

~~‘Synthetic embryology’~~: A new window on mammalian development

with Prof Ali Brivanlou (Lab Molecular embryology and stem cells)
Rockefeller University

Aryeh Warmflash (Rice), Benoit Sorre (Paris 7)

Fred Etoc, Mijo Simunovic, Jakob Metzger
Iain Martyn, Anna Yoney, Tien Phan

‘Kat’ Hadjantonakis MSKCC

Sophie Morgani, Clayton Schwartz

Why should an embryologist work with hESC?

Embryo \Rightarrow pattern(time) \Rightarrow cell communication (\neq Gene Reg. Network)

Genetics \Rightarrow 'parts', ... function?

Tool kit:

Secreted activators (BMP, WNT, NODAL....)

Secreted inhibitors

Apical-Basal polarized epithelia (\Leftrightarrow mesenchymal .. EMT-MET),

Understanding \Leftrightarrow Build it!

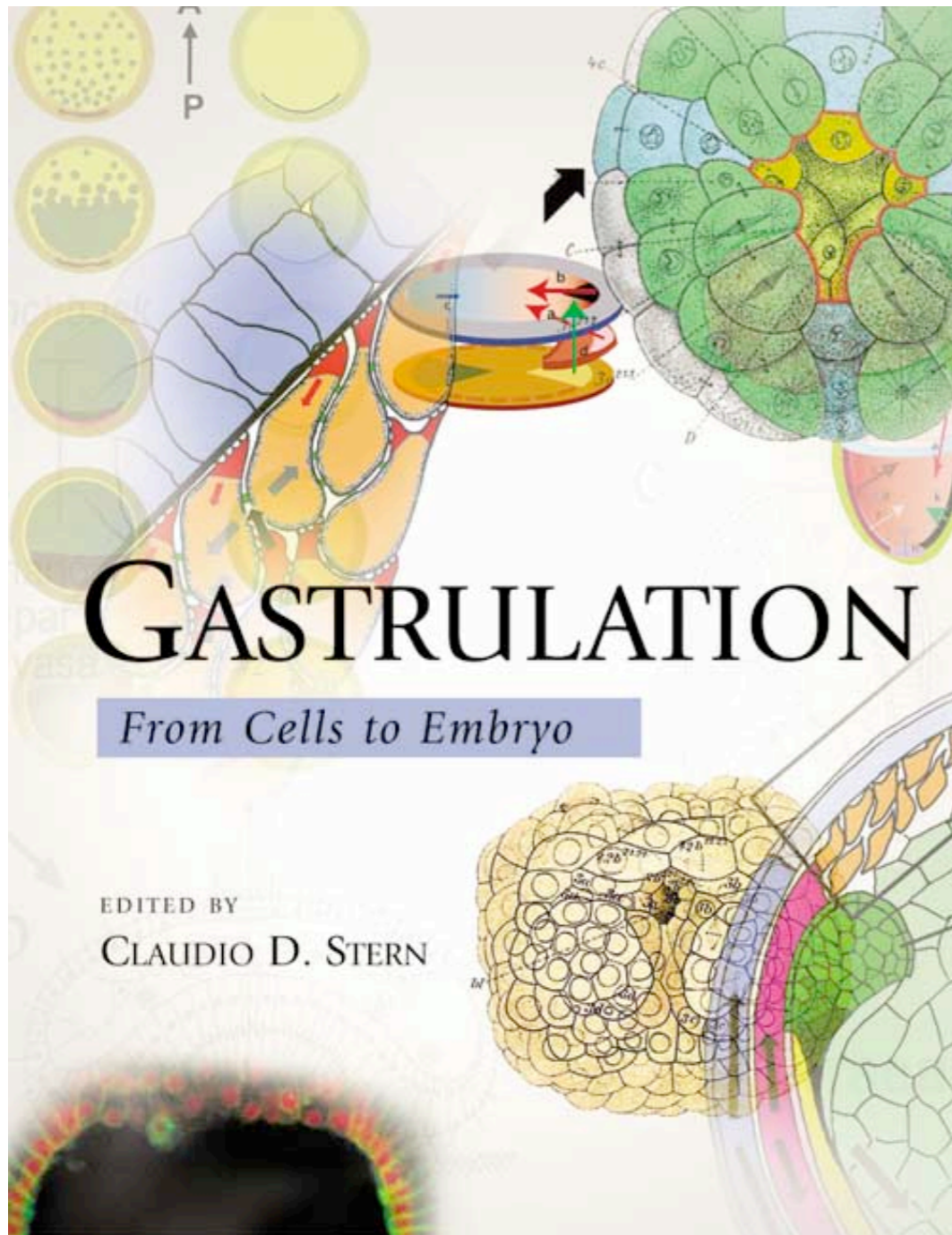
(~ vitro reconstitution for the biochemist)

Particularly for human, synthetic systems essential

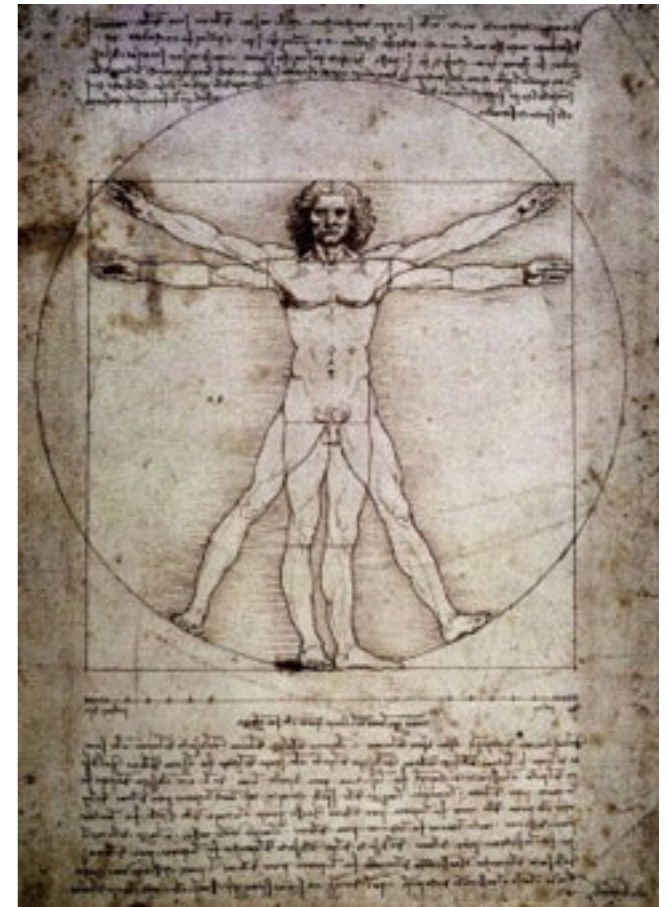
ethical experimentation

regenerative medicine needs engineering.

Human embryology?



Chapter:
'Other Mammals'

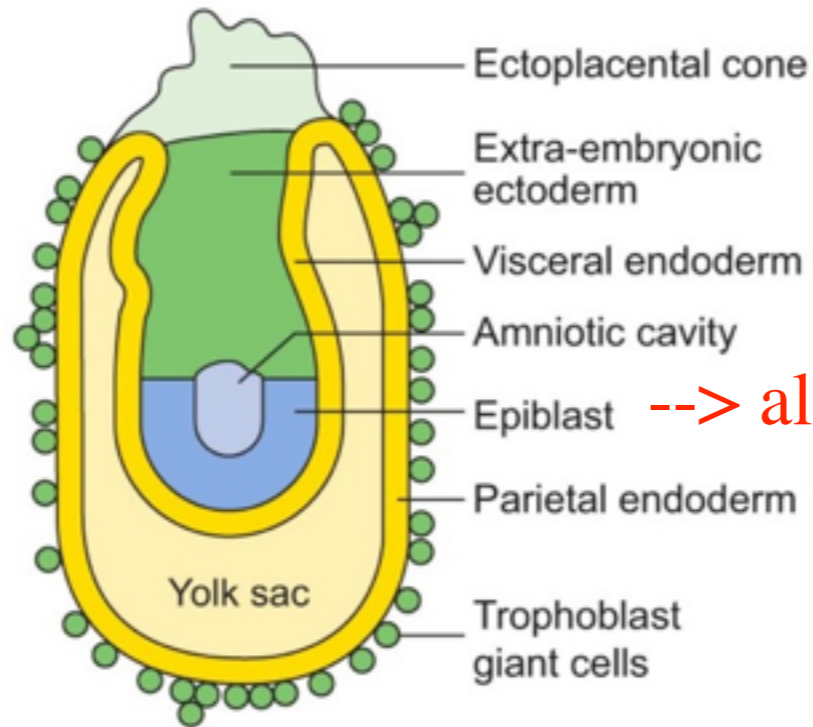
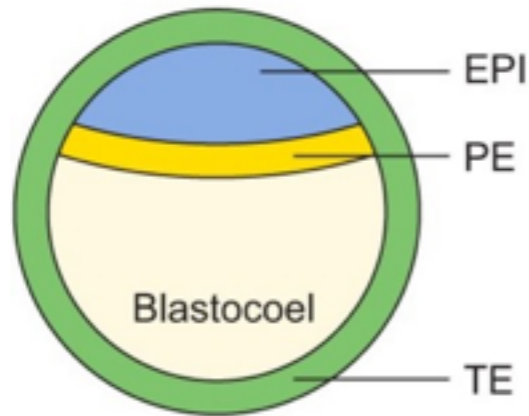


Human vs mouse

blastocyst

implanted

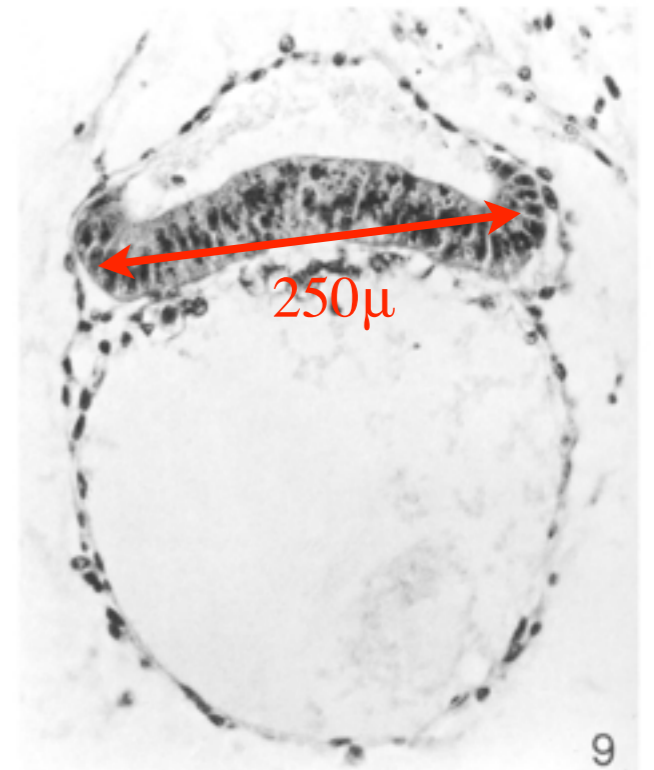
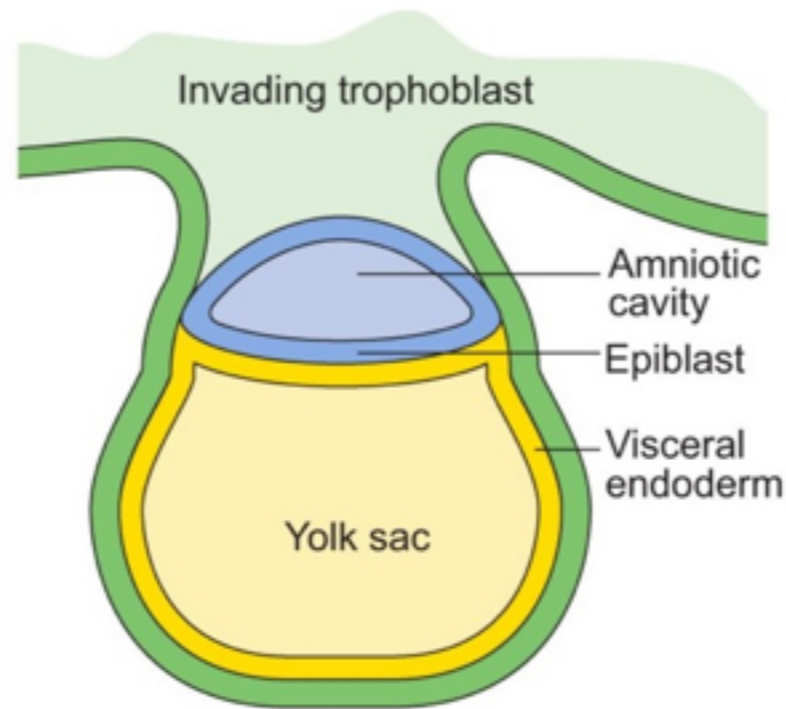
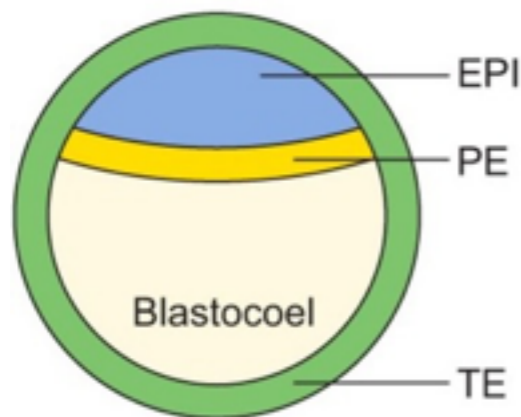
A Mouse



--> all cells fetus: an epithelium!

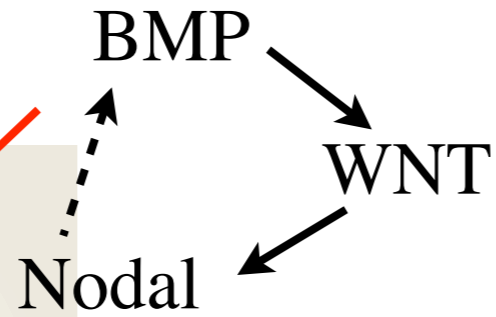
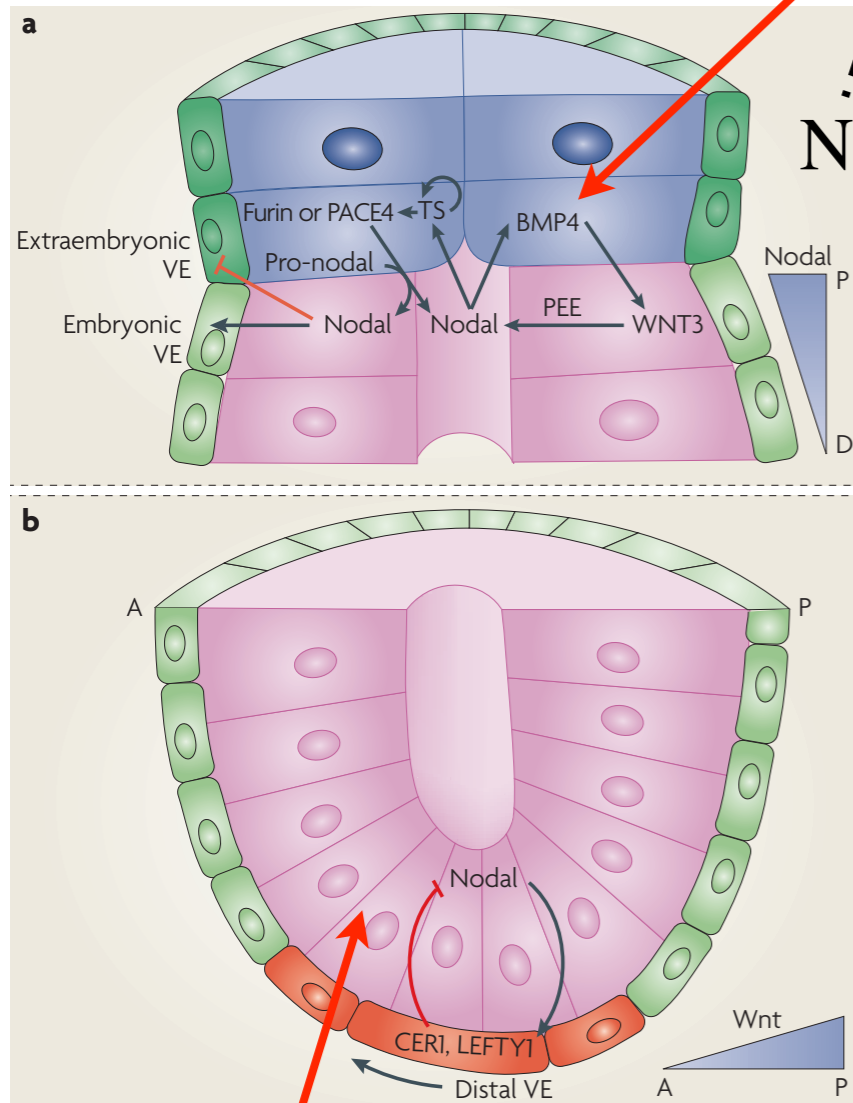
Carnegie Stage 6
early gastrulation
~ 14days

B Human

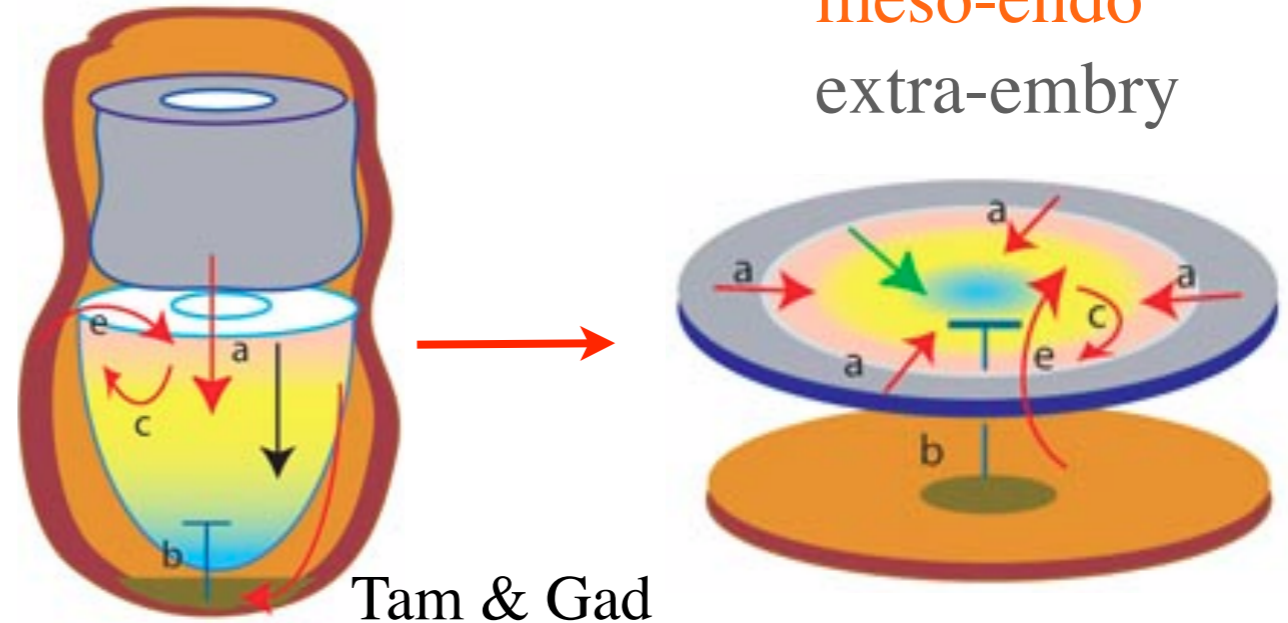


The assay

Arnold & Robertson 2009



ectoderm
meso-endo
extra-embry



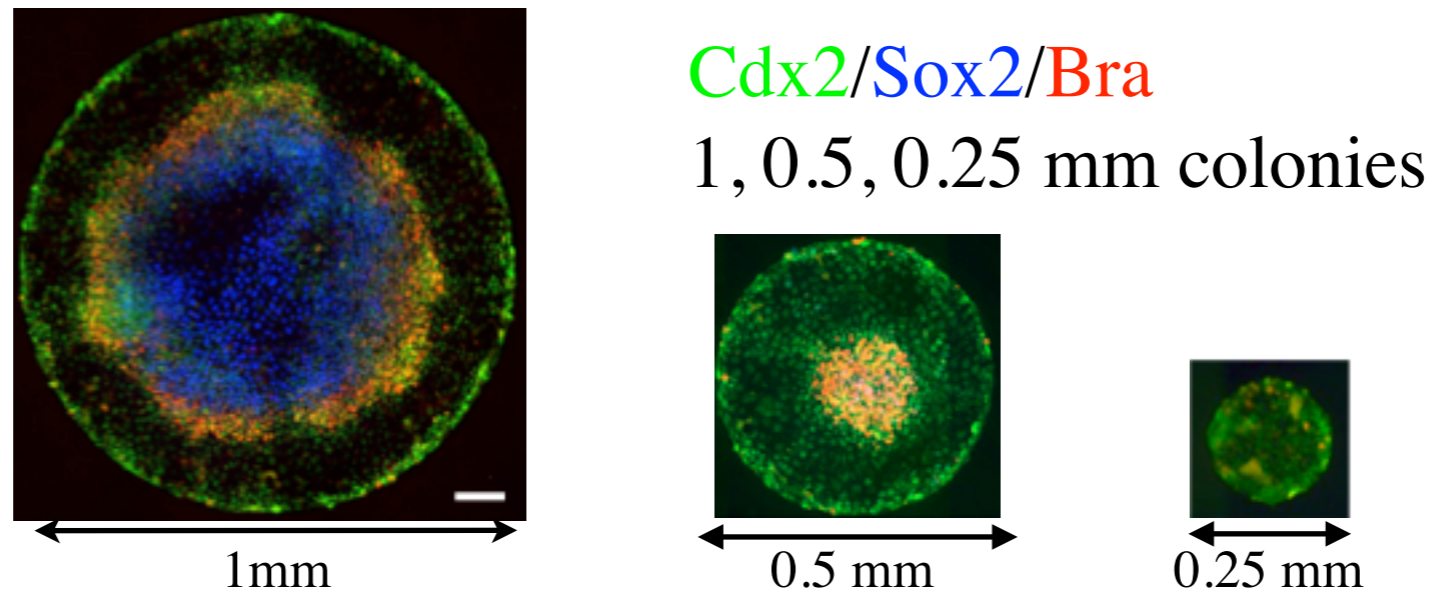
Tam & Gad
Gastrulation 2004

Epiblast (epithelium)
--> embryo

Decouple cell fates and signals from
Anterior-Posterior axis formation

Properties:

- Colonies define fates by distance from boundary: loose center in smaller colonies



Mechanism for localizing BMP signaling to edges:

- Receptors baso-lateral, not accessible to apical ligands, except at boundaries
- Secreted inhibitor Noggin high in center, low edges.....

NB Zhang...Ramanathan experiment 7/22

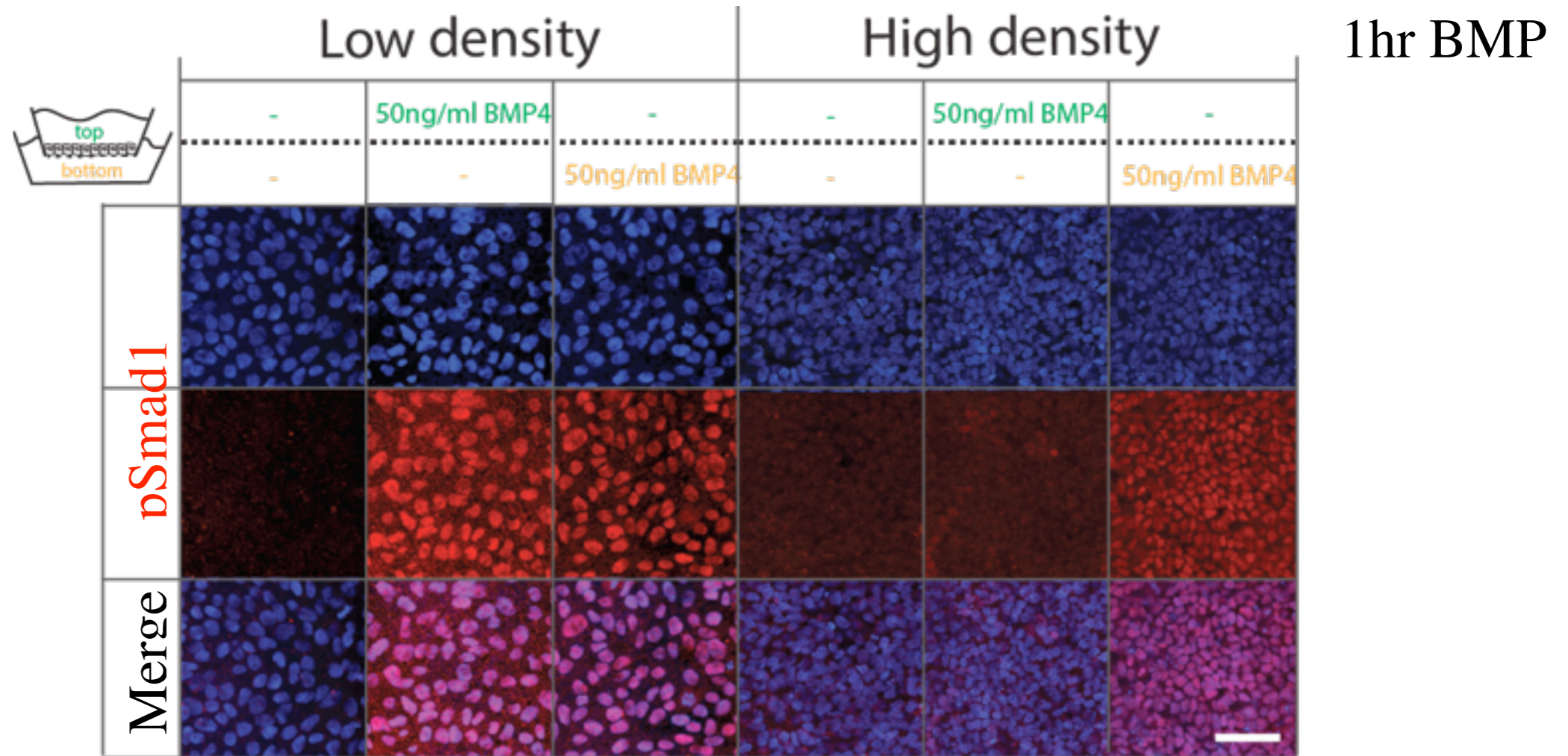
Warmflash, *Nat Meth* 2014,
EtoC, *Dev Cell* 2016

Don't forget cell biology when doing development!

Signaling in apical-basal polarized epithelia &

First Turing pair: $\text{BMP} \rightarrow \text{Noggin} \dashv \text{BMP}$

High density colonies respond only to BMP from bottom

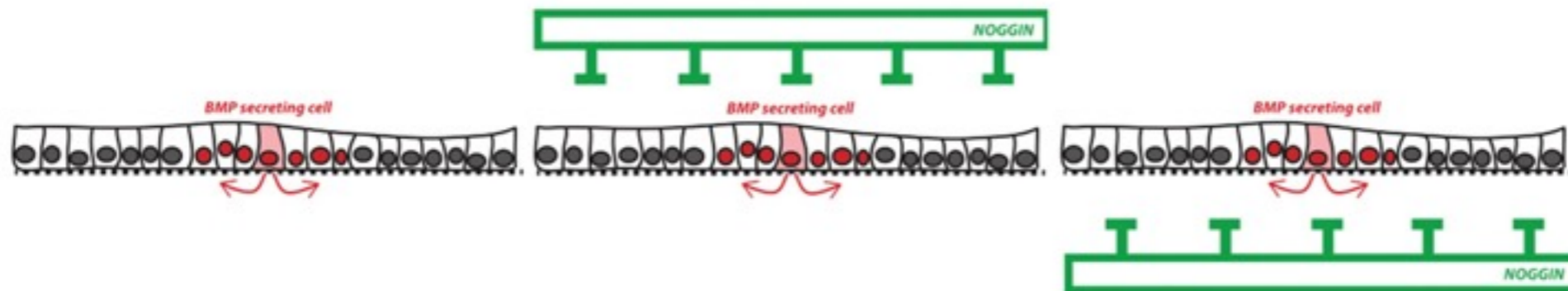
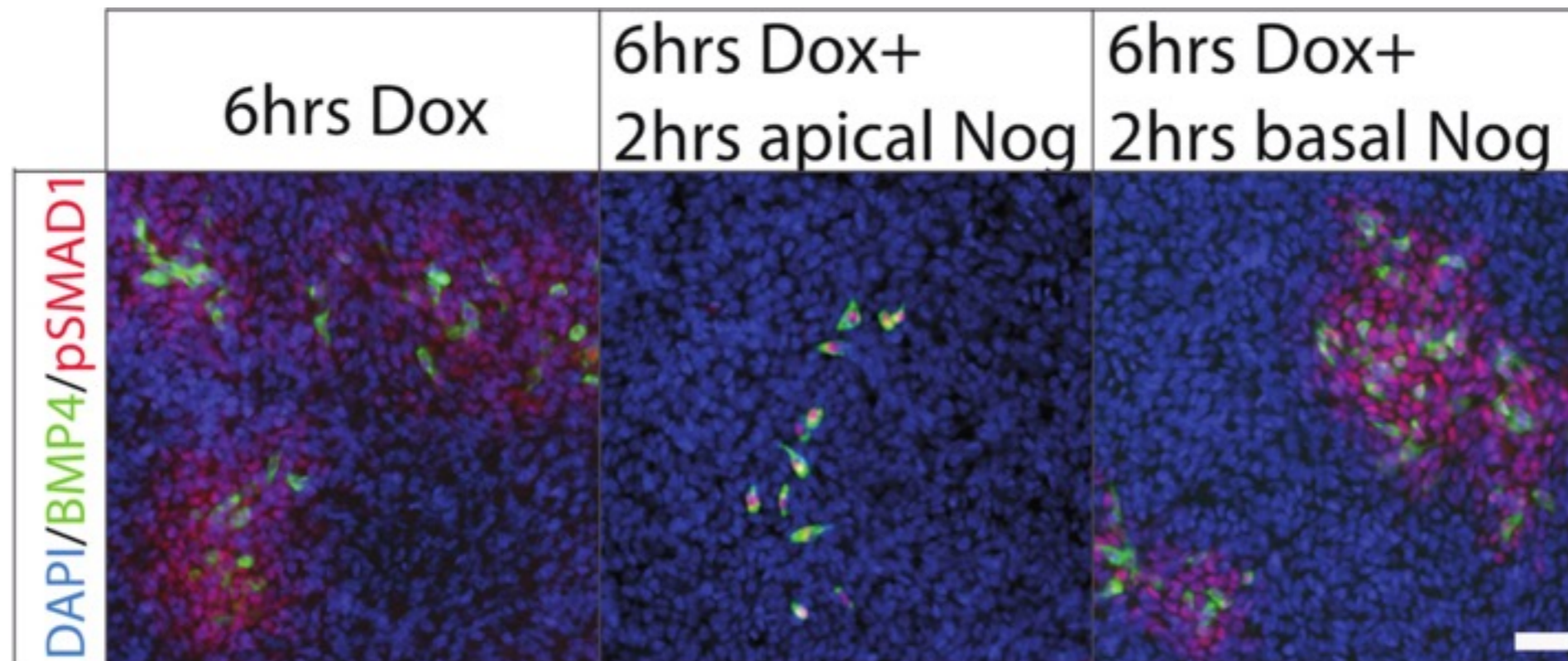


BMP-->pSmad1 Nuc (1h). High density cells respond to basal but not apical BMP

Receptors baso-lateral localized, not accessible to apical ligands, except at edges **

Cell polarity defines sensitivity to Noggin

pSmad1 response to isolated BMP4 secreting cells. Noggin apical vs basal



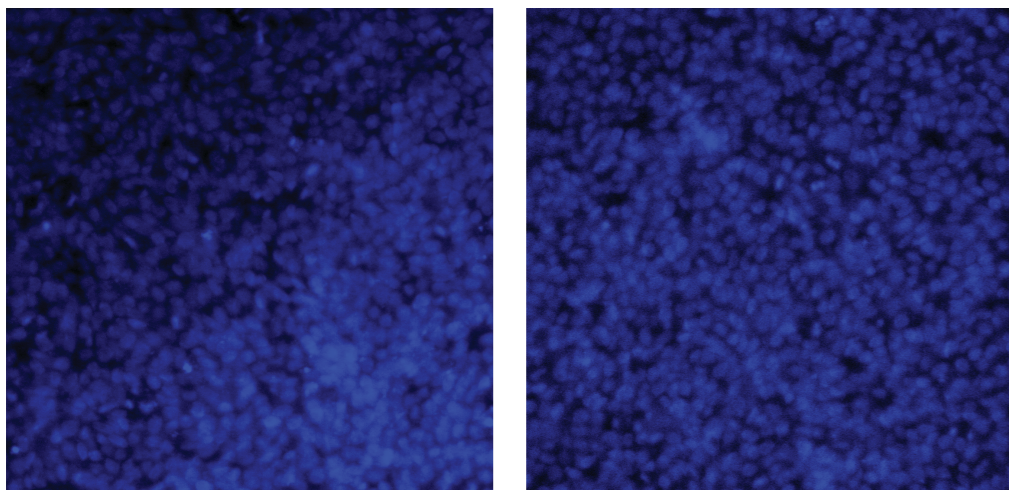
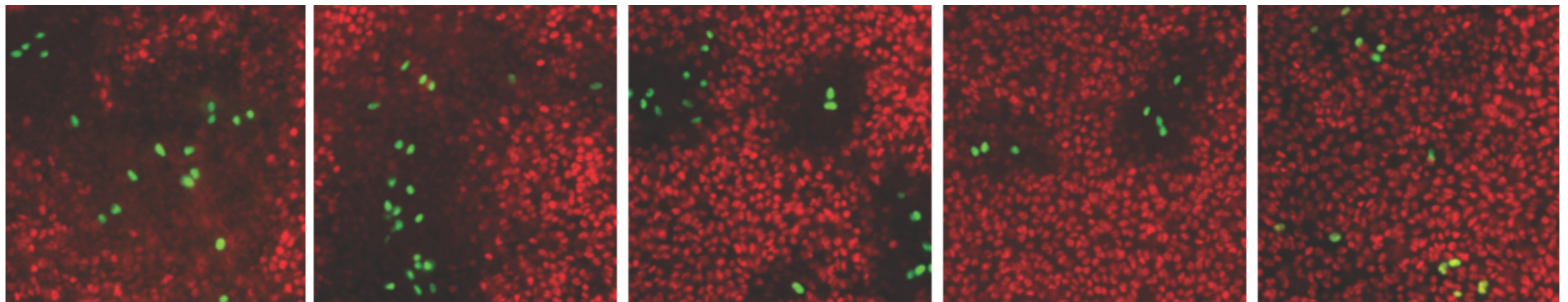
Filter assay for inhibitor & activator

DOX: **Noggin** secreting cells
—| (BMP → **pSmad1**)

Noggin secreted apically via Westerns
for cells on filters

localized Noggin secreting cells block basal BMP4

BMP4:



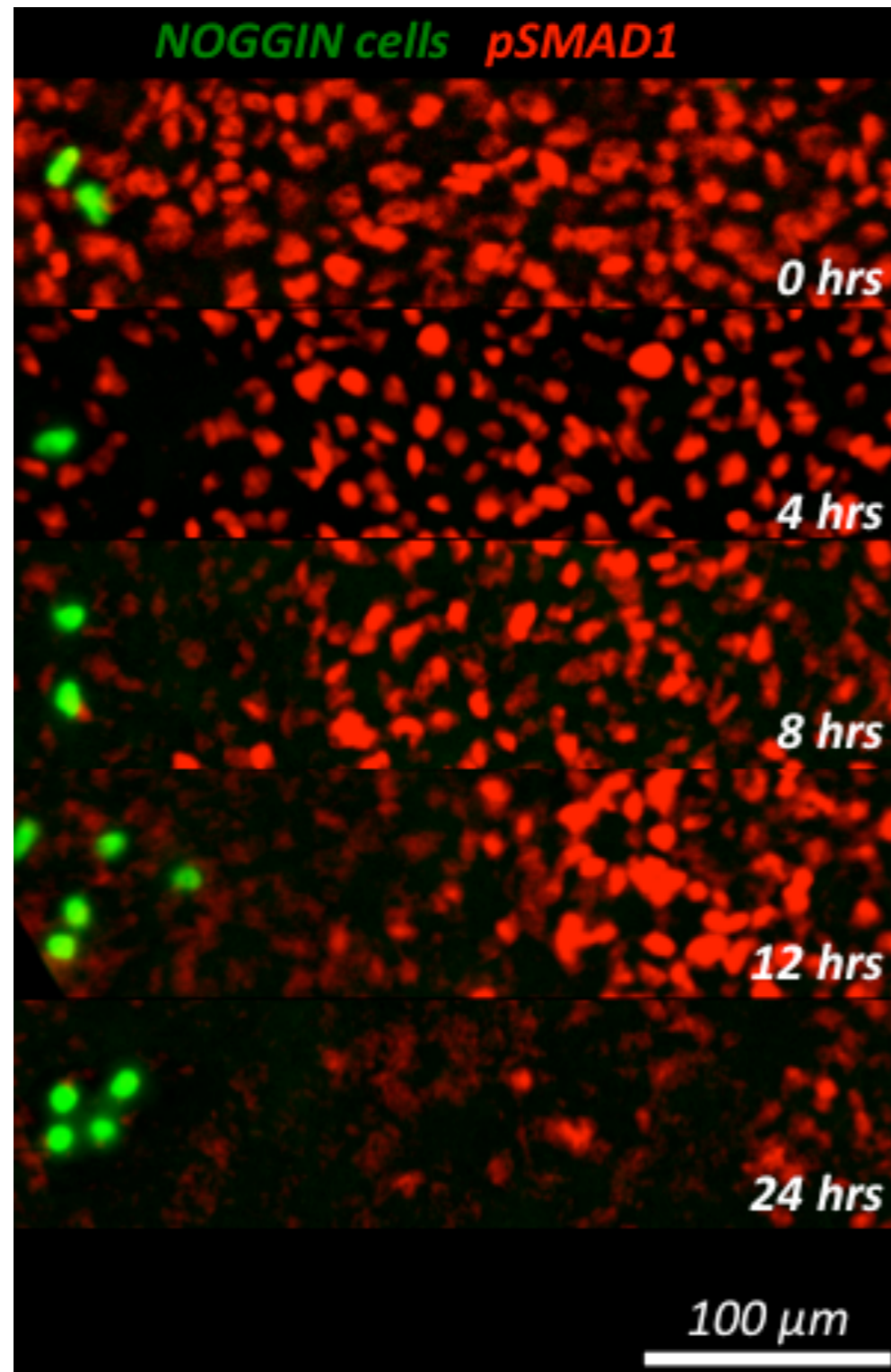
DAPI

BMP titrates range of inhibition

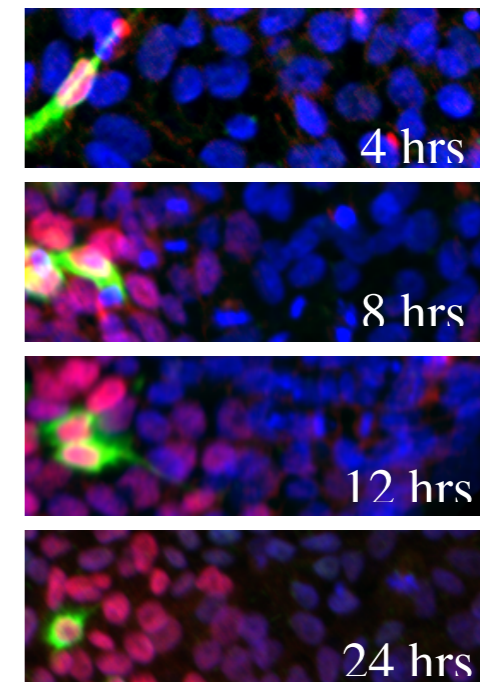
Noggin is a long range inhibitor, BMP short ranged activator

(DOX)BMP (pSMAD1 → Nucl) → Noggin

(DOX)Noggin —| BMP

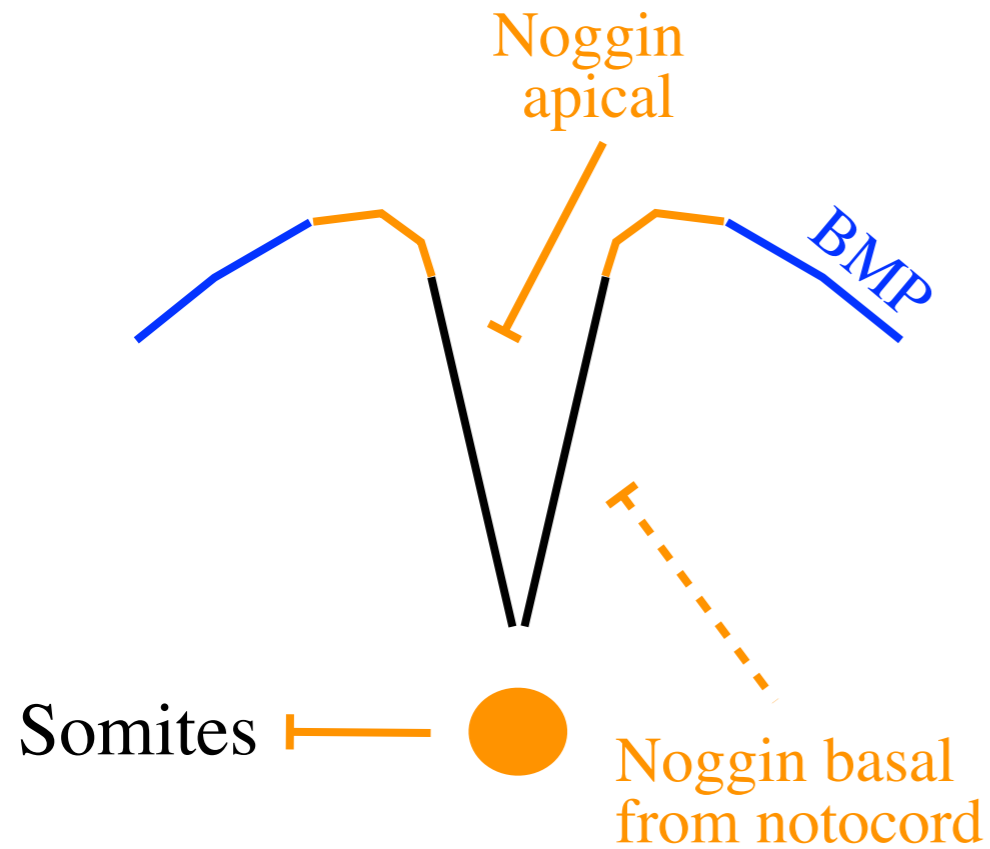


BMP4 cells, pSMAD1



(same scale)

A-B polarity of folding neural plate could affect Noggin access



Ybot-Gonzalez, *Devel*, 2007

Xenopus explants or chick to demonstrate Apical-Basal polarity??

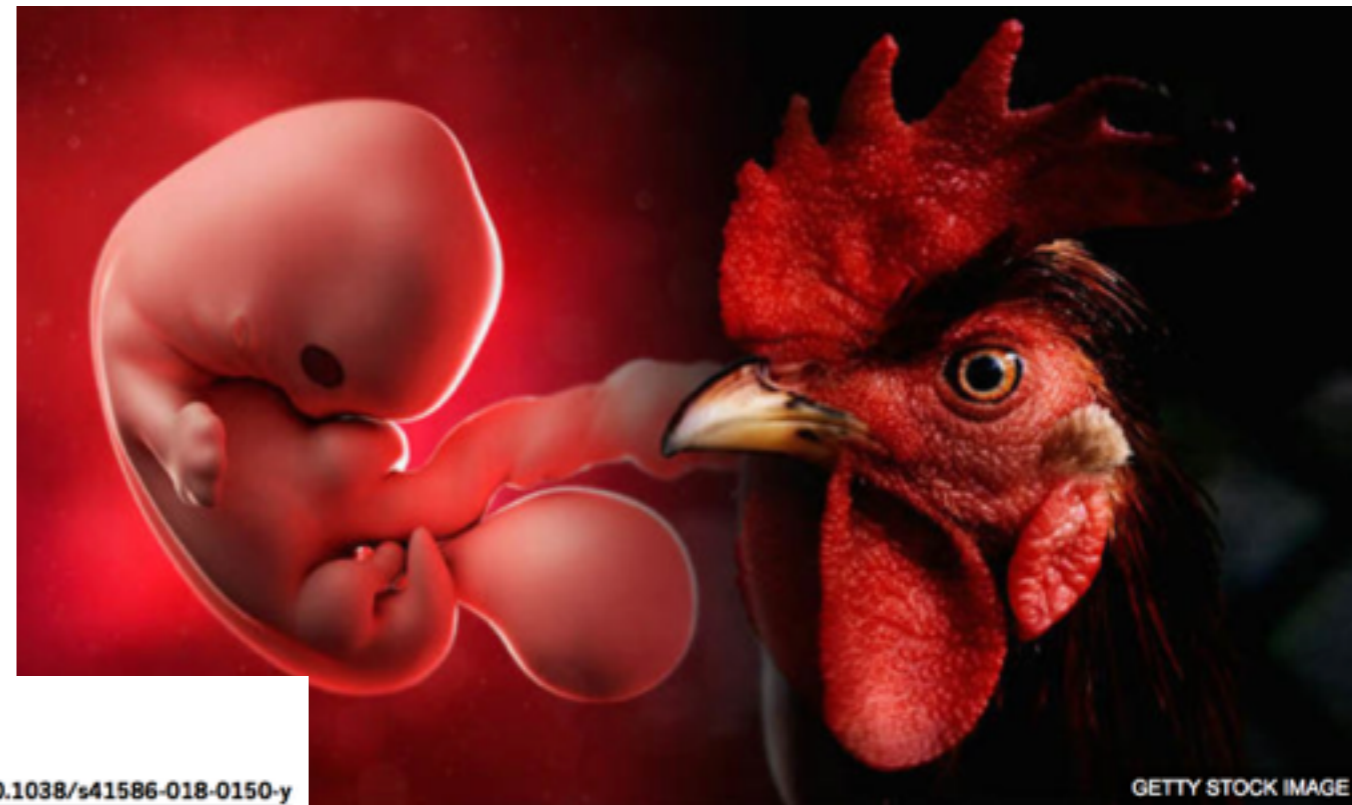
Public outreach?

Hybrid human chicken embryos: HALF HUMAN – HALF CHICKEN abomination created in US lab**

May 28, 2018

A TEAM of stem cell researchers have done the seemingly impossible and successfully combined artificial human cells with the embryo of a chicken in a shock new experiment aimed at trying to better understand developing life.

Fig 3



GETTY STOCK IMAGE

<https://doi.org/10.1038/s41586-018-0150-y>

LETTER**

Self-organization of a human organizer by combined Wnt and Nodal signalling

I. Martyn^{1,2,3}, T. Y. Kanno^{1,3}, A. Ruzo¹, E. D. Siggia^{2*} & A. H. Brivanlou^{1*}

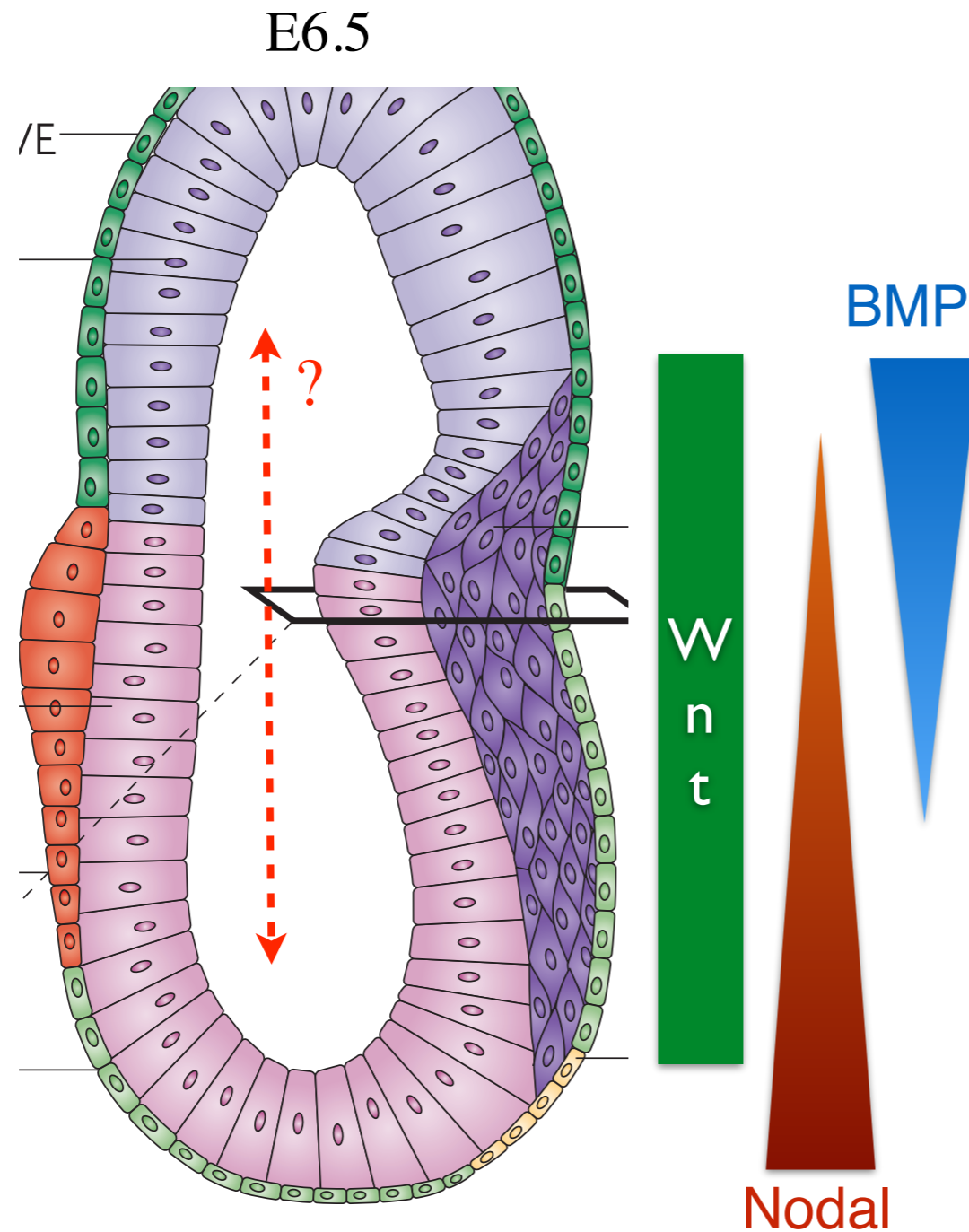
Extensions of signaling in epithelia

Are apically secreted inhibitors affected by flow?? (yes)

Is there a receptor for inhibitors, how are they internalized (no? dynamin dependent)

Trafficking to meet up with basolateral BMP (EM, markers for the endosome soup)

What about Wnt? (I. Martyn)

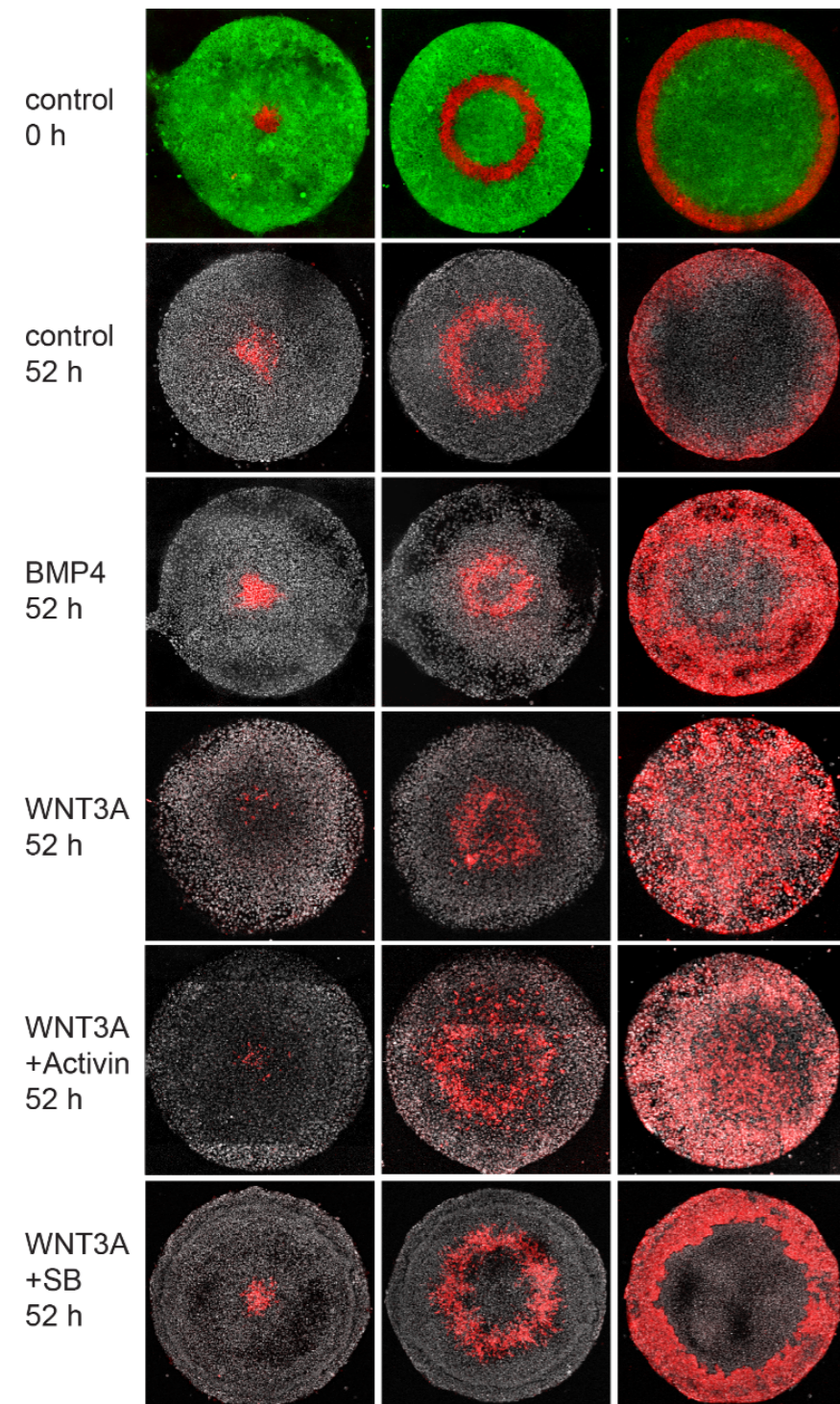


Primitive streak requires Wnt
and defines posterior

opposing BMP, Nodal gradients
define fates

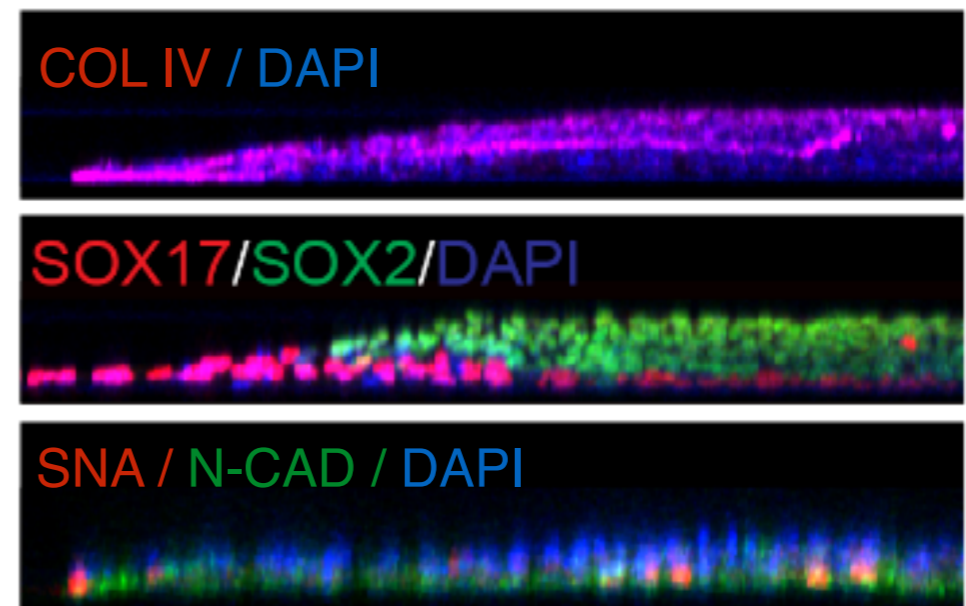
Integrating the signals for a human fate map

Photoactiv: to see movement vs morpho



Germ layer markers (radius, z).....

WNT3A 52 h

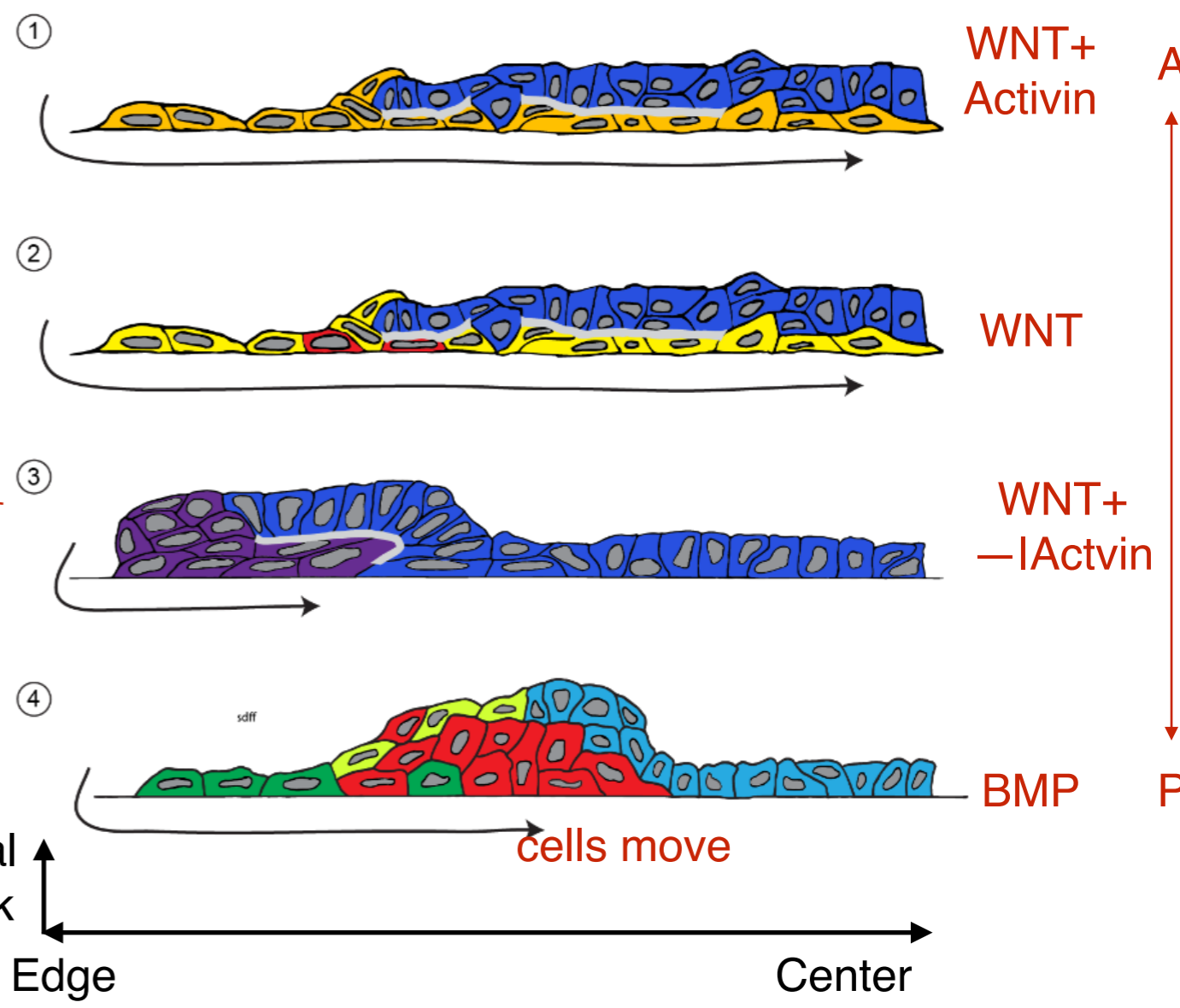
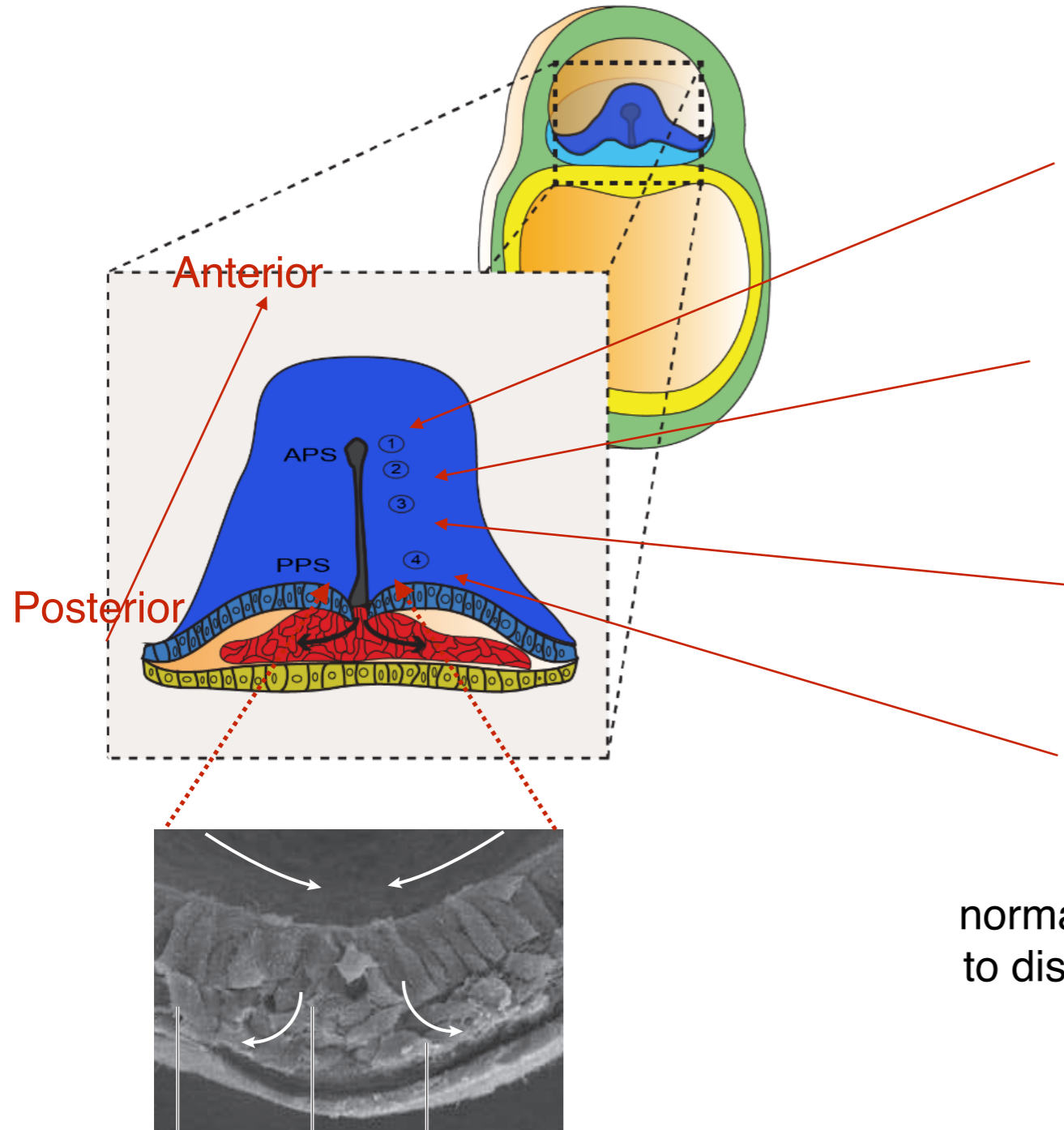


normal
to disk

Edge

Center

Micropatterns to embryo: morphogenesis?



Cells converge in sheet, dive under, and spread

Epiblast, Anter Endoderm, ExEmbMeso, ...

Summary of (other) Wnt data:

Get edge localized primitive streak

- for dkk1- EMT occurs as a wave from edge to center
- Wnt expression [geometry & forces ??]

Similar patterning phenomenology to BMP, very different molecules:

- Edge sensitive prepatterning (E-cad + mechanics based, not receptors)
- Wnt → Dkk1 secreted inhibitor, high center, low edges

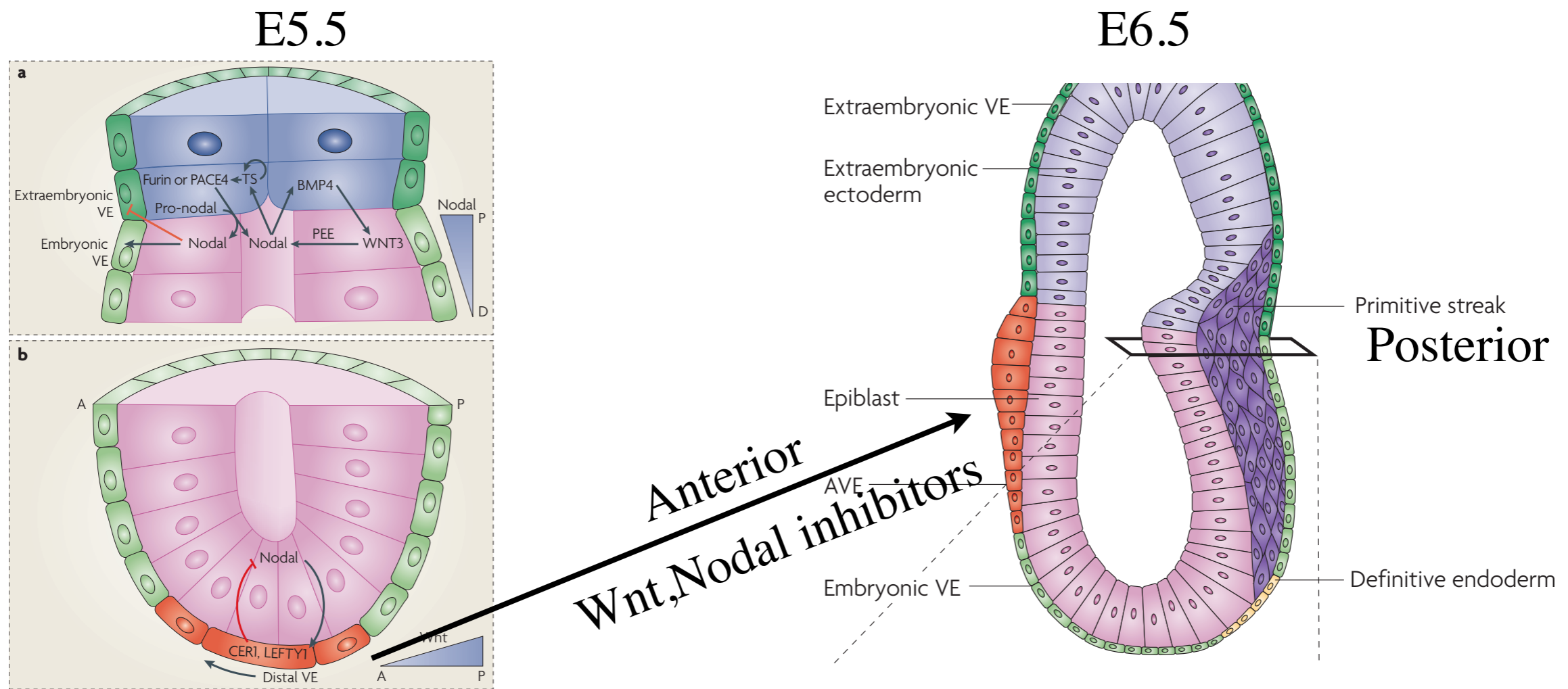
Comparison with mouse (embryos vs human stem cell colonies)

- Same inhibitors different phenotypes
- Mouse micropatterns... (Morgani et al eLIFE 2018)

Colonies without edges?

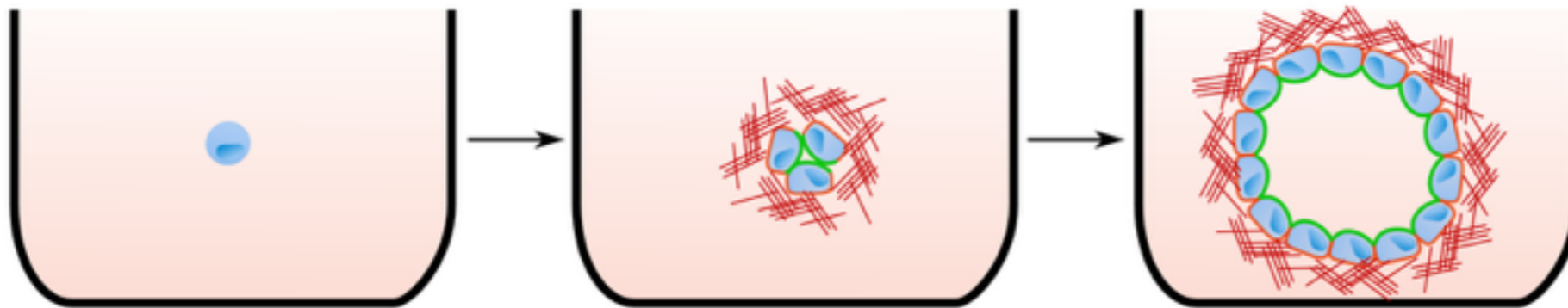
Can the epiblast alone make an Anterior-Posterior Axis??

Sozen et al Nature Cell Bio 2018: Epi + PE + TE \rightarrow gastrulation

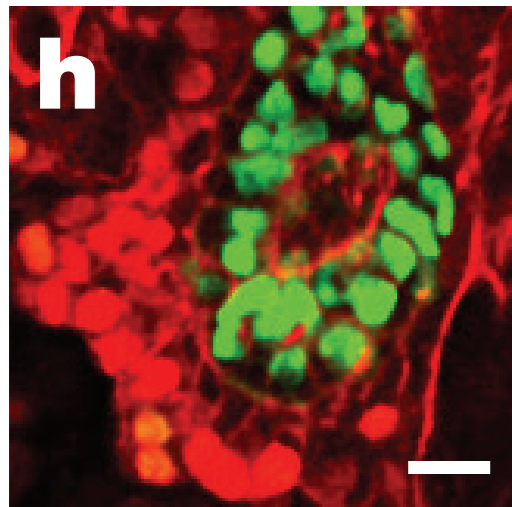


Colonies without edges?

Grow cells in 3D gel matrix: they cavitate and grow as closed shell, basal out



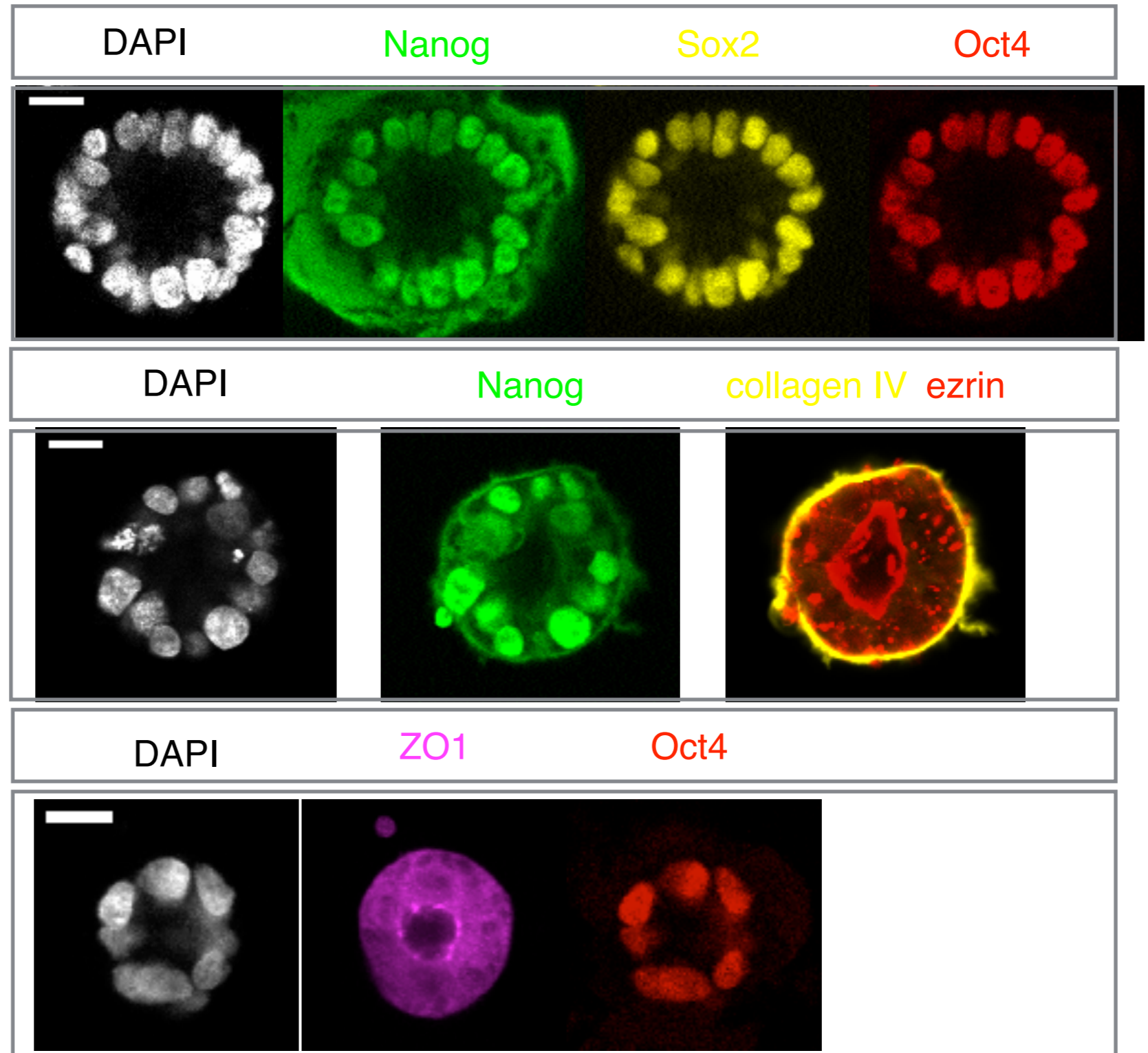
Cyst polarized, Size ~ post-implantation pre-gastrulation embryo



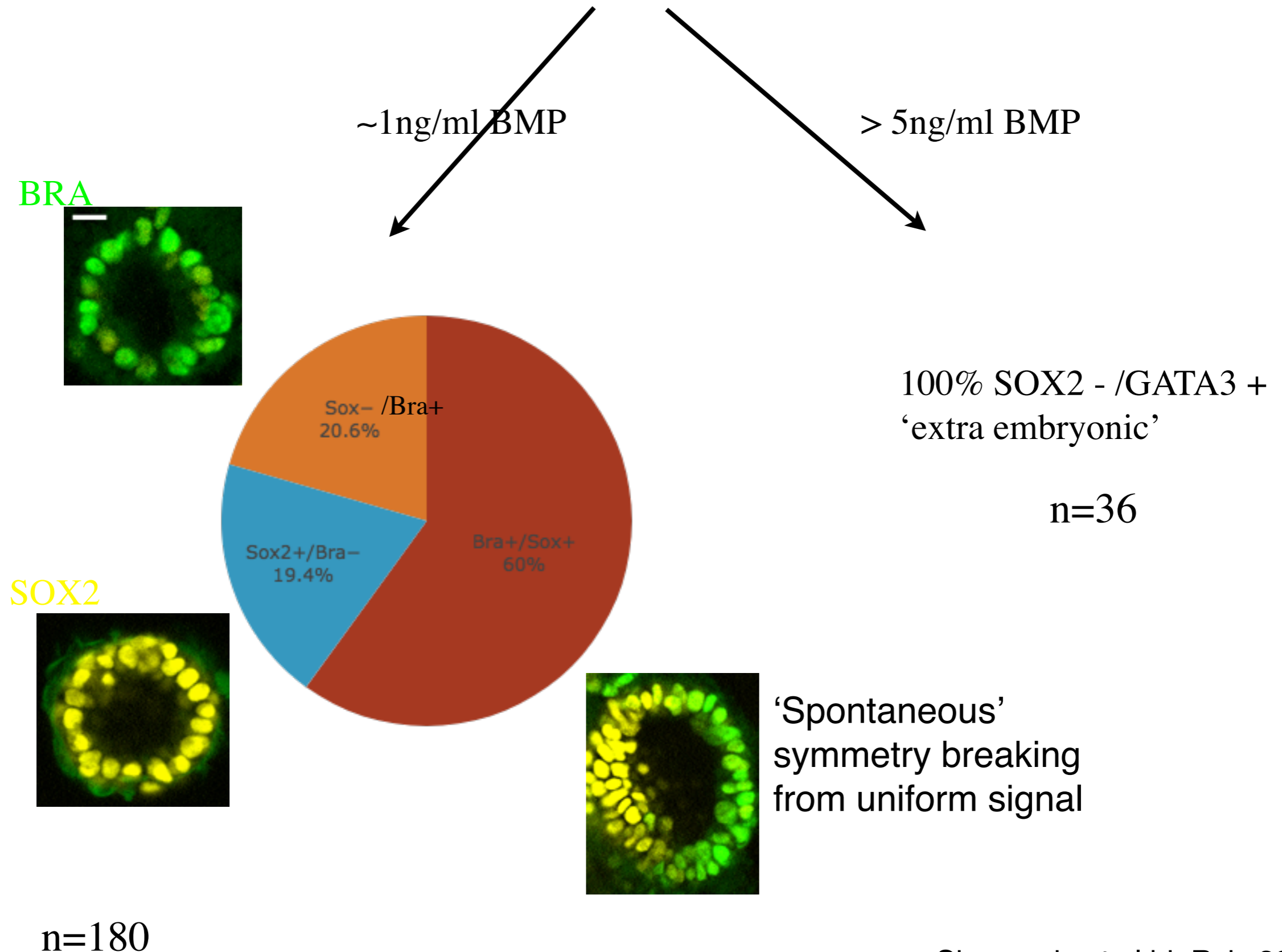
OCT4 **GATA6** +
Phalloidin

Deglincerti et al
Nature 2016, Vitro
implantation human
embryo ~10dpf

Scale bars 20 μ



2 Day BMP causes differentiation



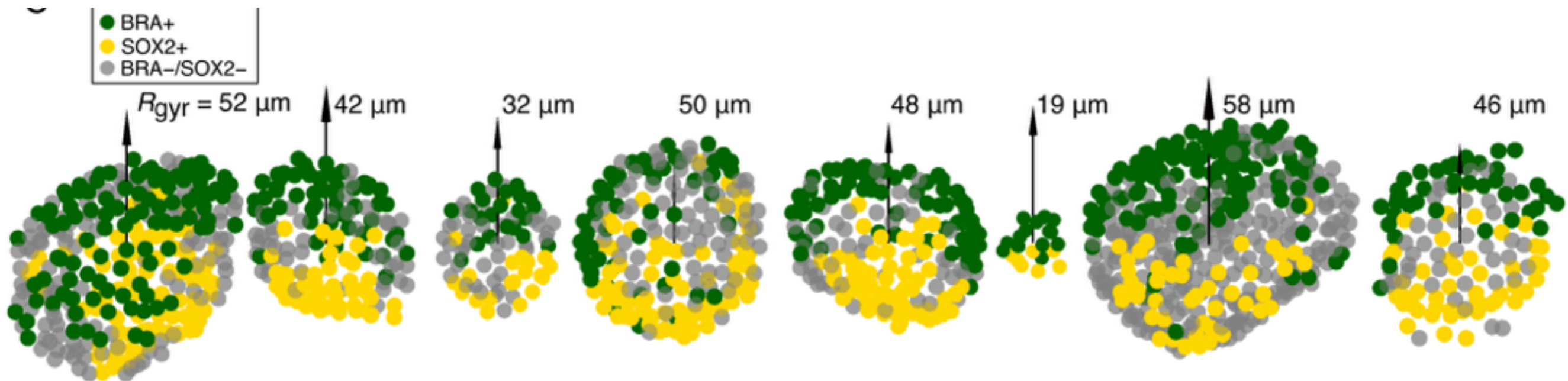
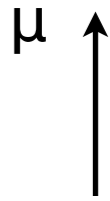
Polarization occurs independent of radius

'dipole moment' $\mu = \text{Sum}(\text{charge} * \text{position})$

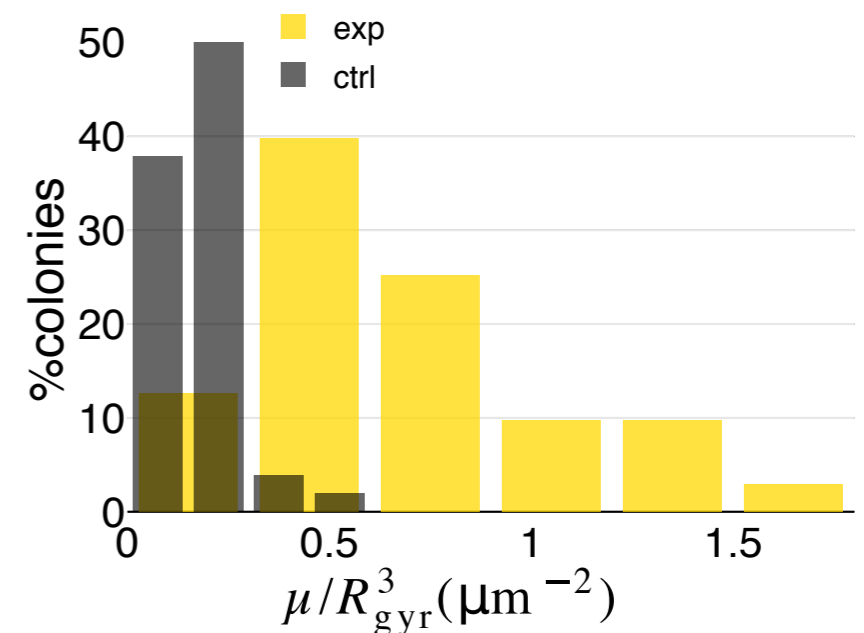
Bra(normalized) $\sim +$, Sox2(normalized) $\sim -$

Normalize μ / R^3 (normalize: charge \sim area, position \sim radius)

Rotate sphere to place μ up

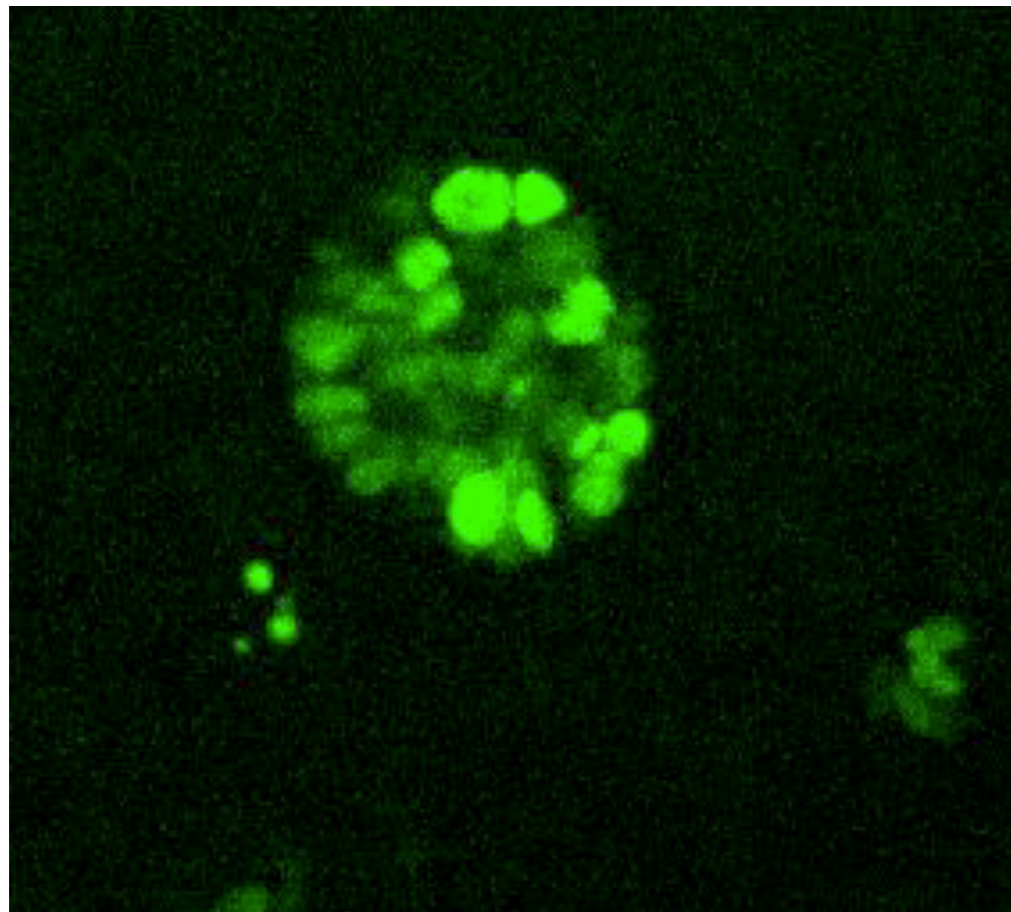


Scramble cells among positions
Recompute μ as control

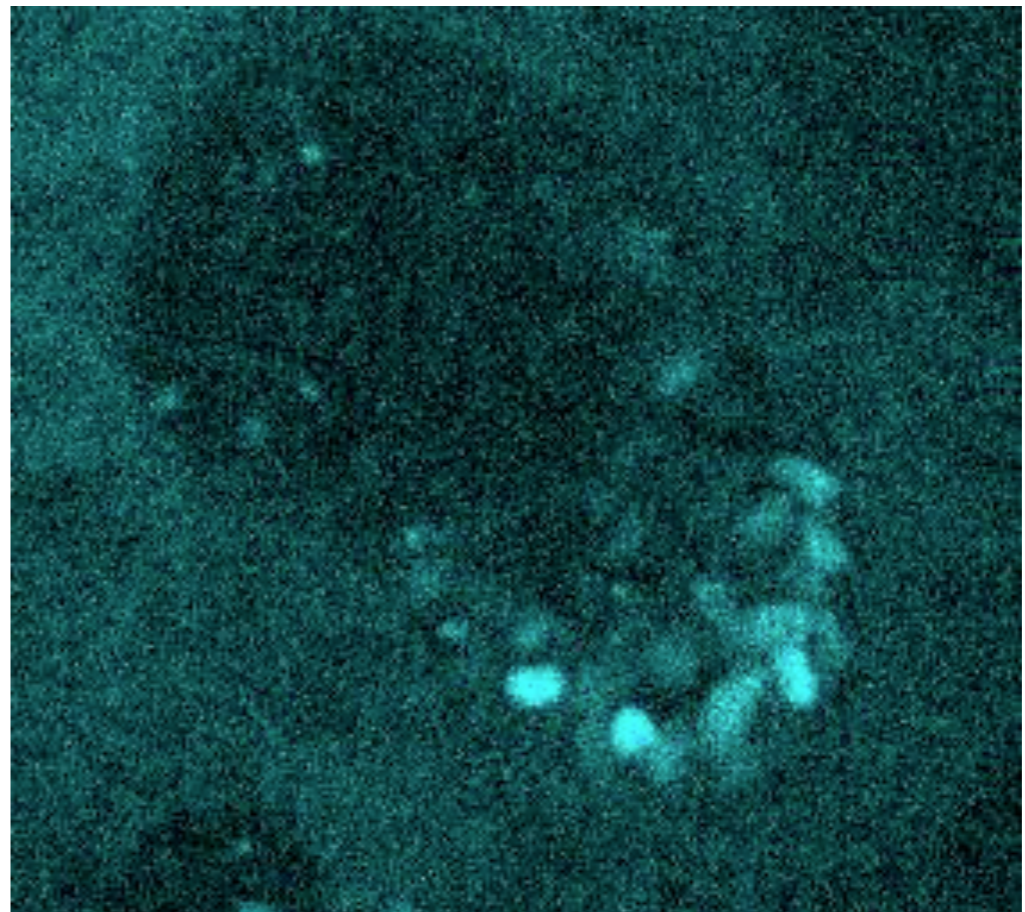


Symmetry breaking colony-level signal (not cell sorting)

Sox2



Bra



Start: 5 hrs after BMP addition, total time ~40 hrs
Some of Bra+ cells escape into gel, also see Sox17

'Gastrulation' in 3D cysts.

Break down of basement membrane (Col IV)

Change in cell contacts (E-CAD to N-CAD)

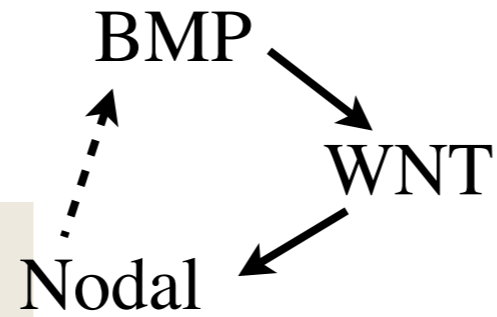
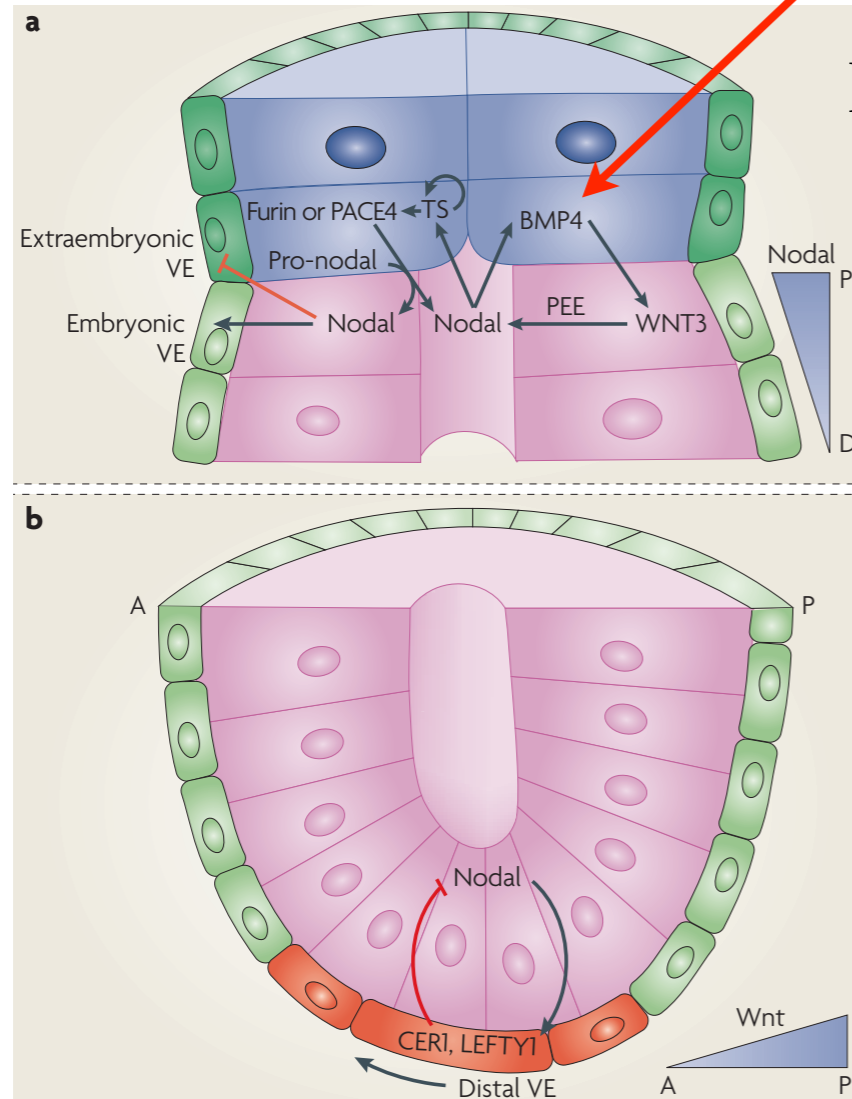
Epithelial to mesenchymal (Sna, T, ..)

Mesenchymal cells unconfined

Wnt, Dkk1, Nodal... expressed in the 'streak' cells

Remember signaling hierarchy

Arnold & Robertson 2009

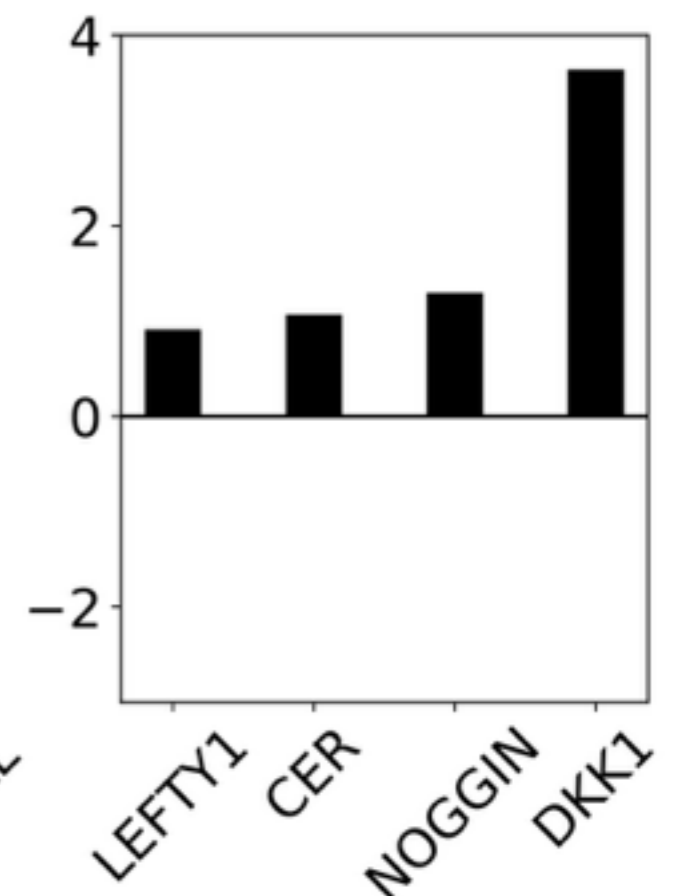
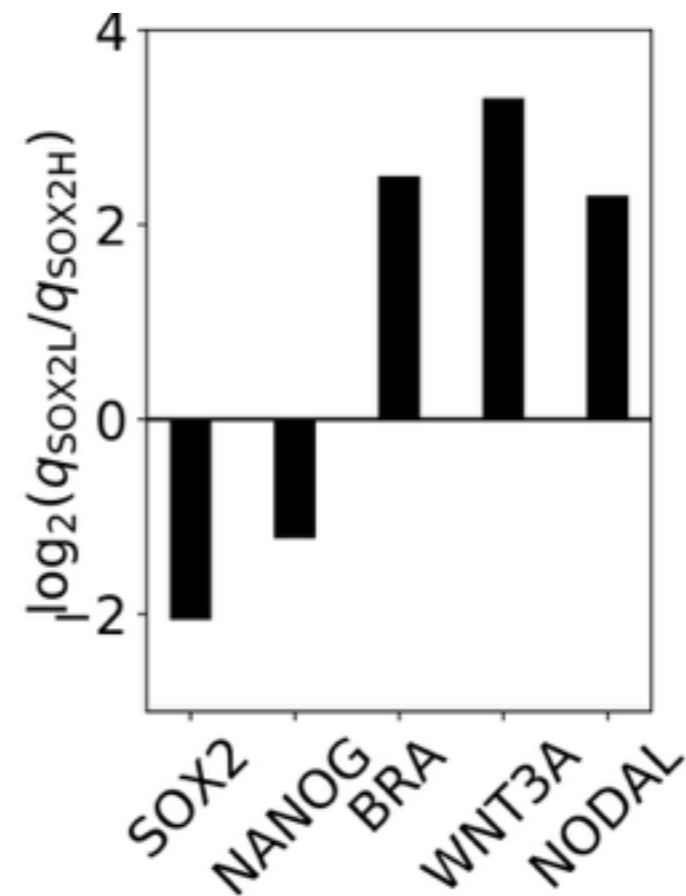
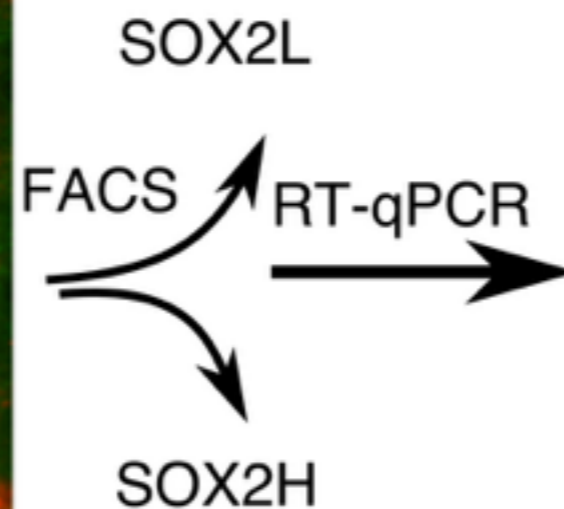
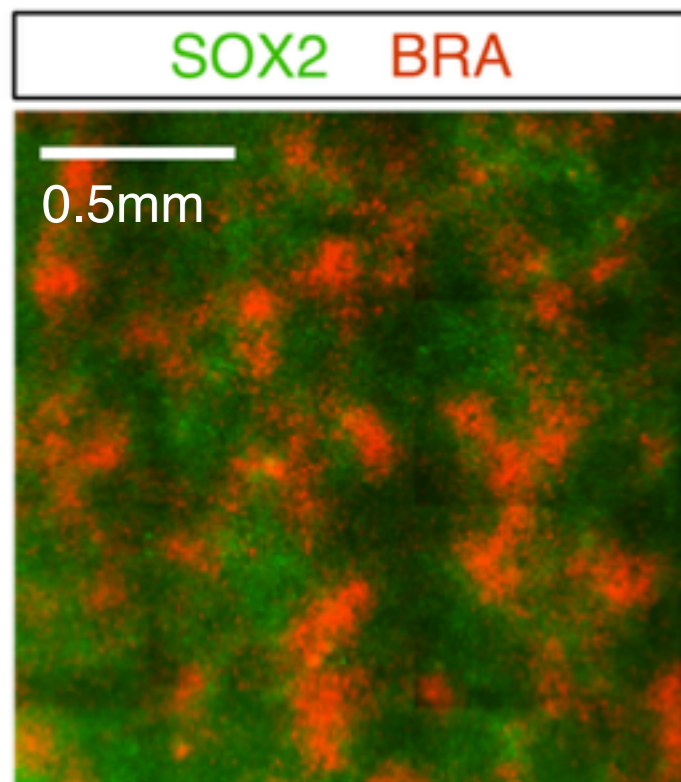


We supply BMP4 to cells,
which pathway responsible for
symmetry breaking.??

Initial response to BMP4 uniform
(nuclear Smad1 live)

Mechanism of symmetry breaking

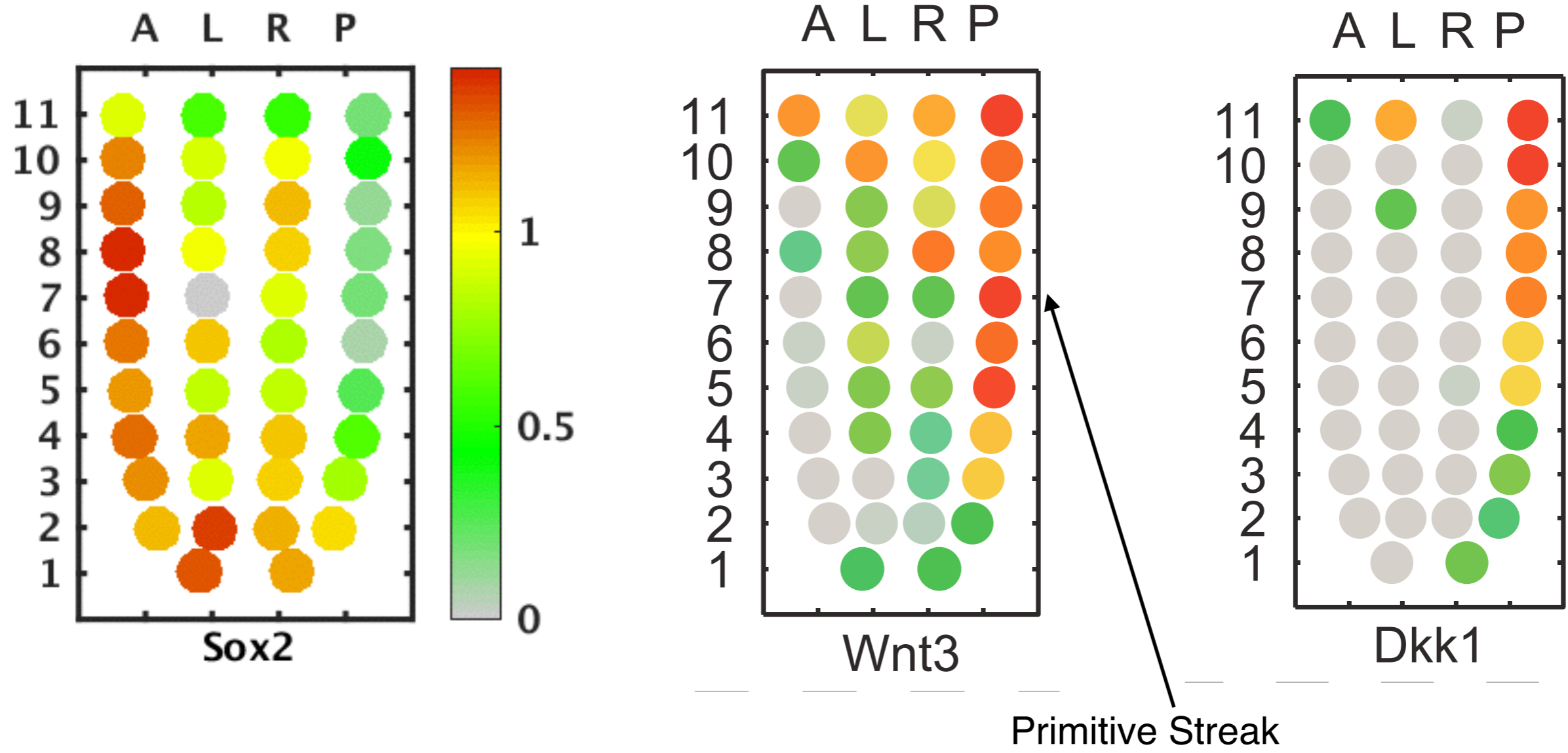
Cells on filters, moderate BMP4 from below, uniform stimulation
Same symmetry breaking as in 3D



NB: DKK1 (Wnt inhibitor) high where WNT is high!

Mouse ss-RNA-seq: Dkk1 high where Wnt3 is hi.

(Anterior, left, right, post.)



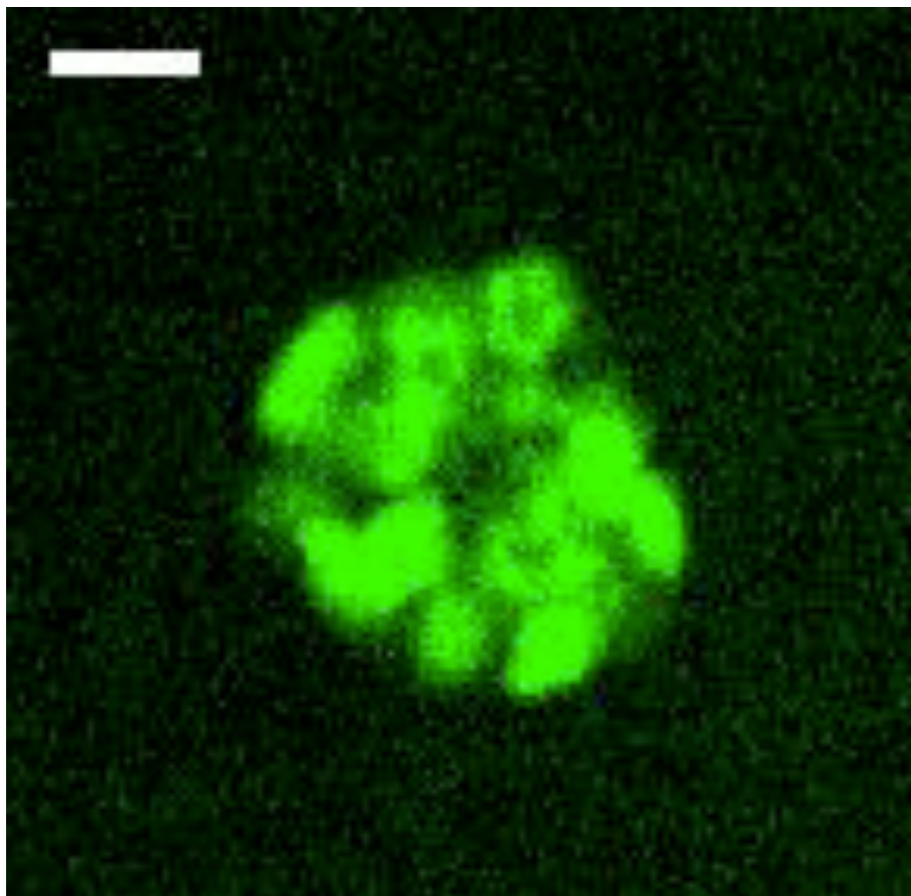
Mechanism of symmetry breaking

WNT required: (BMP4 + IWP2 (\neg WNT secretion) \Rightarrow symmetric)

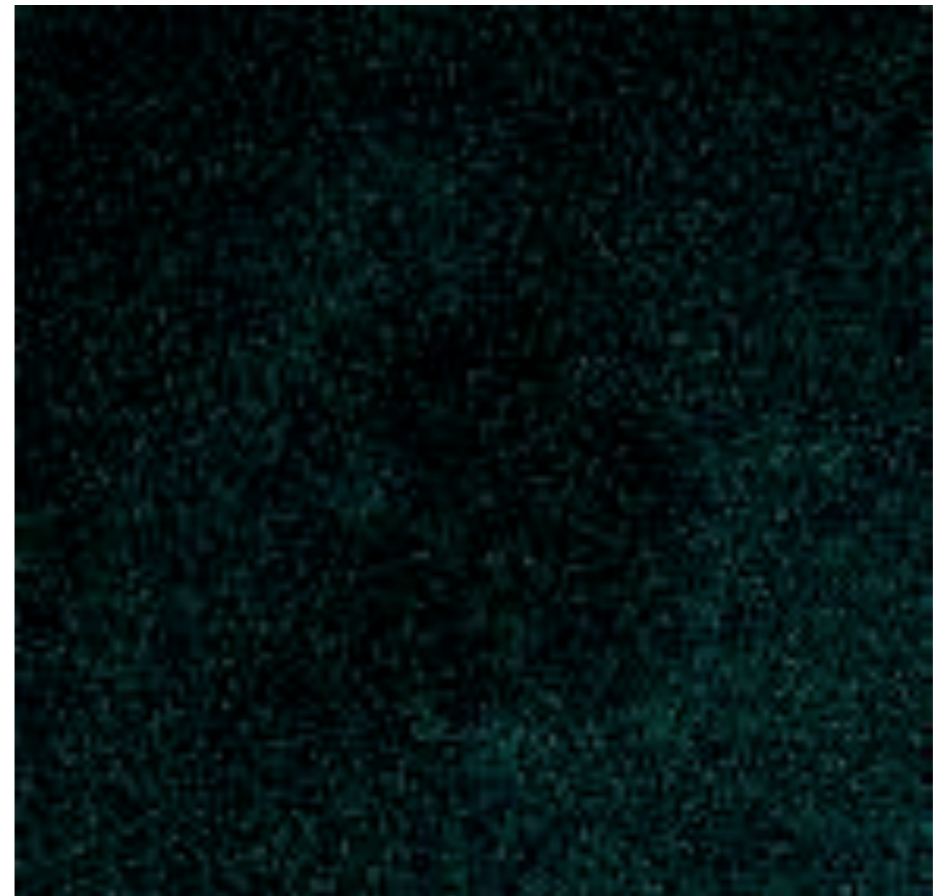
KO inhibitors of BMP & Activin/Nodal pathways: no effect
(NOGGIN $-/-$, CER $-/-$ & LEFTY1 $-/-$)

DKK1 $-/-$ cysts entirely BRA+ & transform early. \rightarrow Turing system

Sox2



Bra



Cysts in 3D gel — conclusions

Symmetric BMP4 induces spontaneous sym breaking: localized gastrulation
(Break down of Col IV, E-CAD—> N-CAD, EMT Sna, T....)

Symmetry breaking a colony property, not spatial sorting of 2 populations

System level signaling feedbacks closed compartment,

Life is all down hill: a theorem

With David Rand, Francis Corson, Archishman Raju

Waddington landscape == Morse-Smale system

(Ignore periodic orbits.. technical) M-S \rightarrow run dynamics forward and backward in time and all points tend to finite set of non degenerate critical points: sources, saddles (# stable, unstable directions), sinks + technical stuff

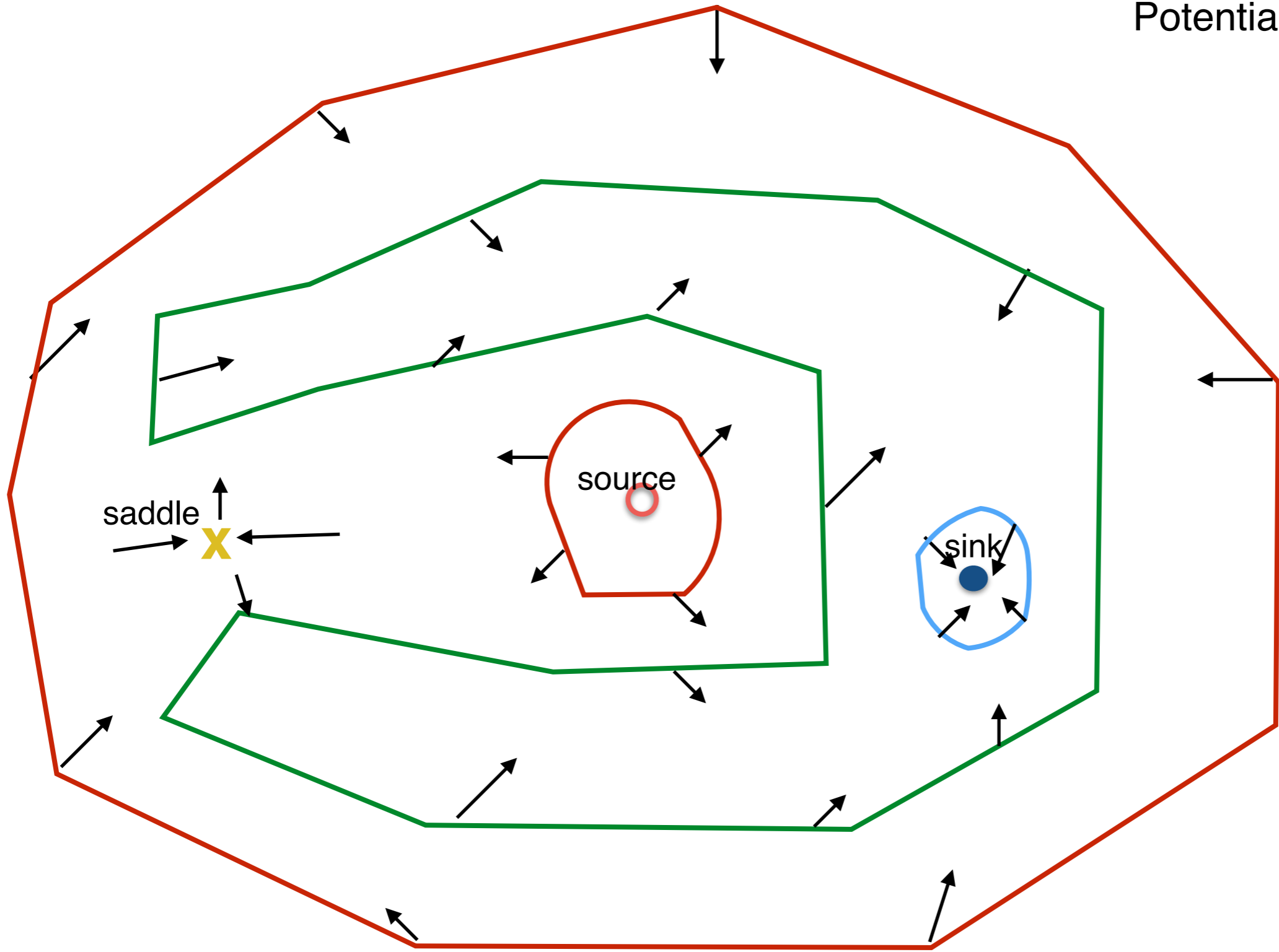
Morphogens 'tilt' landscape, cells 'make decisions'

Impose by hand:

- competence - at given time cells see signal
- commitment - at given time cells are stuck at fixed points (and can't tilt out)

Flows become landscapes

Potential ↓ ● ● ● ●



the glove!

Is this useful?

vector field $v_i = -g_{ij} \text{Grad}_j \text{Potential}$

Critical points & signature \longleftrightarrow potential + linearization

Connections \sim metric (very redundant)

Poincare conjecture and evolution

Any closed N -dim manifold with topology of a sphere (loops \rightarrow trivial) can be smoothly deformed into a sphere.

$D \geq 5$ Smale 1961

$D = 4$ Freedman 1982

$D = 3$ Perelman 2002

Machinery of Smale proof + Morse \rightarrow

$D \geq 6$ the deformation of manifold into sphere can be done by inverse saddle-node bifurcations (Rand).

Thus all Morse-Smale dynamics can be realized by saddle-node bifurcations!

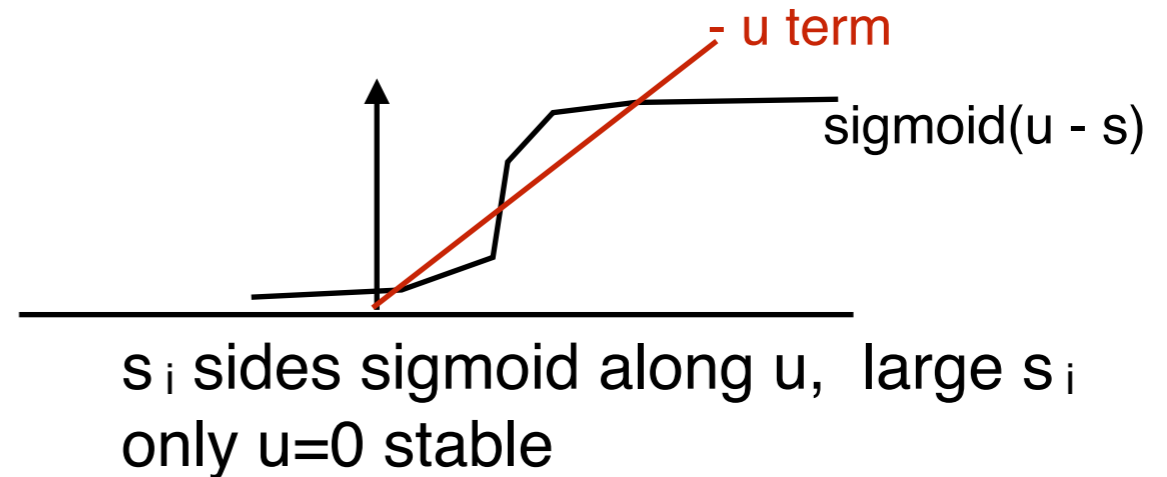
$D < 6$??

Is any of this useful?

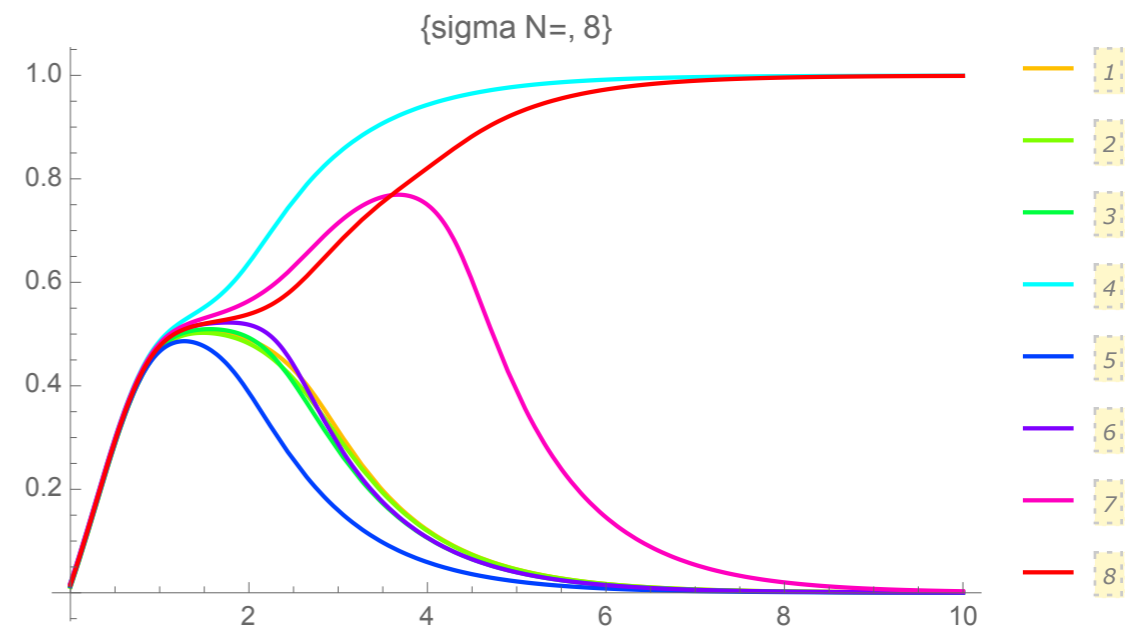
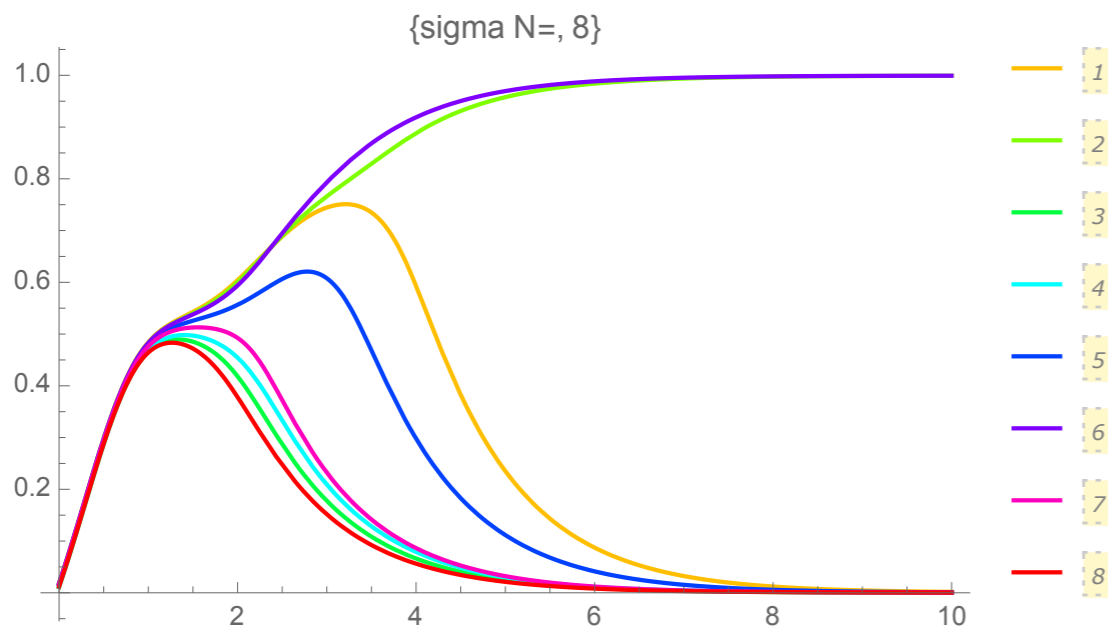
Corson et al Science 2017: Sparse hexagonal pattern of bristles on fly notum due to intermediate range N-DI signaling.

$$\dot{u}_i = \sigma(u_i - s_i) - u_i$$

$$s_i = \sum_{j \neq i} e^{-(i-j)^2} f_n(u_j)$$



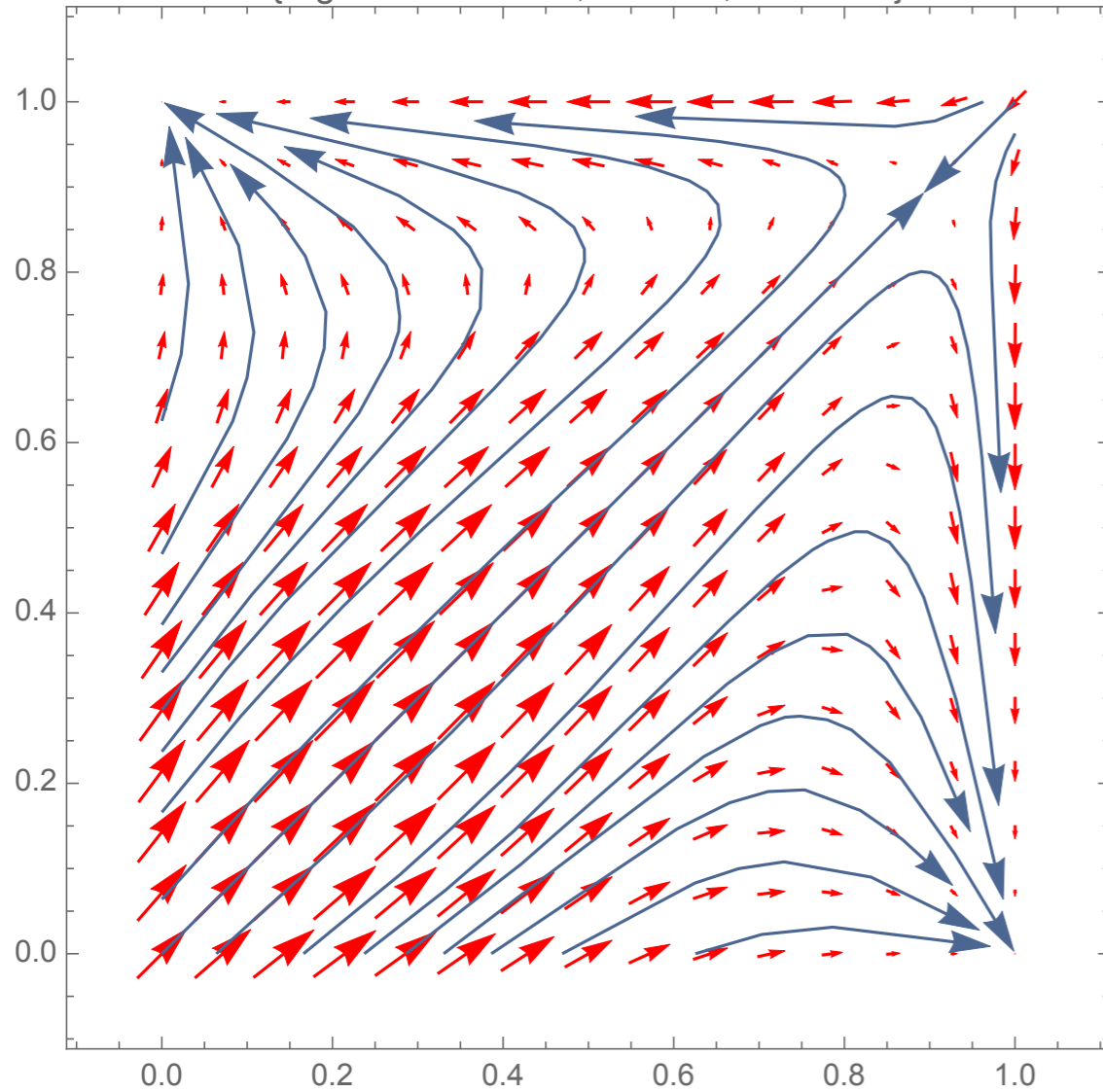
8 cells on ring, start at 0 + noise, only ~opposite cells on ring are stable ~1



Geometry of flows

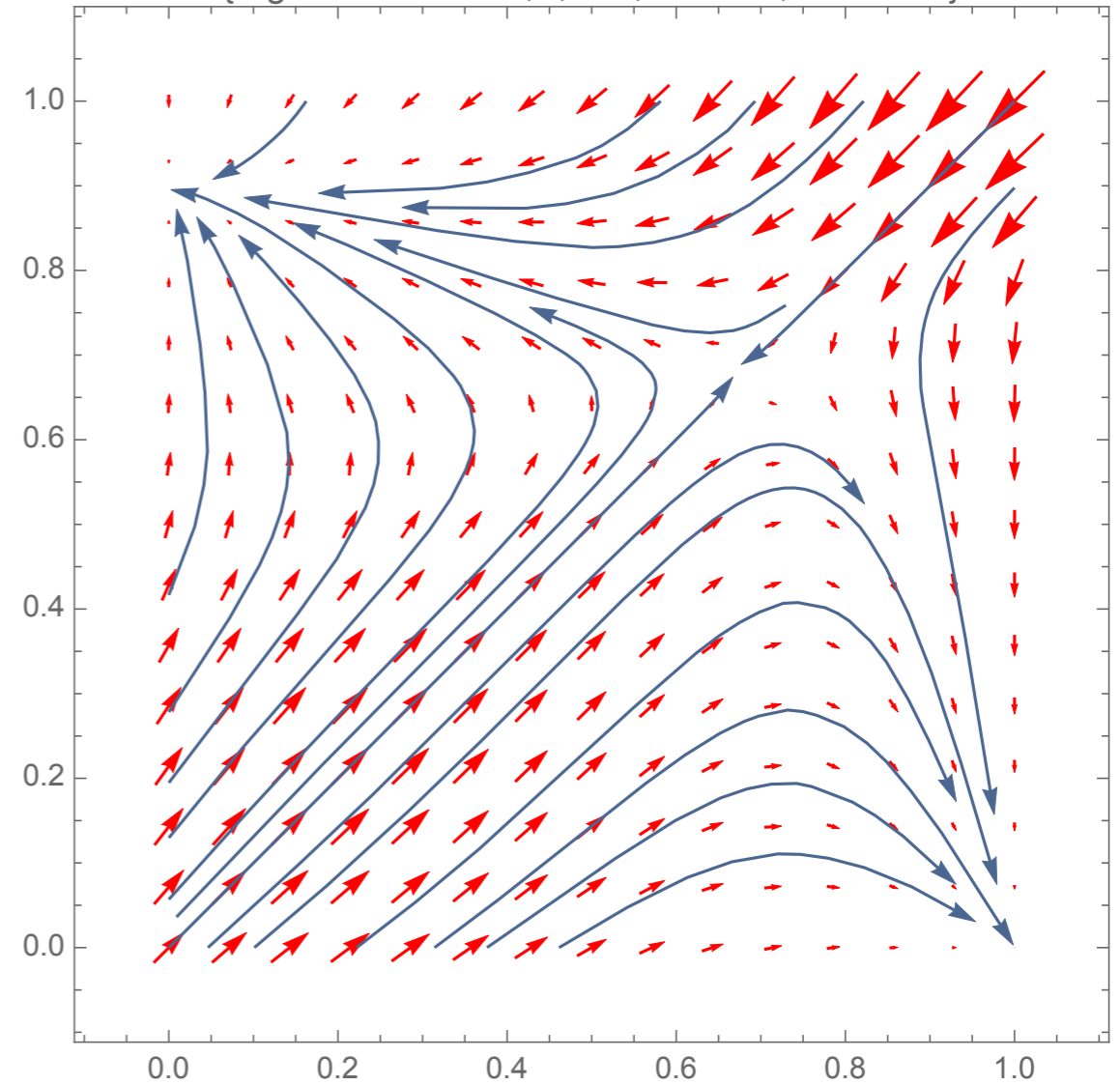
2 cells;

{Sigma model N=2, saddle=, 0.892865}



3 cells: $u_1 == u_2$, u_3

{Sigma model N=3, X,Z=Y, saddle=, 0.677357}

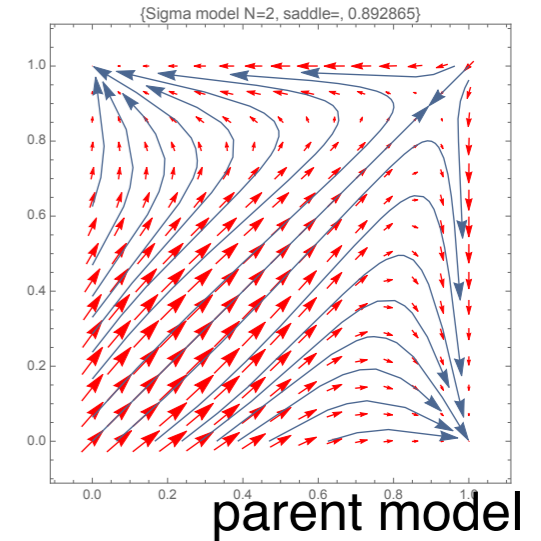


Saddle on diagonal: 1 stable, N-1 unstable directions
 $u_3 = 0$, saddle: 2 stable, 1 (N-2) directions.

Potential model

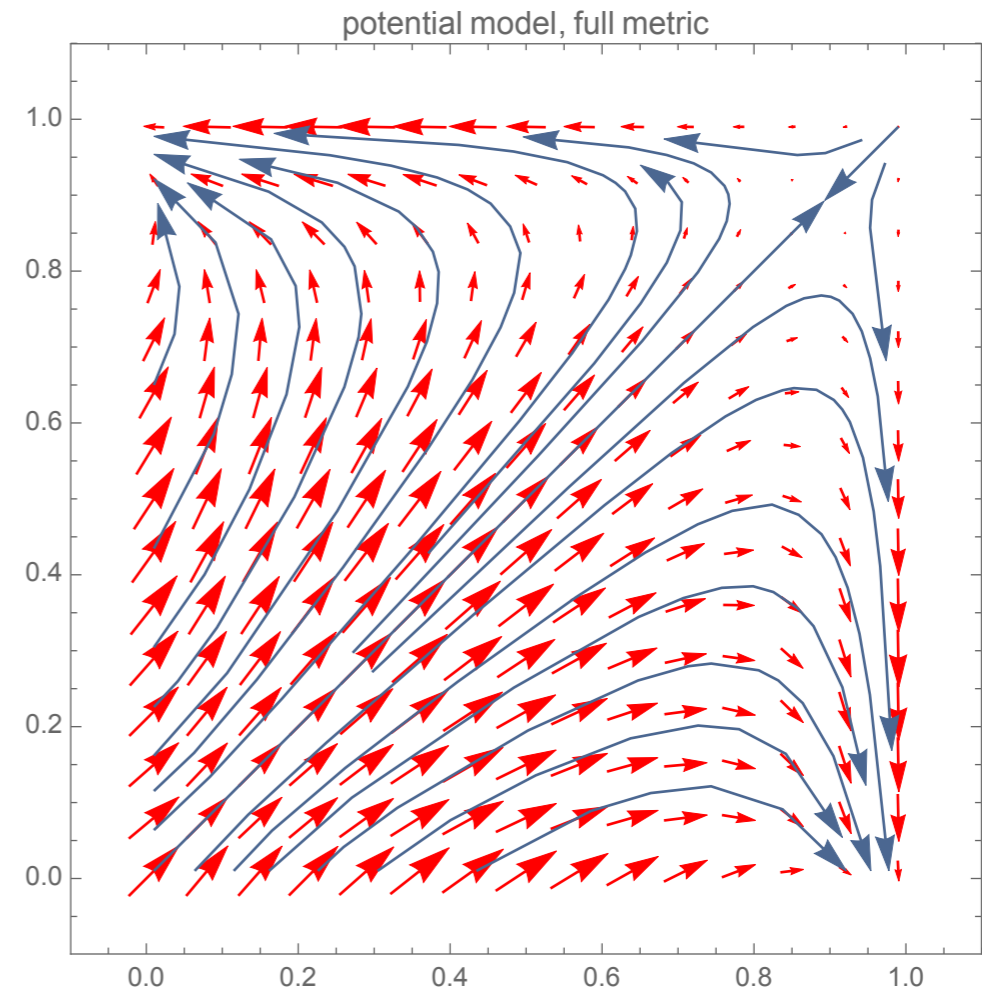
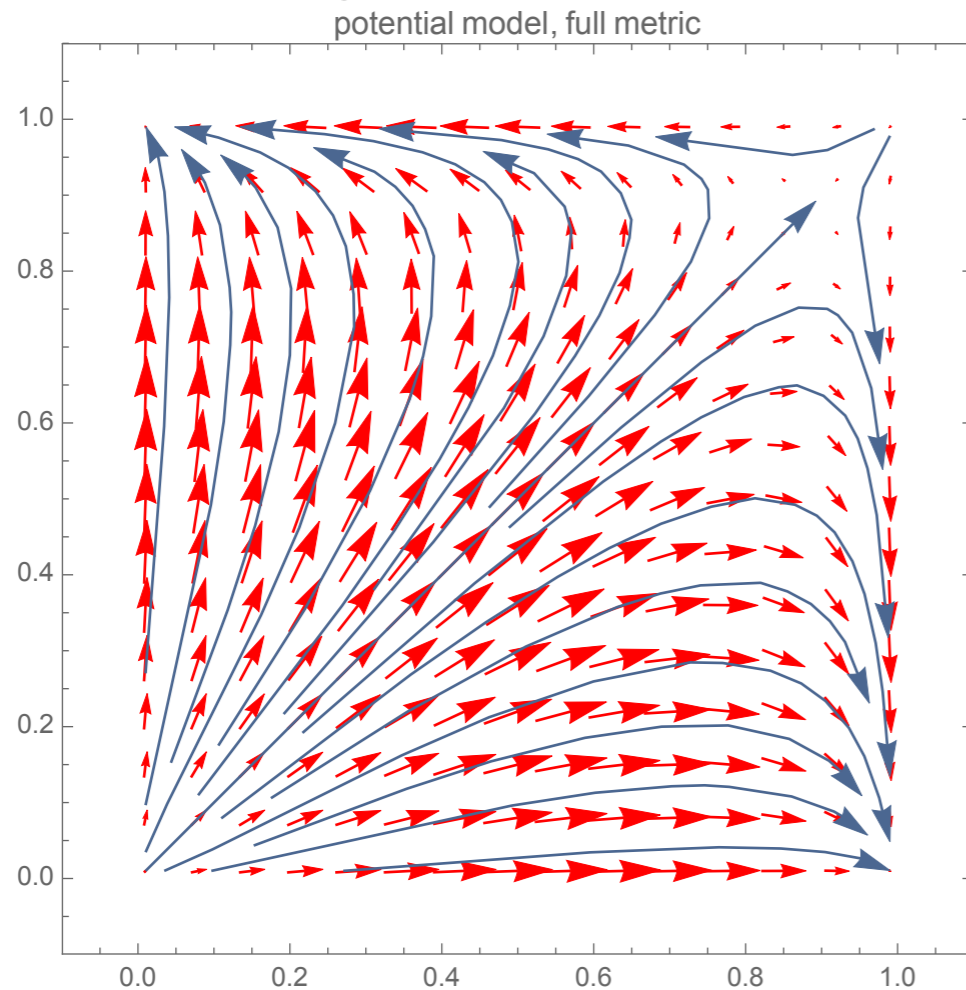
$$V = \sum_i h(u_i) + \frac{1}{2} \sum_{i \neq j} e^{-(i-j)^2} f_n(u_i) f_n(u_j)$$

$$\dot{u}_i = -g_{i,i}(u_i) \nabla_i V$$

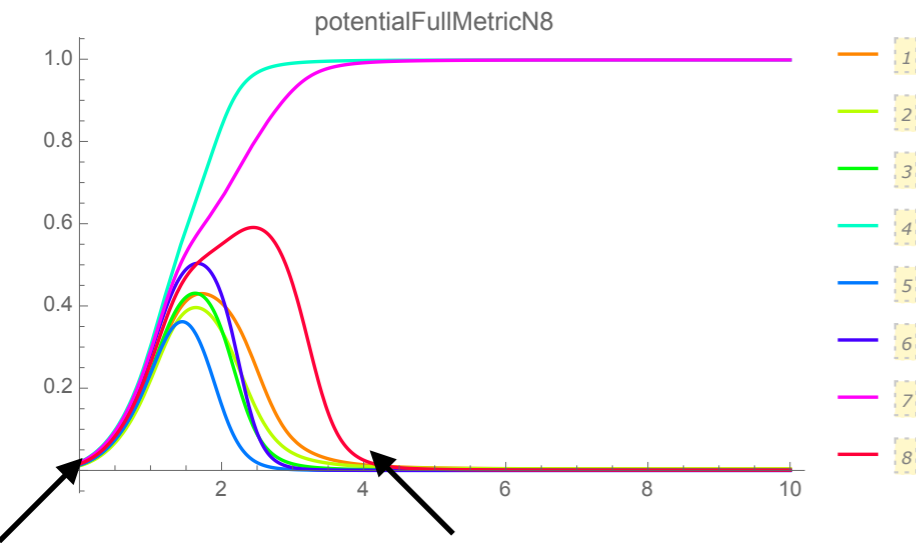


Fit g, h such that saddle on diagonal and Jacobian agree with exact model

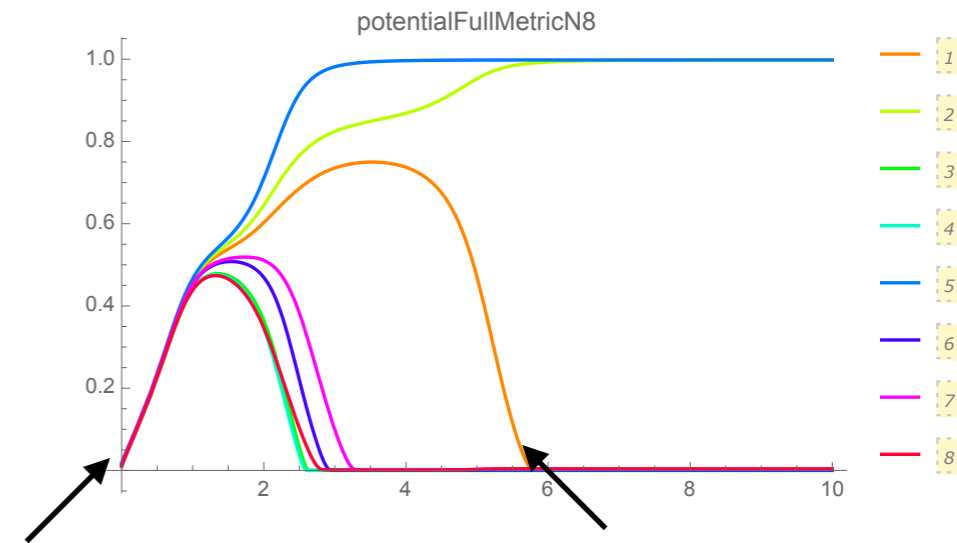
Correct metric around the origin: problems around $[0, 1]$



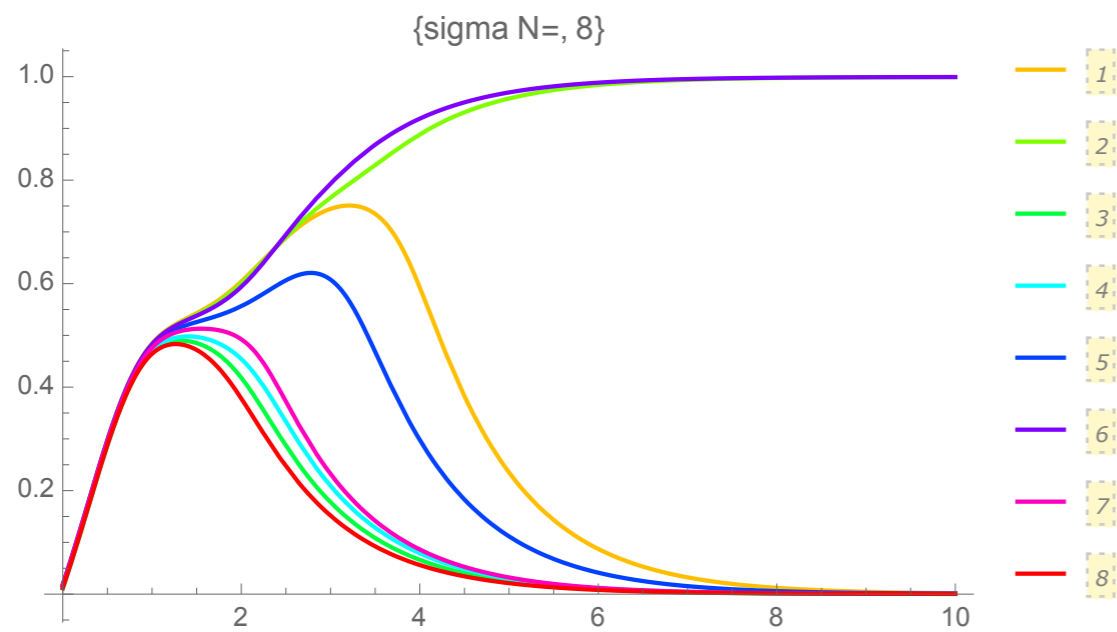
Potential model off, but good enough for the data



fix potential at origin
mess up flows at [0,1]



'Reality'



Why do synthetic embryology?

Analogy with mammalian cell culture ~50's (Eagle, Dulbecco, Puck

Unpack interactions in embryo:

Potential of epiblast alone to pattern, (vs + ExEmbryonic in embryo...

Quant assays for signaling and self-organization

Implications for embryo: receptors, polarity of signaling, cell biology
(Reagents: labeled Smads 1,2,4 β -Cat , 10's KO lines....)

Human!

Problems:

Extra Embryonic cell lines for human ??

Bypassing the blastocyst? assembly of 2 layer systems

Morphogenesis & mechanics

Embryos

“To anyone with his normal quota of curiosity, developing embryos are perhaps the most intriguing objects that nature has to offer. If you look at one quite simply and without preconceptions what you see is a simple lump of jelly that begins changing in shape and texture, developing new parts, sticking out processes, folding up in some regions and spreading out in others, until it eventually turns into a recognizable small plant or worm or insect...

Nothing else that one can see puts on a performance which is both so apparently simple and spontaneous and yet, when you think about it, so mysterious.”

C.H.Waddington 1966 *Principles of Devel. Differentiation*
(Current Concepts in Bio. Series)