

AOP-based ontologies for developmental toxicity

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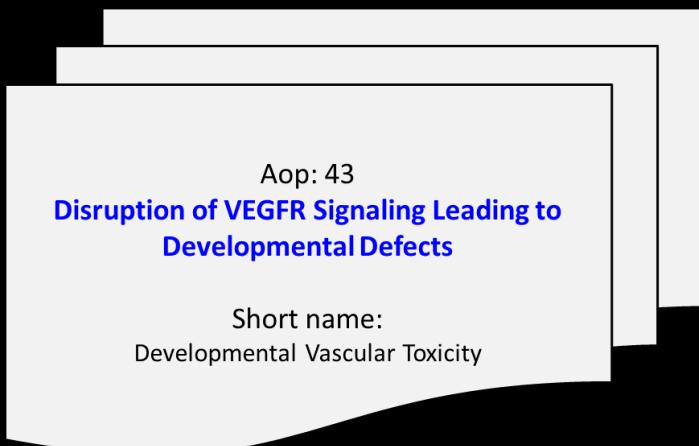
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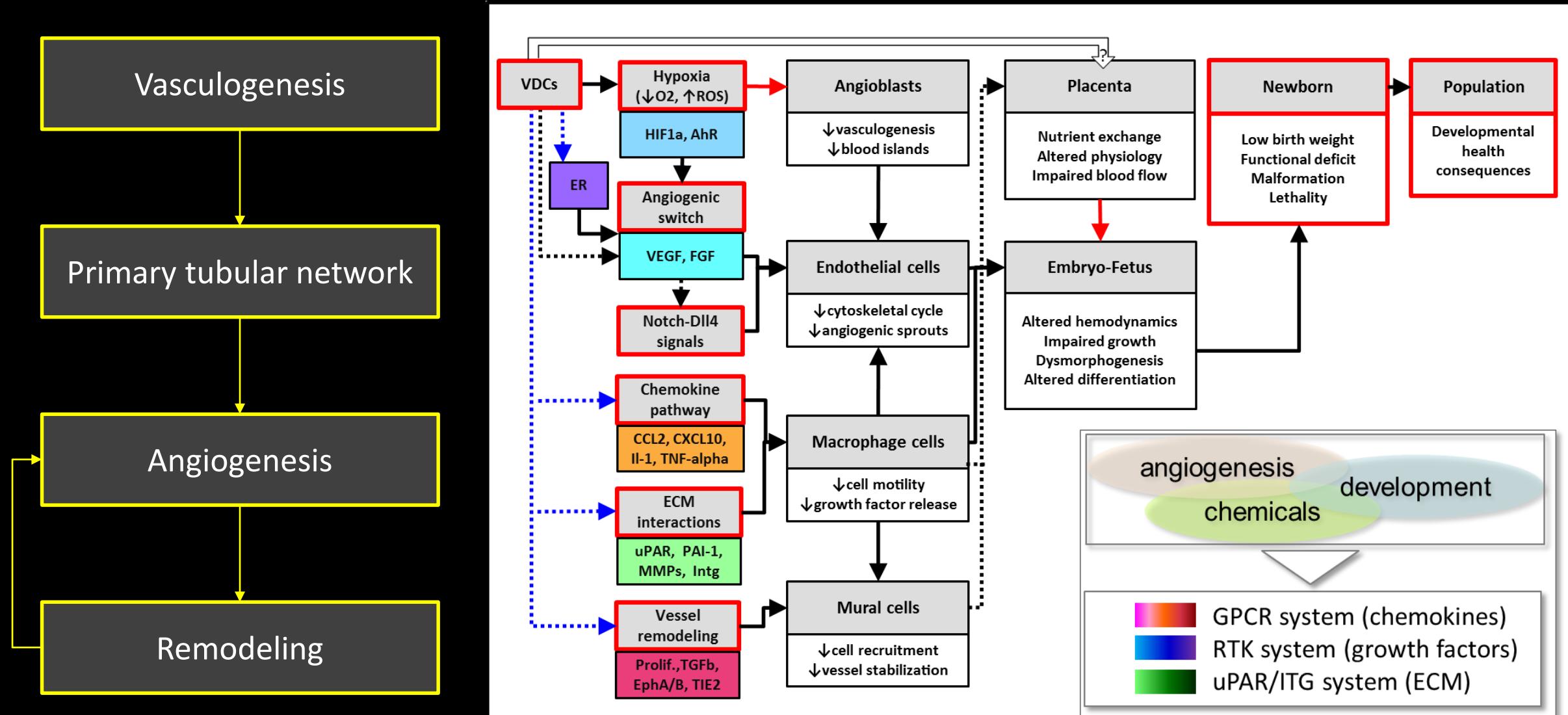
Vascular Development



- 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>].



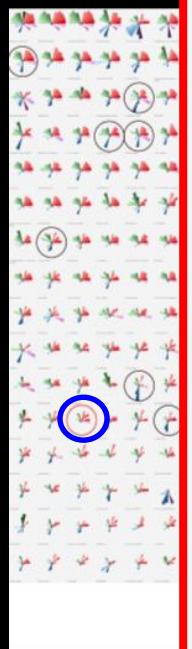
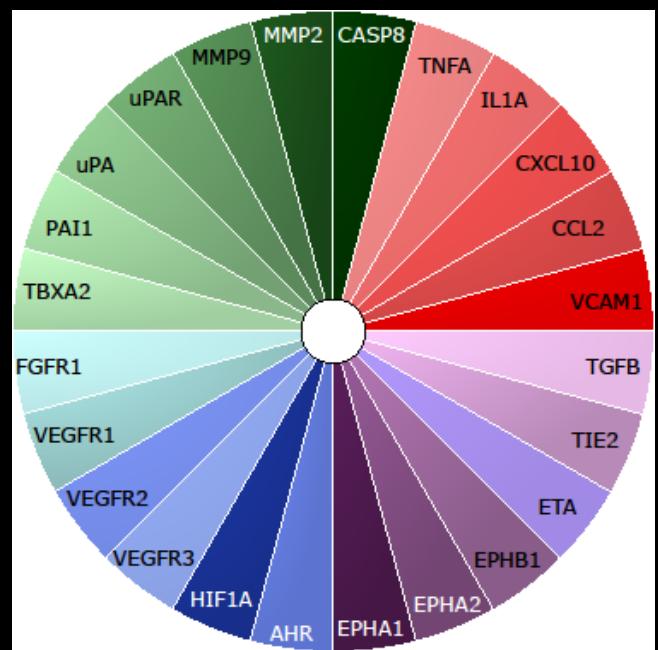
AOP framework: developmental vascular toxicity (DVT)



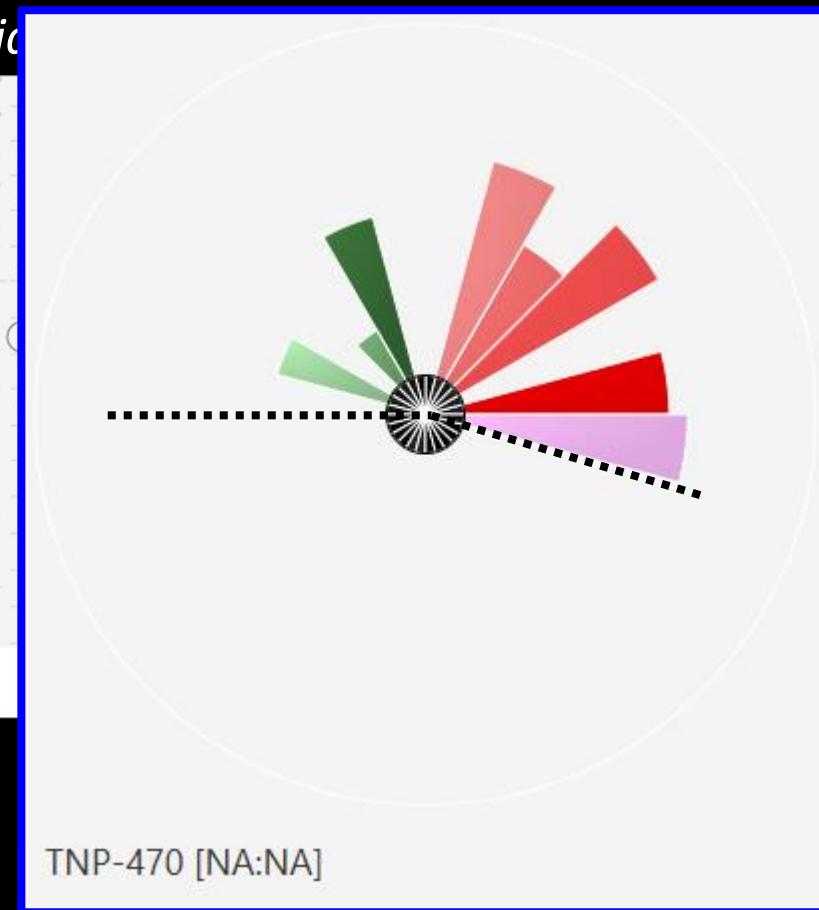
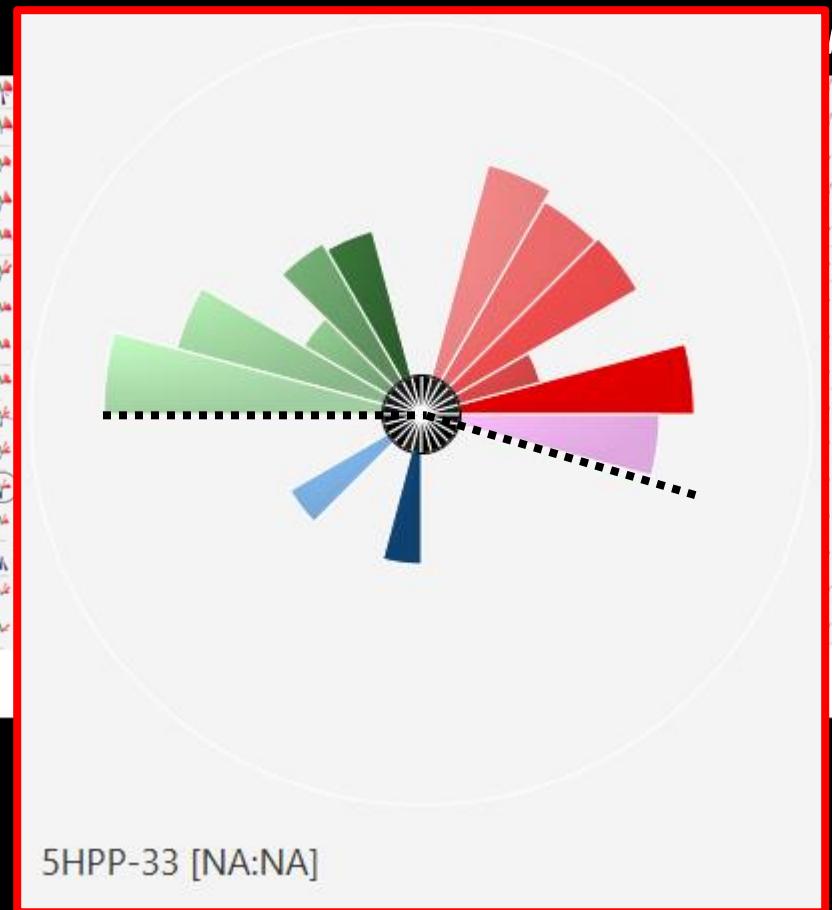
SOURCE: Knudsen and Kleinstreuer (2011) Birth Defects Res

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

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

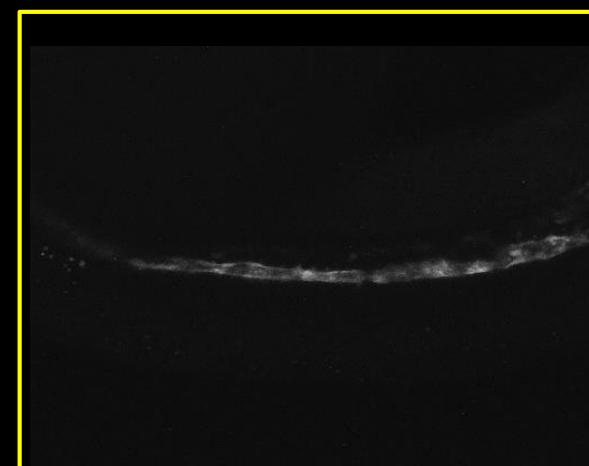
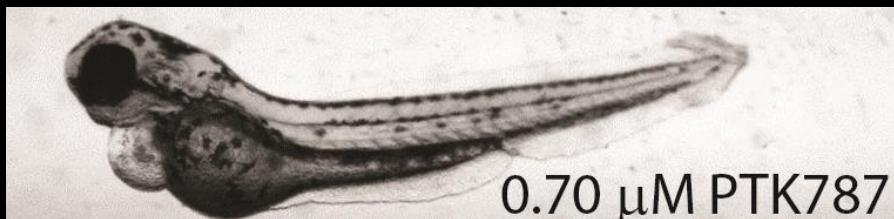
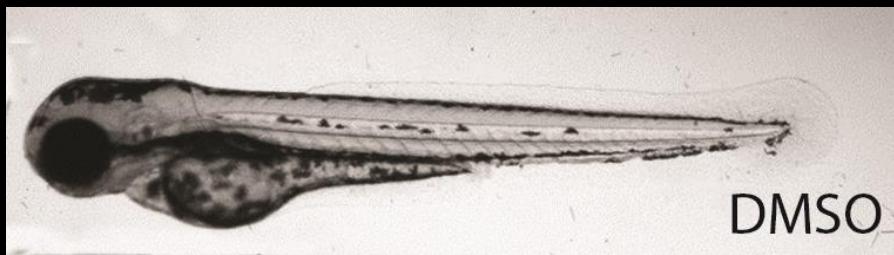


1058 *ToxCast* chemicals ranked by *pVDC ToxPi*

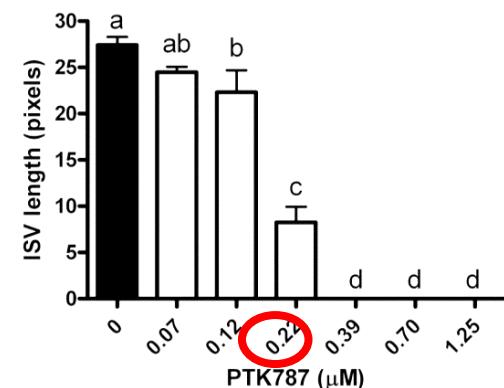


SOURCE: Kate Saily, NCCT

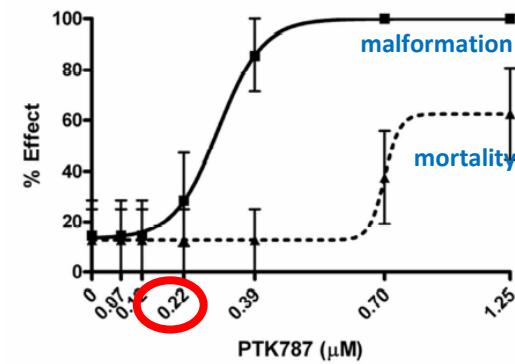
VEGFR2 inhibition (PTK787)



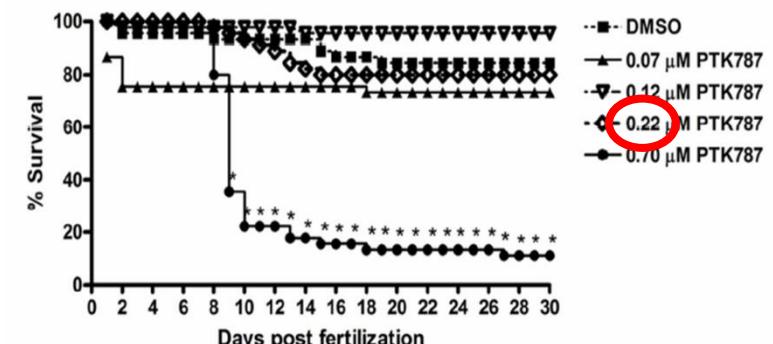
ISV length
(72 hpf)



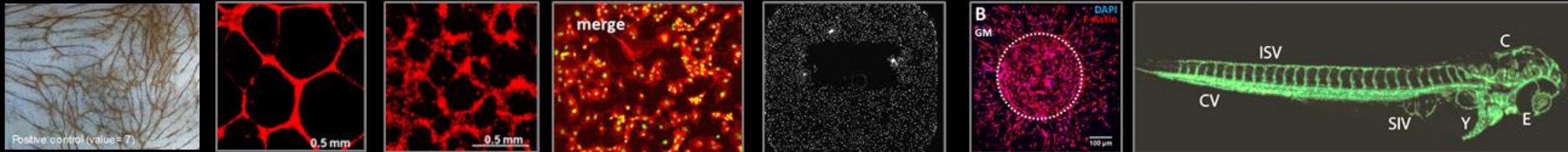
Terata
(120 hpf)



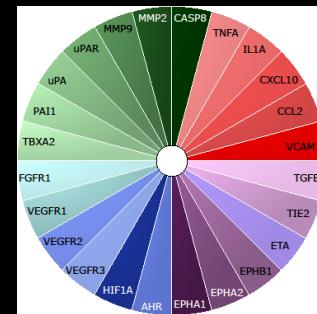
Lifespan
(10 dpf)



SOURCE: Tal et al. (2014) Reprod Toxicol



Vasculogenesis



Primary tubular network

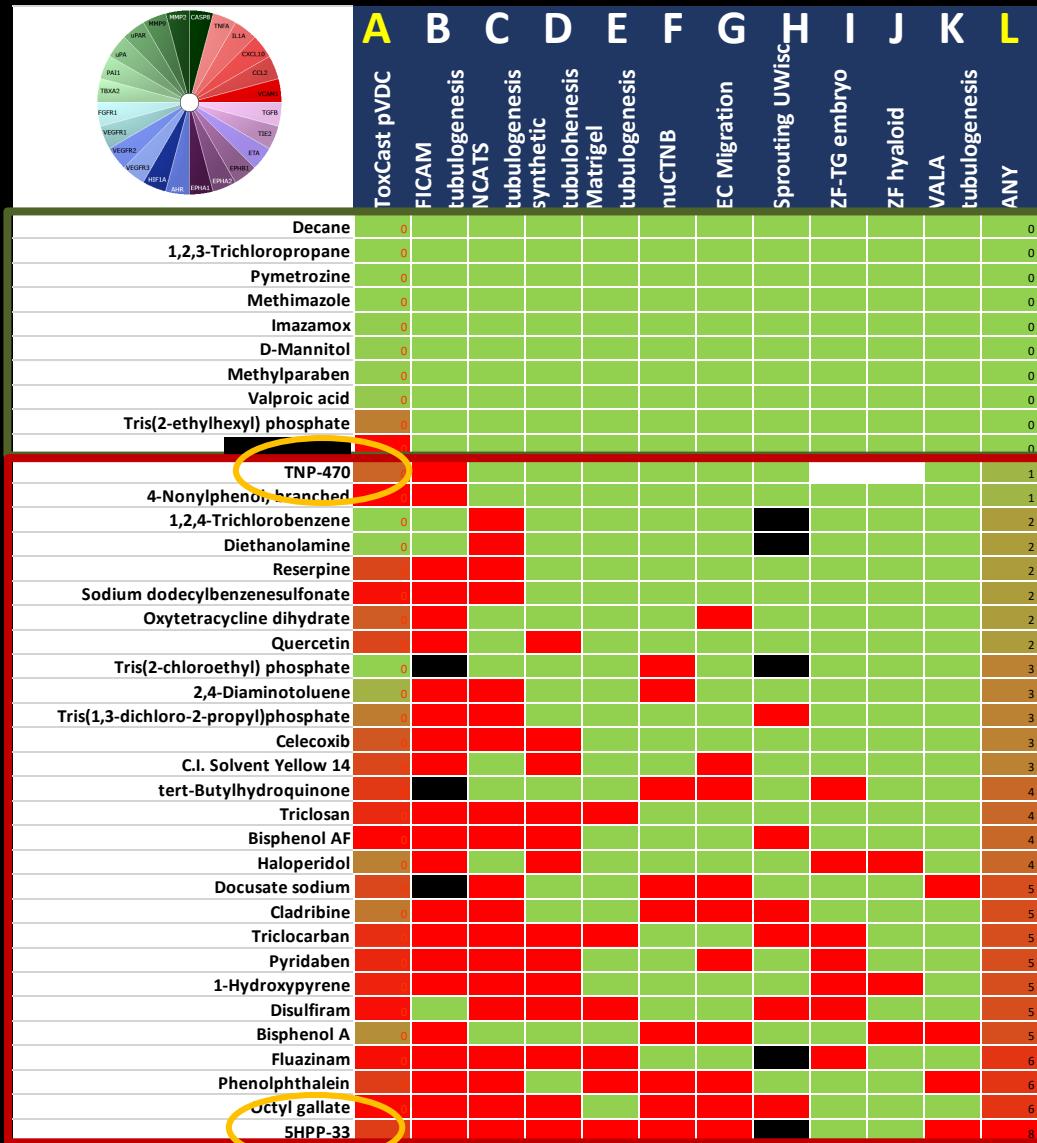
Angiogenesis

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)

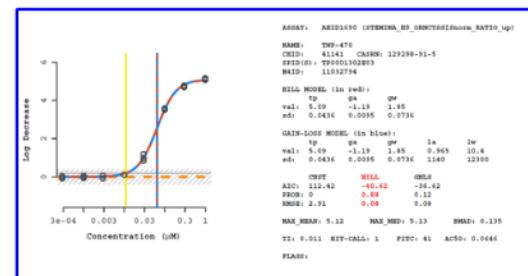
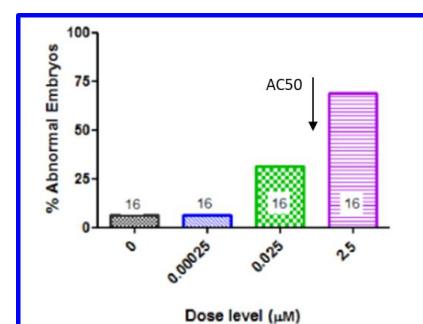
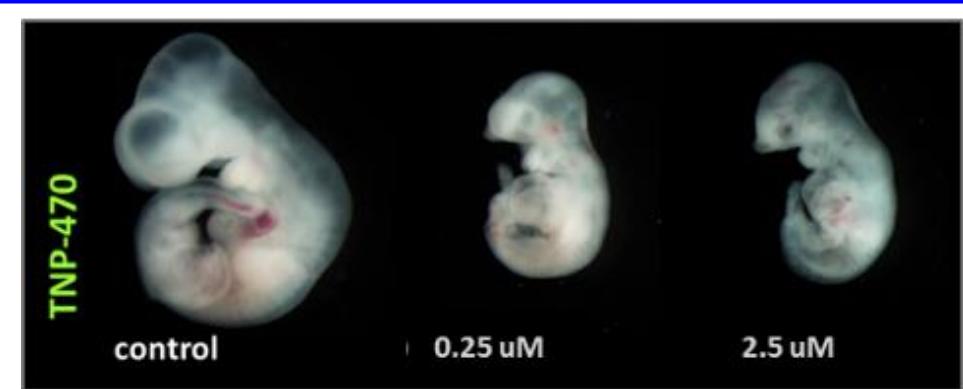
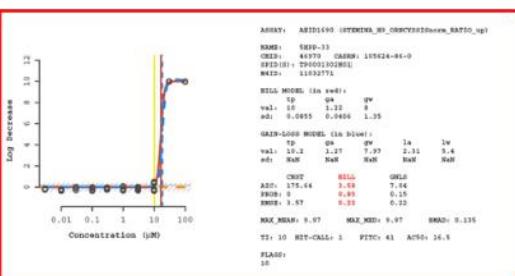
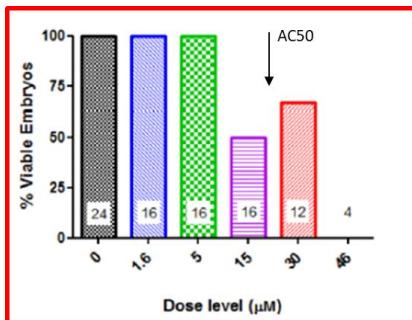
J reporter zebrafish (UDUBLIN)

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

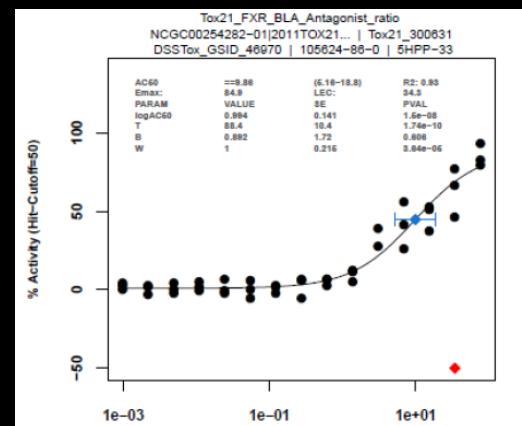
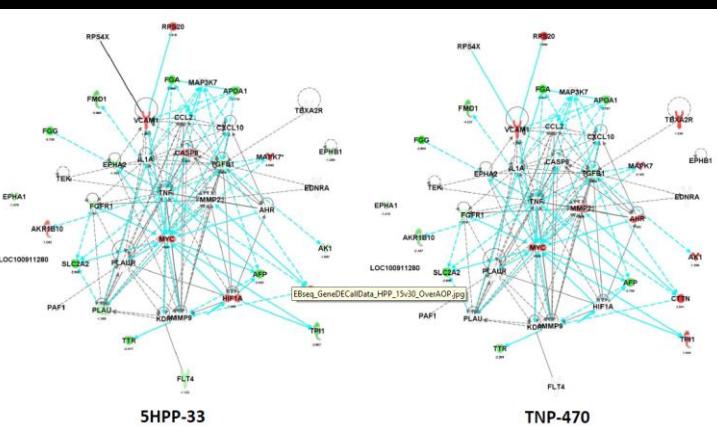
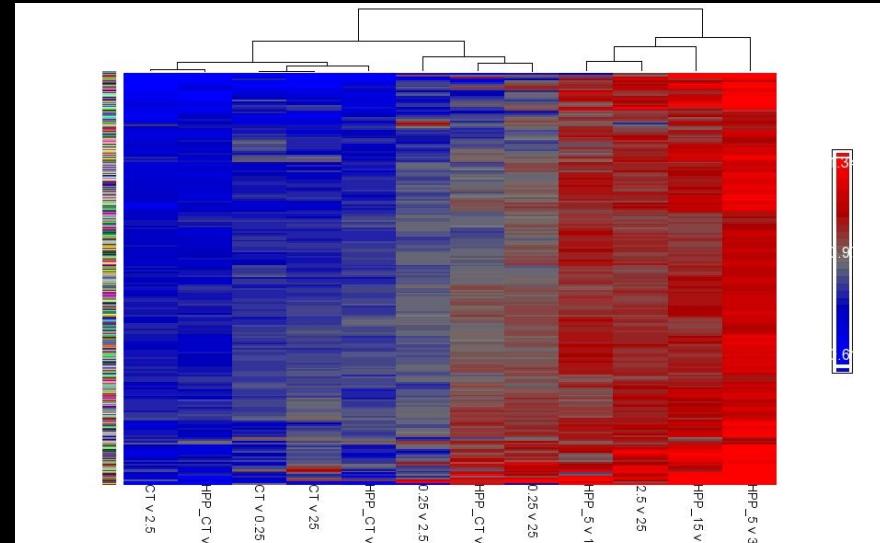
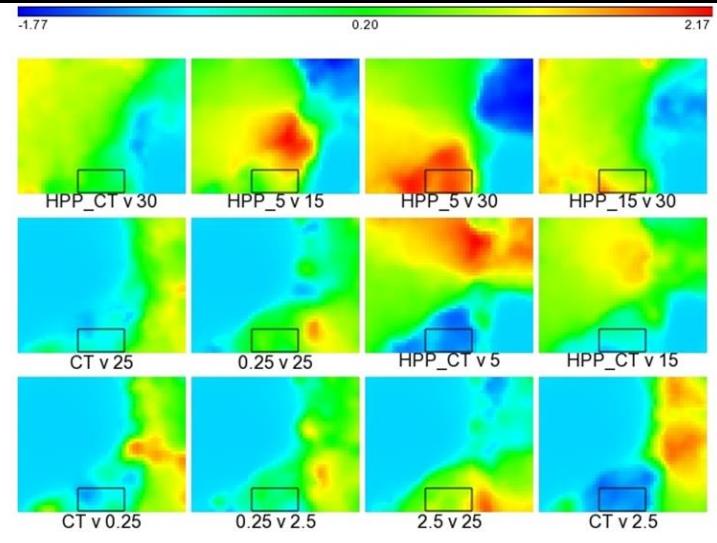
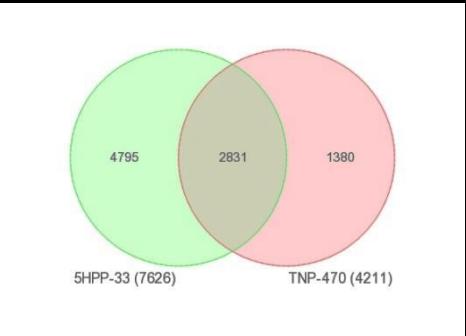
- synthetic thalidomide analog
- microtubule disruptor
- ↓ endothelial networks
- critical effect - embryo viability
- AC50 = 21.2 μM
- TI threshold from hESC = 9.5 μM

TNP-470

- synthetic fumagillin analog
- MetAP II inhibitor
- non-canonical WNT signaling
- critical effect - dysmorphogenesis
- AC50 = 0.038 μM
- TI threshold from hESC = 0.01 μM

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

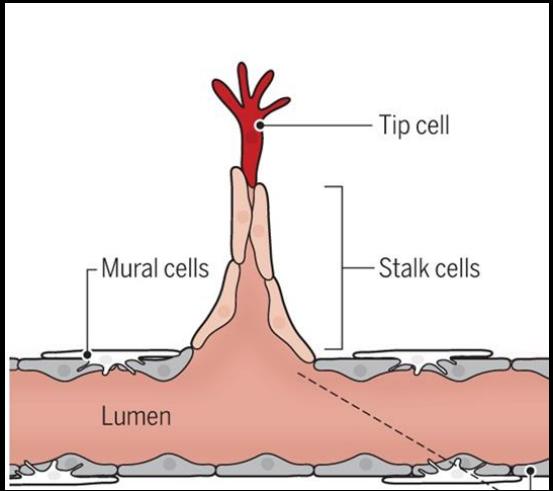
2831 DEGs overlap → SOM (464 genes in ROI box) → ROI clusters



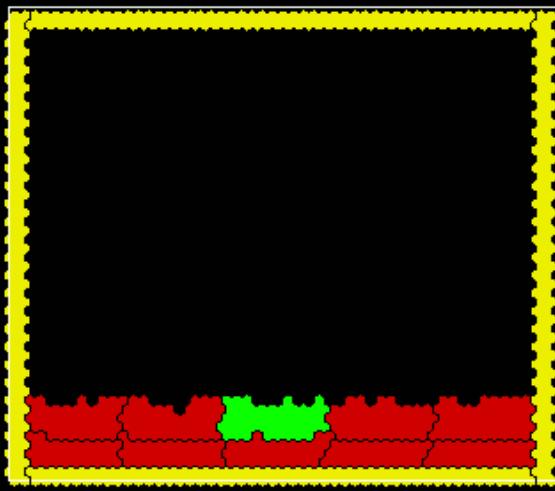
- FXR and LXR pathways common to 5HPP-33 and TNP-470 response.
- FXR (+) and LXR (-) pathways may be key events via RXR heterodimerization.

- splicesome and RNA metabolism
- proteosome and ubiquitination

Computer simulation: cell agent-based models



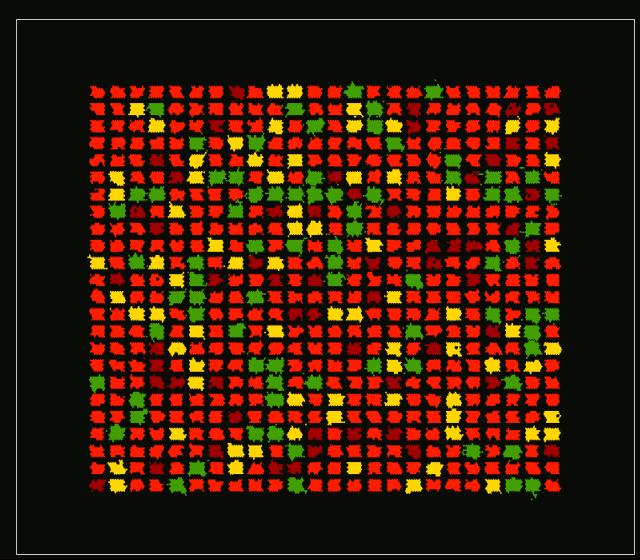
Li and Carmeliet (2018) Science



Nicole Kleinstreuer

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

Network assembly



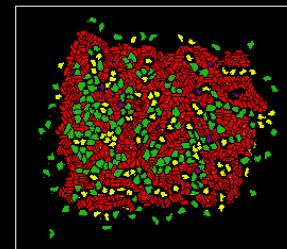
Kleinstreuer et al. (2013) PLoS Comp Biol

- ✿ Endothelial Stalk
- ❀ Endothelial Tip
- Mural Cell
- Inflammatory Cell

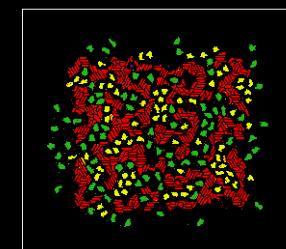
SOFTWARE: www.CompuCell3D.org

BioComplexity Institute, Indiana U

control

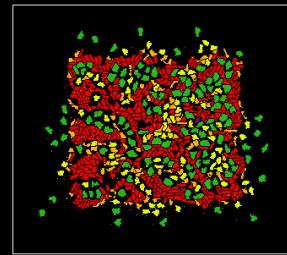


↓ VEGF

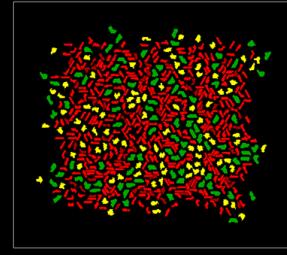


Simulated (*in silico*) profiling

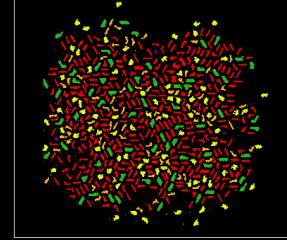
Imazamox



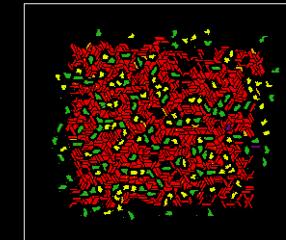
Pyridaben



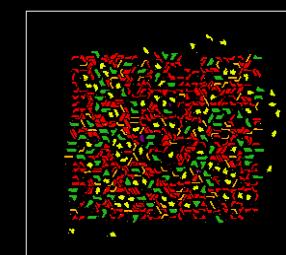
Disulfiram



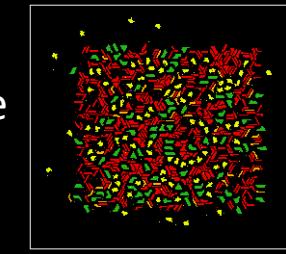
Bisphenol A



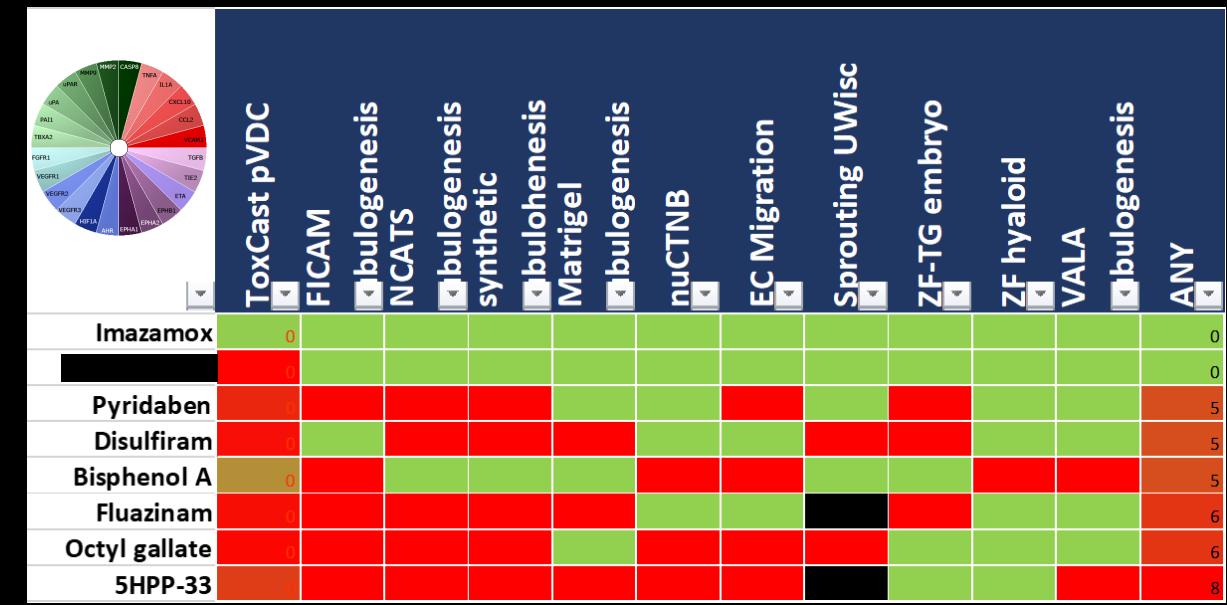
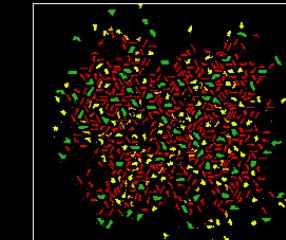
Fluazinam



Octyl gallate

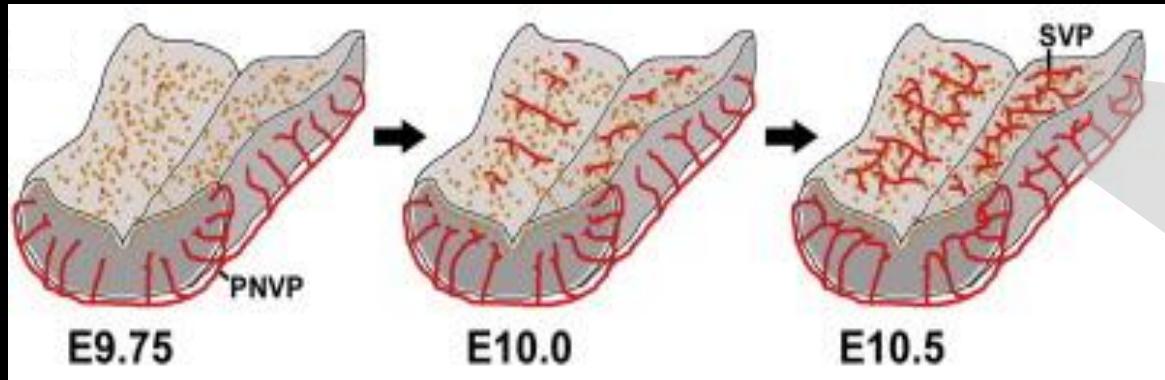


5HPP-33

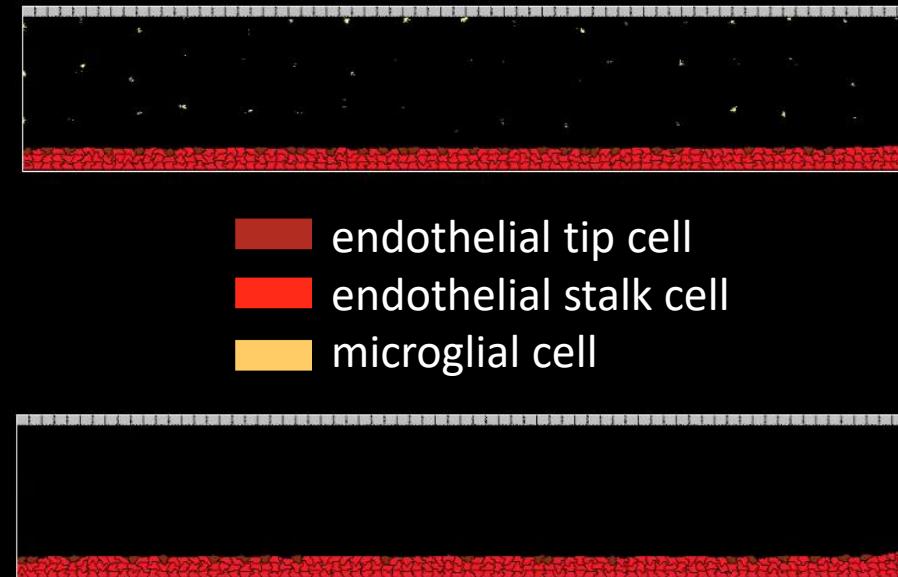
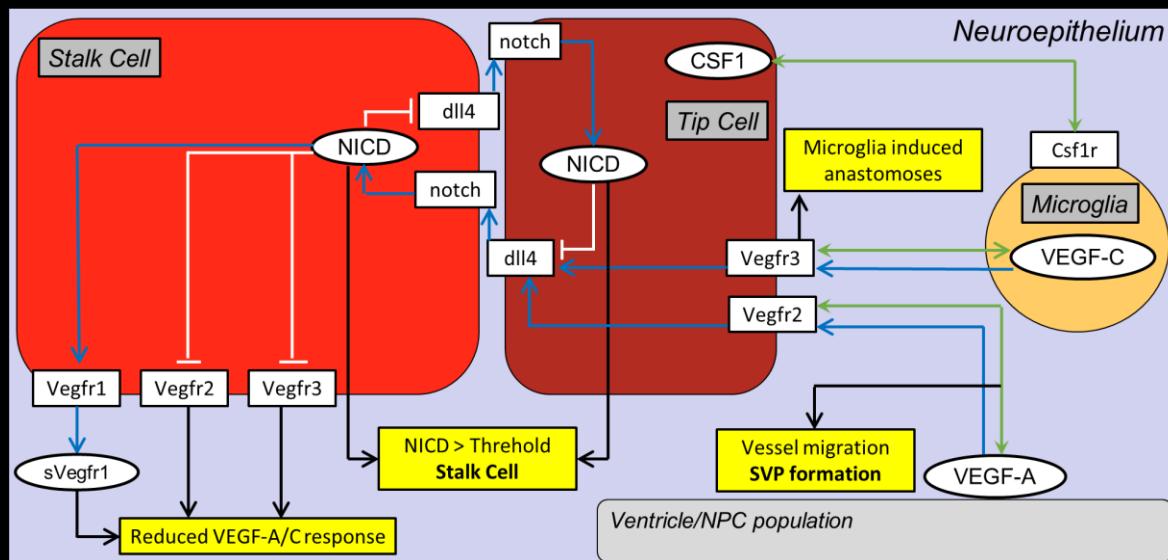
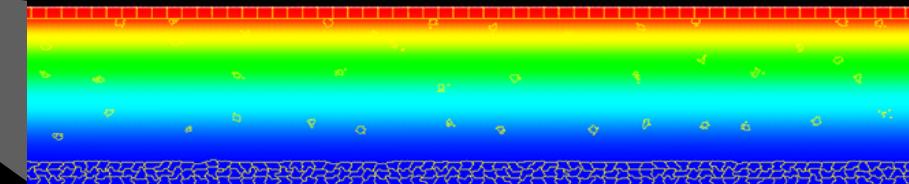


Neural tube vascularization

Tata et al. (2015) Mechanism Devel

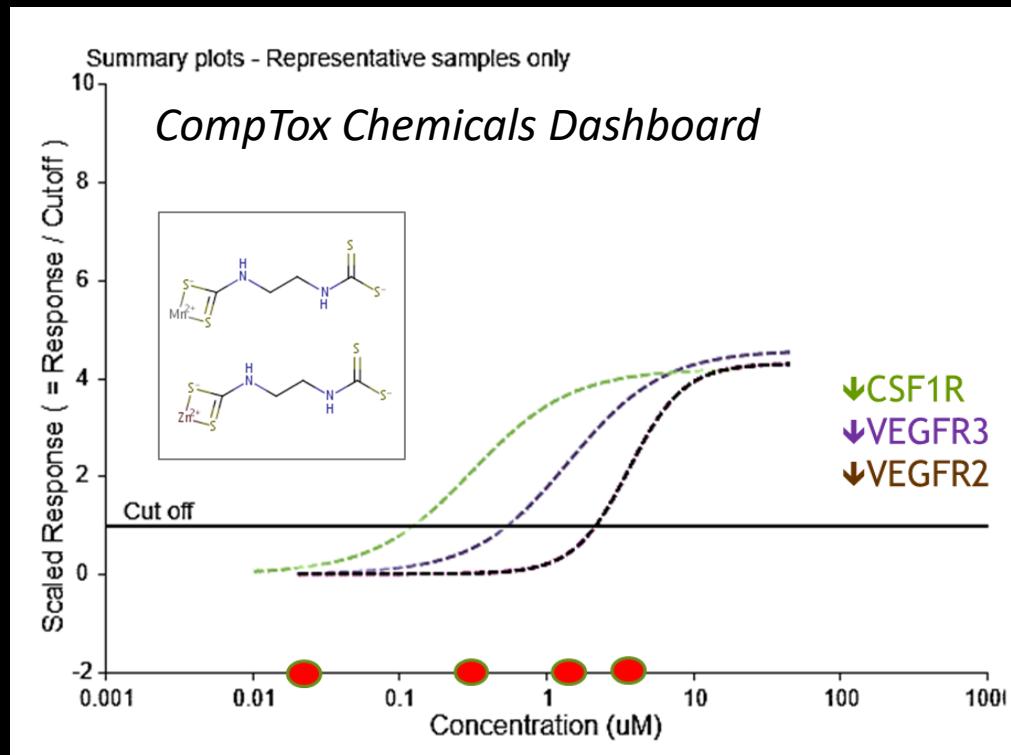


VEGF-A gradient: NPCs in subventricular zone

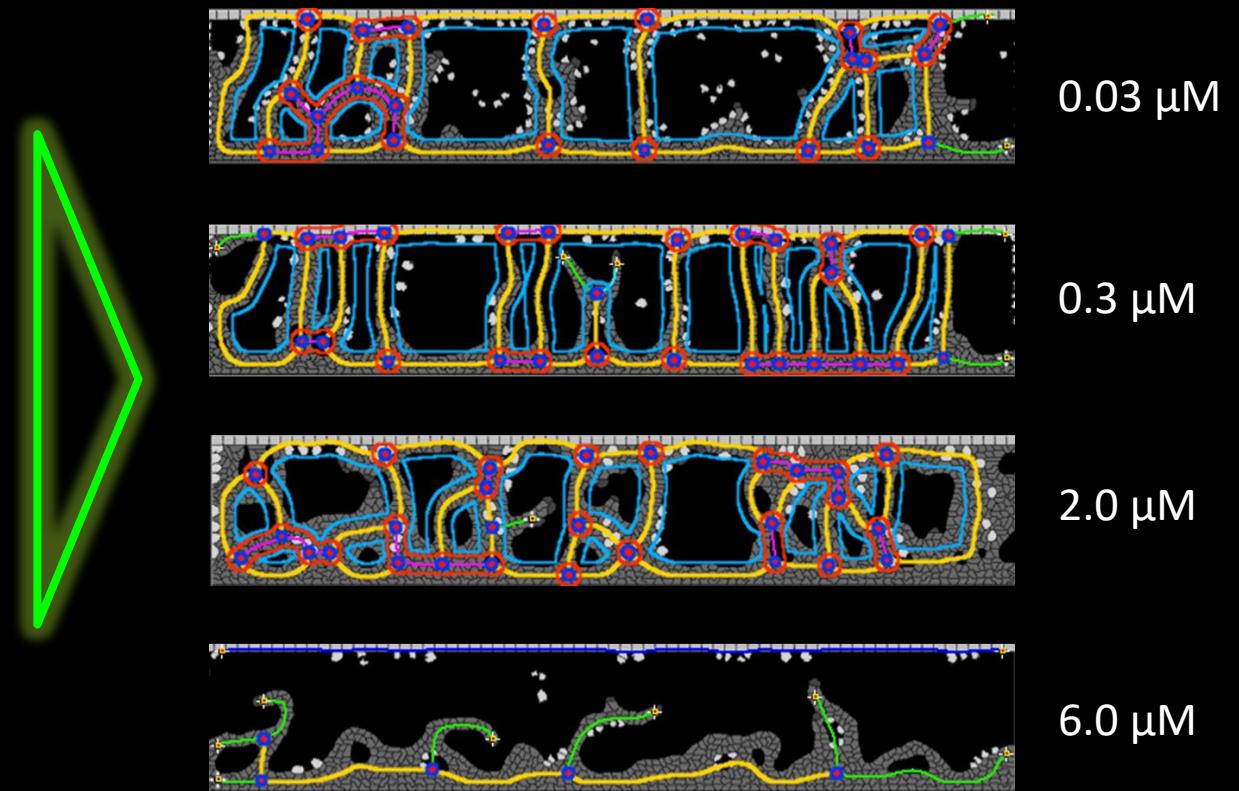


SOURCE: Zurlinden et al. (2018), NCCT

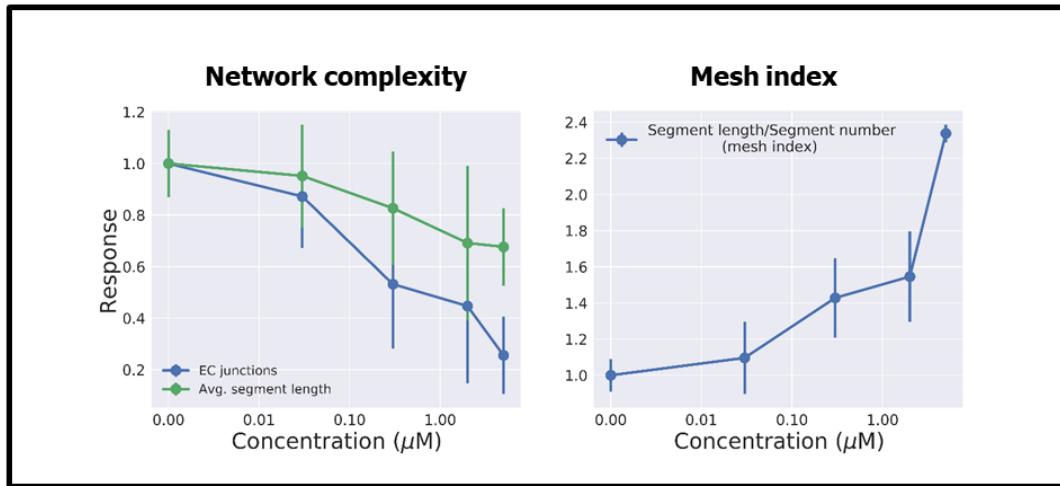
Simulated dose-response: brain angiogenesis from *in vitro* HTS data (ToxCast)



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



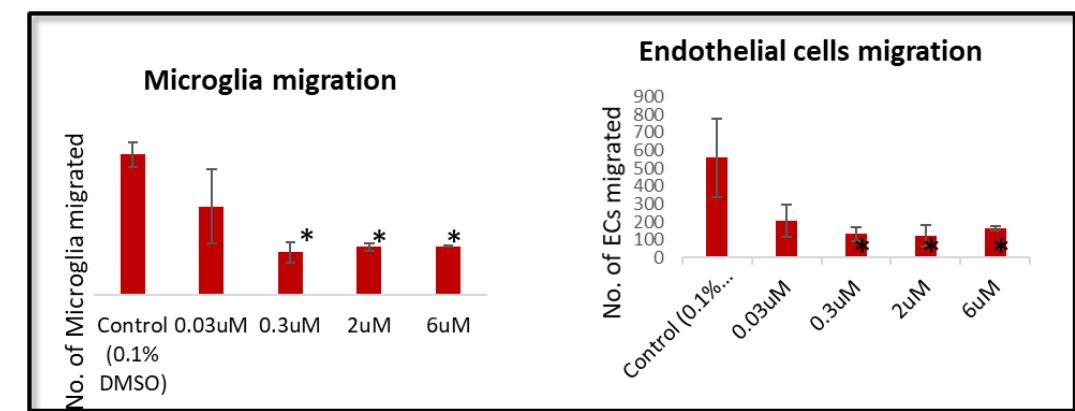
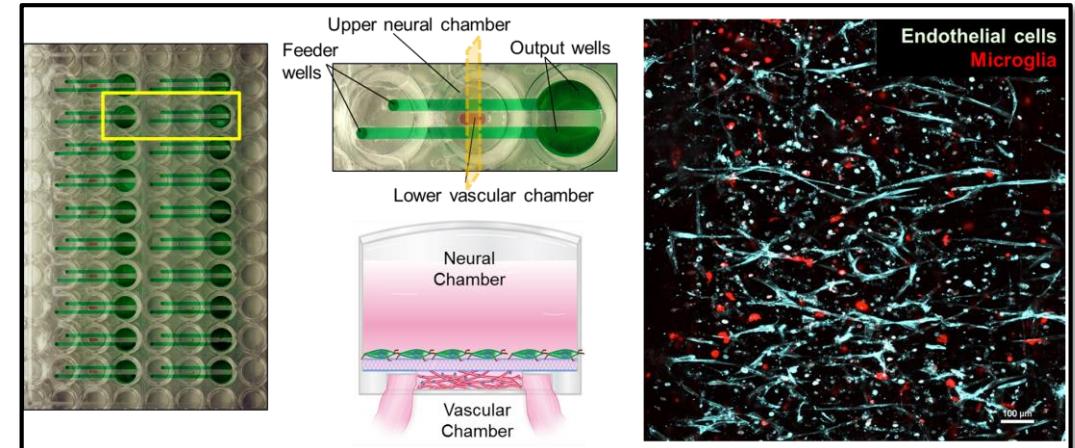
Computational prediction (cNVU)



Critical concentration:

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

Biomimetic reconstruction (hNVU)

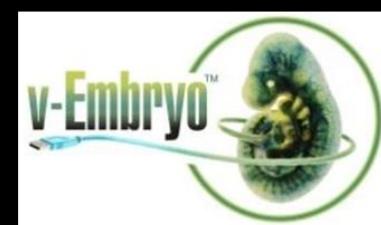


Summary: *decoding the toxicological blueprint of vascular development*

- 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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- Todd Zurlinden – NCCT
- BeiBei Cai – Vala Sciences
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- William Murphy – U Wisconsin (HMAPS)
- William Daly – U Wisconsin (HMAPS)
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- David Belair – NHEERL/TAD (now CellGene)
- Florent Ginhoux – A*STAR/SIgN
- 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

