



Molecular characterization of rabbit ES and iPS cells

Pierre Osteil, Murielle Godet, Suzy S. Markossian, Yann Tapponnier, Thierry Joly, Pierre Savatier, Marielle Afanassieff

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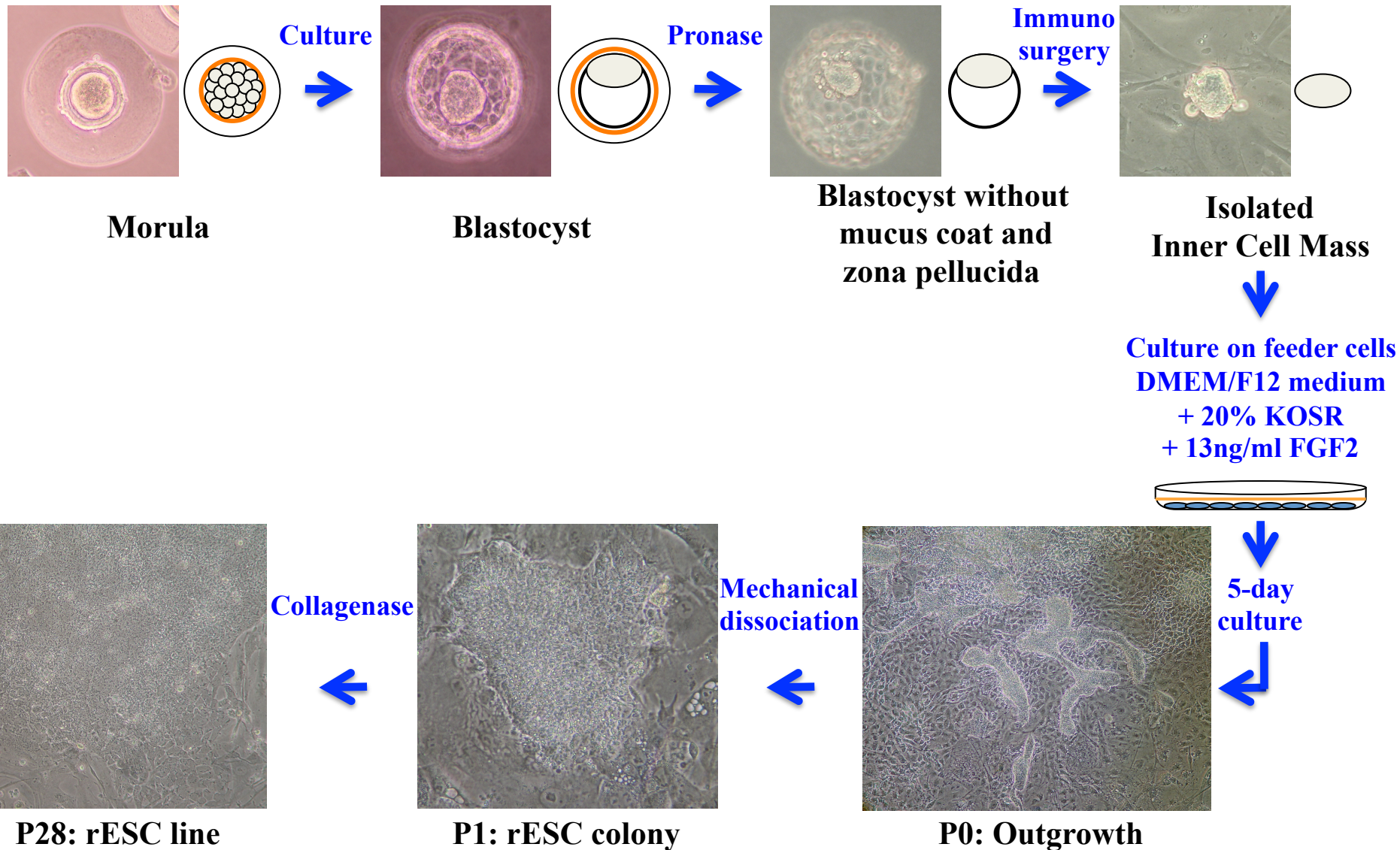
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2nd Plurabbit meeting
Gödöllő Hongria
27/11/2010

***Molecular characterization
of rabbit ES and iPS cells***

**Pierre Osteil, Murielle Godet, Suzy Markossian,
Yann Taponnier, Thierry Joly,
Pierre Savatier and Marielle Afanassieff**

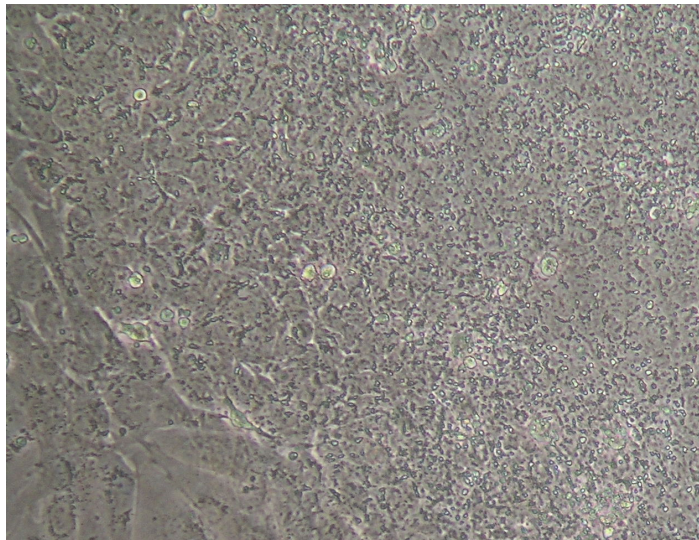
Derivation of rabbit ES cells



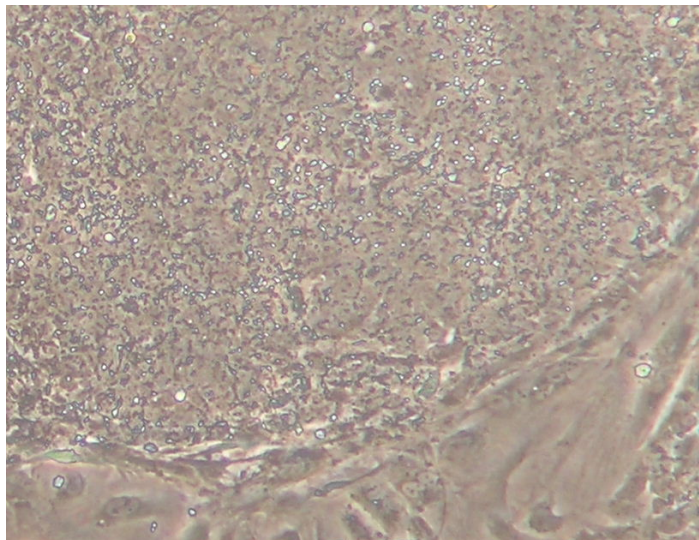
Rabbit ES cell (rESC) lines



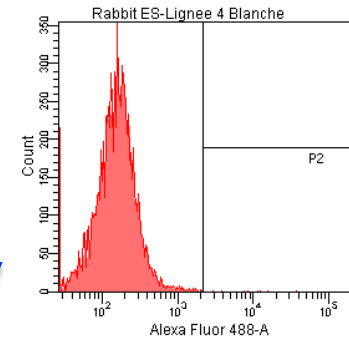
$\sigma\text{GFP}^{+/-}$



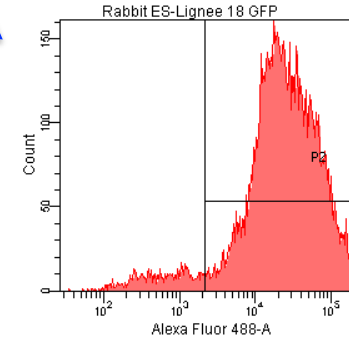
FGF2-dependant rESC



LIF-dependant rESC



rESC GFP⁻
F4, F8, F19



rESC GFP⁺
F17, F18, F24



rESC
L1 and L2

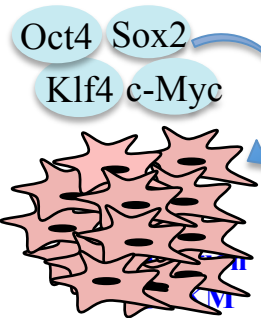
Generation of rabbit iPS cells

Differentiated
cells

Loss of
morphology

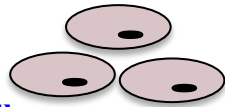
Colony
formation

Pluripotent
stem cells

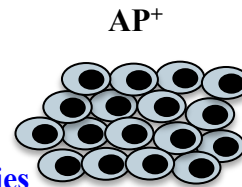


Overexpression

Transfer on feeder cells
+ iPS culture medium

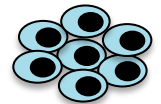


Picking colonies
+ amplification



Oct4⁺ Sox2⁺ Nanog⁺

Analysis at P6



Rabbit
iPS cells

D0/D2

D5/D6

D16

D27

D40/D55

D60

D70

D80

D90

D100

D110

D120

D130

D140

D150

D160

D170

D180

D190

D200

D210

D220

D230

D240

D250

D260

D270

D280

D290

D300

D310

D320

D330

D340

D350

D360

D370

D380

D390

D400

D410

D420

D430

D440

D450

D460

D470

D480

D490

D500

D510

D520

D530

D540

D550

D560

D570

D580

D590

D600

D610

D620

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D650

D660

D670

D680

D690

D700

D710

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D770

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D790

D800

D810

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D830

D840

D850

D860

D870

D880

D890

D900

D910

D920

D930

D940

D950

D960

D970

D980

D990

D1000

D1010

D1020

D1030

D1040

D1050

D1060

D1070

D1080

D1090

D1100

D1110

D1120

D1130

D1140

D1150

D1160

D1170

D1180

D1190

D1200

D1210

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D1250

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D1500

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D1800

D1810

D1820

D1830

D1840

D1850

D1860

D1870

D1880

D1890

D1900

D1910

D1920

D1930

D1940

D1950

D1960

D1970

D1980

D1990

D2000

D2010

D2020

D2030

D2040

D2050

D2060

D2070

D2080

D2090

D2100

D2110

D2120

D2130

D2140

D2150

D2160

D2170

D2180

D2190

D2200

D2210

D2220

D2230

D2240

D2250

D2260

D2270

D2280

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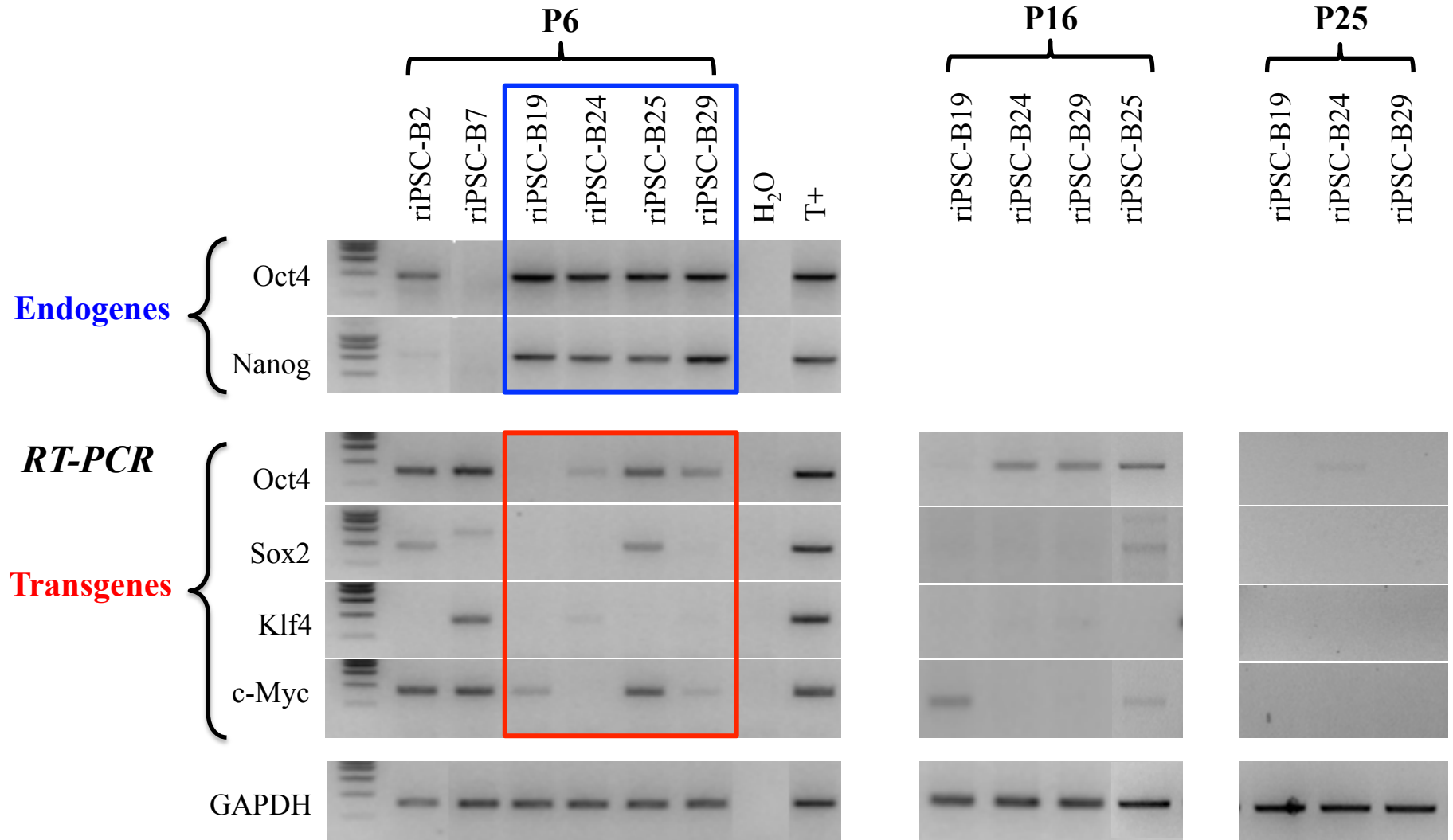
D3010

D3020

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D3040

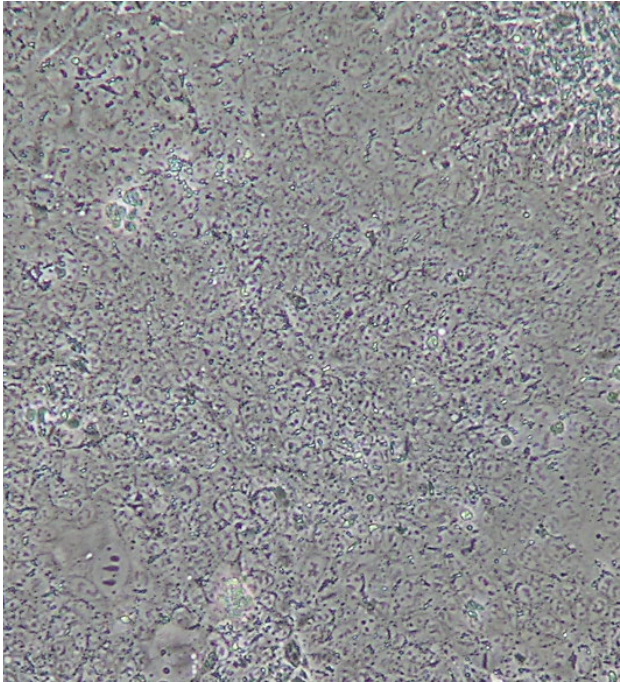
Rabbit iPSC cell (riPSC) lines



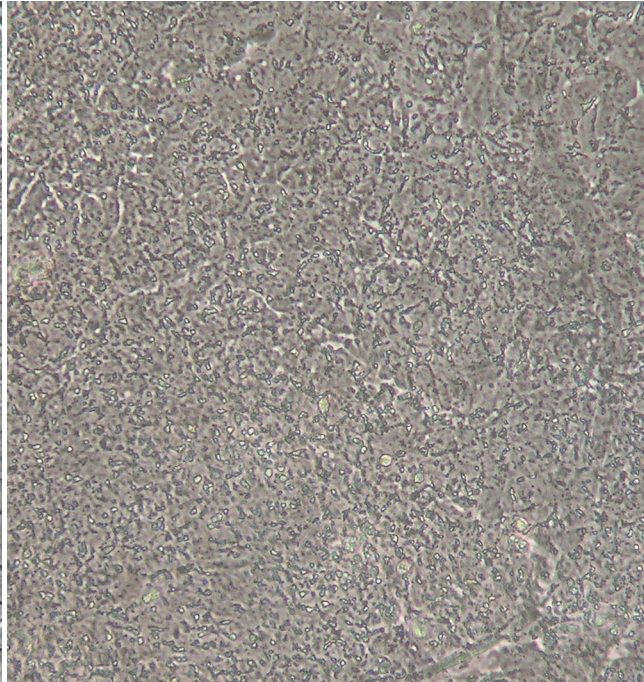
4 lines of FGF2-dependant riPSC: B19, B24, B25 and B29

Morphology of rESC and riPSC

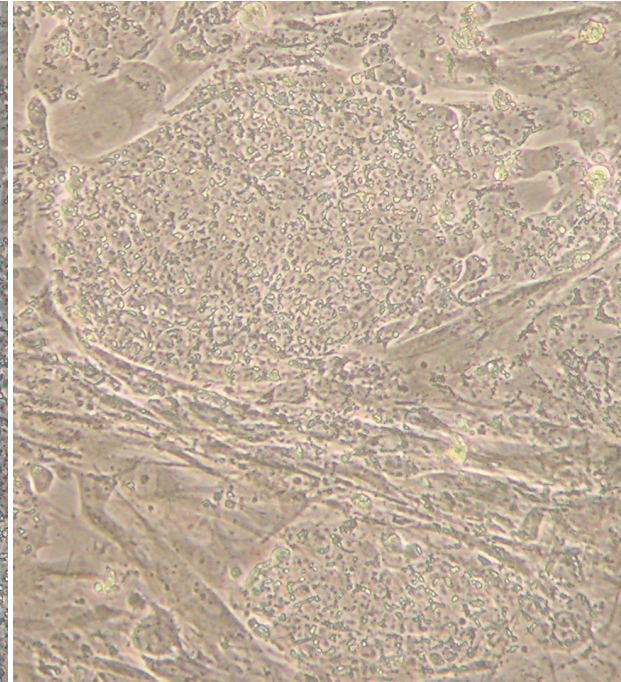
FGF2-dependant rESC



LIF-dependant rESC

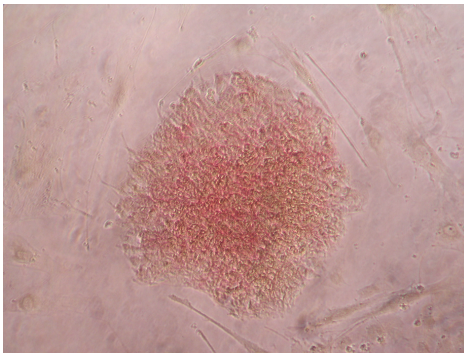


FGF2-dependant riPSC

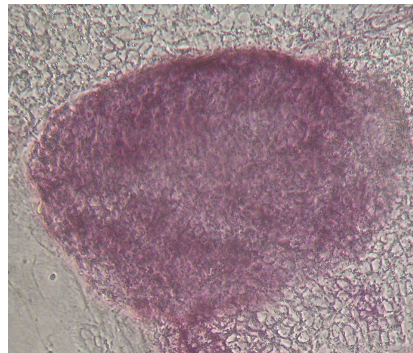


Alkaline phosphatase activity

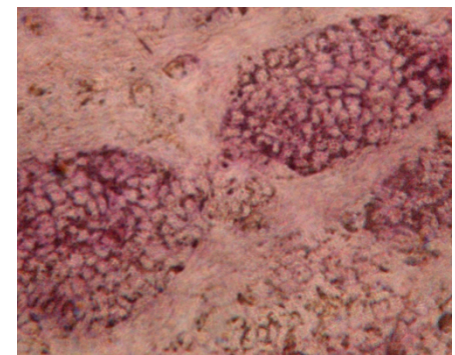
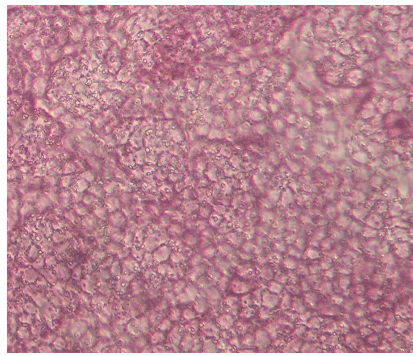
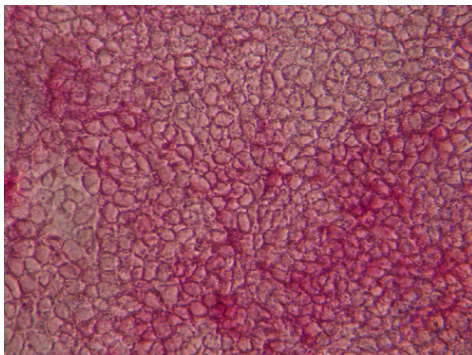
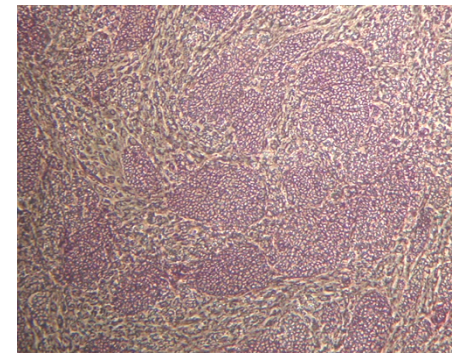
FGF2-dependant rESC



LIF-dependant rESC



FGF2-dependant riPSC



Expression of pluripotency markers

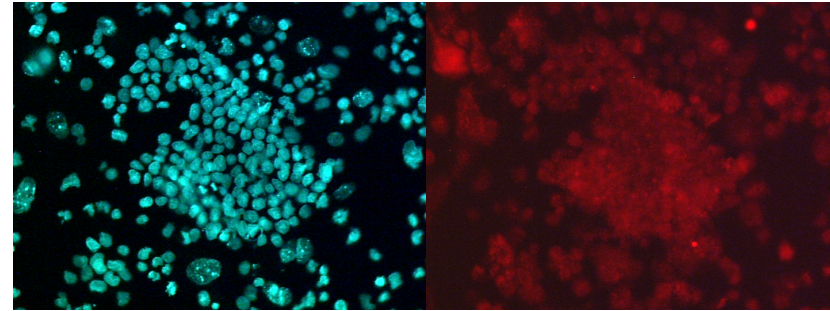
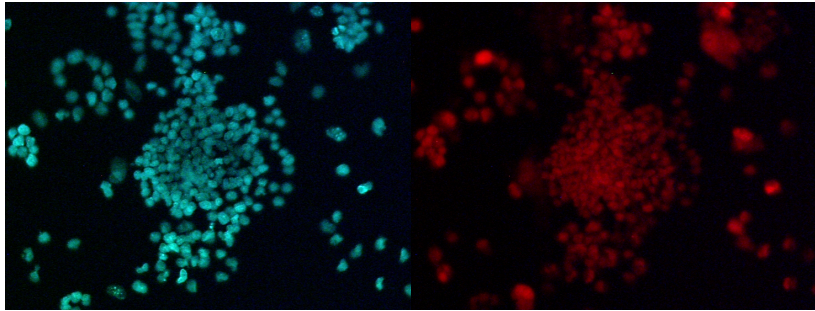
Hoechst

Oct-4

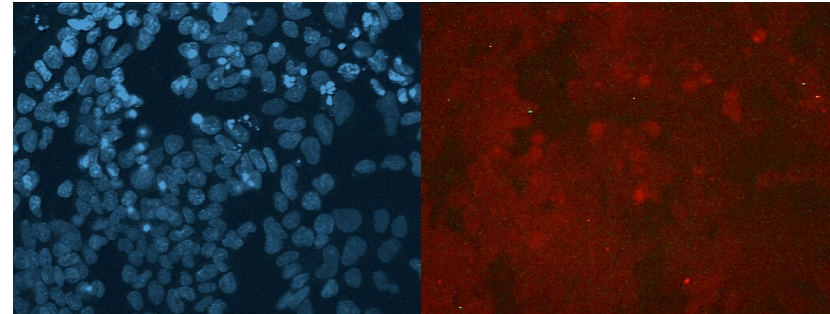
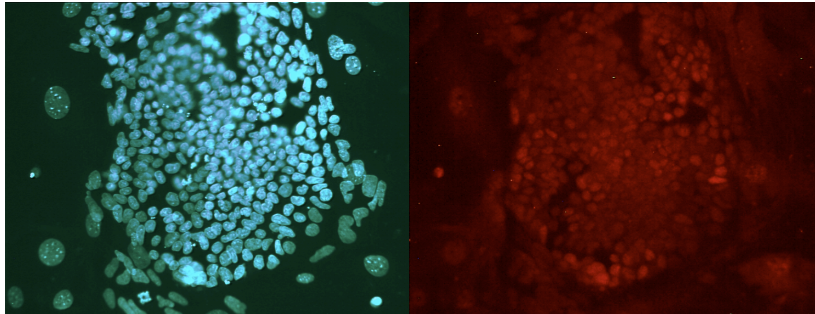
Hoechst

Nanog

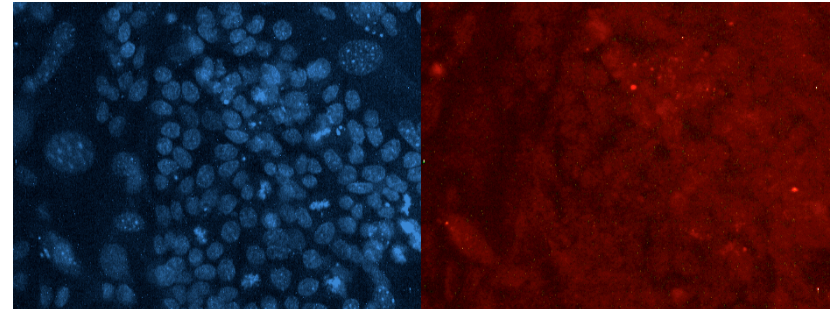
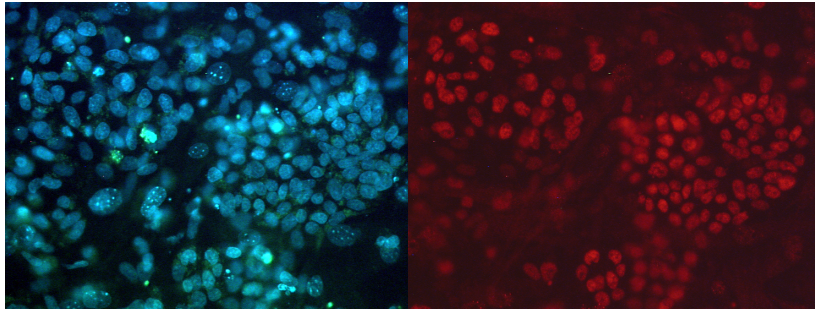
*FGF2-
dependant
rESC*



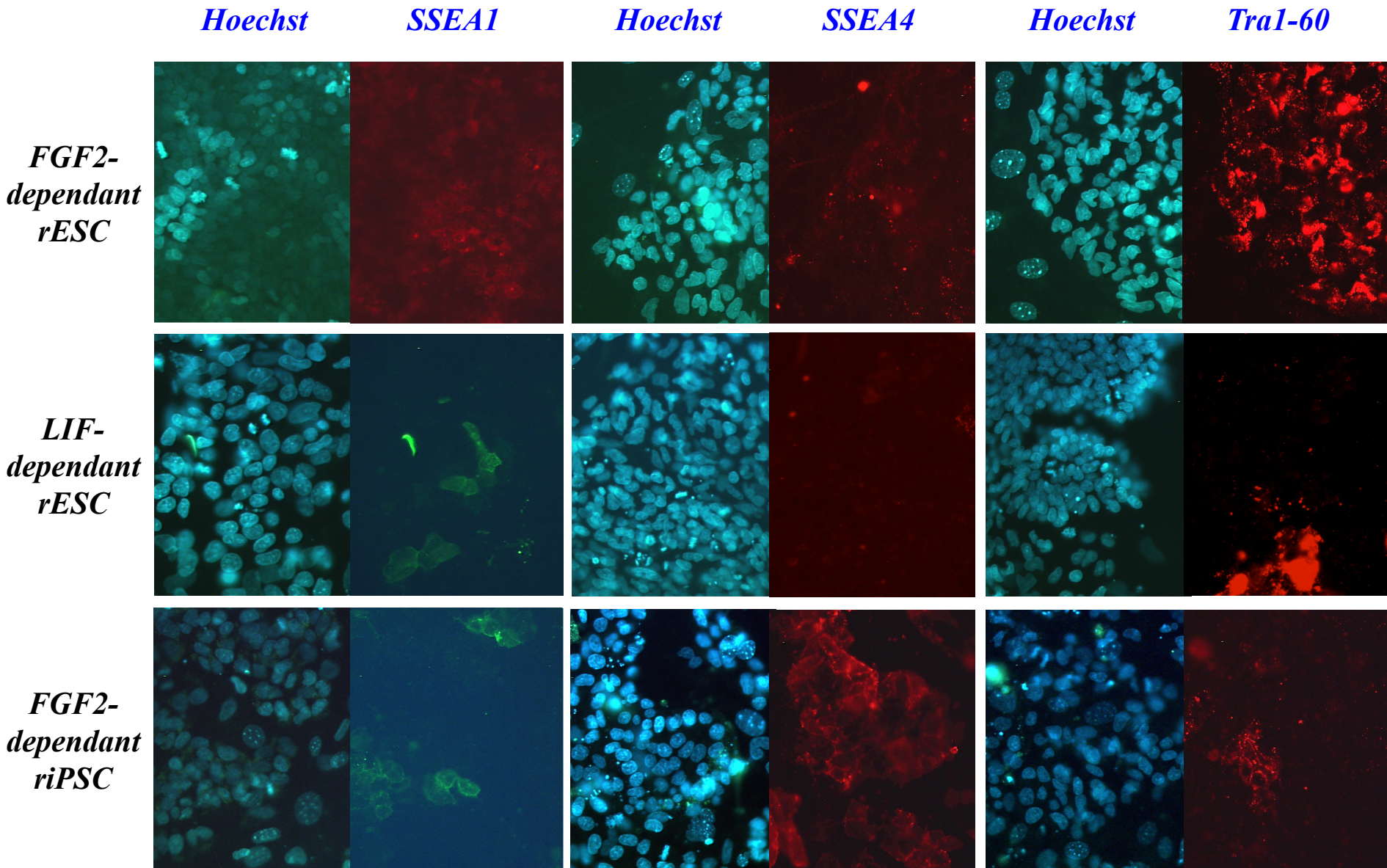
*LIF-
dependant
rESC*



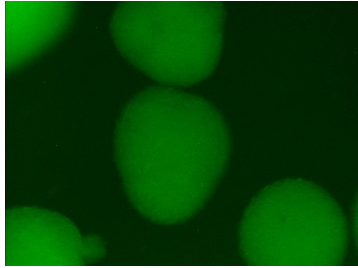
*FGF2-
dependant
riPSC*



Expression of cell-surface markers

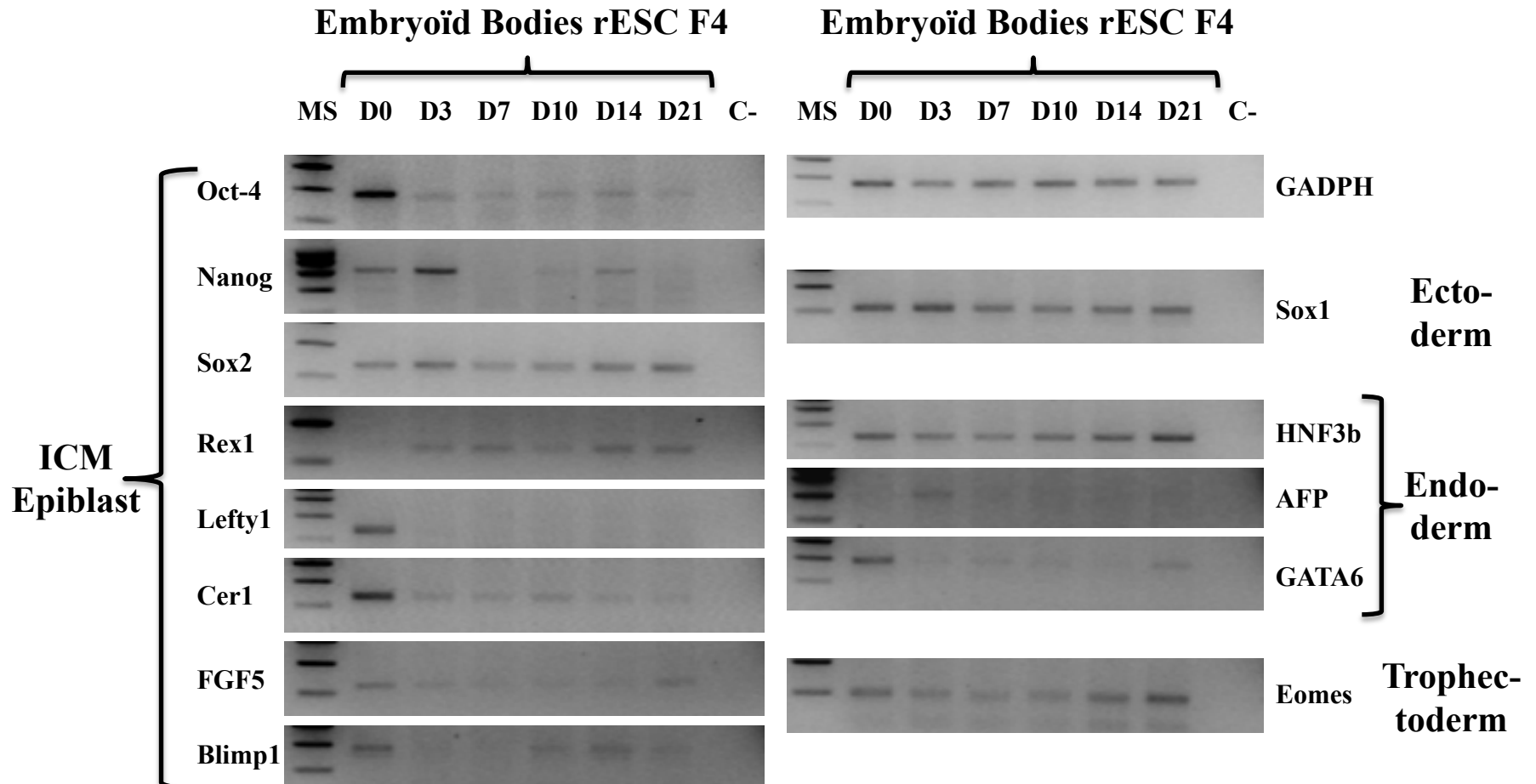
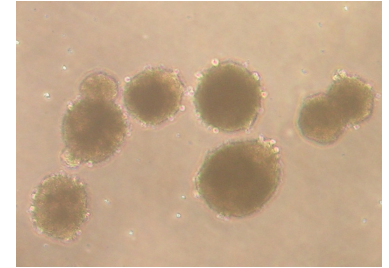


rESC F18 GFP+



Formation of embryoïd bodies

riPSC B29 GFP-



Production of teratoma



rESC F18



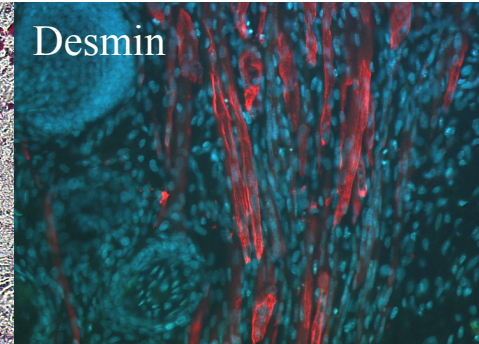
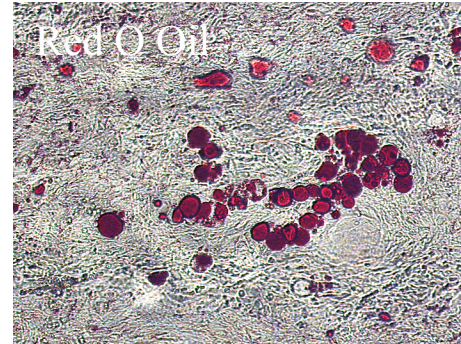
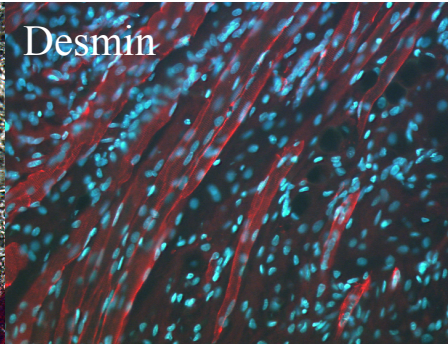
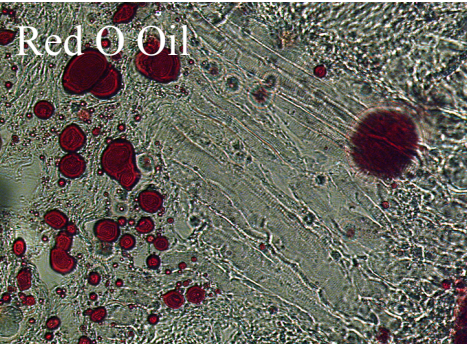
*Injection of $5 \cdot 10^6$ cells under
the kidney capsule of SCID mice*



riPSC B29

Mesoderm

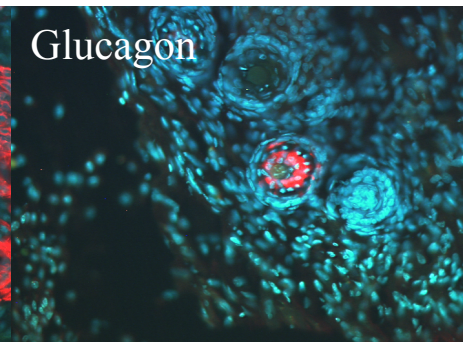
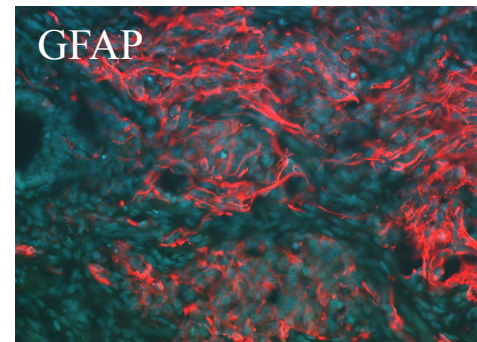
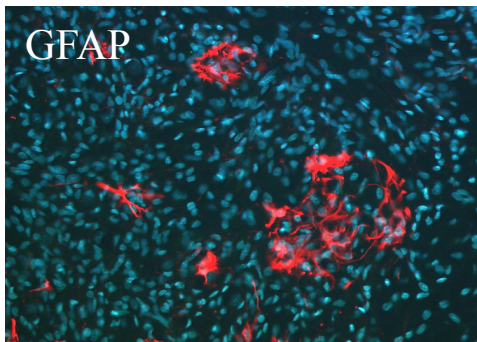
Mesoderm



Ectoderm

Ectoderm

Endoderm



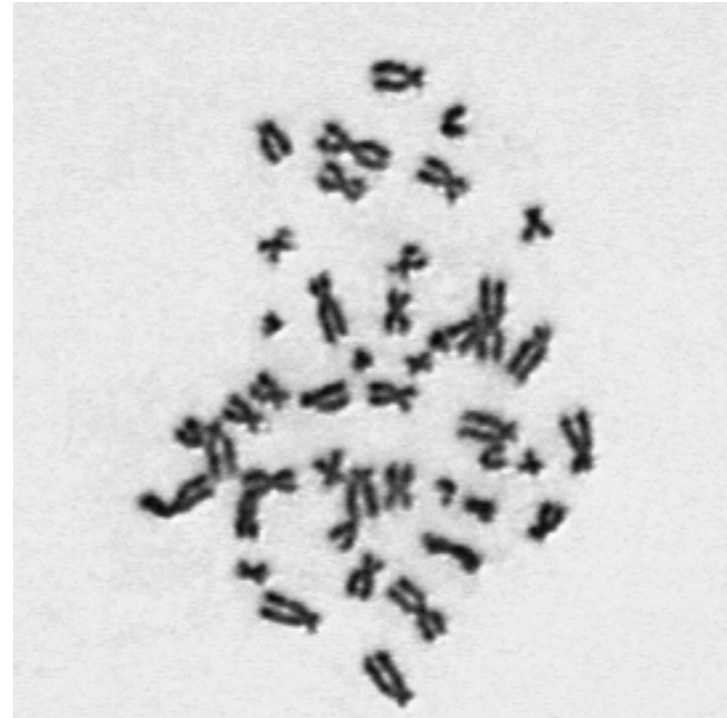
Analysis of karyotype

rESC F18



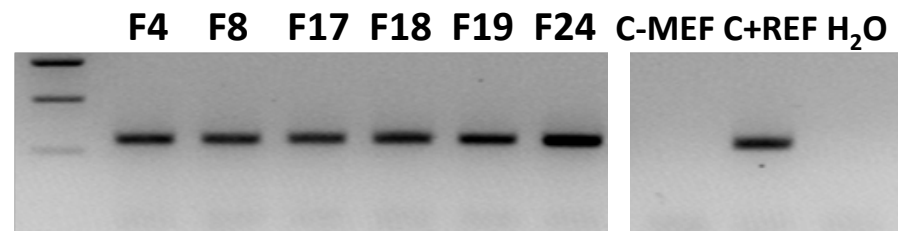
44 XY

riPSC B19



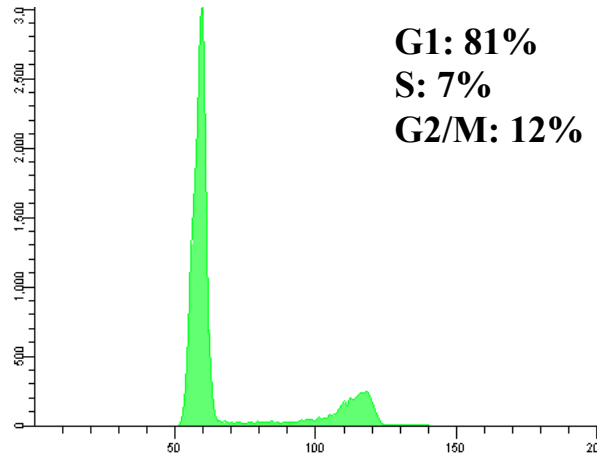
44 XX

*SRY gene detection by PCR
on FGF2-dependant rESC lines*

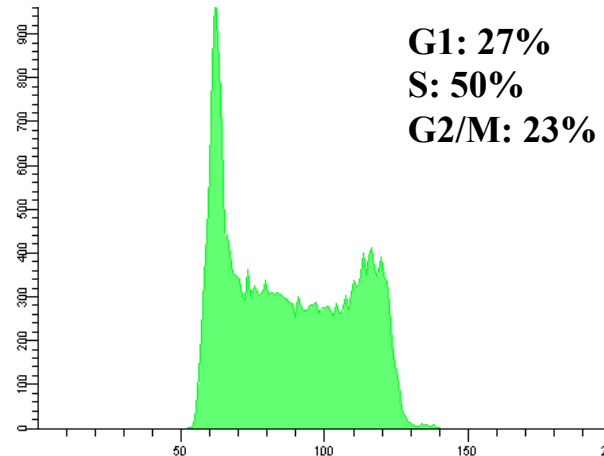


Cell cycle profiles

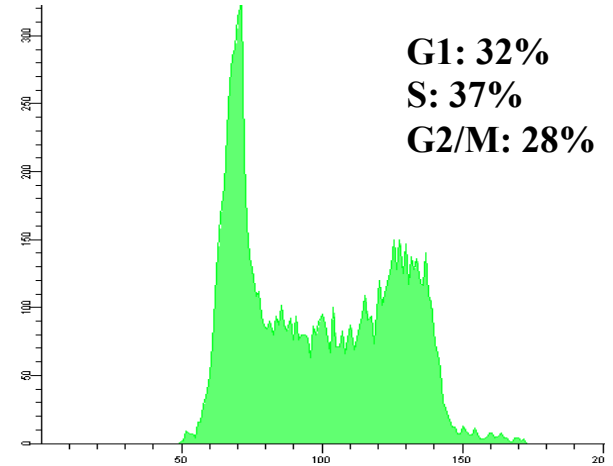
Rabbit Fibroblasts



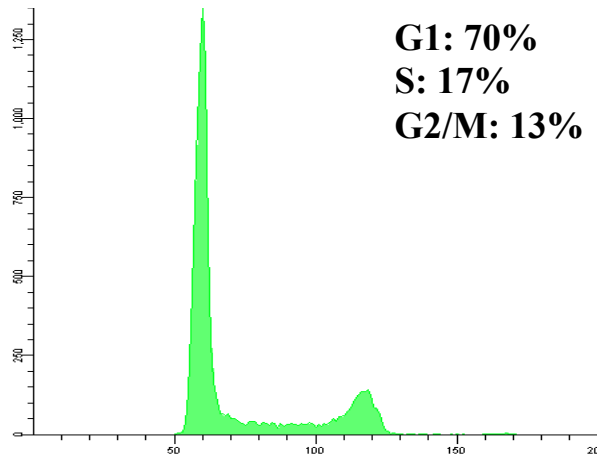
Mouse ESC



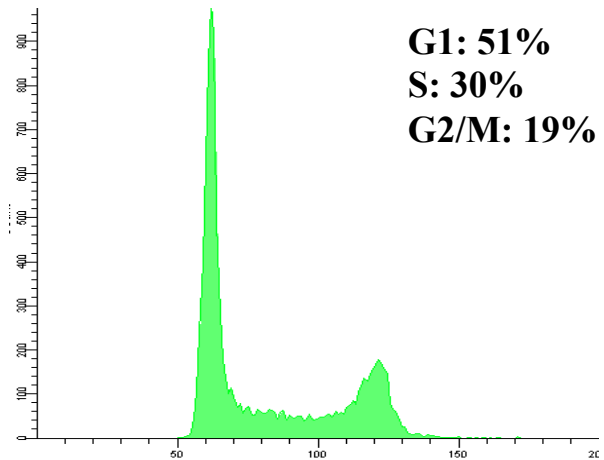
Rhesus ESC



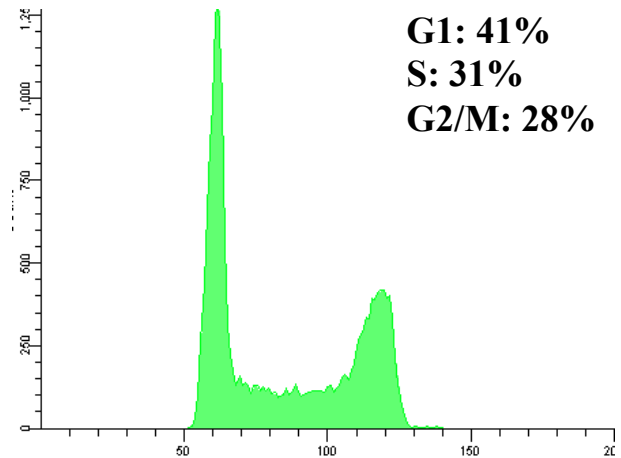
rESC F18



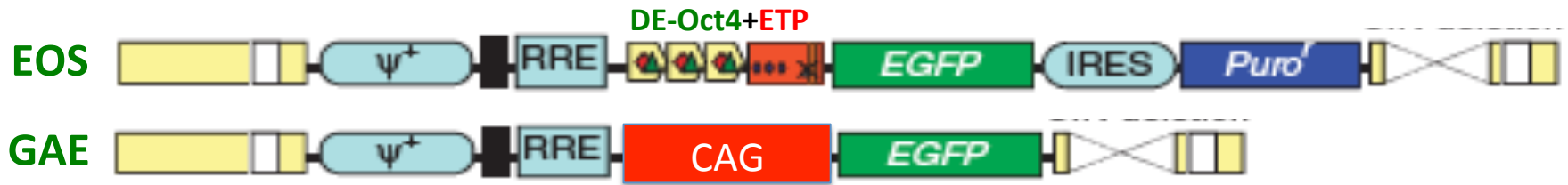
rESC L2



riPSC B19



Activity of mouse Oct-4 distal enhancer

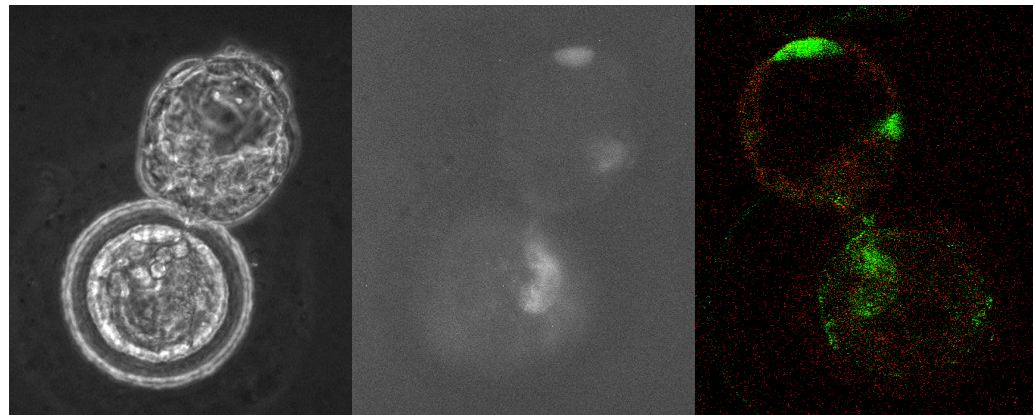


Bright light

GFP
fluorescence

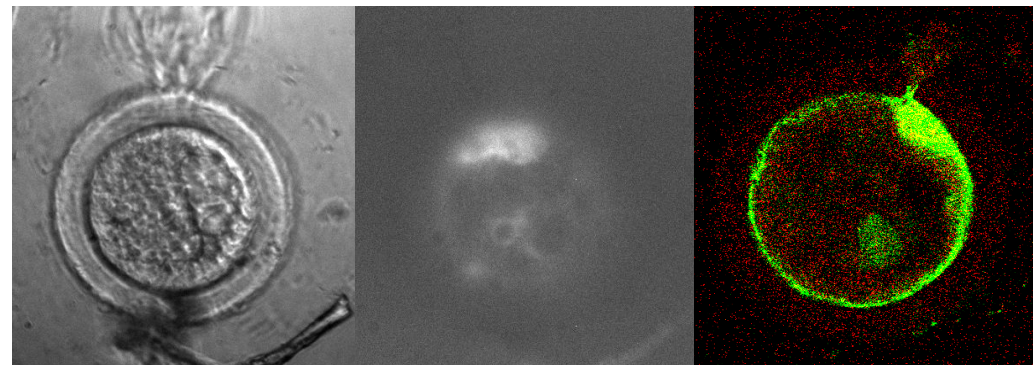
GFP
immuno-labelling

GAE



*Infection of 16-cell
embryos and analysis
at blastocyst stage*

EOS



Expression of EOS vector in rESC and riPSC

C⁺ mESC

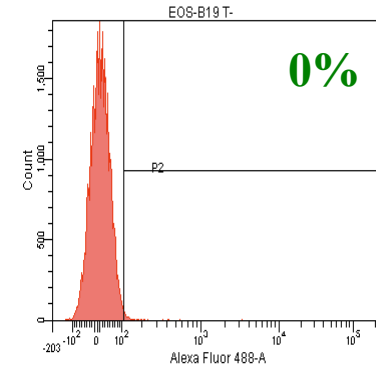
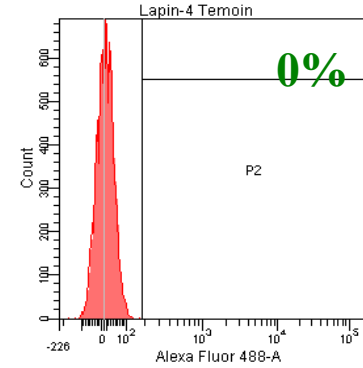
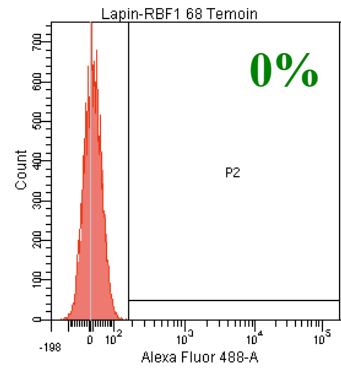
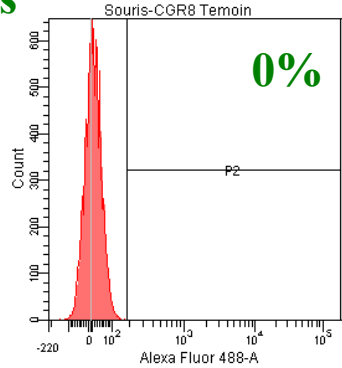
C⁻ rbF

rESC F4

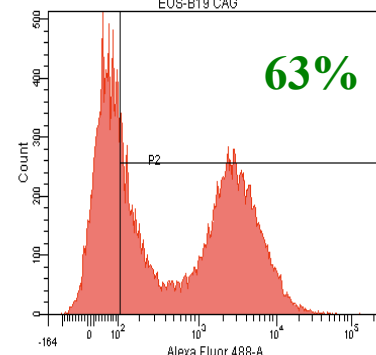
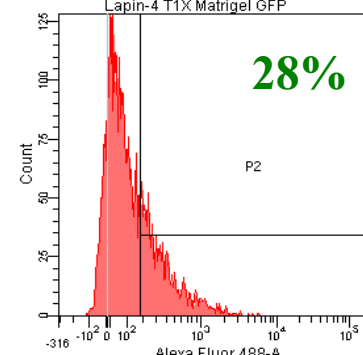
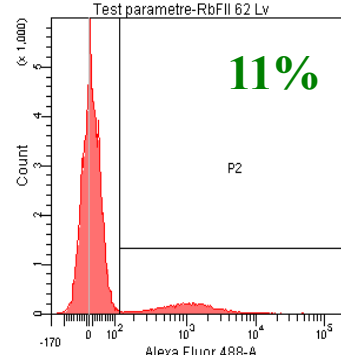
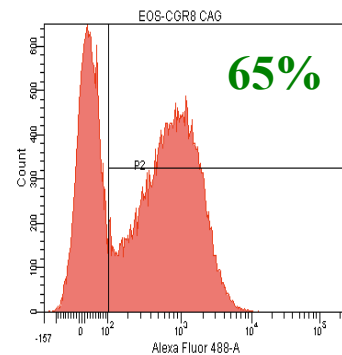
riPSC B19

% of GFP⁺ Cells

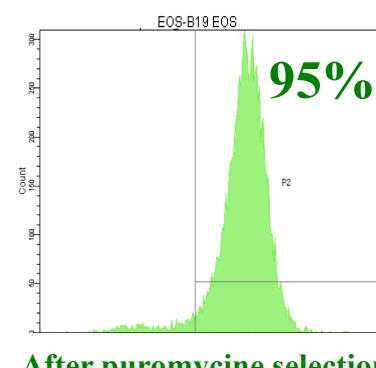
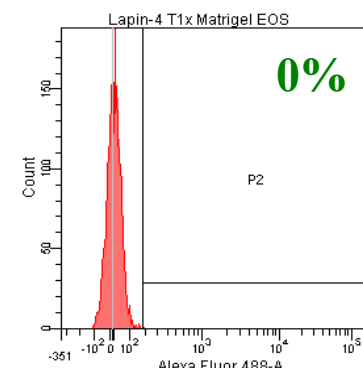
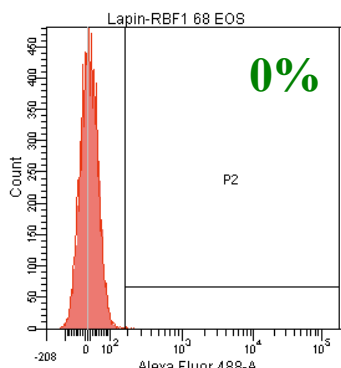
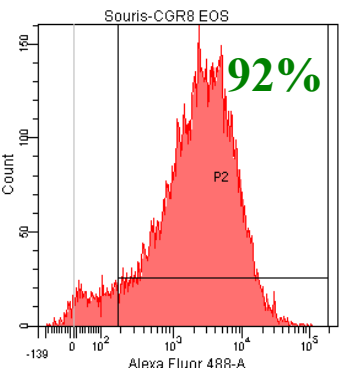
Non infection



GAE infection



EOS infection



After puromycin selection

Loss of mouse Oct-4 distal enhancer during rESC derivation

*Infection of 16-cell embryos with GAE or EOS lentiviruses
and analysis of GFP⁺ cells in ICM-derived outgrowths*

Non infected

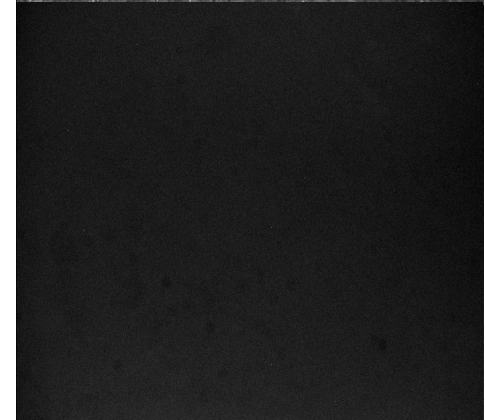
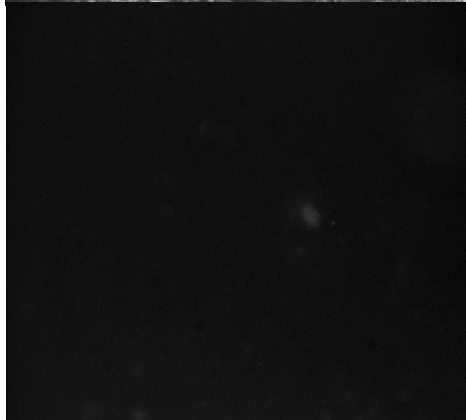
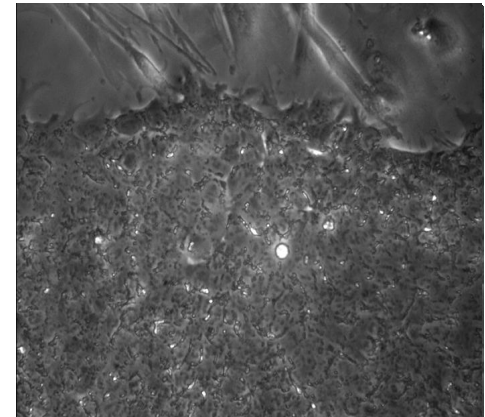
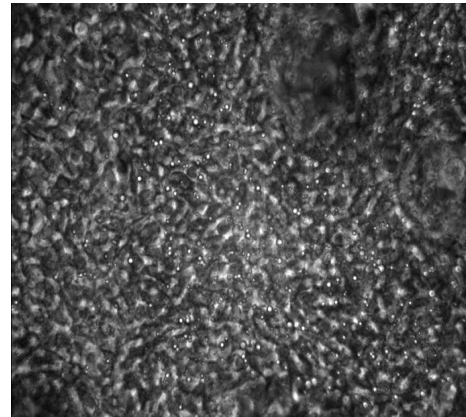
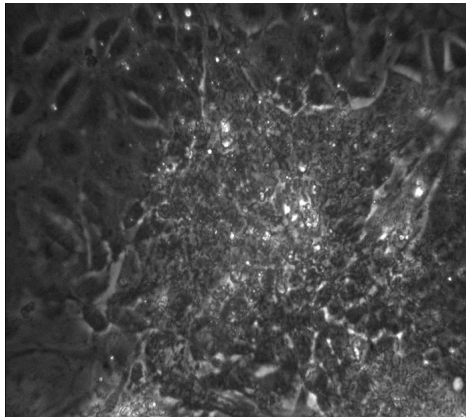
GAE

EOS

Bright Light

*66 days
post-
infection*

GFP
Fluorescence



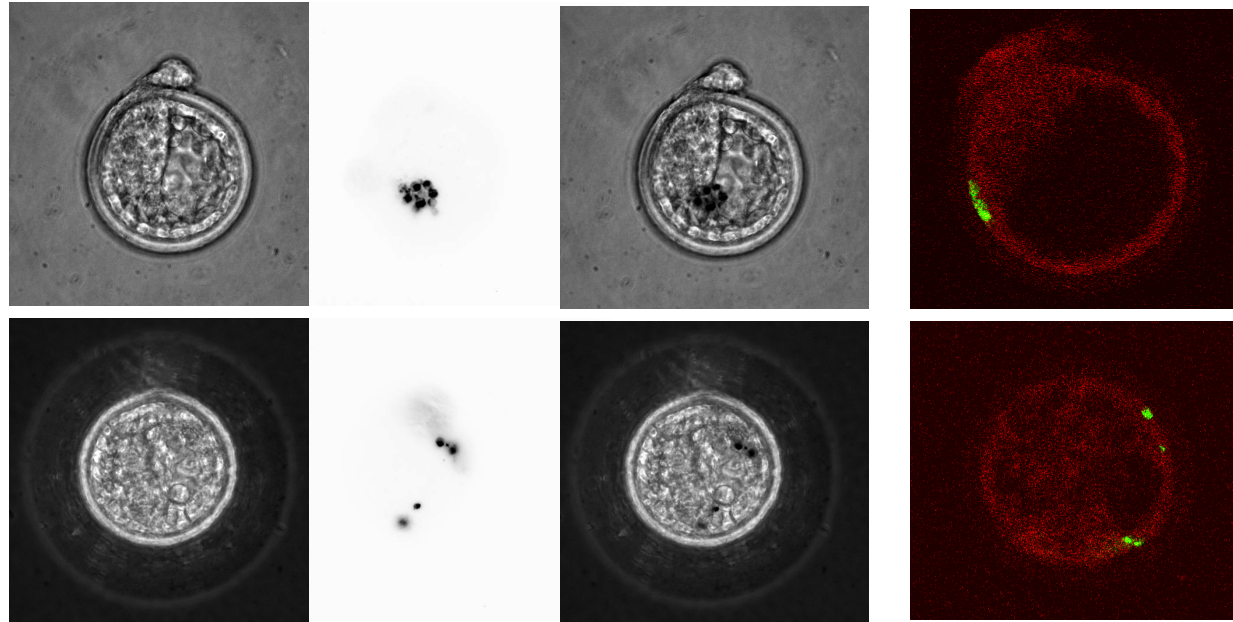
Production of chimera

Bright Light *GFP*
Fluorescence *Merged* *Confocal*
analysis

*analysis of fluorescence
at blastocyst stage (E72h)*

*Injection of
GFP⁺ rESC in
8-cell embryos*

In vitro



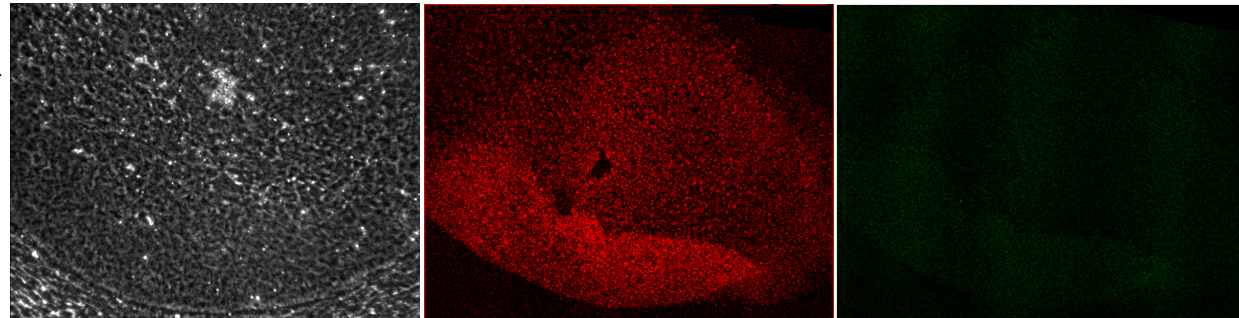
Bright Light

TOPRO-3

GFP
Fluorescence

In vivo

*analysis of fluorescence
at eggcylinder stage (E6.5d)*



Conclusion

- ***Rabbit pluripotent cell lines:***

- Six FGF2-dependant ESC lines

- Two LIF-dependant ESC lines

- Four FGF2-dependant iPSC lines

- ***All these lines show:***

- morphology of primate ESC
 - high alkaline phosphatase activity
 - expression of pluripotency genes Oct4 and Nanog
 - ability to differentiate into the three embryonic lineages
 - normal 2N karyotype

- ***These lines differ by:***

- signaling pathways which sustain self-renewal
 - expression of hESC-specific cell surface markers
 - activity of mouse Oct4 distal enhancer
 - cell cycle profile

- ➔ *pluripotency state?*

- ➔ *ability to colonize host blastocysts and to produce chimera?*

Naïve and primed pluripotent states

