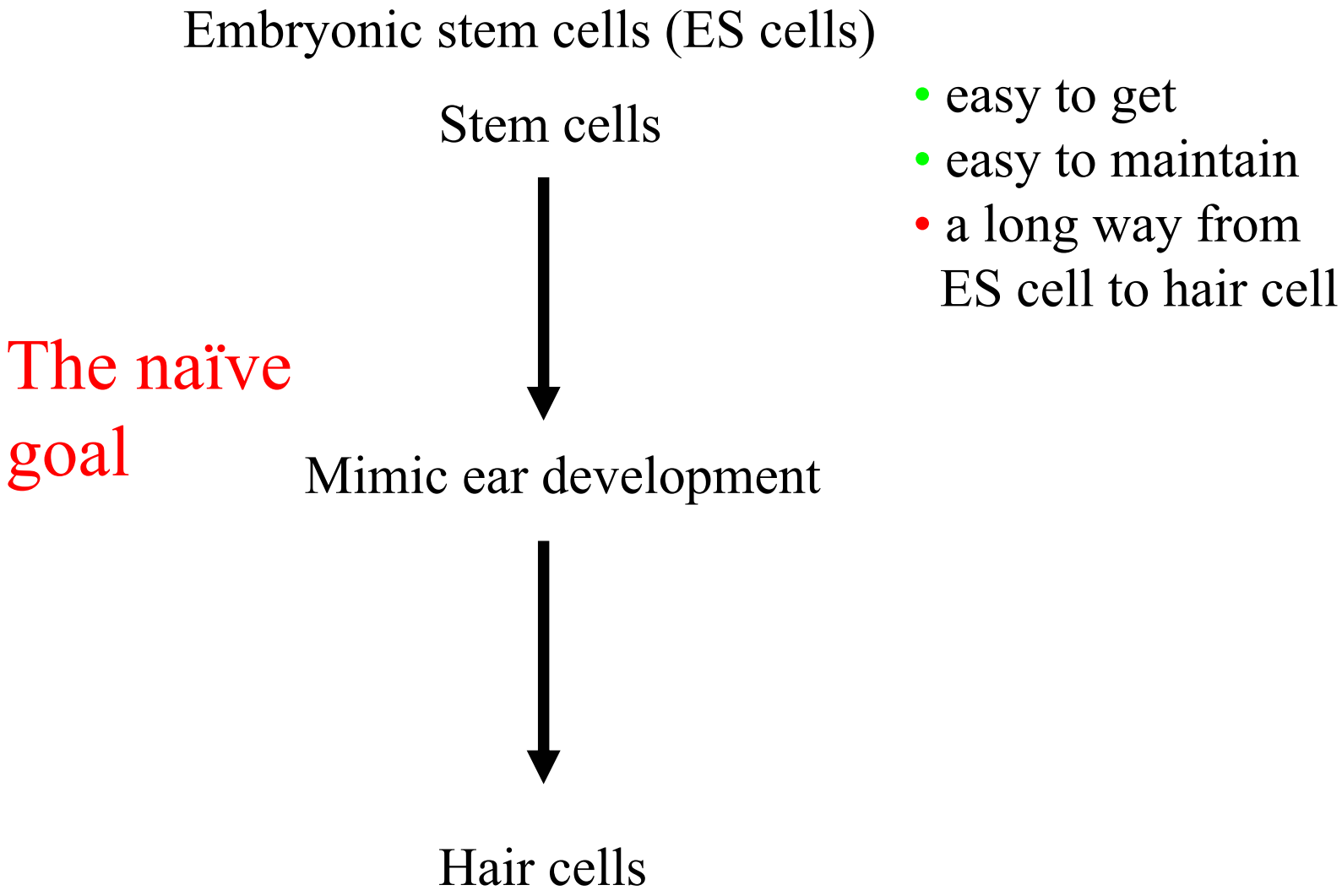
Stem cell: 1) They can make identical copies of themselves (long-term self renewal).
2) They can give rise to mature cell types with characteristic morphologies and functions.

Embryonic stem cells: originate from the inner cell mass of the blastocyst.

Adult stem cells: Reside in organs and can replace cells that die because of injury or disease; they are rare: for example only 1 in 10,000 cells in the bone marrow is a hematopoietic (blood-forming) stem cell.


Embryonic stem cells (ES cells)
Stem cells
• easy to get
• easy to maintain
• a long way from ES cell to hair cell
The naïve goal
Mimic ear development
Hair cells

Inner ear stem cells?

Adult stem cells

Progenitor cells

Hair cells

Ultrastructural Evidence for Hair Cell Regeneration in the Mammalian Inner Ear

Andrew Forge,* Lin Li, Jeffrey T. Corwin, Graham Nevill

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A permanent source for adult stem cells:

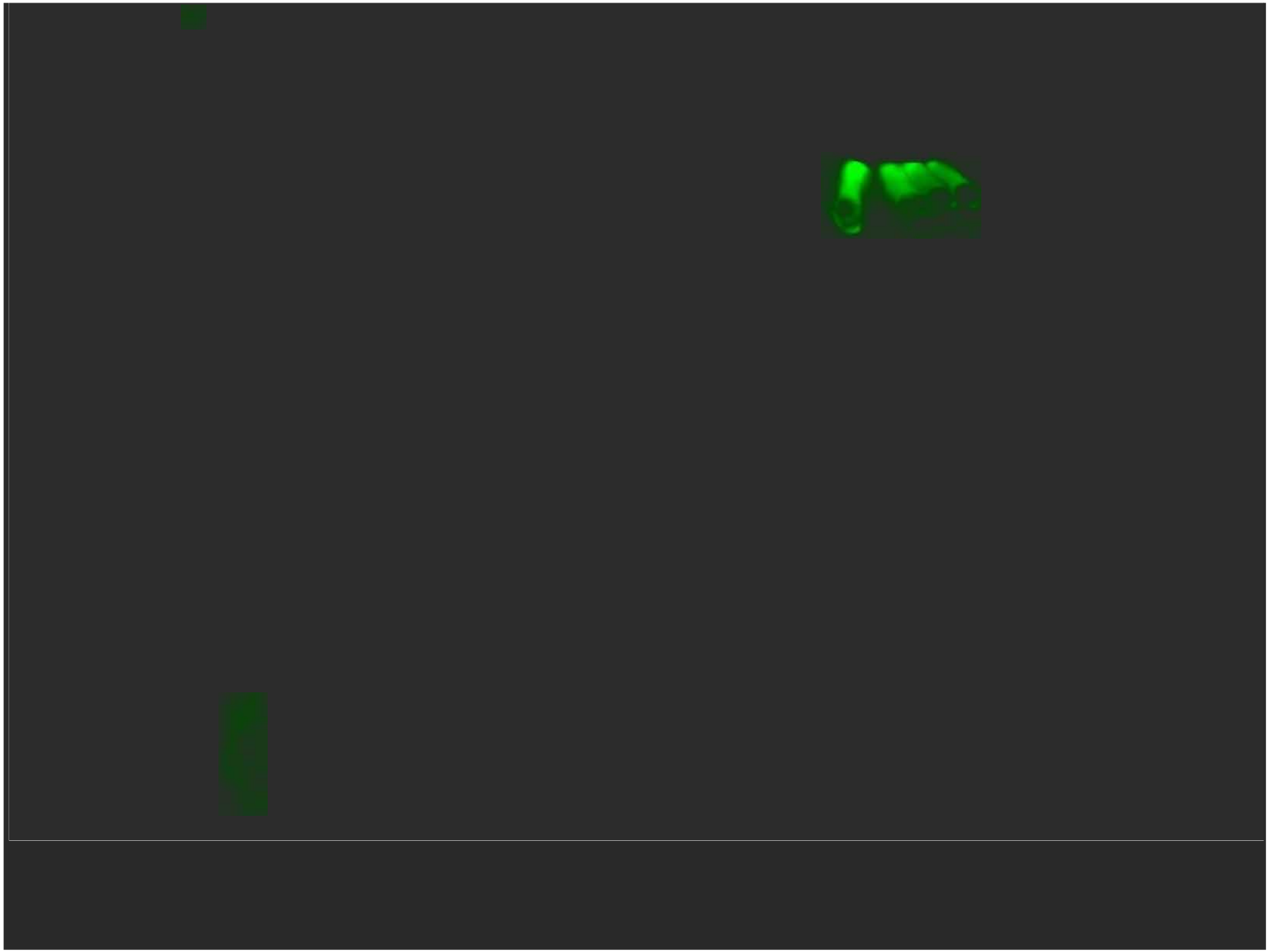
Adult stem cells from the skin

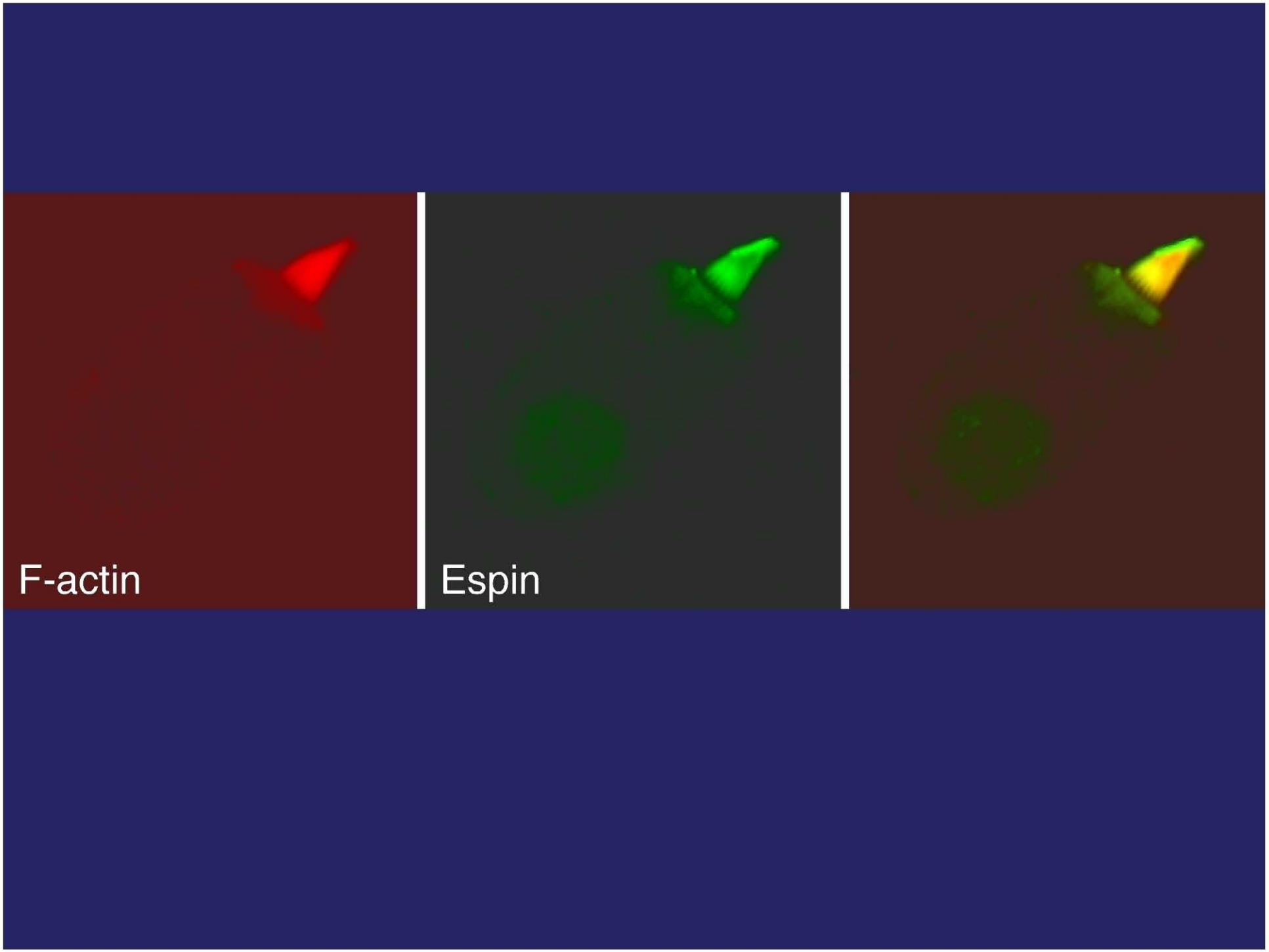
Inner ear progenitor cells

Hair cells

Embryonic development: Marker genes can reveal cell types

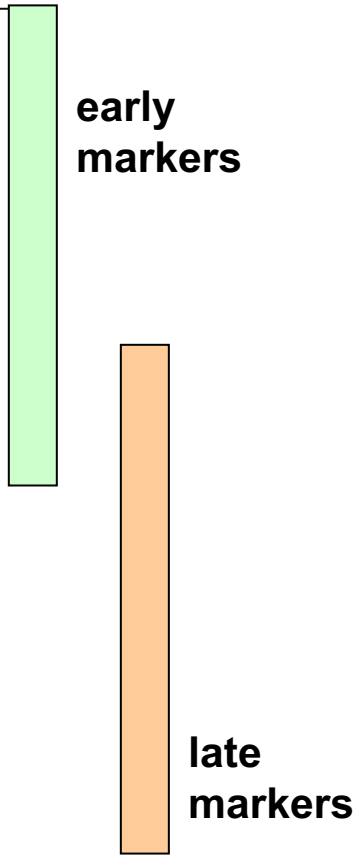
Ectoderm ⟶ Otic placode → Otic vesicle ⟶ Sensory patches → hair cells




F-actin
Espin

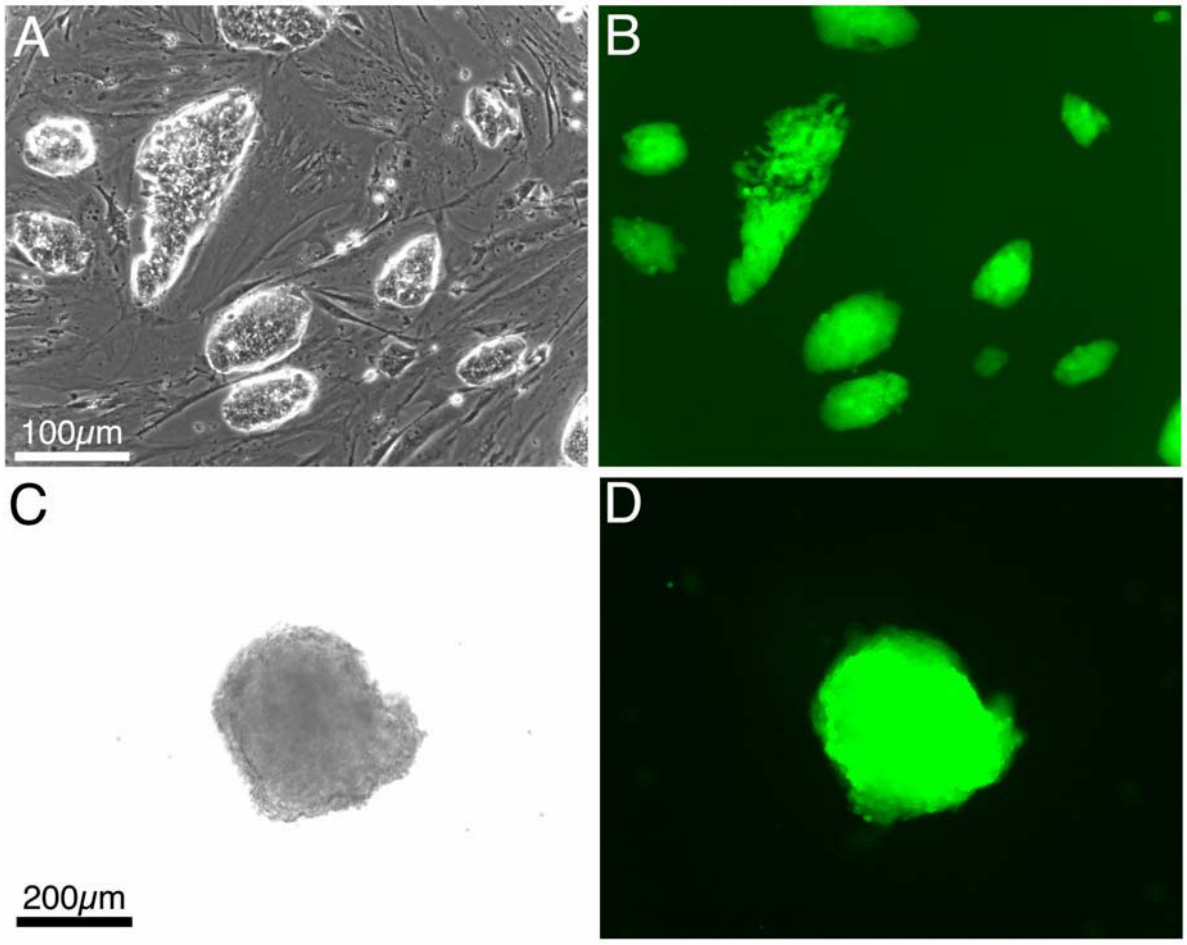
Collection of inner ear marker genes

MARKER GENE	EXPRESSION
Otx2	undifferentiated ES cells, neuroectoderm, otic vesicle
Pax2	otic placode, developing midbrain, hindbrain, and eye
Sox3	otic placode
BMP4, BMP7	otic placode, otic vesicle
Hmx3	developing vestibular inner ear
Nkx5-1, Nkx5-2	developing inner ear non-sensory epithelia, nascent stria vascularis
Sox10	otic vesicle, developing inner ear sensory epithelia, supporting cells
Math1	developing inner ear sensory epithelia
Notch1	otic vesicle, developing inner ear sensory epithelia
Delta1	developing inner ear sensory epithelia, nascent hair cells
Jagged1	developing inner ear sensory epithelia, supporting cells
Jagged2	developing inner ear sensory epithelia
Brn3.1	developing inner ear sensory epithelia, mature hair cells
Pv3	early hair cells, mature hair cells
Myo VIIA	otic vesicle, olfactory epithelium, retina, hair cells
Espin	hair cells, Sertoli cells
AchR α9, α10	cochlear and vestibular hair cells
Mehc1	inner hair cells, mature photoreceptor cells
Tmc1	hair cells
Cx26	inner ear non-sensory cells, skin
TectA	developing inner ear
Coch	inner ear fibrocytes
Pax6	developing eye, developing central nervous system
Nestin	Neuronal progenitors
NCAM	Neuronal progenitors
GAPDH	ubiquitously expressed housekeeping gene



Embryonic development: Marker genes can reveal cell types

Ectoderm ⟶ Otic placode → Otic vesicle ⟶ Sensory patches → hair cells



ES cells

Embryoid bodies (Ectoderm, Endoderm, Mesoderm)

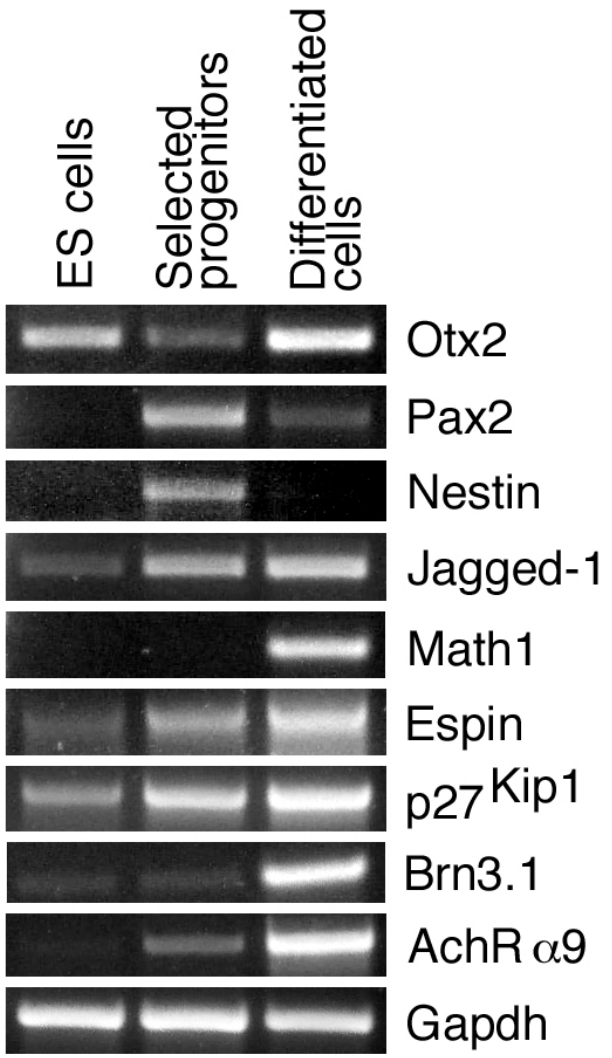
Embryonic development: Marker genes can reveal cell types

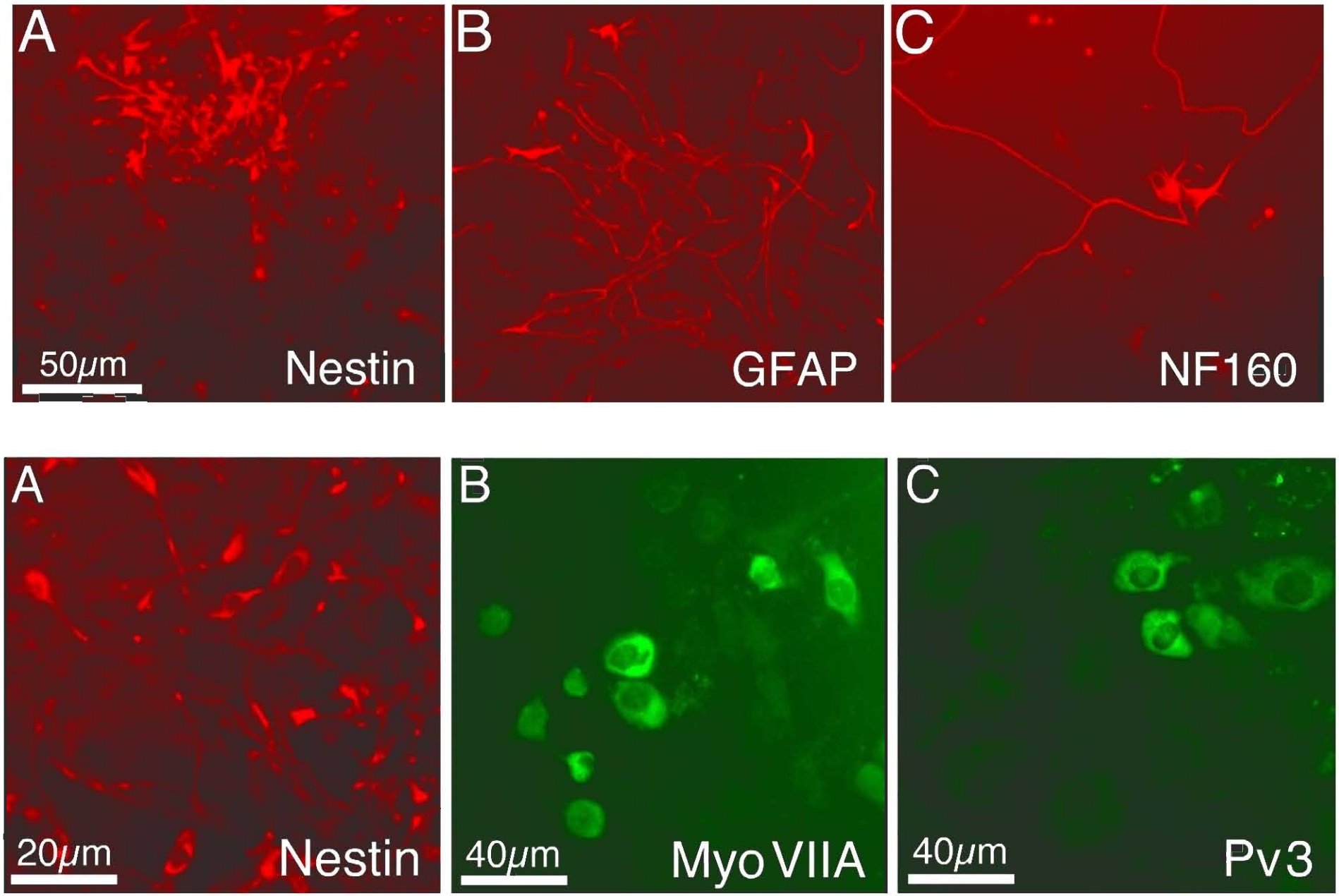
Ectoderm → Otic placode → Otic vesicle → Sensory patches → hair cells

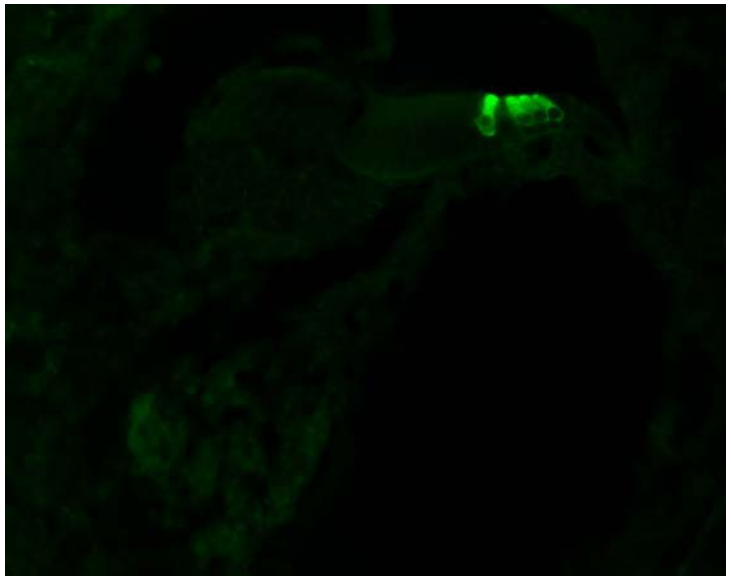
Embryoid bodies
(Ectoderm, Endoderm,
Mesoderm)

↓ Factors that promote selective survival of otic progenitors

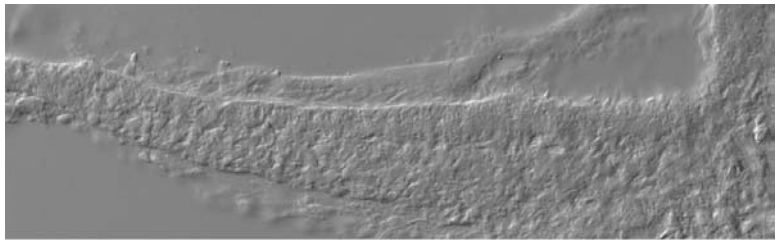
Cell types of the otic placode:
cells that express placode markers

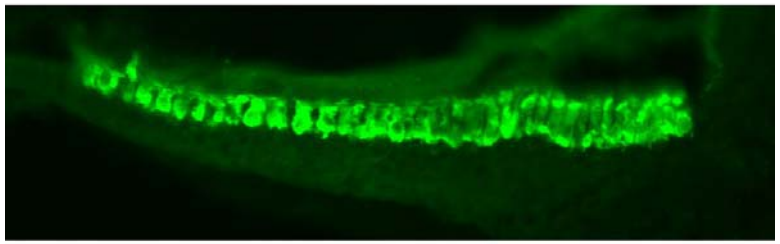



A
50μm
Nestin
B
GFAP
C
NF160
A
20μm
Nestin
B
40μm
Myo VIIA
C
40μm
Pv3

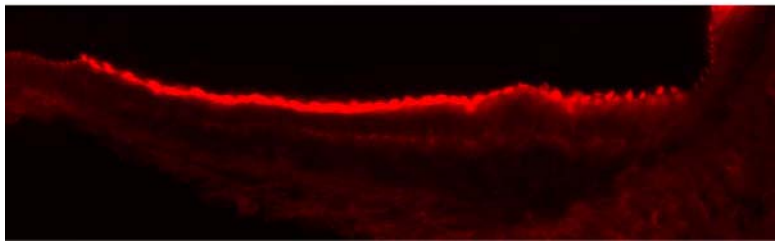


Myosin VIIA





Pv3



F-actin

What is next:

a) Are the hair cell-like cells really hair cells?
b) Are these cells able to integrate into a developing ear?
c) Are these cells able to integrate into an injured ear?
d) Can we “heal” a deaf mouse with these cells?

Inner ear stem cells?

Adult stem cells

Progenitor cells

Hair cells

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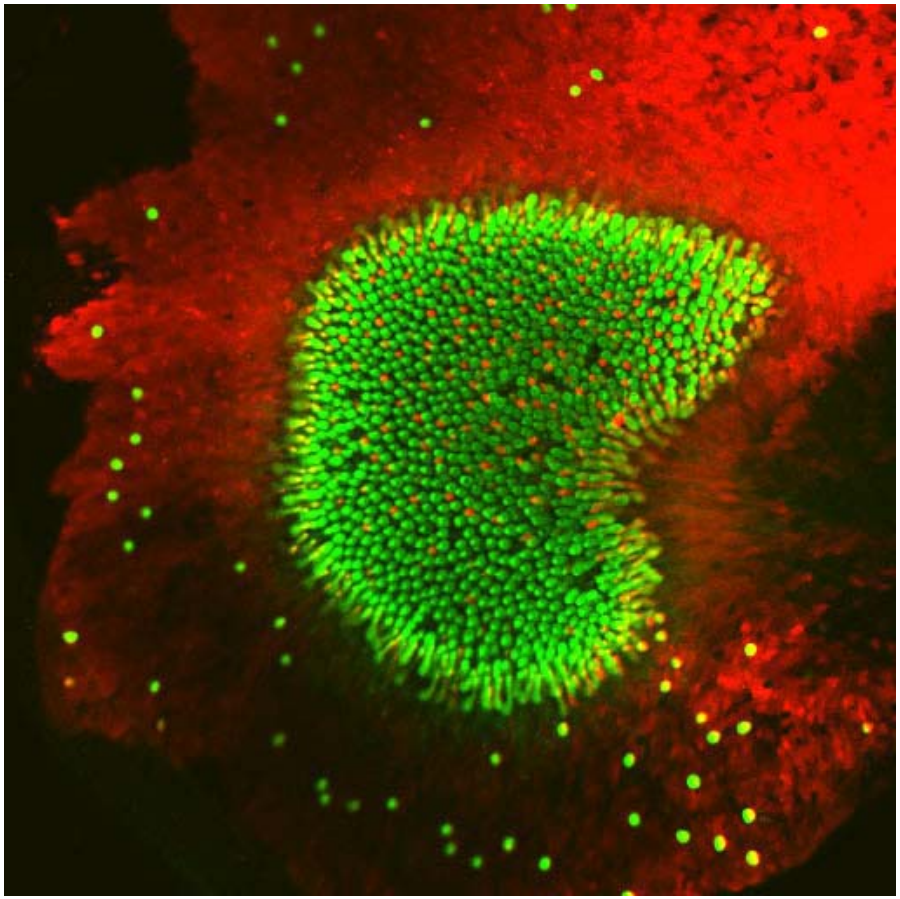
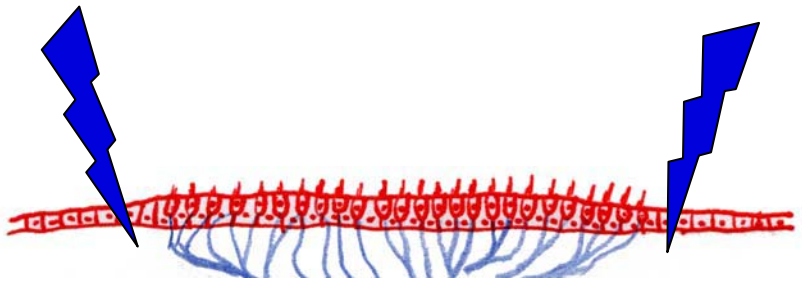
Are there adult stem cells in the inner ear?

- Hair cell regeneration happens to a small degree in the mouse utricle (Forge et al., 1993, Science 259)





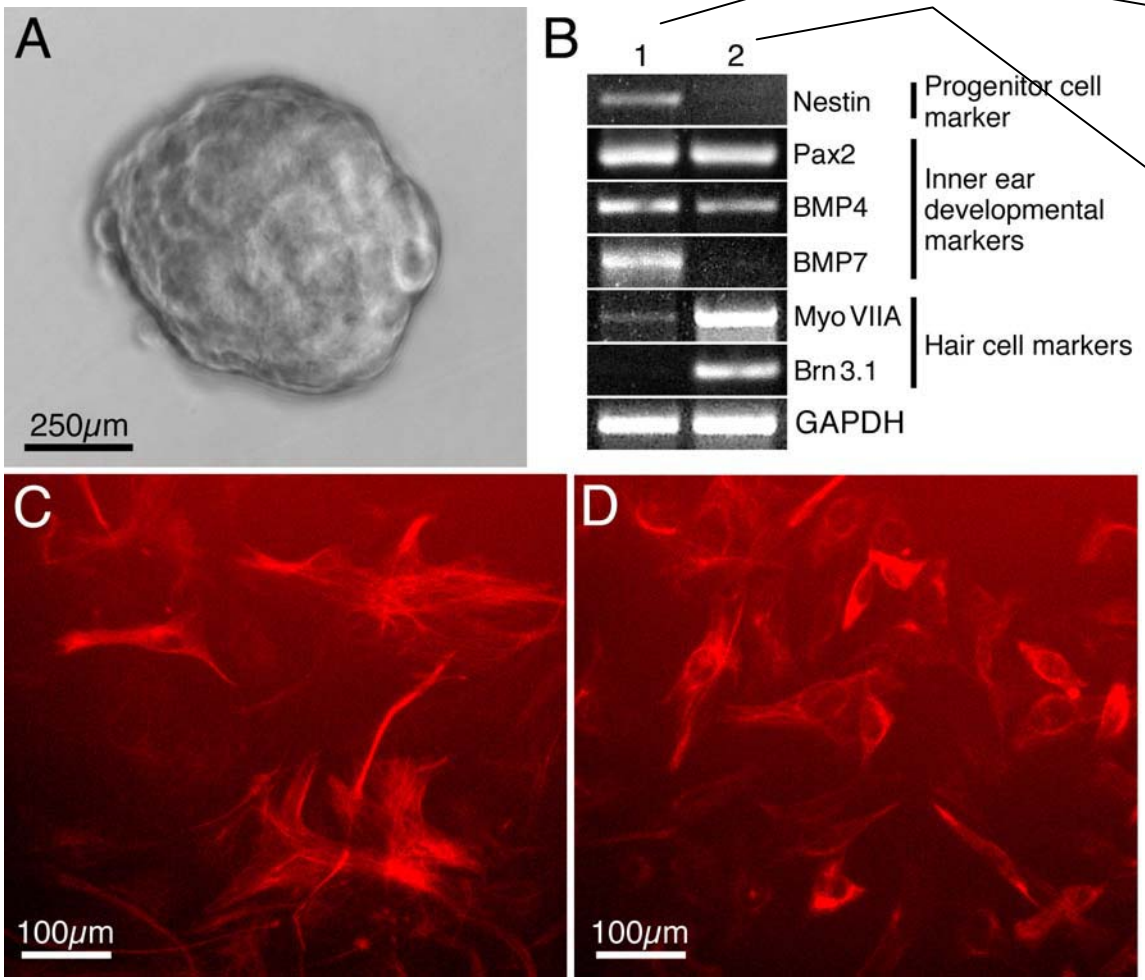
Principal strategy

In neural stem cell world, this would be called a neurosphere

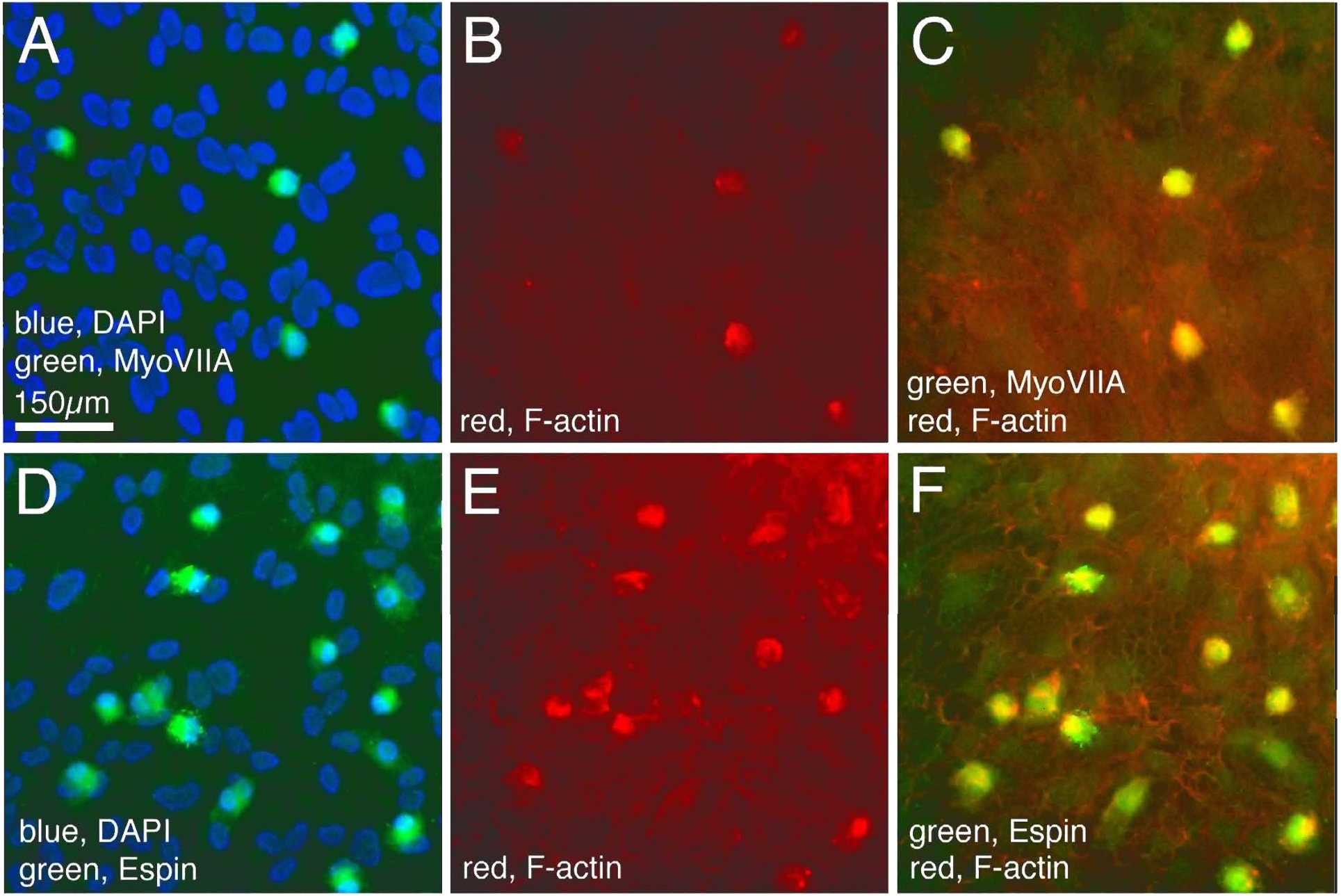
Mouse utricle → Low density single cell culture → Sphere formation

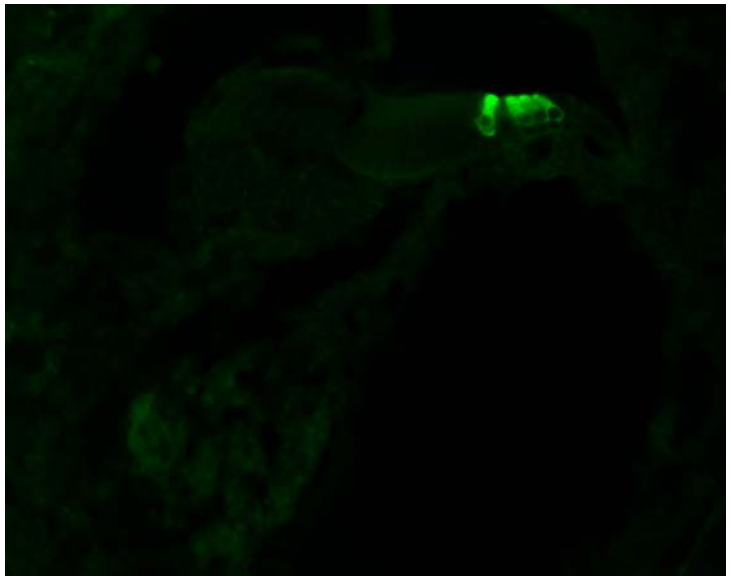
Selection of progenitor cells

Hair cells and supporting cells by *in vitro* differentiation

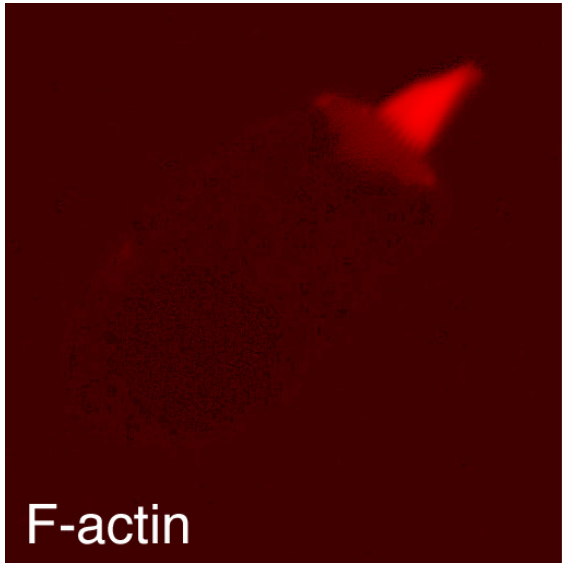


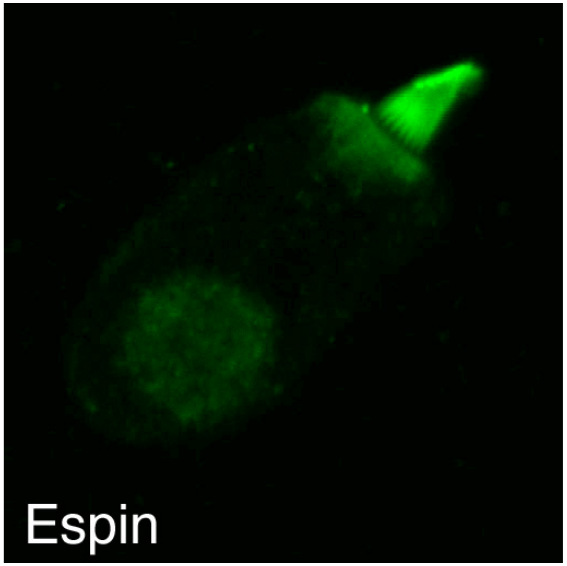
Immunostaining for the progenitor cell marker nestin in neural stem cells (C) and in inner ear spheres (D)

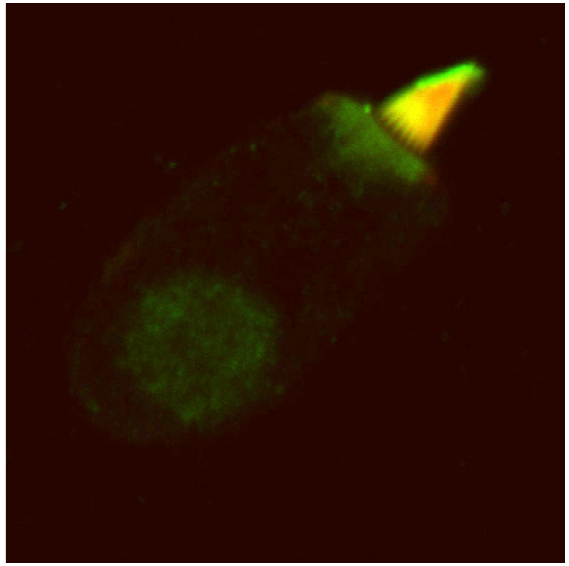

A
blue, DAPI
green, MyoVIIA
150μm
B
red, F-actin
C
green, MyoVIIA
red, F-actin
D
blue, DAPI
green, Espin
E
red, F-actin
F
green, Espin
red, F-actin



Myosin VIIA







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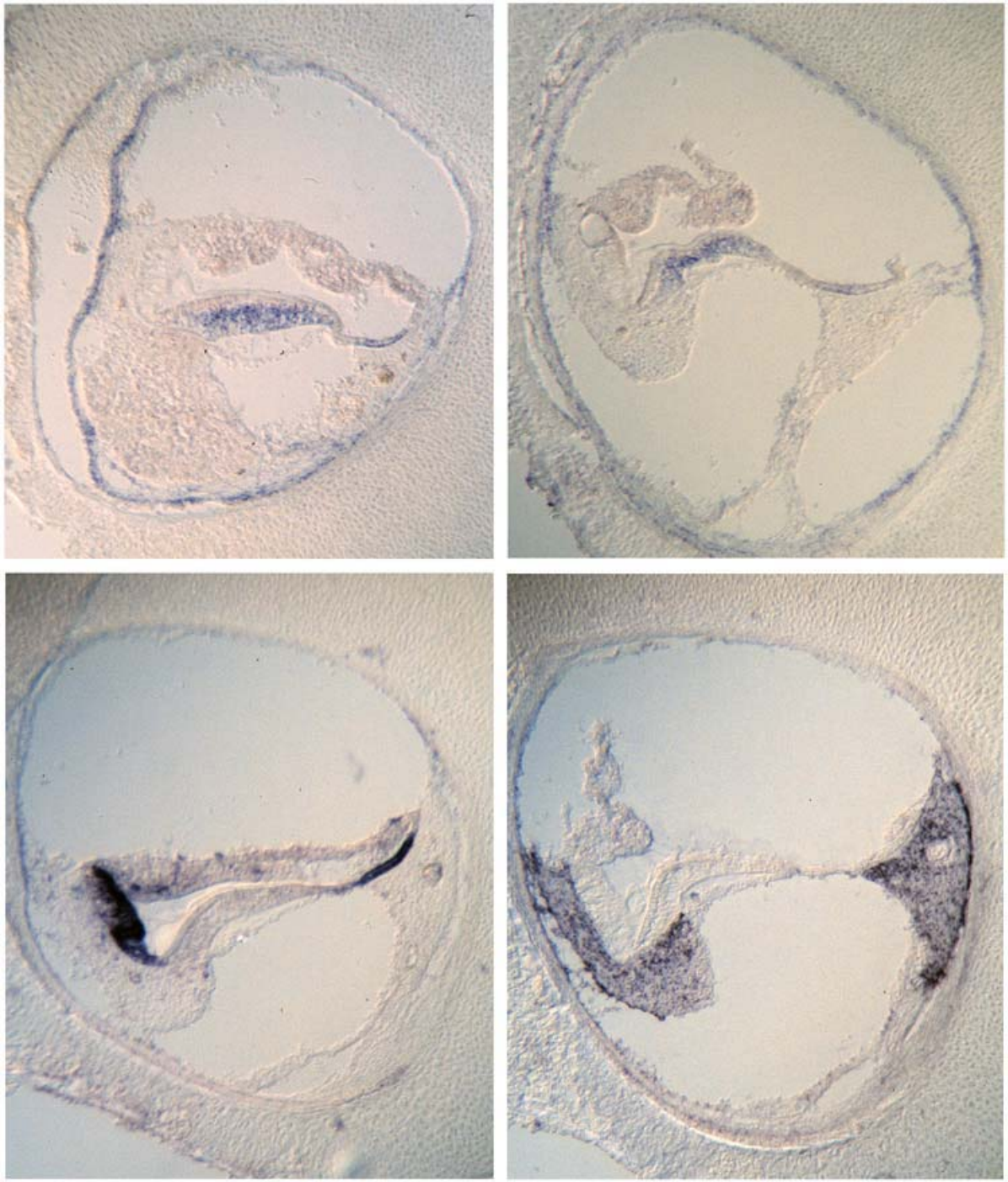
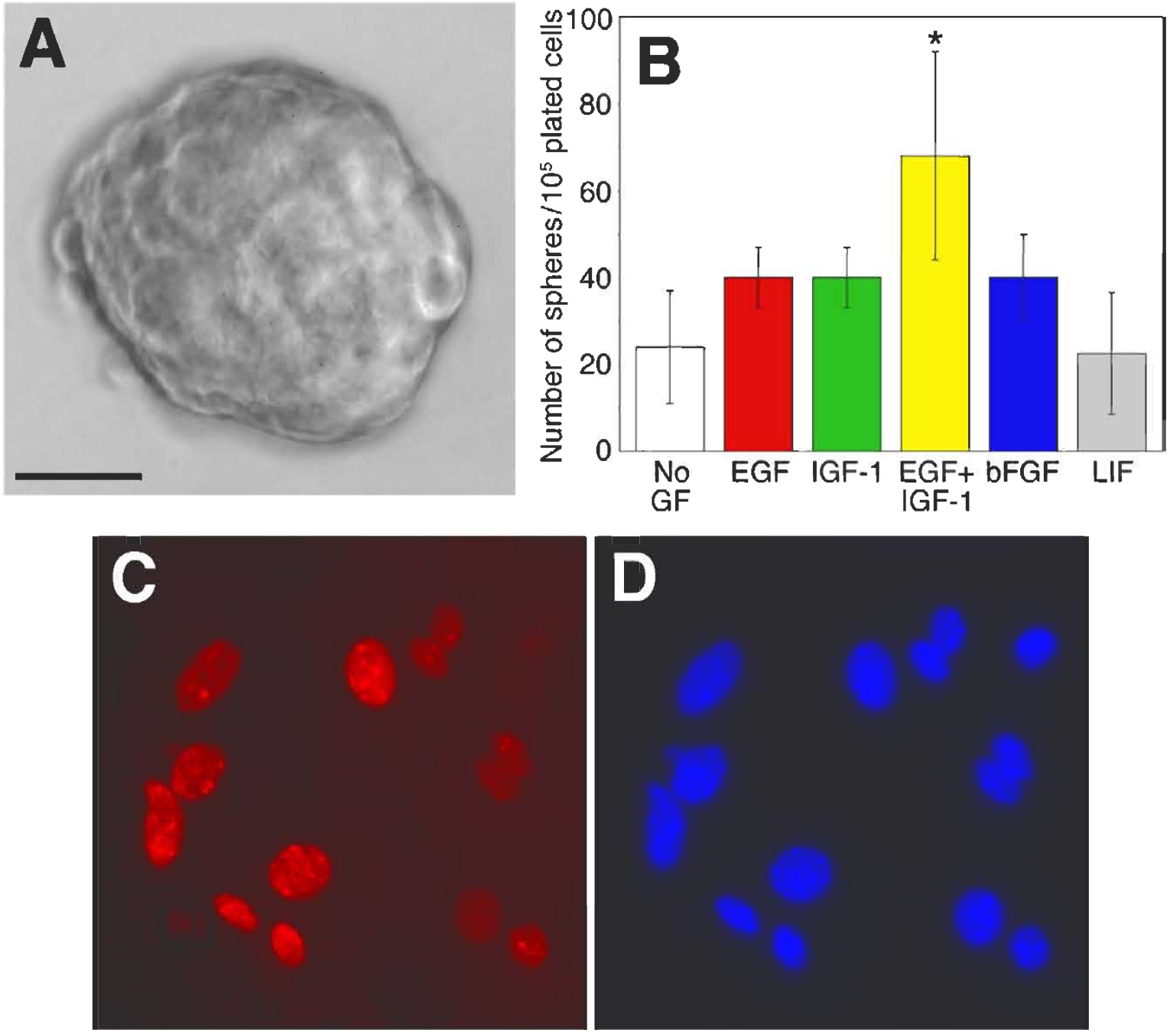
c) Are these cells able to integrate into an injured ear?
d) Can we “heal” a deaf mouse with these cells?
e) Can we generate other cell types as well?
   For example auditory neurons:
   Do these neurons (re-)innervate a cochlea?

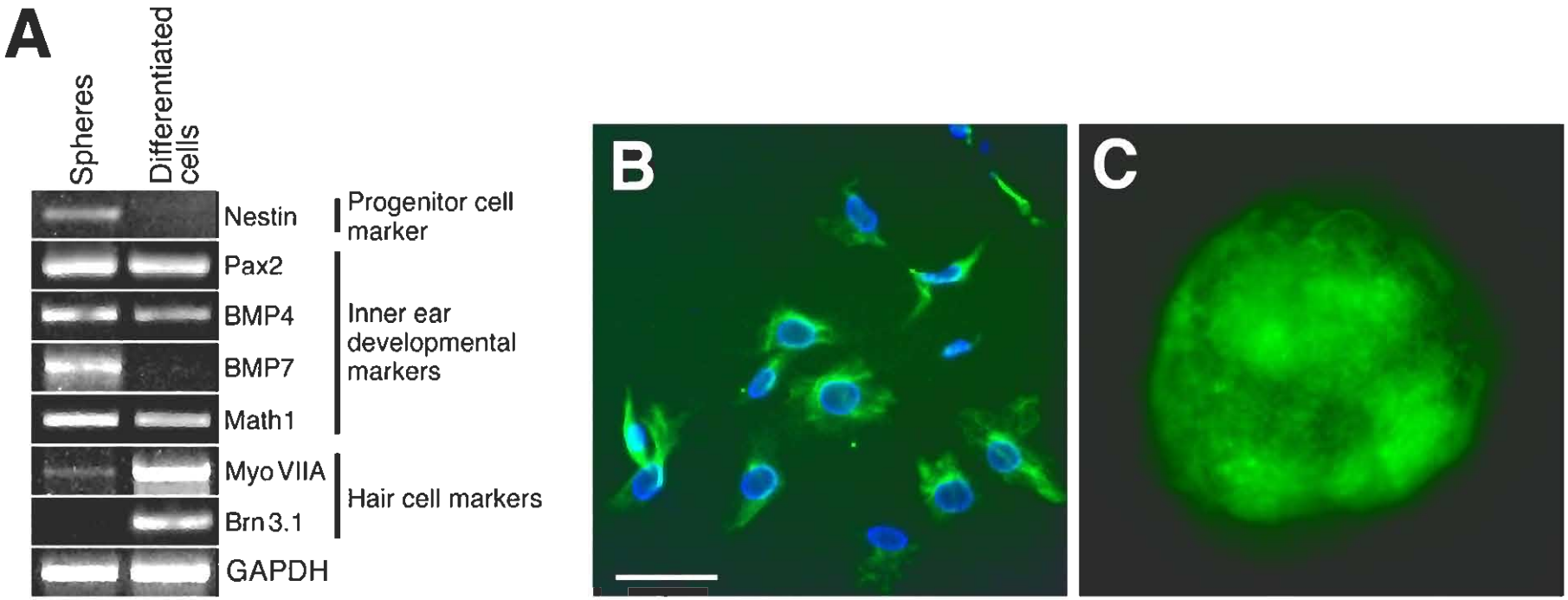


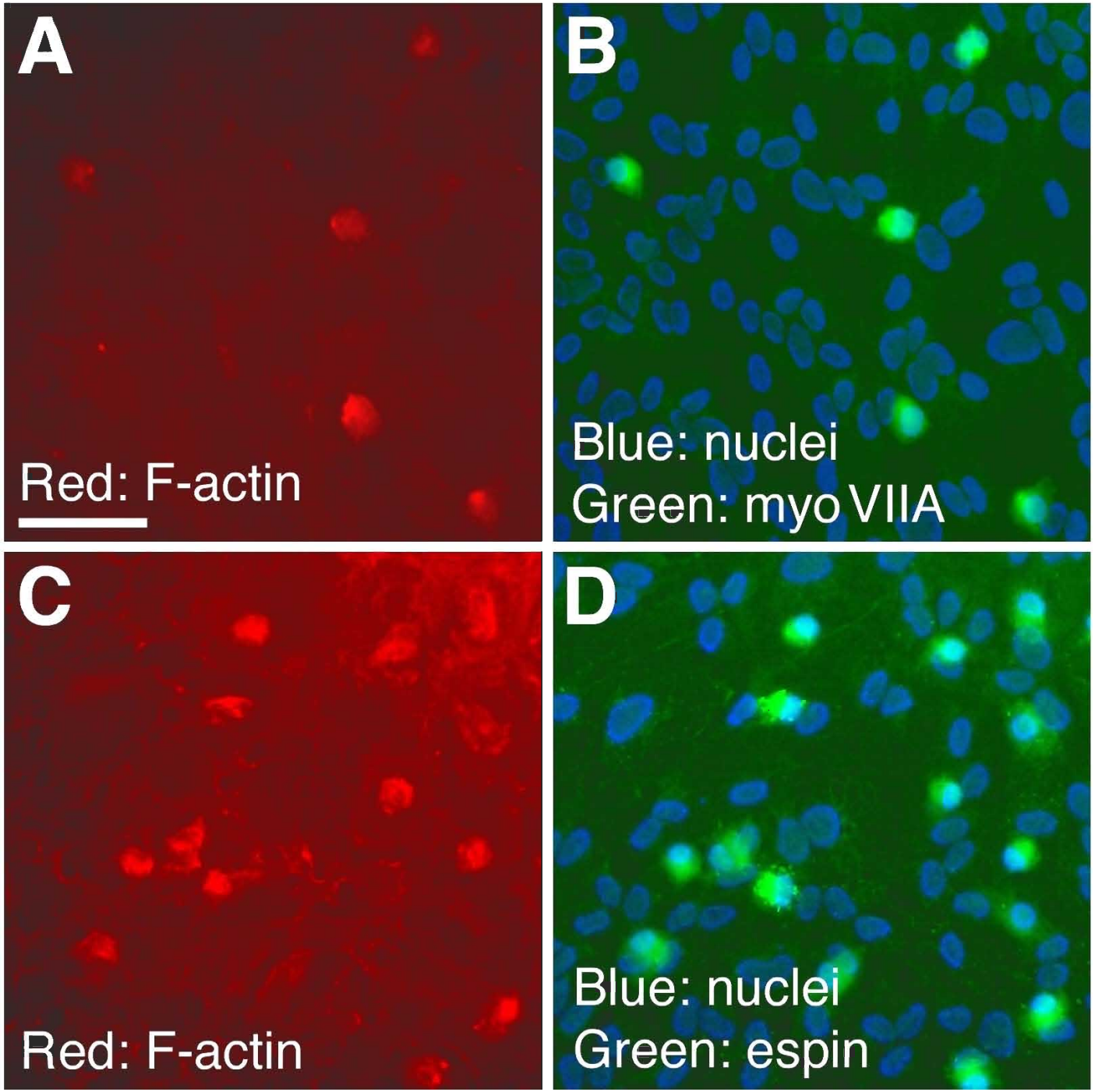
Elimination of the auditory ganglion with a neurotoxin - followed by “repair” with progenitor cells selected from ES or adult stem cells.

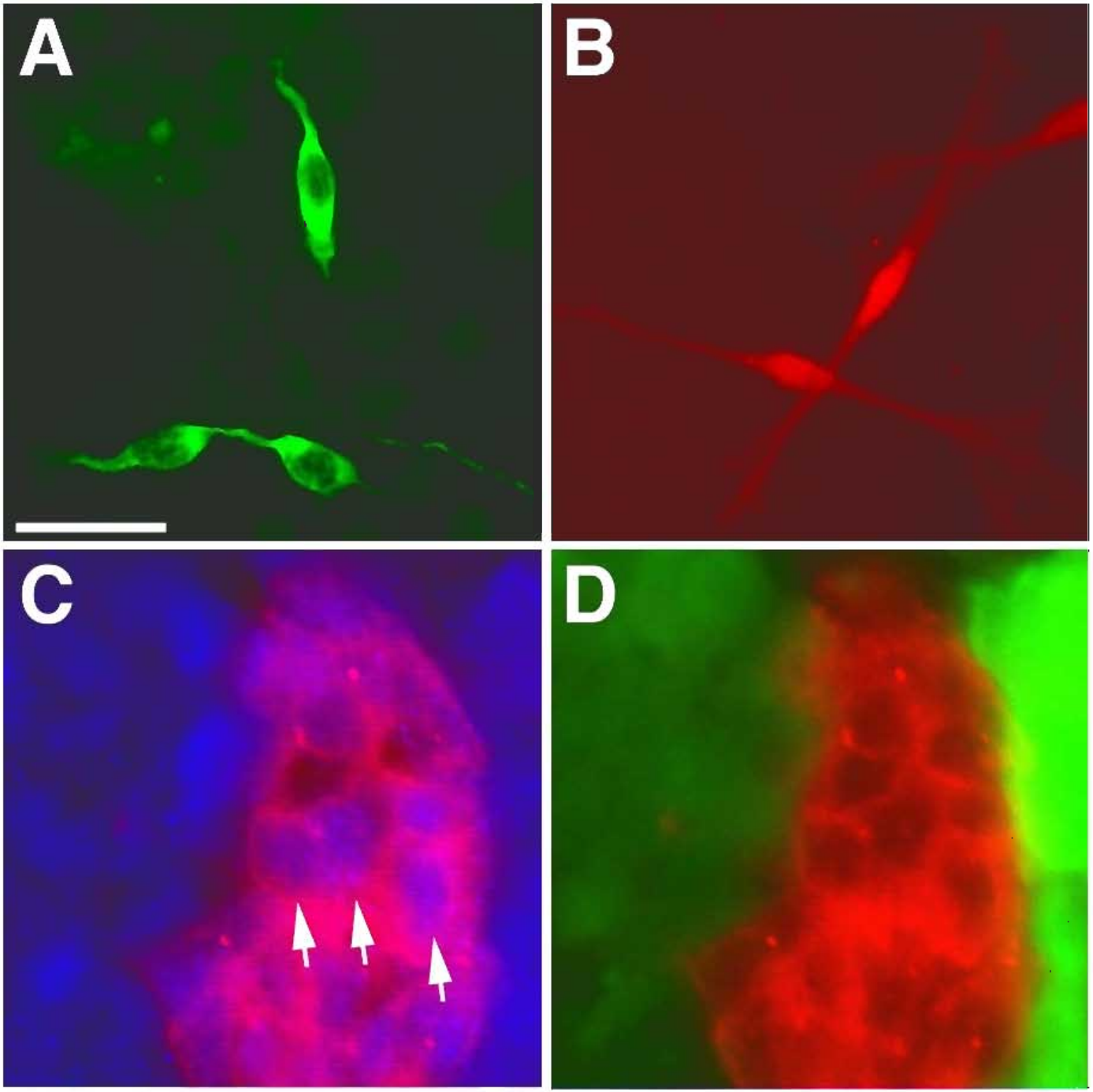
A) TypeII collagen
B) TypeII collagen after elimination
C) Homogenin after elimination
D) Coch after elimination

=> No change in other cell types.


A
B
Number of spheres/10⁵ plated cells
100
80
60
40
20
0
*
No GF
EGF
IGF-1
EGF+ IGF-1
bFGF
LIF
C
D


A
Spheres
Differentiated cells
Nestin
Pax2
BMP4
BMP7
Math1
Myo VIIA
Brn3.1
GAPDH
Progenitor cell marker
Inner ear developmental markers
Hair cell markers
B
C


A
Red: F-actin
B
Blue: nuclei
Green: myo VIIA
C
Red: F-actin
D
Blue: nuclei
Green: espin


A
B
C
D

Further Reading

From Groves and Bronner-Fraser, 2000