Reverse engineering signalling networks in cancer

Defense *for the academical degree*
Doctor rerum naturalium (Dr. rer. nat.)

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submitted to the Faculty of Life Sciences *of*
Humboldt Universität zu Berlin

26/09/2022

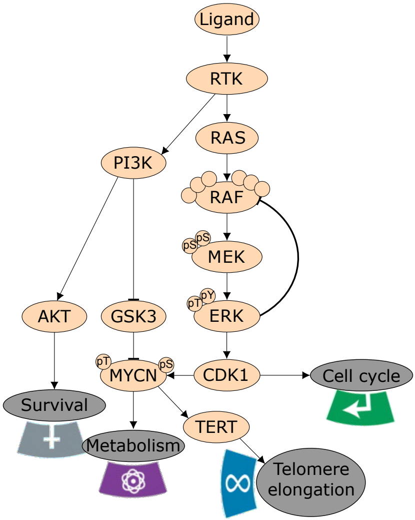
Section 1

Cancer and signalling

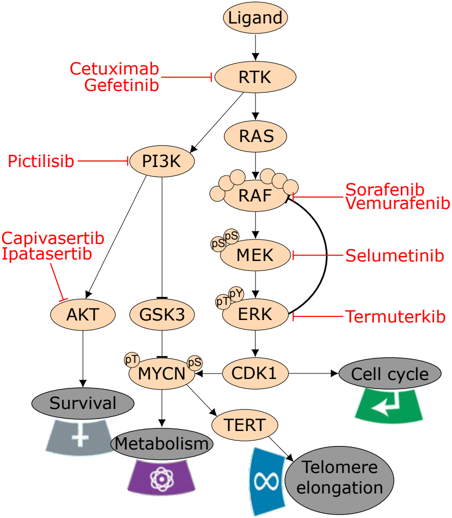
Cancer is a signalling disease

Hanahan and Weinberg (2011)

Cancer is a signalling disease



Cancer is a signalling disease



Section 2

Modelling of signalling networks

Differential equation describe the evolution of a biological system:

$$\dot{x} = f(x, p)$$

Modelling biological systems

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Modelling biological systems

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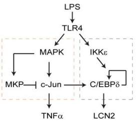
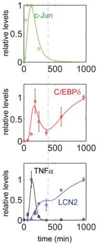
Variable	Name	Differential Equations
x_1	c-Jun	$\frac{dx_1}{dt} = \beta_1 \left(\frac{K_1 \cdot x_3}{1 + \left(\frac{x_2}{k_{12}}\right)^{n_{12}}} - x_1 \right)$
x_2	MKP-1	$\frac{dx_2}{dt} = \beta_2 \left(\frac{x_3^{n_{23}}}{k_{23}^{n_{23}} + x_3^{n_{23}}} - x_2 \right)$
x_3	MAPK	$\frac{dx_3}{dt} = \beta_3 (LPS - x_3)$
x_4	TNFα	$\frac{dx_4}{dt} = \beta_4 \left(\frac{K_4 \cdot x_1^{n_{41}}}{k_{41}^{n_{41}} + x_1^{n_{41}}} - x_4 \right)$
x_5	IKKε	$\frac{dx_5}{dt} = \beta_5 (LPS - x_5)$
x_6	C/EBPδ	$\frac{dx_6}{dt} = \beta 6 \left(\frac{K_{61} \cdot x_1^{n_{61}}}{k_{61}^{n_{61}} + x_1^{n_{61}}} + \frac{K_{66} \cdot x_6^{n_{66}}}{k_{66}^{n_{66}} + x_6^{n_{66}}} \cdot \frac{x_5^{n_{65}}}{k_{65}^{n_{65}} + x_5^{n_{65}}} - x_6 \right)$
x_7	LCN2	$\frac{dx_7}{dt} = \beta_7 \left(\frac{K_7 \cdot x_6^{n_{76}}}{k_{76}^{n_{76}} + x_6^{n_{76}}} - x_7 \right)$





Glaros et al. (2012)

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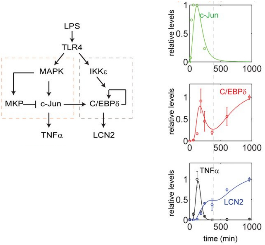

Parameter	Value	Description
β_1	2.1×10^{-2}	Degradation rate of x_1 (based on Western Blot data)
β_2	1.0×10^{-3}	Degradation rate of x_2
β_3	1.5×10^{-2}	Degradation rate of x_3
β_4	5.0×10^{-2}	Degradation rate of x_4 (based on RT-PCR data)
β_5	1.1×10^{-3}	Degradation rate of x_5
β_6	1.1×10^{-2}	Degradation rate of x_6, based on (2)
β_7	2.9×10^{-3}	Degradation rate of x_7 (based on RT-PCR data)
K_1	2.1	Weighted factor
K_4	11.0	Weighted factor
K_{61}	8.6	Weighted factor
K_{66}	1.3	Weighted factor
K_7	1.2	Weighted factor
k_{12}	1.2×10^{-1}	Threshold of x_2 to inhibit x_1
k_{23}	1.5×10^{-2}	Threshold of x_3 to activate x_2
k_{41}	1.7	Threshold of x_1 to activate x_4
k_{61}	1.4	Threshold of x_1 to activate x_6
k_{66}	1.7×10^{-1}	Threshold of x_6 to activate x_6 (auto-regulation)
k_{65}	4.6×10^{-1}	Threshold of x_5 to activate x_6
k_{76}	3.0×10^{-1}	Threshold of x_6 to activate x_7
n_{12}	4	Coefficient of nonlinearity for x_2 to inhibit x_1
n_{23}	4	Coefficient of nonlinearity for x_3 to activate x_2
n_{41}	4	Coefficient of nonlinearity for x_1 to activate x_4
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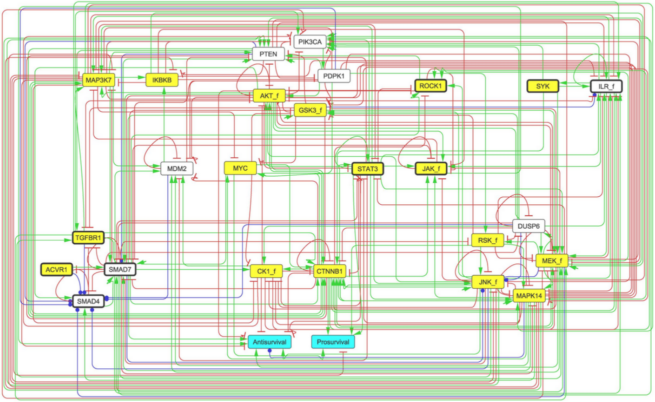

Niederdorfer et al. (2020)

Differential equation describe the evolution of a biological system:

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Modular Response Analysis

Differential equation describe the evolution of a biological system:

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$$\frac{p_j}{x_i}\frac{dx_i}{dp_j} = \frac{p_j}{x_i}\frac{\delta x_i}{\delta p_j} + \sum_{k \neq i} \frac{x_k}{x_i}\frac{\delta x_i}{\delta x_k}\frac{p_j}{x_k}\frac{dx_k}{dp_j}$$

Kholodenko et al. (2002), Klinger et al. (2013)

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Global response coefficient: $R_{kj} = \frac{p_j}{x_k}\frac{dx_k}{dp_j} = \frac{d\log(x_k)}{d\log(p_j)}$

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Sensitivity to perturbation: $s_{ij} = \frac{p_j}{x_i}\frac{\delta x_i}{\delta p_j} = \frac{d\log(x_i)}{d\log(p_j)}$

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$$-s_{ij} = -R_{ij} + \sum_{k \neq j} r_{ik} R_{kj} = \sum r_{ik} R_{kj}$$

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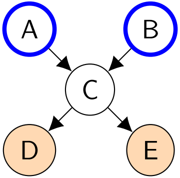
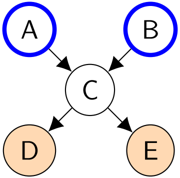
$$R = -r^{-1}S$$

Kholodenko et al. (2002), Klinger et al. (2013)

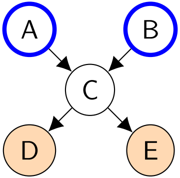
Using Modular Response Analysis





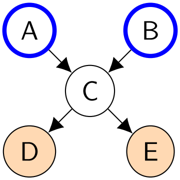
$$\mathbf{r} = \begin{array}{c} \\ A \\ B \\ C \\ D \\ E \end{array} \begin{array}{c} \begin{array}{ccccc} A & B & C & D & E \end{array} \\ \begin{pmatrix} -1 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 \\ r_{CA} & r_{CB} & -1 & 0 & 0 \\ 0 & 0 & r_{DC} & -1 & 0 \\ 0 & 0 & r_{DE} & 0 & -1 \end{pmatrix} \end{array}$$

Using Modular Response Analysis



$$
-\mathbf{r}^{-1} = \begin{array}{c} \\ A \\ B \\ C \\ D \\ E \end{array}
\begin{array}{c}
\begin{array}{ccccc} A & B & C & D & E \end{array} \\
\begin{pmatrix}
1 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 \\
r_{CA} & r_{CB} & 1 & 0 & 0 \\
r_{CA} r_{DC} & r_{CD} r_{DB} & r_{DC} & 1 & 0 \\
r_{CA} r_{EC} & r_{CB} r_{EC} & r_{DE} & 0 & 1
\end{pmatrix}
\end{array}
$$

Using Modular Response Analysis



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Maximum likelihood MRA

$$-log(\mathcal{L}) = RSS = \sum_{i,j,p} \left(\frac{-r_{ij}^{-1} \Delta p - R_{ij,p}^{\text{measured}}}{\text{s.e.m}_i} \right)^2$$

Klinger et al. (2013)

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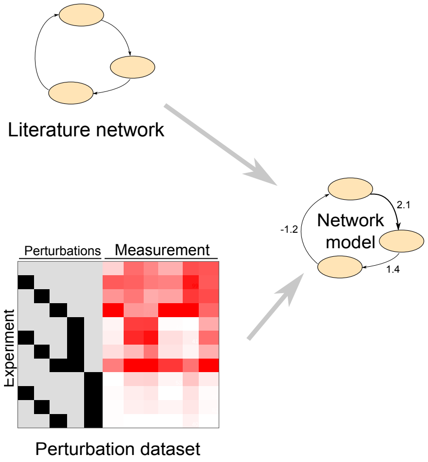
$$\text{abs}(RSS_{complete} - RSS_{reduced}) \sim \chi^2(rank_{complete} - rank_{reduced})$$

Klinger et al. (2013)

STASNet helps generating and analyzing MRA models



Dorel et al. (2018)

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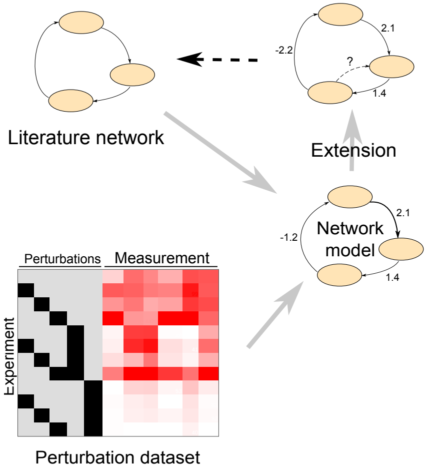

Dorel et al. (2018)

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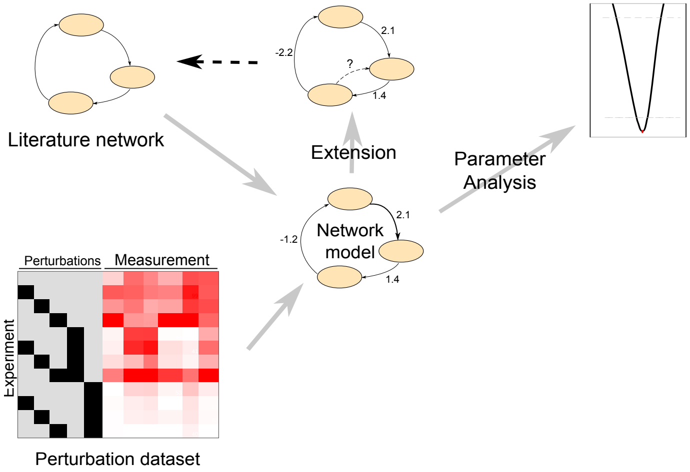

Raue et al. (2009), Dorel et al. (2018)

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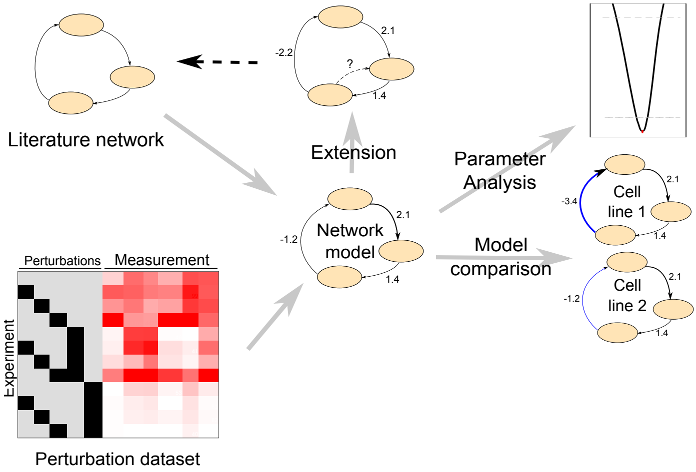

Raue et al. (2009), Dorel et al. (2018)

Section 3

Reverse engineering neuroblastoma signalling pathways

Neuroblastoma

- Most common extracranial tumor in childhood (6-10% of childhood cancers)

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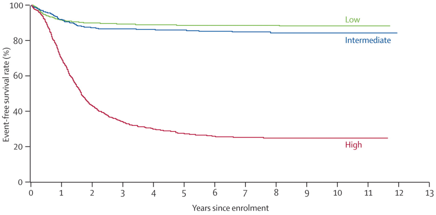
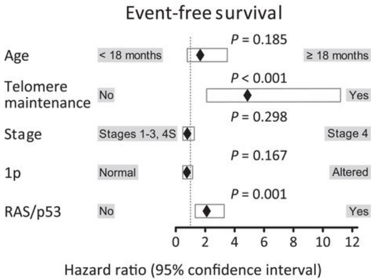
- Most lethal childhood cancer (15% of cancer death in children)
- Spontaneous regression in about 50% of cases
- High risk disease have less than 40% survival rate



Maris et al. (2016)

Neuroblastoma risk factors



Ackermann et al. (2018)

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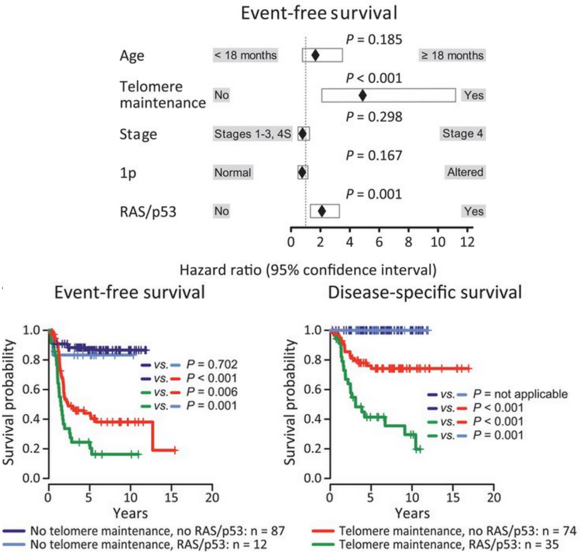
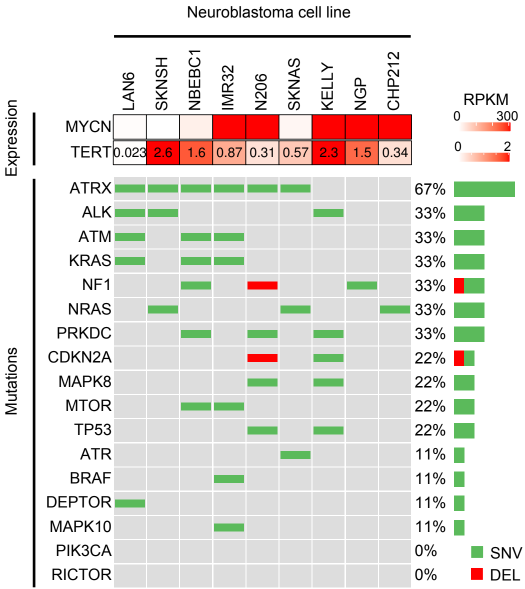


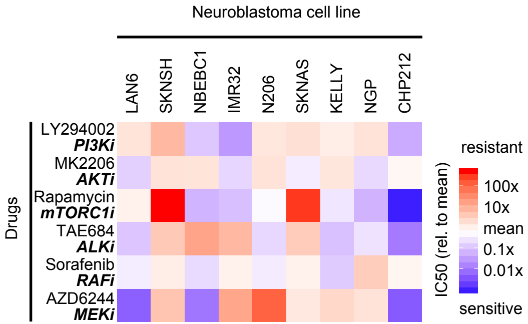
Ackermann et al. (2018)

Neuroblastoma cell lines represent high risk tumors

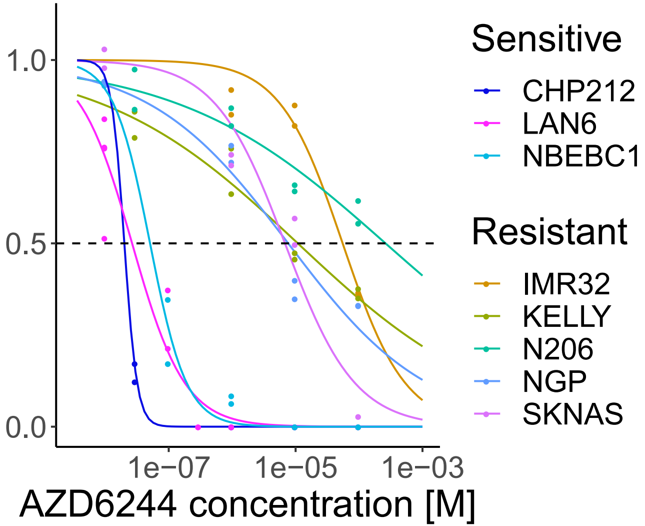


Collaboration: Joern Toedling and Matthias Zhiem

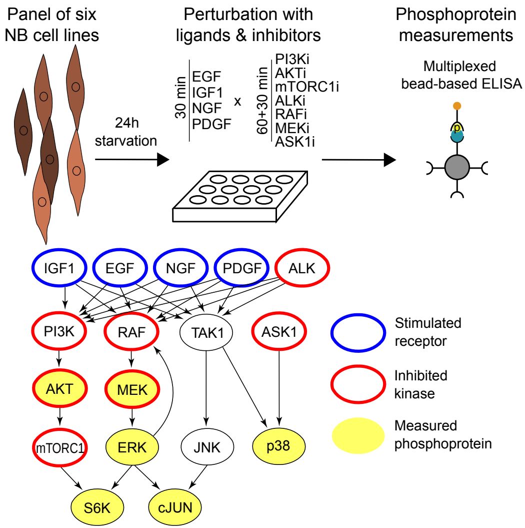
Neuroblastoma cell lines show heterogeneous response to MEK, ALK and mTORC1 inhibitions



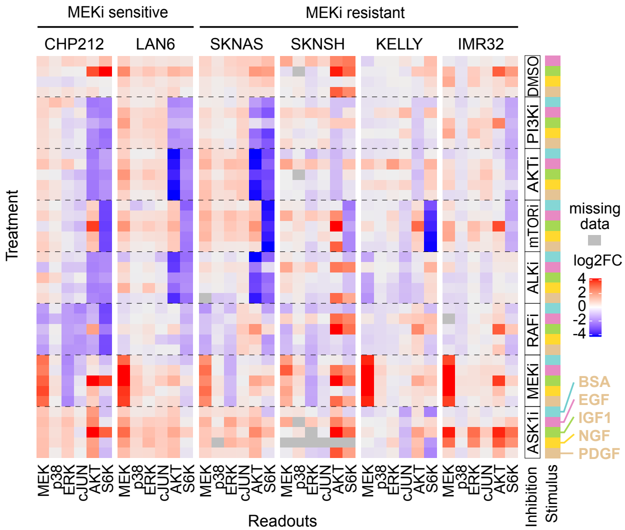
MEK inhibition sensitivity is bimodal



A perturbation panel was used to investigate drug resistance in neuroblastoma



The panel of neuroblastoma cell lines shows high variability in perturbation response



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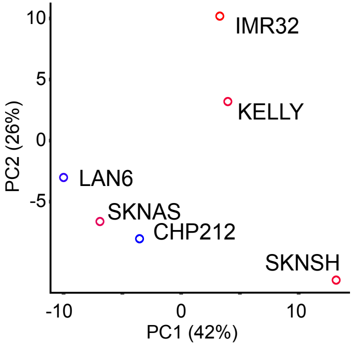
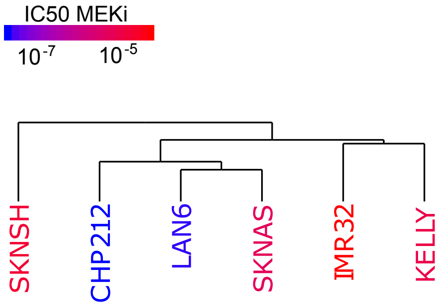
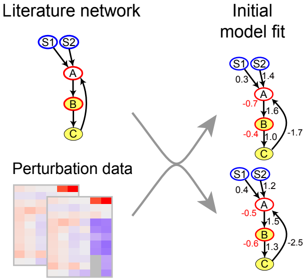


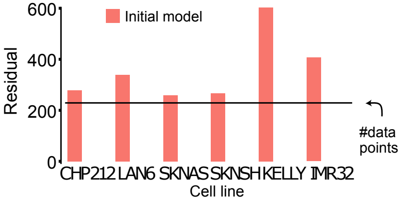
Principal Component Analysis



Hierarchical clustering

A fixed parameter strategy to homogenize the models despite different topologies





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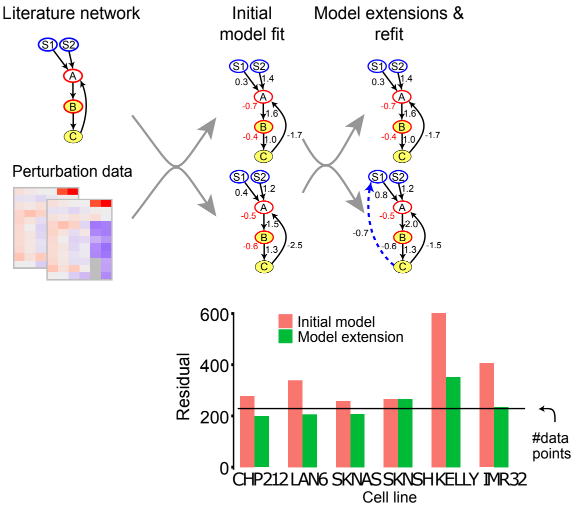

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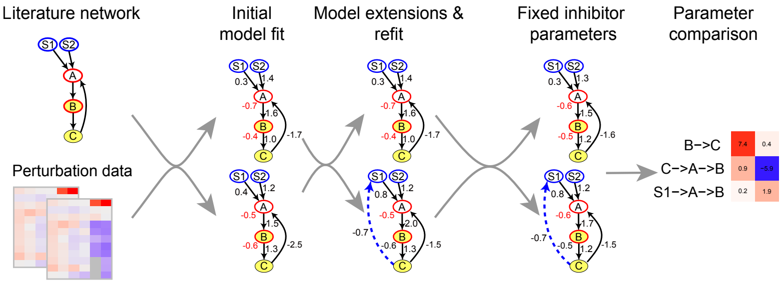
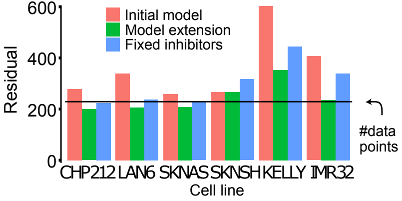
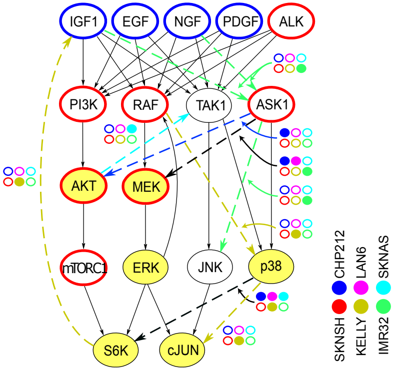




ERK→RAF feedback intensity varies between cell lines



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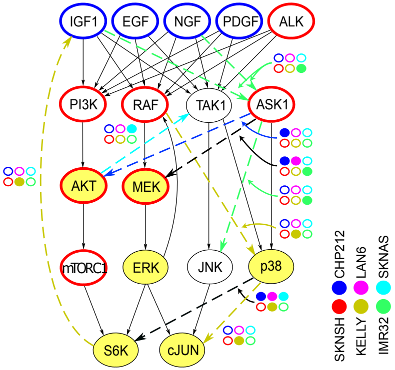
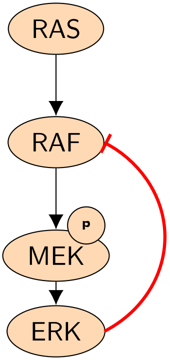


Model parameters

Intracellular signalling

	CHP212	LAN6	SKNAS	KELLY	SKNSH	IMR32
RAF–>ERK–>RAF	−2.9	−2.6	−1.4	−8	−1.8	−8.1
PI3K–>AKT	1.4	1.4	1.4	0.87	1	0.26
p38–>S6K	−9.5	−4.6	−4.6	−110	0	0
mTORC1–>S6K	0.9	0.93	1.3	1.9	0.9	0.74
MEK–>ERK	1.8	0.43	0.83	0.49	1.2	0.75
ERK–>S6K	0.59	2.4	1	5.7	0.49	0.21
ERK–>cJUN	0.28	0.63	−0.010	0.98	0.29	0.093
ASK1–>p38	−0.038	−0.048	−0.023	−0.003	−0.047	−0.27*
ASK1–>MEK	−0.23	−0.51	0	0	0	−20*
AKT–>mTORC1	1.1	1.3	0.73	0.1	0.75	0.82

Cell lines resistant to MEK inhibition tend to have a strong MEK feedback



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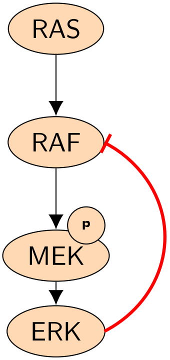
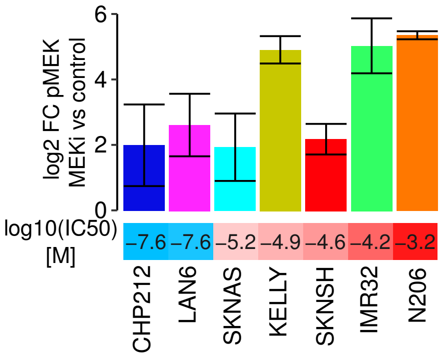
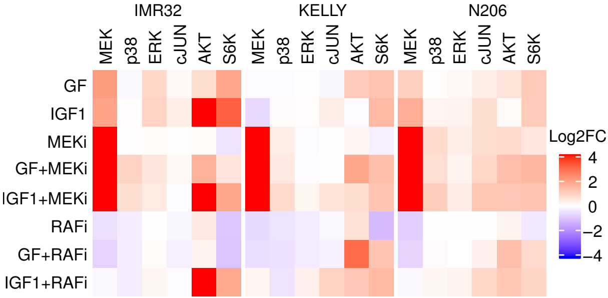




Targeted experiment to probe MEK feedback activation



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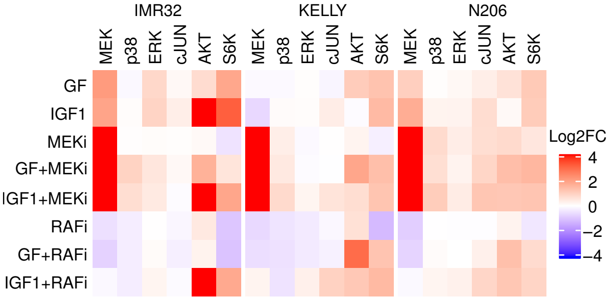

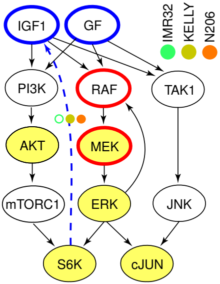

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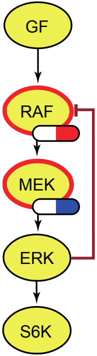

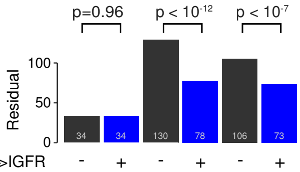

	IMR32	KELLY	N206
ERK->RAF->MEK	−8.4	−29	−8
S6K->IGFR->RAF->MEK	0.026	−7.6	−17
S6K->IGFR->PI3K->AKT	0.014	−1.7	−3.7

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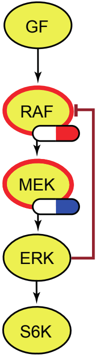

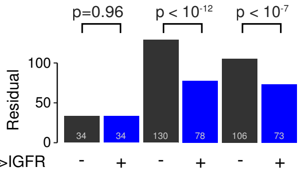

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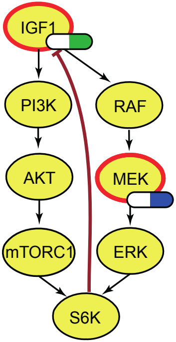
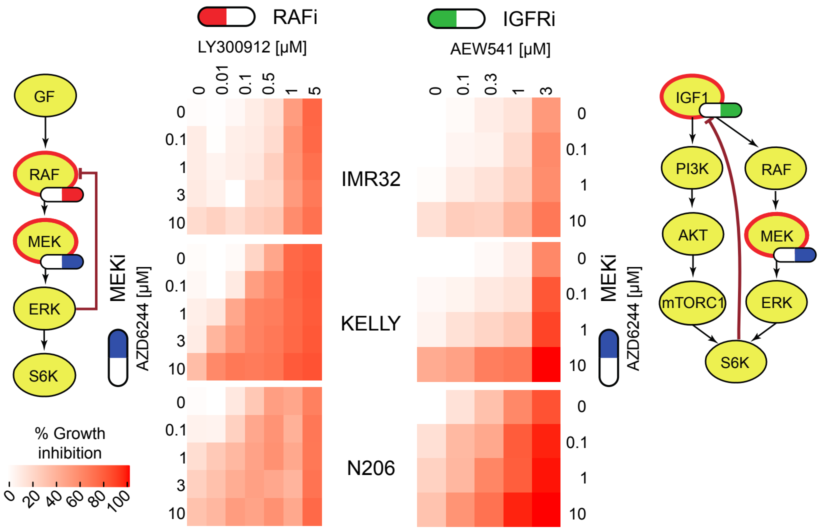


Vertical inhibition can break the feedback-mediated resistance



Conclusion: Neuroblastoma signalling (Dorel et al. (2021))

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- Neuroblastoma cell lines represent very high risk tumors.
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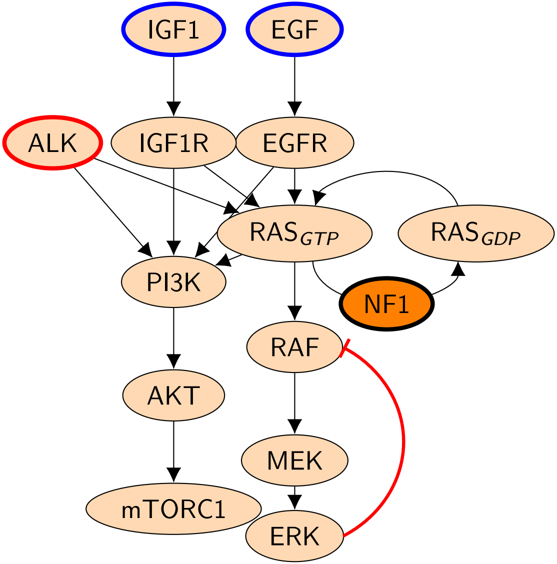
- Sensitivity to MEK inhibition seems related to the strength of the ERK feedbacks.
- MEK inhibitor resistance can be overcome with IGFR or RAF vertical inhibition.

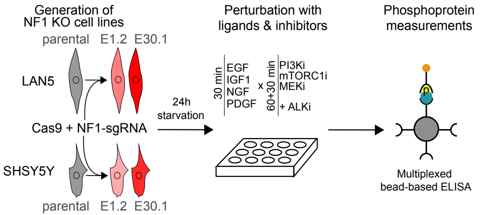
Section 4

Role of NF1 in neuroblastoma

NF1 is RAS GTPase-activating protein



An NF1 KO isogenic panel sheds light on the role of NF1 in neuroblastoma



Collaboration with Mareike Berlak.

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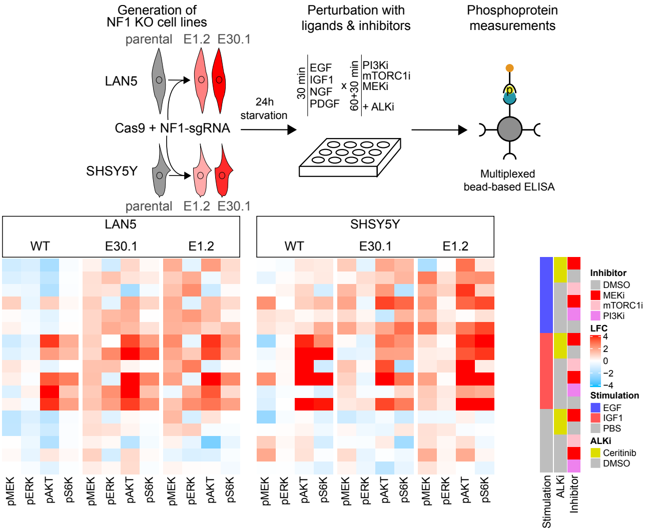

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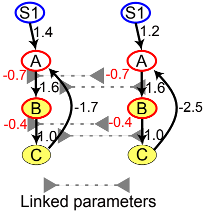
NF1 deletion weakens the ERK→RAF feedback



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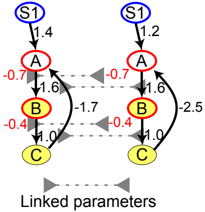

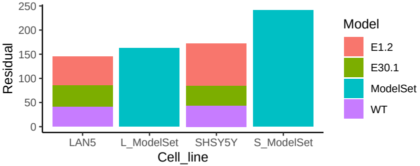

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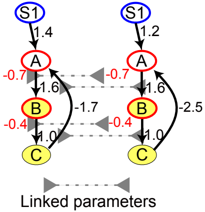
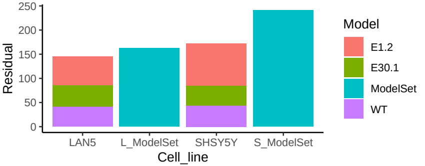
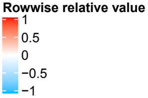
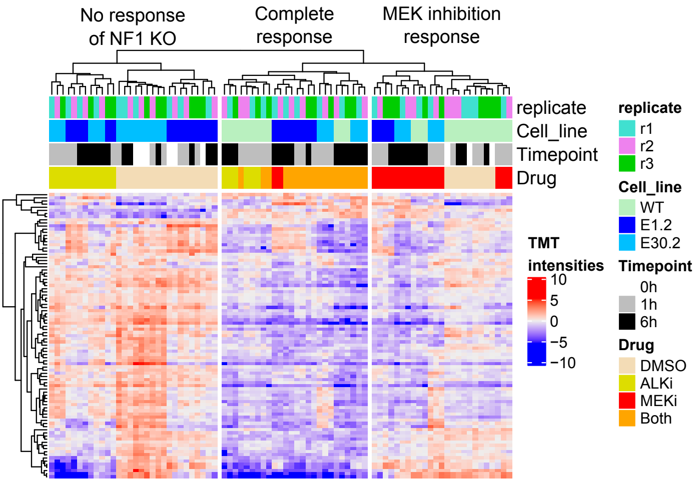




LAN5 E1.2	LAN5 E30.1	LAN5 WT		SHSY5Y E1.2	SHSY5Y E30.2	SHSY5Y WT
2.31	2.31	2.31	AKT−>mTORC1	2.14	2.14	2.14
−0.30	−0.07	−1.53	ALKi->PI3K	−2.06	−2.06	−2.06
0.20	0.13	−3.35	ALKi->RAS->RAF->MEK	−0.03	−0.03	−0.03
1.38	0.99	−0.30	EGF−>PI3K	7.29	5.68	2.82
0.87	0.49	−0.95	EGF−>RAS−>RAF−>MEK	1.99	1.99	1.99
−0.07	−0.13	−4.84	ERK−>RAF−>MEK	−1.30	−4.31	−71.52
0.02	0.11	−0.68	ERK−>S6K	1.13	1.13	1.13
4.42	4.42	4.42	IGF1−>PI3K	12.08	7.65	11.68
0.89	0.89	0.89	IGF1−>RAS−>RAF−>MEK	0.85	0.85	0.85
1.51	1.74	0.52	MEK−>ERK	0.42	0.42	0.42
0.63	0.63	0.63	PI3K−>AKT	0.29	0.29	0.29
0.24	0.24	0.24	mTORC1−>S6K	0.43	0.43	0.43



NF1 deletion desensitizes to ALK inhibition but increases sensitivity to MEK inhibition



Collaboration with Mareike Berlak and Tomasso Mari.

Conclusion: NF1 KO in neuroblastoma (Berlak, Tucker, Dorel et al. (2021))

- NF1 KO decrease MEK feedback strength.

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- MAPK pathway is desensitized to ALK inhibition by the loss of NF1.
- ALK inhibitor resistance can be overcome by an additional MEK inhibition.

Section 5

Conclusion

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During this thesis, I :

- Developed an R package called STASNet to build and analyze MRA models

  Dorel et al. Bioinformatics (2018).

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- Suggested new combinations to sensitize high risk neuroblastoma to MEK inhibition
  Dorel et al. PLoS Computational Biology (2021).

- Helped elucidate how NF1 inactivation leads to ALK inhibitor resistance but also induces MEK inhibitor sensitivity

  Berlak, Tucker, Dorel et al. Molecular Cancer (2022).

Outlook

- Improve STASNet performance to model larger networks.

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- Characterize the MAPK feedbacks and associated resistance in more neuroblastoma cell lines as well as patient samples.
- Investigate how exactly knockout of NF1 weakens the ERK$\rightarrow$RAF feedback.
- Screen how neuroblastoma could overcome MEK combination therapies.

Thank you!

TerminateNB consortium

Computational Modelling in Medicine

Nils Blüthgen
Bertram Klinger
Anja Sieber
and the whole Blüthgen group

CHARITÉ UNIVERSITÄTSMEDIZIN BERLIN

Jasmin Wünschel (Deubzer lab)
Jörn Tödling
Mareike Berlak
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Michal Nadler-Holly
Tomasso Mari
Matthias Selbach (MDC)

Thank you!

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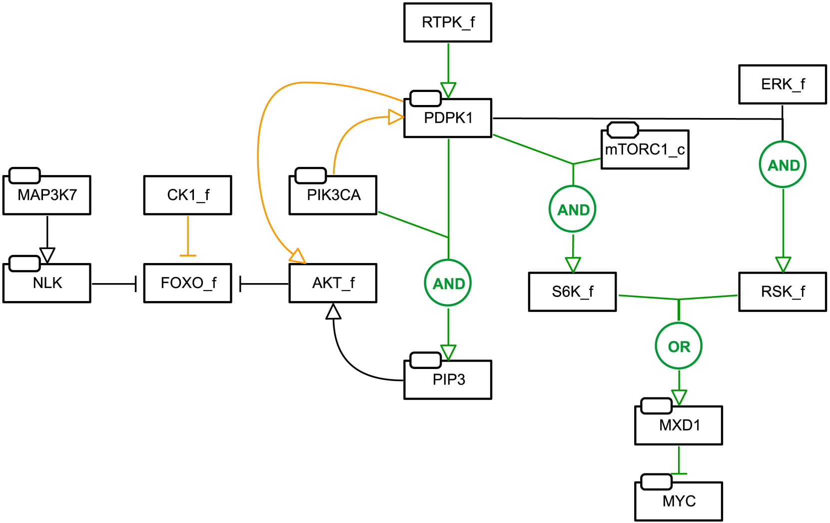
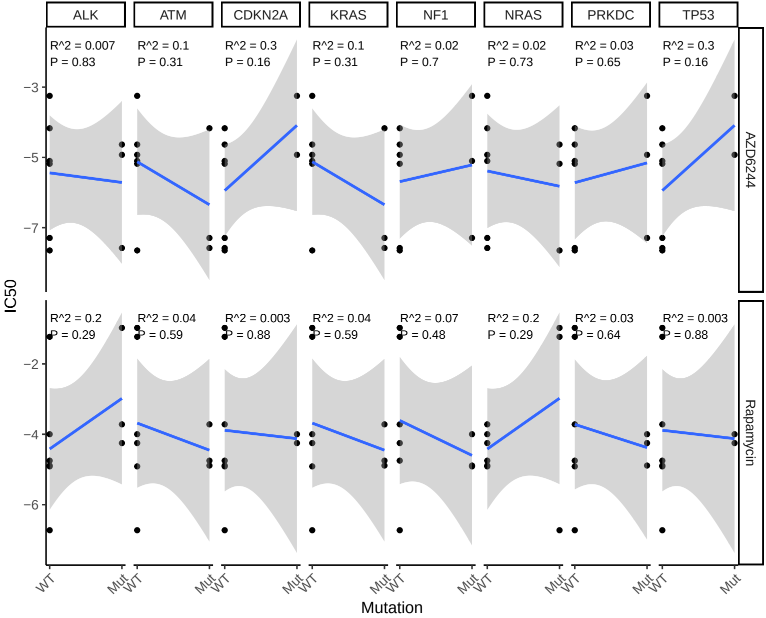
Boolean networks

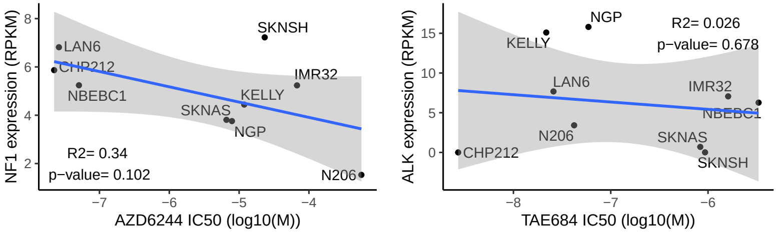


Niederdorfer et al. (2020)

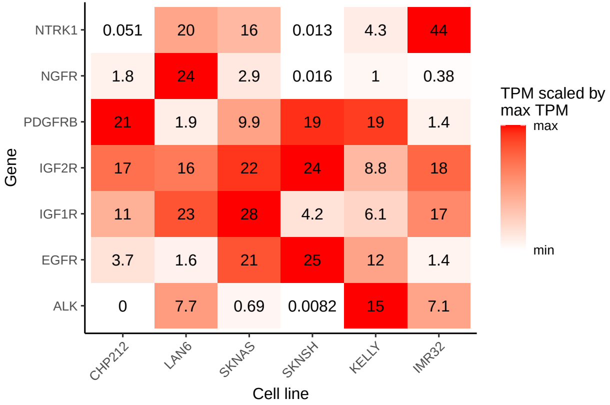
Correlation between drug resistance and selected mutations



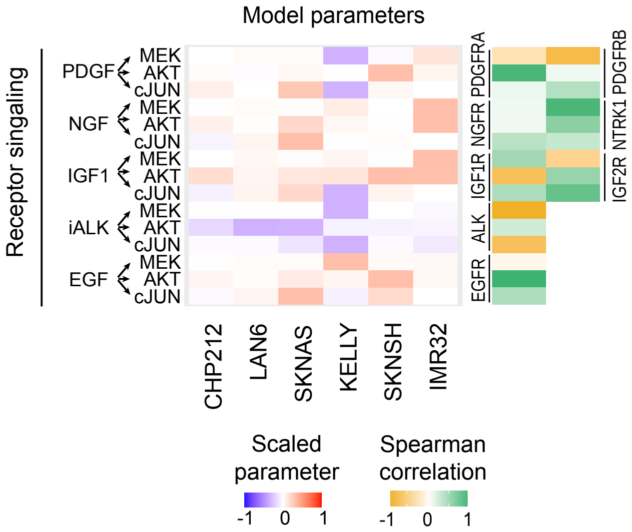
Correlation between drug resistance and selected gene expression



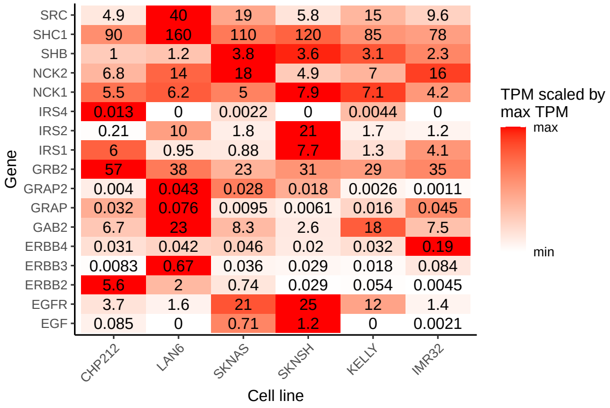
Receptor expression in the neuroblastoma cell lines panel



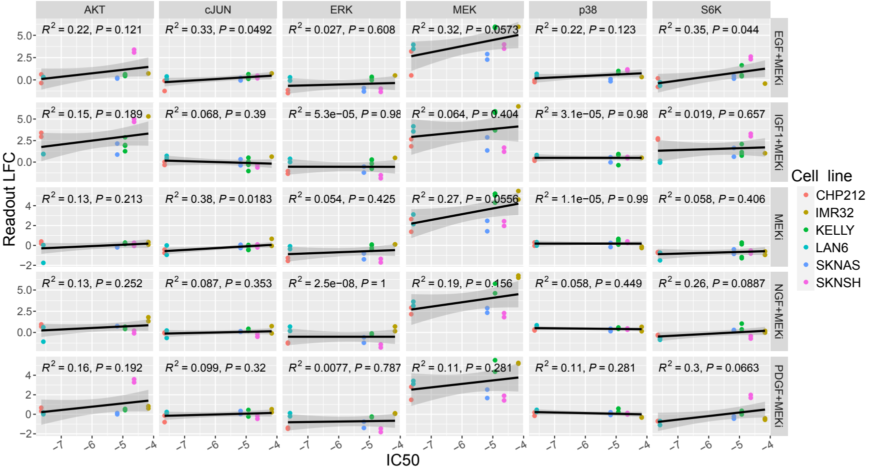
Receptor stimulations are the main source of variation between cell lines



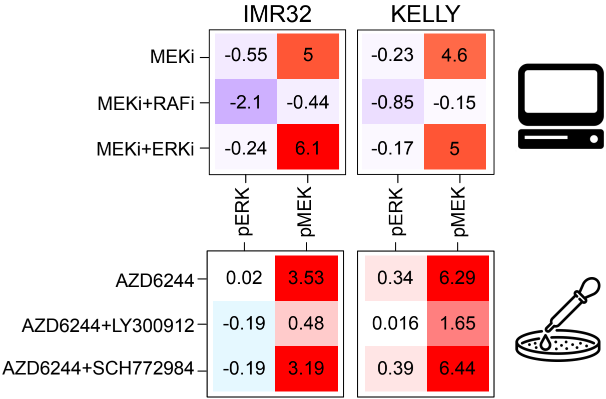
Adapters and ERBB receptor family expression in the neuroblastoma cell lines panel



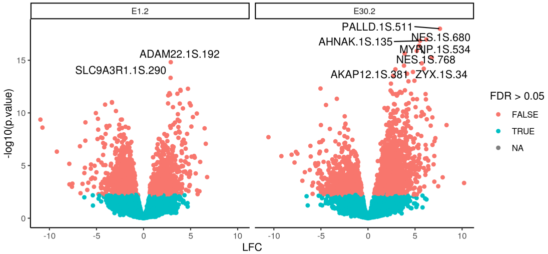
Response of pMEK to MEK inhibition correlates with MEK inhibitor sensitivity



Combination of MEK and RAF inhibition does bring down pMEK and pERK



NF1 deletion induces proliferation and replicative stress



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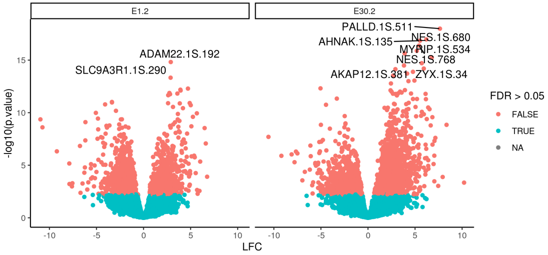

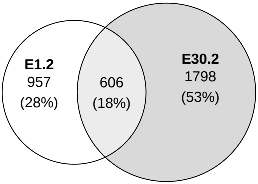

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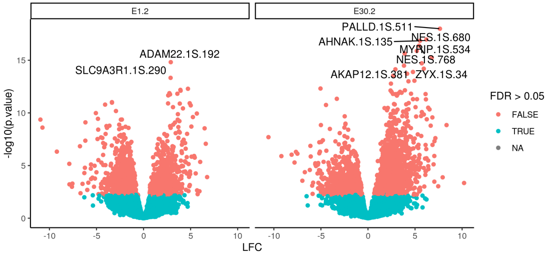

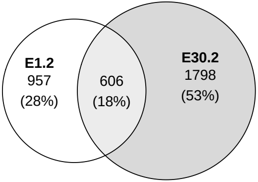

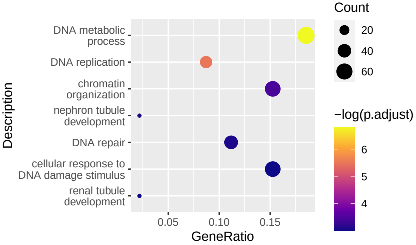

Collaboration with Mareike Berlak and Tomasso Mari.

Removing structural non identifiability

$$P_l = \prod_{j,k} (r_{jk})^{a_{jkl}}$$

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$$[A, -I] \times (\log r_{11}, r_{12}, \ldots, \log r_{NN}, \log P_1, \ldots, logP_M)^T = 0$$

Klinger et al. (2013)

Removing structural non identifiability

$$\mathbf{R} = \begin{array}{cc} & \begin{array}{cc} \boxed{S_A} & \boxed{S_A + S_B} \end{array} \\ \begin{array}{c} D \\ E \end{array} & \begin{pmatrix} \boxed{r_{CA} r_{DC}} & \boxed{r_{CA} r_{DC}} + \boxed{r_{CD} r_{DB}} \\ \boxed{r_{CA} r_{EC}} & \boxed{r_{CA} r_{EC}} + \boxed{r_{CB} r_{EC}} \end{pmatrix} \end{array} = \begin{pmatrix} \boxed{P_1} & \boxed{P_2} + \boxed{P_3} \\ \boxed{P_4} & \boxed{P_5} + \boxed{P_6} \end{pmatrix}$$

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$$\mathbf{A} = \begin{array}{c c} & \begin{array}{cccc} r_{CA} & r_{CB} & r_{DC} & r_{EC} \end{array} \\ \begin{array}{c} P_1 \\ P_2 \\ P_3 \\ P_4 \\ P_5 \\ P_6 \end{array} & \begin{pmatrix} 1 & 0 & 1 & 0 \\ 0 & 1 & 0 & 1 \\ 0 & 1 & 1 & 0 \\ 0 & 0 & 1 & 1 \\ 1 & 0 & 0 & 1 \\ 0 & 1 & 0 & 1 \end{pmatrix} \end{array}$$

Removing structural non identifiability

$$G = \left[\begin{array}{c|c} A' & G_1 \\ \hline 0 & G_2 \end{array}\right] \tag{1}$$

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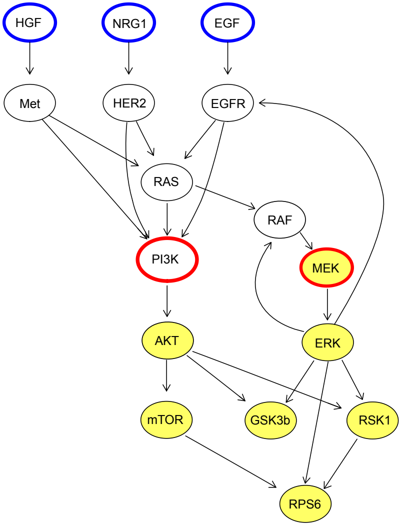
$$G = \left[\begin{array}{c|c} A' & G_1 \\ \hline 0 & G_2 \end{array}\right] \tag{1}$$

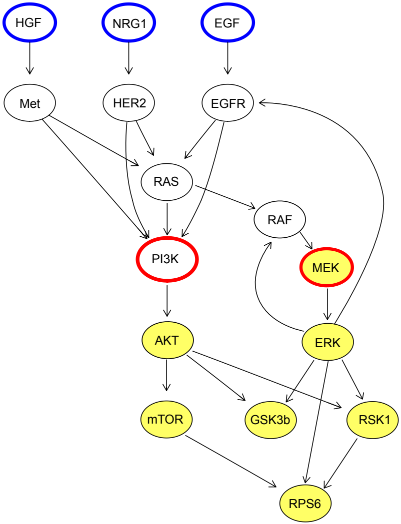
$$G = \begin{array}{c} \\ P_1 \\ P_2 \\ P_3 \\ P_4 \\ P_5 \\ P_6 \end{array} \begin{array}{c} \begin{array}{cccccccccc} r_{CA} & r_{CB} & r_{DC} & r_{EC} & P_1 & P_2 & P_3 & P_4 & P_5 & P_6 \end{array} \\ \left(\begin{array}{cccc|cccccc} 1 & 0 & 1 & 0 & 0 & 0 & -1 & 0 & 1 & 1 \\ 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 1 \\ \hline 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & -1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & -1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 \end{array}\right) \end{array}$$

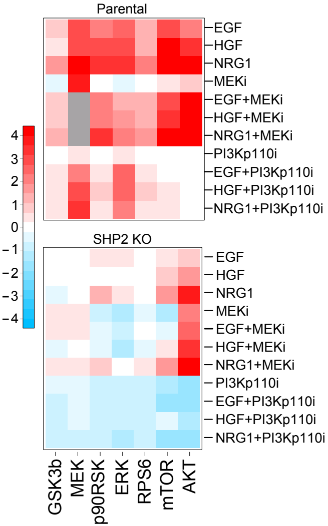
$$\mathbf{R} = \begin{array}{c} \\ D \\ E \end{array} \begin{array}{c} \begin{array}{cc} \boxed{S_A} & \boxed{S_A + S_B} \end{array} \\ \begin{pmatrix} \dfrac{P_5 P_6}{P_3} & \dfrac{P_5 P_6}{P_3} + P_3 \\ P_5 & P_5 + P_6 \end{pmatrix} \end{array}$$

SHP2 KO show a differential activation pattern of the MAPK and PI3K pathways

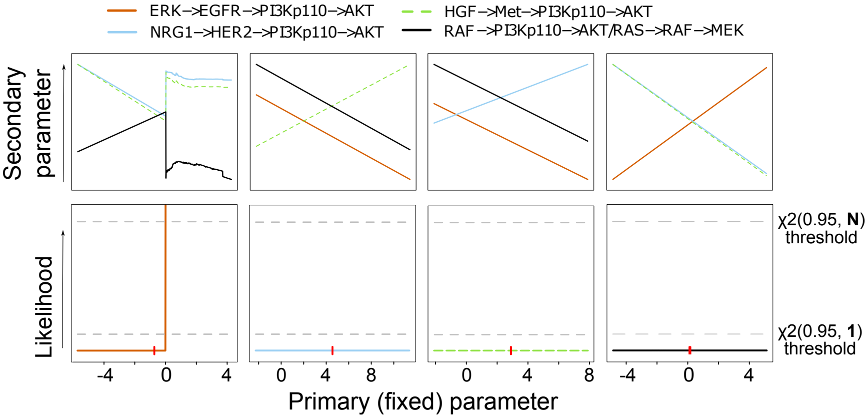


SHP2 KO show a differential activation pattern of the MAPK and PI3K pathways





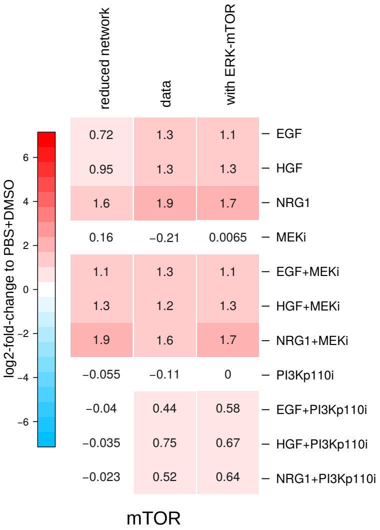
STASNet helps solve structural non identifiability



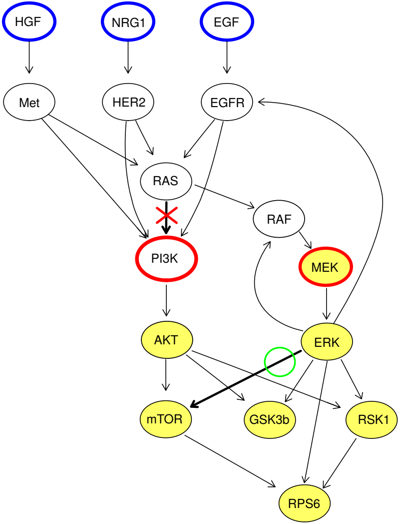
An activation of mTOR by ERK suggested by STASNet improves the quality of the model

from	to	value	residual	adj_pval
RPS6	mTOR	1.25	48.25	2.23E-02
ERK	mTOR	0.24	48.25	2.23E-02
MEK	mTOR	0.21	48.25	2.23E-02
p90RSK	mTOR	1.09	48.25	2.23E-02

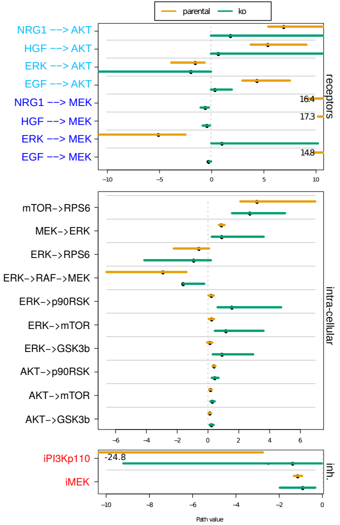
An activation of mTOR by ERK suggested by STASNet improves the quality of the model

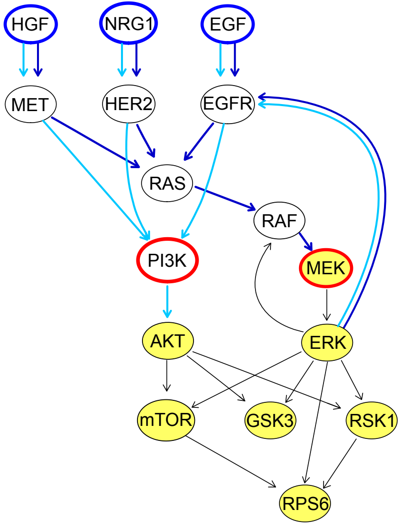


An activation of mTOR by ERK suggested by STASNet improves the quality of the model

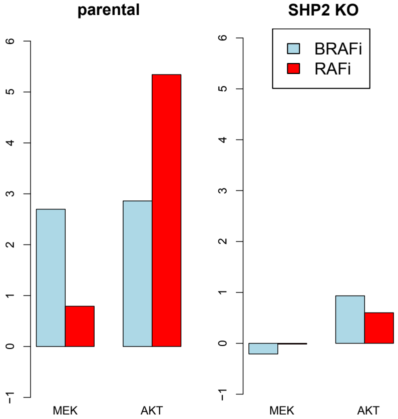


SHP2 KO weakens MEK/ERK signalling, including the feedback, but not PI3K/AKT



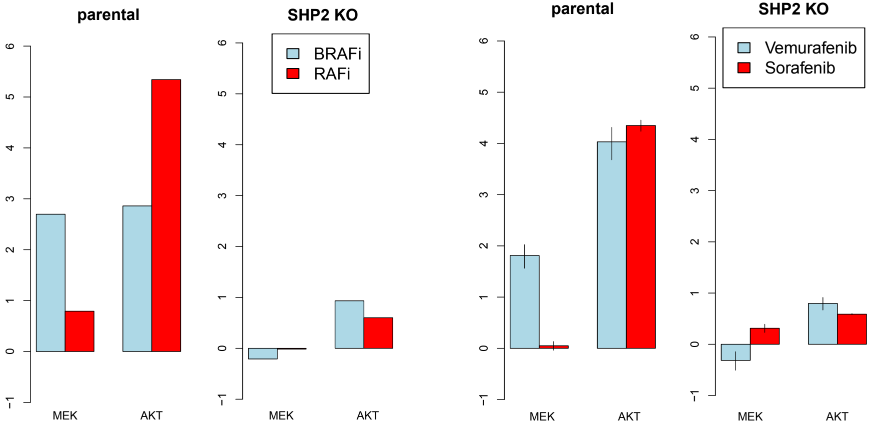


STASNet quantitatively predicts the effect of different RAF inhibitors



STASNet simulation

STASNet quantitatively predicts the effect of different RAF inhibitors



STASNet simulation

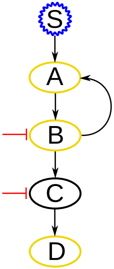
Experimental measurements

Modular response analysis

$$-r^{-1} \quad = \quad R$$

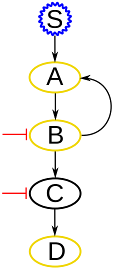
Modular response analysis

$$
-\begin{array}{c c}
 & \begin{array}{ccccc} S & A & B & C & D \end{array} \\
\begin{array}{c} S \\ A \\ B \\ C \\ D \end{array} &
\begin{pmatrix}
-1 & 0 & 0 & 0 & 0 \\
r_{SA} & -1 & r_{BA} & 0 & 0 \\
0 & r_{AB} & -1 & 0 & 0 \\
0 & 0 & r_{BC} & -1 & 0 \\
0 & 0 & 0 & r_{CD} & -1
\end{pmatrix}^{-1}
\end{array}
= \qquad R
$$



Modular response analysis

$$
-\begin{array}{c|c}
 & \begin{array}{ccccc} S & A & B & C & D \end{array} \\
\begin{array}{c} S \\ A \\ B \\ C \\ D \end{array} &
\begin{pmatrix}
-1 & 0 & 0 & 0 & 0 \\
r_{SA} & -1 & r_{BA} & 0 & 0 \\
0 & r_{AB} & -1 & 0 & 0 \\
0 & 0 & r_{BC} & -1 & 0 \\
0 & 0 & 0 & r_{CD} & -1
\end{pmatrix}^{-1}
\end{array}
=
\begin{array}{c|c}
 & \begin{array}{ccccc} S & A & B & C & D \end{array} \\
\begin{array}{c} S \\ A \\ B \\ C \\ D \end{array} &
\begin{pmatrix}
1 & 0 & 0 & 0 & 0 \\
\frac{r_{SA}}{1-r_{AB}r_{BA}} & \frac{1}{1-r_{AB}r_{BA}} & \frac{r_{BA}}{1-r_{AB}r_{BA}} & 0 & 0 \\
\frac{r_{SA}r_{AB}}{1-r_{AB}r_{BA}} & \frac{r_{AB}}{1-r_{AB}r_{BA}} & \frac{1}{1-r_{AB}r_{BA}} & 0 & 0 \\
\frac{r_{SA}r_{AB}r_{BC}}{1-r_{AB}r_{BA}} & \frac{r_{AB}r_{BC}}{1-r_{AB}r_{BA}} & \frac{r_{BC}}{1-r_{AB}r_{BA}} & 1 & 0 \\
\frac{r_{SA}r_{AB}r_{BC}r_{CD}}{1-r_{AB}r_{BA}} & \frac{r_{AB}r_{BC}r_{CD}}{1-r_{AB}r_{BA}} & \frac{r_{BC}r_{CD}}{1-r_{AB}r_{BA}} & r_{CD} & 1
\end{pmatrix}
\end{array}
$$



Modular response analysis

$$
-\begin{array}{c}
 \\ S \\ A \\ B \\ C \\ D
\end{array}
\begin{array}{c}
\begin{array}{ccccc} S & A & B & C & D \end{array} \\
\begin{pmatrix}
-1 & 0 & 0 & 0 & 0 \\
r_{SA} & -1 & r_{BA} & 0 & 0 \\
0 & r_{AB} & -1 & 0 & 0 \\
0 & 0 & r_{BC} & -1 & 0 \\
0 & 0 & 0 & r_{CD} & -1
\end{pmatrix}^{-1}
\end{array}
=
\begin{array}{c}
S \\ A \\ B \\ C \\ D
\end{array}
\begin{array}{c}
\begin{array}{ccccc} S & A & B & C & D \end{array} \\
\begin{pmatrix}
1 & 0 & 0 & 0 & 0 \\
\frac{r_{SA}}{1-r_{AB}r_{BA}} & \frac{1}{1-r_{AB}r_{BA}} & \frac{r_{BA}}{1-r_{AB}r_{BA}} & 0 & 0 \\
\frac{r_{SA}r_{AB}}{1-r_{AB}r_{BA}} & \frac{r_{AB}}{1-r_{AB}r_{BA}} & \frac{1}{1-r_{AB}r_{BA}} & 0 & 0 \\
\frac{r_{SA}r_{AB}r_{BC}}{1-r_{AB}r_{BA}} & \frac{r_{AB}r_{BC}}{1-r_{AB}r_{BA}} & \frac{r_{BC}}{1-r_{AB}r_{BA}} & 1 & 0 \\
\frac{r_{SA}r_{AB}r_{BC}r_{CD}}{1-r_{AB}r_{BA}} & \frac{r_{AB}r_{BC}r_{CD}}{1-r_{AB}r_{BA}} & \frac{r_{BC}r_{CD}}{1-r_{AB}r_{BA}} & r_{CD} & 1
\end{pmatrix}
\end{array}
$$