

# AOP-based ontologies for developmental toxicity

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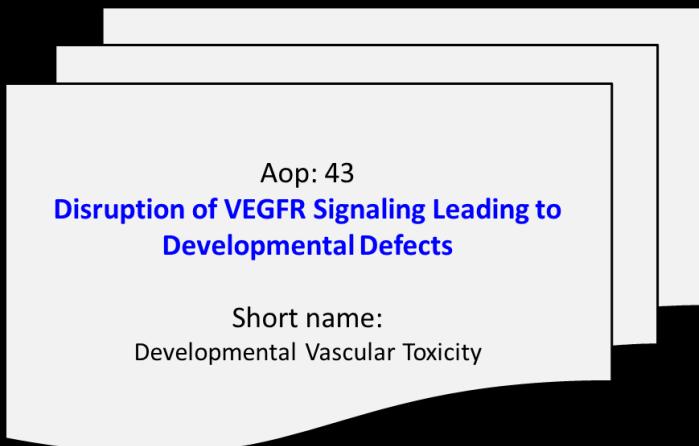
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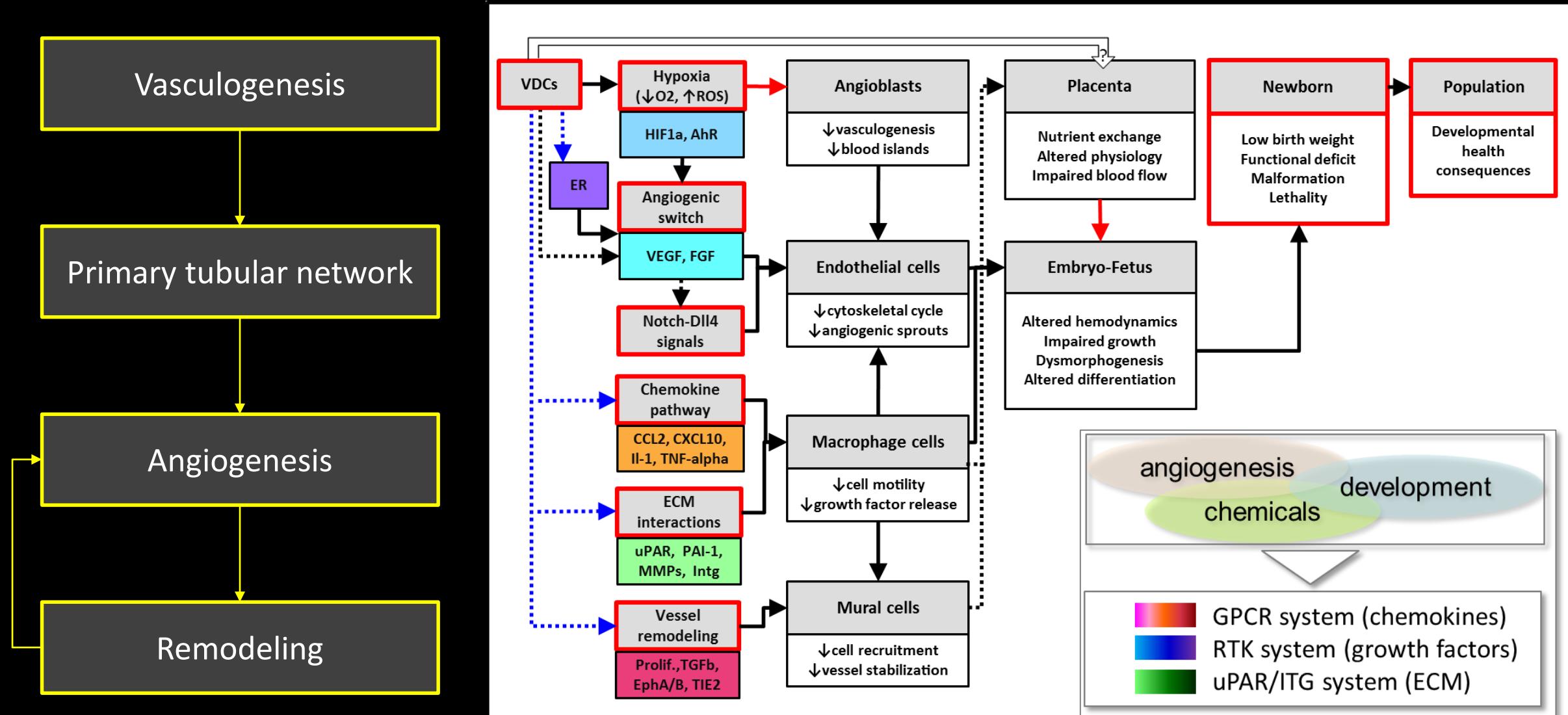
# Vascular Development



- Blood vessel development is essential to the embryo (cardiovascular is first functioning organ system across *Vertebrate* species).
- Vascular insufficiency is tied to many disease processes (stroke, diabetes, pre-eclampsia, neonatal respiratory distress, osteoporosis, Alzheimer's...).
- Aop43: one of 28 AOPs included in the OECD work plan with status ‘open for citation & comment’ [<https://aopwiki.org/wiki/index.php/Aop:43>].



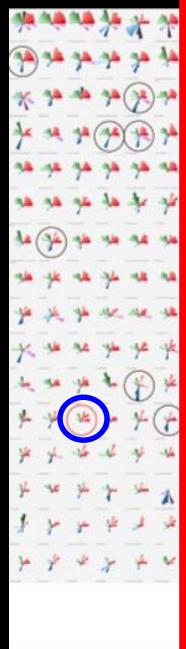
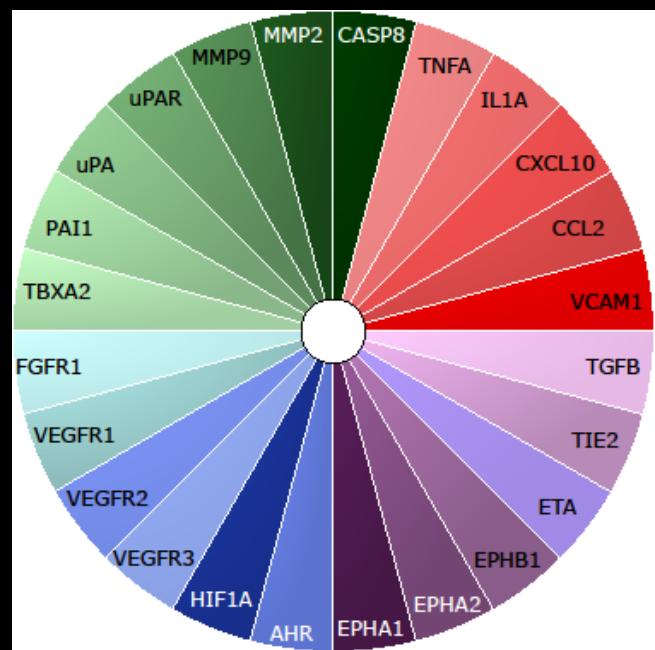
# AOP framework: developmental vascular toxicity (DVT)



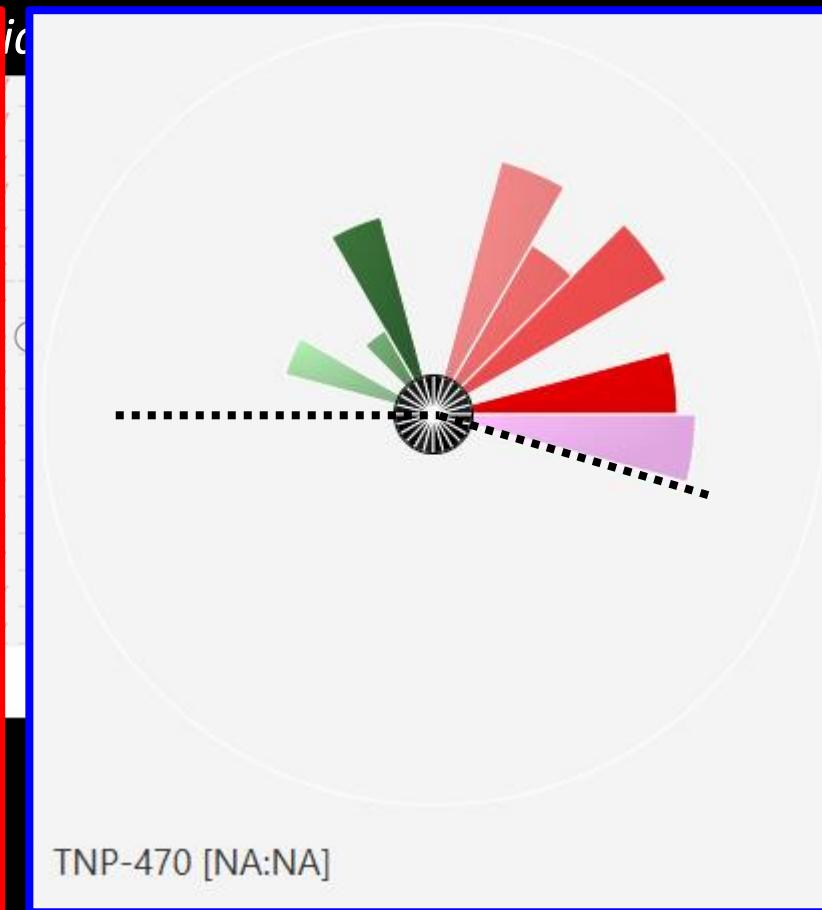
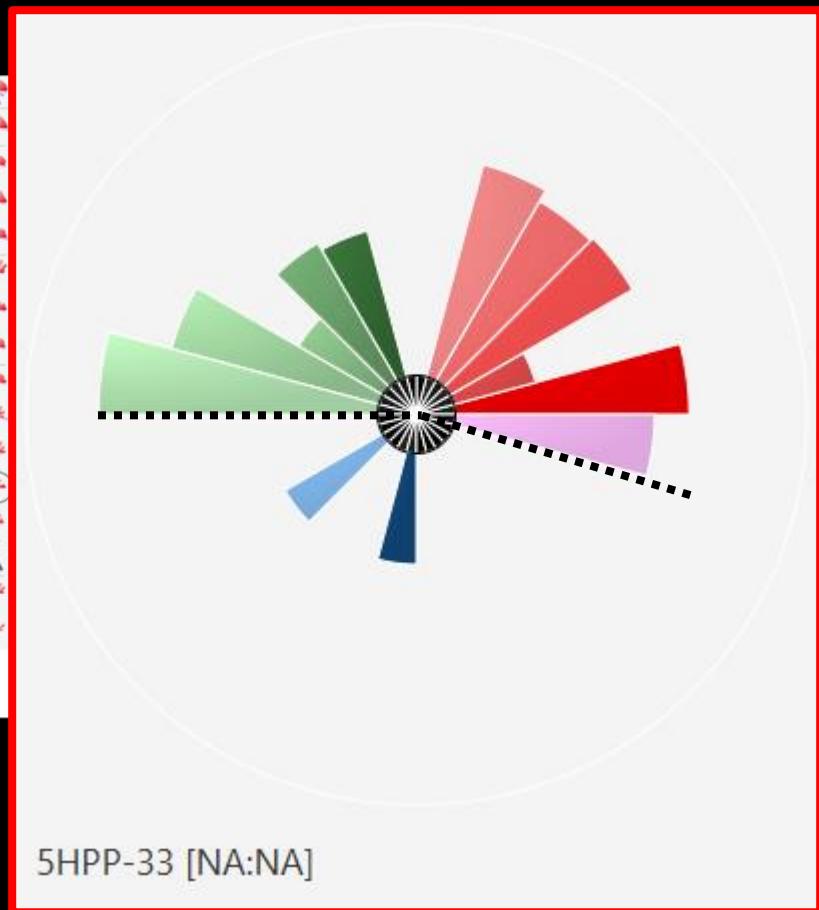
SOURCE: Knudsen and Kleinstreuer (2011) Birth Defects Res

# AOP-based ranking: predicted vascular disrupting chemicals (*pVDCs*)

24 *ToxCast* target assays  
(*pVDC ToxPi*)

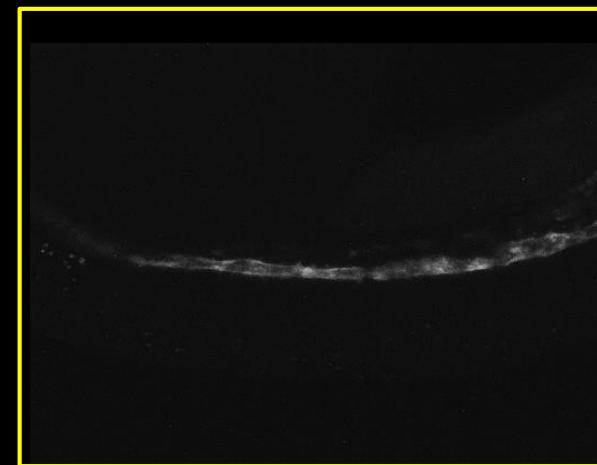
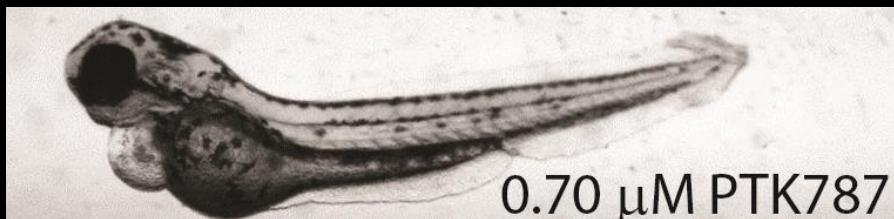
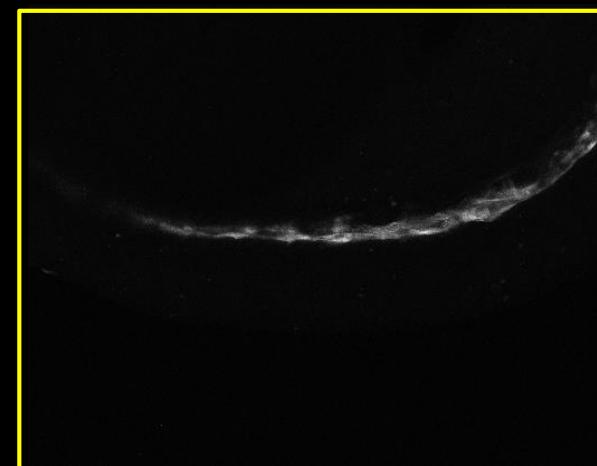
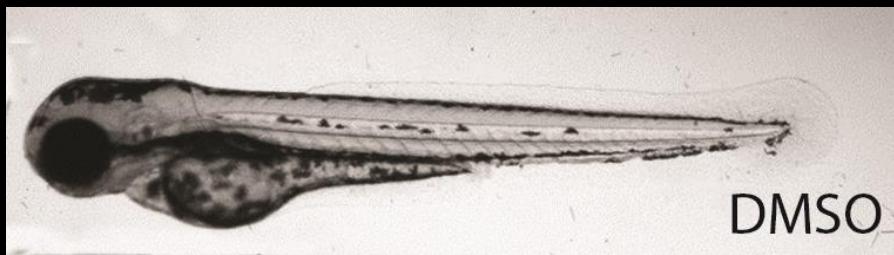


1058 *ToxCast* chemicals ranked by *pVDC ToxPi*

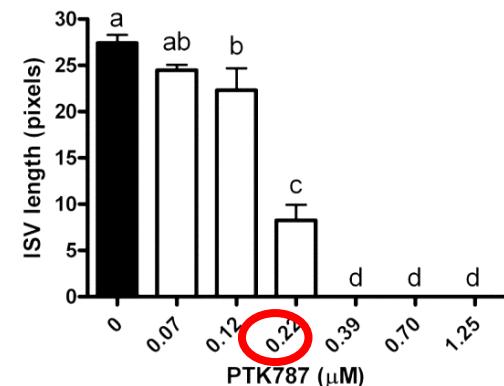


SOURCE: Kate Saily, NCCT

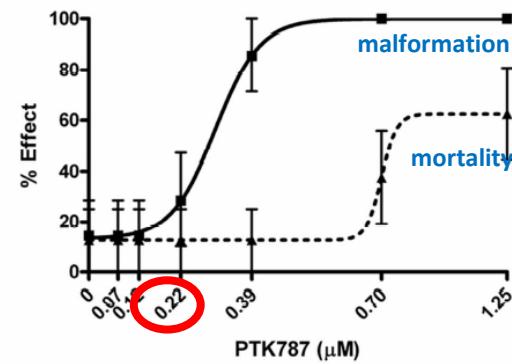
# VEGFR2 inhibition (PTK787)



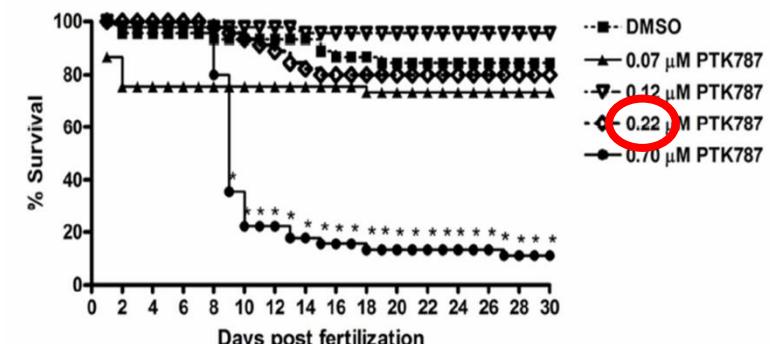
ISV length  
(72 hpf)



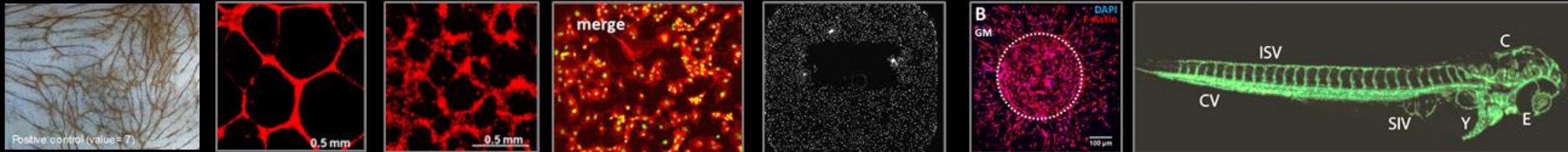
Terata  
(120 hpf)



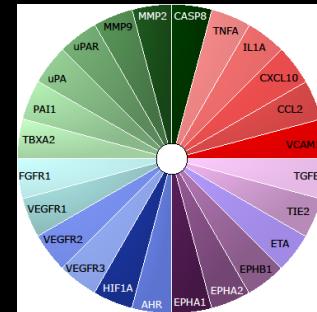
Lifespan  
(10 dpf)



SOURCE: Tal et al. (2014) Reprod Toxicol



## Vasculogenesis



## Primary tubular network

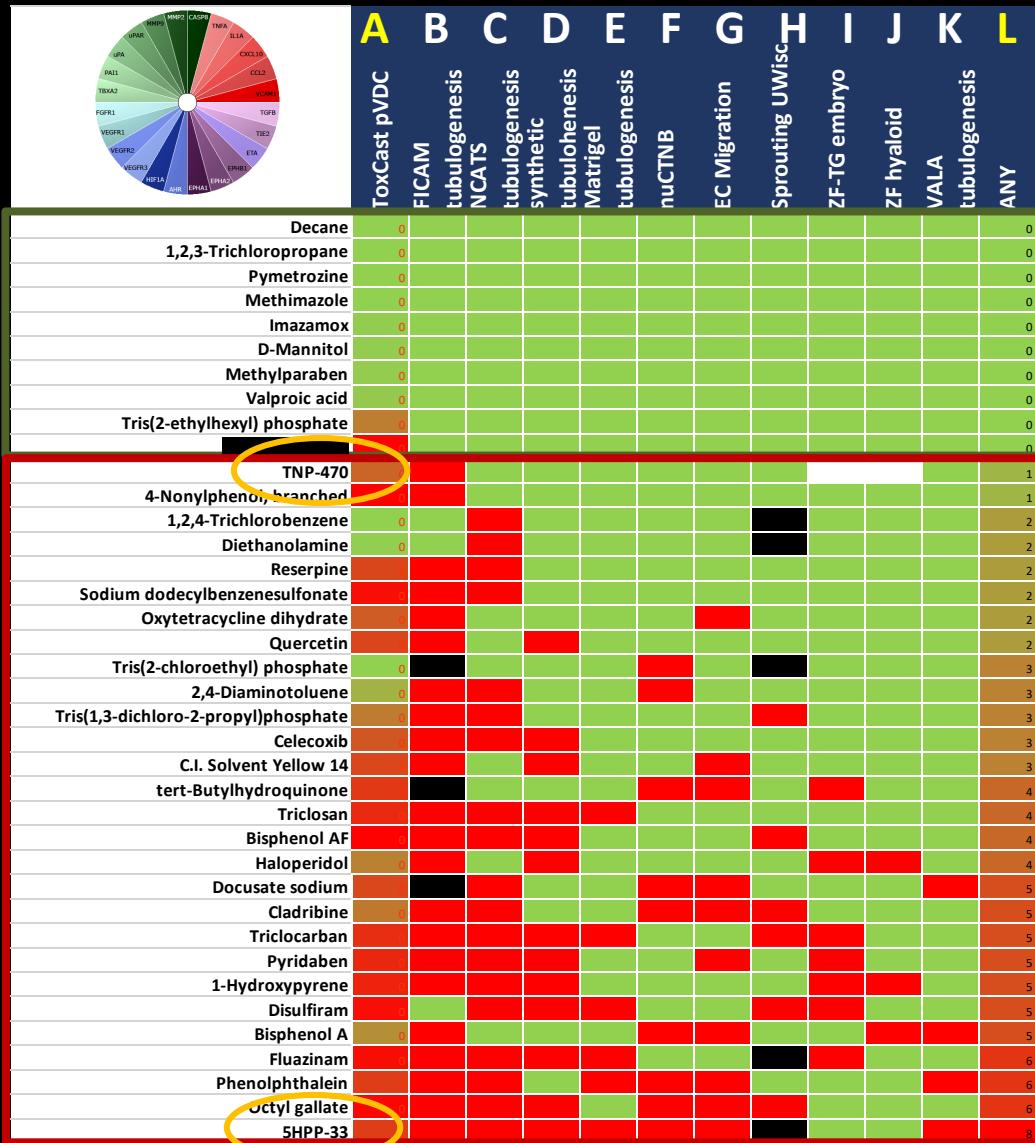
## Angiogenesis

## Remodeling

*How well does ToxCast do predicting endothelial disruption across the angiogenesis cycle?*

- 3D angiogenic sprouting [Belair et al. (2016) Acta Biomat]
- huCTNB and endothelial migration [in preparation]
- HTS tubulogenesis [Li et al. (2018) SLAS Tech]
- endothelial co-culture [in preparation]
- engineered matrices [Nguyen et al. (2017) Nature Bioeng]
- KDR-reporter zebrafish embryos [Tal et al. (2017) Reprod Toxicol]
- rat whole embryo culture [Ellis-Hutchings et al. (2017) Reprod Toxicol]

# 38 chemical test set: qualification of pVDC ToxPi across 9 endothelial behaviors



**A pVDC ToxPi**

**B HUVEC tubulogenesis (FICAM)**

**C HUVEC tubulogenesis (NCATS)**

**D tubulogenesis in synthetic matrices (HMAPS)**

**E tubulogenesis in Matrigel (HMAPS)**

**F nuCTNB biomarker (VALA)**

**G endothelial cell migration (VALA)**

**H iPSC endothelial sprouting (HMAPS)**

**I ISV reporter zebrafish (NHEERL)**

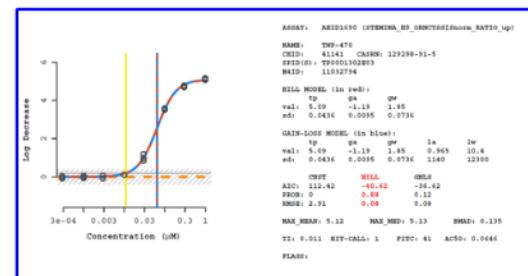
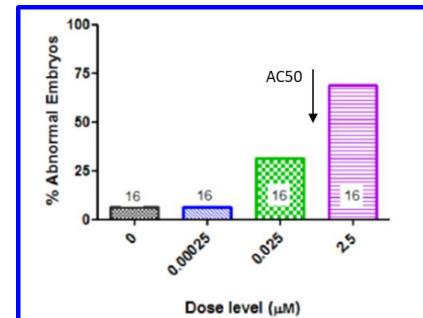
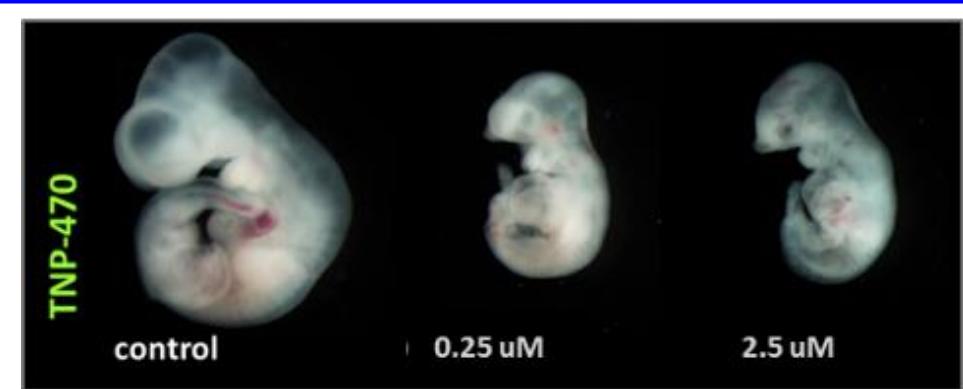
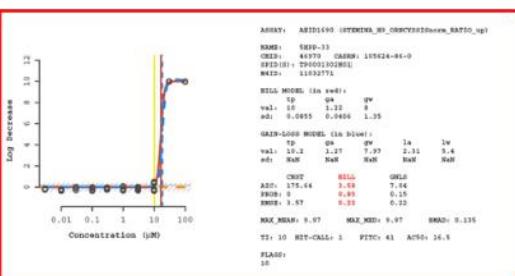
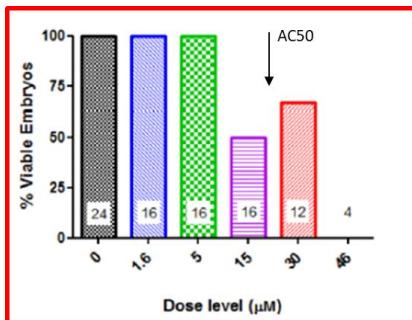
**J reporter zebrafish (UDUBLIN)**

**K HUVEC tubulogenesis (VALA)**

**L ANY (B to K)**

**Sens 0.89, Spec 0.80  
ACC 87% (PPV 93%, NPV 73%)**

# Embryotoxicity: 5HPP-33 vs TNP-470



## 5HPP-33

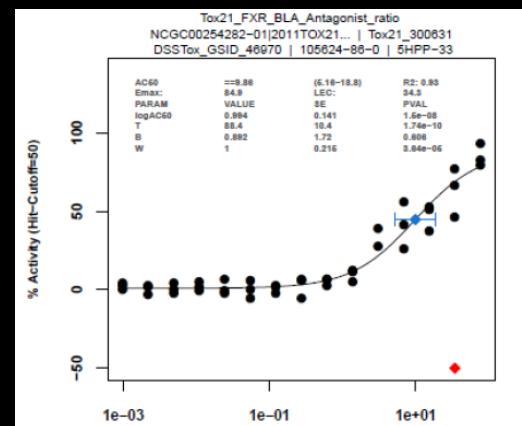
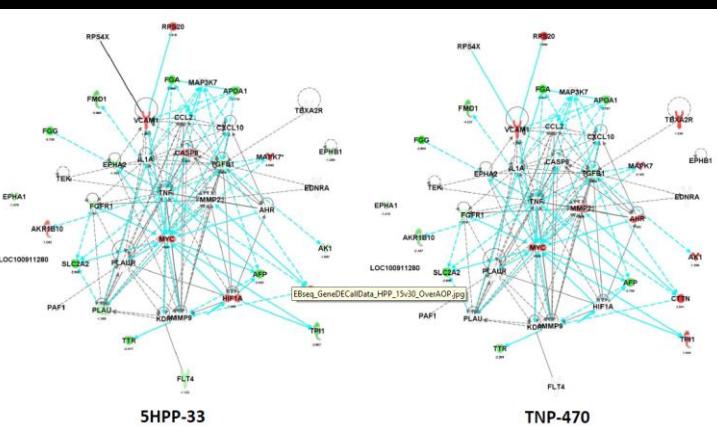
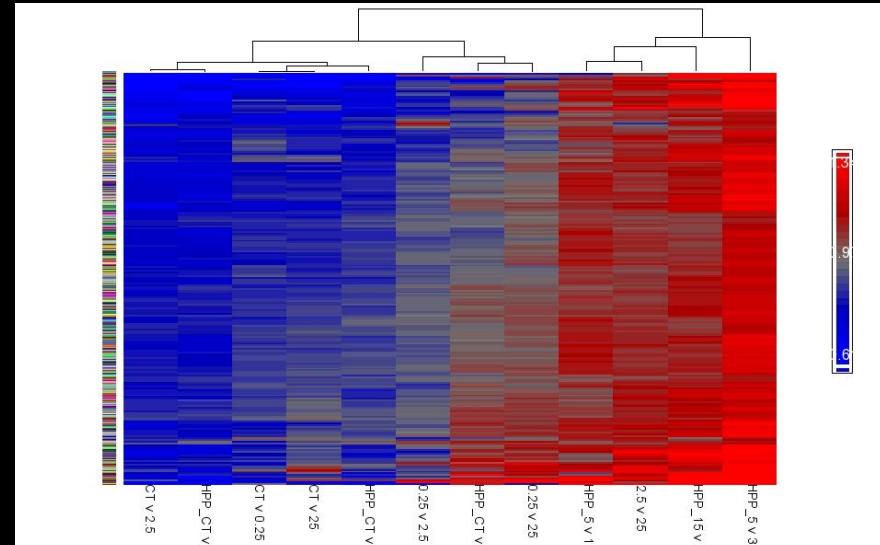
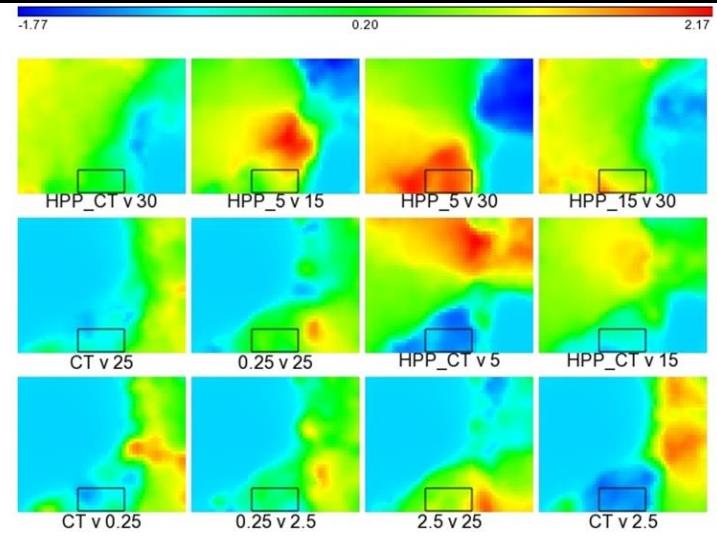
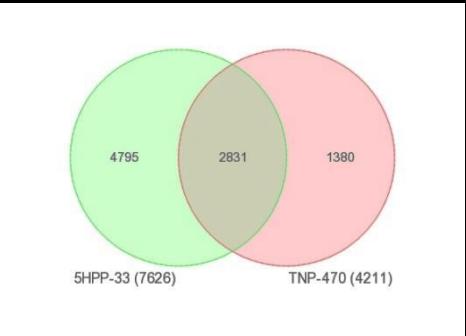
- synthetic thalidomide analog
- microtubule disruptor
- ↓ endothelial networks
- critical effect - embryo viability
- AC50 = 21.2  $\mu\text{M}$
- TI threshold from hESC = 9.5  $\mu\text{M}$

## TNP-470

- synthetic fumagillin analog
- MetAP II inhibitor
- non-canonical WNT signaling
- critical effect - dysmorphogenesis
- AC50 = 0.038  $\mu\text{M}$
- TI threshold from hESC = 0.01  $\mu\text{M}$

# RNAseq: 5HPP-33 vs TNP-470 whole embryo culture

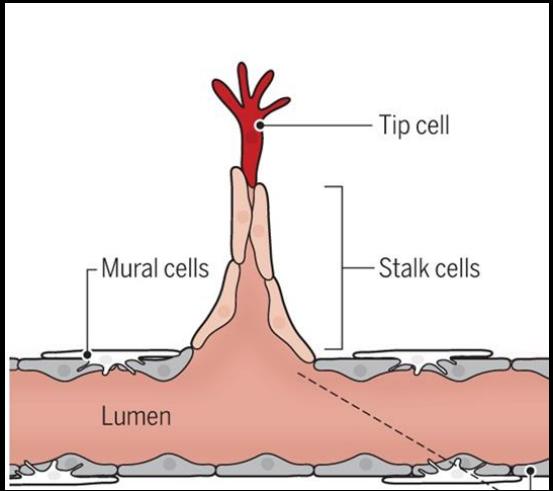
2831 DEGs overlap → SOM (464 genes in ROI box) → ROI clusters



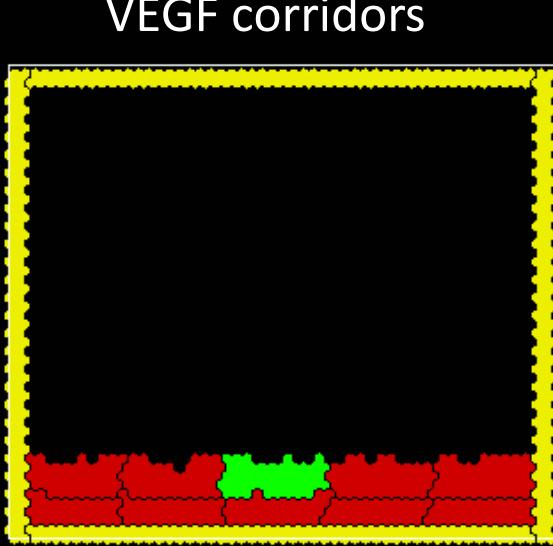
- FXR and LXR pathways common to 5HPP-33 and TNP-470 response.
- FXR (+) and LXR (-) pathways may be key events via RXR heterodimerization.

- splicesome and RNA metabolism
- proteosome and ubiquitination

# Computer simulation: cell agent-based models



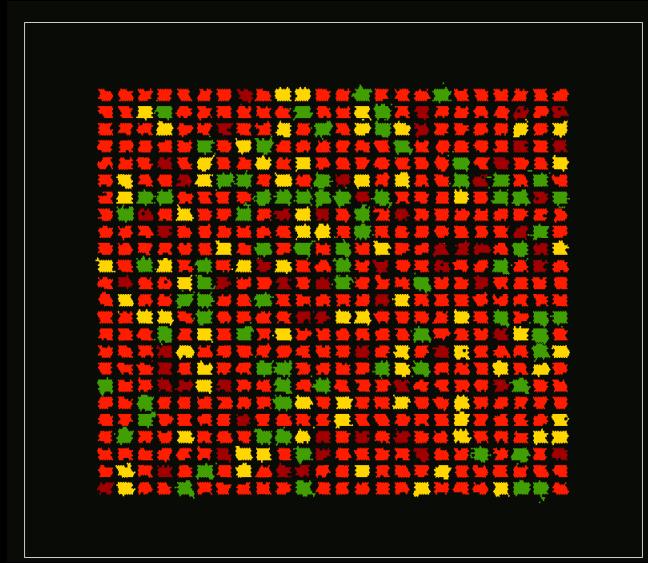
Li and Carmeliet (2018) Science



Nicole Kleinstreuer

**VEGF165**  
**MMPs**  
**VEGF121**  
**sFlit1**  
**TIE2**  
**CXCL10**  
**CCL2**

Network assembly



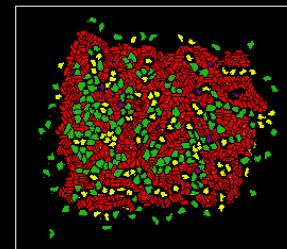
Kleinstreuer et al. (2013) PLoS Comp Biol

- ❖ Endothelial Stalk
- ❖ Endothelial Tip
- ❖ Mural Cell
- ❖ Inflammatory Cell

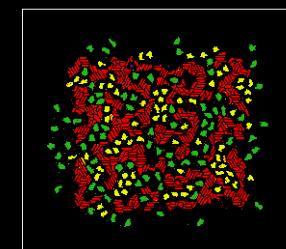
**SOFTWARE:** [www.CompuCell3D.org](http://www.CompuCell3D.org)

BioComplexity Institute, Indiana U

control

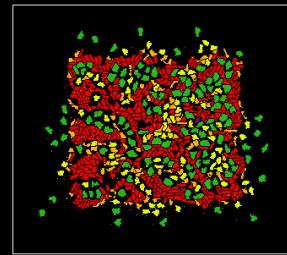


↓ VEGF

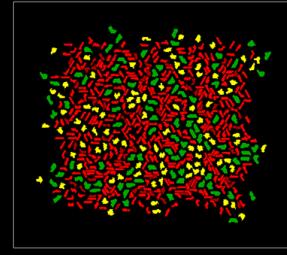


# Simulated (*in silico*) profiling

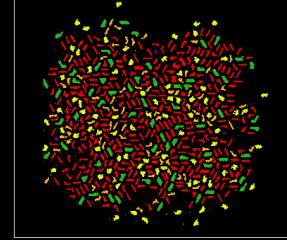
Imazamox



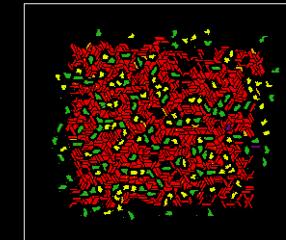
Pyridaben



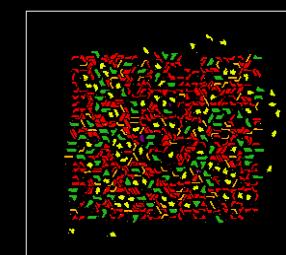
Disulfiram



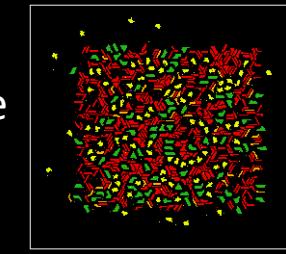
Bisphenol A



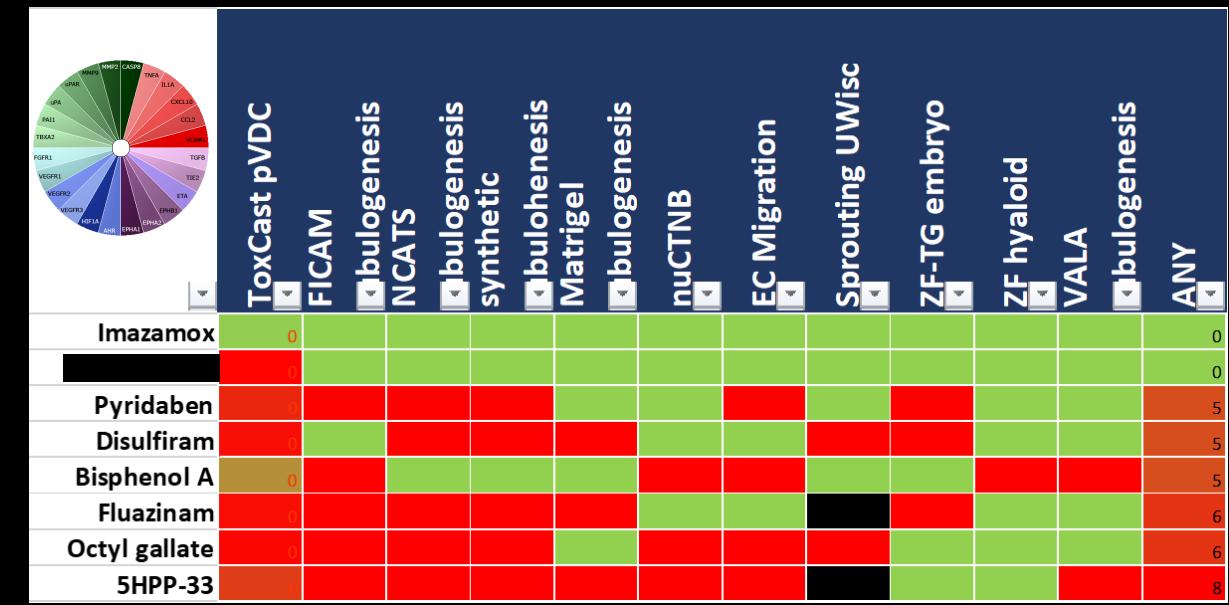
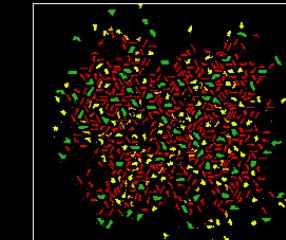
Fluazinam



Octyl gallate

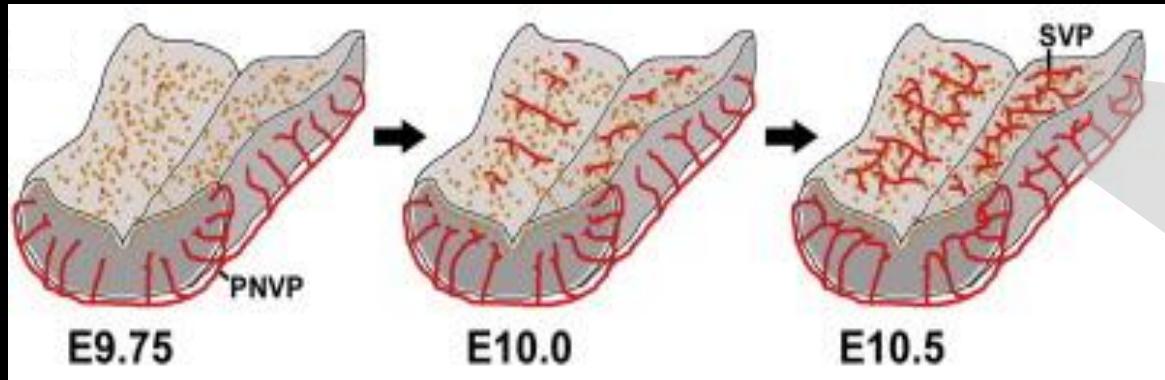


5HPP-33

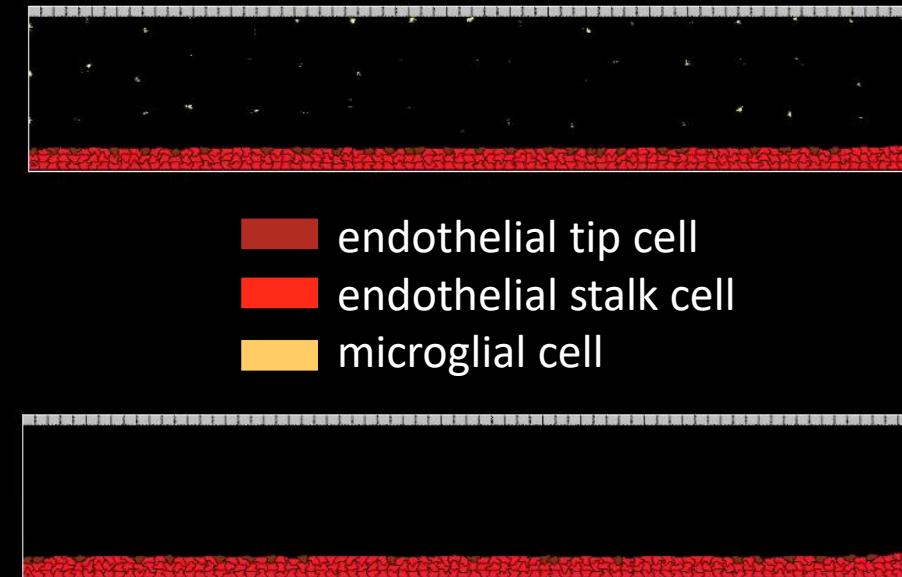
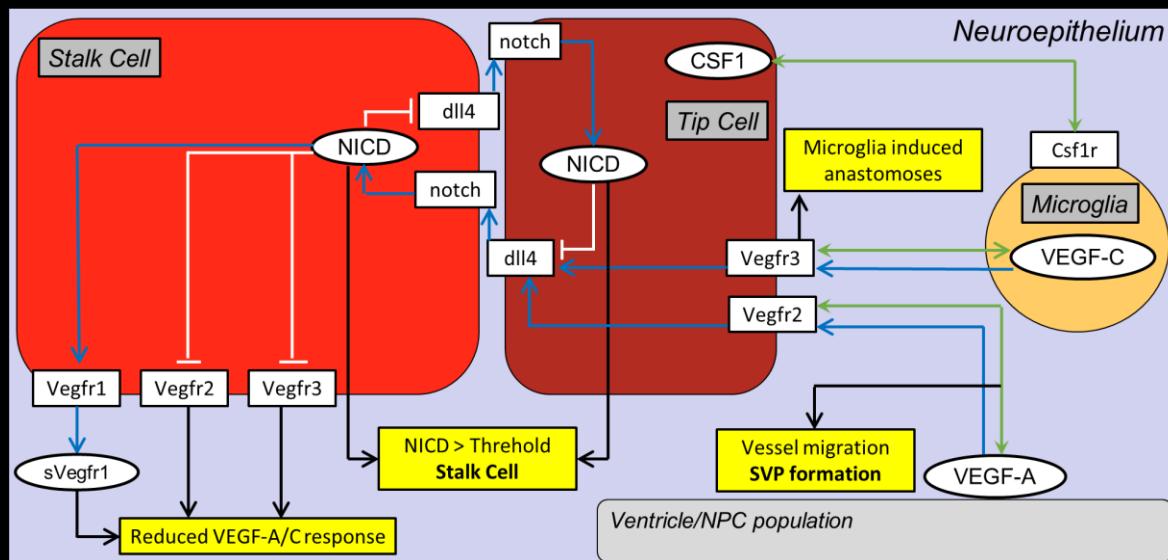
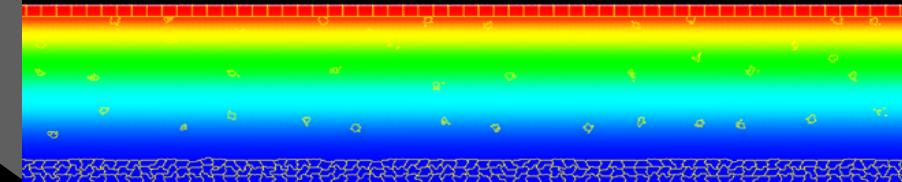


# Neural tube vascularization

Tata et al. (2015) Mechanism Devel

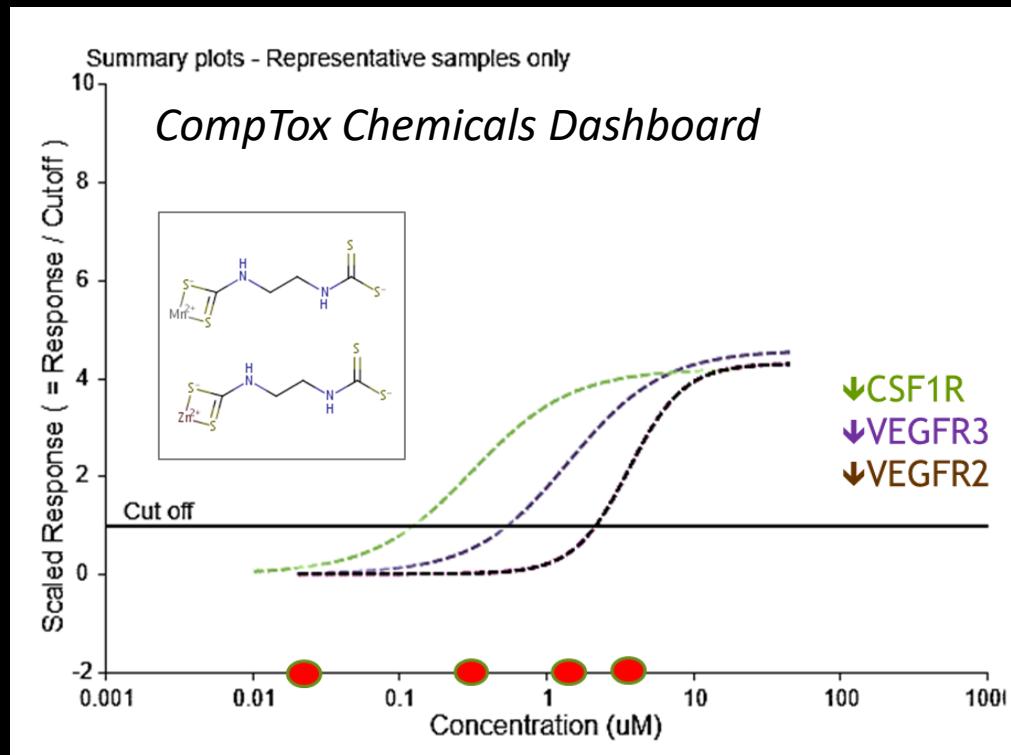


VEGF-A gradient: NPCs in subventricular zone

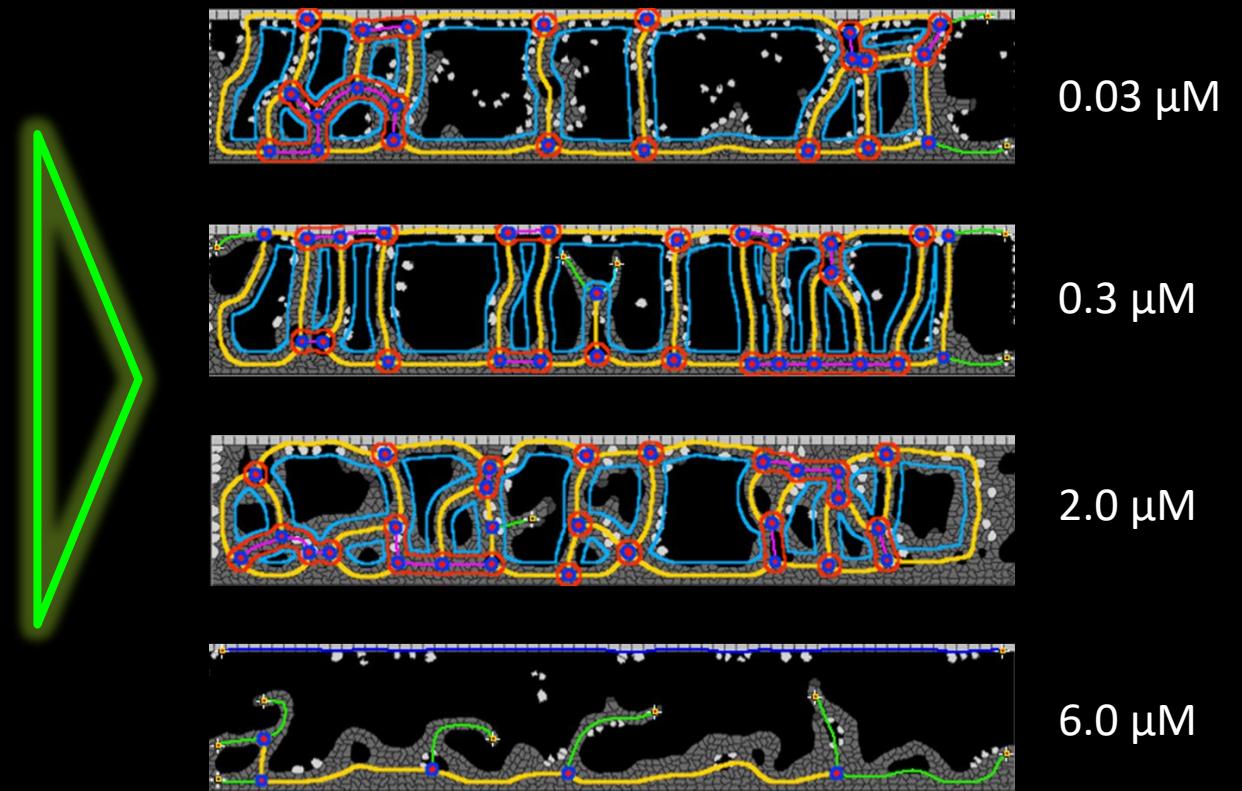


SOURCE: Zurlinden et al. (2018), NCCT

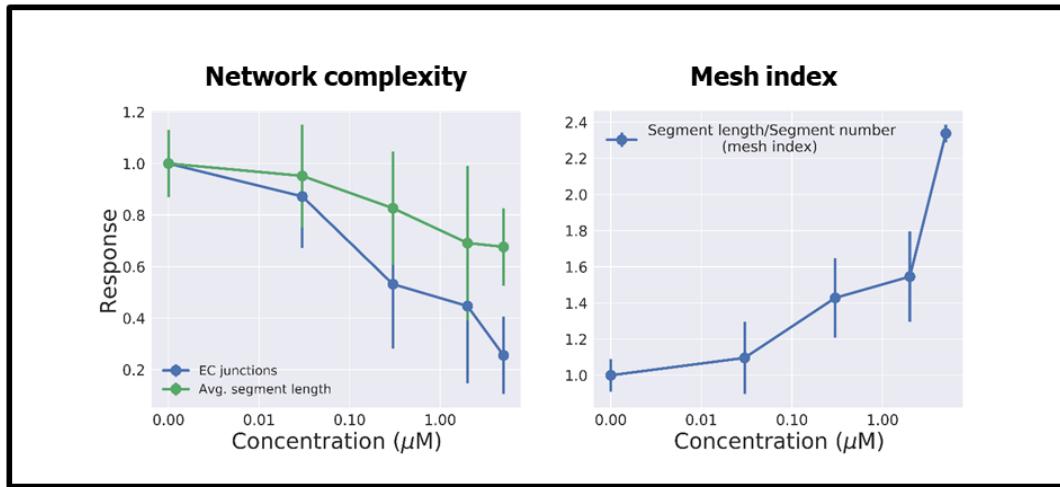
# Simulated dose-response: brain angiogenesis from *in vitro* HTS data (ToxCast)



<https://www.epa.gov/chemical-research/toxcast-dashboard>



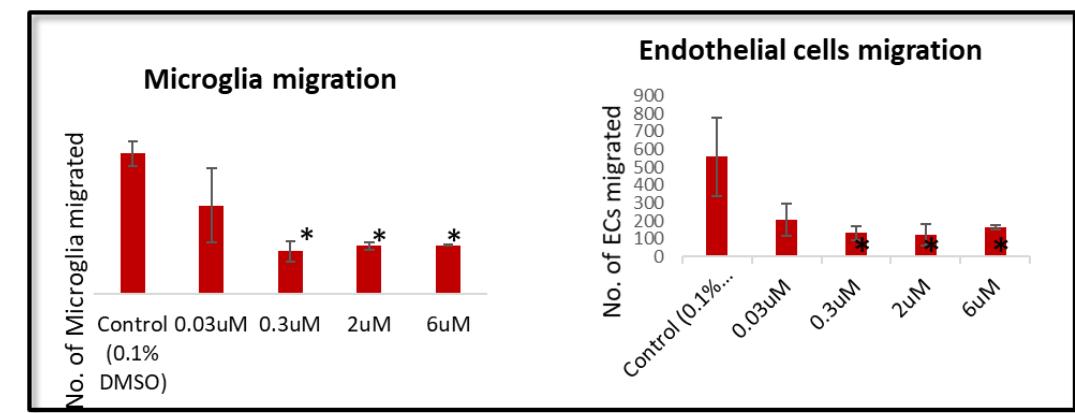
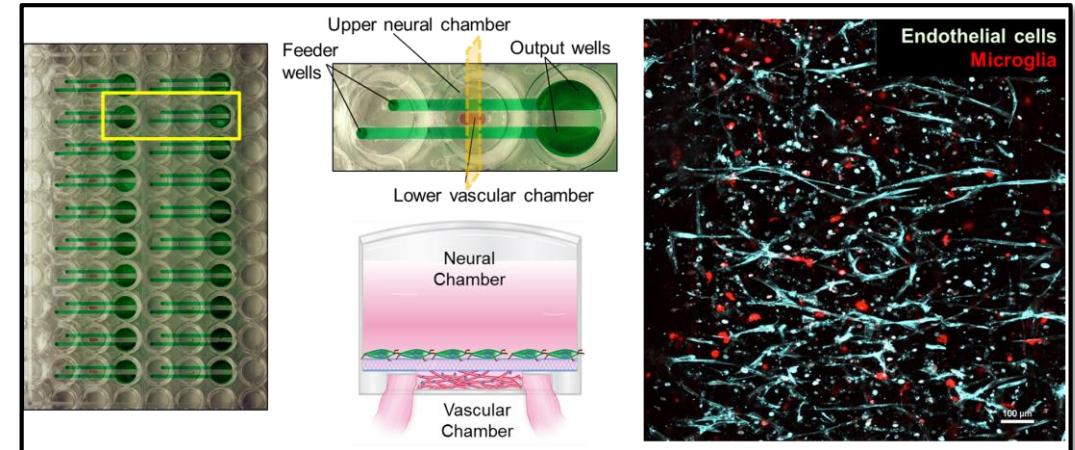
## Computational prediction (cNVU)



### Critical concentration:

- predicted *in silico*  $\sim 0.5 \mu\text{M}$
- observed *in vitro*  $\sim 0.3 \mu\text{M}$

## Biomimetic reconstruction (hNVU)

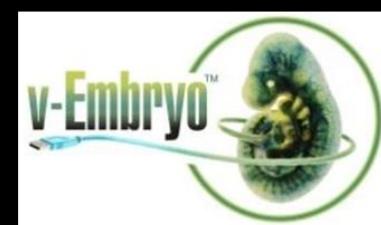


## **Summary:** *decoding the toxicological blueprint of vascular development*

- HTS profiles can assess *in vitro* bioactivity of large numbers of chemicals but translation remains a challenge for complex processes such as DevTox.
- Mapping HTS features to AOPs brings into context the weight of evidence for critical determinants potential invoking the altered phenotype in a self-organizing system.
- AOP-based ontologies provide the necessary structure for quantitative prediction of cellular and tissue responses to molecular perturbation.
- The ‘angiogenic cycle’ is responsive to genetic and physiological signals in the embryonic microenvironment, and can be useful for predictive toxicology.
- For DevTox, this can be demonstrated by an AOP network for embryonic vascular disruption represented in the OECD AOP-KB (Aop43).

# Acknowledgements

- Nicole Kleinstreuer - NCCT (now NTP/NICEATM)
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- Rob Ellis-Hutchings – Dow Chemical Company
- Raj Settivari – Dow Chemical Company
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- Todd Zurlinden – NCCT
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- Jill Franzosa – NCCT (now CSS)
- Eric Nguyen – U Wisconsin (HMAPS)
- Guarav Kaushick – U Wisconsin (HMAPS)
- William Murphy – U Wisconsin (HMAPS)
- William Daly – U Wisconsin (HMAPS)
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- David Belair – NHEERL/TAD (now CellGene)
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- Aymeric Silvin – A\*STAR/SIgN



Virtual Tissue Models: Predicting How Chemicals Impact Human Development

[http://www2.epa.gov/sites/production/files/2015-08/documents/virtual\\_tissue\\_models\\_fact\\_sheet\\_final.pdf](http://www2.epa.gov/sites/production/files/2015-08/documents/virtual_tissue_models_fact_sheet_final.pdf)

