Predictive logical modelling of cell fate decision networks

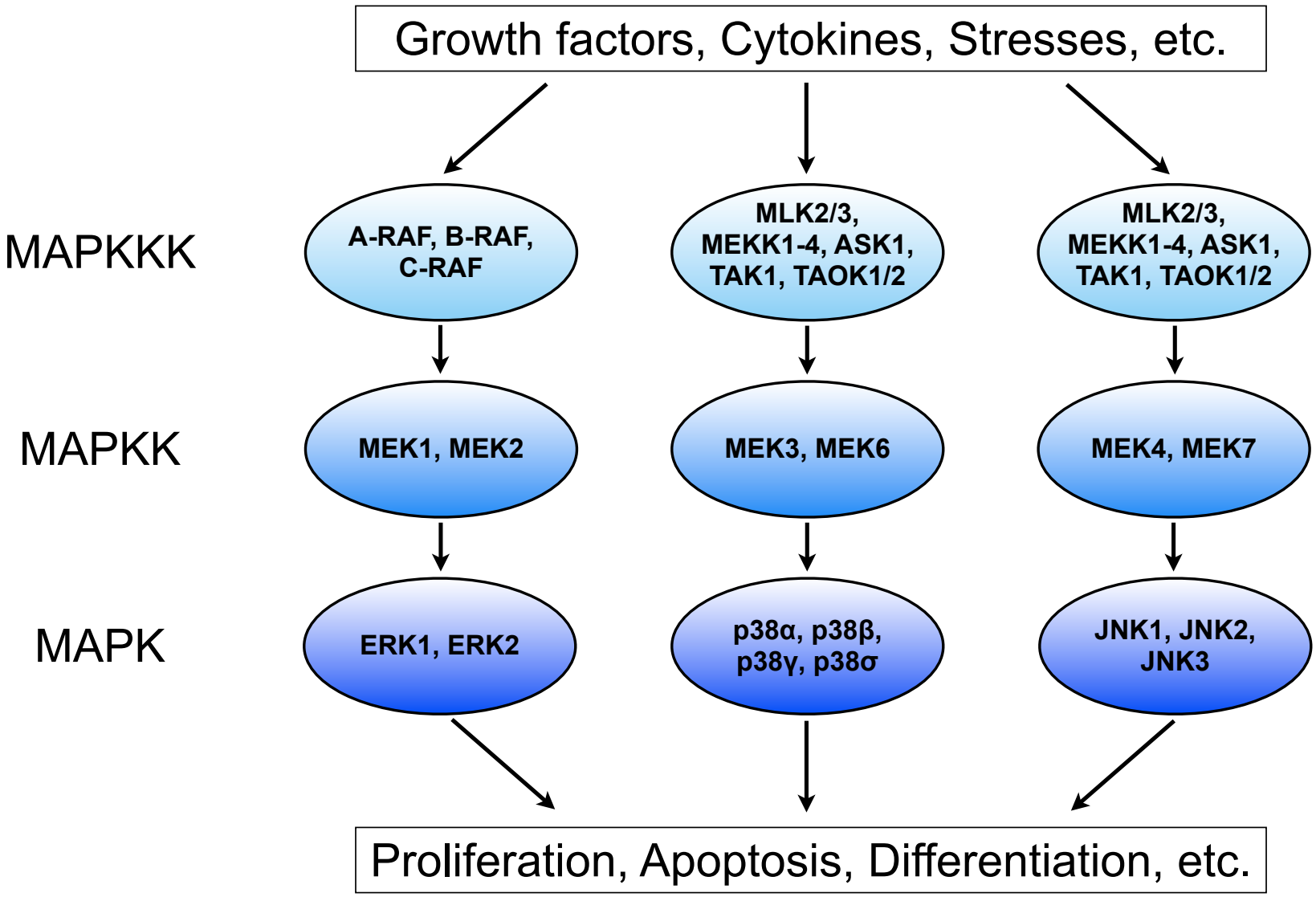
Denis Thieffry

Contents

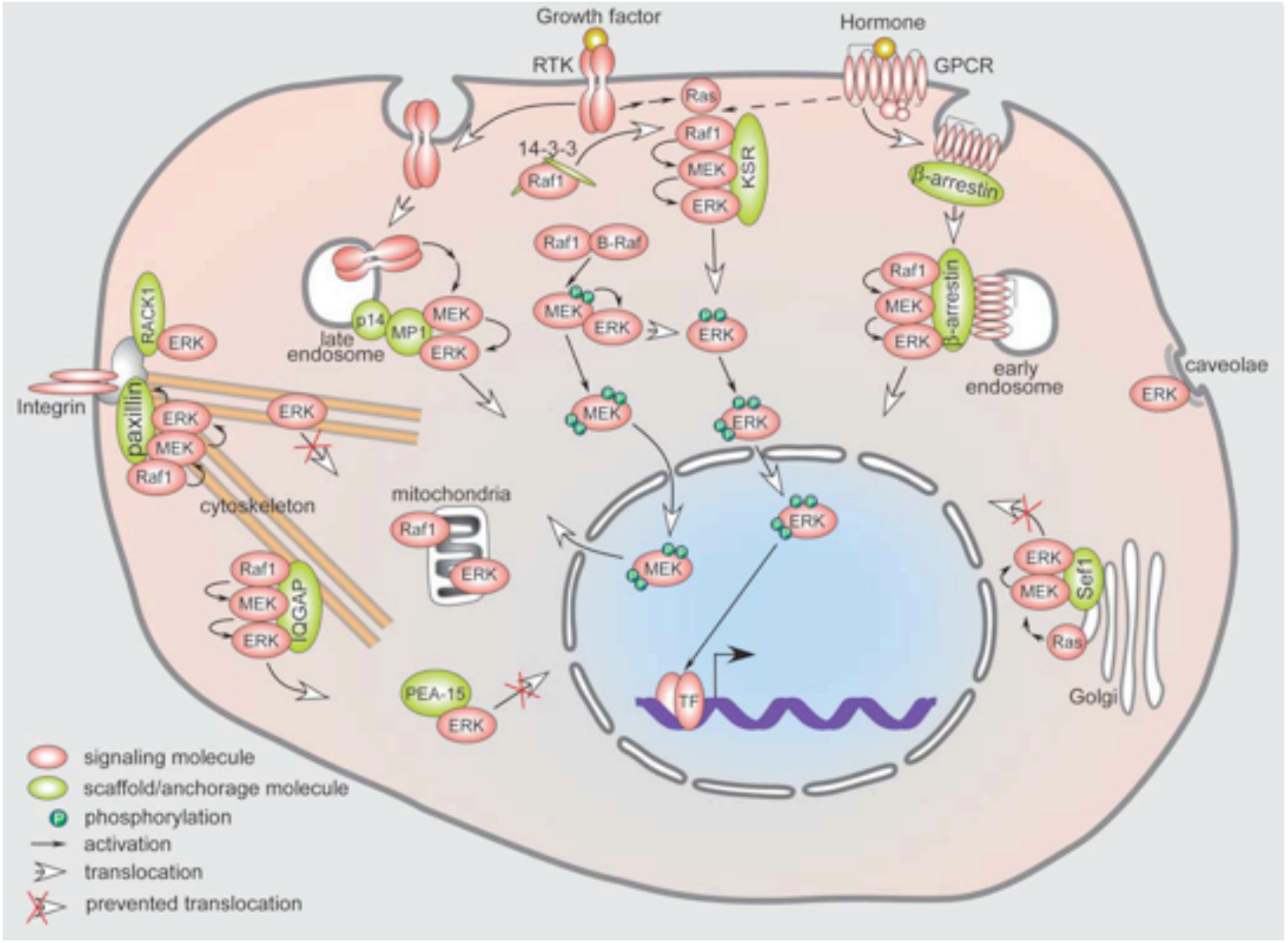
- MAPK pathways
- Molecular mapping of MAPK network
- Logical modelling of MAPK network in bladder cancer cells
- Predictive modelling of drug synergies in gastric cancer cells
- Conclusions and prospects

BioNetVisA Workshop, Singapore, November 25th, 2015

MAPK pathways (simplistic view)



Specificity factors



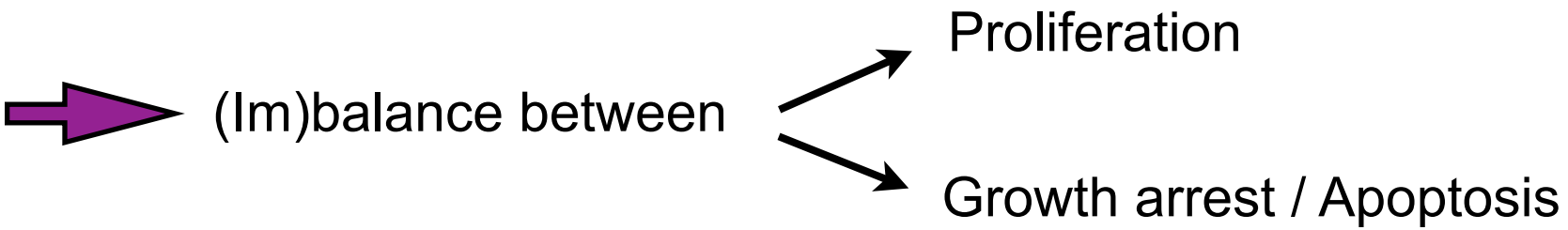
- Different stimuli
- Protein isoforms
- Sub-cellular localisation
- Scaffold proteins
- Phosphatases
- Feedbacks
- Cross-talks

Yao and Seger, *BioFactors,* 2009

Biological motivation

Generic scope

Study the role(s) of MAPK signalling deregulations
in cancer cell fate decision

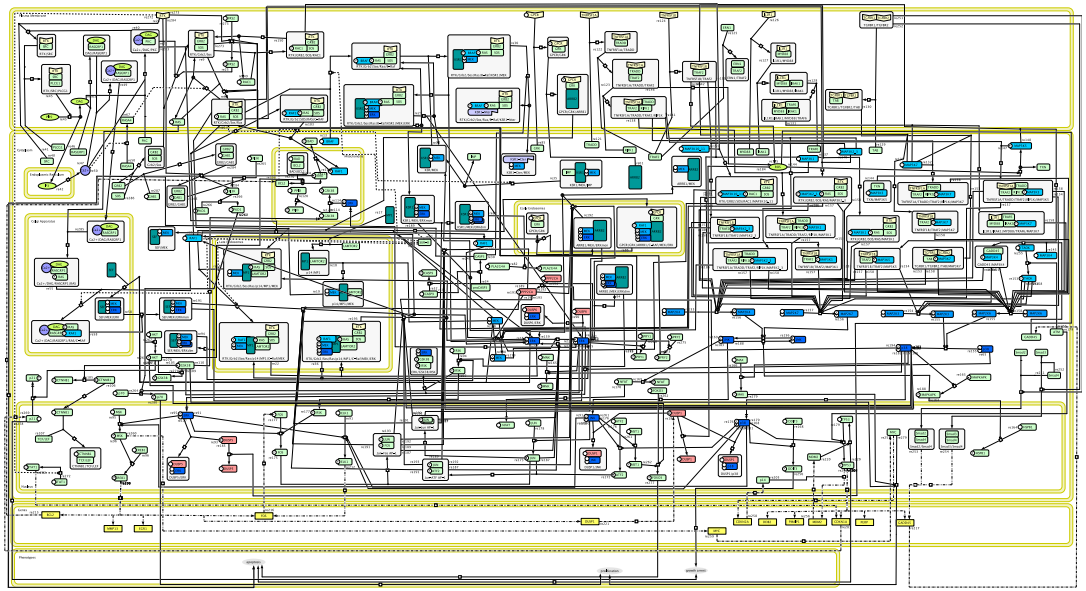


Specific aims

- Identification of **key players** for the transduction of proliferative signals in bladder cancer
- **Understand the mechanisms** governing varying MAPK activity in urinary bladder cancer subtypes

MAPK reaction map

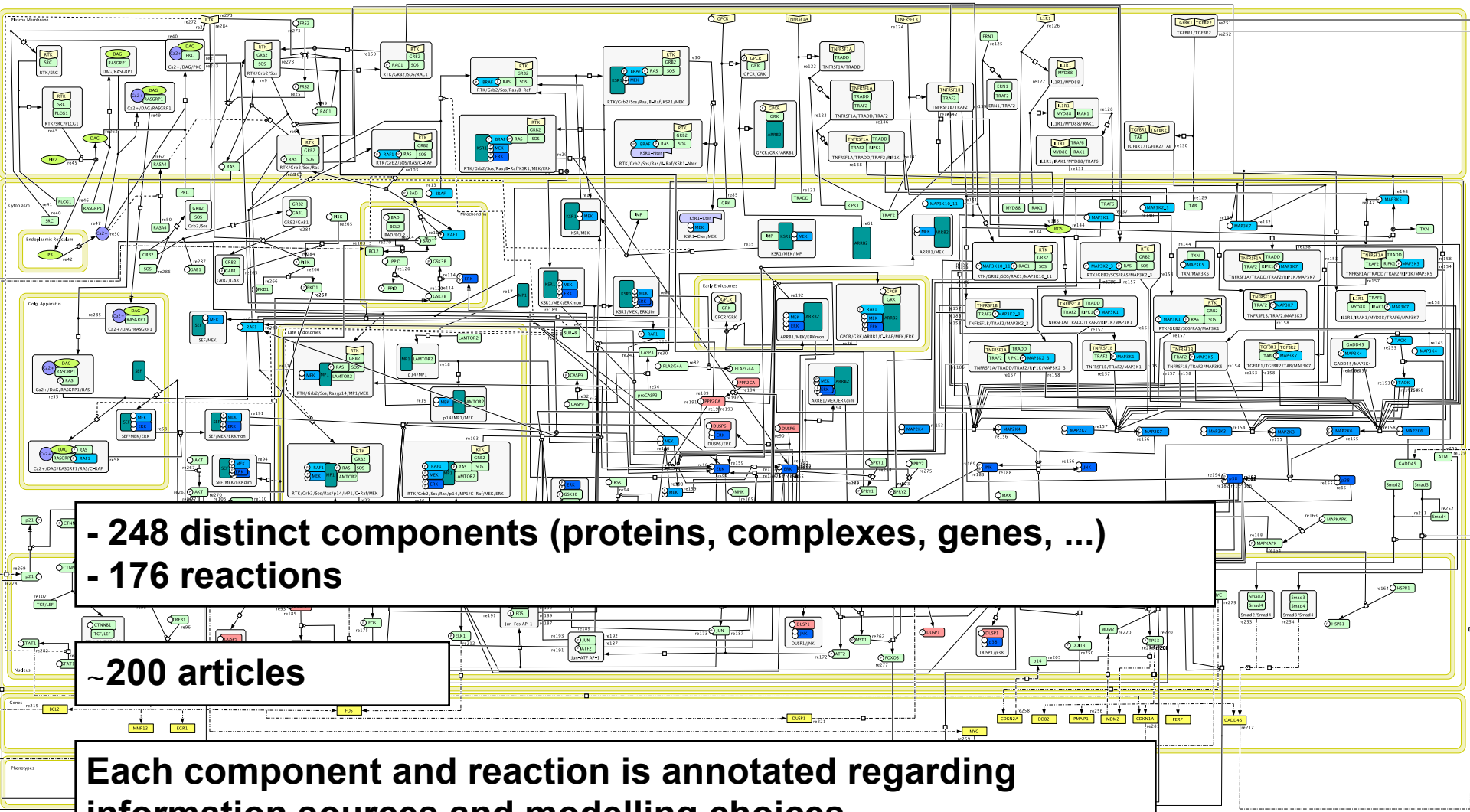
Luca GRIECO
now at UCL, UK



Integration of relevant information into a detailed reaction map

- **CellDesigner** software (www.celldesigner.org)
- Literature-derived information
- Emphasis on specificity factors
- Generic map: several human/mouse cell types

The MAPK reaction map

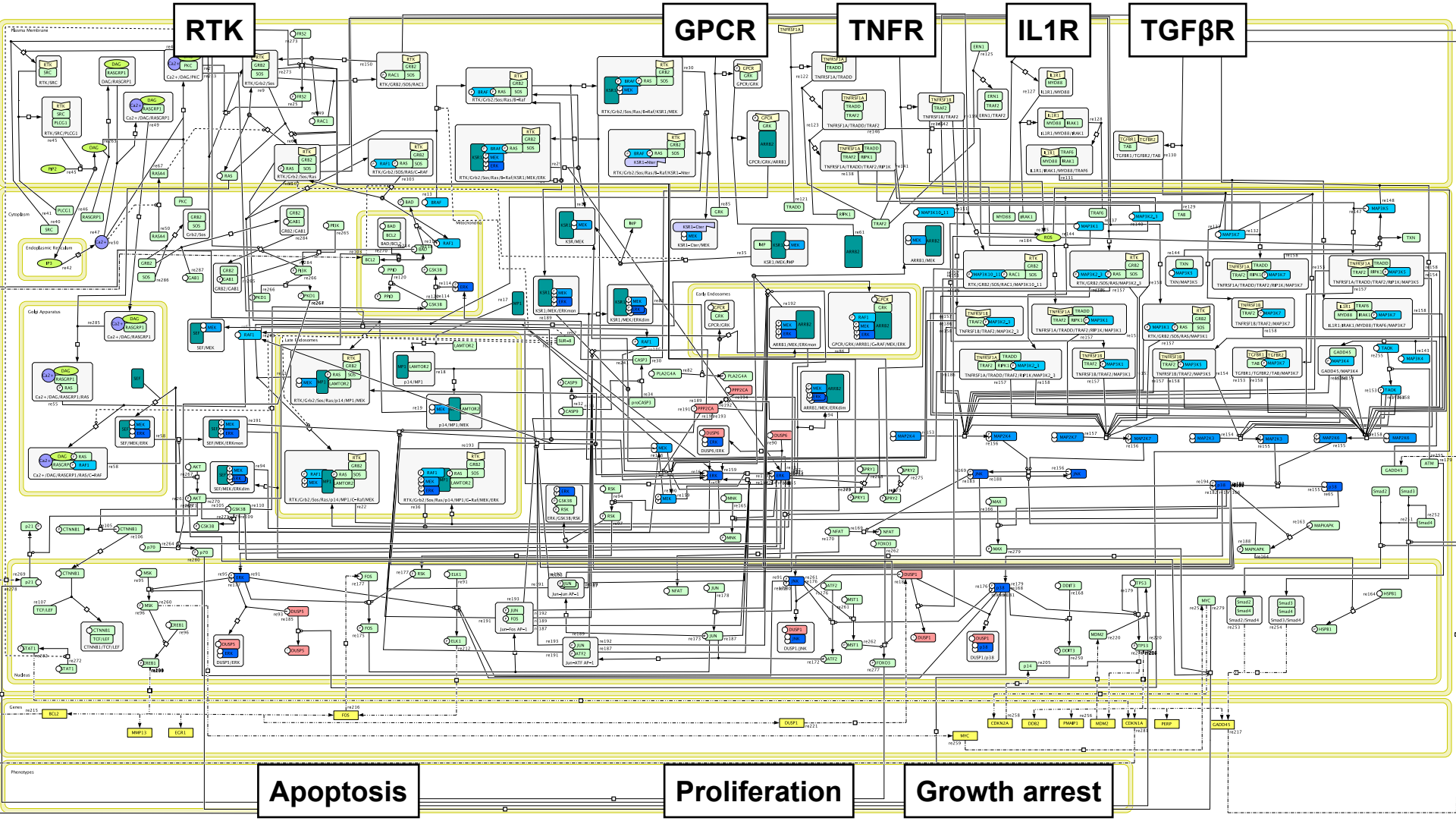


- 248 distinct components (proteins, complexes, genes, ...)
- 176 reactions

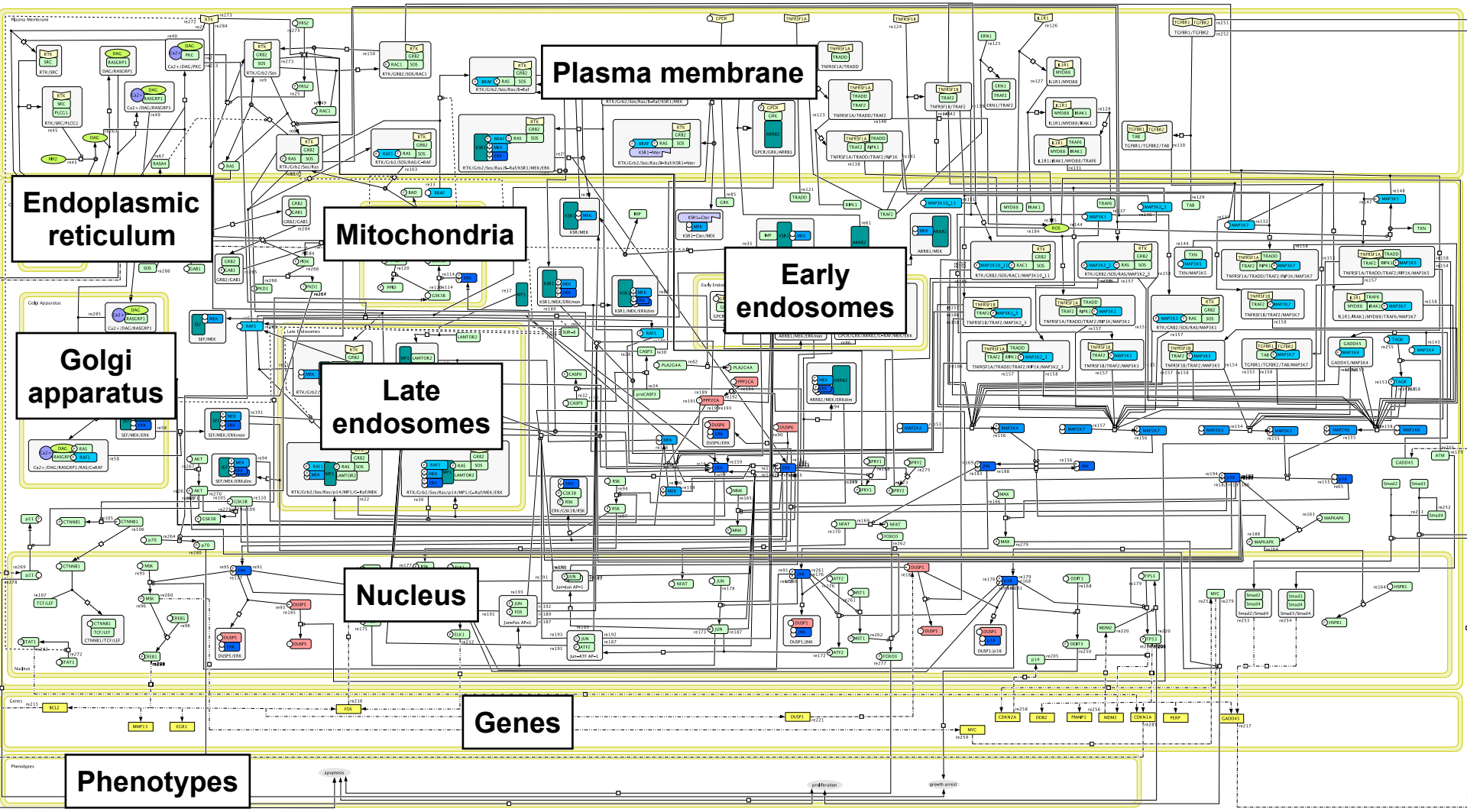
~200 articles

Each component and reaction is annotated regarding information sources and modelling choices

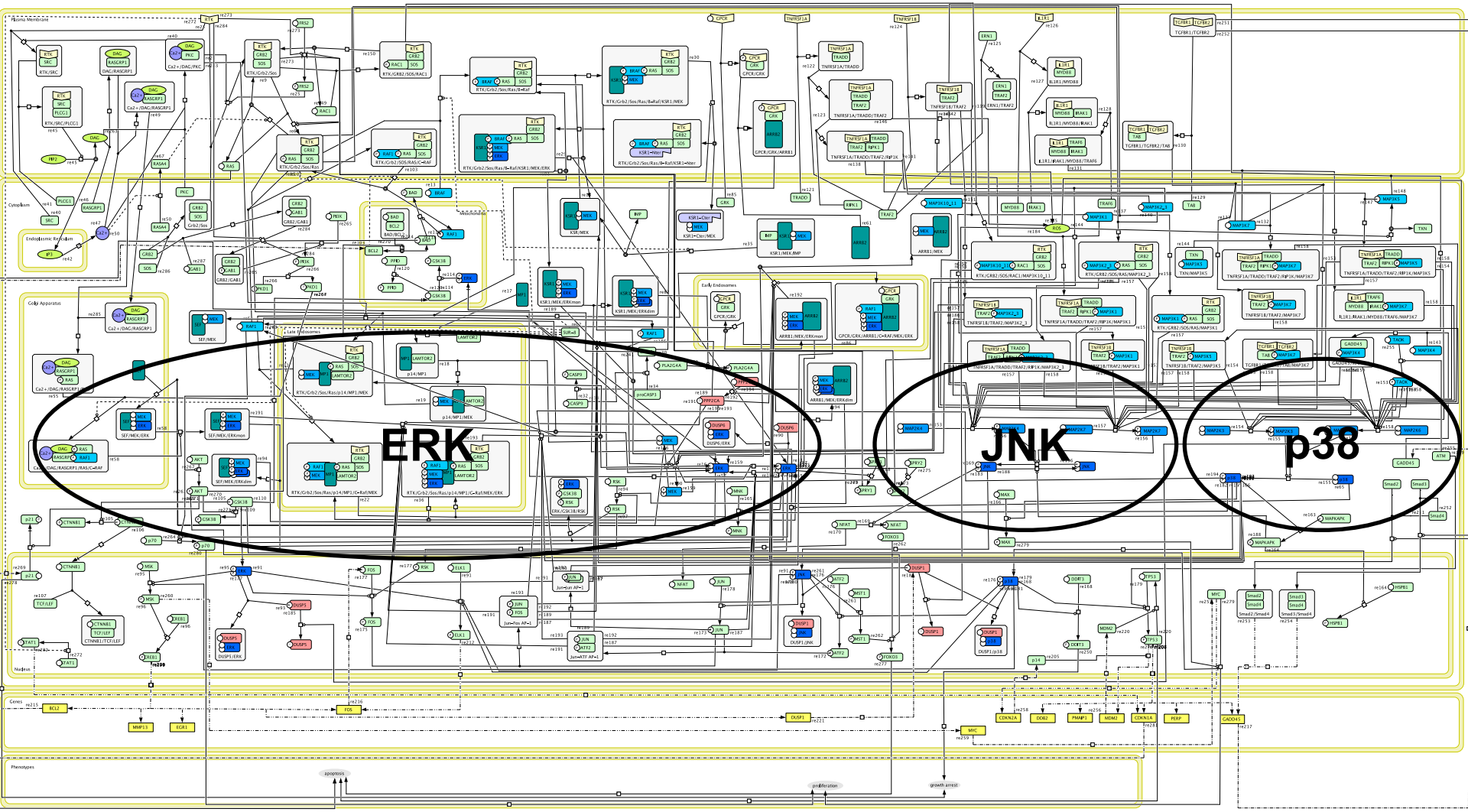
External stimuli and phenotypes



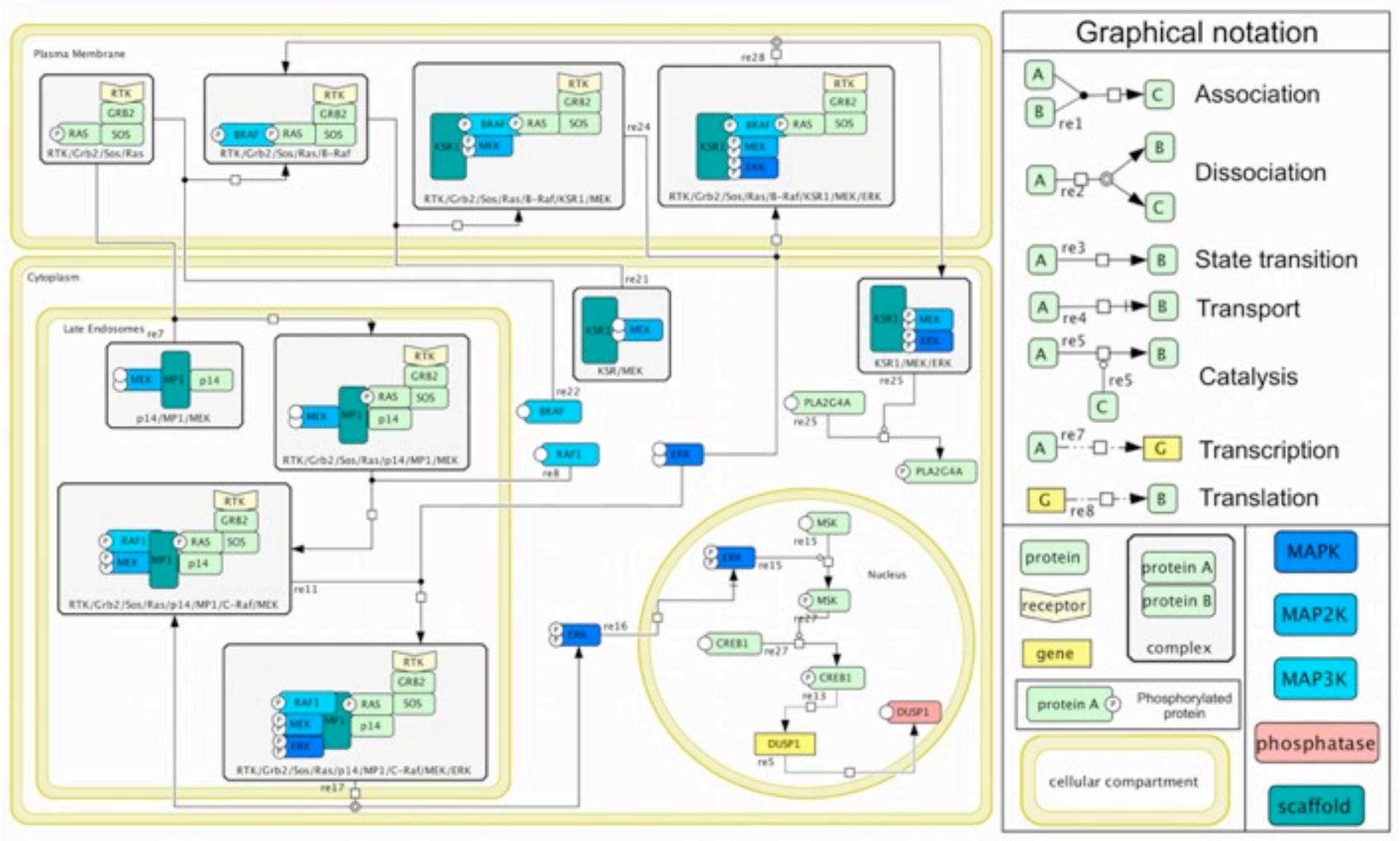
Sub-cellular compartments



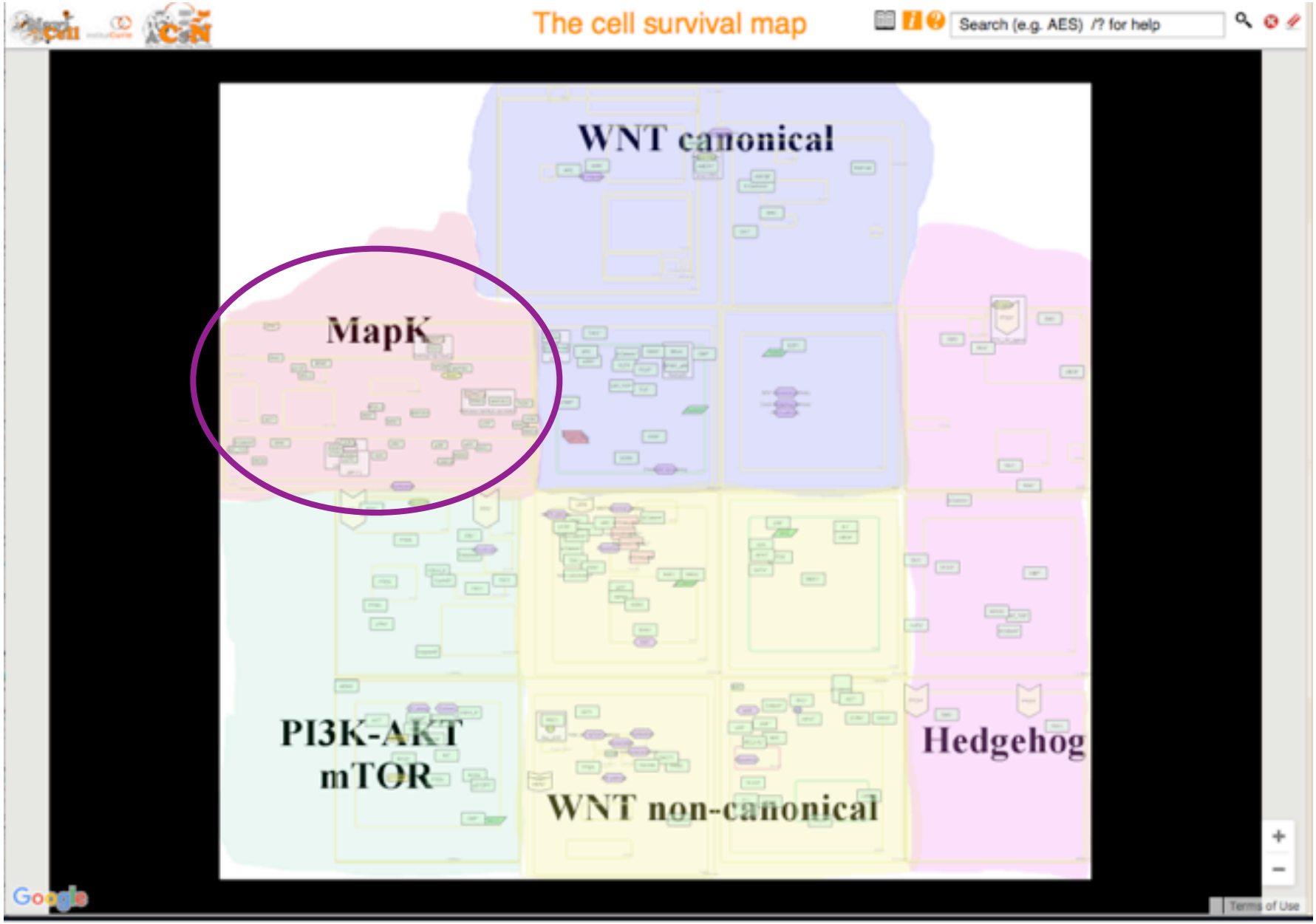
Main MAPK pathways and cross-talks



Example: ERK activation

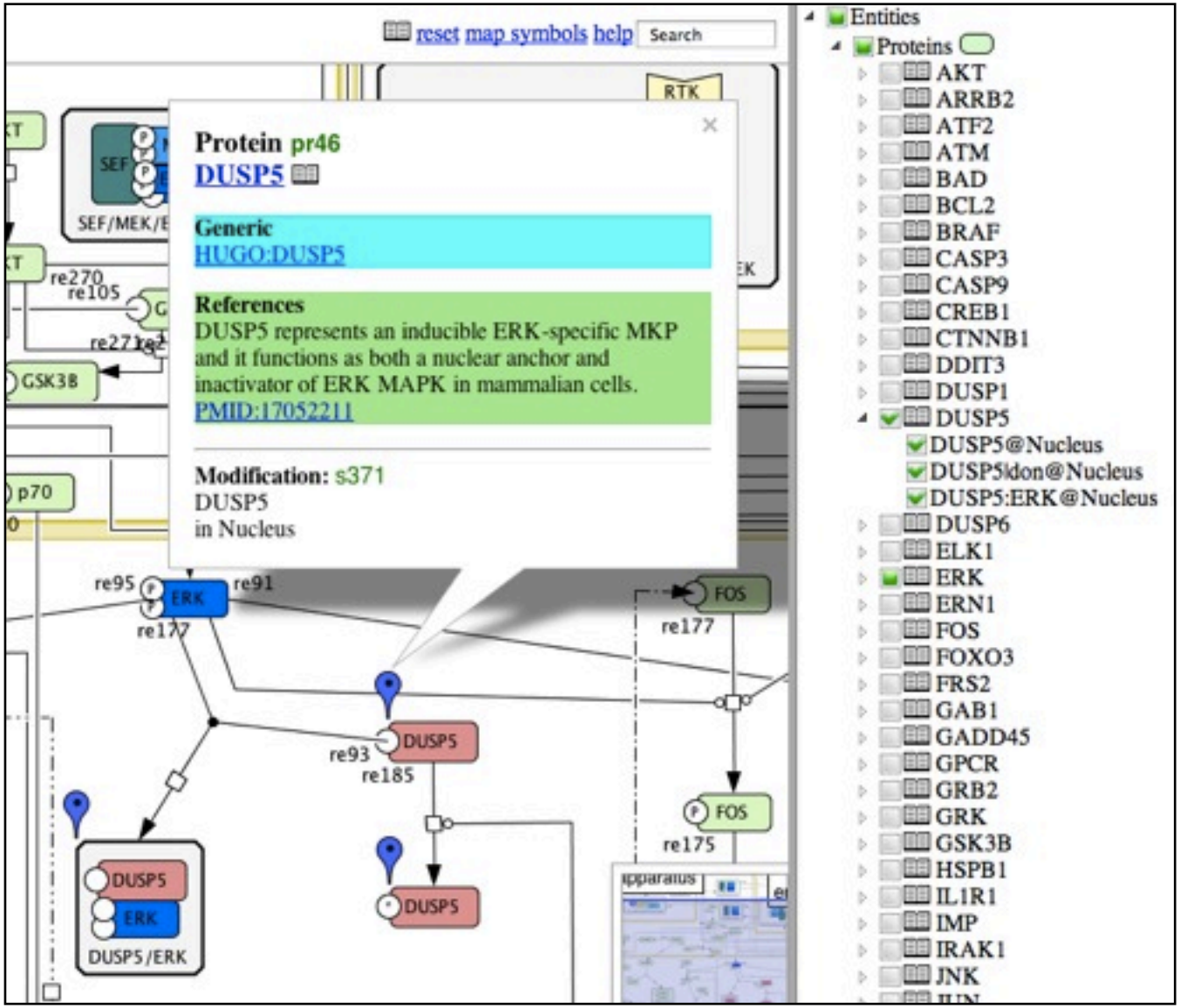


Clickable map => Atlas Of Cancer Signalling Networks



https://acsn.curie.fr/

Clickable map => Atlas Of Cancer Signalling Networks



https://acsn.curie.fr/

Bladder cancer

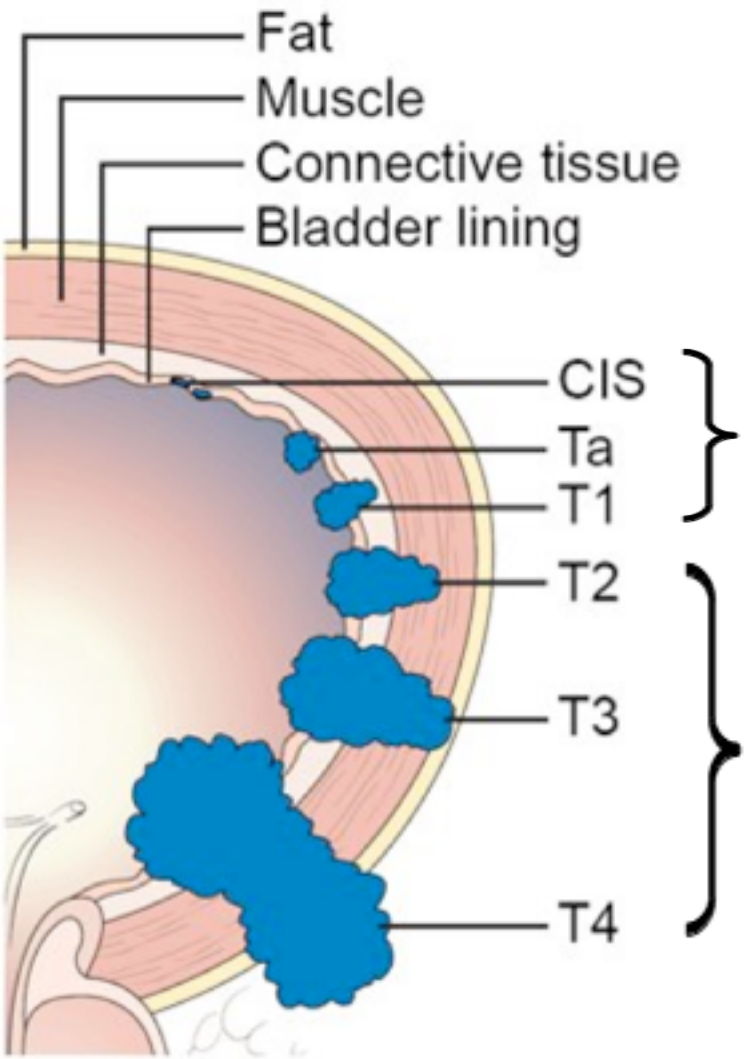


Diagram showing the T stages of bladder cancer
© CancerHelp UK

Non-invasive
associated with
FGFR3 activating mutation

Invasive
associated with
EGFR over-expression

Logical modelling of MAPK network in bladder cancer using GINsim

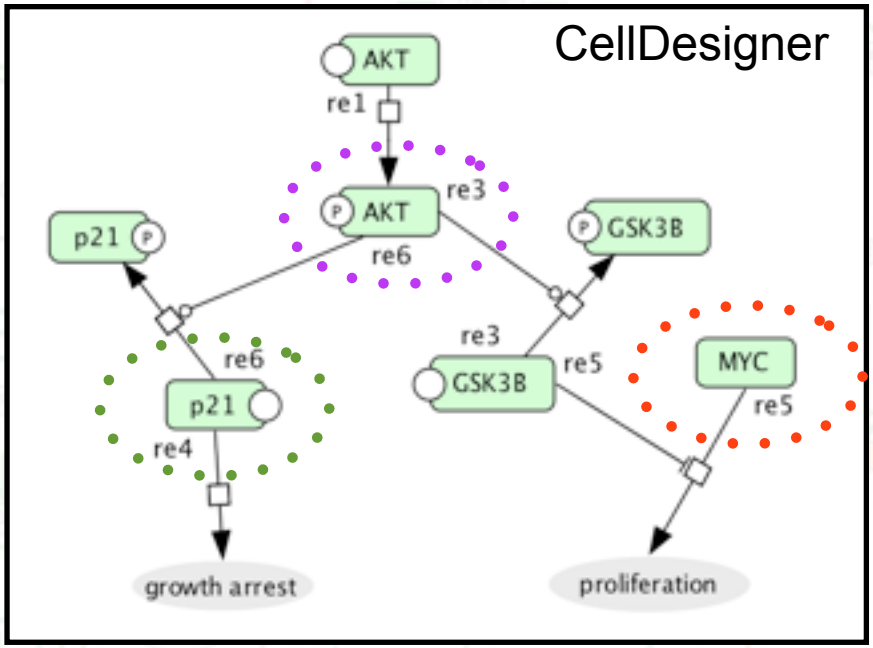
Bladder cancer deregulations

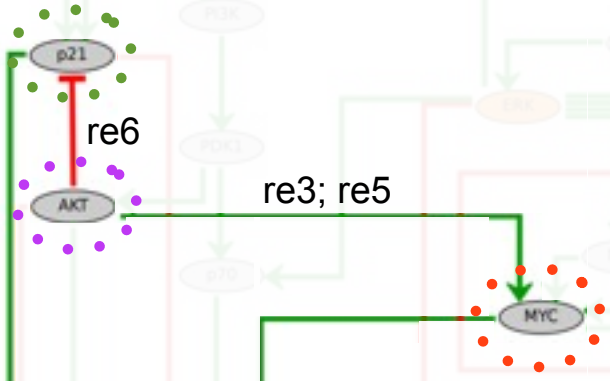
- **FGFR3** activating mutation → Ta & **less aggressive**
- **EGFR** over-expression → **Invasive** (>T1) & high proliferation rate
- **Both receptors activate MAPKs**

Aims

1. Recapitulate this differential behaviour with a dynamical model
2. Decipher the underlying mechanisms

From molecular map to logical model (abstraction)





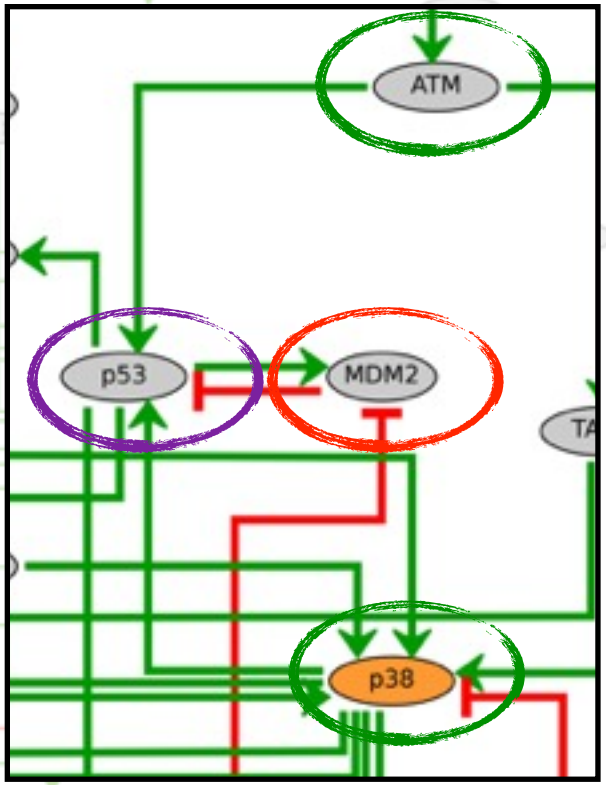
MAPK logical model: defining the logical rules

WILD TYPE

p53=1 **iff** (ATM=1 **and** p38=1)

or ((ATM=1 **or** p38=1) **and** MDM2=0)

p53=0 **otherwise**



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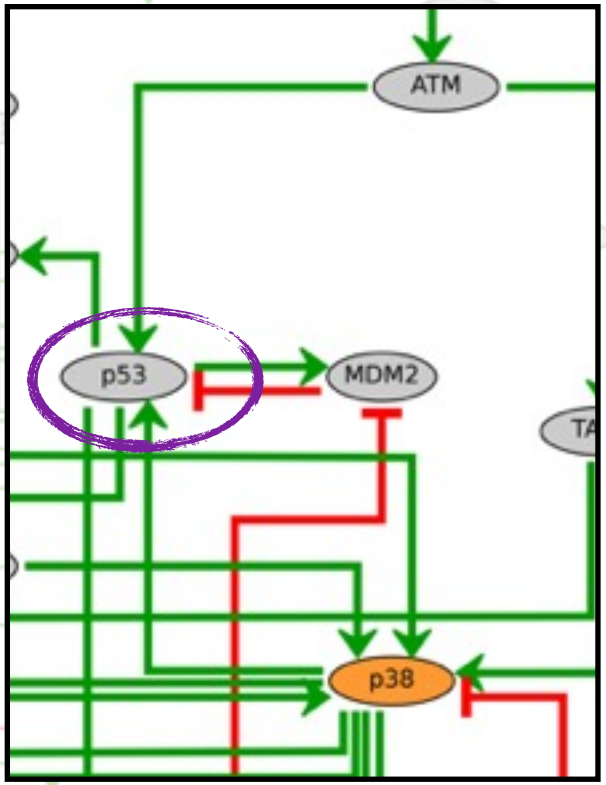
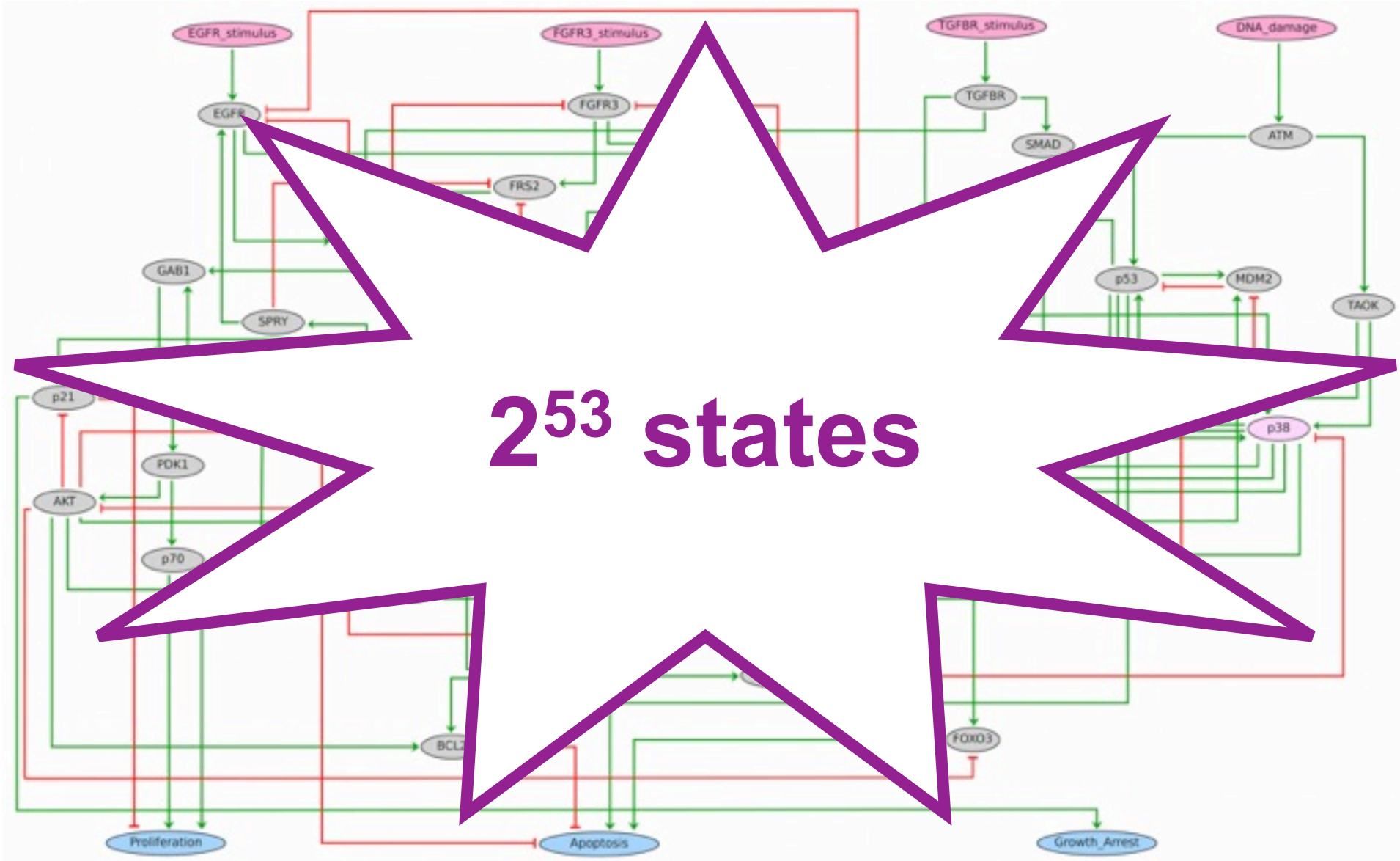
p53=0 **otherwise**

PERTURBATIONS

- *p53 loss-of-function:* p53=0 **always**
- *p53 gain-of-function:* p53=1 **always**



MAPK logical model



Regulatory graph encompassing 53 components, 552 circuits

Coping with the exponential growth of logical state transition graphs

- Focus on attractors and their reachability
- Model reduction (based on user specifications)
- Compaction of state transition graphs
- Temporisation (e.g. priorities, delays, etc.)
- Model checking (NuSMV, Petri nets toools, ...)
- Delineation of the roles of regulatory circuits/modules

MAPK model reduction

	Reduction 1	Reduction 2	Reduction 3
Inputs	EGFR_stimulus, FGFR3_stimulus, TGFBR_stimulus, DNA_damage		
Phenotypes	Proliferation, Apoptosis, Growth_Arrest		
Selected observables	EGFR, FGFR3, p53, p14, PI3K, AKT, PTEN, ERK	EGFR, FGFR3, RAF, RAS, ERK, AKT, p53, p21	JNK, p38, GADD45, ERK, RAS
Auto-regulated components	FRS2, MSK	GRB2, PI3K, p38	GRB2, PLCG, PI3K, MDM2

Three reductions of MAPK models

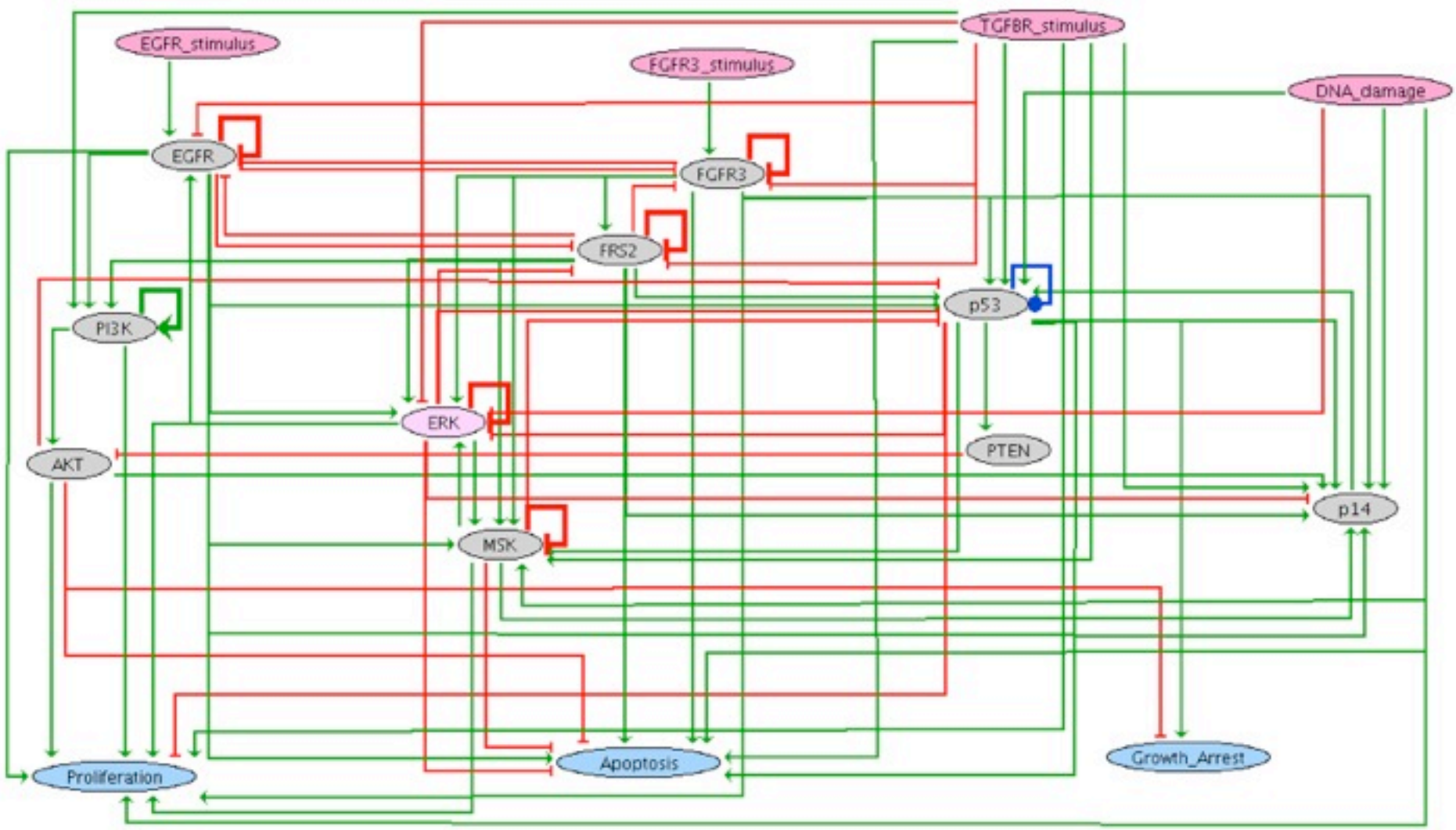
Each reduction preserves the input and phenotype components.

Additional components were kept depending on the simulations performed.

Apparition of auto-regulations impede further component reduction.

Conservation (compression) of regulatory circuit ensure the preservation of the main dynamical properties.

MAPK reduced model (version 1)



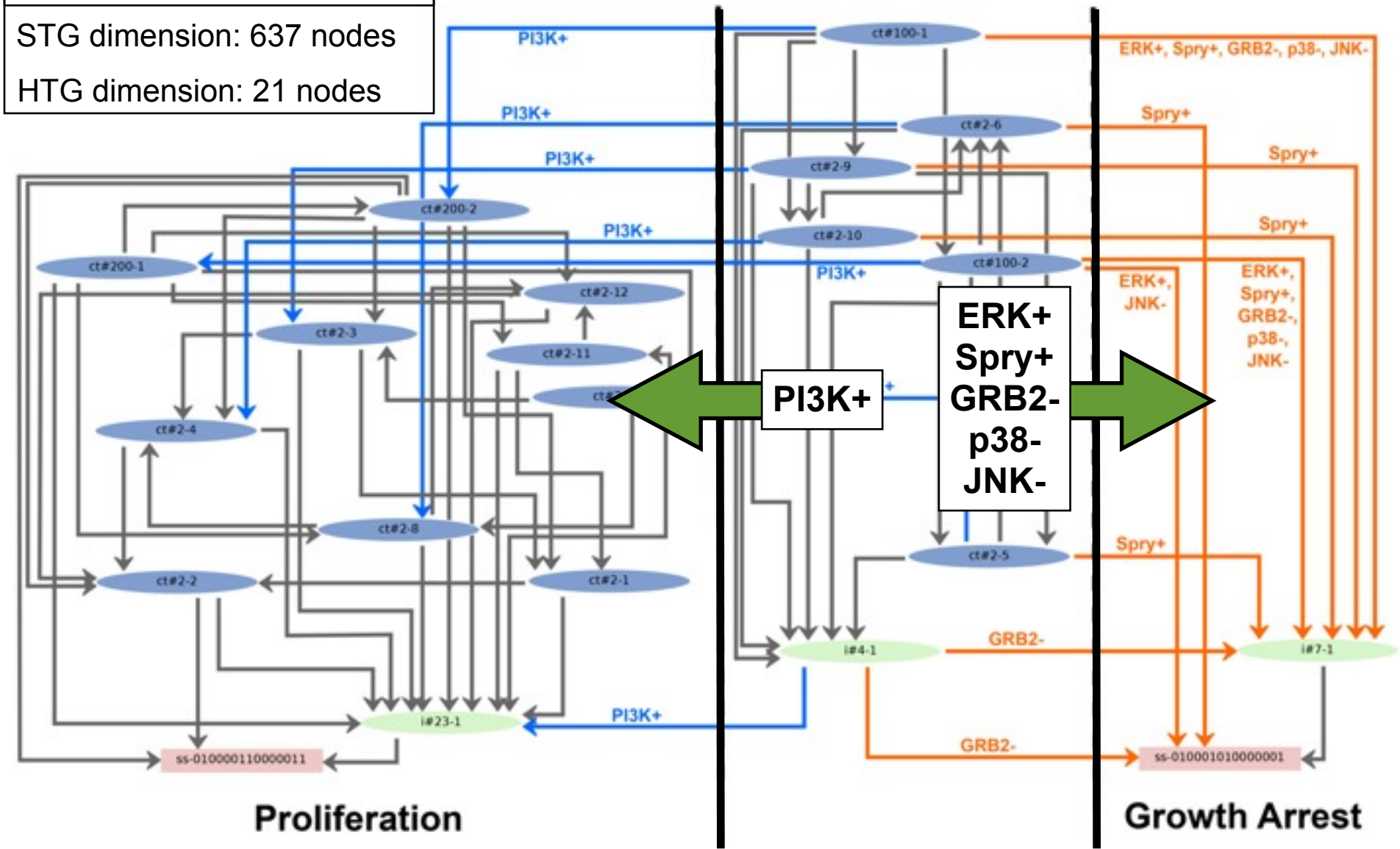
17 components (including 4 inputs and 3 outputs), **128 circuits**
Functional circuits: **1 positive**, **5 negative**, **1 dual**

Asynchronous simulation for p53 KO
Hierarchical State Transition Graph (STG)

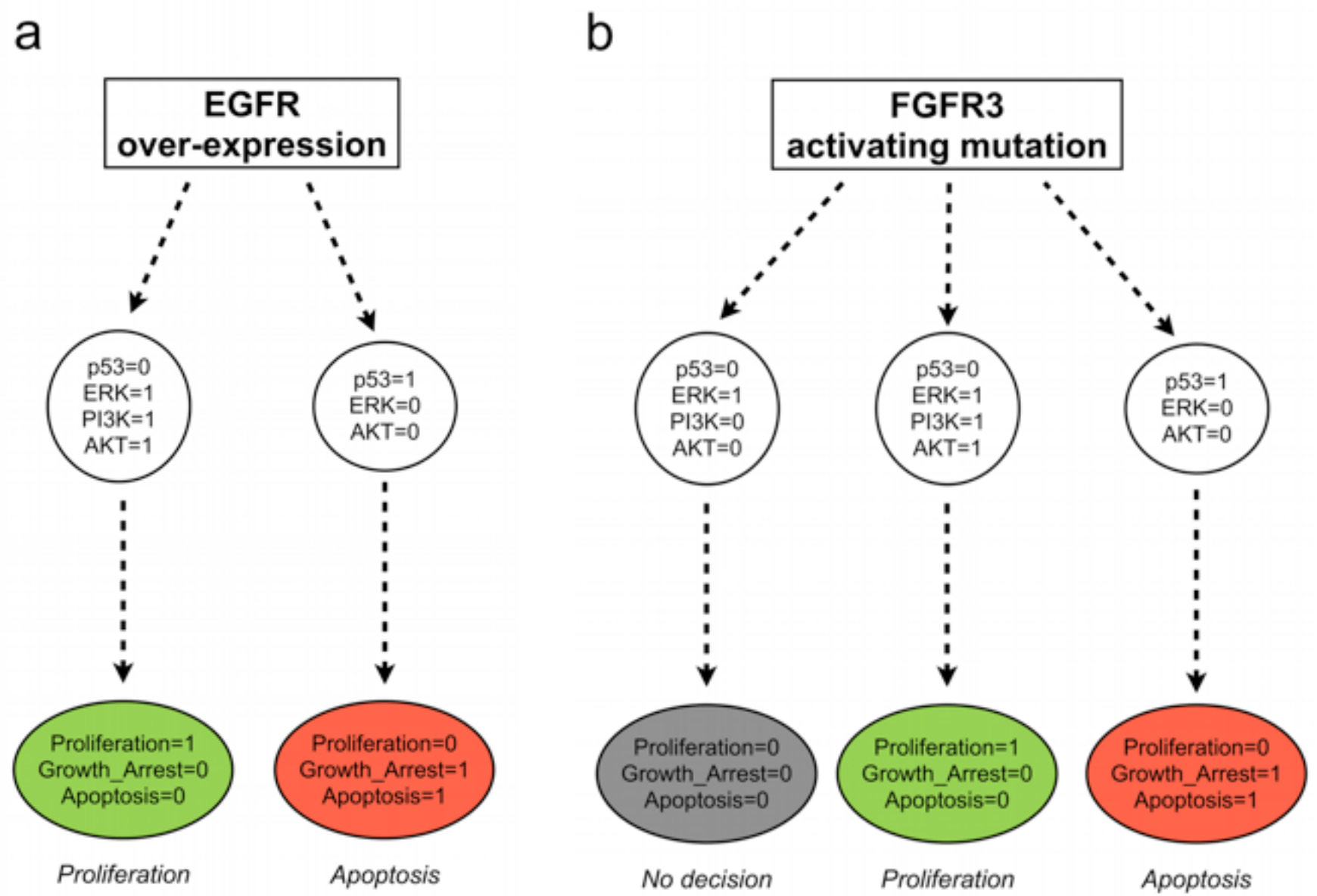
Init. cond.: **FGFR3_stim = 1**

STG dimension: 637 nodes

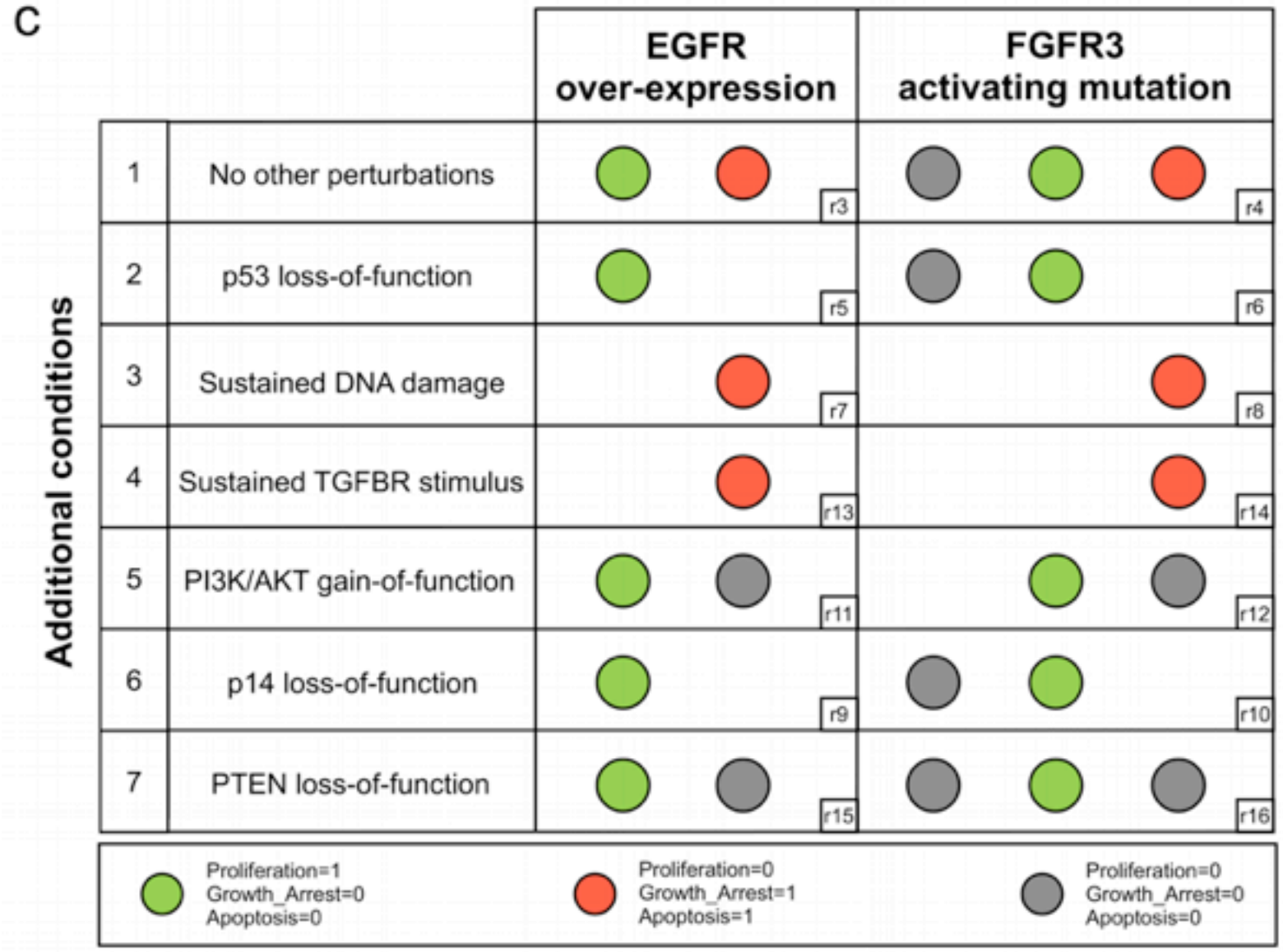
HTG dimension: 21 nodes



Simulations of EGFR vs FGFR3 activating mutations



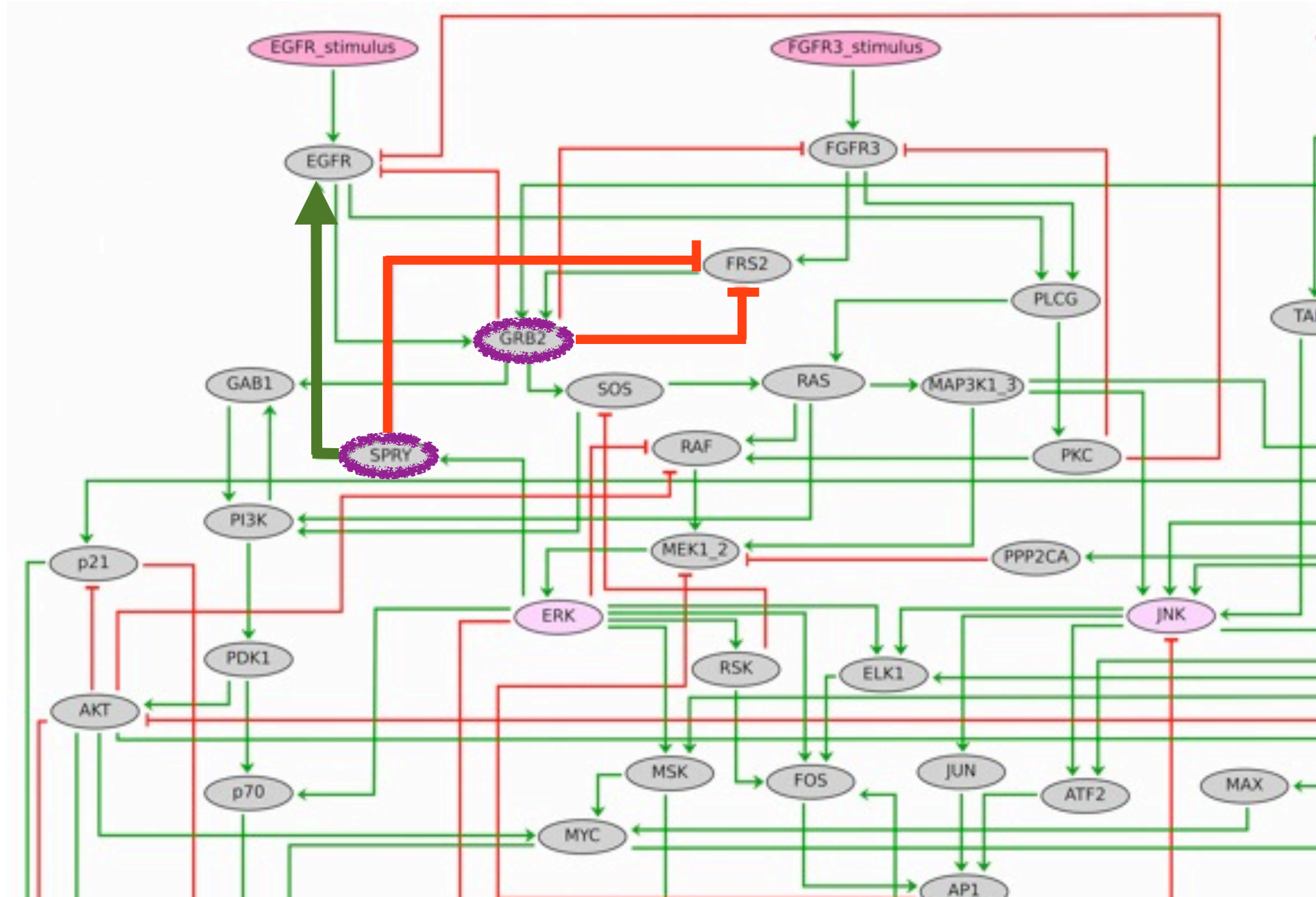
Simulations of documented perturbations



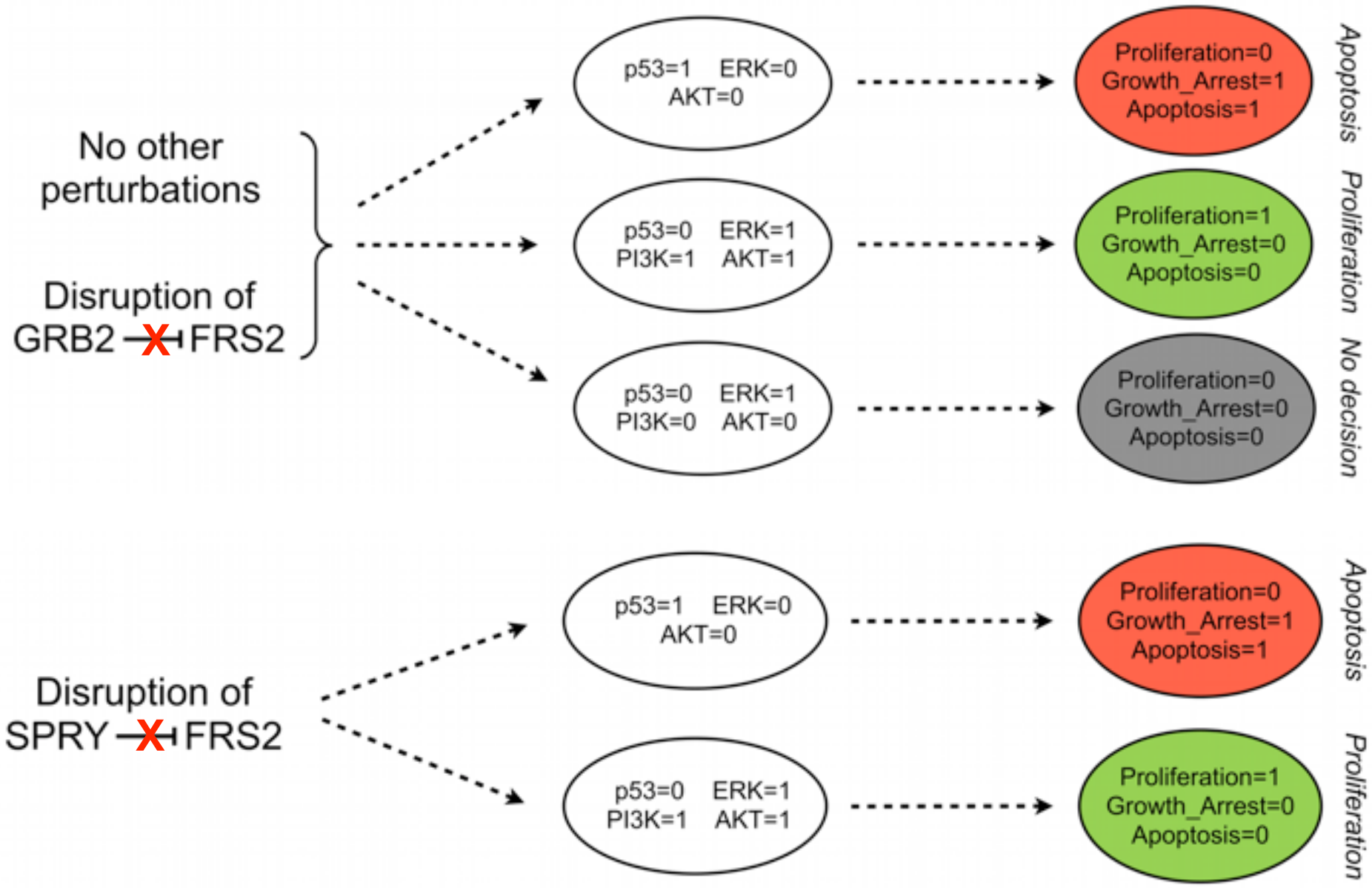
Coherence of simulations with published data

Biological data	Model behaviour
RAF or RAS over-expressions can lead to constitutive activation of ERK.	In absence of inputs, constitutive activity of RAF or RAS can lead to permanent ERK activation, associated with proliferation.
HSP90-inhibitor disrupts RAF, AKT and EGFR, leading to successful cancer treatment.	Concomitant RAF, AKT, EGFR deletions abrogate the proliferative stable states, in the case of EGFR over-expression and in the case of FGFR3 activating mutation.
Patients with p53-altered/p21-negative tumors demonstrated a higher rate of recurrence and worse survival compared with those with p53-altered/p21-positive tumors.	Following either EGFR over-expression or FGFR3 activating mutation, concomitant p21 and p53 loss-of-functions correspond to a phenotype characterised by apoptosis escape, with the possibility to attain proliferation. Association of p53 loss-of-function and p21 gain-of-function leads to growth arrest attractors, without proliferation.
p38 and JNK play important roles in stress responses, such as cell cycle arrest and apoptosis.	In presence of either DNA_damage or TGFBR_stimulus, growth arrest/apoptosis stable states are all lost in the p38/JNK-deleted model.
p38 and JNK have been shown to induce apoptotic cell death.	When p38/JNK are constitutively active, apoptotic attractors are obtained in the absence of other stimuli.
p38 plays its tumour suppressive role by promoting apoptosis and inhibiting cell cycle progression.	Under JNK constitutive activation, p38 loss-of-function determines loss of apoptotic attractors obtained in r26.
JNK may contribute to the apoptotic elimination of transformed cells by promoting apoptosis.	Under p38 constitutive activation and JNK loss-of-function, apoptotic attractors are lost.
Epigenetic gene silencing of GADD45 family members has been frequently observed in several types of human cancers.	In presence of DNA_damage), Growth_Arrest and Apoptosis components permanently oscillate when GADD45 is silenced, suggesting less propensity to cell death. Apoptotic stable states are still reached in presence of TGFBR_stimulus
ERK increases transcription of the cyclin genes and facilitates the formation of active Cyc/CDK complexes, leading to cell proliferation.	ERK gain-of-function always leads to proliferative attractors, in the absence of other stimuli.
ERK disrupts the anti-proliferative effects of TGFβ.	TGFBR_stimulus leads to an apoptotic stable state, but coupling of TGFBR_stimulus with ERK gain-of-function leads to growth arrest.
JNK might reduce RAS-dependent tumour formation by inhibiting proliferation and promoting apoptosis.	In absence of other stimuli, JNK constitutive activation completely abrogates RAS-dependent proliferation following RAS over-expression. Instead, apoptotic attractors are always reached.

Using the model to analyse feedback mechanisms

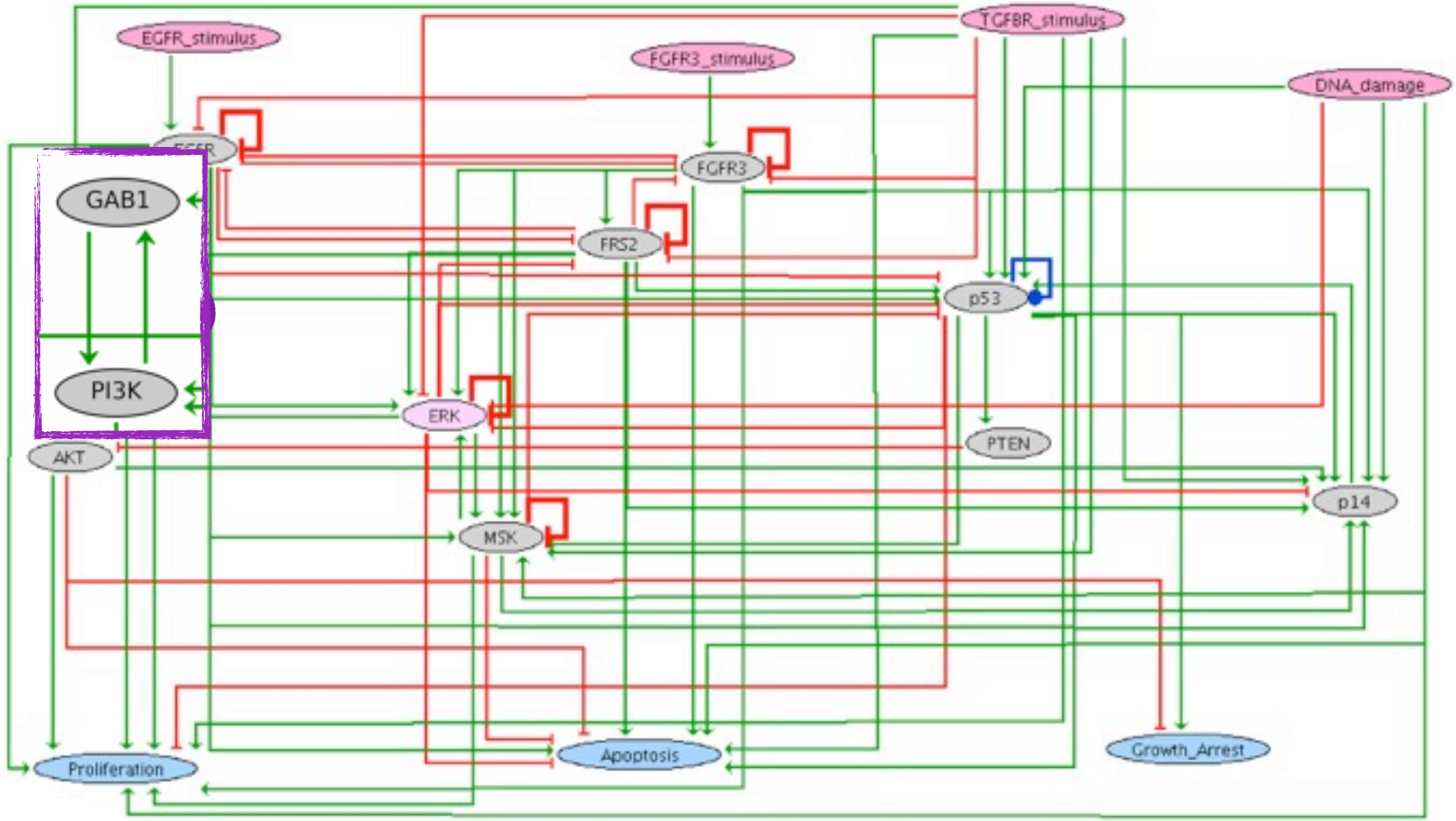


Simulation of the disruption of GRB2 or Sprouty feedback on FRS2 under FGFR3 gain-of-function



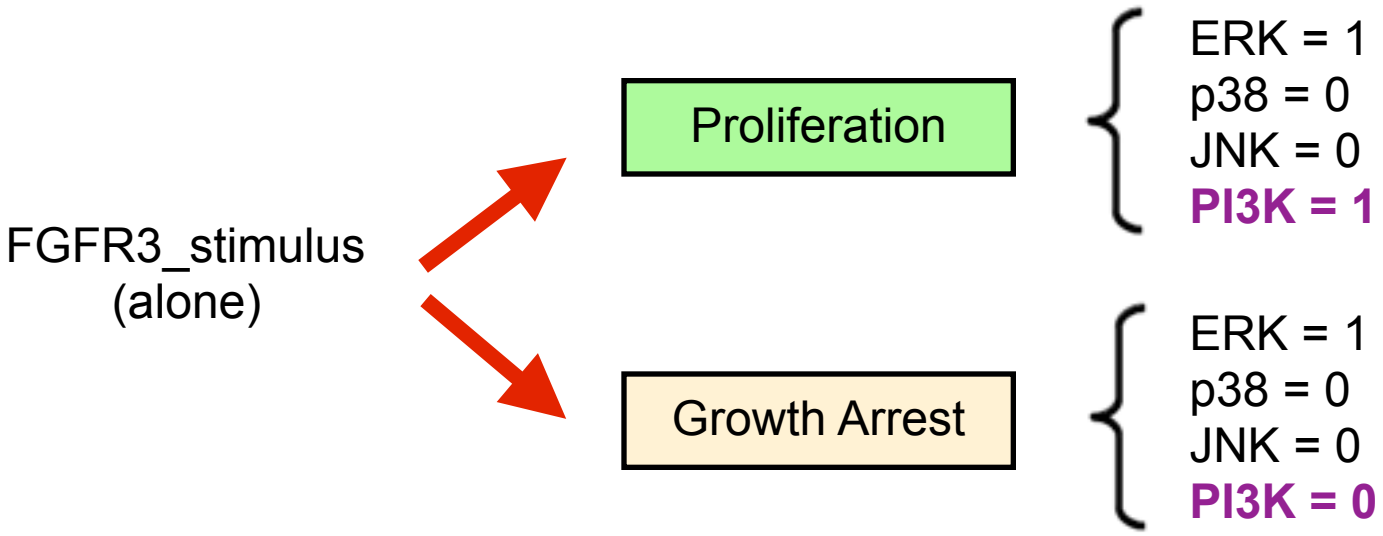
FGFR3 stimulation and multistability

Apoptosis	Growth_Arrest	Proliferation	ERK	p53	EGFR	FGFR3	FRS2	PI3K	AKT	MSK	p14	PTEN
0	0	**1**	1	0	0	1	0	1	1	1	1	0
0	0	0	1	0	0	1	0	0	0	1	0	0



Comparison with experimental data

PI3K activation tentatively influences the switch between proliferation and growth arrest following **FGFR3** stimulus:



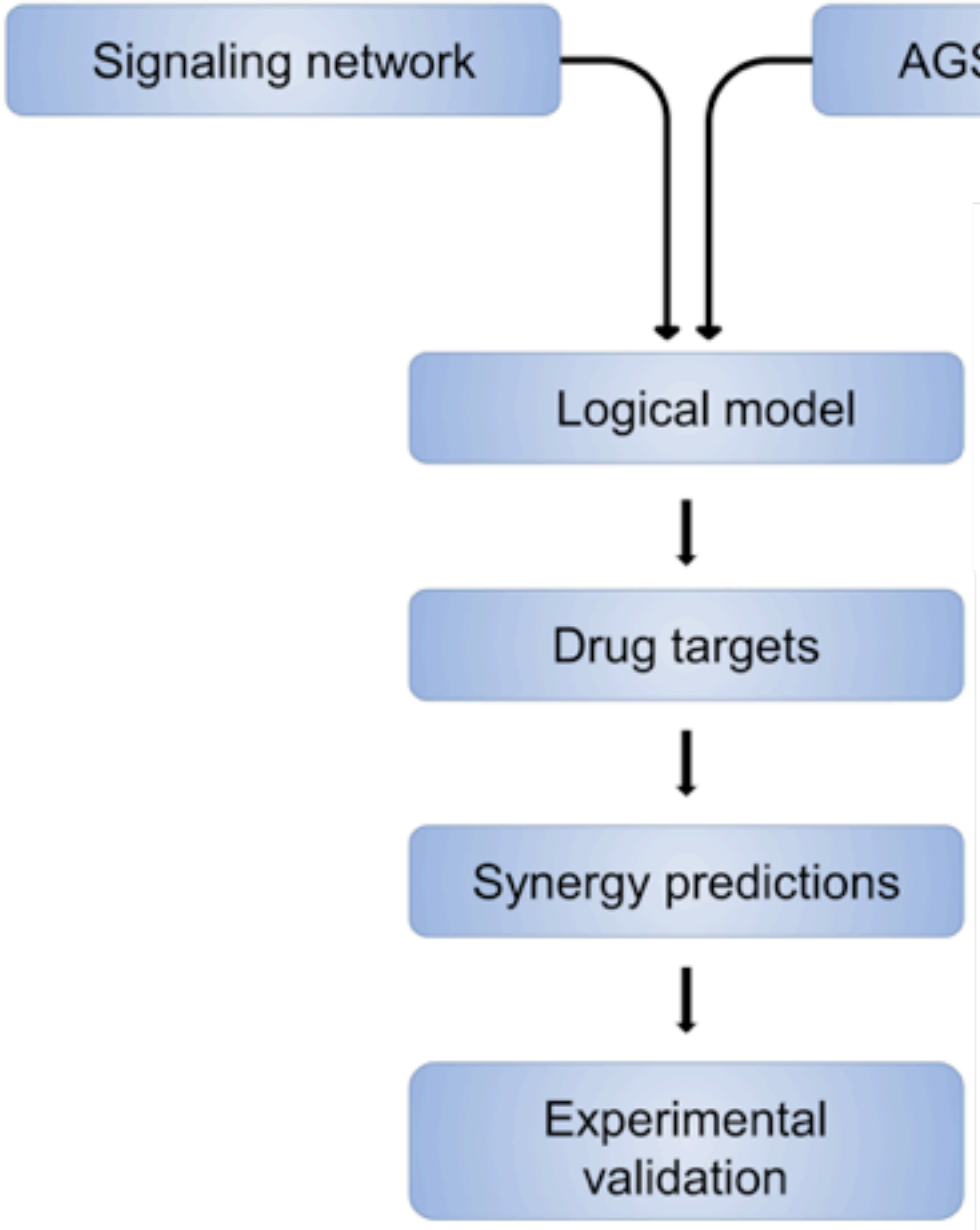
In **FGFR3-mutated bladder cancer cell lines**, **PI3K** activation (in contrast with MAPKs) is **determinant for proliferation** (Radvanyi's group, Institut Curie).

Outlook - MAPK and bladder cancer cell decisions

- **Qualitative recapitulation** of known effects of MAPK network on cancer cell fate decision, following specific stimuli
- **Insights** into the role of MAPK network and of specific components in different bladder cancer types
- **Novel hypotheses** concerning the mechanisms underlying the different effects of EGFR/FGFR3 deregulations
  - Feedbacks via Sprouty
  - PI3K switch following FGFR3 stimulus
- **Adaptation/extension of MAPK model for other cell types**
  => Analysis of drug synergies in a gastric cell line

Grieco *et al* (2013) *PLoS Comp Biol* **9**: e1003286

Prediction of drug synergies in gastric cancer cells

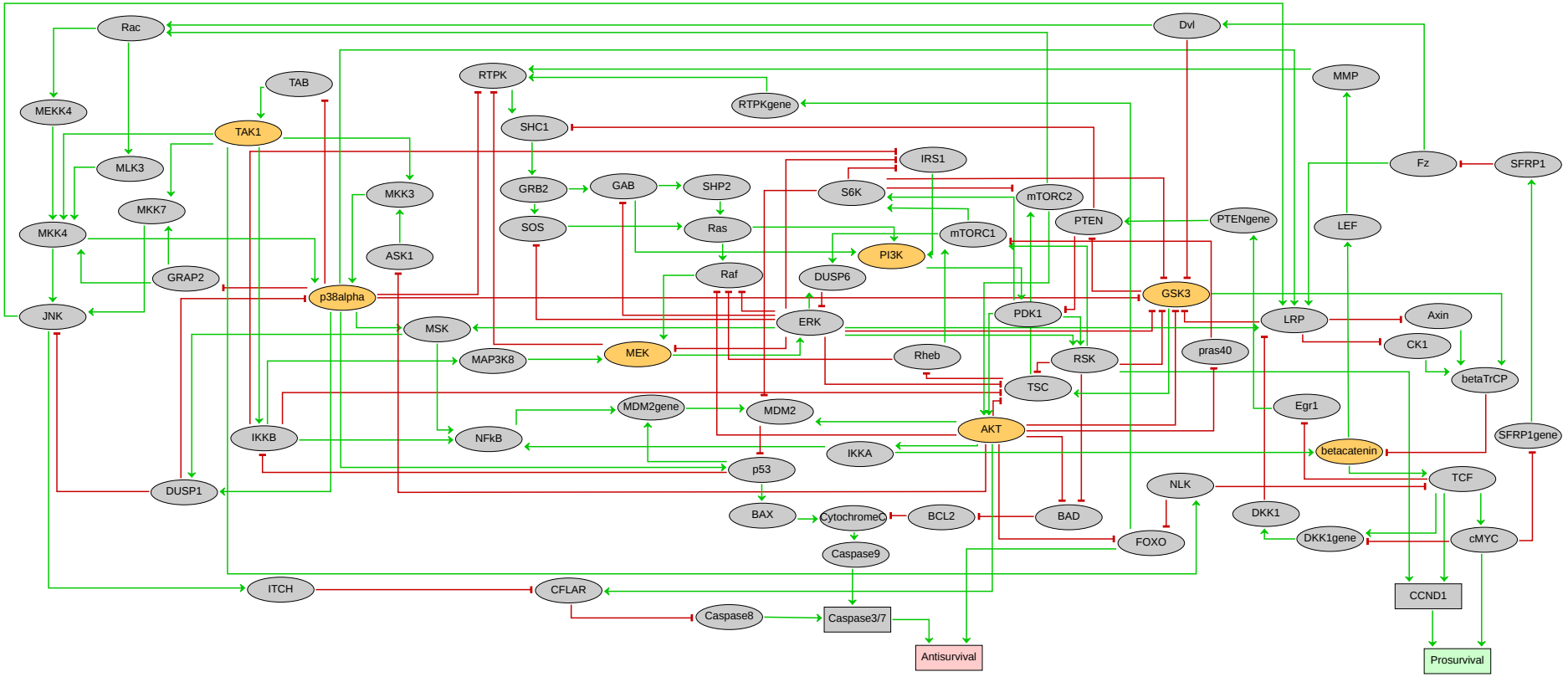


Asmund Flobak
NTNU, Norway

In collaboration with
Liv Thommesen, **Martin Kuiper** and **Astrid Lægreid** at NTNU, Trondheim, Norway
Anaïs Baudot and **Elisabeth Remy** at IML, Marseille, France

Flobak A et al (2015)
PLoS Comp Biol **11**: e1004426

Knowledge-based logical model for cell fate decision in AGS gastric adenocarcinoma cells

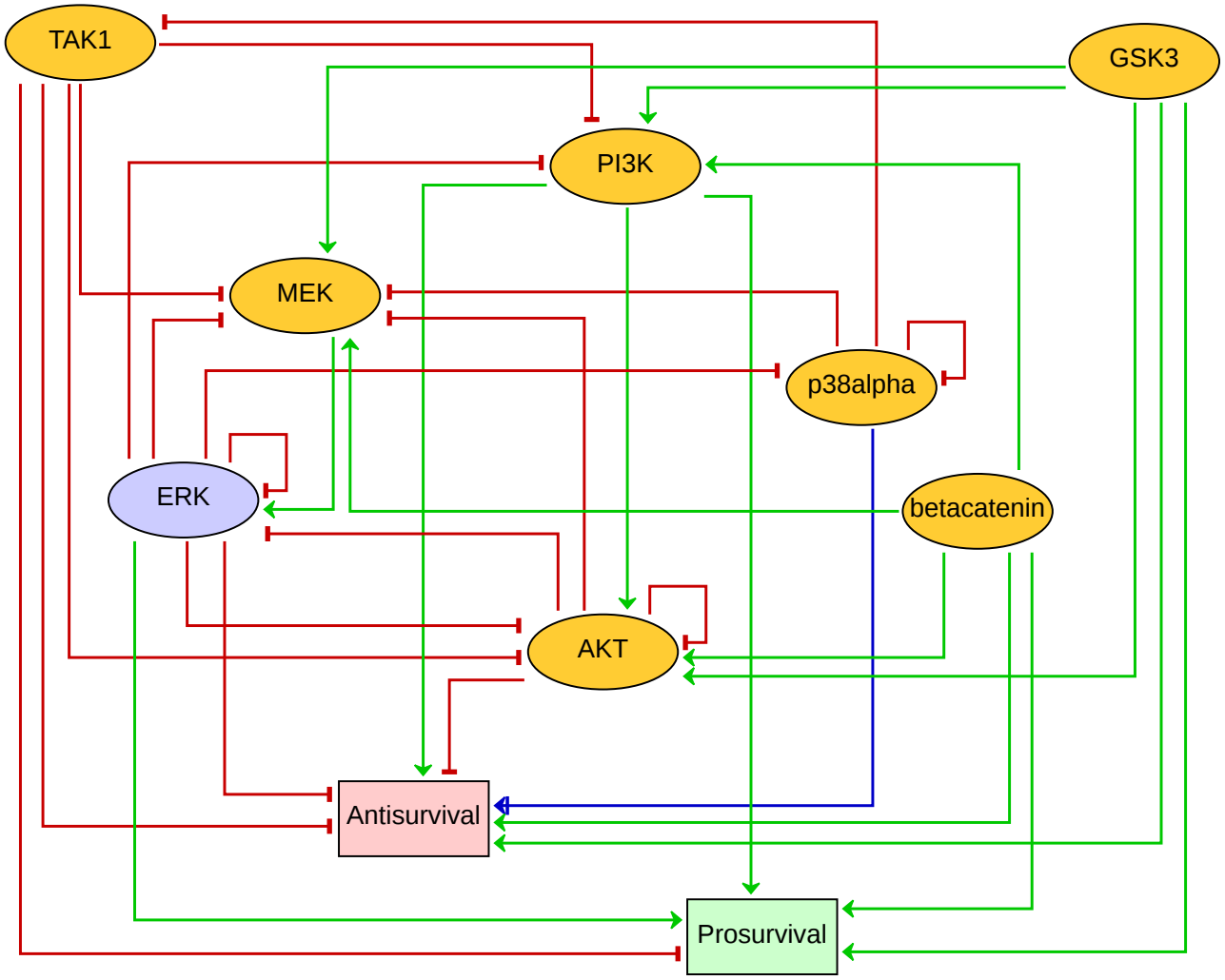


Logical network calibrated for actively growing AGS cells

Based on 72 scientific publications and 219 experiments providing information on model protein activities.

77 components, no input, single (proliferative) attractor = stable state

Reduced model for AGS cell growth



Reduced logical model, keeping 7 drug targets + 1 non reducible node (ERK) and two outputs => extensive perturbation analysis

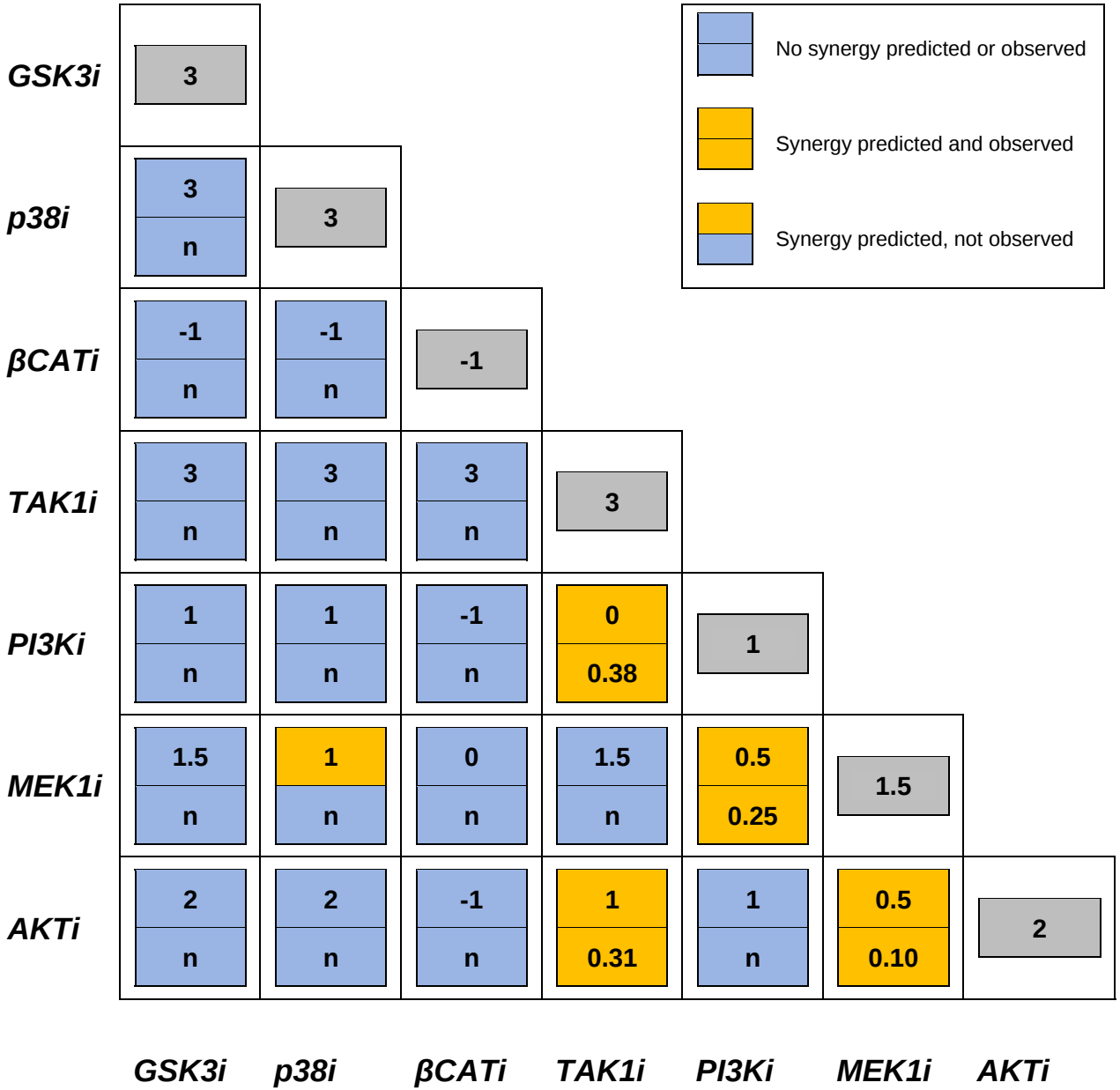
Chemical inhibitors and their targets.

Chemical inhibitor	*Target name*	*Target HGNC symbol*	*GI50**
(5Z)-7-oxozeaenol	TAK1	MAP3K7	0.5 μM
AKTi-1,2 (AKT inhibitor VIII)	AKT1/2	AKT1, AKT2	10 μM
BIRB0796	p38 MAPK	MAPK14	N/A (5 μM used) **
CT99021	GSK3	GSK3A, GSK3B	N/A (5 μM used) **
PD0325901	MEK	MAP2K1, MAP2K2	35 nM
PI103	PI3K	PIK3CA	0.7 μM
PKF118-310	β-catenin	CTNNB1	150 nM

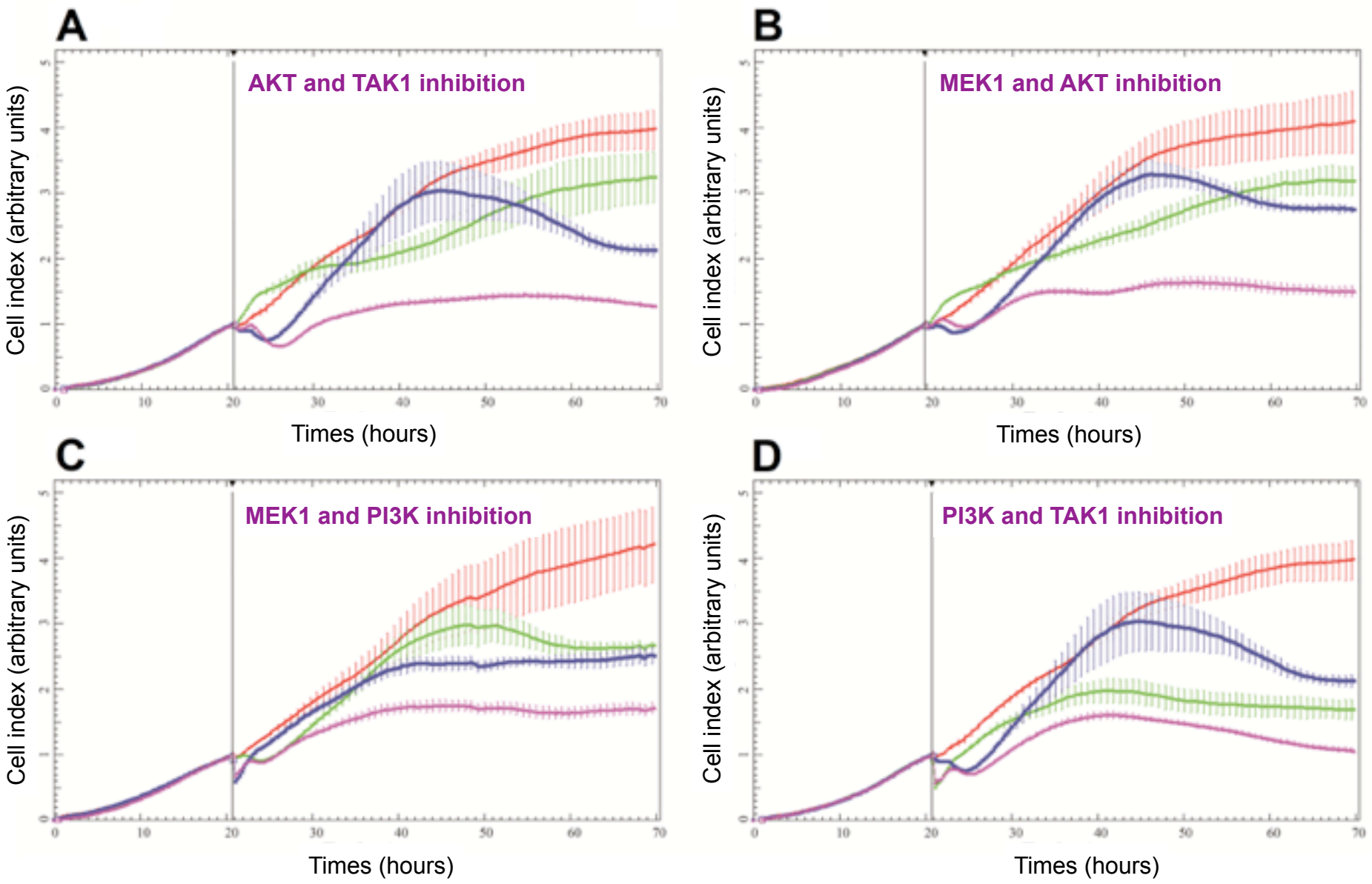
* Experimentally determined concentration that inhibits AGS cell growth by 50% (GI50).

** For the two inhibitors BIRB0796 and CT99021 no GI50 could be obtained, and 5 μM was chosen as a concentration that is expected affect their target in our experimental setup, based on observed effects in similar cell systems [27]. See Supporting Information S1 for further documentation of inhibitor properties.

Prediction of Drug Synergies in AGS Cells



Assessing predicted Drug Synergies in AGS cells



Outlook - prediction of drug synergies

- Prediction of synergistic action of pairs of drugs on AGS cell growth
- All **16** predicted non synergetic drug pairs => confirmed in AGS cells
- **4** of the **5** predicted synergies => confirmed in AGS cells
  - Known effects of combined MEK-AKT or MEK-PI3K inhibitions
  - Novel synergistic effects of TAK1-AKT and TAK1-PI3K inhibitions
- Combinatorial drug effects can be inferred from background knowledge on unperturbed and proliferating cancer cells
- Generalization of this approach => large pannel of cancer cell lines
- From in vitro to in vivo drug synergy assessment (xenografts)

Collaborations & supports

- ★ **ENS (Paris)**
  - Wassim Abou-Jaoudé
  - Samuel Collombet
  - Jérome Feret
  - Morgane Thomas-Chollier
  - Pauline Traynard
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  - Isabelle Bernard-Pierrot
  - Laurence Calzone
  - Francois Radvanyi
  - Andrei Zinovyev
- ★ **NTNU (Trondheim)**
  - **Åsmund Flobak**
  - Liv Thommesen
  - Martin Kuiper
  - Astrid Lægreid
- ★ **TAGC (Marseille)**
  - **Luca Grieco** (=> UCL, London)
  - Brigitte Kahn-Perlès
  - Aurélien Naldi (=> Univ. Montpellier)
  - Jacques van Helden
- ★ **IML (Marseille)**
  - Anaïs Baudot
  - Elisabeth Rémy
- ★ **IGC (Lisboa)**
  - Claudine Chaouiya
  - Pedro Monteiro

Belgian Inter-university Attraction Pole

Bioinformatics and Modelling : from Genomes to Networks

Further reading

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