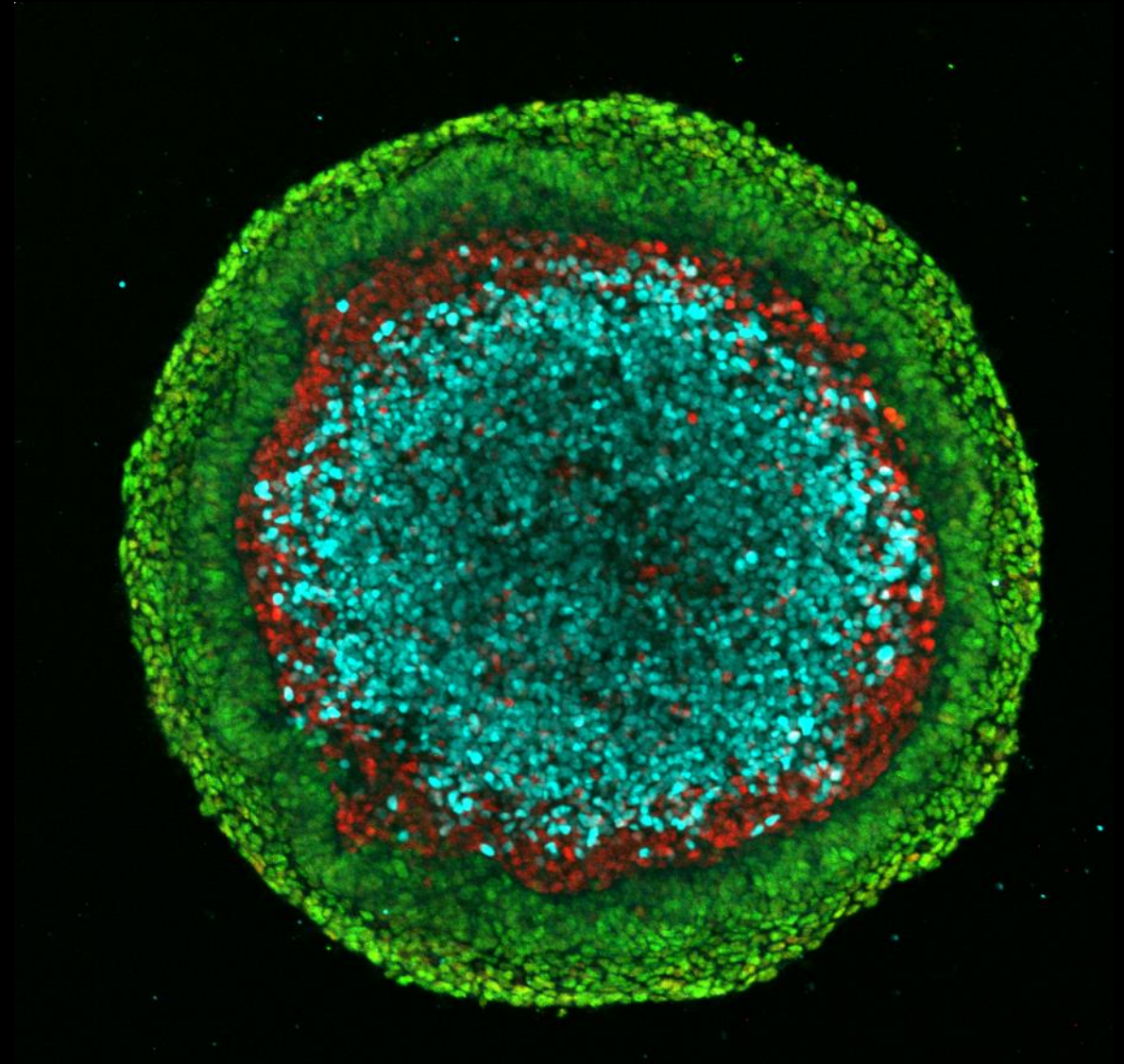
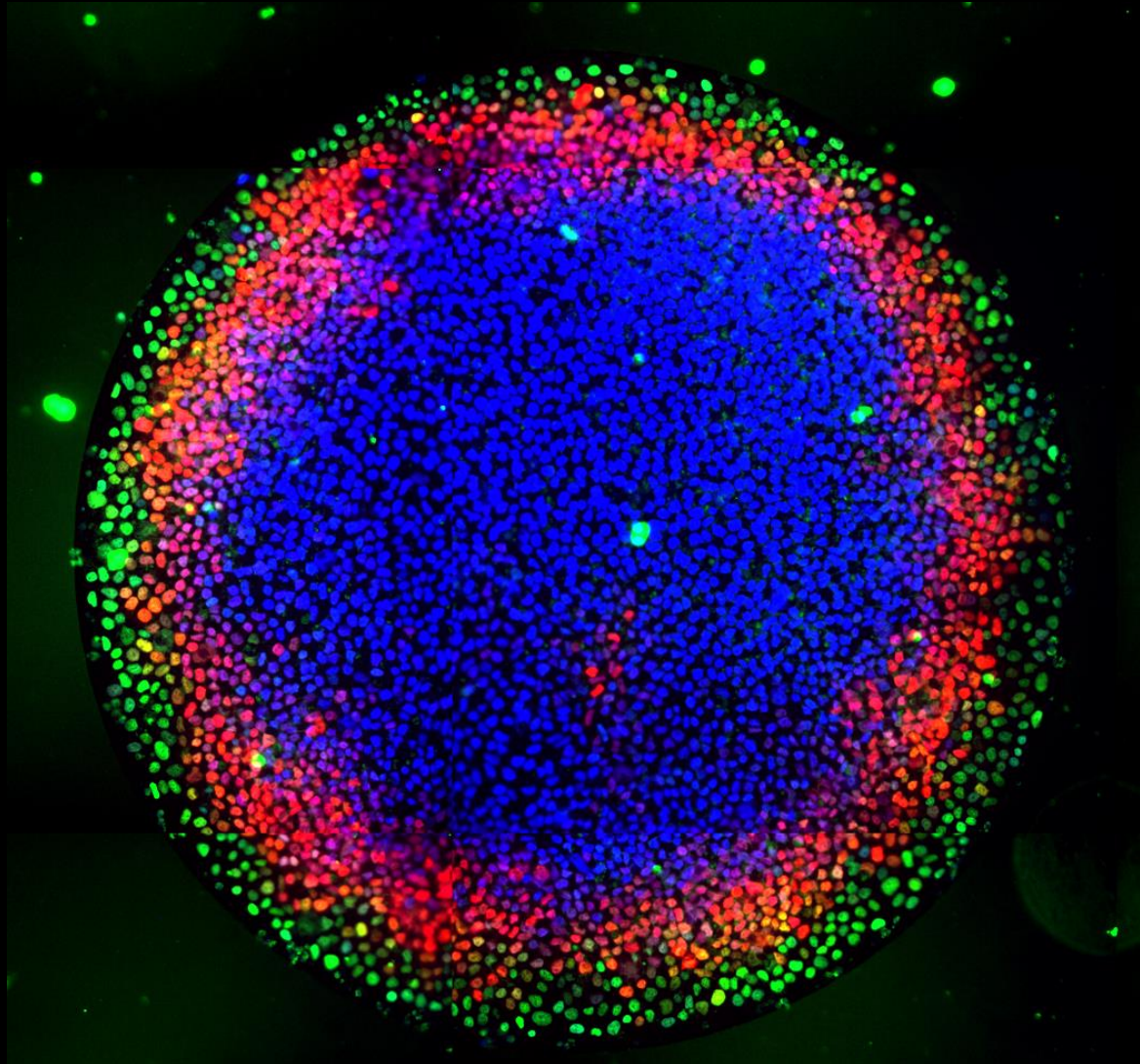


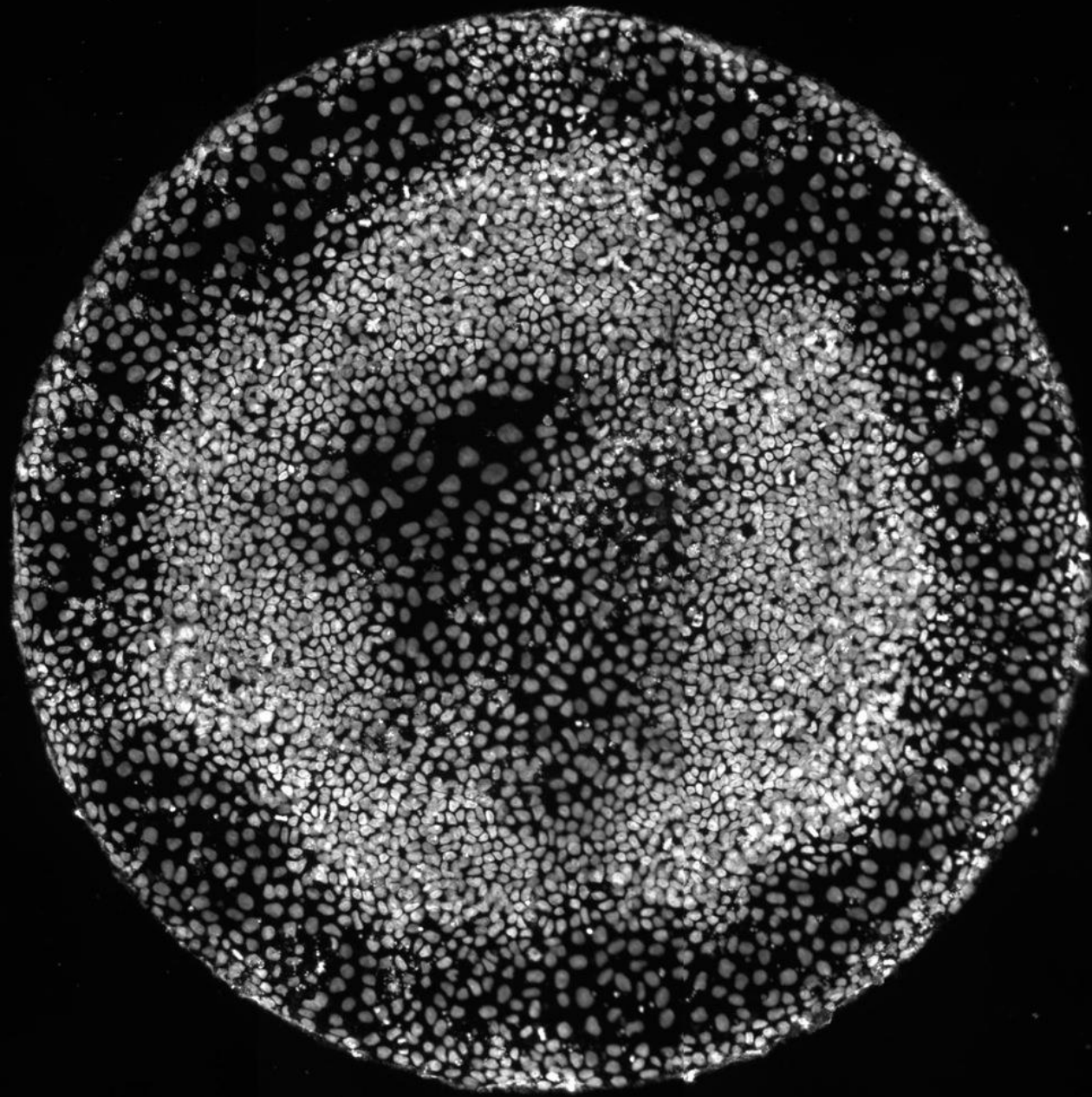
Modeling human development in two dimensions with micropatterns



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

Geometric confinement triggers self-organized patterning along the colony axis

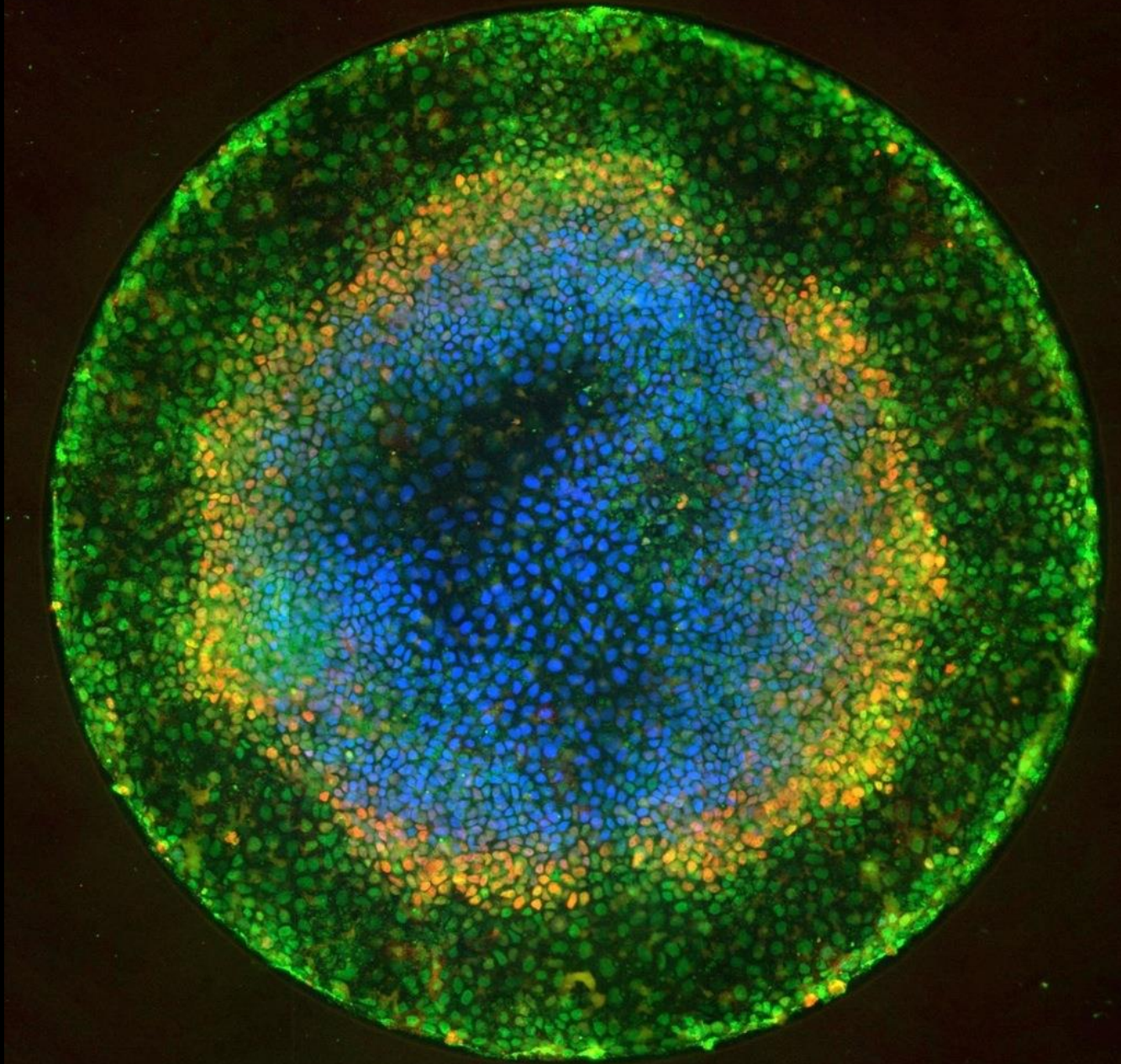
Nuclei (DAPI)



Fate Markers

Sox2/Bra/Cdx2

Ectoderm/Mesoderm/Extraembryonic



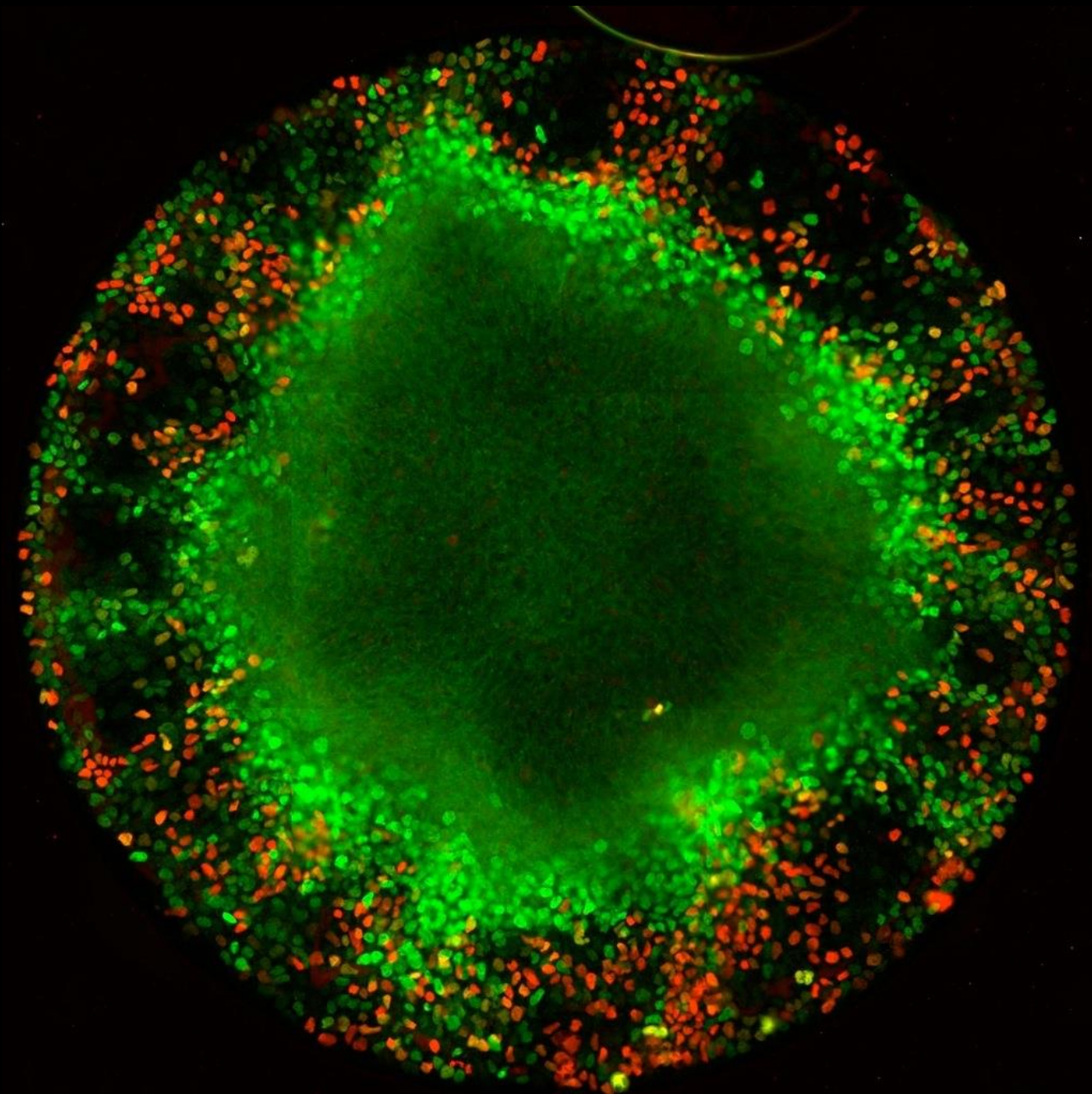
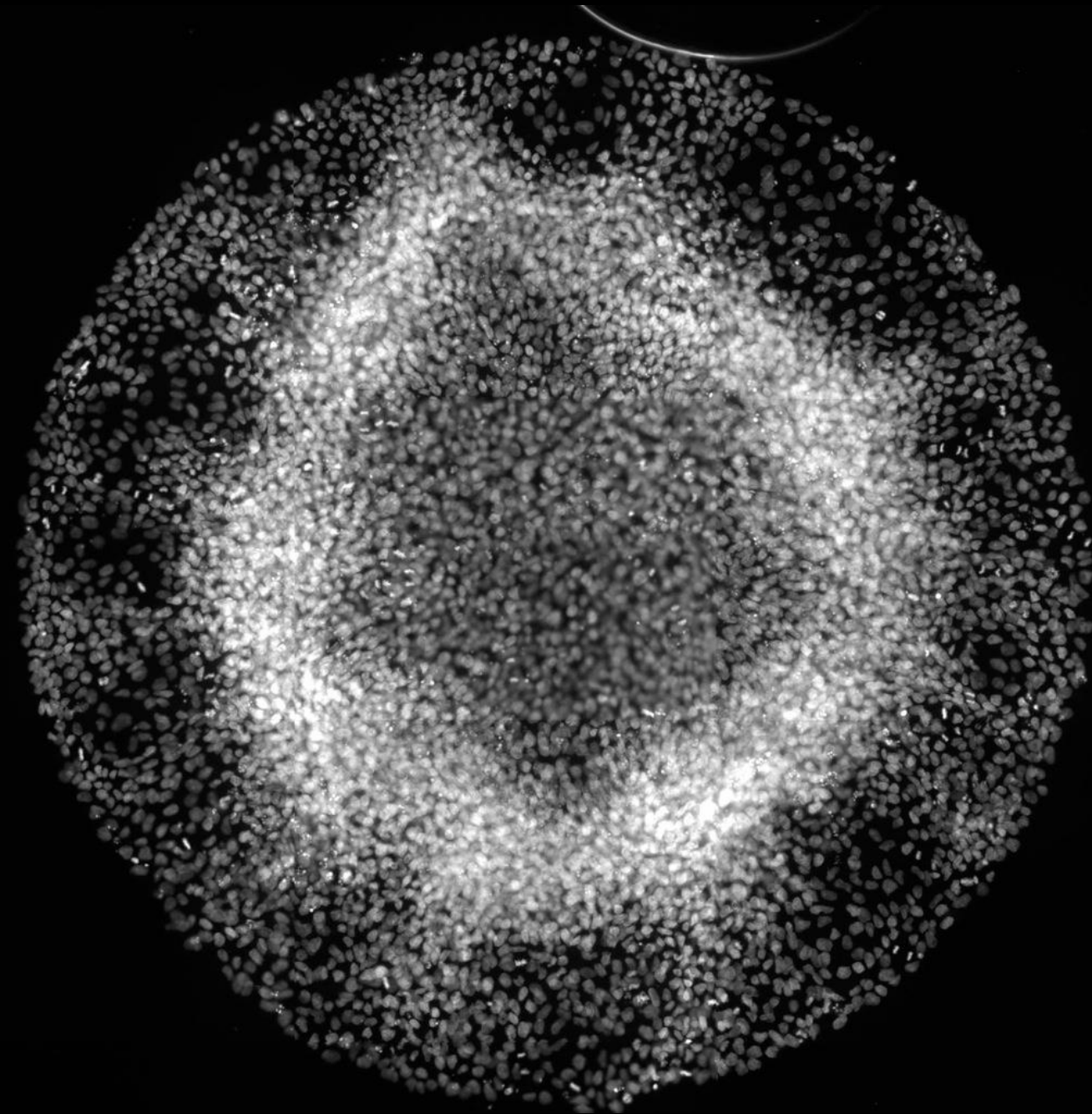
Geometric confinement triggers self-organized patterning along the colony axis

Nuclei (DAPI)

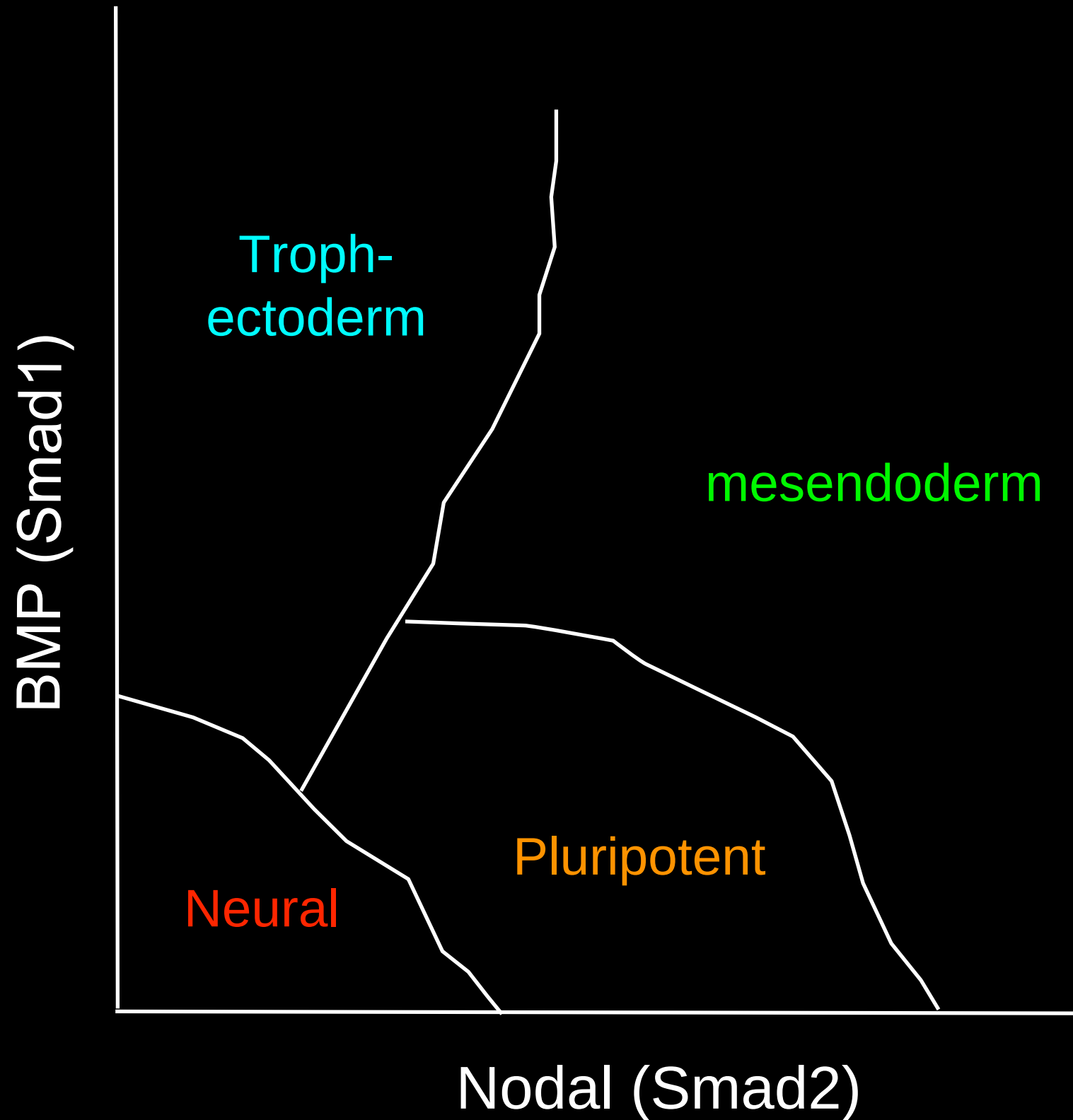
Fate Markers

Eomes/Sox17

Mesoendoderm/Endoderm



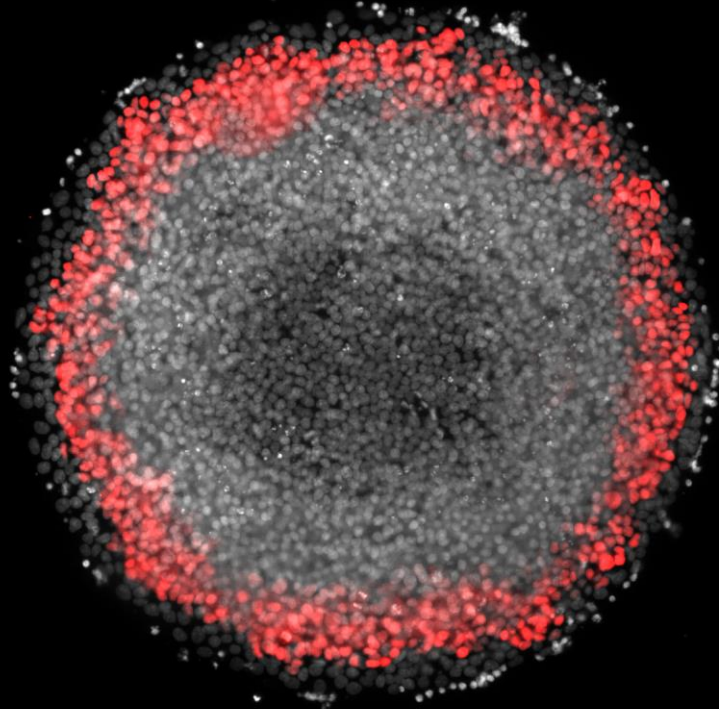
TGF- β signals and fates in hESCS



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

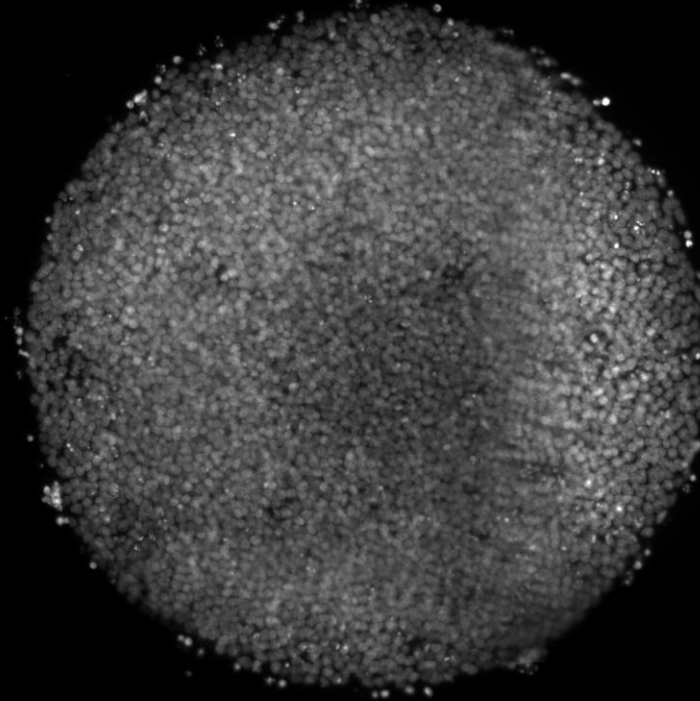
BRA/DAPI

Control



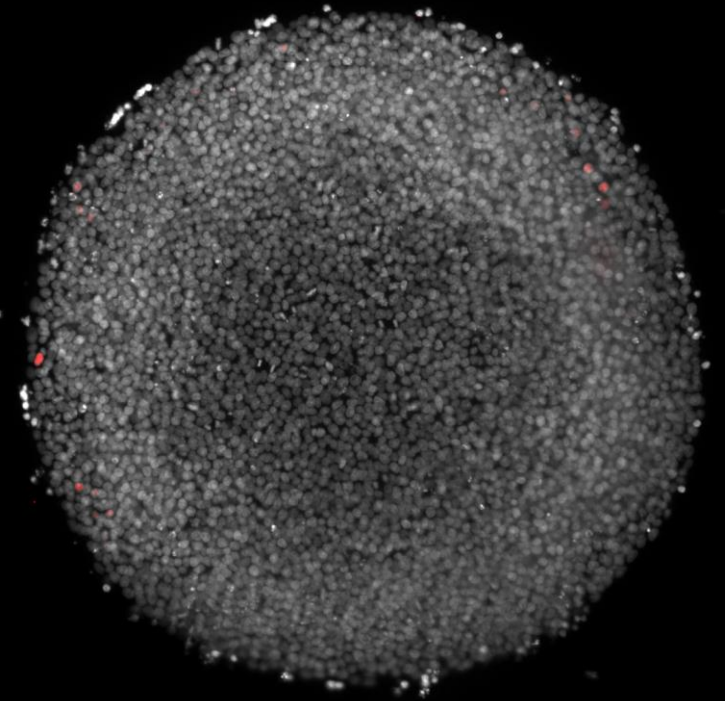
BMP4

Inhibit Wnt



BMP4+IWP2

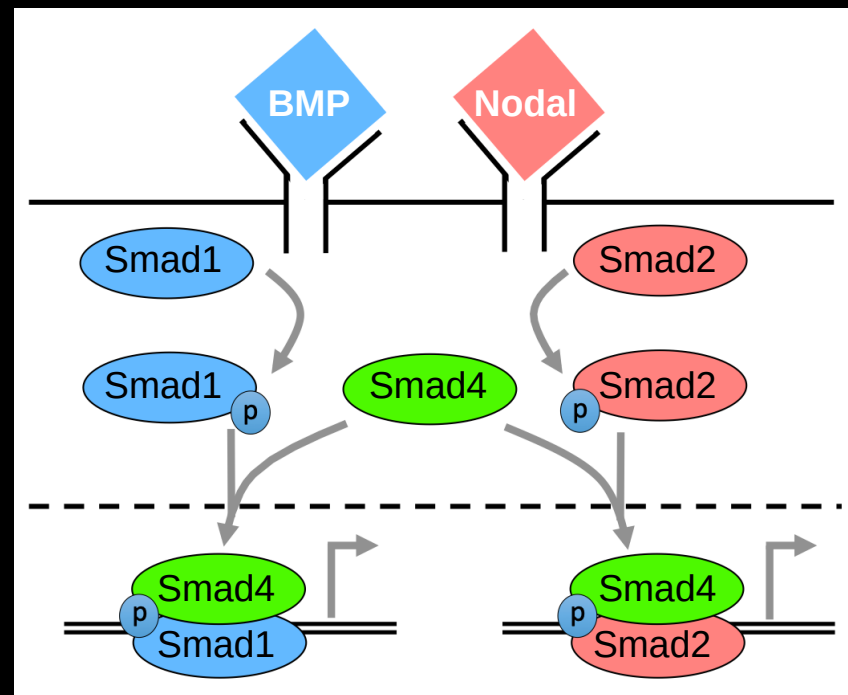
Inhibit Nodal



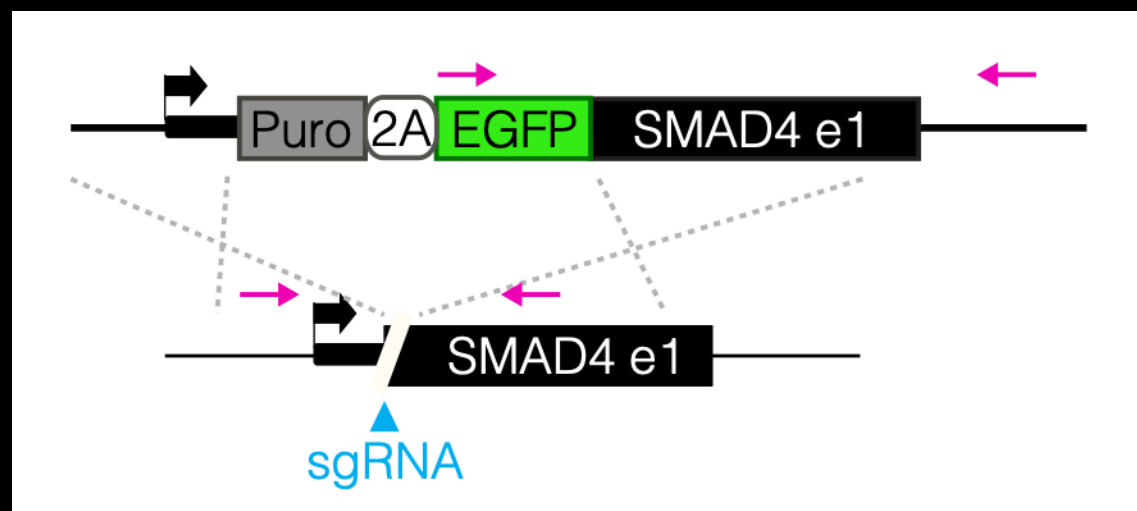
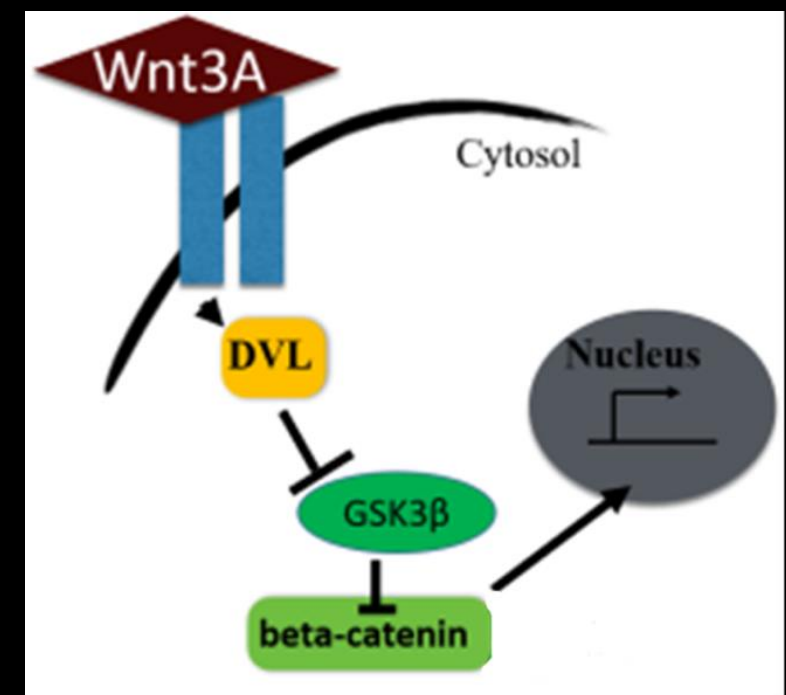
BMP4+SB

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

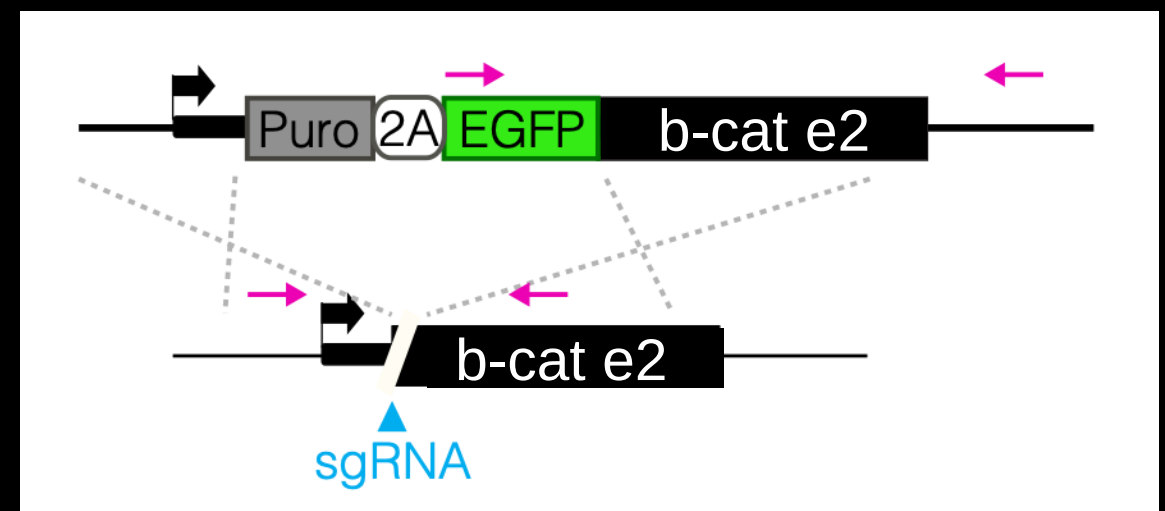
TGFb superfamily (BMP/NODAL)



Wnt pathway



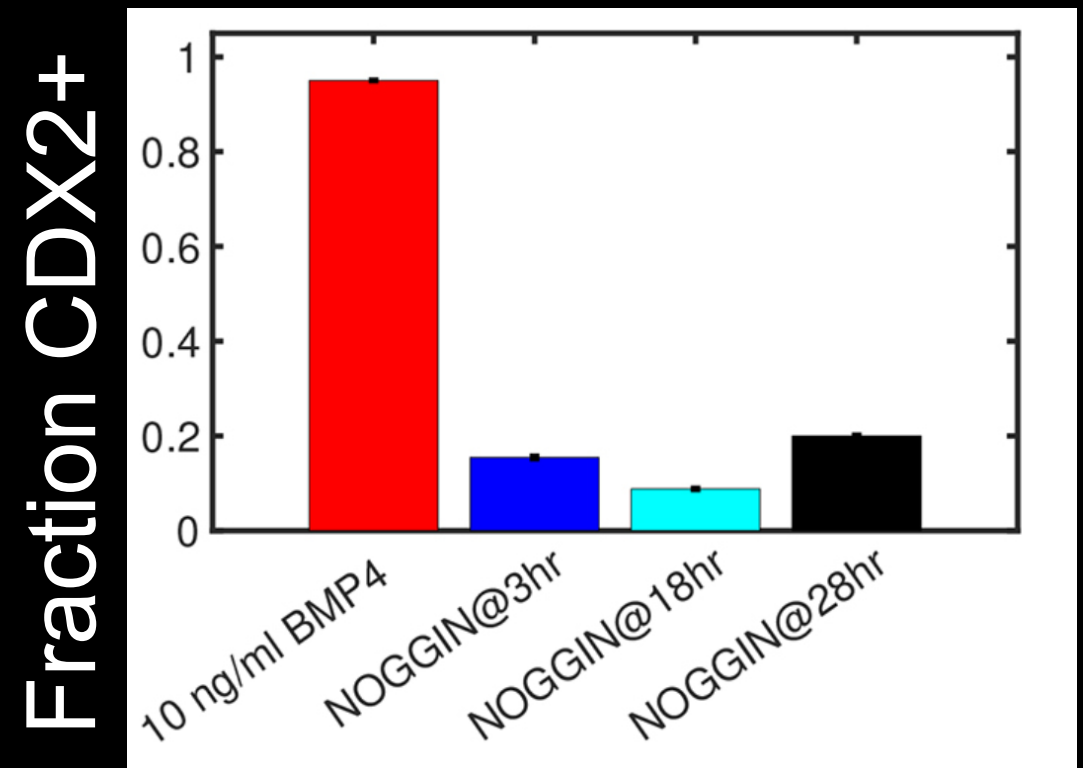
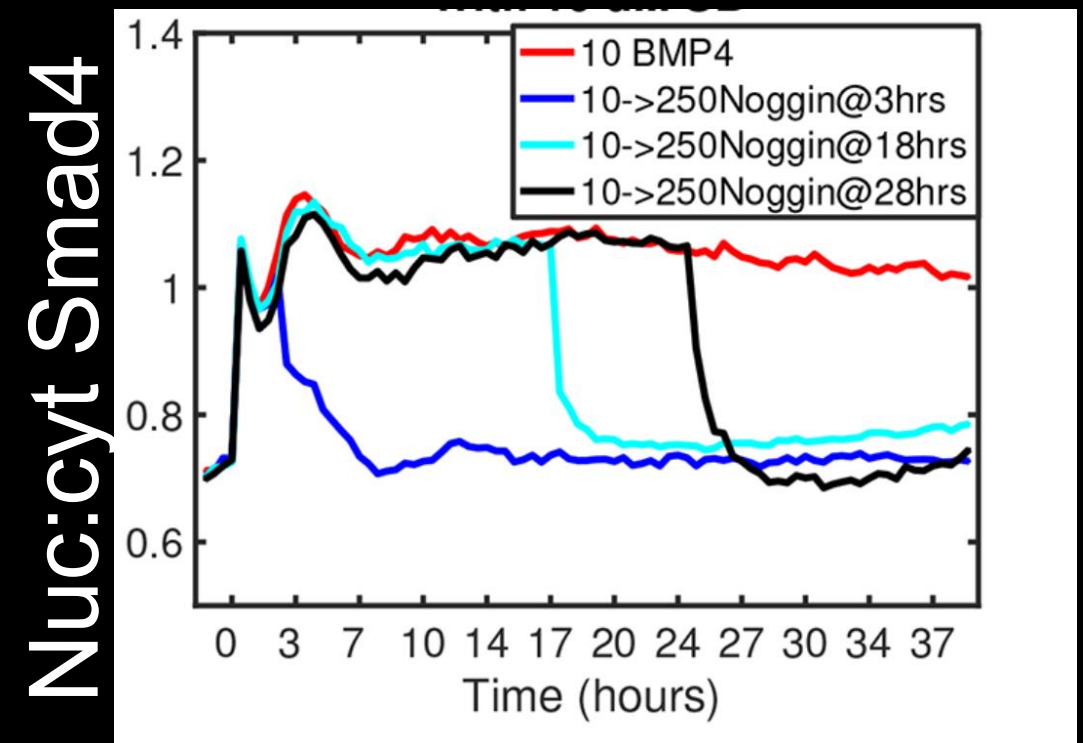
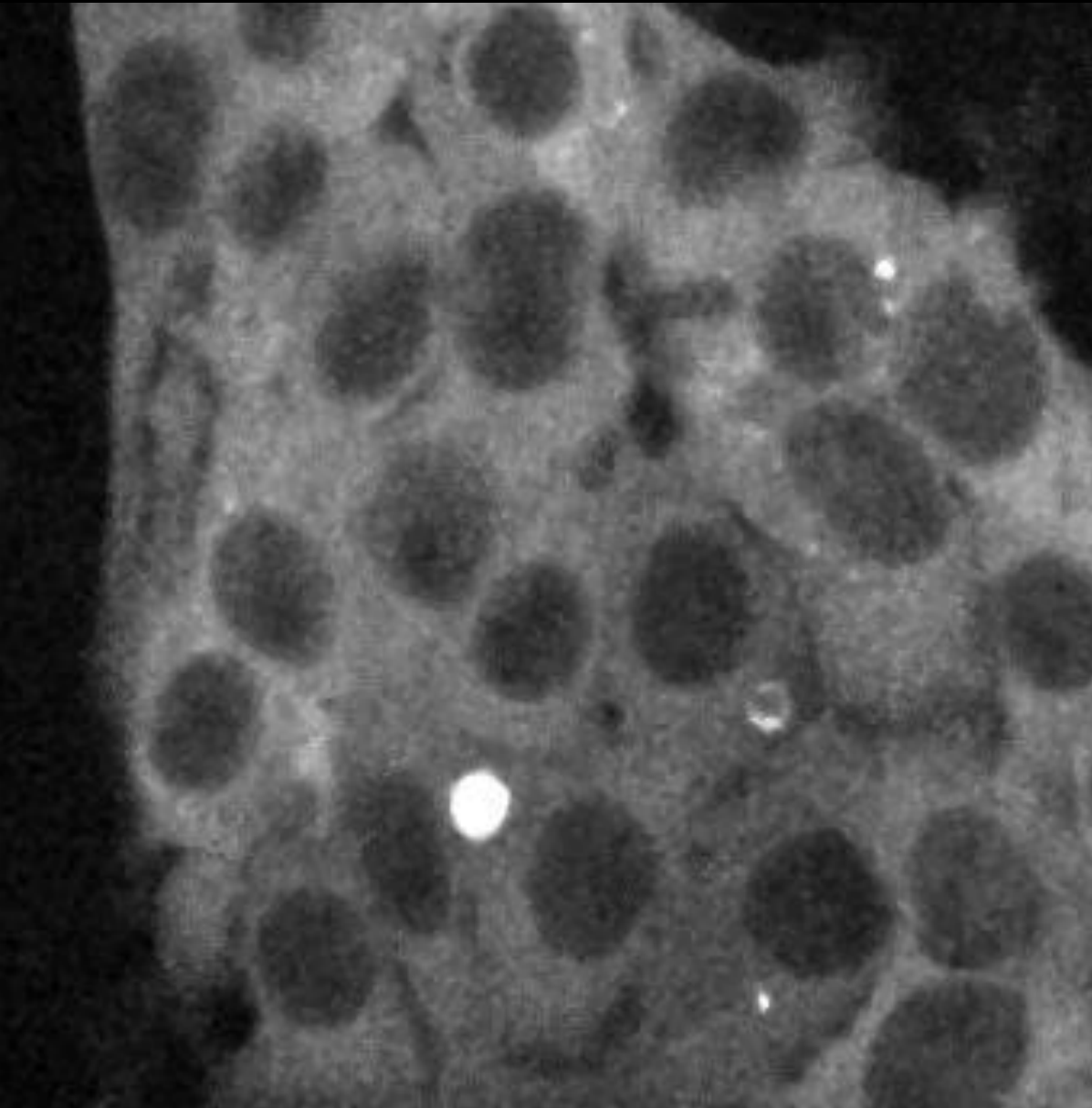
Nemashkalo et al Development 2017
Heemskerk et al. eLife 2019



Massey et al. PNAS 2019

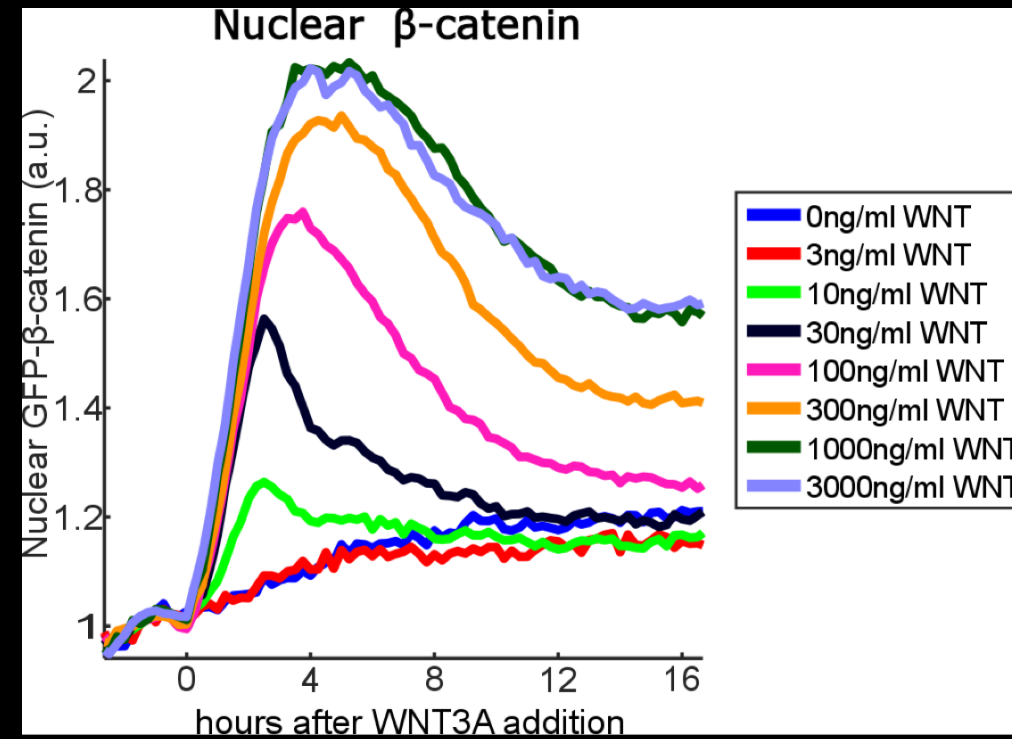
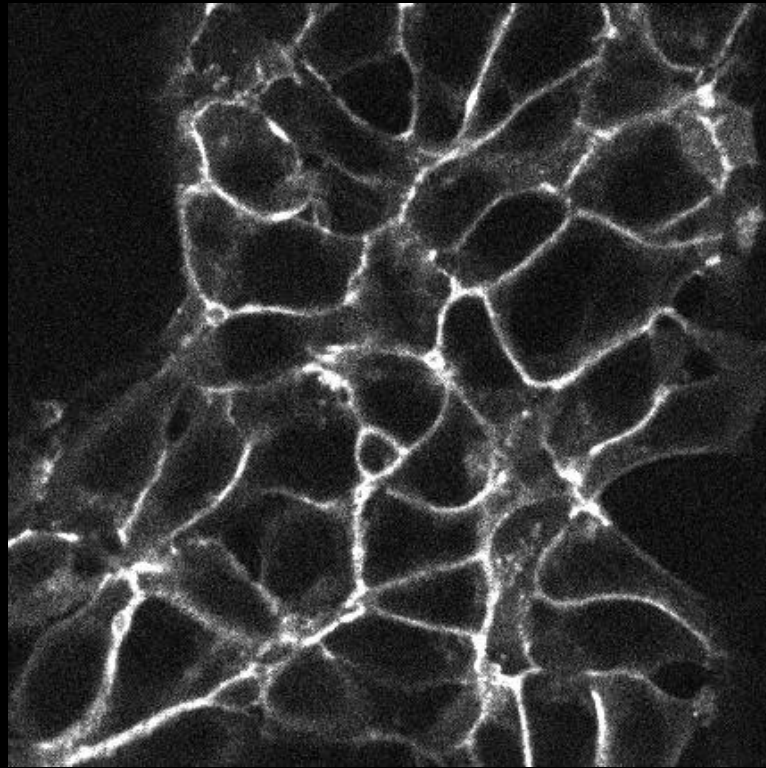
BMP signaling is sustained and duration of signaling is required for differentiation to CDX2+ fates

GFP-SMAD4: BMP response



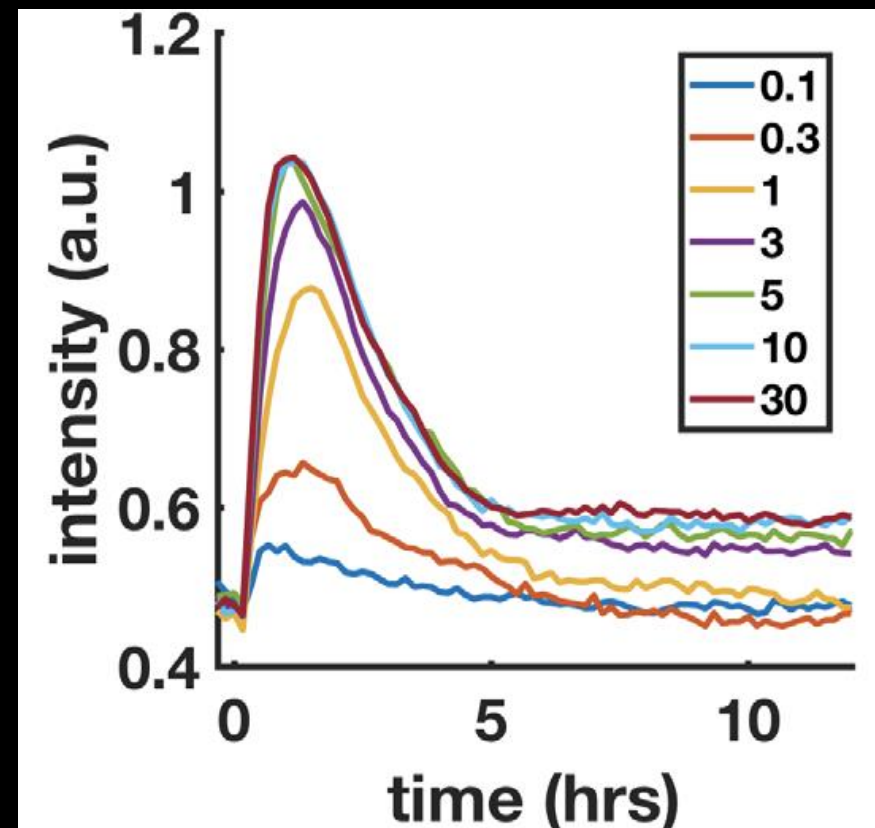
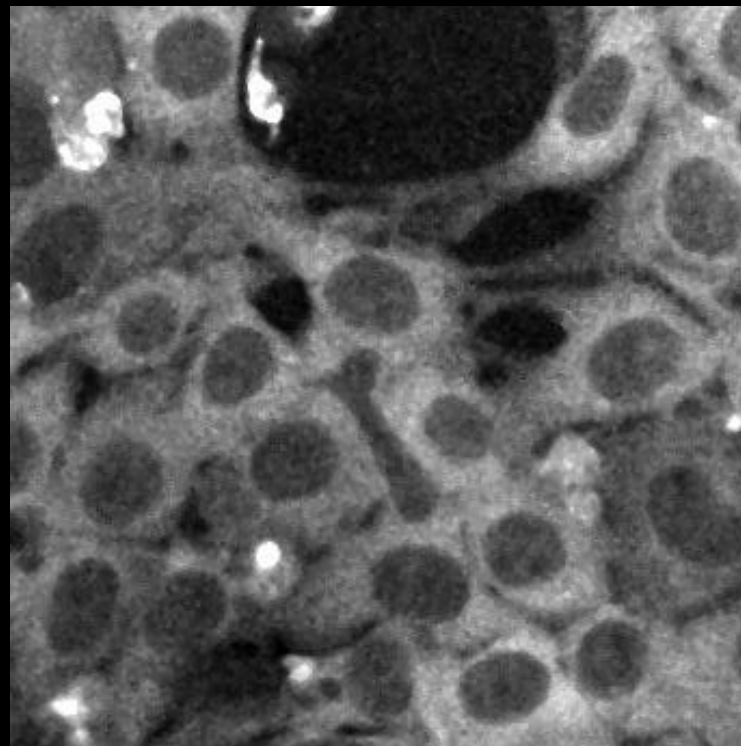
Wnt and Nodal signaling both respond adaptively to ligand simulation

Wnt response
GFP- β -catenin



Massey et al. PNAS (2019)

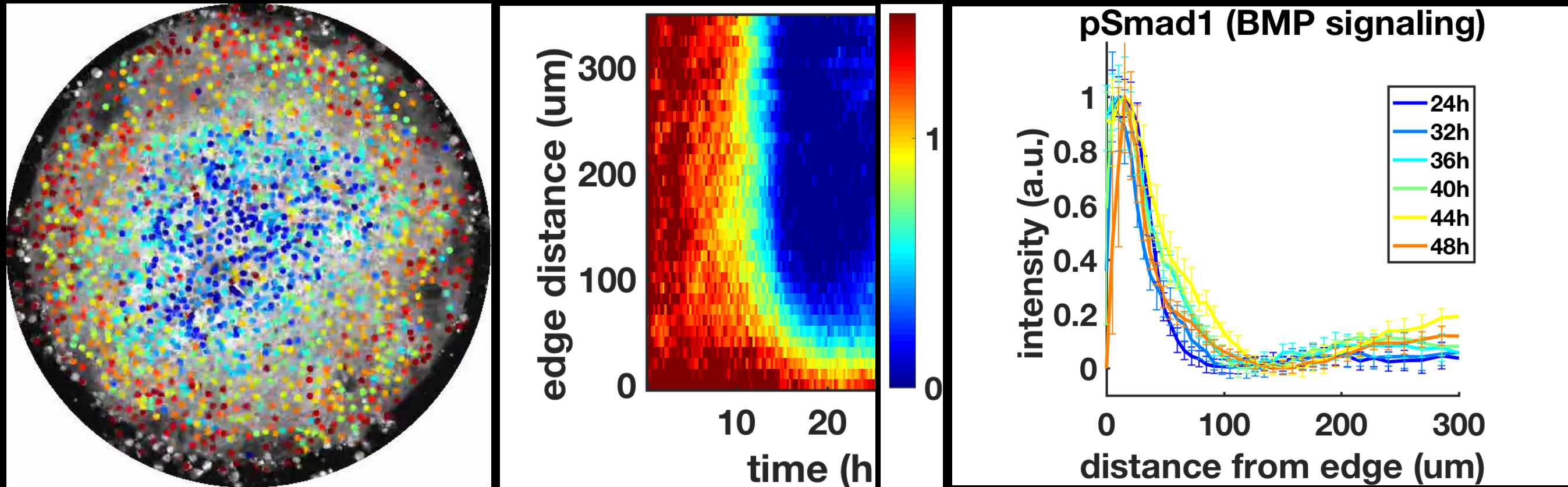
Activin/Nodal response
GFP-SMAD4



Heemskerk et al eLife (2019)

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

Smad4 signaling in BMP4 treated colony:

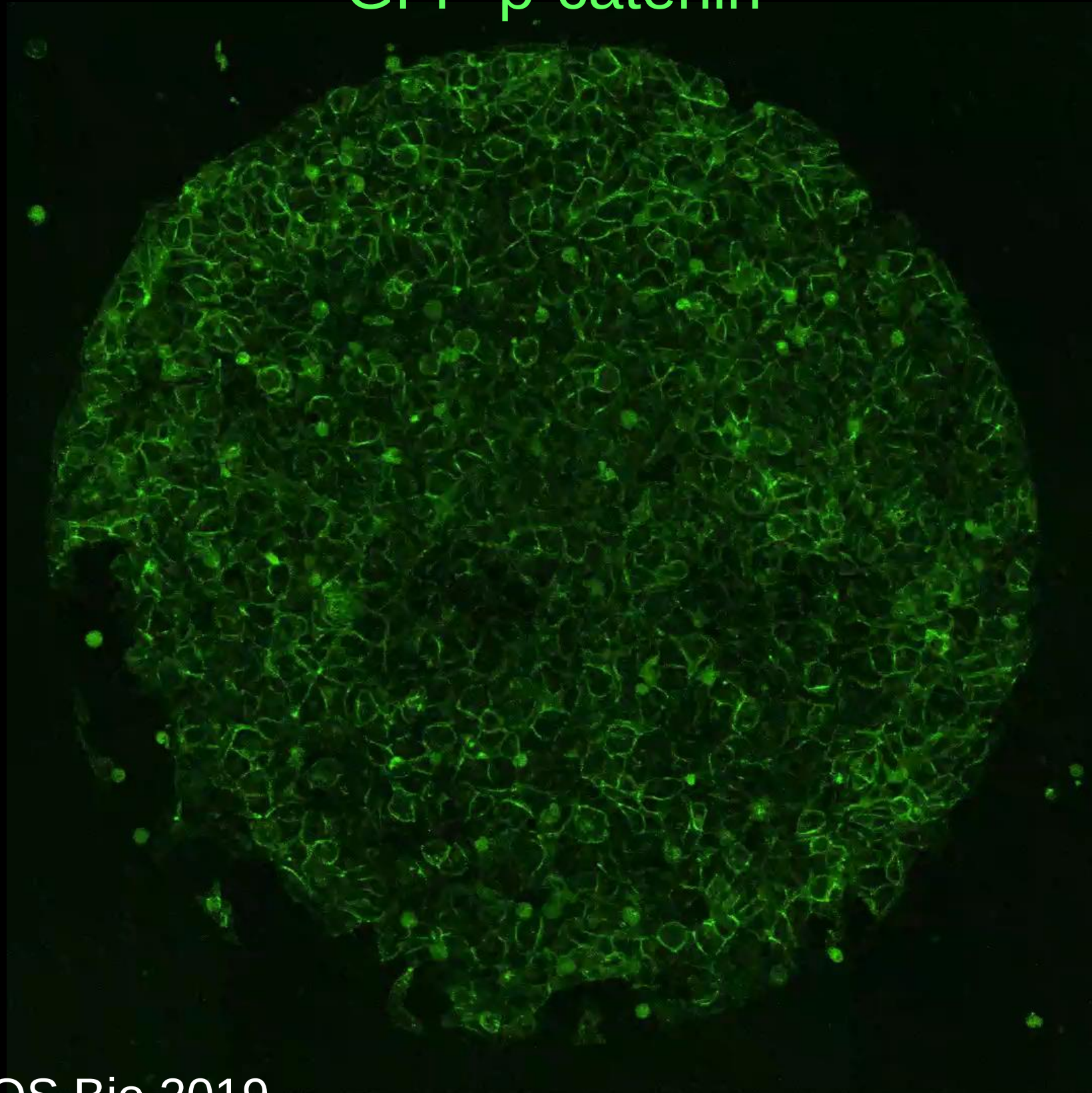


Heemskerk et al eLife (2019)

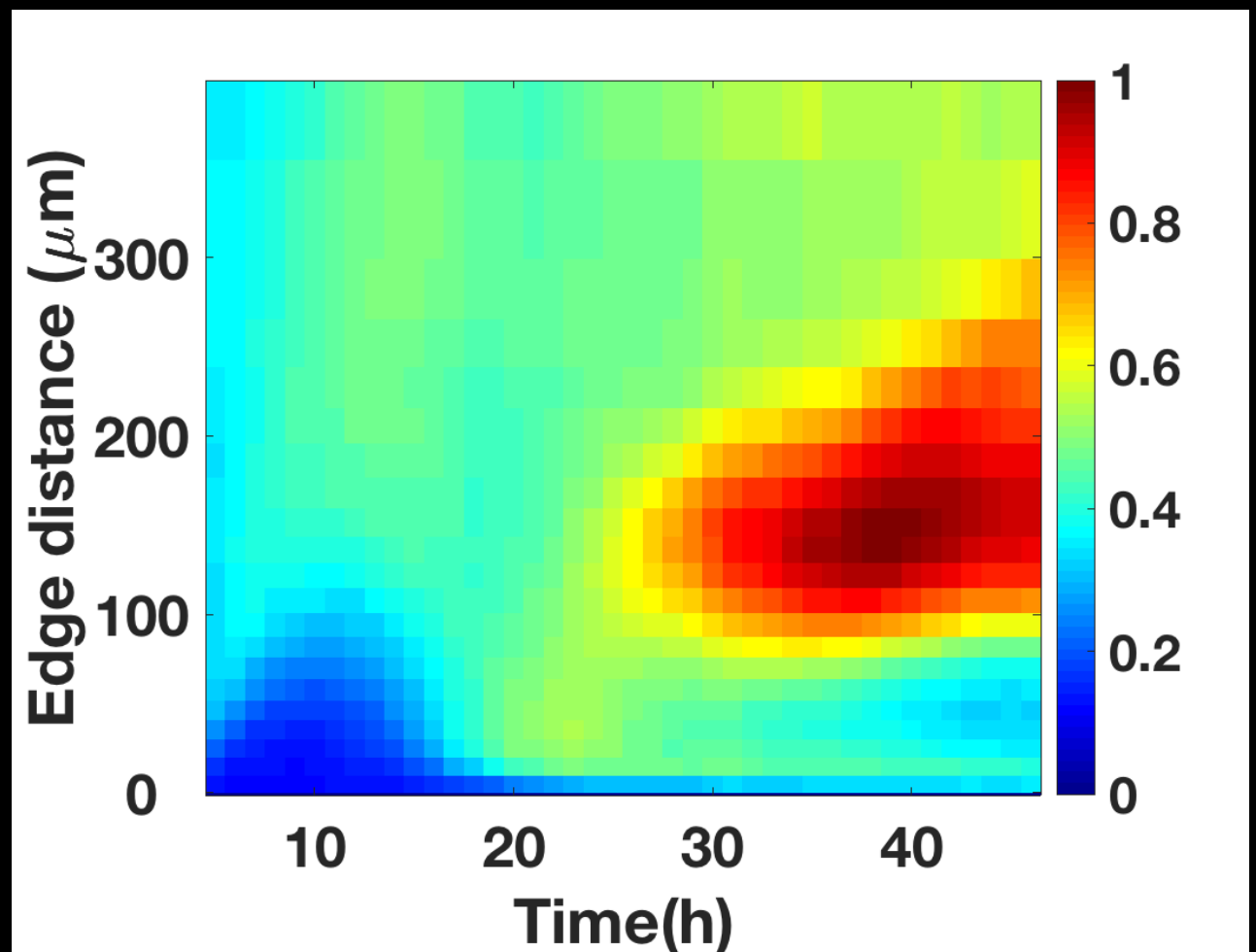
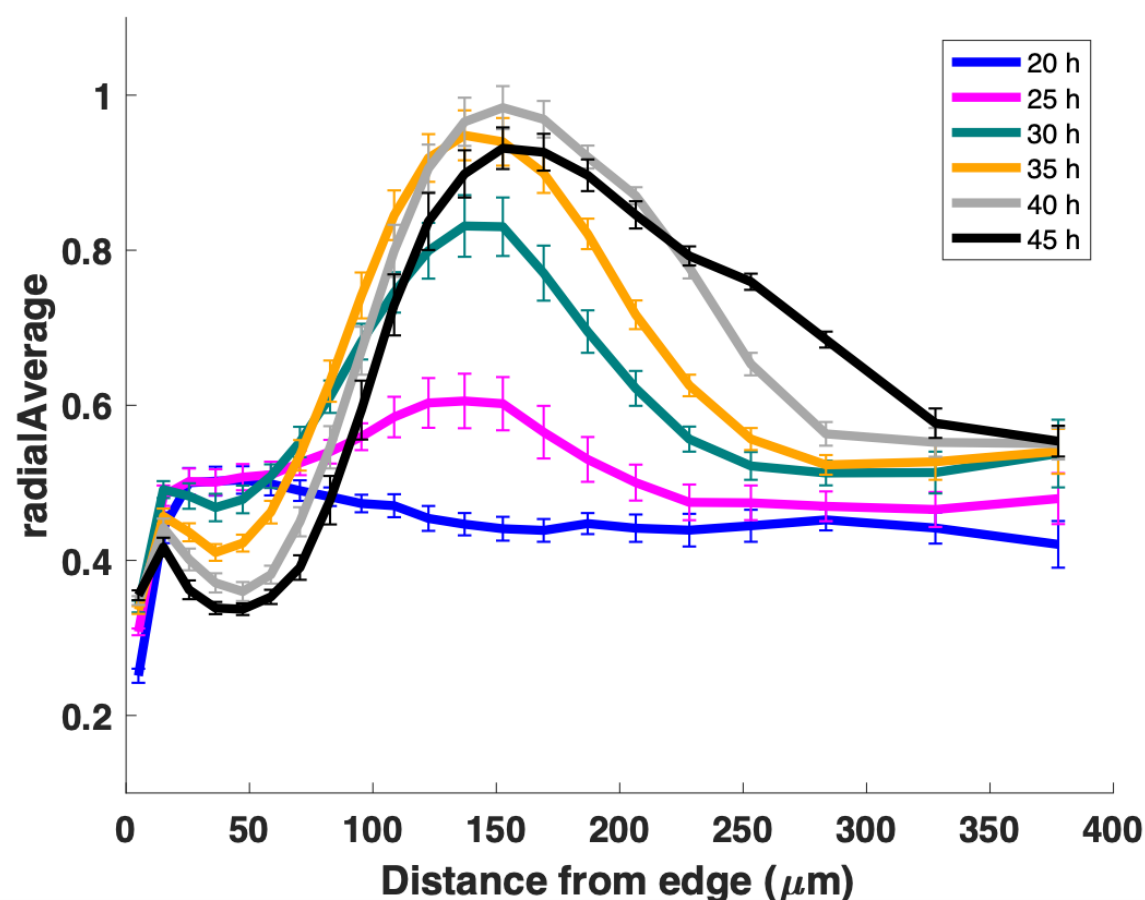
Consistent with the need for sustained BMP signaling in generating the CDX2⁺ border fates

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

GFP- β -catenin

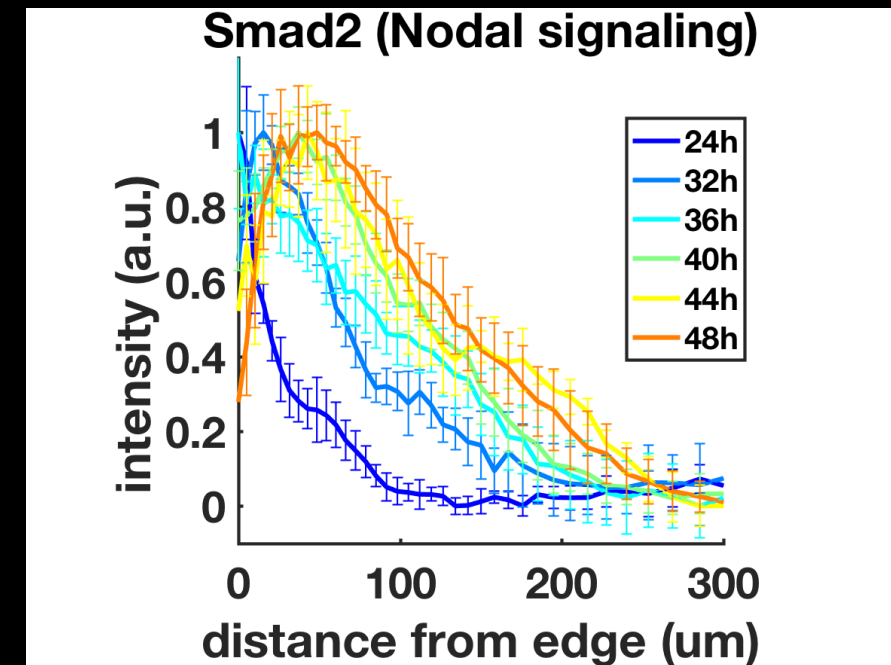
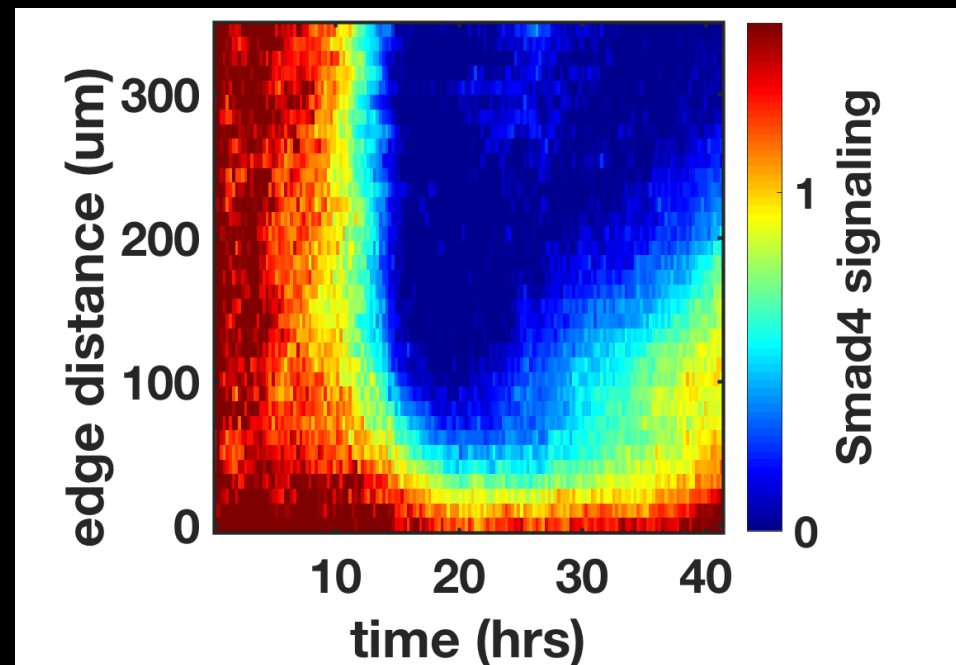
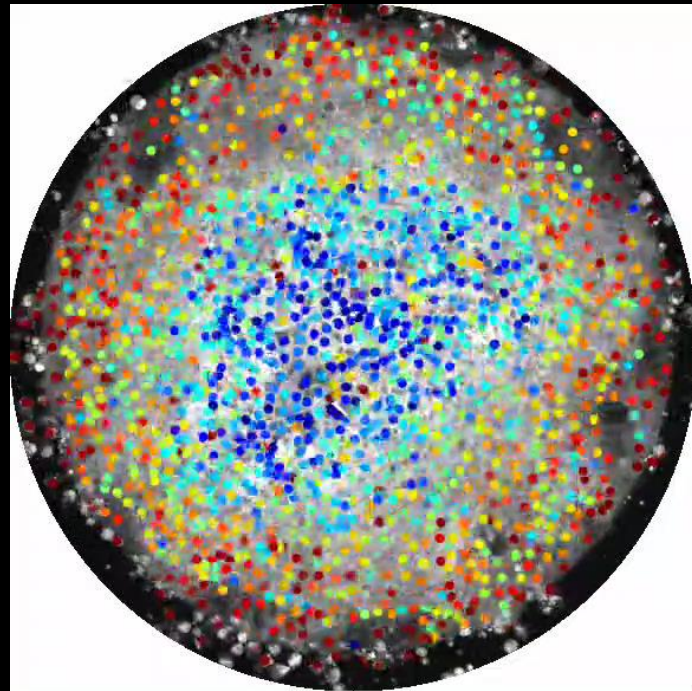


WNT is initially activated close to the edge and spreads inward at a constant rate

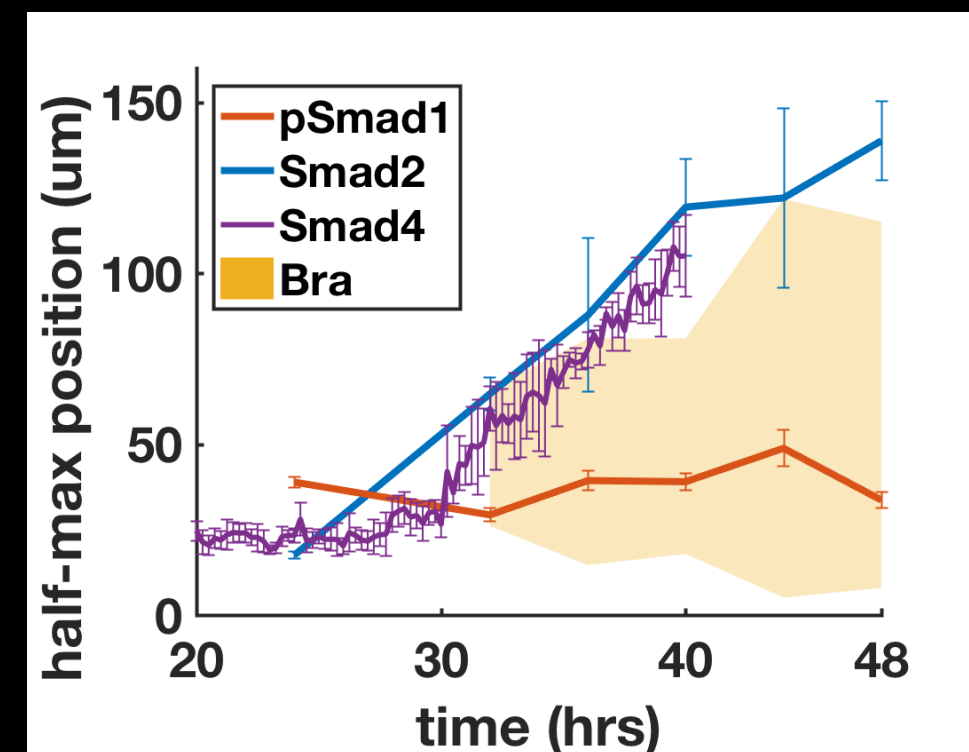
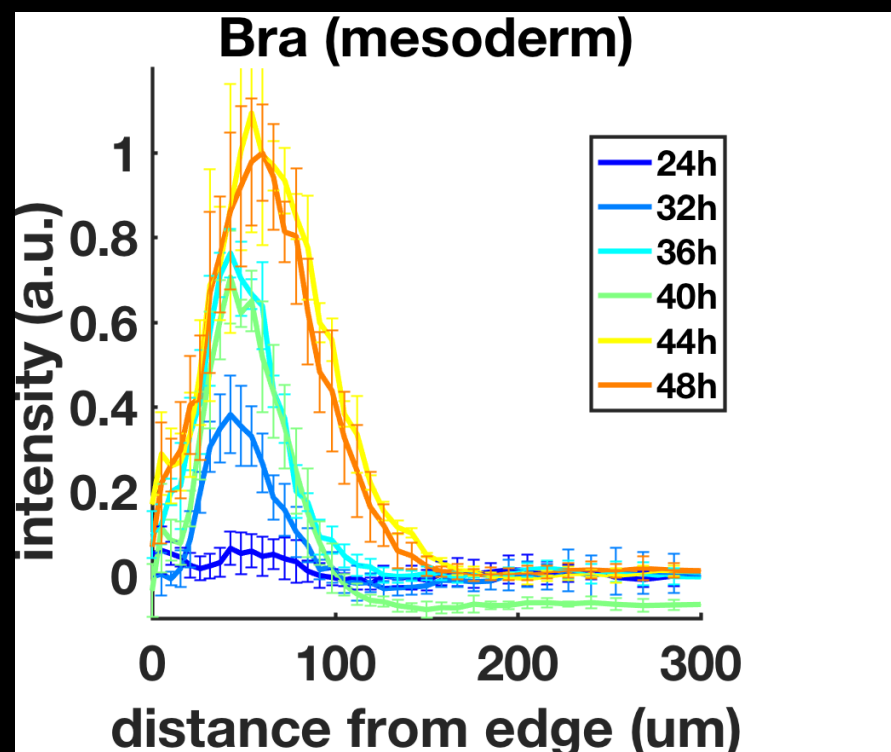


Note: WNT signaling reach well beyond the region of mesoderm differentiation

A wave of Nodal signaling follows the wave of Wnt signaling

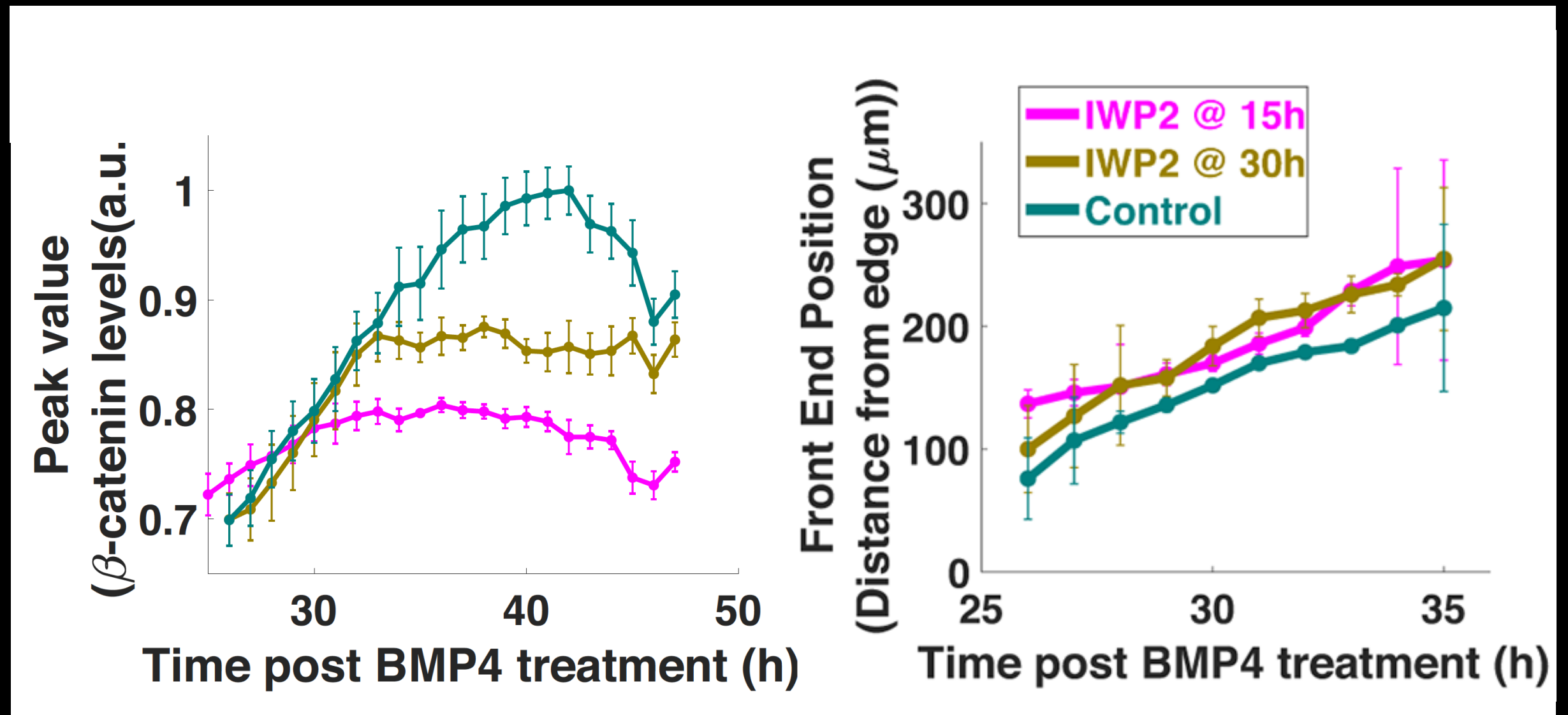


...And induces mesodermal differentiation in its wake



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

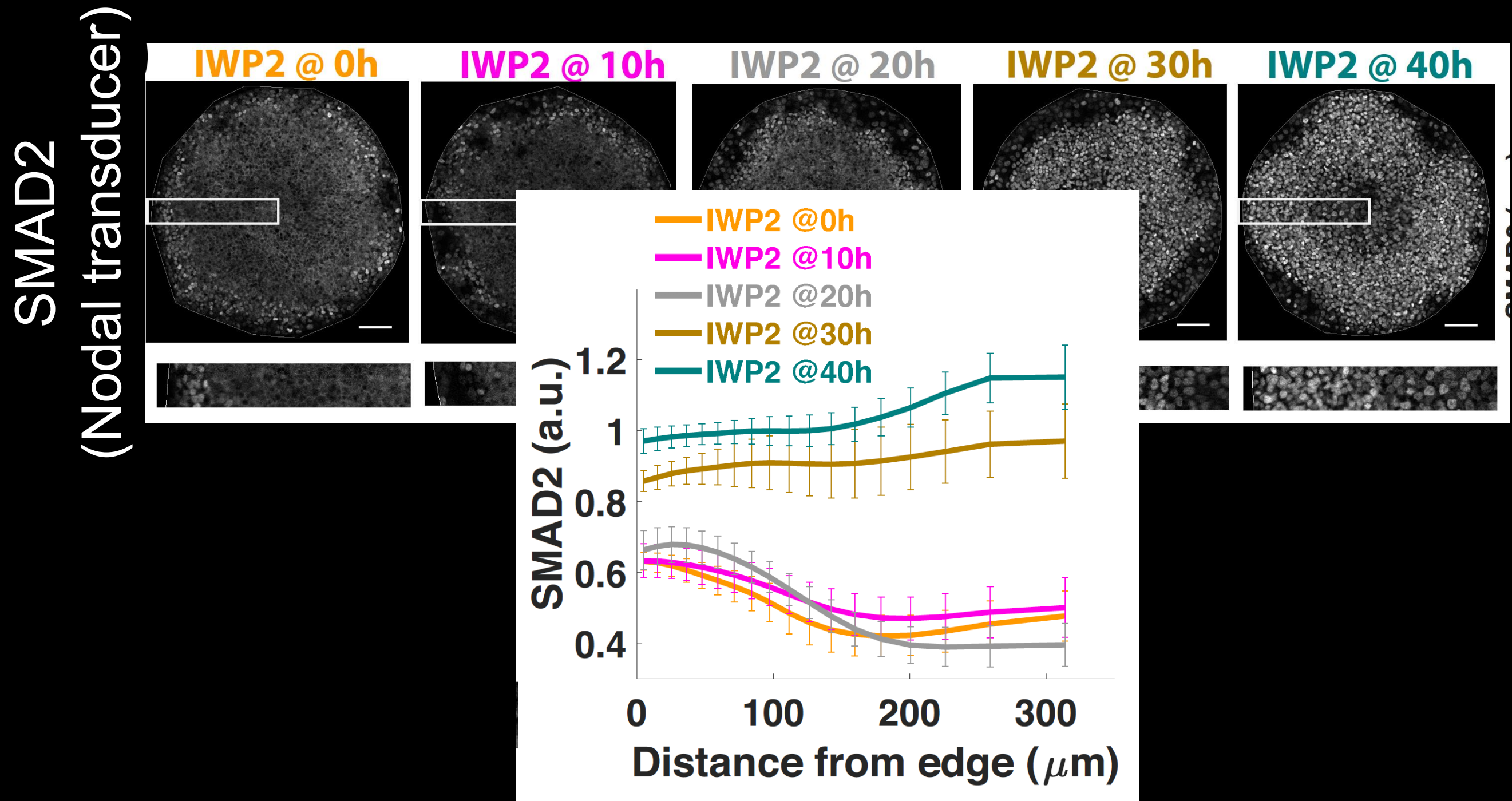
IWP2 = small molecule inhibitor of WNT secretion



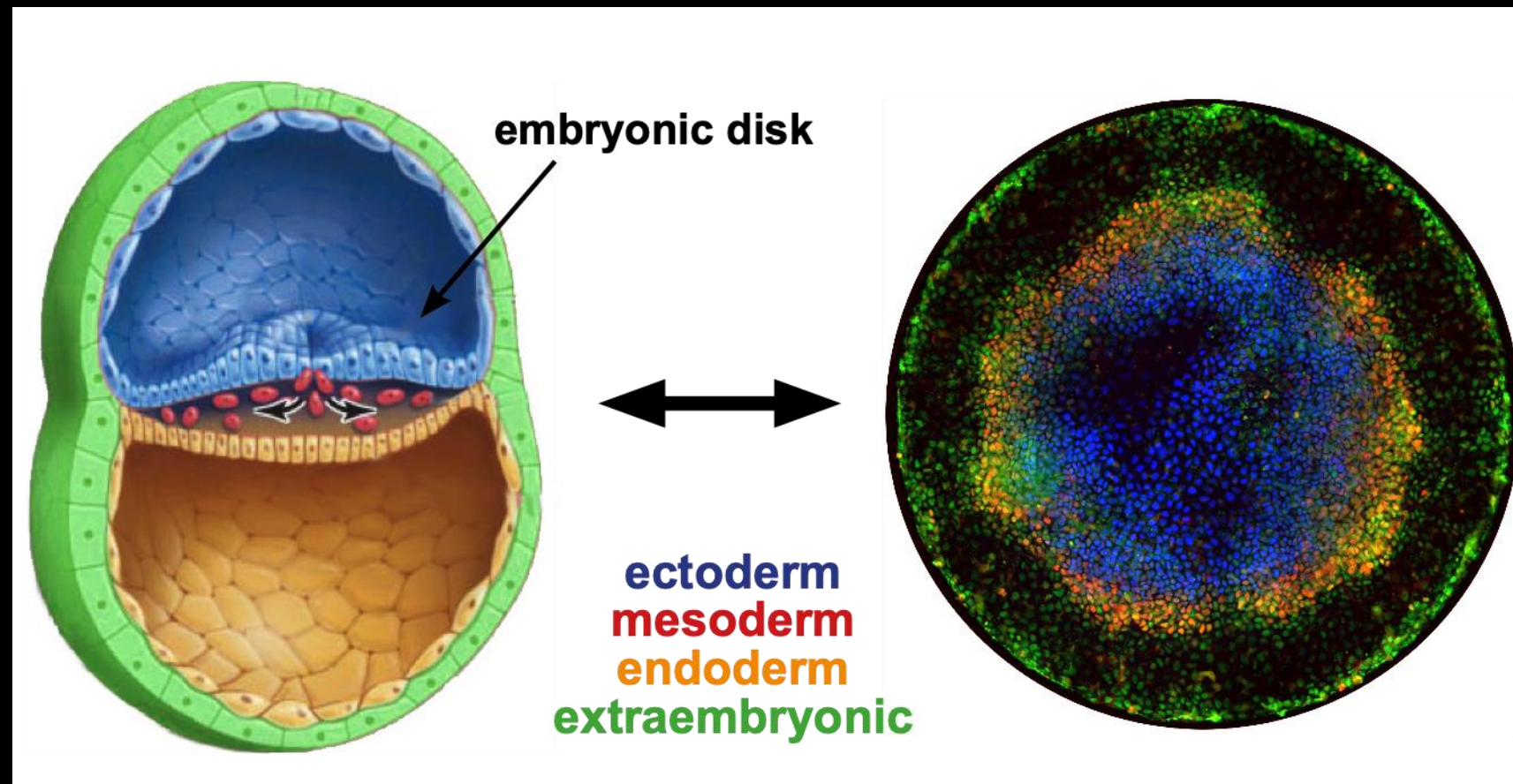
Suggests long range diffusion of WNT ligands

WNT initiated Nodal rapidly becomes independent of upstream pathways

IWP2 = small molecule inhibitor of WNT secretion



Differences between micropatterned cultures and the embryo



1. Germ “layers” are adjacent in the same 2D-plane, rather than layered in 3D
2. Failure to break symmetry: primitive streak forms as a ring
3. Some extra-embryonic lineages are missing. Trophectoderm-like cells are present, but no visceral endoderm cells.
4. Glass surface constrains movements of cells resulting in loss of order as cells delaminate

Would this system develop further if implanted? NO!

Perspective: where should synthetic embryology go?

-Is our goal to build embryos from stem cells? NO!

1. Given our current state of knowledge and technical capability, we need simplified systems where we can quantitatively manipulate, measure, and model.
2. We still have a lot to learn from 2D simplified systems
3. To learn about later stages or other aspects of development, new systems can be created that focus on relevant aspects without modeling the entire embryo.

Patterns can be made at other developmental stages with more complex protocols



DAPI
(nuclear)

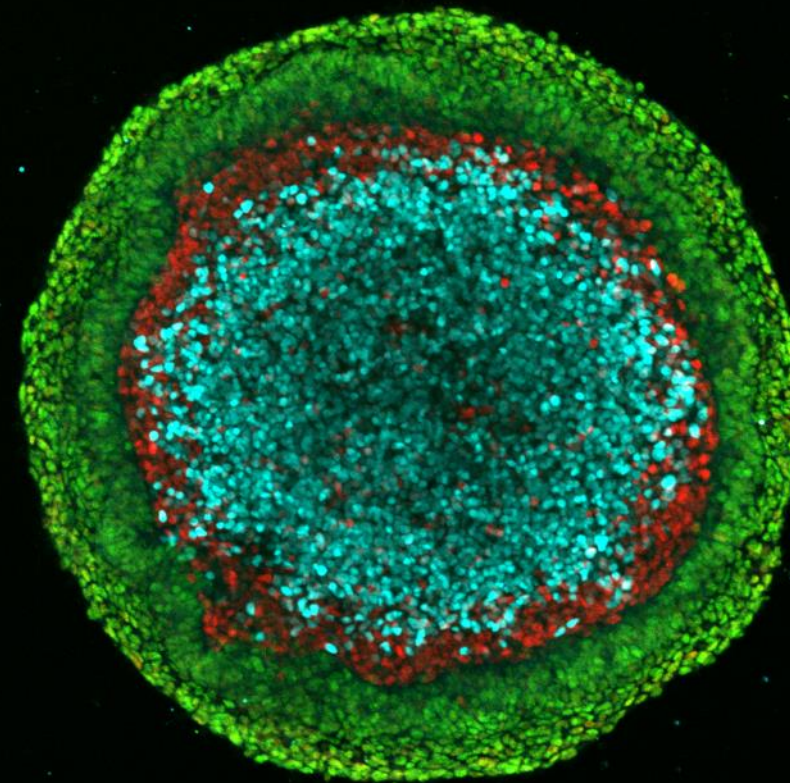
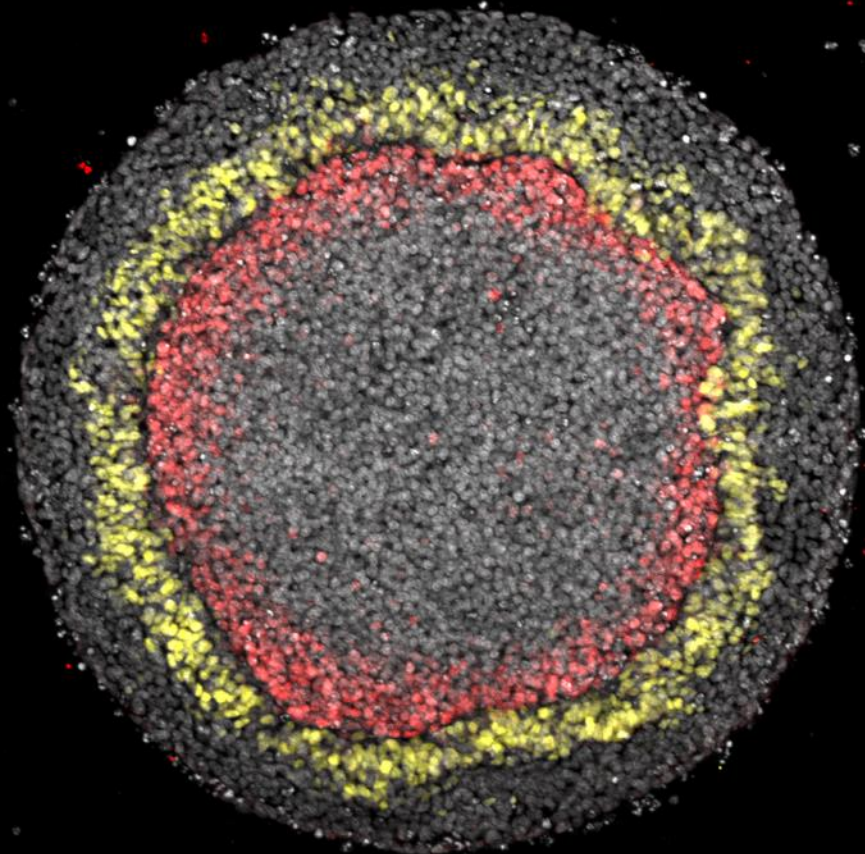
SIX1
(placode)

PAX3
(neural crest)

PAX6
(neural)

ISL1
(epidermis)

SOX9
(neural crest)



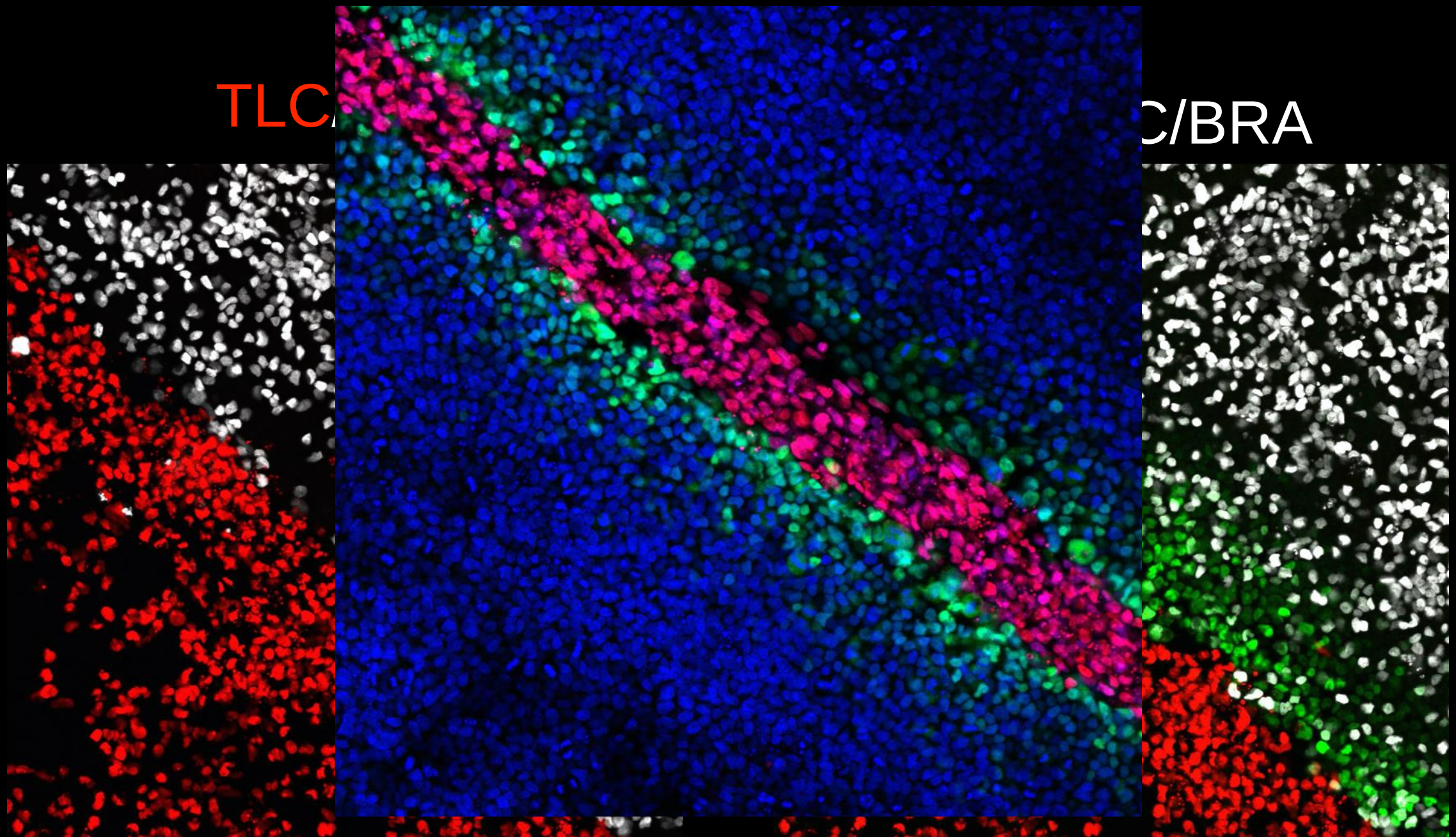
Britton et al Development 2019

Epidermal/Placode/Neural Crest/Neural



Studying interactions between embryonic and extraembryonic cells

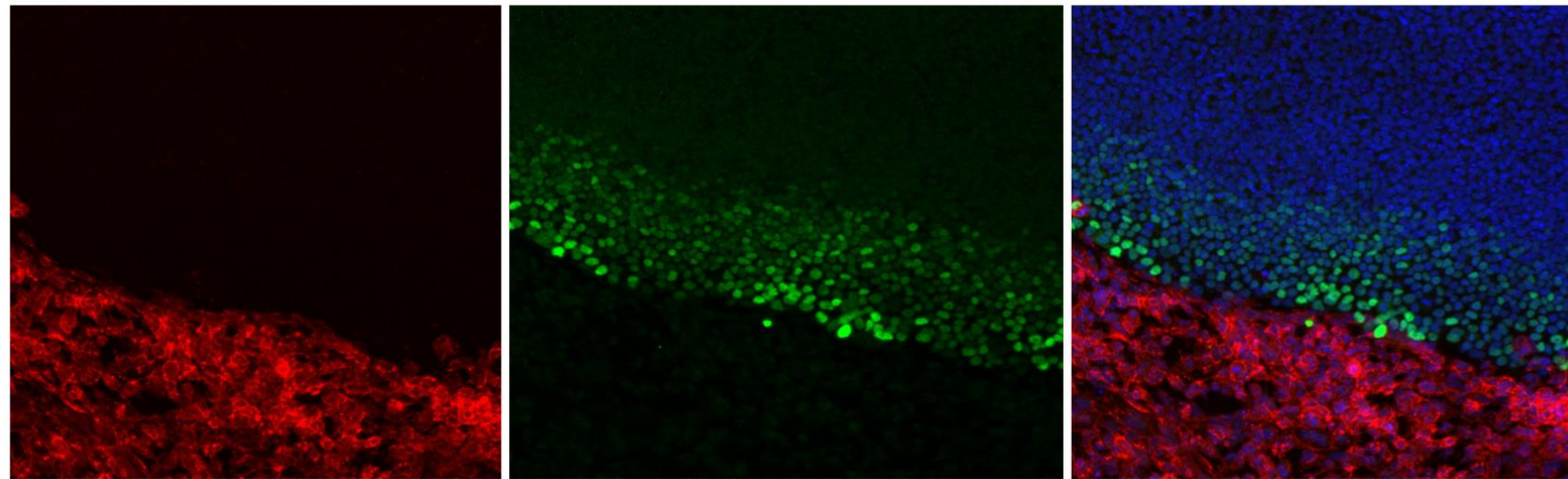
ulation with BMP4 mimics interaction with trophectoderm. Can we replace with trop



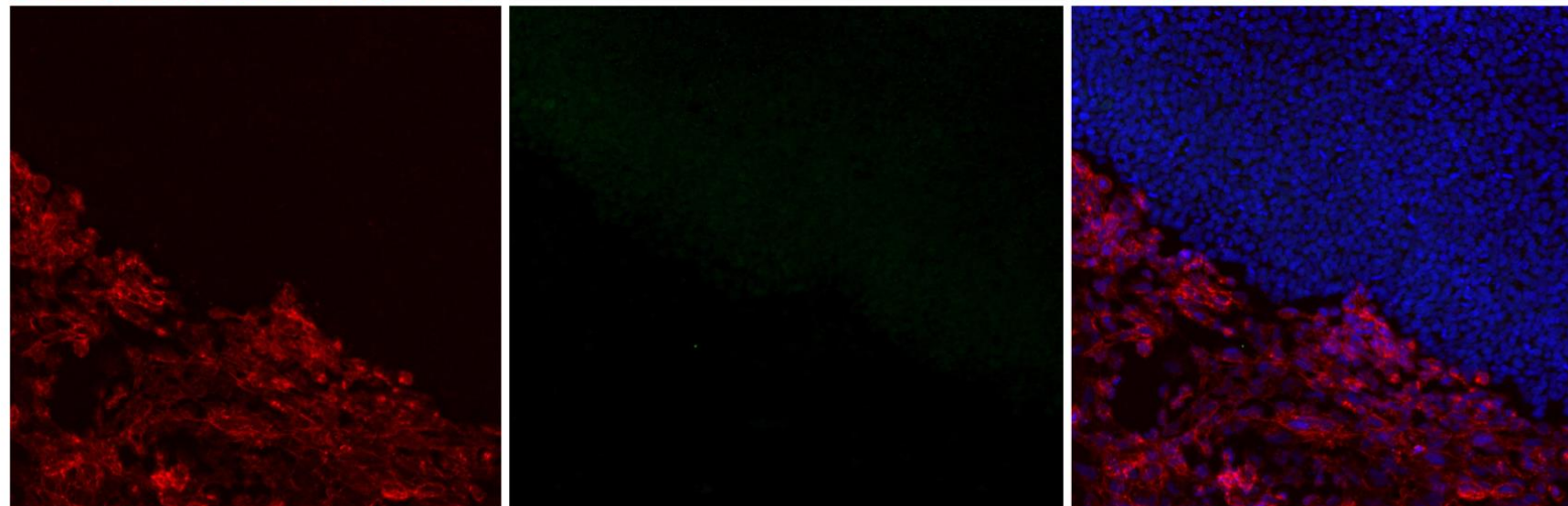
Induction of BRA by cell juxtaposition depends on endogenous BMP and WNT

TLC/BRA/DAPI

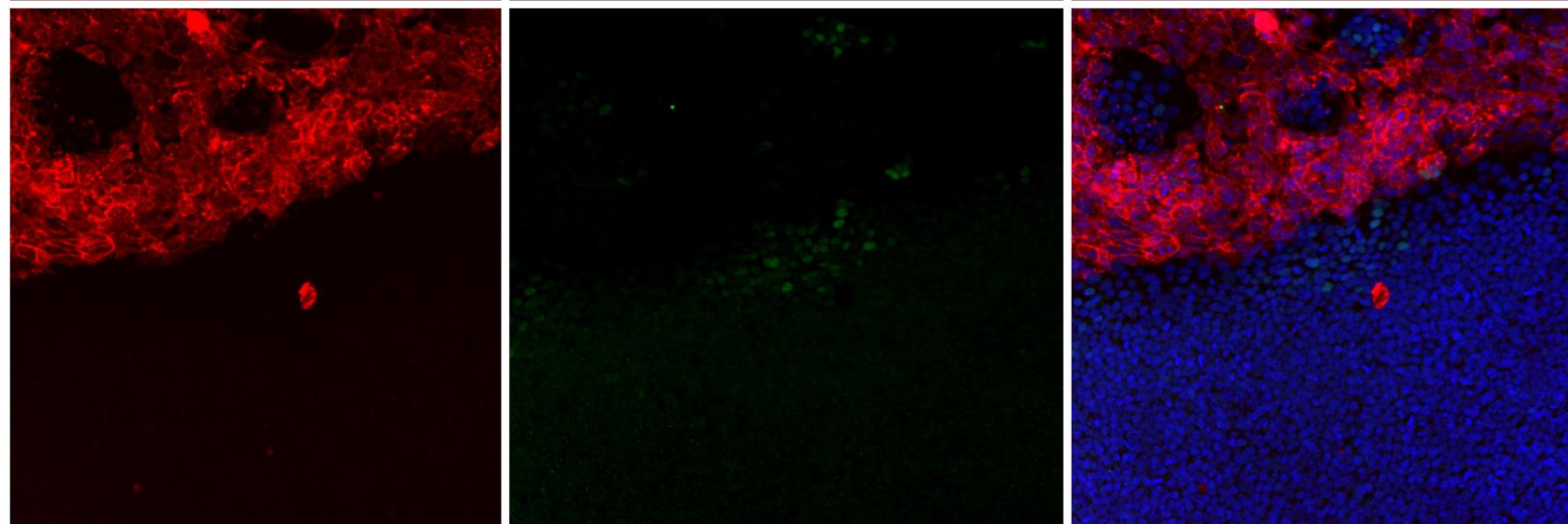
Control



+IWP2
(WNT
Inhibitor)



+Noggin
(BMP
Inhibitor)



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Warmflash lab

Current

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