Basic research on early human development
-gene editing is one of the tools

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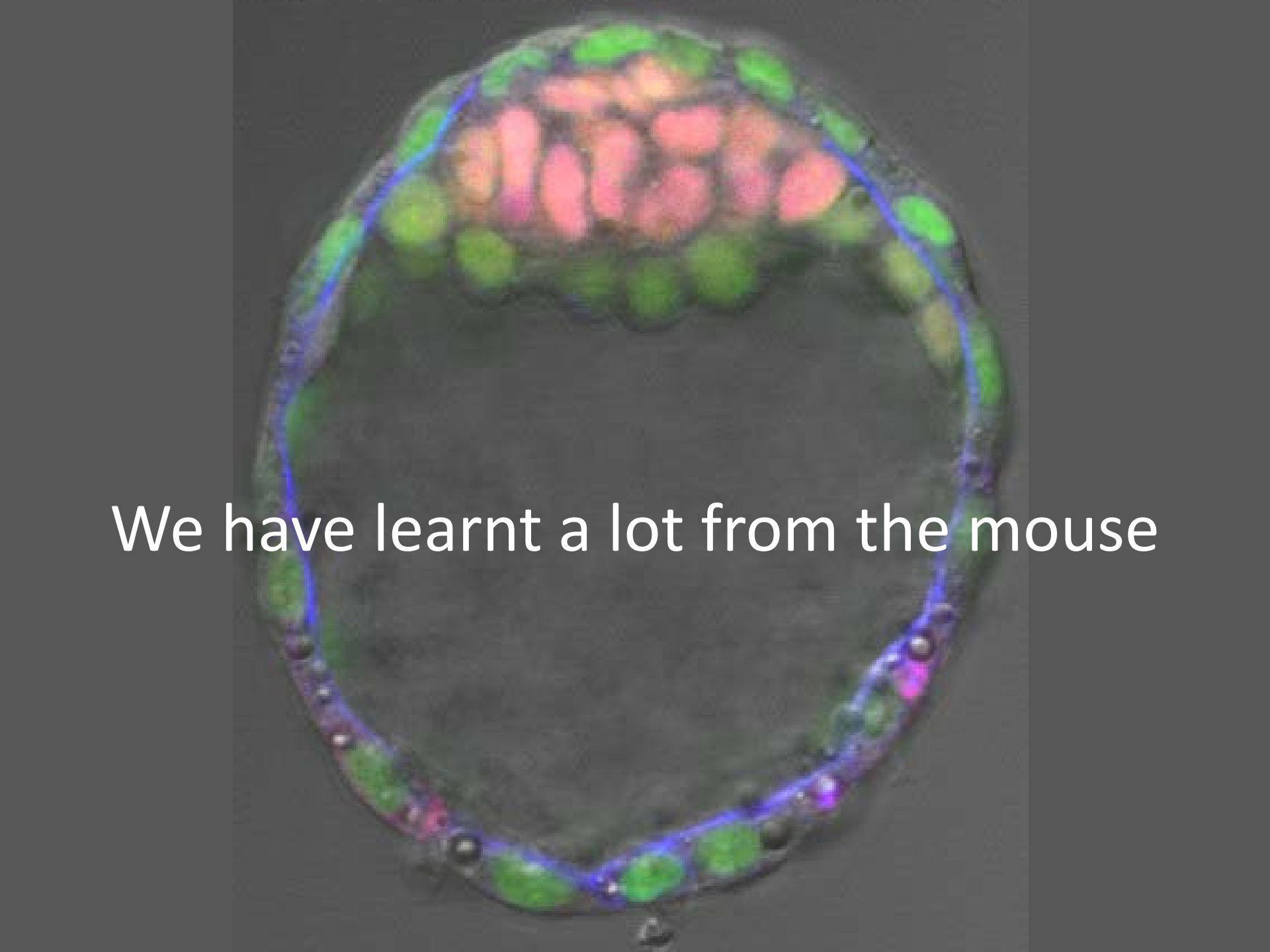
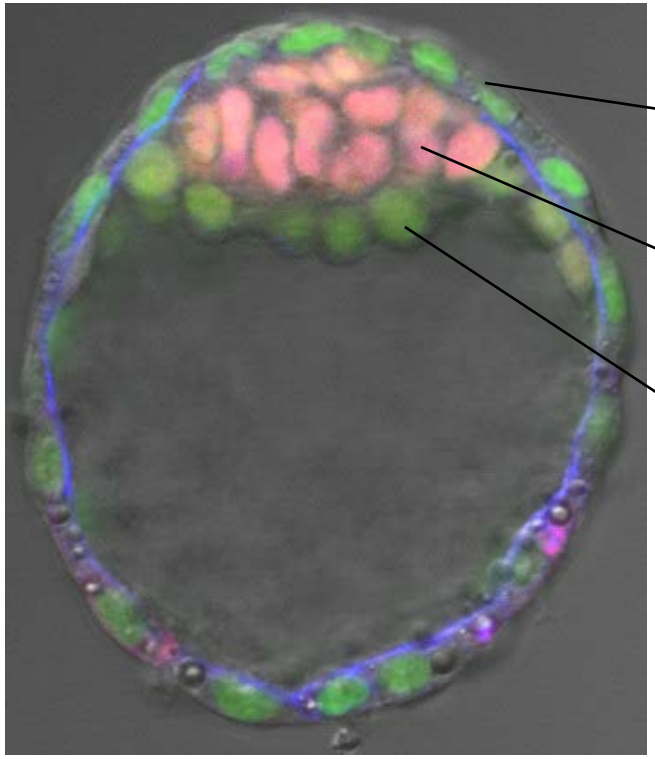
Why understanding the development of the human early embryo is important

- Fundamental knowledge of early beginnings of human development
- Improvements in IVF and other reproductive technologies
- Prevention of early pregnancy losses
- Understanding the origins of pluripotency and the placenta and how to translate into stem cells


We have learnt a lot from the mouse

Lineages from the blastocyst



trophoblast

epiblast

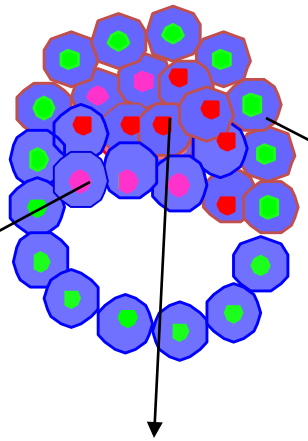
primitive endoderm

placenta

fetus

yolk sac

Three lineage-specific stem cell lines from the mouse blastocyst



trophectoderm

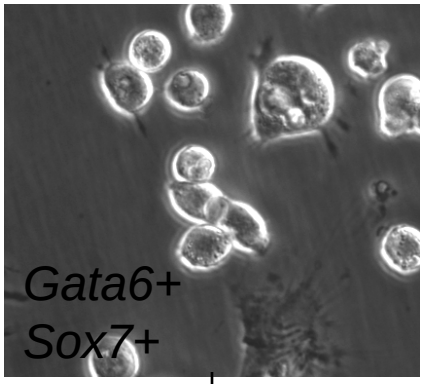
epiblast progenitor

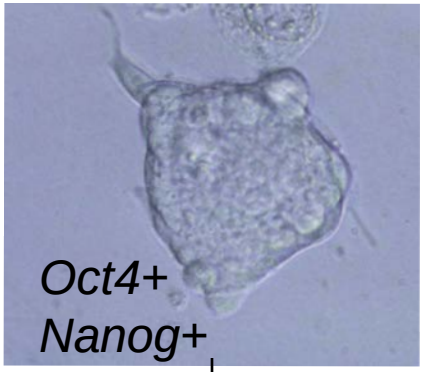
primitive endoderm progenitor

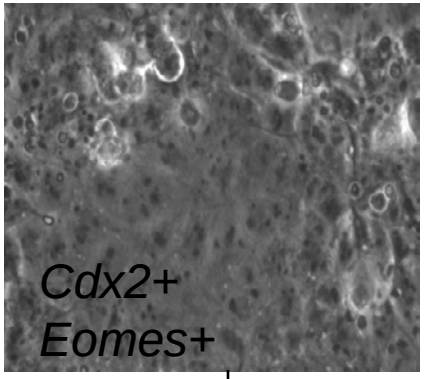
XEN

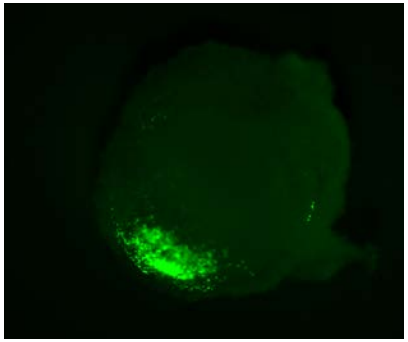
ES

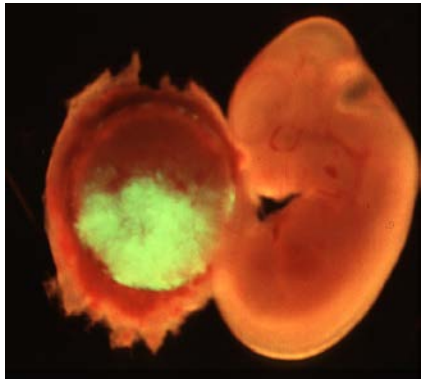
TS



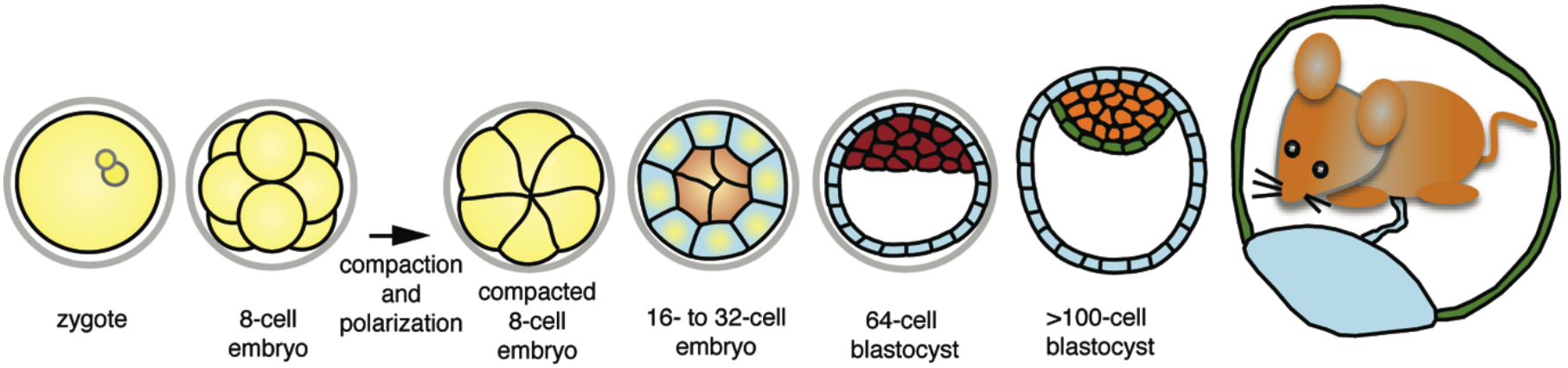






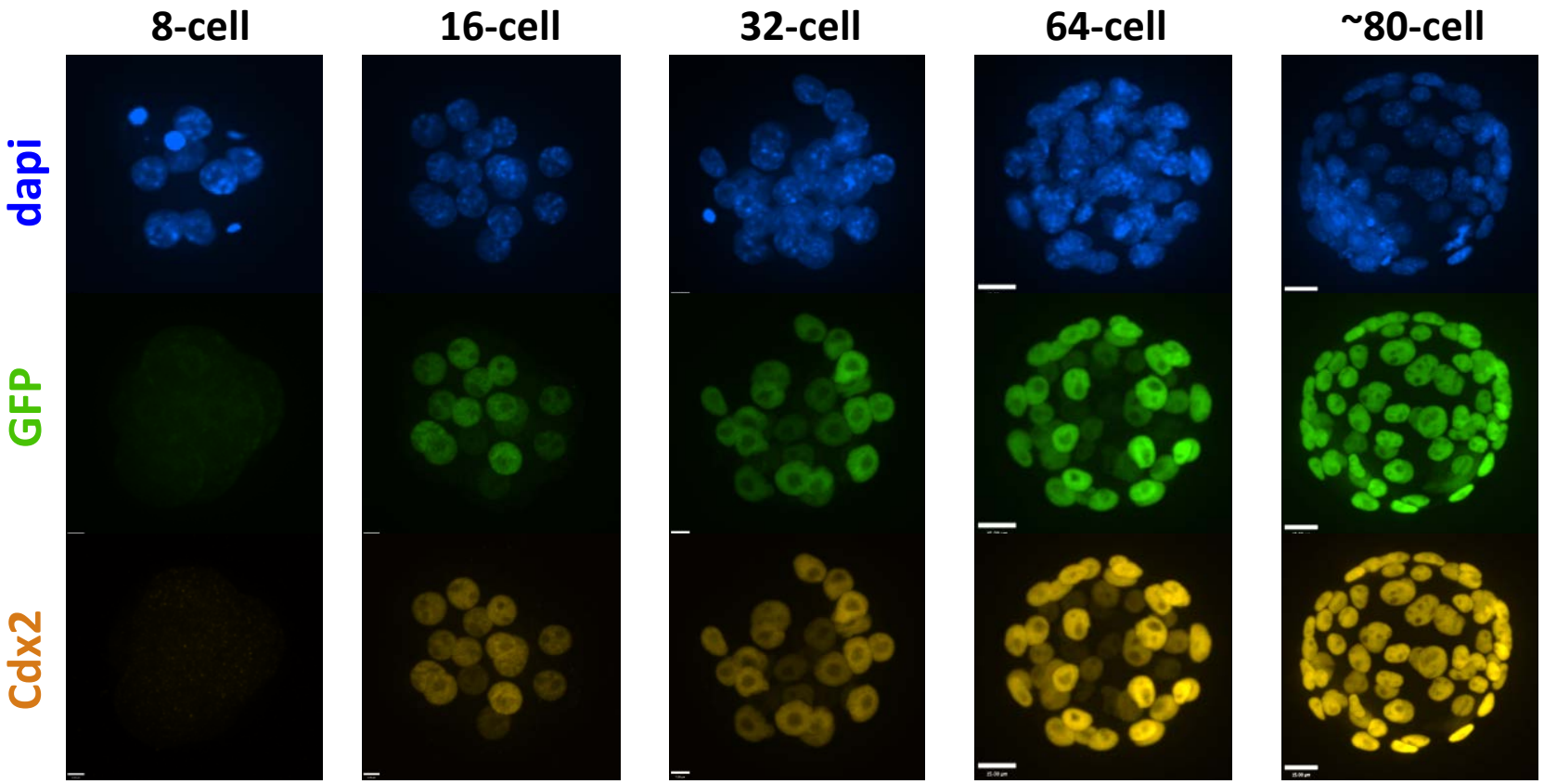


Developmental potential is gradually restricted during pre-implantation development



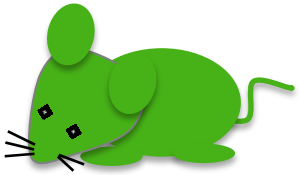
When exactly do blastomeres lose their totipotent potential and what are the molecular mechanisms behind it?

Cdx2-eGFP reporter: an early marker of the developing TE lineage



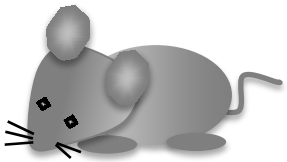
Reporter line from: *McDole and Zheng 2012 Genesis*

Experimental outline for testing the plasticity of blastomeres
Eszter Posfai

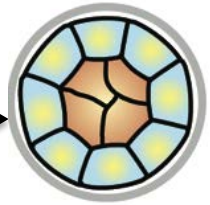


Cdx2-eGFP

x



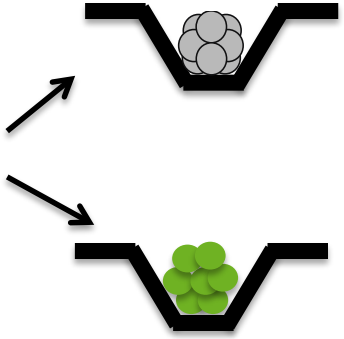
Wild type



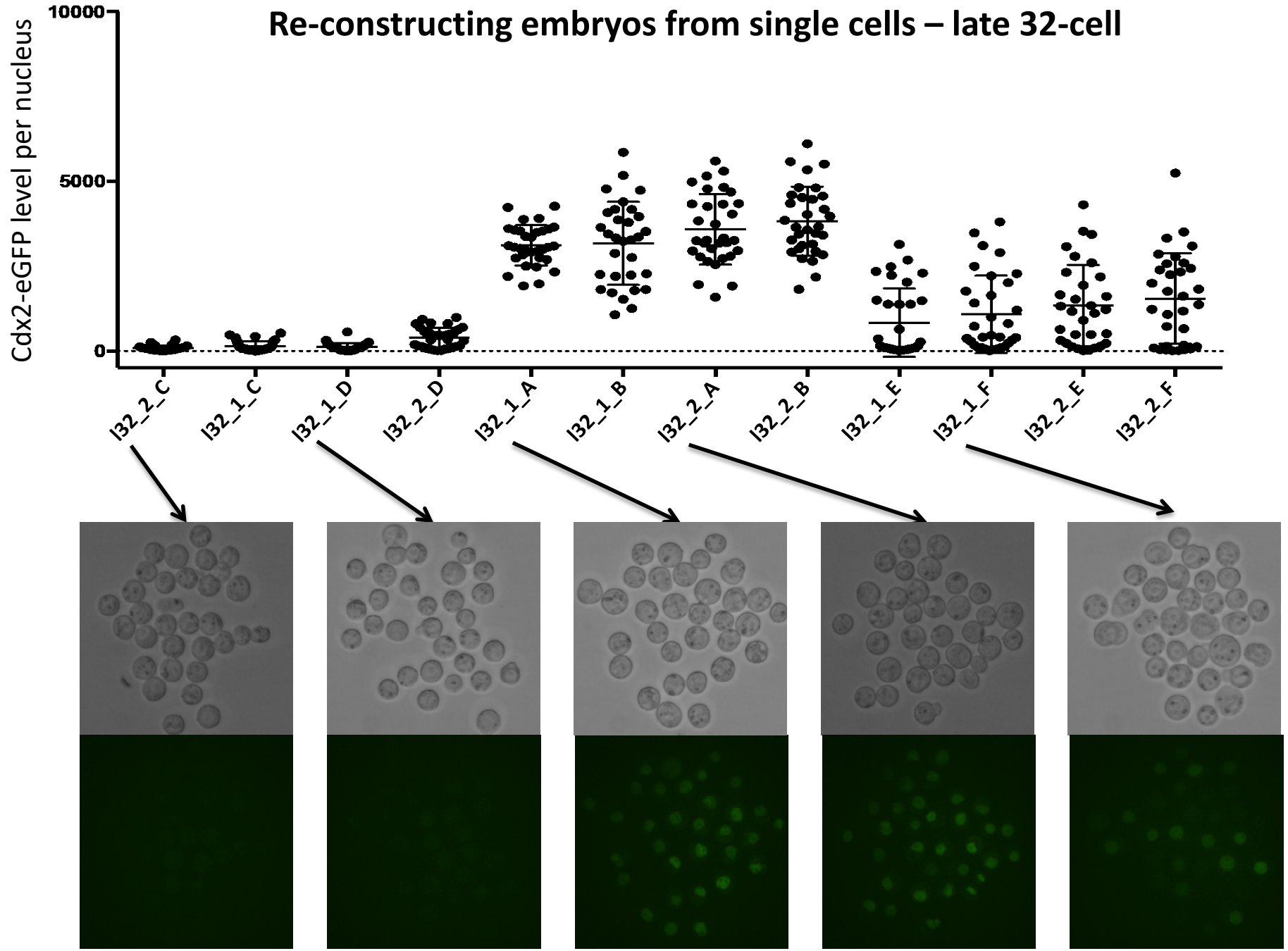
harvest embryos
at different stages

dissociation to
single cells

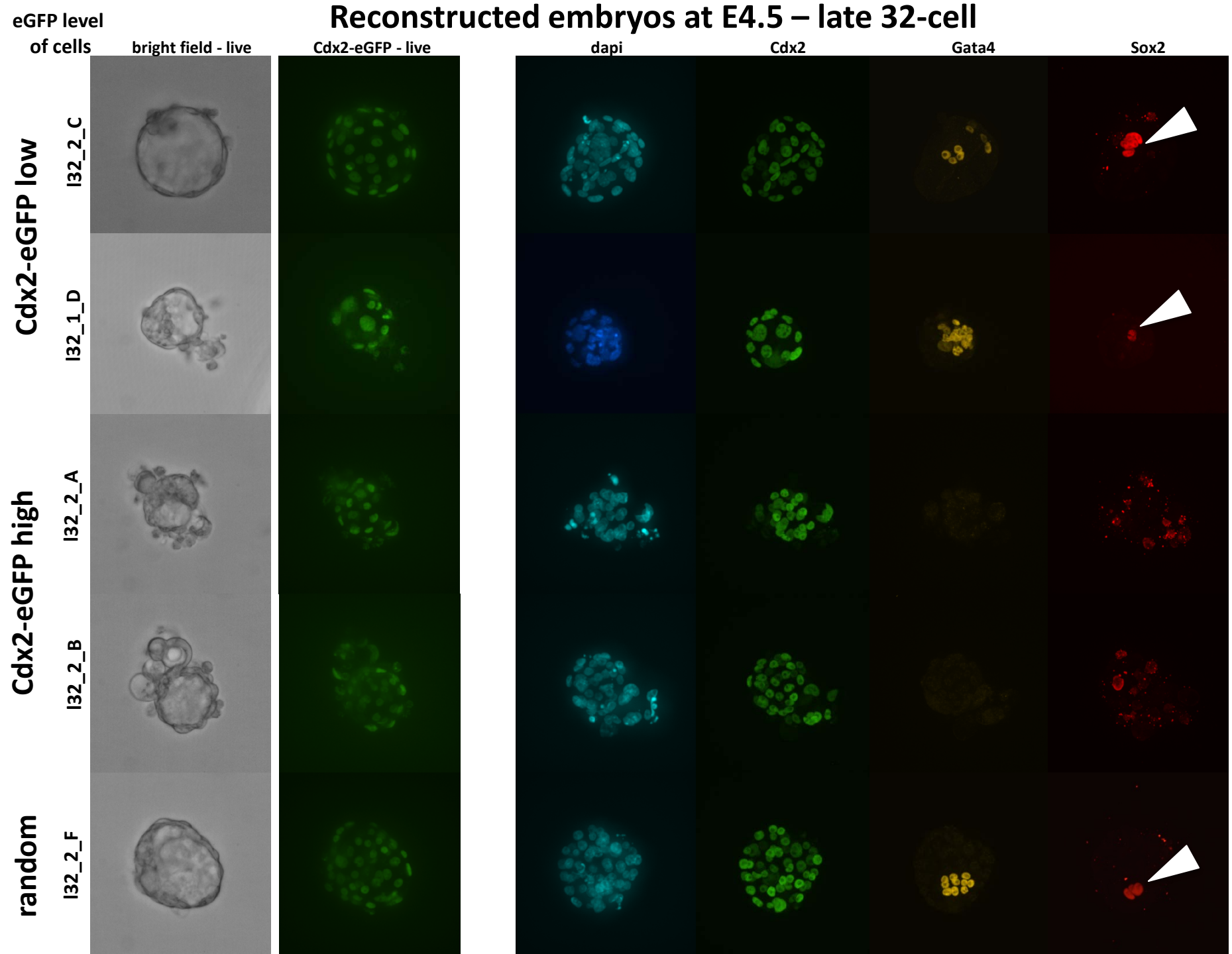
GFP quantification with
confocal microscope



Reaggregate
GFPhi or lo cells

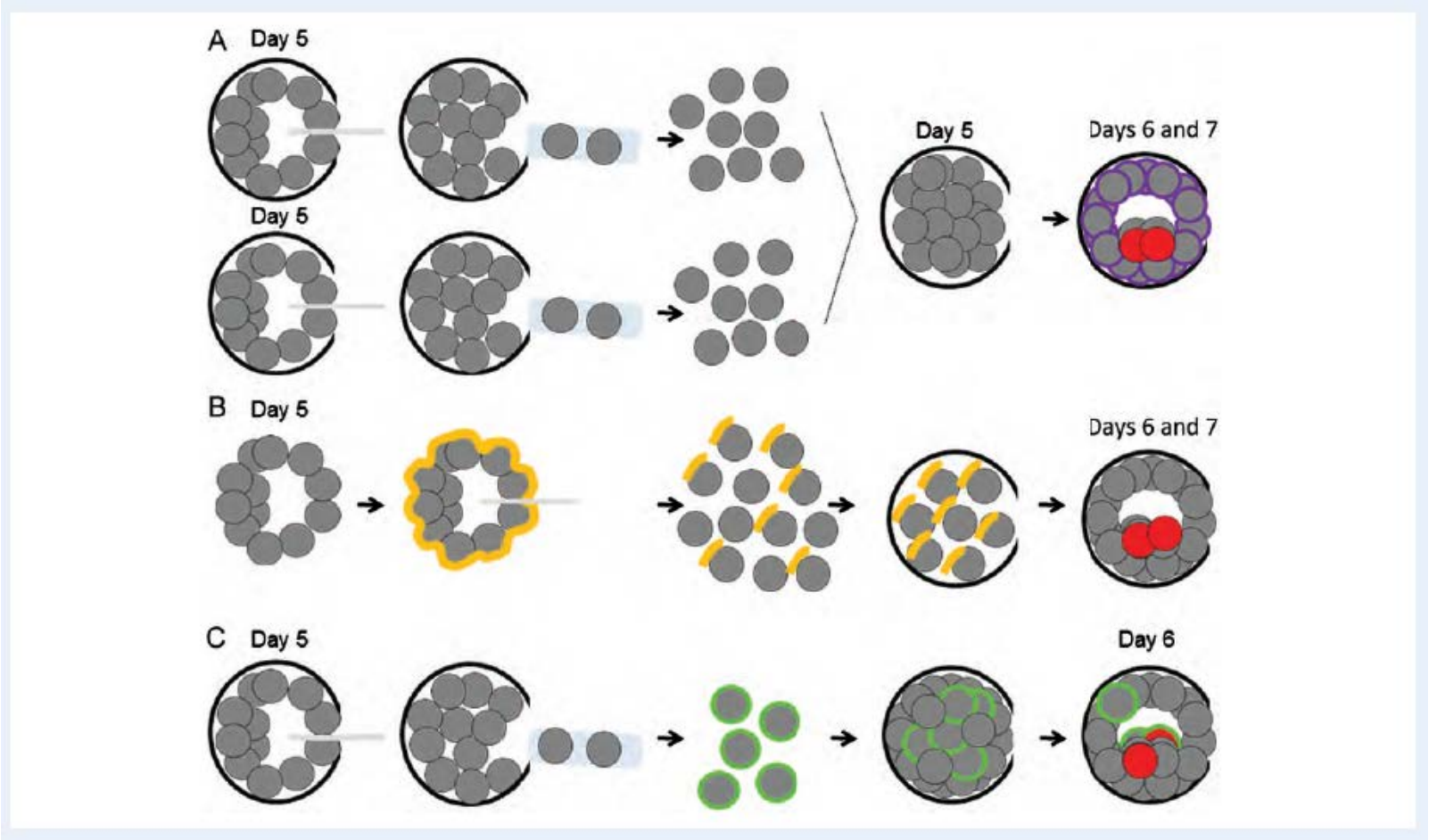

Re-constructing embryos from single cells – late 32-cell
Cdx2-eGFP level per nucleus
10000
5000
0
l32_2_C
l32_1_C
l32_1_D
l32_2_D
l32_1_A
l32_1_B
l32_2_A
l32_2_B
l32_1_E
l32_1_F
l32_2_E
l32_2_F

Reconstructed embryos at E4.5 – late 32-cell

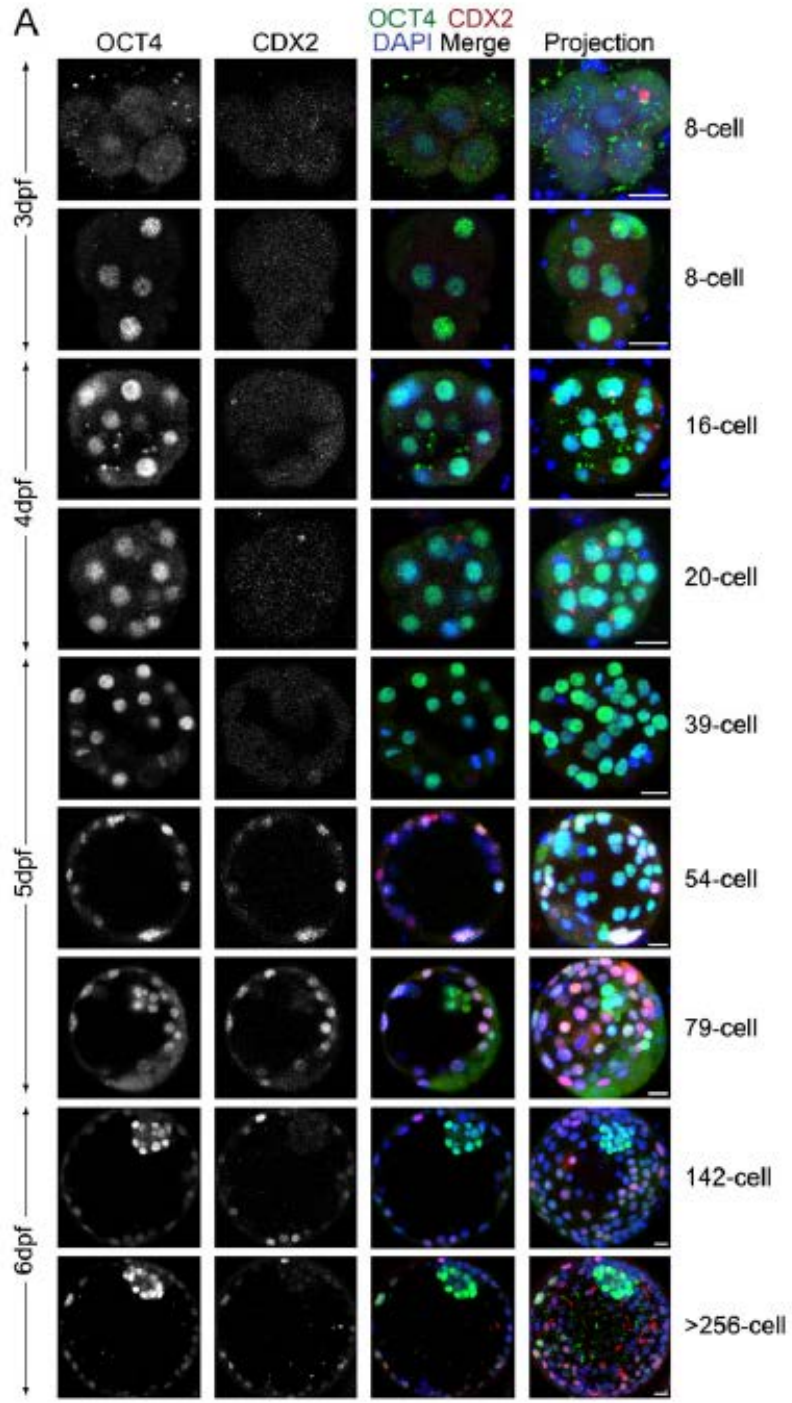


- Cdx2 +ve outside cells committed to trophectoderm by late 32-cell stage
- Inside cells can still regenerate trophectoderm
- Mouse lineage restriction is progressive but complete by blastocyst stage

Human early blastocyst cells are uncommitted to lineage



De Paepe et al (2013) Human Reprod. 28: 740-789



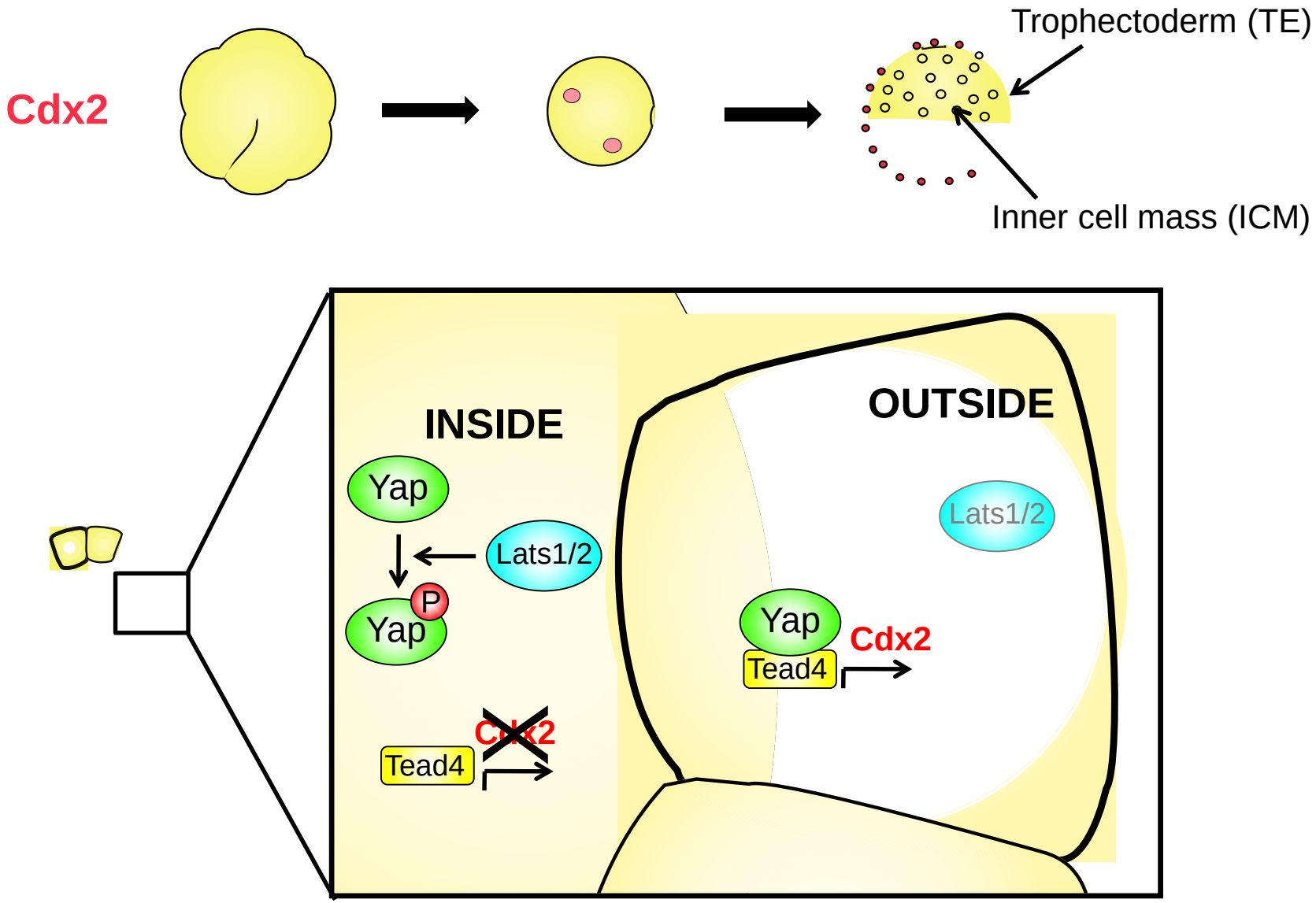
Is this related to timing of expression of lineage specifiers in human embryo?

Cdx2 does not begin expression until blastocyst stage and Oct4 not restricted to ICM till very late blastocyst.
Niakan and Eggan (2013)
Dev Biol 375: 54-64

What restricts Cdx2 to the outside cells in mouse?

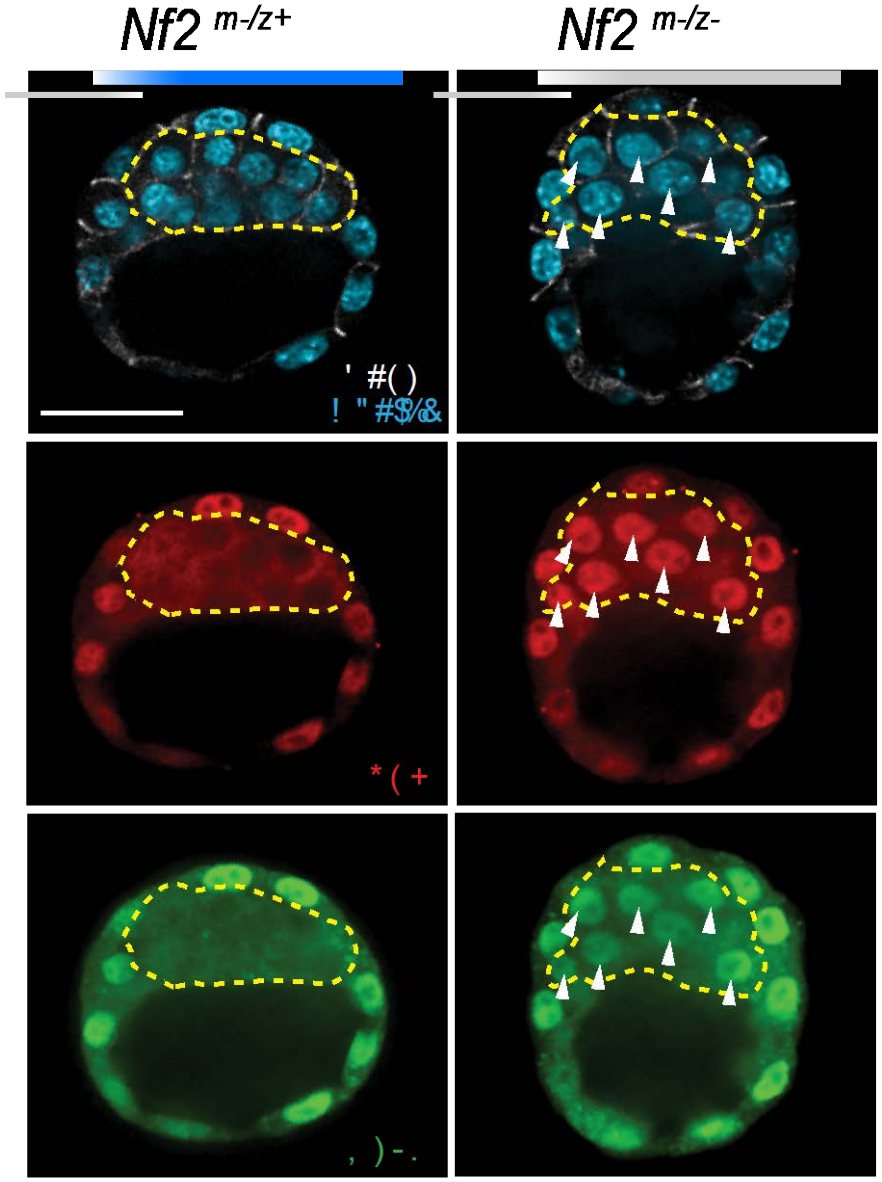
Hippo signaling activation in inside cells

Lats1/2 and Yap control Cdx2 expression in the preimplantation embryo



Nishioka et al, Dev Cell, 2009

Genetic deletion of required Hippo signaling components like Nf2 turns inside cells into TE

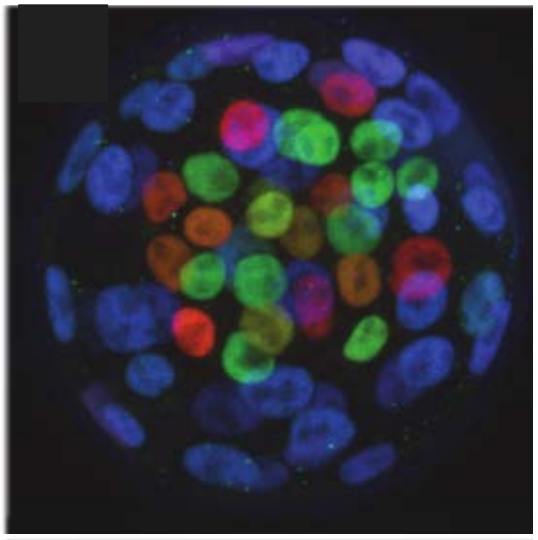


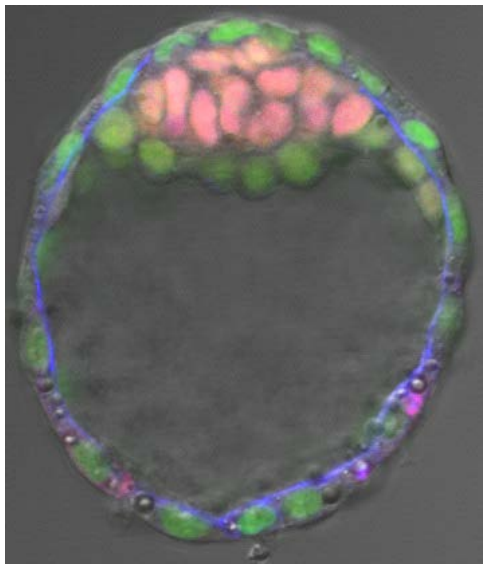
Cockburn et al (2013) Curr Biol

- Is Hippo signaling involved in later lineage restriction in human embryos?

Next step- making epiblast and primitive endoderm

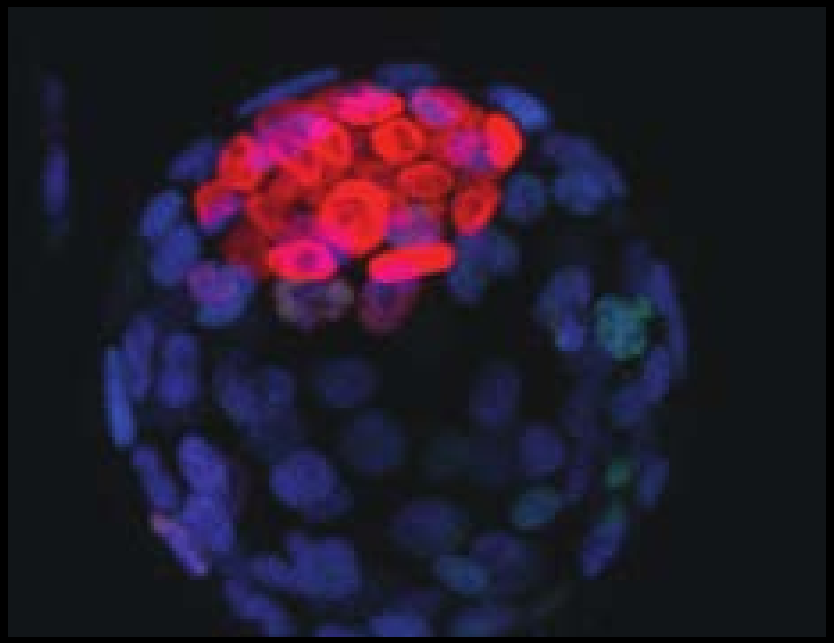
Cdx2
Gata6
Nanog



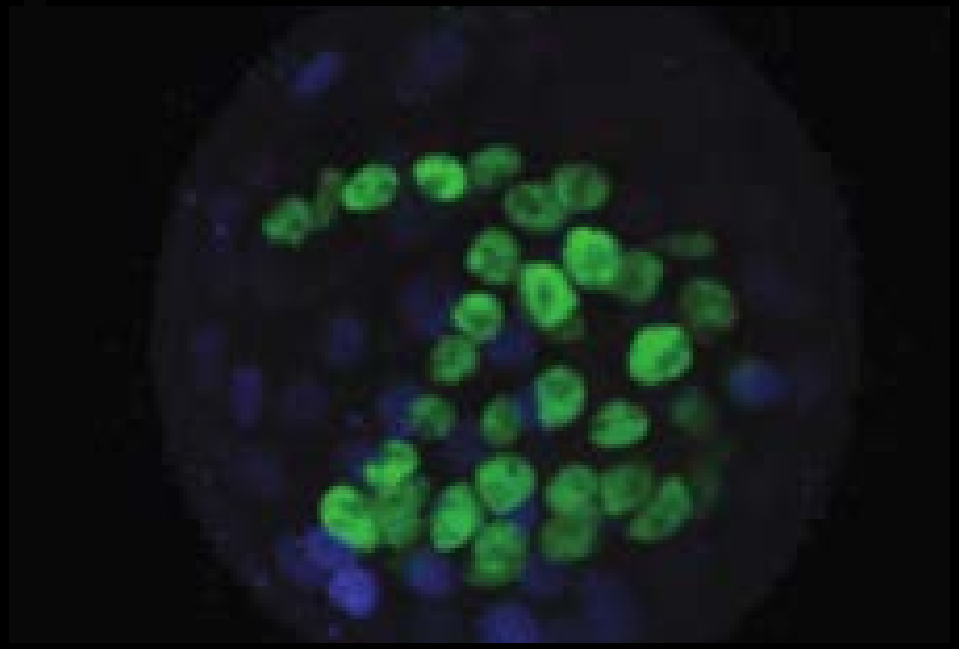


Chazaud et al, 2006, Dev Cell

Levels of FGF signaling during blastocyst stage influence EPI versus PE formation



Block FGF/Erk signaling;
All ICM cells make epiblast



Stimulate FGF/Erk signaling;
All ICM cells make primitive endoderm

Yamanaka et al, 2010, Development

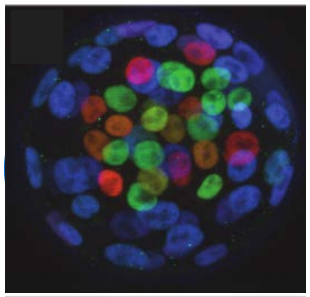
But recent evidence suggests that FGF may not play the same role in human blastocyst development

- Treatment of human blastocysts with FGF or ERK inhibitors does not affect primitive endoderm formation

  (Roode et al, 2011, Dev Biol 361, 358;

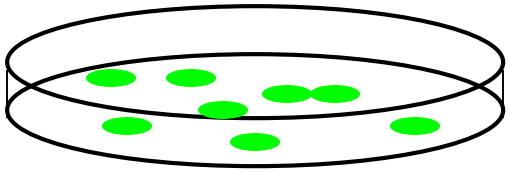
  Kuijk et al, 2012, Development 139, 871)

FGF signaling levels drive stem cell development from mouse blastocyst



Add FGF4

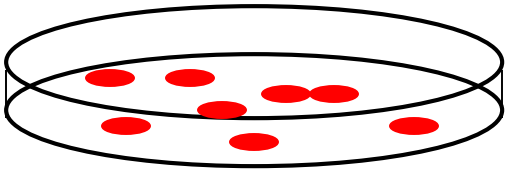
XEN cells



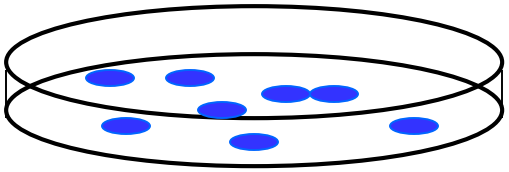
+ cond medium

(Kunath et al,Development 2007)

‘naïve’
ES cells



Block FGF/GSK3
(Ying et al, Nature 2008)



TS cells

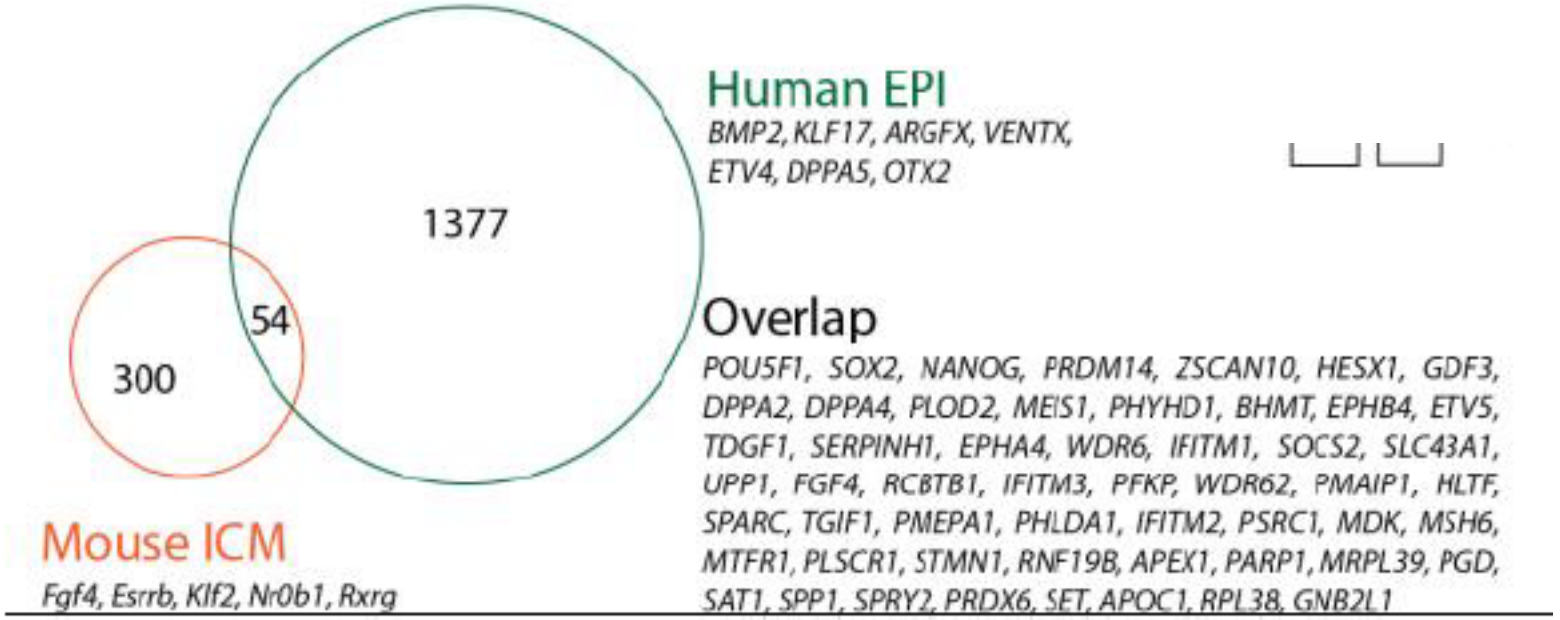
Add FGF4
+ activin
(Tanaka et al,Science, 1998)

Human- different again?

- Treatment of human blastocysts with FGF promotes isolation of human ES cells
- Generation of ‘naïve’ human ES cells requires complex conditions
- Cannot derive human TS cells from human blastocysts by activating FGF signaling

Single cell RNA seq identifies conservation and divergence between mouse and human

Galan et al (2010) PLoS One 5, e13615
Xue et al (2013) Nature 500:593-597
Yan et al (2013) Nature Struct. Mol. Biol. 20: 1131-1139
Tohonen et al (2015) Nature Comms. 6:8207

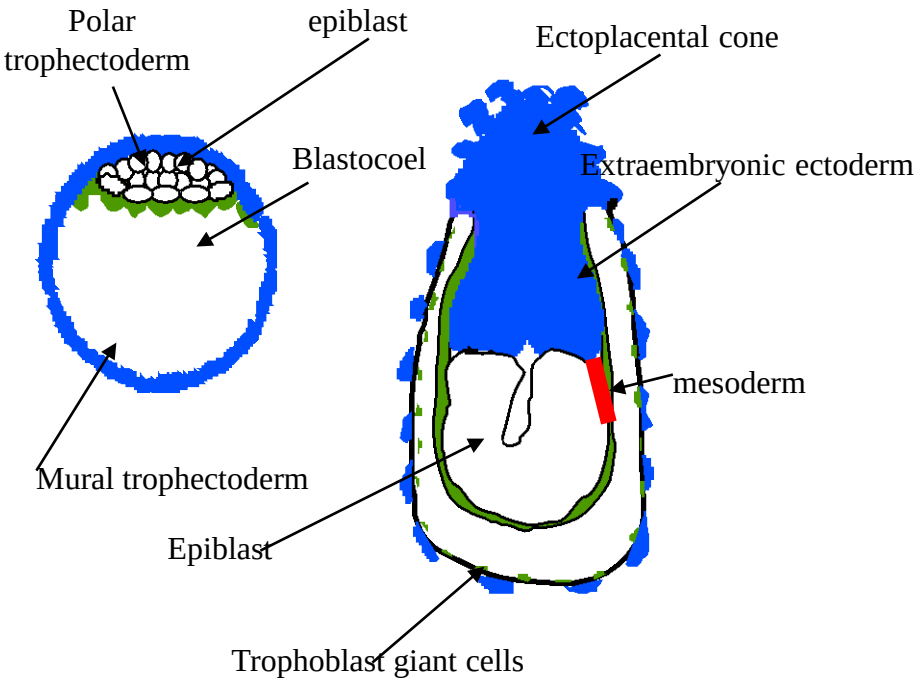


Blakeley et al (2015) Development 142:3151-65

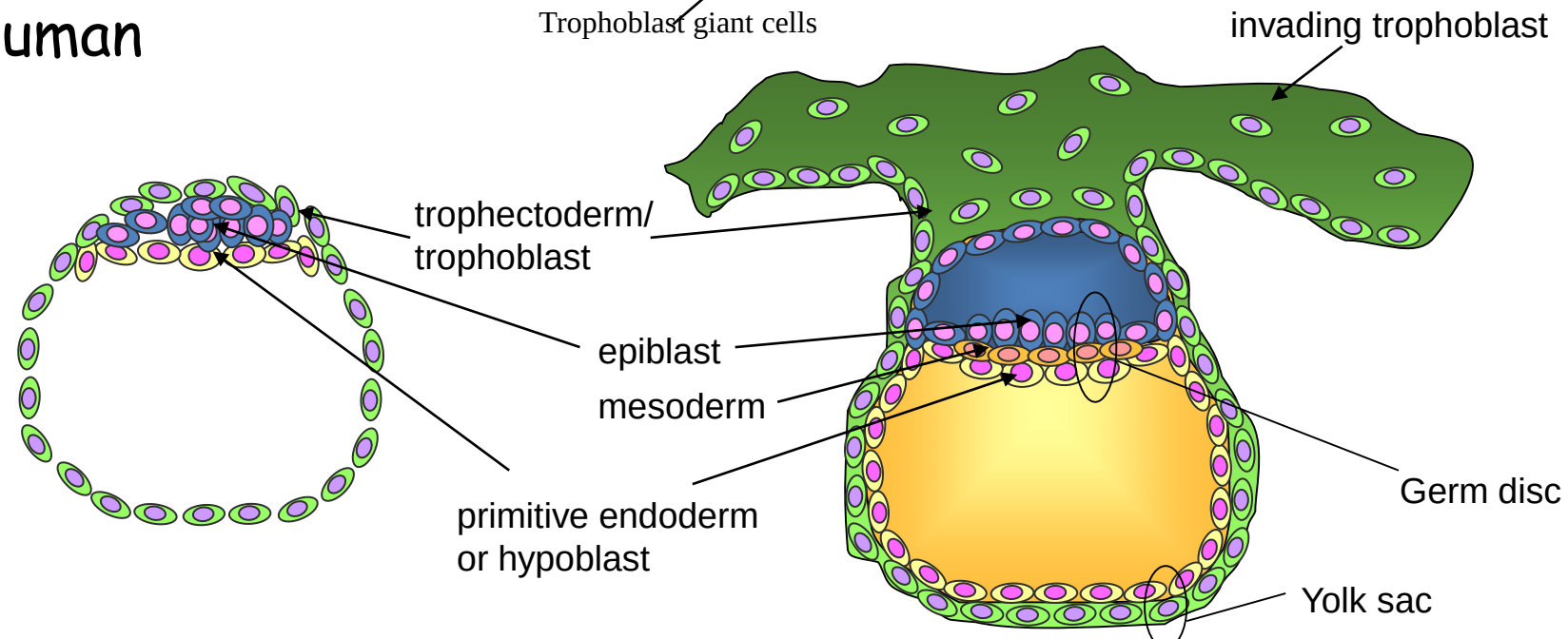
Overall events and timelines of preimplantation development differ in mouse and human

Early postimplantation development is different too

mouse



Human



Many questions unanswered

- Is Hippo/Yap/Cdx2 important for TE formation in human blastocyst?
- What is role of FGF signaling?
- Does TGFβ/Nodal signaling play an important role in lineage specification in human?
- Why can't we derive human TS cells?
- Is there actually a naïve pluripotent state in human development?
- Can we develop better culture systems to study early postimplantation development up to 14 days?

Need for more functional analysis of human preimplantation development

- Experimental assessment of cell fate
- Inhibitor and growth factor treatments
- siRNA and dominant negative mRNA approaches
- CRISPR-Cas9 guided gene activation or inactivation
- CRISPR-Cas9 gene editing

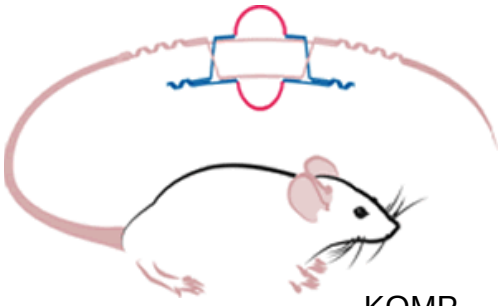
  - All experiments to be analyzed in vitro at blastocyst or early outgrowth stage

Gene editing in human embryos need not lead inevitably to germline modification

CRISPR-Cas9 gene editing is changing the face of large-scale mouse mutagenesis projects

International Knockout Mouse Consortium (2006 on)

- Generation of a resource of targeted mutations across the mouse genome
- Over 15,000 targeted cell lines available
- Collaboration between NIH-KOMP, EUCOMM and NorCOMM



KOMP

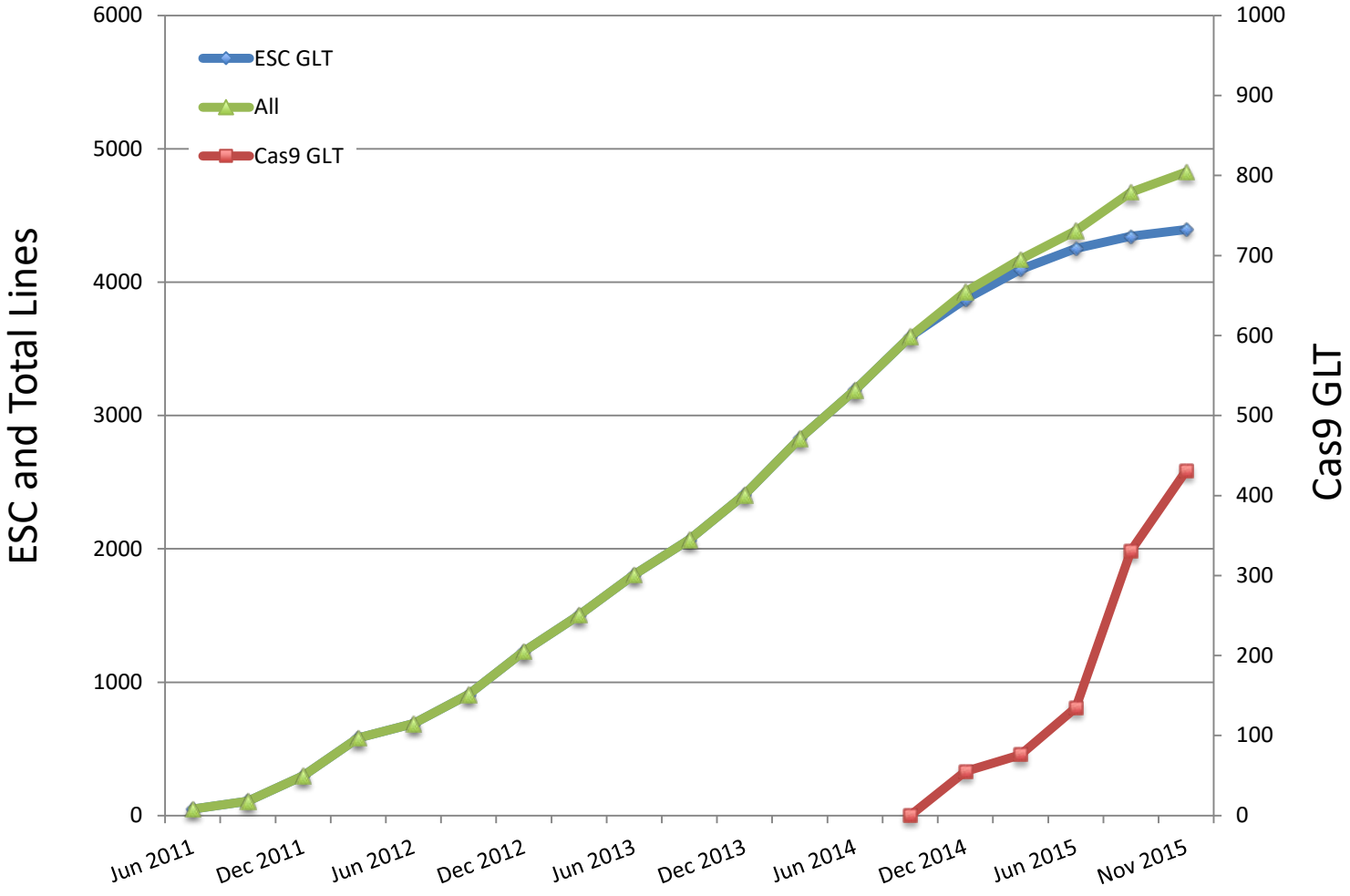
IMPC

- **International Mouse Phenotype Consortium**

- The goal of the International Mouse Phenotyping Consortium (IMPC) is to discover functional insight for every gene by generating and systematically phenotyping 20,000 knockout mouse strains.
  - Current data online for 1,855 genes
  - Phenotyping pipeline provides clinical phenotyping for existing mutations and strains as well

www.mousephenotype.org

Mouse line production for IMPC



New mouselines made by IMPC centres, Jun 2011 to Nov 2015

Cas9 Type	Type	#injected	#weaned	#G0 by allele type		%G0 by desired allele	
				indel	desired	weaned	injected
Cas9 mRNA	indel	1450	236	100	-	42%	6.90%
Cas9n mRNA		924	148	38	-	26%	4.11%
Cas9 mRNA	PM	2349	295	99	52	18%	2.21%
Cas9 protein		812	165	47	16	10%	1.97%
Cas9 mRNA	2G Δexon	1668	220	2	41	19%	2.46%
Cas9n mRNA		856	154	2	12	8%	1.40%
Cas9 mRNA	MTI Δexon	984	96	58	37	39%	3.76%
Cas9 mRNA	4G Δexon	364	68	-	21	31%	5.77%

Summary of founder production at TCP using Cas9

- note that the overall success per injected embryo is still quite low