human Embryonic Stem Cells (hESC) recapitulate early embryonic patterning

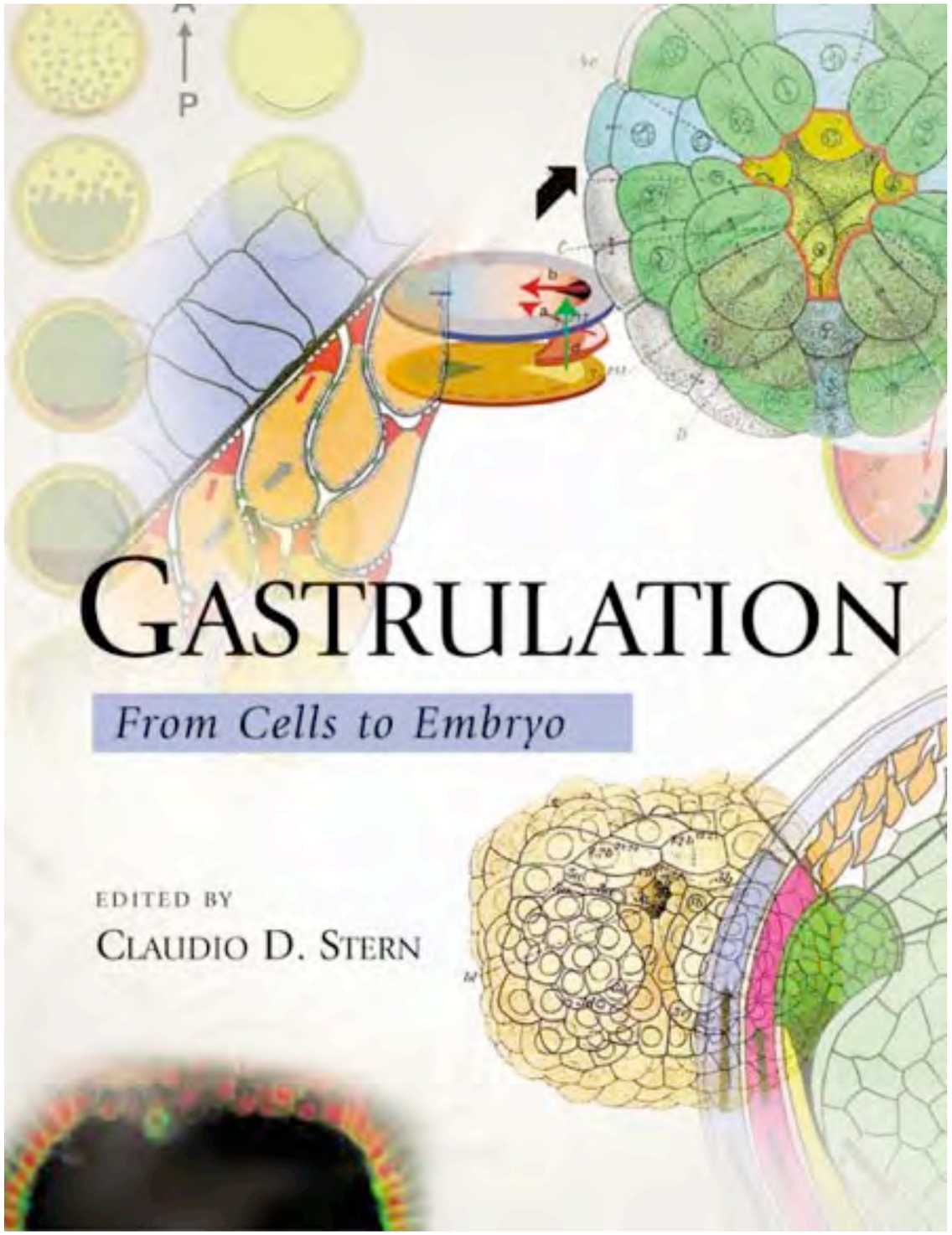
[Aryeh Warmflash (Rice) Benoit Sorre (U. Paris 7)]

Fred Etoc, Christoph Kirst, Iain Martyn, Jakob Metzger, Mijo Simunovic, Anna Yoney

with Ali Brivanlou, Lab Mol. Embryology Rockefeller.

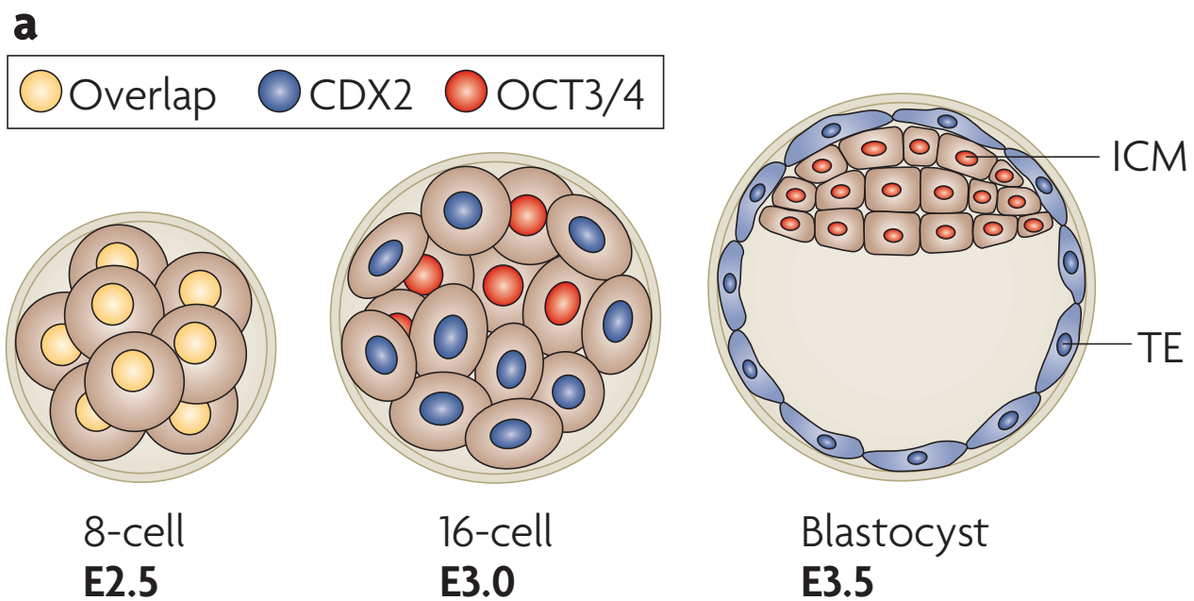
(ref: Warmflash etal *Nature Meth*, 2014)

Human embryology?



Humans in chapter on
“Other Mammals”,
equal billing with ....

Mouse embryo structured



Arnold & Robertson, *Nat Rev Mol Cell Bio*, 2009

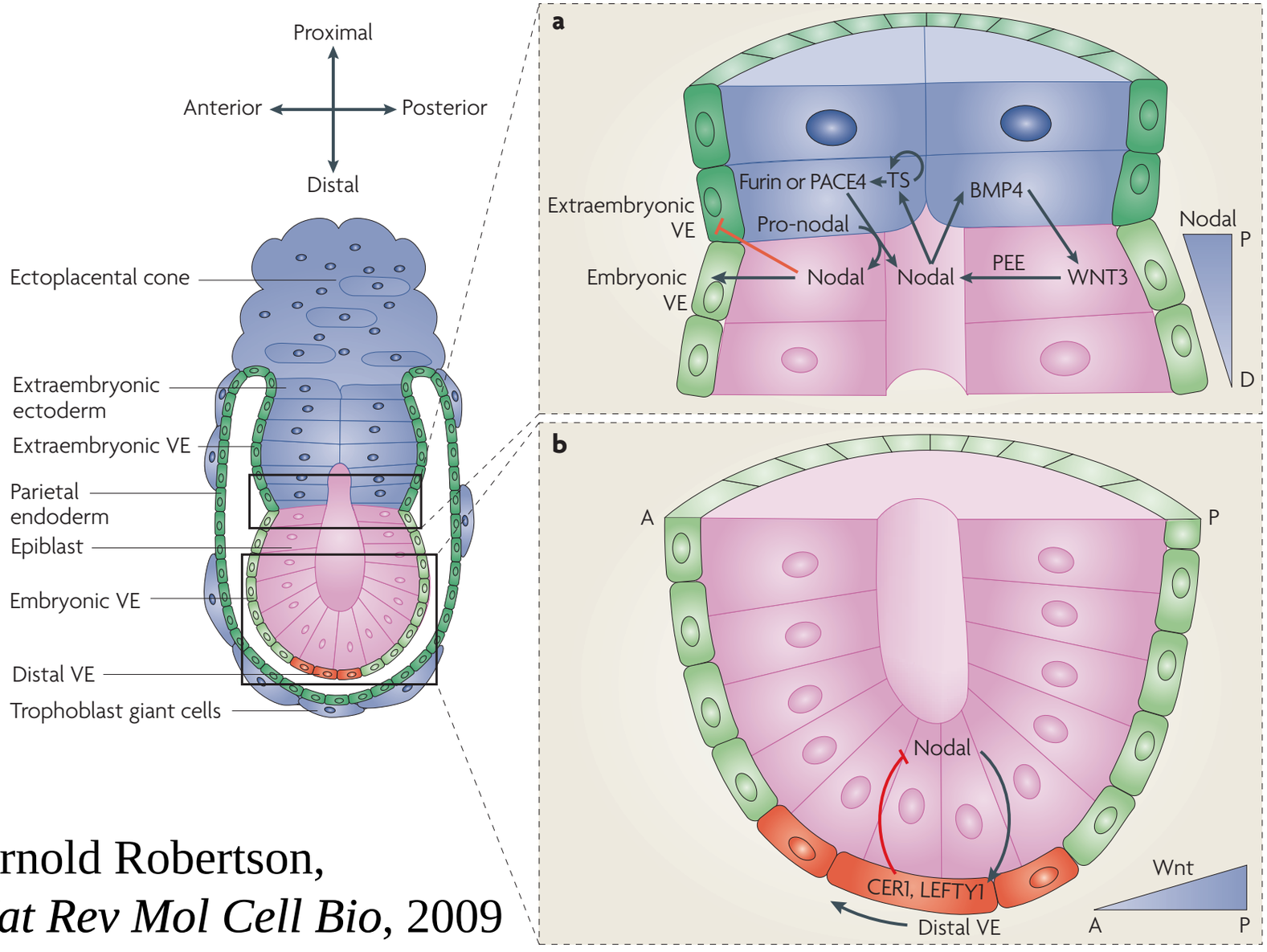
Blastocyst ≠ embryoid body

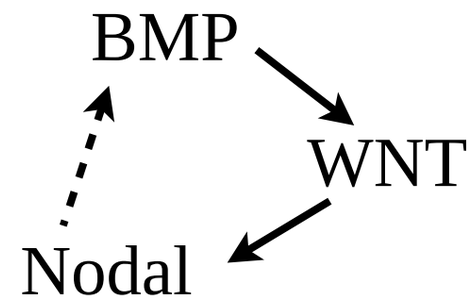
Structure from 8 cell state..

"Understanding the molecular mechanisms of early patterning and cell fate allocation will be of key importance in efforts towards inducing the directed differentiation of (ES)cells in research and medicine. "

Mouse embryo at implantation (hESC ~ epiblast)

E 5.5 Proximal-Distal axis defined, ~ implantation

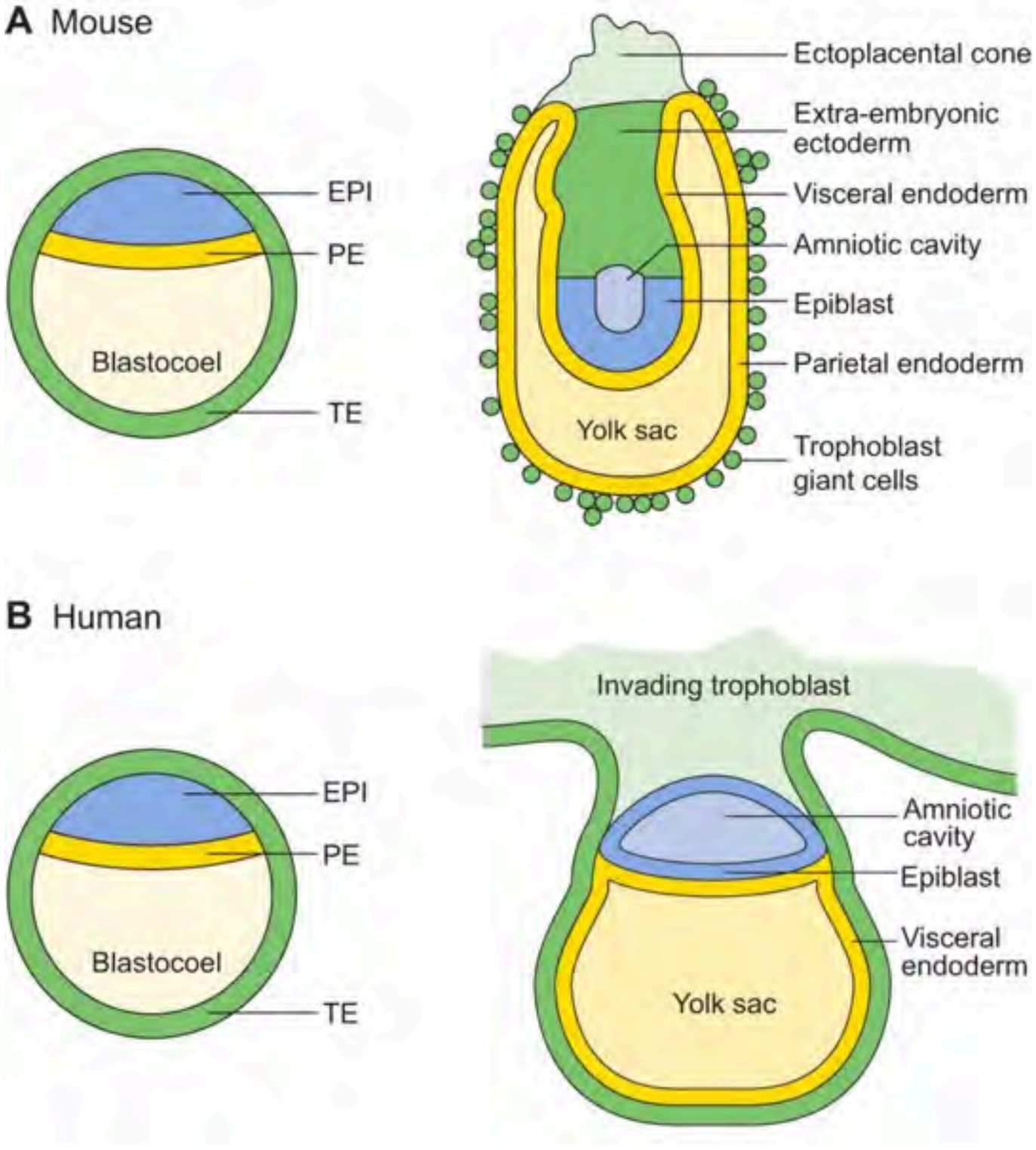




Arnold Robertson,
Nat Rev Mol Cell Bio, 2009

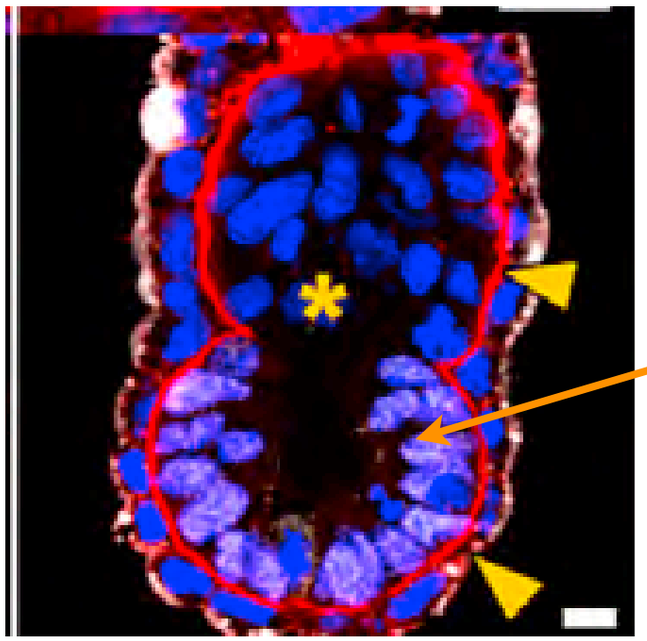
How do signals propagate in the embryo?

Human vs mouse

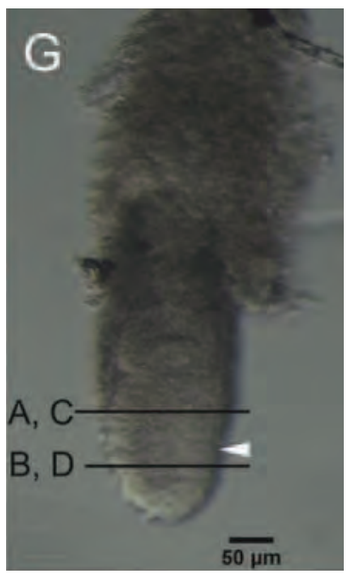


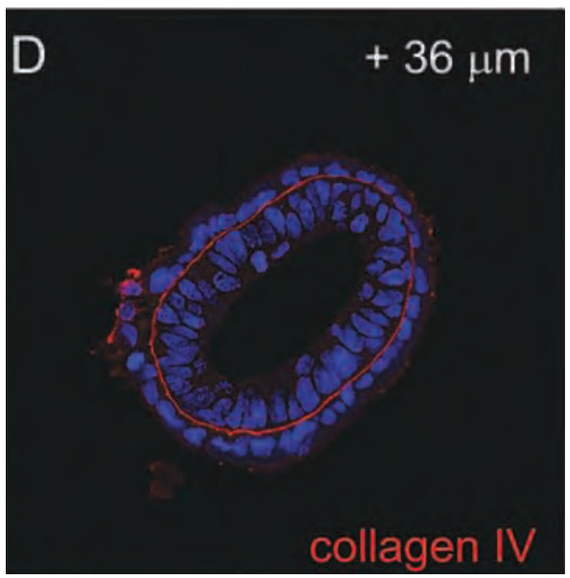
Rossant *Devel* 2015

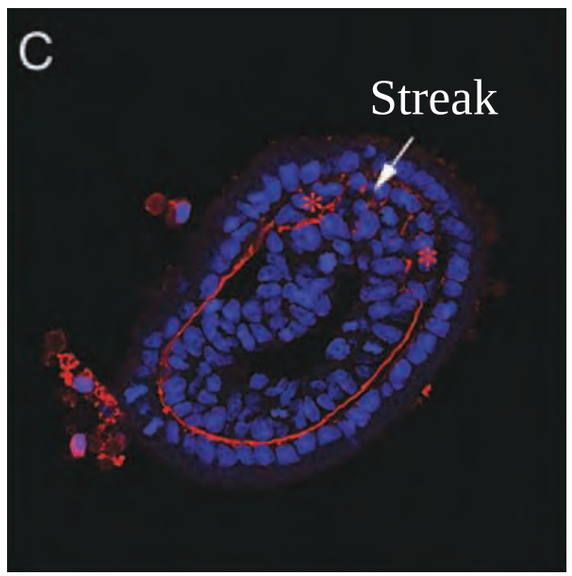
Embryo : Two opposed epithelia



E5.5 Laminin membrane surrounds both ExE & epiblast (Oct4 stain White, DAPI), Bedzhov, *Cell*, 2014





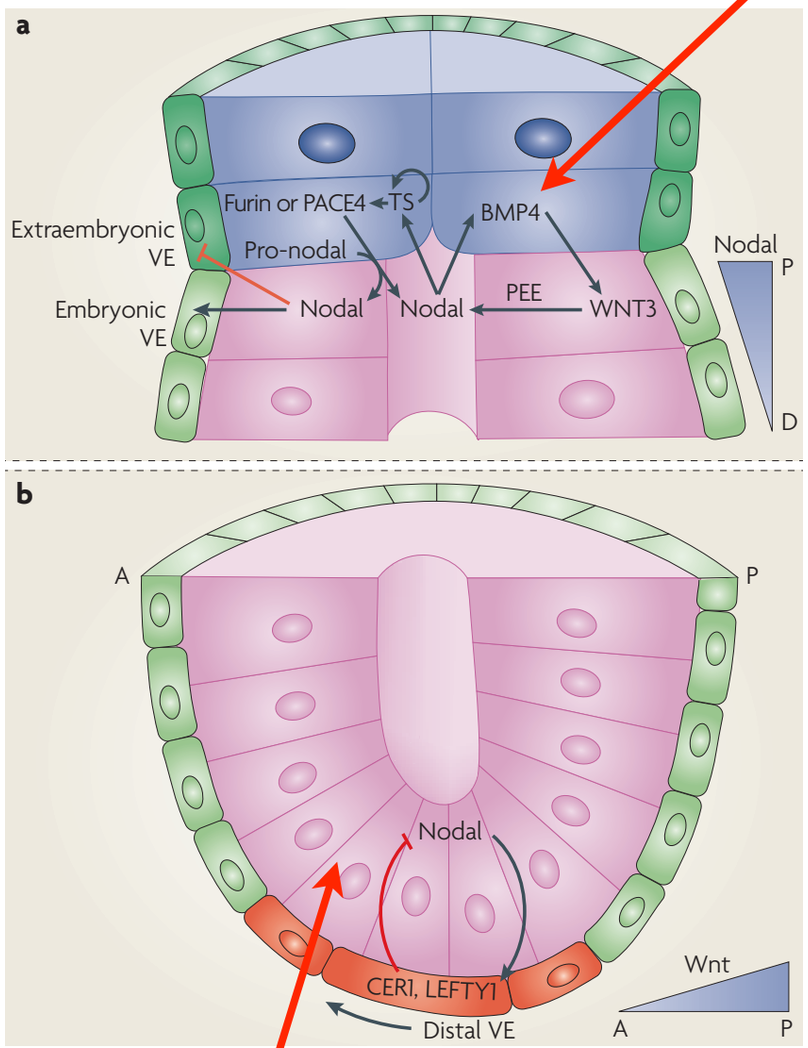


Early streak (E6.5):
laminin ~ Col-IV basal
(PKA apical, E-cad breakdown)
Williams etal *Dev Dyn* 2012

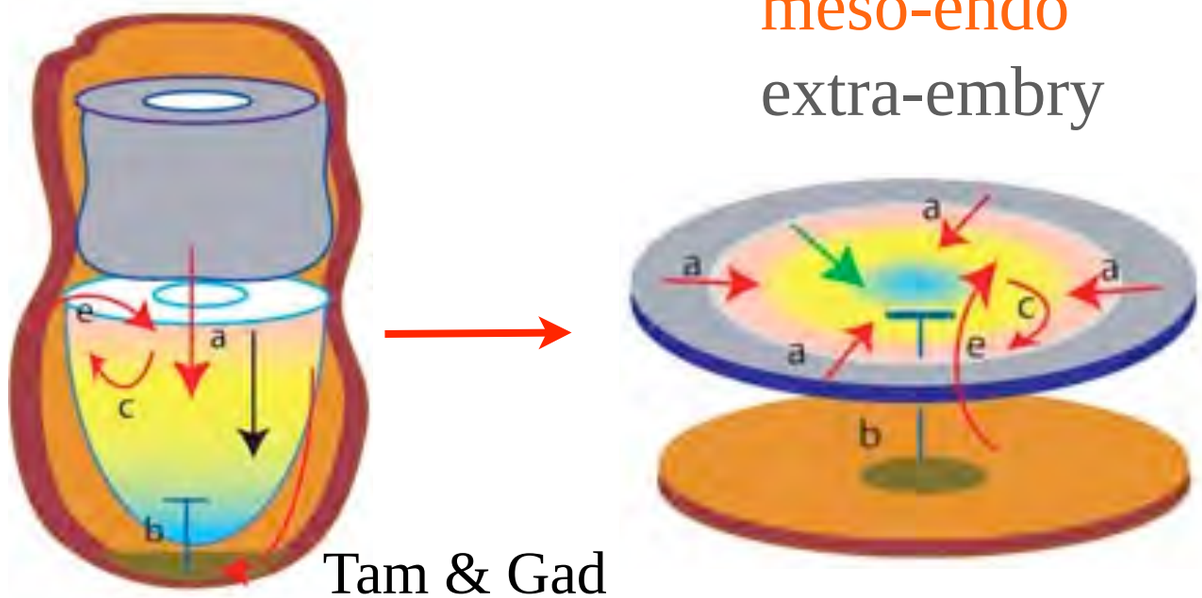
The assay

Arnold & Robertson 2009

BMP4



Epiblast (epithelium)
--> embryo

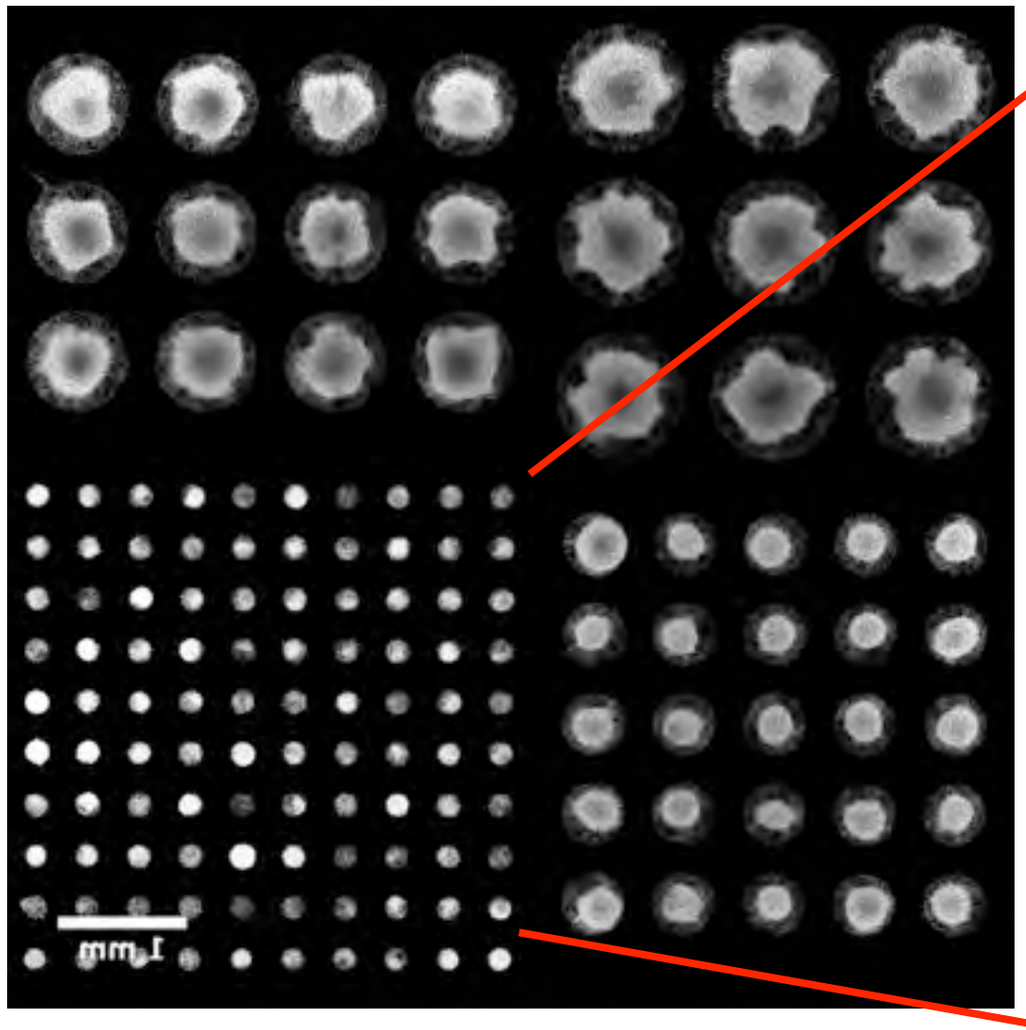


ectoderm
meso-endo
extra-embry

Tam & Gad
Gastrulation 2004

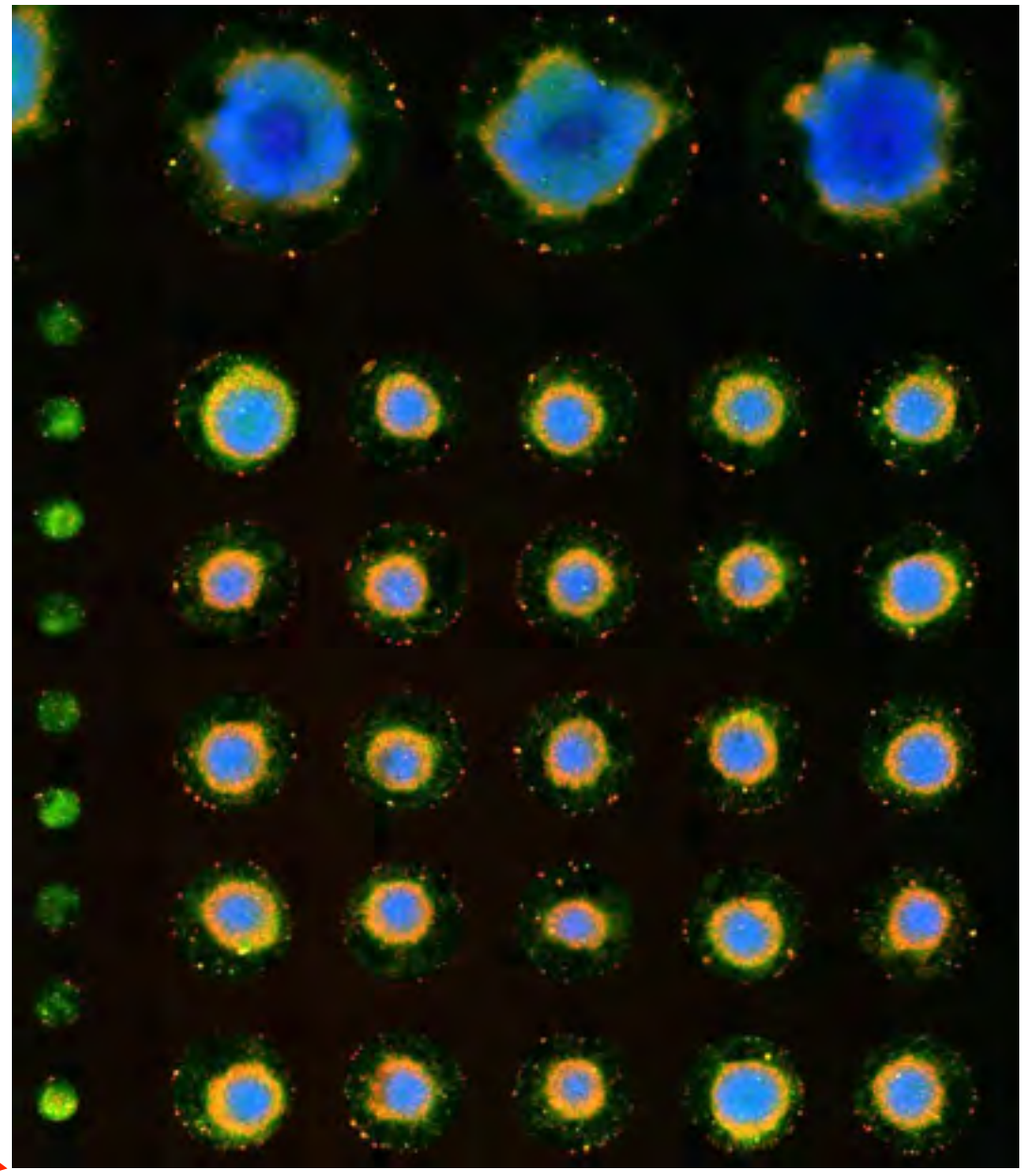
Differentiate hESC with BMP4
A. Warmflash, et al. *Nat. Methods* 2014

Work on micropatterned substrates



DAPI stained hESC
each chip 2x2 this area

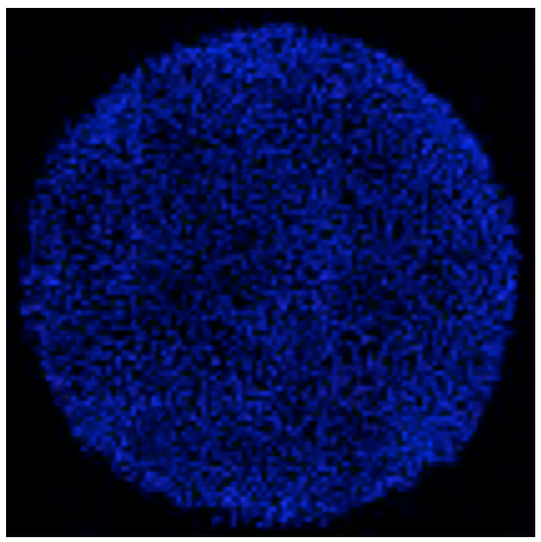
+BMP



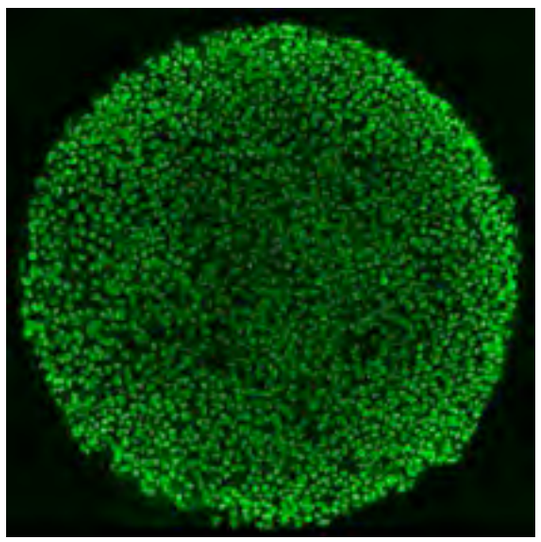
IF: Cdx2, Bra, Sox2
or Movies..

Seed, grow 1 day: pluripotency markers (1mm diam.)

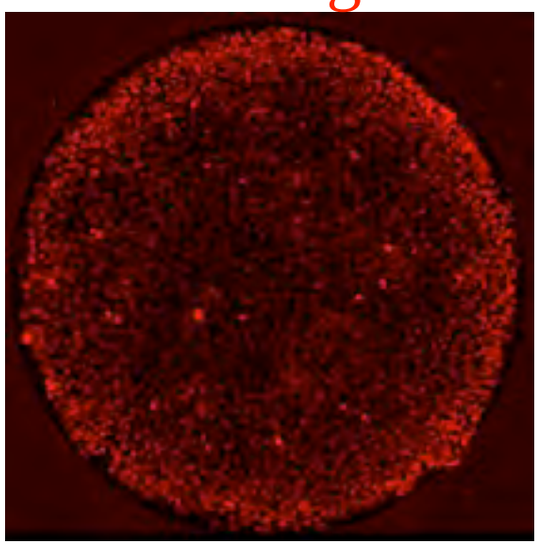
DAPI



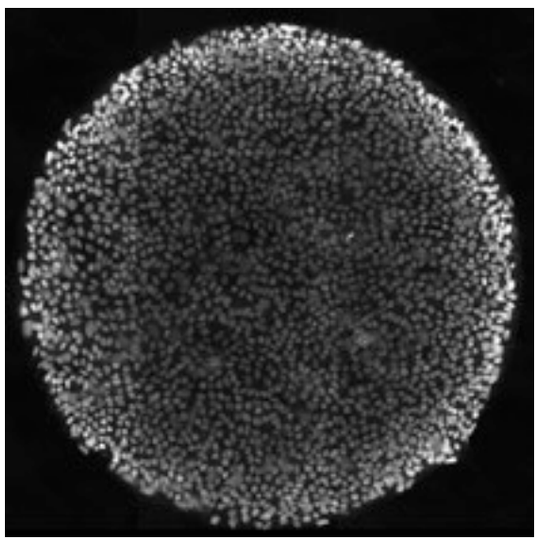
Oct4



Nanog

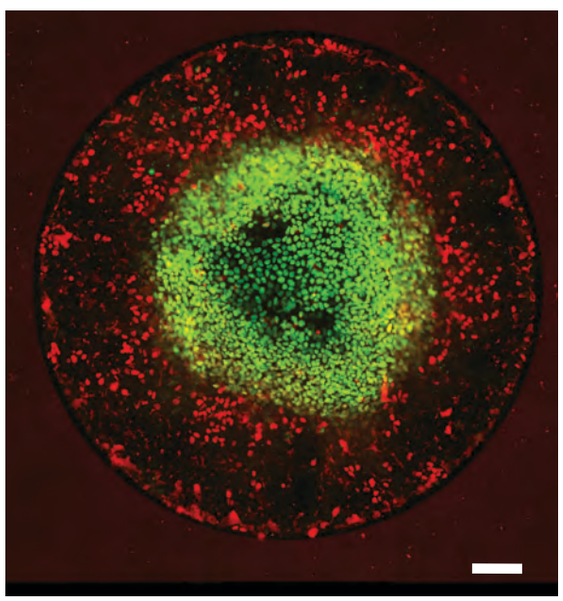


Sox2



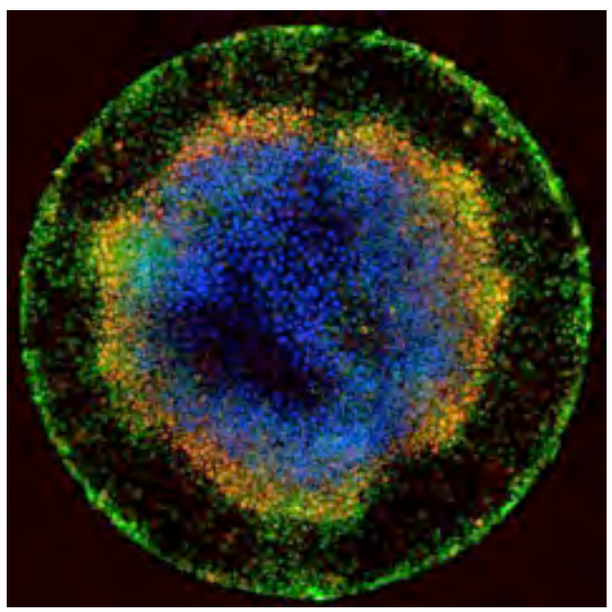
42 hrs BMP4: Germ layers

Nanog/Sox2



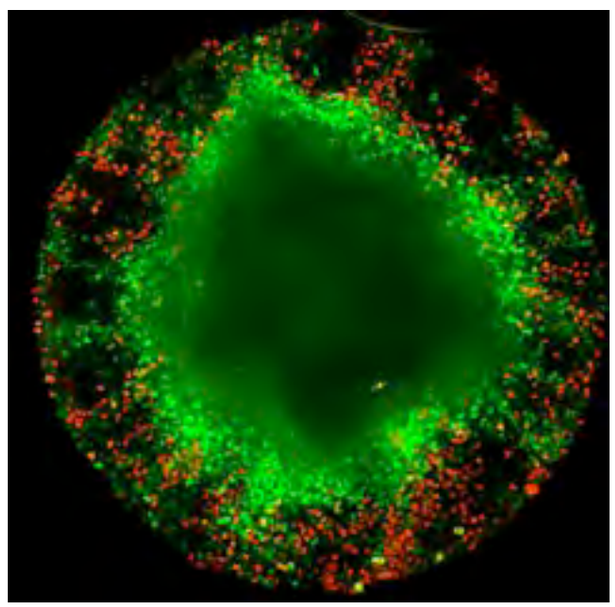
Sox2 --> ectoderm,

Cdx2/ Bra/ Sox2

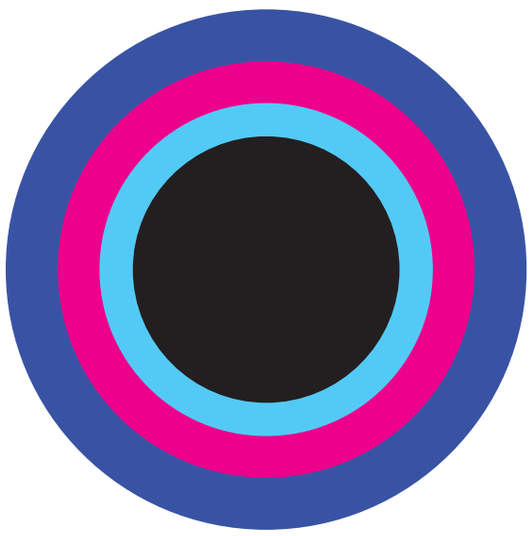


mesoderm, bra~eomes..

Sox17/Eomes



Sox17 --> def. endoderm

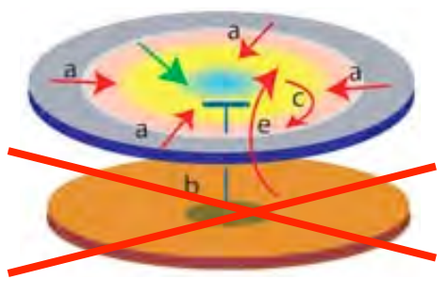


TE-like=CDX2+BRA-SOX17-SOX2-

Endoderm=SOX17+NANOG+SOX2-

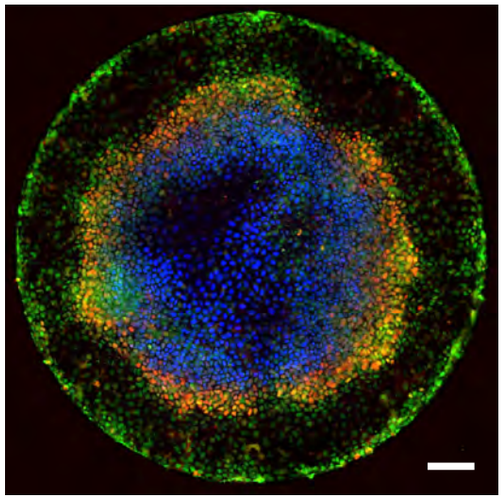
PS/mesoderm=BRA+NANOG+SOX17-SOX2-

Ectoderm=SOX2+NANOG-BRA-SOX17-



Properties:

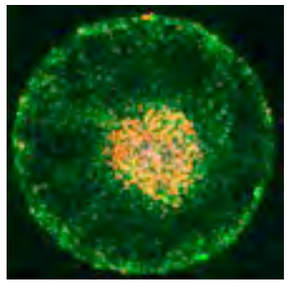
- Colonies define fates by distance from boundary: loose center in smaller colonies

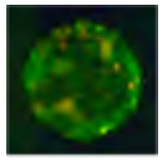


1mm

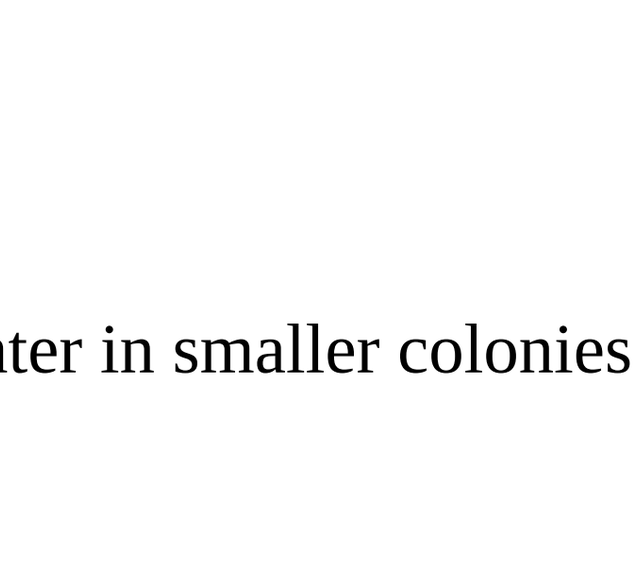
Cdx2/Sox2/Bra

1, 0.5, 0.25 mm colonies





- BMP4 --> [Wnt, Nodal] signaling,...



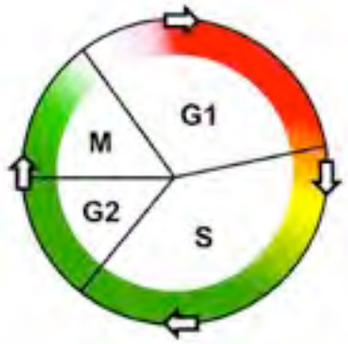
- Primitive streak markers + layering

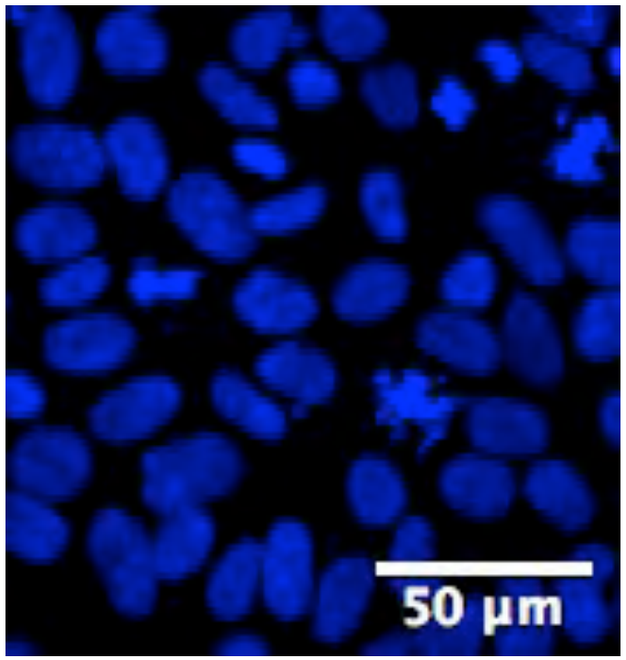
- BMP, Nodal secreted inhibitors, exclude signal from center

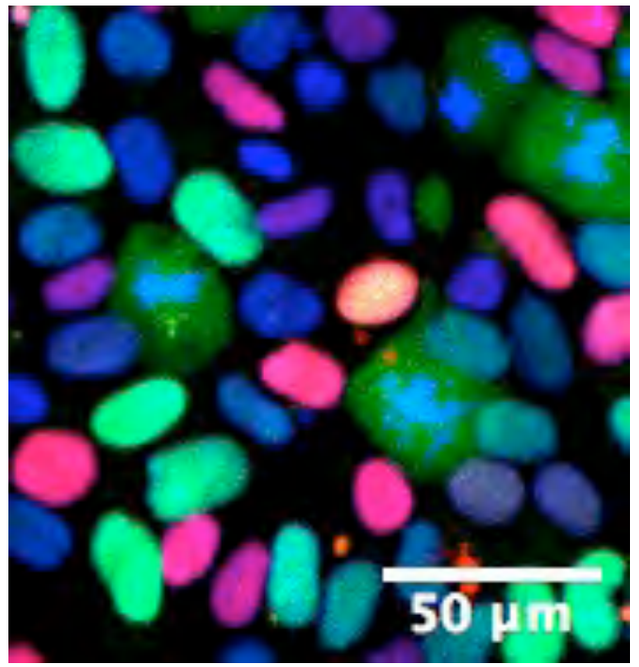
How uniform is cell division on colonies?

Cell cycle uniform over colony

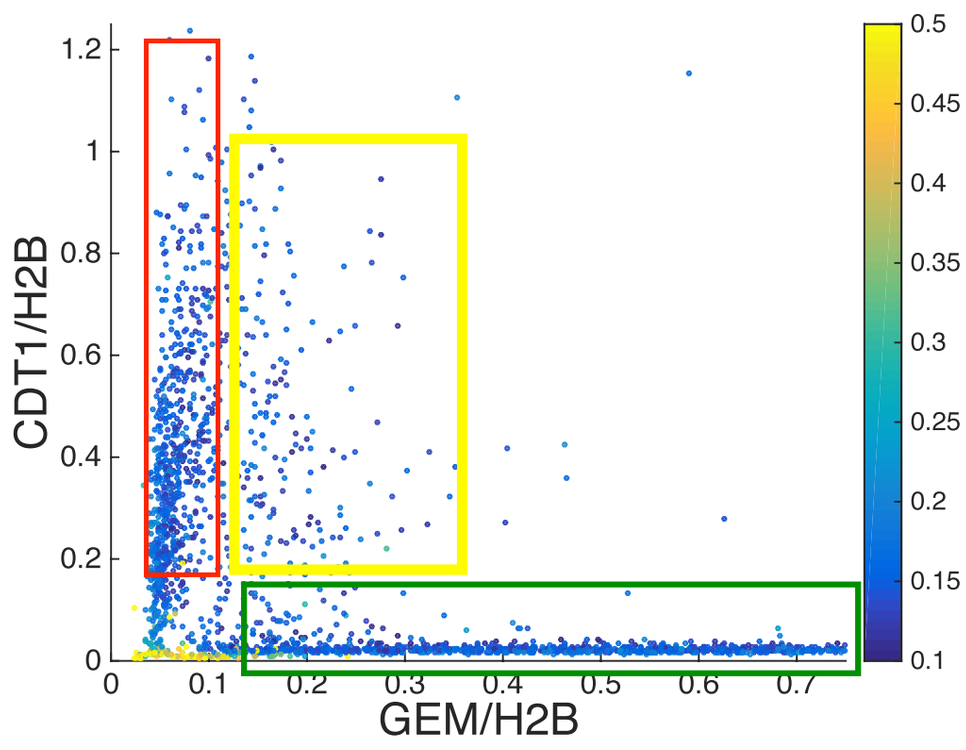
FUCCI stably integrated RUES2



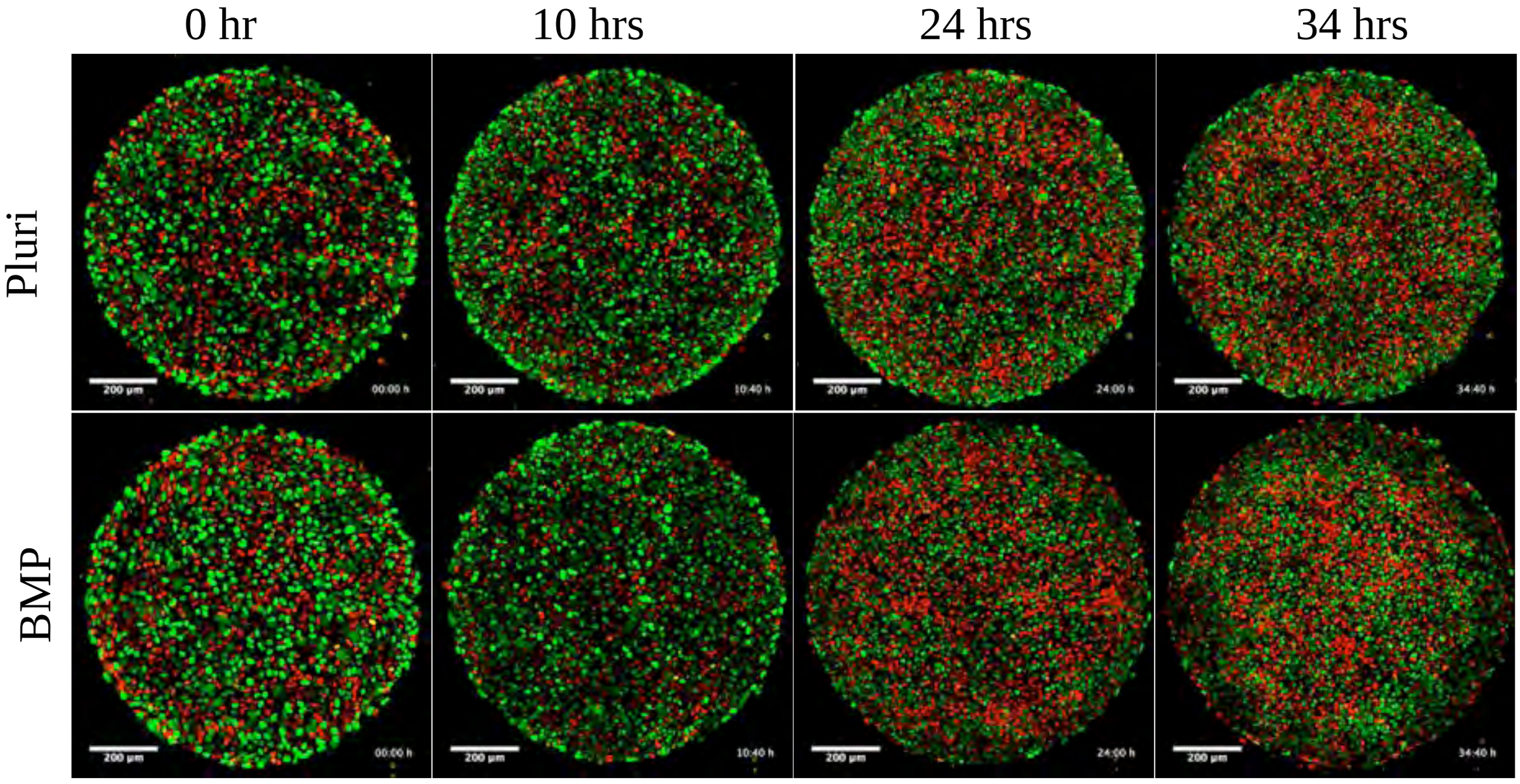




H2B nuclear marker + Gem + Cdt1



Pluri cells grow ~ BMP4

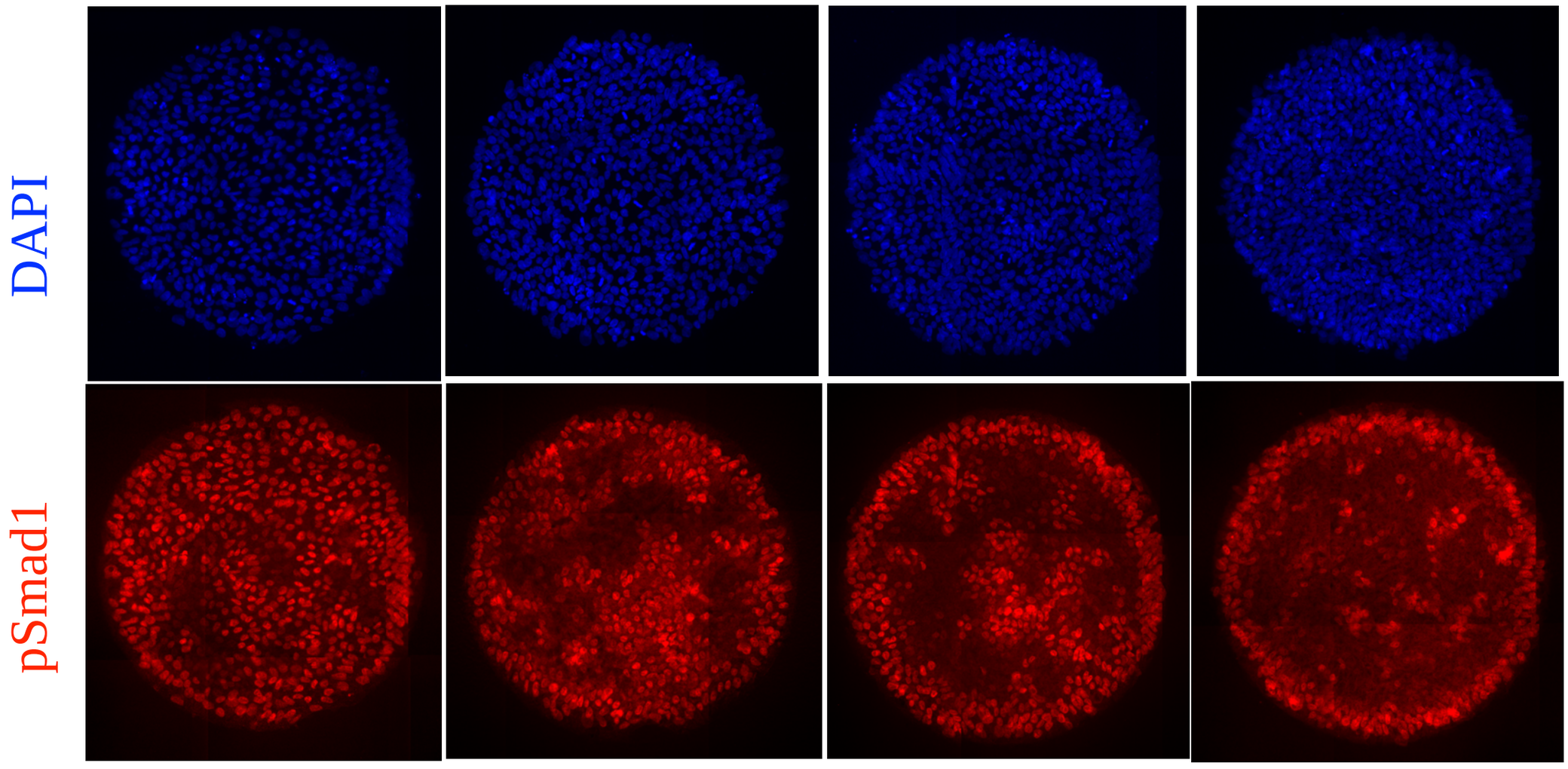


Quantified: proliferation uniform in radius, slows down with density.

Density effects in pluripotent colonies--> prepattern

Immediate (1hr) response to BMP depends on density

#cells:	700	835	925	1162
%Smad1:	90%	73%	64%	20%



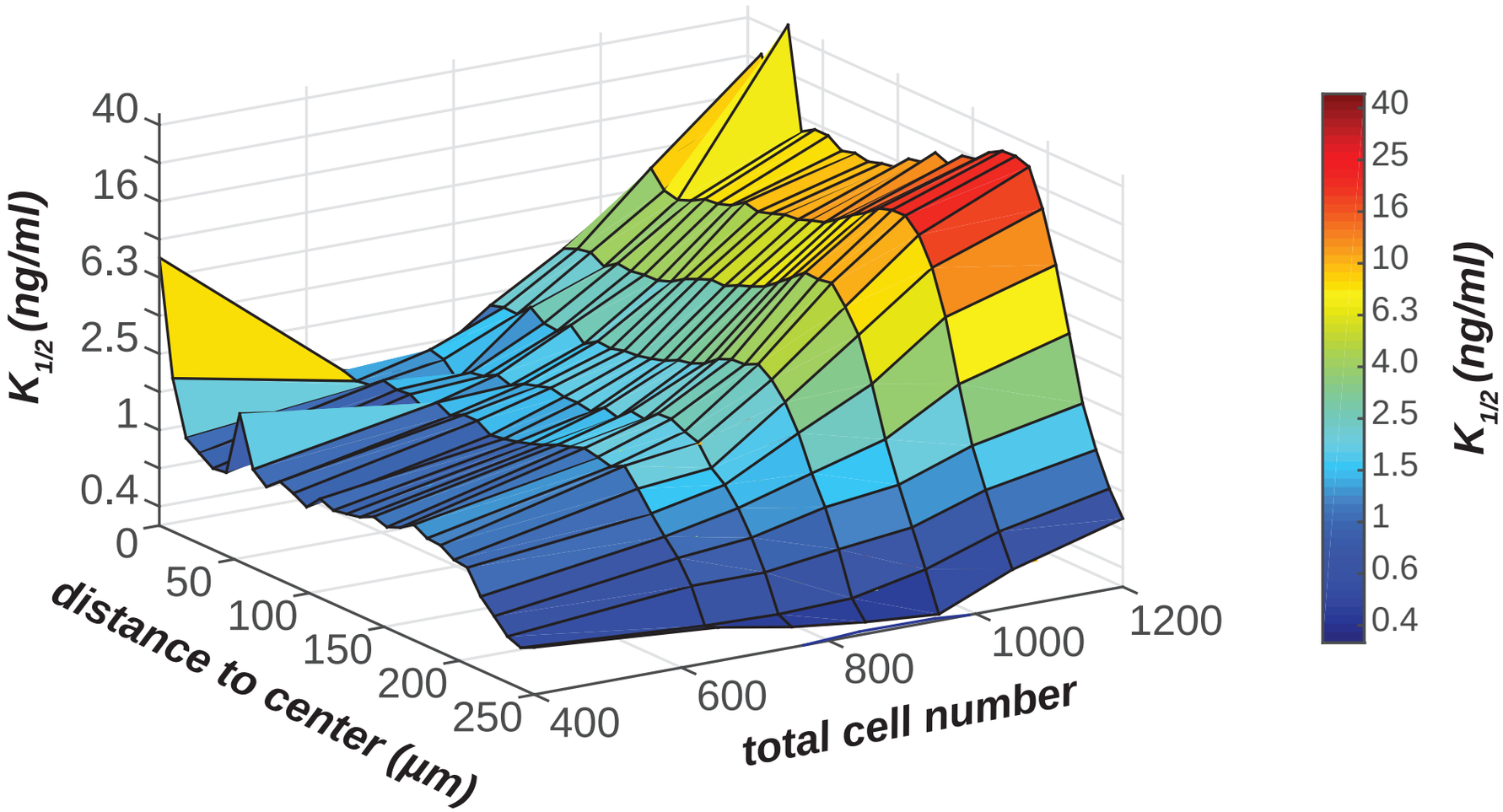
no change with CHX --> immediate early response --> pluri colonies

(Same for Activin / Smad2)

Smad1(radius, BMP, cell density)

$$[\text{pSmad1}] \sim \frac{[\text{BMP}]}{K(R,N) + [\text{BMP}]}$$

Edge always sensitive K small
High density:
colony center K large



Edge Sensitivity: Signal transduction in epithelium?

deplete Ca: uniform response all densities

Where are the receptors? (Nallet-Staub *Dev Cell* 2015)

RNA-seq on microcolony cells (A. Ruzo)

DOX induce tagged... BMPr1a, BMPr2, Actr1b, Actr2b

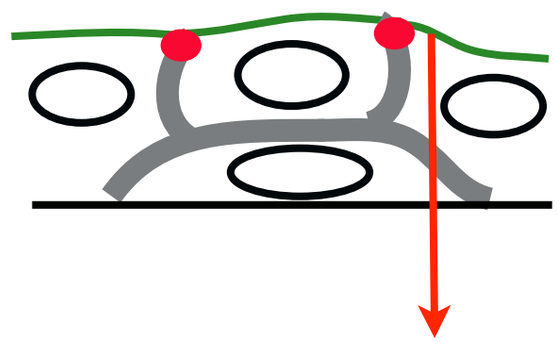
(Minimal involvement of Hippo in linking density --> signaling)

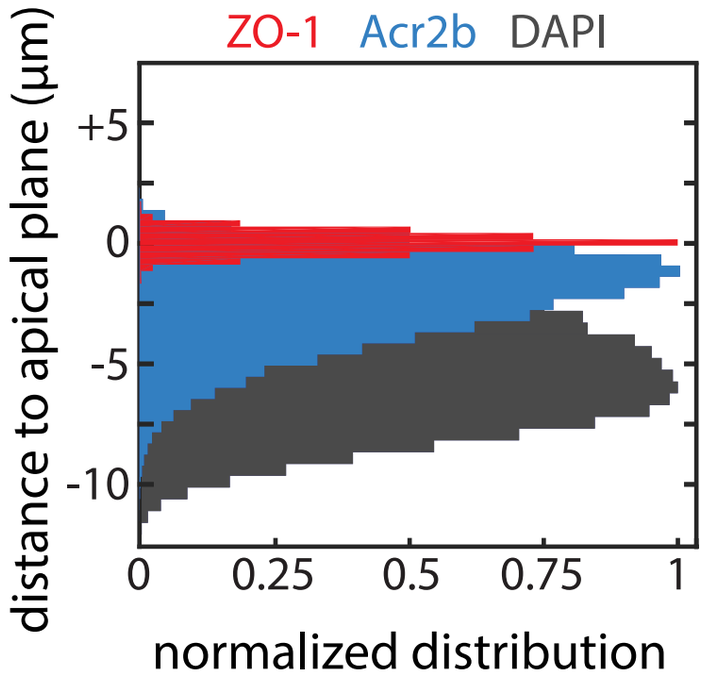
Where are the receptors?

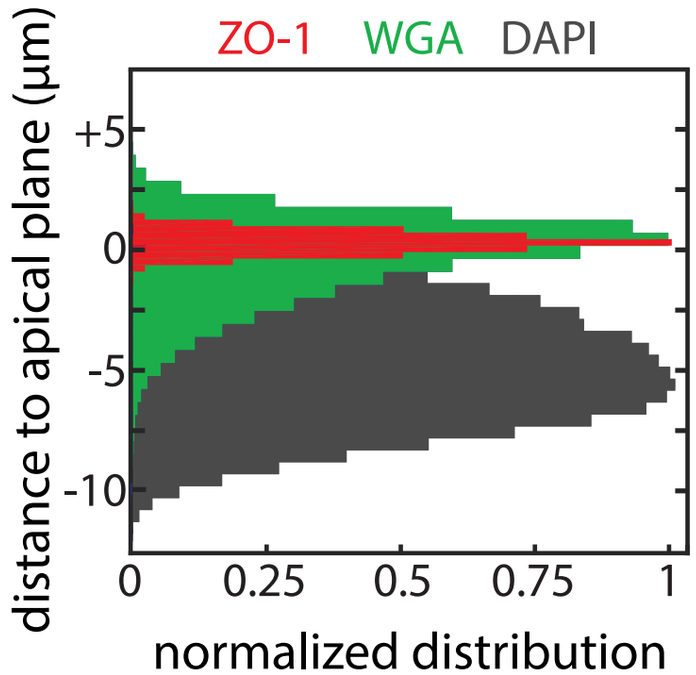
Receptor localization eg Acr2b (high density)

DAPI/ZO-1/Acr2b/WGA

X Z slice

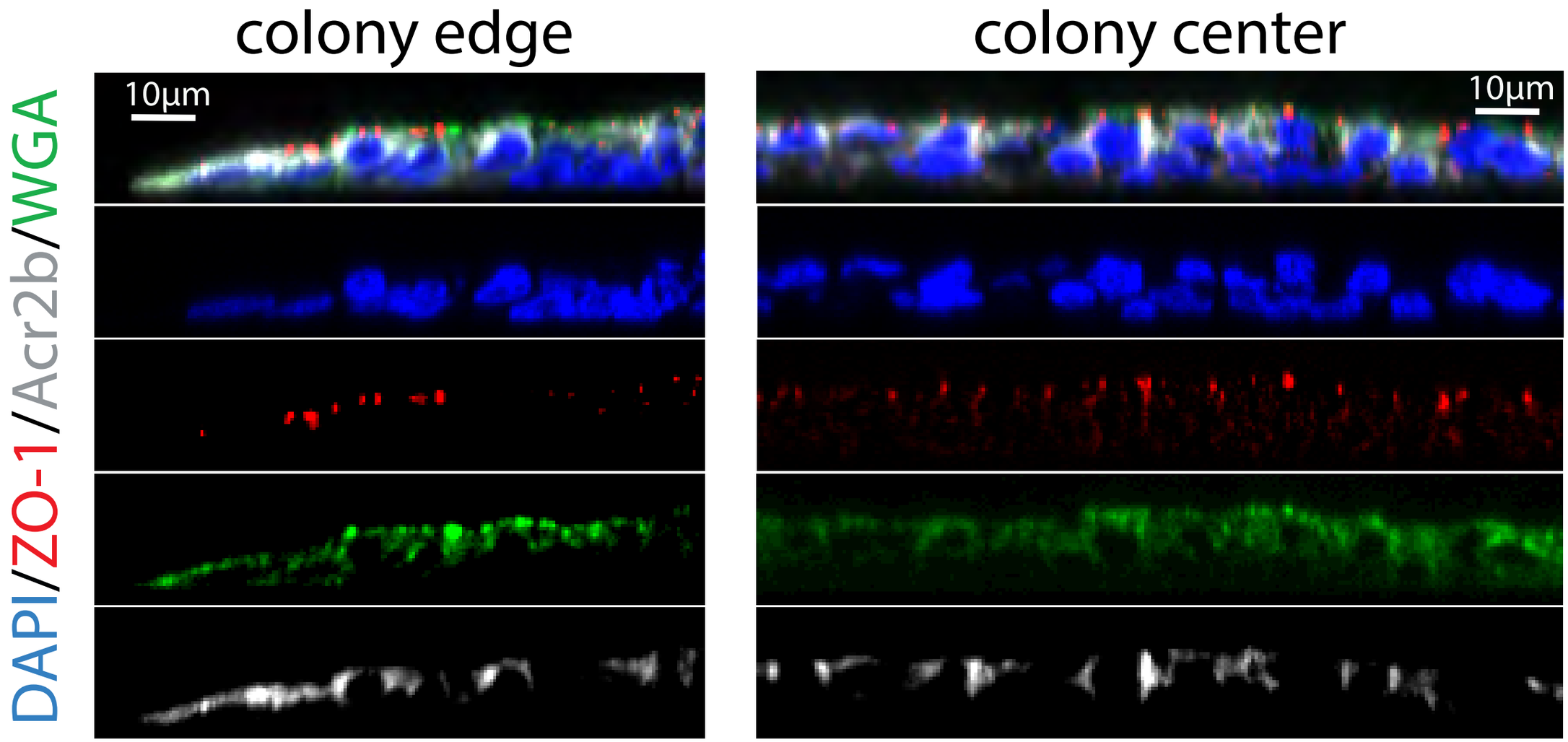




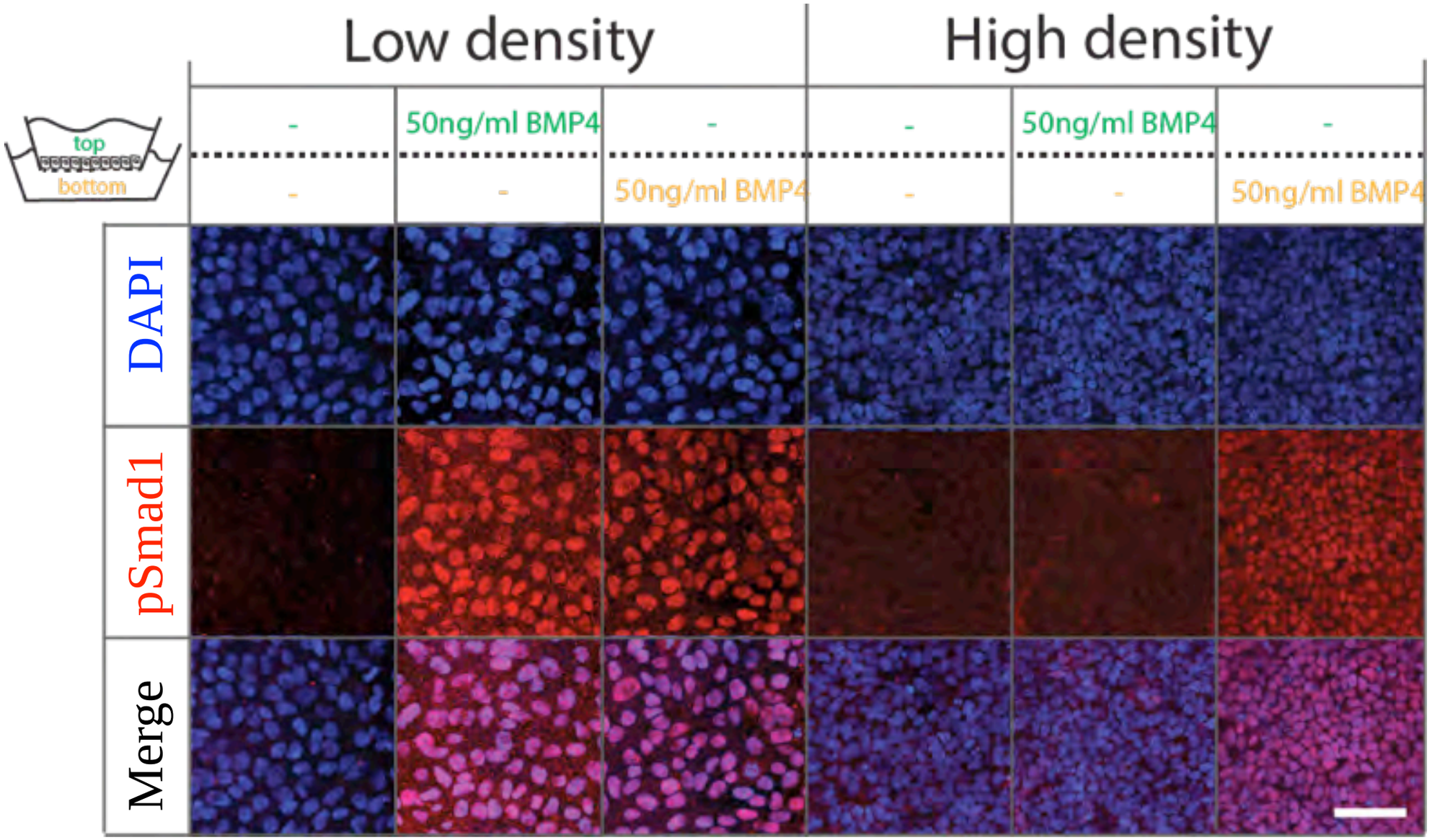


Apical distance:
relative to ZO-1 surf.
(computed from pts
in 3D)

Receptors exposed at edge vs center



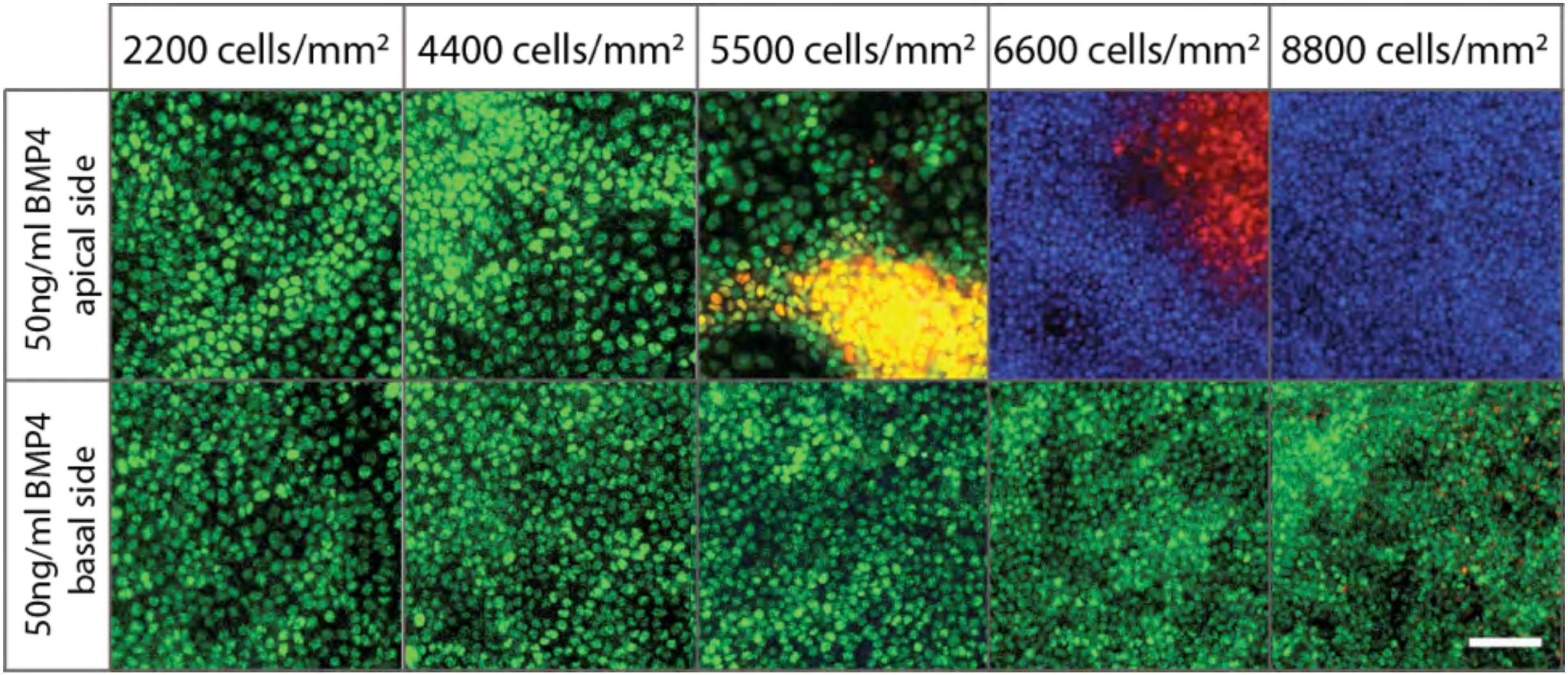
Grow cells on filters:
High density colonies respond only to BMP from bottom



immediate response 1hr BMP

Asymmetry in signaling: filters(1hr) --> fates (48 hr)

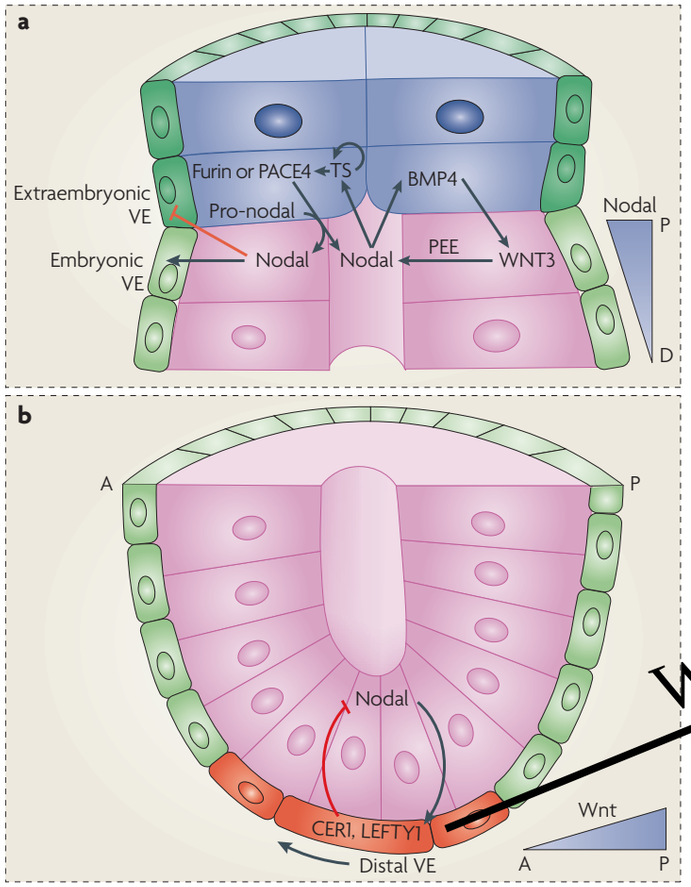
Cdx2 Bra Sox2



(*tech*: stimulate cells on filters from below to generate pure populations)

Embryonic signaling via basal surface

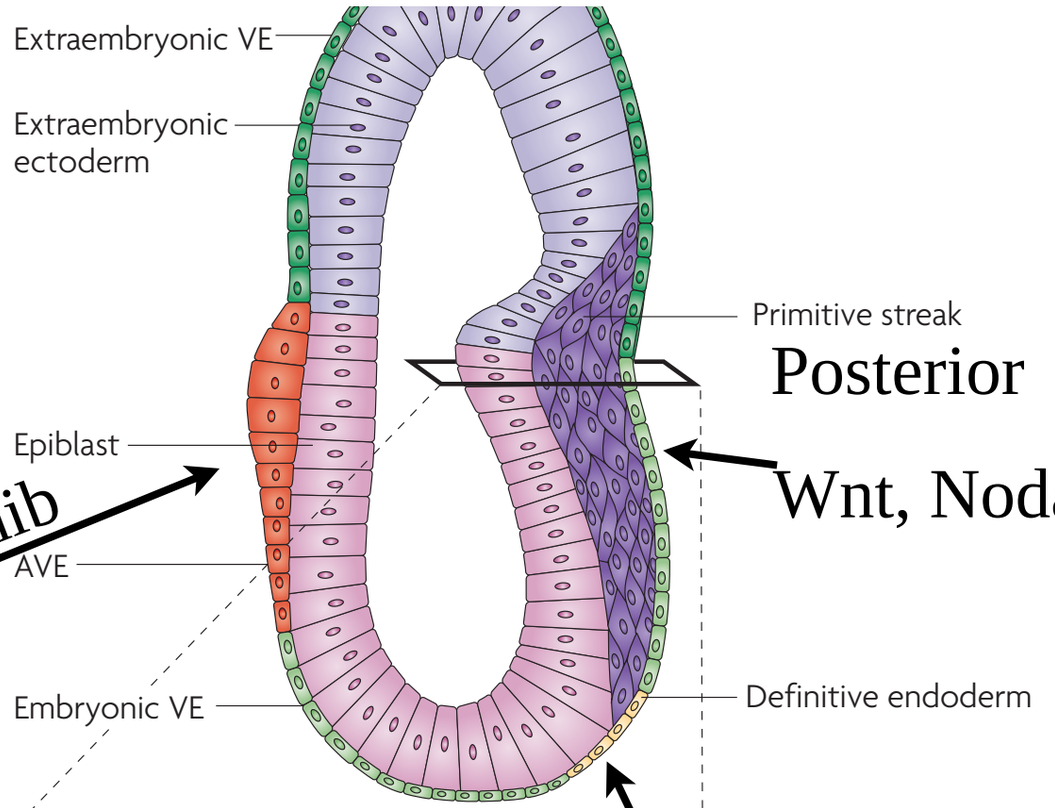
E5.5



Wnt, Nodal Inhib
Anterior

Arnold 2009

E6.5

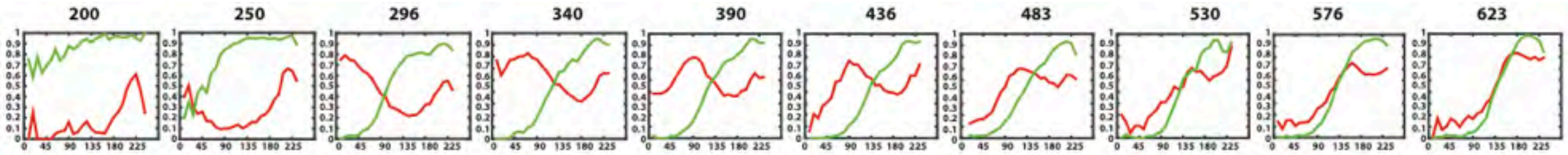


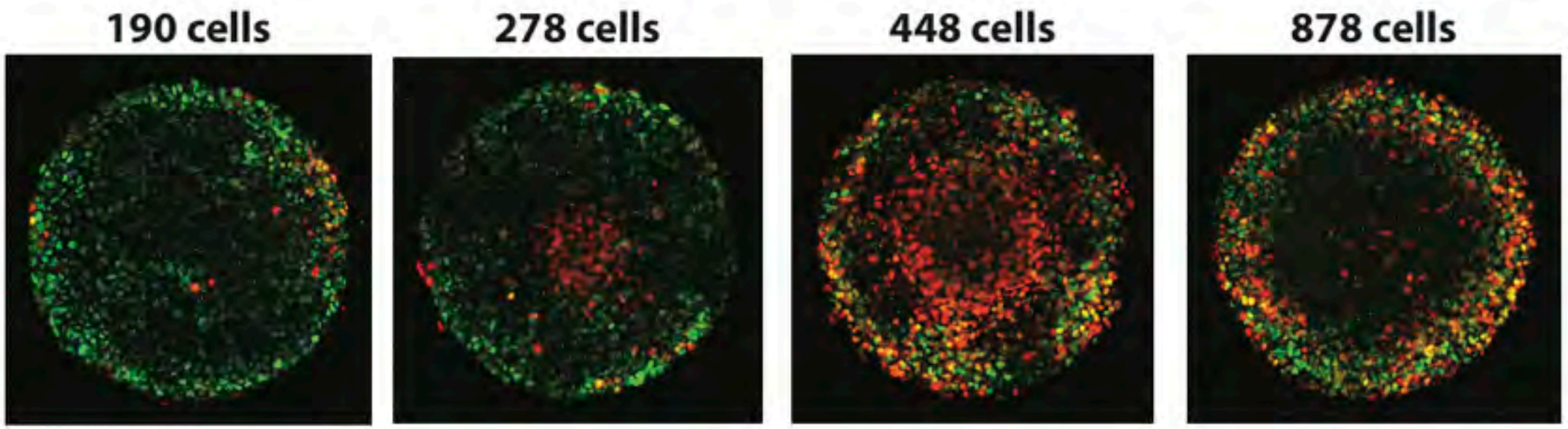
Node/organizer:
limits prim. streak
BMP inhibitors

Fates: 48hrs BMP vs density (2)

Published work here

Cdx2 Bra vs radius

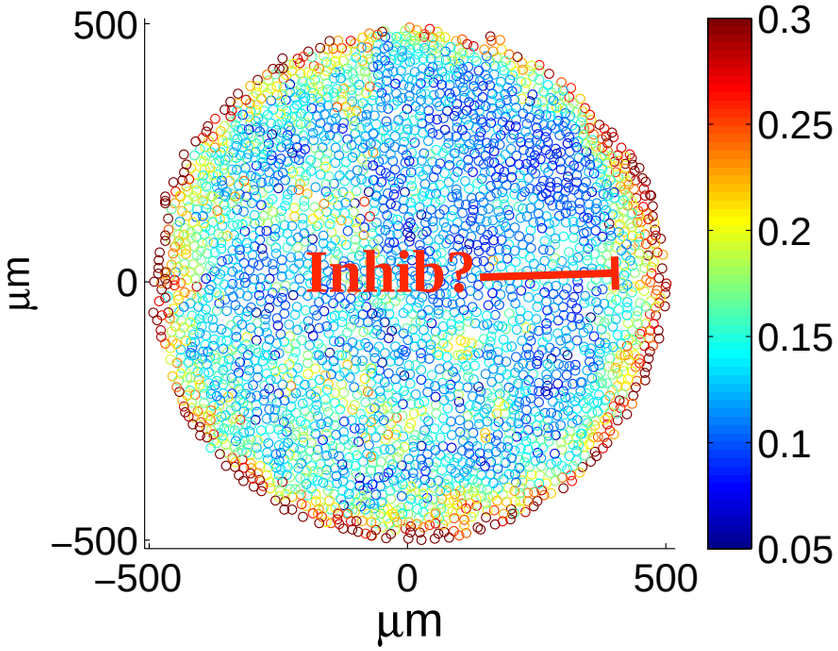




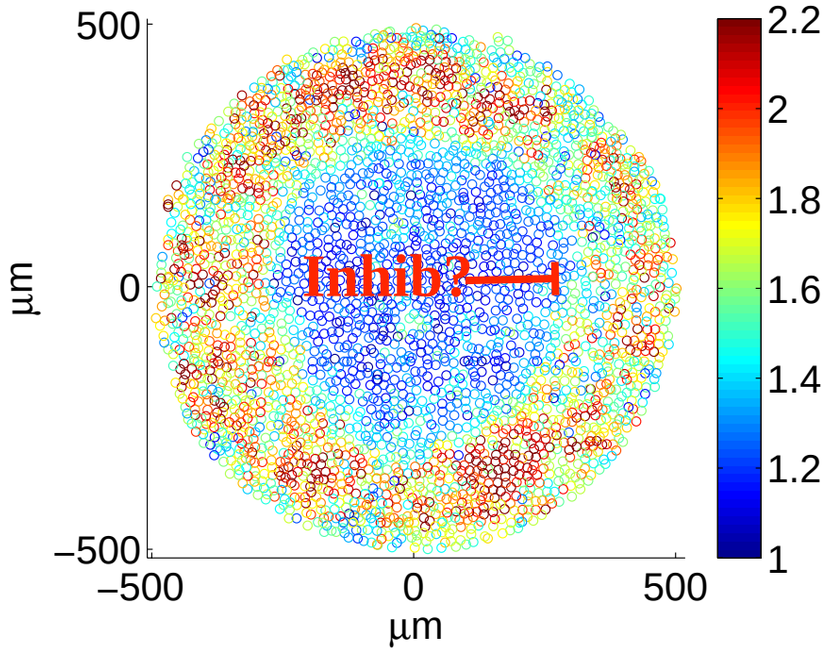
Role of Inhibitors on microcolonies (long times)?

24hrs BMP → Wnt → Nodal

pSmad1



Smad2



Early times: (high density) receptor accessibility

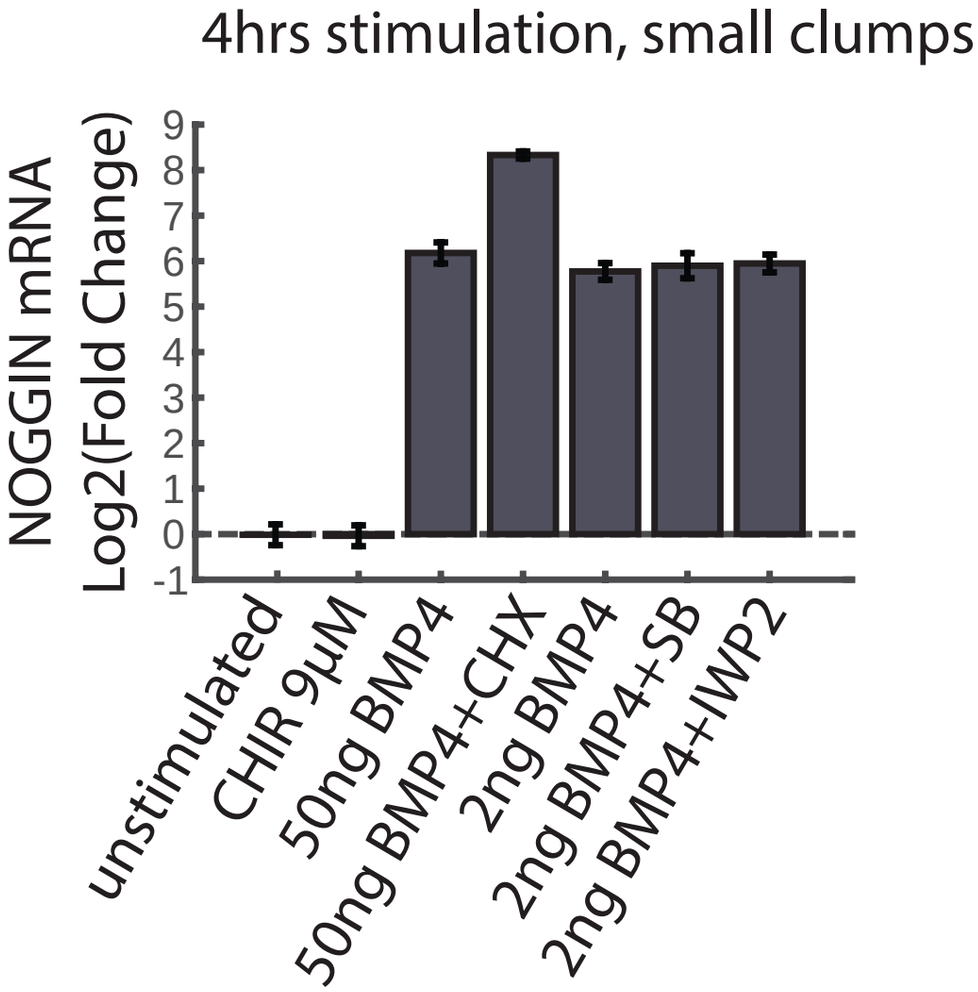
Late times (lo/hi densities) do inhibitors explain the fate pattern??

BMP inhibitors ?

RNA-seq: BMP stimulated colonies 0-24hrs + phenotypes of KO in mice --> Noggin

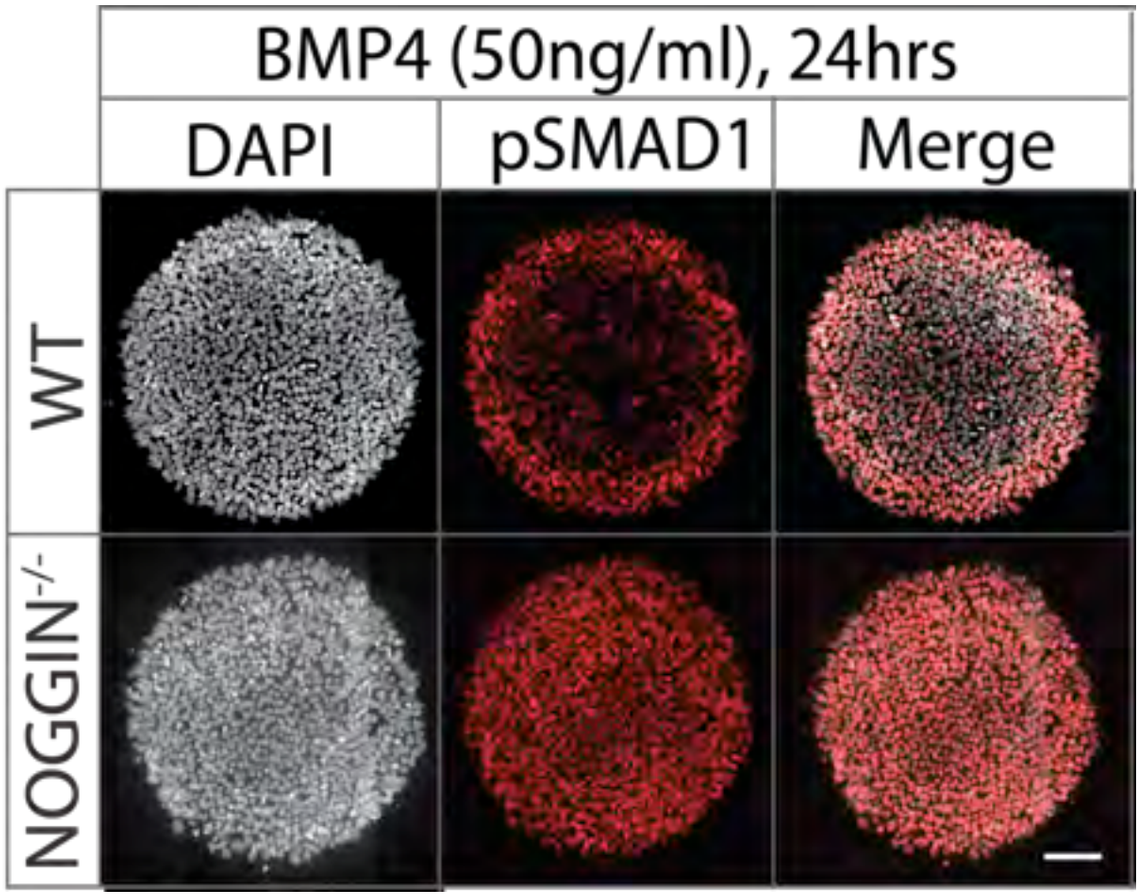
- Noggin direct target of BMP:

qPCR + inhibitors of Wnt, Nodal

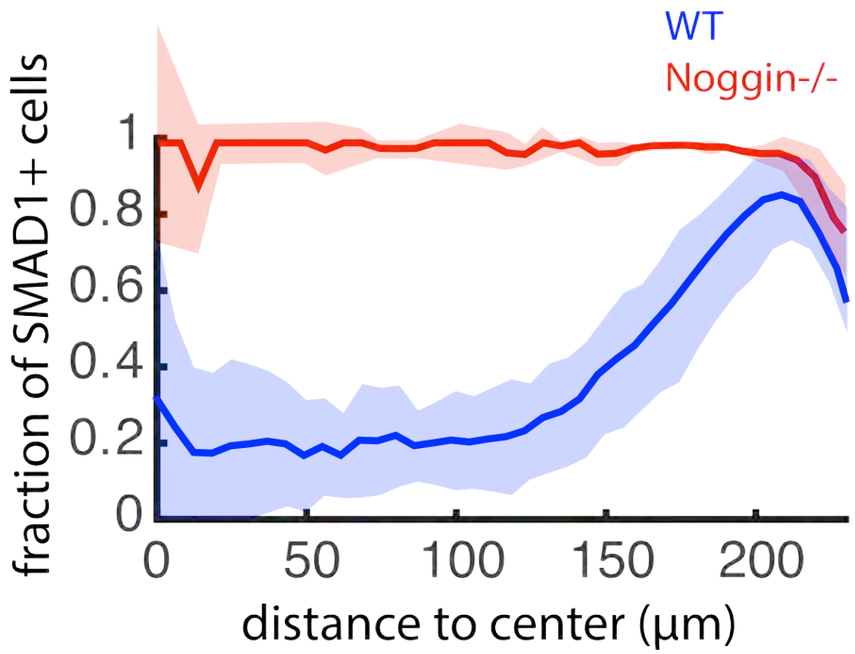


- Low density long time: noggin-/- pSmad1 uniform --> Noggin necessary
- Force expression of Noggin --> restrict pattern to edges --> Noggin sufficient

Low density colonies, long time: Noggin necessary

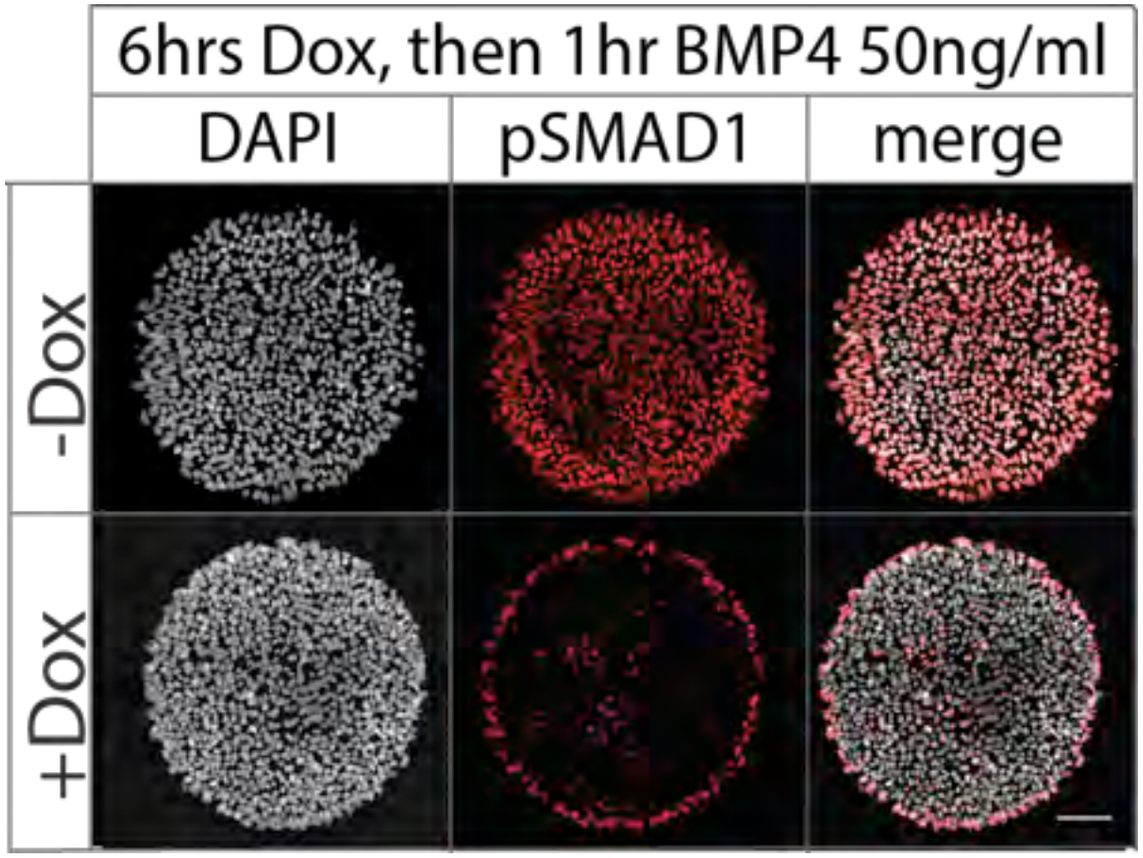


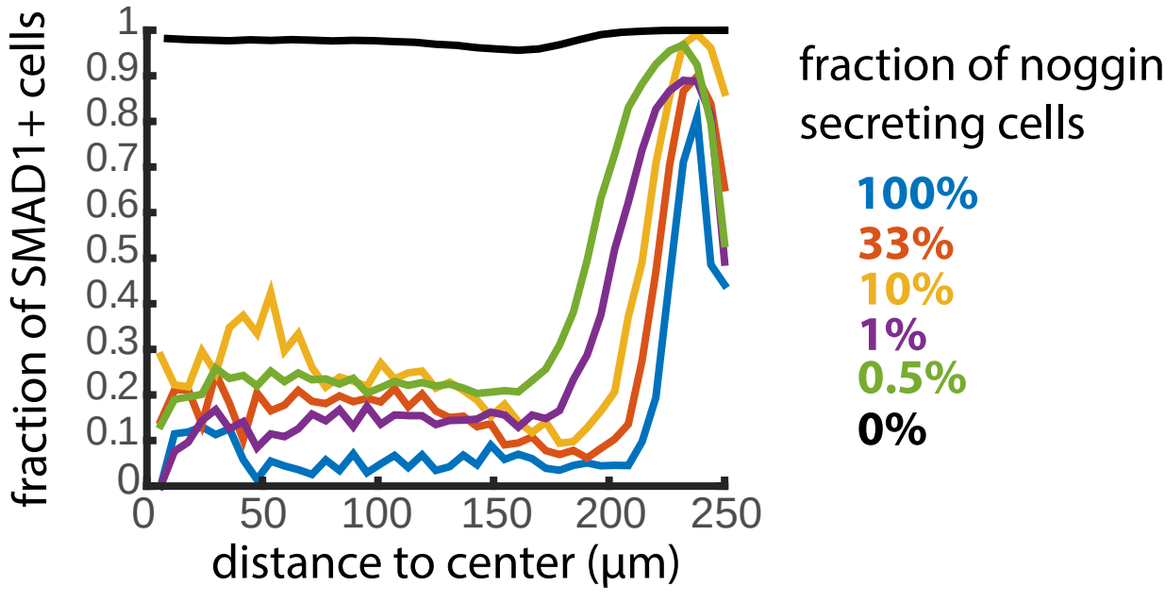
Uniform response without Noggin



Quantify many colonies

Dilute Noggin secreting cells in parent





Turn on Noggin with Dox, apply BMP
100% Noggin Secreting Cells

As dilute cells: broaden Smad1 at edge

Model (radius, time, density, [BMP])

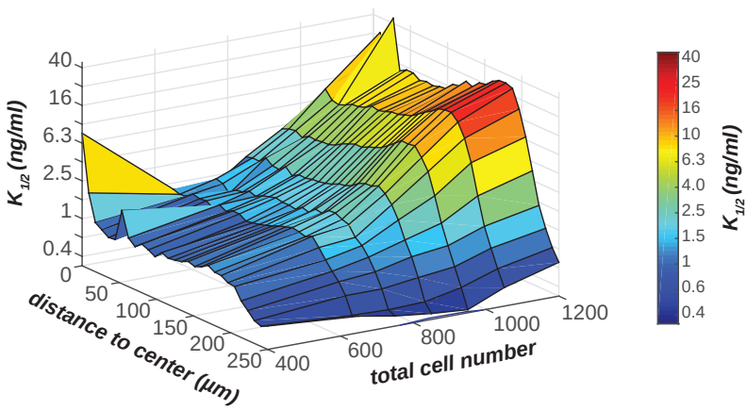
1. Time = 0: Cell Density --> Receptor(radius) --> BMP reception(level)

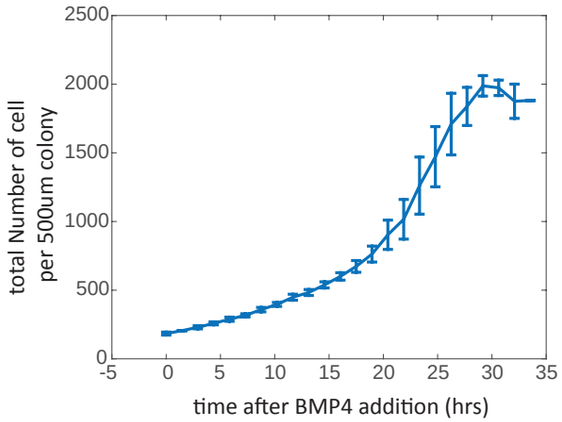
2. Cells grow (0-48hrs) 3x

3. Induce inhibitors, diffuse

4. Transcribe fate markers

1 + **2** + **3**





BMP(r,t) --> Nog

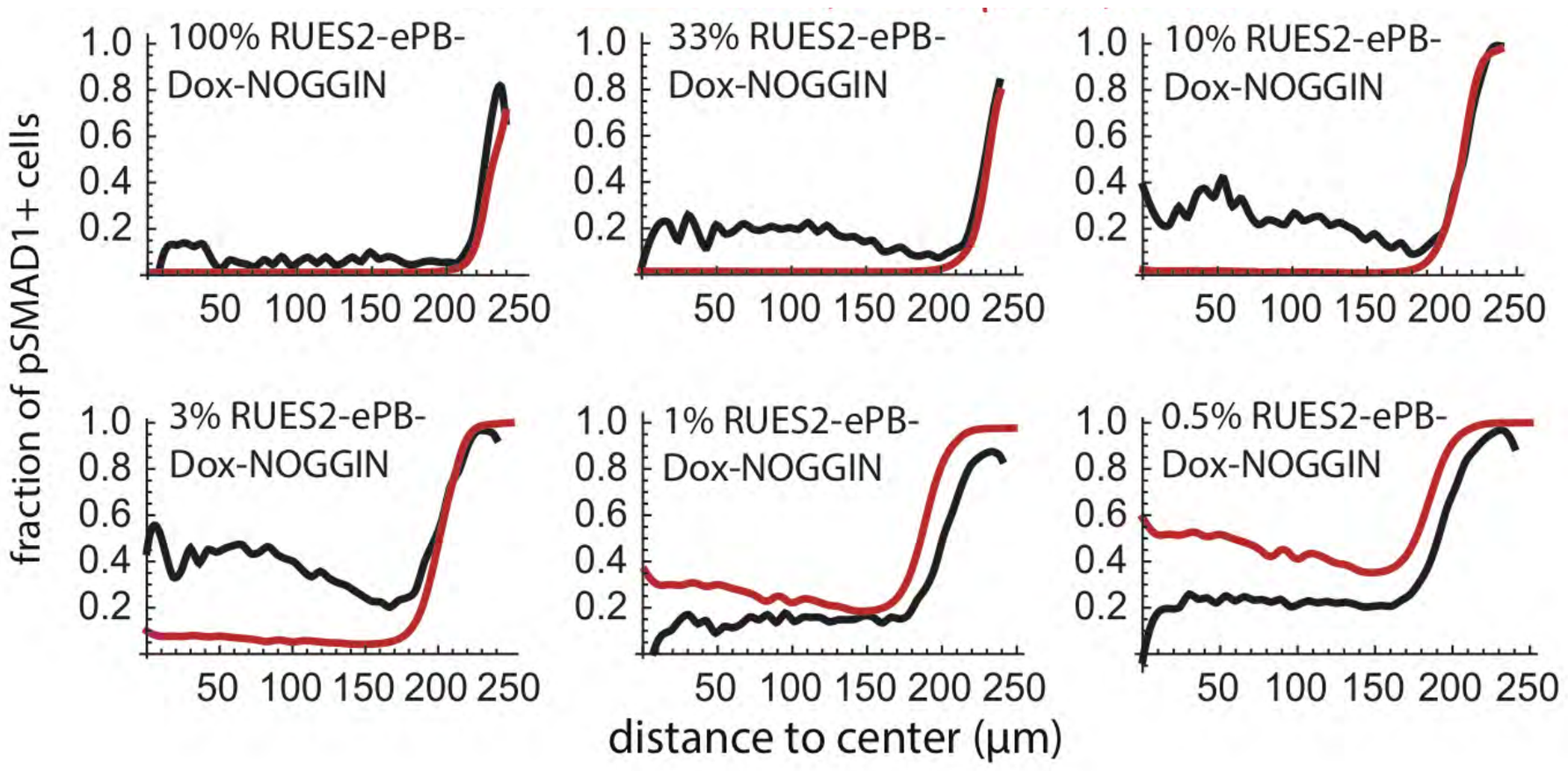
$$\partial_t N = D\nabla^2 N + prod$$

(Both receptors and inhibitors restrict response to edge)

Fit 200x range [Noggin]

1 free parameter merges receptor prepattern
to reaction diffusion

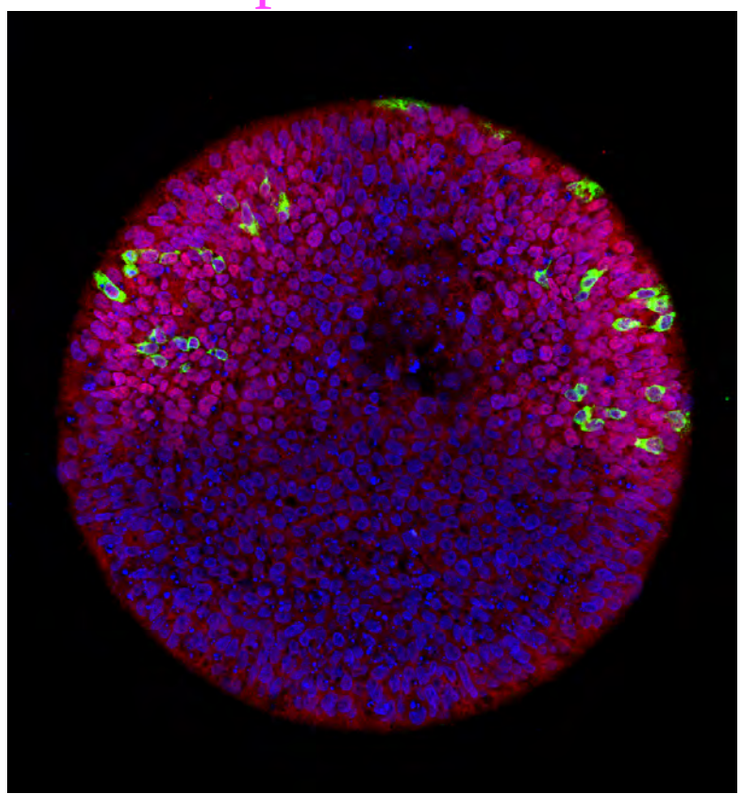
Model Data



Localized expression of activators & inhibitors

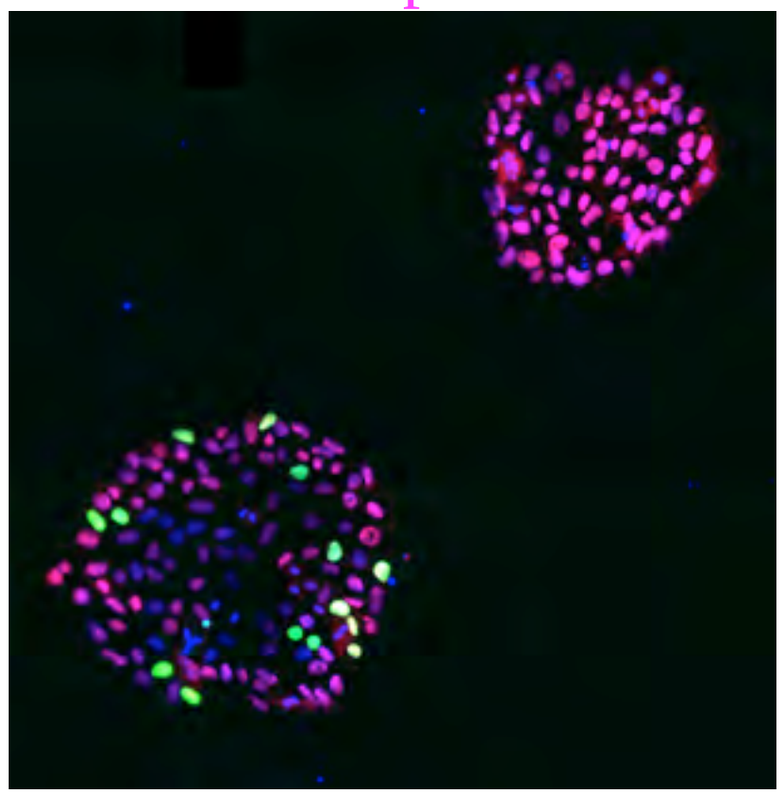
DOX --> MYC tagged BMP4 --> pSmad1 response in naive isogenic cells

BMP4 pSmad1 DAPI



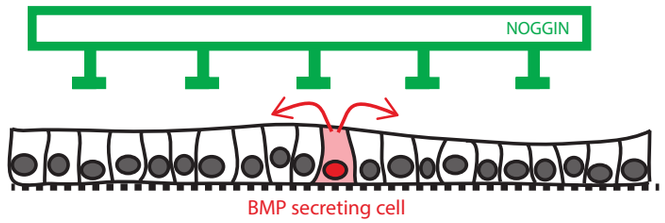
DOX -> Noggin (+GFP-H2B) --| [BMP --> pSmad1]

Dish culture: pSmad1 DAPI



Cell biology of signaling in epithelial layer

Filter assay:Polarity defines sensitivity to inhibitors

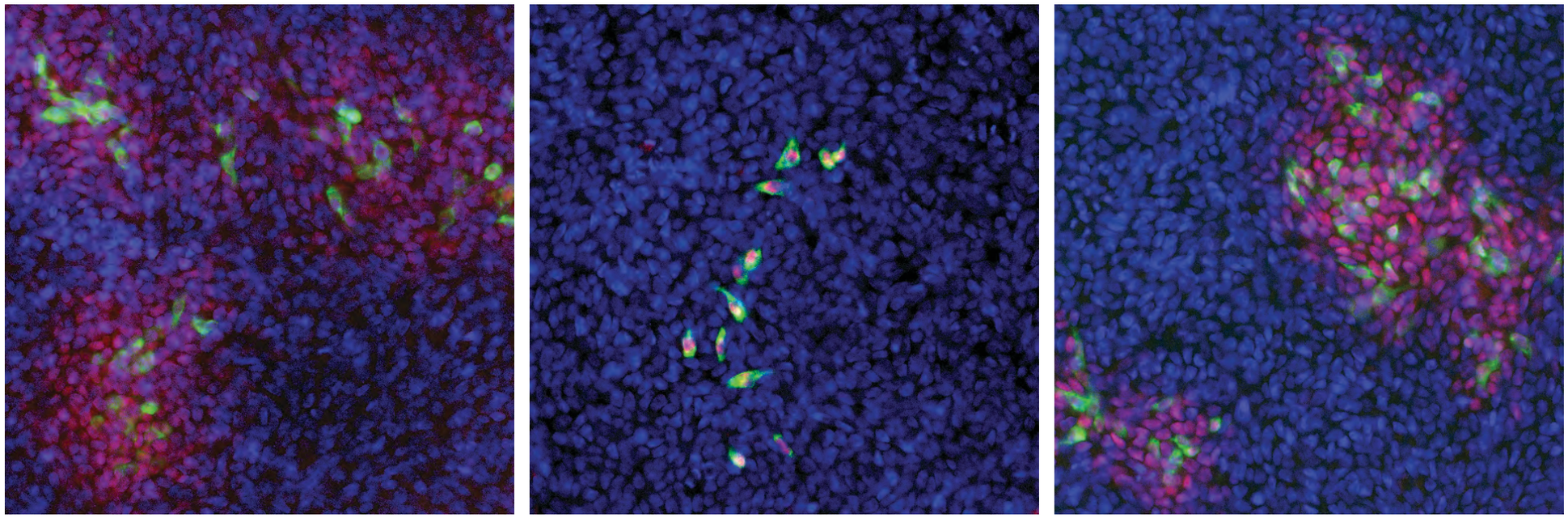


DAPI BMP4 pSmad1

Induce BMP4 in layer probe pSmad1

Noggin TOP inhibits BMP4

Noggin BOTTOM no effect on pSmad1



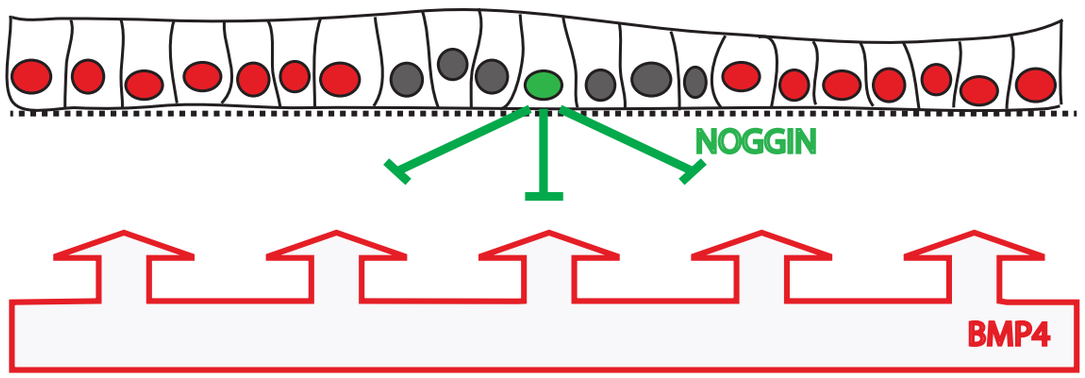
(NB Autocrine survives, LDN kills it.)

(NB for these densities sensitive to BMP4 from Bottom only!)

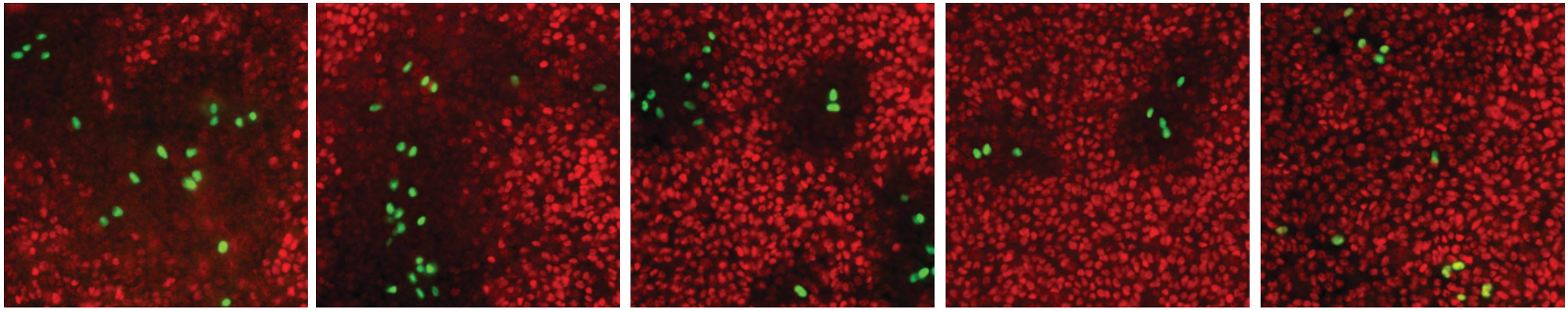
Filter assay for inhibitor & activator

Dox: Noggin secreting cells

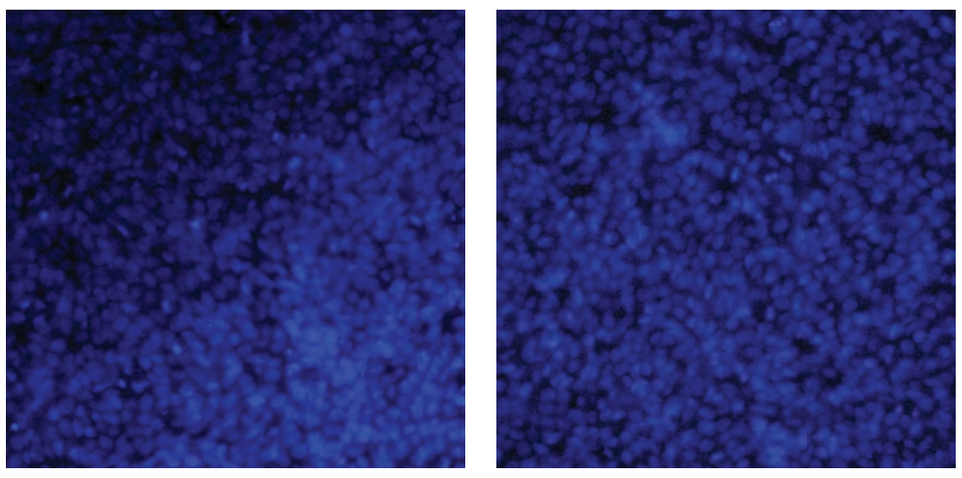
⊣ (BMP ⟶ pSmad1)



BMP4: ⟶



DAPI



BMP titrates range of inhibition

Summary:

0. Solution: morphogen (BMP) + inhibitor (Noggin) --> *dead*

1. Noggin only active from apical side (internalized & dynamin dependent)
   (Noggin penetrates filter, laminin coat?)

2. BMP only active from basal side
   (when made in layer, secreted to both sides)

Mechanism?

Noggin inhibits BMP+receptors in endosomes
basolateral BMP traffics to apical side
Noggin gets around Z0-1 junctions
apical Noggin interferes with BMP endocytosis on basolateral surface

What about Wnt?

- Signaling restricted to edge at hi density in pluri state by E-cad
  (but no apical-basal polarity in signaling on filters... contrary to BMP)

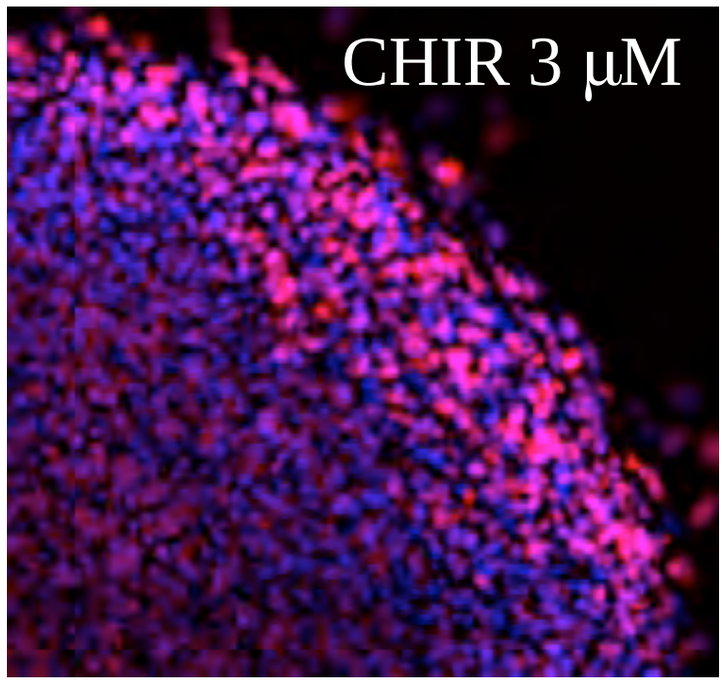
- RNA-seq + KO: Dkk1 restricts pattern at later times

Vary Wnt +- Nodal get axial vs more lateral primitive streak fates

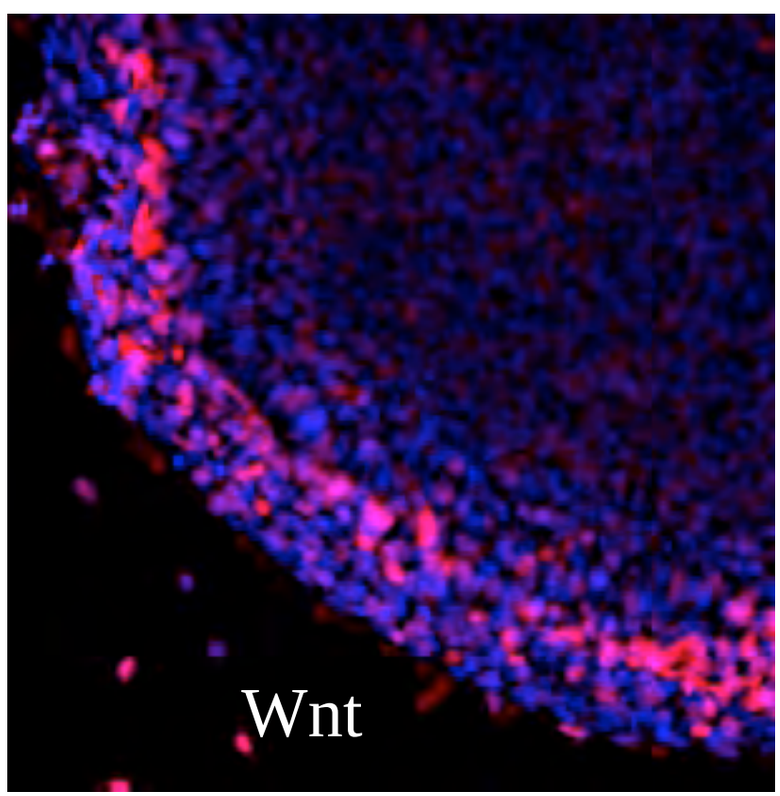
CHIR ≠ Wnt3a (48 hrs)

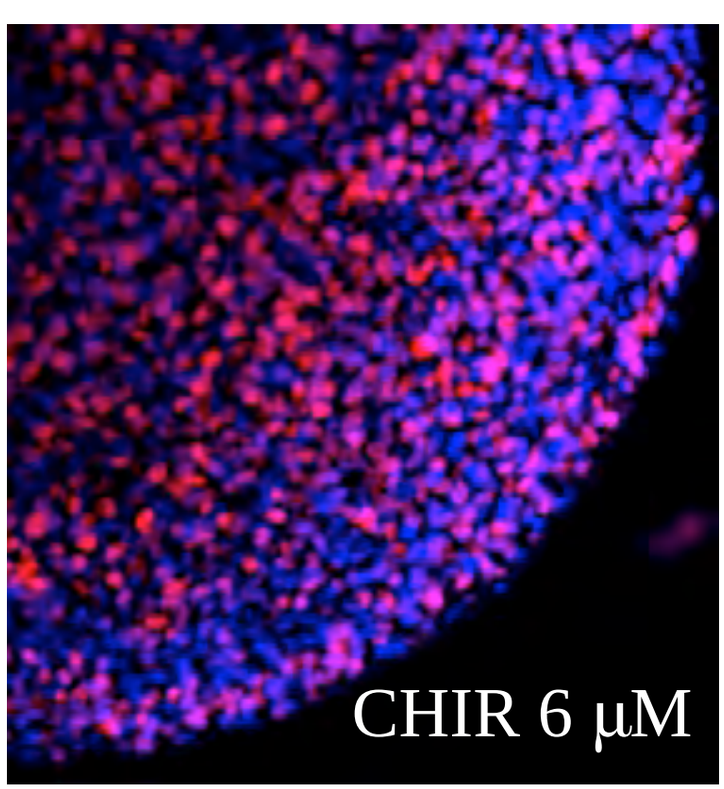
Wnt sensitive to secreted inhibitor

CHIR (--| GSK3b) circumvents receptors



Bra DAPI

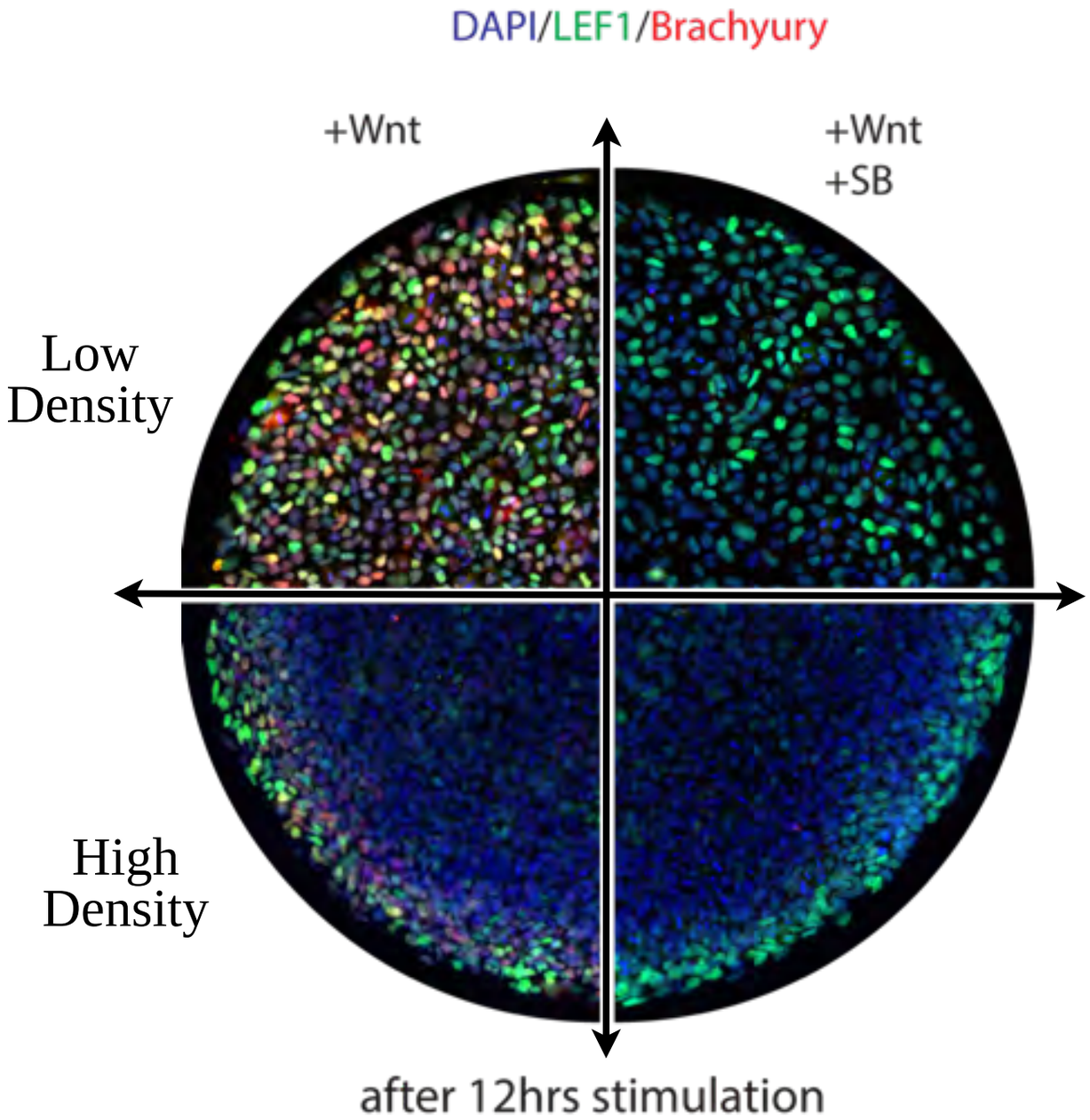




Wnt signaling density dependent

Wnt --> LEF1(direct)

Wnt (--> Nodal) Bra



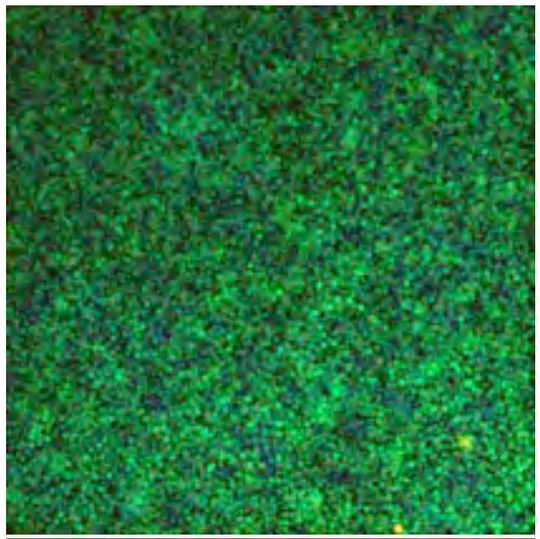
Wnt signaling is NOT apical-basal polarized via transwell

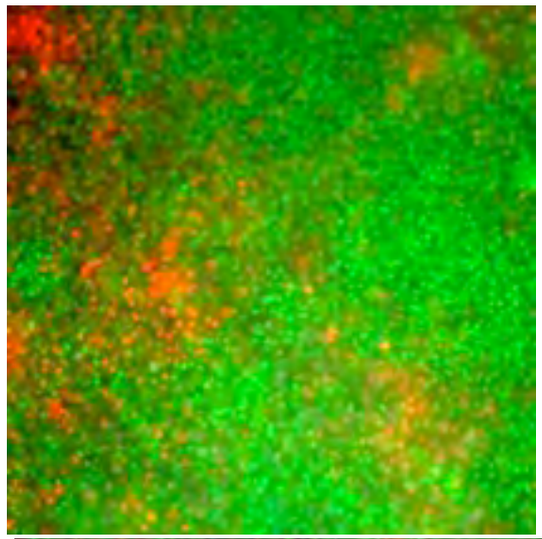
BMP 16k cells
DAPI/Sox2/Cdx2

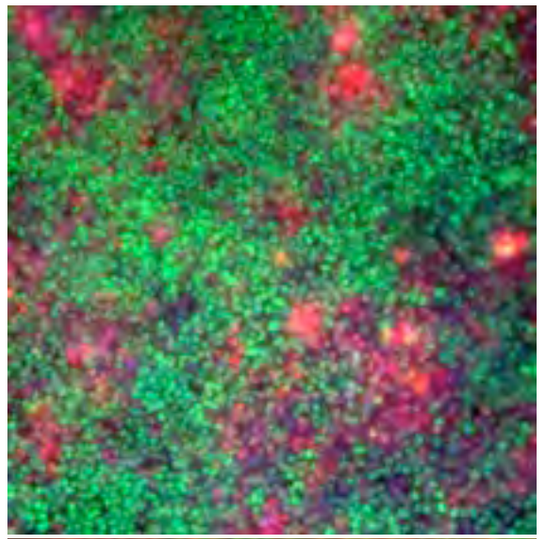
Wnt 16k cells
DAPI/Sox2/Bra

Wnt 32k cells
DAPI/Sox2/Bra

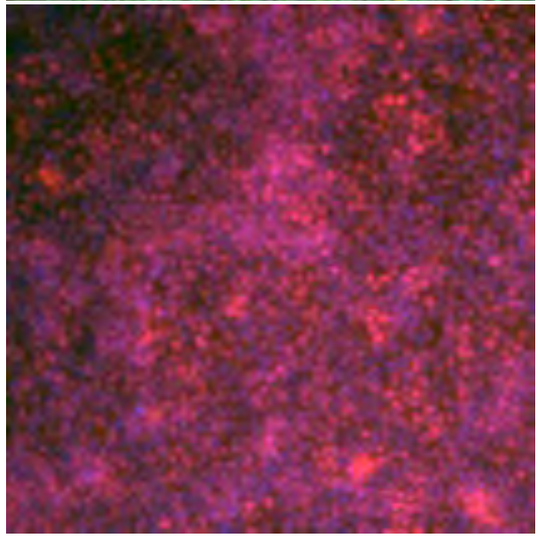
Top

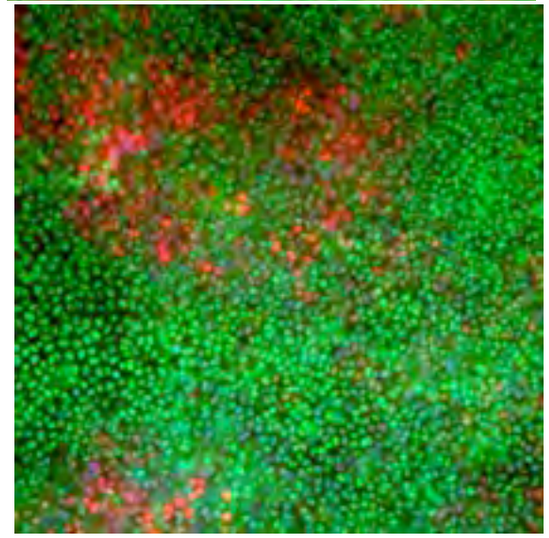


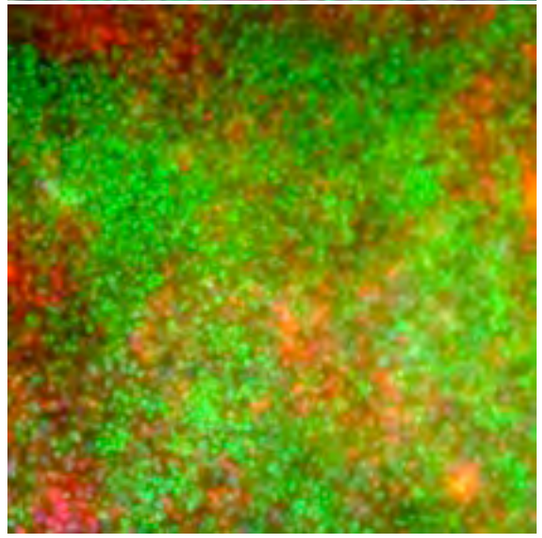




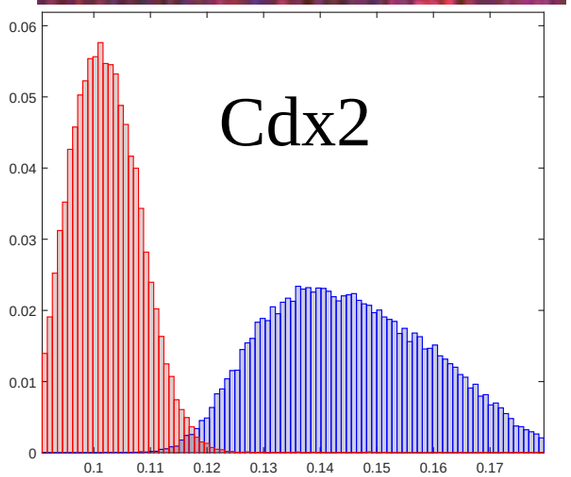
Bottom

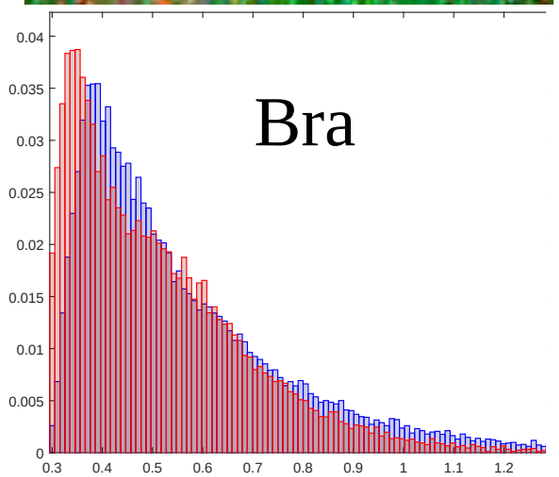


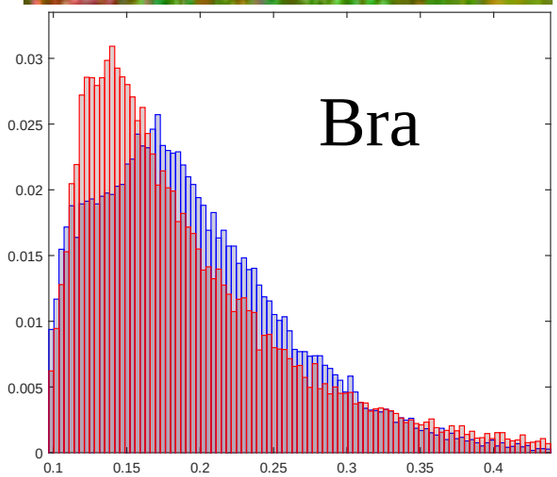




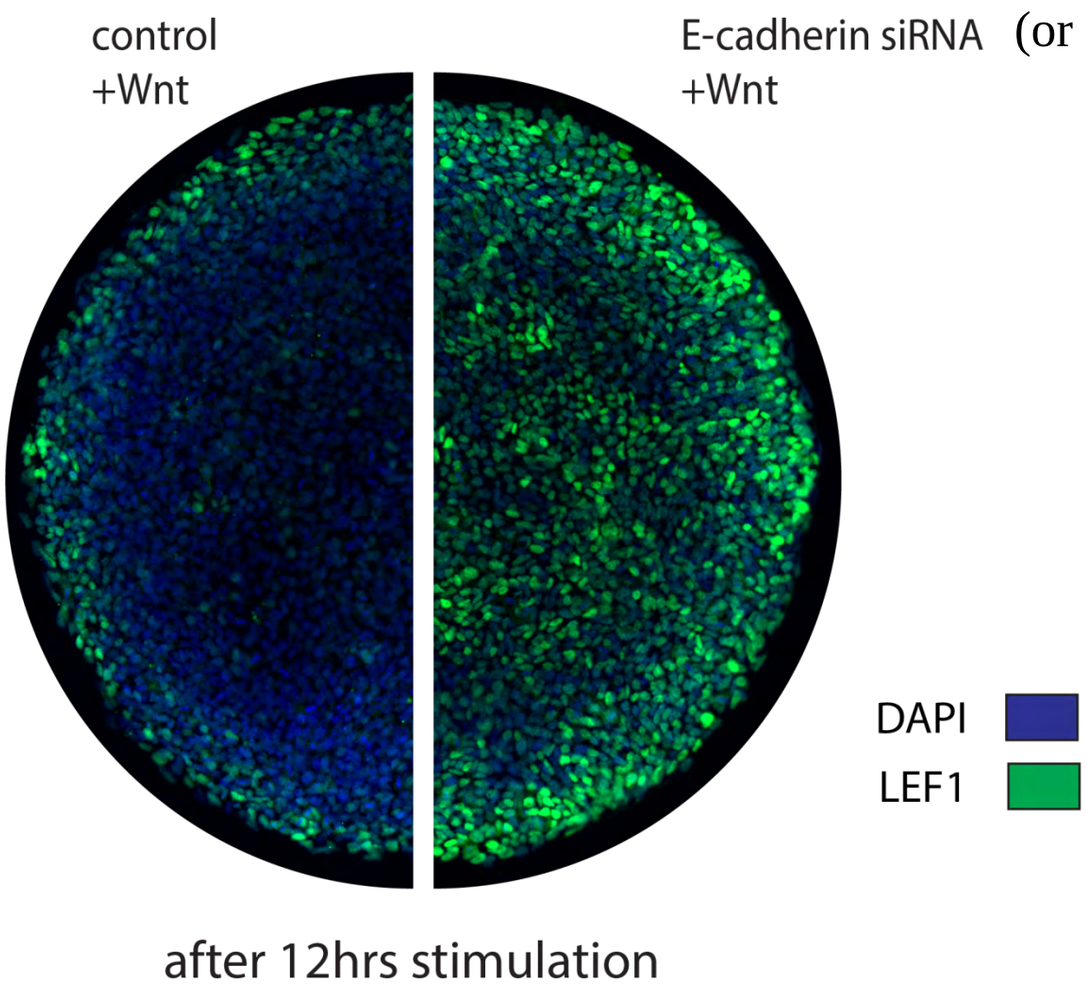
Hist. Nuc I.







Early Wnt restriction due to E-cadherin

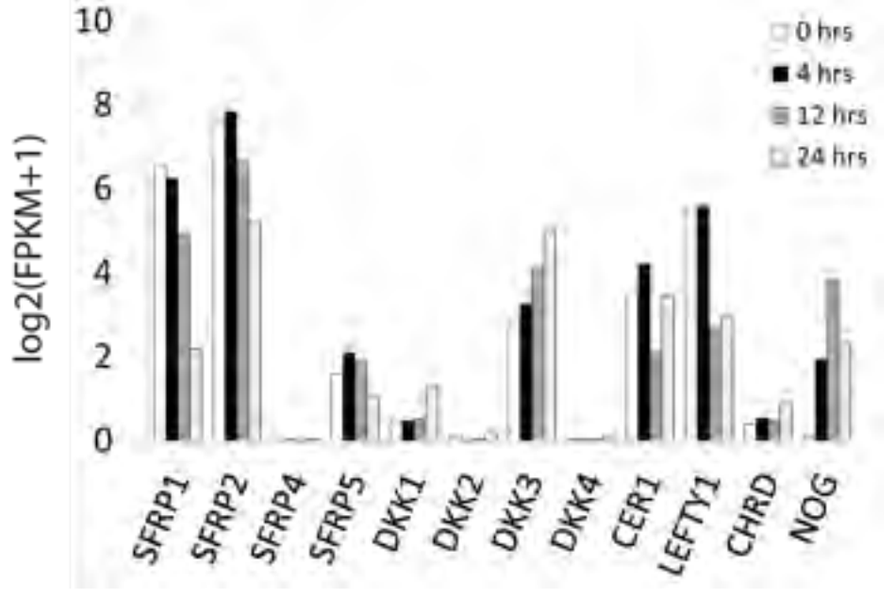


E-cad --| Wnt signaling

Drosophila 1990's &
Ciruna-Rossant 2001

Canonical Inhibitors:

RNA-seq, BMP4 on Colonies 0 - 24 hrs



homozygous CRISPR KO lines (*and in progress*):

E-cad (+ Dkk1) ✓ (~ RNAi)

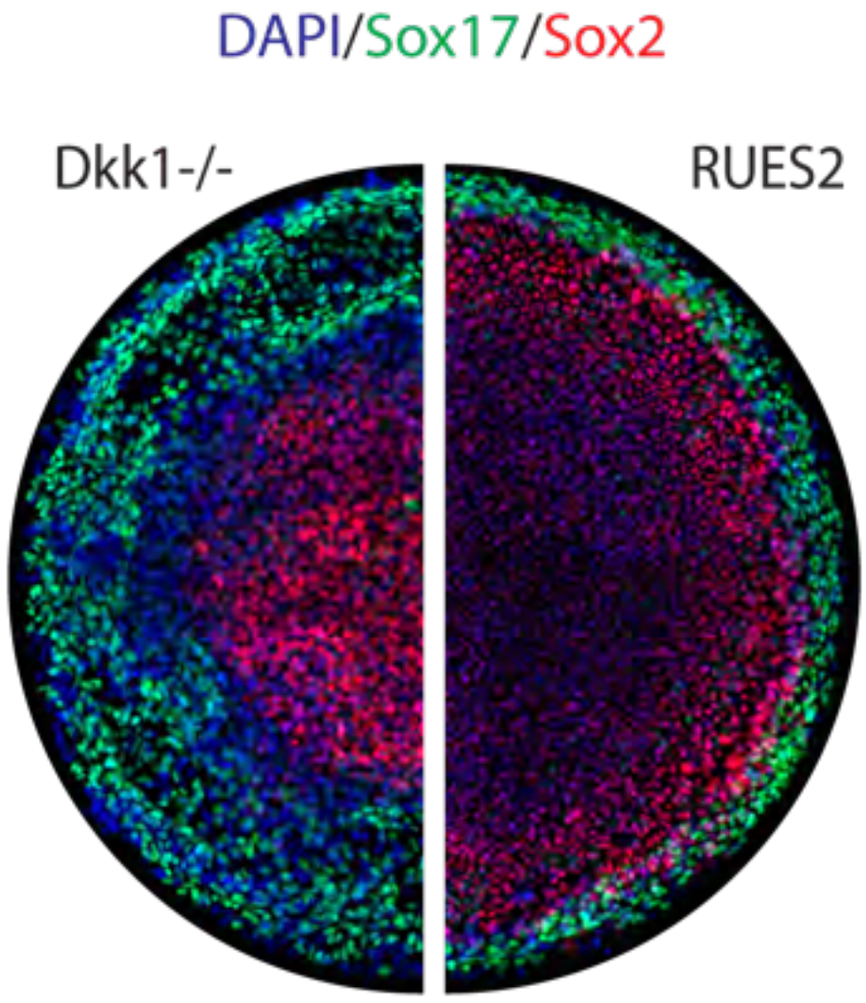
Dkk1, 3 (*dbl)* ✓

SFRP1+2 (minimal phenotype)

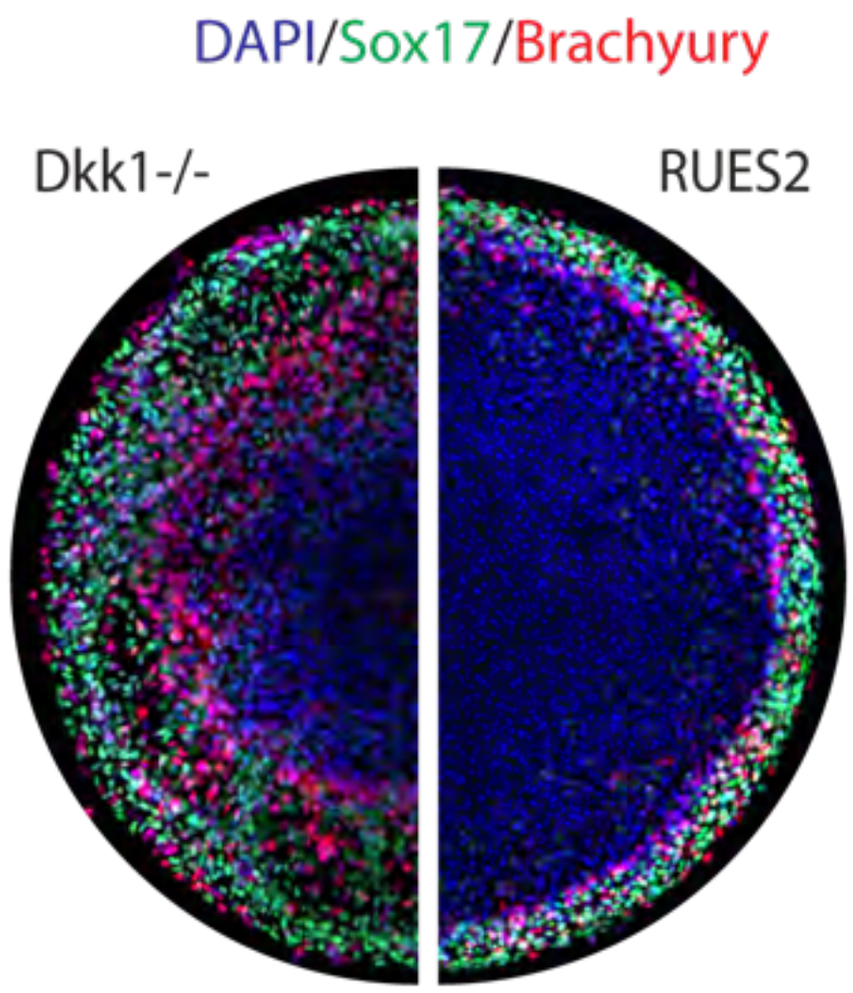
Cer1 (+ *Lefty1,2*) (minimal phenotype)

Dkk1-/- expands band of Wnt expression

Dkk1 (TIME) here **48** hrs



Wnt 48hrs

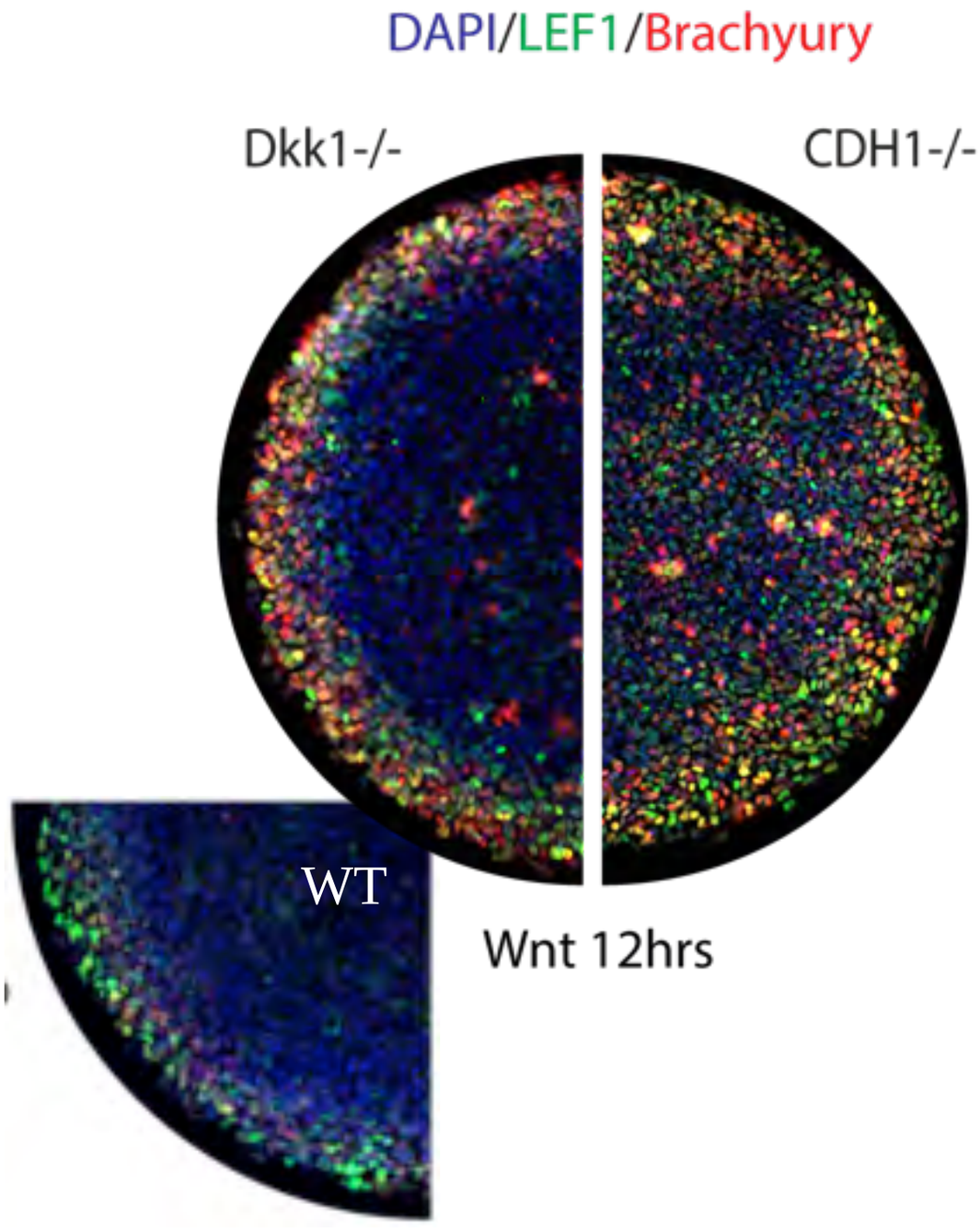


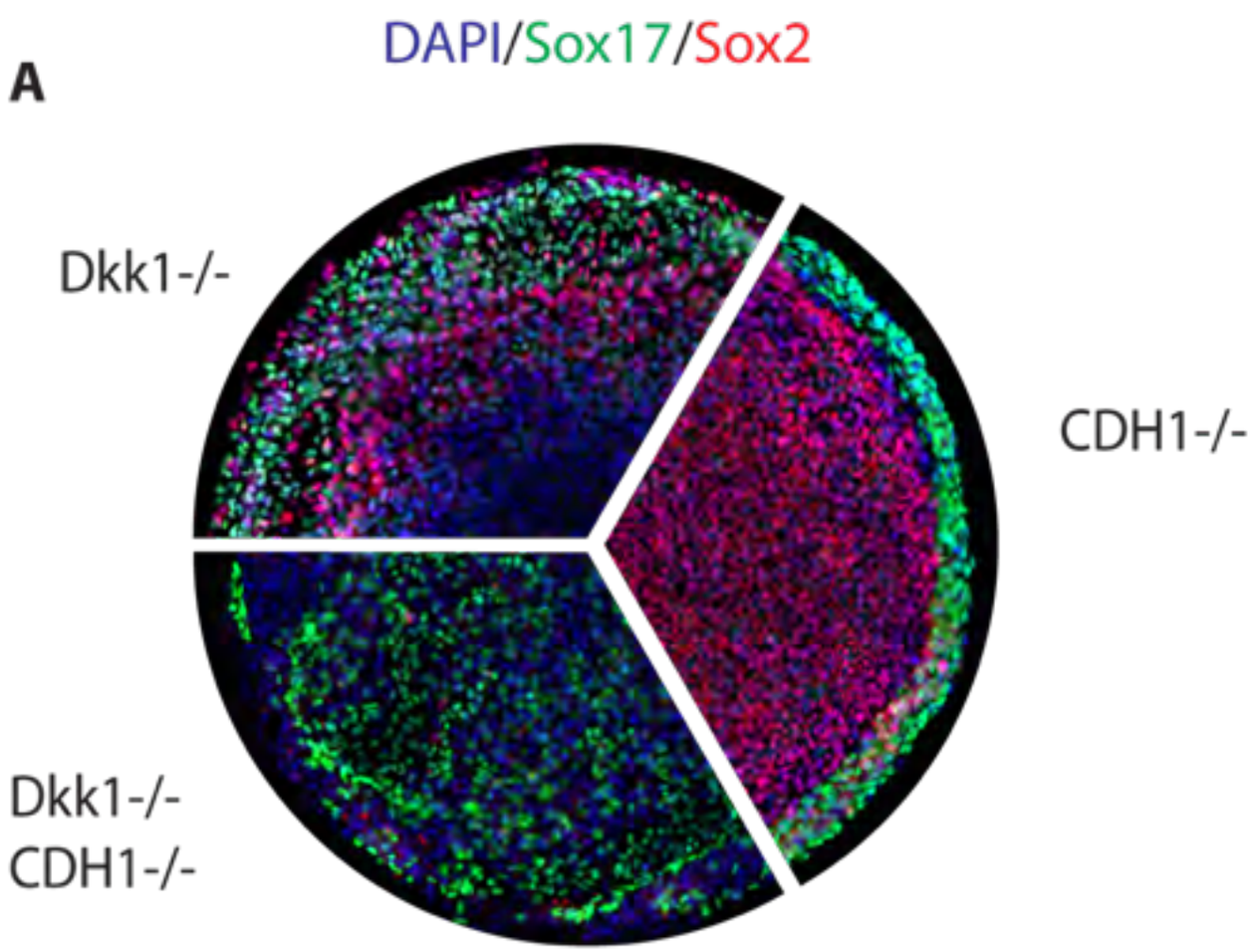
Wnt 48hrs

TIME:: Dkk1-/- & Ecad (aka CDH1) -/-

E-cad dominant at 12 hrs

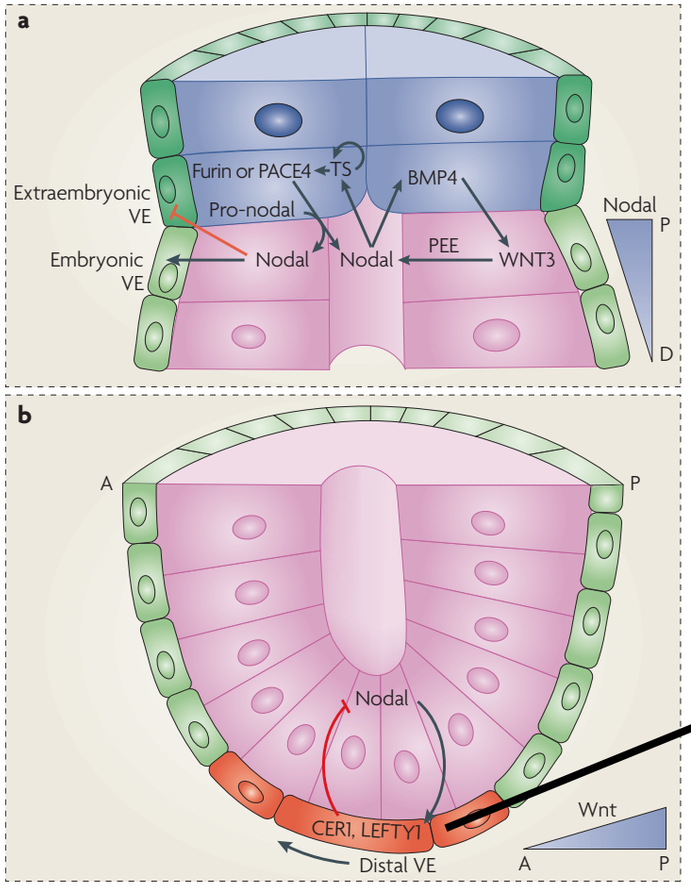
Dkk1 dominant inhibitor 48 hrs.





Anterior Posterior axis?

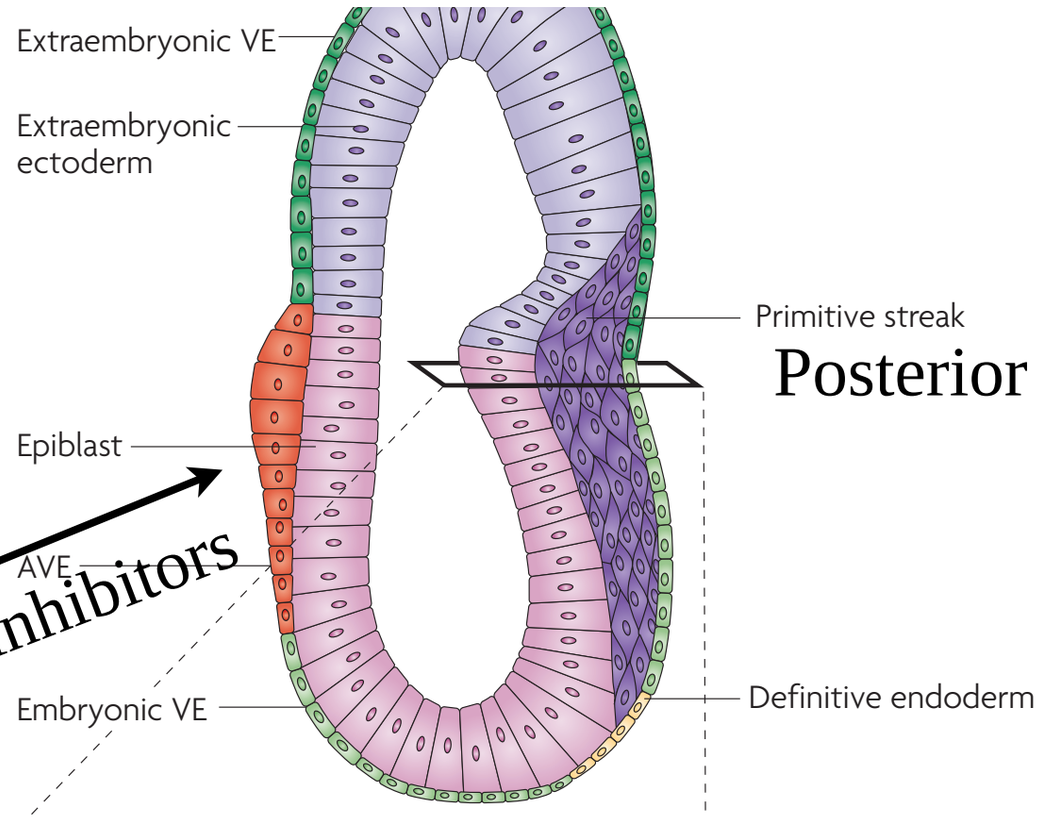
E5.5



Anterior

Wnt,Nodal inhibitors

E6.5



Geometric asymmetry does **not** induce AP: edges rule

Are two epithelial layers needed?

One layer: (force expression of Wnt (P) or inhibitors (A), Turing?)

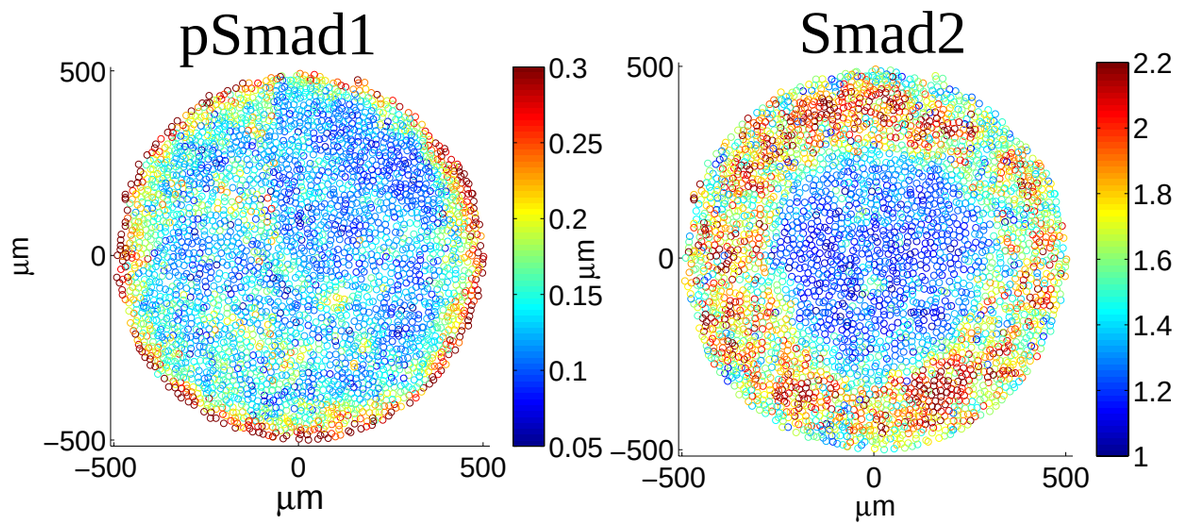
Morphogenic Symmetry breaking ?

Minimal reaction diffusion model:

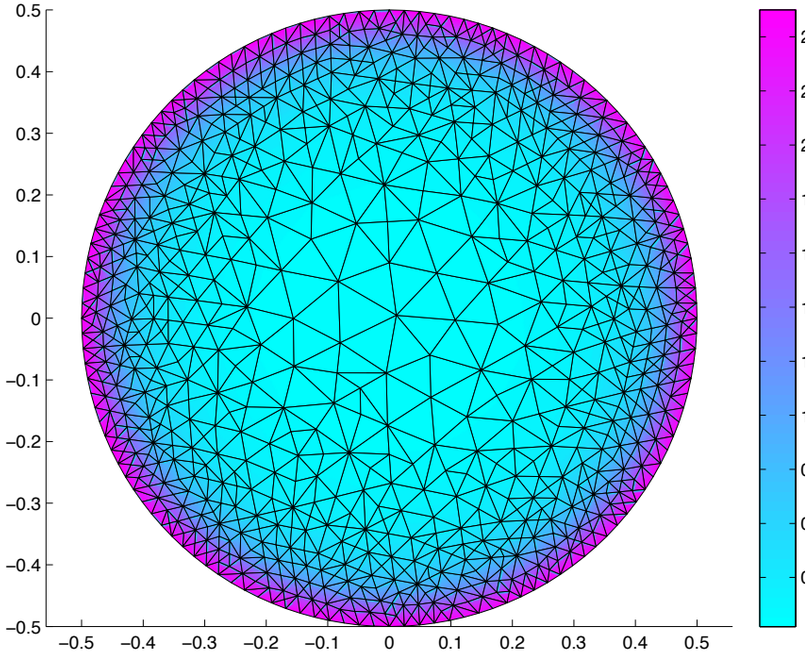
BMP --> Smad1, Nodal, Inhibitor,

Inhibitor --| BMP, Nodal, Inhib.,

Nodal, Inhibitor diffuse, leak out at edge

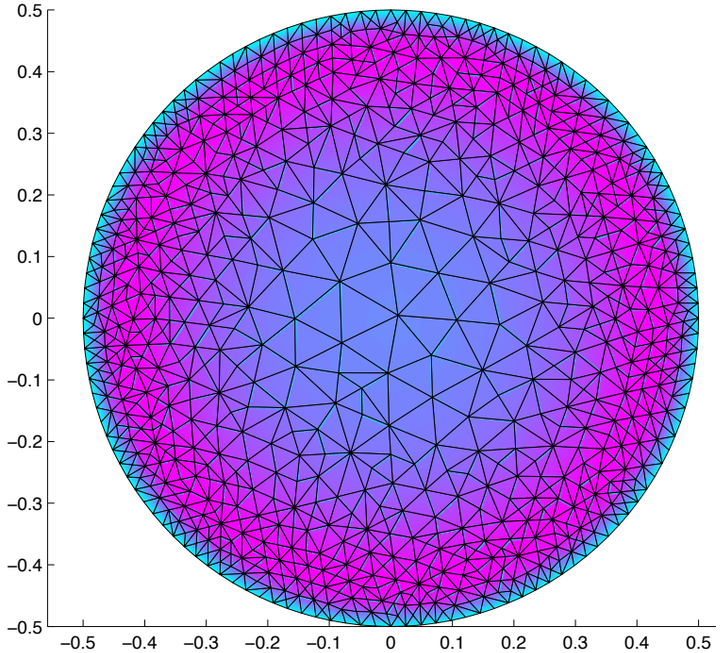


pSmad1~ Inhibitor(RNA)



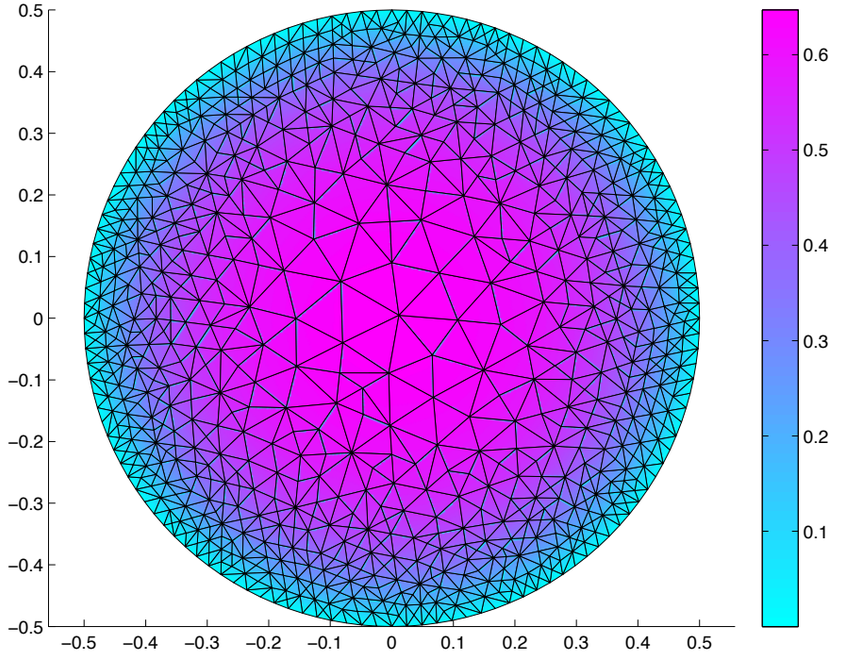
$$\frac{\partial S_1}{\partial t} = \frac{B}{1+I^2} - d_1 S_1$$

Nodal (Smad2)



$$\frac{\partial N}{\partial t} = D_N \nabla^2 N + \frac{B}{1+I^2} - d_N N$$

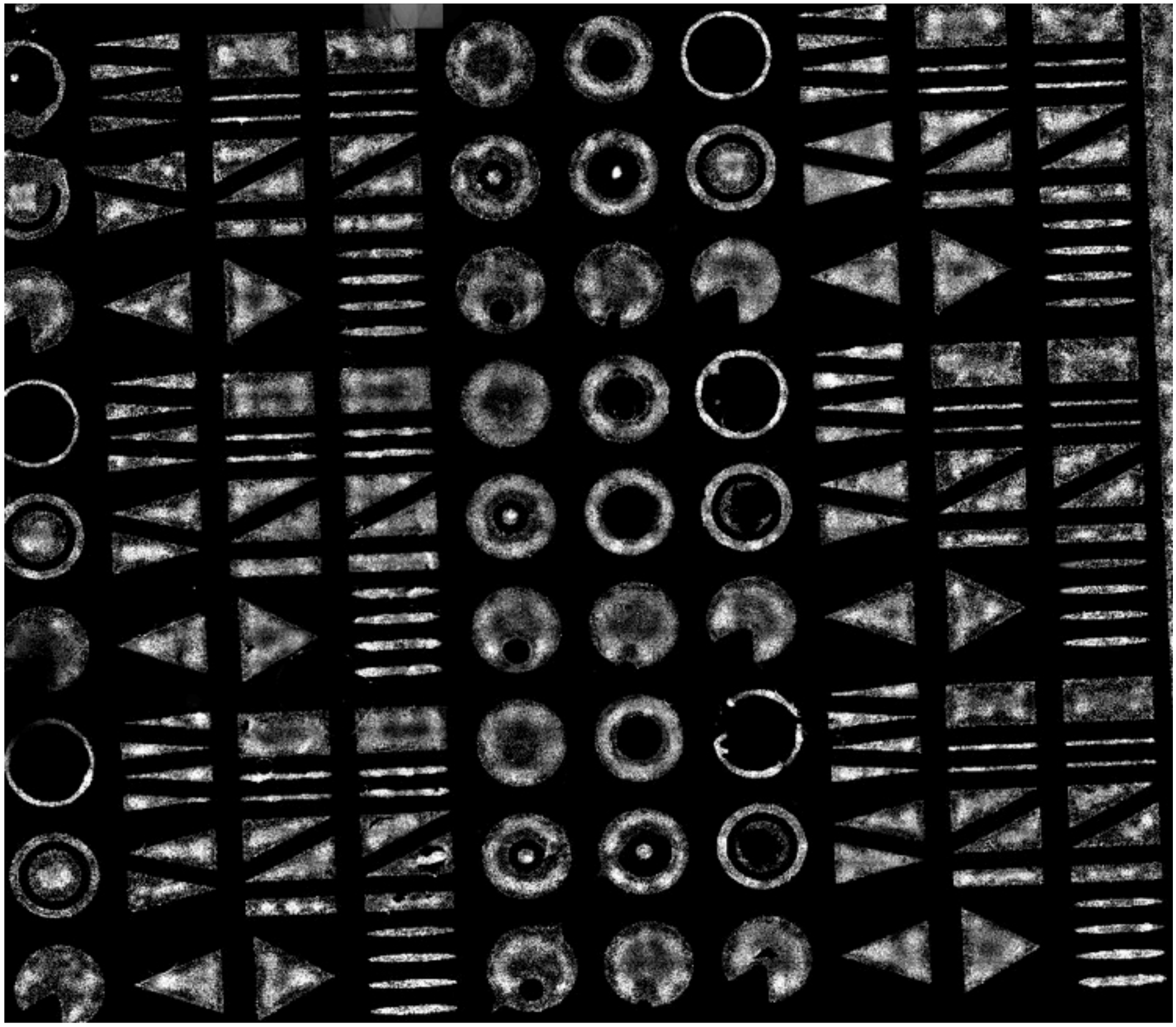
Inhibitor(protein)



$$\frac{\partial I}{\partial t} = D_I \nabla^2 I + \frac{B}{1+I^2} - d_I I$$

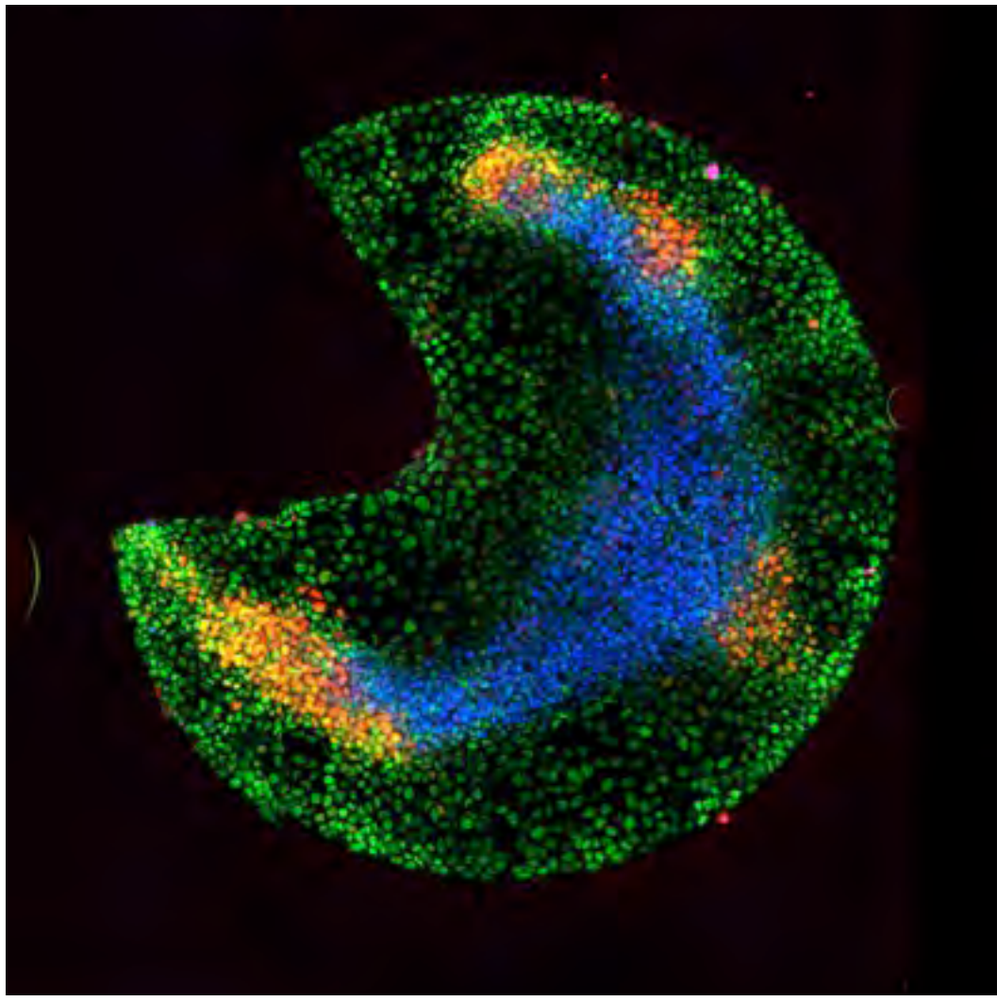
Galerie de paléontologie et d'anatomie comparée

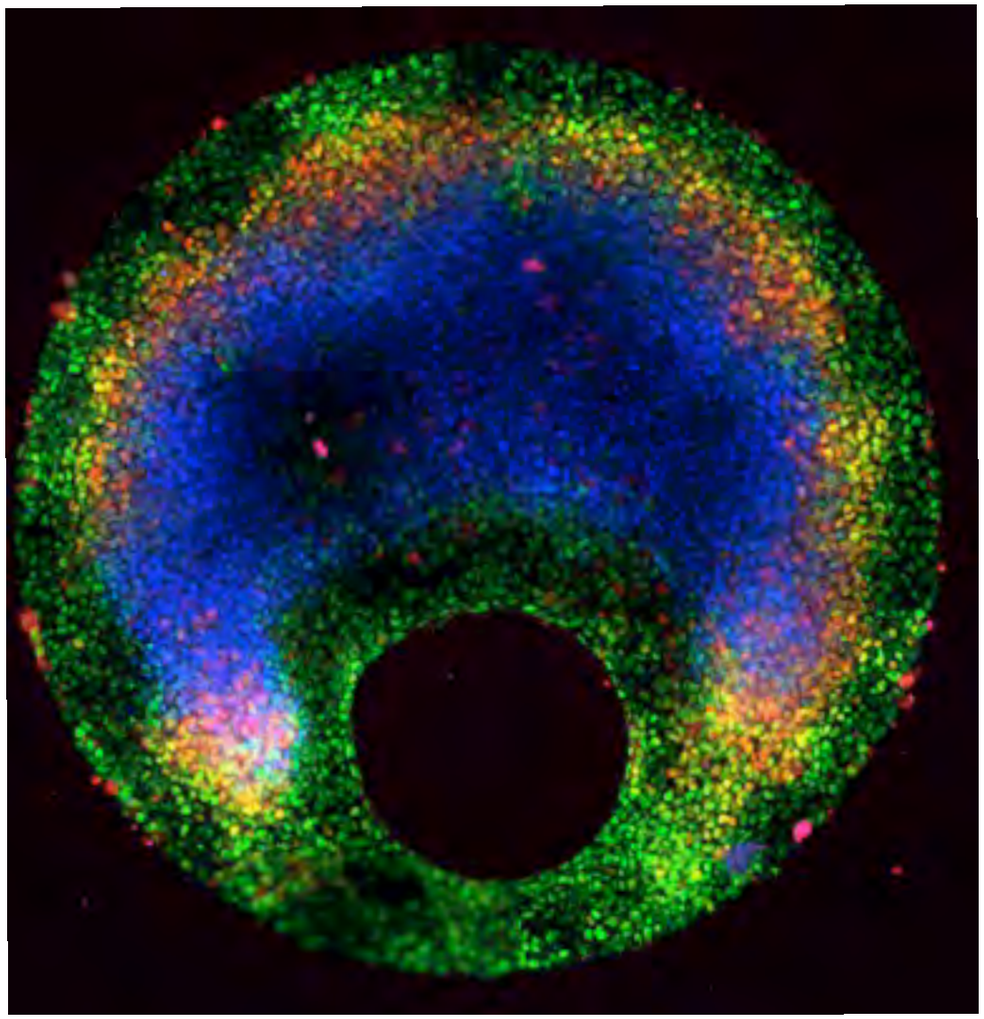
Our Zoo in DAPI



Cells compute fate relative to nearest boundary

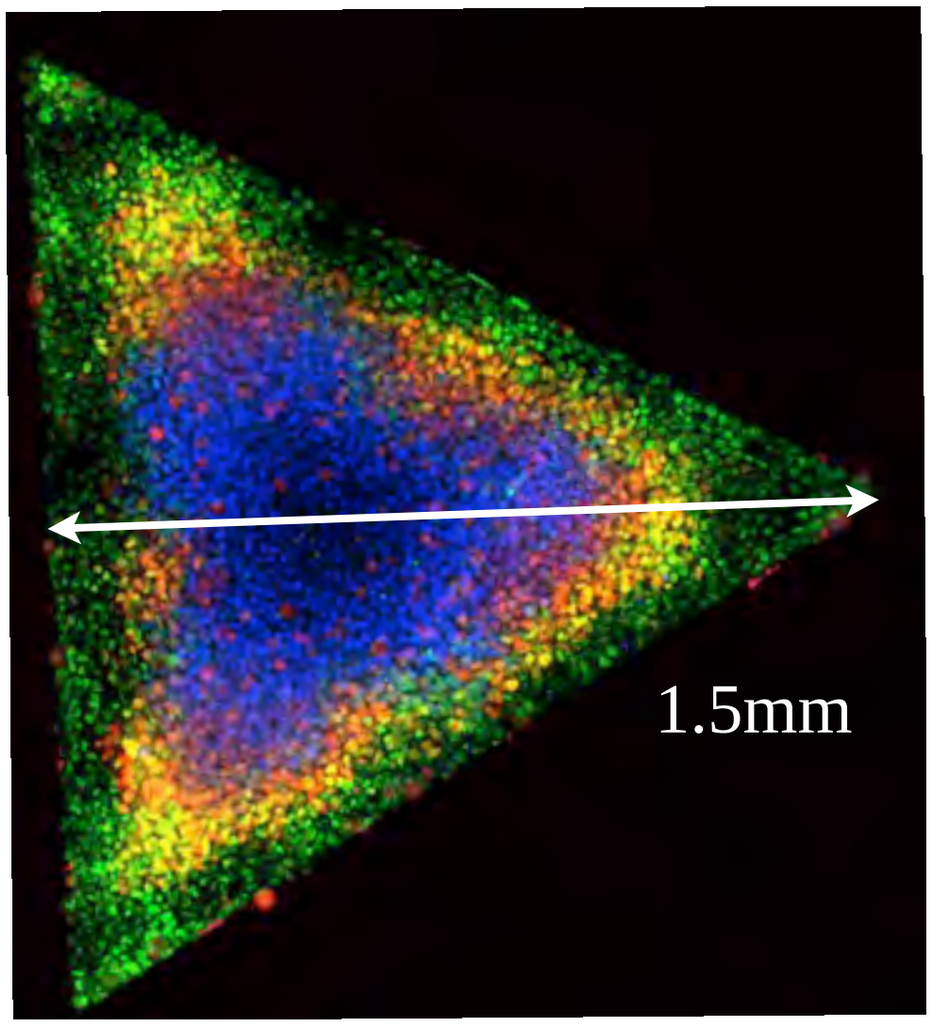
Sox2/Bra/Cdx2

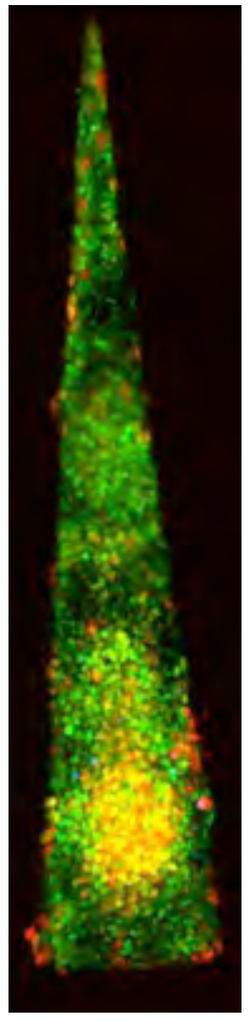


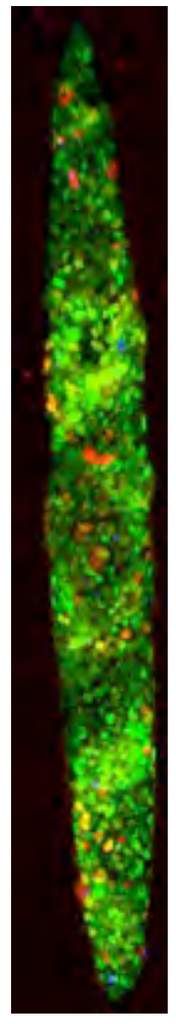


Do disks explain other shapes?

Sox2/Bra/Cdx2

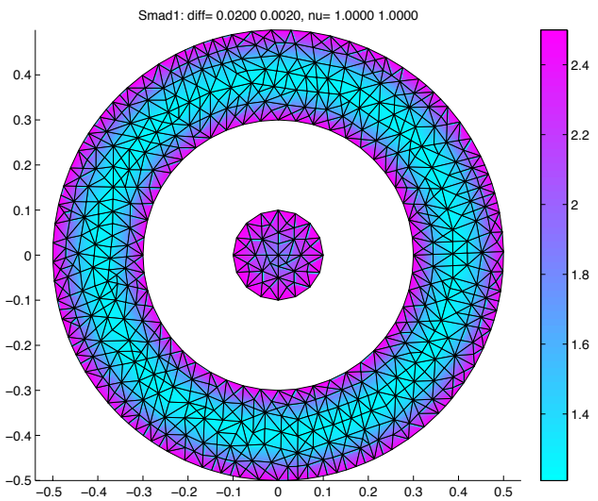




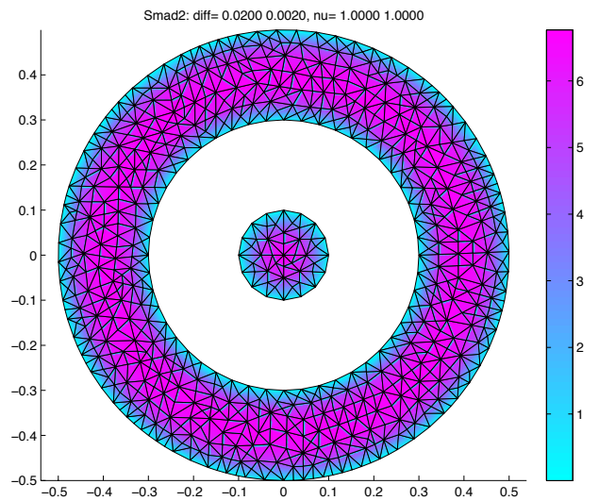


Predictions for other patterns

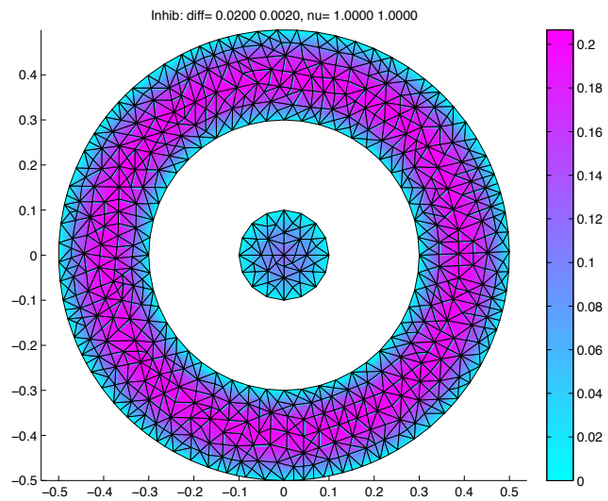
Smad1

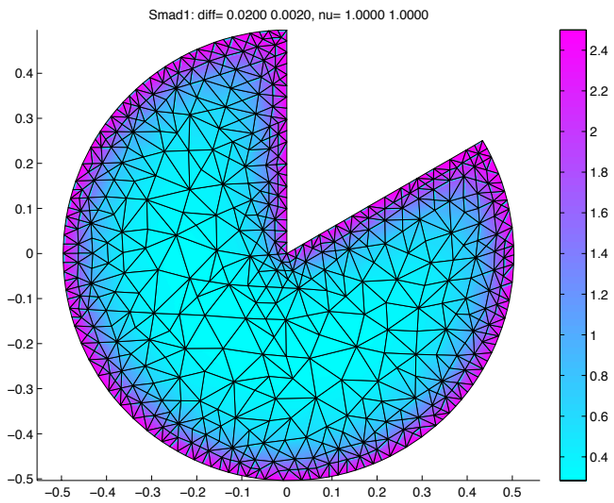


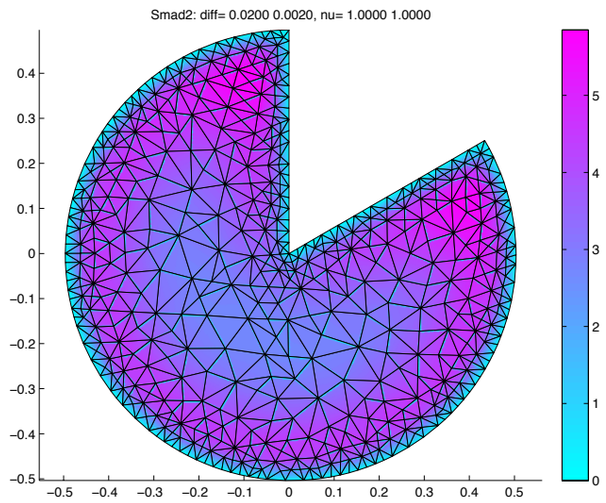
Smad2

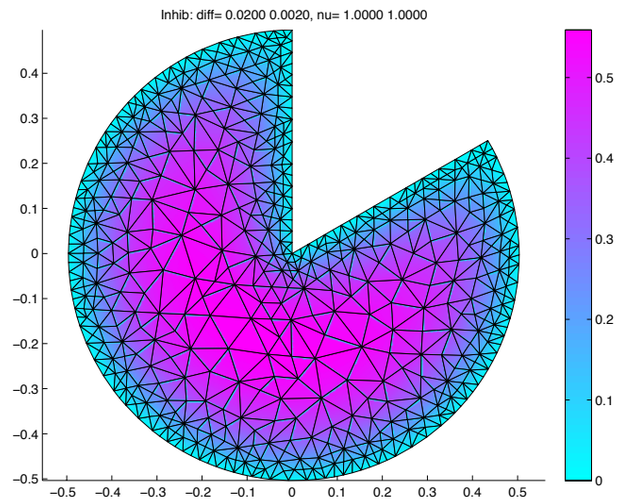


Inhibitor



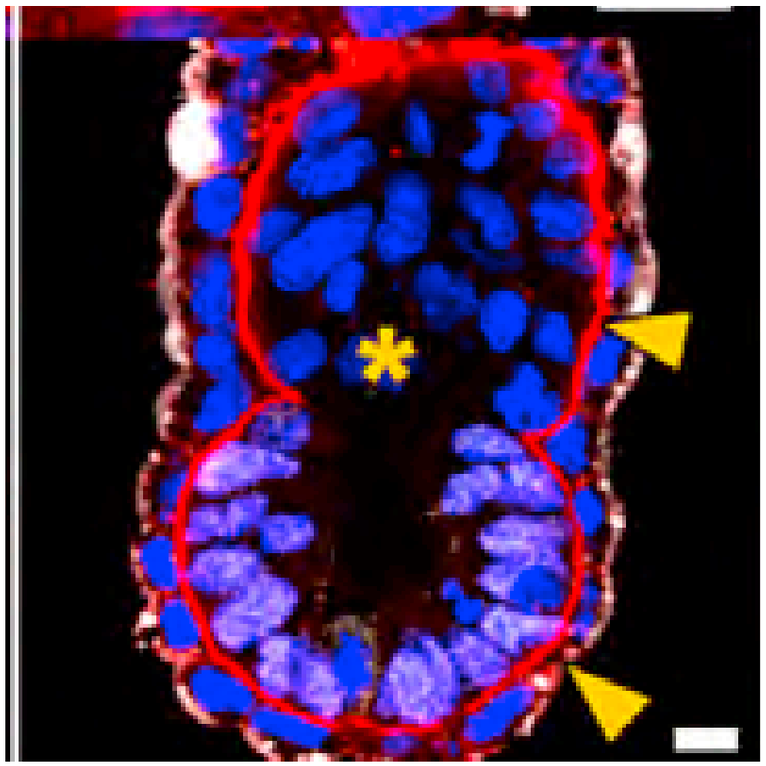






Shapes of patterns are roughly correct but are fates right? Need to look at signaling on shapes...

Experiments of M. Zernicka-Goetz (Cambridge)



E5.5 Laminin membrane surrounds both ExE & epiblast (Oct4 stain White, DAPI), Bedzhov, *Cell*, 2014

How to make an AP axis?

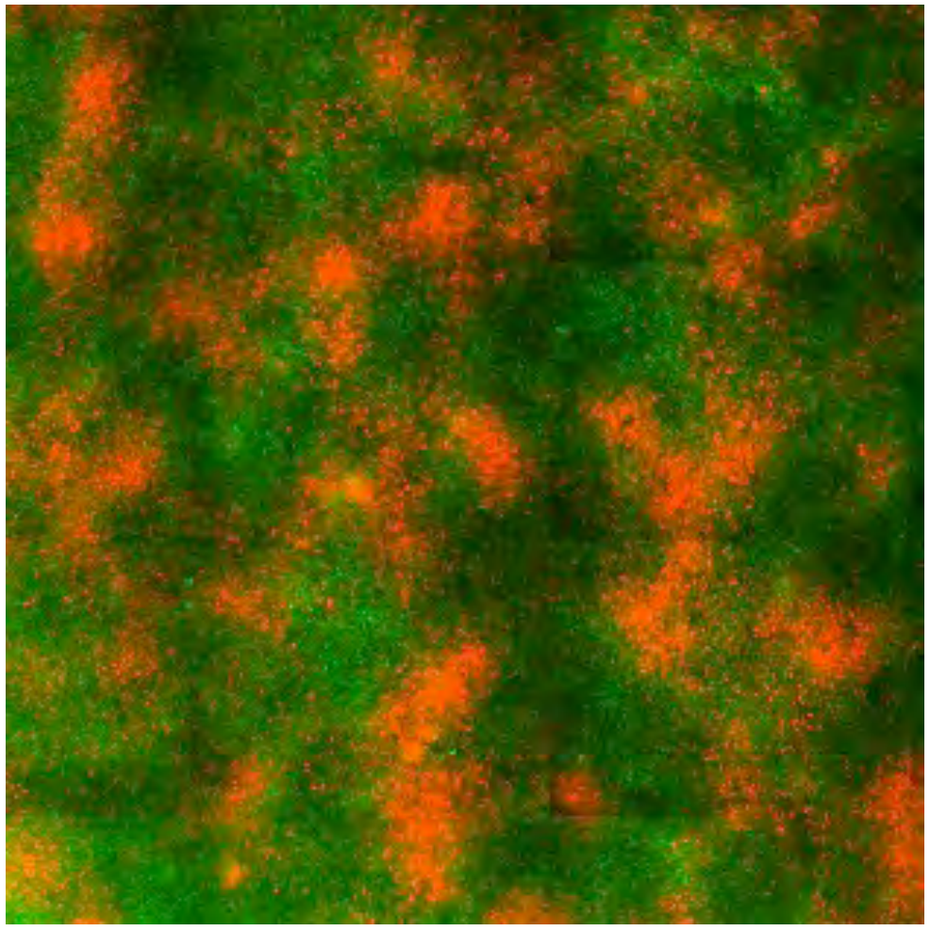
~~Geometric asymmetry does **not** induce AP:~~

Are two epithelial layers needed? NO

One layer: (force expression of Wnt (P) or inhibitors (A), Turing?)

Morphogenic Symmetry breaking ?

Turing patterns when remove boundaries?....



Sox2, Bra 42hrs,
filters, moderate BMP from bottom

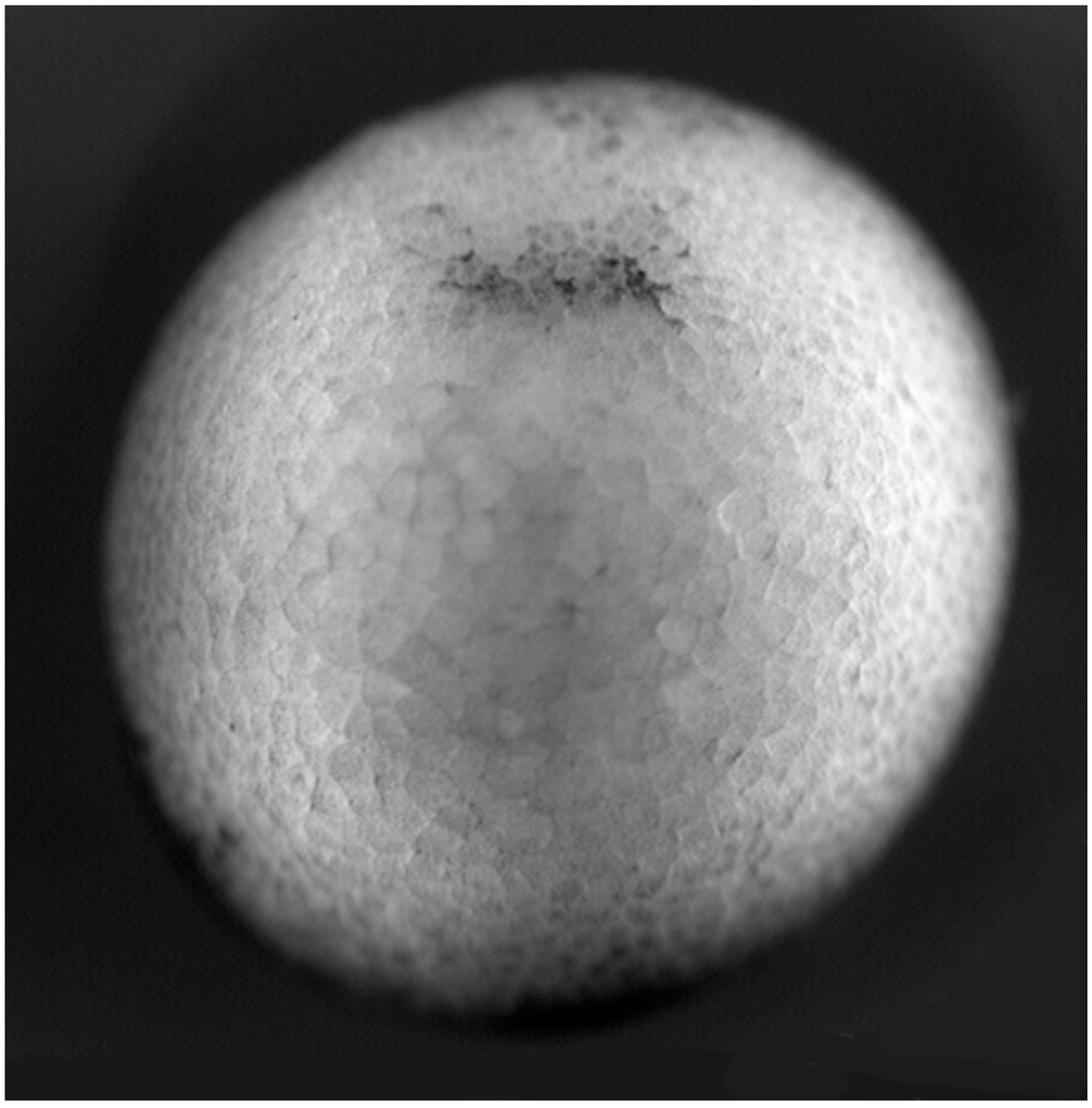
Inhibitor Noggin?
repeat Noggin-/- line
One Bra patch in finite size colony?
microcontact print on filters
Subsequent development?

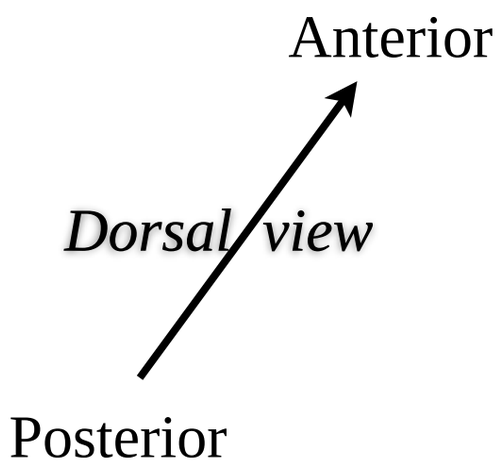
[Wnt , dkk1] patterns?

Xenopus gastrulation

1.2mm egg

17hrs @23C Movie





Why work with hESC on micropatterns?

Minimal spatial confinement, open system --> flattened embryo

Assay for paracrine signaling.. vs genetics

Genome encodes r-t structure,.. how? (build it-->understand)

Gastrulation/Primitive Streak

Builds 3D structure

Epithelial to mesenchymal transition: access to signaling (cooperative?

Cell biology of patterning

Polarized epithelia: receptors.

Production and movement of inhibitors.

--> Questions to check in mouse embryo

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)