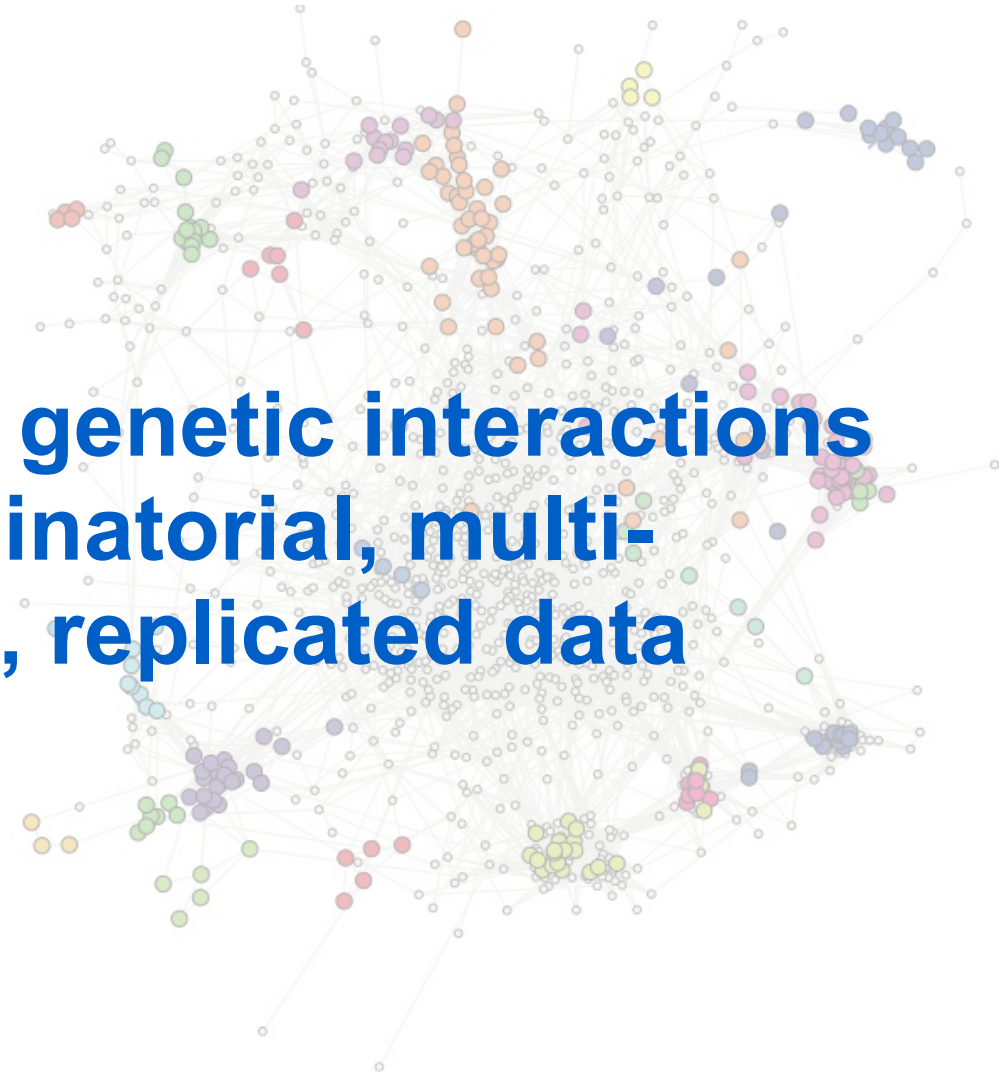
19.11.2016

Inferring directional genetic interactions from combinatorial, multi-parametric, replicated data

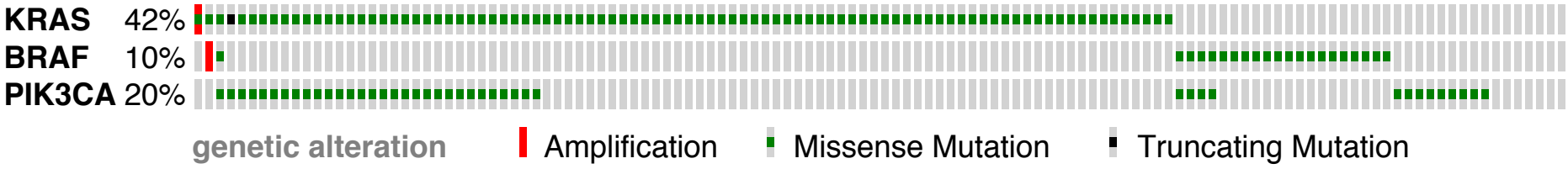


Bernd Fischer

dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

50 Years – Research for A Life Without Cancer

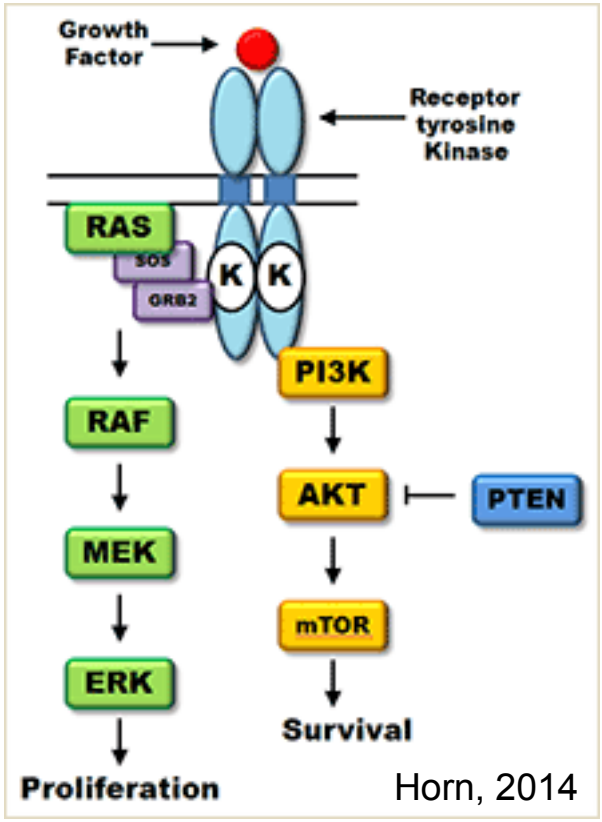
Mutual exclusivity / co-occurrence



Colorectal Adenocarcinoma (TCGA, Nature 2012)

KRAS-BRAF: mutational exclusive
odds-ratio: 0.22, p < 0.001 (Fisher's exact test)

KRAS-PIK3CA: co-occurrent
odds-ratio: 2.70, p < 0.001 (Fisher's exact test)



Horn, 2014

Statistical Power!

Assume two genes are mutually exclusive
odds-ratio: 1/4; each gene mutated in 3% of cases

How many samples to obtain a p-value of 0.01?

9924 samples with Fisher's exact test
multiple testing not yet considered!!!

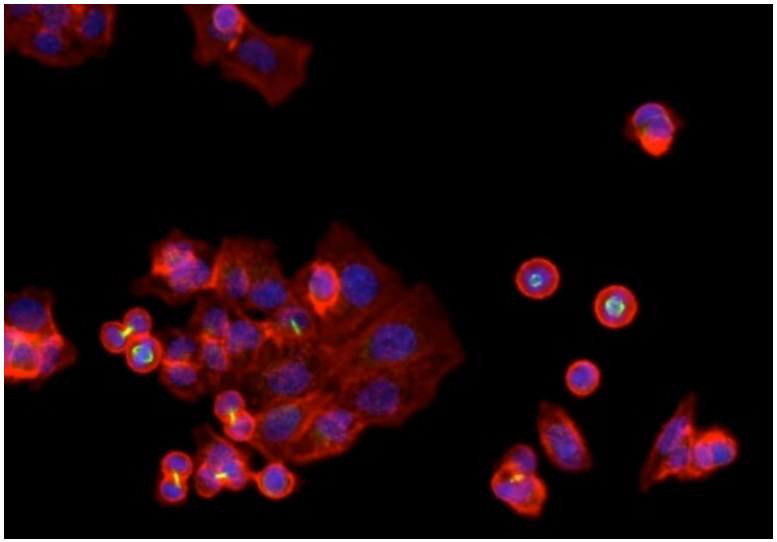
What is the order of the genes in the pathway?

Systematic mapping of multivariate genetic interactions via RNAi

all pairwise knock-downs of

1367 genes x 72 genes

image-based readout



Interventions

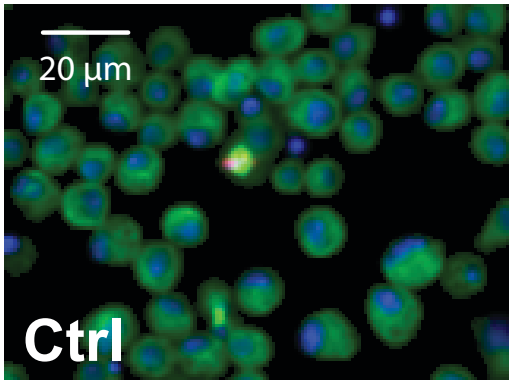
Phenotypes

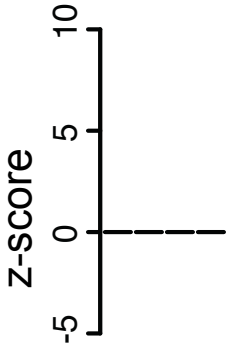
with Michael Boutros, DKFZ

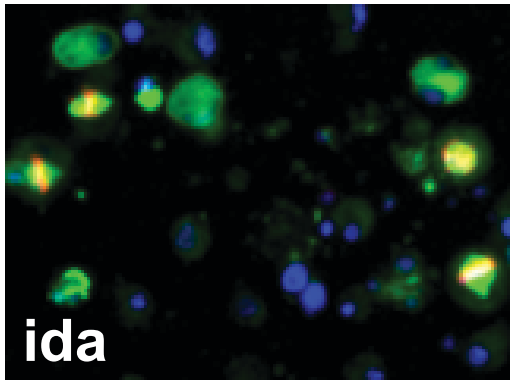
Fischer*, Sandmann*, Horn*, Billmann*, eLife, 2015 (Directional Interactions)

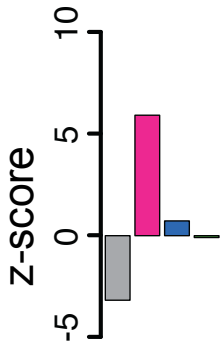
Horn*, Sandmann*, Fischer*, Nat. Methods, 2011

Single knock down phenotypes



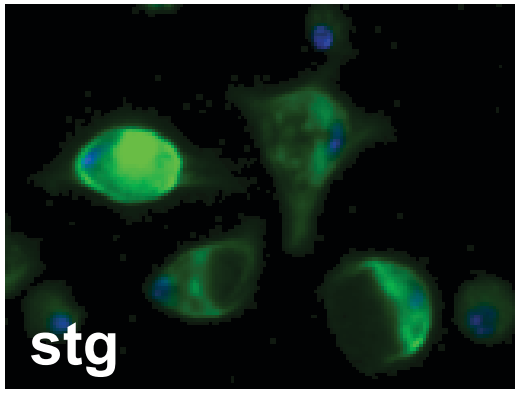


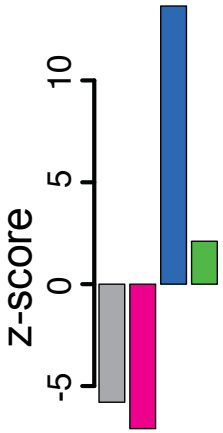


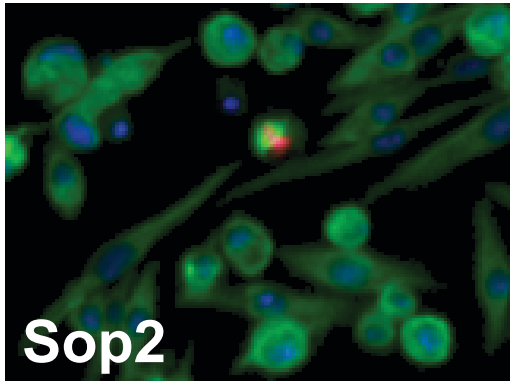


ida is member of the APC

number cells
mitotic index
nuclear area
eccentricity







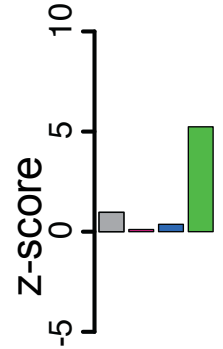
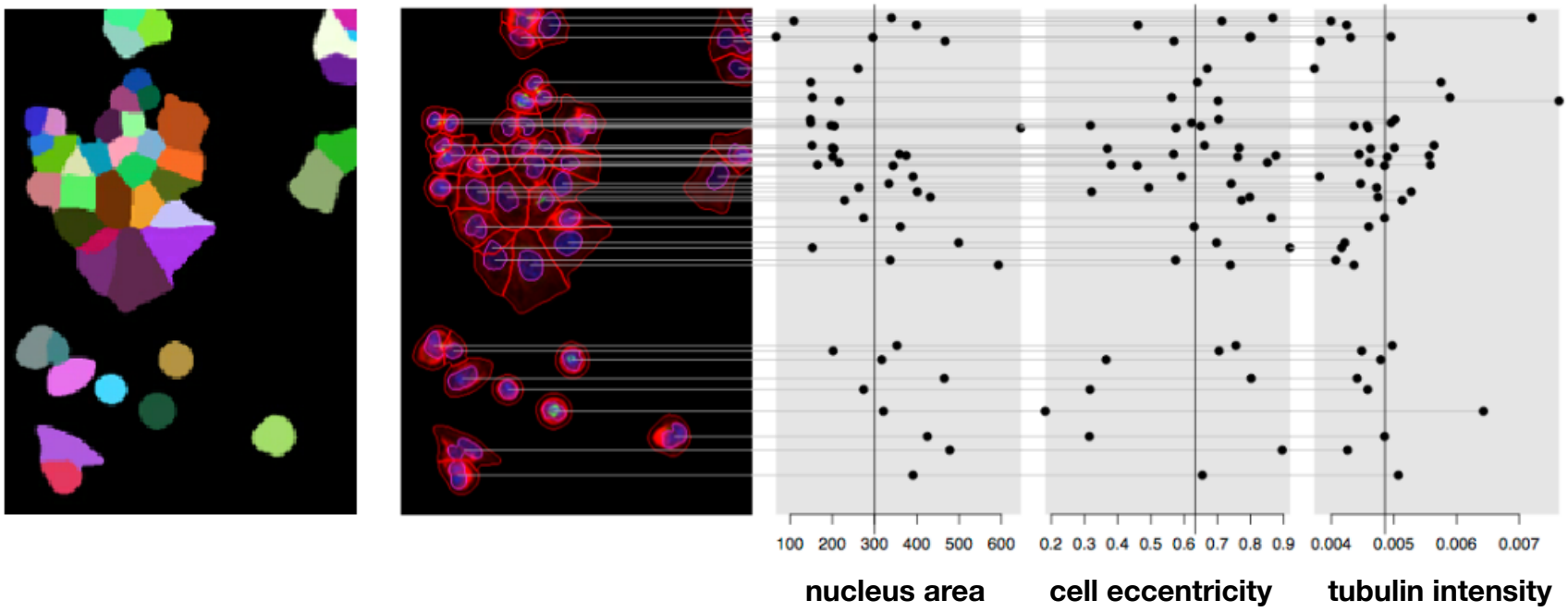


Image segmentation and feature extraction



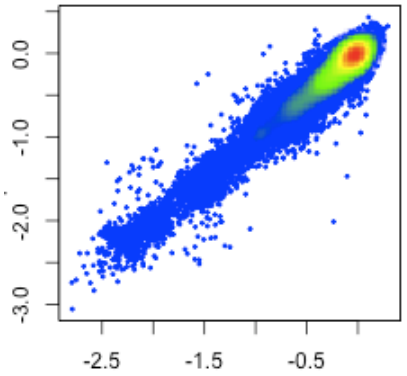
- features: number cells, ratio of mitotic cells, local cell density, radius, area, shape, texture (Haralick)
- **21 non-redundant features selected**

Bioconductor OPEN SOURCE SOFTWARE FOR BIOINFORMATICS packages EBImage / imageHTS / rhdf5

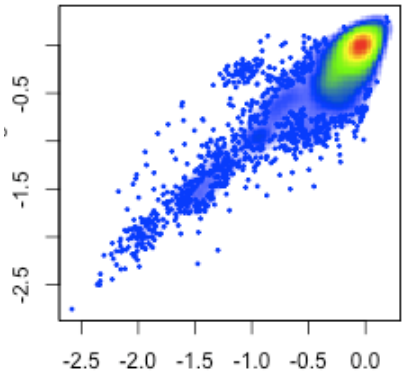
(Laufer*, Fischer*, Nat. Methods, 2013)

Quality control and normalization

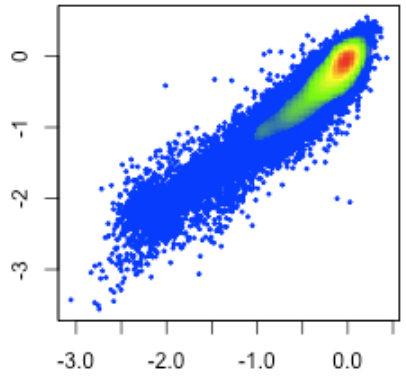
within screen replicates (cor=0.968)



independent dsRNA designs (cor=0.902)

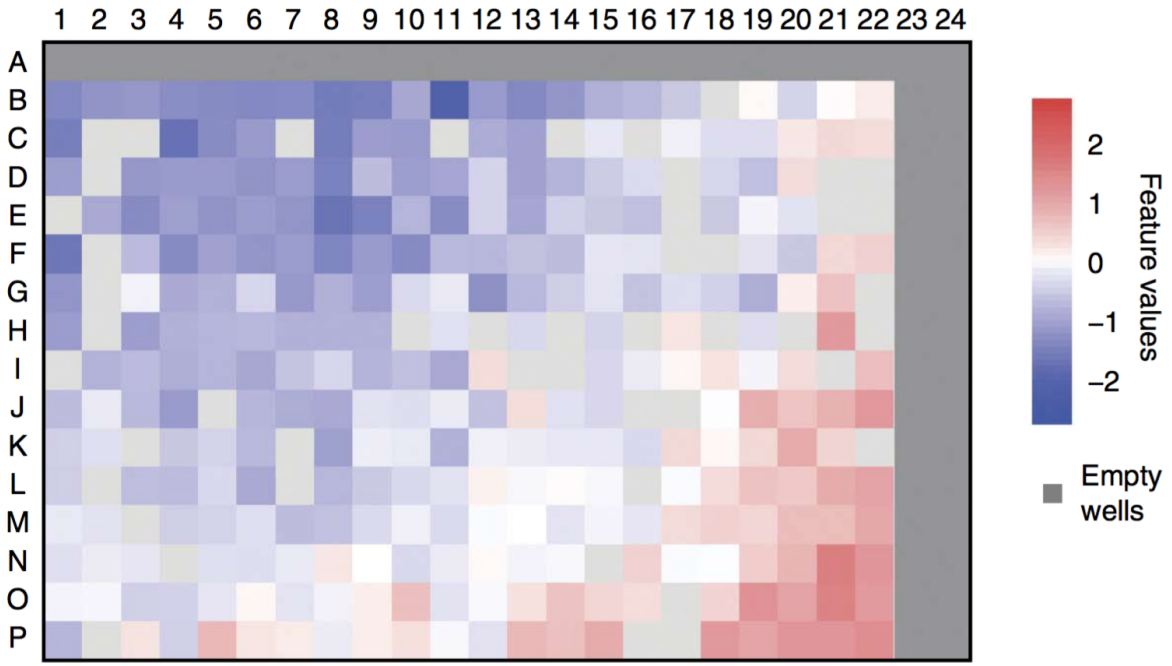


between screen replicates (cor=0.948)



Quality control:
Filter irreproducible data (irreproducible features, off-target effects)

Normalize for known technical artefacts: spatial effects, time effects, …



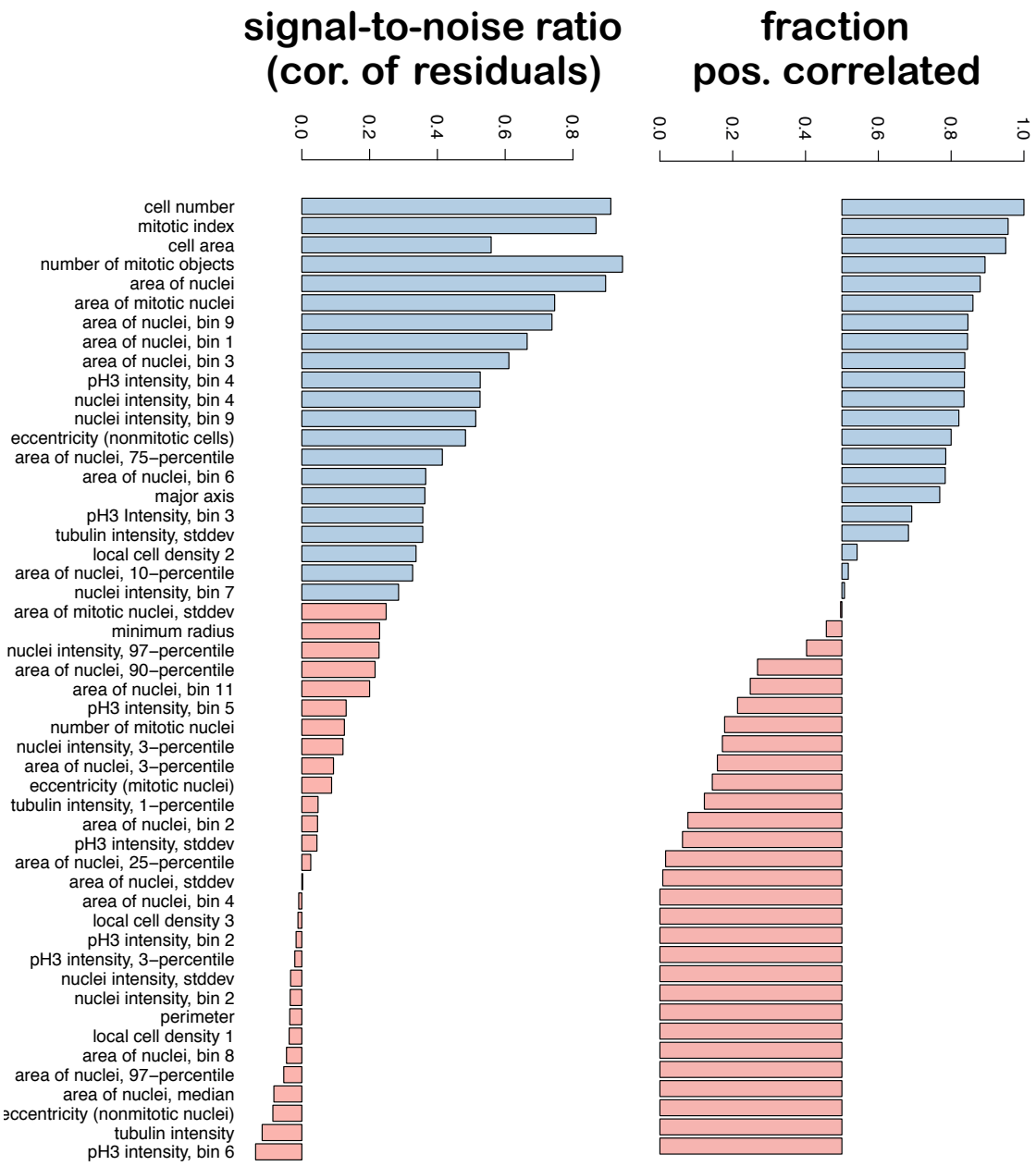
21 non-redundant features

Selection procedure:

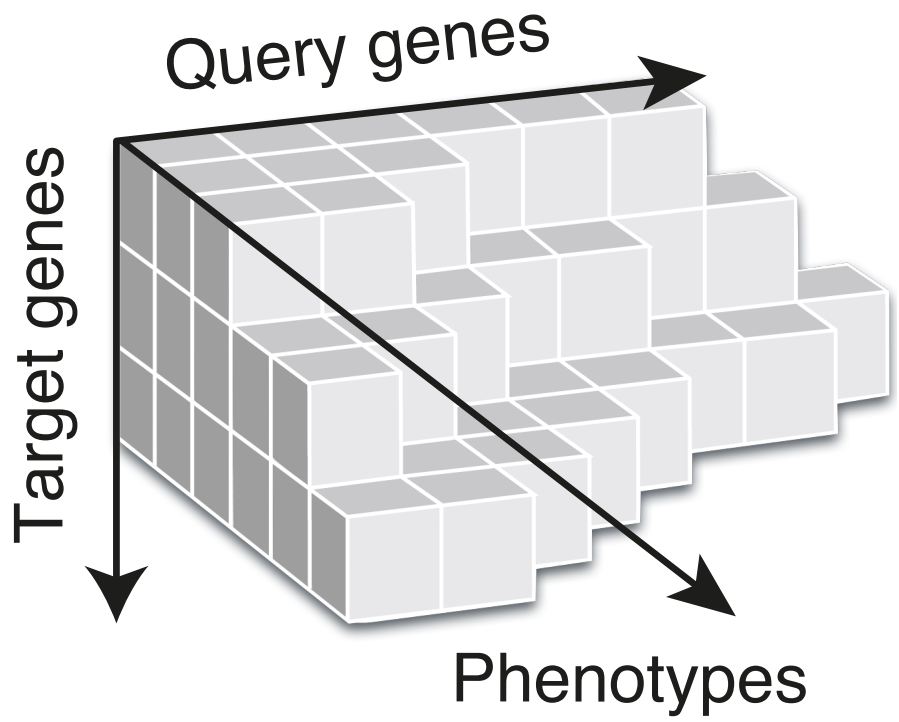
- Fit a **linear model** for each feature as a function of the previously selected features

- Select the feature among all remaining ones with **highest correlation of residuals** between replicates

- **Stop criterium:** equal proportion of pos. and neg. residual correlations



Raw data cube



1293 target genes

x 72 query genes

x 21 feature

(statistical) genetic interactions

- For many phenotypes, the main effects (single gene) are multiplicative for non interacting genes i, j: $d_{ij} = \varpi \cdot \mu_i \cdot \mu_j$ **R.A. Fisher, 1918**

- Additive on logarithmic scale

$$\log d_{ijk} = w + m_i + m'_j + g_{ij} + \varepsilon_{ijk}$$

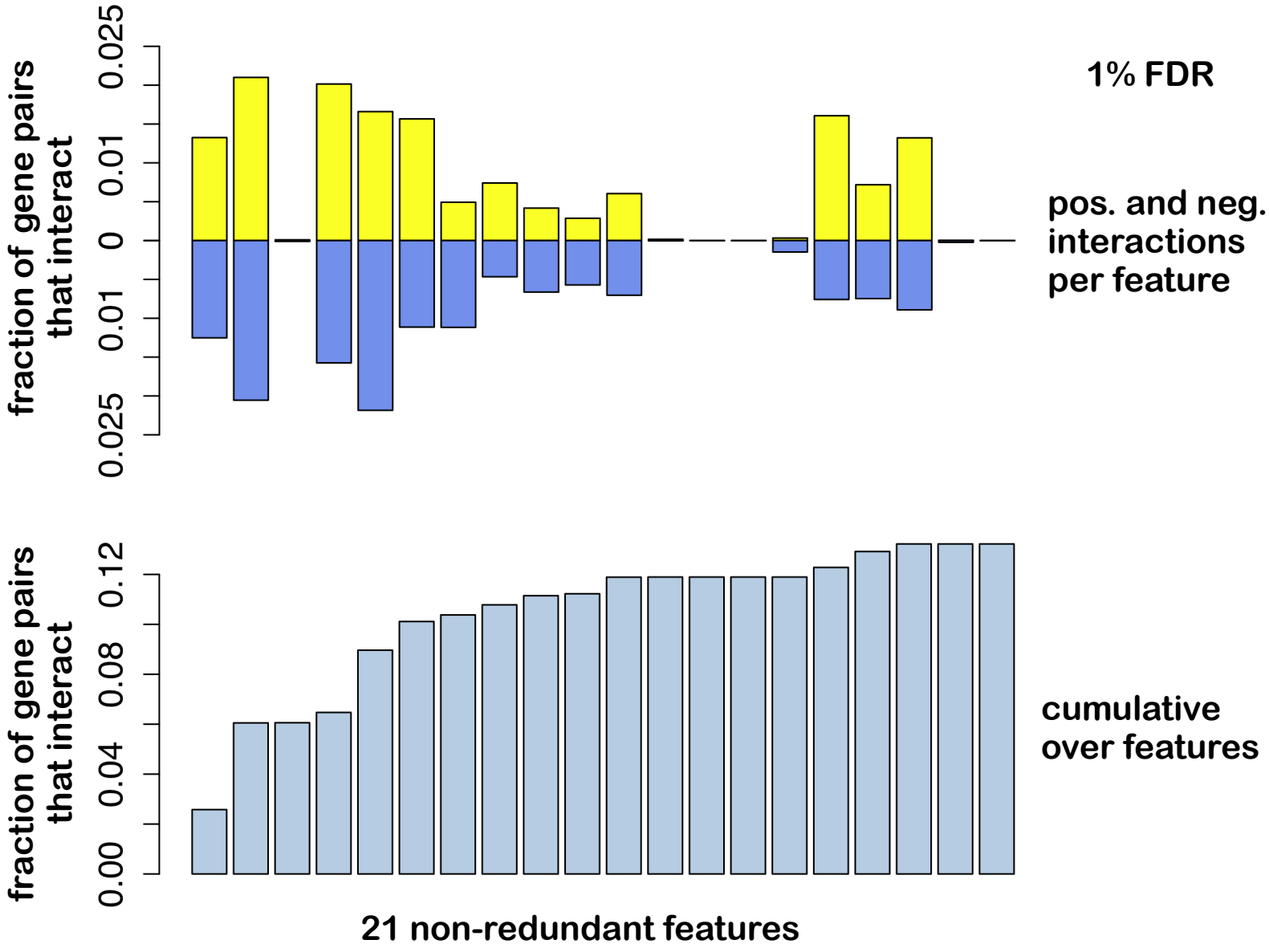
- $\log d_{ijk}$: measurement (nr cells, growth rate, ...)
- w: effect of control
- m_i: main effect of dsRNA i
- m'_j: main effect of dsRNA j
- g_{ij}: interaction term; 0, for non interacting genes; ≠0, for interacting genes
- ε_{ijk}: error term

- Estimation of main effects (assume that interactions are rare)

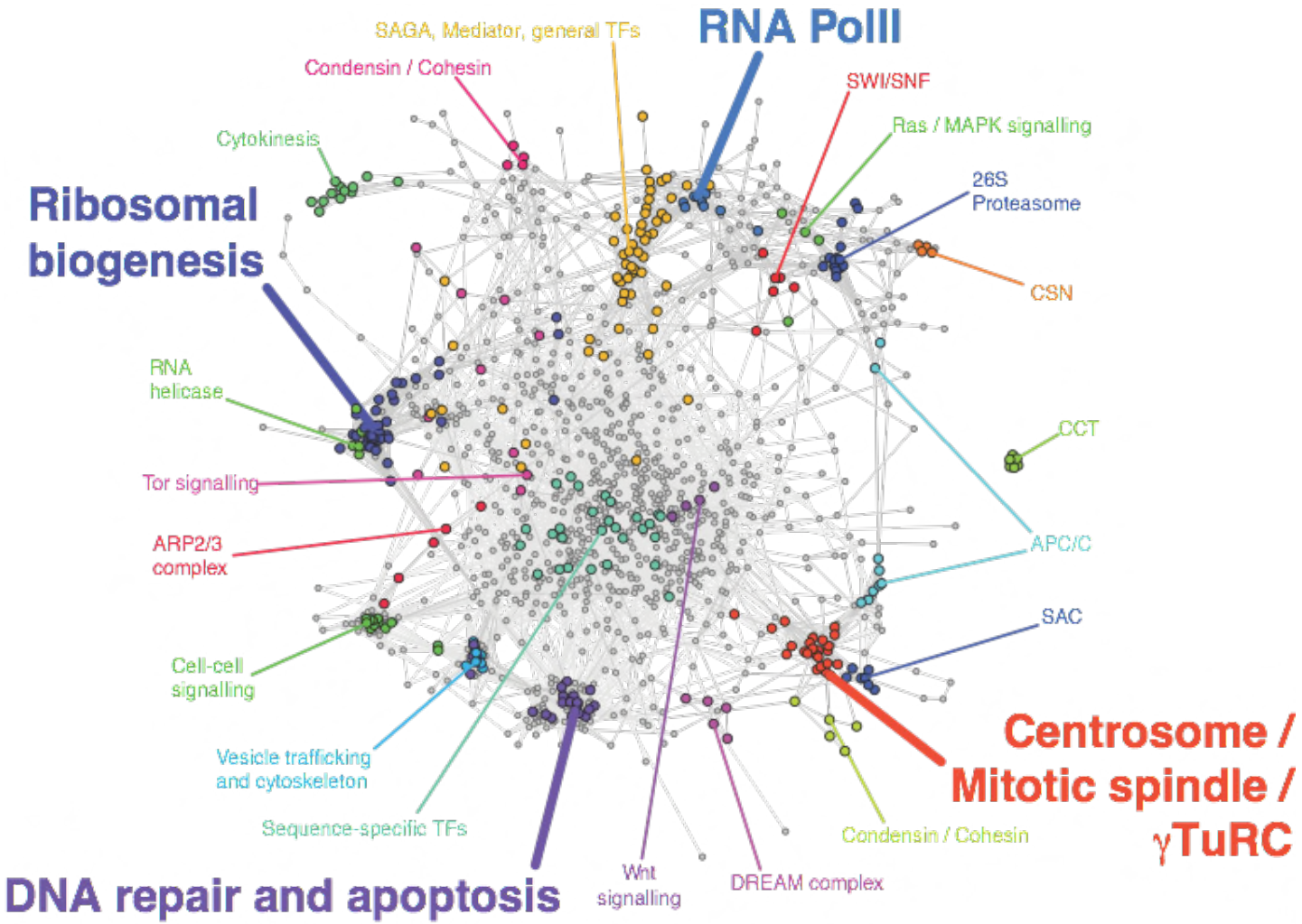
$$(\hat{m}, \hat{m}') = \arg\min_{m,m'} \sum_{i,j} \left\| \log d_{ij} - w - m_i - m_j \right\|_1^1$$

- Detect Genetic Interactions: Compare $\log d_{ijk}$ to $w - \hat{m}_i - \hat{m}_j$ (t-test)

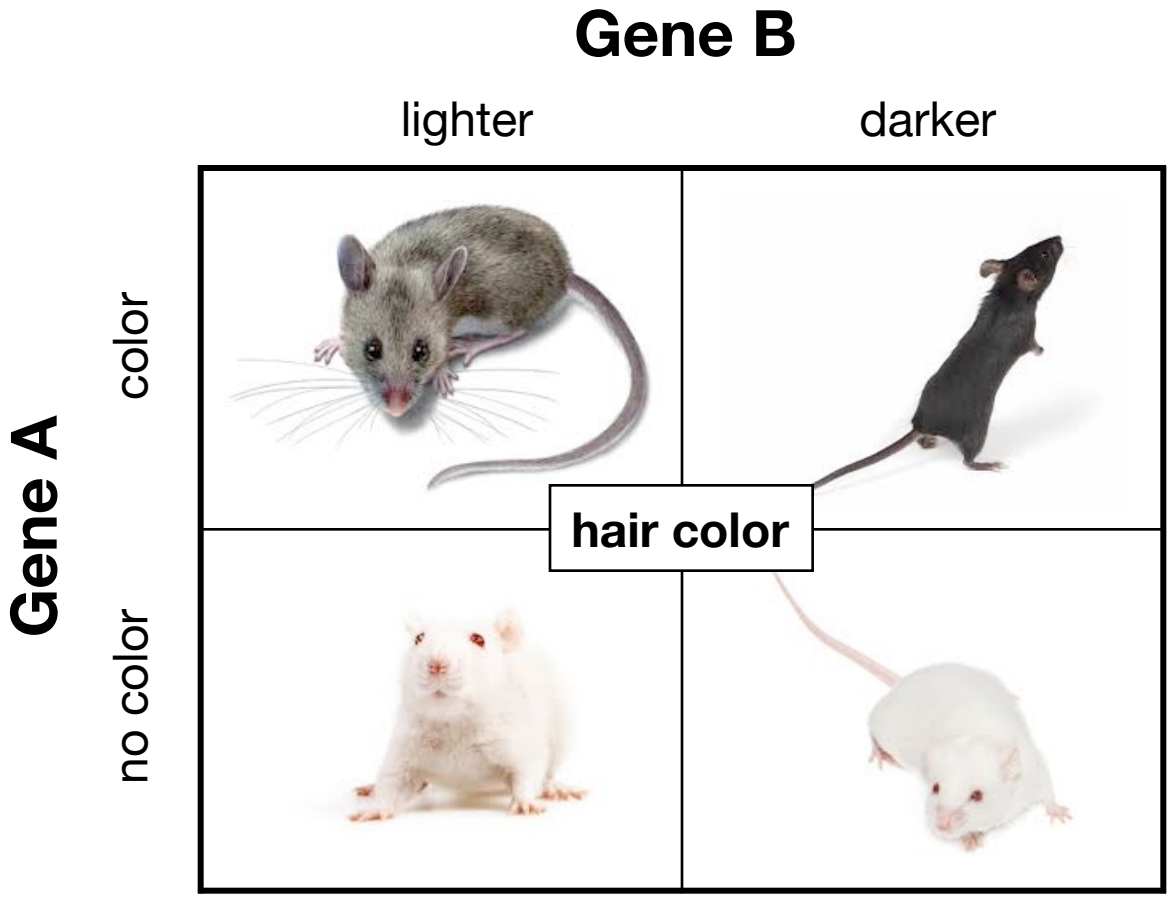
Number of interactions



Functional prediction from large-scale image-based genetic interaction screens



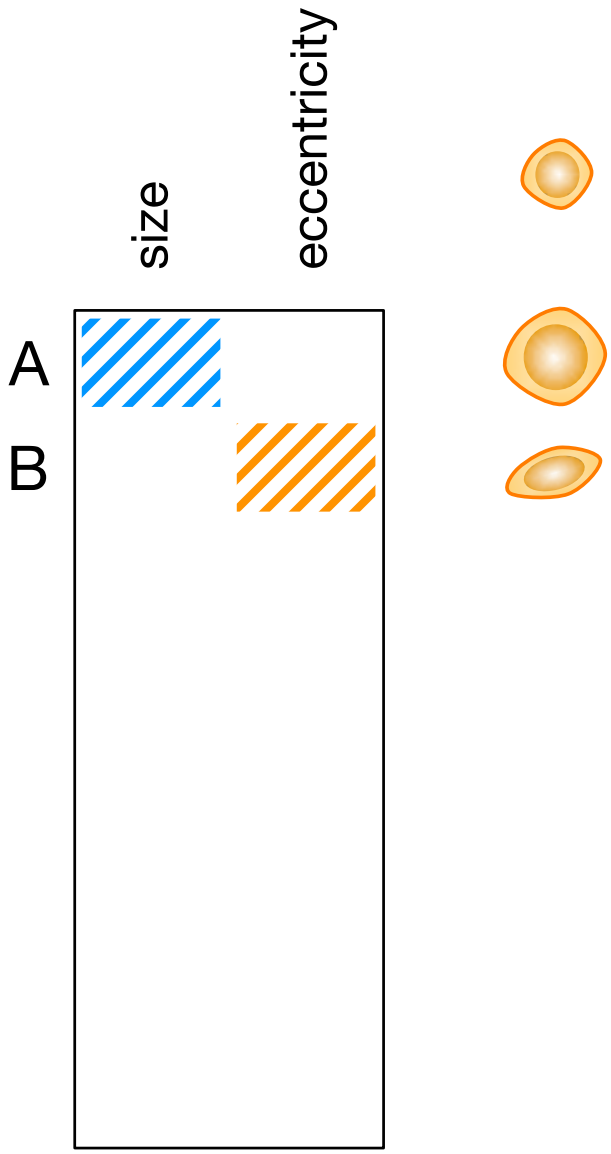
Epistasis



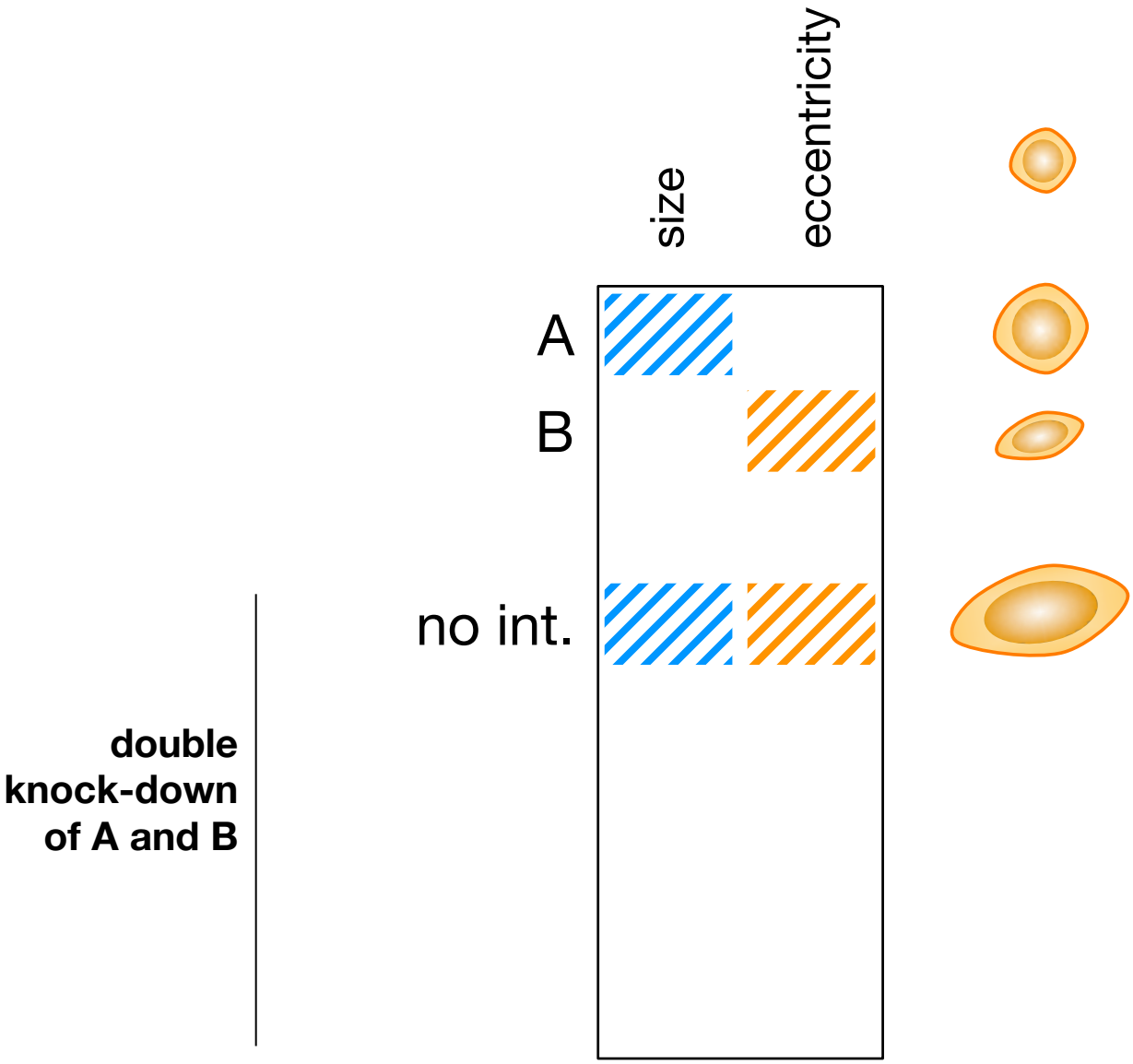
A is epistatic to B

W. Bateson, 1907

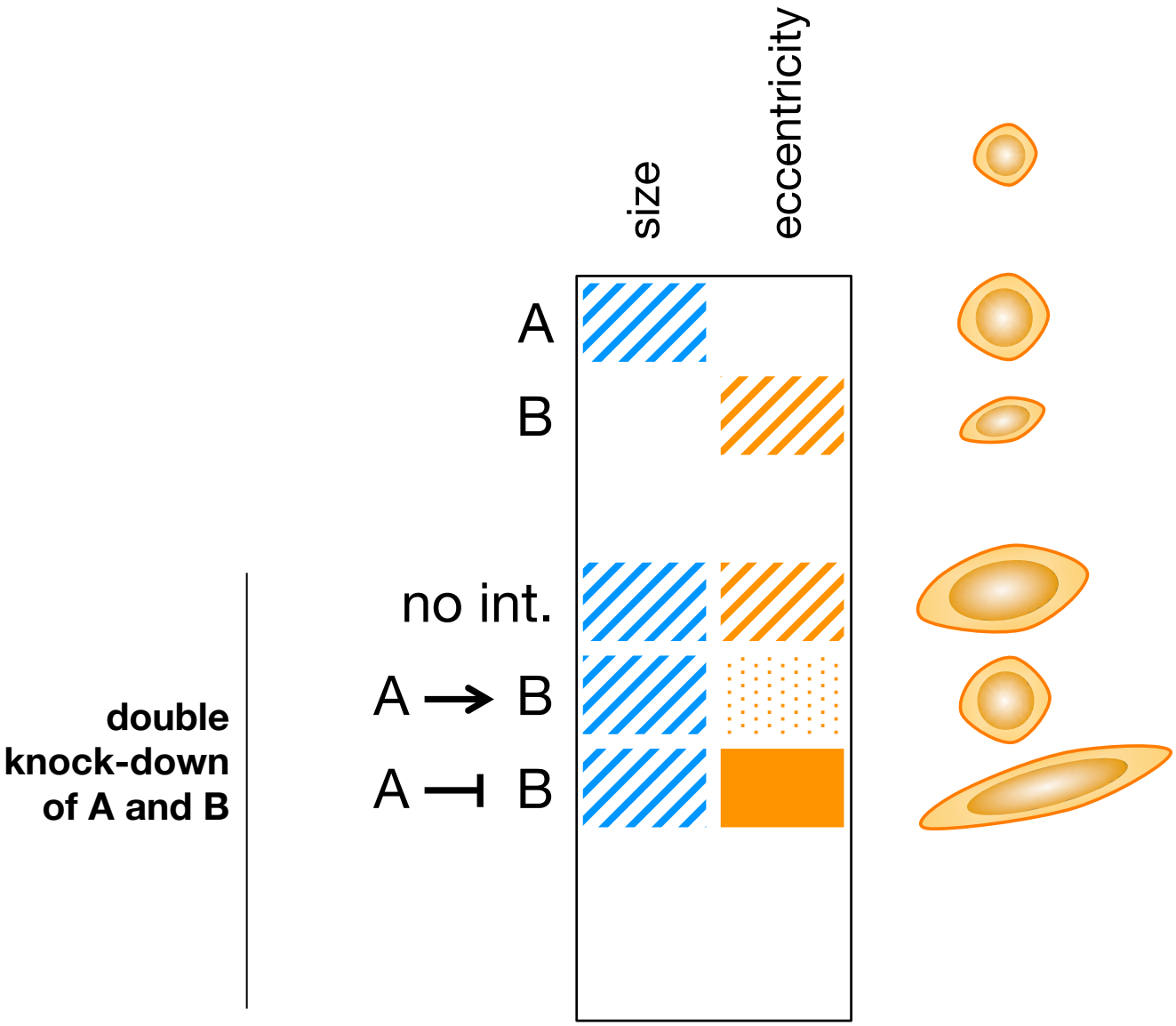
Directional, epistatic interactions



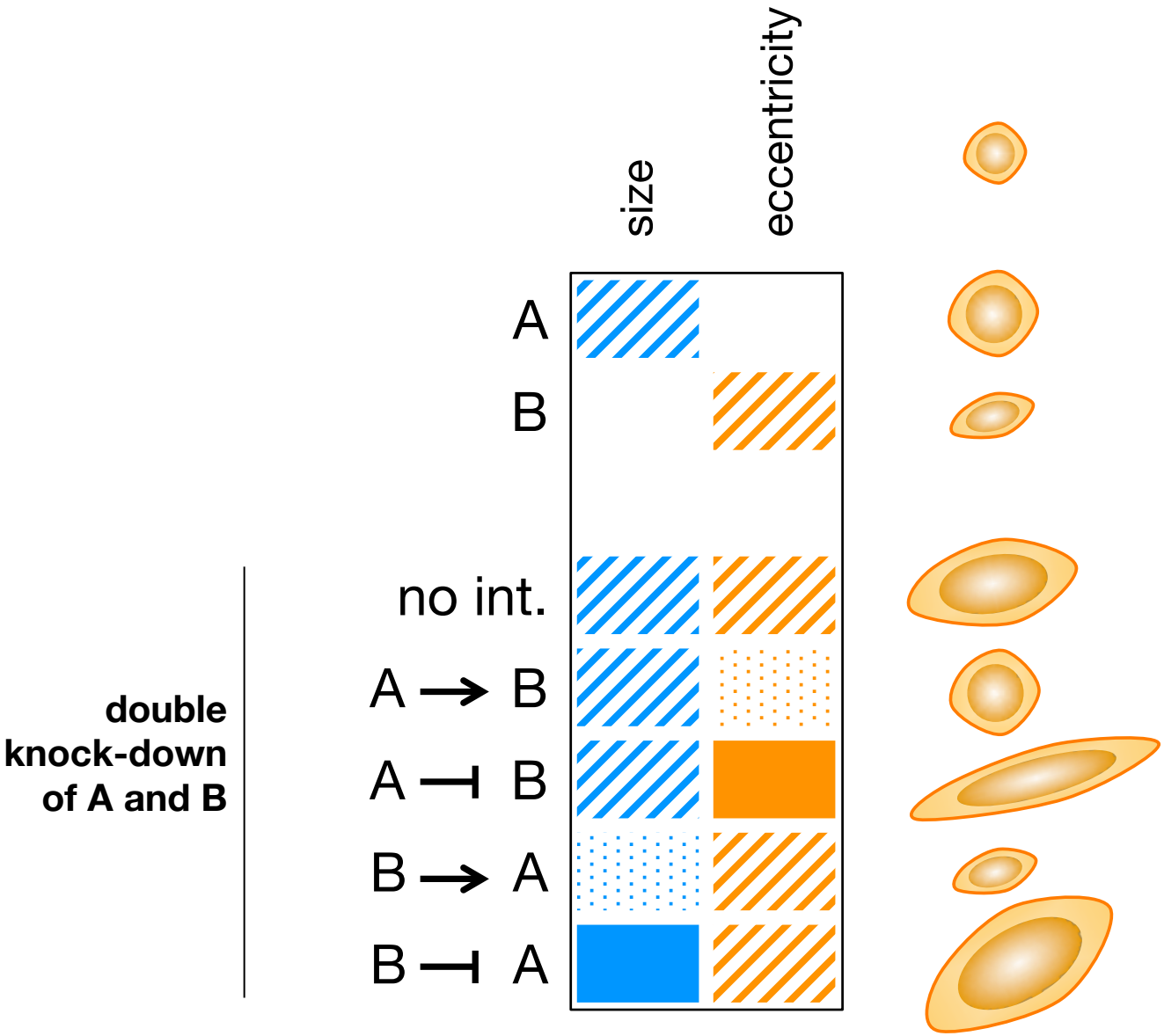
Directional, epistatic interactions



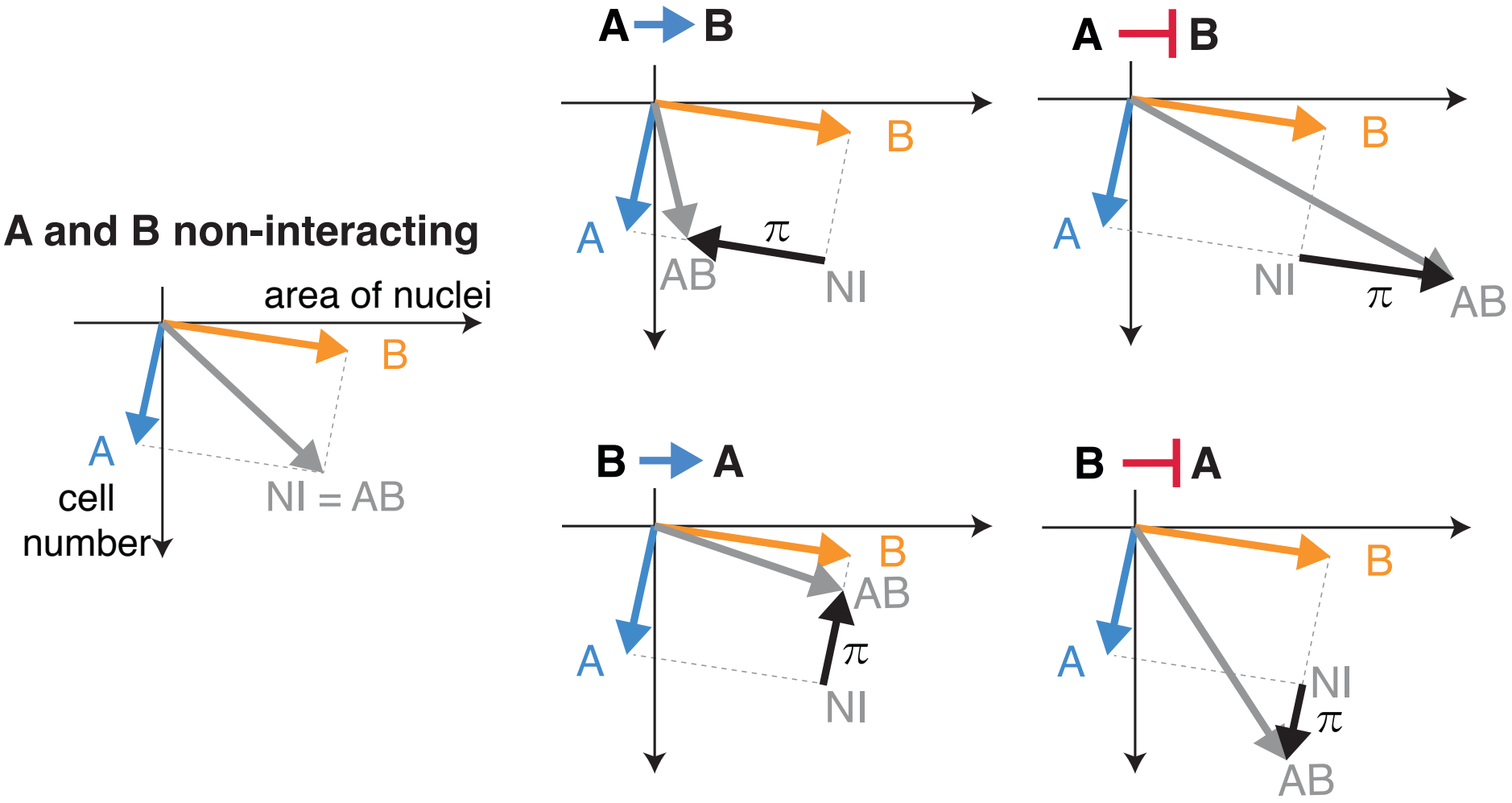
Directional, epistatic interactions



Directional, epistatic interactions



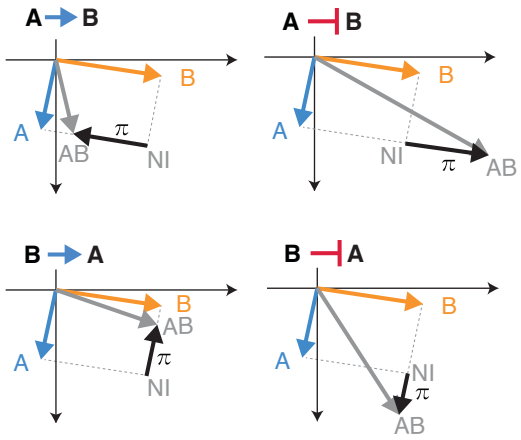
Directional, epistatic interactions



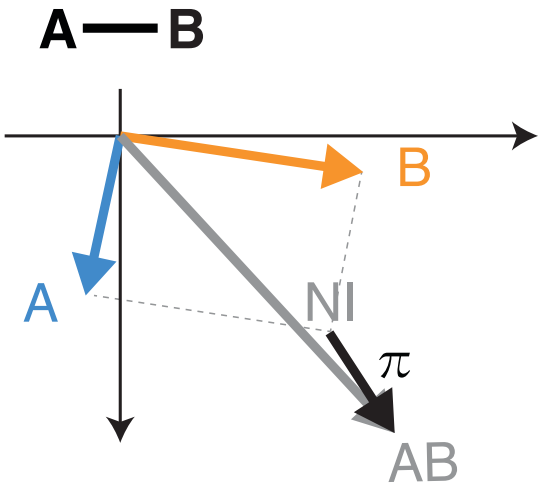
Requires multiple phenotypes

Special cases

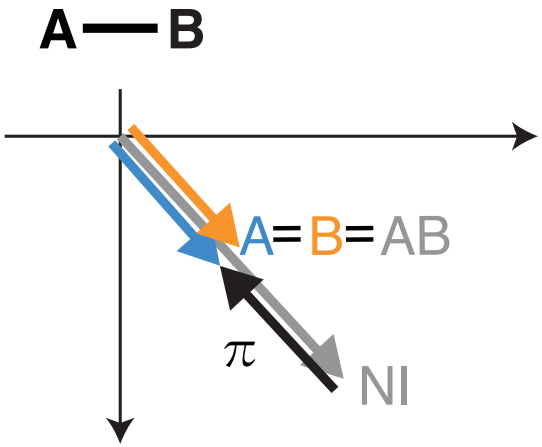
Directional interactions



No direction detectable

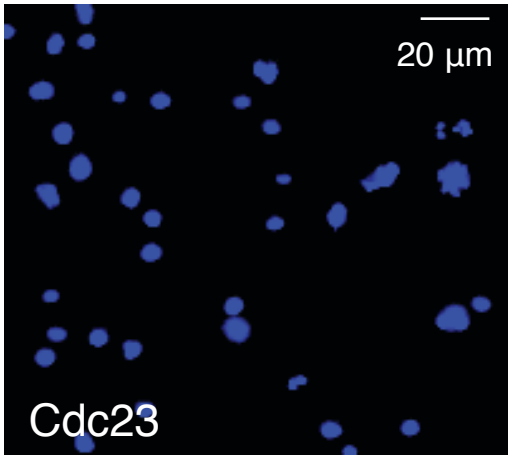


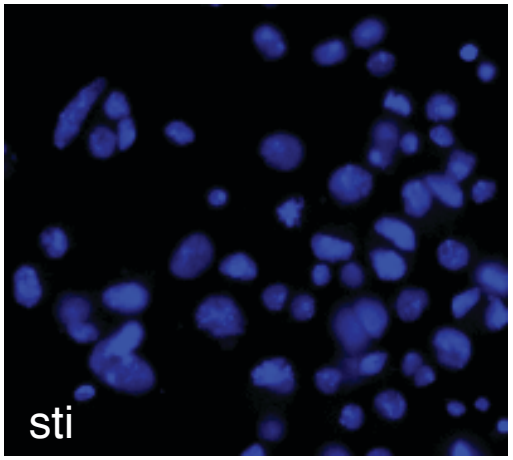
Mixed Epistasis?

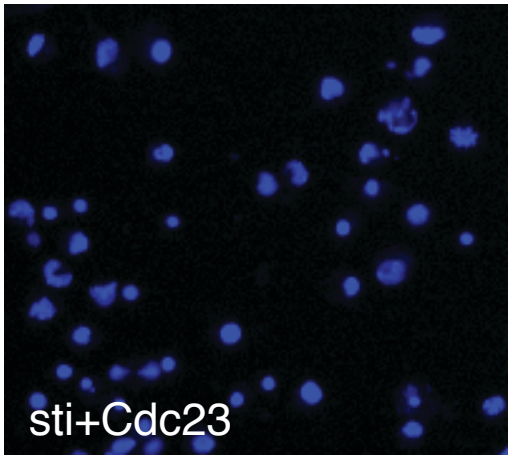


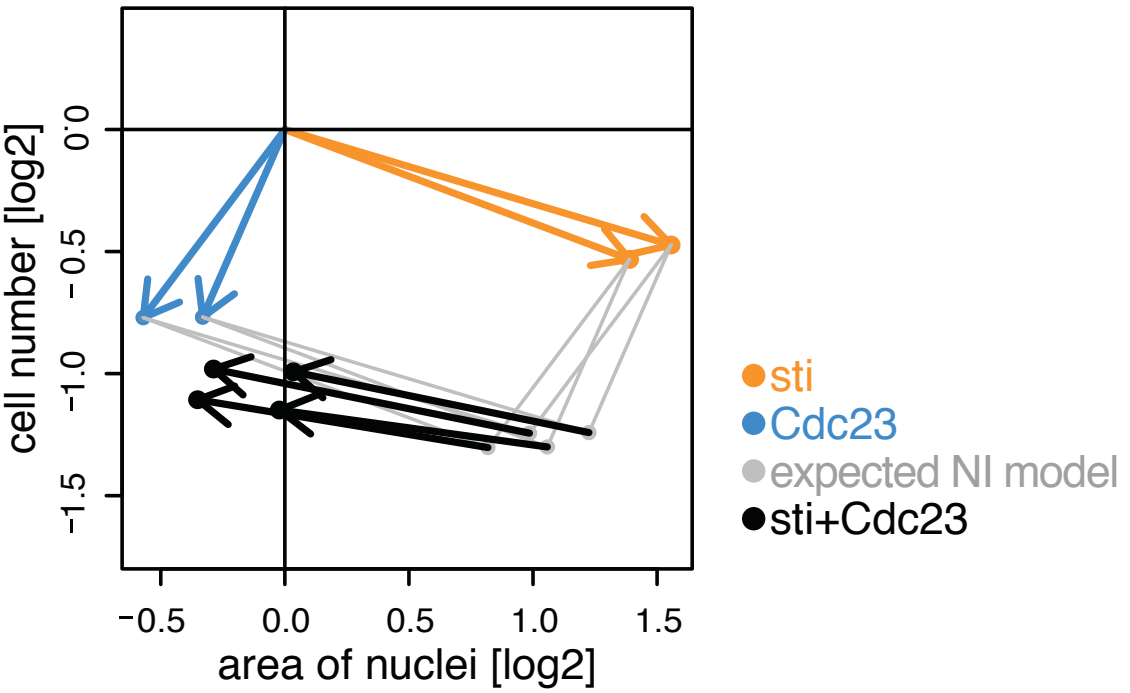
protein complexes

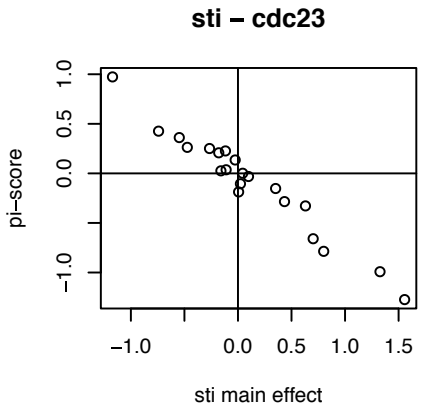
sti depends on cdc23

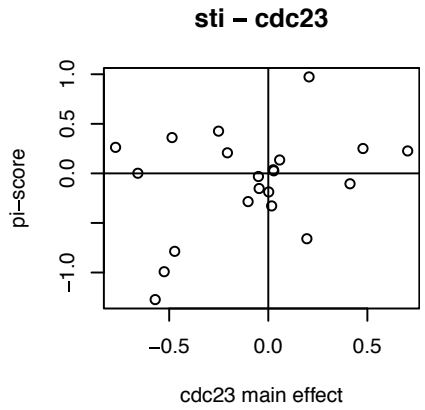


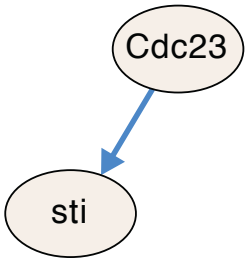




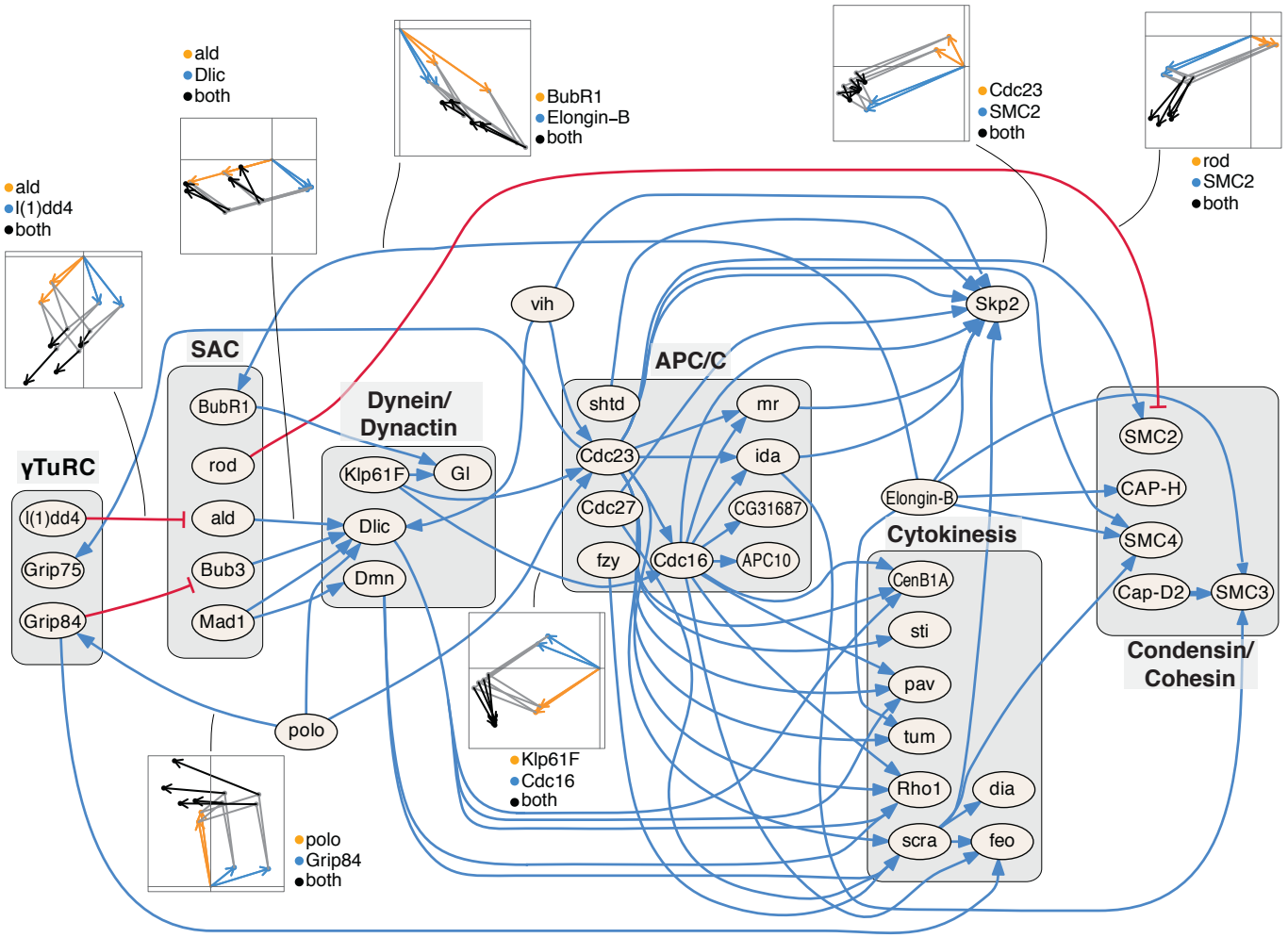








Directional interactions in mitosis

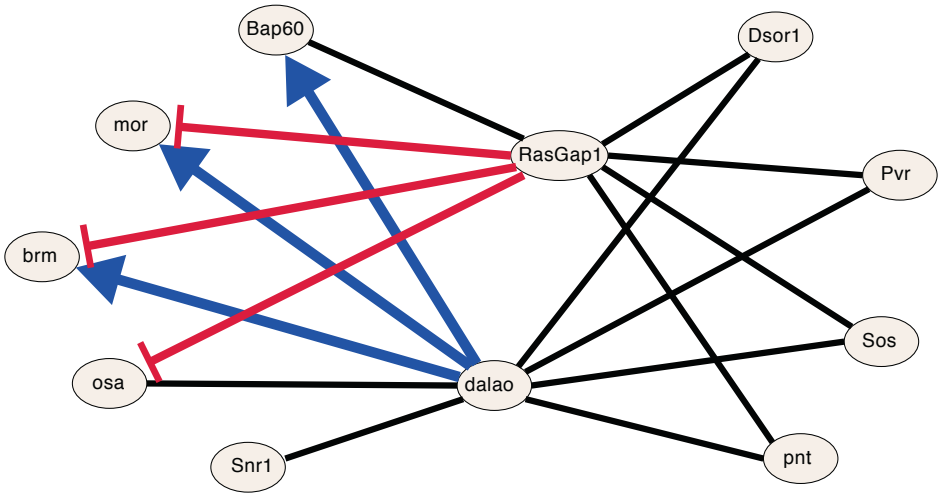


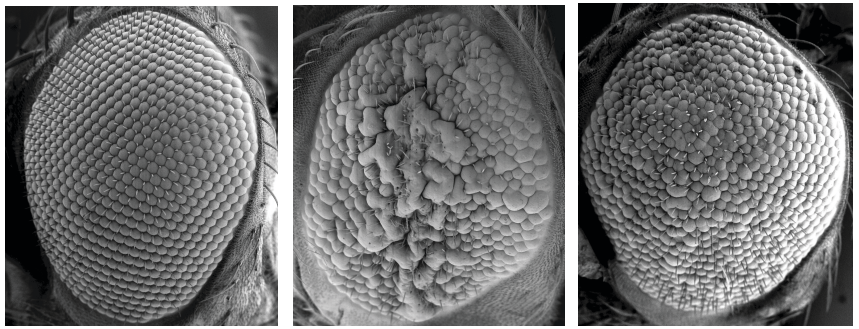
Observed directional interactions

→ activating (alleviating)

⊣ inhibiting (aggravating)

SWI/SNF interacts with Ras signaling

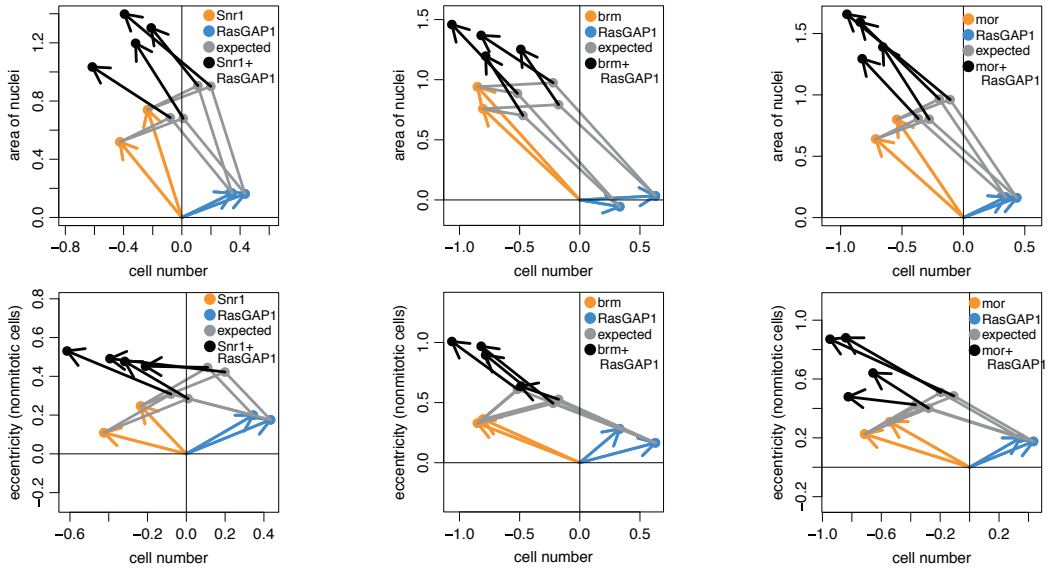




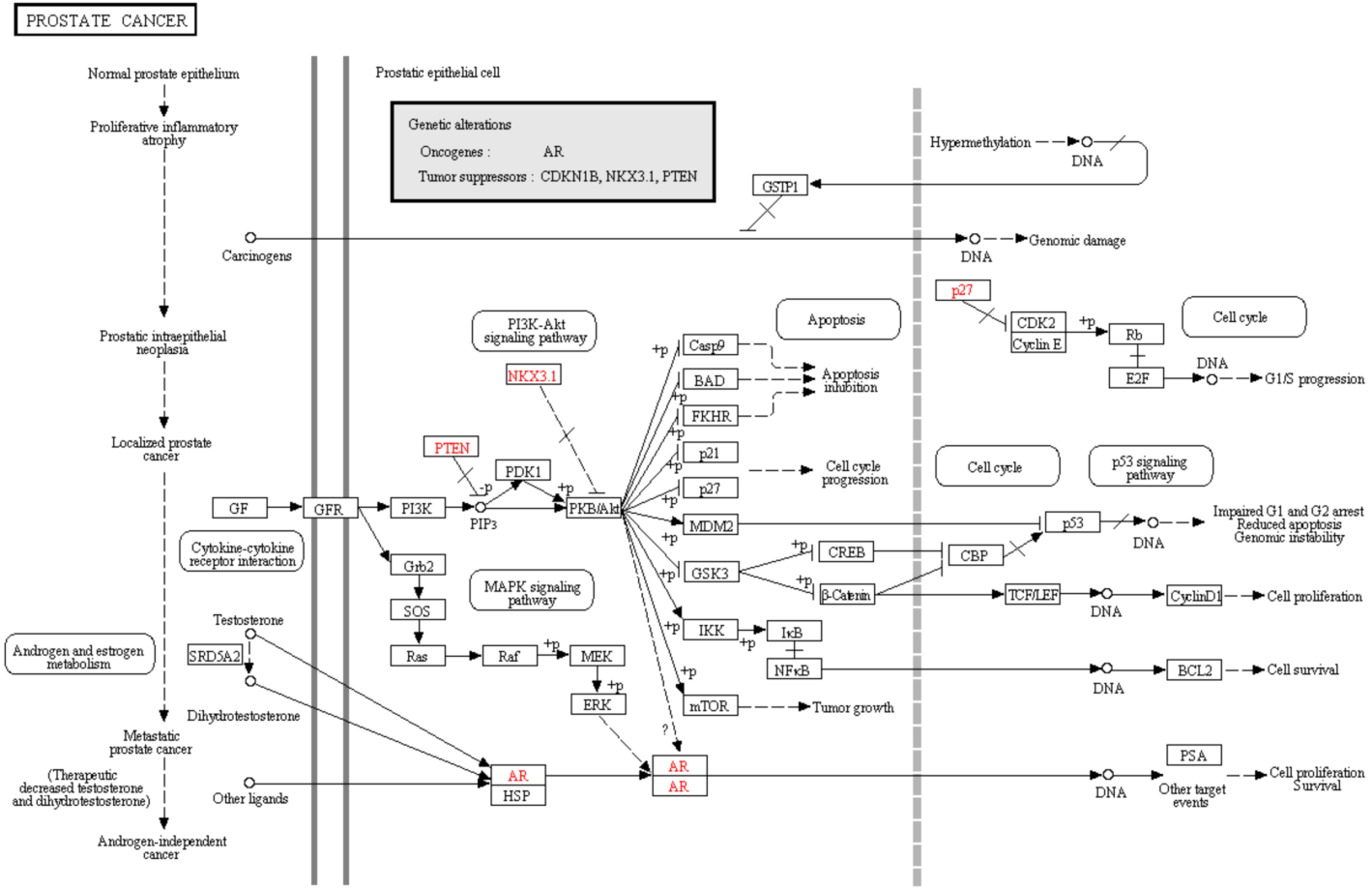
Control
+/+

sev-Ras^{v12}/+

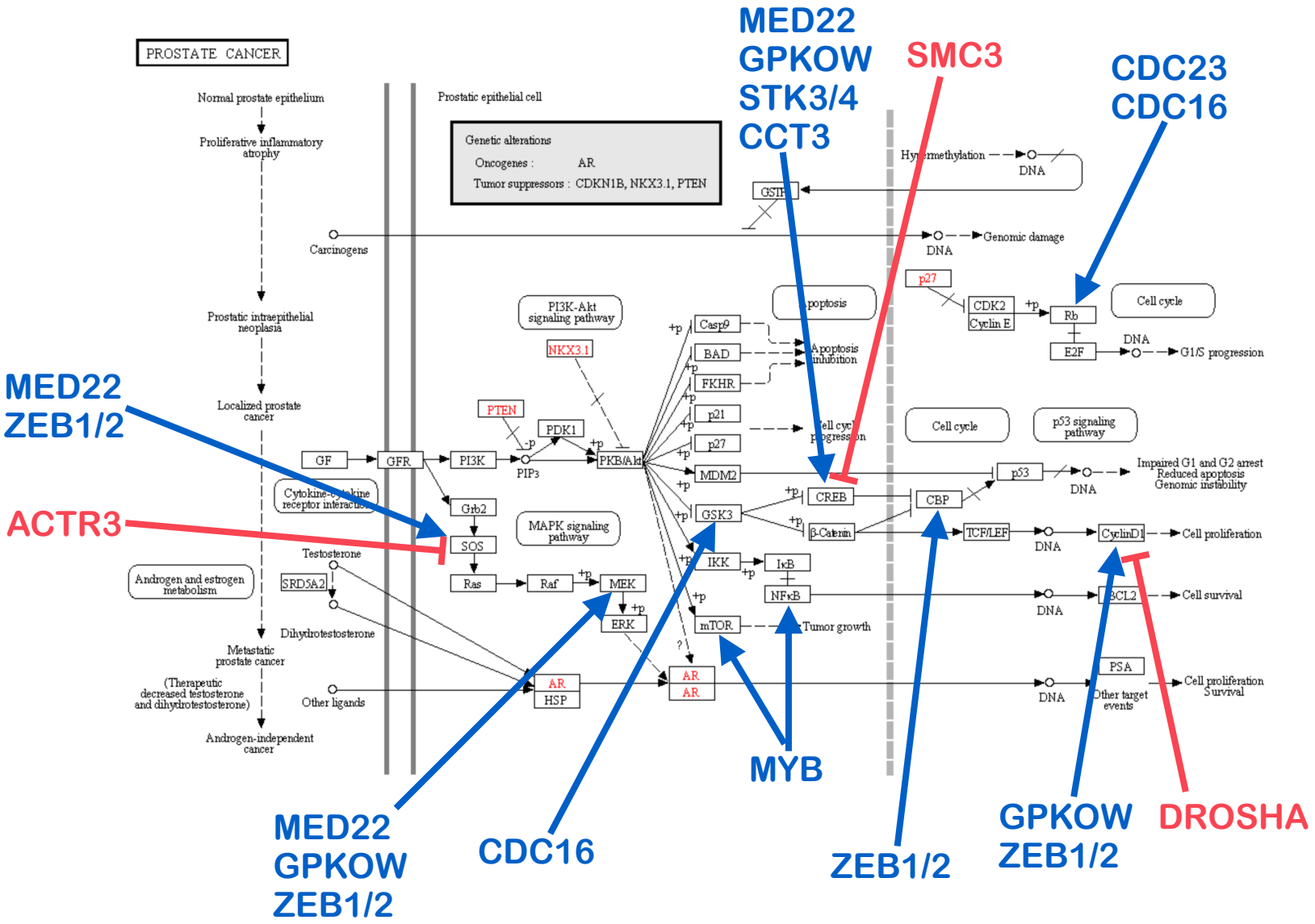
sev-Ras^{v12}/ *osa*^{2}



KEGG Prostate Cancer Pathway



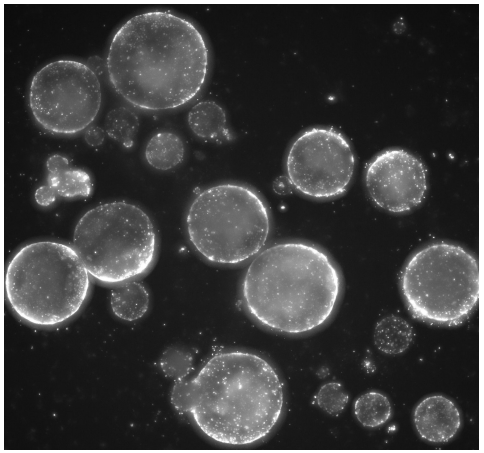
KEGG Prostate Cancer Pathway



Outlook

3D Organoids to model environment

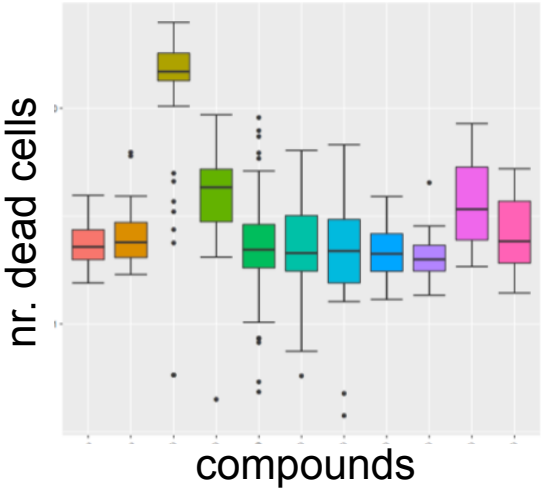
new challenge
in image analysis



Deep learning for
organoid detection
and feature extraction

drug-sensitivity screens with patient cells

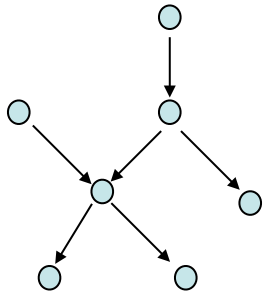
test treatment
outcome in vitro



models to predict
treatment outcome
from genomic data

knockdown screens in drug-resistant cells

new combinatorial
drug treatments



overcome acquired
drug-resistance

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Niklas Rindtorff

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Tomaz Curk
Julian Gehring
Bernd Klaus
Felix Klein
Andrzej Oles
Malgorzata Oles
Gregoire Pau
Aleksandra Pekowska
Paul Theodor Pyl
Alejandro Reyes

dkfz.
GERMAN CANCER RESEARCH CENTER
IN THE HELMHOLTZ ASSOCIATION

50 Years – Research for
A Life Without Cancer