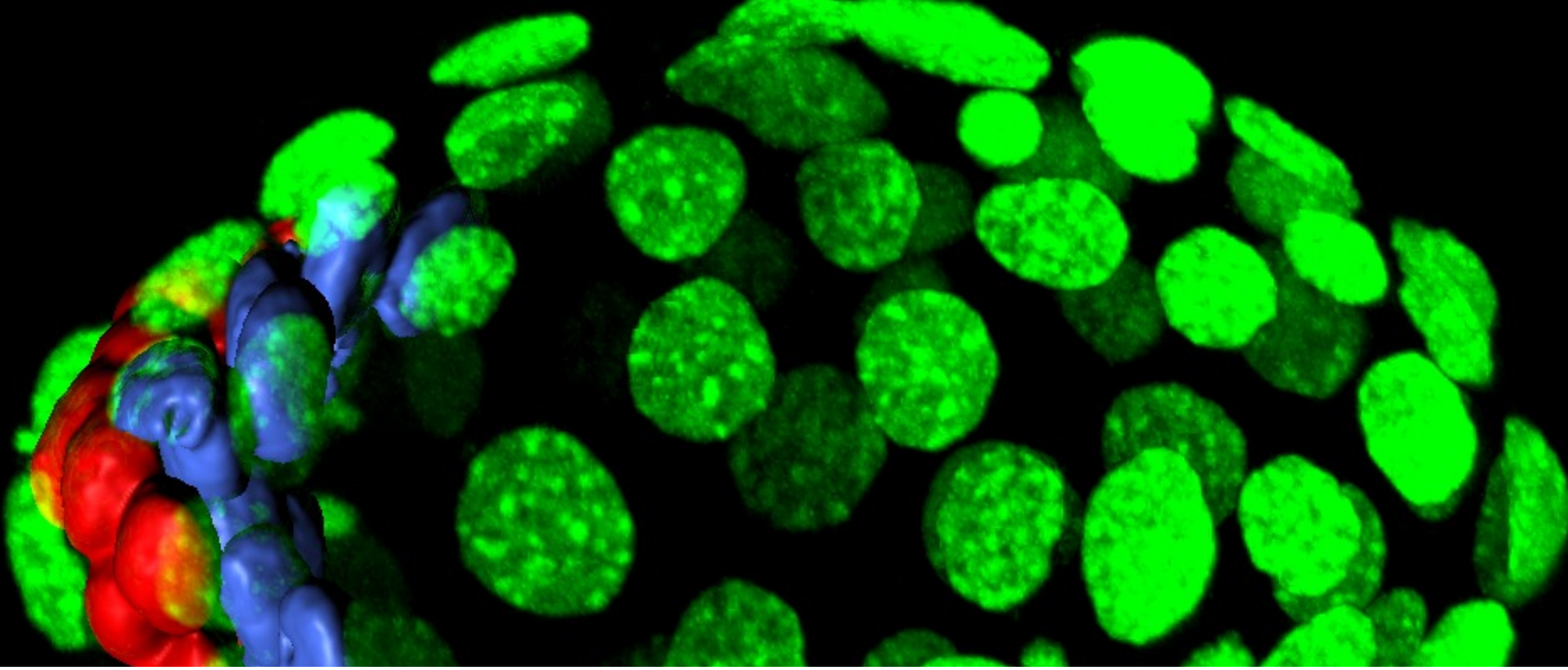
Early mammalian embryonic development

from pre-implantation to gastrulation

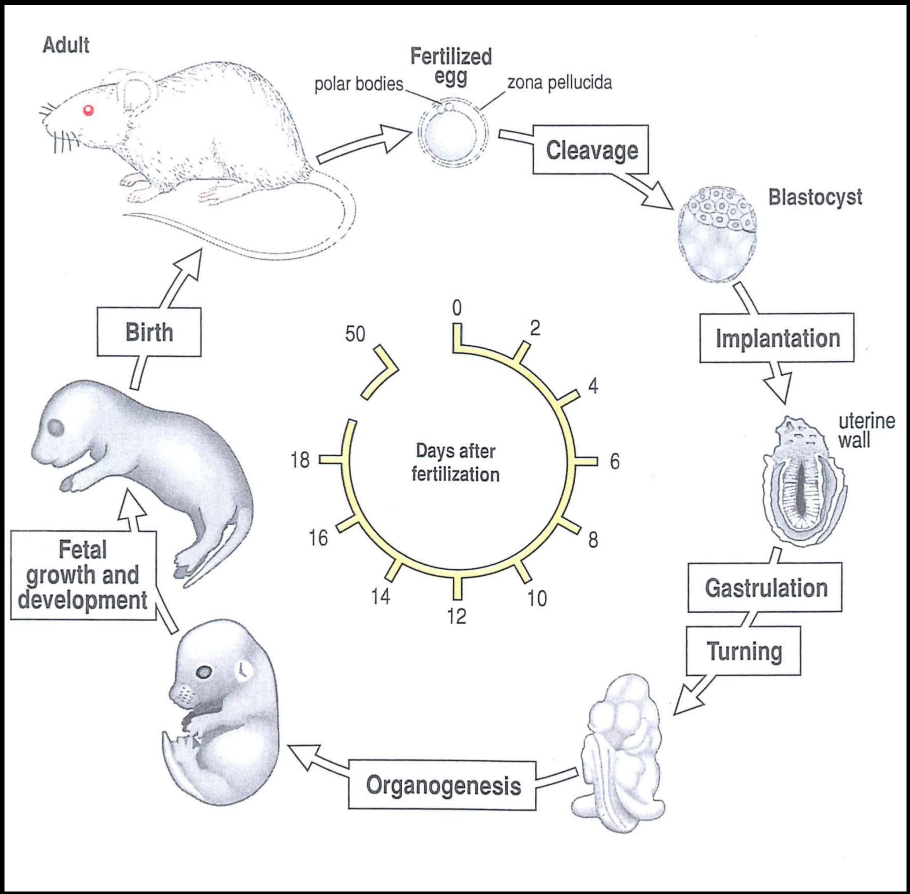
GSK Core Course (Lecture 59), Monday November 24th 2025

Kat Hadjantonakis - hadj@mskcc.org



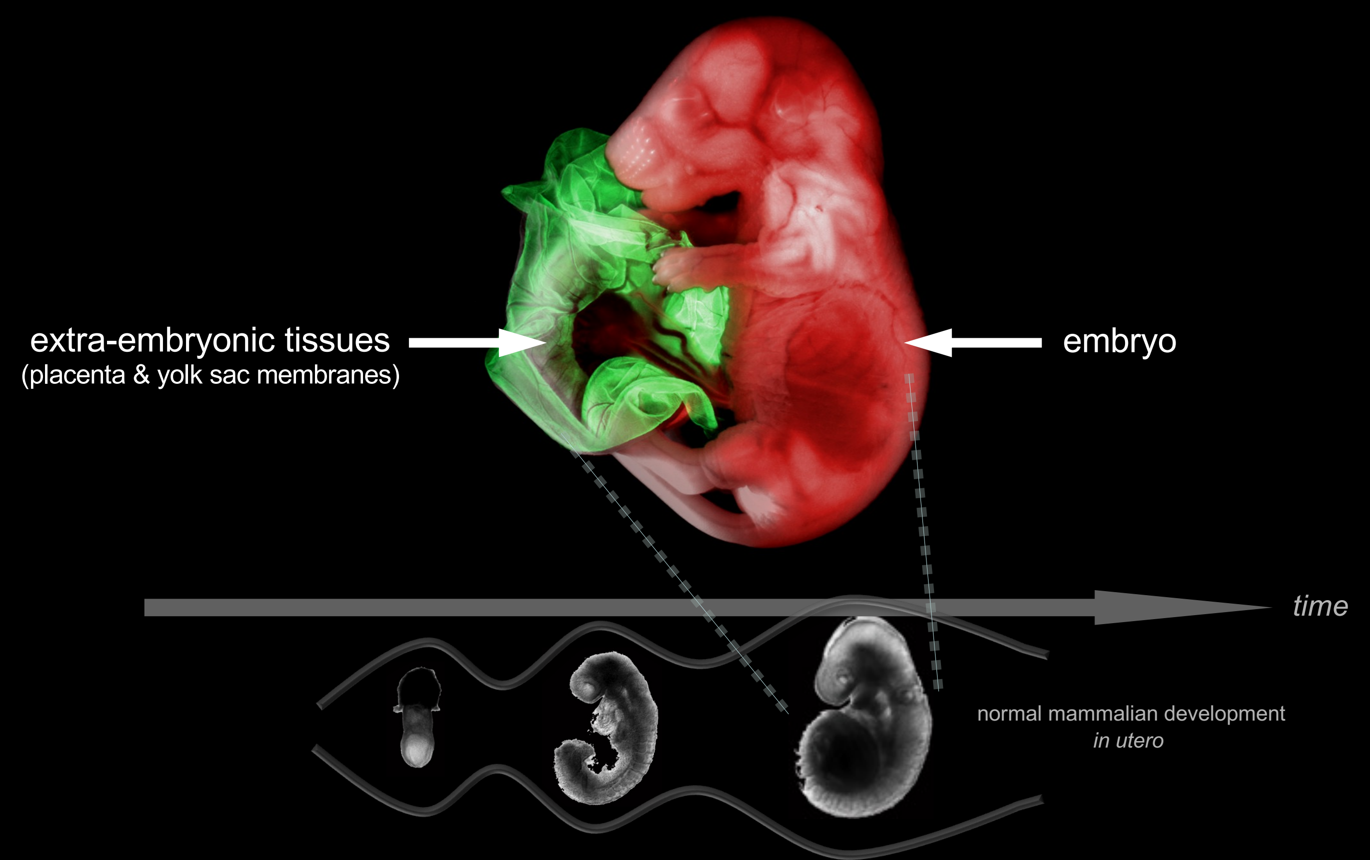
The life cycle of the mouse

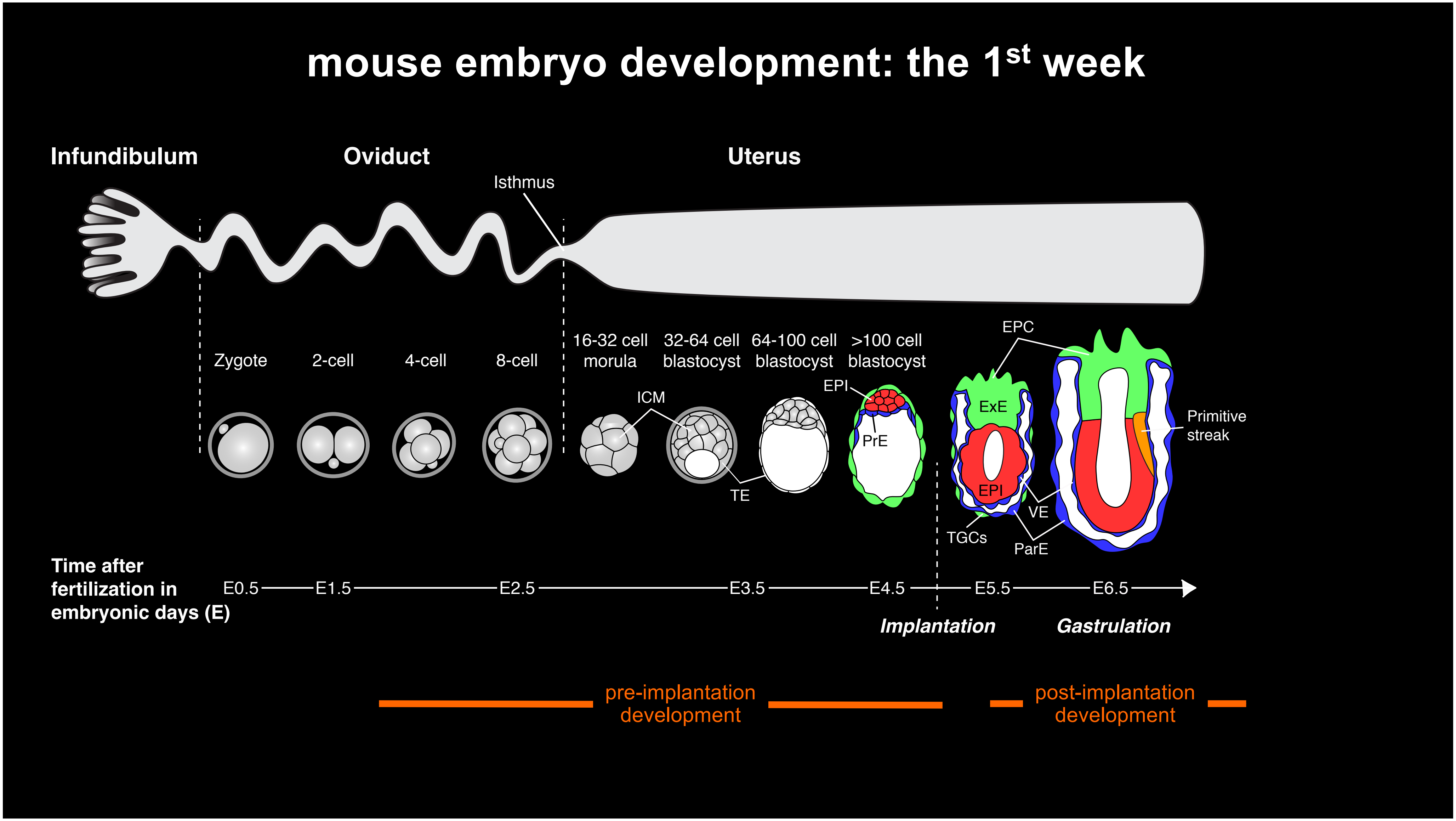
- ~ 3 week gestation period
- small litter size: ~4-10 embryos/female



taken from Wolpert L., et al., Principles of Development

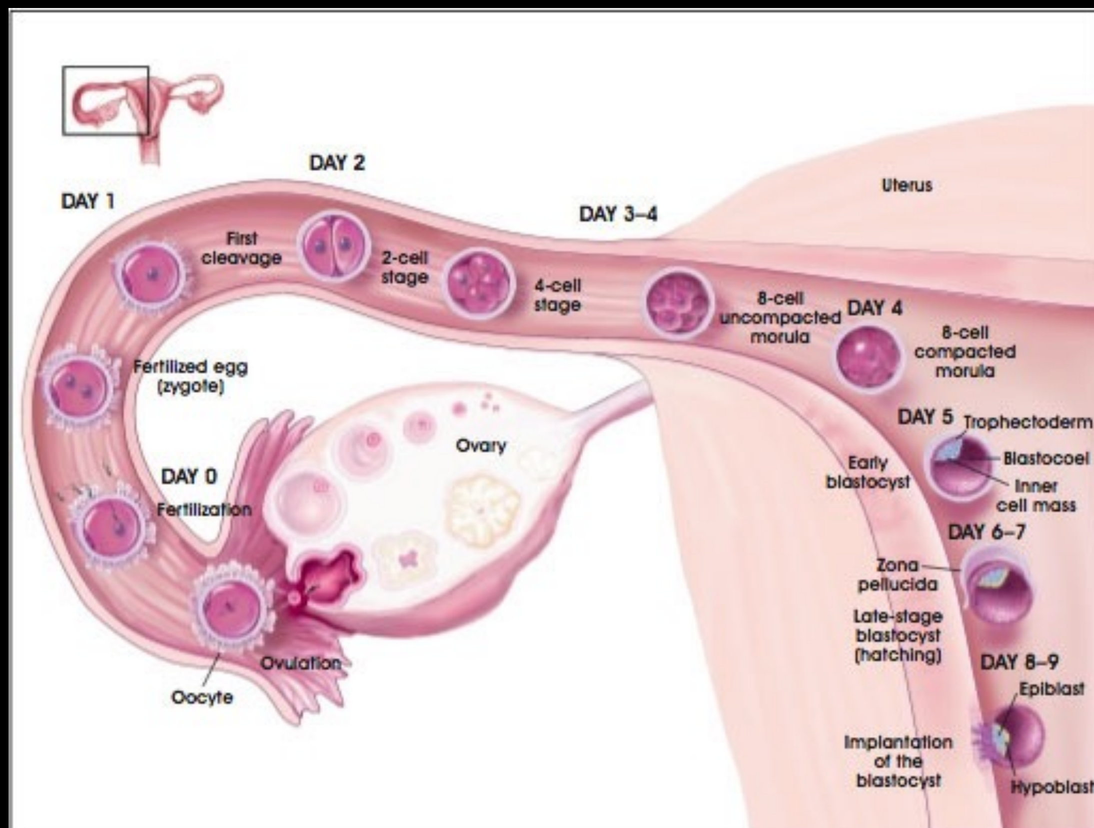
mammalian development takes place *in utero*





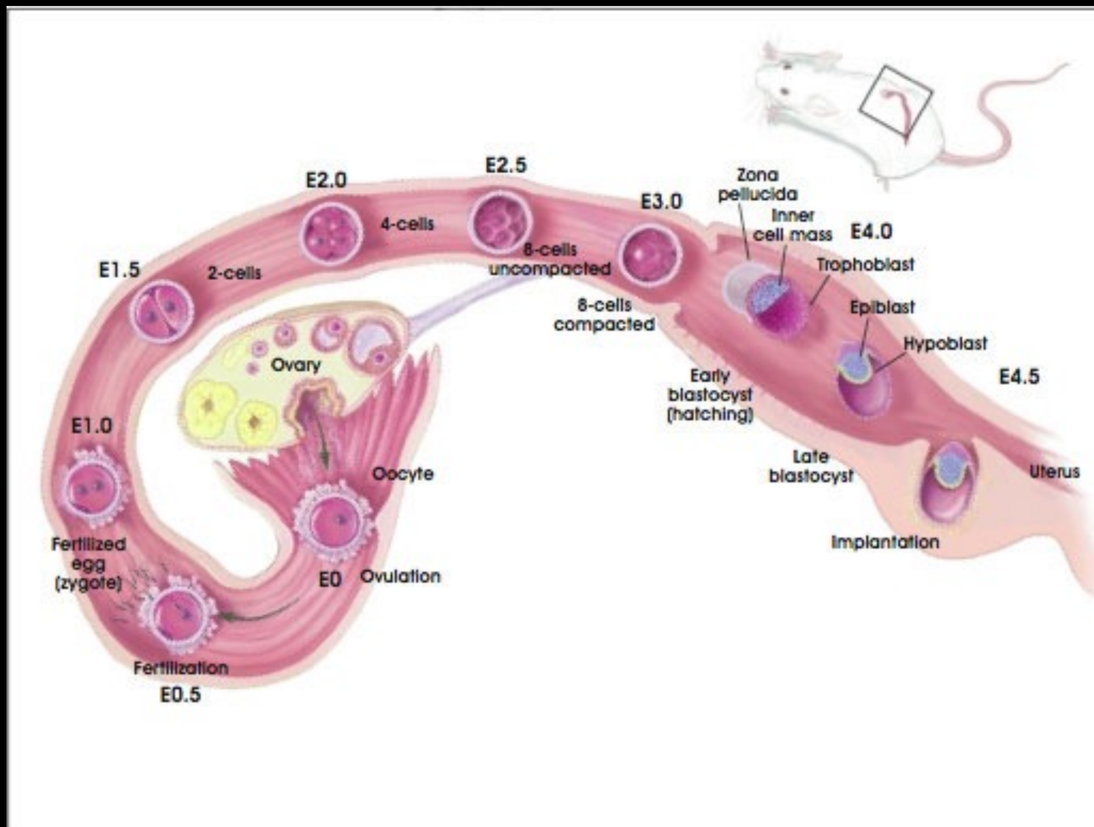
mouse embryo development: the 1st week
Infundibulum
Oviduct
Uterus
Isthmus
Zygote
2-cell
4-cell
8-cell
16-32 cell morula
32-64 cell blastocyst
64-100 cell blastocyst
>100 cell blastocyst
ICM
TE
EPI
PrE
EPC
ExE
EPI
VE
TGCs
ParE
Primitive streak
Time after fertilization in embryonic days (E)
E0.5
E1.5
E2.5
E3.5
E4.5
E5.5
E6.5
Implantation
Gastrulation
pre-implantation development
post-implantation development

Development of the Pre-implantation Blastocyst in Humans

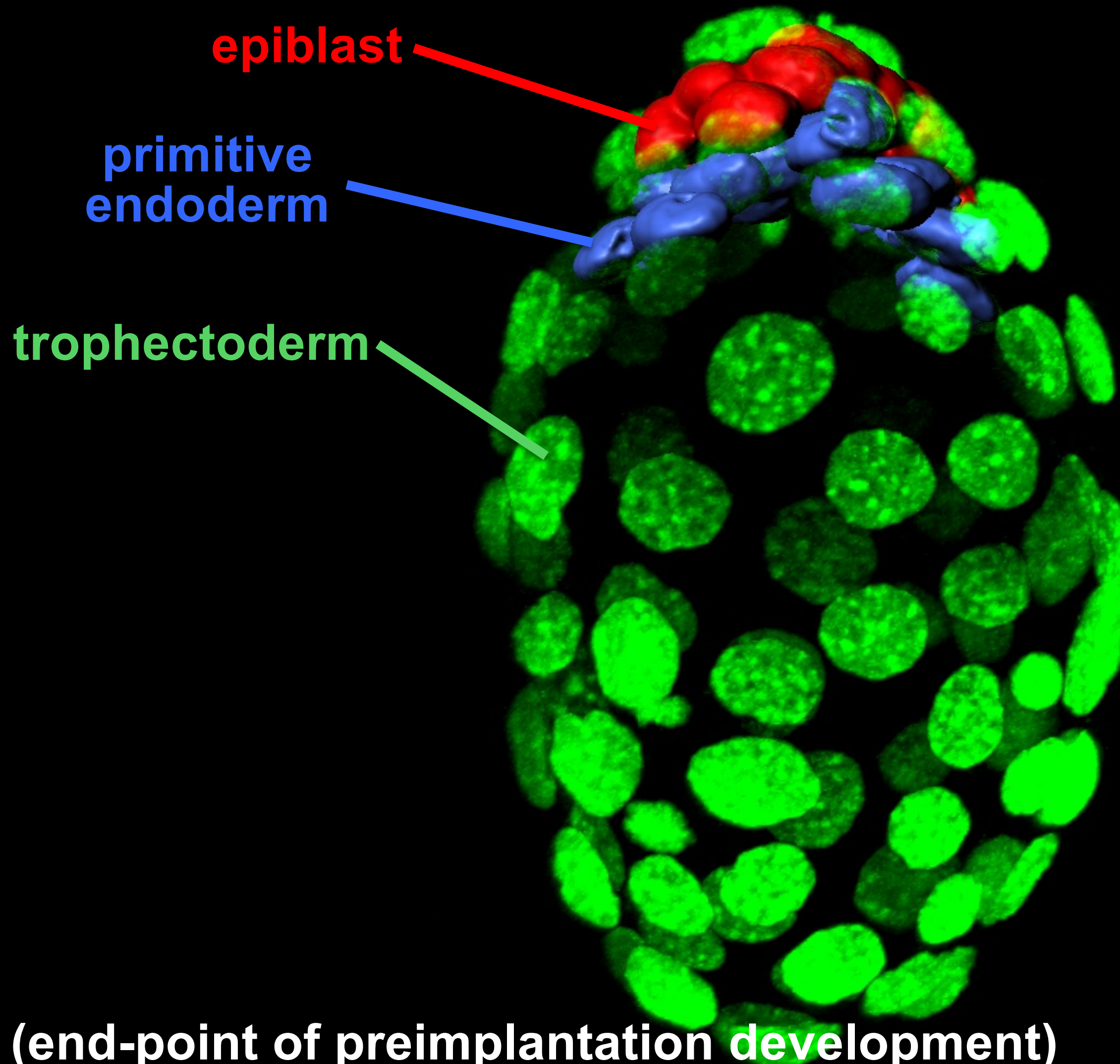


https://stemcells.nih.gov/info/2001report/appendixA.htm

Development of the Pre-implantation Blastocyst in Mice

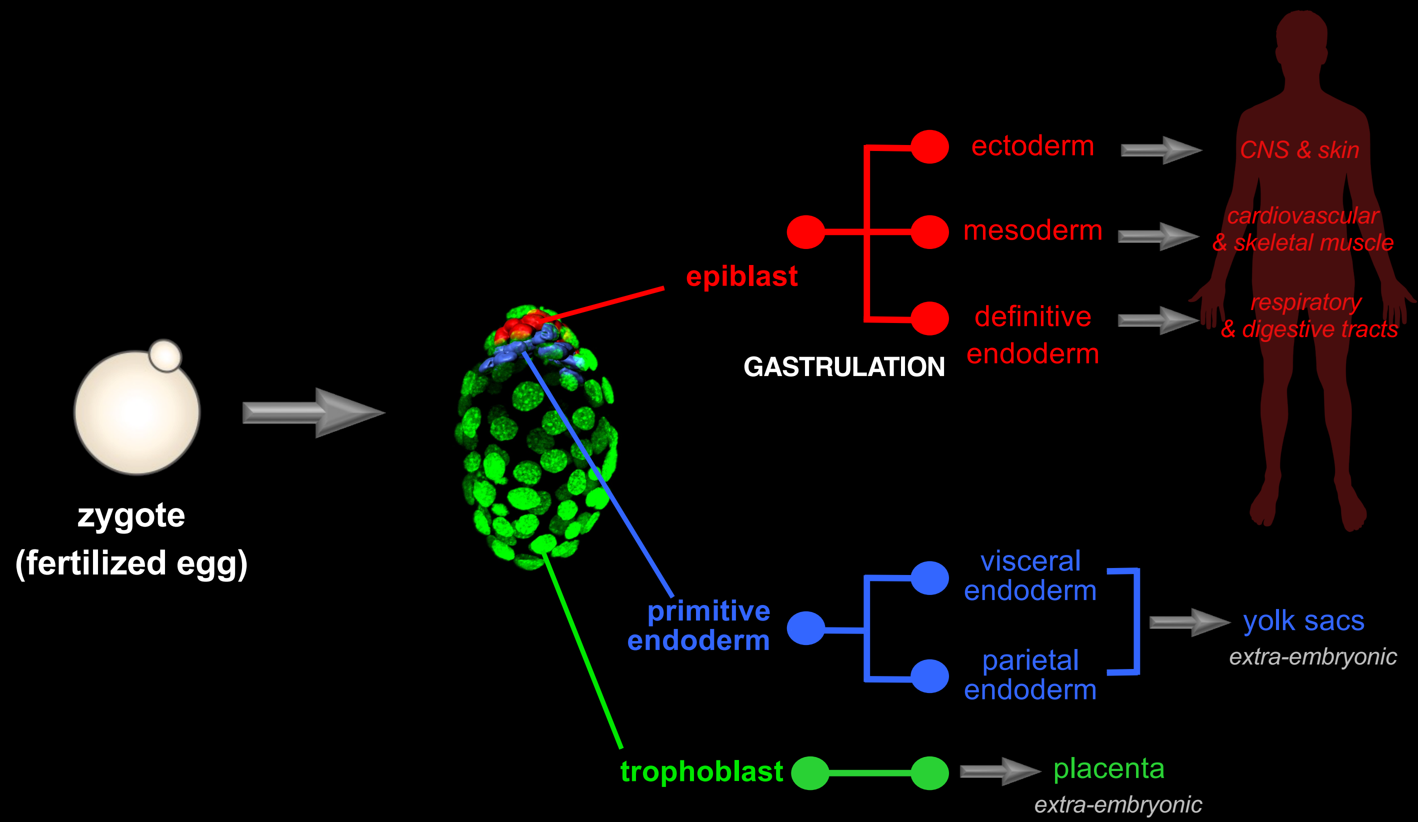


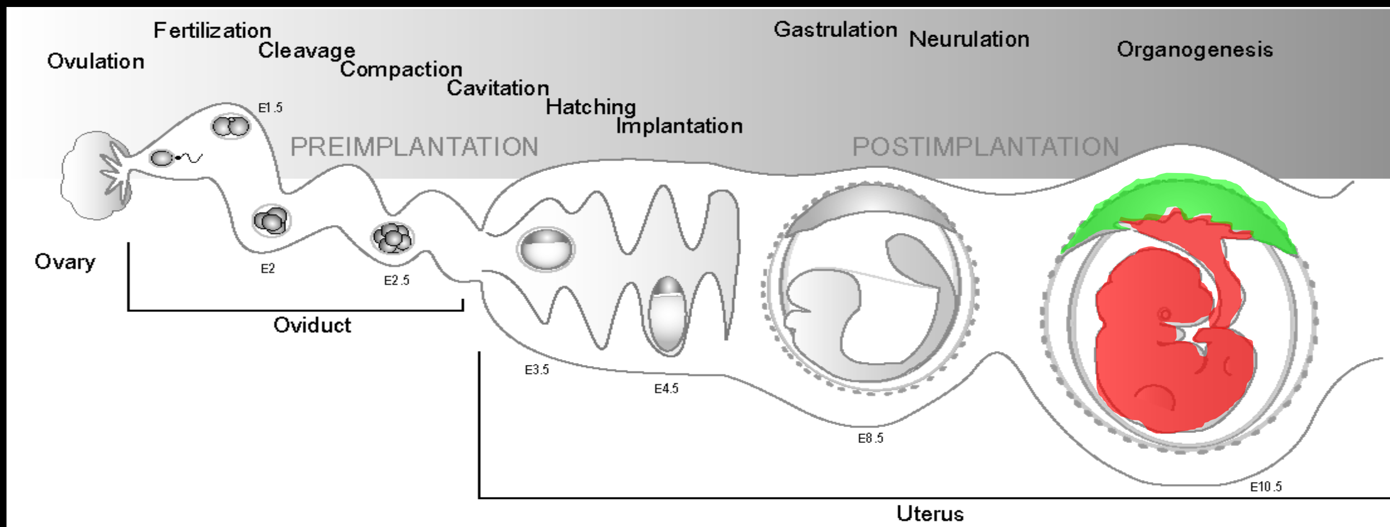
https://stemcells.nih.gov/info/2001report/appendixA.htm



the mammalian blastocyst (end-point of preimplantation development)

Lineage contributions of the mammalian blastocyst





- Mouse genetically tractable mammalian model. Can modify the genome at base pair resolution.

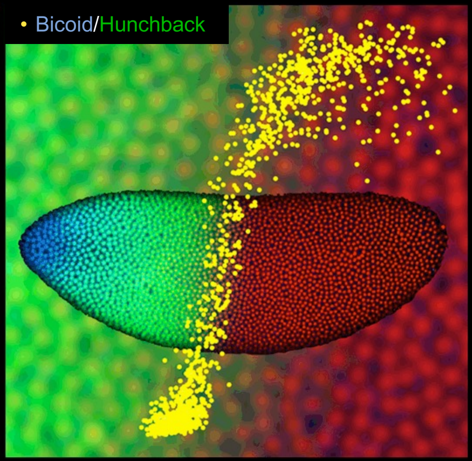
- Preimplantation development mirrors other mammalian embryos (e.g. duration of the cell cycles and the timing of specific events differs).

- Preimplantation development can take place *in vitro*. Therefore early mammalian embryos are easily accessible to experimental manipulation.

- Oocytes and embryos of a defined stage are easily obtained. This fact in particular has lead to the use of the mouse model in experimental research using embryo micromanipulation techniques, including the generation of transgenic and knock-out mice.

Axis determination in the *Drosophila* embryo depends on localized maternal components

Bicoid Hunchback DNA

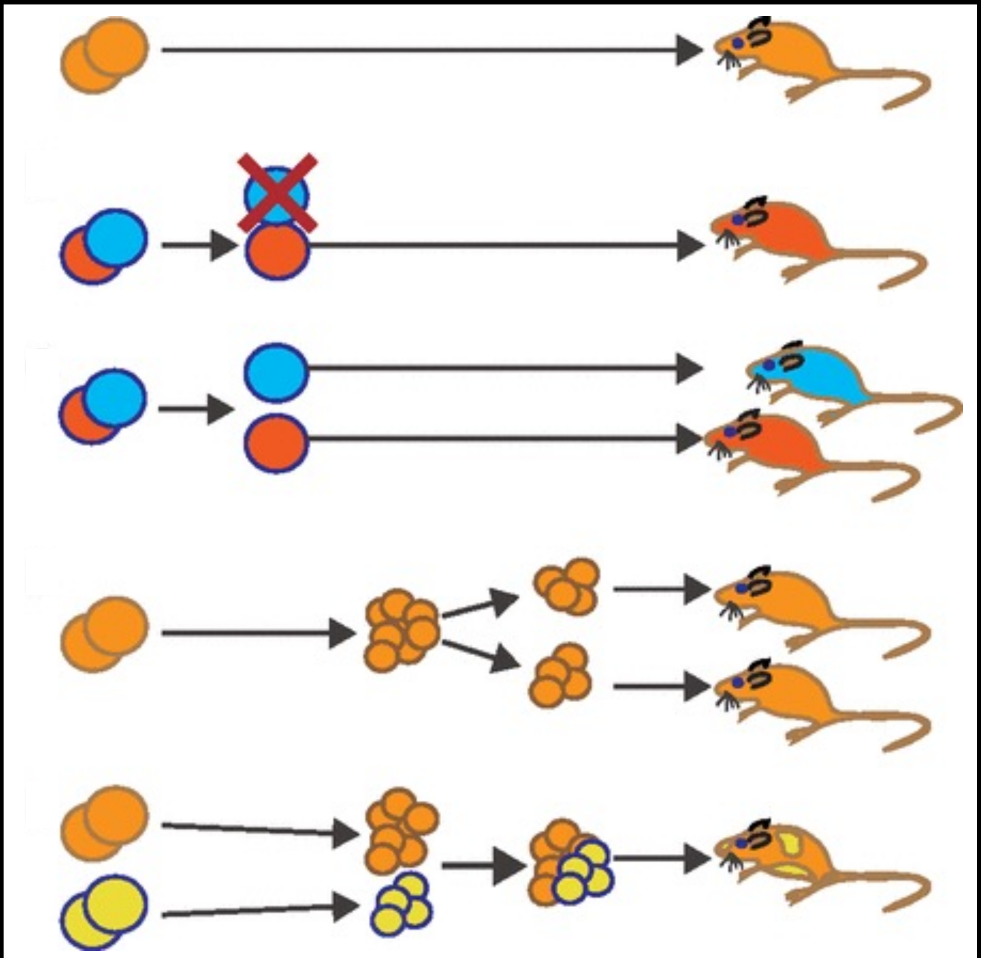


Gregor T. *et al.*, Cell 2007

Mammalian development does not depend on maternal products

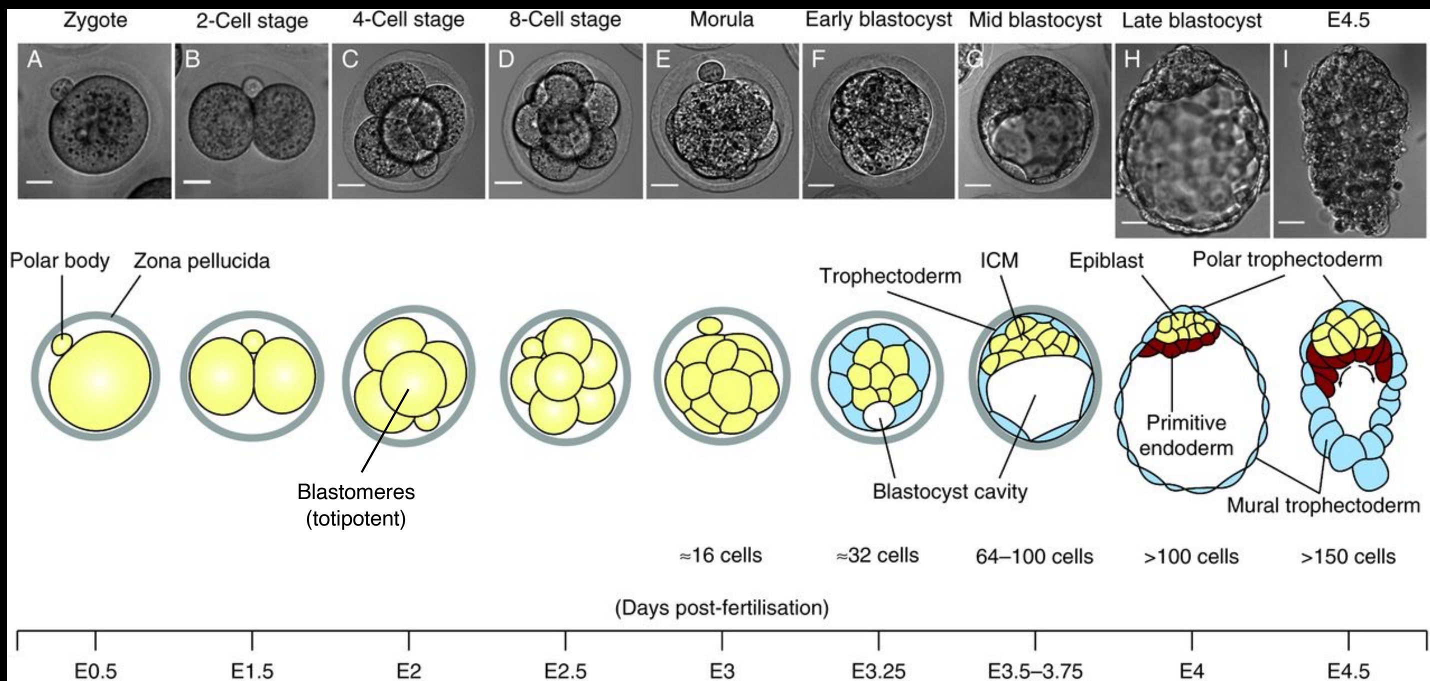
	Drosophila	Zebrafish	Mouse
Time of onset of zygotic transcription	3 hours	4 hours	>24 hours
Time of early patterning	4 hours	5 hours	~5 days

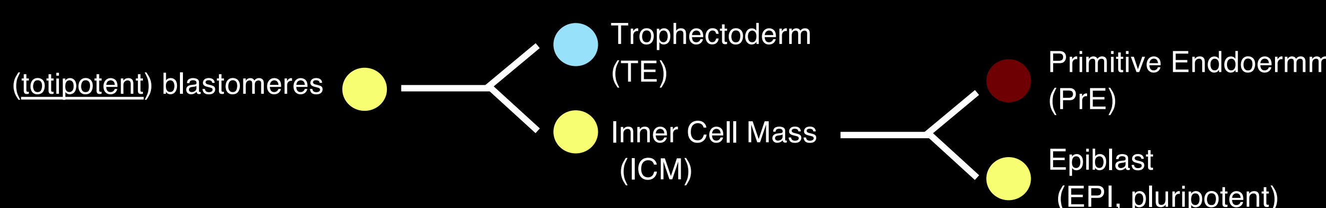
normal development



Martinez-Arias (2013) *Development*

The mammalian blastocyst
is a paradigm of self-organization and regulative development





The mammalian blastocyst
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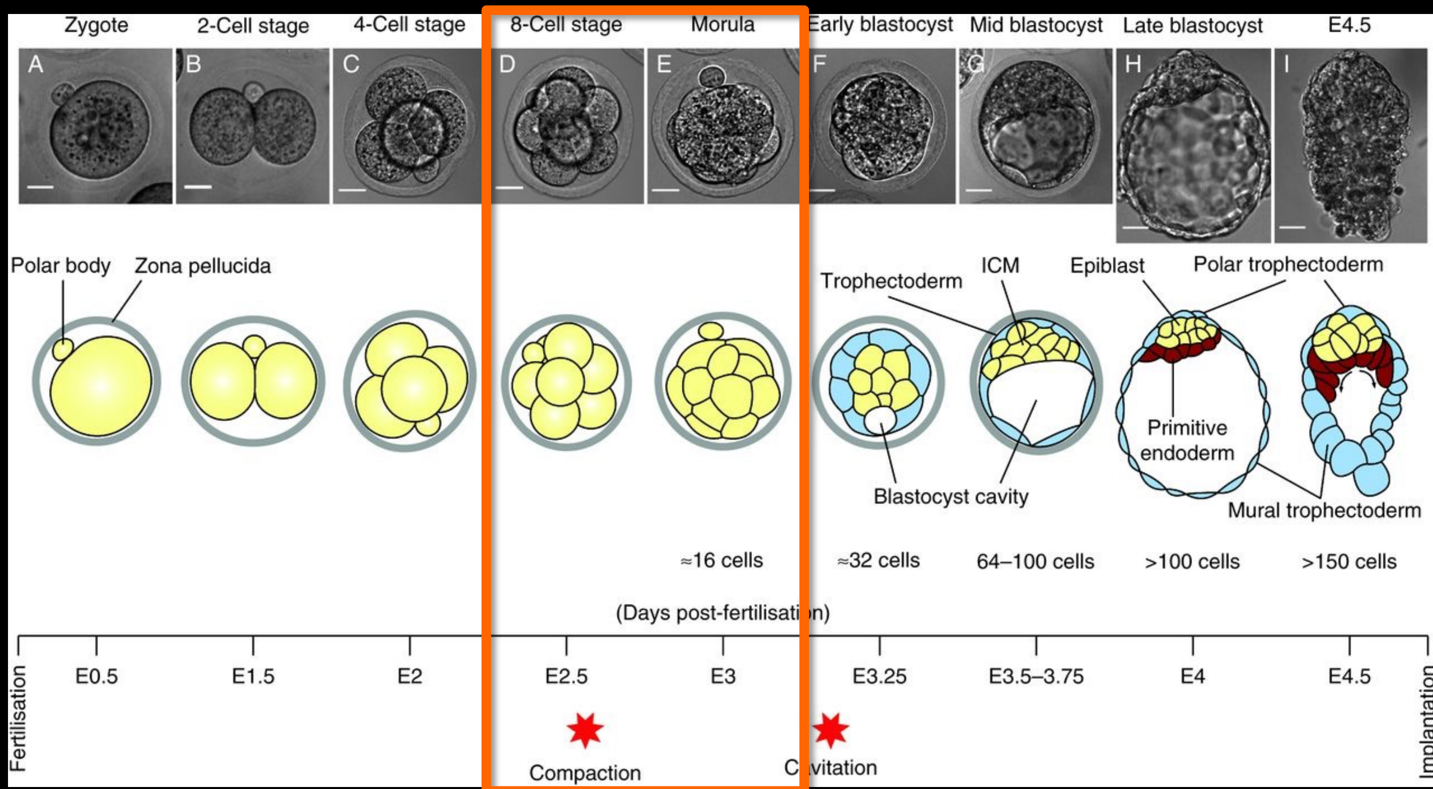
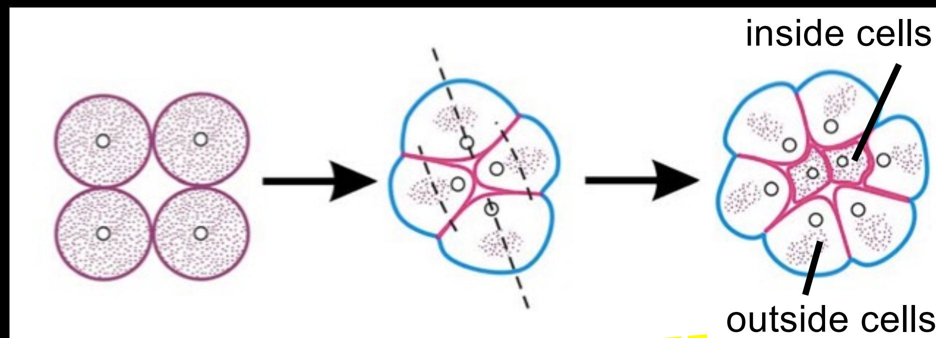
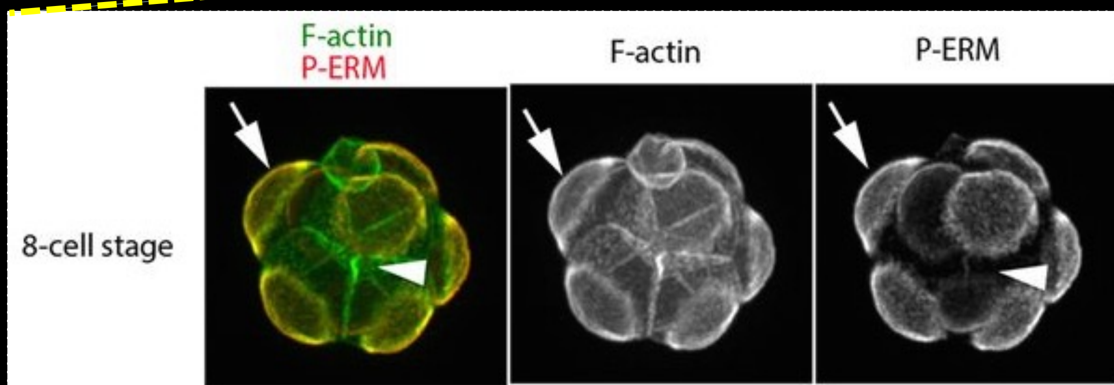


How are the lineages established?

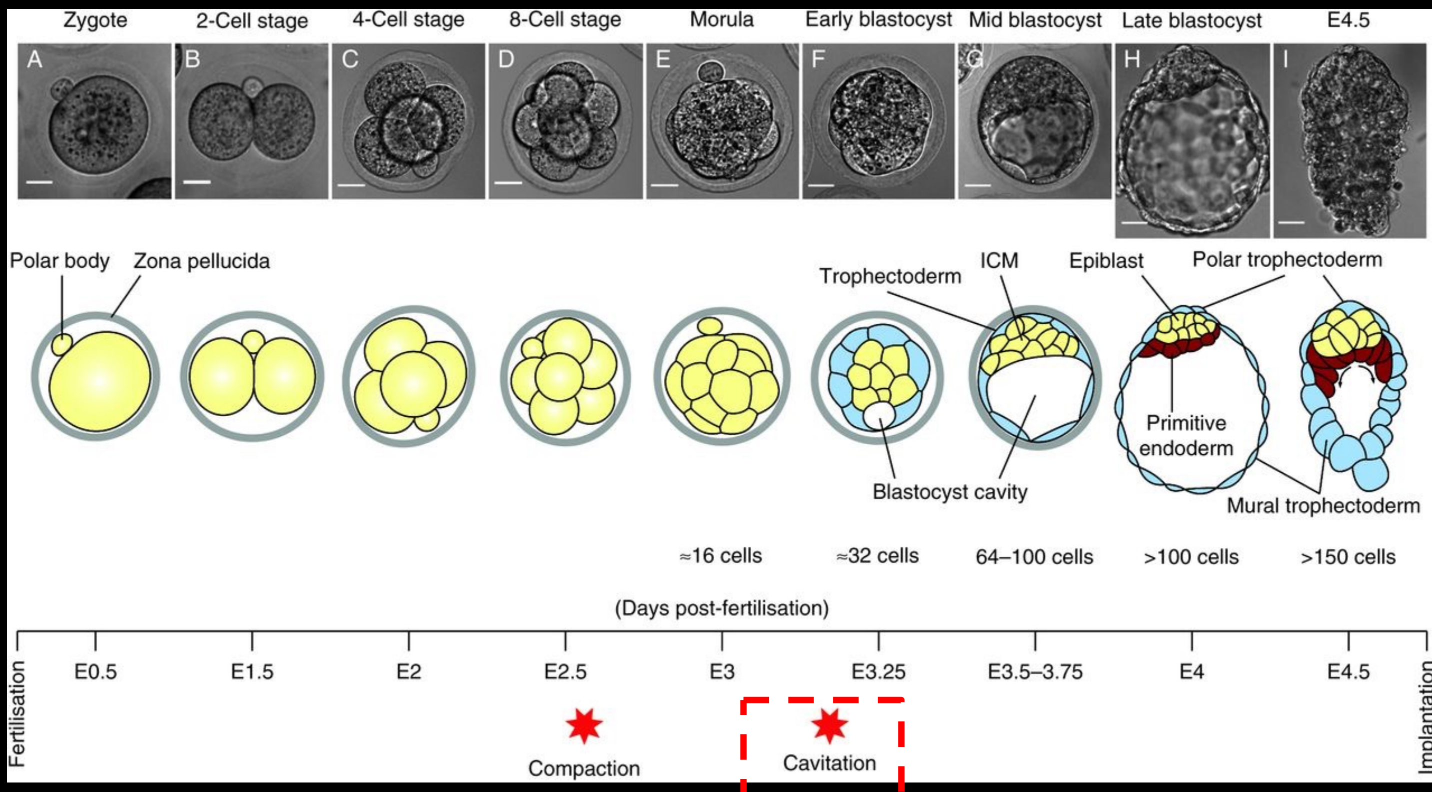
Polarized cell divisions drive epithelialization of blastocyst



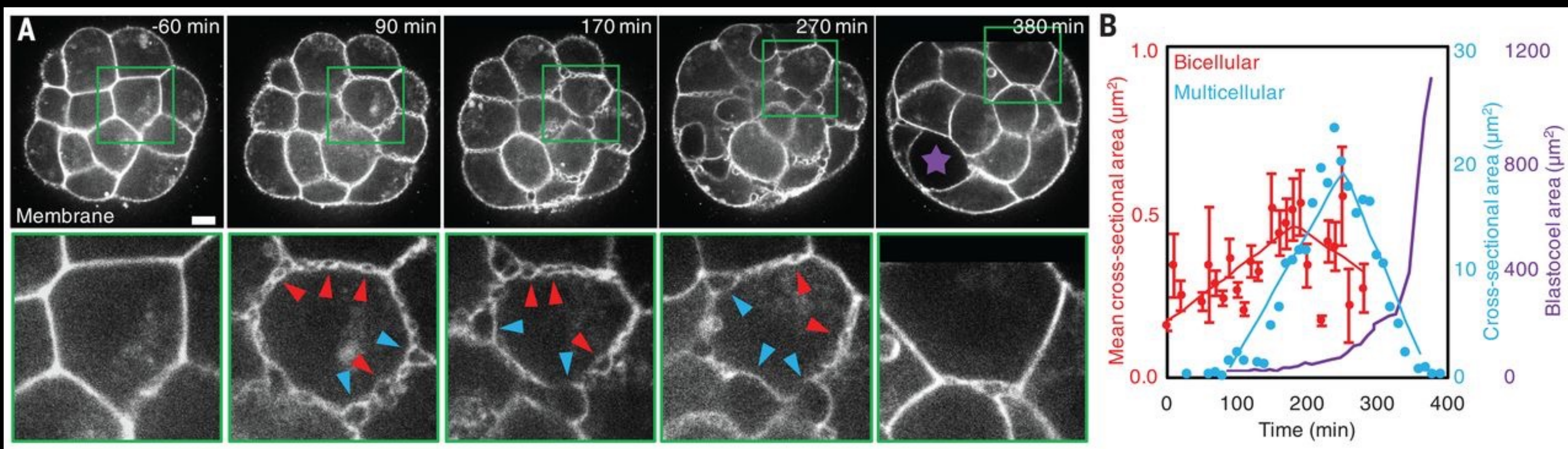


Johnson & McConnell, Seminars Cell Dev. Bio. 2004
Anani *et al.*, Development 2014
Ziomek & Johnson, Cell 1980
… *et al*

The mammalian blastocyst
is a paradigm of self-organization and regulative development

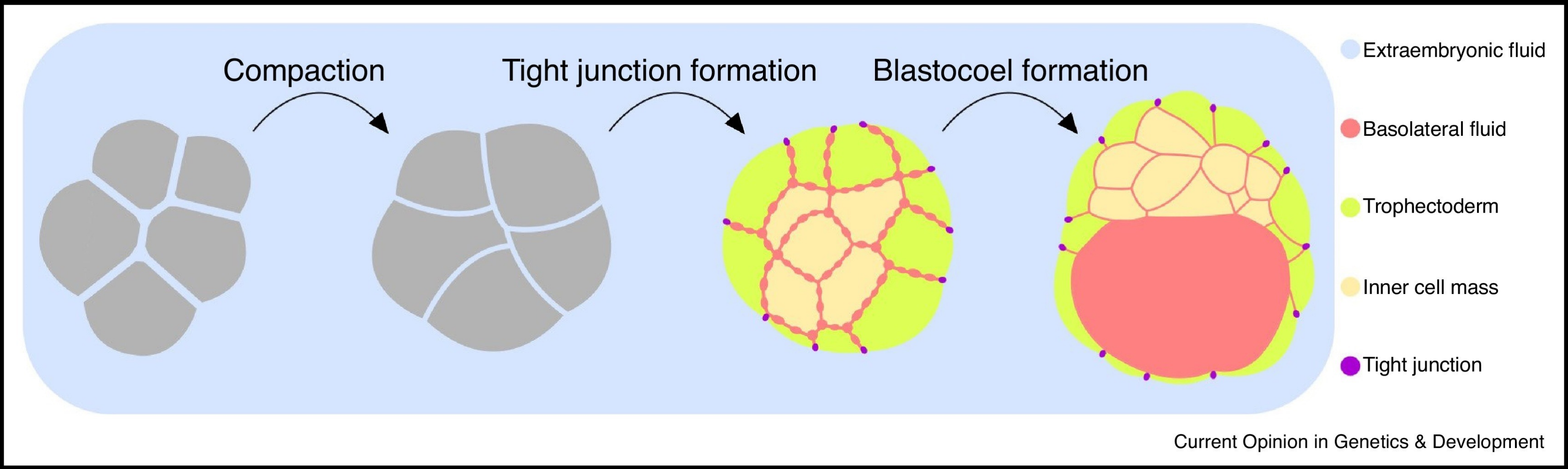


Blastocyst forms by swelling and discharge of microlumens



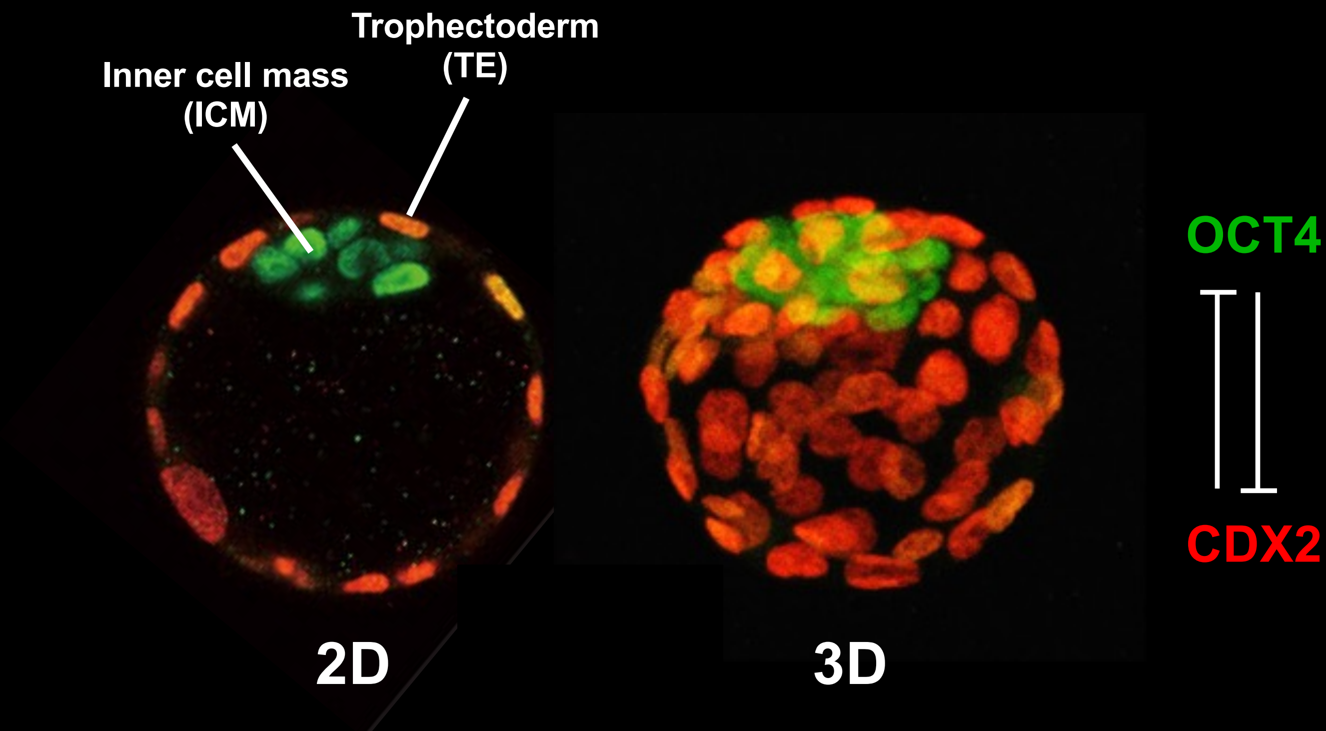
Dumortier *et al.*, Science 2019

Blastocyst forms by swelling and discharge of microlumens



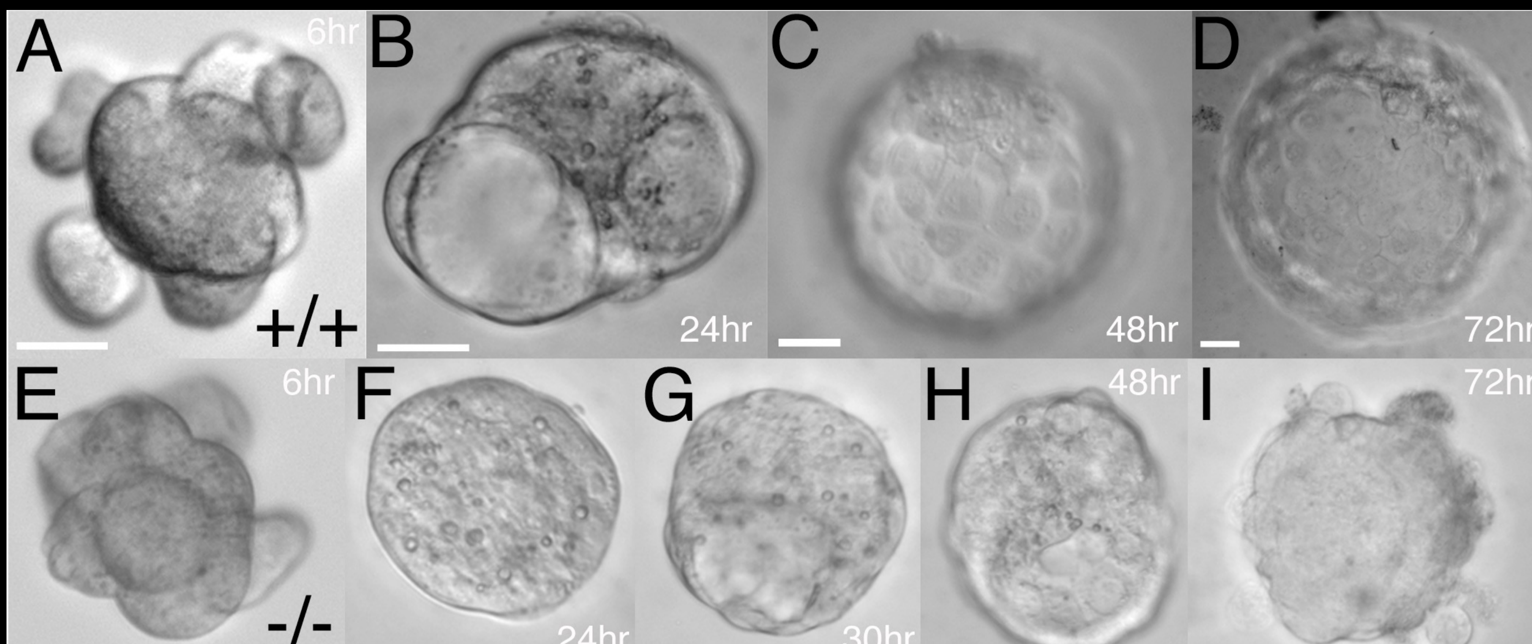
Schliffka & Maitre Current Opinion in Genetics & Development 2019

CDX2 & OCT4 expression is reciprocal in Trophectoderm / ICM



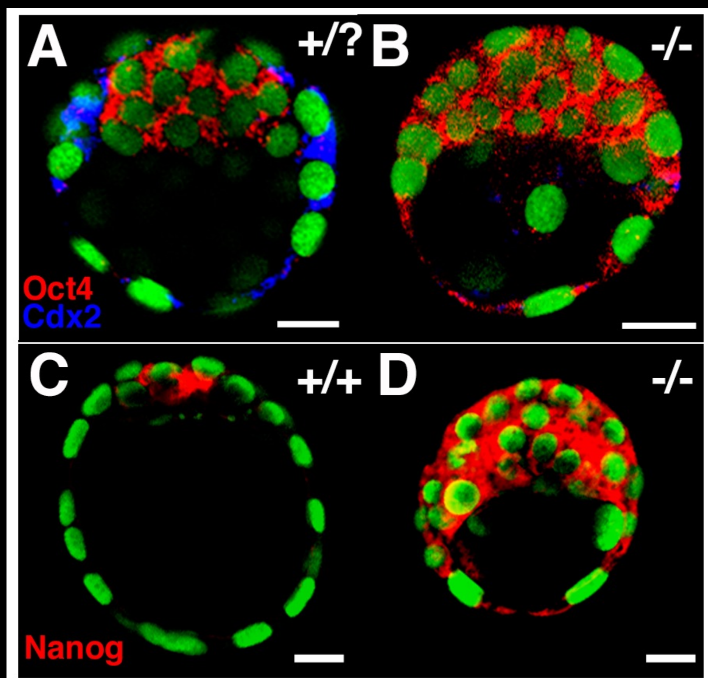
Niwa H. *et al.*, Cell 2005
Marikawa & Alarcon Mol. Reprod. Dev. 2009
and others

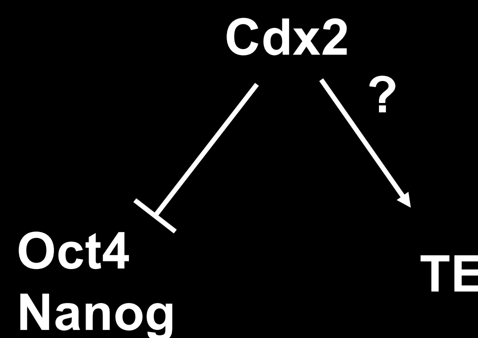
Cdx2 mutants (try but) fail to make expanded blastocysts



Strumpf *et al.*, Development 2005

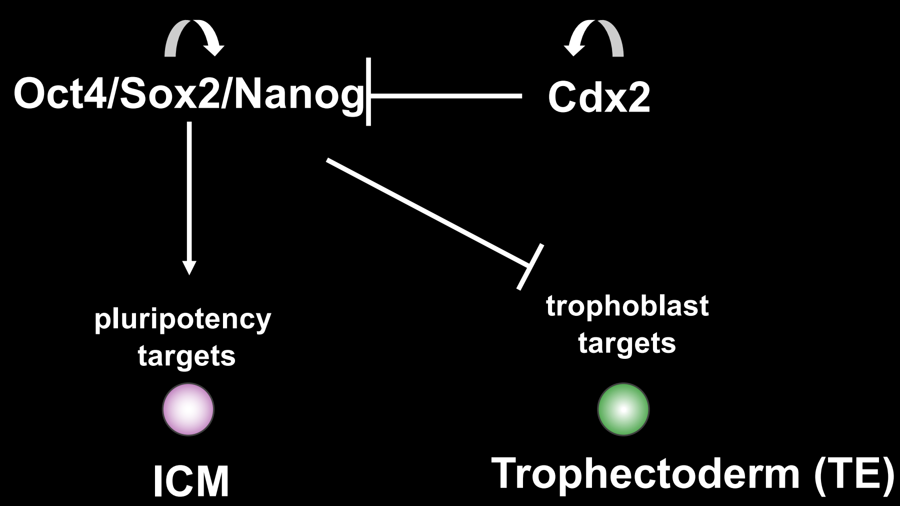
Cdx2 is required for trophoblast cell fate specification



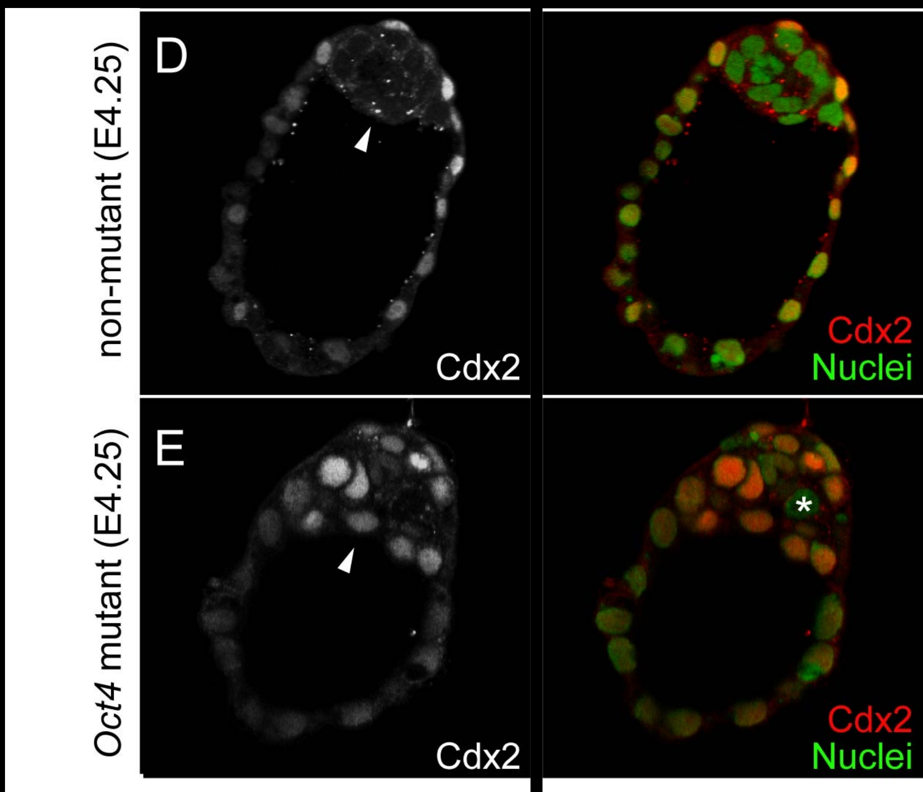


Strumpf *et al.*, Development 2005

- At blastocyst stage **Cdx2** is required to
  - downregulate Oct4/Nanog
  - maintain trophectoderm epithelial integrity
  - promote trophoblast differentiation
- Conversely, **Oct4** is required to maintain ICM



OCT4 (*Pou5f1*)is required to maintain an ICM fate



- Loss of OCT4 – blastocyst forms, but ICM not maintained
- Inside cells take up trophectoderm fate

Nichols et al., Cell 2008
Ralston et al, Dev Bio 2010
Frum et al., Dev. Cell 2013
Le Bin et al., Development 2013
Stiraparo et al., PNAS 2021
and others…

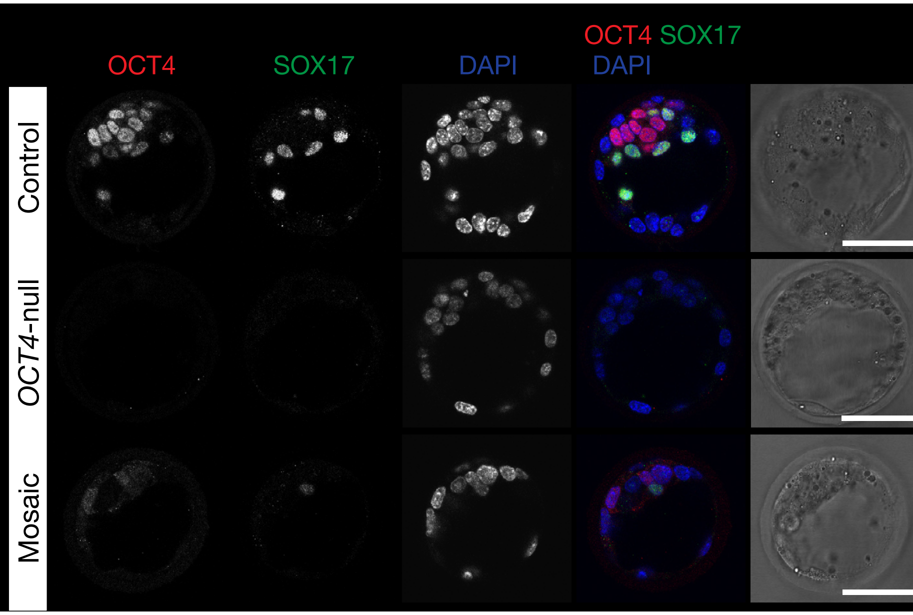
A conserved role for OCT4 in the human embryo

ARTICLE

doi:10.1038/nature24033

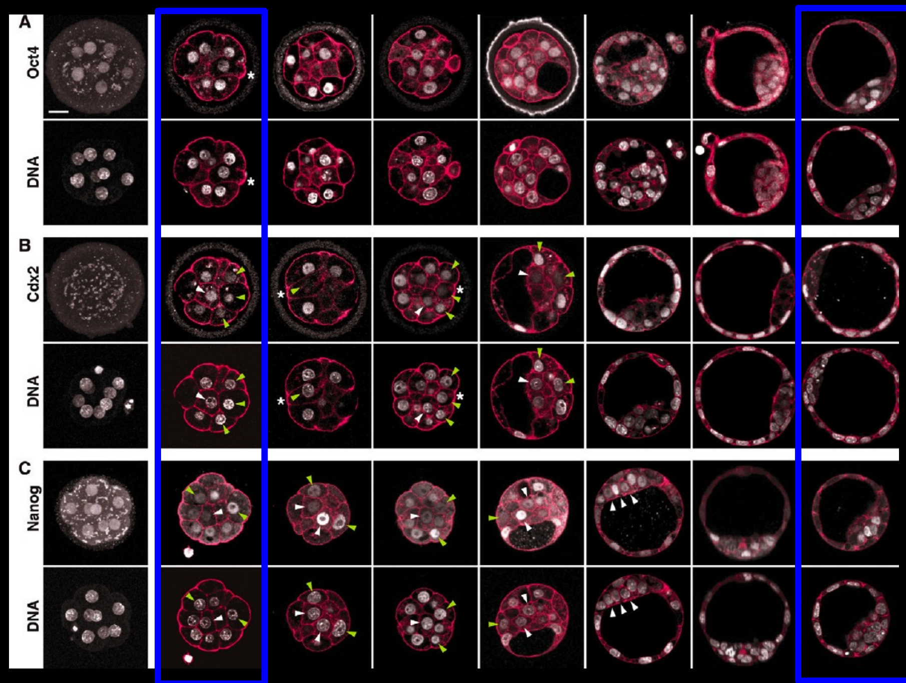
Genome editing reveals a role for OCT4 in human embryogenesis

Norah M. E. Fogarty[1], Afshan McCarthy[1], Kirsten E. Snijders[2], Benjamin E. Powell[3], Nada Kubikova[4], Paul Blakeley[1], Rebecca Lea[1], Kay Elder[5], Sissy E. Wamaitha[1], Daesik Kim[6], Valdone Maciulyte[3], Jens Kleinjung[7], Jin-Soo Kim[6,8], Dagan Wells[4], Ludovic Vallier[2,9,10], Alessandro Bertero[10]†, James M. A. Turner[3] & Kathy K. Niakan[1]



Fogarty et al., Nature 2017

Initially lineage-specific transcription factors are expressed by all cells

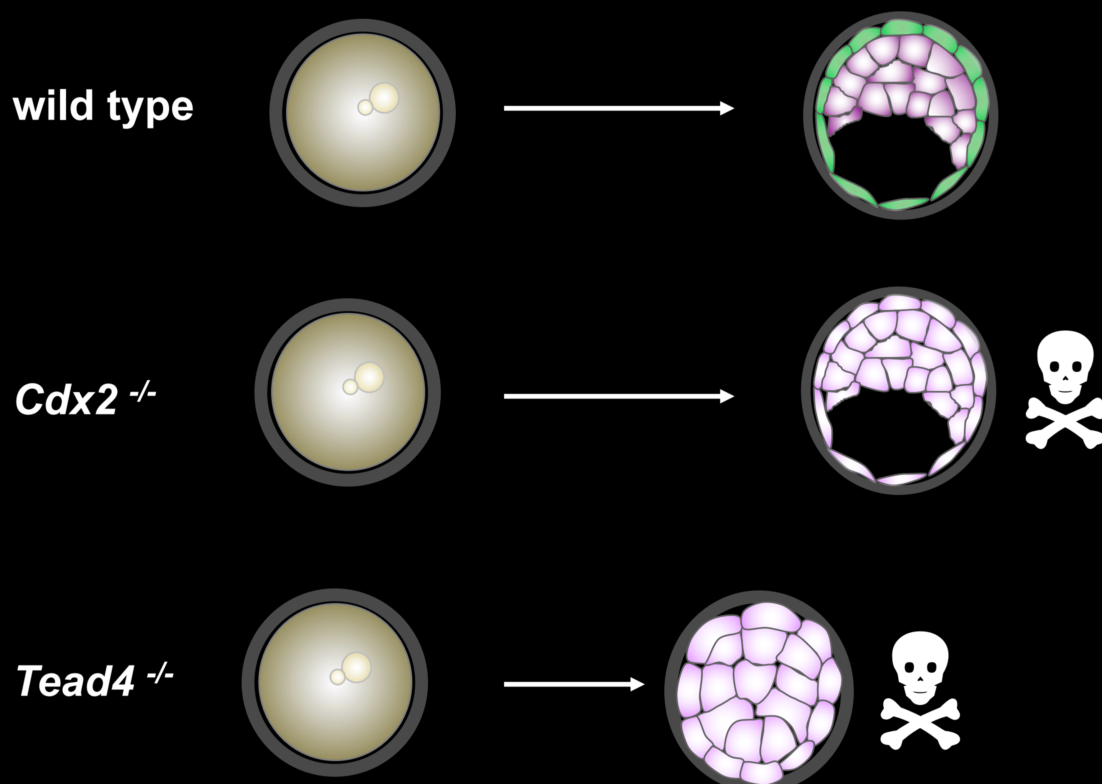


- There is initially no reciprocal relationship between **CDX2 (TE)** and **OCT4 (ICM)** !!
- What are the molecular mechanisms that initially interpret inside *vs*. outside positional information within the embryo?

Dietrich and Hiiragi, Development 2007
Plusa et al., Development 2008

What is upstream of Cdx2?

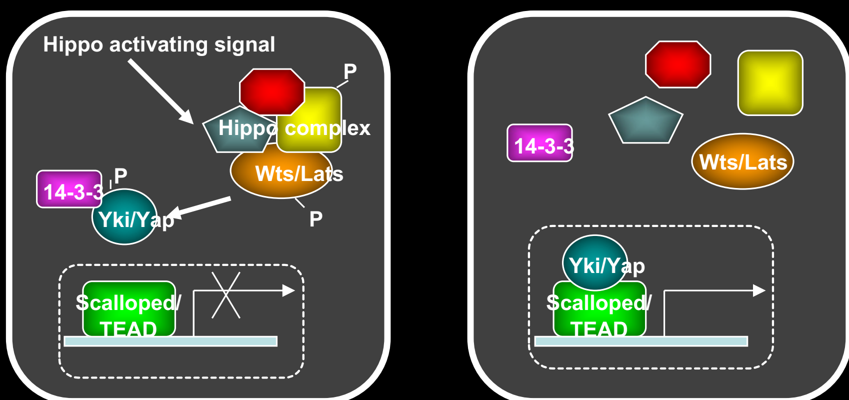
Hierarchy of trophectoderm (TE) transcription factors



Strumpf *et al.*, Development 2005
Yagi *et al.*, Development 2007
Nishioka *et al.*, MOD 2008

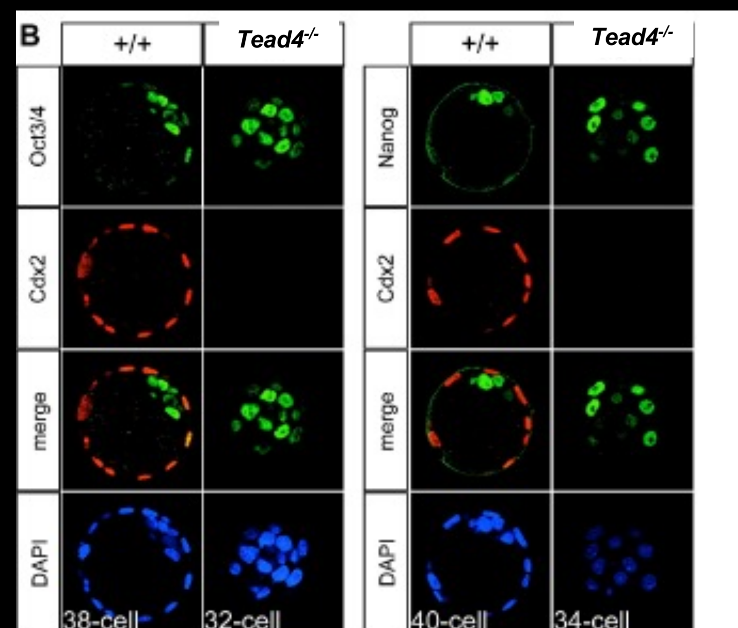
Outside cells of the preimplantation embryo form the trophectoderm (TE)

a process requiring the transcription factor Tead4



Wrts/Lats active:
Scalloped/Tead inactive

Wrts/Lats inactive:
Scalloped/Tead active



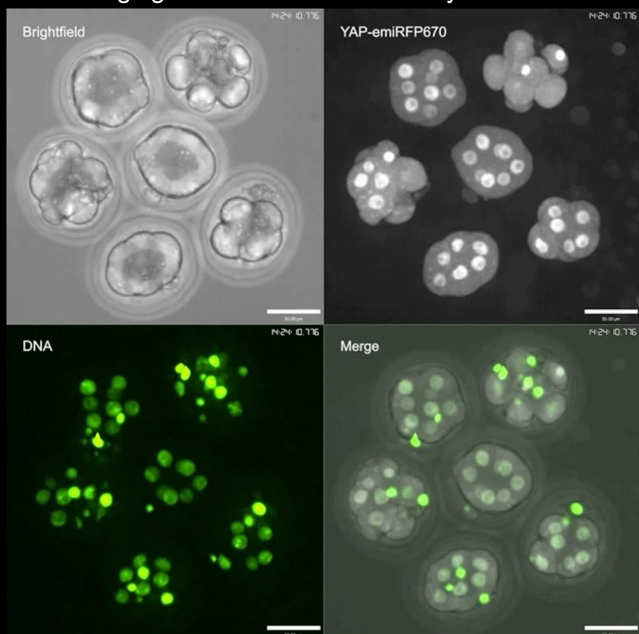
Yagi et al., Development 2007
Nishioka et al., Dev Cell 2008
Nishioka et al., Mechanisms of Development 2008
Wicklow et al., PLOS Genet. 2014
Posfai et al., eLife 2017
Frum et al., eLife 2018
Frum et al., Development 2019
and others…

- Tead4 is genetically upstream of *Cdx2* during TE formation in the embryo
- Tead4 coactivator protein Yap localizes to nuclei of outside cells
- Tead4/Yap required for Cdx2 expression in outside cells
- Hippo signaling proposed to be controlled by cell polarity/contact in embryos

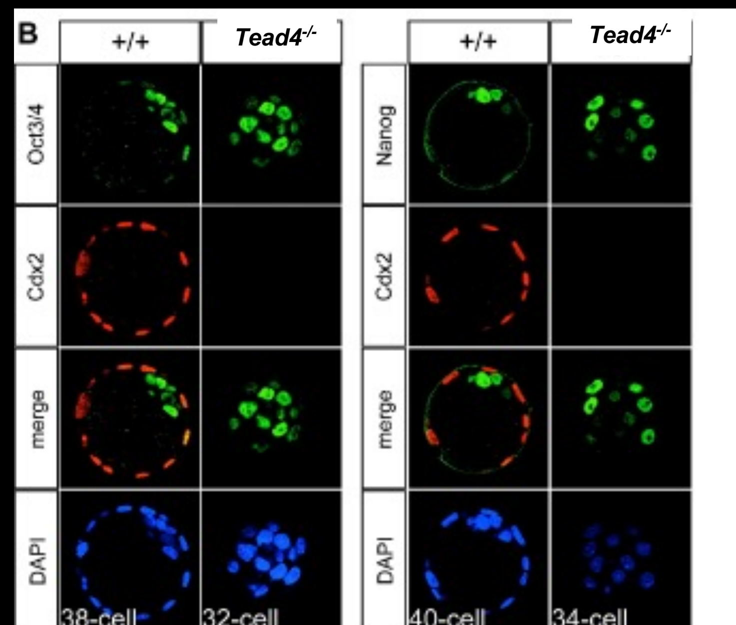
Outside cells of the preimplantation embryo form the trophectoderm (TE)

a process requiring the transcription factor Tead4

Live imaging YAP in 8-to-16 cell embryos



Gu et al., Open Biol. 2022



Nishioka et al., Dev Cell 2008
Nishioka et al., Mechanisms of Development 2008
Yagi et al., Development 2007

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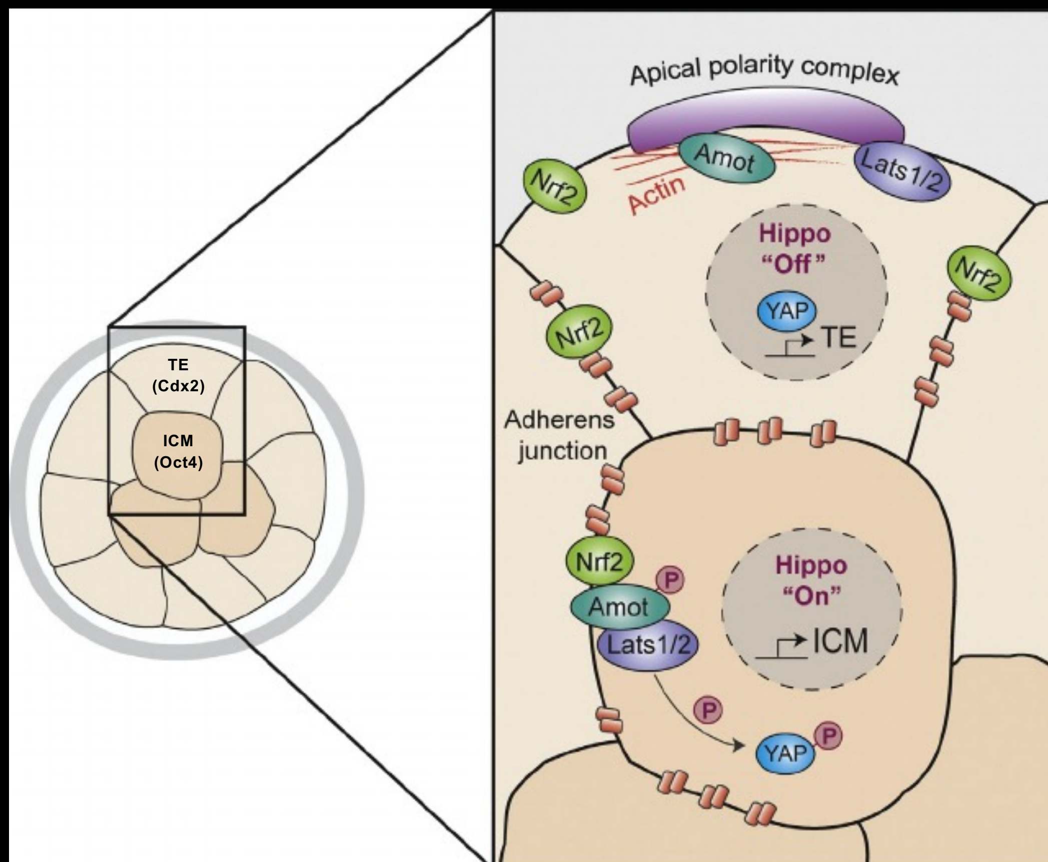
Model of TE specification in the mouse embryo



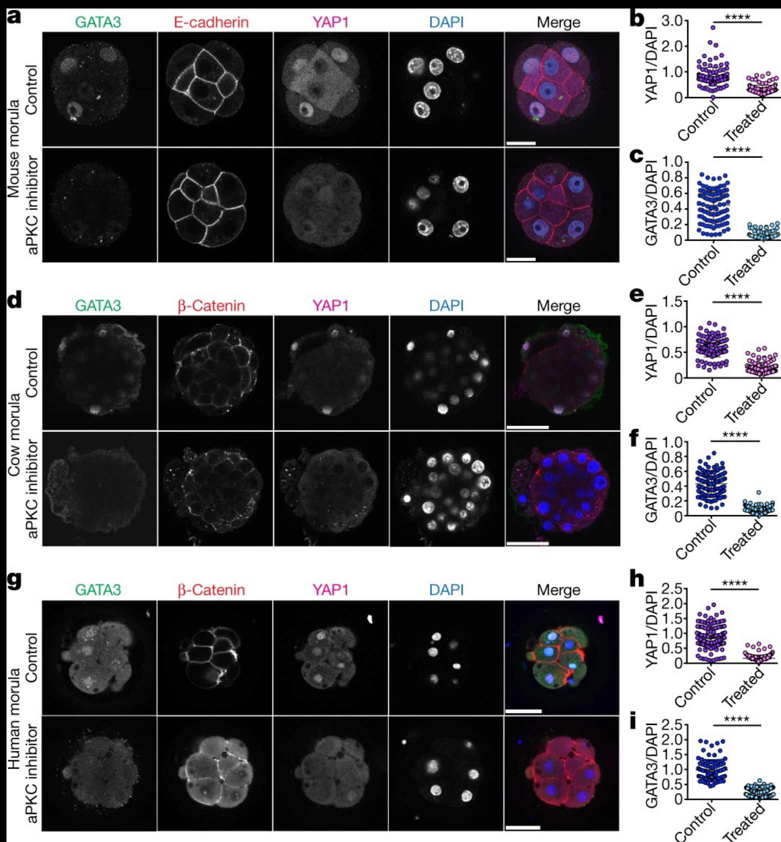
White et al., Current Topics in Dev Bio 2015

A conserved trophectoderm (TE) program in human, cow & mouse embryos

mouse

cow

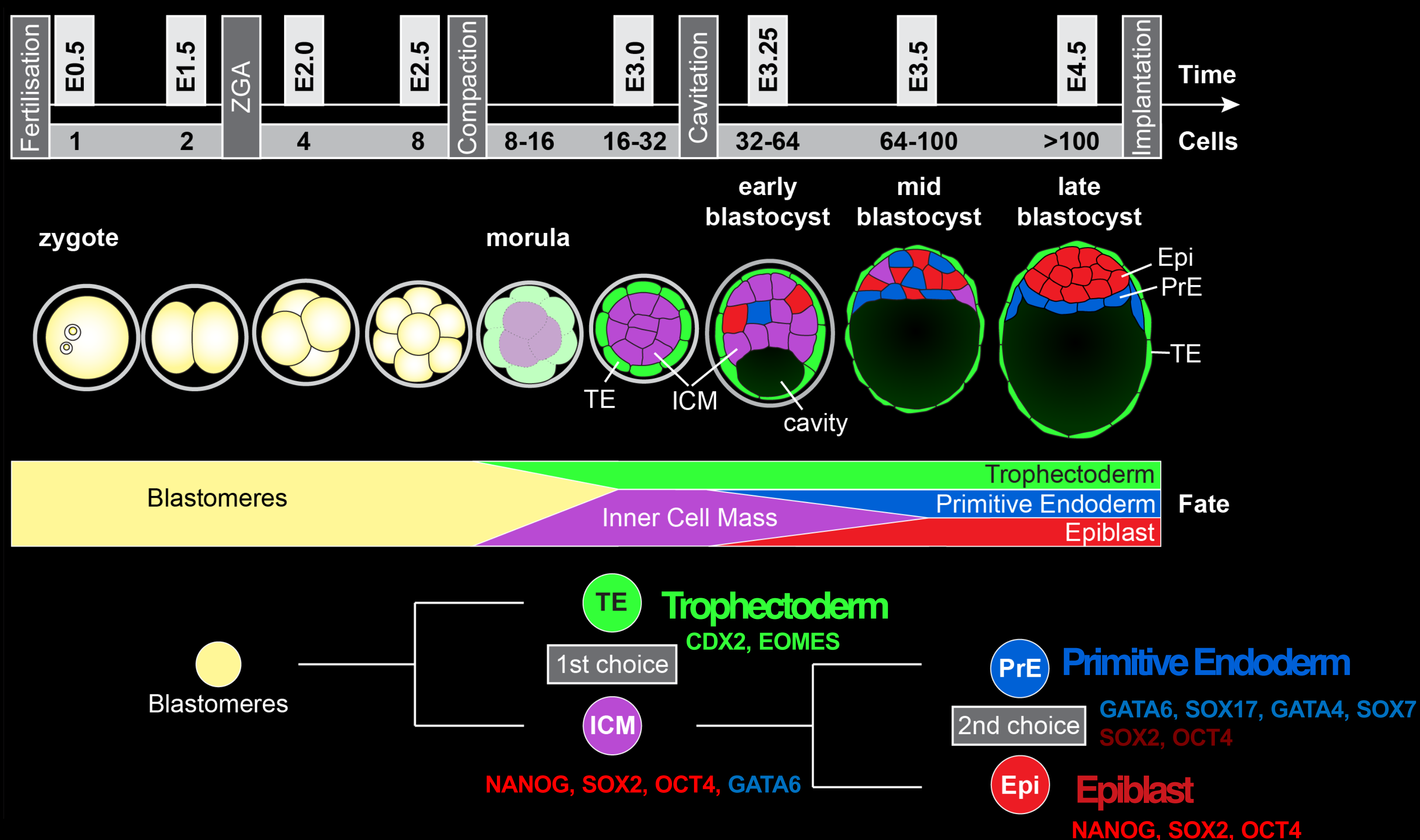
human



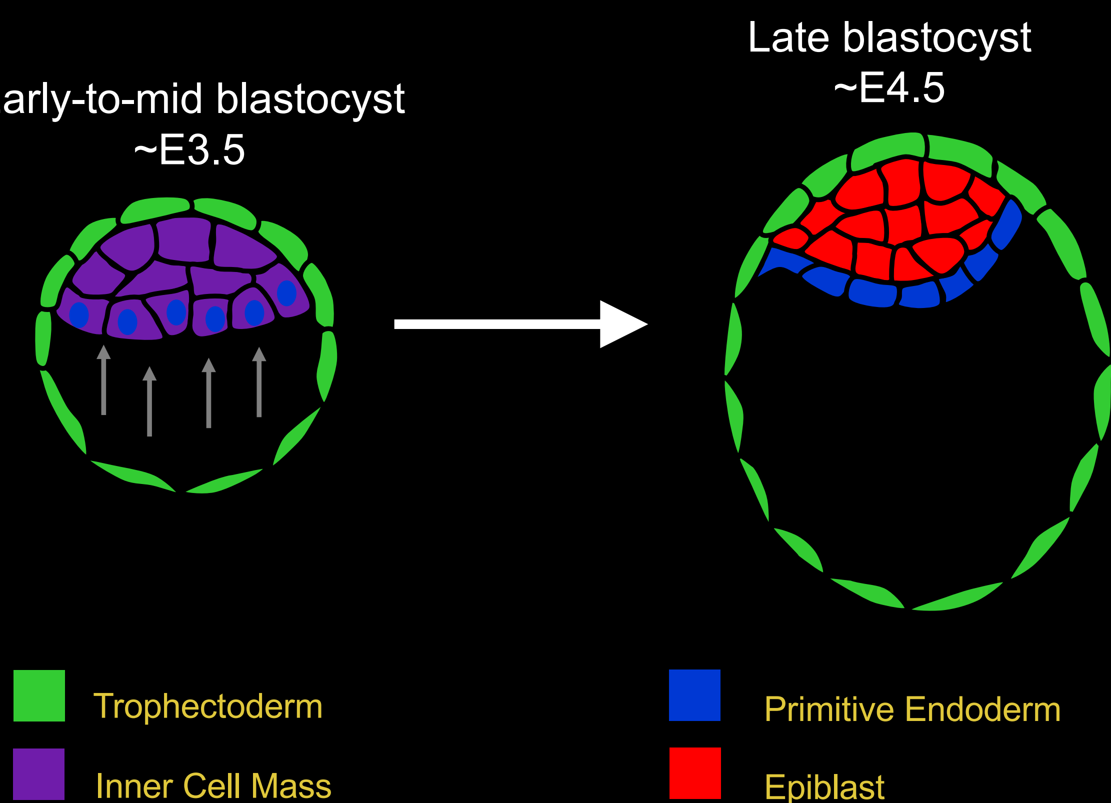
aPKC activity is required for YAP1 and GATA3 (TE marker) expression in mouse, cow and human morula stage embryos

Gerri et al., Nature 2020

2nd lineage decision in the mammalian embryo - Epi *vs.* PrE

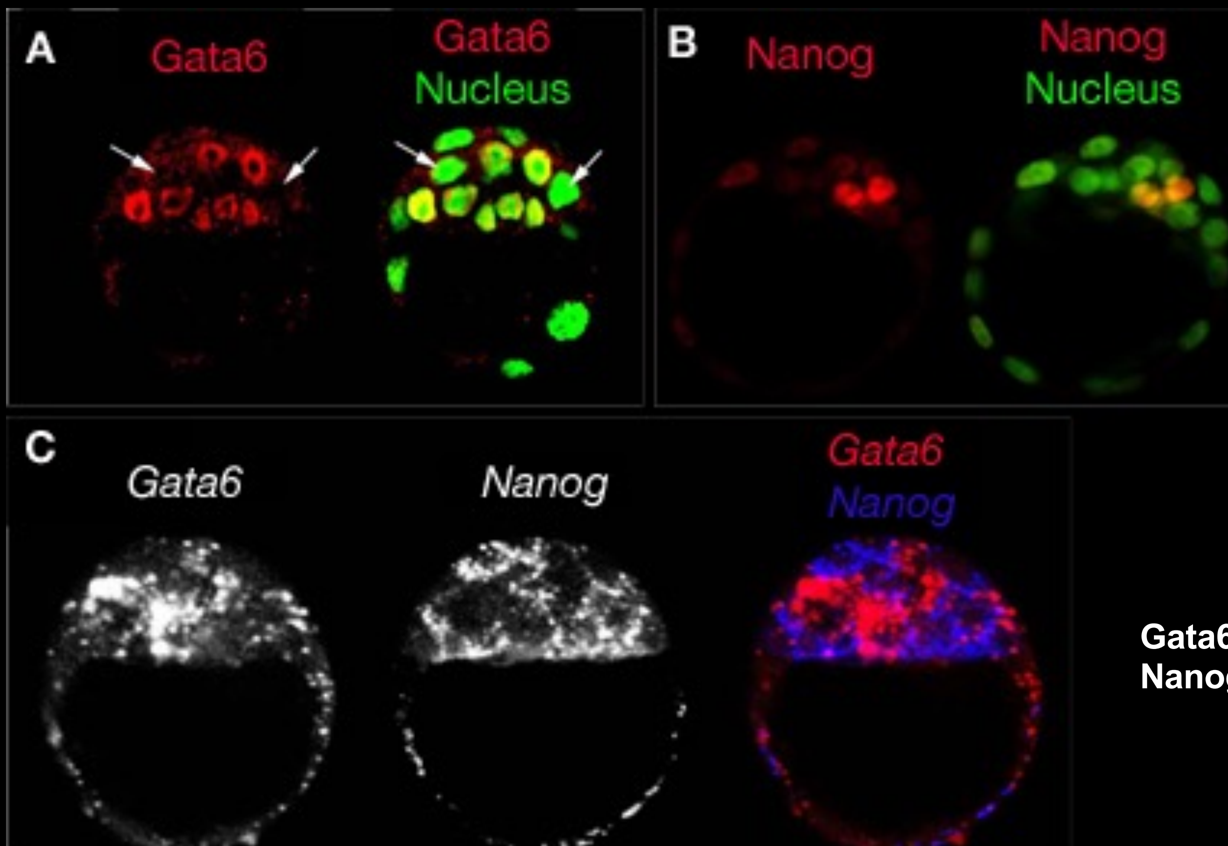


Position-dependent model for PrE *vs.* EPI specification within the ICM



Gardner & Rossant, JEEM 1979
Gardner, JEEM 1982; 1984

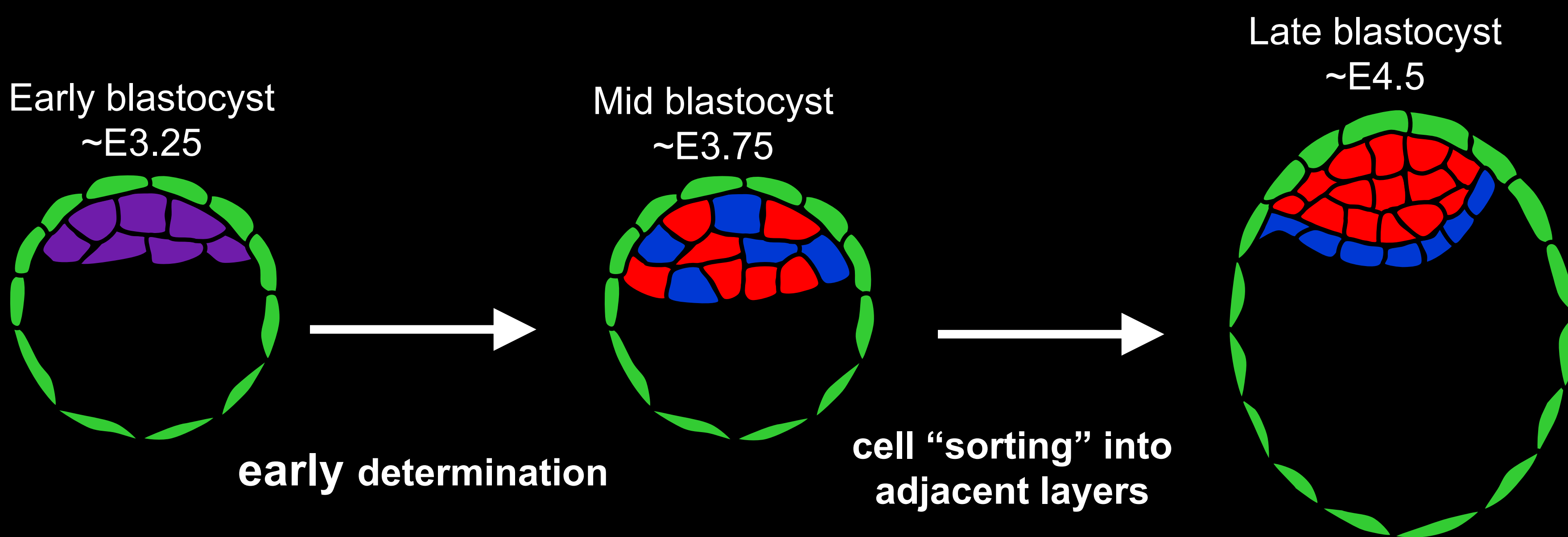
Salt-and-Pepper expression of Nanog and Gata6 in E3.5 blastocyst ICM



Gata6 = primitive endoderm (PrE) marker
Nanog = Epiblast (EPI) marker

Chazaud *et al*., Dev. Cell 2006

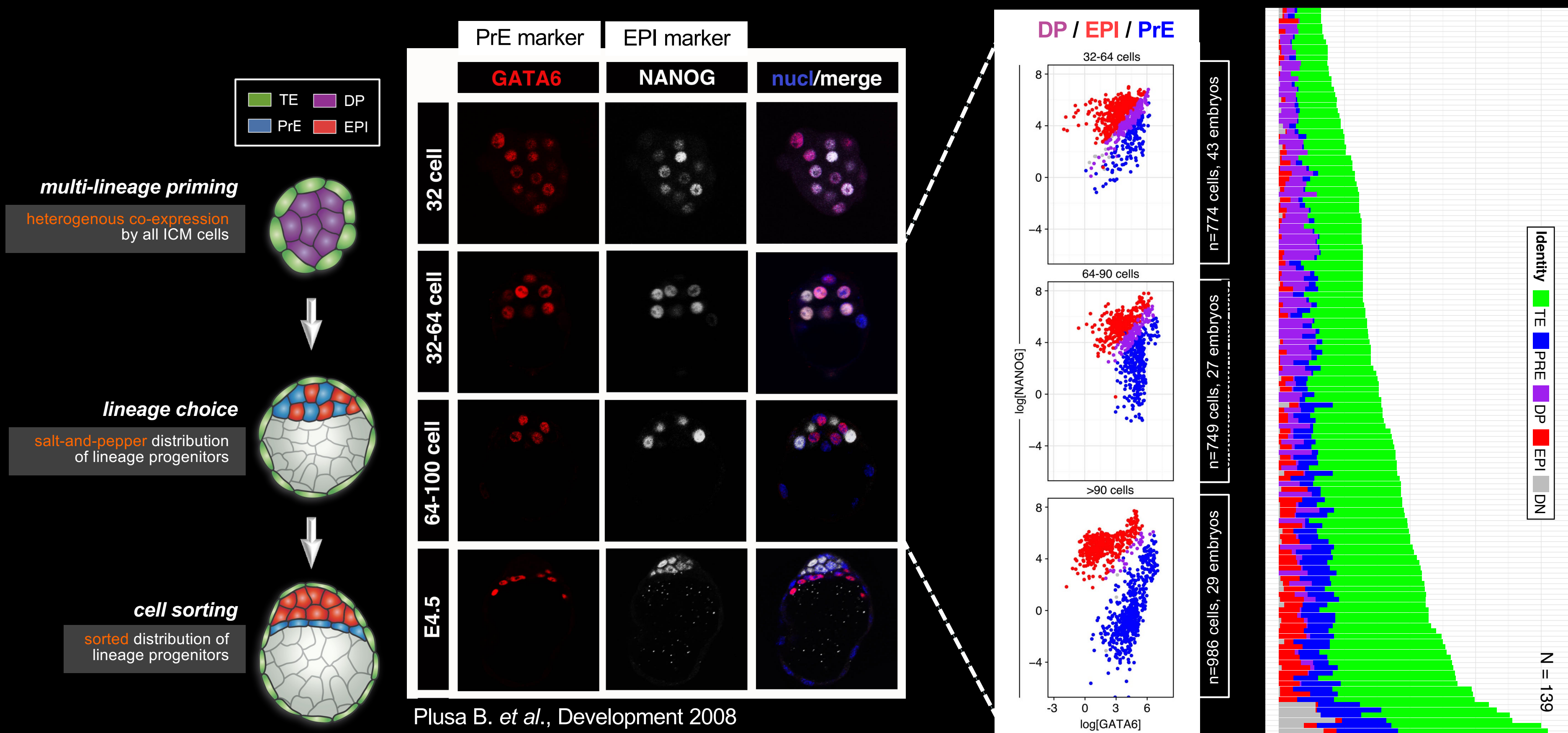
Current model for ICM differentiation



- Trophectoderm
- Inner Cell Mass
- Primitive Endoderm
- Epiblast

Chazaud et al., Developmental Cell 2006
Plusa et al., Development 2008
Meilhac et al., Dev. Biology 2009
Ohnishi et al., Nature Cell Biol. 2014
and many others

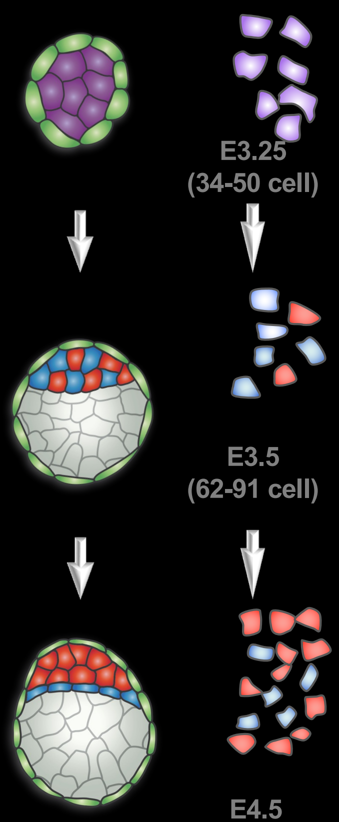
Restriction of NANOG and GATA6 correlates with EPI/PrE cell fate choice

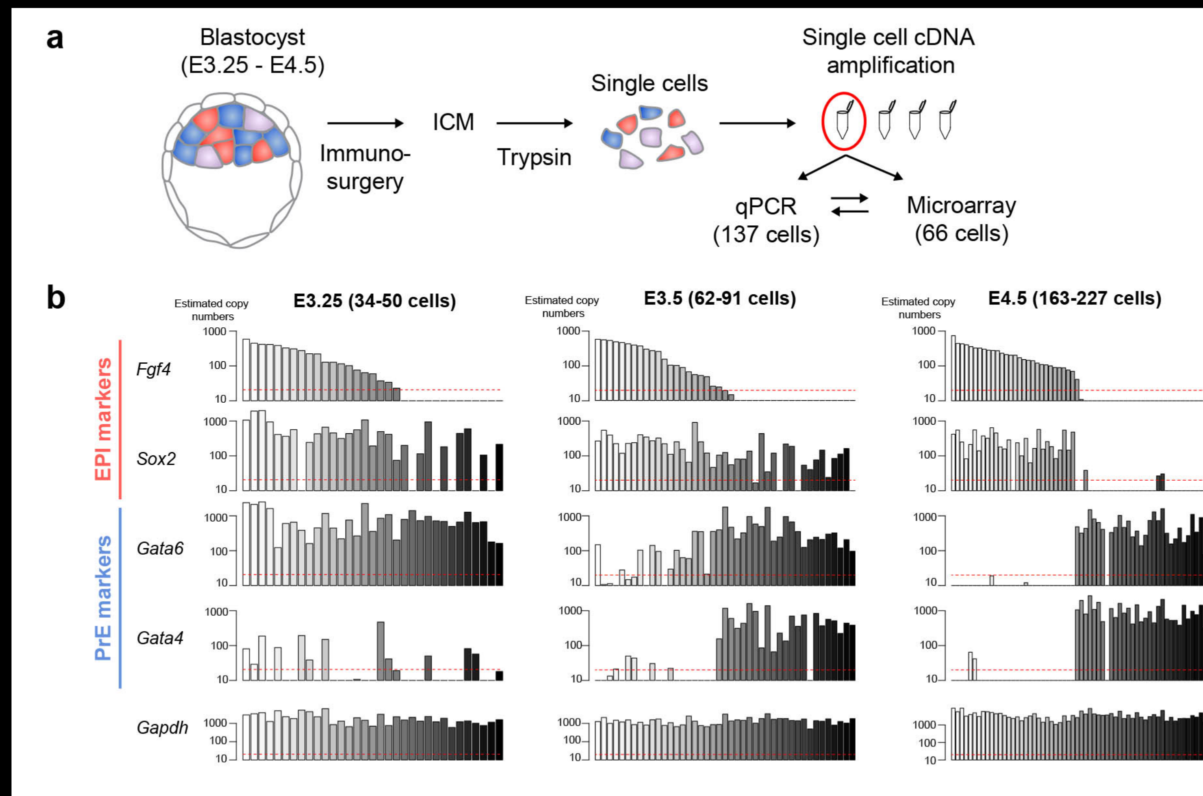


Plusa B. *et al*., Development 2008

Saiz N. *et al*., Nature Comms. 2016

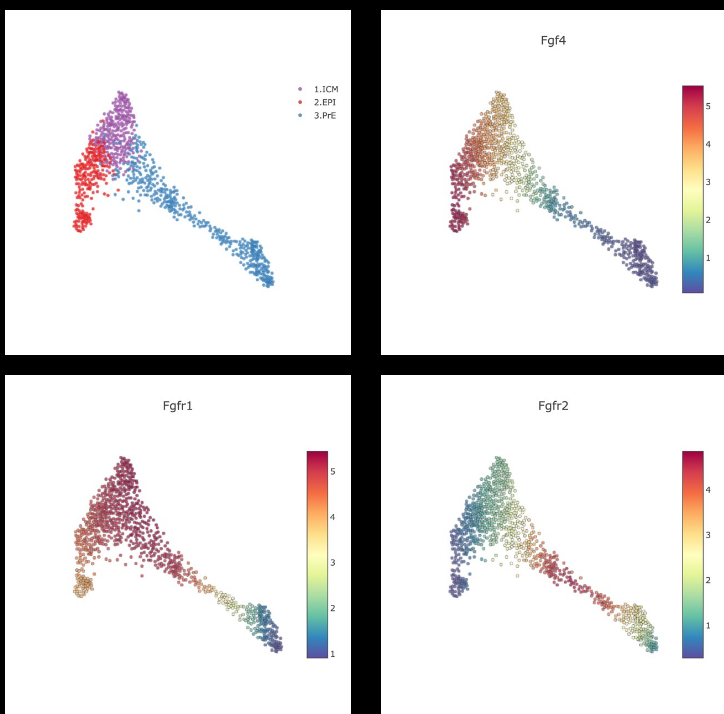
Fgf4 is the first marker to be asymmetrically expressed within the ICM



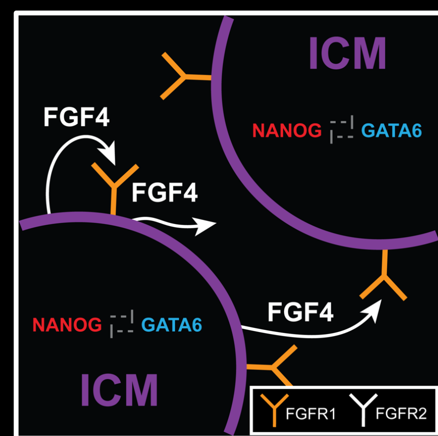


Ohnishi *et al.* Nature Cell Biol. 2014

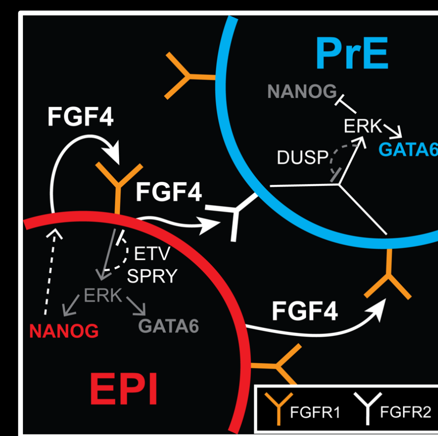
Fgfr1 and Fgfr2 expression in ICM



Nowotschin et al., Nature 2019

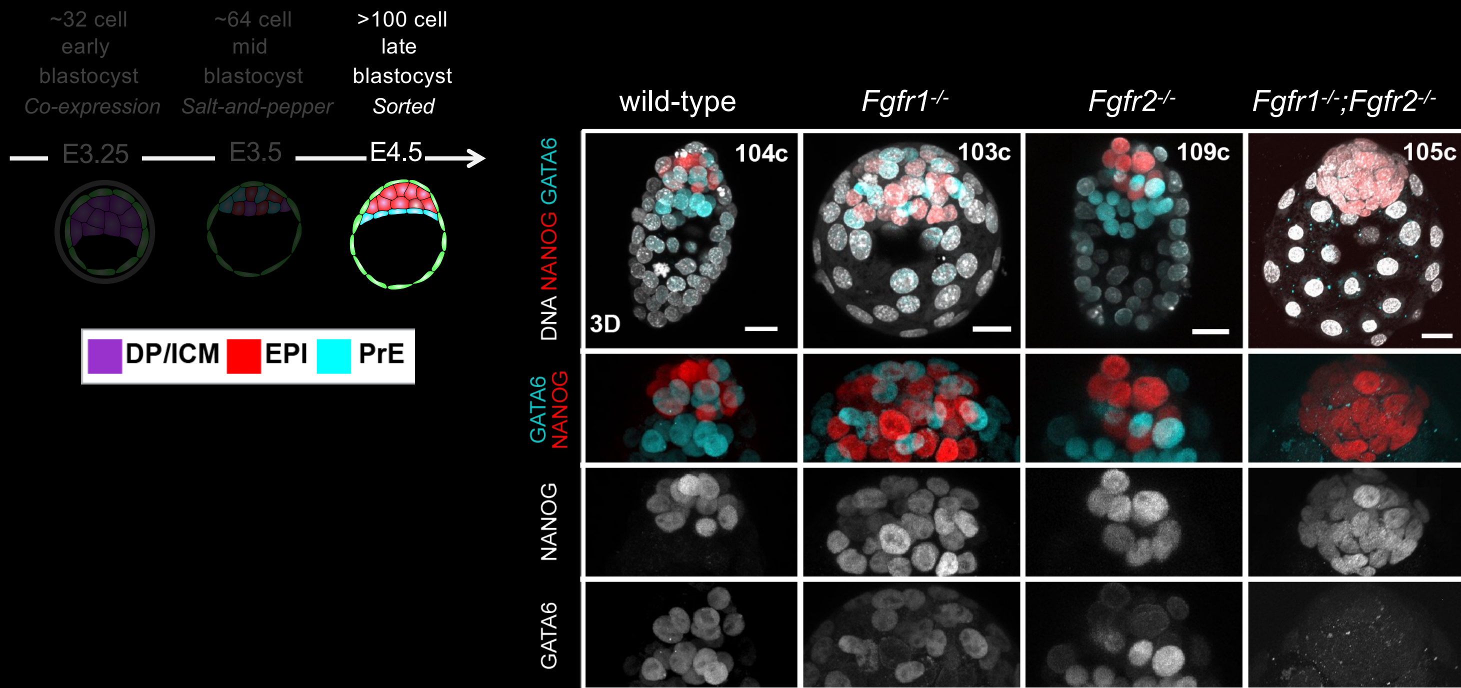


E3.25 (early blastocyst)



E3.5 (mid blastocyst)

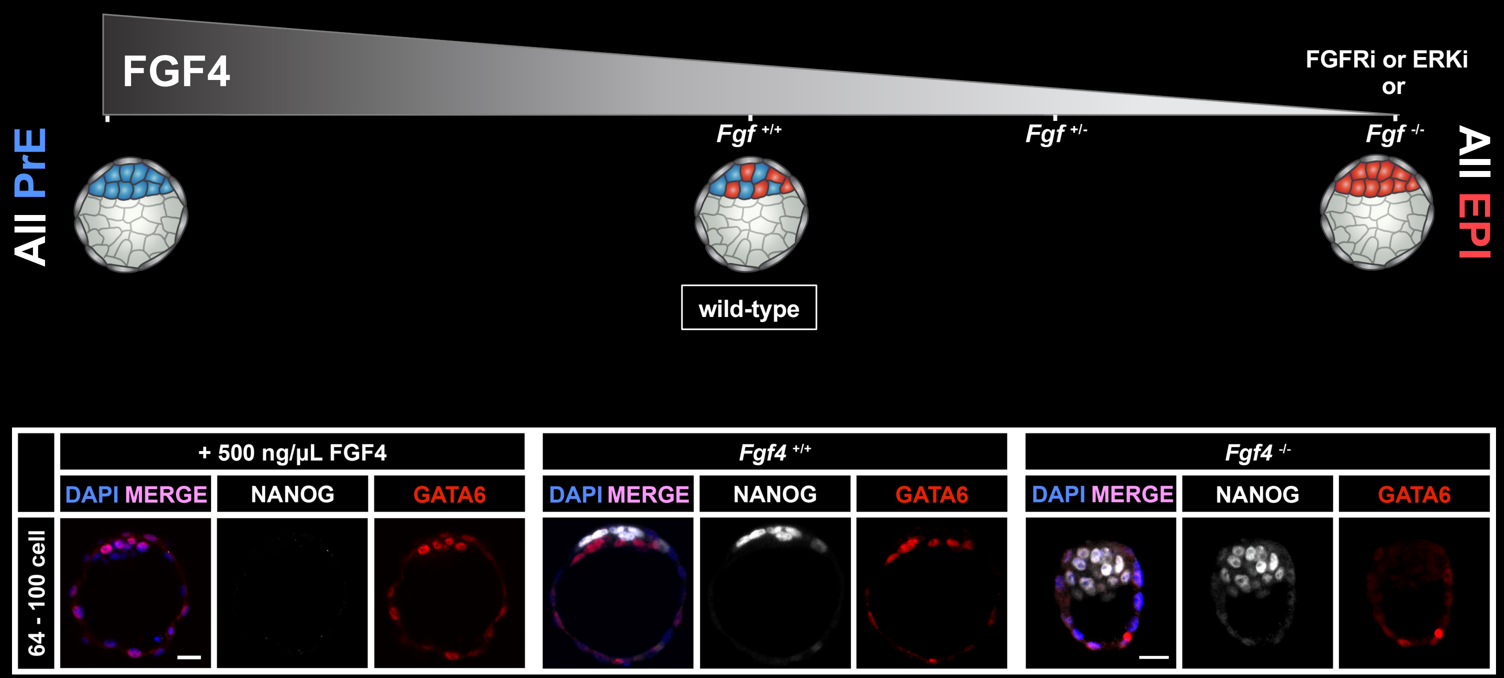
Fgfr1$^{-/-}$*;Fgfr2*$^{-/-}$ embryos fail to form PrE lineage



- *Fgf4* mutants phenocopy *Fgfr1 ; Fgfr2* double mutants (Kang et al., 2013; Krawchuk et al., 2014)

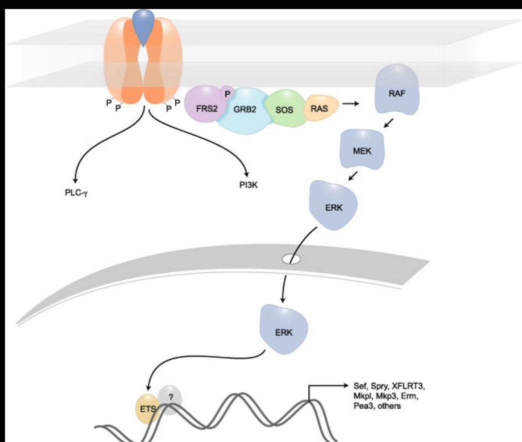
Kang *et al.*, Dev. Cell 2017
Molotkov *et al.*, Dev. Cell 2017

Modulation of FGF signaling influences ICM cell lineage specification

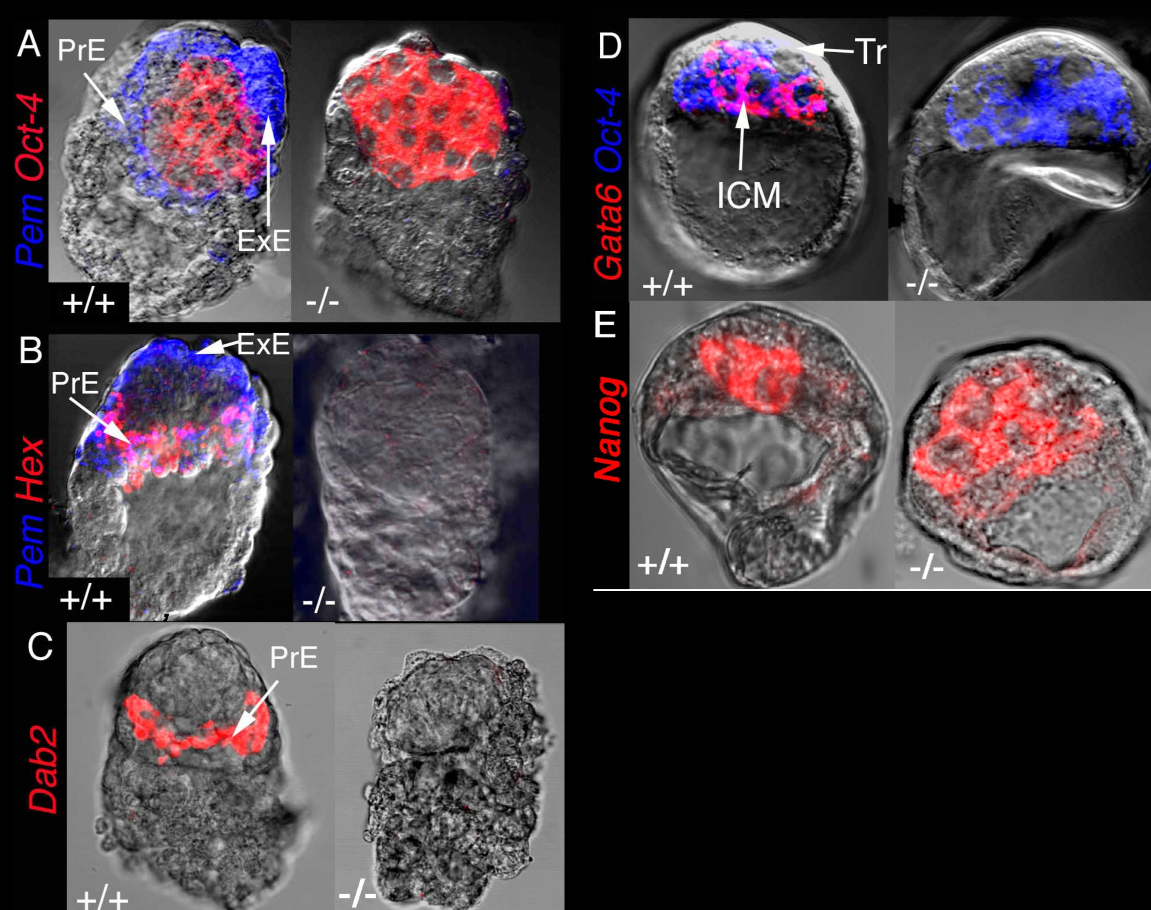


Chazaud *et al*., Dev. Cell 2006; Nichols *et al.,* Development 2009; Yamanaka *et al*., Development 2010; Kang *et al.* Development 2013; Krawchuk *et al.,* Dev. Bio 2014; Saiz et al., Nature Comms 2016; and others…

Grb2$^{-/-}$ mutants lack PrE, all ICM cells transform to Nanog-positive Epi cells

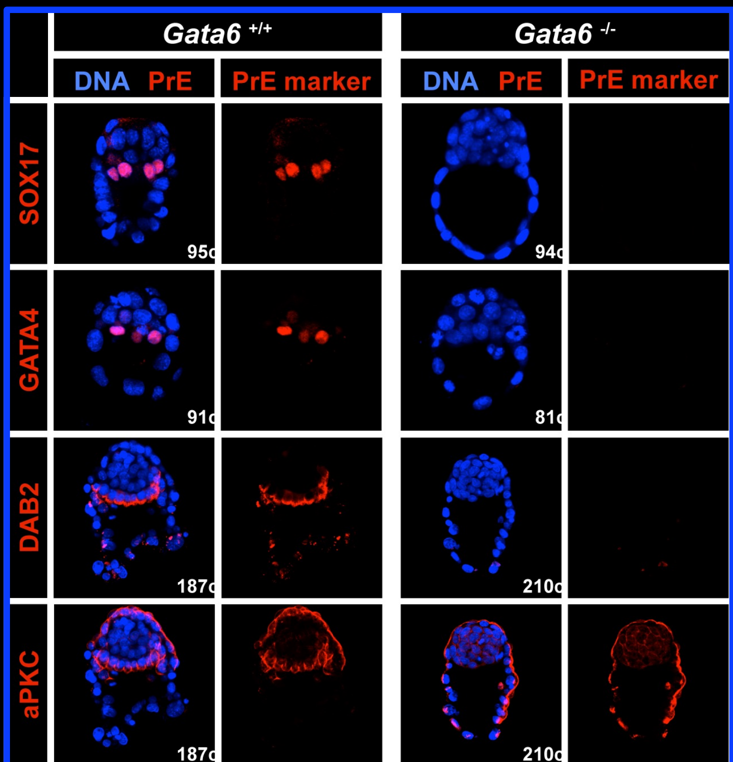


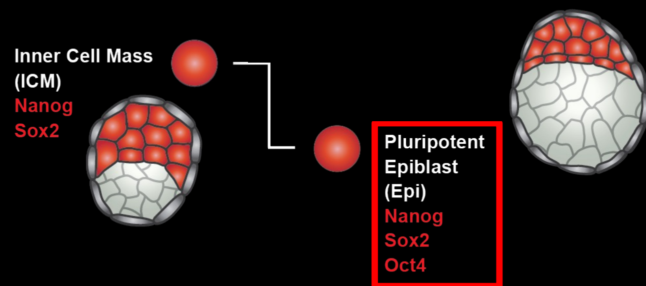
Tsang et al., Science Signaling 2004

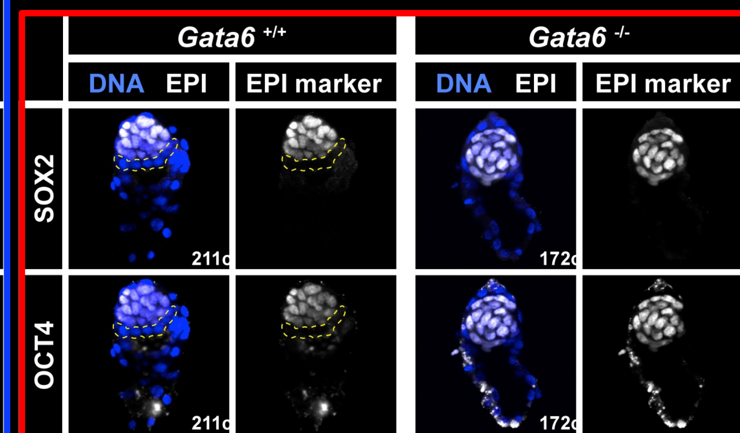


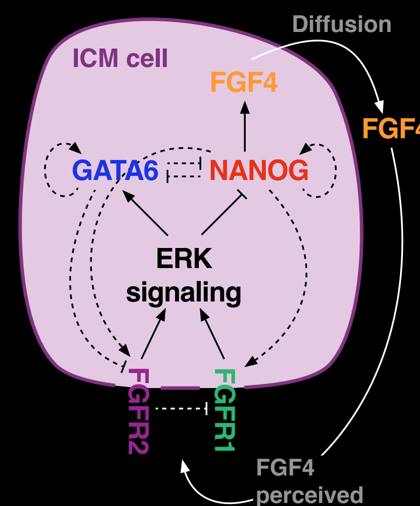
Chazaud et al., Developmental Cell 2006

Like FGF signaling mutants, Gata6$^{-/-}$ embryos fail to specify primitive endoderm (PrE), all ICM cells adopt a pluripotent epiblast (EPI) identity



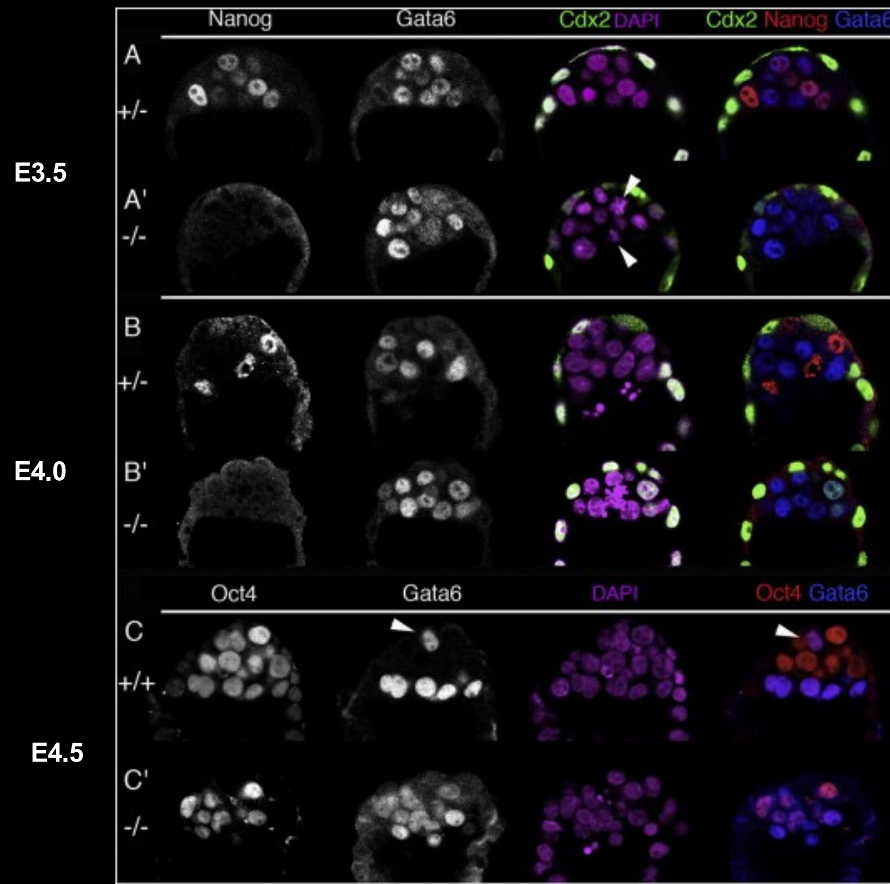


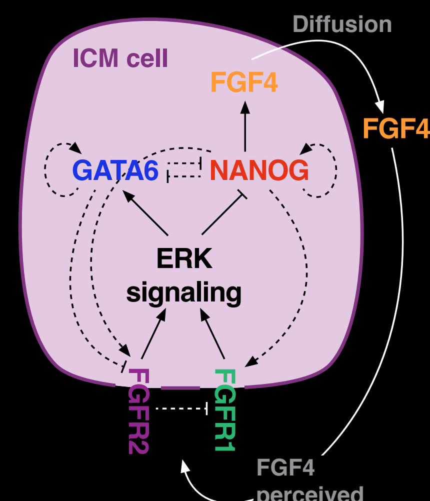




Schrode et al., Developmental Cell 2014
Bessonard et al., Development 2015
Saiz et al., eLife 2020

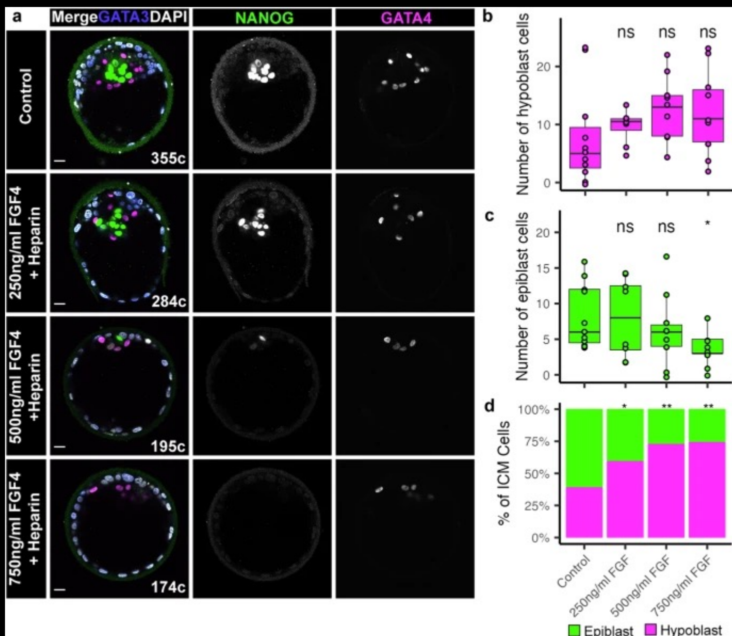
Nanog$^{-/-}$ embryos fail to specify epiblast (EPI), *all ICM cells adopt a pluripotent Primitive Endoderm (PrE) identity*



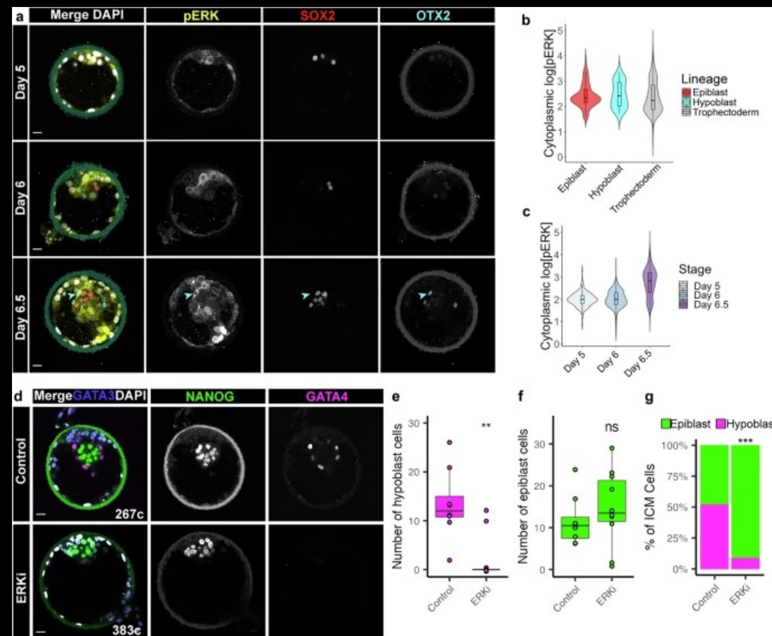


Frankenberg et al., Developmental Cell 2011
Allegre et al., Nature Comms. 2023

FGF/ERK signaling drives PrE vs. EPI specification in human embryos



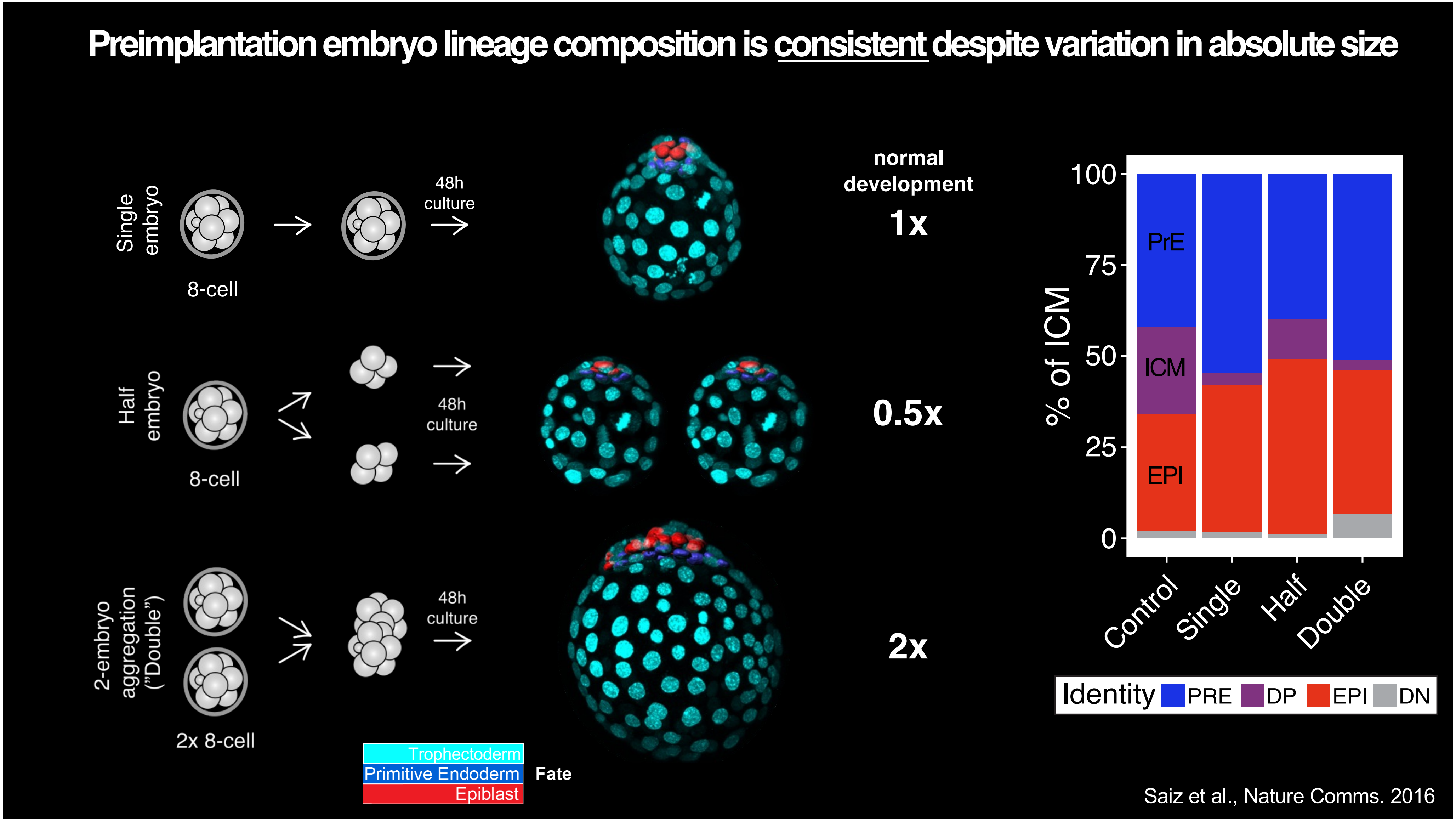
Exogenous FGF sufficient to drive human hypoblast specification



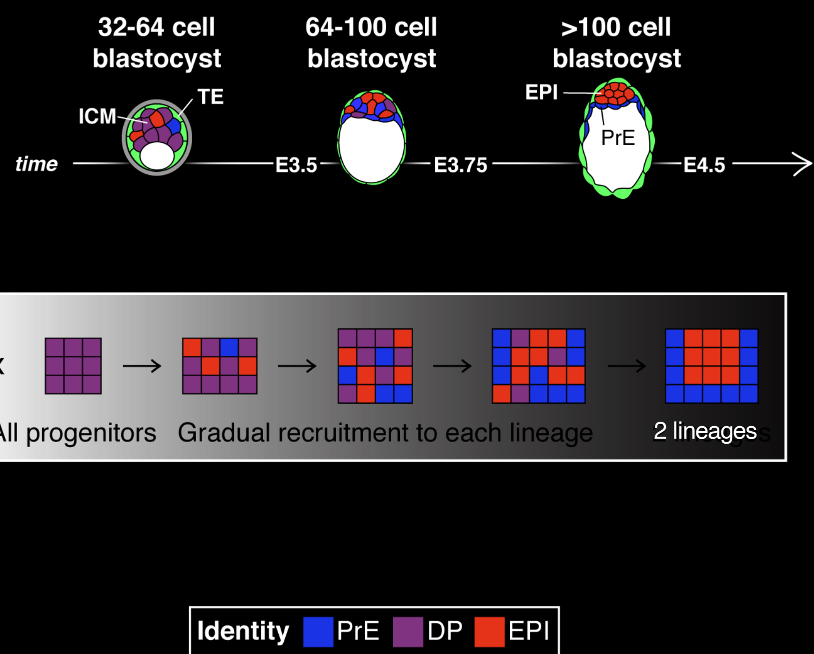
ERK inhibition blocks hypoblast formation

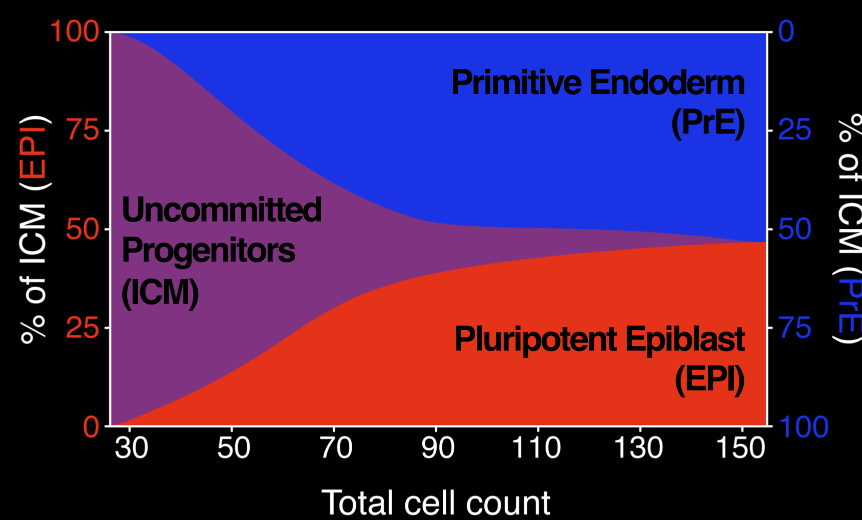
primitive endoderm = hypoblast in higher mammals

Dattani et al., Cell Stem Cell 2024
Simon et al., Nature Comms. 2025

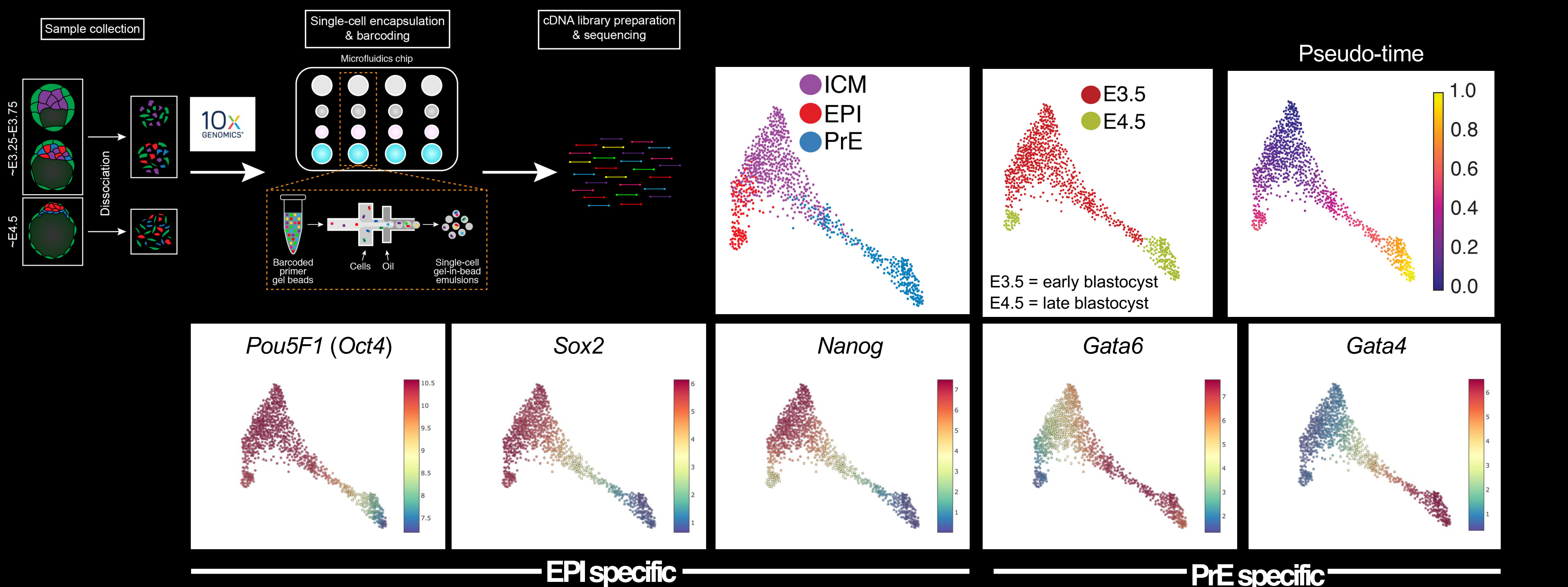

Preimplantation embryo lineage composition is consistent despite variation in absolute size
Single embryo
8-cell
48h culture
normal development
1x
Half embryo
8-cell
48h culture
0.5x
2-embryo aggregation ("Double")
2x 8-cell
48h culture
2x
Trophectoderm
Primitive Endoderm
Epiblast
Fate
% of ICM
100
75
50
25
0
PrE
ICM
EPI
Control
Single
Half
Double
Identity
PRE
DP
EPI
DN
Saiz et al., Nature Comms. 2016

Asynchronous fate decisions by single cells collectively ensure a consistent lineage composition



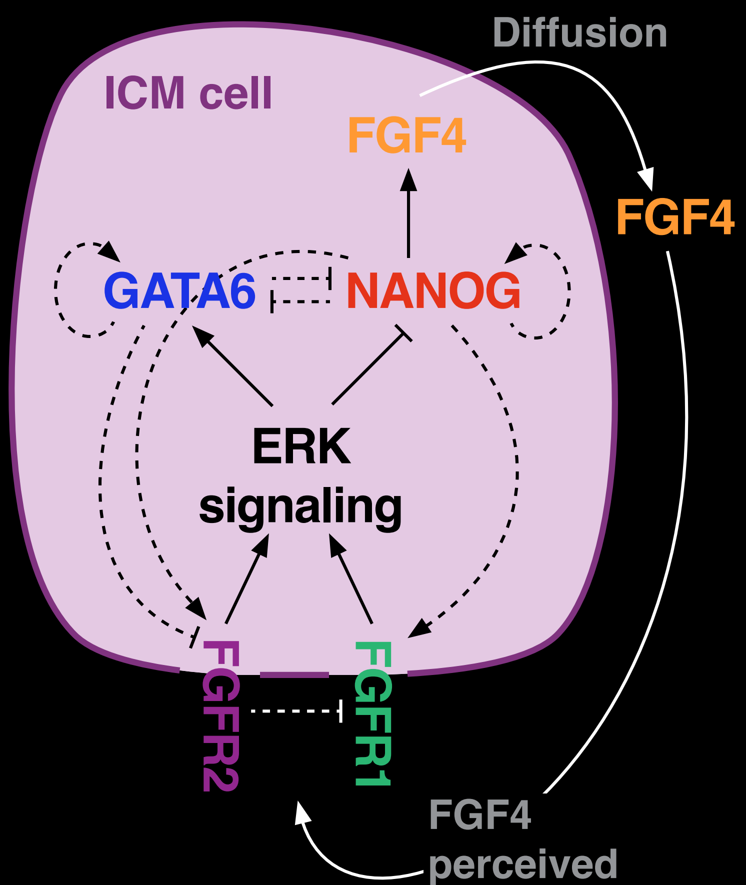


Asynchronous fate decisions by ICM cells



Nowotschin et al., Nature 2019

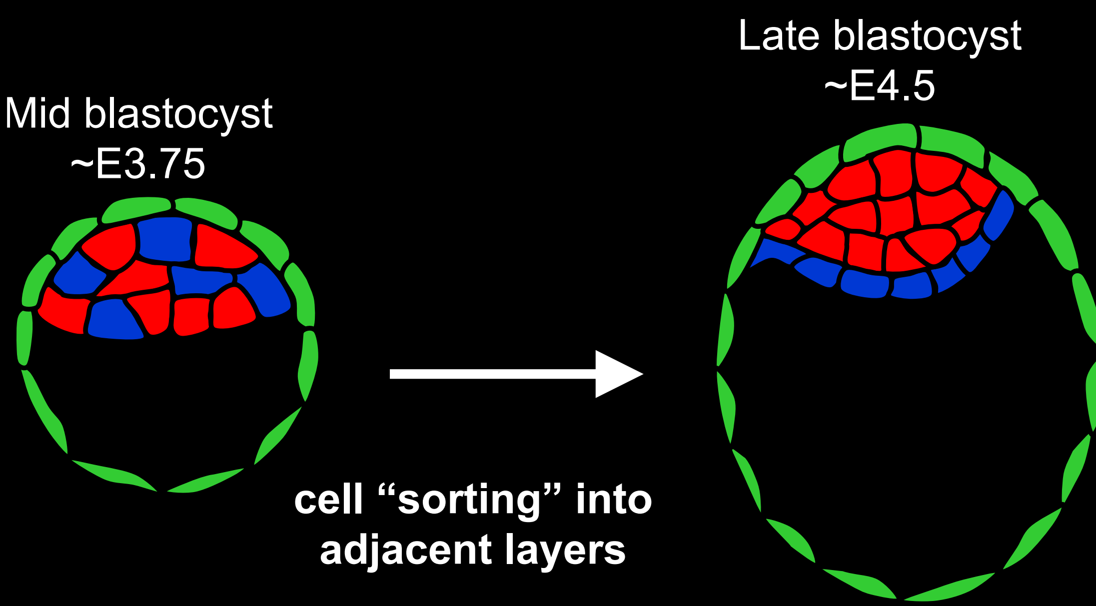
The cell counting mechanism involves FGF?



- Cell fate choice in the ICM of the mouse blastocyst is determined by tissue size (scaling)
- Bipotent ICM progenitors respond to perturbations and ensure an appropriate lineage composition (regulative development)
- Feedback control is achieved via availability of growth factor (FGF4), which acts as a dynamic read-out of tissue size

Saiz et al., eLife. 2020
Moghe et al., Nature Cell Biol. 2025

Current model for ICM differentiation



Trophectoderm

Inner Cell Mass

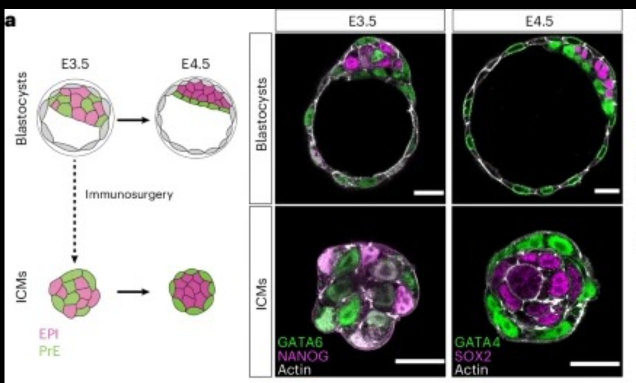
Primitive Endoderm

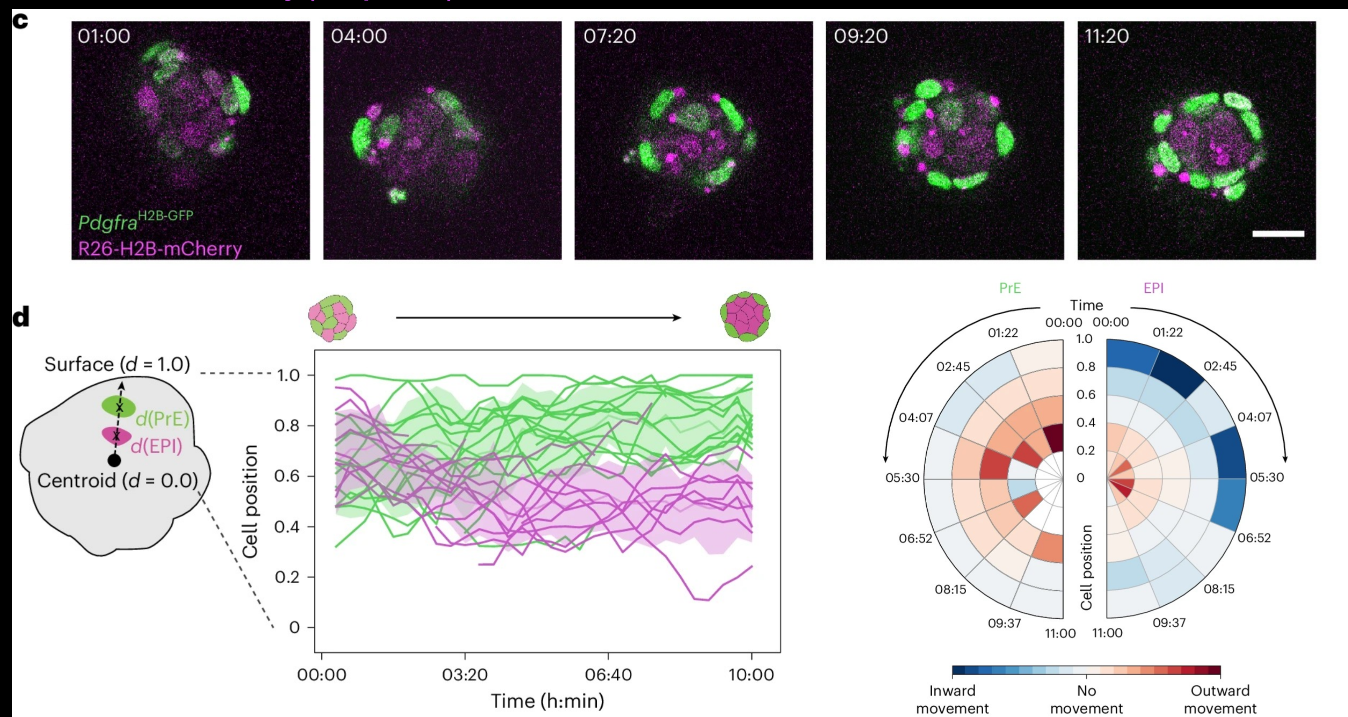
Epiblast

Chazaud et al., Developmental Cell 2006
Plusa et al., Development 2008
Meilhac et al., Dev. Biology 2009
Ohnishi et al., Nature Cell Biol. 2014
Moghe et al., Nature Cell Biol. 2025
and many others

Differential cell movements between epiblast (EPI) and primitive endoderm (PrE) contribute to fate segregation in the ICM

Pdgfra$^{H2B\text{-}GFP}$ (PrE lineage-specific)
R26-H2B-mCherry (ubiquitous)



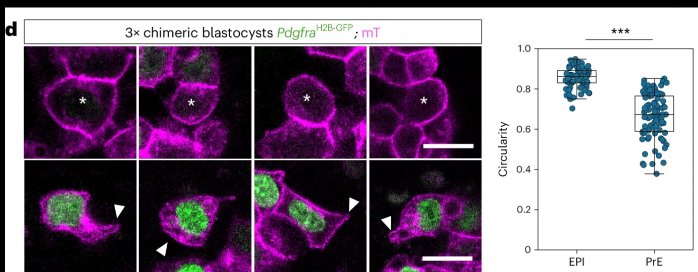


single-cell tracking of EPI and PrE cells from isolated ICMs

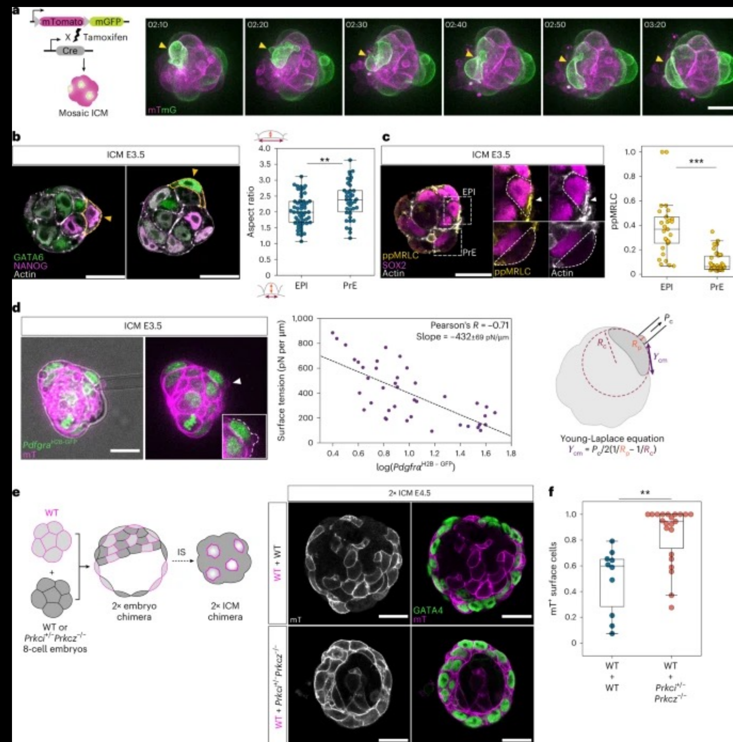
Polar plots indicating preferential direction of cell movements among PrE and EPI

Moghe et al., Nature Cell Biol. 2025

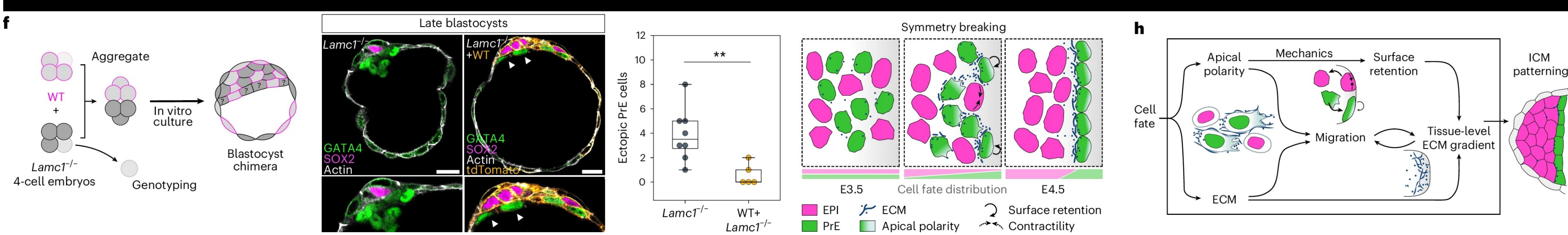
Cell sorting involves active directed migration of PrE cells towards the surface via actin-mediated protrusions



Acquisition of apical domain decreases surface tension and is sufficient for retaining PrE cells at fluid interface

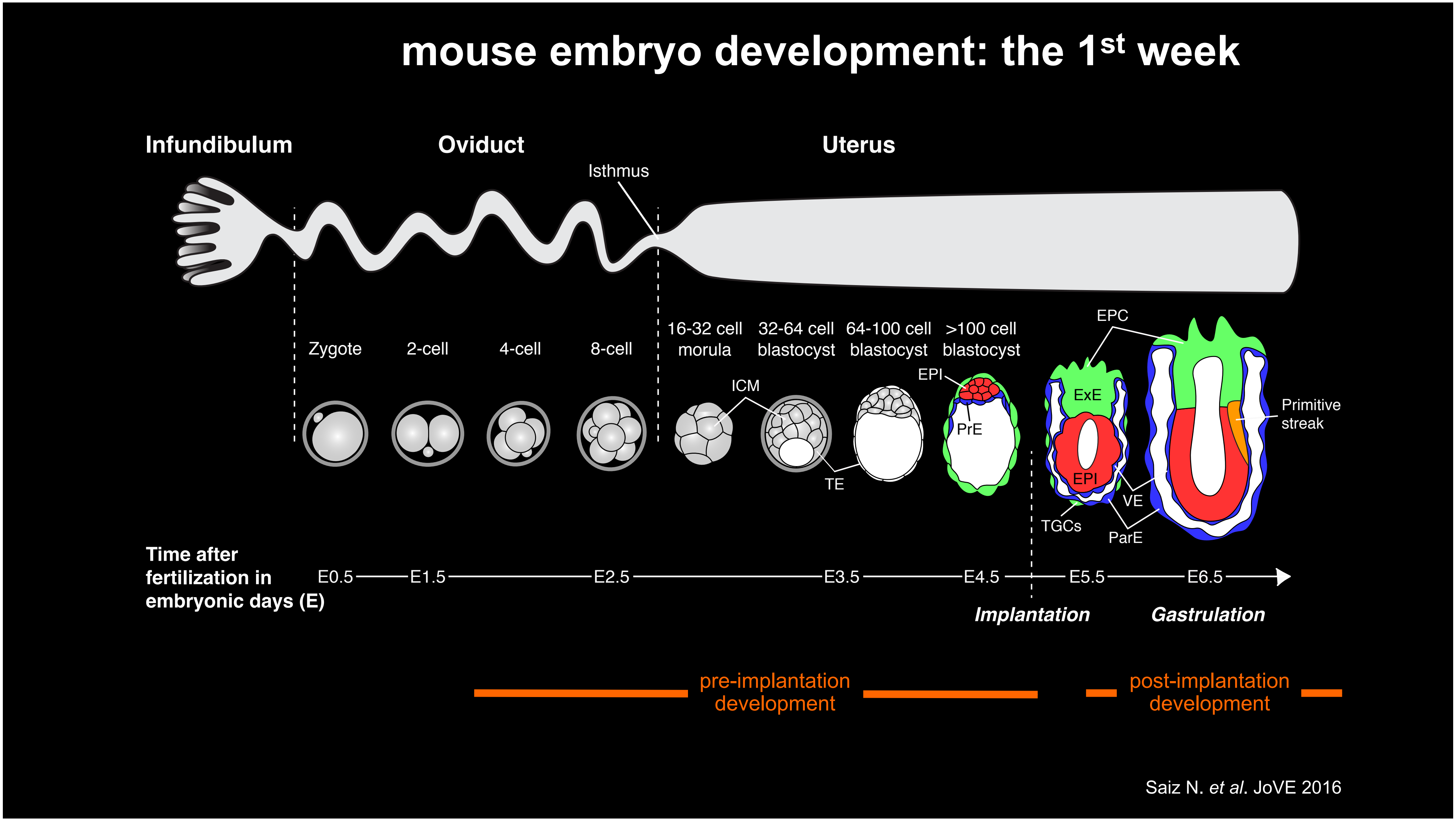


ECM deposited by PrE cells builds a gradient and guides PrE cell migration



Moghe et al., Nature Cell Biol. 2025

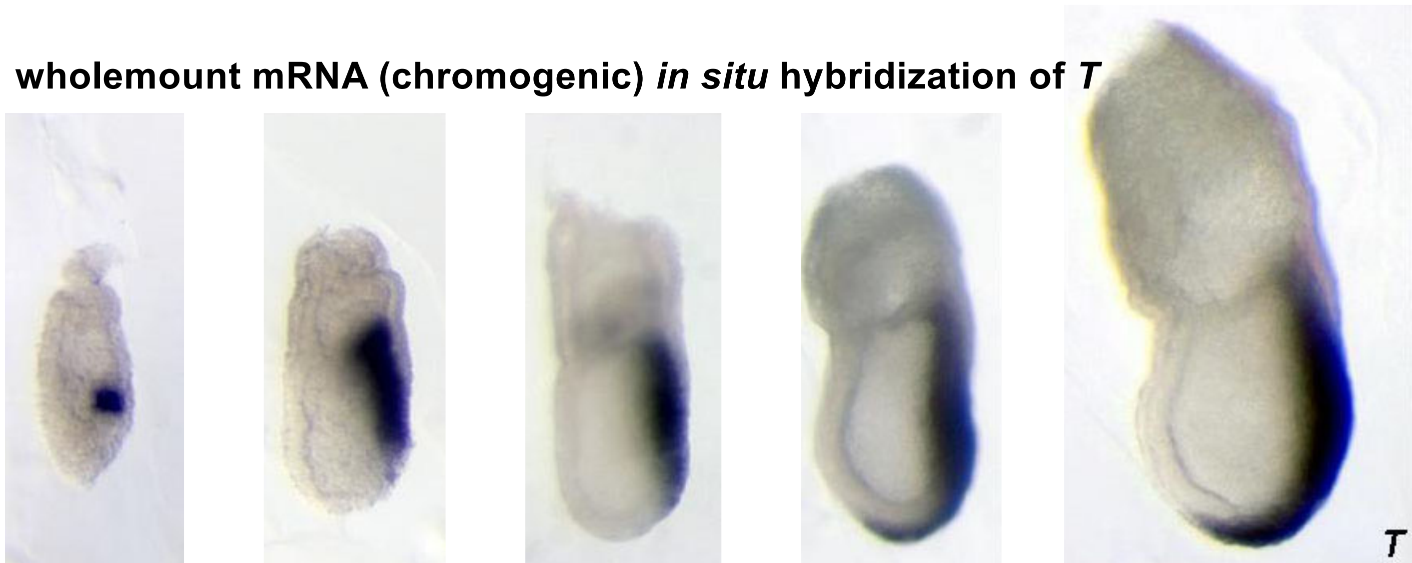
time for a break?


mouse embryo development: the 1st week
Infundibulum
Oviduct
Uterus
Isthmus
Zygote
2-cell
4-cell
8-cell
16-32 cell morula
32-64 cell blastocyst
64-100 cell blastocyst
>100 cell blastocyst
ICM
TE
EPI
PrE
EPC
ExE
EPI
VE
TGCs
ParE
Primitive streak
Time after fertilization in embryonic days (E)
E0.5
E1.5
E2.5
E3.5
E4.5
E5.5
E6.5
Implantation
Gastrulation
pre-implantation development
post-implantation development
Saiz N. et al. JoVE 2016

The Body Axes of the Mouse Embryo

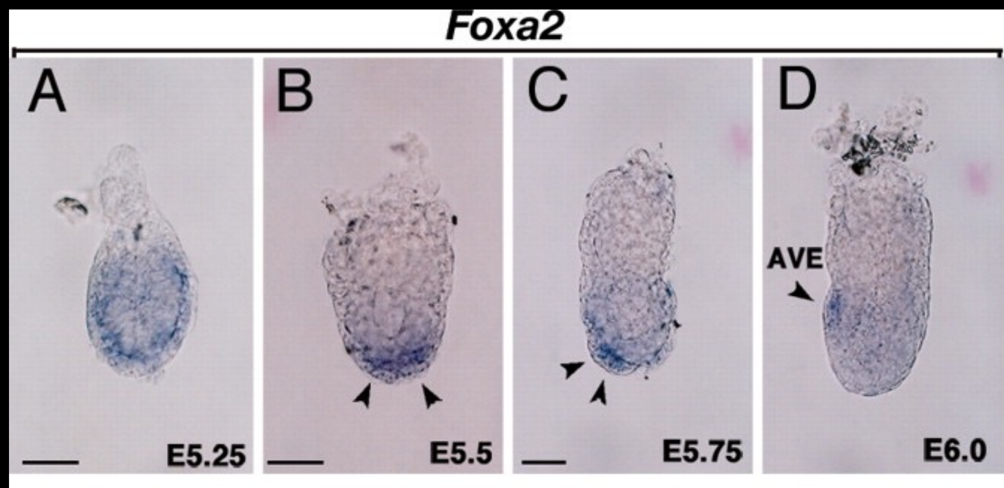
Brachyury (*T*): a T-box transcription factor is a marker of the primitive streak

wholemount mRNA (chromogenic) *in situ* hybridization of *T*



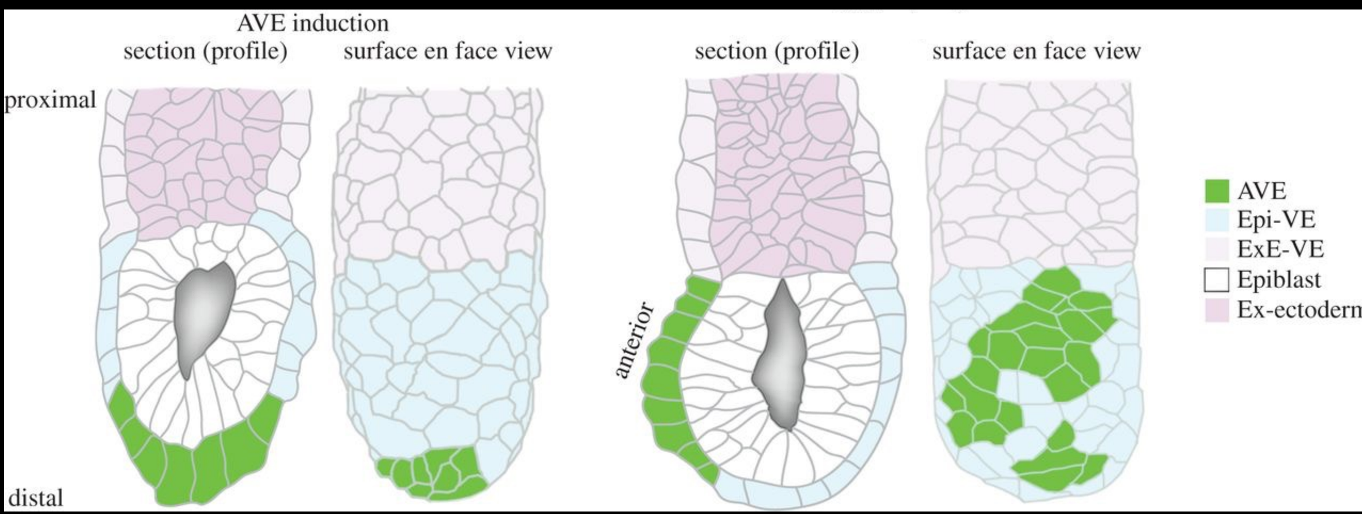
Embryonic day (E) 6.25 → E7.5

Anterior pattern in the visceral endoderm prior to gastrulation



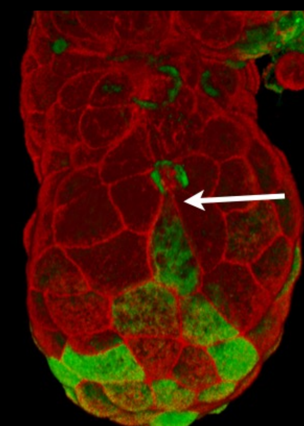
Chikara-Yoshida PNAS 2007

How is the proximal-distal (P-D) axis converted to an anterior-posterior (A-P) axis?



Stower M. and Srinivas S. Phil Trans Royal Soc. 2014

wild-type, E5.75

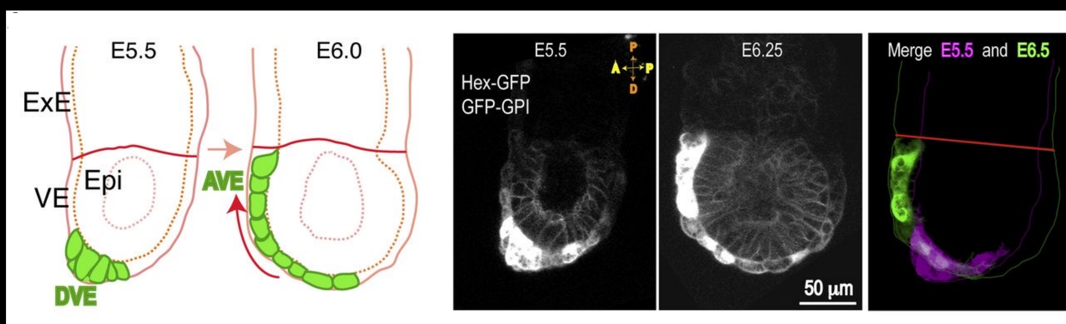


F-actin (phalloidin)

Hex-GFP

Migeotte et al., PLOS Biol 2010

Anterior Visceral Endoderm (AVE) cells exhibit collective migration in the early mouse embryo

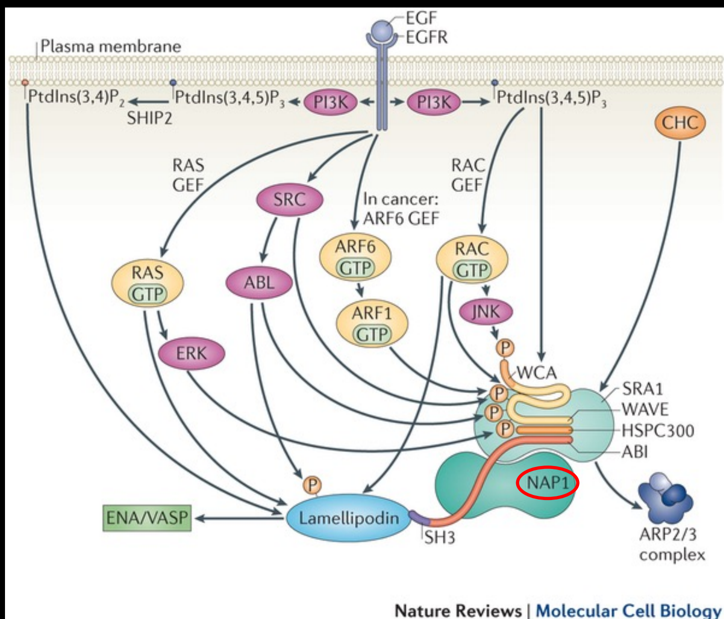


Hex-*GFP* marks the collective migration of AVE cells

single optical section /time
E5.5-E6.5

Omelchenko, T. *et al.*, Genes & Dev. 2014

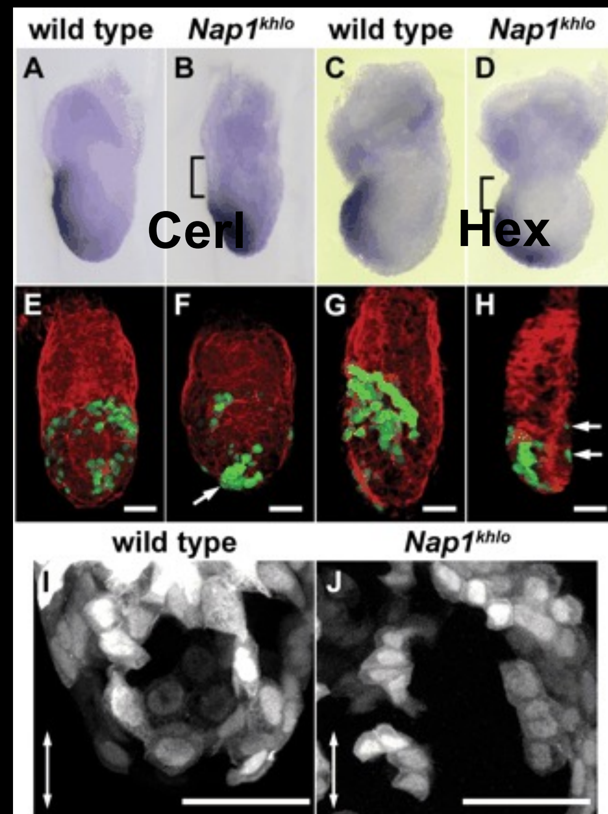
Axis specification (movement of prospective AVE) in the mouse embryo requires Nap1, a regulator of WAVE-mediated actin branching



Krause M. and Gautreau A. Nature Rev. Cell Biol. 2014

Membrane protrusions (lamellipodia) at the leading edge of cells, drive cell migration in many normal and pathological situations

Hex-GFP transgenic mouse embryos

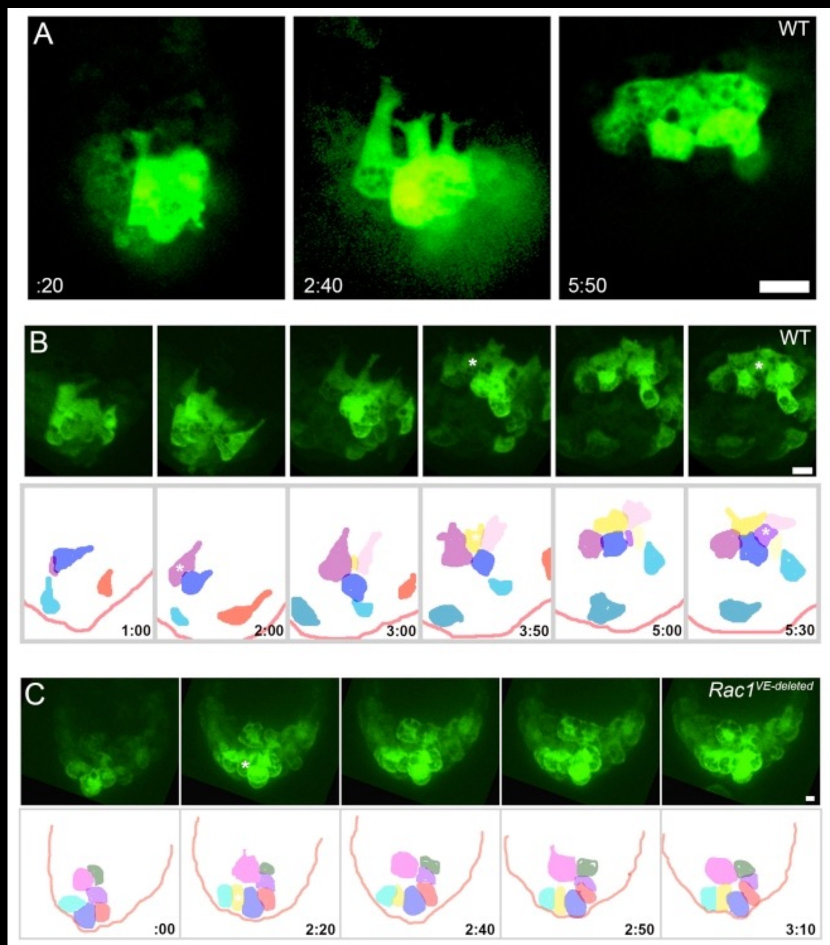


Rakeman, A., and Anderson, K.V. Development 2006

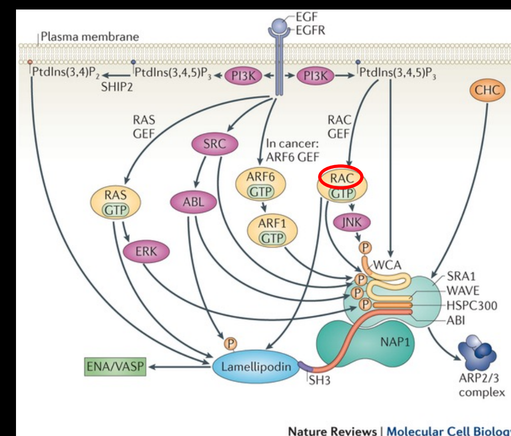
AVE cells extend lamellipodia that depend on Rac1 (a Rho GTPase)

WT

Rac1



migrating AVE cells, *Hex-GFP*



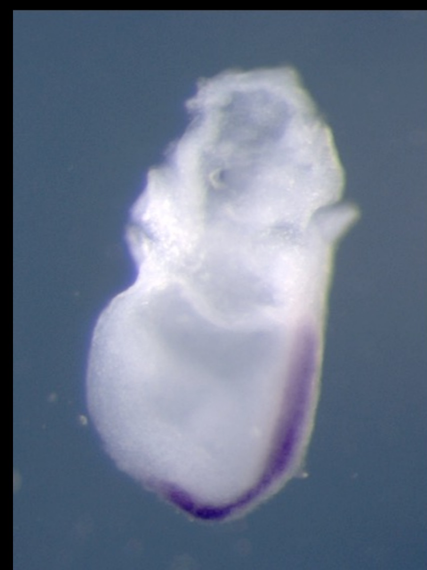
Krause M. & Gautreau A. Nature Rev. Cell Biol. 2014

Migeotte I. *et al.*, PLoS Biology 2010

Genes required for AVE migration affect the AP axis (axis duplications)

M1un: an allele of *Pten*

Brachyury, E7.5

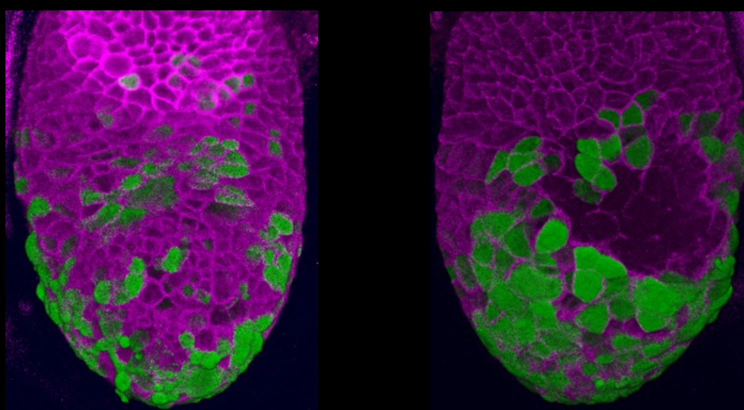


wt

$Pten^{-/-}$

PTEN required for AVE migration affect the Anterior-Posterior axis (axis duplications)

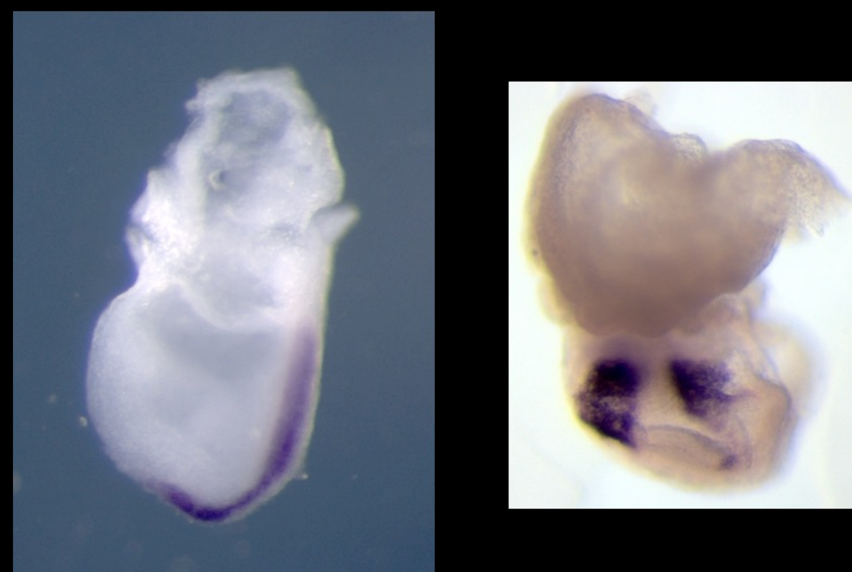
Pten$^{-/-}$



Anterior Posterior

Hex-GFP E-cadherin

Brachyury, E7.5



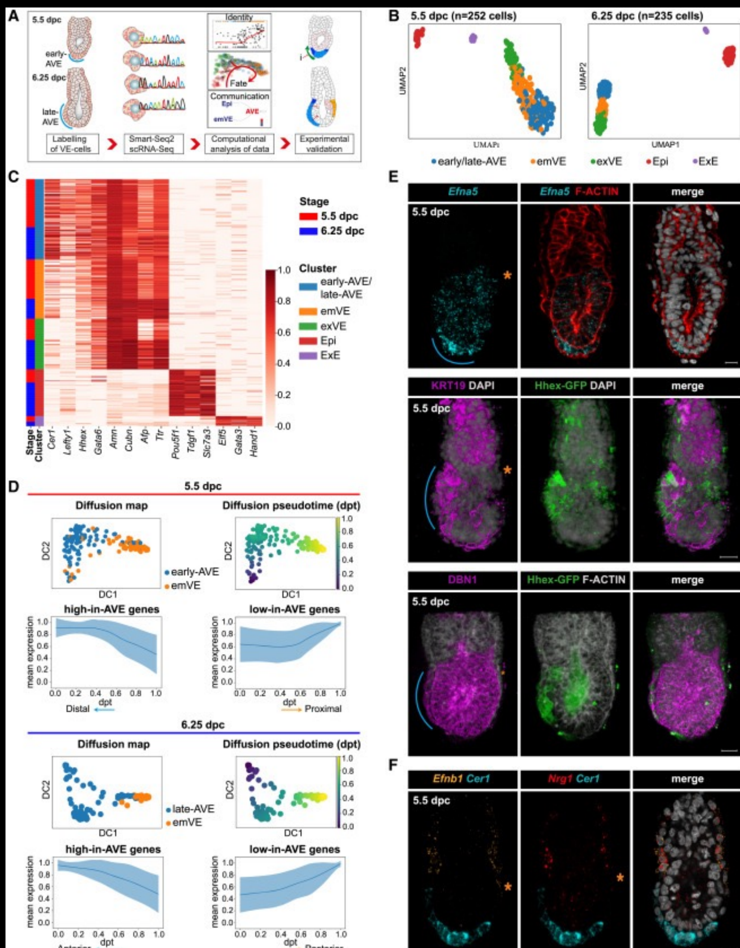
wt *Pten*$^{-/-}$

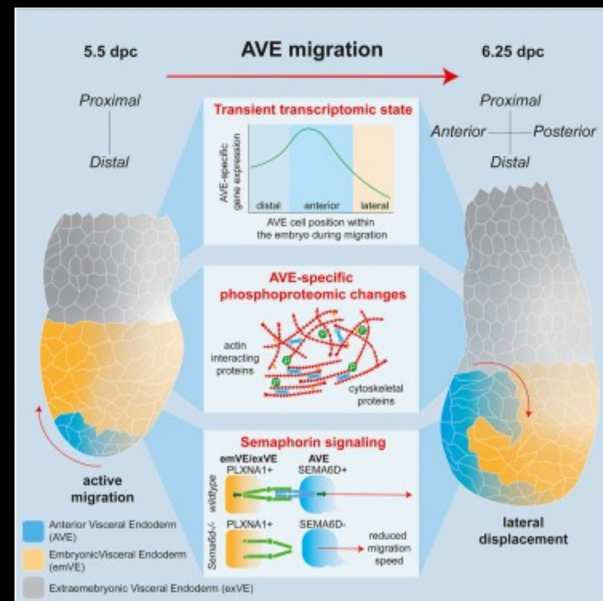
CONCLUSIONS SO FAR

PTEN is required for directional migration
of single cells and during collective cell migration

Loss of directional migration of the AVE
can cause AP axis duplication

Integrated approach identifies molecular underpinnings of Anterior Visceral Endoderm (AVE) migration

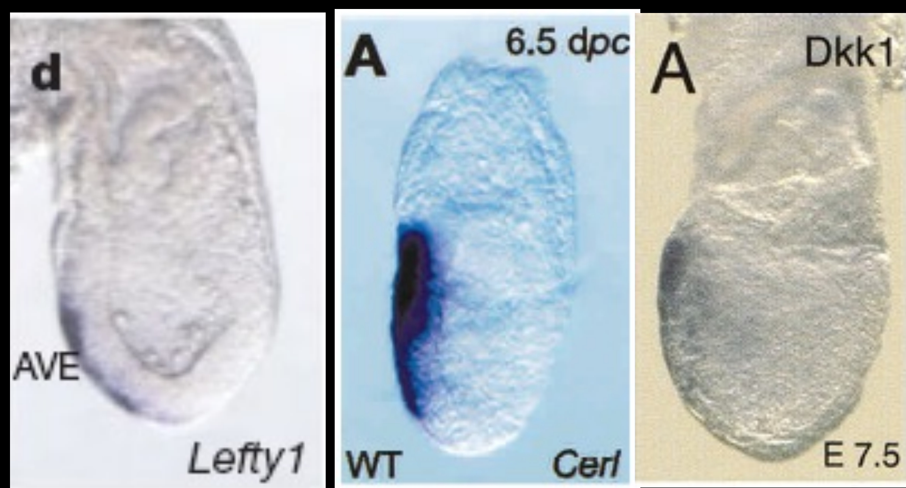




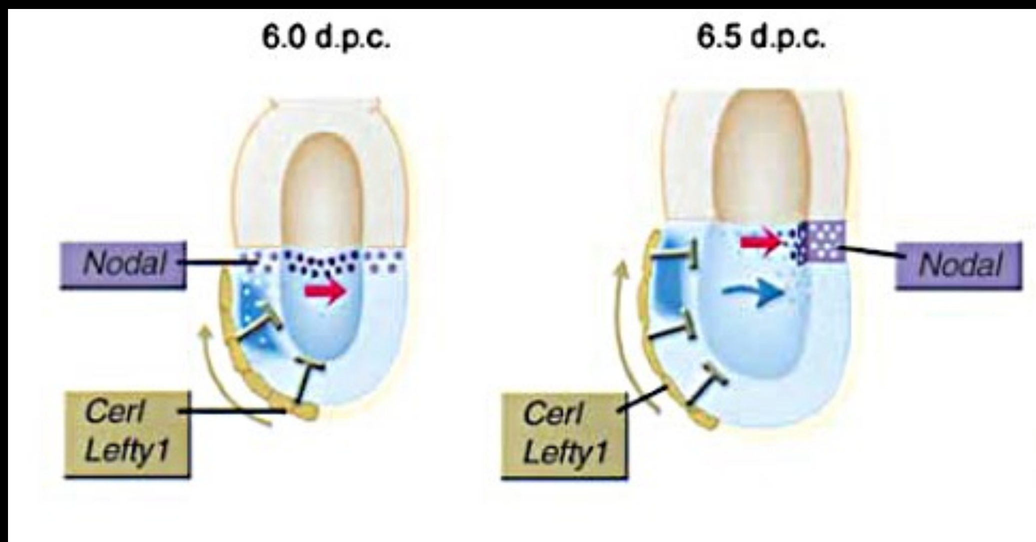
- AVE is composed of transcriptionally and spatially distinct sub-populations
- AVE transcriptional state is downregulated upon its lateral displacement
- Cytoskeletal proteins and modulators are subjected to AVE-specific modifications
- Semaphorin 6D-mediated signaling is necessary for proper AVE migration

Thowfeequ *et al.*, Developmental Cell 2024

Inhibitors of Nodal and Wnt signaling expressed in AVE

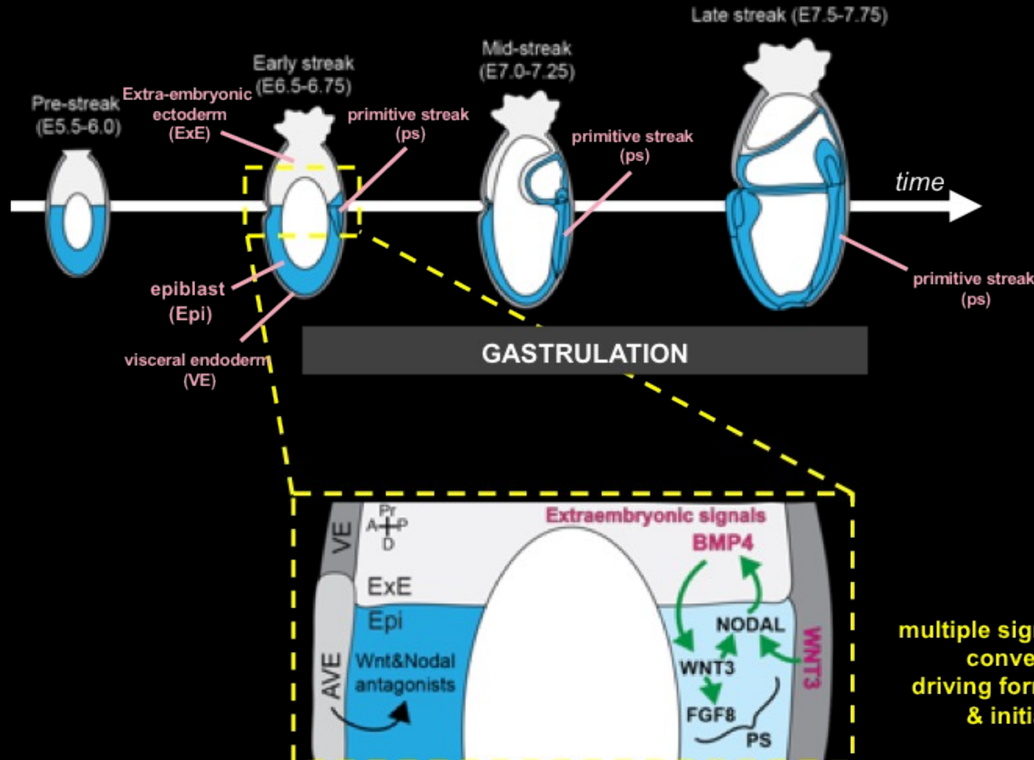


Inhibitors from the AVE localize the primitive streak to the posterior



Nodal (TGFβ), Wnt and BMP (another TGFβ) signals required for gastrulation initiation

GASTRULATION: driven by multiple signals converging on posterior epiblast cells

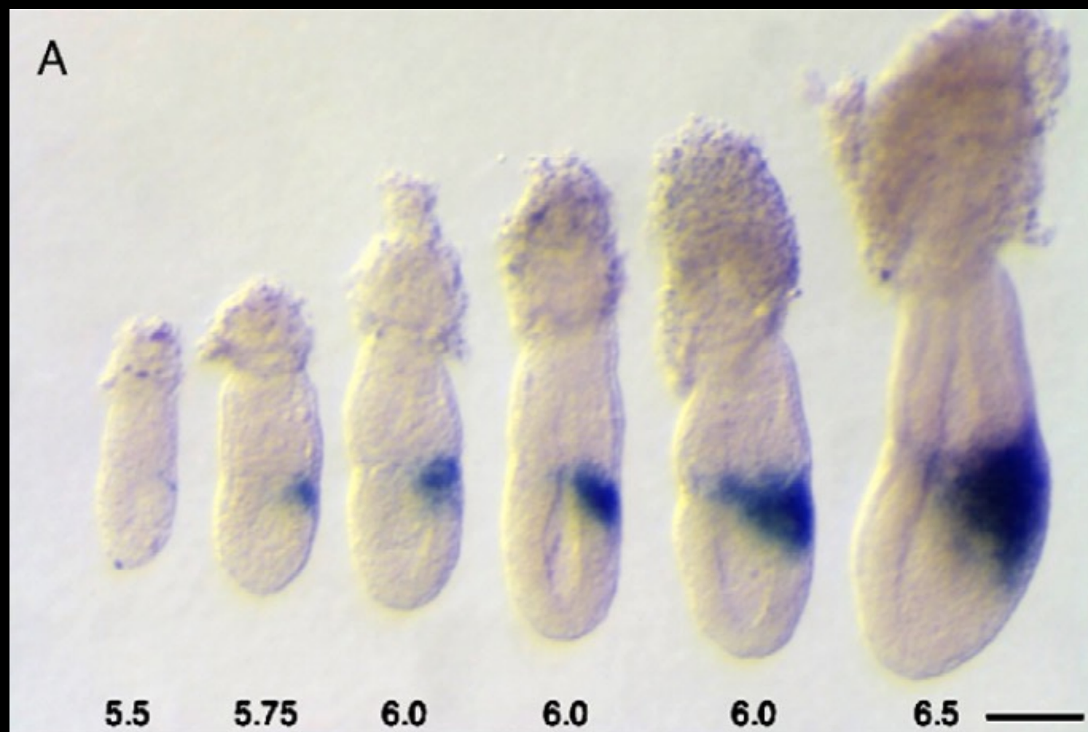


multiple signals (BMP, NODAL, WNT, FGF) converge on posterior epiblast driving formation of PRIMITIVE STREAK & initiation of GASTRULATION

Gastrulation is:

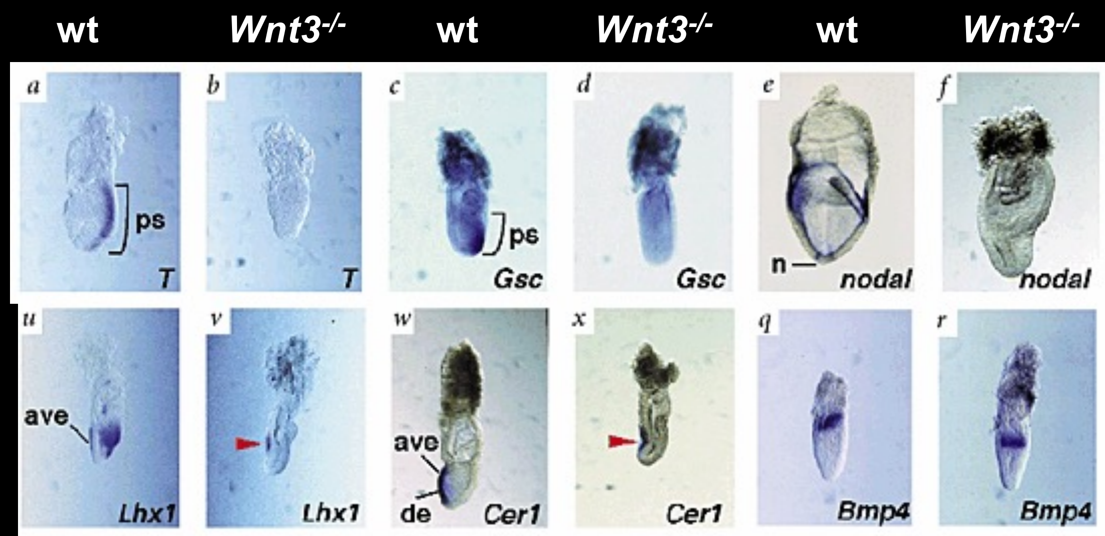
- formation of definitive germ layers (mesoderm and endoderm) and elaboration of the axes (A-P, D-V and L-R).
- coordination of cell fate specification (concomitant loss of pluripotency) and tissue morphogenesis.
- an *in vivo* platform for developing a detailed mechanistic understanding of the mechanisms of EMT (&MET).

Wnt3 expression restricted to the posterior of the embryo



Rivera-Perez and Magnuson, 2005

Wnt3 is required for primitive streak formation

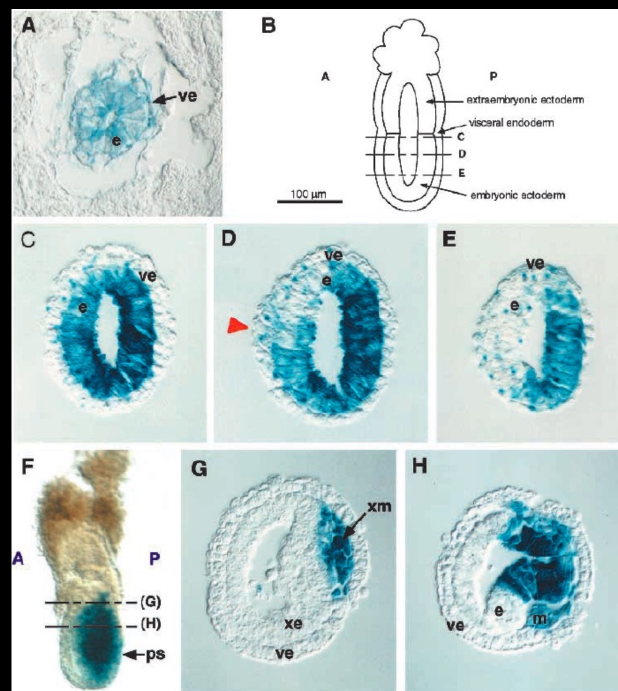


Liu, P. *et al.*, Nature Genetics 1999

Bmp4 expression in the mouse embryo

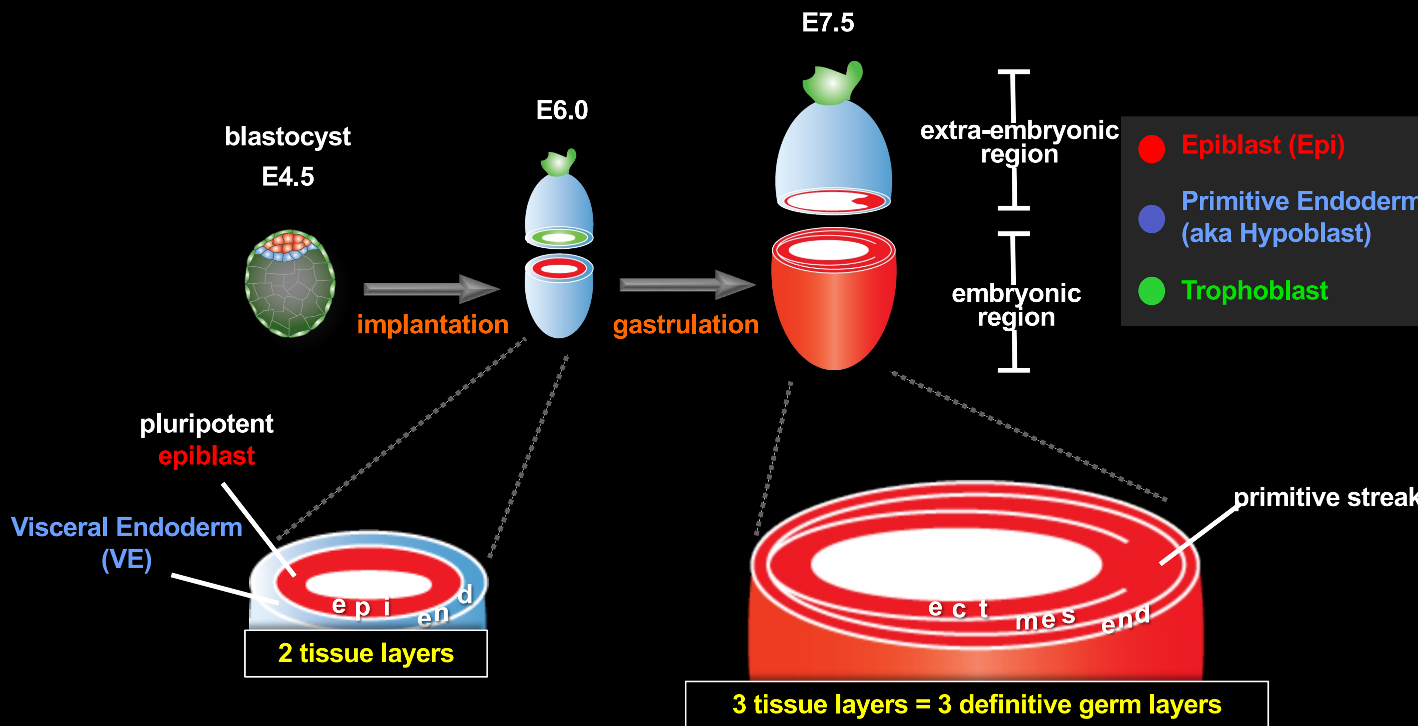
Lawson et al., Genes & Development 1999

Nodal expression in the mouse gastrula as revealed by *lacZ* knock-in

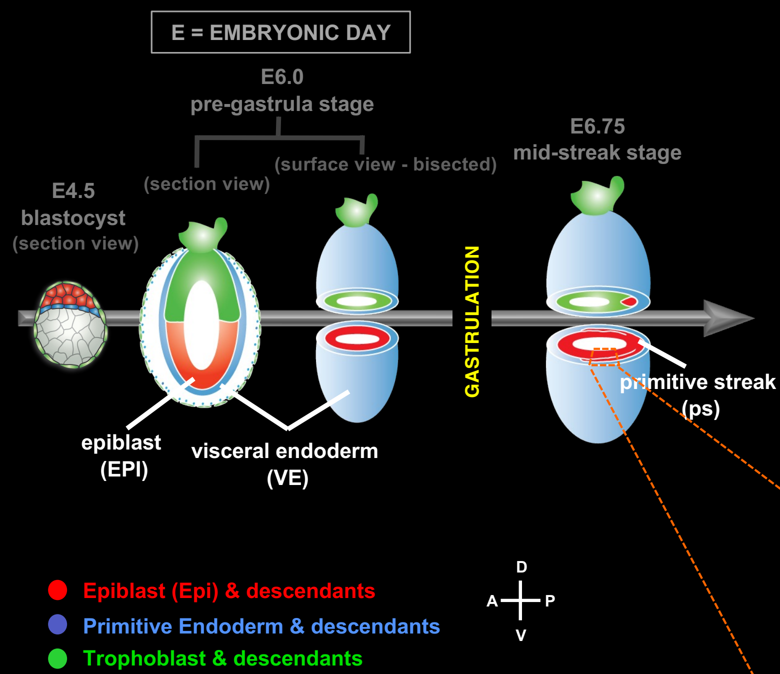


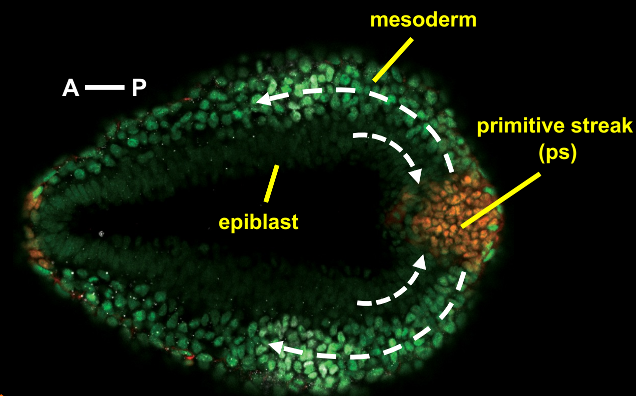
Varlet, I. *et al*., Development 1997

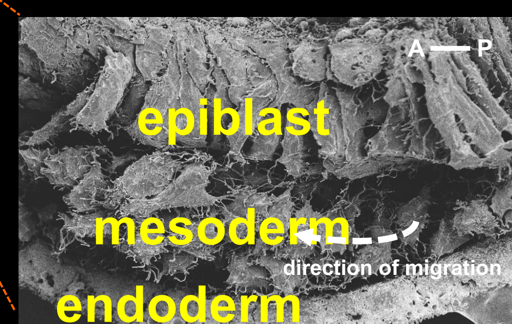
Gastrulation in the mouse embryo: transformation of 2 tissue layers into 3



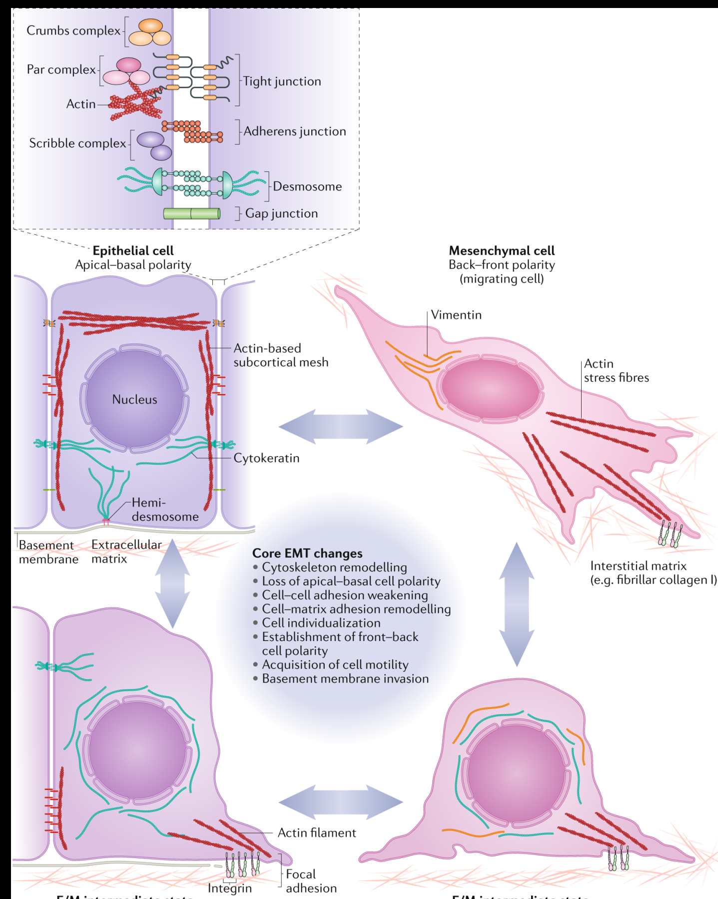
Gastrulation in the Mouse: EMT at the primitive streak





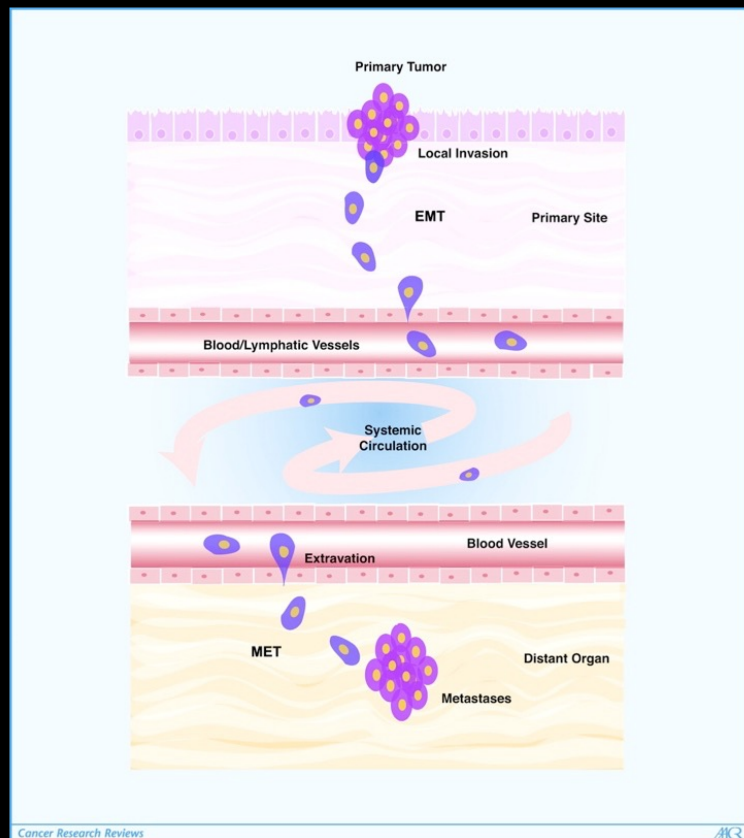


Epithelial-Mesenchymal transitions (EMTs)

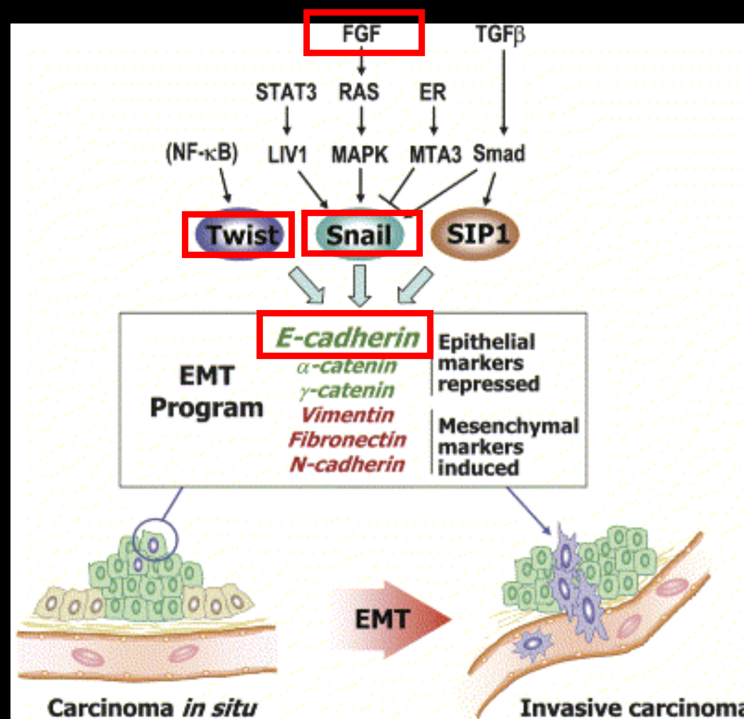


Yang et al. Nat Rev Mol Cell Biol 2020

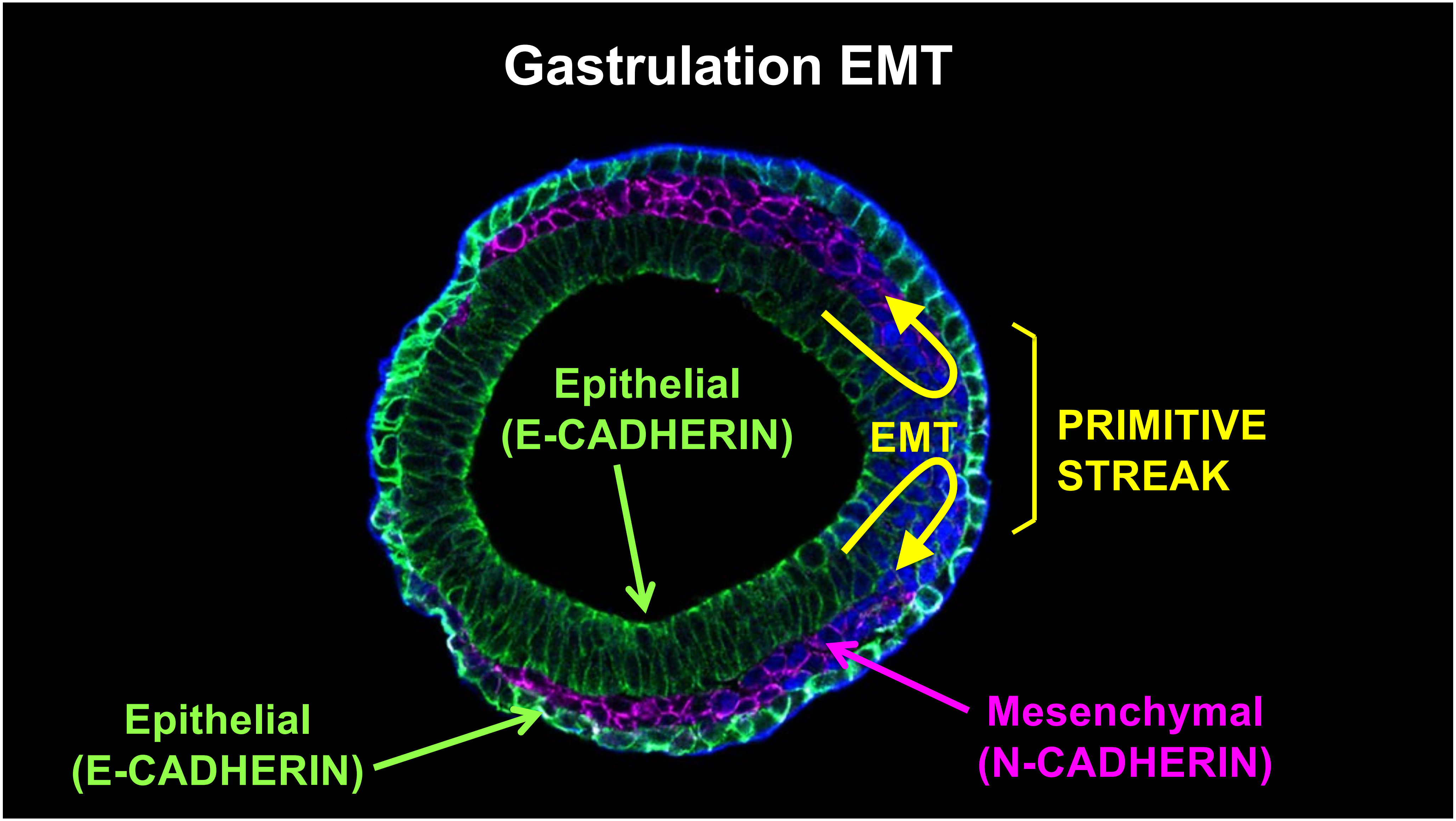
EMT (and MET) in metastasis



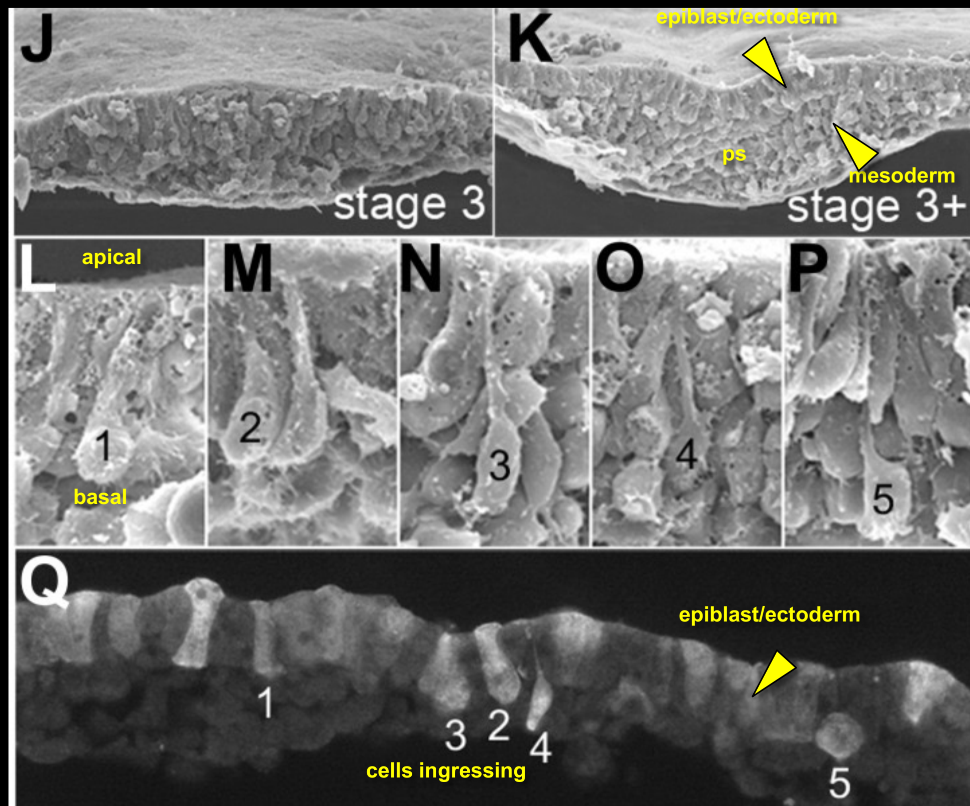
Yang J. et al., Cancer Research 2006



Kang, Y. and Massague, J. Cell 2004

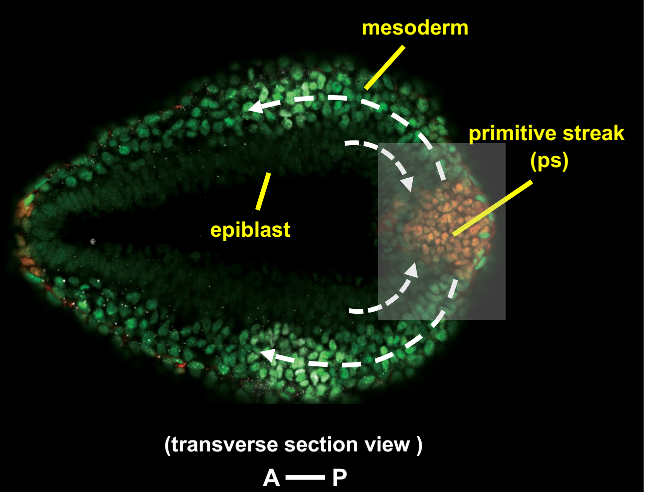

Gastrulation EMT
Epithelial
(E-CADHERIN)
EMT
PRIMITIVE
STREAK
Epithelial
(E-CADHERIN)
Mesenchymal
(N-CADHERIN)

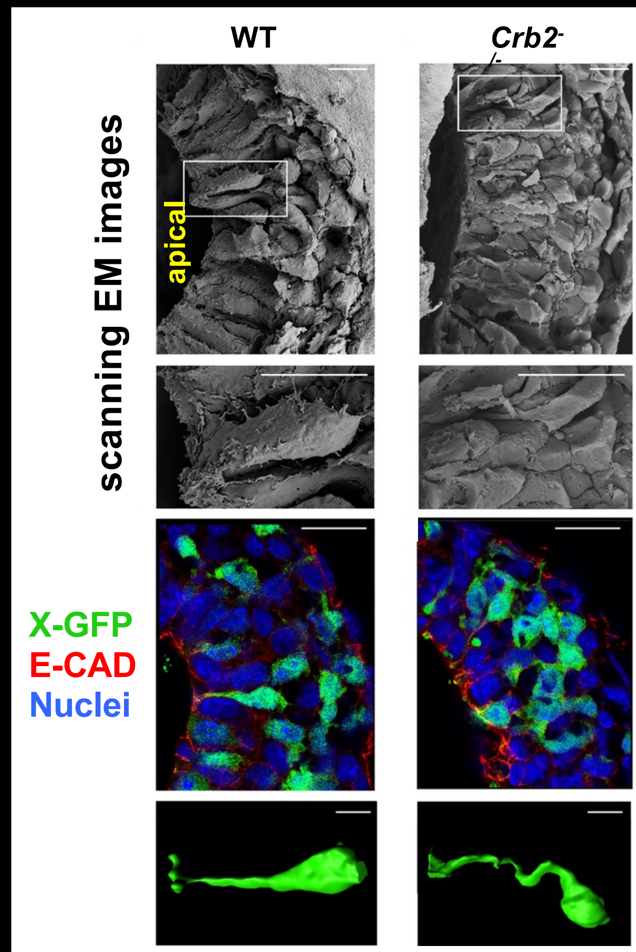
Gastrulation in chick: cells ingress individually at primitive streak (ps)



Voiculesku O. *et al.*, eLife 2014

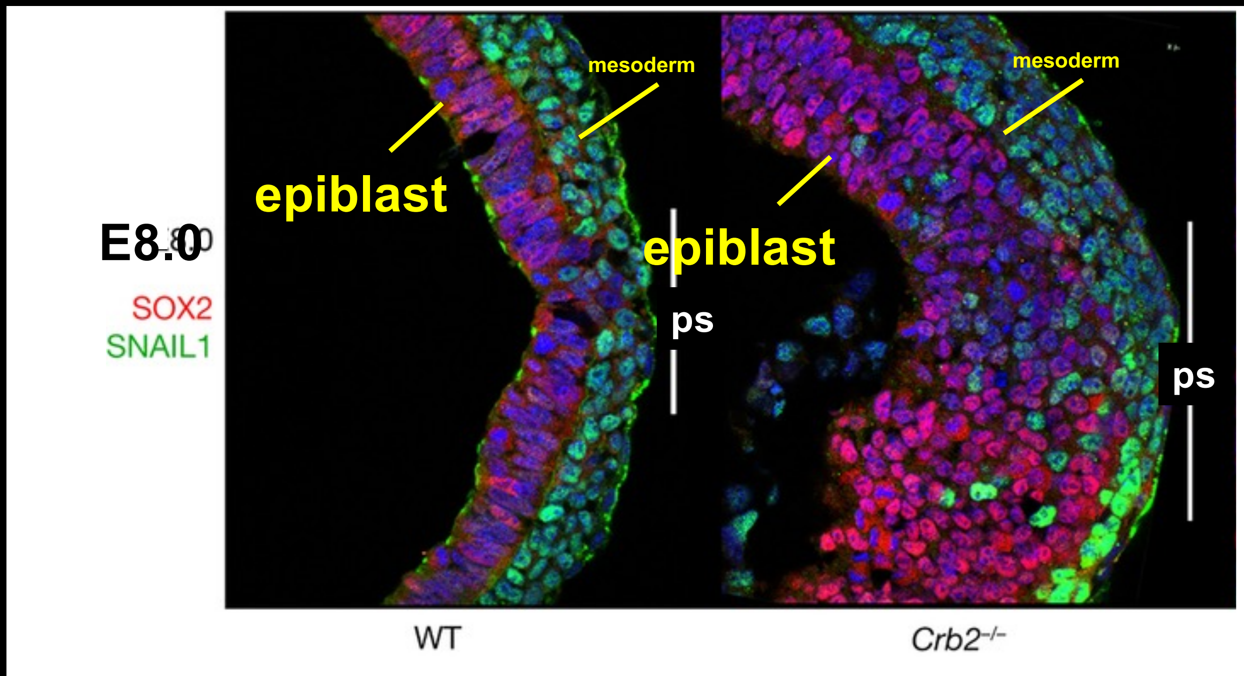
Gastrulation in mouse: cells ingress individually at primitive streak (ps)





Ramkhumar N. *et al.*, Nature Cell Biol. 2016

Gastrulation defective: Cells accumulate at the primitive streak (ps) of *Crumbs2* mutants

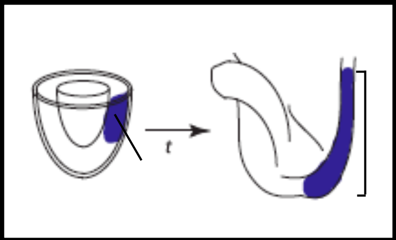


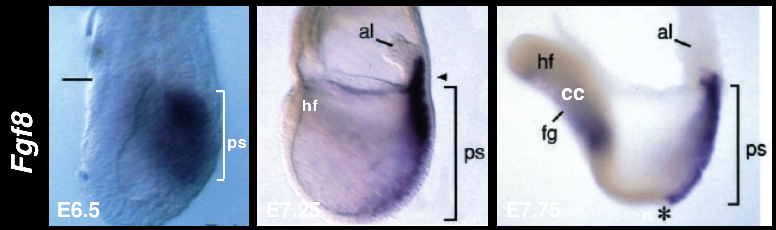
(transverse section view)

A — P

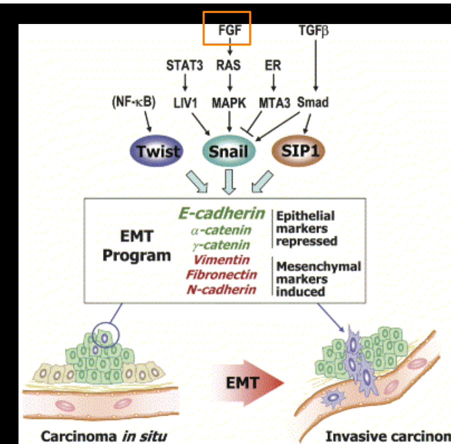
Fgf signaling is required for the gastrulation EMT

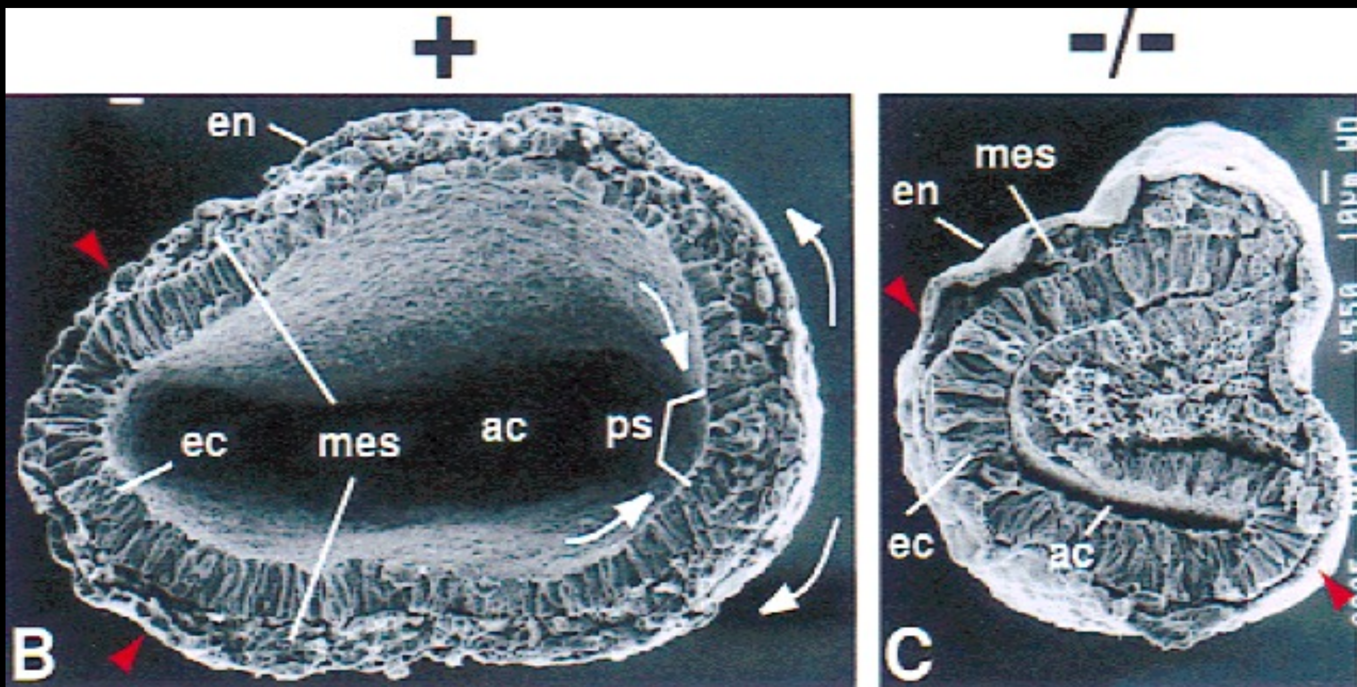
Fgf8 is expressed in the primitive streak





Fgf8 is required for the gastrulation EMT





wild-type
(E7.0 embryo)

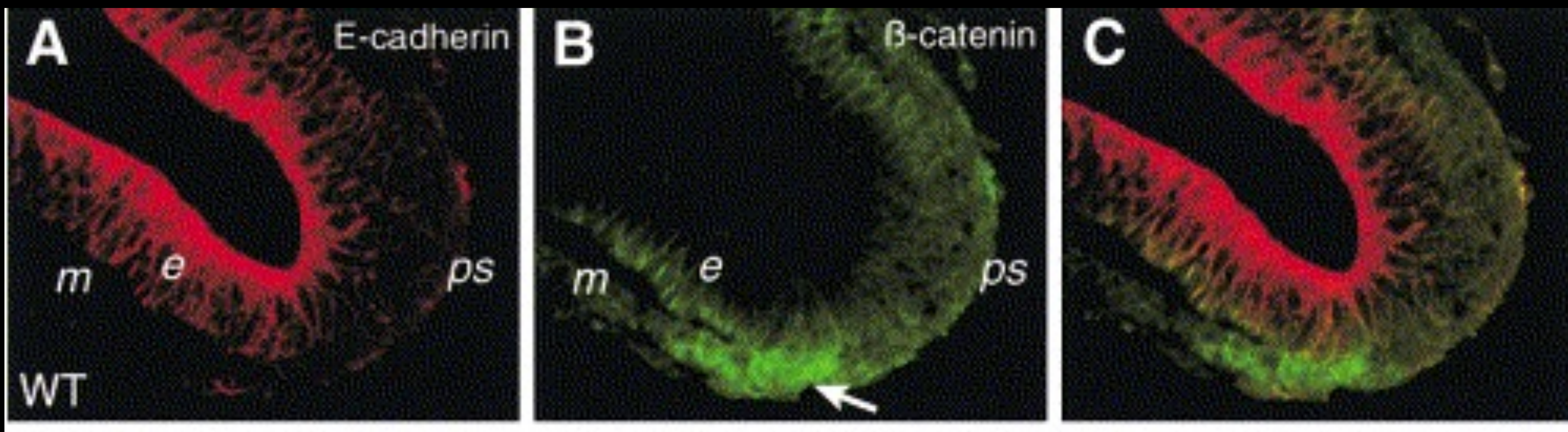
Fgf8 mutant

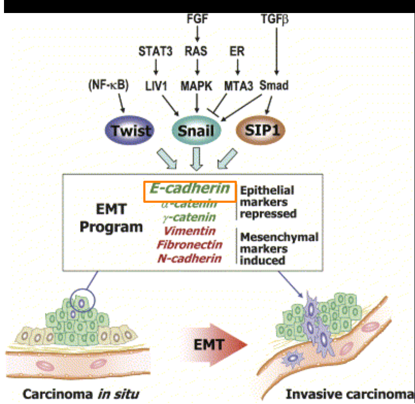
Accumulation of cells
at primitive streak
-
'constipated gastrulation'
phenotype

-
Fgf8 required for
gastrulation EMT

Sun X. *et al*., Genes & Dev 1999

E-cadherin is downregulated as cells traverse the primitive streak

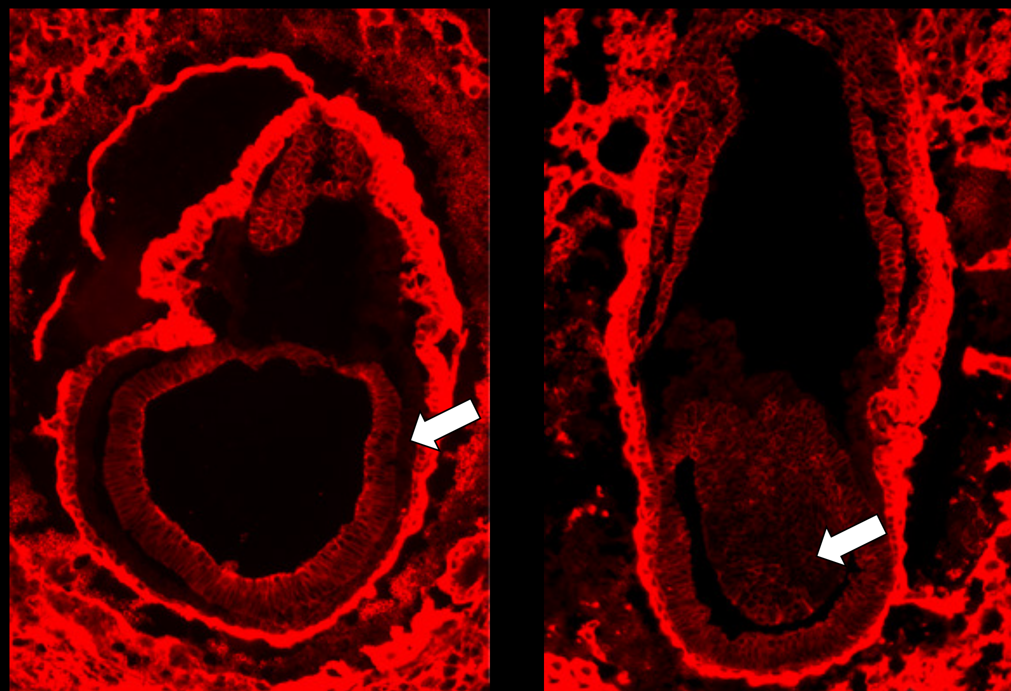




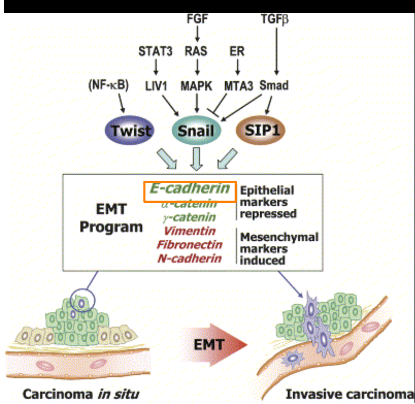
mesoderm is E-cadherin -ve

Ciruna, B. & Rossant, J. Dev. Cell 2001

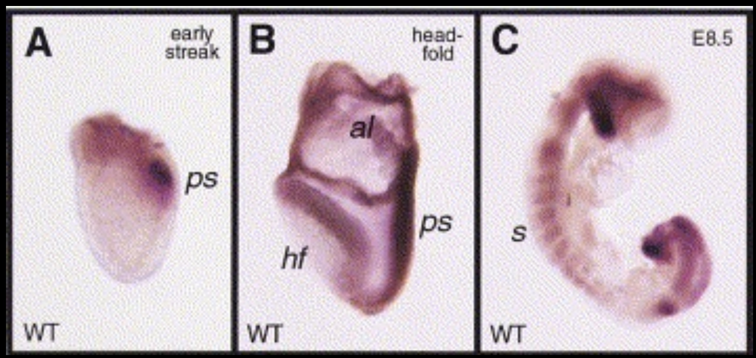
E-cadherin is not down-regulated in Fgf signaling mutants

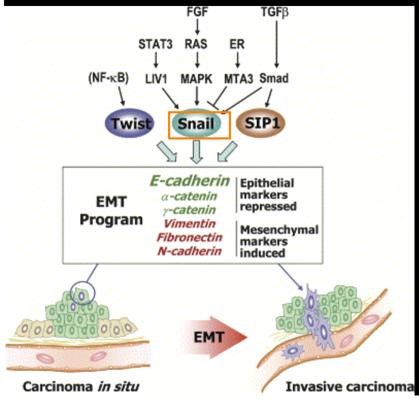


wild-type   *Fgf8*$^{-/-}$



Snail is expressed in the primitive streak

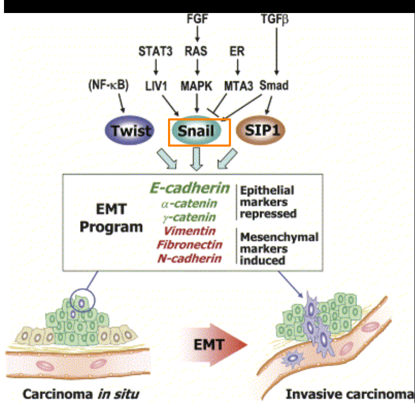


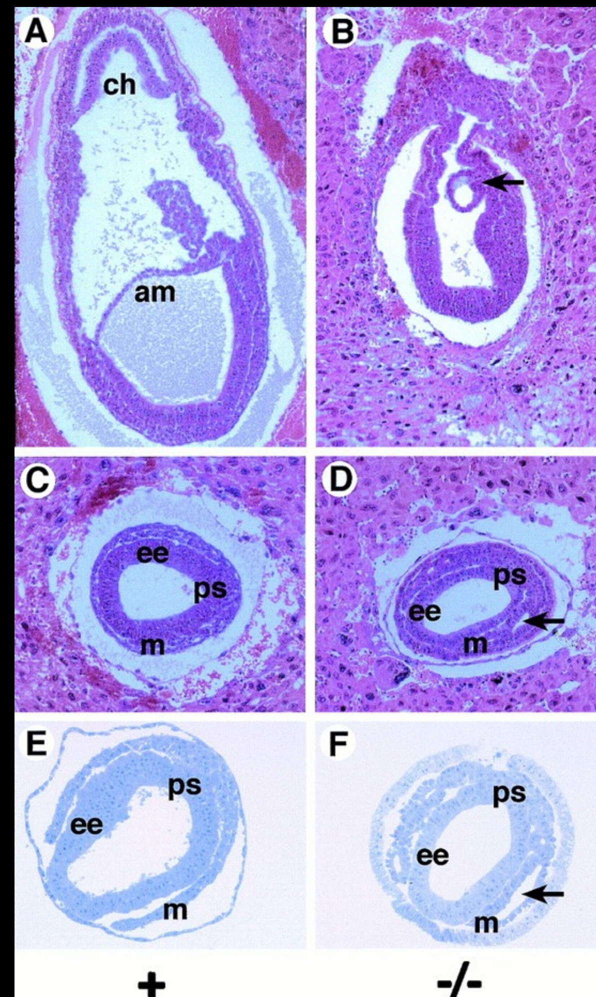


Ciruna, B. and Rossant, J. Dev Cell 2001

The Mouse Snail Gene Encodes a Zn Finger Transcription Factor
-
A Key Regulator of the EMT

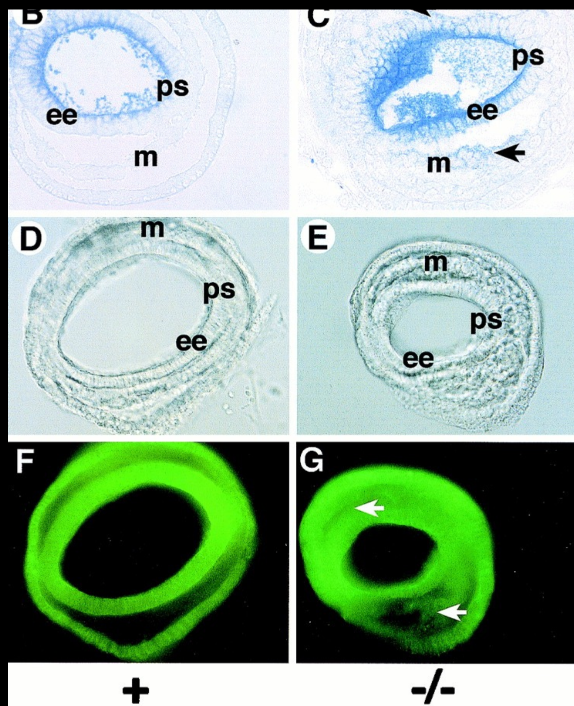
Snail mutants exhibit a failure of EMT at gastrulation

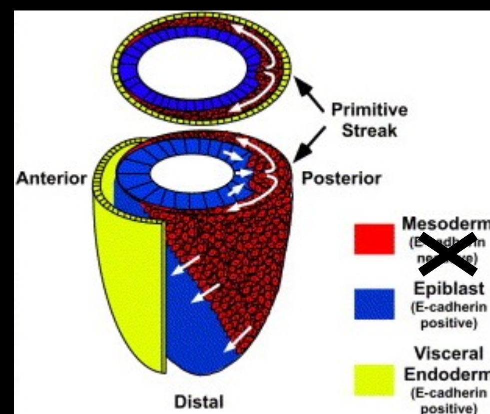




Carver, E.A. *et al.*, MCB 2001

E-cadherin expression is retained in *snail* mutant embryos



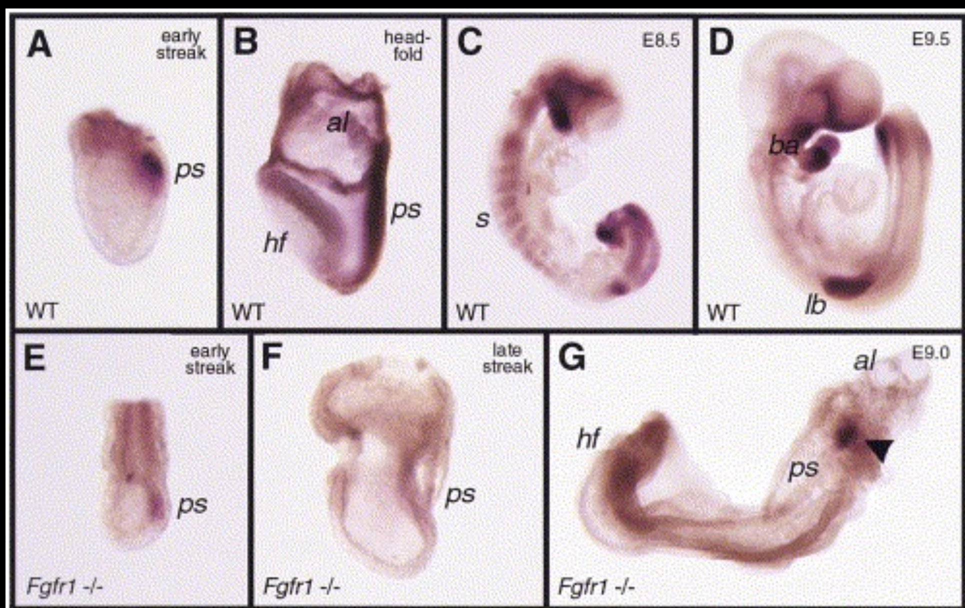


Snail

⊥

E-cadherin

Fgf signaling is required for *snail* expression



Ciruna, B. and Rossant, J. Dev Cell 2001

FGF

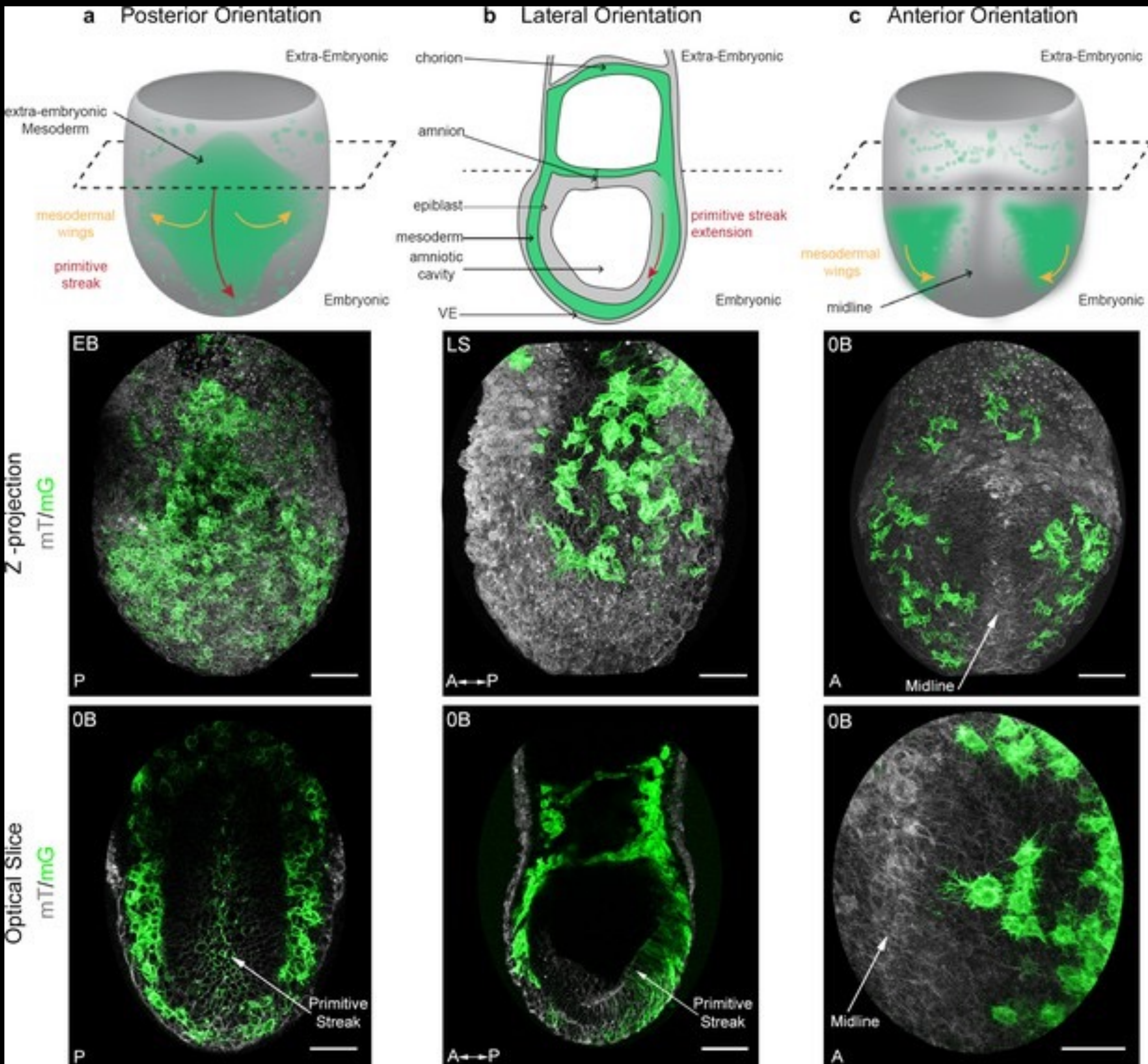
↓

Snail

⊥

E-cadherin

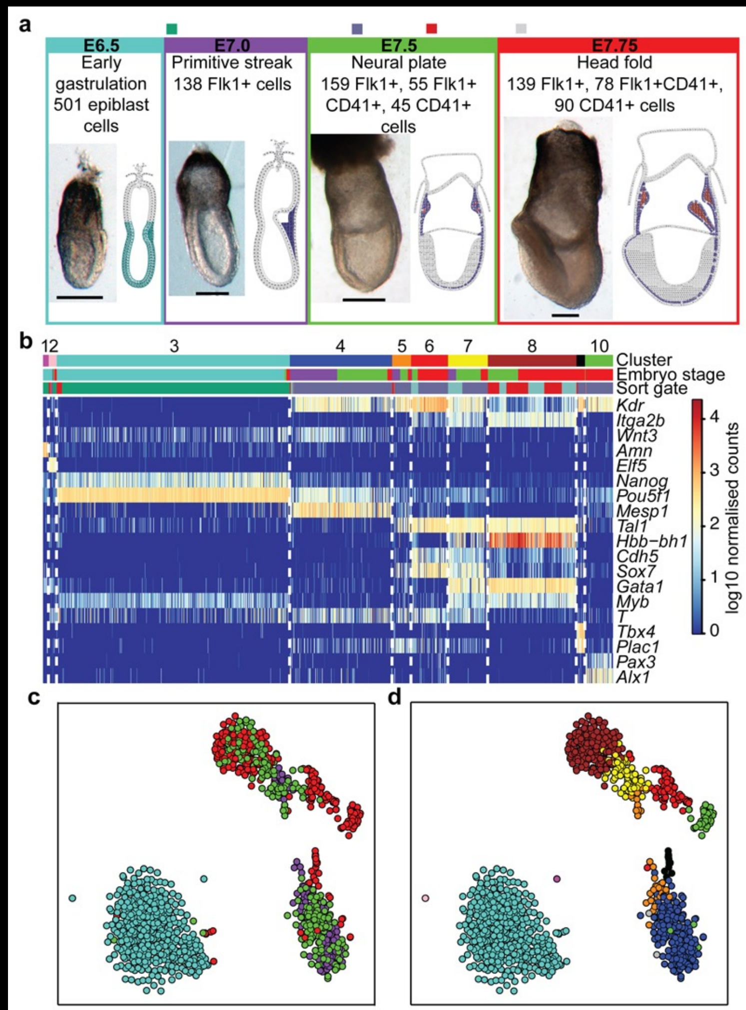
EMT pathway in gastrulation (and in metastasis)



Live imaging reveals distinct mesoderm migration phenotypes in extra-embryonic and embryonic regions of the early mouse embryo

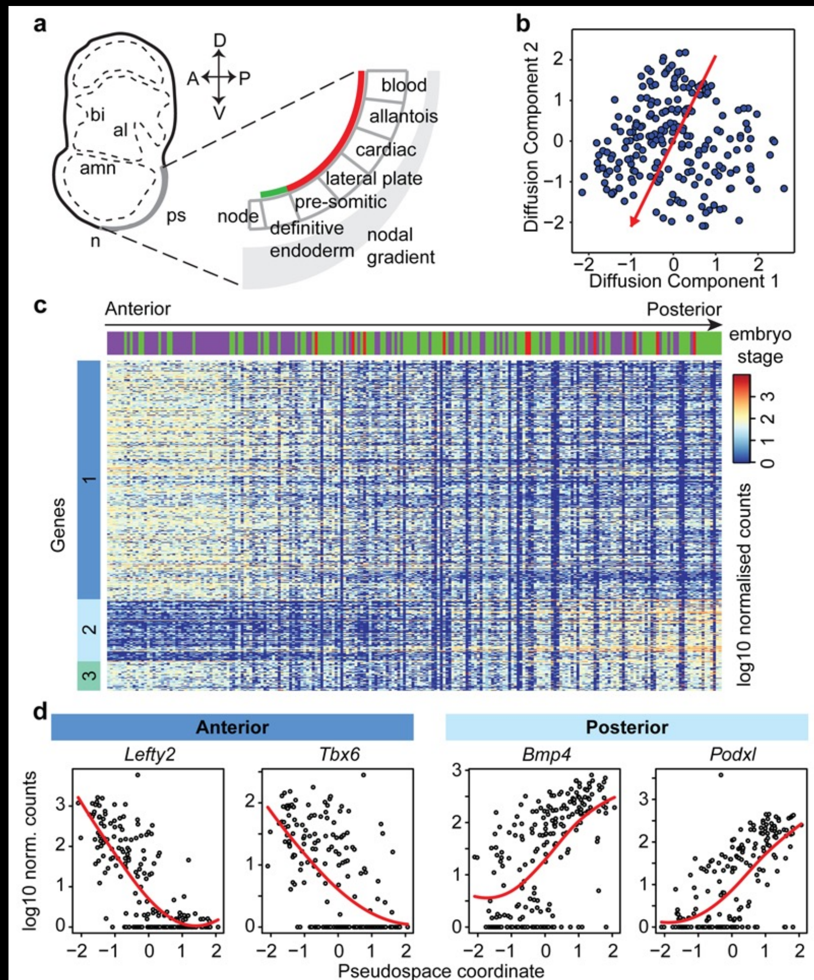
Saykali *et al.*, eLife 2019

Single cell transcriptomics identifies 10 populations relevant to early mesodermal development



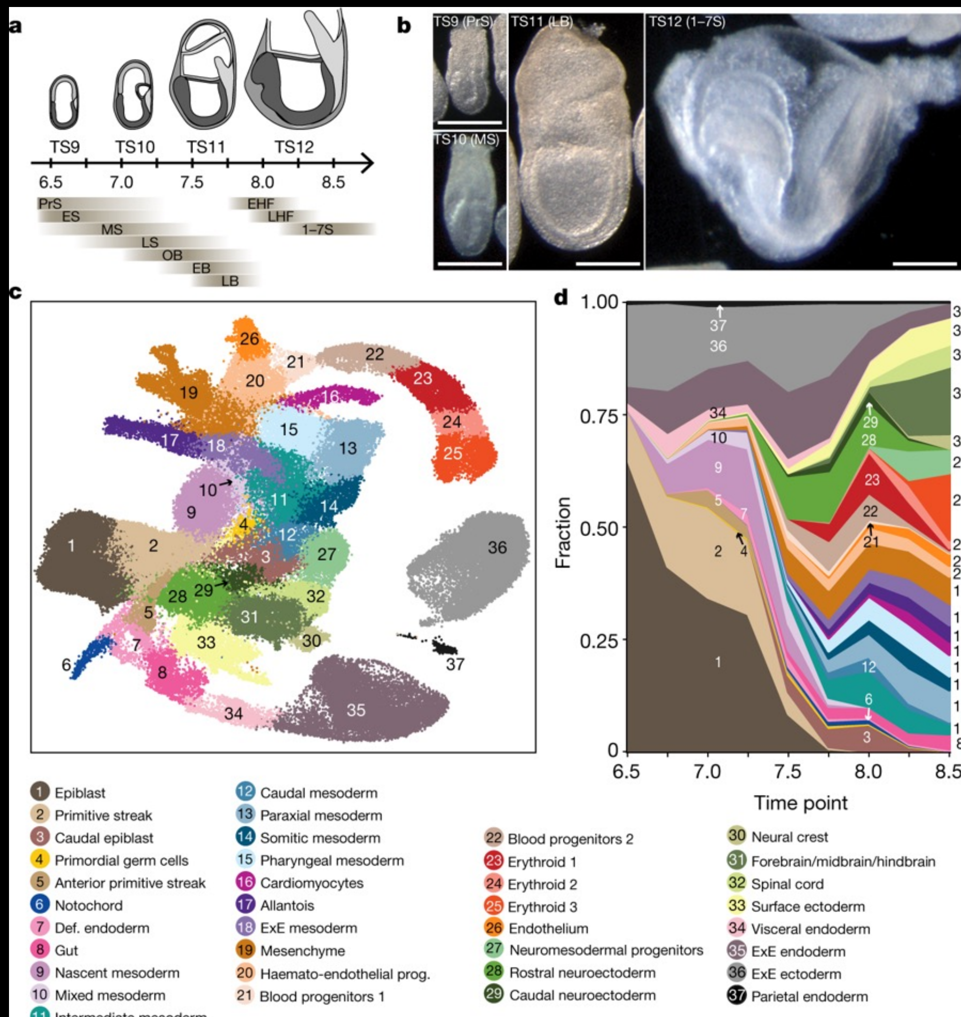
Scialdone A. et al., Nature 2016

Single cell transcriptomics reveal that fate of mesoderm dictated spatially and temporally



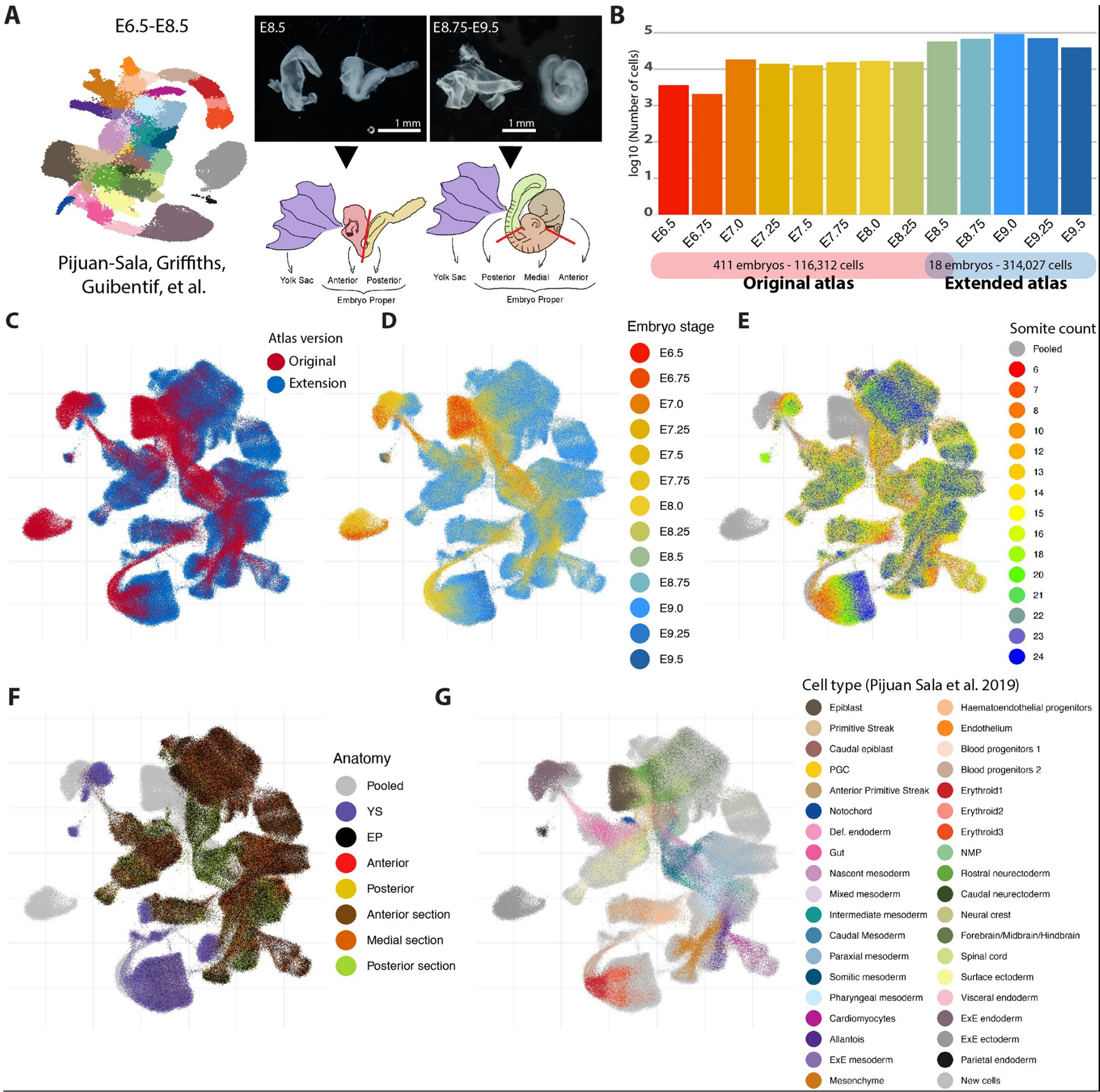
Scialdone A. et al., Nature 2016

A single-cell resolution atlas of mouse gastrulation



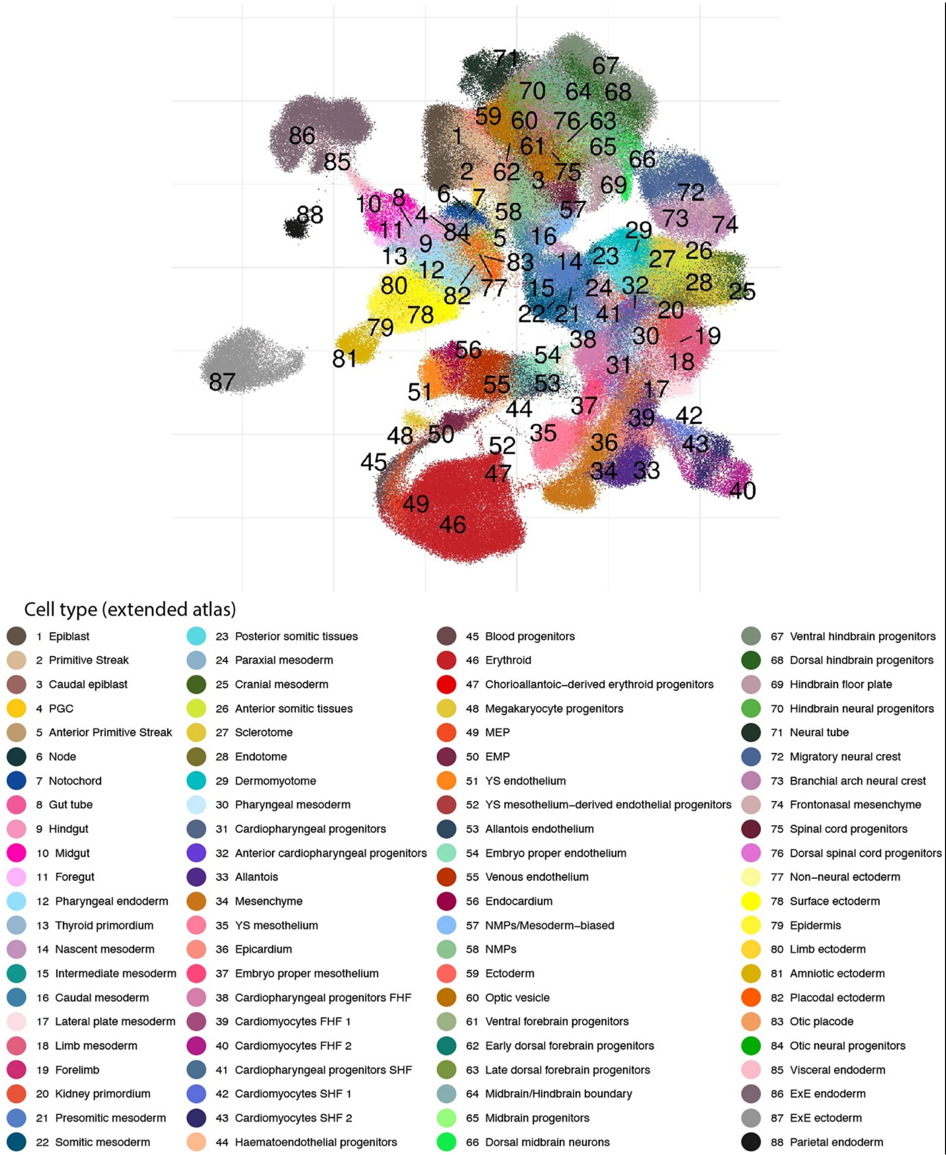
Pichuan-Sala *et al.*, Nature 2019

see also Nowotschin *et al.*, Nature 2019
Chen *et al.*, Nature 2019



Tracking early mammalian organogenesis - prediction and validation of differentiation trajectories at whole organism scale

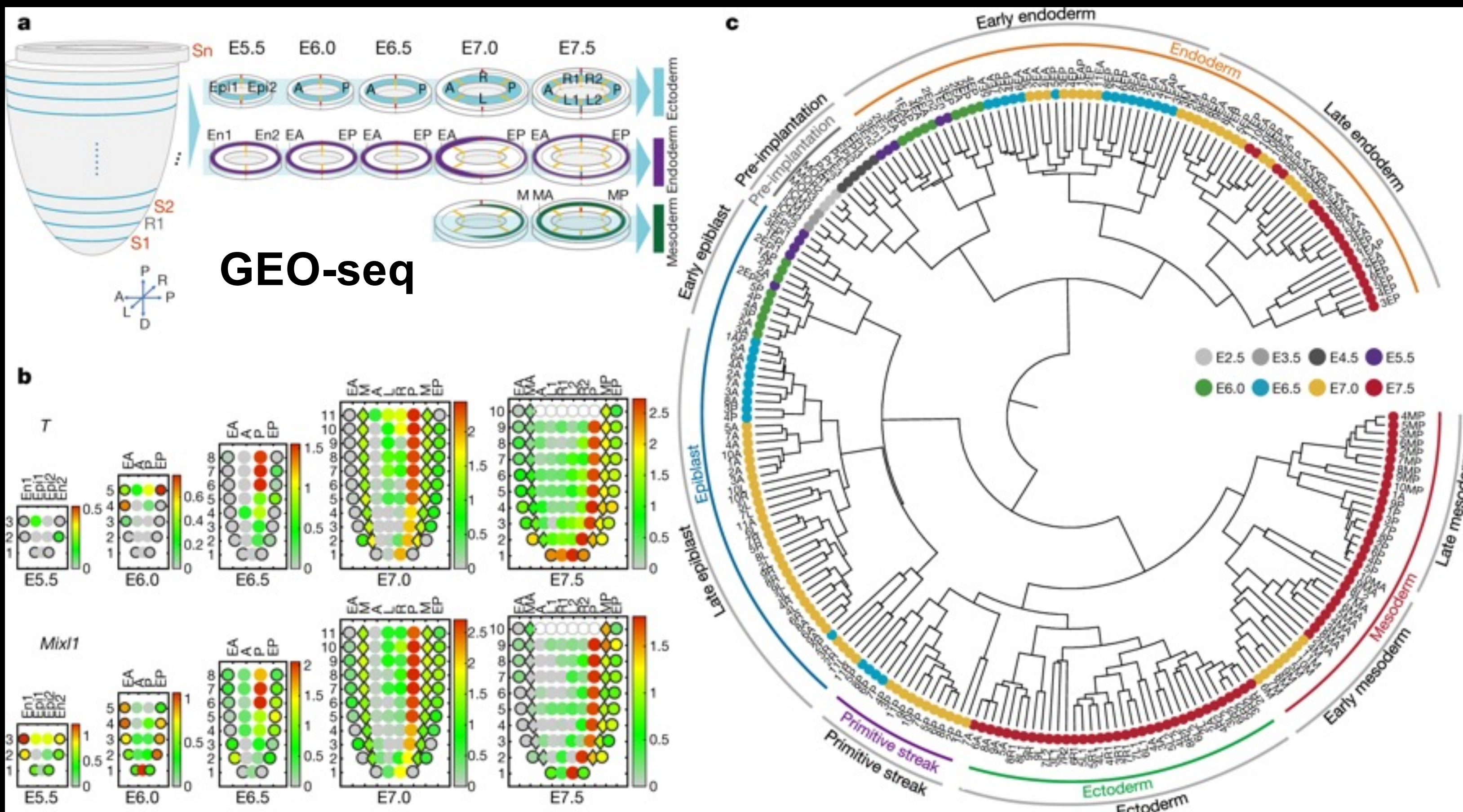
Imaz-Rosshandler *et al.*, Development 2024



An extended transcriptional atlas of mouse gastrulation and early organogenesis

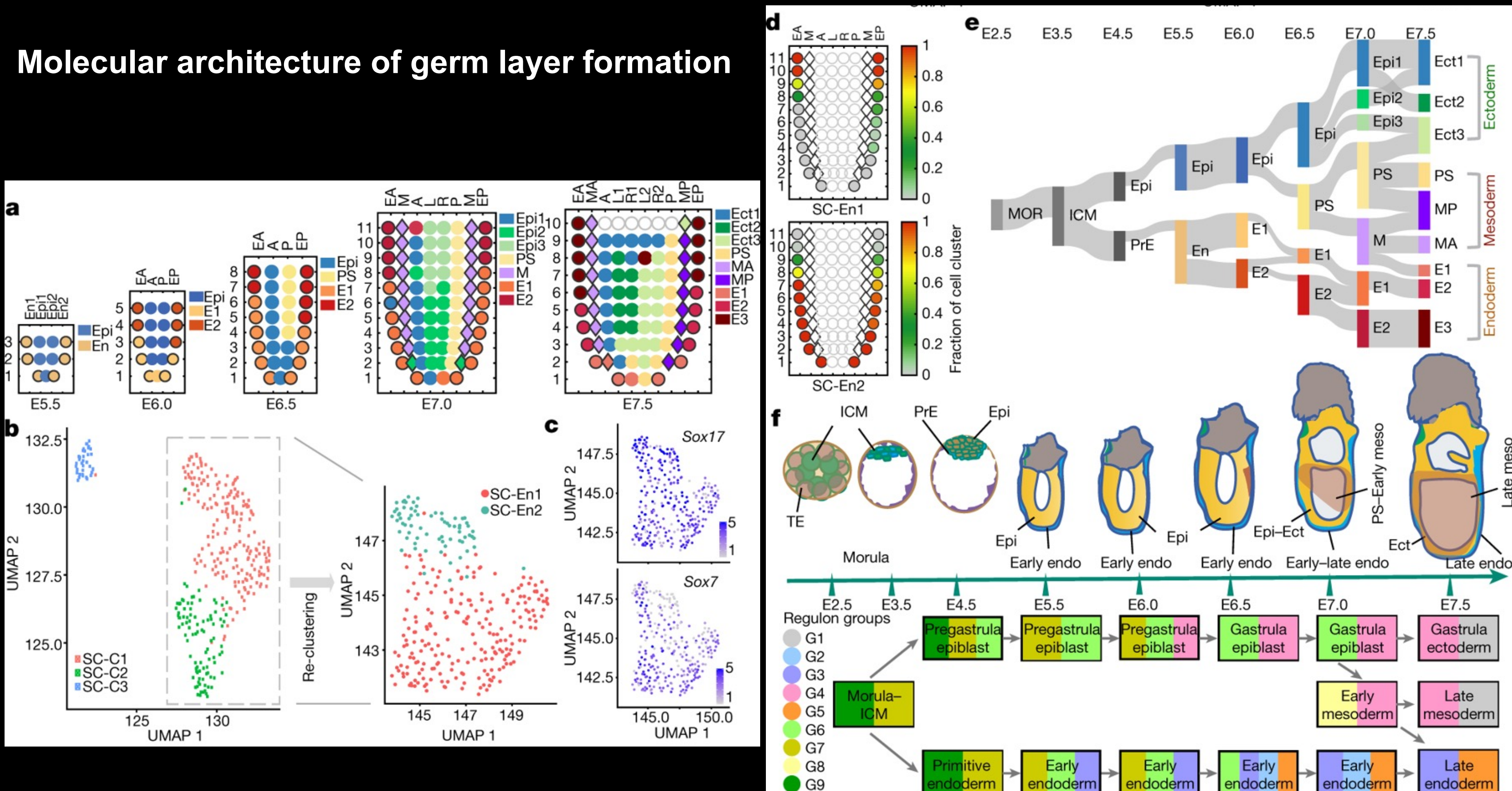
Imaz-Rosshandler *et al.*, Development 2024

Spatial transcriptomics: molecular architecture of lineage allocation & tissue organization in mouse embryo

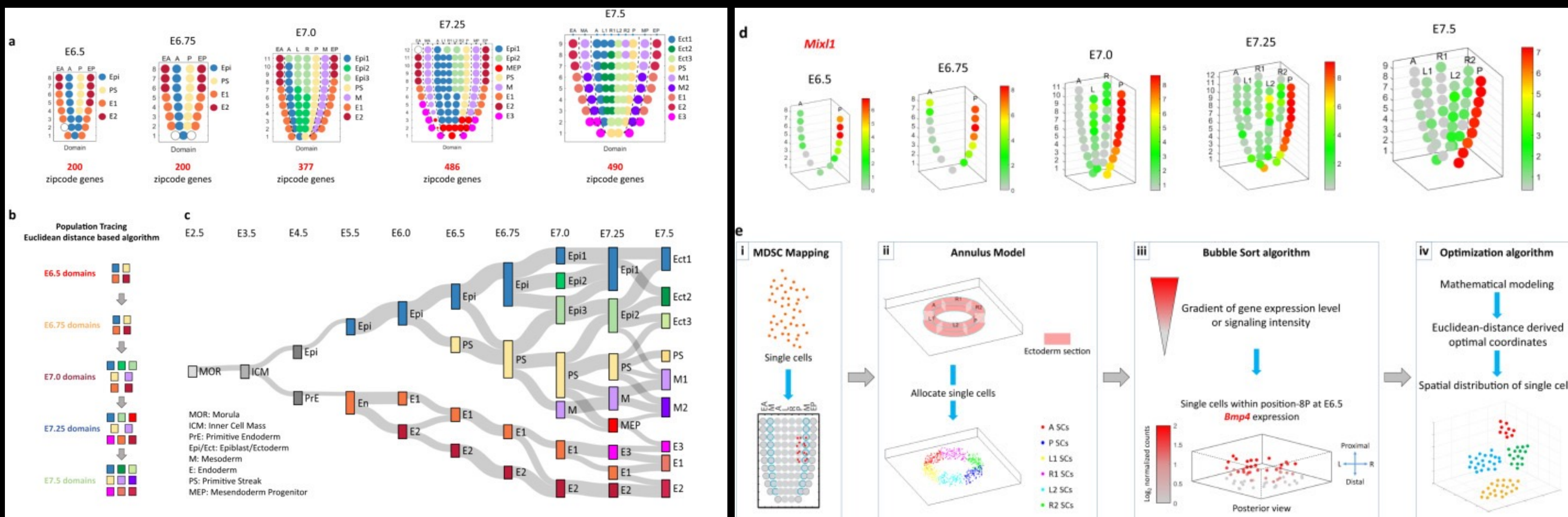


Peng *et al.*, Nature 2019

Molecular architecture of germ layer formation



Time space and single-cell resolved tissue lineage trajectories and laterality of body plan at gastrulation



Wang *et al*., Nature Comms 2023