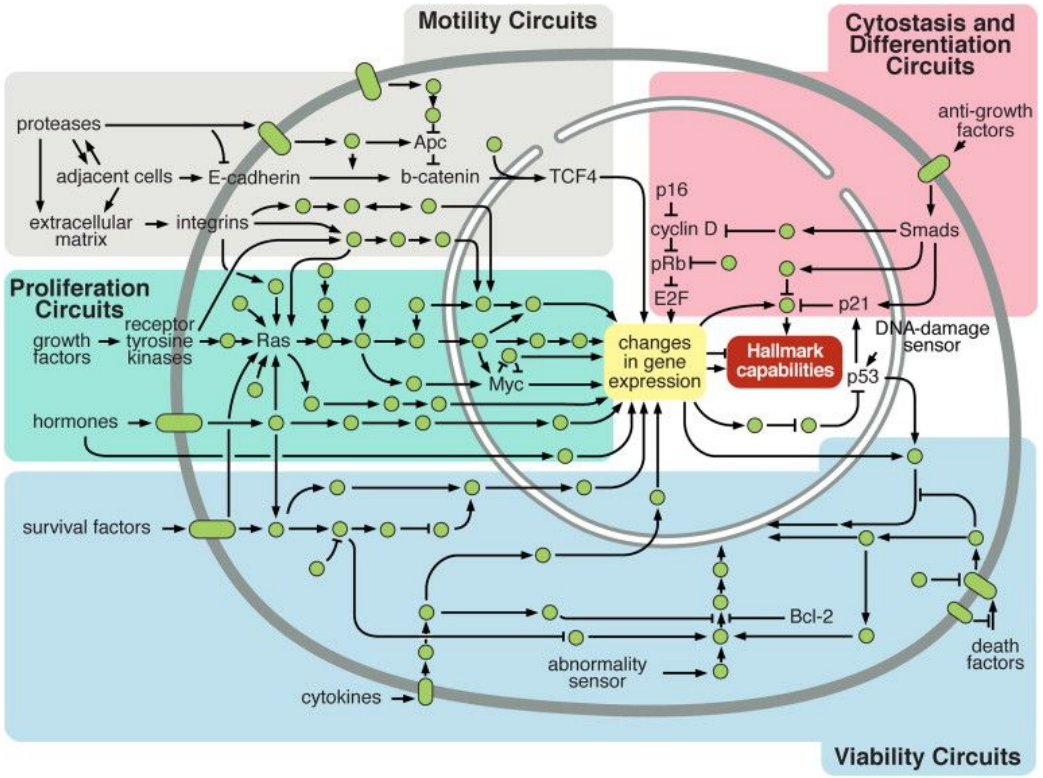
Design principles of complex cellular enabling phenotypic plasticity in cancer

Mohit Kumar Jolly, PhD
Cancer Systems Biology Laboratory
Associate Professor,
Department of Bioengineering,
Indian Institute of Science (IISc), Bangalore, India
mkjolly@iisc.ac.in

Editor-in-Chief, NPJ Systems Biology & Applications

Modularity in Biological Systems Working Group | Feb 2026

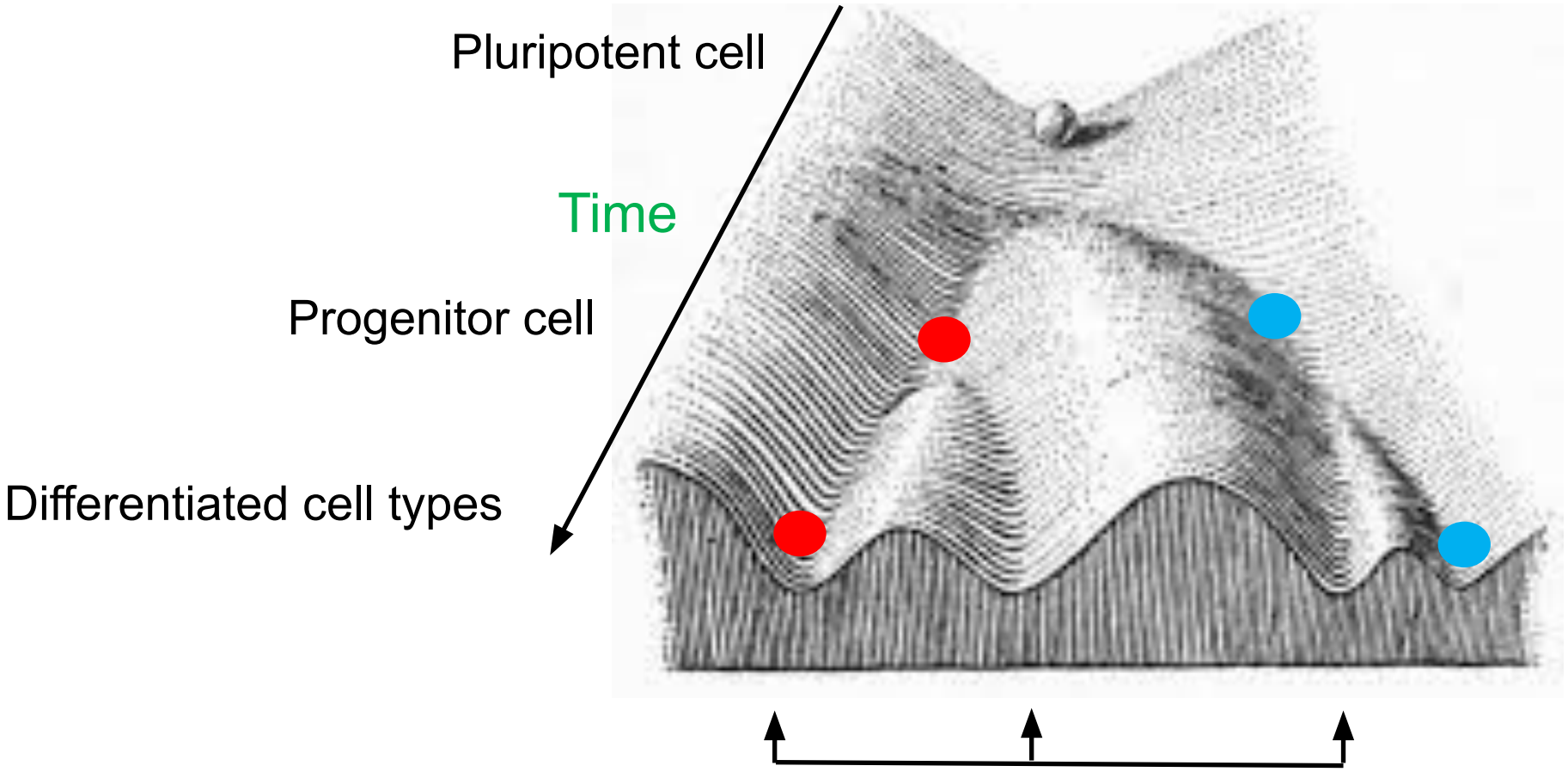
Cellular decision-making



- Cells receive diverse biophysical/chemical signals varying in (x, t).
- Cells in a population can respond differently to the same signals.
- Cellular decision-making is driven by interconnected complex networks.

Hanahan & Weinberg, Cell 2011

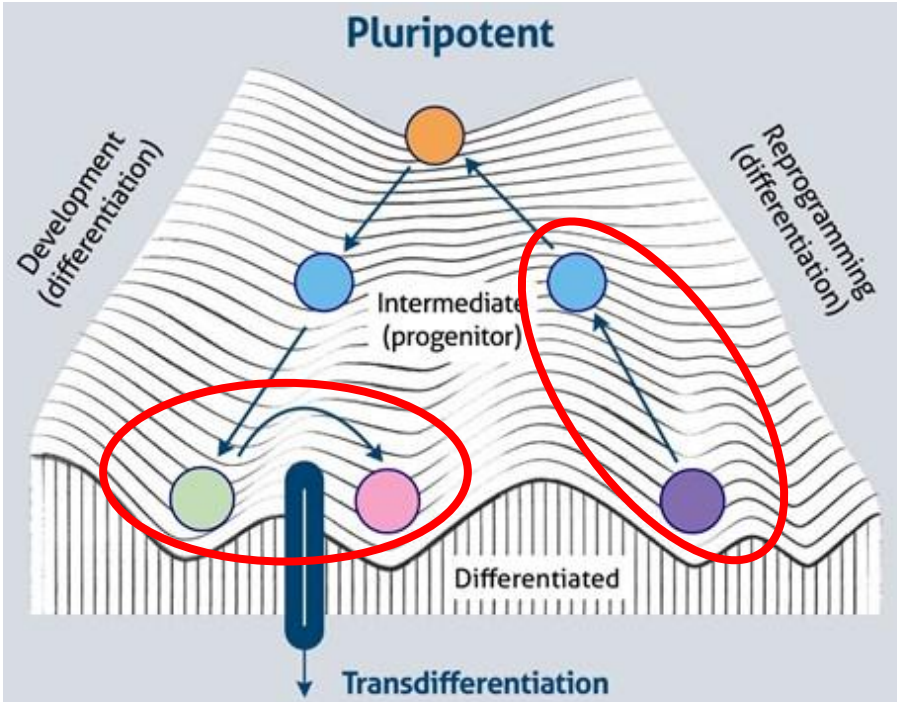
From 1 cell to 200 cell-types: the developmental trajectory



“Final” or “terminal” cell-fates
(a liver cell is not allowed to become a heart cell)

Waddington landscape (1940)

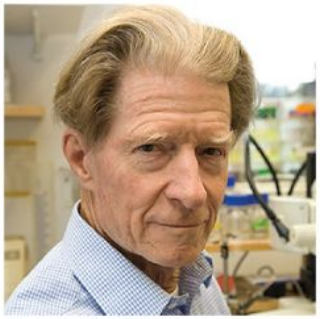
Cell-state changes: bidirectional, reversible



Granados *et al.* Int J Mol Sci 2020

2012 Nobel Prize in Physiology or Medicine

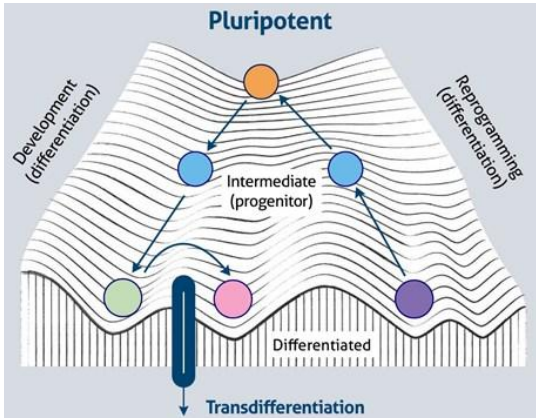
Shinya Yamanaka
University of Kyoto, Japan

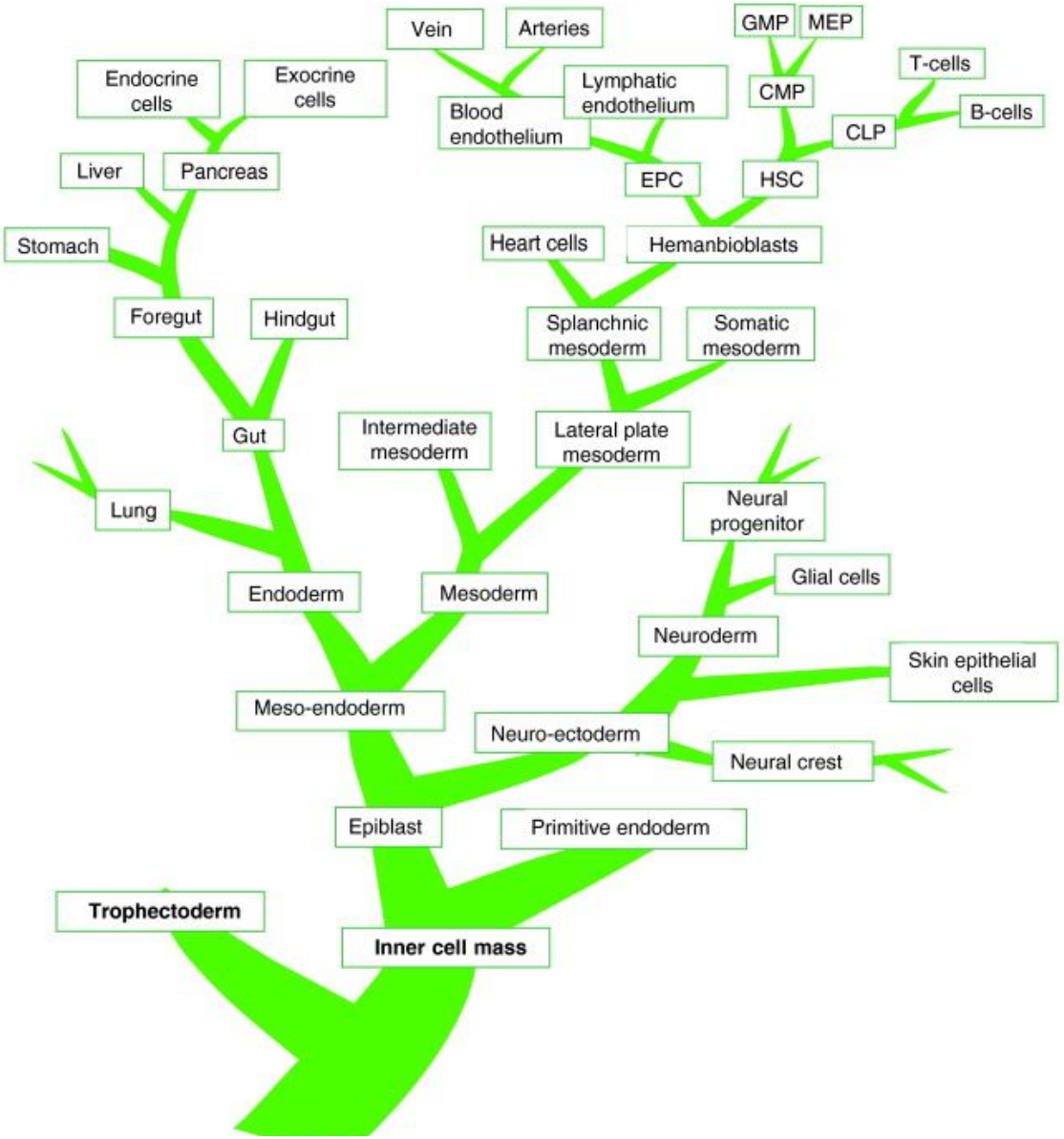


John B. Gurdon
Gurdon Institute in Cambridge, UK

Cells can also reversibly change their identity => "Controlled enthusiasm"

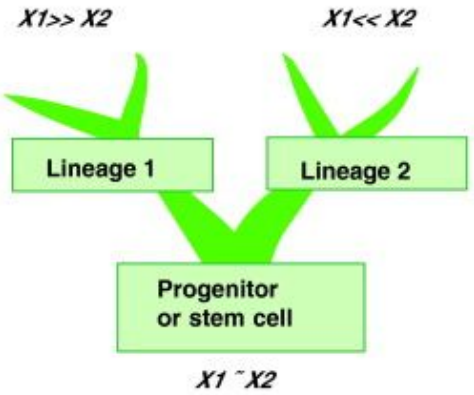
The ‘bifurcating’ cell-state tree

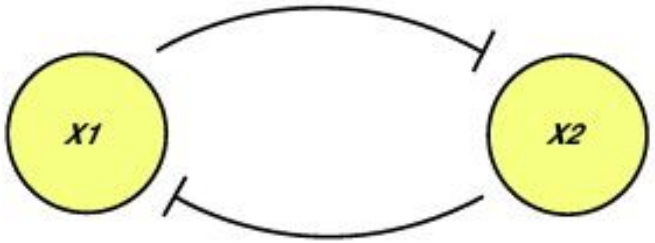




Zhou & Huang, Trends Genet 2011

Toggle switch: a motif for bifurcating cell-states





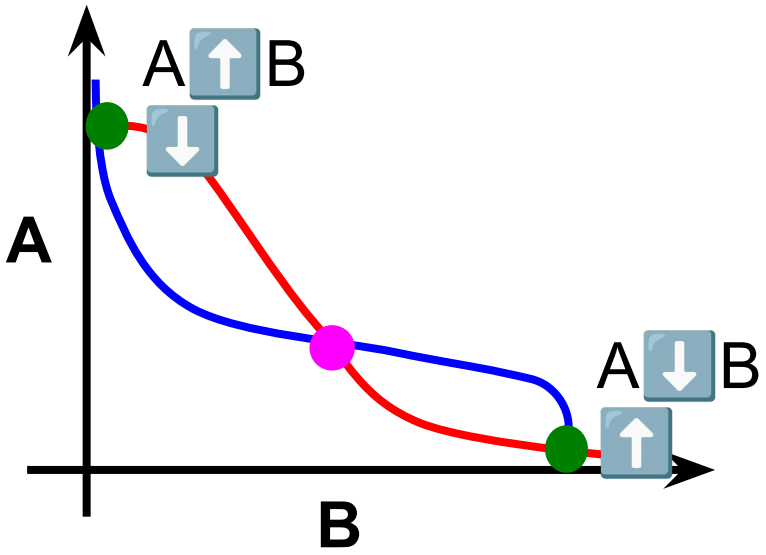
Cells	Transcription factors		Cell fates		
	X1	X2	X1> X2	X1~ X2	X1< X2
Early embryo	Cdx2	Oct4	Trophectoderm	Totipotent embryo	Inner cell mass
Embryo ICM	GATA6	Nanog	Primitive endoderm	Inner cell mass	Epiblast
Blood	GATA1	PU.1	Erythroid cells	Common myeloid progenitor	Myeloid cells
Pancreas	Ptf1a	Nkx6	Exocrine cells	Pancreatic progenitor	Endocrine cells
Somite	Pax3	Foxc2	Myogenic cells	Dermomyotome progenitor	Vascular cells

Zhou & Huang, Trends Genet 2011

Bi-stability in toggle switch

Bistability
(A, B) = (high, low)
(A, B) = (low, high)

Huang, PloS Biology 2013
Gardner *et al.* Nature 2000



$$\frac{dA}{dt} = g_A \frac{(B_0)^{n_B}}{(B_0)^{n_B} + B^{n_B}} - k_A A$$

$$\frac{dB}{dt} = g_B \frac{(A_0)^{n_A}}{(A_0)^{n_A} + A^{n_A}} - k_B B$$

Production Regulation Degradation

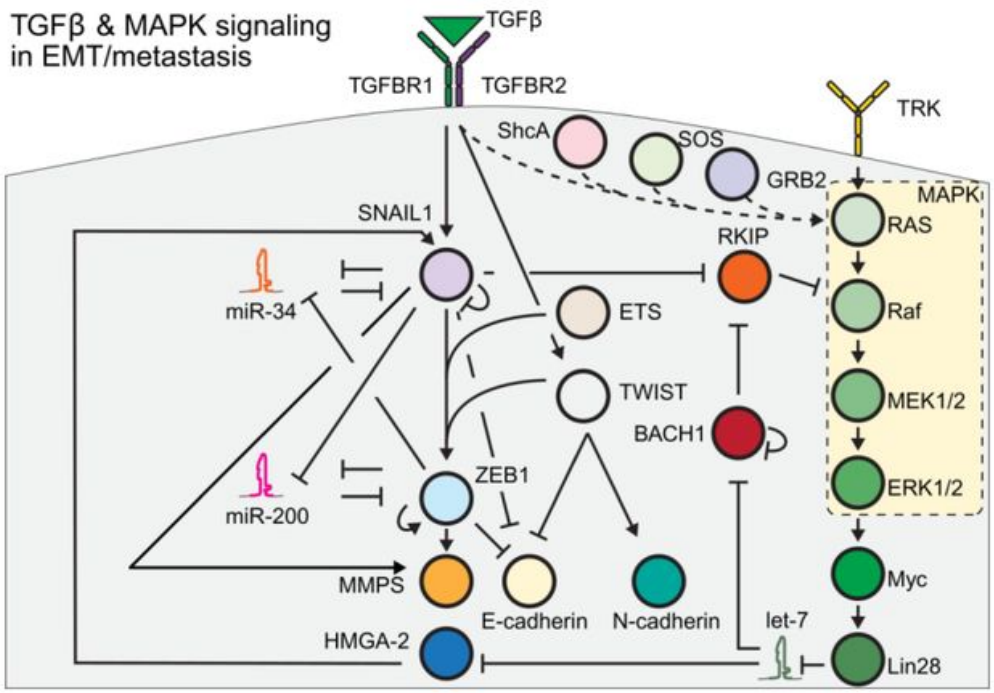
Construction of a genetic toggle switch in *Escherichia coli*

Timothy S. Gardner*†, Charles R. Cantor* & James J. Collins*†

** Department of Biomedical Engineering, † Center for BioDynamics and ‡ Center for Advanced Biotechnology, Boston University, 44 Cummington Street, Boston, Massachusetts 02215, USA*

Gardner et al. Nature 2000

Dynamics of decision-making in larger networks



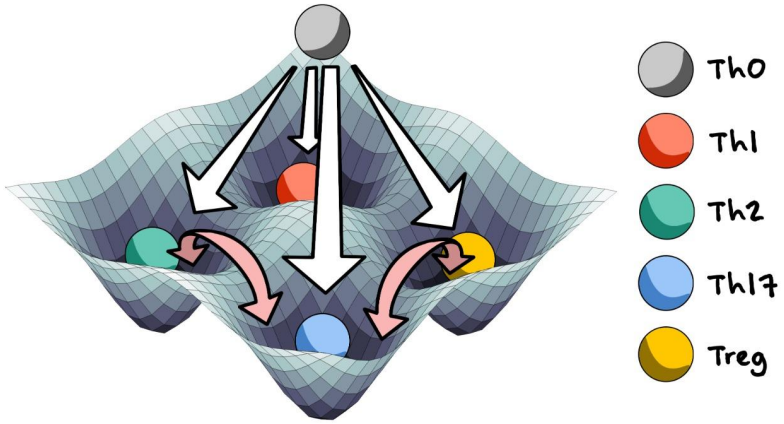
Zanudo *et al.* Phys Biol 2019

How do regulatory networks govern cellular decision-making?
(Dynamical principles)

Why are regulatory networks designed the way they are?
(Topological hallmarks)

What rules/principles cells follow in decision-making?

CD4+ T-cell differentiation



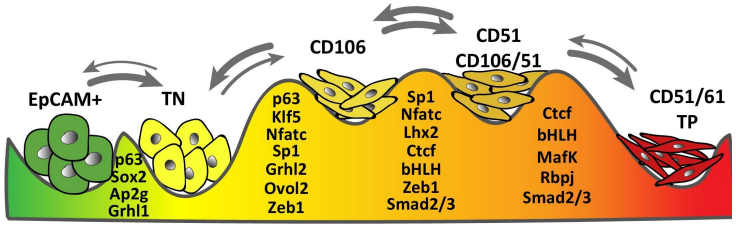
Atchuta Duddu

Sarthak Sahoo

Pradyumna Harlapur

Kishore Hari

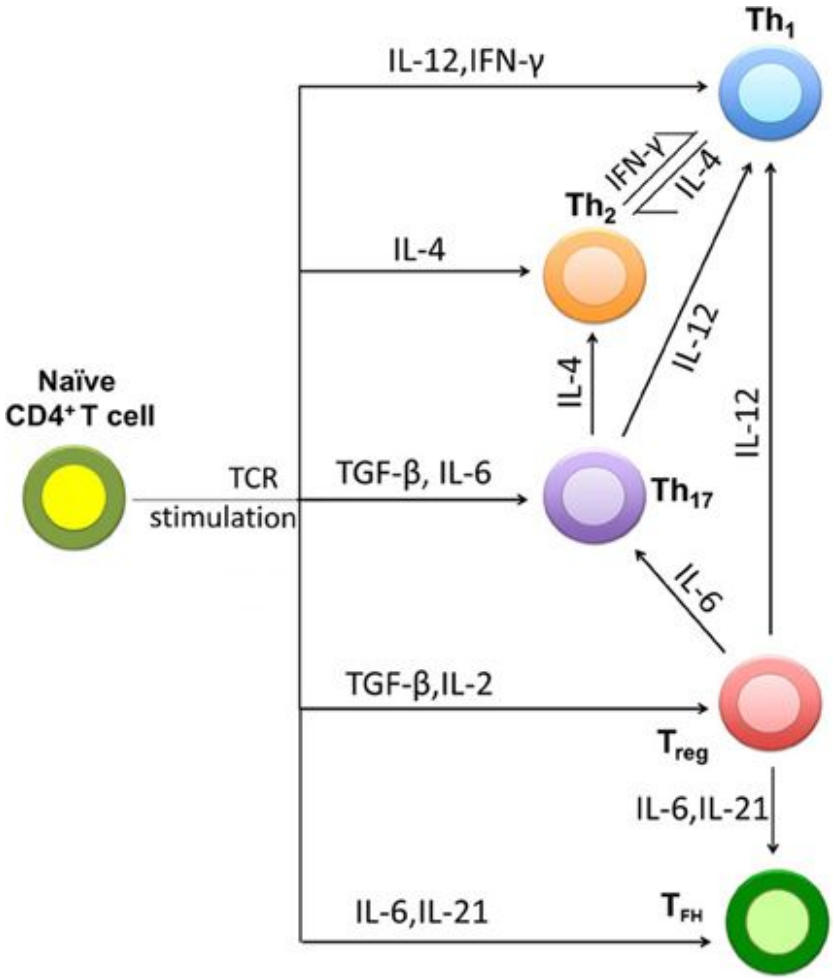
Epithelial-Mesenchymal Transition

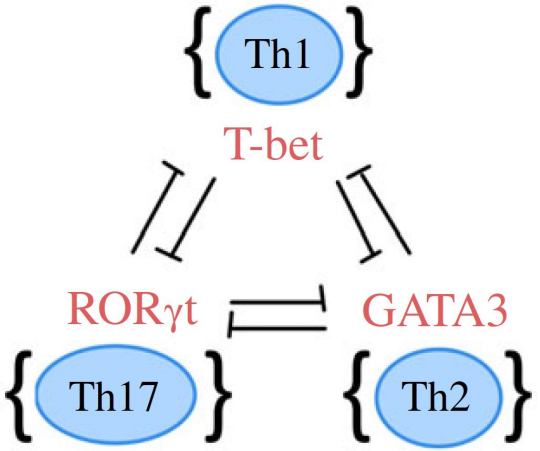


	Epithelial tumor cells	Early hybrid EMT state	Hybrid EMT state	Late hybrid EMT state	Mesenchymal tumor cells
Proliferation	+++++	++++	+++	++	+
Invasion	+	++	+++	++++	+++++
Plasticity	+	++	+++	++++	++
Stemness	+	+++	+++	+++	+++
Metastasis	+	++++	++++	++	+

Hari*, Harlapur* et al. iScience 2025
Duddu *et al.* NPJ Sys Biol Appl 2024
Hari *et al*. eLife 2022
Harlapur *et al.* Biomolecules 2022
Sahoo *et al.* NAR Cancer 2021
Duddu *et al.* J R Soc Interface 2020

Differentiation of naïve CD4+ T cells into > 2 subsets





Th1 | { **T-bet high**, GATA3 low, RORγT low }

Th2 | { T-bet low, **GATA3 high**, RORγT low }

Th17 | { T-bet low, GATA3 low, **RORγT high** }

Can this 'toggle triad' explain T-cell differentiation?

Magombedze *et al.* Front Physiol 2013; Duddu *et al.* J R Soc Interface 2020

ODEs governing the dynamics

$$\frac{d[X]}{dt} = g_X \left(\frac{1 + \lambda_{YX}[Y/T_{YX}]^{n_{YX}}}{1 + [Y/T_{YX}]^{n_{YX}}} \right) - k_X[X]$$

$$\frac{d[Y]}{dt} = g_Y \left(\frac{1 + \lambda_{XY}[X/T_{XY}]^{n_{XY}}}{1 + [X/T_{XY}]^{n_{XY}}} \right) - k_Y[Y]$$

Here,

g_X and g_Y are the production rates of A and B

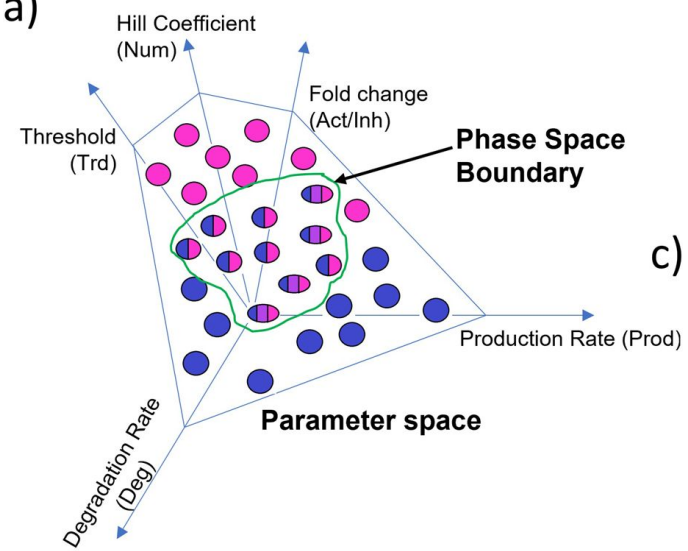
k_X and k_Y are the degradation rates of A and B

λ_{XY} and λ_{YX} is the fold change in interaction

n_{XY} and n_{YX} is the hill's coefficient in interaction

T_{XY} and T_{YX} is threshold value of the interaction

a)



c)

Huang *et al.* PloS Comp Biol 2017

Network topology (say, a toggle triad)

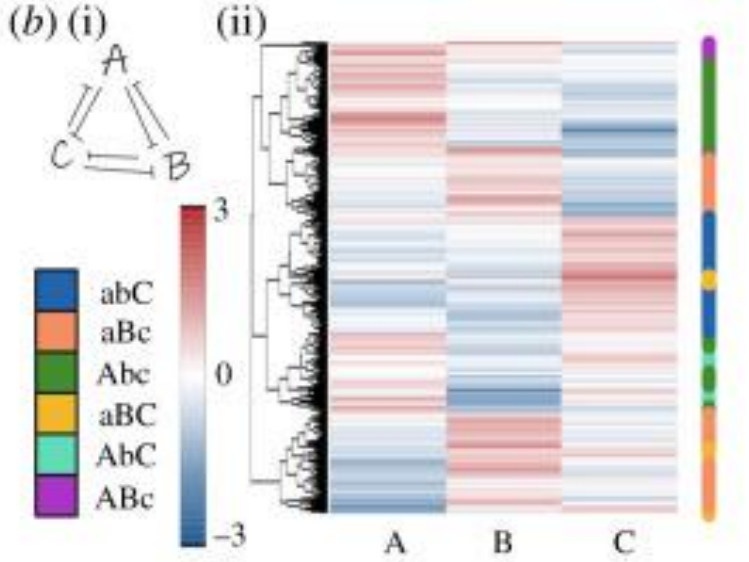
+

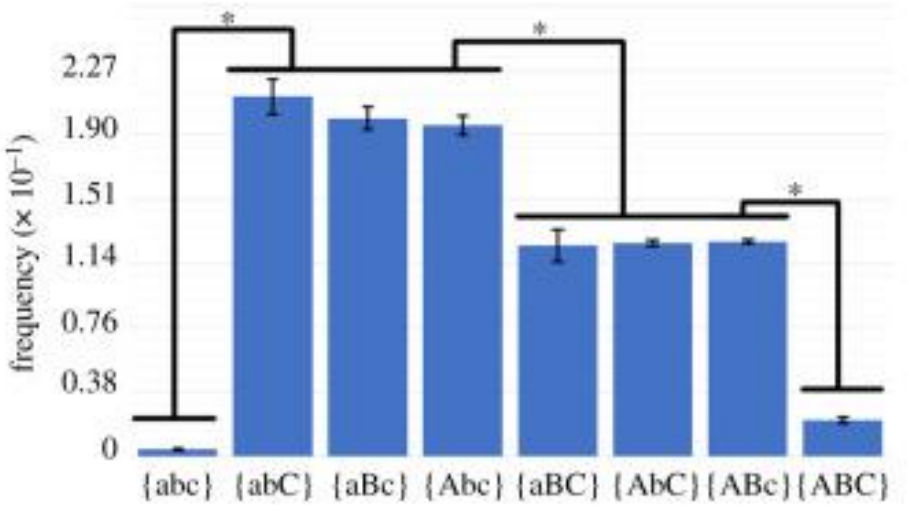
Ensemble of kinetic models over a biologically relevant parameter space

=

"Possibility space" of phenotypes

Multistability in toggle triad





Differentiated states:

Abc | { **A high**, B low, C low }

aBc | { A low, **B high**, C low }

ABc | { A low, B low, **C high** }

Hybrid states:

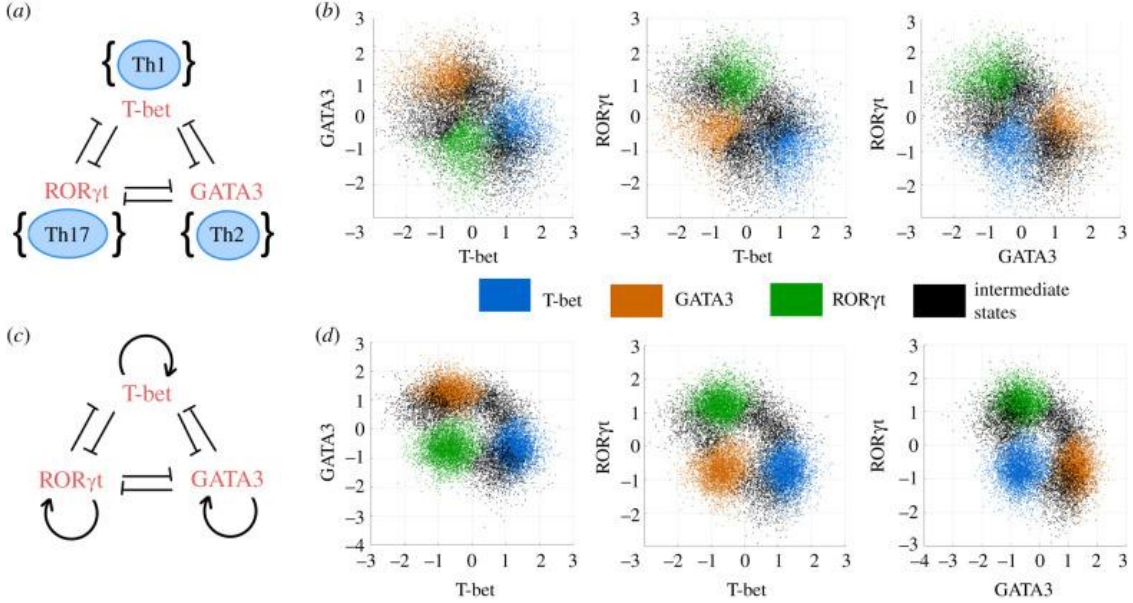
ABc | { **A high**, **B high**, C low }

AbC | { **A high**, B low, **C high** }

aBC | { A low**, B high, C high** }

These dynamical traits were unique to this 3-node network topology.

Duddu *et al.* J R Soc Interface 2020



Model predictions

Existence of three 'single-positive' states

Existence of three 'double-positive

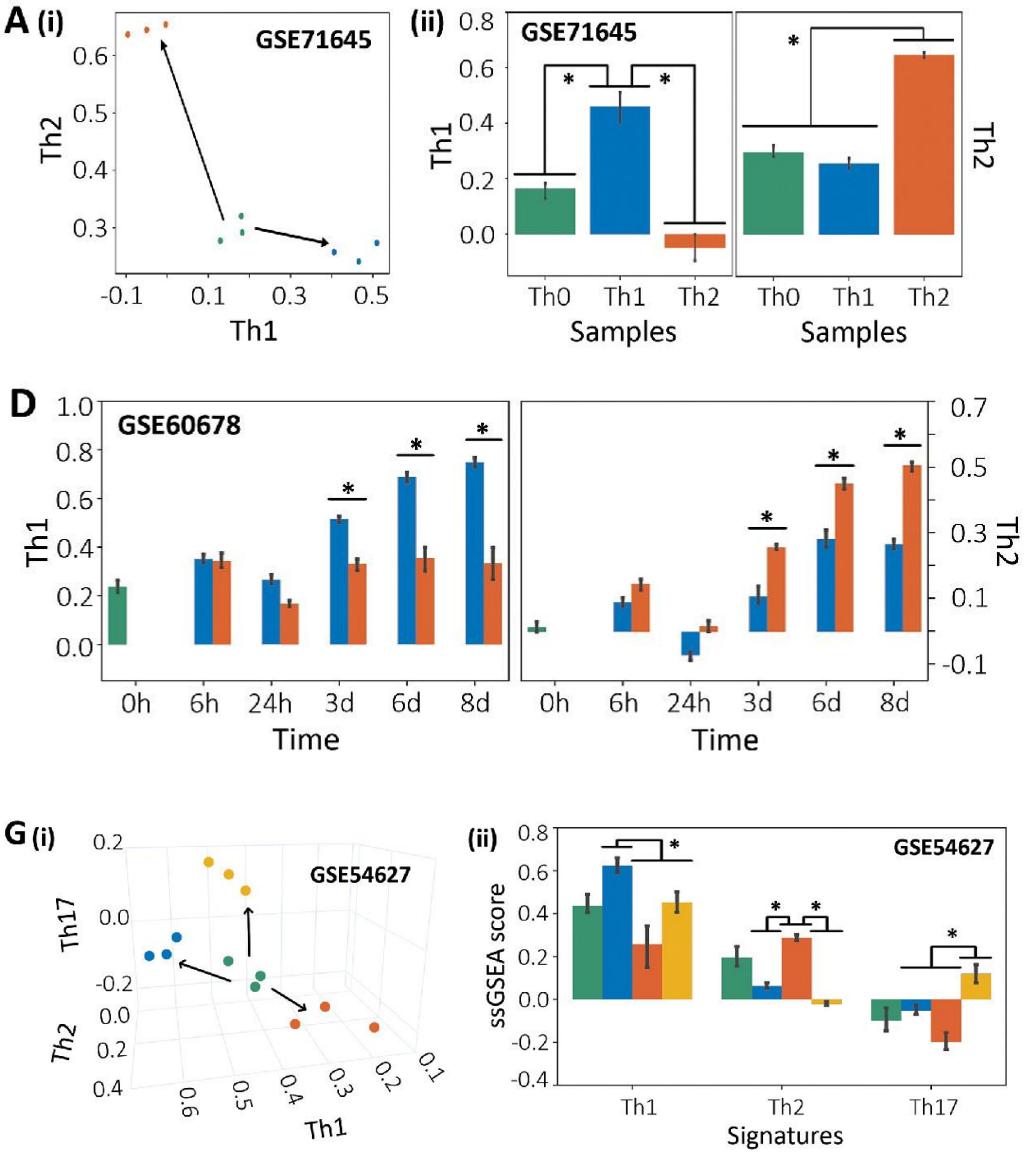
Experimental validations

The three states have been well established

Antebi *et al.*, PLoS Biol. 2013

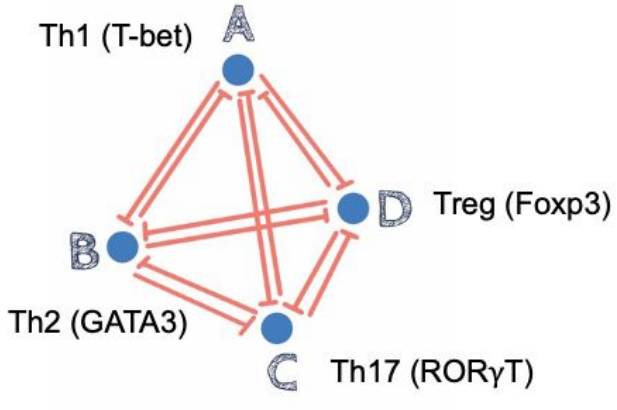
Chatterjee *et al.,* Cell Metab.

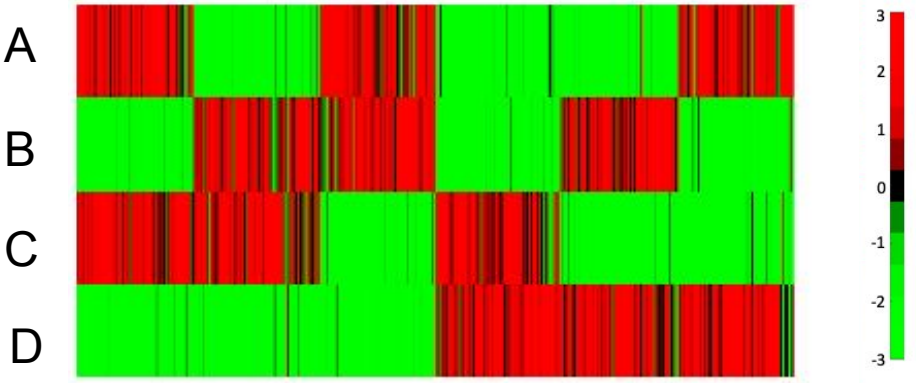
RNA-seq data validates our model predictions

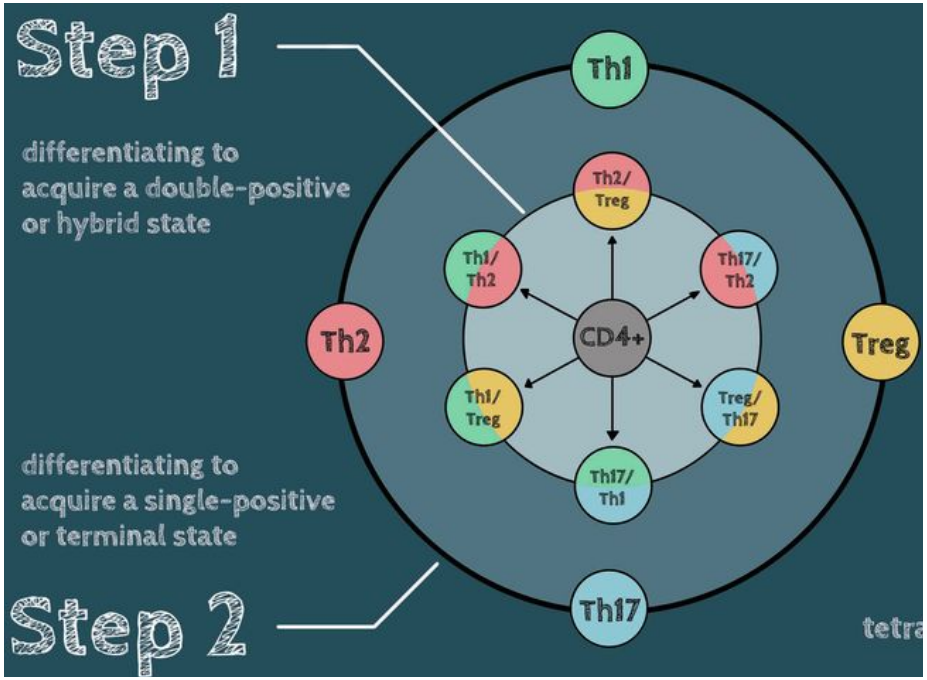


Duddu *et al.* Mol Biol Cell 2022

Toggle tetrahedron => Predominant ‘hybrid’ states



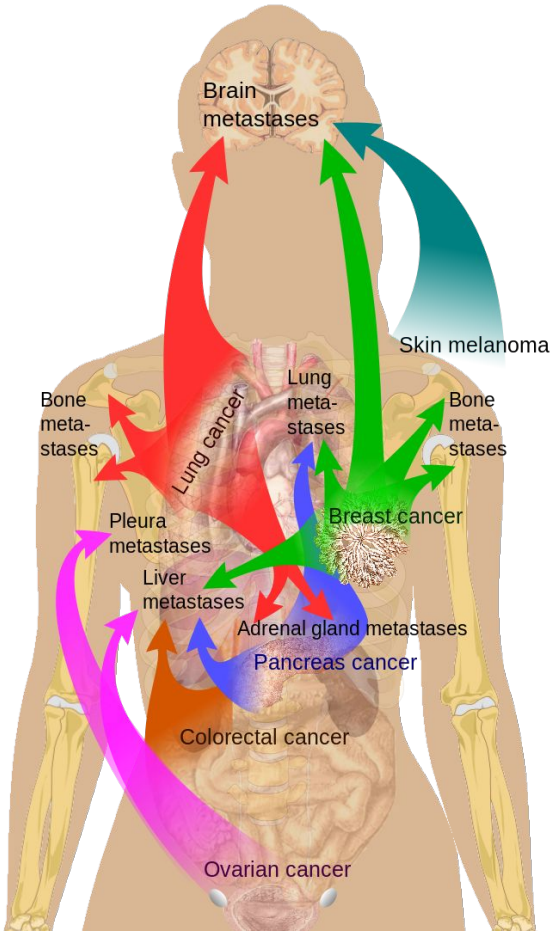


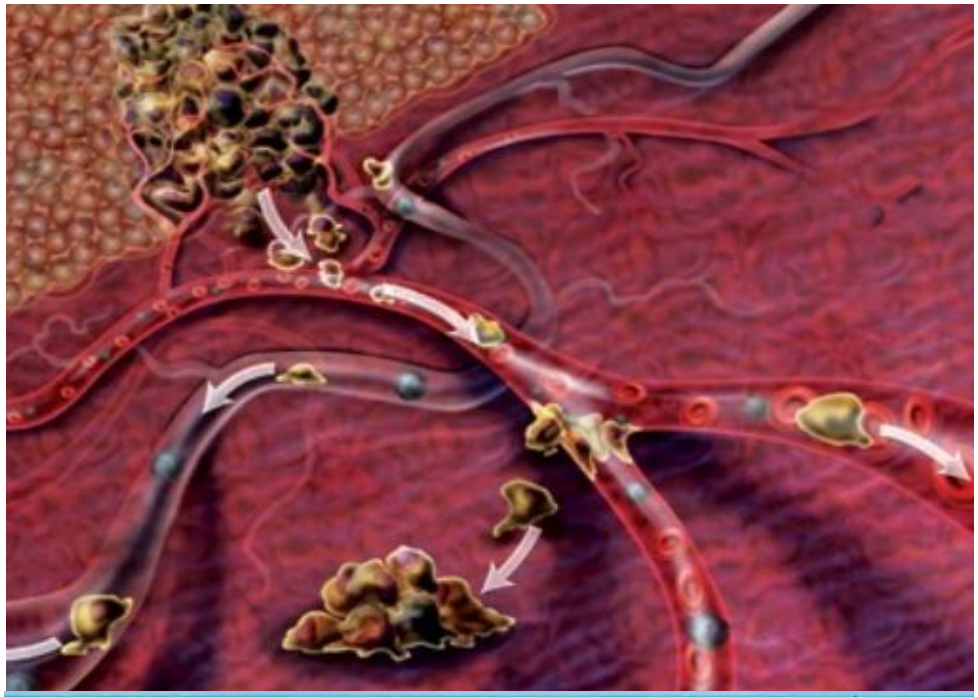


- None of the 4-node network topologies allows for ‘single-positive’ (1,0,0,0) states as predominant.
- ‘Single-positive’ states not the predominant ones for toggle-n (n > 3) states.

Duddu *et al.* NPJ Syst Biol Appl 2024
Harshavardhan et al. J R Soc Interface 2025

Metastasis : the cause of 90% of all cancer deaths





No unique mutational signatures yet identified for metastasis.

Metastasis is a highly inefficient process (<0.02%)

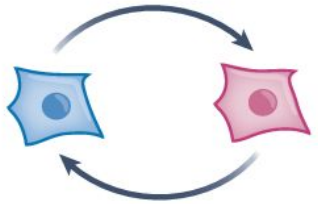
Challenges in developing anti-metastatic drugs

The dynamics of metastasis is poorly understood.

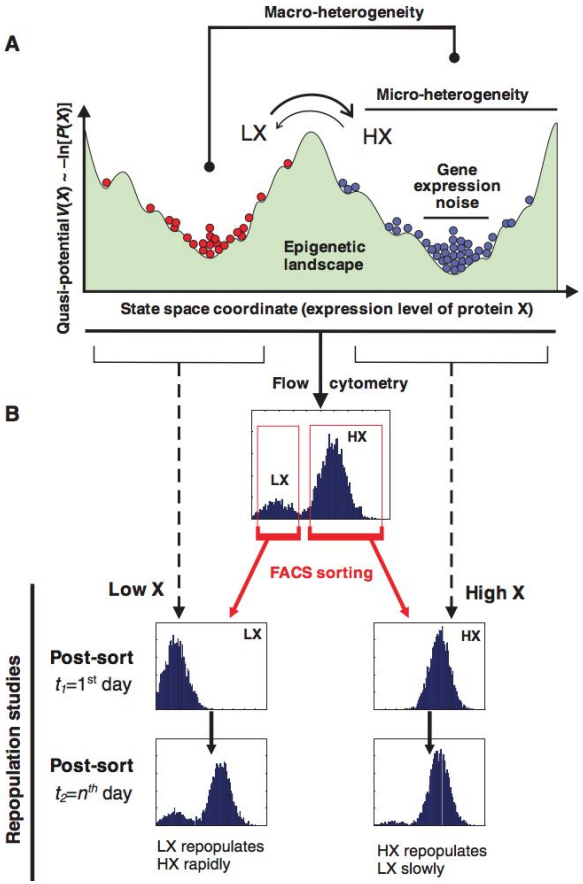
- When and why do cells leave the primary tumor?
- Do they leave as individual cells or as groups? Does it matter?
- Which organs do they stop by, and which organs do they settle in?
- How much time it takes for them to grow a clinically notable metastasis?

(Harry Potter and the Philosopher's Stone; Harry Potter and the Deathly Hallows)

Phenotypic plasticity : hallmark of cancer metastasis



Phenotypic plasticity:
Ability of cells to switch their 'state'
reversibly in response to environmental conditions

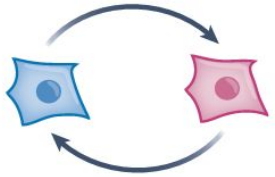


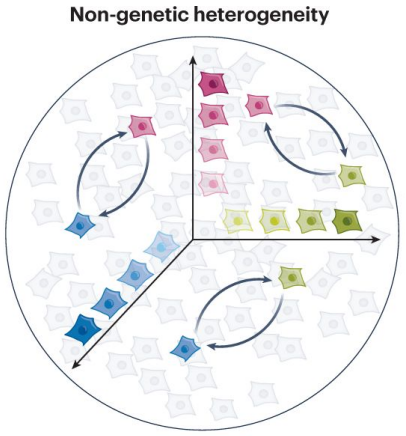
	Genetic mutations	Phenotypic plasticity
Timescale	Slow (months)	Fast (hours)
Reversibility	Irreversible	Reversible (can be transiently heritable too)

No unique mutational signatures
have been yet identified for metastasis.

Huang *et al.* Development 2009; Pillai *et al.* Nat Comp Sci 2023

Phenotypic plasticity in metastasis and drug resistance





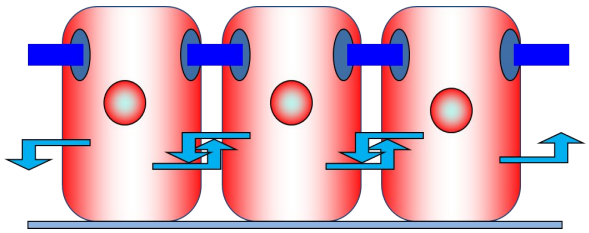
Mathematical models of phenotypic plasticity:

- Intracellular mechanistic biochemical network models
- Population-level models for inferring cell-state transitions
- Agent-based models for the impact of TME on plasticity
- Statistical approaches (infer cell-state trajectories etc.)

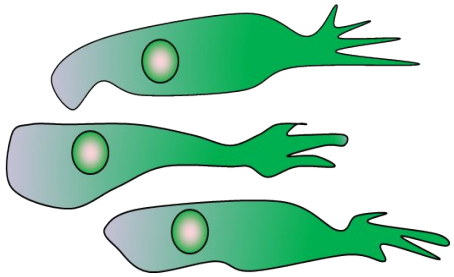
Our focus:

- Develop mechanistic models to understand cancer cell-state transitions
- Analyse single-cell omics data to validate mechanistic dynamical models
- Predict therapeutic strategies based on better dynamical understanding

EMT/MET: The engine of metastasis

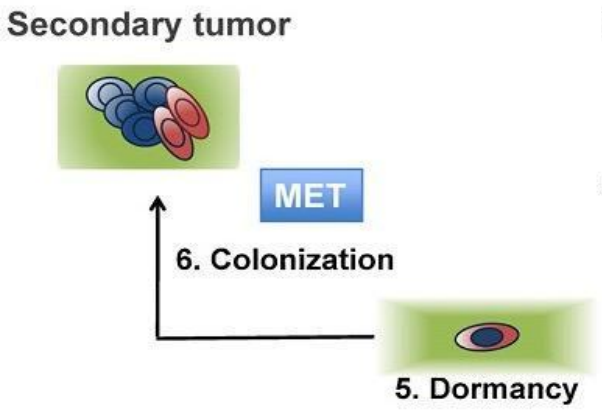


Adhere to neighbors
Do NOT migrate or invade
Epithelial (E)

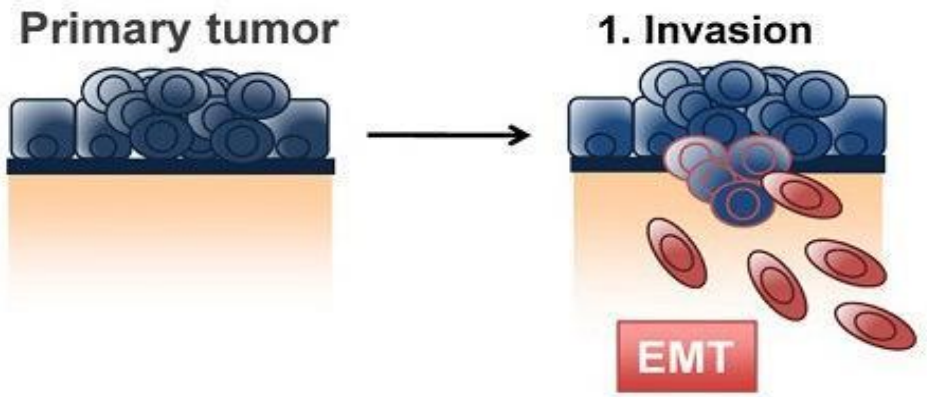


Do NOT adhere to neighbors
Migrate and invade
Mesenchymal (M)

Mesenchymal-to-Epithelial Transition (MET)

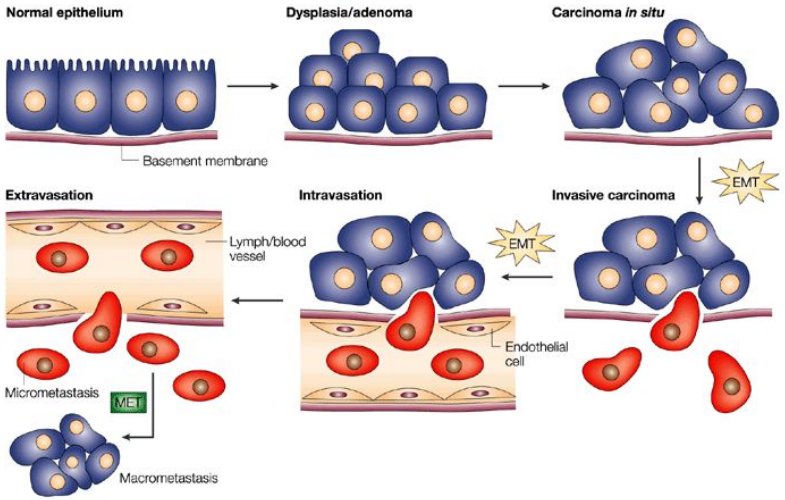


Epithelial-to-Mesenchymal Transition (EMT)

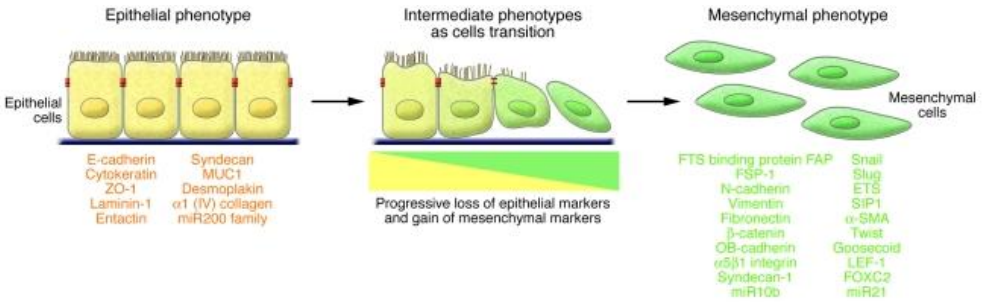


Scheel & Weinberg, Semin Cancer Bio 2012

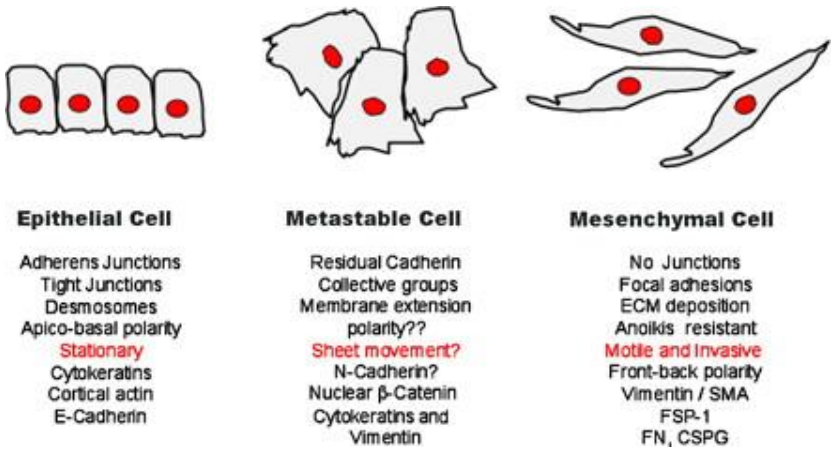
EMT 'model' in cancer (2002 – 2012)



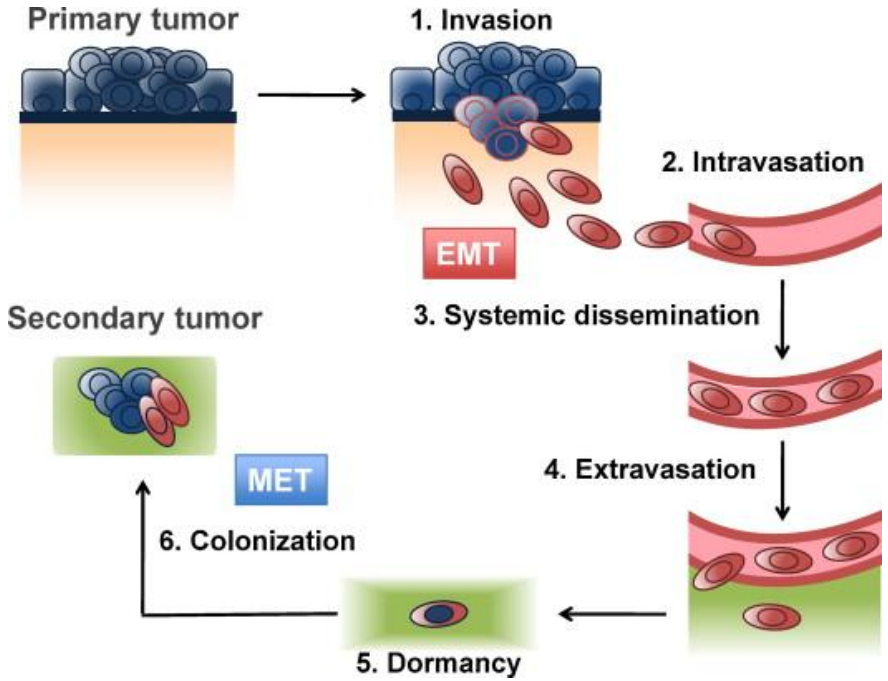
Thiery JP, Nat Rev Cancer 2002



Kalluri & Weinberg, J Clin Invest 2009

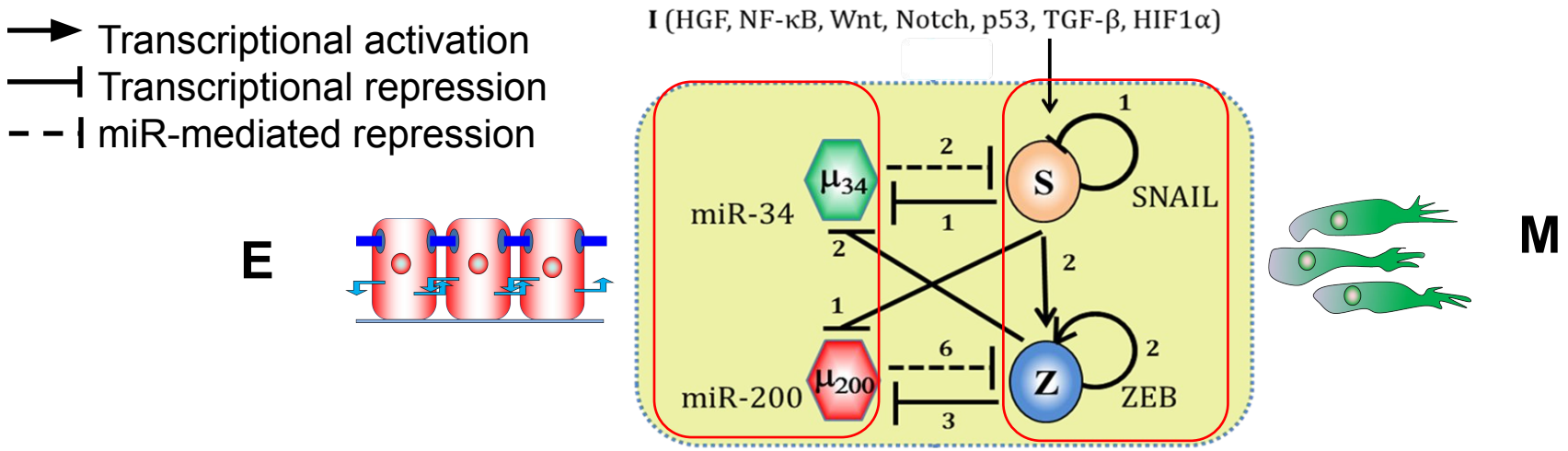


Lee *et al.* J Cell Biol 2006



Scheel & Weinberg, Semin Cancer Biol 2012

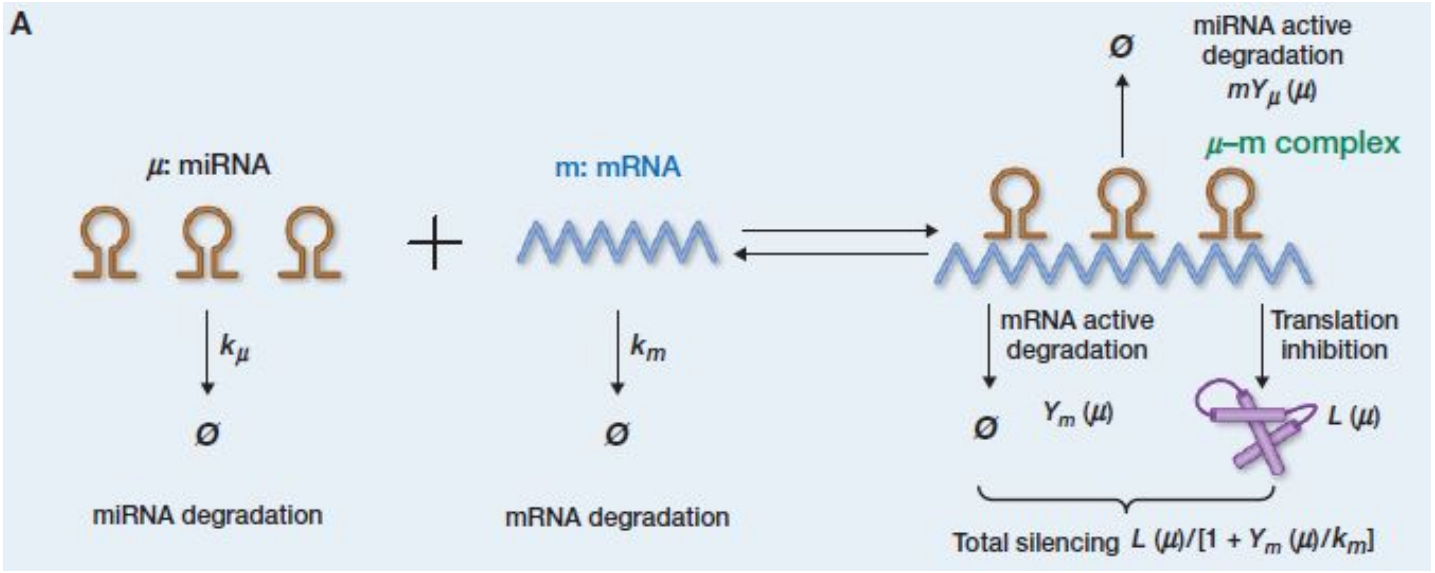
Network that controls EMT/MET



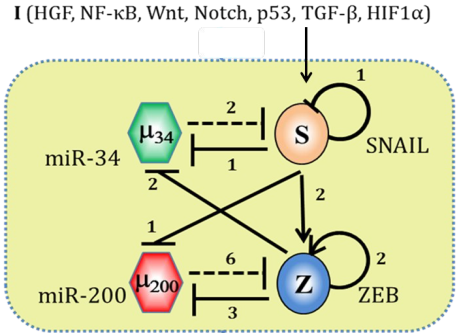
- Each arrow/bar indicates a quantitative input-output relationship.
- Such models have been extensively built for simpler microorganisms.
- Can we decode the emergent properties of these nonlinear interactions?

Lu*, Jolly* *et al.* PNAS 2013

Mathematical model formulation



Lu*, Jolly* *et al.* PNAS 2013



Production

Degradation

miR regulation

TF regulation

$$\frac{d\mu_{200}}{dt} = g_{\mu_{200}} H^S(Z,\lambda_{Z,\mu_{200}}) H^S(S,\lambda_{Z,\mu_{200}}) - m_Z Y_\mu(\mu_{200}) - k_{\mu_{200}}\mu_{200}$$ miR-200

$$\frac{dm_Z}{dt} = g_{m_Z} H^S(Z,\lambda_{Z,m_Z}) H^S(S,\lambda_{S,m_Z}) - m_Z Y_m(\mu_{200}) - k_{m_Z} m_Z$$ ZEB mRNA

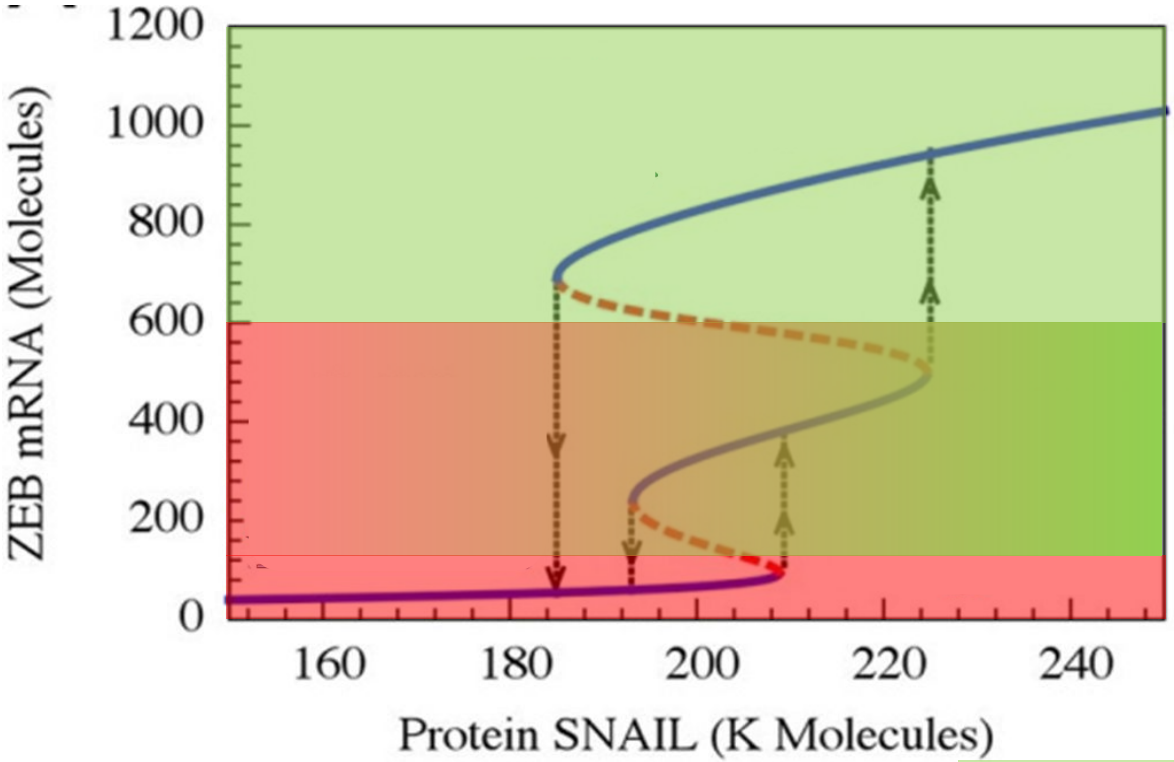
$$\frac{dZ}{dt} = g_Z m_Z L(\mu_{200}) - k_Z Z$$ ZEB

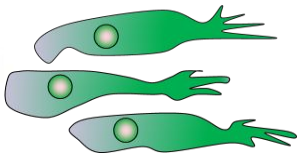
$$\frac{d\mu_{34}}{dt} = g_{\mu_{34}} H^S(Z,\lambda_{Z,\mu_{34}}) H^S(S,\lambda_{Z,\mu_{340}}) - m_S Y_\mu(\mu_{34}) - k_{\mu_{34}}\mu_{34}$$ miR-34

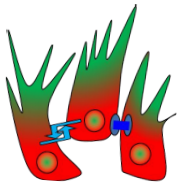
$$\frac{dm_S}{dt} = g_{m_S} H^S(S,\lambda_{S,m_S}) H^S(I,\lambda_{I,m_S}) - m_S Y_m(\mu_{34}) - k_{m_S} m_S$$ SNAIL mRNA

$$\frac{dS}{dt} = g_S m_S L(\mu_{34}) - k_S S$$ SNAIL

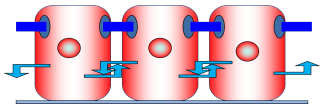
Model prediction: EMT is NOT binary

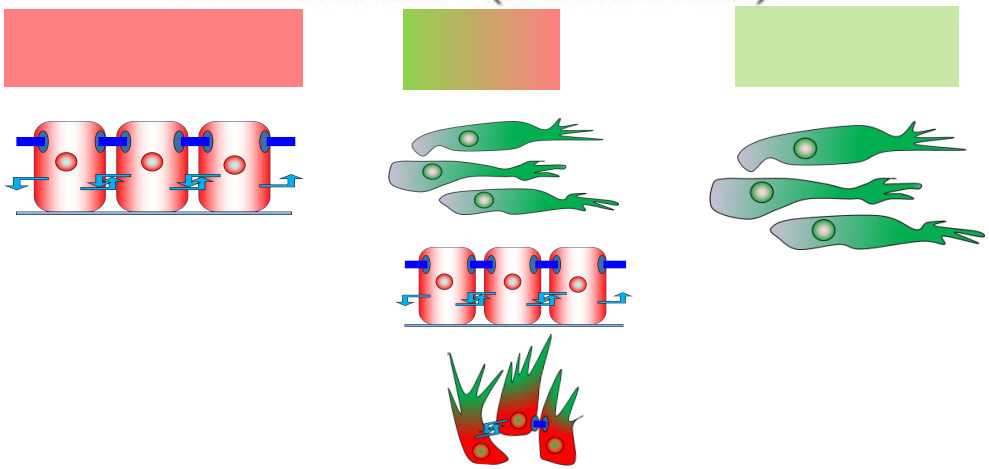


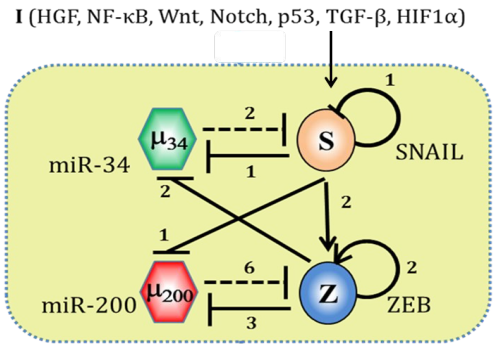




Hybrid E/M
Adhere AND
migrate collectively

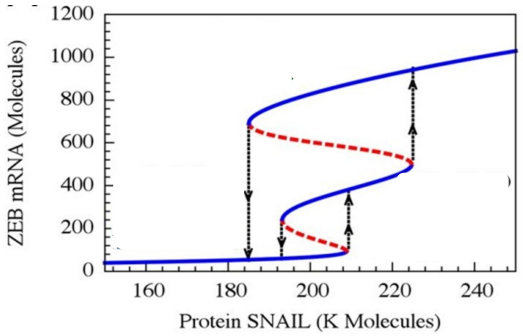






Lu*, Jolly* *et al.* PNAS 2013

Mathematical modeling for EMT dynamics

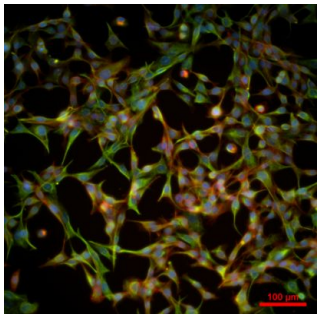


Predictions from mathematical model:

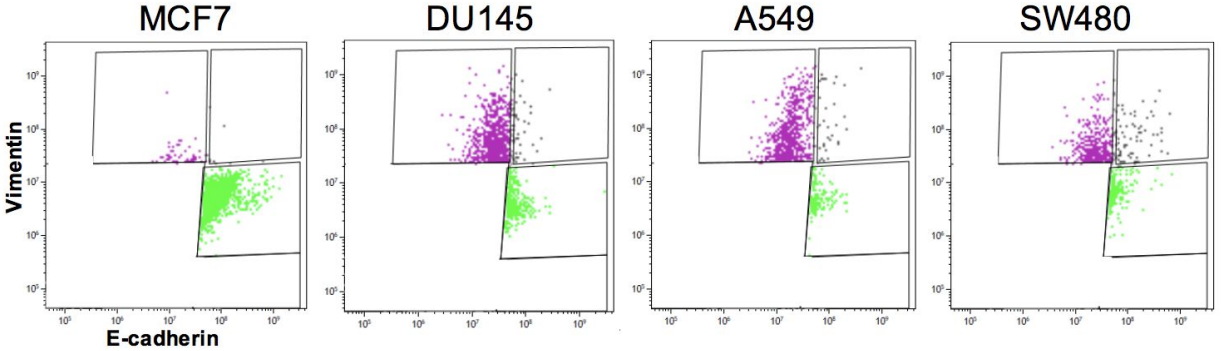
1. Cells can stably exist in hybrid E/M state
2. Isogenic cells can exist in different EMT states
3. Cells can 'spontaneously' switch their states

Lu*, Jolly* *et al.* PNAS 2013

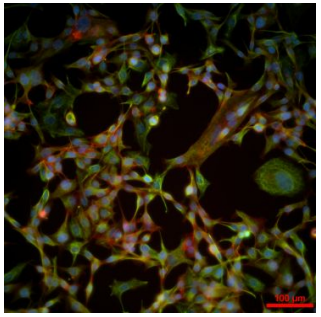
Experimental validation:



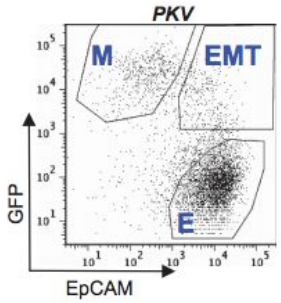
H1975, T=0

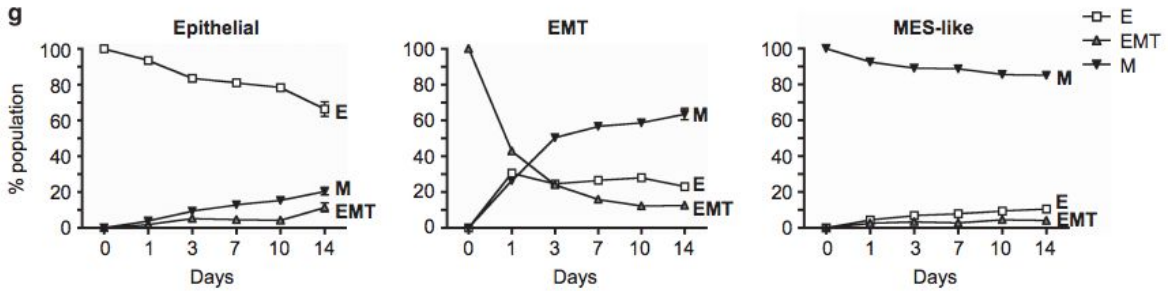


George*, Jolly* *et al.* Cancer Res 2017



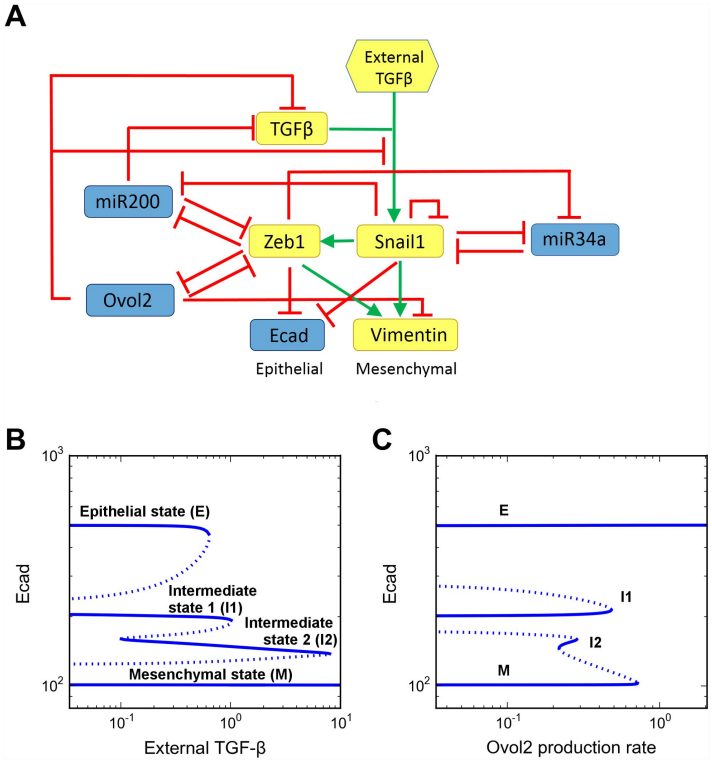
H1975, T=2 months

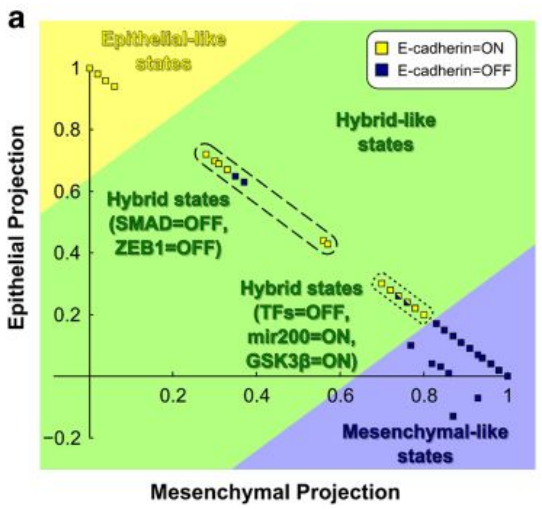


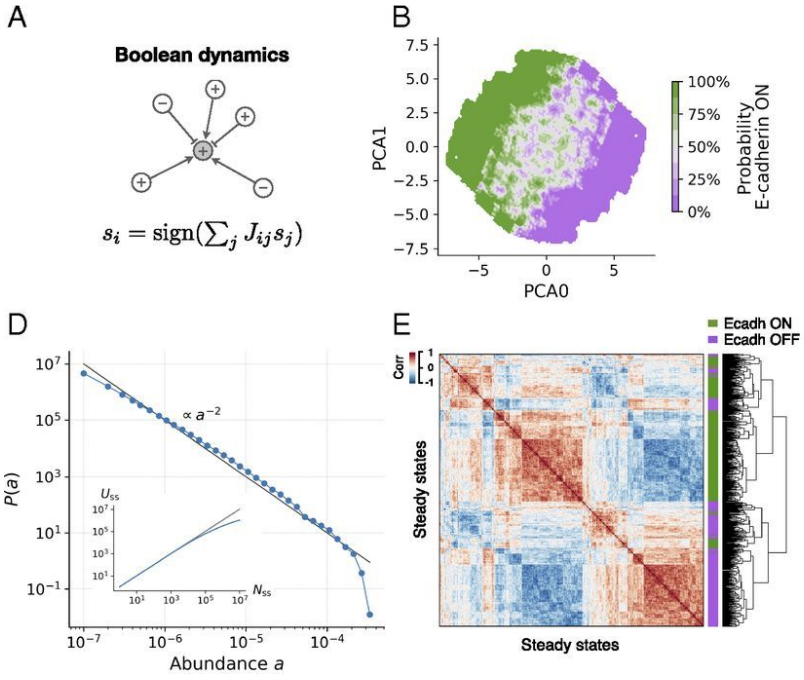


Jolly *et al.* Oncotarget 2016; Ruscetti *et al.* Oncogene 2016

Hybrid E/M phenotype(s) seen in other math models too

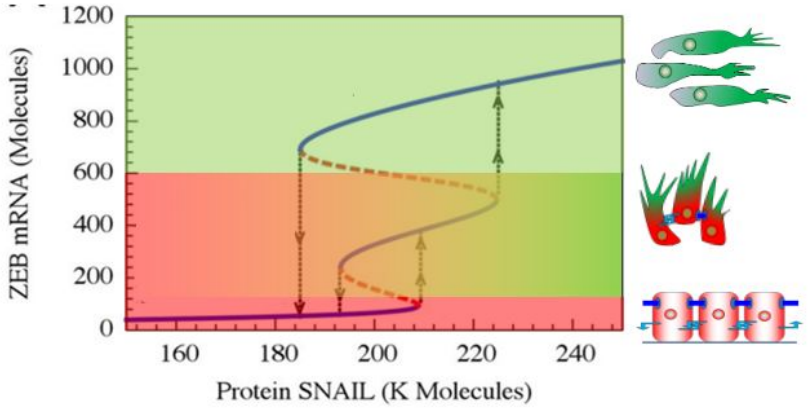




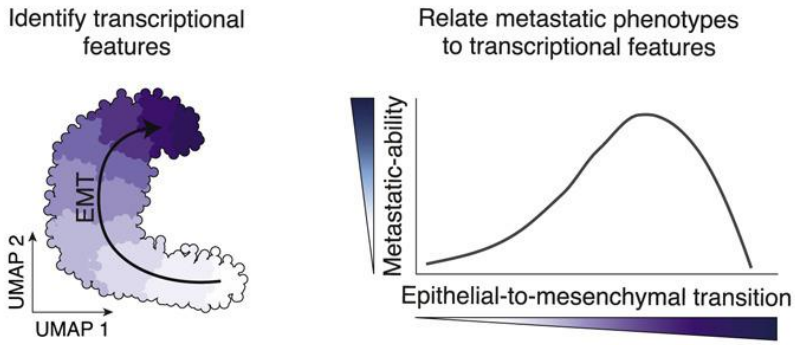


Xing *et al.* Biophys J 2013
Steinway *et al.* Cancer Res 2014, NPJ Sys Biol Appl 2015
Hong *et al.* PLoS Comp Biol 2015
Jolly *et al.* Oncotarget 2016
Huang *et al.* PLoS Comp Biol 2017, BMC Sys Biol 2018
Font-Clos *et al.* PNAS 2018, Cell Systems 2021
Silveira *et al.* FEBS J 2020, J R Soc Interface 2020
Hari *et al.* NPJ Sys Biol Appl 2020, PLoS Comp Biol 2022
Sullivan *et al.* iScience 2024
McDermott *et al.* NPJ Sys Biol Appl 2024

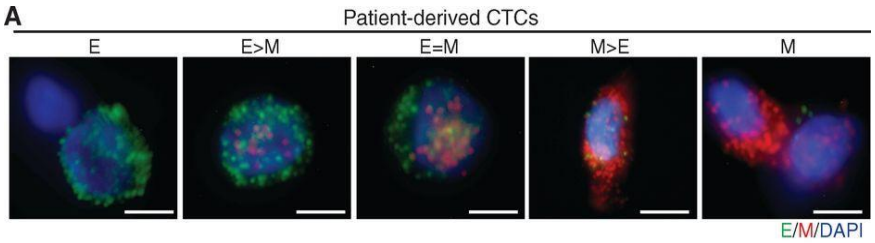
From EMT (2002-2012) to EMP (Epi-Mes Plasticity; 2013-now)



Lu[#], Jolly[#] *et al.* PNAS 2013



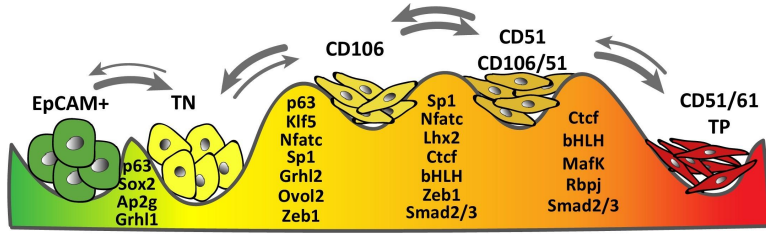
Simeonov *et al.* Cancer Cell 2021



Acquisition of a hybrid E/M state is essential for tumorigenicity of basal breast cancer cells

Cornelia Kröger[a], Alexander Afeyan[a,b], Jasmin Mraz[a,c], Elinor Ng Eaton[a], Ferenc Reinhardt[a], Yevgenia L. Khodor[d], Prathapan Thiru[a], Brian Bierie[a], Xin Ye[a,e], Christopher B. Burge[d], and Robert A. Weinberg[a,f,g,1]

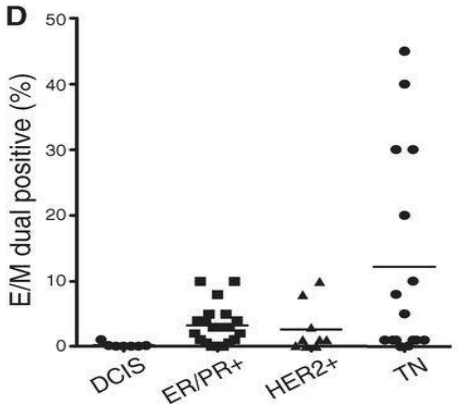
Yu *et al.* Science 2013
Kroger *et al.* PNAS 2019



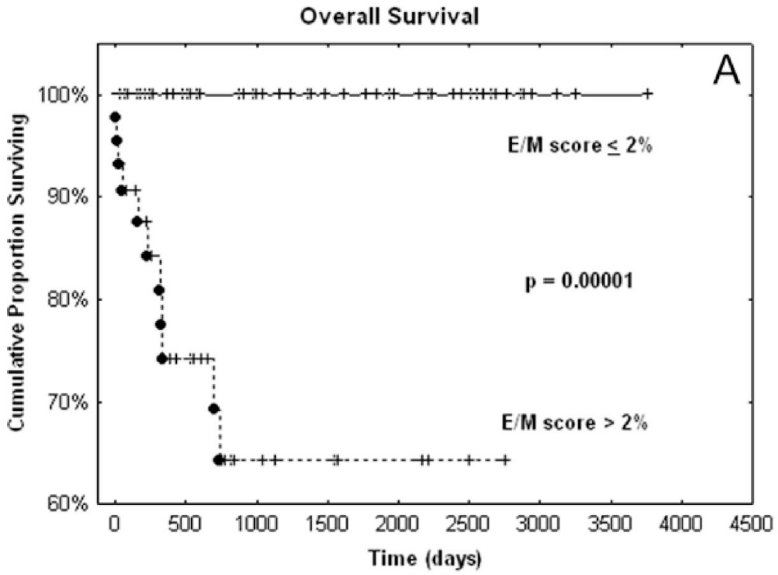
	Epithelial tumor cells	Early hybrid EMT state	Hybrid EMT state	Late hybrid EMT state	Mesenchymal tumor cells
Proliferation	+++++	++++	+++	++	+
Invasion	+	++	+++	++++	+++++
Plasticity	+	++	+++	++++	++
Stemness	+	+++	+++	+++	+++
Metastasis	+	++++	++++	++	+

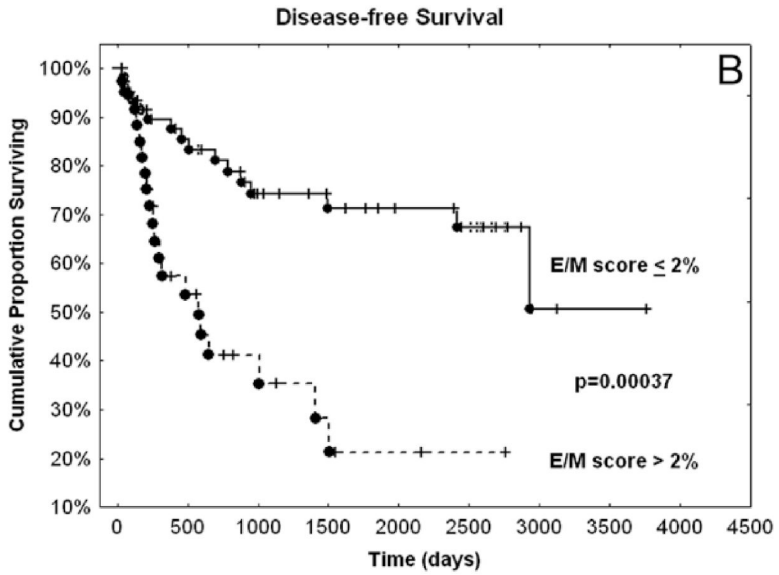
Pastushenko & Blanpain, Trends Cell Biol 2019
Pastushenko *et al.* Nature 2018

Clinical relevance of hybrid E/M phenotype(s)



- Single-cell migration is very rare, if any, in cancer
- A small (2-5%) hybrid E/M cells can associate with worse survival

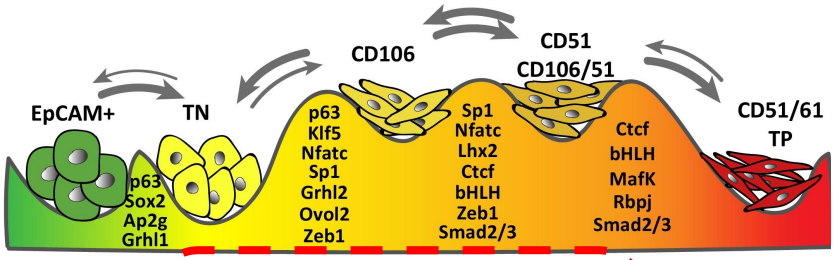




Godin *et al.* Cancers 2021; Bronsert *et al.* J Pathol 2014; Yu *et al.* Science 2013

Why are hybrid E/M cells the ‘fittest’?

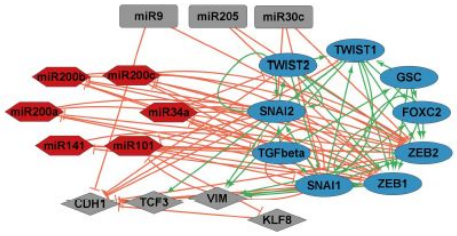
Kishore Hari



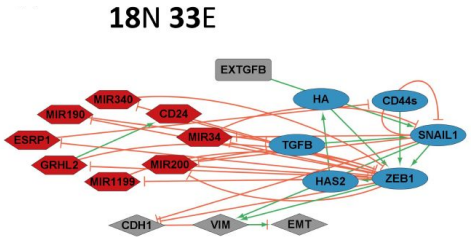
	Epithelial tumor cells	Early hybrid EMT state	Hybrid EMT state	Late hybrid EMT state	Mesenchymal tumor cells
Proliferation	+++++	++++	+++	++	+
Invasion	+	++	+++	++++	+++++
Plasticity	+	++	+++	++++	++
Stemness	+	+++	+++	+++	+++
Metastasis	+	++++	++++	++	+

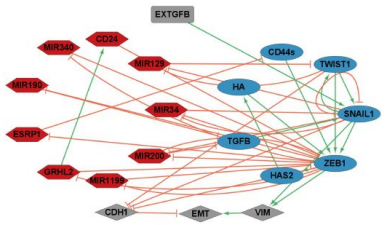
$$s_i(t+1) = \begin{cases} +1, \sum_j Adj_{ij} s_j(t) > 0 \\ -1, \sum_j Adj_{ij} s_j(t) < 0 \\ s_i(t), \sum_j Adj_{ij} s_j(t) = 0 \end{cases}$$

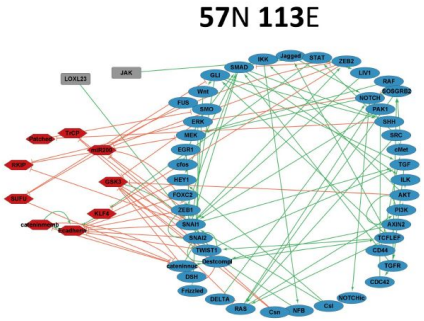
Is the “high plasticity” behavior of hybrid E/M a feature of underlying regulatory networks?



22N 82E



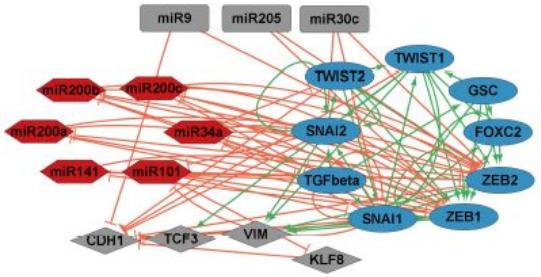




Nodes: Epithelial, Mesenchymal.

Edges: Activation, Inhibition

EMT networks consist of two “teams” of players



22N 82E

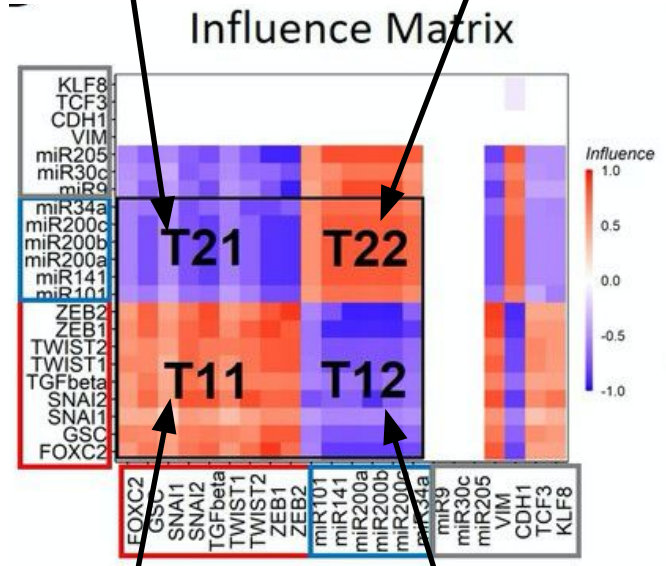
$$Infl = \frac{\sum_{l=1}^{lmax} \frac{Adj^l}{Adj^l_{max}}}{lmax}$$

$$T_{KL} = \frac{\sum_{i \in T_K} \sum_{j \in T_L} Infl_{ij}}{n_{KL}}, K, L \in \{1,2\}$$

$$Ts = \frac{\sum_{K,L \in \{1,2\}} |T_{KL}|}{4}$$

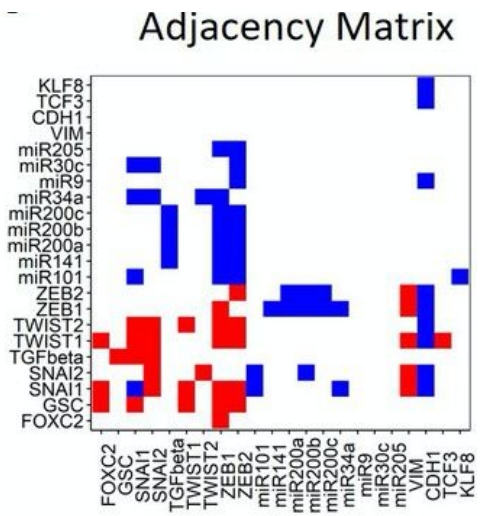
All Epi nodes effectively **inhibit** all Mes nodes.

All Epi nodes effectively **activate** all Epi nodes.



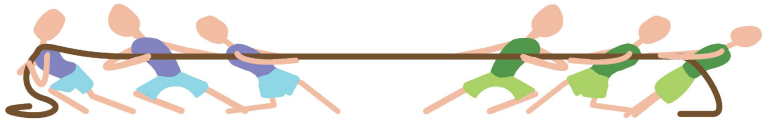
All Mes nodes effectively **activate** all Mes nodes.

All Mes nodes effectively **inhibit** all Epi nodes.

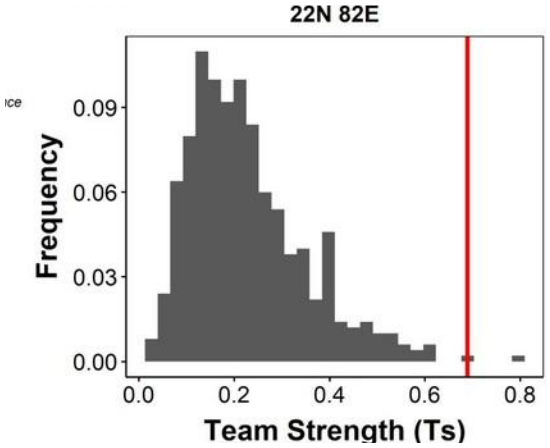


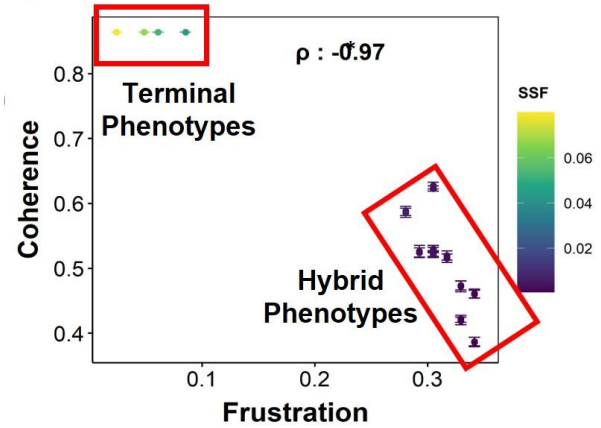
Row => Source; Column => Target

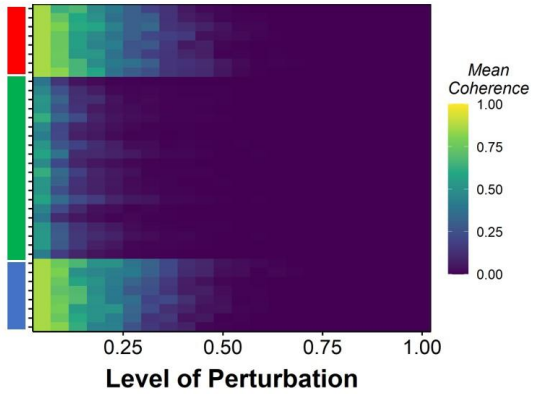
Red (Activation), Blue (Inhibition)

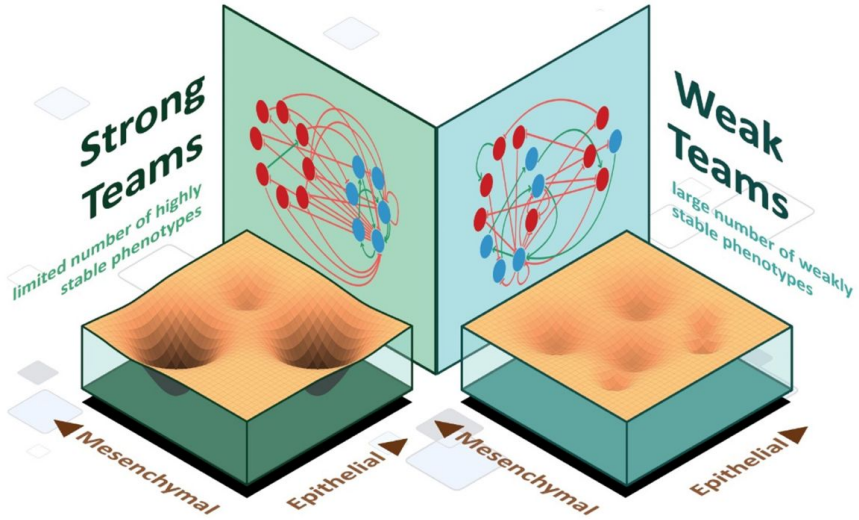


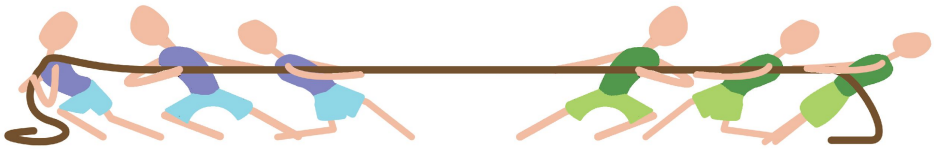
“Teams” offer “resistance” to transition out of E, M











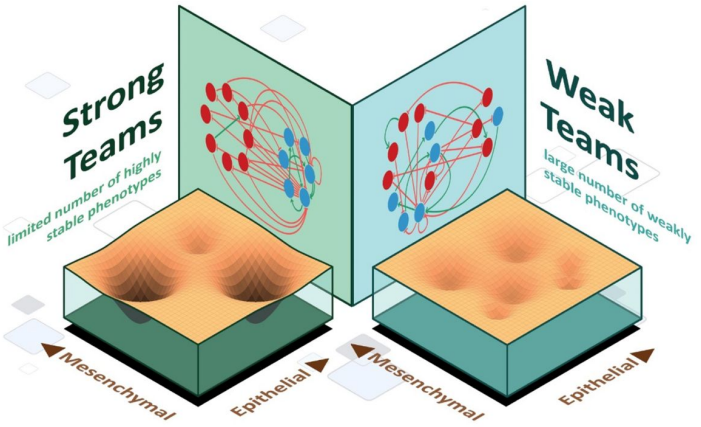
Absence of any “teams” supporting the hybrid E/M phenotypes makes them the ‘fittest’ for metastasis.

Hari *et al.* eLife 2022

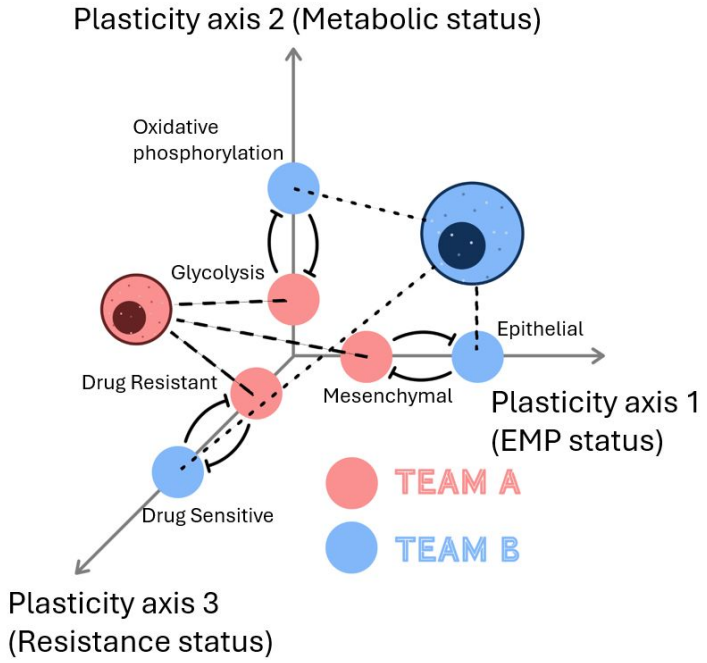
Why do “teams” exist?

To shape the phenotypic plasticity landscape

=> Control the rates of cell-state transitions

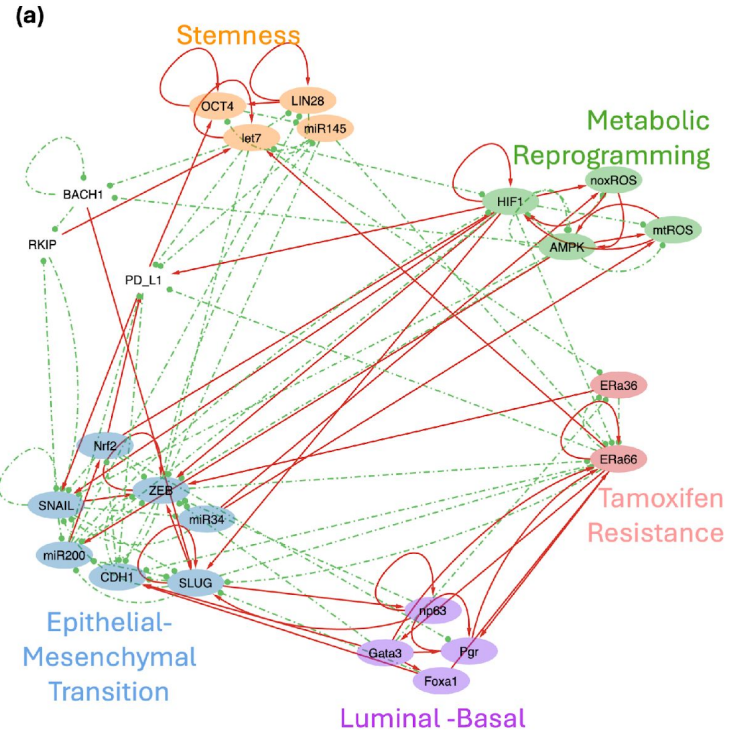


To couple the multiple axes of phenotypic plasticity
=> Increase the ‘fitness’ of metastasizing cells



Sahoo *et al.* Curr Opin Cell Biol 2024

A gene regulatory network (GRN) for interconnected axes of phenotypic plasticity in ER+ breast cancer



Ritesh Meena
(BS/MS, IISc)

Prior building blocks:

a) EMT-stemness (Jolly *et al.* 2015)
b) EMT-metabolism (Galbraith *et al.* 2022)
c) EMT-Tamoxifen Res (Sahoo *et al.* 2021)
d) EMT-luminal-basal (Sahoo *et al.* 2024)
e) EMT-PD-L1 (Sahoo *et al.* 2021; Shyam *et al.* 2023)

Meena *et al.* bioRxiv 2025: 688595

Individual axes of plasticity show multi-modal behavior

Definition of scores:

EMT = (ZEB + SLUG – CDH1 – miR200) / 4

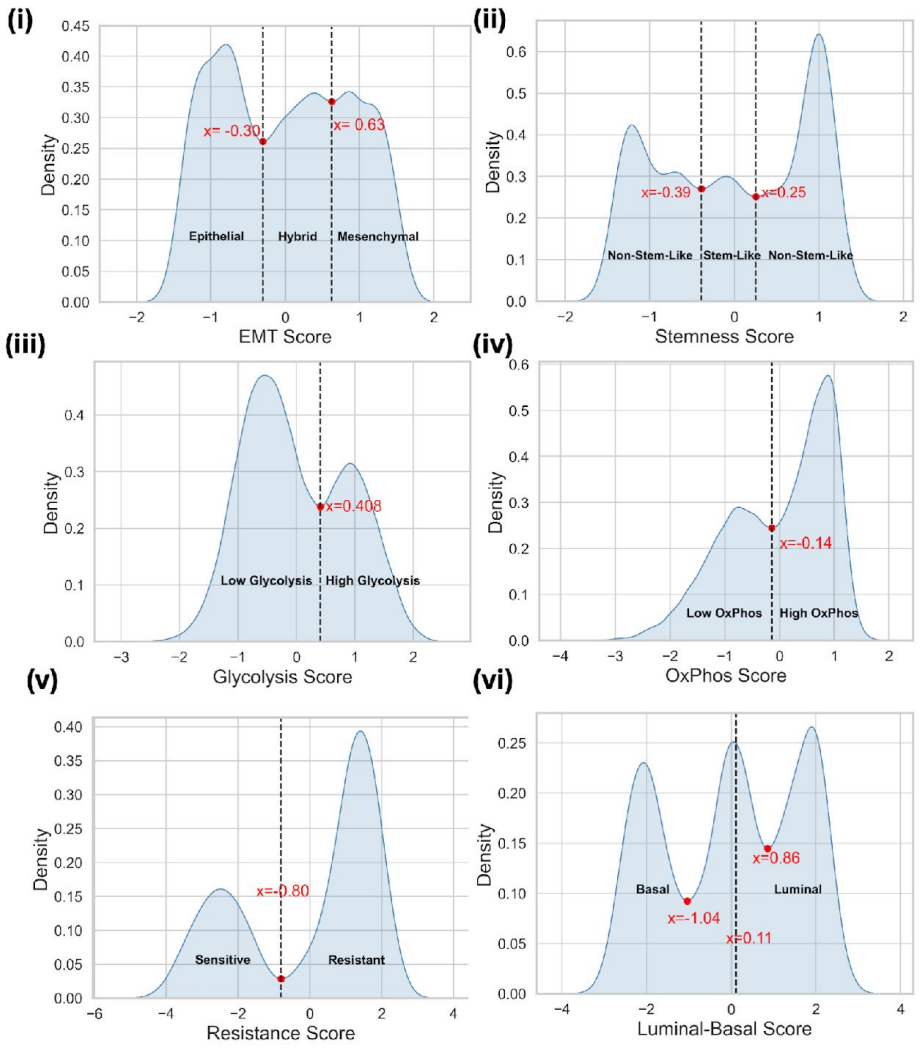
SN = (LIN28 + OCT4 – let-7 – miR145) / 4

Glycolysis = (noxROS + HIF1) / 2

OXPHOS = (mtROS + AMPK) / 2

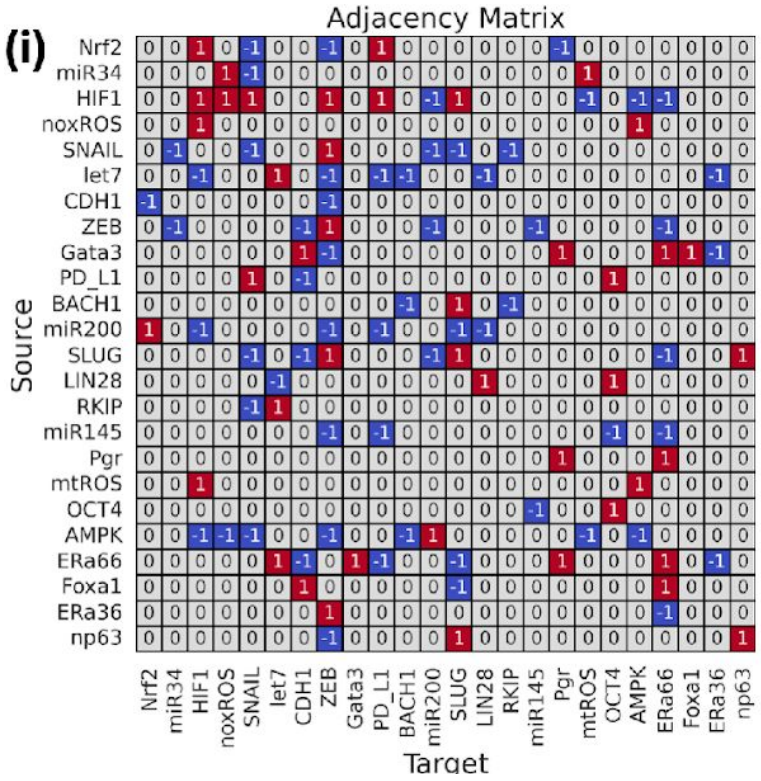
Res = (ERa36 – ERa66) / 2

L-B = (ERa66 + GATA3 + PGR + FOXA1)/4 – (NP63 + SLUG) / 2



Meena *et al.* bioRxiv 2025: 688595

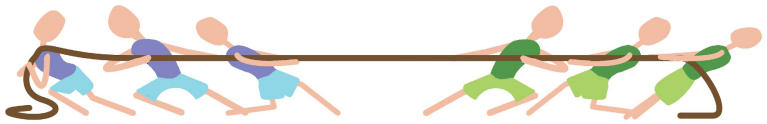
Any topological patterns in this GRN?



$$Infl = \frac{\sum_{l=1}^{lmax} \frac{Adj^l}{Adj^l_{max}}}{lmax}$$

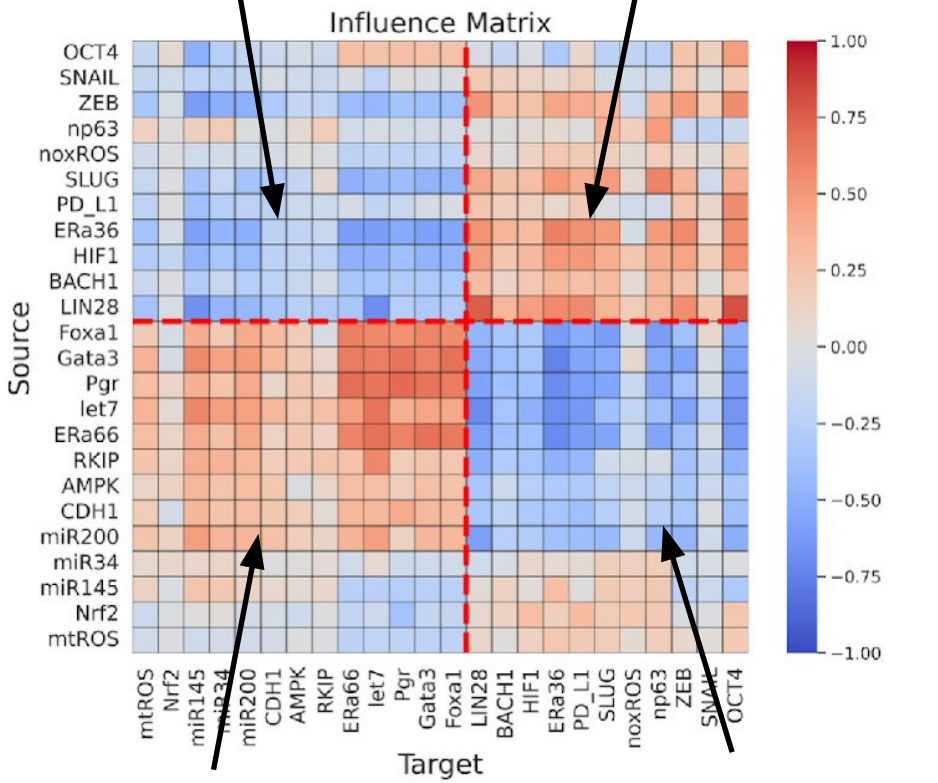
$$T_{KL} = \frac{\sum_{i \epsilon T_K} \sum_{j \epsilon T_L} Infl_{ij}}{n_{KL}}, K, L \in \{1,2\}$$

$$Ts = \frac{\sum_{K,L \epsilon \{1,2\}} |T_{KL}|}{4}$$



Team 1 members effectively **inhibit** Team 2 members.

Team 1 members effectively **activate** Team 1 members.



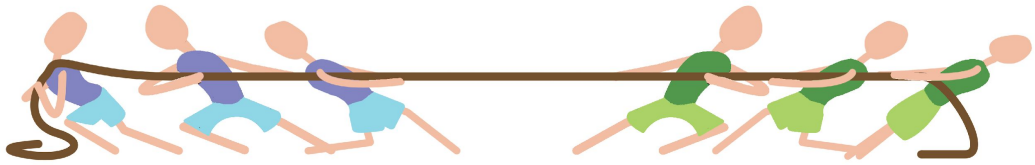
Team 2 members effectively **activate** Team 2 members.

Team 2 members effectively **inhibit** Team 1 members.

Meena *et al.* bioRxiv 2025: 688595

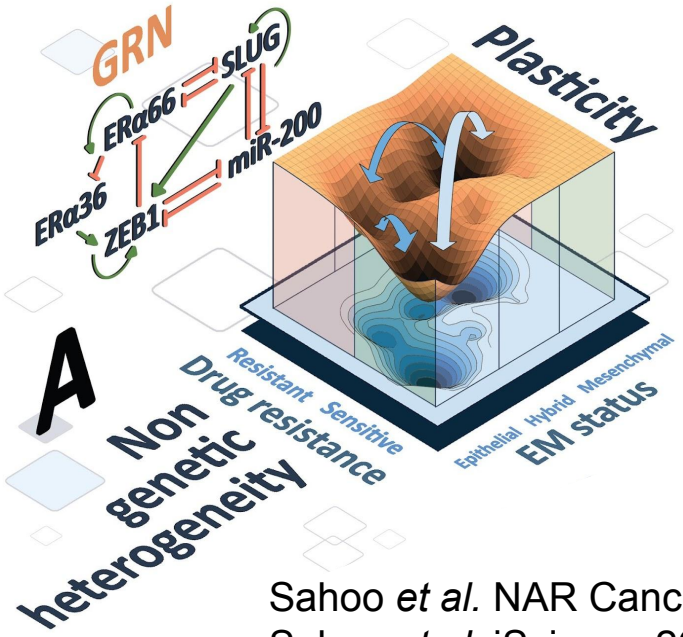
Mutually opposing two-team behavior across plasticity axes

Epithelial
Non-stem like
Tam-Sensitive
Luminal-like
OXPHOS

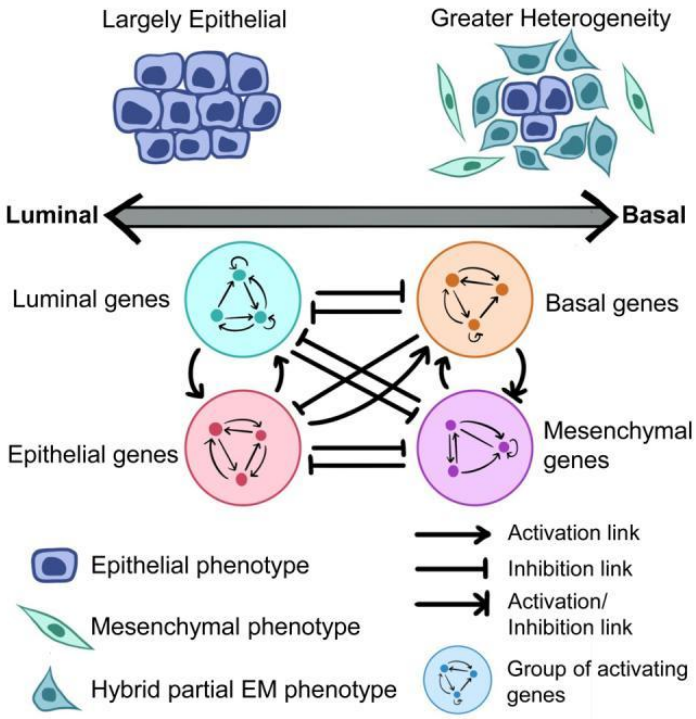


Mesenchymal
Stem-like
Tam-Resistant
Basal-like
Glycolysis

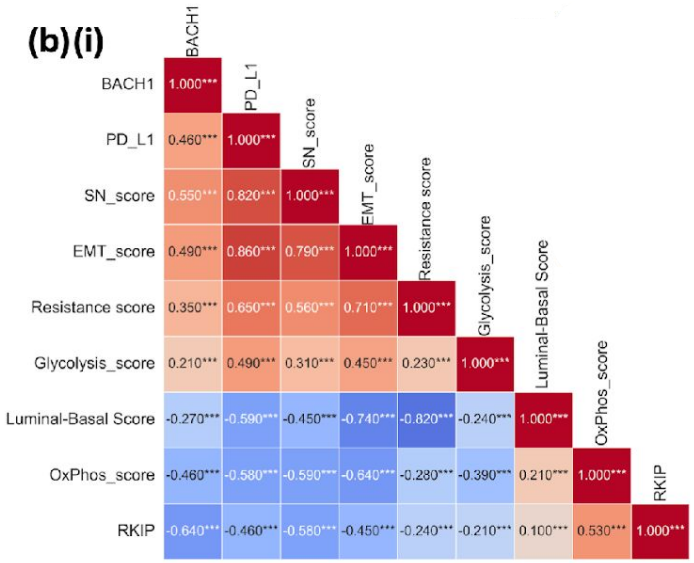
Network topology explains why we had noticed specific patterns in simulation, omics data



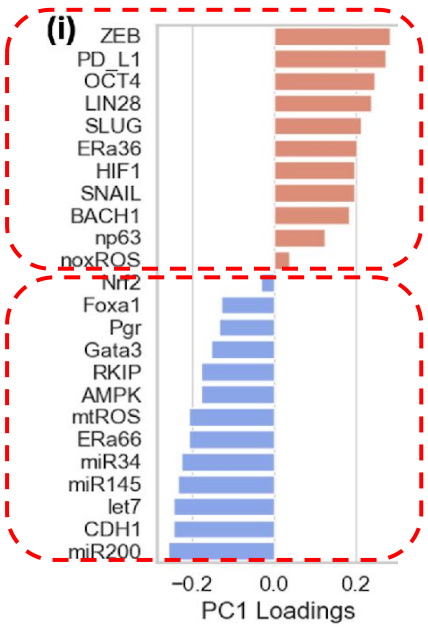
Sahoo *et al.* NAR Cancer 2021
Sahoo *et al.* iScience 2024

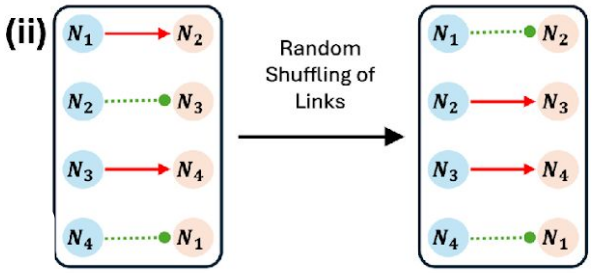


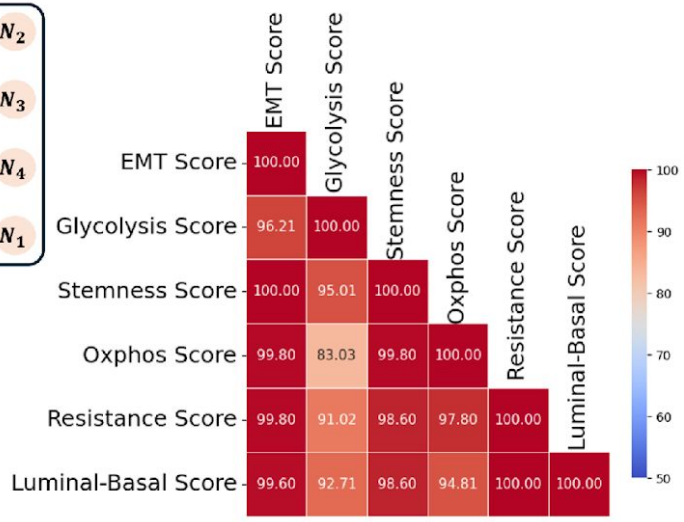
GRN simulations confirm the two-team dynamics across axes



- Phenotypic plasticity is a tightly coordinated multi-axis systems-level process in cancer.
- This coordination is enabled by functional cellular programs forming two opposing ‘teams’.
- ‘Teams’ => evolutionary conserved mechanism?



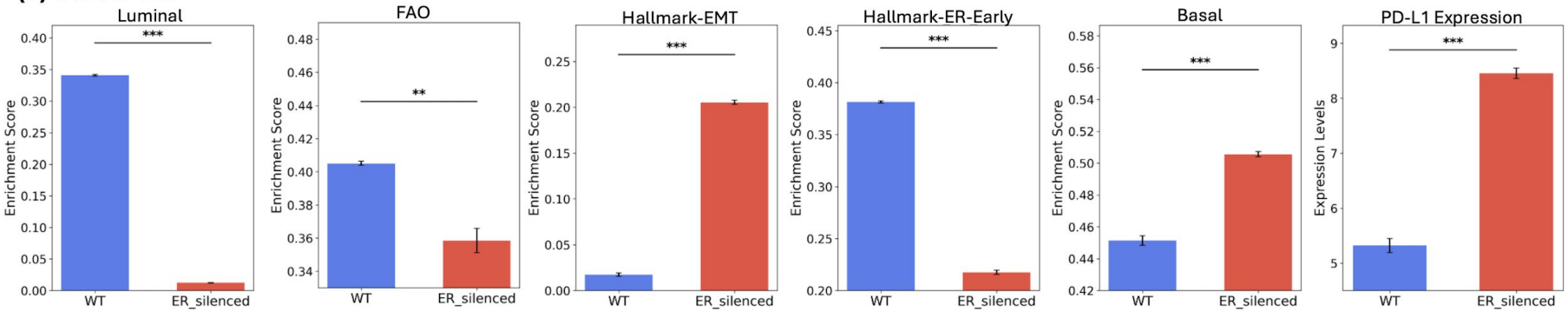




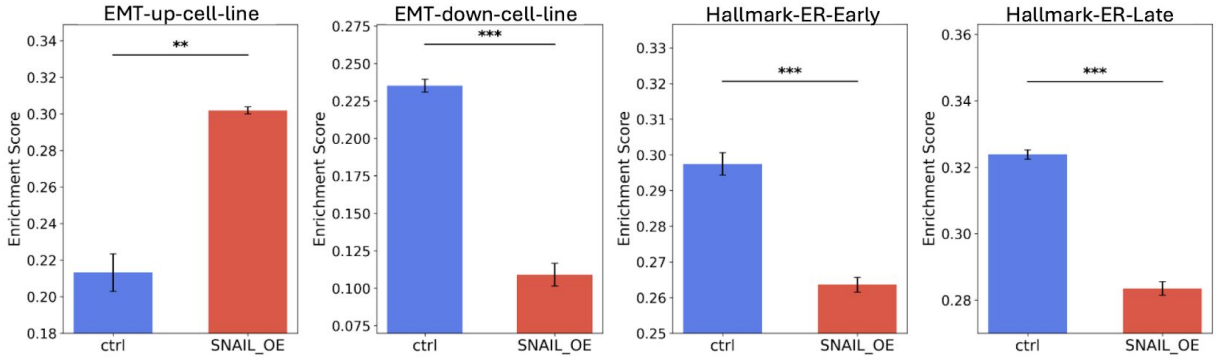
Meena *et al.* bioRxiv 2025: 688595

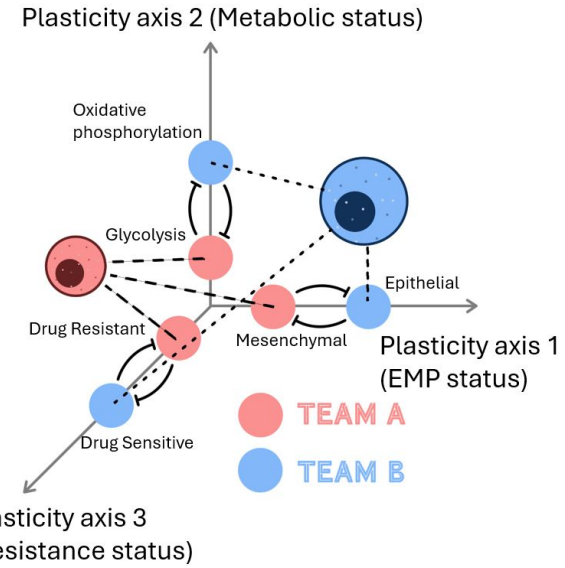
RNA-seq analysis suggests coordinated cell-state transitions

(a) GSE27473



(c) GSE81929

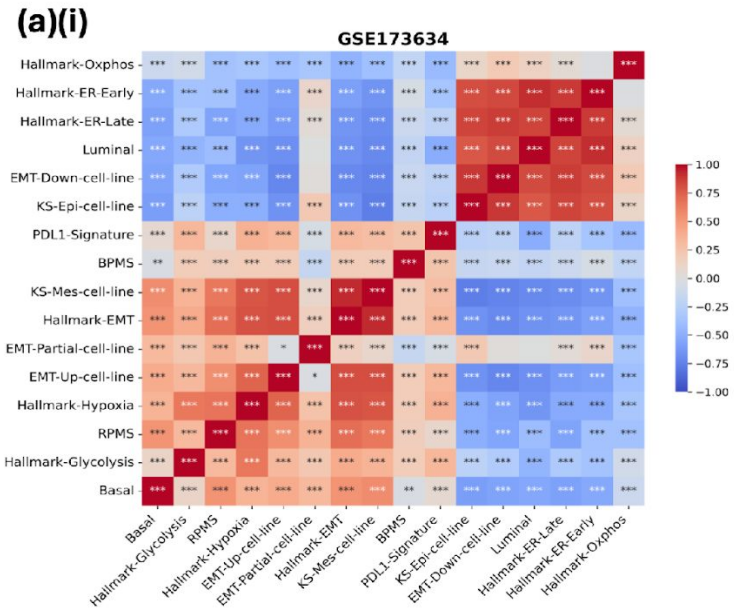




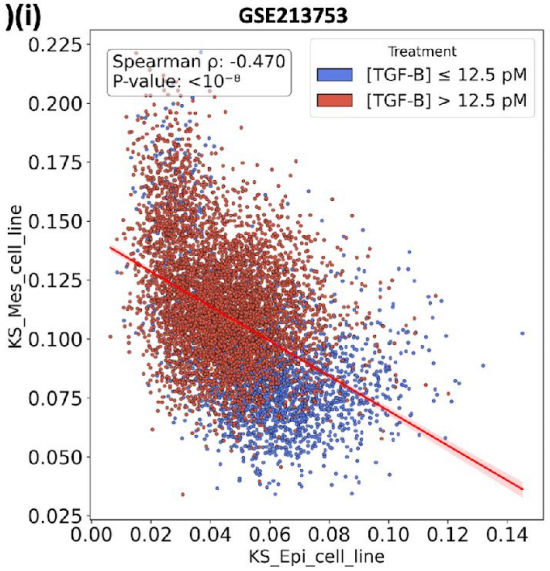
The presence of two opposing ‘teams’ can drive the coordinated cellular responses in specific directions.

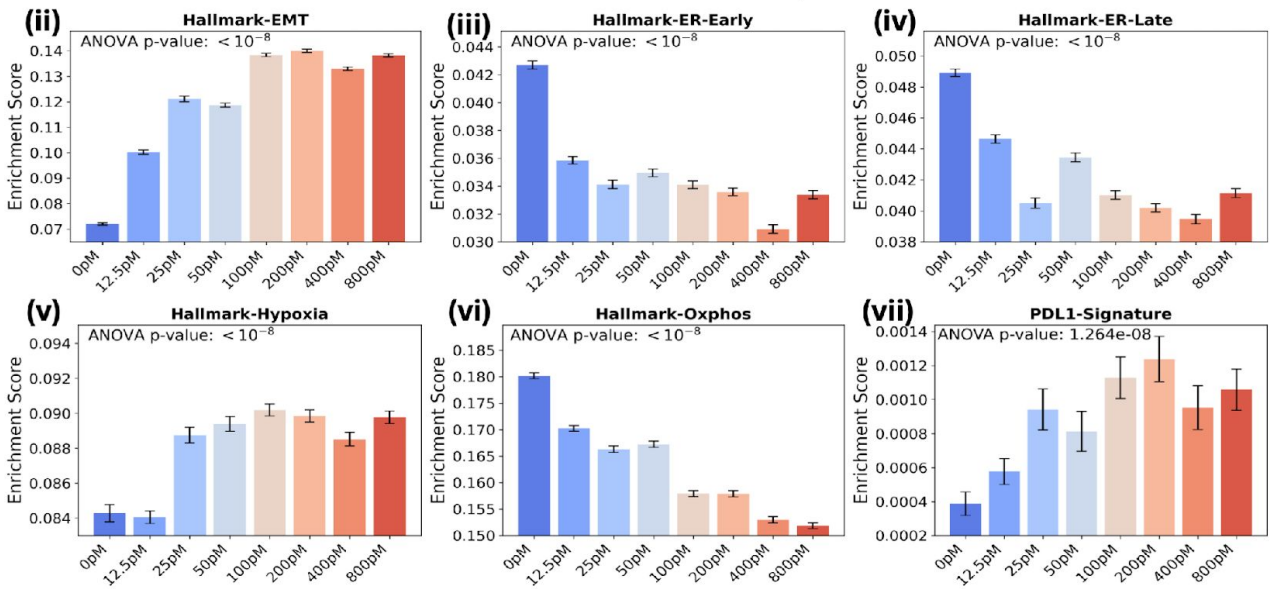
Meena *et al.* bioRxiv 2025: 688595

scRNA-seq data recapitulates systemic plasticity patterns



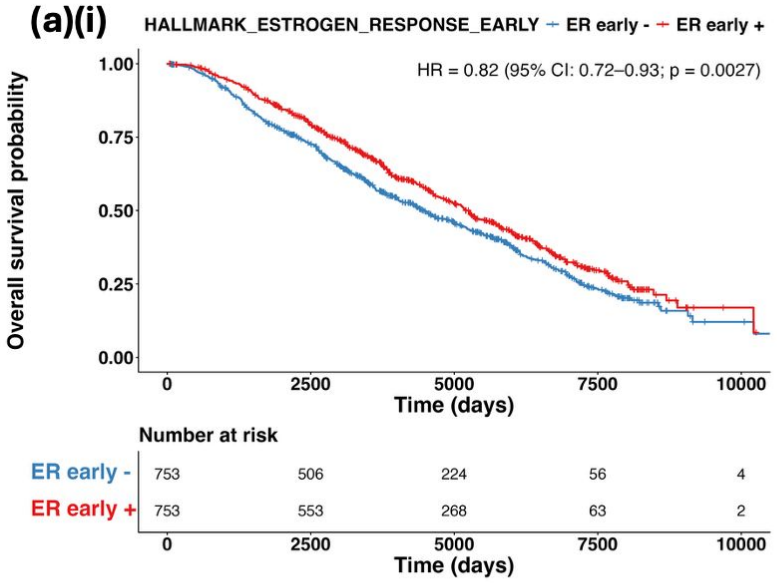
EMT progression usually proceeds with the suppression of ER signaling and increased glycolysis in ER+ breast cancer samples.

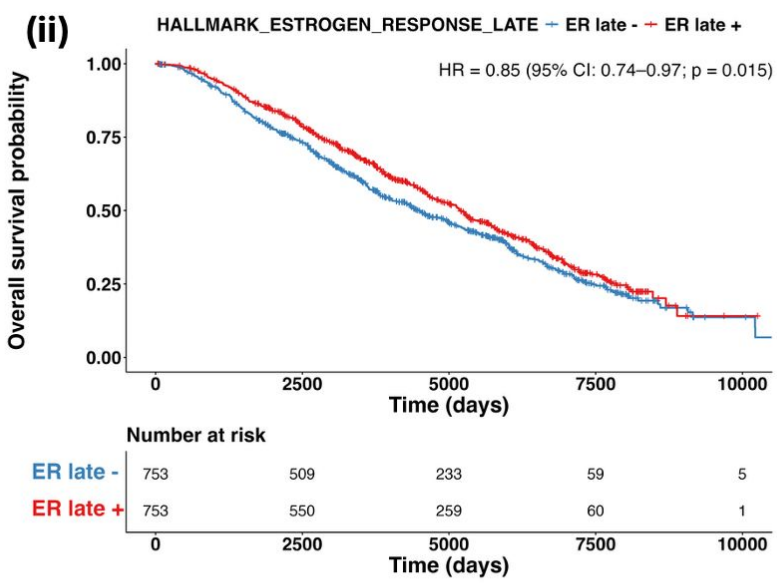




Meena *et al.* bioRxiv 2025: 688595

Any clinical relevance of coordinated cell-state transitions?



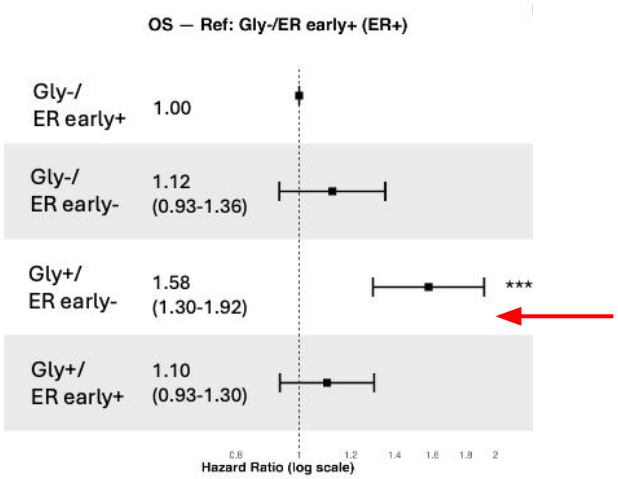


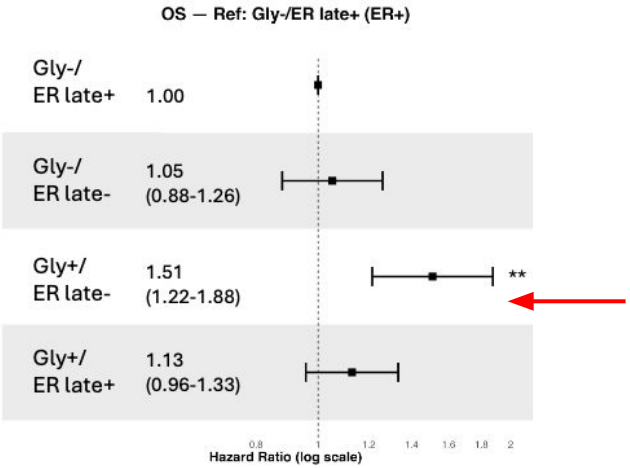
Suppression of ER signaling associated with worse clinical outcomes in ER+ BCa (TCGA, METABRIC cohorts).

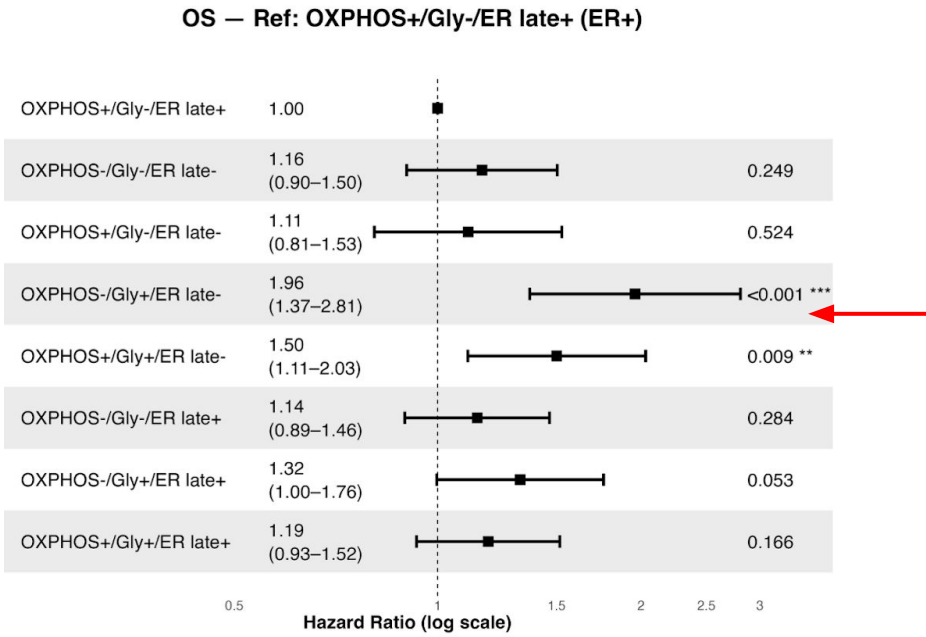
Does suppression of ER AND increased glycolysis or PD-L1 makes it even worse?

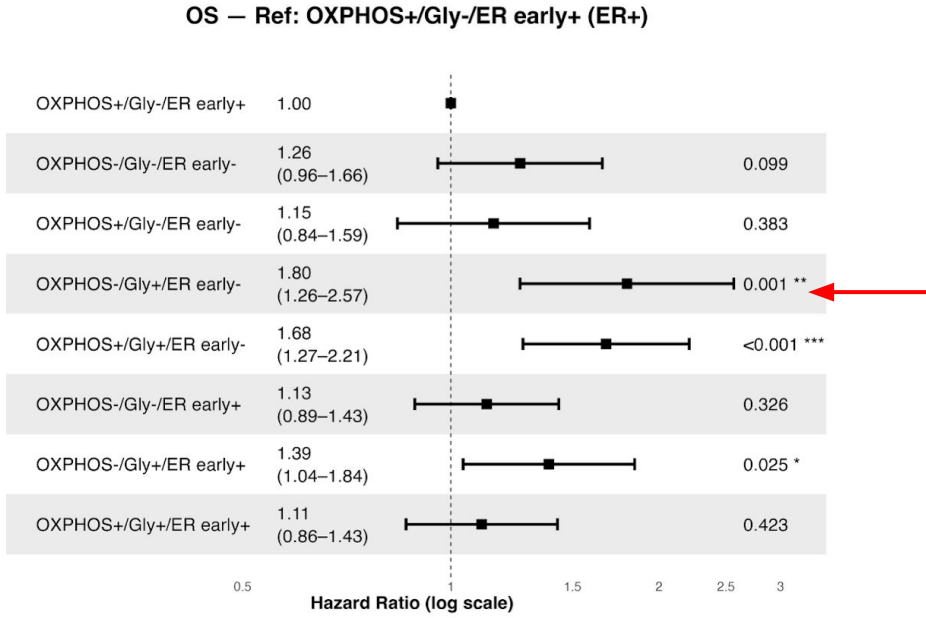
Meena *et al.* bioRxiv 2025: 688595

Coordinated plasticity leads to worse clinical outcomes in TCGA, METABRIC cohorts









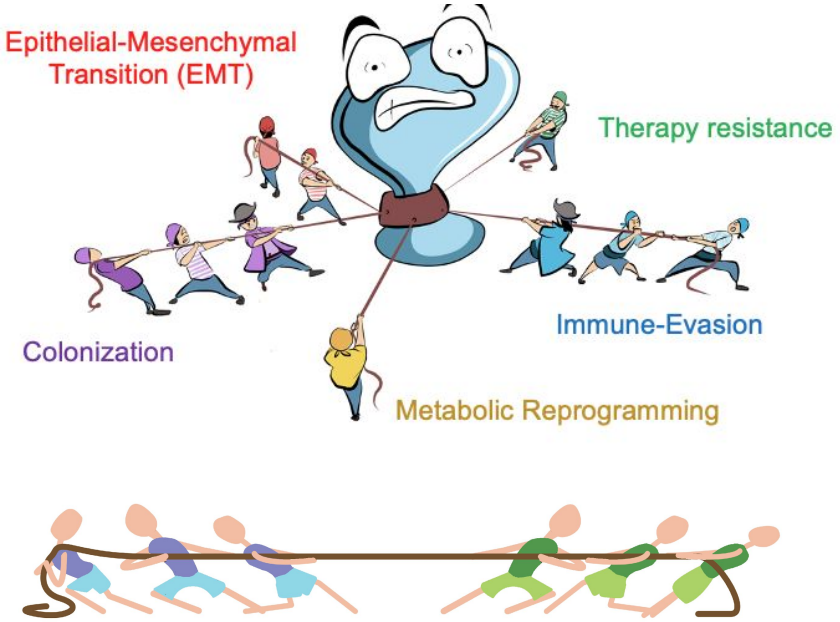
Meena *et al.* bioRxiv 2025: 688595

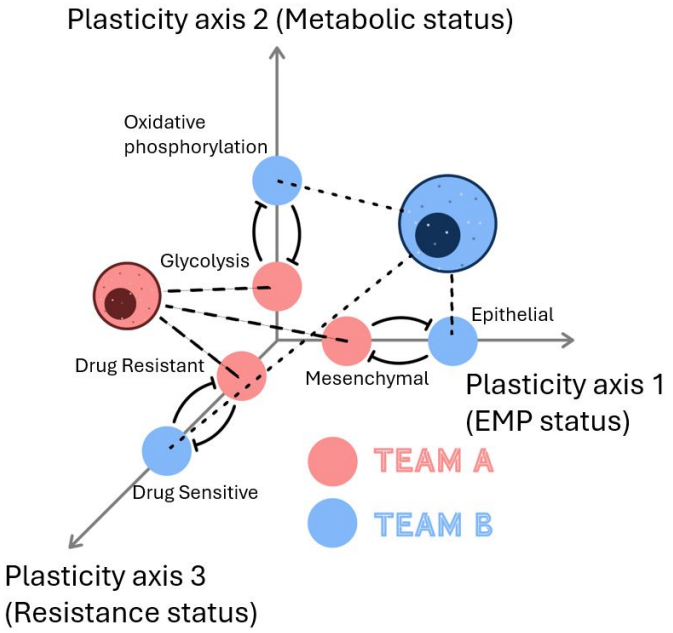
Summary

- ‘Design principles’ of regulatory networks driving cell-state switching:

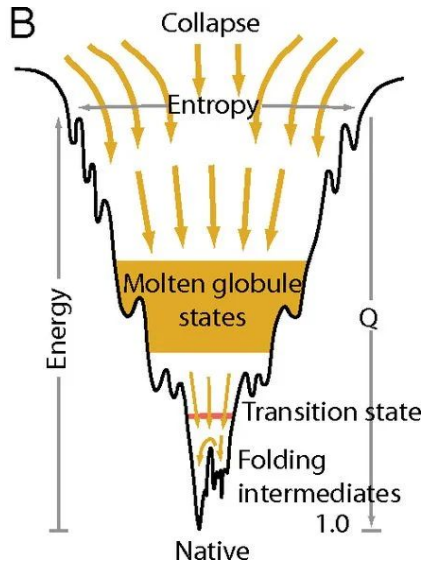
1. Multistable dynamics => Phenotypic plasticity & heterogeneity
2. Existence of “teams” enables coordination across axes of plasticity

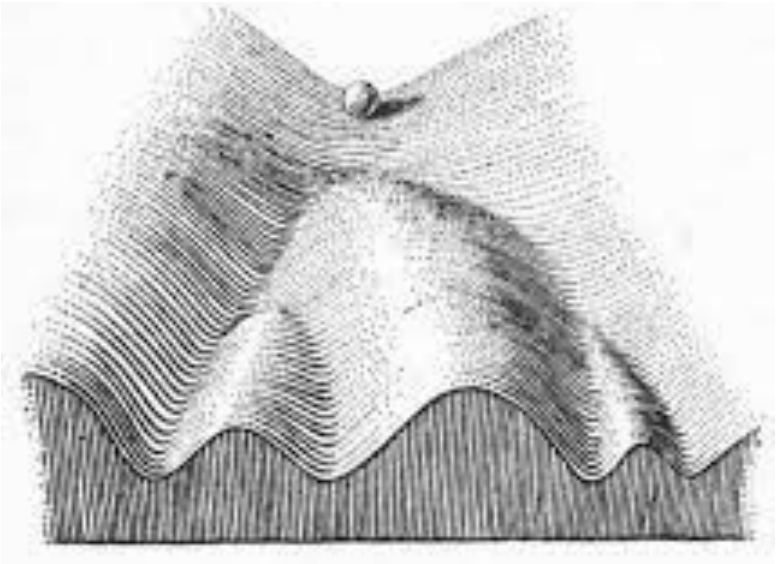
- Coordinated cell-state transitions increase cancer cell fitness and associate with worse patient survival





“Teams” ~ a driving principle of cell-fate canalization?



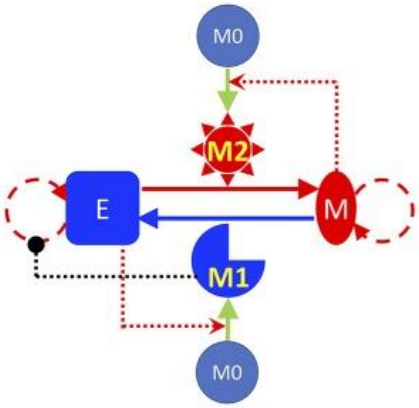


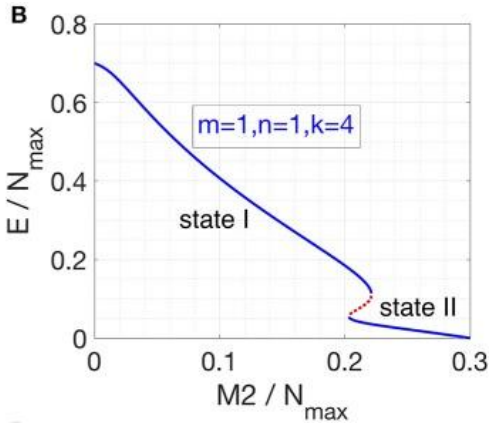
Englander & Mayne, PNAS 2014; Waddington, 1942

“Teams” can minimize “frustration” in EMT networks,
allowing only a limited number of trajectories and cell-states.

Open questions

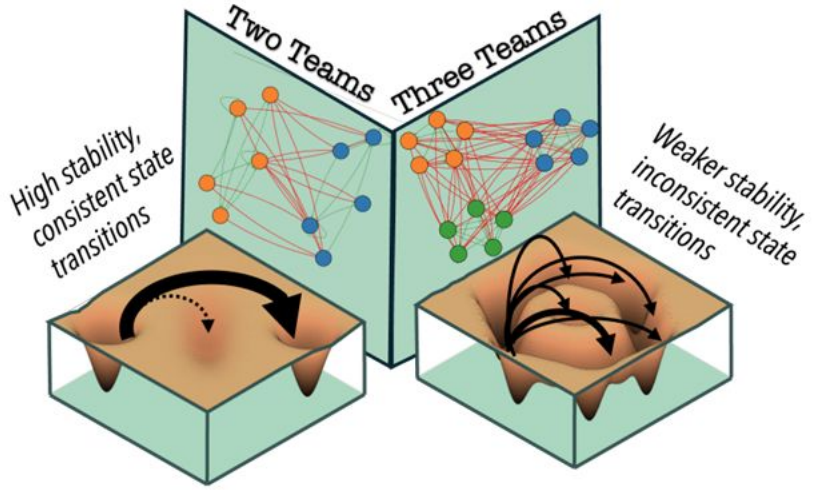
- Can ‘teams’ be defined for cell types in a tissue, similar to our definition of ‘teams’ within a cell?
- How does spatial interplay impact team formation?
- Is the composition of ‘teams’ similar in other epithelial cancers as well?





Li*, Jolly* *et al.* Front Oncol 2019

- How is the behavior of 3 team networks different than 2 team ones?
- Can we predict the number and the composition of teams in a network without calculating influence matrix?
- How to build multi-scale models for ‘teams’ within cells and in a tissue?



Haldar*, Anand* *et al.* bioRxiv 2025: 679762

Acknowledgements: Our "team"

∫ Oncologists
Clinicians
Mathematicians
Physicists
Chemists
Engineers

∫ Biotechnology
Electrical Engineering
Bioinformatics
Physics
Mathematics
Cancer Biology

Funding agencies

Open for new collaborations : mkjolly@iisc.ac.in