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Examining the state of the Science of Mammalian Embryo Model System

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Models of human embryos and embryonic tissues

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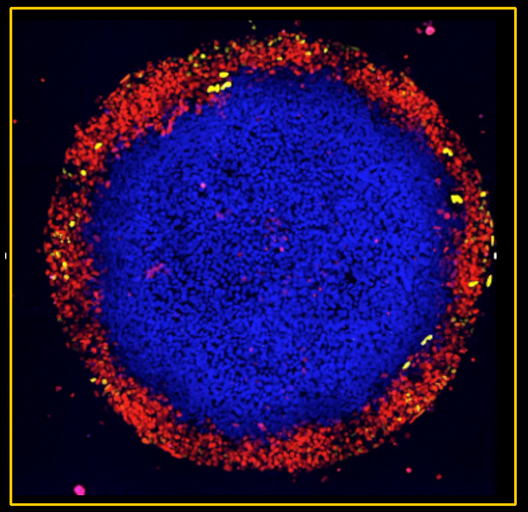
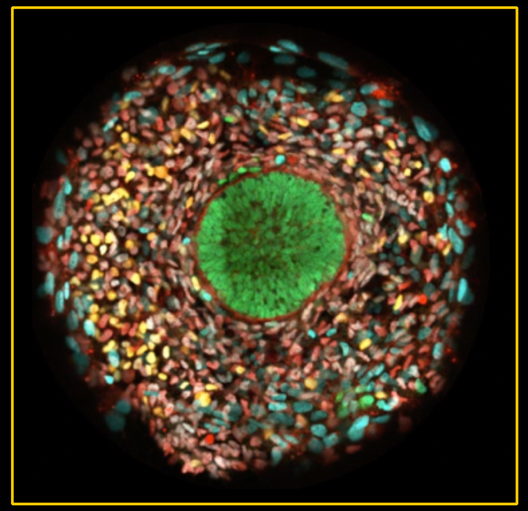
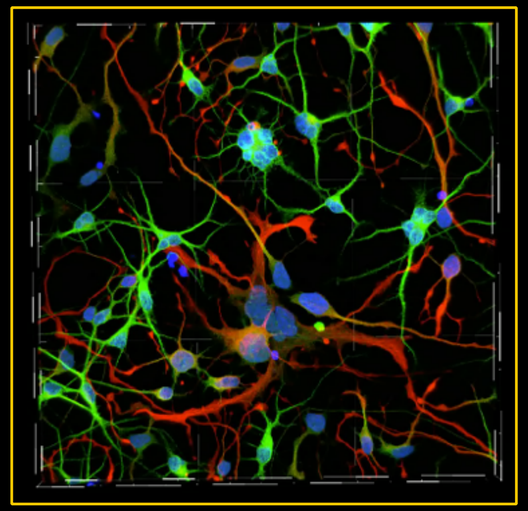
Gastruloids



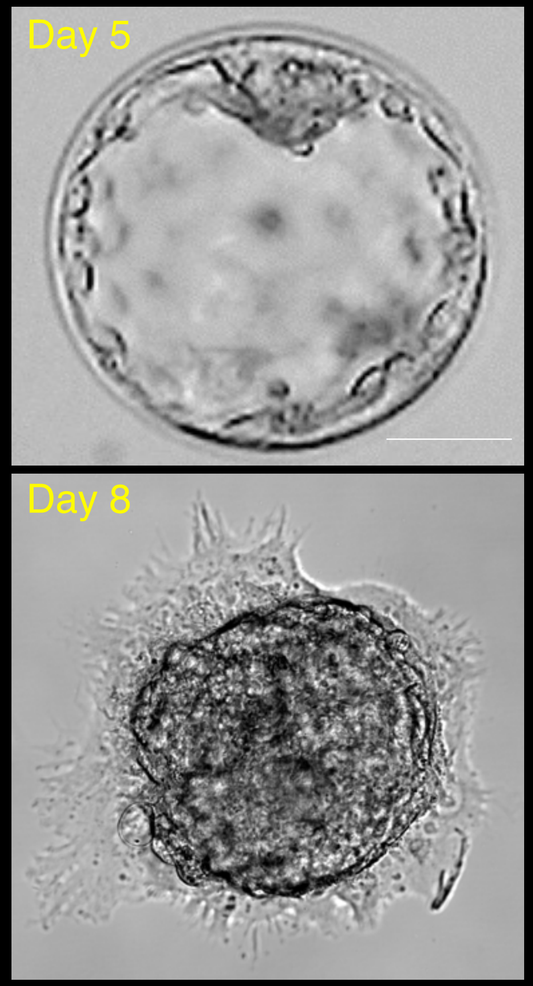
Neuruloids



Disease modeling



in vitro attachment and self-organization in real (natural) embryos



- Discovery of a novel human cell type: Yolk sac trophectoderm (ysTE): $OCT4^{LO}$ $GATA6^{LO}$ $GATA3^{+}CDX2^{+}$

  (Confirmed by sc-RNASeq: Zhou et al., 2019)

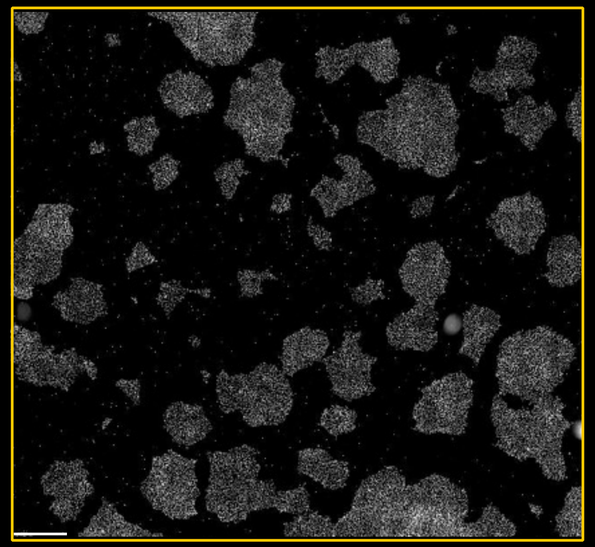
(Deglincerti; Croft, Pietila, Zernicka-Goetz, Siggia, Brivanlou, Nature 2016)
(Fang et al, Nature 2019)

Human embryo models: "*gastruloids*" derived from *human embryonic stem cells*

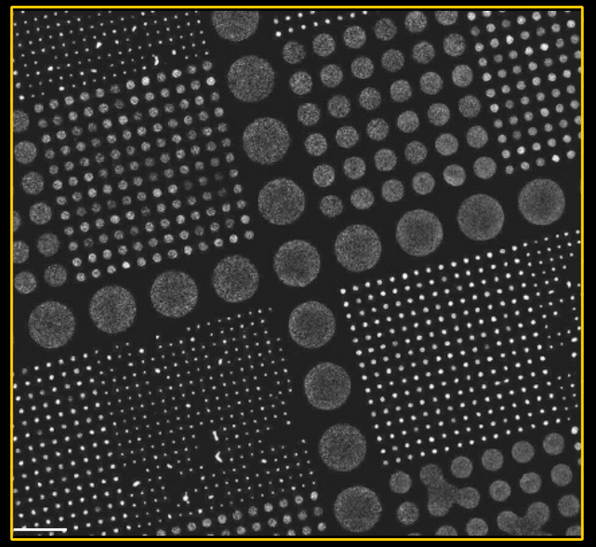
Rationale for human embryo models

- Understanding of our own origins is limited by availability of biological material
- Specie-specific differences that cannot be studied in model systems
- Model human embryos allow clinical breakthroughs without the need of using natural human embryos

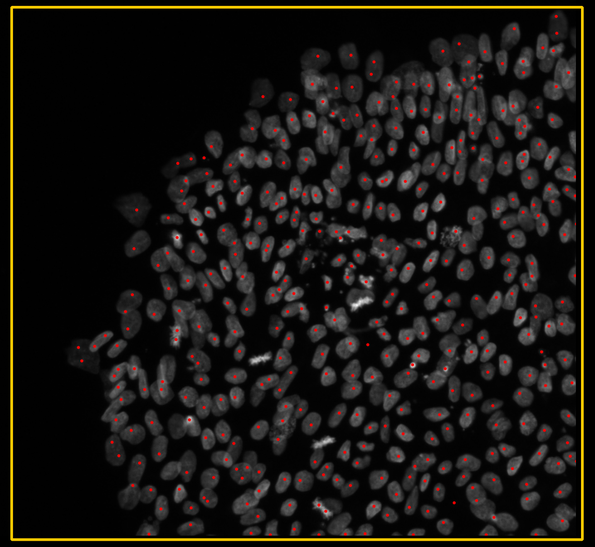
Random geometries



Imposed architecture



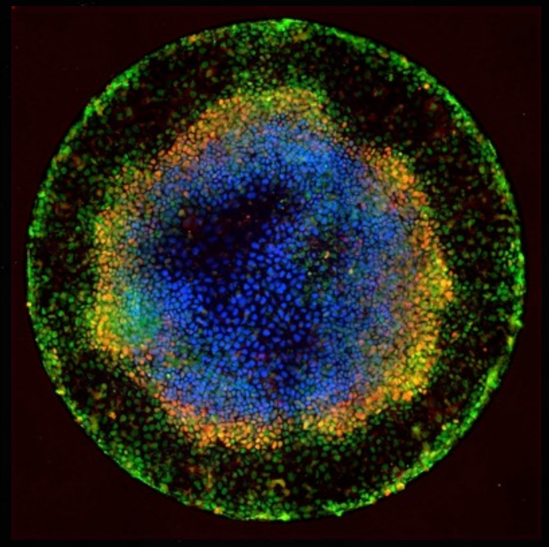
Single cell quantification



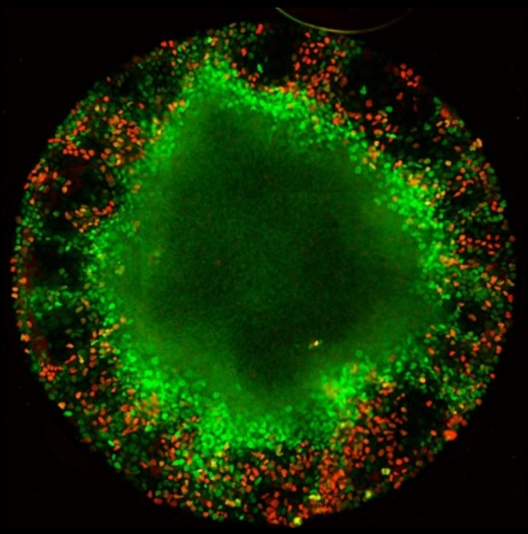
RUES2

(Warmflash, Sorre, Etoc, Siggia, and Brivanlou, Nature Methods 2014)

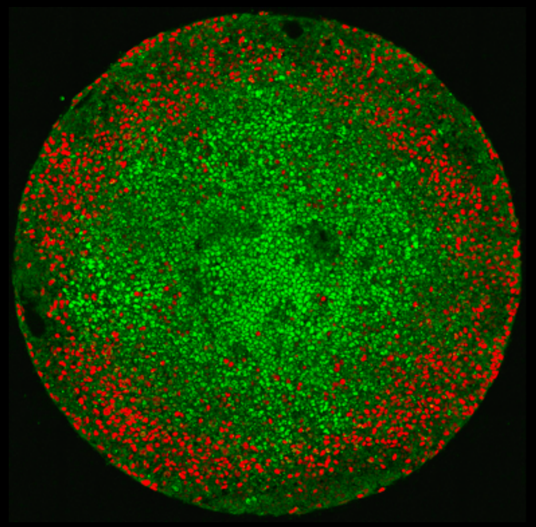
Human embryo models: "*gastruloids*" derived from *human embryonic stem cells*



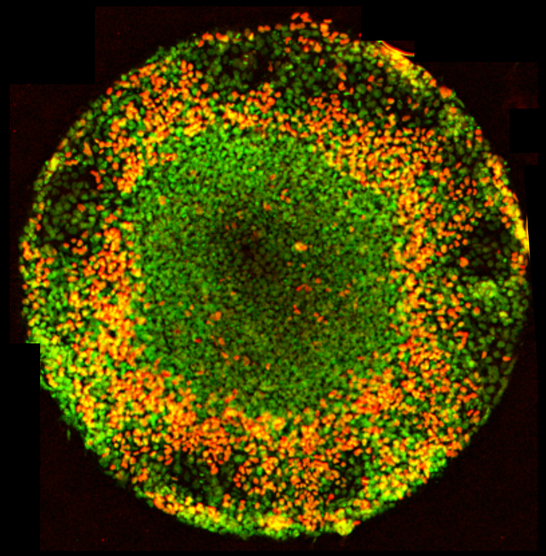
SOX2 / BRA / CDX2
Ectoderm / Mesoderm / Trophectoderm



EOMES / SOX17
Endoderm



SOX2 / SNAIL
Primitive streak



DAPI / BLIMP1
PGCs

- Geometrical confinement is sufficient to induce self-organization of gastruloids in response to BMP4
- Eliminates inherent variabilities from natural embryos

(Warmflash, Sorre, Etoc, Siggia, and Brivanlou, Nature Methods 2014)

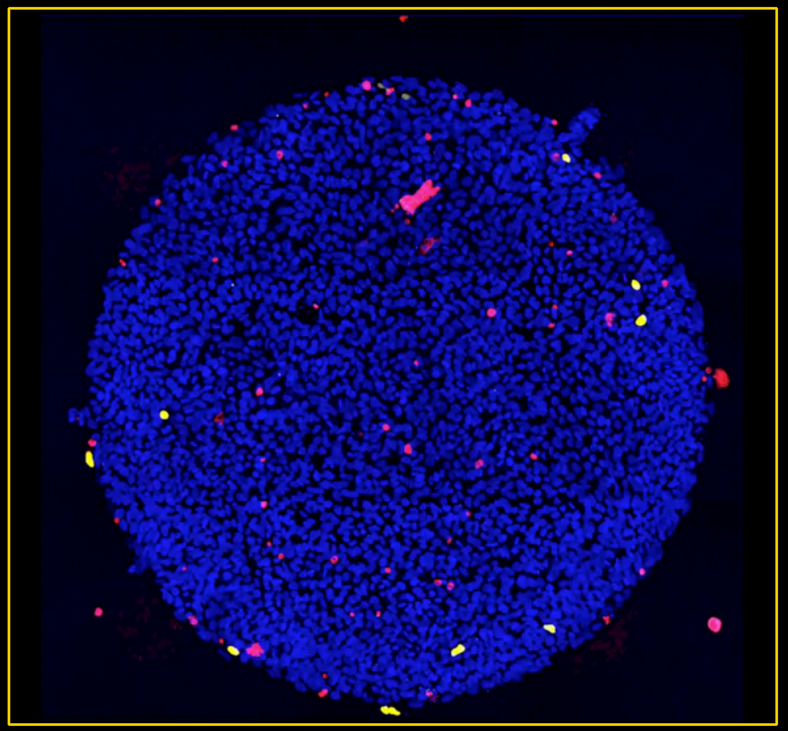
Lessons from human *gastruloids* otherwise impossible to learn from the human embryo

- Mechanism of self-organization of gastruloids: edge sensing and reaction diffusion
  (Etoc F, Metzger J, Ruzo A, Kirst C, Yoney A, Ozair MZ, Brivanlou AH, Siggia ED., Developmental Cell 2016)

- WNT signaling memory is required for ACTIVIN to function as a morphogen
  (Yoney A, Etoc F, Ruzo A, Carroll T, Metzger JJ, Martyn I, Li S, Kirst C, Siggia ED, Brivanlou AH. eLife 2018)

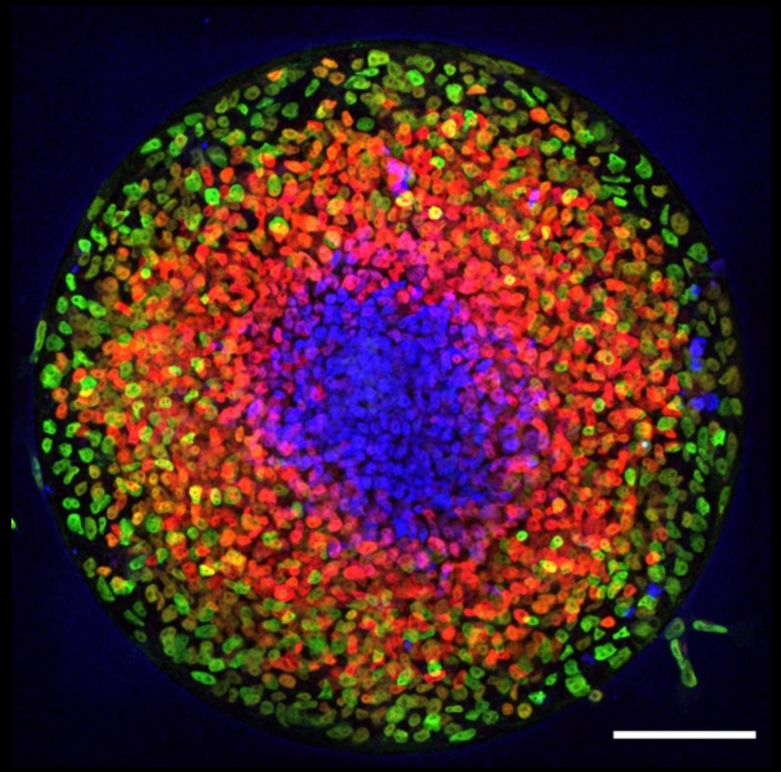
- A wave of WNT signaling controls primitive streak formation downstream of BMP4
  (Iain Martyn, Ali H. Brivanlou, and Eric D. Siggia. Development 2019)

CRISPR/Cas9 + ePB genome editing
Germ Layer Reporters:
RUES2-GLR



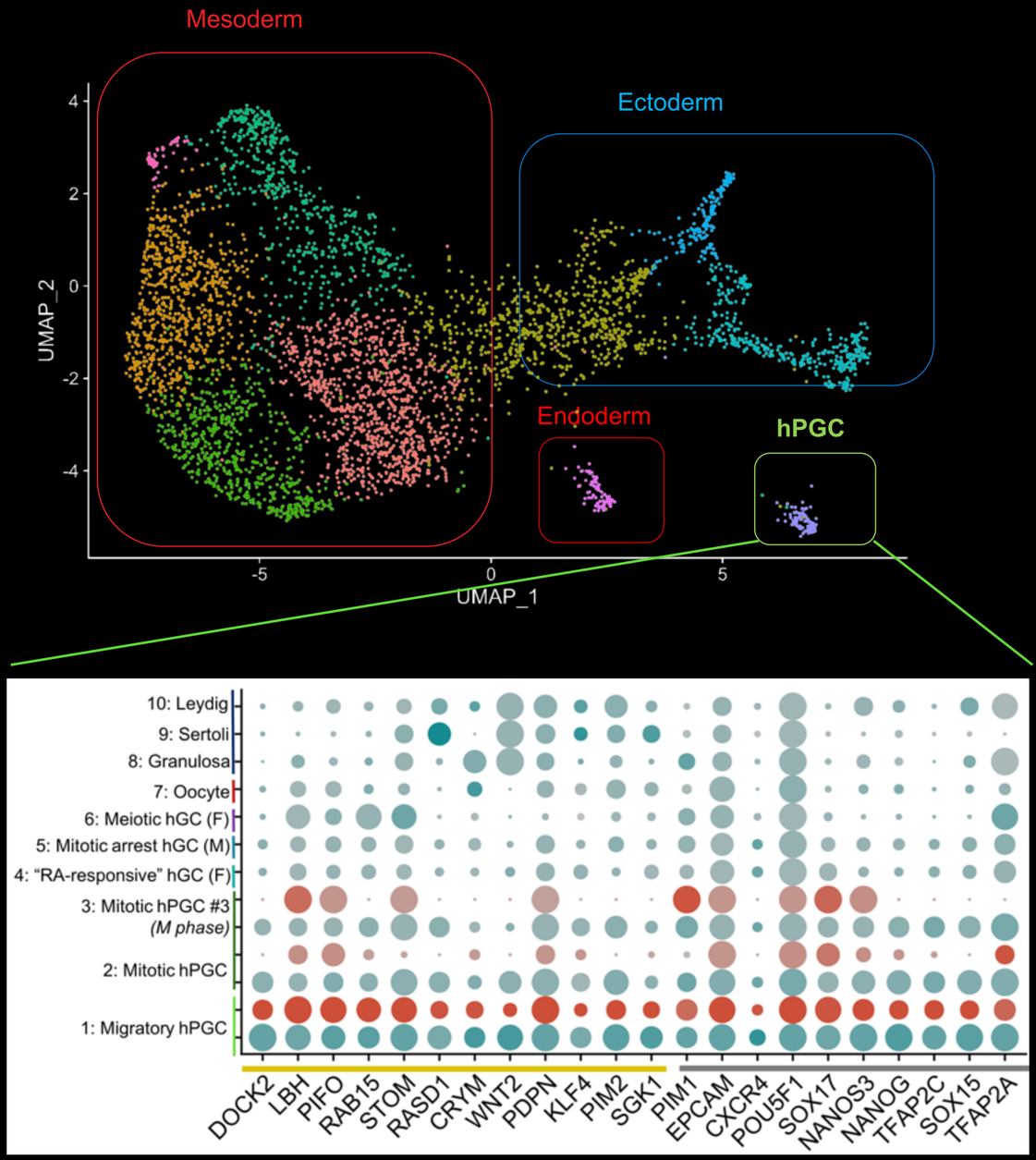
Sox2 / BRA / Sox17
Ectoderm / Mesoderm / Endoderm

Single cell RNA-seq of *gastruloids* reveals novel human-specific molecular signatures



SOX2 / BRA / GATA3

Ectoderm / Mesoderm / Trophectoderm

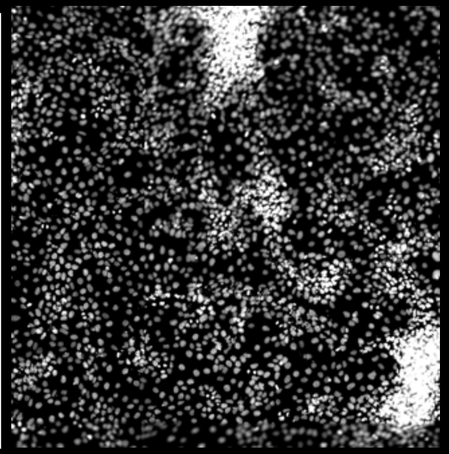


(Soman, Kanno, and Brivanlou, Submitted)

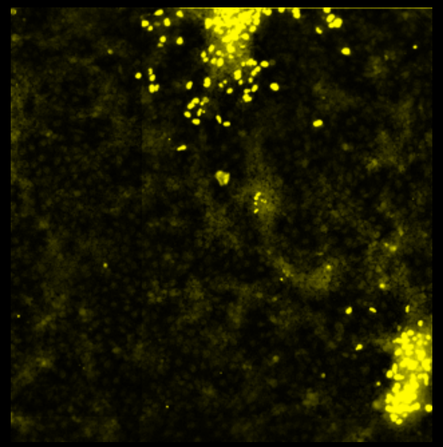
3D *gastruloids* and symmetry breaking

Differentiation:
(Reprograming to enhanced pluripotency or change in substrate stiffness)

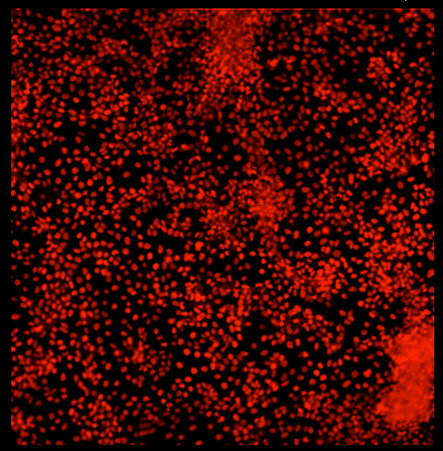
2D-RUES2



DAPI

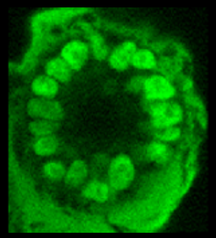


GATA6 (primitive endoderm)



GATA3 (trophectoderm)

→ Dissociation + 3D-Epiblast



OCT4 (model epiblast)

(Simunovic, Metzger, Etoc, Yoney, Ruzo, Martyn, Croft, You, Brivanlou, and Siggia. Nature Cell Biology 2019)

Modeling human disease in *gastruloids*

Huntington's Disease (HD)

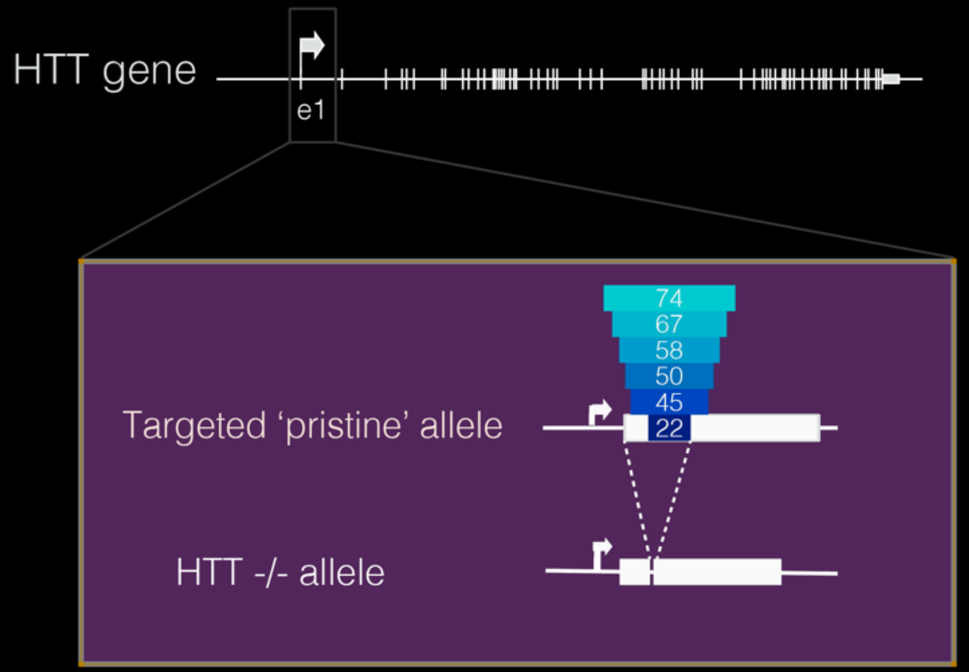
- Dominant mutation: Caused by the expansion of polyQ repeat in Huntingtin gene *(HTT)*
- Considered a neurodegenerative disease with no therapy
- Failure of model systems to accurately model

RESEARCH ARTICLE

Discovery of Novel Isoforms of Huntingtin Reveals a New Hominid-Specific Exon

Albert Ruzo, Ismail Ismailoglu, Melissa Popowski, Tomomi Haremaki, Gist F. Croft, Alessia Deglincerti, Ali H. Brivanlou*

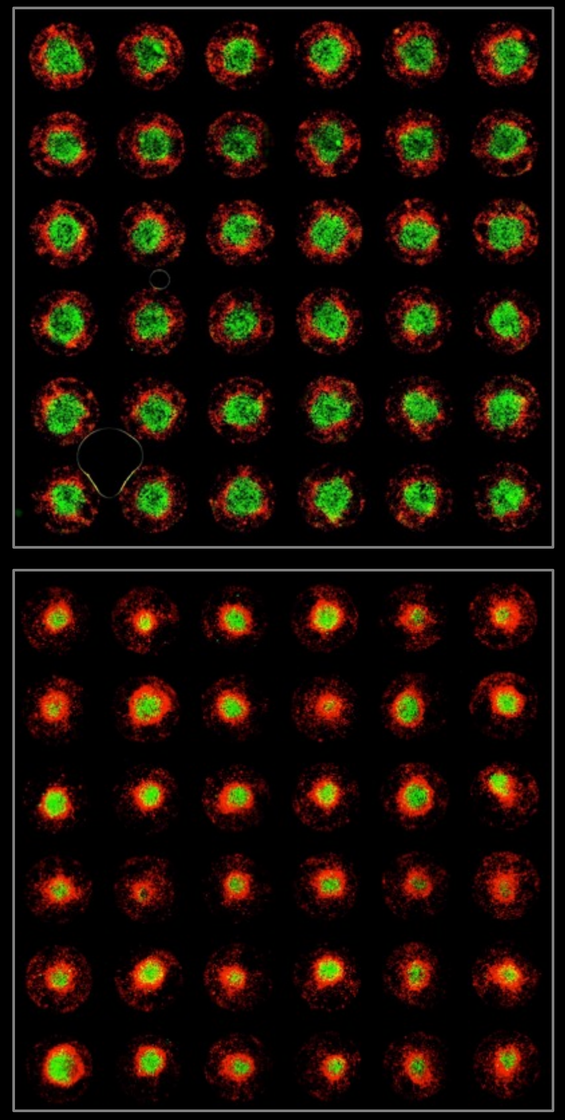
CRISPR-edited isogenic HD RUES2

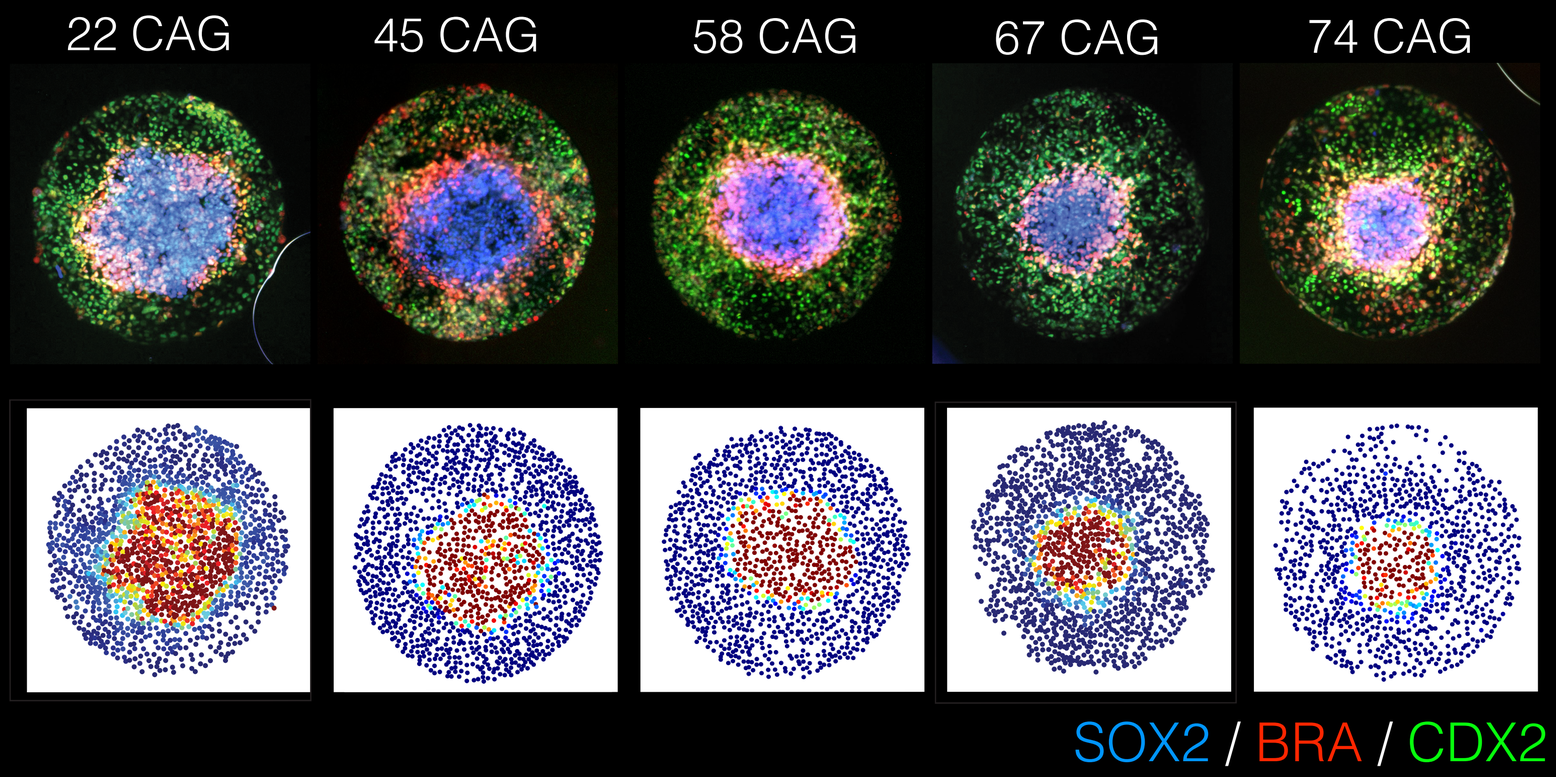


(Ruzo, Croft, Metzger, Galgoczi, Gerber, Pellegrini, Wang, Fenner, Tse, Marks, Nchako, Brivanlou. Development 2018)

(Galgoczi, Ruzo, Markopoulos, Yoney, Phan-Everson, Siggia, Etoc, Brivanlou. Submitted)

HD phenotype in *gastruloids*

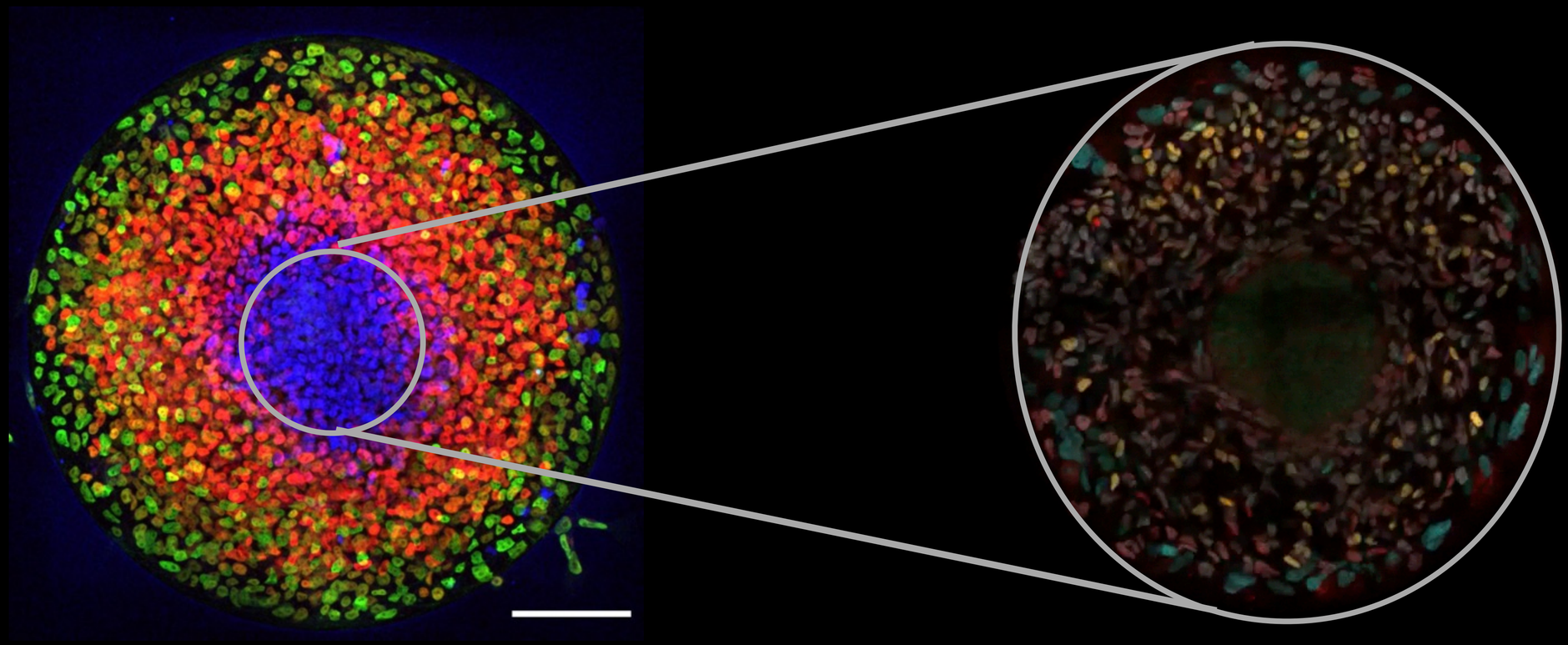




- CAG-length dependent phenotypes
- HD is a developmental disease

(Galgoczi, Ruzo, Markopoulos, Yoney, Phan-Everson, Siggia, Etoc, Brivanlou. Submitted)

Self-organization of the ectodermal germ layer into "*neuruloids*" brain, sensory places, neural crest and epidermis



SOX2 / BRA / GATA3
Ectoderm / Mesoderm / Trophectoderm

PAX6 / SOX10 / SIX1 / TFAP2A

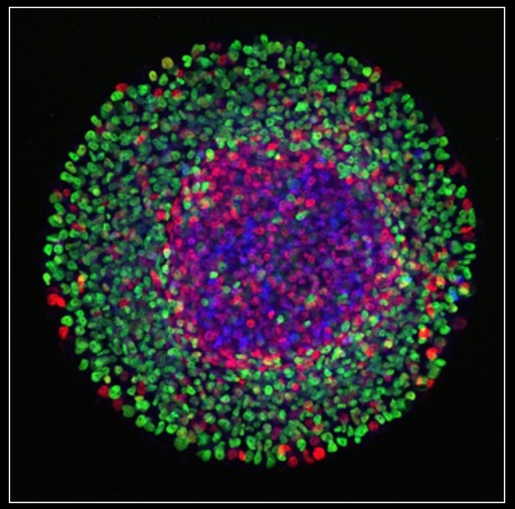
(Haremaki, Metzger, Rito, Ozair, Etoc, and Brivanlou. Nature Biotechnology 2019)

HD phenotype in *neuruloids*

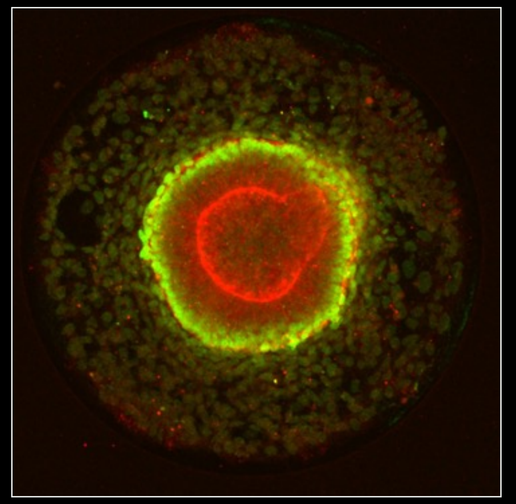
Gastruloids

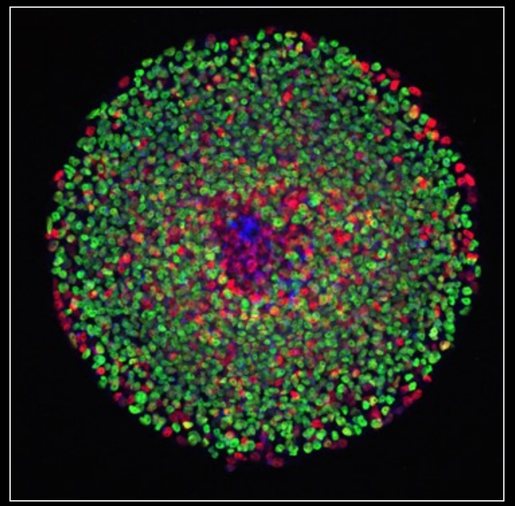
Neuruloids

SOX2 / CDX2

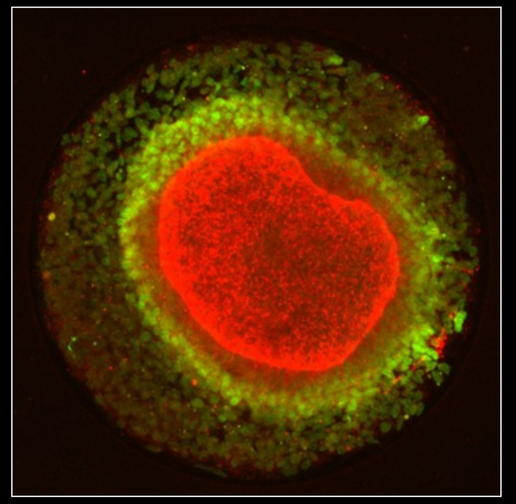


non-HD
22-CAG





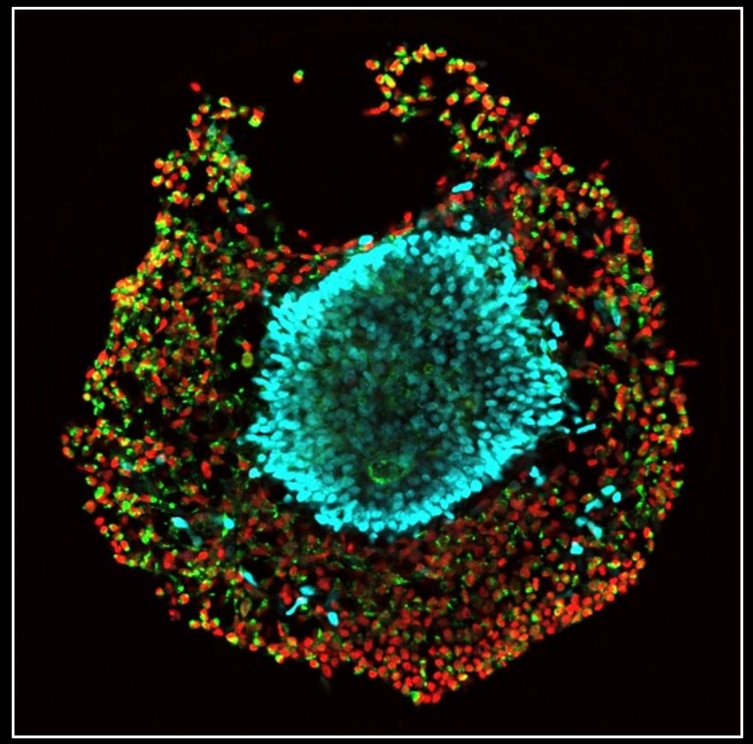
HD
56-CAG



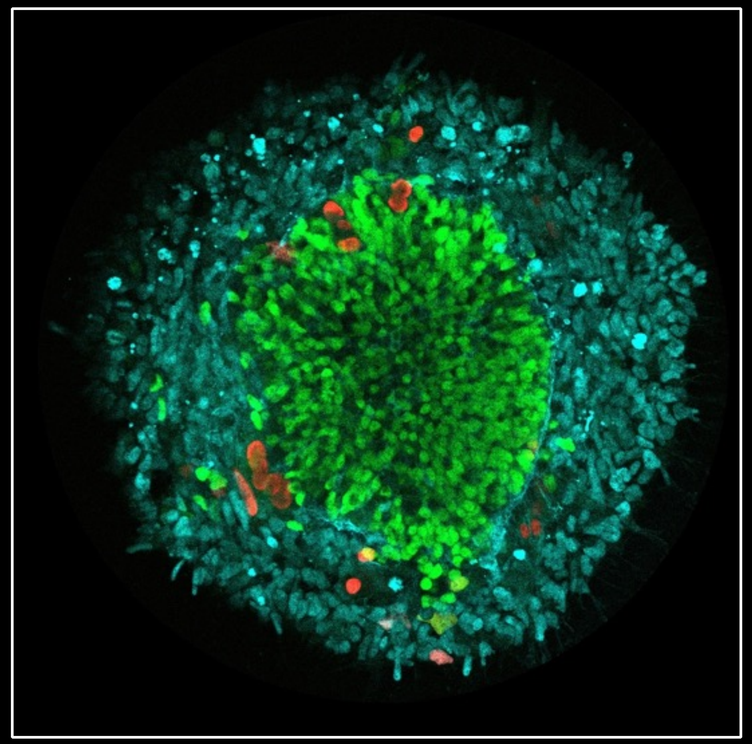
PAX6 / Collagen IV

(Haremaki, Metzger, Rito, Ozair, Etoc, and Brivanlou. Nature Biotechnology 2019)

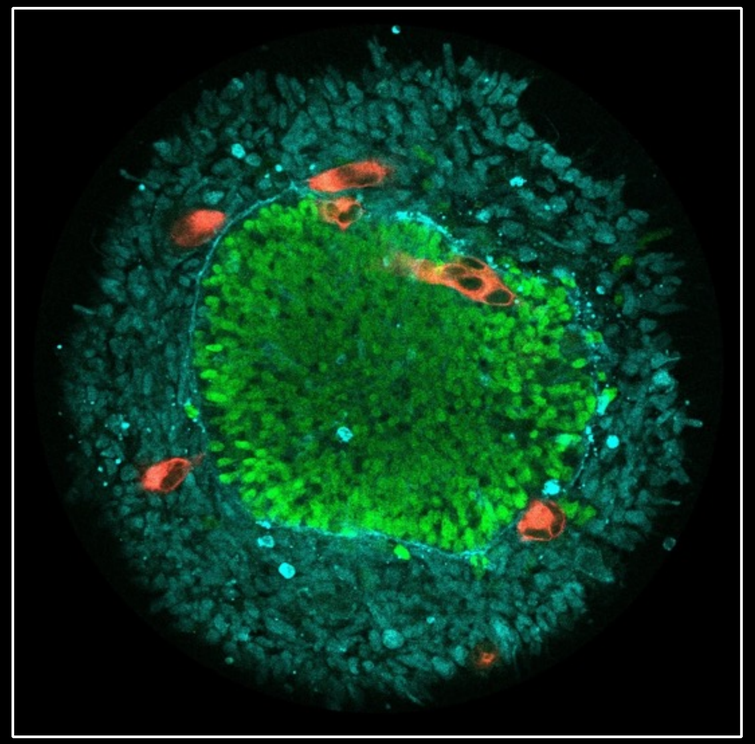
Modeling infectious diseases in *gastruloids* and *neuruloids*



Zika Infection



CMV1 Infection



CMV2 Infection

(In collaboration with Brandon Razooky and Charlie Rice)

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Edwin Rosado-Olivieri
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Fred Etoc
Mijo Simunovic
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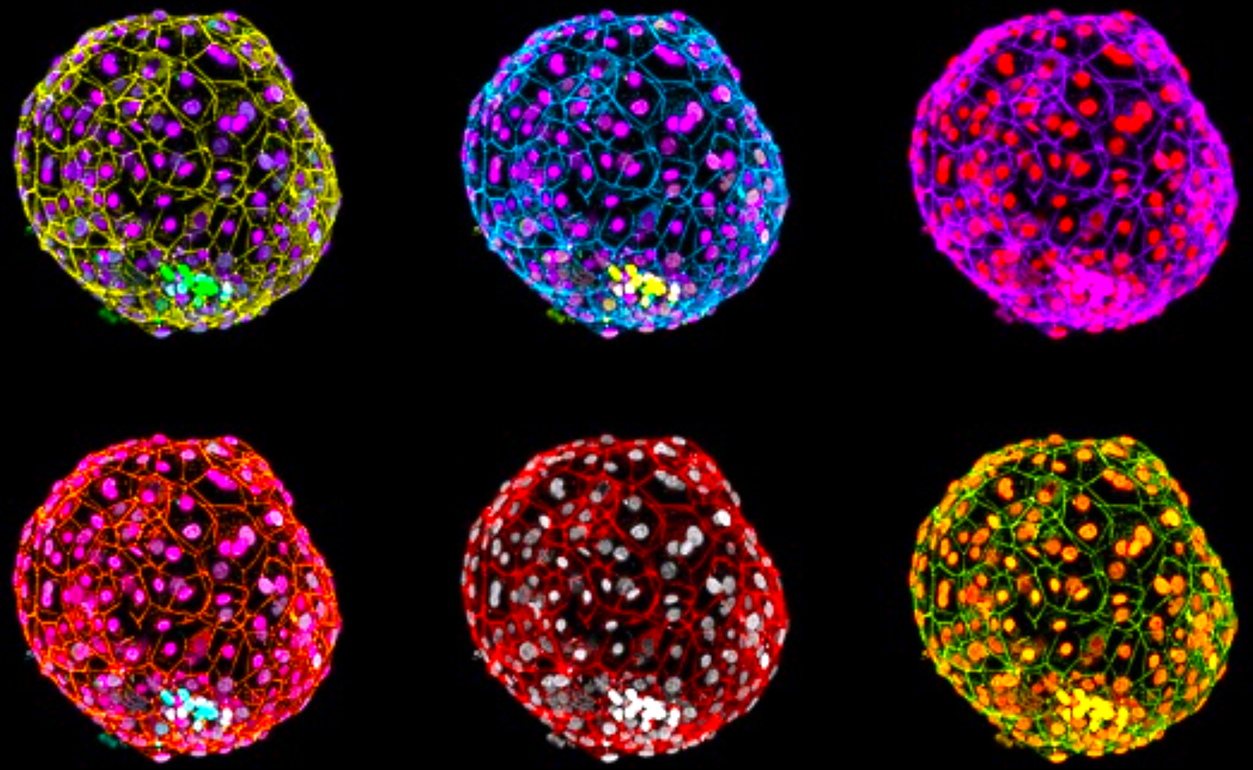

In collaboration with Eric Siggia

Technical support

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