



NTP
National Toxicology Program

Embryonic Vascular Disruption and Adverse Prenatal Outcomes

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ILS/NICEATM

AOP Workshop, Bethesda, MD

3 September 2014



Vascular Developmental Processes

❖ endothelial proliferation & cell migration

- growth factors
- chemokine signaling

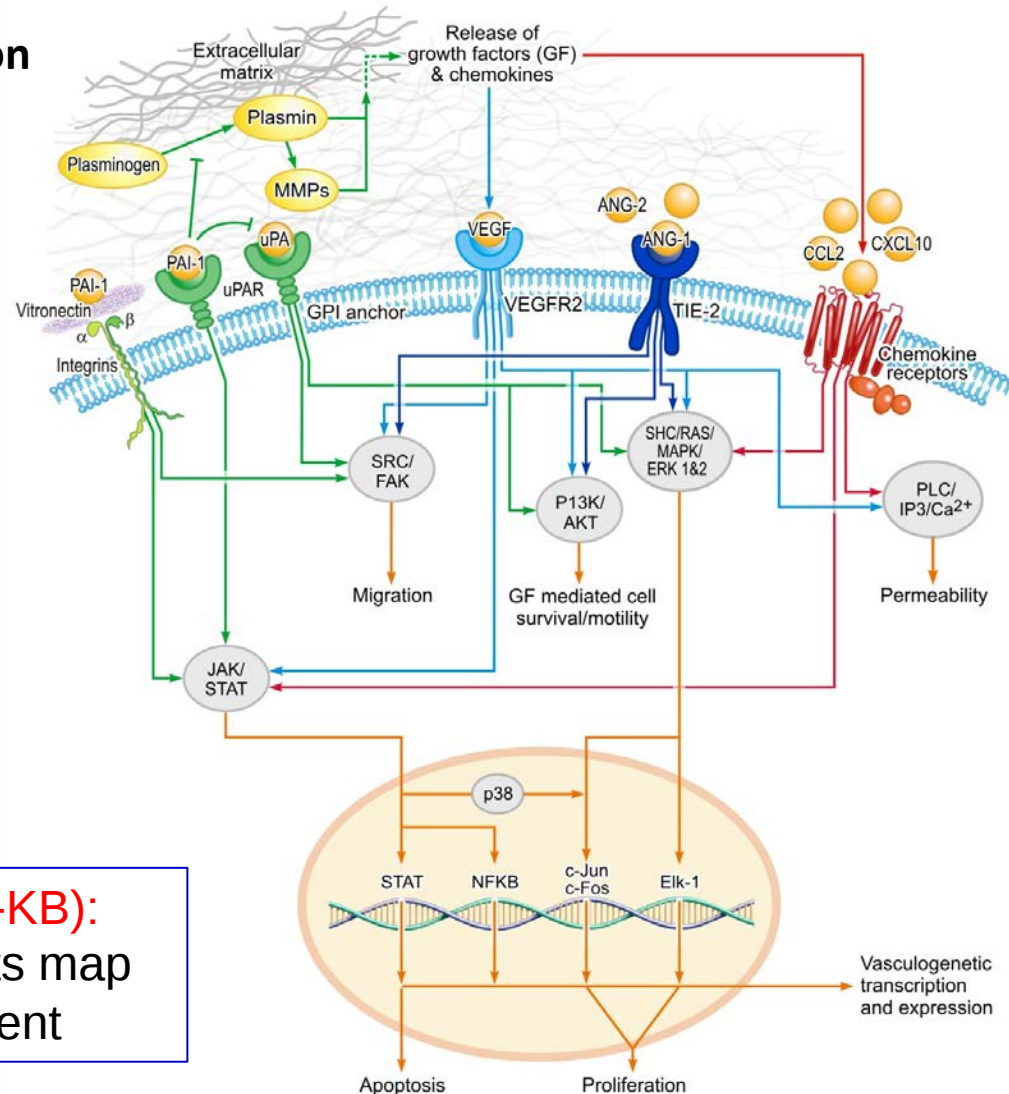
• extracellular matrix degradation

- plasminogen activating system
- matrix metalloproteinases

❖ neovascular stabilization

- Ang/Tie2 signaling
- vascular remodeling

Virtual Tissues-Knowledge Base (VT-KB):
~100 distinct ToxCastDB assay targets map to key systems in vascular development



ToxCastDB: 700+ HTS Assays

Assay Provider

ACEA
Apredica
Attagene
BioSeek
CellzDirect
NCGC/Tox21
NHEERL MESC
NHEERL NeuroTox
NHEERL Zebrafish
NovaScreen
Odyssey Thera

Biological Response

cell proliferation and death
cell differentiation
mitochondrial depolarization
protein stabilization
oxidative phosphorylation
reporter gene activation
gene expression (qNPA)
receptor activity
receptor binding

Target Family

Response Element
Transporter
Cytokines
Kinases
Nuclear Receptor
CYP450 / ADME
Cholinesterase
Phosphatases
Proteases
XME metabolism
GPCRs
Ion Channels

Assay Design

viability reporter
morphology reporter
conformation reporter
enzyme reporter
membrane potential reporter
binding reporter
inducible reporter

Readout Type

Single
Multiplexed
Multiparametric

Cell Format

Cell free
Cell lines
Primary cells
Complex cultures
Free-living embryos

Species

Human
Rat
Mouse
Zebrafish
Sheep
Boar
Rabbit
Cattle
Guinea pig

Tissue Source

Lung	Breast
Liver	Vascular
Skin	Kidney
Cervix	Testis
Uterus	Brain
Intestinal	Spleen
Bladder	Ovary
Pancreas	Prostate
Inflammatory	Bone

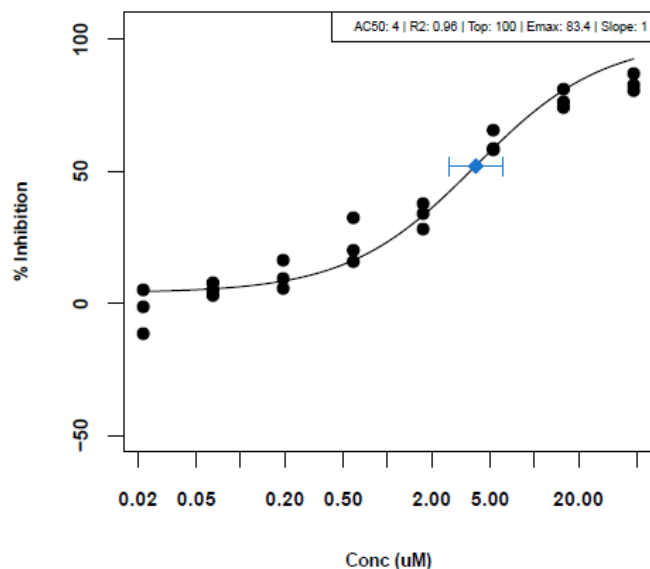
Detection Technology

qNPA and ELISA
Fluorescence & Luminescence
Alamar Blue Reduction
Arrasyscan / Microscopy
Reporter gene activation
Spectrophotometry
Radioactivity
HPLC and HPEC
TR-FRET

<http://actor.epa.gov/actor/faces/ToxCastDB>

ToxCastDB

<http://actor.epa.gov/actor/faces/ToxCastDB/DataCollection.jsp>



AC50 concentration producing a 50% change
LEC lowest effect concentration

ToxCastDB
 You are here: EPA Home » National Center for Computational Toxicology » ToxCastDB » Data Collection

Home | Basic Info | Data Collection List | Chemical List | Genes Associated with Assays | Help

Data Collection: Novascreen

Name: Novascreen
 Description: Novascreen - Calcium - receptor binding and enzyme inhibition assays
 Number of Chemicals: 379
 Number of Assays: 273
 Number of Data Points: 93440

Name	Assay Name	Description	Substances	Components	Species	Gene
NVS_ENZ_XTA2	Novascreen Human TxA2	Human TxA2 Fluorescein-peptide	300	1	Homo sapiens	TXA2
NVS_ENZ_XTAA	Novascreen Human TAA	Human TAA Fluorescein-labeled peptide	300	2	Homo sapiens	NTN3
NVS_ENZ_VEGFR1	Novascreen Human VEGFR1	Human VEGFR1 Fluorescein-labeled peptide	300	2	Homo sapiens	FLT1
NVS_ENZ_VEGFR2	Novascreen Human VEGFR2	Human VEGFR2 Fluorescein-labeled peptide	300	1	Homo sapiens	KDR
NVS_ENZ_VEGFR3	Novascreen Human VEGFR3	Human VEGFR3 Fluorescein-labeled peptide	300	1	Homo sapiens	FLT4
NVS_ENZ_ZAP70	Novascreen Human ZAP70	Human ZAP-70 Fluorescein-peptide	300	1	Homo sapiens	ZAP70
NVS_ENZ_ACO1	Novascreen Oase COX1	Oase COX1 Acarboxylic acid-TMPD	300	1	Ovis aries	PTCD1
NVS_ENZ_OOG2	Novascreen Oase COG2	Oase COG2 Acarboxylic acid-TMPD	300	1	Ovis aries	PTGDS-2
NVS_ENZ_MTHFR	Novascreen Pig MTHFR	Pig MTHFR S-(14C)-MethF	300	1	Sus scrofa	MTHFR
NVS_ENZ_RALG12	Novascreen RalG12	RalG12 G12-SP1	300	1	Oryzotetes carmelite	
NVS_ENZ_ACF Binding	Novascreen Rat ACF Binding	Rat ACF Binding 3H-Forskolin	300	1	Rattus norvegicus	Adcy5

ToxCastDB
 You are here: EPA Home » National Center for Computational Toxicology » ToxCastDB » Assay

Home | Basic Info | Data Collection List | Chemical List | Genes Associated with Assays | Help

Assay: Novascreen Human VEGFR2

Assay Id: 978
 Source: Novascreen
 Source Name AID: NVS_ENZ_hVEGFR2
 Name: Novascreen Human VEGFR2
 Description: Human VEGFR2 Fluorescein-labeled peptide
 Number of Substances: 320
 Number of Components: 1
 Species: Homo sapiens

Parameter	Value
CATALOG NUMBER	200-0768
ASSAY CATEGORY	Enzyme Inhibition
ASSAY CATEGORY	In vitro (Biochemical)
ASSAY TARGET	VEGFR2
ASSAY TARGET FAMILY	Kinase
ASSAY TARGET SOURCE	Recombinant
ASSAY TARGET SOURCE TYPE	amino acid 805 to 1356
ASSAY GENE ID	3791
ASSAY GENE NAME	KDR
ASSAY REFERENCE COMPOUND	Staurosporine
ASSAY NOTE	KINASE
ASSAY SUBSTRATE NAME	receptor tyrosine kinase
ASSAY ATP CONCENTRATION (M)	NCCT_v2
ASSAY ENZYME AFFINITY ATP KM (M)	Fluorescein-labeled peptide
ASSAY LIGAND NAME	1.50E-06
ASSAY LIGAND CONCENTRATION (M)	1.20E-05
ASSAY BMAX	Fluorescein -peptide + ATP -> fluorescein -phosphopeptide + ADP

Name	CASRN	NVS_ENZ_hVEGFR2 (uM)
Mancozeb	8018-01-7	5.9
Maneb	12427-38-2	31.0
Metiram-zinc	9006-42-2	45.0
Oxytetracycline dihydrate	6153-64-6	19.0

- Gene Ontology (GO) and Mammalian Phenotype (MP) browsers of MGI database (<http://www.informatics.jax.org/>) for **neovascularization**:
 - abnormal vasculogenesis [MP:0001622; 72 genotypes, 73 annotations]
 - abnormal angiogenesis [MP:0000260; 610 genotypes, 894 annotations]
- 65 genes with roles in vasculogenesis or angiogenesis linked to ToxCast assays, 50 had evidence of abnormal embryonic vascular development in MGI

Overlap between ToxCast assay targets and abnormal vascular phenotypes from genetic mouse models.

<u>ToxCast</u> Gene Target *	MP Annotated Term	<u>ToxCast</u> Assays
AHR	patent ductus venosus, abnormal vascular regression	<u>ATG_Ahr_CIS</u> , <u>NCGC_Ahr</u>
BMPR2	decreased angiogenesis	ATG_BRE_CIS
CASP8	abnormal vitelline vasculature morphology	NVS_ENZ_hCASP8
CCL2	decreased angiogenesis, abnormal physiological <u>neovascularization</u> , <u>choroidal neovascularization</u>	BSK_3C_MCP1, BSK_4H_MCP1, BSK_KF3CT_MCP1, BSK_LPS_MCP1, BSK_SAg_MCP1, BSK_SM3C_MCP1
CEBPB*	abnormal <u>vasculogenesis</u> , absent organized vascular network	ATG_C_EBP_CIS, ATG_CRE_CIS

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Environ Health Perspect. 2011 Nov;119(11):1596-603. doi: 10.1289/ehp.1103412. Epub 2011 Jul 25.

Environmental impact on vascular development predicted by high-throughput screening.

Kleinstreuer NC, Judson RS, Reif DM, Singh NS, Singh AV, Chandler KL, Dawarshan P, Dix DJ, Kaulsky BJ, Kauden TP

Author information

Abstract

BACKGROUND: Understanding the diverse chemical landscape and pa Agency (EPA) ToxCast™ project pr in vitro assays in phase I (complete diverse biological targets and build developmental health and disease

Birth Defects Research, Part C

Embryo Today: Reviews

Review

Disruption of embryonic vascular development in predictive toxicology†

Thomas B. Knudsen, Nicole C. Kleinstreuer

Issue

Article first published

DOI: 10.1002/bd

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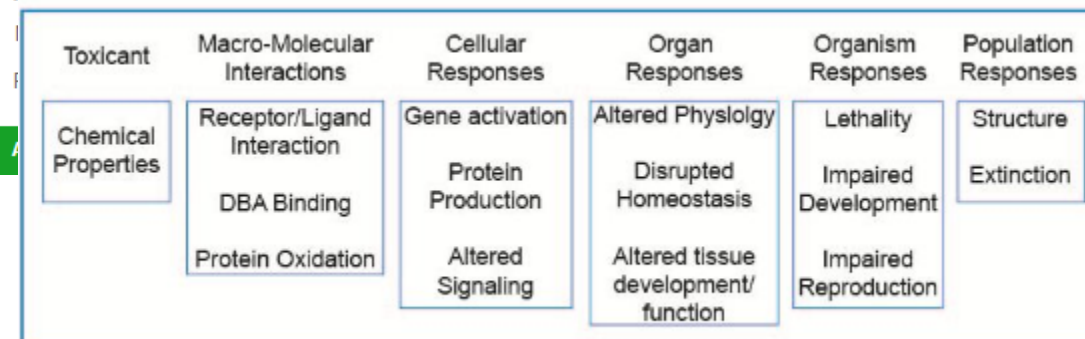
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CITATIONS

19

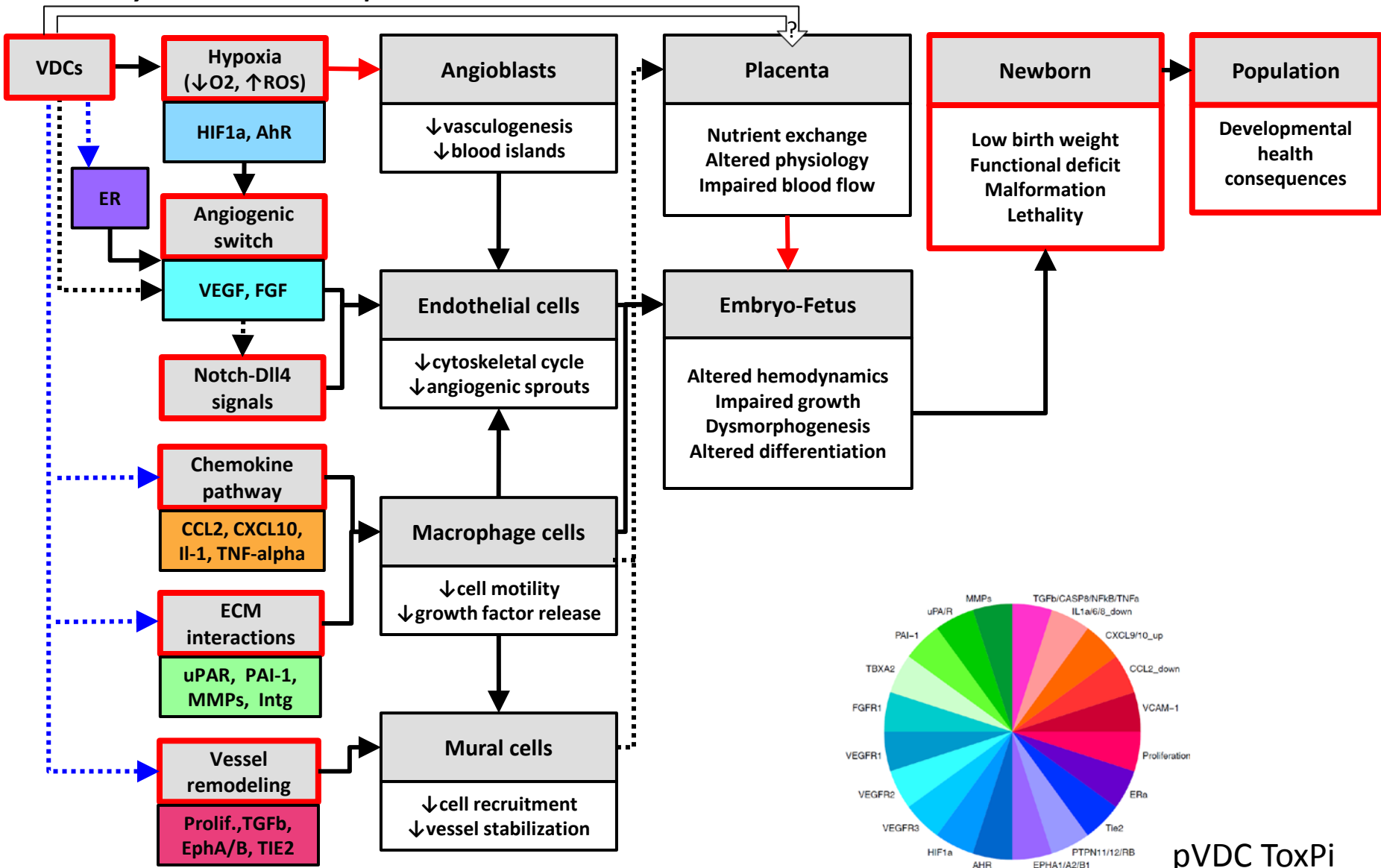
SAVES

A Computational Model Predicting Disruption of Blood Vessel Development



Adverse Outcome Pathway Framework

AOP: Embryonic Vascular Disruption



→ Established mechanistic linkage with quantitative or semi-quantitative data

... Predictive model linkages based on quantitative concentration-response data

Test the pVDC signature

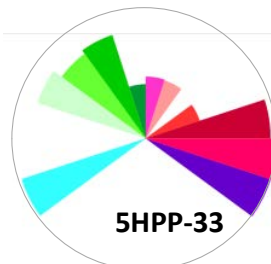
Developmental vascular toxicity predictive signature



Rank order 1060 ToxCast compounds based on putative ability to disrupt developmental angiogenesis

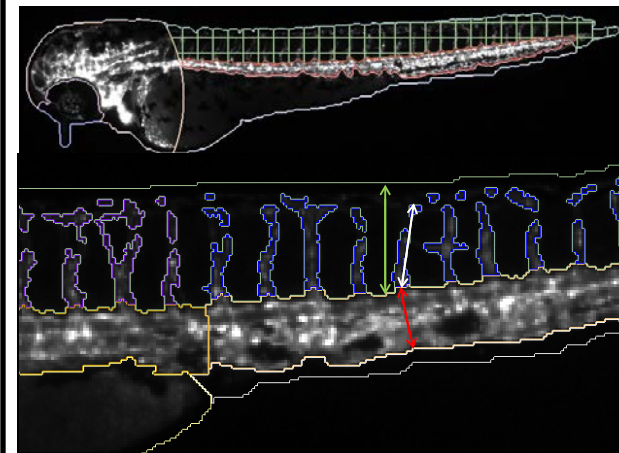
Poster I-4-550:
Human cell-based
functional 3D-angiogenesis
test for identification of
inhibitors of angiogenesis
T. Toimela, O. Huttala, J-R.
Sarkanen, T. Heinonen

ToxPi

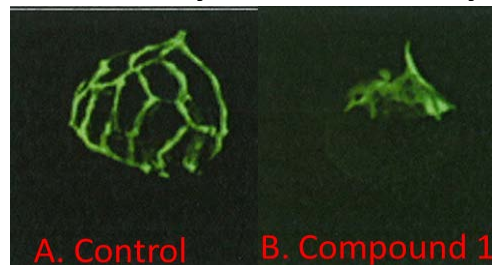


5HPP-33
Thalidomide analog

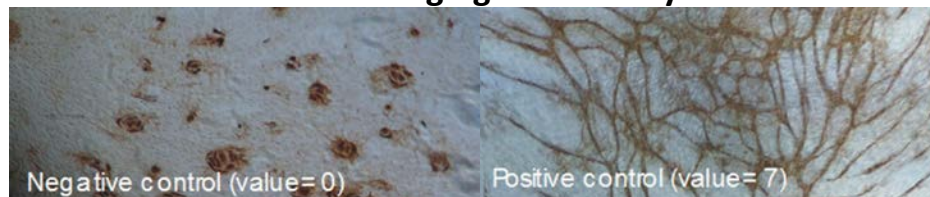
Zebrafish trunk vessel assay



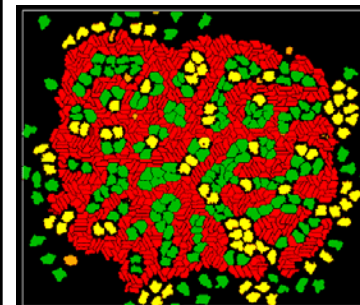
Zebrafish hyaloid vessel assay

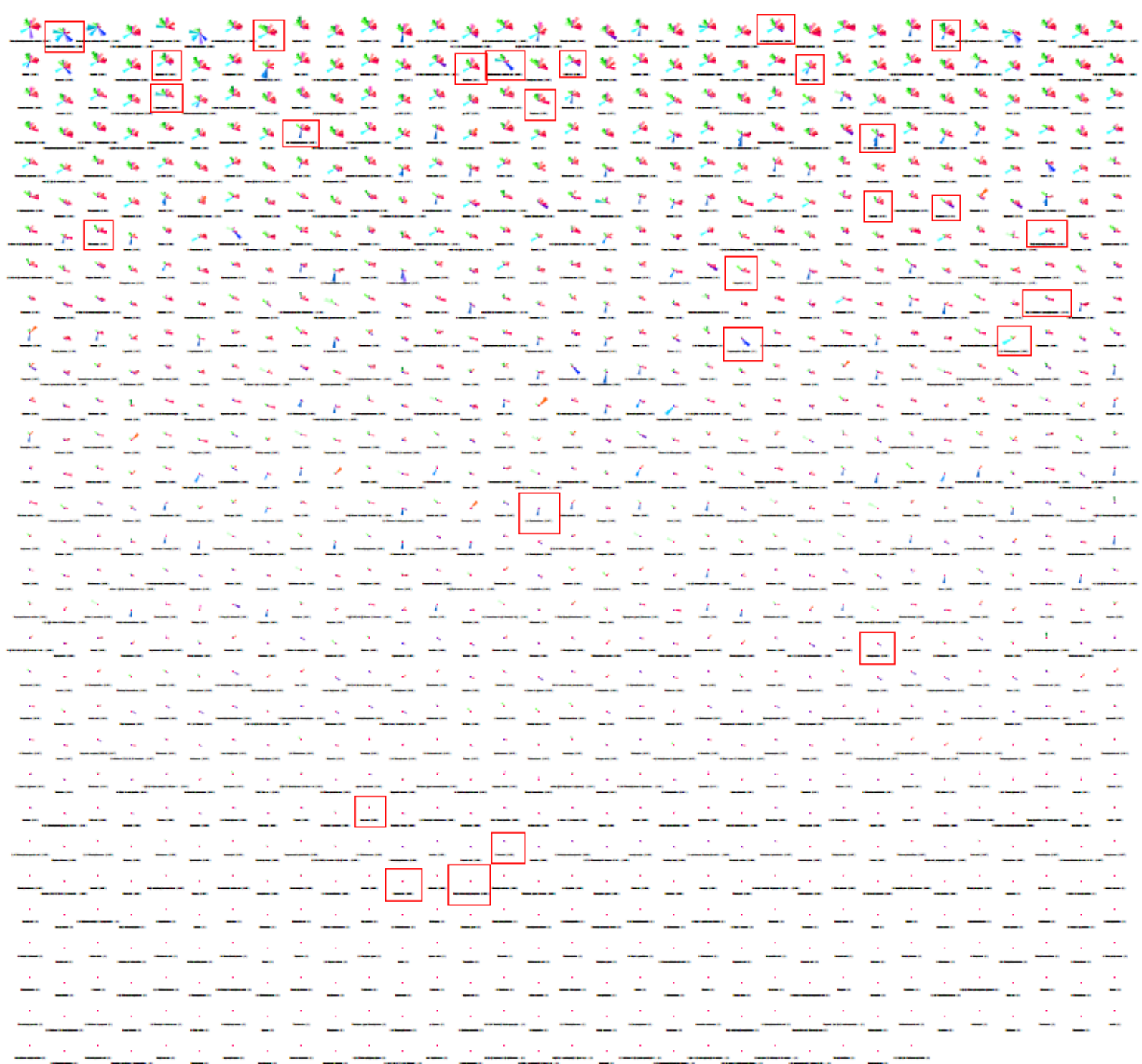


Human angiogenesis assay

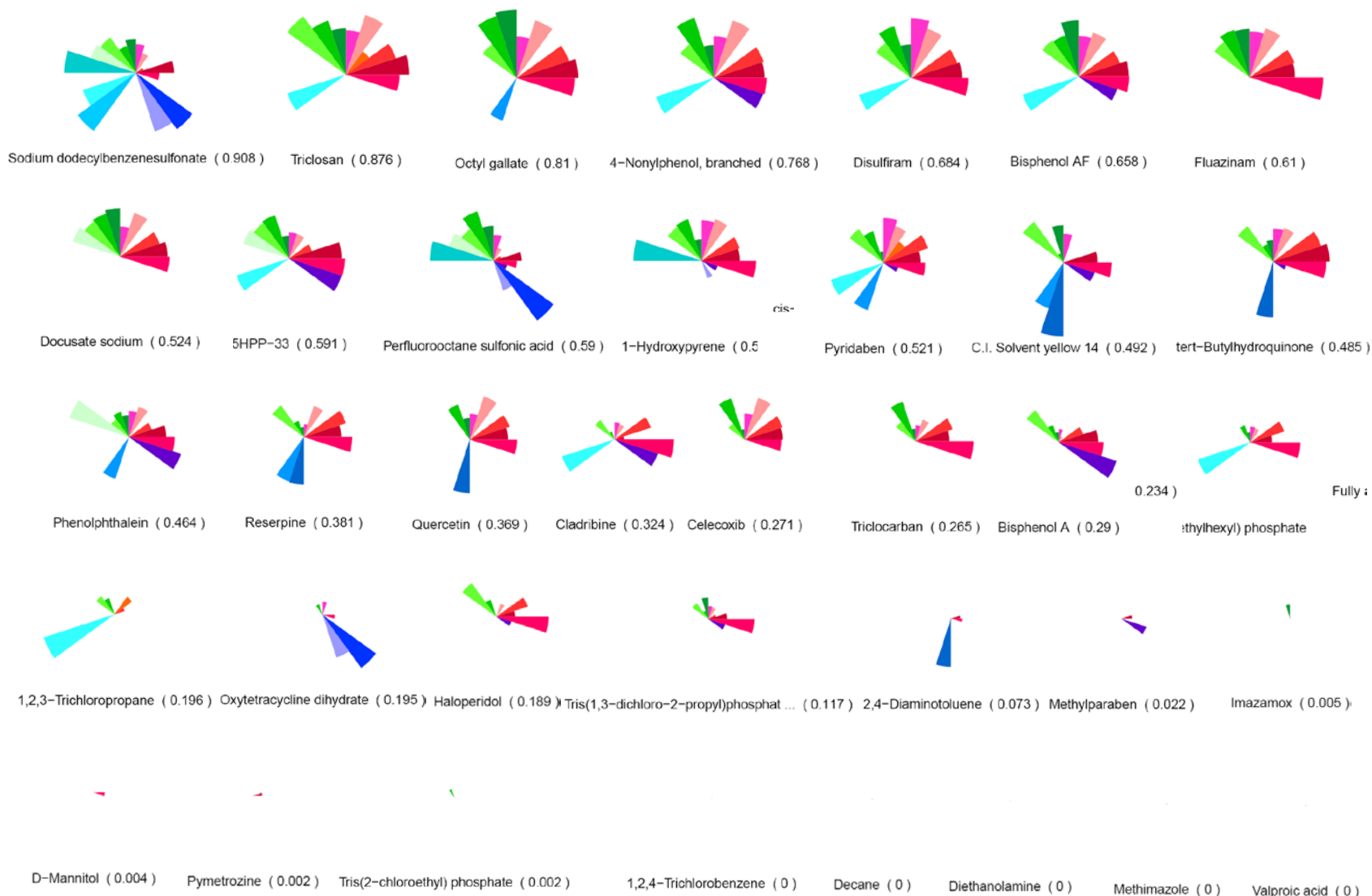


in silico model of vascular plexus formation





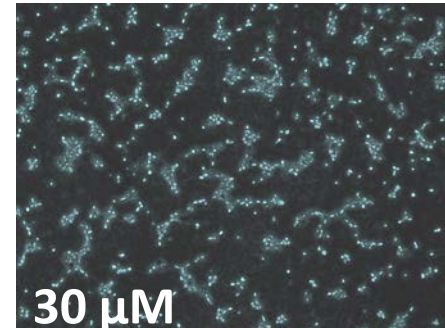
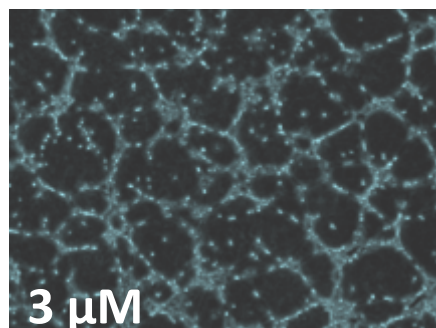
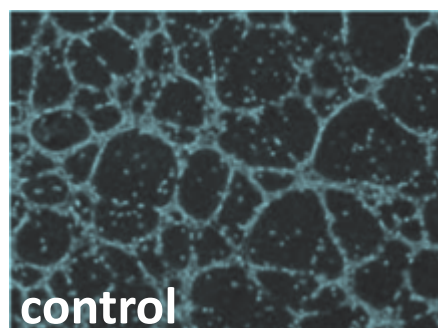
Chemical selection: Test the pVDC signature



Validation of vascular disruption AOP by orthogonal assays: *in vitro*, *in silico*, and *in situ*

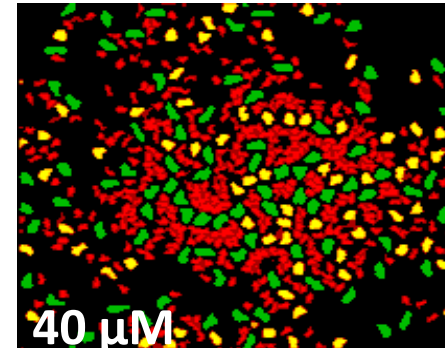
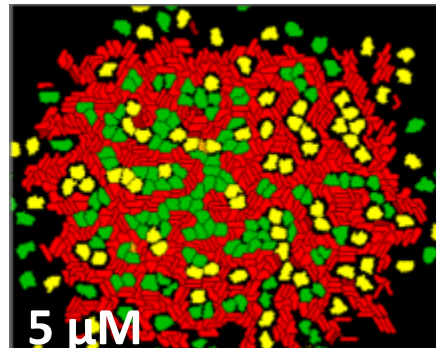
In vitro (HUVEC)

Noguchi et al. 2005,
Bioorg Med Chem Lett.



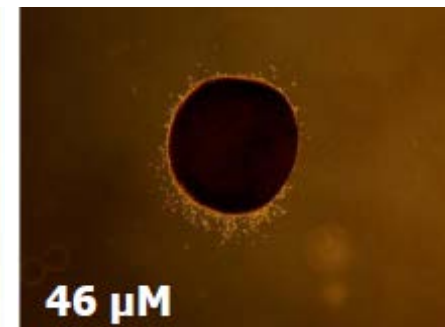
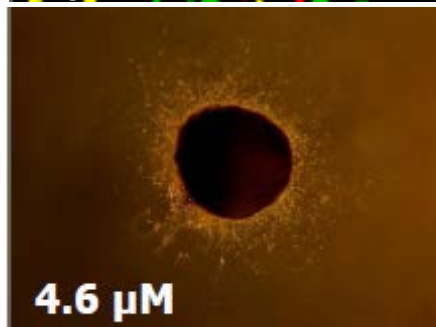
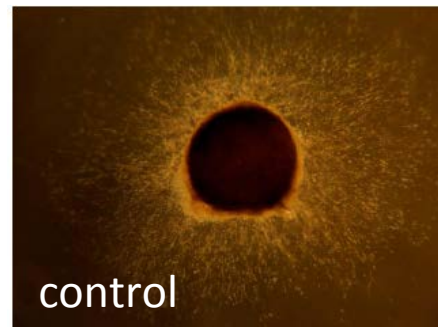
In silico (virtual tissue)

Kleinstreuer et al (2013)
PLoS Comp Biol 9(4):
e1002996



In situ (Aortic explant)

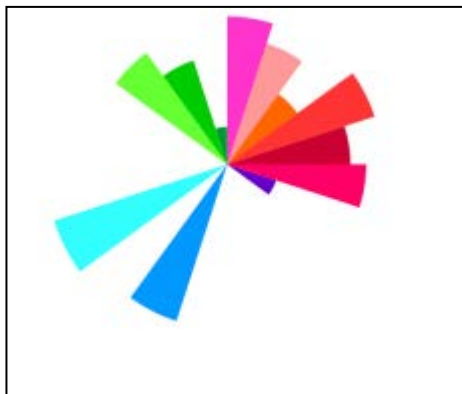
Carney & Ellis-Hutchings,
Dow Chemical Co.
(manuscript in prep)



5HPP-33 exposure disrupts angiogenesis *in vitro*, *in silico*, and *in situ*

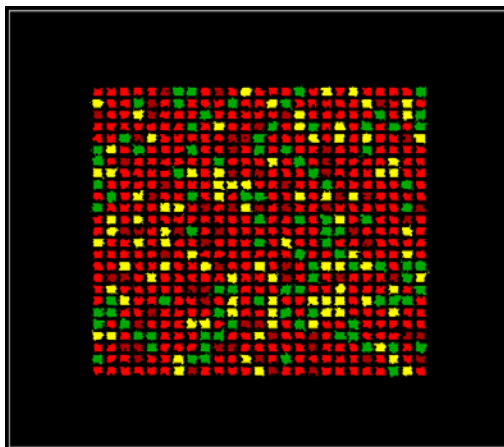
Virtual Tissues & Human Cell Based Tubulogenesis

ToxCast prediction

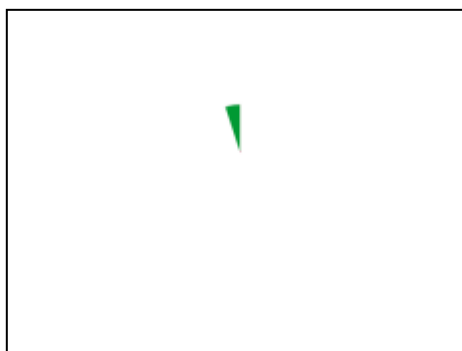
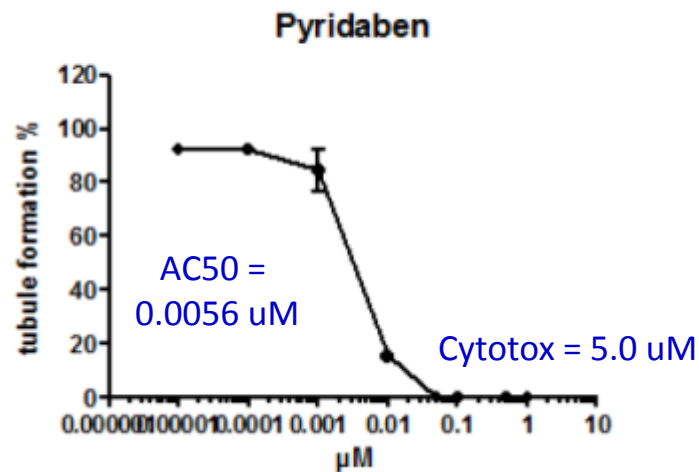


Pyridaben

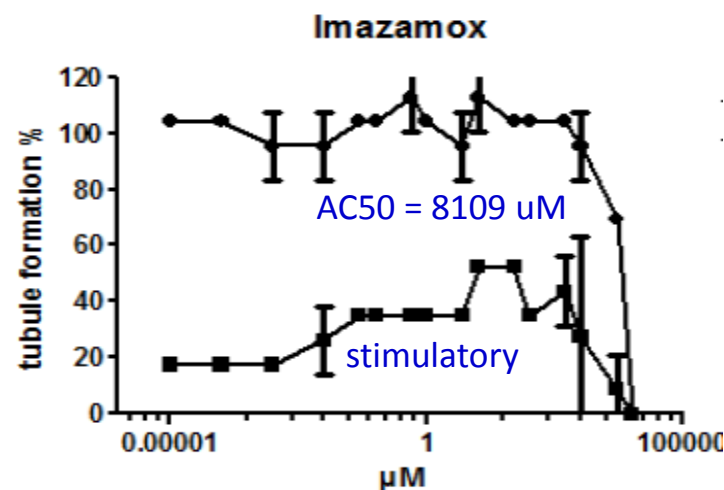
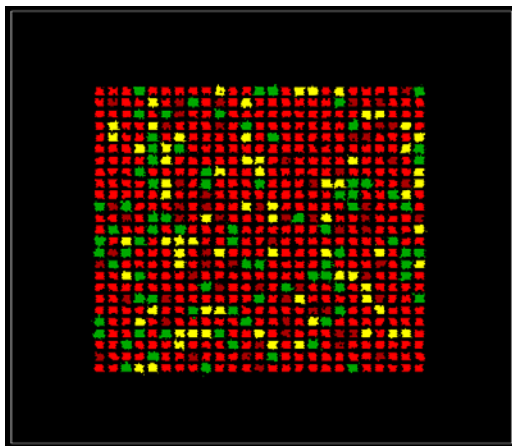
Virtual Tissue model



In vitro qualification



Imazamox

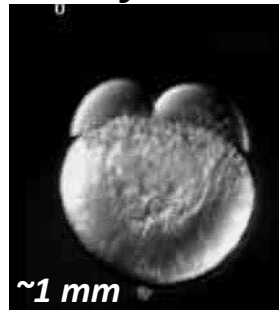




Zebrafish embryogenesis: A Quantitative AOP Model?

- ❖ a biologically complex system
 - - vascular developmental toxicity
- ❖ conserved pathways
 - - 75% of genes have human homologs
- ❖ embryo is transparent
 - - amenable to quantitative imaging
- ❖ transgenic reporter lines
 - - map vasculature across space-time
- ❖ rapid and scalable platform
 - - amenable to automation and HTS

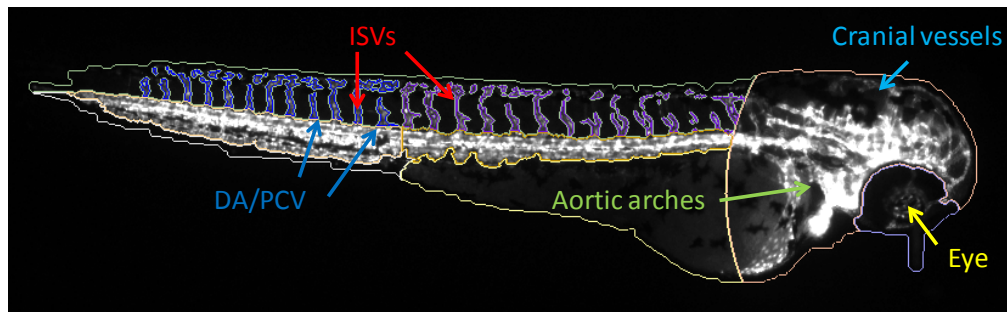
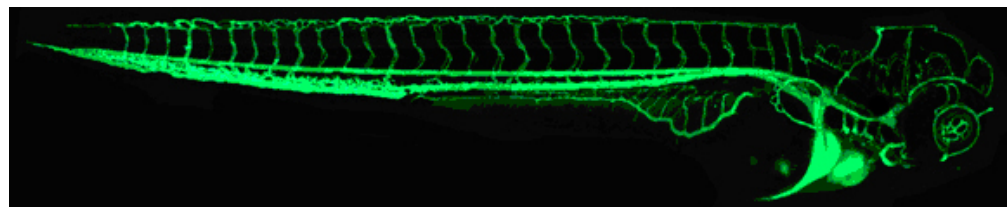
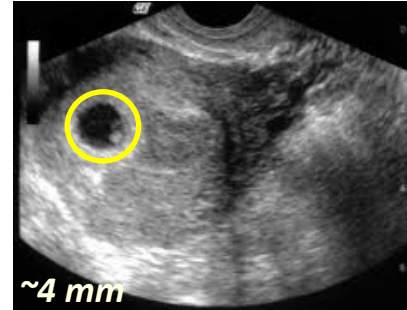
Zebrafish



Mouse



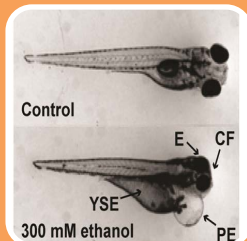
Human



SOURCE: Tamara Tal, EPA/NHEERL-ISTD

Zebrafish trunk vessel assay

Screening strategy



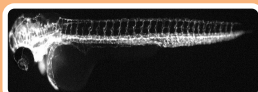
Define overt toxicity

- Start at 80 μ M
- Semi-log spacing
- 8 concentrations
- n=1, 2 embryos/rep
- Chlopyrifos positive control (8 + 80 μ M)

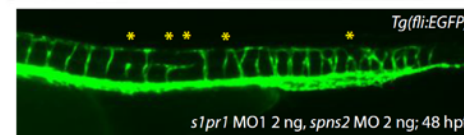
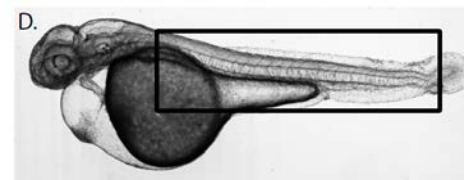
Criteria for inclusion

- <15% controls abnormal
- Positive controls on each plate

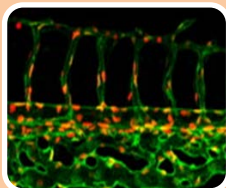
Screen for developmental vascular toxicity



- Start at LOEL
- Quarter-log spacing
- 4 concentrations
- n=2, 2 embryos/rep
- PTK787 positive control (4 μ M)



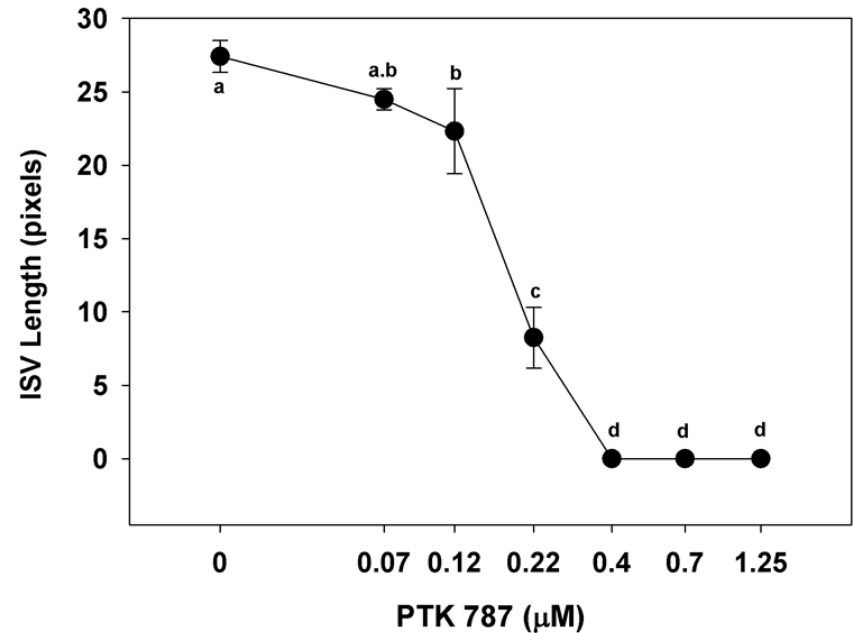
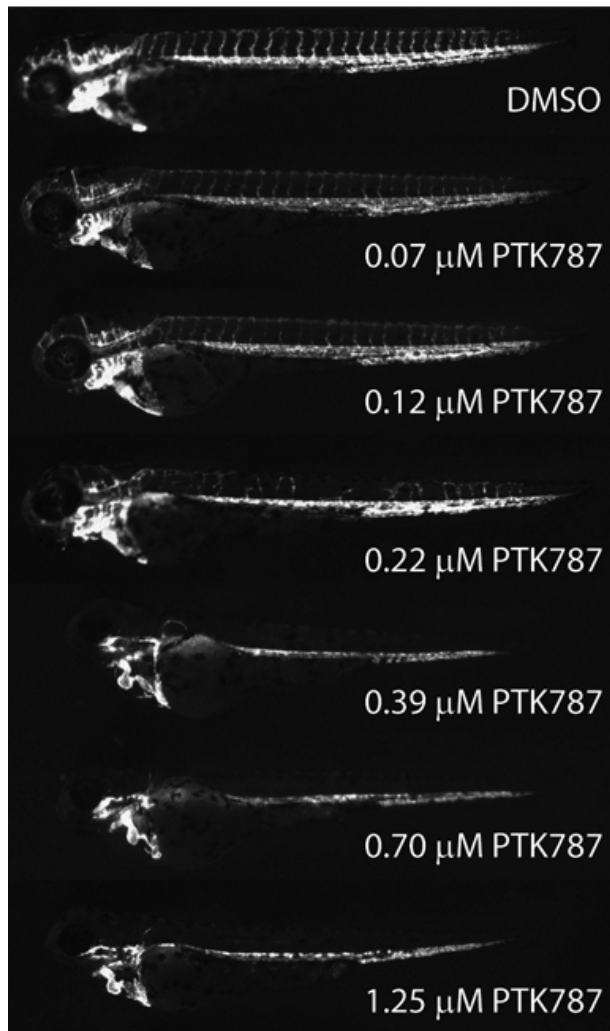
Quantify vascular toxicity



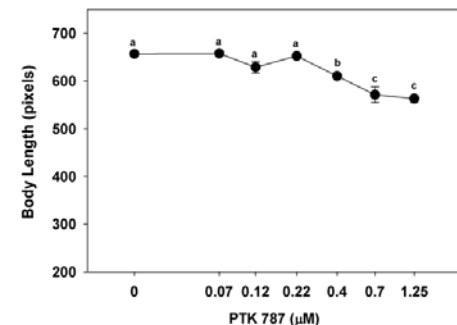
- For hits and subset of
- negative compounds
- Start at LOEL
- Quarter-log spacing
- 8 concentrations
- n=3, 2 embryos/rep
- PTK787 positive control (4 μ M)

Source: Mendelson et al. 2012.

Impaired angiogenesis in larvae exposed to PTK787 (VEGFR2 inhibitor)

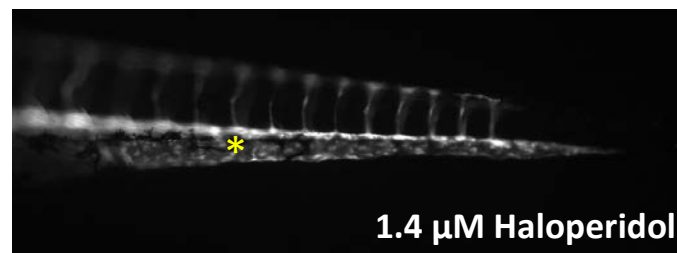
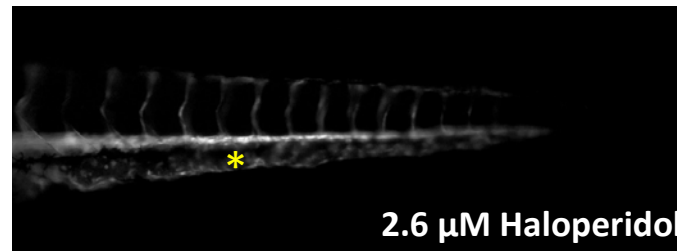
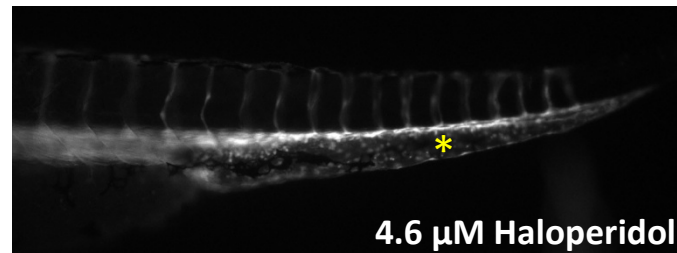
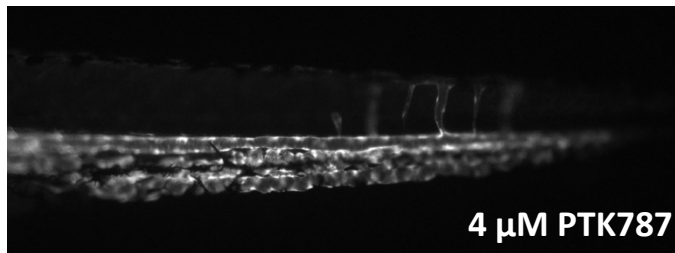
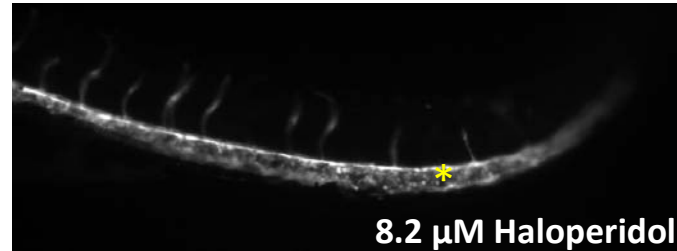
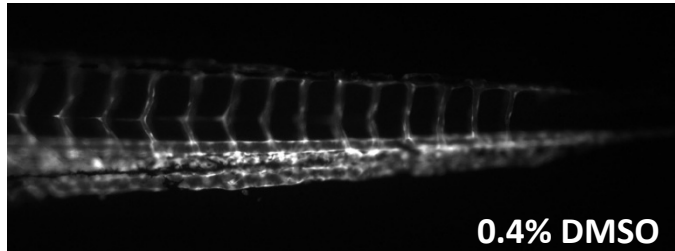


**Overt Toxicity:
(Body Length)**



Source: Tal et al. 2014

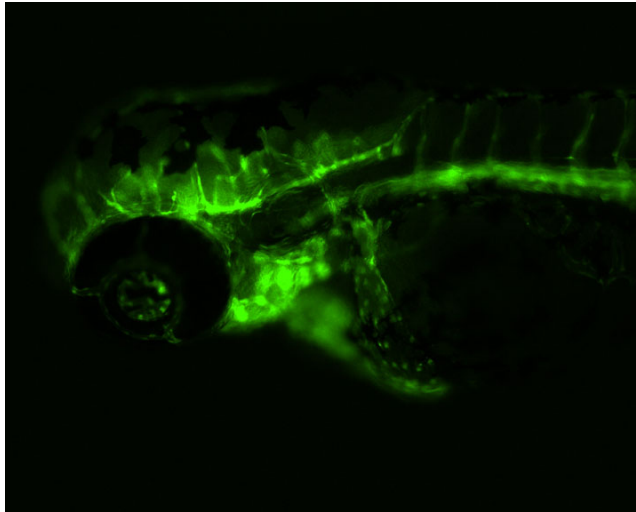
Predominant CVP phenotype: Haloperidol



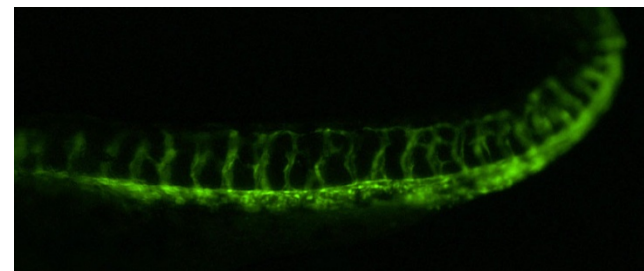
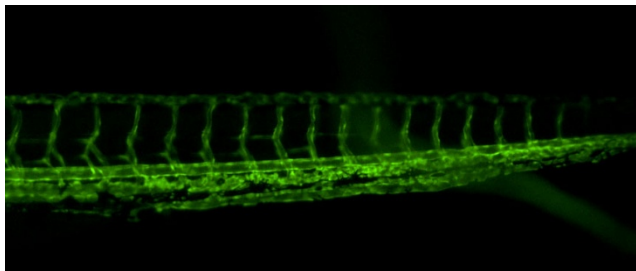
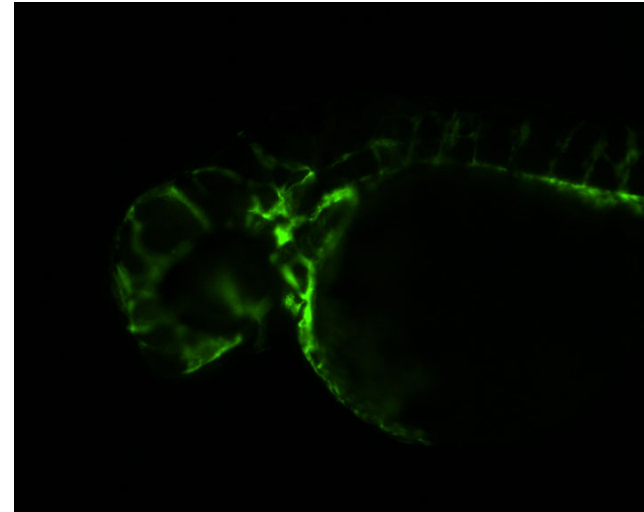
Cranial vascular phenotype: Fluazinam



0.4% DMSO

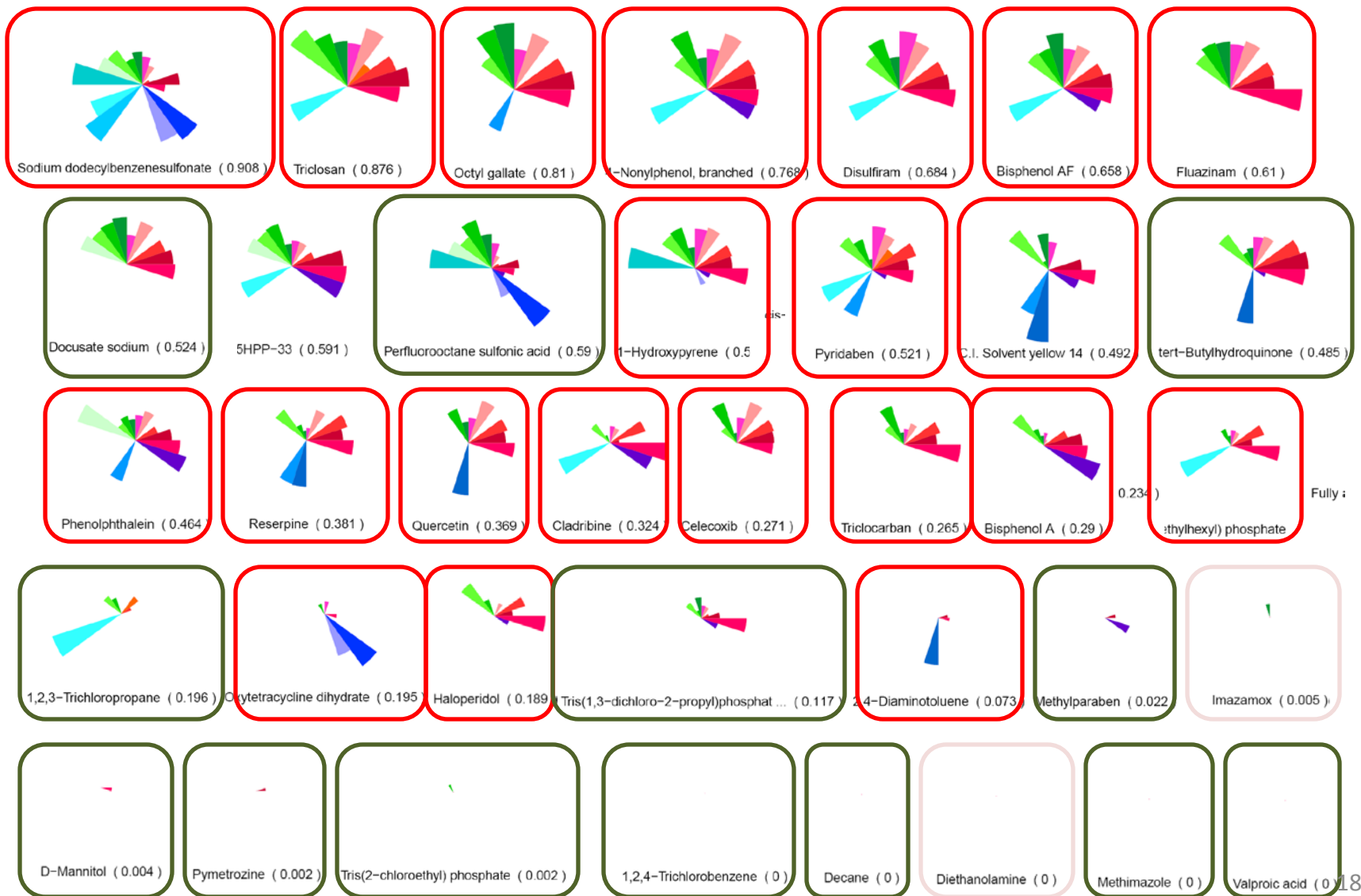


0.47 μ M Fluazinam



Vascular Toxicity Results

(**Red**: hit in at least one assay, **Green**: no hit)

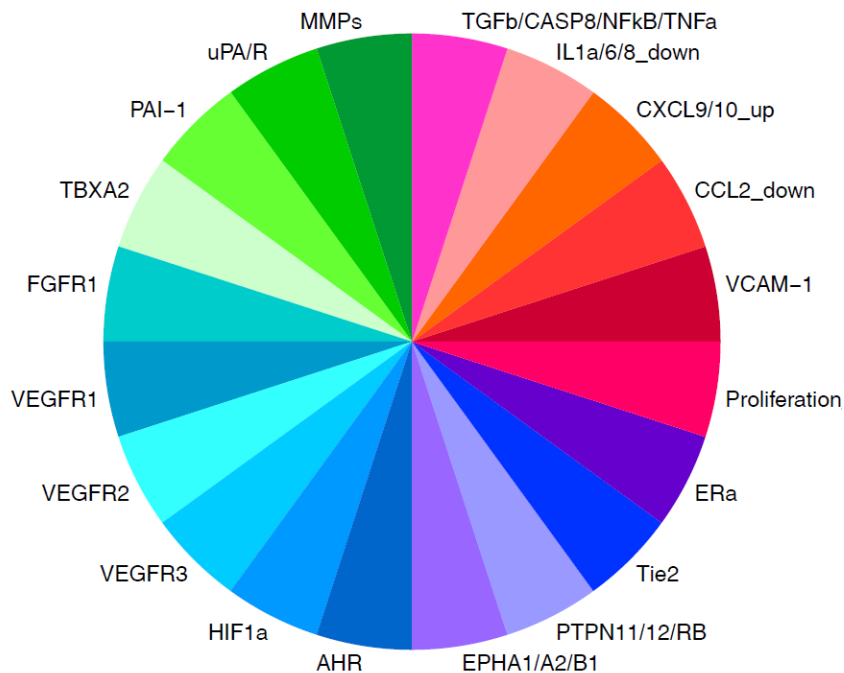


SUMMARY

- Quantitative AOPs using HTS dose response data allow for hypothesis generation, modeling and testing of MIEs and cellular interactions that may lead to toxicities.
- AOP validation is facilitated via orthogonal assays, small model organisms such as zebrafish, and other scientifically relevant information.
- Our group has generated and evaluated a vascular development screening tool utilizing phenotypic endpoints.
- Preliminary data shows that chemical rankings are generally well correlated among the predictive signature, zebrafish overt toxicity and *in vitro* tubulogenesis assays.
- Validated AOPs enable chemical prioritization, high throughput risk assessments, and probabilistic frameworks.

Future directions: Leveraging diverse data streams to improve the developmental vascular toxicity signature

1. Are all of the assays relevant and how should they be weighted?
2. What non-ToxCast targets are needed?
3. Should other ToxCast assays be included?
4. Are we taking full advantage of the zebrafish model?
5. What other orthogonal assays should be used to test pVDC predictions?



Acknowledgements

Vascular Toxicity Team/ Virtual Embryo Group

- Barbara Abbot (TAD)
- Nancy Baker (NCCT)
- Maria Bondesson (UH)
- Ed Carney (Dow)
- Gabrielle Daniels (GED)
- Rob Ellis-Hutchings (Dow)
- Hisham El Masri (ISTD)
- Jill Franzosa (NCCT)
- Peggy Harris (GED)
- Michael Hemmer (GED)
- Karl Jensen (TAD)
- Breandán Kennedy (UD)
- Claire Kilty (UD)
- Tom Knudsen (NCCT)
- Catherine McCollum (UH)
- Kimberly Nelson (GED)
- Jeanene Olin (ISTD)
- Stephanie Padilla (ISTD)
- Raja Settivari (Dow)
- **Tamara Tal (ISTD)**
- Sherry Vickery (GED)
- Charles Wood (ISTD)

FICAM

- Tarja Toimela
- Riina Sarkanen
- Tuula Heinonen

Padilla Lab

- Shad Mosher
- Alisha Palekar

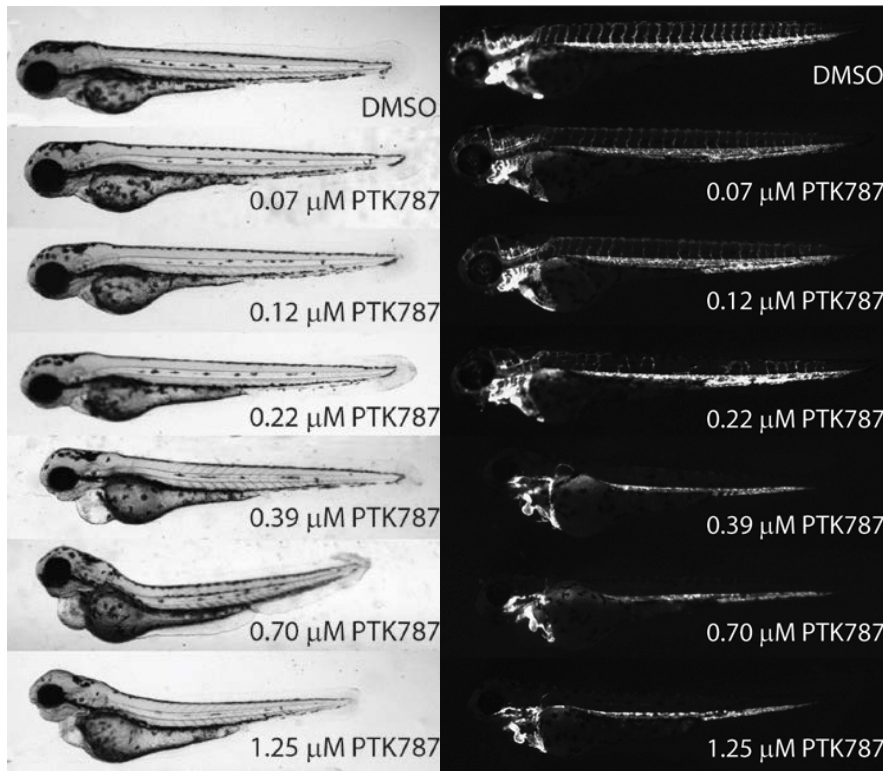
EPA Animal Facility

- Kim Howell
- Ned Collins
- Crystal Walden
- Leslie Martin

Questions?

Functional consequence of vascular disruption during development

A.



B.

