Computational Systems Biology:
translational tools for data integration and modeling

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DISCLAIMER: The views expressed are those of the presenters and do not reflect Agency policy.

Conflict of Interest Statement

The author declares no conflict of interest.

Abbreviations

ToxCast EPA's *Toxicity Forecaster* generates data and predictive models on thousands of chemicals of interest to the as part of the Tox21 federal collaboration. https://www.epa.gov/chemical-research/toxicity-forecasting

HTS ToxCast/Tox21 use *high-throughput screening* and computational modeling to rank chemicals based on their *in vitro* bioactivity profiles. https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data.

ToxRefDB EPA's *Toxicity Reference Database* provides results from across thousands of animal toxicity studies effects and endpoints. https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data

AOP *Adverse outcome pathway* is a formal representation of steps linking a molecular initiating event (MIE) to an adverse outcome (AO) relevant to risk assessment. https://aopwiki.org/aops/15

ABM *Agent-based model* is an *in silico* approach to simulate the dynamics of autonomous agents (eg, cells) that individually assess their situation and make decisions based on encoded rules and constraints.

CC3D *CompuCell3D* is an open-source simulation environment to execute cellular ABMs with a view to assessing critical state transitions and emergent properties in a self-organizing system. http://www.compucell3d.org/

Problem statement

- Automated *in vitro* assays enable high-throughput screening (HTS) to ‘decode the toxicological blueprint of active substances’ that interact with pregnancy.
- Vast HTS data (ToxCast/Tox21) in hand [https://comptox.epa.gov/dashboard], the need arises for predictive models of developmental toxicity.
- Key challenge: model ‘critical phenomena’ in self-organizing embryonic systems that compute with complex genetic circuits and multi-cellular networks.

Outline

Two examples will demonstrate the predictive power of ToxCast HTS data when integrated with developmentally-conserved cell signaling pathways:

1. Profiling the ToxCast library with a pluripotent human (H9) embryonic stem cell assay

Objective: increase the diversity and relevance of assays in ToxCast that can be used to profile chemicals for potential adverse effects on human embryonic development.

2. Translating biomolecular lesion(s) into quantitative phenotypes for predictive developmental toxicology

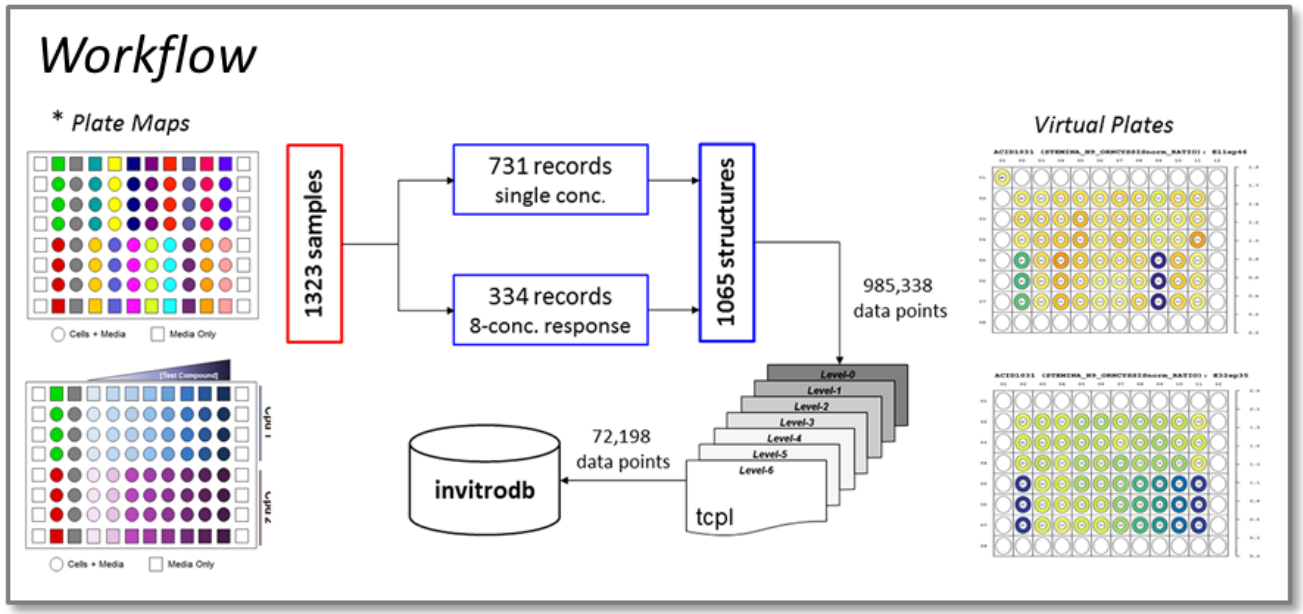
Objective: build and test computer models of complex tissues that advance critical phenomena (specificity, canalization, plasticity) for quantitative prediction for virtual screening and *in silico* testing.

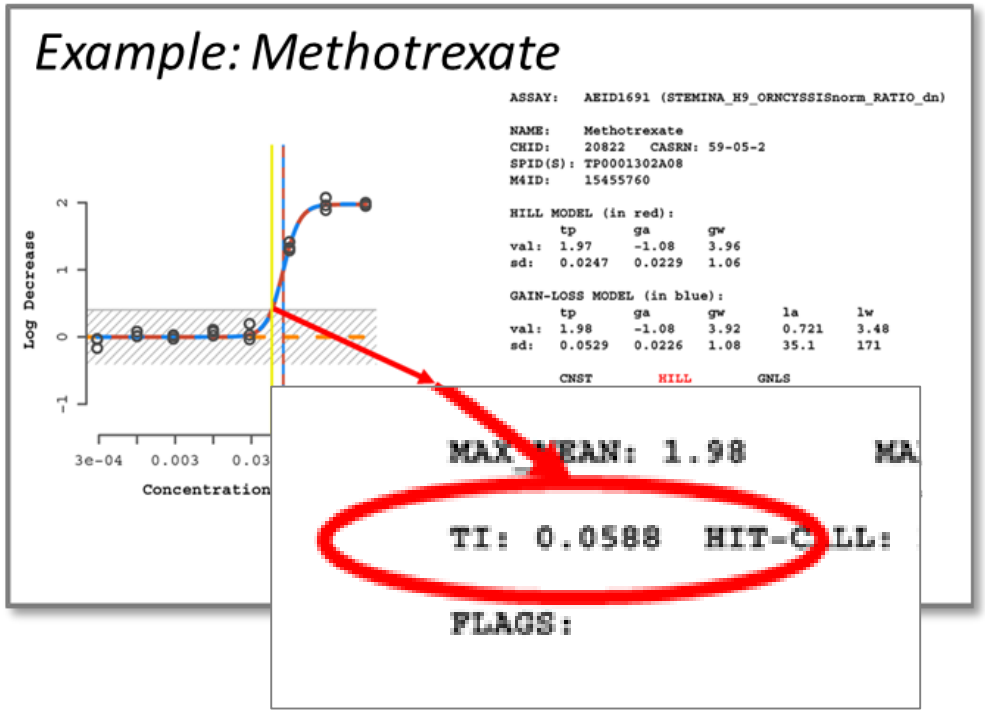
1. Profiling the ToxCast library with a pluripotent human (H9) embryonic stem cell assay

Objective: increase the diversity and relevance of assays in ToxCast that can be used to profile chemicals for potential adverse effects on human embryonic development.

ToxCast_STM assay

- devTOX^{qP} assay from Stemina Biomarker Discovery [Palmer et al. 2013]
- pluripotent stem cells exposed for 3-days
- critical drop in ornithine : cystine ratio is the targeted readout
- **Key point:** 183 of 1065 (17%) ToxCast chemicals tested positive





SOURCE: Zurlinden et al. (manuscript)

ToxCast_STM assay

- *in vitro* accuracy anchored to *in vivo* DevTox studies (ToxRefDB)
- **Key point**: balanced accuracy improves with evidence for DevTox

		Stringency Filter Applied to DevTox Anchor			
	Condition[2]	***Base***	***Low***	***Medium***	***High***
	TP	85	60	35	19
	FP	14	37	23	9
	FN	217	127	51	11
	TN	116	208	176	88
	n	432	432	285	127
	sensitivity	0.281	0.321	0.407	0.633
	specificity	0.892	0.849	0.884	0.907
	PPV	0.859	0.619	0.603	0.679
	NPV	0.348	0.621	0.775	0.889
	ACC	46.5%	62.0%	74.0%	84.3%
	MCC	0.190	0.202	0.332	0.554
		any dLEL rat OR rabbit	SOME evidence rat OR rabbit	CLEAR evidence rat OR rabbit	CLEAR evidence rat AND rabbit

in vivo

in vitro

TP	FP
FN	TN

STM versus rat WEC

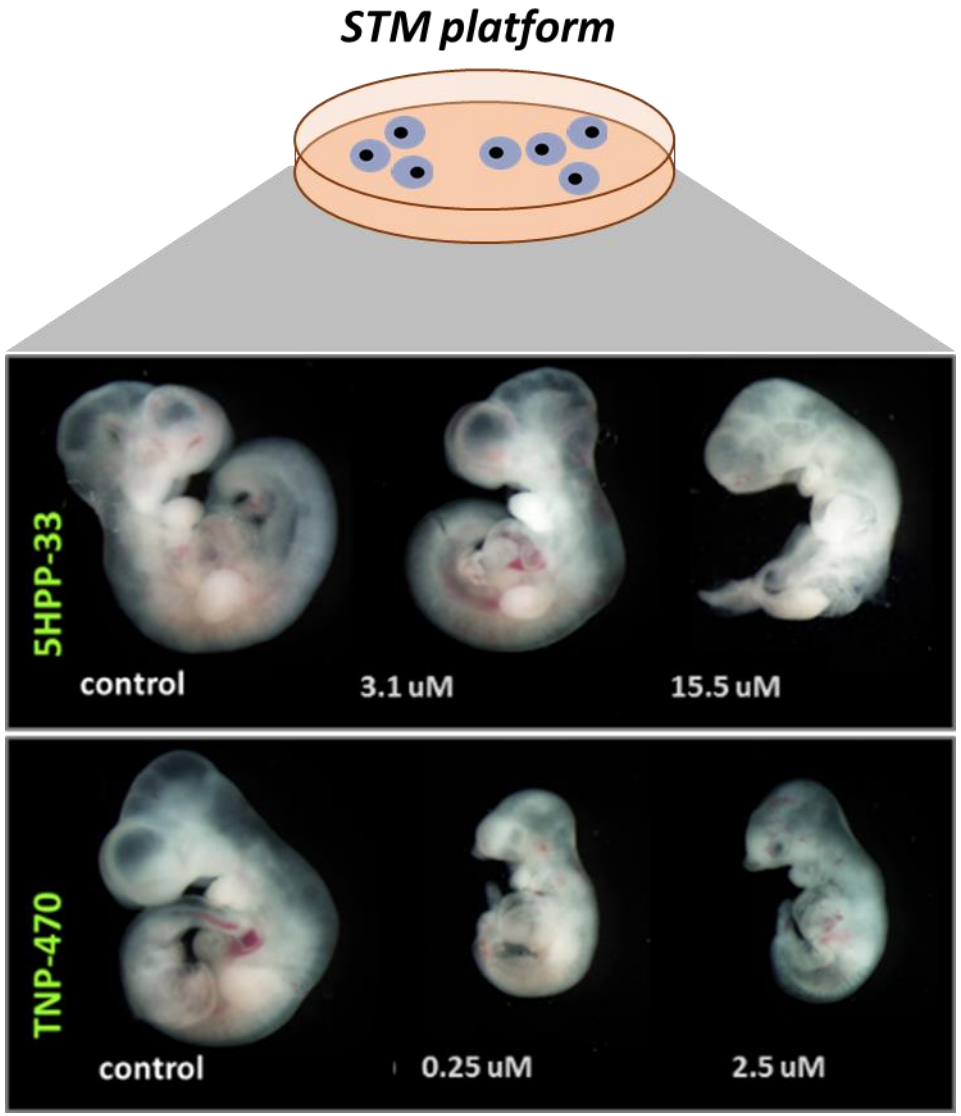
5HPP-33: *synthetic thalidomide analog*

- T.I. predicted 9.5 µM
- AC50 observed 21.2 µM (embryo viability)

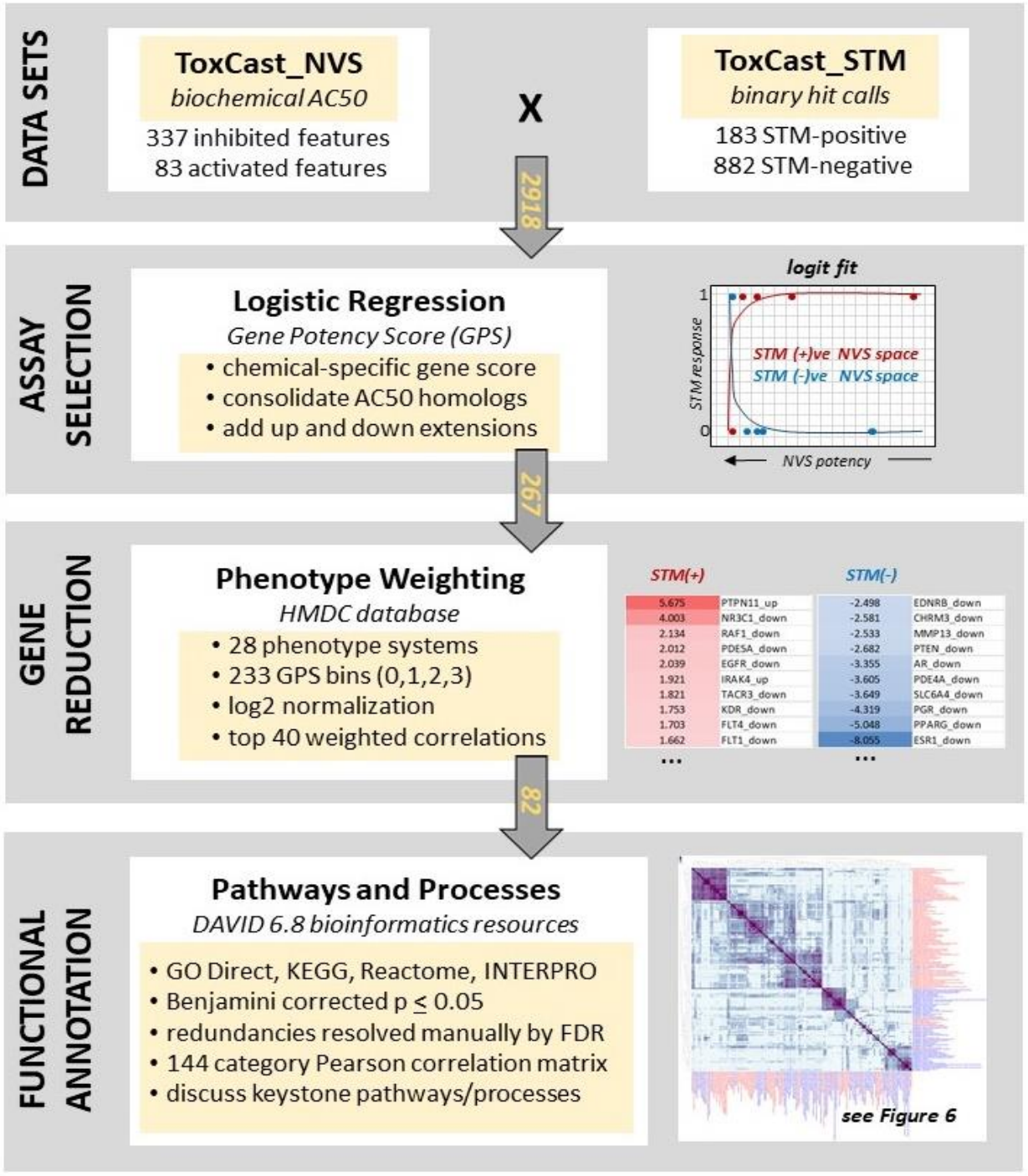
TNP-470: *synthetic fumagillin analog*

- T.I. predicted 0.01 µM
- AC50 observed 0.04 µM (dysmorphogenesis)

Key point: exposure-based potential for DevTox predicted by STM assay (quantitative prediction).



SOURCE: Ellis-Hutchings et al. (2017) Reprod Toxicol



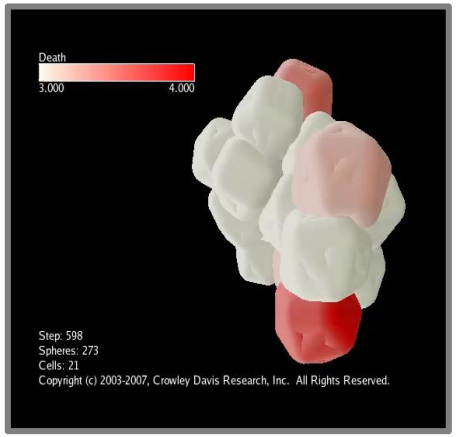
Keystone Pathways

- Functional annotations inferred from mining STM response against biochemical (NVS) features.
- What we can and cannot say about the applicability domain with regards to biochemical targets:

  - sensitive: regulation of PI3K signaling, FoxO signaling pathway, and focal adhesion pathway.

  - insensitive domain: GPCR signaling through G(q) and steroid hormone mediated signaling pathways.

2. Translating biomolecular lesion(s) into quantitative phenotypes for predictive developmental toxicology



Objective: build and test computer models of complex tissues that advance critical phenomena (specificity, canalization, plasticity) for quantitative prediction for virtual screening and *in silico* testing.

SOURCE: Andersen, Newman and Otter (2006) Am. Assoc. Artif. Intel.

Cell agent-based models (ABMs)

- **Approach:** build and test self-organizing morphogenetic systems *in silico* using CC3D modeling environment (www.compucell3d.org).
- **Input:** A.I. cast into mathematically-defined cells (agents), synthetic gene circuits, and viscoelastic properties to emulate developmental progression.
- **Emergence:** simulation resolves into normal or perturbed phenotypes reading *in vitro* data input from specific ToxCast assays (cybermorphs).
- **Output:** probabilistic rendering of where, when and how a developmental defect might occur (critical phenomena).

AOP framework: *cleft palate as an example*

ToxCast Chemicals

500 chemicals summarized by ToxCast gene score and chemotype for machine-learning

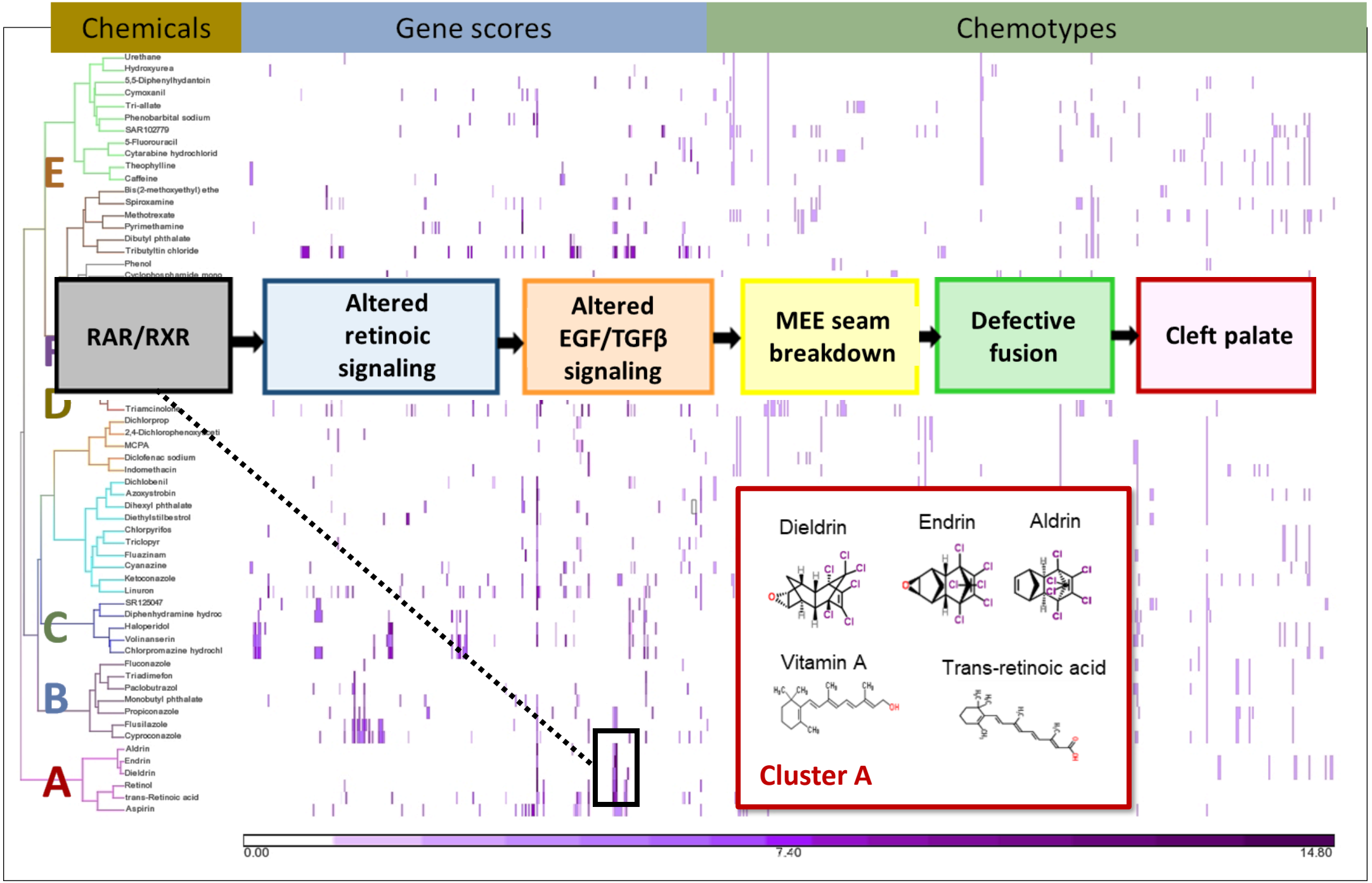
Animal studies

63 chemicals associated with cleft palate in ToxRefDB or open literature

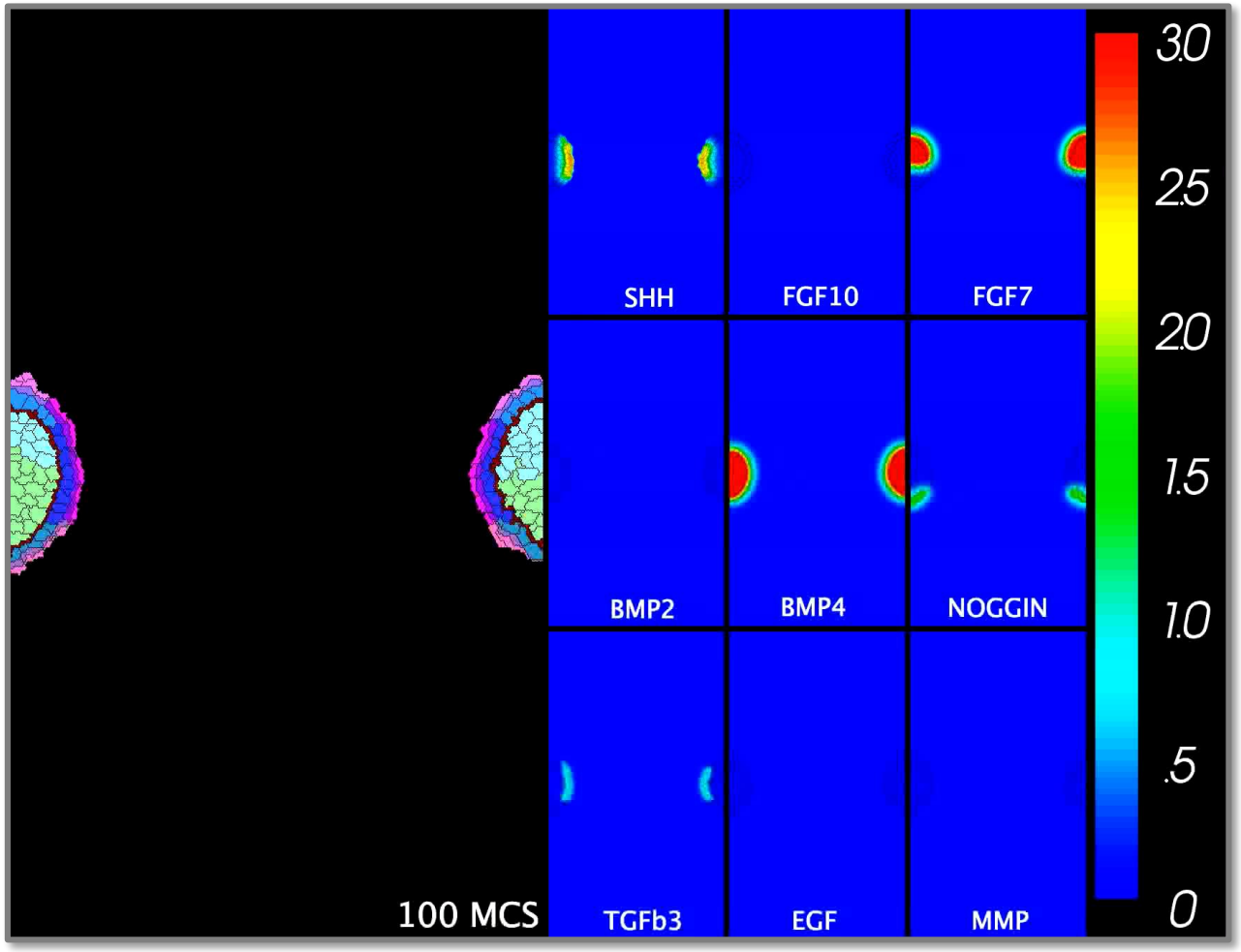
AOP clusters

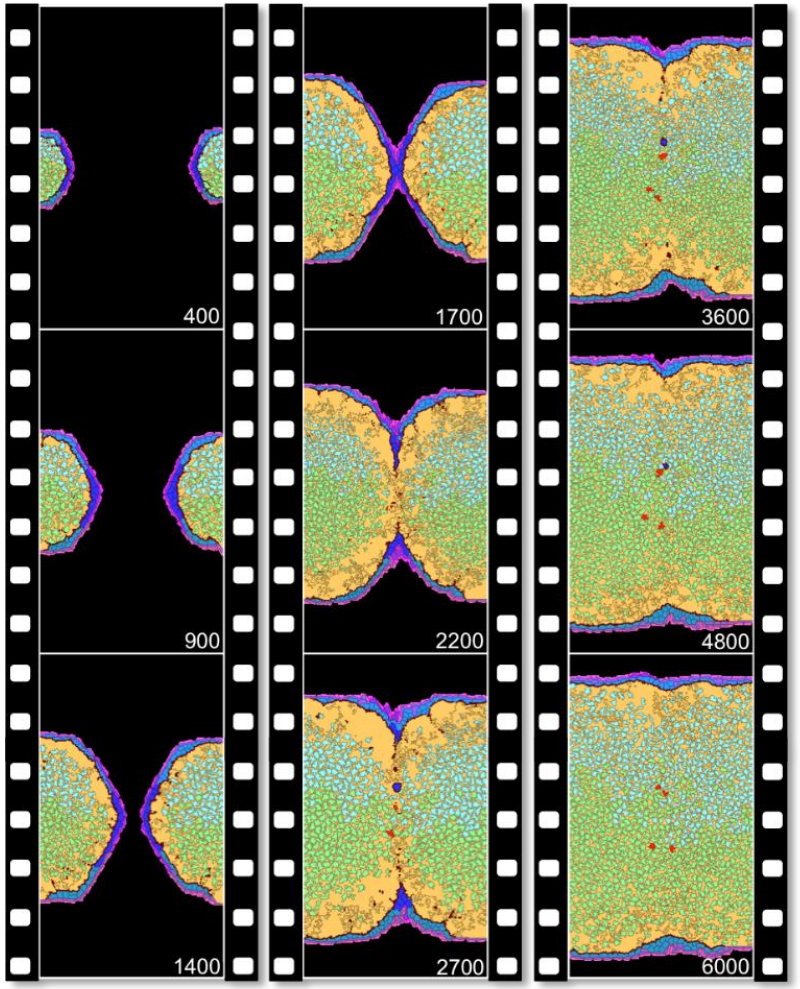
6 mechanistic pathways inferred from integration of HTS data with chemical structure.

SOURCE: Baker et al. (manuscript)


Chemicals
Gene scores
Chemotypes
Urethane
Hydroxyurea
5,5-Diphenylhydantoin
Cymoxanil
Tri-allate
Phenobarbital sodium
SAR102779
5-Fluorouracil
Cytarabine hydrochlorid
Theophylline
Caffeine
Bis(2-methoxyethyl) ethe
Spiroxamine
Methotrexate
Pyrimethamine
Dibutyl phthalate
Tributyltin chloride
Phenol
E
RAR/RXR
Altered retinoic signaling
Altered EGF/TGFβ signaling
MEE seam breakdown
Defective fusion
Cleft palate
Triamcinolone
Dichlorprop
2,4-Dichlorophenoxyacti
MCPA
Diclofenac sodium
Indomethacin
Dichlobenil
Azoxystrobin
Dihexyl phthalate
Diethylstilbestrol
Chlorpyrifos
Triclopyr
Fluazinam
Cyanazine
Ketoconazole
Linuron
SR125047
Diphenhydramine hydroc
Haloperidol
Volinanserin
Chlorpromazine hydrochl
C
Fluconazole
Triadimefon
Paclobutrazol
Monobutyl phthalate
Propiconazole
Flusilazole
Cyproconazole
B
Aldrin
Endrin
Dieldrin
Retinol
trans-Retinoic acid
Aspirin
A
Dieldrin
Endrin
Aldrin
Vitamin A
Trans-retinoic acid
Cluster A
0.00
7.40
14.80

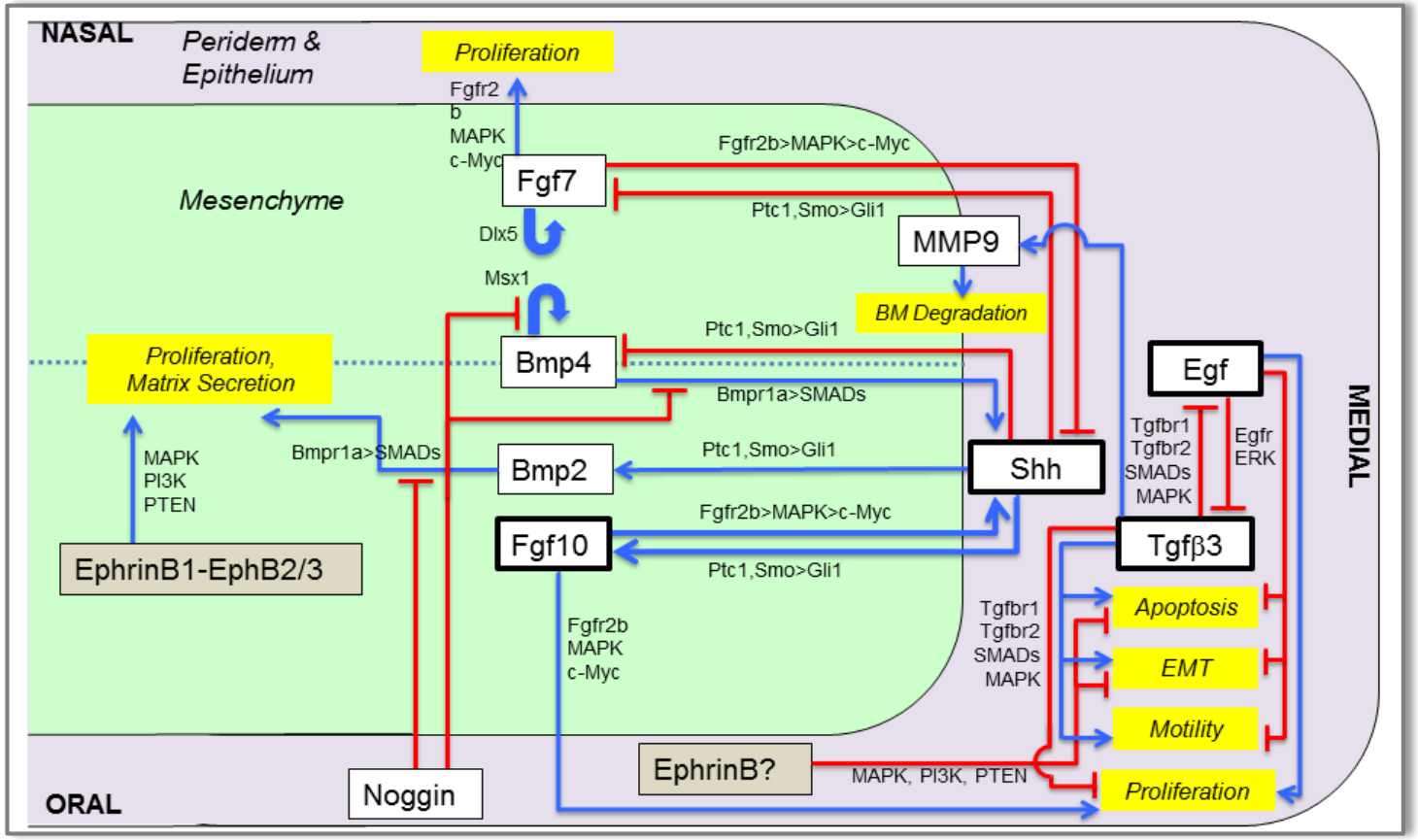
Epithelial seam breakdown and mesenchymal confluence during palatal fusion

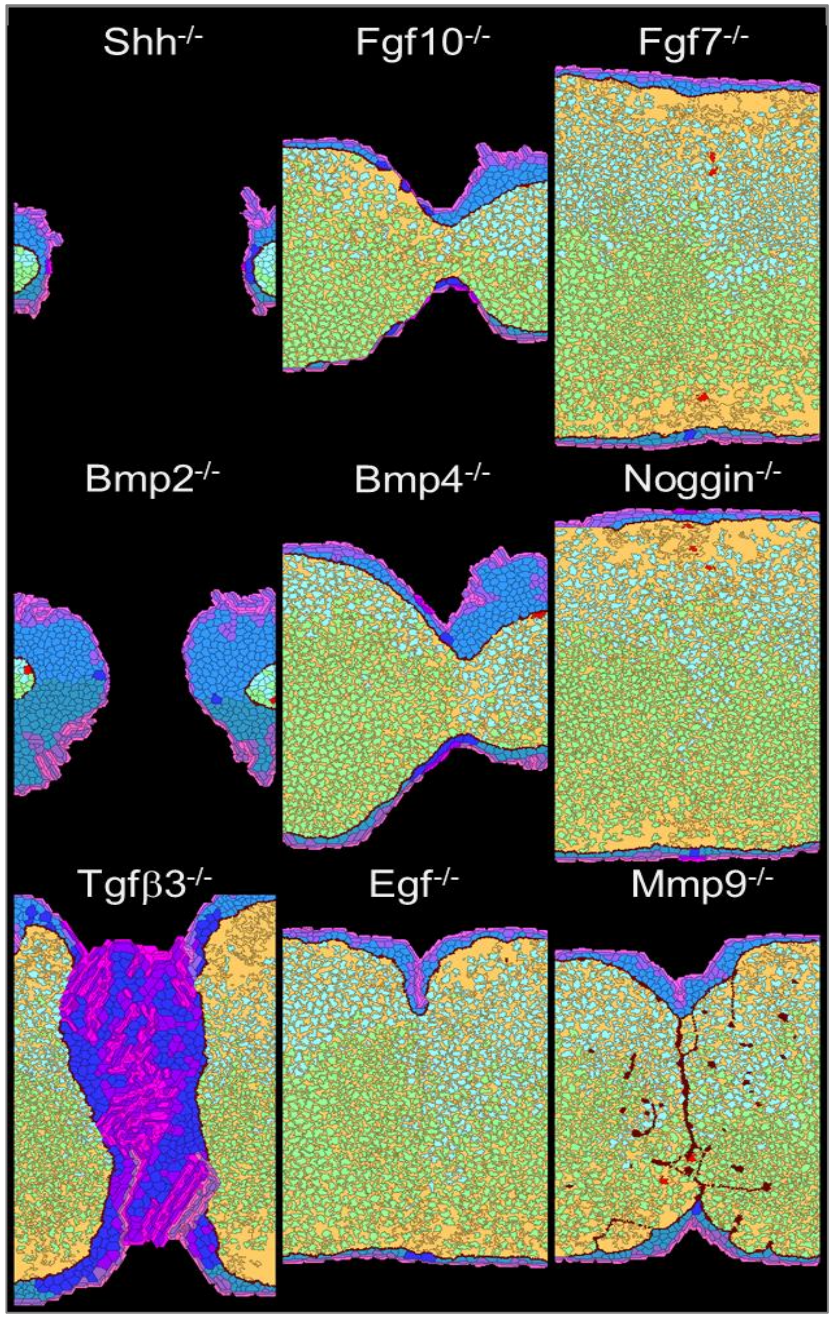




SOURCE: Hutson et al. (2017) Chem Res Toxicol

Hacking the control network

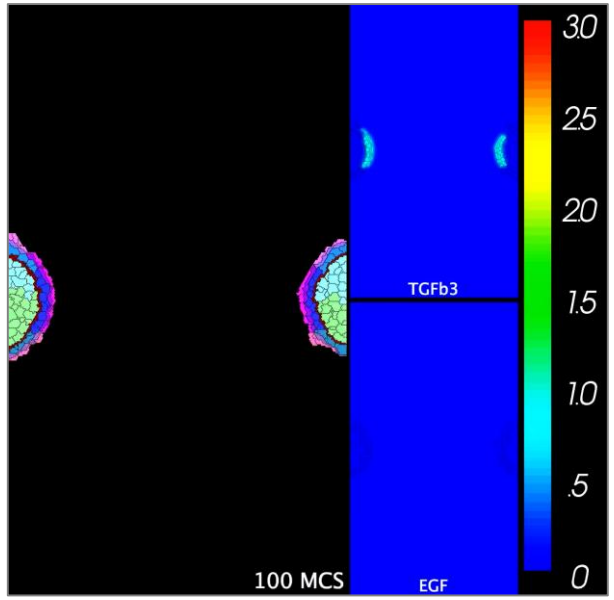


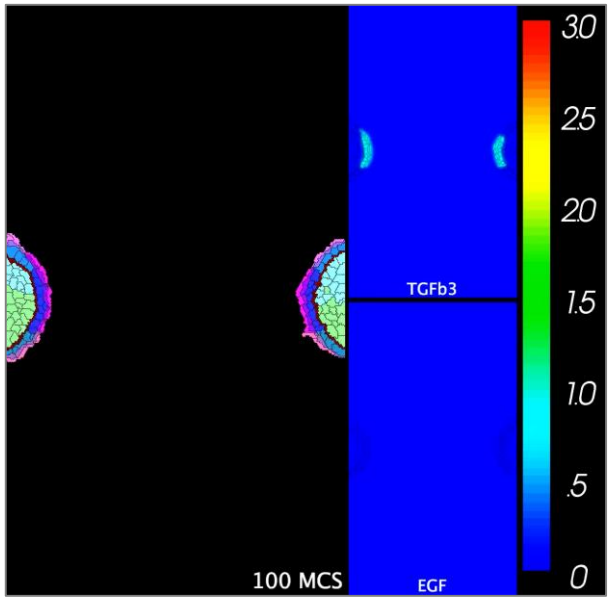


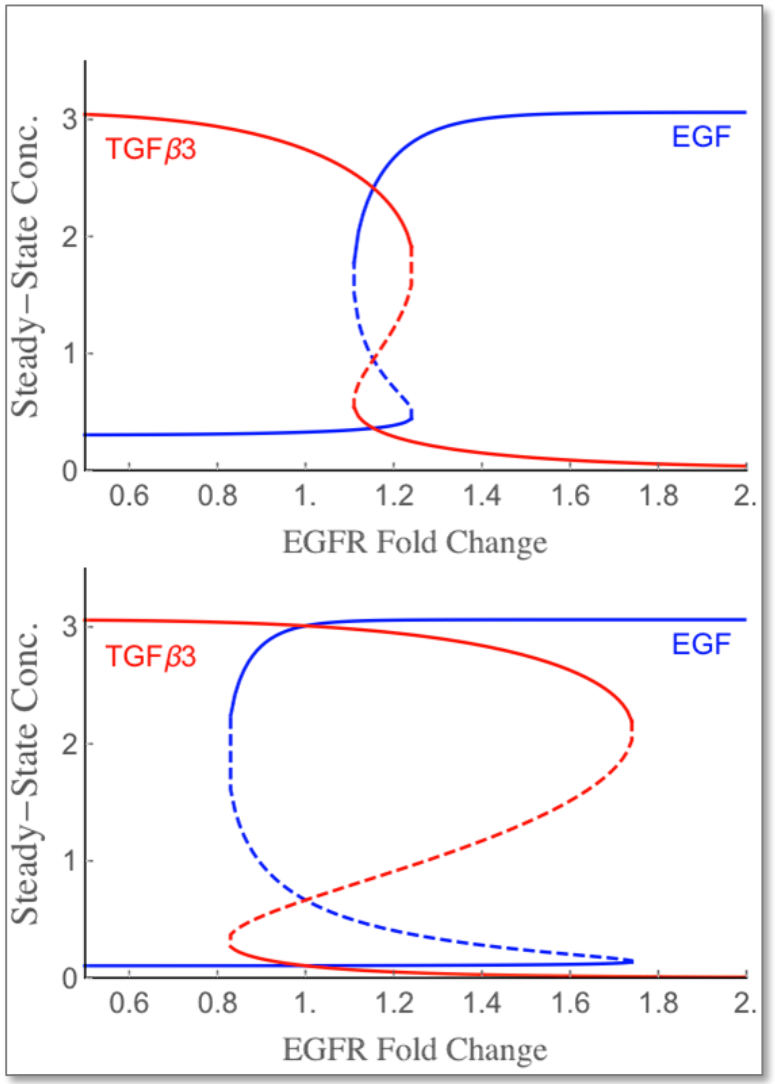
A.I. = synthetic cell signaling networks

Cybermorphs = simulated loss of function

Messin' with the switch: *two scenarios for bistable dynamics*







Narrow hysteresis:
less resilient but reversible

Broad hysteresis:
more resilient but irreversible

ToxCast dataset

(-) ToxRefDB_DEV

(+) ToxRefDB_DEV

ChemicalName	FR_up(A	GFR_up(	1_down	b1_dow	ToxRef(lo
Methylene bis(thiocyanate)	1.14	2.13	5.93	4.26	NEG
Zoxamide	14.22	1.85	17.37	9.69	NEG
2-(Thiocyanomethylthio)benzothiazole	2.28	1.54	6.48	7.21	NEG
Diphenylamine	32.71	1.49	5.95	1.63	NEG
Azamethiphos	0.89	1.81	1000.00	1000.00	NEG
Bromacil	20.50	1.57	1000.00	1000.00	NEG
Forchlorfenuron	0.02	1.53	1000.00	1000.00	NEG
Methyl isothiocyanate	4.60	1.44	1000.00	1000.00	NEG
Diuron	16.51	1.44	1000.00	1000.00	NEG
Rotenone	0.82	1.42	1000.00	1000.00	NEG
Captan	4.59	2.57	7.15	7.25	POS
Triflumizole	32.71	2.48	19.88	19.88	POS
Butachlor	32.71	2.47	17.85	17.85	POS
Captafol	1.02	2.20	3.76	3.25	POS
Thiram	4.45	1.96	6.95	5.38	POS
Raloxifene hydrochloride	12.40	1.91	15.94	10.94	POS
Fluazinam	2.39	1.61	2.48	4.84	POS
Carbaryl	0.07	1.55	1000.00	1000.00	POS
Linuron	10.91	1.46	1000.00	1000.00	POS
Maneb	0.01	1.46	1000.00	1000.00	POS
Bendiocarb	8.75	1.43	1000.00	1000.00	POS
Fipronil	1.18	1.43	1000.00	1000.00	POS
Propoxur	1.67	1.43	1000.00	1000.00	POS
TNP-470	7.78	1.57	3.97	3.61	x
1-(2,3,8,8-Tetramethyl-1,2,3,4,5,6,7,8-octal	8.33	2.10	9.74	1.88	x
Trimethylolpropane triacrylate	2.02	1.80	5.17	1.41	x
Diiodomethyl 4-methylphenyl sulfone	3.15	1.77	3.74	17.68	x
1,2-Benzisothiazolin-3-one	8.22	1.74	11.91	14.70	x
Tralopyril	18.30	1.68	0.87	1.08	x
Bis(trichloromethyl)sulfone	1.95	1.61	4.49	5.74	x
N,N,N-Trimethyloctadecan-1-aminium chl	2.22	1.56	1.77	1.45	x
beta-Nitrostyrene	7.12	1.52	2.01	2.34	x
4,5-Dichloro-3H-1,2-dithiol-3-one	2.71	1.47	6.42	6.56	x
Tri-o-cresyl phosphate	8.95	1.45	9.54	1.56	x
Isobornyl methacrylate	13.66	1.44	21.86	1.97	x
SAR102779	0.05	1.43	12.95	14.97	x
PharmaGSID_48511	12.19	1.37	11.22	17.33	x
Perfluoroundecanoic acid	6.81	1.35	4.76	5.04	x
FR167356	17.65	2.06	1000.00	1000.00	x
Monobutyl phthalate	0.01	1.35	1000.00	1000.00	x
Niclosamide	0.58	2.14	1000.00	1000.00	x
Tripropylene glycol diacrylate	26.52	2.09	1000.00	1000.00	x
CP-457920	3.50	1.92	1000.00	1000.00	x
Trimethylolpropane trimethacrylate	32.85	1.81	1000.00	1000.00	x
alpha-Terpinyl acetate	39.18	1.64	1000.00	1000.00	x
3-(4-tert-Butylphenyl)-2-methylpropanal	35.26	1.62	1000.00	1000.00	x
1,4-Dinitrobenzene	2.95	1.54	1000.00	1000.00	x
SB281832	34.72	1.54	1000.00	1000.00	x
2-(Morpholin-4-yldithio)-1,3-benzothiazol	5.61	1.52	1000.00	1000.00	x
Tolclofos-methyl	7.71	1.49	1000.00	1000.00	x
1,1':3',1''-Terphenyl	11.98	1.38	1000.00	1000.00	x
Estrone	0.03	1.35	1000.00	1000.00	x

	↑EGFR		↓TGFβ1		ToxRefDB
µM effect in vitro	*AC50*	*top*	*AC50*	*top*	*DevTox*
Captan	4.59	2.57	7.15	7.25	POS
Triflumizole	32.71	2.48	19.88	19.88	POS
Butachlor	32.71	2.47	17.85	17.85	POS
Captafol	1.02	2.20	3.76	3.25	POS
Thiram	4.45	1.96	6.95	5.38	POS
Raloxifene hydrochloride	12.40	1.91	15.94	10.94	POS
Fluazinam	2.39	1.61	2.48	4.84	POS

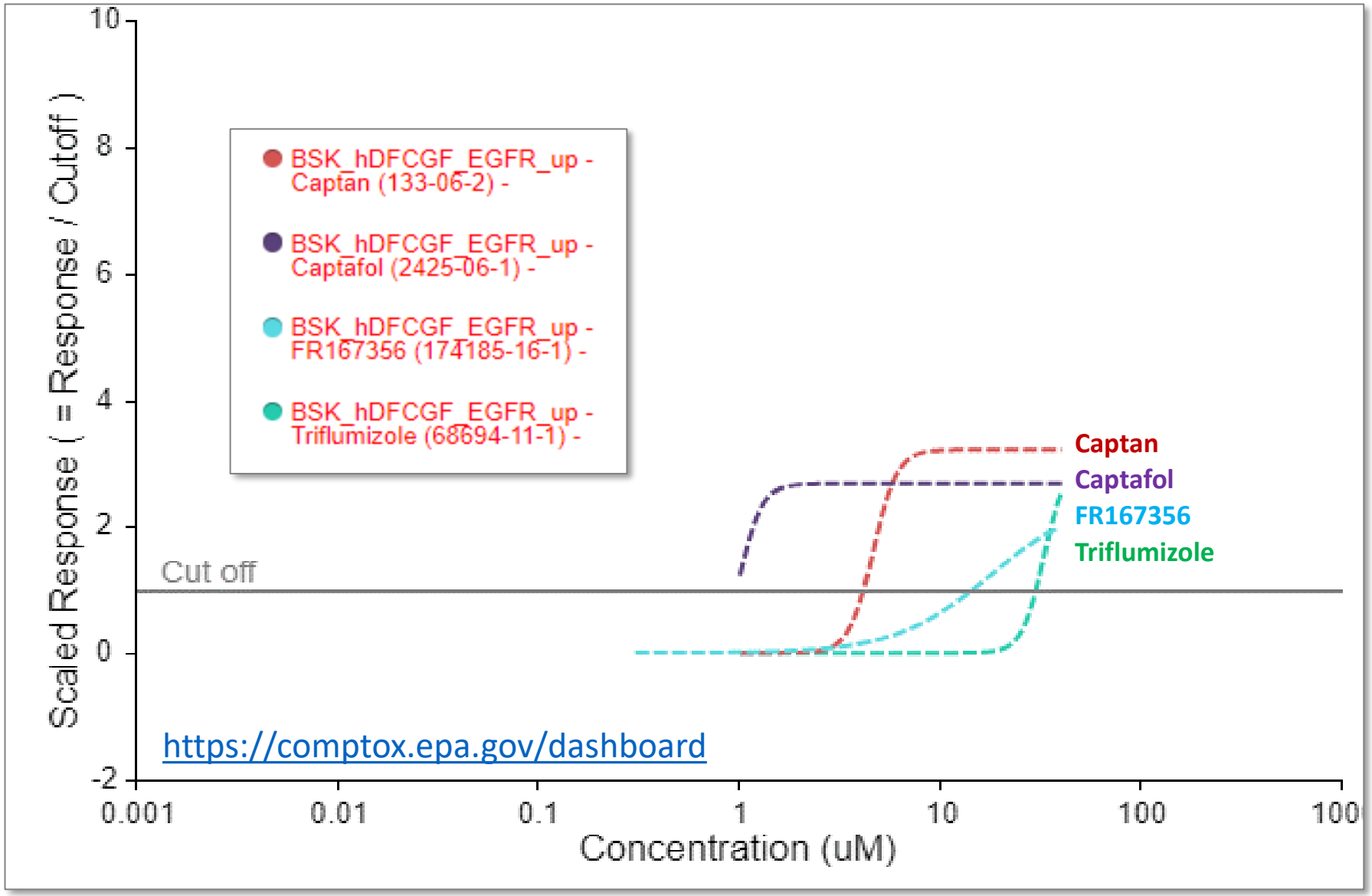
- *54 chemicals ↑EGFR density*
- *some also ↓TGF-beta signaling*

negative for developmental toxicity in ToxRefDB

positive for developmental toxicity in ToxRefDB

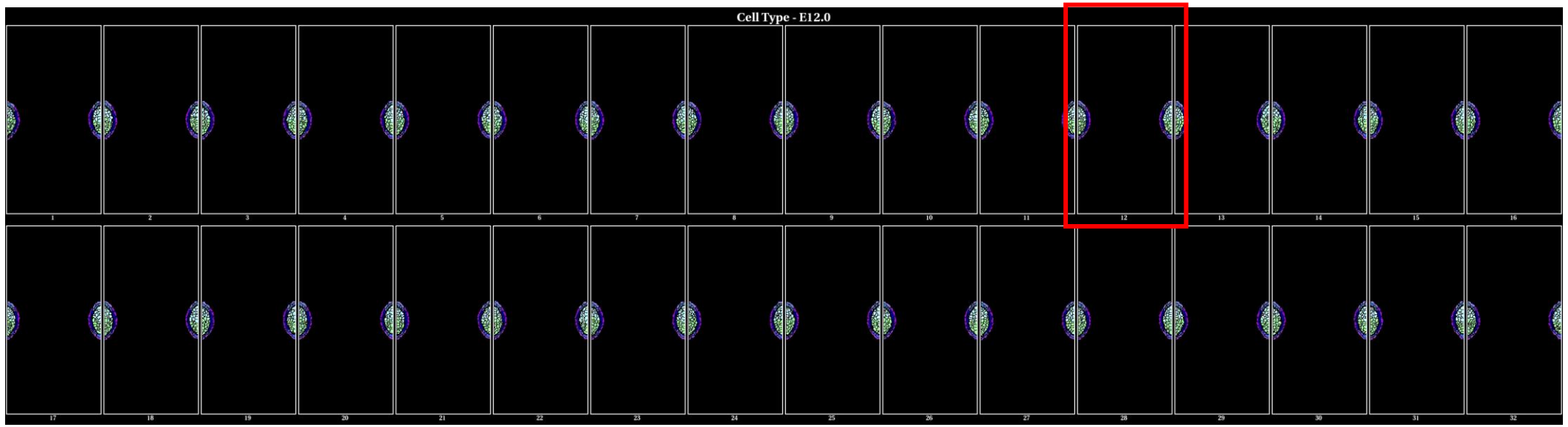
positive for developmental toxicity in ToxRefDB

ToxCast dataset: EGFR: ↑ immunoreactivity relative to DMSO



***In silico* dose-response:** translating ↑*EGFR conc. profile into a critical dose*

Captan



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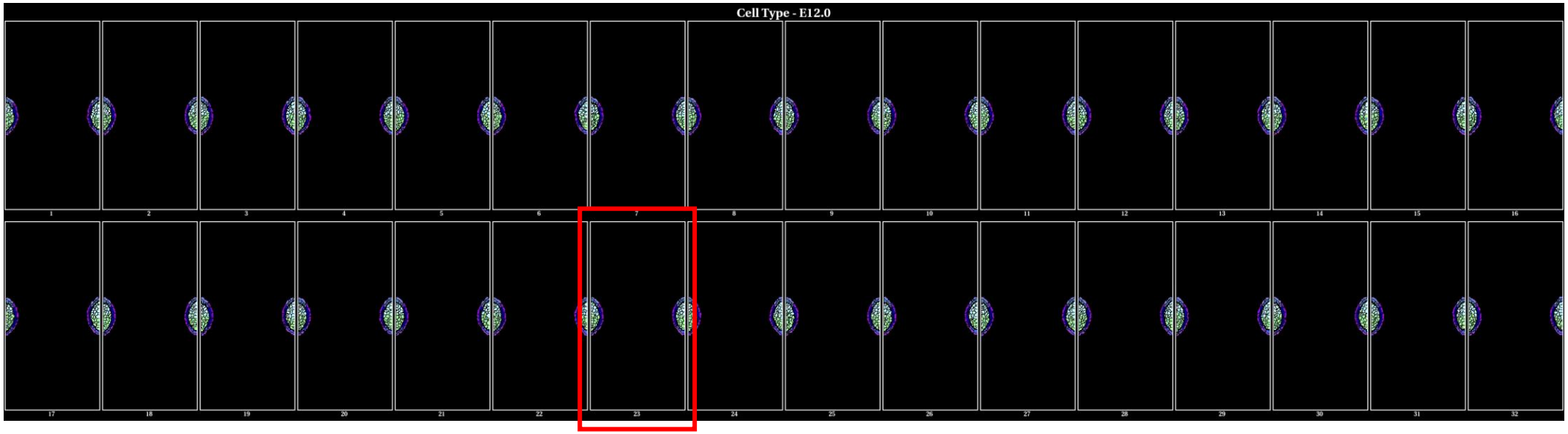
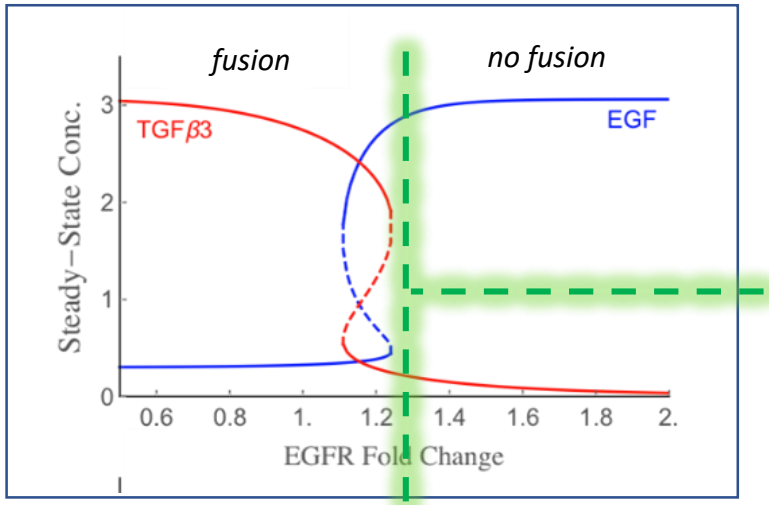


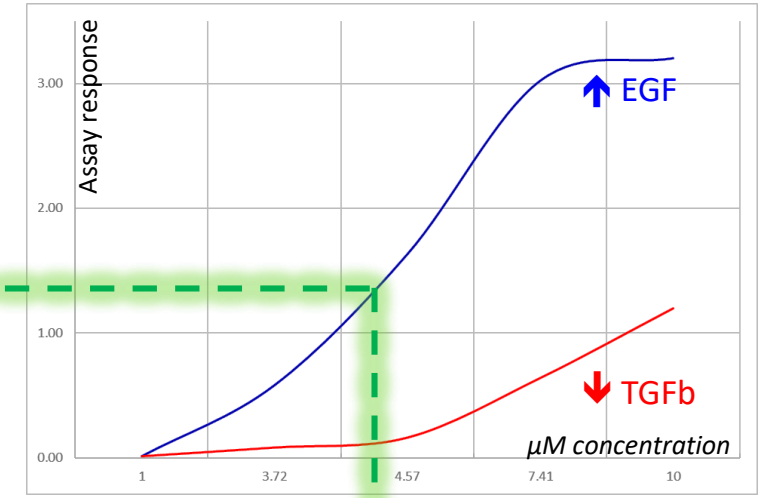
Predictive model: *modeling the critical phenomenon*

INPUT: switch dynamics

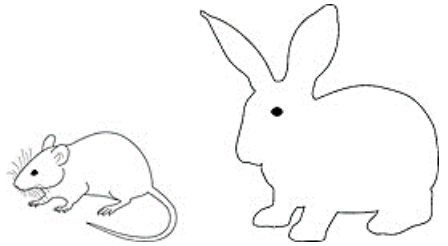


tipping point predicted by computational dynamics (hysteresis switch)

Captan in ToxCast



OUTPUT: tipping point mapped to concentration response (4 µM)



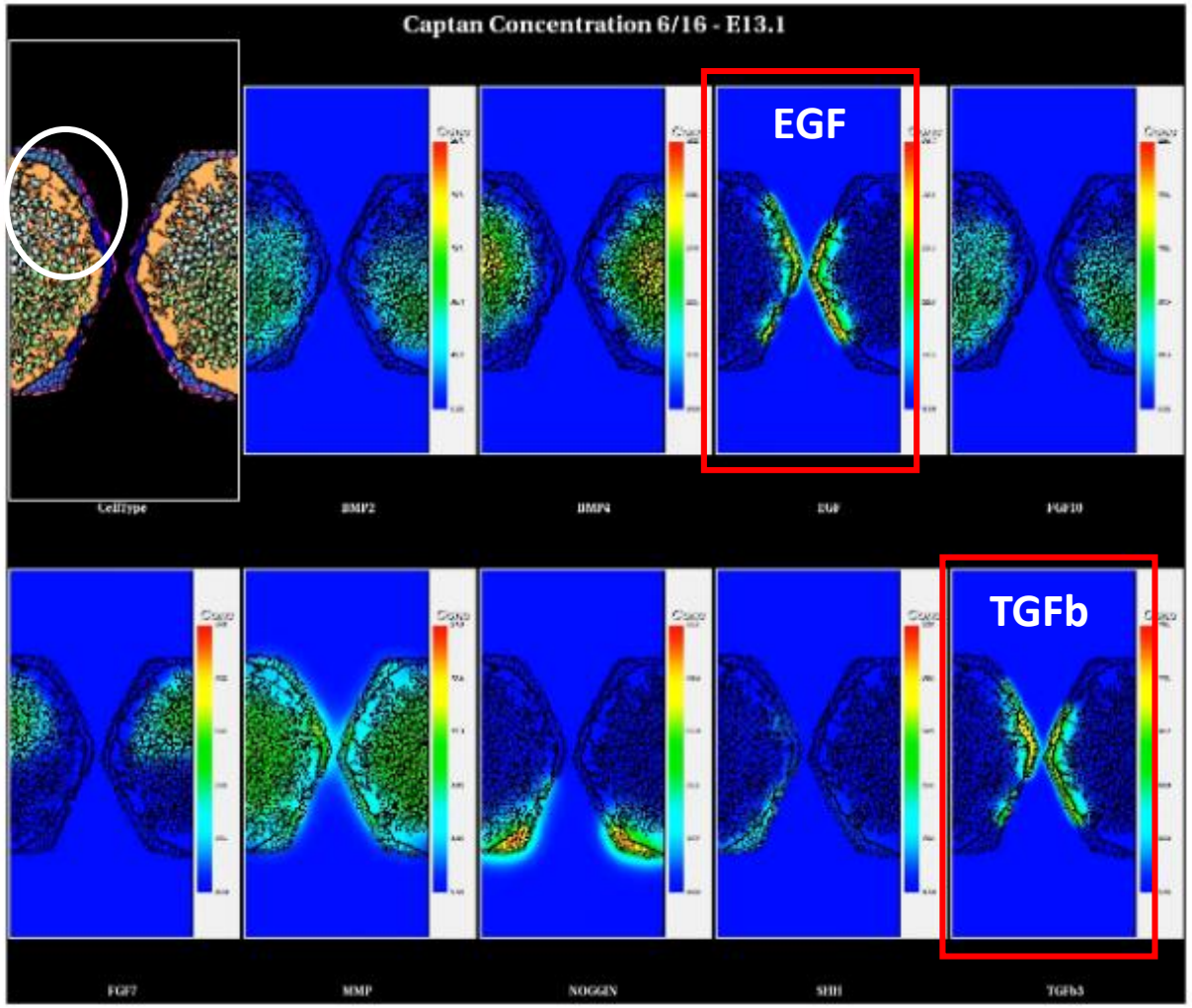
Captan in ToxRefDB
NEL = 10 mg/kg/day
LEL = 30 mg/kg/day

human HTTK model
2.39 mg/kg/day would achieve a steady state of 4 µM in fetal plasma

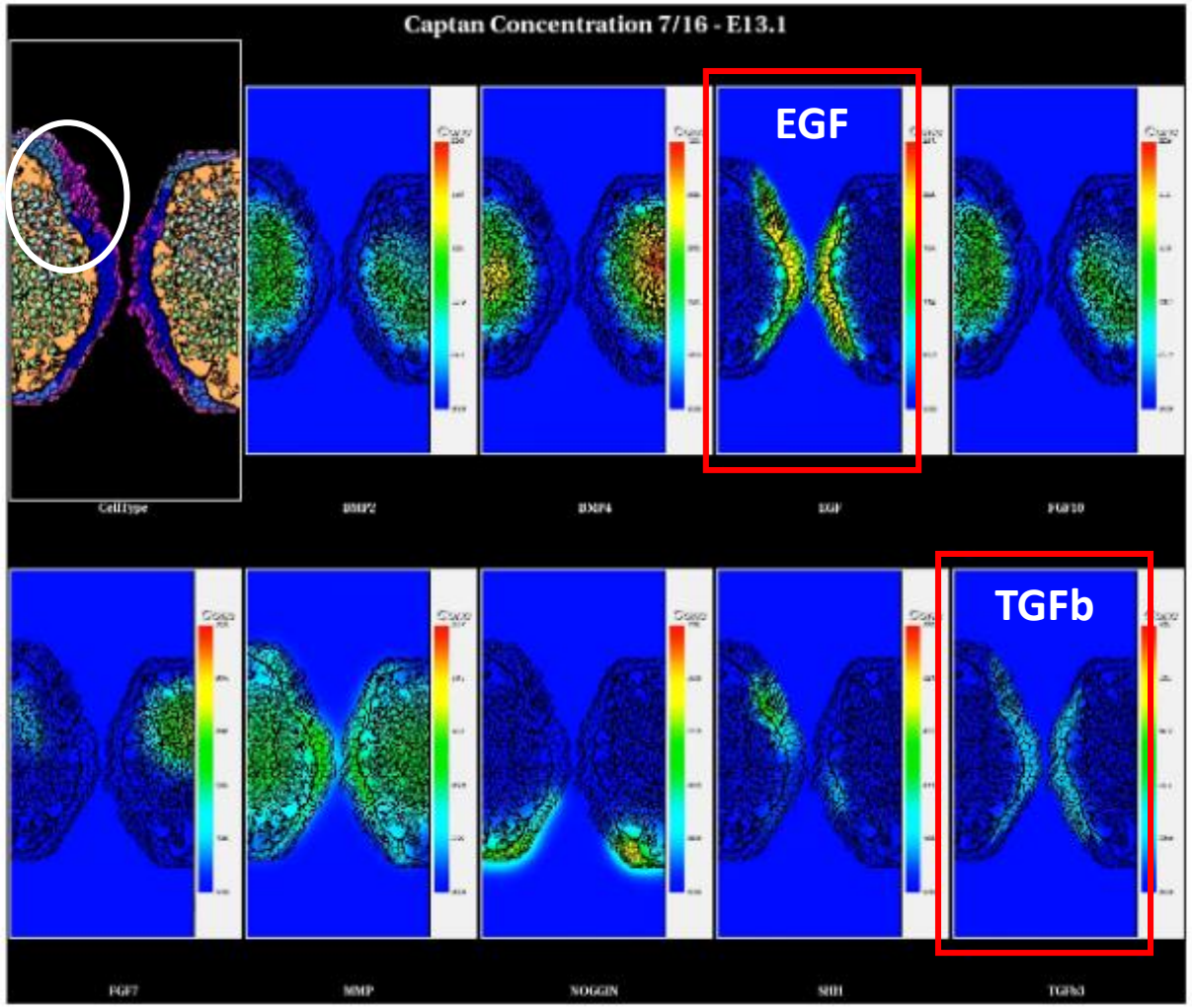
CompTox Chemicals Dashboard exposure prediction
0.88×10^{-7} mg/kg/day

Pathogenesis: *simulating the prefusion alterations*

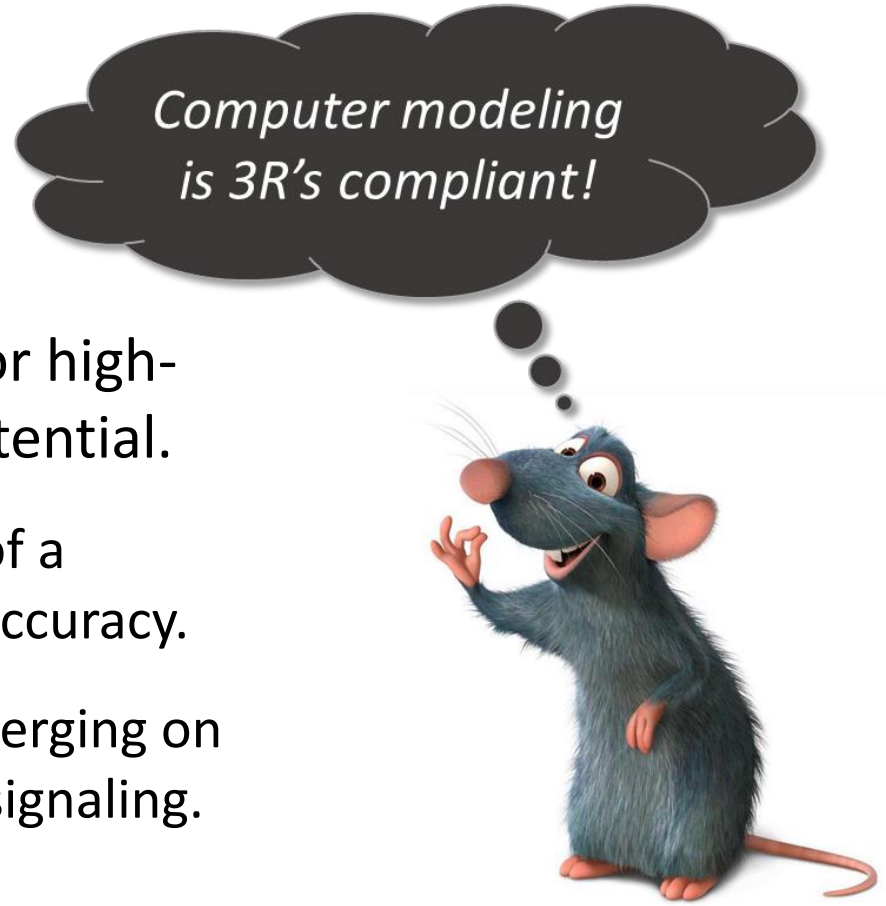
pre-critical dose



post-critical dose



Summary and Conclusions



1. Several new approach methods (NAMs) are available for high-throughput screening chemical inventories for DevTox potential.

- STM assay in ToxCast gives an exposure-based readout of a chemical's DevTox hazard potential with 84% balanced accuracy.
- Assay sensitivity predicted high for kinase signaling converging on FoxO signaling but weak for estrogenic (ESR1) and G(q) signaling.

2. Cell ABMs recapitulate morphogenesis cell-by-cell and interaction-by-interaction as an embryonic system advances in time.

- Computer models simulate key events in AOPs to render mechanistic predictions and critical phenomena for DevTox.

Special Thanks

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