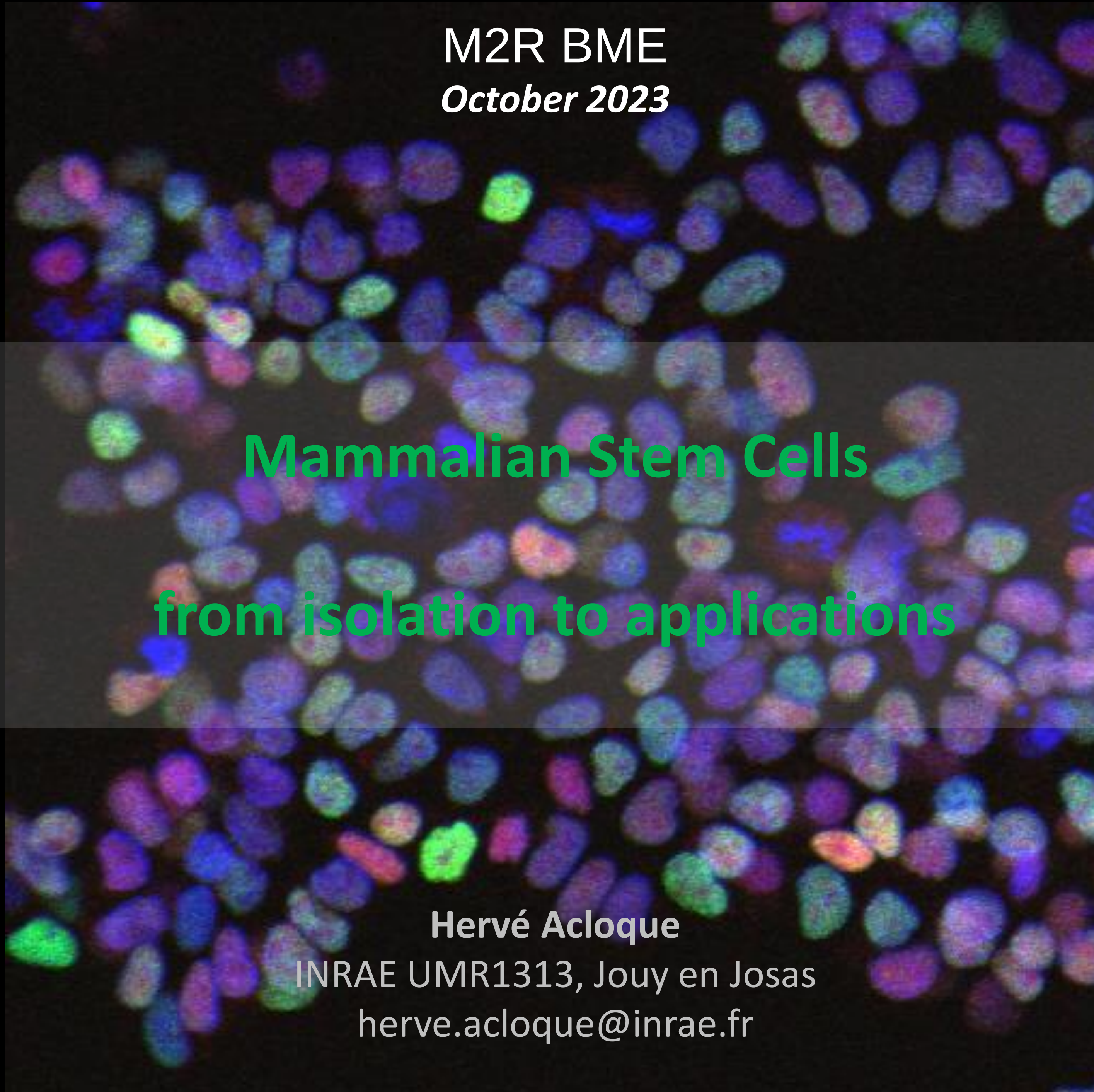
# Mammalian Stem Cells: from isolation to applications

Hervé Acloque

M2R BME

***October 2023***



# Mammalian Stem Cells

# from isolation to applications


**Hervé Acloque**

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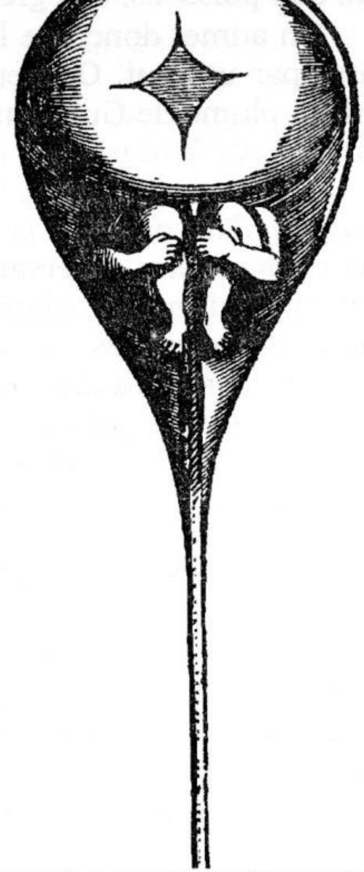
# Mammalian stem cells

- Terminology and definition
- The quest for adult pluripotent stem cells
- Non rodent pluripotent stem cells: production and applications
- Interspecies chimera: why, why not, how

# Terminology and definition

# The concept of celular potential is quite novel: a biologically fixed cell fate ?

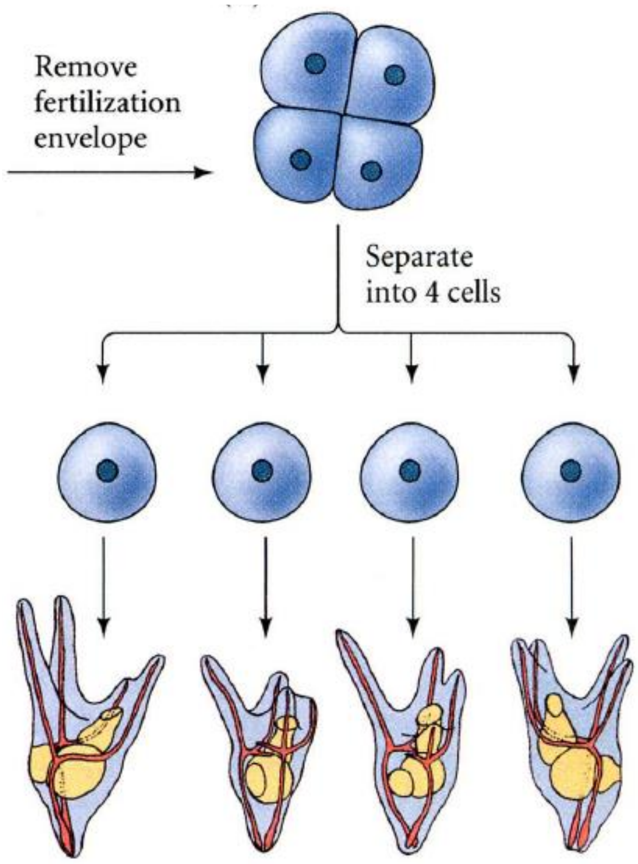
Au reſte, l'œuf n'eſt à proprement parler que ce qu'on appelle *placenta*, dont l'enfant, aprés y avoir demeuré un certain temps tout courbé & comme en peloton, briſe en s'étendant & en s'allongeant le plus qu'il peut, les membranes qui le couvroient, & poſant ſes pieds contre le *placenta*, qui reſte attaché au fond de la matrice, ſe pouſſe ainſi avec la tête hors de ſa priſon ; en quoi il eſt aidé par la mere, qui agitée par la douleur qu'elle en ſent, pouſſe le fond de la matrice en bas, & donne par conſequent d'autant plus d'occaſion à cet enfant de



**Human Fœtus into a spermatozoide*: The homunculus* (Nicolas Hartsoeker, 1694)**

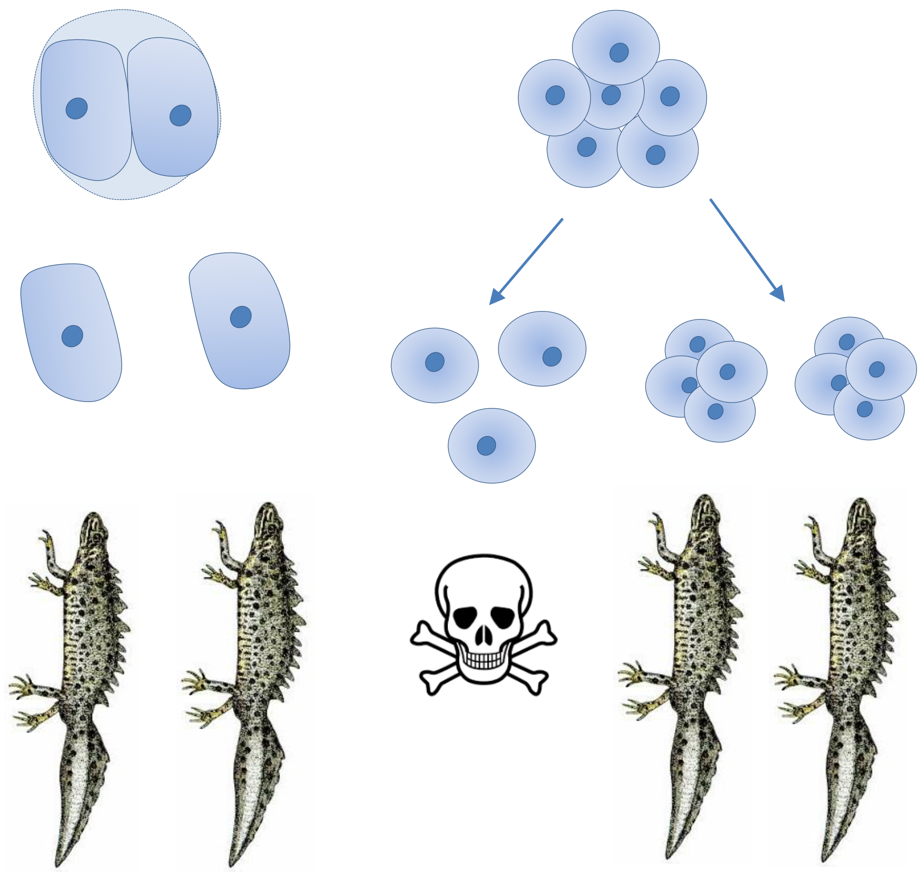
# The concept of pluripotency is quite novel
# Experimental studies in aquatic species

Driesch experiments in 1891 on sea urchin blastomeres at the 2-cell and 4—cell stages: totipotency

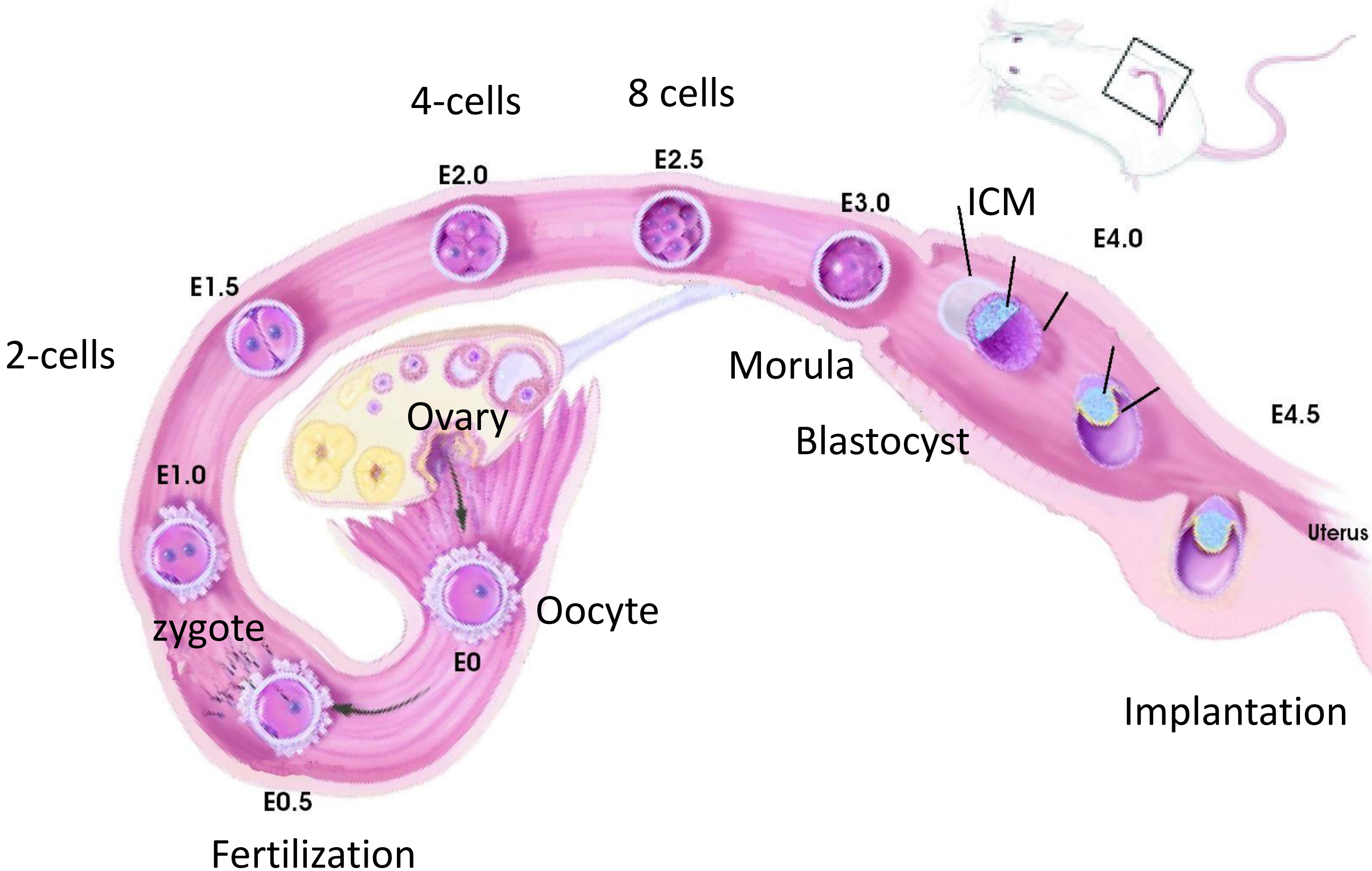



Plutei developed from single cells of 4-cell embryo

Speeman's experiments in 1902 on triton's blastomeres blastomeres: totipotency and pluripotency

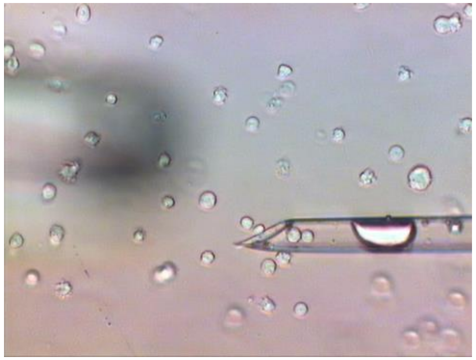


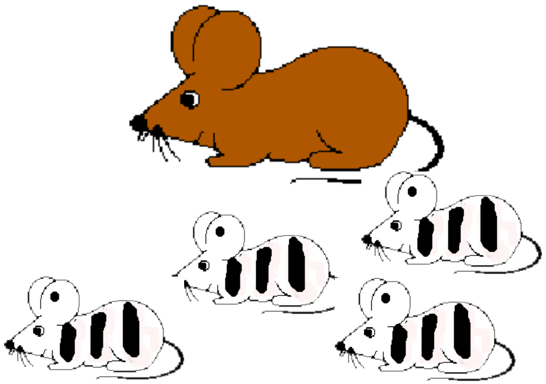
# Pluripotency in mammals: the mouse model



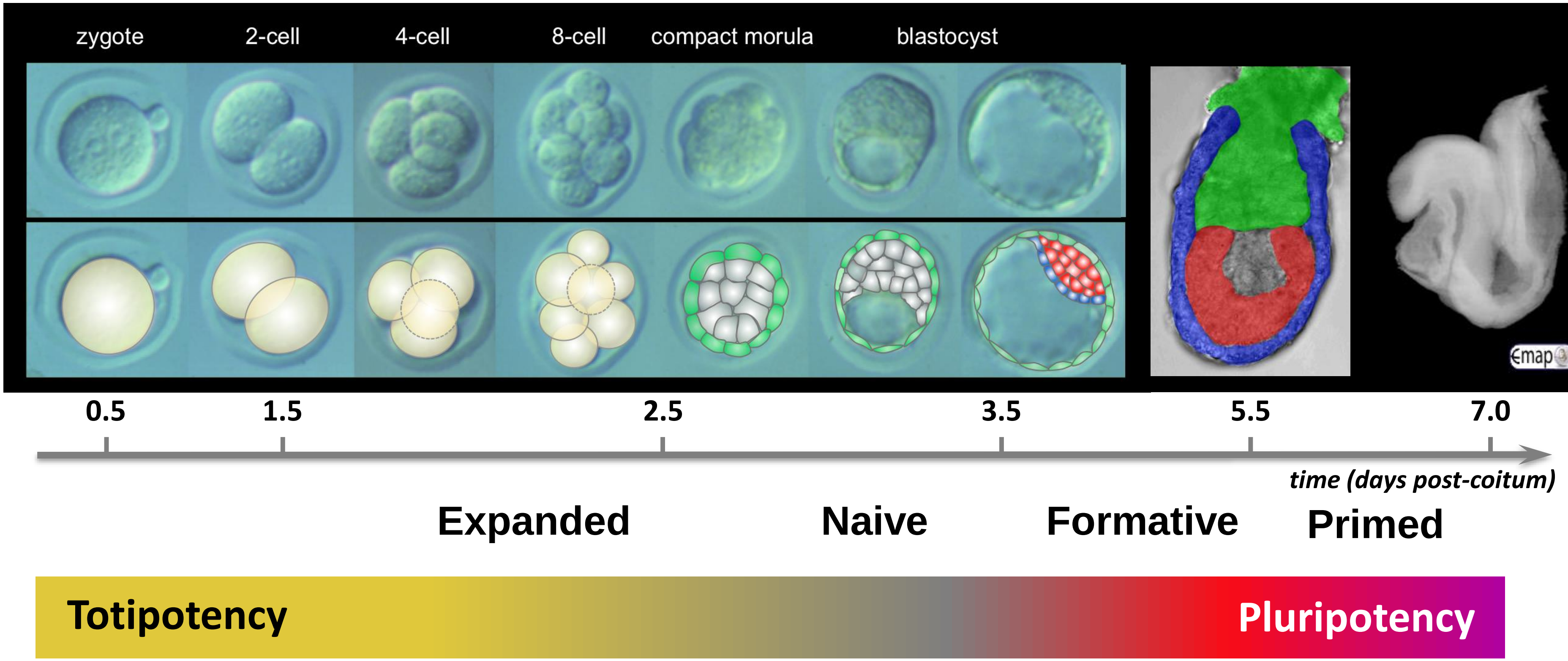
# Pluripotency in mammals: the mouse model

## Aggregation chimeras (Tarkowski 1962) and injection chimera (Gardner 1968)

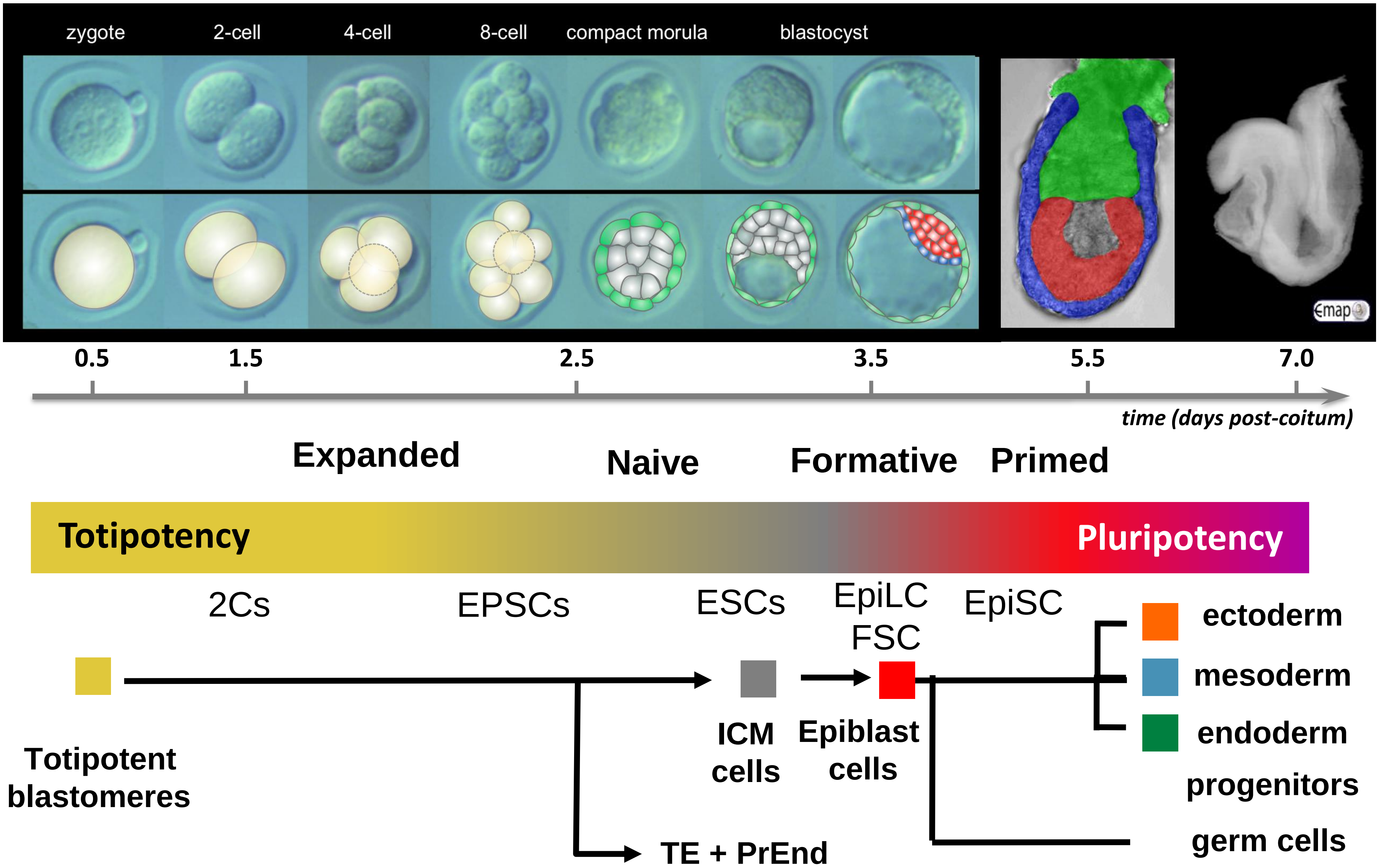
# Pluripotency in mammals: the mouse model




Totipotent: sufficient to form entire organism.

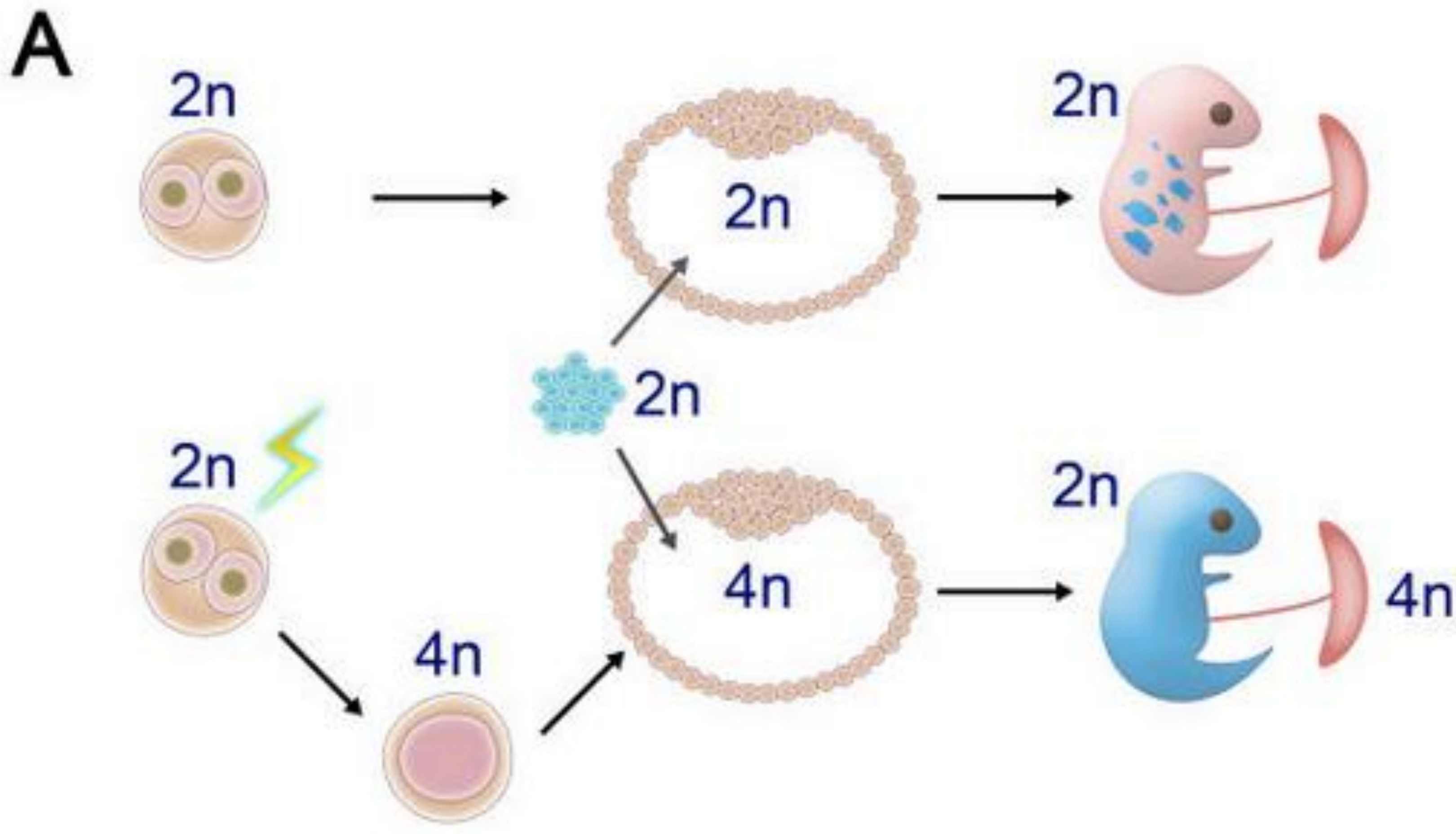
Pluripotent: able to form all the body cell lineages, including germ cells.

# Pluripotency in mammals: the mouse model



# Pluripotency in mammals: the mouse model

## The ultimate demonstration of pluripotency in mammals: tetraploid complementation



## Totipotency

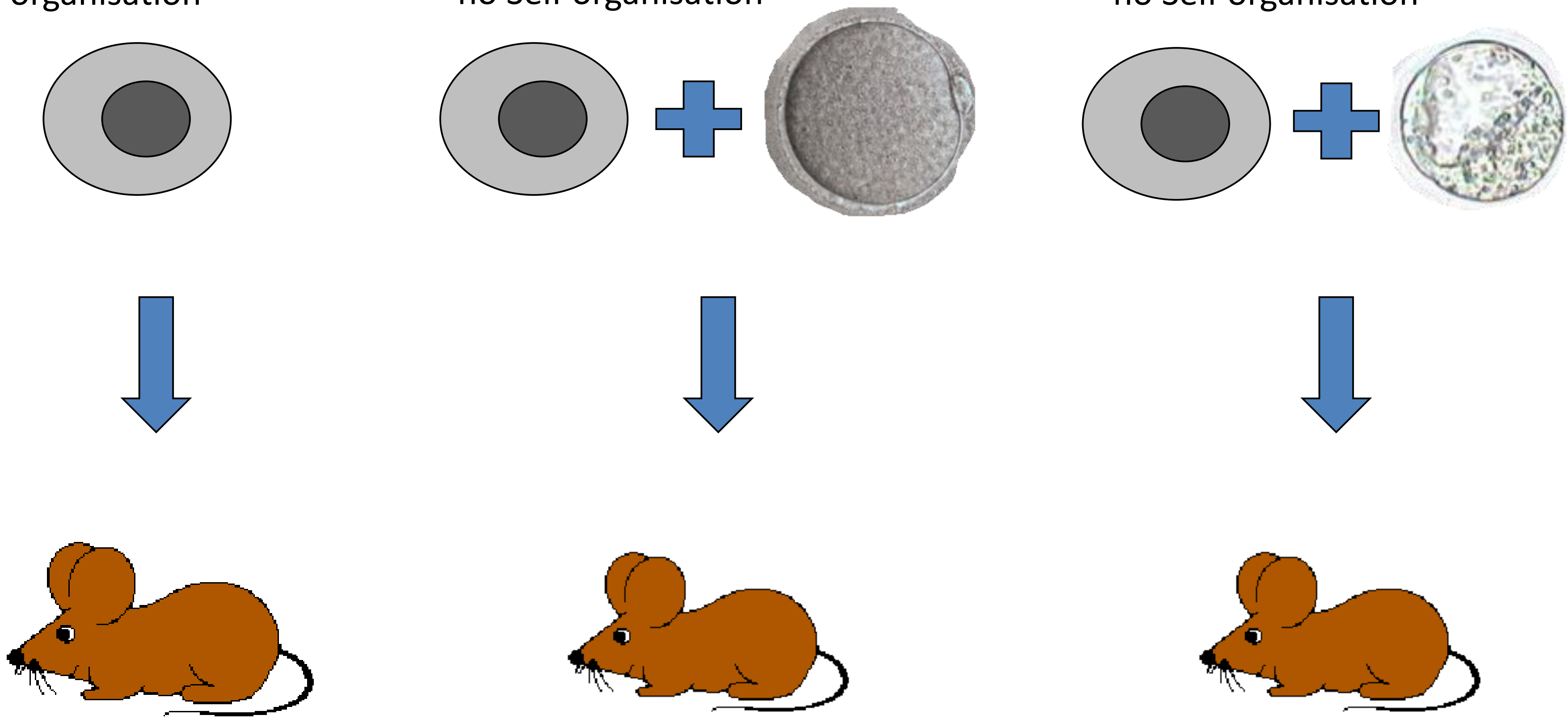
Full differentiation potential
(ExtraEmb + Emb)
+ Self organisation

## Expanded pluripotency

Full differentiation potential
(ExtraEmb + Emb)
no Self organisation

## Pluripotency

Full embryonic differentiation potential
no Self organisation



## Totipotency

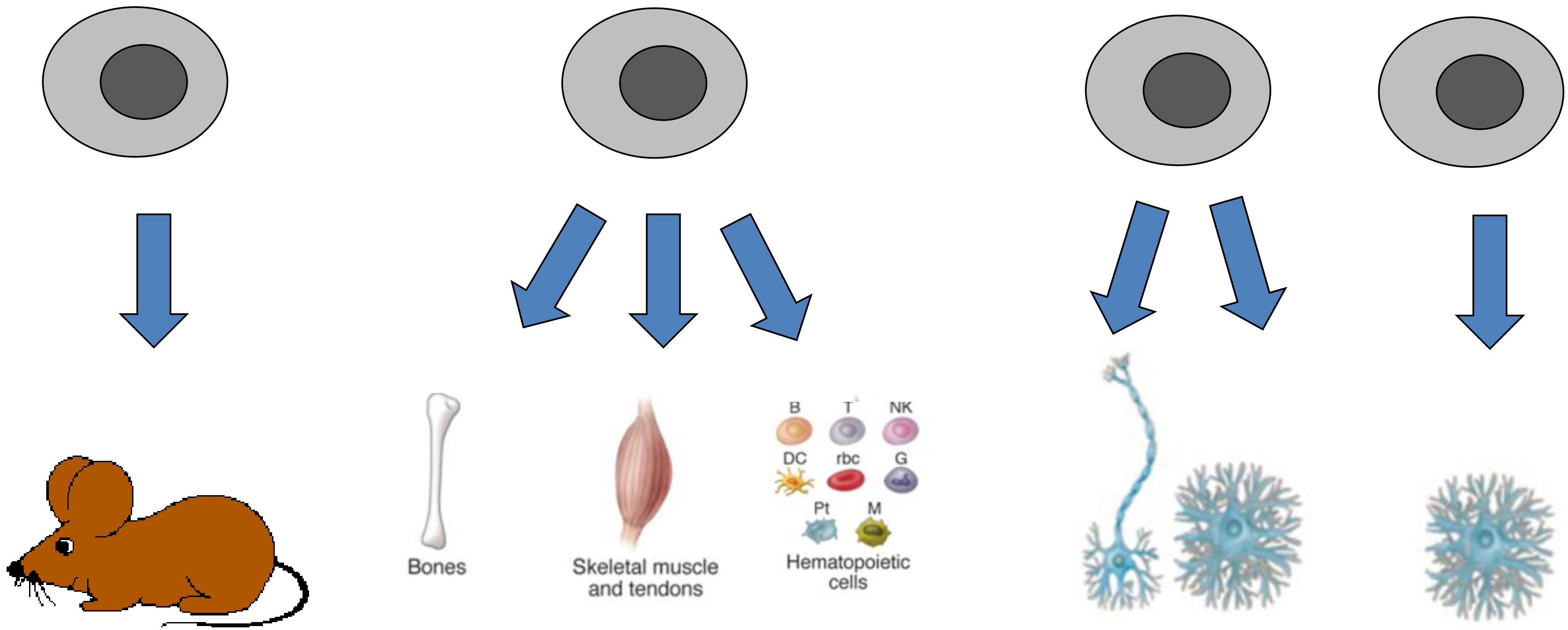
Full differentiation potential
+ Self organisation

## Multipotency

Multiple but restricted
differentiation potential
Self organisation: somehow
possible (ie organoïds)

## Bipotency / Unipotency

Restricted differentiation potential




**Neural Crest Cells (NCCs)**
**Hematopoïetic stem cells (HSCs)**
**Mesenchymal Stem Cells (MSCs)**

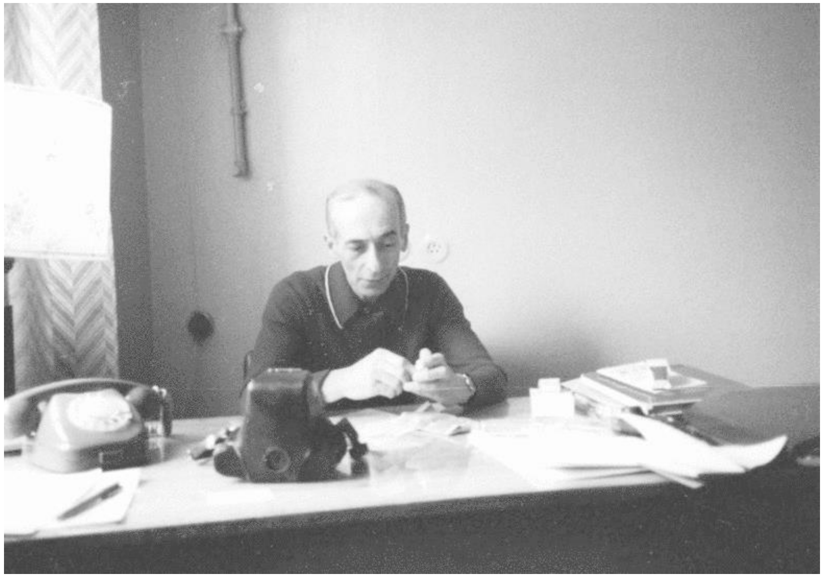
# The quest for adult pluripotent stem cells

# Adult pluripotent stem cells: do they exist ?

## The discovery of mesenchymal stem cells (MSCs)

The first description of adult multipotent stem cells: HSCs

In the 1960s, working on graft of bone marrow cells, Alexander Friedenstein discovered that some cells possess an osteogenic potential.



Alexander Friedenstein (1924-1998)
URSS

# Adult pluripotent stem cells: do they exist ?

## The discovery of mesenchymal stem cells (MSCs)

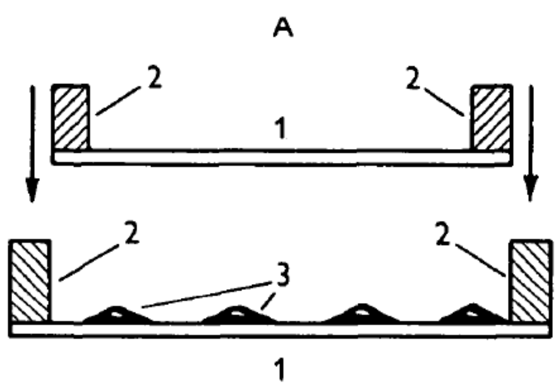
*J. Embryol. exp. Morph., Vol. 16, 3, pp. 581–390, December 1966* 381
*With 5 plates*
*Printed in Great Britain*

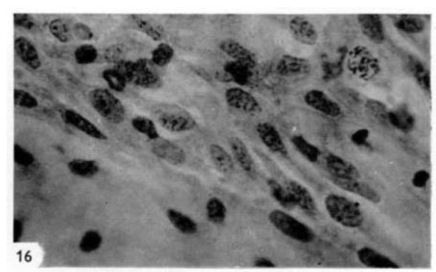
**Osteogenesis in transplants of bone marrow cells**

By A. J. FRIEDENSTEIN[1], I. I. PIATETZKY-SHAPIRO[1] & K. V. PETRAKOVA[1]

*From the Laboratory of Immunomorphology, Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the U.S.S.R., and Laboratory of Mathematical Methods in Biology, University of Moscow*

**BM**



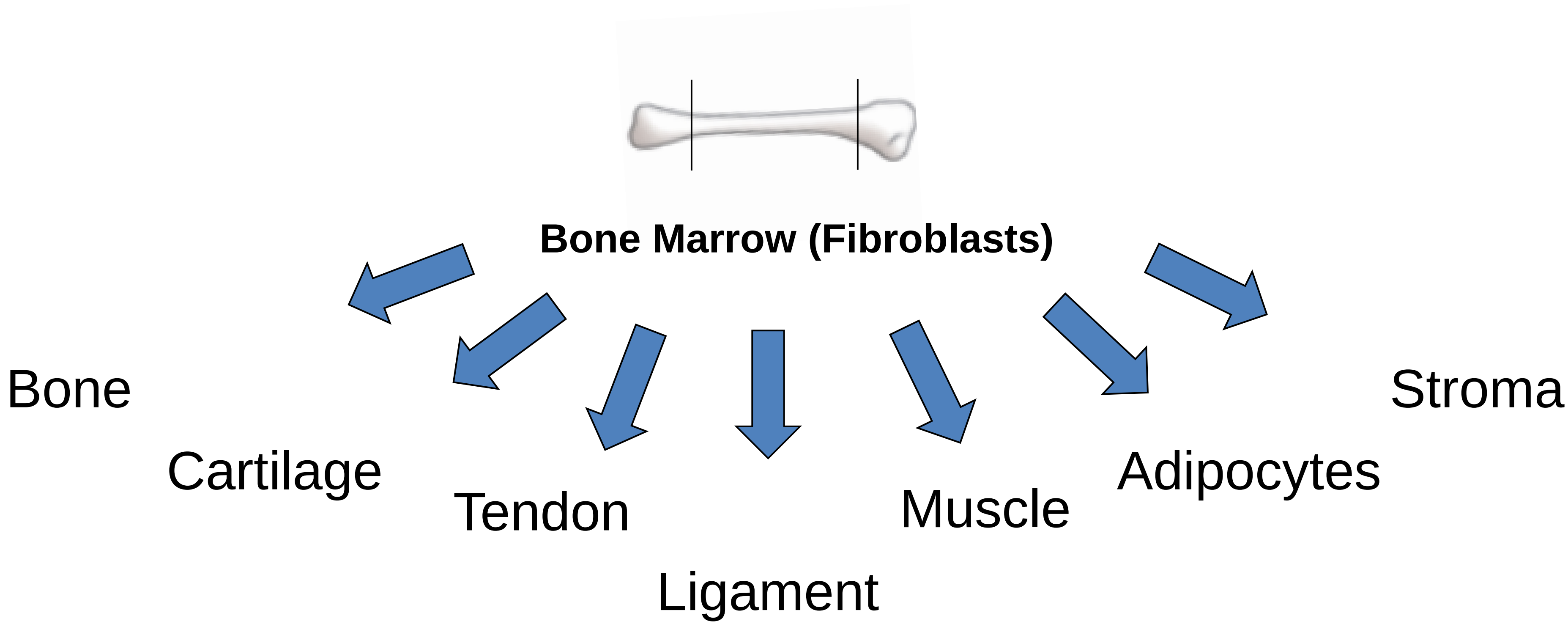

**5 days**

**Bone**

**Colony Forming Cells (CFU-F)**

# Adult pluripotent stem cells: do they exist ?

## The discovery of mesenchymal stem cells (MSCs)

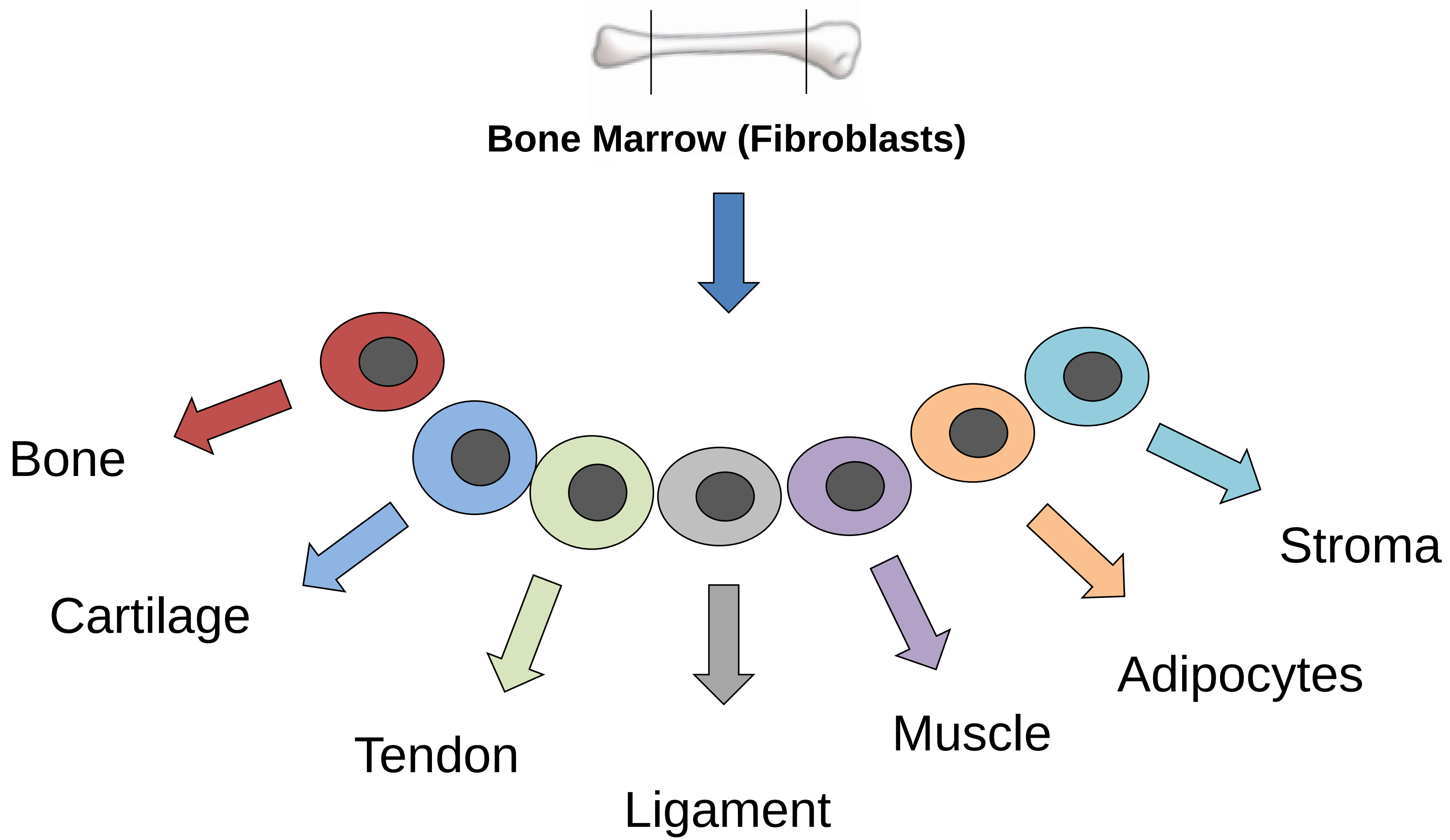



Friedenstein et al. 1987, Ashton et al. 1980, Bab et al. 1984 and others

# Adult pluripotent stem cells: do they exist ?

## The discovery of mesenchymal stem cells (MSCs)

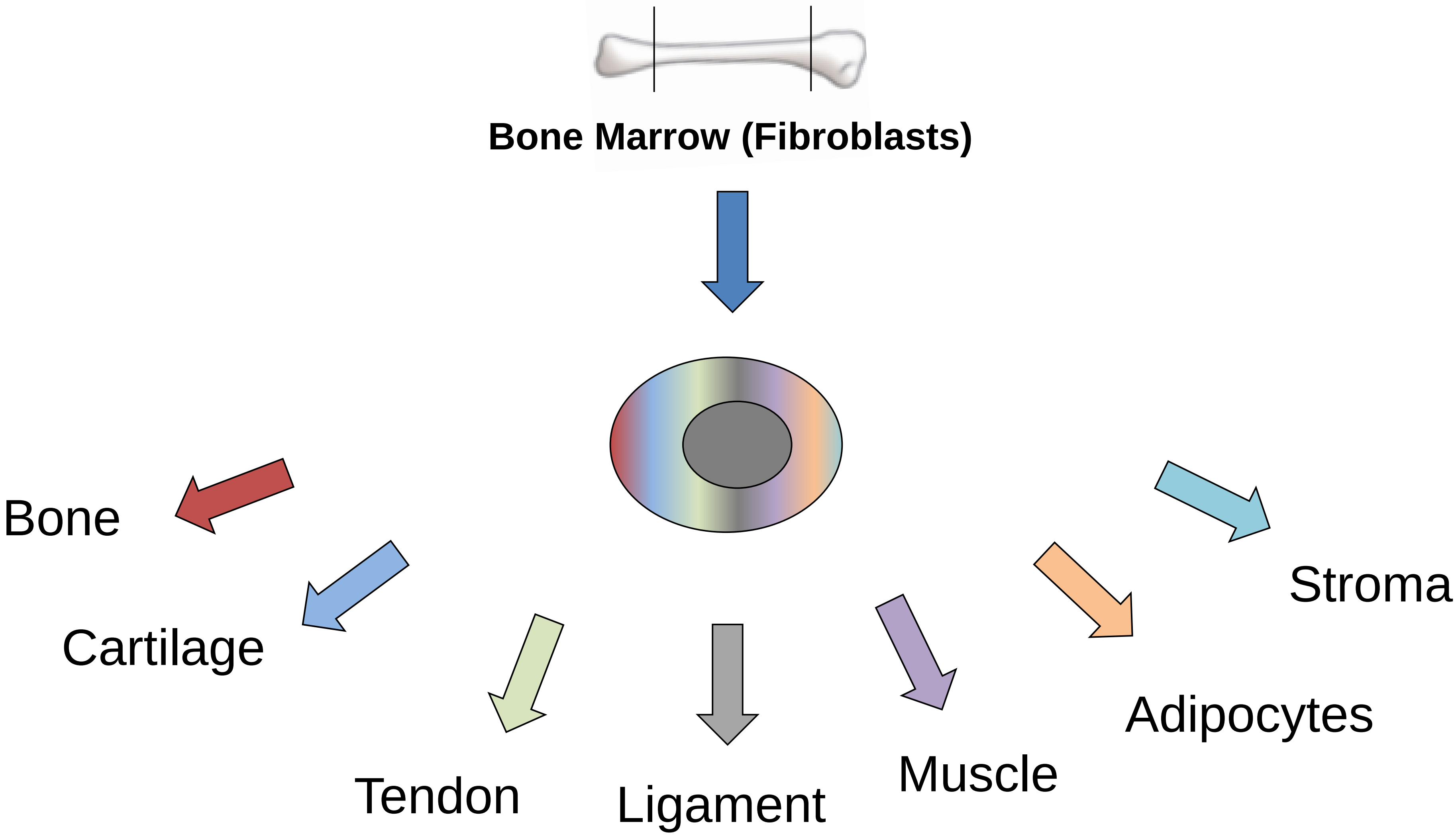
## Many different stem cells with restricted potency ?



# Adult pluripotent stem cells: do they exist ?

## The discovery of mesenchymal stem cells (MSCs)

## One unique multipotent stem cells with enlarged potency ?



# Adult pluripotent stem cells: do they exist ?

## The discovery of mesenchymal stem cells (MSCs)

One unique multipotent stem cells with enlarged potency

# Multilineage Potential of Adult Human Mesenchymal Stem Cells

Mark F. Pittenger,[1*] Alastair M. Mackay,[1] Stephen C. Beck,[1] Rama K. Jaiswal,[1] Robin Douglas,[1] Joseph D. Mosca,[1] Mark A. Moorman,[1] Donald W. Simonetti,[1] Stewart Craig,[1] Daniel R. Marshak[1,2]

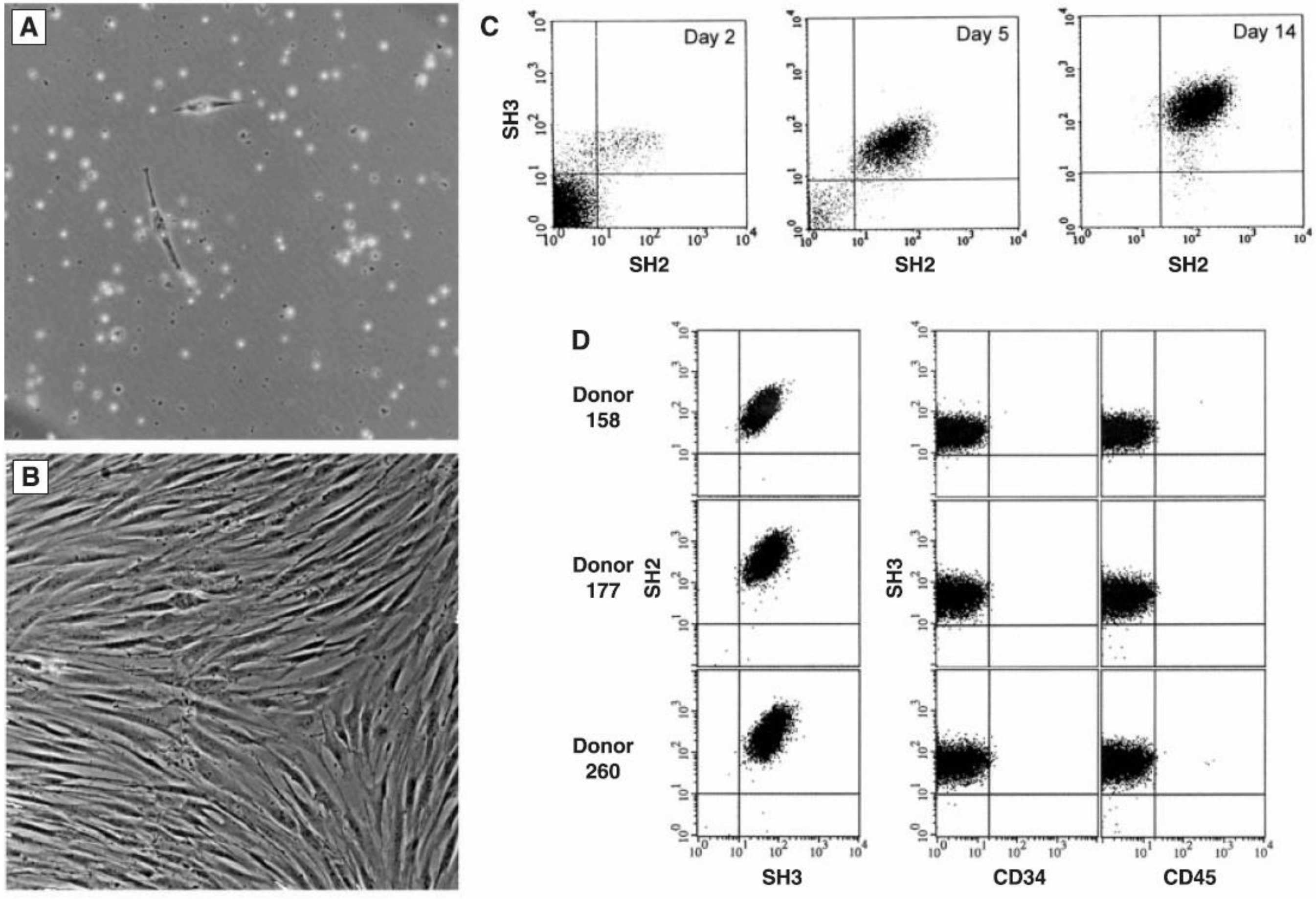
**Nature 1999**

# Adult pluripotent stem cells: do they exist ?

## The discovery of mesenchymal stem cells (MSCs)

One unique multipotent stem cells with enlarged potency

CD73



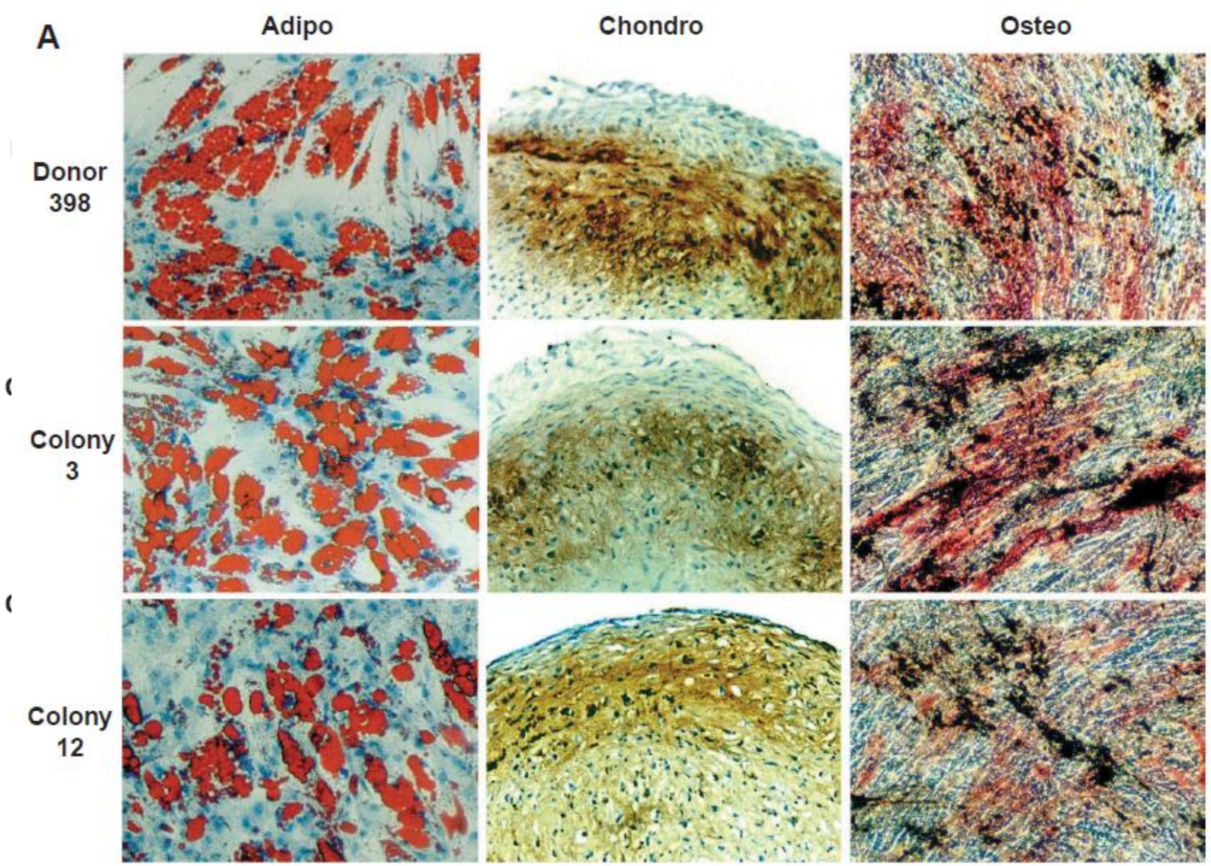

CD105

# Adult pluripotent stem cells: do they exist ?

## The discovery of mesenchymal stem cells (MSCs)

One unique multipotent stem cells with enlarged potency

Colonies from single cell



# Adult pluripotent stem cells: do they exist ?

## The discovery of mesenchymal stem cells (MSCs)

### One unique multipotent stem cells with enlarged potency

- Adherent cells in 2D culture (Fibroblasts)
- Cells positives for the surface determinants CD73, CD90 and CD105
- Cells negative for hematopoïetic markers :CD34-, CD45-, CD14-, CD79a-, HLA-DR-
- Multipotency *in vitro* and *in vivo* at least for osteoblastic, chondroblastic and adipocytic lineages (mesodermal)

# Adult pluripotent stem cells: do they exist ?

## The discovery of mesenchymal stem cells (MSCs)

One unique multipotent stem cells with enlarged potency

- Originally discovered in the Bone Marrow
- Can be isolated from cord blood, placenta, circulating blood, bone marrow, dental pulp, cartilage, adipose tissue, skeletal muscle, blood vessel (pericytes) etc
- MSCs seem to be elsewhere in the organism but in extremely low proportion (0,01% - 0,001% in BM MNCs)

# Adult pluripotent stem cells: do they exist ?

## The discovery of adult pluripotent stem cells

- Inspired by the discovery of adult multipotent stem cells in the bone marrow, laboratories worldwide demonstrated the existence of adult pluripotent stem cells in the 2000s

# Adult pluripotent stem cells: do they exist ?

## The discovery of adult pluripotent stem cells

### Generalized Potential of Adult Neural Stem Cells

Diana L. Clarke,[1] Clas B. Johansson,[1,2] Johannes Wilbertz,[1] Biborka Veress,[1] Erik Nilsson,[1] Helena Karlström,[1] Urban Lendahl,[1] Jonas Frisén[1]*

### From Marrow to Brain: Expression of Neuronal Phenotypes in Adult Mice

Timothy R. Brazelton, Fabio M. V. Rossi, Gilmor I. Keshet, Helen M. Blau*

Science 2000

articles

### Pluripotency of mesenchymal stem cells derived from adult marrow

Yuehua Jiang*†, Balkrishna N. Jahagirdar*†‡, R. Lee Reinhardt§, Robert E. Schwartz*, C. Dirk Keene||, Xilma R. Ortiz-Gonzalez||, Morayma Reyes*, Todd Lenvik*, Troy Lund*, Mark Blackstad*, Jingbo Du*, Sara Aldrich*, Aaron Lisberg*, Walter C. Low||, David A. Largaespada¶ & Catherine M. Verfaillie*‡

** Stem Cell Institute, ‡ Division of Hematology, Oncology and Transplantation, Department of Medicine, § Department of Microbiology, Center for Immunology, || Department of Neurosurgery, and ¶ Department of Genetics, Cell Biology and Development, University of Minnesota Medical School, Minneapolis, Minnesota 55455, USA*

*† These authors contributed equally to this work*

Nature 2002

# Adult pluripotent stem cells: do they exist ?

## The discovery of adult pluripotent stem cells

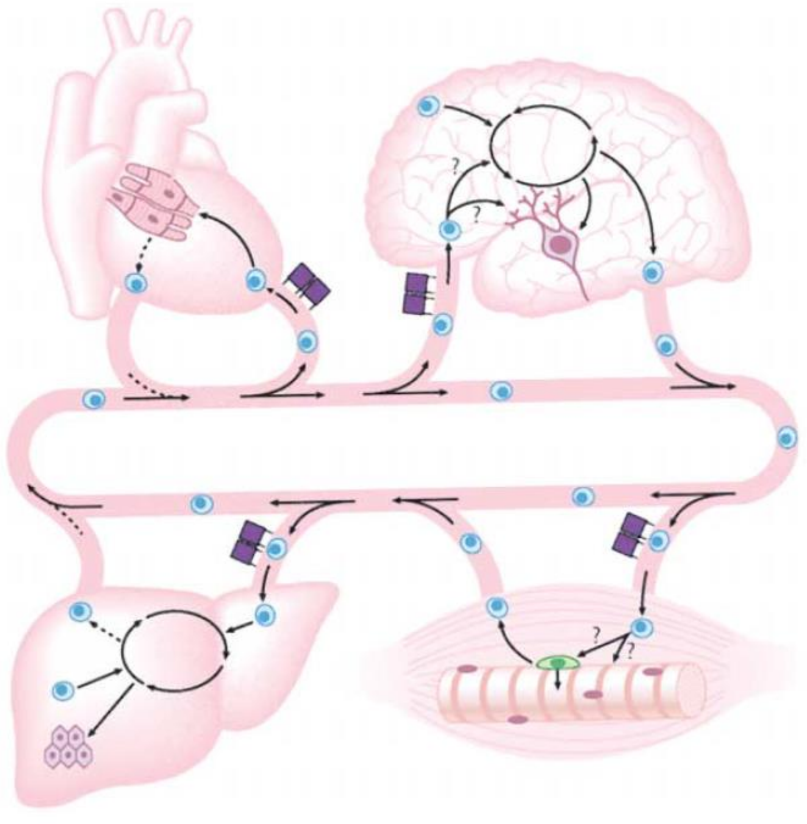


Figure 1. Evolving Concepts of Stem Cell Plasticity

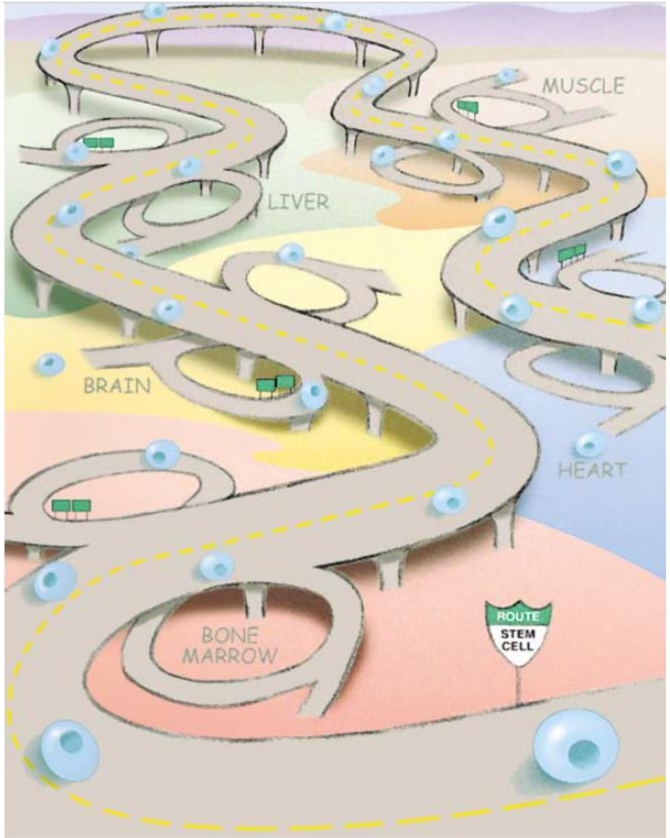


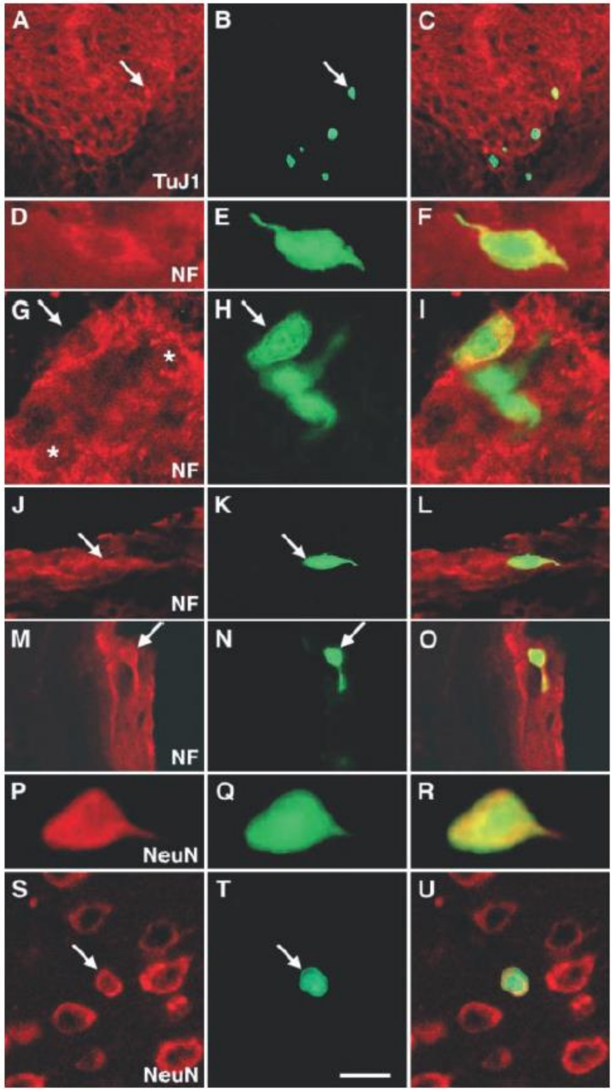

Figure 8. Route Stem Cell

Blau et al. 2001 Cell

# Adult pluripotent stem cells: do they exist ?

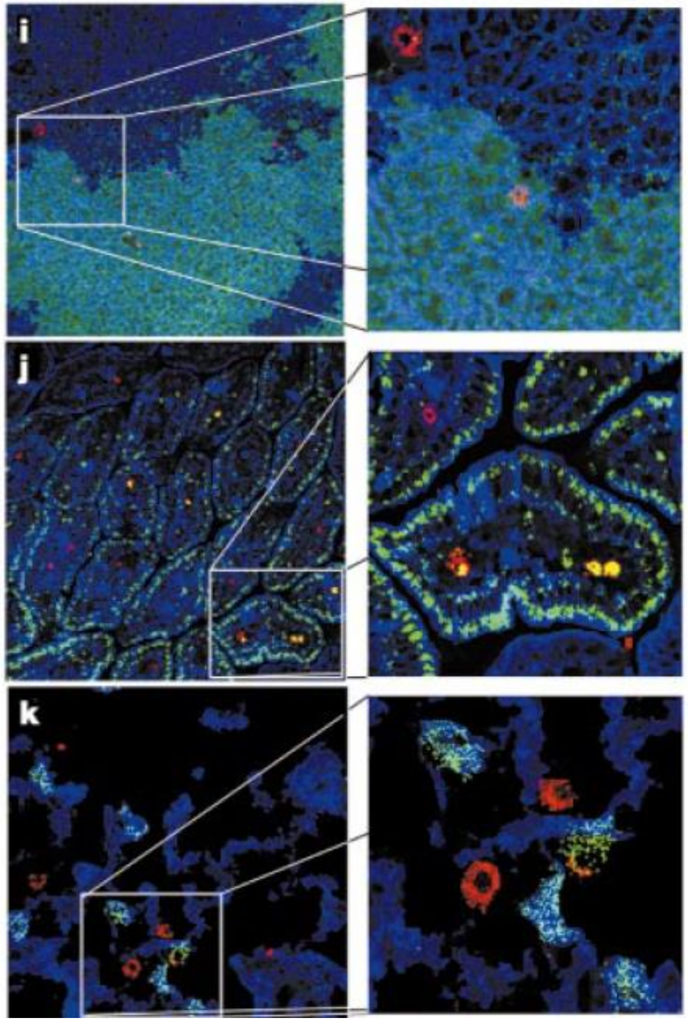
## The discovery of adult pluripotent stem cells

In all these studies, only very scarce cells show a pluripotent potential:




neurons

Blau et al. 2000




Liver

intestine

lung

Jiang et al. 2002

# Adult pluripotent stem cells: a scientific mistake

## Adult pluripotent stem cells result from rare events of cell fusion

**Changing potency by spontaneous fusion**

**Qi-Long Ying*, Jennifer Nichols*, Edward P. Evans† & Austin G. Smith***

** Centre for Genome Research, University of Edinburgh, The King's Buildings, West Mains Road, Edinburgh EH9 3JQ, UK*
*† Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK*

Nature 2002

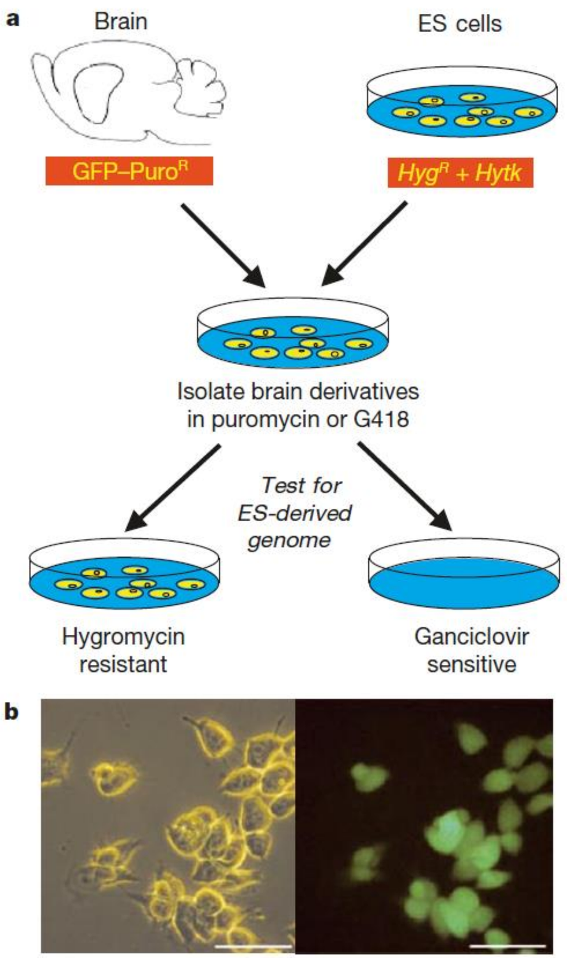
**Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes**

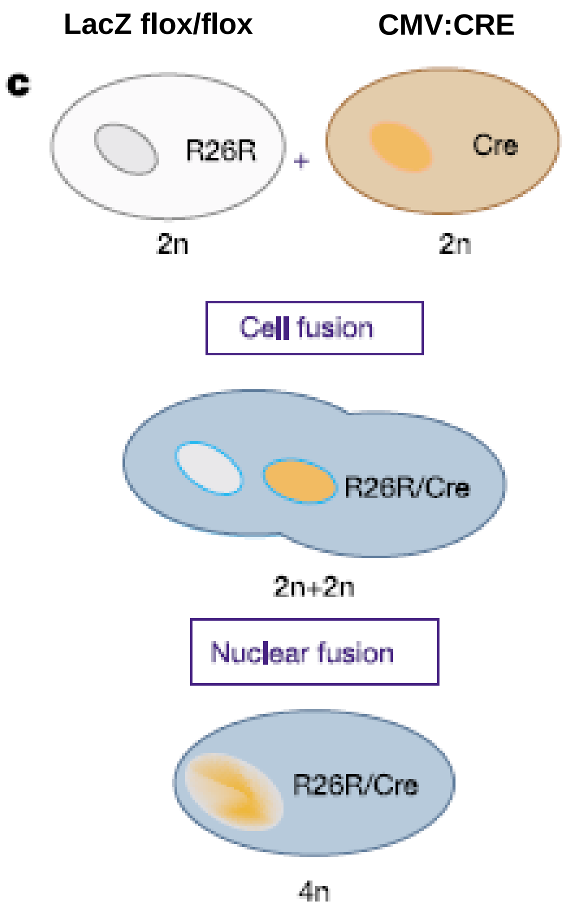
**Manuel Alvarez-Dolado[1], Ricardo Pardal[2], Jose M. Garcia-Verdugo[3], John R. Fike[1], Hyun O. Lee[2], Klaus Pfeffer[4], Carlos Lois[5], Sean J. Morrison[2] & Arturo Alvarez-Buylla[1]**

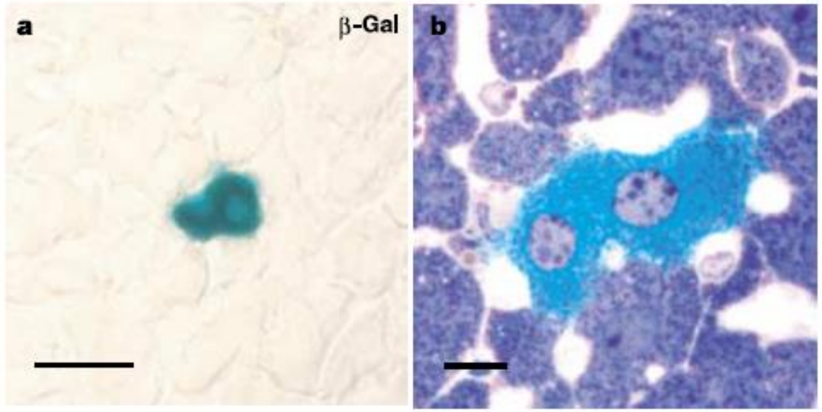
Nature 2003

# Adult pluripotent stem cells: a scientific mistake

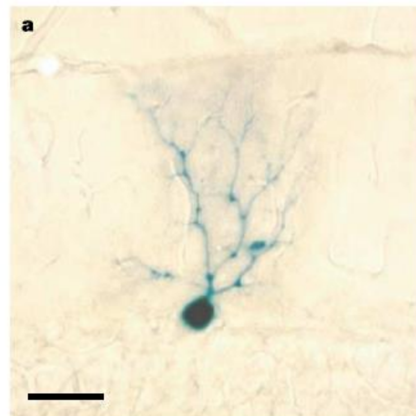
## Adult pluripotent stem cells result from rare events of cell fusion



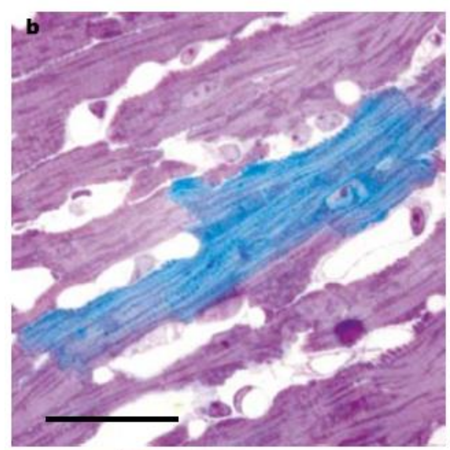






Hepatocytes




Purkinje cells




Skeletal muscle

# Adult pluripotent stem cells: a scientific mistake

## Adult pluripotent stem cells result from rare events of cell fusion from MSCs with differentiated cells

**Differentiation, cell fusion, and nuclear fusion during *ex vivo* repair of epithelium by human adult stem cells from bone marrow stroma**

**Jeffrey L. Spees*, Scott D. Olson*, Joni Ylostalo*, Patrick J. Lynch*, Jason Smith*, Anthony Perry*, Alexandra Peister*, Meng Yu Wang†, and Darwin J. Prockop*‡**

*Center for Gene Therapy, Tulane Health Sciences Center, New Orleans, LA 70112; and †Department of Tumor Biology, Institute for Cancer Research, Norwegian Radium Hospital, University of Oslo, 0310 Oslo, Norway

Contributed by Darwin J. Prockop, December 30, 2002

Fusion of MSCs with differentiated somatic cells is not a rare events

# Adult pluripotent stem cells: a scientific mistake

- MSCs are multipotent cells from adult tissues
- MSCs mostly act through paracrine actions on adjacent cells and tissues
- MSCs may contribute to tissue regeneration

- The existence of pluripotent adult stem cells has never been demonstrated to date

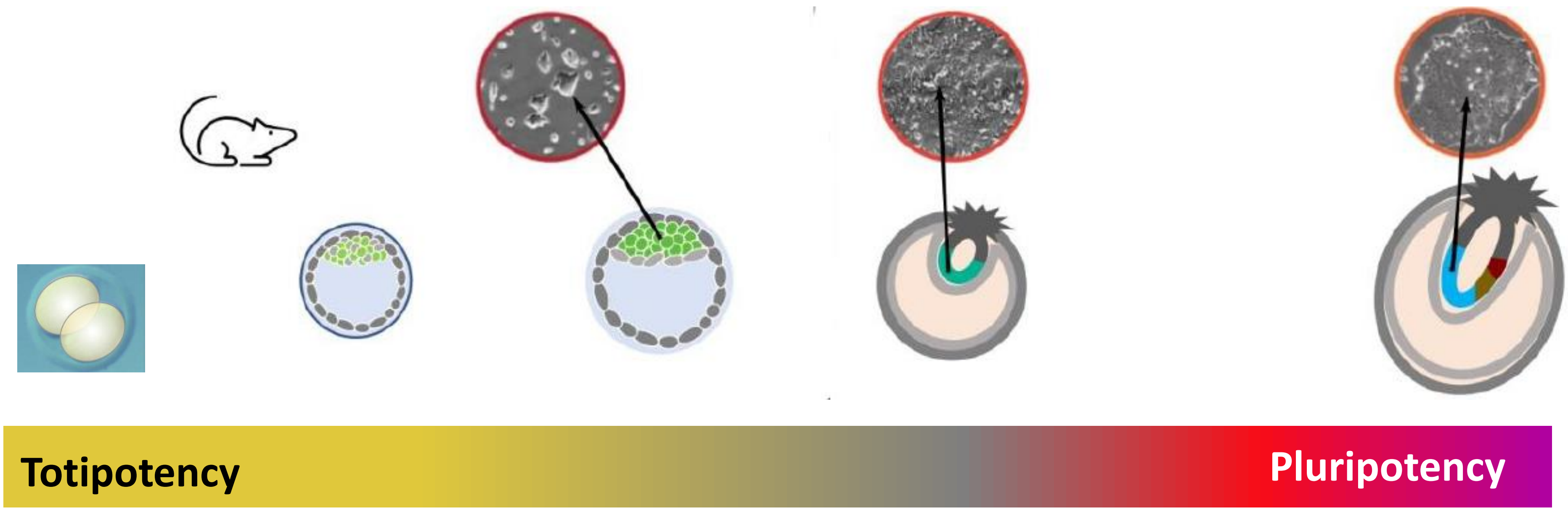
# Non rodent pluripotent stem cells:
# Production and applications

# Non rodent mammalian pluripotent stem cells
## *the three main properties of true ESCs*

- Infinite self-renewal in culture with stable diploid caryotype and symetrical division
- ***in vitro* pluripotency**: ability to differentiate toward cell types from the three embryonic germ-layers and the germ line.
- ***in vivo* pluripotency**: production of chimerae, germ line transmission and tetraploid complementation

# Non rodent mammalian pluripotent stem cells

The work made to characterize distinct pluripotent states in mouse embryos helped to derive PSCs in other species




| 2Cs | EPSCs | ESCs Naive | ESCs Mixte | EpiLC FSCs | EpiSC |
|---|---|---|---|---|---|
| | | LIF<br>MEKi<br>GS3Ki | LIF<br>serum | Activin low<br>Wnt/hippo inh. | Activin<br>FGF2 |

But it took decades to success!!!

# Non rodent mammalian pluripotent stem cells

**Monkey**

BIOLOGY OF REPRODUCTION 55, 254–259 (1996)

**Pluripotent Cell Lines Derived from Common Marmoset (*Callithrix jacchus*) Blastocysts[1]**

**James A. Thomson,[2,3] Jennifer Kalishman,[3] Thaddeus G. Golos,[3,4] Maureen Durning,[3] Charles P. Harris,[6] and John P. Hearn[3,5]**

*The Wisconsin Regional Primate Research Center,[3] Departments of Obstetrics and Gynecology[4] and Physiology,[5] School of Medicine, and Cytogenetics Laboratory,[6] State Hygiene Laboratory, University of Wisconsin, Madison, Wisconsin 53715–1299*

**Human**

**Embryonic Stem Cell Lines Derived from Human Blastocysts**

James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, Jeffrey M. Jones

**1998 Science**

Cell

**Rat**

**Capture of Authentic Embryonic Stem Cells from Rat Blastocysts**

Mia Buehr,[1,2] Stephen Meek,[1,2] Kate Blair,[3,4] Jian Yang,[3,5] Janice Ure,[1] Jose Silva,[3,4] Renee McLay,[1] John Hall,[3,4] Qi-Long Ying,[1,6] and Austin Smith[3,4,*]

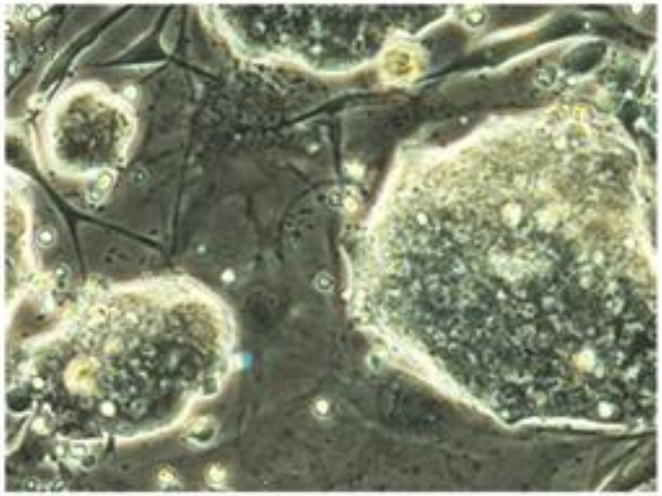
**2008 Science**

# Human embryonic stem cells are equivalent to mouse EpiSCs (epiblast stem cells)

- Non-human primate (1994) and human (1998) pluripotent stem cells have been derived from blastocysts
- Normal caryotype but unstable X inactivation
- Permanent cultures (no "crisis") but slow growth rate
- High telomerase activity
- Express specific "embryonic" antigens but relies on Activin and FGF signalling
- Formation of teratomas (injection into SCID mice) : tissues derived of all three embryonic germ layers
- *In vitro* differentiation
- No evidence for chimera

# Rat embryonic stem cells are similar to mouse and can be used for intra and interspecies chimerae

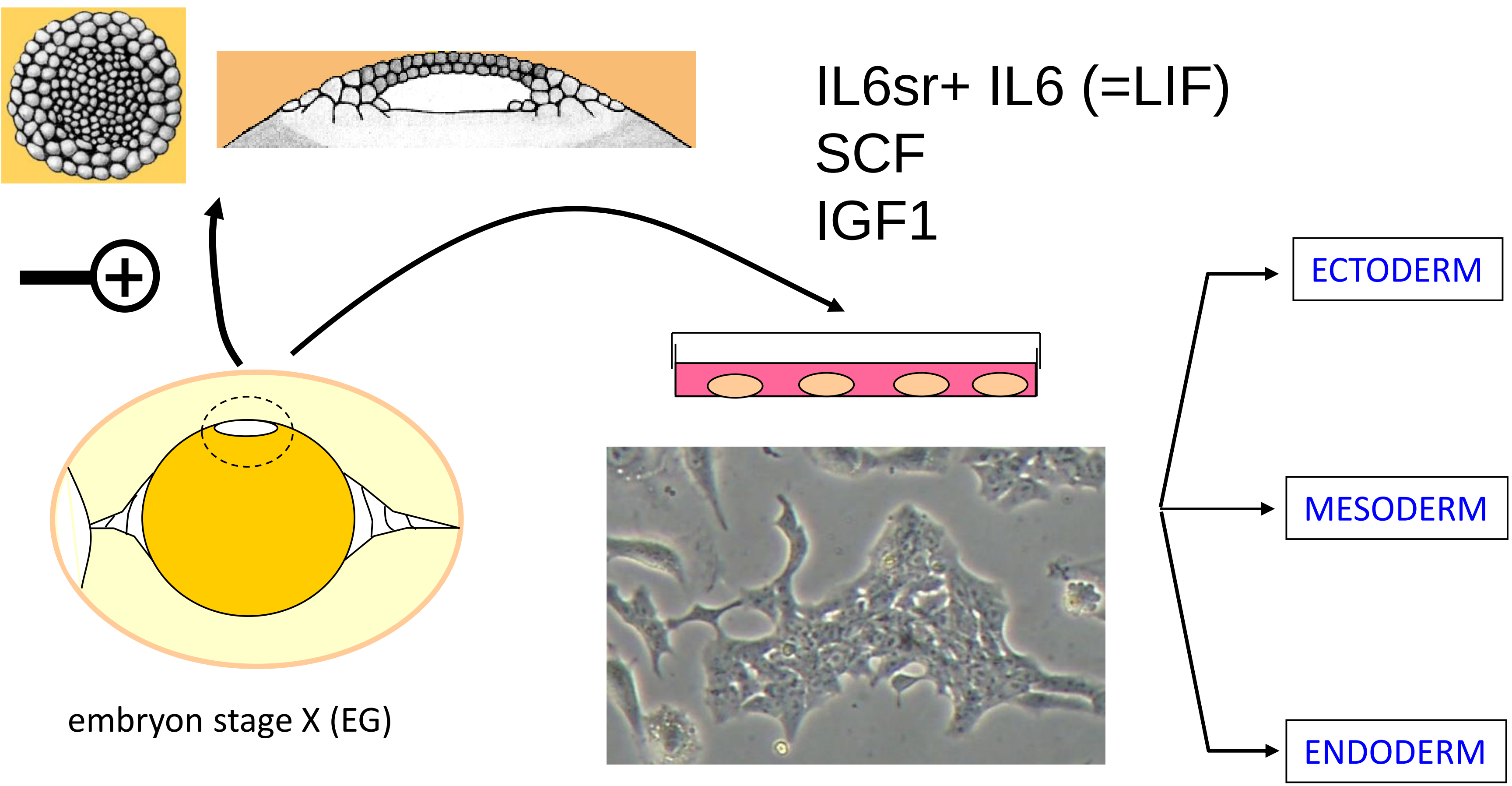
**2i(+LIF) culture condition allow ES cells derivation from mice and rat**



Buehr et al, Cell 2008

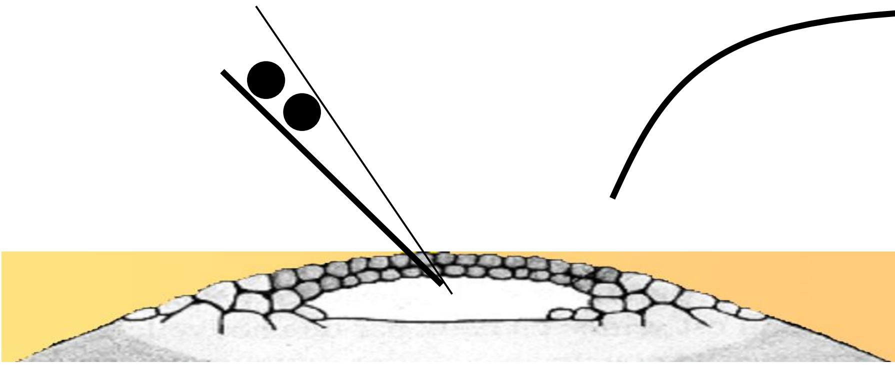
Li et al, Cell 2008

# Chicken embryonic stem cells contribute poorly to the germline

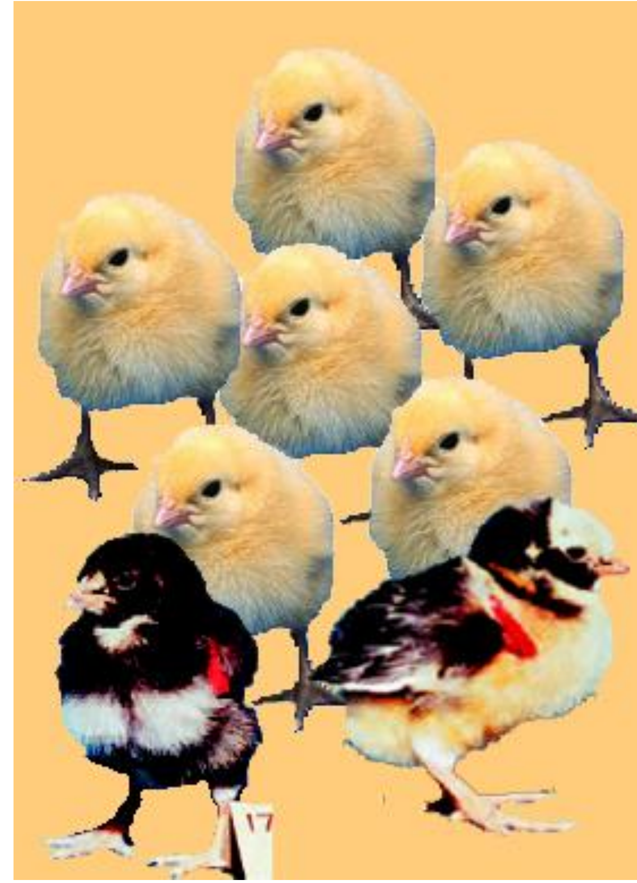



Pain et al. 1996

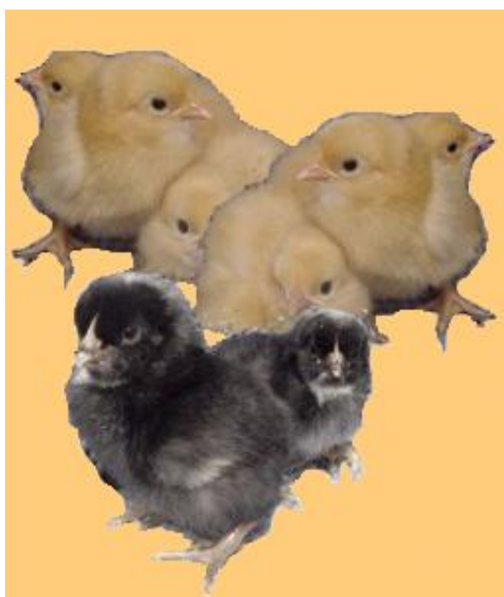
21 days of incubation



Embryo injection with ES cells from different feather colors



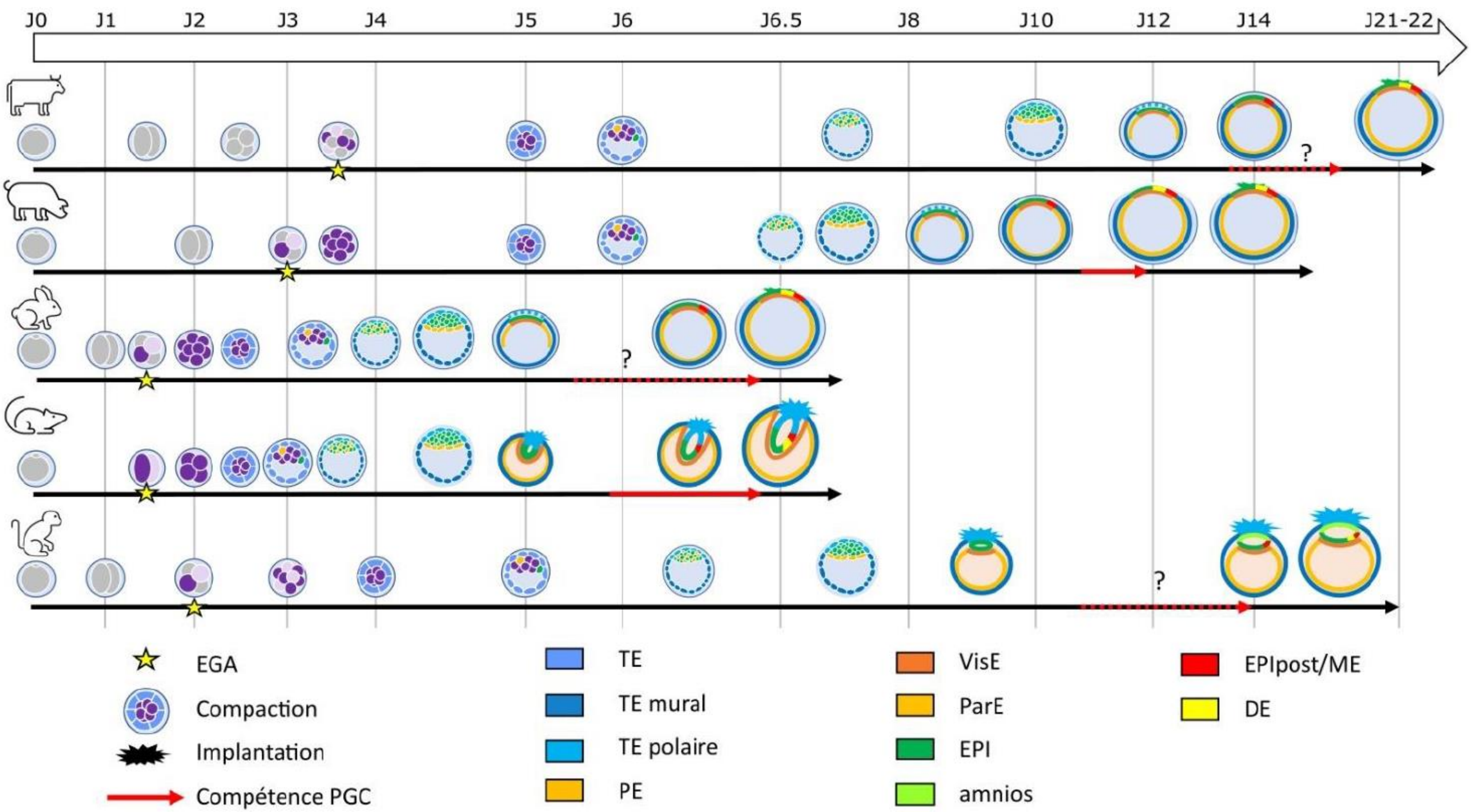
Cross the chimeara



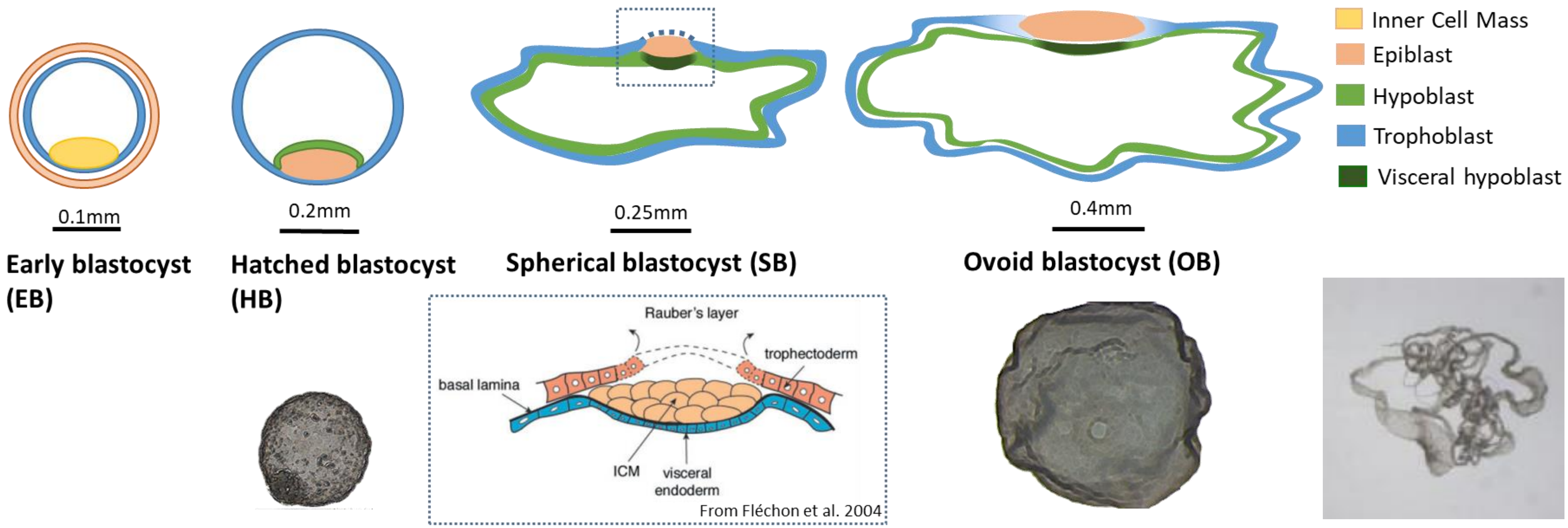
Black chicks derived from ES-differentiated germ cells

Pain et al. 1996

# But it remains difficult to produce TRUE PSCs from many other mammalian species: a developmental issue ?



# Challenges to produce TRUE mammalian PSCs: a developmental issue ?




| | EB | HB | | SB | OB | Time post fertilisation (days) |
|---|---|---|---|---|---|---|
|  | 6 | 7-8 | | 9-10 | 11-12 | 16 = implantation |
|  | 6-7 | 8 | | 9-10 | 11-13 | 20 = implantation |
|  | 3.5 | 4 | 5.5 = implantation | | | |
|  | 5.5 | 6.5 | 8 = implantation | | | |

# How to tackle these developmental barriers ?

- A better knowledge of *in vivo pluripotency :*
  - molecular characterization of the different pluripotent states
  - Active signalling pathways in PSCs in vivo
  - Startpoint of PSCs proliferation: when + how
  - Composition of uterine fluids during preimplantatory development

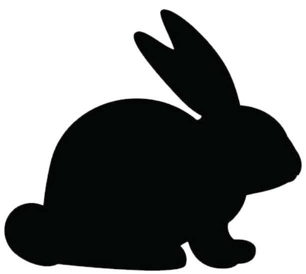
- The production of faithfull reporter systems to efficiently track **endogenous** pluripotency

- Cell culture optimisation by
  - Media optimization from transcriptomics and proteomics studies
  - HTS screening of small molecules based using reporter systems

# Producing TRUE PSCs from many other mammalian species

Using these tools some research groups succeed in producing true PSCs in different species

## Producing rabbit ESCs



Stem Cell Reports
**Article**
OPEN ACCESS

A Panel of Embryonic Stem Cell Lines Reveals the Variety and Dynamic of Pluripotent States in Rabbits

2017: empiric approach

# These rabbit ESCs poorly contribute to chimera

C


D

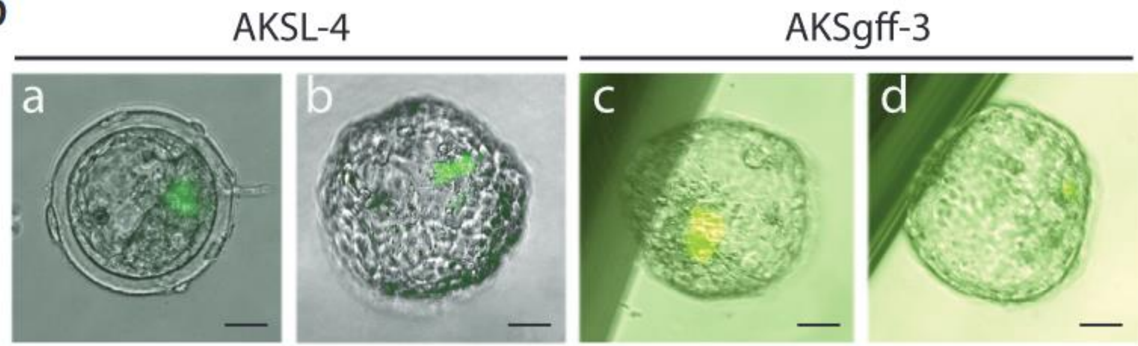


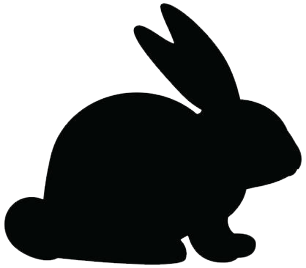

**Table 1. Colonization of Rabbit Blastocysts by AKSL-GFP and AKSgff-GFP Cells**

| | CKF | AKF | | AKSF | | AKSgff | | AKSL | |
|---|---|---|---|---|---|---|---|---|---|
| Line no. | 18 | 5 | 20 | 19 | 26 | 3 | 62 | 4 | 8 |
| No. of injected embryos | 87 | 82 | 84 | 78 | 82 | 61 | 56 | 75 | 71 |
| No. of blastocysts | 74 | 68 | 77 | 64 | 66 | 39 | 40 | 64 | 61 |
| No. of blastocysts with GFP-positive cells | 0 | 0 | 0 | 0 | 0 | 4 | 2 | 12 | 6 |
| No. of blastocysts with colonized ICM | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 12 | 5 |

Only with LIF culture rbESCs

Osteil et al. 2017

A: Accutase
C: Collagenase
F: bFGF
L: LIF
S: SVF
K: KOSR



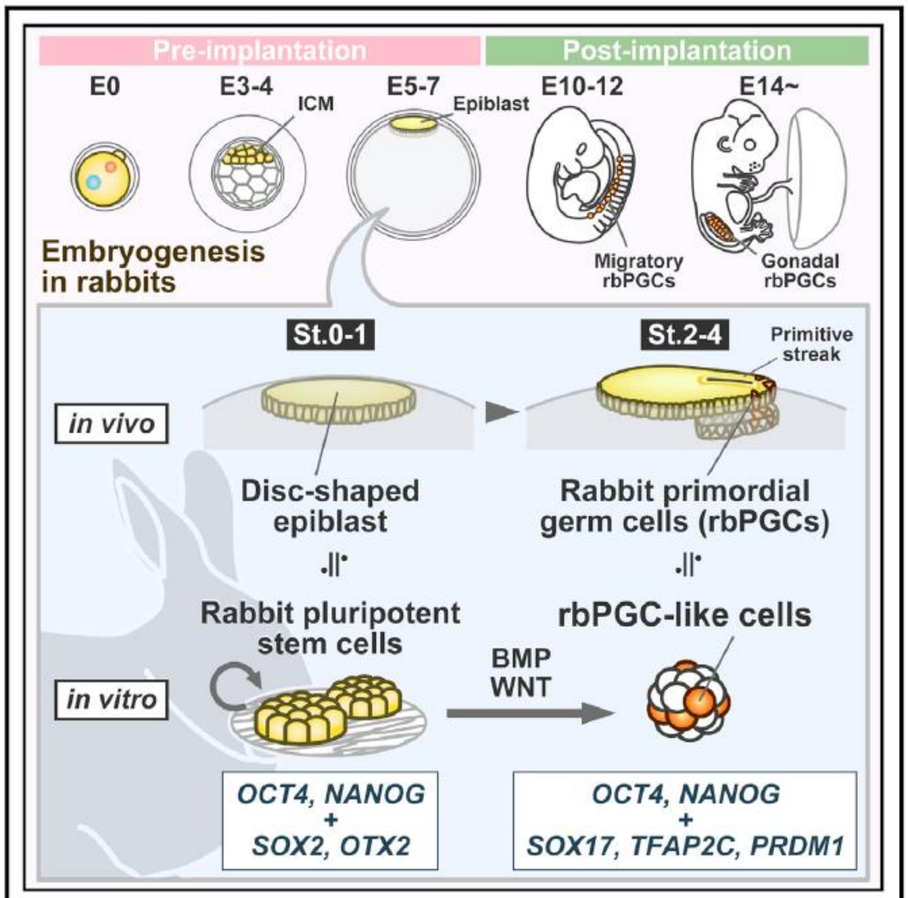
Cell Reports

CellPress OPEN ACCESS

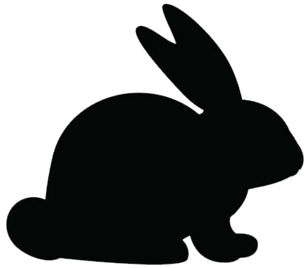
Article

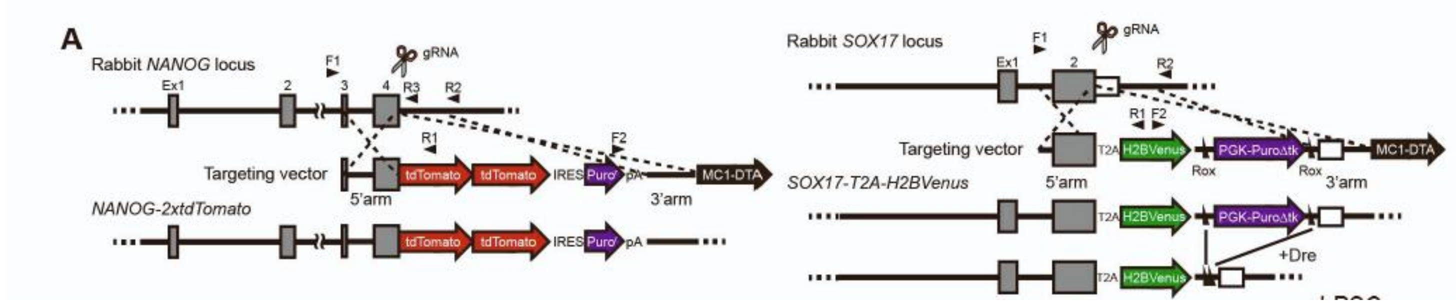
# Tracing the emergence of primordial germ cells from bilaminar disc rabbit embryos and pluripotent stem cells

Toshihiro Kobayashi,[1,2,12,*] Aracely Castillo-Venzor,[3,4,5] Chris A. Penfold,[4] Michael Morgan,[6,7] Naoaki Mizuno,[8] Walfred W.C. Tang,[3,4] Yasuyuki Osada,[9] Masao Hirao,[9] Fumika Yoshida,[2] Hideyuki Sato,[8] Hiromitsu Nakauchi,[8,10] Masumi Hirabayashi,[2,11,*] and M. Azim Surani[3,4,*]

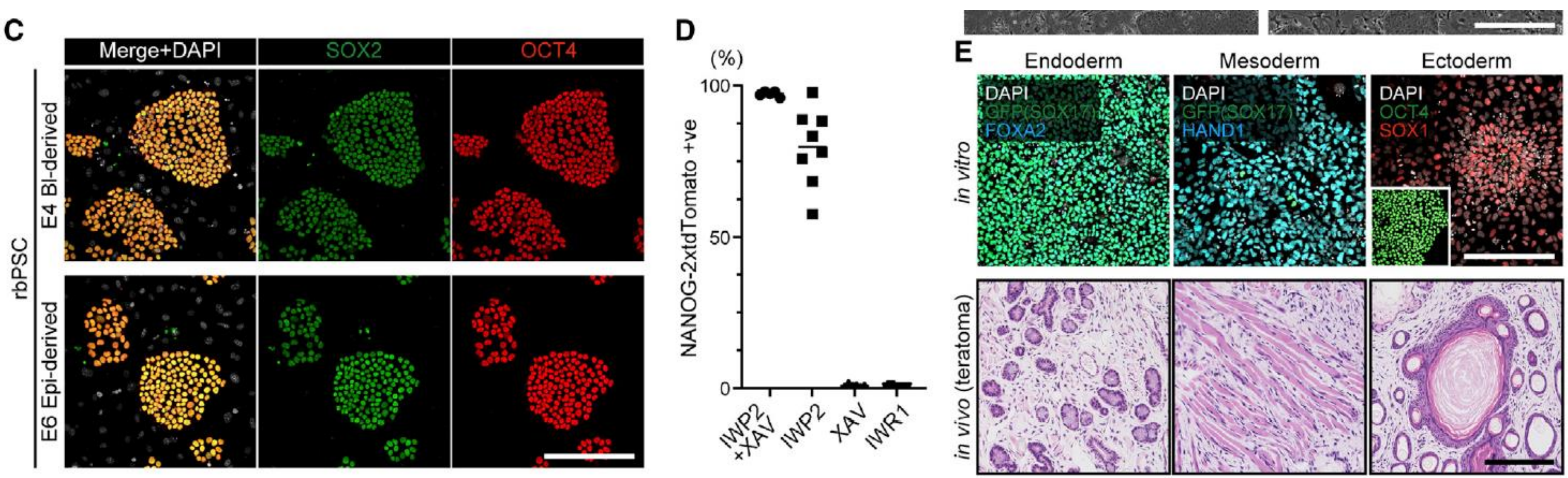



2021: with reporters, scRNAseq from embryos
Signalling pathway screening



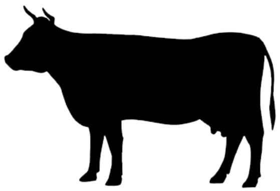



Essential 8 medium (ITS + FGF2 + TGFb) WNT inhibition (IWP2 non canonical) + XAV939 (canonical)




But no chimeras were produced

# Recent progress for the production of ungulate PSCs



**PNAS 2018**

## Efficient derivation of stable primed pluripotent embryonic stem cells from bovine blastocysts

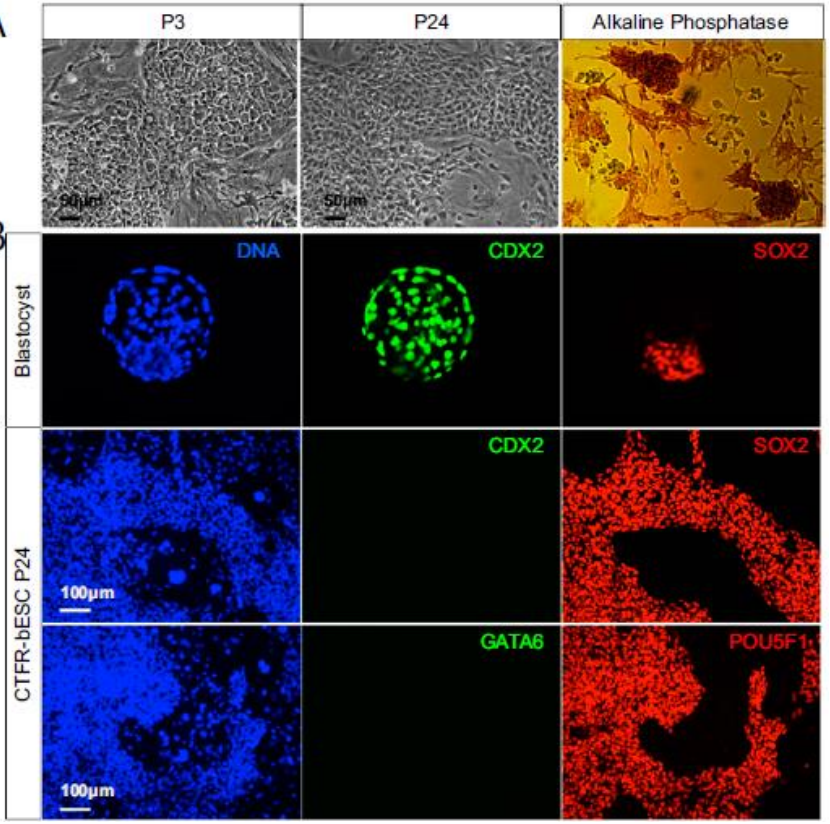
Yanina Soledad Bogliotti[a,1], Jun Wu[b,c,d,e,1,2], Marcela Vilarino[a], Daiji Okamura[d,f], Delia Alba Soto[a], Cuiqing Zhong[e], Masahiro Sakurai[b,c,d,e], Rafael Vilar Sampaio[a], Keiichiro Suzuki[e], Juan Carlos Izpisua Belmonte[e,2], and Pablo Juan Ross[a,2]

[a]Department of Animal Science, University of California, Davis, CA 95616; [b]Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390; [c]Hamon Center for Regenerative Science and Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75390; [d]Universidad Católica San Antonio de Murcia, 30107 Guadalupe, Murcia, Spain; [e]Gene Expression Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037; and [f]Department of Advanced Bioscience, Graduate School of Agriculture, Kindai University, 631-8505 Nara, Japan

Edited by R. Michael Roberts, University of Missouri, Columbia, MO, and approved January 3, 2018 (received for review September 13, 2017)

**Canonical Wnt inhibition (IWR1) + bFGF2**

A

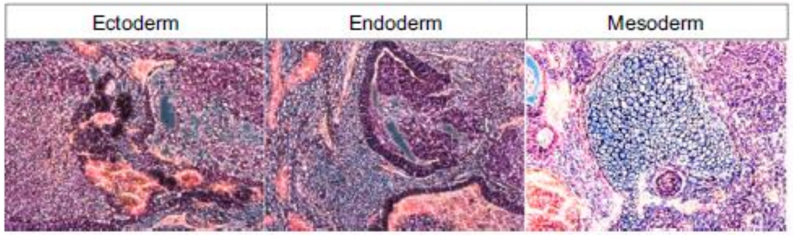



C

| | Gene | CTFR-bESC | Whole blastocyst | Fibroblast |
|---|---|---|---|---|
| ICM | *POU5F1* | 186.95 | 52.25 | 0 |
| | *NANOG* | 3.35 | 5.75 | 0 |
| | *SOX2* | 149.55 | 2.95 | 1.05 |
| | *LIN28B* | 60.8 | 0.75 | 0 |
| | *DNMT3B* | 88.05 | 27.25 | 0.25 |
| | *UTF1* | 7.15 | 0.15 | 0 |
| | *SALL4* | 54.85 | 5.7 | 0 |

| | Gene | CTFR-bESC | Whole blastocyst | Fibroblast |
|---|---|---|---|---|
| TE | *CDX2* | 0 | 36.2 | 0 |
| | *GATA2* | 0.05 | 4.65 | 2.5 |
| | *GATA3* | 0.1 | 14.4 | 0.1 |
| | *ELF3* | 0.45 | 1.4 | 0.4 |
| | *FGF4* | 0 | 0.55 | 0 |
| | *TFAP2A* | 0.4 | 0.95 | 16.65 |
| PE | *GATA6* | 0.15 | 17.6 | 0 |
| | *HNF4A* | 0 | 6.45 | 0 |
| | *PDGFRA* | 1.7 | 10.85 | 57.55 |

D



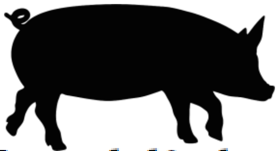

# Bovine primed ESCs

A

| | Gene | CTFR-bESC | | Gene | CTFR-bESC |
|---|---|---|---|---|---|
| Naïve ESC markers | *FGF4* | 0 | Primed ESC markers | *SOX6* | 0.1 |
| | *DNMT3L* | 0 | | *HOXB3* | 0.15 |
| | *DPPA3* | 0 | | *POU3F2* | 0.15 |
| | *HORMAD1* | 0 | | *RFX4* | 0.6 |
| | *TFCP2L1* | 0 | | *MYC* | 0.85 |
| | *DPPA2* | 0 | | *DLL1* | 0.95 |
| | *ZFP42* | 0.1 | | *MEIS1* | 1.65 |
| | *TBX3* | 0.15 | | *LMO2* | 2.15 |
| | *MAEL* | 0.55 | | *TET3* | 3.45 |
| | *DUSP10* | 1.4 | | *SOX1* | 6.1 |
| | *DUSP5* | 1.85 | | *ZIC1* | 7.05 |
| | *DUSP3* | 1.9 | | *CD47* | 7.65 |
| | *KLF4* | 2.2 | | *MEIS2* | 9.6 |
| | *CD44* | 2.85 | | *ZNF521* | 12.3 |
| | *NANOG* | 3.35 | | *NCAM1* | 16.55 |
| | *TET2* | 4.55 | | *TET1* | 18.5 |
| | *TEAD4* | 5.05 | | *DUSP6* | 30.75 |
| | *KLF5* | 5.45 | | *ZIC3* | 33.15 |
| | *DUSP14* | 6.35 | | *DNMT3A* | 49.1 |
| | *STAT3* | 7.05 | | *DNMT3B* | 88.05 |
| | *TFE3* | 32.35 | | *ZIC2* | 127.35 |
| | *CD9* | 301.55 | | *OTX2* | 129.95 |

B

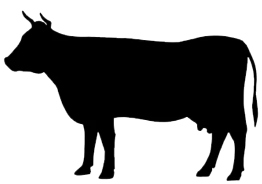
# Recent progress for the production of ungulate PSCs



Nat Cell Biol 2019

## Establishment of porcine and human expanded potential stem cells

Xuefei Gao[1,2,23], Monika Nowak-Imialek[3,4,23], Xi Chen[2,23], Dongsheng Chen[5,6], Doris Herrmann[3,4], Degong Ruan[1,7], Andy Chun Hang Chen[8], Melanie A. Eckersley-Maslin[9], Shakil Ahmad[10], Yin Lau Lee[8], Toshihiro Kobayashi[11], David Ryan[2], Jixing Zhong[5,6], Jiacheng Zhu[5,6], Jian Wu[1], Guocheng Lan[12], Stoyan Petkov[3,4,20], Jian Yang[2,21], Liliana Antunes[2], Lia S. Campos[2], Beiyuan Fu[2], Shengpeng Wang[5,6], Yu Yong[2], Xiaomin Wang[7], Song-Guo Xue[13], Liangpeng Ge[14], Zuohua Liu[14], Yong Huang[14], Tao Nie[7], Peng Li[7], Donghai Wu[7], Duanqing Pei[7,15], Yi Zhang[16], Liming Lu[17], Fengtang Yang[2], Susan J. Kimber[18], Wolf Reik[9], Xiangang Zou[12], Zhouchun Shang[5,6], Liangxue Lai[7], Azim Surani[11], Patrick P. L. Tam[19], Asif Ahmed[10], William Shu Biu Yeung[8], Sarah A. Teichmann[2], Heiner Niemann[3,4,22*] and Pentao Liu[1,2*]

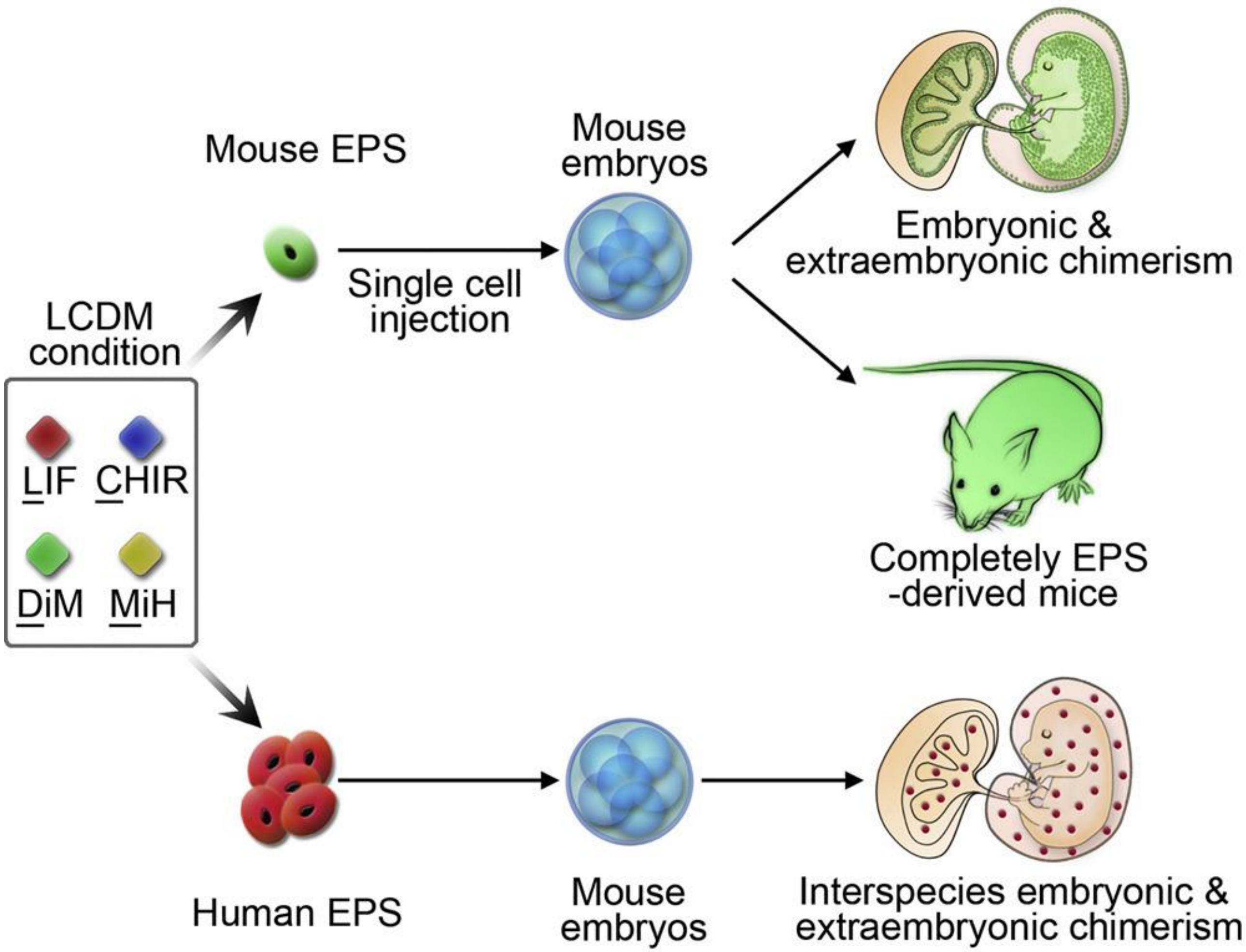


PNAS 2021

## Establishment of bovine expanded potential stem cells

Lixia Zhao[a,b,c,1], Xuefei Gao[d,e,f,1], Yuxuan Zheng[g,1], Zixin Wang[c], Gaoping Zhao[c], Jie Ren[g], Jia Zhang[a,b], Jian Wu[f], Baojiang Wu[a,b,c], Yanglin Chen[a,b], Wei Sun[b,c], Yunxia Li[b,c], Jie Su[c,h], Yulin Ding[i], Yuan Gao[c], Moning Liu[h], Xiaochun Bai[d,j], Liangzhong Sun[k], Guifang Cao[h], Fuchou Tang[g,l,m], Siqin Bao[a,b], Pentao Liu[f,n,2], and Xihe Li[a,b,c,2]

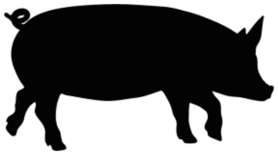
# ESCs with Expanded potential (EPSCs)



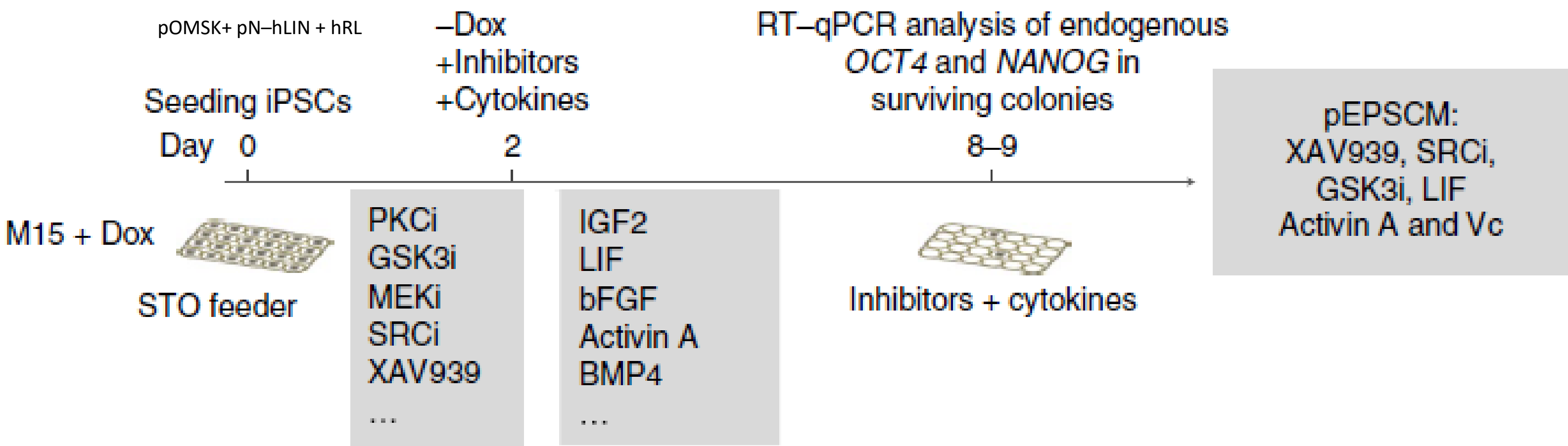

LCDM:

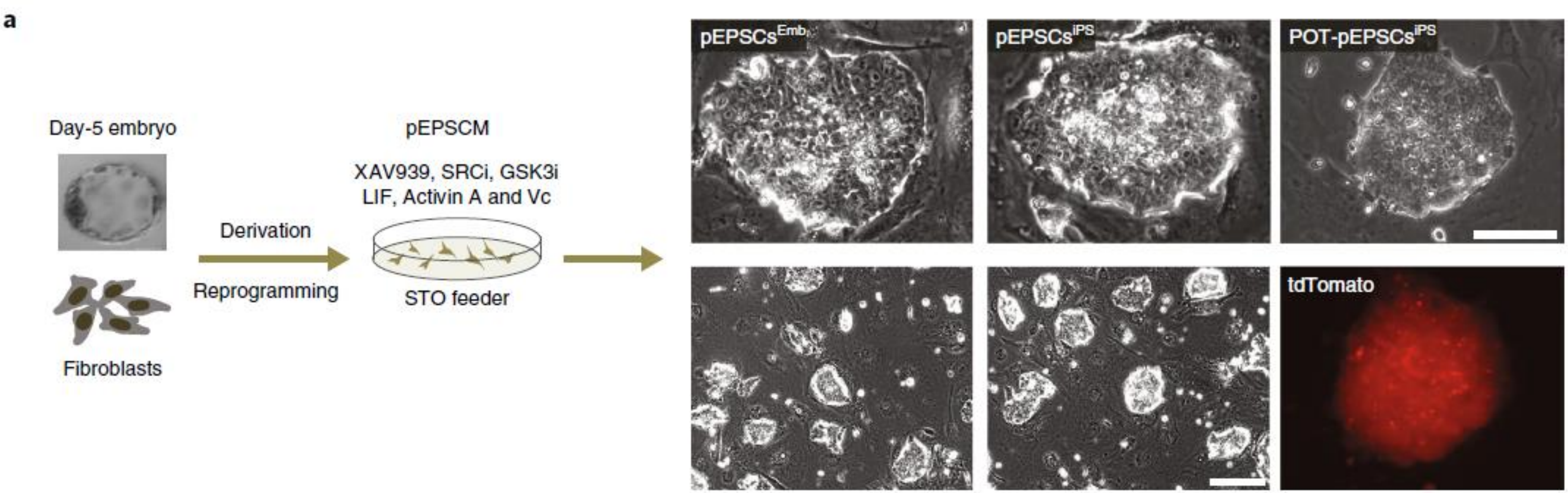
- LIF
- GSK3b inh.
- DiM: Gprot-receptor
- MiH: PARP1 inh.

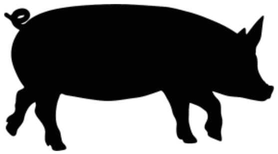
Yang et al. 2017 Cell



# Producing pig ESCs (2019)

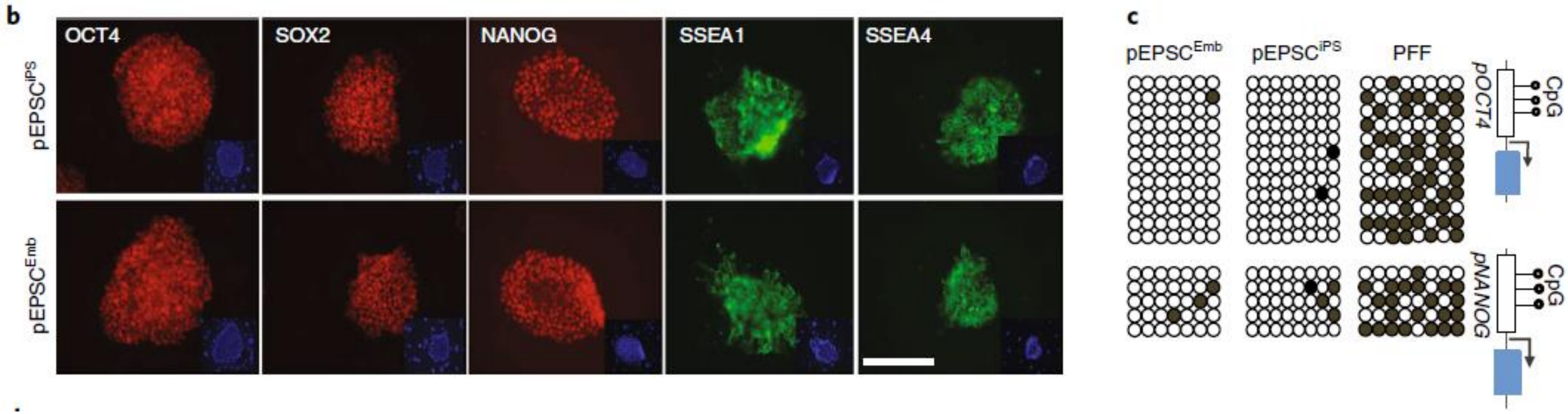


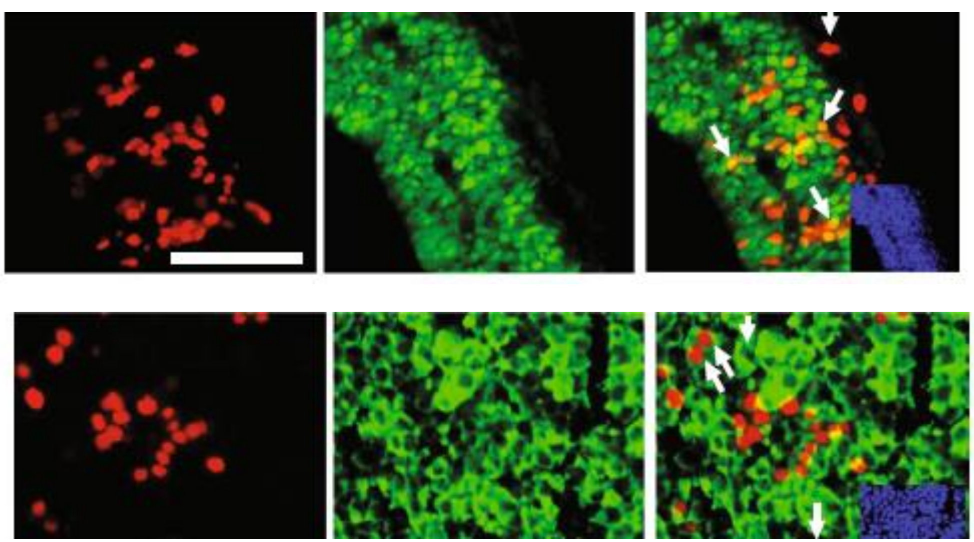


# Producing pig ESCs (2019)

## Pluripotency markers

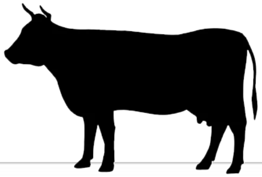



## Chimera

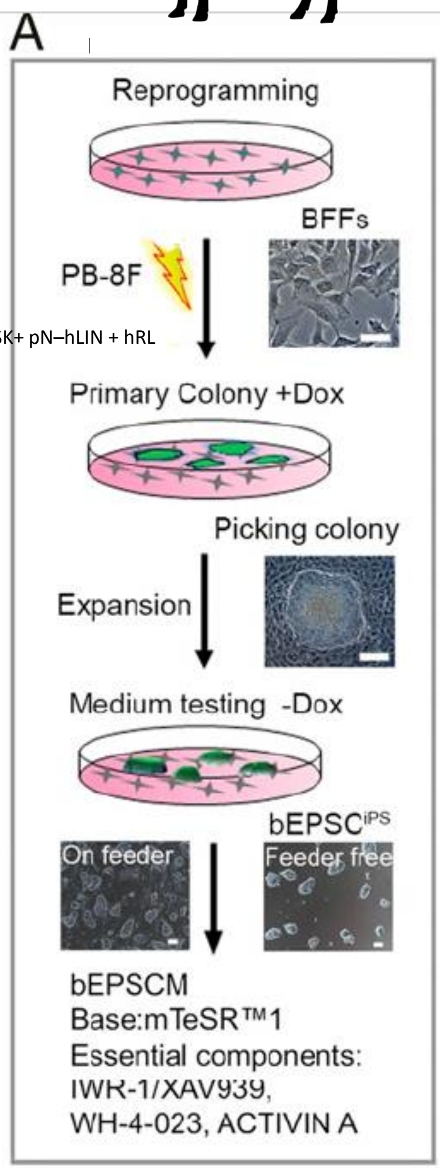


Cortex
SOX2
dTomato

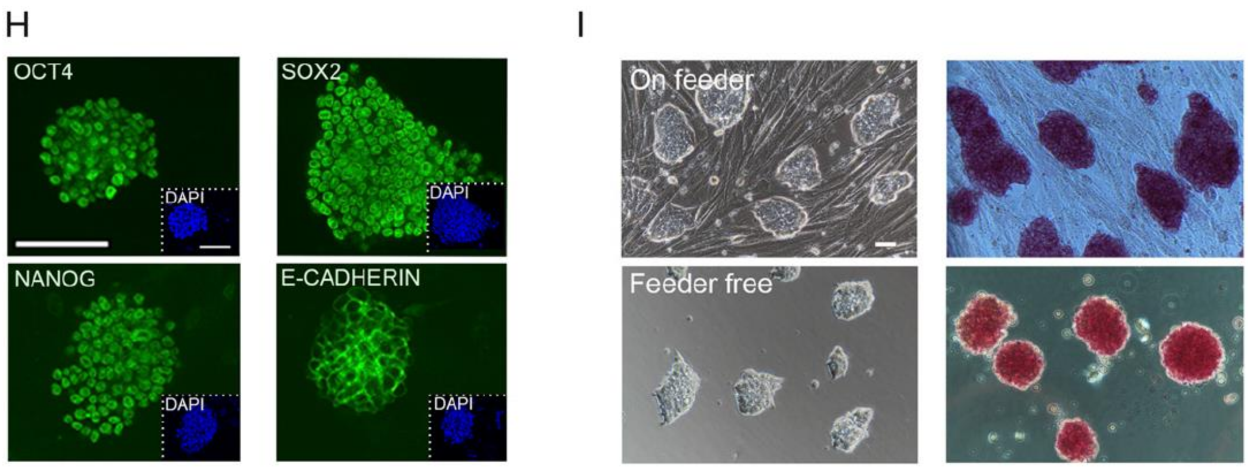
Liver
AFP
dTomato

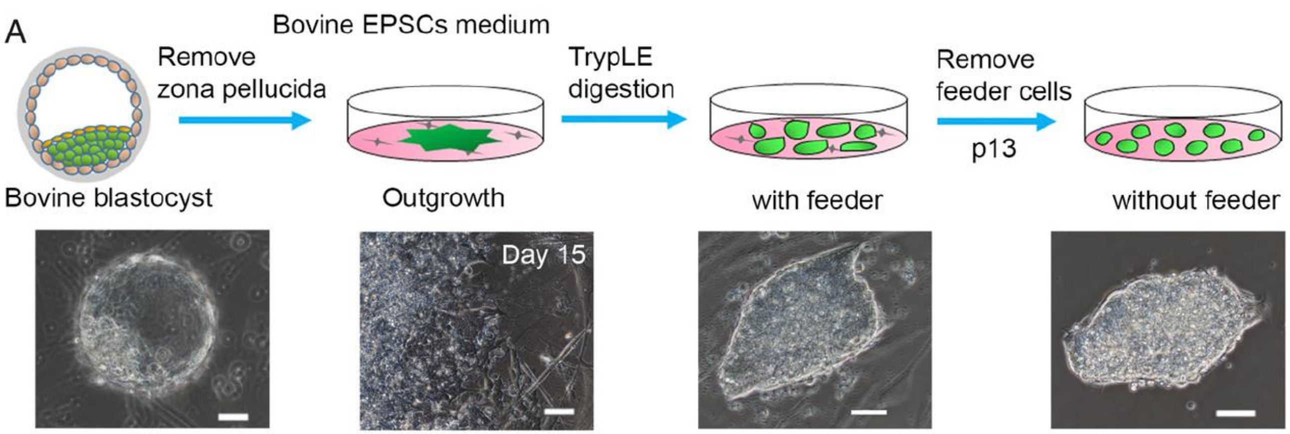


# Producing bovine ESCs (2021)

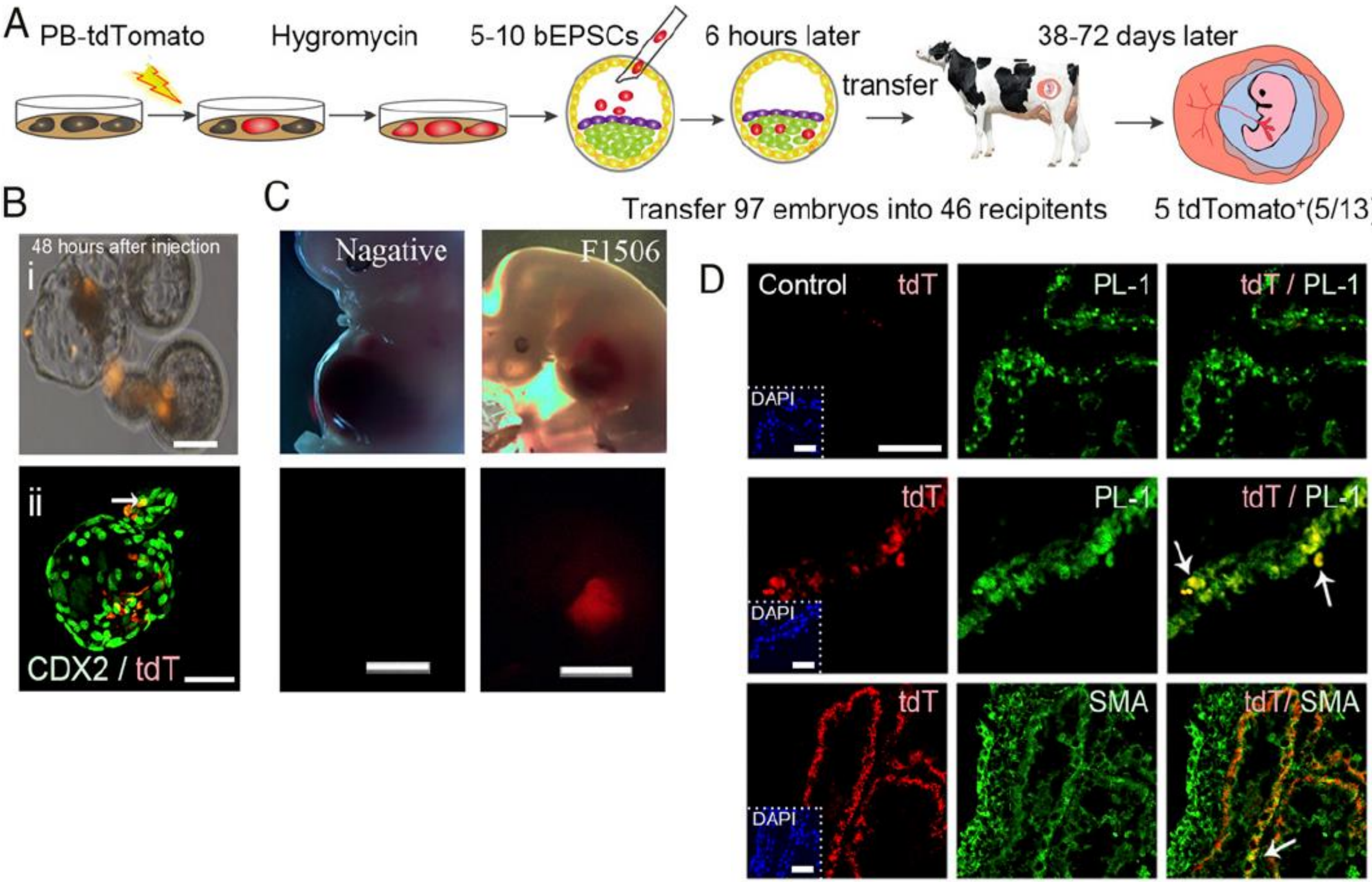



pOMSK+ pN–hLIN + hRL



# Producing bovine ESCs: chimera

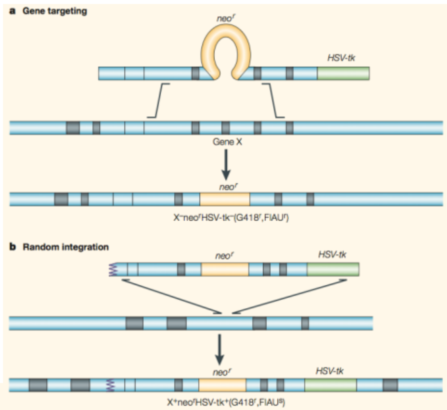


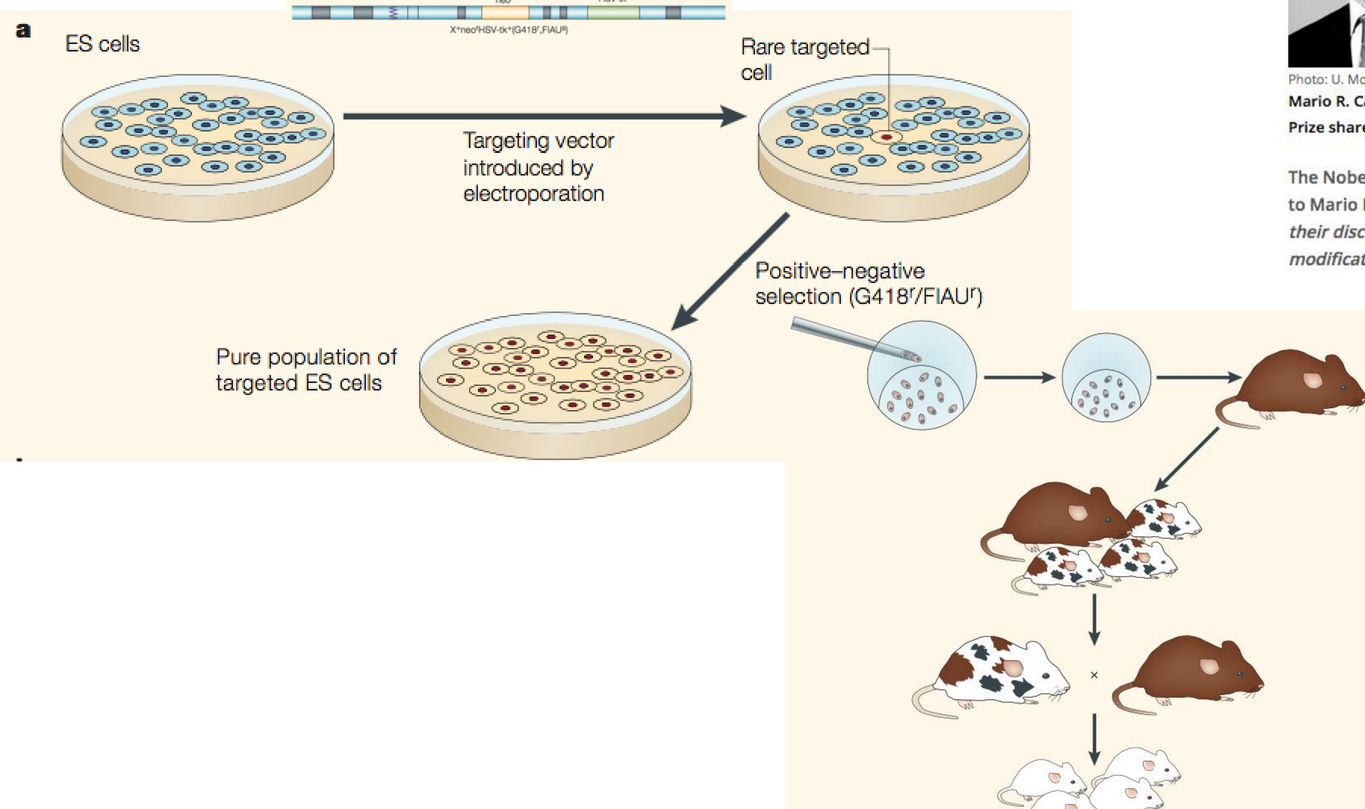

Placenta

Smooth muscle

# Some applications of mammalian ESCs

# 1. Gene targeting (before CRISPR/Cas9) (30 years of functional genomics in mice




## The Nobel Prize in Physiology or Medicine 2007

Photo: U. Montan
**Mario R. Capecchi**
**Prize share: 1/3**

Photo: U. Montan
**Sir Martin J. Evans**
**Prize share: 1/3**

Photo: U. Montan
**Oliver Smithies**
**Prize share: 1/3**

The Nobel Prize in Physiology or Medicine 2007 was awarded jointly to Mario R. Capecchi, Sir Martin J. Evans and Oliver Smithies *"for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells".*

Capecchi *et al.*, 2005 Nat Rev Gen

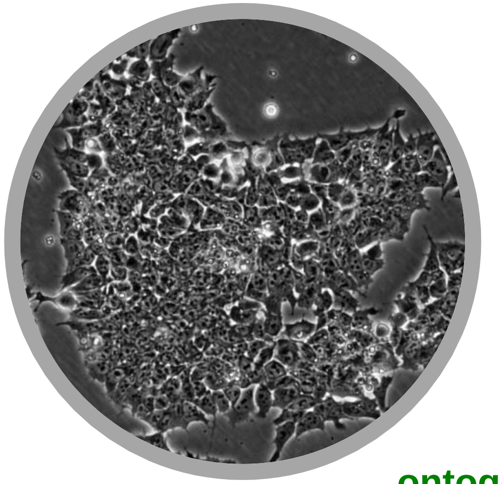
# 2. Modelling disease from pluripotent stem cells

**PSC** → **HSC**

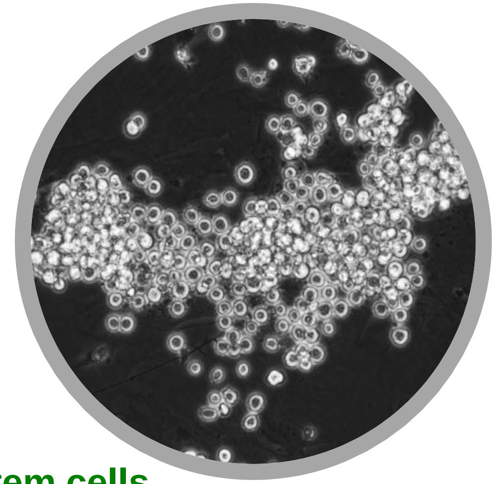
RUNX1, HOXA5, HOXA9, HOXA10, ERG, LCOR, SPI1

HoxB4, Low Notch

## Modelling chronic myeloid leukemia in a dish from PSCs



Inducible expression → of BCR-ABL

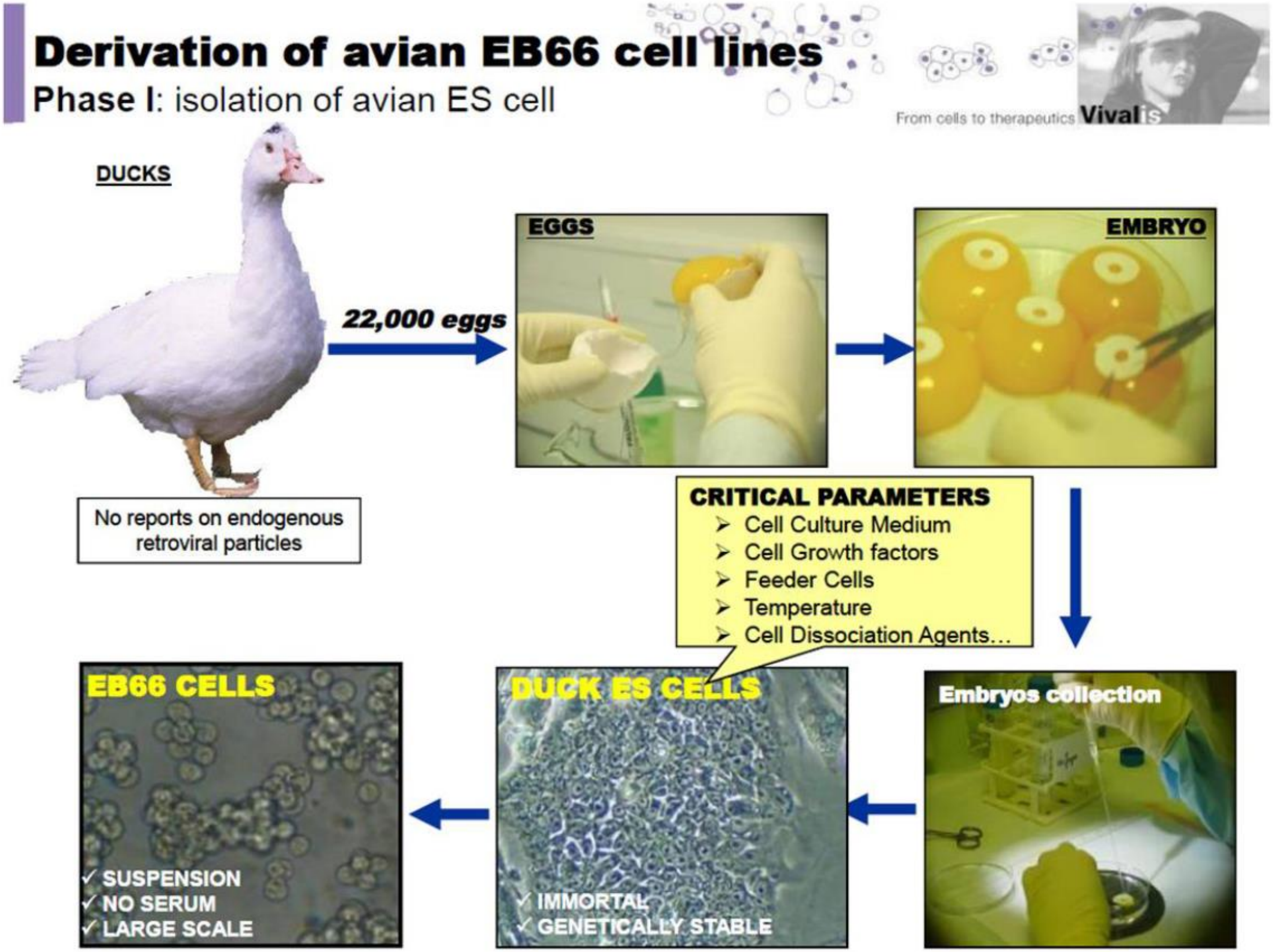


**ontogeny of the cancer leukemic stem cells**
**disease progression**
**resistance to chemotherapy**

**Impact on the differentiation** **Identity and stem cell potential**

marker expression : FACS, qRT-PCR, immunofluorescence…
self-renewal / differentiation (*in vitro/in vivo*)

# 3. Amplyfying virus for vaccine and screening antiviral drugs



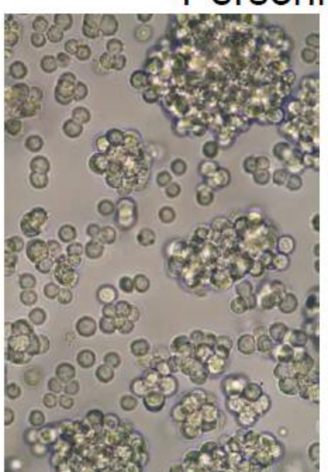

Valneva

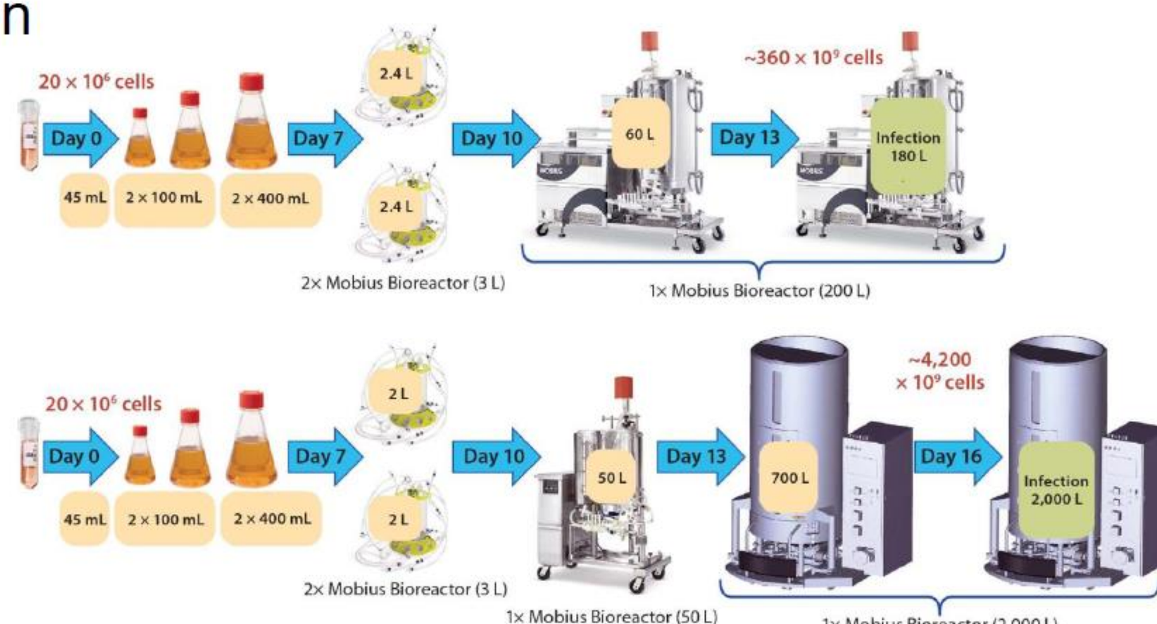
# 3. Amplyfying virus for vaccine and screening antiviral drugs

## Process at Scale

- Grow cells
  - Suspension efficient for scale-up
    - Seed on scaffolds
    - Use directly
- EB66 duck embryonic stem cells
  - Bioreactor
  - Contamination
    - Personnel

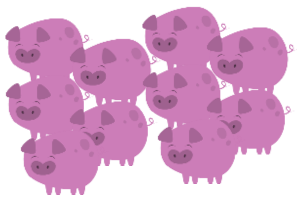
Valneva

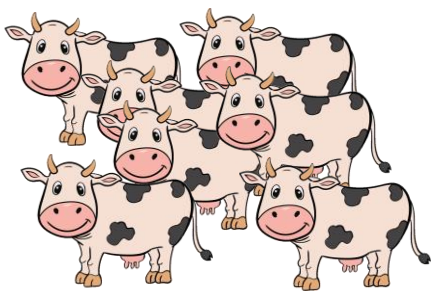




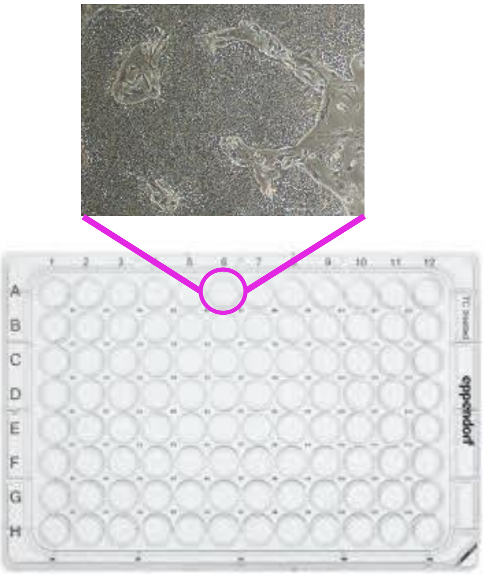
# 4. High-througput phenotyping for genetic studies

**Use of cell populations instead of animals/patients**





Reference (x1000-x10,000 x1M …) individuals



Reference (x1000-x10,000 x1M …) Cell lines

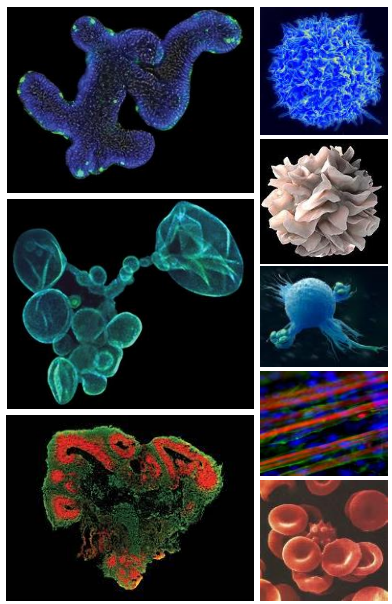
**Bio-bank of PSCs**

# 4. High-througput phenotyping for genetic studies

1 cell line

Genome editing

**In vitro differentiation**



**Phenotyping**:

Biomecanics
Gene expression
Proteome
Metabolome
Absorption
Endocytosis
Exocytosis
Phagocytosis
Toxicity
Infections
….

**X1000 lines**

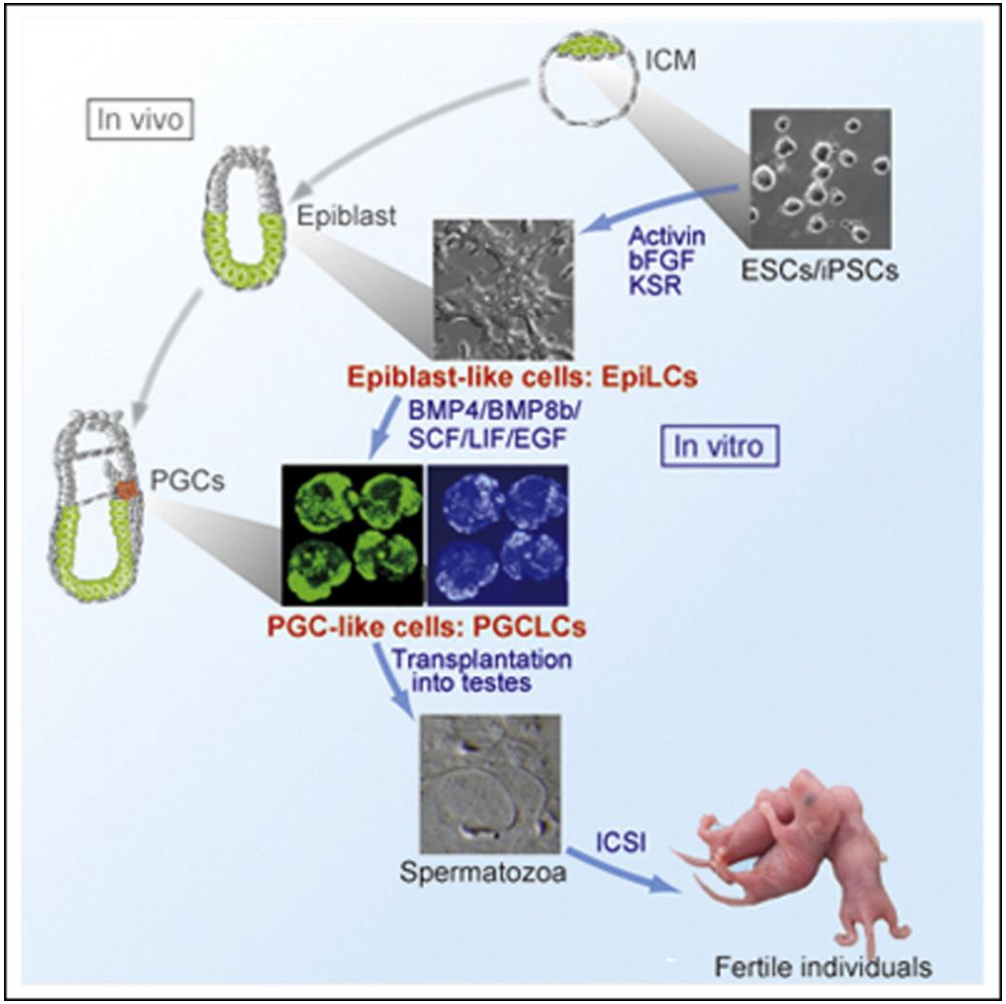
**HTS**

**GWAS**

**Big data**

# 5. Using pluripotent stem cells for reproduction
# Producing functional gametes

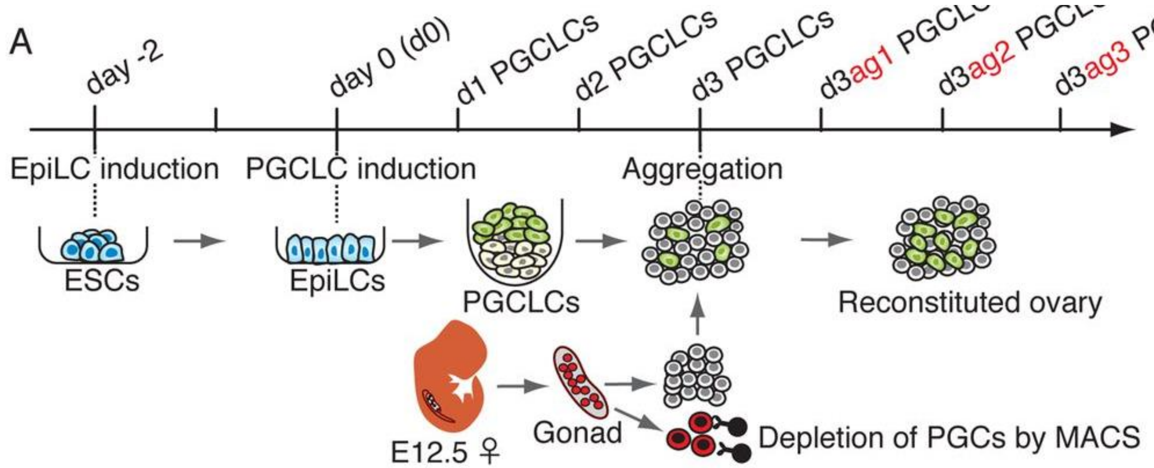
## *in vitro differentiation: Male primordial germ cells*



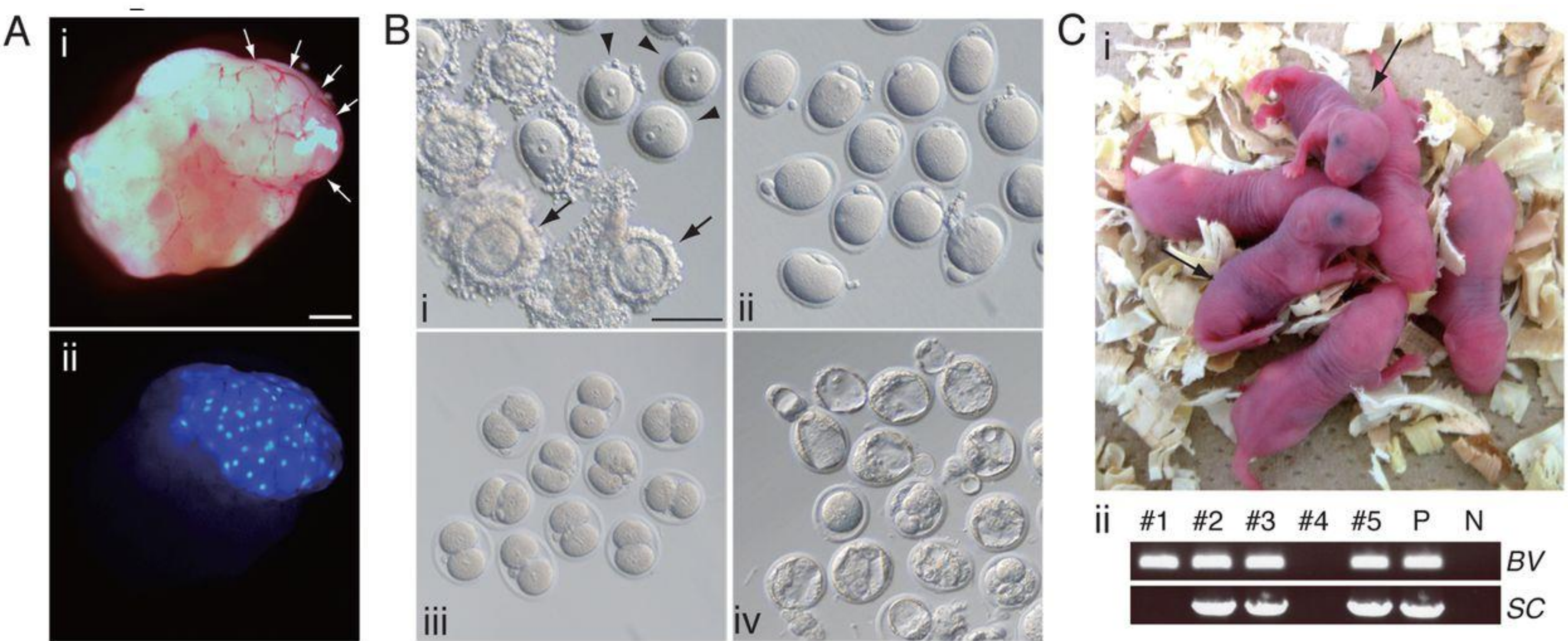

Hayashi et al.
Cell 2011

# 5. Using pluripotent stem cells for reproduction

*in vitro differentiation: female primordial germ cells*

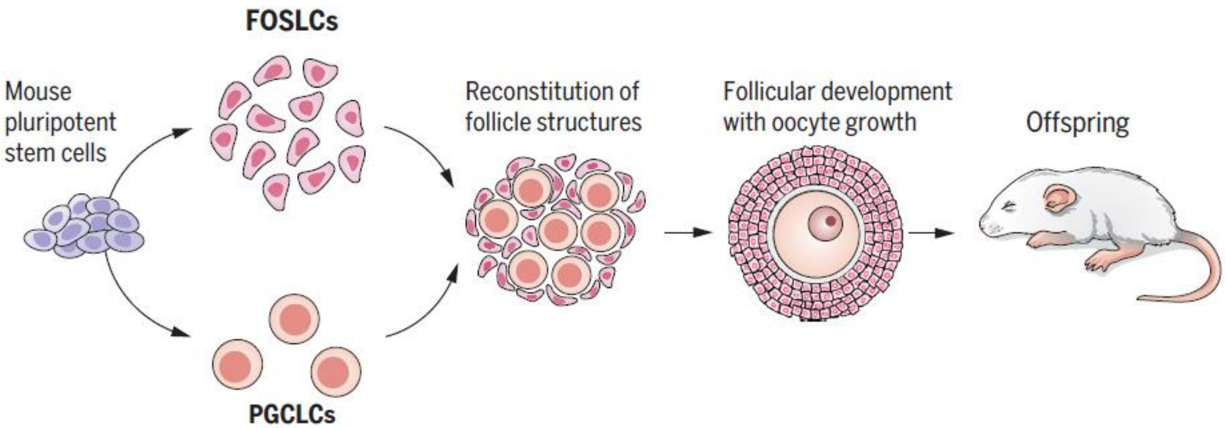


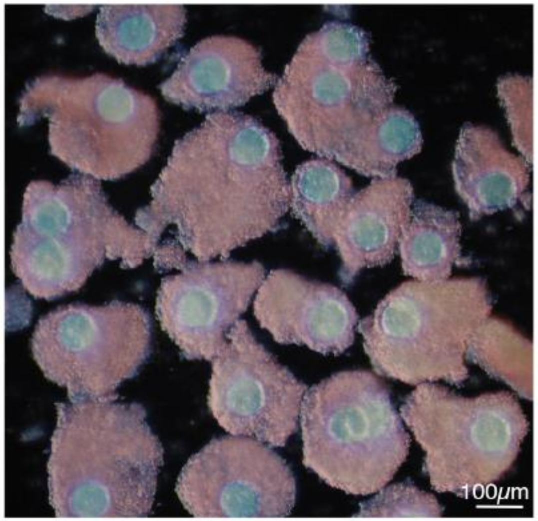

Hayashi et al.
Science 2012



# 5. Using pluripotent stem cells for reproduction

## *in vitro differentiation: fully derived oocytes from PSCs*




Yoshino et al. Science 2021

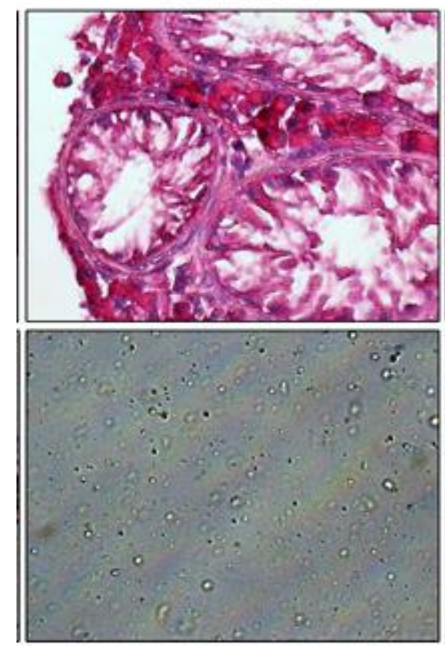
# *Producing functional gametes in host gonads*

Nanos mutant and testis complementation

## Generation of germline ablated male pigs by CRISPR/Cas9 editing of the *NANOS2* gene

Ki-Eun Park[1,2,3,*], Amy V. Kaucher[4,*], Anne Powell[2], Muhammad Salman Waqas[4], Shelley E.S. Sandmaier[1,2], Melissa J. Oatley[4], Chi-Hun Park[1,2], Ahmed Tibary[4], David M. Donovan[2], Le Ann Blomberg[2], Simon G. Lillico[5], C. Bruce A. Whitelaw[5], Alan Mileham[6], Bhanu P. Telugu[1,2,3] & Jon M. Oatley[4]

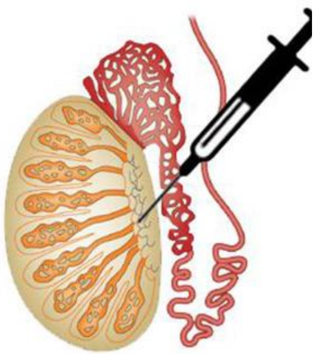
Boar #146-/-



Also Dazl mutant in chicken

# 5. Using pluripotent stem cells for reproduction

## *Producing functional gametes in host gonads*



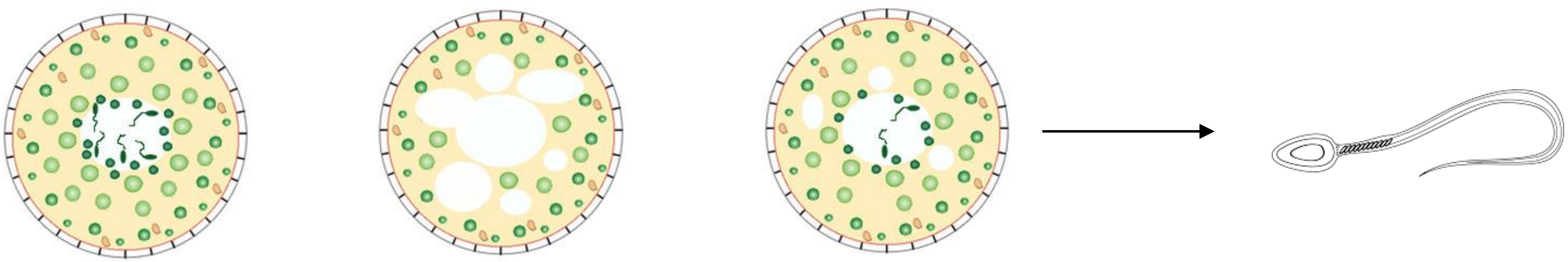
Genetically modified PGCLs injection in Nanos-/- testis

wild type

Nanos KO

Nanos KO + ePGCLs injection

Genetically modified sperm cells



normal tubule

spermatocyte depletion

phenotype rescued

# 5. Using pluripotent stem cells for reproduction

## In vitro breeding to speed-up genomic selection in livestock

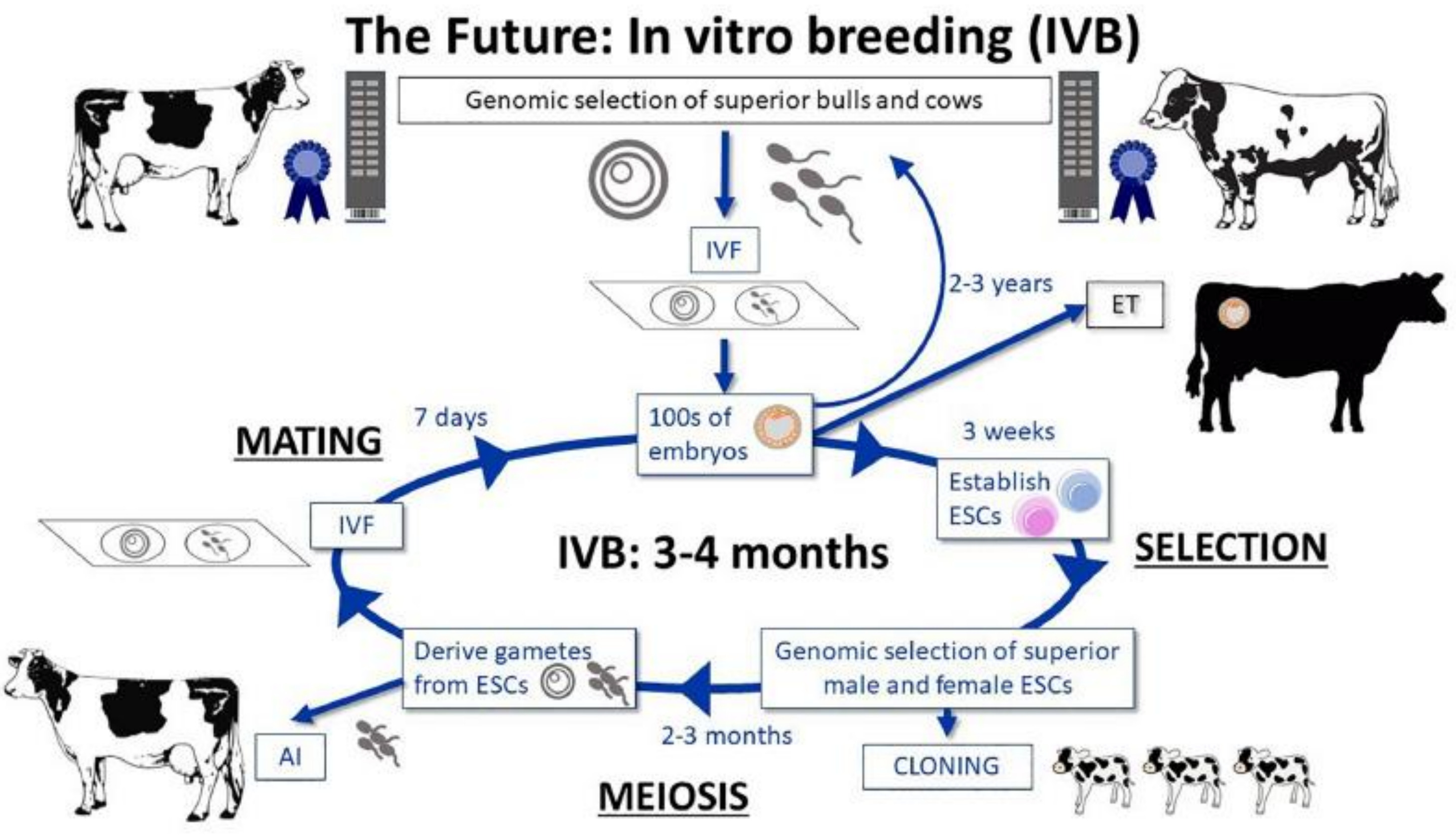


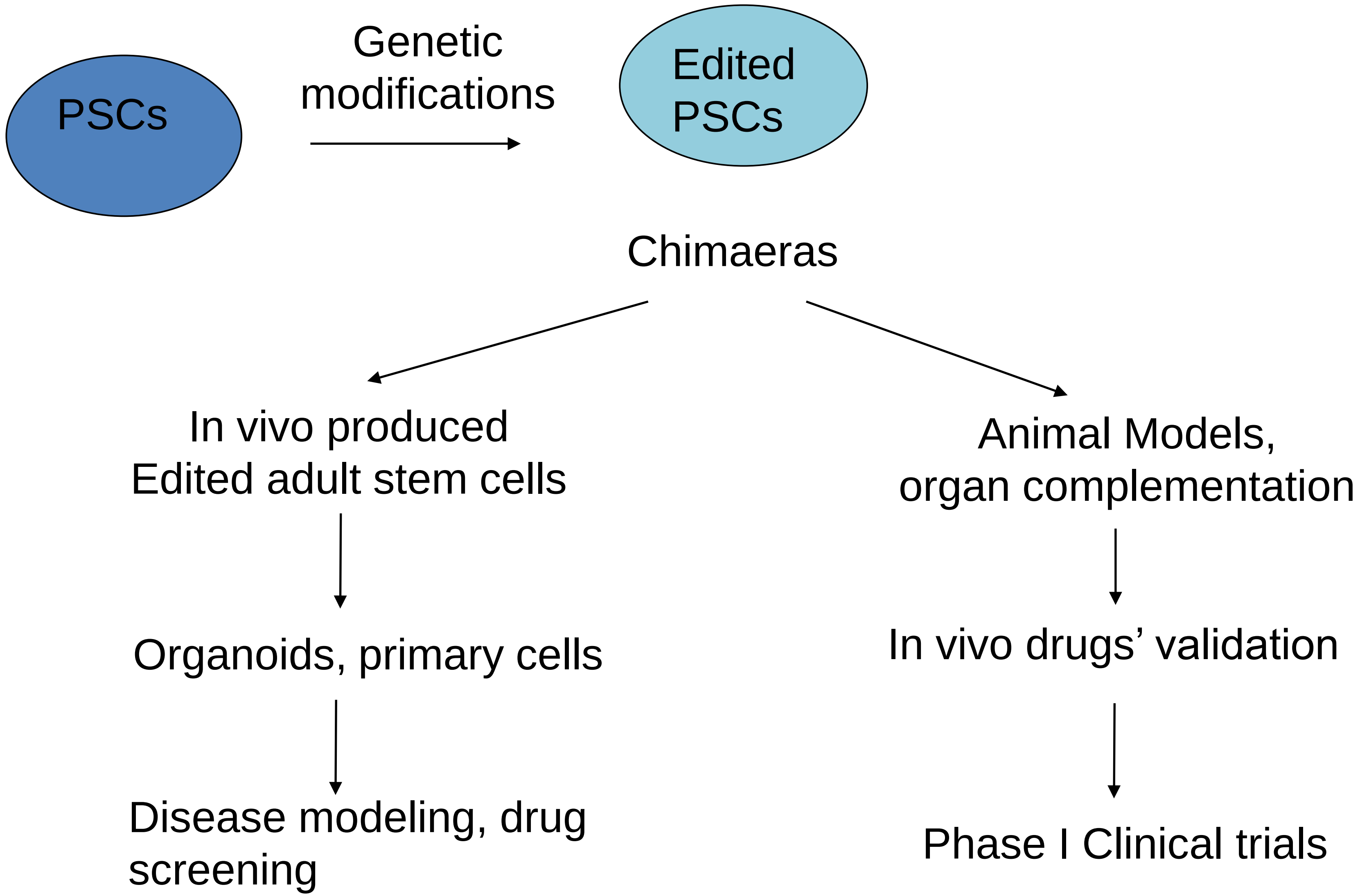

**FIGURE 10 |** Efficient isolation of pluripotent embryonic stem cells (ESCs) from cattle embryos allows the development of *in vitro* breeding schemes based on an embryo-stem cell-gamete cycle, including an intermediate genomic selection to provide directional selection of genetic progress. If such a scheme could be accomplished, it would significantly decrease the generation interval and allow for increased selection intensity leading to accelerated genetic progress. IVF, *in vitro* fertilization; ET, Embryo transfer. Image from Van Eenennaam (2018). Reproduced with permission from the author's entry in the *Encyclopedia of Food Security and Sustainability*, Ferranti et al. (2018).

**Rexroad et al. 2019**

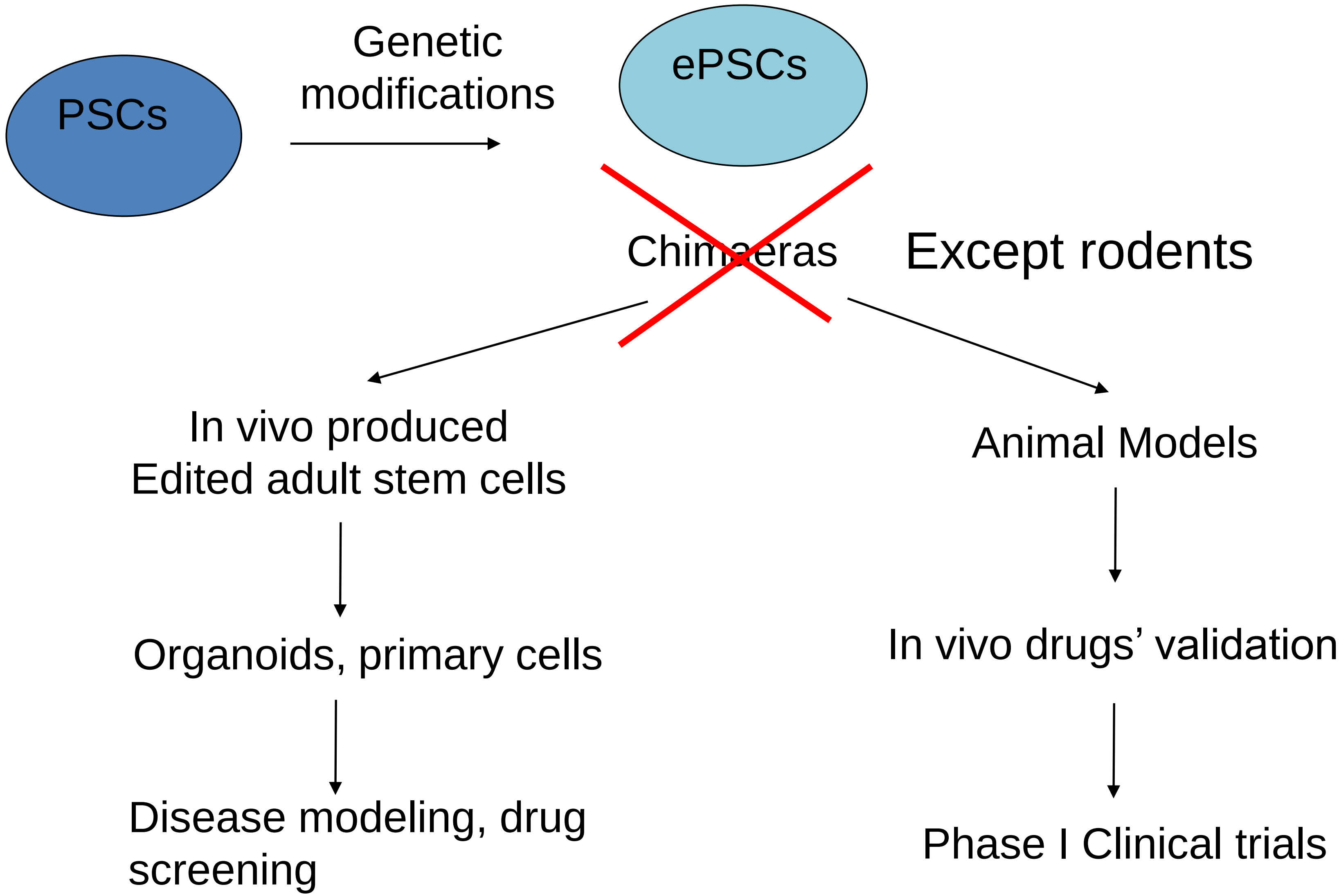
## 6. To produce organs

## Chimera and interspecies chimera

# Chimera: a powerful tool to model disease and organs in mammalian species (except human)

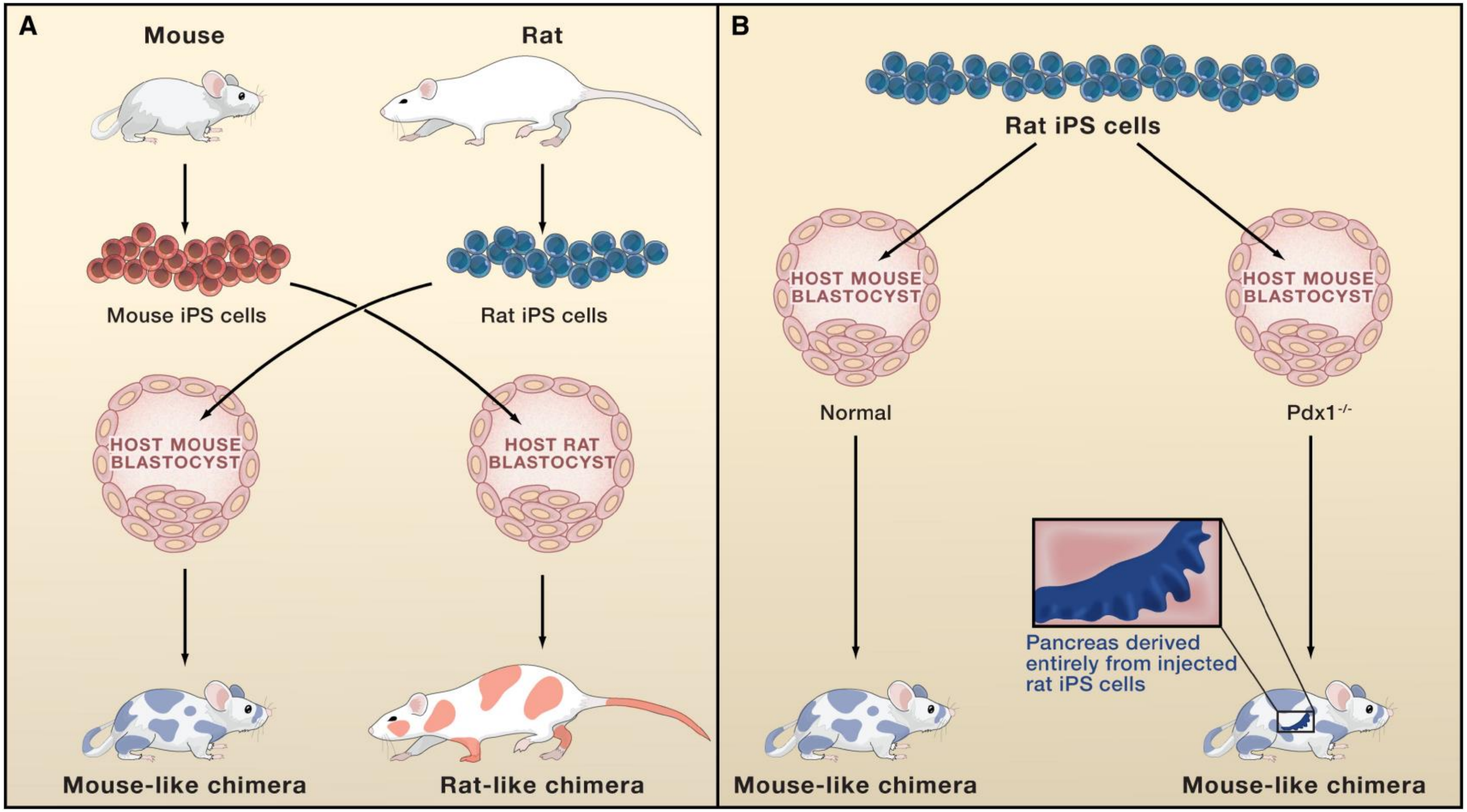


# Chimera: a powerful tool to model disease and organs in mammalian species (except human)



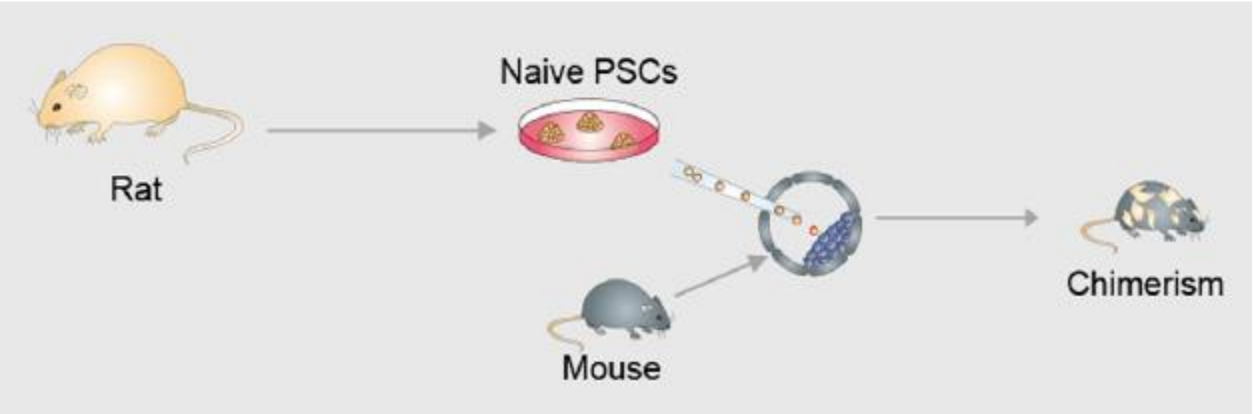
**That’s why studies on chimera and interspecies chimera are mostly done on rodents**

# Producing organs using chimera:
# Organ complementation by interspecies chimeras

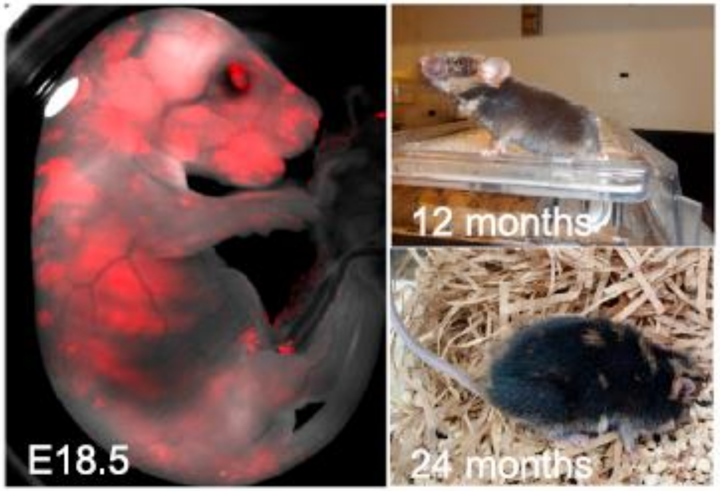



Solter et al. 2010

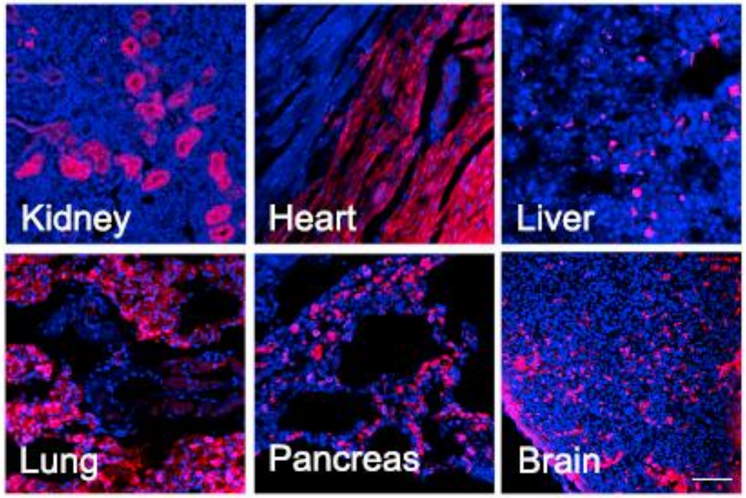
# Organ complementation by interspecies chimeras




A

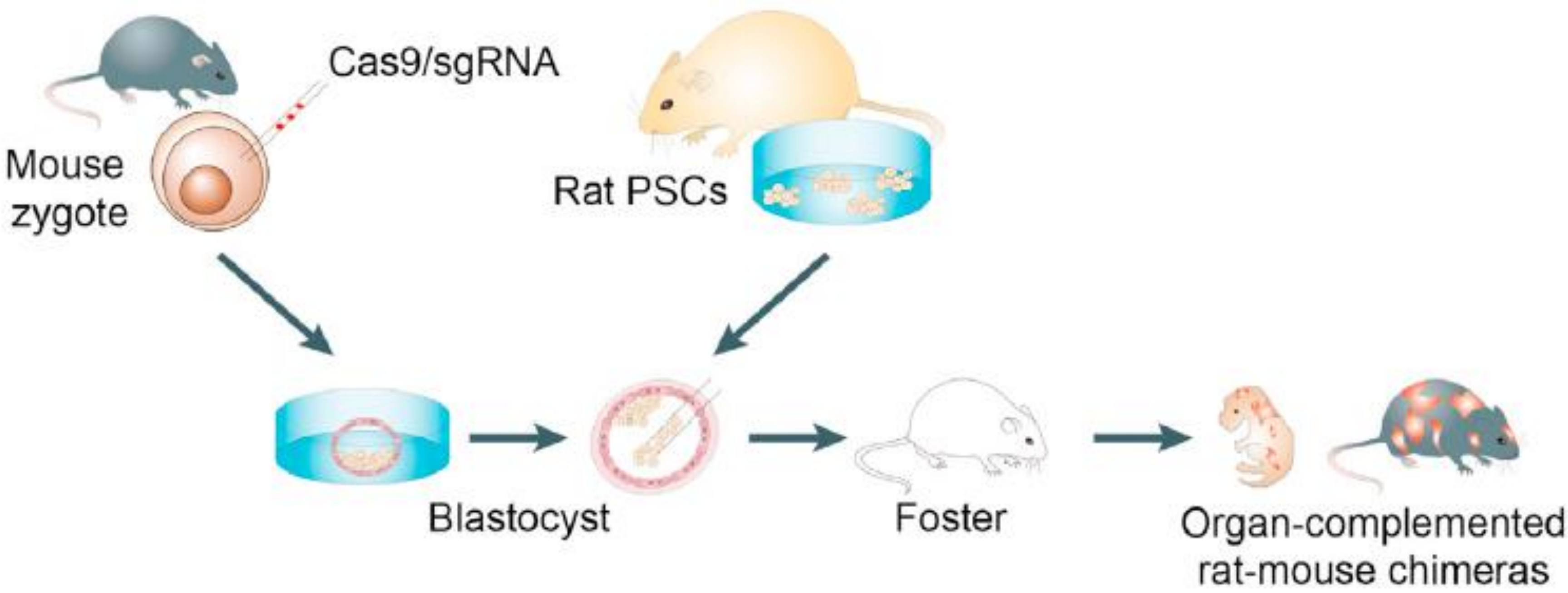



C



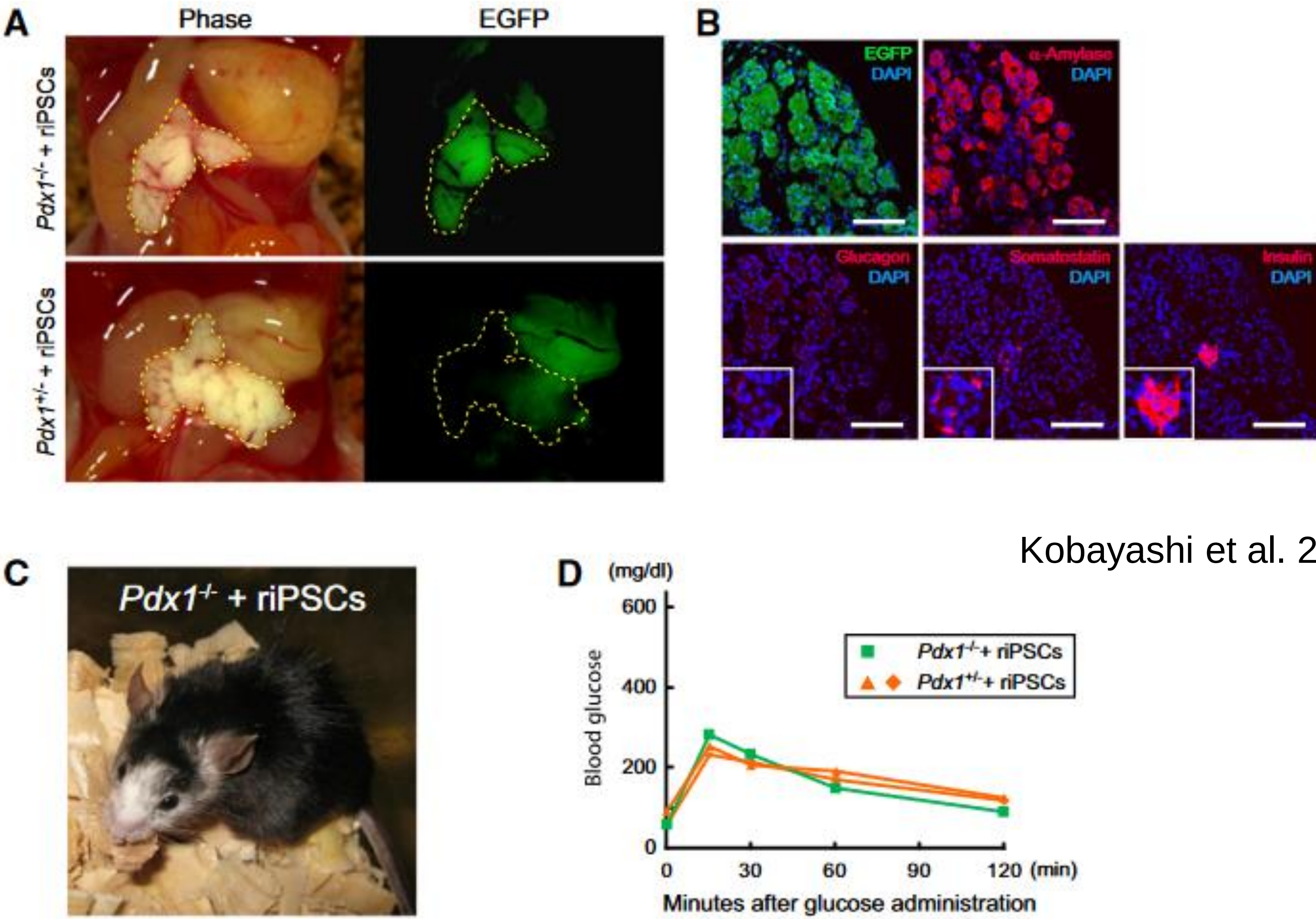

Wu et al. Cell 2017

# Organ complementation by interspecies chimeras



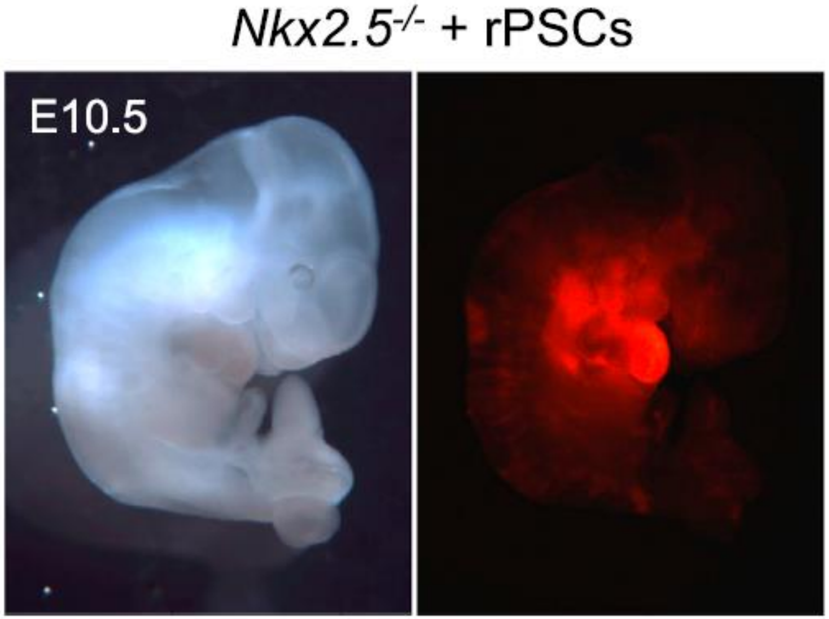

Wu et al. Cell 2017

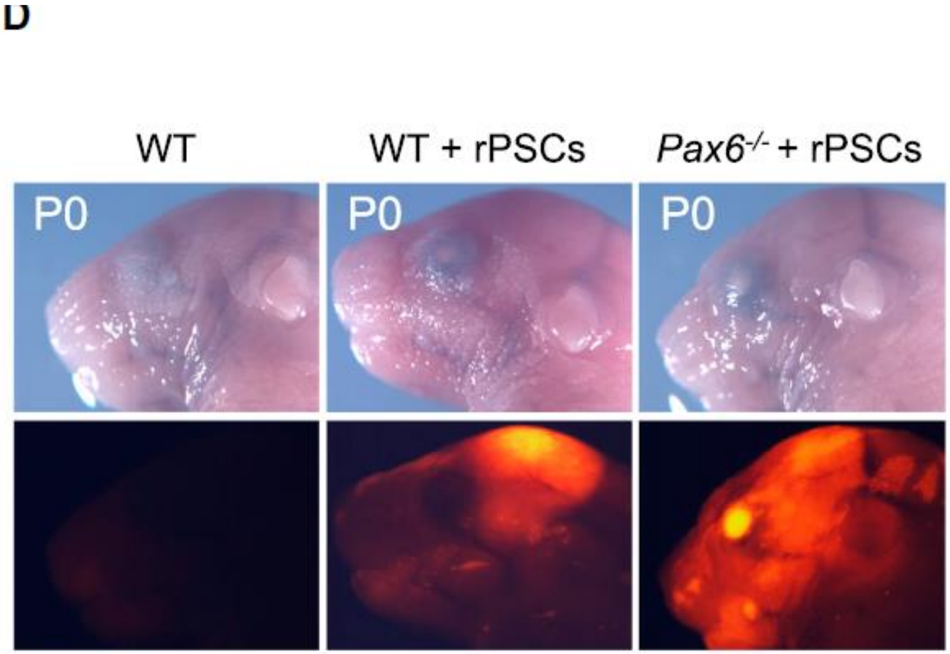
# Organ complementation by interspecies chimeras
# Pancreas




Kobayashi et al. 2010

# Organ complementation by interspecies chimeras
# Heart and eyes




Heart

Retina

Wu et al. Cell 2017

Producing human organs in non human species: Xenografts and organ complementation

The interest for the pig species

# Grafting pig organs in human :
# Xenografts and organ complementation

## The interest for the pig species

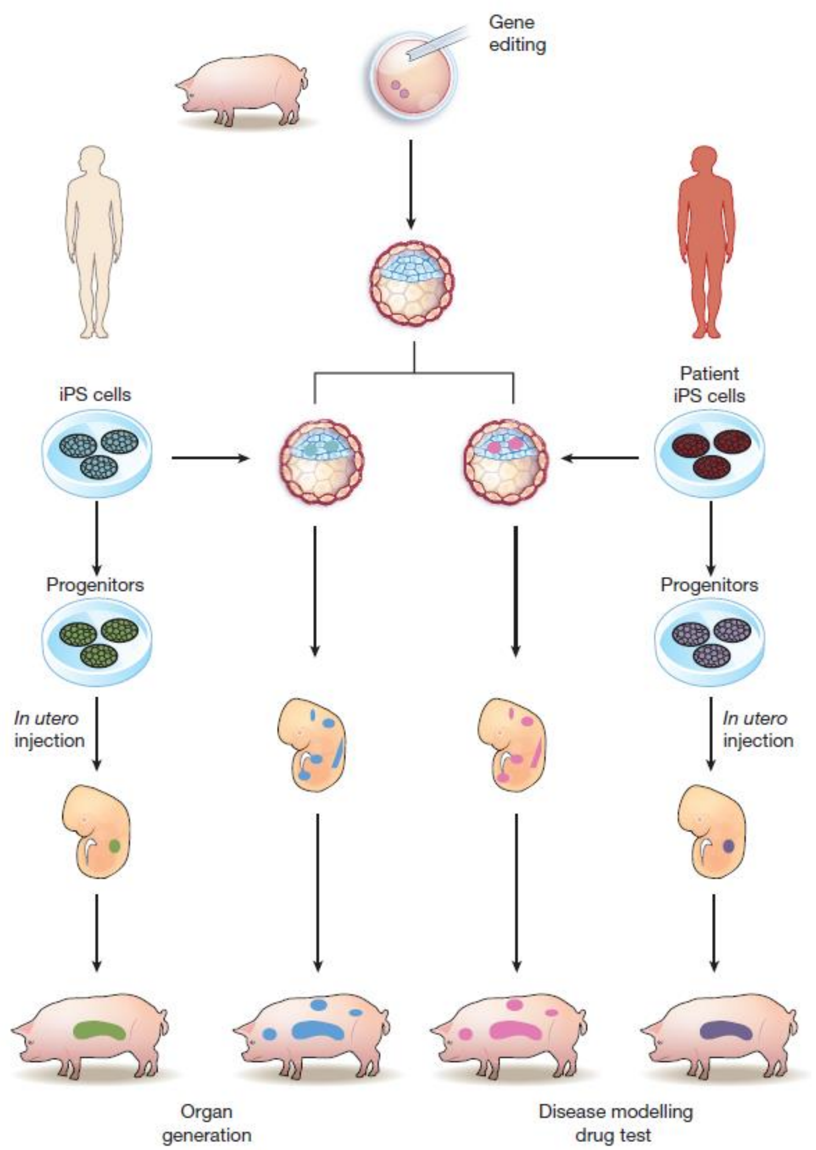
BRIEF REPORT

### Genetically Modified Porcine-to-Human Cardiac Xenotransplantation

Bartley P. Griffith, M.D., Corbin E. Goerlich, M.D., Ph.D., Avneesh K. Singh, Ph.D., Martine Rothblatt, Ph.D., Christine L. Lau, M.D., Aakash Shah, M.D., Marc Lorber, M.D., Alison Grazioli, M.D., Kapil K. Saharia, M.D., Susie N. Hong, M.D., Susan M. Joseph, M.D., David Ayares, Ph.D., and Muhammad M. Mohiuddin, M.D.

NEJM, June 2022

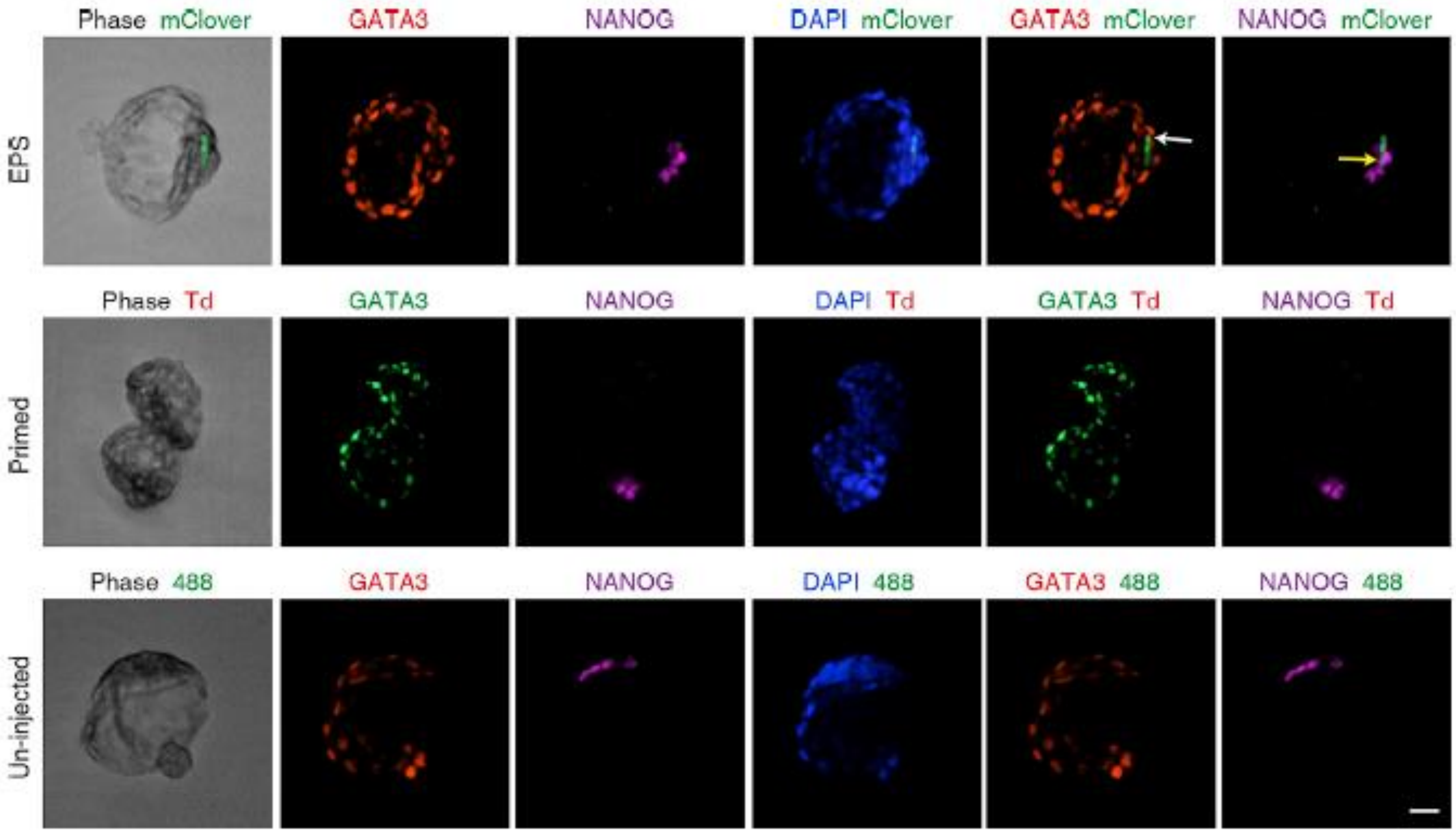
# Producing human organs in non human species: Organ complementation by interspecies chimearas




Wu et al. 2016

# Producing human organs in non human species: Optimisation of interspecies chimearas

## Human EPSCs in mouse embryo

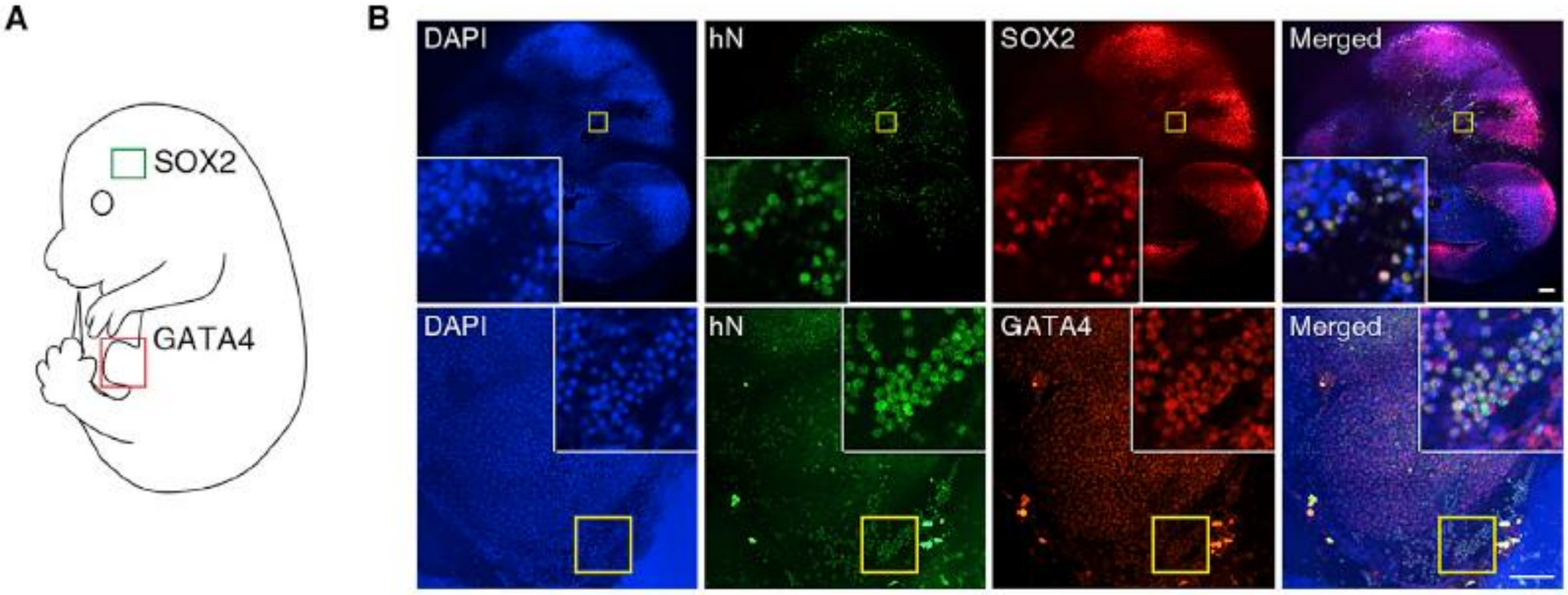



Yang et al. 2017 Cell

# Producing human organs in non human species: Optimisation of interspecies chimearas

Human EPSCs in mouse embryo

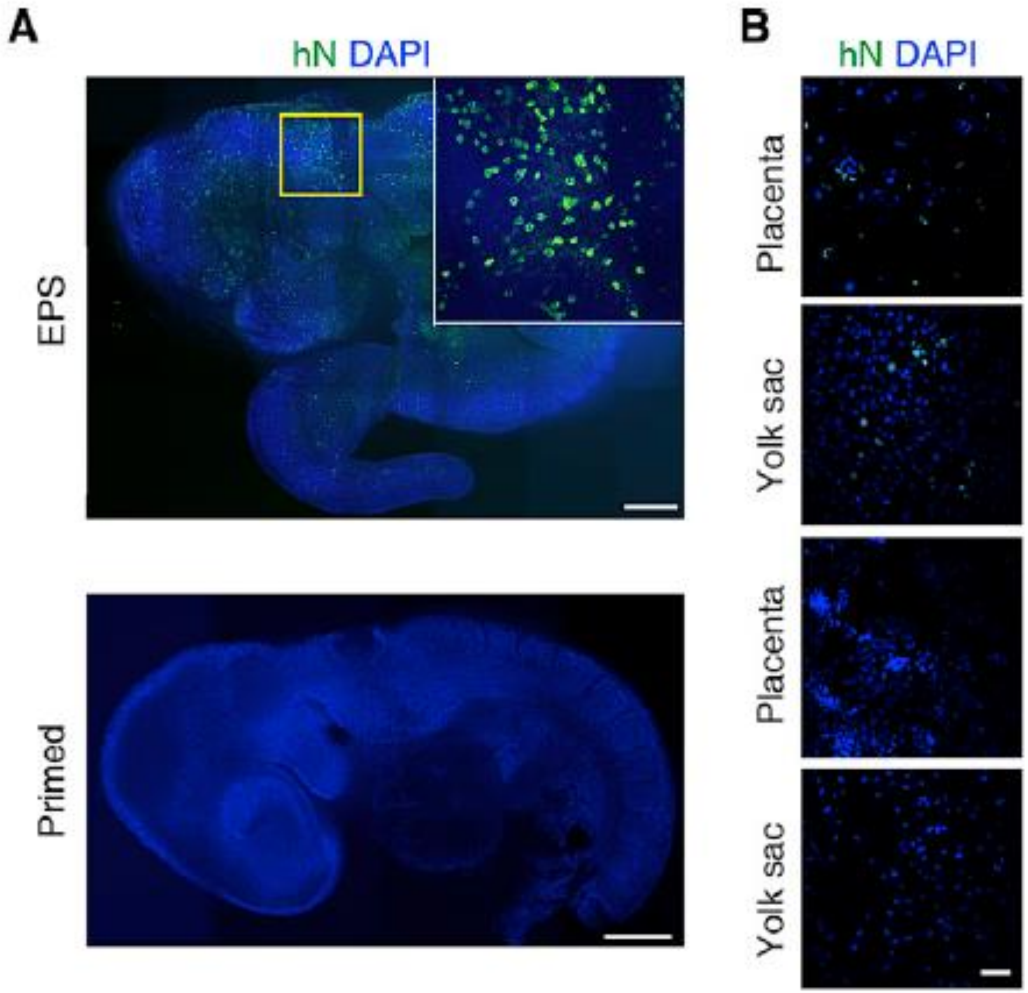
Embryonic tissue




Yang et al. 2017 Cell

# Producing human organs in non human species: Optimisation of interspecies chimearas

## Human EPSCs in mouse embryo




Extra- Embryonic tissue

Yang et al. 2017 Cell

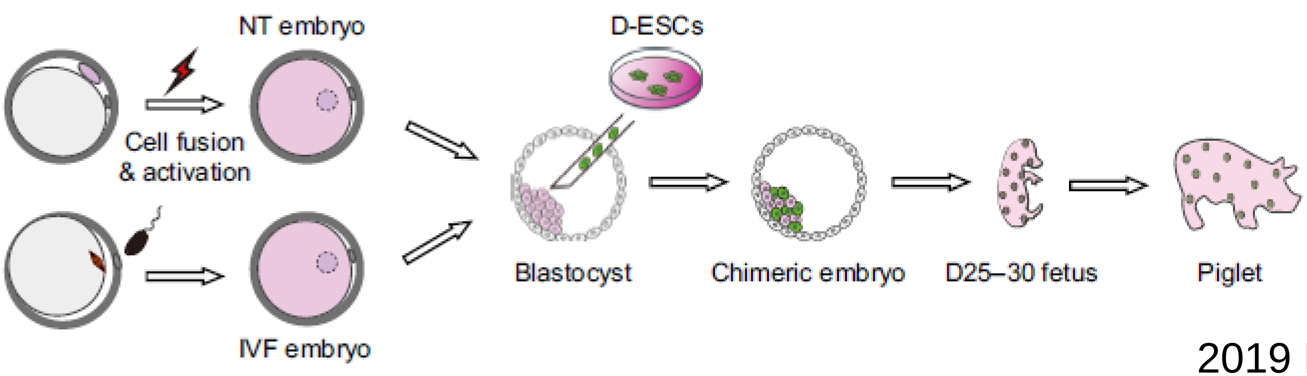
# Producing human organs in non human species: Optimisation of interspecies chimeras


## Monkey cells in pig embryos

RESEARCH ARTICLE

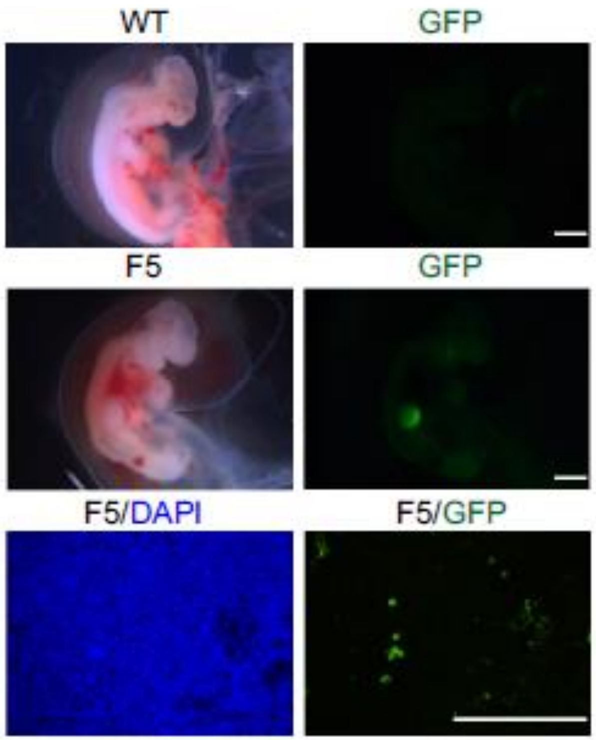
# Domesticated cynomolgus monkey embryonic stem cells allow the generation of neonatal interspecies chimeric pigs

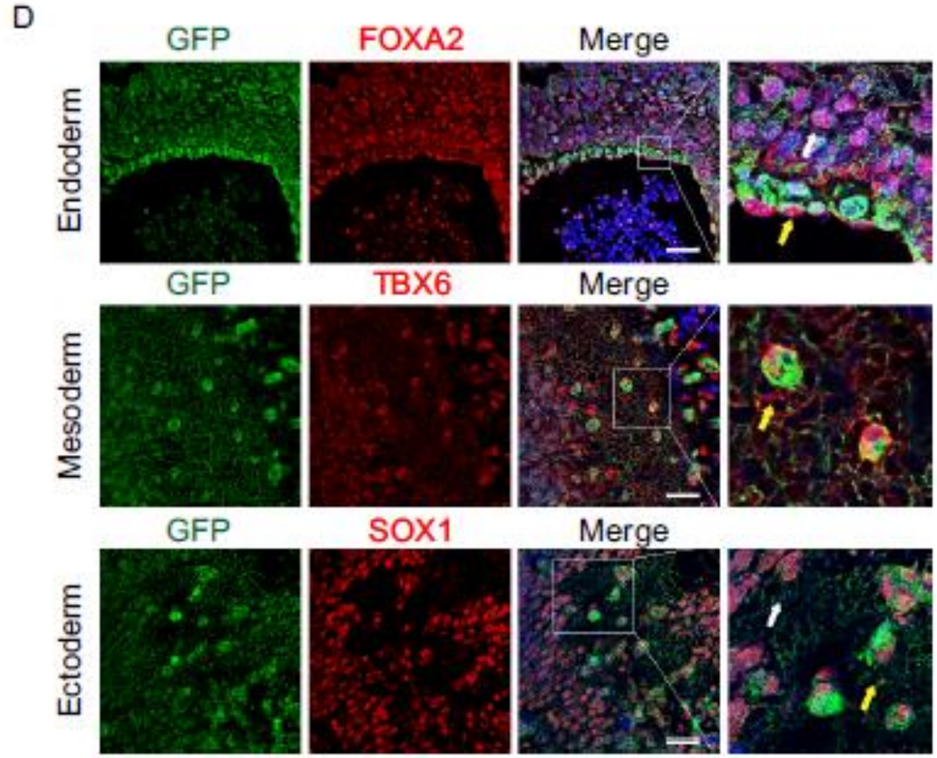
Rui Fu[1,2], Dawei Yu[1,2], Jilong Ren[1,2], Chongyang Li[1,2,3], Jing Wang[1,2,3], Guihai Feng[1,2], Xuepeng Wang[1,2], Haifeng Wan[1,2], Tianda Li[1,2], Libin Wang[1,2], Ying Zhang[1,2,3], Tang Hai[1,2]✉, Wei Li[1,2,3]✉, Qi Zhou[1,2,3]✉

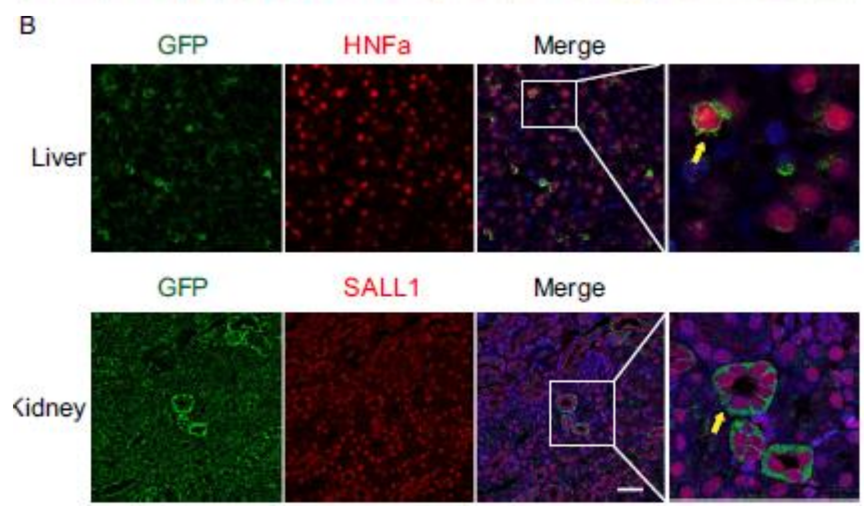



2019 Protein Cell

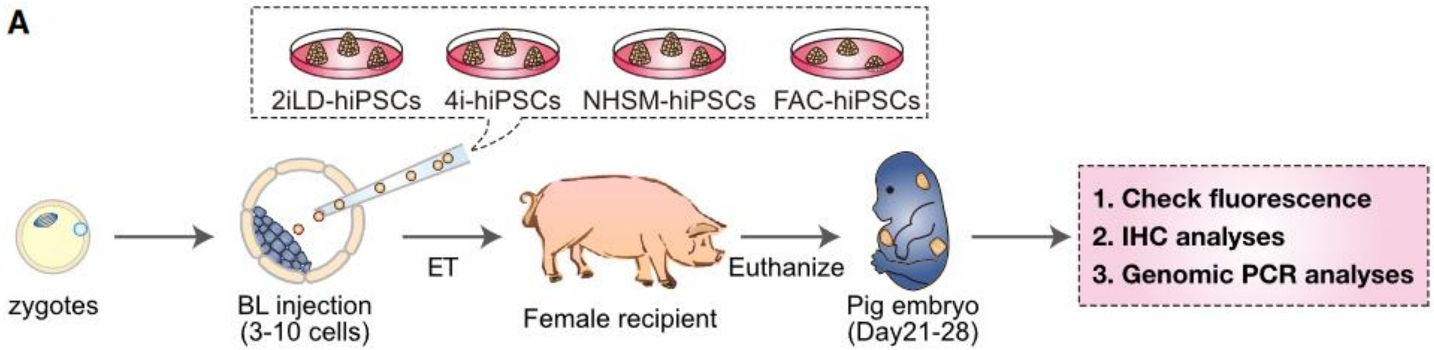
# Monkey cells in pig embryos > chimera

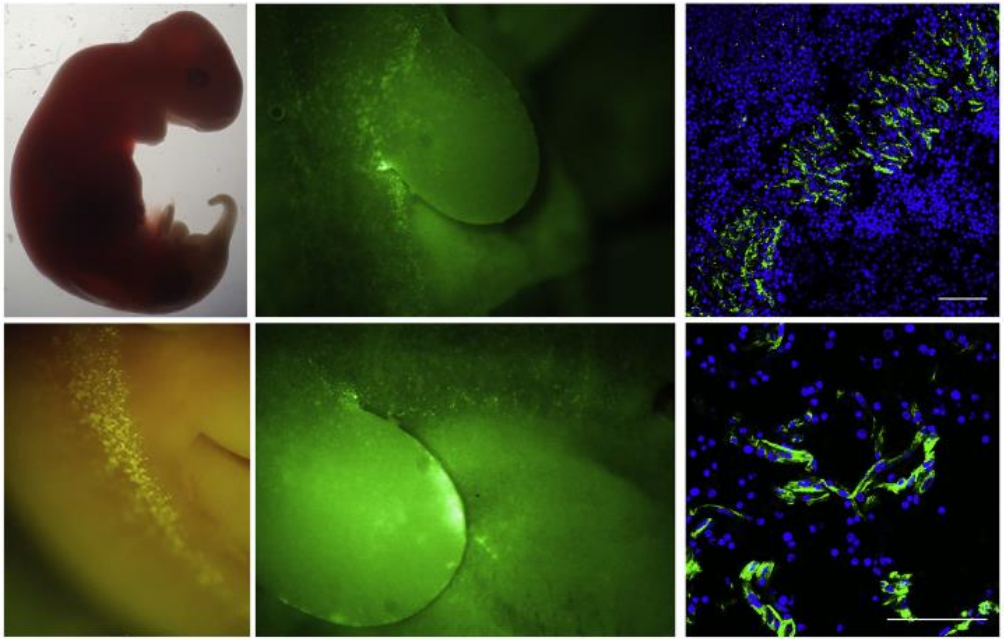


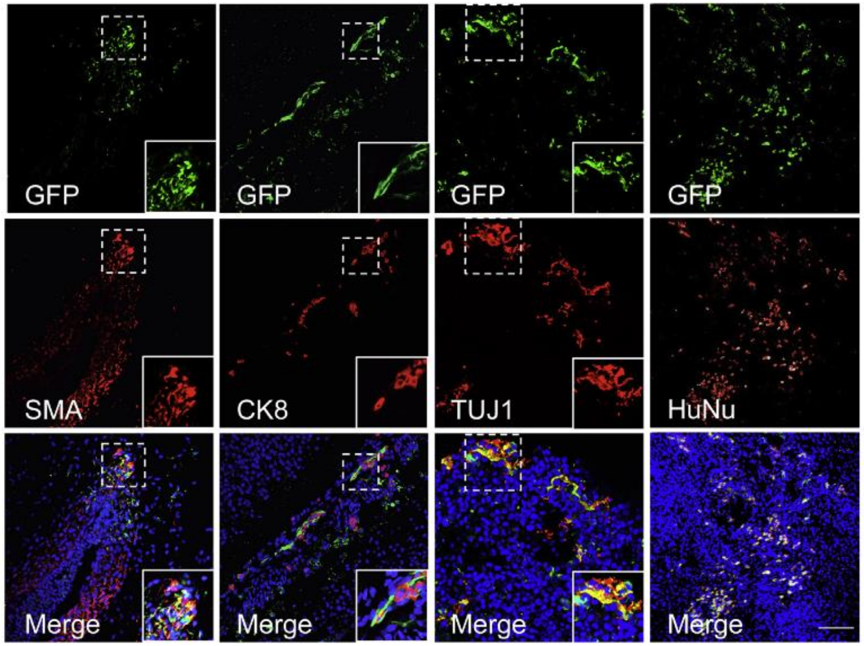



# Producing human organs in non human species: First step: human cells in pig embryos > chimera






Wu et al. 2017 Cell

# Organ complementation by interspecies human/pig chimeras:

## Production of human endothelial cells in pigs

nature biotechnology

LETTERS

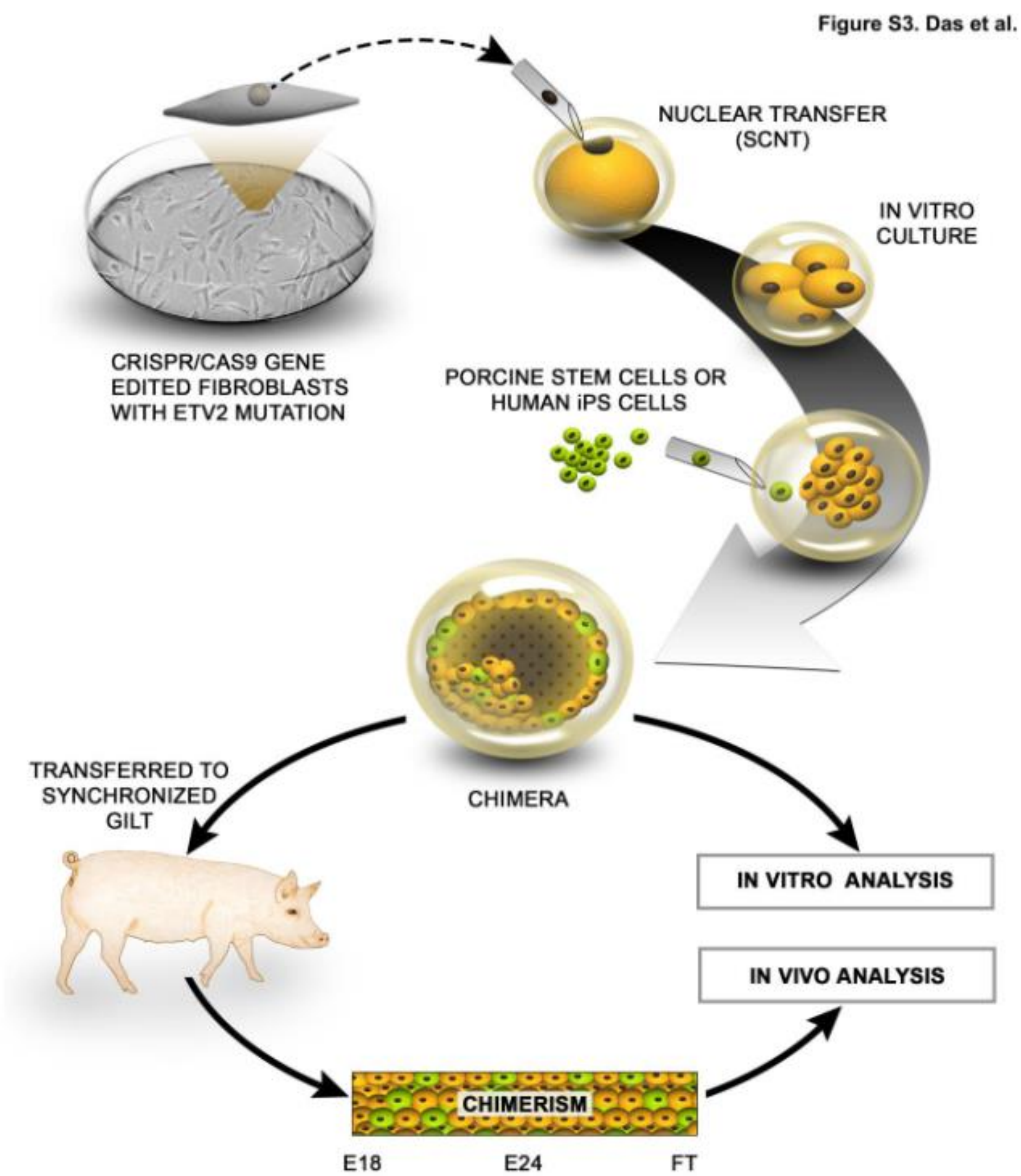
https://doi.org/10.1038/s41587-019-0373-y

# Generation of human endothelium in pig embryos deficient in *ETV2*

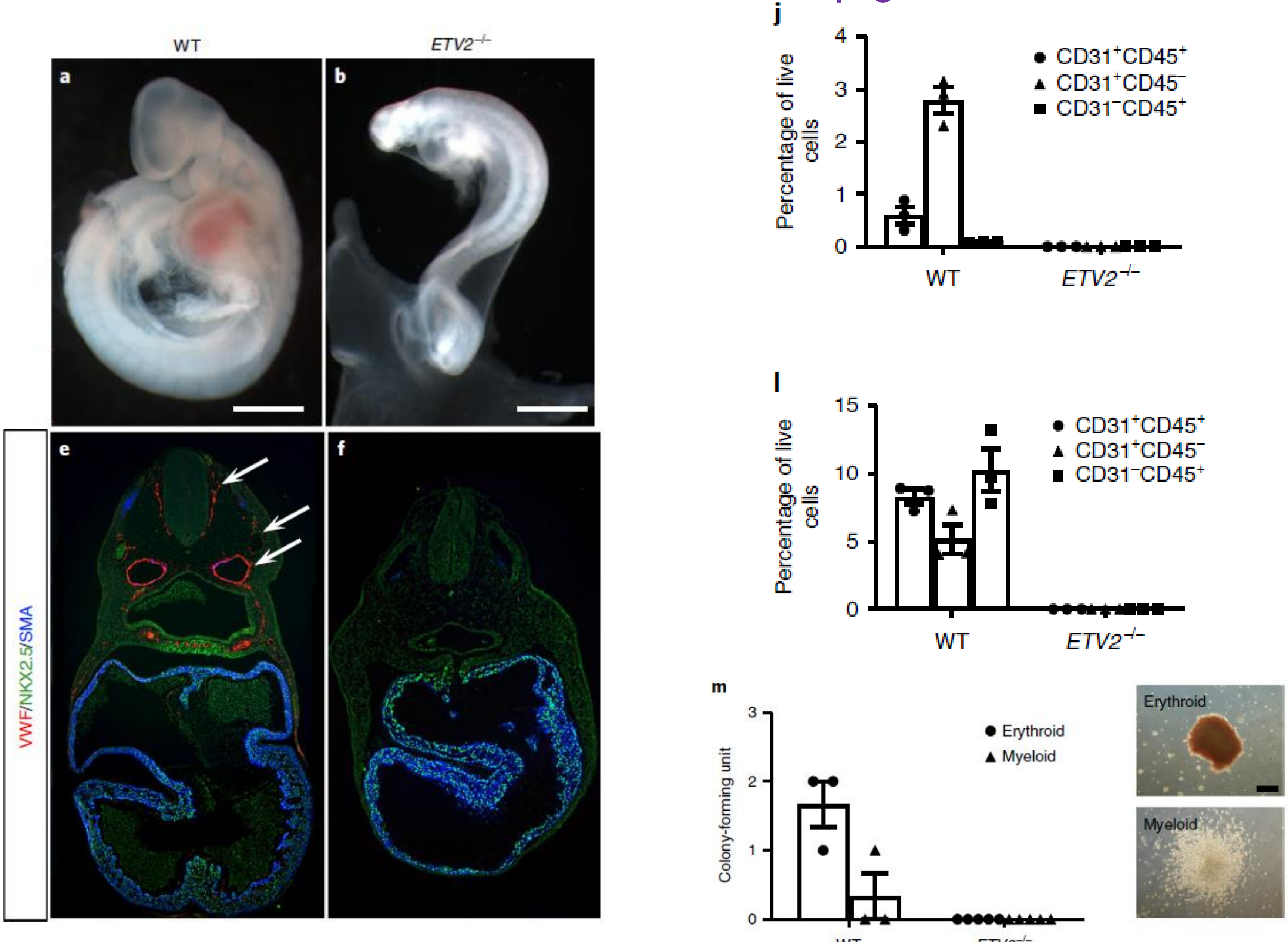
Satyabrata Das[1,6], Naoko Koyano-Nakagawa[1,2,6], Ohad Gafni[1], Geunho Maeng[1], Bhairab N. Singh[1], Tara Rasmussen[1], Xiaoyan Pan[1], Kyung-Dal Choi[1], Daniel Mickelson[1], Wuming Gong[1], Pruthvi Pota[1], Cyprian V. Weaver[1], Stefan Kren[1], Jacob H. Hanna[3], Demetris Yannopoulos[1,4], Mary G. Garry[1,4,5,7*] and Daniel J. Garry[1,2,4,5,7*]

**2020**

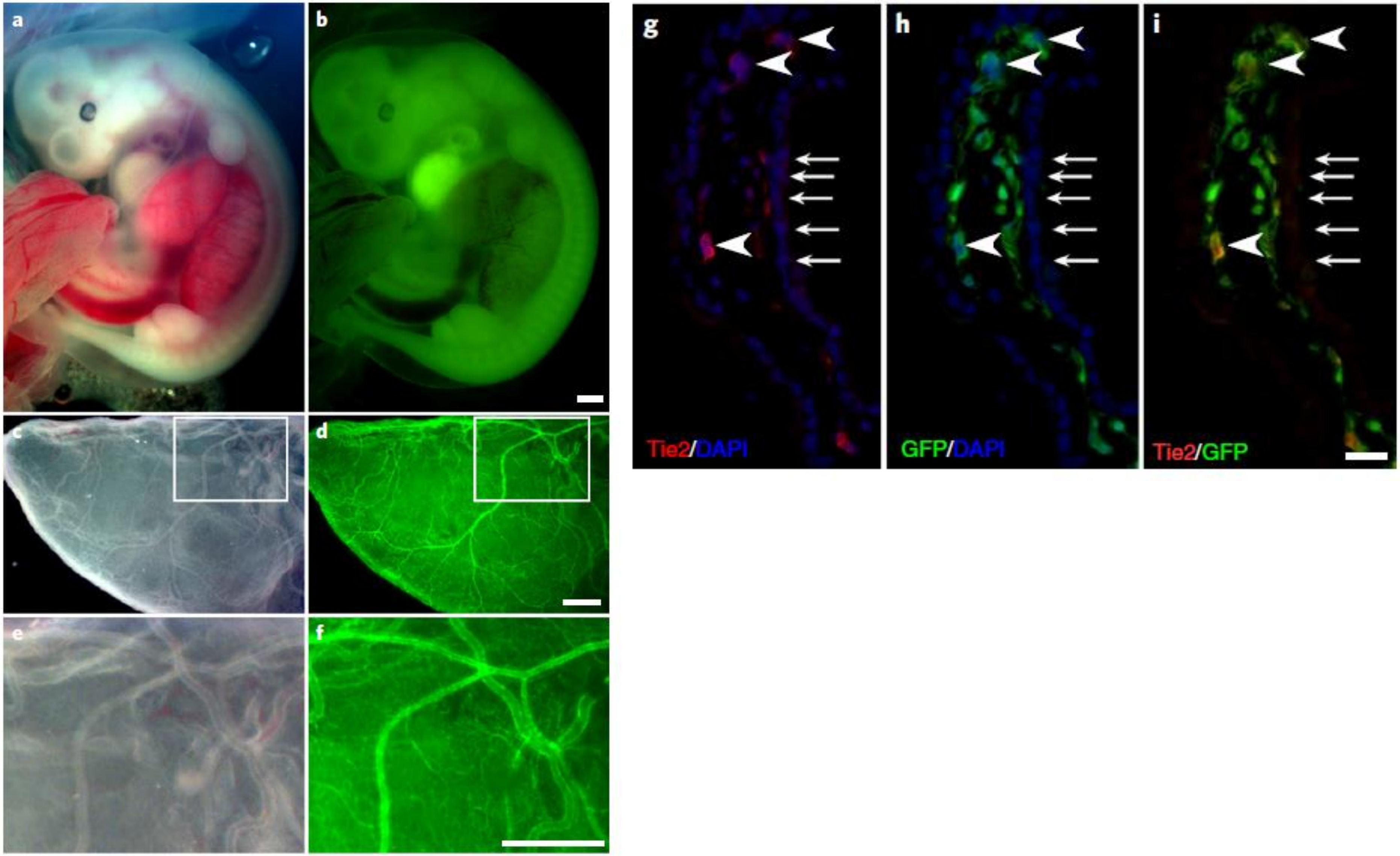
# Organ complementation by interspecies pig/human chimearas



# Characterization of ETV2-/- pig fetuses

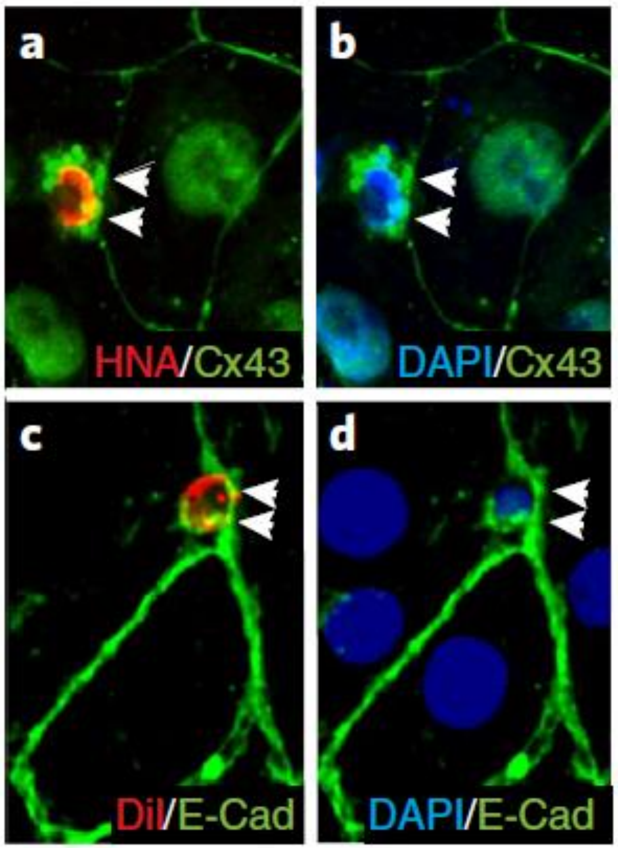


# Complementation of ETV2-/- pig fetuses by pig embryonic cells Intra-species chimera

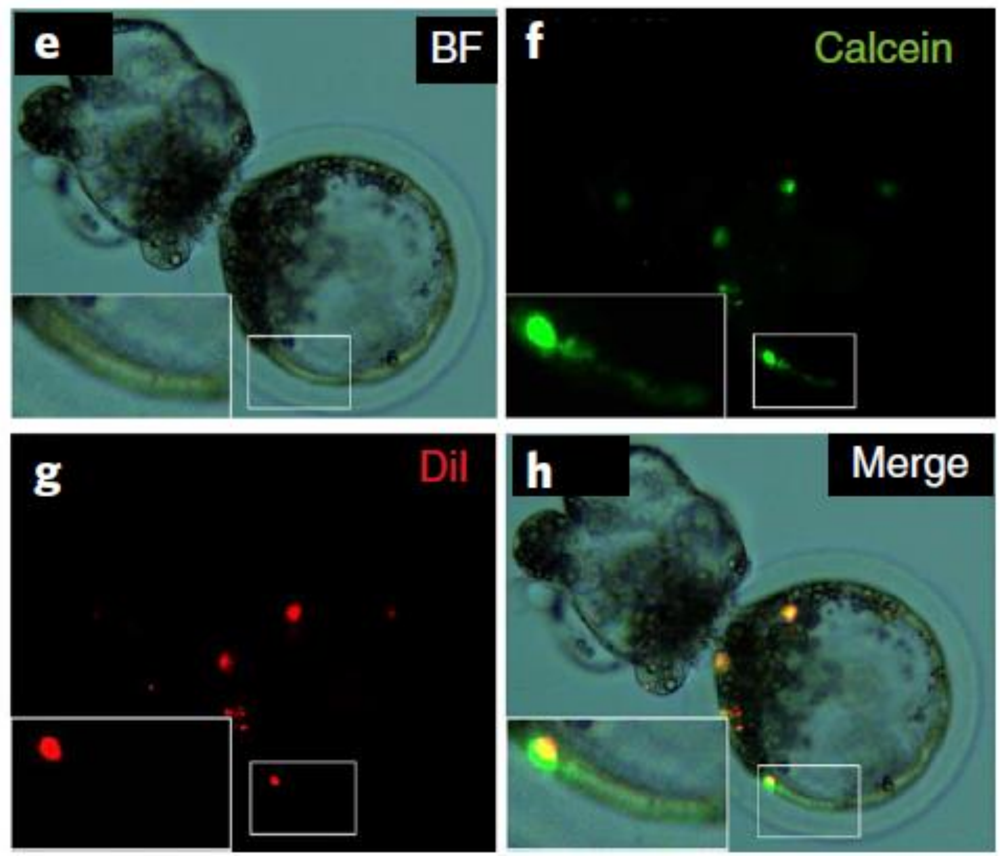


# Complementation of ETV2-/- pig fetuses with human iPSCs
# Inter-species chimera

## Blastocyst colonization




n=400

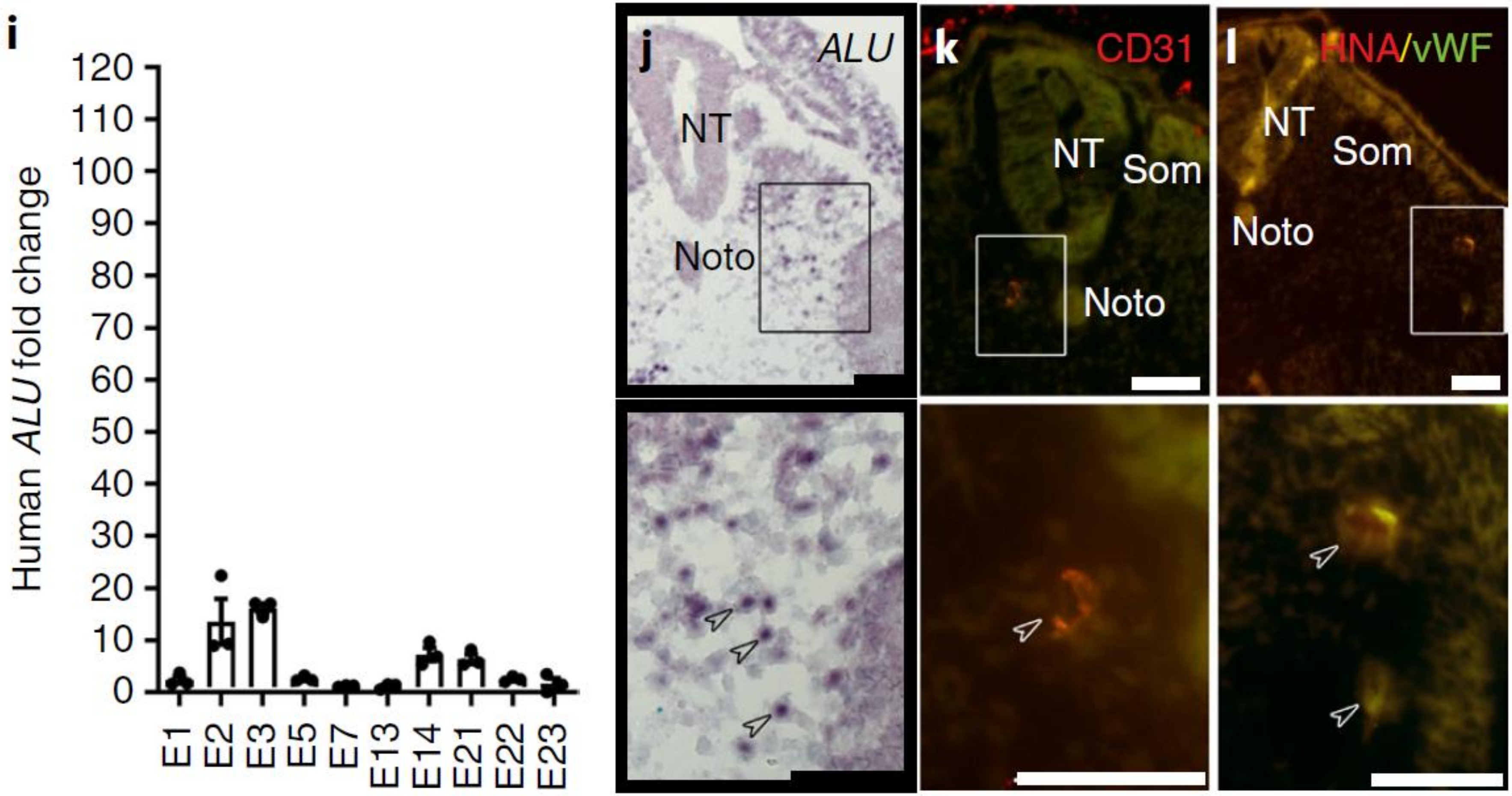



n=300

The best pictures you can get from 400 injected embryos

# Complementation of ETV2-/- pig fetuses with human iPSCs
# Inter-species chimera

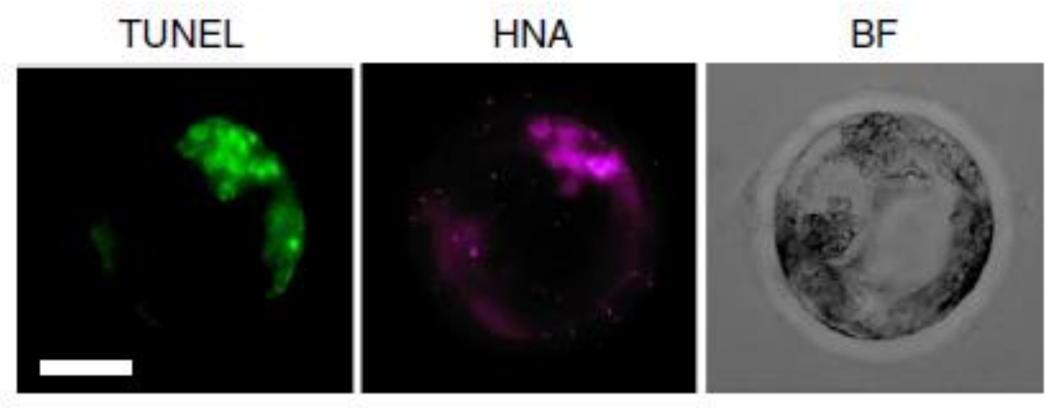
## Fetus colonization




Top 10 over 1700 embryos

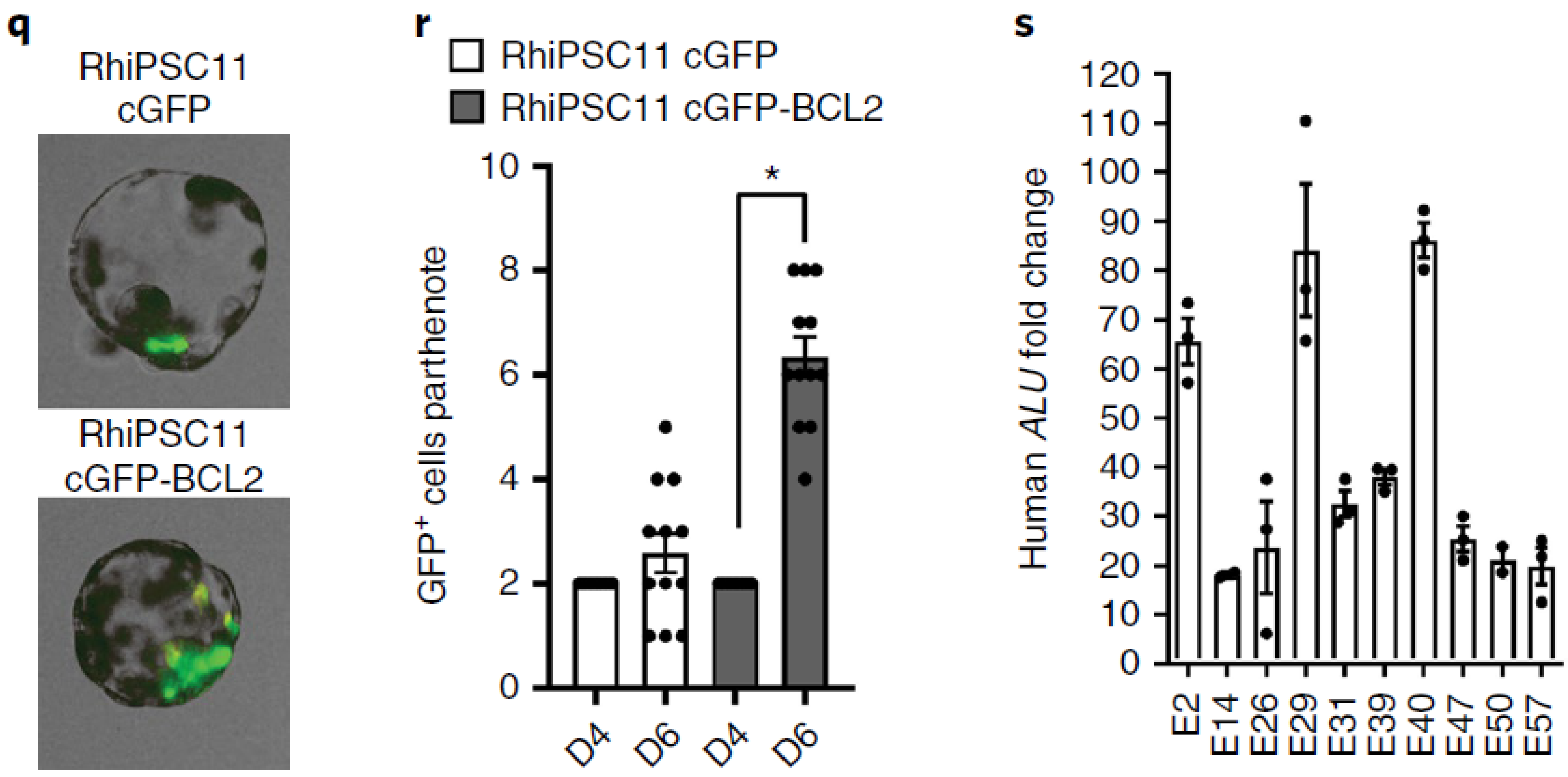
# Complementation of ETV2-/- pig fetuses with human iPSCs
# Inter-species chimera

Low chimera efficiency due to high apoptosis levels



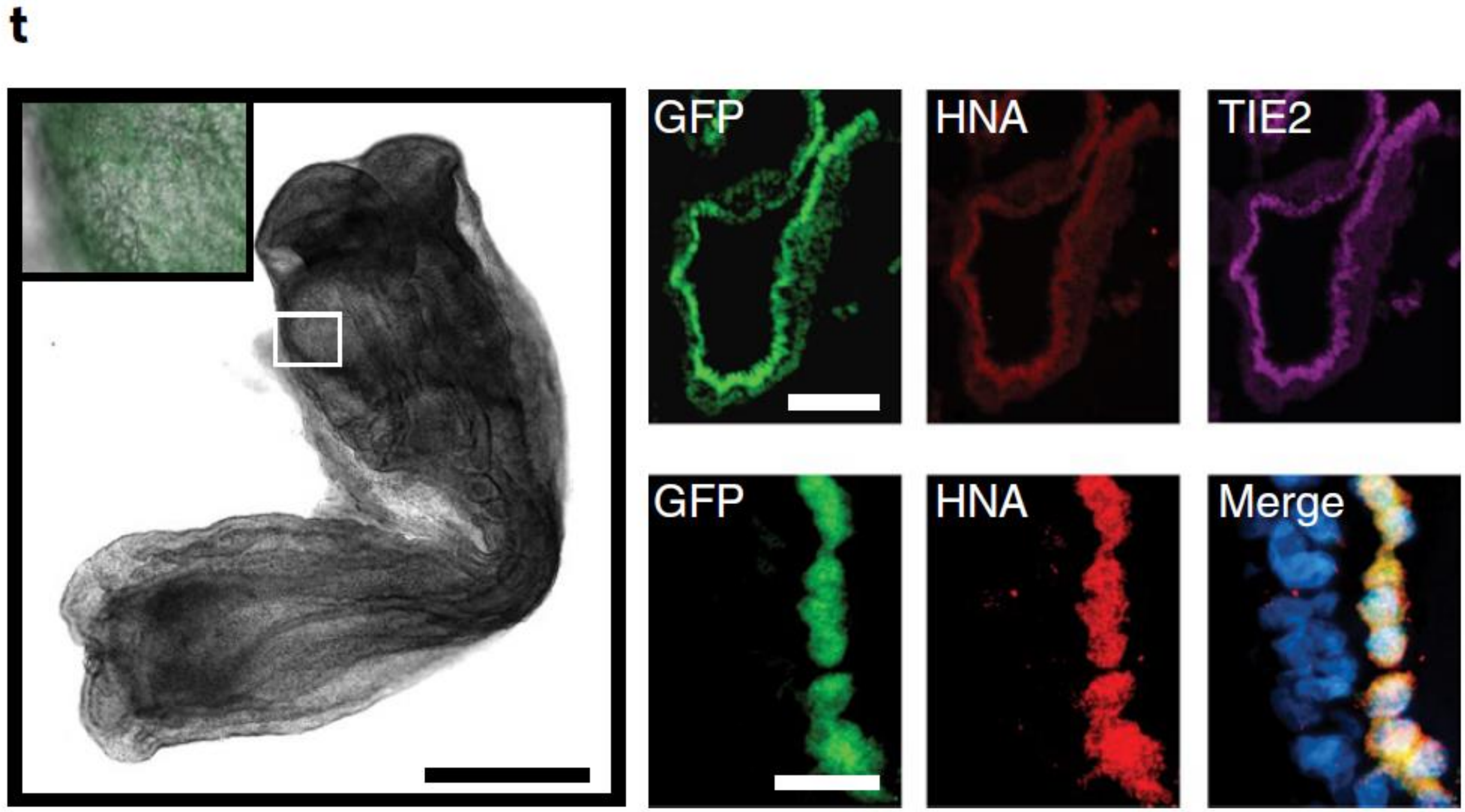
# Complementation of ETV2-/- pig fetuses with human iPSCs
# Inter-species chimera

OE of BCL2 in human iPSCs to decrease apoptosis levels and increase colonization



# Complementation of ETV2-/- pig fetuses with human iPSCs
# Inter-species chimera

OE of BCL2 in human iPSCs favors complementation of ETV2-/- pig embryos



Complementation of MYF5/MYOD/MYF6 -/- pig fetuses with human iPSCs (TP53-/-)
Inter-species chimera

nature biomedical engineering

ARTICLES
https://doi.org/10.1038/s41551-021-00693-1

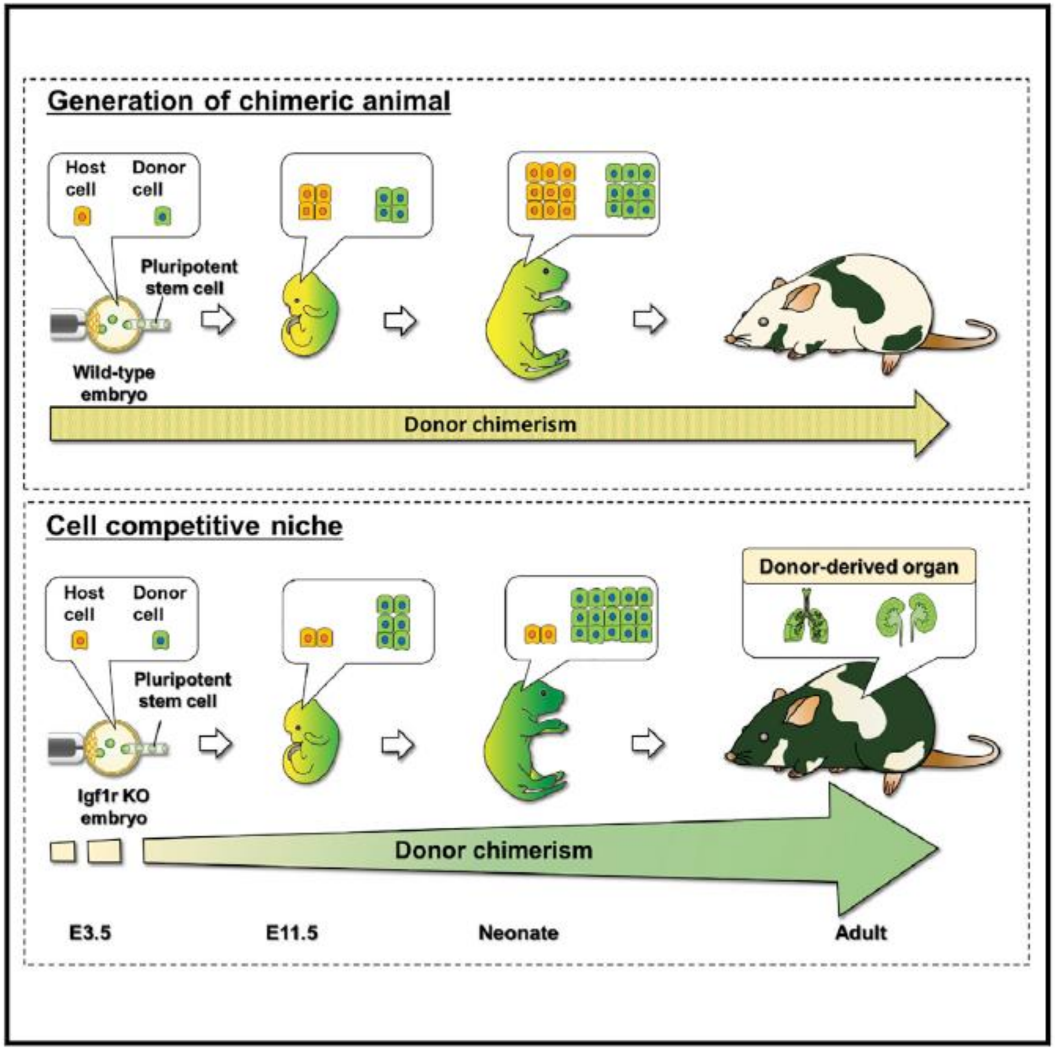
Check for updates

# Humanized skeletal muscle in *MYF5/MYOD/MYF6*-null pig embryos

Geunho Maeng[1,6], Satyabrata Das[1,6], Sarah M. Greising[2], Wuming Gong[1], Bhairab N. Singh[1], Stefan Kren[1], Daniel Mickelson[1], Erik Skie[1], Ohad Gafni[1], Jacob R. Sorensen[2], Cyprian V. Weaver[1], Daniel J. Garry[1,3,4,5] ✉ and Mary G. Garry[1,4,5] ✉

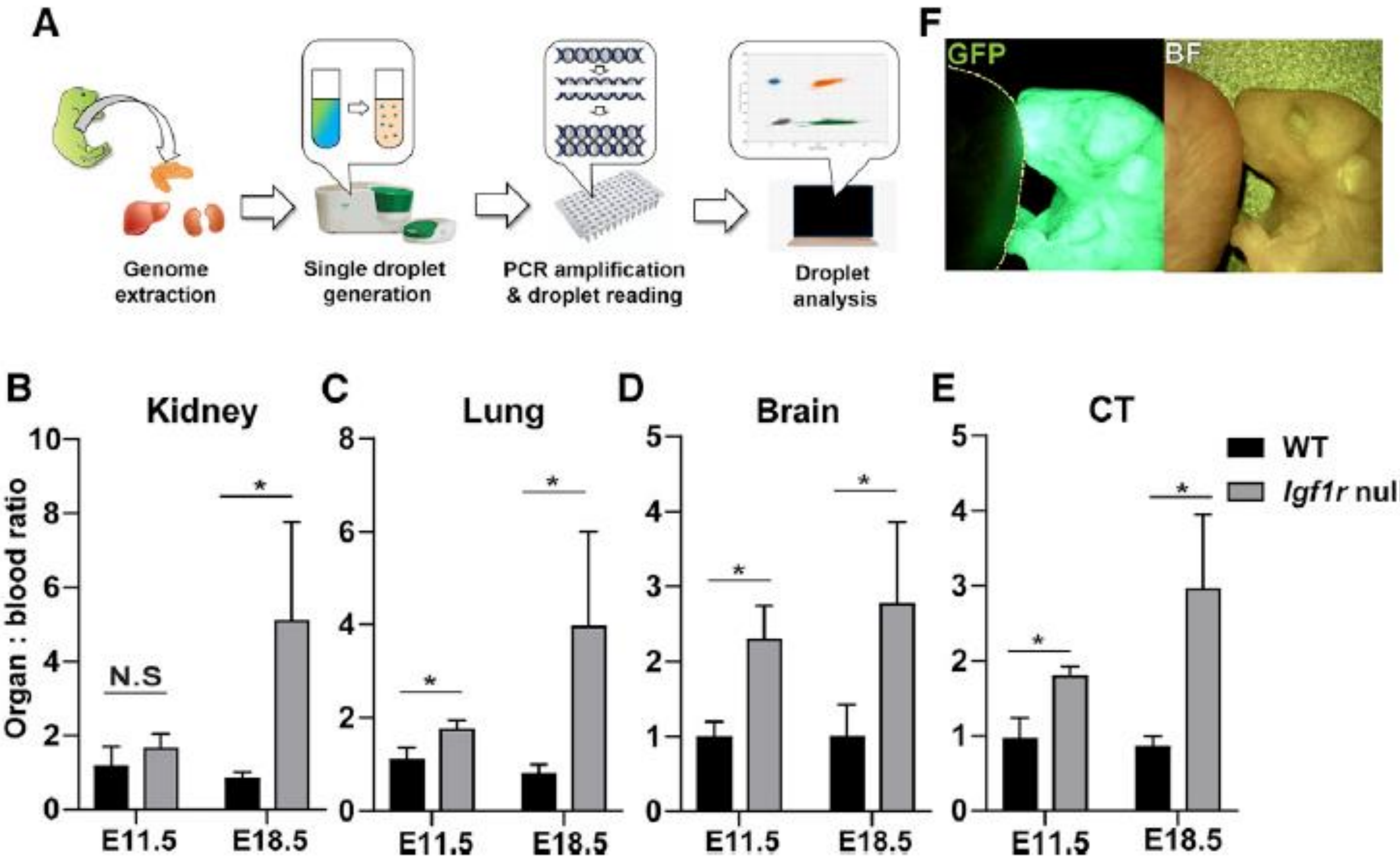
2021

# Balancing cell competition to improve niche receptivity in inter-species chimera

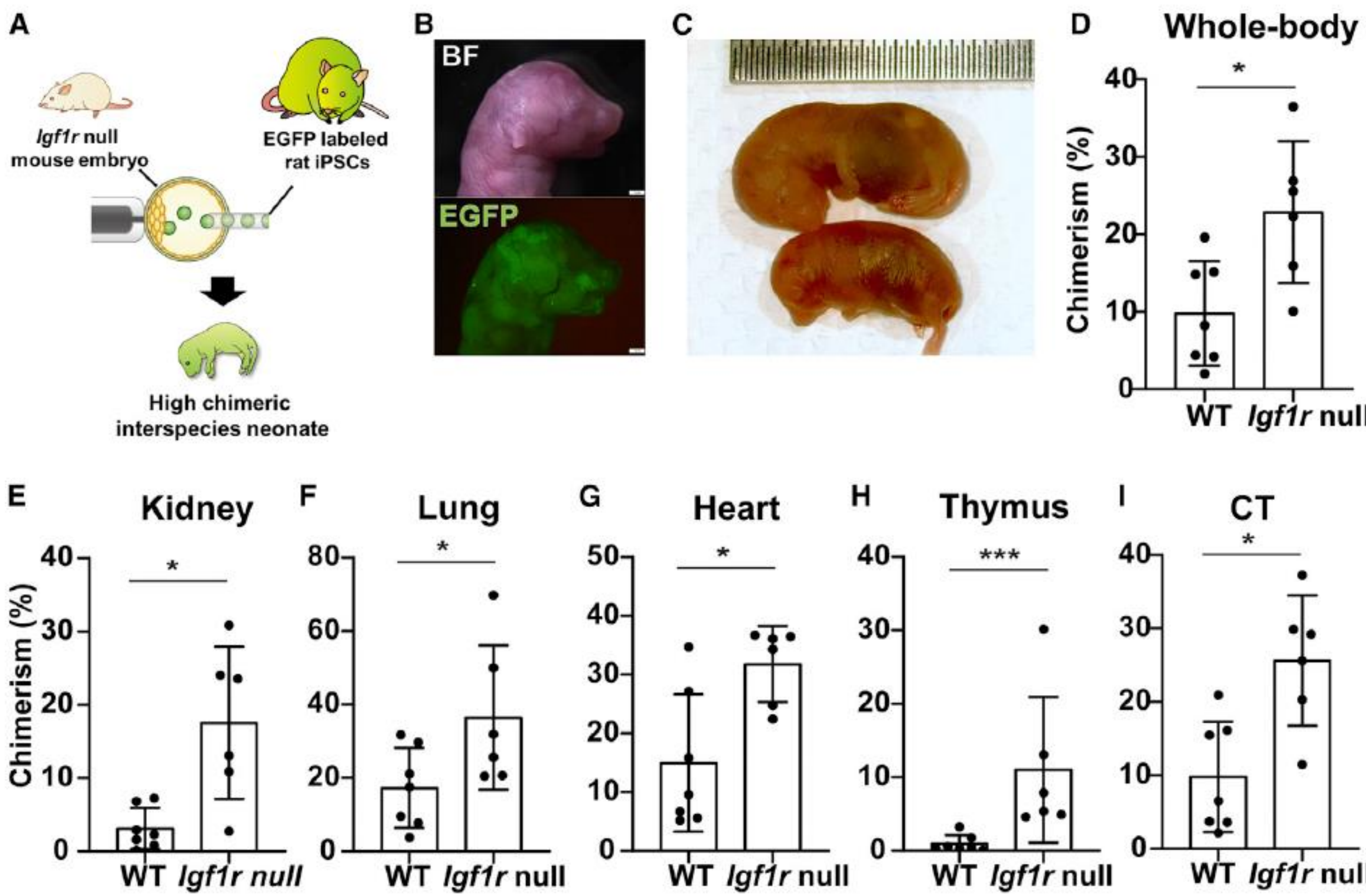



Nishimura et al. Cell Stem Cell 2020

# Balancing cell competition to improve niche receptivity: Mouse in mouse chimera



# Balancing cell competition to improve niche receptivity: Rat in mouse chimera



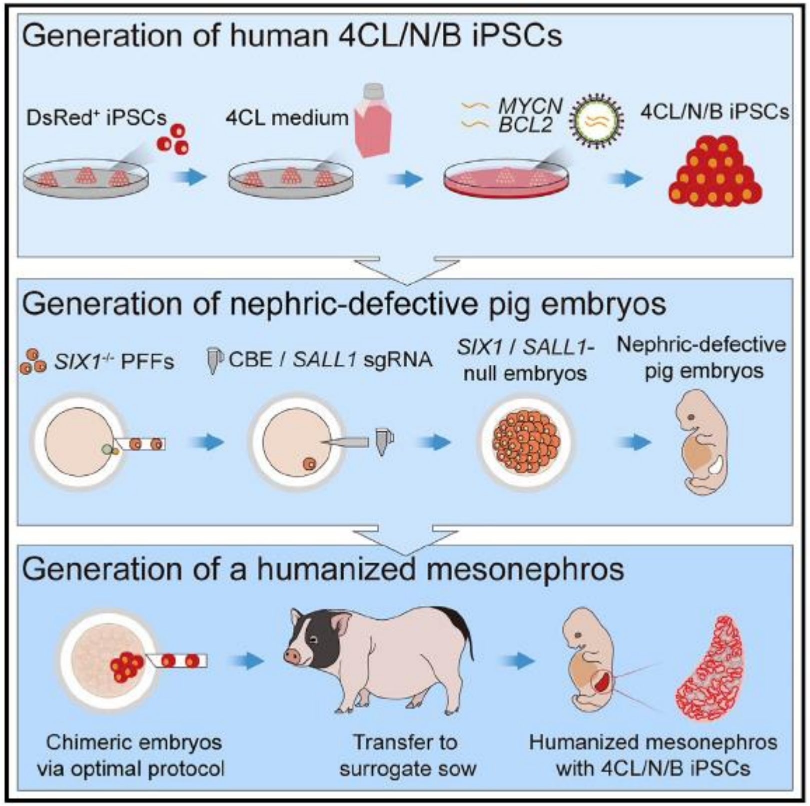
# 2023: Amazing progress toward human organs in pigs

Cell Stem Cell

Short article

## Generation of a humanized mesonephros in pigs from induced pluripotent stem cells via embryo complementation

### Graphical abstract




### Authors

Jiaowei Wang, Wenguang Xie, Nan Li, ..., Miguel A. Esteban, Zhen Dai, Liangxue Lai

### Correspondence

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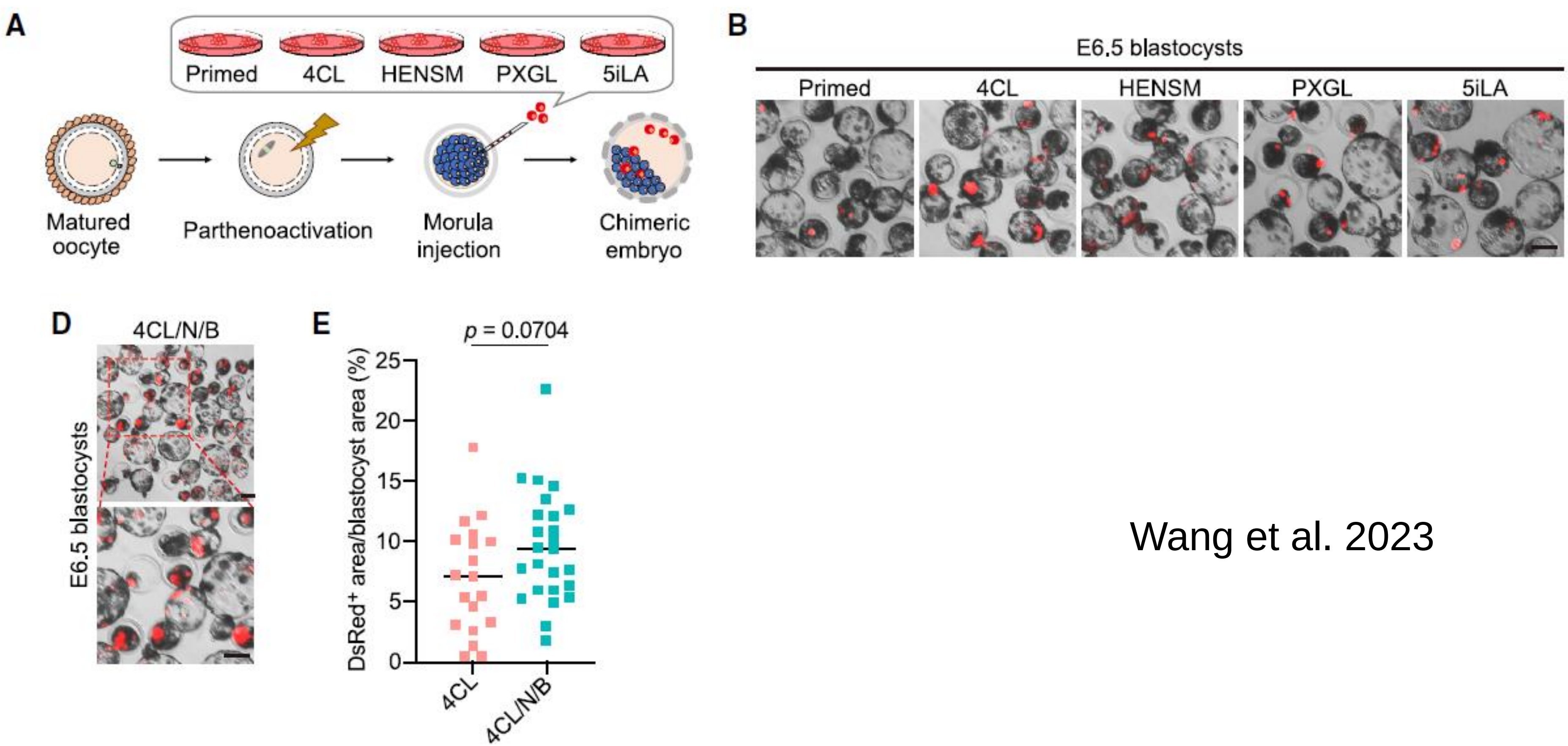
miguel@gibh.ac.cn (M.A.E.),
dai_zhen@gibh.ac.cn (Z.D.),
lai_liangxue@gibh.ac.cn (L.L.)

### In brief

Wang and colleagues show that 4CL medium and MYCN/BCL2 overexpression generate human iPSCs with superior interspecies chimeric potential in pig embryos. This led to the successful formation of a humanized mesonephros in nephric-defective pig embryos via early embryo complementation, paving the way for growing a human kidney in pigs.

# 2023: Amazing progress toward human organs in pigs

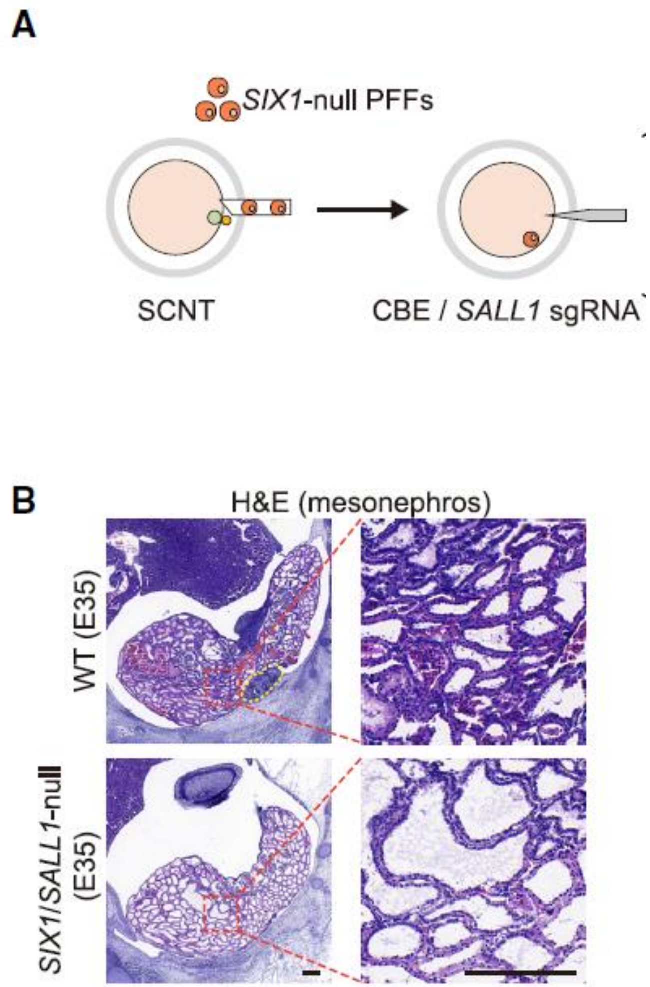
Test chimera contribution of hPSCs with different naive human PSC media + OE MYCN/BCL2




Wang et al. 2023

# 2023: Amazing progress toward human organs in pigs

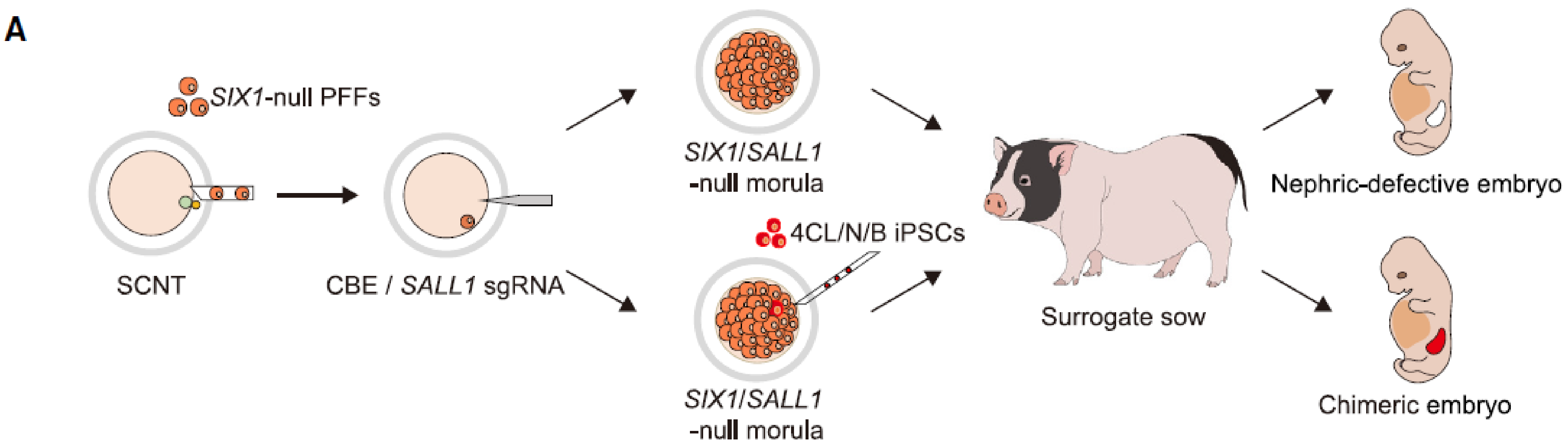
## Pigs mutated for SIX1 and SALL1: no kidney




Wang et al. 2023

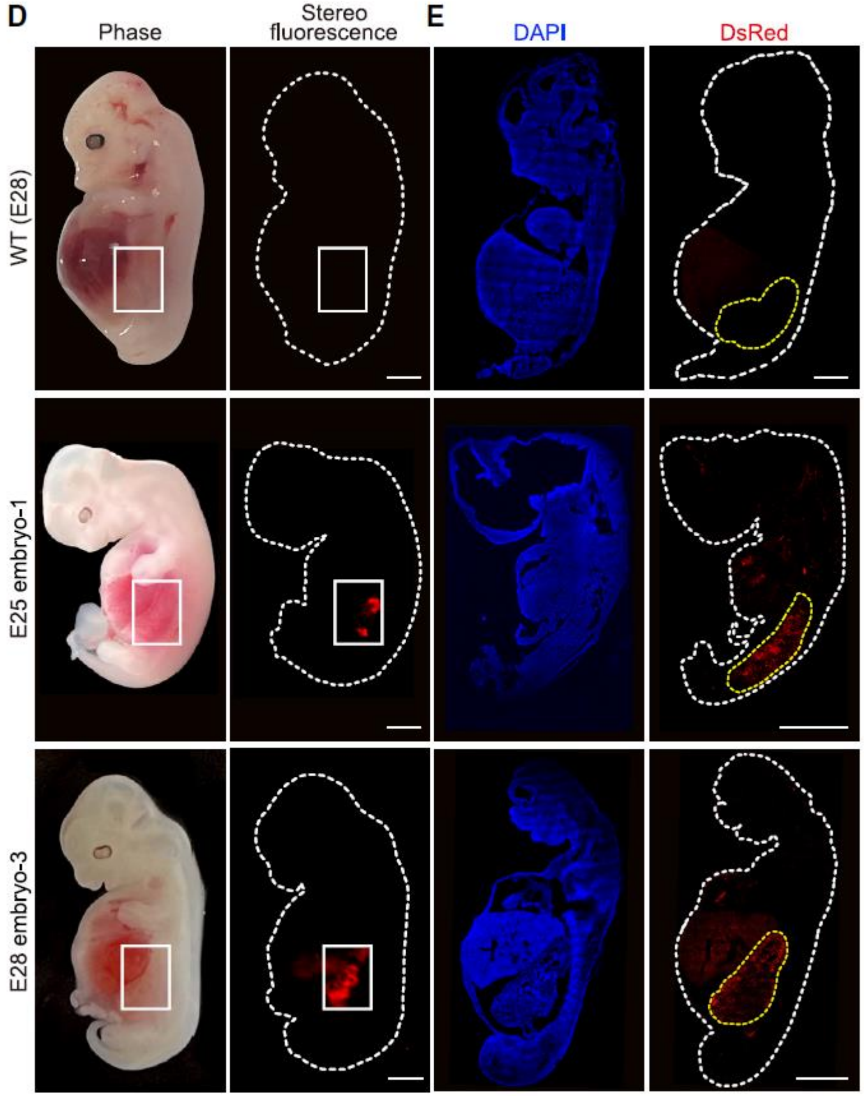
# 2023: Amazing progress toward human organs in pigs

## Complementation of Pig kidney with human PSCs



# 2023: Amazing progress toward human organs in pigs

## Complementation of Pig kidney with human PSCs



# 2023: Amazing progress toward human organs in pigs

But still with really low efficiency….

**C**

| Cell line | | Transferred embryos | | Transferred sows | | Pregnant sows | |
|---|---|---|---|---|---|---|---|
| 4CL/N/B | | 1820 | | 13 | | 6 | |
| Total embryos | | | | DsRed+ | | | |
| Normal | | Retarded | | Normal | | Retarded | |
| E25 | E28 | E25 | E28 | E25 | E28 | E25 | E28 |
| 2 | 3 | 4 | 0 | 2 | 3 | 1 | 0 |

# Non rodent mammalian pluripotent stem cells

- All non rodent PSCs are close to a primed state of pluripotency
- Until recently, few true PSCs from non primate species
- Intraspecies chimera production remains complicated with this cells:
- Interspecies chimera in non-rodent species are at their beginning > close to Science Fiction but …