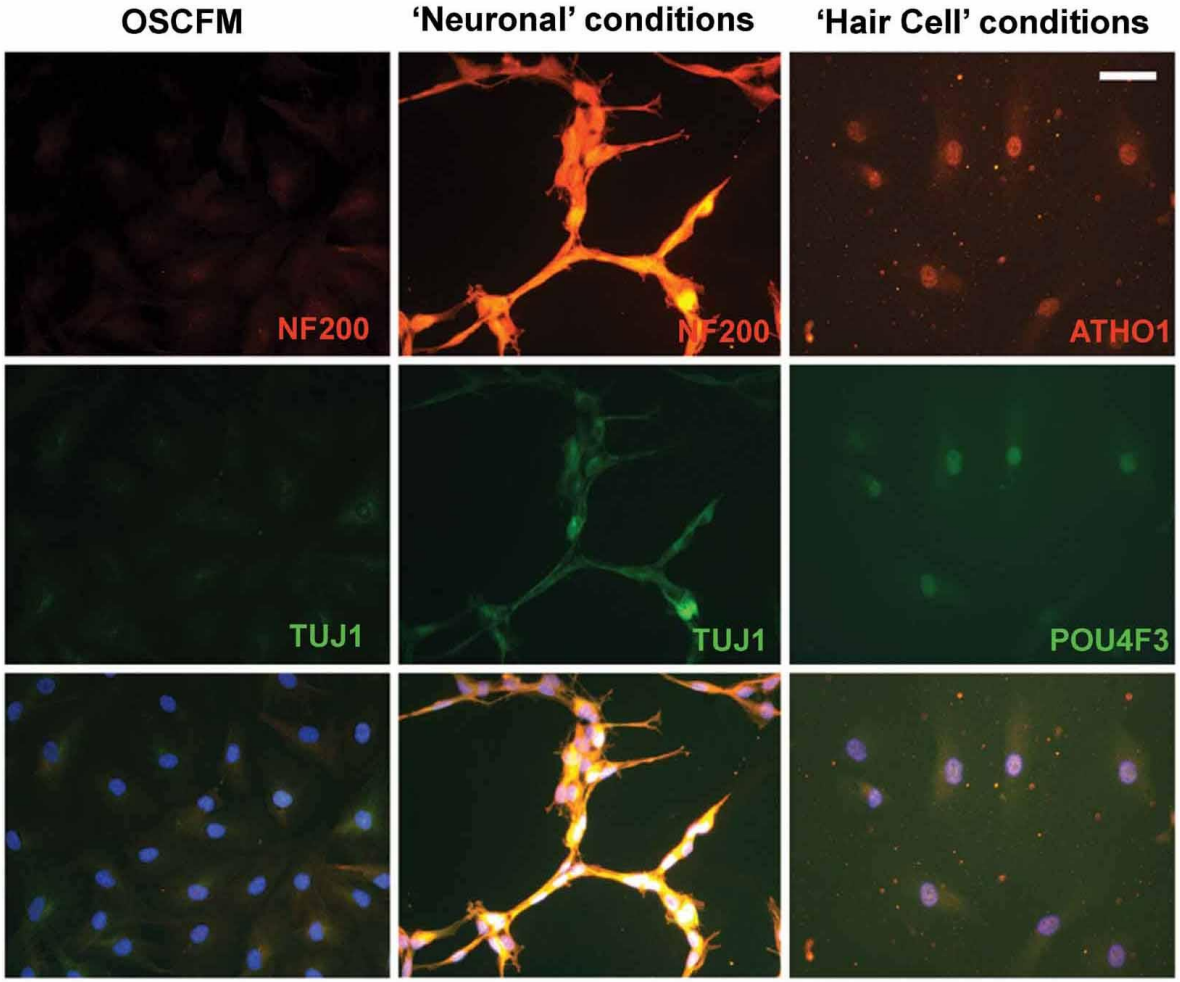


hFASCs express stem cell and progenitor markers

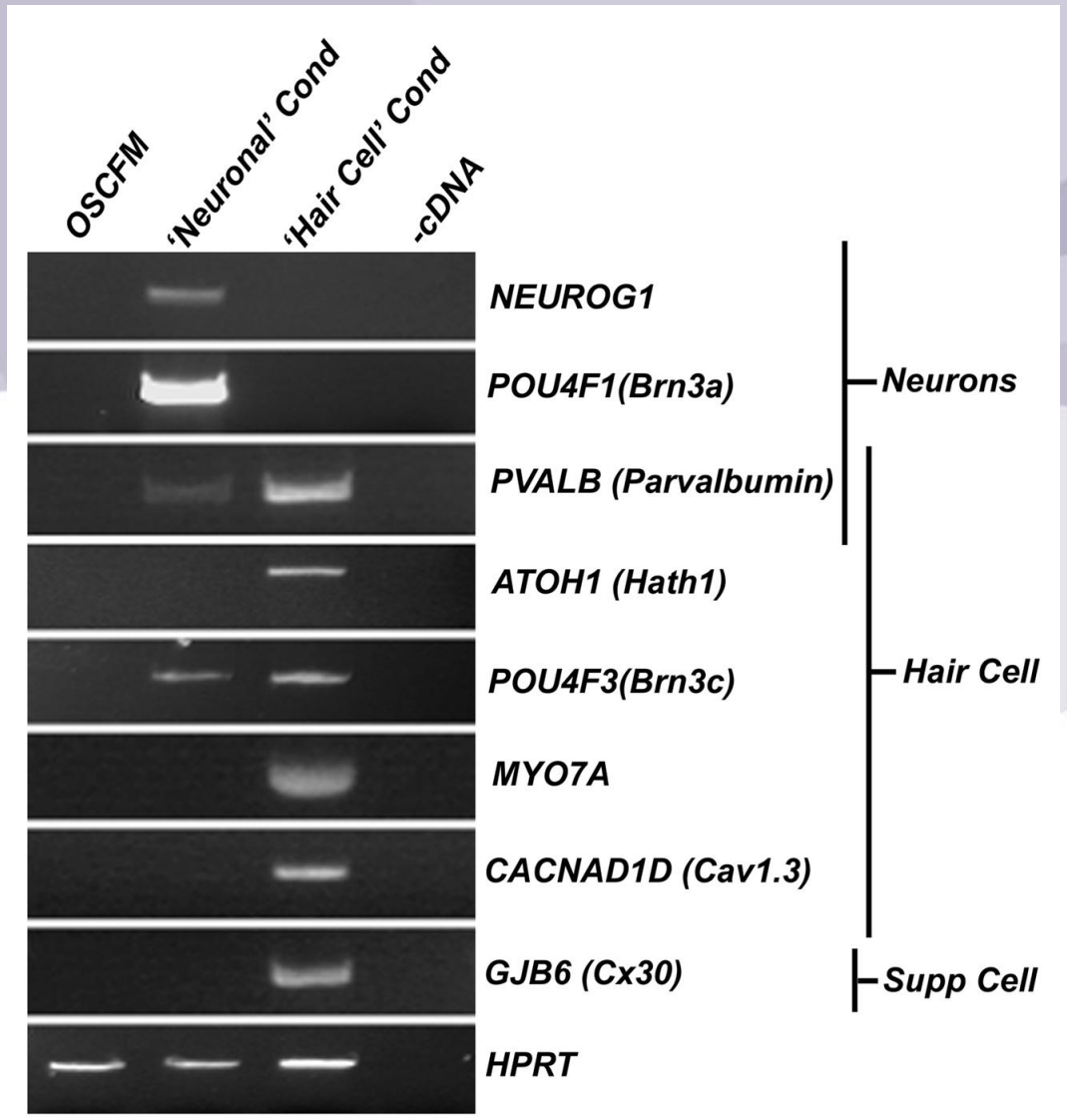



SOX2
NESTIN
OCT4
NESTIN

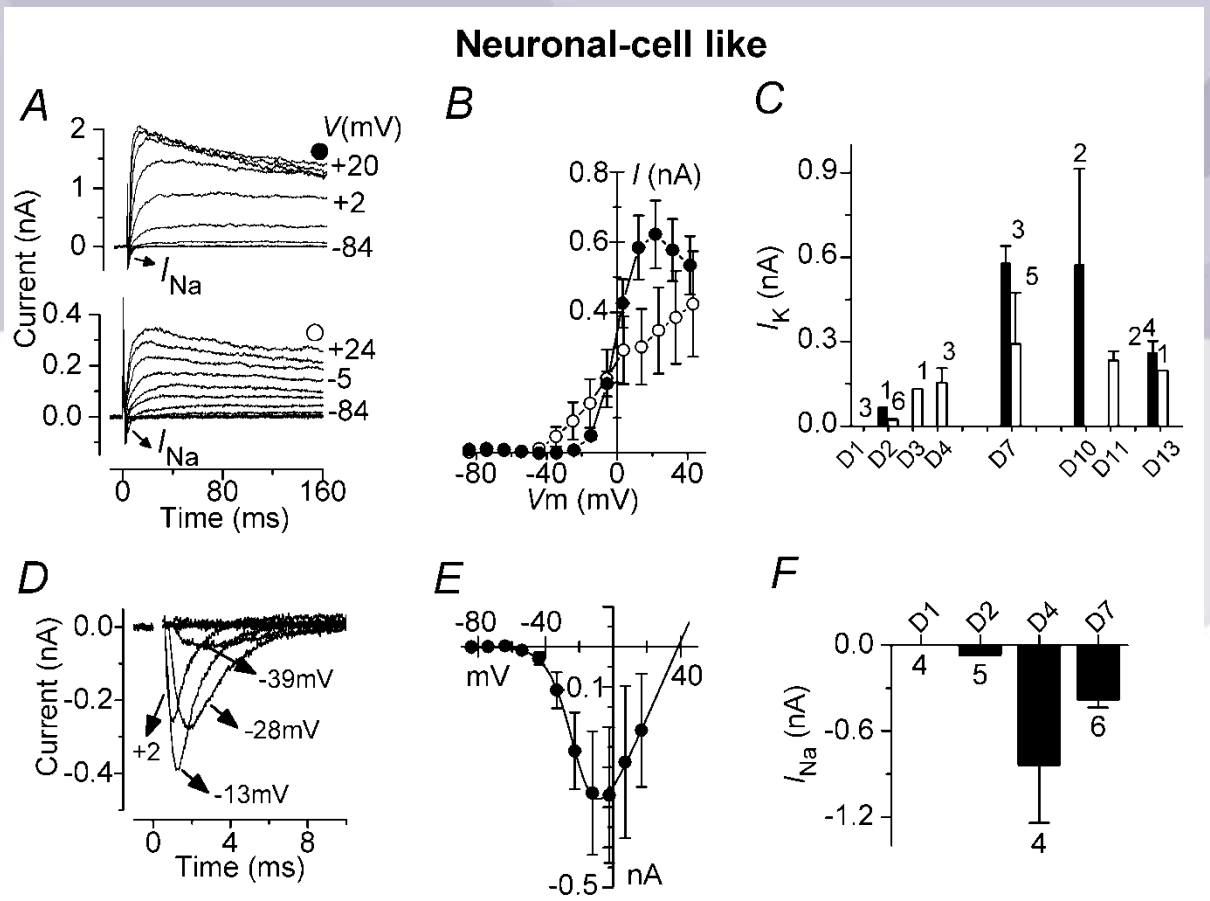

A
B
C


OSCFM
'Neuronal' conditions
'Hair Cell' conditions
NF200
NF200
ATHO1
TUJ1
TUJ1
POU4F3

RT-PCR profile of lineage markers of differentiating hFASCs

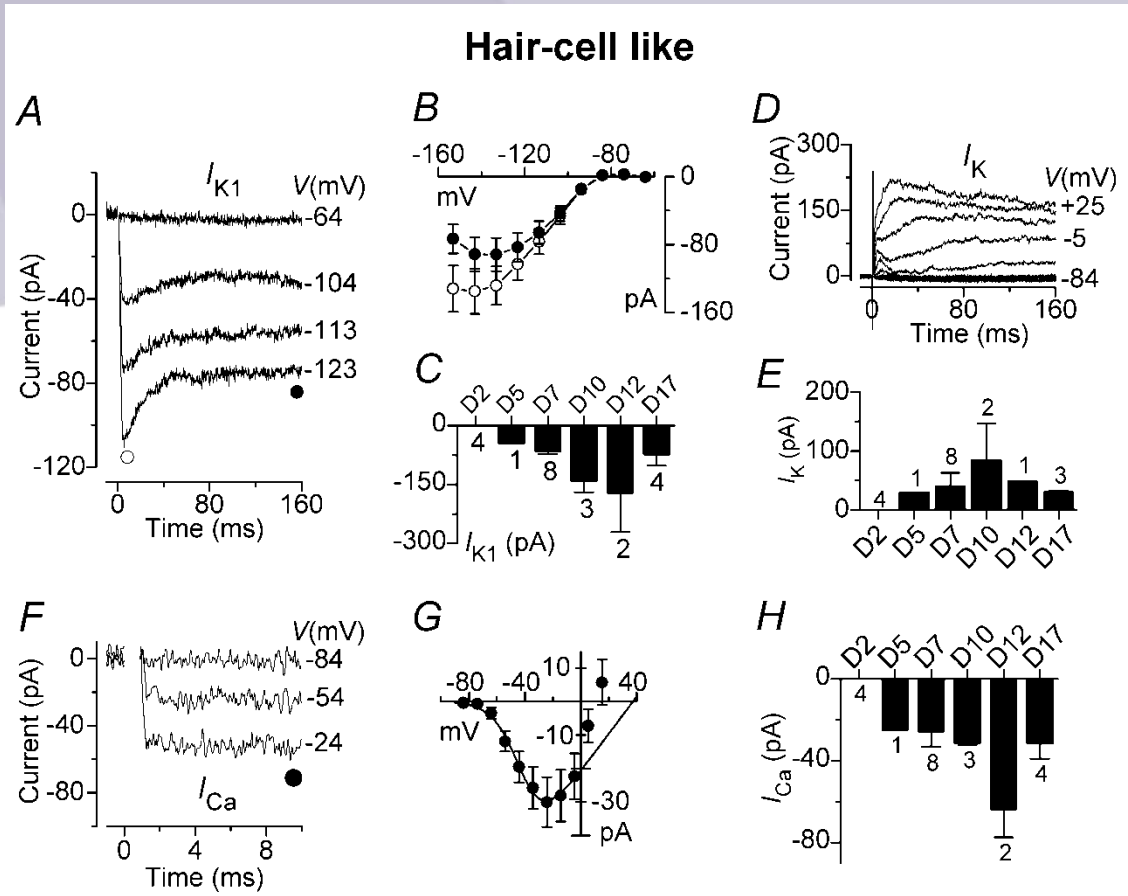


The neuralizing protocol induced bipolar cells that displayed potassium delayed rectifiers and voltage-gated sodium currents



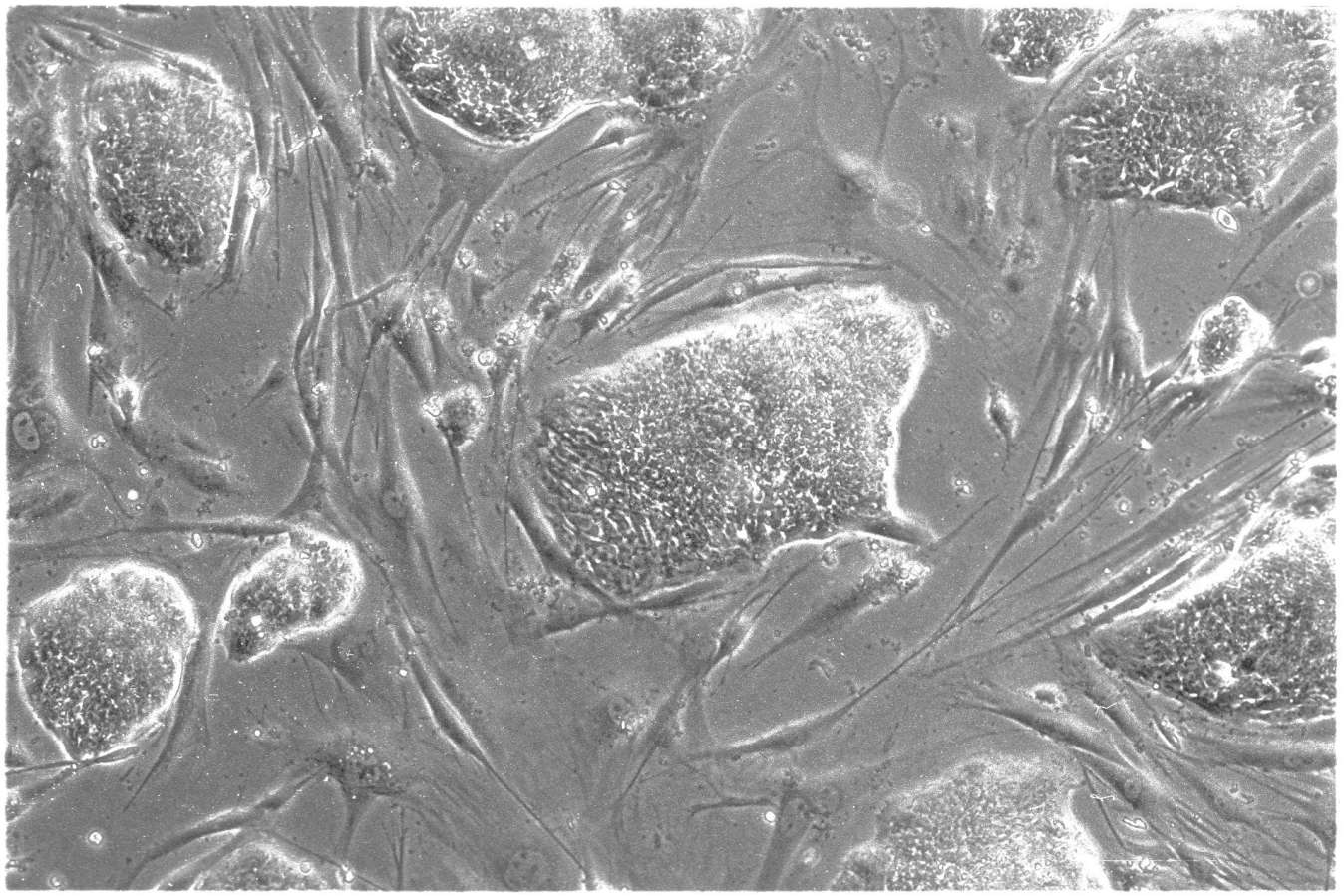
Johnson, Marcotti

After inducing hair cell differentiation, cells displayed inward potassium and calcium currents, characteristics of hair cells

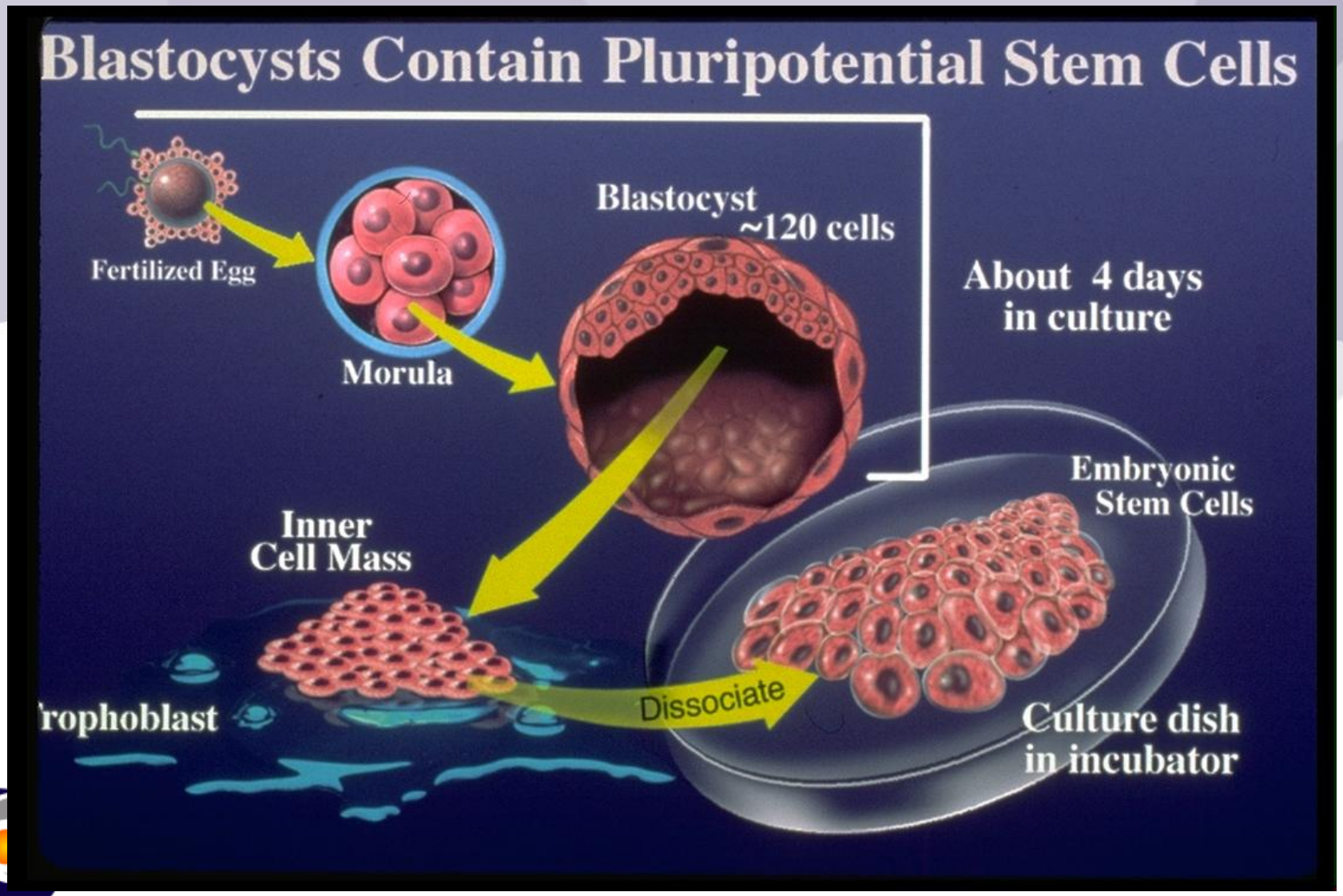


Johnson, Marcotti

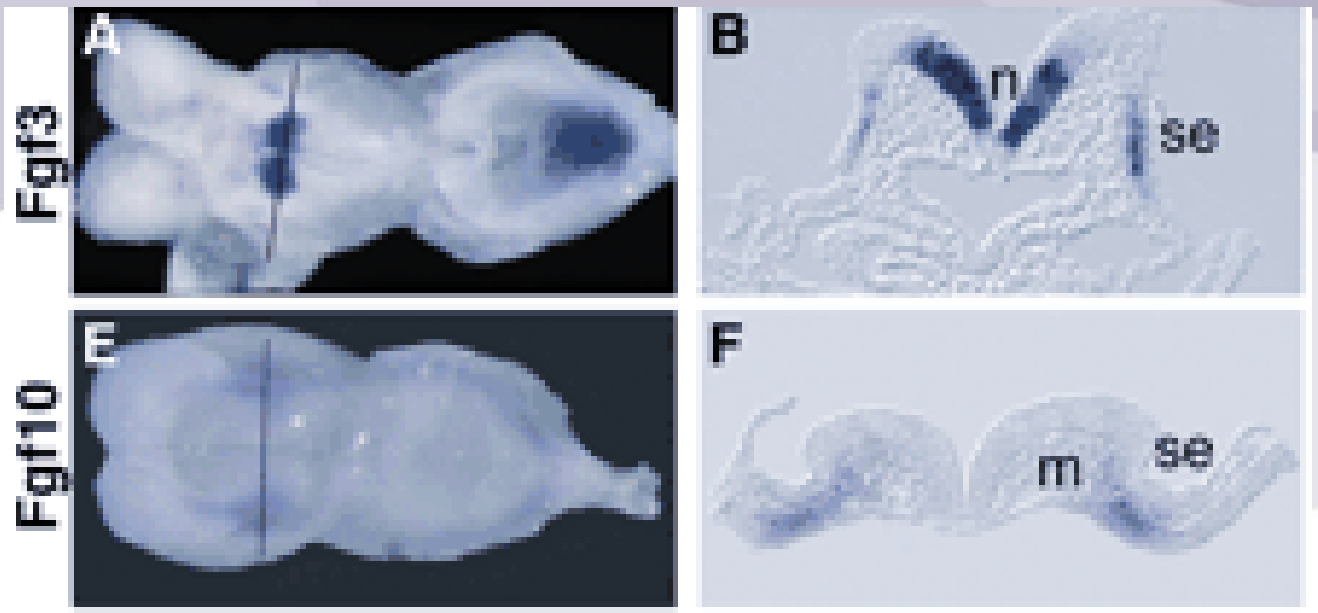
Derivation of Otic Progenitors from Human Embryonic Stem Cells (hESCs)

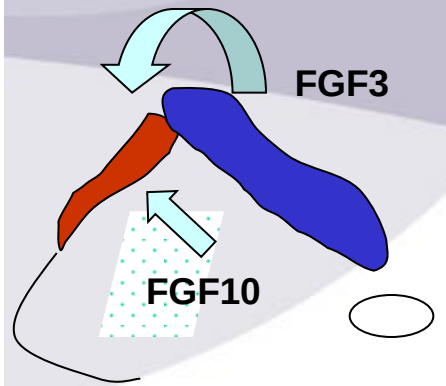


Embryos created by IVF have pluripotent stem cells

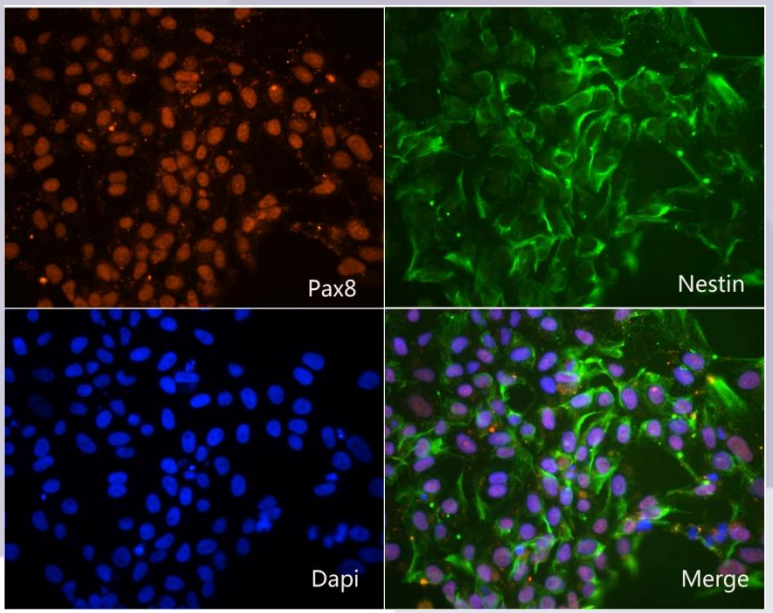


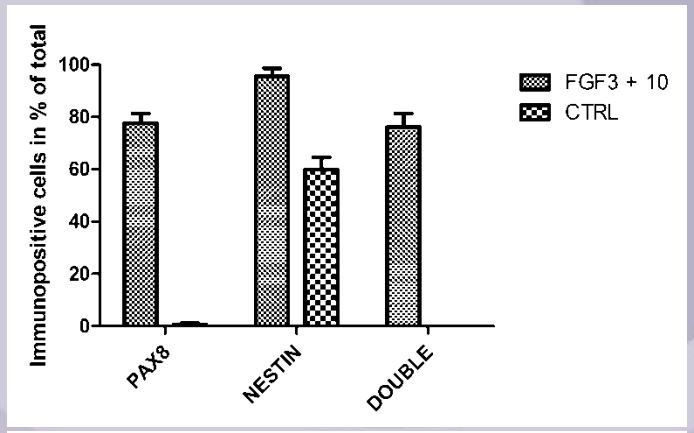
Fgf3 and Fgf10 are necessary for otic placode specification in mice

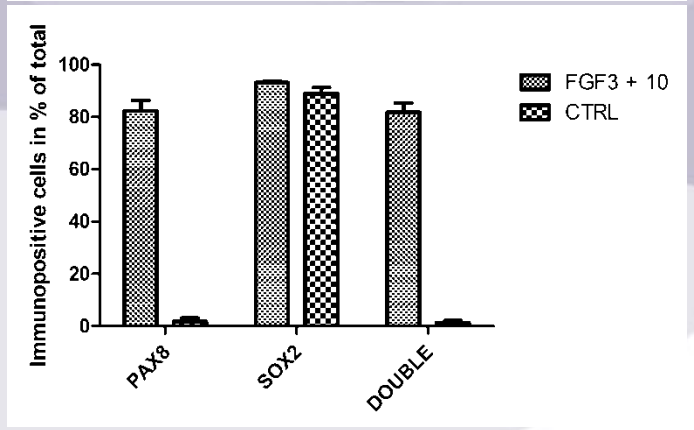


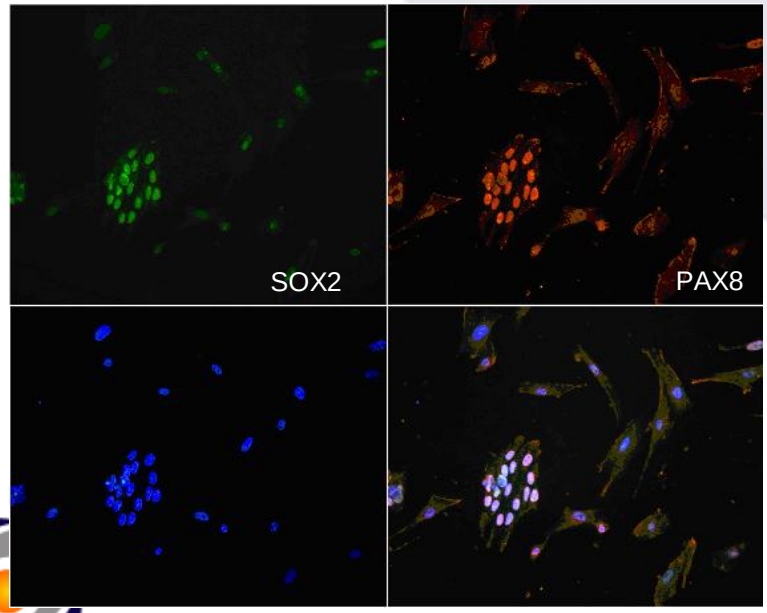


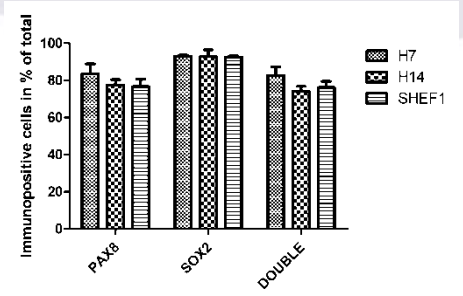
Wright and Mansour, Development 130, 3379 2003

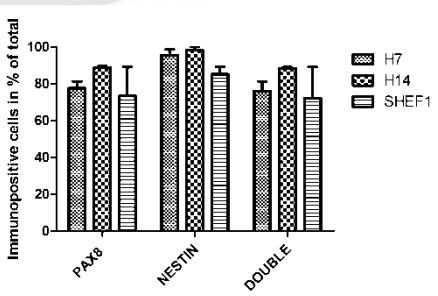

Pax8
Nestin
Dapi
Merge


Immunopositive cells in % of total
100
80
60
40
20
0
PAX8
NESTIN
DOUBLE
FGF3 + 10
CTRL

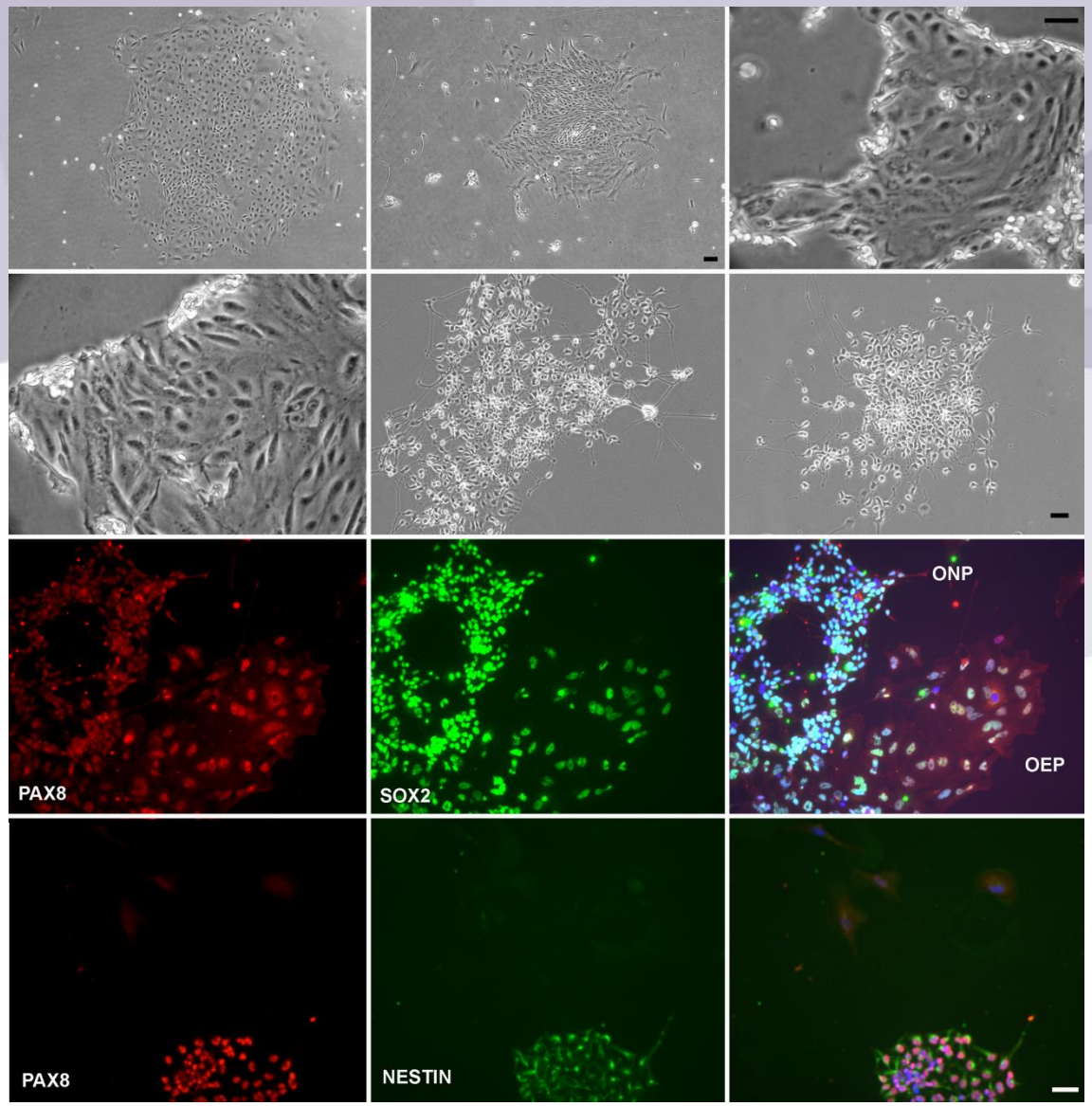

Immunopositive cells in % of total
100
80
60
40
20
0
PAX8
SOX2
DOUBLE
FGF3 + 10
CTRL


SOX2
PAX8

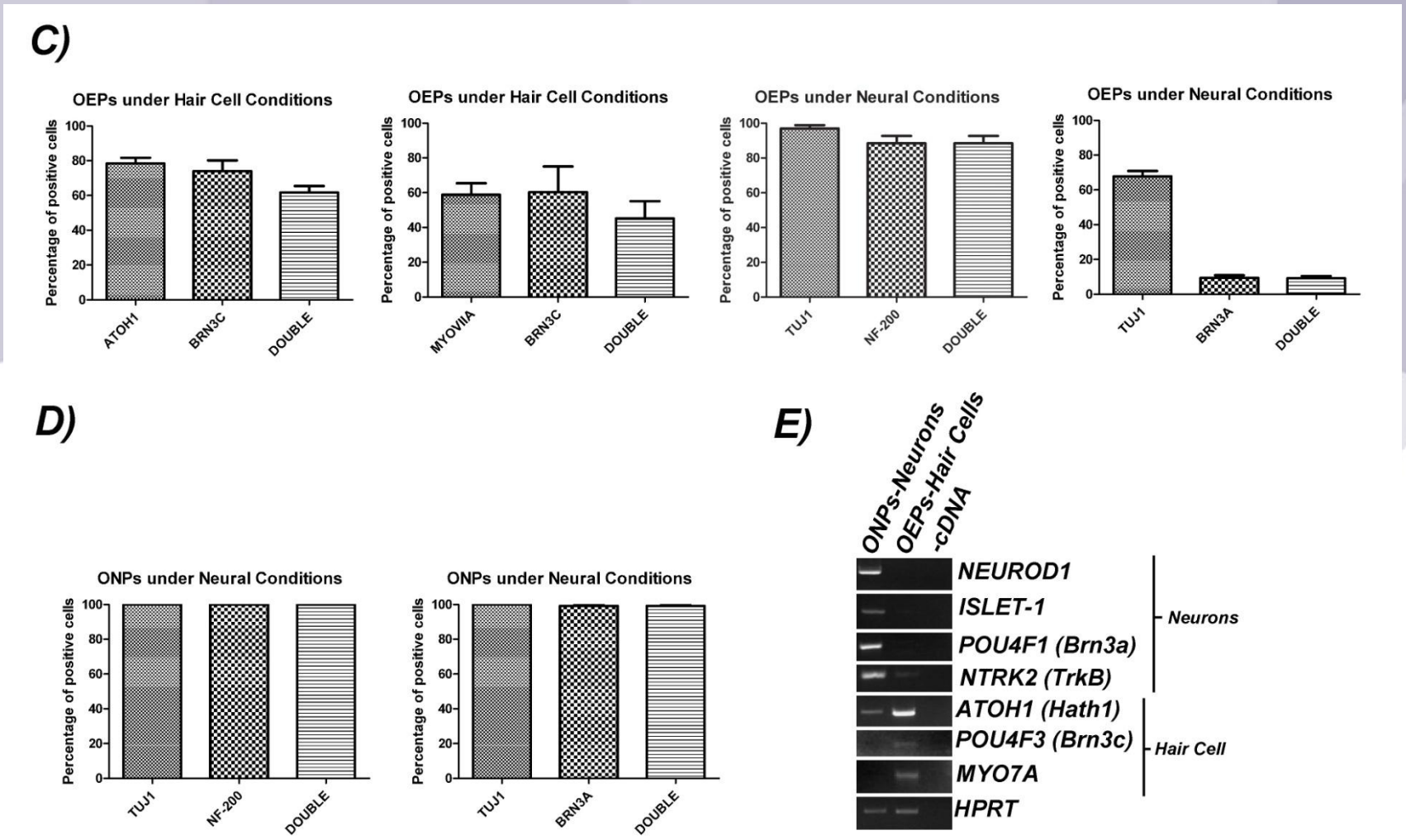

Immunopositive cells in % of total
100
80
60
40
20
0
PAX8
SOX2
DOUBLE
H7
H14
SHEF1


Immunopositive cells in % of total
100
80
60
40
20
0
PAX8
NESTIN
DOUBLE
H7
H14
SHEF1

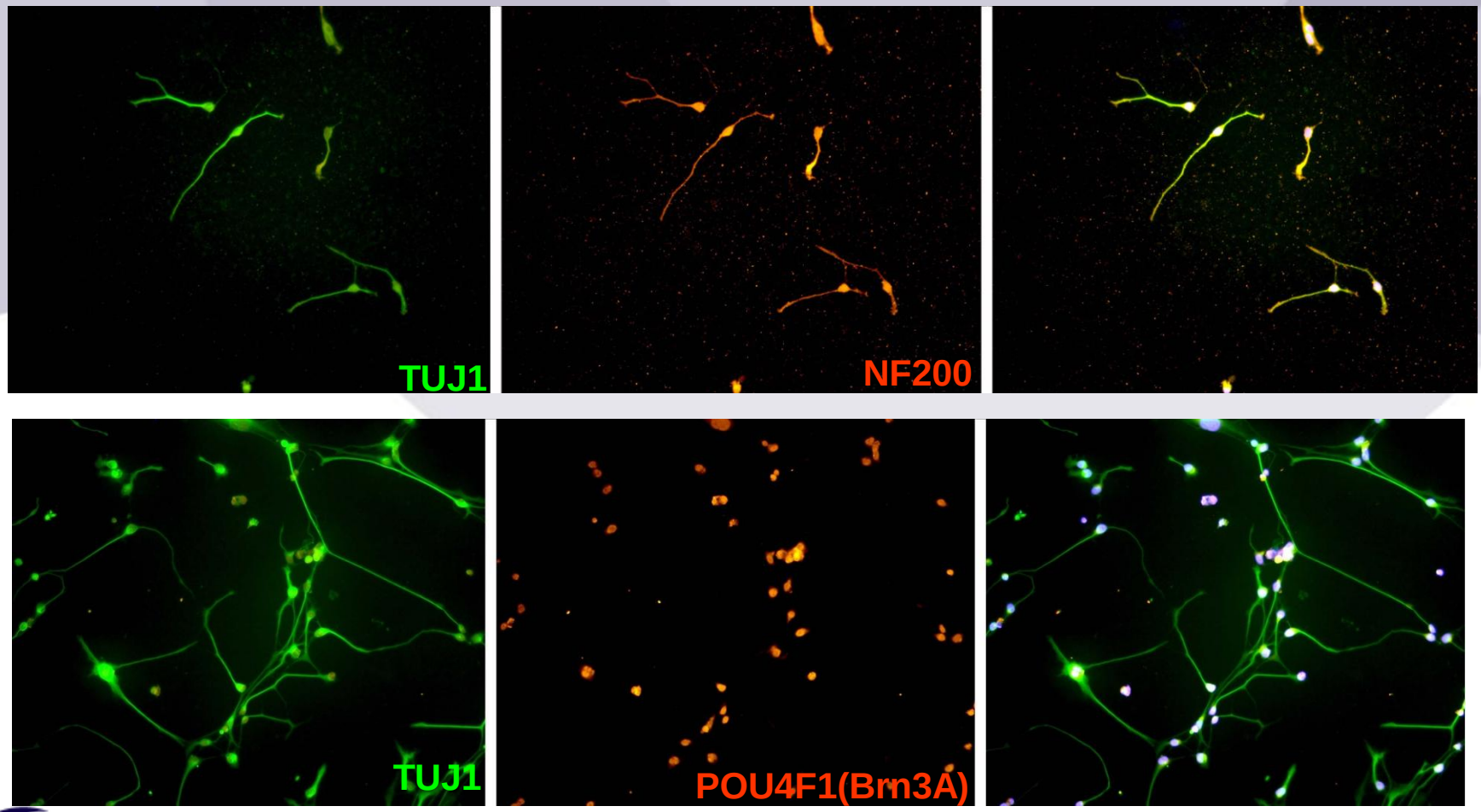
Two types of colonies are induced: Otic Epithelial progenitors (hOEPs) and Otic Neuroprogenitors (hONPs)



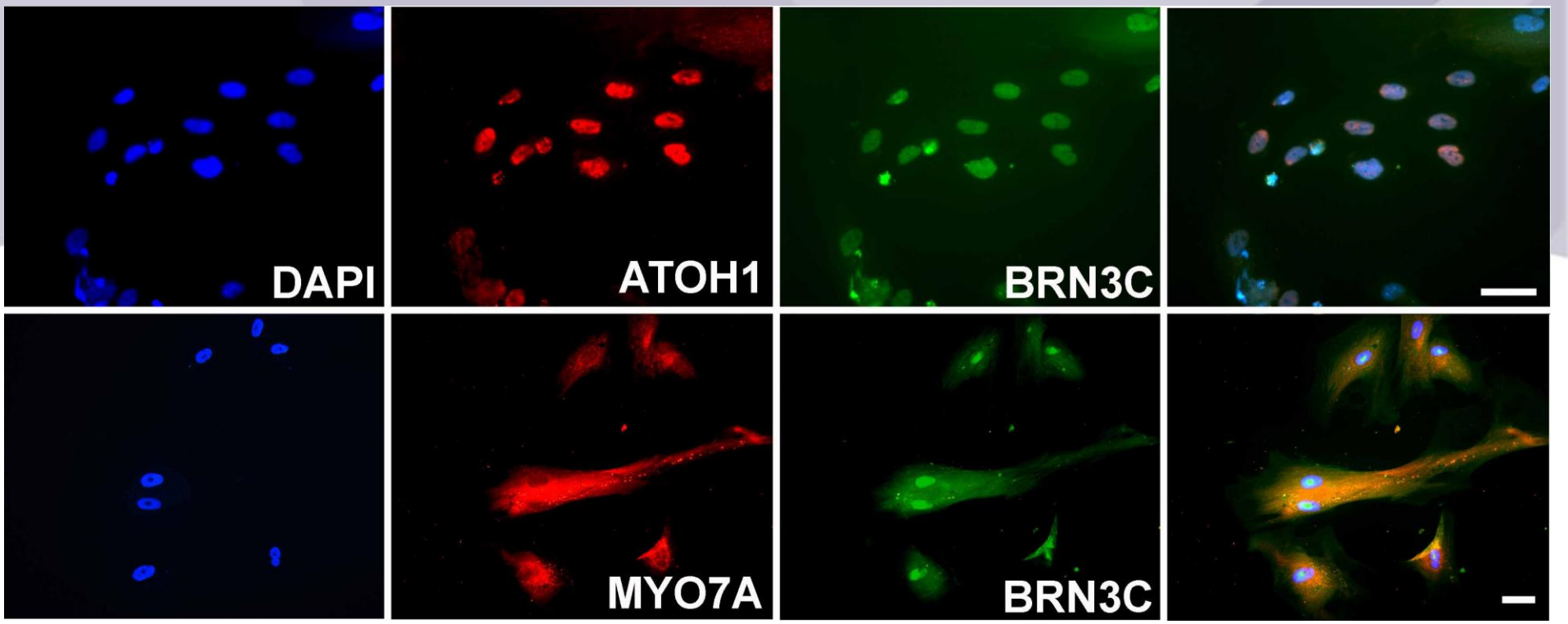
Generation of hair cell and neuronal phenotypes from hESC-OEPs and hESC-ONPs



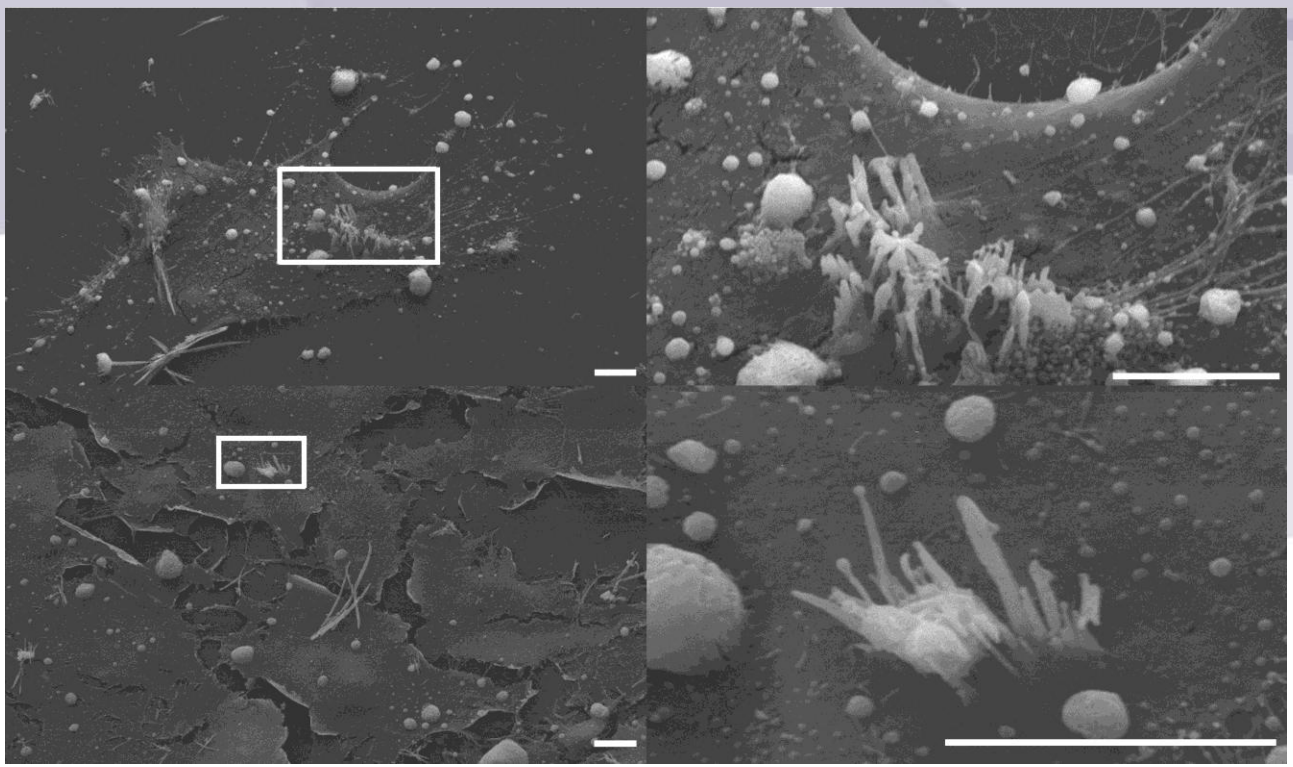
Neuronal differentiation from hESC-induced otic progenitors



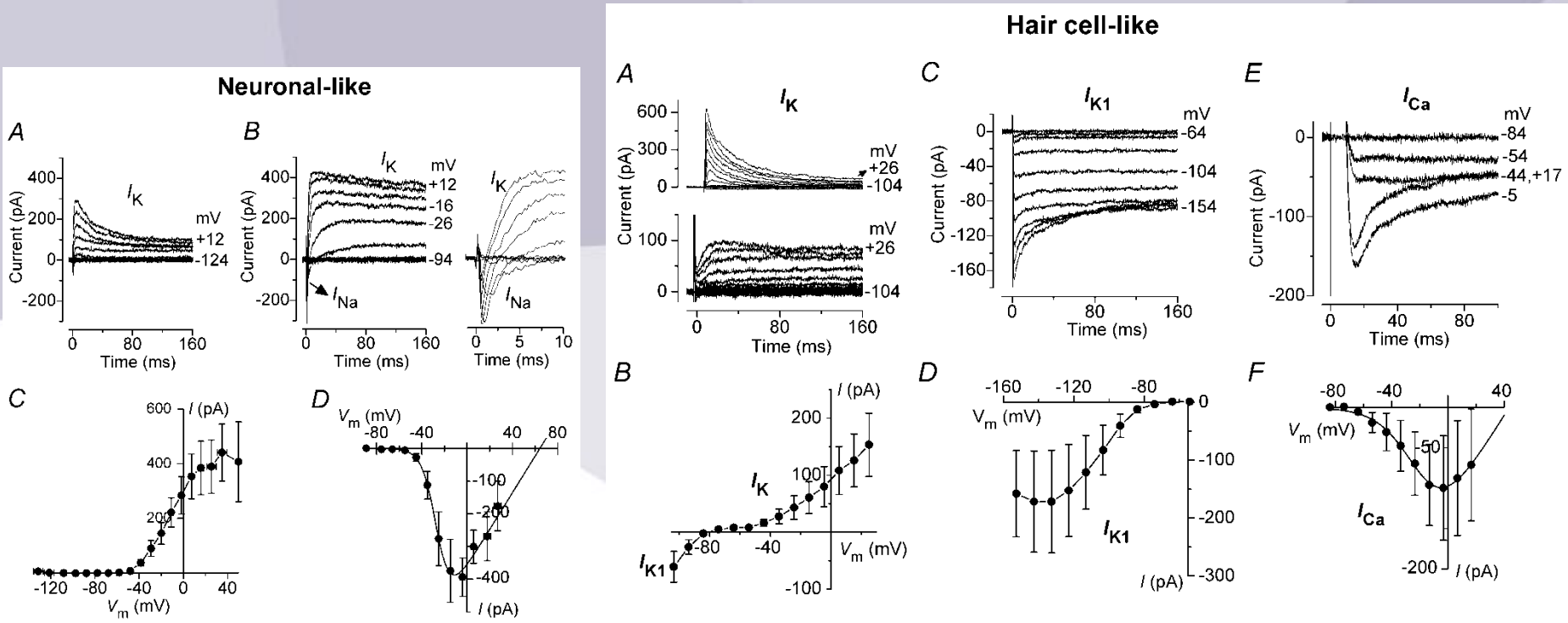
Hair Cell differentiation from hESC-induced otic progenitors



Apical differentiation of a proto stereociliary bundle



Functional properties of hESC-derived hair cells and neurons

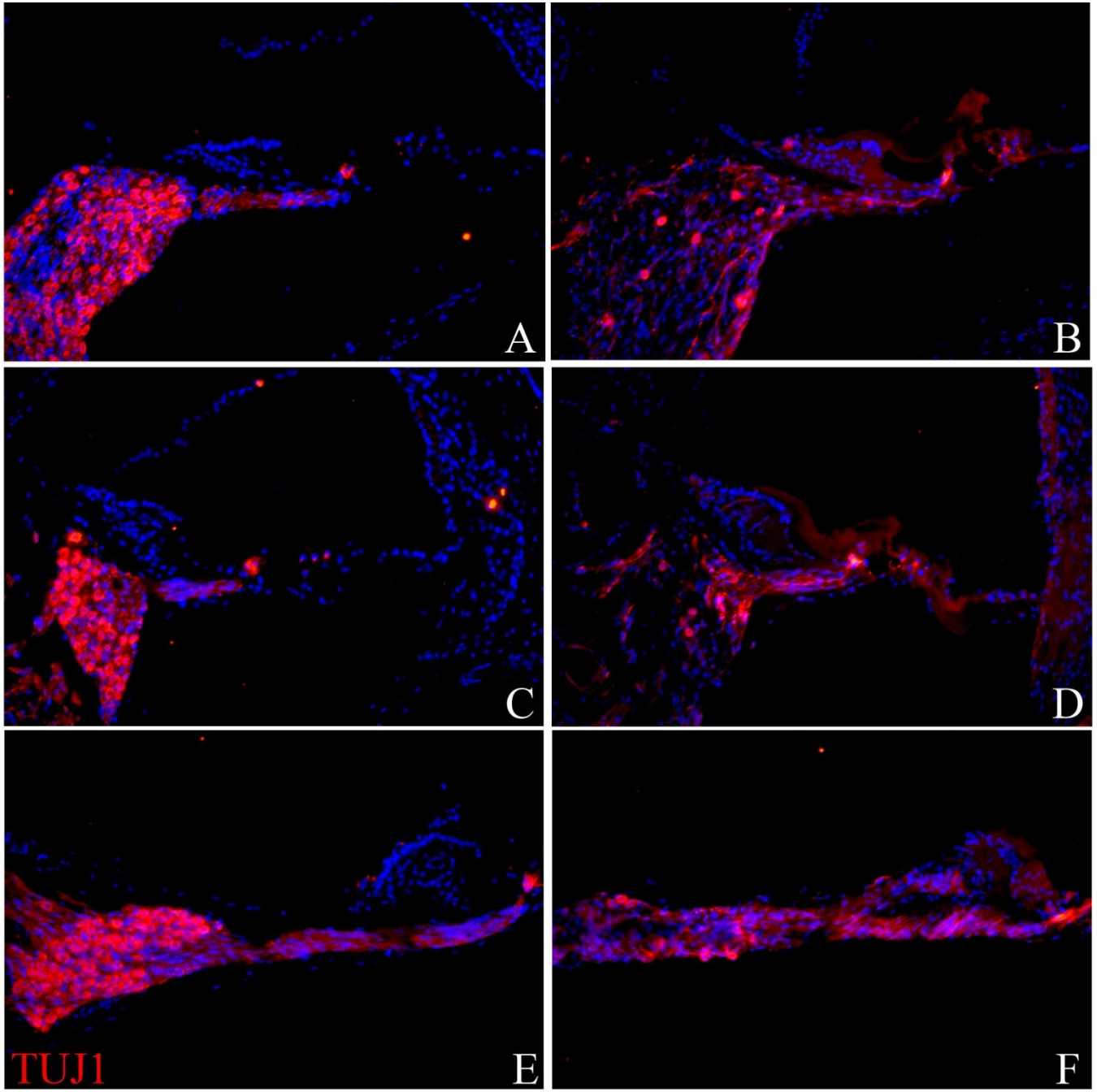


Johnson, Kuhn and Marcotti

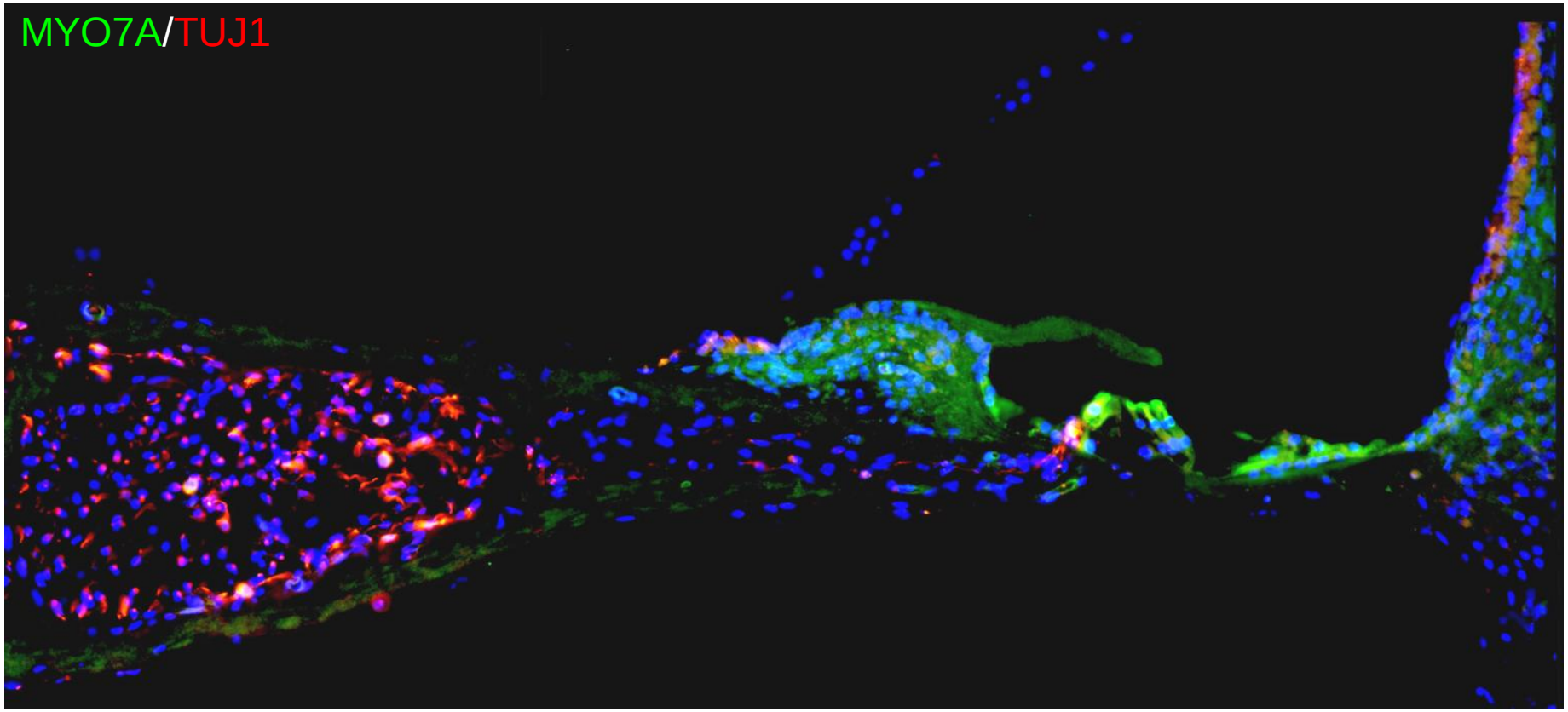
- Transplantation into a model of auditory neuropathy

Application of ouabain into the RW of gerbils produce the death of spiral ganglion neurons *(Schmiedt et al 2002; Lang et al 2005, 2008)*

Comparison between Ouabain Treated and Untreated Cochlea

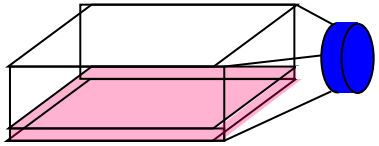


Persistence of Hair cells in Ouabain treated cochlea



Ouabain induces apoptotic cell death in type I spiral ganglion neurons.

Induction of Otic Neuroprogenitors from hESCs

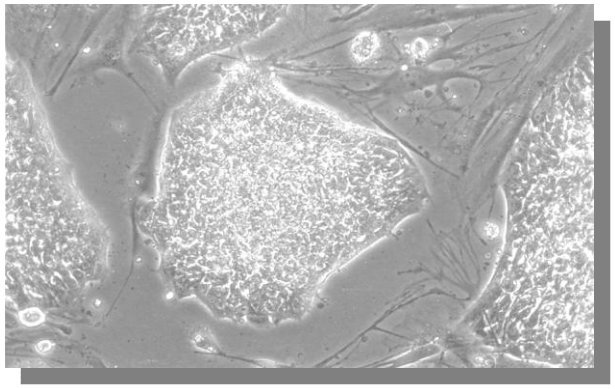


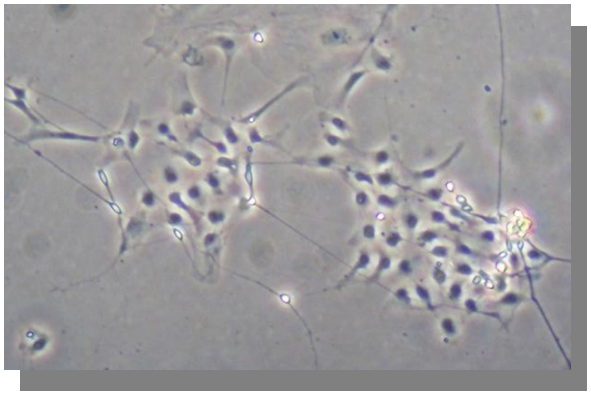
Harvested by trypsinization

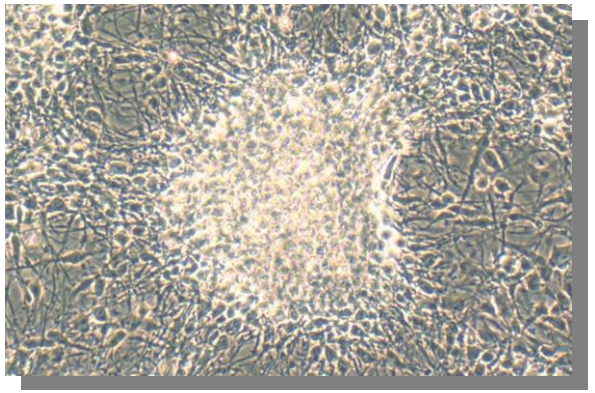
Enrichment for hONP

Shef1 GFP	FGF 3 & FGF 10 Induction	Expanded in OSCFM

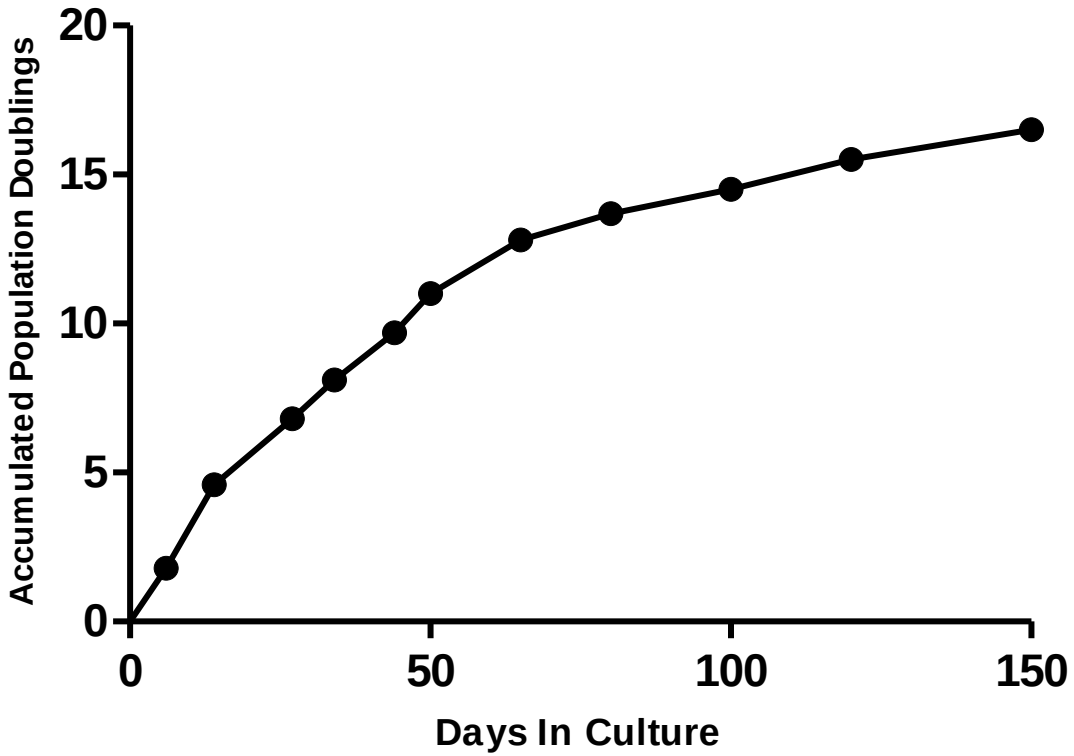
Day 0 Day 1 Day 10

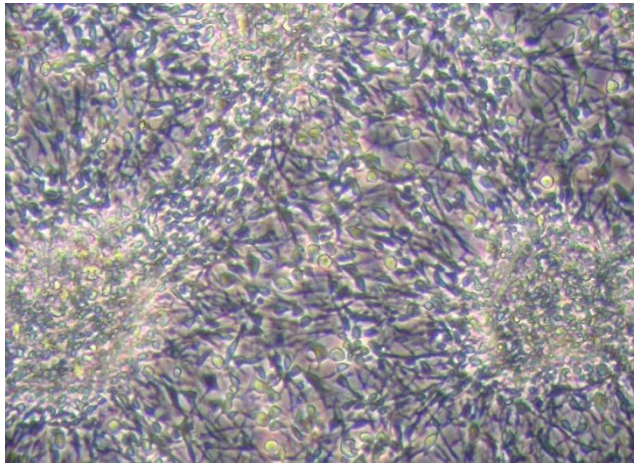


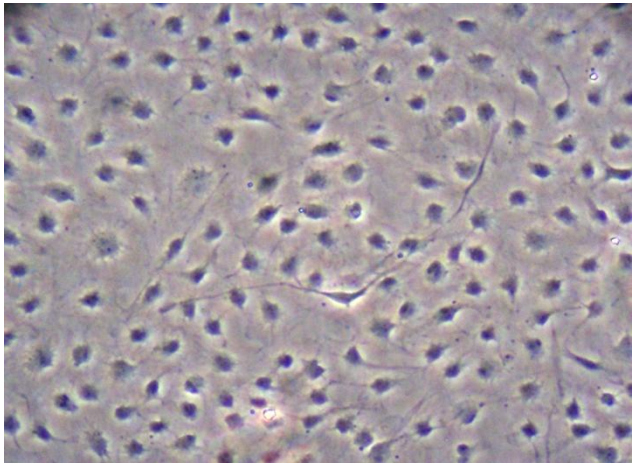


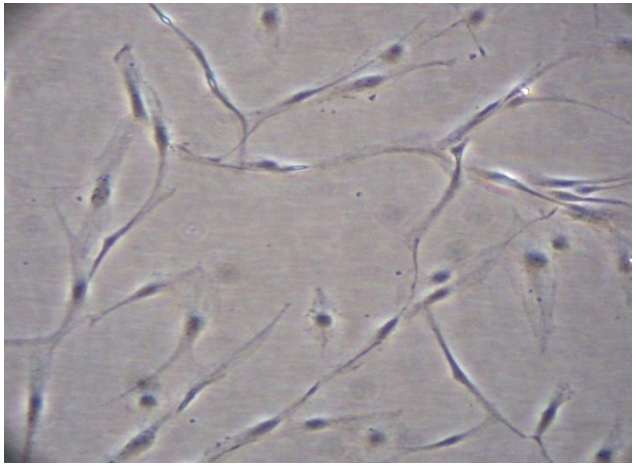


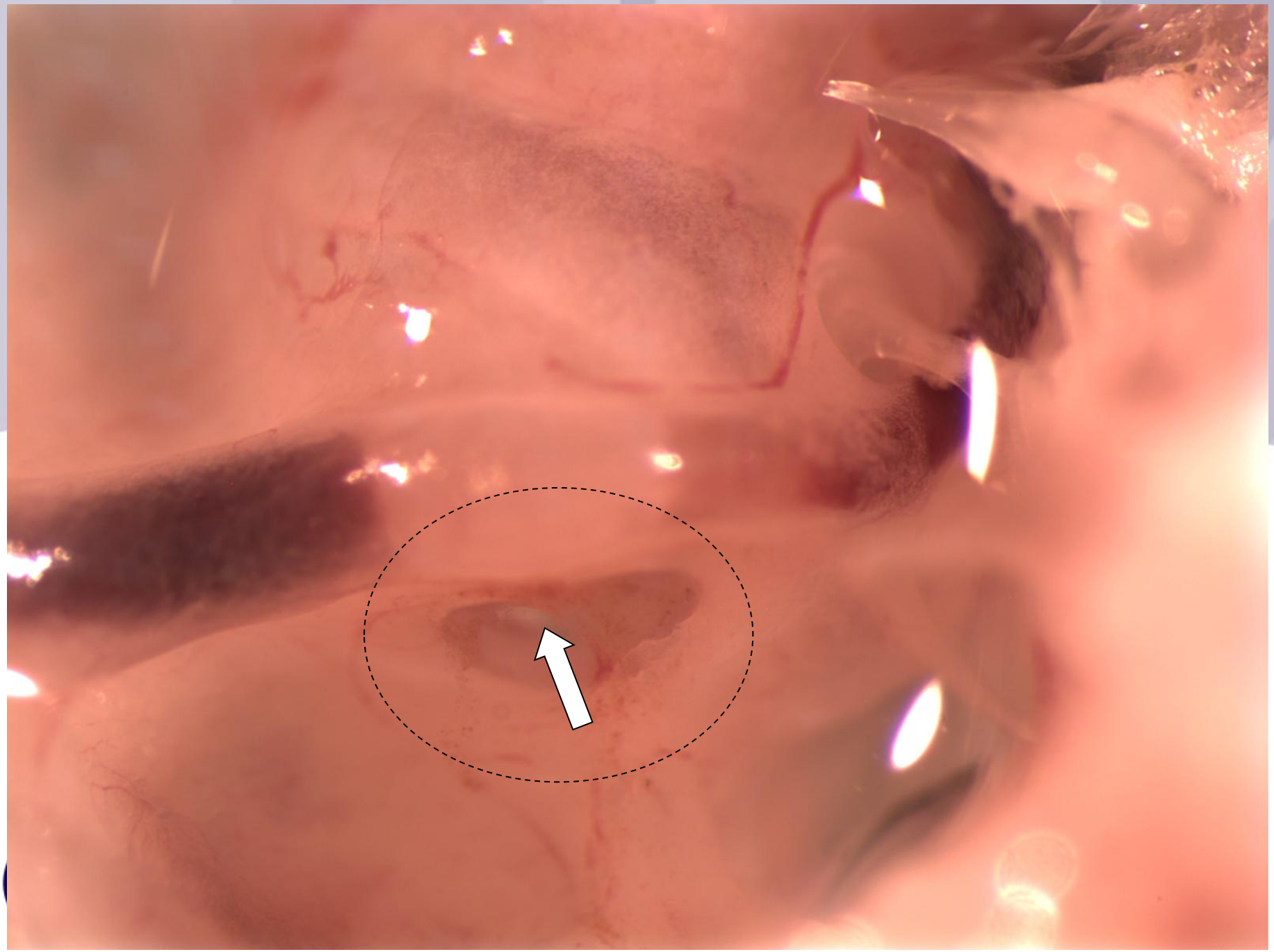
Population Doublings of hESC-derived ONPs



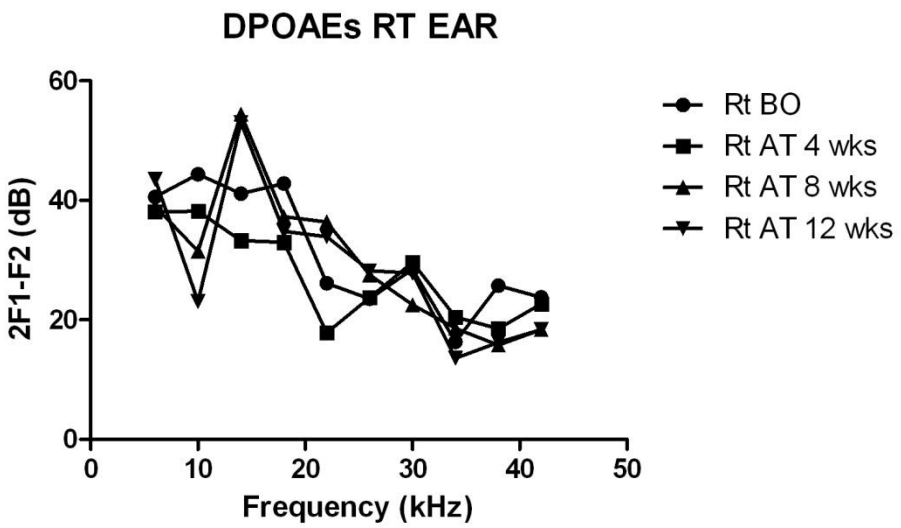


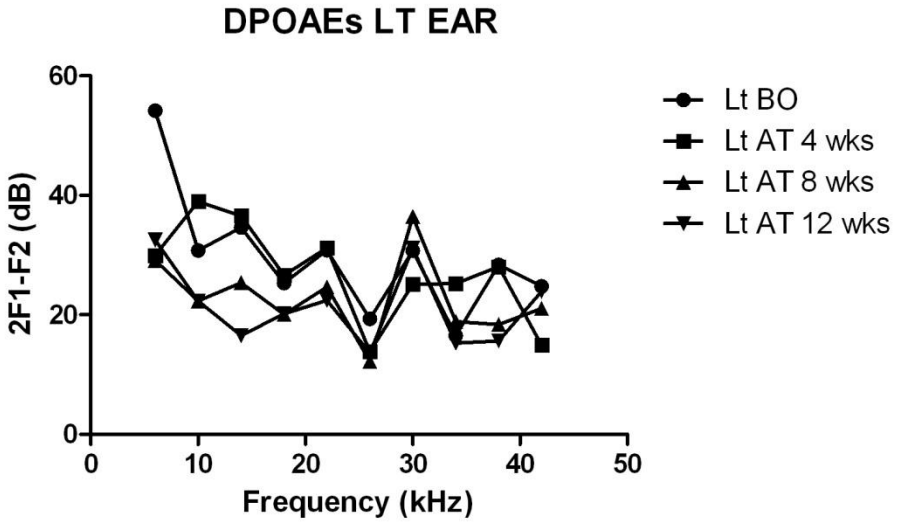


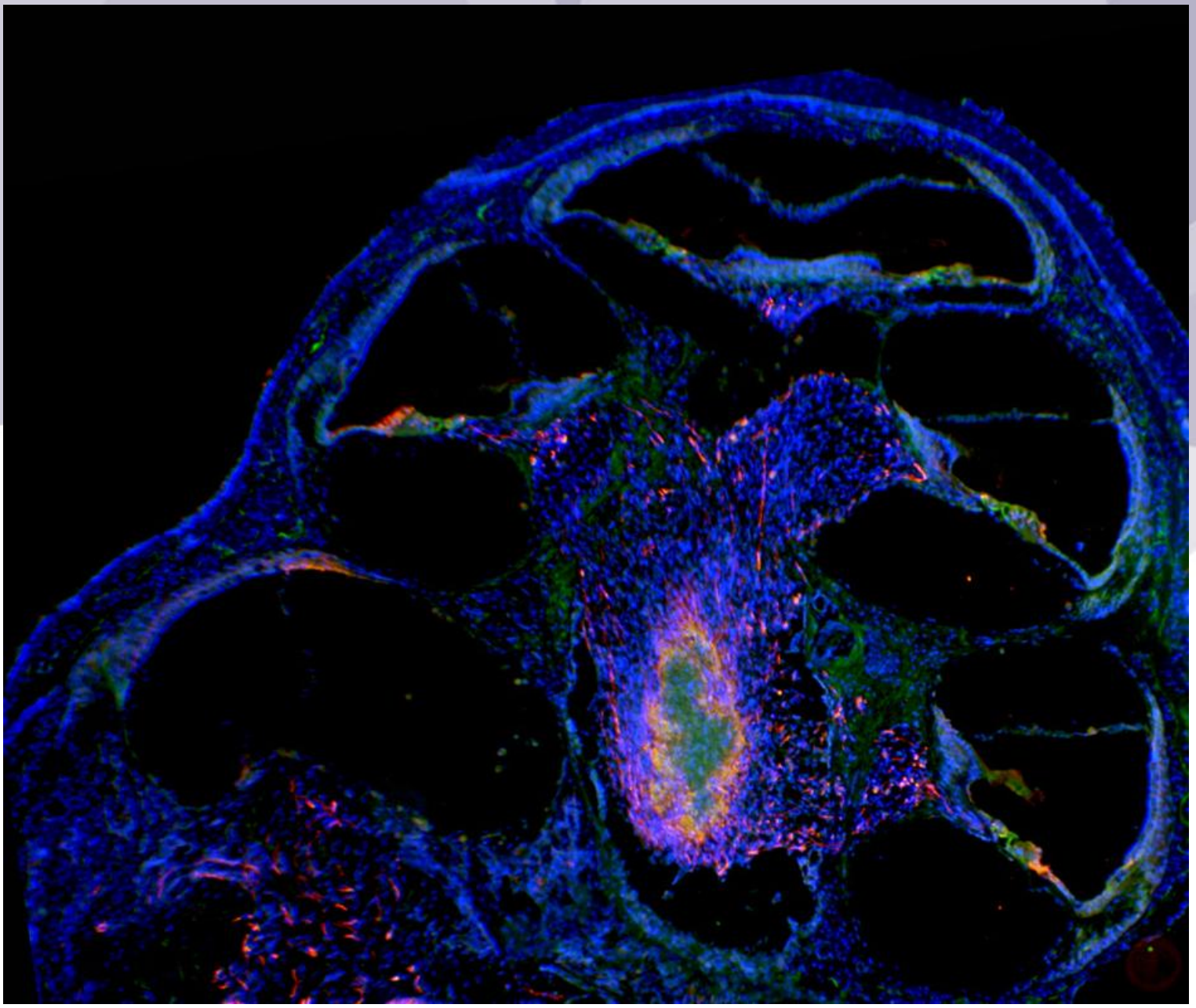




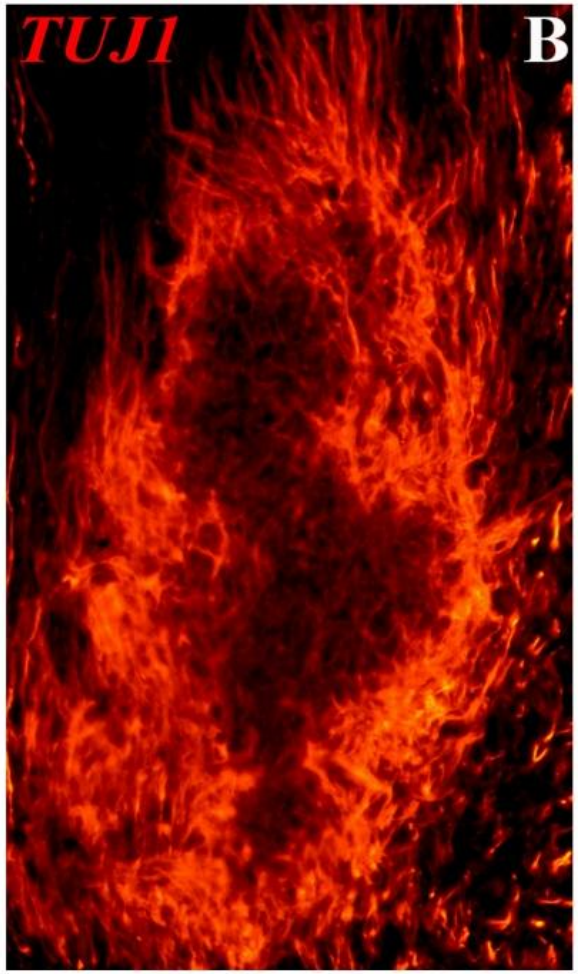
DPOAE pre and post treatment showing preservation of the organ of Corti

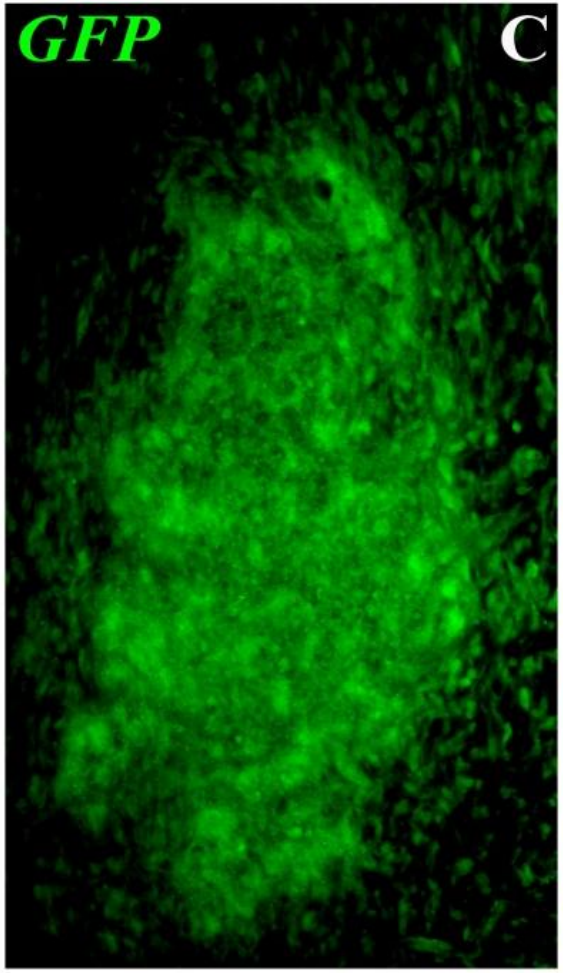


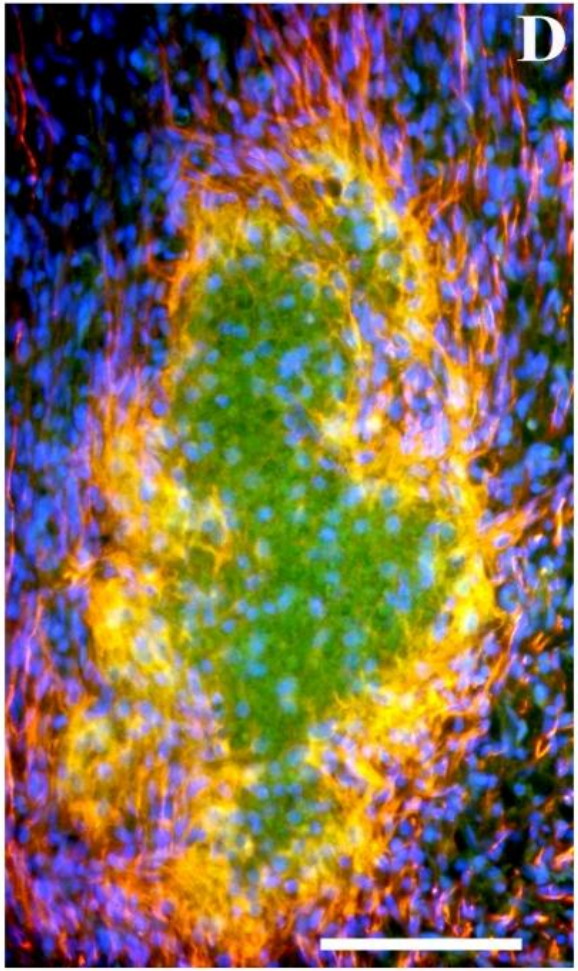


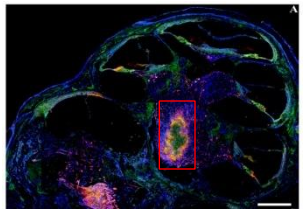


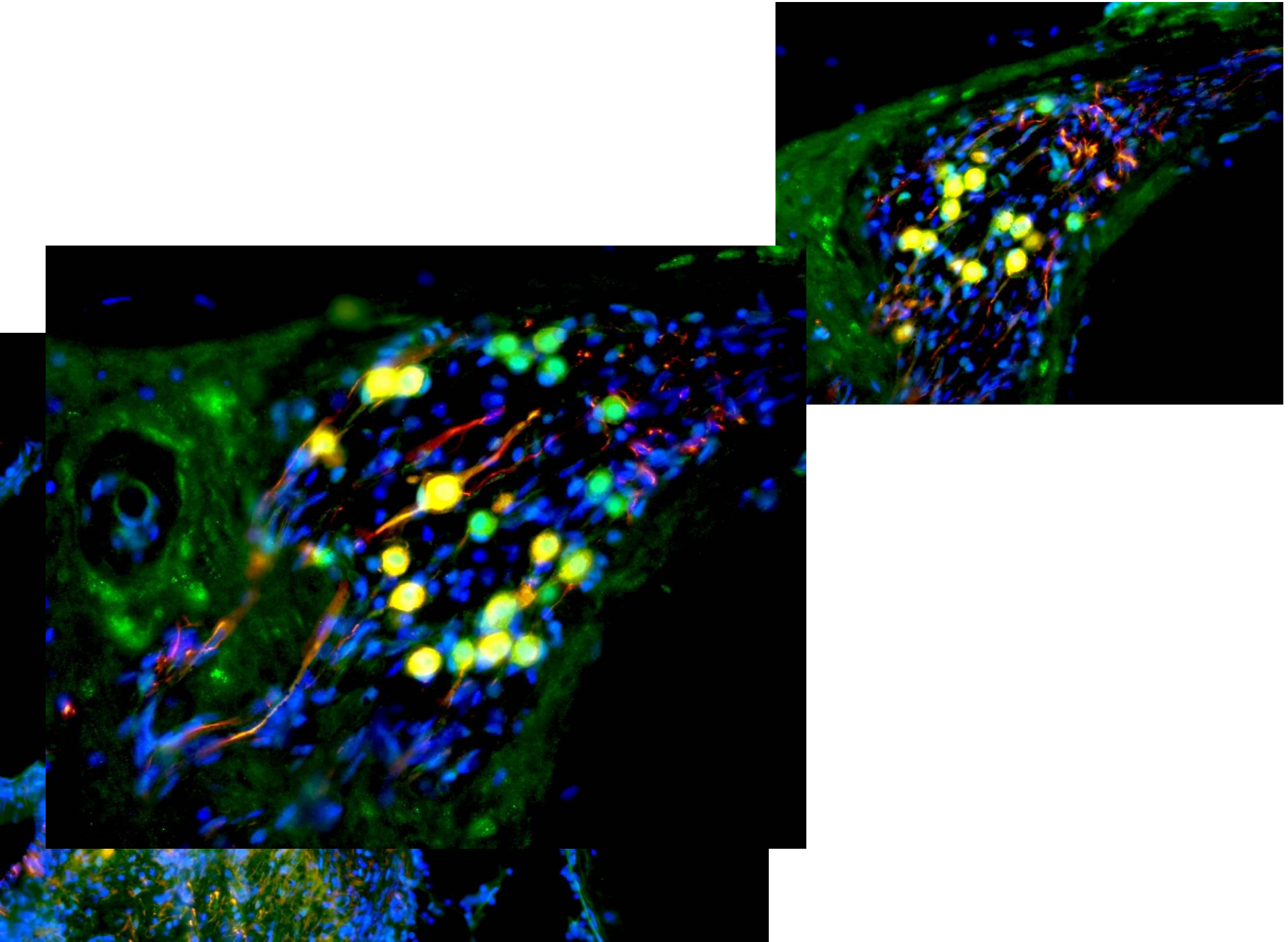
Detailed view of ectopic ganglion



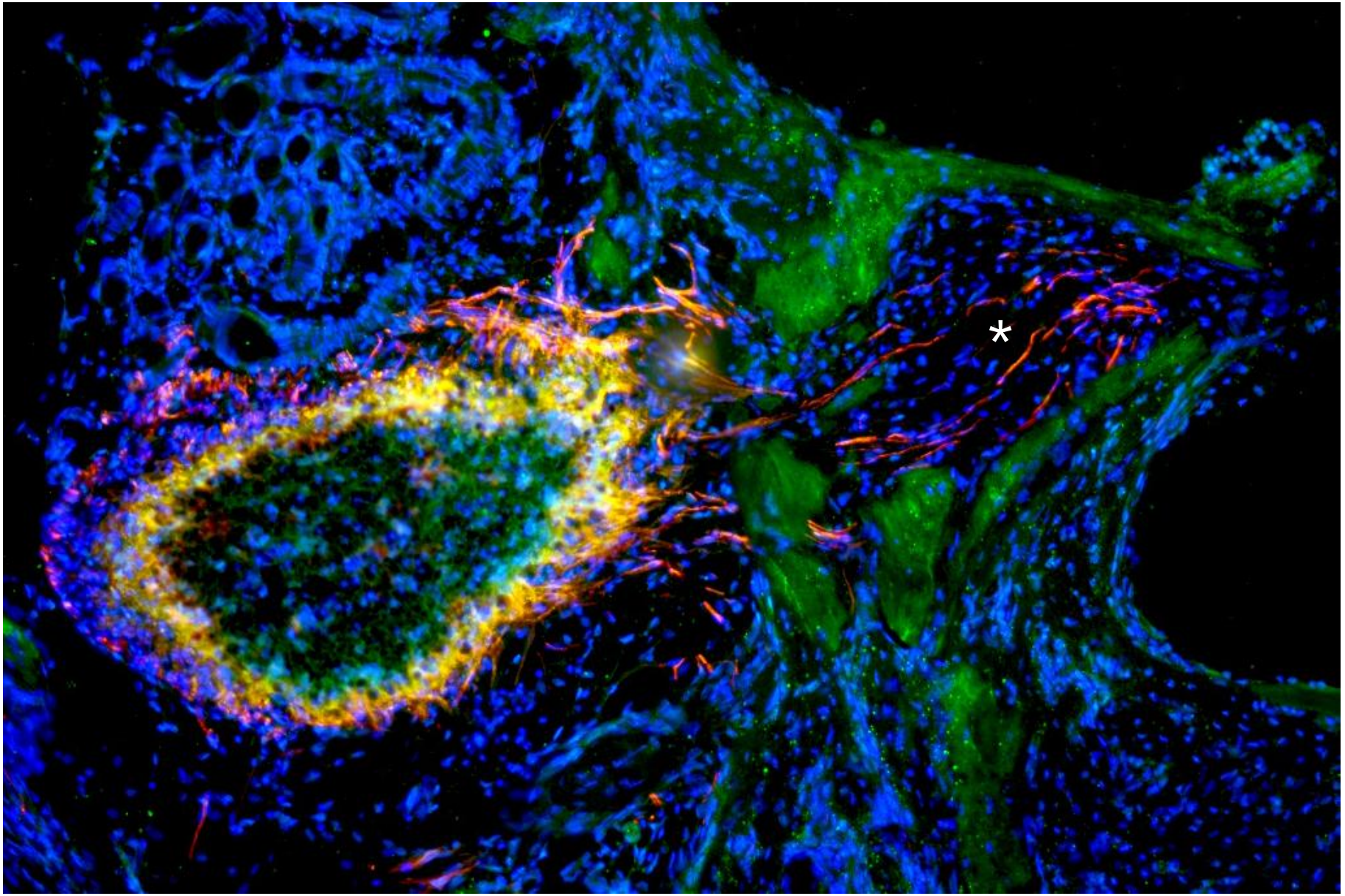


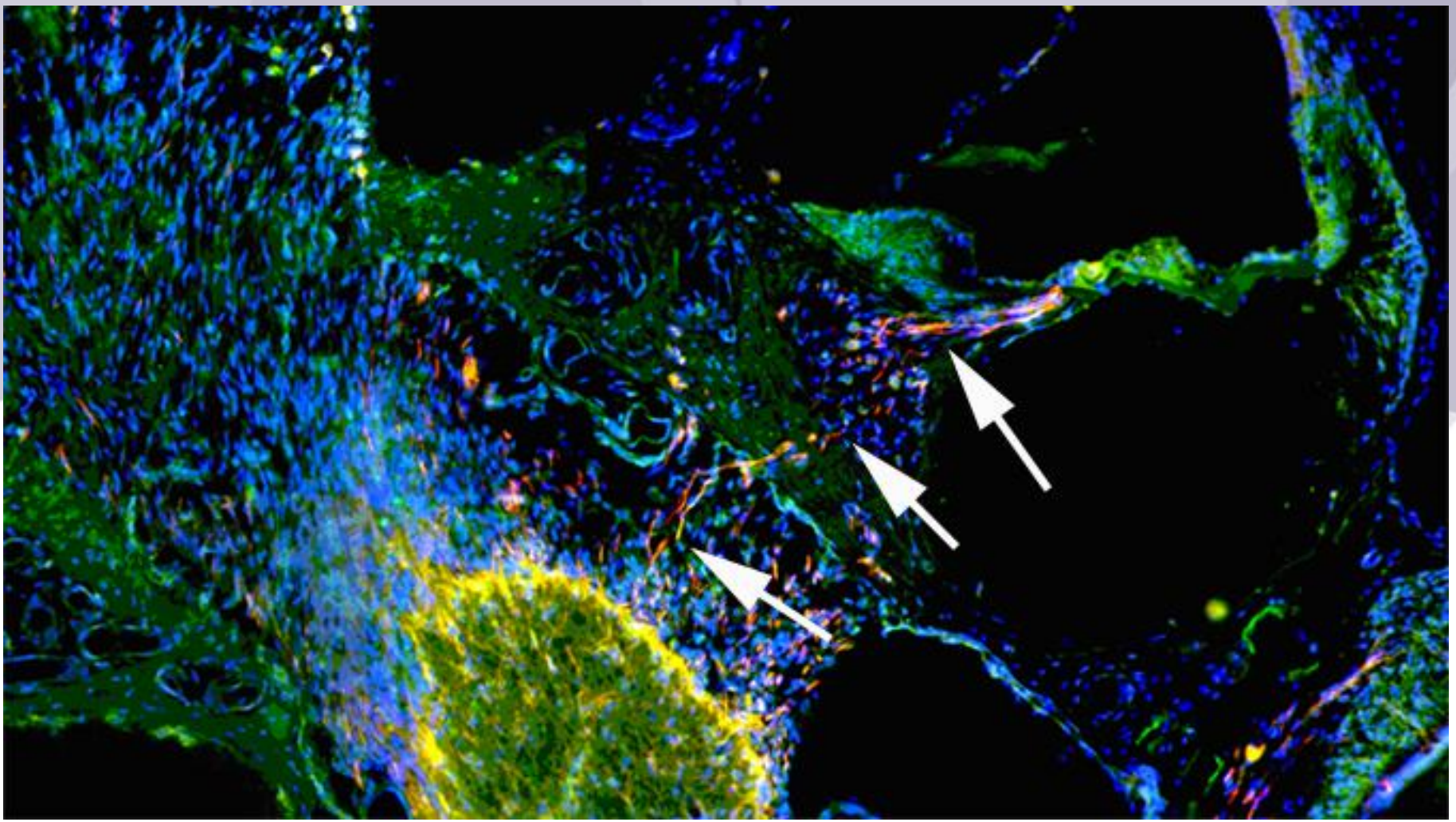


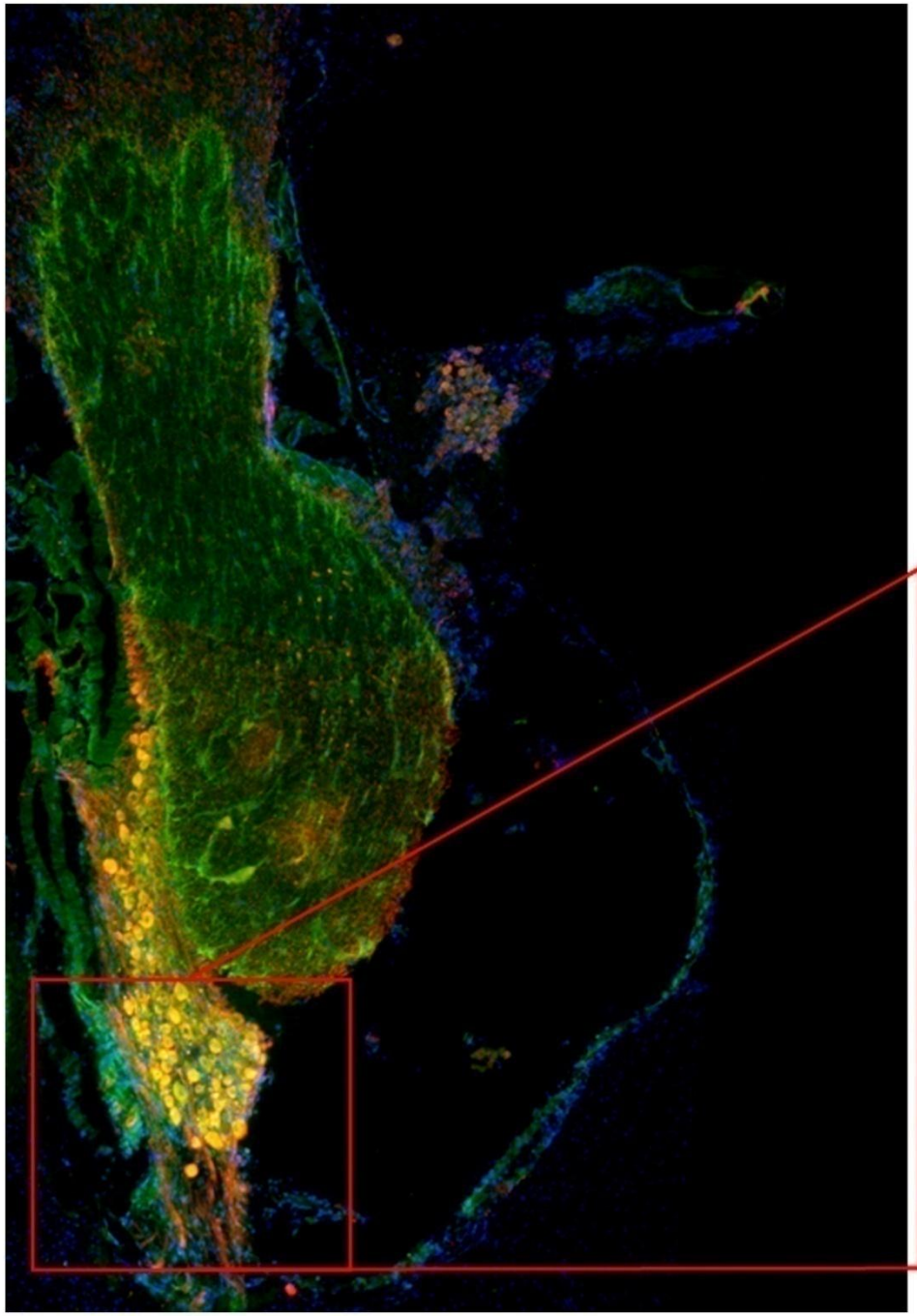


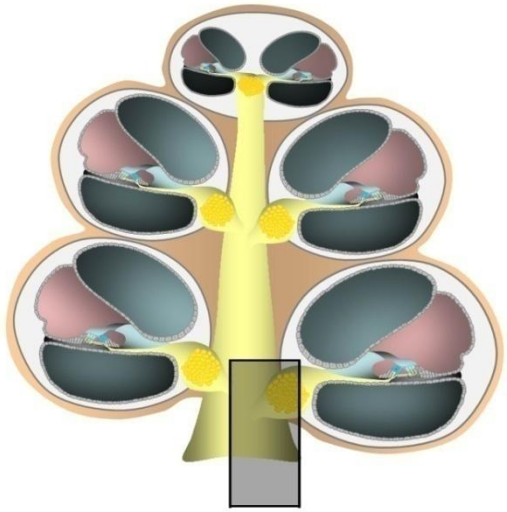


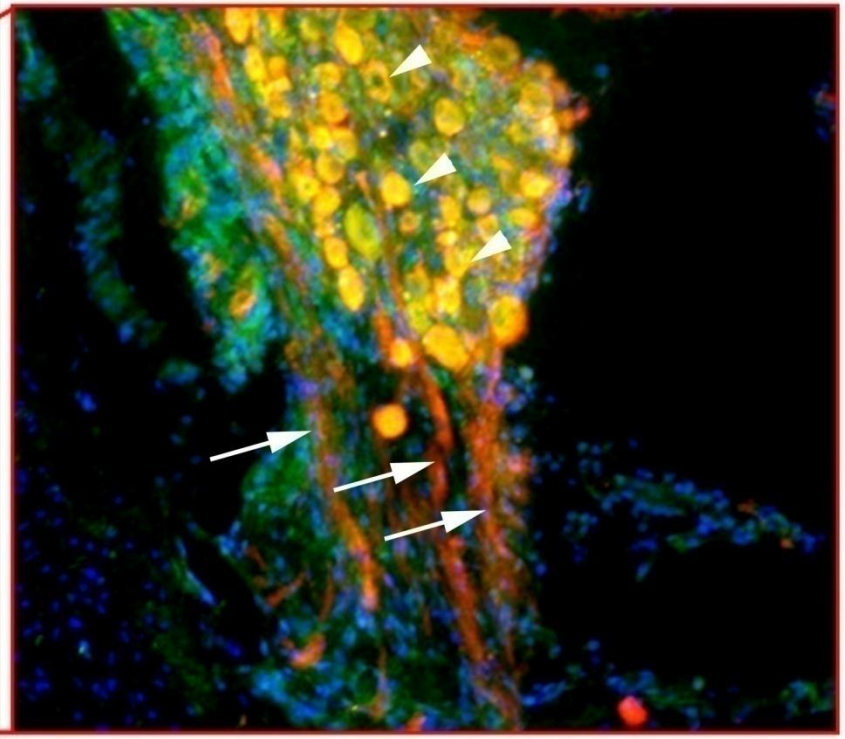
Fibers projecting from the transplanted cells towards the OC





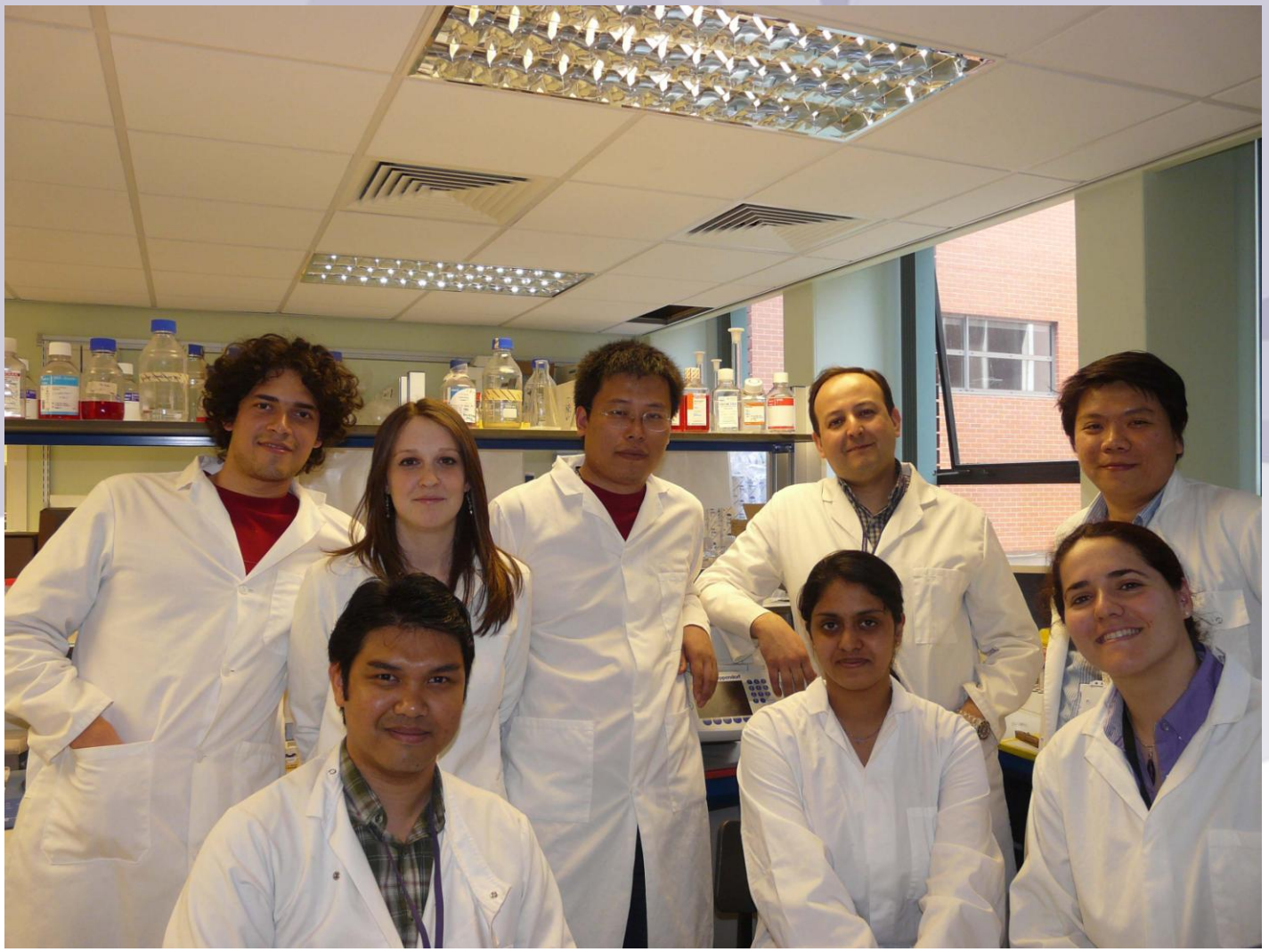






In summary…

- We have established several lines of human auditory stem cells that have the potential to generate sensory cell types in vitro.
- Inner ear progenitors can be derived from hESCs by using a protocol that resembles normal development. These progenitors have the capacity to differentiate into sensory hair cells-like cells and neurons in vitro and in vivo and are beginning to show the potential to induce functional recovery.



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