

Identification of Diagnostic and Therapeutic Markers in Tumor Invasion using Logic-based Modeling

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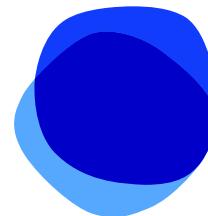
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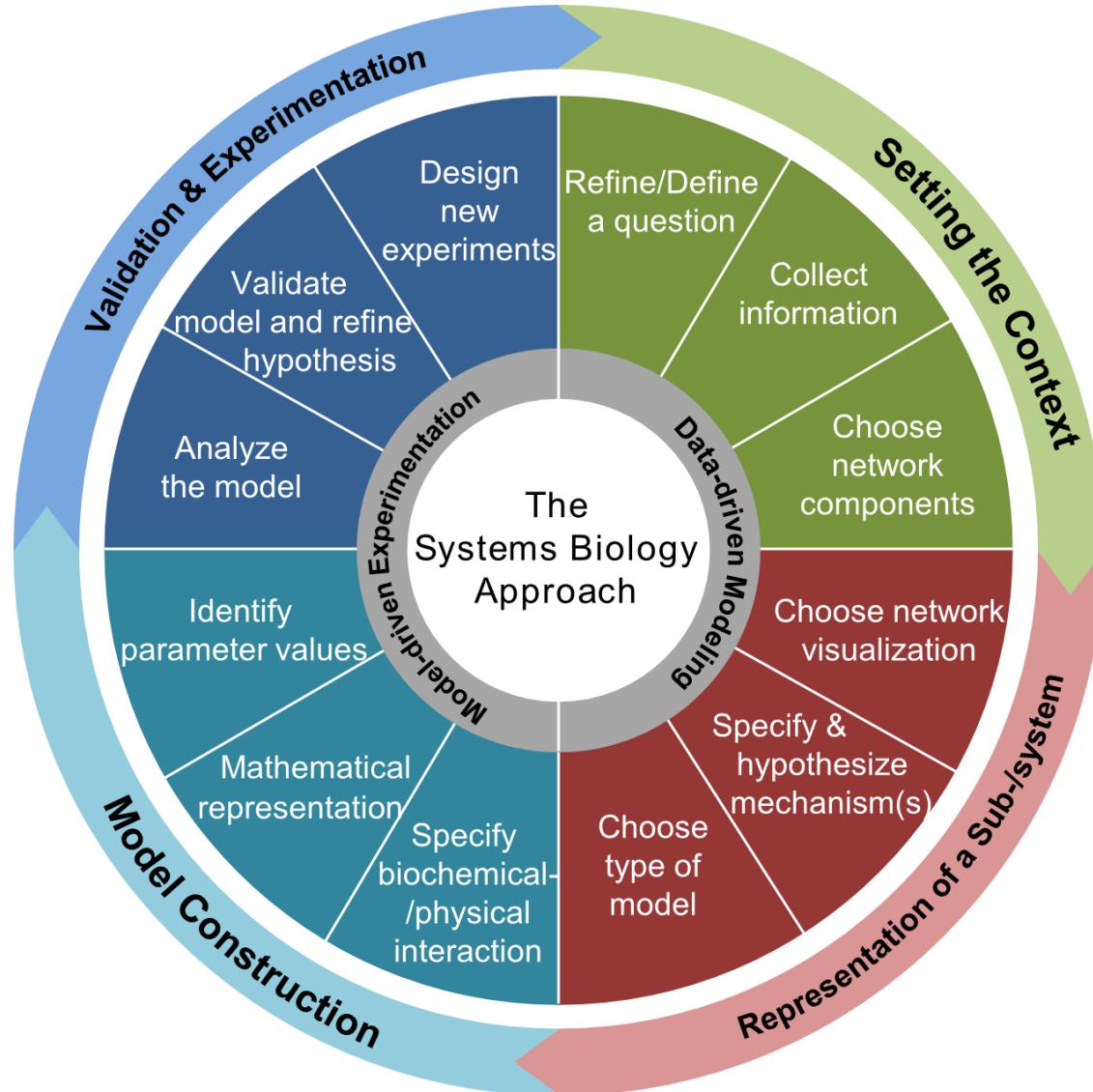
Traditio et Innovatio

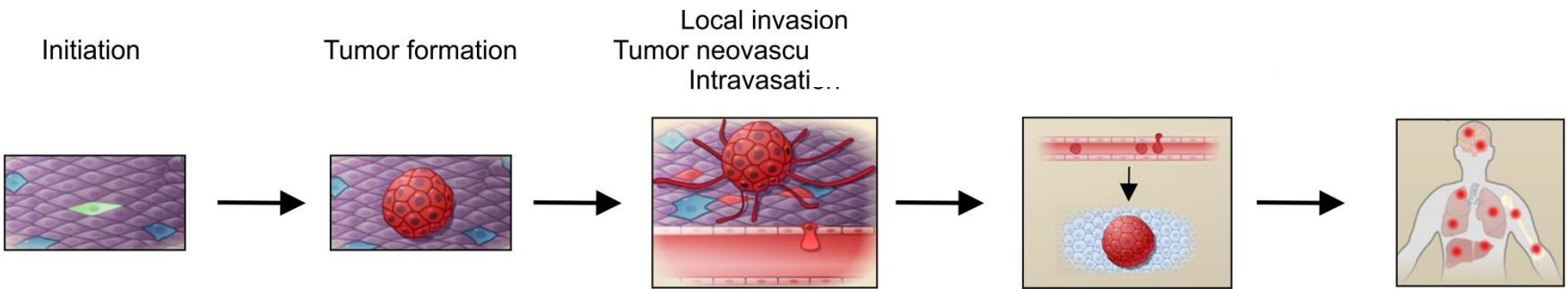


SYSTEMS BIOLOGY
BIOINFORMATICS
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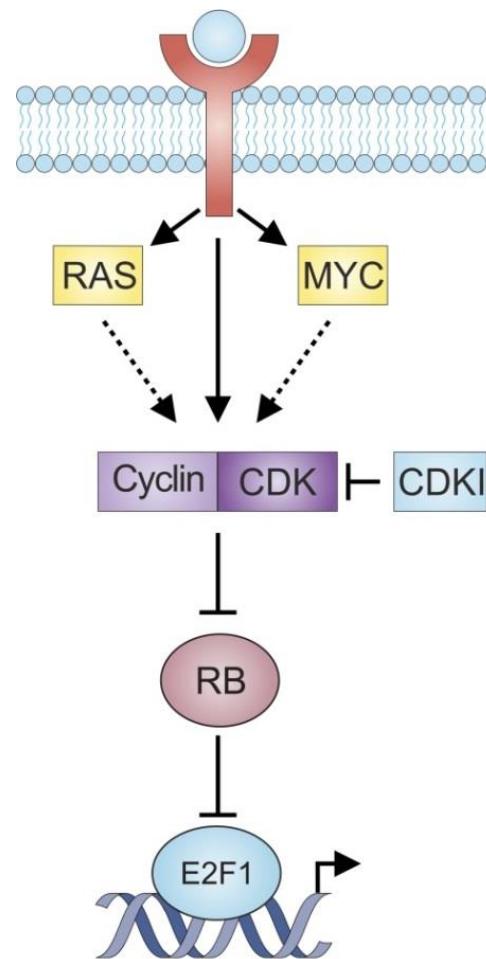
- The systems biology approach to complex diseases
- Biological question
- Workflow for identification diagnostic and therapeutic markers
 - Construction and analysis of E2F1 map
 - Identification of tumor specific core-regulatory network(s)
 - Dynamical analysis of core-regulatory network(s)
 - Stimulus response behavior
 - Perturbation analysis
 - Experimental and patient data validation of model predictions

The Systems Biology Approach





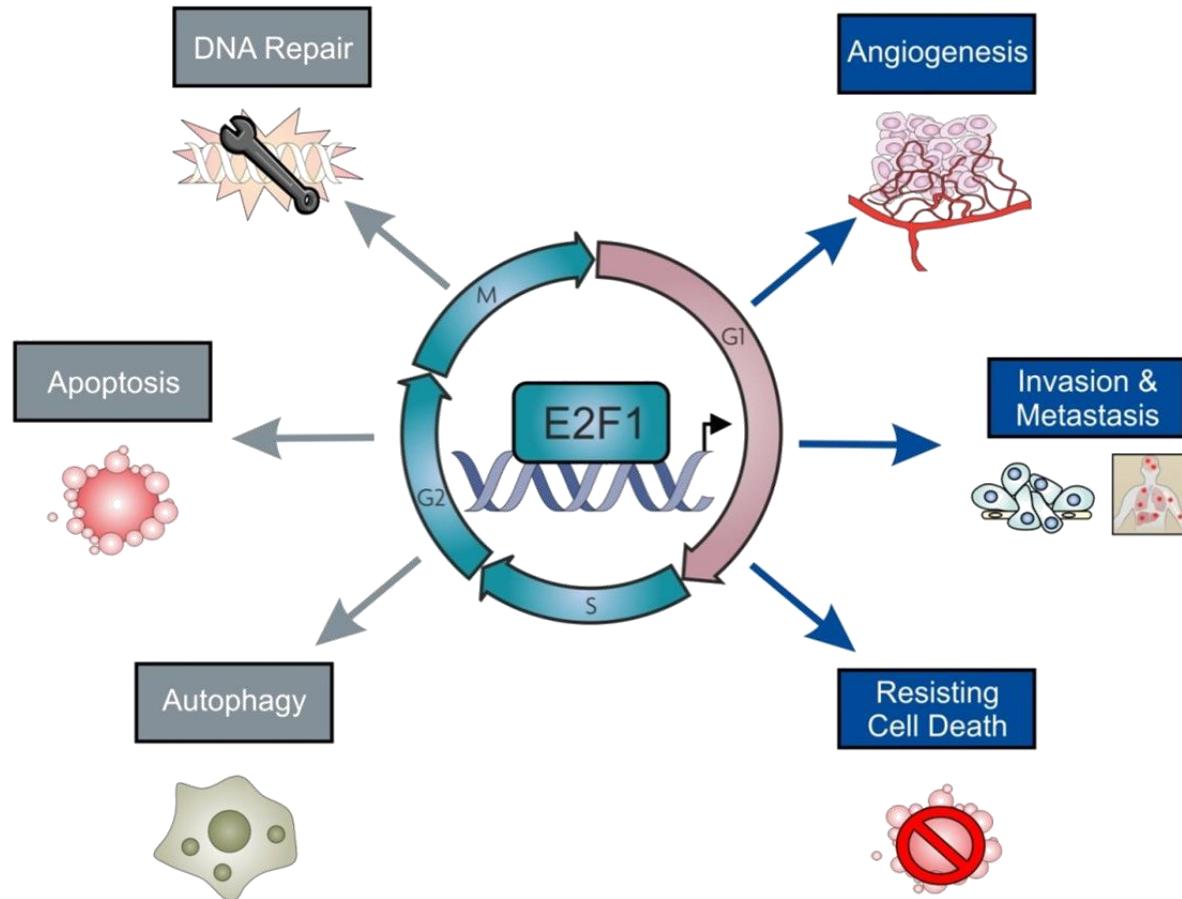
- Malignant tumors and metastasis are frequently resistant to chemotherapy.
- Therapy-induced resistance will result in recurrence and further disease progression.
- The **transcription factor** E2F1 has recently been identified as a key regulator in tumor invasiveness and metastasis by switching duties during carcinogenesis.



The dual role of the E2F1 in carcinogenesis

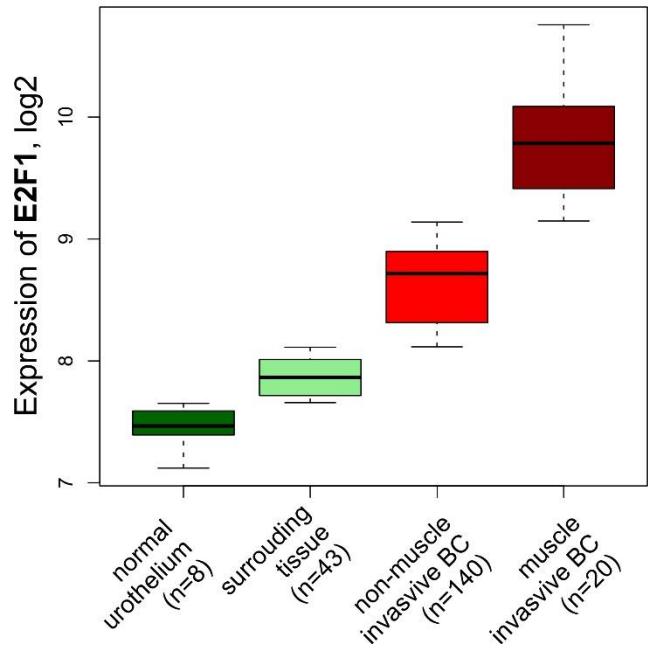
... as a tumor suppressor

... as a metastasis inducer

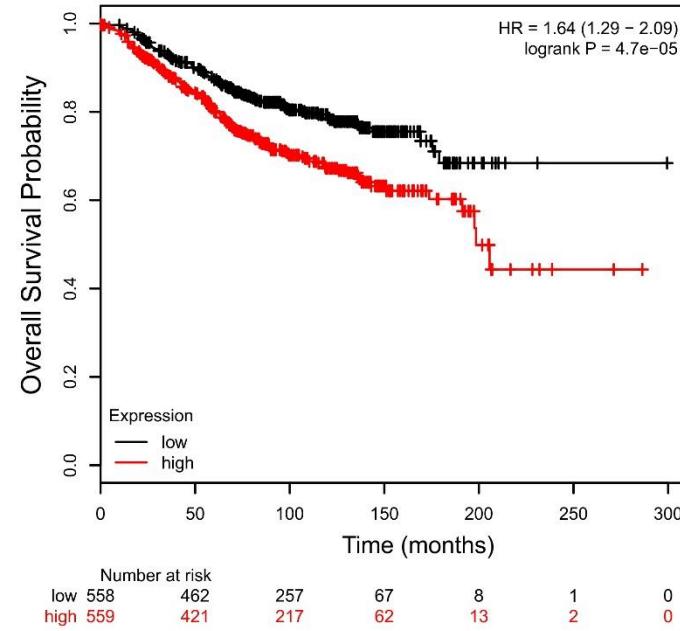




Bladder cancer

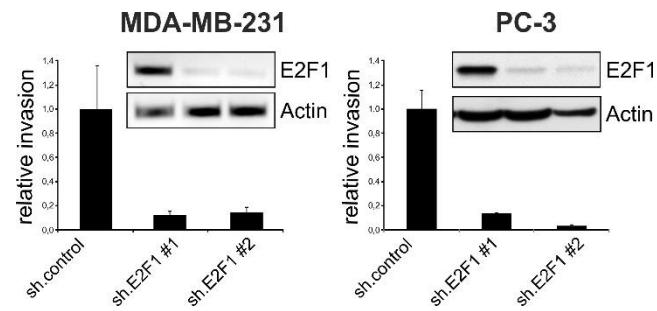
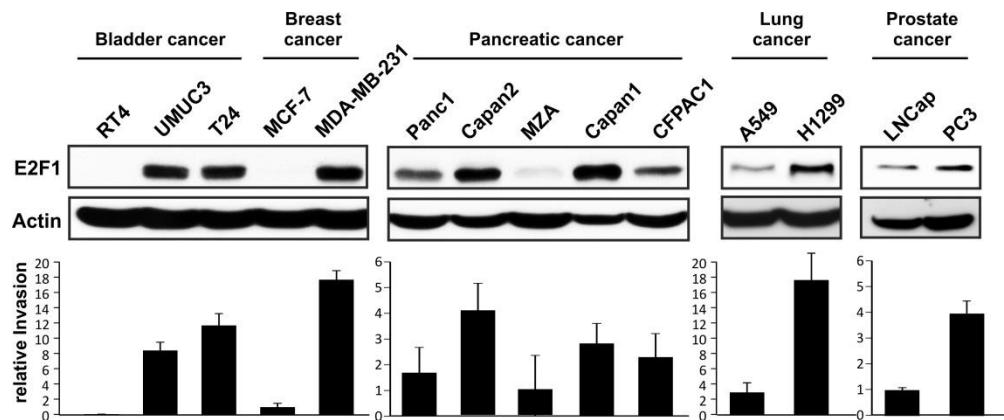


Breast Cancer

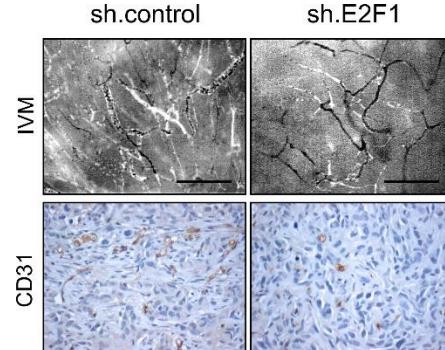
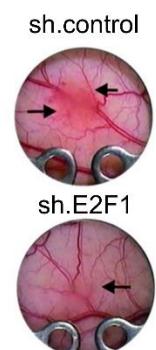
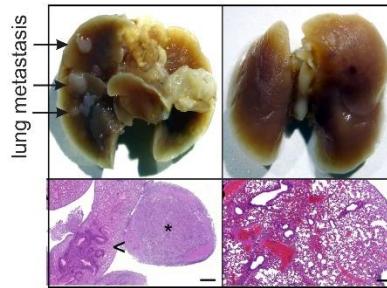
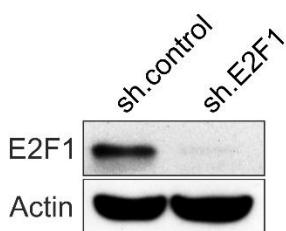


E2F1 promotes tumor progression

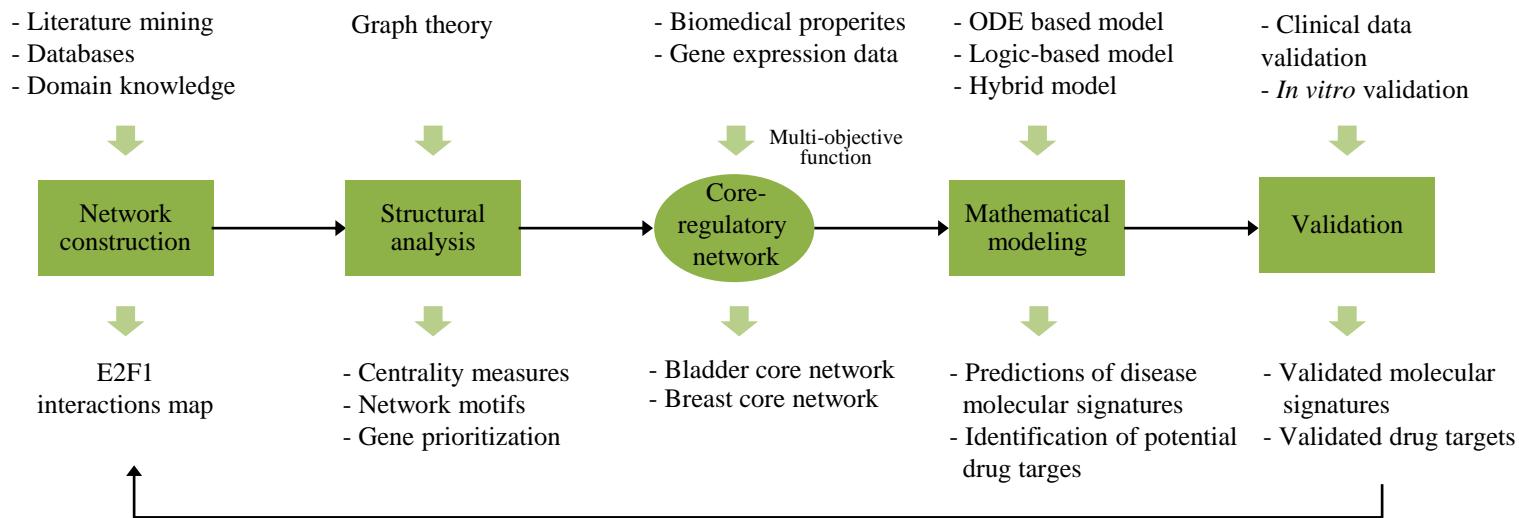
Cell migration and invasion



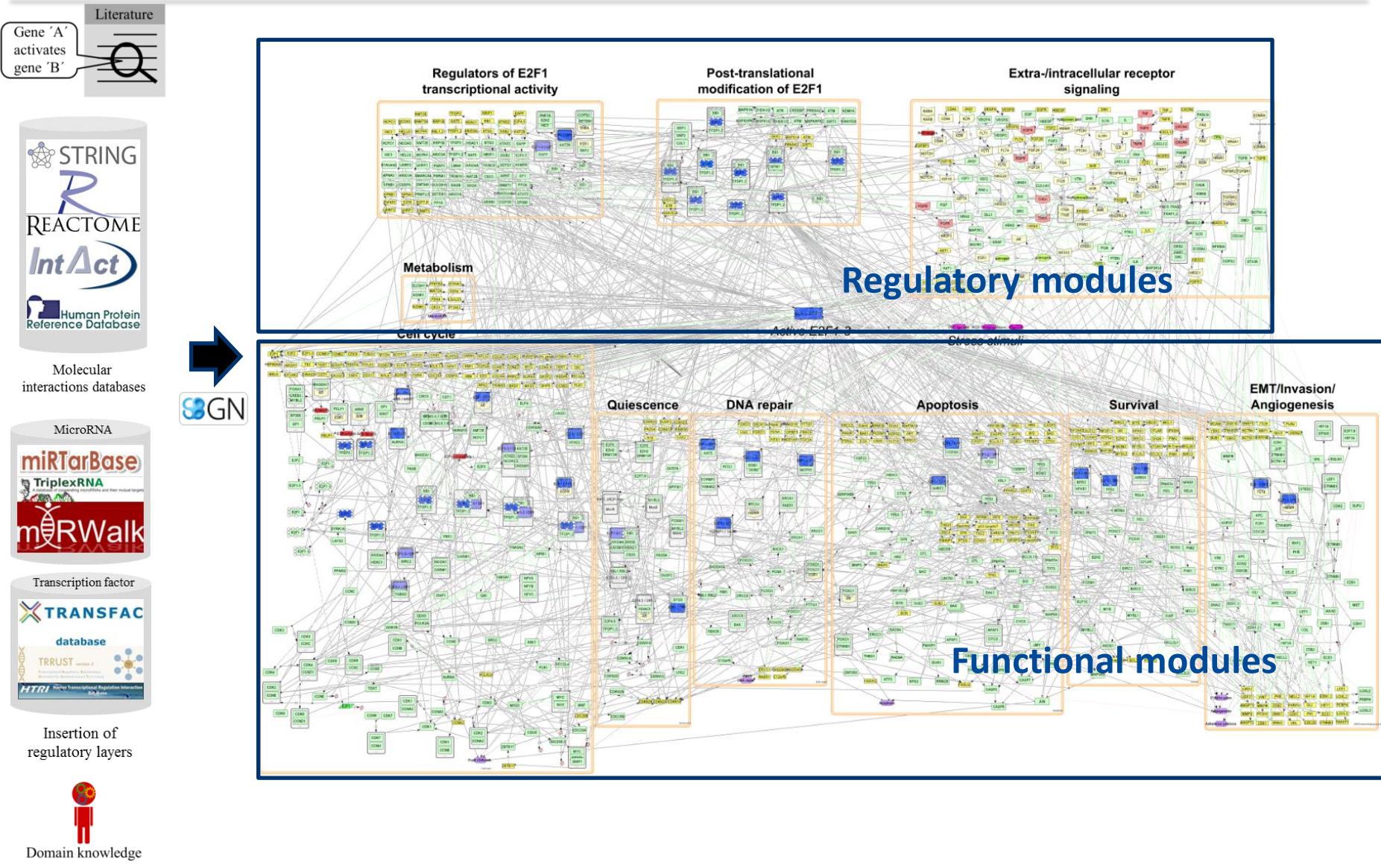
Tumor angiogenesis and metastasis



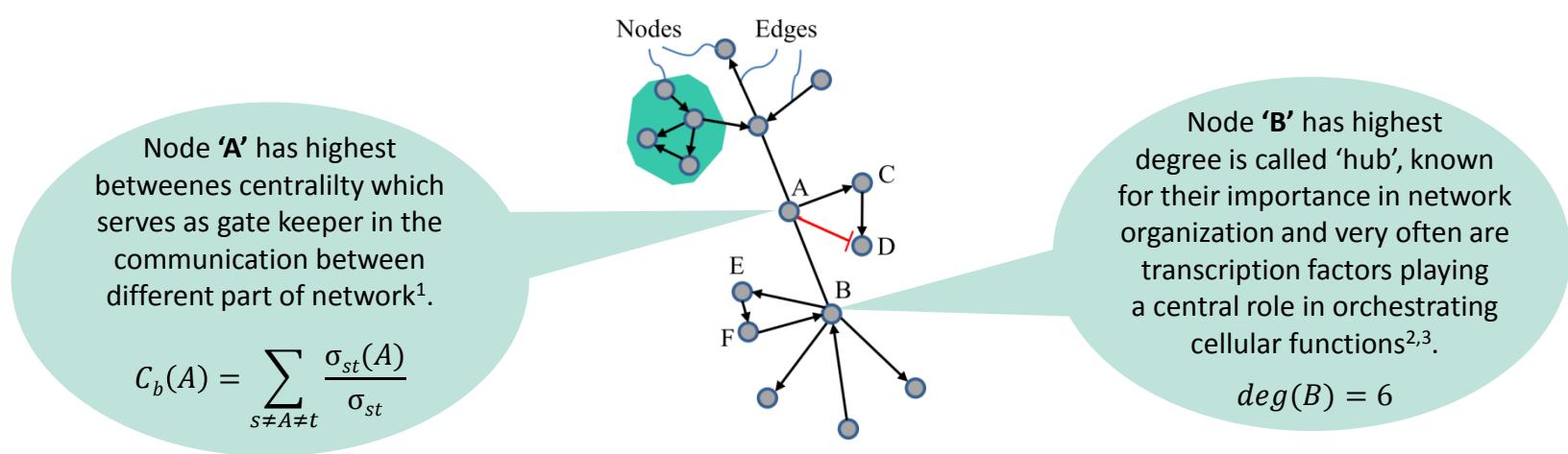
- Which gene signatures promote the malignant phenotype?
- What the possible therapeutic candidates that can render invasive phenotype to non-invasive
- What are the mechanisms underlying E2F1 mediated drug resistance?



Construction of modularized map of E2F1 in tumor progression



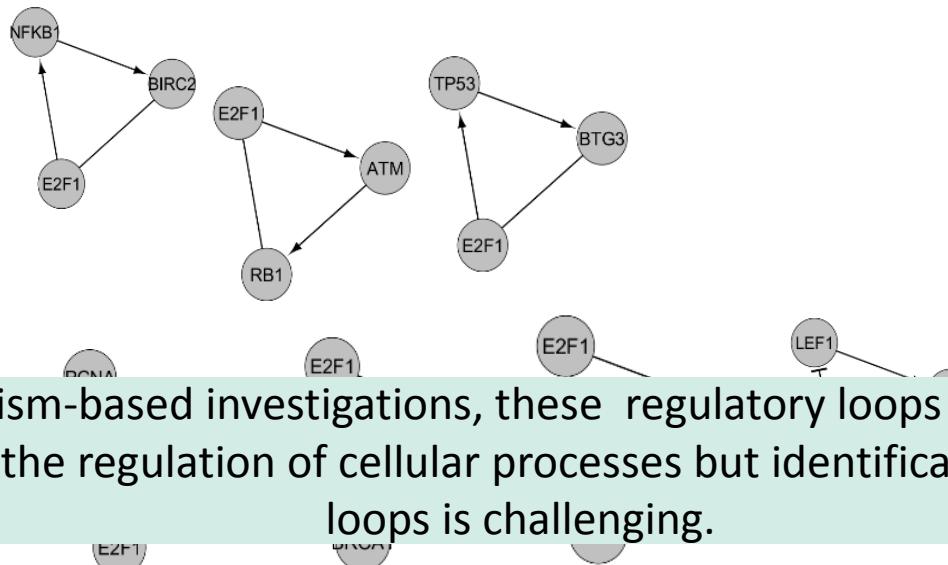
- Network characterization through topological properties (e.g., node degree (ND) and betweenness centrality (BC)) which provide useful information regarding the network architecture¹.
- **Node degree** is the number of edges connected to a node, and
- **Betweenness centrality** is the number of shortest paths from all nodes to all others that pass through that node.



1. Barabasi et al., Nature reviews genetics. 2004 Feb;5(2):101.
2. He et al., PLoS genetics. 2006 Jun 2;2(6):e88.
3. Jeong et al., Nature. 2001 May;411(6833):41.

- Tool used: Cytoscape plugin NetworkAnalyzer

- Biological networks are enriched in recurring structural patterns called network motifs including feedback/feedforward loops¹.
- They induce non-intuitive behavior and play a crucial role in system dynamics^{1,2}.

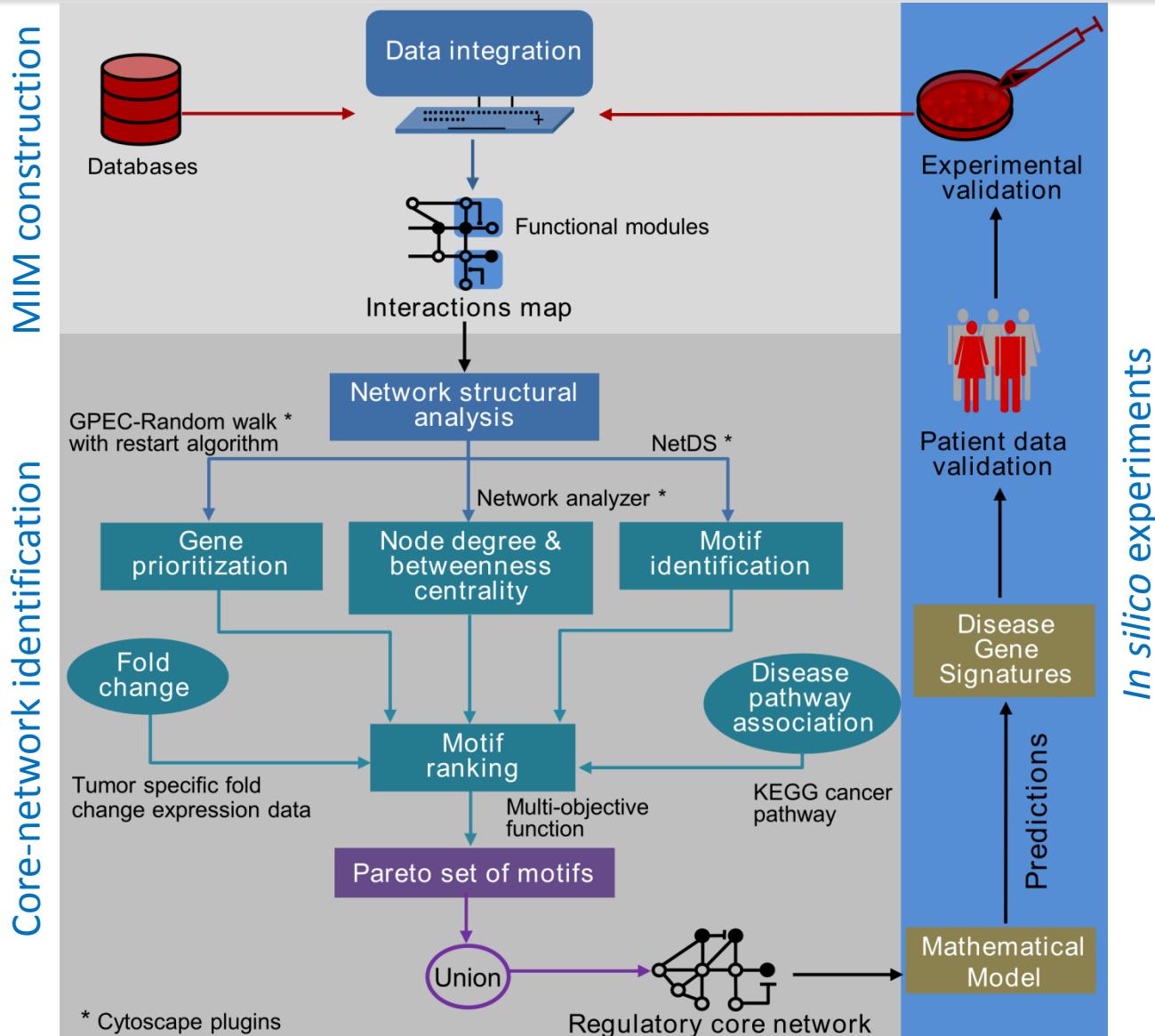


For mechanism-based investigations, these regulatory loops provide valuable insights into the regulation of cellular processes but identification of important loops is challenging.

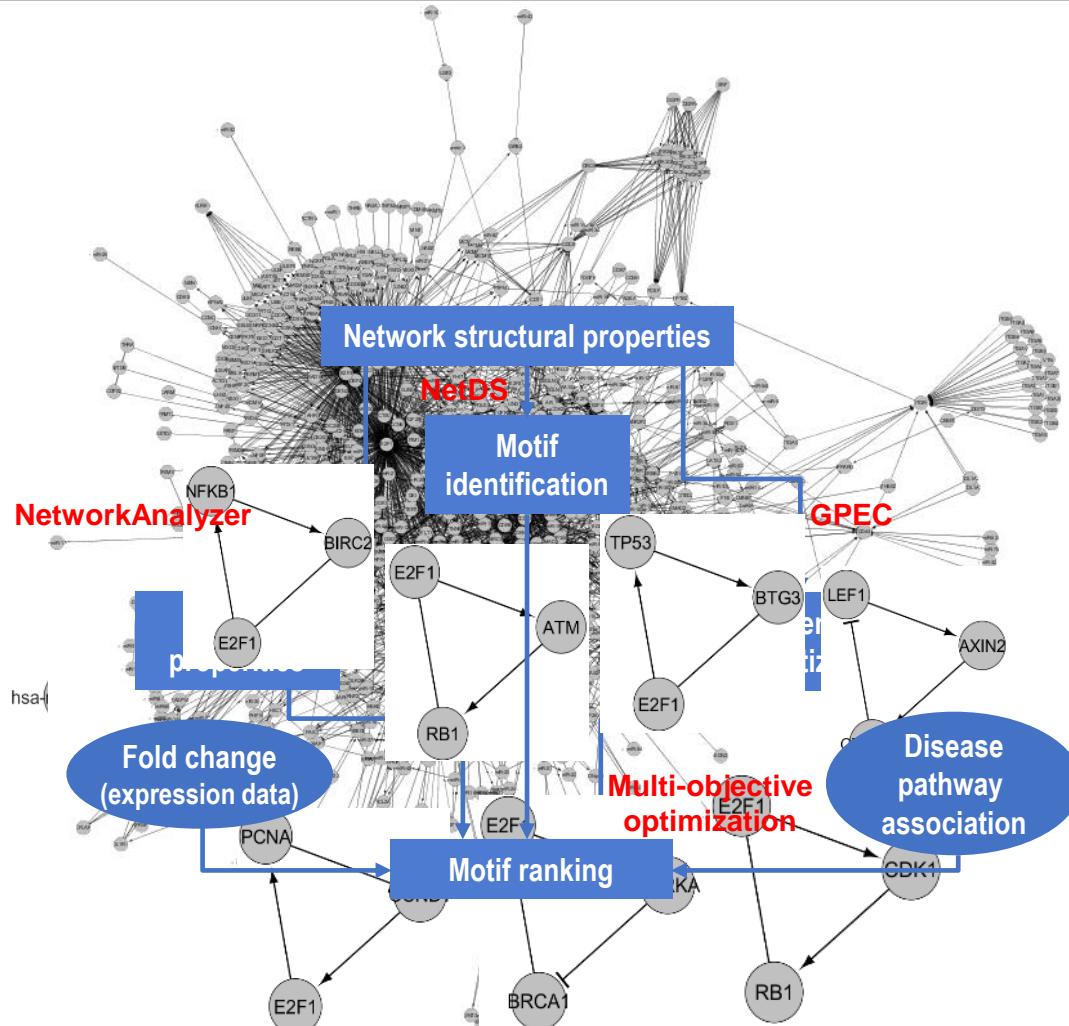
- Tool used: Cytoscape plugin NetDS

1. Alon U. Nature Reviews Genetics. 2007 Jun;8(6):450.
2. Yeger-Lotem et al., PNAS genetics. 2004 Apr 20;101(16):5934-9.

Integrative workflow

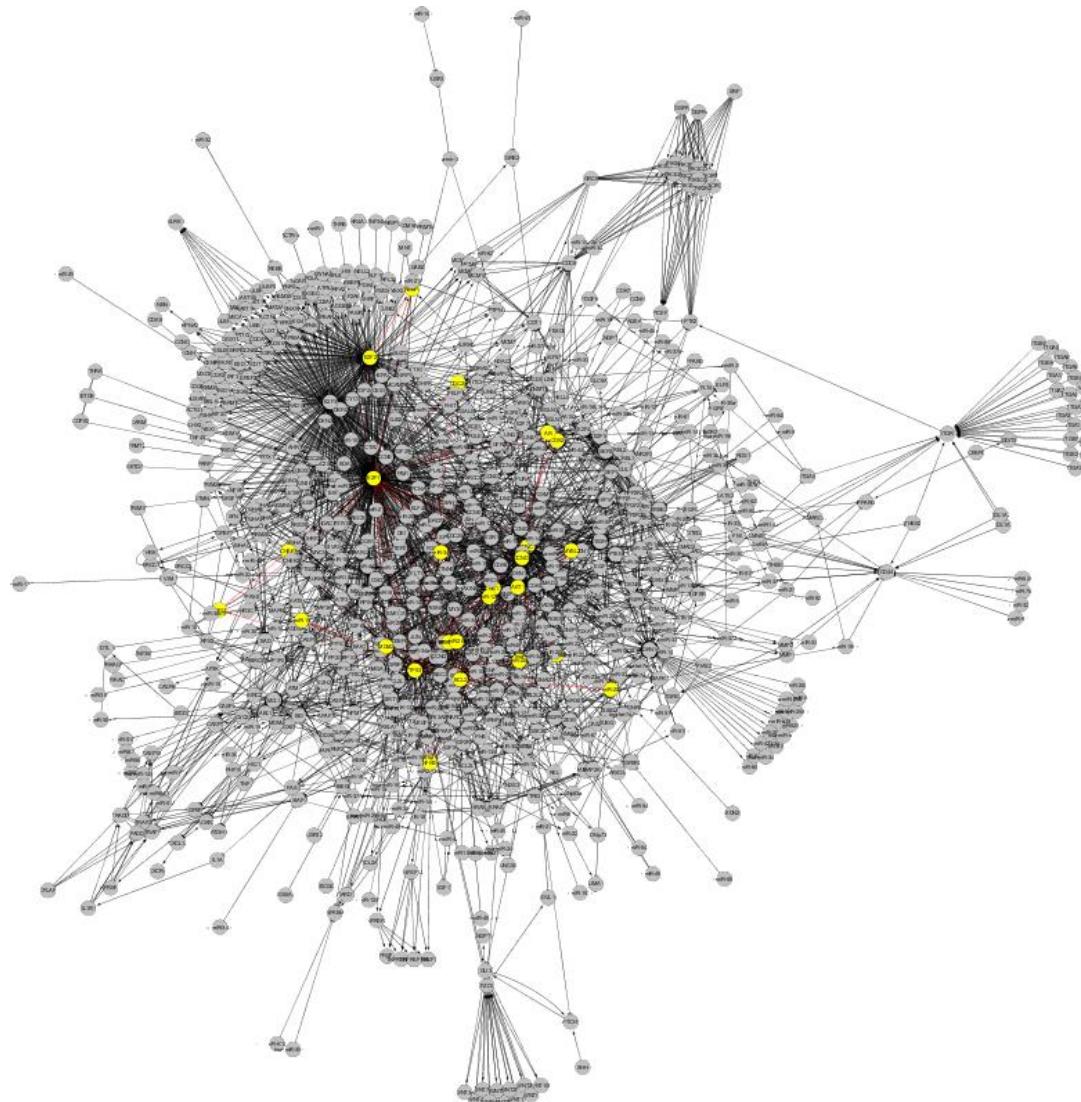


Identification of the regulatory core: Motifs ranking

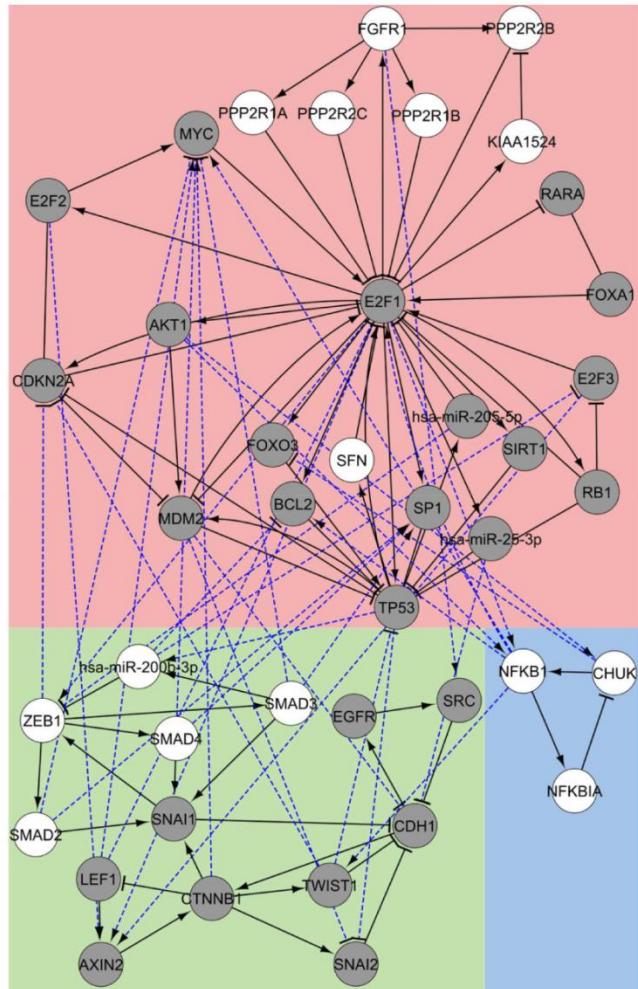


$$F_{M_{jk}} = \frac{w_{1k}}{2} \cdot \frac{\langle ND \rangle_j}{\max(ND)} + \frac{w_{1k}}{2} \cdot \frac{\langle BC \rangle_j}{\max(BC)} + w_{2k} \cdot \frac{\langle GP \rangle_j}{\max(GP)} + w_{3k} \cdot \frac{\langle |FC| \rangle_j}{\max(|FC|)} + w_{4k} \cdot \frac{\langle DP \rangle_j}{\max(DP)}$$

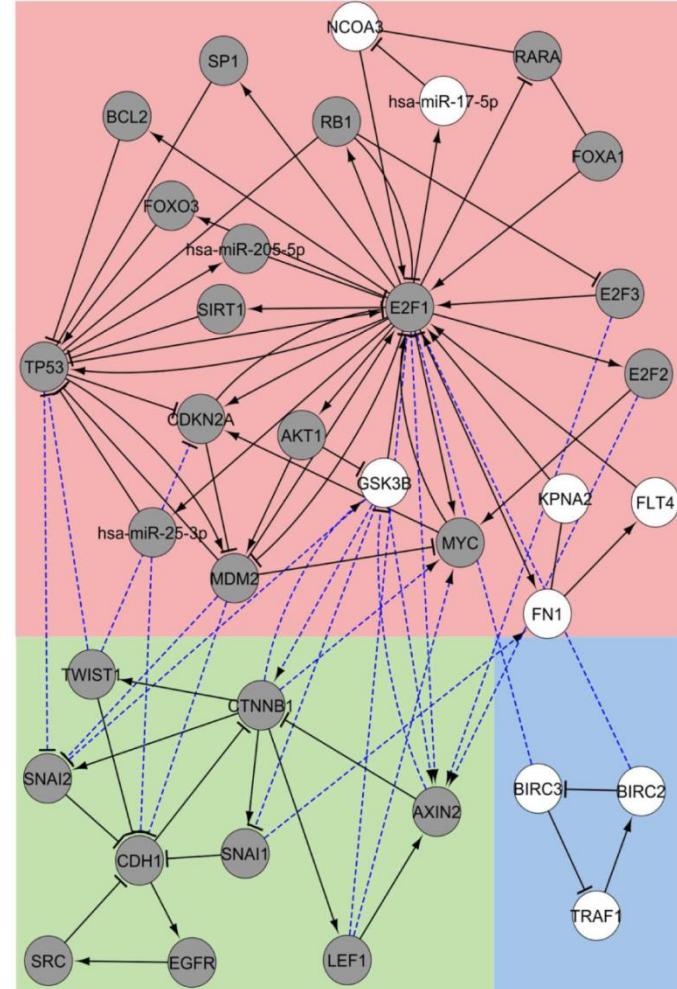
Identification of a regulatory core from the large network



Bladder cancer



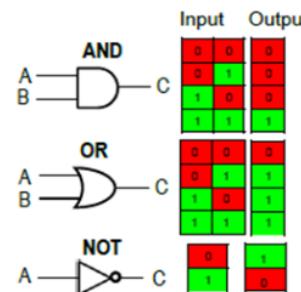
Breast cancer



- Stimulus response behavior
- Perturbation analysis

- Simple yet powerful modelling formalism in explaining the in-put/out-put behavior of large biochemical systems
- Large logic-based models are easier to analyze, compared to large systems of differential equations.
- Best choice when detailed quantitative information is not available
- Boolean models are simplest logical modeling approach

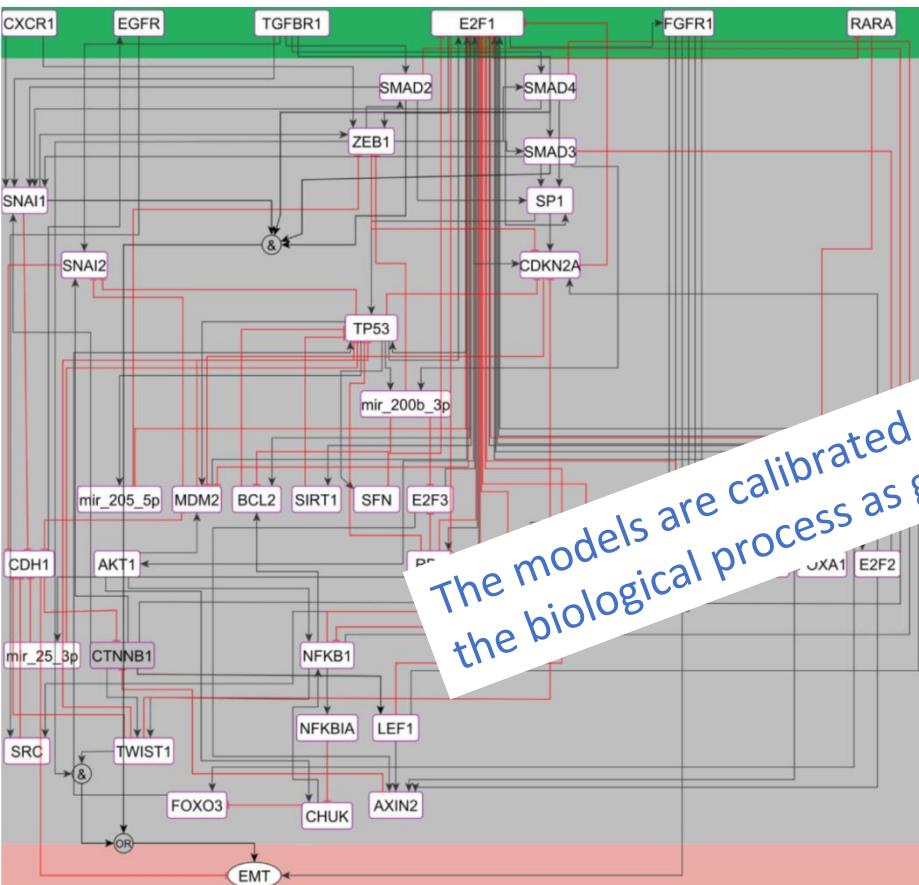
 1: ON or  0: OFF



	Input	Output
AND	0 0	0
	0 1	0
	1 0	0
	1 1	1
OR	0 0	0
	0 1	1
	1 0	1
	1 1	1
NOT	0	1
	1	0

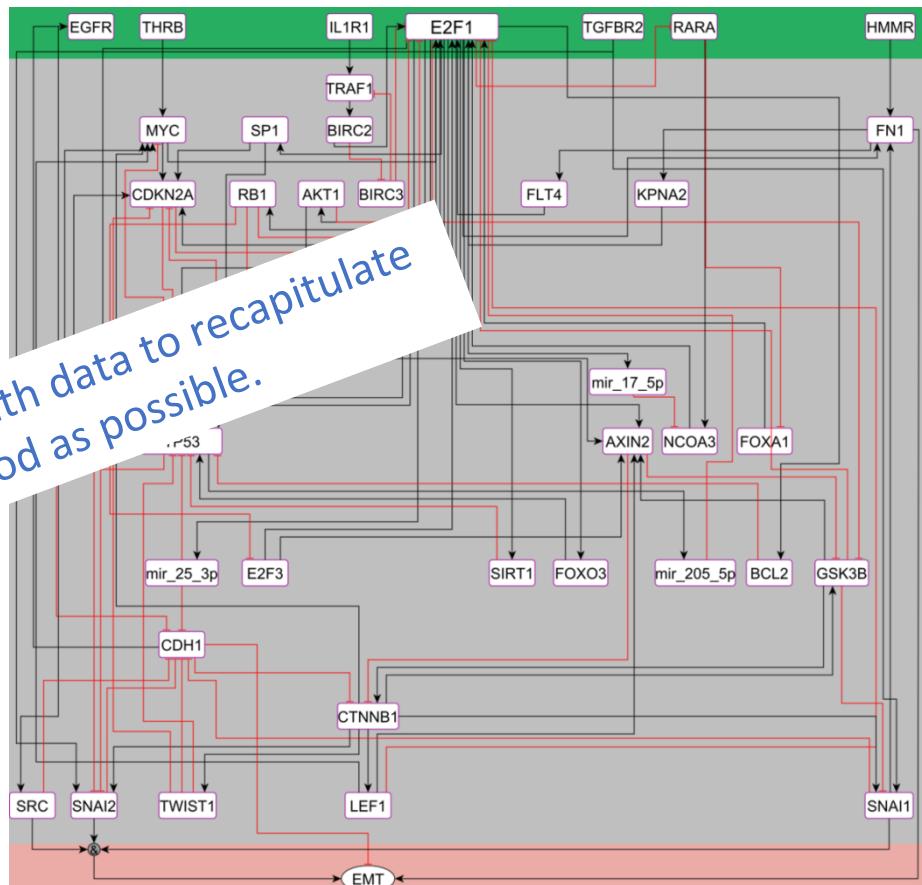
Logic-based model of bladder cancer

Bladder cancer



The models are calibrated with data to recapitulate the biological process as good as possible.

Breast cancer



Tool used:

- ProMoT for model development; S.Mirschel et al. 2009
- yEd for graphical visualization
- CellNetAnalyzer for model simulation ; Klamt et al. 2007



Model simulation results

BLADDER CANCER								
E2F1	TGFBR1	FGFR1	EGFR	CXCR1	RARA	EMT		
0	0	0	0/1	0/1	0/1	0		
0	0	1	0/1	0/1	0/1	1		
0	1	0	0/1	0/1	0/1	1		
0	1	1	0/1	0/1	0/1	2		
1	0	0	0/1	0/1	0/1	1		
1	0	1	0/1	0/1	0/1	2		
1	1	0	0/1	0/1	0/1	2		
1	1	1	0/1	0/1	0/1	3		
BREAST CANCER								
E2F1	TGFBR2	EGFR	HMMR	VEGF	THRB	IL1R1	RARA	EMT
0	0	0	0/1	0/1	0/1	0/1	0/1	0
0	0	1	0/1	0/1	0/1	0/1	0/1	1
0	1	0	0/1	0/1	0/1	0/1	0/1	1
0	1	1	0/1	0/1	0/1	0/1	0/1	2
1	0	0	0/1	0/1	0/1	0/1	0/1	1
1	0	1	0/1	0/1	0/1	0/1	0/1	2
1	1	0	0/1	0/1	0/1	0/1	0/1	2
1	1	1	0/1	0/1	0/1	0/1	0/1	3

EMT Phenotype

0	Non invasive
1	Less invasive
2	Moderately invasive
3	Highly invasive



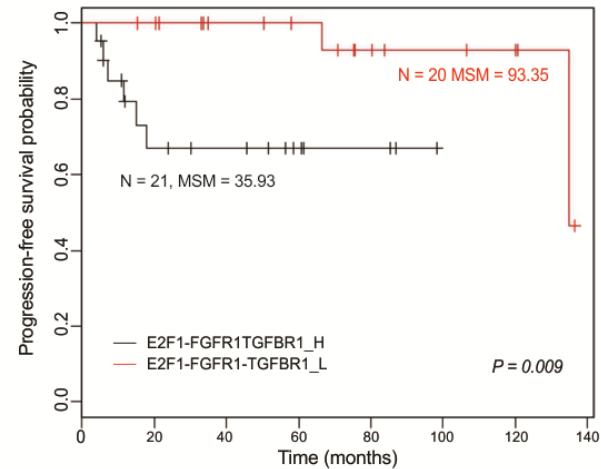
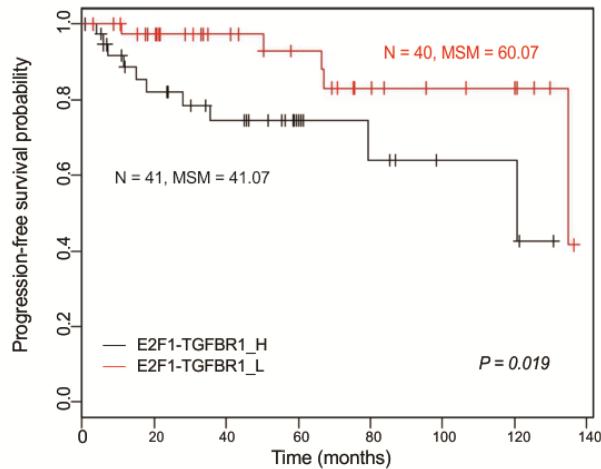
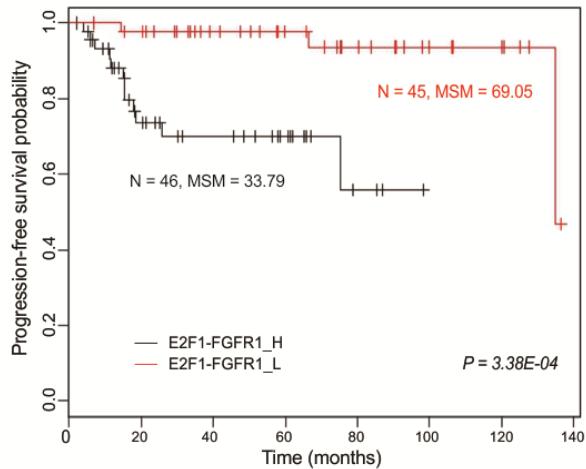
EMT model findings

- Our simulation results suggest that
 1. When E2F1, TGFBR1 and FGFR1 are simultaneously active bladder cancer cells become highly invasive ($EMT = 3$).
 2. A similar effect was observed in breast cancer when E2F1, TGFBR2 and EGFR are simultaneously active.

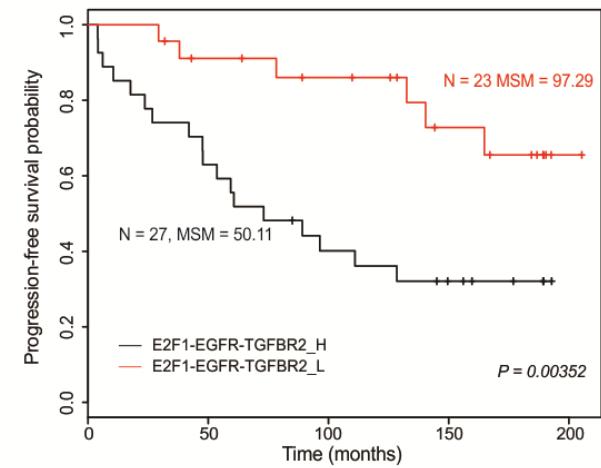
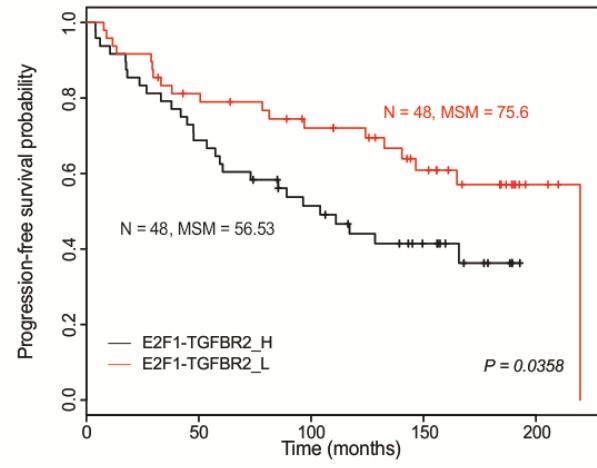
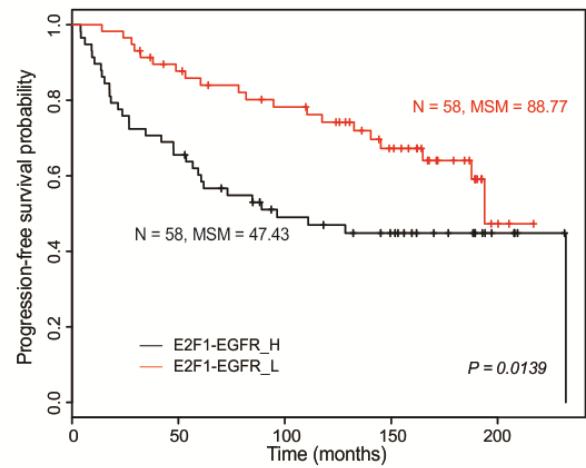
Model Validation



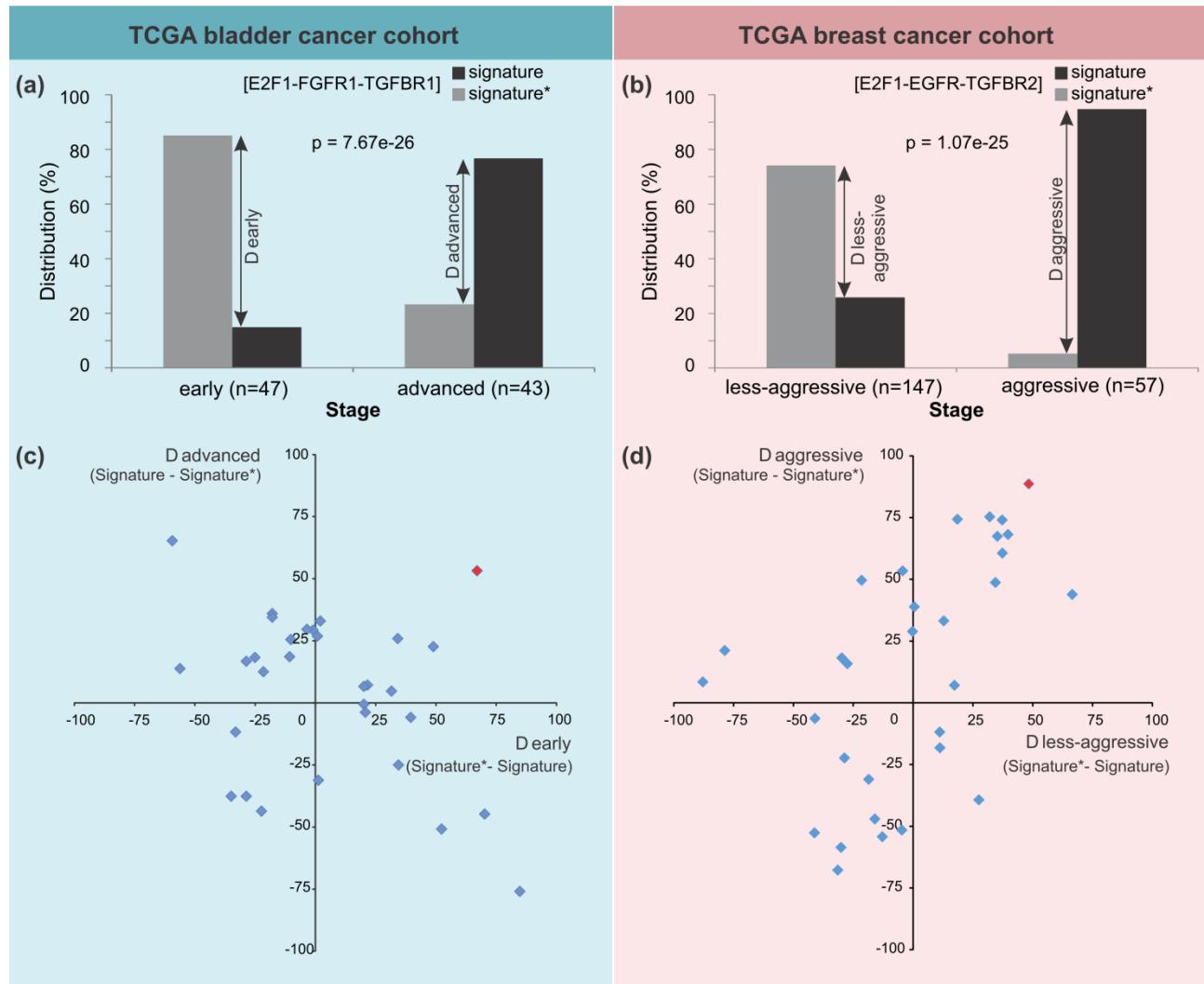
bladder cancer patients



breast cancer patients



Classification of patients based on proposed vs. random signatures

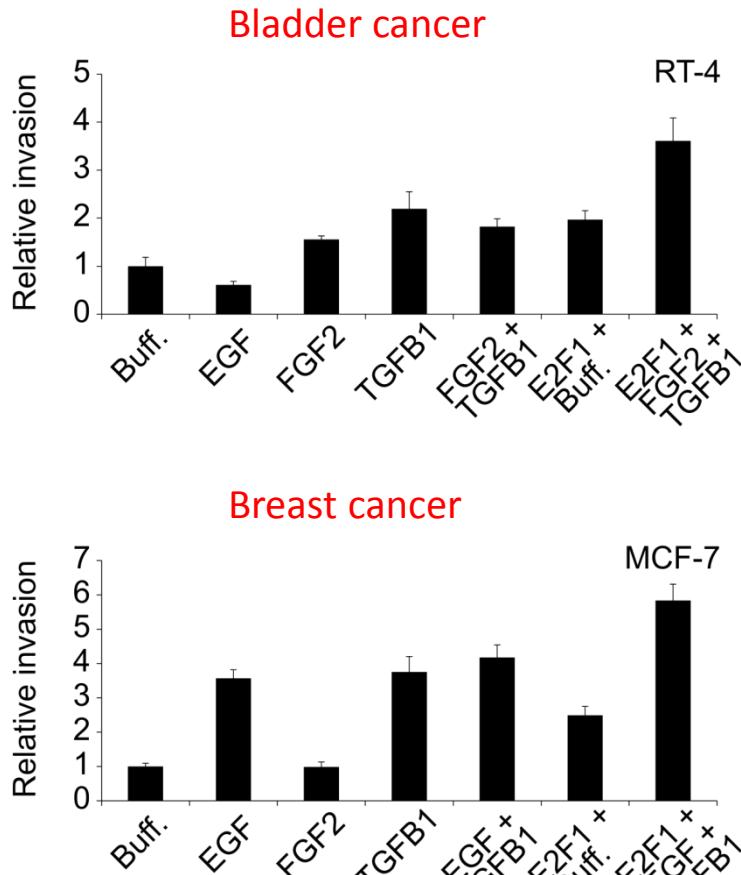


◆ Proposed signatures

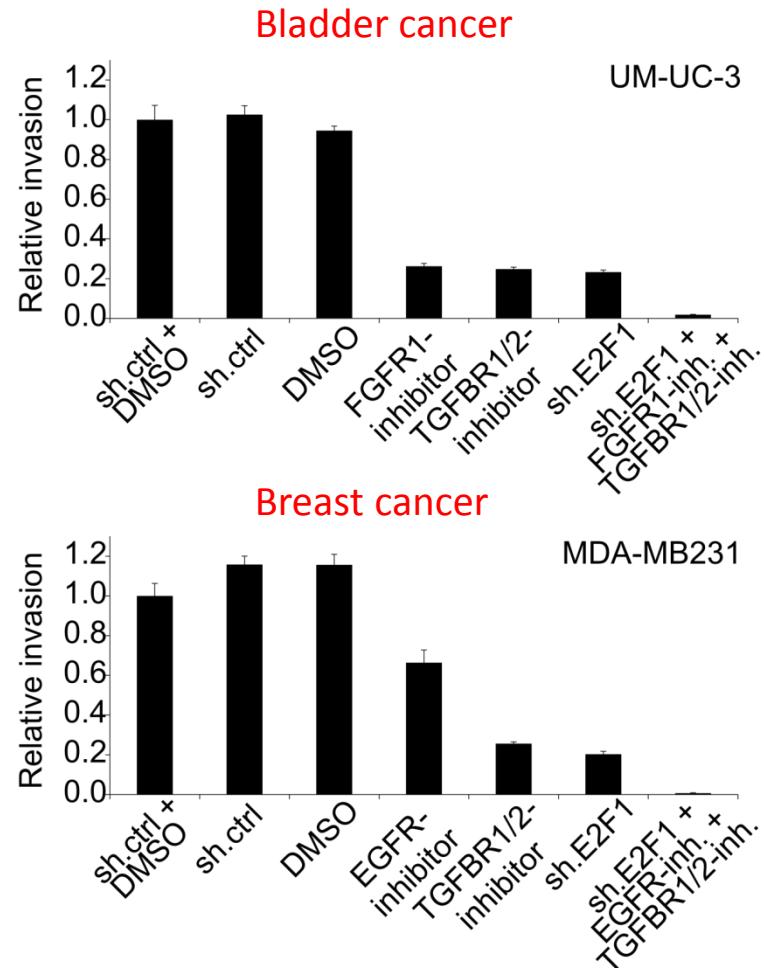
◆ Random signatures

Model driven experimentation

Non-invasive cell lines



Invasive cell lines



In silico perturbation for drug targets



(a) Bladder cancer									
Signature			Double <i>in silico</i> perturbations in regulatory layer						Output
E2F1	TGFBR1	FGFR1	ZEB1	TWIST1	SNAI1	NFKB1	SMAD2,3,4	CDH1	EMT
1	1	1	1	1	1	1	1	0	3
1	1	1	0	0	1	1	0	1	1
1	1	1	0	1	0	1	0	1	1
1	1	1	0	0	1	0	0	1	1
1	1	1	0	1	1	1	0	1	1
1	1	1	1	0	1	1	0	1	1
1	1	1	1	0	1	0	0	1	1

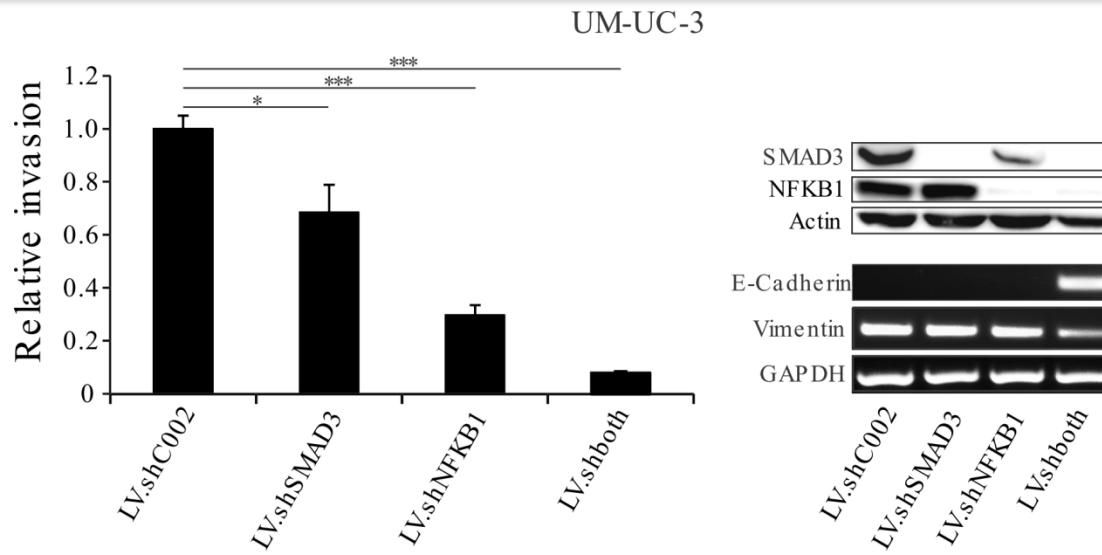
(b) Breast cancer									
Signature			Double <i>in silico</i> perturbations in regulatory layer						Output
E2F1	TGFBR2	EGFR	SRC	FN1	SNAI1	SNAI2	CDH1		EMT
1	1	1	1	1	1	1	0		3
1	1	1	0	1	1	1	1		1
1	1	1	0	0	1	1	0		1
1	1	1	0	1	0	1	1		1
1	1	1	0	1	1	0	1		1
1	1	1	1	0	1	1	1		1
1	1	1	1	1	0	1	1		1
1	1	1	1	1	1	0	1		1
1	1	1	1	0	0	1	0		1
1	1	1	1	0	1	0	0		1

Model driven experimentation

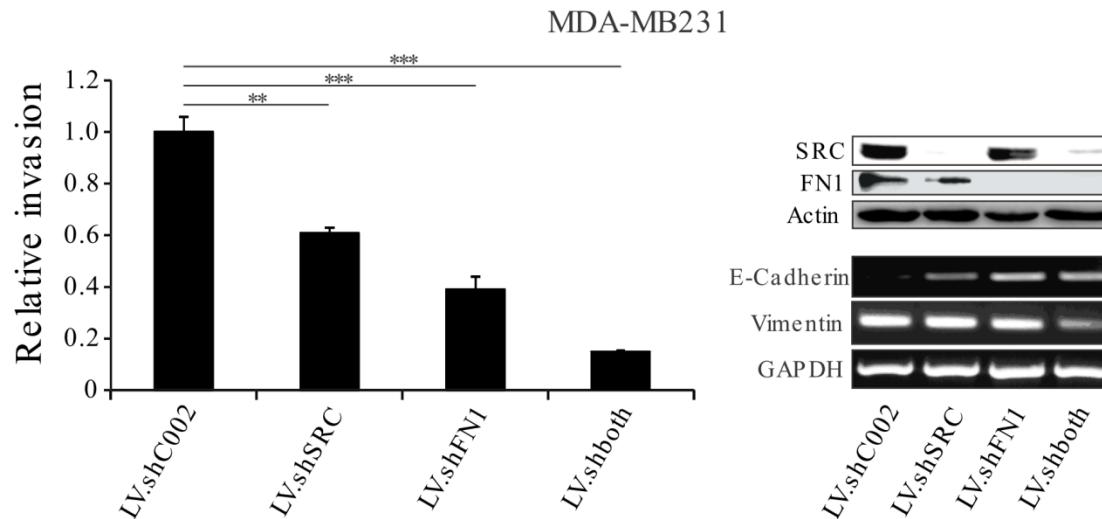


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Bladder cancer



Breast cancer



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