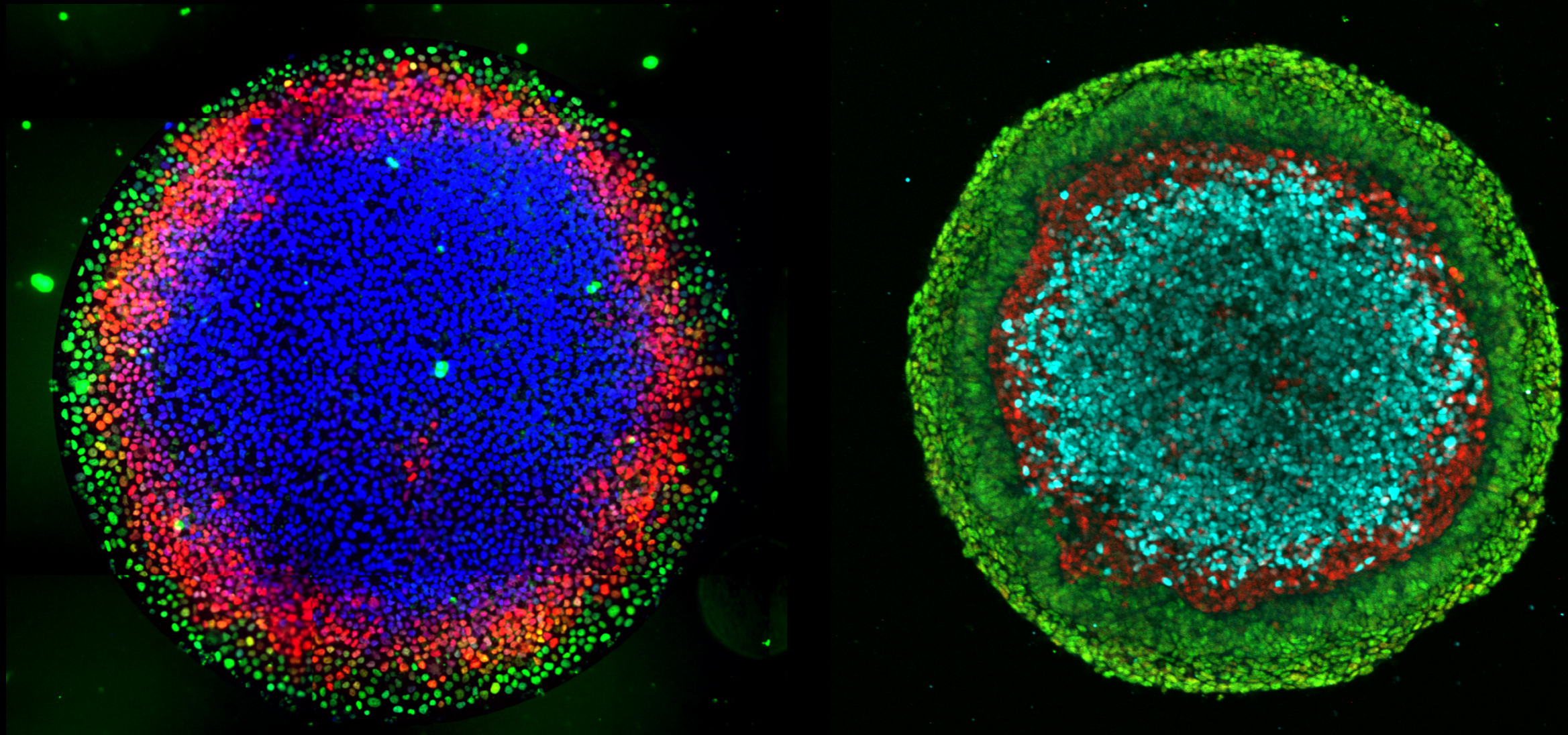


Self-organized patterning in human embryonic stem cells



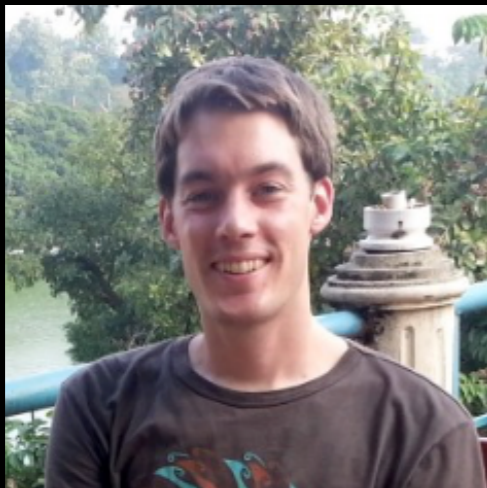
Aryeh Warmflash
Department of Biosciences
Rice University
<http://stemcell.rice.edu>
@WarmflashLab

Outline:

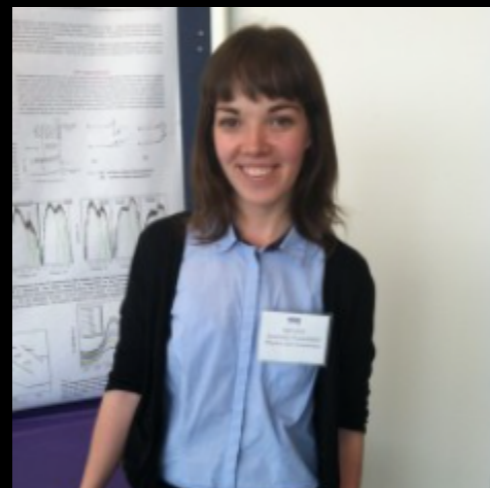
1. Understanding patterning during mammalian gastrulation in hESCs
2. Ectoderm patterning: similarities and differences with gastrulation

Outline:

1. Understanding patterning during mammalian gastrulation in hESCs
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Idse
Heemskerk



Anastasiia
Nemashkalo

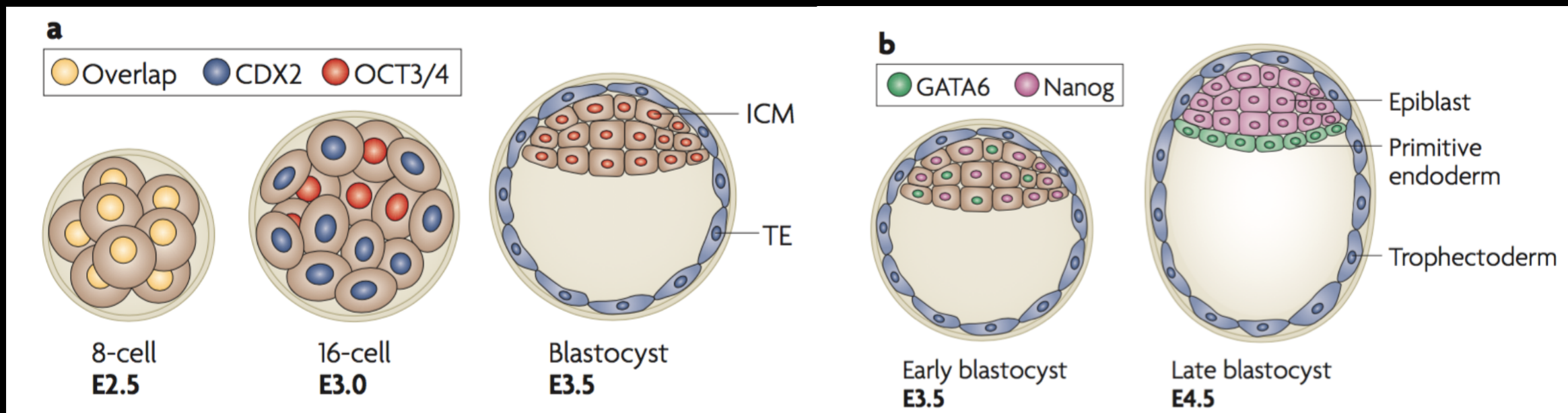


Sapna
Chhabra

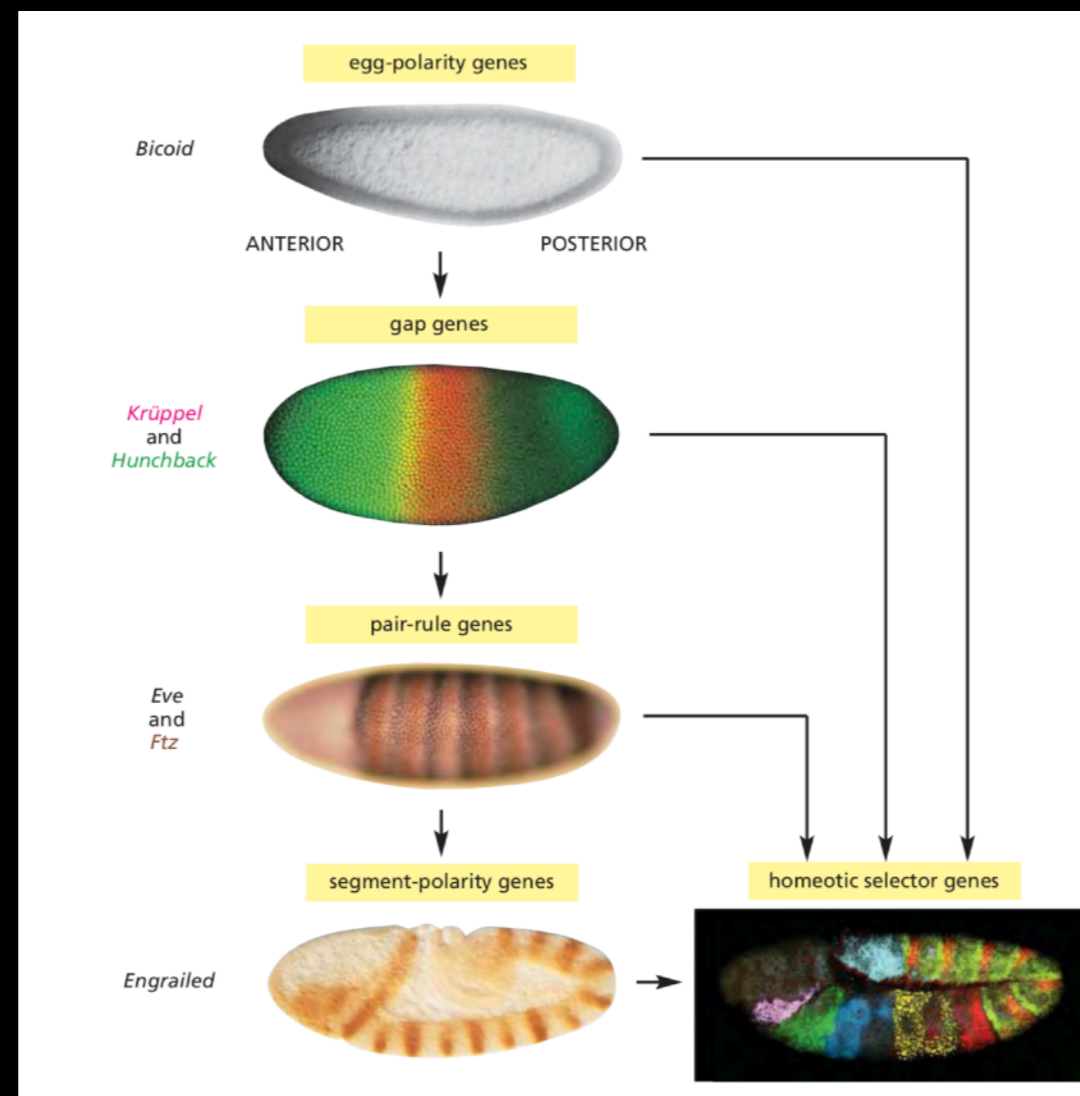
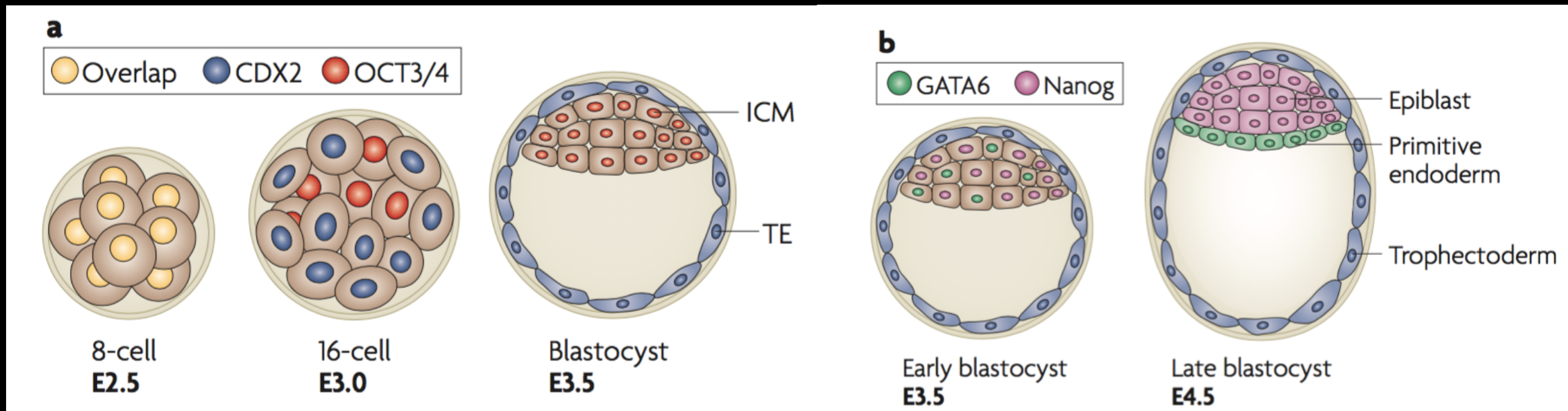


Joseph
Massey

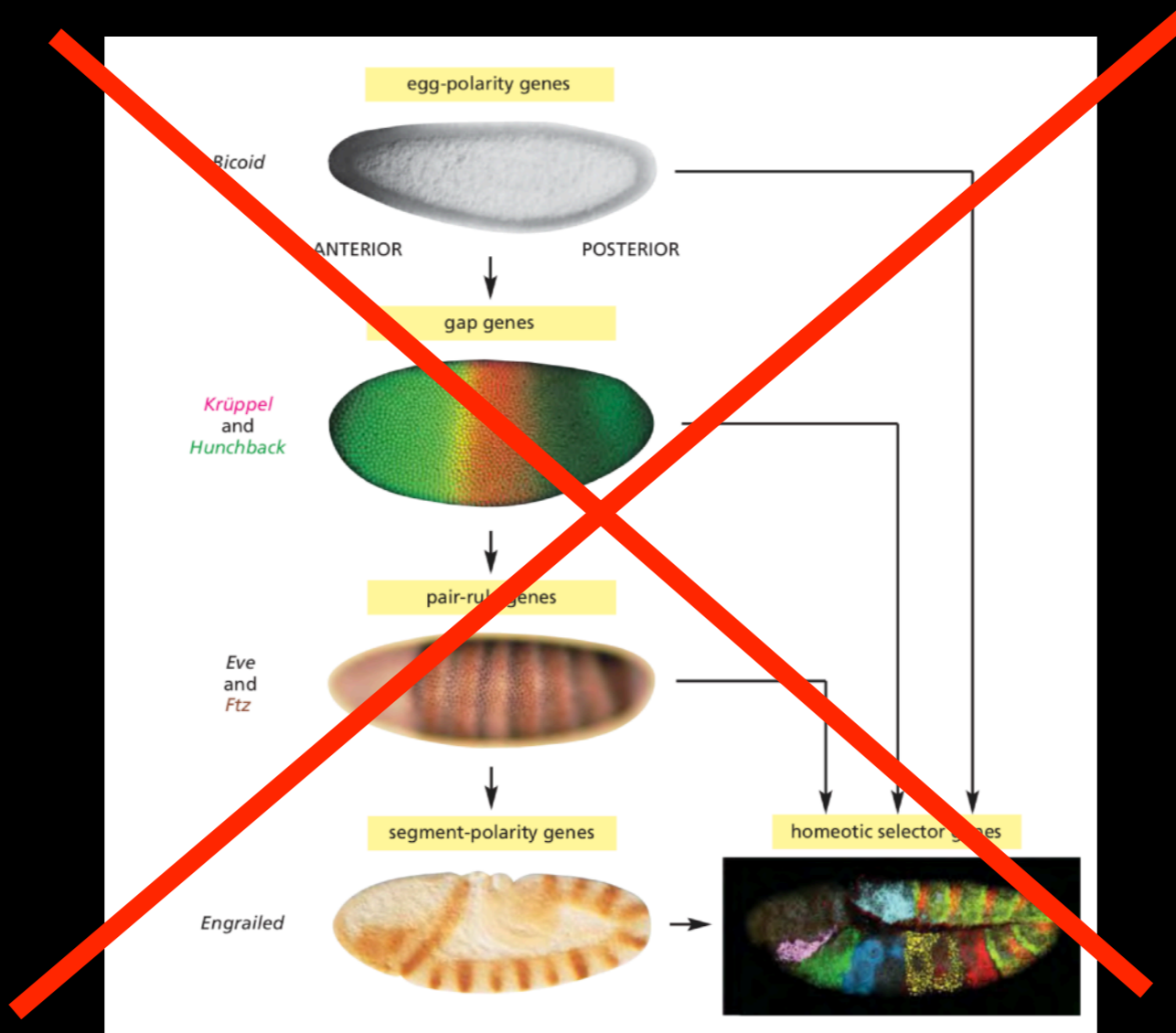
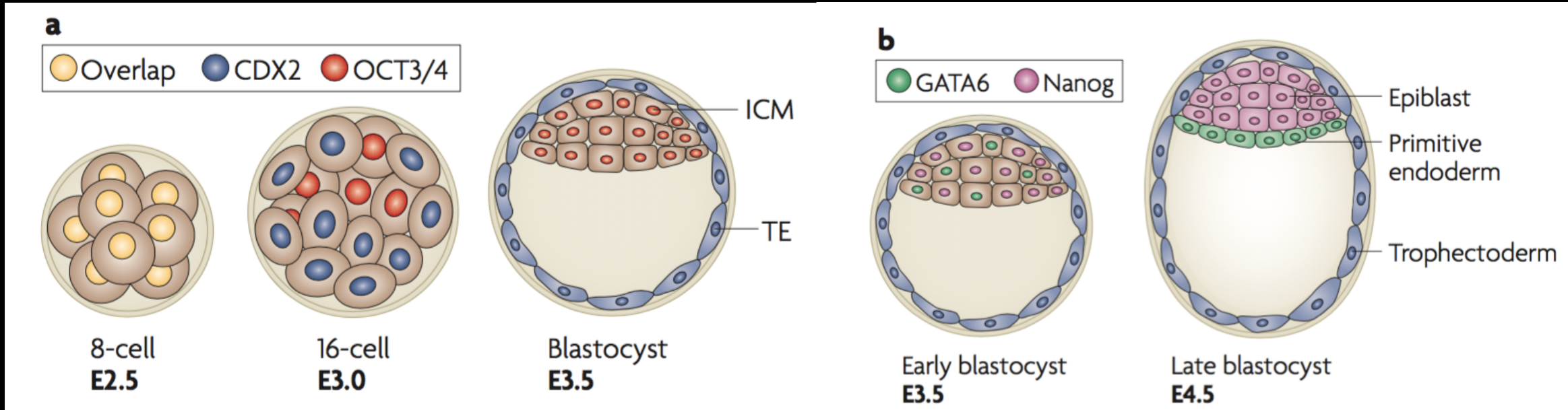
How is the mammalian embryo patterned at gastrulation?



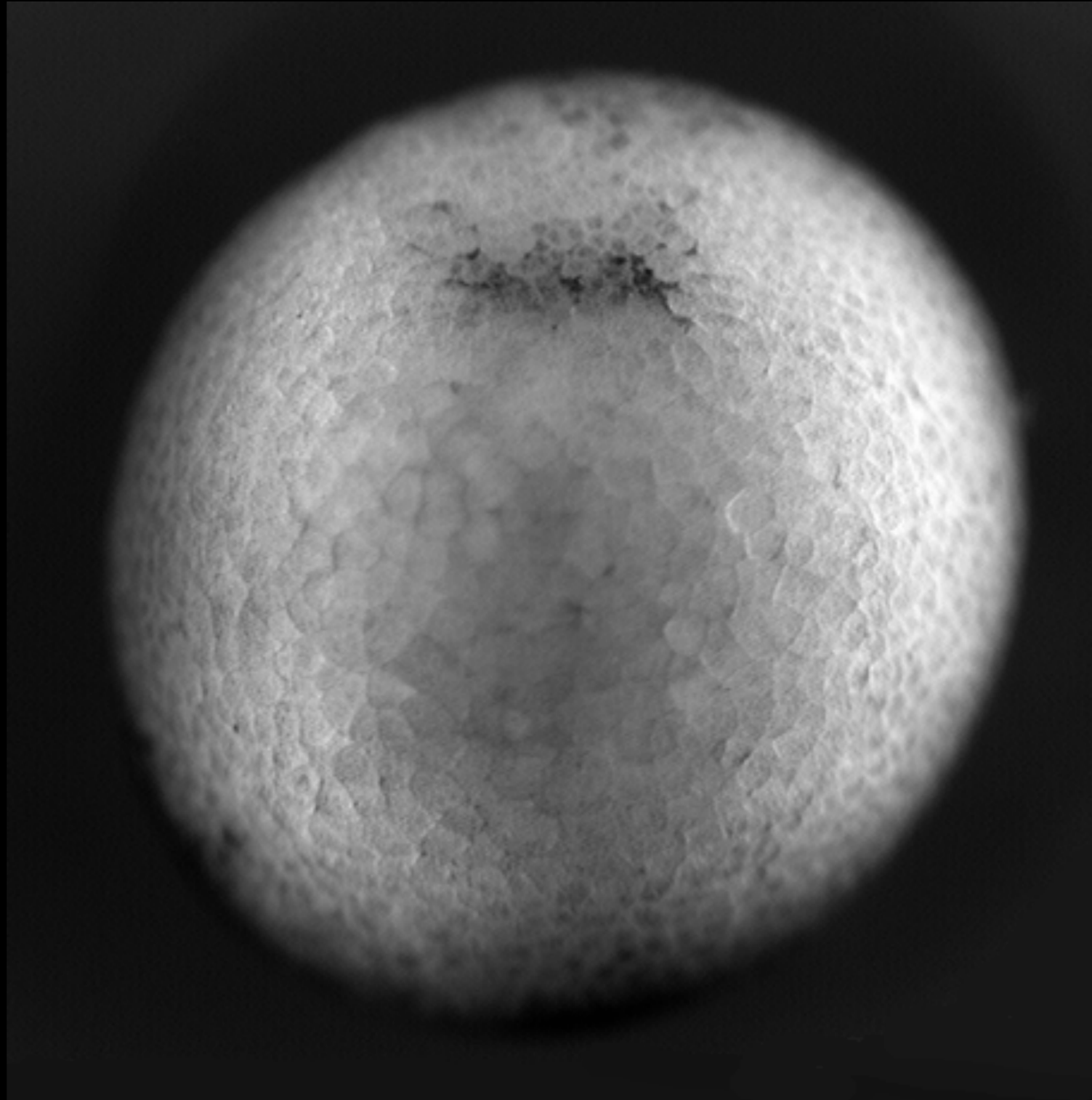
How is the mammalian embryo patterned at gastrulation?



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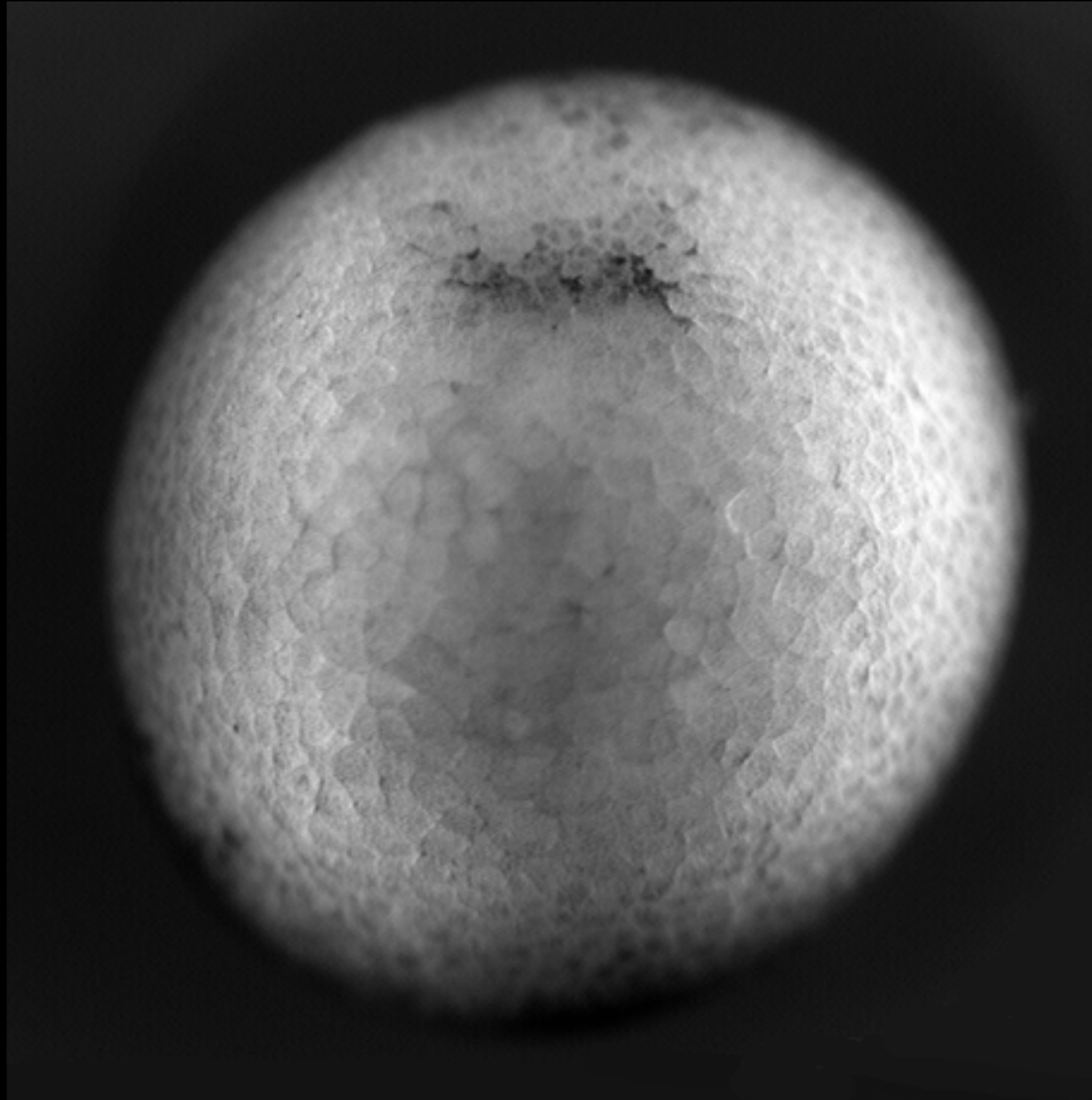


Gastrulation



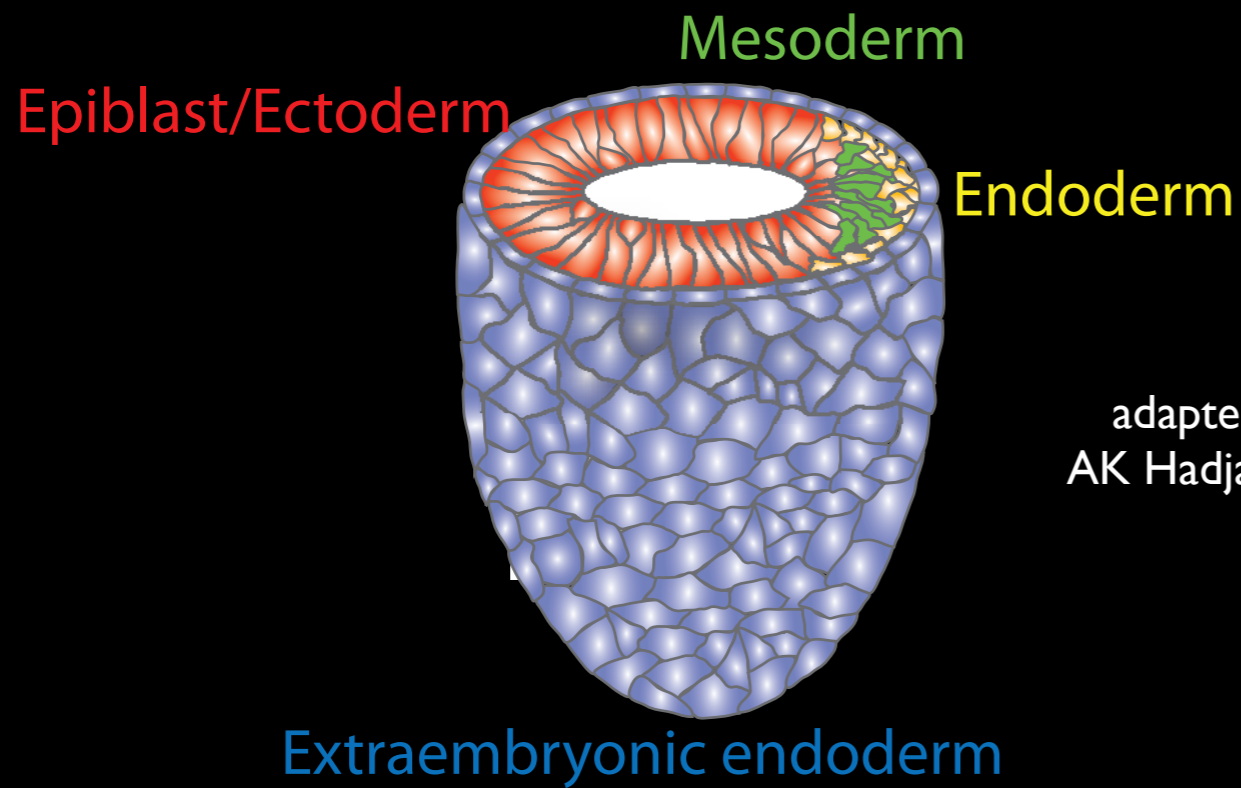
D Shook

Gastrulation

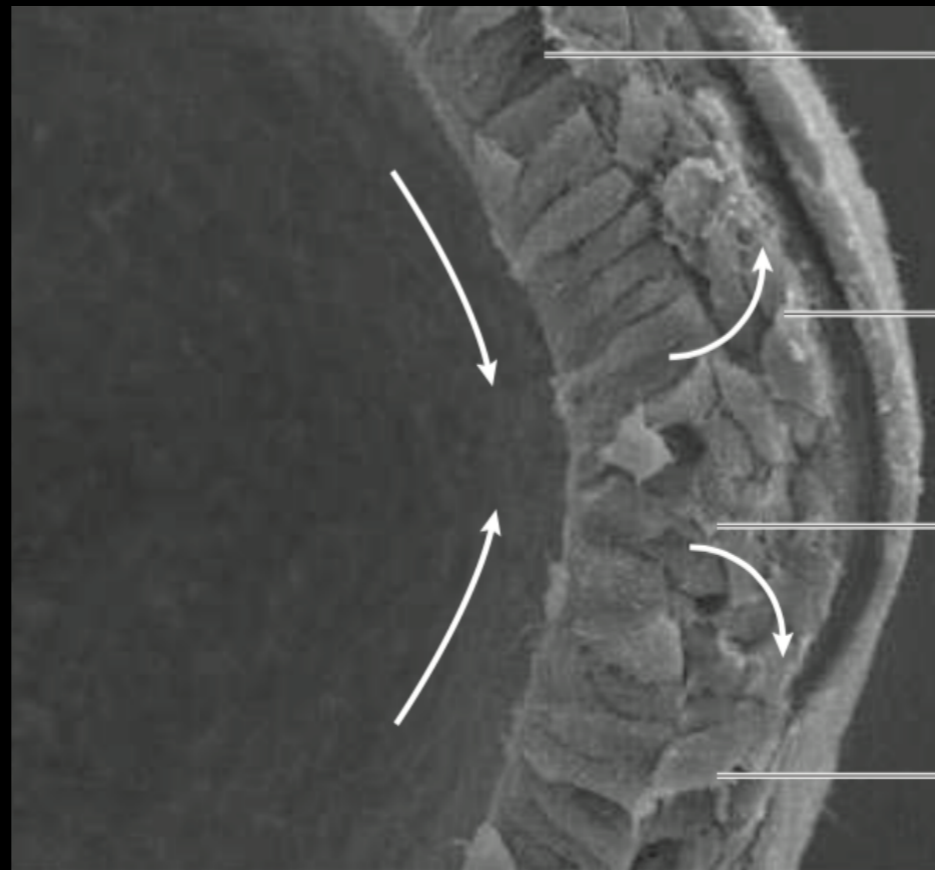
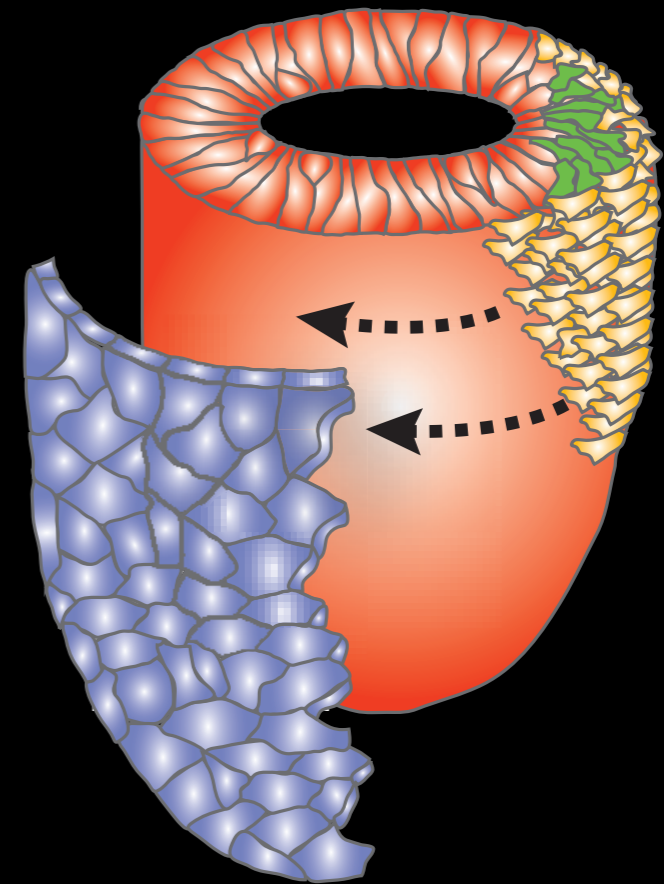


D Shook

Gastrulation - creating the three germ layers



adapted from
AK Hadjantonakis



Arnold & Robertson
Nat Rev Mol Cell Biol 2009

How is the embryo patterned during gastrulation?

Review

Dose-dependent Nodal/Smad signals pattern the early mouse embryo



Elizabeth J. Robertson*

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK

What is the evidence for this?

1. Expression of morphogens

Nodal^{lacZ} reporter allele



Brennan et al Nature 2001

How is the embryo patterned during gastrulation?

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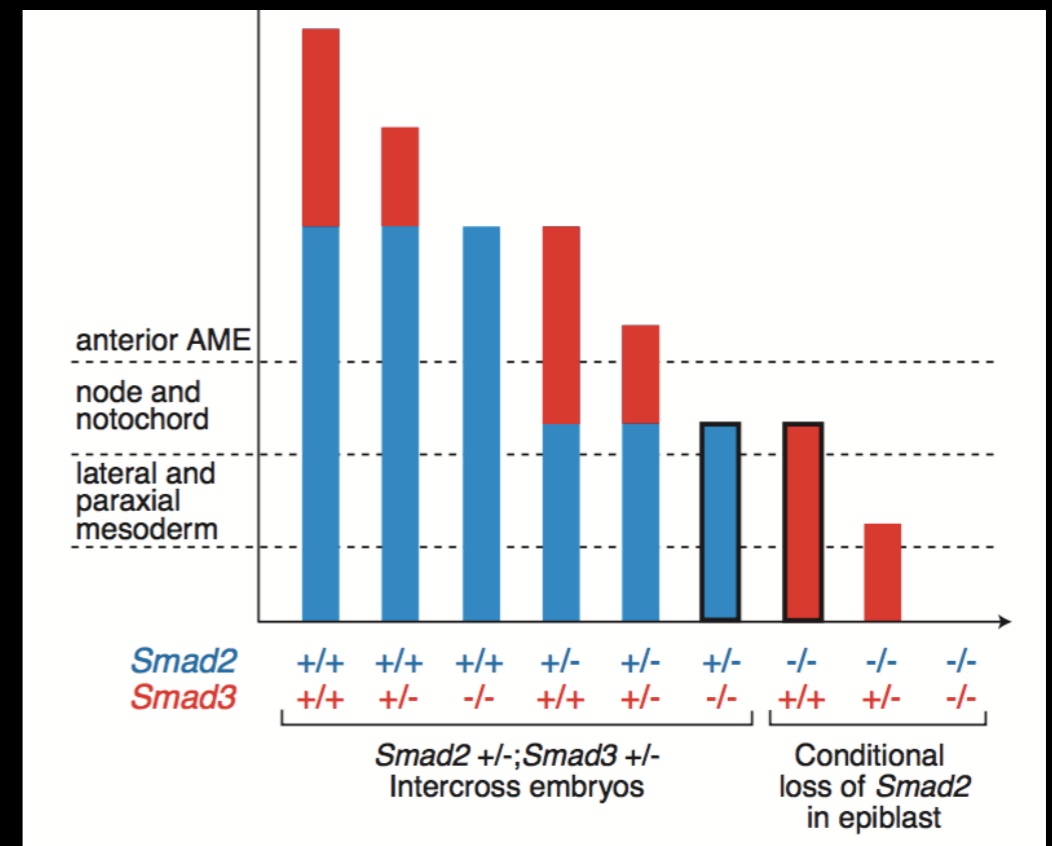
1. Expression of morphogens

2. Phenotypes of knockouts

Nodal^{lacZ} reporter allele

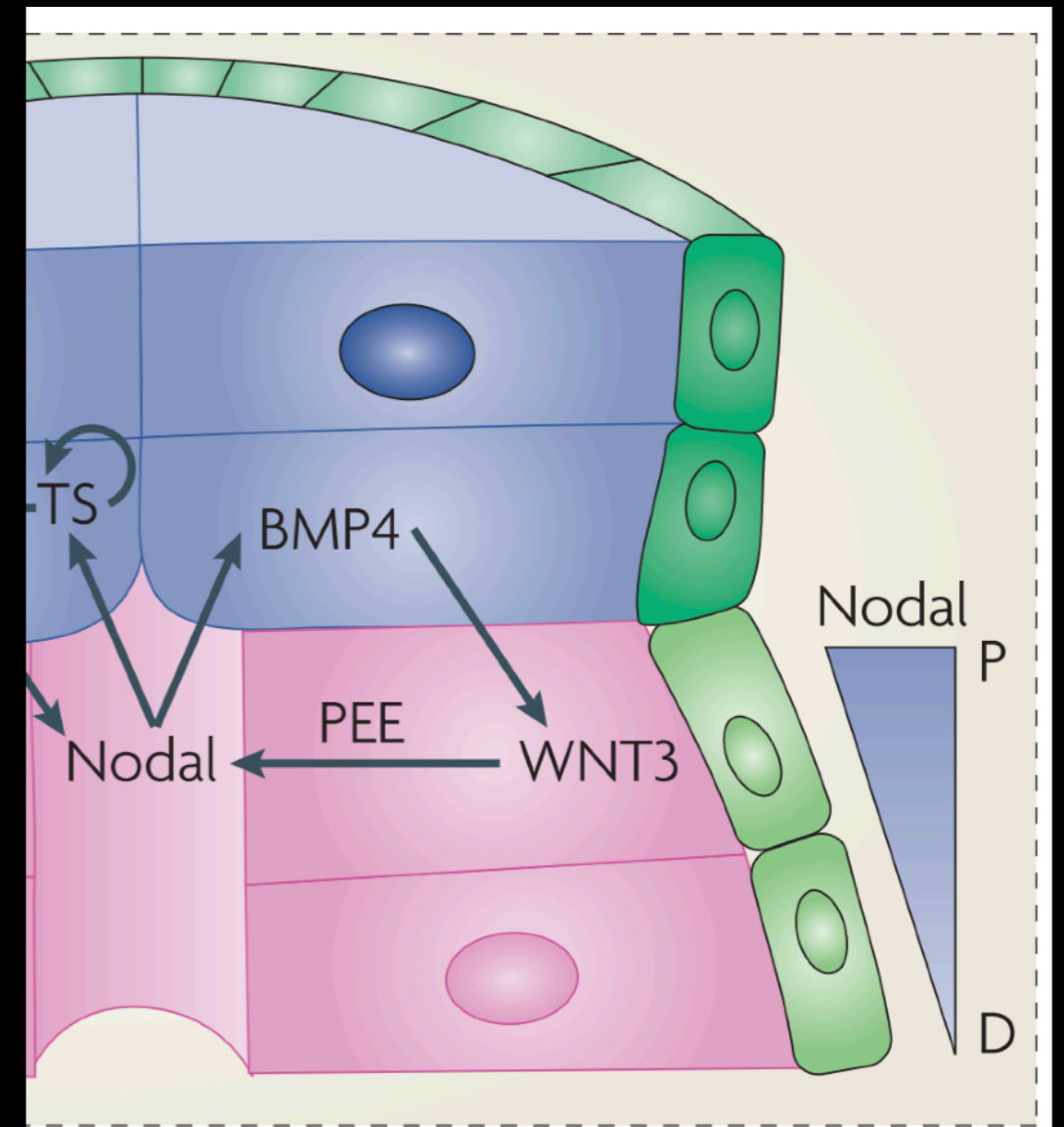
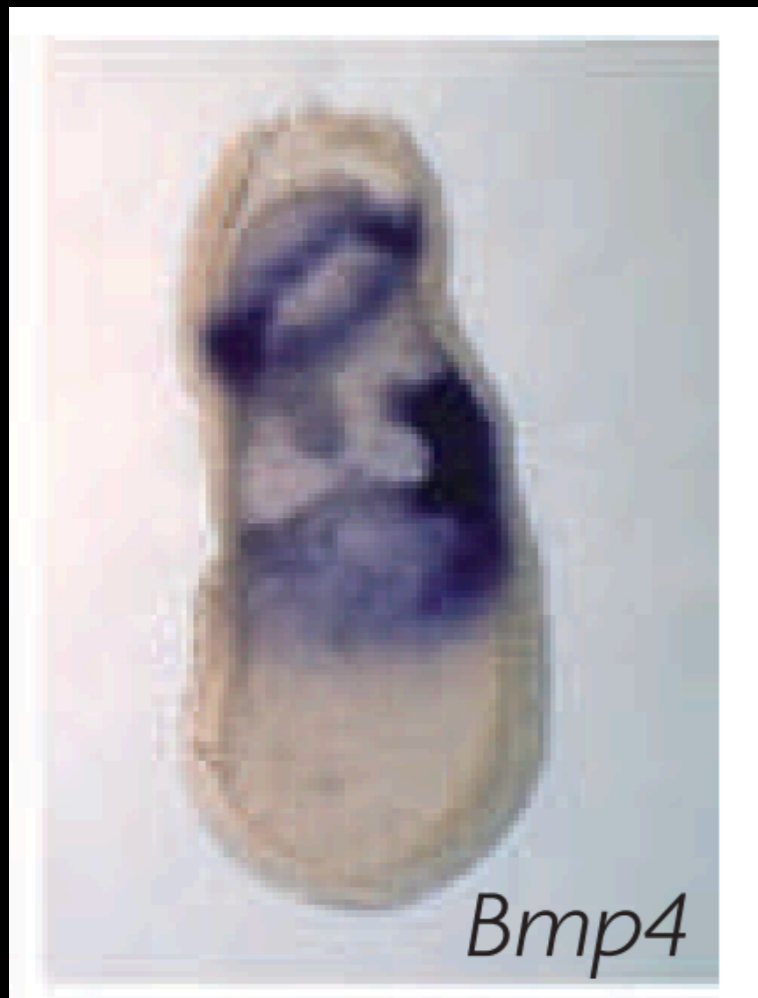


Brennan et al Nature 2001

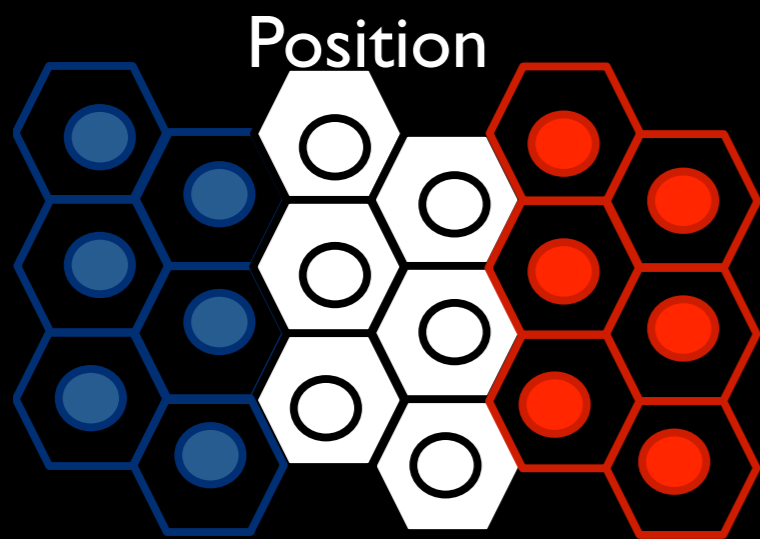
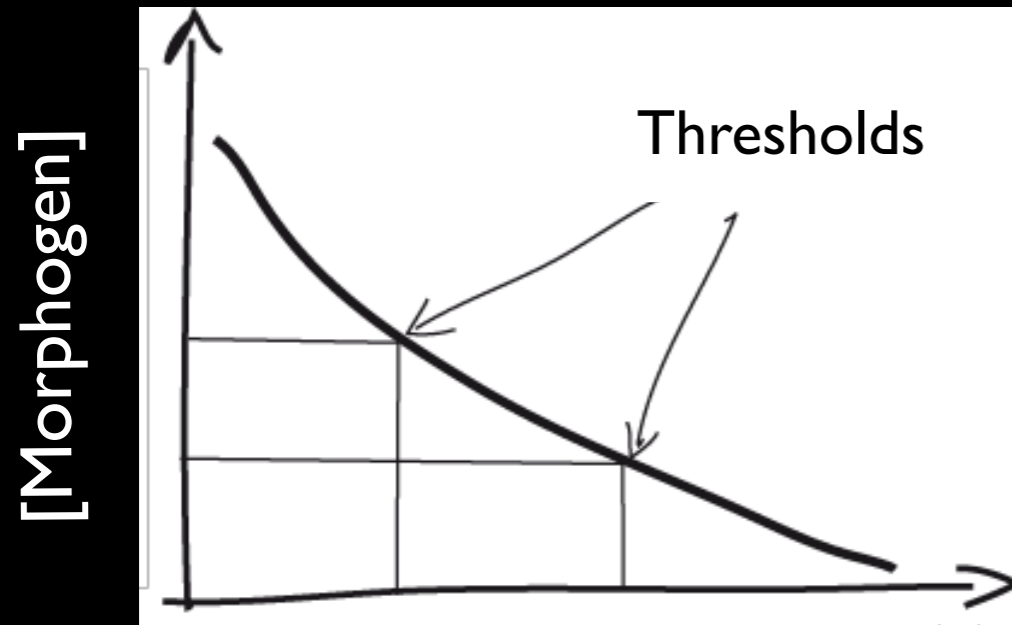


Dunn et al Development 2004

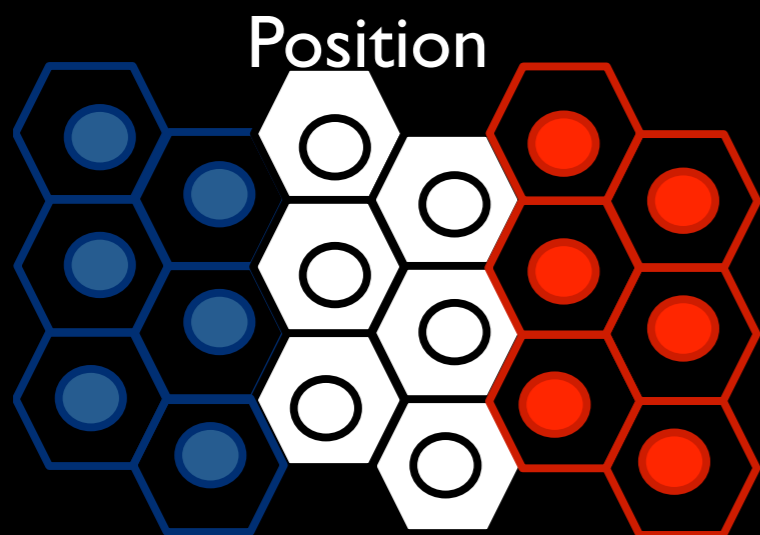
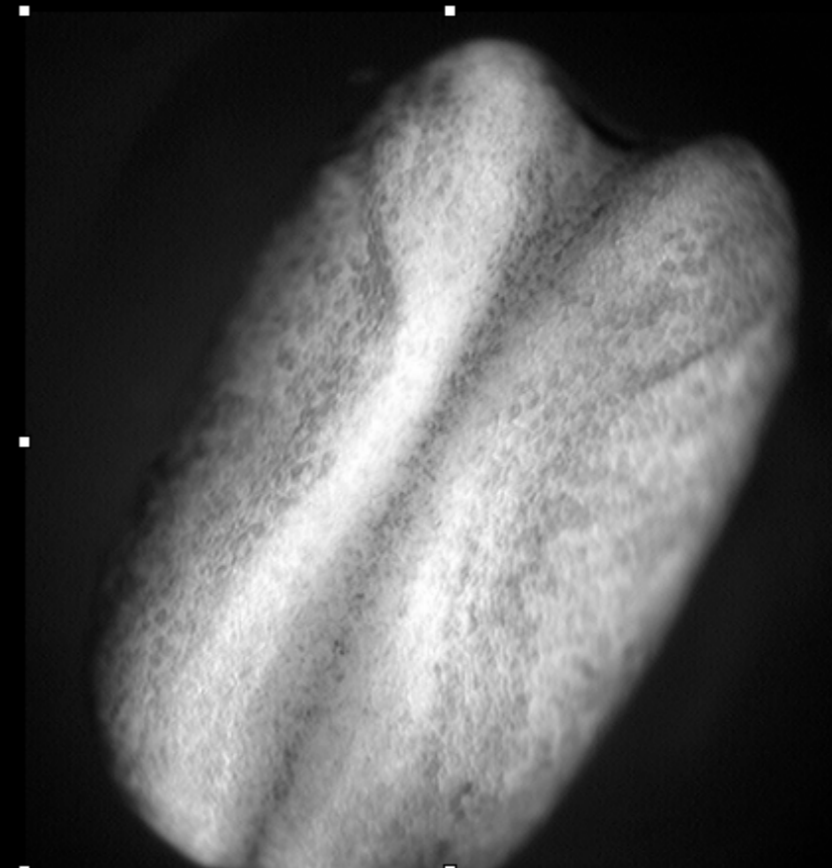
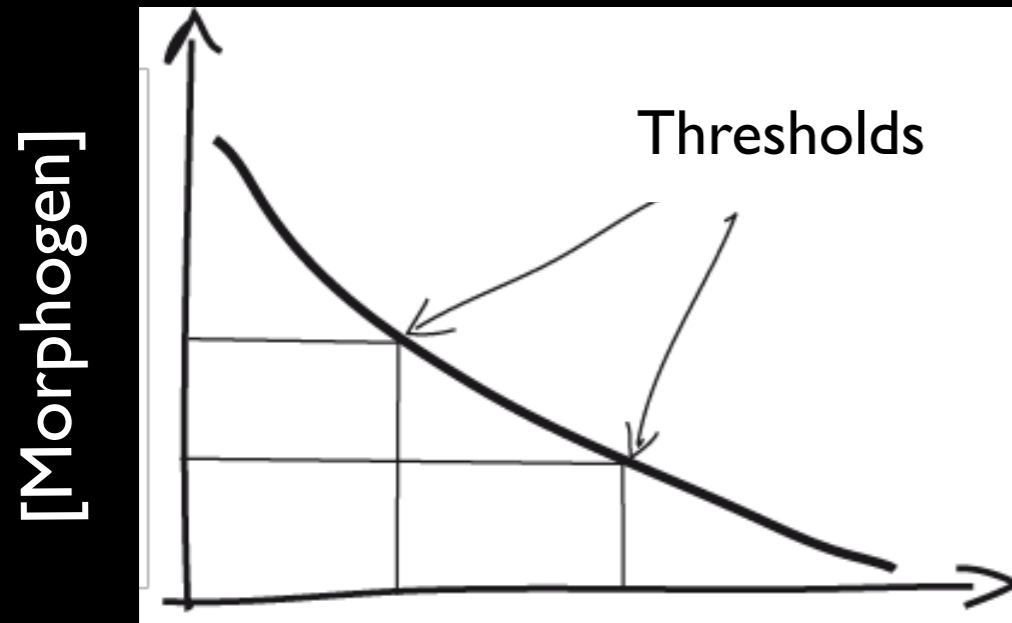
Nodal is one of three pathways essential for patterning at gastrulation



This data could be consistent with a simple French flag model,
but also with many other models



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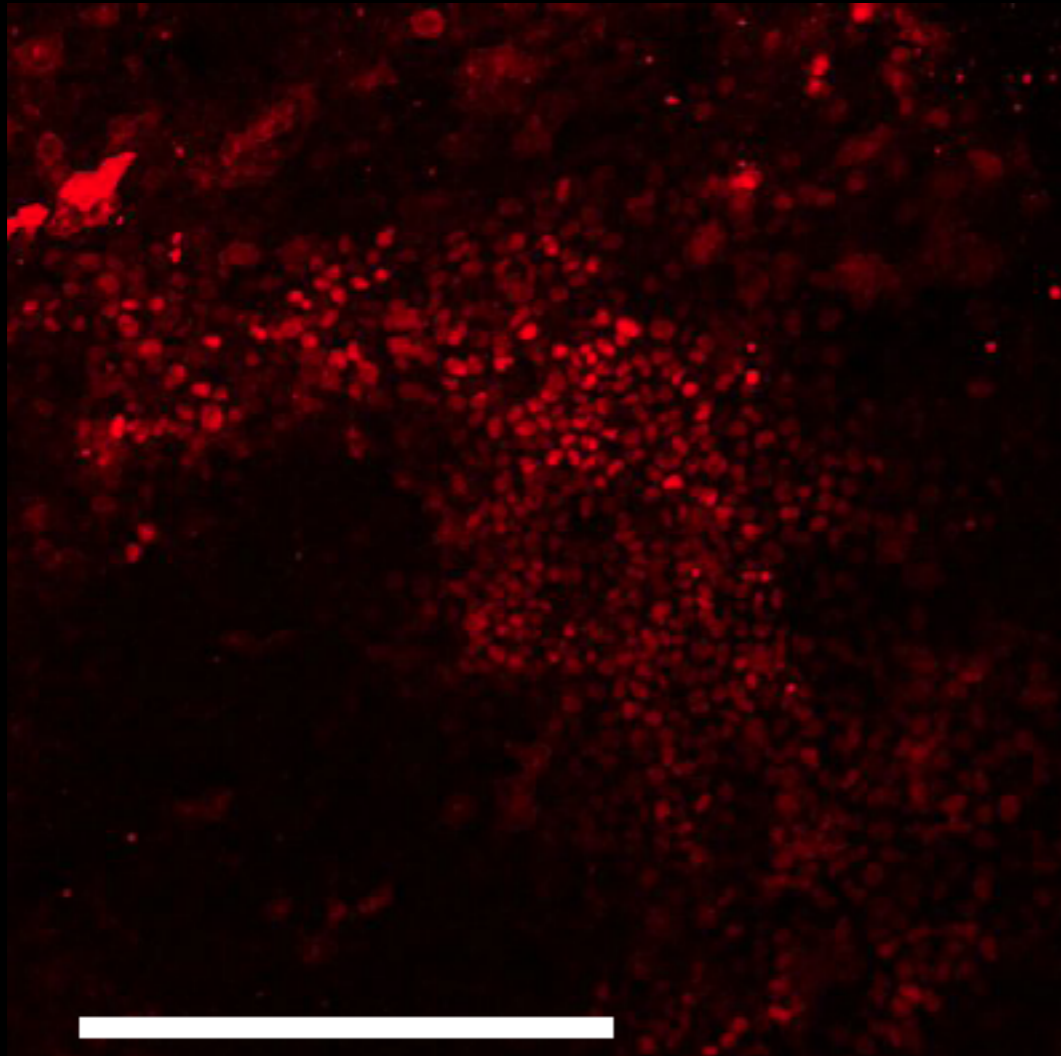


How do you put a French flag on a gastrulating embryo?

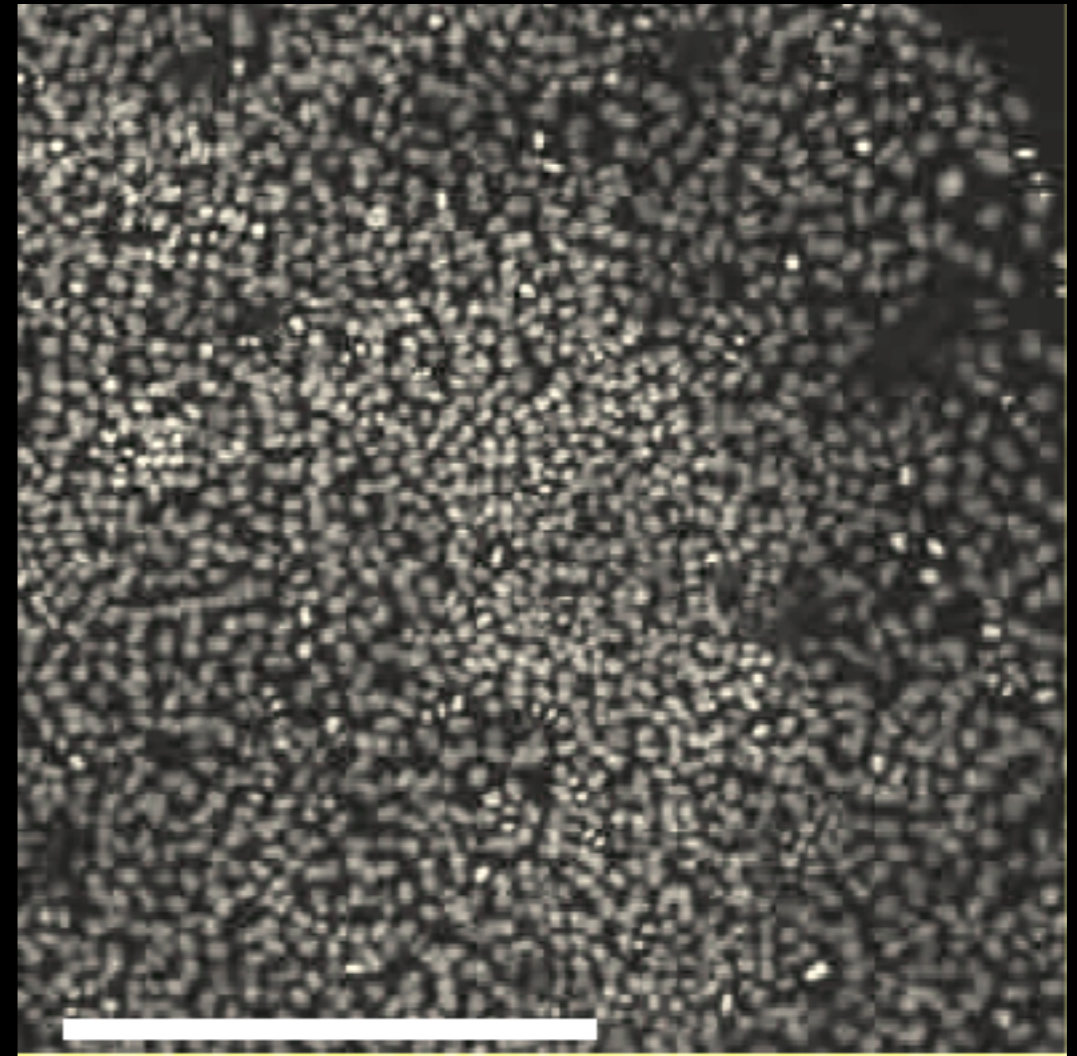
...but patterning, morphogenesis, and cell division all occur simultaneously.

Simple application of BMP4 produces spatially disorganized differentiation

Brachyury (mesoderm)



DAPI



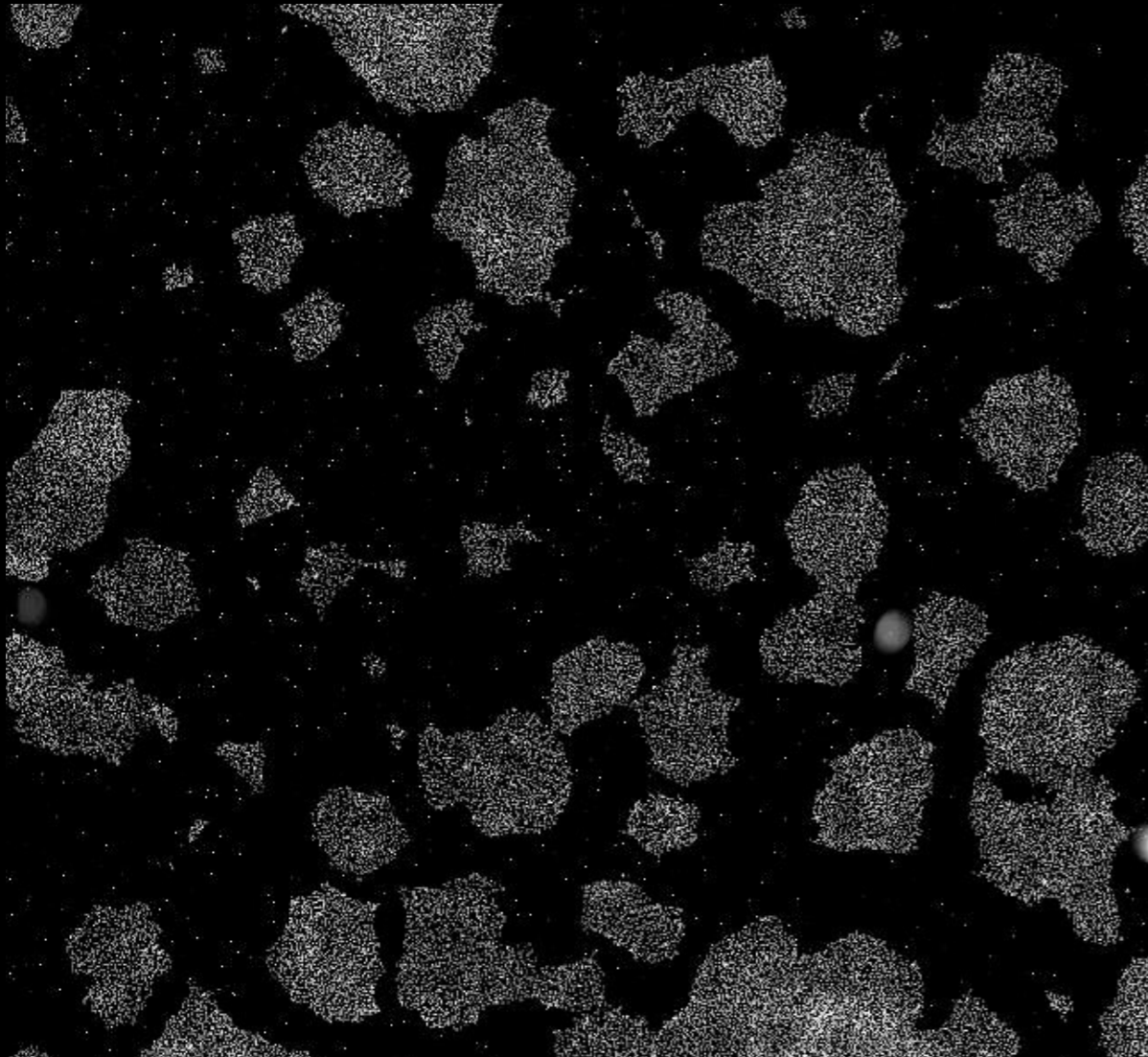
48 hours BMP4 treatment. What is the source of this variability?

Micropatterned surfaces can be used to grow colonies of defined size and shape

DAPI

Standard culture

1mm

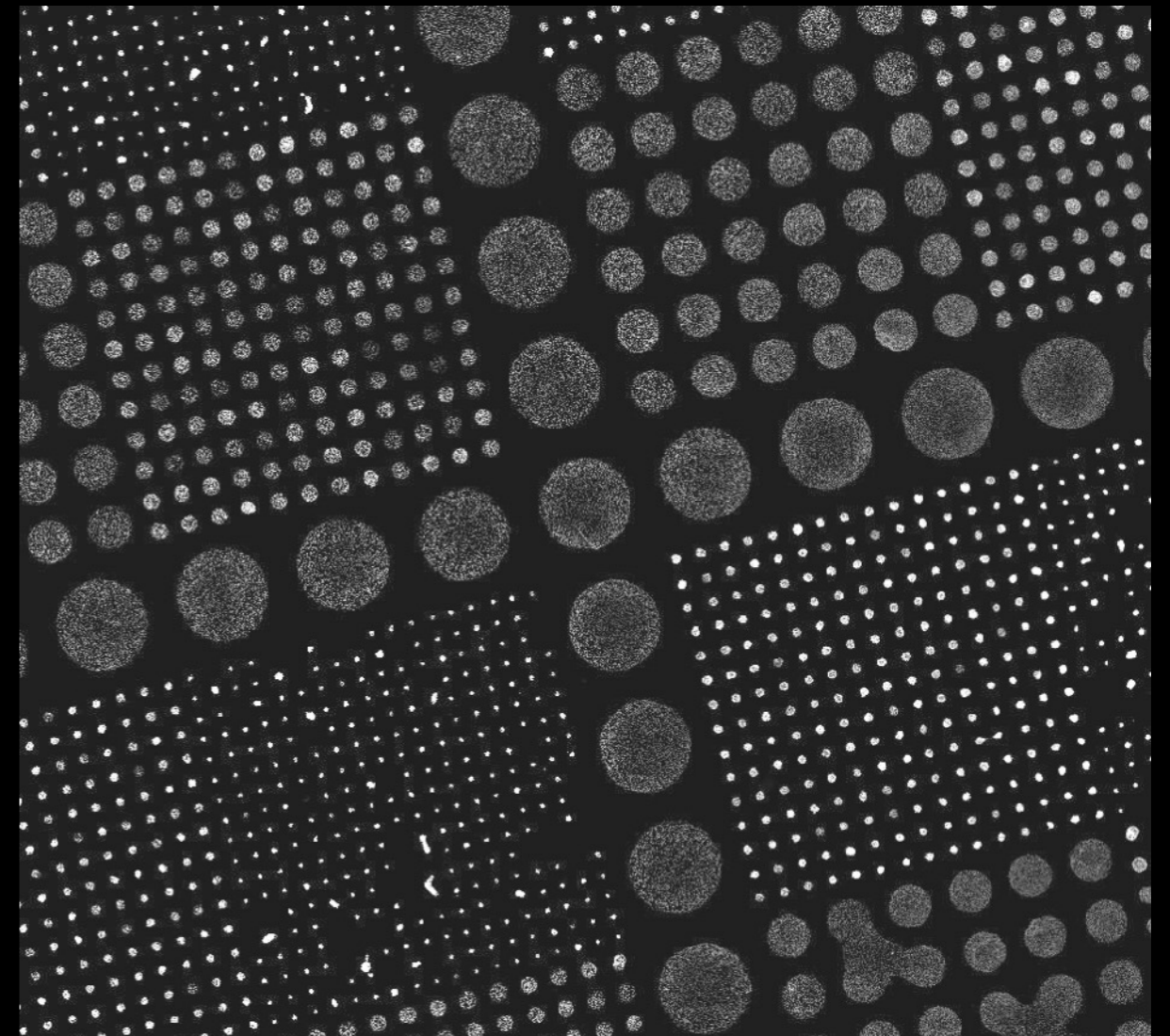
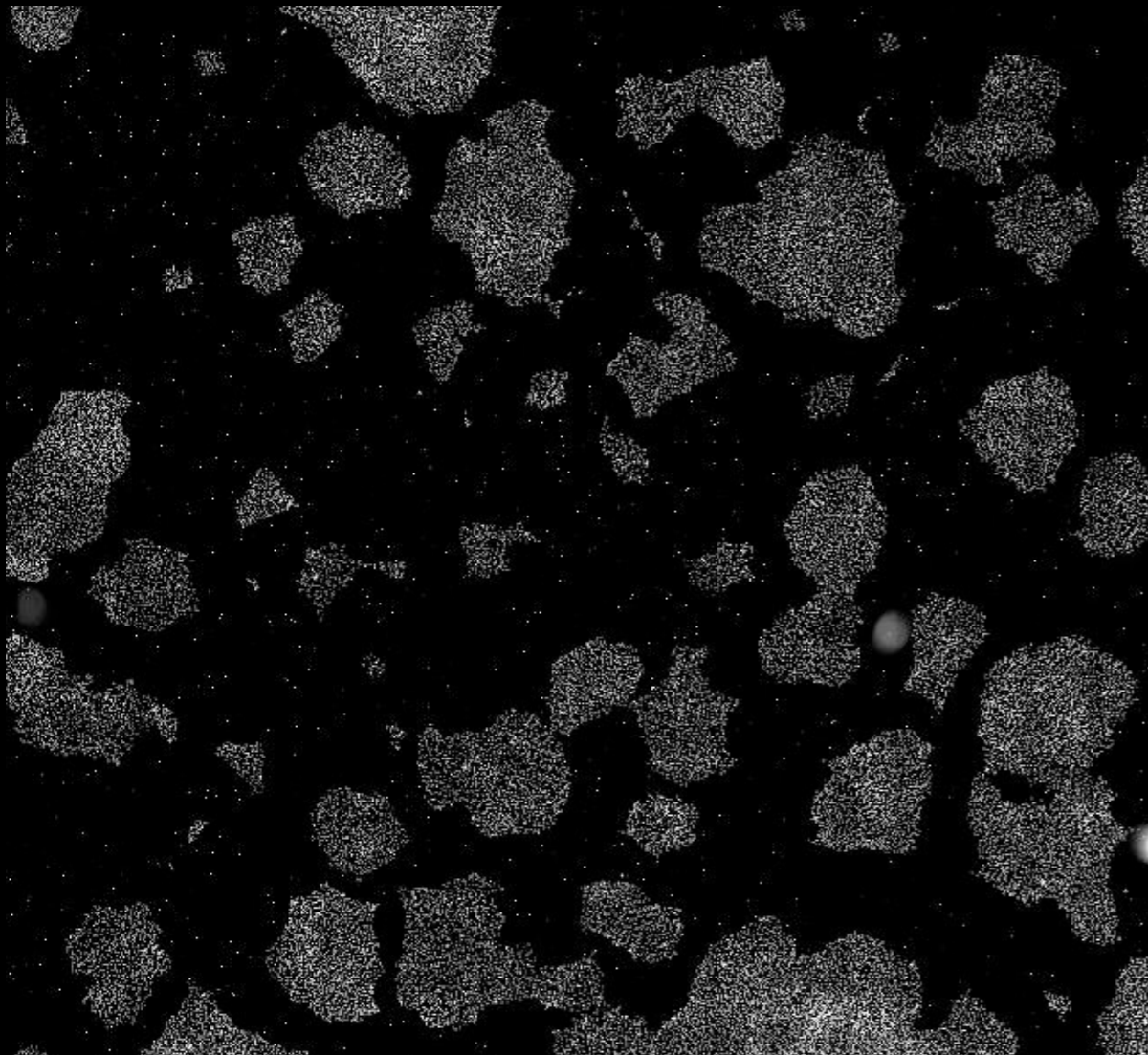
Micropatterned surfaces can be used to grow colonies of defined size and shape

DAPI

Standard culture

1mm

Micropatterned culture



Colony sizes ranging from 80-1000um

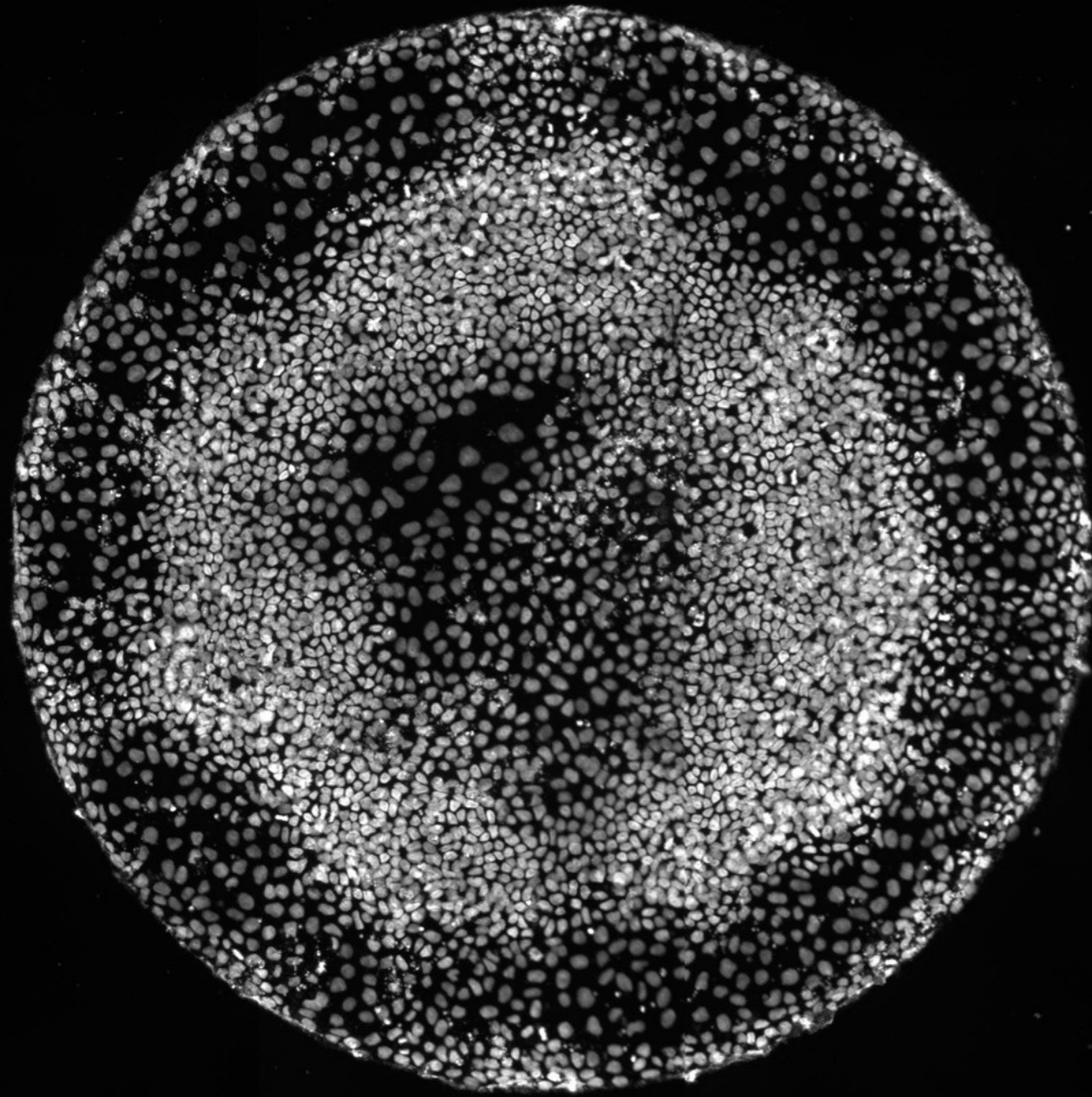
Geometric confinement triggers self-organized patterning along the radial axis

Nuclei (DAPI)

Fate Markers

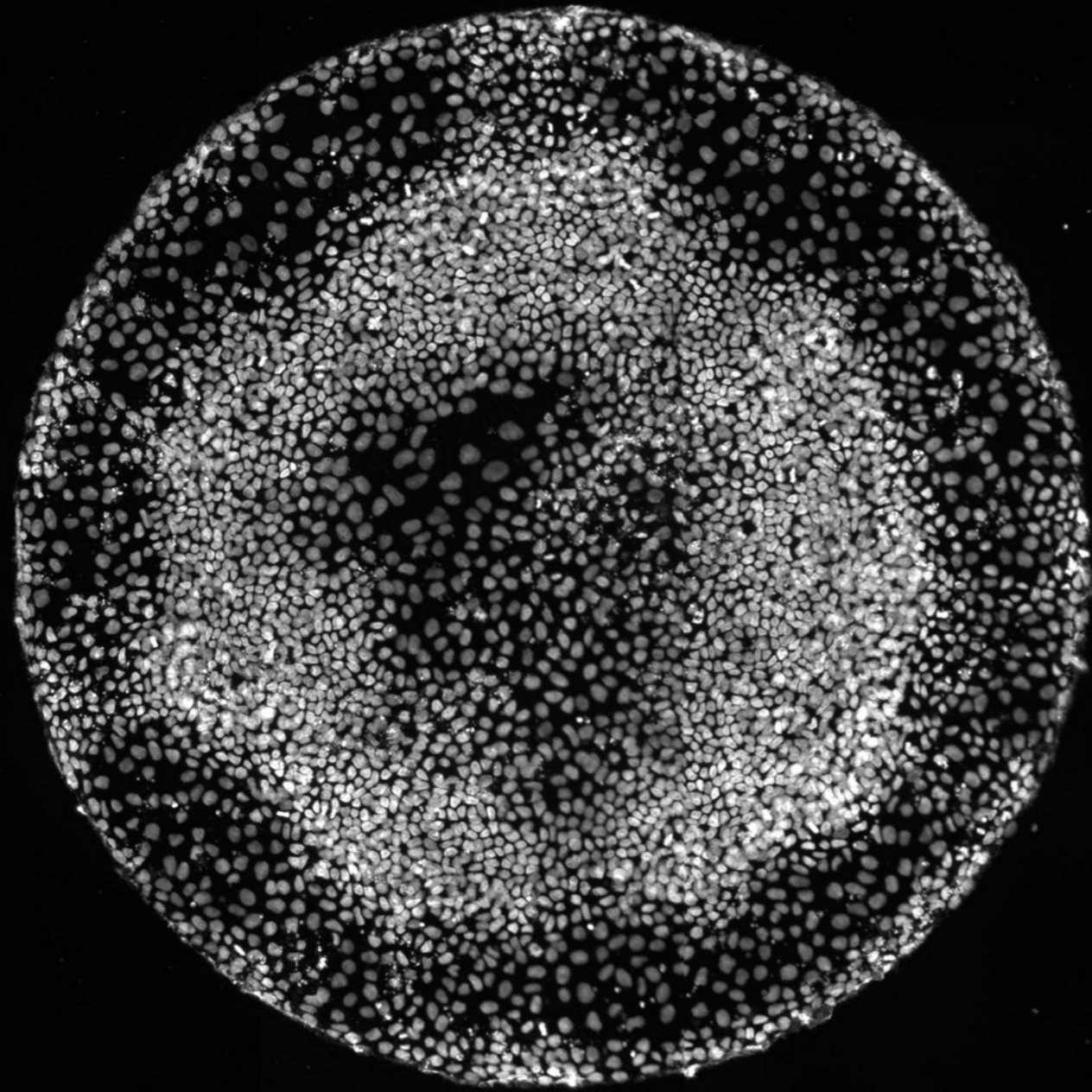
Sox2/Bra/Cdx2

Ectoderm/Mesoderm/Extraembryonic



Geometric confinement triggers self-organized patterning along the radial axis

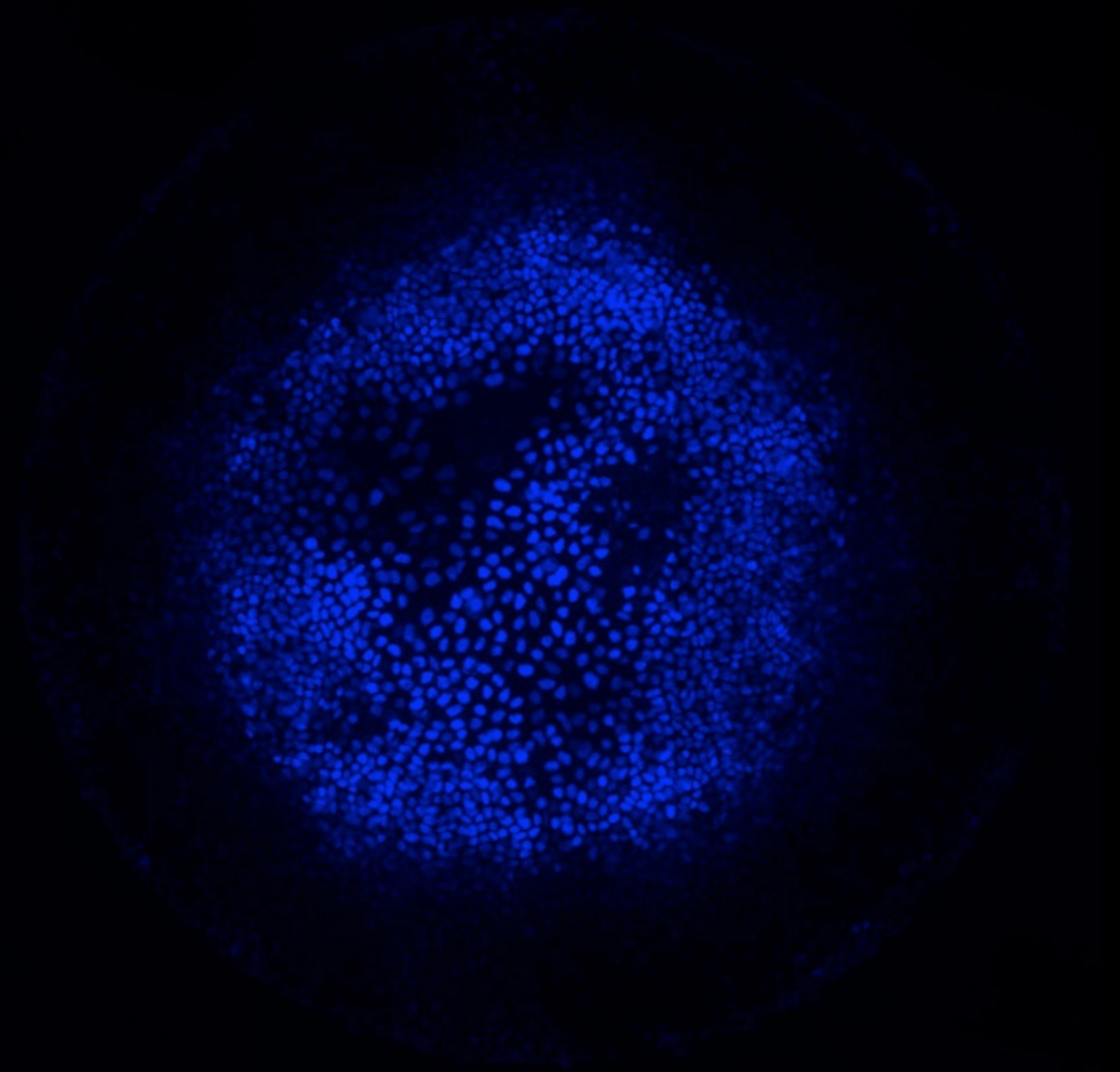
Nuclei (DAPI)



Fate Markers

Sox2/Bra/Cdx2

Ectoderm/Mesoderm/Extraembryonic



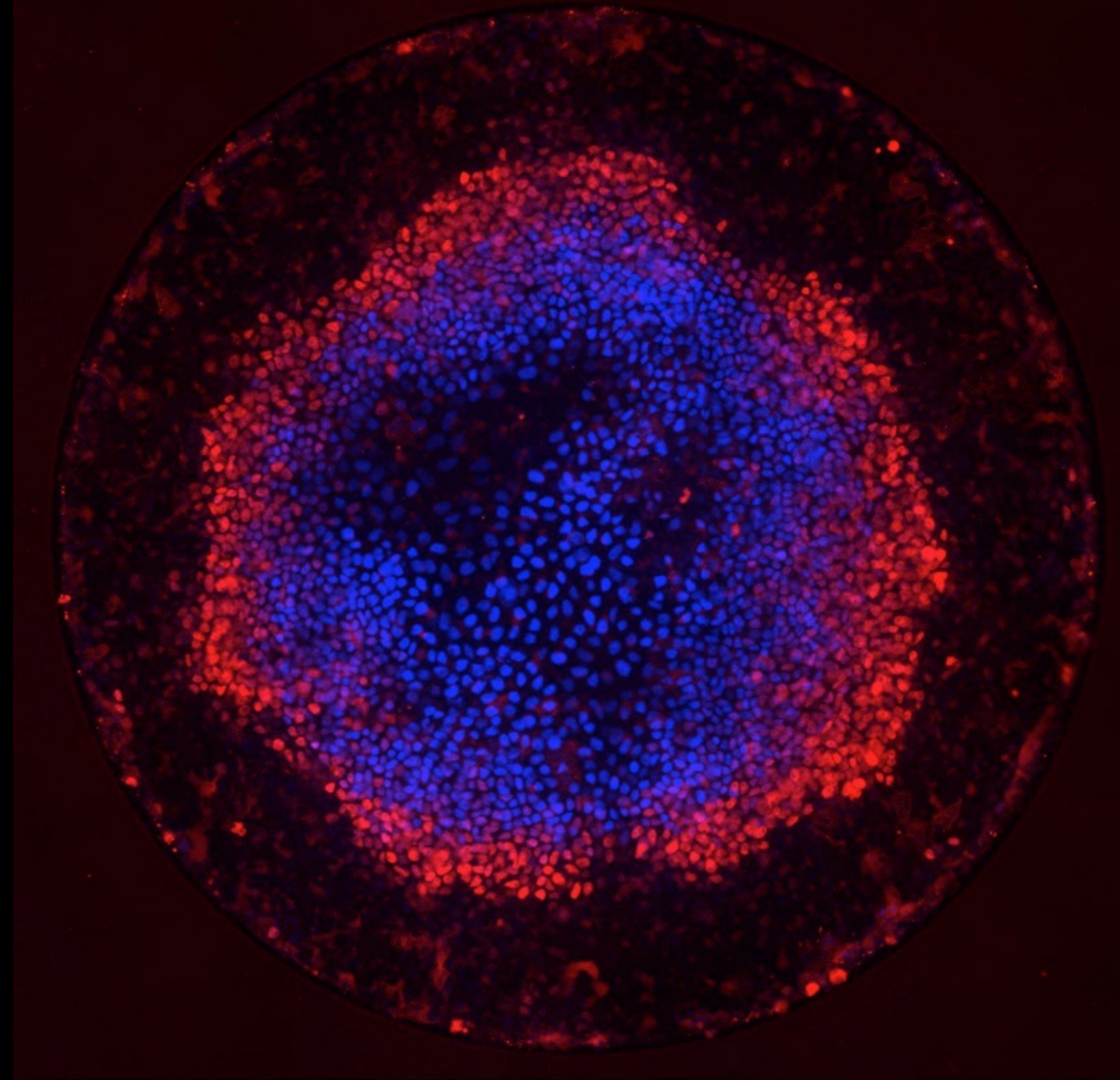
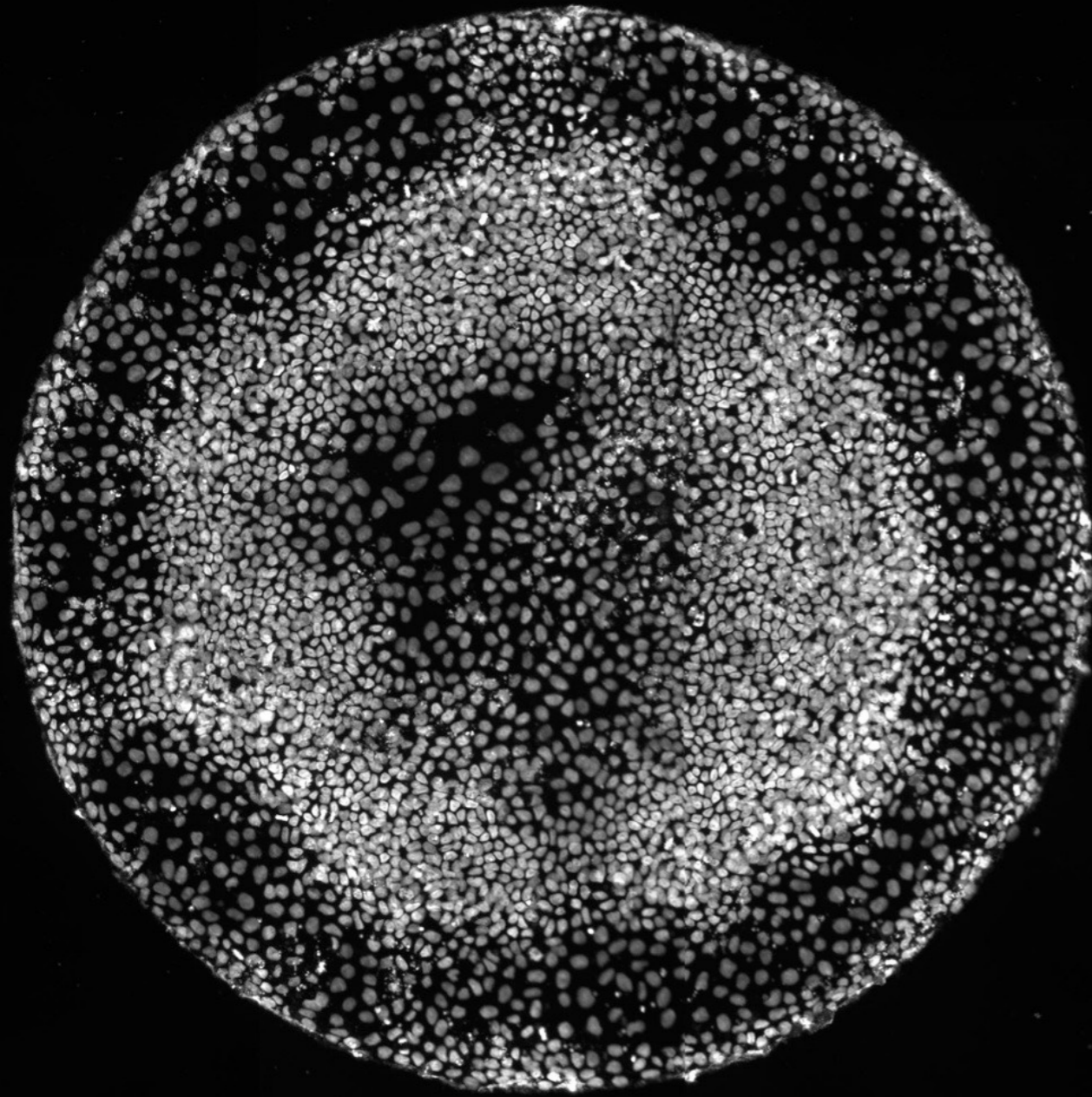
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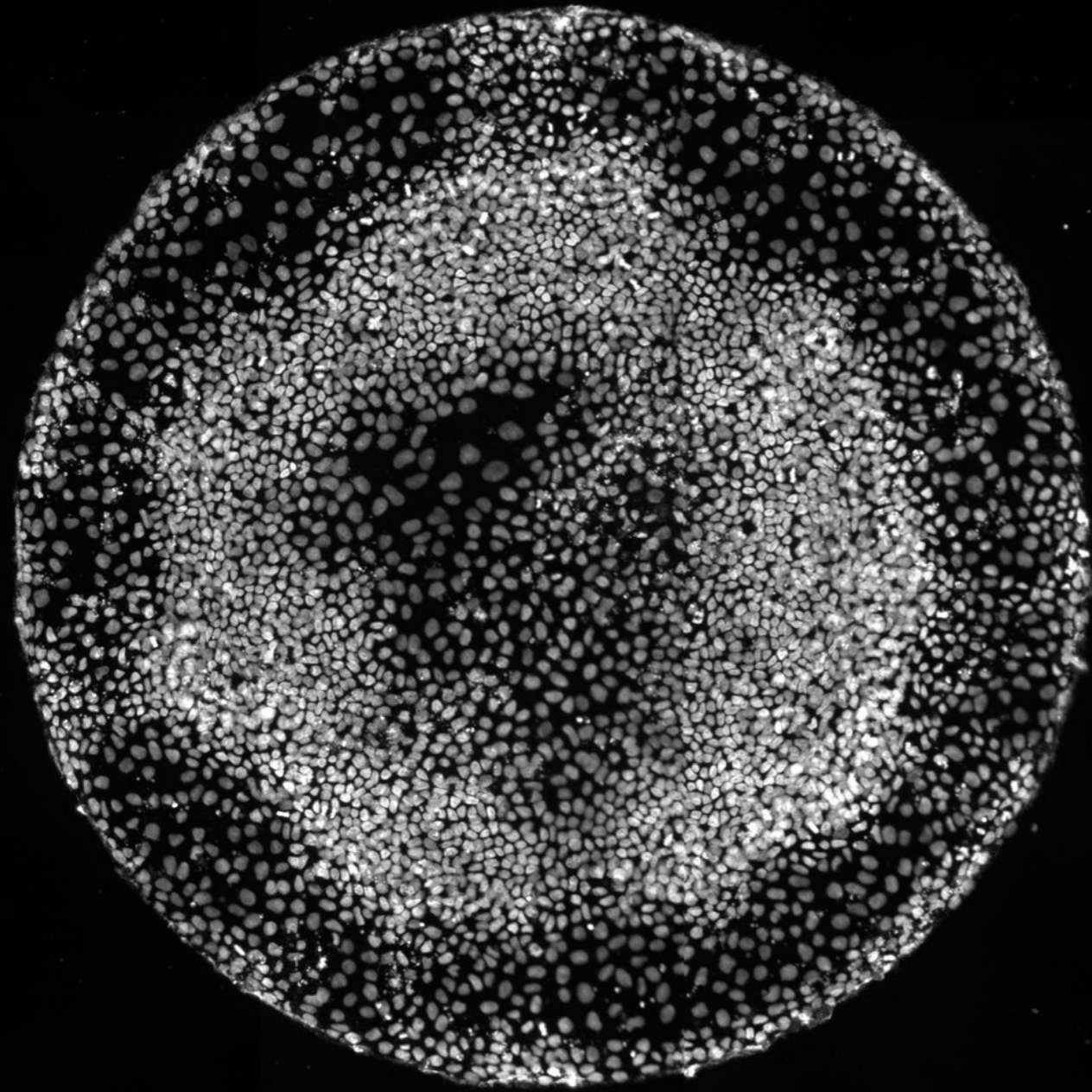
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Geometric confinement triggers self-organized patterning along the radial axis

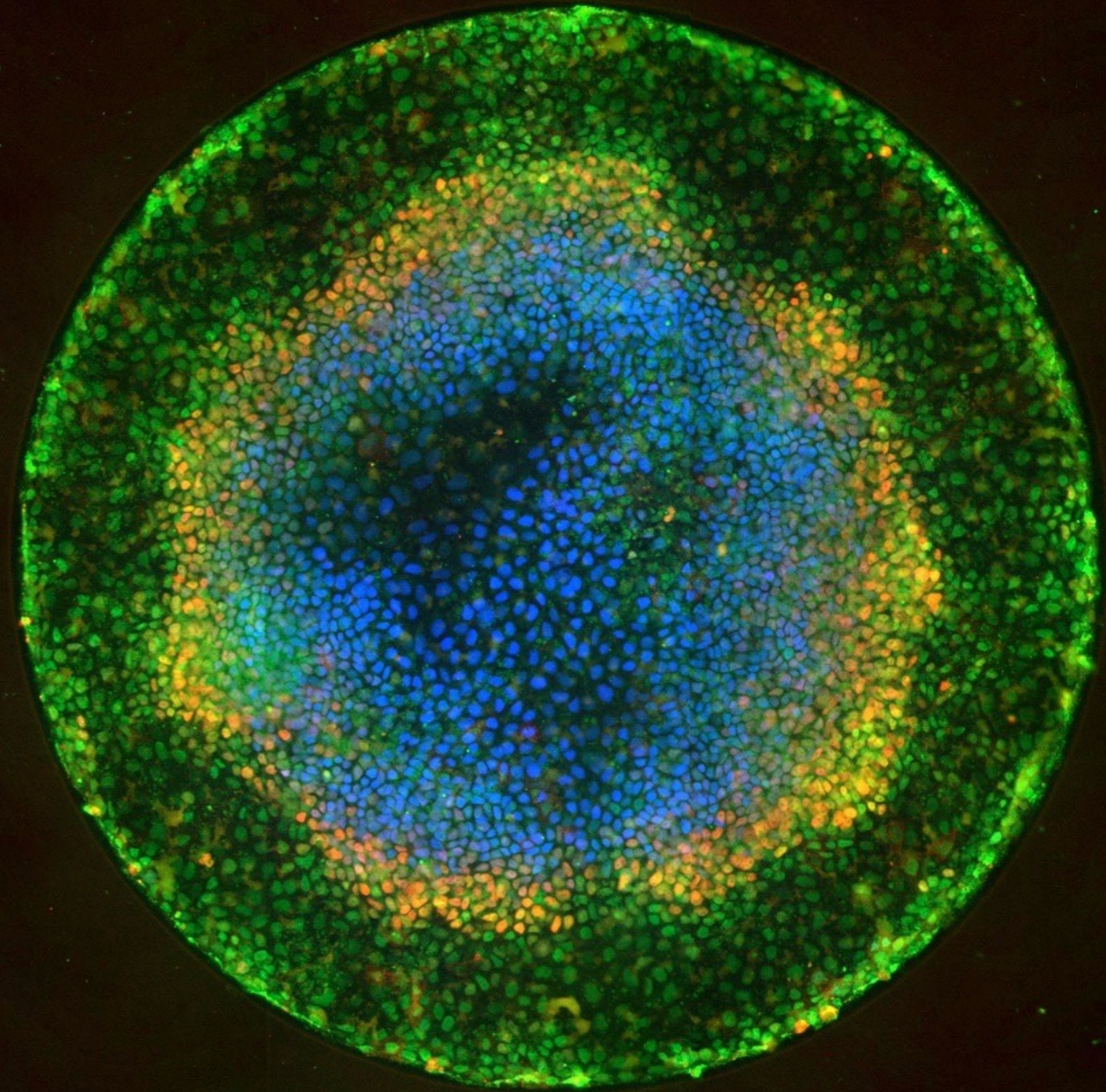
Nuclei (DAPI)



Fate Markers

Sox2/Bra/Cdx2

Ectoderm/Mesoderm/Extraembryonic



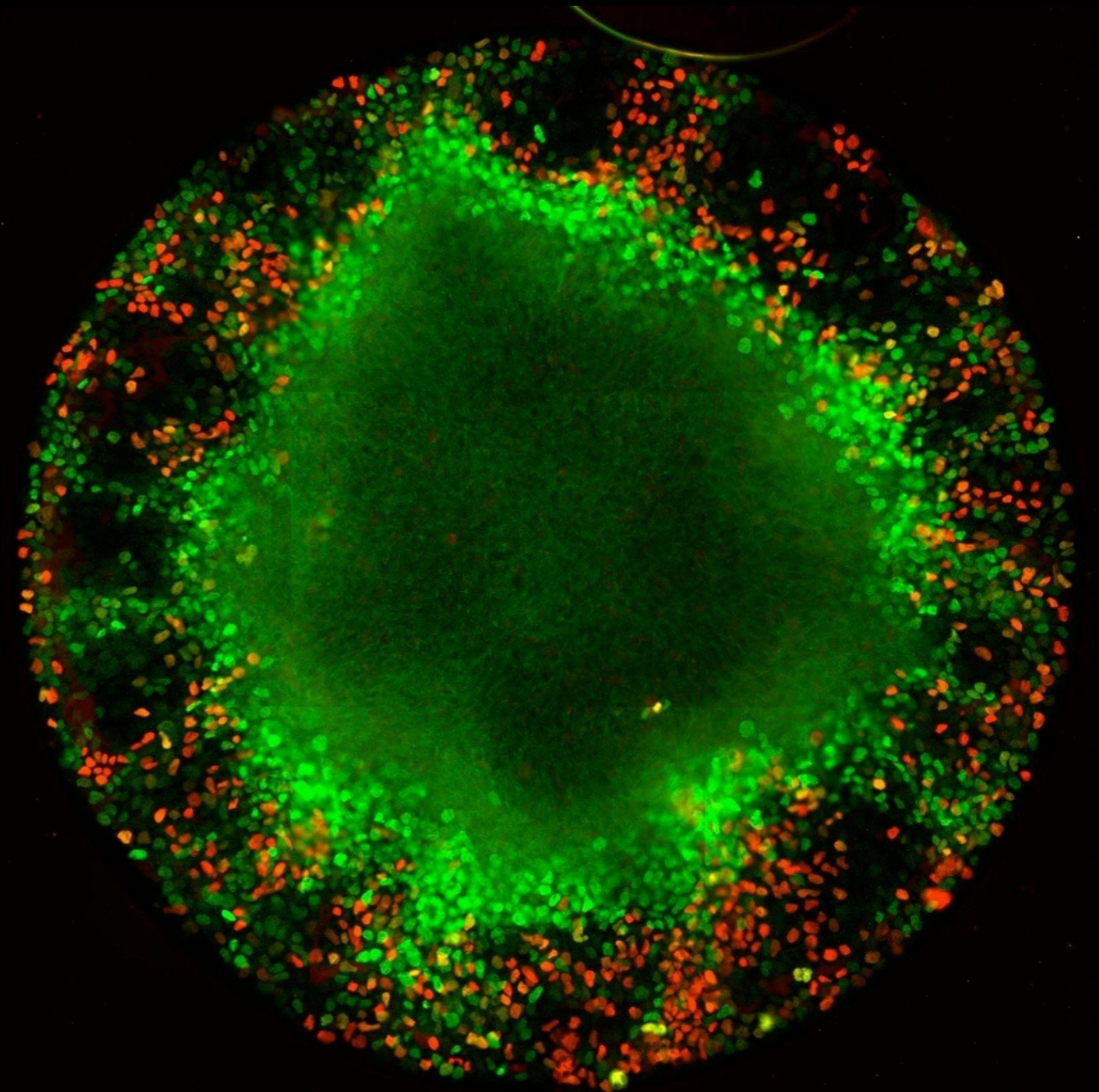
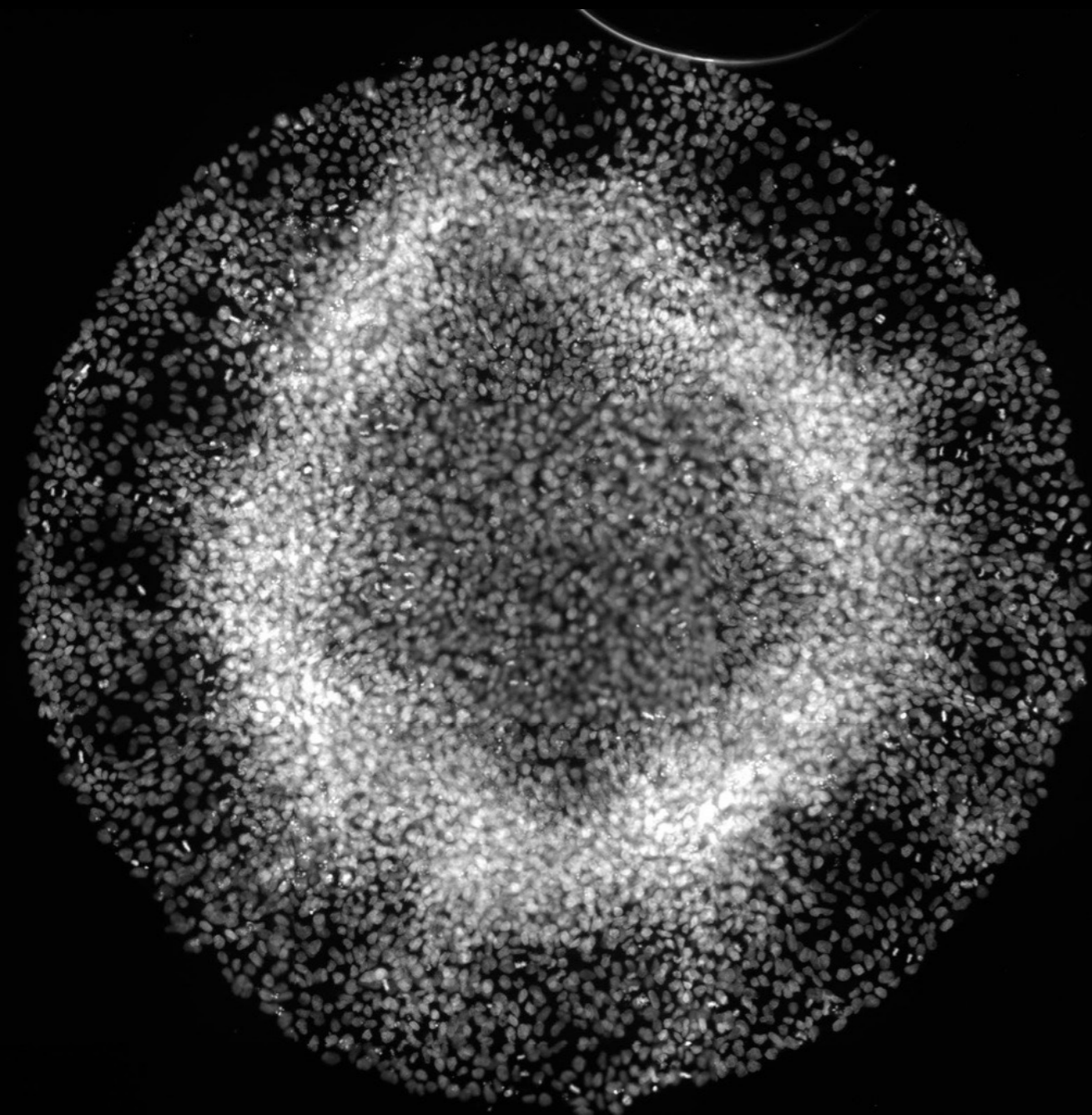
Germ layers along the radial axis

Nuclei (DAPI)

Fate Markers

Eomes/*Sox17*

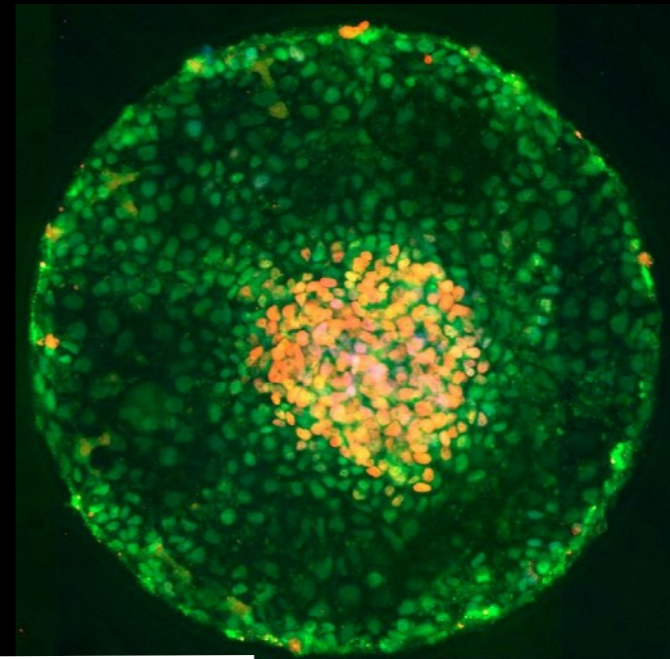
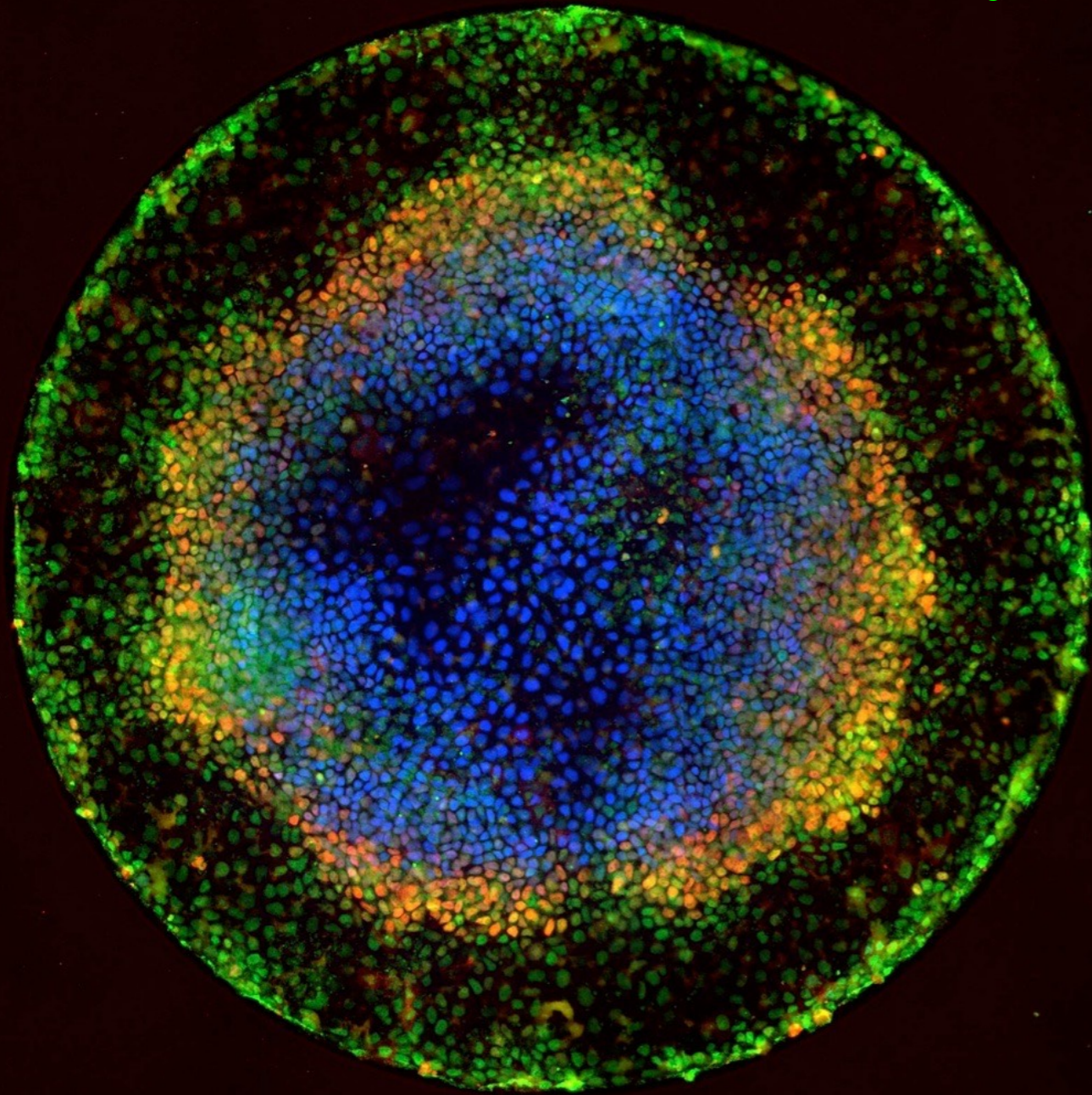
Mesoendoderm/*Endoderm*



Cells measure distance from the colony edge

Sox2/Bra/Cdx2

Ectoderm/Mesoderm/Extraembryonic

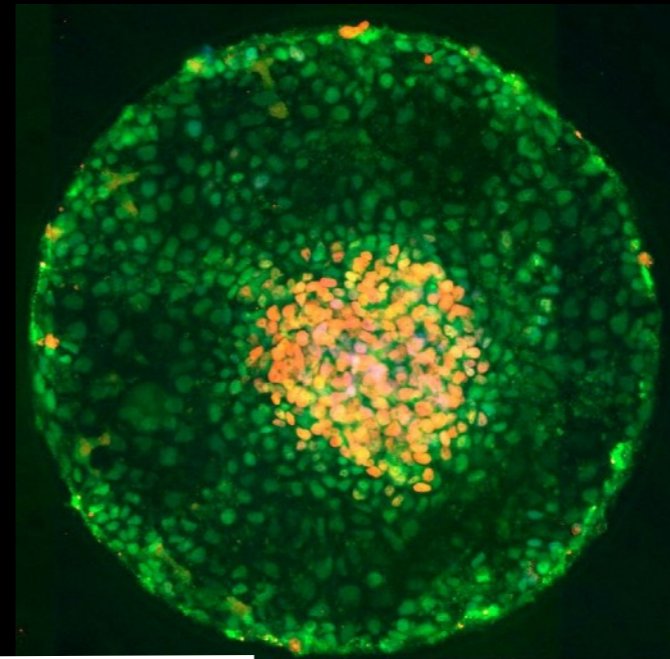
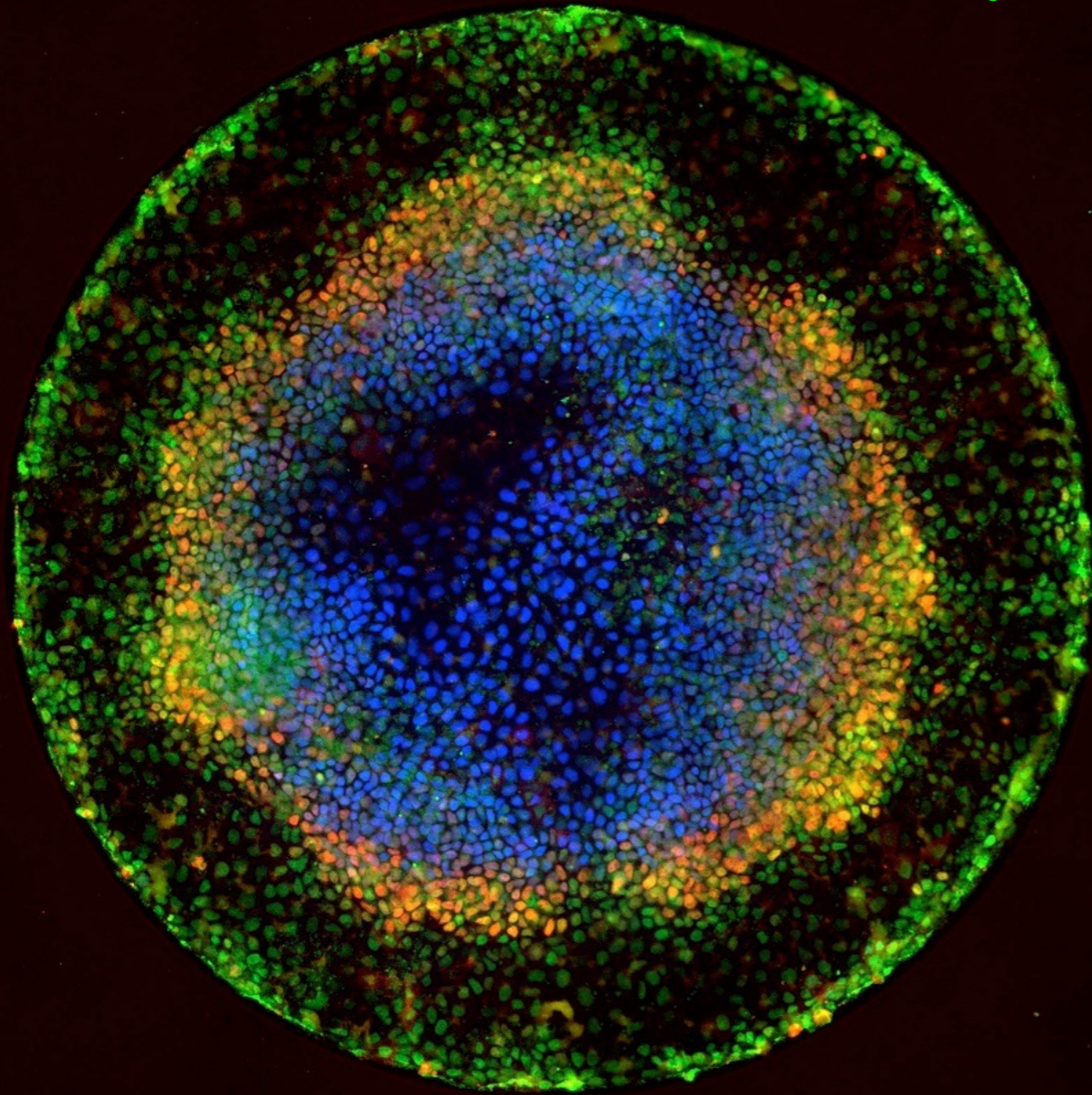


200µm

Cells measure distance from the colony edge

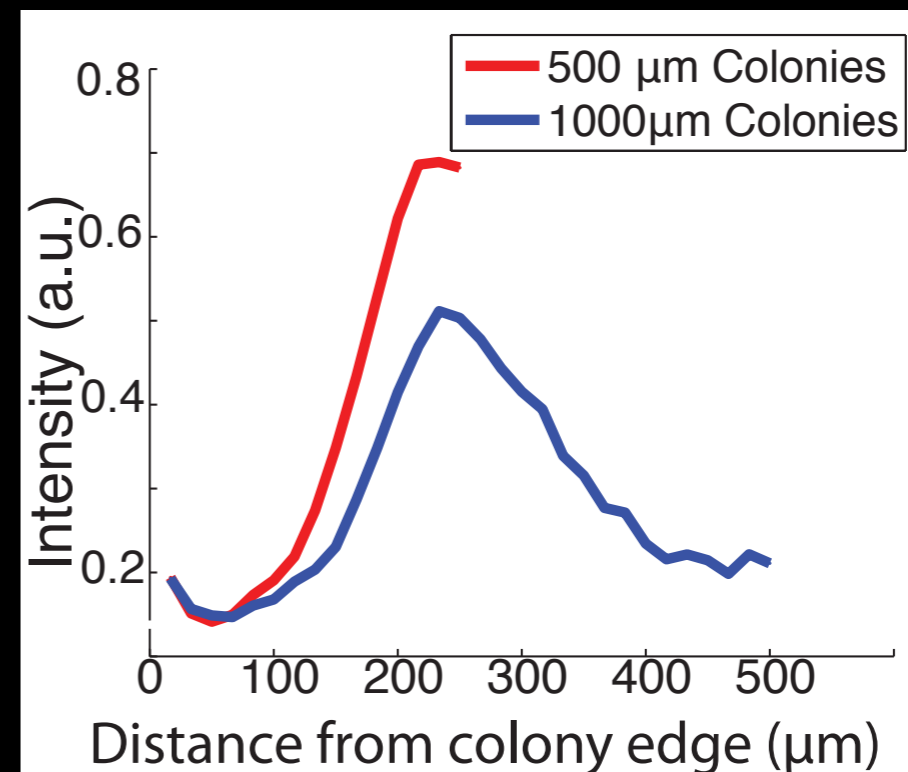
Sox2/Bra/Cdx2

Ectoderm/Mesoderm/Extraembryonic

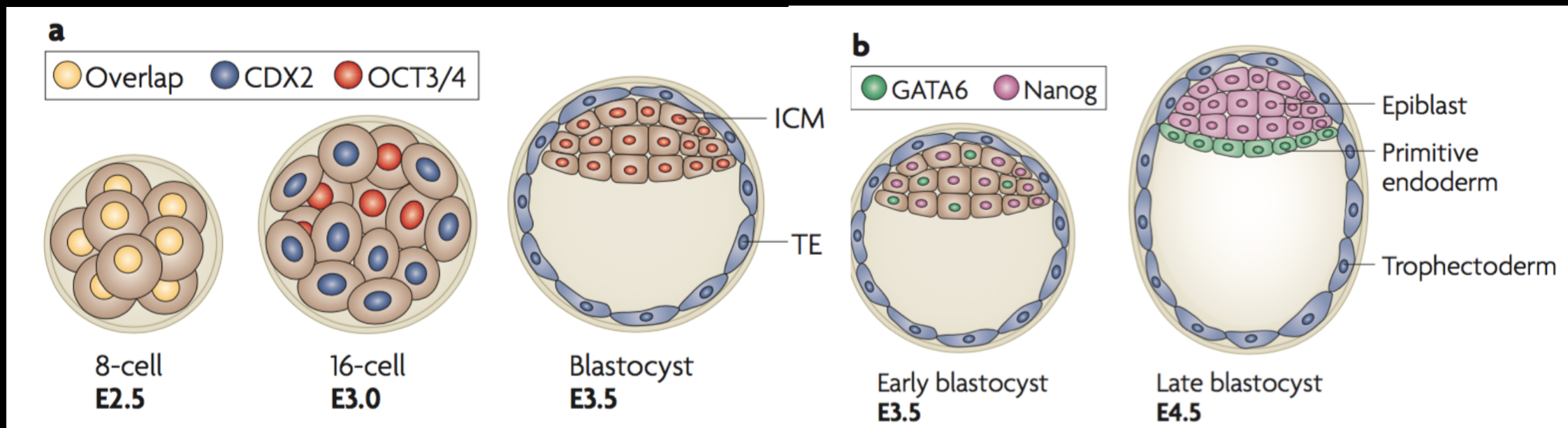


200 μ m

Bra

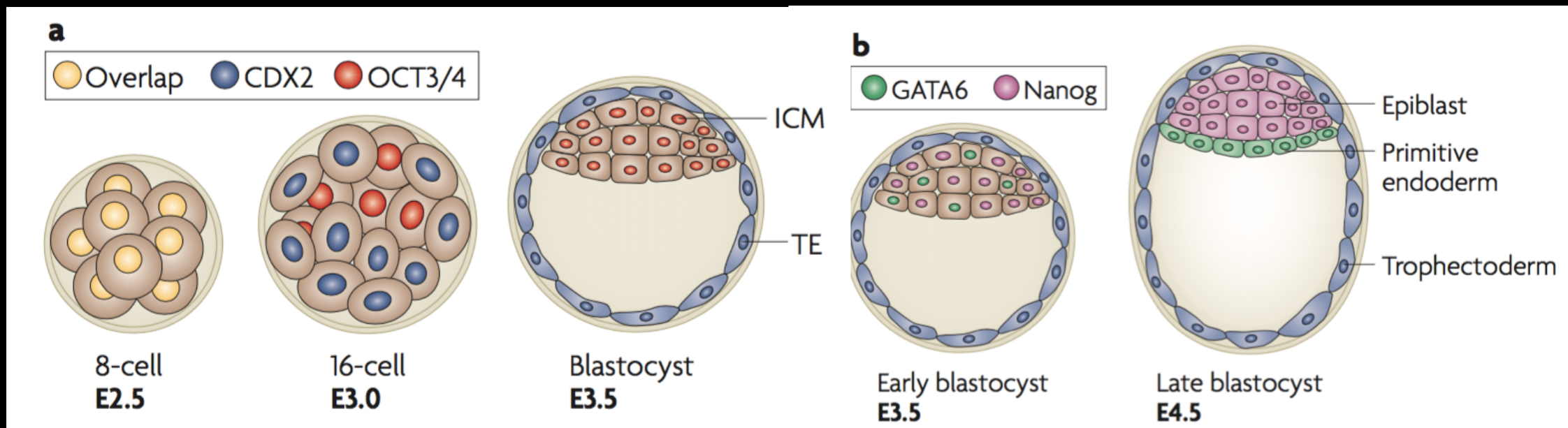


Aside: extraembryonic cells at the exterior are transcriptionally similar to in vivo human trophoctoderm

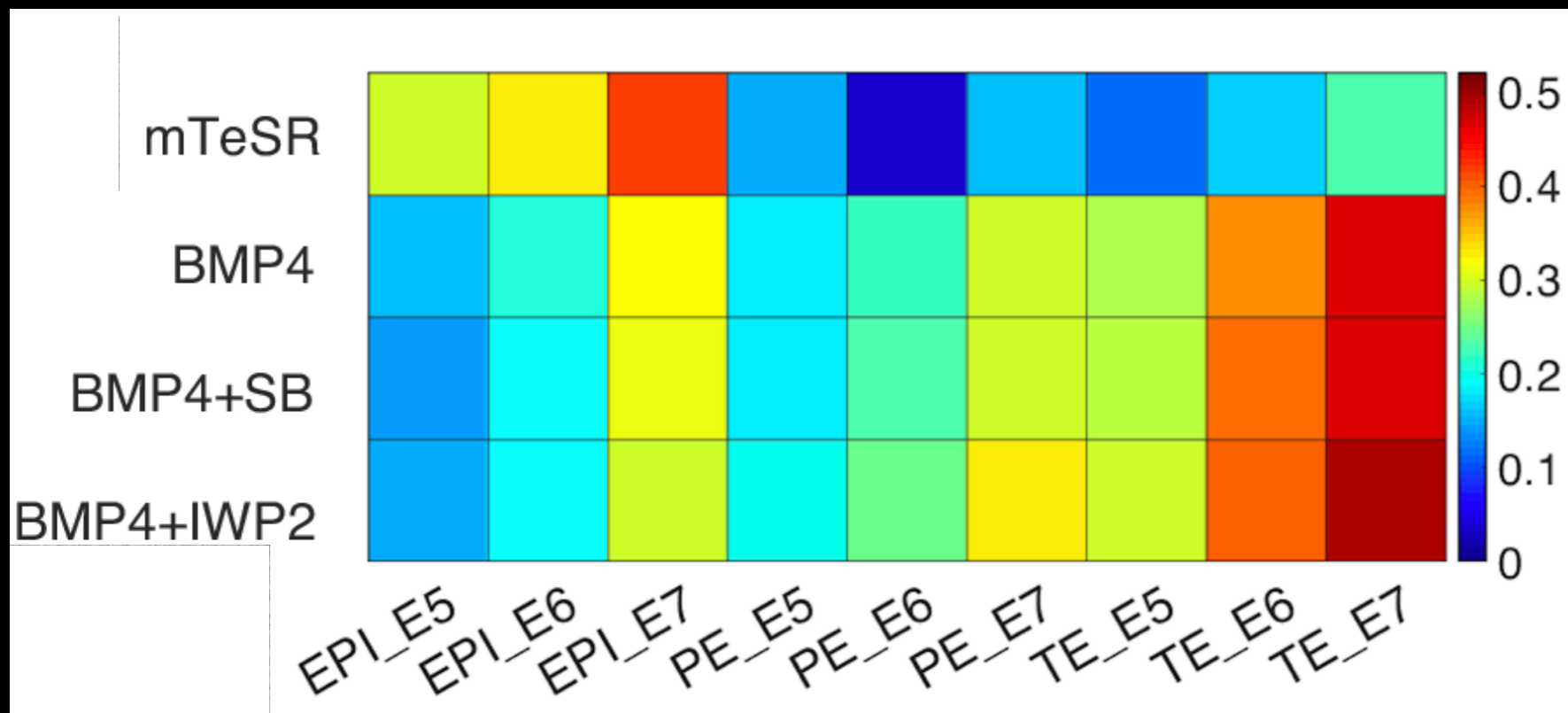


Arnold & Robertson
Nat Rev Mol Cell Biol 2009

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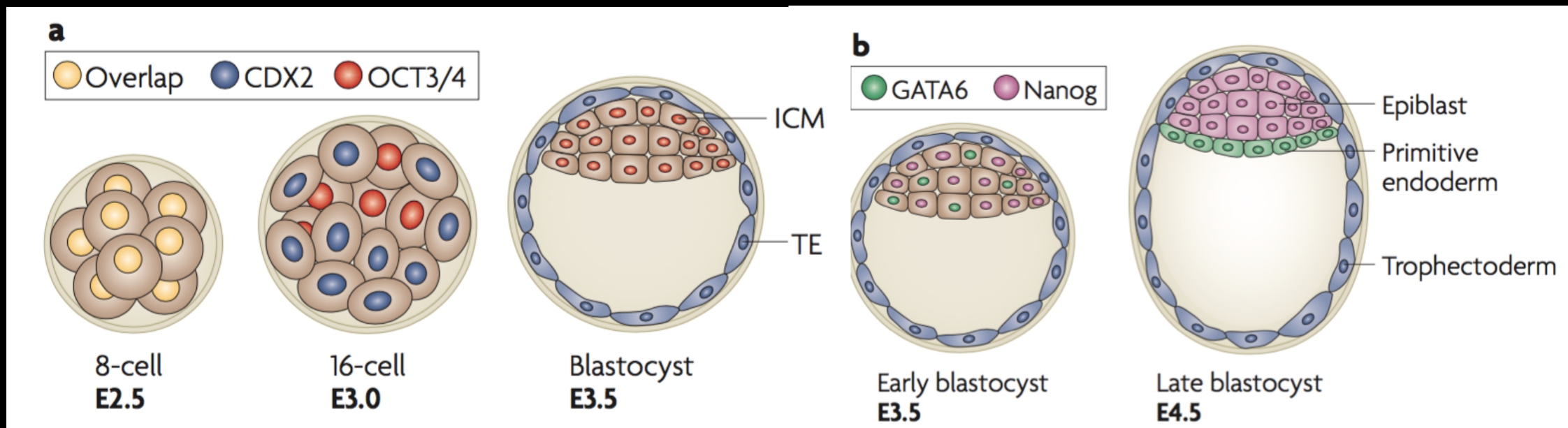
Arnold & Robertson
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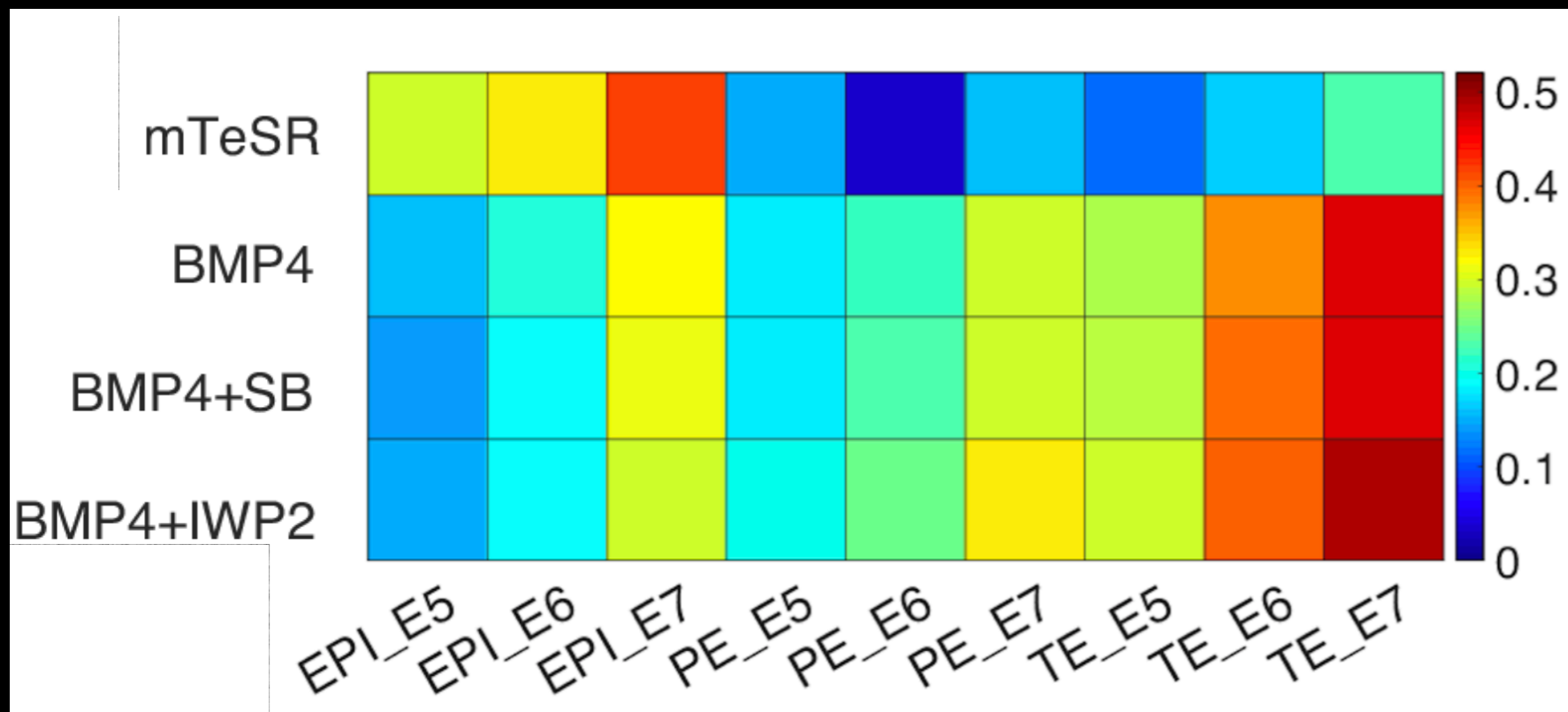
Pearson correlation over genes that discriminate between the three lineages in vivo

Chhabra et al. 2019 (submitted)

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Nat Rev Mol Cell Biol 2009

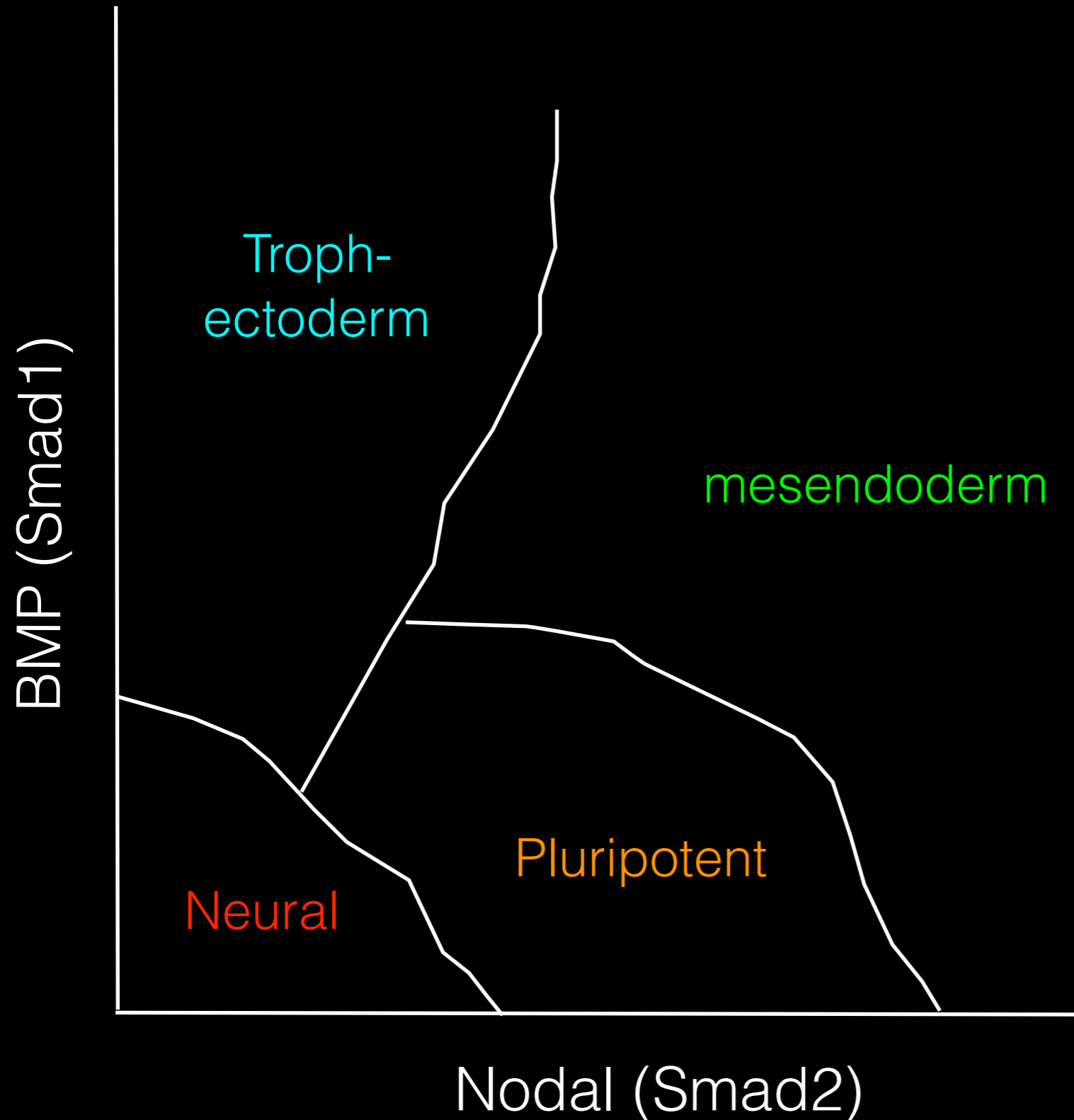


Note: BMP treated samples are as correlated with TE as ESCs are with Epi

Pearson correlation over genes that discriminate between the three lineages in vivo

Chhabra et al. 2019 (submitted)

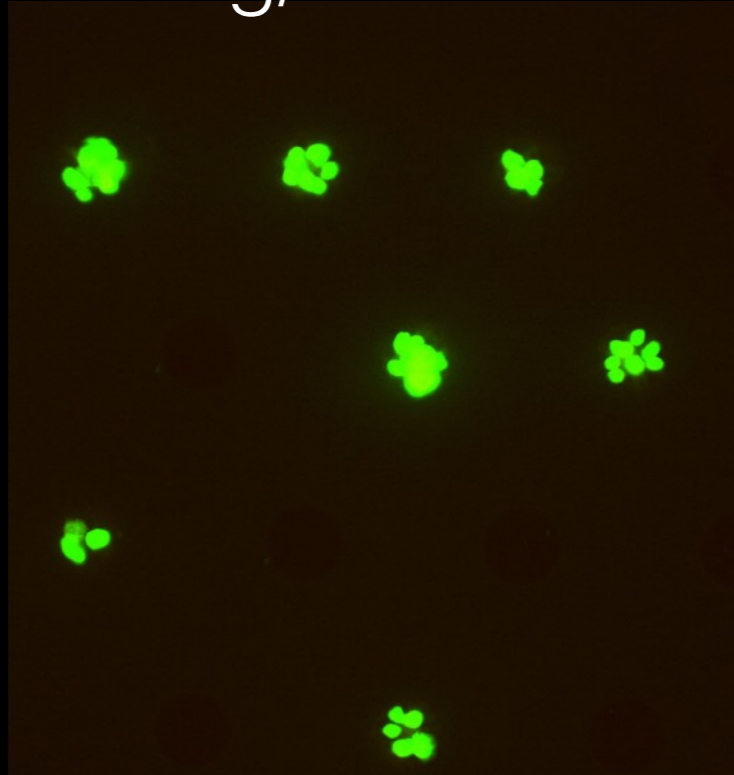
TGF- β signals and fates in hESCS



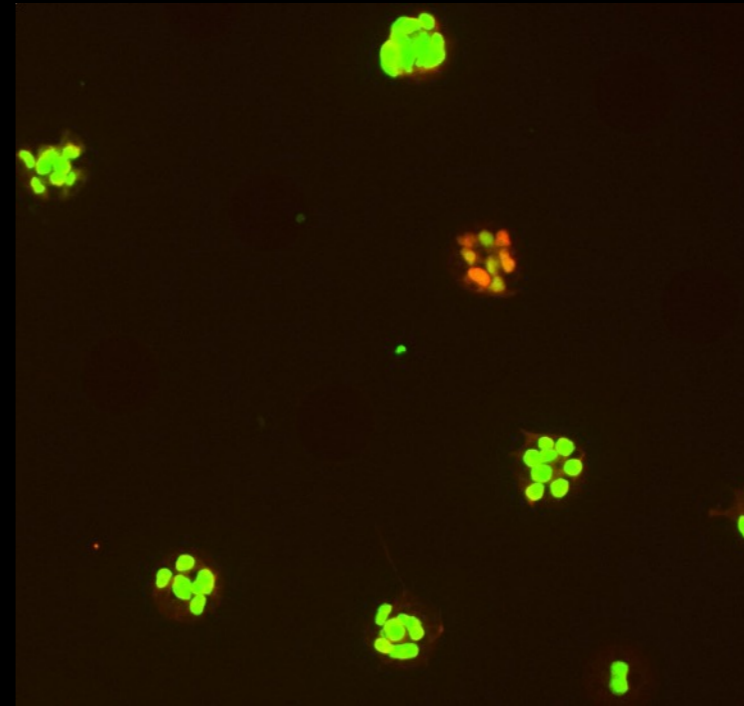
Without secondary signals, BMP4 induces a single fate

Sox2/Cdx2
Pluripotent/Trophectoderm

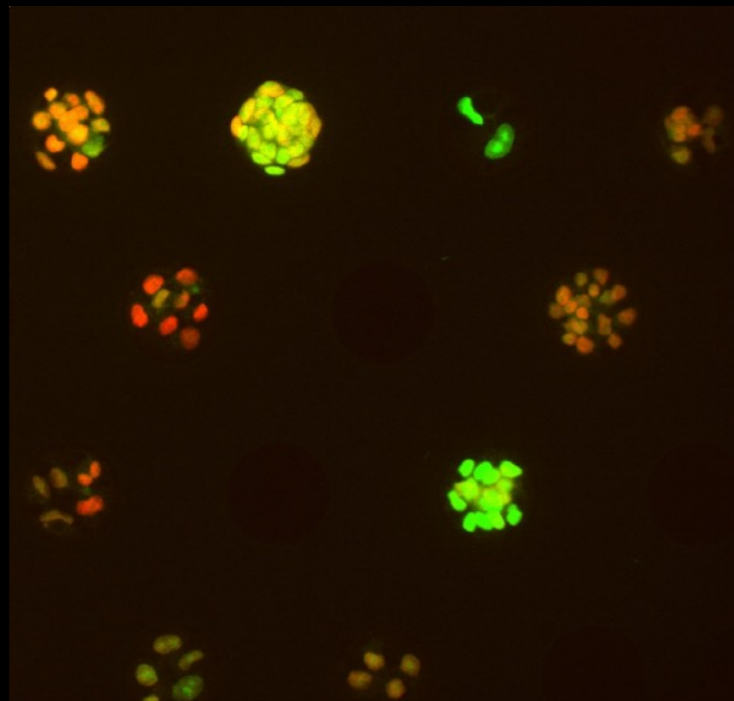
0 ng/ml BMP4



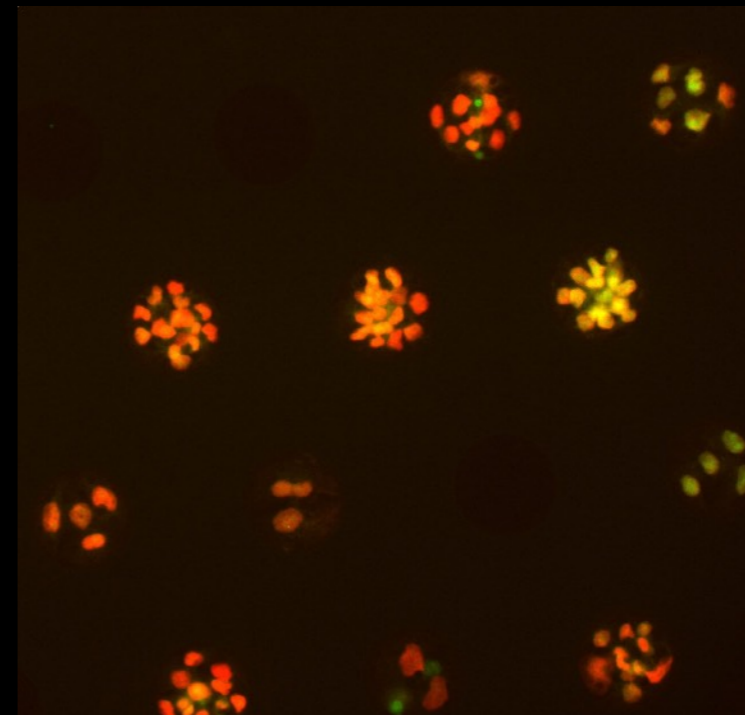
0.1 ng/ml BMP4



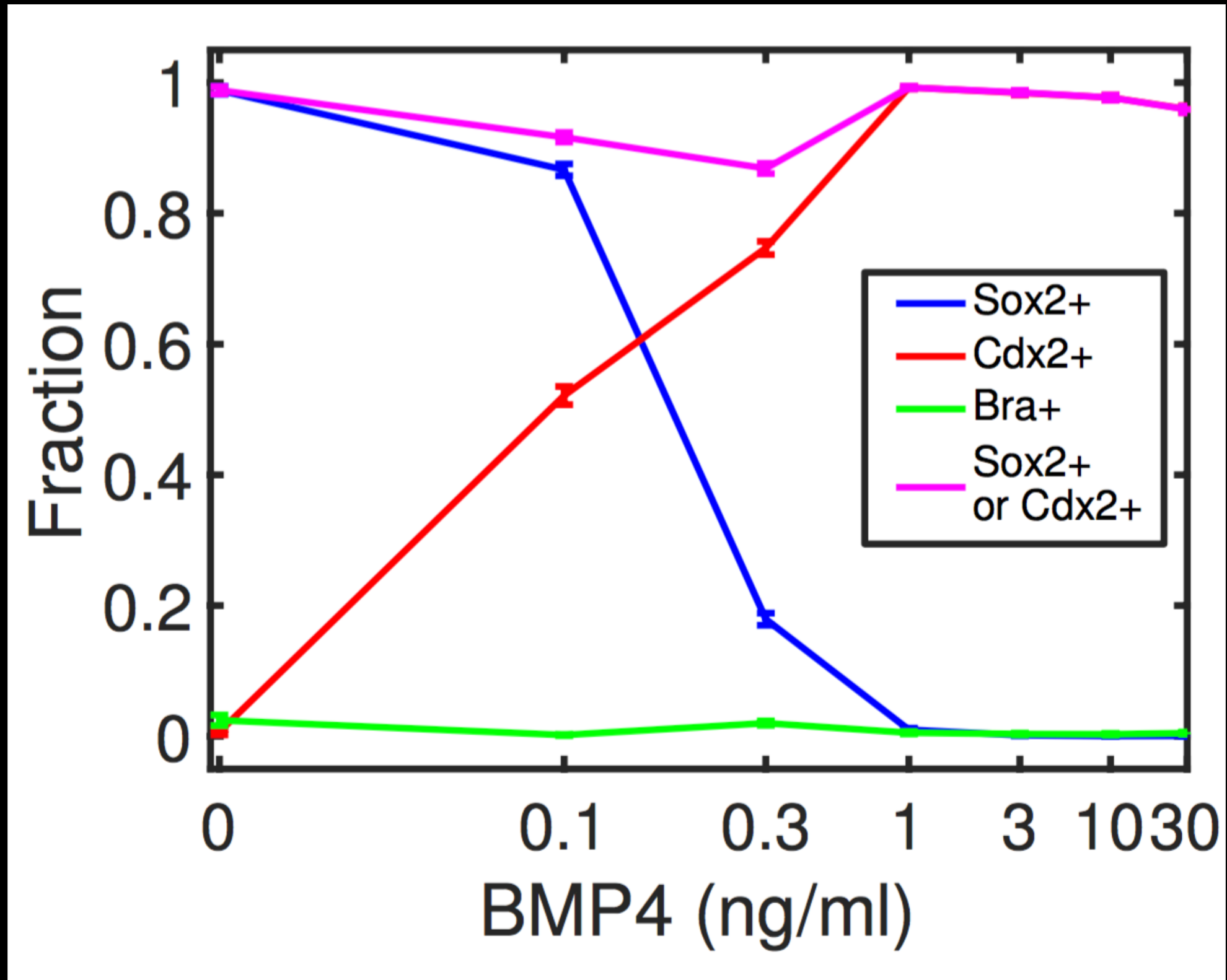
0.3 ng/ml BMP4



1 ng/ml BMP4

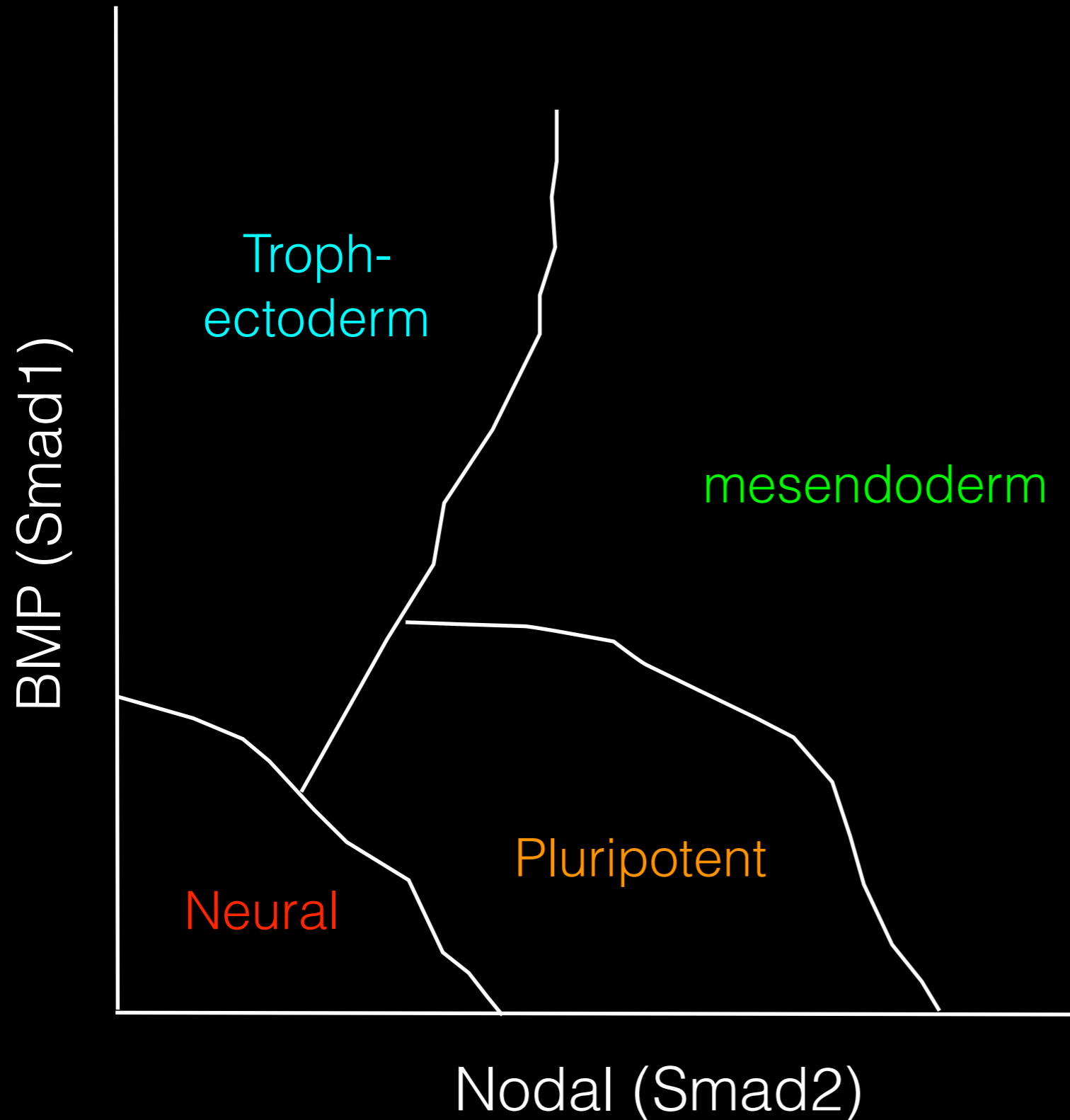


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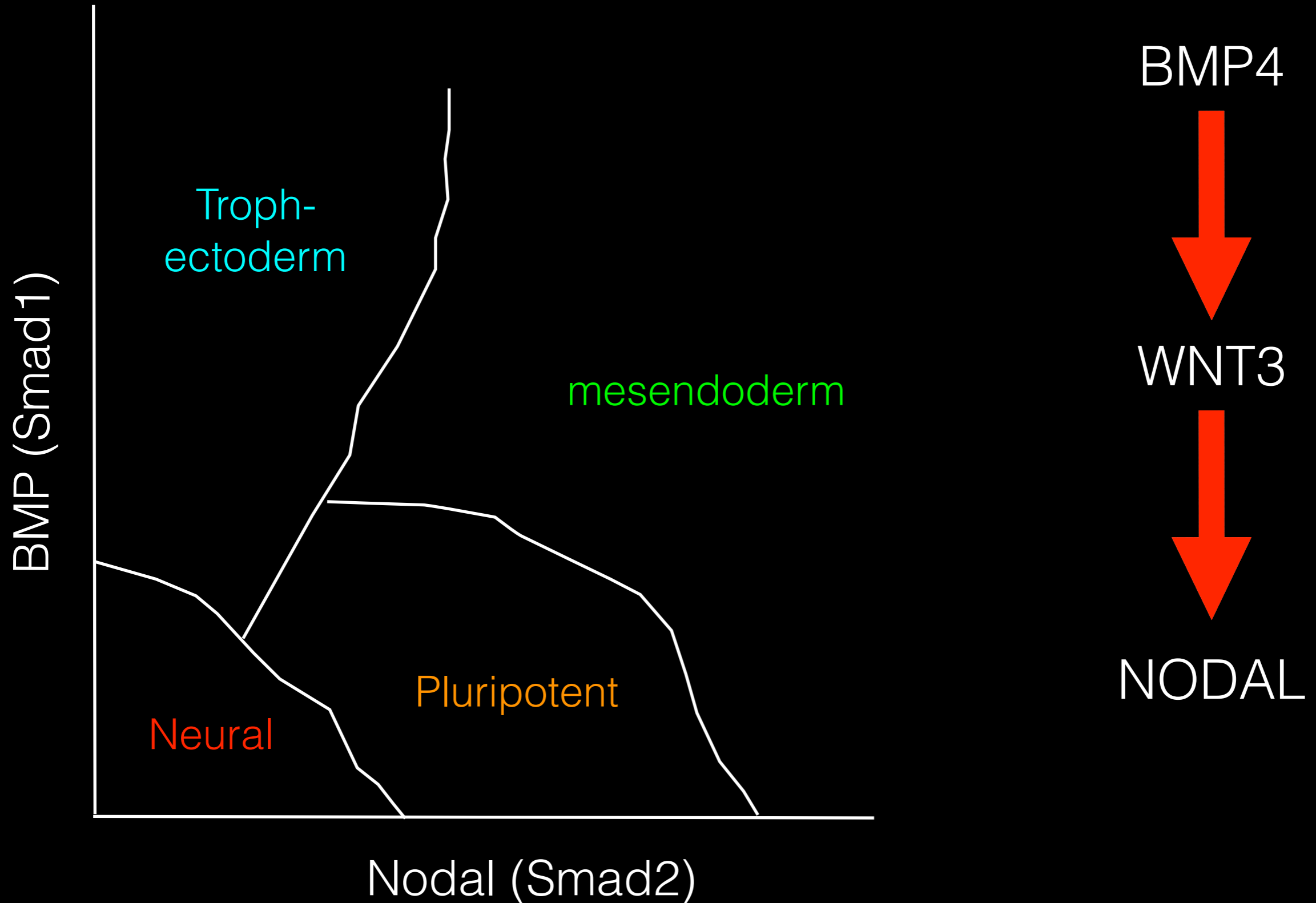


Only two fates account for all cells. BMP does not act as a morphogen in this system.

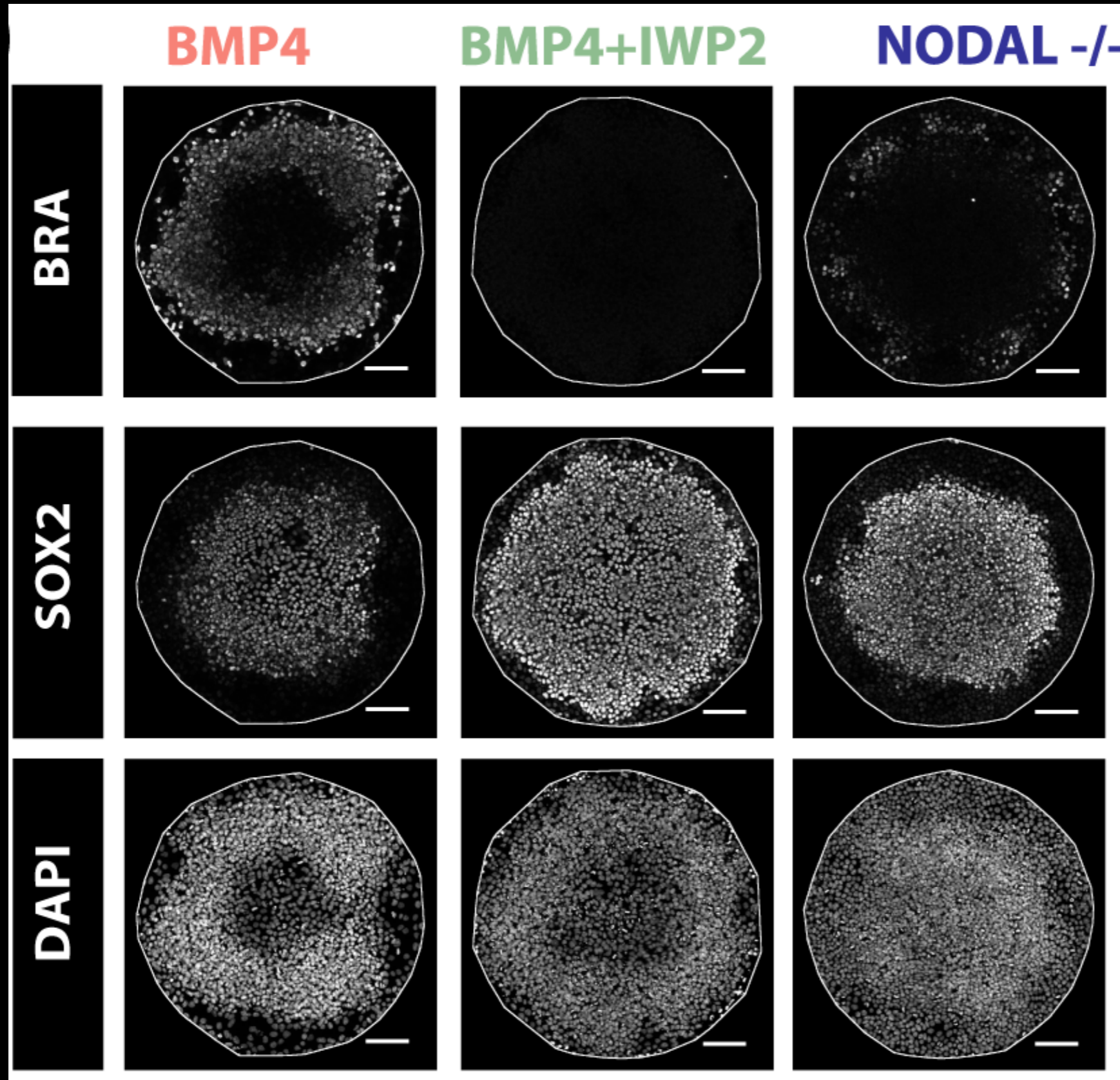
TGF- β signals and fates in hESCS



TGF- β signals and fates in hESCS

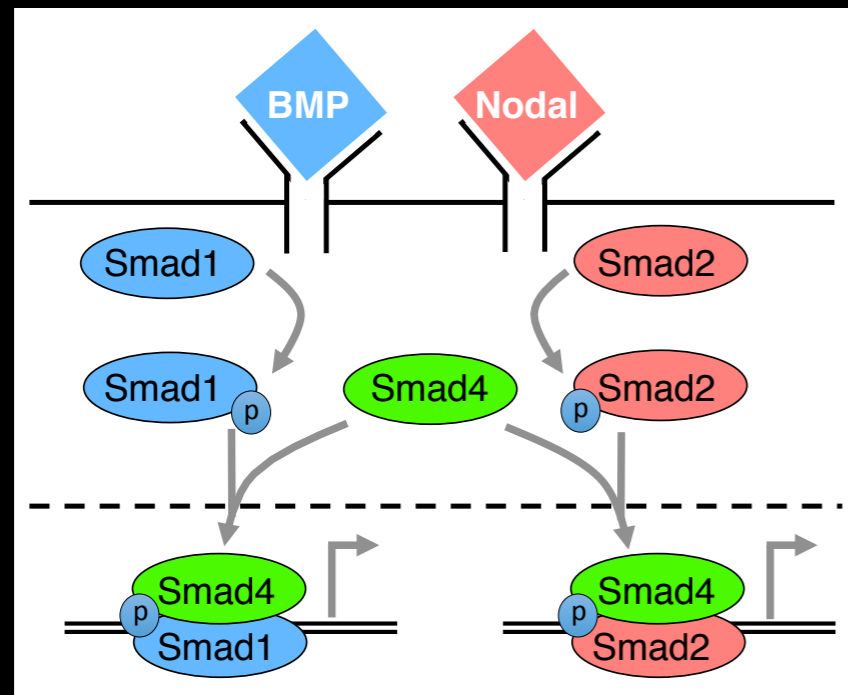


Paracrine Wnt and Nodal signaling are required downstream of BMP4 to pattern the gastruloid

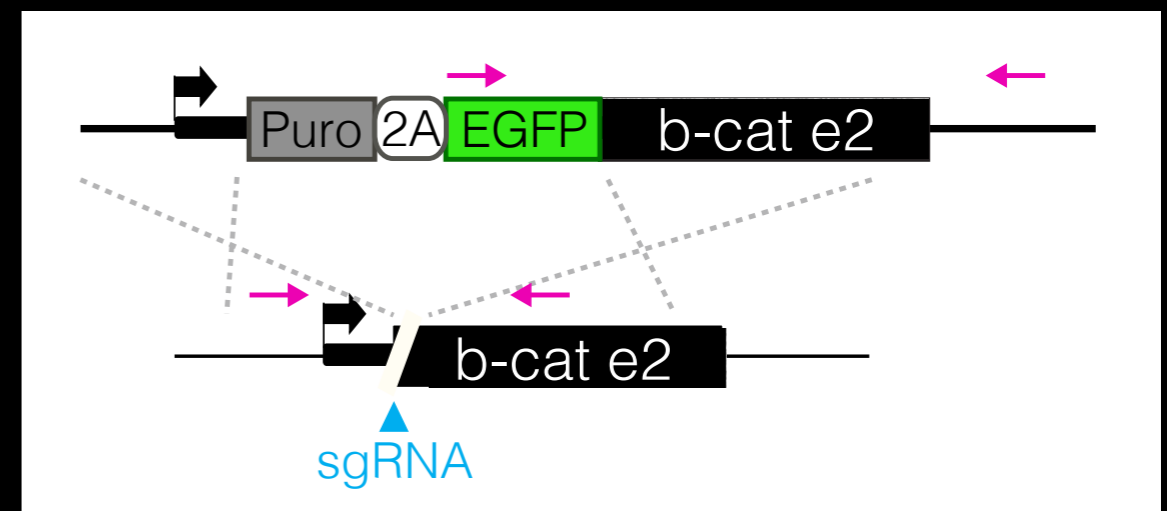
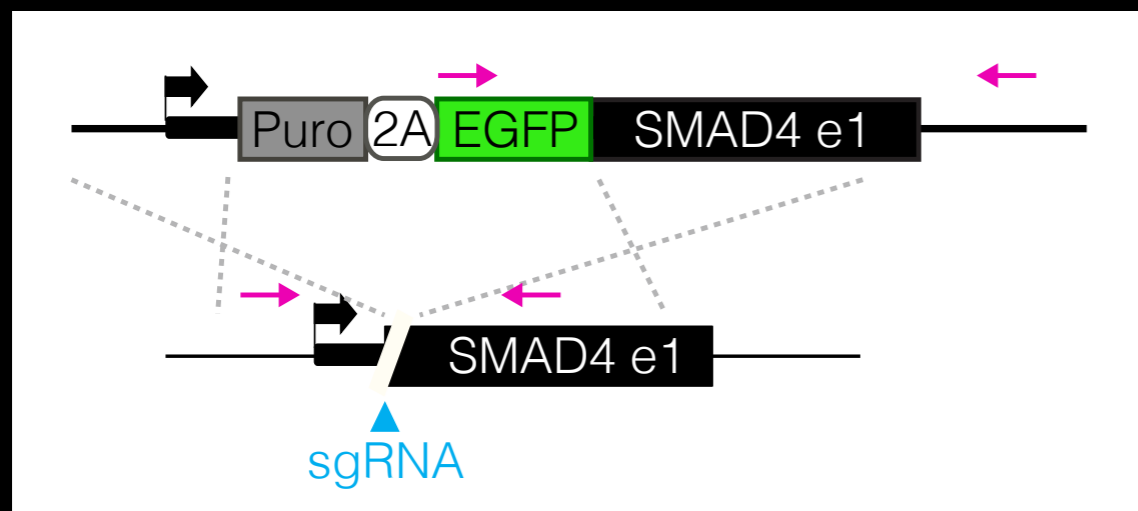
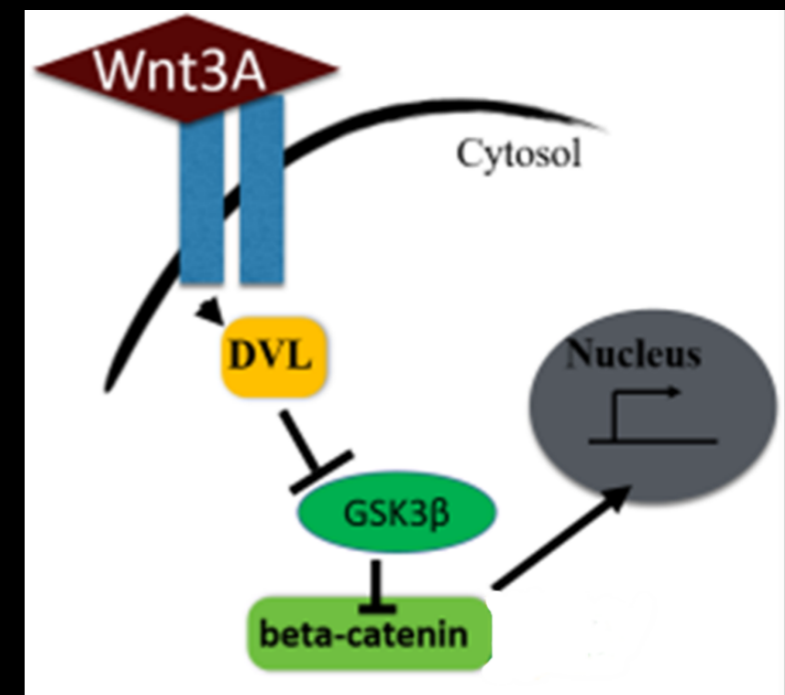


Measuring signaling dynamics of key morphogen pathways with CRISPR-engineered reporters

TGFb superfamily (BMP/NODAL)



Wnt pathway

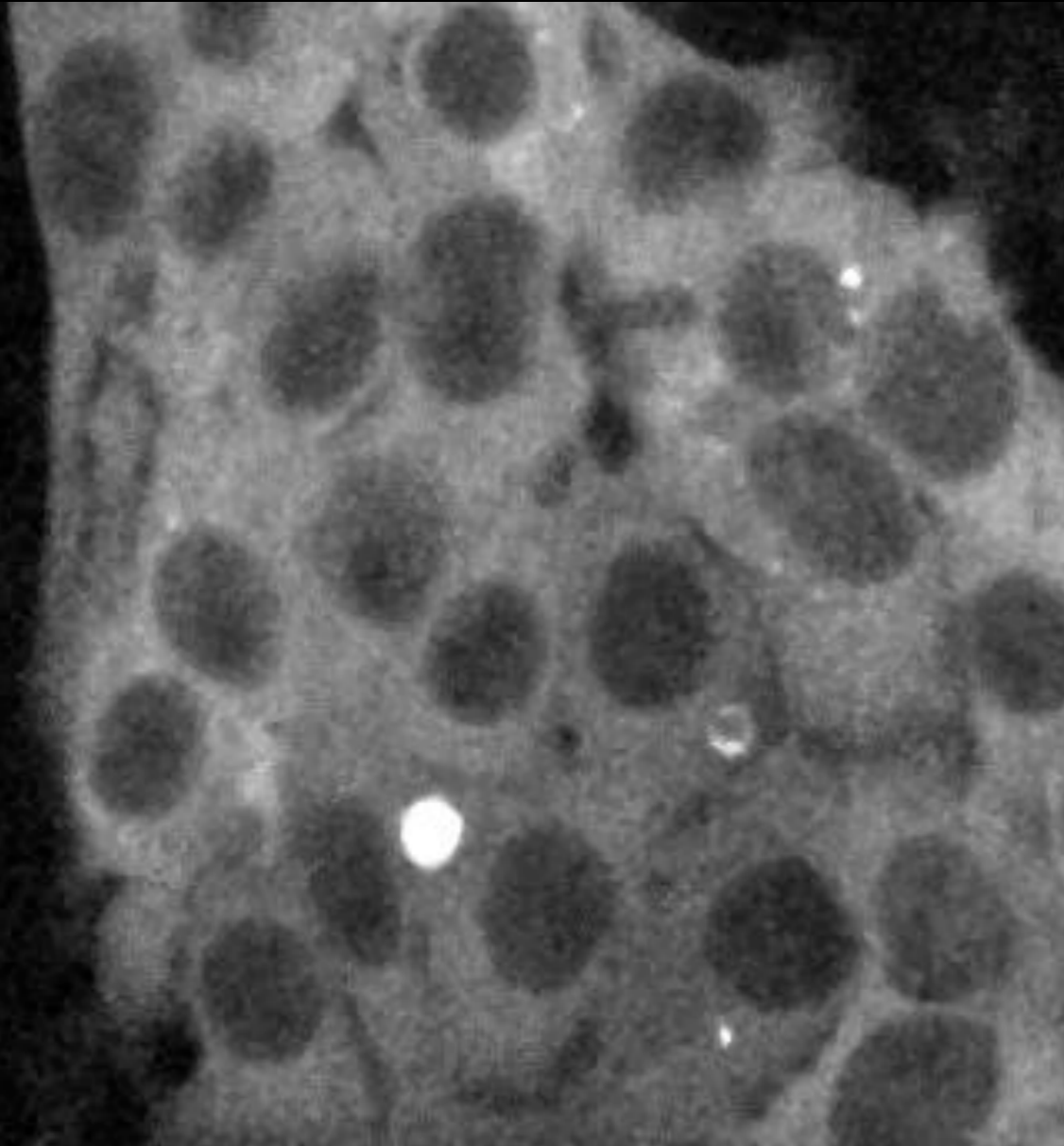


Nemashkalo et al Development 2017
Heemskerk et al. eLife 2019

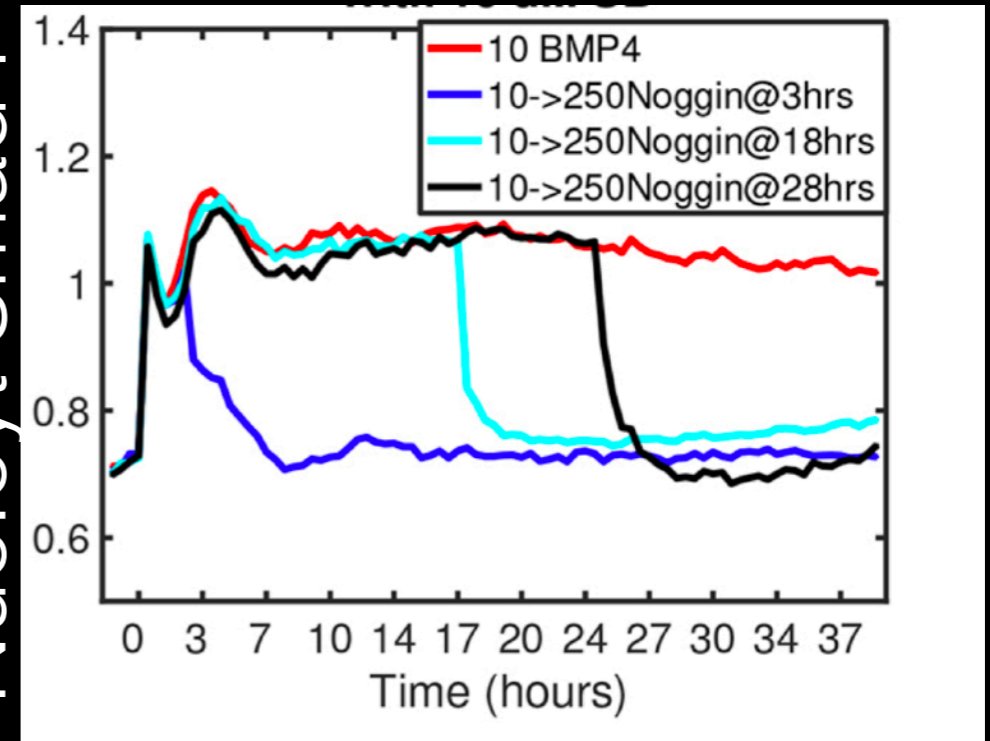
Massey et al. PNAS 2019

BMP signaling is sustained and duration of signaling is required for differentiation to CDX2+ fates

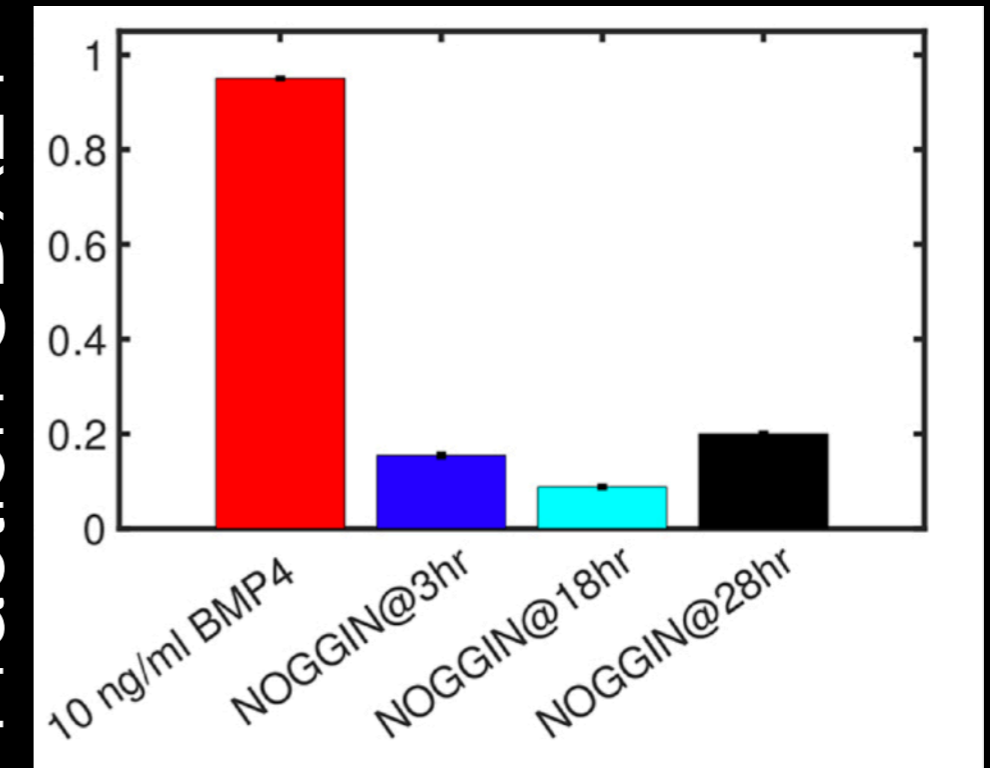
GFP-SMAD4: BMP response



Nuc:cyt Smad4

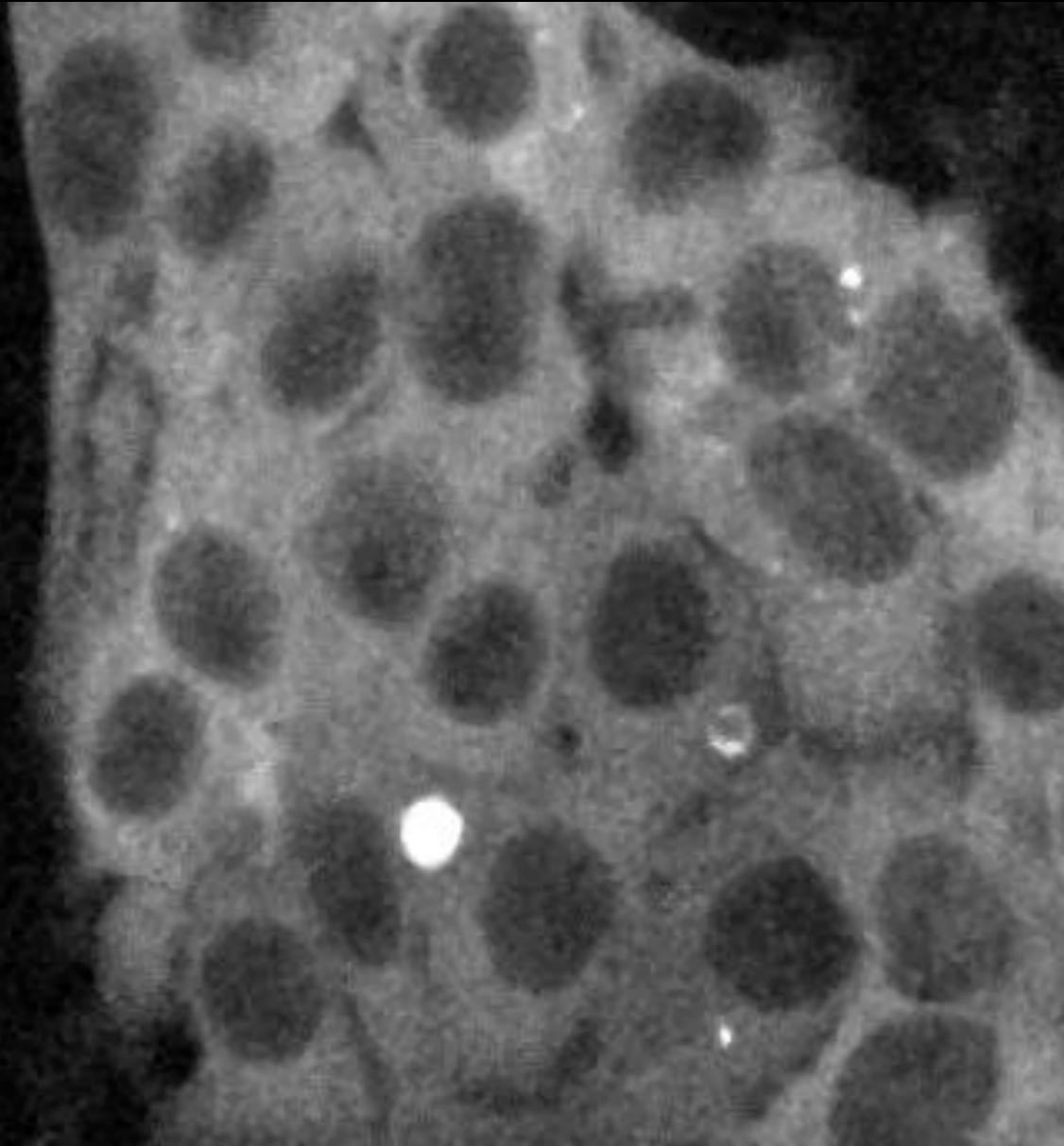


Fraction CDX2+

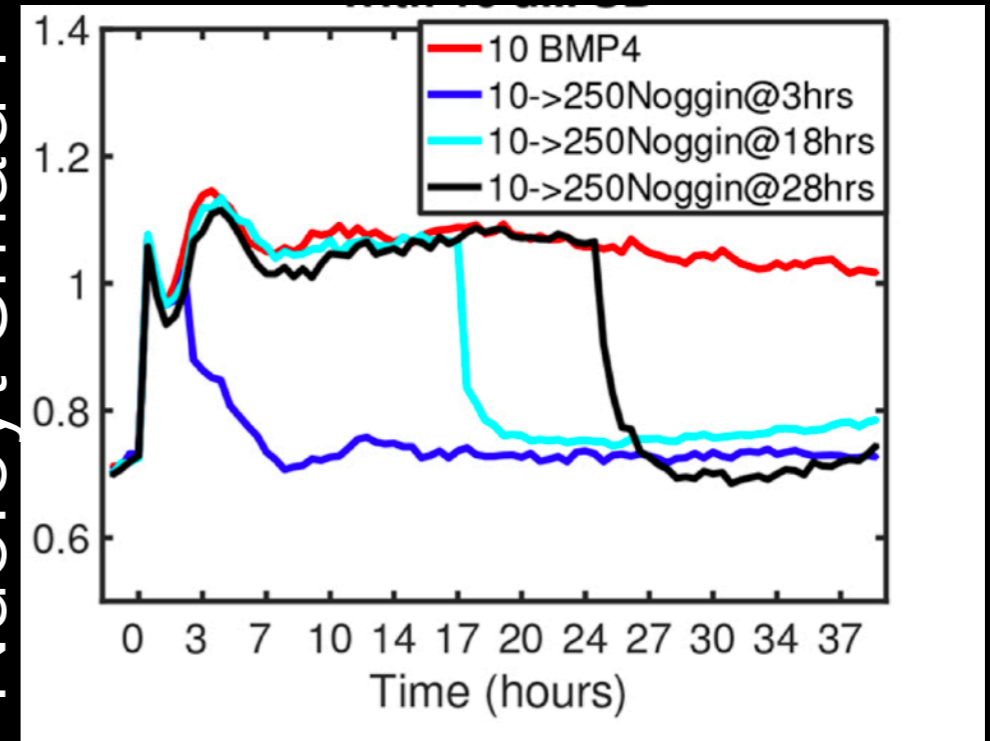


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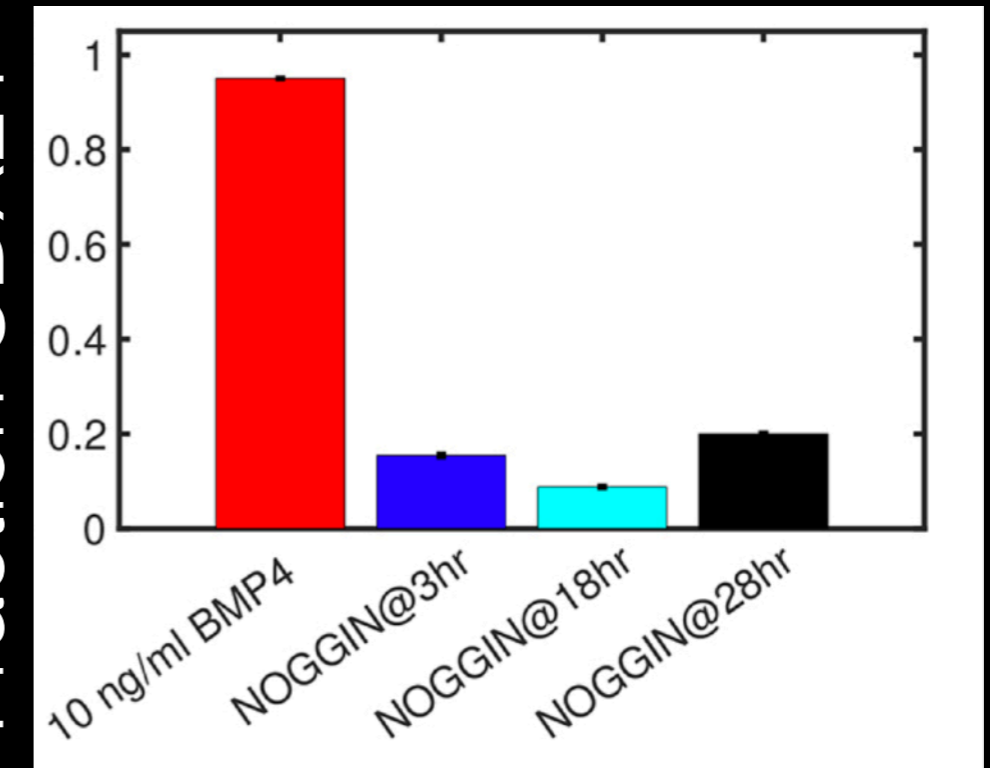
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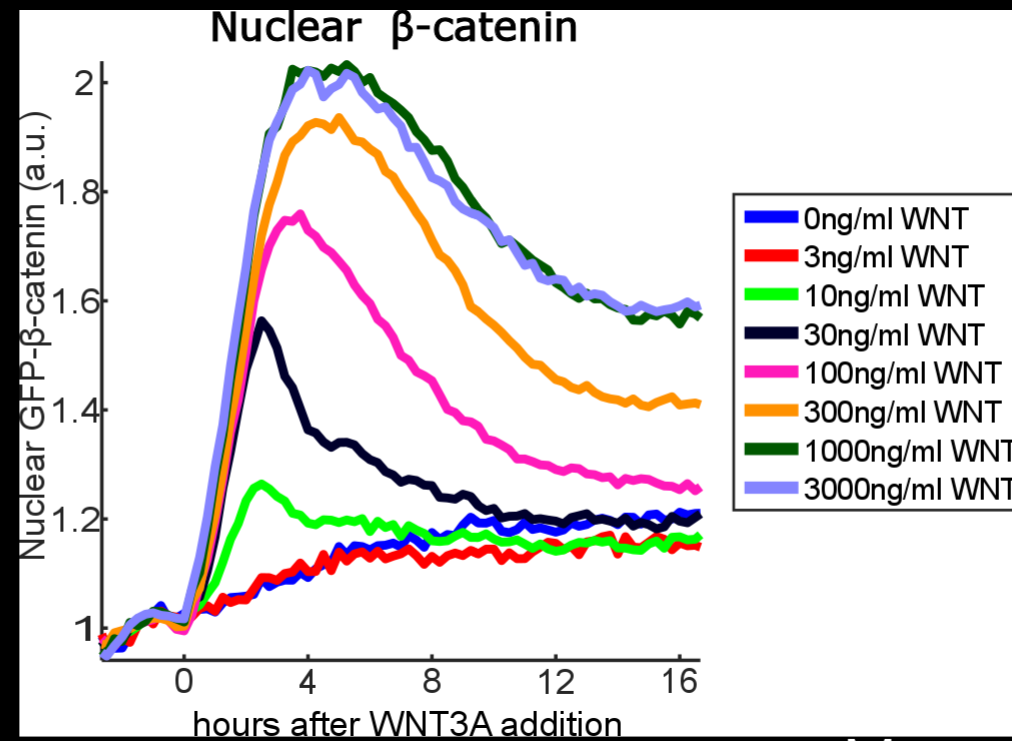
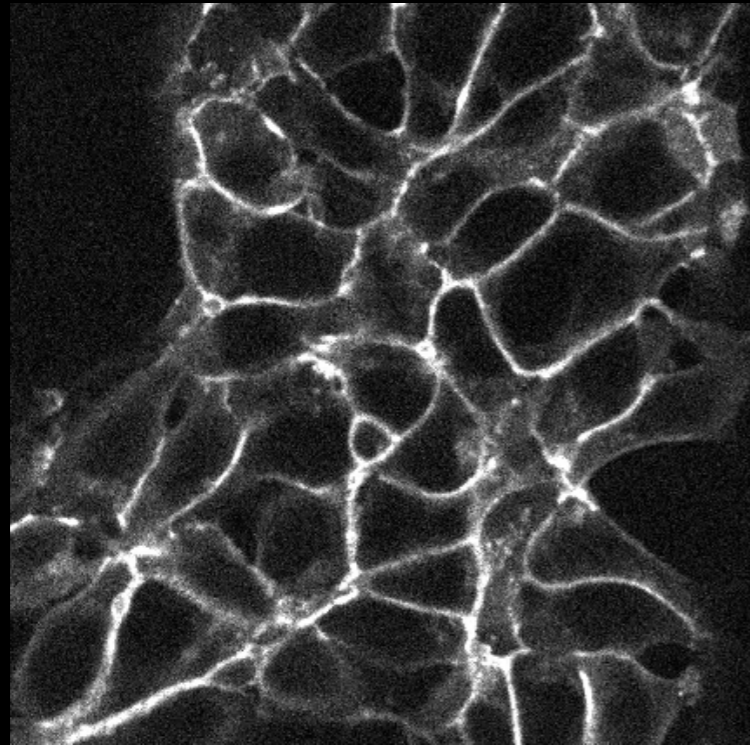


Fraction CDX2+



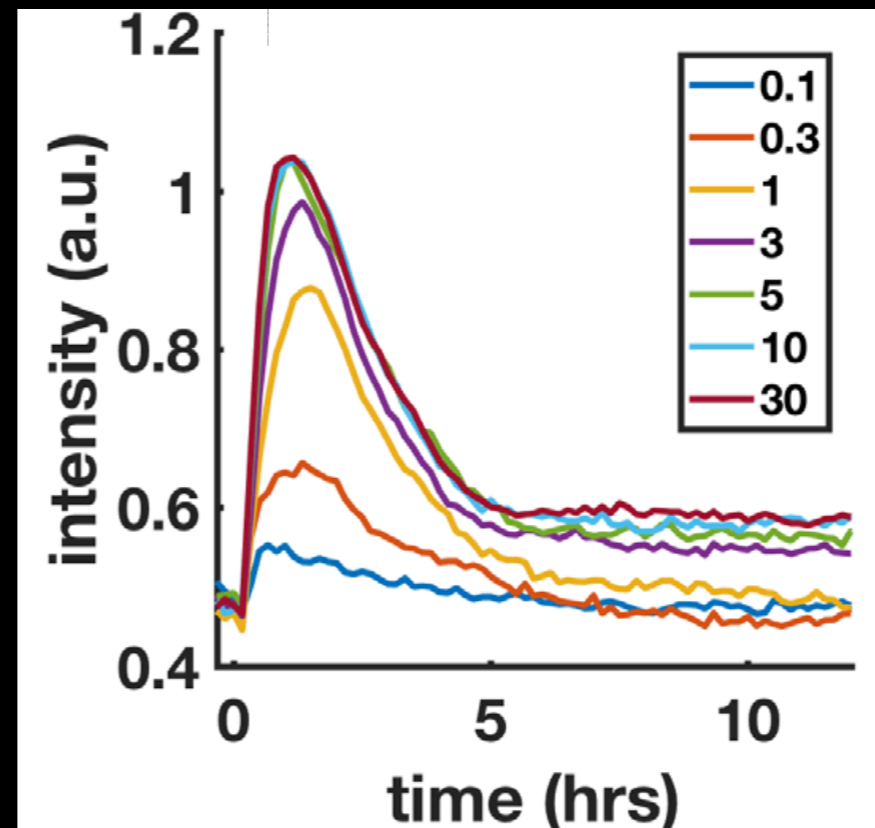
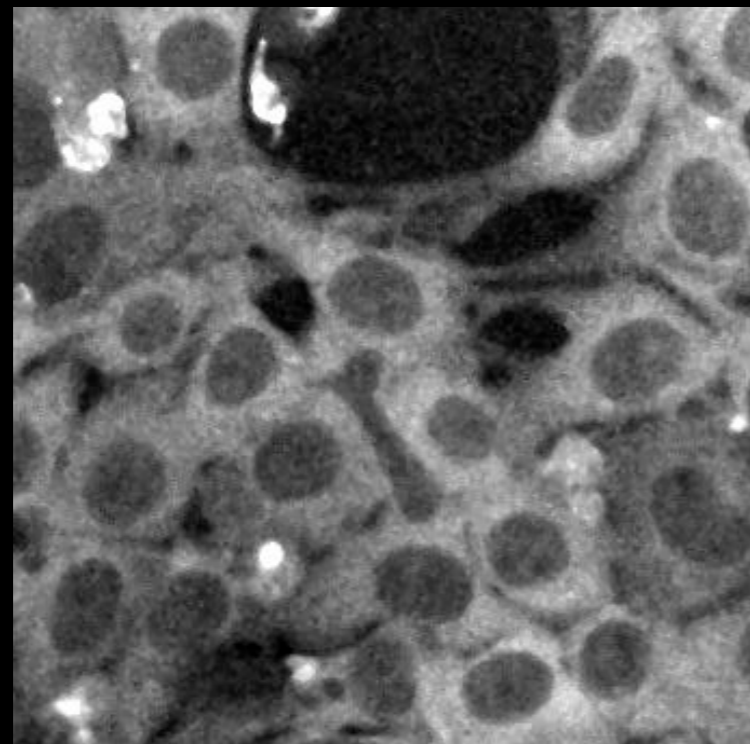
Wnt and Nodal signaling both respond adaptively to ligand simulation

Wnt response
GFP- β -catenin



Massey et al. PNAS (2019)

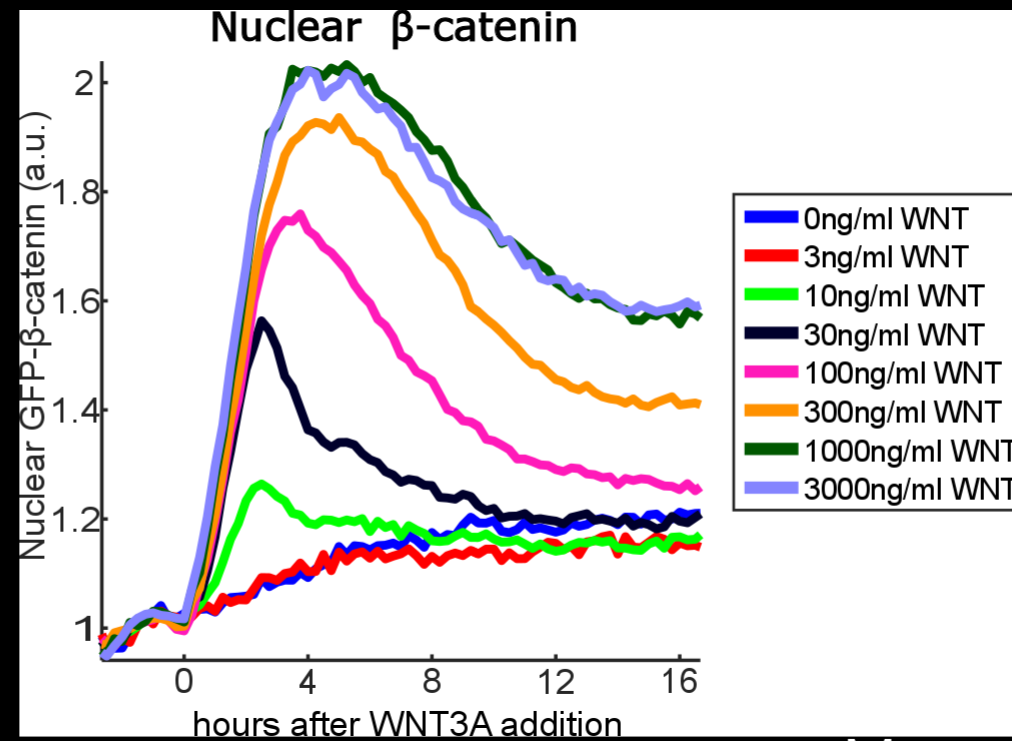
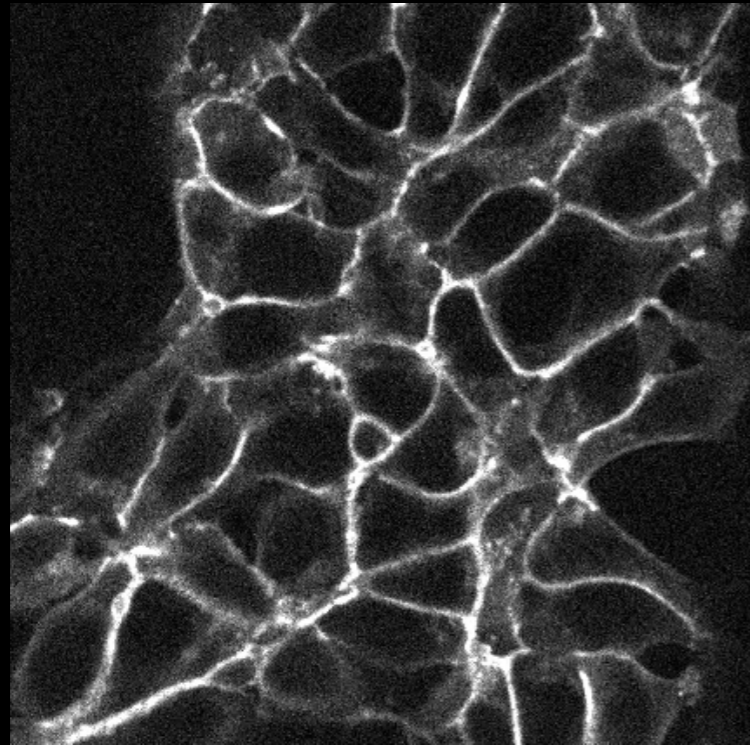
Activin/Nodal response
GFP-SMAD4



Heemskerk et al eLife (2019)

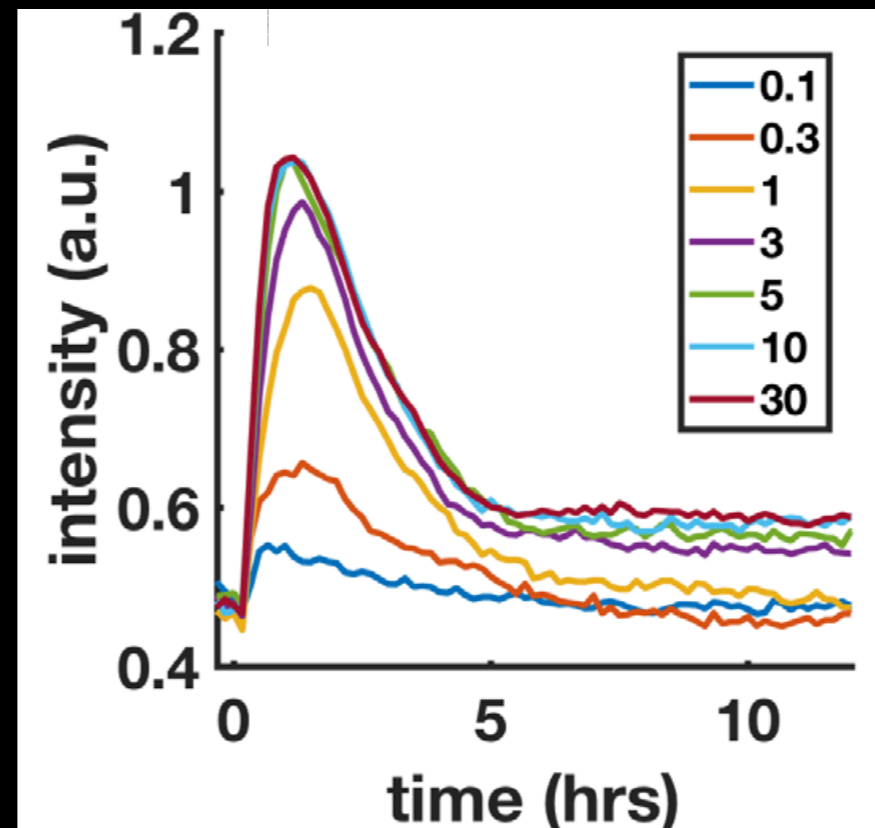
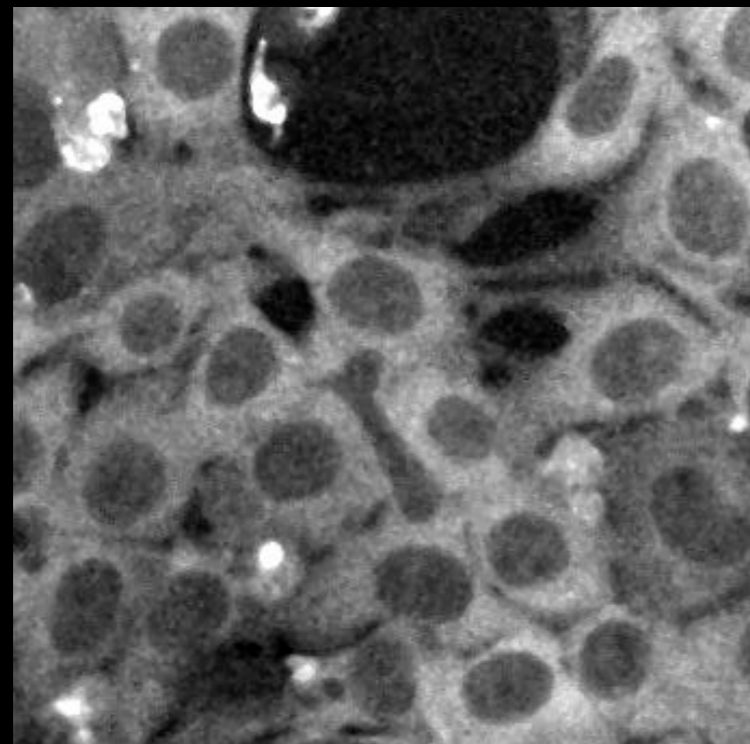
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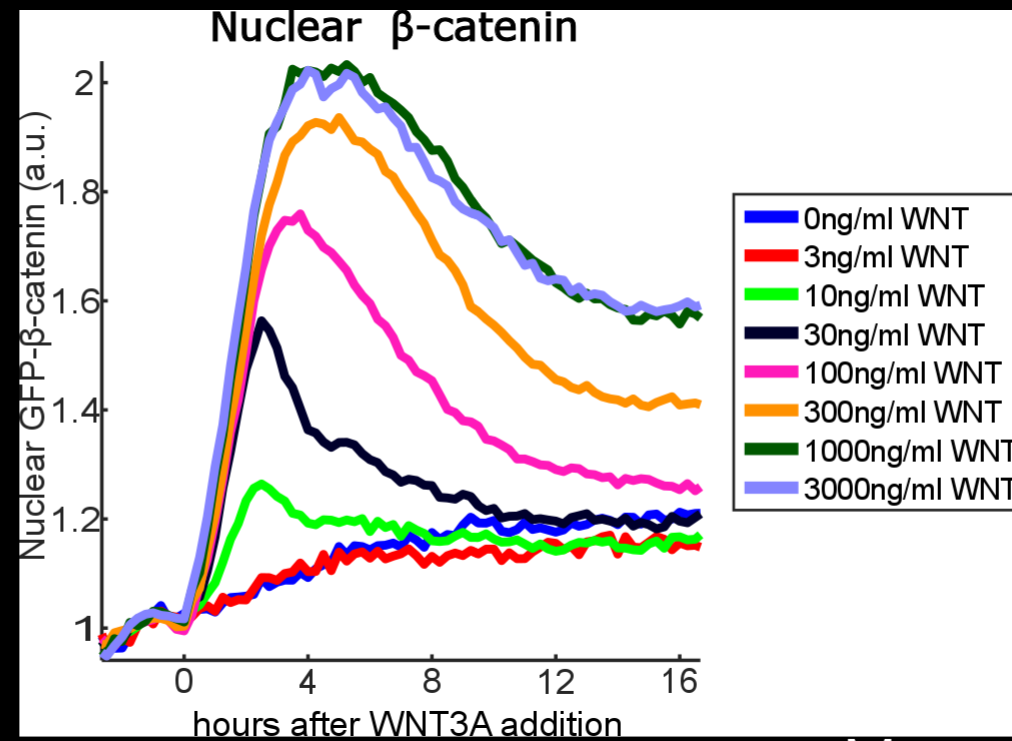
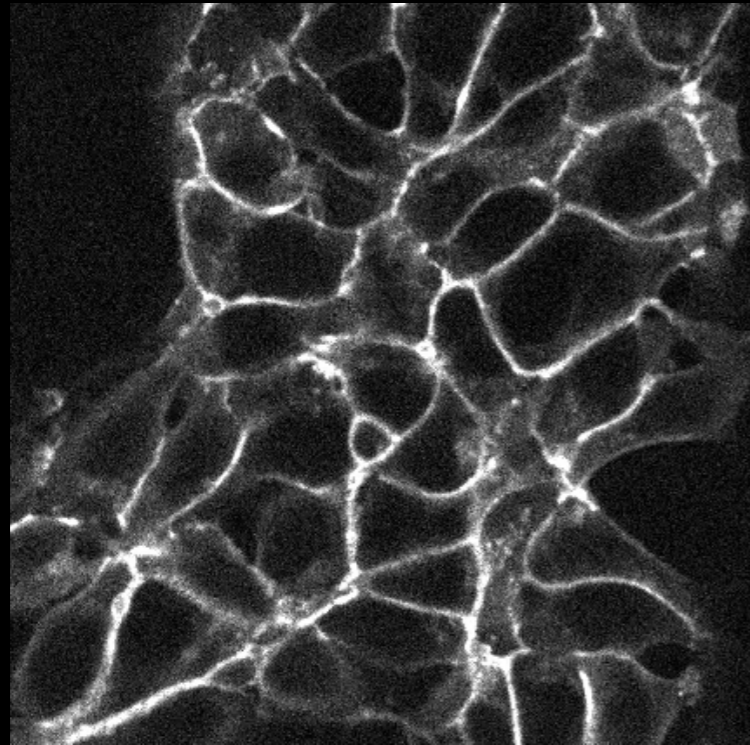
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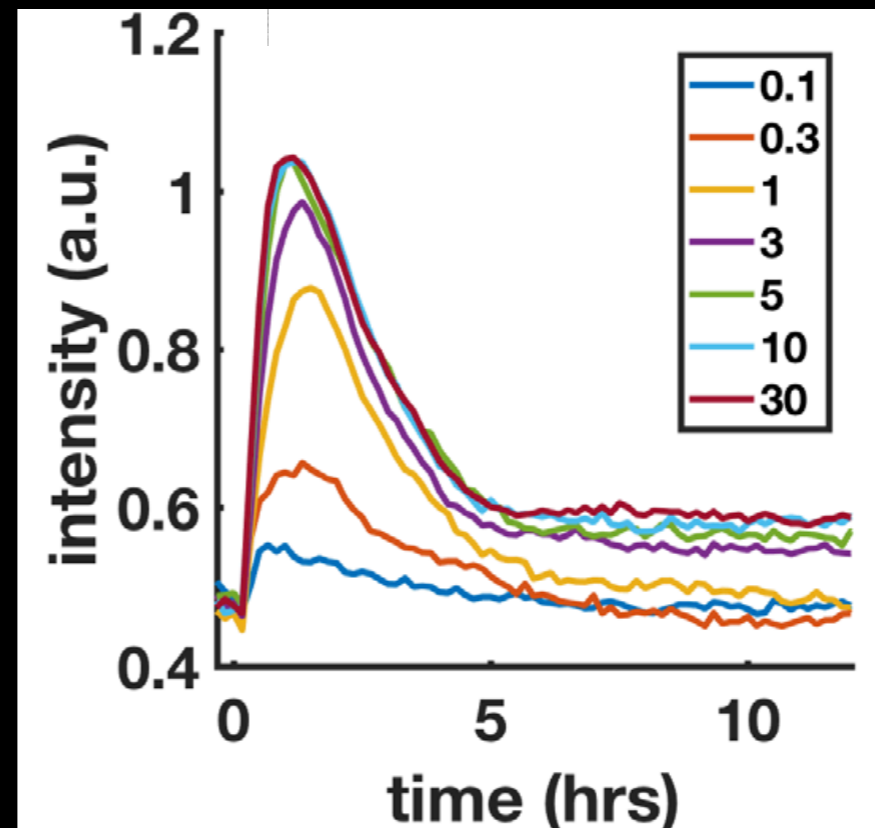
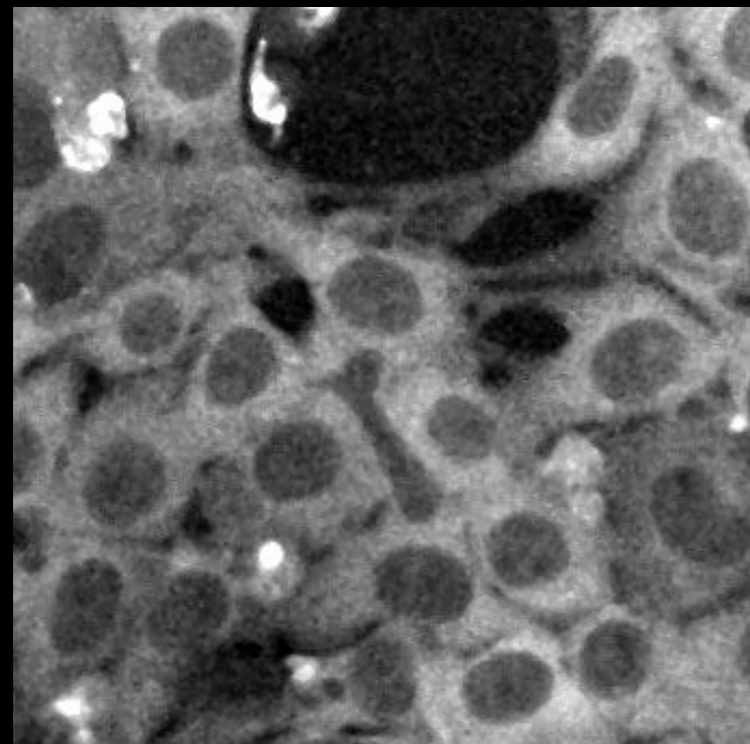
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Wnt response
GFP- β -catenin



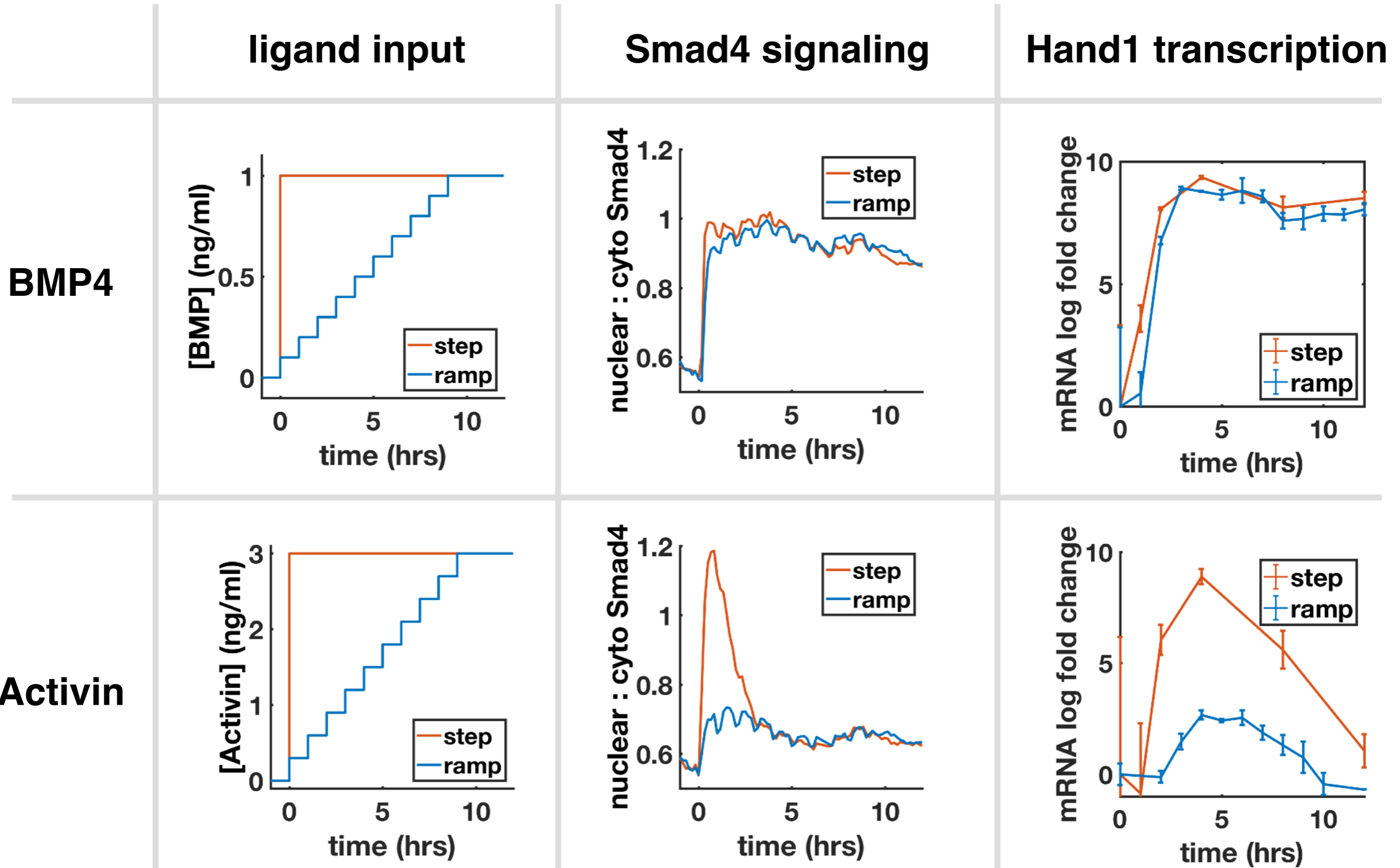
Massey et al. PNAS (2019)

Activin/Nodal response
GFP-SMAD4

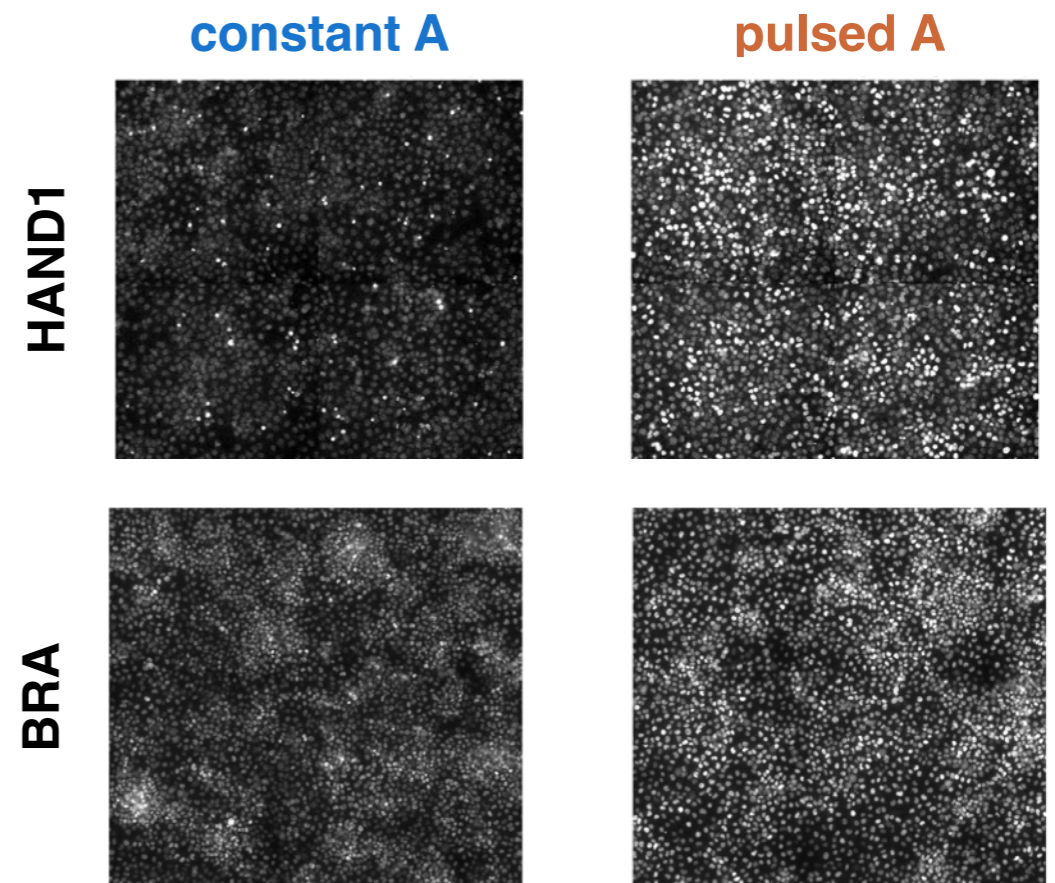
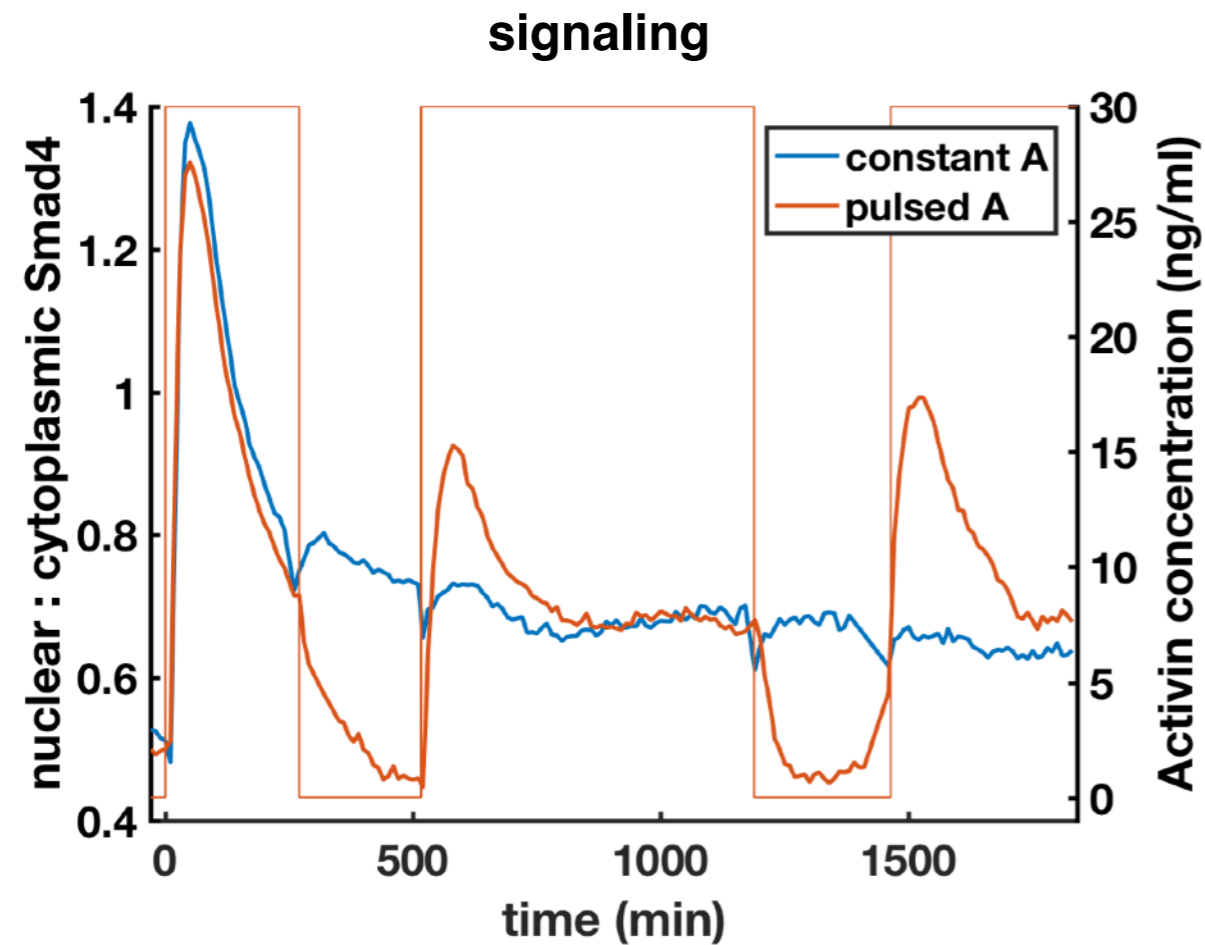


Heemskerk et al eLife (2019)

Adaptive pathway responds to the rate of ligand change



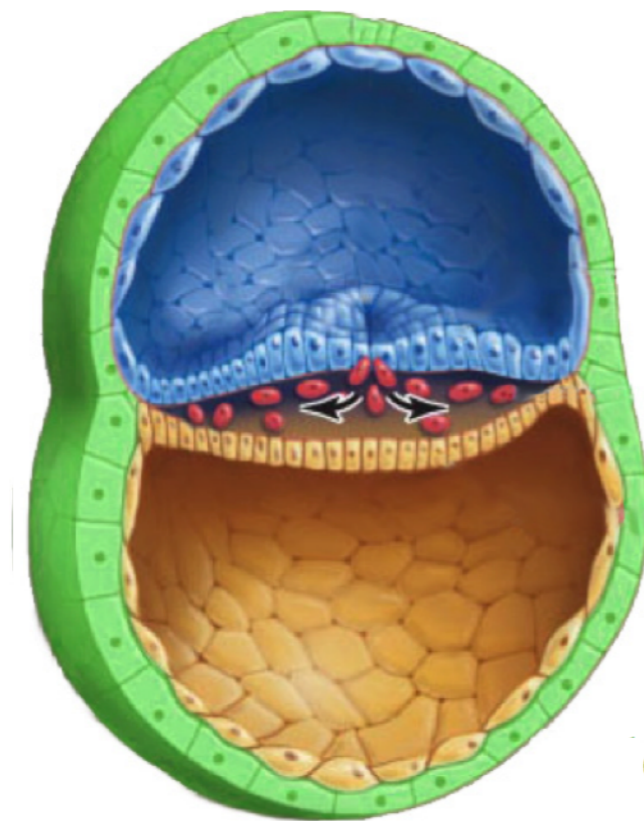
Pulsed Activin increases expression of adaptive genes



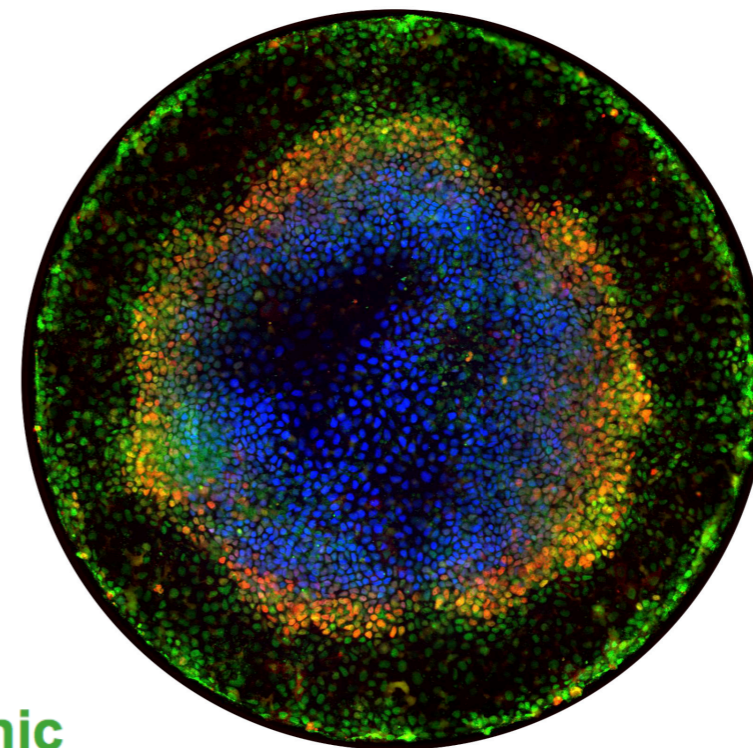
36h under differentiation conditions

How do these pathways work during patterning?

human embryo



micropatterned hESCs
42h after BMP4

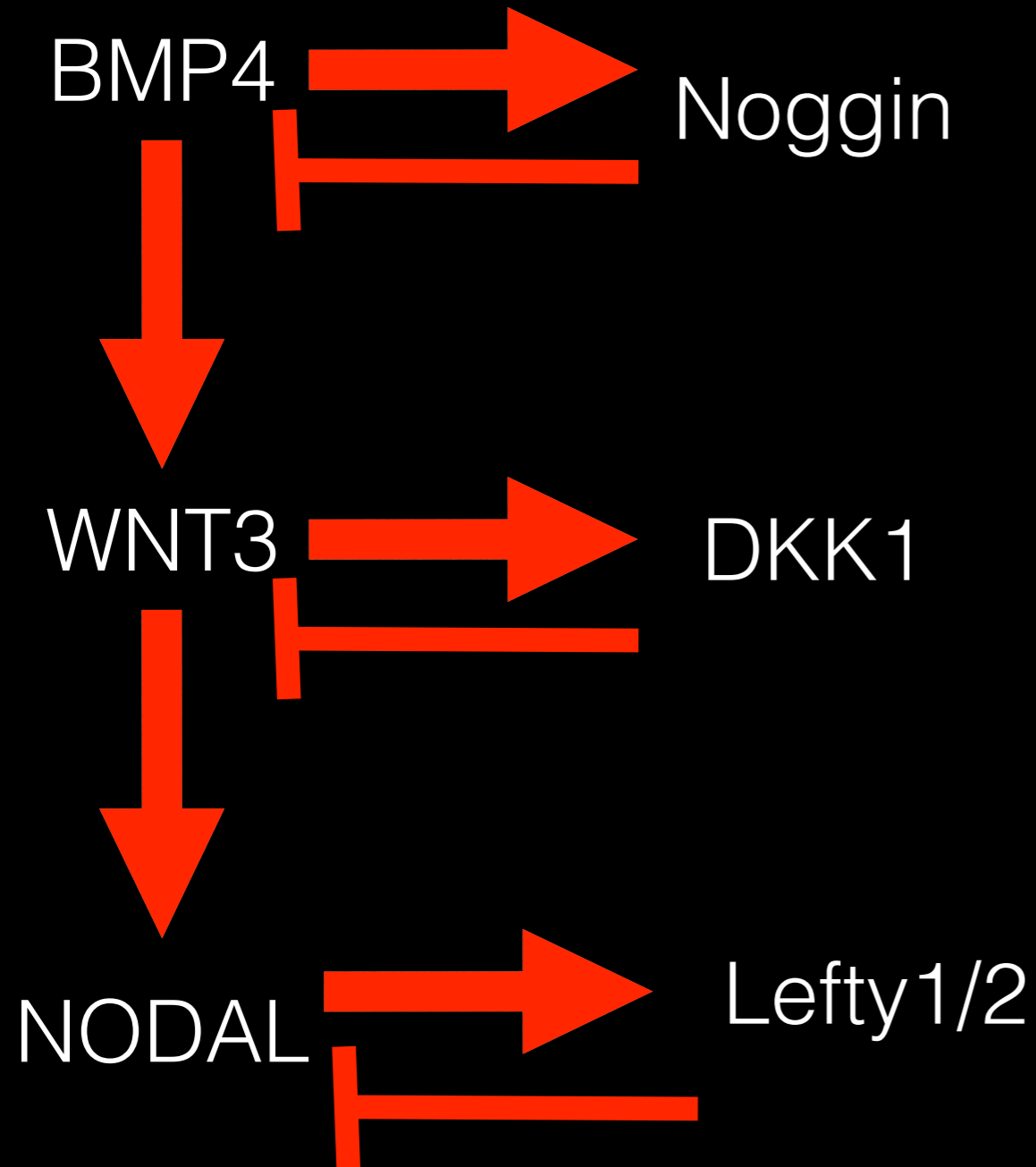


ectoderm
mesoderm
endoderm
extraembryonic

There are activator inhibitor pairs at each level in the patterning cascade

Activators

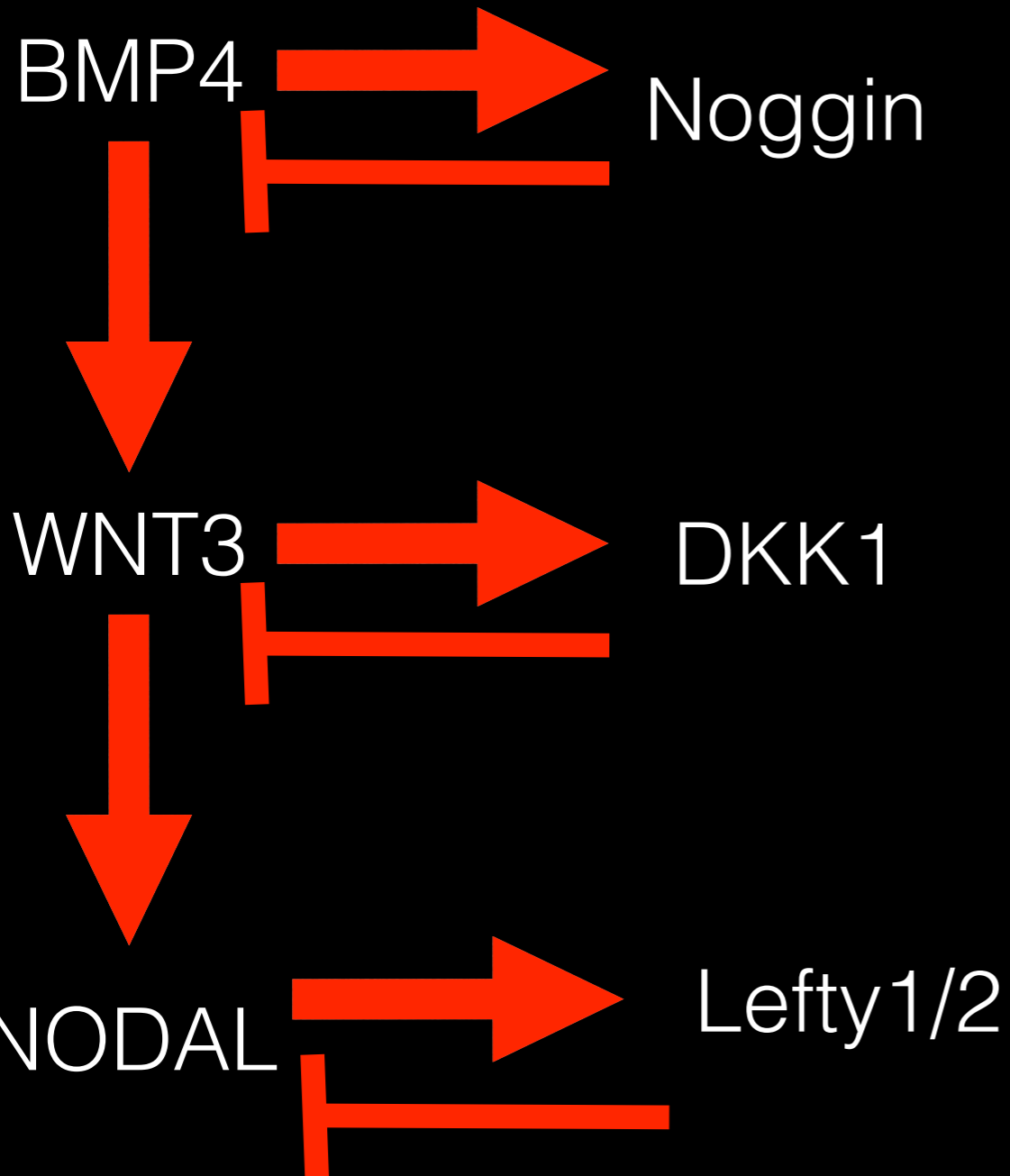
Inhibitors



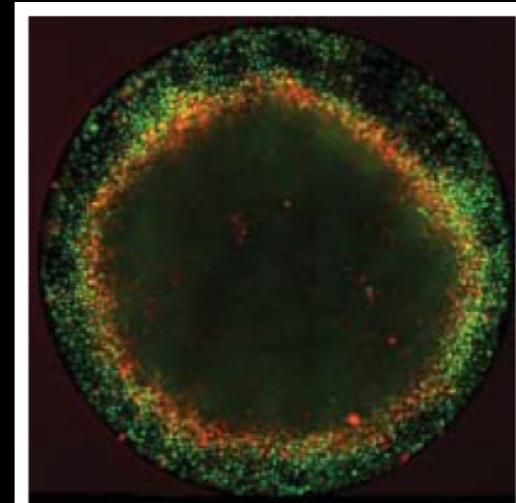
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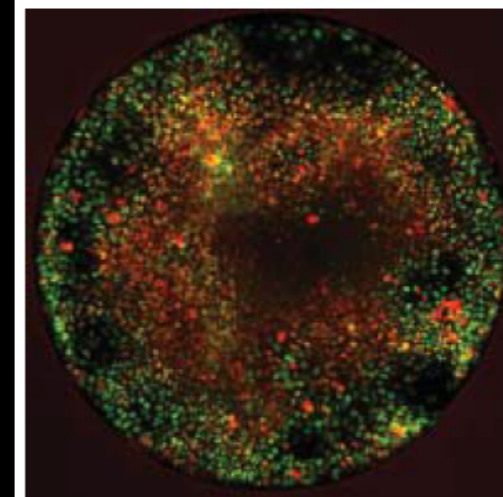
Inhibitors



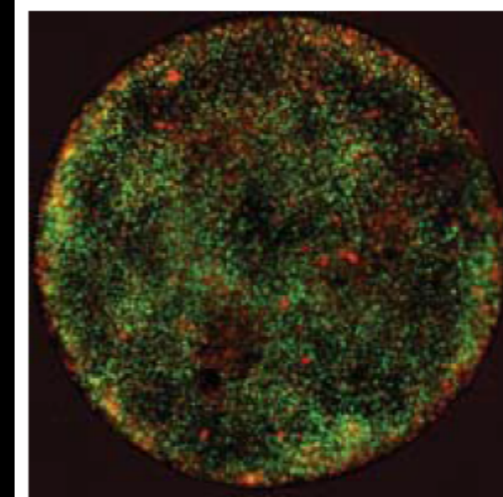
CDX2/BRA



Control



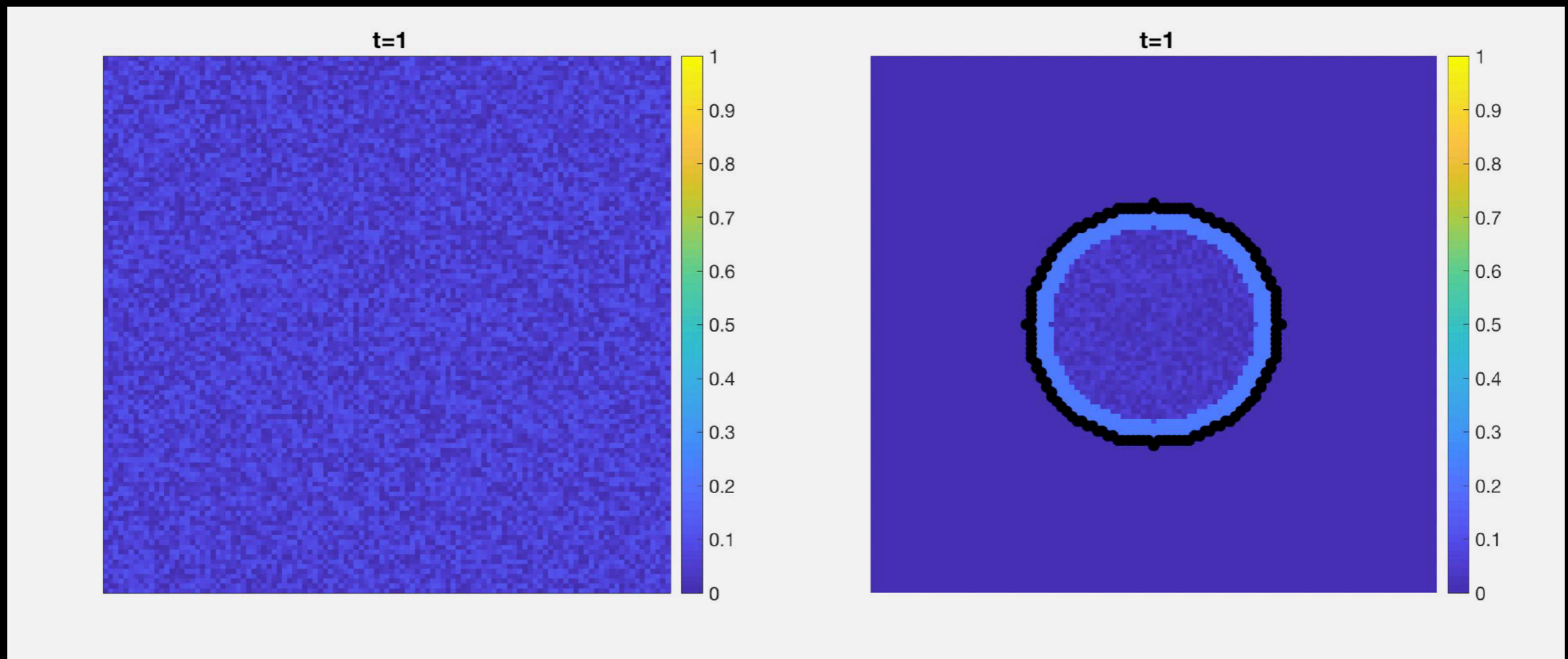
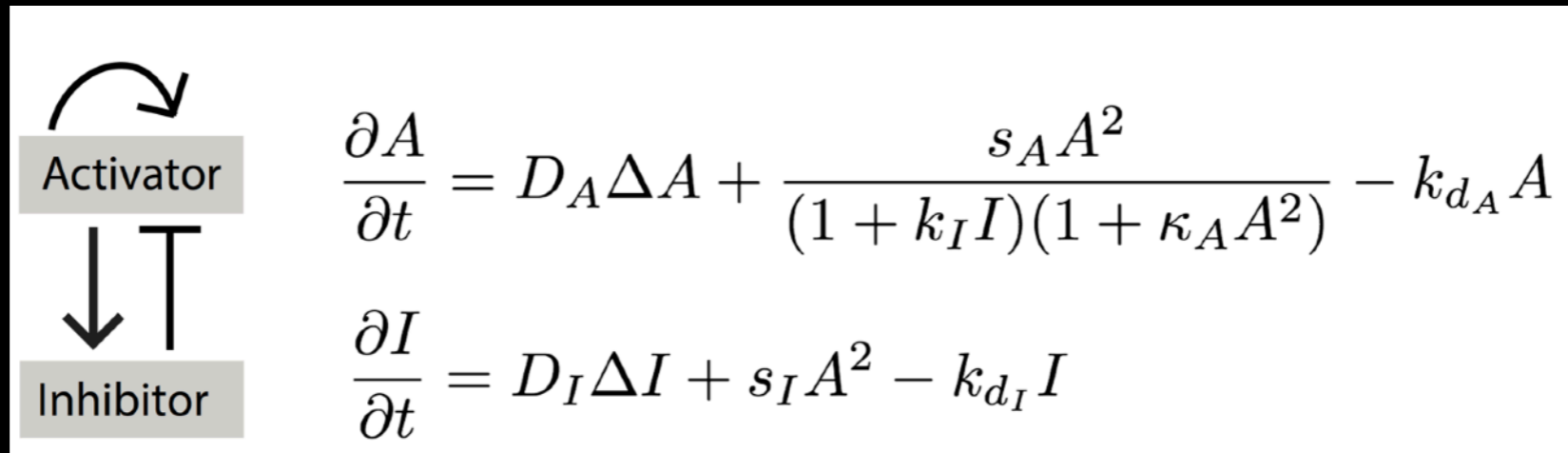
Knockdown
BMP
Inhibitors



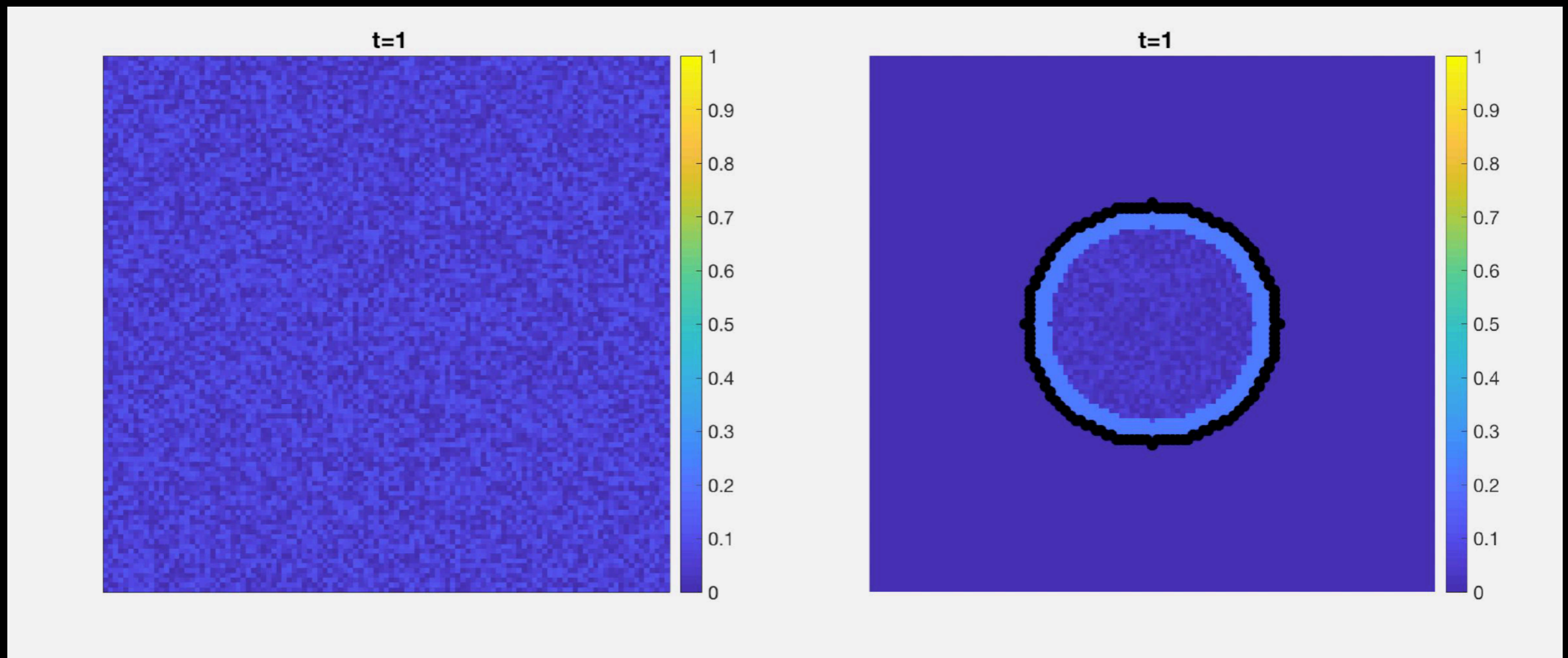
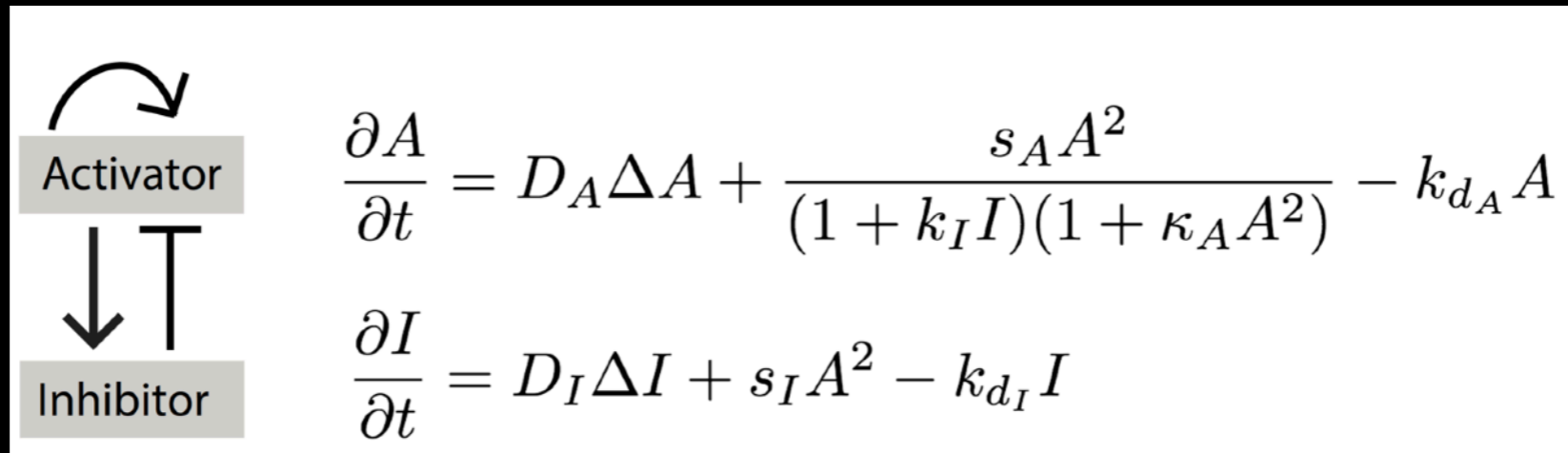
Knockdown
Nodal
Inhibitors

Inhibitors play a role in patterning

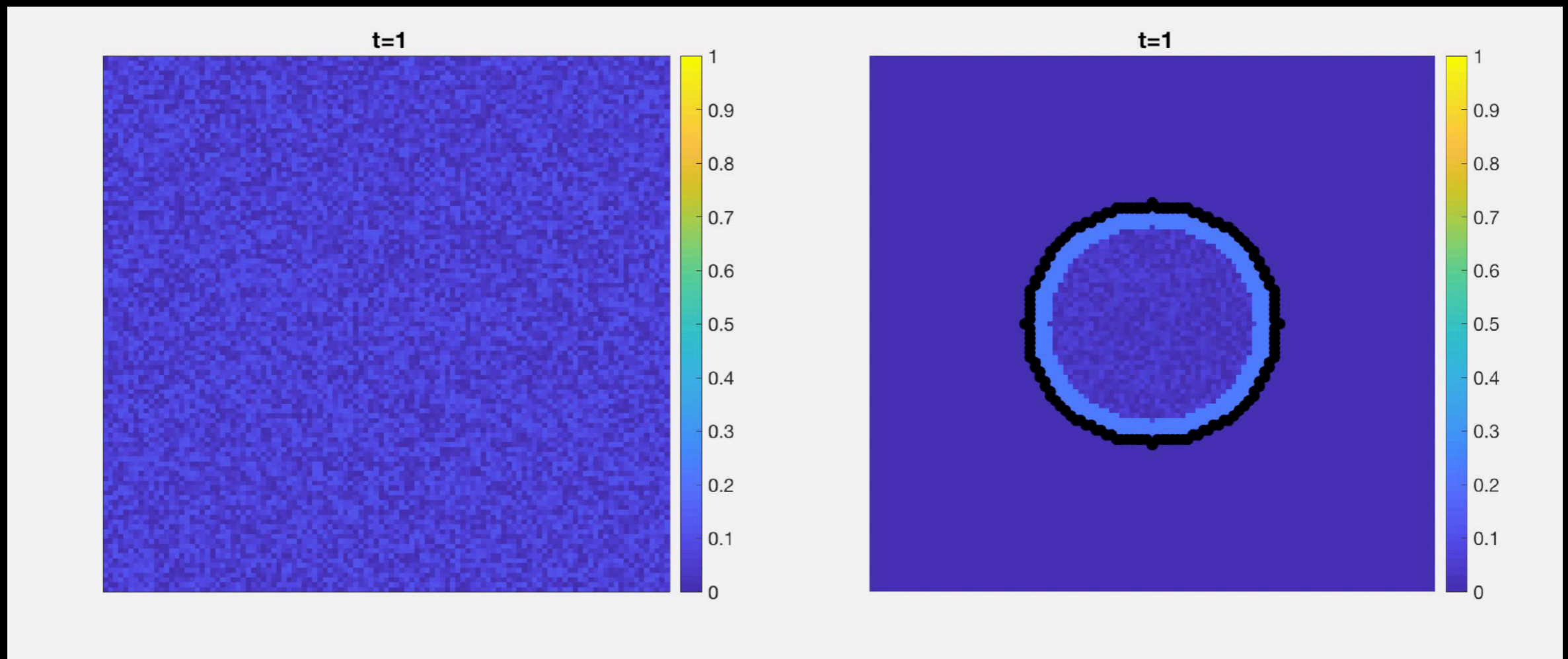
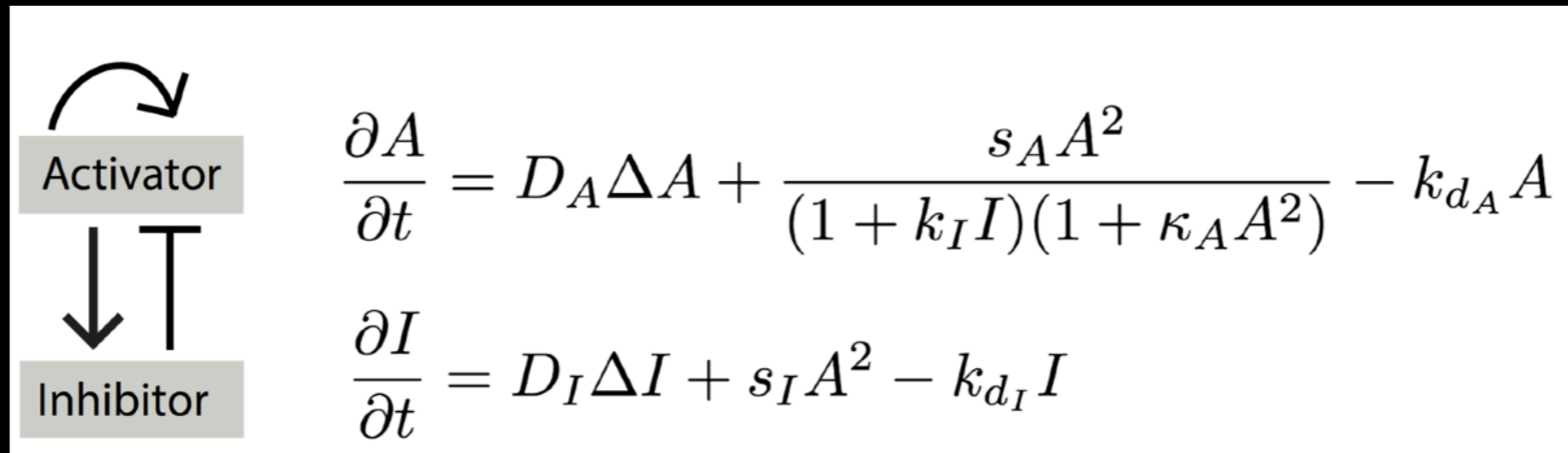
What to expect from a activator-inhibitor Turing system?



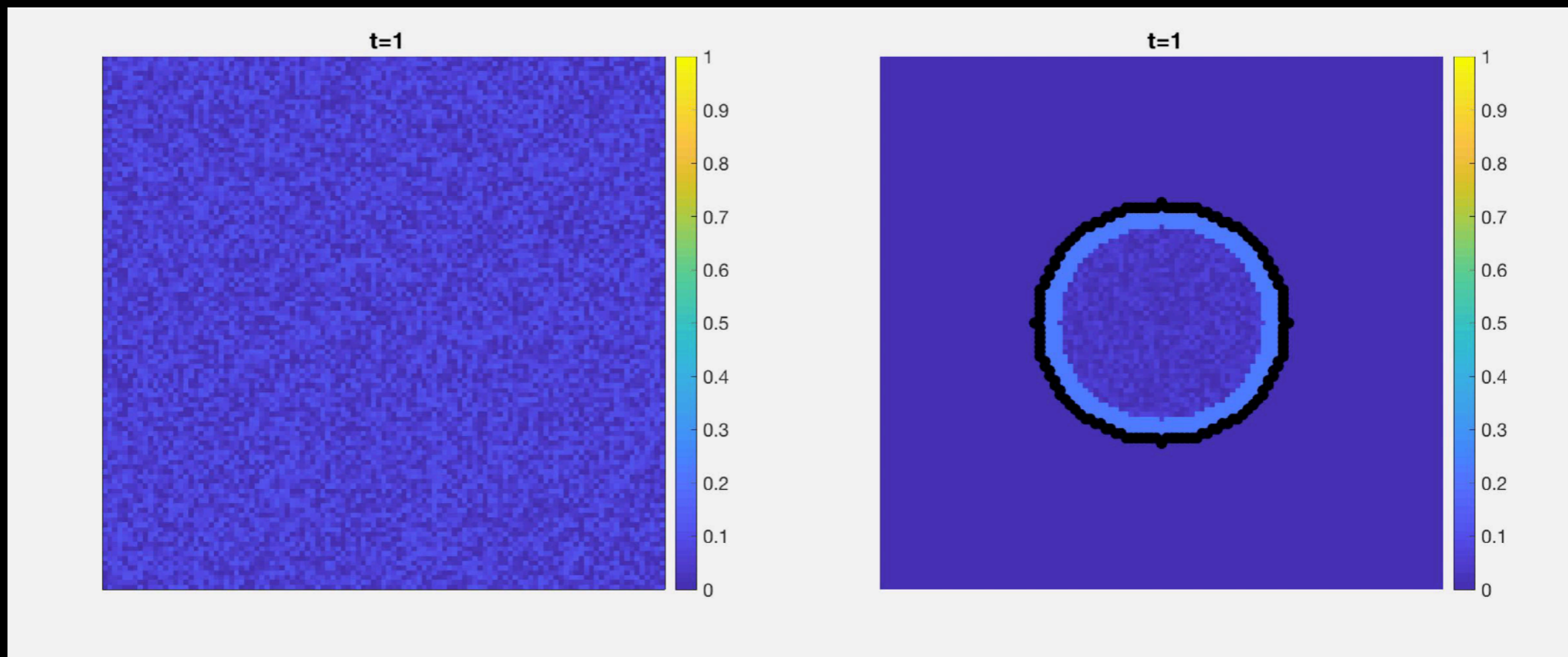
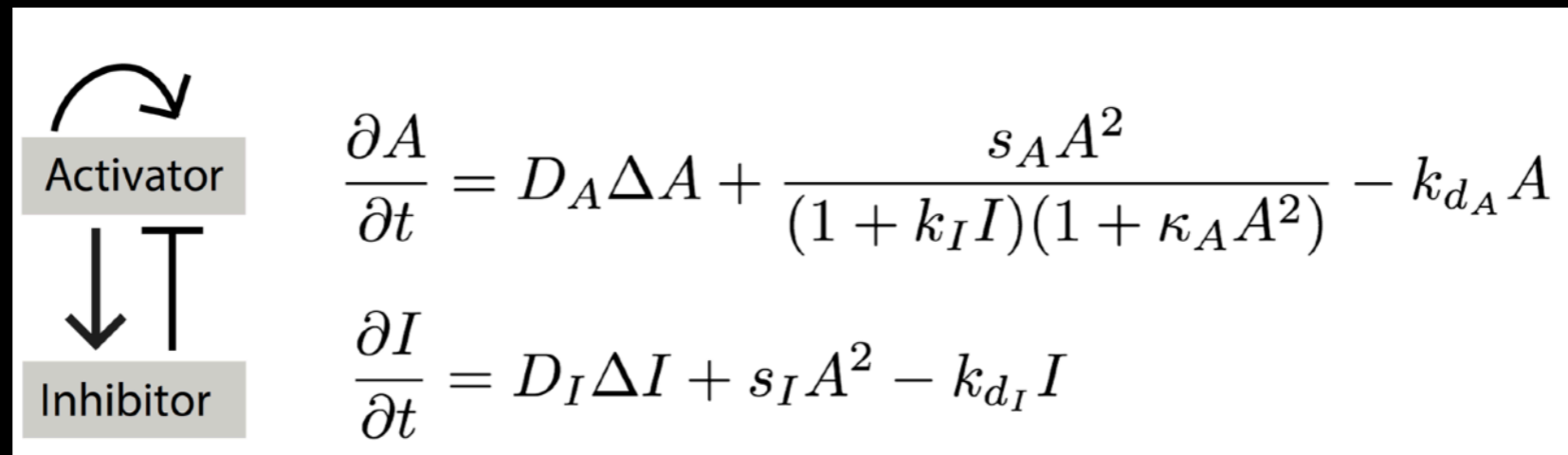
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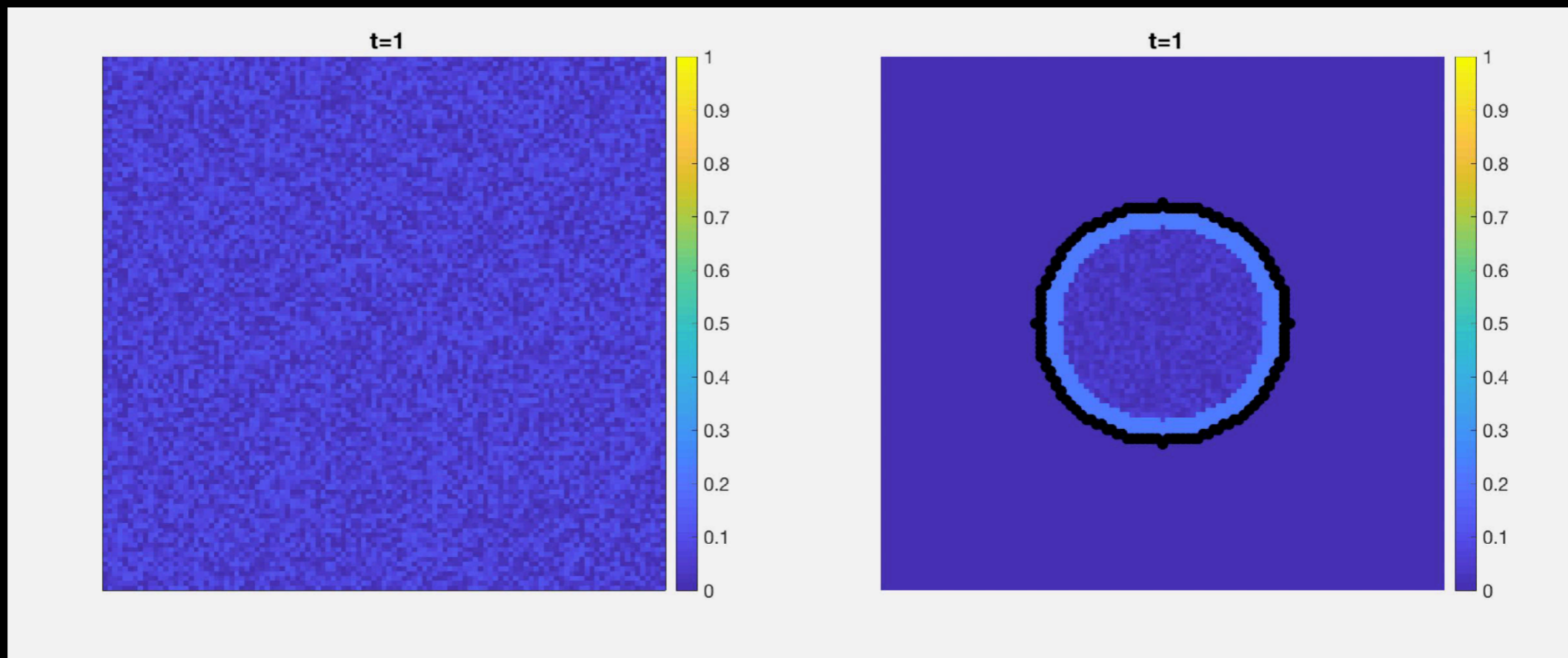
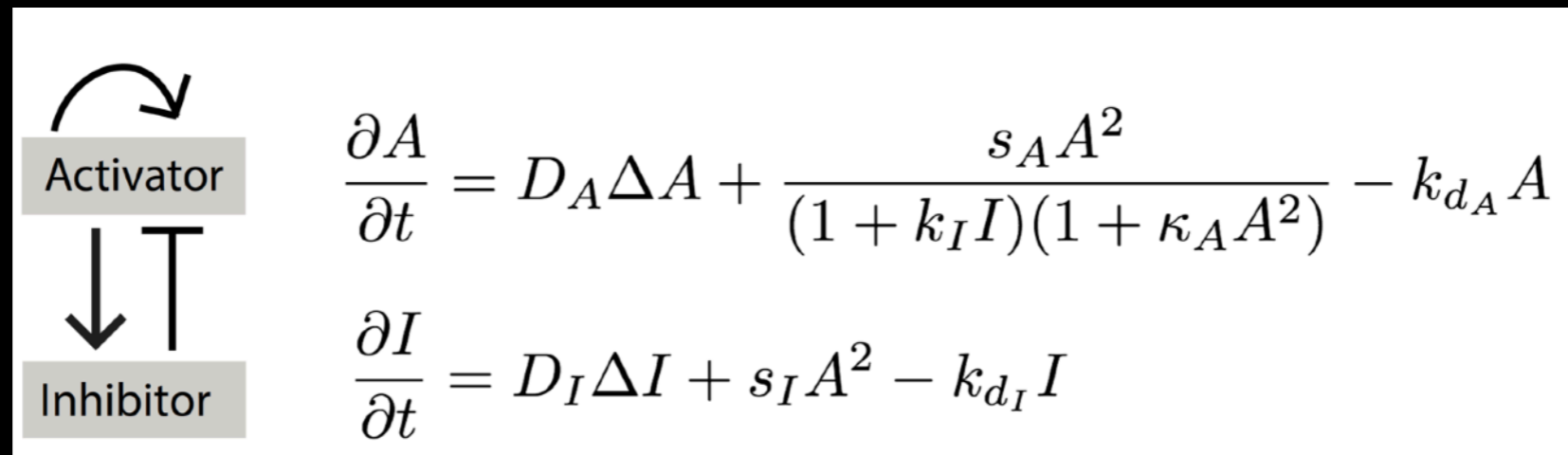
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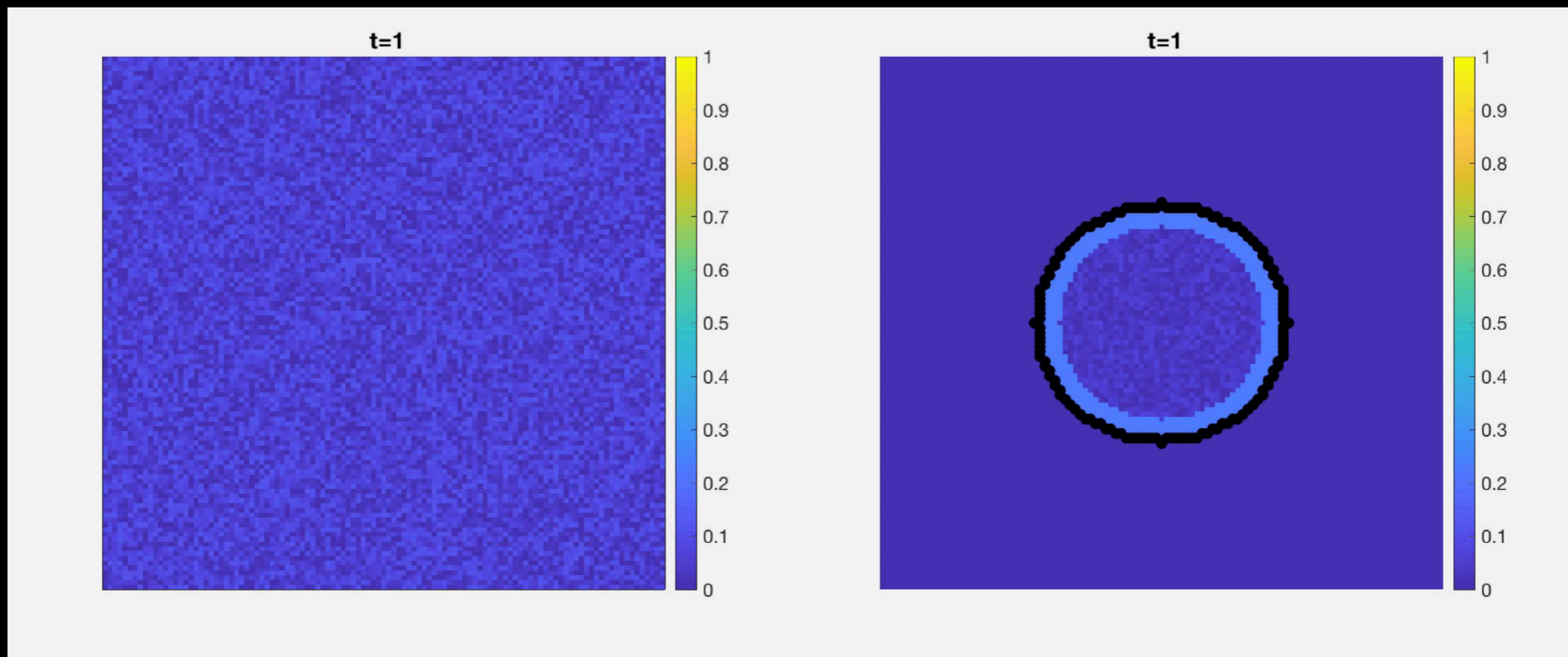
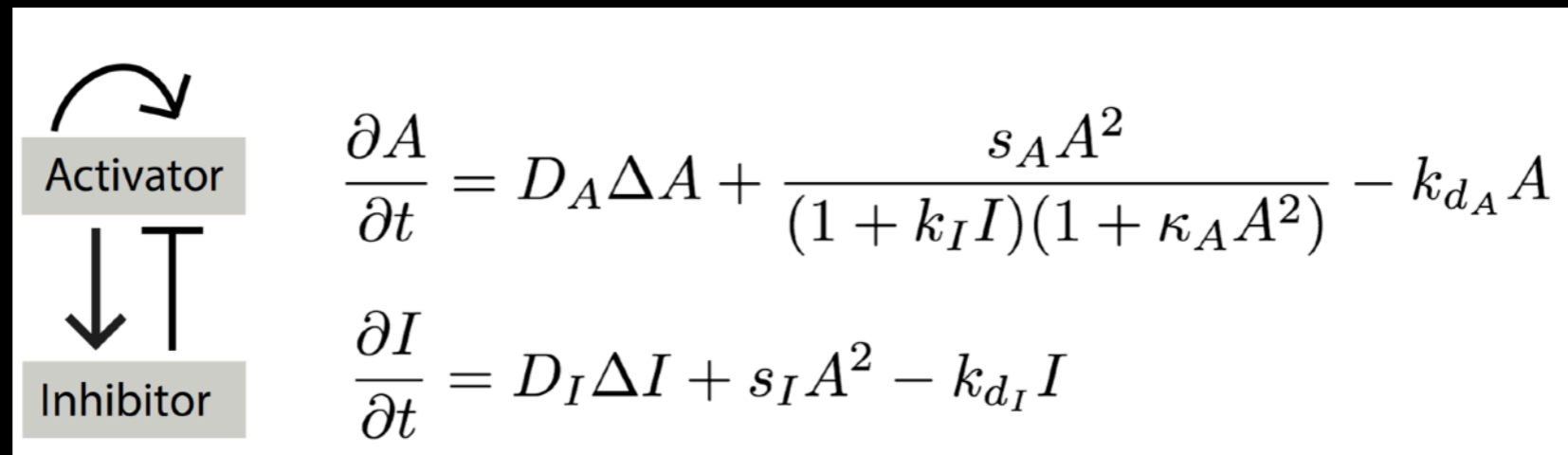
Outside the Turing regime, pattern results from auto-activation and diffusive loss



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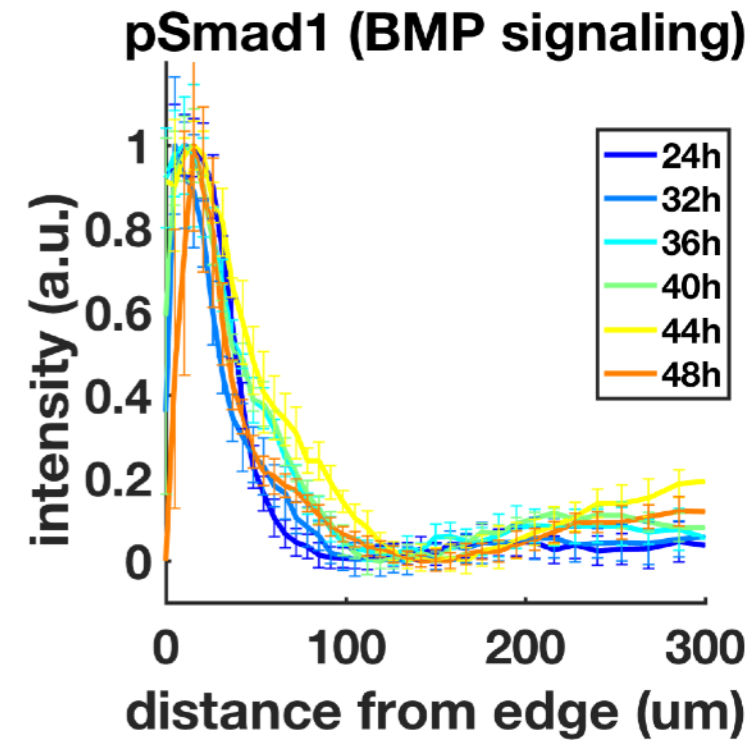
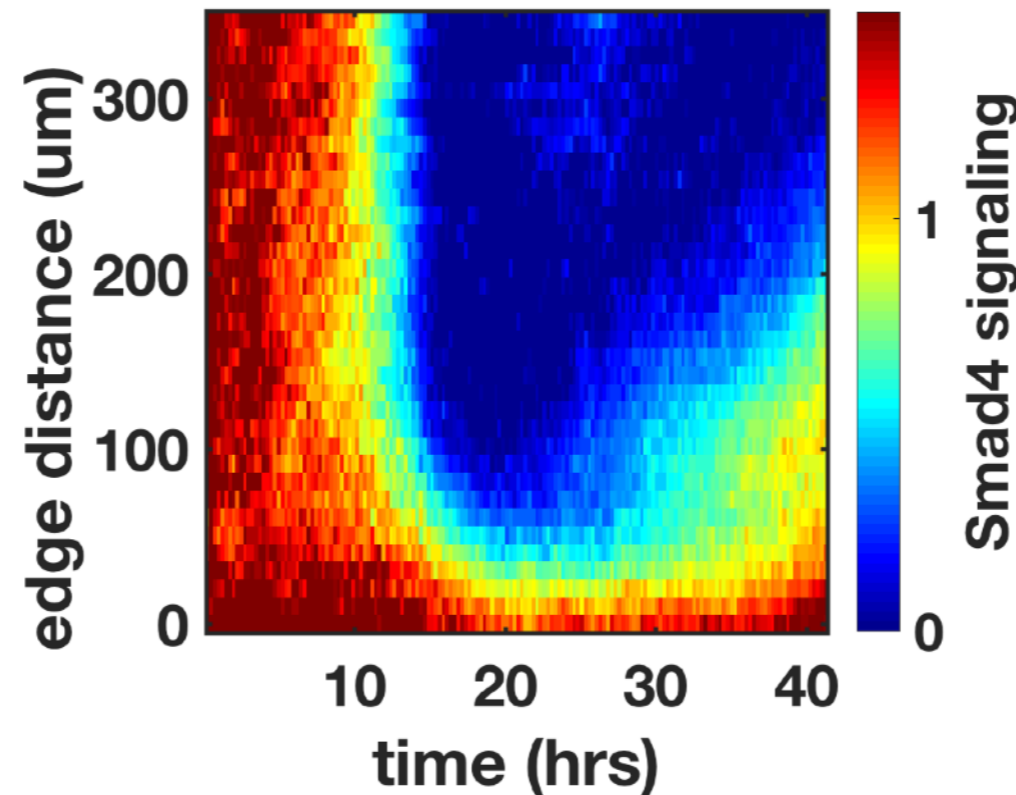
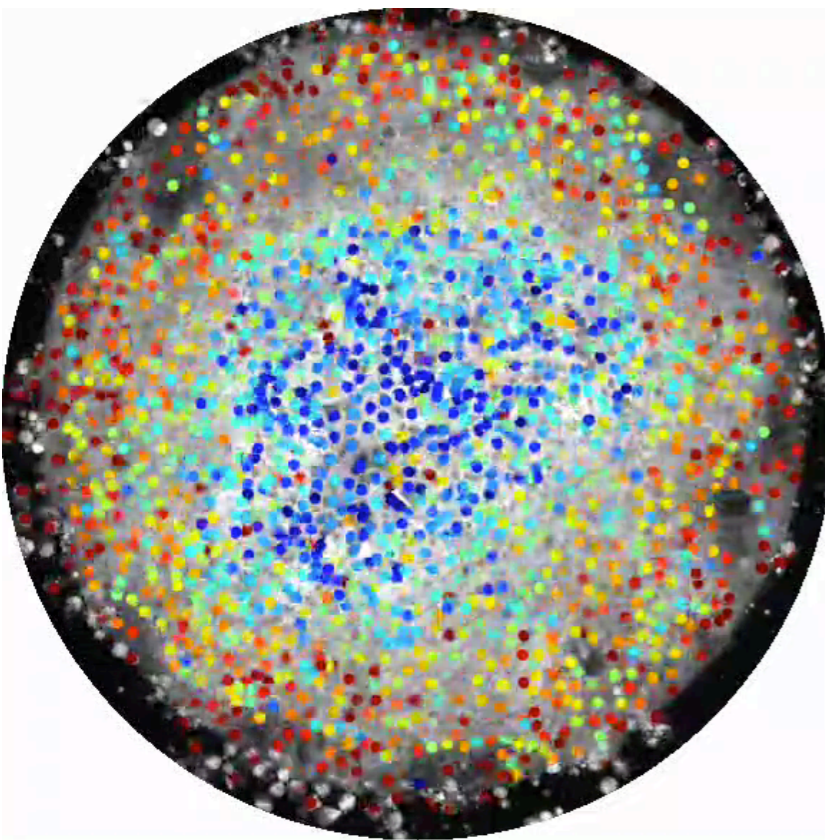


Outside the Turing regime, pattern results from auto-activation and diffusive loss



BMP signaling is initially widespread and then restricted to the colony border where it is sustained.

Smad4 signaling in BMP4 treated colony:

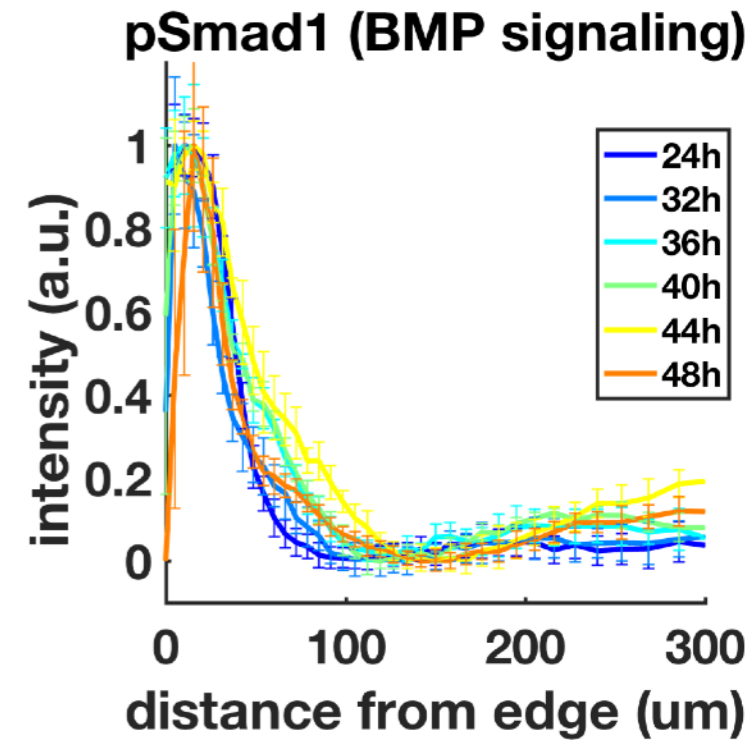
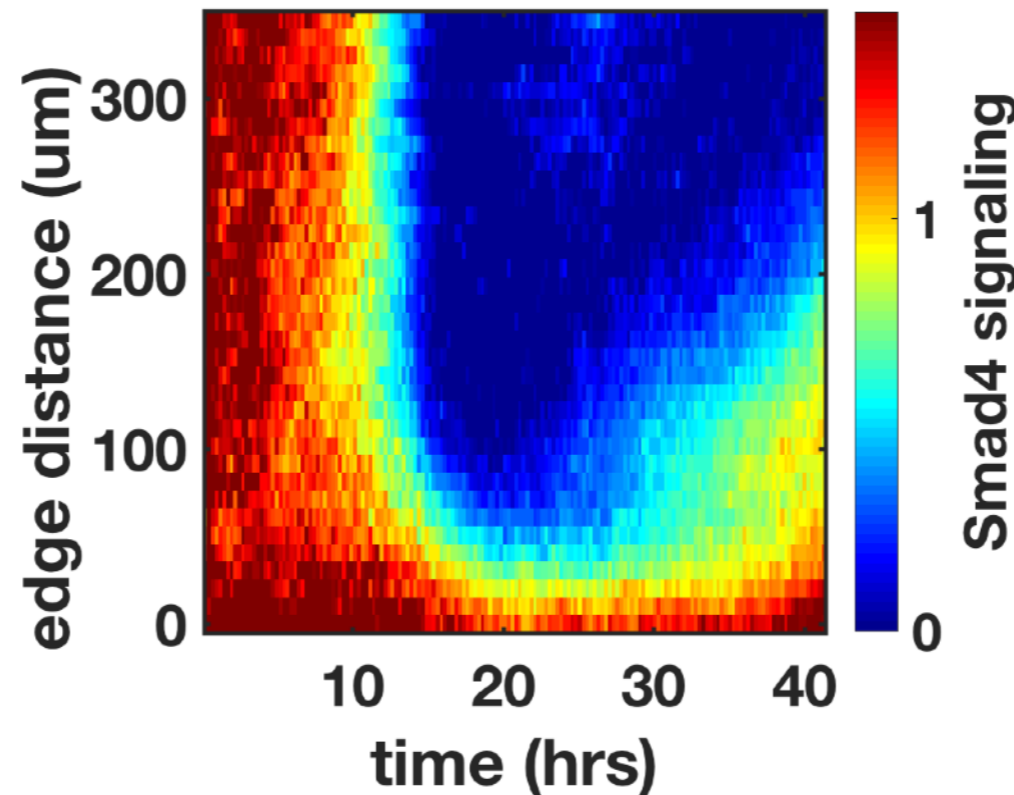
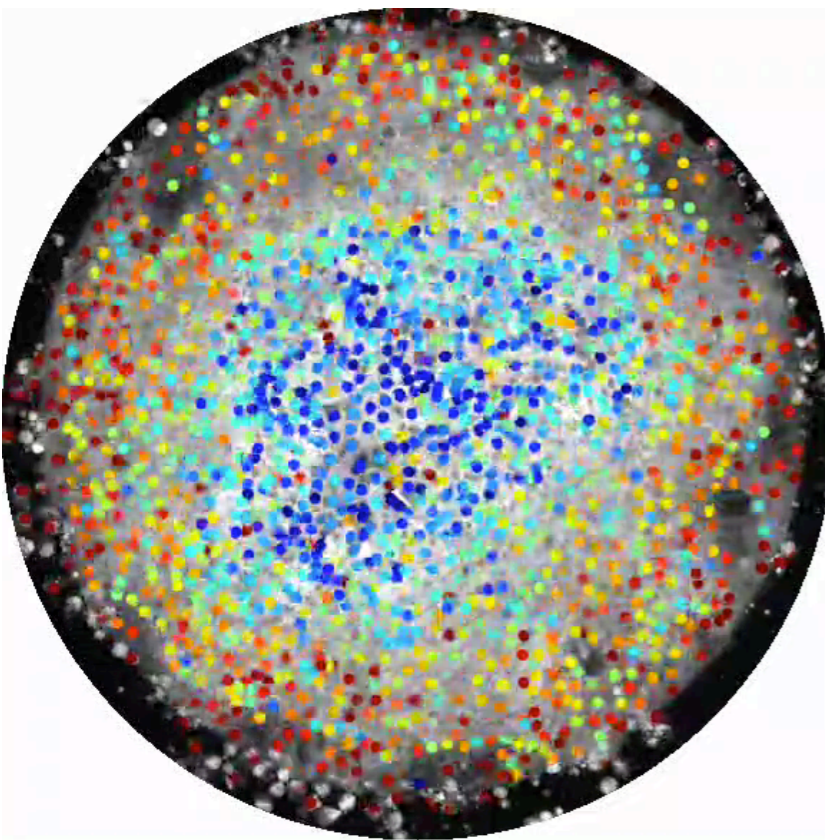


Heemskerk et al eLife (2019)

Consistent with the need for sustained BMP signaling in generating the CDX2+ border fates

BMP signaling is initially widespread and then restricted to the colony border where it is sustained.

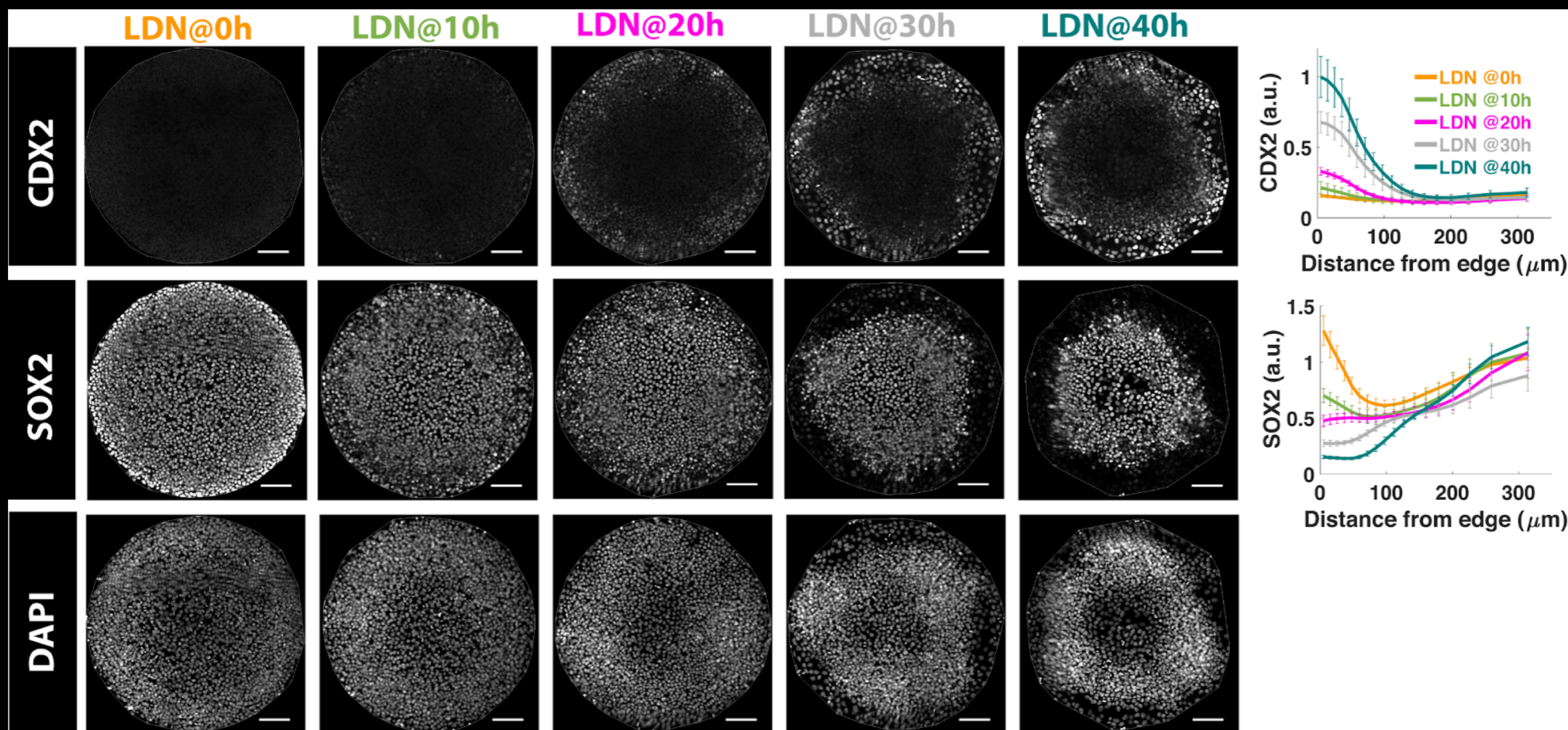
Smad4 signaling in BMP4 treated colony:



Heemskerk et al eLife (2019)

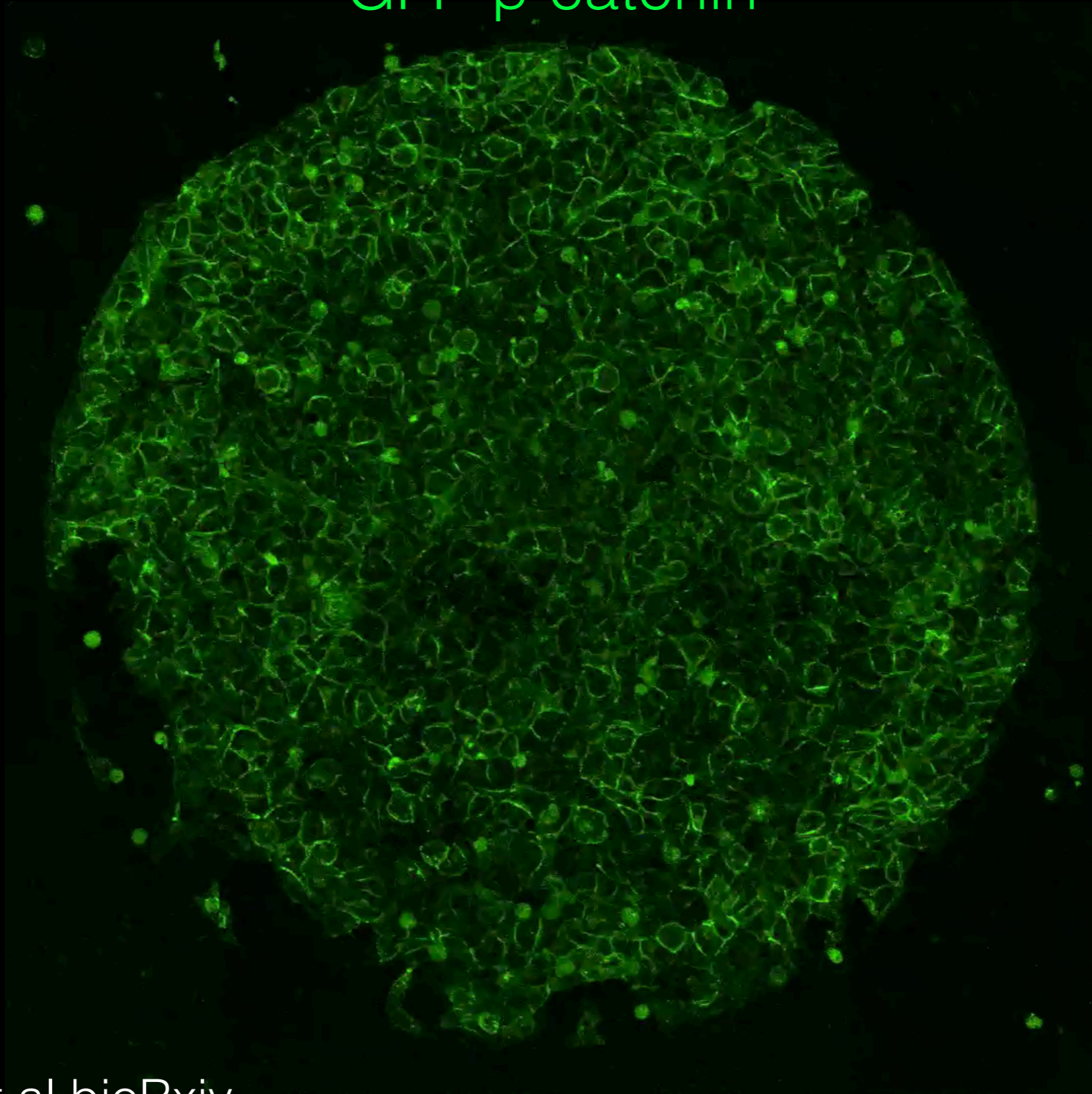
Consistent with the need for sustained BMP signaling in generating the CDX2+ border fates

Duration of BMP signaling at the colony edge determines extra-embryonic differentiation



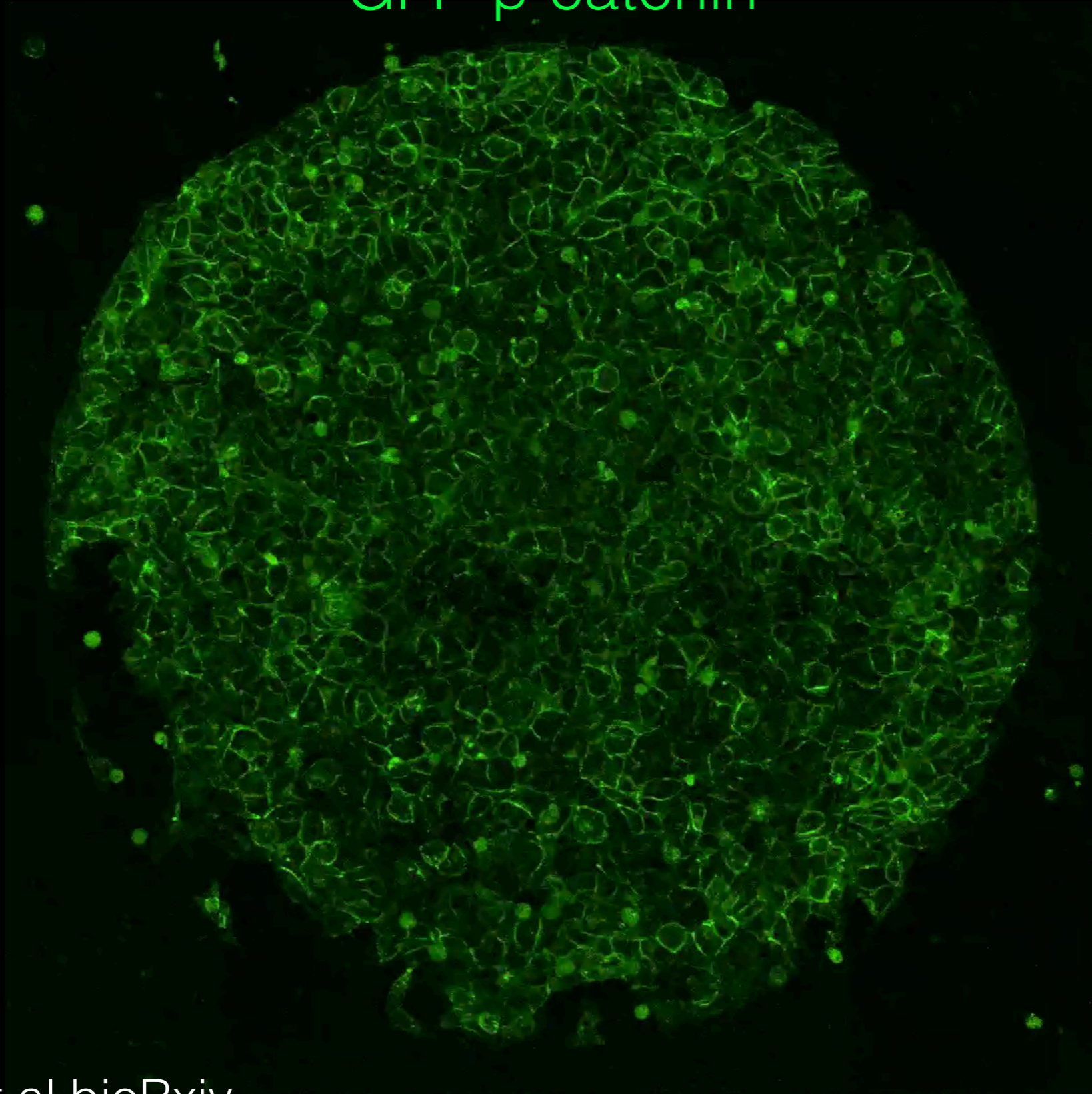
A wave of Wnt signaling moves through the colony from the edge inward

GFP- β -catenin

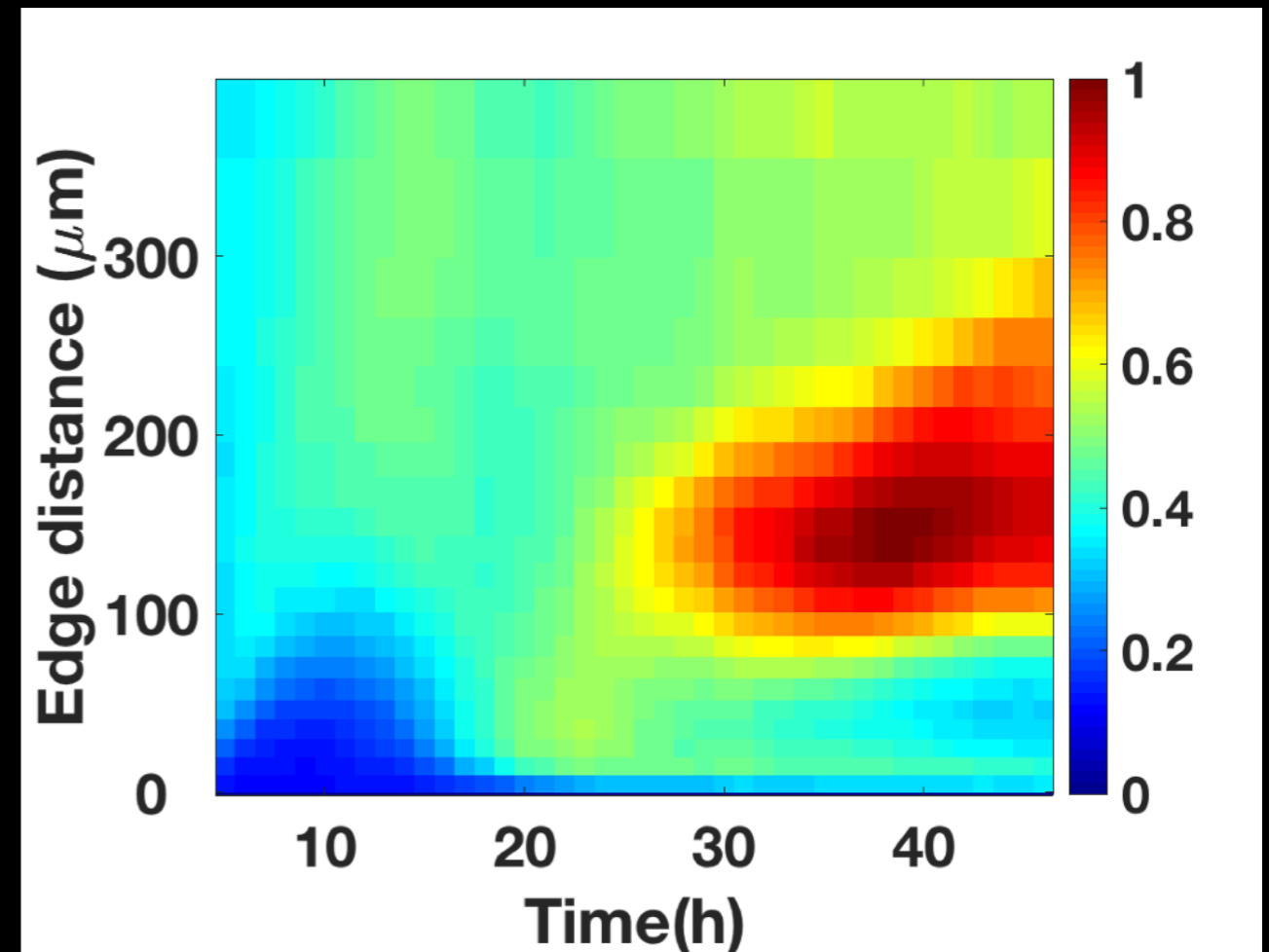
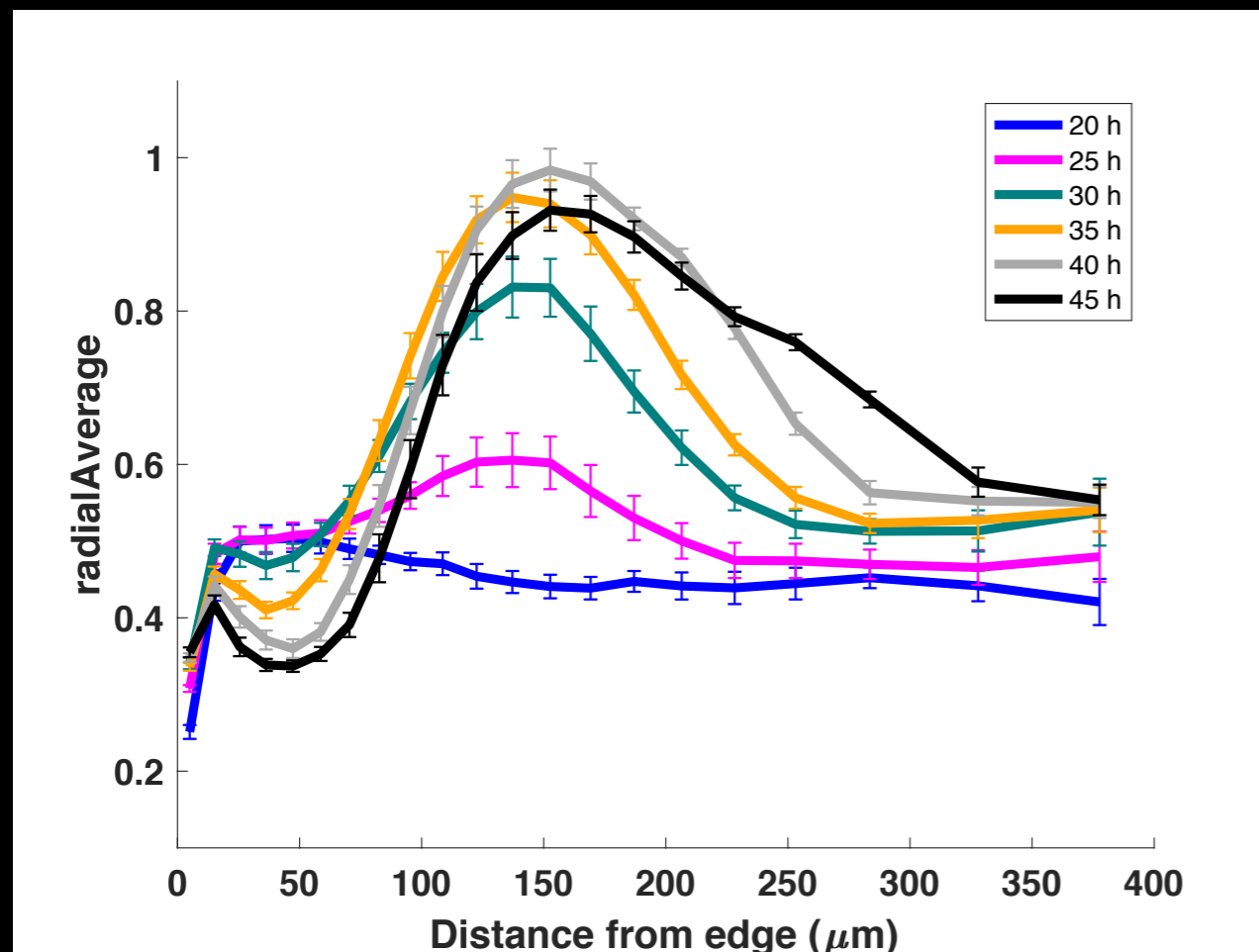


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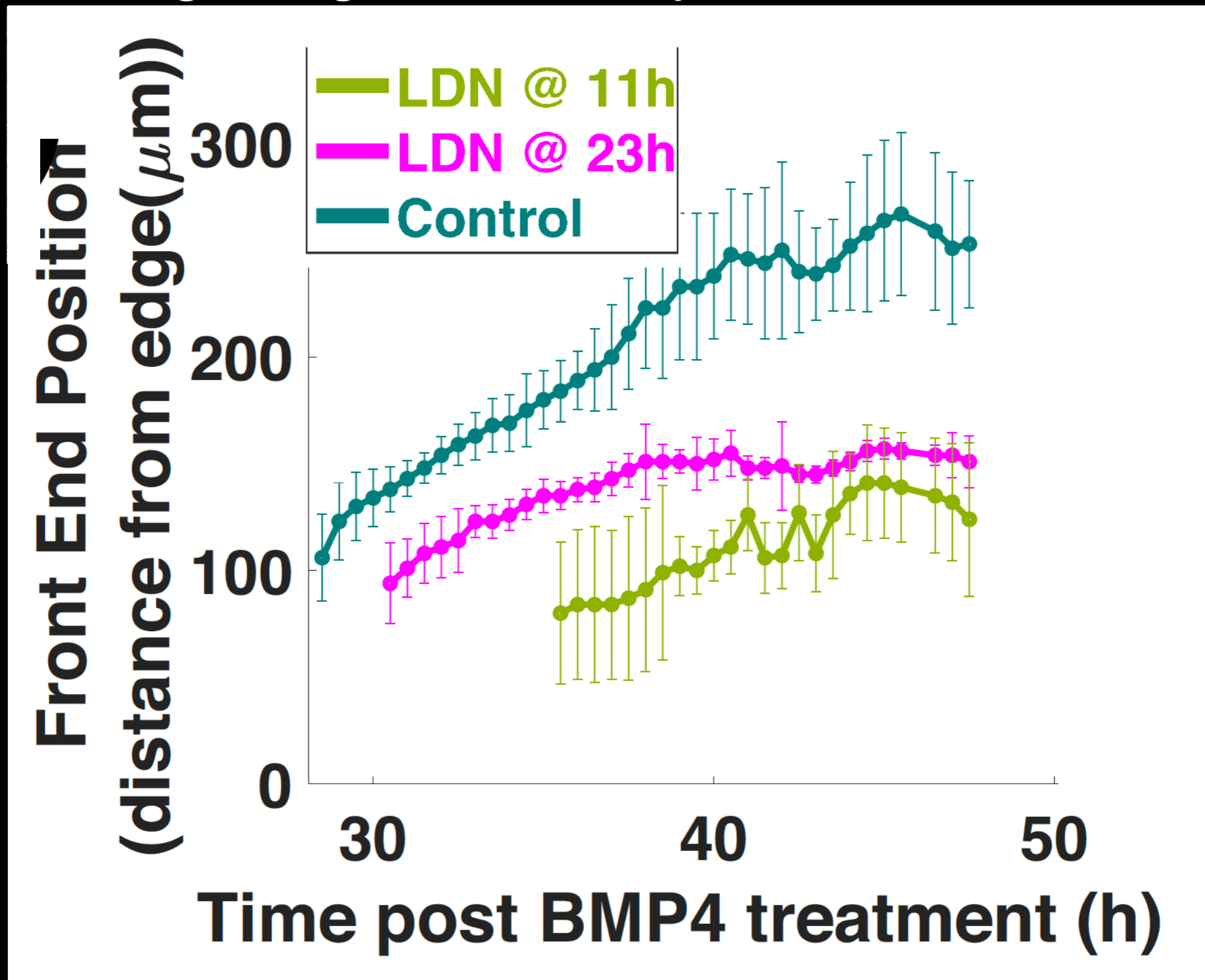


WNT is initially activated close to the edge and spreads inward at a constant rate



Note: WNT signaling reach well beyond the region of mesoderm differentiation

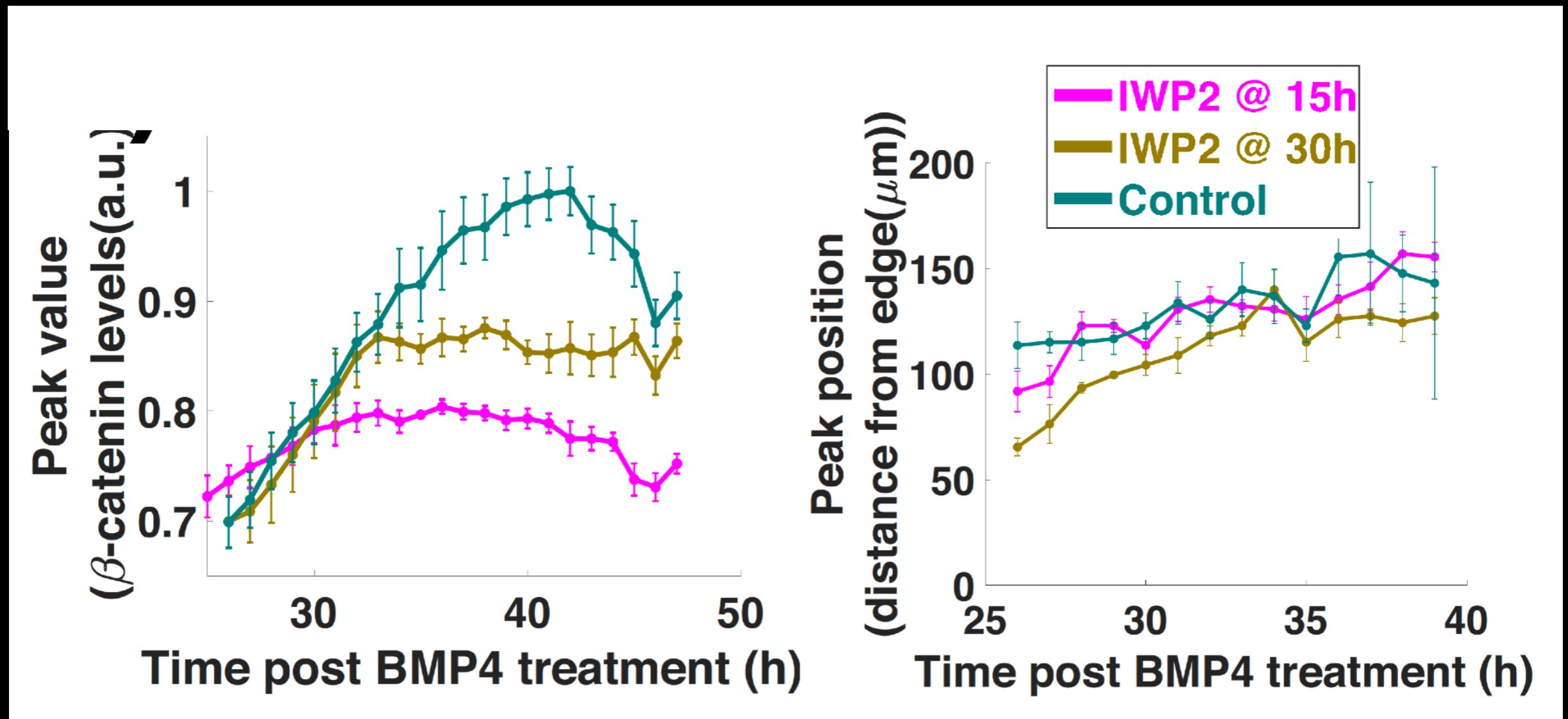
BMP signaling continuously drives WNT inwards



LDN = small molecule BMP pathway inhibitor

Secretion of new WNT ligands is dispensable for movement of WNT signaling but increases WNT levels

IWP2 = small molecule inhibitor of WNT secretion



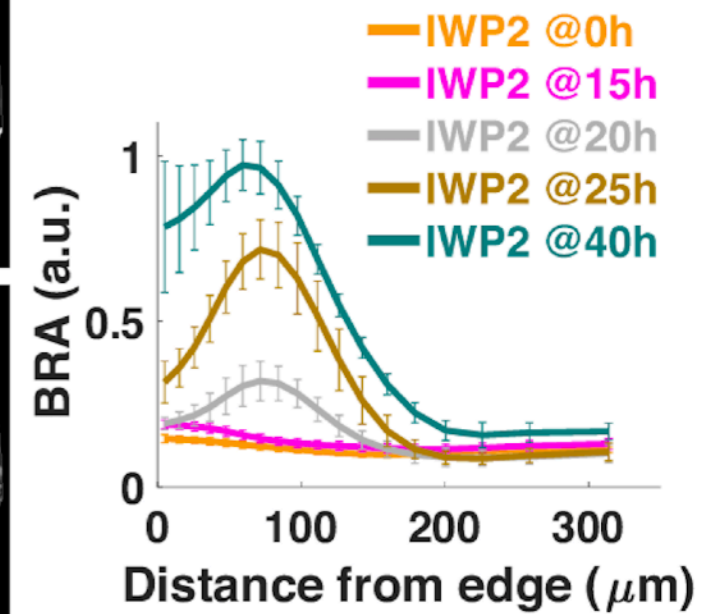
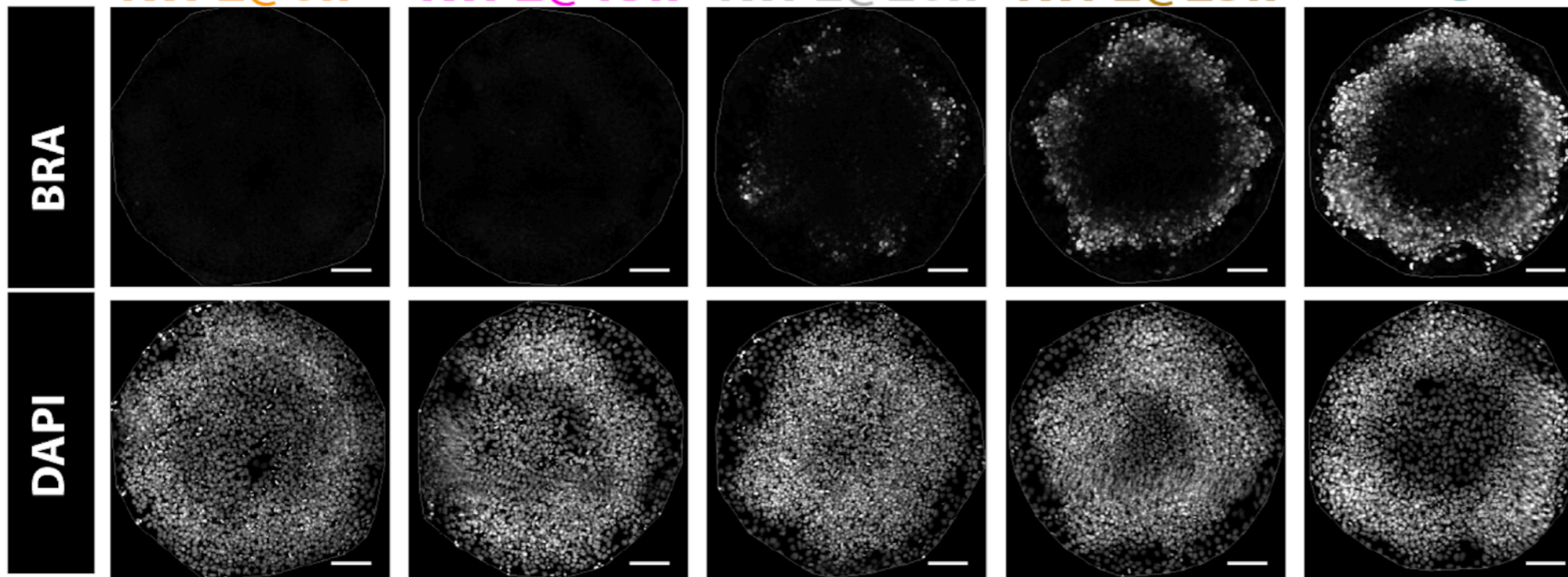
Suggests long range diffusion of WNT ligands

Mesoderm increases with duration of WNT

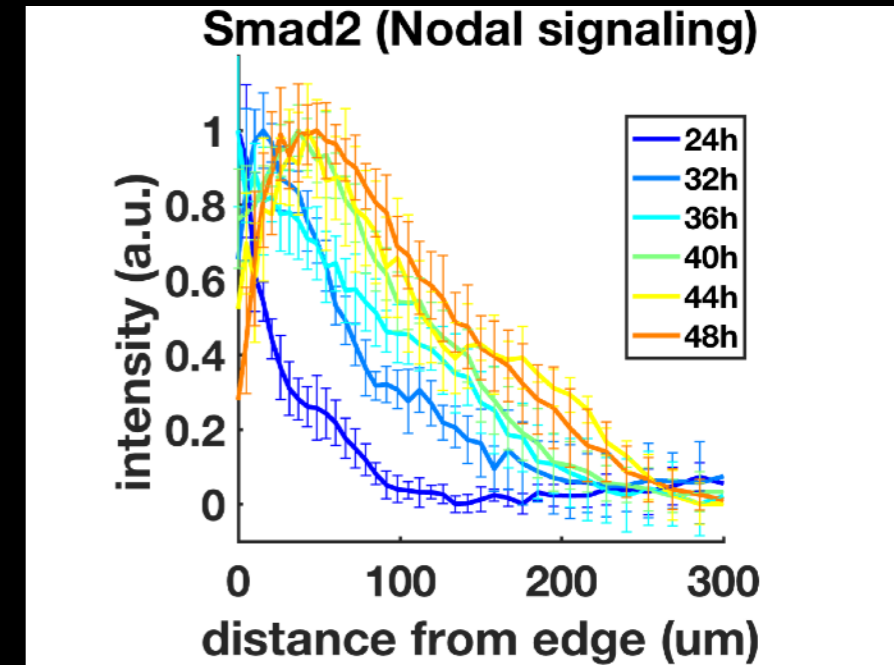
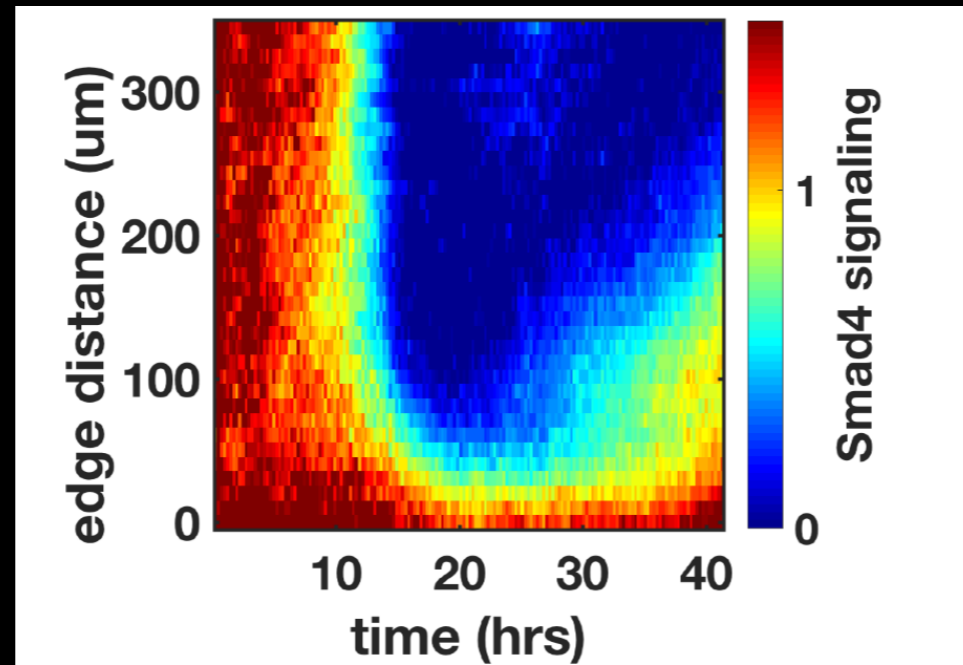
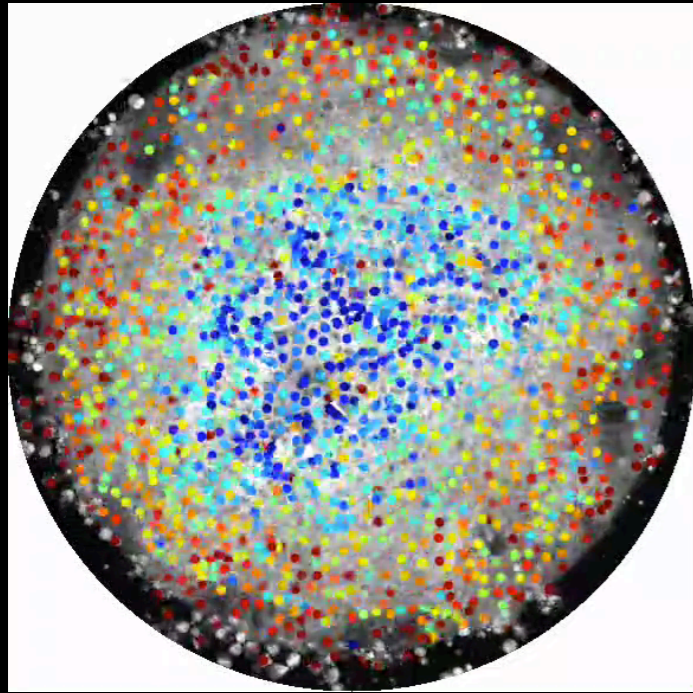
Inhibit Wnt at different times

B)

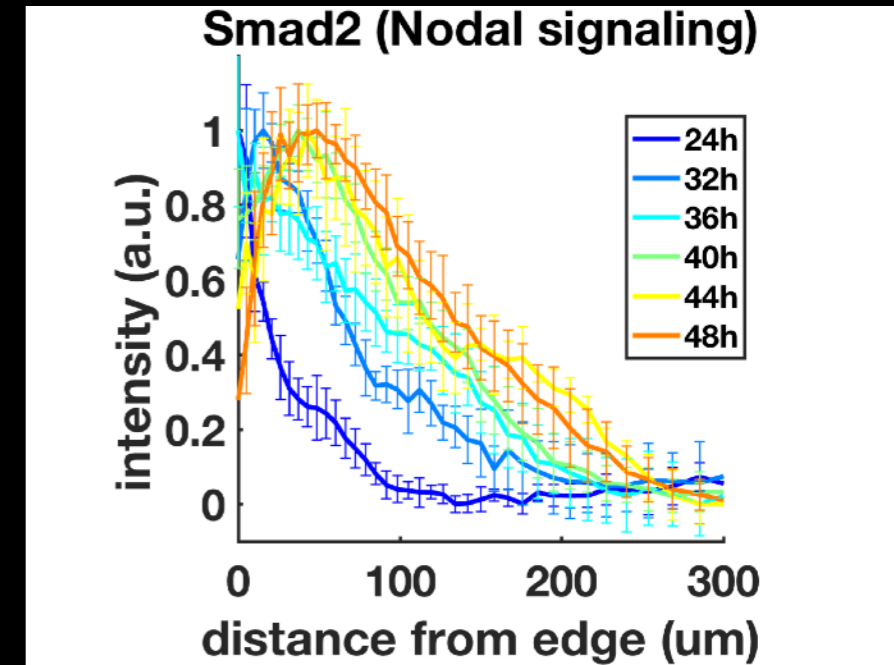
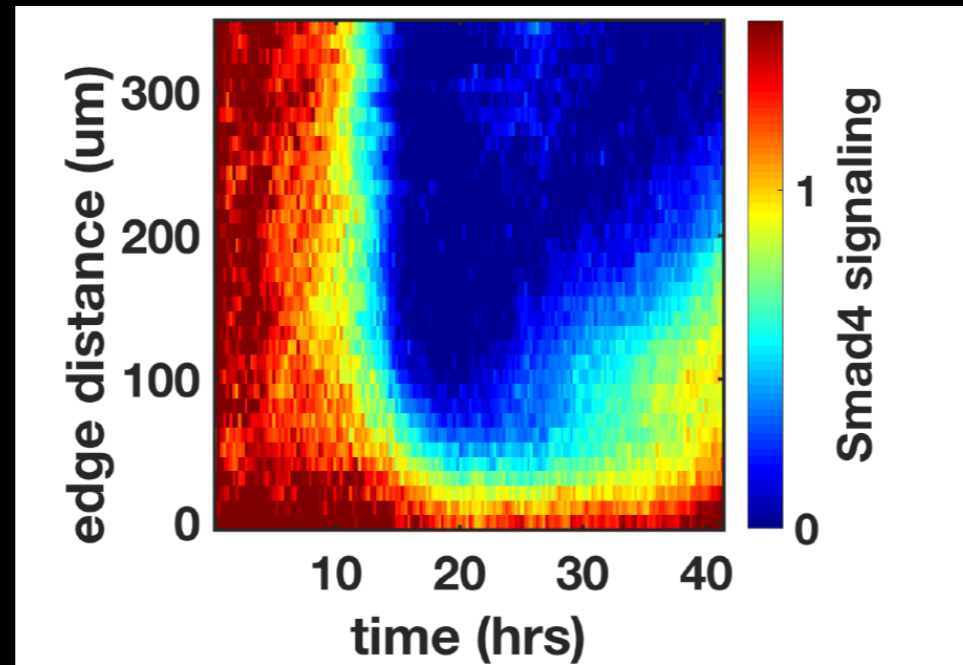
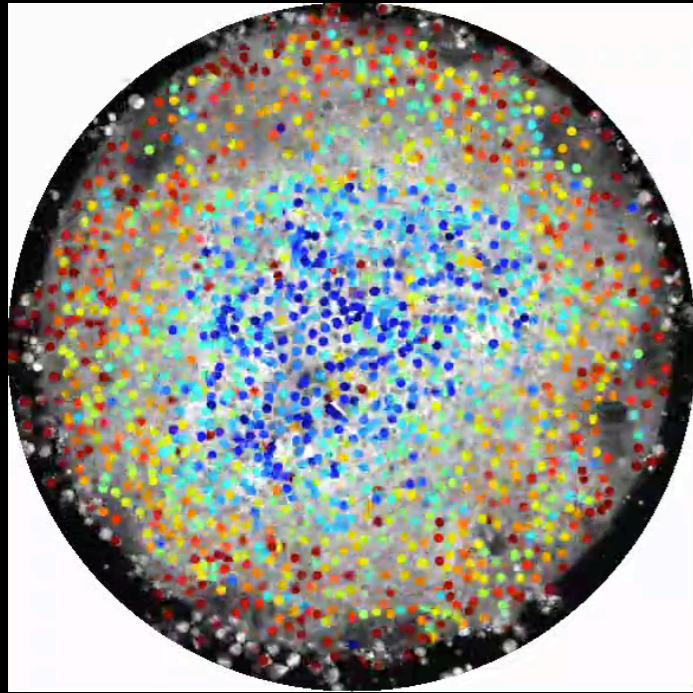
IWP2@0h IWP2@15h IWP2@20h IWP2@25h IWP2@40h



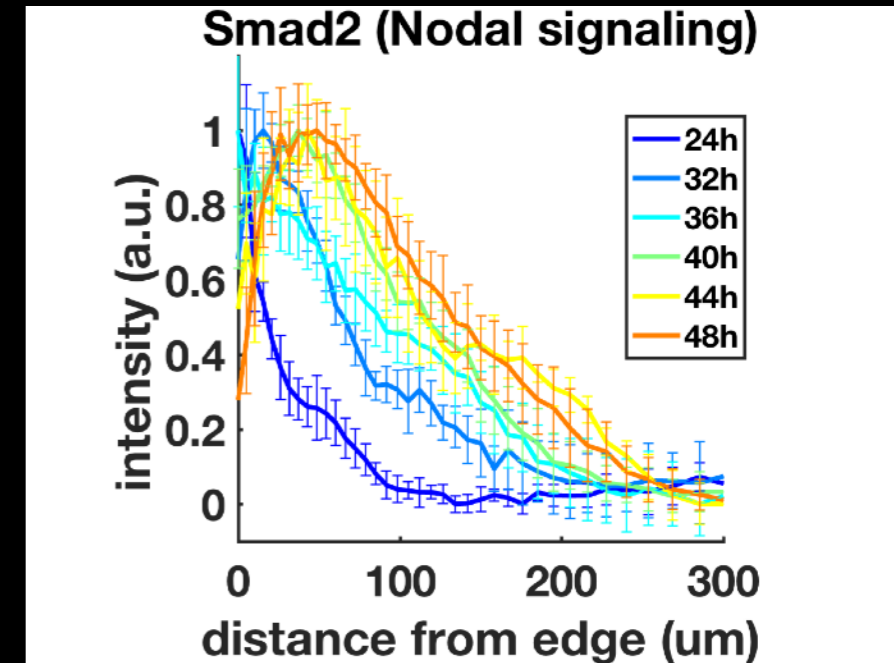
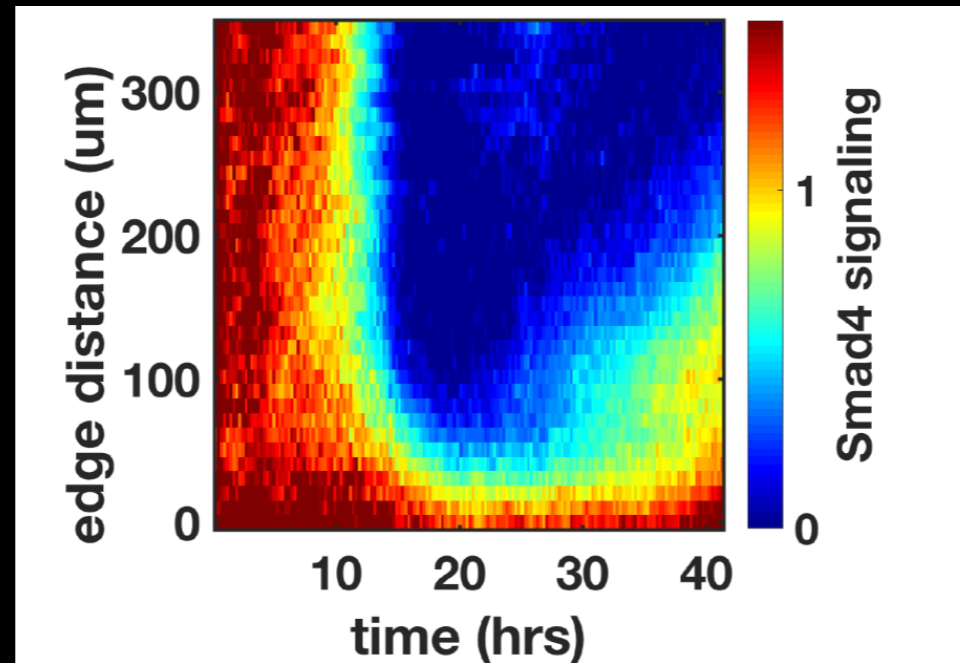
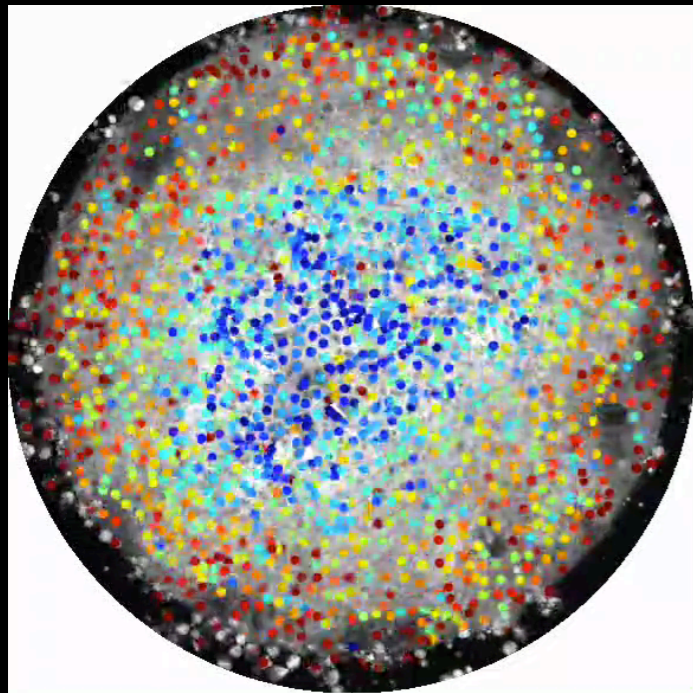
A wave of Nodal signaling follows the wave of Wnt signaling



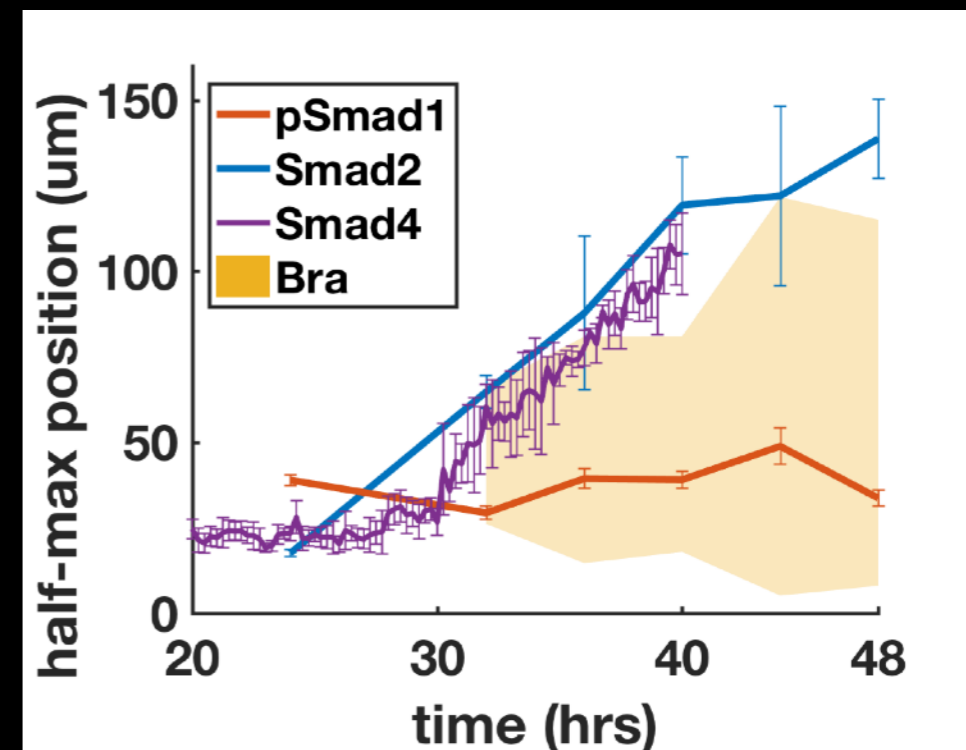
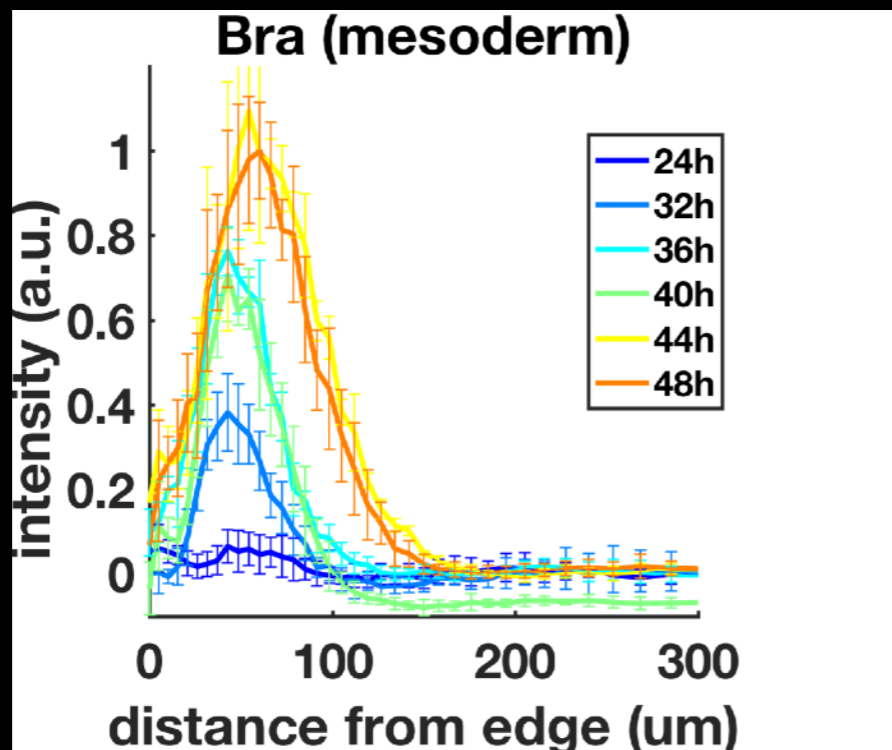
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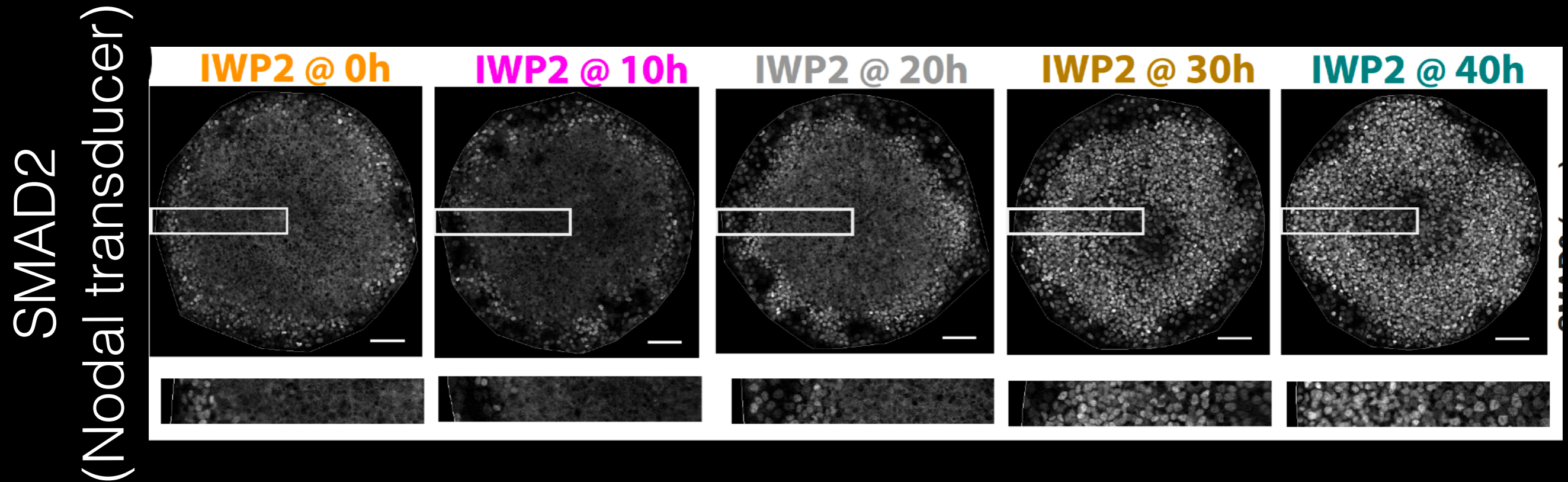


...And induces mesodermal differentiation in its wake



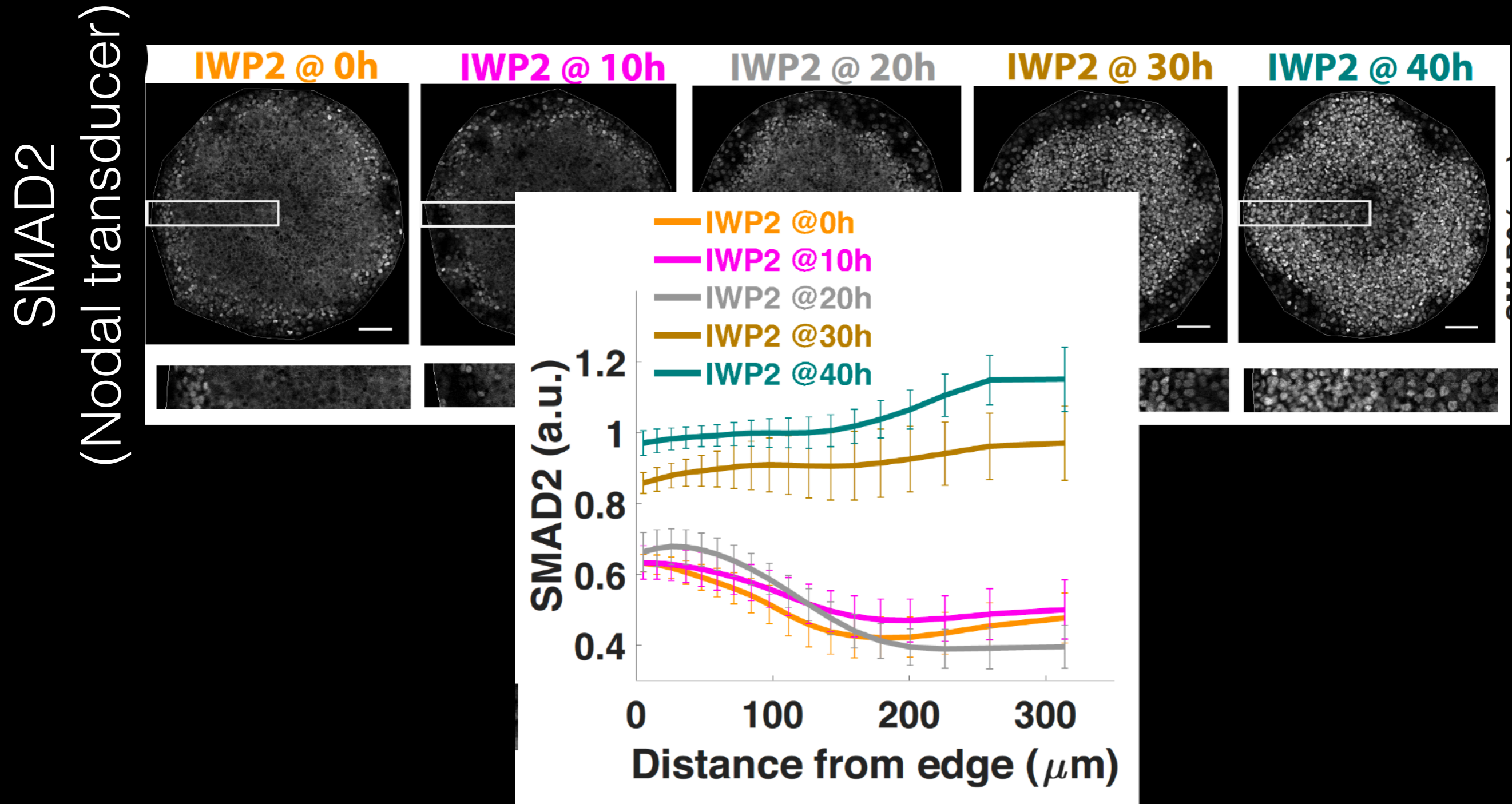
WNT initiated Nodal rapidly becomes independent of upstream pathways

IWP2 = small molecule inhibitor of WNT secretion



WNT initiated Nodal rapidly becomes independent of upstream pathways

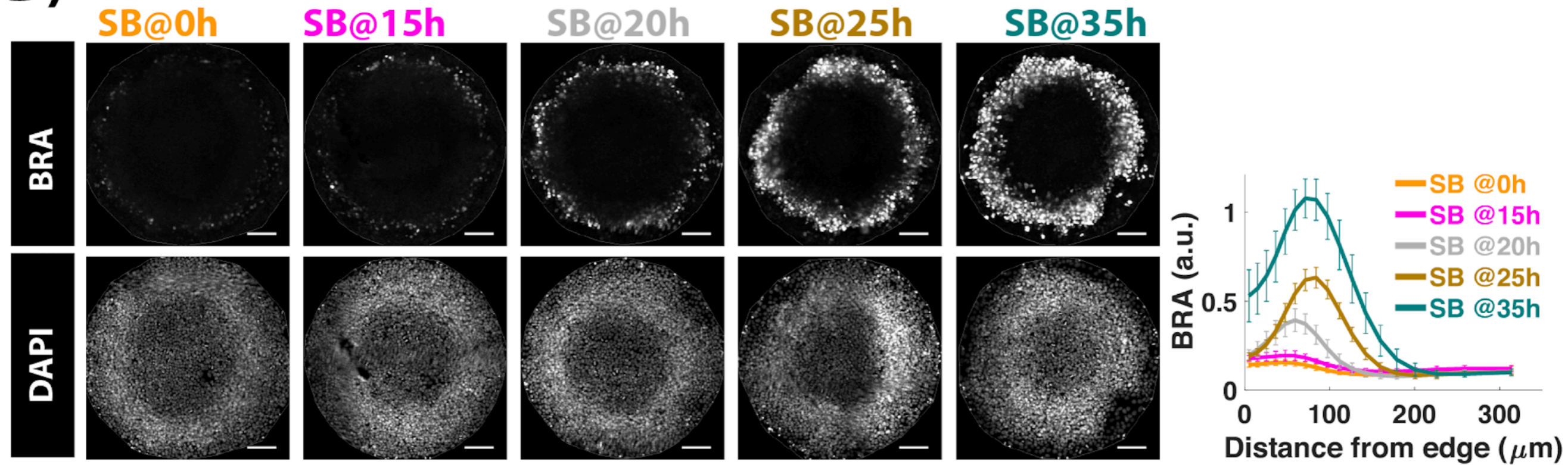
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Mesoderm increases with duration of Nodal

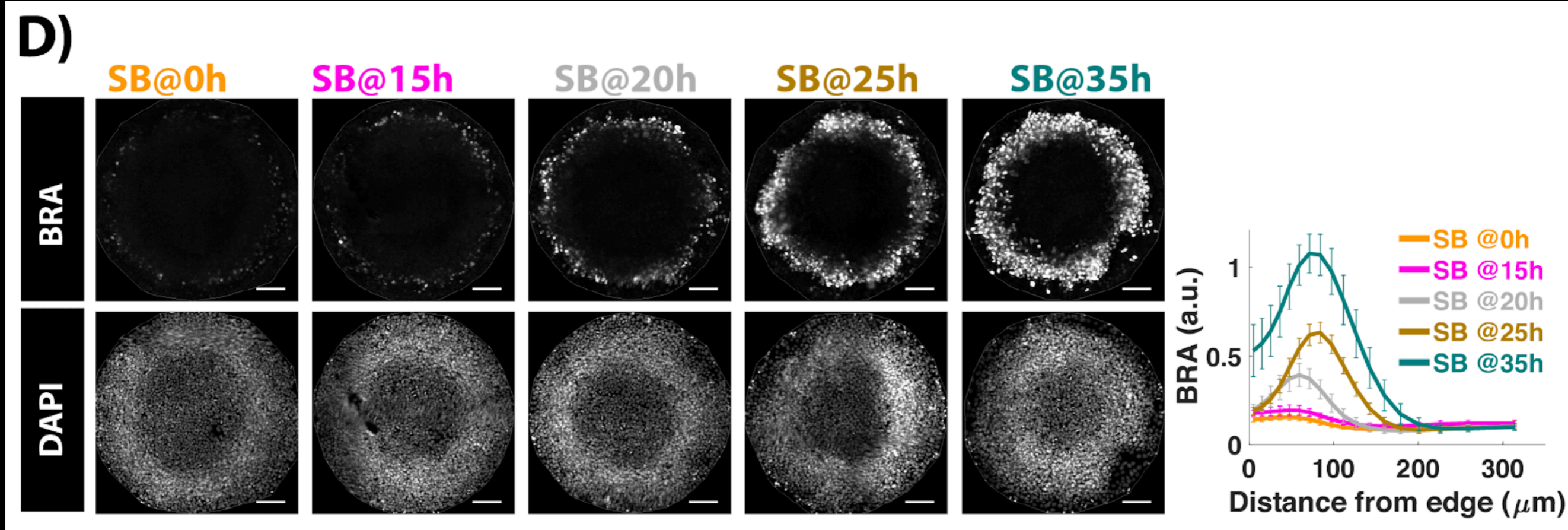
Inhibit Nodal at different times

D)



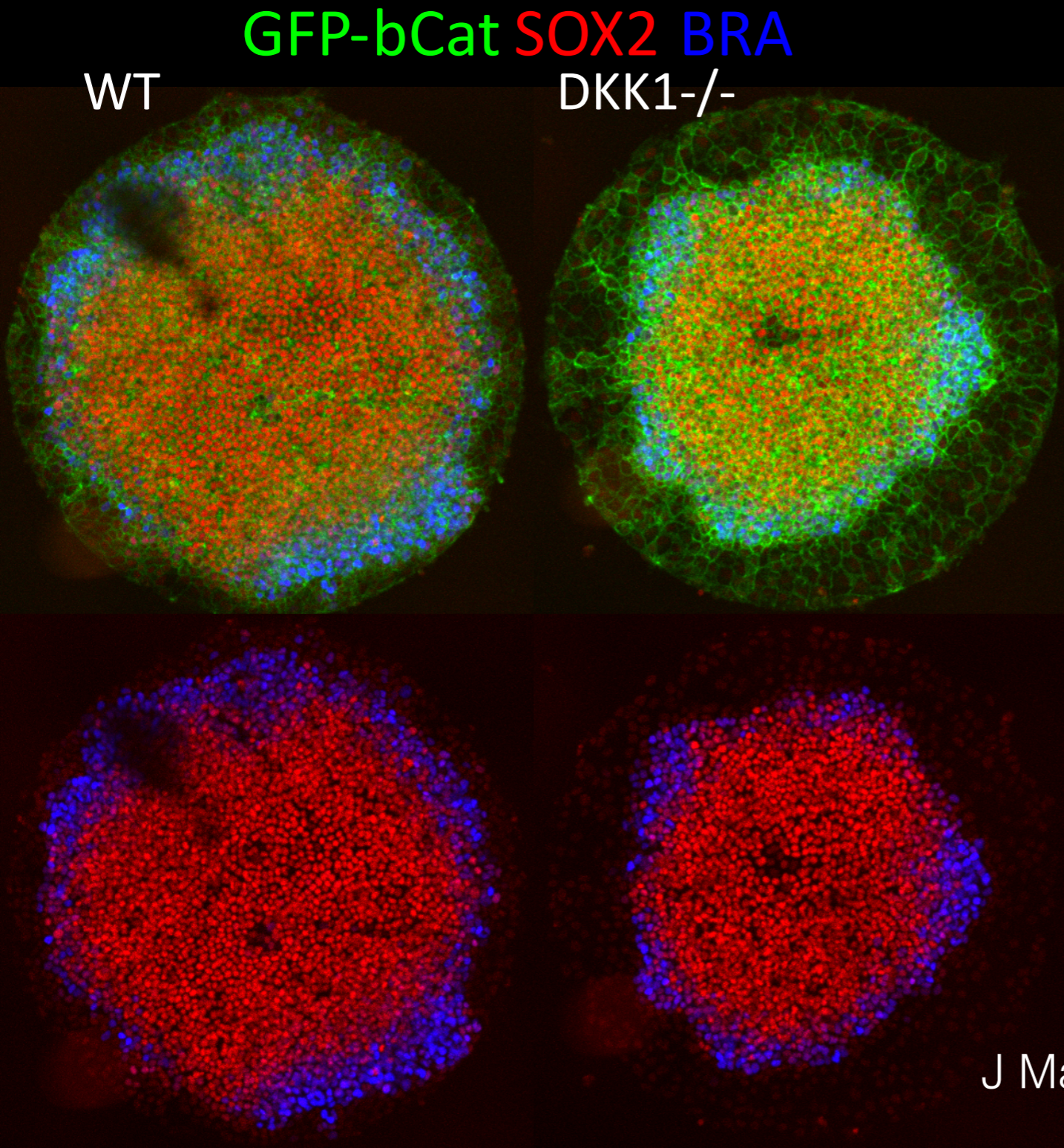
Mesoderm increases with duration of Nodal

Inhibit Nodal at different times



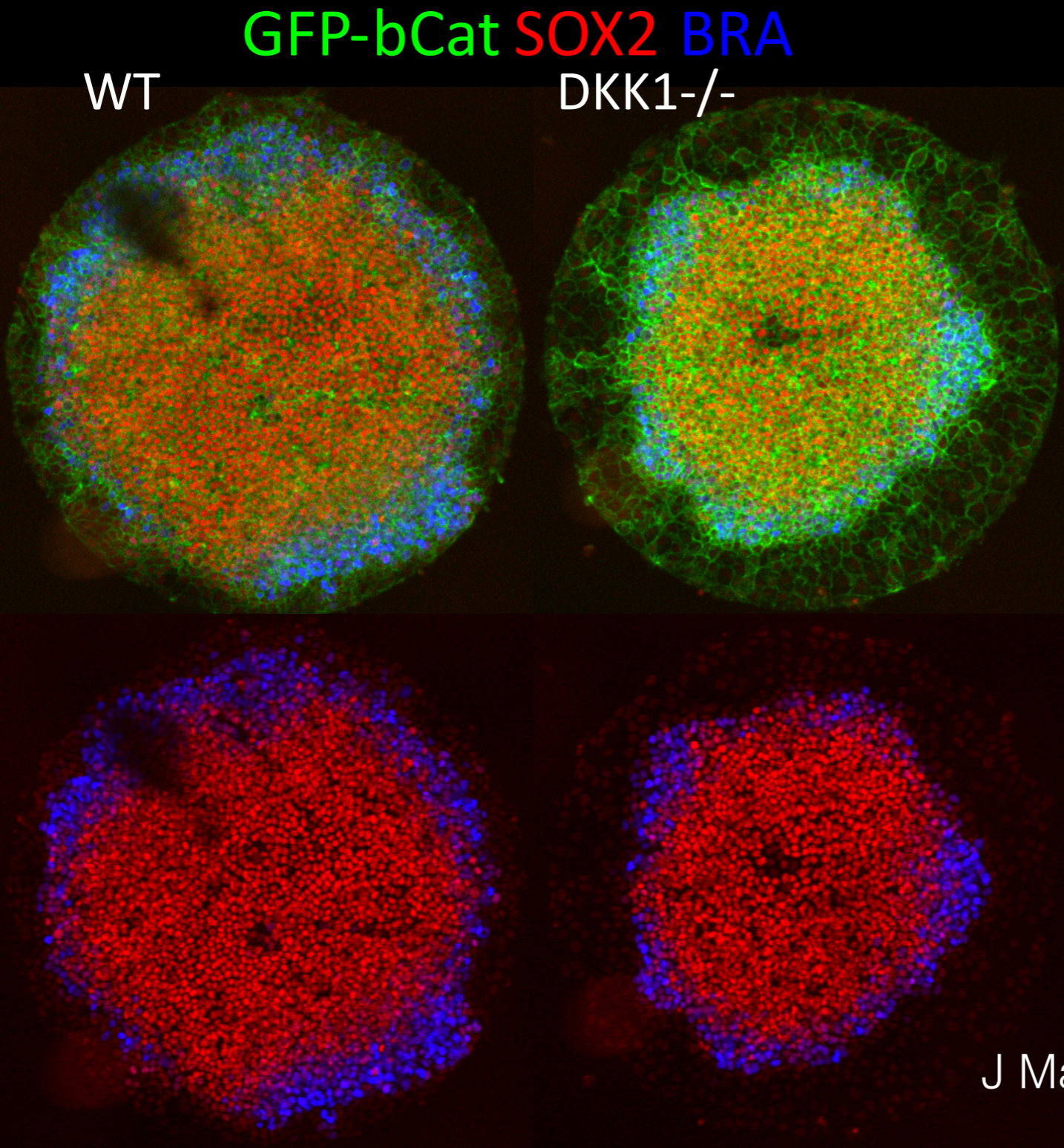
What positions the ring of mesoderm?

DKK1-KO shifts mesodermal ring inward without affecting its width



J Massey unpublished

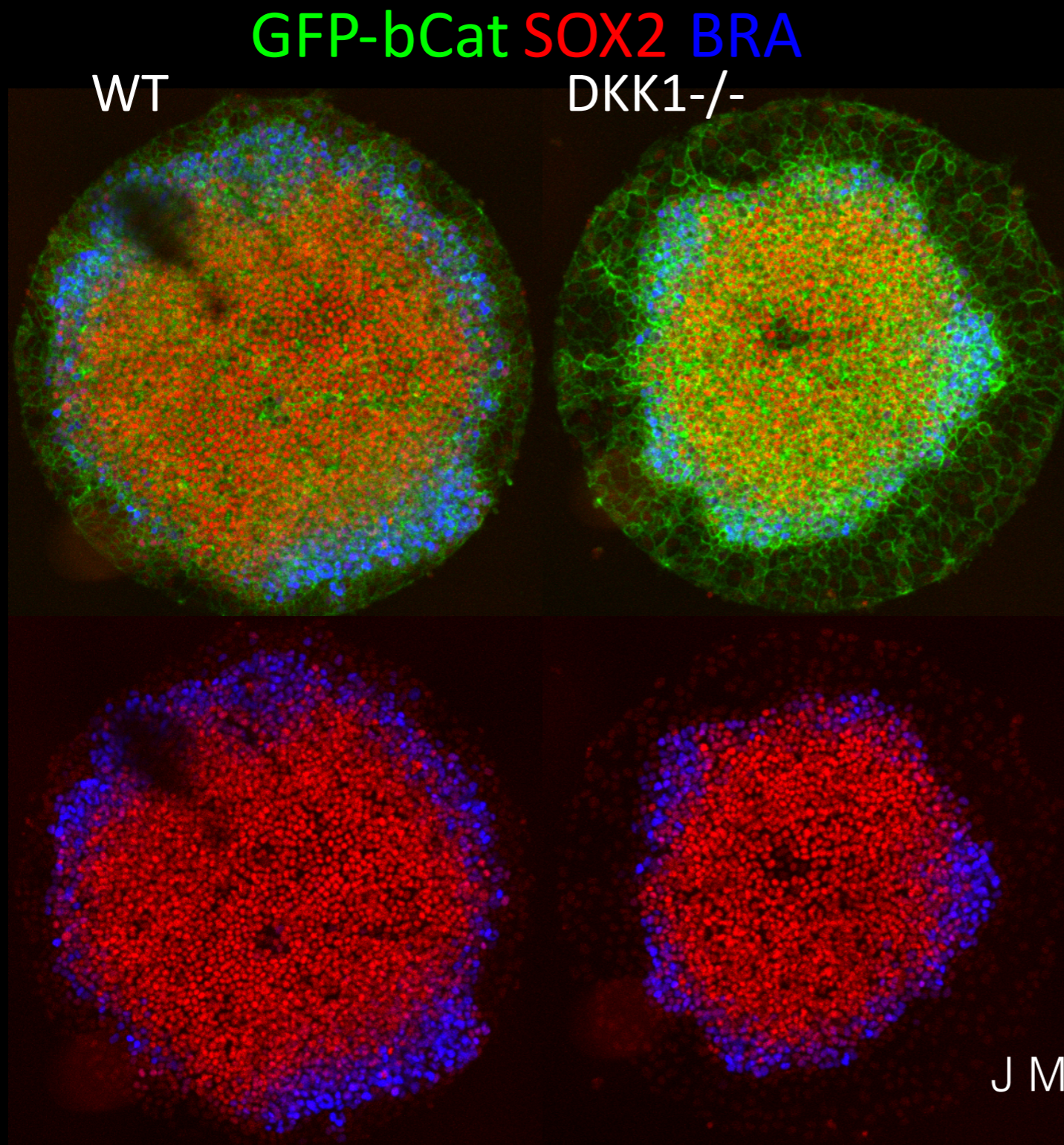
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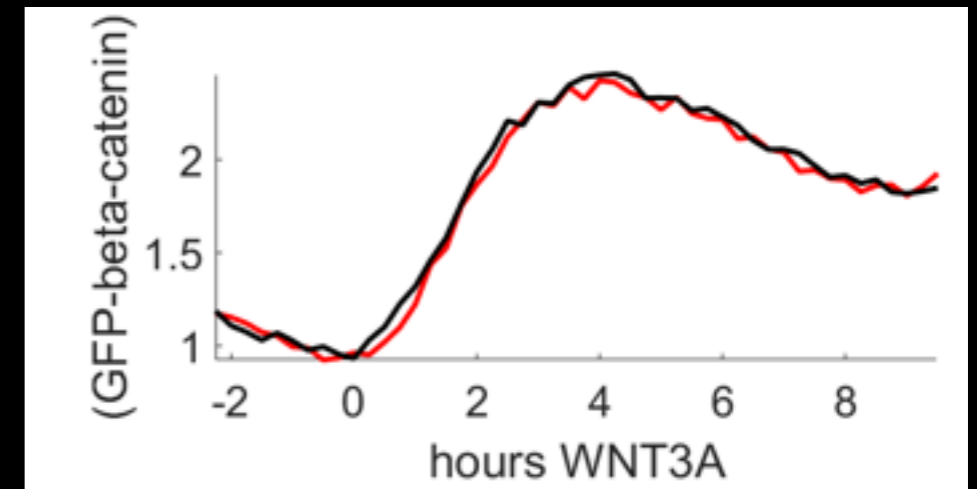
J Massey unpublished

What is different about the signaling dynamics?

DKK1-KO shifts mesodermal ring inward without affecting its width



Not apparent in response to exogenous Wnt



J Massey unpublished

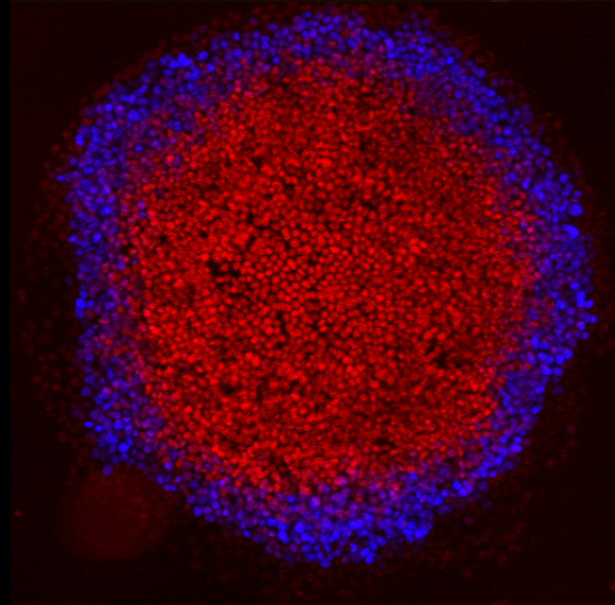
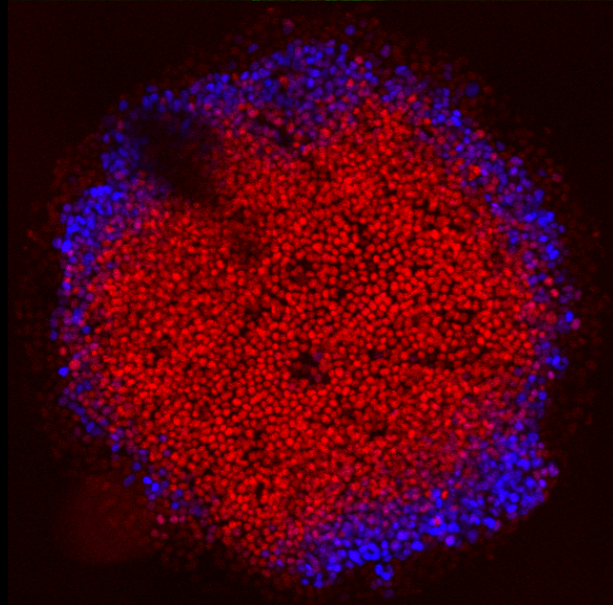
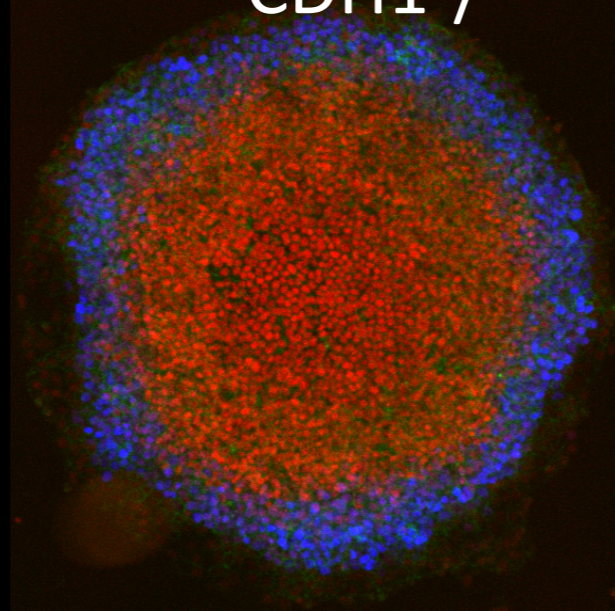
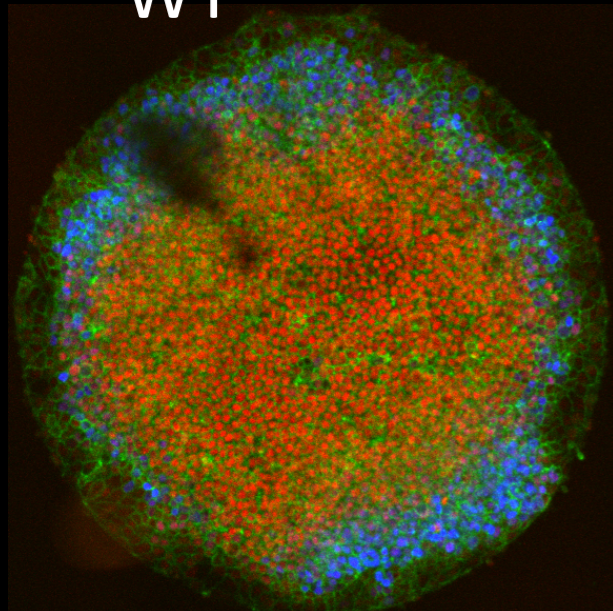
What is different about the signaling dynamics?

E-CAD knockout has a wider mesodermal ring at a similar

GFP-bCat SOX2 BRA position

WT

CDH1^{-/-}

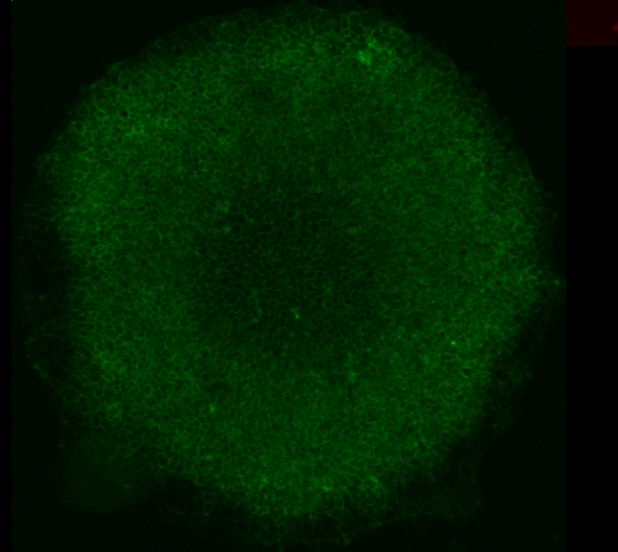
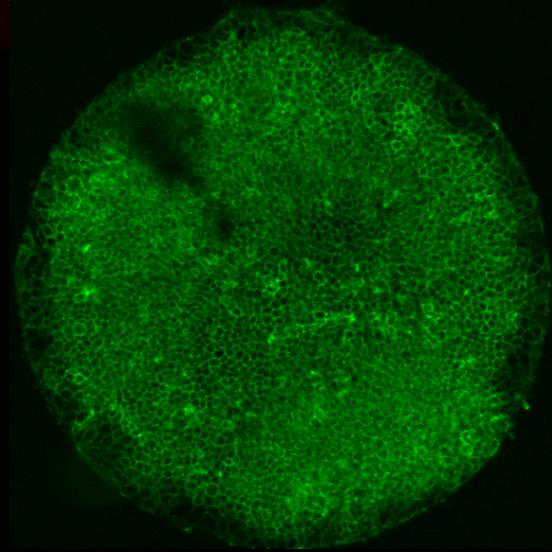
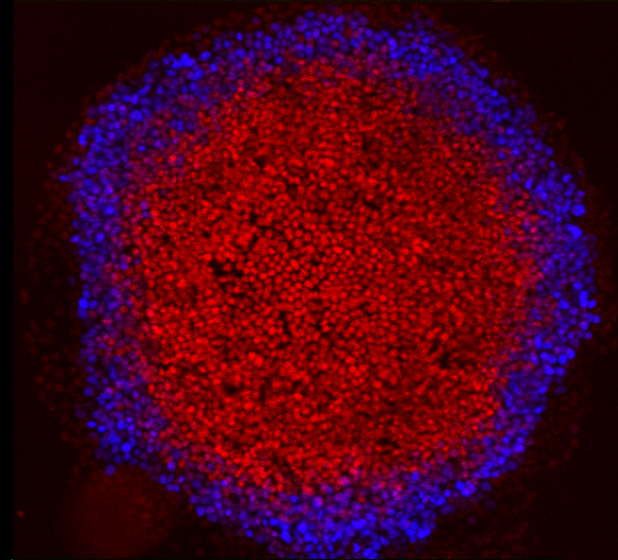
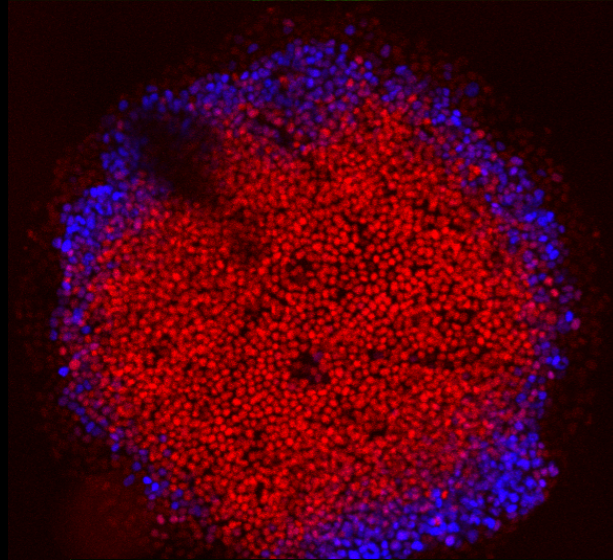
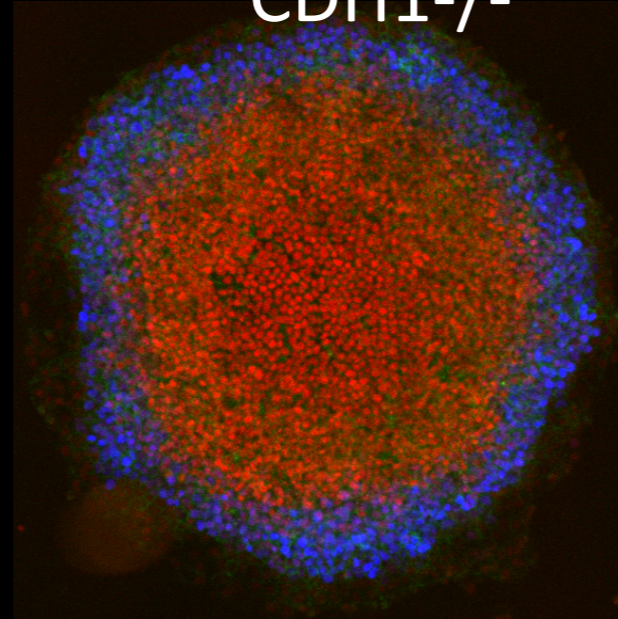
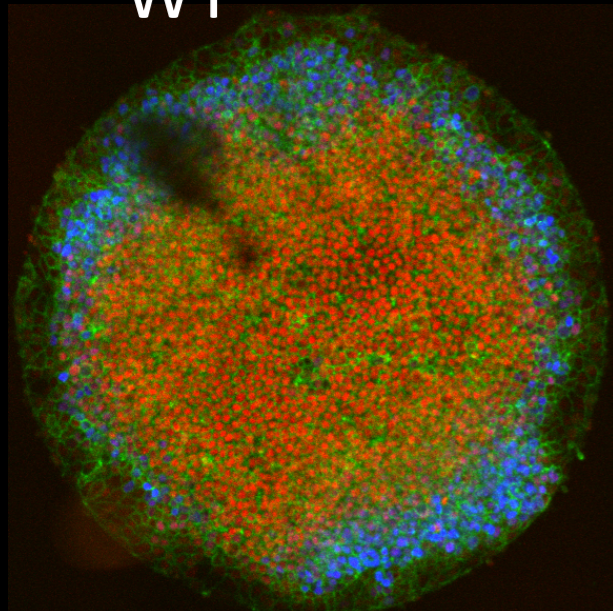


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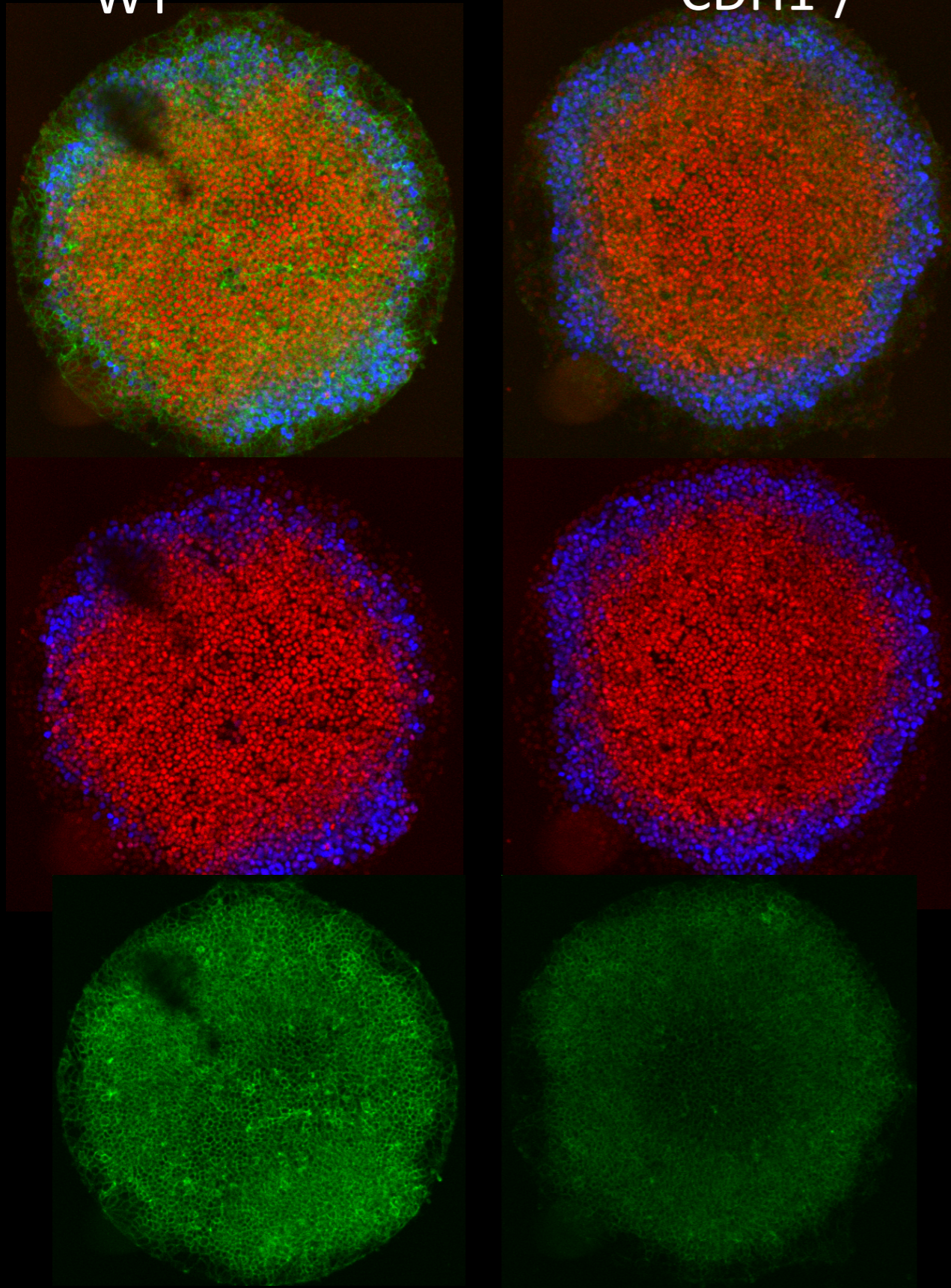


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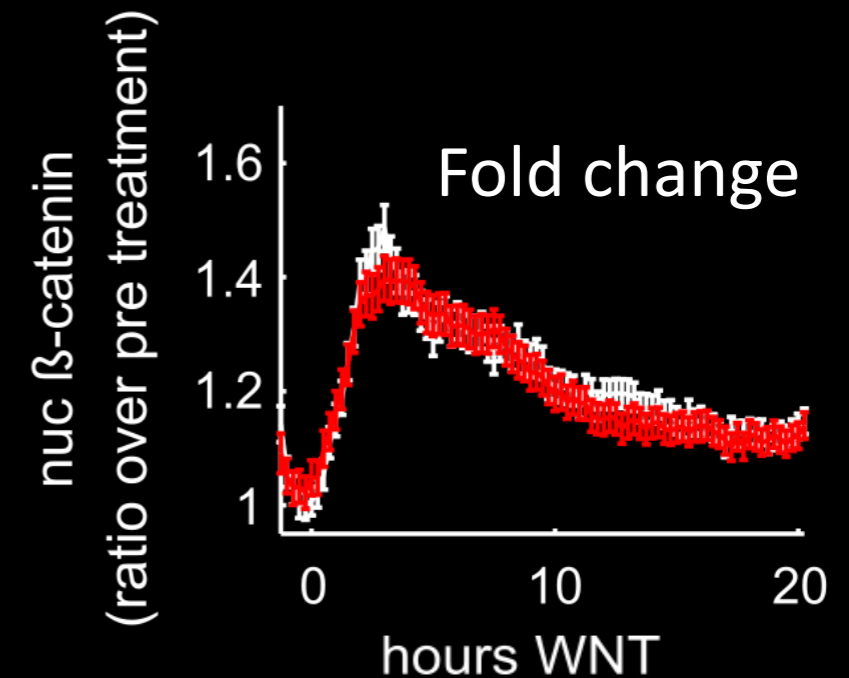
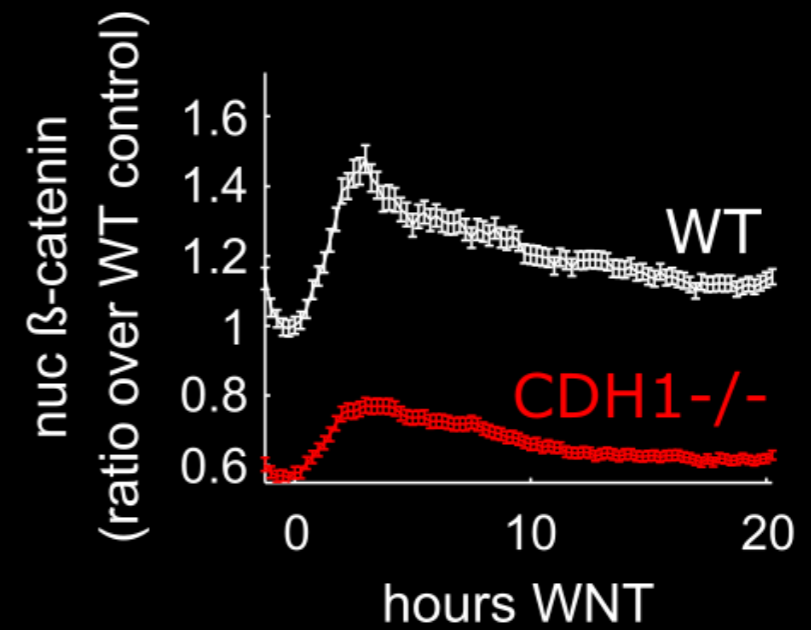
GFP-bCat SOX2 BRA position

WT

CDH1^{-/-}



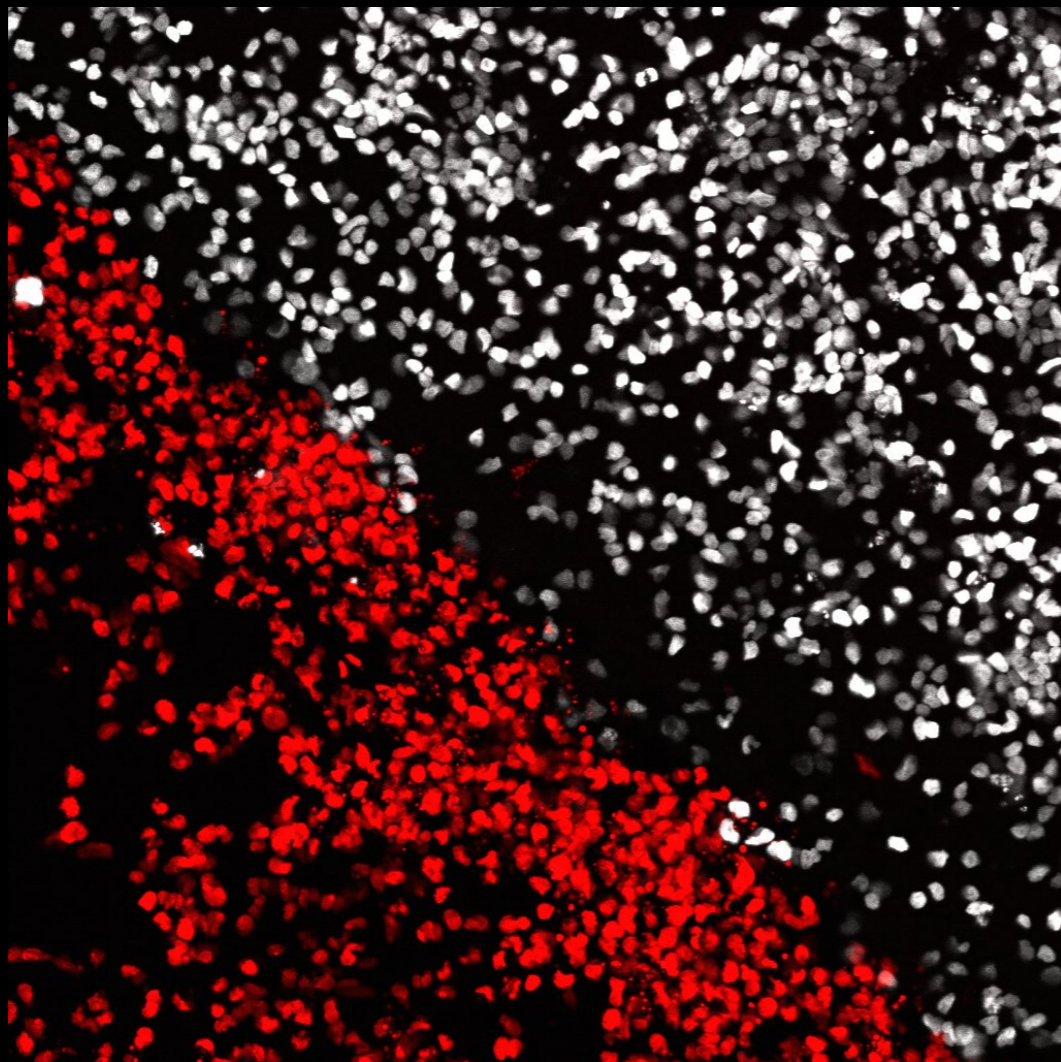
Total b-catenin is lower but fold change is preserved in response to exogenous Wnt



Studying interactions between embryonic and extraembryonic cells

In our gastrulation model, stimulation with BMP4 mimics interaction with trophectoderm. Can we replace with trophectoderm-like cells (TLCs)?

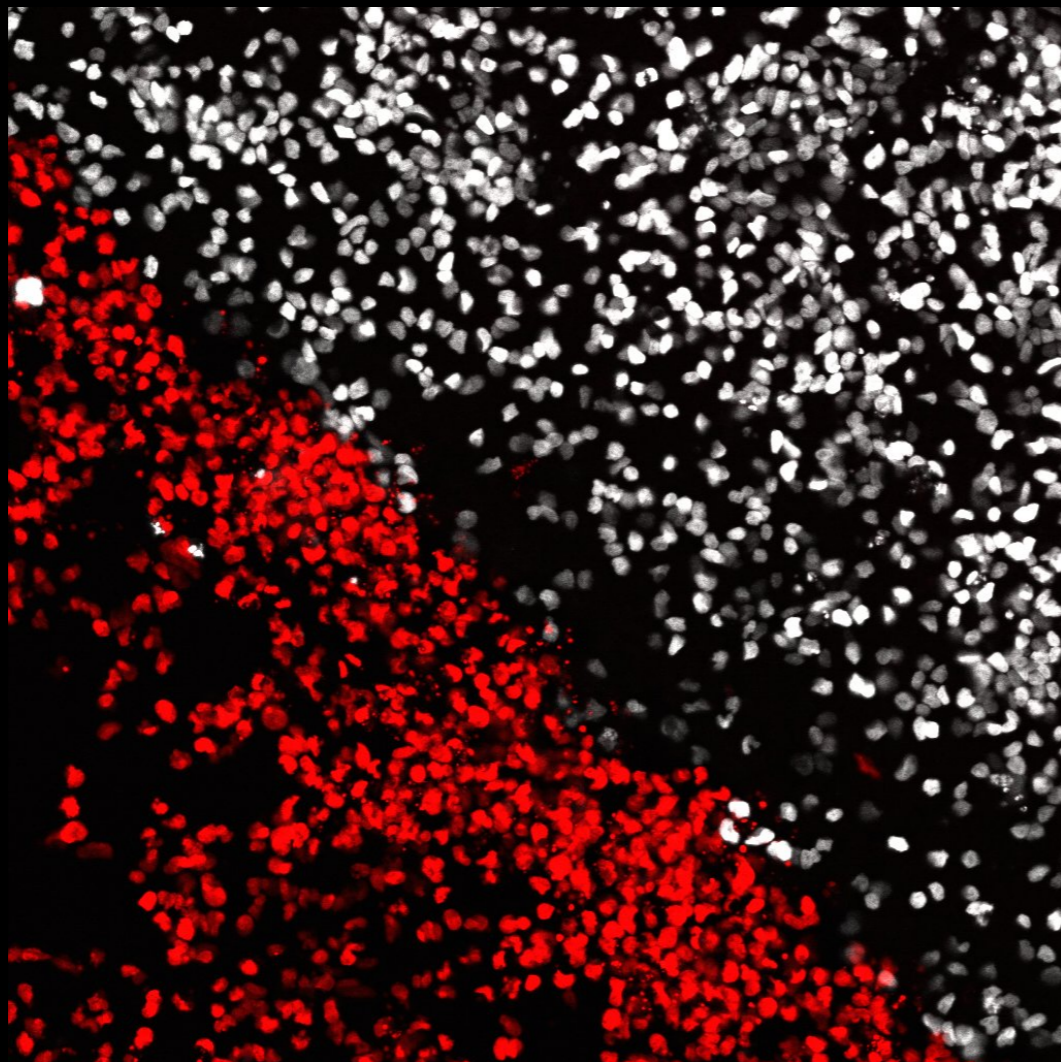
TLC/ESC



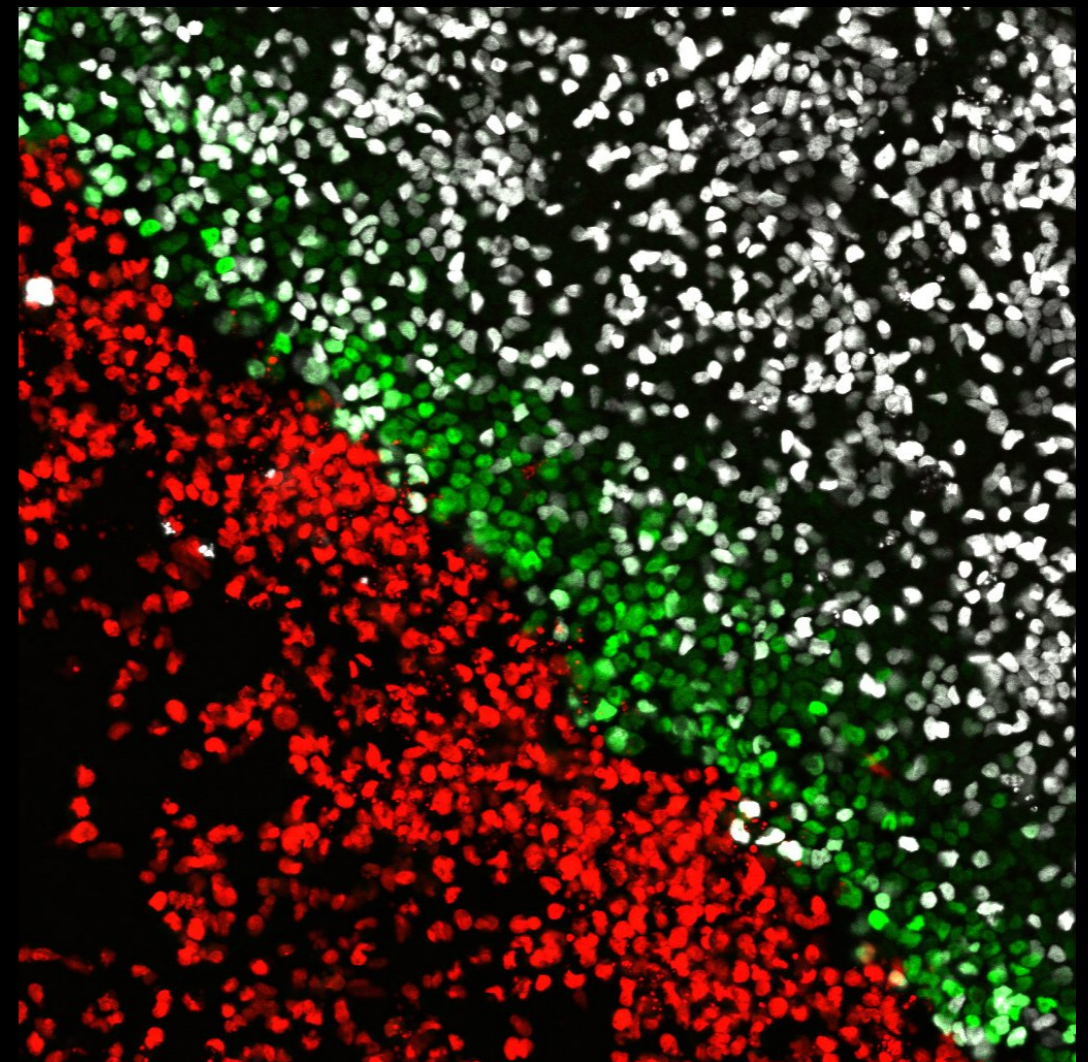
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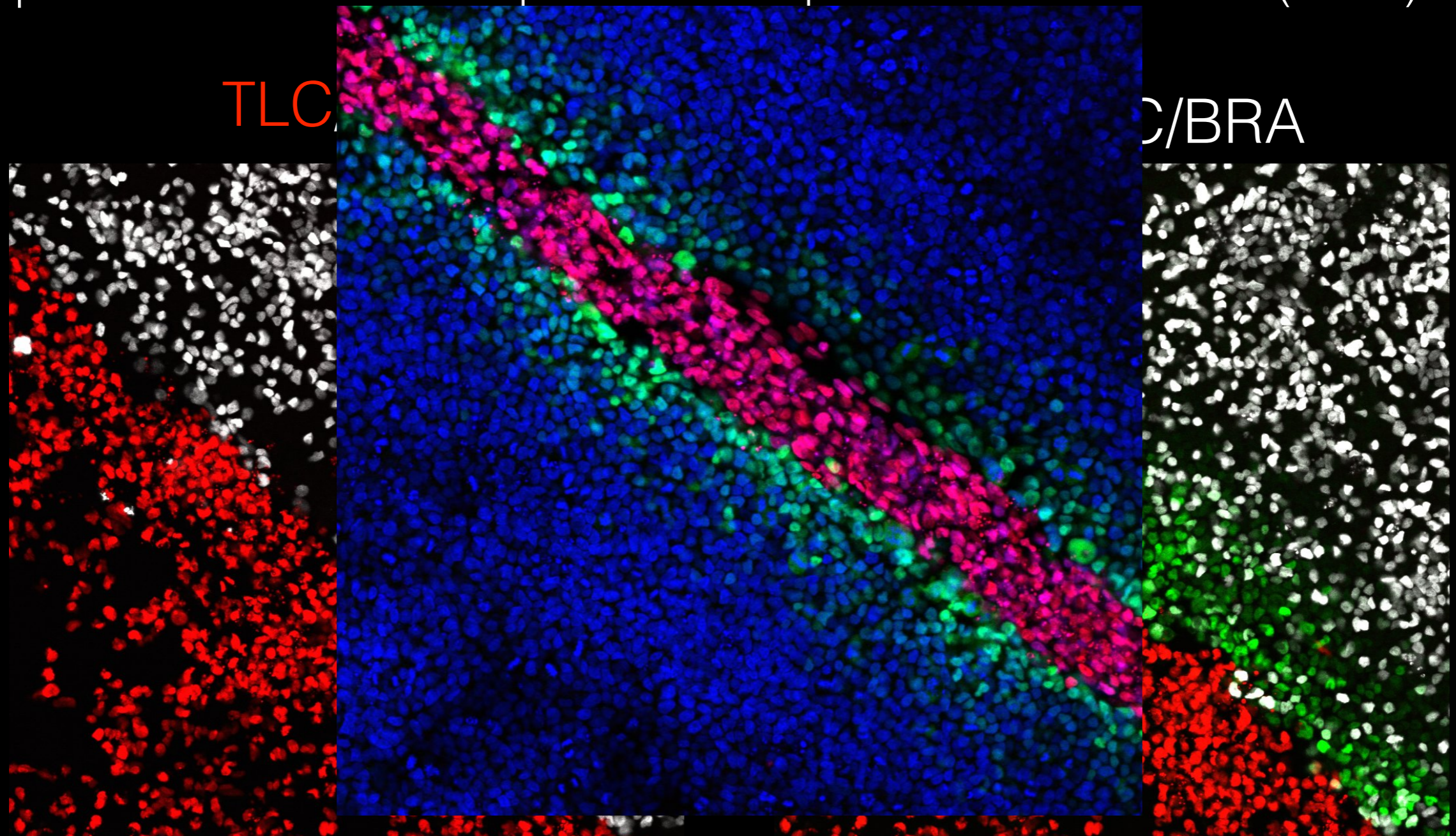


TLC/ESC/BRA



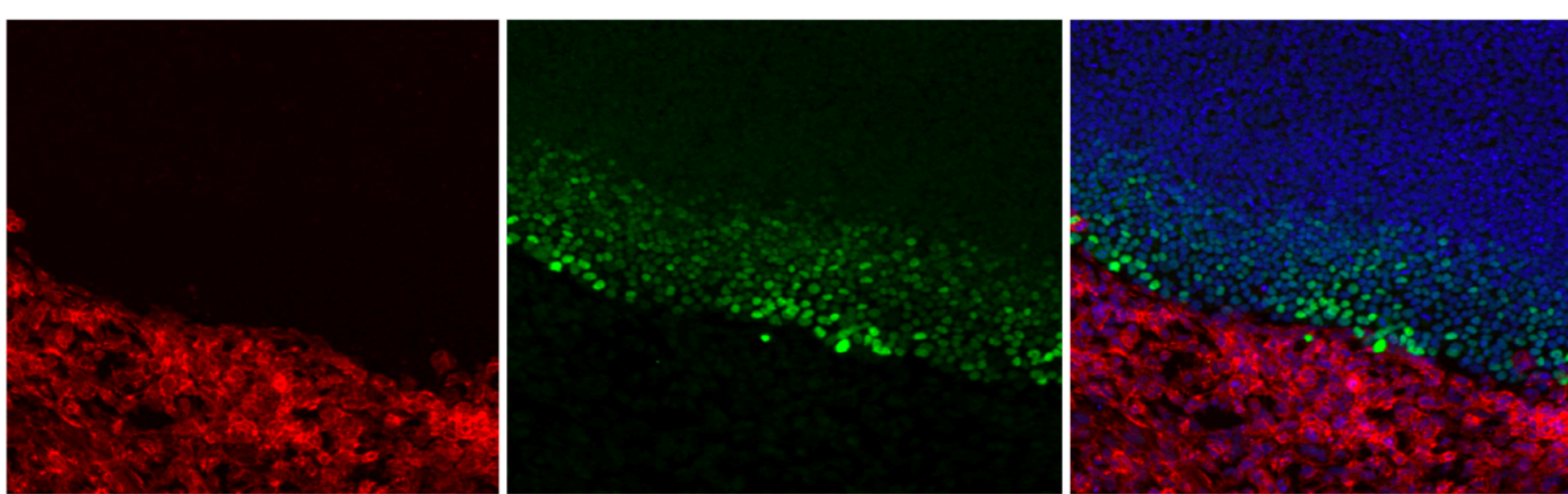
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Induction of BRA by cell juxtaposition depends on
endogenous BMP and WNT
TLC/BRA/DAPI

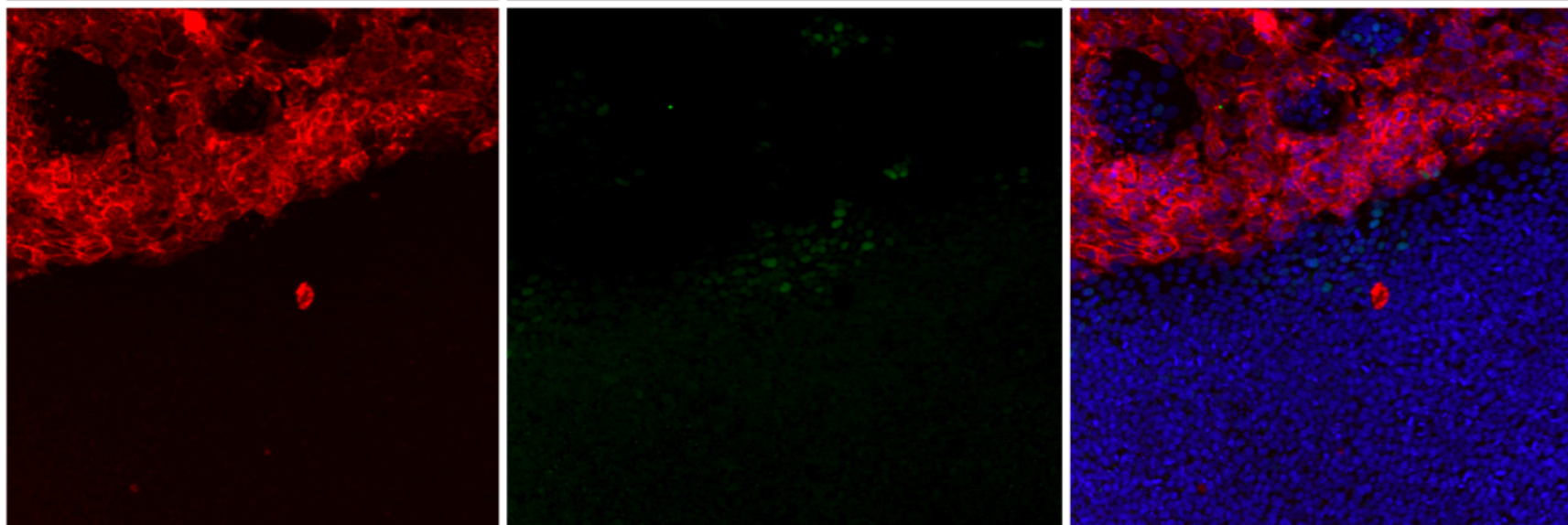
Control



+IWP2
(WNT
Inhibitor)



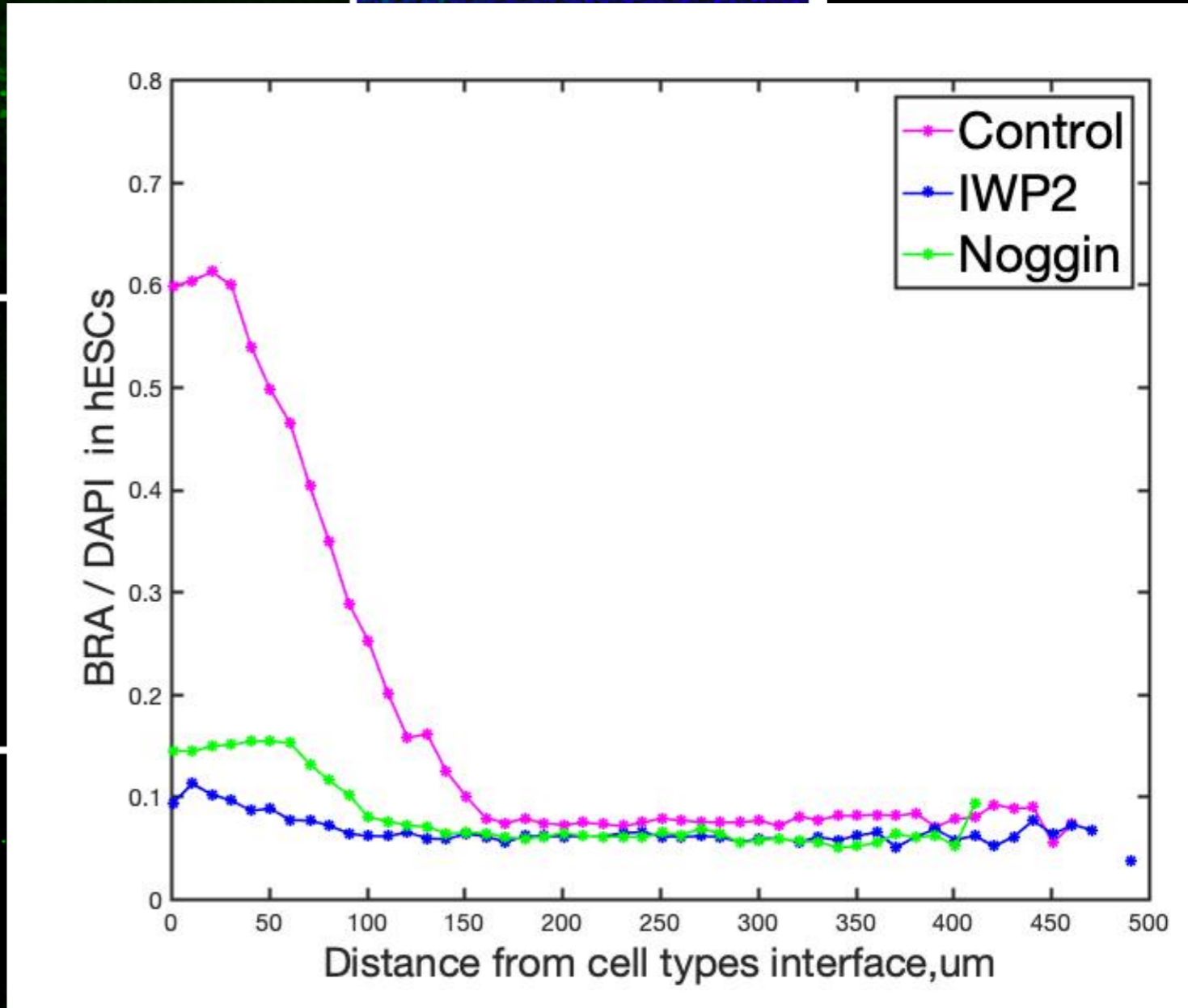
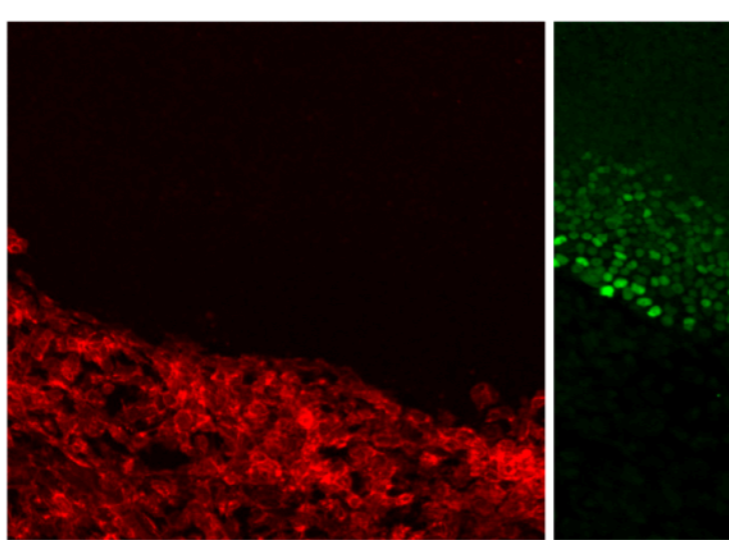
+Noggin
(BMP
Inhibitor)



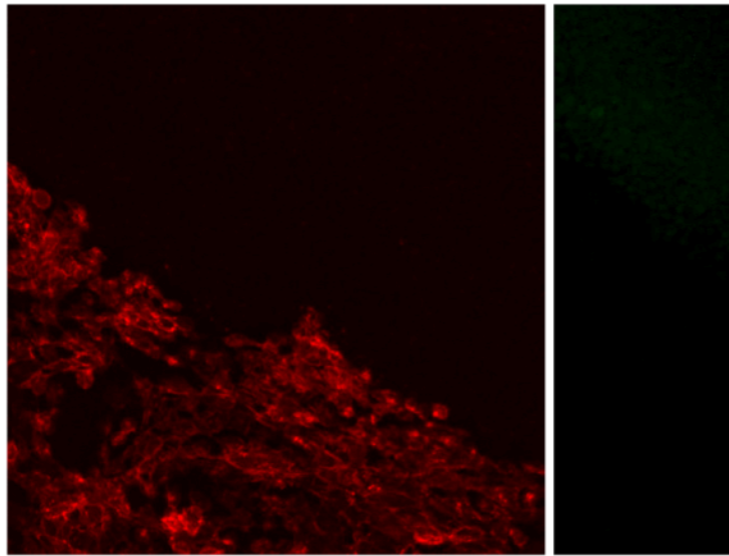
Induction of BRA by cell juxtaposition depends on endogenous BMP and WNT

TLC/BRA/DAPI

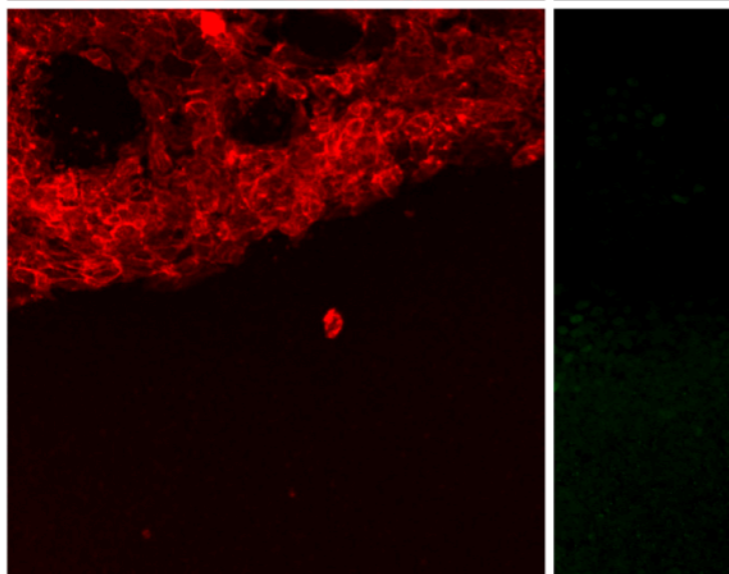
Control



+IWP2
(WNT
Inhibitor)



+Noggin
(BMP
Inhibitor)



Summary

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1. BMP signaling is sustained in a stable pattern at the edge

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5. This signaling system specifies fates. Levels and durations do not specify positions. Best hypothesis is relative timing of signals.

Aside: Do these pathways function as Turing systems in other contexts

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Almost certainly yes:

Dev Cell 2006:

Generation of Robust Left-Right Asymmetry in the Mouse Embryo Requires a Self-Enhancement and Lateral-Inhibition System

Tetsuya Nakamura,^{1,4} Naoki Mine,^{1,4,5}
Etsushi Nakaguchi,^{2,4,*} Atsushi Mochizuki,³
Masamichi Yamamoto,¹ Kenta Yashiro,¹
Chikara Meno,^{1,6} and Hiroshi Hamada^{1,*}

¹ Developmental Genetics Group
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Other examples: digit patterning, palette ridge formation

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Patterning and growth control by membrane-tethered Wntless

Cyrille Alexandre^{1*}, Alberto Baena-Lopez^{1*} & Jean-Paul Vincent¹

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Direct visualization of a native Wnt in vivo reveals that a long-range Wnt gradient forms by extracellular dispersal

Ariel M Pani^{1,2*}, Bob Goldstein^{1,2}

Outline:

1. Understanding patterning during mammalian gastrulation in hESCs
2. Ectoderm patterning: similarities and differences with gastrulation



George Britton

Can we make patterns representing later developmental stages?

Trophectoderm

Endoderm

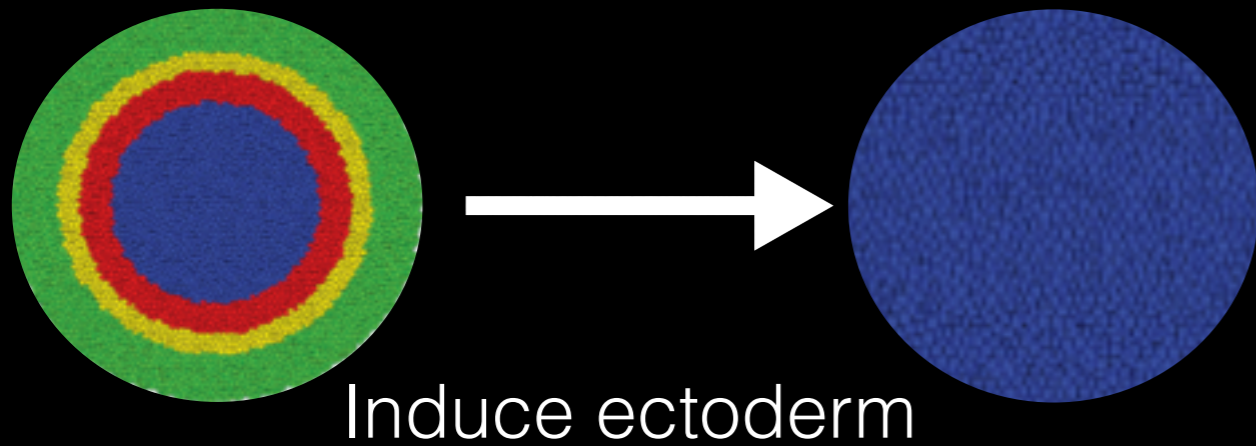
Mesoderm

Ectoderm



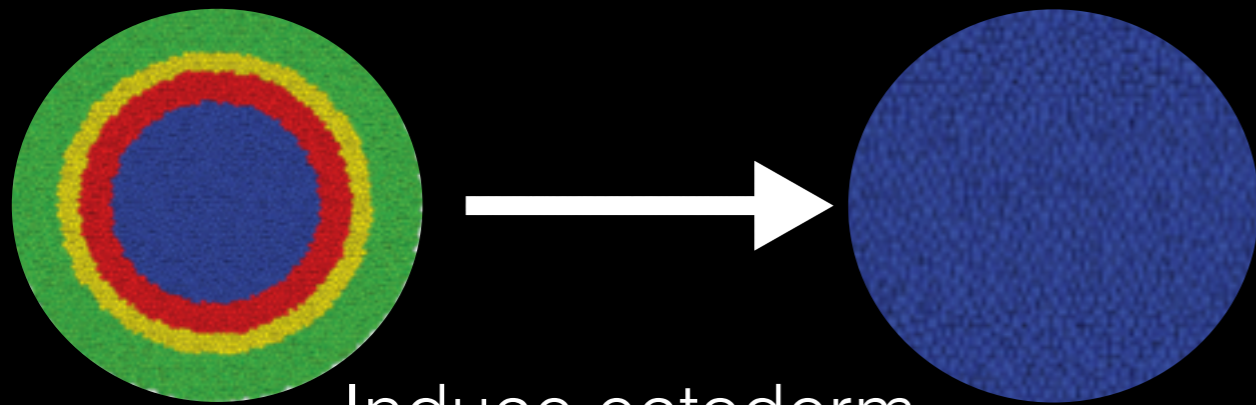
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Trophectoderm
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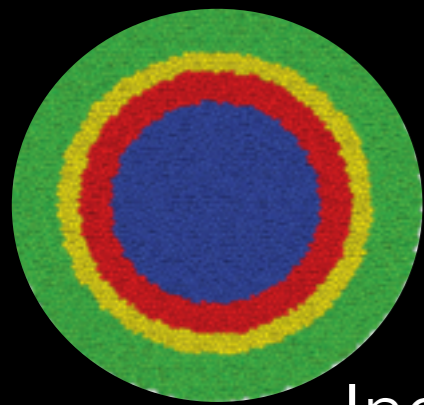
Trophectoderm
Endoderm
Mesoderm
Ectoderm



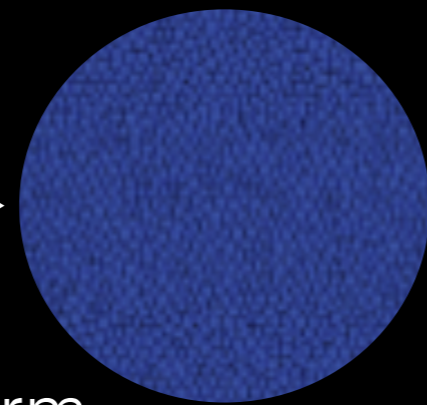
Induce ectoderm
Nodal inhibition

Can we make patterns representing later developmental stages?

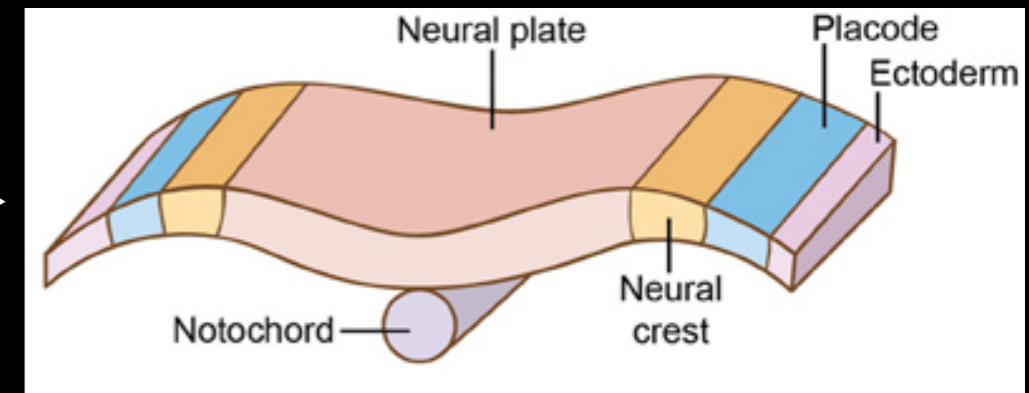
Trophectoderm
Endoderm
Mesoderm
Ectoderm



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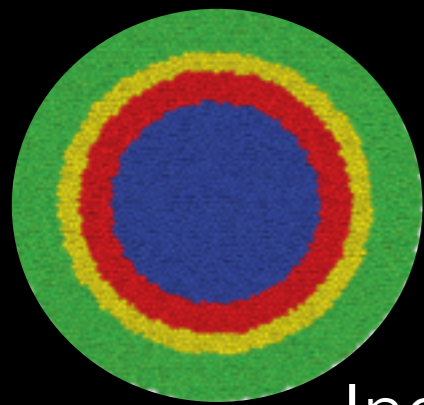


Pattern the ectoderm

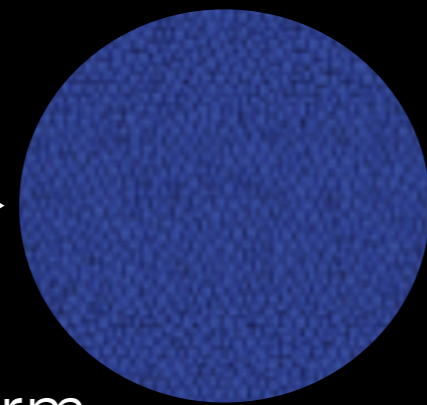


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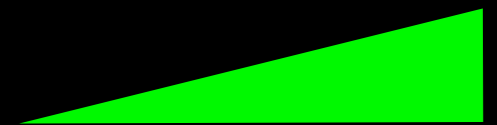
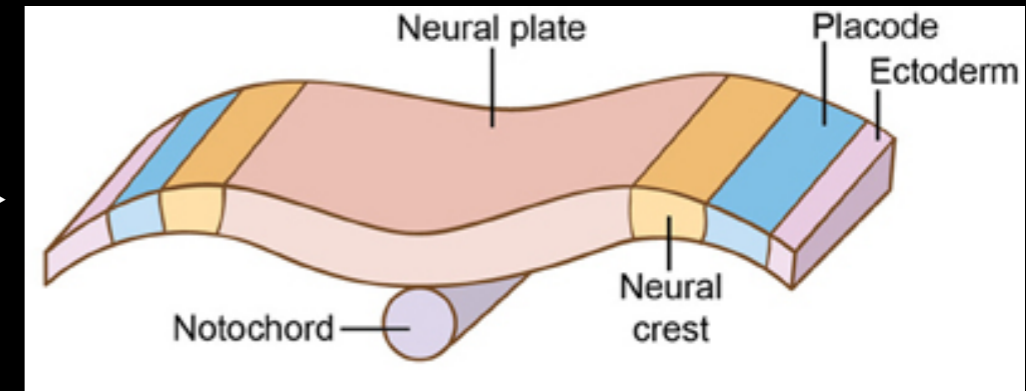
Trophectoderm
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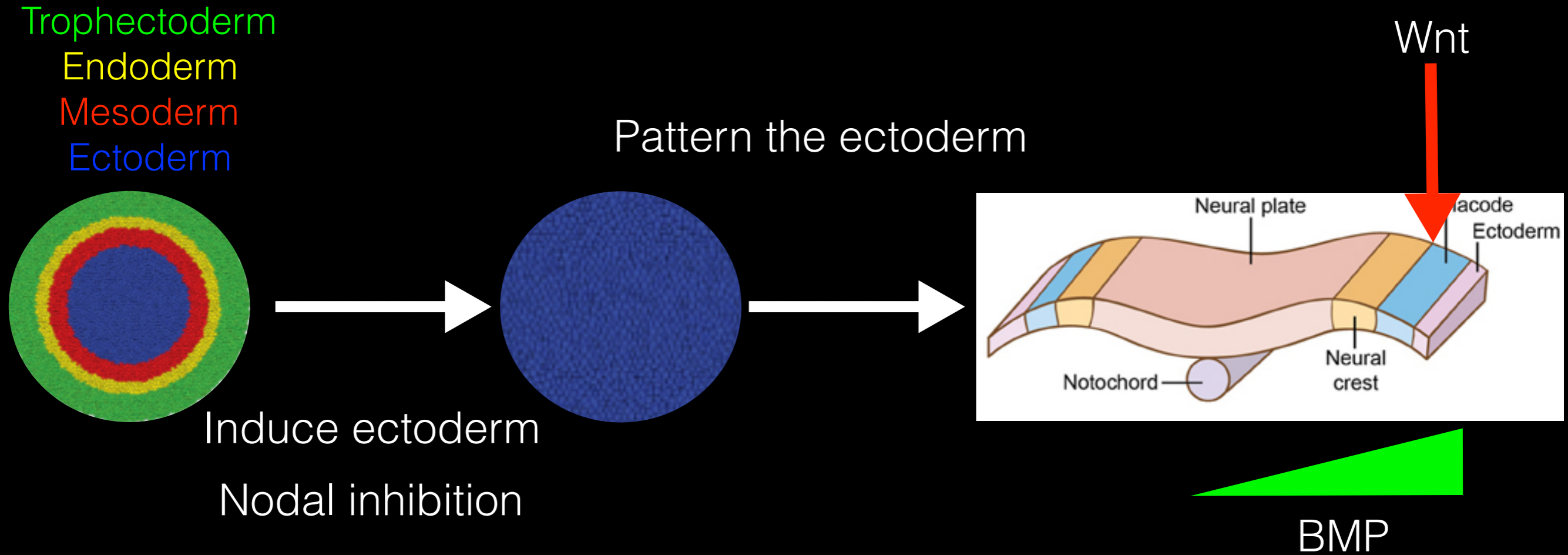


Pattern the ectoderm

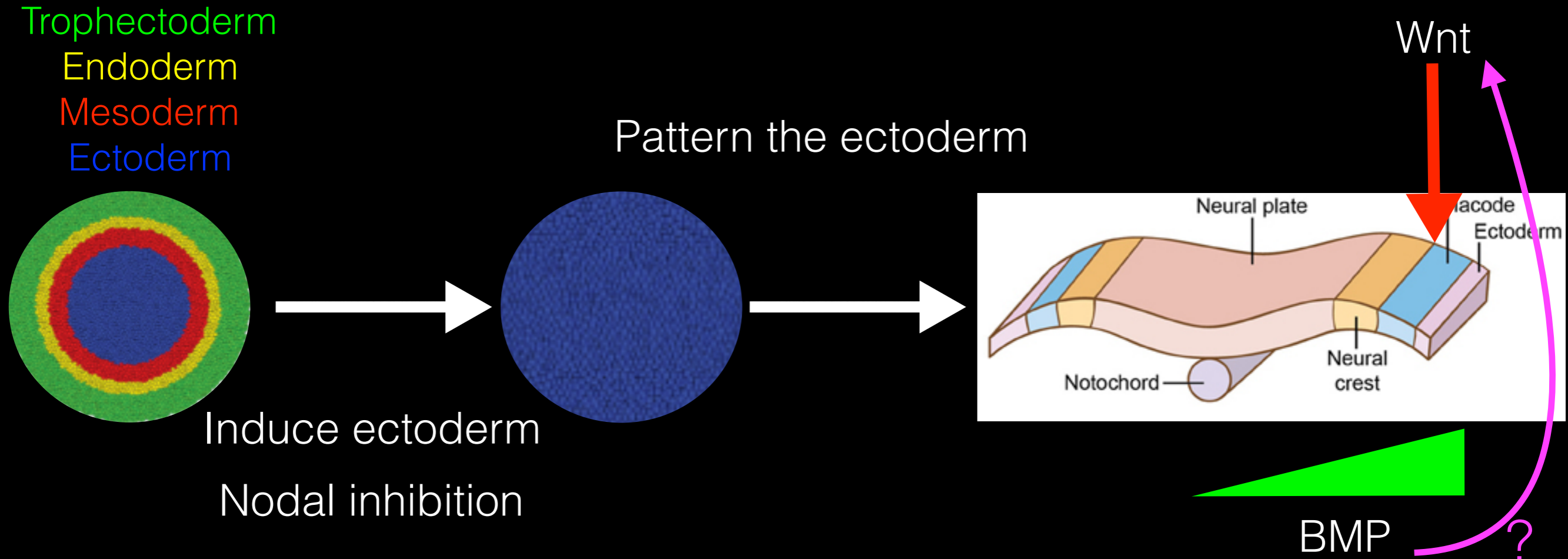


BMP

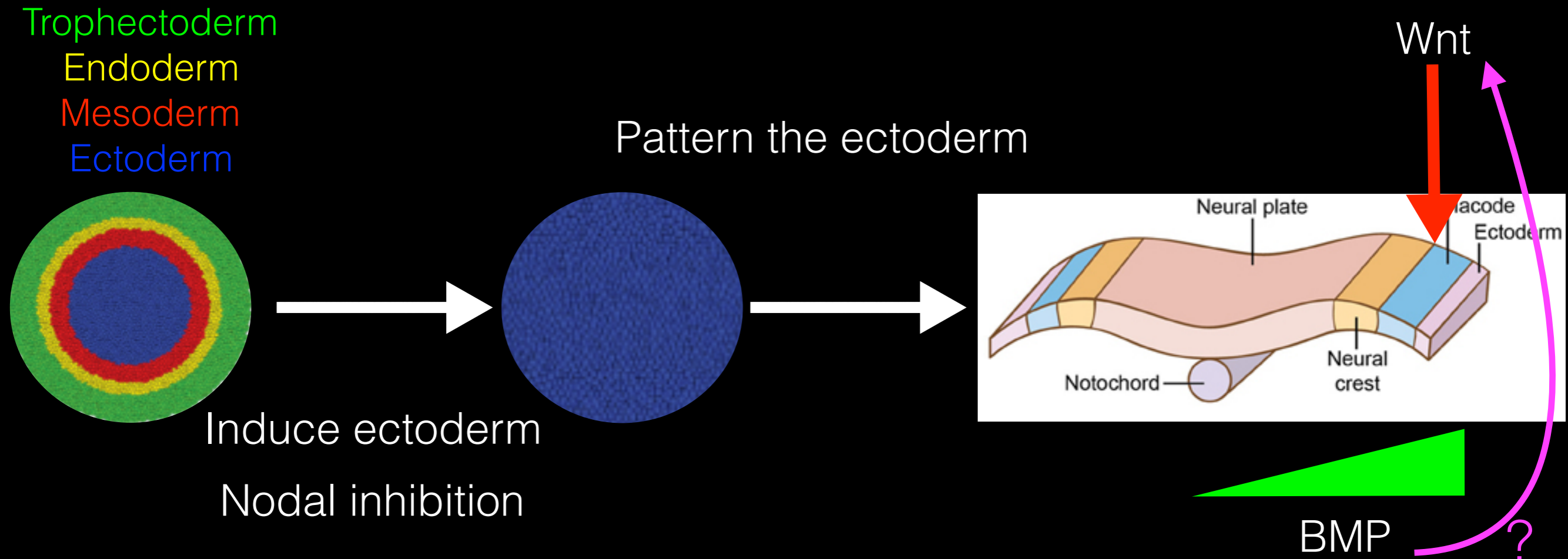
Can we make patterns representing later developmental stages?



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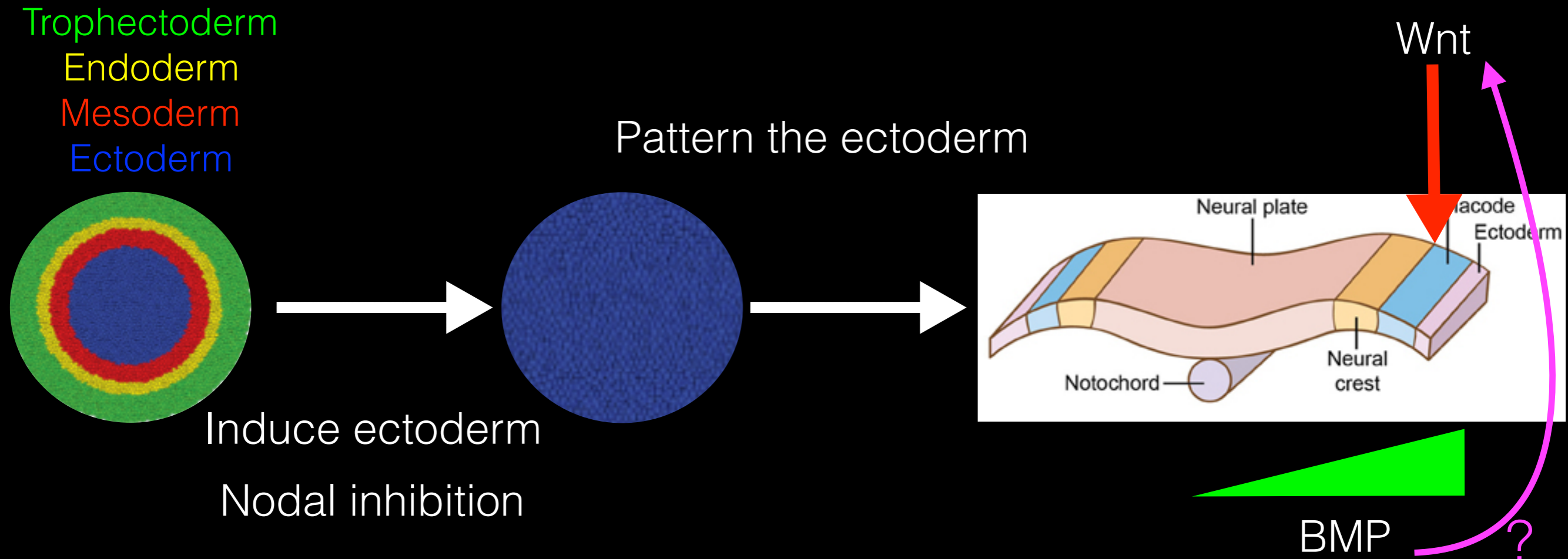


Can we make patterns representing later developmental stages?



The same signaling pathways are recycled slightly later in development.

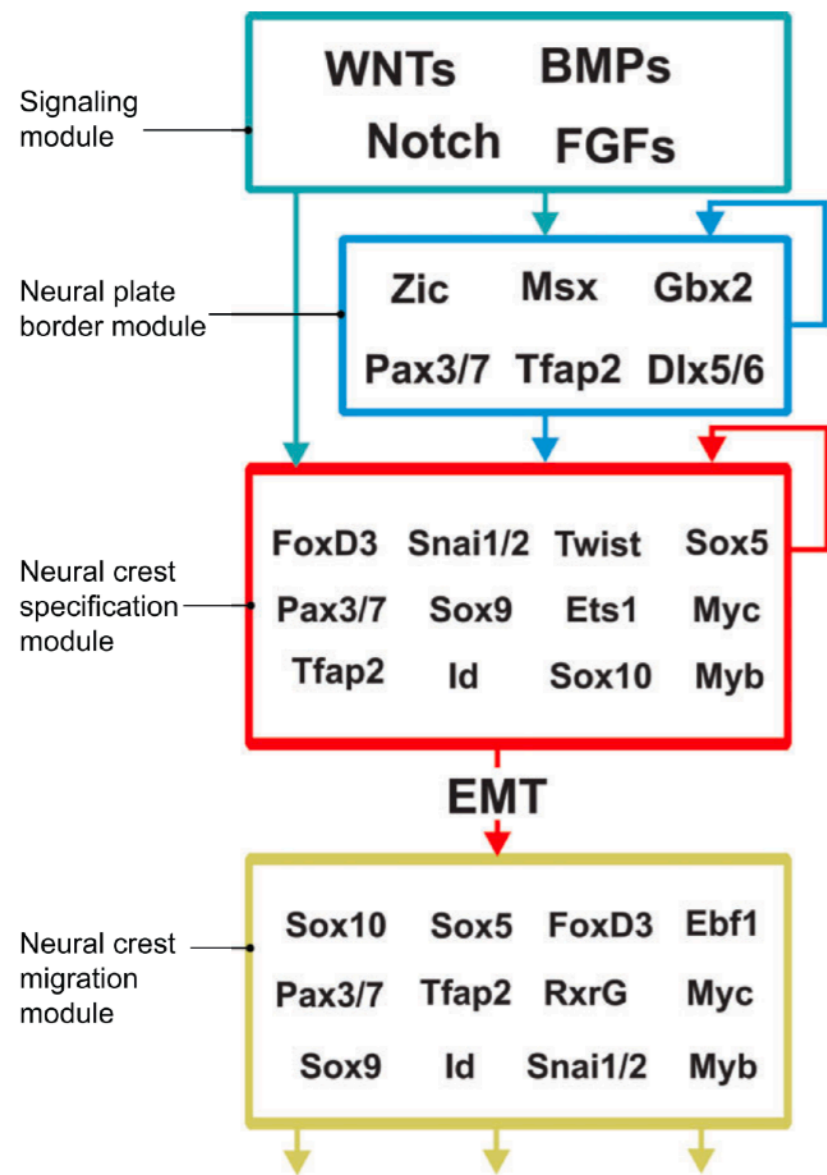
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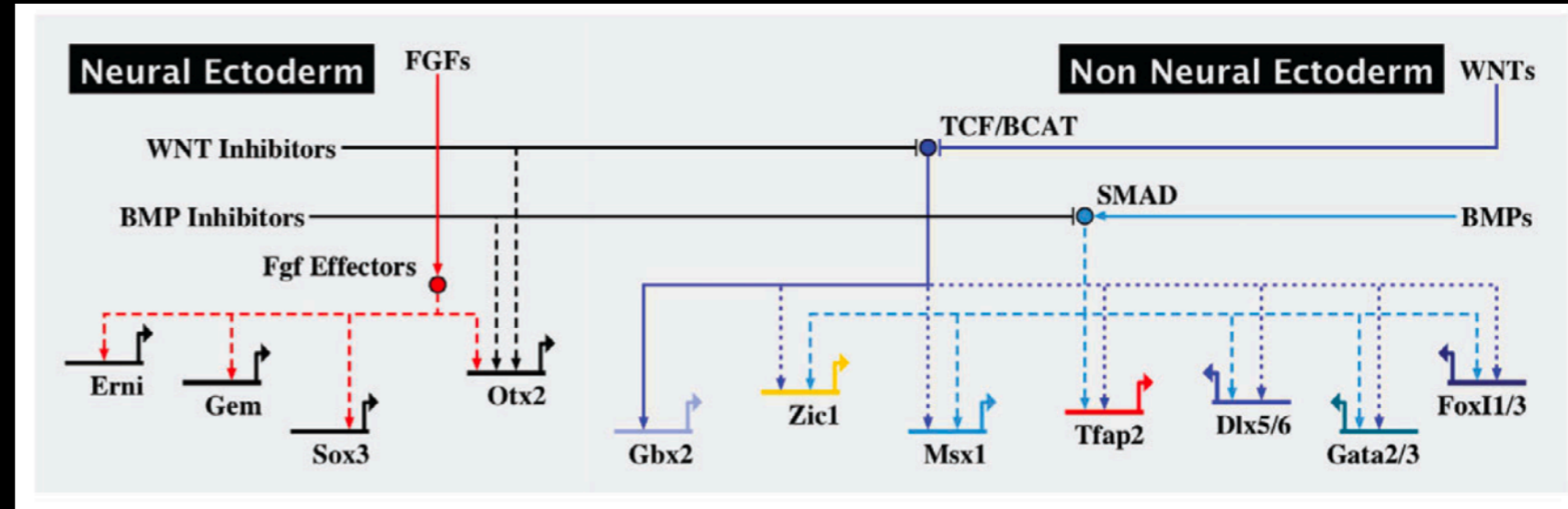
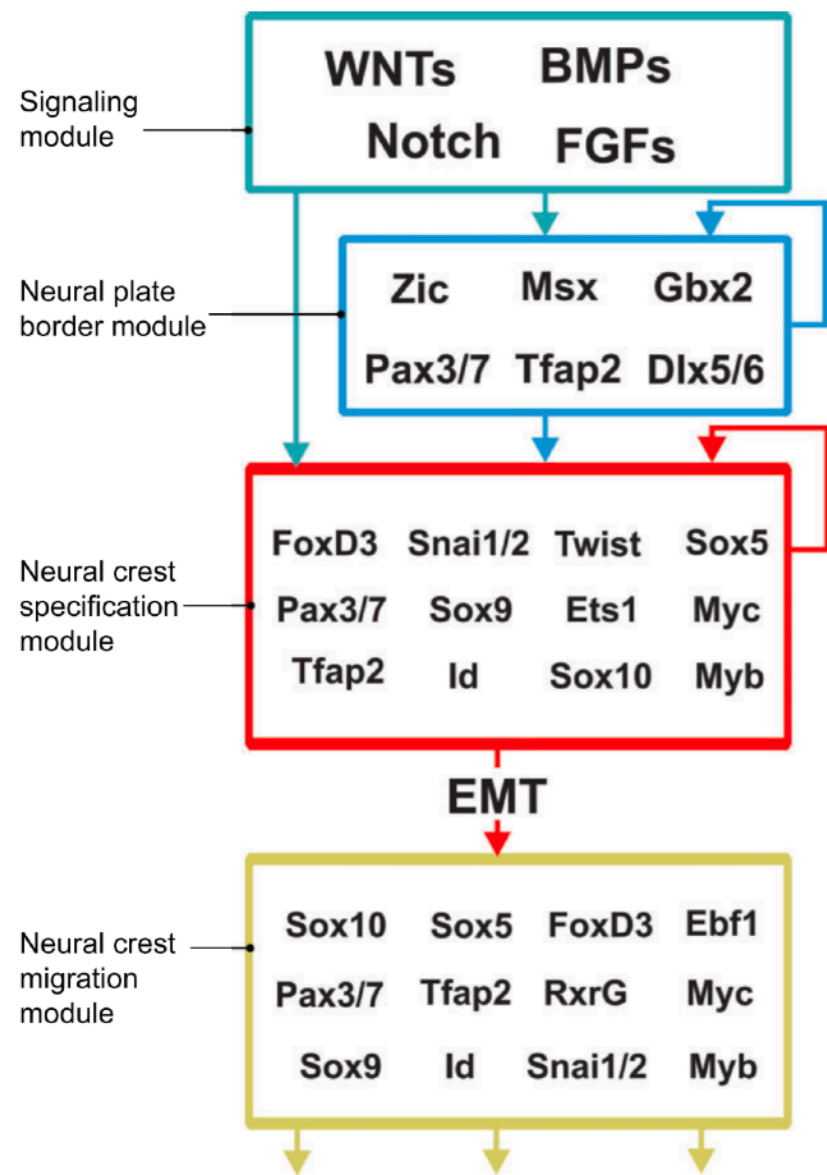
Does the signaling change, the competence of the cells or both?

Complexity of in vivo systems has made it difficult to determine relationships between signaling and differentiation in the ectoderm



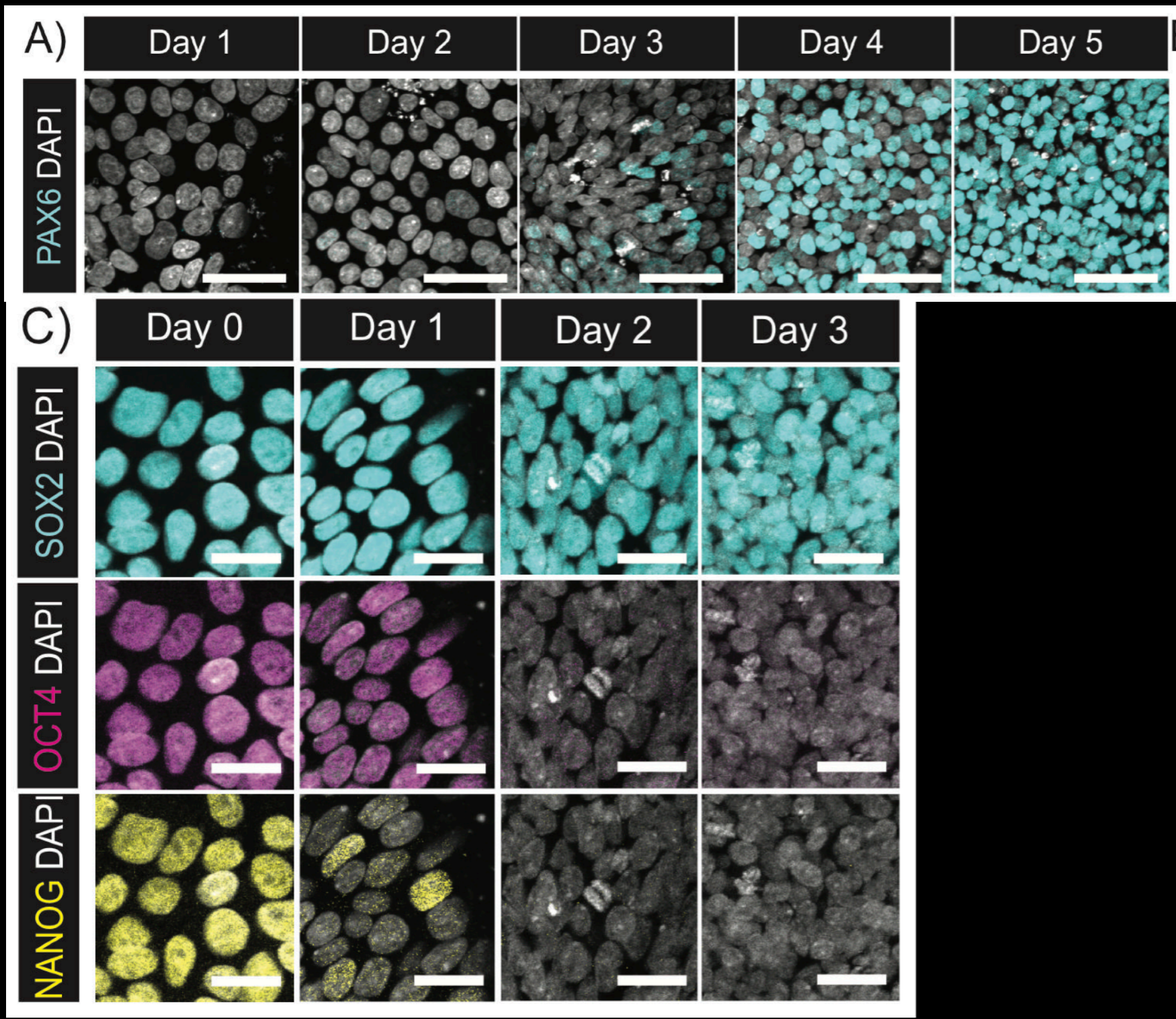
Simões-Costa, M., and Bronner, M.E. (2015). Establishing neural crest identity: a gene regulatory recipe. *Development* 142, 242–257.

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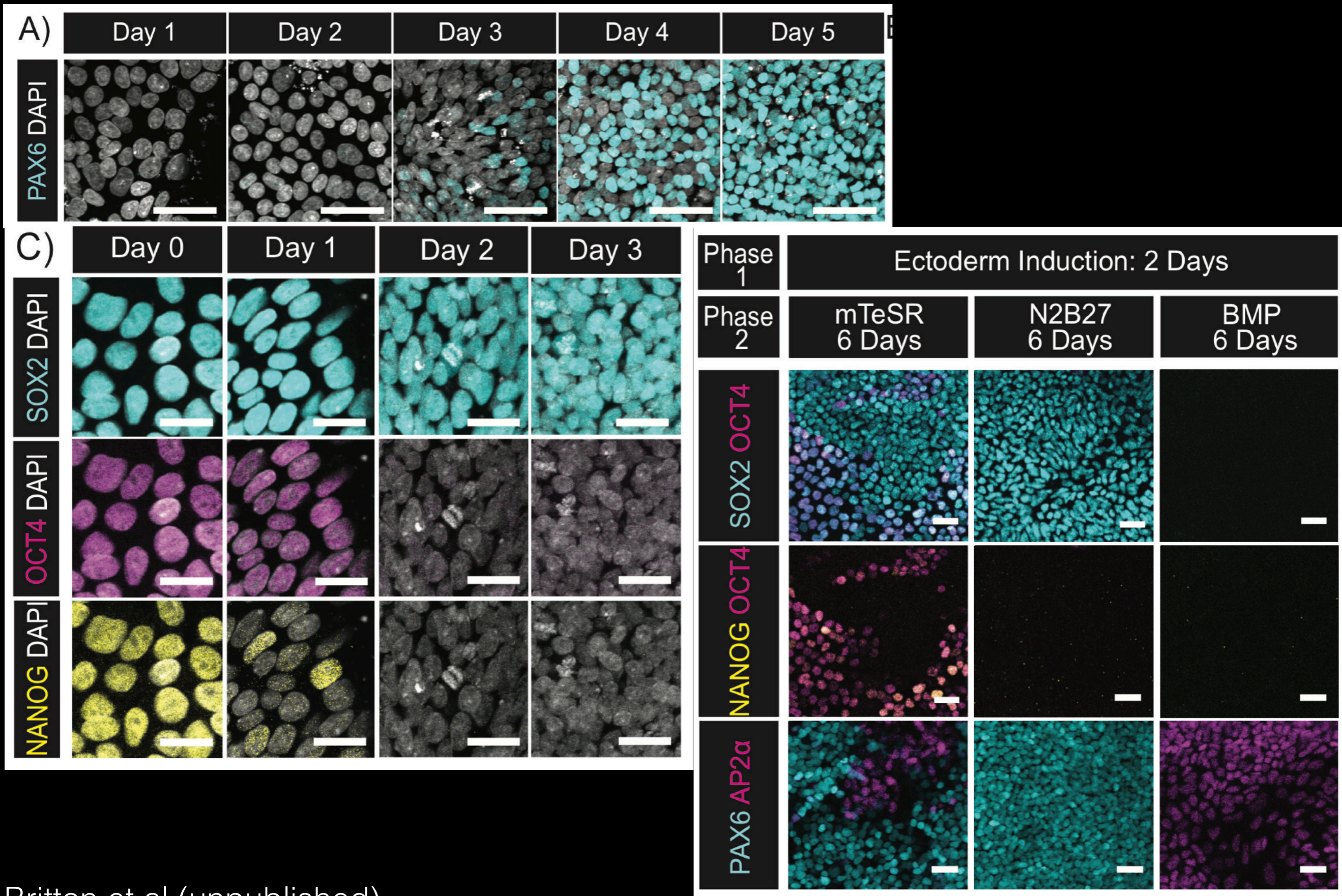


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Determining a window of competence for ectoderm patterning



Determining a window of competence for ectoderm patterning



Britton et al (unpublished)

A three phase protocol makes sharp patterns of four fates



DAPI
(nuclear)

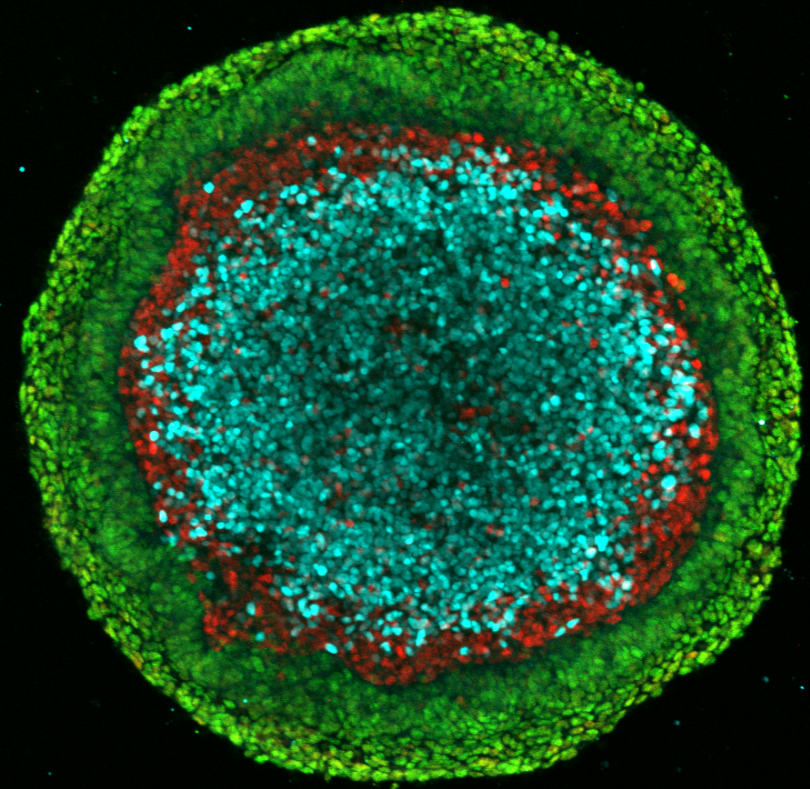
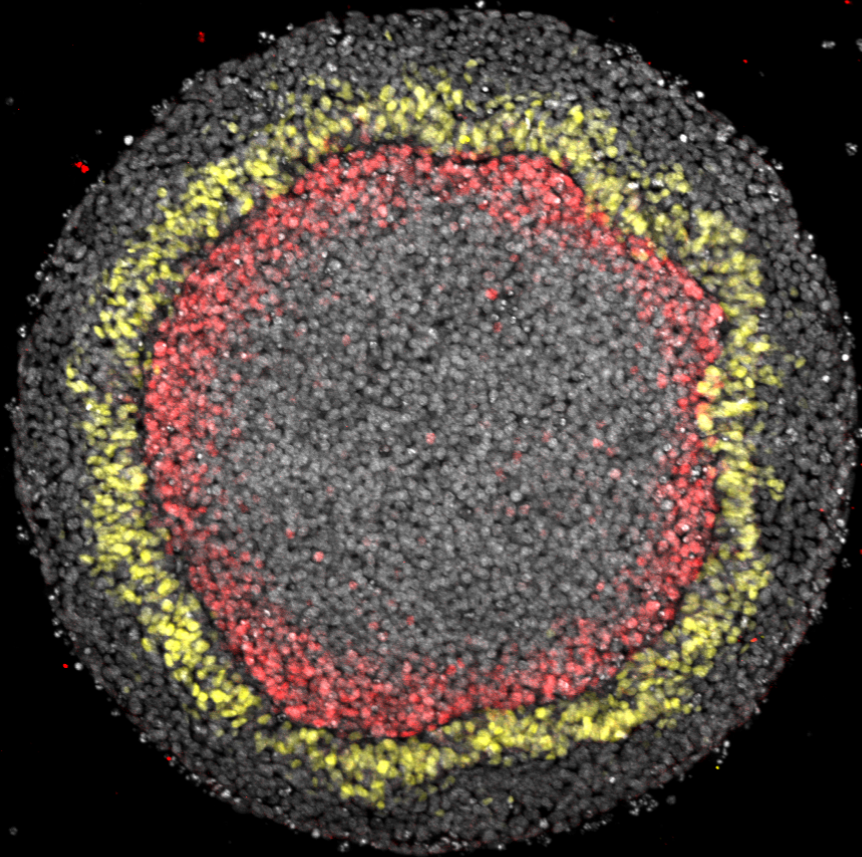
SIX1
(placode)

PAX3
(neural crest)

PAX6
(neural)

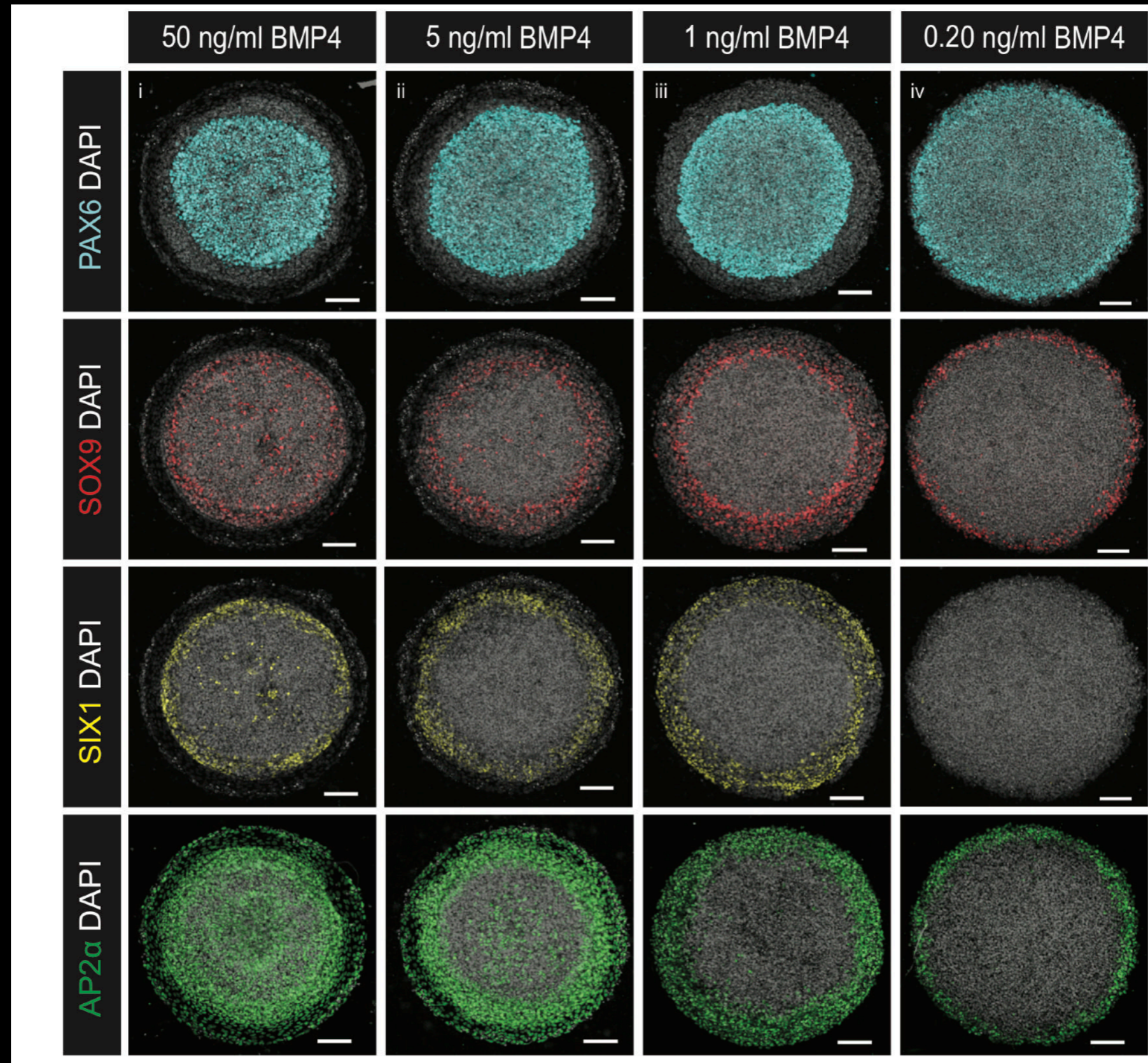
ISL1
(epidermis)

SOX9
(neural crest)

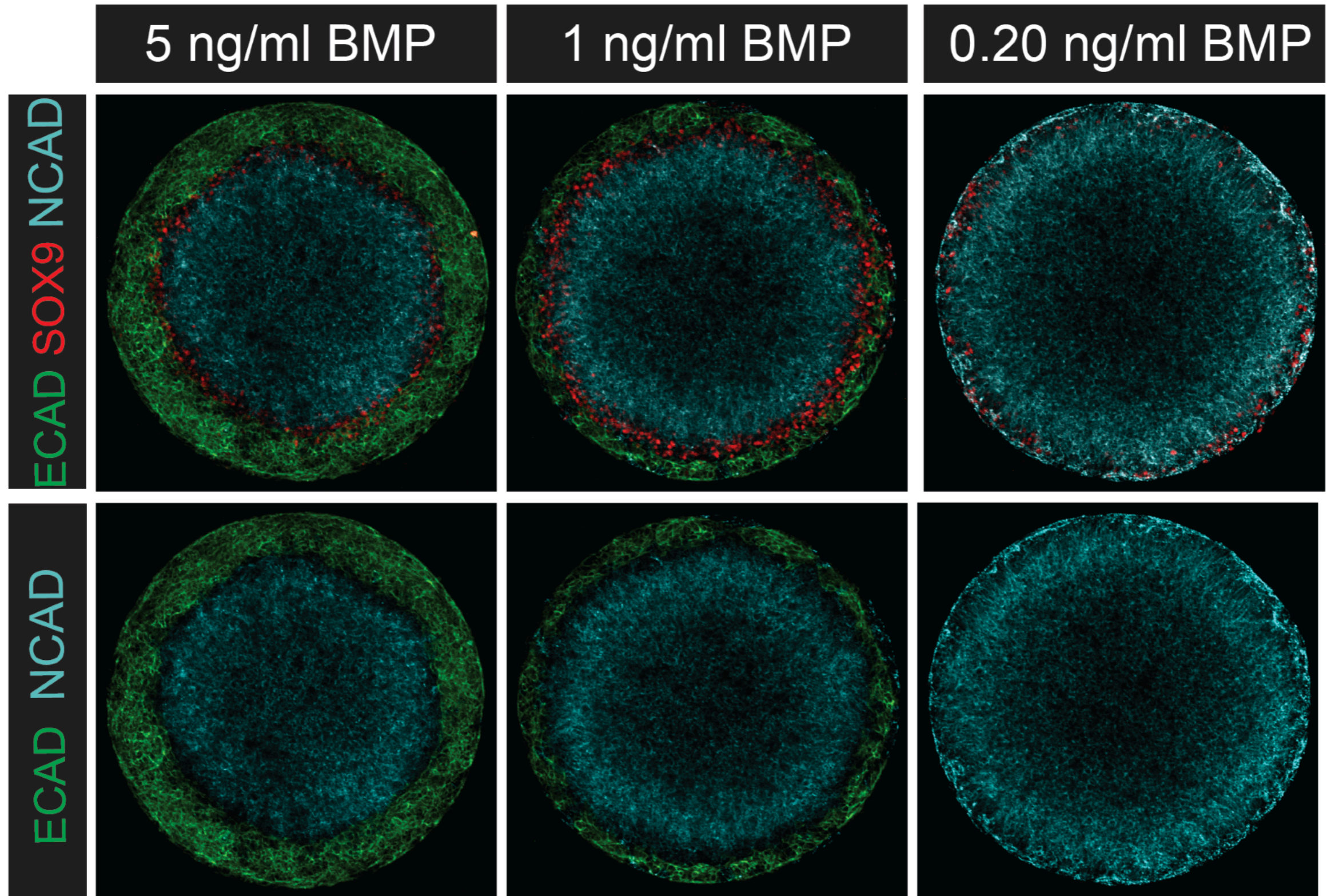


Epidermal/Placode/Neural Crest/Neural

BMP is required for outer (epidermal) fates. More BMP = wider region



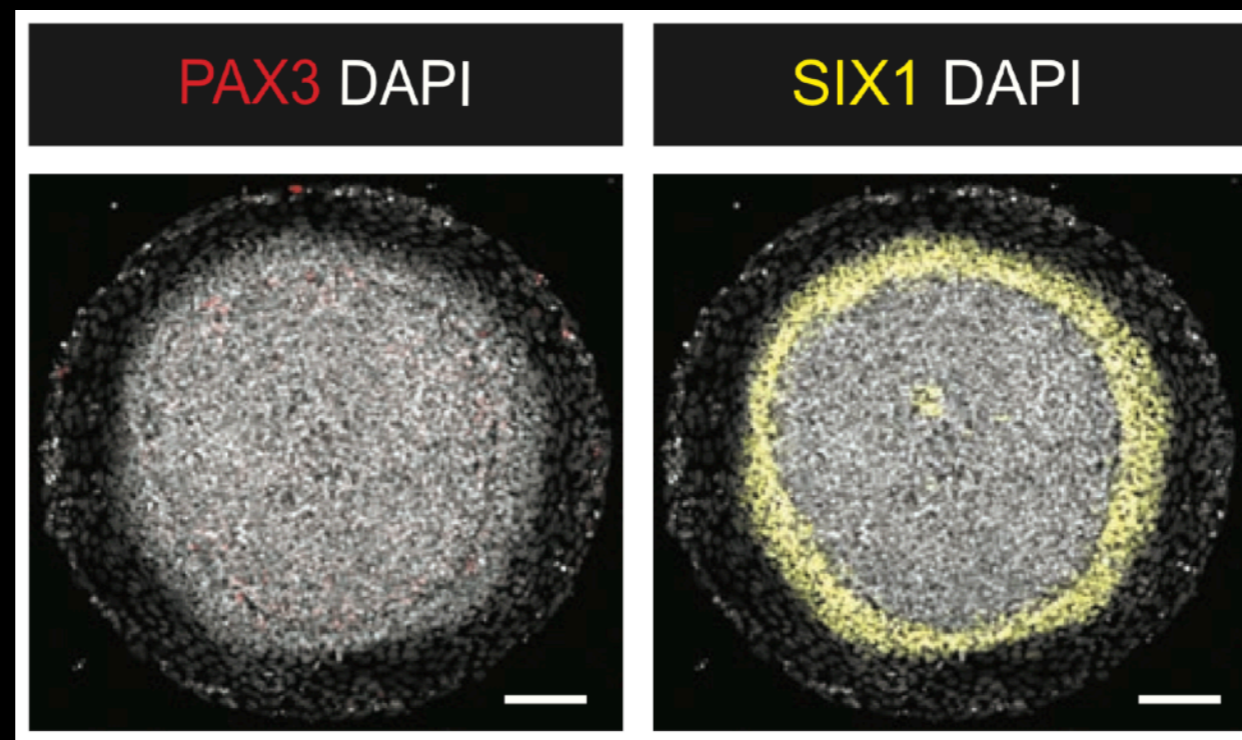
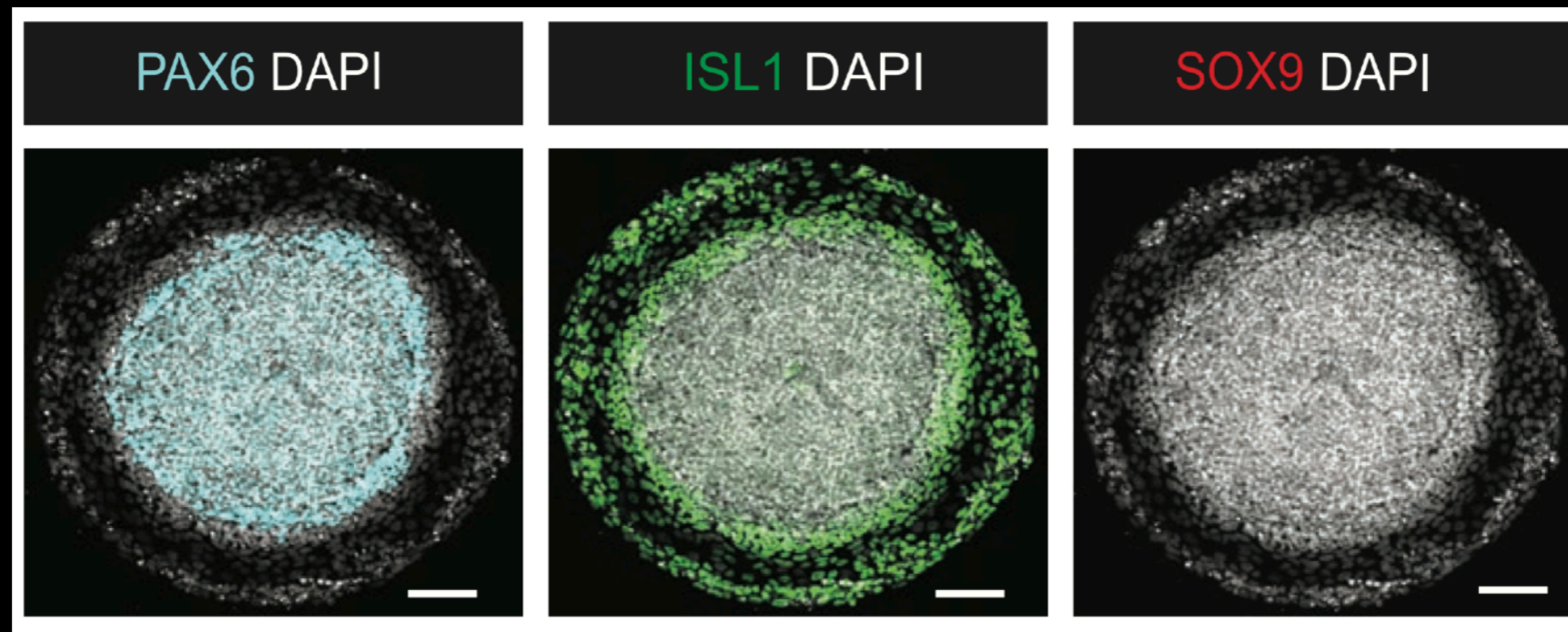
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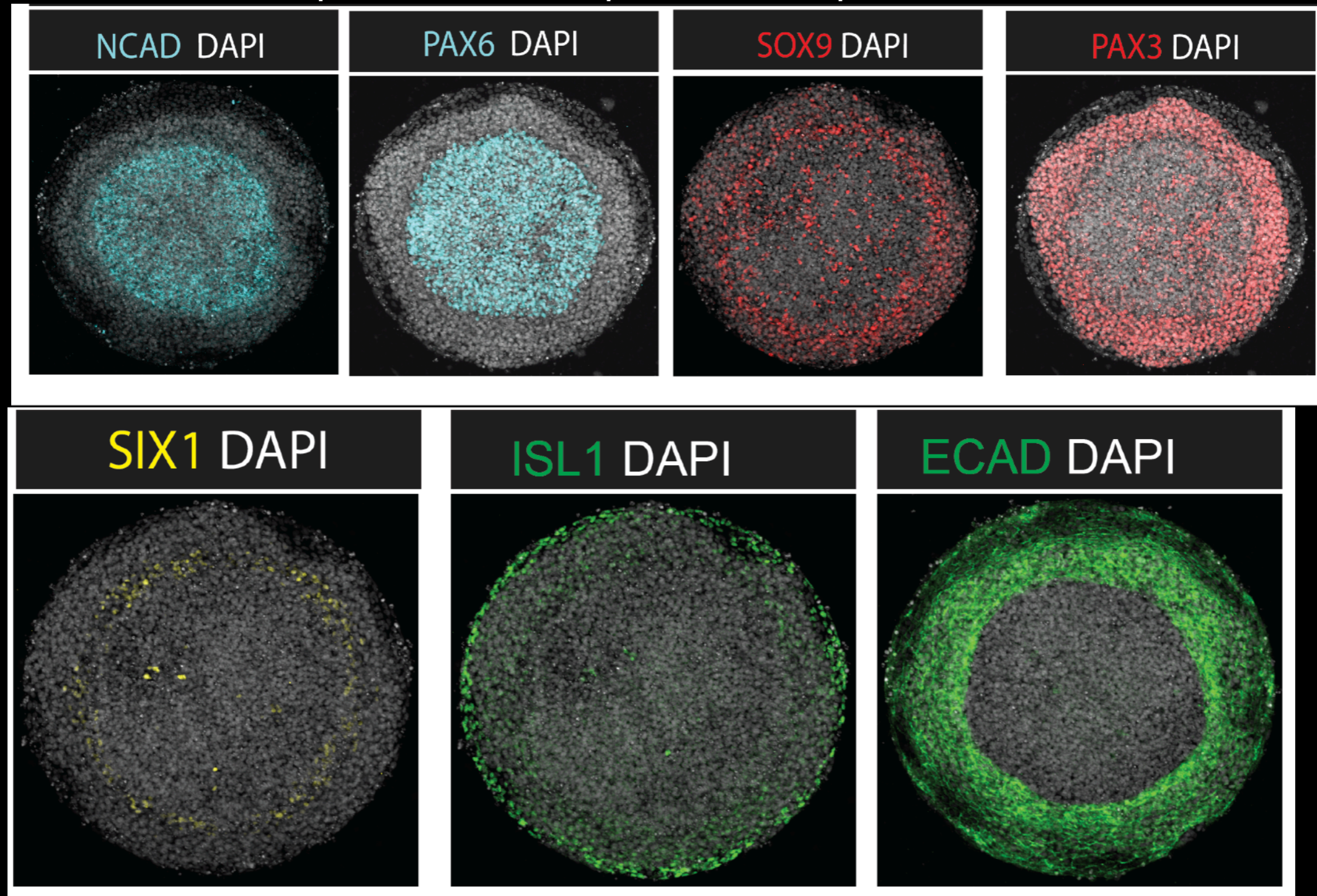
Wnt is required for intermediate (neural crest) fate

With IWP2 (Wnt inhibitor) throughout

Epidermal/Placode/Neural Crest/Neural

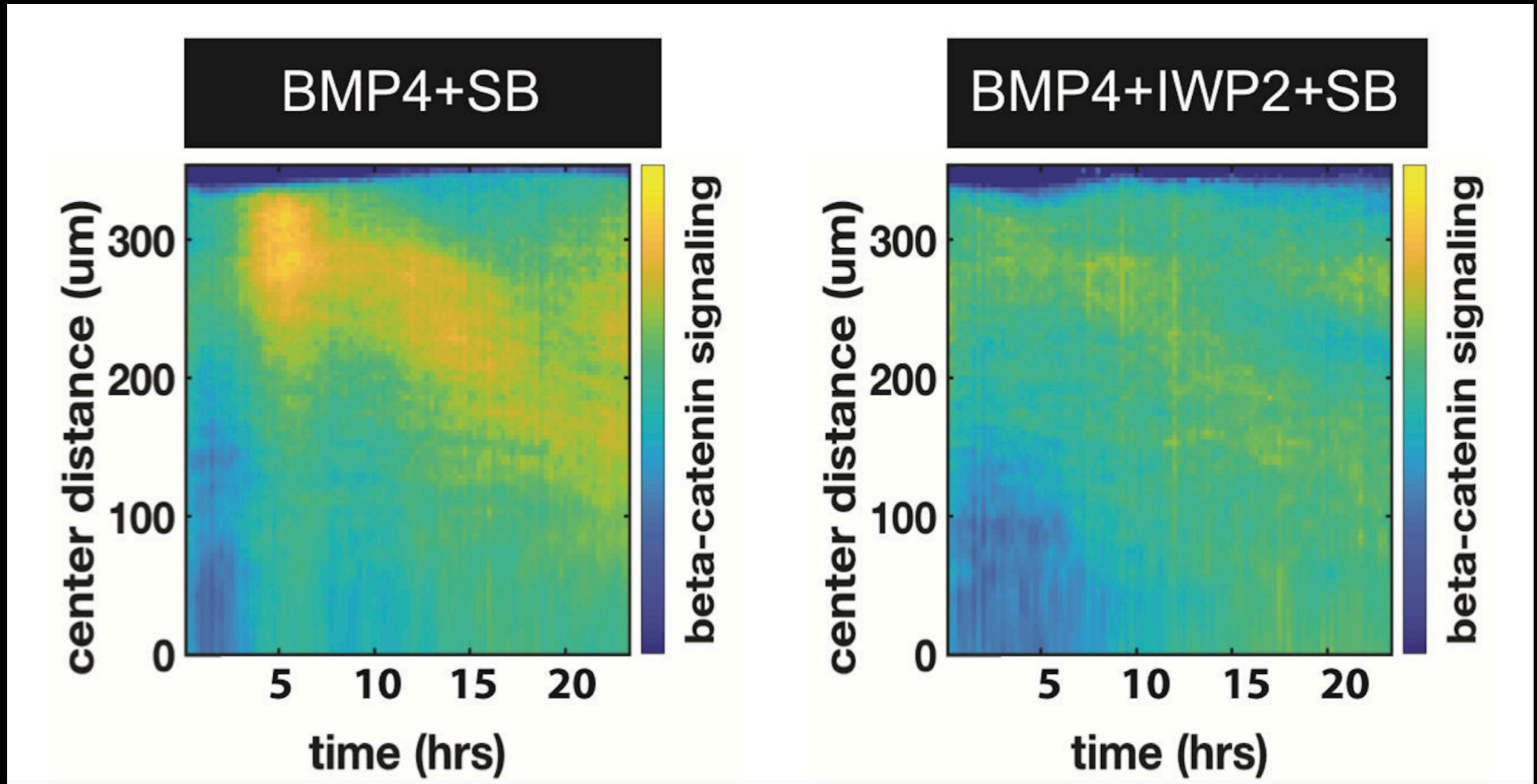


A two phase protocol has messier boundaries with neural crest expanded in place of placodes



Range of signaling response is internally specified at gastrulation but needs external modulation for ectoderm

Wnt signaling dynamics show a propagating wave rather than a spreading front

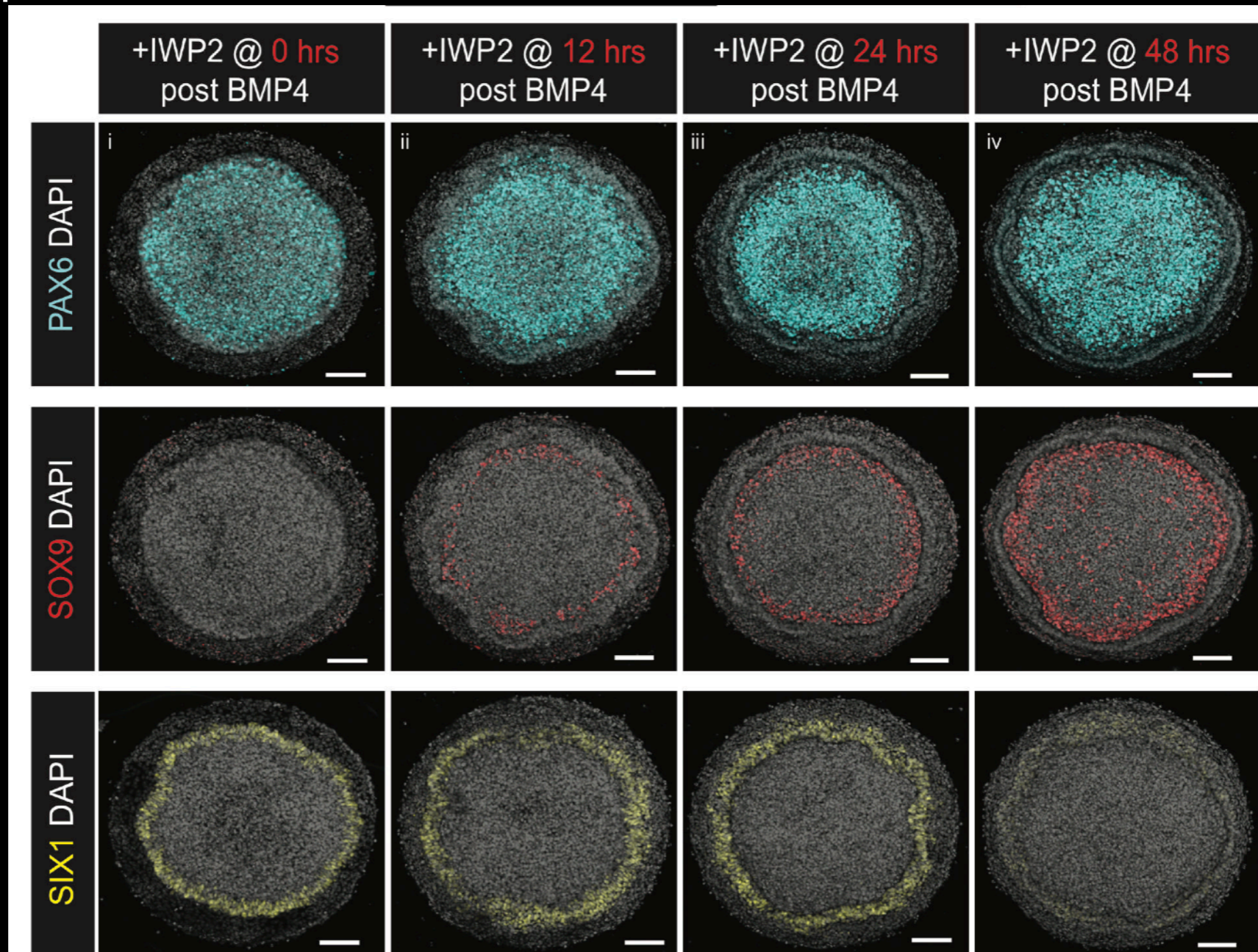


Duration of WNT signaling modulates neural crest vs placode differentiation

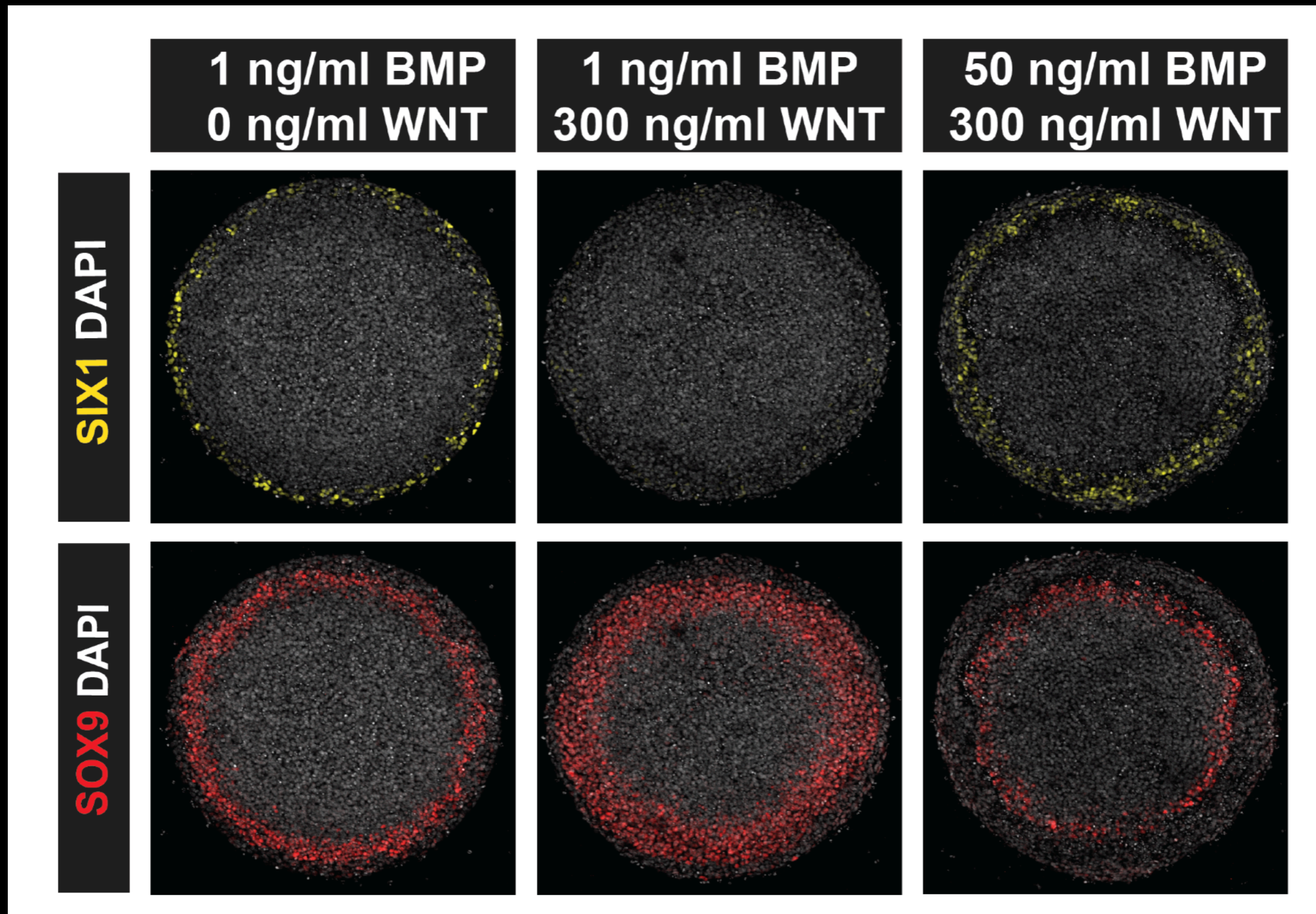
Longer
WNT
Inhibition



Shorter
WNT
Inhibition



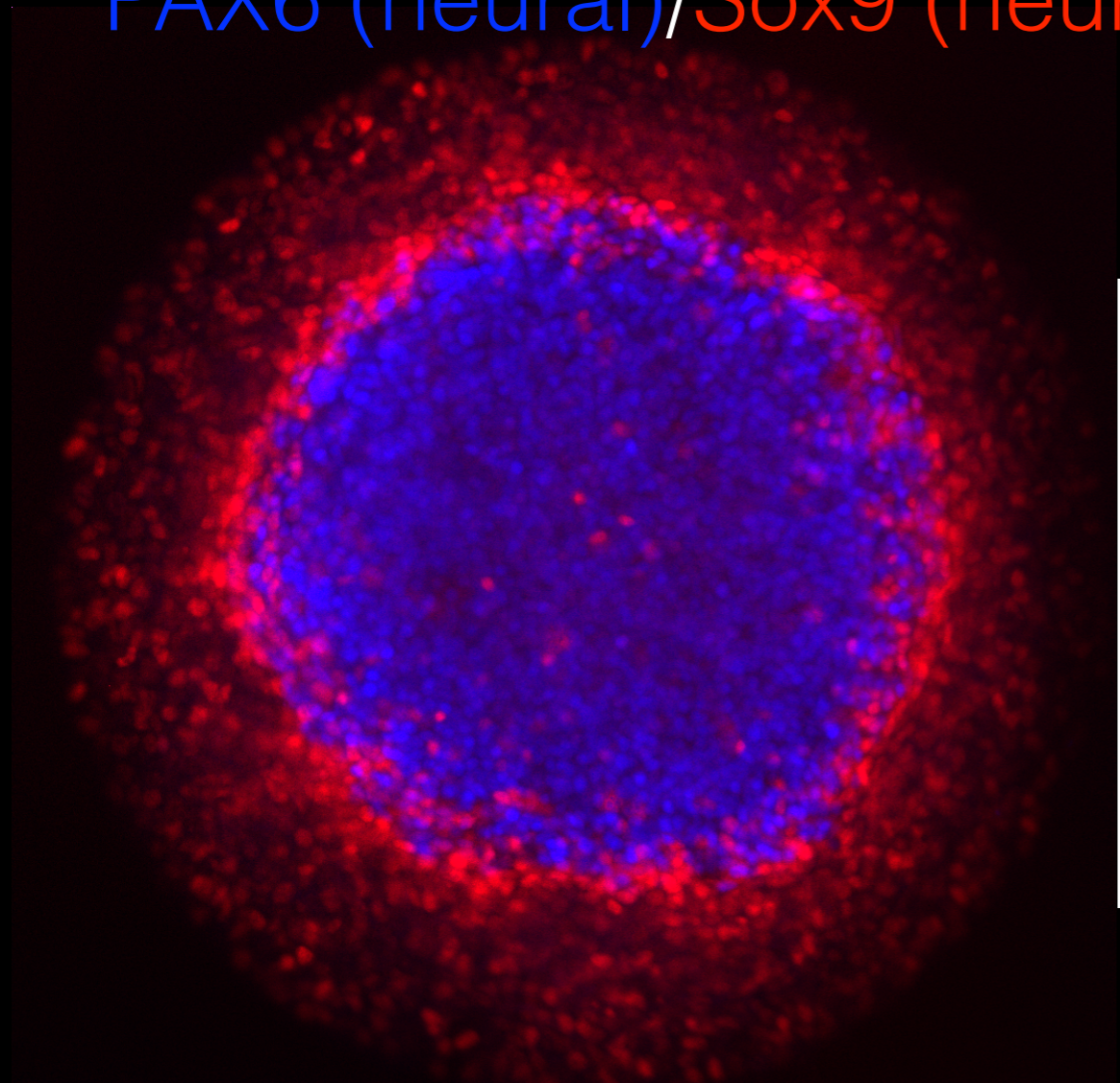
Cell fates are defined by tradeoff between BMP and WNT



The competency of cells is set during the early phase of differentiation

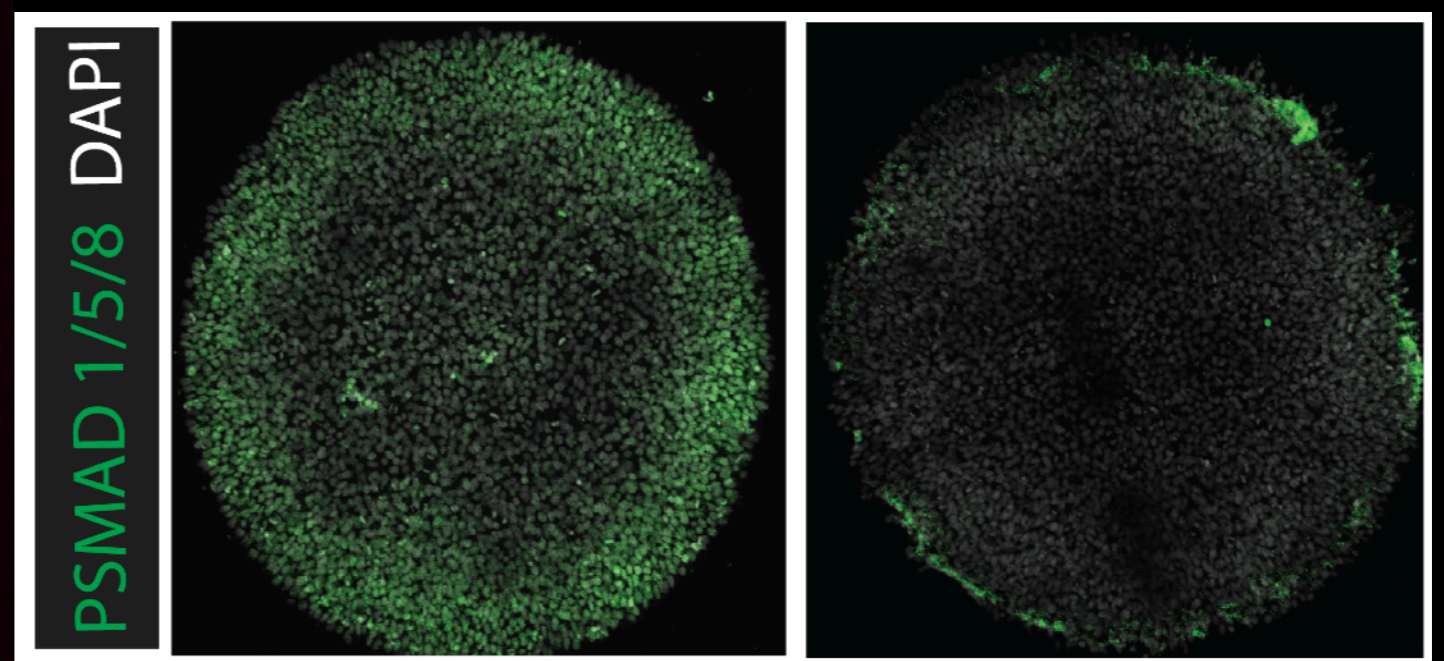


PAX6 (neural)/Sox9 (neural crest)



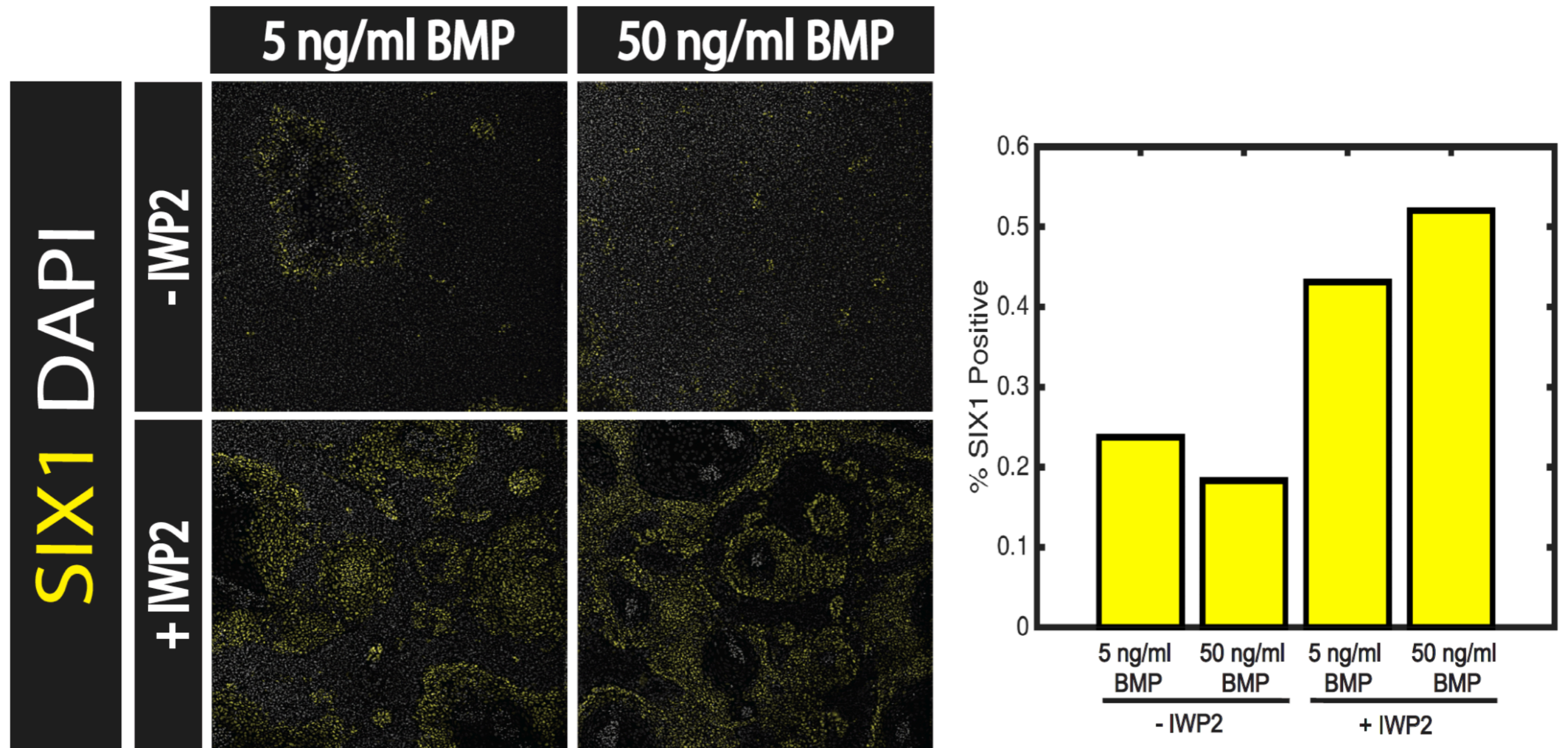
Day 3

Day 3+BMP-I



Endogenous BMP signaling is required to maintain epidermal/placodal competence

WNT inhibition dramatically improves placode differentiation protocols



Warmflash lab

Acknowledgements

Current:

Cecilia Guerra
Elena Camacho Aguilar
Lizhong Liu
Joseph Massey
George Britton
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Siqi Du

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Idse Heemskerk
Anastasiia Nemashkalo
Kinshuk Mitra
Kari Burt
Teresa Saez
Yida Liu
Matthew Schmerer

Postdoc mentors: Ali Brivanlou, Eric Siggia



Funding:

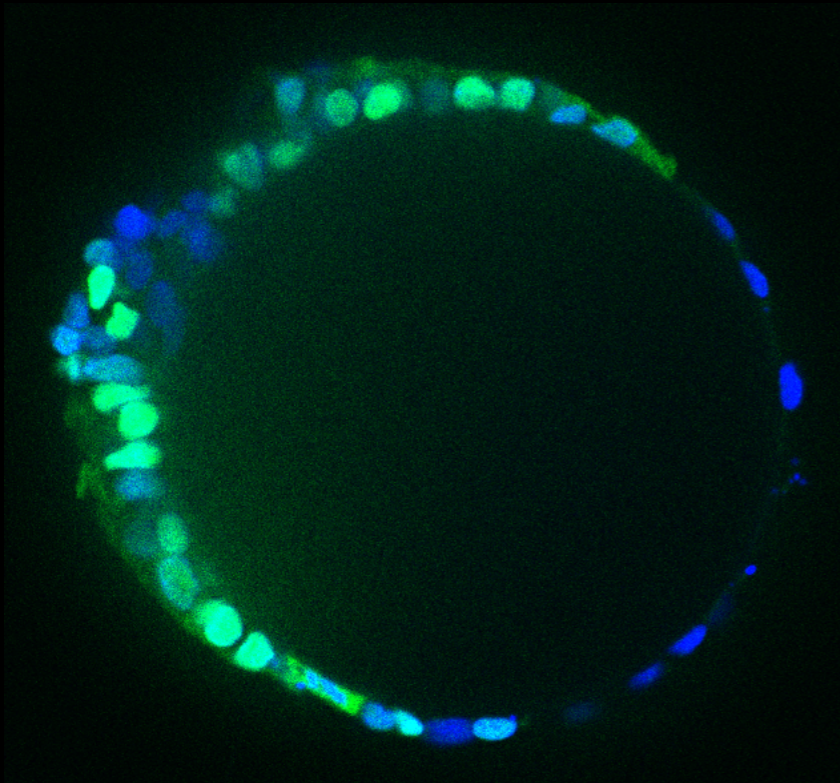


Patterning without a boundary suggests a self-organizing system

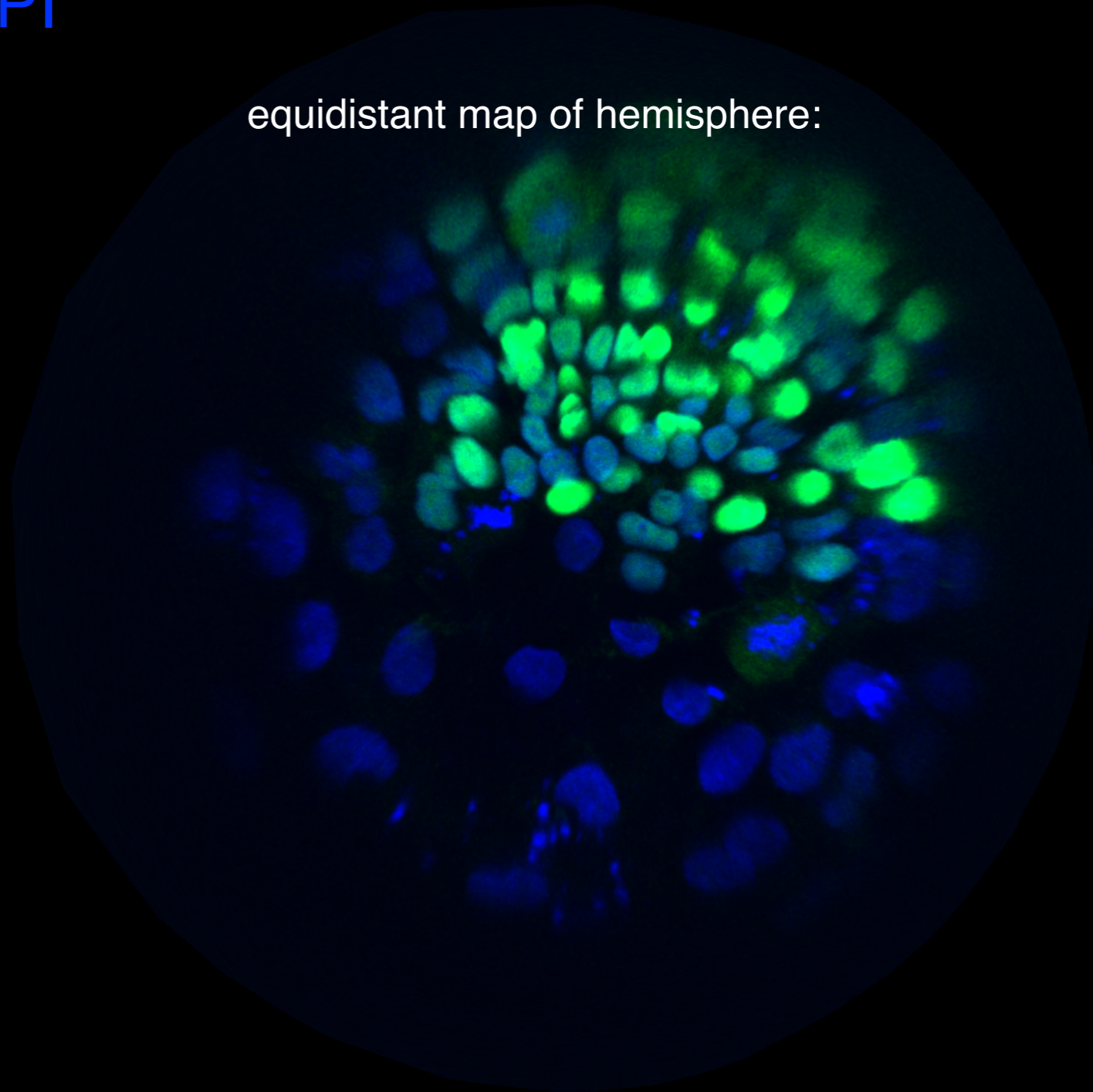
Brachyury

DAPI

Cross section



equidistant map of hemisphere:



“Double beads” pattern with mesoderm in between the beads

Sox2 / Bra/ Cdx2

