EPA
United States
Environmental Protection
Agency

Thomas Jefferson University
Philadelphia, May 24, 2016

Computer Simulation of Embryonic Systems

What can a virtual embryo teach us about developmental toxicity?

v-Embryo™

Thomas B. Knudsen, PhD
Developmental Systems Biologist
National Center for Computational Toxicology
US EPA, Research Triangle Park, NC 27711
knudsen.thomas@epa.gov

Anatomical homeostasis in a self-regulating multicellular system

SOURCE: Tim Otter, – with permission
Andersen, Newman and Otter (2006) Am. Assoc. Artif. Intel.

Can a computer model of the developing embryo translate cellular disruptions into a prediction of dysmorphogenesis?

and if so …

How might such models be used with high-performance computing **analytically** (to understand) and **theoretically** (to predict) adverse developmental outcomes for different exposure scenarios?

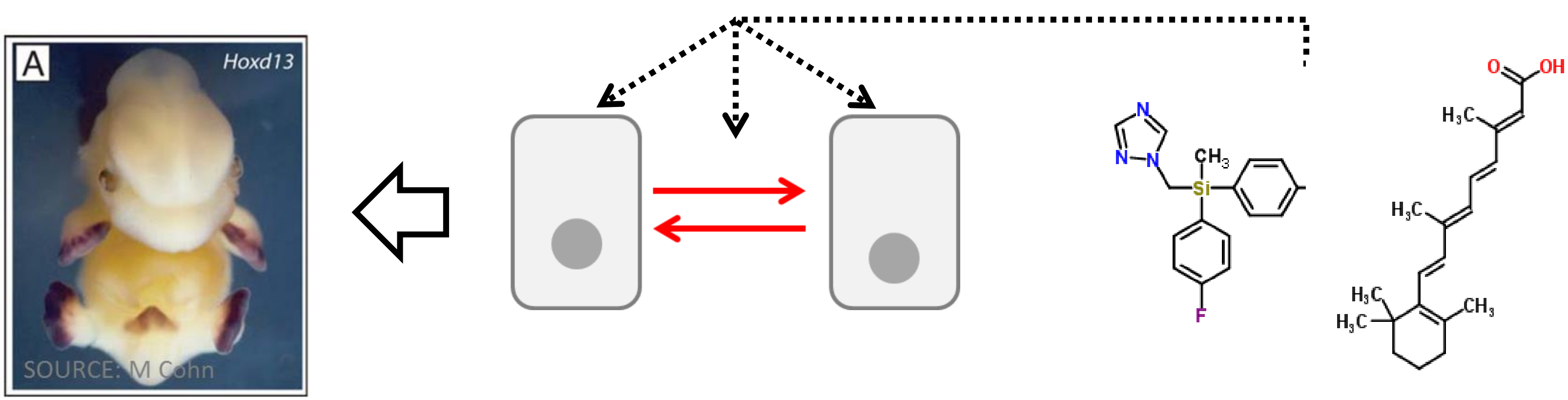
e.g., chemicals, non-chemical stressors, drugs, mixtures, lifestages, …

Organizing Principles

1. In patterning the embryo, genetic signals setup spatial information that cells then translate into a coordinated biological response.

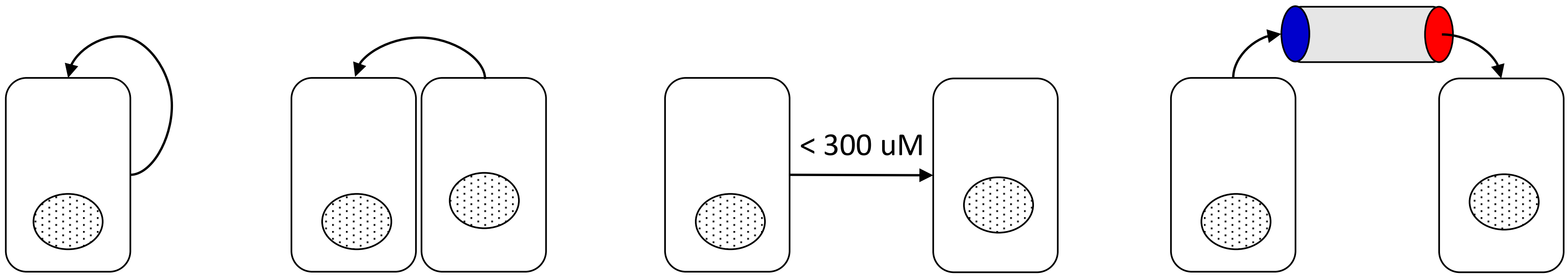
2. A hallmark of multicellular organization is the ability of cells to interact with one another via well-conserved signaling pathways.

3. Just as ‘the Cell’ is the fundamental unit of biology, so too should it be the computational unit (‘Agent’) for modeling embryogenesis.



Cell Signaling Domains

- Kinematics: range of activity varies by distance and geometry.



Autocrine

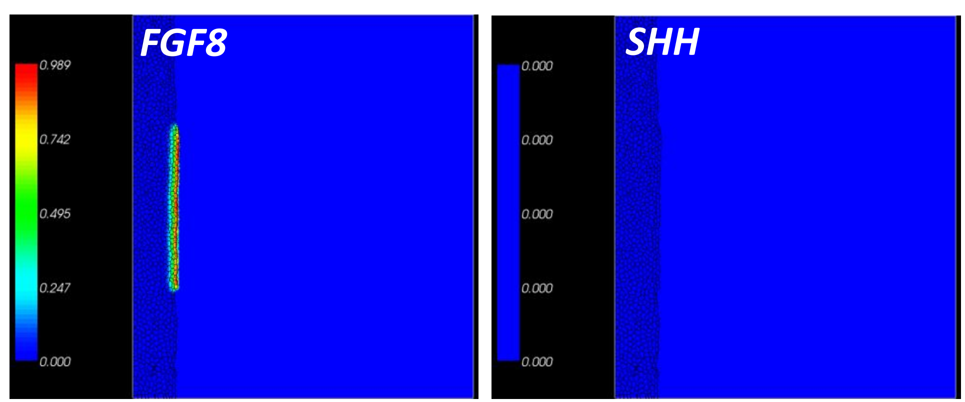
Lateral
(e.g., *Notch-Delta*)

Paracrine
(e.g., *FGF, BMP, SHH*)

Endocrine
(e.g., *estrogen, androgen*)

- Dynamics: spatial relationships in a developing system vary over time and space.

This complexity can be captured in a 'virtual tissue'.



Cellular Agent-Based Models (ABMs)

- Built from the known biology of an embryological system and structured to recapitulate key cell signals and responses.
- Running the models with real (*in vitro*) or synthetic (*in silico*) data can be used to predict emergent responses to perturbation.
- Simulated outcomes can be validated against experimental phenotypes to assess model performance and analyze sensitivity.
- Models can help translate screening-level data from chemical-biology into predictive toxicology of a developmental hazard.

Predictive Toxicology & Human Development

- Evaluating and assessing impacts to development is an Agency priority – *EPA's Children's Environmental Health (CEH) Research Roadmap.*

- Too many chemicals (~80K) to test each by traditional animal-based methods (cost, time, 3Rs).

- Profile the 'human exposure universe' of chemicals *in vitro* with high-throughput (HTS) assays (ToxCast/Tox21).

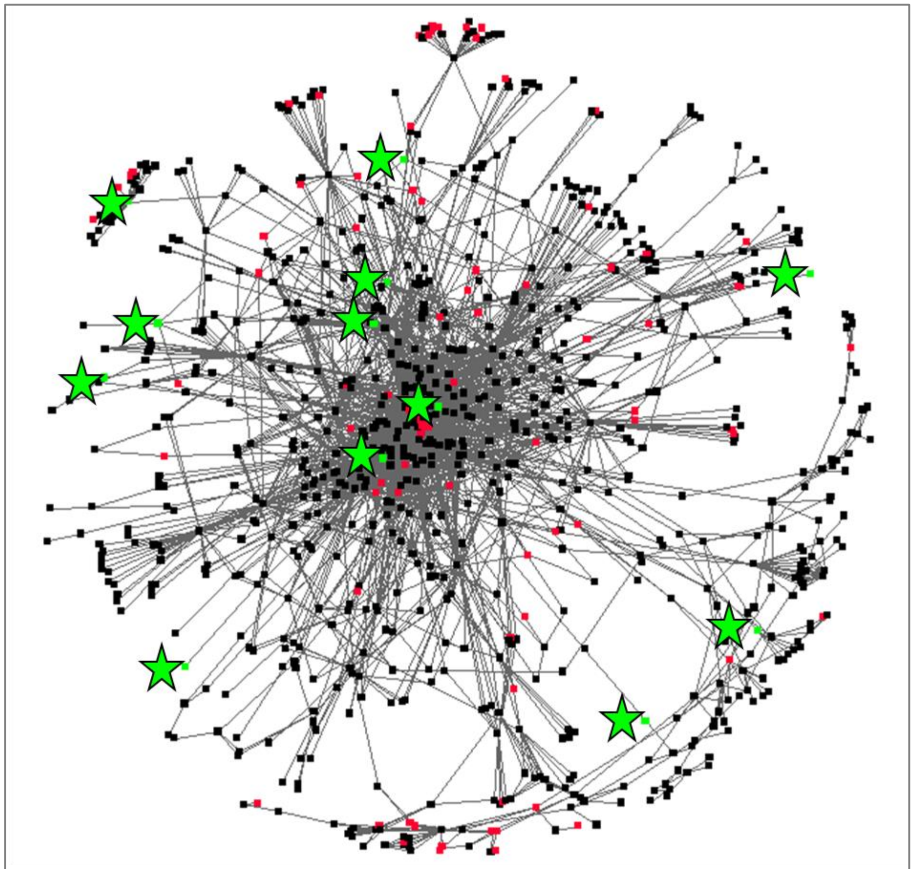
- ToxCast >1060 chemicals evaluated in over 600 assays; >27M data points and ~1.7M concentration response curves.

http://actor.epa.gov/dashboard/

1. ToxCast Predictive Signature: *developmental toxicity*

univariate DevTox features

★ *angiogenesis*



Sipes et al (2011) Toxicol Sci 124

136 of 1065 ToxCast chemicals tested (12.8%) were positive in an embryonic stem cell assay predicting teratogenicity in a human system

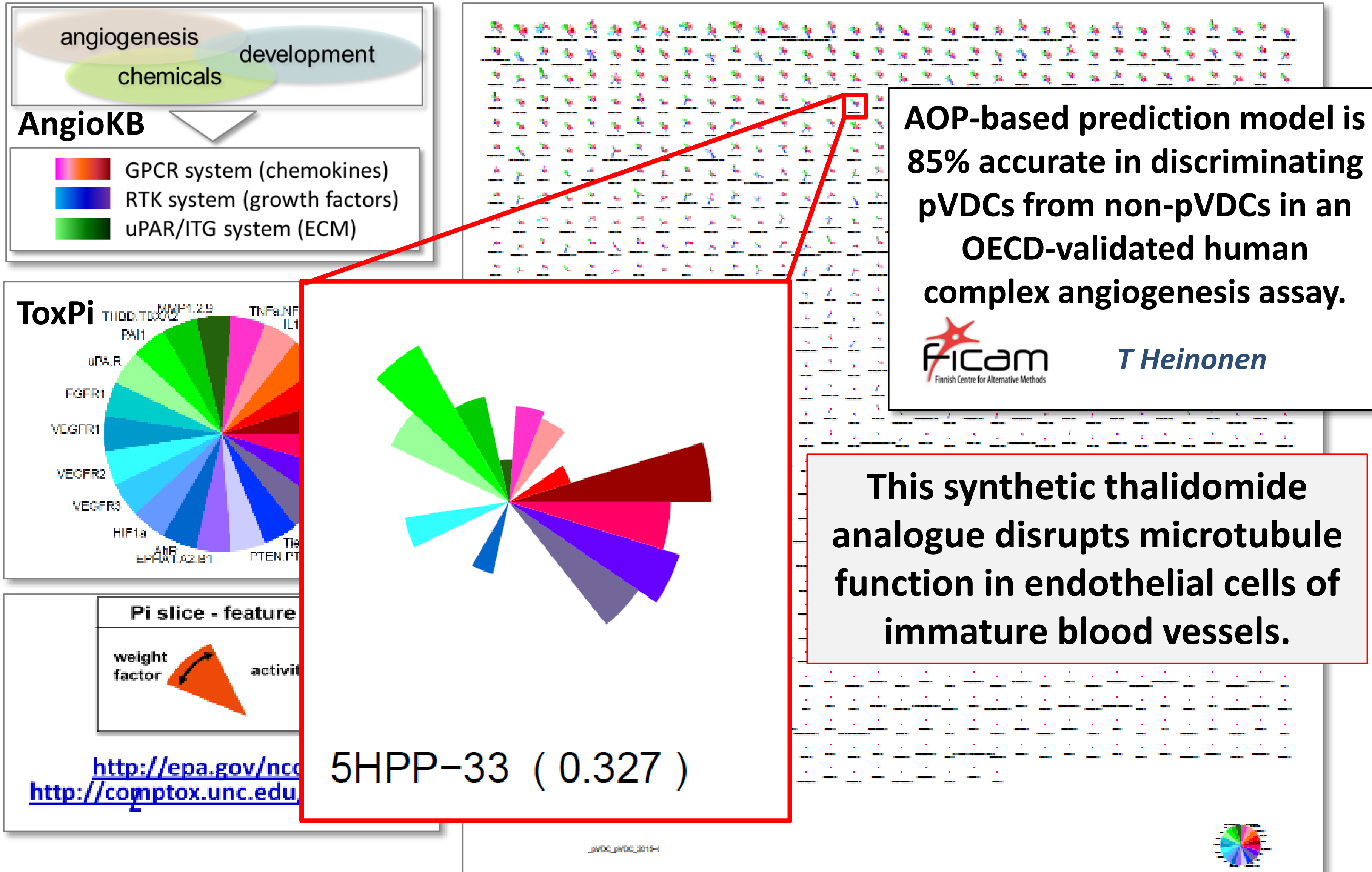
Chemical Name	OC	CV
trans-Retinoic acid	0.000048	1000.000000
PharmaGSID_47333	0.000748	1000.000000
trans-Retinoic acid	0.000975	1000.000000
3'-Azido-3'-deoxythymidine	0.047045	1000.000000
Thalidomide	0.078349	1000.000000
Mirex	0.117320	1000.000000
Aplaviroc hydrochloride	0.430095	1000.000000
Spiroxamine	0.533445	1000.000000
SAR150640	0.644029	1000.000000
Rifampicin	1.105449	1000.000000
7,12-Dimethylbenz(a)anthracene	1.340670	1000.000000
Carbamazepine	1.421311	1000.000000
Etridiazole	1.465742	1000.000000
Tridemorph	1.561177	1000.000000
CP-409092	1.573462	1000.000000
Dihexyl phthalate	1.797381	1000.000000
Nitrofurazone	1.815341	1000.000000
Carbaryl	1.900012	1000.000000
AVE8488	2.367389	1000.000000
GW473178E methyl benzene sulphonic acid	2.382346	1000.000000
Darbufelone mesylate	2.511774	1000.000000
Besonprodil	3.024379	1000.000000
Amiodarone hydrochloride	3.048013	1000.000000
Diethanolamine	3.144575	1000.000000
Elzasonan	3.160128	1000.000000
Volinanserin	3.583474	1000.000000
PharmaGSID_47259	3.610267	1000.000000
Nitrilotriacetic acid	3.615839	1000.000000
SAR102608	4.528155	1000.000000
2-tert-Butyl-5-methylphenol	4.617006	1000.000000
Tributyl phosphate	4.779370	1000.000000
Carbendazim	5.380144	1000.000000
Lovastatin	5.826556	1000.000000
Cycloate	6.077669	1000.000000
N,N-Dimethyldecylamine oxide	11.451464	1000.000000
Pirinixic acid	13.391406	1000.000000
Isazofos	14.149359	1000.000000
Atrazine	15.622259	1000.000000
2-Methoxy-5-nitroaniline	15.694327	1000.000000
Tricresyl phosphate	17.980392	1000.000000
Dinoseb	20.997202	1000.000000
Diallyl phthalate	21.045472	1000.000000
2,4,7,9-Tetramethyl-5-decyne-4,7-diol	22.670906	1000.000000
Cyhalofop-butyl	26.359875	105.172964
Isopropyl triethanolamine titanate	29.417908	1000.000000
Clomazone	29.770828	1000.000000
N-Nitrosodiphenylamine	32.997148	1000.000000
Diuron	52.867066	1000.000000
1,3-Propane sultone	69.899428	1000.000000

Chemical Name	OC	CV
Fenpyroximate (Z,E)	0.000000	0.034565
FR073317	0.000023	0.000551
Colchicine	0.005630	0.016543
Tributyltin methacrylate	0.006368	0.713209
Triphenyltin hydroxide	0.008626	0.288356
TNP-470	0.009958	0.110224
Pyridaben	0.010285	0.119996
TNP-470	0.010981	0.095629
Tributyltin chloride	0.016769	0.642458
PharmaGSID_48511	0.019382	1.985294
Clomiphene citrate	0.024992	7.488947
Rotenone	0.031103	0.501587
Cytarabine hydrochloride	0.036753	0.267927
Triglycidyl isocyanurate	0.040291	1.062350
Methotrexate	0.046665	0.086651
Diquat dibromide monohydrate	0.055567	8.995770
Prometryn	0.064709	0.083605
Tebufenpyrad	0.064958	2.120929
SAR115740	0.138578	1.665954
PharmaCode_0119307	0.155186	2.014392
Chlorpromazine hydrochloride	0.156237	4.006364
Phenylmercuric acetate	0.170702	0.357751
Captafol	0.180977	5.577974
Cladribine	0.202951	0.770262
Sodium (2-pyridylthio)-N-oxide	0.217232	7.219203
Disulfiram	0.265362	0.315611
Diphenhydramine hydrochloride	0.387290	21.716431
PharmaGSID_48519	0.509305	2.568090
Ketoconazole	0.514342	4.328221
Mercuric chloride	0.579816	8.230124
Pyraclostrobin	0.671383	0.501531
Fluridone	0.676795	63.245553
Cycloheximide	0.695669	2.559216
Dodecyltrimethylammonium chloride	0.719288	7.159614
SR271425	0.740679	5.094216
Famoxadone	0.997196	3.442388
6-Thioguanine	1.000000	ND
Difenoconazole	1.013564	6.276471
5-Fluorouracil	1.080186	3.219272
Busulfan	1.123890	23.003161
MK-274	1.190975	0.731067
Octyl gallate	1.221169	1.844842
Propazine	1.318655	4.945733
SR125047	1.357789	4.455654
Octyl gallate	1.455710	2.306594
SB236057A	2.156641	10.627490
Nitrofurantoin	2.164410	4.215680
Azathioprine	2.430280	4.814399

Azathioprine	2.430280	4.814399
N,N,N-Trimethyl(oxiran-2-yl)methanaminium chl	3.411829	13.999363
1,3-Dinitrobenzene	6.203772	64.196654
SSR241586	6.357735	8.772243
PharmaCode_0343701	6.467789	9.731276
1,2-Phenylenediamine	7.774040	2.972275
4-Methylaniline	7.830403	44.816816
SHPP-33	9.009209	13.589654
Flutamide	9.757821	64.864682
Diisobutyl phthalate	10.000000	ND
Flusilazole	10.256422	48.544794
Ametryn	11.004570	13.868057
Flumetralin	14.137651	54.271359
Butyl benzyl phthalate	14.222819	33.843403
Thidiazuron	17.305152	79.977247
4-Vinyl-1-cyclohexene dioxide	17.626518	66.534209
Fluazifop-P-butyl	17.659025	43.919159
4-(2-Methylbutan-2-yl)phenol	18.348295	79.886918
4,4'-Oxydianiline	19.320515	43.018932
Methyl methanesulfonate	21.425038	73.494677
Norethindrone	27.649764	72.035048
Linuron	27.725507	89.346226
N,N'-Methylenebisacrylamide	27.987139	61.474359
2,4-Dinitrotoluene	29.138000	87.276363
Diazinon	29.922180	74.424923
2,4-Diaminotoluene	30.210145	65.000000
Ethofumesate	30.450193	46.072468
Propetamphos	31.805323	90.260952
Malathion	53.773177	68.904109
Flumioxazin	56.687903	45.583782
Phenanthrene	59.970166	74.563154
Corticosterone	60.666132	88.093797
Indomethacin	64.572031	69.191857
Myclobutanil	66.222967	65.542607
Prometon	100.000000	ND
L-Tartaric acid	100.000000	ND
2',3'-Dideoxyinosine	100.000000	ND
Tetracycline	100.000000	ND
Cyanazine	100.000000	ND
3,3'-Dimethylbenzidine	100.000000	ND
Stavudine	100.000000	ND
3,4-Dimethylphenol	100.000000	ND
Methyleugenol	100.000000	ND

Knudsen et al (2016) in preparation

pVDCs: chemicals sorted for potential vascular disruption (pVDCs)



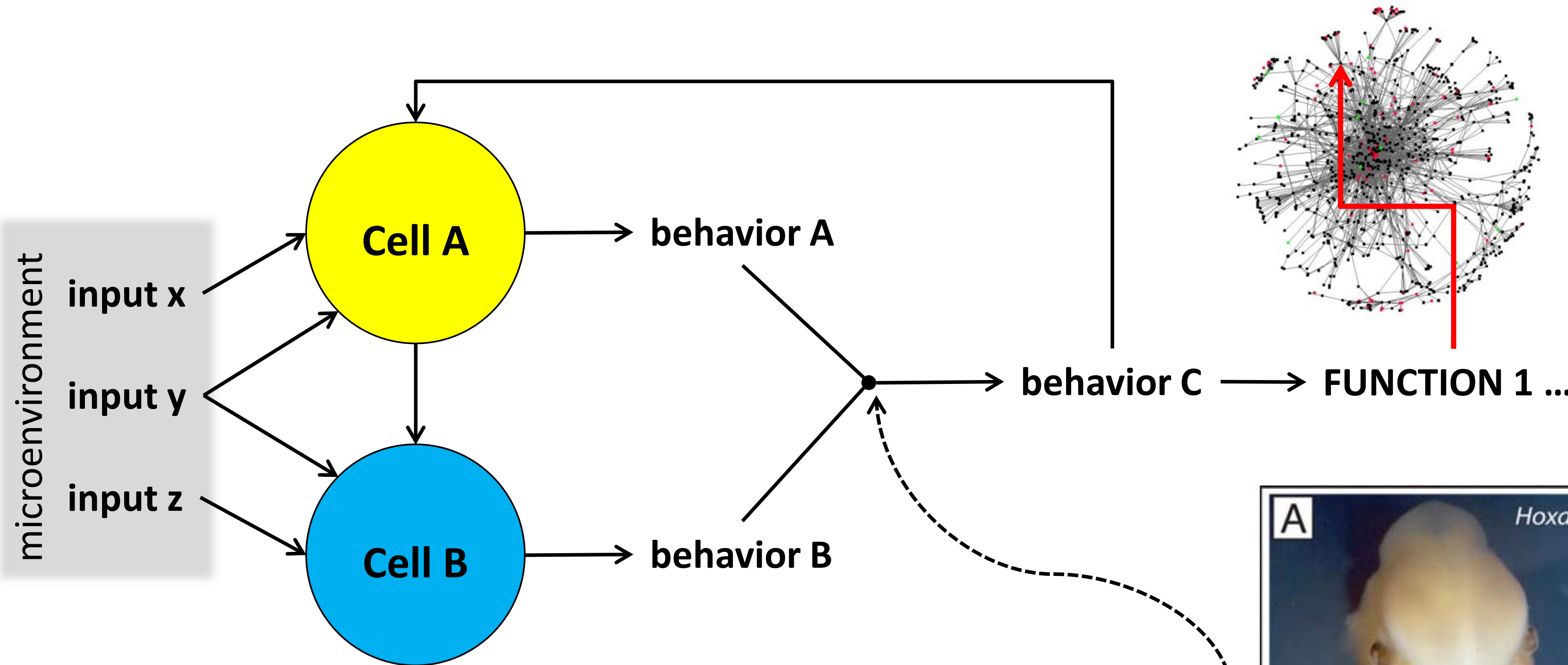
AOP-based prediction model is 85% accurate in discriminating pVDCs from non-pVDCs in an OECD-validated human complex angiogenesis assay.

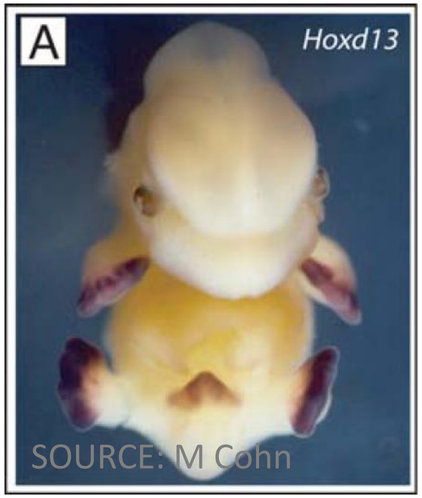
FICAM Finnish Centre for Alternative Methods

T Heinonen

This synthetic thalidomide analogue disrupts microtubule function in endothelial cells of immature blood vessels.

Cellular Response Network (CRN): how cellular systems translate spatial information into higher-order function



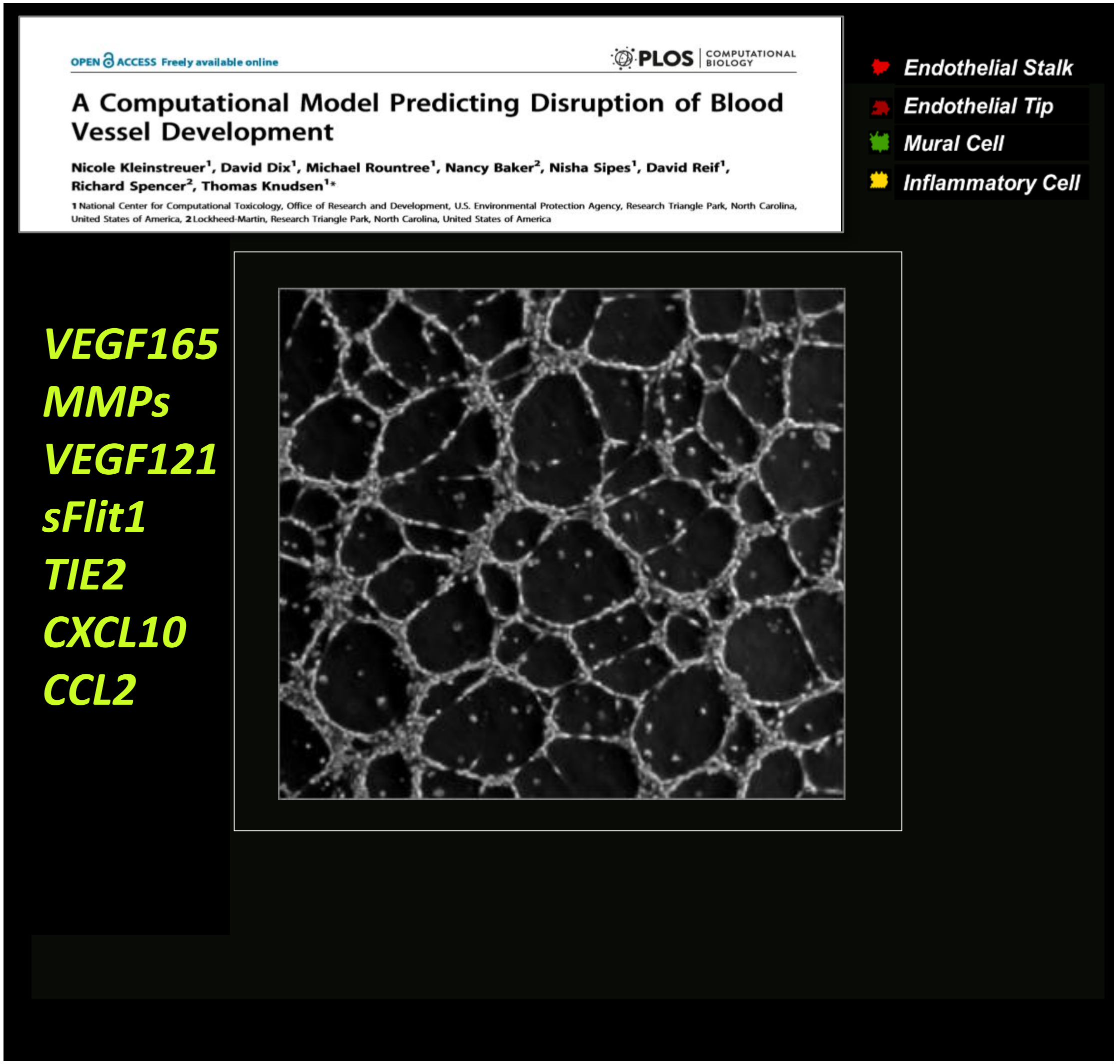


Capturing this mathematically:

$$\sum_\sigma \lambda_V(\sigma)\big(v(\sigma)-V_t(\sigma)\big)^2+\sum_\sigma \lambda_S(\sigma)\big(s(\sigma)-S_t(\sigma)\big)^2+\sum_{i,j}J\big(\tau(\sigma(\vec{i})),\tau(\sigma'(\vec{j}))\big)\Big(1-\delta\big(\sigma(\vec{i}),\sigma'(\vec{j})\big)\Big)-\lambda_{chem}\big(c(\vec{i})-c(\vec{j})\big)$$

10

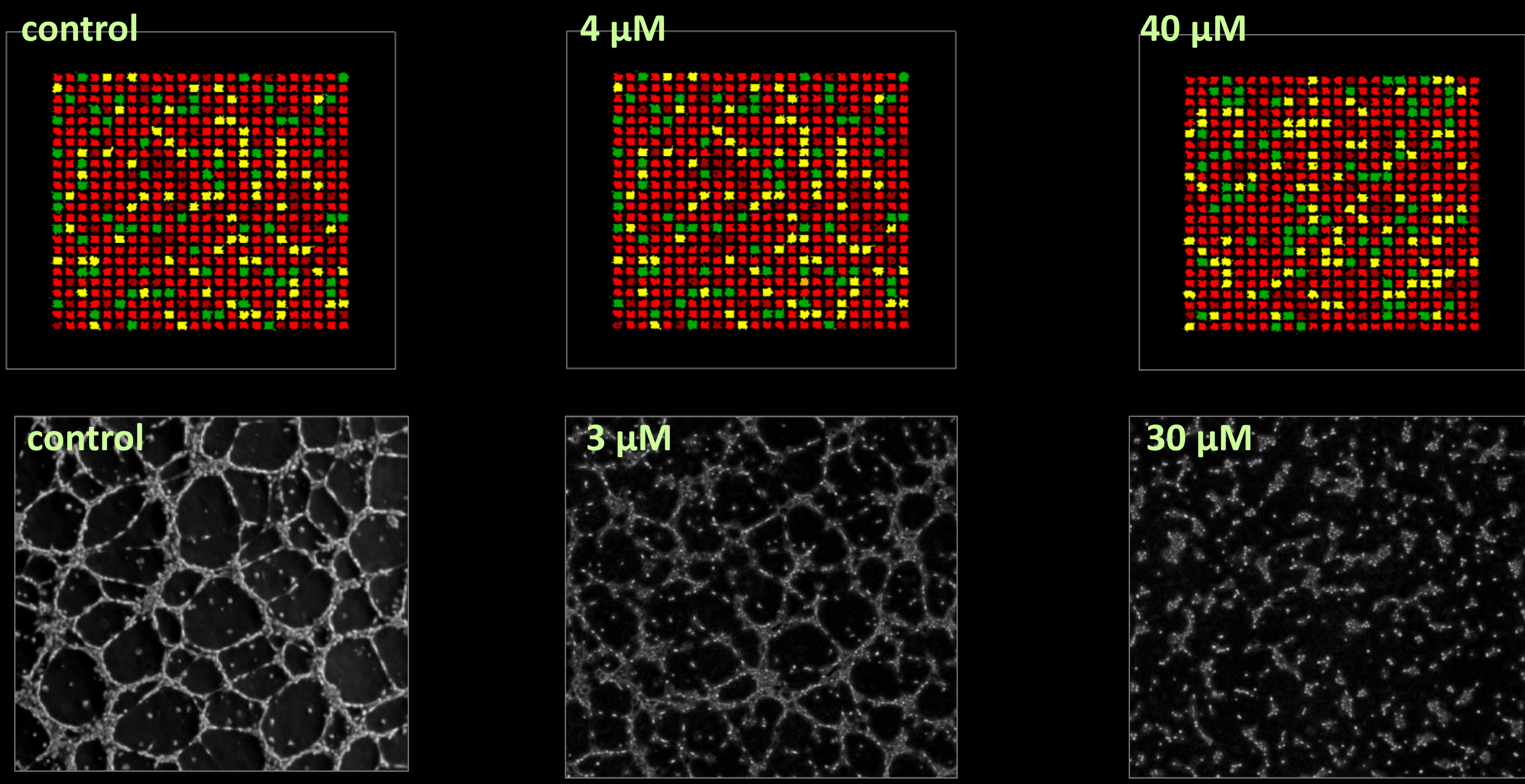
Cellular Agent-Based Models (ABMs)



- individual rules are assigned to low-level ‘agents’ (here = cells)
- agents then interact in a shared environment (CompuCell3D*)
- running the simulation executes this biology (emergence)
- models run differently each time (stochastic)
- each run reveals one possible solution (outcome)

** CompuCell3D.org is an open-access environment for cell-oriented modeling developed at Indiana University by J Glazier and colleagues*

5HPP-33 concentration response predicted *in silico from* ToxCast and demonstrated *in vitro* with a human endothelial cell assay



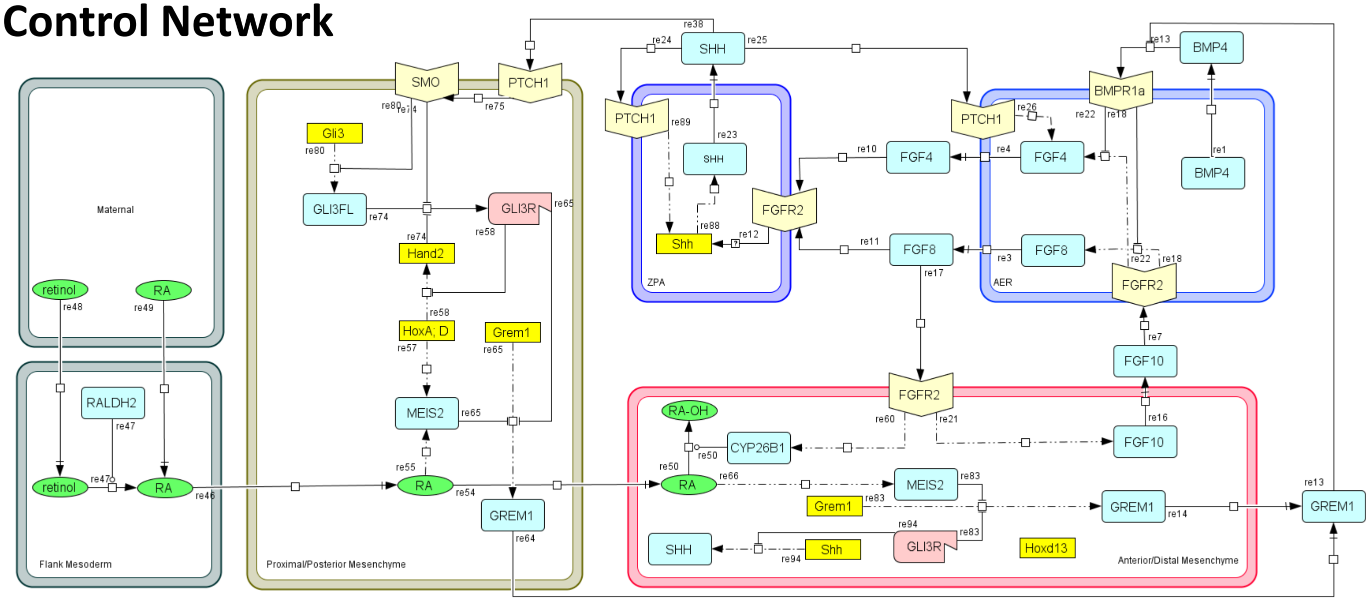
SOURCE: Kleinstreuer et al. (2013) PLoS Comp Biol

2. Limb Development

Stage 16	Stage 18	Stage 19	Stage 20	Stage 21
GD9	GD10	GD12	GD12.5	GD13

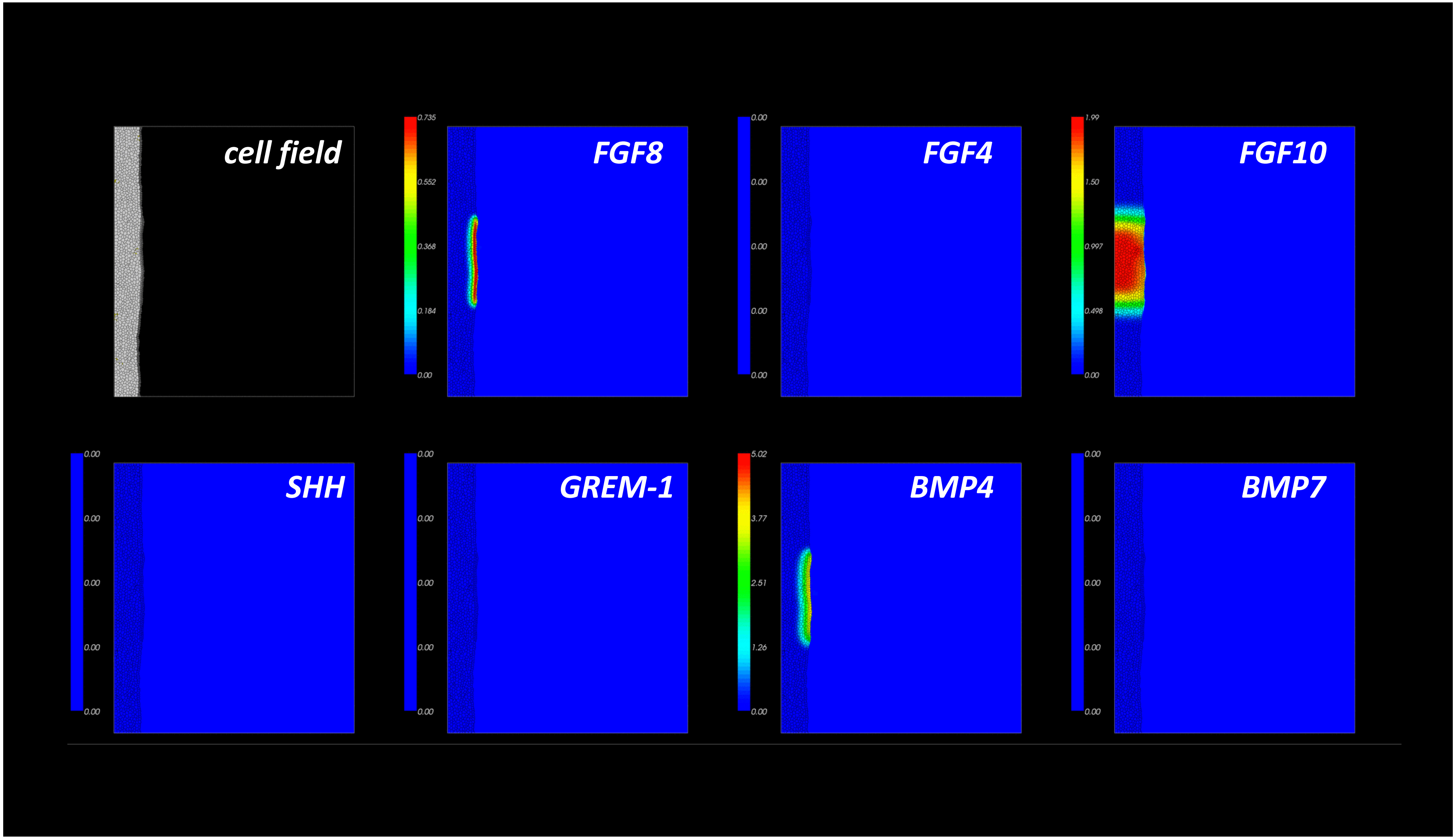
Boehm et al. (2011) Development 138

Control Network

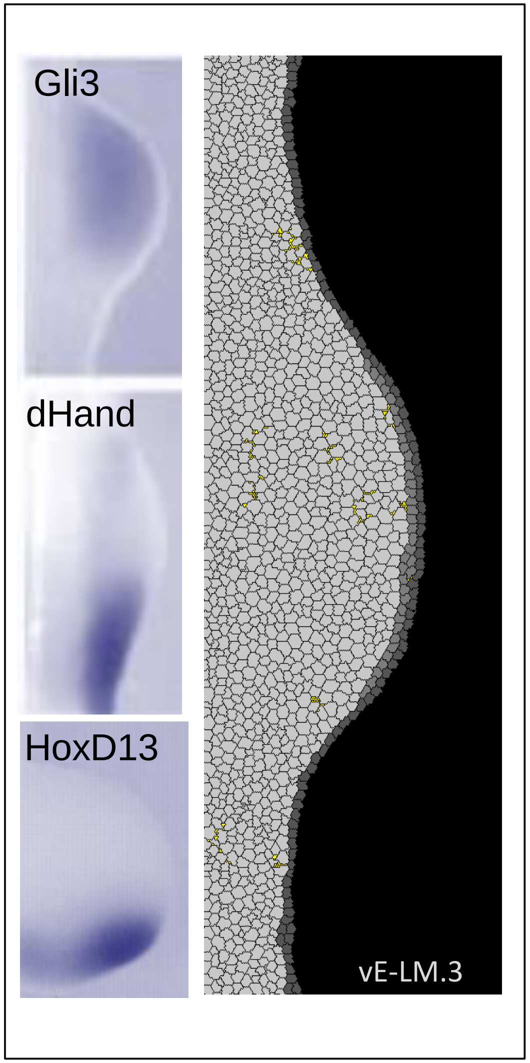


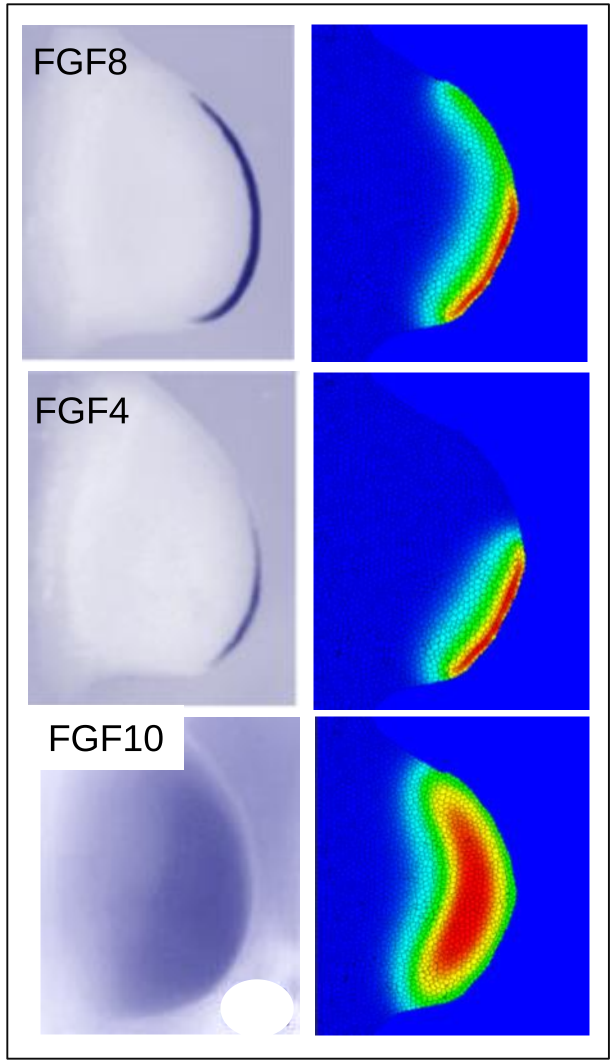
Dewoskin – NCCT, unpublished

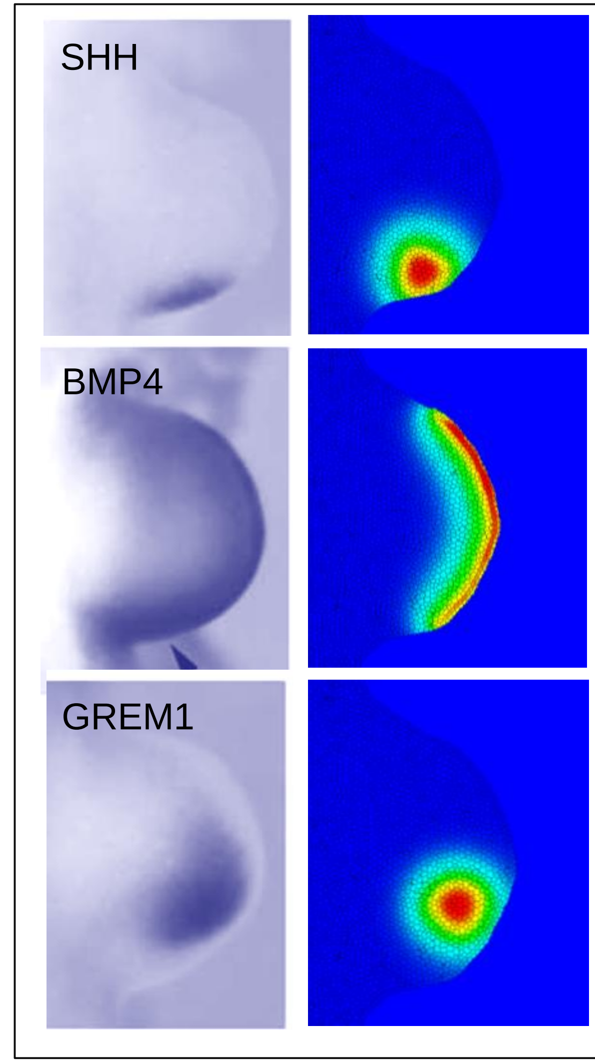
Spatial dynamics: patterning early limb outgrowth



SOURCE: unpublished, manuscript in preparation





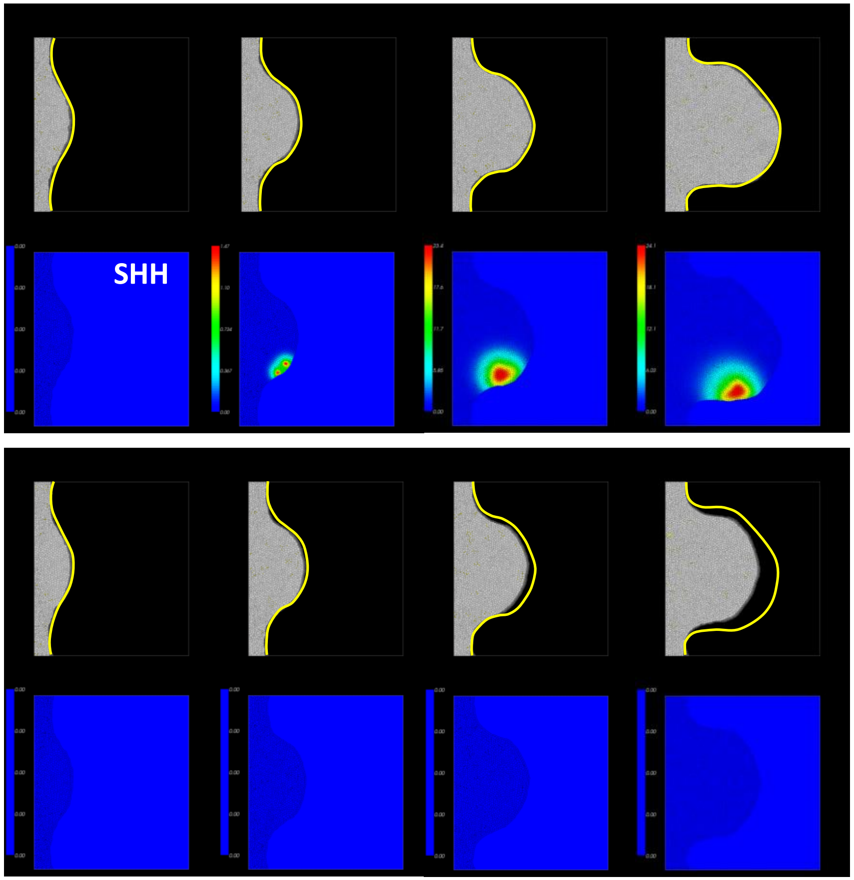


ISH (mouse literature) vs ABM

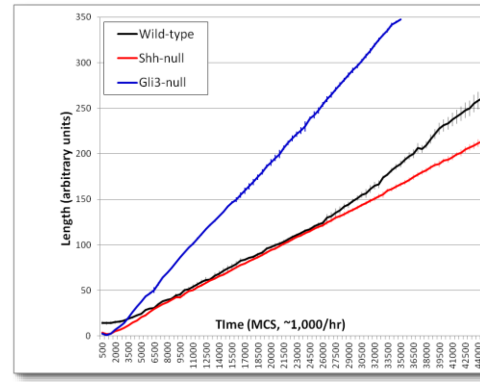
Failability: exposures may disrupt signaling domains directly

Wild-type

Shh-null



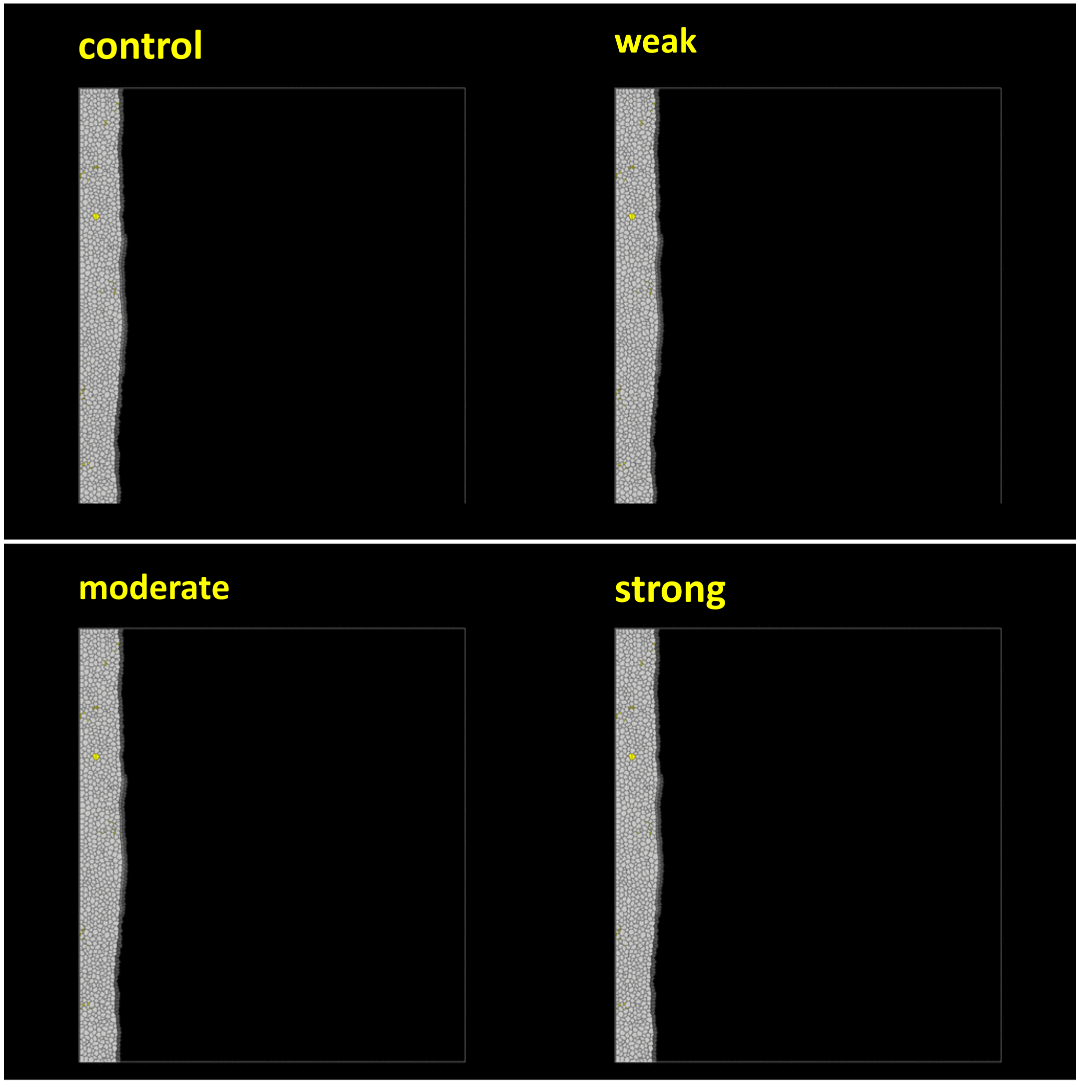
Rate of outgrowth (n=5)

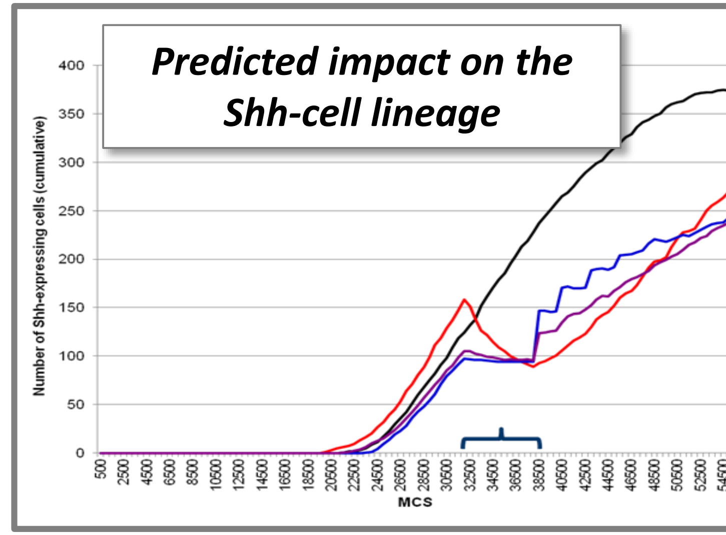


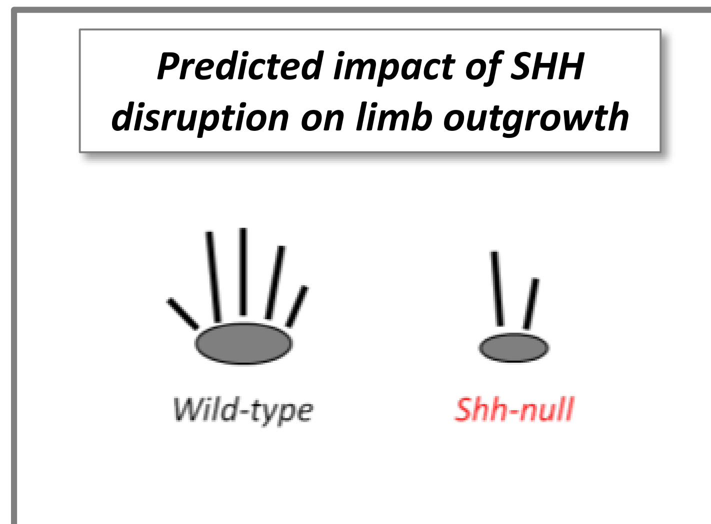
Predicted impact of SHH disruption on limb outgrowth

Wild-type *Shh-null*

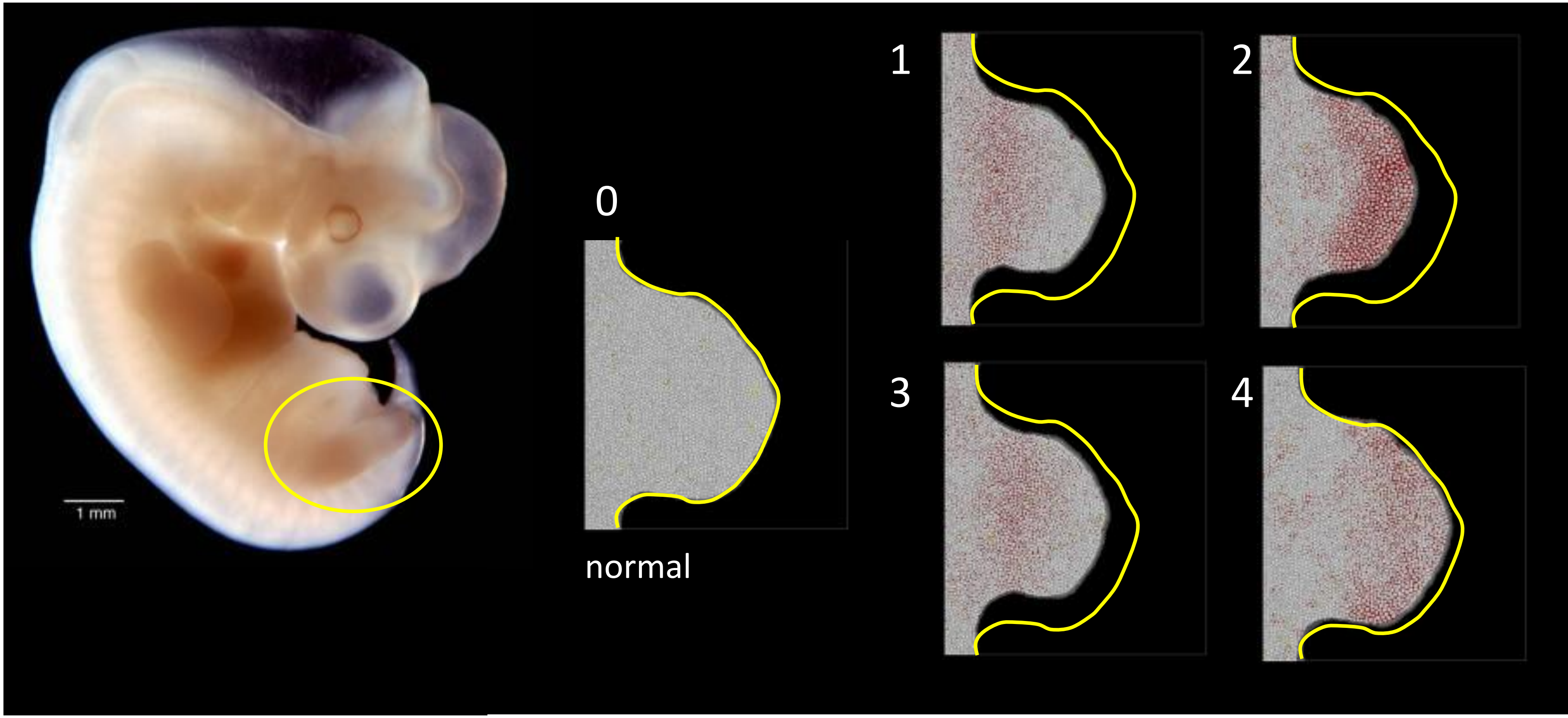
Failability: exposures may disrupt signaling domains indirectly

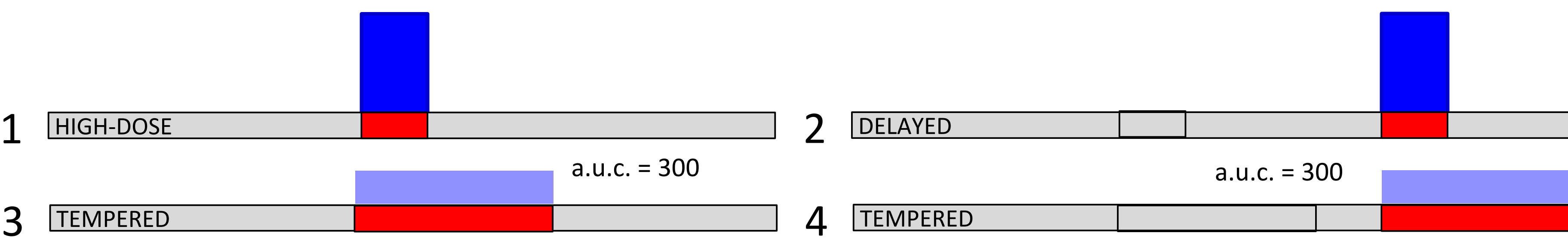






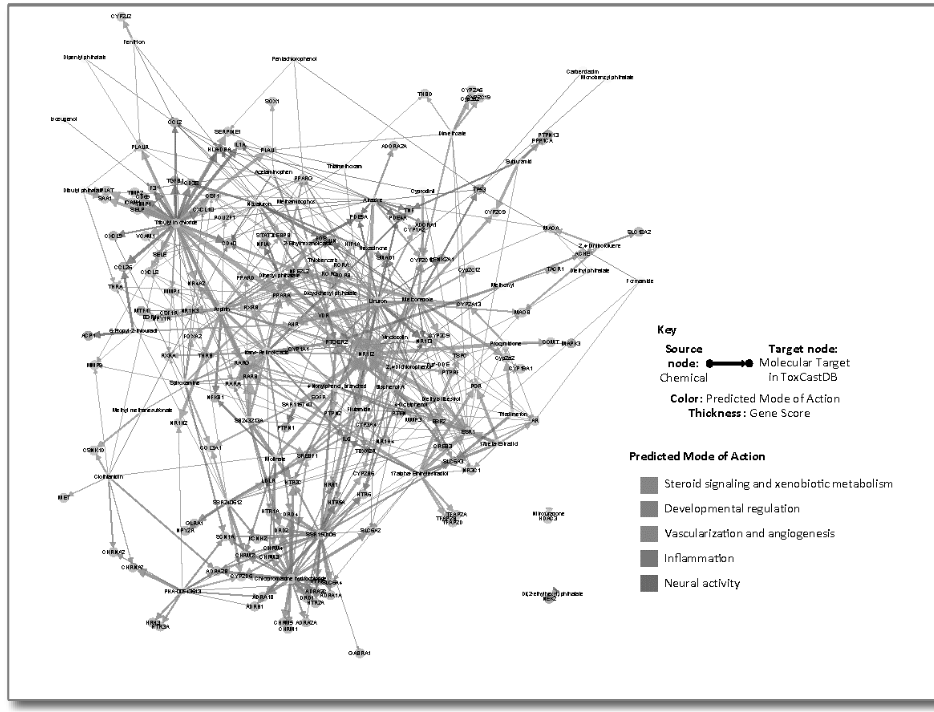
‘What-if’? simulating different exposure scenarios

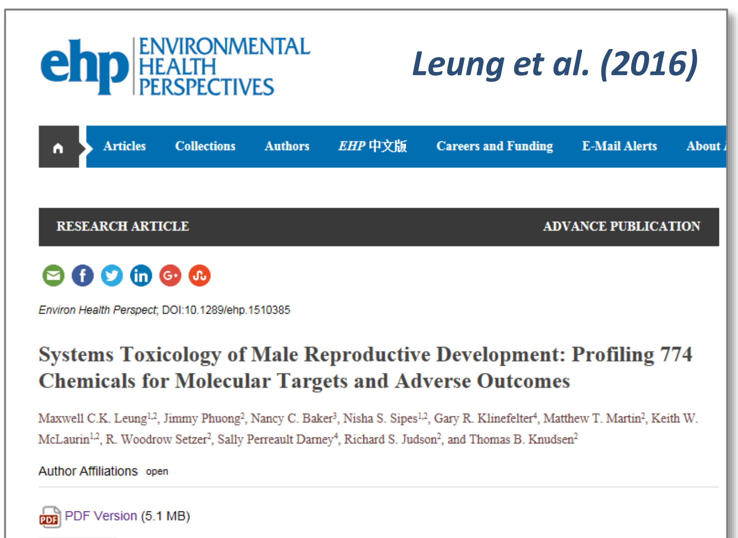




3. Chemical-Target Bipartite Network: translates ToxCast bioactivity profiles into predicted mode-of-action for an AOP

- BPN for 54 ToxCast chemicals that produce male developmental toxicity.
- Functional annotation revealed 4-5 target biological processes.

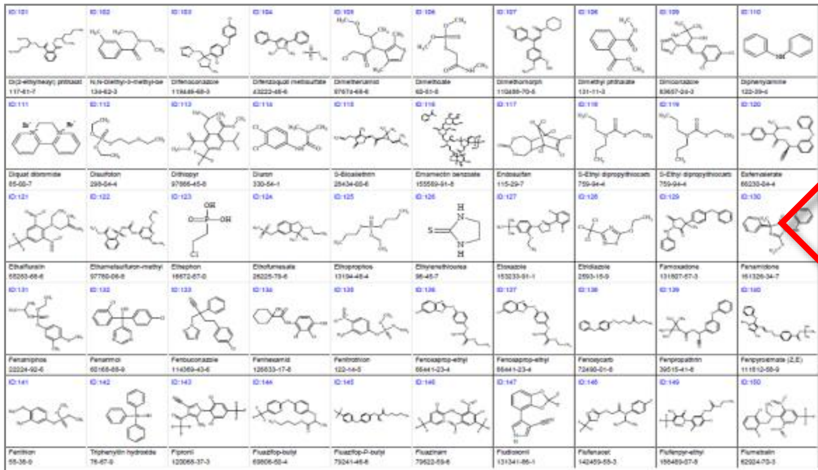


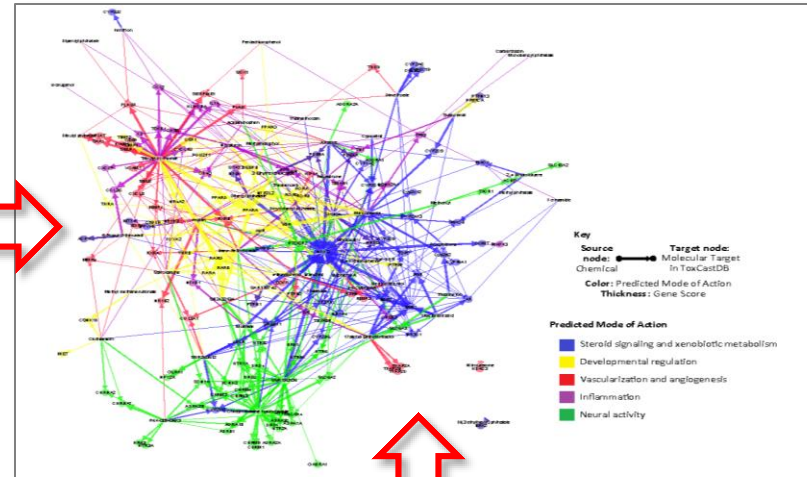


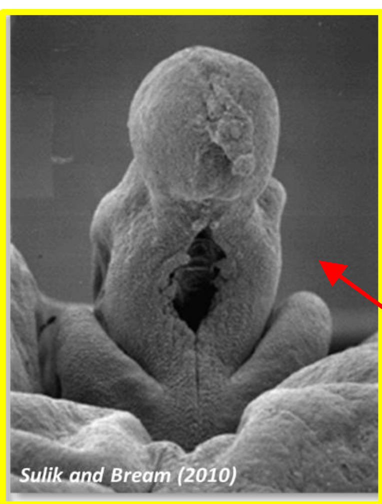
How do chemical-bioactivity *bipartite networks* interact with *control networks* in disrupting development?

EXAMPLE: hypospadias, a urethral closure defect

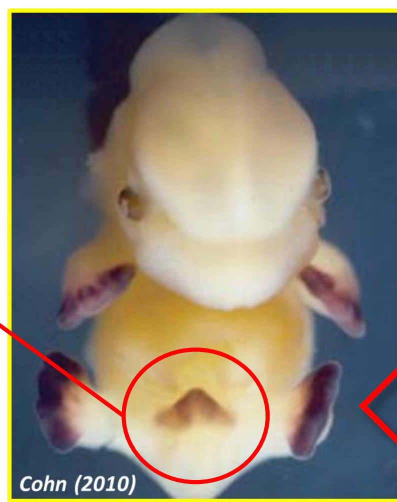
Leung et al. (in preparation)



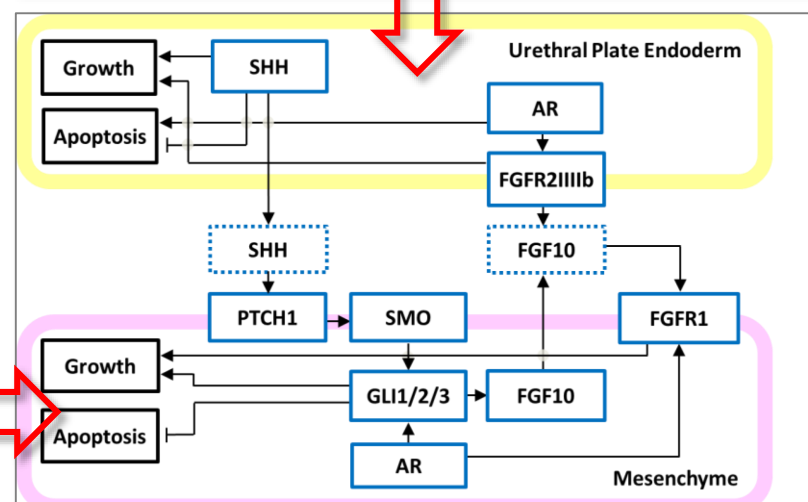




Sulik and Bream (2010)



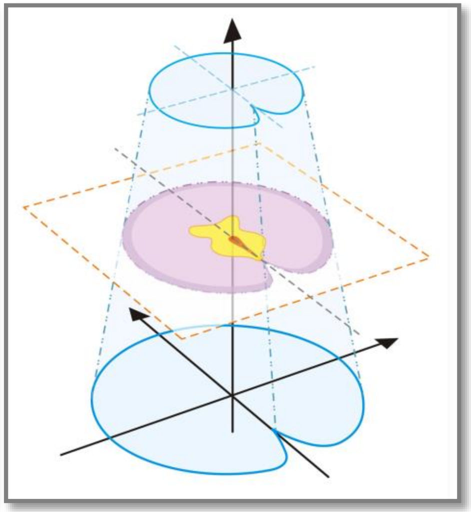
Cohn (2010)



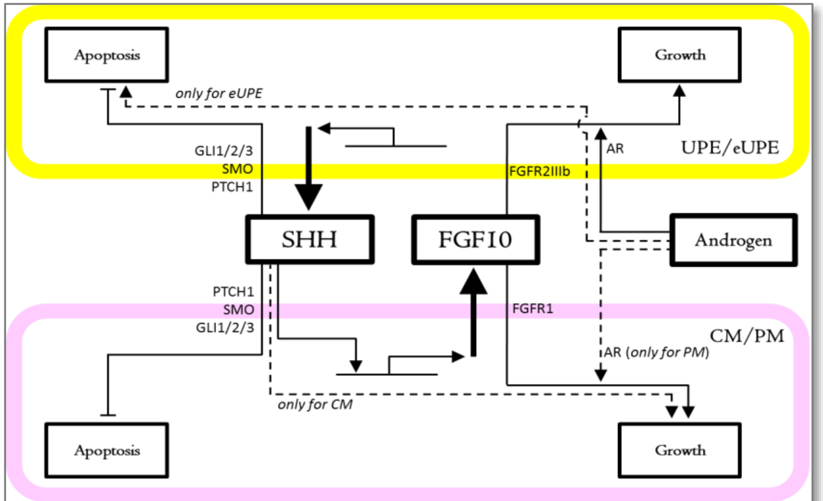
Genital Tubercle (GT) development

Embryonic GT

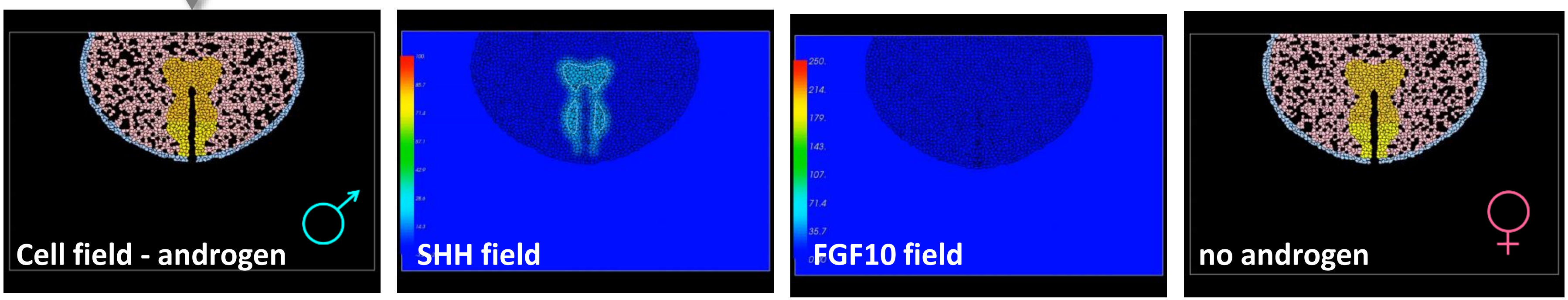
Abstracted GT



Control Network (mouse)



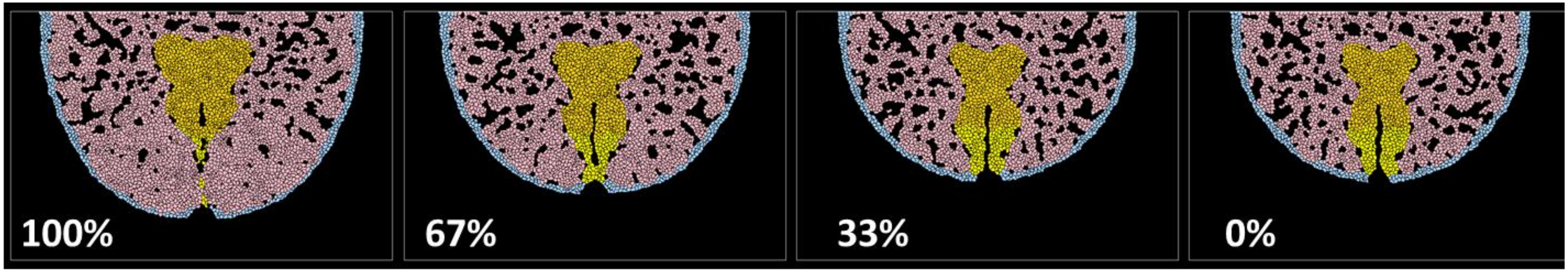
ABM simulation for sexual dimorphism (MCS 4000 = GD13.5 – 17.5)



- sexually indifferent at MCS 0 (GD13.5)
- androgen production by fetal testis introduced at MCS 2000 (GD15.5)
- sexual dimorphism evaluated at MCS 4000 (GD17.5)

Urethral Closure

- Driven by urethral **endoderm** (contact, fusion apoptosis) and preputial **mesenchyme** (proliferation, condensation, migration).
- Disruption of SHH, FGF10, or AR signaling leads to urethral closure defects (e.g., hypospadias).



Androgenization *(n = 10 sims)*	***Phenotype (MCS 4000)*** Septation	Fusion	Conden.	Closure Index
100%	6/10	8/10	10/10	0.80
67%	2/10	5/10	10/10	0.57
33%	0/10	4/10	0/10	0.13
0%	0/10	2/10	0/10	0.07

Programmed Fusion of Opposing Surfaces

- Disruption is a key event in AOPs for many human birth defects:
  NTDs, coloboma, cleft palate, valvuloseptal defects, hypospadias, gastroschisis, …

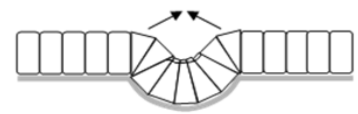
- Emergent property orchestrated by CRNs: EMT, apoptosis, epithelial cell adhesion / migration / intercalation are recurring themes.

Cellular primitives

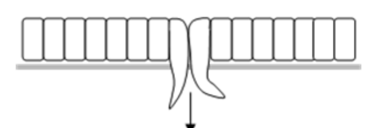
- growth (proliferation)
- programmed cell death (apoptosis)
- genetic signals and responses
- differentiation
- cell adhesion
- shape (geometry)
- motility (cell migration)
- ECM (remodeling)

Tissue movements

- folding
- epiboly

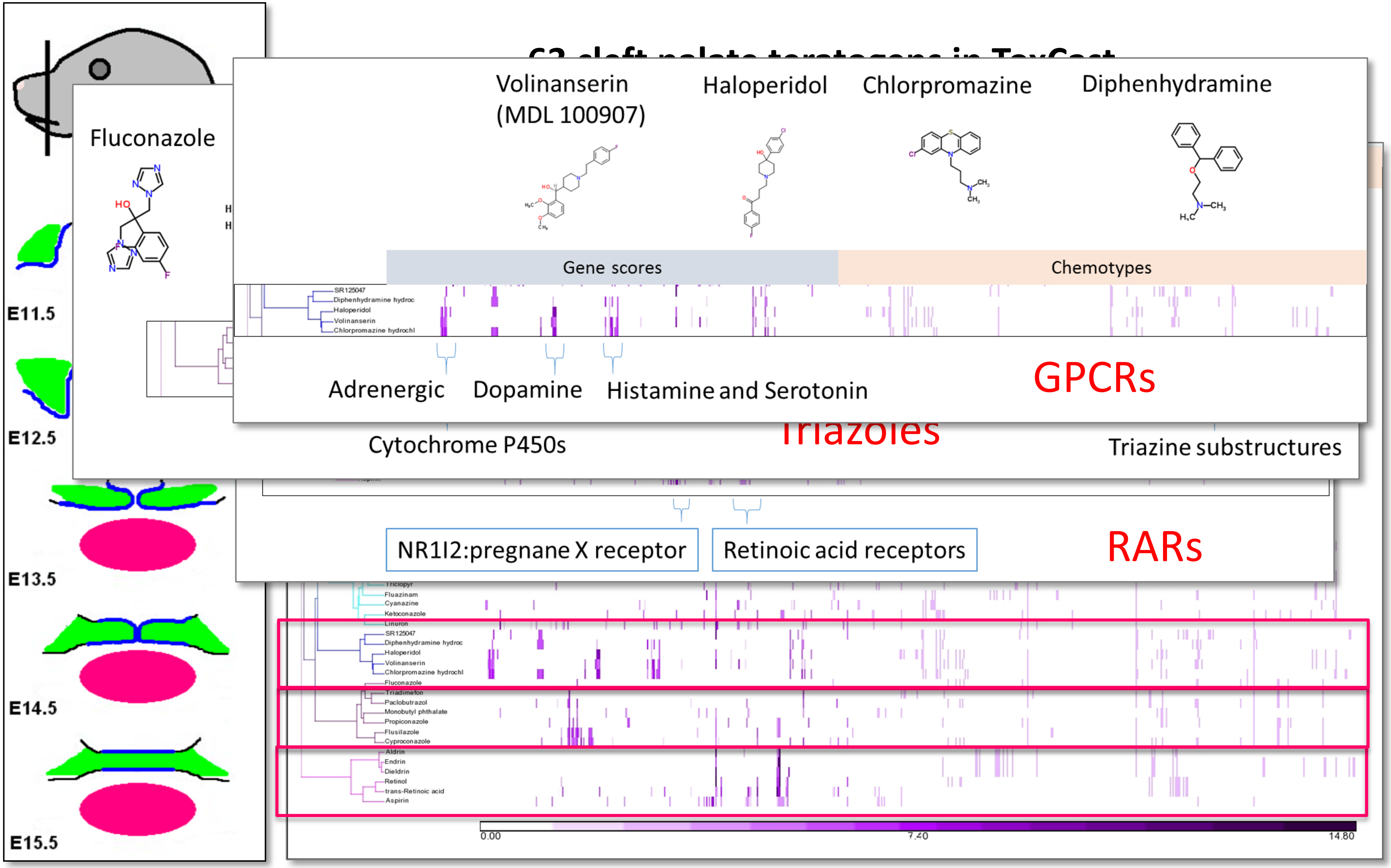


- convergent extension
- branching morphogenesis
- cell condensation
- cell sorting
- trans-differentiation (EMT)
- cavitation
- involution

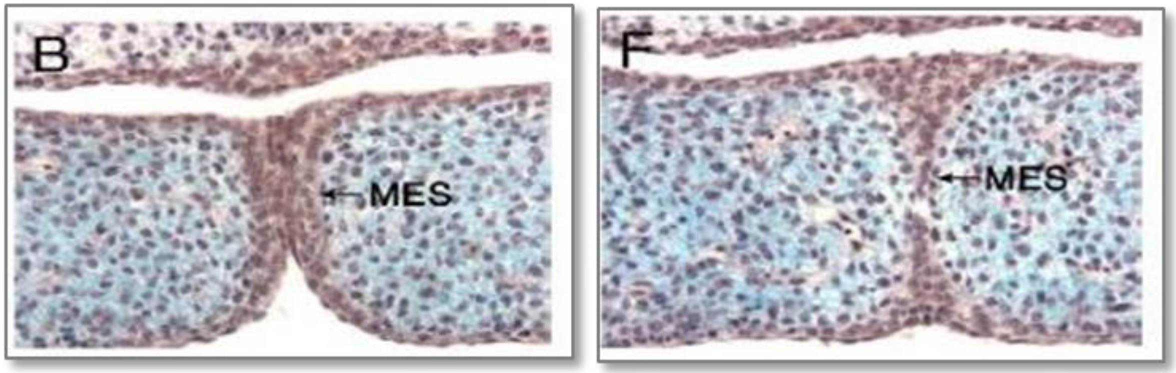


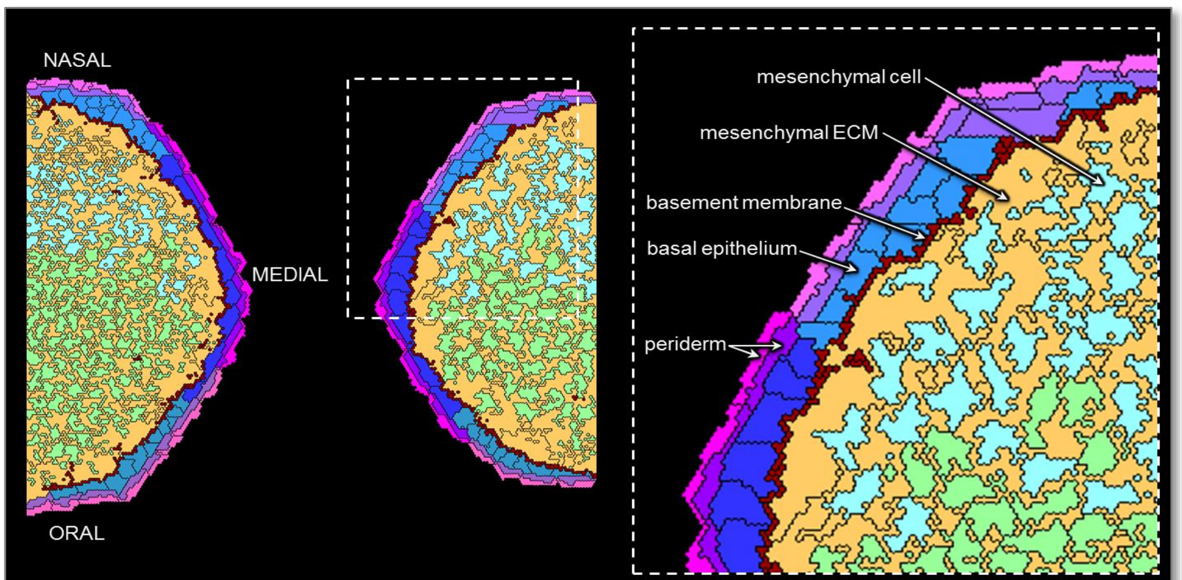
- tractional forces
- …

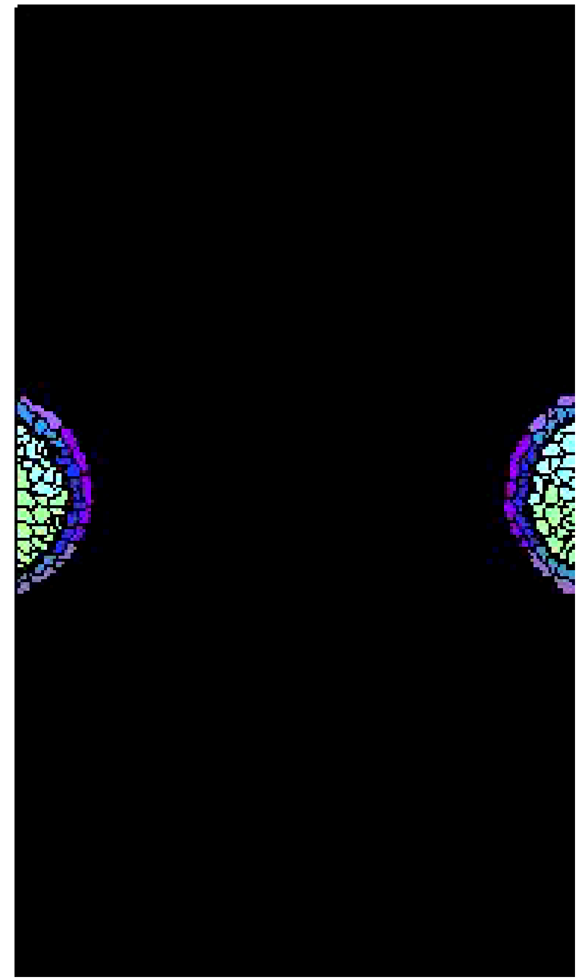
4. Palatal Closure: complex process disrupted in 'cleft palate'.



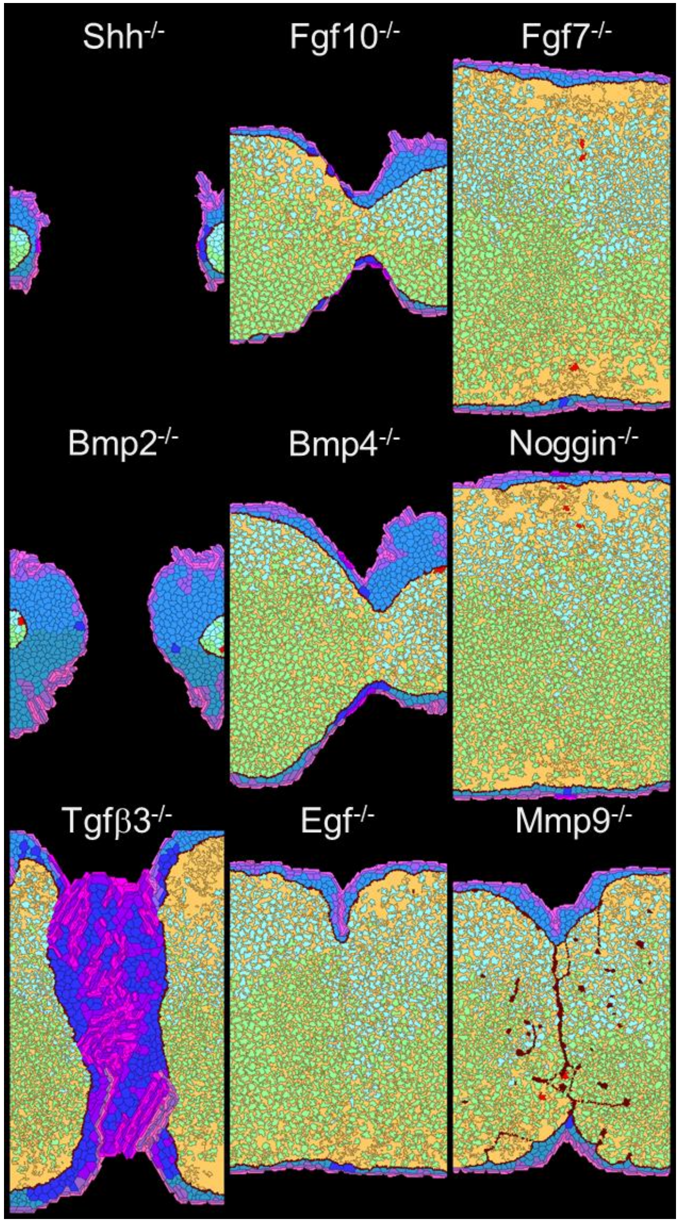
Palatal Closure: ABM recapitulating cellular dynamics of Medial Edge Epithelium (MEE) contact and seam (MES) breakdown.

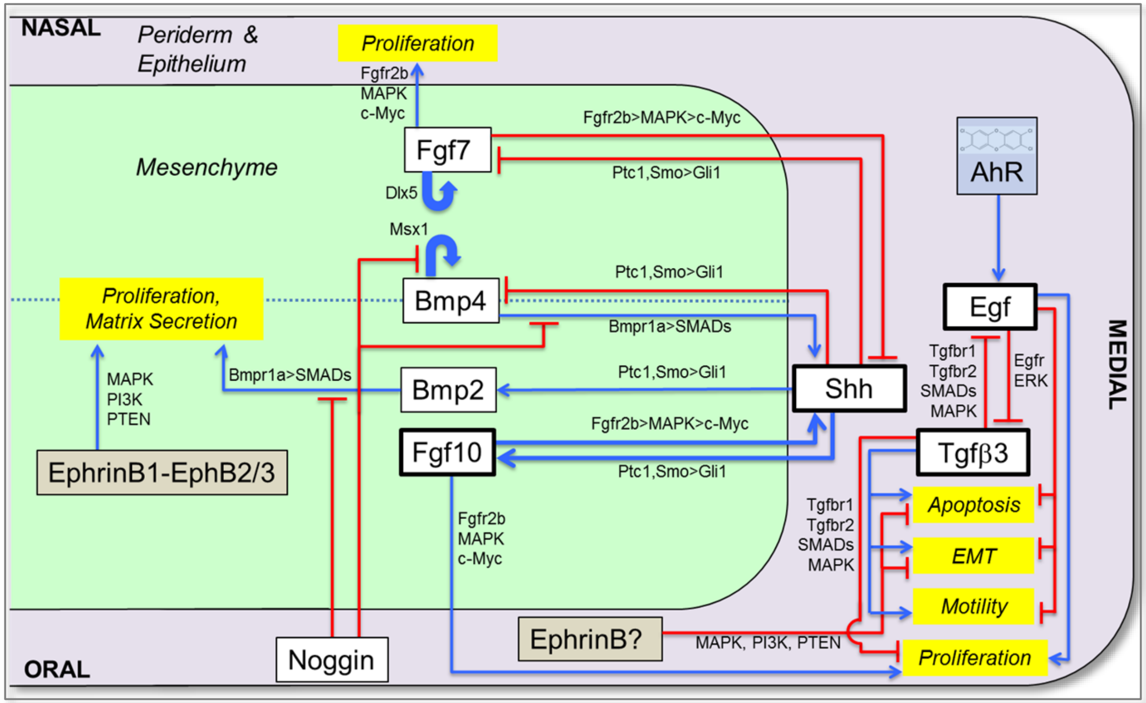






Hacking the Control Network

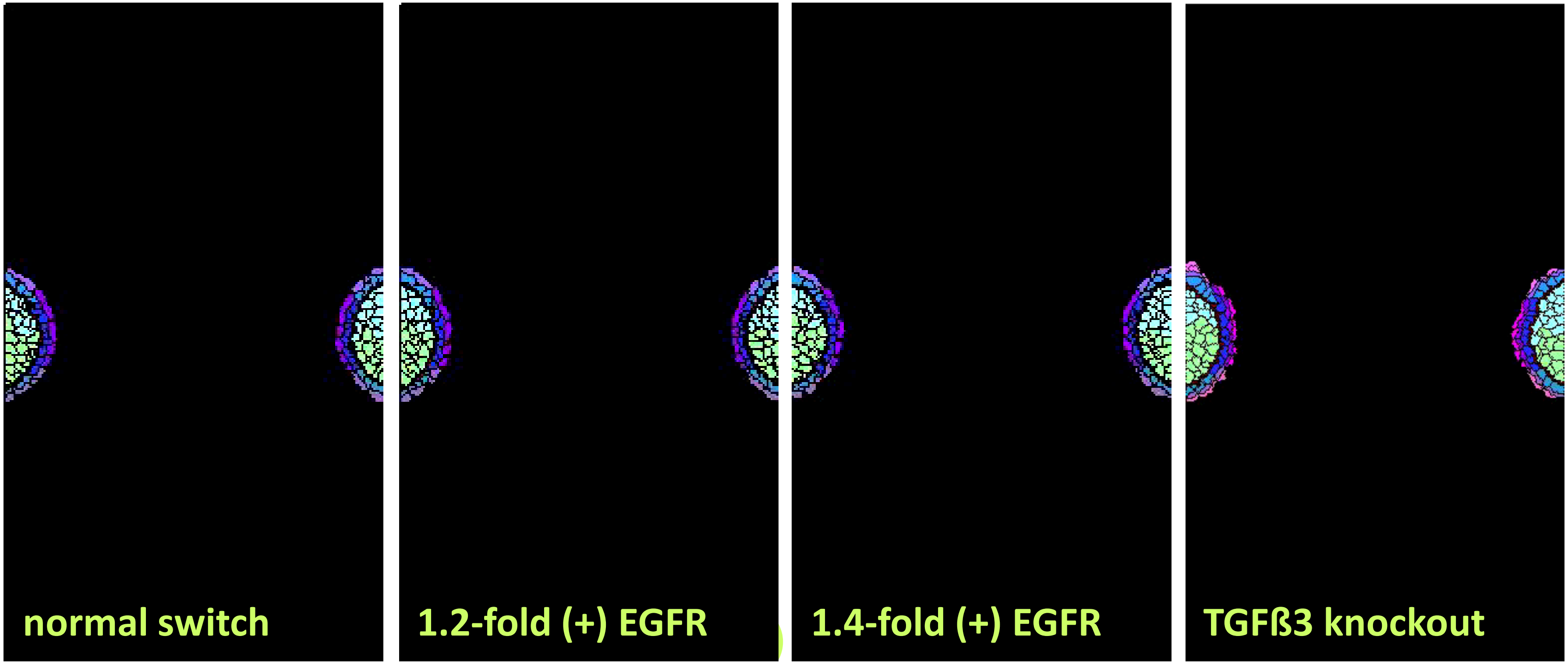


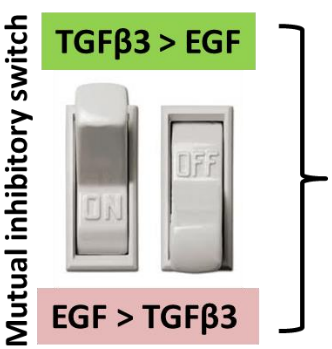


- *in silico* knockouts of elements in the underlying signaling network
- predicted impacts on MEE contact and seam breakdown (critical events)

Hutson et al., *unpublished*

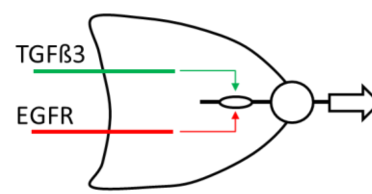
MES Differentiation: TGFβ3/EGF switching





primary biological switch for MEE seam breakdown

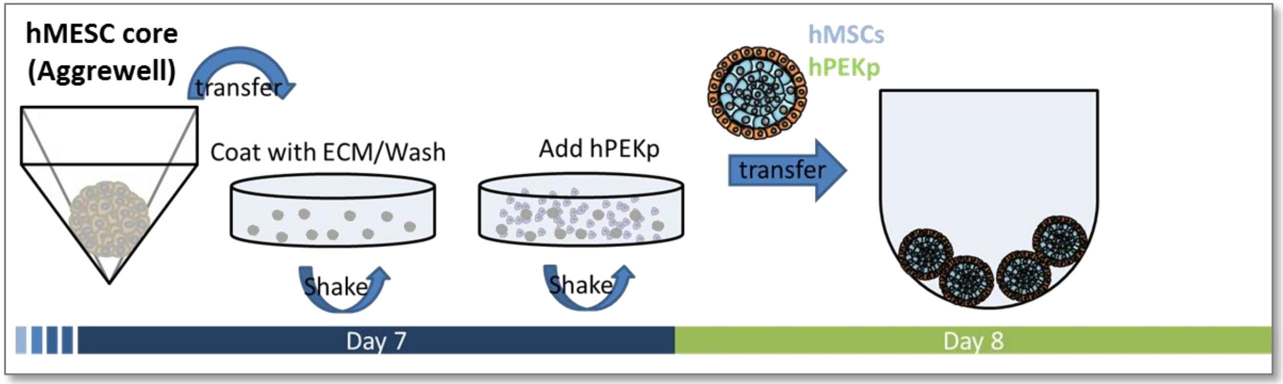
$$k_{EGF} = 15 + \mathrm{Random}[0,1] - 12\frac{[Tgf\beta3]^4}{[Tgf\beta3]^4 + AC_{50}^4} + k_{EGF-AhR}$$

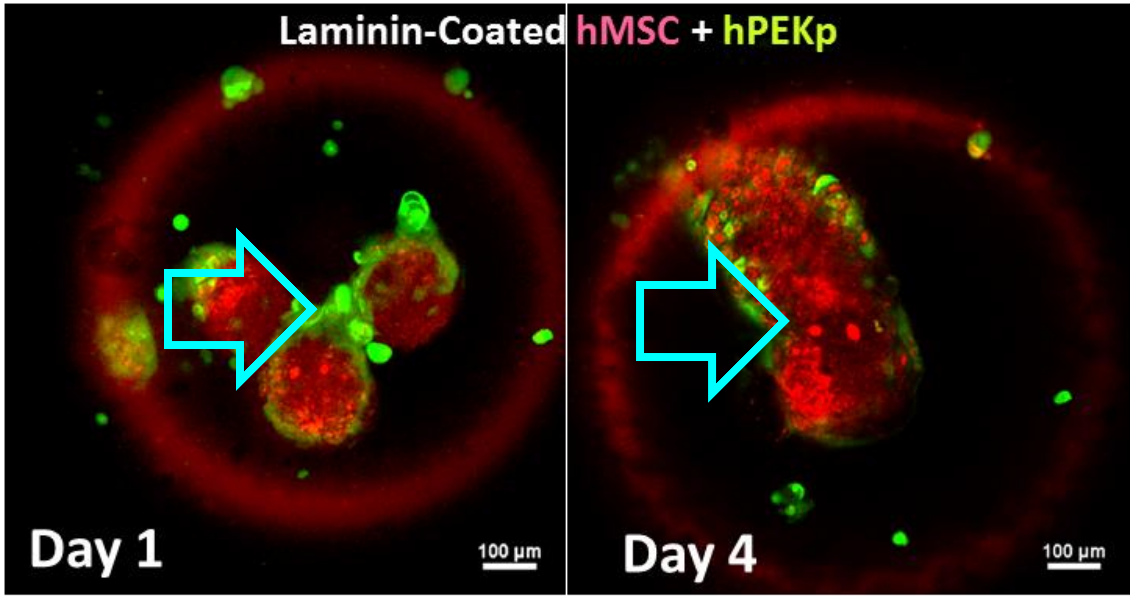


TGFß3	EGFR	CP
1.0	1.0	0
0	1.0	1
1.0	1.2	0
1.0	1.4	1
1.0	1.5	1

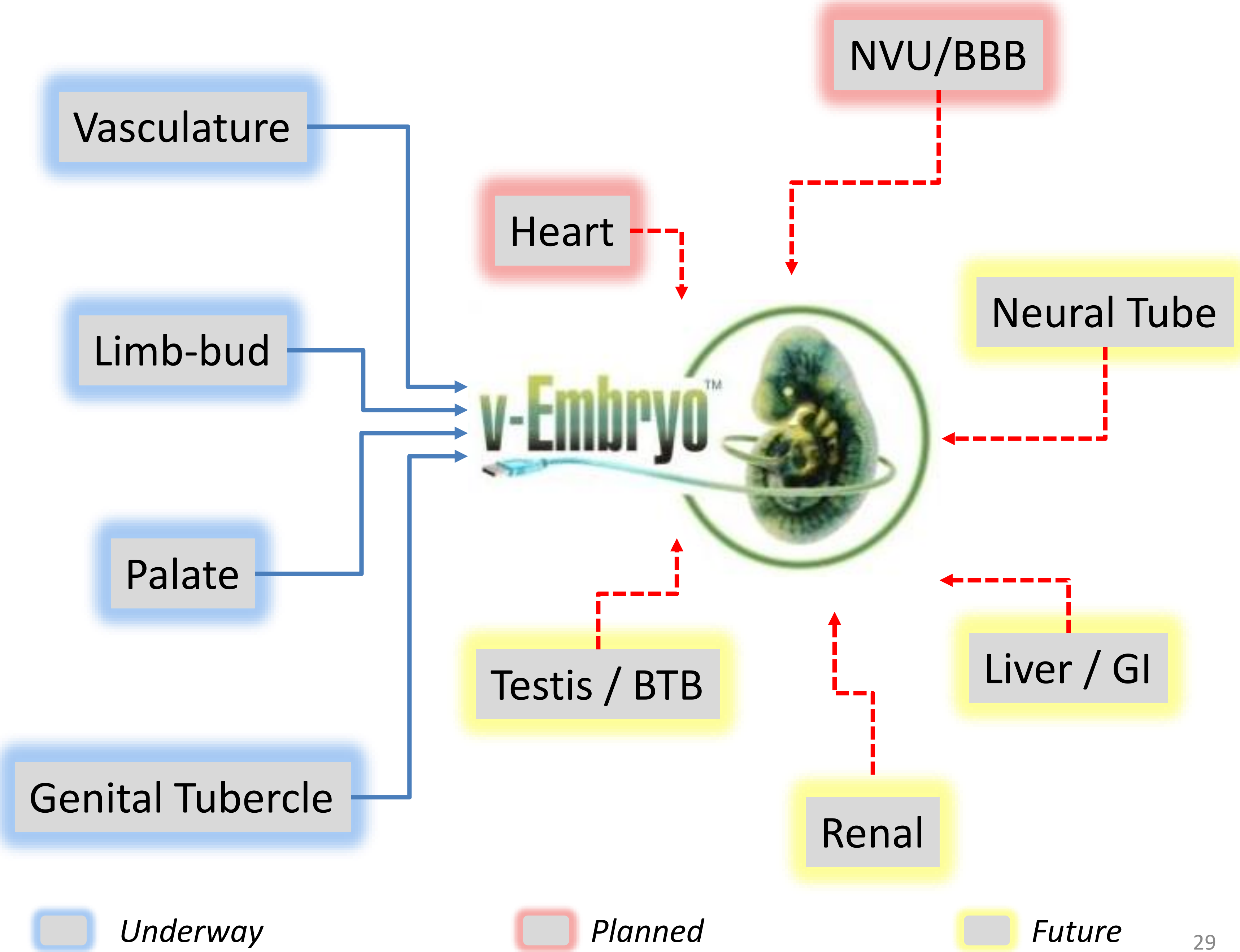
Predicted imbalance disrupting the timing of MES differentiation

Fusion-Competent hMSC Spheroids: engineering a HTS human MES breakdown assay from pluripotent human cell lines





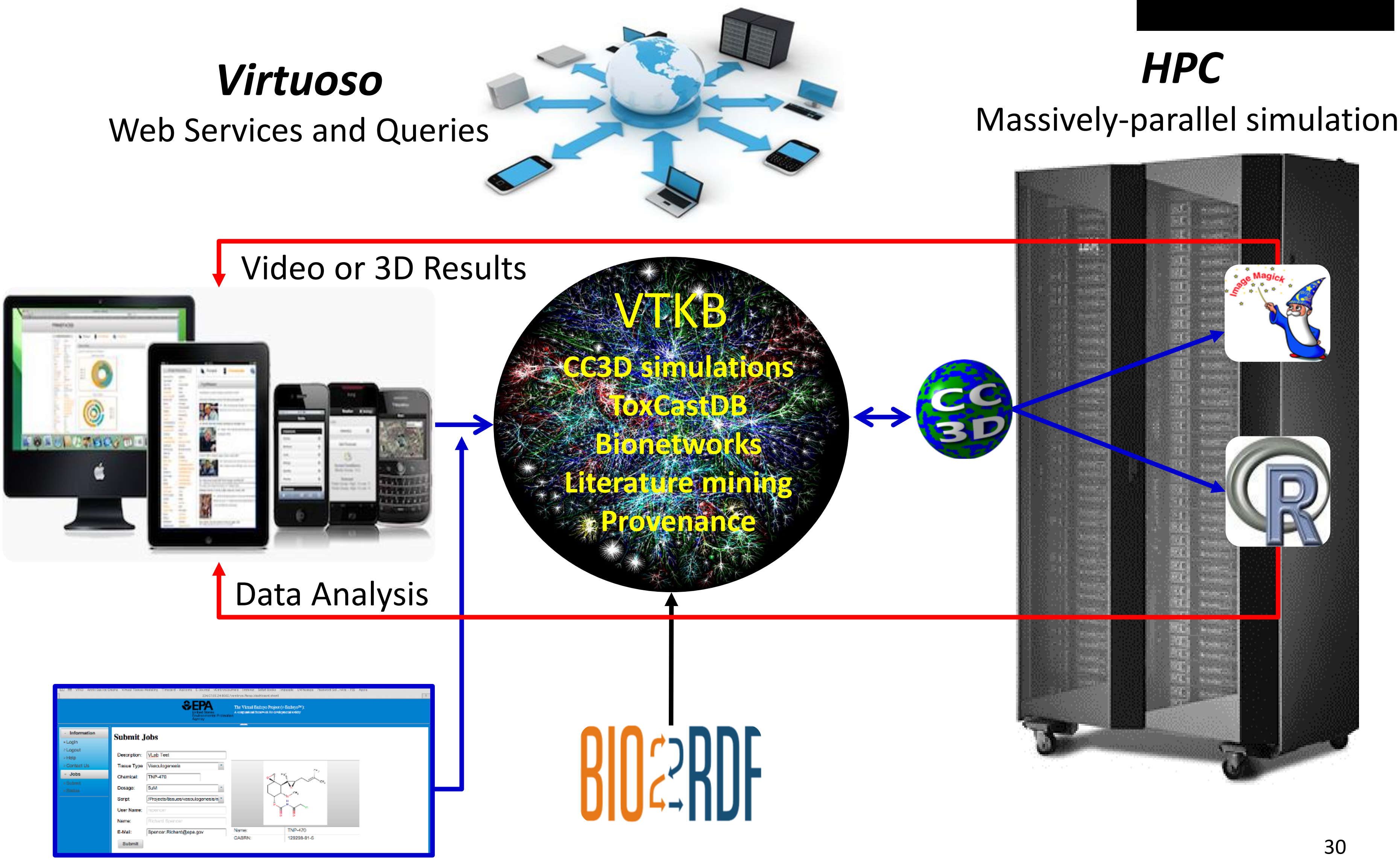
D Belair, C Wolf, B Abbott - NHEERL, *preliminary*


Vasculature
Limb-bud
Palate
Genital Tubercle
Heart
NVU/BBB
v-Embryo™
Neural Tube
Testis / BTB
Renal
Liver / GI
Underway
Planned
Future

Virtual Tissues Laboratory System

LOCKHEED MARTIN

VIRTUAL TISSUES LABORATORY SYSTEM



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