Using perturbation data for ensemble modeling to infer vulnerabilities in colon cancer cells

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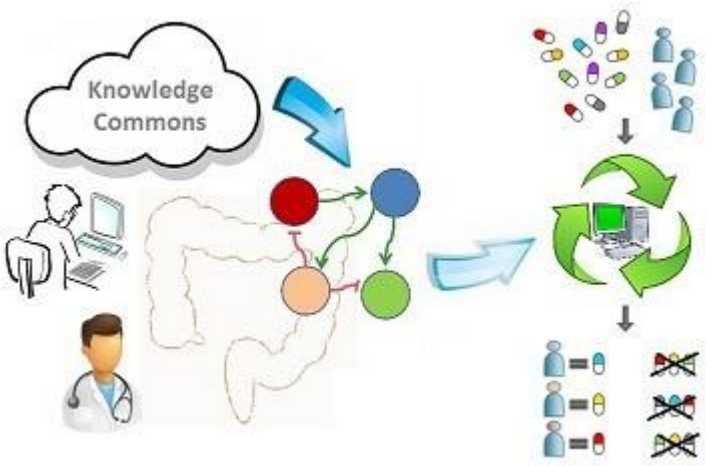
Mathematical Perspectives in the Biology and Therapeutics of Cancer

12/07/2018

Drug resistance in colon cancer

COLOSYS

A systems approach to preventing drug resistance in colon cancer



Project description:

"Current treatment involves chemotherapy combined with anti-EGFR [...] drugs and radiotherapy. First-line combinations of chemotherapy and EGFR-inhibitors for patients being RAS wild-type have led to an increase in overall survival to more than 30 months.

However, most patients develop ***RAS mutations*** *under anti-EGFR therapy, or do not respond to EGFRi for unknown reasons. The majority of patients develop resistance and succumb to the disease."*

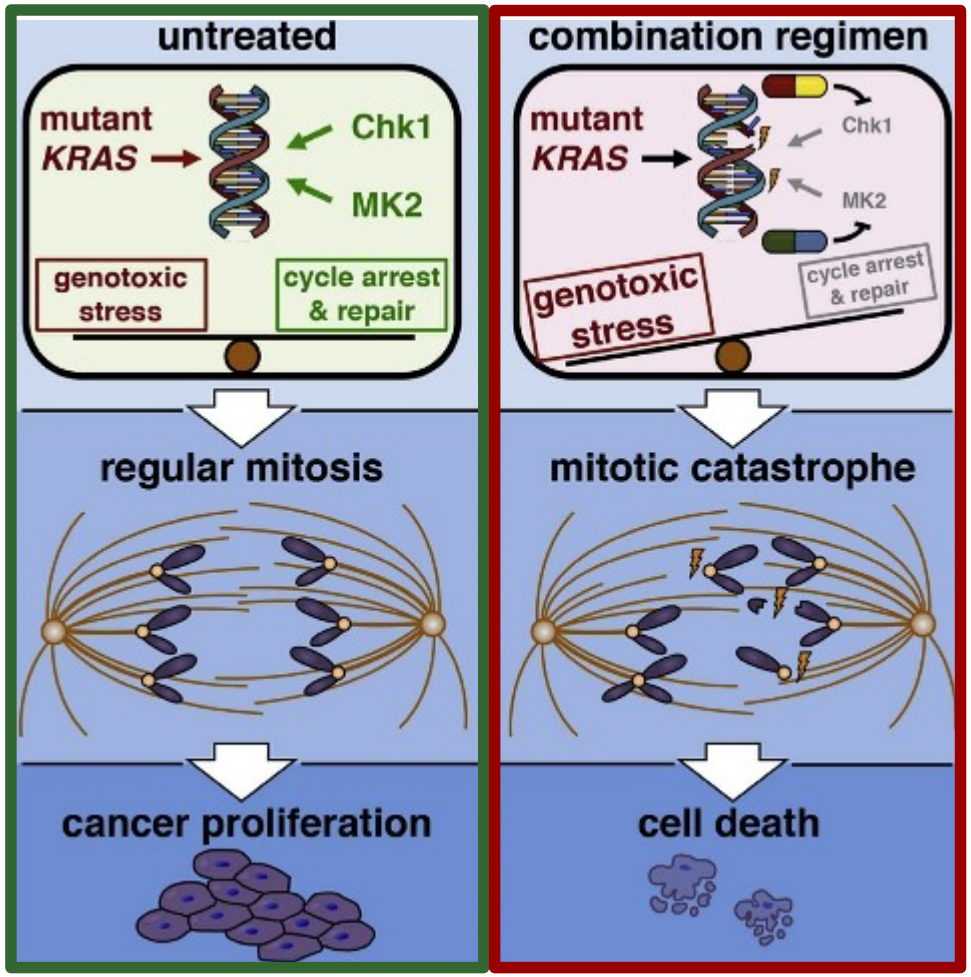
Combinatorial drug effects in colon cancer cells

A Synergistic Interaction between Chk1- and MK2 Inhibitors in *KRAS*-Mutant Cancer

Cell

Dietlein et al., 2015, Cell *162*, 146–159
July 2, 2015 ©2015 Elsevier Inc.
http://dx.doi.org/10.1016/j.cell.2015.05.053

- KRAS mutants: high DNA damage
- intact DNA repair mechanism: cell cycle arrest → resumed proliferation
- parallel blocking of MK2 (MAPK) and CHK1 (DNA repair) leads to mitotic catastrophe
- single inhibition of MK2 or CHK1 does *not* lead to killing



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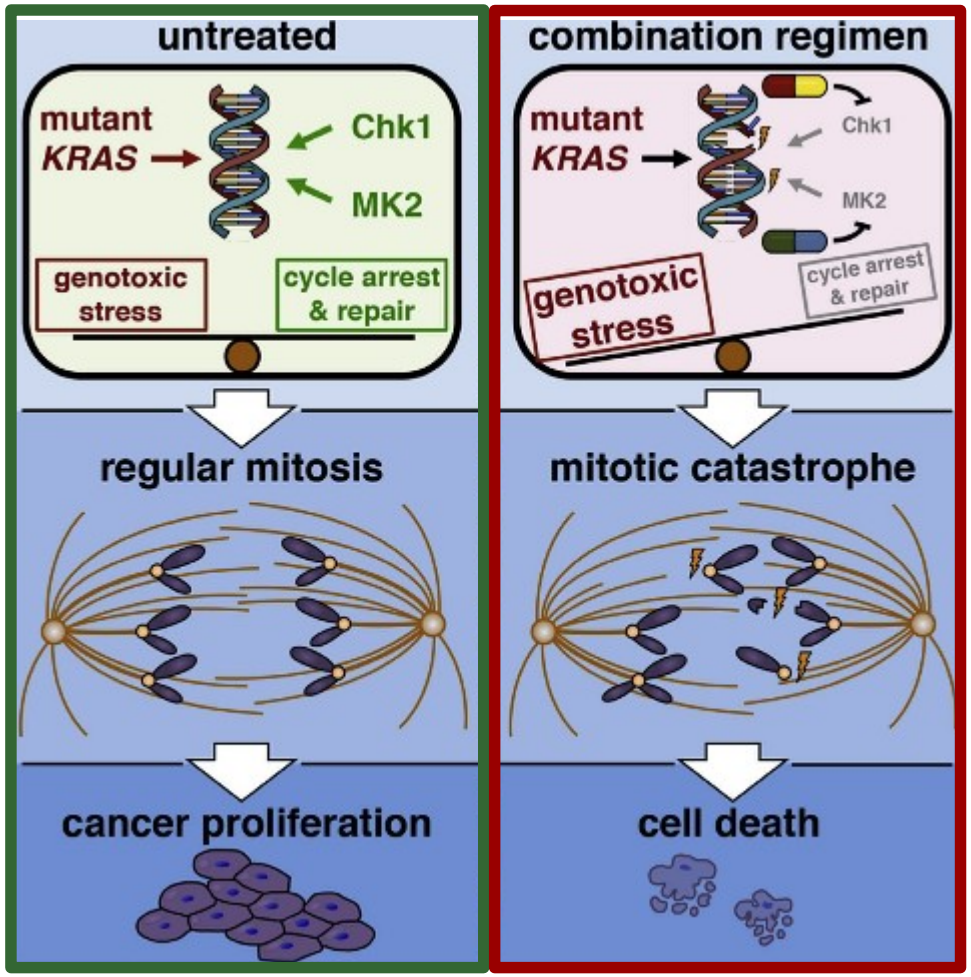
Use modeling to mechanistically explain combinatorial effect in KRAS/BRAF mutants & find further vulnerabilities



Consortium data sources

CHARITÉ
UNIVERSITÄTSMEDIZIN BERLIN

Institute of Pathology

Prof. Christine Sers
Dr Markus Morkel
Natalie Bubitz

Perturbations of DNA damage-related cell cycle arrest proteins (MK2, CHEK1) in different concentrations – **readout**:

Growth

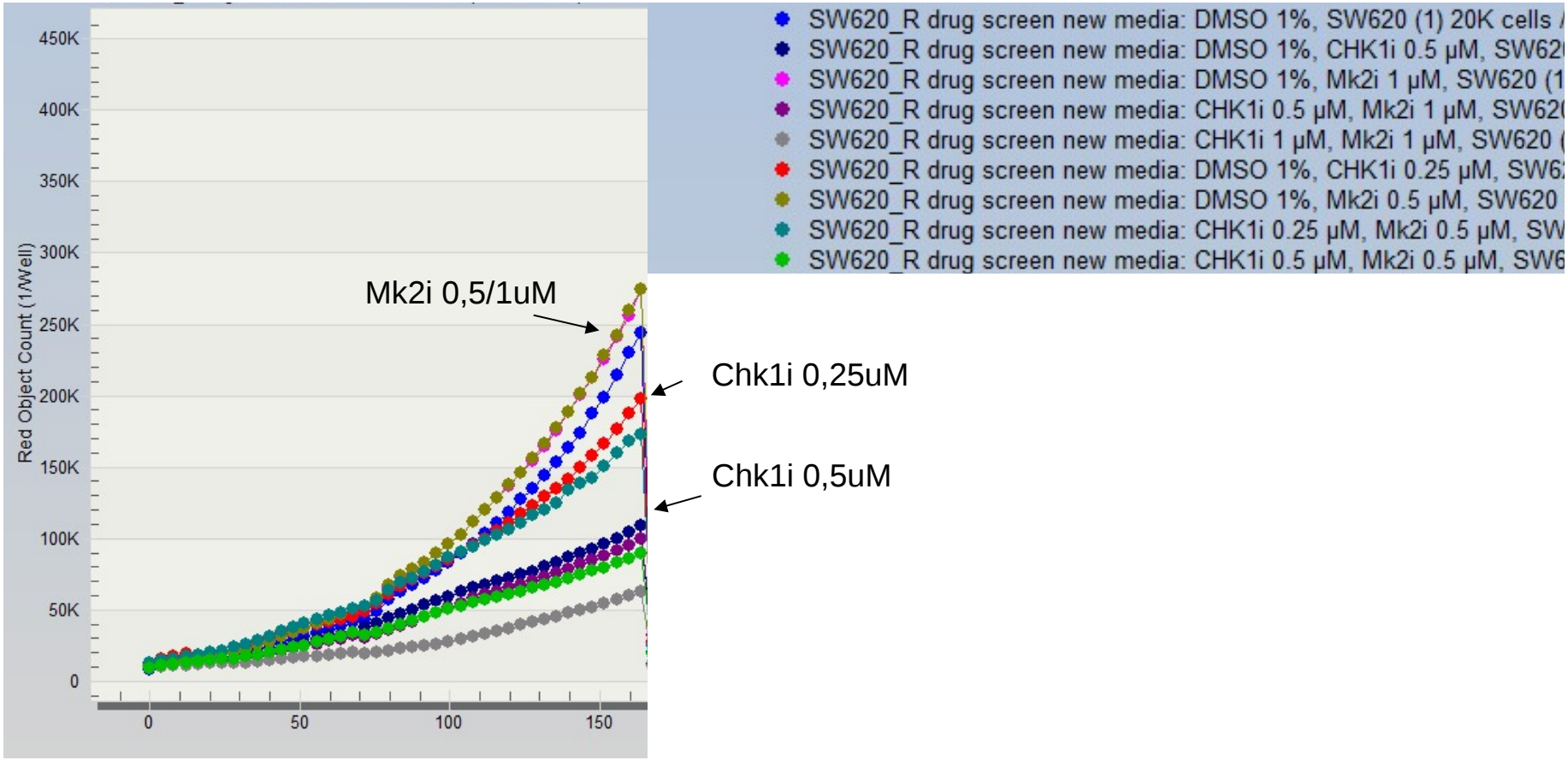
	summary all	PF477736					
	SW620 KRAS G12V	Chk1i 0µM	Chk1i 0,1µM	Chk1i 0,25µM	Chk1i 0,5µM	Chk1i 1µM	Chk1i 2,5µM
PF3644022	Mk2i 0µM	1.00	1.10	1.05	0.97	0.62	0.29
	Mk2i 0,5µM	1.01	0.98	0.98	0.86	0.52	0.24
	Mk2i 1µM	0.85	0.76	0.76	0.70	0.45	0.22
	Mk2i 2,5µM	0.76	0.78	0.73	0.58	0.34	0.21

Growth rate of cell line SW620 under different [Chk1i] and [MK2i]

Consortium data sources

Perturbations of DNA damage-related cell cycle arrest proteins (MK2, CHEK1) in different concentrations – **readout**:

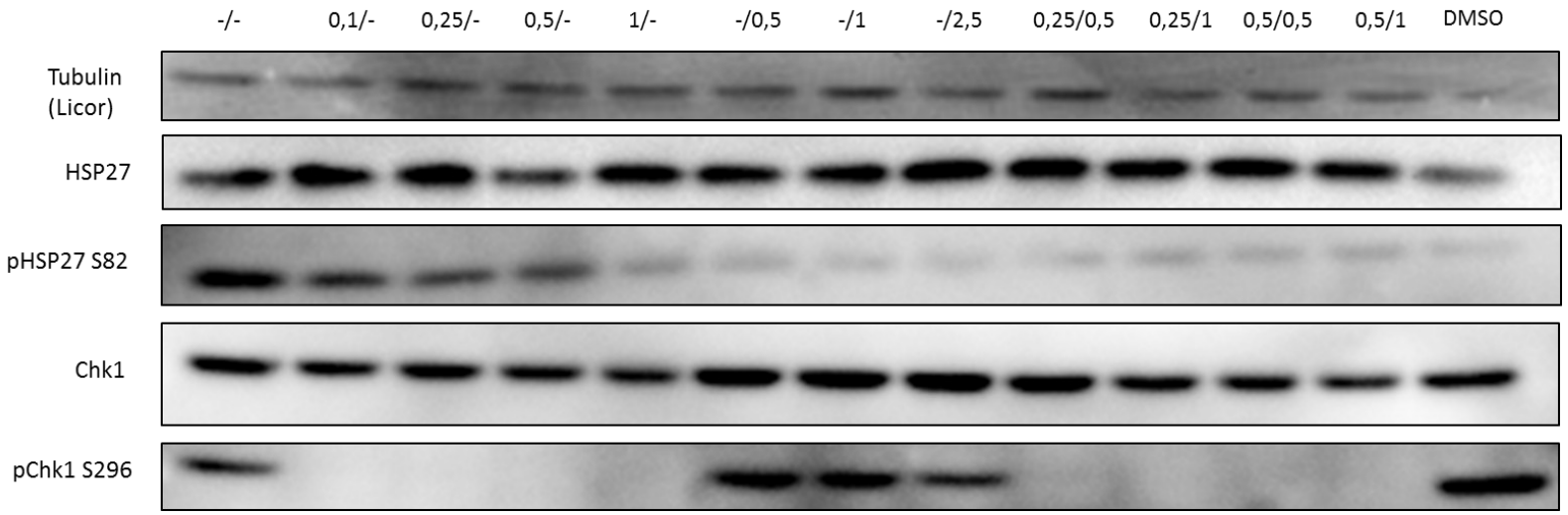
Growth



Consortium data sources

Perturbations of DNA damage-related cell cycle arrest proteins (MK2, CHEK1) in different concentrations – **readout**:

Phosphoprotein concentrations by WES™
(capillary immunoassay / quantifiable 'Western')



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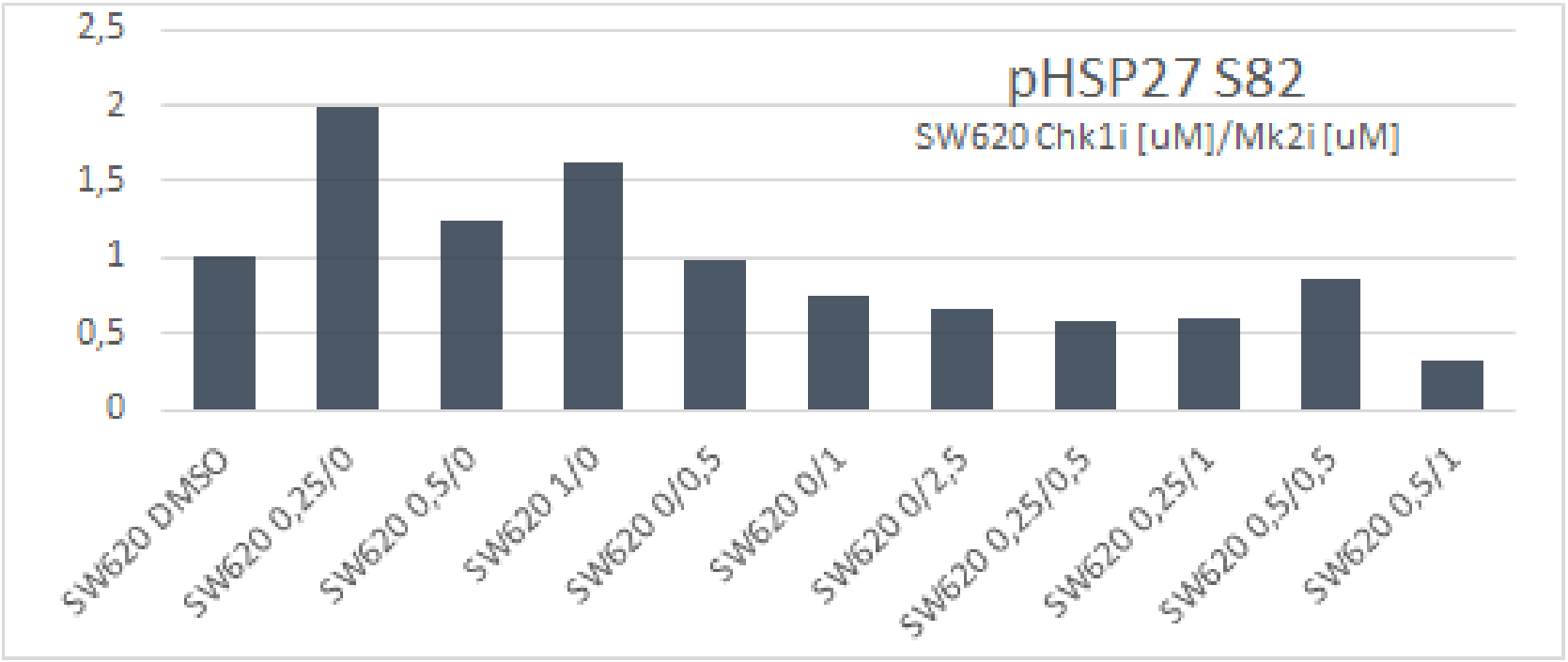
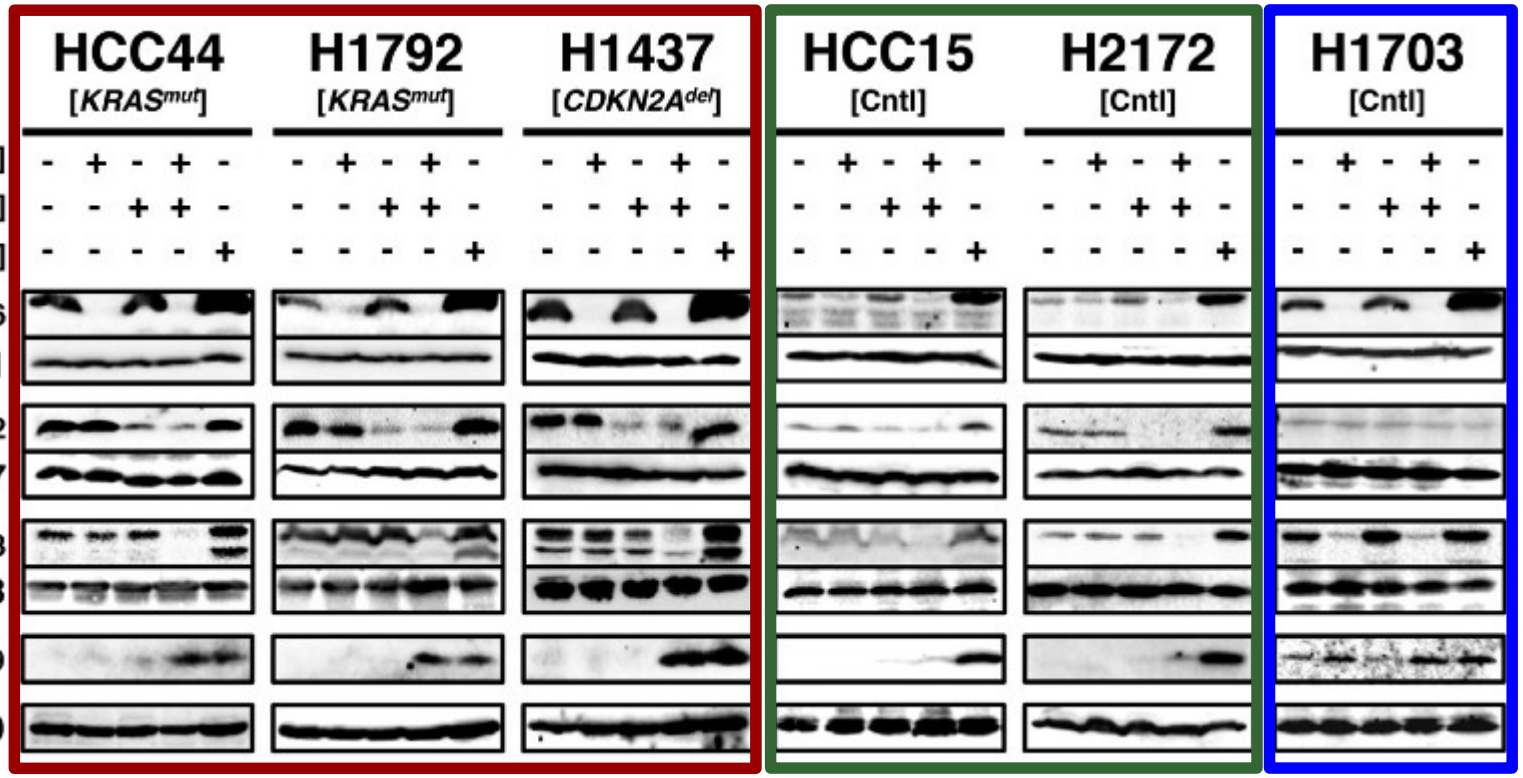


Data from literature

- Western blots on activation state of key components
- 'wild type' cell lines: no DNA damage, no effect of MK2i/CHK1i
- cell lines with DNA damage, but no KRAS/BRAF mutation (HT1703)
- BRAF, CDKN2A, KRAS mutant cell lines: killing only by MK2i & CHK1i

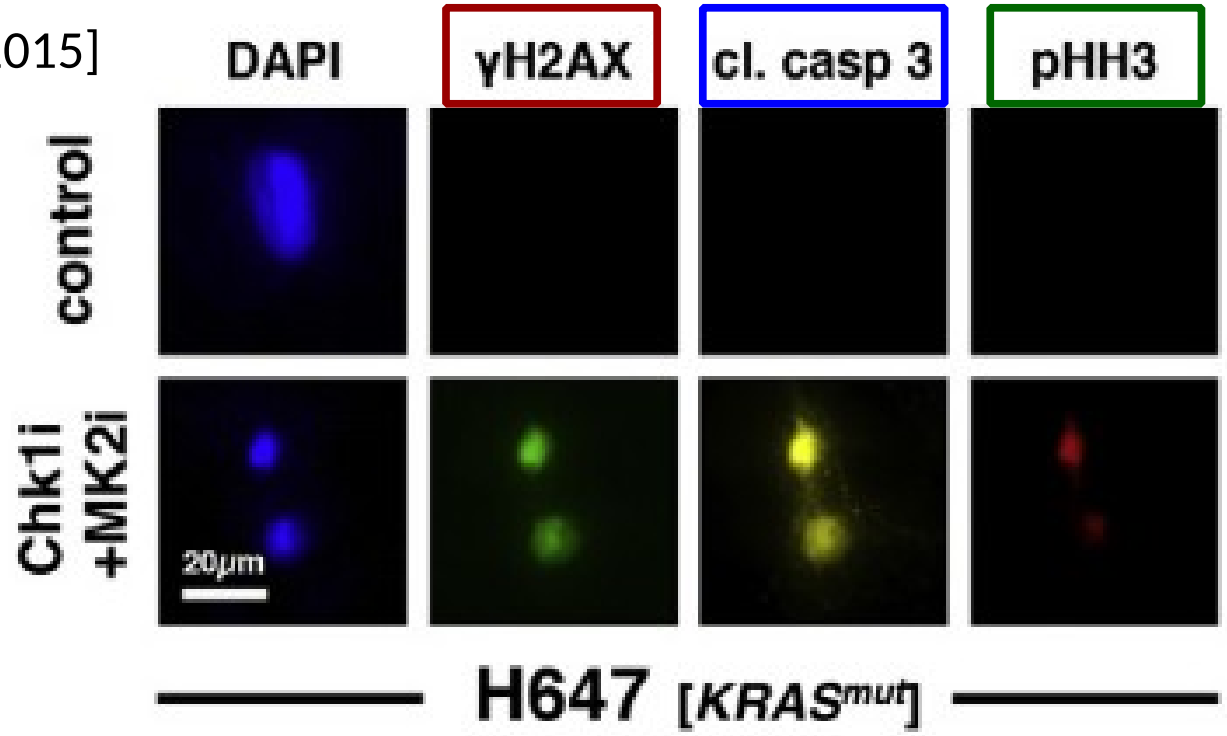
[Dietlein 2015]



Combinatorial drug effects in colon cancer cells

- 3 markers of *mitotic catastrophe* = γH2AX (DNA damage) + CC3 (cleaved caspase, apoptotic cell death) + pHH3 (histone, mitosis)
- Triple positivity indicates cell death *in mitosis*

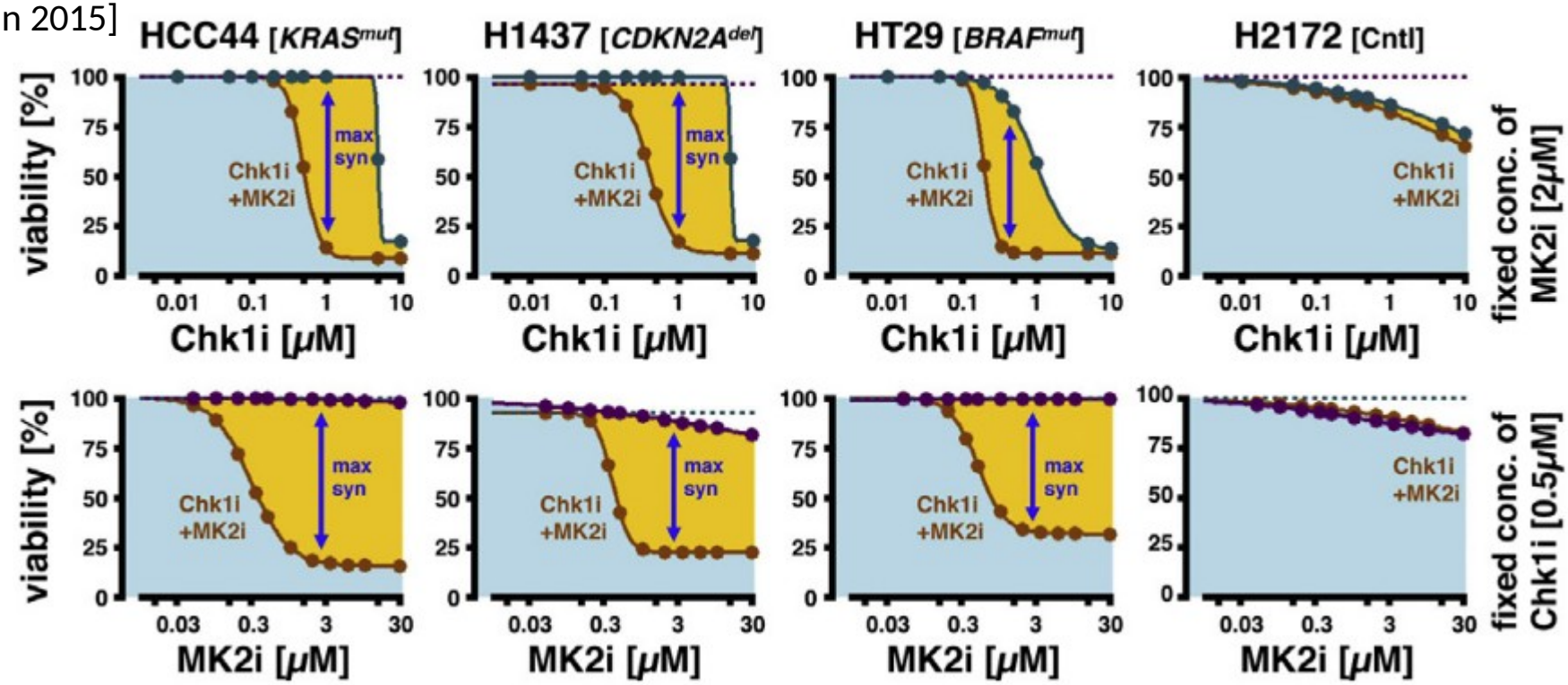
[Dietlein 2015]



Combinatorial drug effects in colon cancer cells

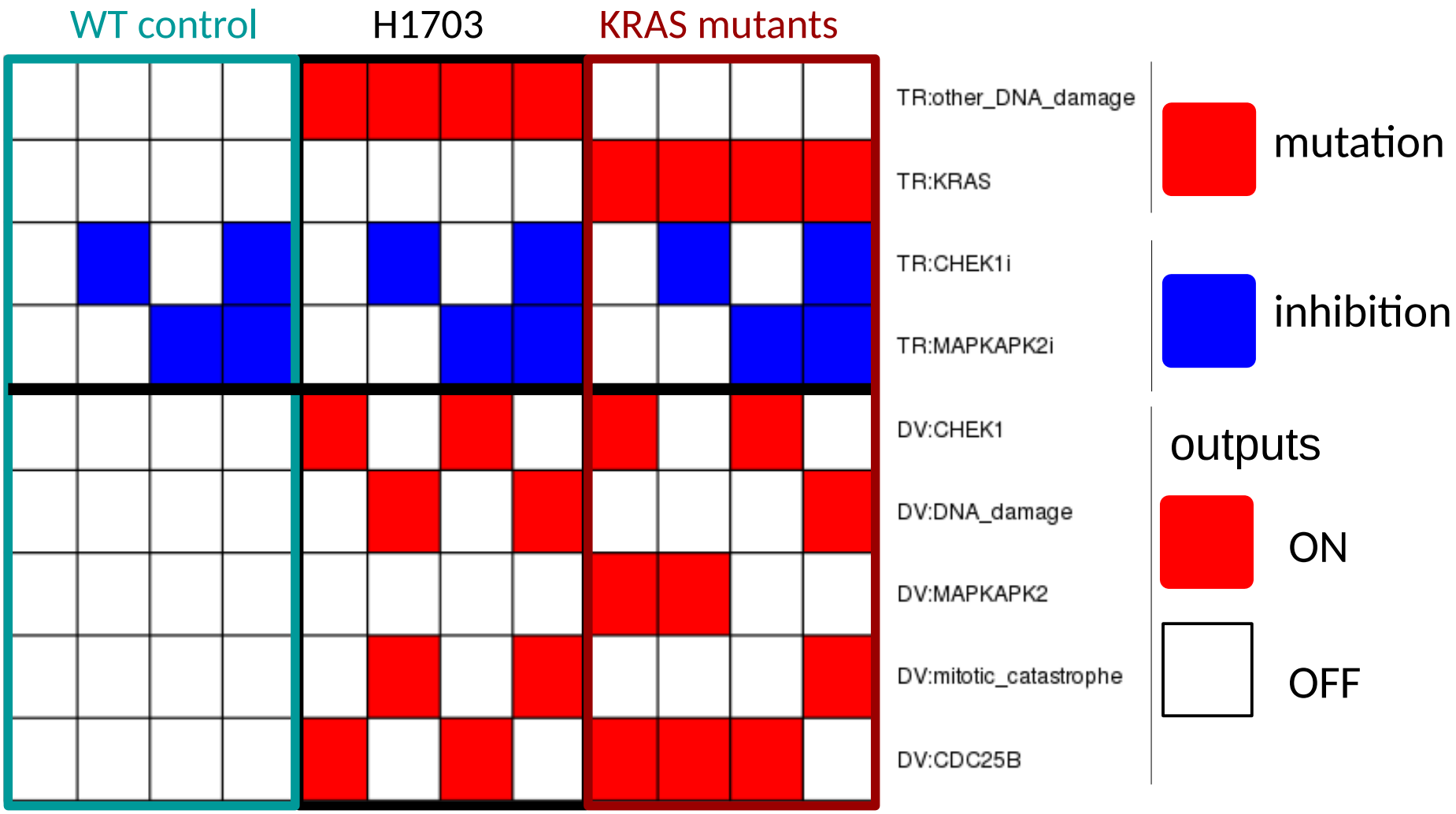
- 3 markers of *mitotic catastrophe* = γH2AX (DNA damage) + CC3 (cleaved caspase, apoptotic cell death) + pHH3 (histone, mitosis)
- Triple positivity indicates cell death *in mitosis*
- Growth experiments showing synergistic effect

[Dietlein 2015]



Using modeling to hypothesize mechanisms

Western Blots + conditions (mutations, inhibitors) as binary constraints:

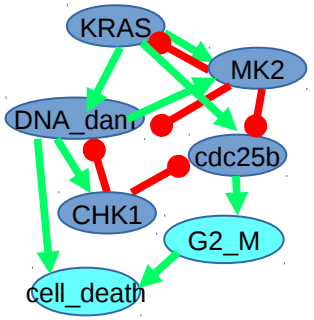


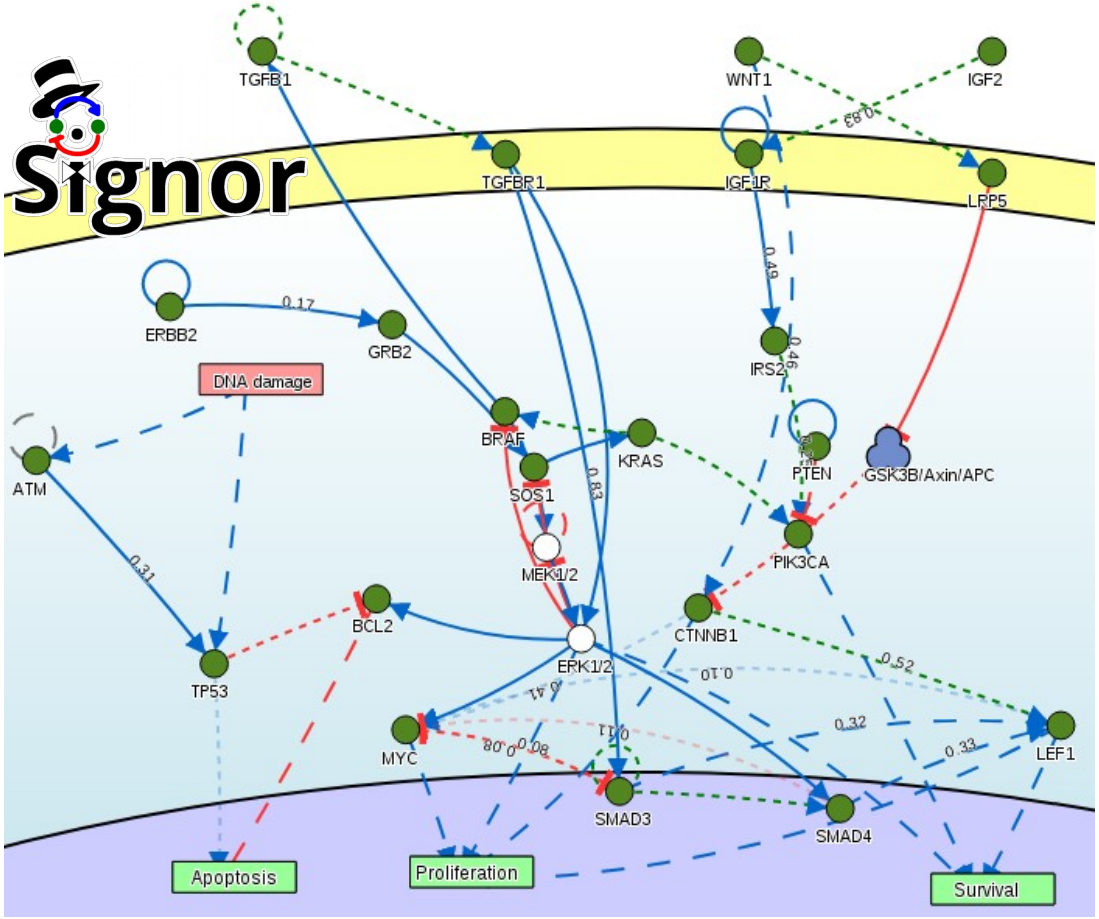
Using modeling to hypothesize mechanisms

influence network
(databases / literature)

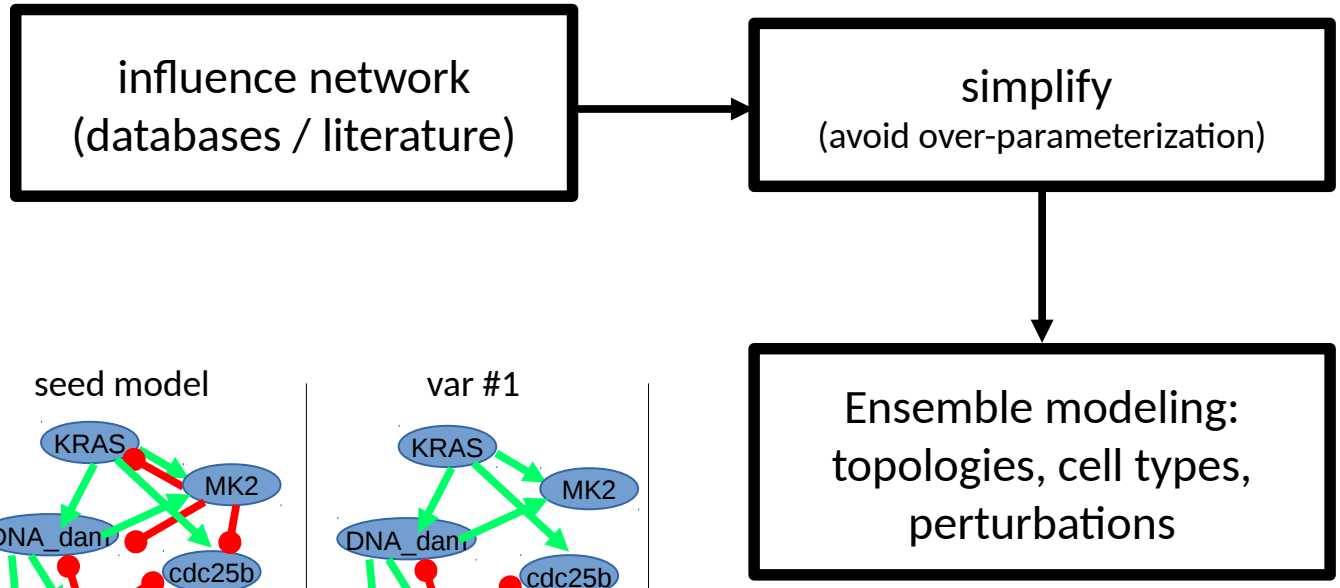
simplify
(avoid over-parameterization)

seed model





Using modeling to hypothesize mechanisms

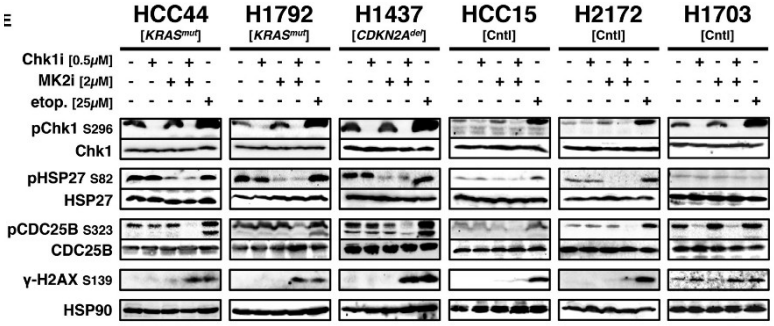


Using modeling to hypothesize mechanisms

influence network
(databases / literature)

simplify
(avoid over-parameterization)

Ensemble modeling:
topologies, cell types,
perturbations



comparison
with data

Experimental
conditions
and readouts

Structural analysis
of ranked models

Using modeling to hypothesize mechanisms

influence network
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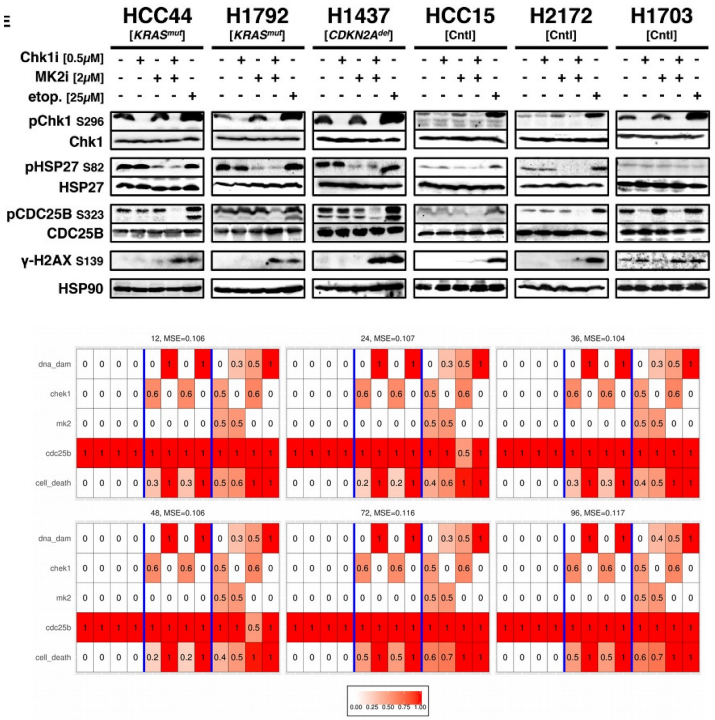
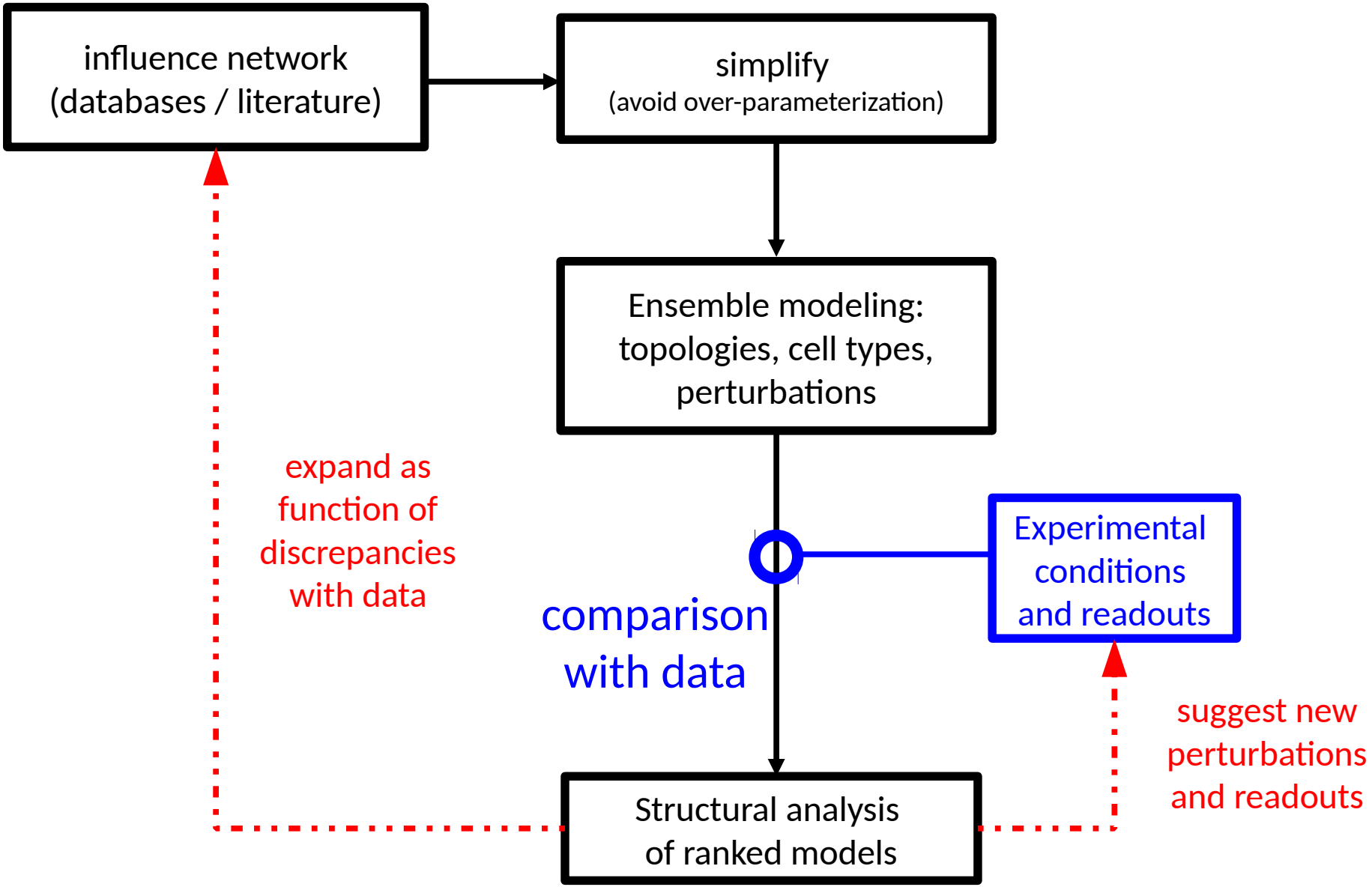
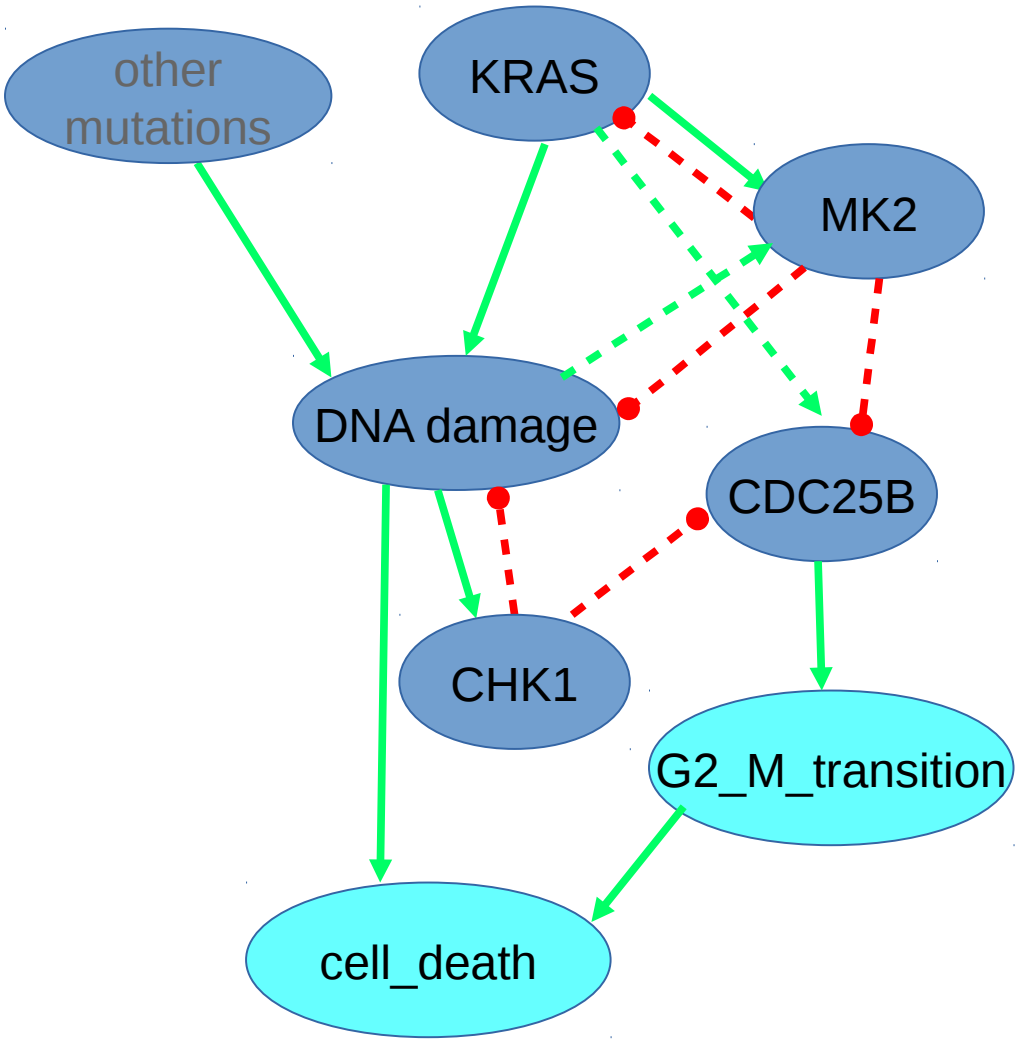


Using modeling to hypothesize mechanisms



Ensemble modeling

Generate *biologically justified* topologies



variable edges

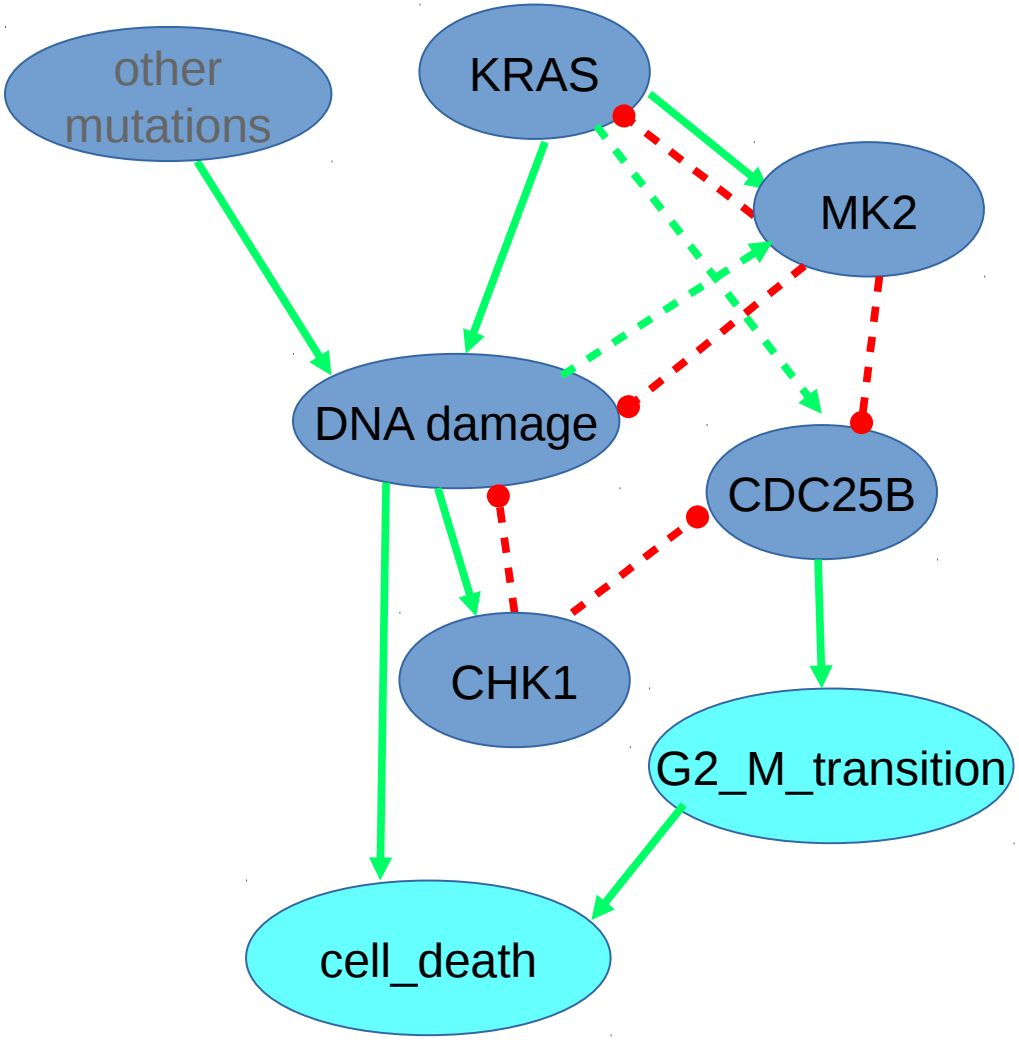
120 possible topologies
3 cell types
4 conditions (perturbations)

1440 simulations

(intermediate nodes omitted, CHK1 is not a direct repair protein etc.)

Ensemble modeling

Generate *biologically justified* topologies

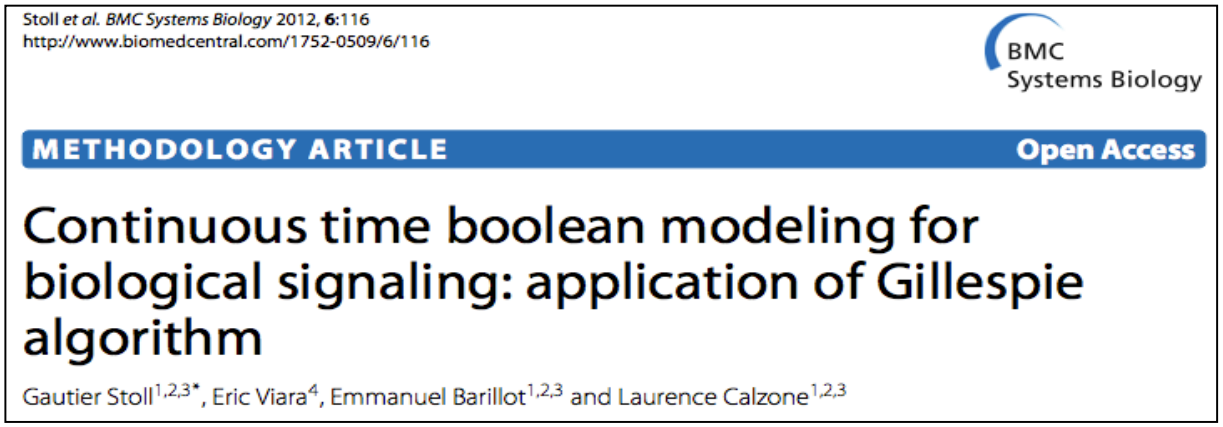


variable edges

'Variable' edges *supported* by literature / database:

- MK2, CHEK1 activated by DNA damage
- MK2, CHEK1 implicated in DNA repair
- MK2 negative feedback loop to upstream MAPK elements

MaBoSS: stochastic logical modeling



Stoll *et al. BMC Systems Biology* 2012, **6**:116
http://www.biomedcentral.com/1752-0509/6/116

BMC Systems Biology

METHODOLOGY ARTICLE Open Access

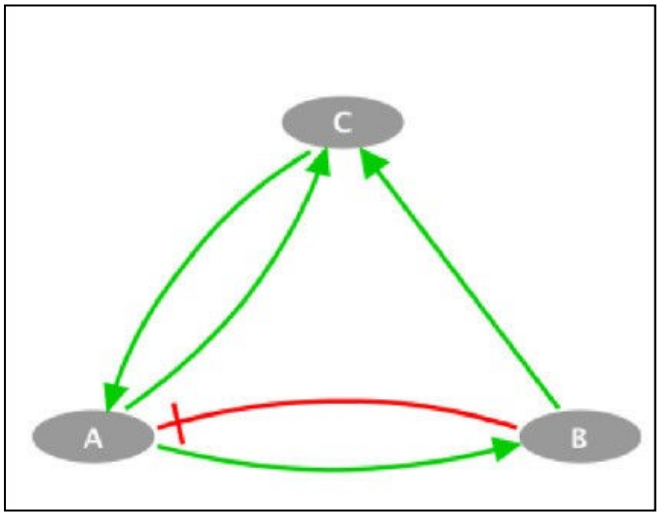
Continuous time boolean modeling for biological signaling: application of Gillespie algorithm

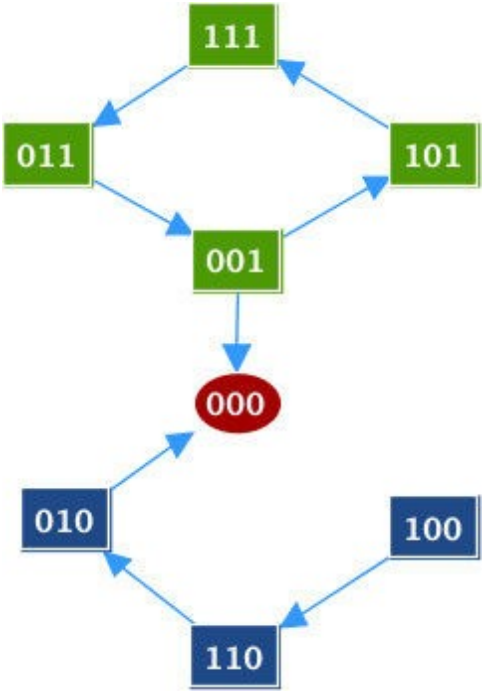
Gautier Stoll[1,2,3*], Eric Viara[4], Emmanuel Barillot[1,2,3] and Laurence Calzone[1,2,3]

https://maboss.curie.fr

- stochastic, continuous time implementation of logical model

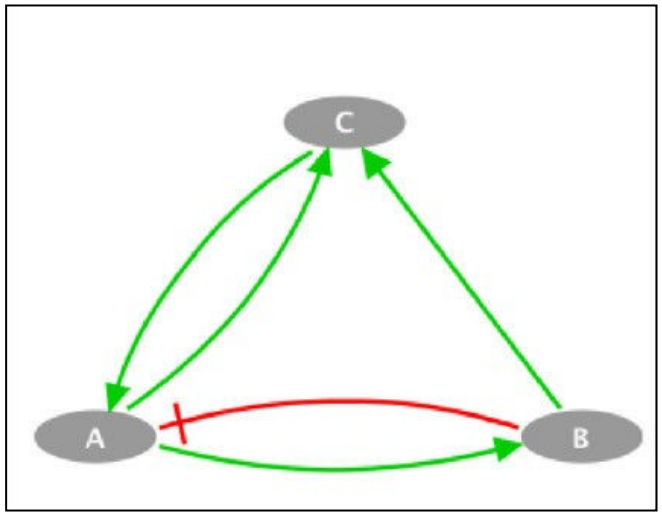
MaBoSS: stochastic logical modeling

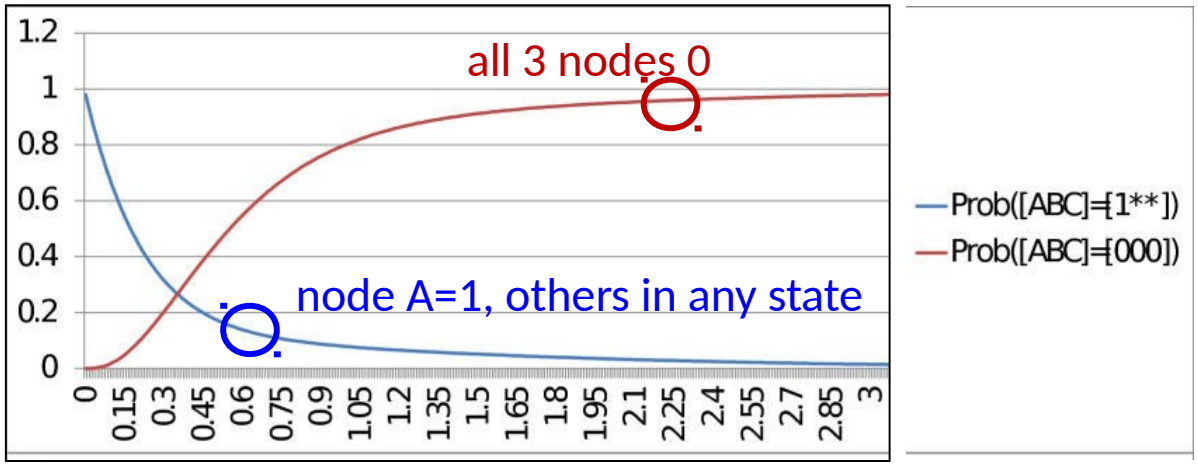




- stochastic, continuous time implementation of logical model
- one node selected for updating: one simulation is a sample trajectory

MaBoSS: stochastic logical modeling





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- fraction of given states across *many* sample trajectories

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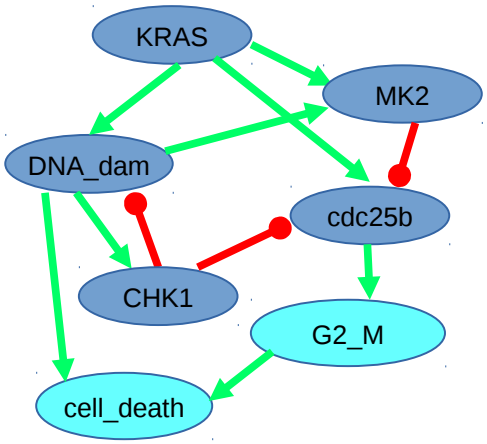


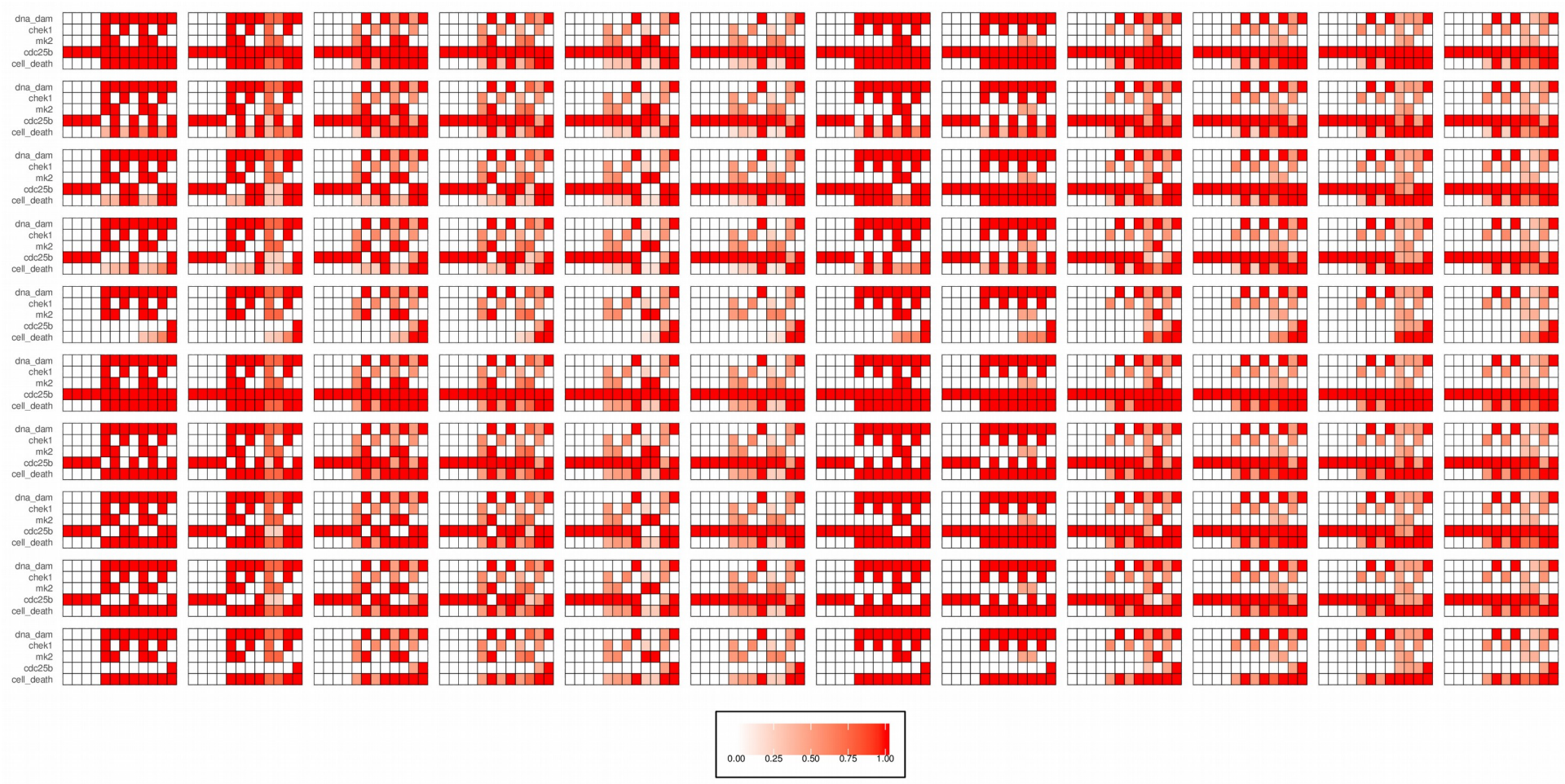
perturbations

	-/-		+/-		-/+		+/+					
dna_dam	0	0	0	0	0	1	0	1	0	0.3	0.5	1
chek1	0	0	0	0	0.6	0	0.6	0	0.5	0	0.6	0
mk2	0	0	0	0	0	0	0	0	0.5	0.5	0	0
cdc25b	1	1	1	1	1	1	1	1	1	1	1	1
cell_death	0	0	0	0	0.3	1	0.3	1	0.5	0.6	1	1

wt control | h1703 | kras mutant

- stochastic, continuous time implementation of logical model
- one node selected for updating: one simulation is a sample trajectory
- fraction of given states across *many* sample trajectories

Ensemble modeling



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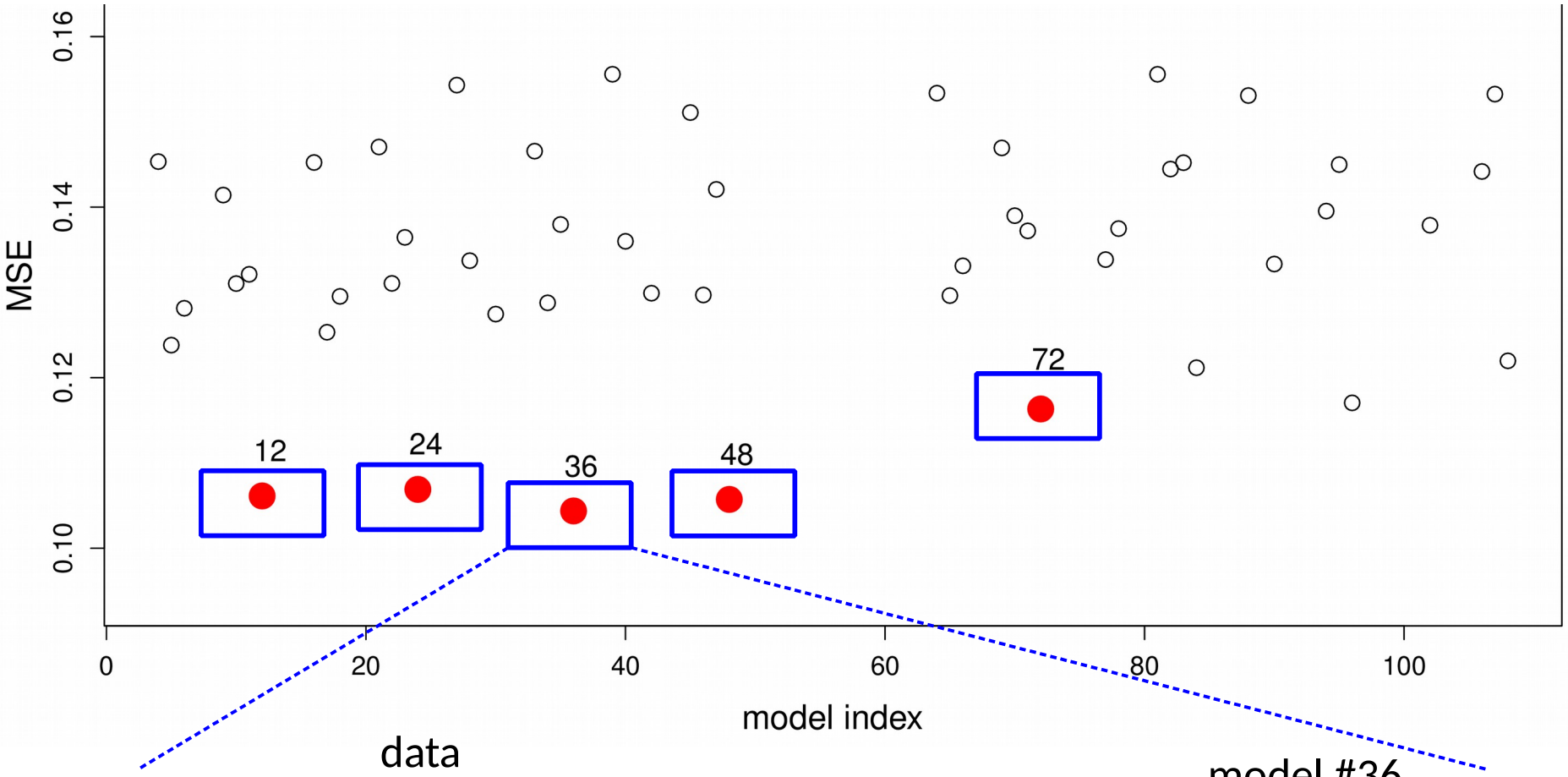
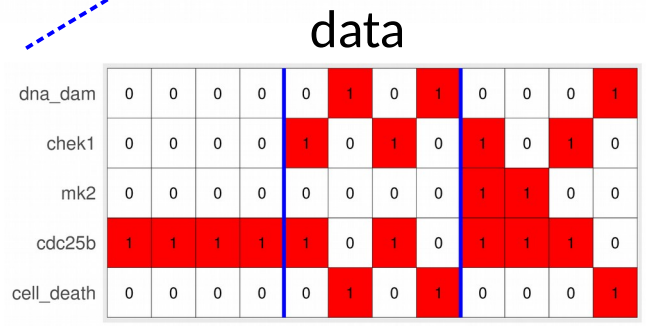
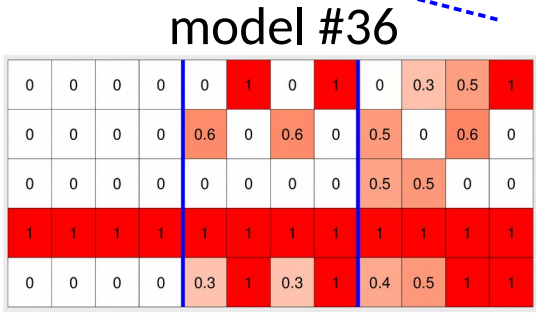




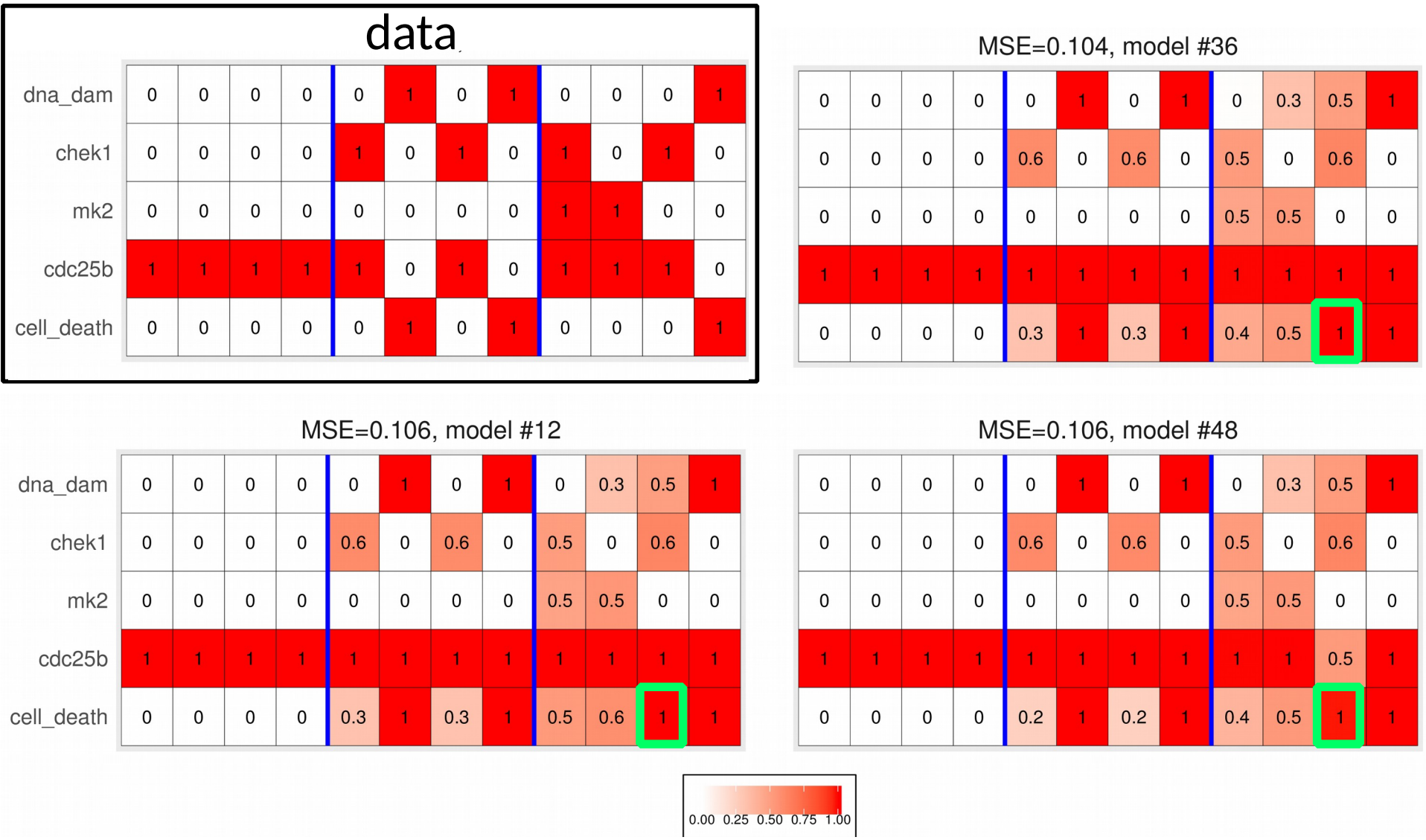
distance



Ensemble modeling

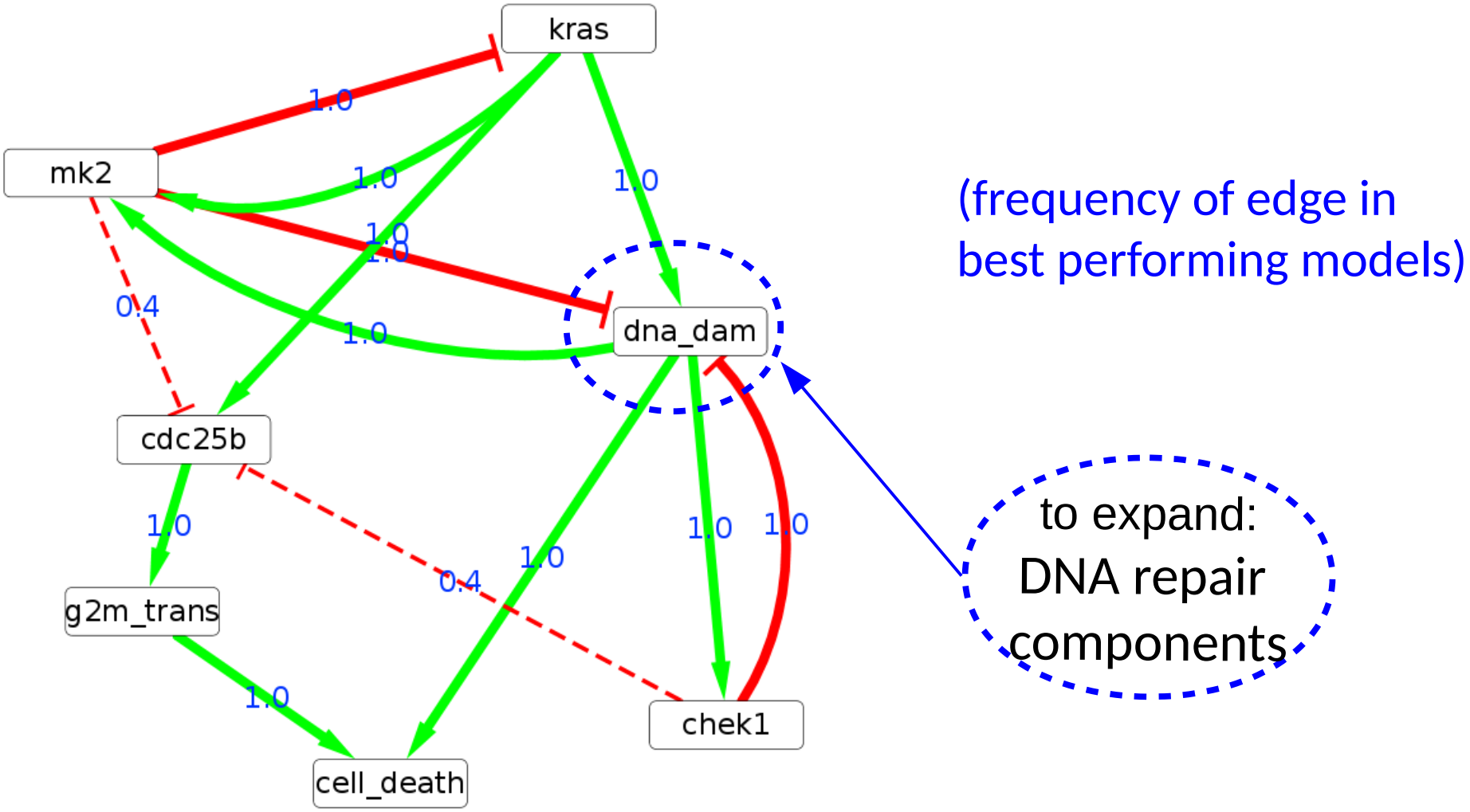
Best topologies

Ensemble modeling



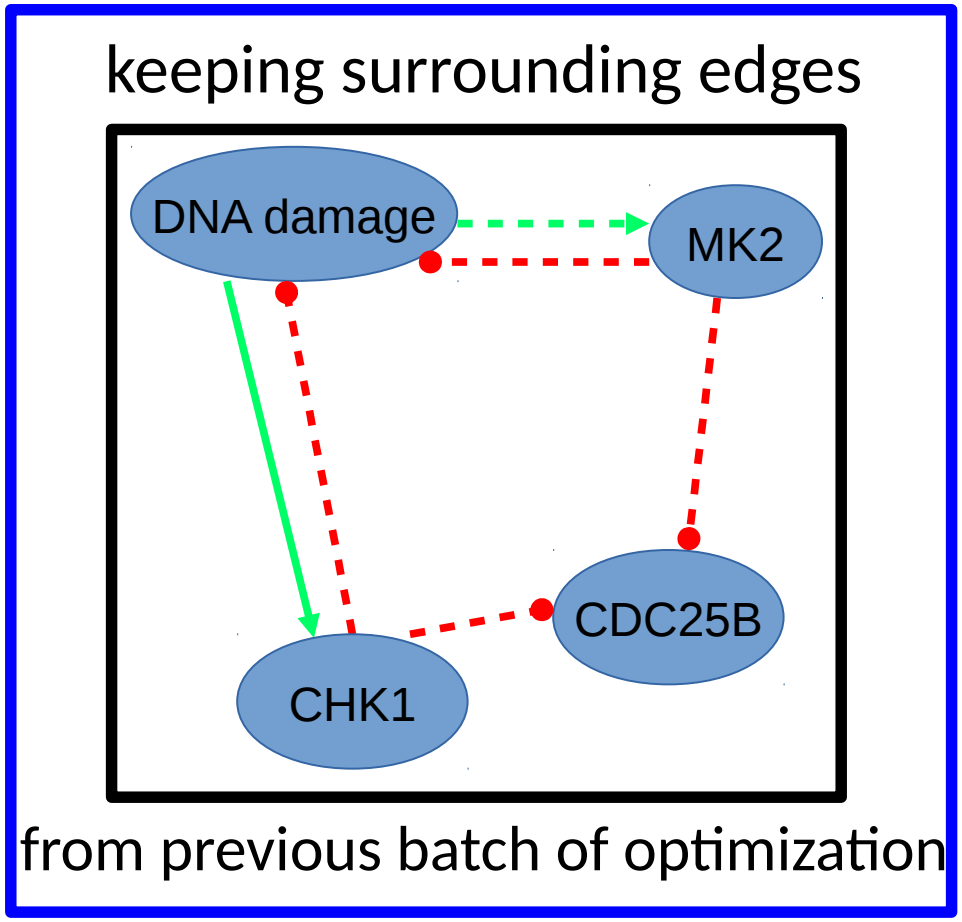
Discrepancy: unexpected killing for MK2i

Ensemble modeling: model expansion



Ensemble modeling: model expansion

Adding DNA repair module (CHEK1 and MK2 not directly implicated in DNA repair)



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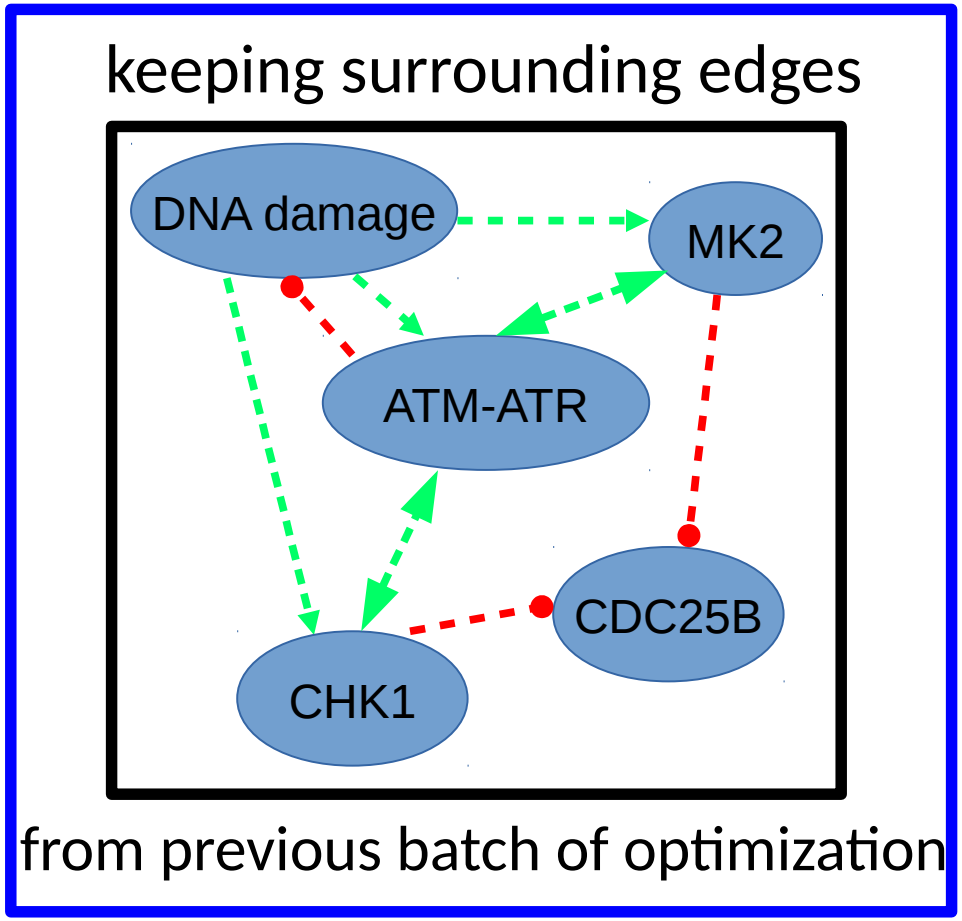
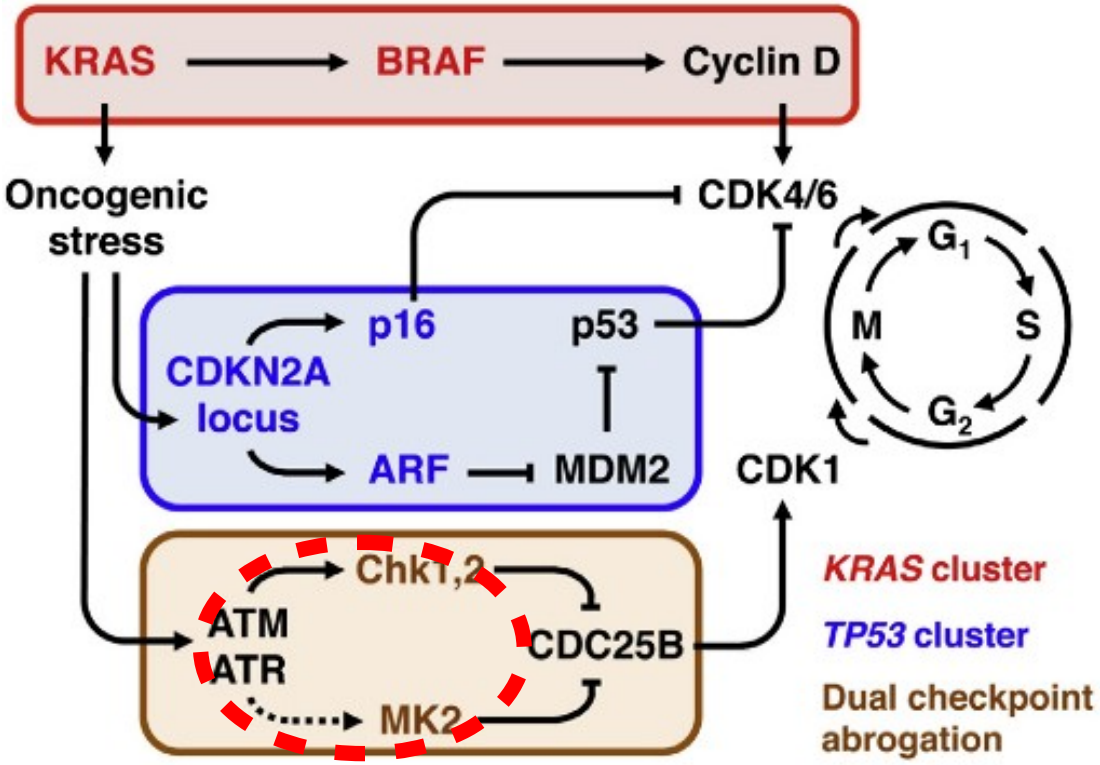


Direction of causality:
CHEK1/MK2 ←?→ DNA

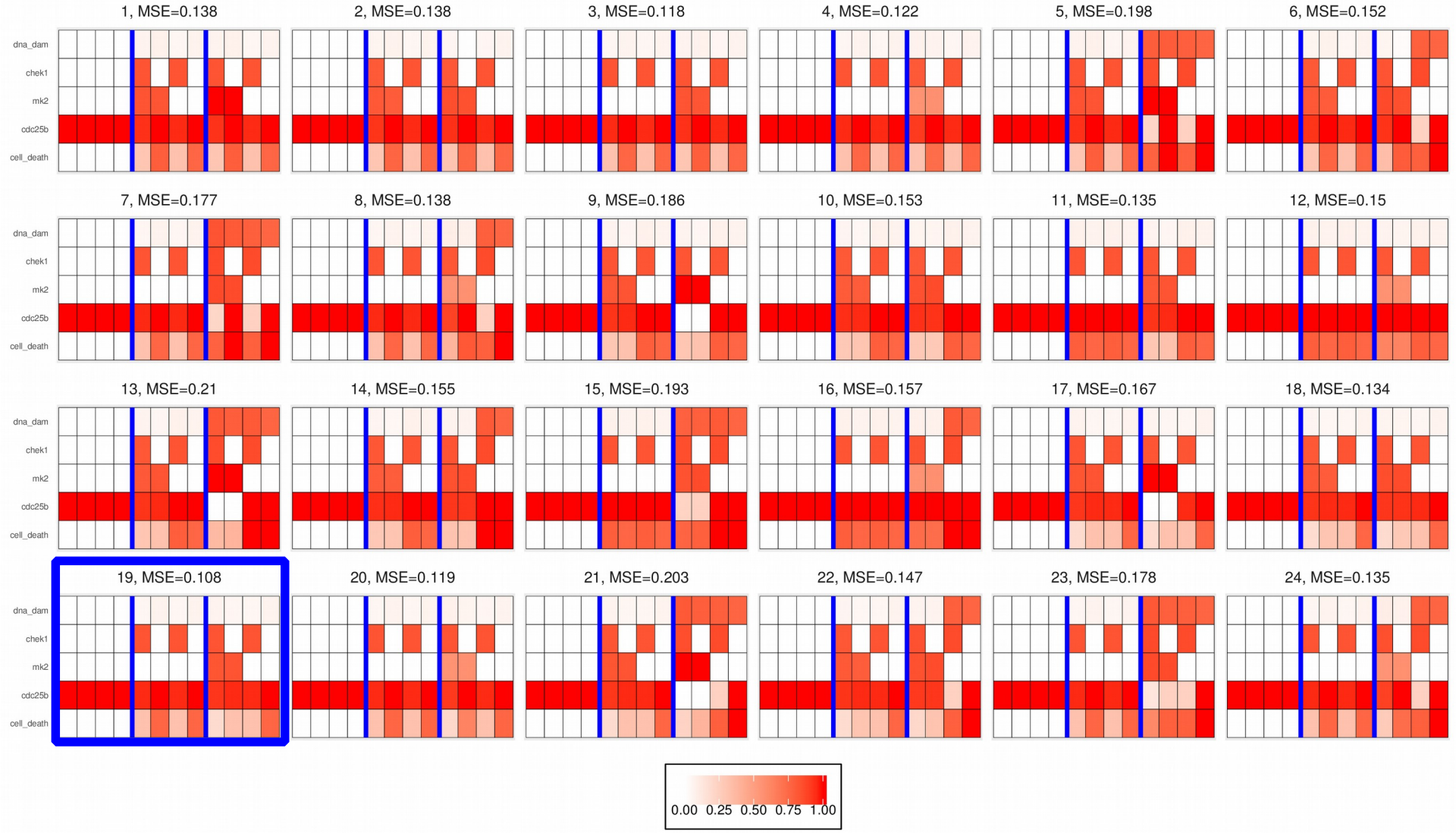
Inferring causality and identifying mechanisms

[Dietlein 2015]

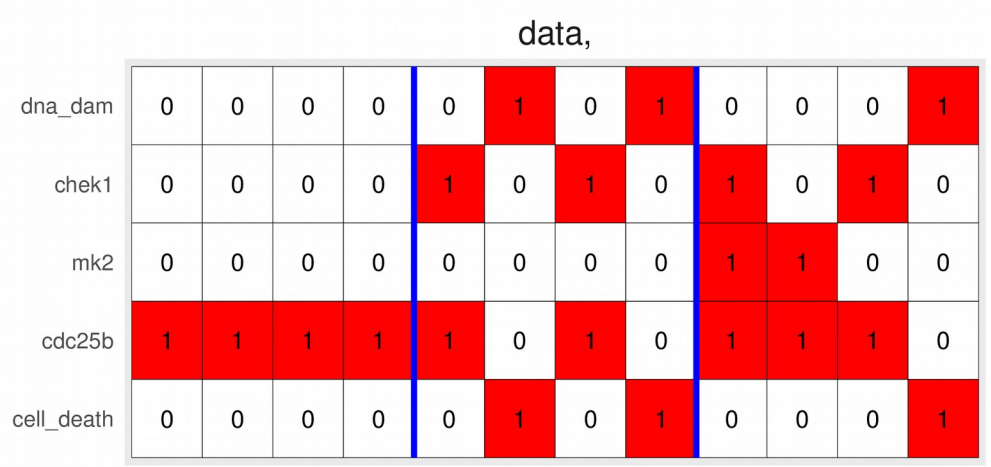


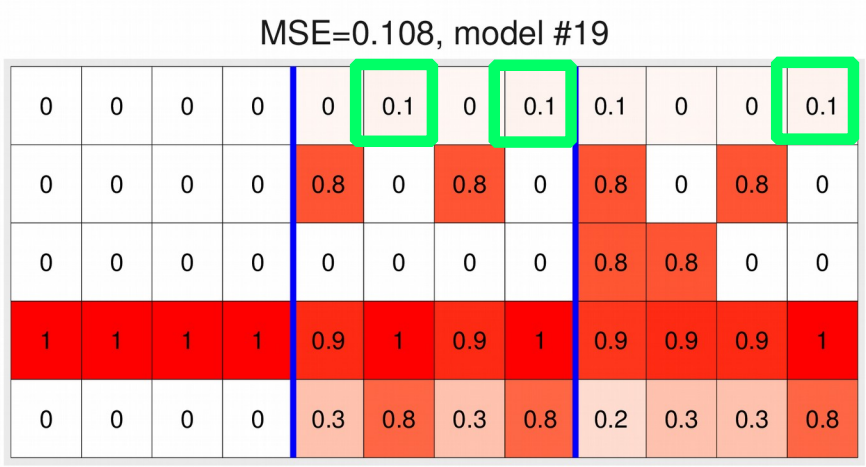
Inferring causality and identifying mechanisms

Ensemble modeling (new edges)



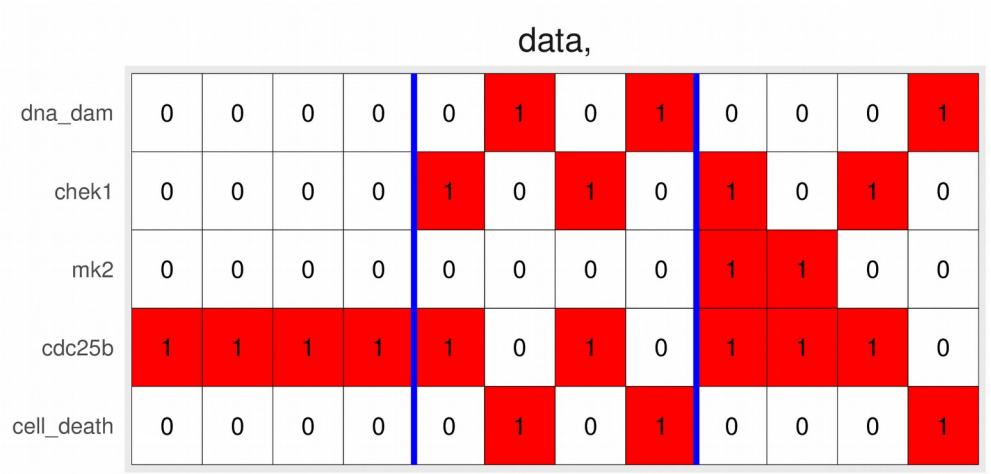
Inferring causality and identifying mechanisms

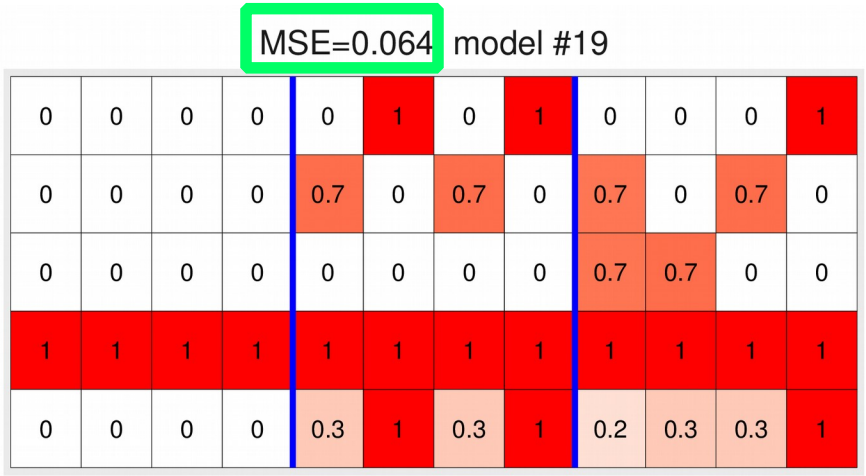




Cell death pattern correct, but dependence of DNA damage-repair on MK2-CHEK1 perturbations not explained by model

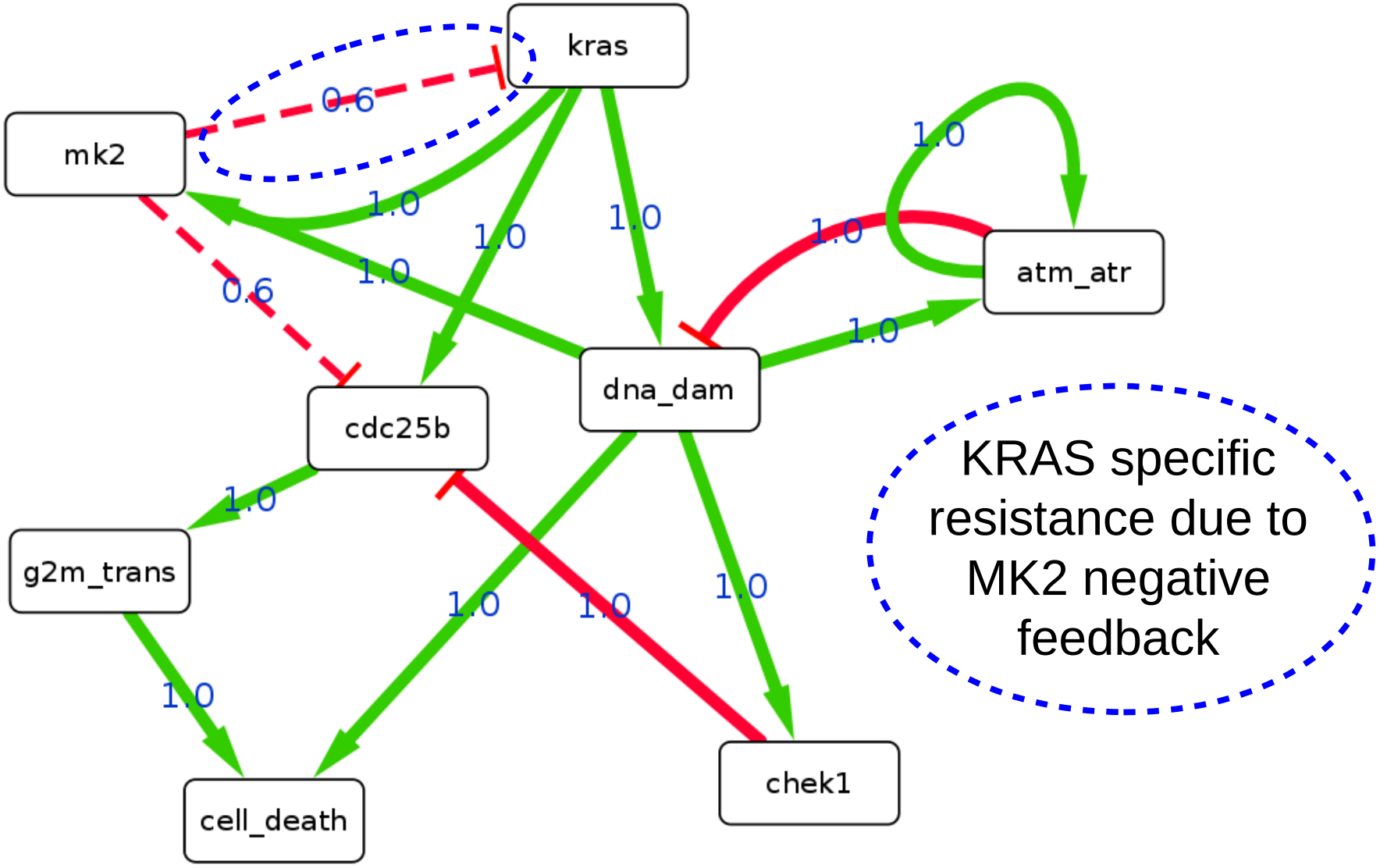
Inferring causality and identifying mechanisms





Allowing for CHK1/MK2 → DNA repair causality can explain DNA damage pattern as a function of perturbations

Inferring causality and identifying mechanisms



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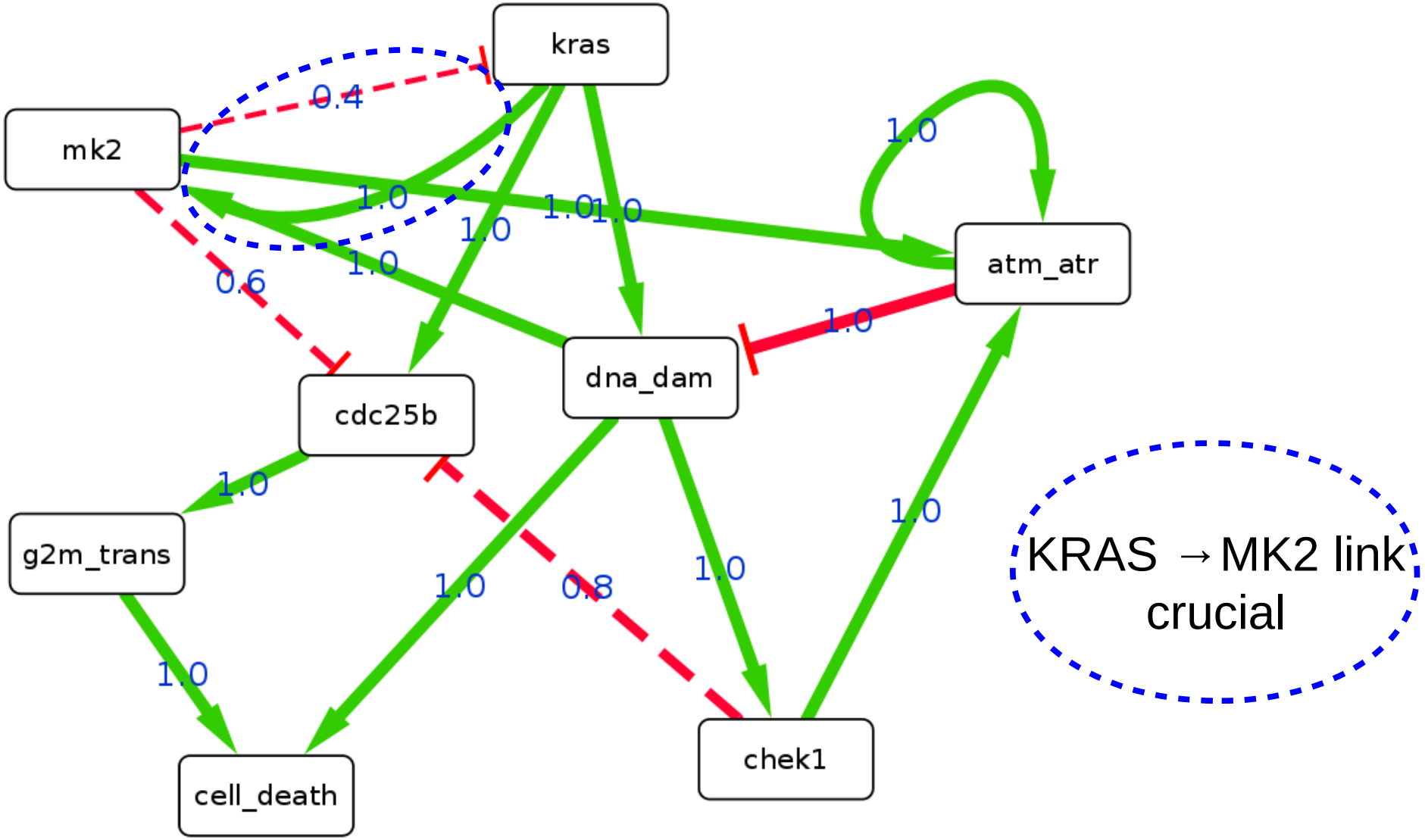
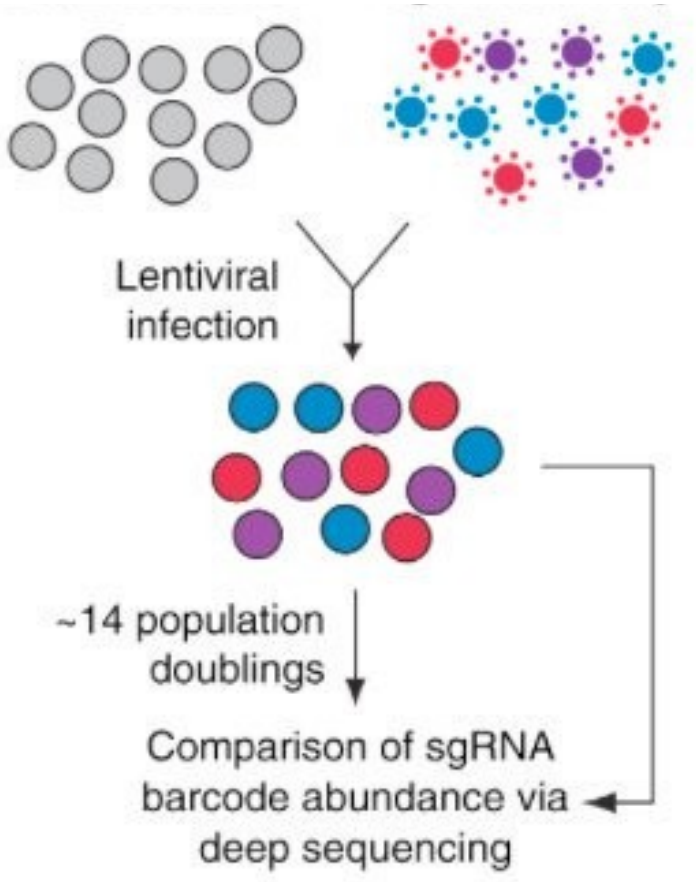
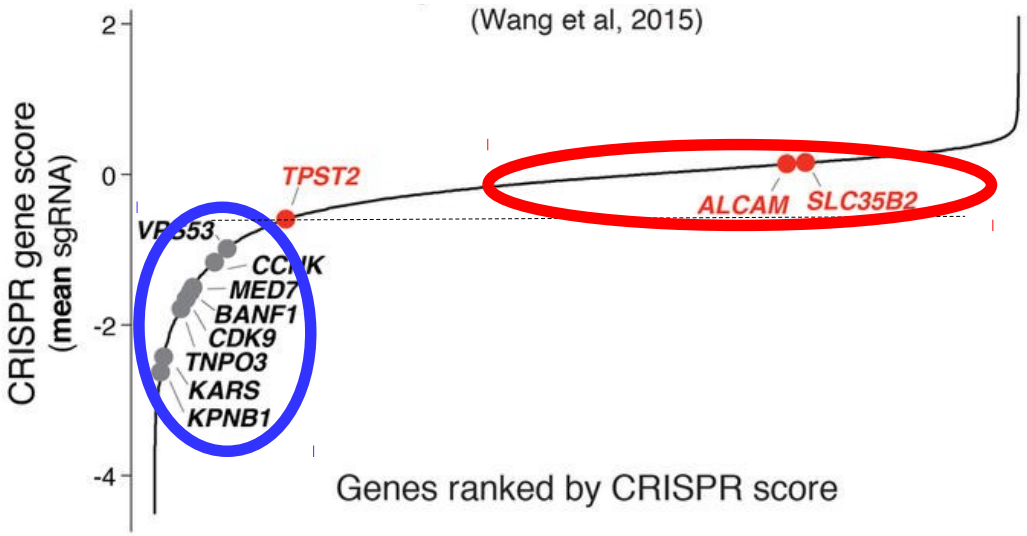


Perspective: CRISPRi screen

High throughput gene-inactivation method, superior to sh/siRNA





Negative CS: relative loss of cells with given deletion

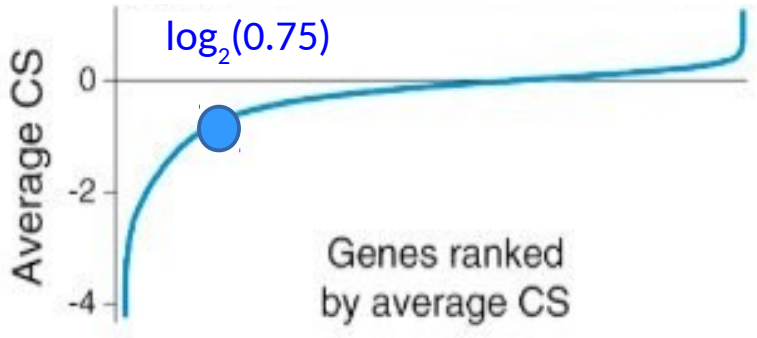
Positive CS: relative increase of cells with given deletion

$$CS(CRISPR\ score) = average\left[\log_2\left(\frac{final\ sgRNA\ abundance}{initial\ sgRNA\ abundance}\right)\right]$$

Perspective: generating hypotheses for CRISPRi

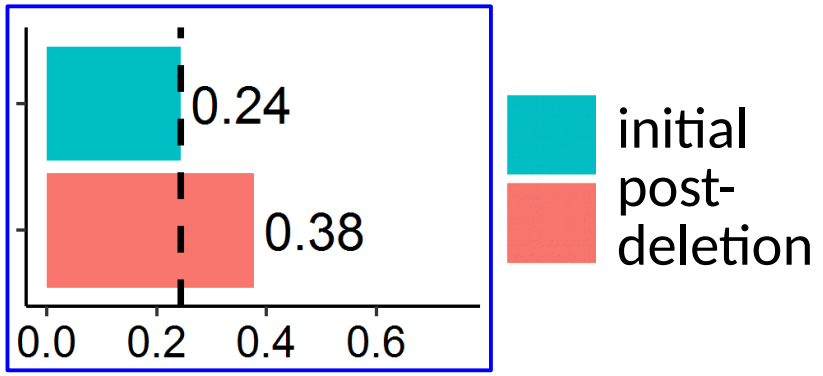
- from model to CRISPRi: target genes
- from CRISPRi to model: CHEK1i-sensitizing deletions help model (resistance mechanism) identification

CRISPRi result

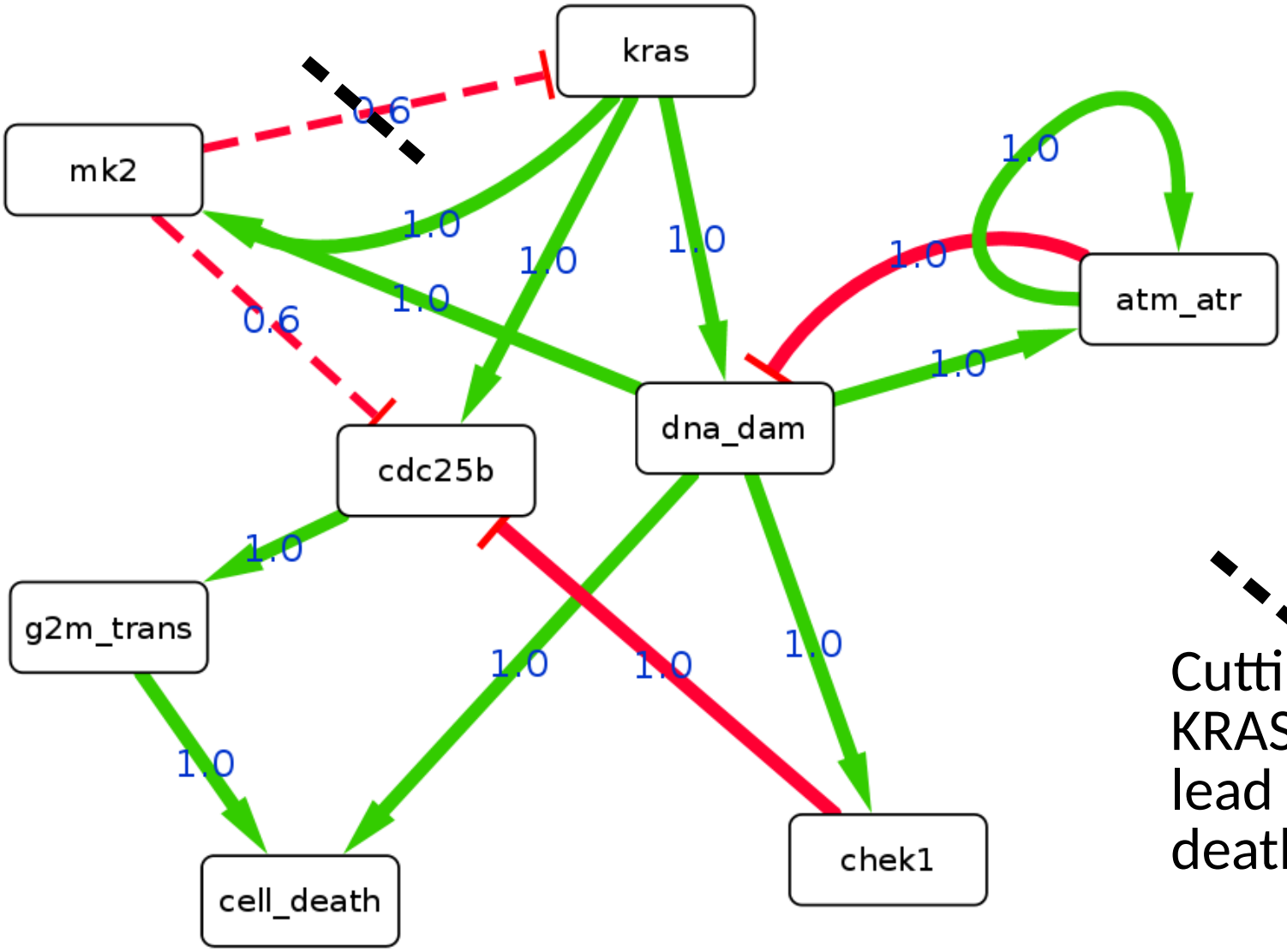


Simulation results

% cell death phenotype

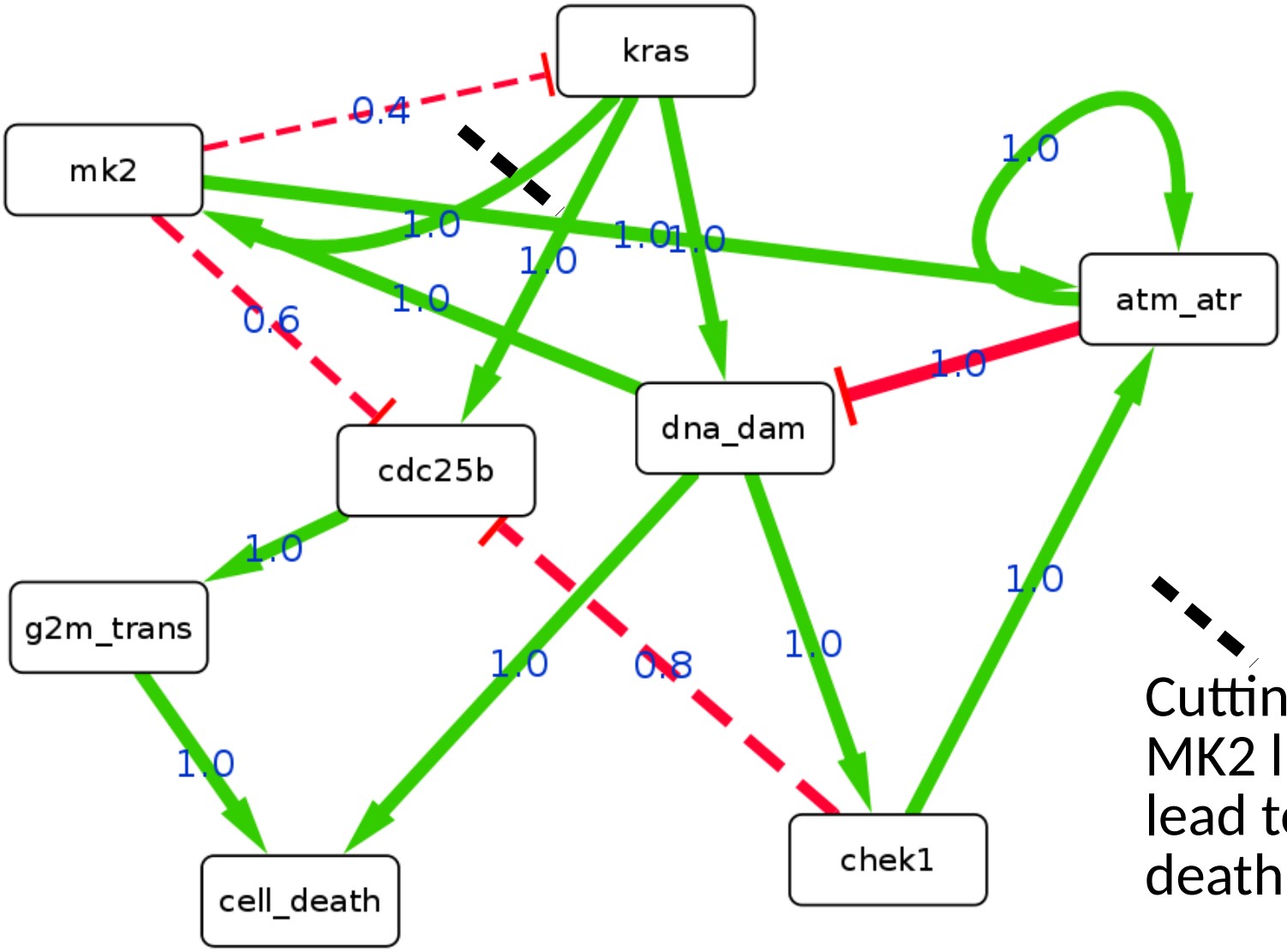


Perspective: generating hypotheses for CRISPRi



Cutting MK2 –| KRAS link should lead to higher cell death, if correct

Perspective: generating hypotheses for CRISPRi



Cutting KRAS → MK2 link should lead to higher cell death, if correct

Conclusions

- Quantitative phosphoproteomics + growth data from perturbation experiments are powerful data sources
- Ensemble modeling of small, well-constrained models ideal to distinguish between mechanisms of drug resistance
- Results of ensemble modeling can be used to propose targets of perturbation experiments
- Small, transparent models still have their merits if our aim is mechanistic understanding, not only reproduction

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