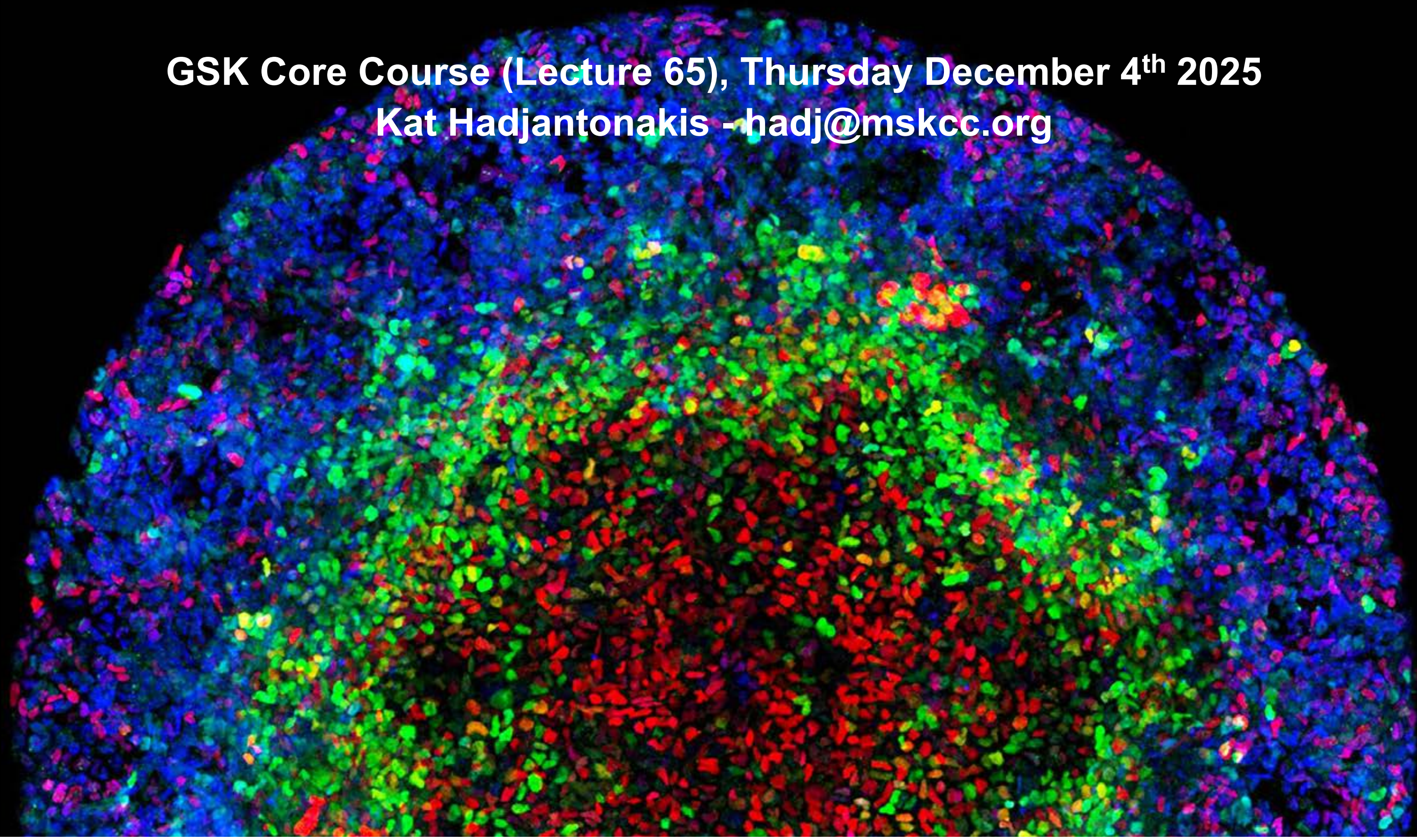
Stem Cells from Early Mammalian Embryos & Stem Cell-Derived Embryo Models

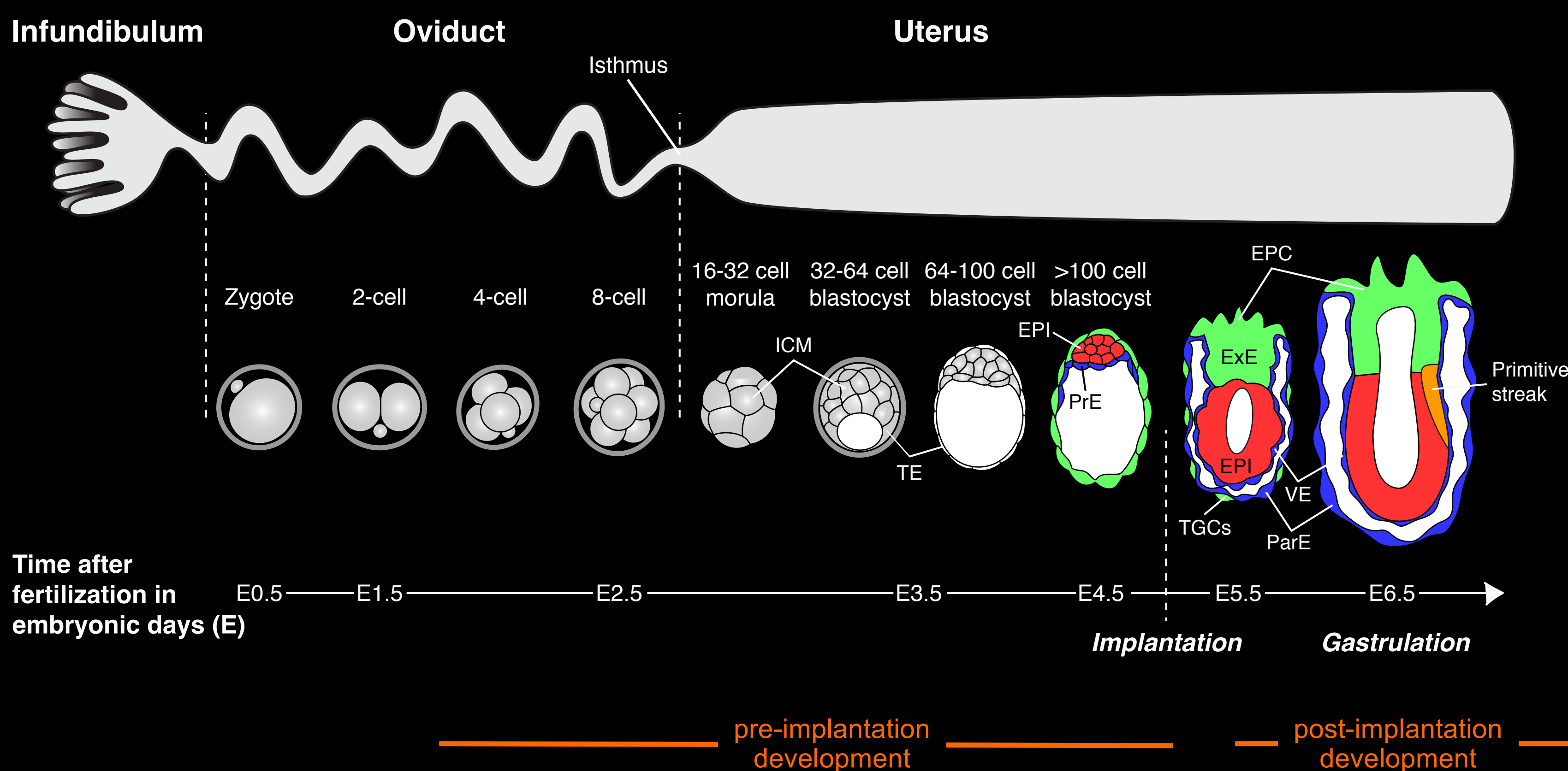
GSK Core Course (Lecture 65), Thursday December 4th 2025

Kat Hadjantonakis - hadj@mskcc.org



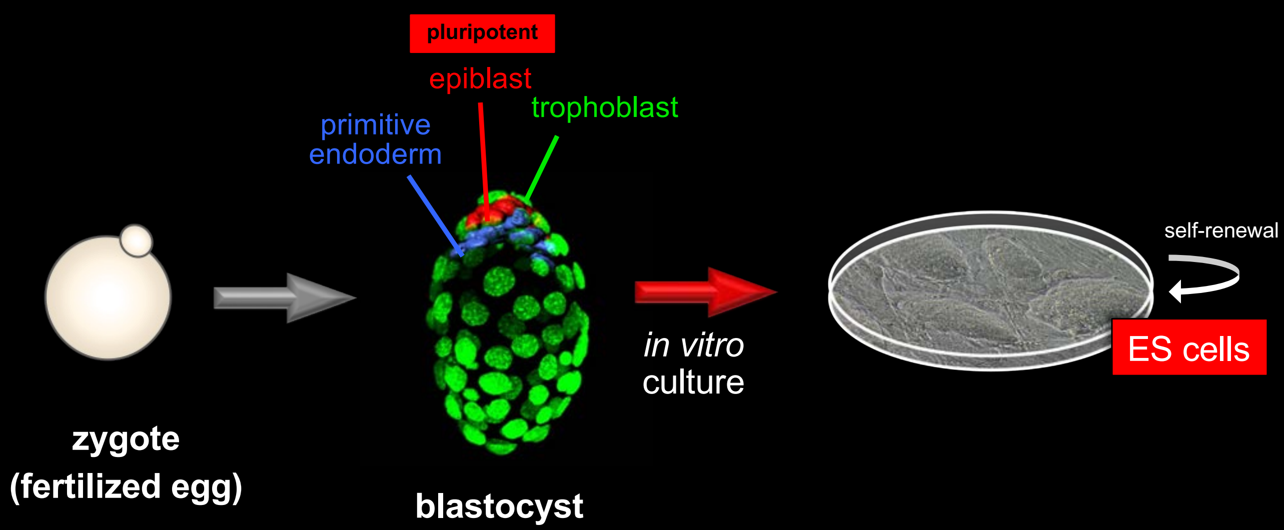
Stem Cells derived from Embryos

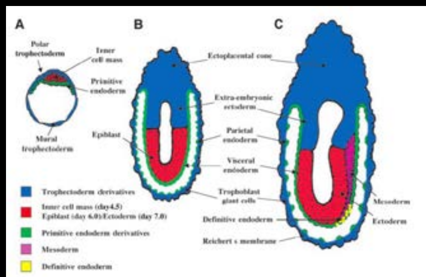
mouse embryo development: the 1^{st} week



Saiz N. *et al*. JoVE 2016

Derivation of embryonic stem (ES) cells from mouse blastocyst stage embryos





ES cells

TS cells

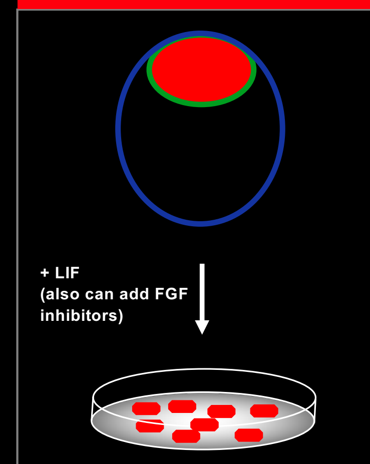
Xen cells

Stem cells

from the mammalian blastocyst

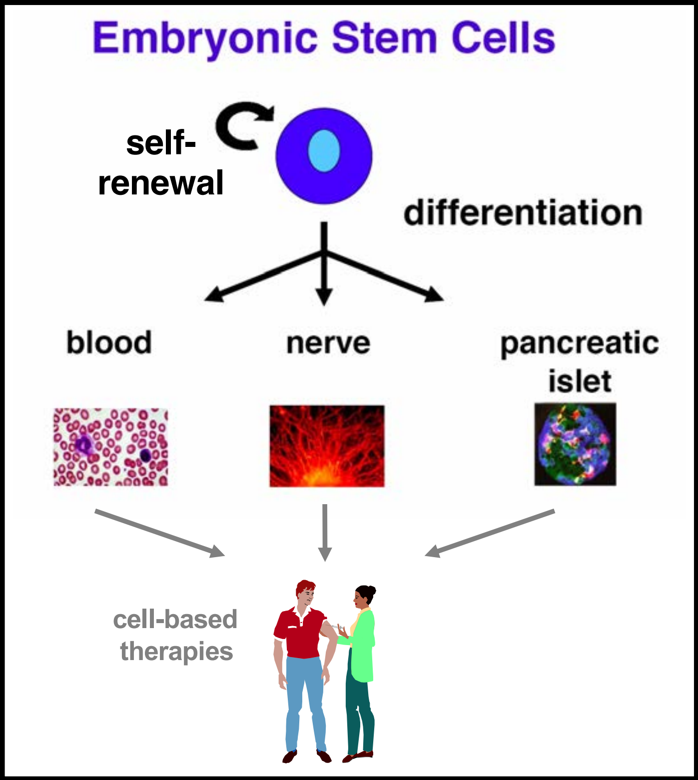
- Derived from the EPIBLAST of the mammalian blastocyst.
- They approximate to the:
  - (1) Morphology of EPIBLAST cells
  - (2) Gene expression profile
  - (3) Developmental potential of EPIBLAST
- ES cells grow indefinitely while maintaining their developmental potential (pluripotency)

ES cells (ESCs)

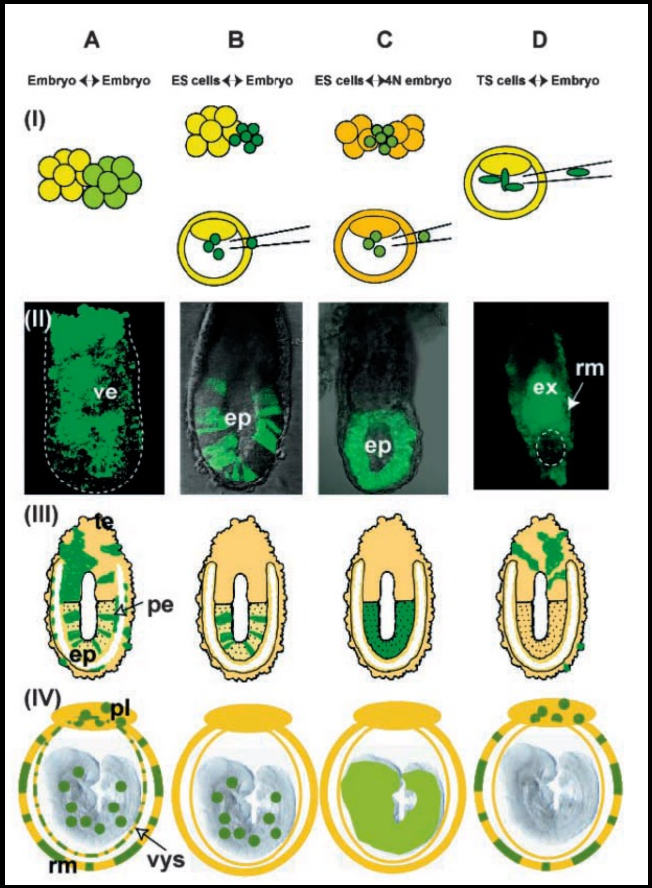


Testing developmental potential *in vitro* and *in vivo*

Developmental potential *In vitro*



Developmental potential *In vivo*



Taken from Tam and Rossant, 2003, *Development* 7:155.

Testing developmental potential *in vivo* chimeras

- From the Greek meaning "she-goat"
- The Chimera was a mythical fire-breathing creature with the body of a goat, the head of a lion and the tail of a serpent

- mouse chimeras can be generated by aggregation or injection
- used as a tool for investigating the developmental potential of cells *in vivo*

mouse chimeras

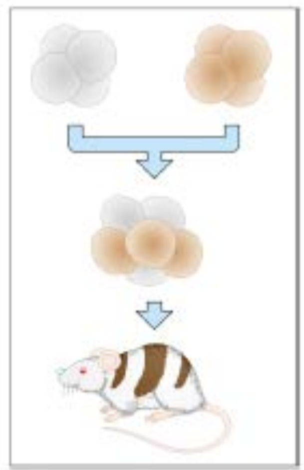
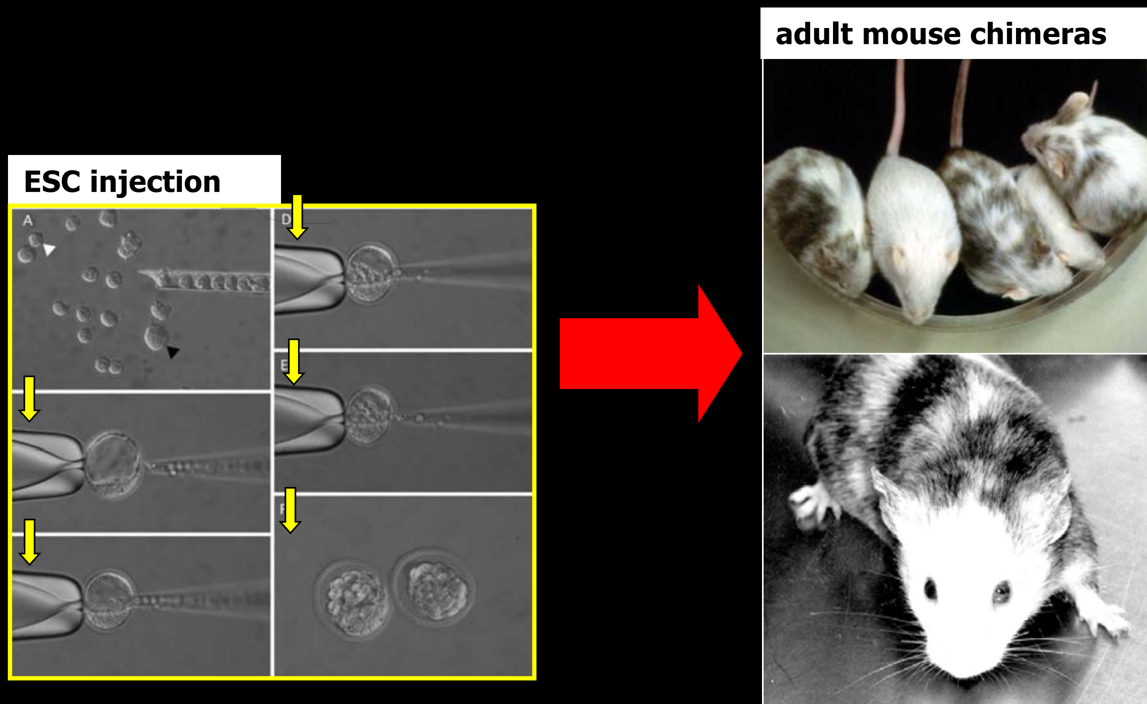


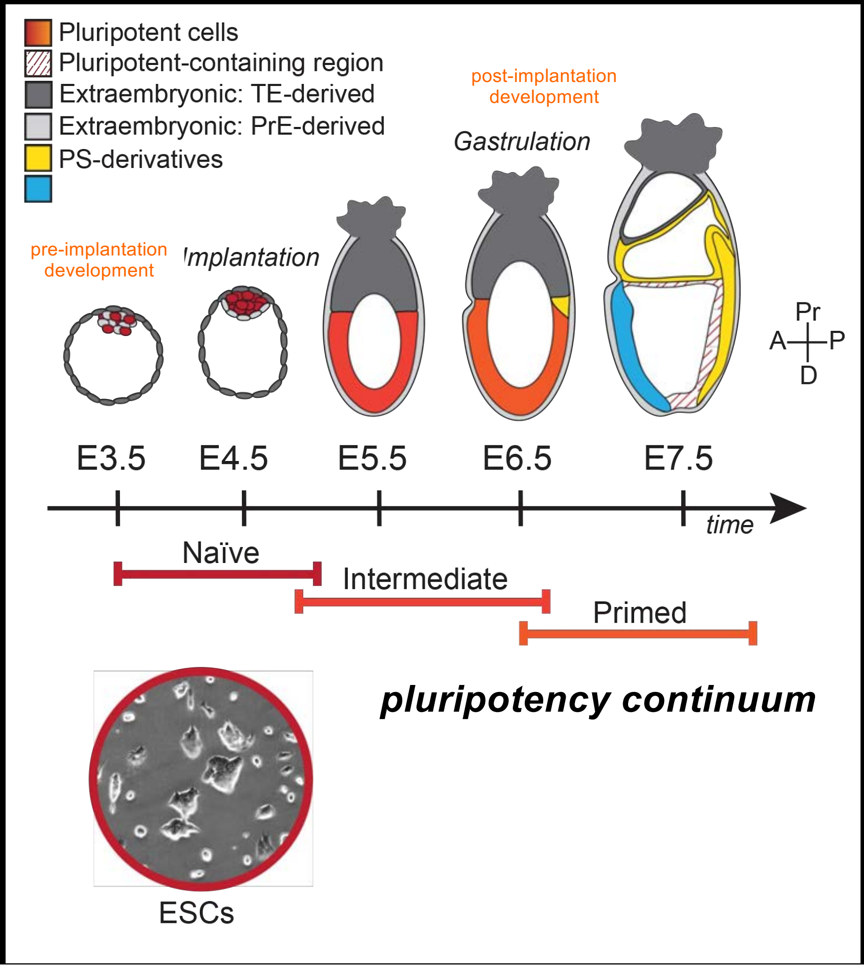
Fig 3.24

- Combine two 8-cell embryos into one 16-cell embryo
- **aggregation chimera** (with 4 parents)
- Cell division slows so that blastocyst is normal size
- Very rare examples of human chimeras (XX--XY) formed by fusion?

Embryonic stem cells (ESCs) form chimeras when injected into pre-implantation embryos

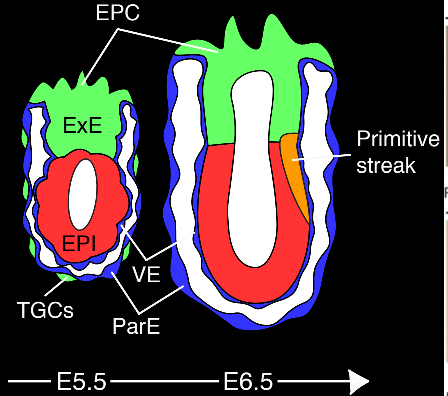


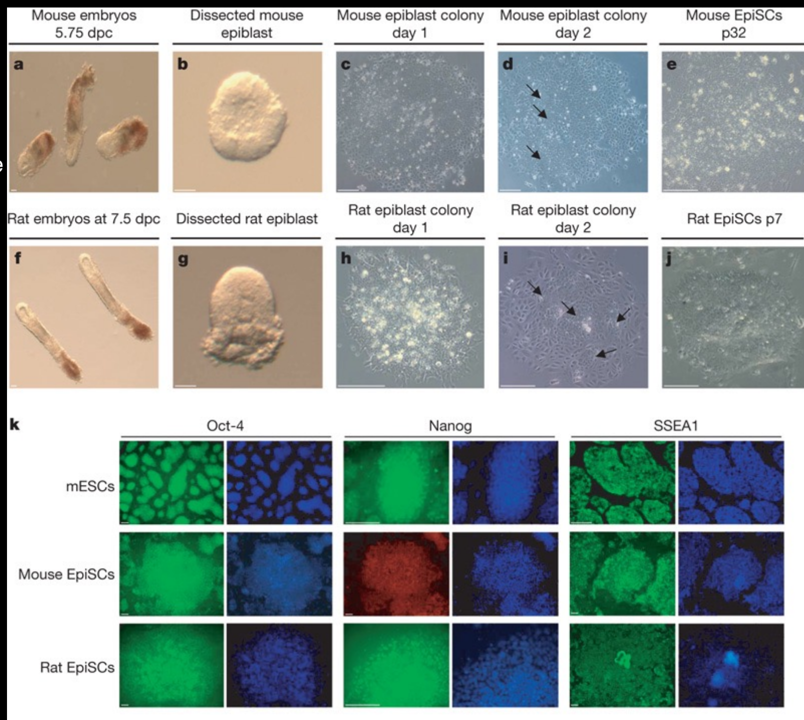
Pluripotent stem cells representing different epiblast states can be isolated from mouse embryos

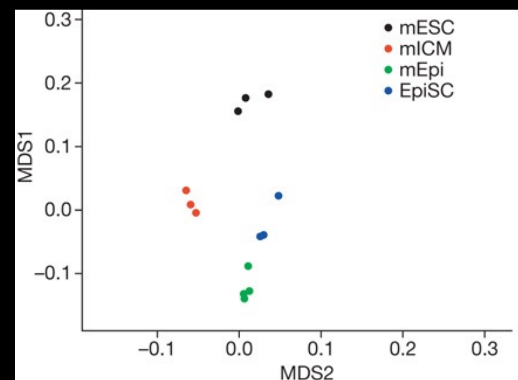


Morgani *et al.*, 2017

Derivation of pluripotent epiblast stem cells (EpiSCs) from postimplantation mouse (& rat) epiblast



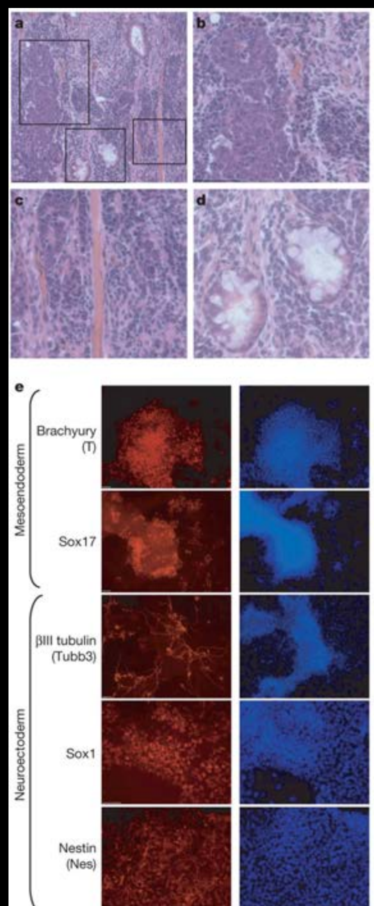




Brons *et al.*, Nature 2007

in vivo (teratomas)

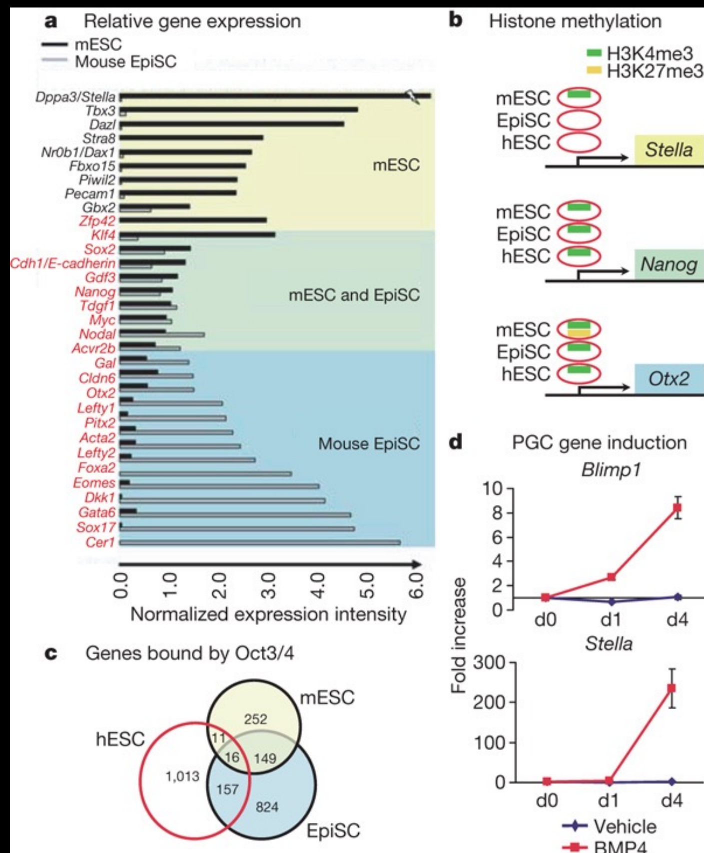
in vitro



EpiSCs are pluripotent: capable of differentiating into the three primary germ layers *in vitro* and *in vivo*

Brons *et al.*, Nature 2007

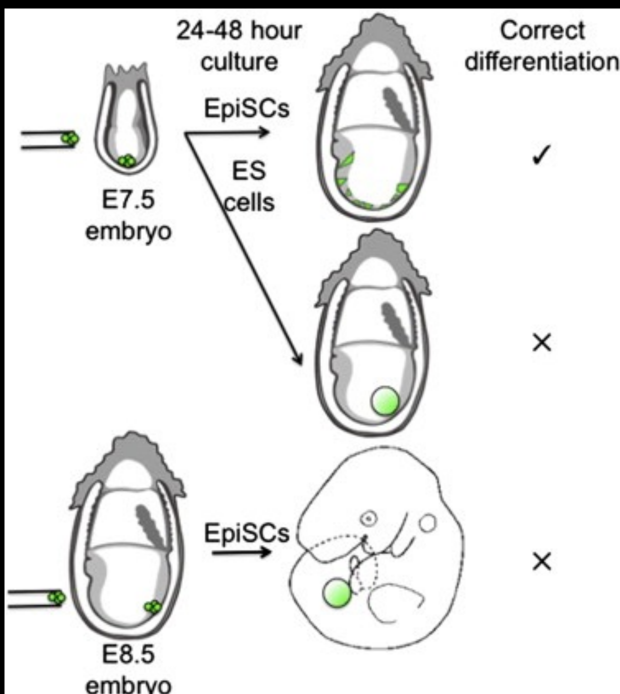
mESCs and mEpiSCs have distinct gene expression

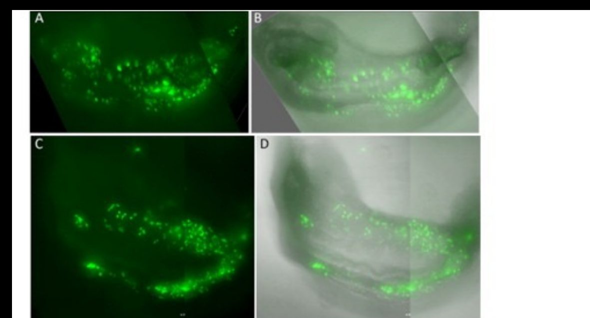


Tesar *et al.*, Nature 2007

In vivo differentiation potential of mEpiSCs revealed by chimeric embryo formation

- **Epiblast stem cells (EpiSCs) form chimeras when injected into post-implantation epiblast**
- **Embryonic stem cells (ESCs) do not form post-implantation chimeras**
- **EpiSCs do not integrate if they are injected after gastrulation**

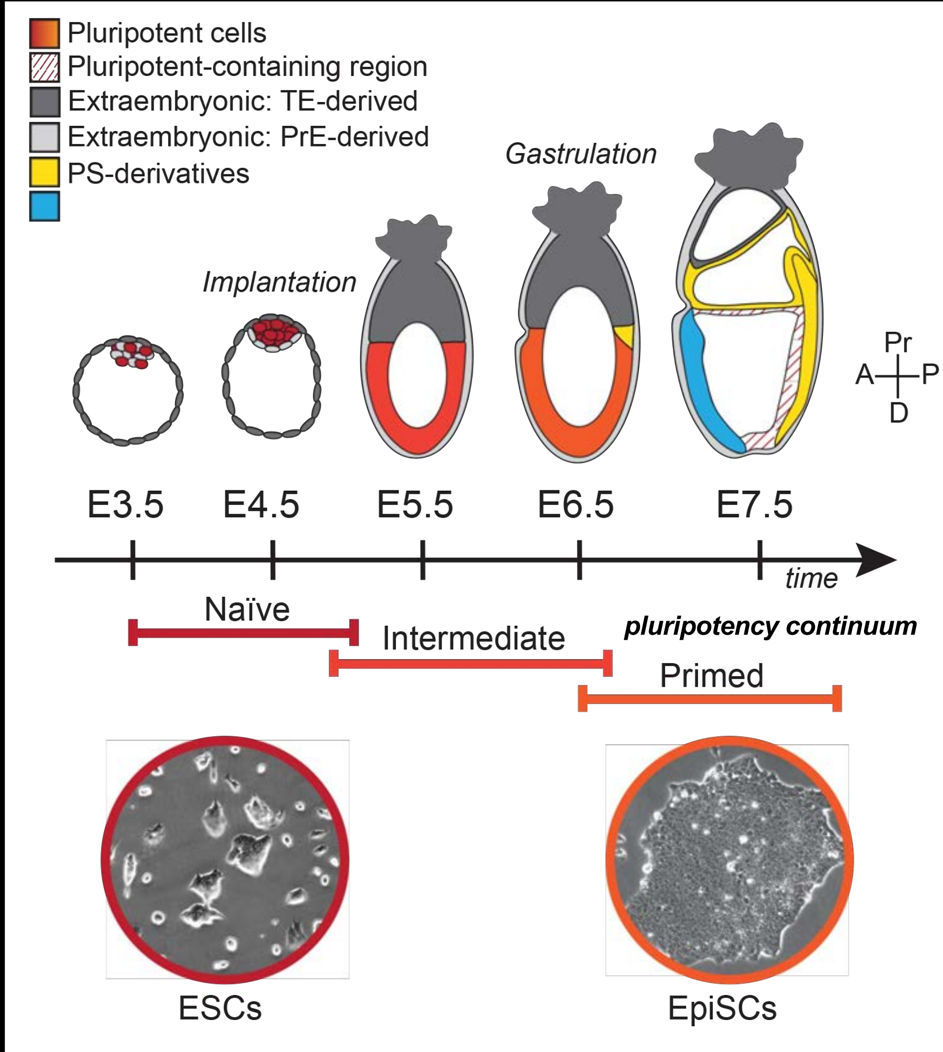




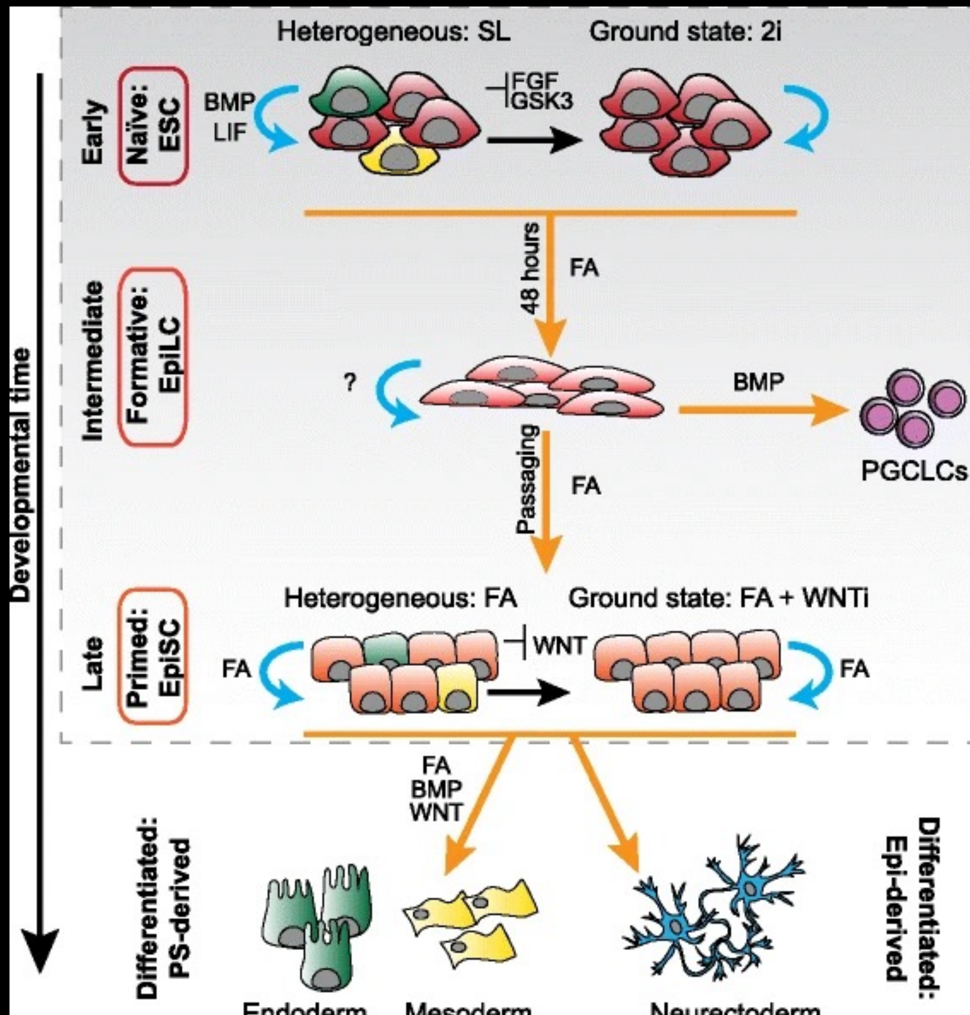
E

Donor cells	Culture time of embryos	Graft site	Stage of recipients	No.of grafted embryos	Dispersed donor cells					Cell spread		
					<12 cells	12-24 cells	24-48 cells	48-96 cells	>96 cells	<1/4	1/4-1/2	>1/2
Emebryo-derived EpiSC (r04-GFP)	24h	MA	M-LS	12	5	7				8	4	
		D	M-LS	8			7	1			6	2
		MP	LS	4	1			3		1	2	1
		PP	M-LS	6	1	1	3	1		1	5	
	48h	D	M-LS	5+(4)			3		2	1+(2)	1+(2)	3
		MP	MS	1			1				1	
		PP	E-MS	4				1	3			4
In vitro derived EpiSC (Tps/tb;HPRT ^{TREDsRed2}-C2)	24h	MA	MS	3	3					3		
		D	M-LS	5	1	3	1				5	
		MP	MS	4			2	2			3	1
	48h	PP	ES	4				1	3		1	3
ES cells (AGFP7)	24h	D	M-LS	7	7					6	1	
		PP	M-LS	6	3	2	1			5	1	

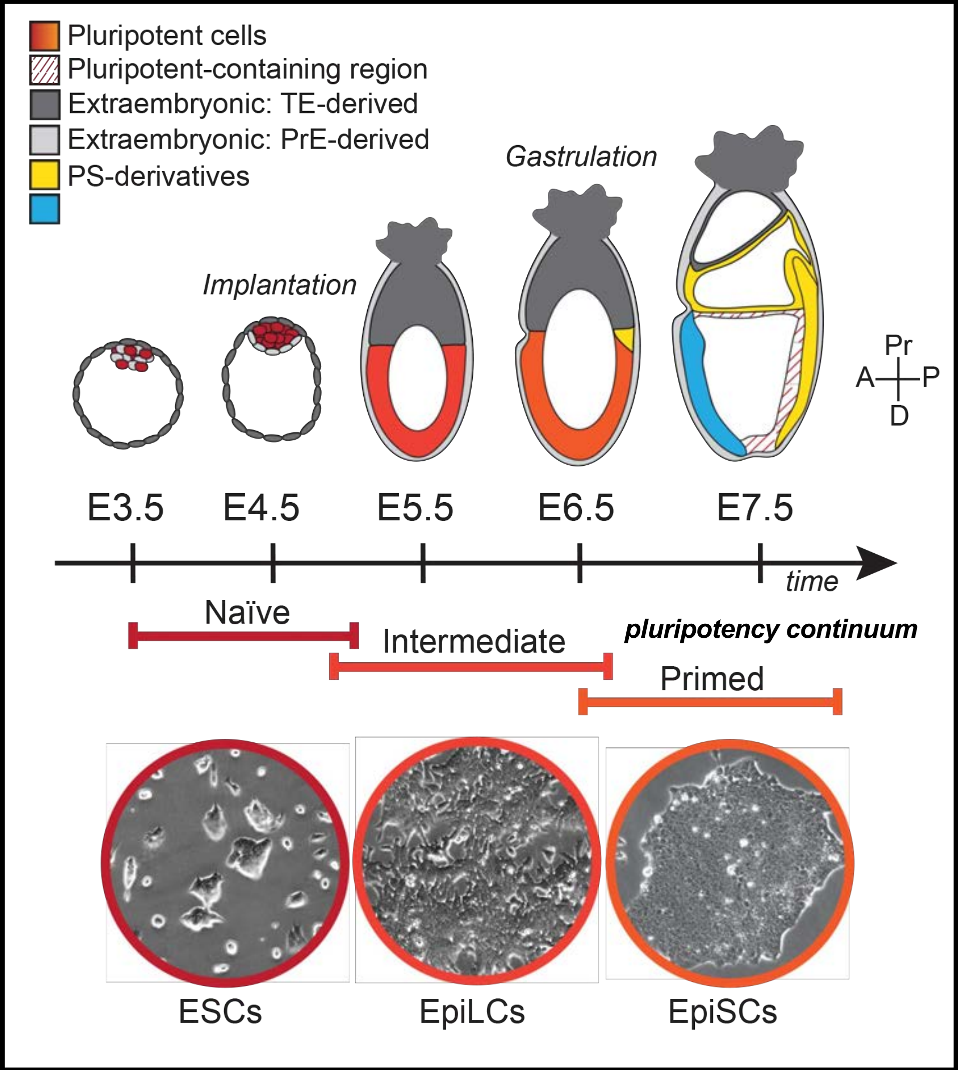
Huang *et al.*, Cell Reports 2012



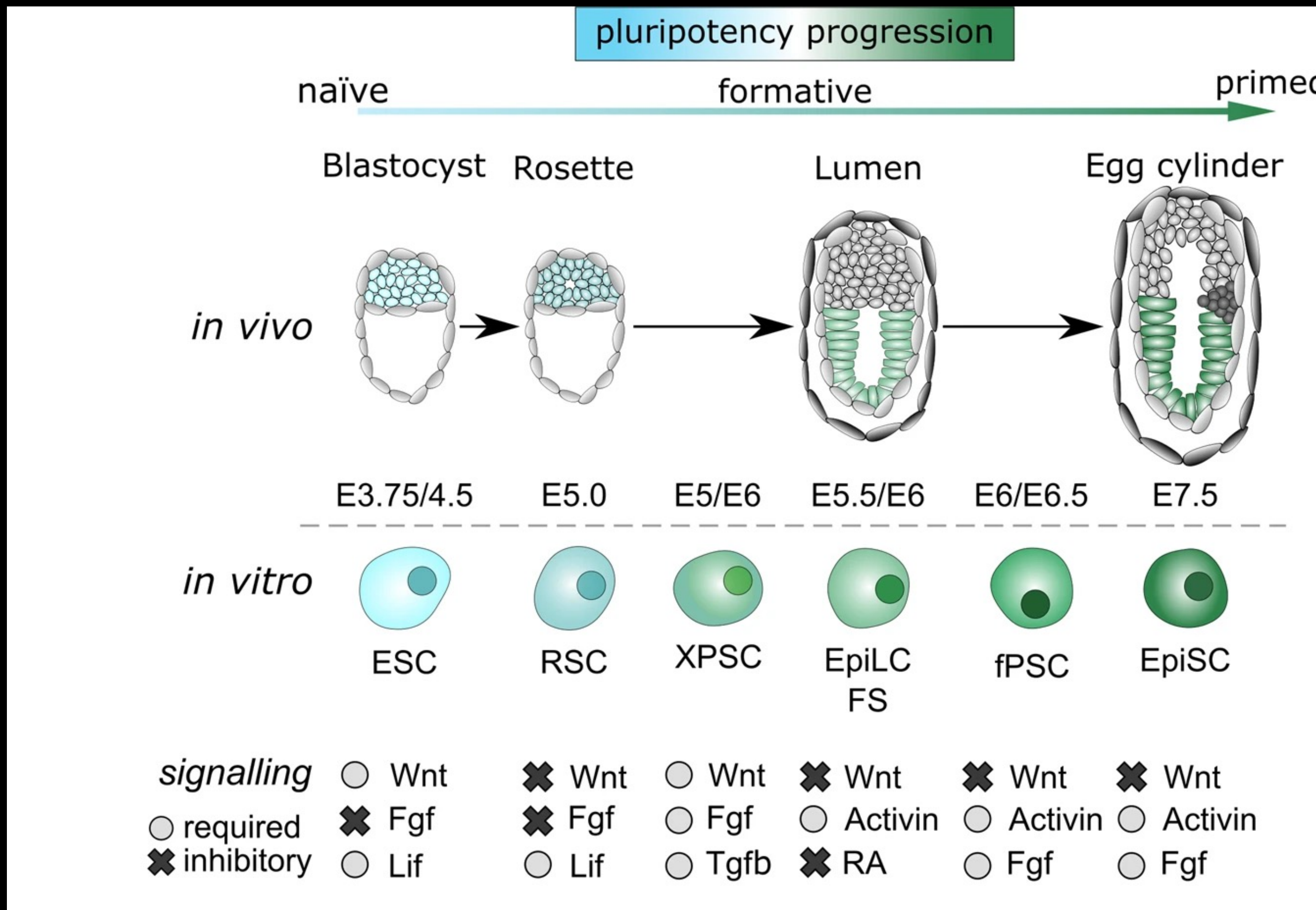
Pluripotent cells
Pluripotent-containing region
Extraembryonic: TE-derived
Extraembryonic: PrE-derived
PS-derivatives
Implantation
Gastrulation
Pr
A
P
D
E3.5
E4.5
E5.5
E6.5
E7.5
time
Naïve
Intermediate
Primed
pluripotency continuum
ESCs
EpiSCs



Heterogeneous: SL
Ground state: 2i
Early
Naïve: ESC
BMP
LIF
FGF
GSK3
48 hours
FA
Intermediate
Formative: EpiLC
?
BMP
PGCLCs
Passaging
FA
Developmental time
Heterogeneous: FA
Ground state: FA + WNTi
Late
Primed: EpiSC
FA
WNT
FA
FA
BMP
WNT
Differentiated: PS-derived
Differentiated: Epi-derived
Endoderm
Mesoderm
Neurectoderm

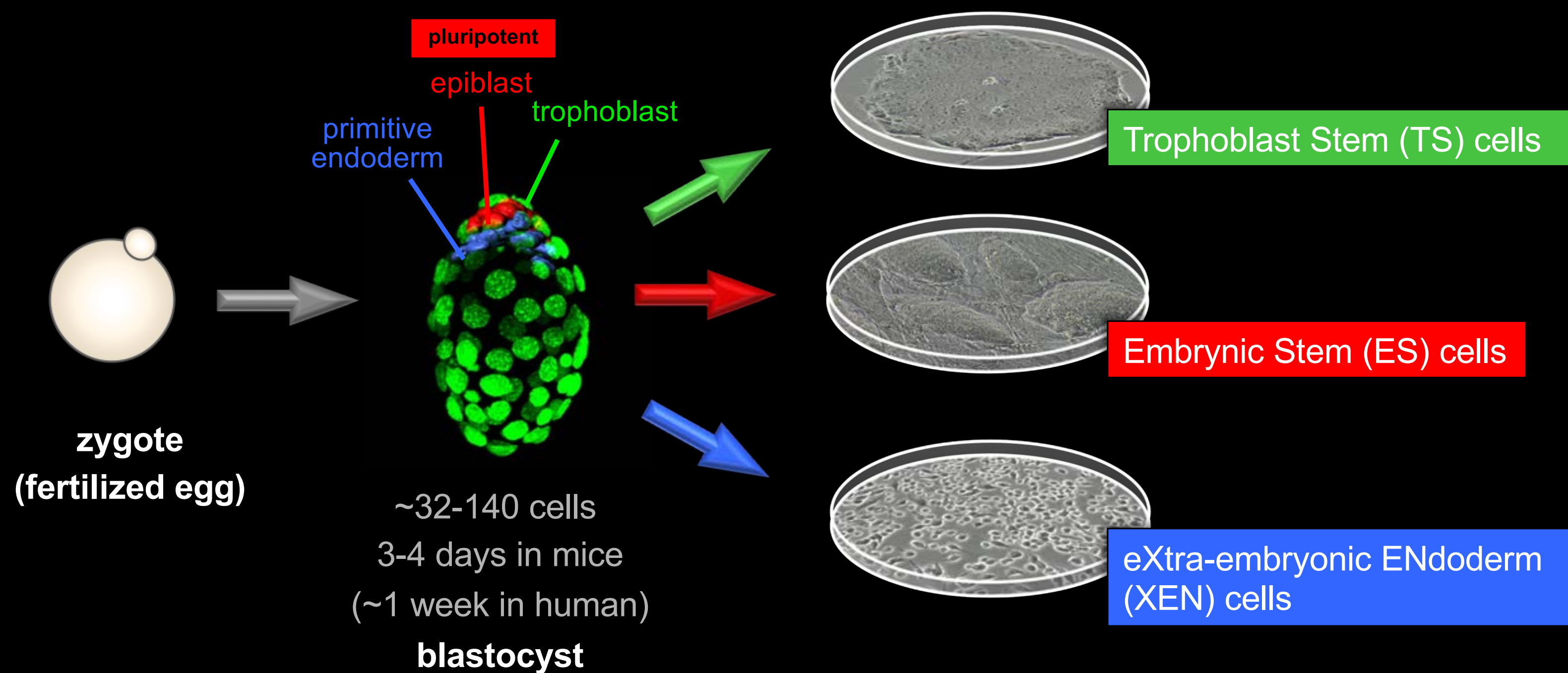


Morgani *et al.*, 2017

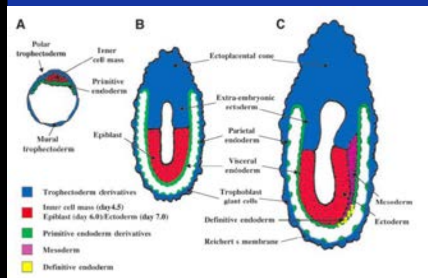


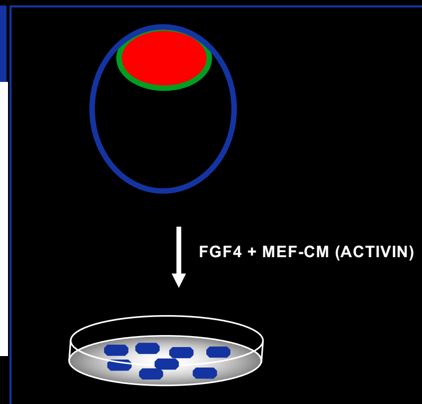
Furlan *et al.*, Nature Comms. 2023

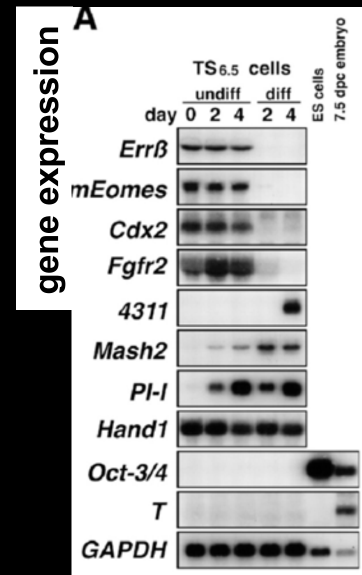
Derivation of stem cells from
all 3 lineages of the mouse blastocyst stage embryos



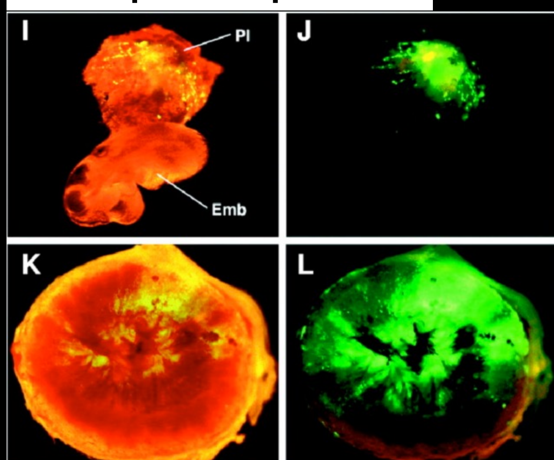
TS cells



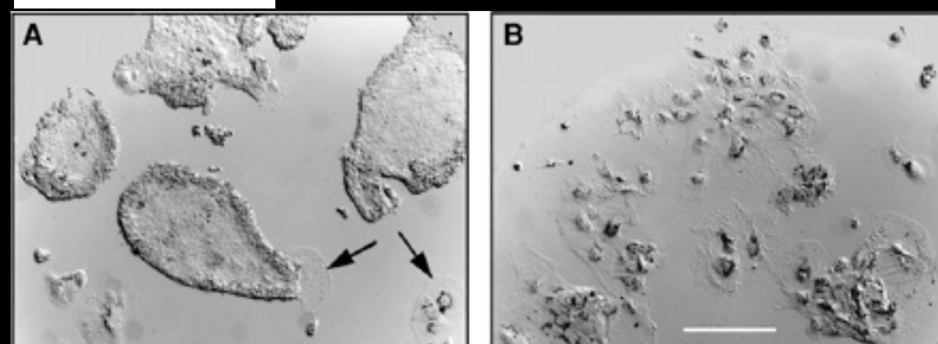




developmental potential

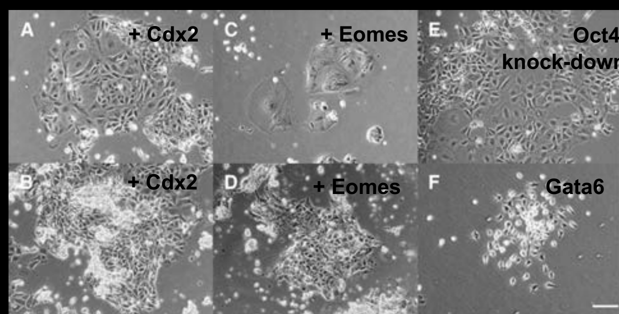


morphology

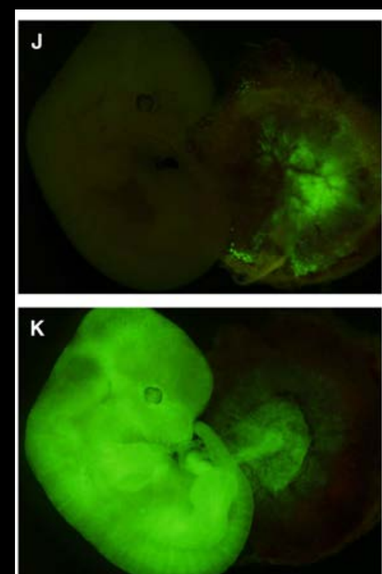


Tanaka *et al.*, 1998. *Science* 282:2072

Overexpression of Cdx2 in ES cells directs them to a TS cell fate



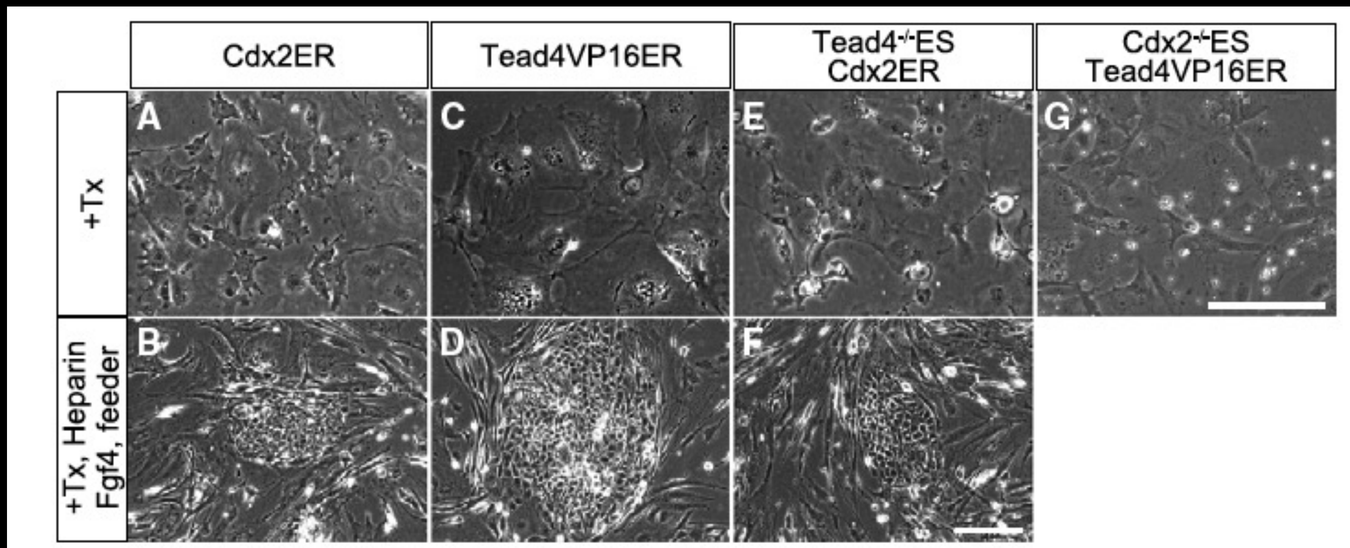
TS cell morphology in ES cells expressing Cdx2



Placental contribution of ES-derived TS cells generated by expression of Cdx2

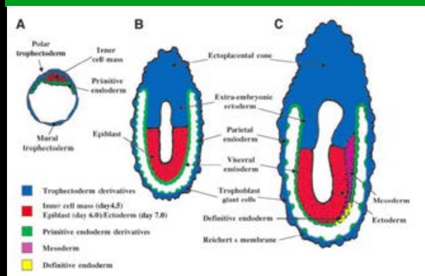
Niwa *et al.*, 2005, *Cell* 123:917-929.

Overexpression of Tead4 in ES cells directs them to a TS cell fate

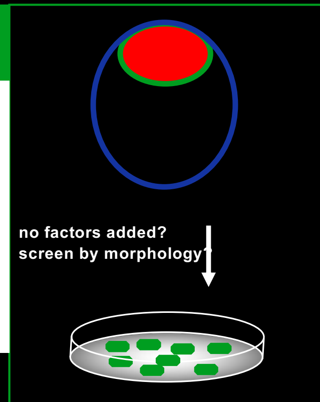


Nishioka *et al.*, 2008, *Dev Cell* 16:398

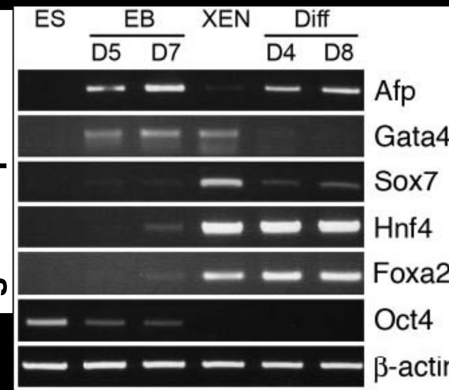
XEN cells



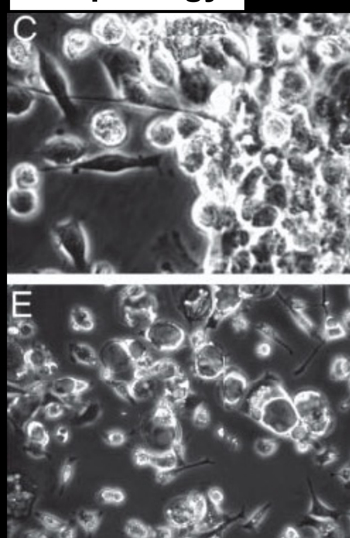
Kunath *et al.*, 2005, *Development* 132:1649-61.



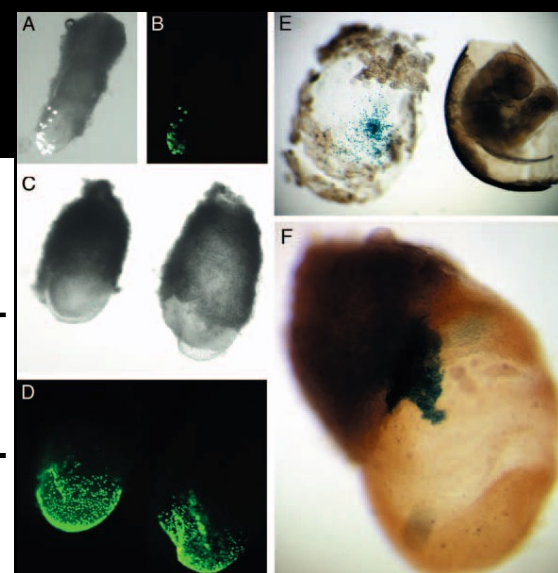
gene expression



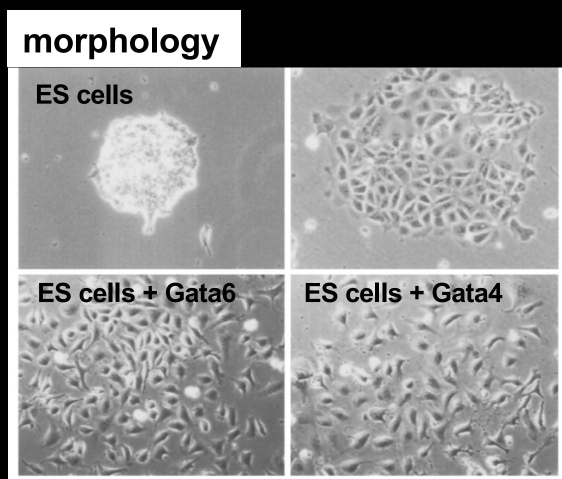
morphology

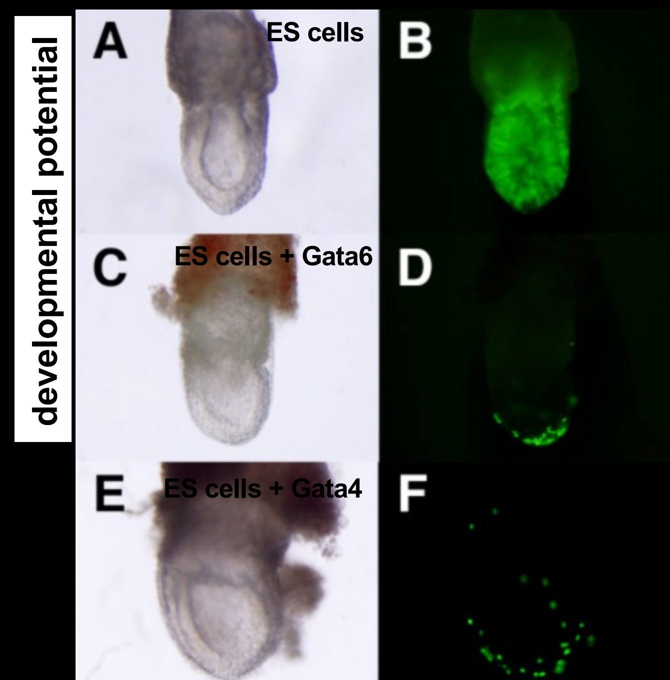


developmental potential



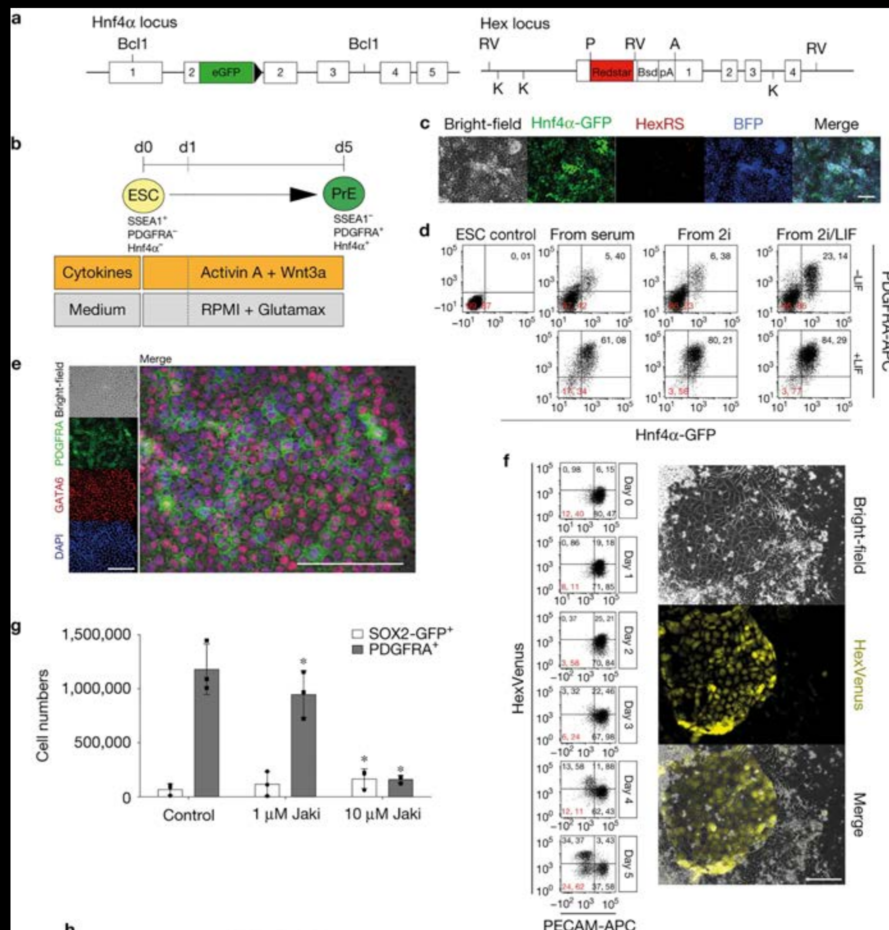
Overexpression of Gata6 (or Gata4) in ES cells directs them to a XEN cell fate



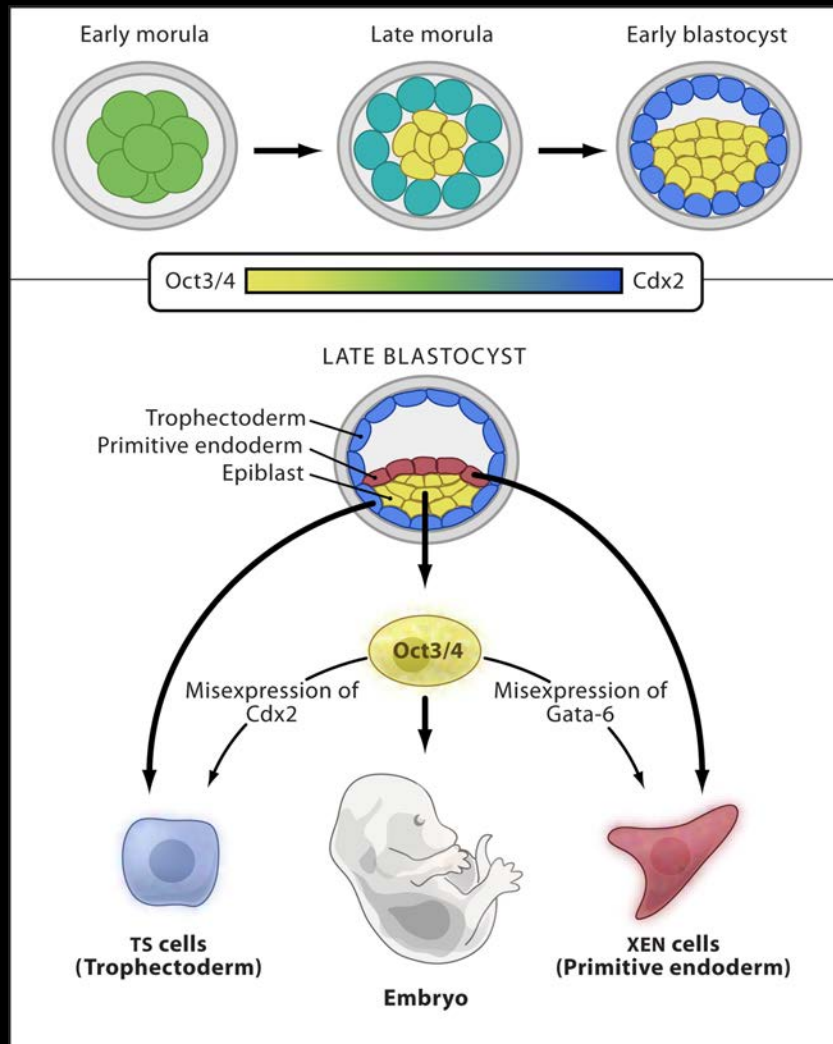


Fujikura *et al.*, 2002, *Genes and Development* 123:917-929.
Niwa *et al.*, 2007, *BMC Developmental Biology* 123:917-929.

Wnt3a and Activin (Nodal) drive primitive endoderm (PrE) differentiation of naive pluripotent cells (ESCs)

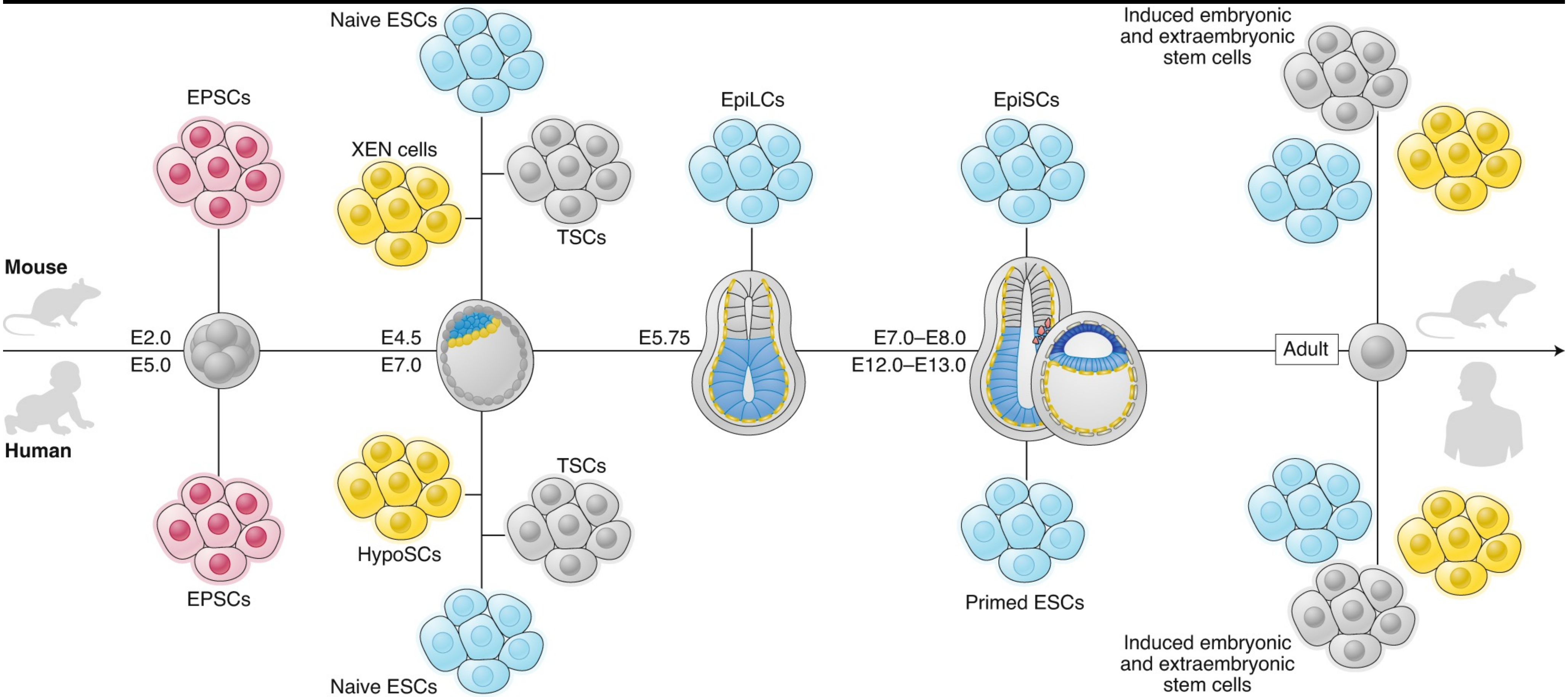


Anderson *et al.*, Nature Cell Biol 2017



Early morula
Late morula
Early blastocyst
Oct3/4
Cdx2
LATE BLASTOCYST
Trophectoderm
Primitive endoderm
Epiblast
Oct3/4
Misexpression of Cdx2
Misexpression of Gata-6
TS cells (Trophectoderm)
Embryo
XEN cells (Primitive endoderm)

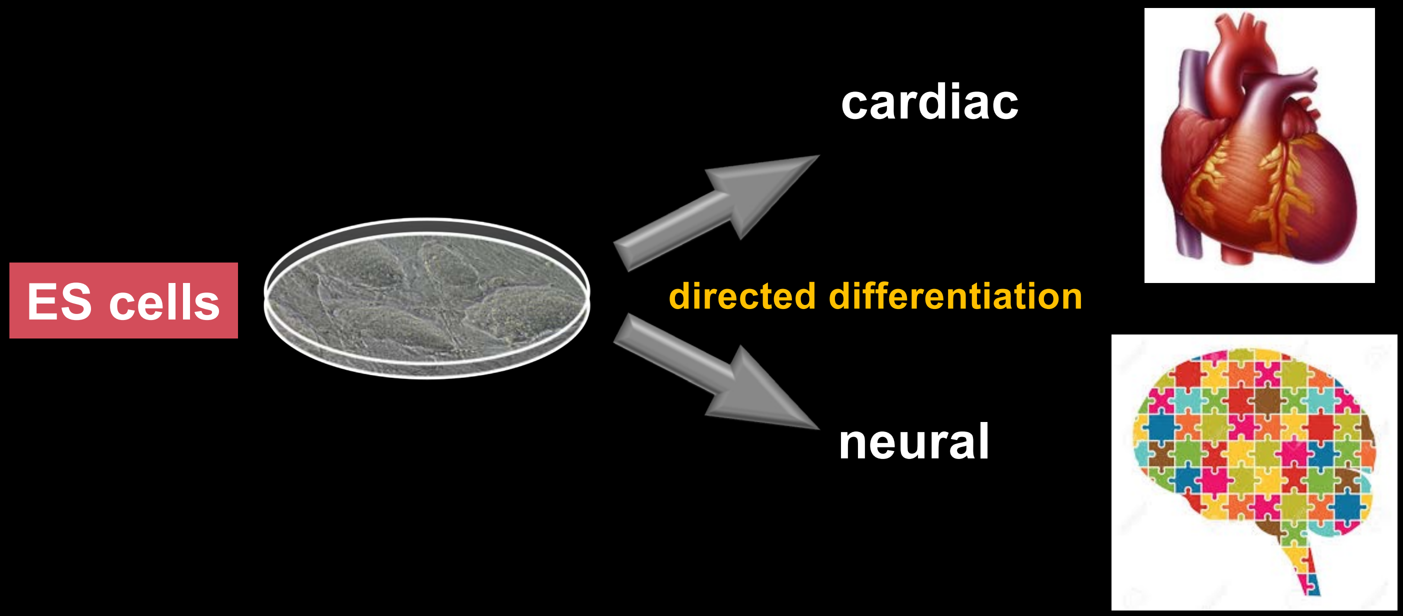
Mouse & human embryonic & extra-embryonic stem cells & their corresponding developmental potencies



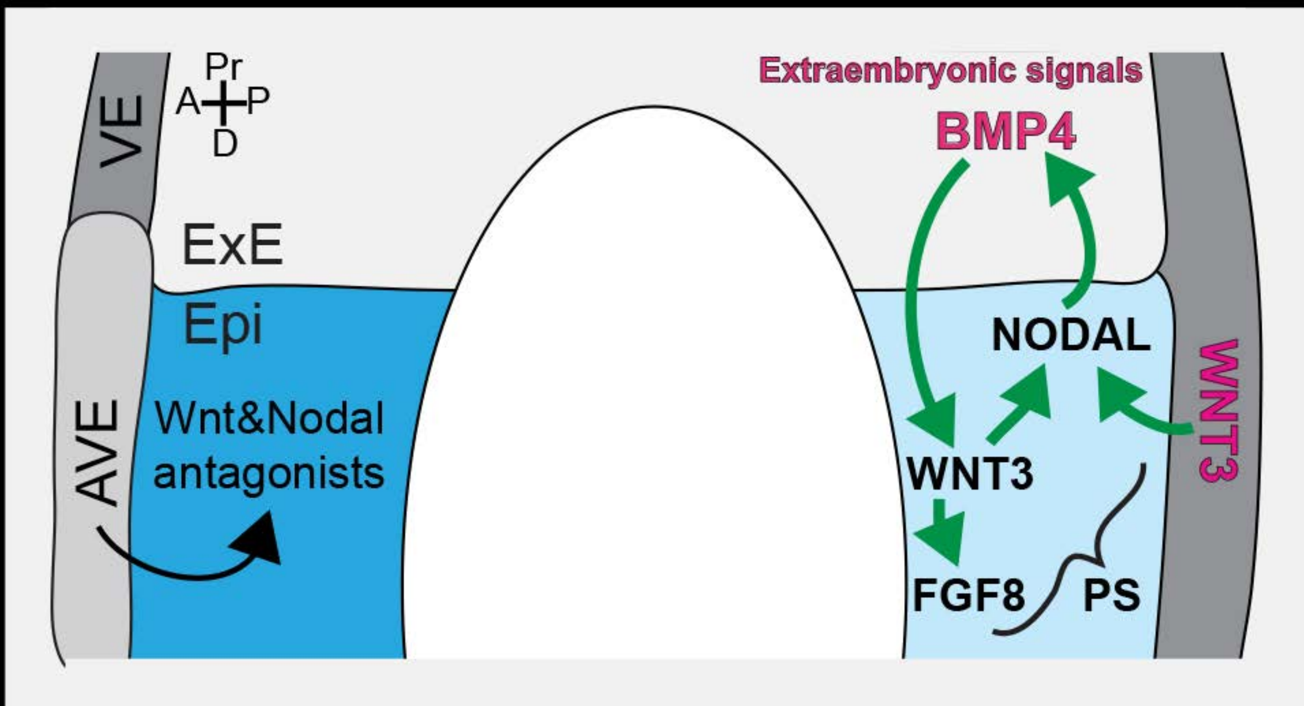
Fu et al., Nature Materials 2020

Stem Cell-derived Embryo Models

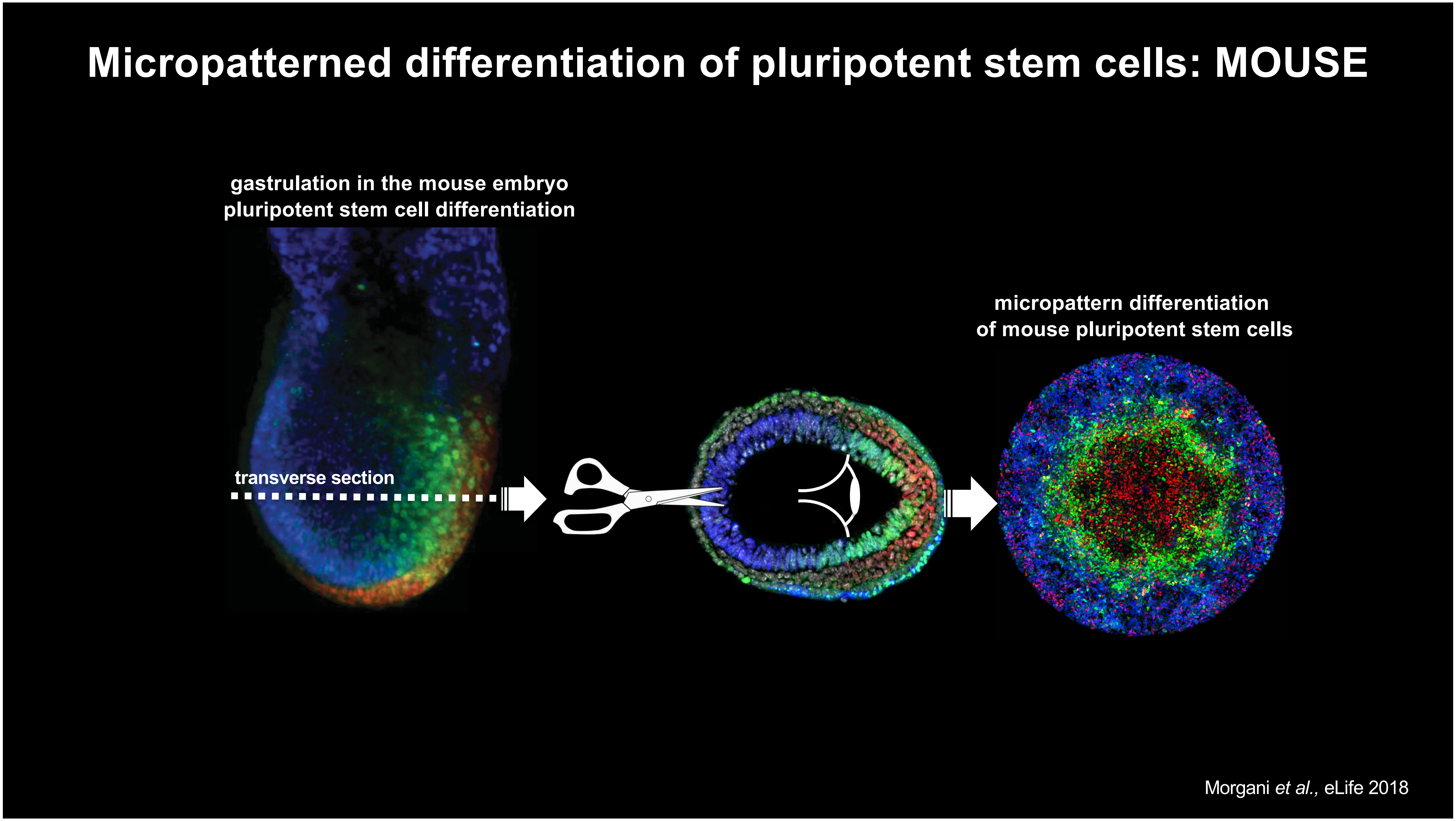
Blastocyst-derived stem cells: applications



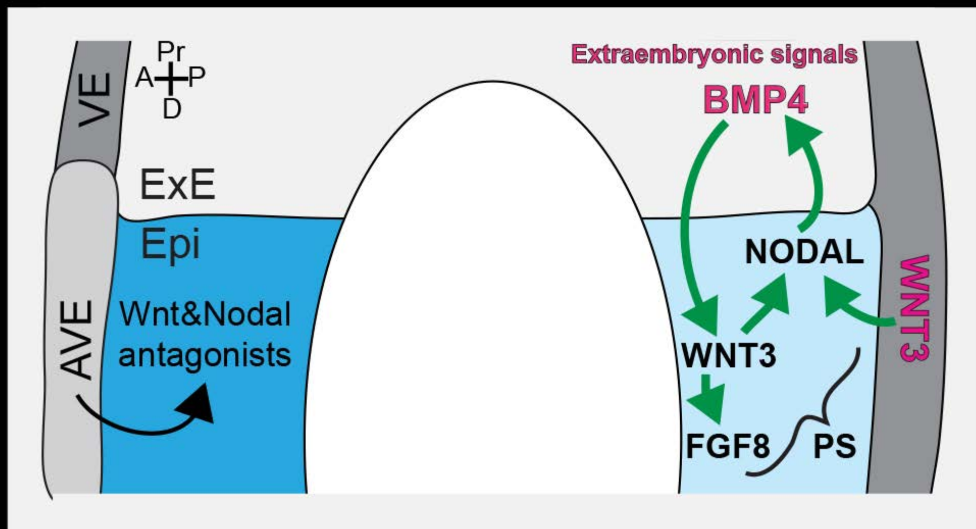
Mimicking the *in vivo* signals driving gastrulation initiation



BMP, NODAL, WNT & FGF
converge on posterior epiblast
driving formation of PRIMITIVE STREAK
& initiation of GASTRULATION


Micropatterned differentiation of pluripotent stem cells: MOUSE
gastrulation in the mouse embryo
pluripotent stem cell differentiation
transverse section
micropattern differentiation
of mouse pluripotent stem cells
Morgani *et al.*, eLife 2018

Mimicking the *in vivo* signals driving gastrulation initiation



BMP, NODAL, WNT & FGF
converge on posterior epiblast
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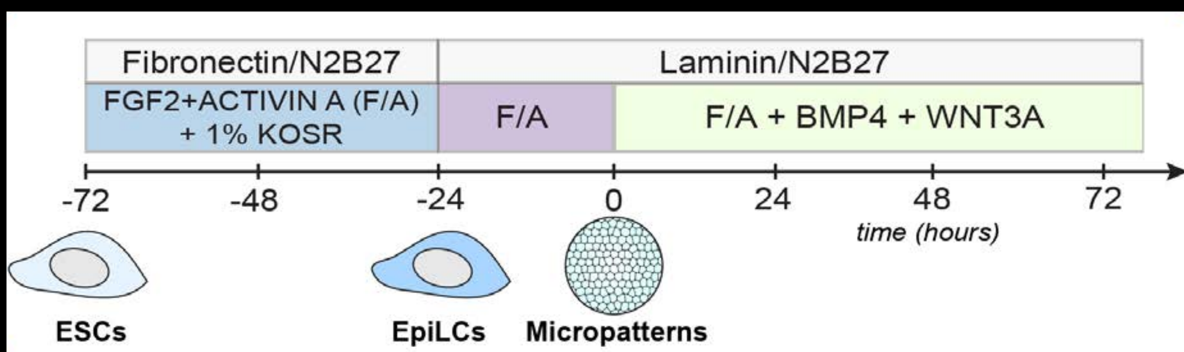
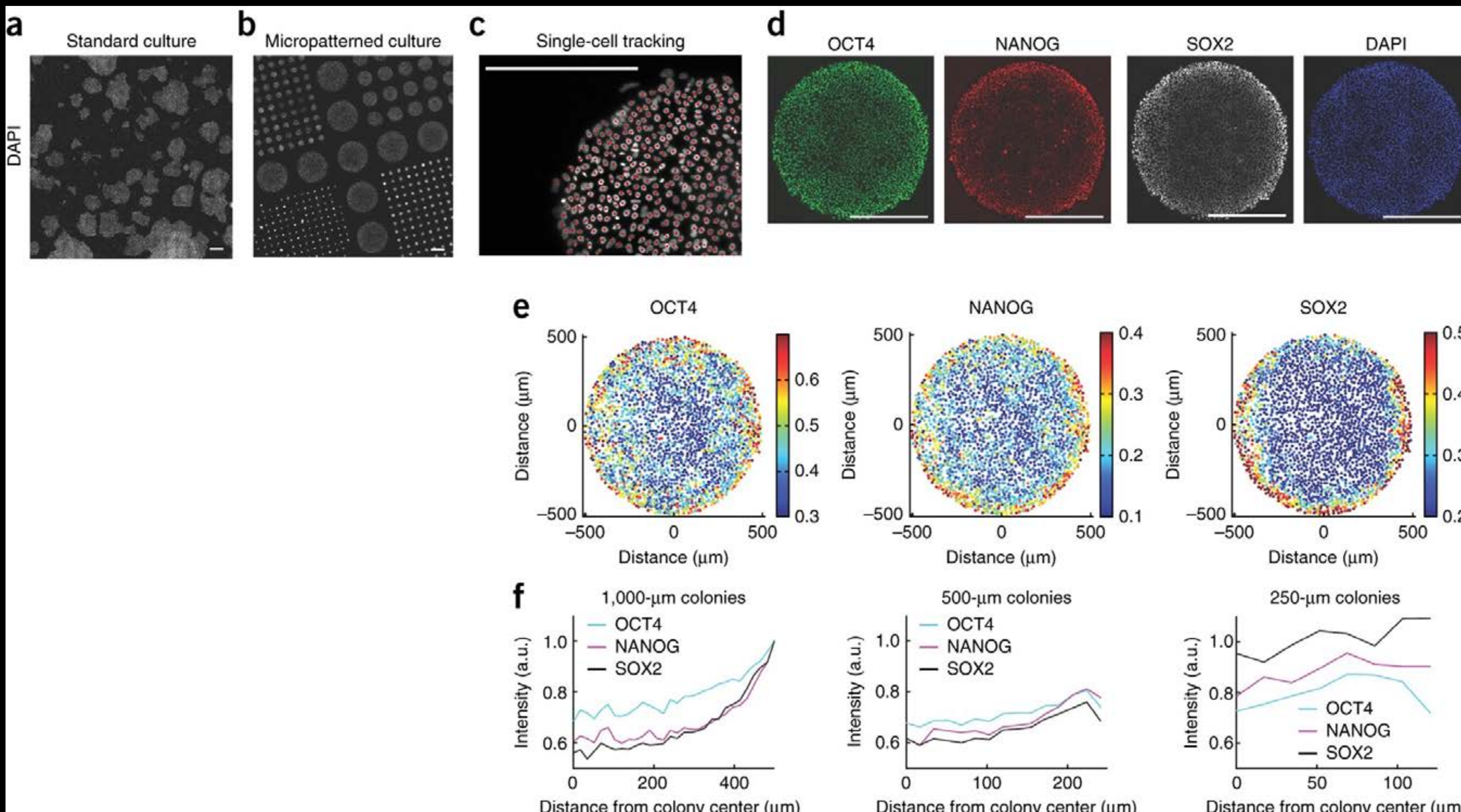
& initiation of GASTRULATION

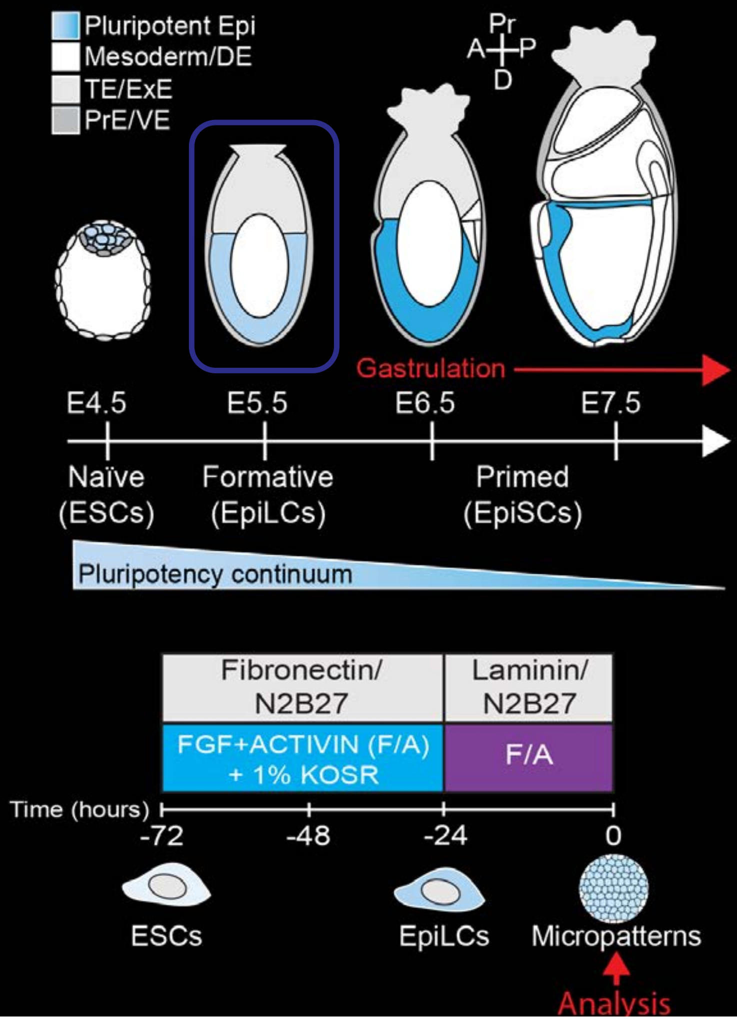


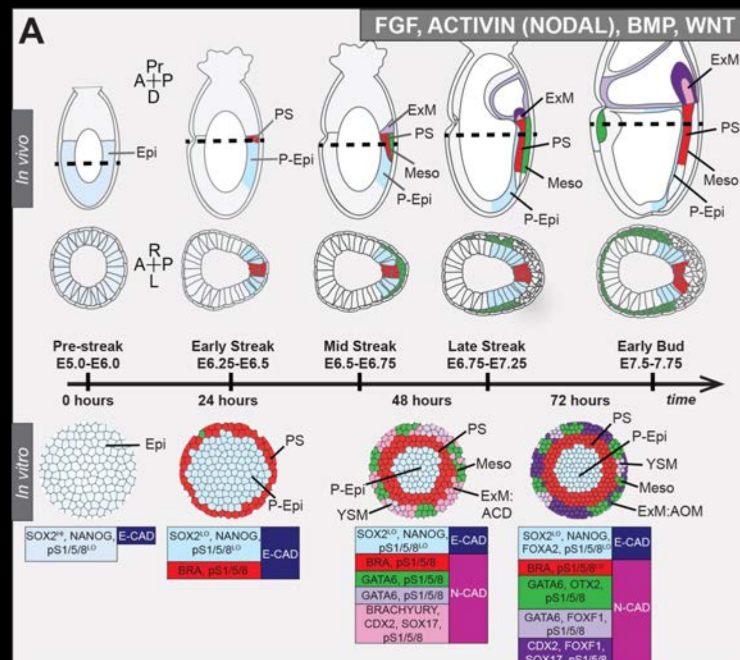
Micropatterned (2D) differentiation of pluripotent stem cells



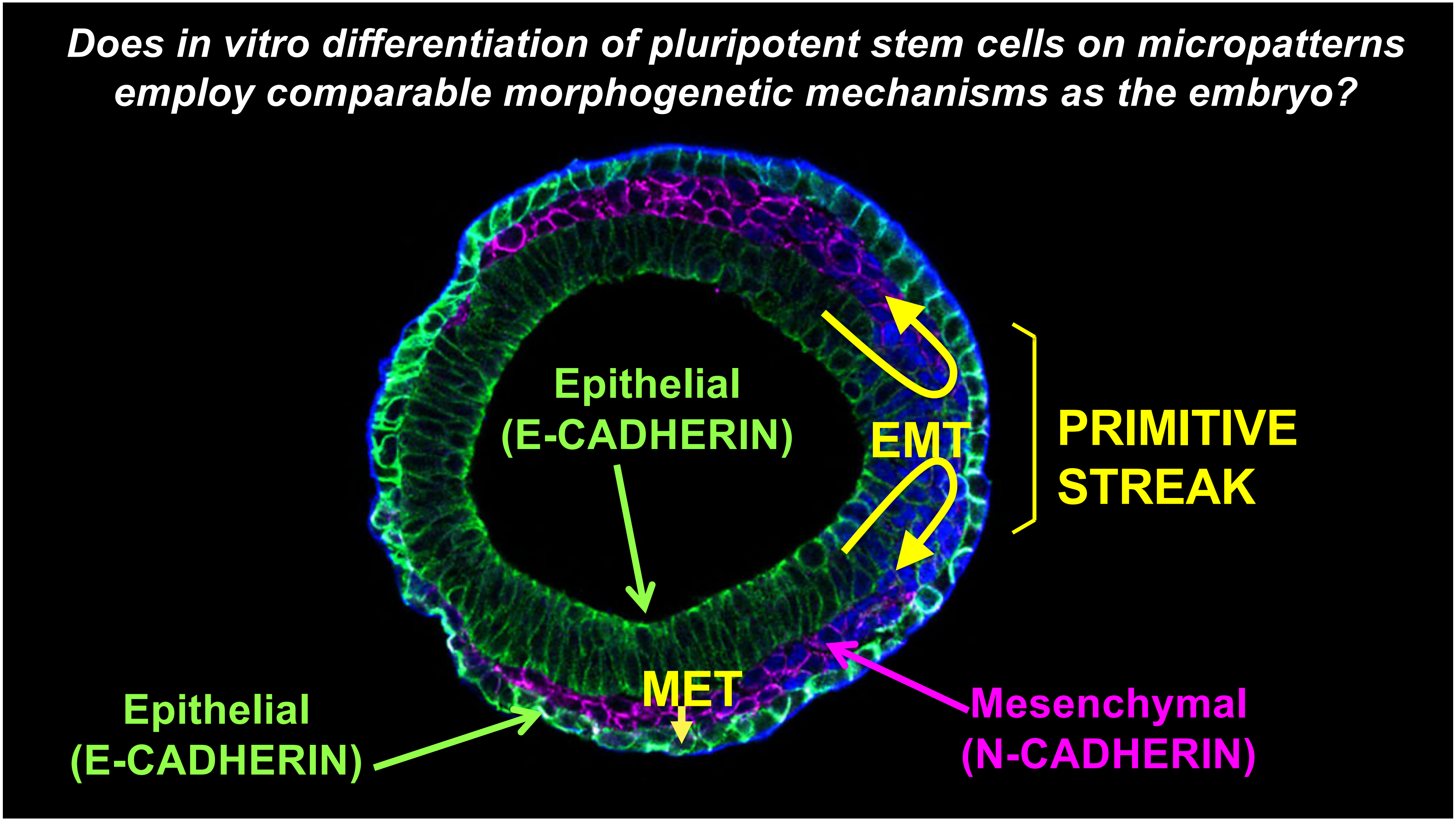
Warmflash *et al.*, Nature Methods 2014

EpiLCs correlate to the pre-gastrulation epiblast

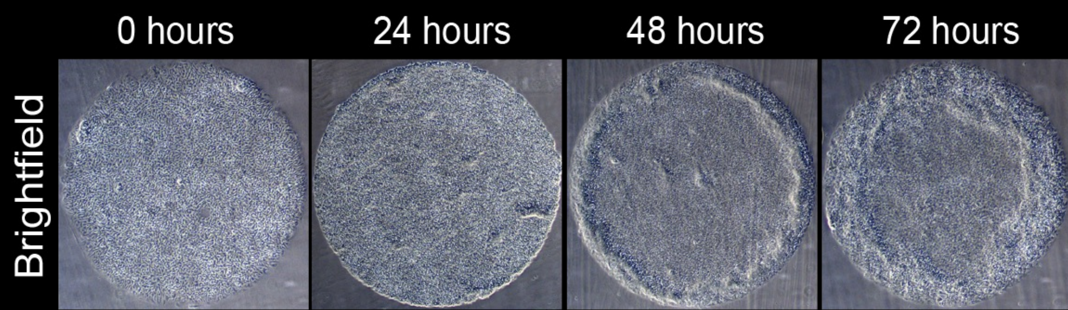


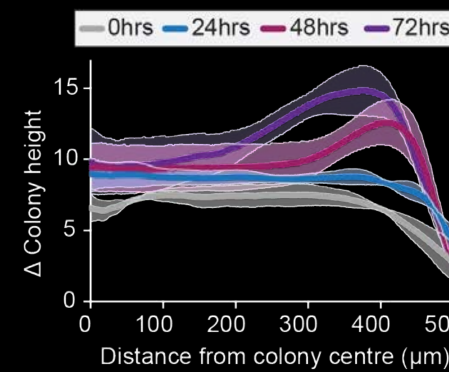


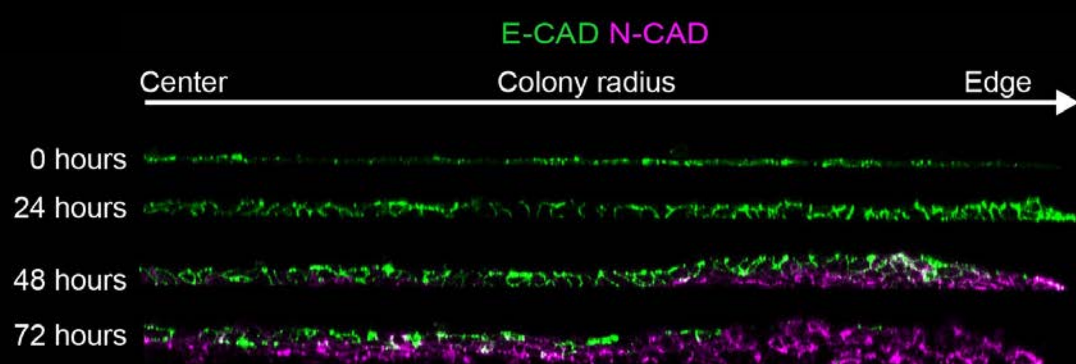
Morgani S. *et al.*, eLife 2018


Does in vitro differentiation of pluripotent stem cells on micropatterns employ comparable morphogenetic mechanisms as the embryo?
Epithelial (E-CADHERIN)
EMT
PRIMITIVE STREAK
MET
Epithelial (E-CADHERIN)
Mesenchymal (N-CADHERIN)

Differentiating EpiLCs undergo EMT





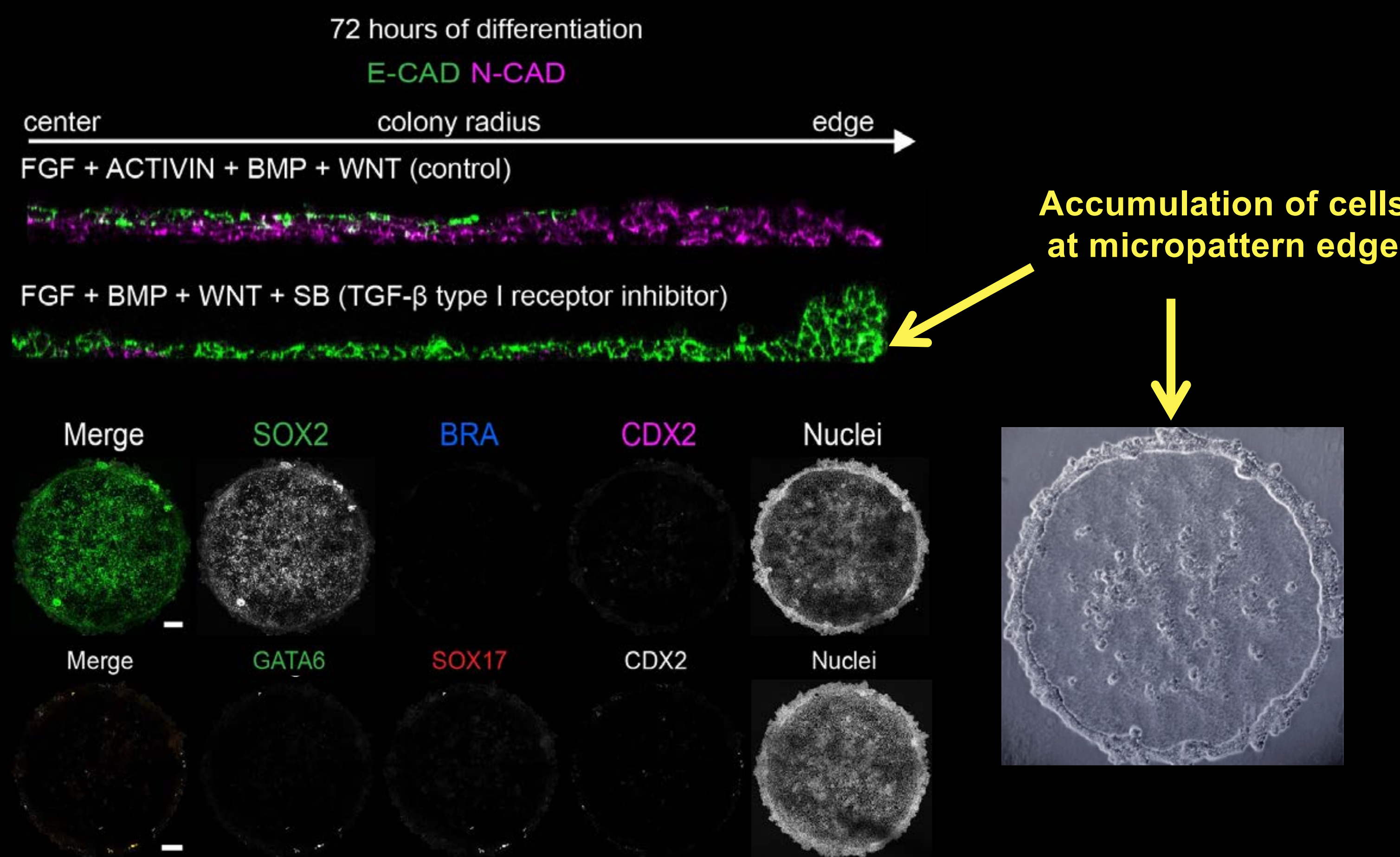


EMT

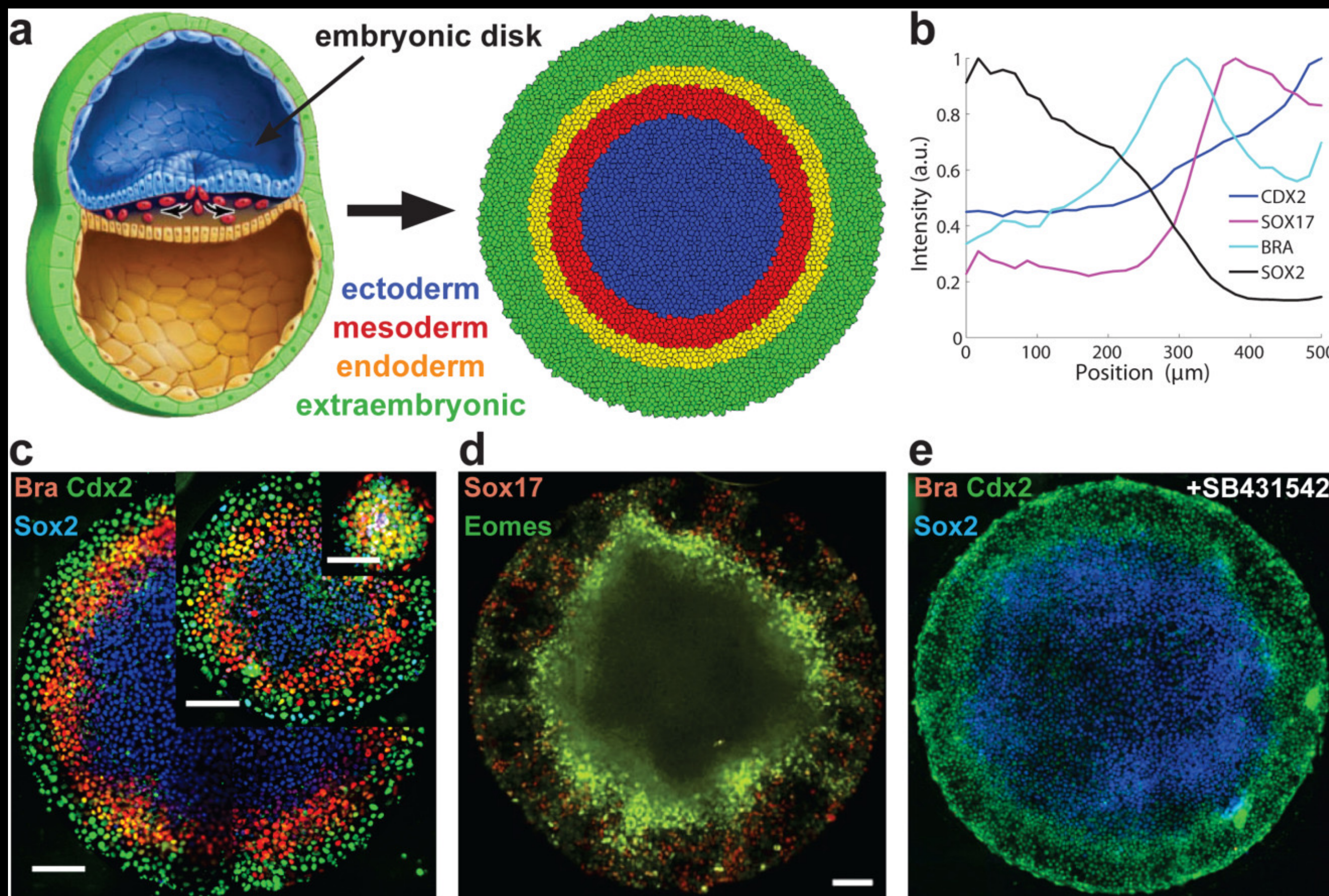
Epithelial → **Mesenchymal**

Posterior Epi → PS → Cell fates

Activin/Nodal signaling inhibition affects EMT (as it does in the embryo)

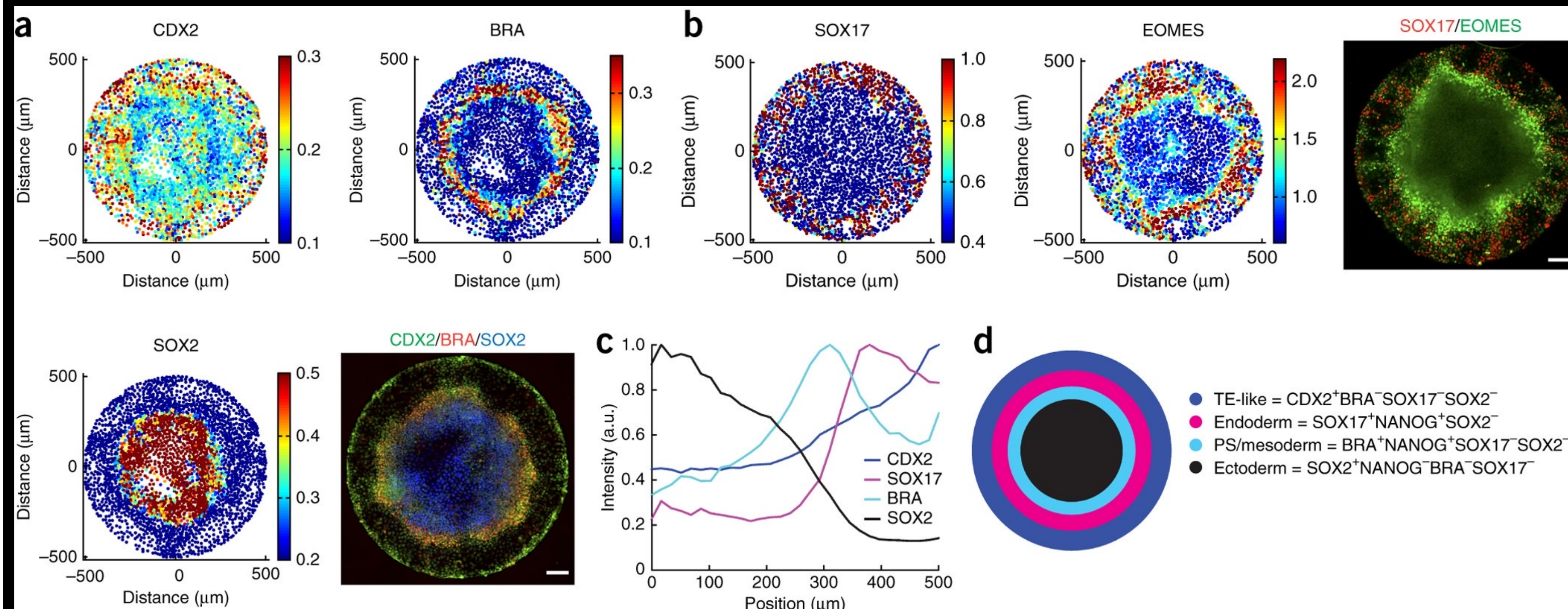


Micropatterned differentiation of pluripotent stem cells: HUMAN 2D GASTRULOIDS



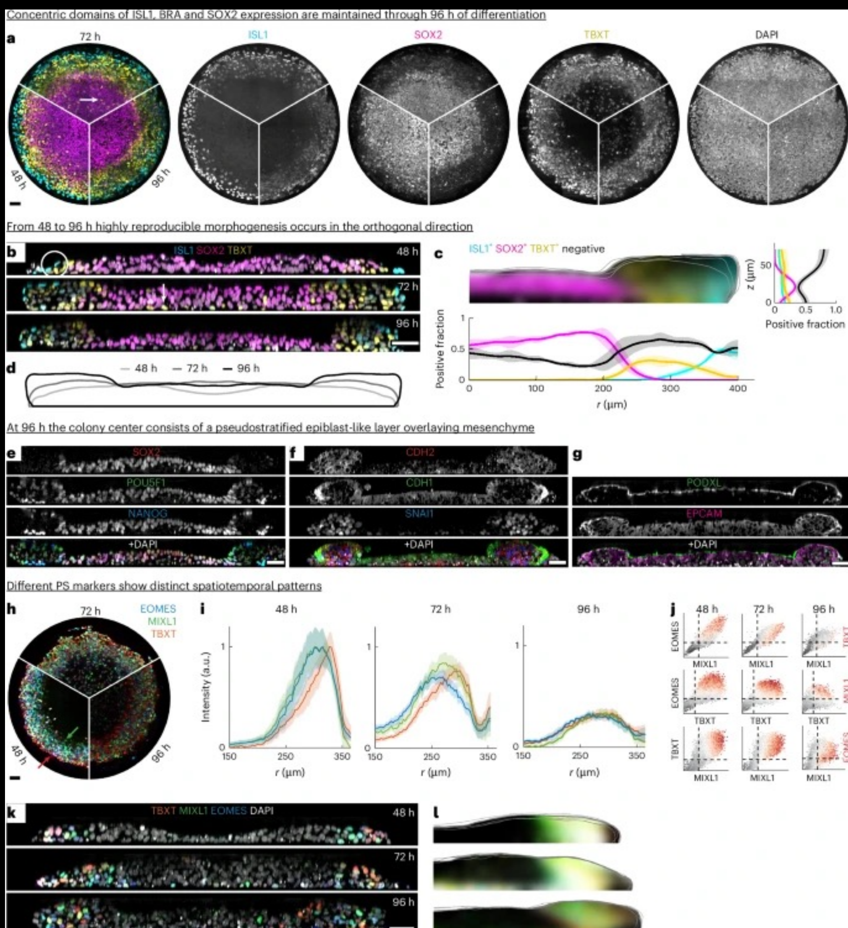
Warmflash *et al.*, Nature Methods 2014
Heemserk & Warmflash *et al.*, Dev Dynamics 2016
Liu *et al.*, Nature Comms. 2022
Chen *et al.*, Nature Methods 2025

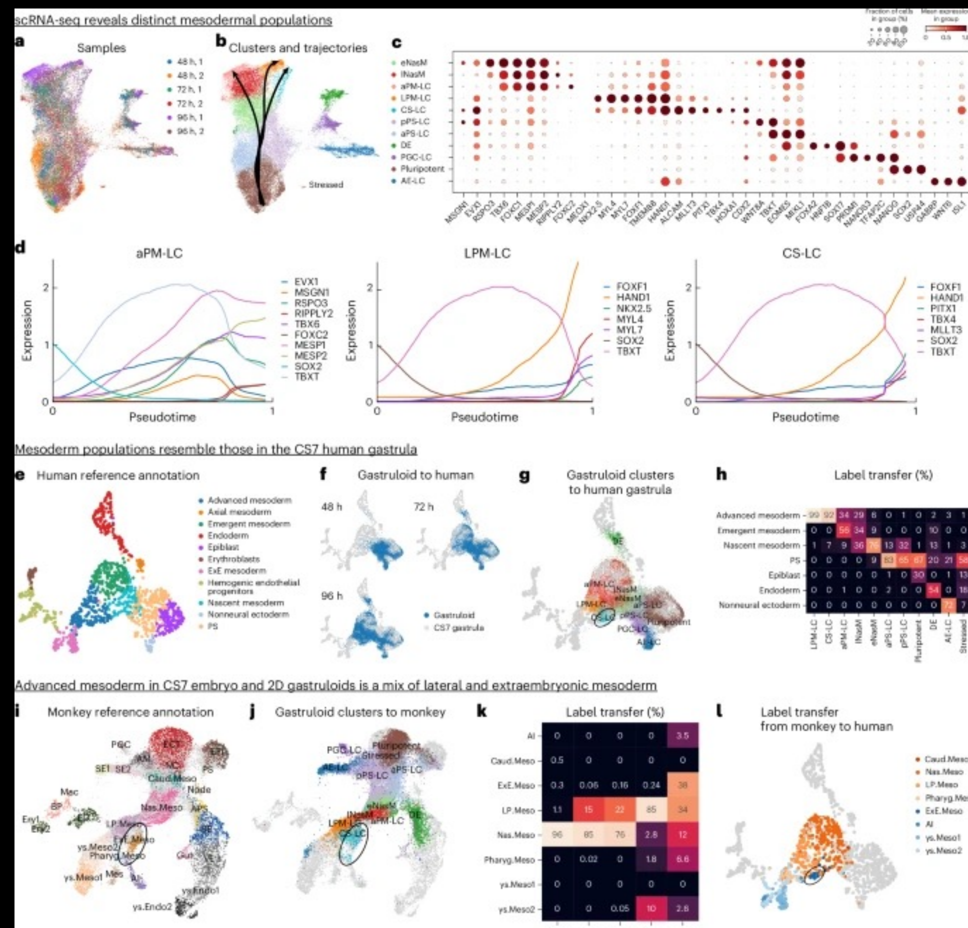
Micropatterned differentiation of pluripotent stem cells: HUMAN 2D GASTRULOIDS



Warmflash *et al.*, Nature Methods 2014

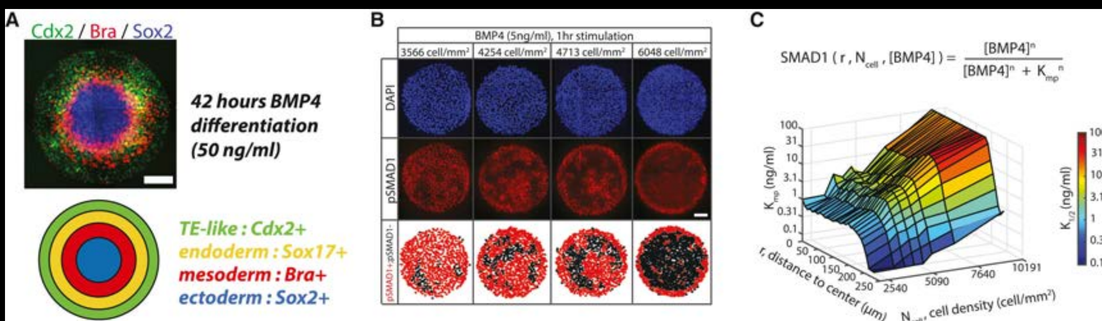
Extended culture of 2D gastruloids to model human mesoderm development

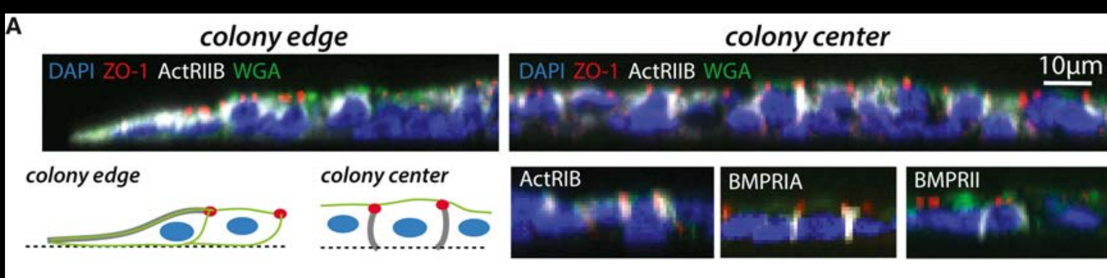


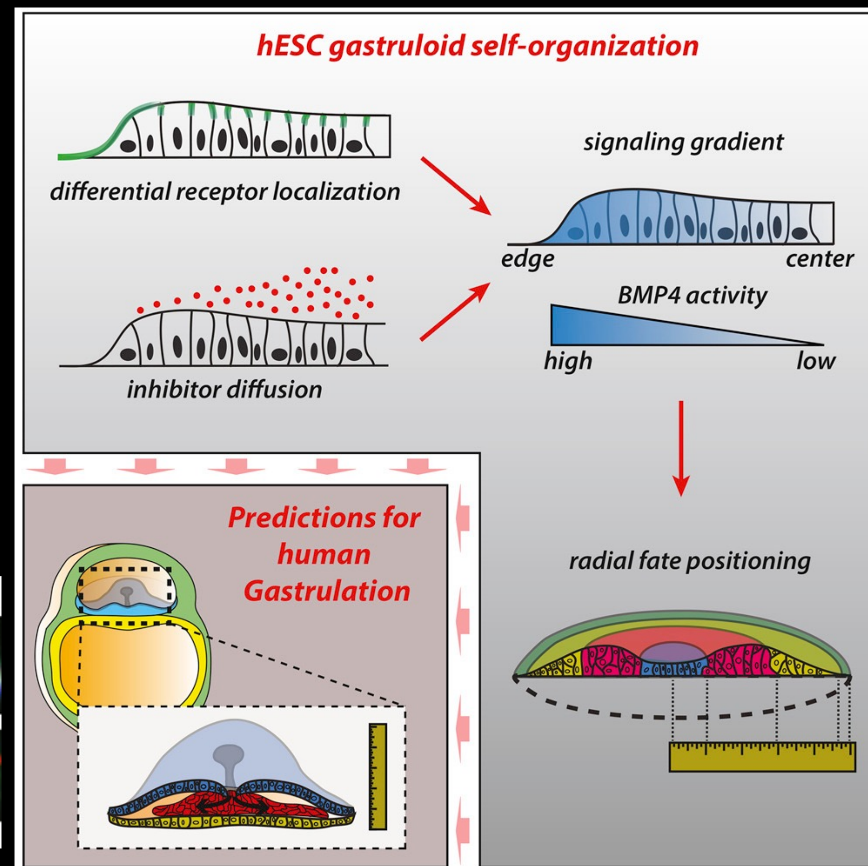


Chen *et al.*, Nature Methods 2025

Differential receptor availability drives micropatterned differentiation

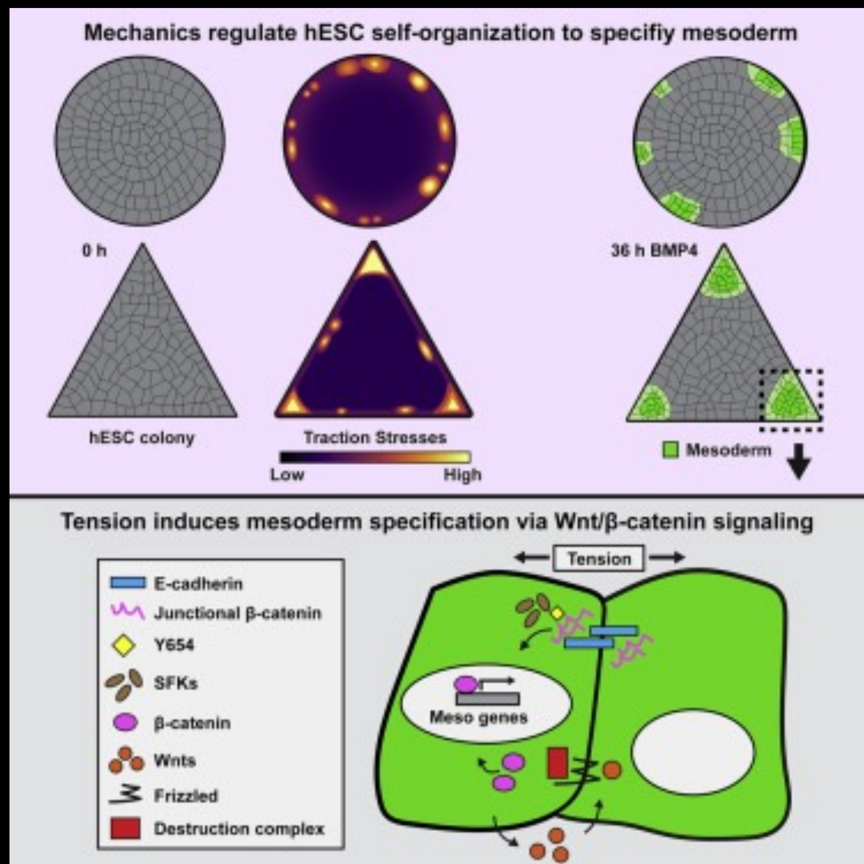






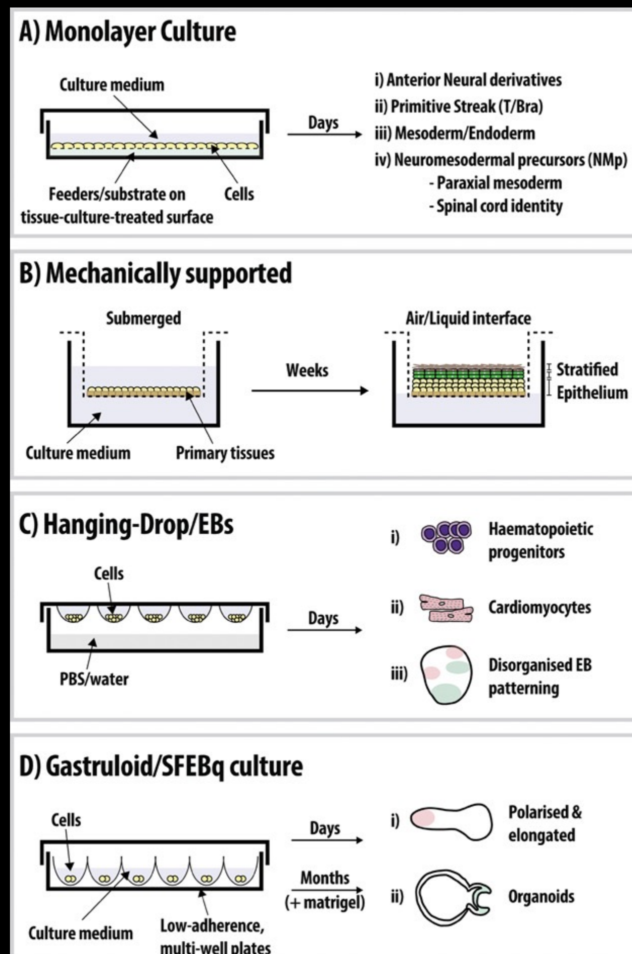
Etoc et al., Developmental Cell 2016

Mechanical Tension Promotes Formation of Gastrulation-like Nodes and Patterns Mesoderm Specification in HESCs



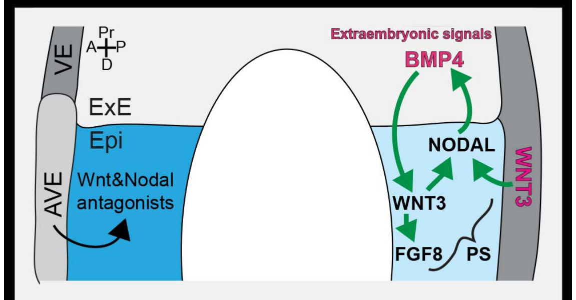
Muncie et al., Developmental Cell 2020
Schwarz & Hadjantonakis Developmental Cell 2020

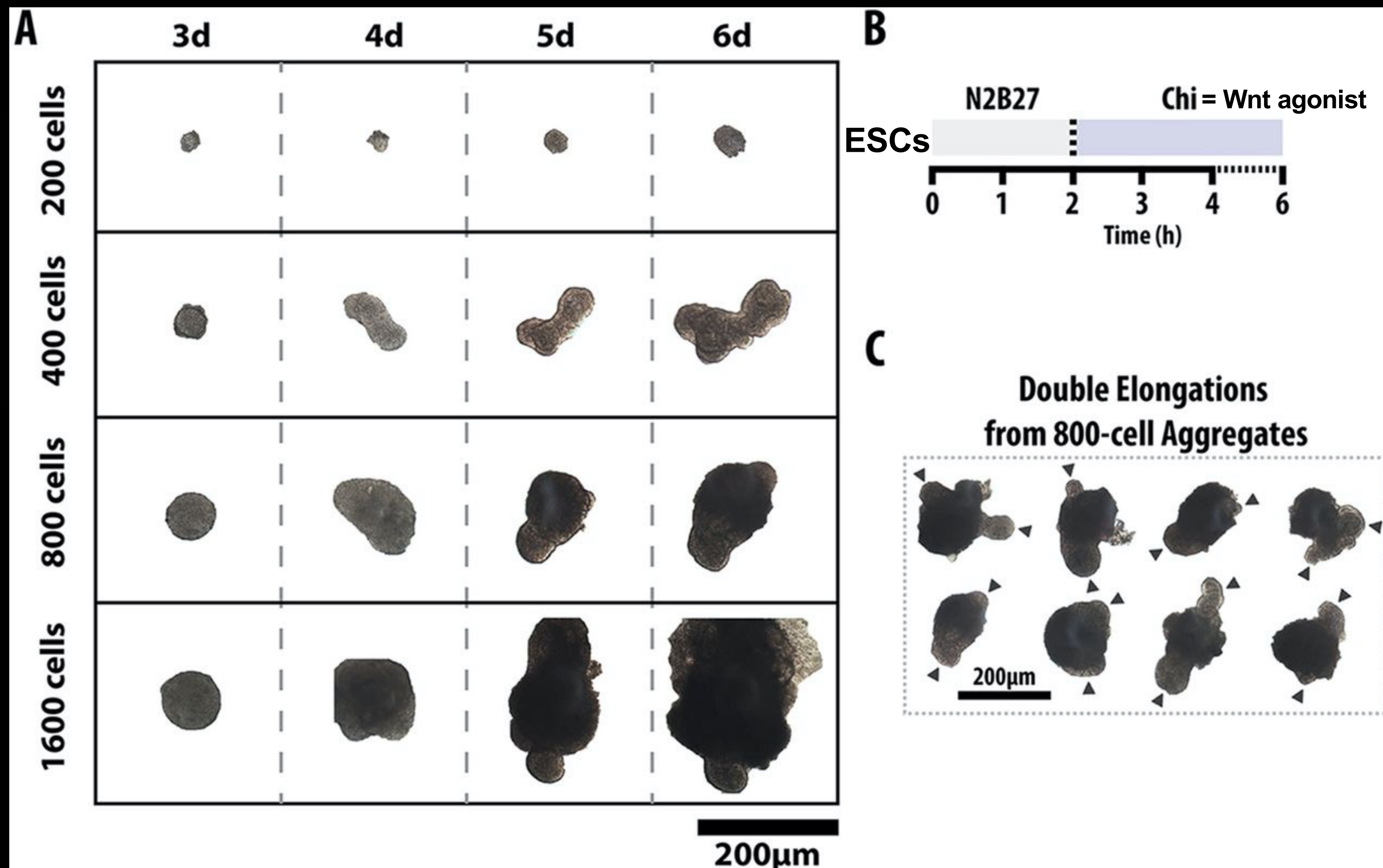
Different differentiation protocols for different ESC-based embryo models



Turner *et al.*, Bioessays 2015

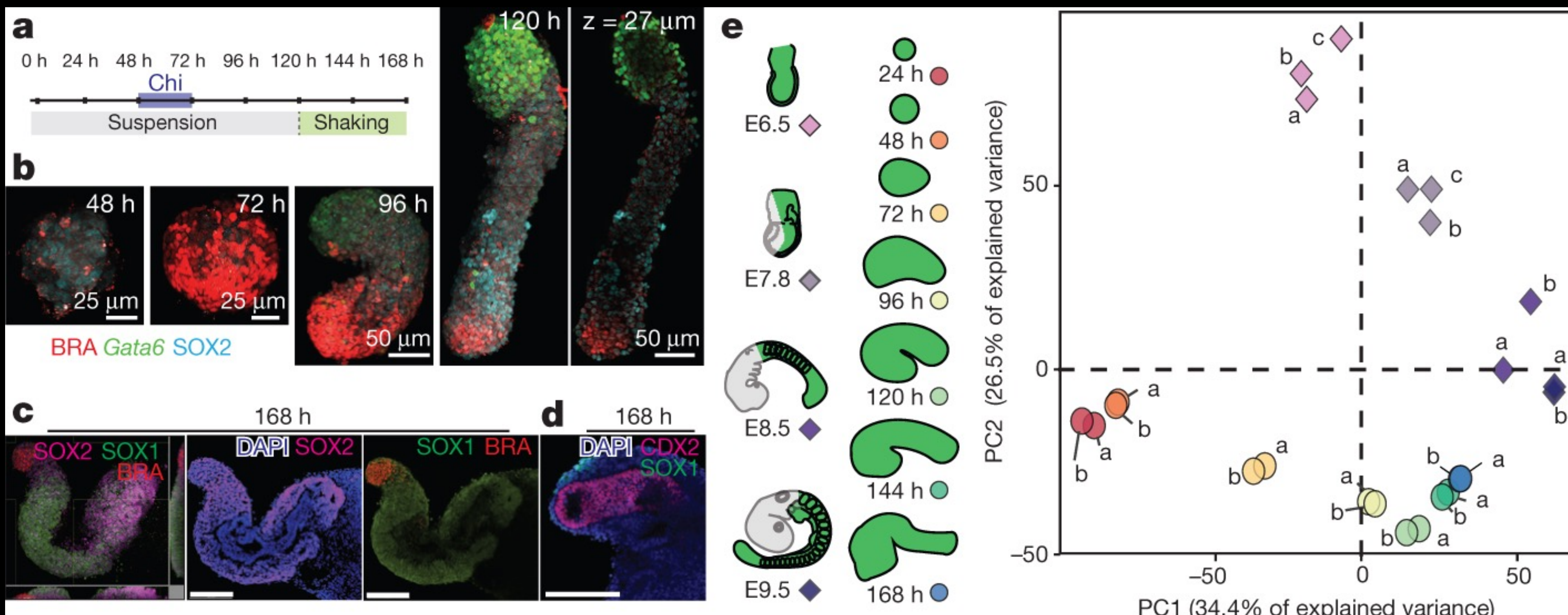
3D GASTRULOIDS: MOUSE





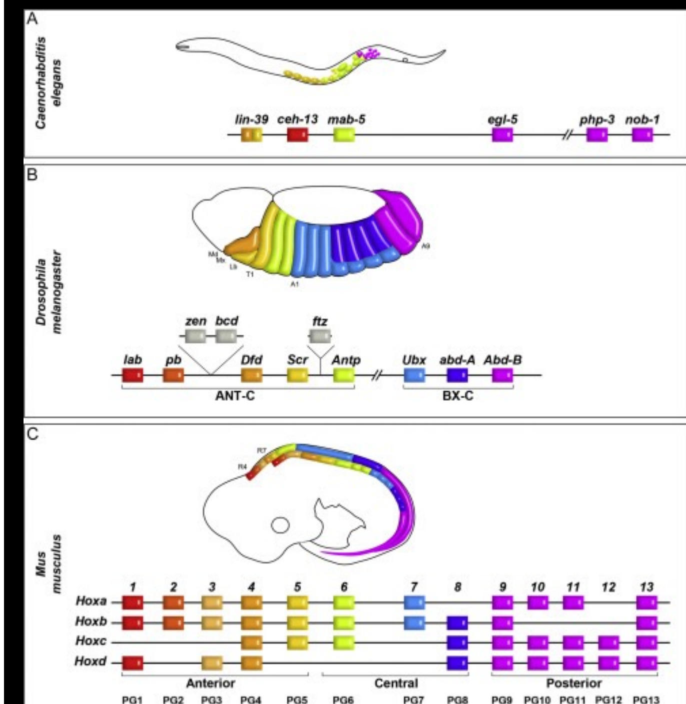
Van den Brink *et al.*, Development 2014

Multi-axial self-organization properties of mouse ESCs into 3D gastruloids

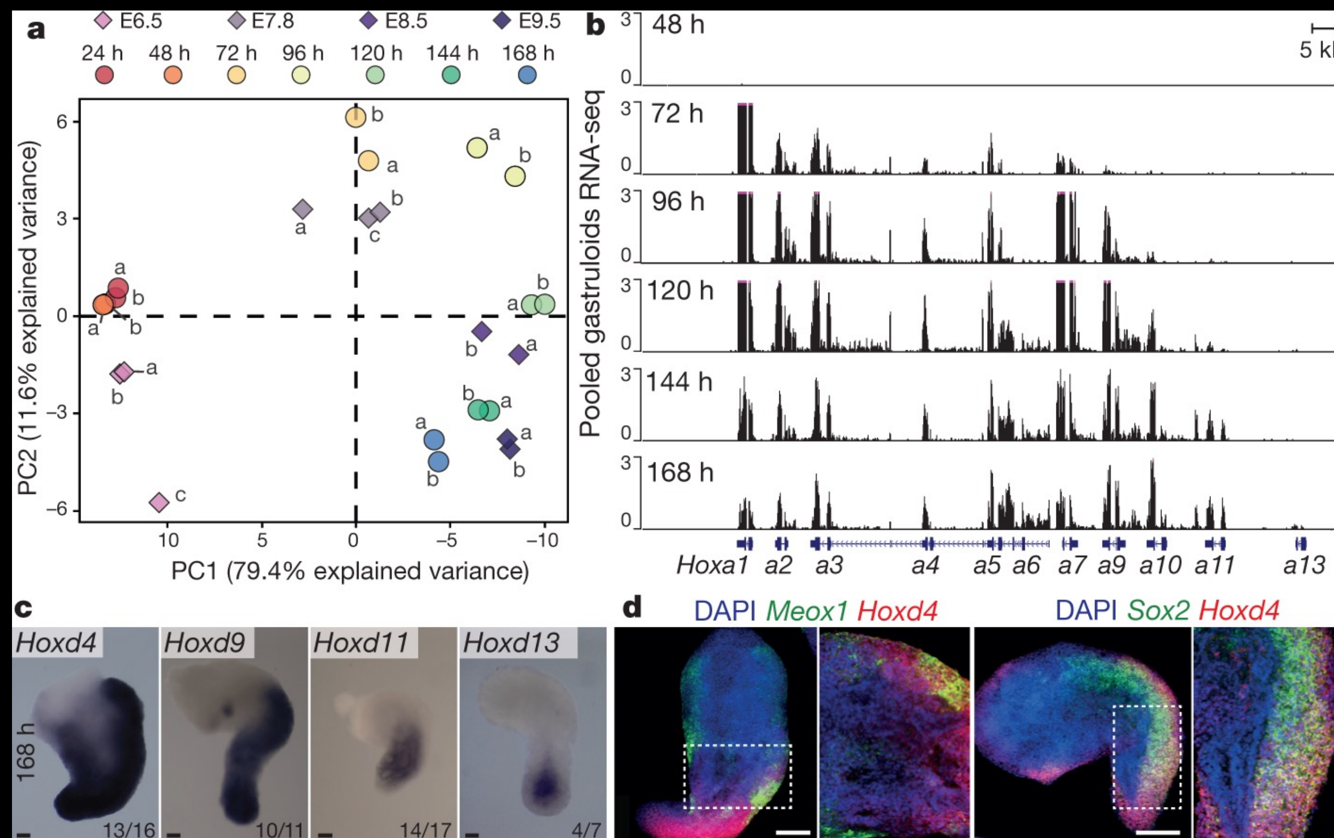


Becari *et al.*, Nature 2018

Collinear *Hox* gene expression in mouse 3D gastruloids

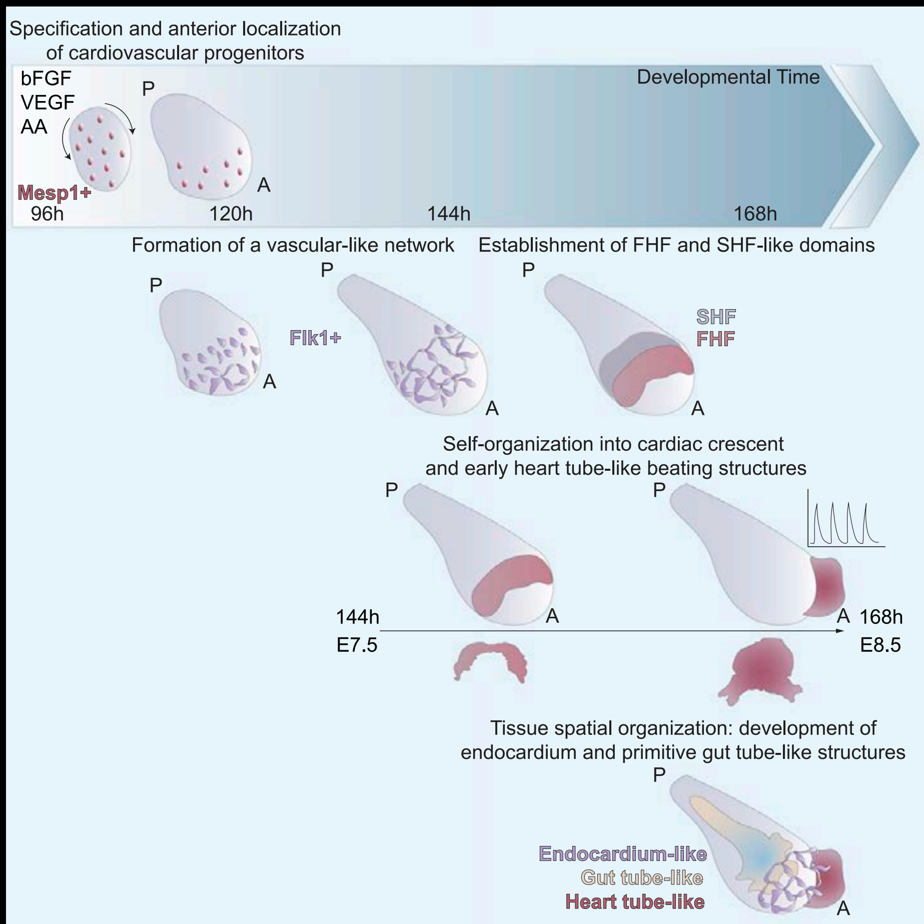


Krumlauf *et al.*, 2013



Becari *et al.*, Nature 2018

3D Gastruloids generate cardiac structures



- Gastruloids generate cardiovascular progenitors and form a vascular-like structure

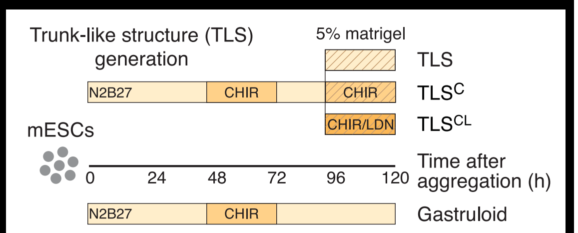
- Both first and second heart field-like progenitors are specified

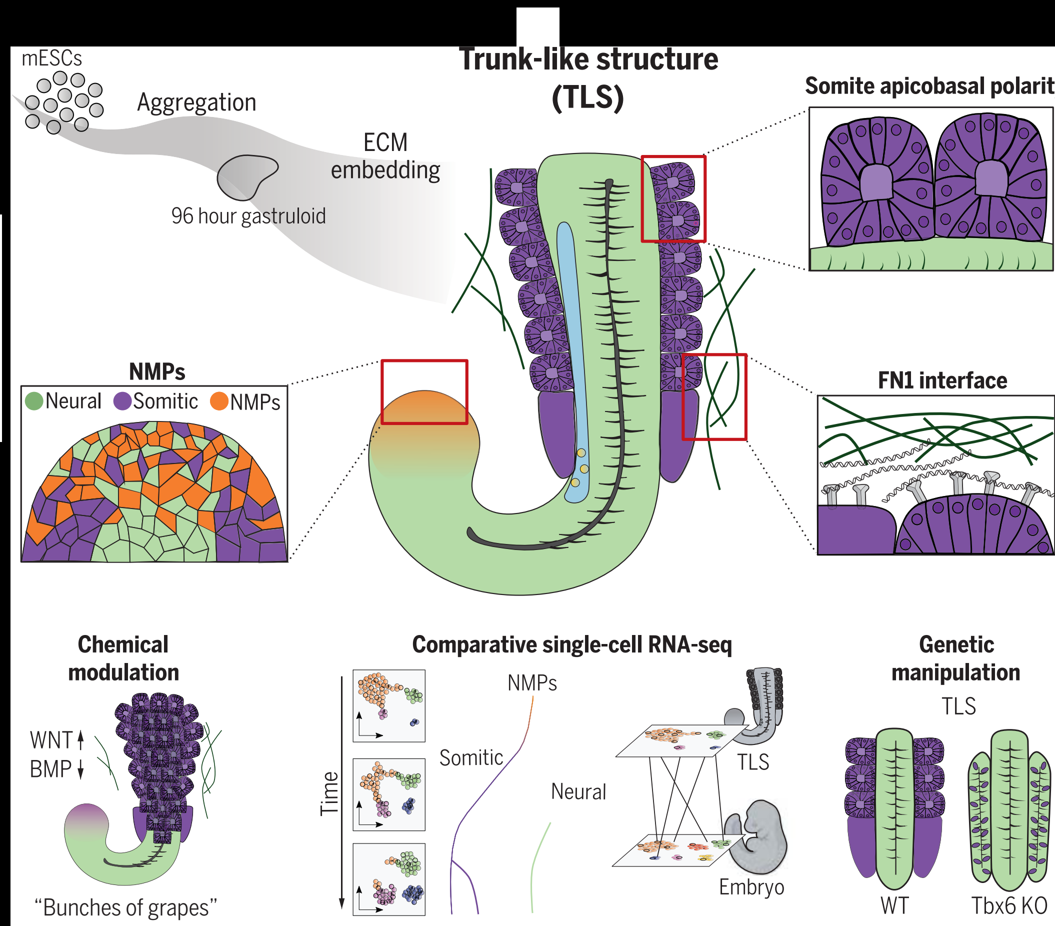
- Cardiac progenitors self-organize into crescent and heart tube-like beating domains

- Cellular diversity and tissue-tissue interactions mimic embryonic development

Rossi *et al.*, Cell Stem Cell 2021

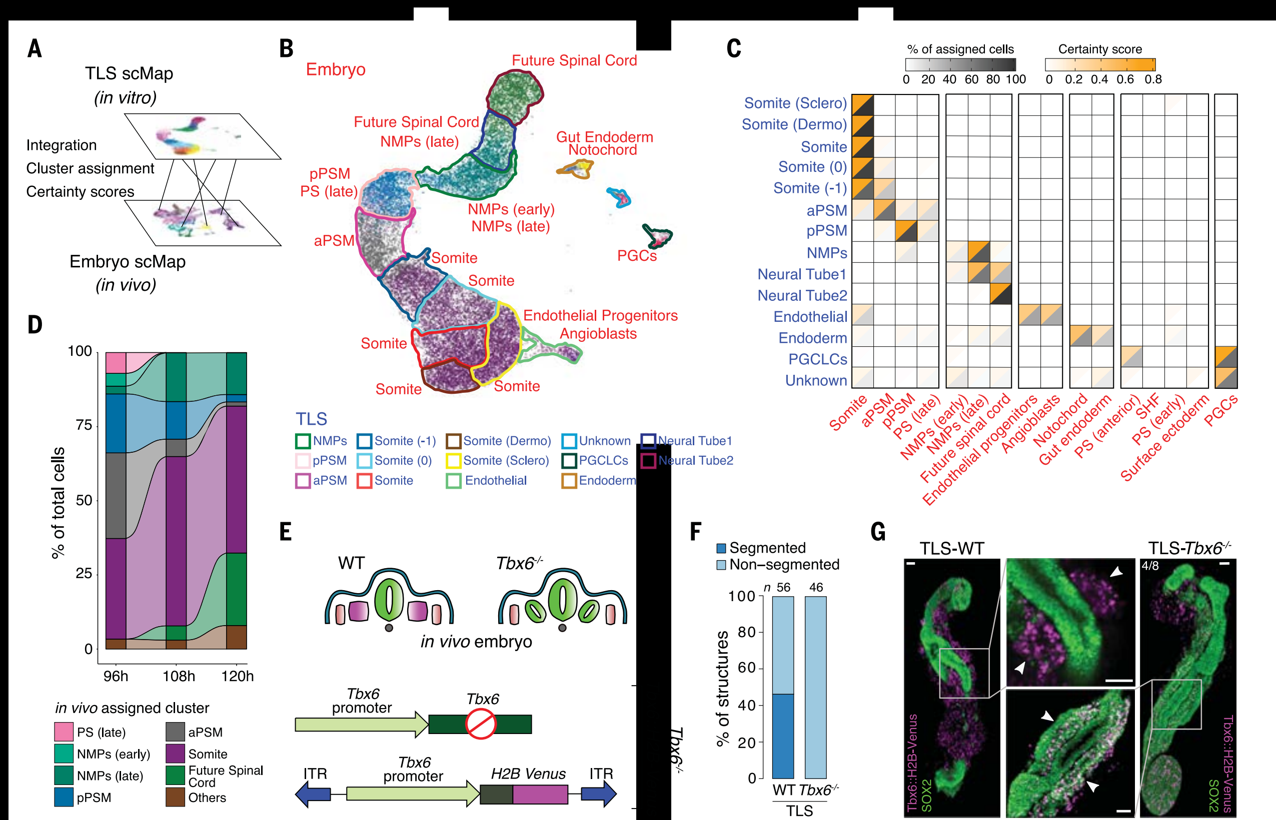
Gastruloids organize into trunk-like structures including neural tube and somites





Veenelevet *et al.*, Science 2020

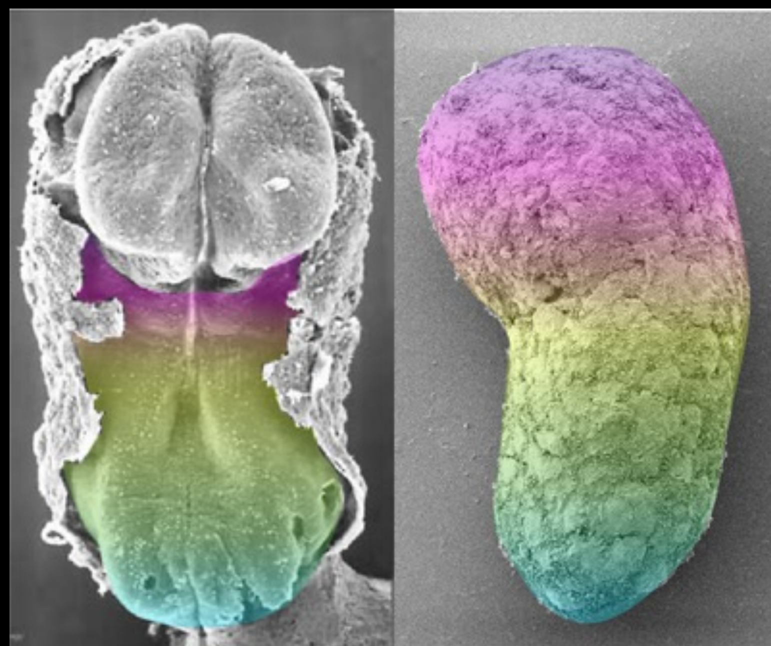
TLS cell states are embryo-like and Tbx6–/– TLSs recapitulate the embryonic knockout phenotype



TLS = tail like structure

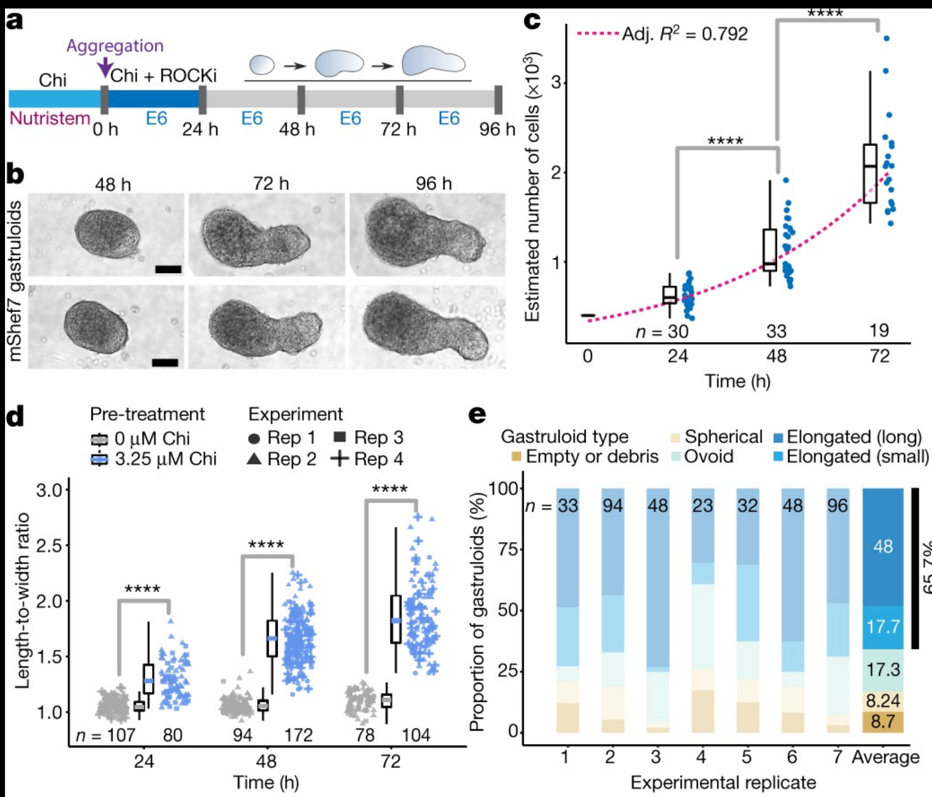
Veenelevet *et al.*, Science 2020

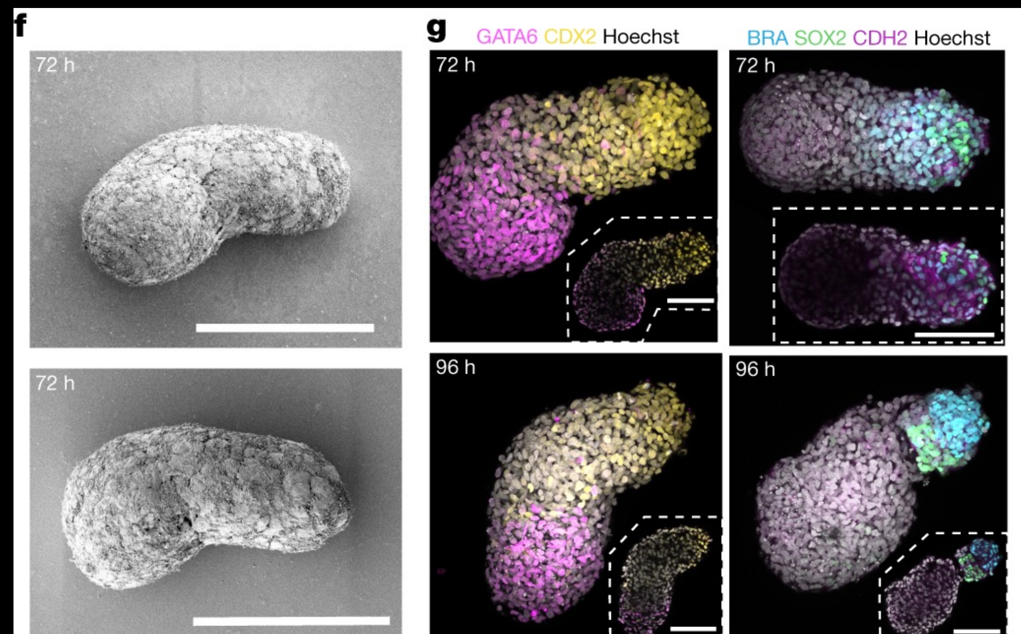
3D GASTRULOIDS: HUMAN



Comparison between a 20 day old human embryo and a human gastruloid.
Left; False-colored Carnegie Stage 9 human embryo, with additional brain/neural folds and extraembryonic tissues (not colored).
Right; False-colored 72h human gastruloid. Coloring indicates estimated similarity of gene expression profiles.

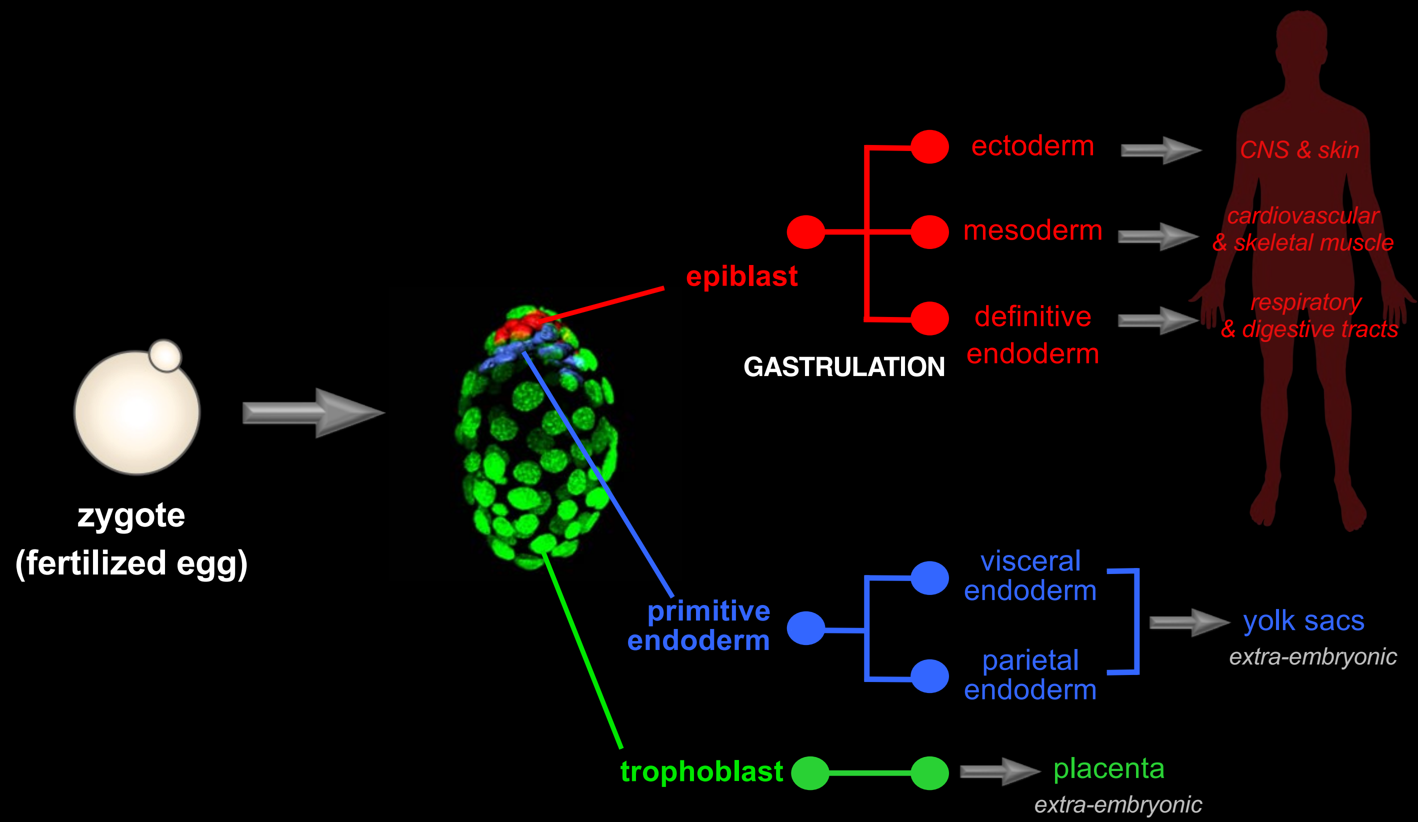
3D GASTRULOIDS: HUMAN





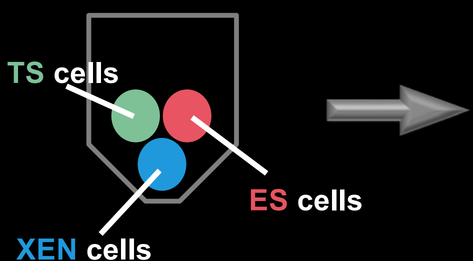
Noris *et al.*, Nature 2020

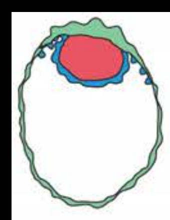
Lineage contributions of the mammalian blastocyst



INTEGRATED SYSTEMS
(comprising more than 1 stem cell type co-cultured)

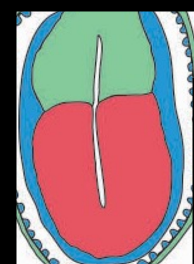
aggregation of different stem cells in a microwell





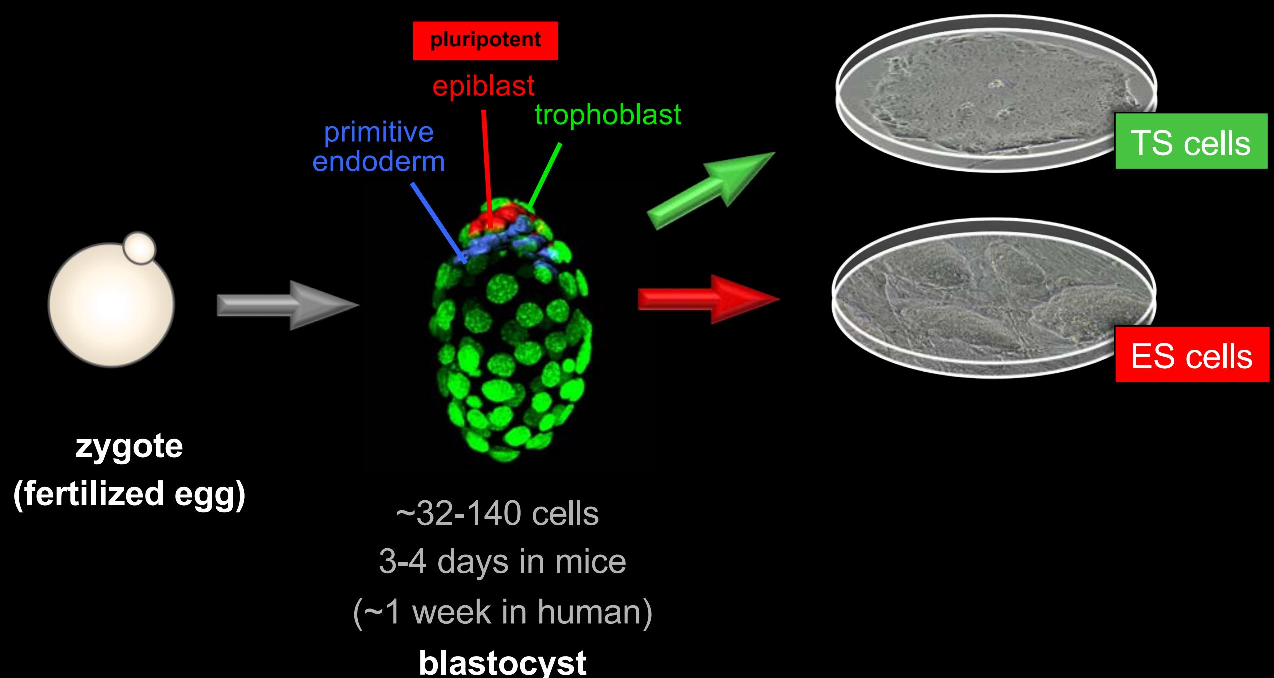
In vitro reconstituted
Blastoids
Rivron *et al*., Nature 2018
Li *et al*., Cell 2019

OR

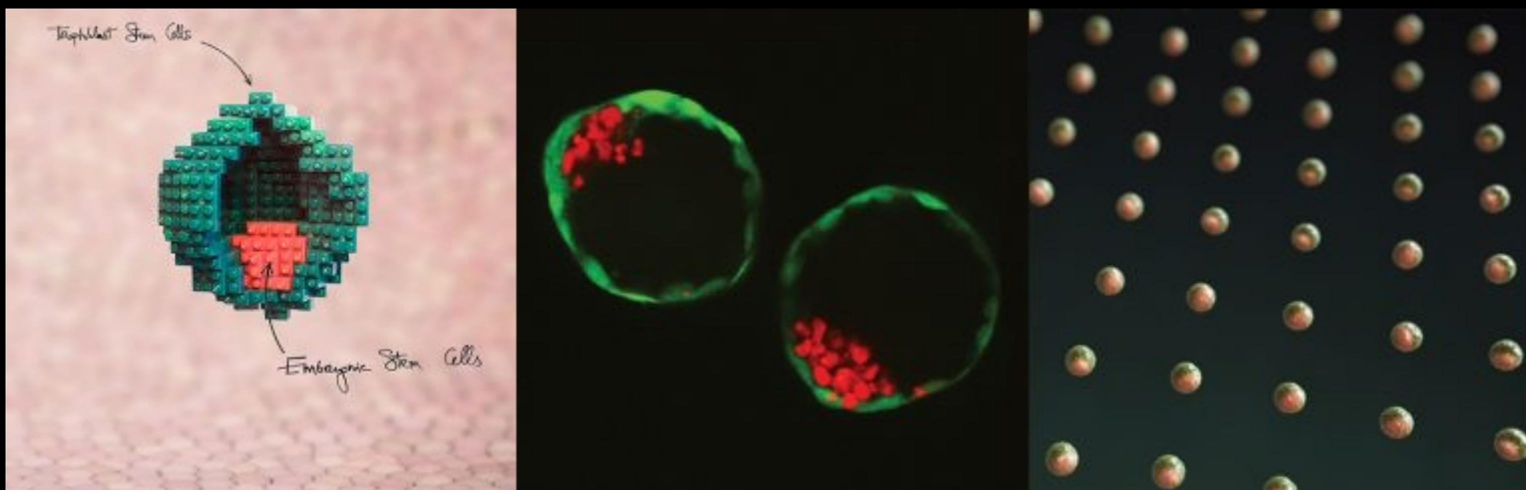


In vitro reconstituted
ETS & ETX 'embryos'
Harrison *et al*., Science 2017
Sozen *et al*., Nature Cell Bio. 2019

Stem cells representing epiblast (ESC) & trophectoderm (TE) of the mammalian blastocyst stage embryo

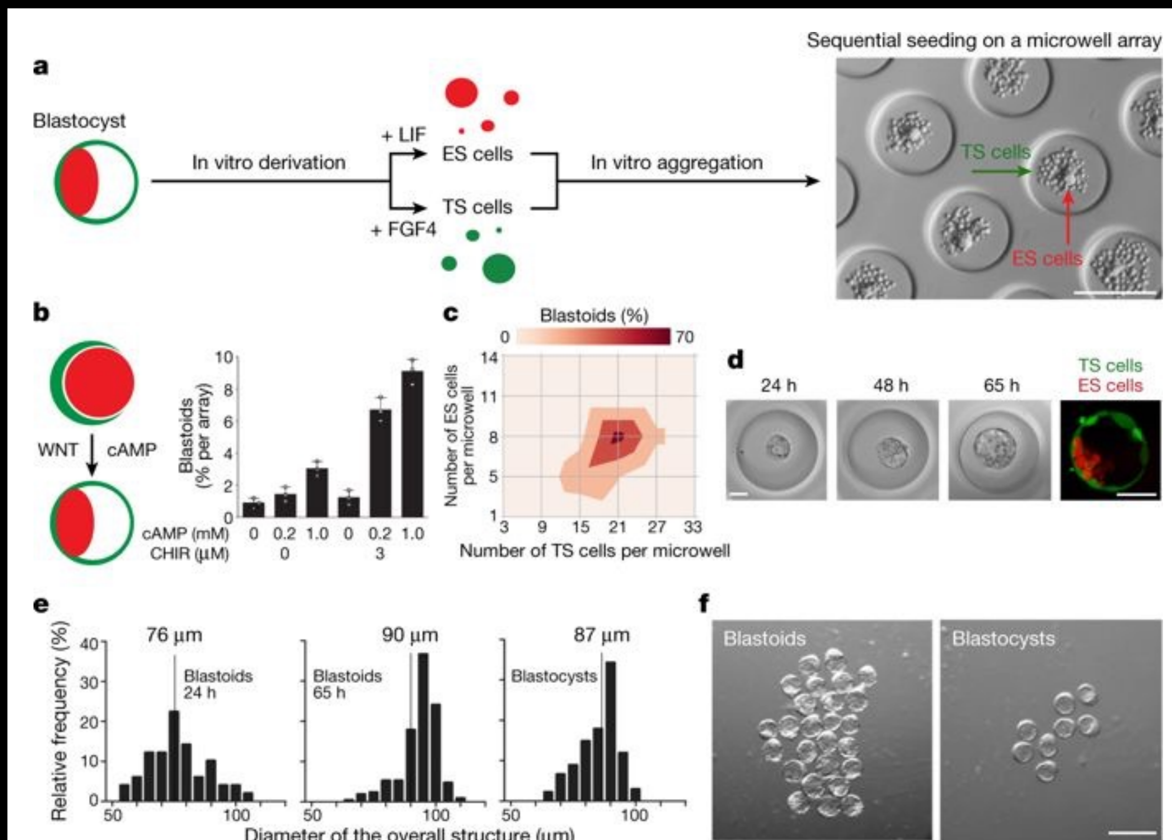


BLASTOIDS: Stem cell-derived blastocyst stage embryo-like structures



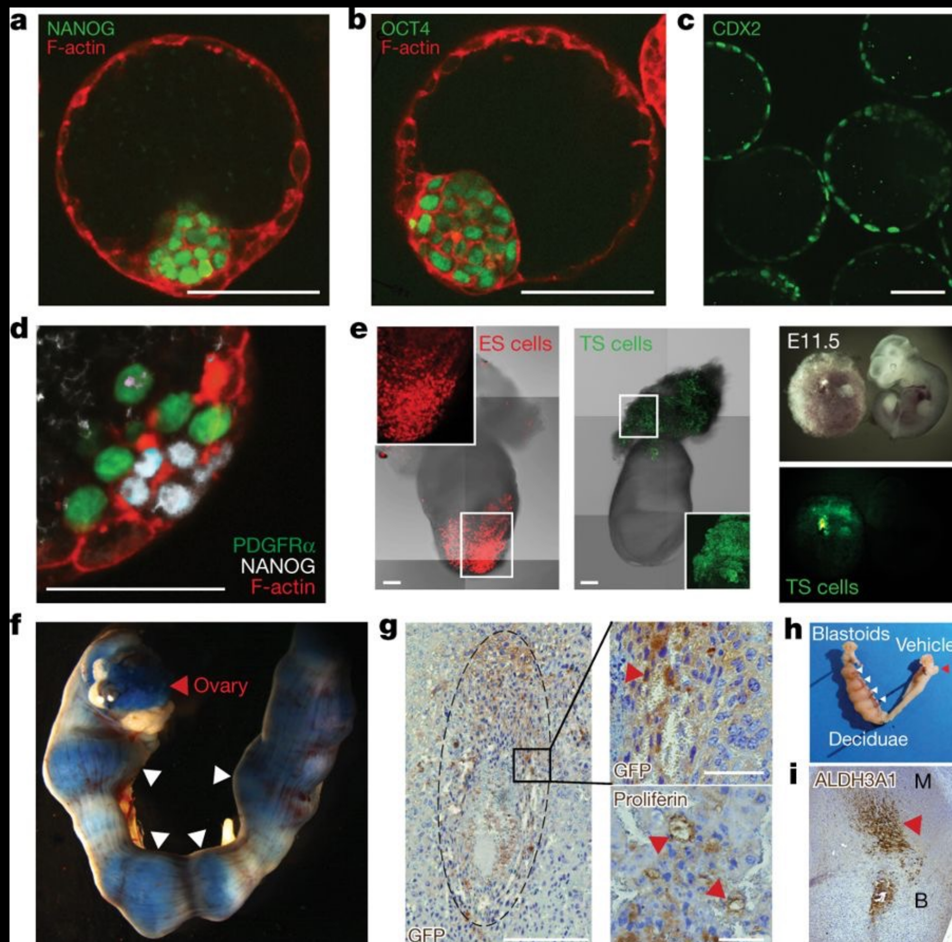
ES cells + TS cells

ES & TS cells can be combined to form a mouse blastocyst-like structure *in vitro*



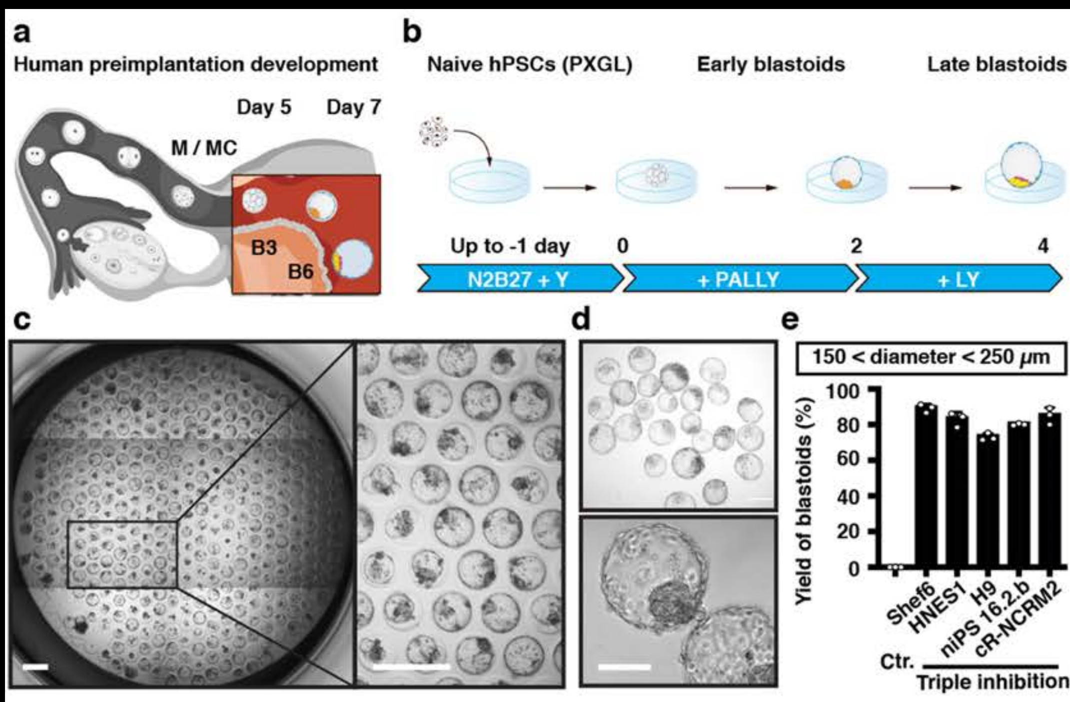
Rivron N. *et al.*, Nature 2018

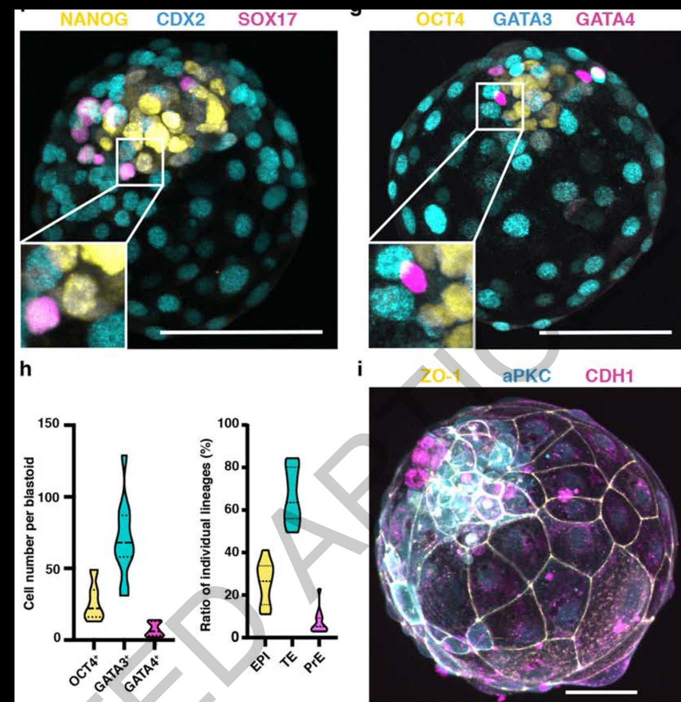
Mouse blastoids implant *in utero* & trigger the formation of deciduae



Rivron N. *et al.*, Nature 2018

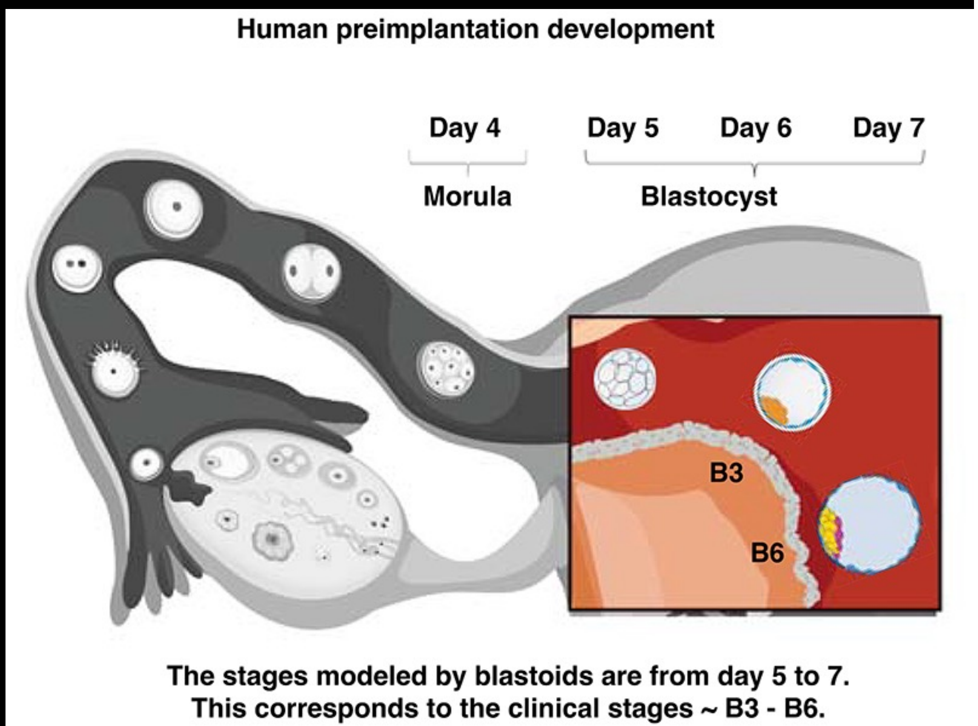
Human blastoids model blastocyst development & implantation

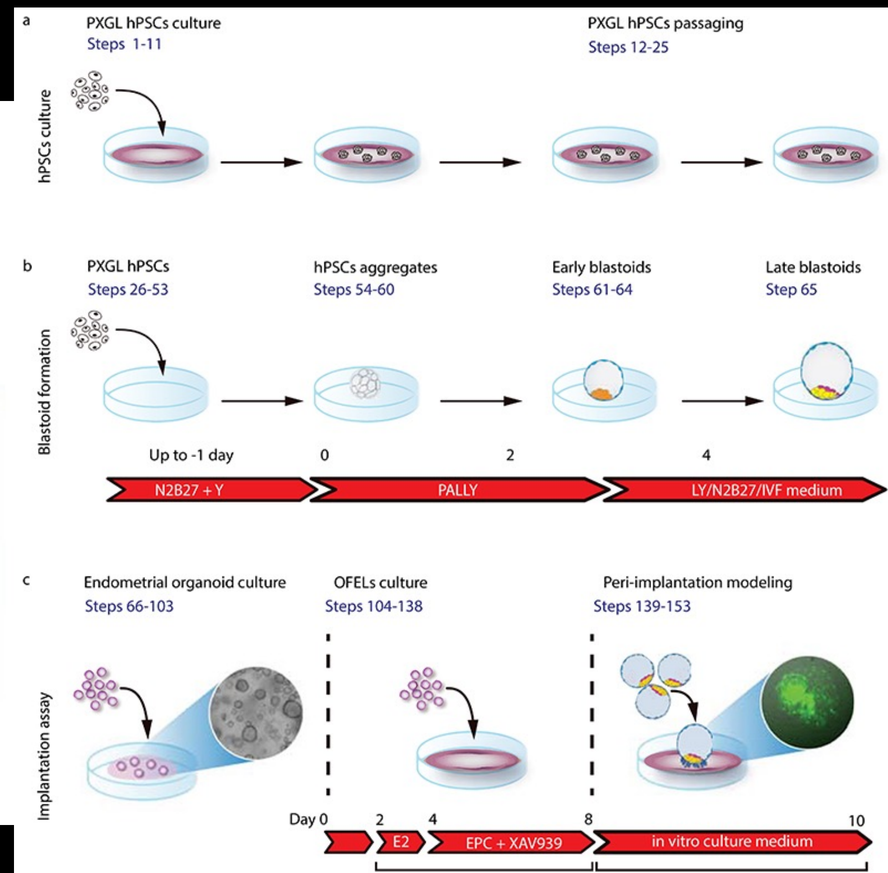




Kagawa *et al.*, Nature 2022

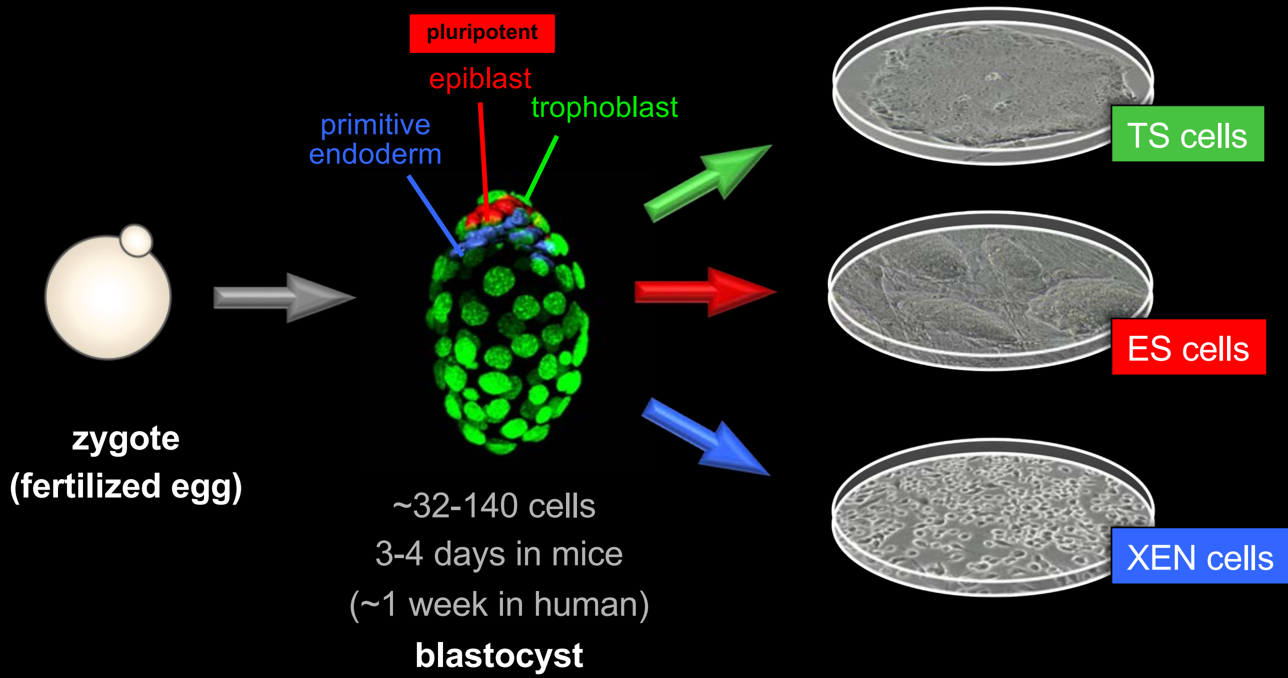
Stem cell based human blastoid and endometrial (implantation) models



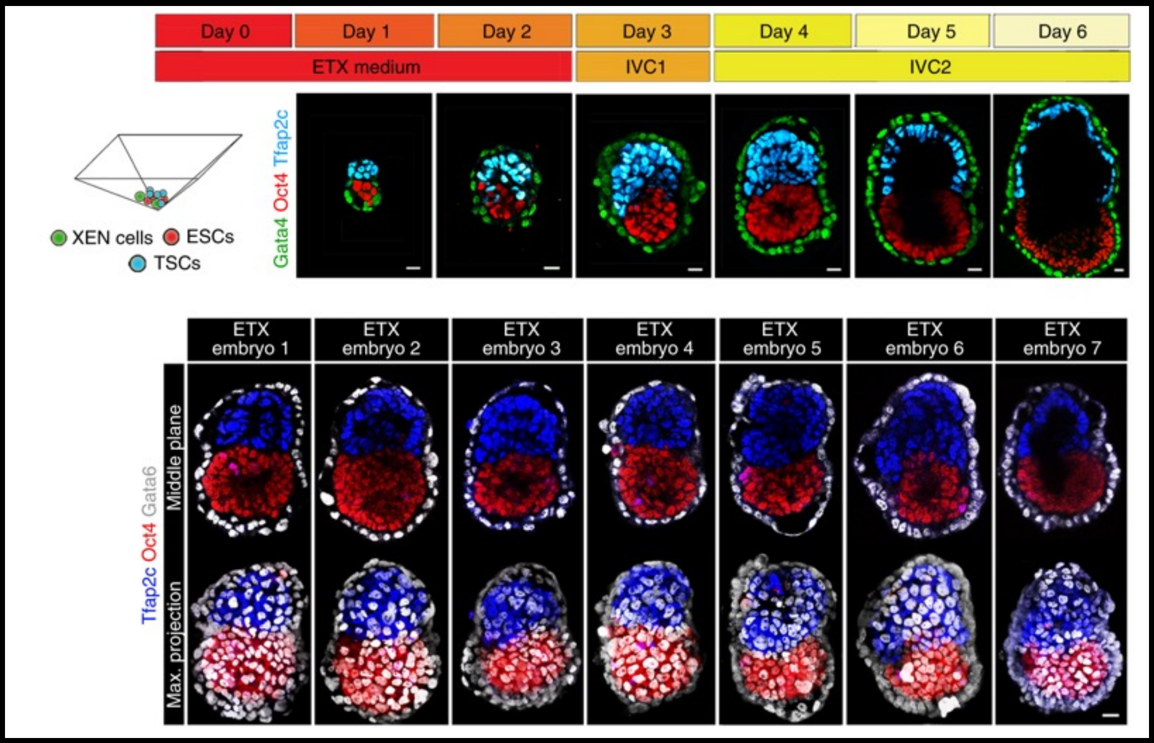


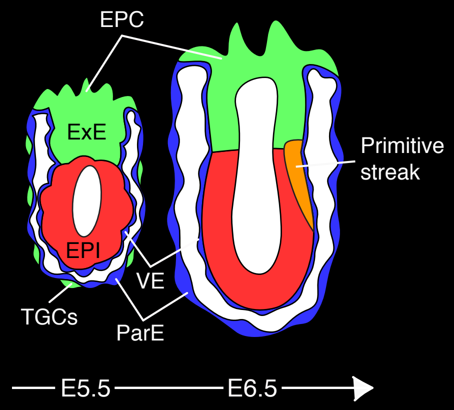
Heidari Kohei *et al.*, Nature Protocols 2023

Derivation of stem cells from all 3 lineages of the mouse blastocyst stage embryos



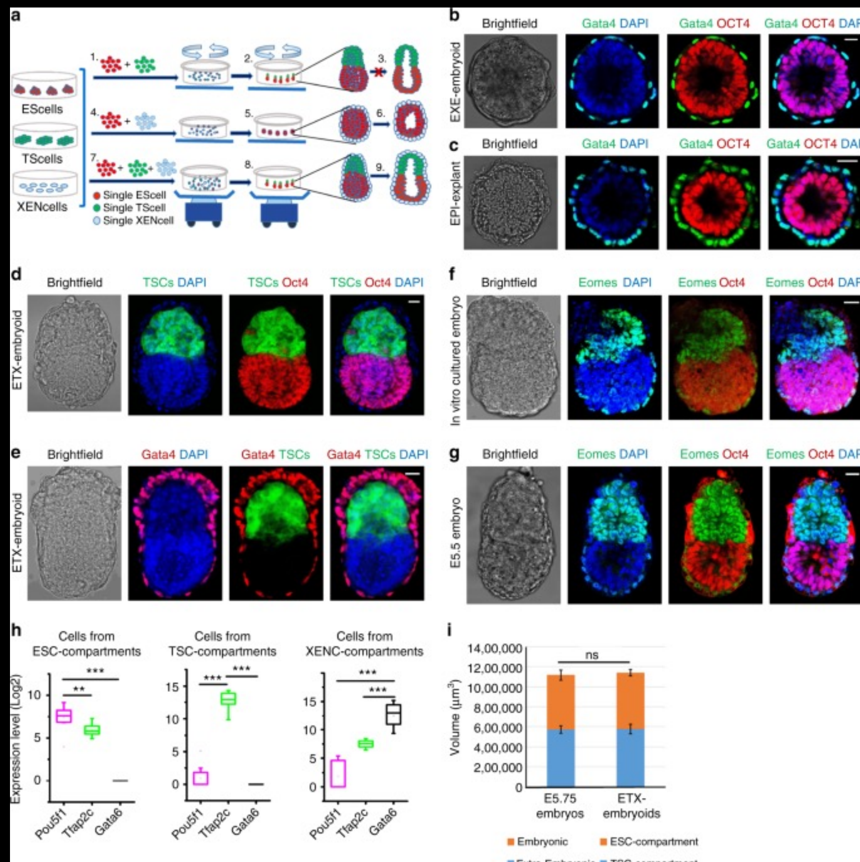
ETX embryos: Synthetic embryos comprised of ES-TS-XEN cells that develop to post-implantation





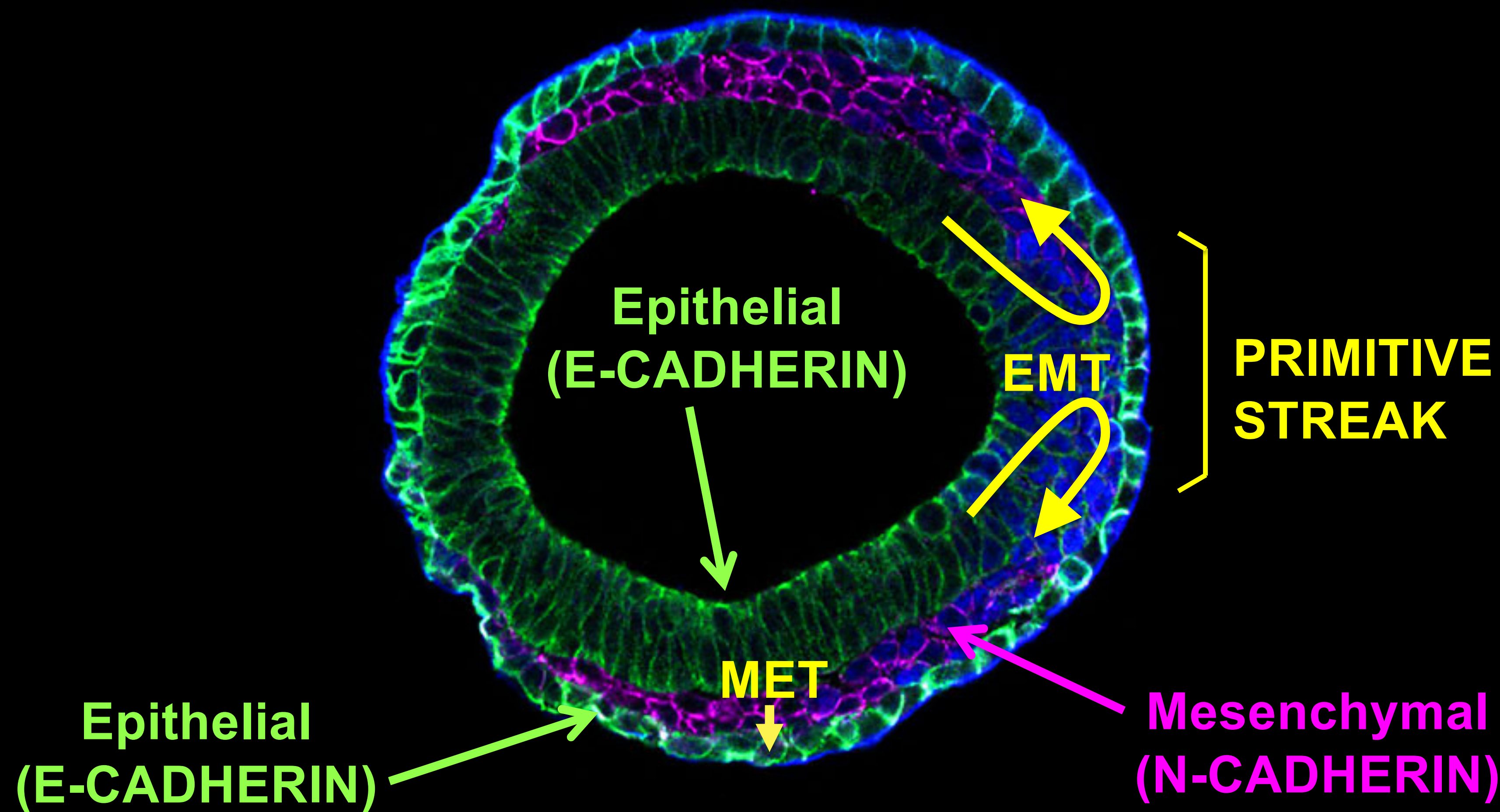
Harrison S. *et al.*, Science 2017
Sozen B. *et al.*, Nature Cell Biology 2018

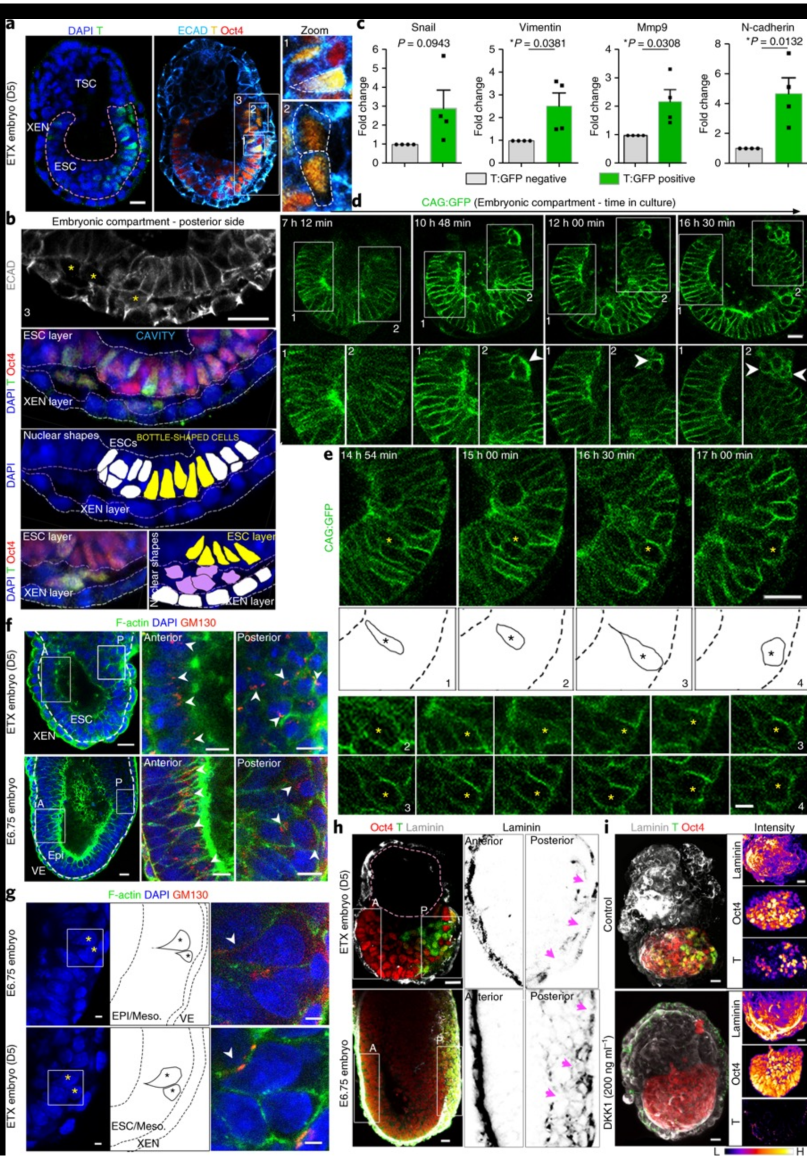
INTEGRATED SYSTEMS: ETX embryos



Zhang *et al*., Nature 2019

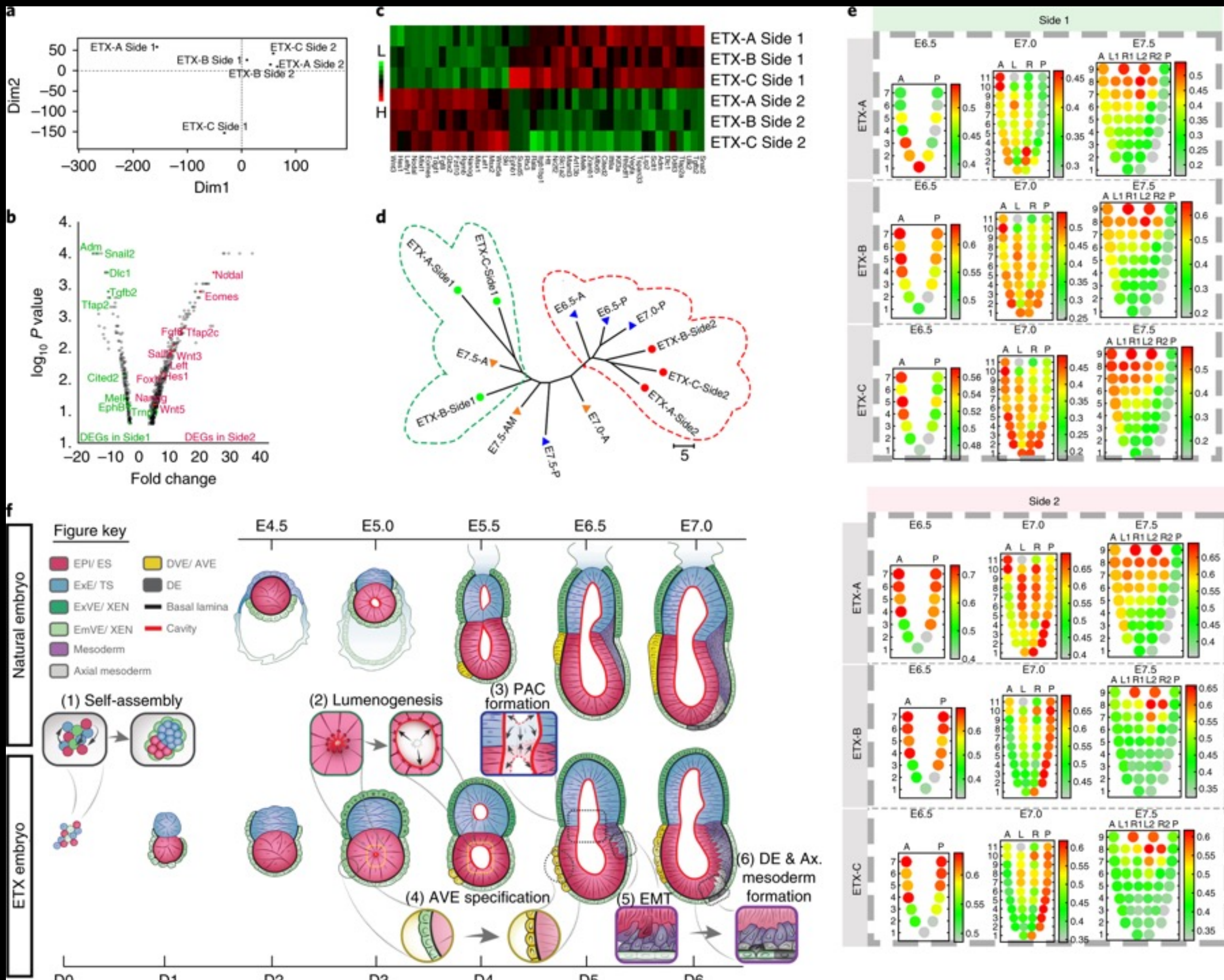
Do ETX embryos employ comparable morphogenetic mechanisms as the natural embryo?





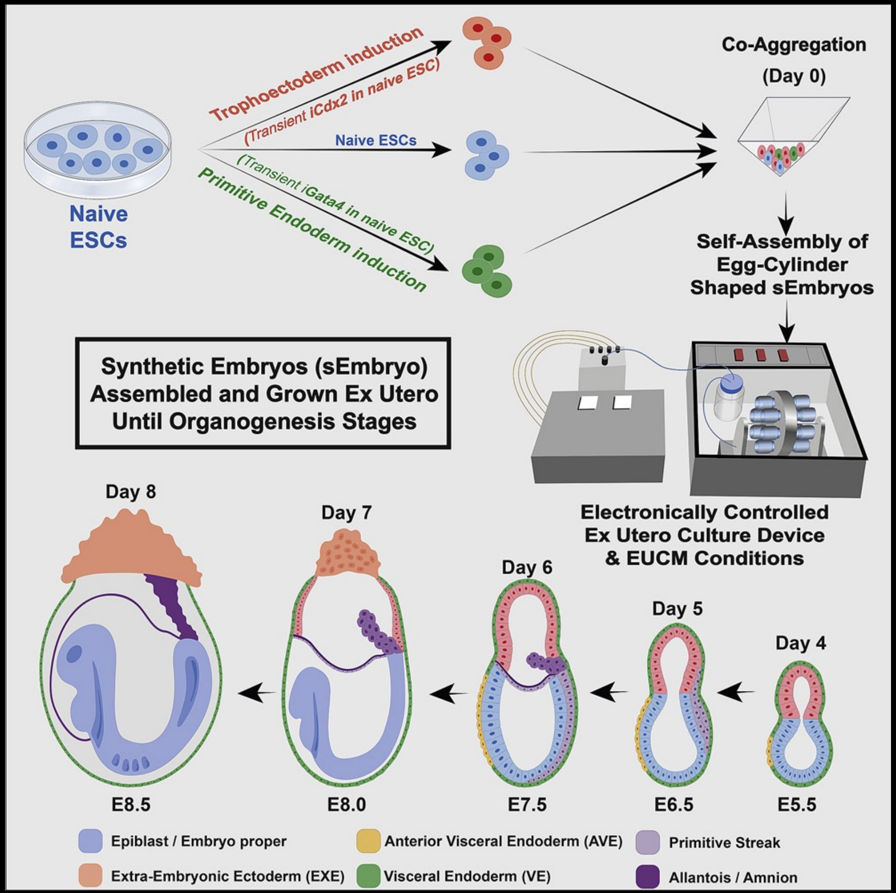
EMT events in ‘gastrulating’ ETX embryos

Sozen B. *et al.*, Nature Cell Biology 2018



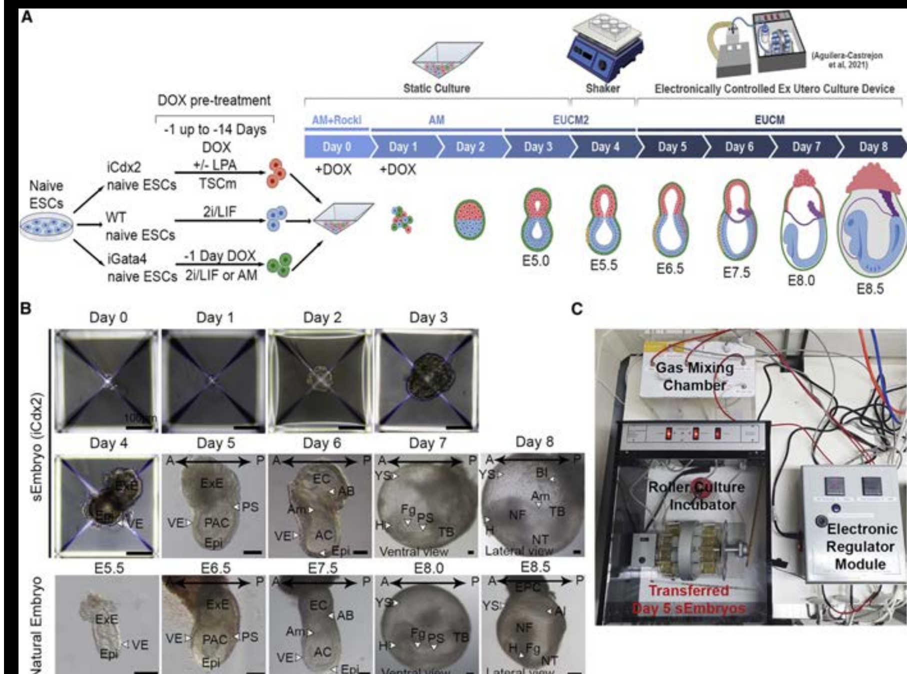
Transcriptional profiling of 'gastrulating' ETX embryos reveals global similarity of anterior–posterior patterning to gastrulating natural embryos

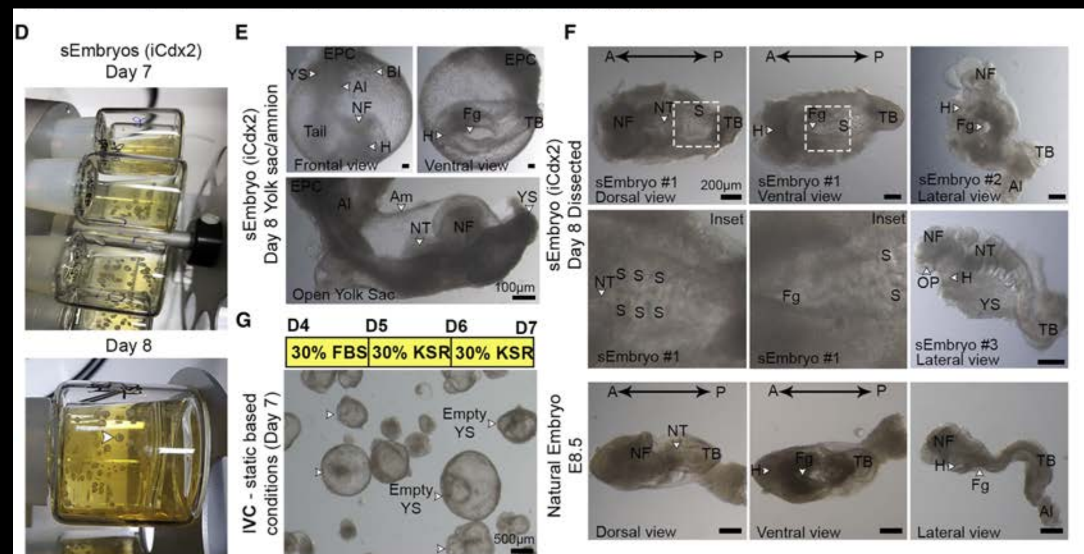
Post-gastrulation synthetic embryos generation *ex utero* from mouse naïve ESCs



Tarazi S. *et al.*, Cell 2022
Niwa H. *et al.*, Cell Stem Cell 2022

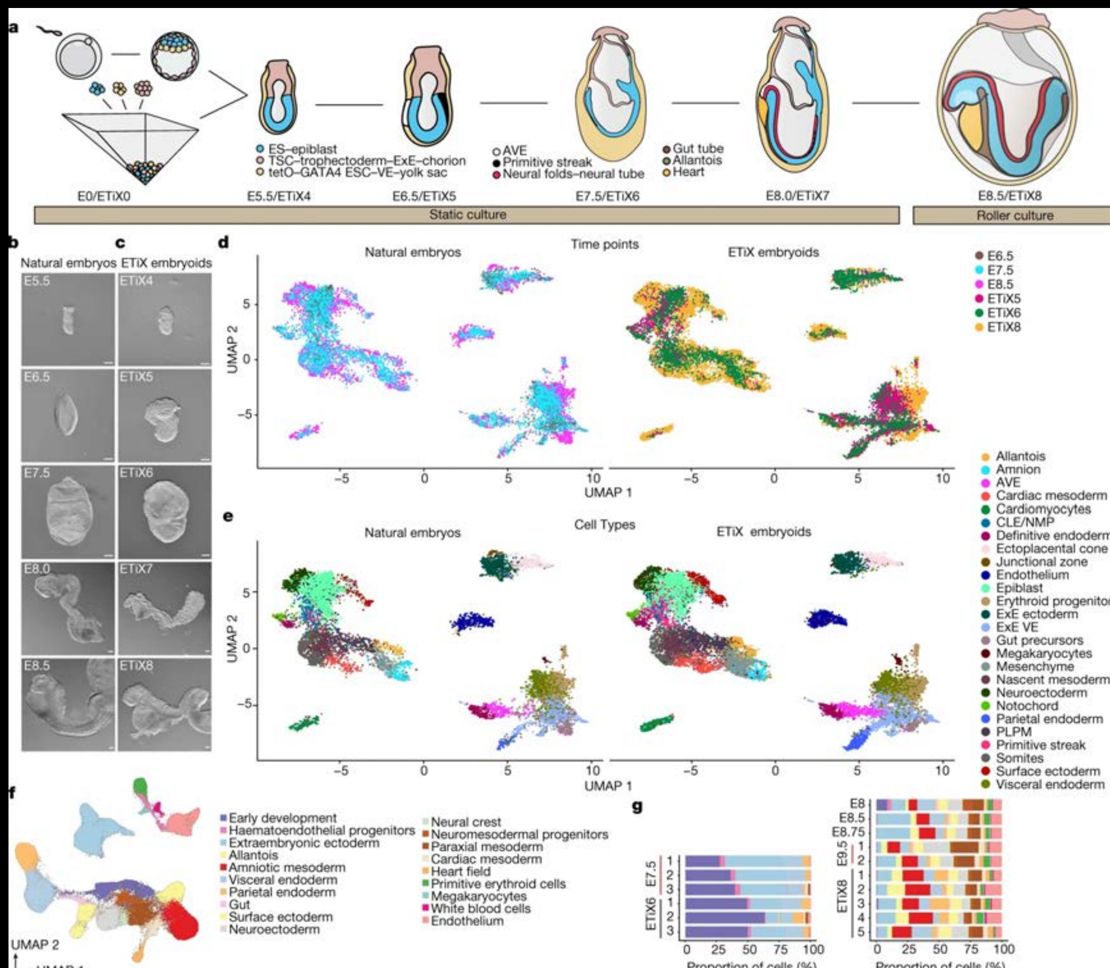
Post-gastrulation stem cell-derived embryo-like models generated *ex utero* from mouse naïve ESCs





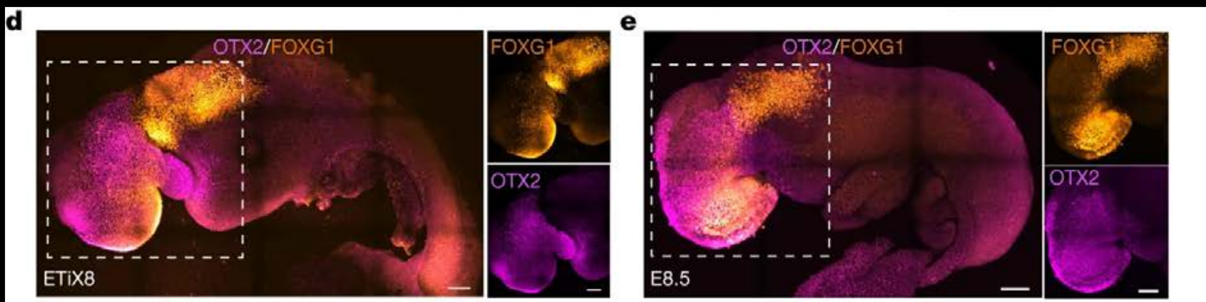
Tarazi S. *et al.*, Cell 2022
Niwa H. *et al.*, Cell Stem Cell 2022

Stem cell-derived embryo-like models complete gastrulation to neurulation & organogenesis (i.e. later development)



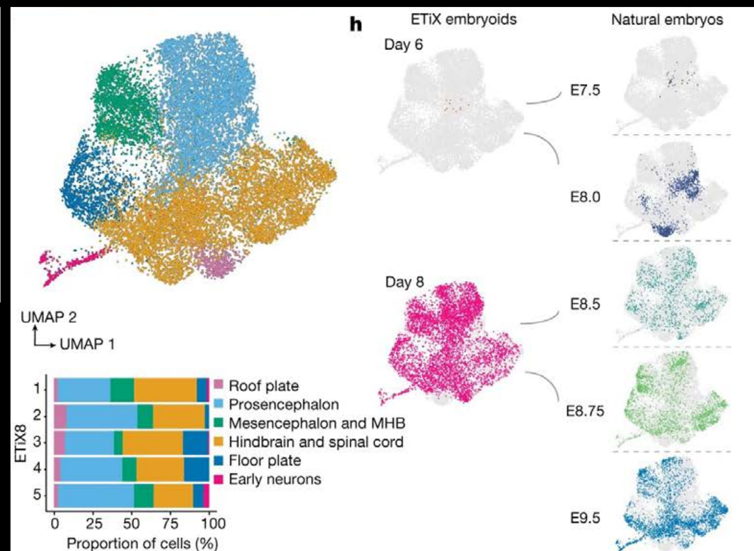
Amadei J. *et al.*, Nature 2022

Stem cell-derived embryo-like models complete gastrulation to neurulation and organogenesis (i.e. later development)



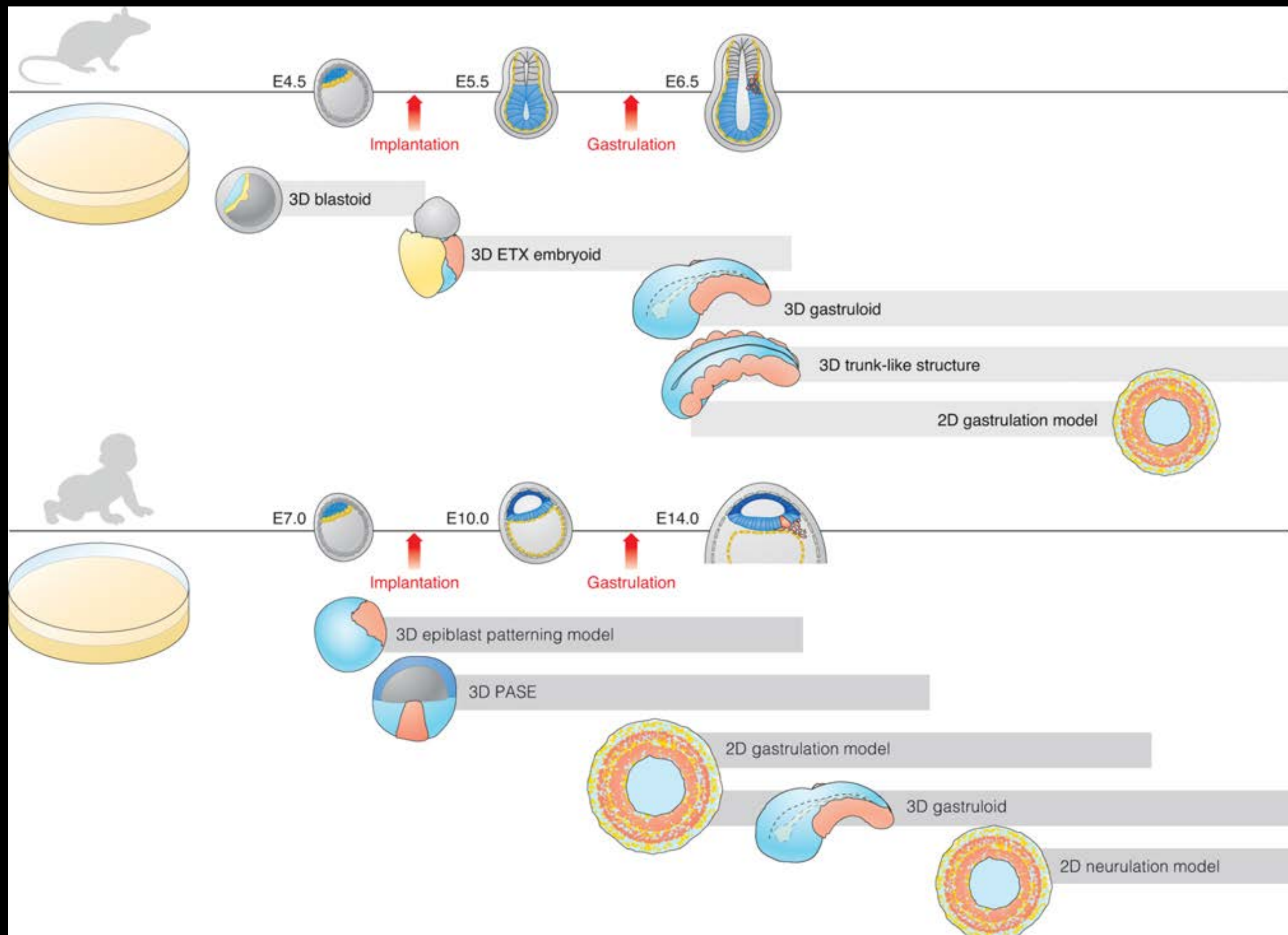
synthetic embryo
(ETiX embryoid)

natural embryo



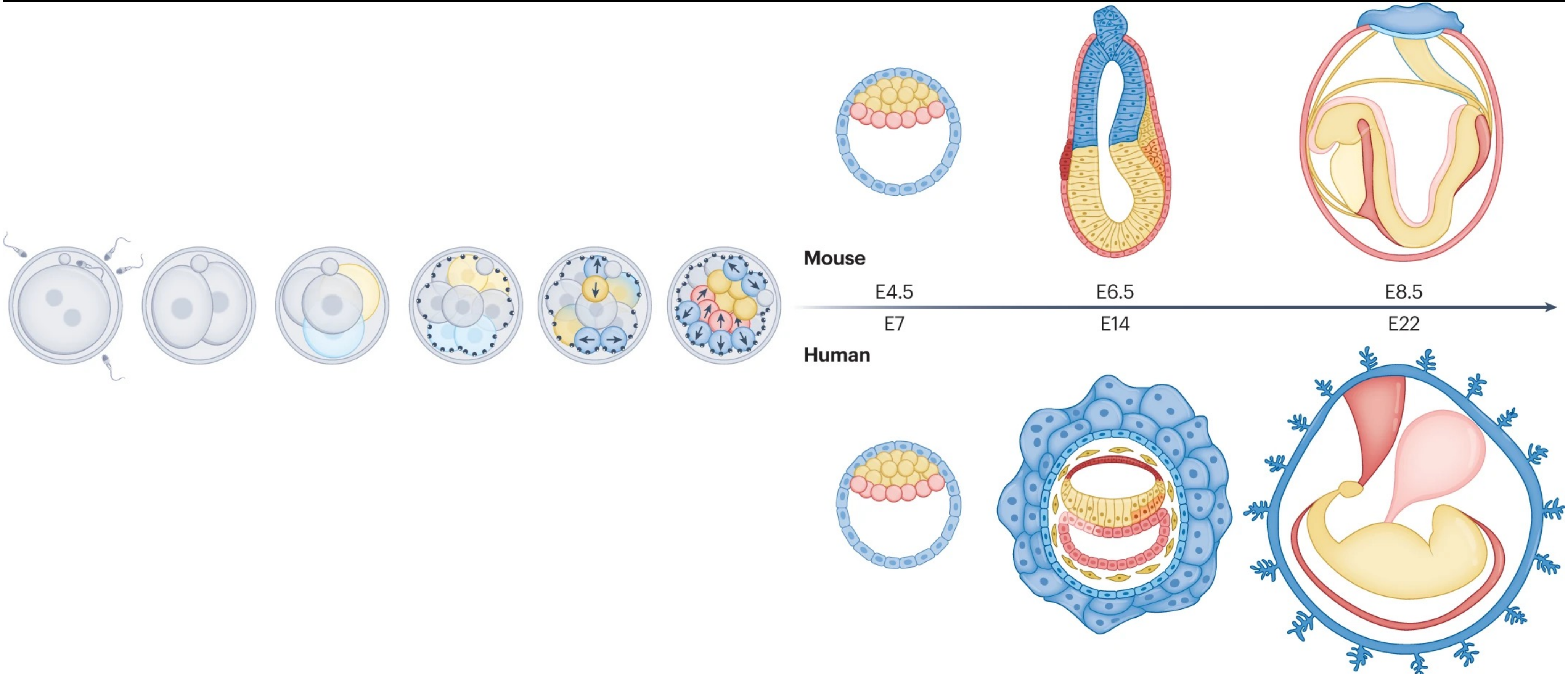
Amadei J. *et al.*, Nature 2022

Embryoid models recapitulate different stages of mouse & human development



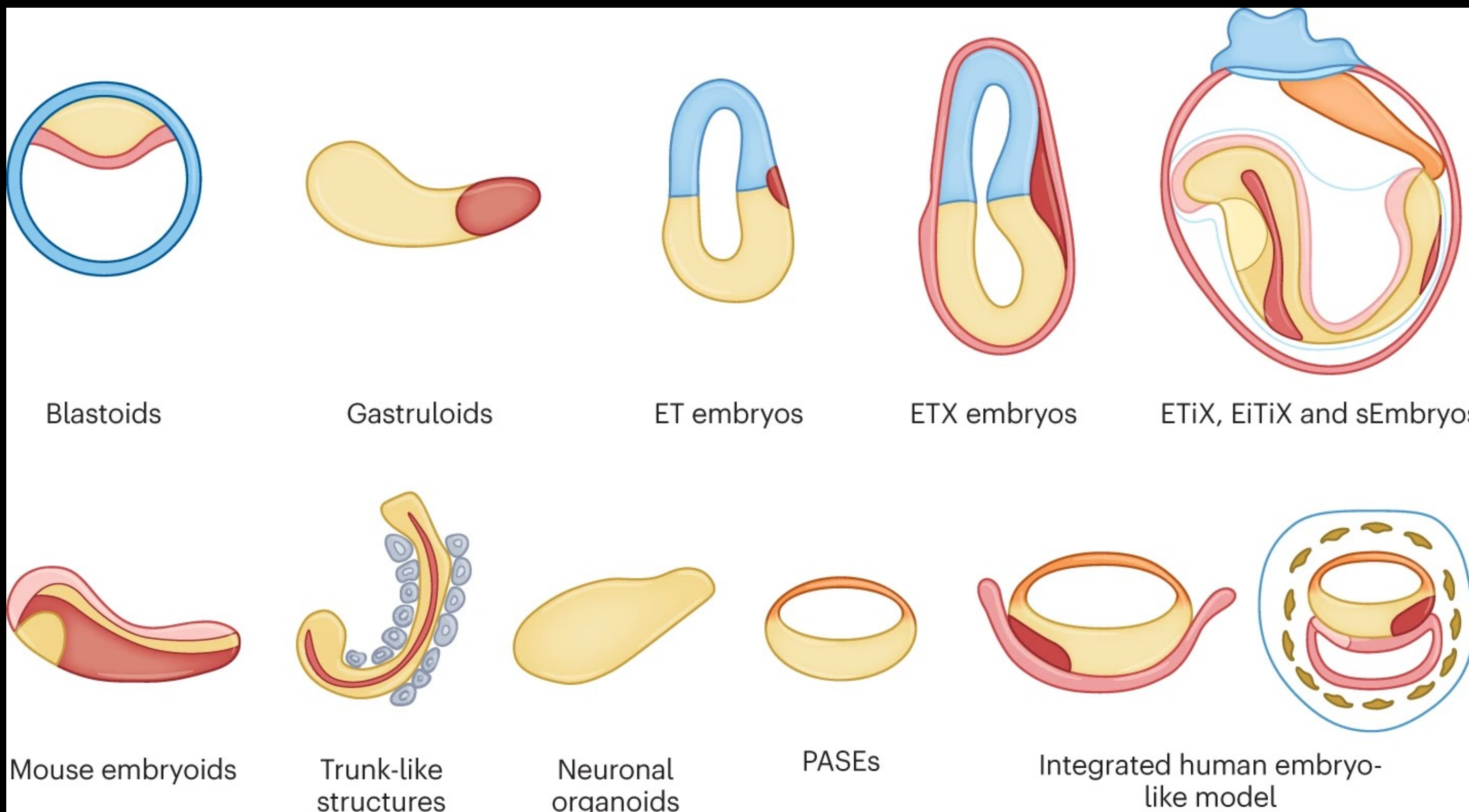
Fu et al., Nature Materials 2020

Mouse and human embryogenesis



Zernicka-Goetz et al., Nature Methods 2023

3D stem cell-based embryo humans in mouse and human



Zernicka-Goetz et al., Nature Methods 2023