



Chemical Genetics

PUBS 2018

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Refresher: Genetics

Gene

Genotype

Phenotype

Fitness

Selection

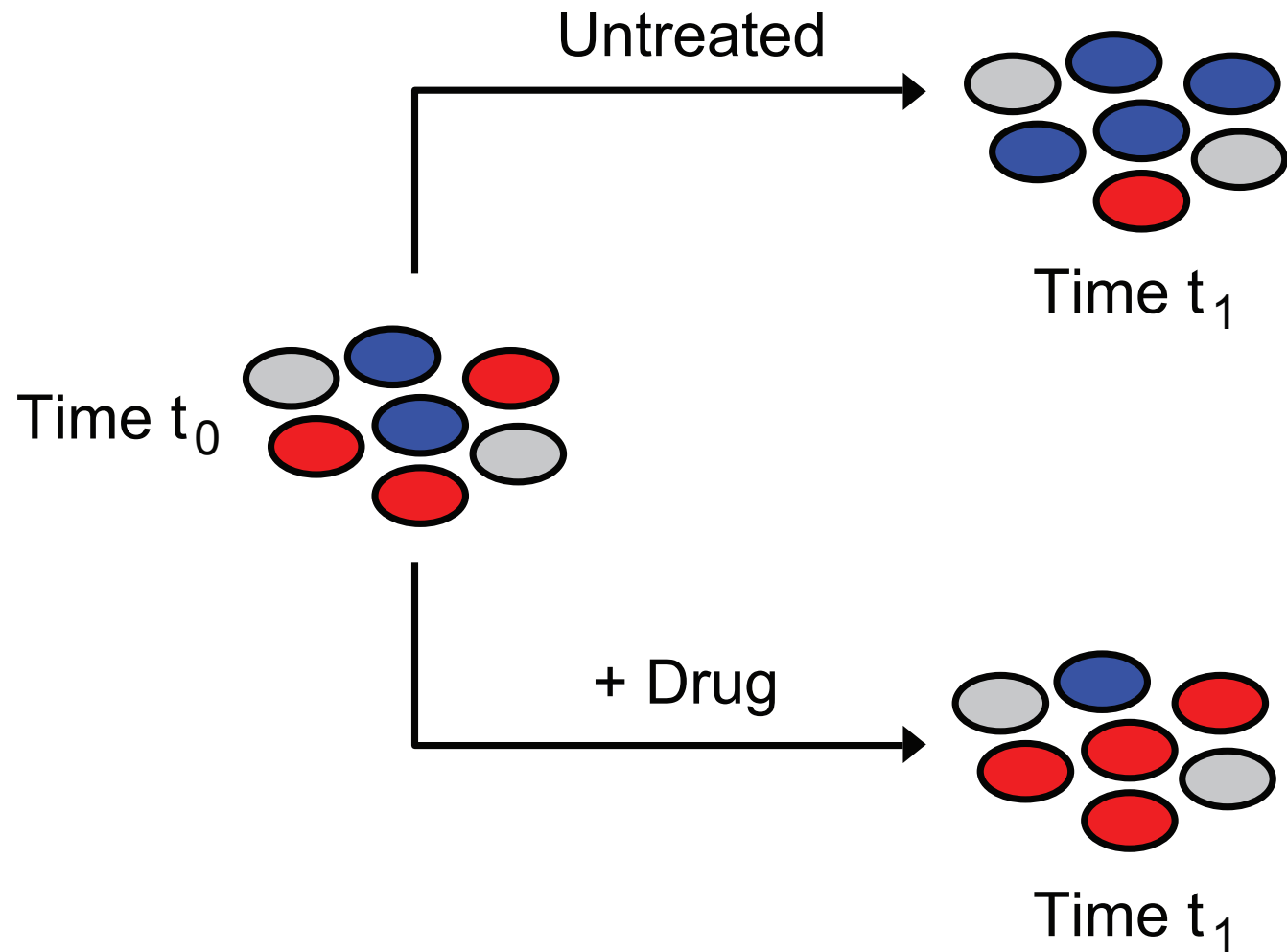
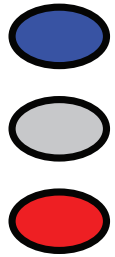
Forward genetics / Reverse genetics

Genetic interaction

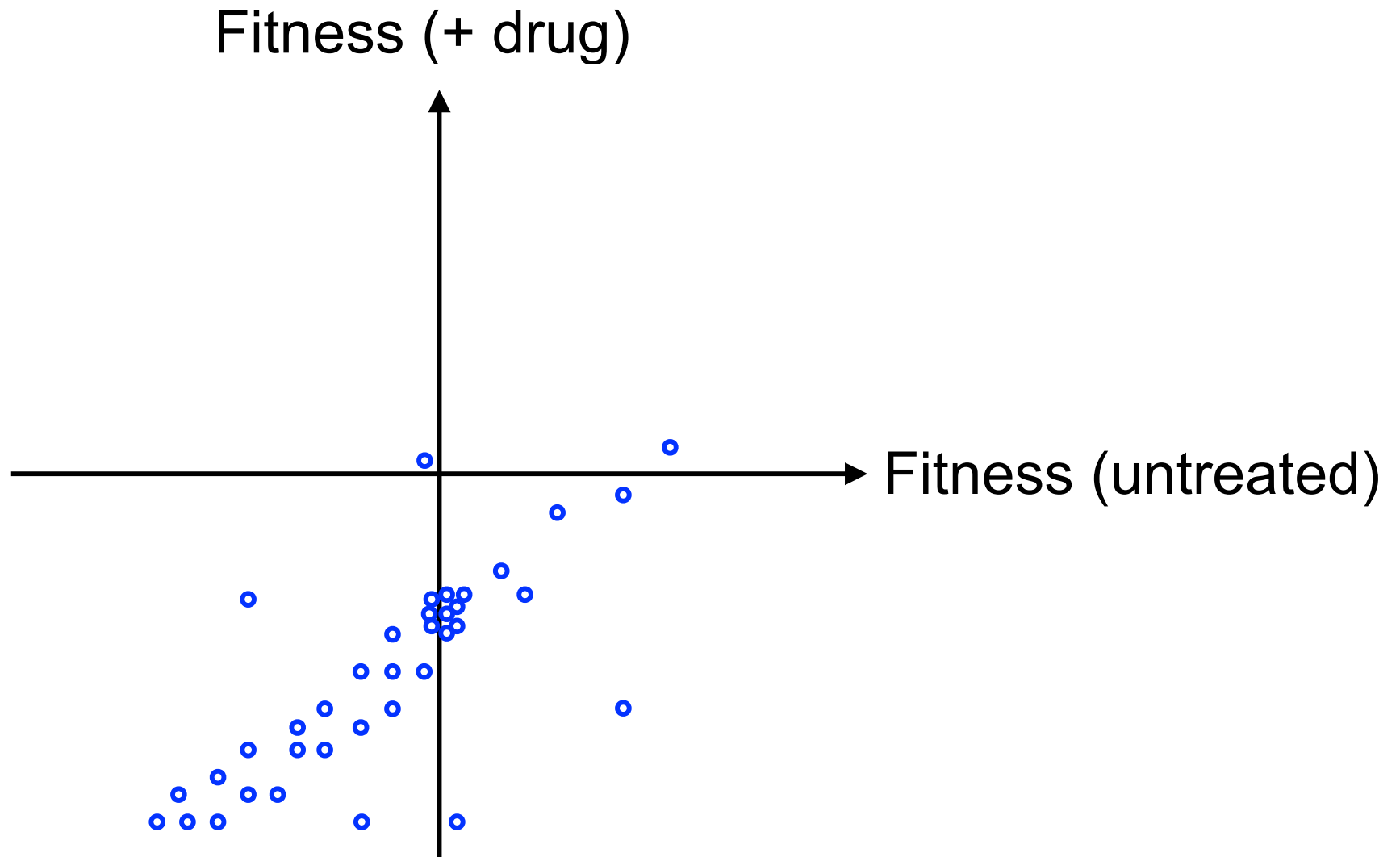
Gene-Drug interaction

Chemical-genetic screen

Genotypes



Gene-drug interactions



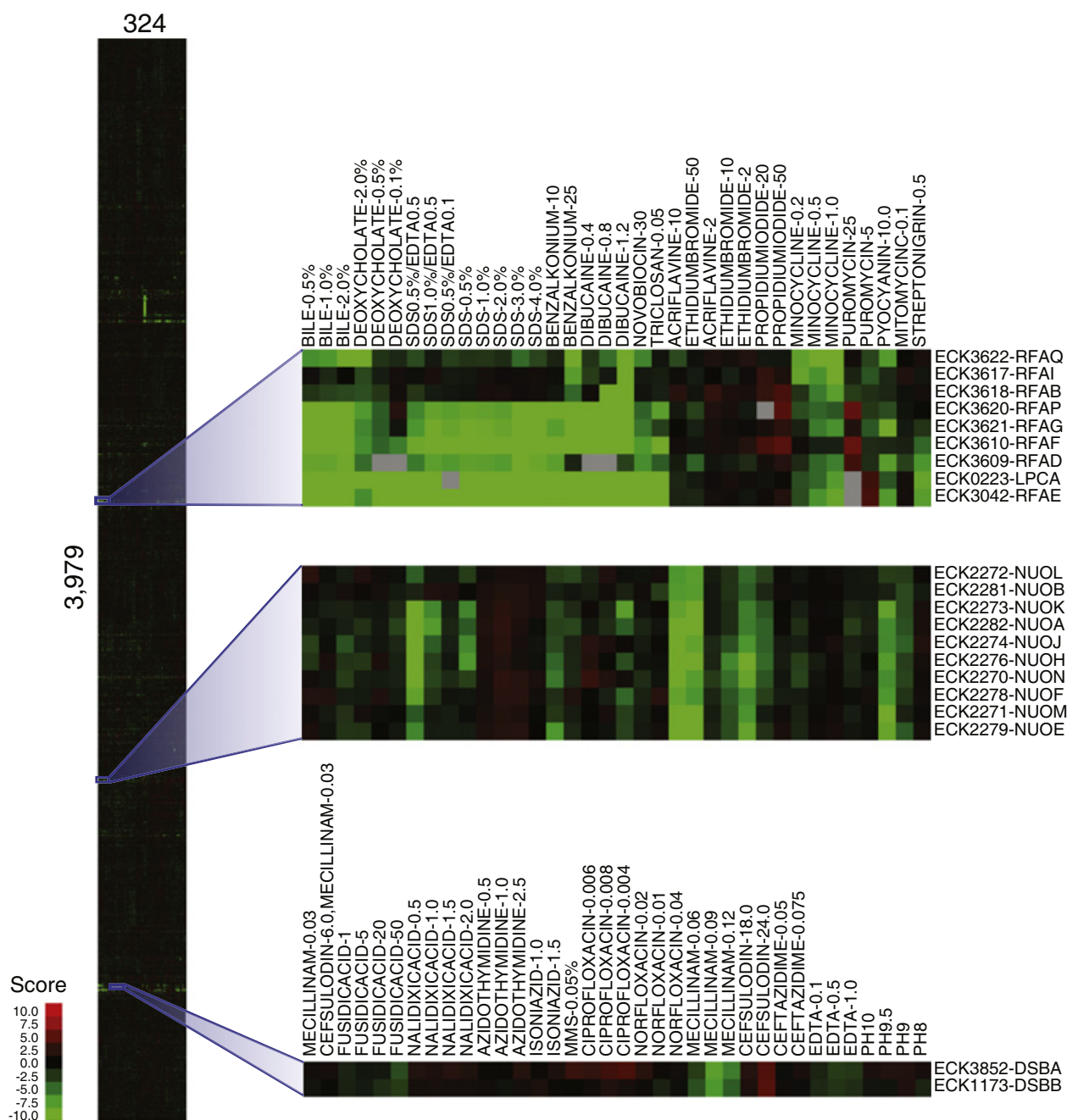
Interpreting Gene-drug interactions

	Knockdown	Knockout	Overexpression
Assay results	HIP ^a	HOP ^b	MSP ^c
Direct molecular target of drug, essential gene	S	NA	R
Direct molecular target of drug, nonessential gene	S	R	R
Essential genes encoding proteins involved in target pathway	S	NA	not S or R
Genes involved in drug detoxification, essential genes	S	NA	R
Genes involved in drug detoxification, nonessential genes	S	S	R

Ericson *et al* (2010) *Methods Enz*

How about point mutants? Neomorphs?

Large-scale chemical genetics: identifying gene function



Chemical genetics: Use cases

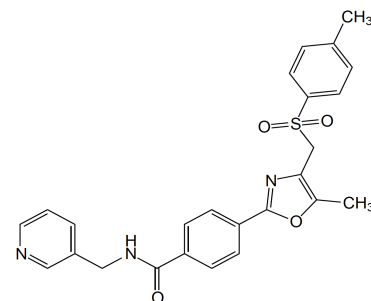
- Drug target ID
- Gene function ID
- Precision medicine:
 - Biomarkers predicting drug response
 - Mechanisms of acquired drug resistance
 - Development of combination therapies

Drug target identification

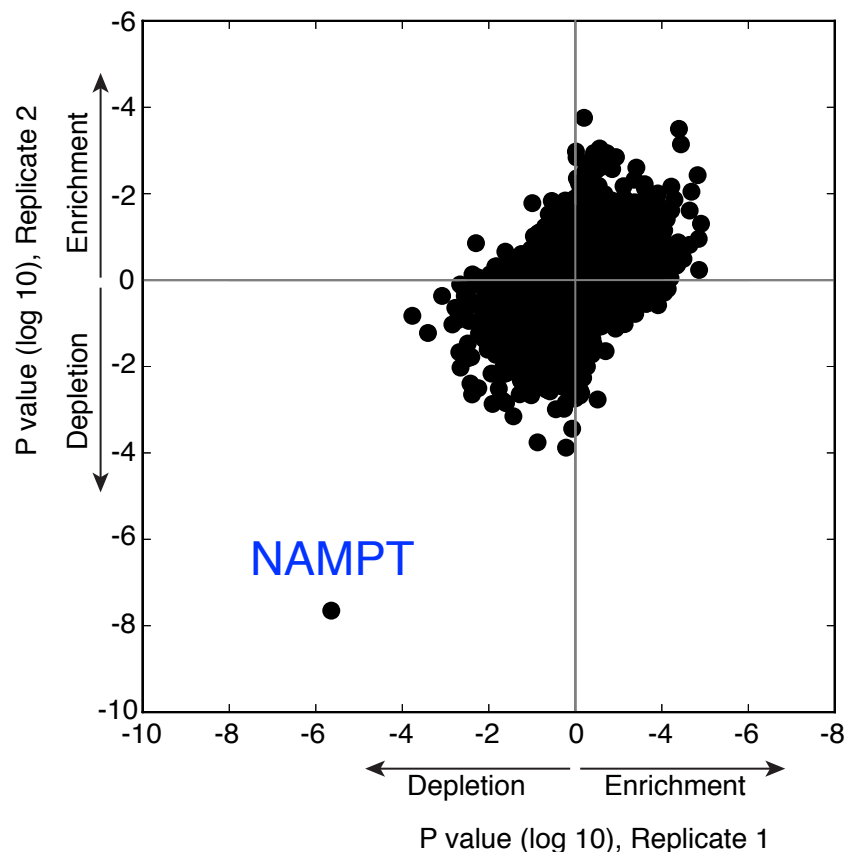
Screen >100k compounds
for selective killing of high-risk
Acute Lymphoblastic Leukemia cells



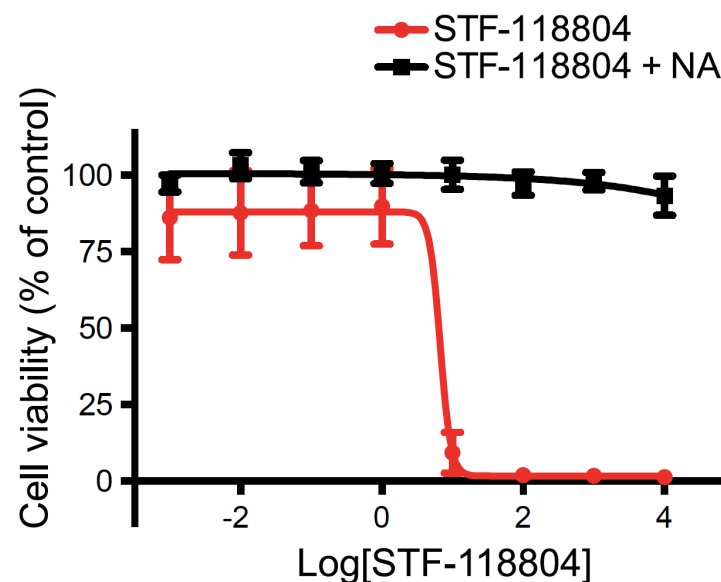
STF-118804



shRNA screen in MV411 cells



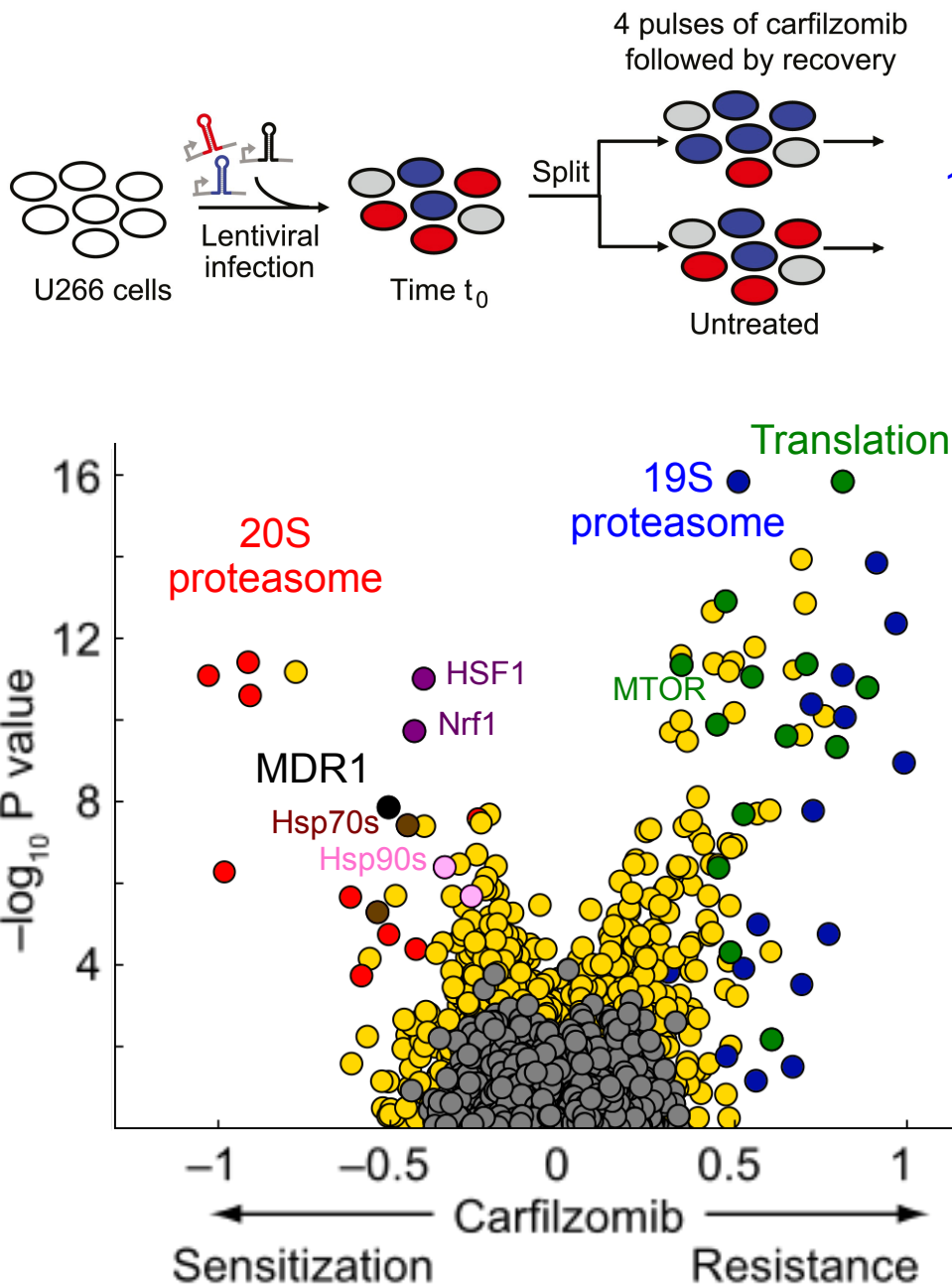
Rescue with nicotinic acid



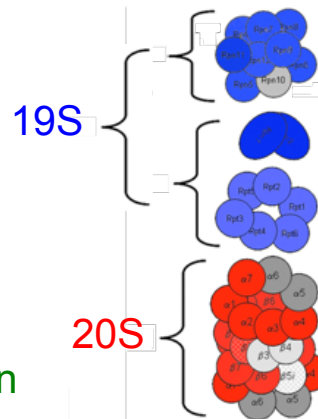
Matheny et al. *Chemistry & Biology* 2013



What determines proteasome addiction in multiple myeloma?

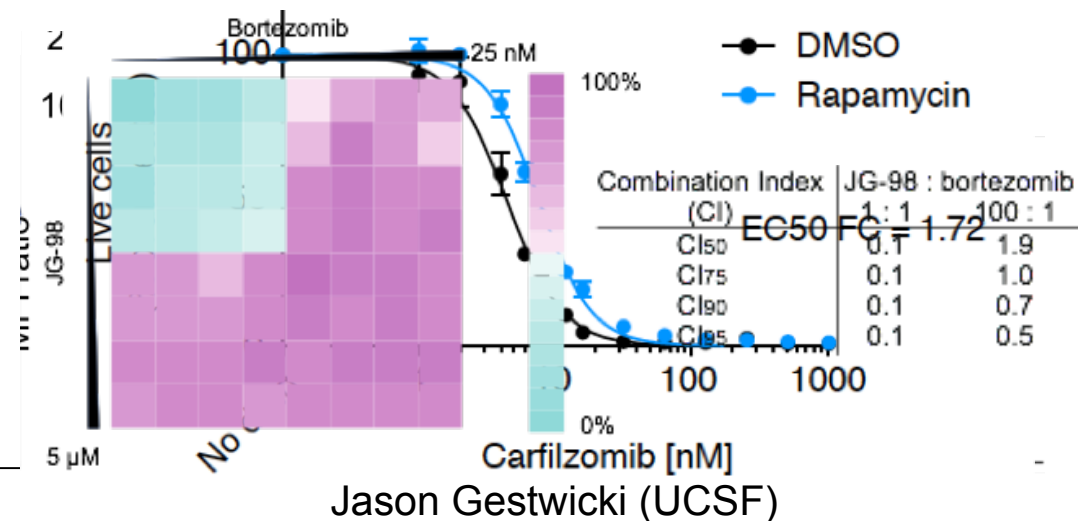


26S proteasome



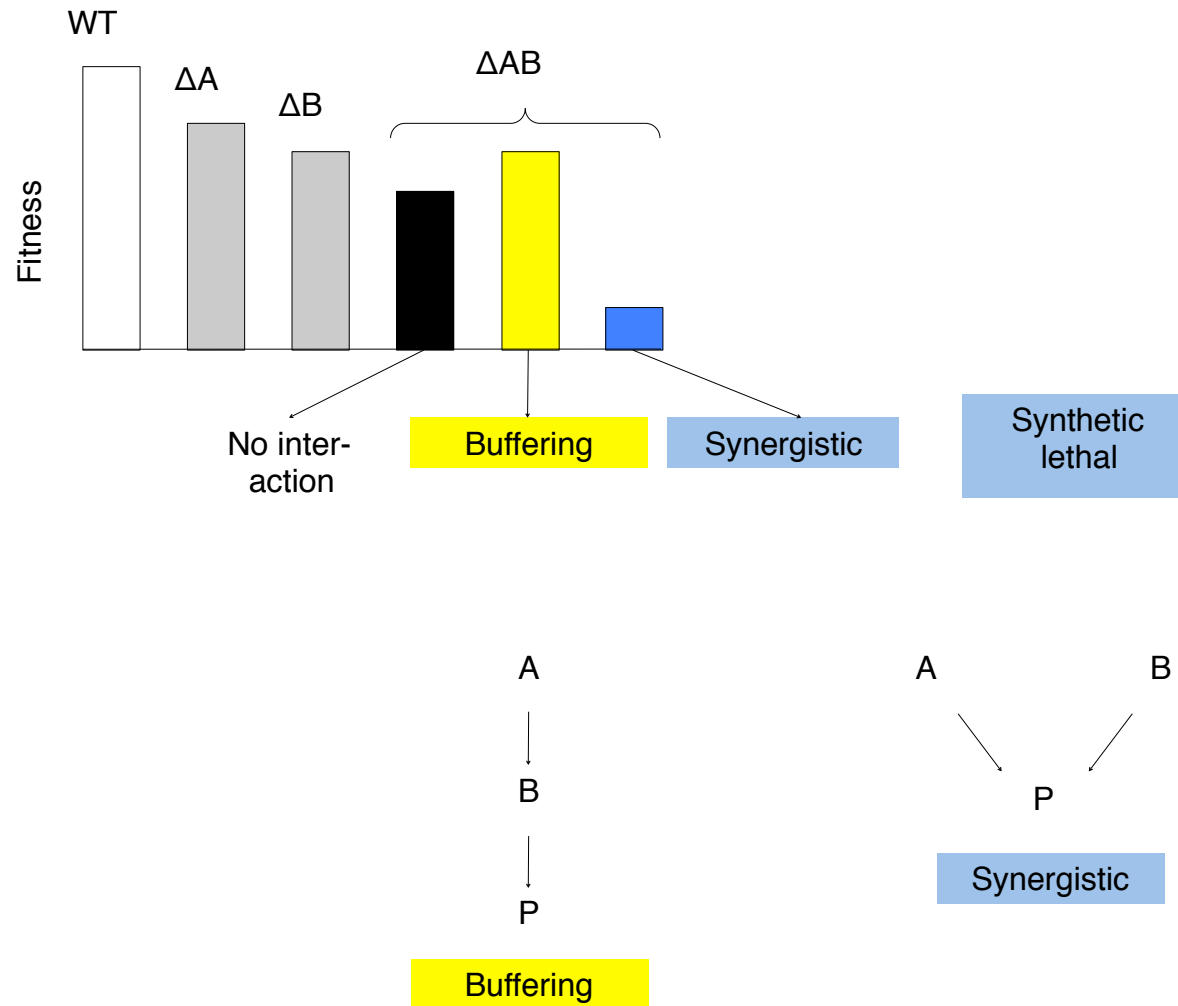
- Therapeutic target in myeloma (Bortezomib, carfilzomib)
- Therapeutic window?
- Biomarkers?
- Resistance mechanisms?
- Synergistic combination therapy?

Heatmap of proteasome inhibition by bortezomib and rapamycin





Genetic interactions reflect functional relationships



Synthetic lethality

Gene 1	Gene 2	
functional	functional	→ viable
compromised	functional	→ viable
functional	compromised	→ viable
compromised	compromised	→ 

BRCA loss + PARP inhibitor synthetic lethality
Farmer et al. *Nature* 2005
Bryant et al. *Nature* 2005



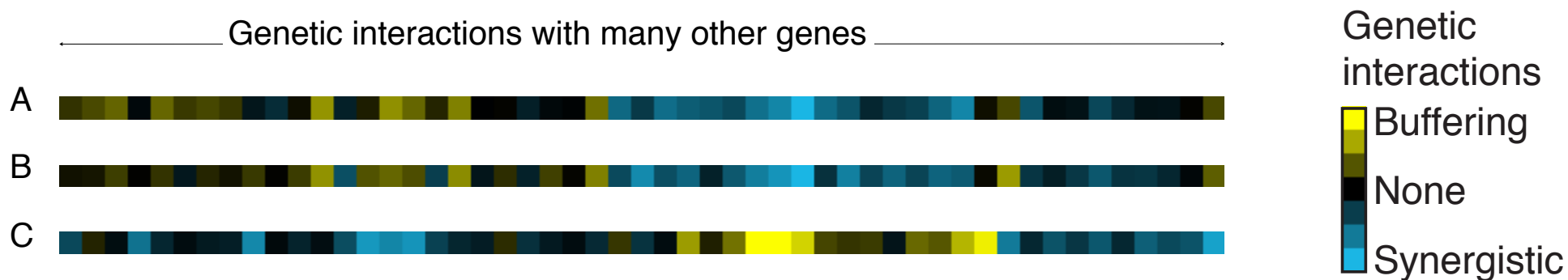
Insights from systematic genetic interaction data

Measure all pairwise genetic interactions for $N \times N$ genes

↓
Genetic interaction map (EMAP)
Yeast: Weissman, Krogan, Boone

1. Unbiased detection of rare gene pairs with strong interactions

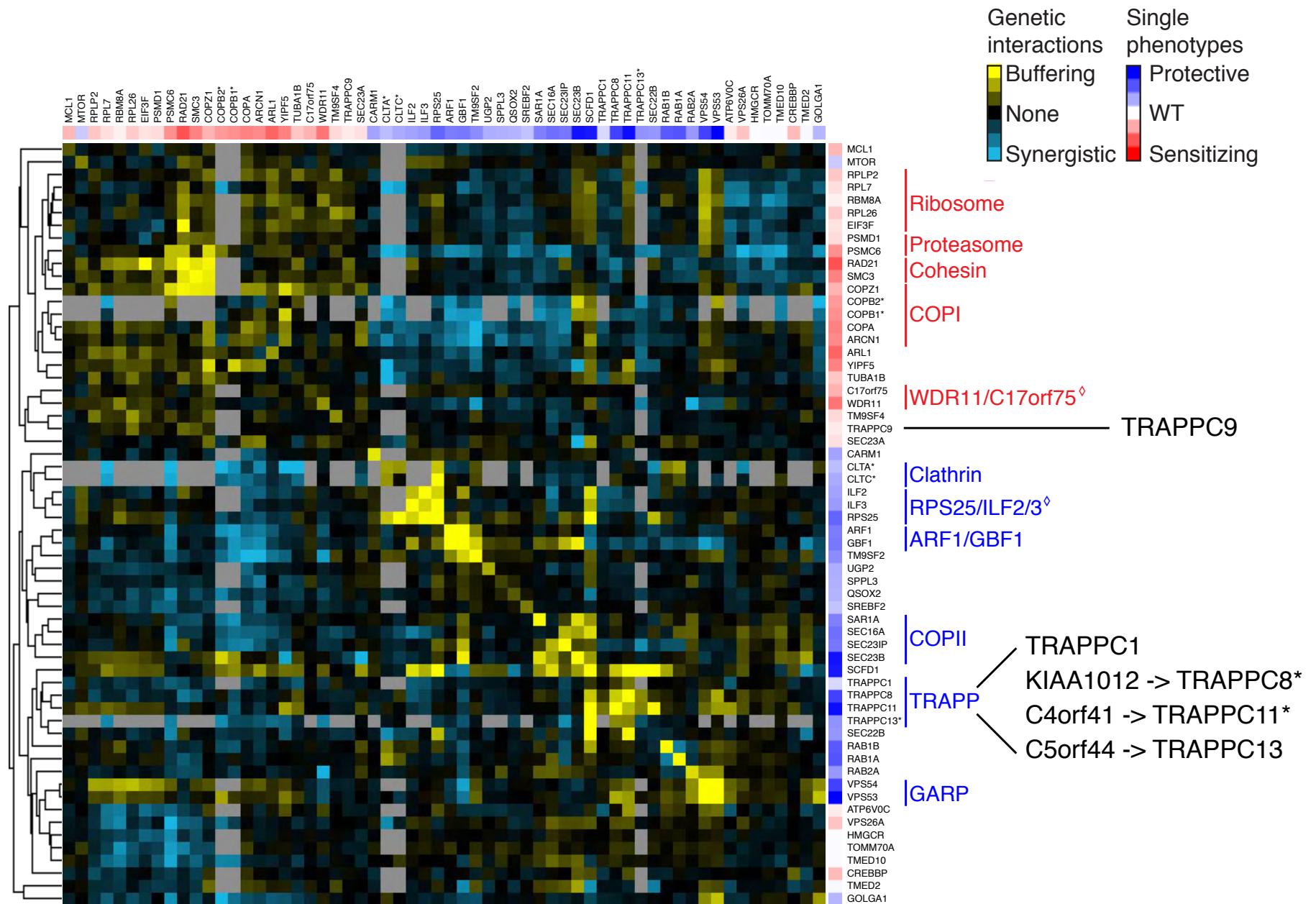
2. Dissection of gene functions and pathways



Correlation of Genetic interaction pattern -> Functional relatedness



Genetic interaction map

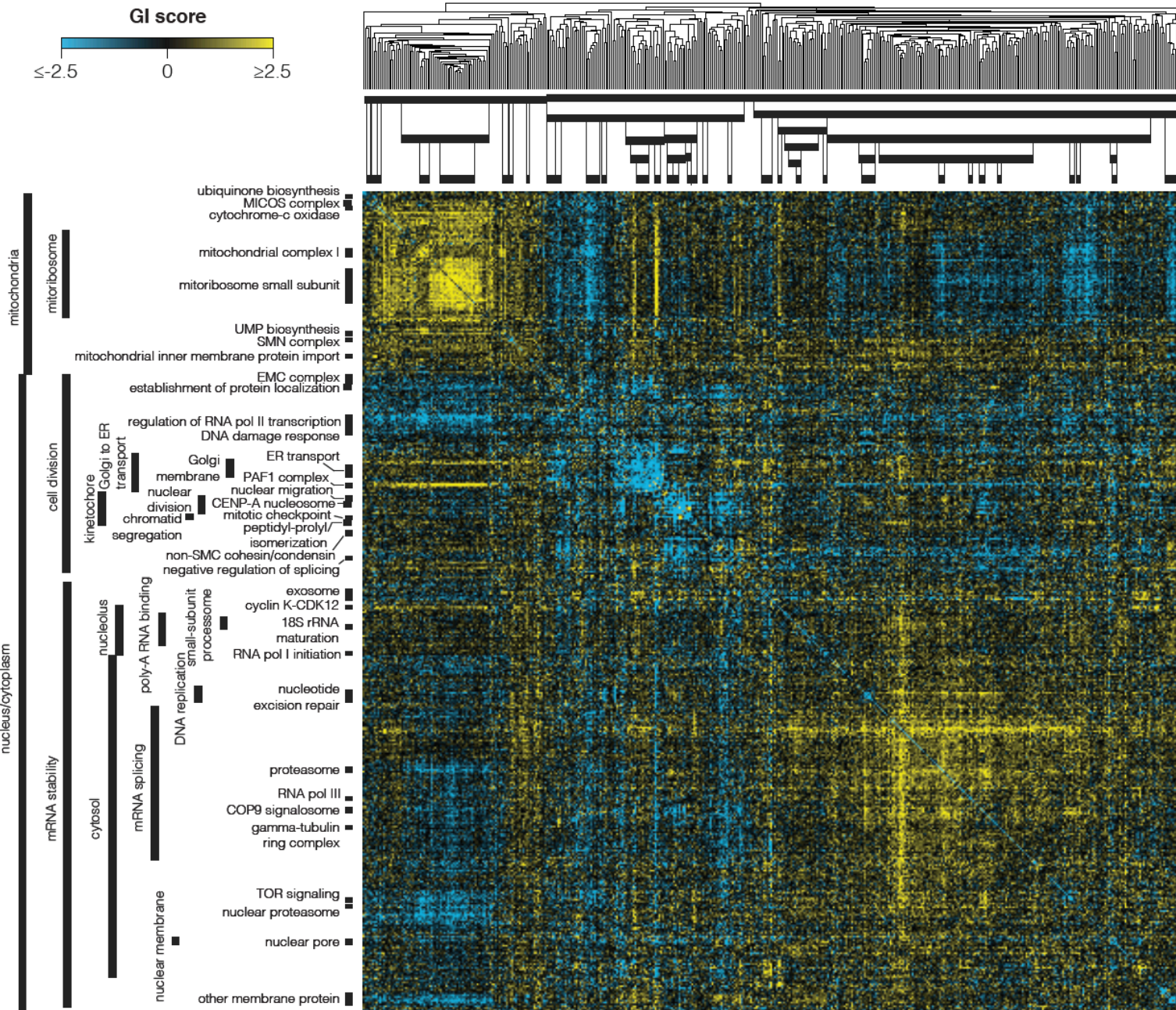


Bassik*, Kampmann* et al *Cell* 2013

Kampmann et al *PNAS* 2013



Genetic interaction map

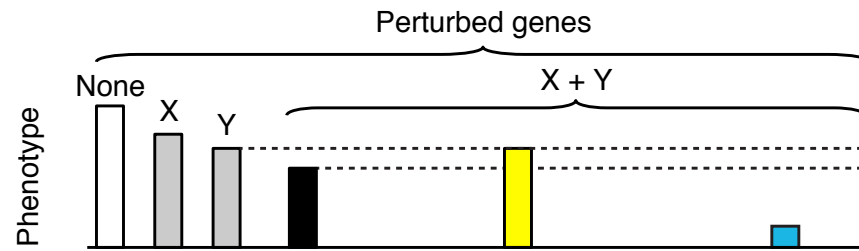


CRISPRi-based GI map

- 472 genes x 472 genes
- 222,784 gene pairs
- > 1 million sgRNA pairs



Directional pathways from genetic interaction maps



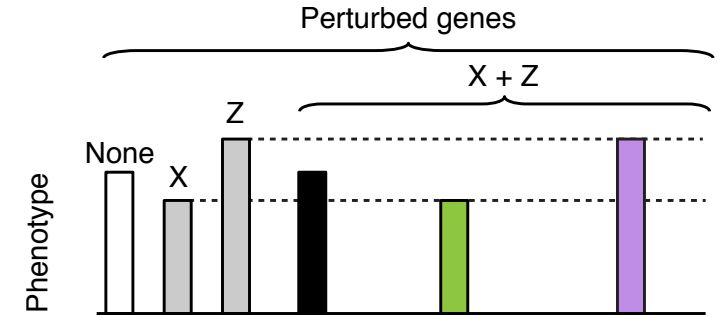
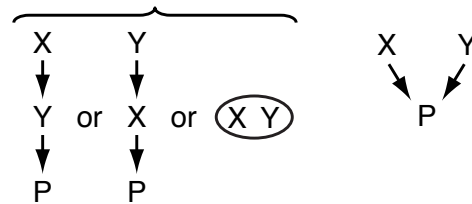
Genetic interaction:

None

Buffering

Synergistic

Possible pathway relationship:



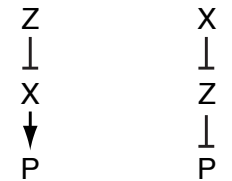
Genetic interaction:

None

X masks Z

Z masks X

Possible pathway relationship:



How to obtain opposing phenotypes for genes in a pathways?



CRISPR-based gene perturbation

CRISPRn

Cas9



Gene editing / inactivation

CRISPRi

dCas9-KRAB

KRAB



TSS

Gene repressed

CRISPRa

dCas9-SunTag

VP64

sfGFP

scFv-GCN4

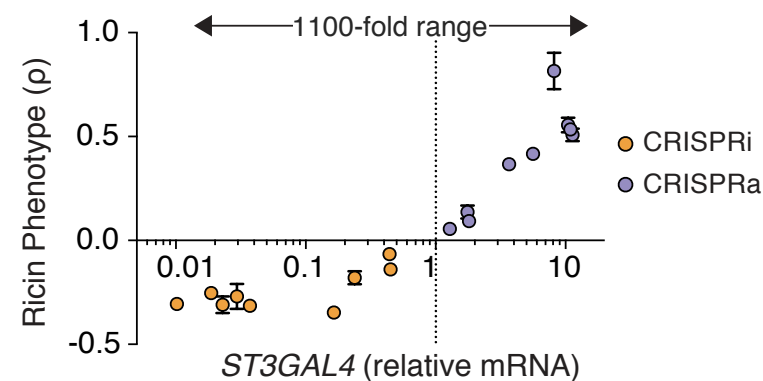


TSS

Gene activated

CRISPRi/a screening platform

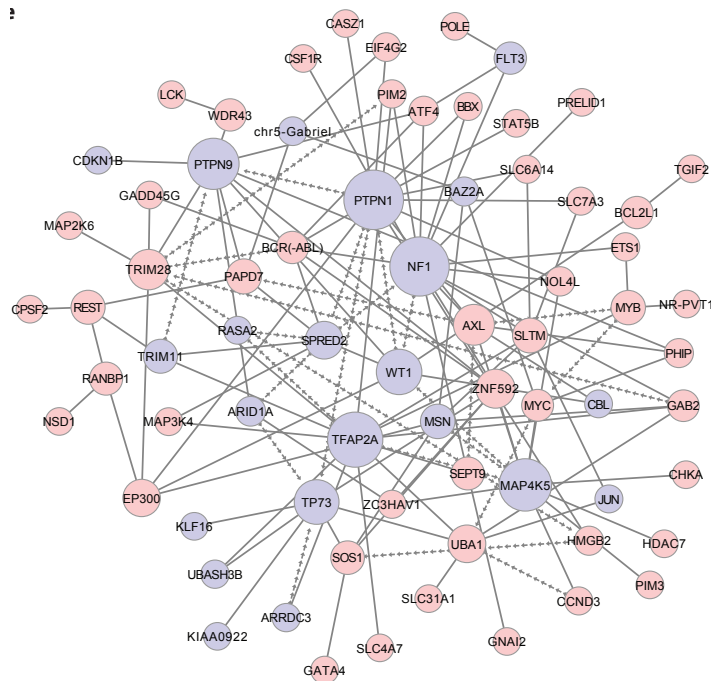
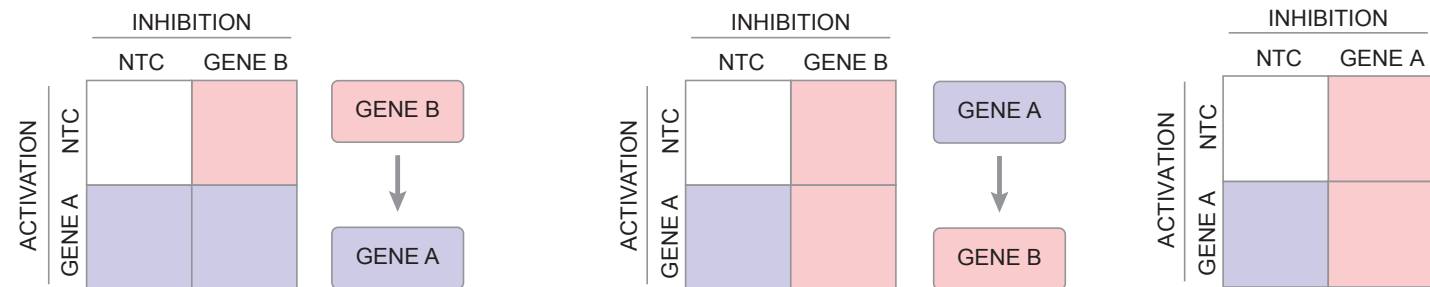
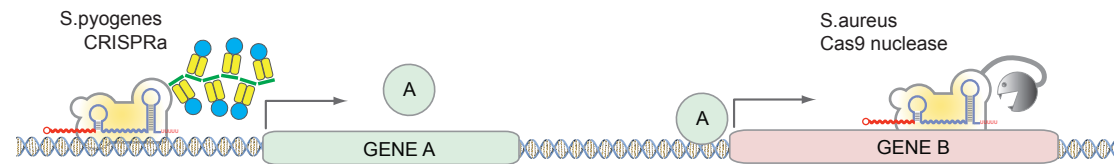
Gilbert ... Kampmann* & Weissman*
Cell (2014)



- Highly specific
- Non-toxic
- Inducible, reversible



Directional genetic interaction map

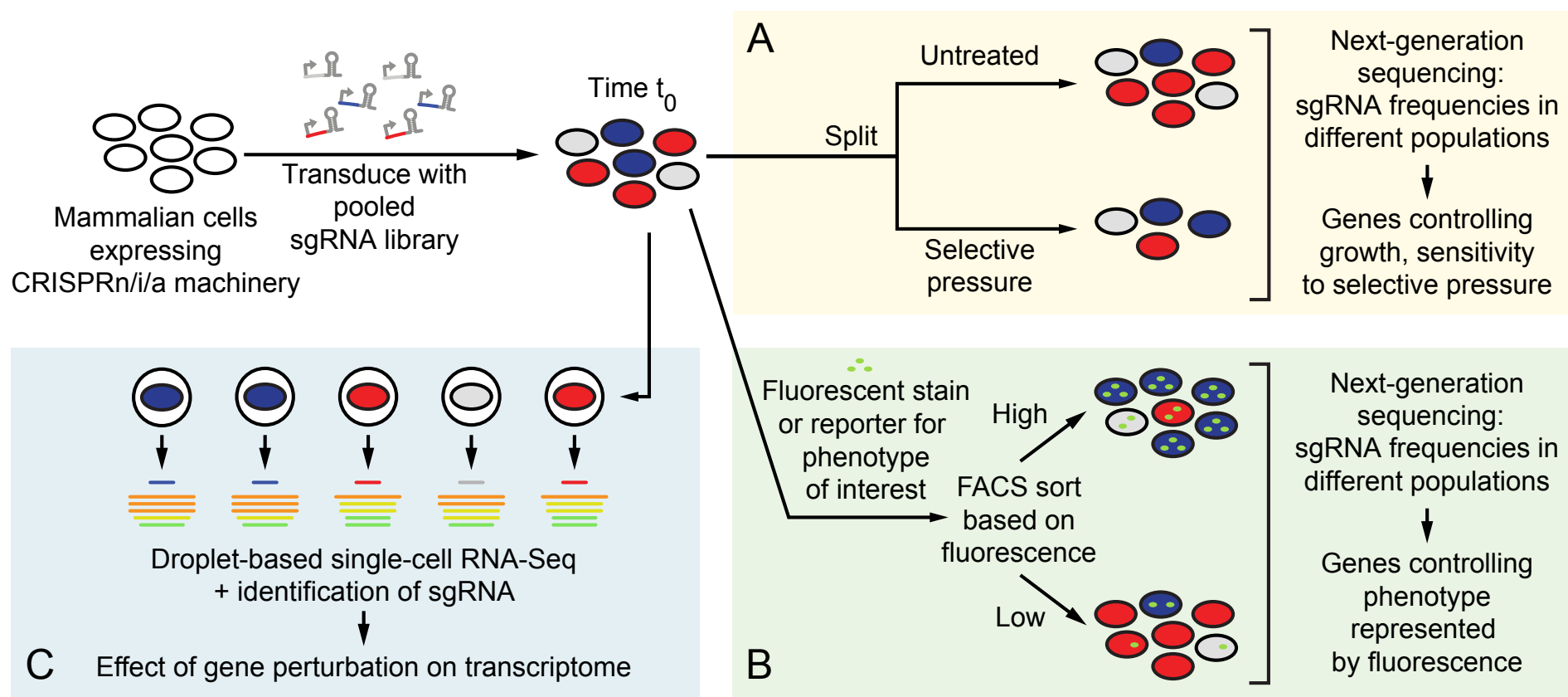


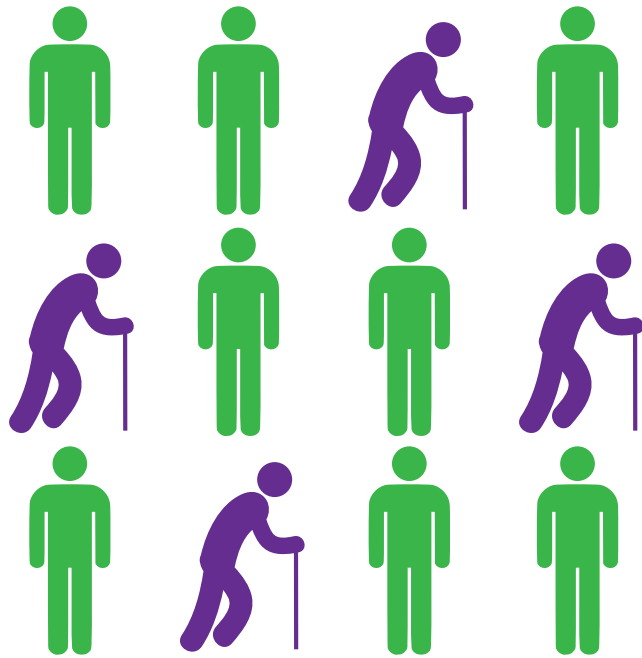
Ruilin Tian

Boettcher, ... Kampmann & McManus
(2018) *Nature Biotech*

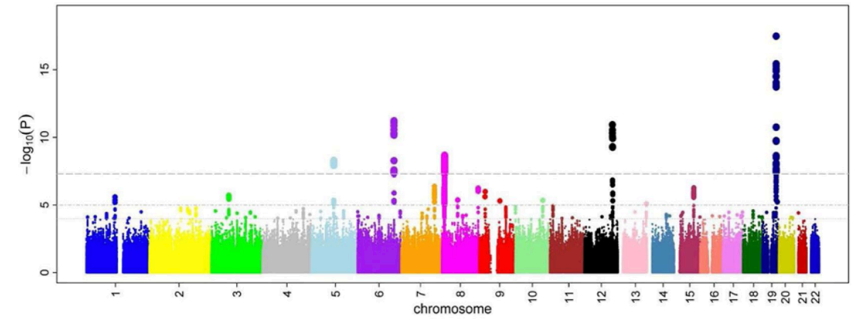


Massively parallel phenotypes in mammalian cells





Human
genetics



Disease-associated genes

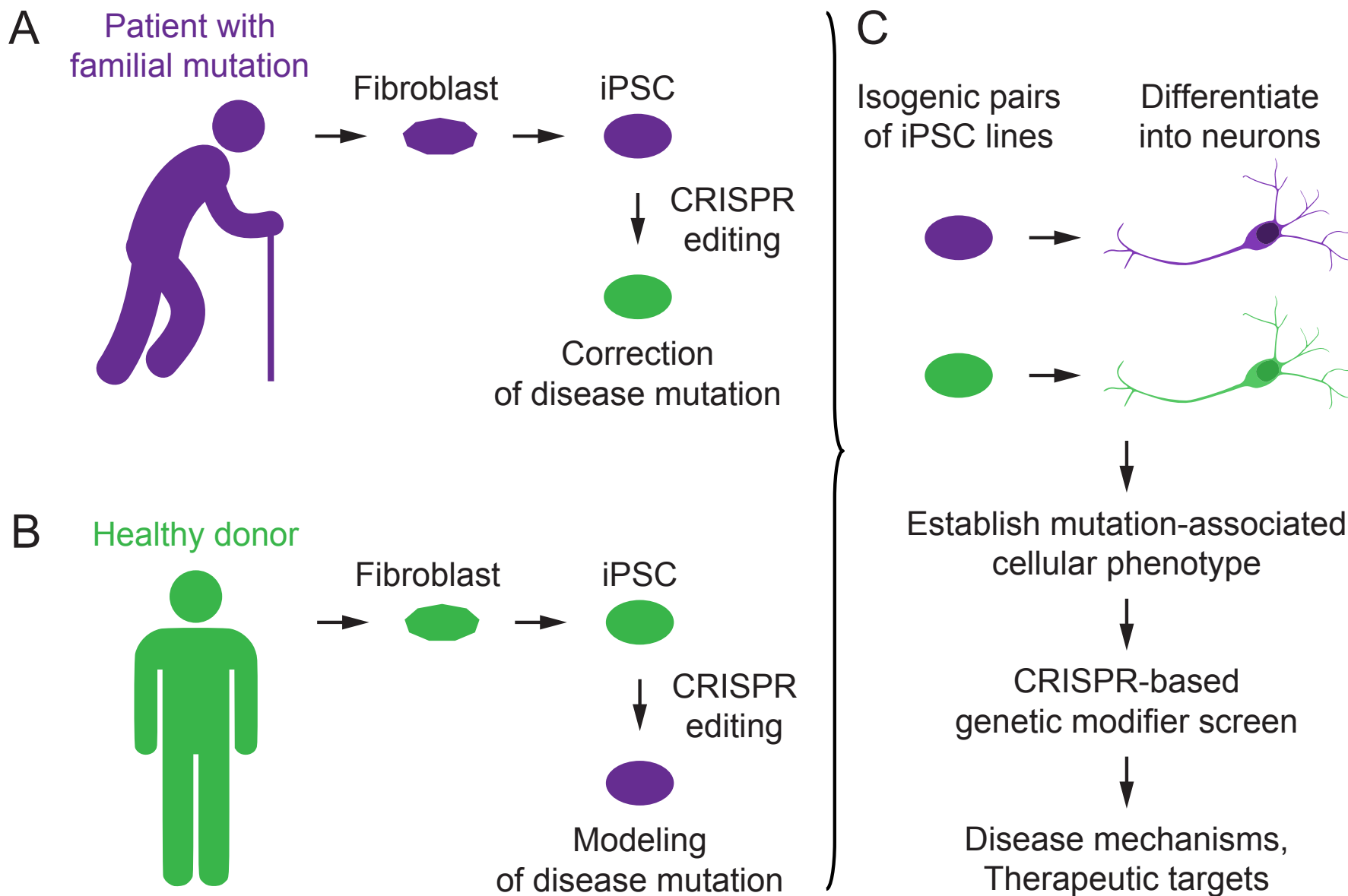
Functional
Genomics

Mechanism?
When?
Where?

Therapeutic
strategy?



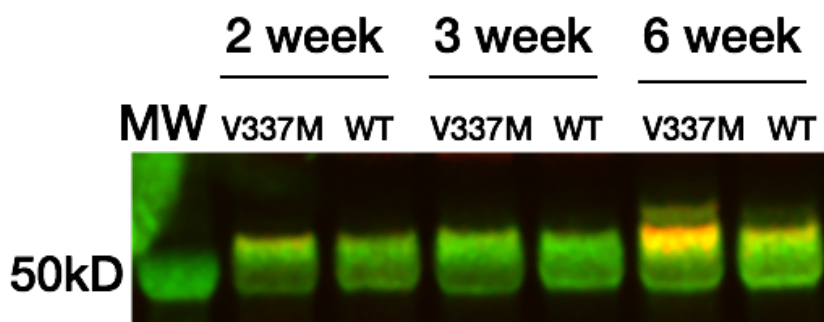
CRISPRi/a in iPSC-derived disease models





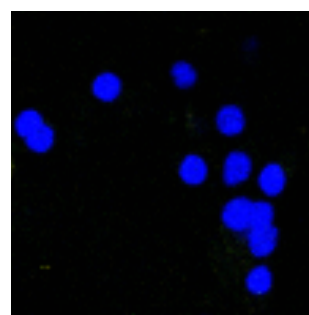
Modifiers of neuronal tau aggregation

- Tau pathology is a hallmark of Alzheimer's and other neurodegenerative diseases
- Mutations in tau (*MAPT*) cause familial diseases such as frontotemporal dementia

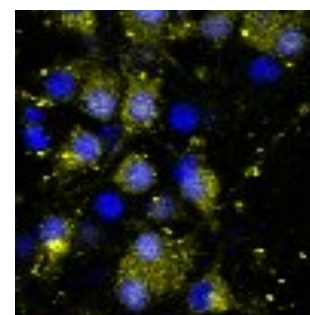


Total tau

Phospho-tau (AT8)



WT



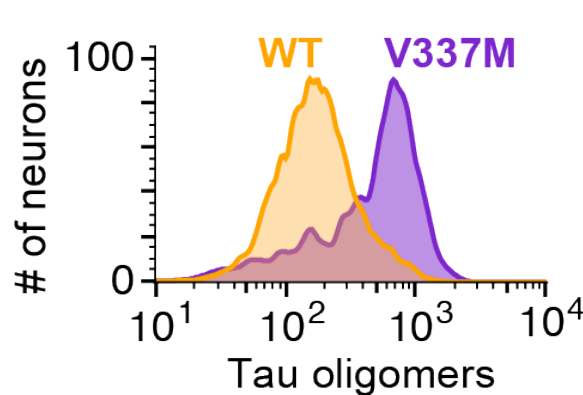
V337M

■ Hoechst

■ T22 (tau oligomers)



Avi Samelson



Pilot CRISPRi screen
(sgRNA library targeting chaperones, co-chaperones)

