In Vitro Data and *In Silico* Models for Computational Toxicology

Thomas B. Knudsen, PhD
Developmental Systems Biologist
US EPA, National Center for Computational Toxicology
Chemical Safety for Sustainability Research Program
Virtual Tissue Models (VTM) project
knudsen.thomas@epa.gov
ORCID 0000-0002-5036-596x

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ILSI HESI Workshop: “Redesigning the Embryofetal Developmental Toxicity Study: Evolution or Revolution?”

DISCLOSURES

FUNDING:

US EPA/ORD Chemical Safety for Sustainability (CSS) Research Program

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EPA/ORD/NCCT contract EP-D-13-053 with Vala Sciences

EPA/ORD/NCCT contract EP-D-13-055 with Stemina Biomarker Discovery

EPA/ORD/NCCT contract EP-D-13-056 with ArunA Biomedical

Organotypic Culture Models for Predictive Toxicology Centers (EPA/STAR)

DISCLAIMER: The views expressed are those of the presenter and do not reflect Agency policy.

CONFLICTS OF INTEREST: none to disclose.

Drivers for Change: *NAS 2007 'Toxicity Testing in the 21st Century'*

- **PROBLEM:** >85,000 chemicals on EPA's inventory under TSCA (amended 2016) that explicitly targets pregnant women and children as key susceptible populations for decision-making.

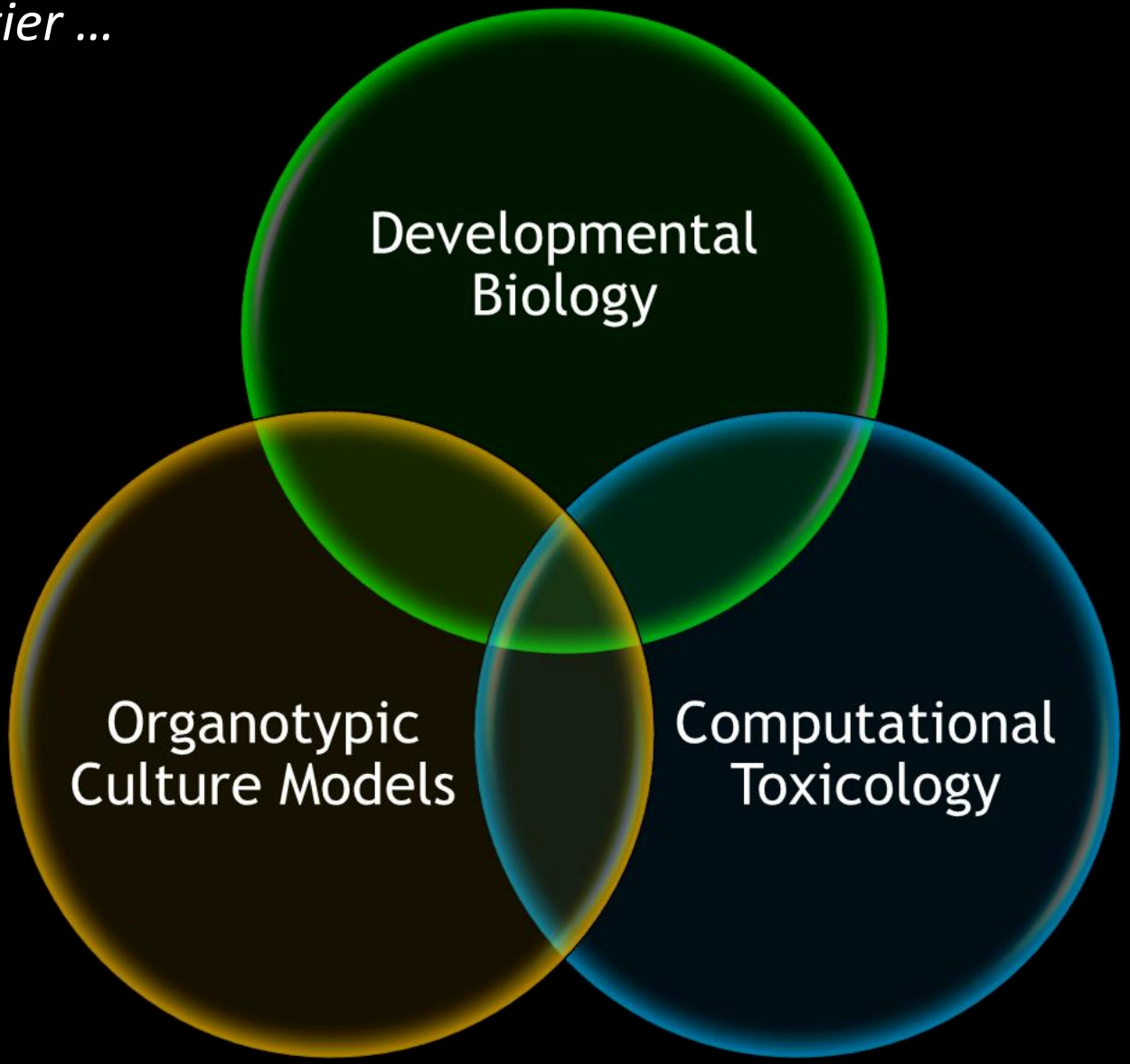
  - current animal-based testing does not have the throughput to scale this problem for children's environmental health protection.

- **TECHNOLOGY:** significant advances in the genome sciences, in automated high-throughput screening (HTS), and in alternative methods for testing now enable rapid profiling of chemical-biology.

  - computational systems models are needed to translate HTS data into human physiology and development.

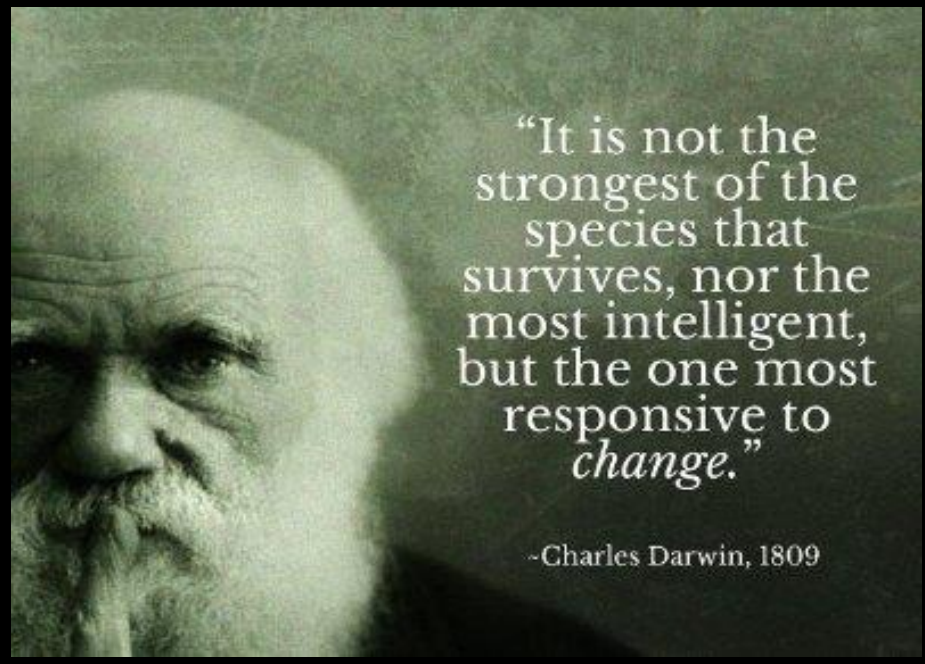
http://www.ncats.nih.gov/

Predictive Toxicology: *the final frontier …*



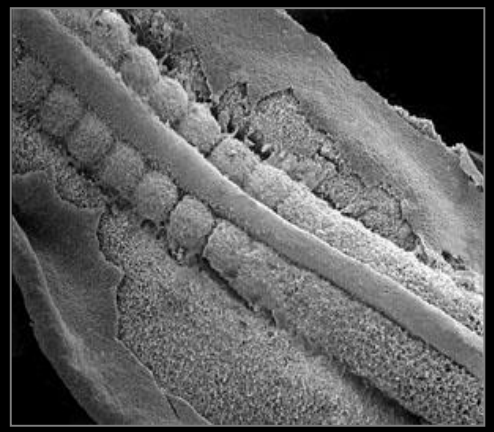
Grand Challenge Questions for ‘Re/volution’

- How do we ethically capitulate ‘human embryology’ at a more physiological level than possible with cell culture?
- How far must ‘computational embryology’ advance to predict developmental toxicity in lieu of animal testing?
- How can ‘synthetic toxicology’ help shift regulatory reliance from animal studies to mechanistic models?



Knudsen TB, Klieforth B and Slikker W Jr (2017) Programming microphysiological systems for children’s health protection. Exp Biol Med (in press)

Somite formation

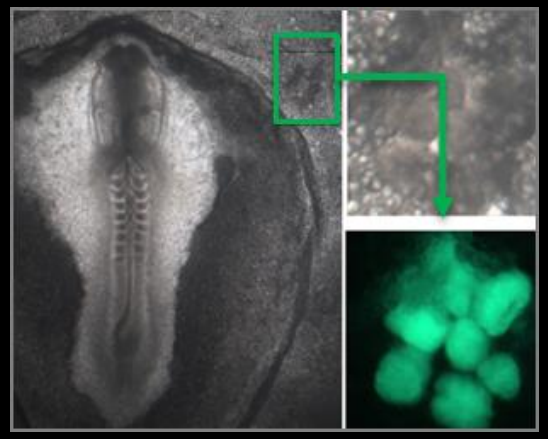


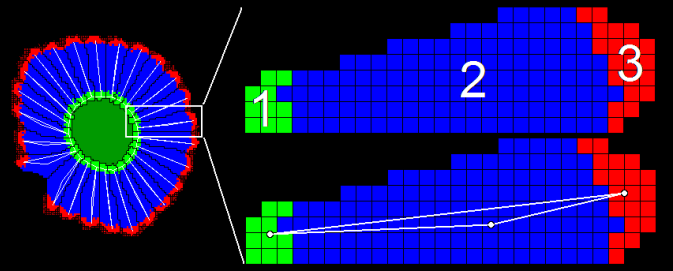
Hes1-EGFP time-lapse (3h)
Masamizu et al. 2006

Clock and Wavefront Model

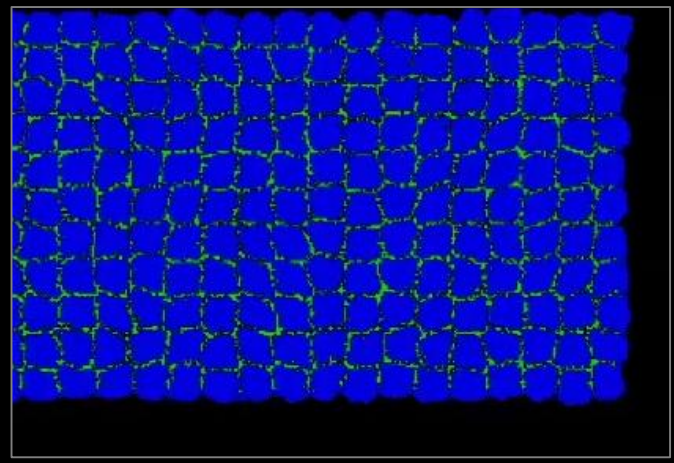
- *oscillating gene expression (eg, Hes1, LNFG)*
- *signal gradients along AP axis (eg, FGF8, RA)*
- *differential cell adhesion (eg, ND, ephrin system)*

SOURCE: Hester et al. (2011) PLoS Comp Biol

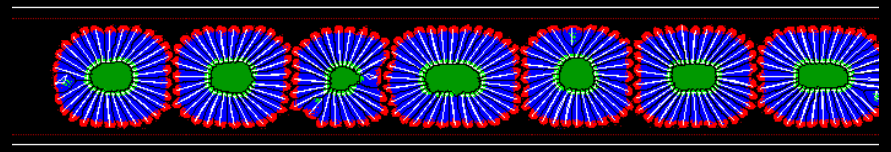




Epithelialization Model

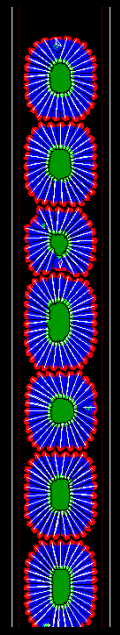


- *adding the wavefront restores sequentiality*
- *adding the clock improves regularity*

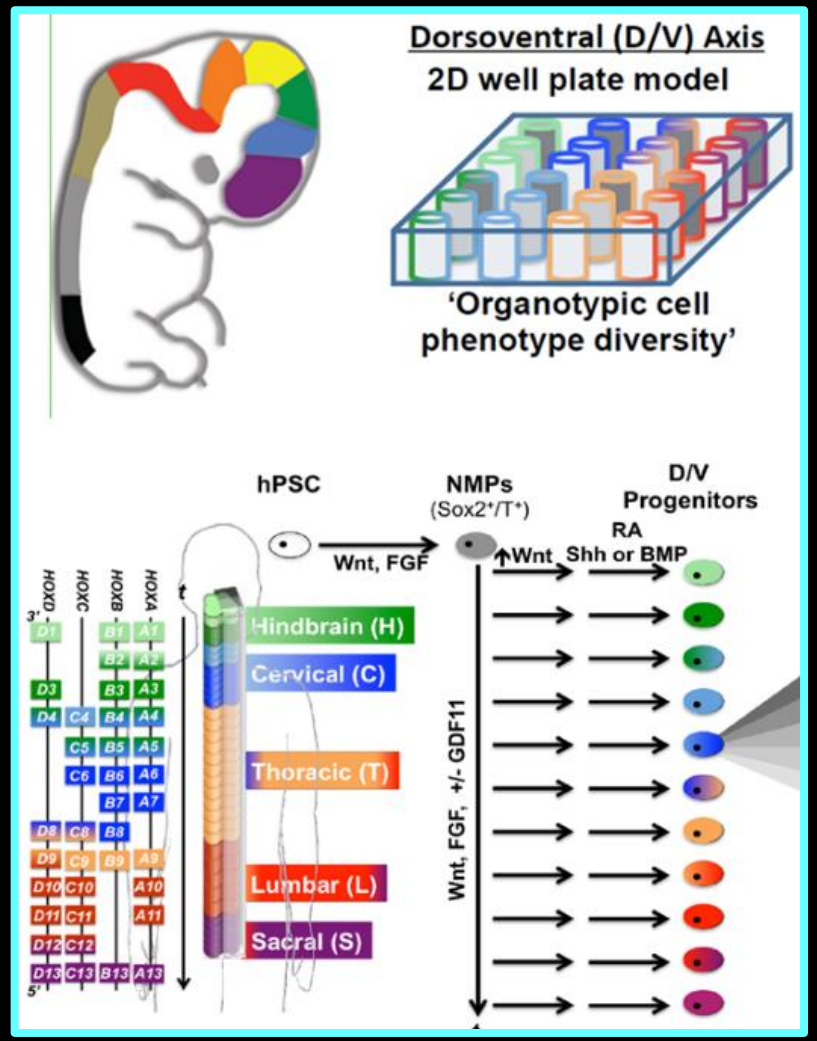


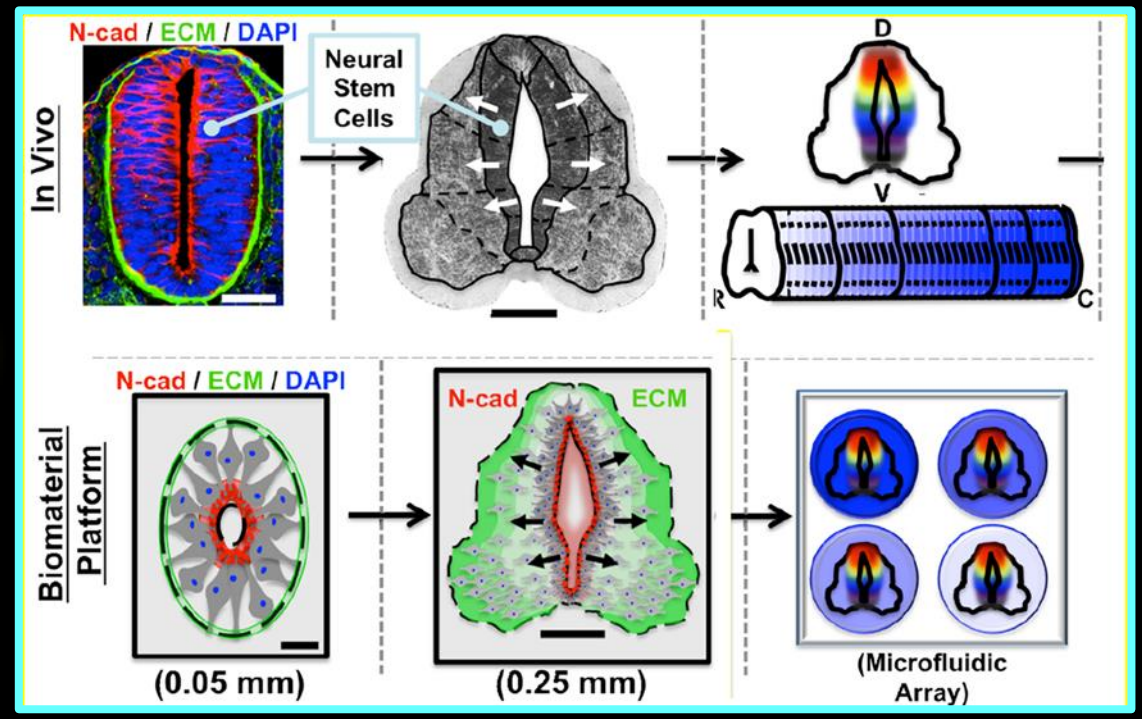
SOURCE: Dias et al. (2014) Science

Micropatterning: *stem cell arrays representing the human neural tube*



retinoic acid signaling

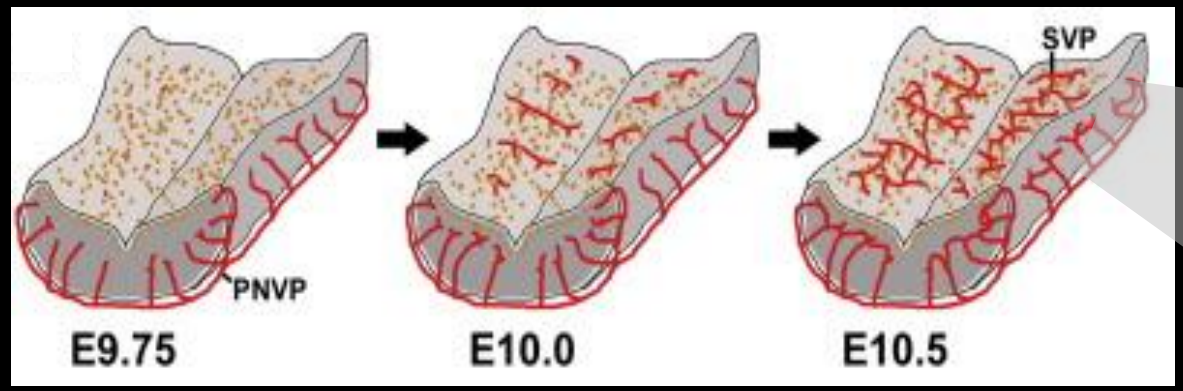




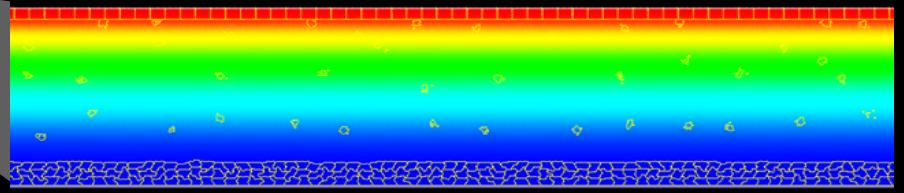
SOURCE: Randolph Ashton, U Wisconsin H-MAPS Center [Lippman et al. 2015; Knight and Ashton 2015]

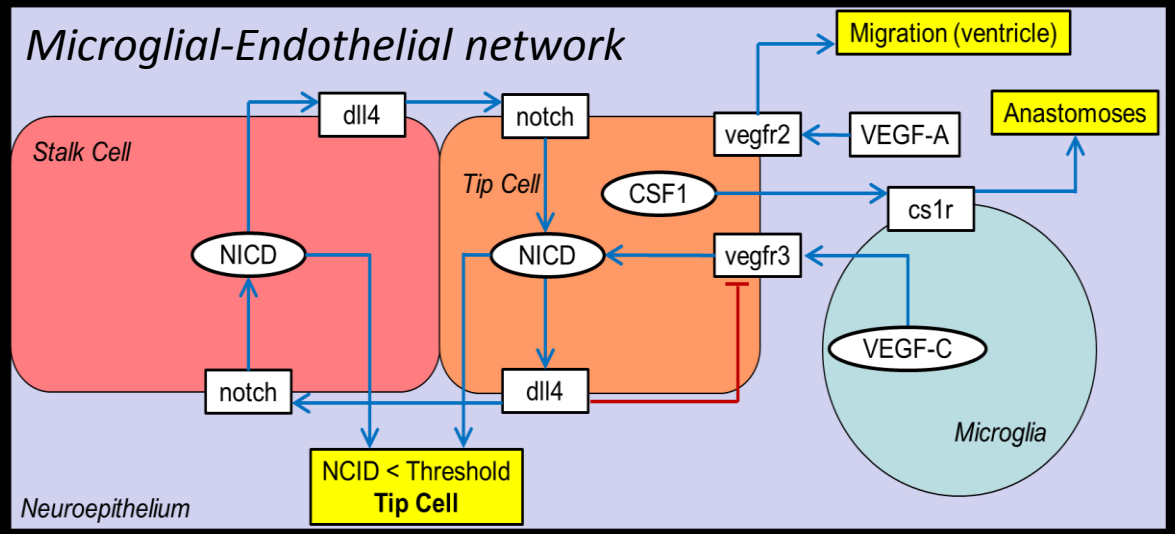
Modeling Brain Angiogenesis: *cellular agent-based model of arborization*

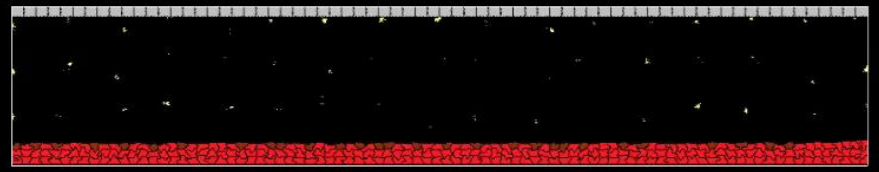
Tata et al. (2015) *Mechansim Devel*



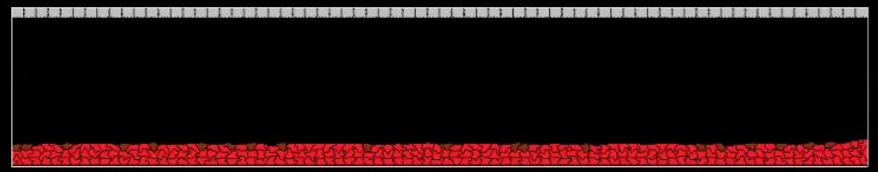
VEGF-A gradient: NPCs in subventricular zone

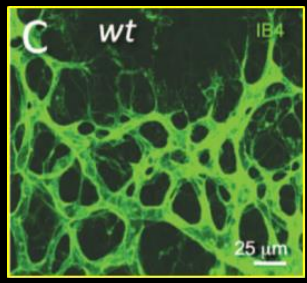


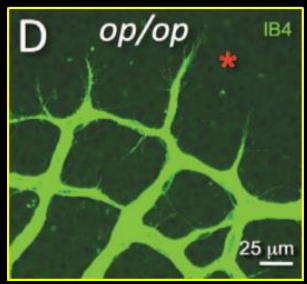




endothelial tip cell
endothelial stalk cell
microglial cell

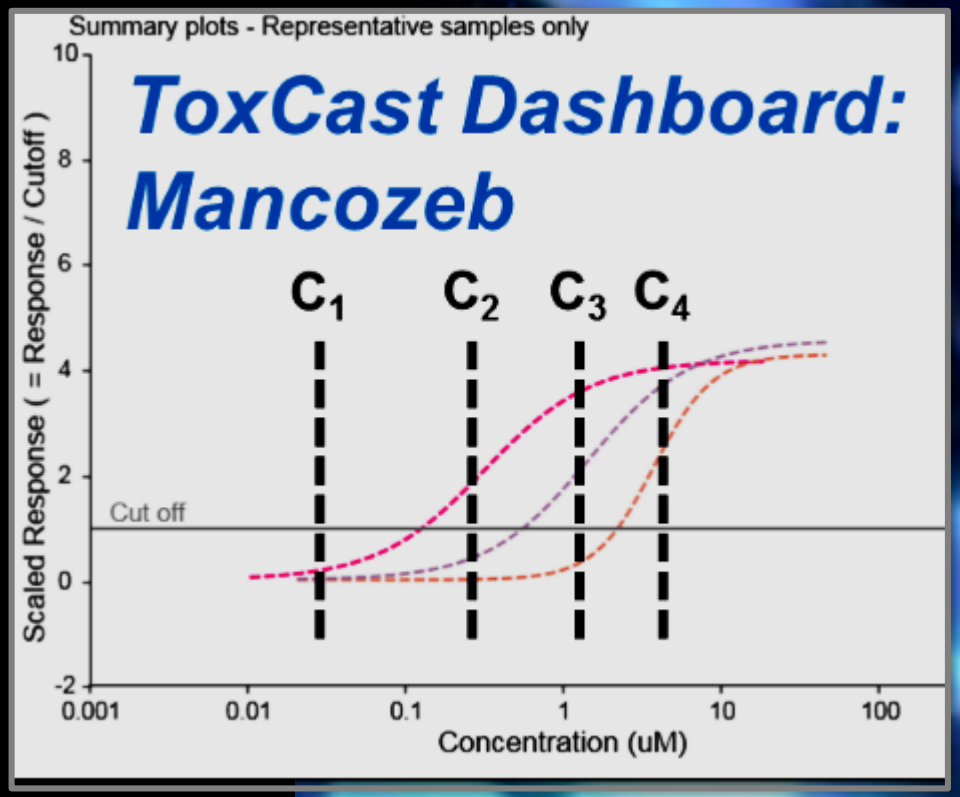


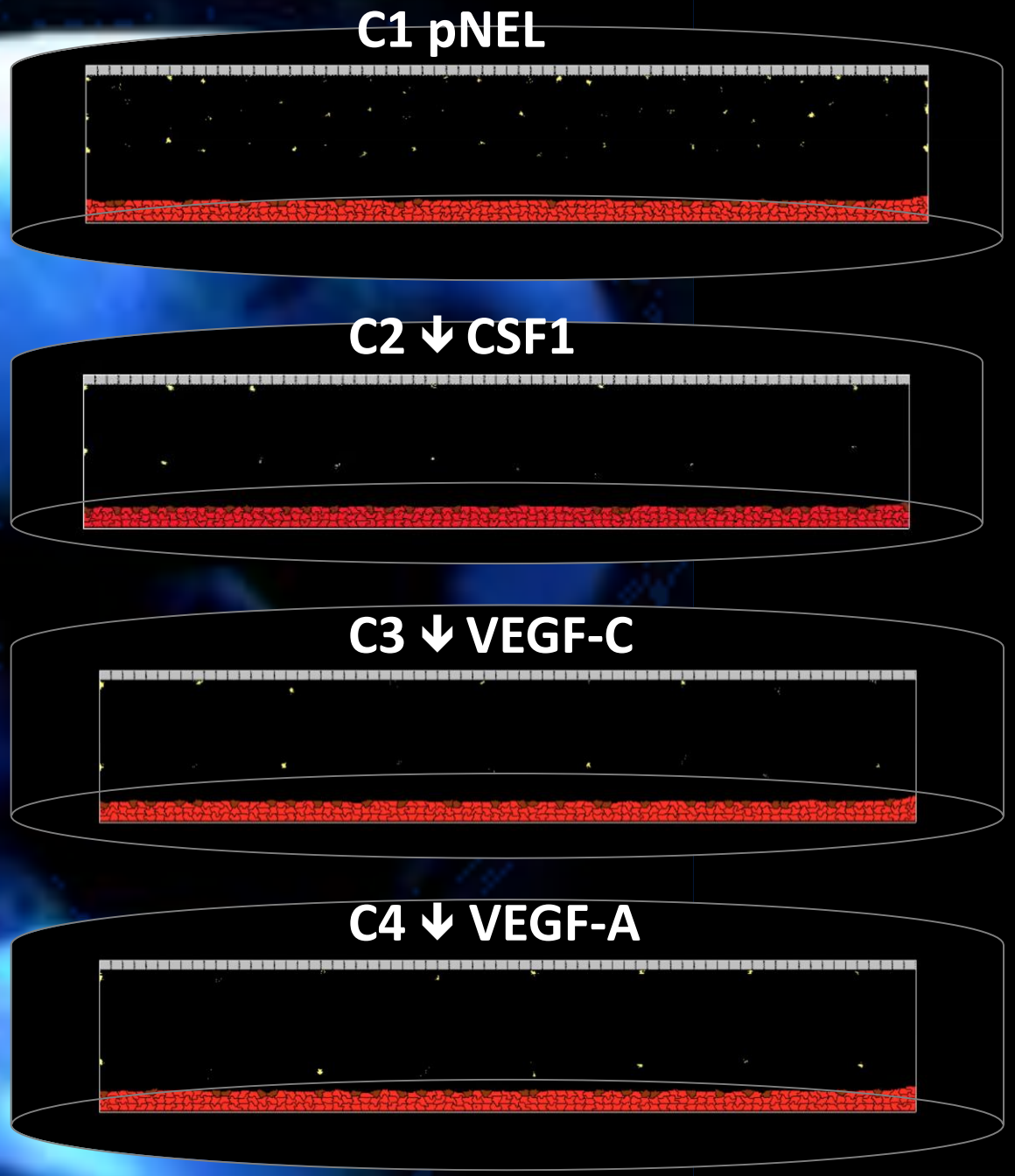




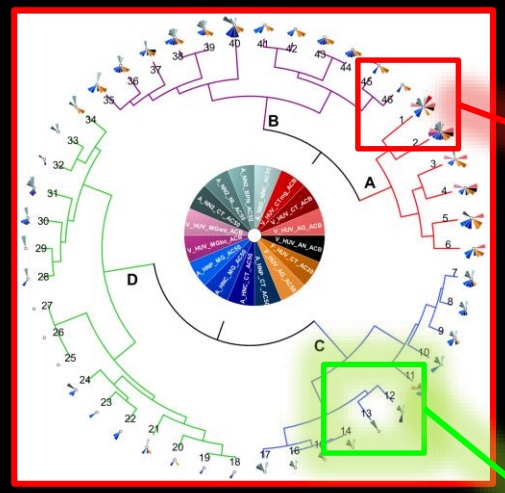
Rymo et al. (2011) PLoS one

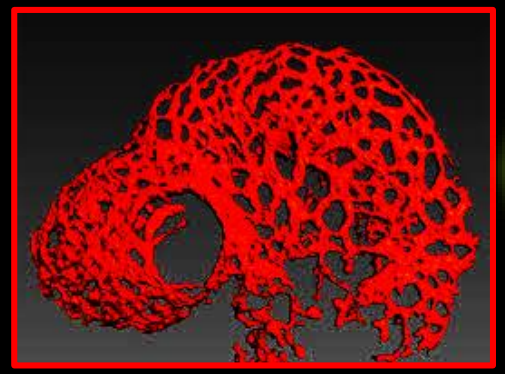
SOURCE: T Zurlinden – NCCT (2017)

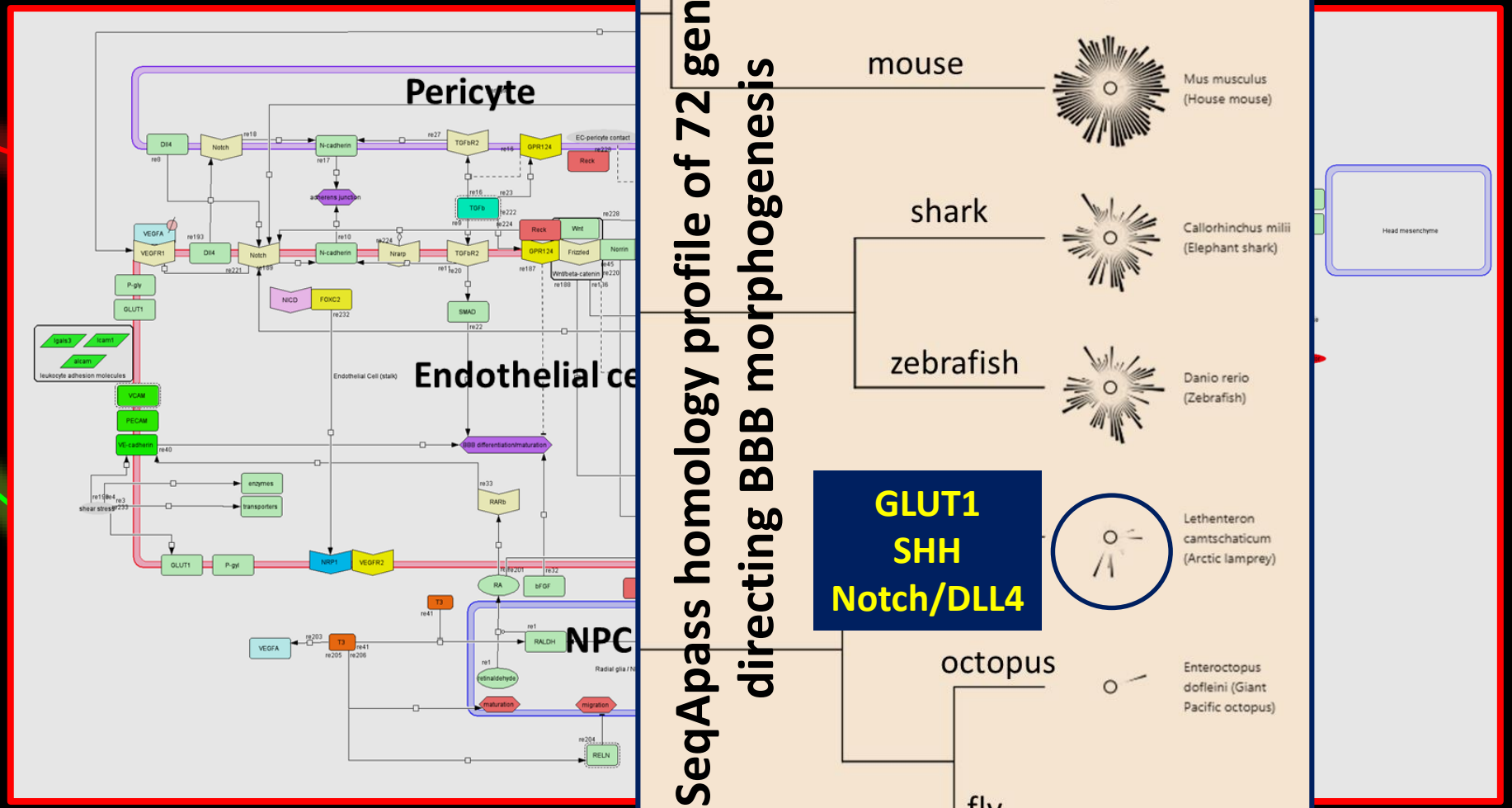

Summary plots - Representative samples only
ToxCast Dashboard:
Mancozeb
C1
C2
C3
C4
Cut off
Scaled Response (= Response / Cutoff)
10
8
6
4
2
0
-2
0.001
0.01
0.1
1
10
100
Concentration (uM)


C1 pNEL
C2 ↓ CSF1
C3 ↓ VEGF-C
C4 ↓ VEGF-A

Vascularization of the Neural tube: *46 ToxCast compounds assessed for in vitro effects on human angiogenesis (Vala) and neurog*



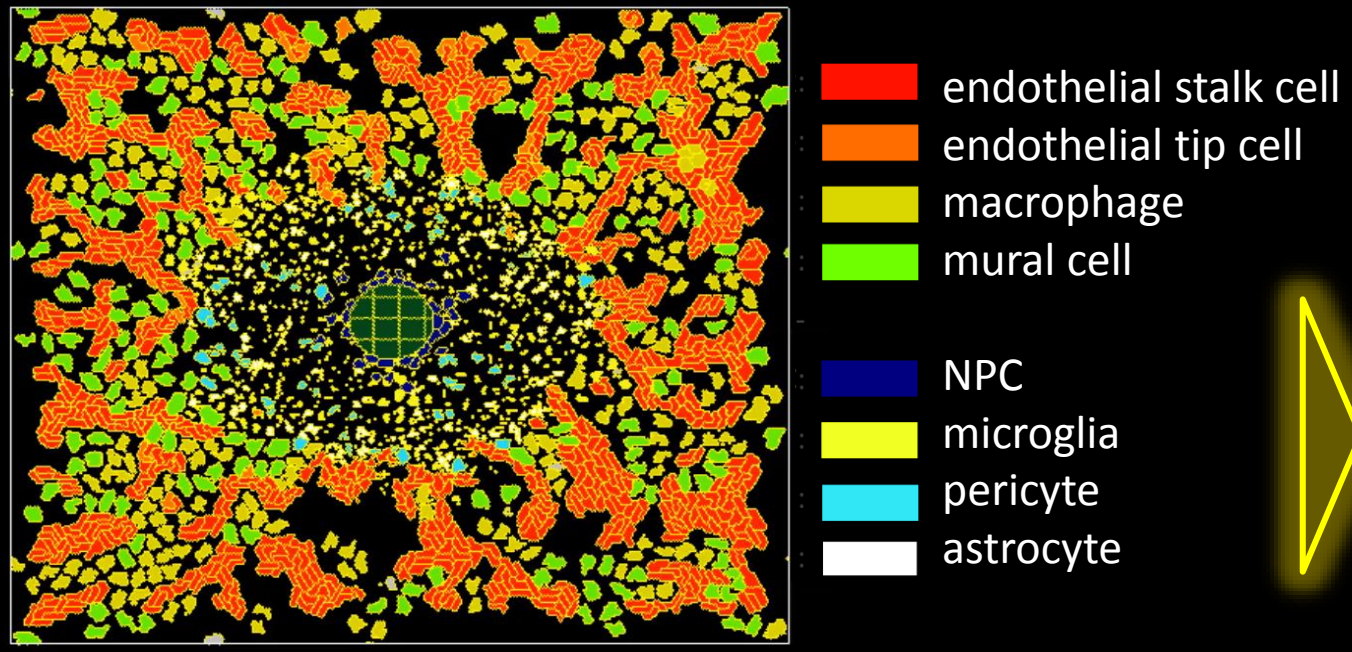




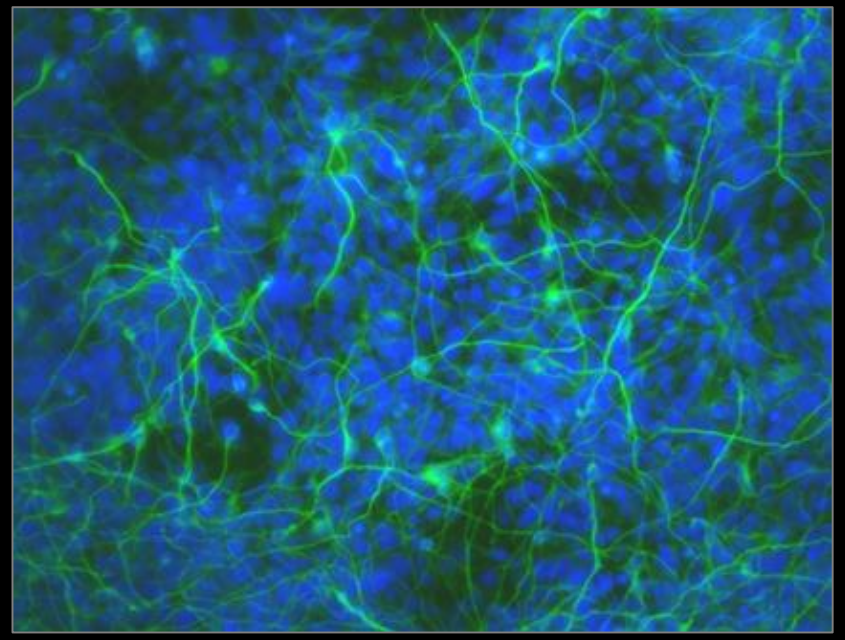
Control network for

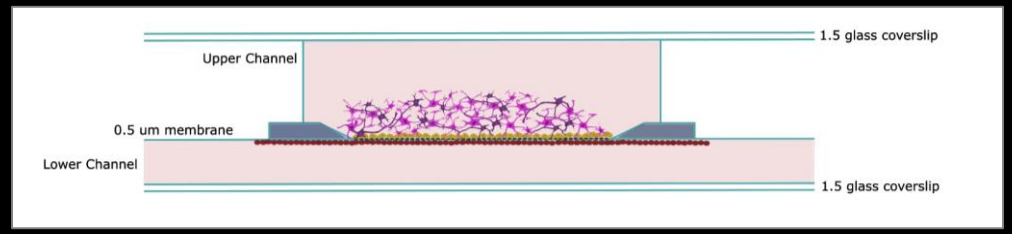
Saili et al. (2017) i

Modeling cell dynamics in the fetal Neurovascular unit (NVU)



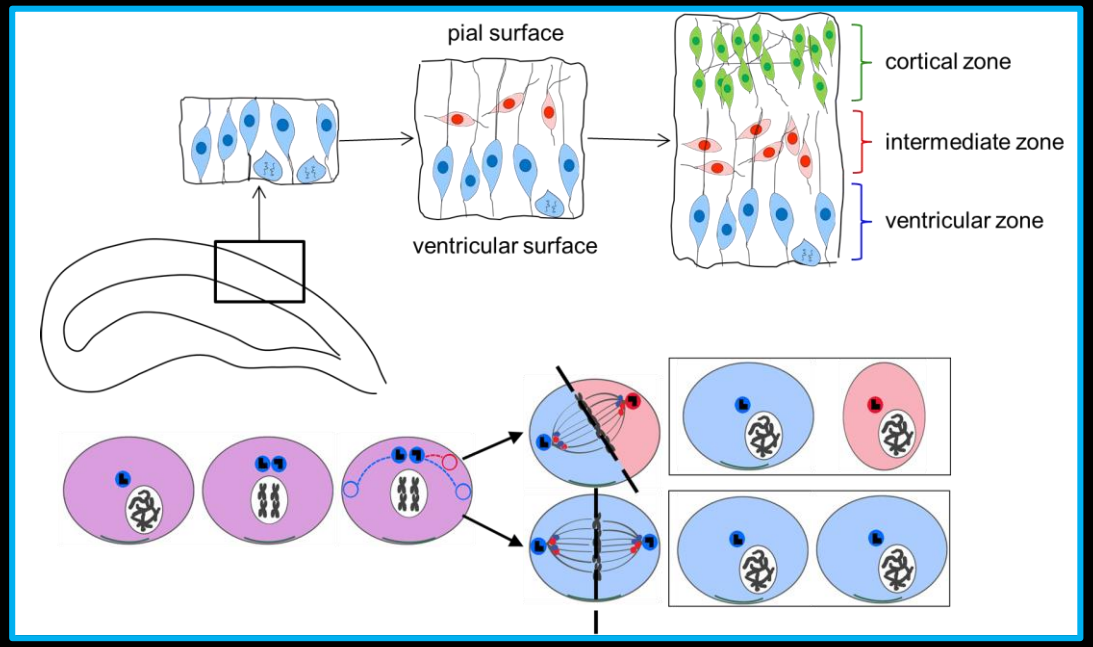
computational NVU (cNVU)
(T Zurlinden, NCCT)

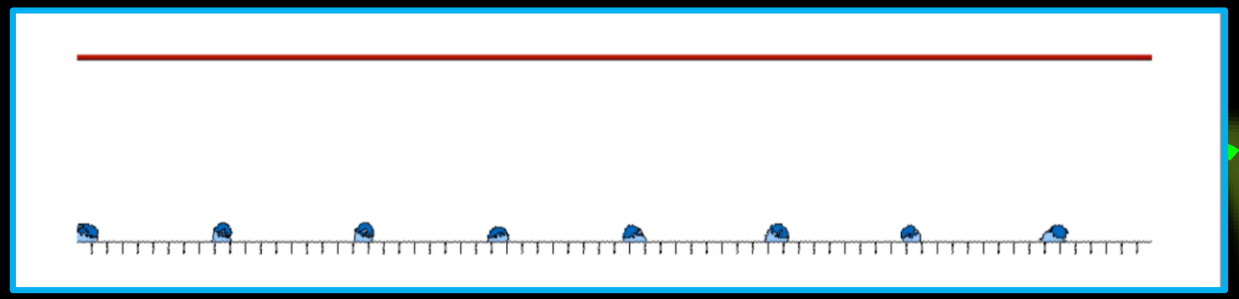


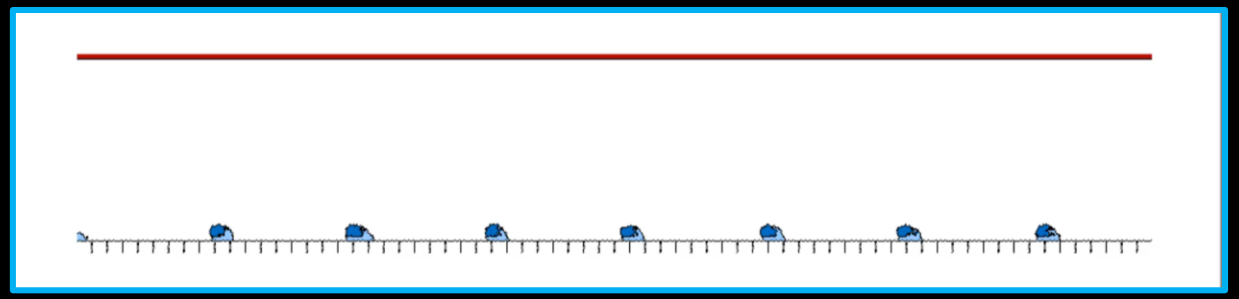


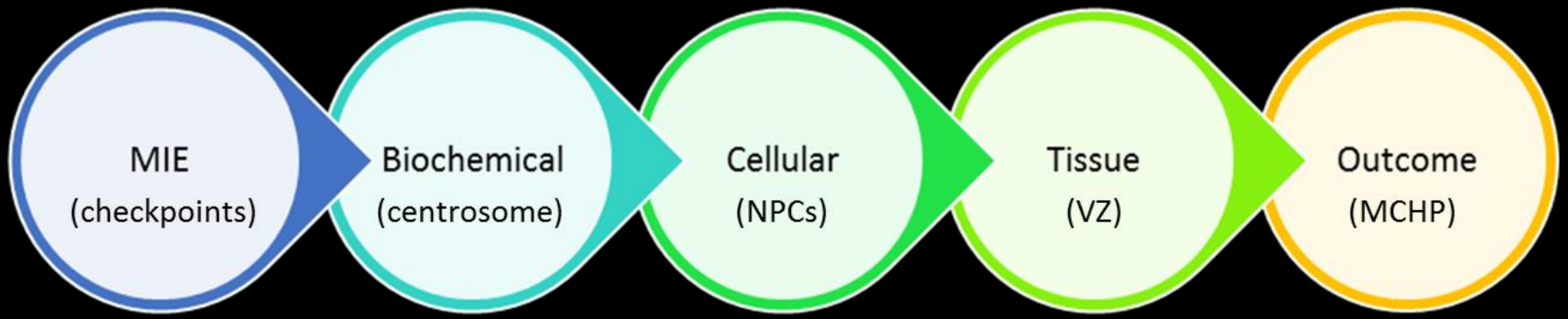
Ibidi hNVU device
(A Schwab / S Hunter – NHEERL)

Microcephaly: *computational model (in silico) and human brain mimic (in vitro)*

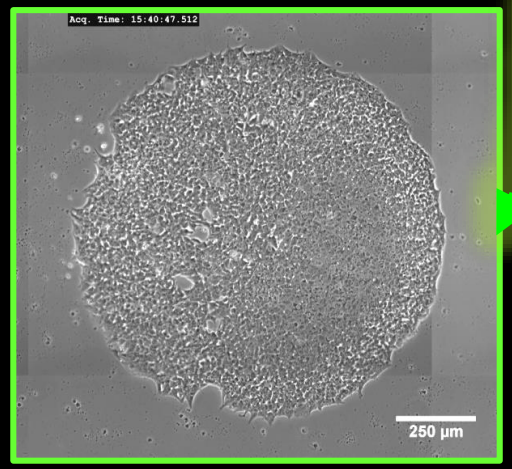








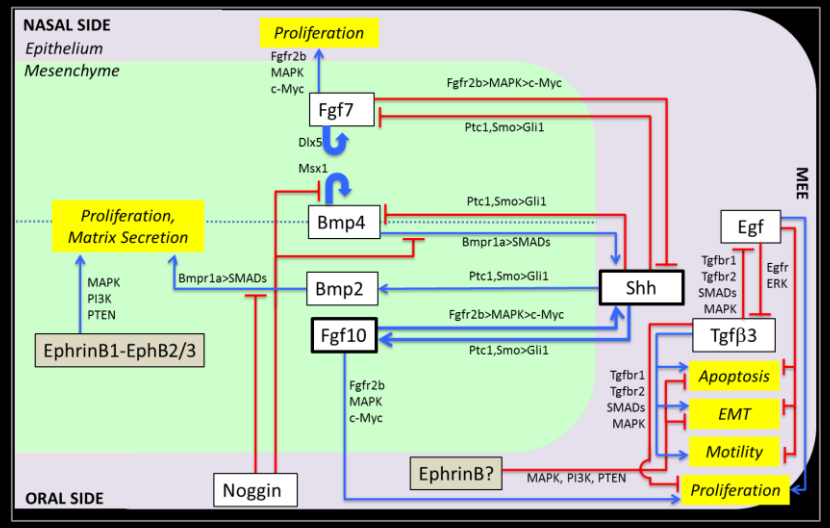
Todd Zurlinden, NCCT



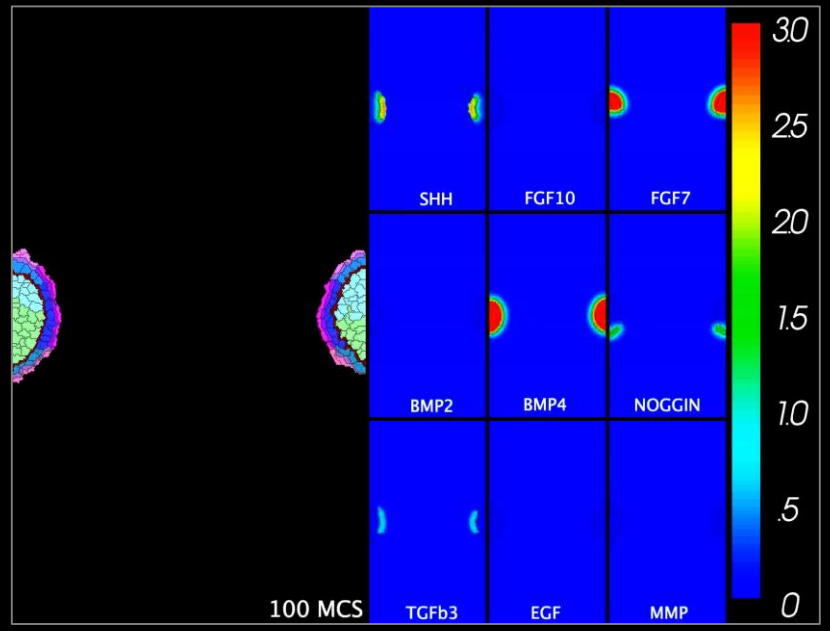
Bill Murphy, H-MAPS Center

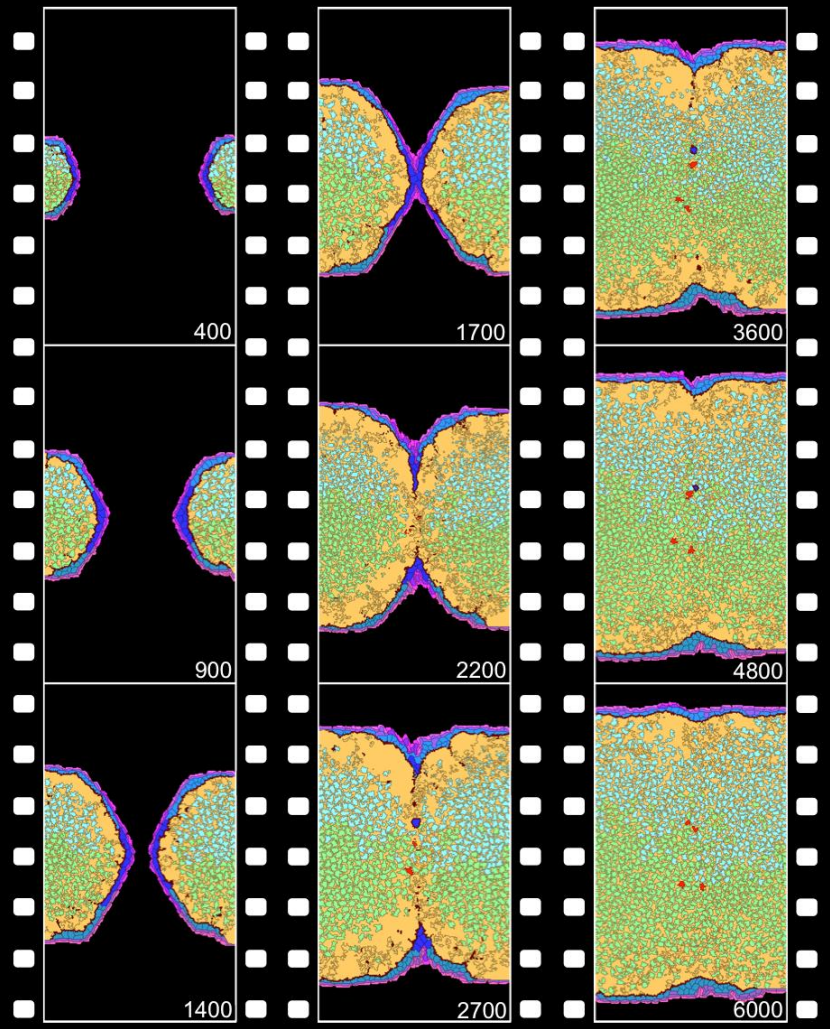
Morphogenetic fusion: *palate development (in vivo)*

Control network



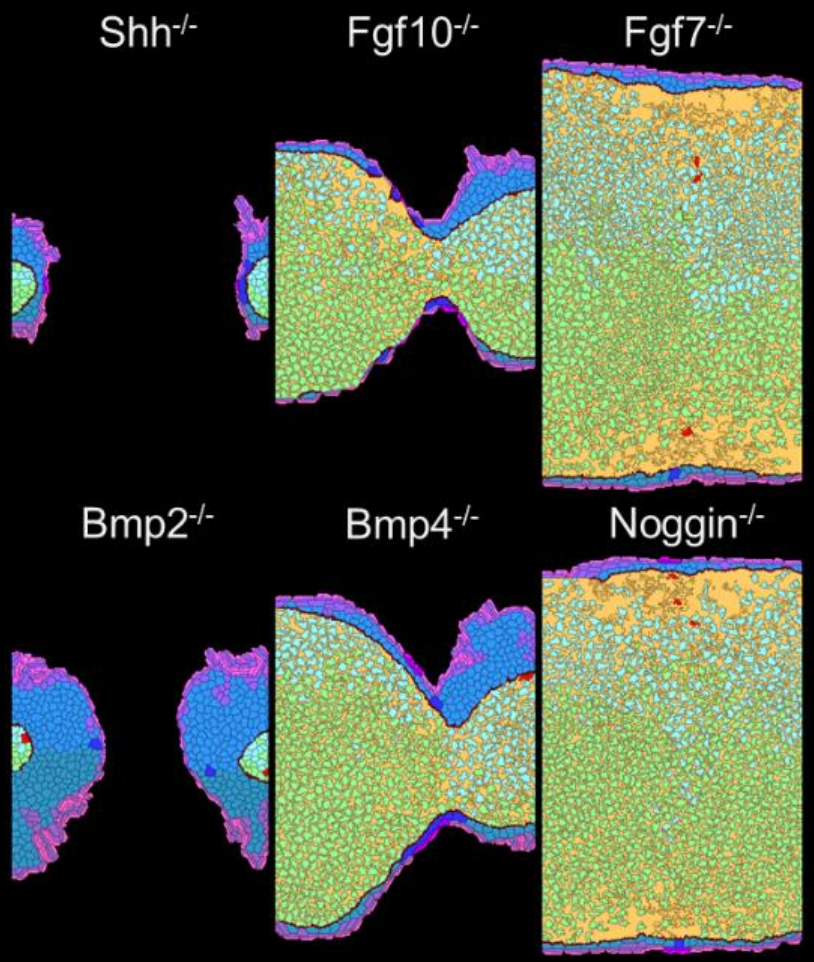
Anterior simulation





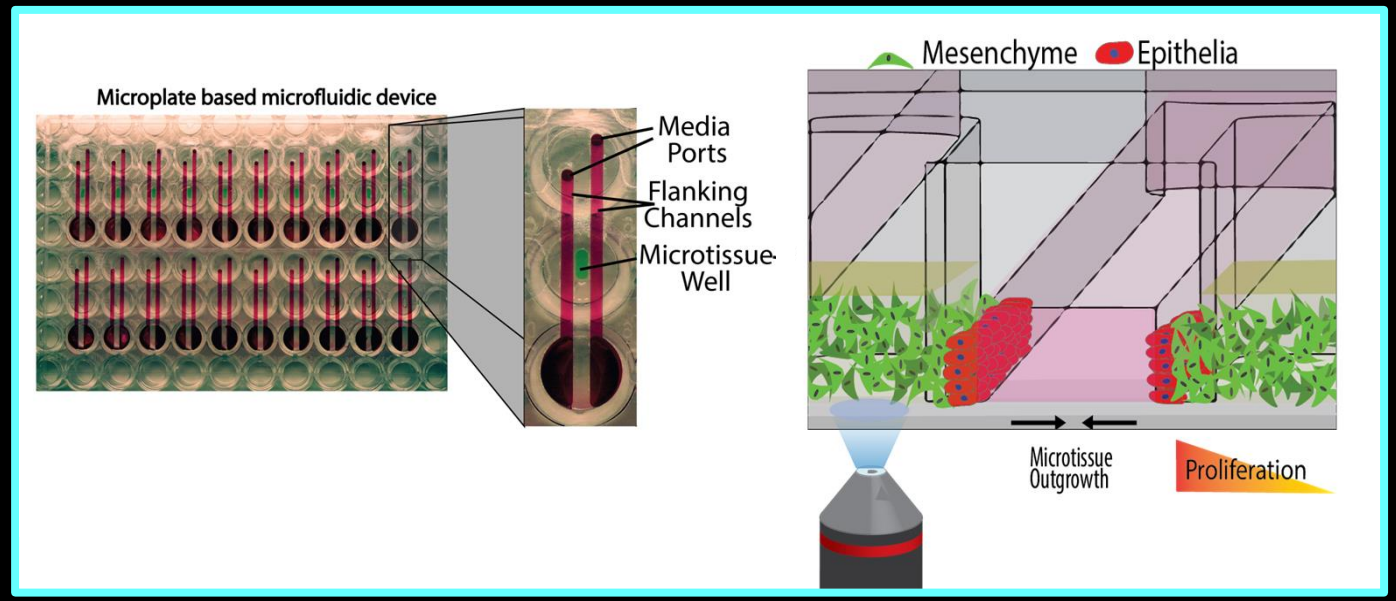
SOURCE: Hutson et al. (2017) Chem Res Toxicol

Hacking the Control Network: *in silico* knockouts → 'Cybermorphs'



SHH signaling drives outgrowth *(MCS 200-2000)*

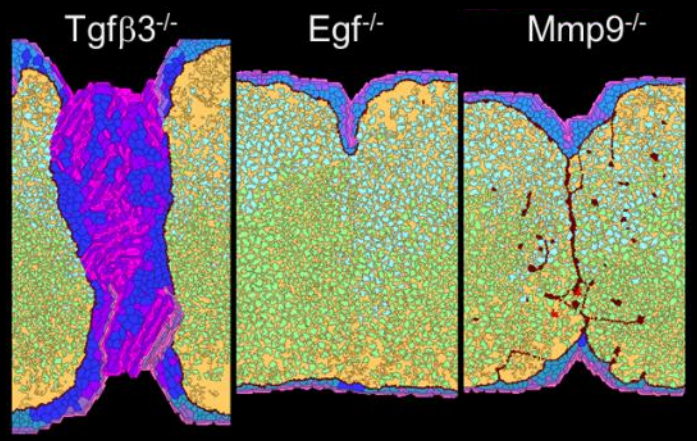
- SHH::FGF and SHH::BMP stimulate mesenchymal cell proliferation and ECM production

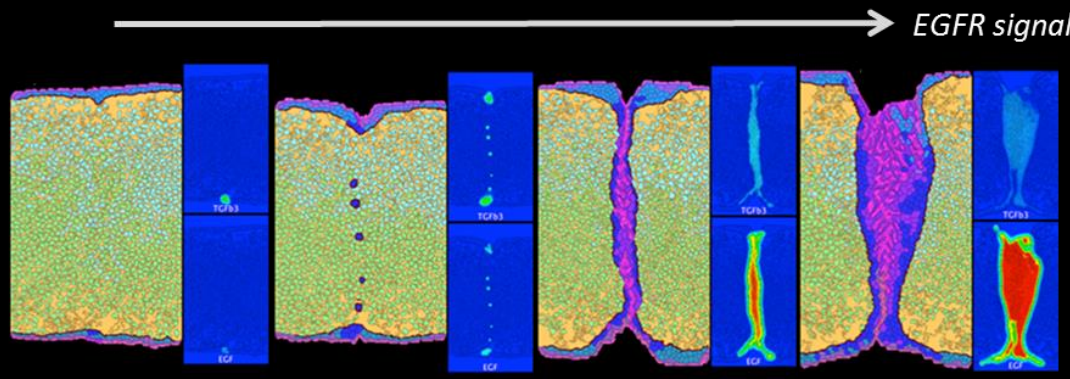


SHH-driven outgrowth

(B Johnson / D Beebe H-MAPS Center, U Wisc)

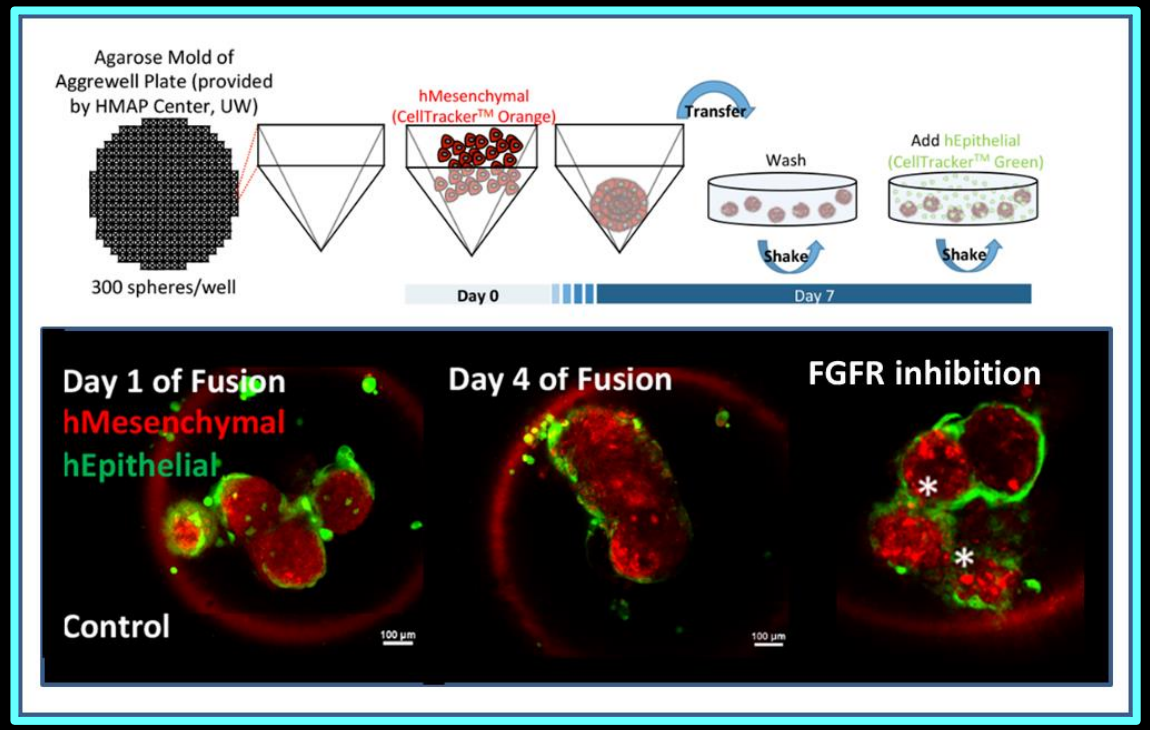
MEE Fusion: *cybermorphs (ABM) and organotypic culture models (OCM)*





TGF-beta signaling drives fusion *(MCS 2000-3000)*

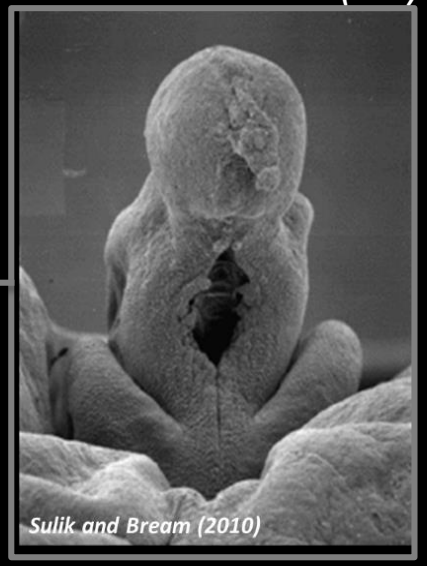
- TGFβ3::EGF signaling switches epithelial cell fate from survival (high EGFR) to regression (low EGFR).

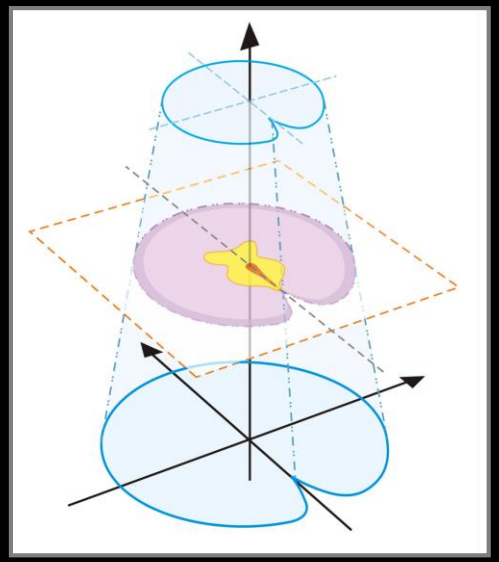


Epithelial fusion
(D Belair / C Wolf / B Abbott – NHEERL)

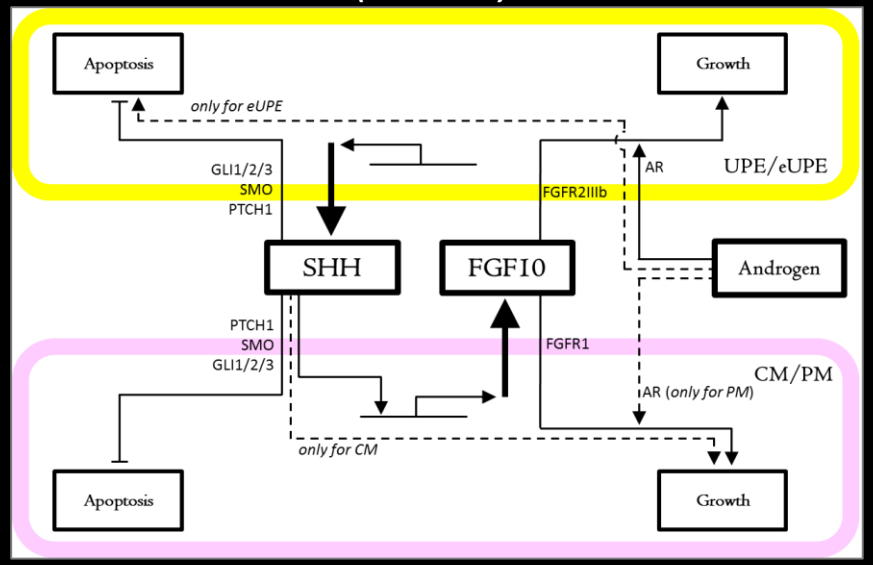
Genital Tubercle Differentiation

Genital tubercle (GT)

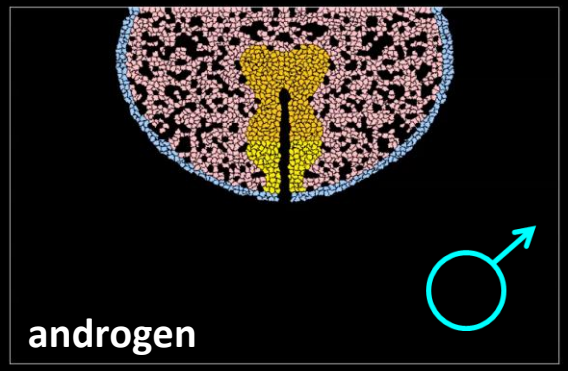


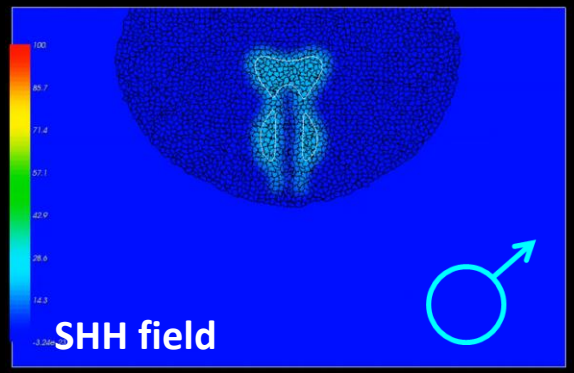


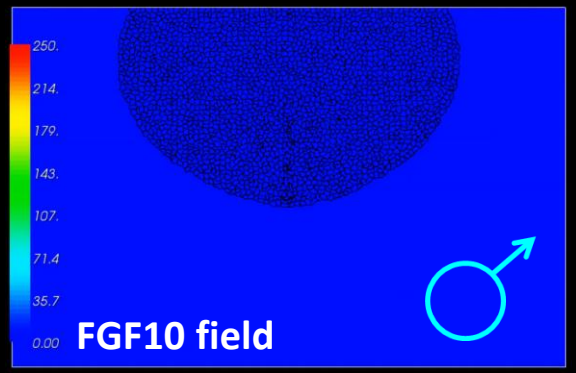
Control Network (mouse)

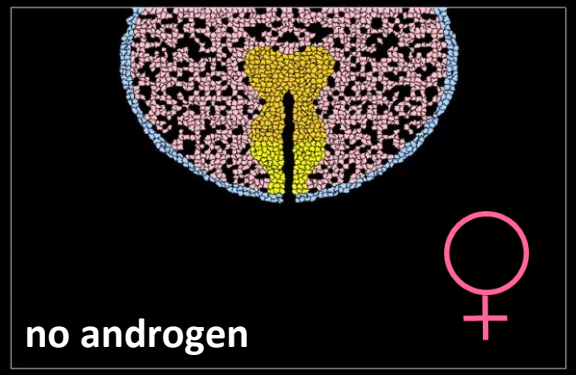


ABM simulation for sexual dimorphism (mouse GD13.5 – 17.5)





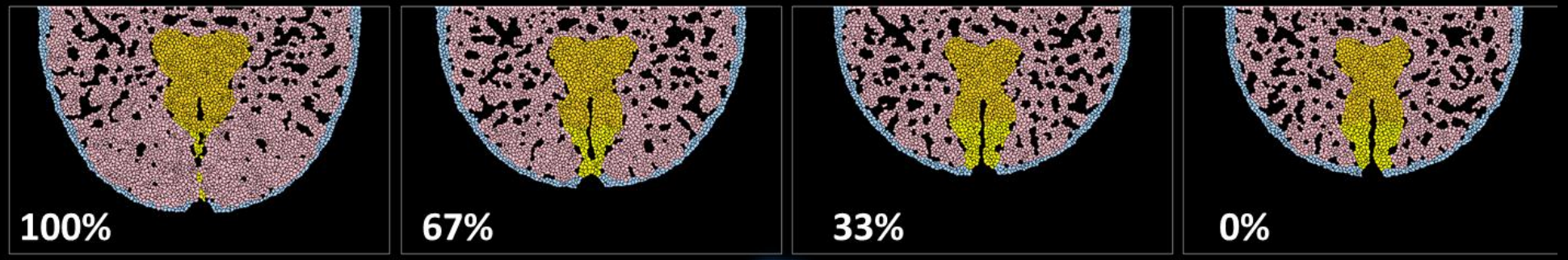




SOURCE: Leung et al. (2016) Reprod Tox

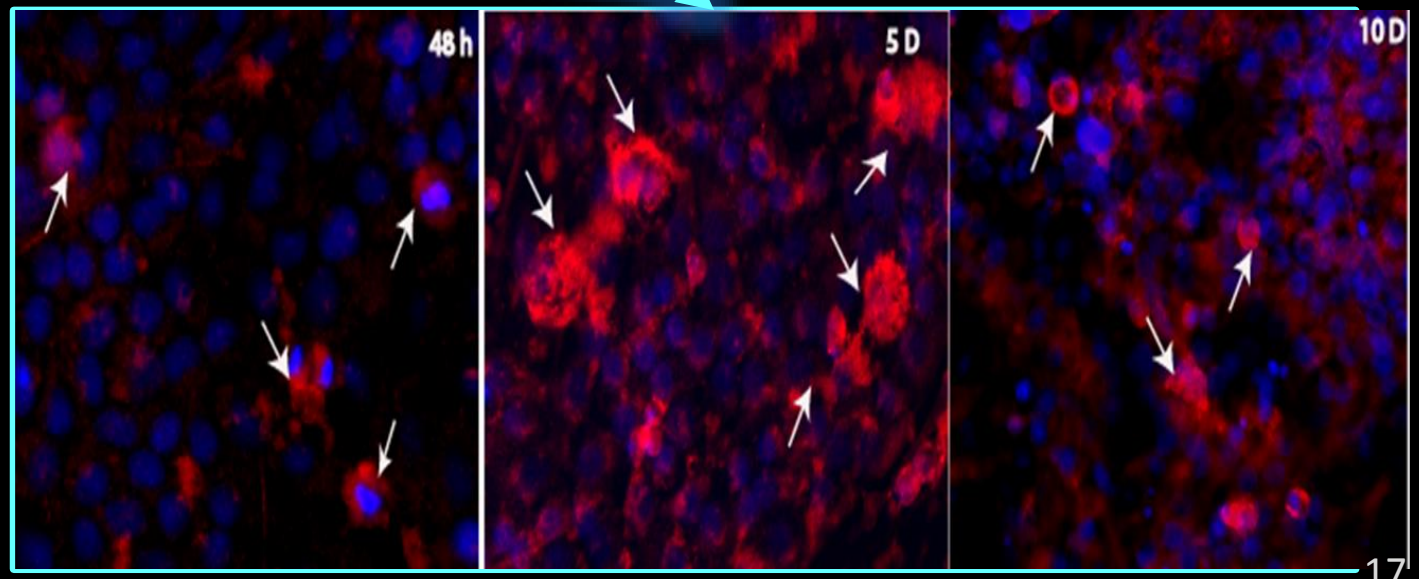
Hypospadias: *recapitulating urethral fusion and disruption in vitro*

Predicted impact of fetal testosterone deficiency on genital tubercle differentiation

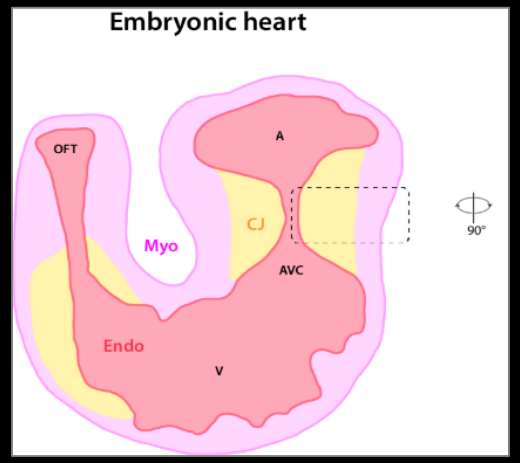


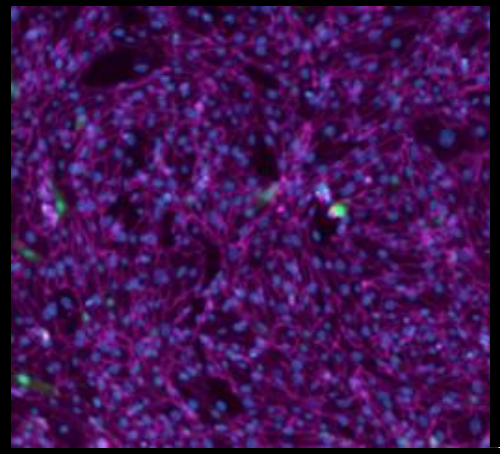
OCM for testicular development

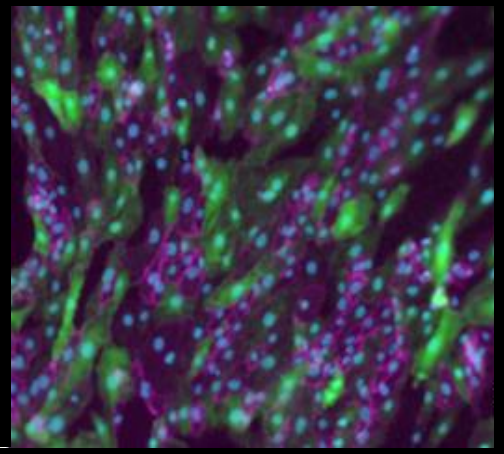
SOURCE: Harris et al. (2016) Toxicol In Vitro



Epithelial-Mesenchymal Transition: *disruption underlies some heart malformations (e.g., valvulo-septal defects)*

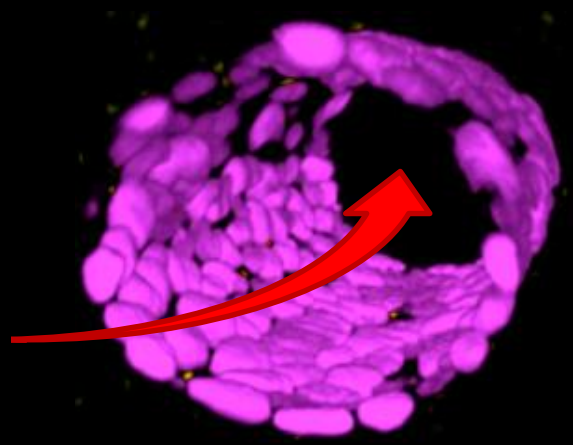


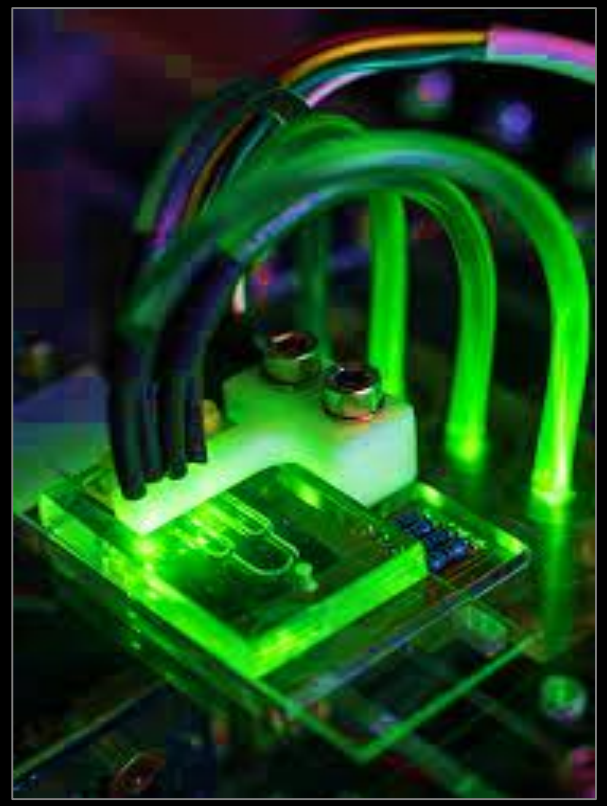




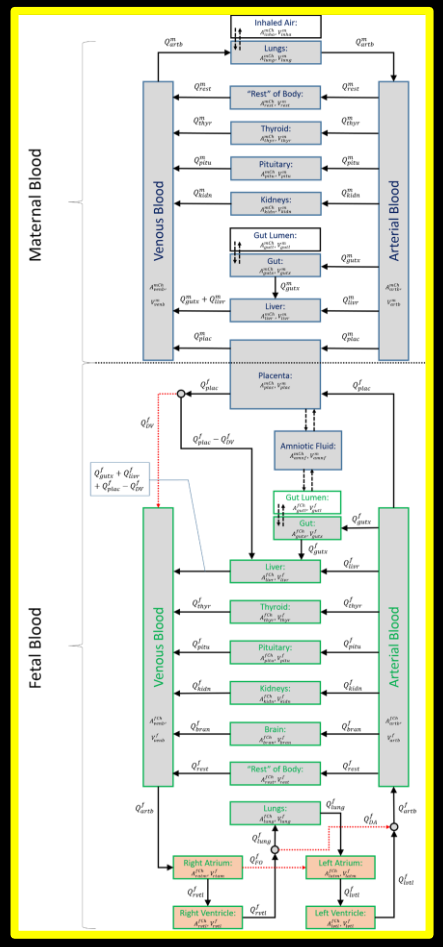
SOURCE: K Grode / S Hunter - NHEERL

… but endocardial EMT does not occur in a static environment: need to "go with the flow" (K Grode / D Belair – NHEERL)



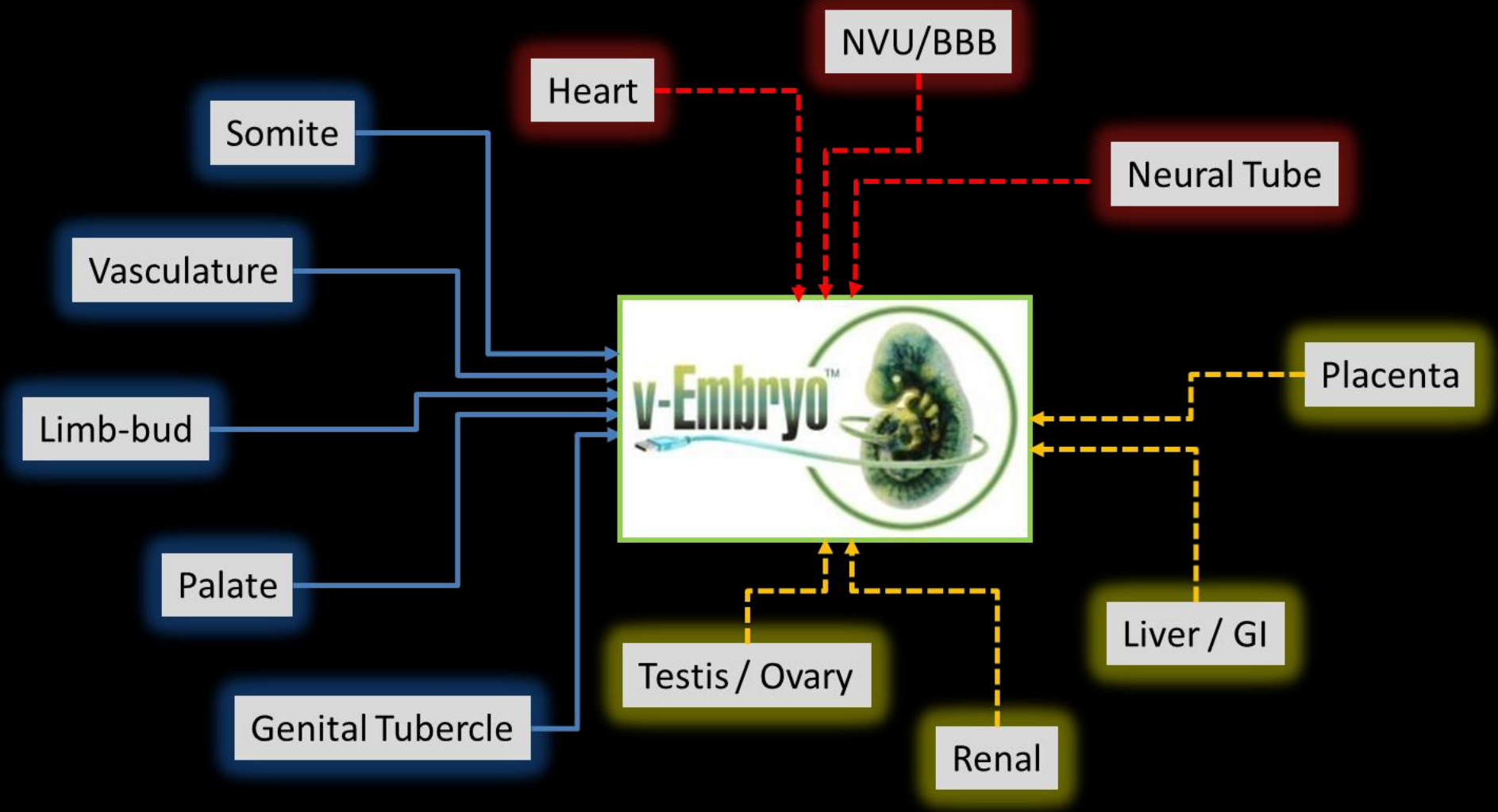


Fetal Microfluidic circuits: ‘testing the homunculus’



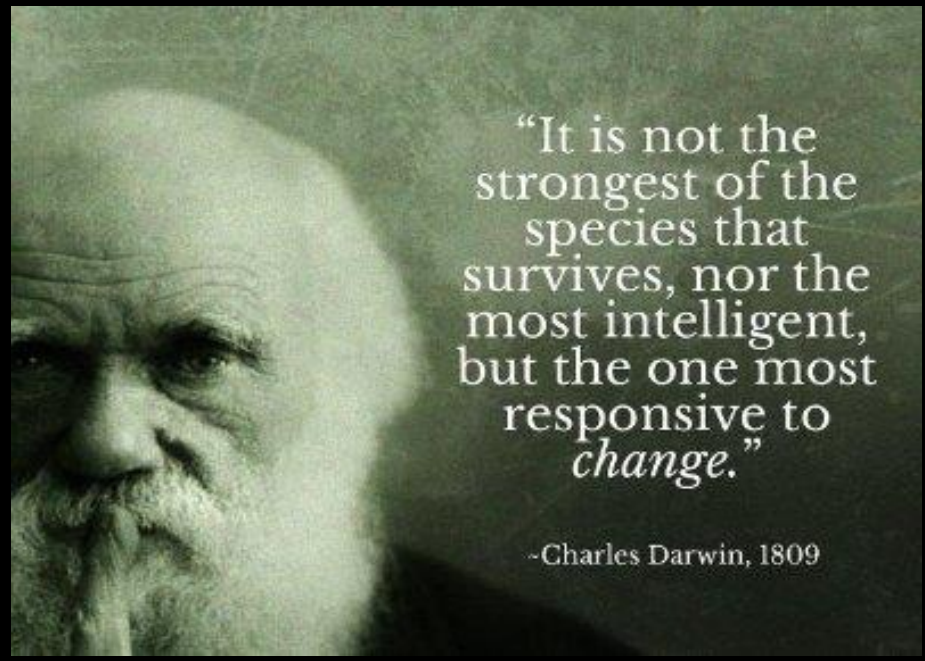
Fetal PBPK model
Kapraun et al. (NCCT)

Grand Challenge: *a predictive 'virtual embryo'*



Grand Challenge Questions for ‘Re/volution’

- How do we ethically capitulate ‘human embryology’ at a more physiological level than possible with cell culture?
- How far must ‘computational embryology’ advance to predict developmental toxicity in lieu of animal testing?
- How can ‘synthetic toxicology’ help shift regulatory reliance from animal studies to mechanistic models?



Knudsen TB, Klieforth B and Slikker W Jr (2017) Programming microphysiological systems for children’s health protection. Exp Biol Med (in press)

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http://www2.epa.gov/sites/production/files/2015-08/documents/virtual_tissue_models_fact_sheet_final.pdf