

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

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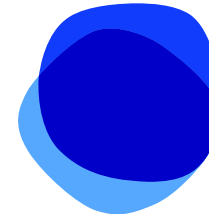
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08.09.2018  
ECCB 18, Athens

**Universität  
Rostock**

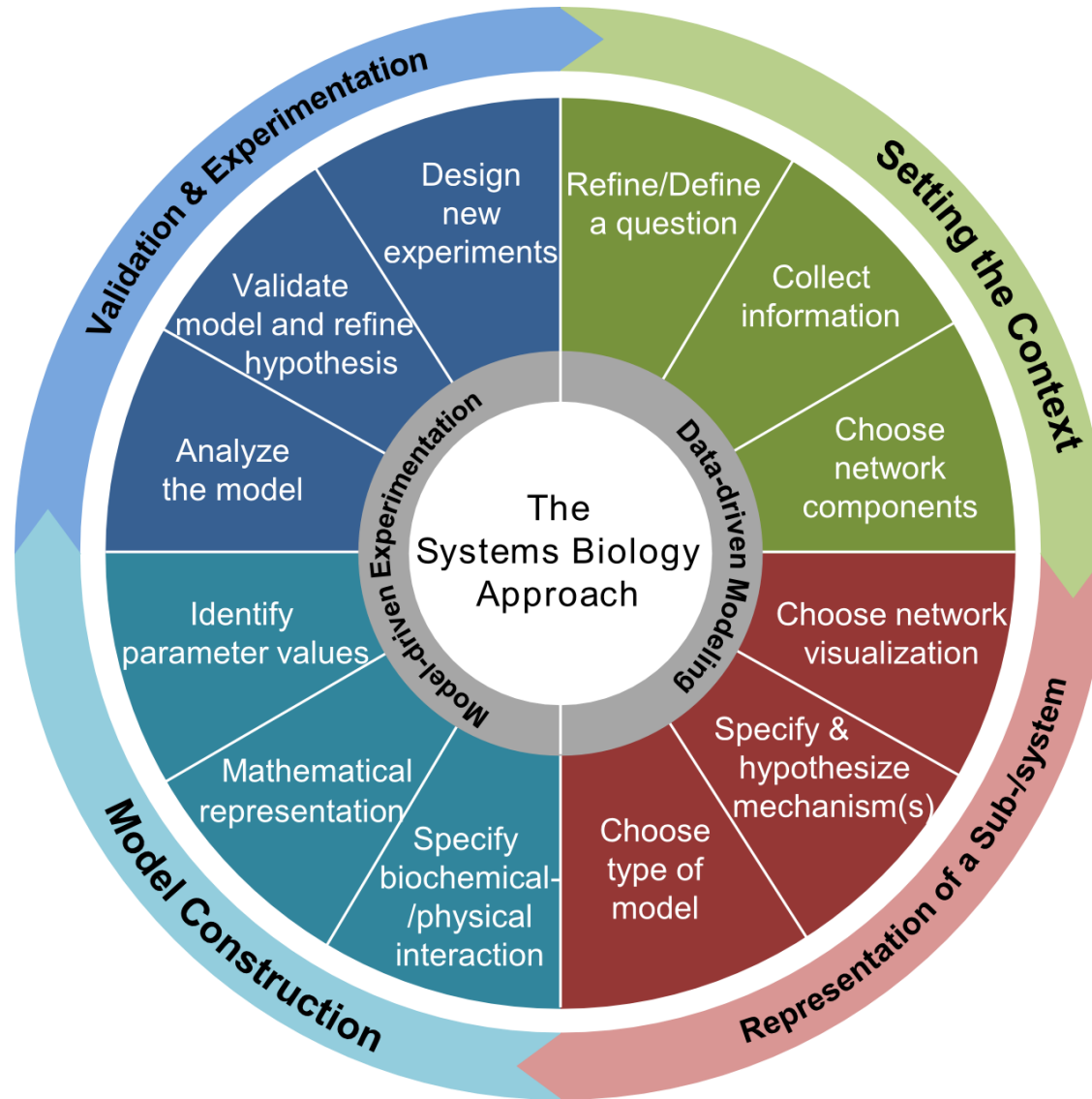


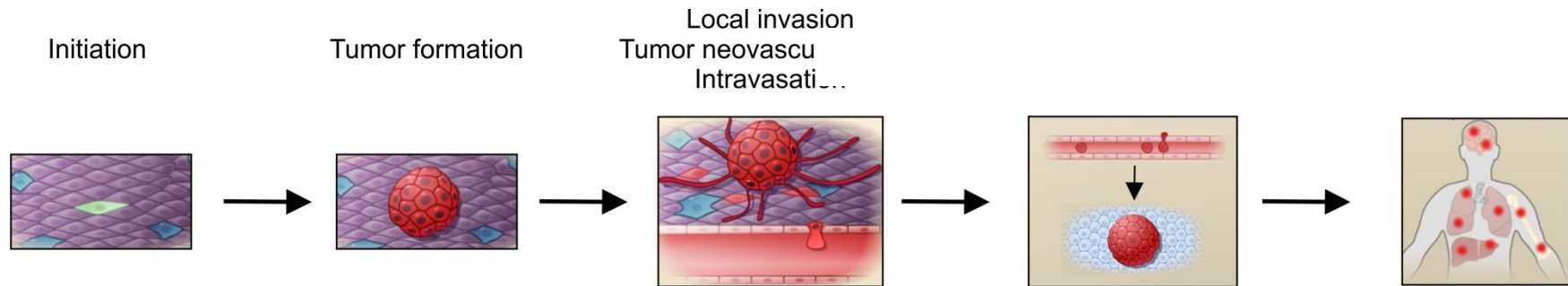
Traditio et Innovatio



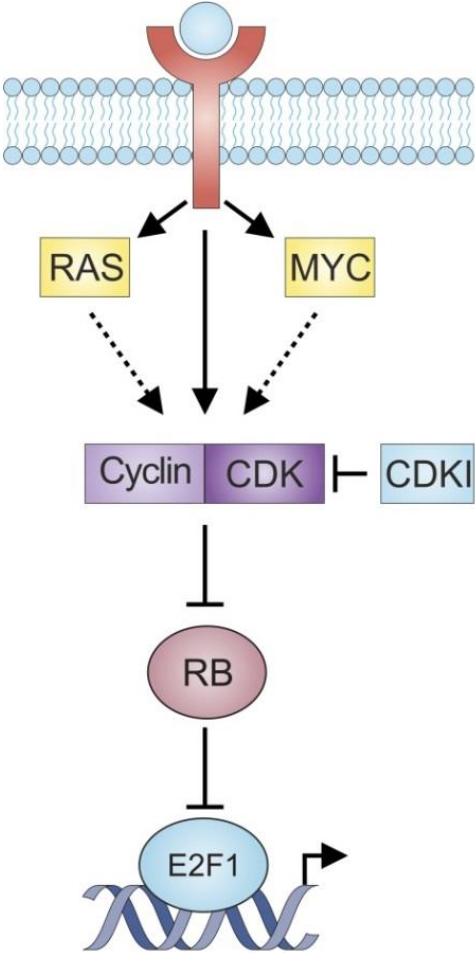
**SYSTEMS BIOLOGY  
BIOINFORMATICS  
ROSTOCK**

- 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





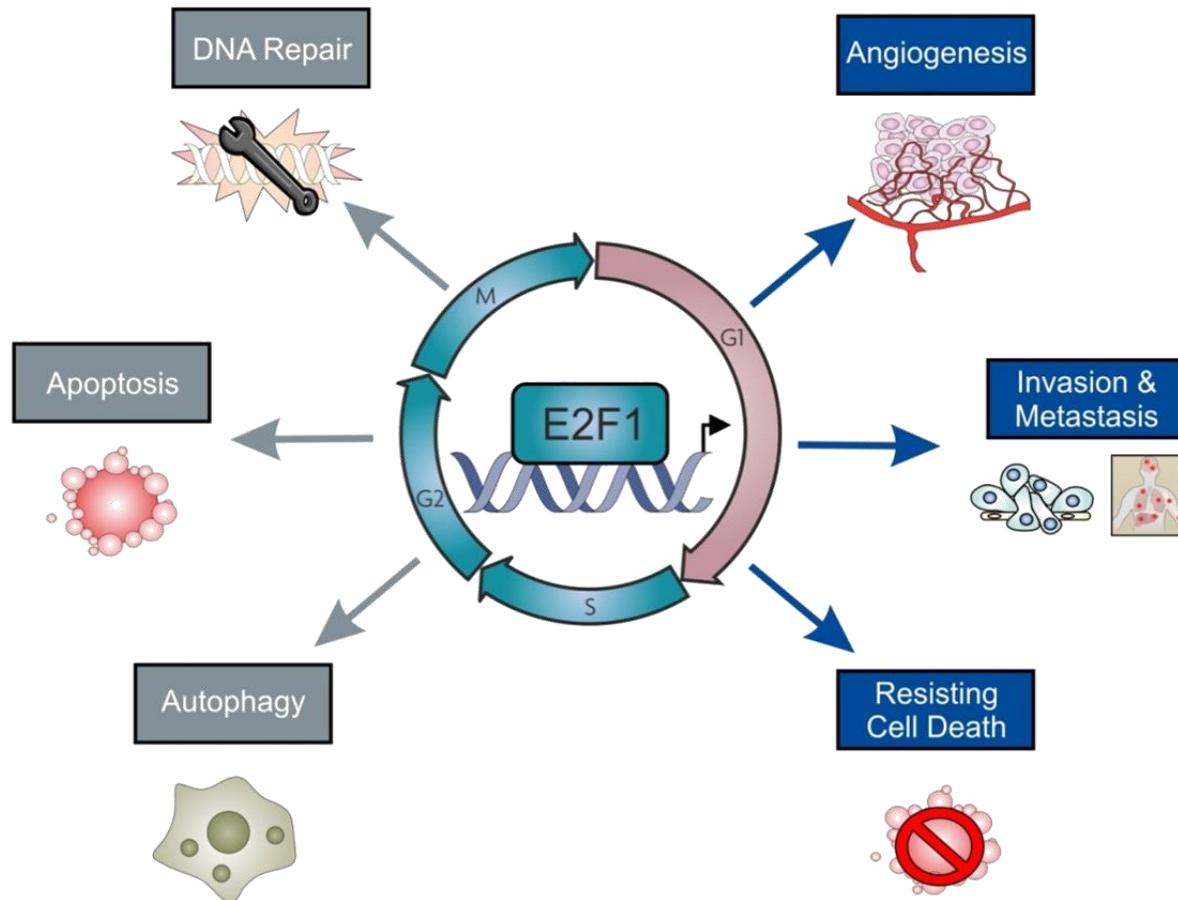
- Malignant tumors and metastasis are frequently resistant to chemotherapy.
- Therapy-induced resistance will result in recurrence and further disease progression.
- The **transcription factor** E2F1 has recently been identified as a key regulator in tumor invasiveness and metastasis by switching duties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



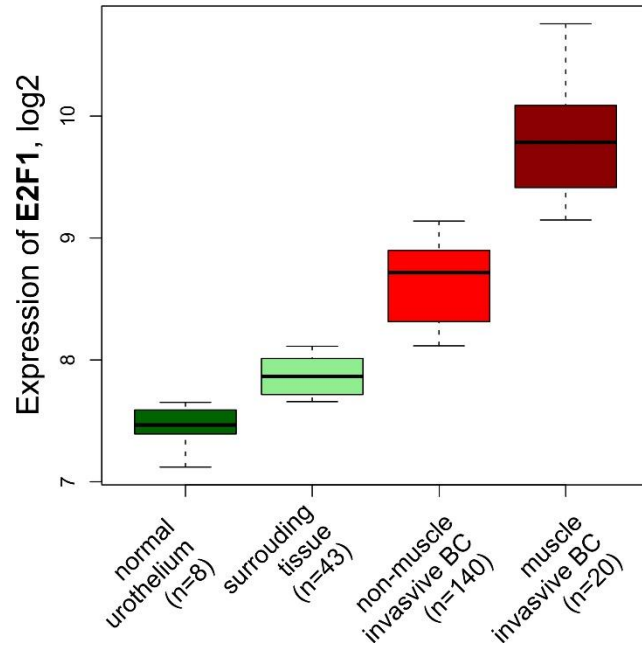
# The dual role of the E2F1 in carcinogenesis

... as a tumor suppressor

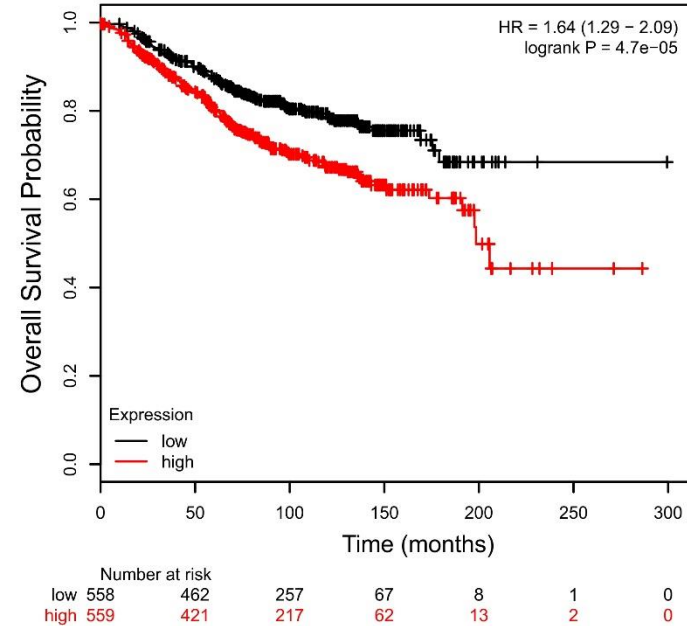
... as a metastasis inducer



## Bladder cancer

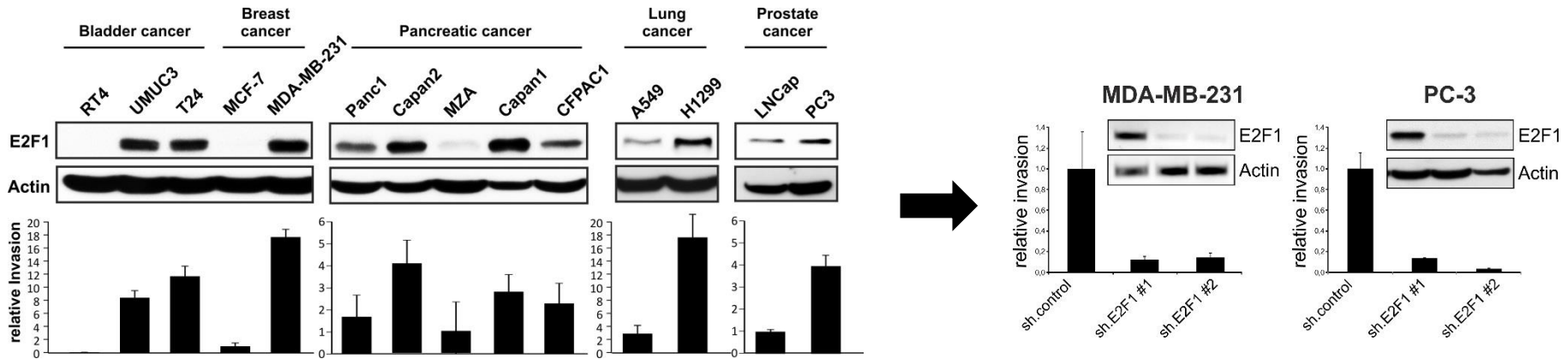


## Breast Cancer

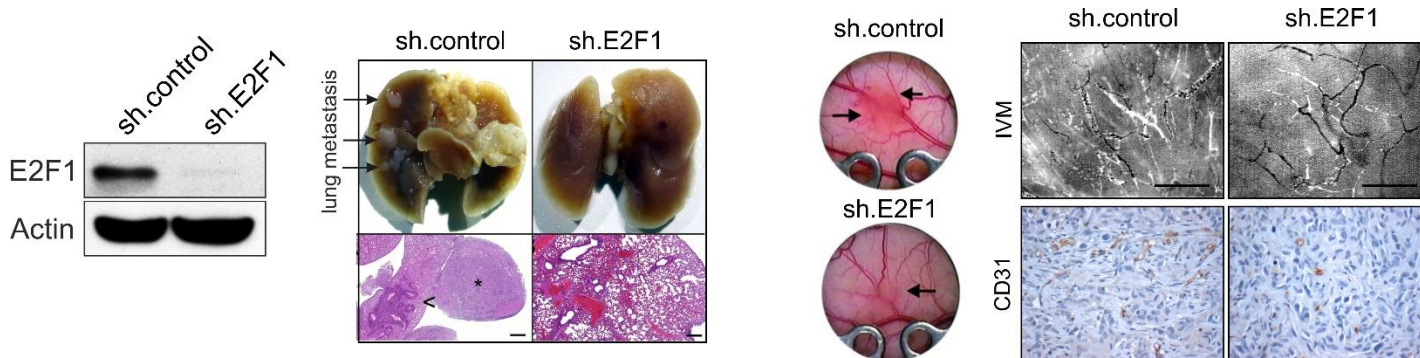


# E2F1 promotes tumor progression

## Cell migration and invasion



## Tumor angiogenesis and metastasis

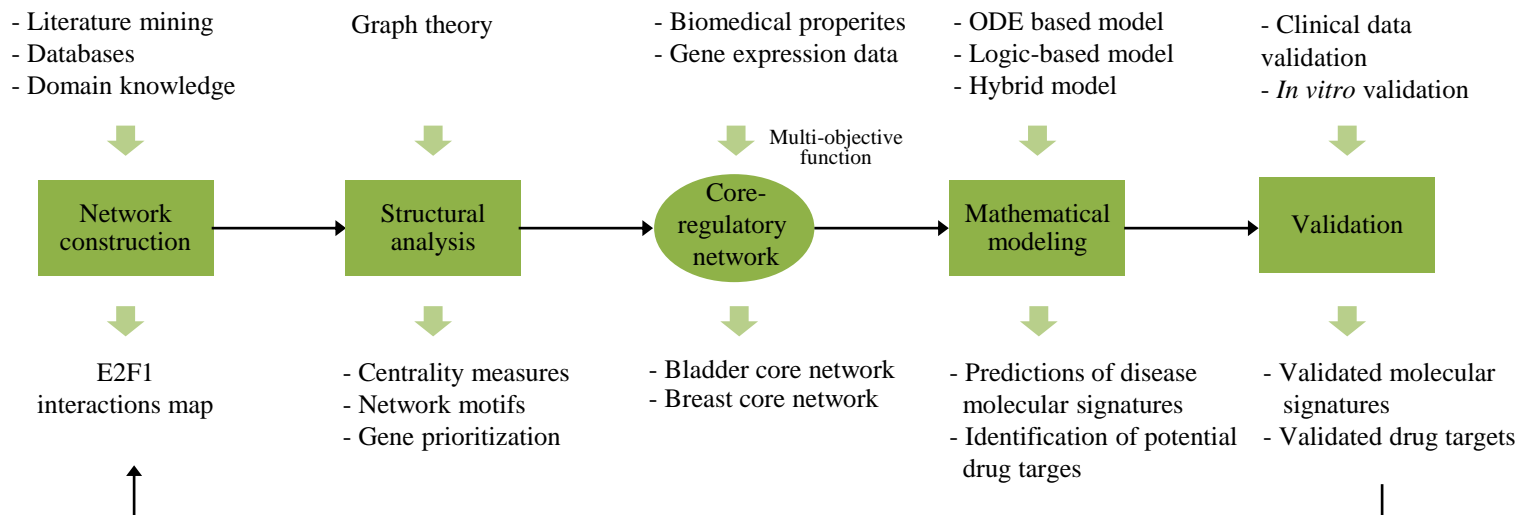


Alla et al., *J. Natl. Cancer Inst.* 2010

Engelmann et al., *J. Mol. Cell Biol.* 2014



- 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

Literature  
Gene 'A' activates gene 'B'

STRING  
REACTOME  
IntAct  
Human Protein Reference Database

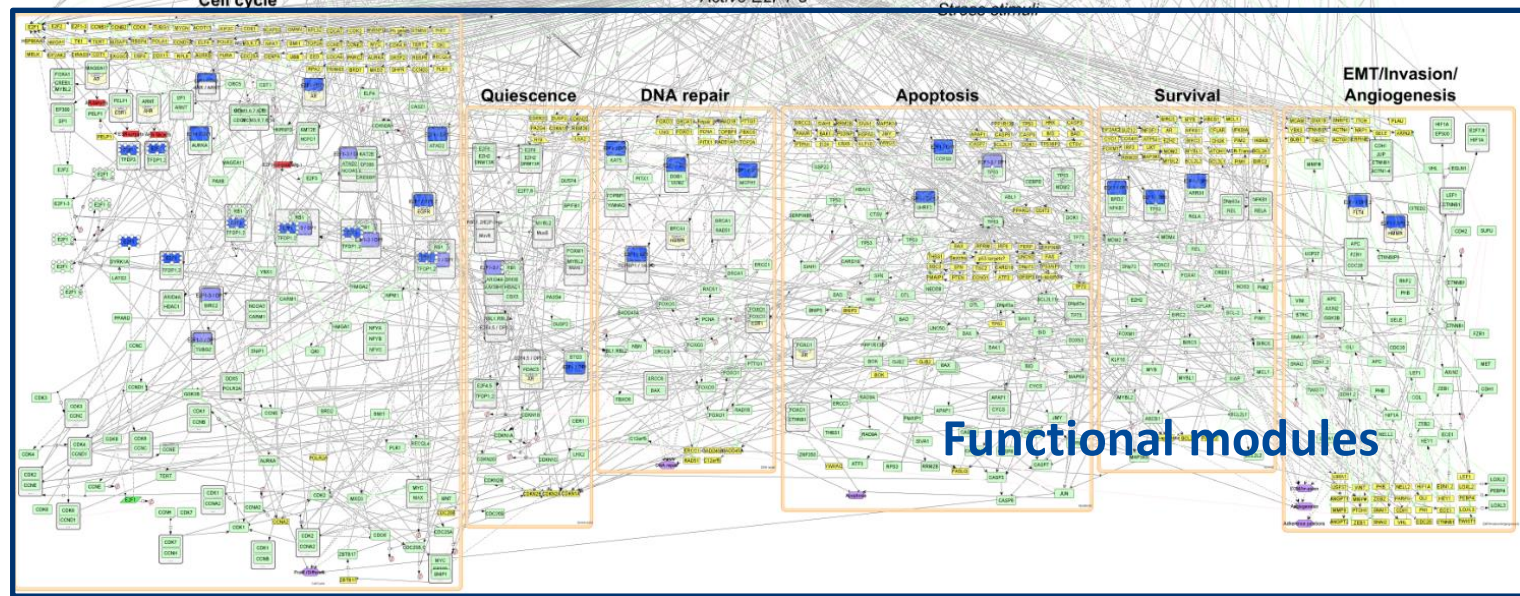
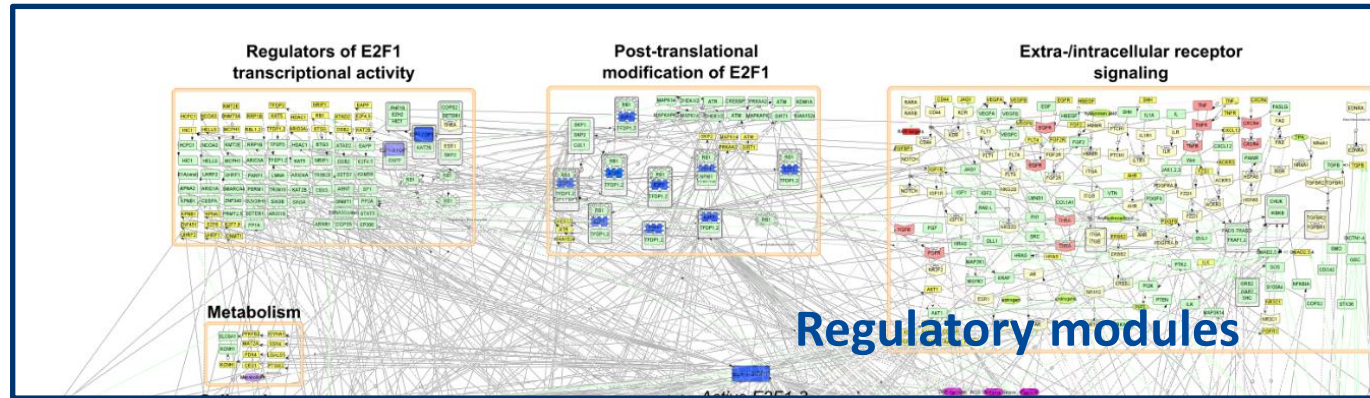
Molecular interactions databases

MicroRNA  
miRtarBase  
TriplexRNA  
MERWalk

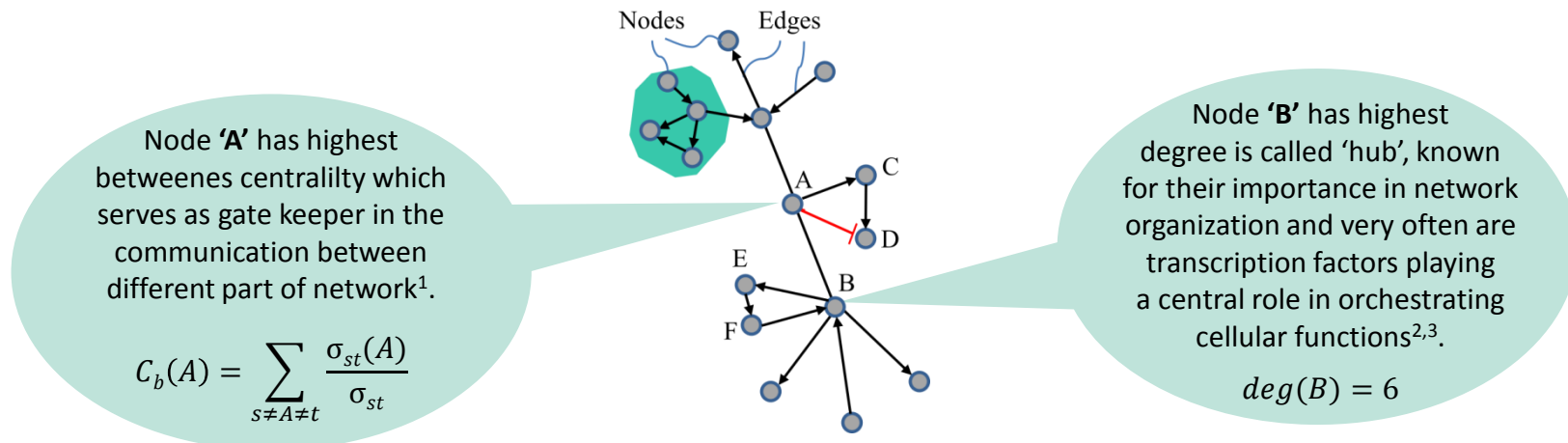
Transcription factor  
TRANSFAC database  
TRRUST  
HTRI

Insertion of regulatory layers

Domain knowledge



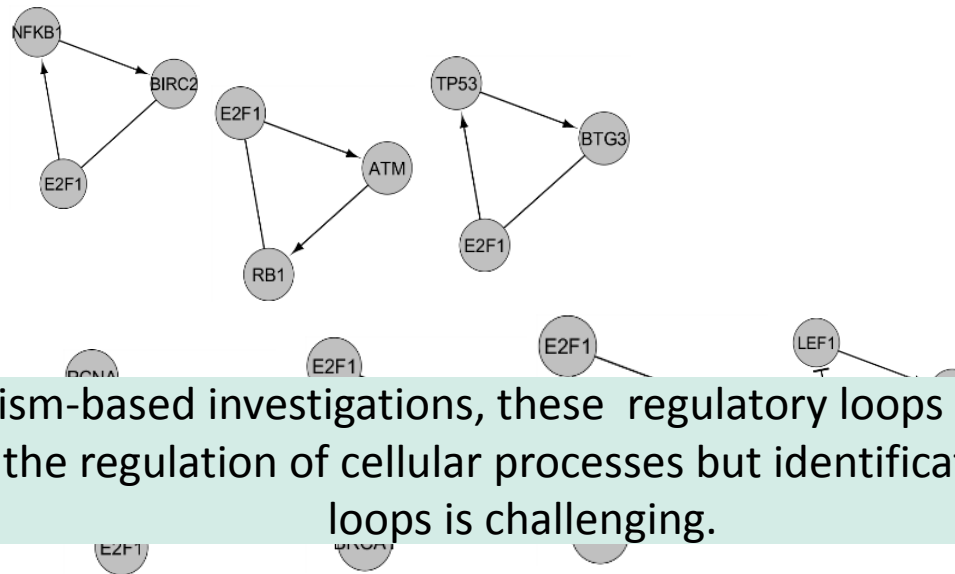
- Network characterization through topological properties (e.g., node degree (ND) and betweenness centrality (BC)) which provide useful information regarding the network architecture<sup>1</sup>.
- **Node degree** is the number of edges connected to a node, and
- **Betweenness centrality** is the number of shortest paths from all nodes to all others that pass through that node.



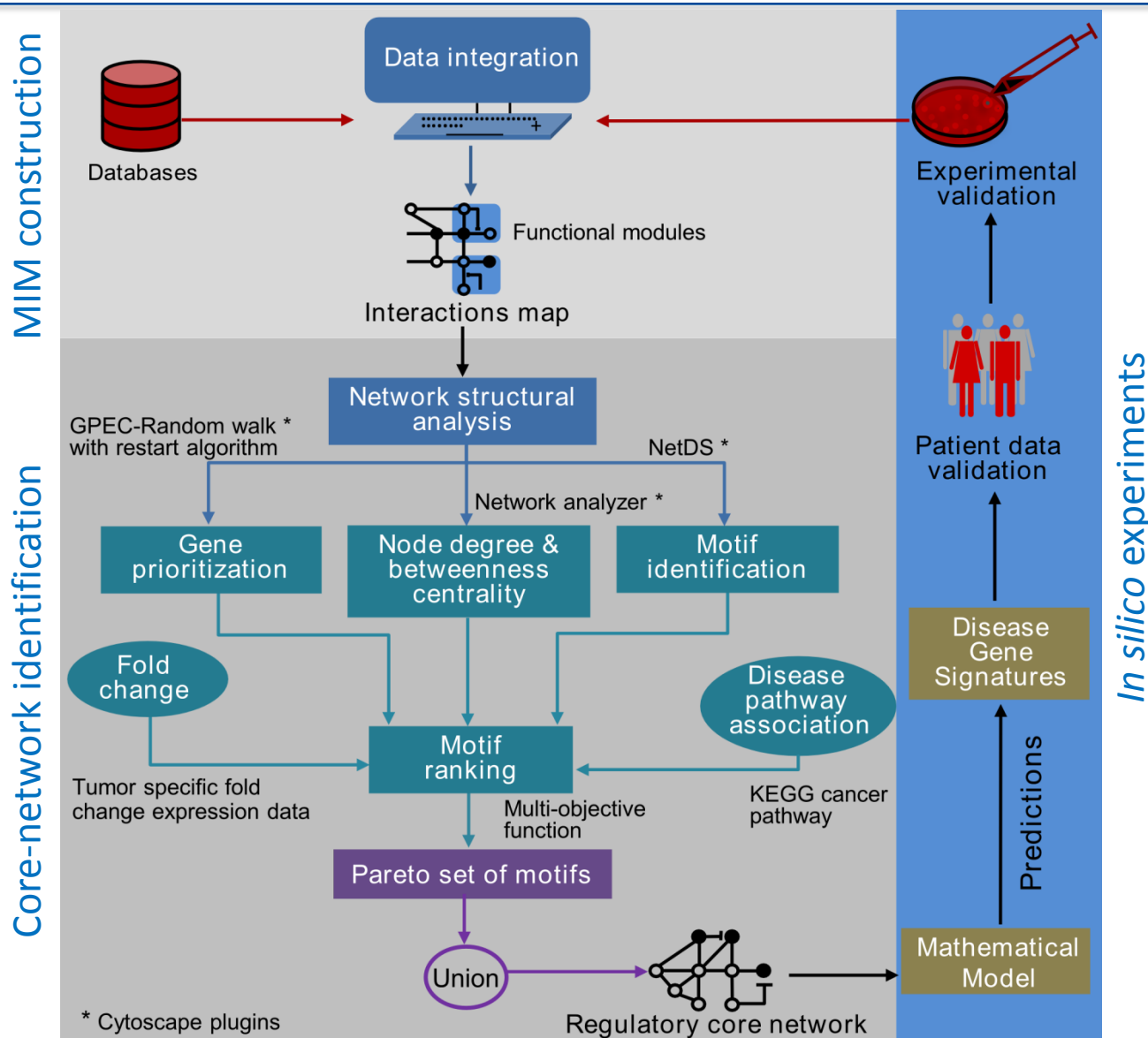
1. Barabasi et al., Nature reviews genetics. 2004 Feb;5(2):101.
2. He et al., PLoS genetics. 2006 Jun 2;2(6):e88.
3. Jeong et al., Nature. 2001 May;411(6833):41.

- Tool used: Cytoscape plugin NetworkAnalyzer

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

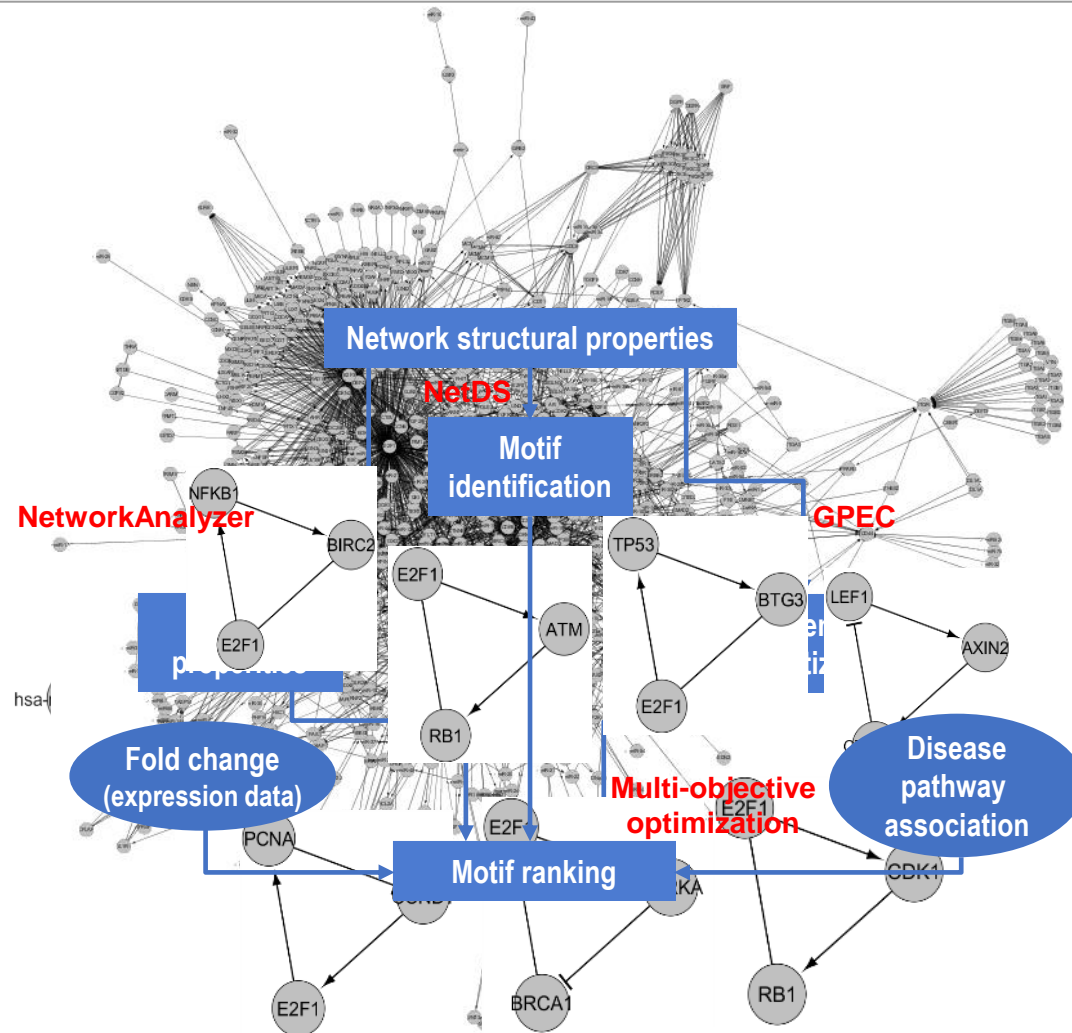


- Tool used: Cytoscape plugin NetDS

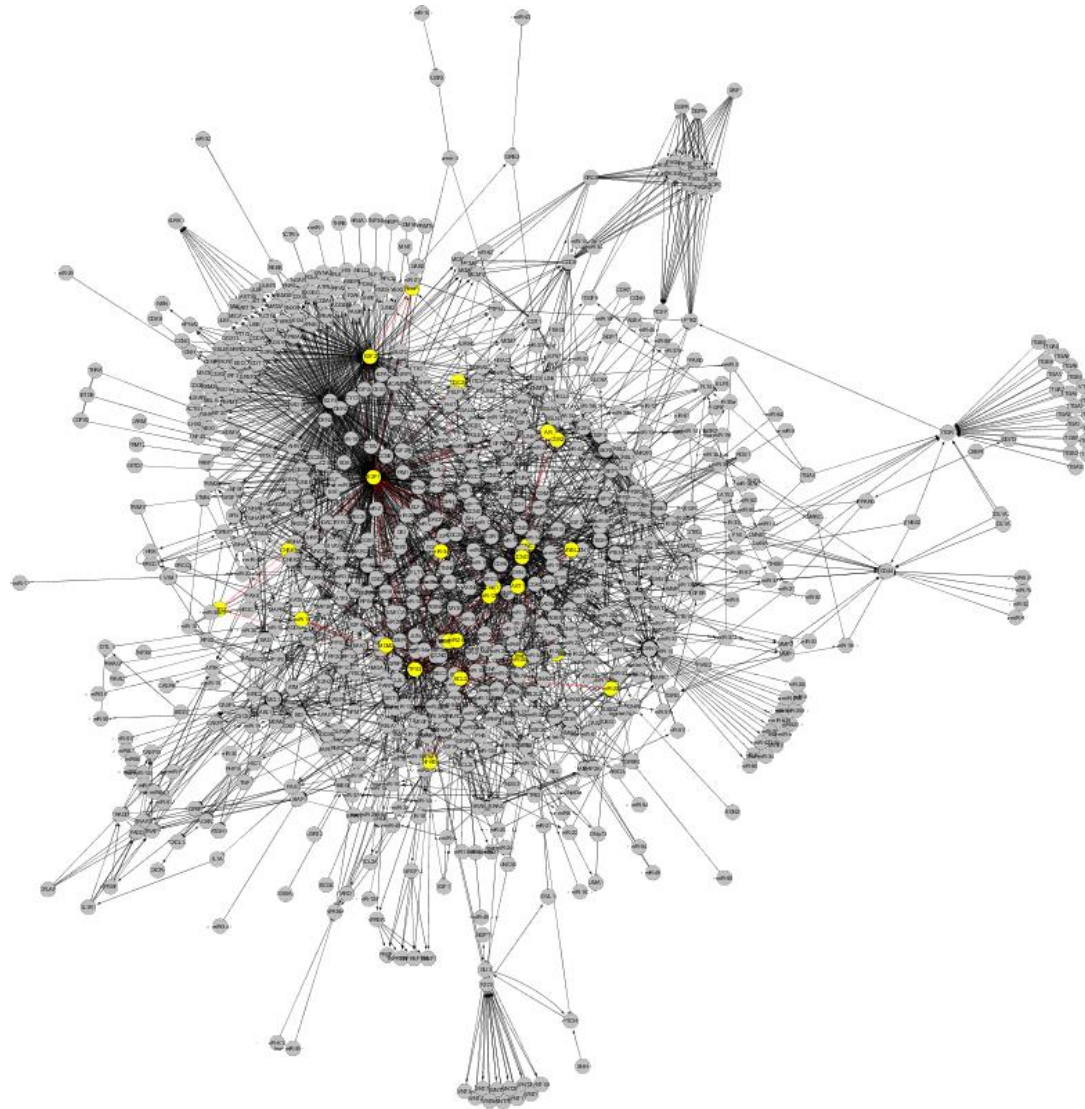




# Identification of the regulatory core: Motifs ranking

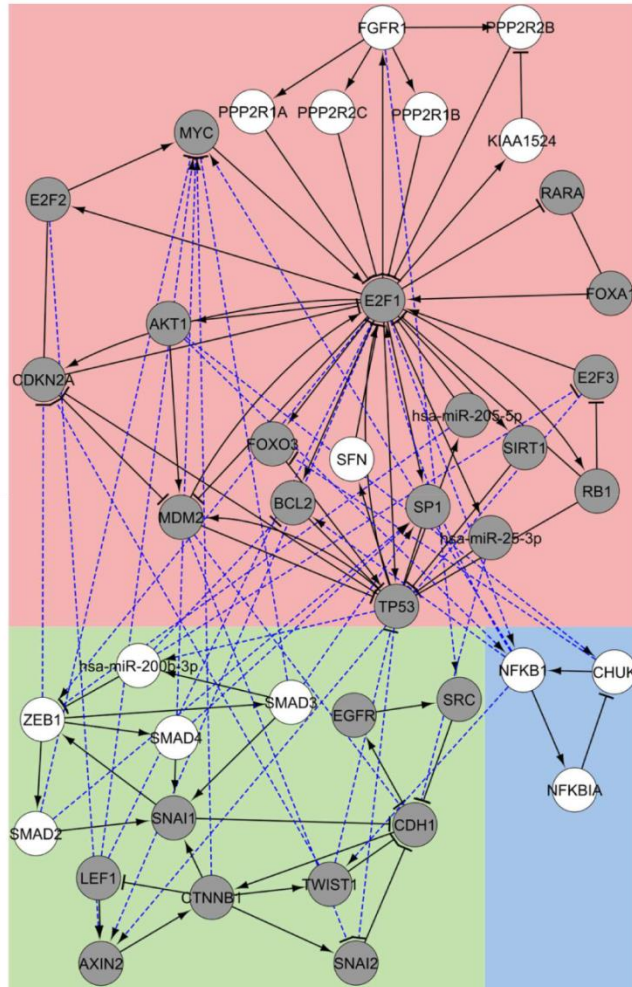


$$F_{M_{jk}} = \frac{w_{1k}}{2} \cdot \frac{\langle ND \rangle_j}{\max(ND)} + \frac{w_{1k}}{2} \cdot \frac{\langle BC \rangle_j}{\max(BC)} + w_{2k} \cdot \frac{\langle GP \rangle_j}{\max(GP)} + w_{3k} \cdot \frac{\langle |FC| \rangle_j}{\max(|FC|)} + w_{4k} \cdot \frac{\langle DP \rangle_j}{\max(DP)}$$

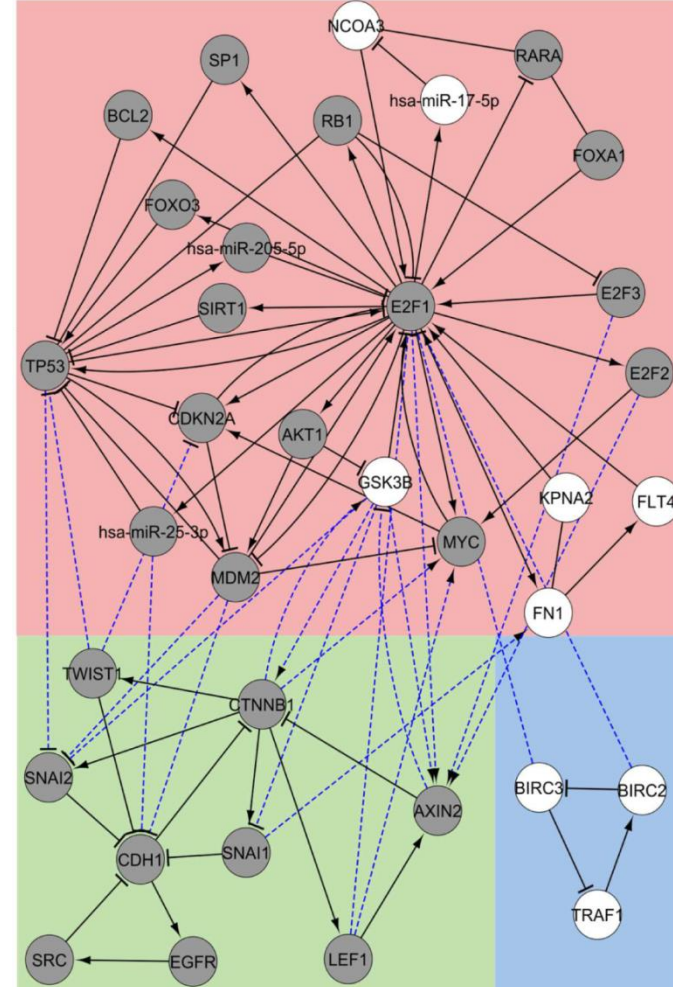




## Bladder cancer

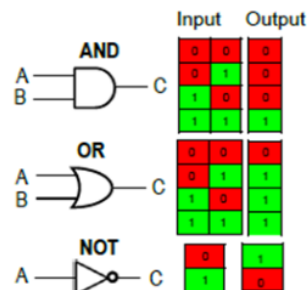
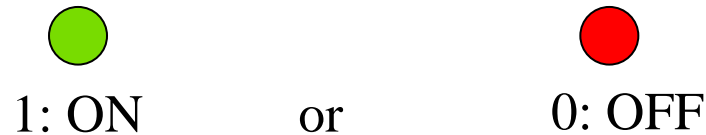


## Breast cancer

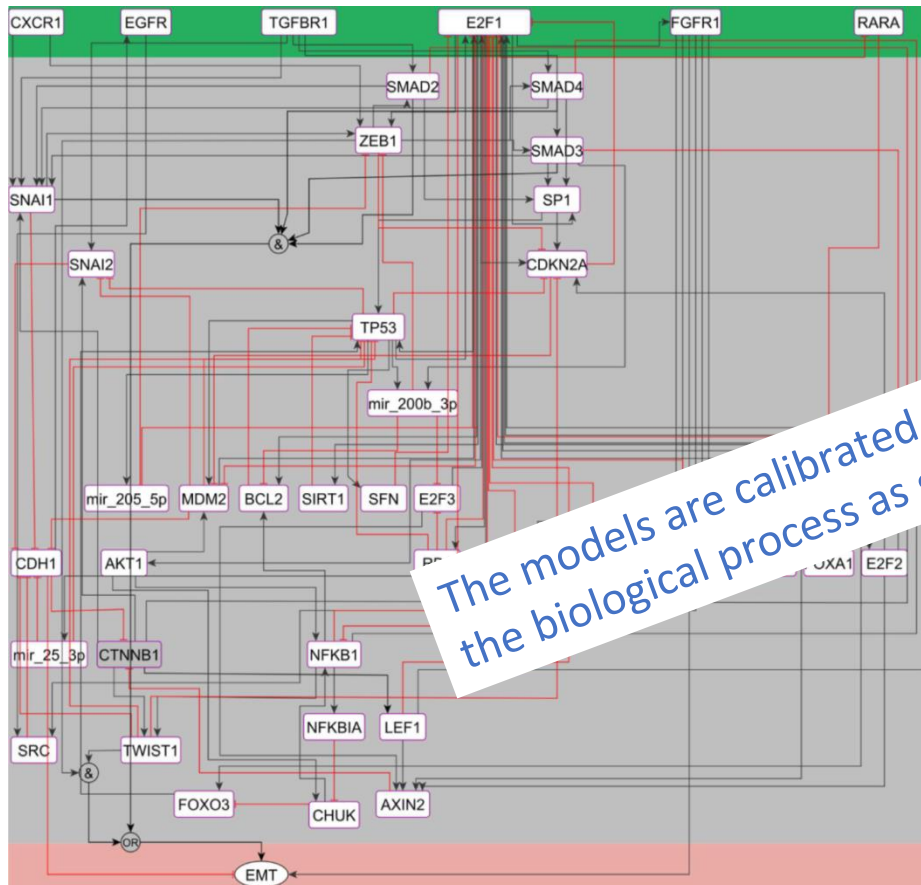


- Stimulus response behavior
- Perturbation analysis

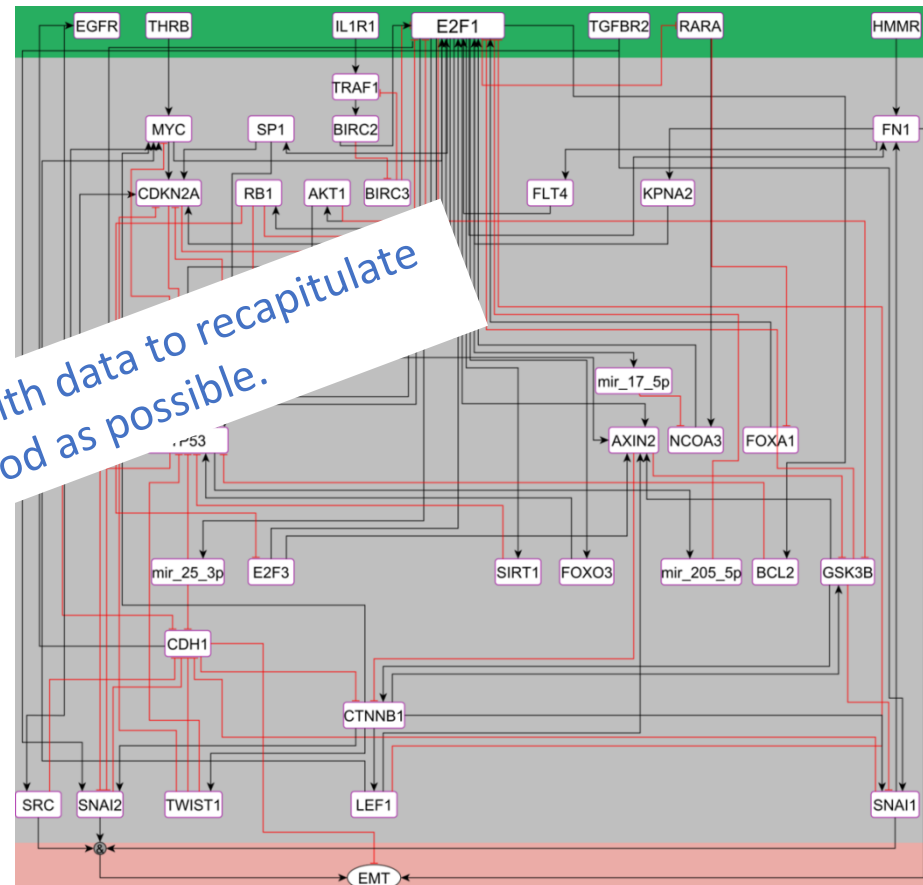
- Simple yet powerful modelling formalism in explaining the in-put/out-put behavior of large biochemical systems
- Large logic-based models are easier to analyze, compared to large systems of differential equations.
- Best choice when detailed quantitative information is not available
- Boolean models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



## Bladder cancer



## Breast cancer



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

### Tool used:

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

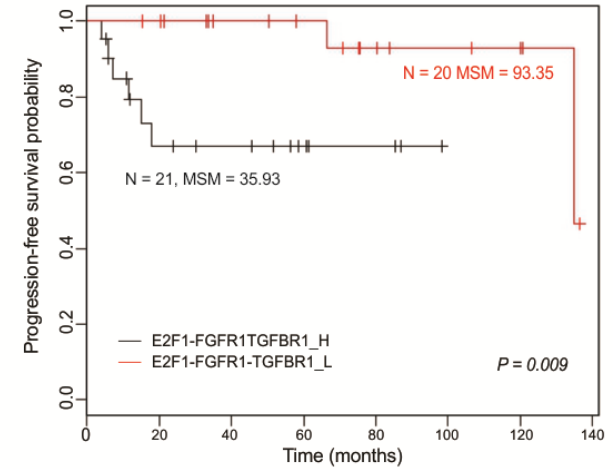
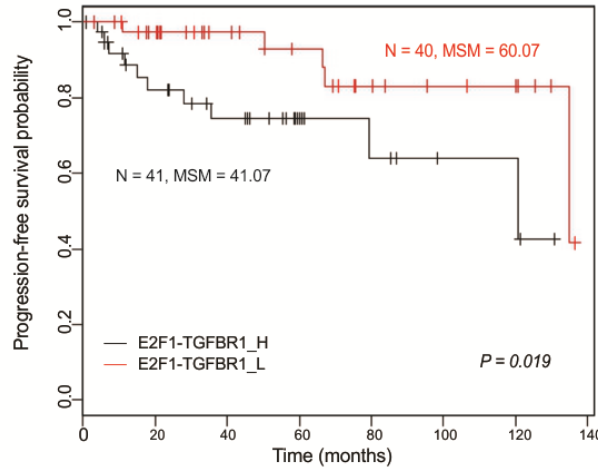
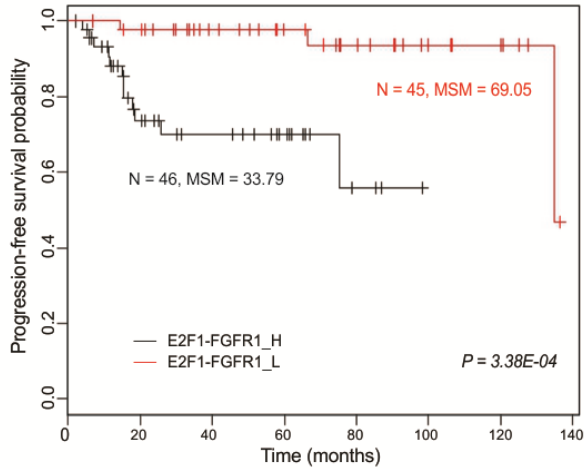
BLADDER CANCER								
E2F1	TGFBR1	FGFR1	EGFR	CXCR1	RARA	EMT		
0	0	0	0/1	0/1	0/1	0		
0	0	1	0/1	0/1	0/1	1		
0	1	0	0/1	0/1	0/1	1		
0	1	1	0/1	0/1	0/1	2		
1	0	0	0/1	0/1	0/1	1		
1	0	1	0/1	0/1	0/1	2		
1	1	0	0/1	0/1	0/1	2		
1	1	1	0/1	0/1	0/1	3		
BREAST CANCER								
E2F1	TGFBR2	EGFR	HMMR	VEGF	THRB	IL1R1	RARA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

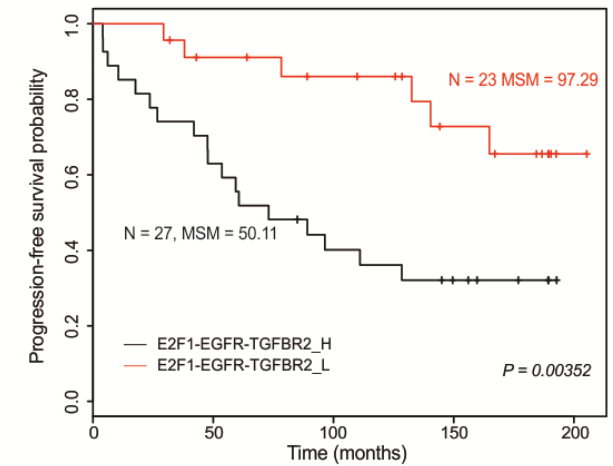
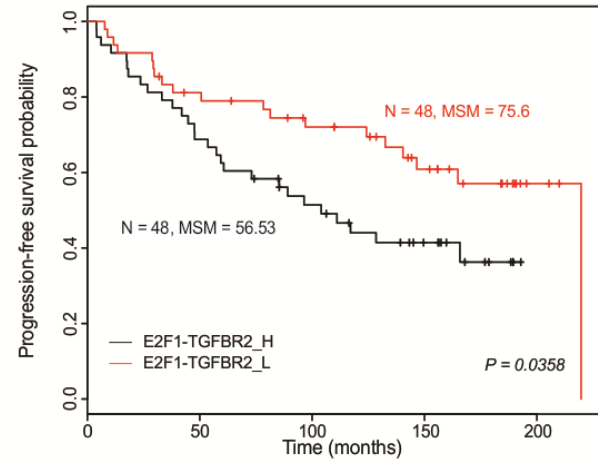
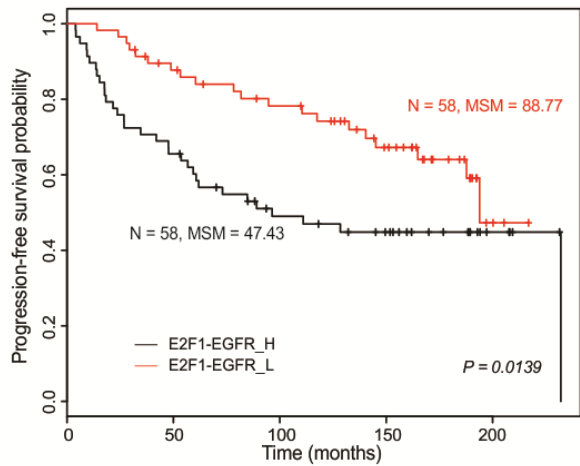
0	Non invasive
1	Less invasive
2	Moderately invasive
3	Highly invasive

- 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.

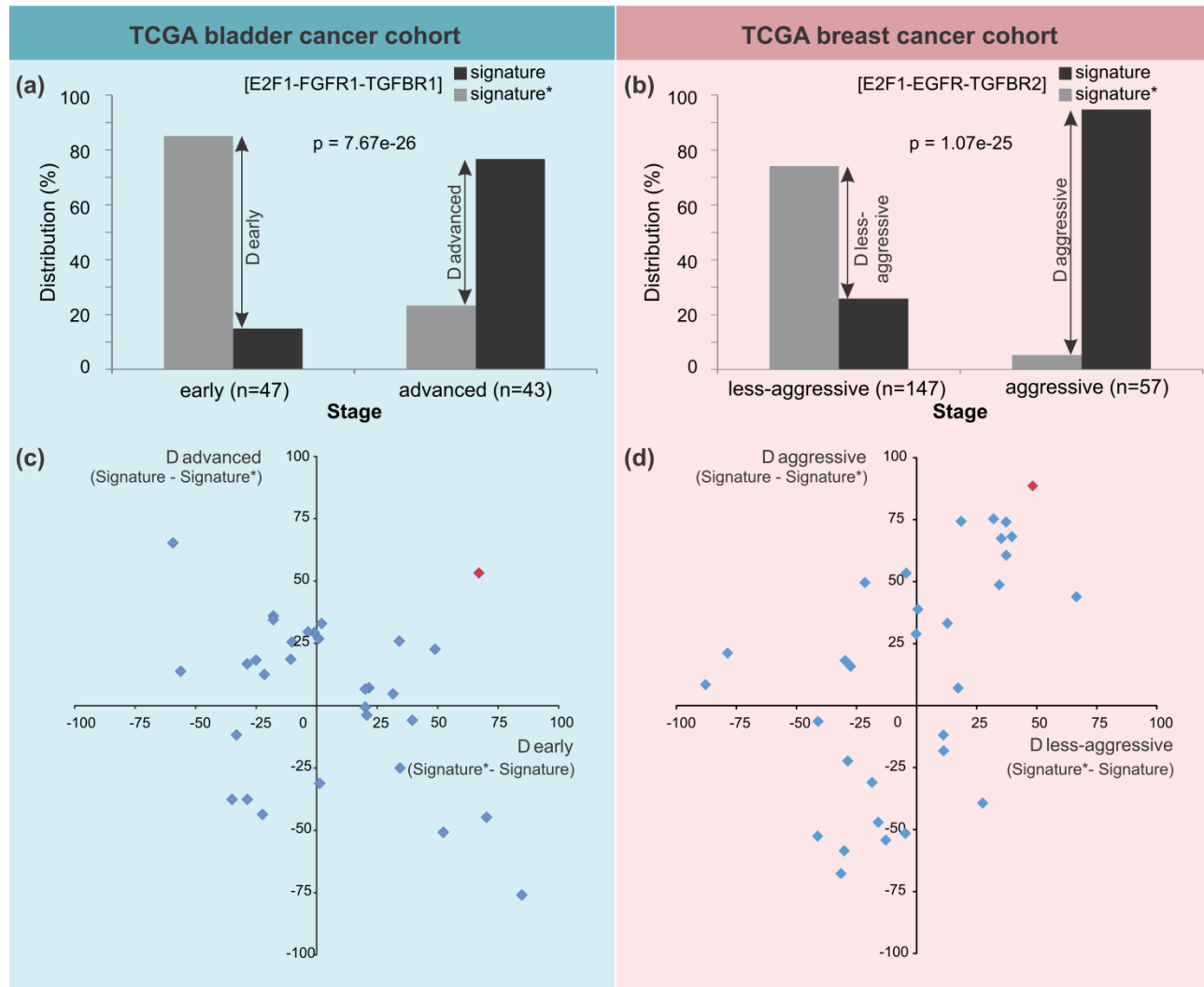
## bladder cancer patients



## breast cancer patients



# Classification of patients based on proposed vs. random signatures

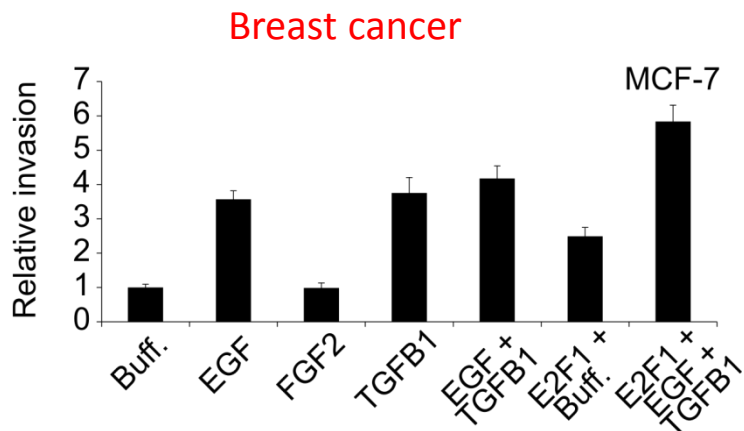
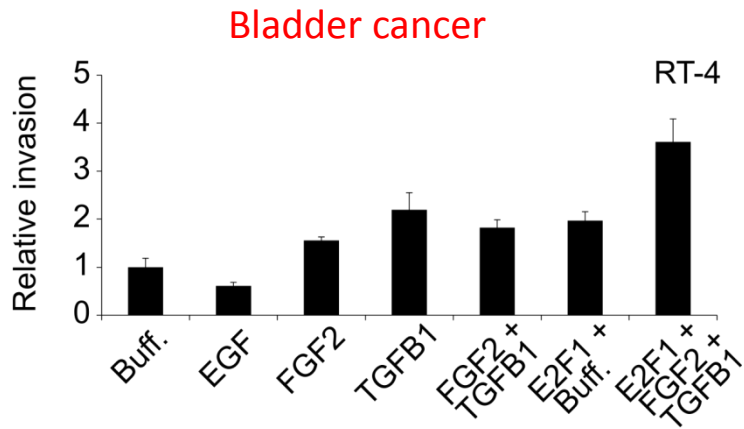


◆ Proposed signatures

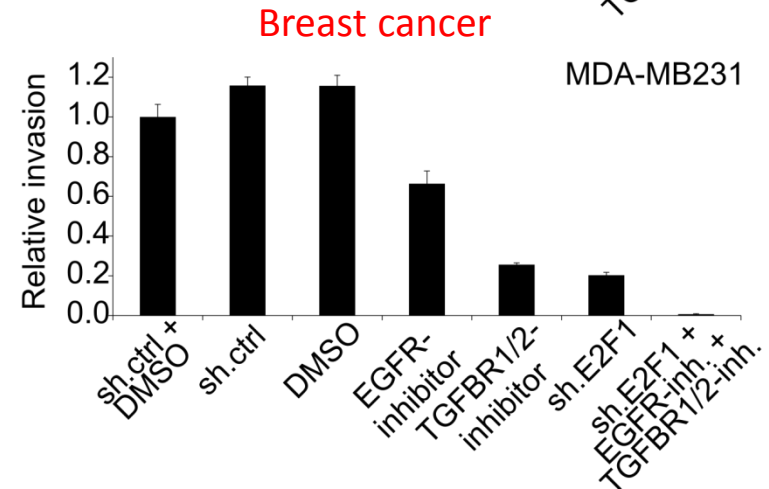
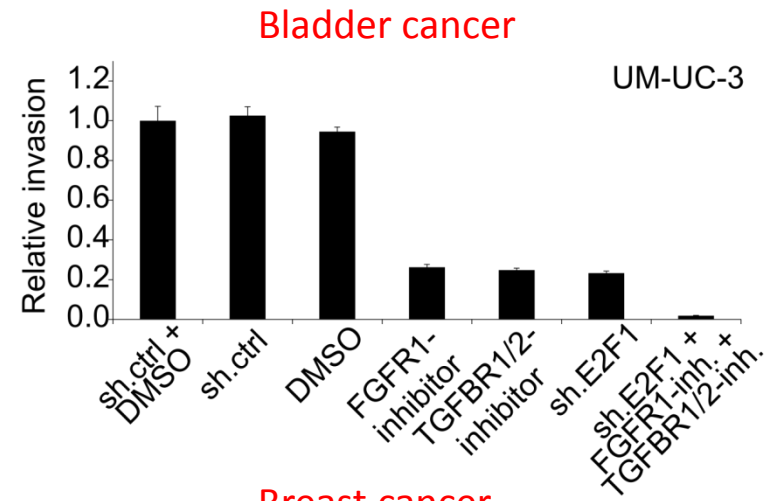
◆ Random signatures



## Non-invasive cell lines



## Invasive cell lines

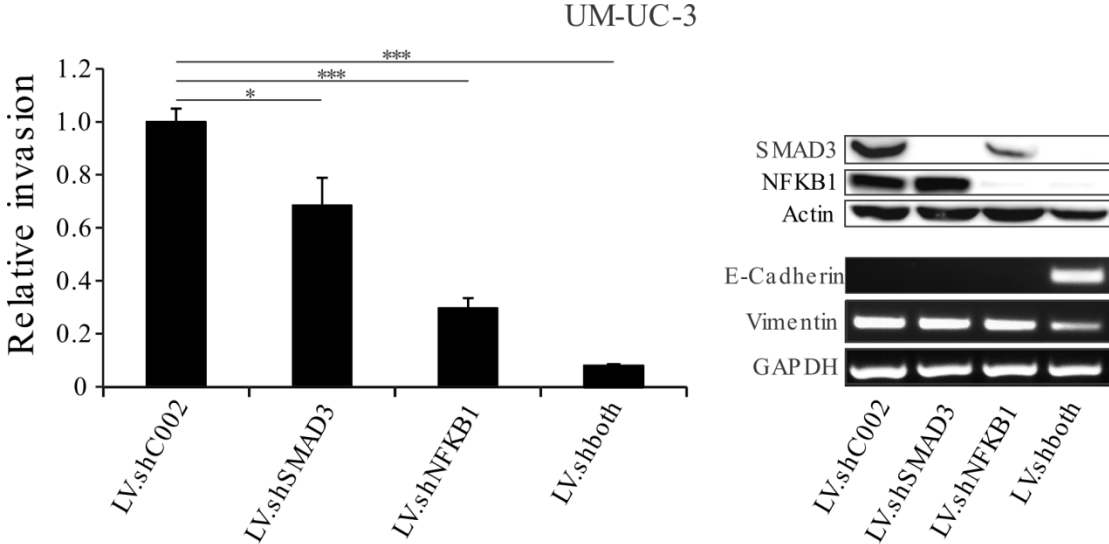


<b>(a) Bladder cancer</b>									
Signature			Double <i>in silico</i> perturbations in regulatory layer						Output
E2F1	TGFBR1	FGFR1	ZEB1	TWIST1	SNAI1	NFKB1	SMAD2,3,4	CDH1	EMT
1	1	1	1	1	1	1	1	0	3
1	1	1	0	0	1	1	0	1	1
1	1	1	0	1	0	1	0	1	1
1	1	1	0	0	1	0	0	1	1
1	1	1	0	1	1	1	0	1	1
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1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

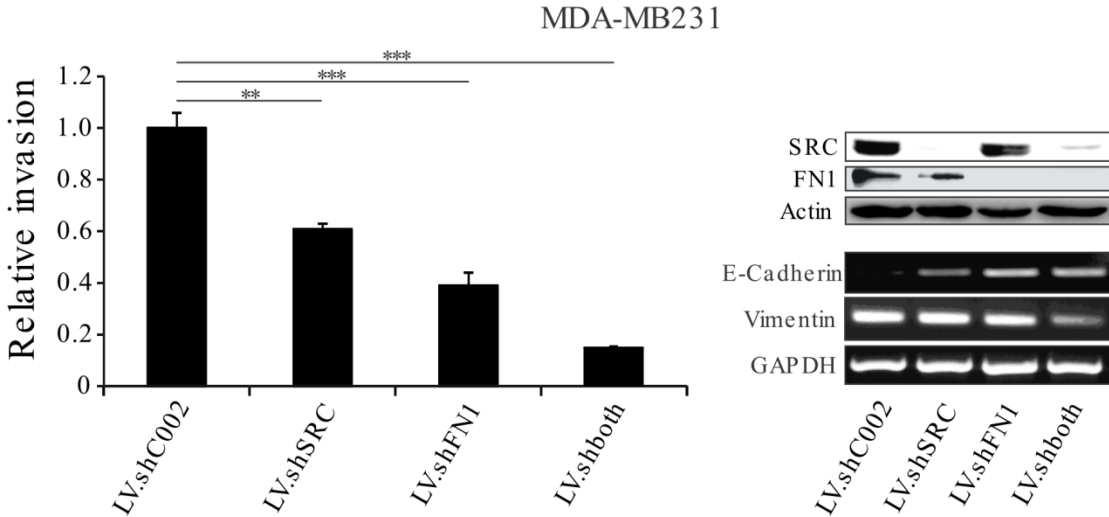
  

<b>(b) Breast cancer</b>									
Signature			Double <i>in silico</i> perturbations in regulatory layer					Output	
E2F1	TGFBR2	EGFR	SRC	FN1	SNAI1	SNAI2	CDH1	EMT	
1	1	1	1	1	1	1	0	3	
1	1	1	0	1	1	1	1	1	
1	1	1	0	0	1	1	0	1	
1	1	1	0	1	0	1	1	1	
1	1	1	0	1	1	0	1	1	
1	1	1	1	0	1	1	1	1	
1	1	1	1	1	0	1	1	1	
1	1	1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

## Bladder cancer



## Breast cancer



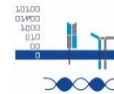
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and Research