Microglia are Required for Anastomosis in a Computational Neurovascular Unit (cNVU)

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US EPA/ORD, National Center for Computational Toxicology (NCCT)

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Scaling Barriers: Cellular Dynamics and Models of Blood-Brain Barrier Developmental Toxicity

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Disclaimers

- The authors have no financial or other interests which pose a conflict of interest.
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- Some of the data presented here were collected by Vala Sciences Inc. or ArunA Biomedical under contract to the US EPA.
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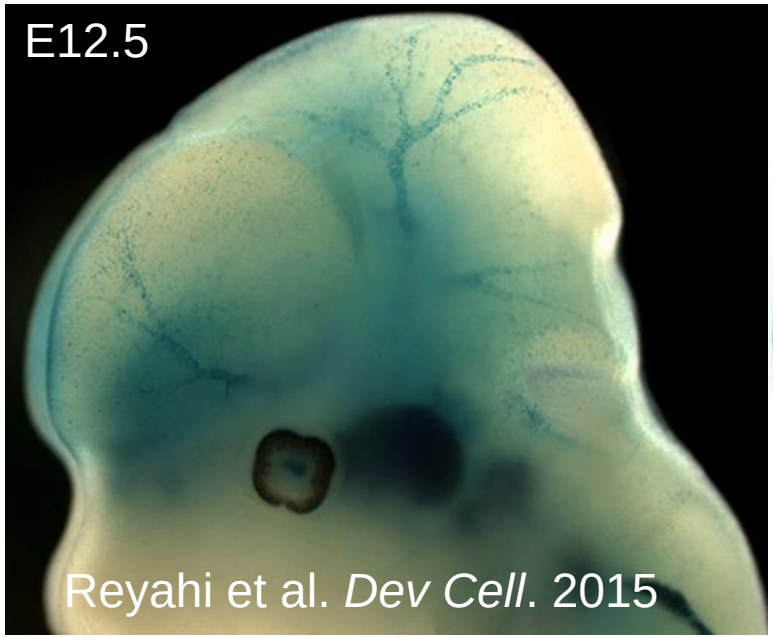
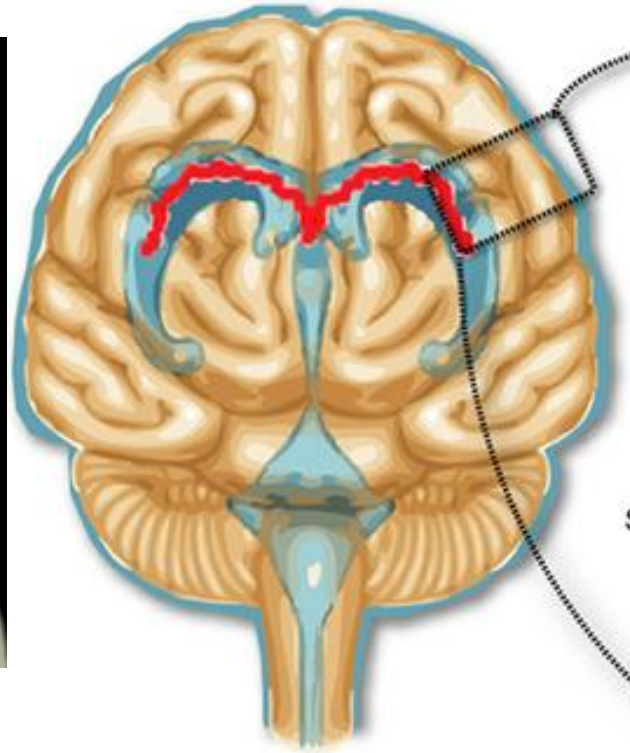
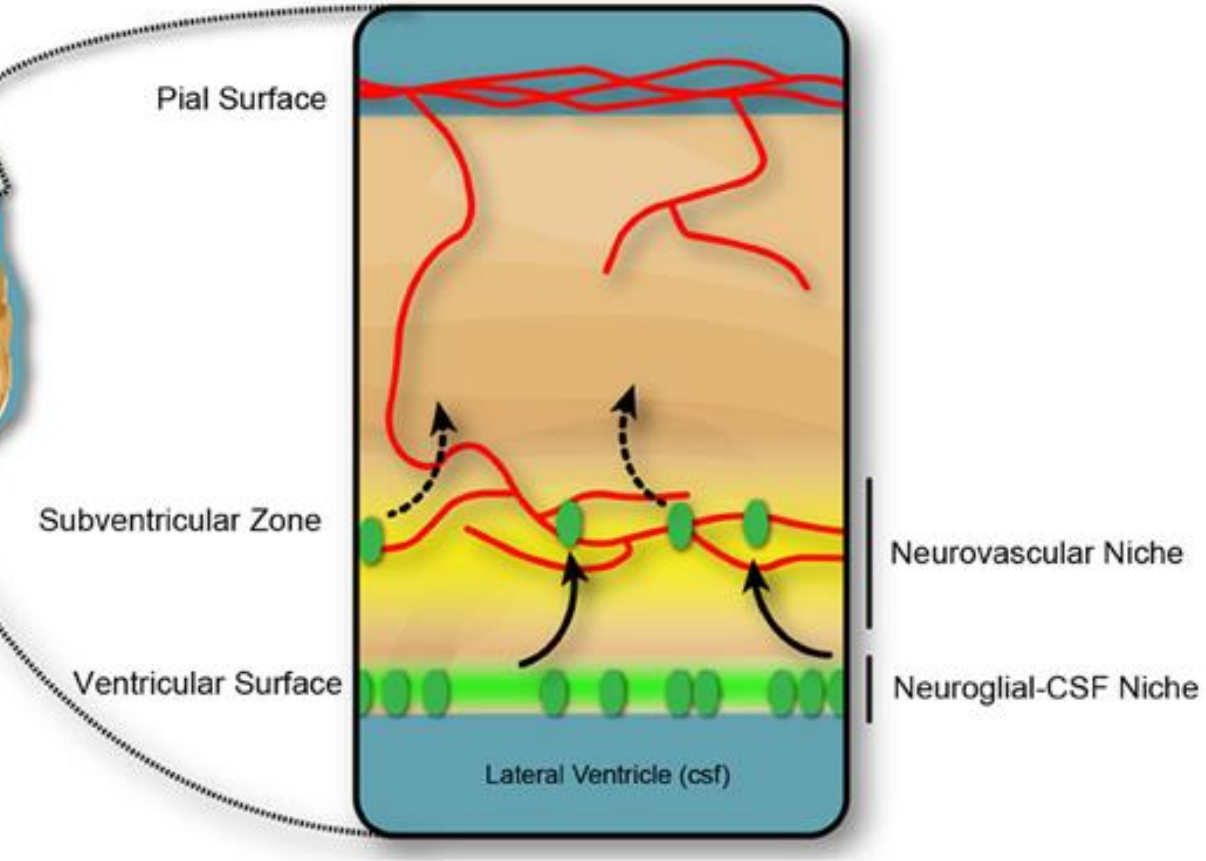
- The views expressed in this presentation do not reflect US EPA policy.

Computational neurovascular unit (cNVU) focus

Chemical signals from the neuroepithelium (eg, VEGF) initiate brain angiogenesis via sprouting from the PNVP.



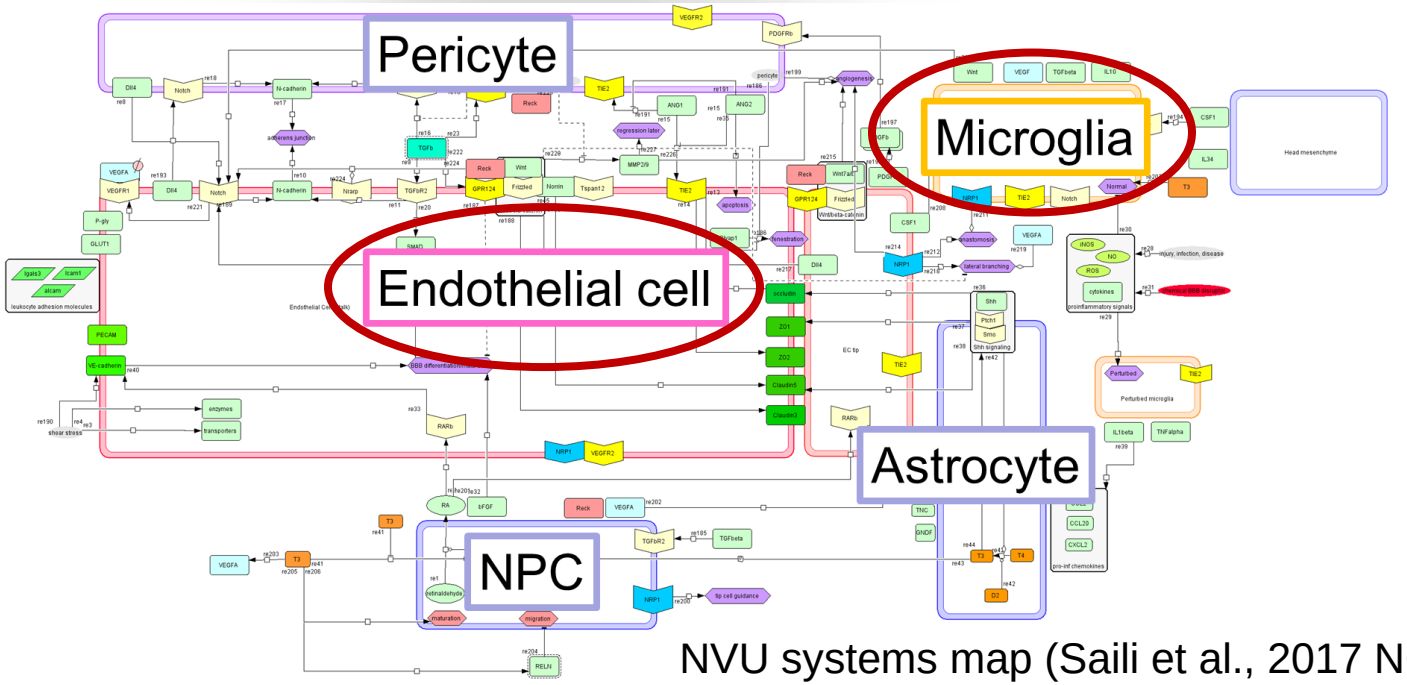




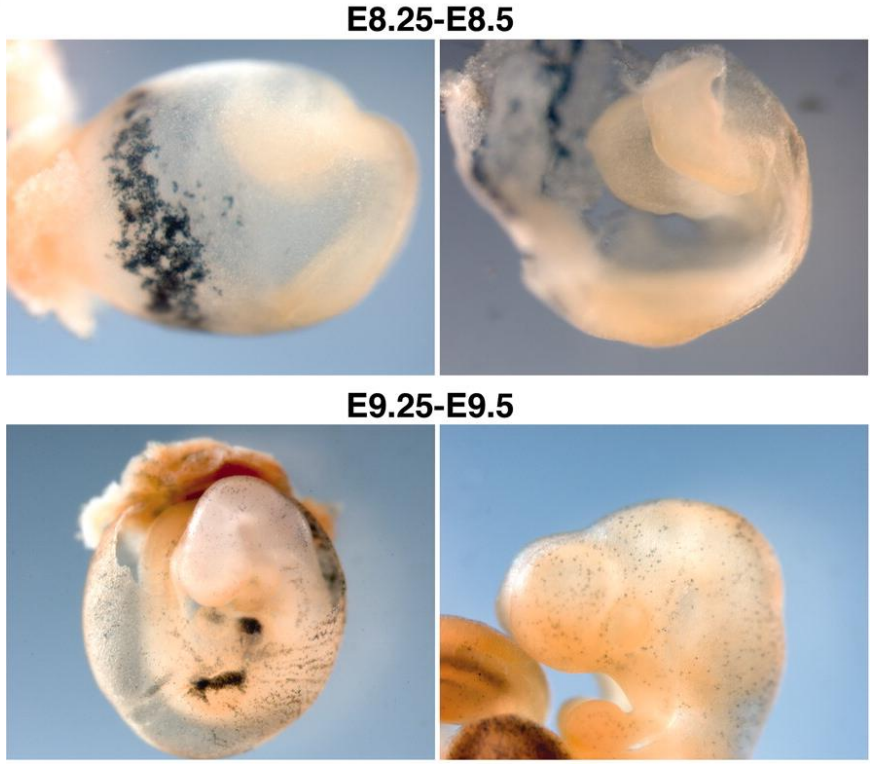
Stolp et al., *Front. Integr. Neurosci*. 2013

Hypothesis: Chemical disruption of NVU development adversely impacts blood-brain-barrier (BBB) formation leading to abnormal brain development and function.

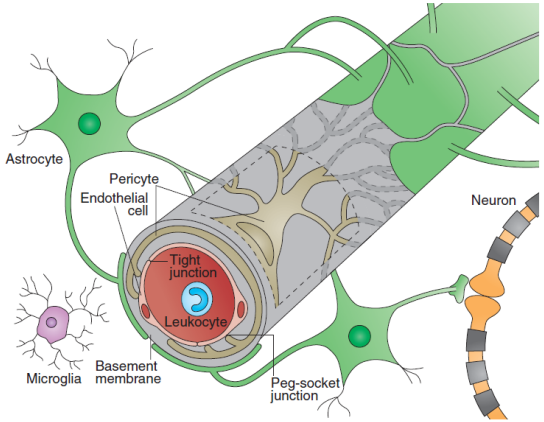
Cell-Cell interactions of the NVU



NVU systems map (Saili et al., 2017 NCCT)



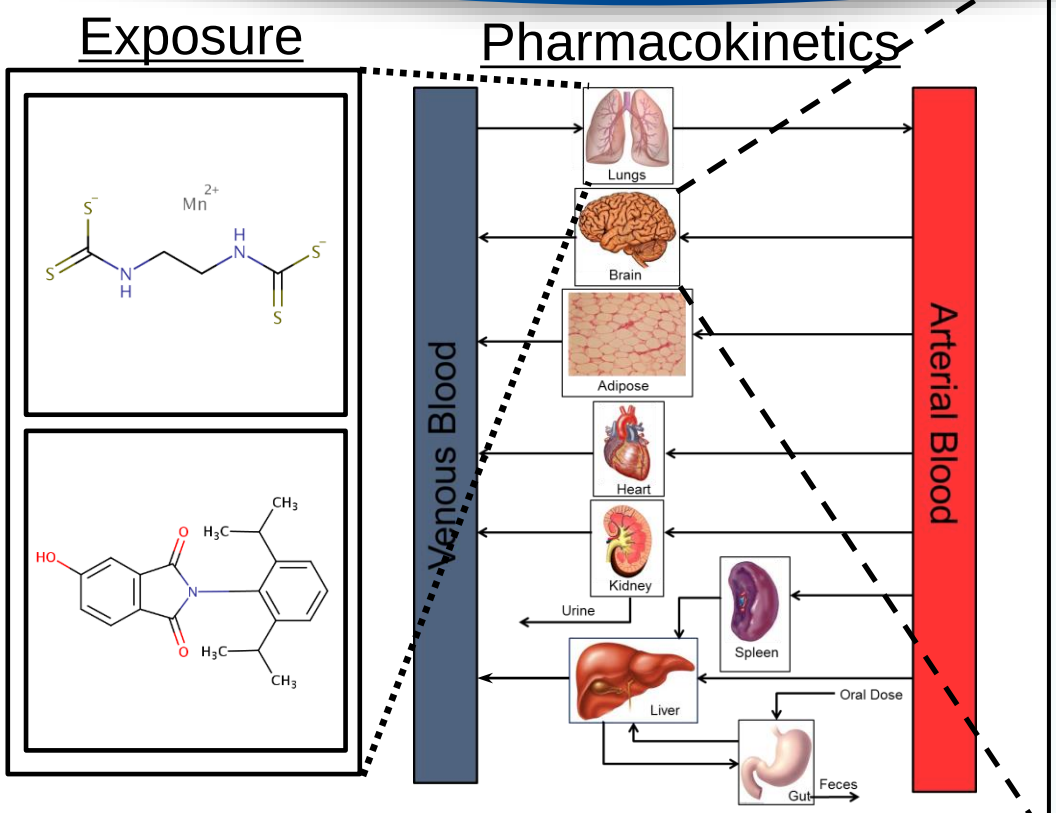
Ginhoux et al., *Science*, 2010



Obermeier et al, 2013

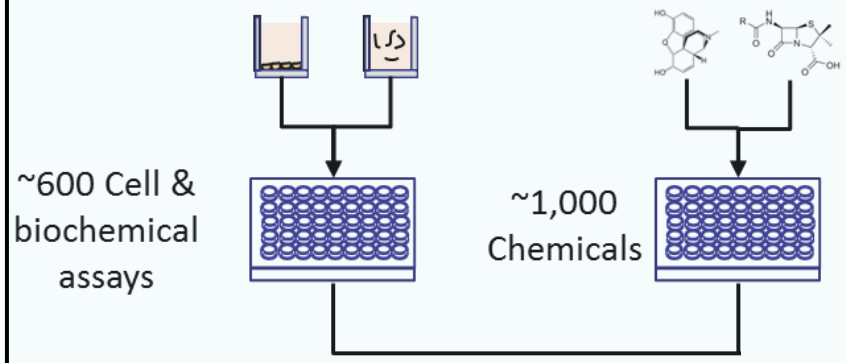
- Microglia, resident macrophages of the brain.
- During development…
  - Orchestrate neurovascular ramifications, surveillance of local injury where hyperactivation can invoke an adverse neuroinflammatory response
  - Are they mediators of developmental toxicity?

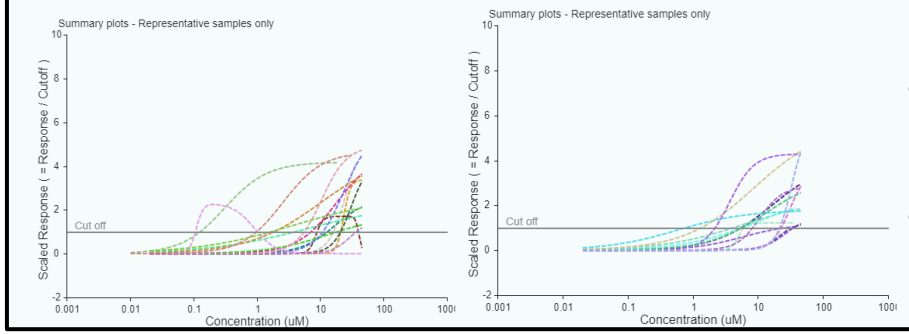
Computational source-to-outcome framework



Translation

In vitro assays





Pharmacodynamics

- Apoptosis
- Blood-brain barrier integrity
- Cell growth
- Dysmorphogenesis
- Endothelial cell migration

Utilize screening techniques to predict a concentration-dependent disruption of neurovascular development.

In vitro: Characterize chemical effects on cell-based phenotypes.

In silico: Use mechanistic information to translate HTS data into cell/tissue predictions.

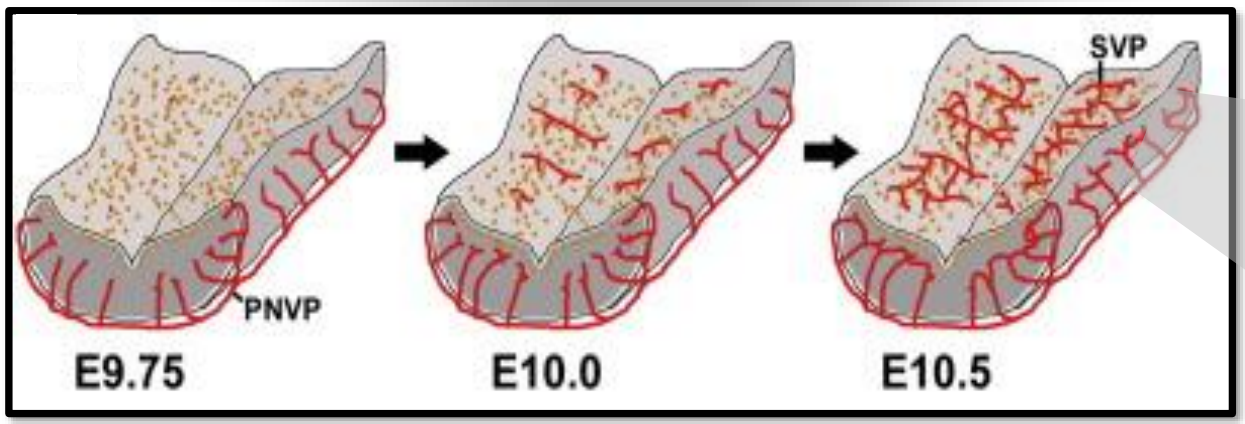
Cell Agent-Based Modeling

- **Agent-Based Modeling and Simulation (ABMS):** a heuristic approach to reconstruct tissue dynamics using knowledge of biochemistry and cell-by-cell interactions.
  - Program each *agent* (cell) to follow specific rules
  - Interactions of agents gives rise to *emergent features* (phenotypic outcomes)
  - Qualify emergent feature with experimentally derived phenotypes (tissue level morphology)
  - Make toxicodynamic predictions by integrating biological knowledge & high throughput data

- **CompuCell3D*:** open source modeling environment
  - Rules (steppables) for distinct cell behaviors (growth, proliferation, apoptosis, differentiation, polarization, motility, ECM, signal secretion, …);
  - Rules coded in Python for cell-autonomous 'agents' that interact in shared microenvironment and self-organize into emergent phenotypes.
  - Methodology applied to past systems: vasculogenesis, genital tubercle, palate fusion, etc.

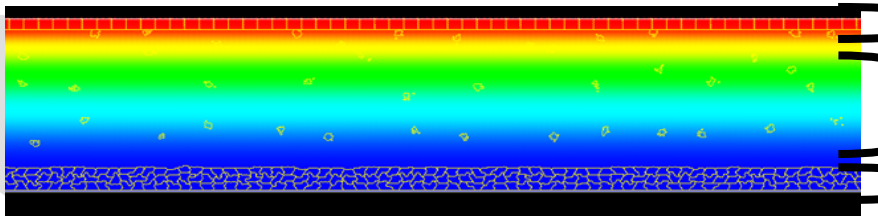
*James Glazier and colleagues, Indiana University

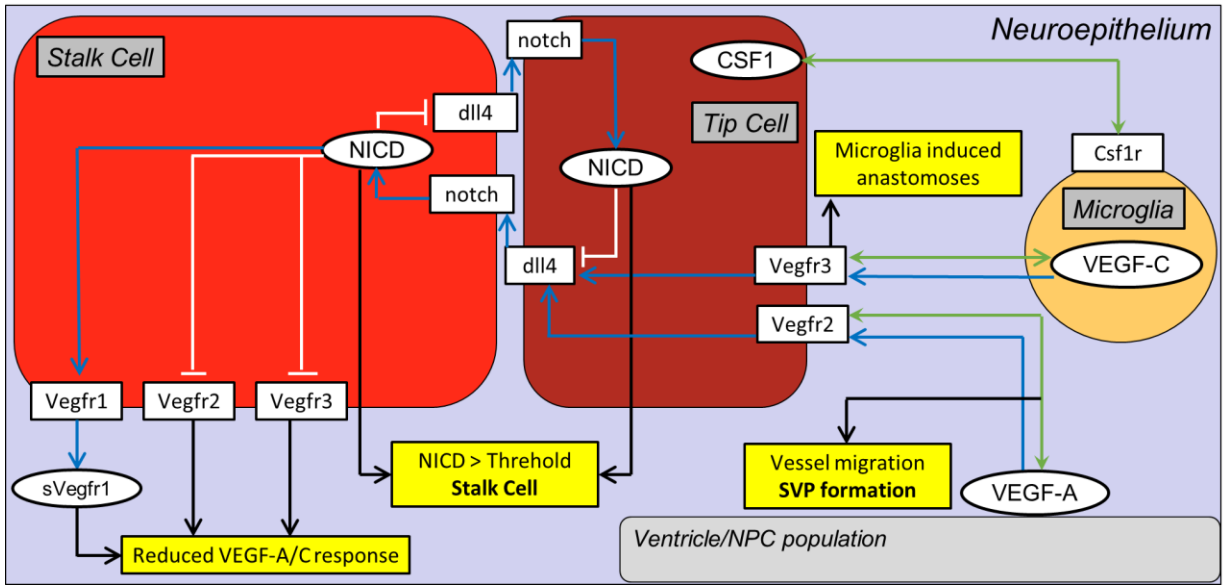
Modeling brain angiogenesis

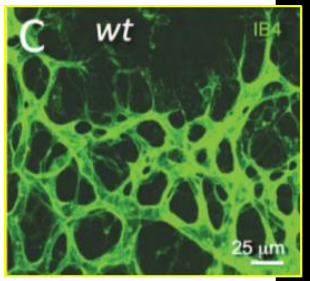


Fantin et al., (2013) Nature Protocols

VEGF-A gradient: NPCs in the subventricular zone

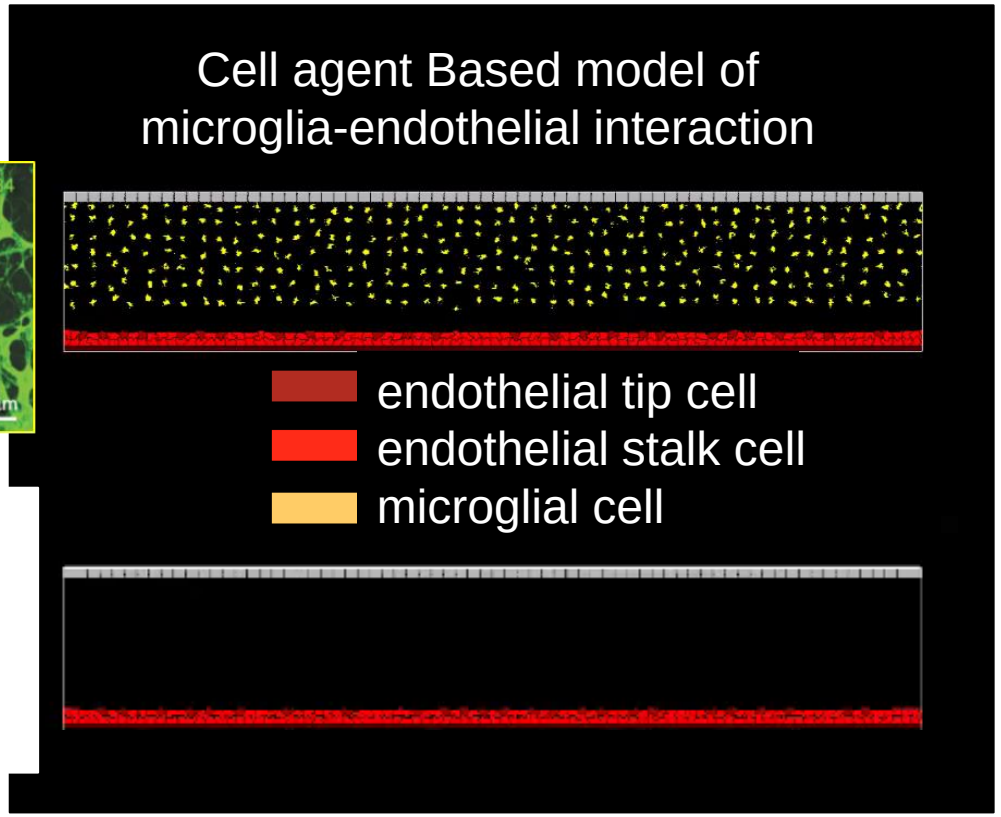




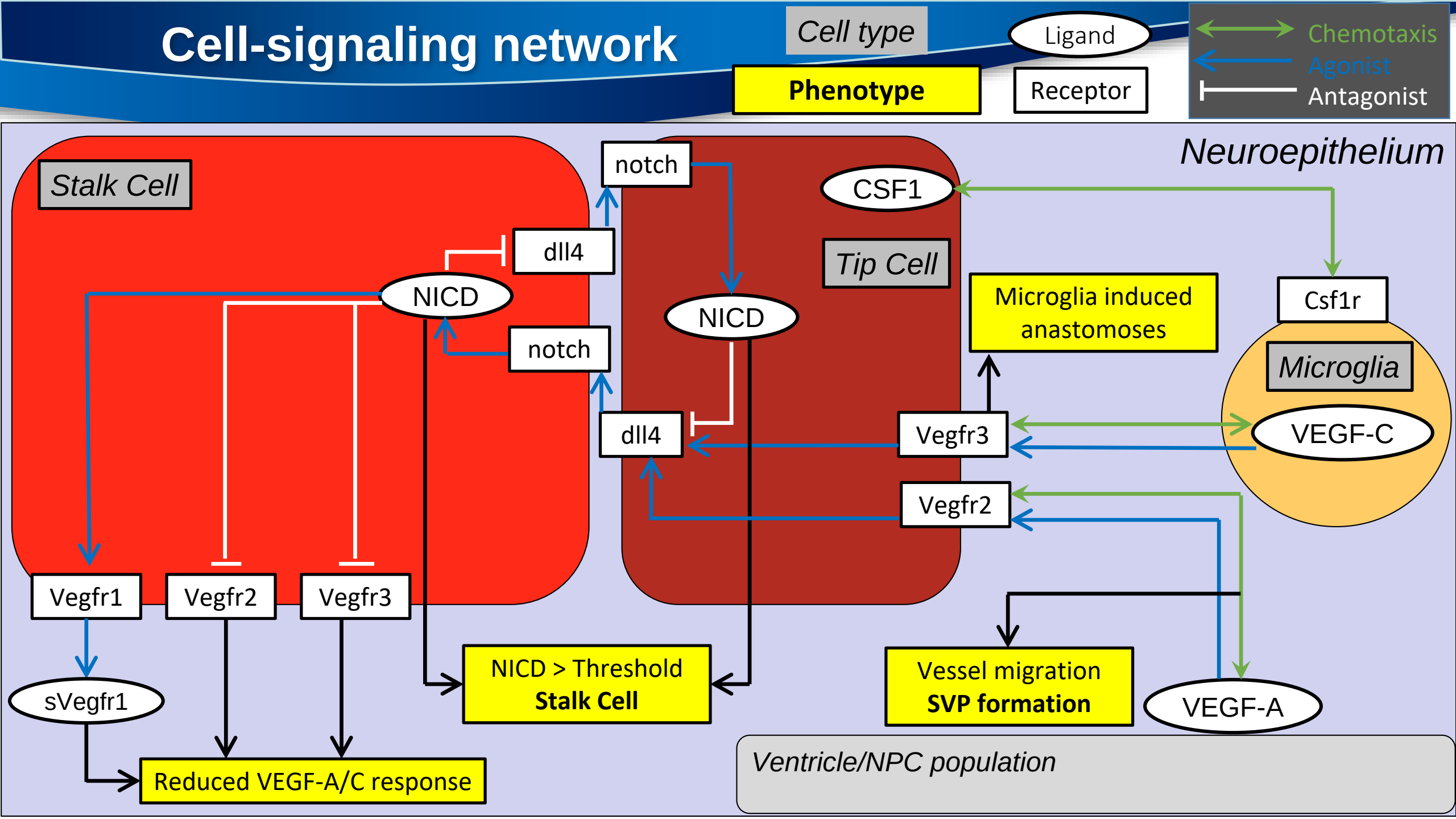


Rymo et al. (2011) PLoS One

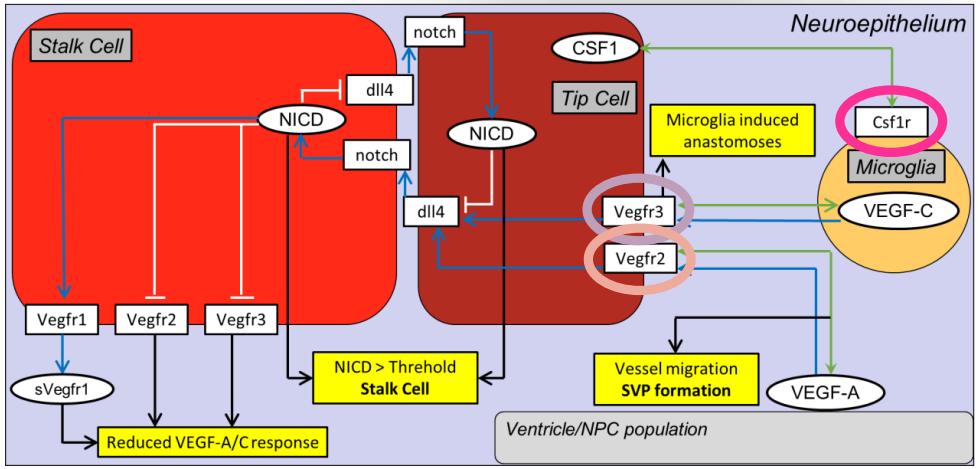
Cell agent Based model of microglia-endothelial interaction

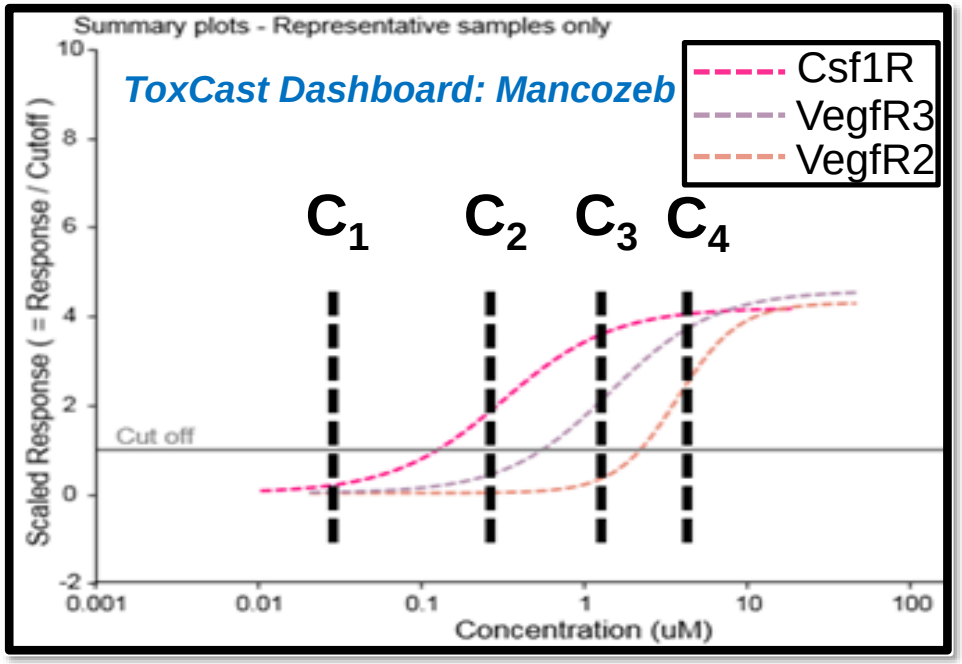


Cell-signaling network

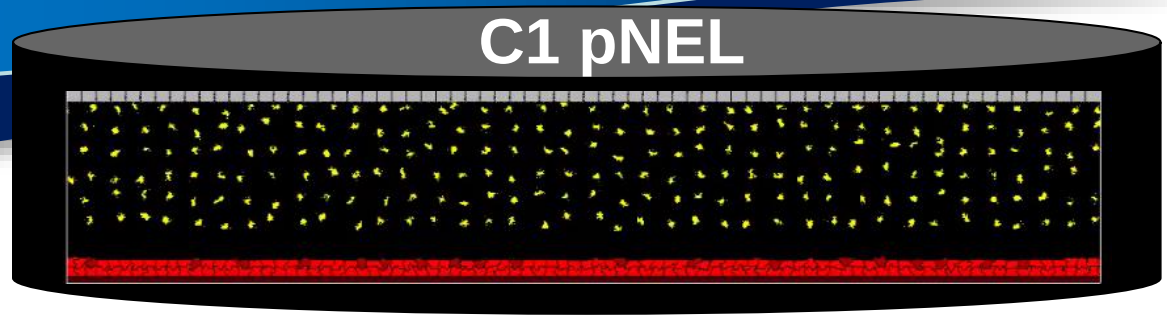


Translating HTS data - mancozeb

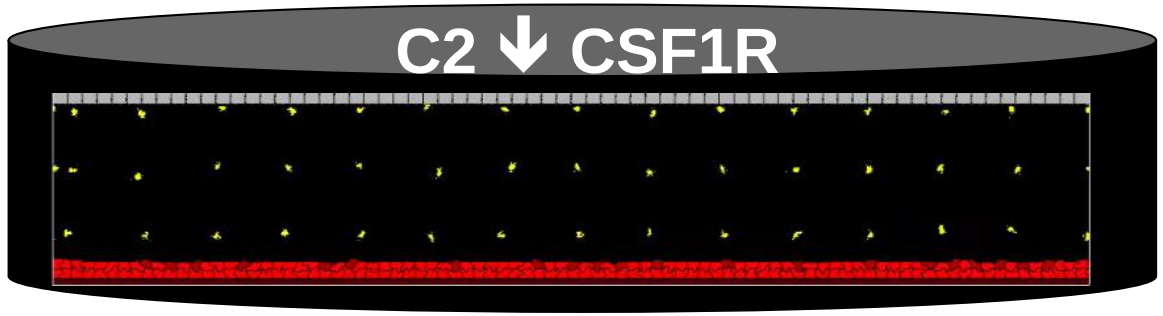




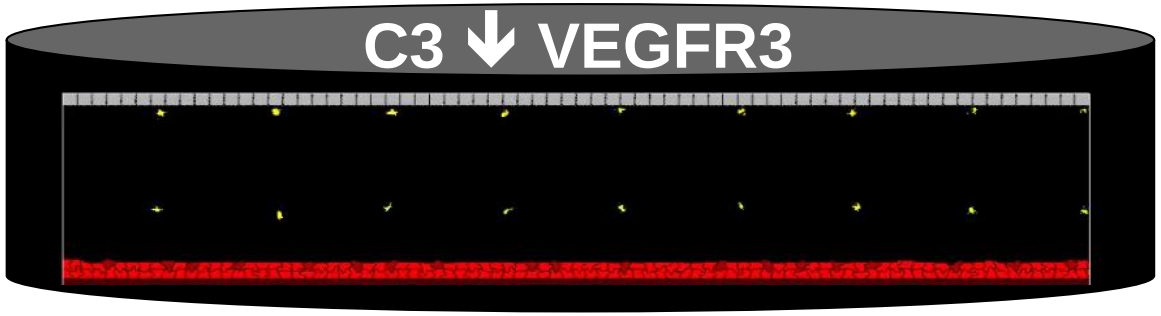
0.03 µM
No significant reduction in any receptor



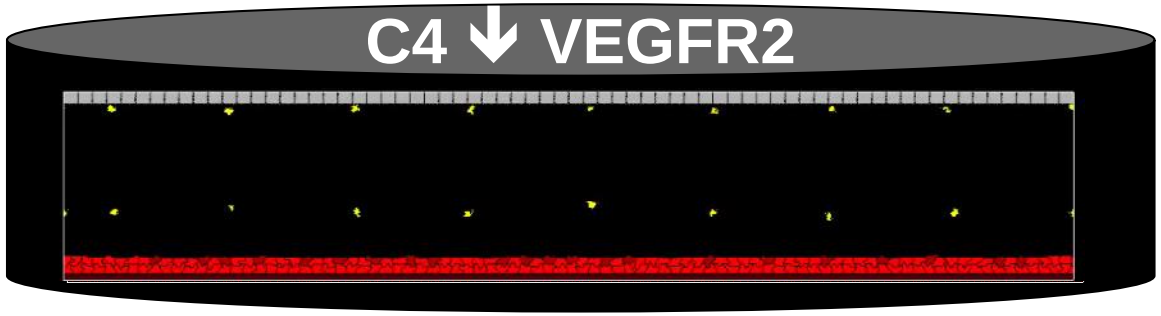
0.3 µM
50% ↓ CSF1R



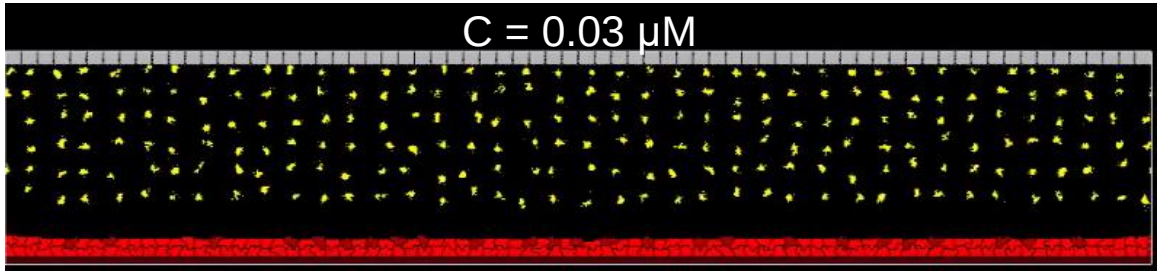
2 µM
50% ↓ VEGFR3
80% ↓ CSF1R

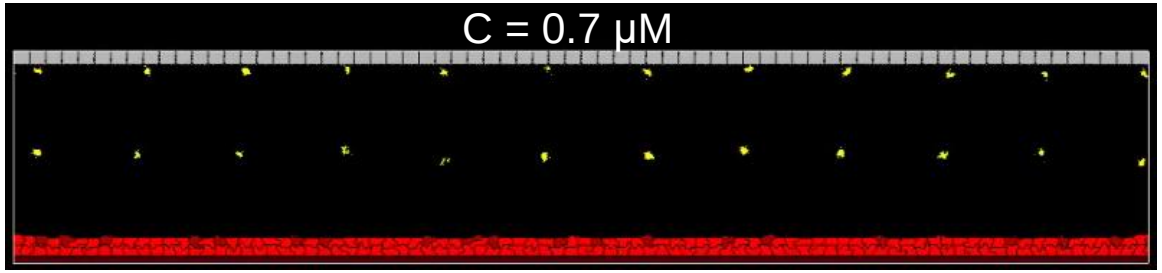


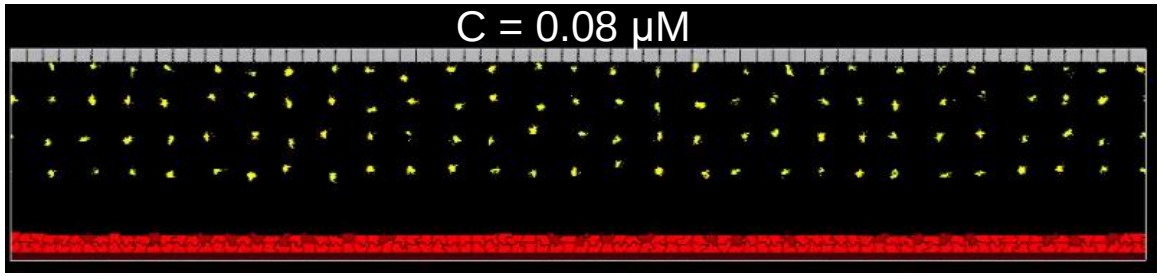
7 µM
50% ↓ VEGFR2
85% ↓ VEGFR3
95% ↓ CSF1R

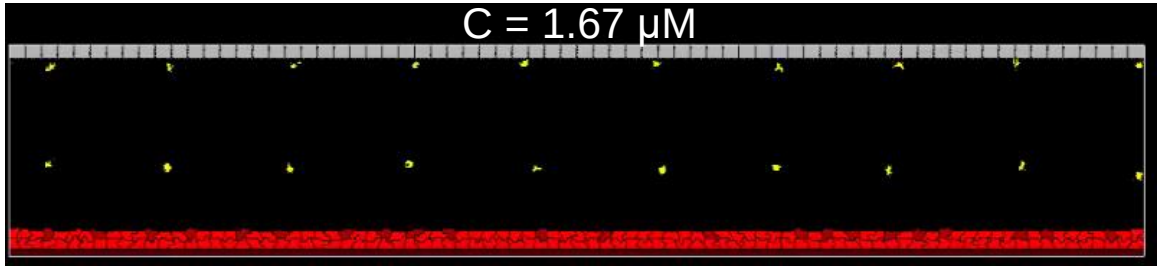


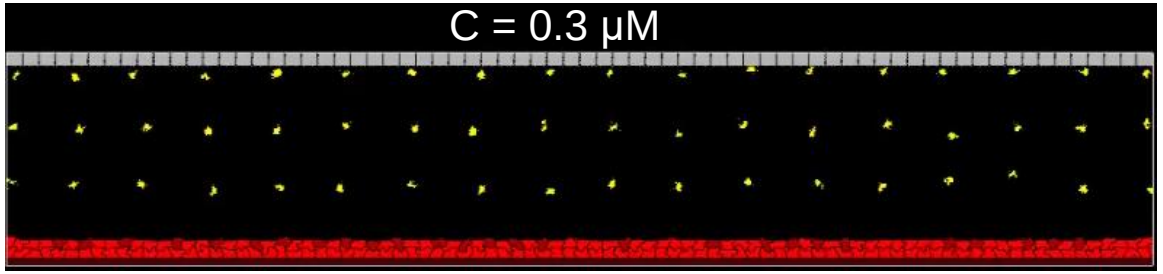
Visualizing the qualitative dose response

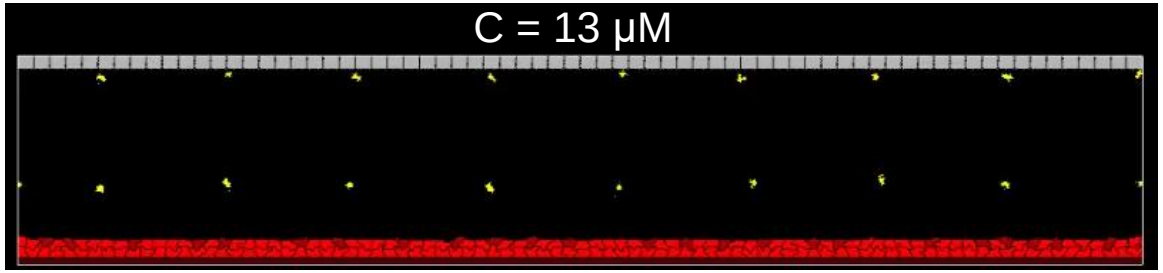








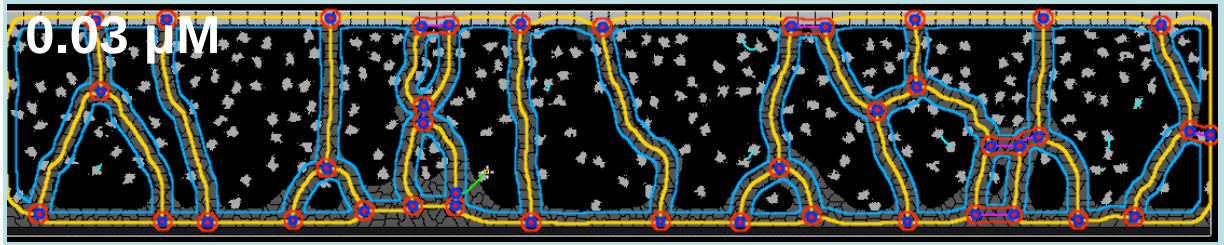




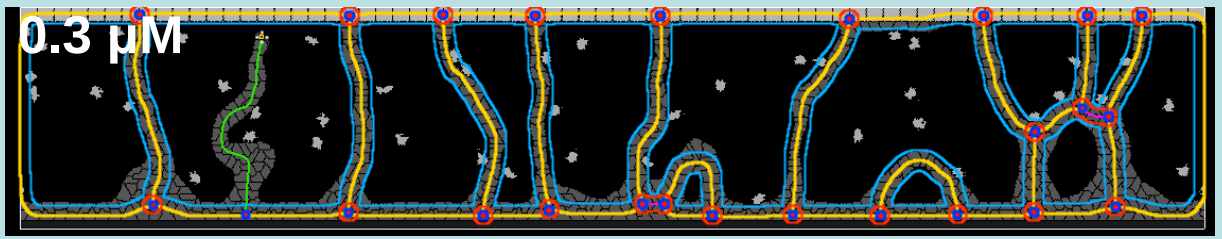
- Changes in vasculature are visualized through escalating dose response translated from ToxCast HTTS data
  - Reduction in microglia
  - Reduction in endothelial cell migration

Vascular quantitation - mancozeb

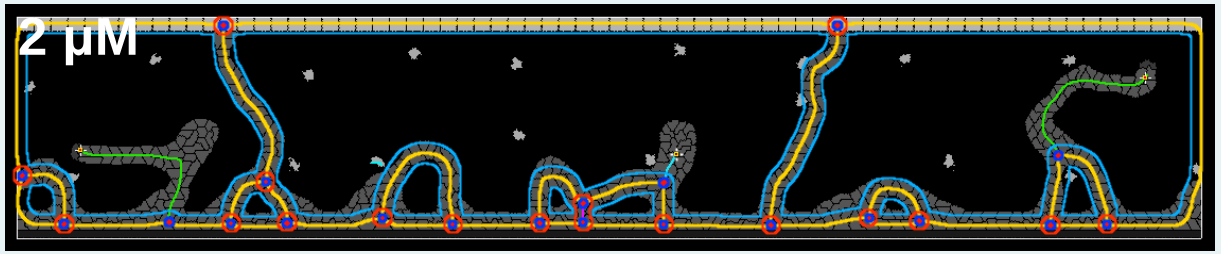
Image analysis in ImageJ: Angiogenesis Analyzer



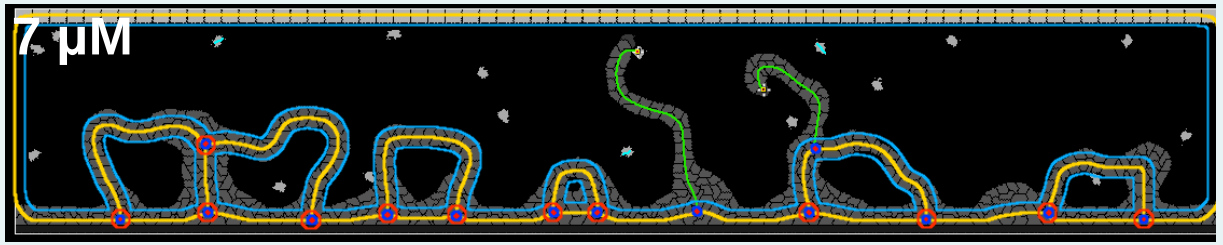
Predicted NEL (pNEL): No changes to vasculature



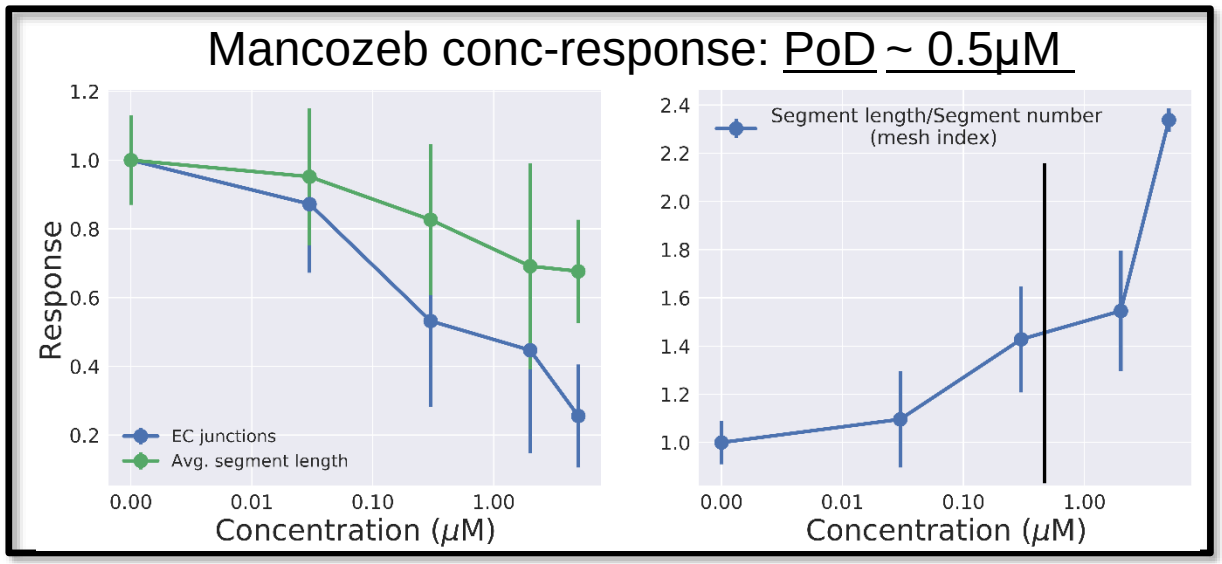
Predicted LEL (pLEL): Reduced tortuosity



Reduction in overall vascular area

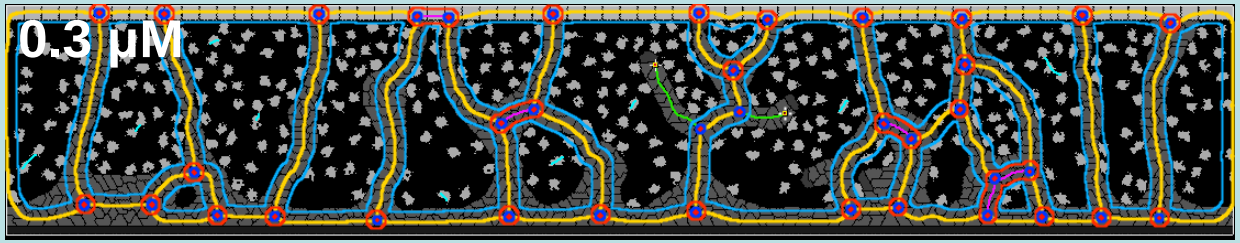


Hypo-vascular angiodysplasia

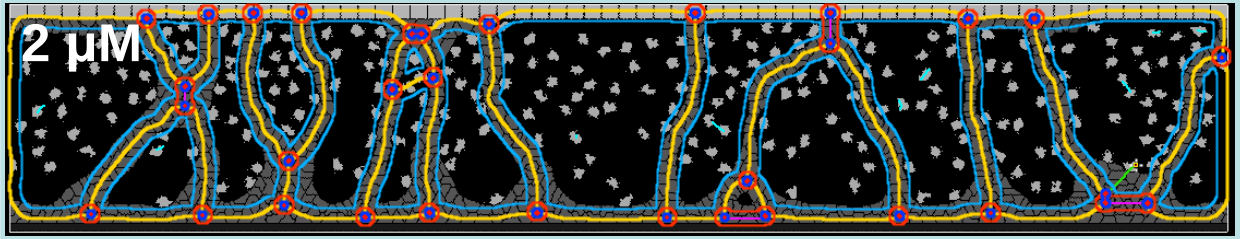


- Quantitate multiple vascular network endpoints in concentration response.
- Running multiple simulations allows us to account for stochastic variability.

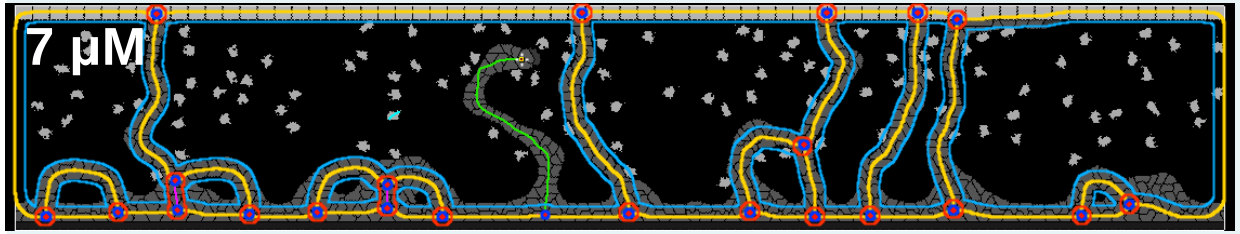
Vascular quantitation – oxytetracycline dihydrate



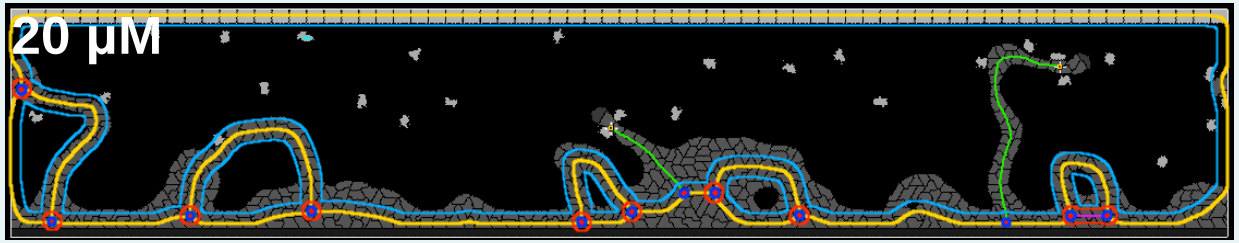
Predicted NEL (pNEL): No changes to vasculature



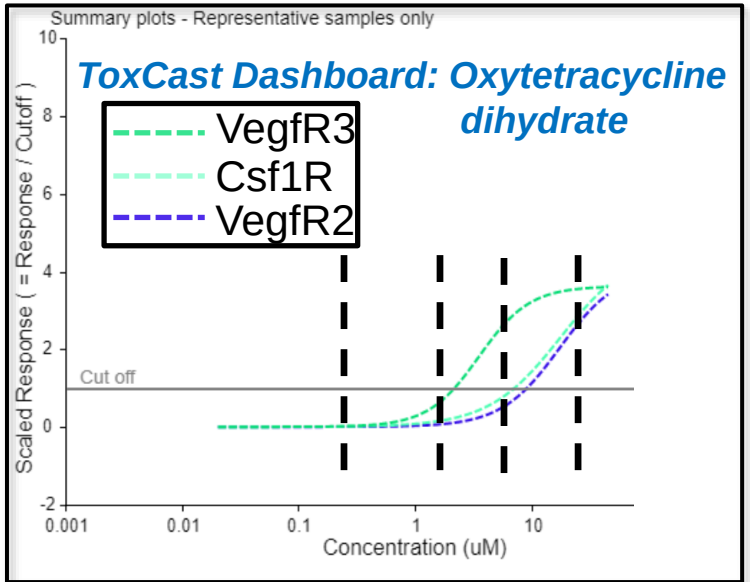
Predicted LEL (pLEL): Reduced vascular area



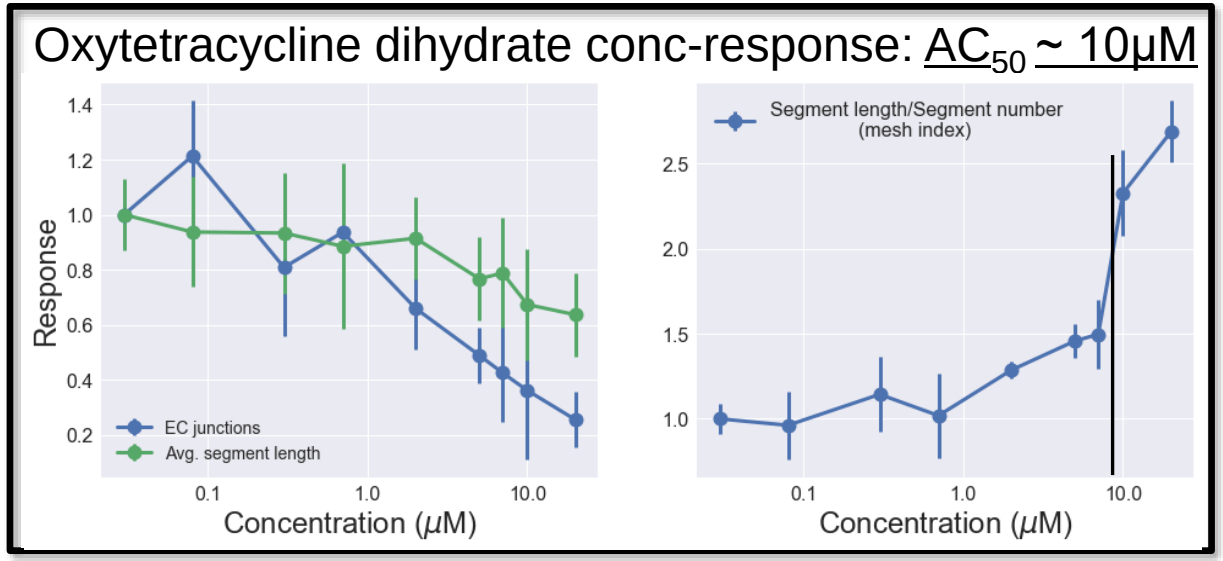
Reduced branching and anastomoses



Hypo-vascular angiodysplasia

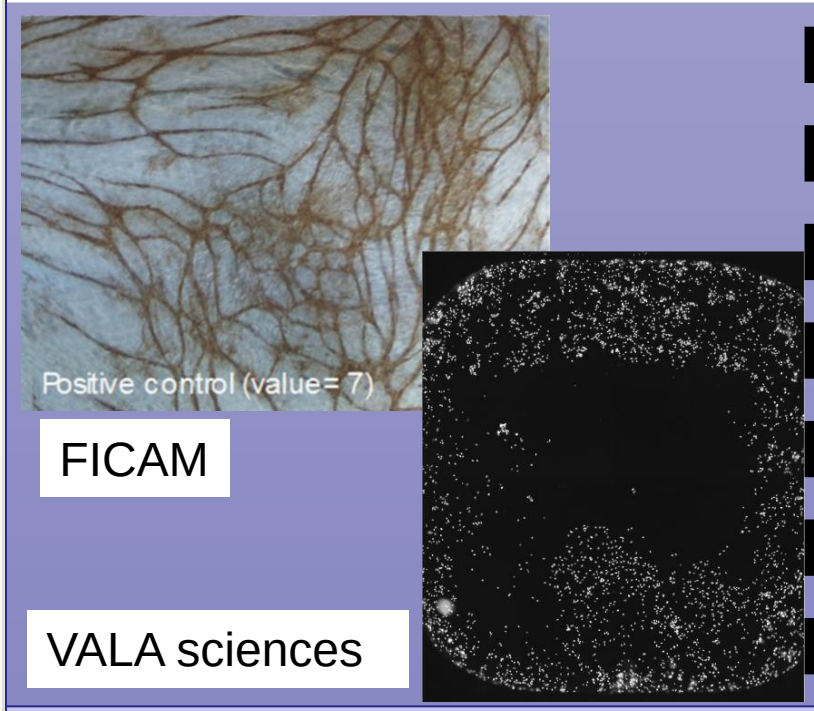


VEGFR3 serves as the more sensitive angiogenesis endpoint for oxytetracycline dihydrate exposure



Experimental comparison

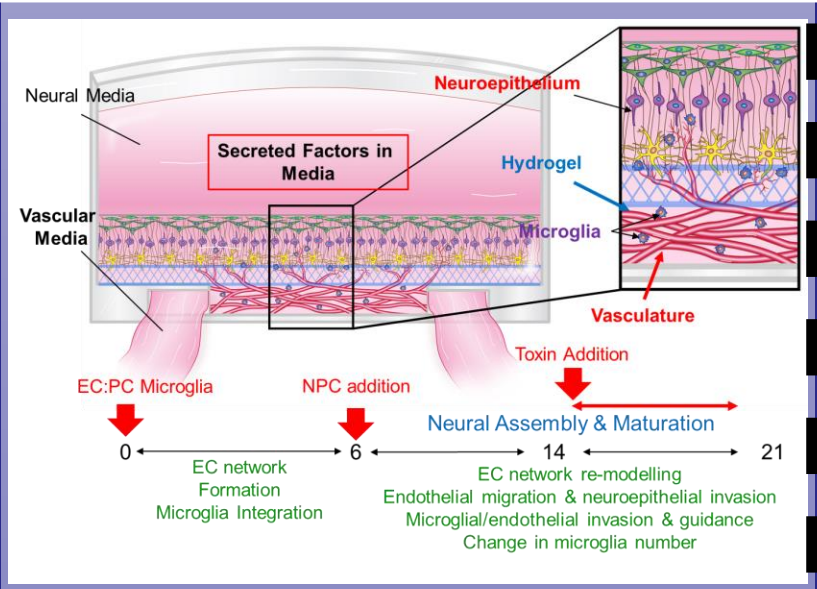
Single cell models



FICAM

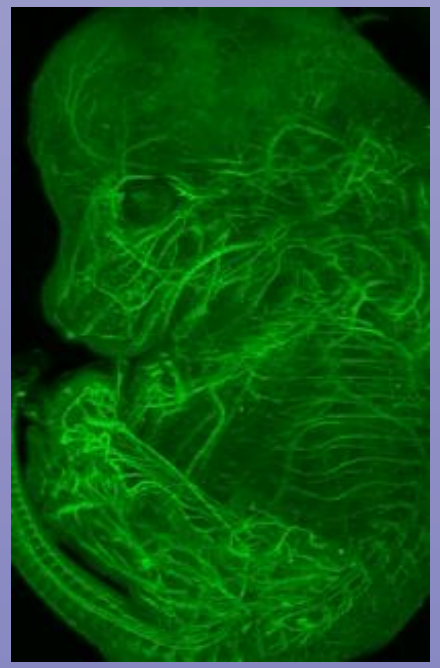
VALA sciences

Organotypic culture models



Kaushik, H-MAP, Wisc

In vivo



<u>Endothelial cell (EC) endpoints</u>

- EC area
- EC protrusions
- EC nodes
- Network formation
- EC migration

<u>Multi-cellular endpoints</u>

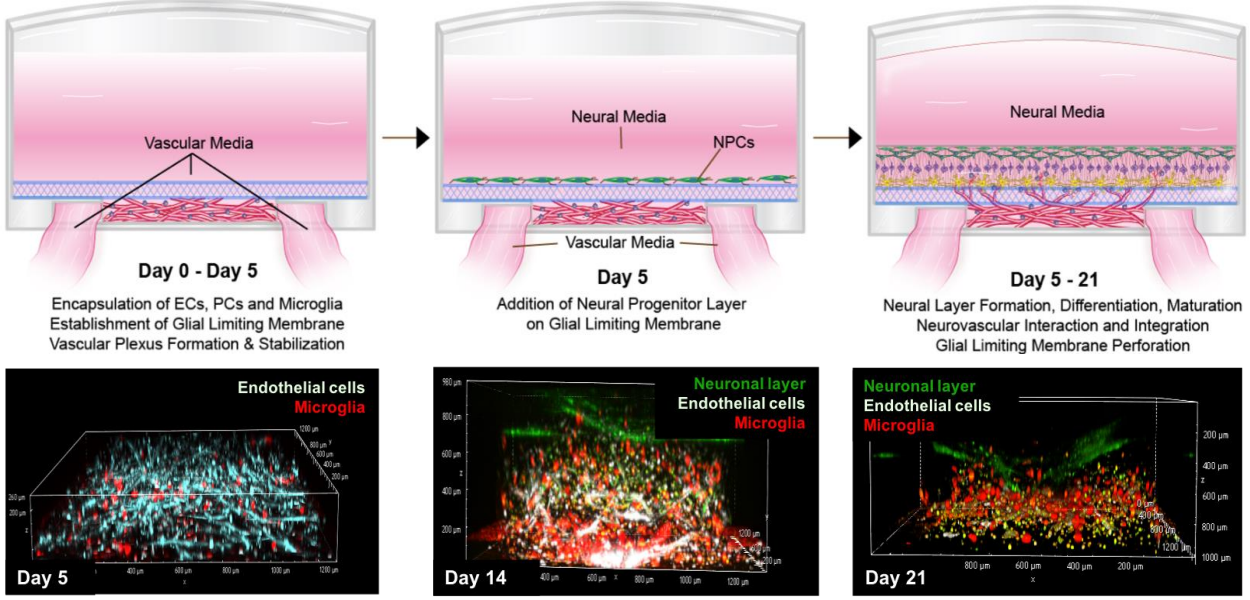
- Microglia migration
- Endothelial cell migration
- Endothelial cell connectivity
- VE-Cadherin

<u>Barrier-specific endpoints</u>

- FITC permeability
- Claudin/Occludin expression
- Neural-specific endpoints

Neurovascular unit OCM

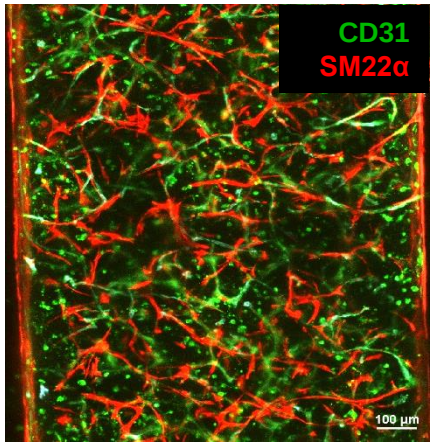
(A) **Time-course development of PNVP model *in vitro***

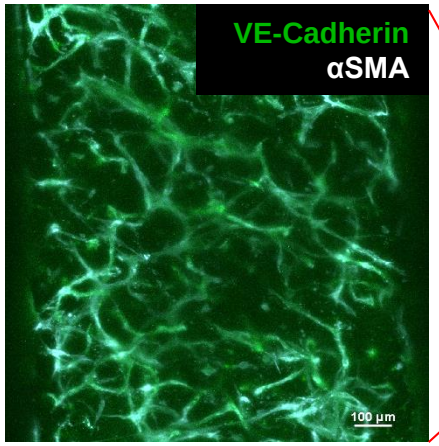


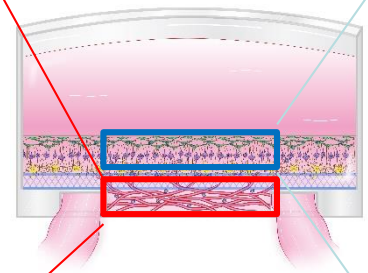
- Time-course invasion of endothelial cells into a neural compartment
- Constructed as a microfluidic device on a 96-well plate for higher throughput testing

G. Kaushik, W. Murphy, W. Daly, UW Madison

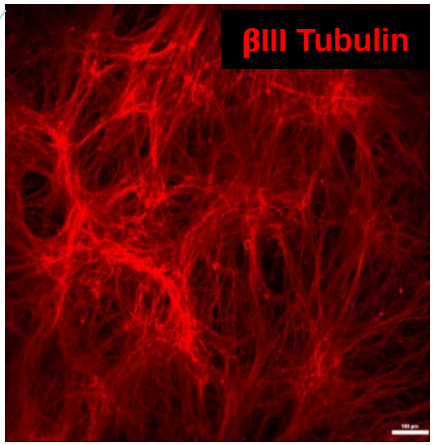
(B) **Vascular network characterization**

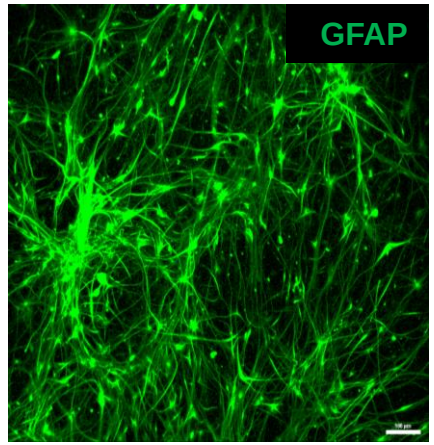






(C) **Neural layer characterization**



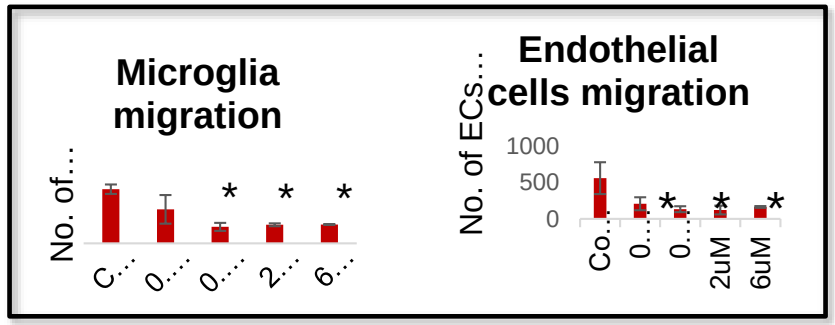


Model evaluation with *in vitro* models

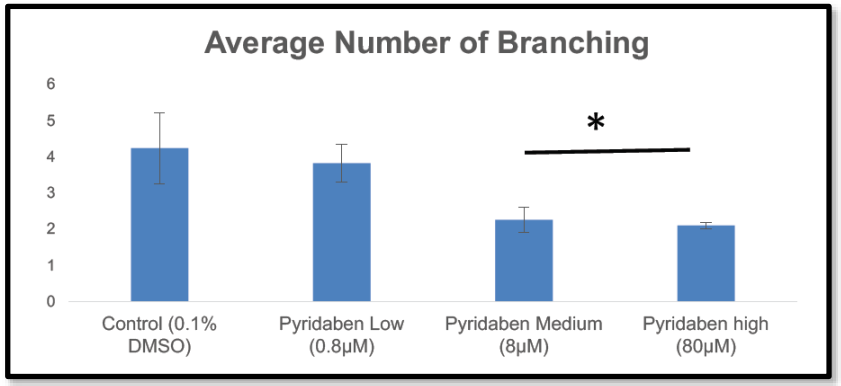
OCM NVU

Chemical response results from *in vitro* NVU OCM to compare to *in silico* endpoints

Mancozeb



Pyridaben



PoD Comparison

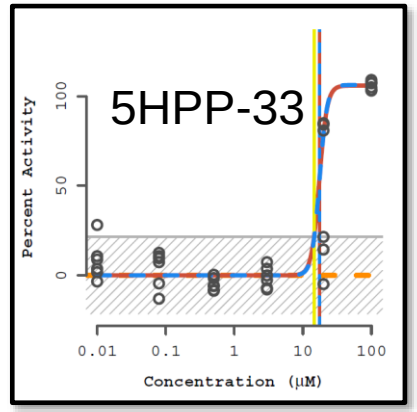
Comparison of predictions across various platforms

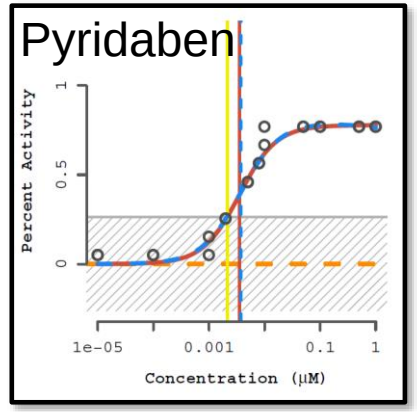
Chemical	*In silico (PoD)*	*OCM (LOEC)*	*EC* (AC_{50})*
Mancozeb	0.5 μM	0.3 μM	9 μM
Maneb	10 μM	20 μM	N/A
Oxytetracycline dihydrate	10 μM	N/A	30 μM
Pyridaben	12 μM	8 μM	0.005 μM
5HPP-33†	80 μM	20 μM	12 μM

*Cell-based high throughput assay
†No effect on microglia population in OCM

HTTS Assays

Chemical response results from HTTS endothelial cell assays to compare to *in silico* endpoints

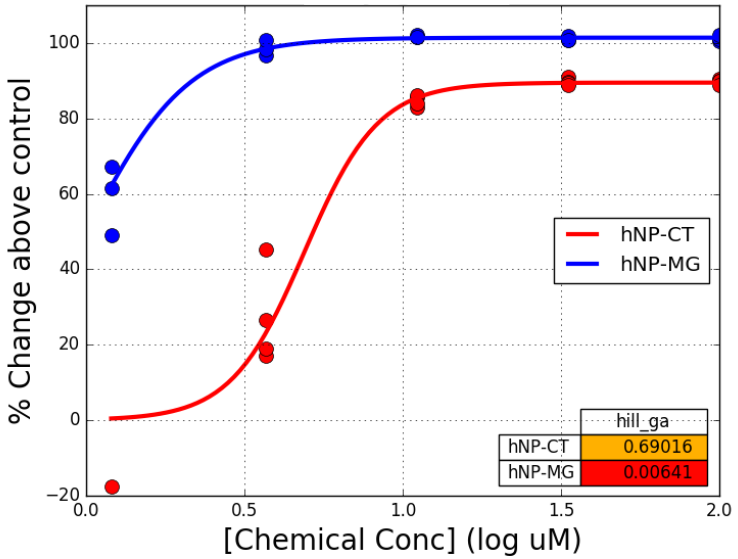




NVU cell-based assays

Zurlinden et al., *in prep*

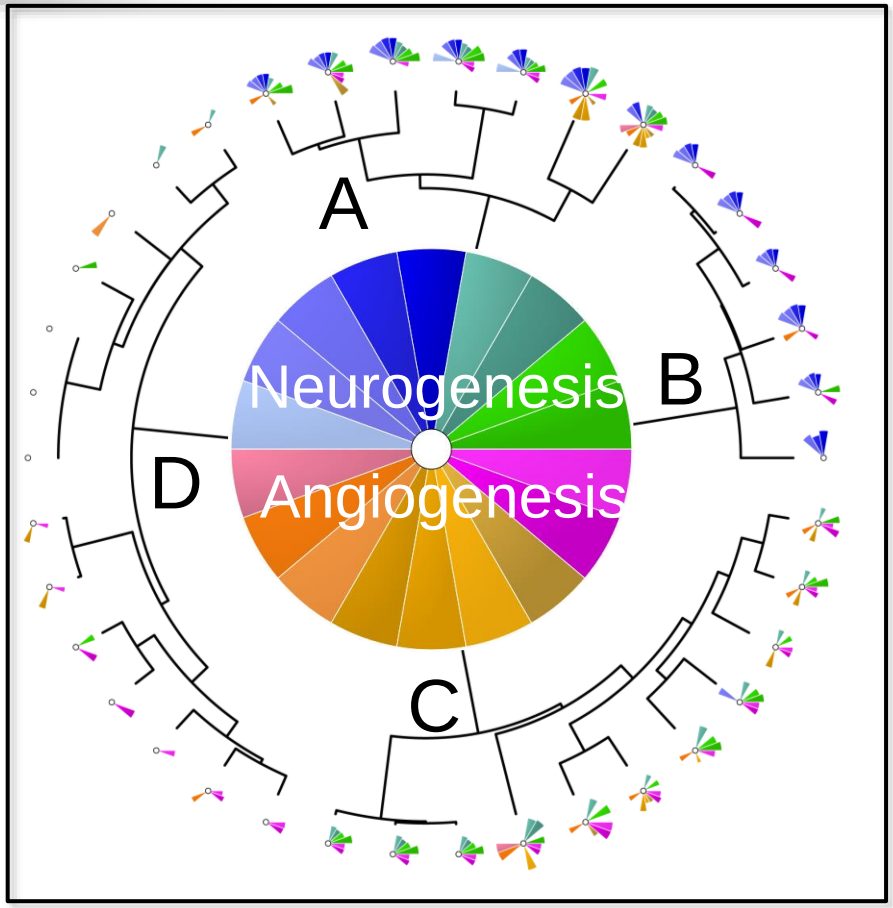
Cell-based Assays



ArunA: Migration/Proliferation
hNP/hNC/hNN cells

VALA: Migration/Proliferation
HUVEC cells

FICAM: Tubulogenesis/Proliferation
HUVEC cells



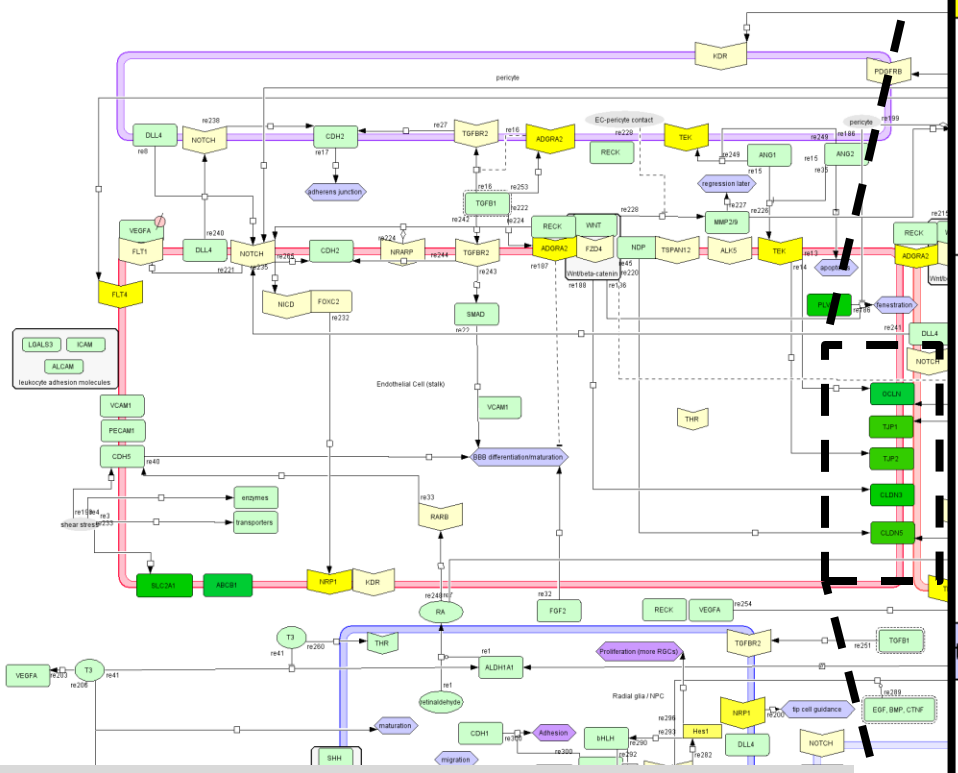
Process data

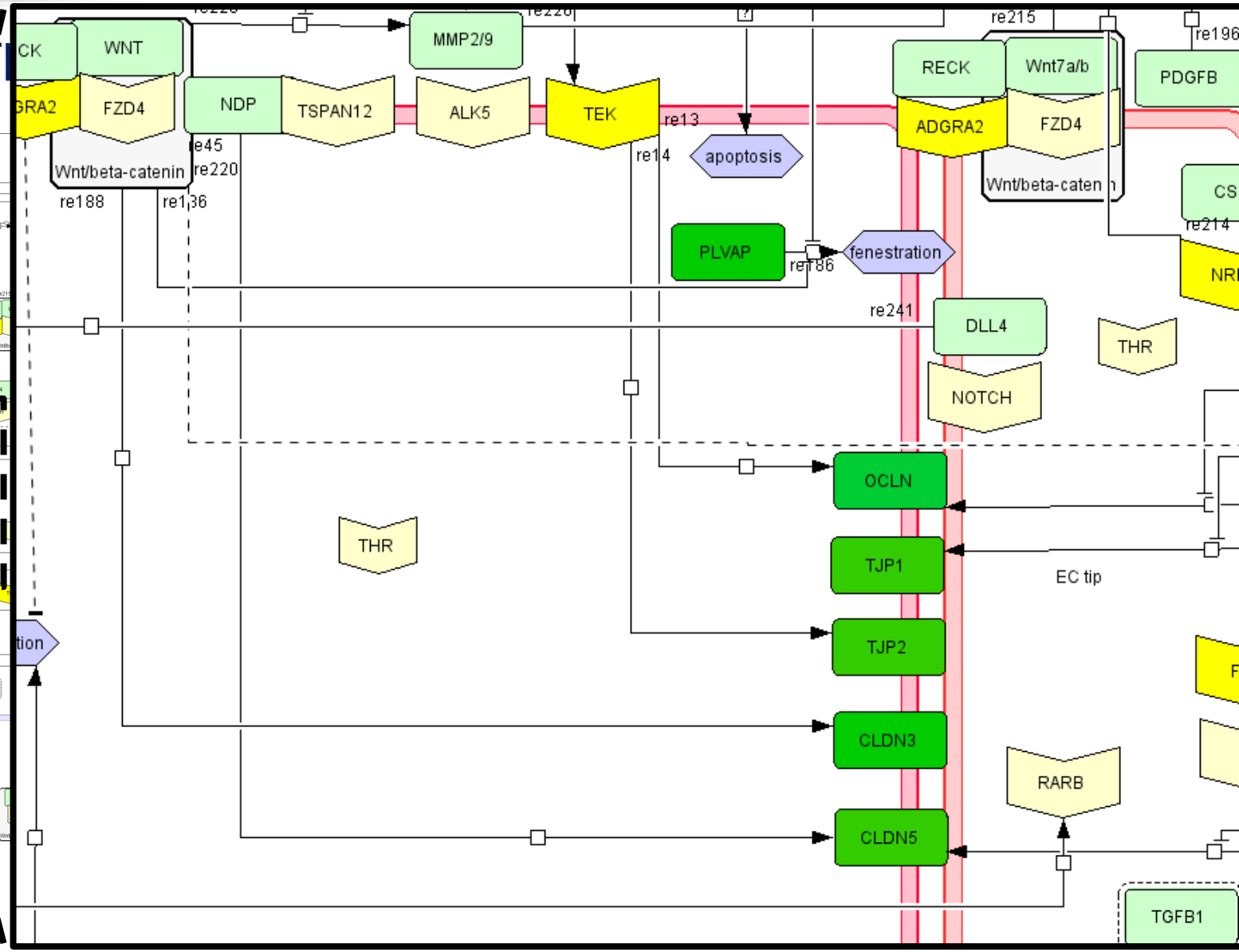
Cluster through ToxPi

Define in literature

Applicability to blood-brain barrier

- Extend predictions of mor

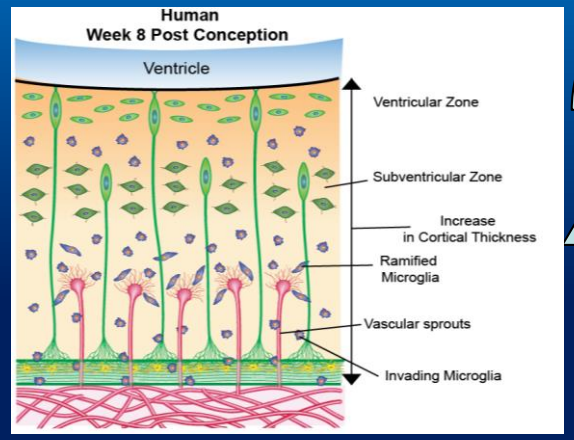


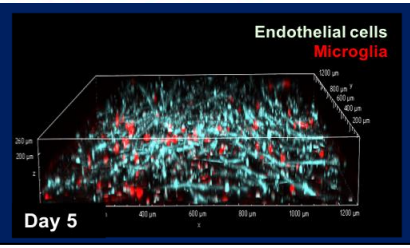


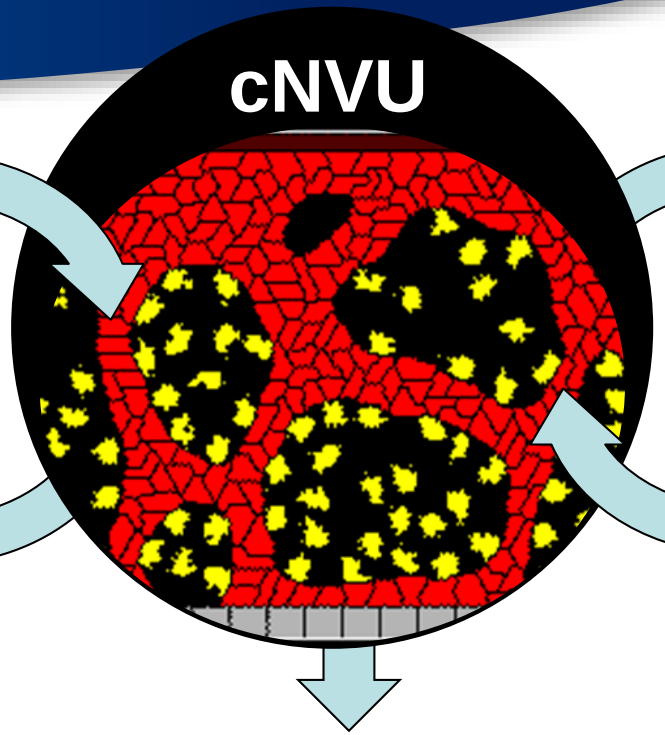
Cell-cell interactions important for establishing barrier. The next step is to leverage predictions of morphology to prediction endpoints of barrier integrity.

Utilize data to update model

In vitro OCM

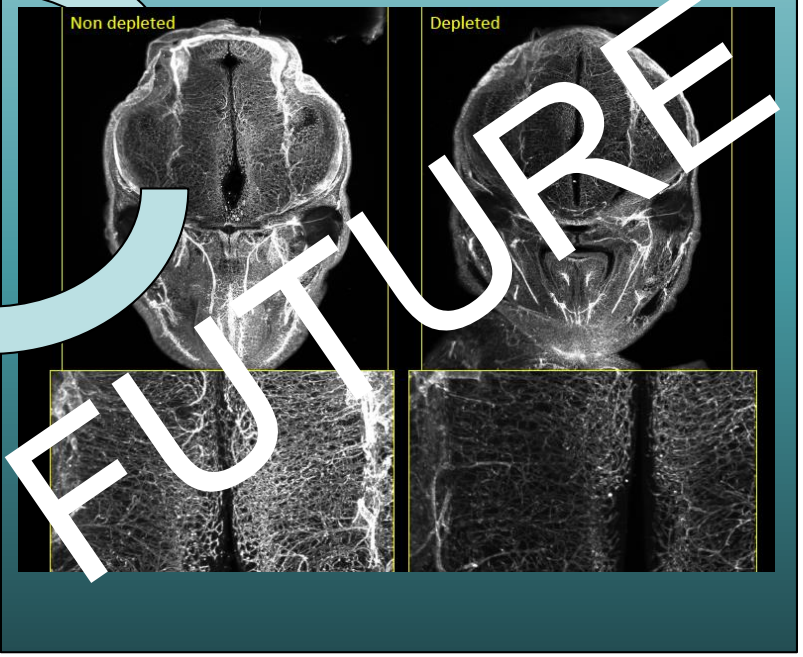






Barrier markers
Vascular morphology

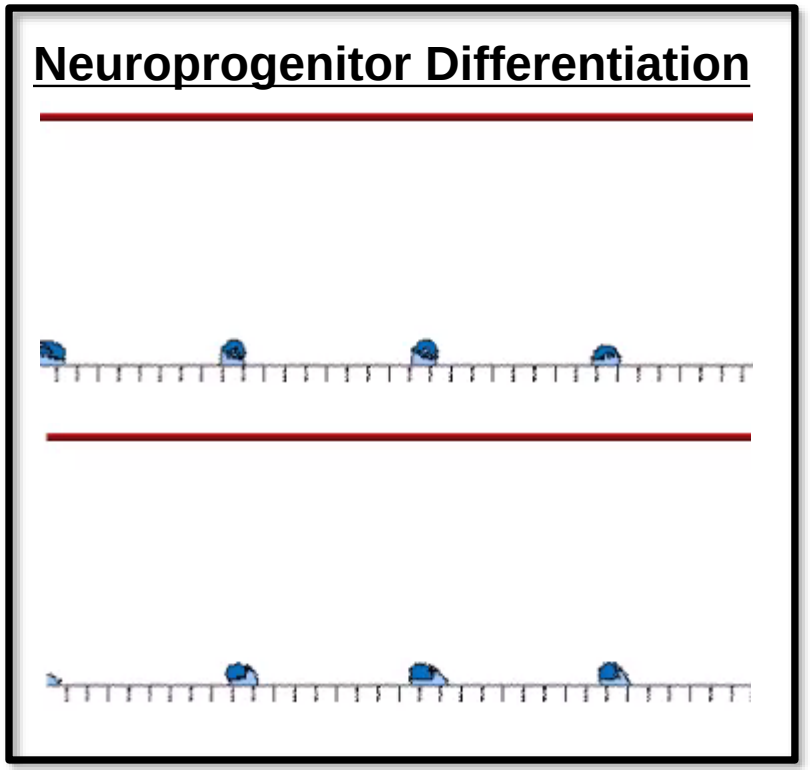
In vivo



- In vitro models of morphogenesis may not capture distinct barrier properties while barrier models may not recapitulate relevant morphology.
- A cNVU approach allows for integration of multiple data streams to produce high throughput, salient predictions of BBB development

Towards a functional cNVU model

- Biological pathway perturbations
  - Predict NVU phenotypes from literature fingerprint and cell-agent based model
  - ‘Cybermorphs’ for investigating single pathway knockouts
  - Continuum response following chemical exposure and resulting receptor inhibition
- Neurogenesis submodel
  - Differentiation/migration to neurons and astrocytes
  - Utilize intracellular signaling pathways (cell/centrosome cycle)
  - Endothelial network interacting with neural network (3D)
- Phenotype quantitation
  - Microglia abundance, vessel branch points, network complexity (cortical angiogenesis)
  - Neuron proliferation/differentiation (neurogenesis)
  - Barrier permeation for chemical distribution to neural compartment (barriergenesis)



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Thank You

Questions?