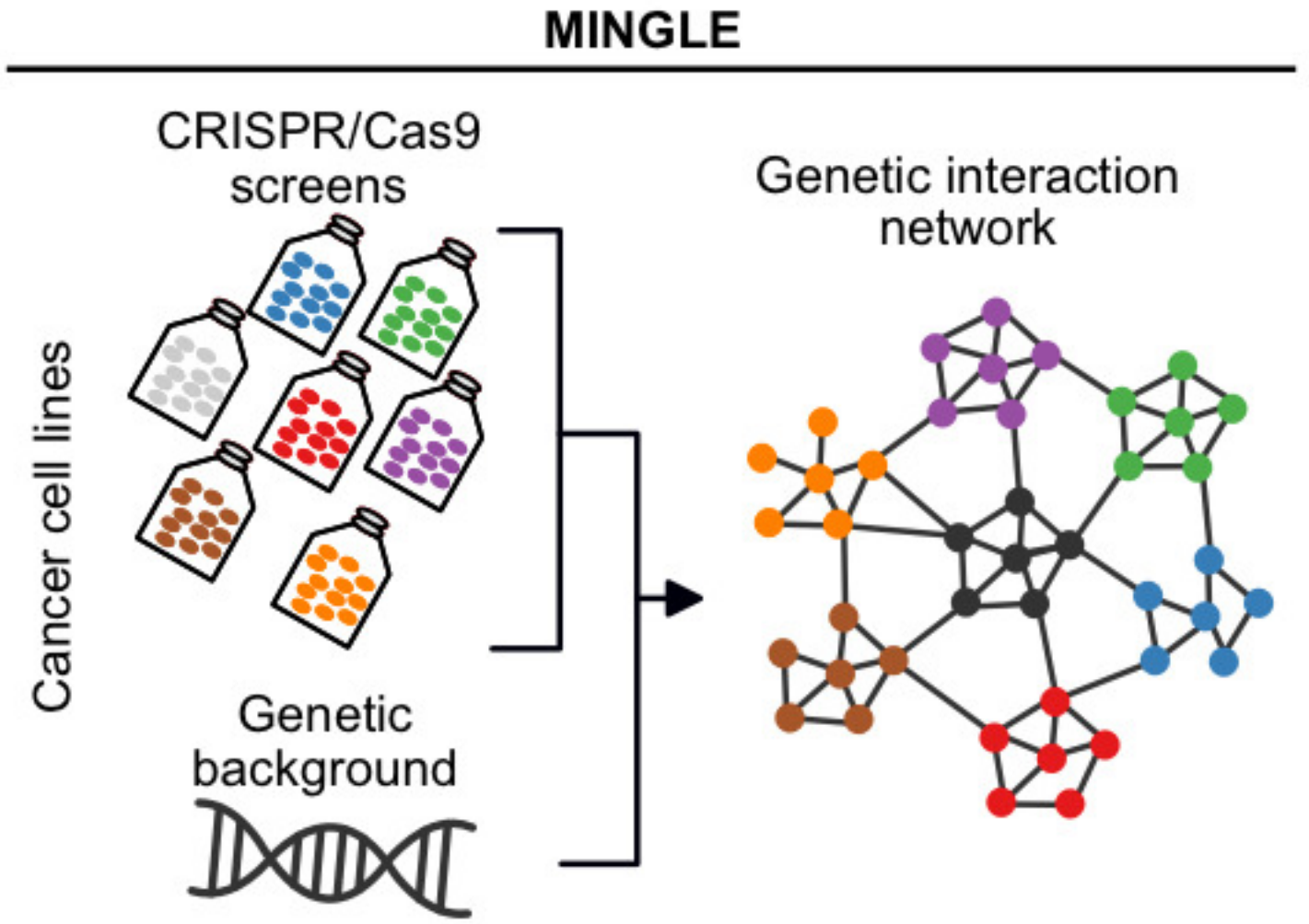
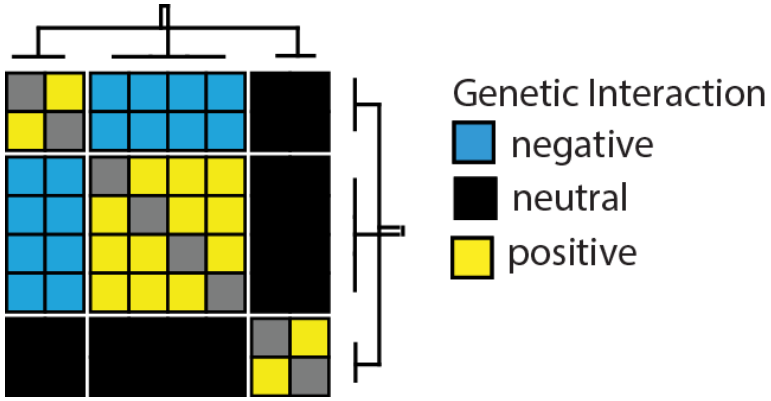


Asvin Lakkaraju

05.10.2020

Genetic Interaction maps

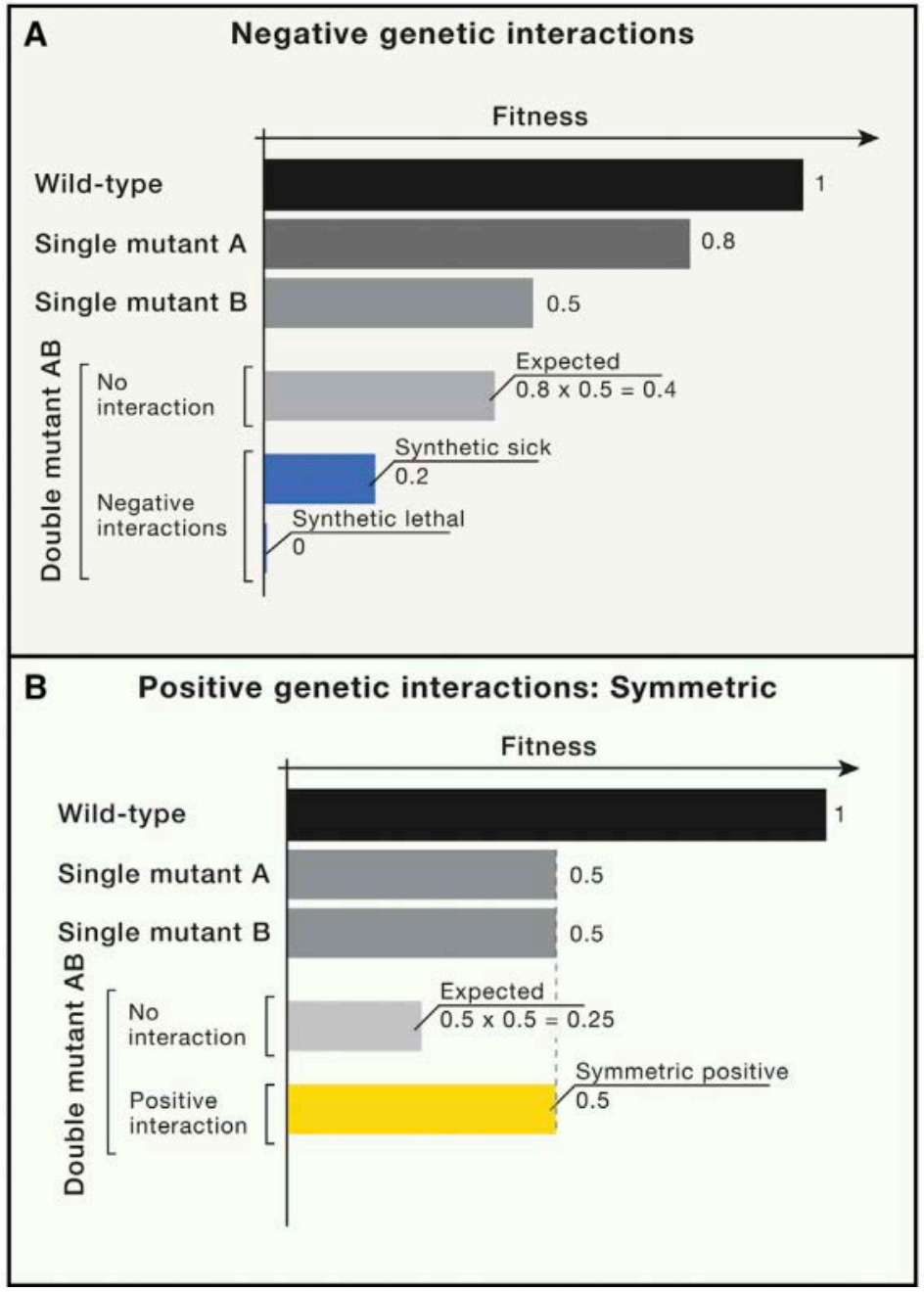


Genetic interaction (GI) mapping, pioneered in the early 2000s, is a powerful technique to systematically reveal functional relationships between genes, which often also reveal the presence of a physical interaction.

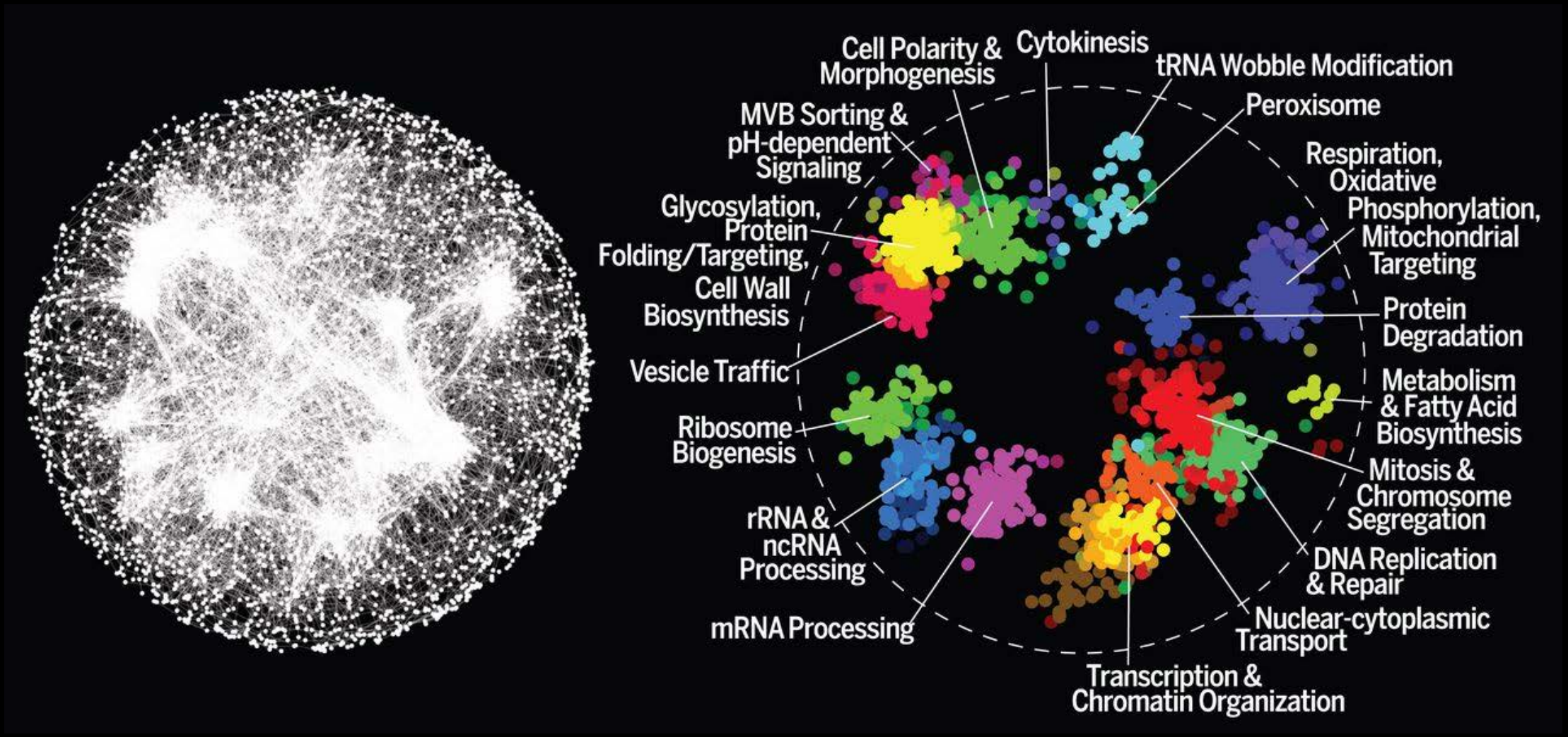
GI mapping involves the pairwise perturbation of genes (e.g. knockout, knockdown or overexpression) in order to elucidate how one gene modulates the phenotype of the other.

Typically, cell viability is used as the phenotypic readout, where GIs that increase cellular fitness are said to be “positive” and GIs that decrease cellular fitness are said to be “negative”.

Genetic Interaction maps: At a glance

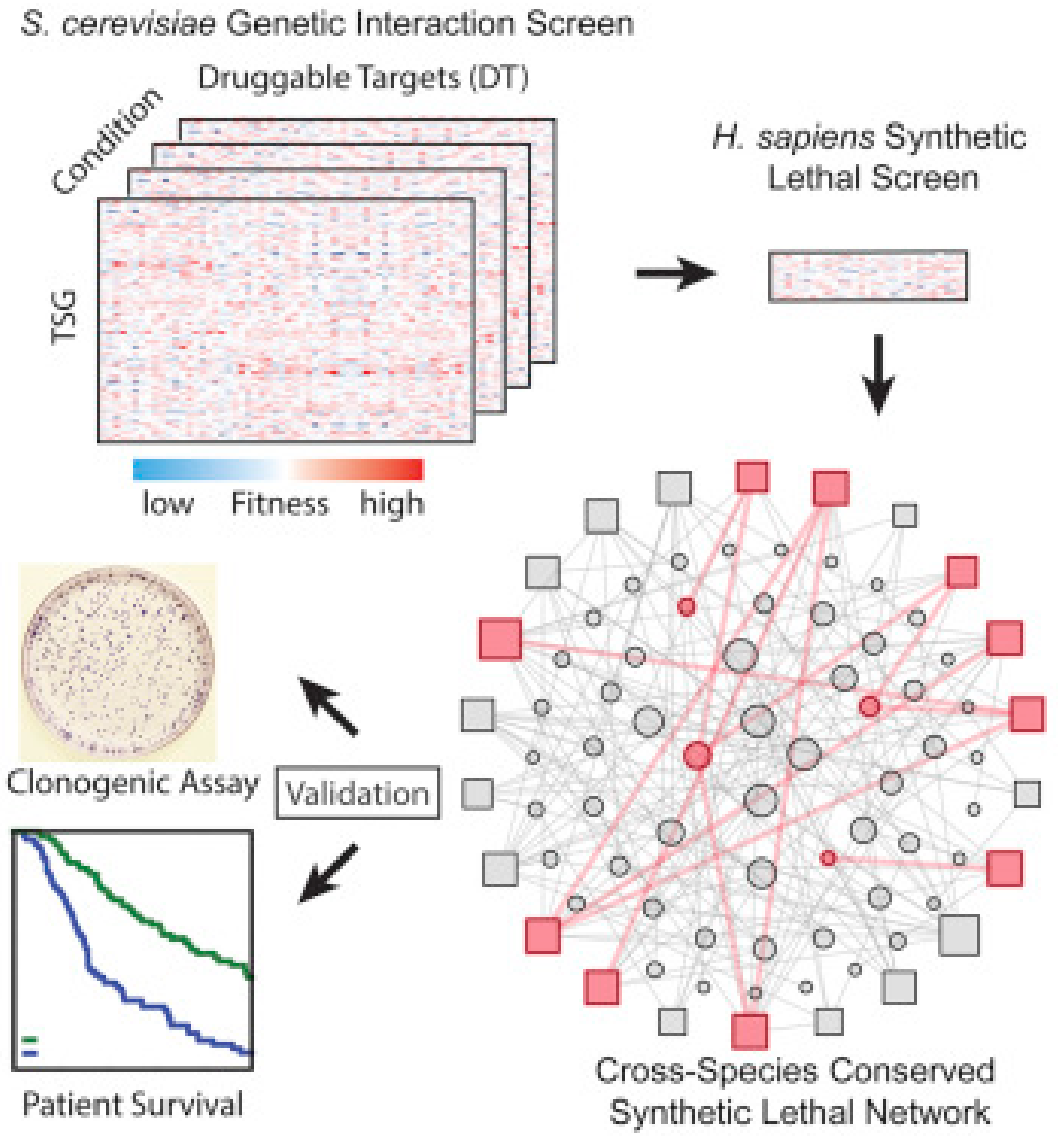


Genetic Interaction maps in Yeast

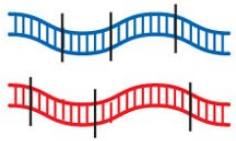
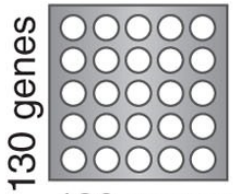
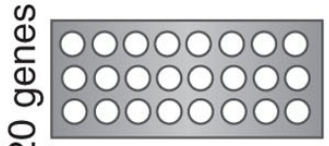
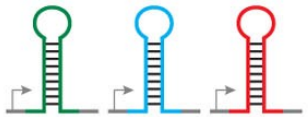
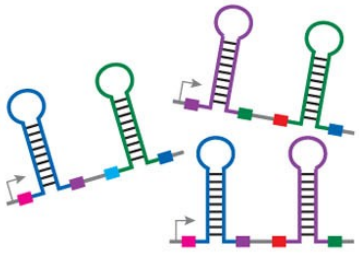


23 million double mutants, identifying about 550,000 negative and about 350,000 positive genetic interactions.

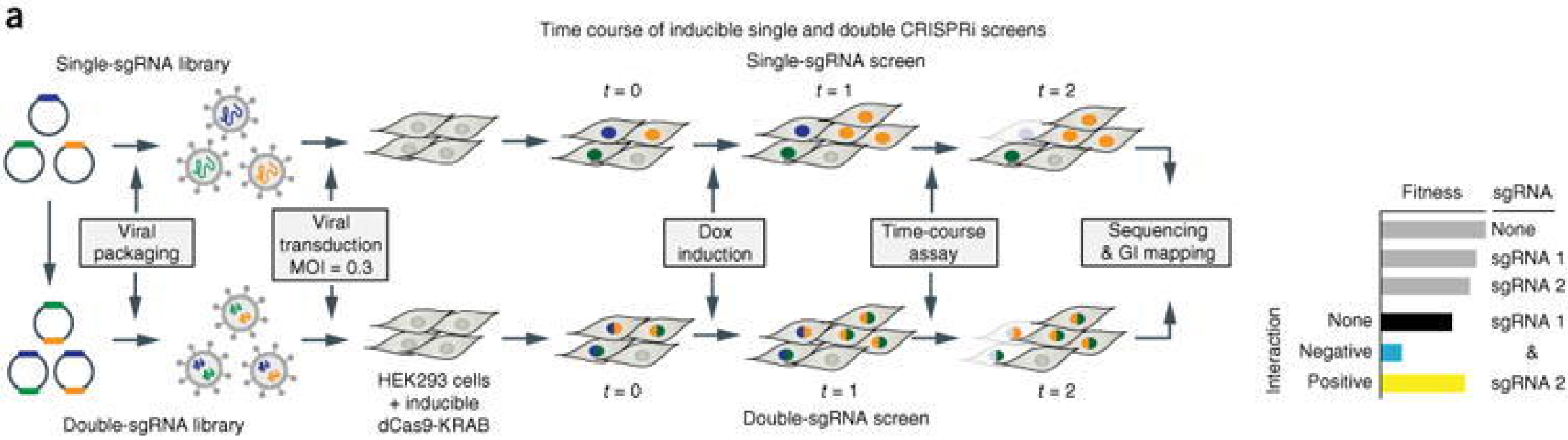
Genetic Interaction maps in mammlian cells



Genetic interaction maps in Mammalian cells: Attempts

	Select genes	Generate library	Perform experiment	Assay	Pros	Cons
Roguev *et al.*[3]	130 genes (chromatin modifiers)	 esiRNA	 130 genes × 130 genes	Cell count	Reagent design	Reproducibility
Laufer *et al.*[2]	323 genes (chromatin modifiers)	 siRNA	 20 genes × 323 genes	High-content microscopy	Reproducibility; deep phenotyping may improve GI calls	Labor-intensive reagent design
Bassik *et al.*[1]	 60 genes (hits from primary screen)	 Dual shRNA	 Pooled library	Sequence depth	Reproducibility; pooled libraries and sequencing most scalable	Labor-intensive reagent design; paired hairpins limit scale

Mammalian Cells: CRISPR based screens



Mammalian cells: Complexities

1. High off target effects and low KD efficiency with RNAi and shRNA

2. Variability among the cell types chosen.

3. Problems with CRISPR methodologies so far: multiple plasmid transfection or cloning steps, large constructs and complex combinations of promoters, which limit its usage in genome-wide genetic screening due to high risk of losing library components during library construction.

4. The application of barcode to label multiple gRNAs has been found result in half of the mismatch of gRNA-barcodes due to lentiviral template switching

3 papers using different CRISPR based screens to map genetic interactions

Paper -1

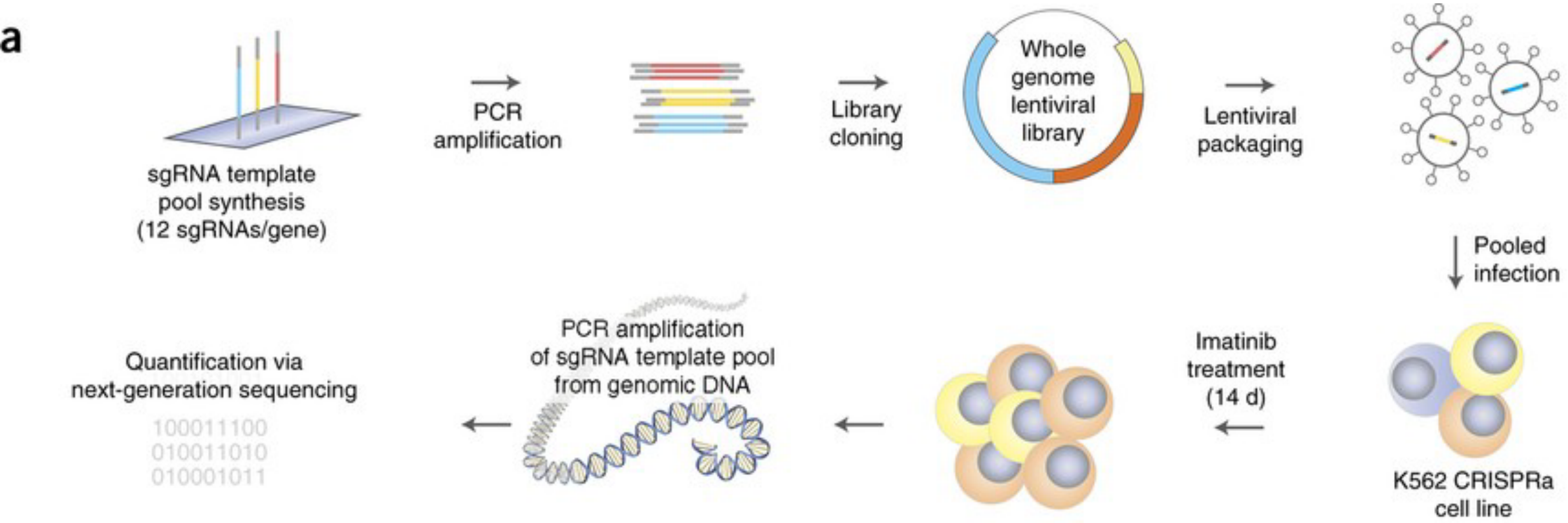
nature › nature biotechnology › articles › article

Published: 15 January 2018

Dual gene activation and knockout screen reveals directional dependencies in genetic networks

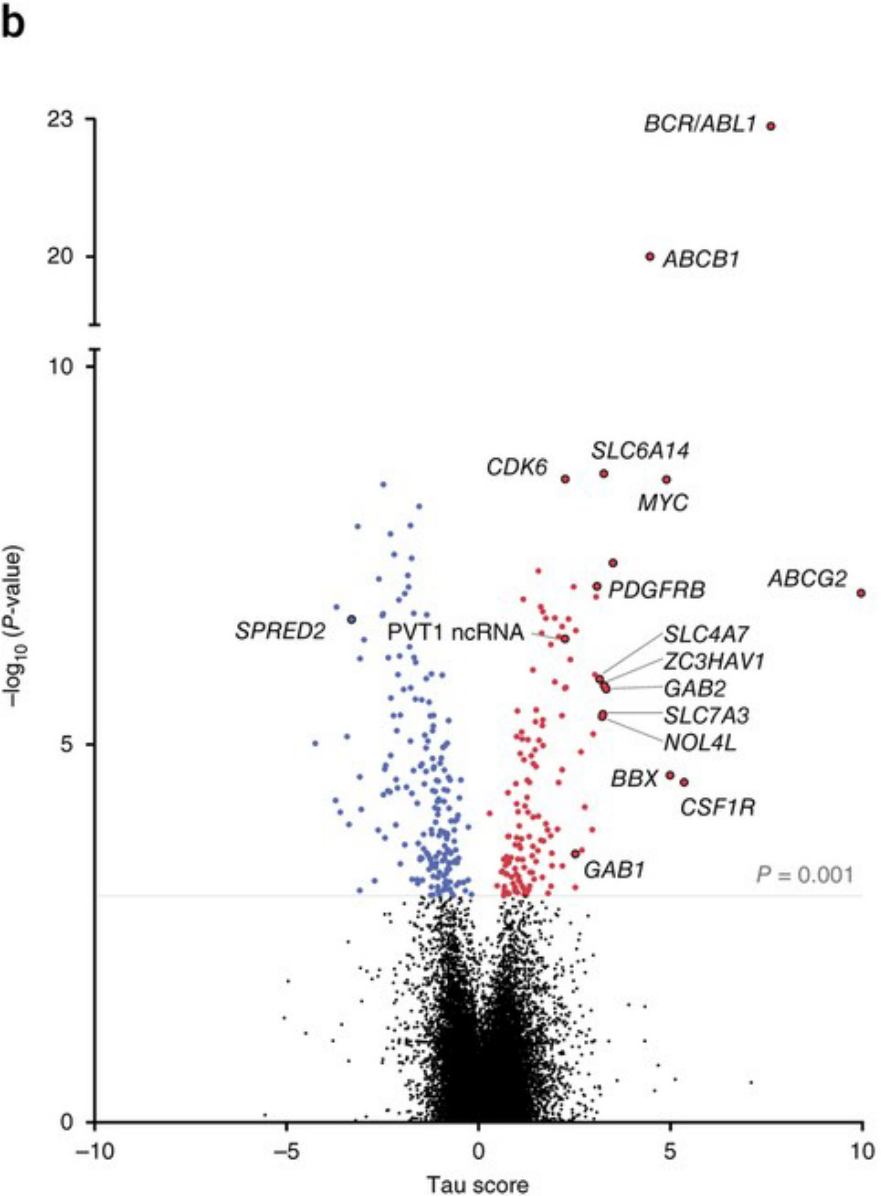
Michael Boettcher, Ruilin Tian, James A Blau, Evan Markegard, Ryan T Wagner, David Wu, Xiulei Mo, Anne Biton, Noah Zaitlen, Haian Fu, Frank McCormick, Martin Kampmann & Michael T McManus

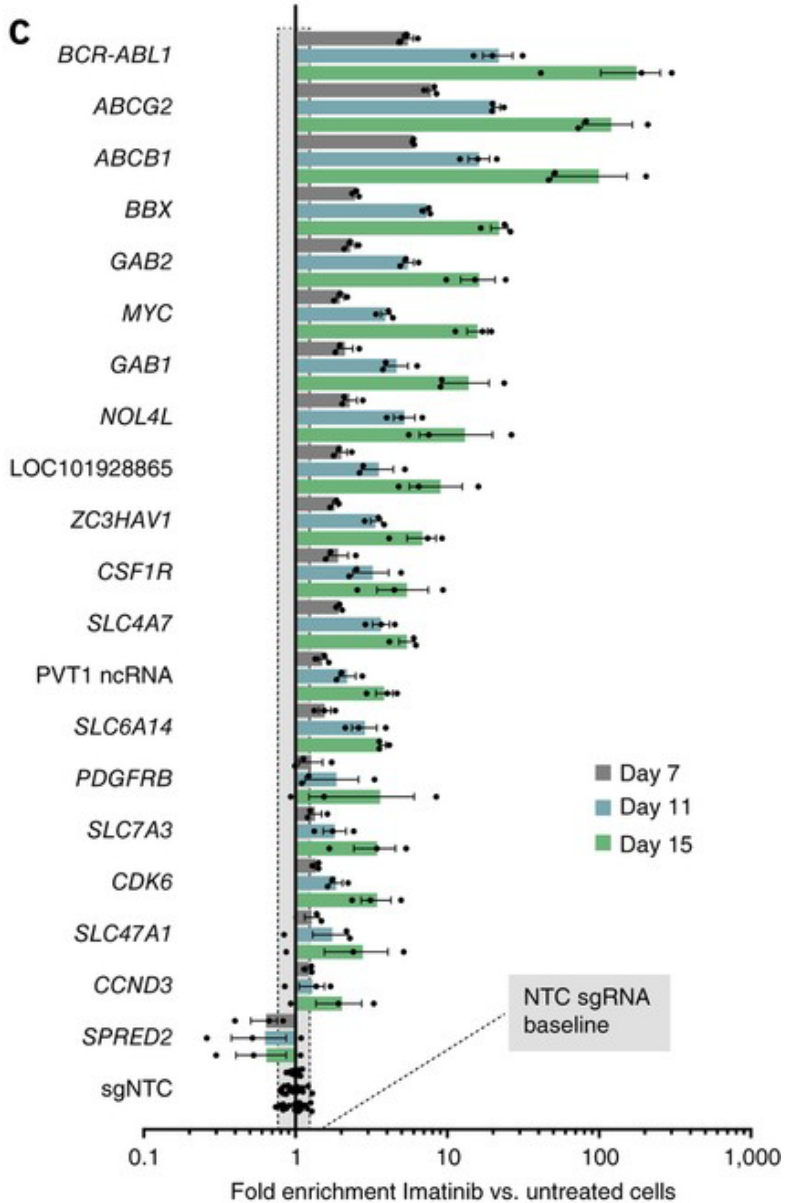
CRISPRa screen: Identify genes involved in cancer signaling pathways



Imatinib is a small molecule kinase inhibitor used to treat certain types of cancer.

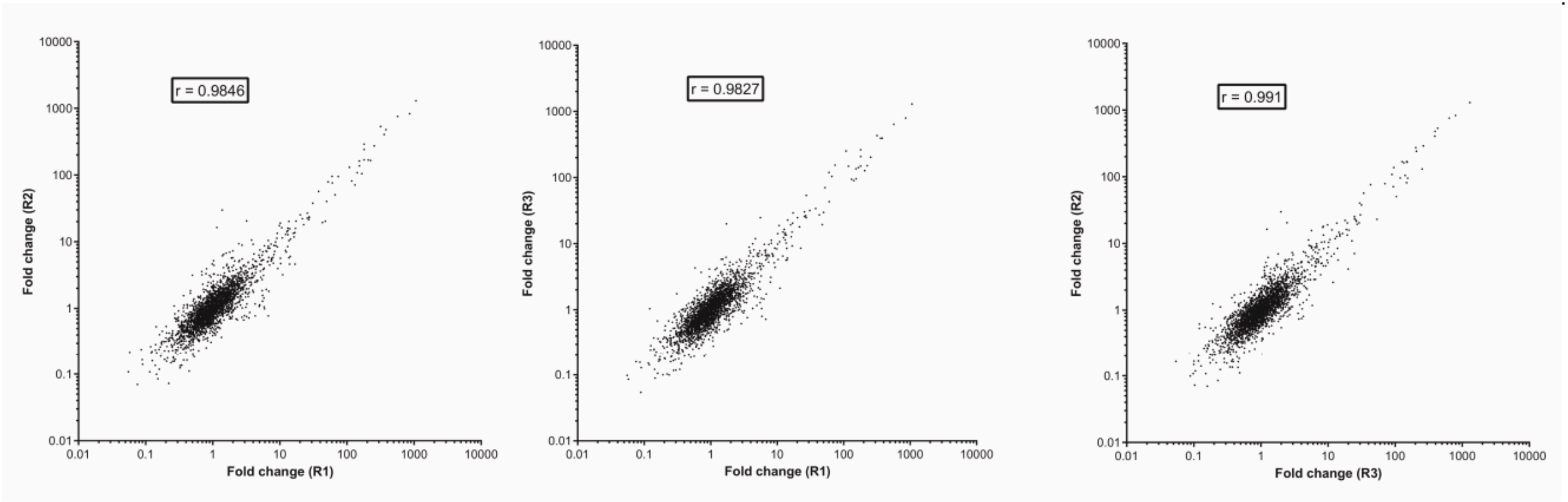
Genes altering sensititvity of cells to Imanitib



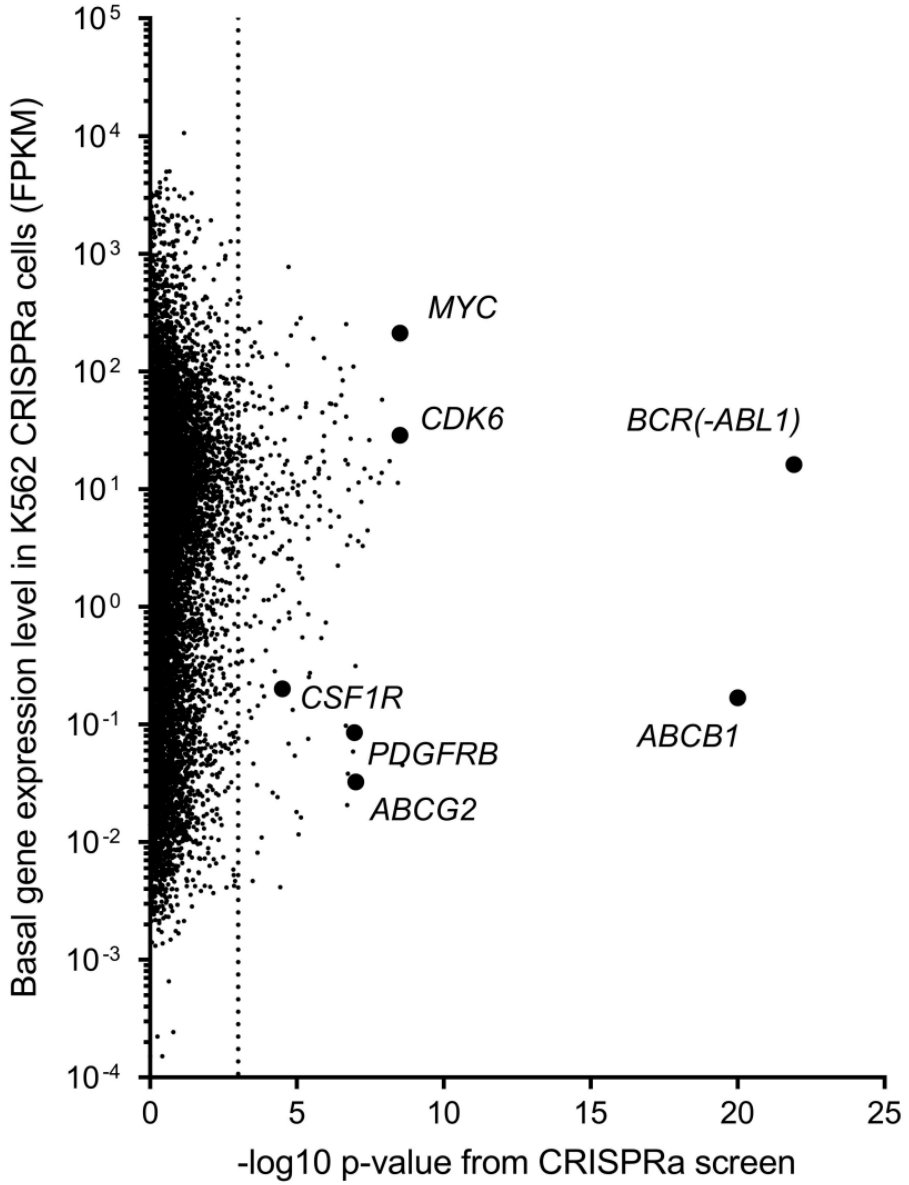


The activation of 332 genes significantly (FDR<0.05, P < 0.001) altered the fitness of imatinib-treated K562 cells, with 57% (188 genes) causing significant depletion (blue) and 43% (144 genes) driving cell enrichment

Biological replicates of the screen



CRISPRa induced phenotypes are independent of endogenous gene expression levels

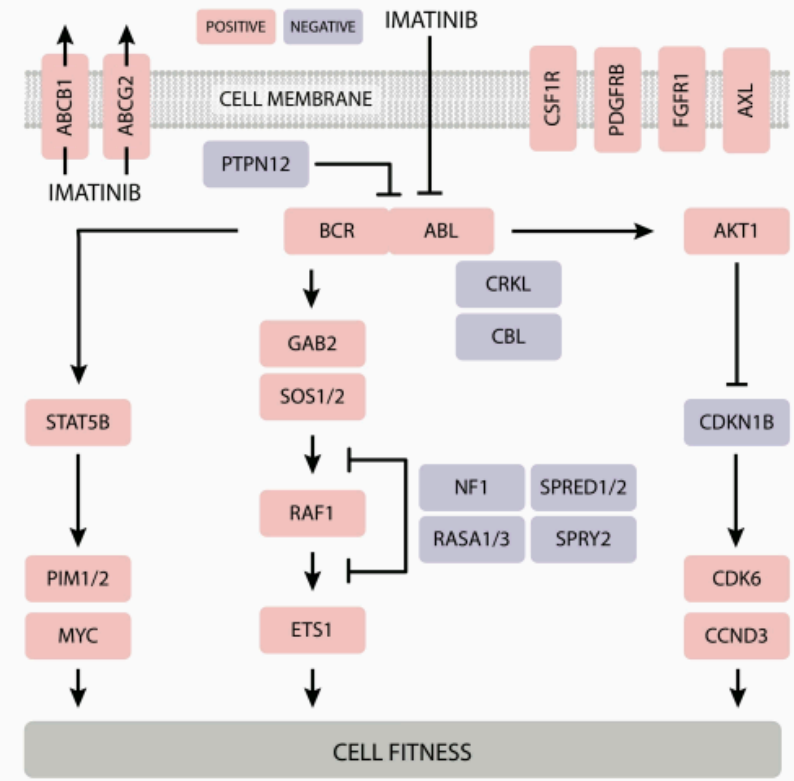


Pathway reconstruction from CRISPRa screen candidate genes

a

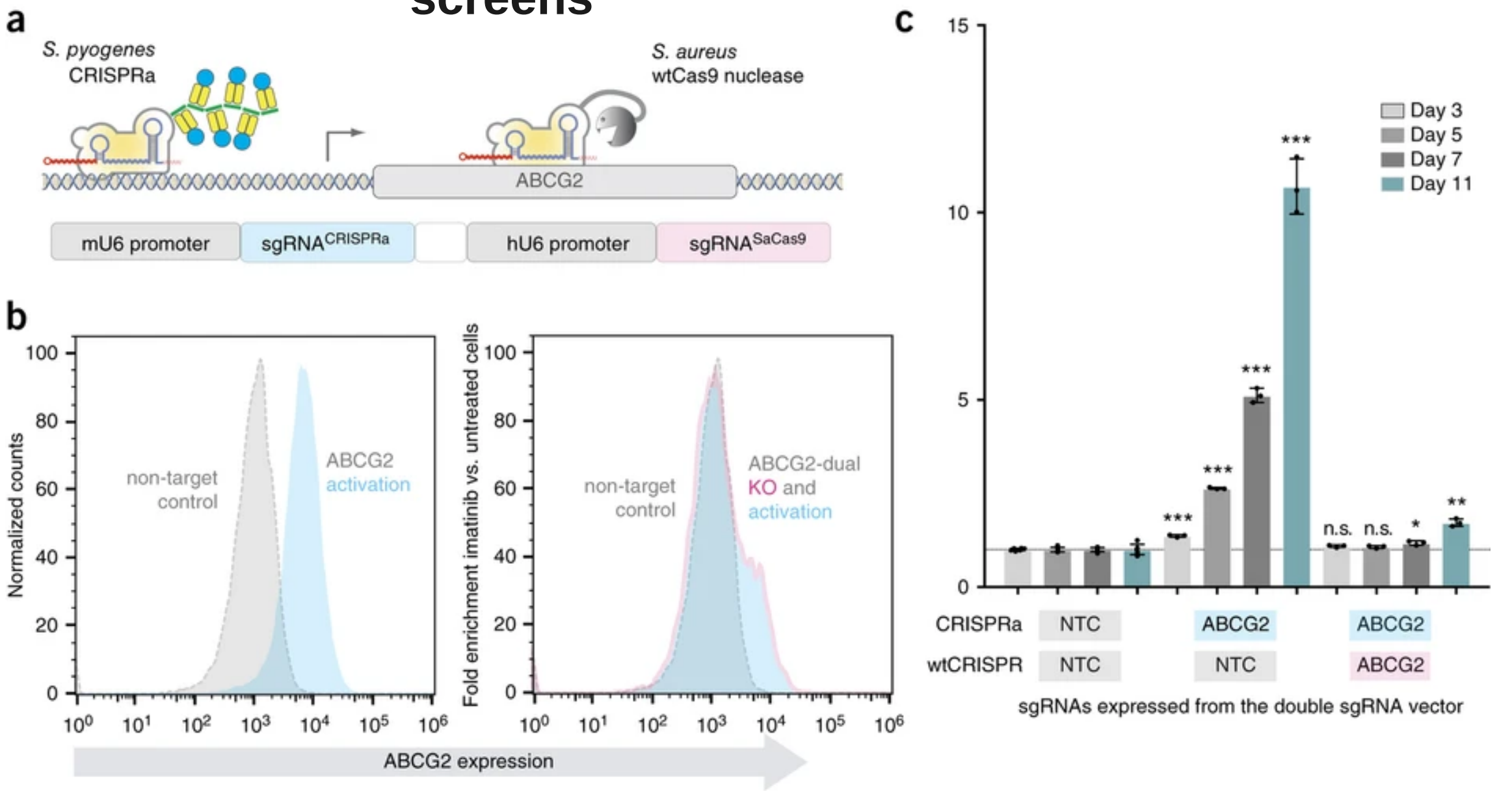
KEGG Pathway	Enrichment	p	FDR
Acute myeloid leukemia	11.54	6.6E-10	8.08E-07
Chronic myeloid leukemia	9.67	1.2E-09	1.45E-06
ErbB signaling pathway	8.00	1.3E-08	1.63E-05
Pathways in cancer	3.42	4.5E-08	5.49E-05
Transcriptional misregulation in cancer	5.03	1.9E-07	2.30E-04
MAPK signaling pathway	3.90	6.0E-07	7.45E-04

b

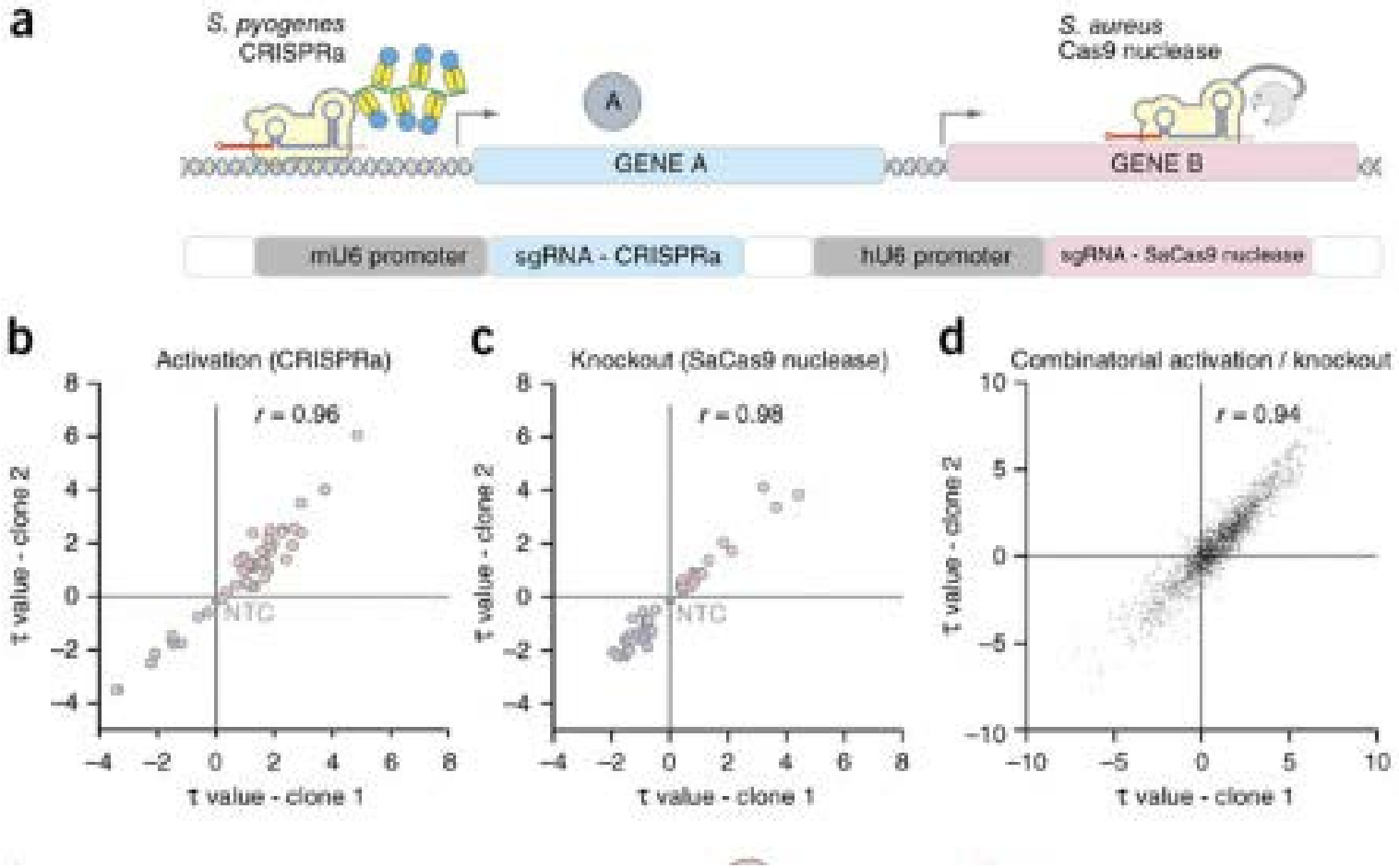


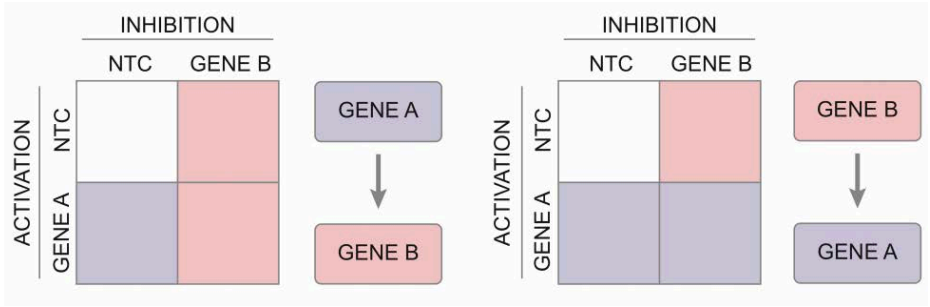
Subset of identified candidate genes mapped onto known signalling pathways (blue = negative, red = positive regulator of fitness)

Orthogonal CRISPR screens

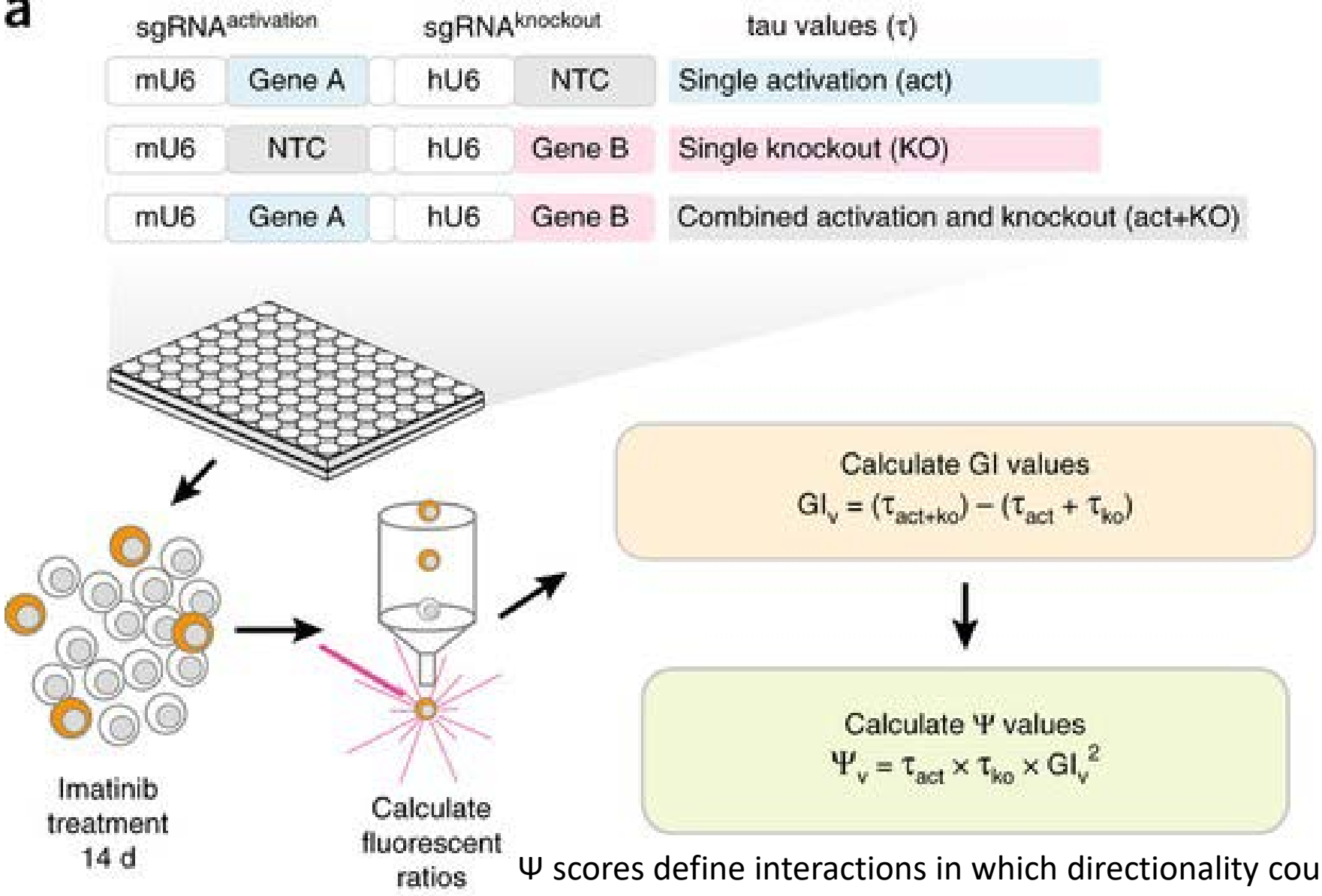


Orthogonal CRISPR screens can quantify directional genetic interactions.



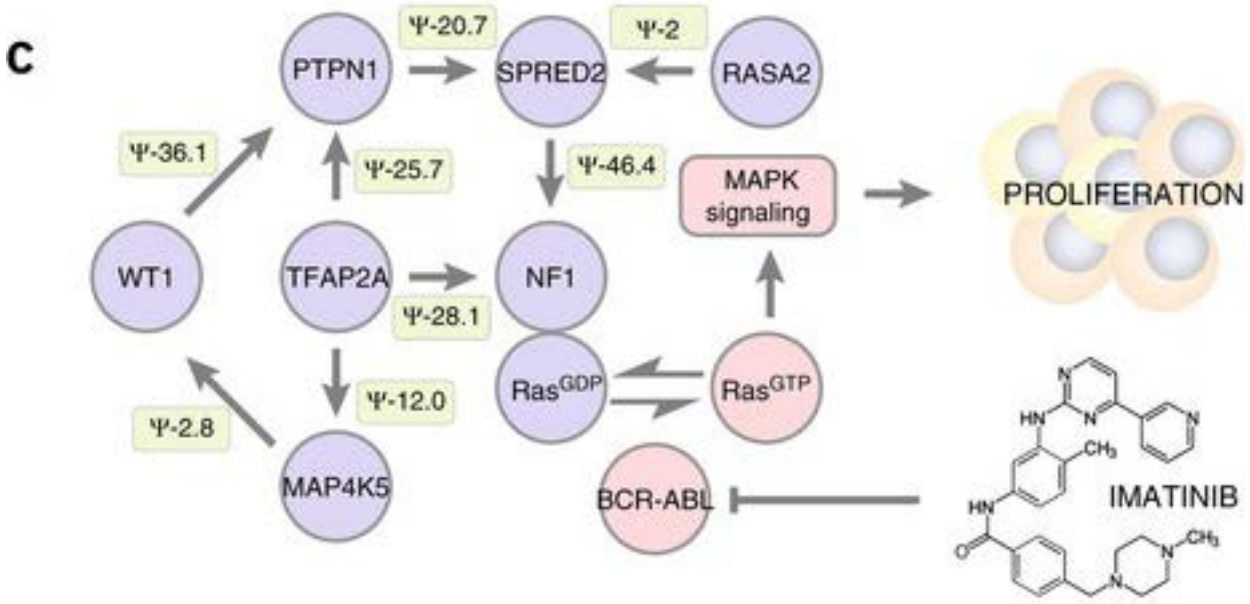


Orthogonal CRISPR screen: Workflow

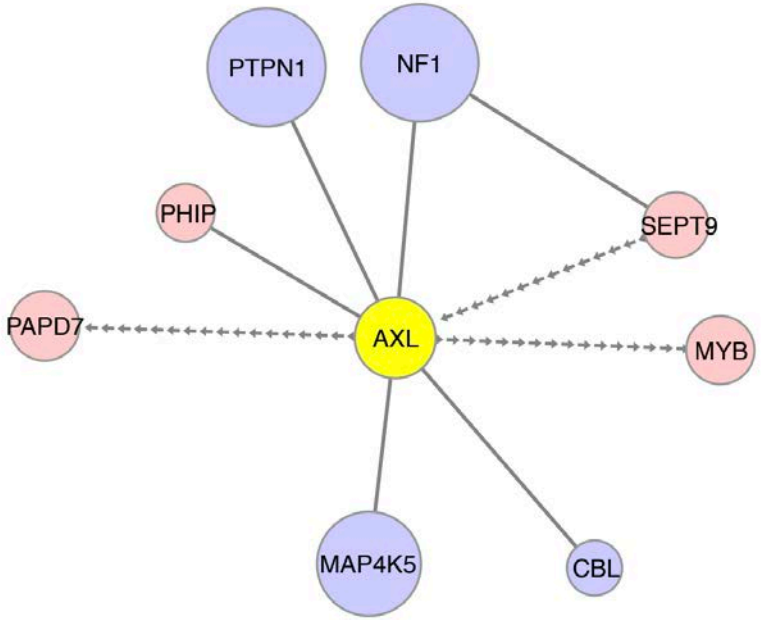


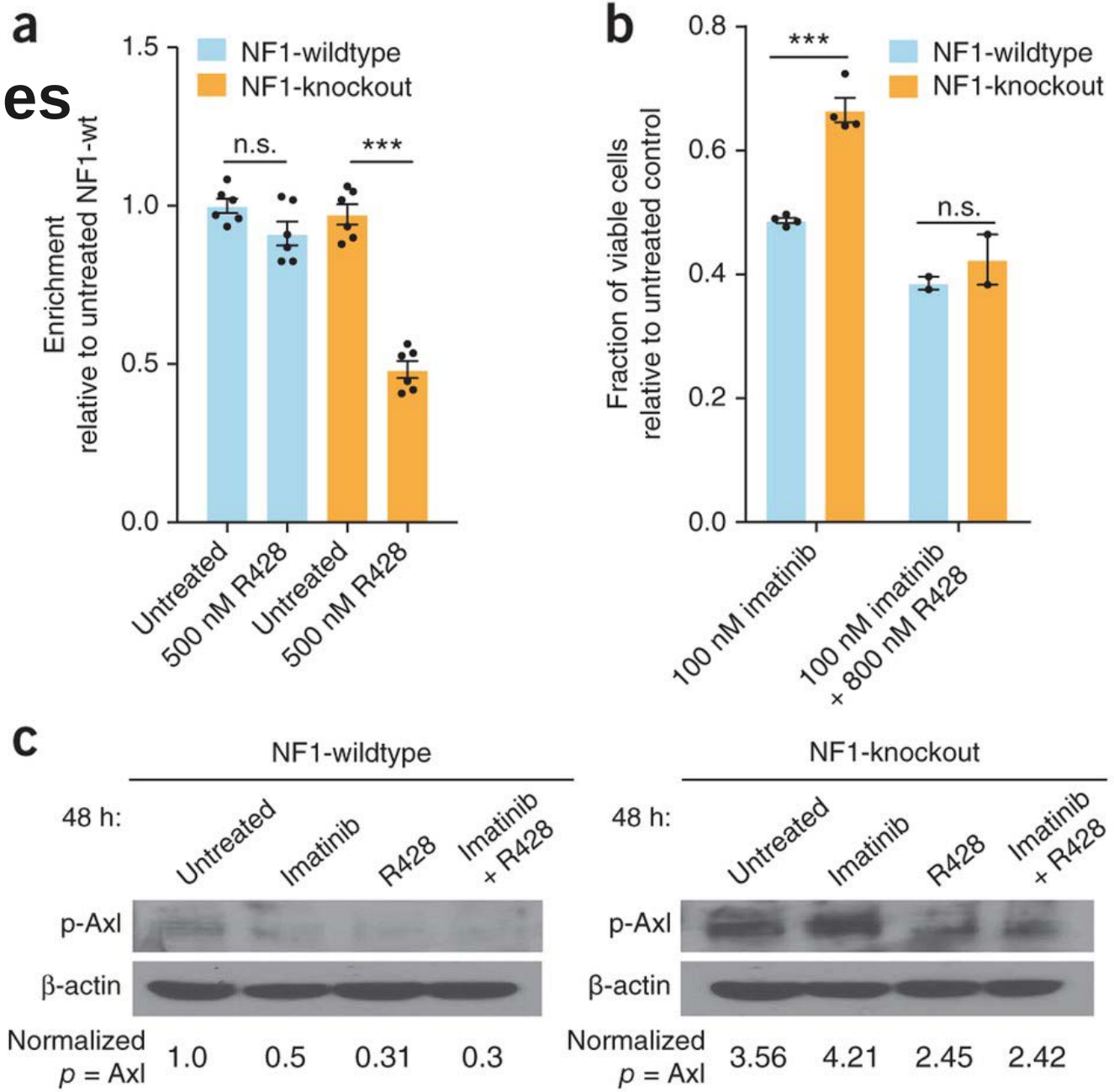
Ψ scores define interactions in which directionality could be inferred

Validation of a directional Ras-centric genetic subnetwork

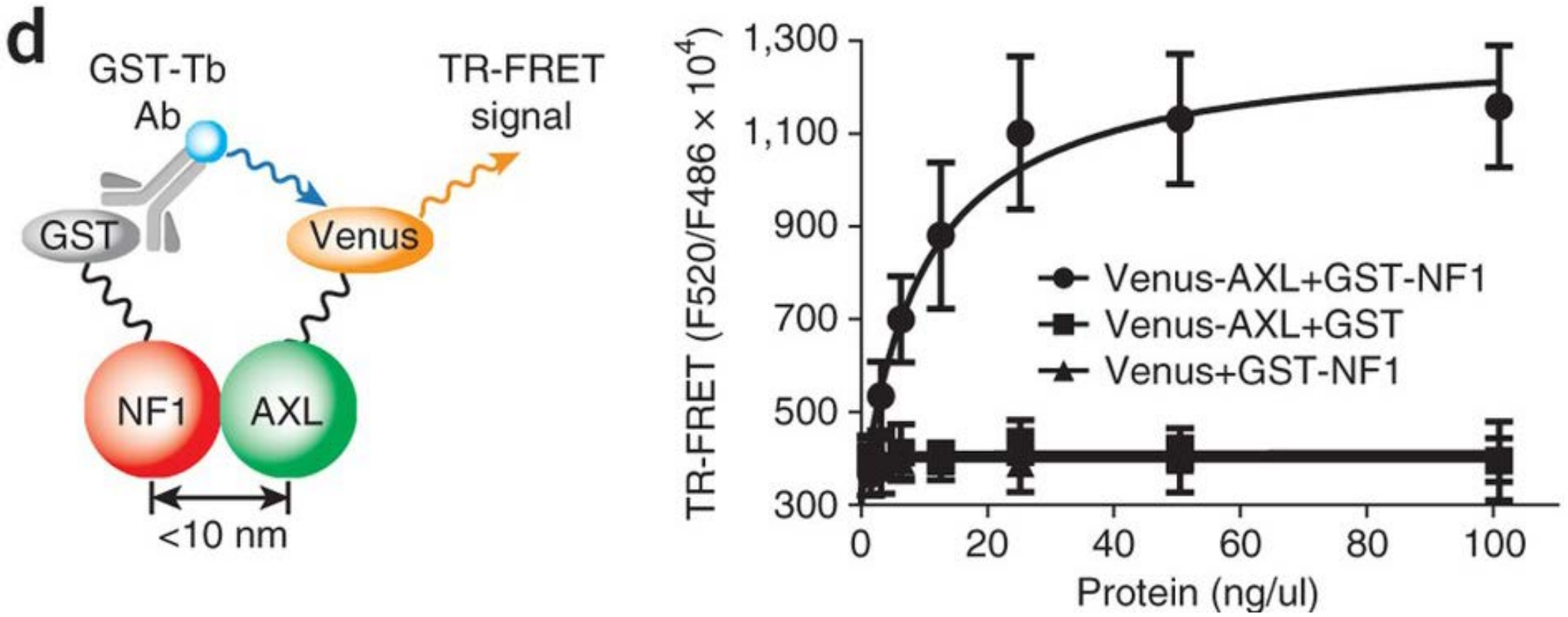


xploiting genetic dependencies for cancer therapy.





Physical interaction between NF1 and AXL



Conclusions

1. Establishment of methods / tools to perform orthogonal screens.

2. Such screens help in identifying directionality for genetic interactions

3. Loss and gain of functions can be quantified from same cells

Paper -2

Article | Open Access | Published: 06 February 2020

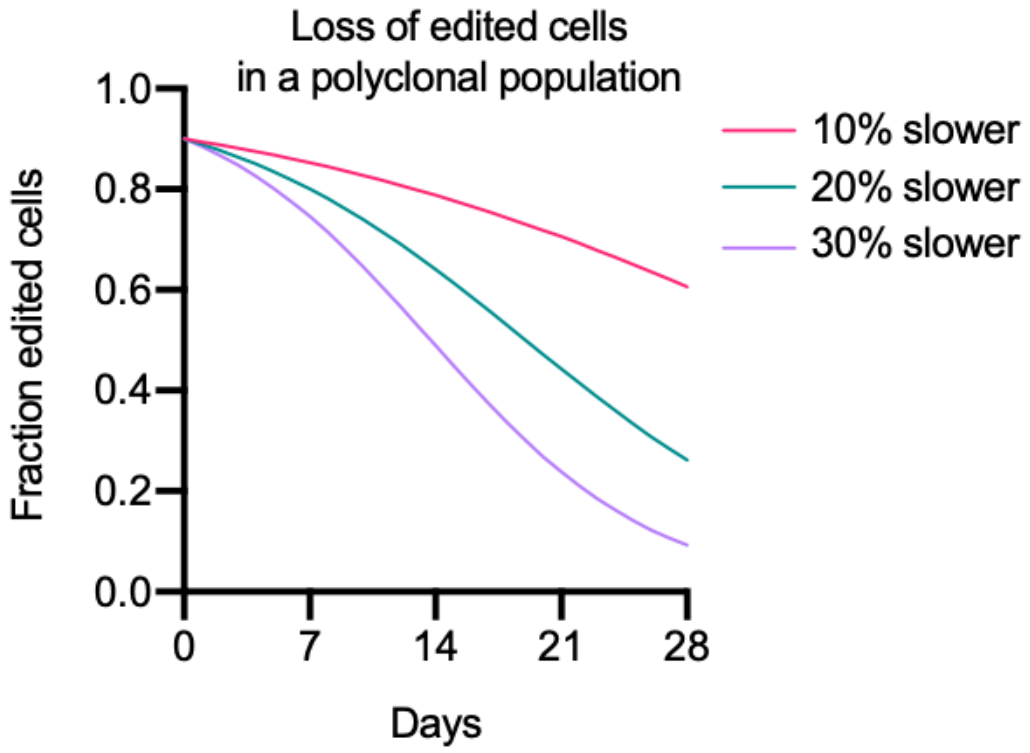
Genetic screens in isogenic mammalian cell lines without single cell cloning

Peter C. DeWeirdt, Annabel K. Sangree, Ruth E. Hanna, Kendall R. Sanson, Mudra Hegde, Christine Strand, Nicole S. Persky & John G. Doench

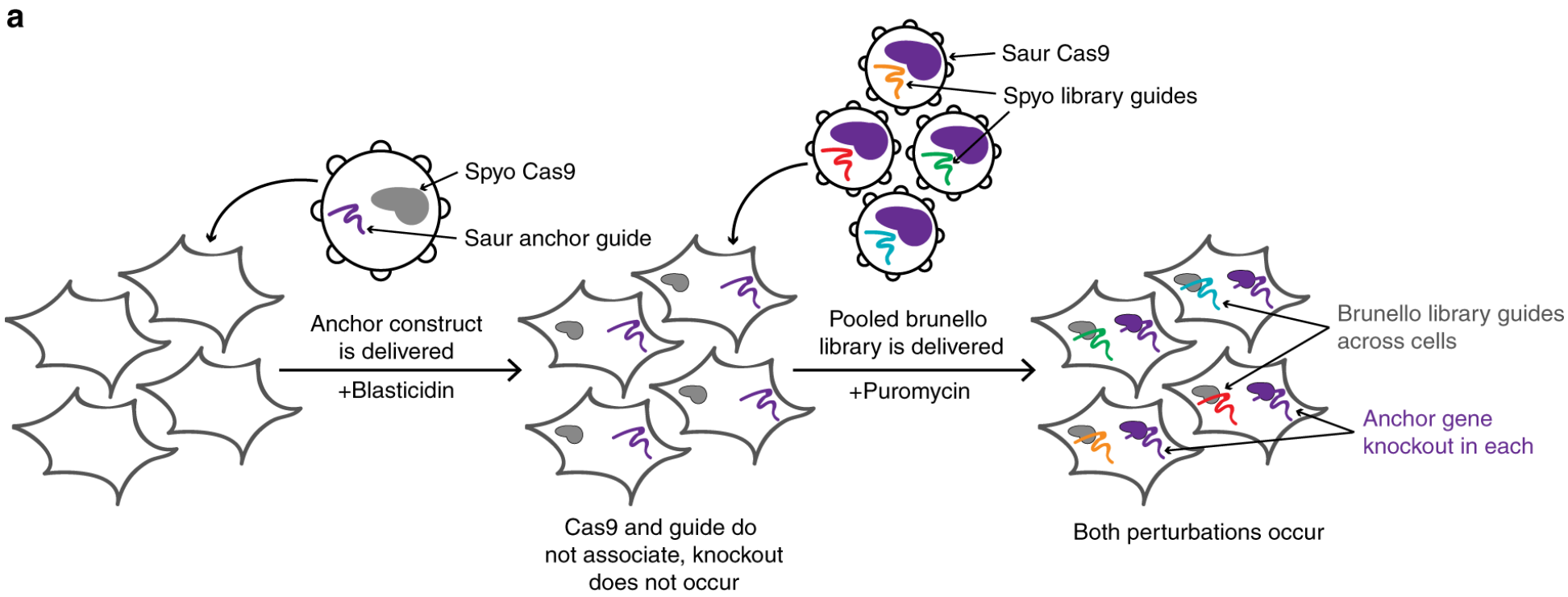
Nature Communications **11**, Article number: 752 (2020) | Cite this article

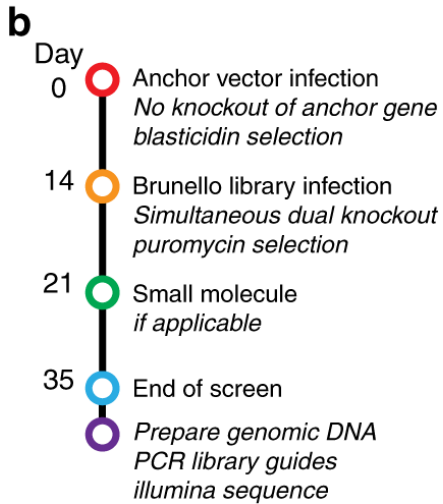
Problems with synthetic lethality screens

1. Selection pressure against ko cells
2. Cells harboring antibiotic resistance but not gRNA

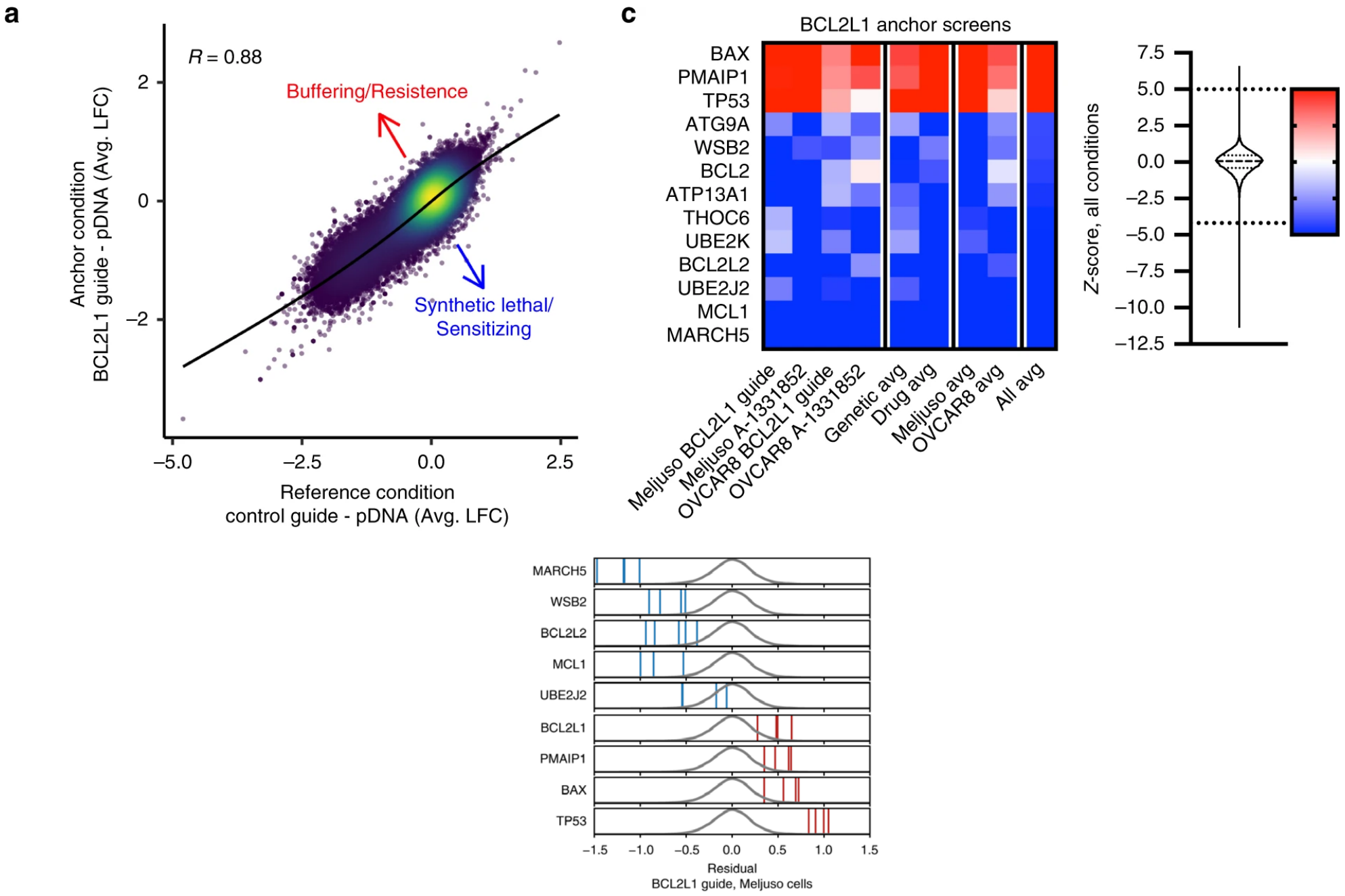


Anchor screen: Methodology

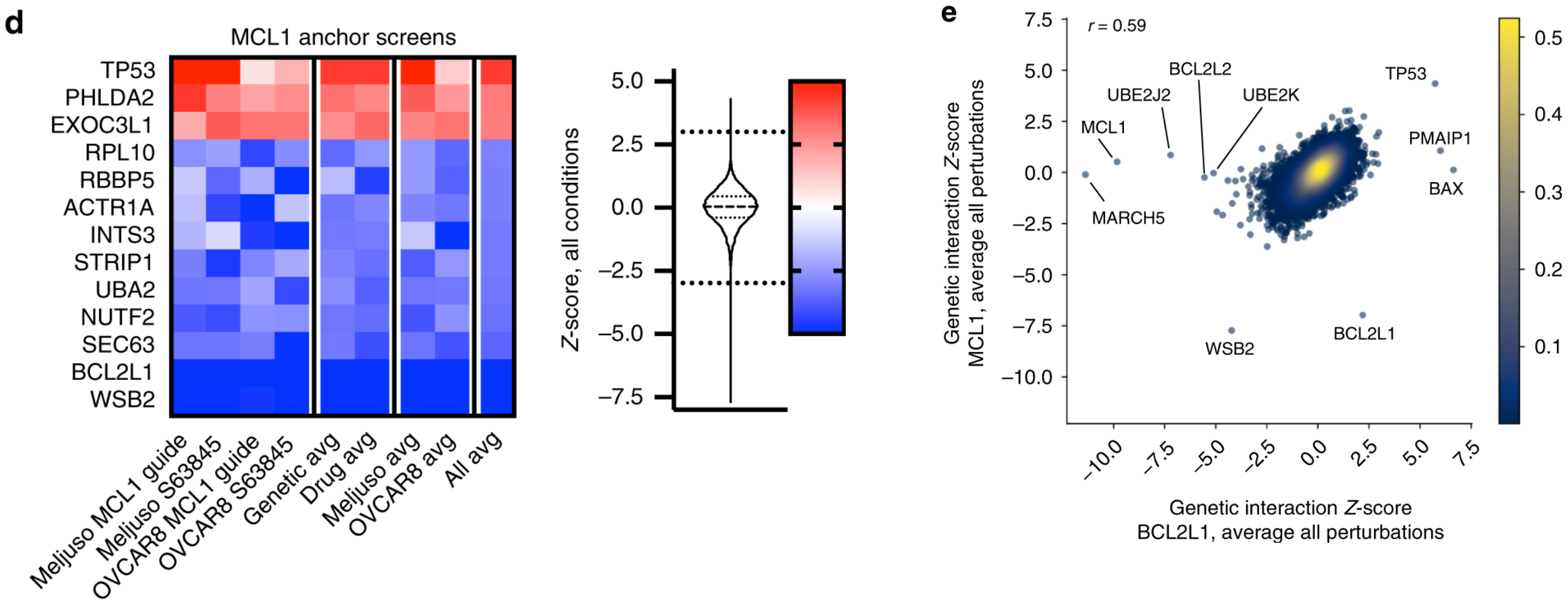


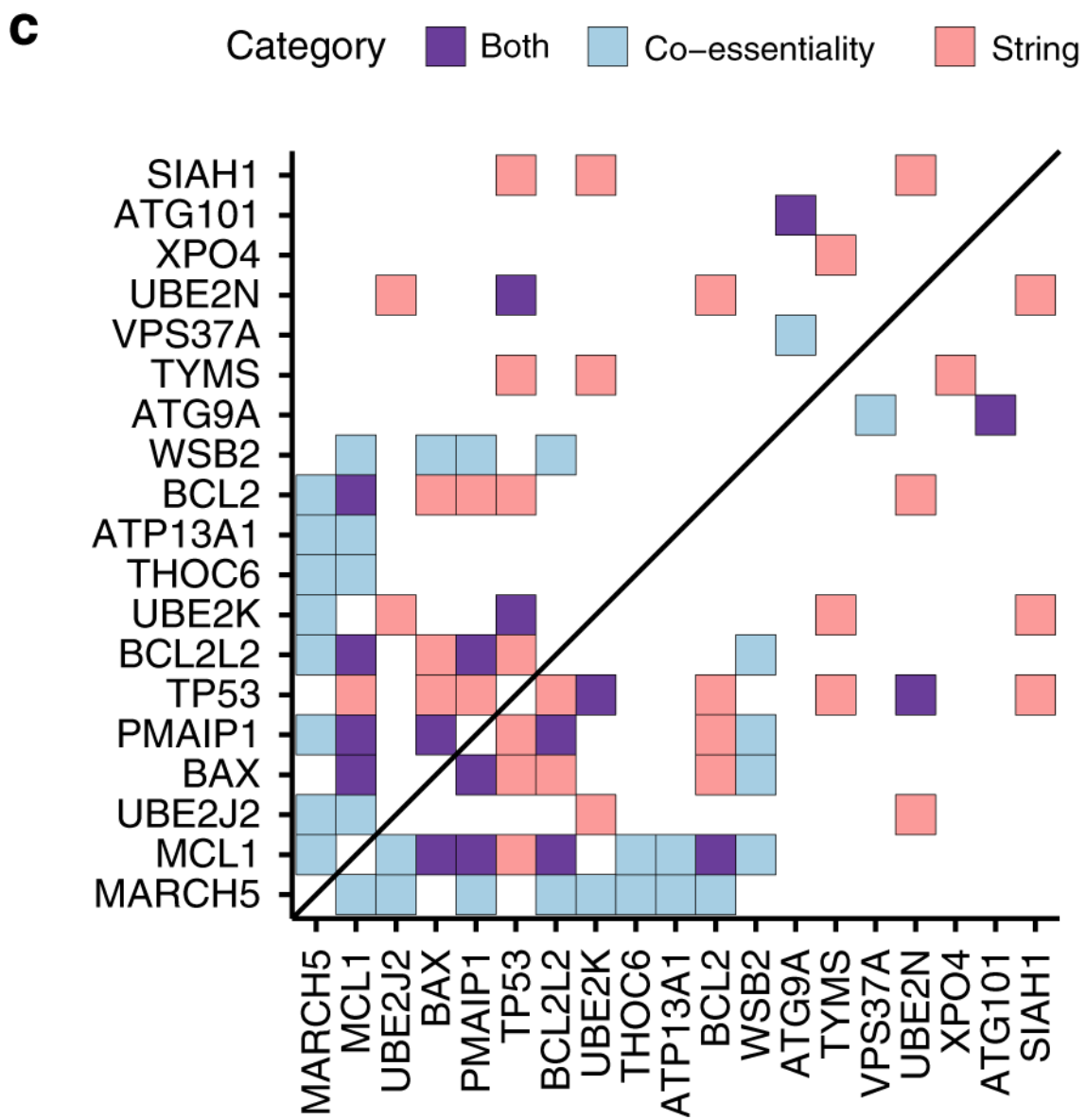


Anchor screen for BCL2L1. Synthetic lethality

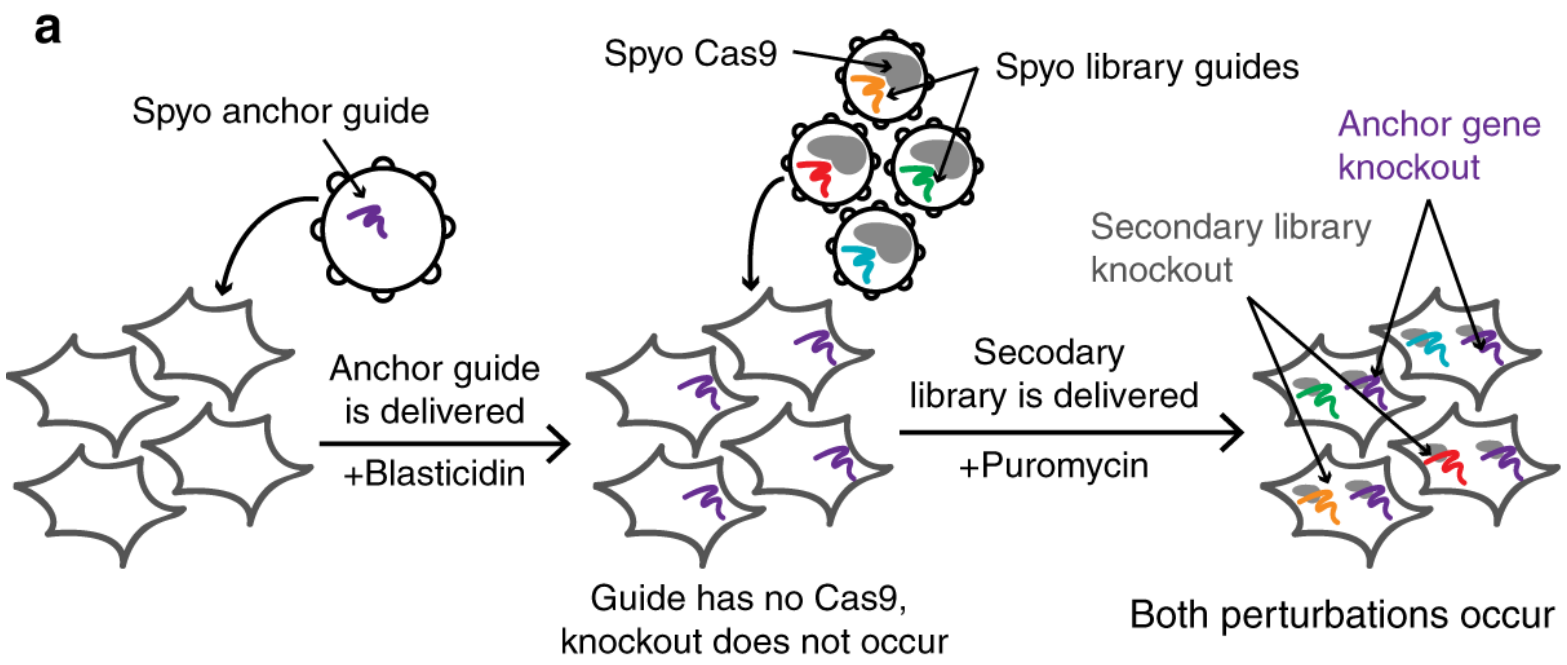


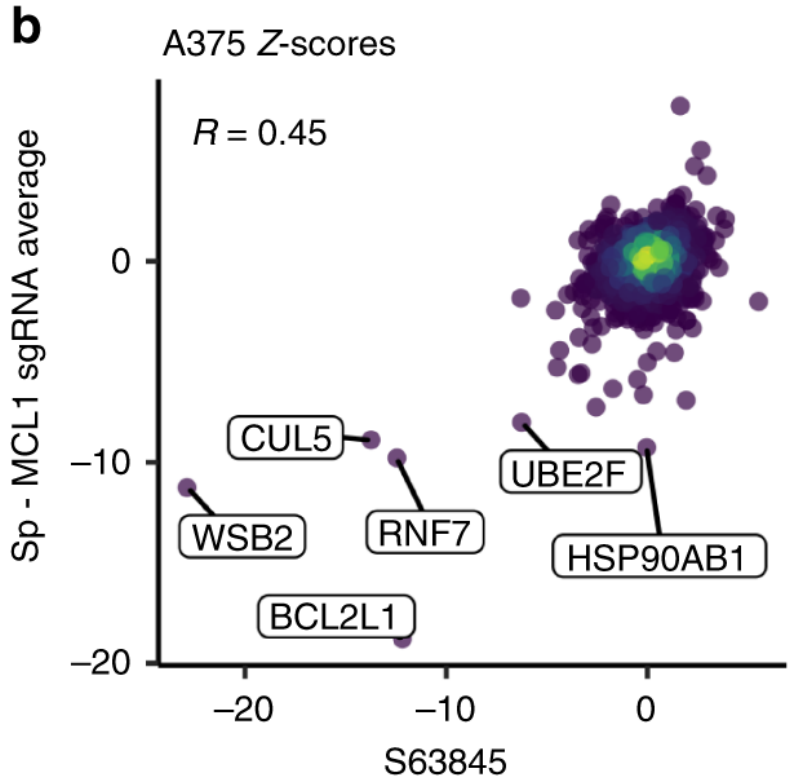
Anchor screen for MCL1. Synthetic lethality




c
Category
Both
Co−essentiality
String
SIAH1
ATG101
XPO4
UBE2N
VPS37A
TYMS
ATG9A
WSB2
BCL2
ATP13A1
THOC6
UBE2K
BCL2L2
TP53
PMAIP1
BAX
UBE2J2
MCL1
MARCH5
MARCH5
MCL1
UBE2J2
BAX
PMAIP1
TP53
BCL2L2
UBE2K
THOC6
ATP13A1
BCL2
WSB2
ATG9A
TYMS
VPS37A
UBE2N
XPO4
ATG101
SIAH1

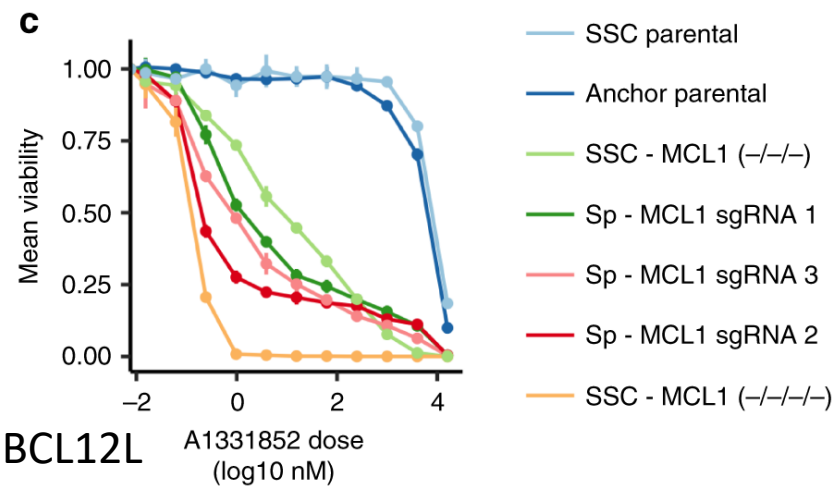
MCL1 secondary screens comparing alternative screening approaches.

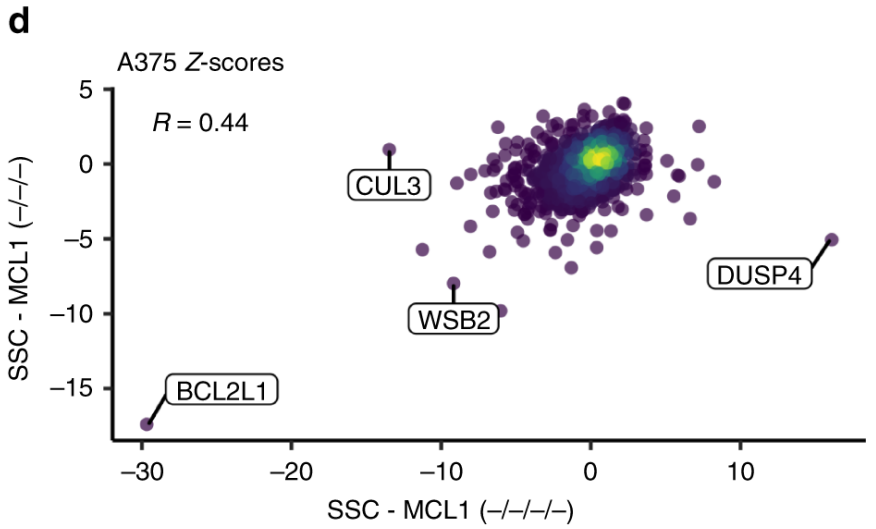


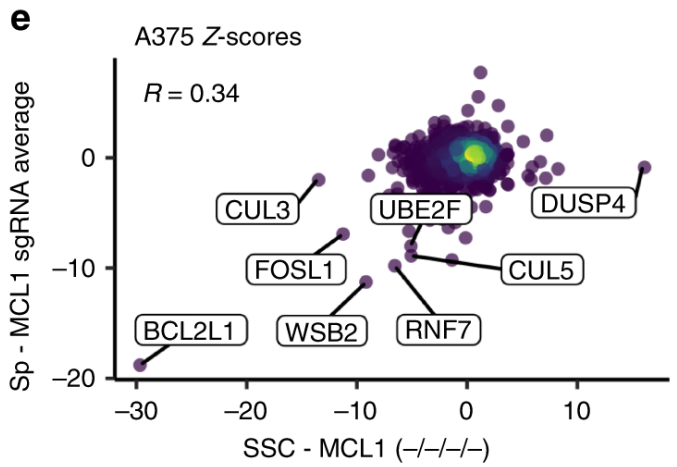


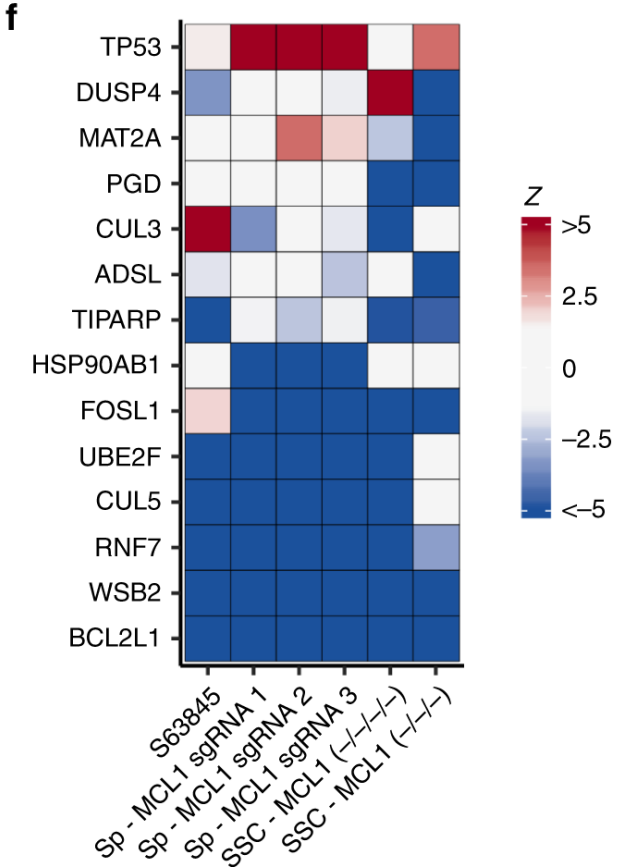
Validation

Inhibitor of BCL12L

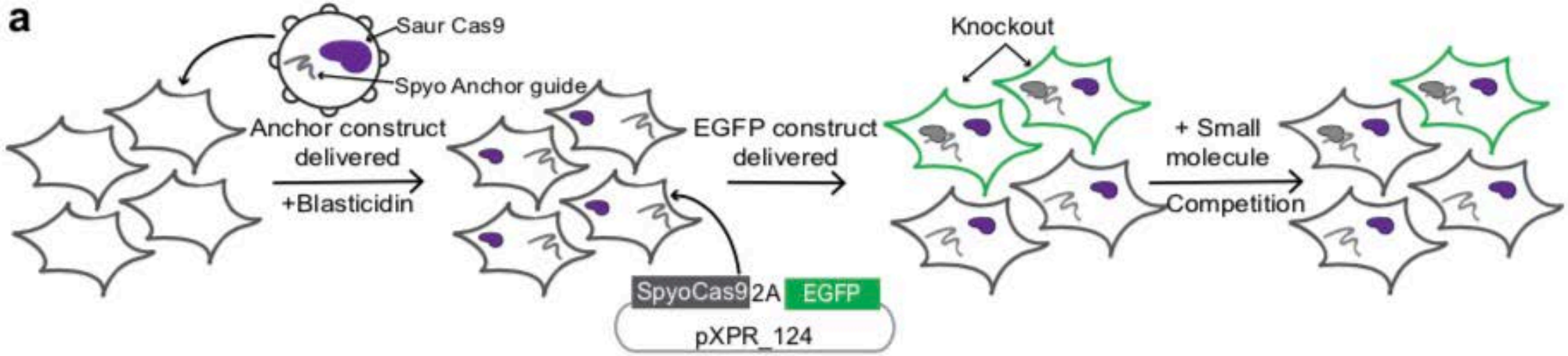


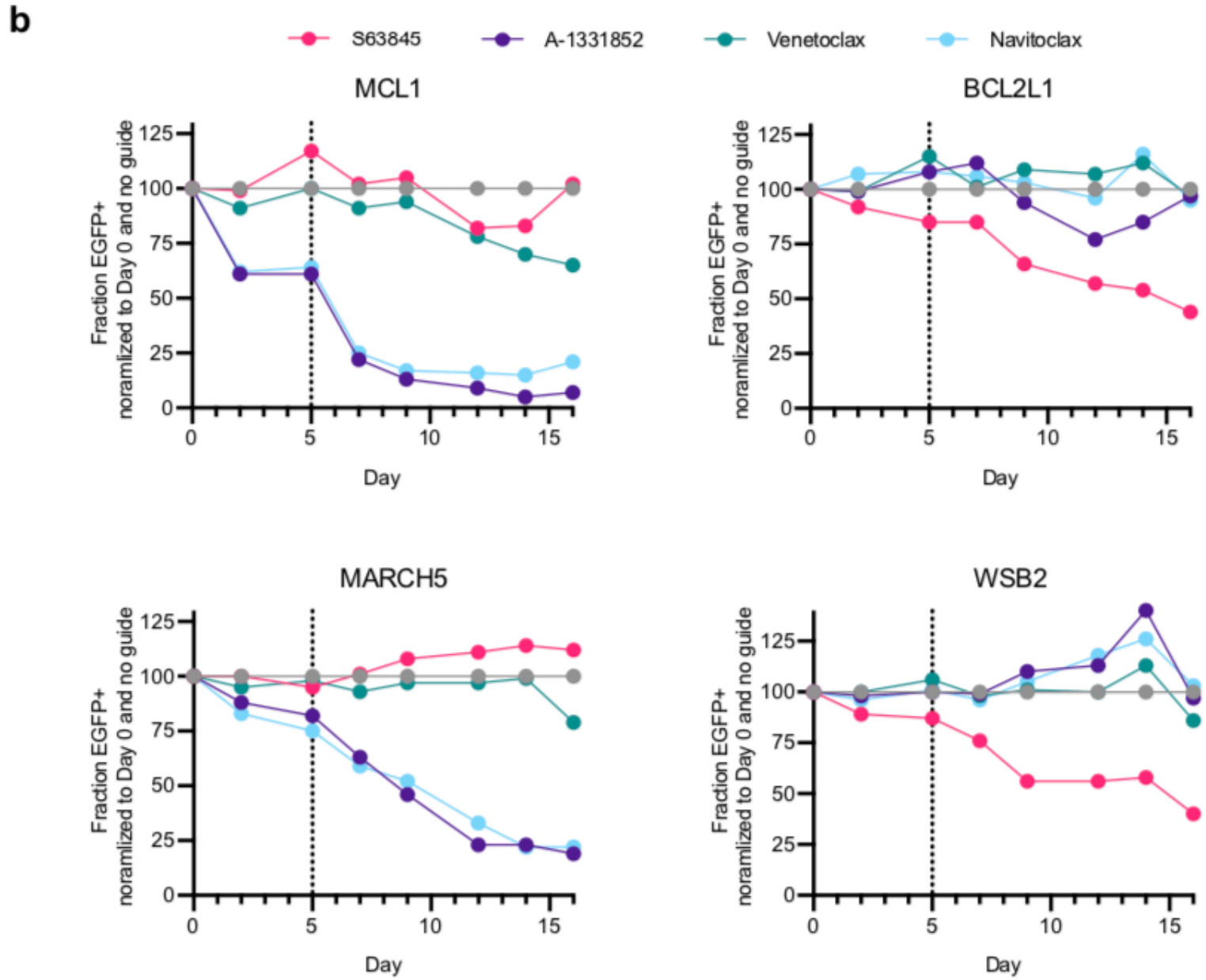






Validation of the data using a competition assay

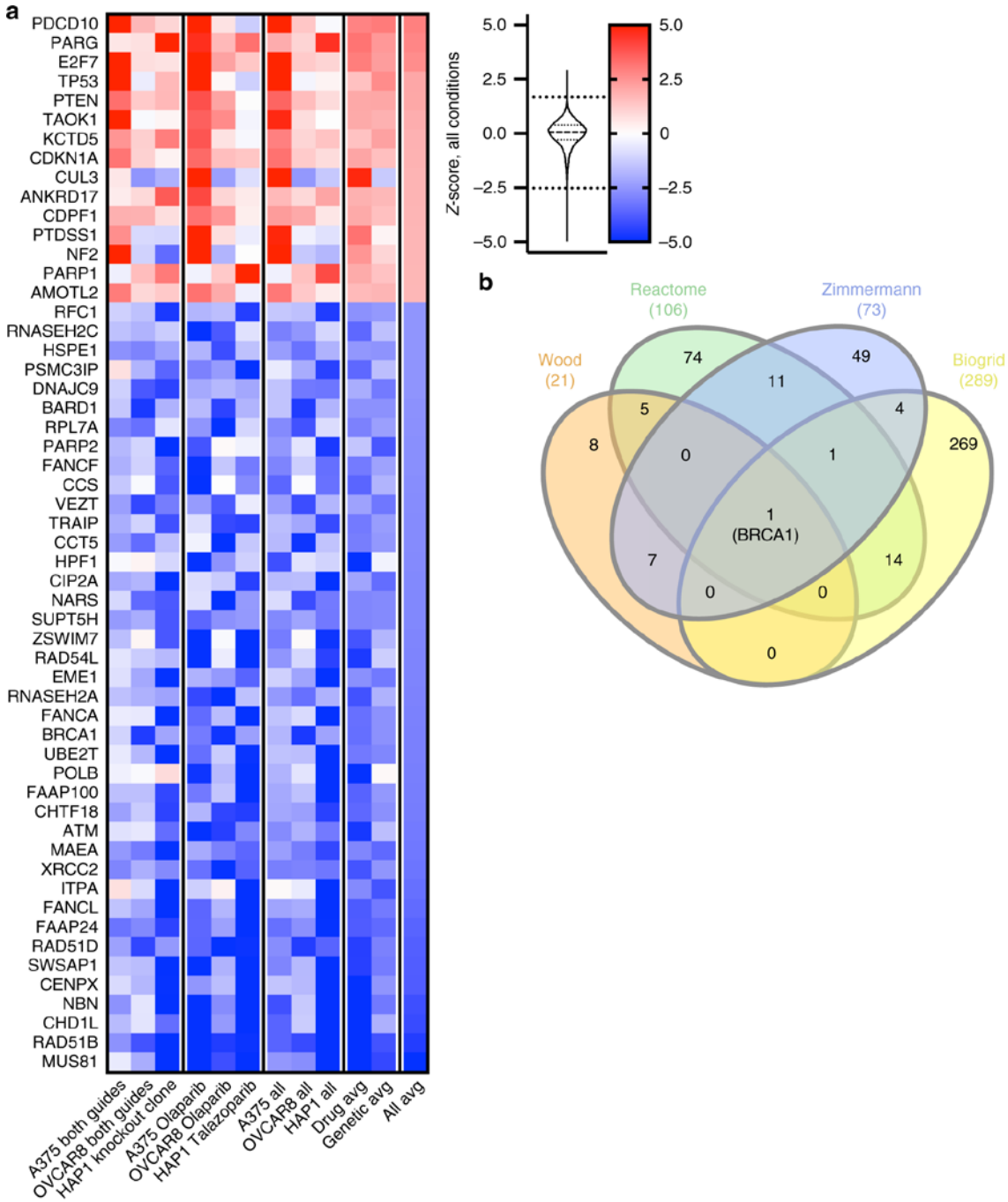



b
S63845
A-1331852
Venetoclax
Navitoclax
MCL1
BCL2L1
MARCH5
WSB2
Fraction EGFP+
noramlized to Day 0 and no guide
Day
0
25
50
75
100
125
0
5
10
15

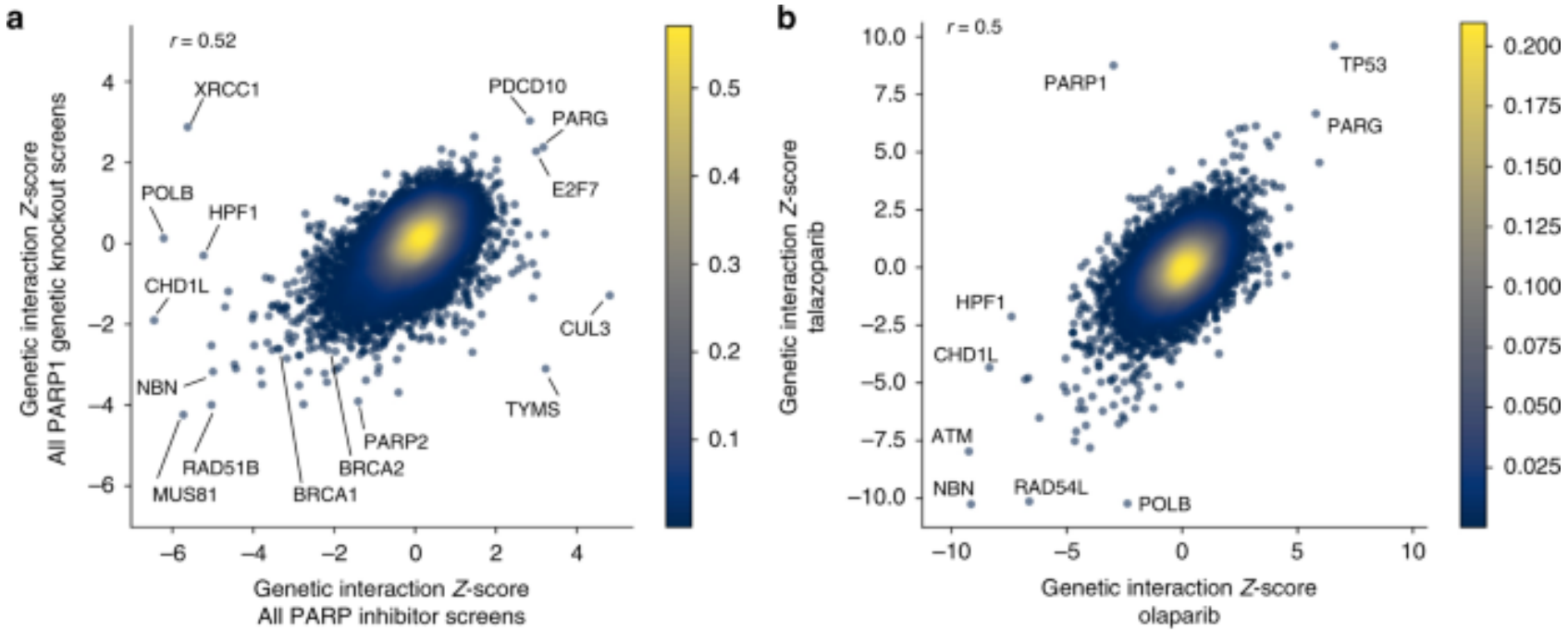
Anchor screen with DNA damage response gene PARP1

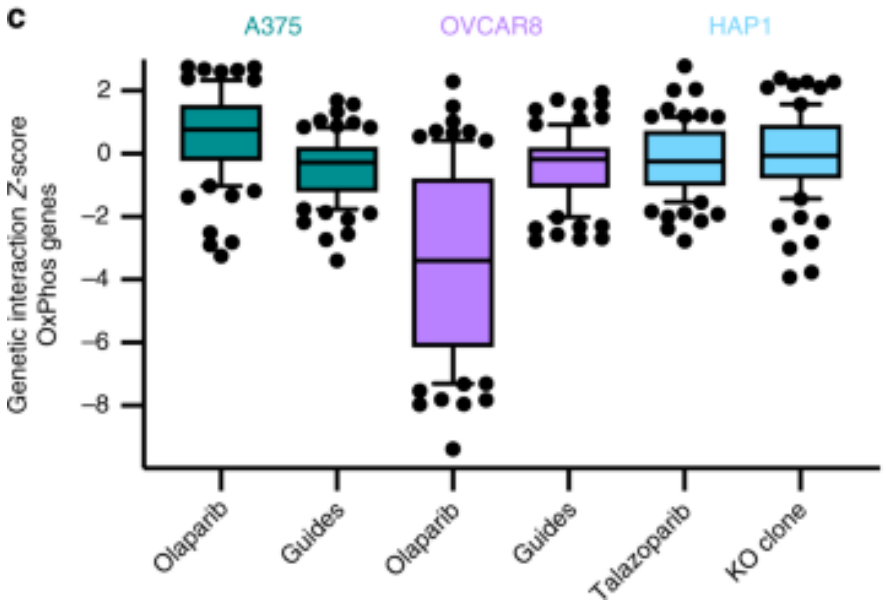
Screen was performed in multiple cell lines.

Also performed with two inhibitors of PARP1

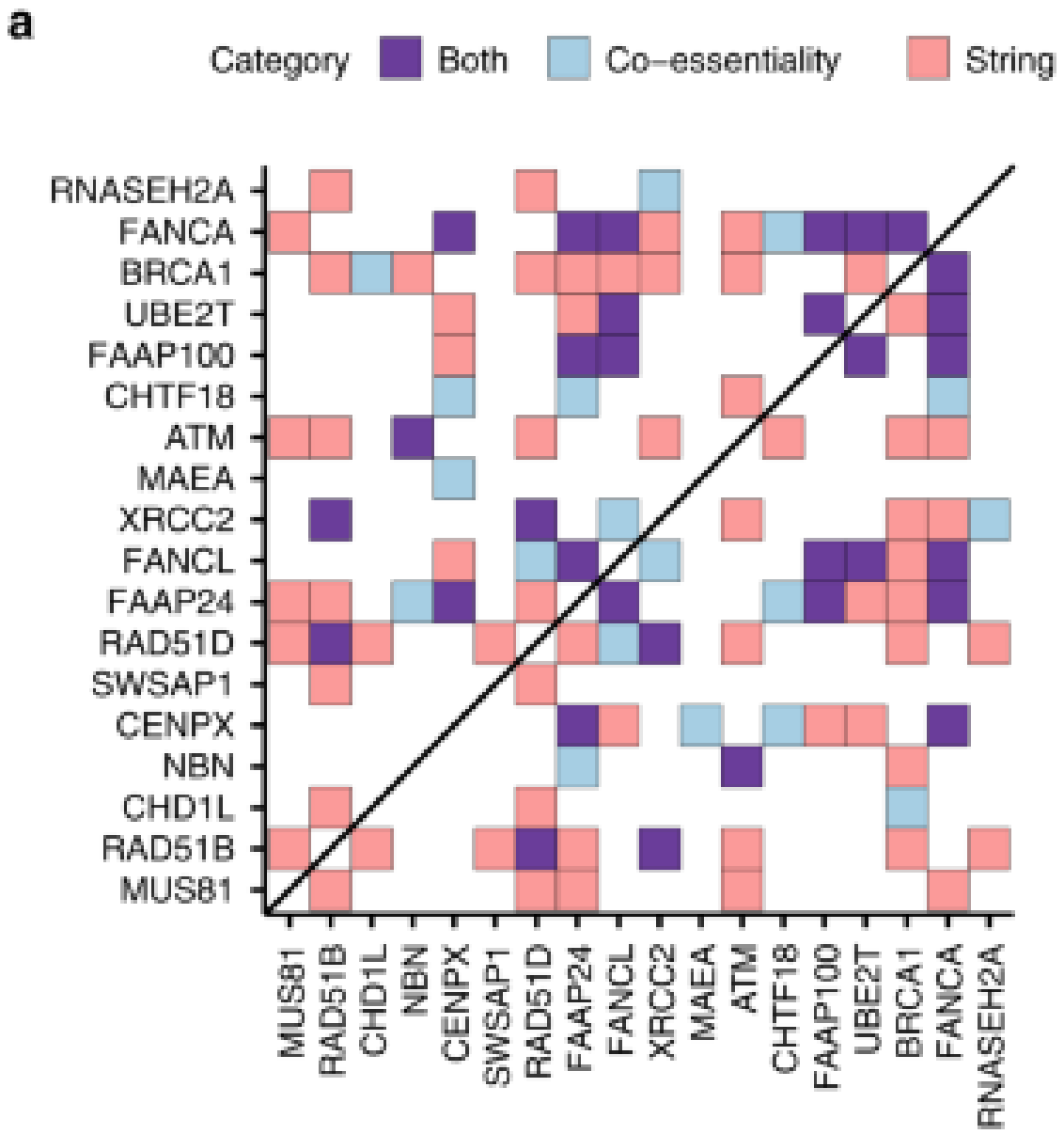


GI Score: Residuals of all the guides/
SD of all the guides





Creating networks form the data obtained



Conclusions

1. A smart way to perform synthetic lethality screens overcoming the need for an inducible system.

2. Coessentiality data can be used to generate genetic interaction maps.

Questions:

1. Stability of the anchor guides?

2. Can we used patient derived cell lines?

Paper -3

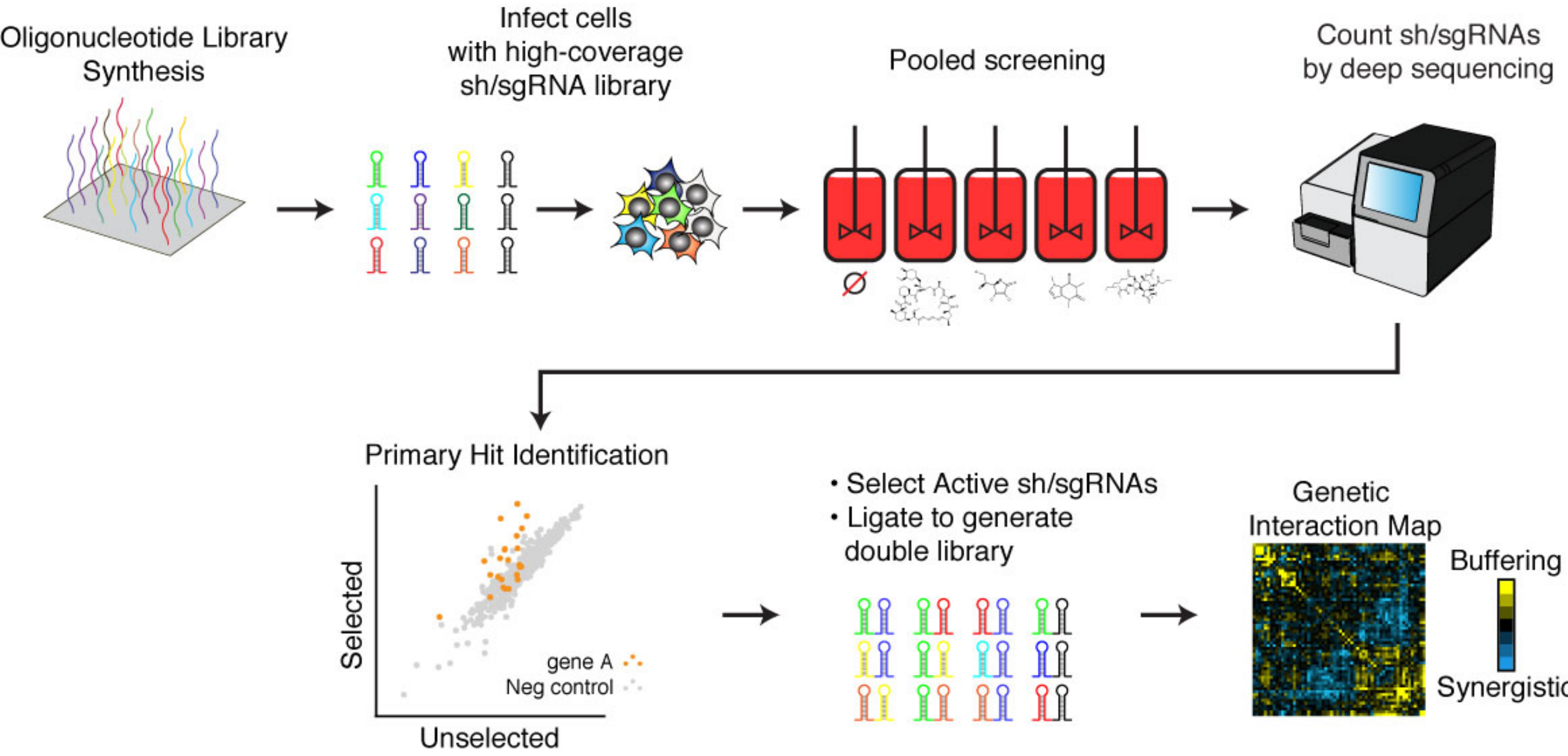
Cell Reports

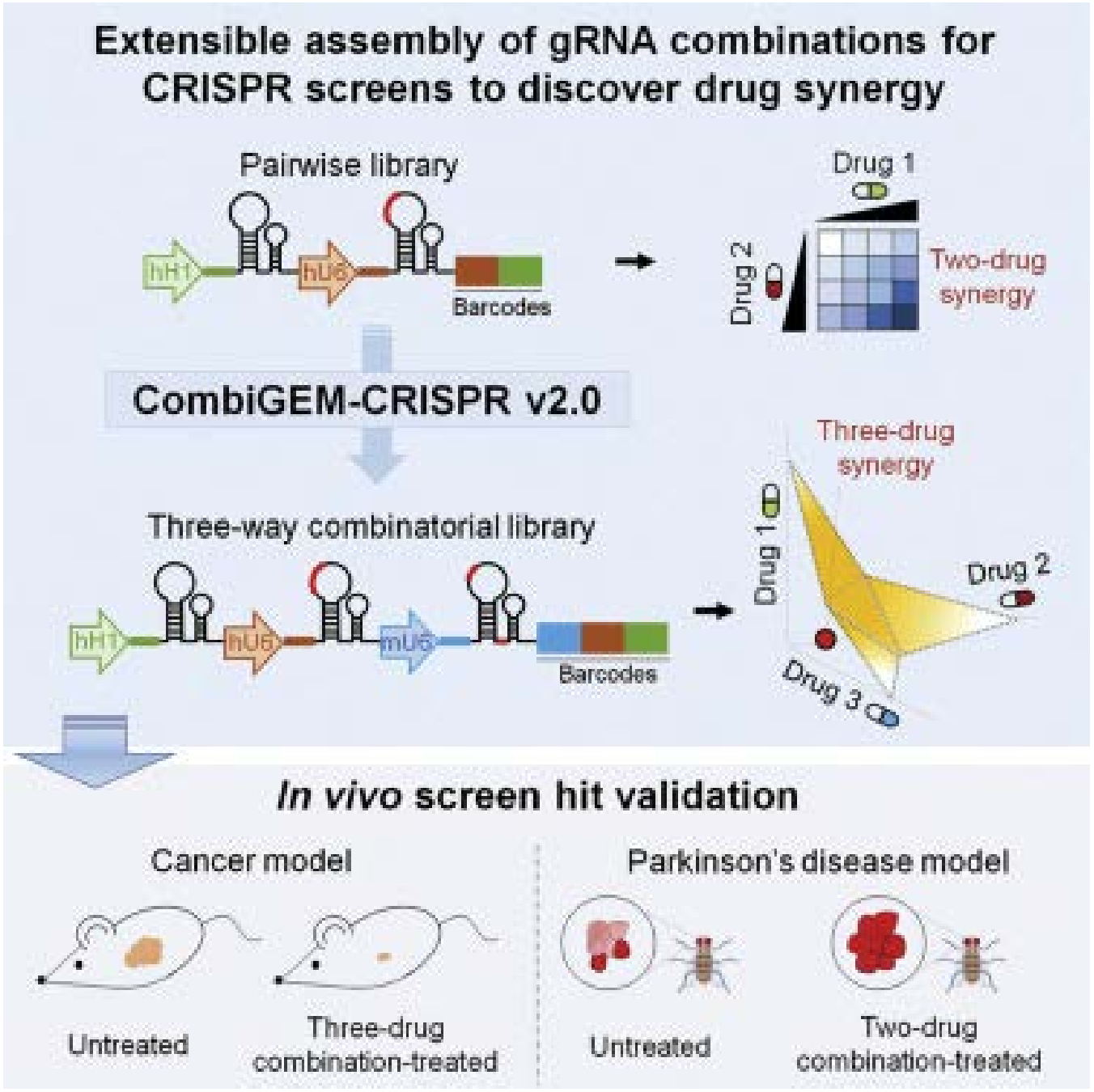
CellPress
OPEN ACCESS

Article

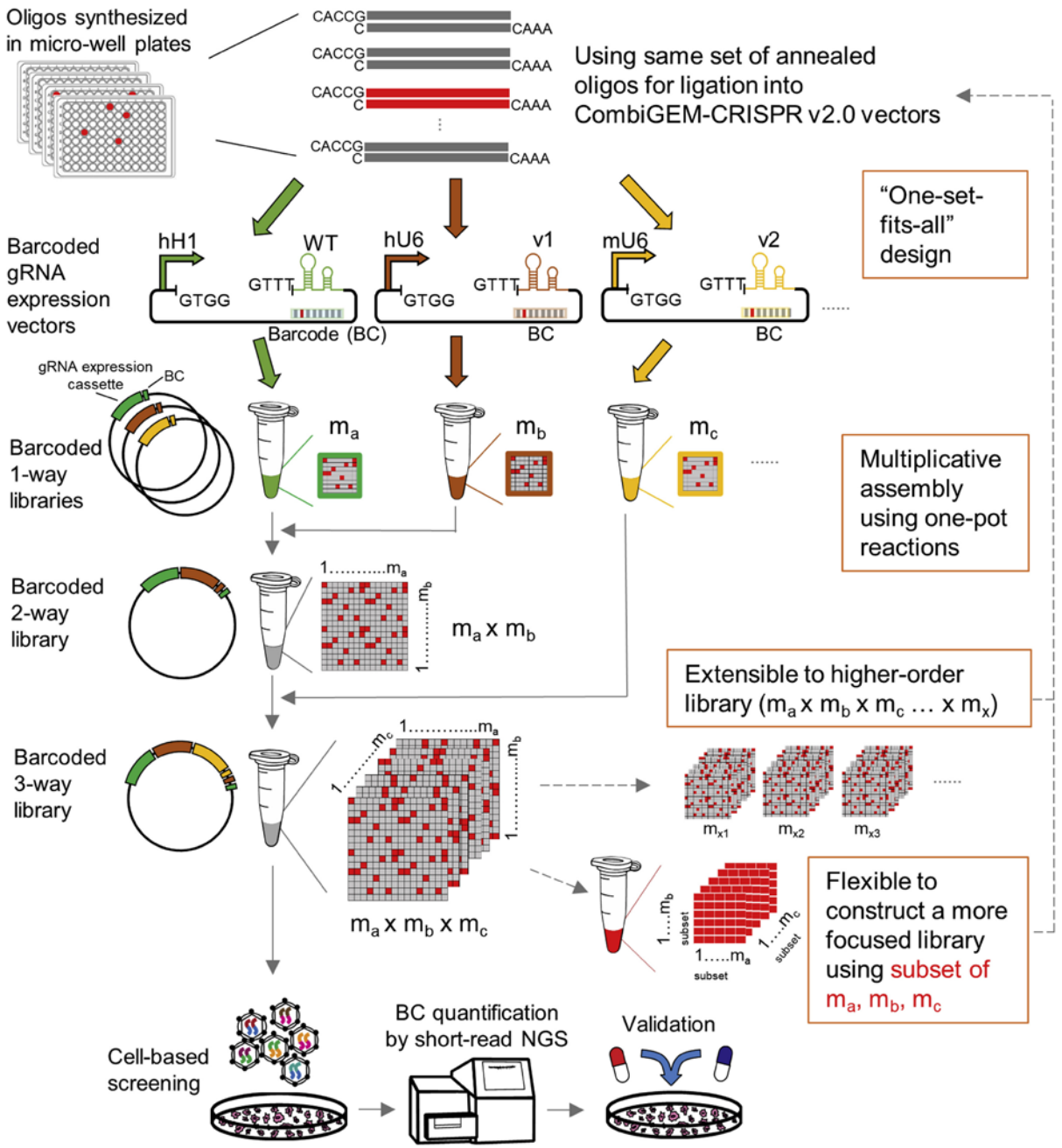
A Three-Way Combinatorial CRISPR Screen for Analyzing Interactions among Druggable Targets

Peng Zhou,[1] Becky K.C. Chan,[1] Yuk Kei Wan,[1] Chaya T.L. Yuen,[1] Gigi C.G. Choi,[1] Xinran Li,[2] Cindy S.W. Tong,[1] Sophia S.W. Zhong,[1] Jieran Sun,[1] Yufan Bao,[3,4] Silvia Y.L. Mak,[3] Maggie Z.Y. Chow,[3] Jien Vei Khaw,[1] Suet Yi Leung,[5,6,7] Zongli Zheng,[3,4,8] Lydia W.T. Cheung,[2] Kaeling Tan,[9,10] Koon Ho Wong,[9,11] H.Y. Edwin Chan,[12,13] and Alan S.L. Wong[1,14,15,*]

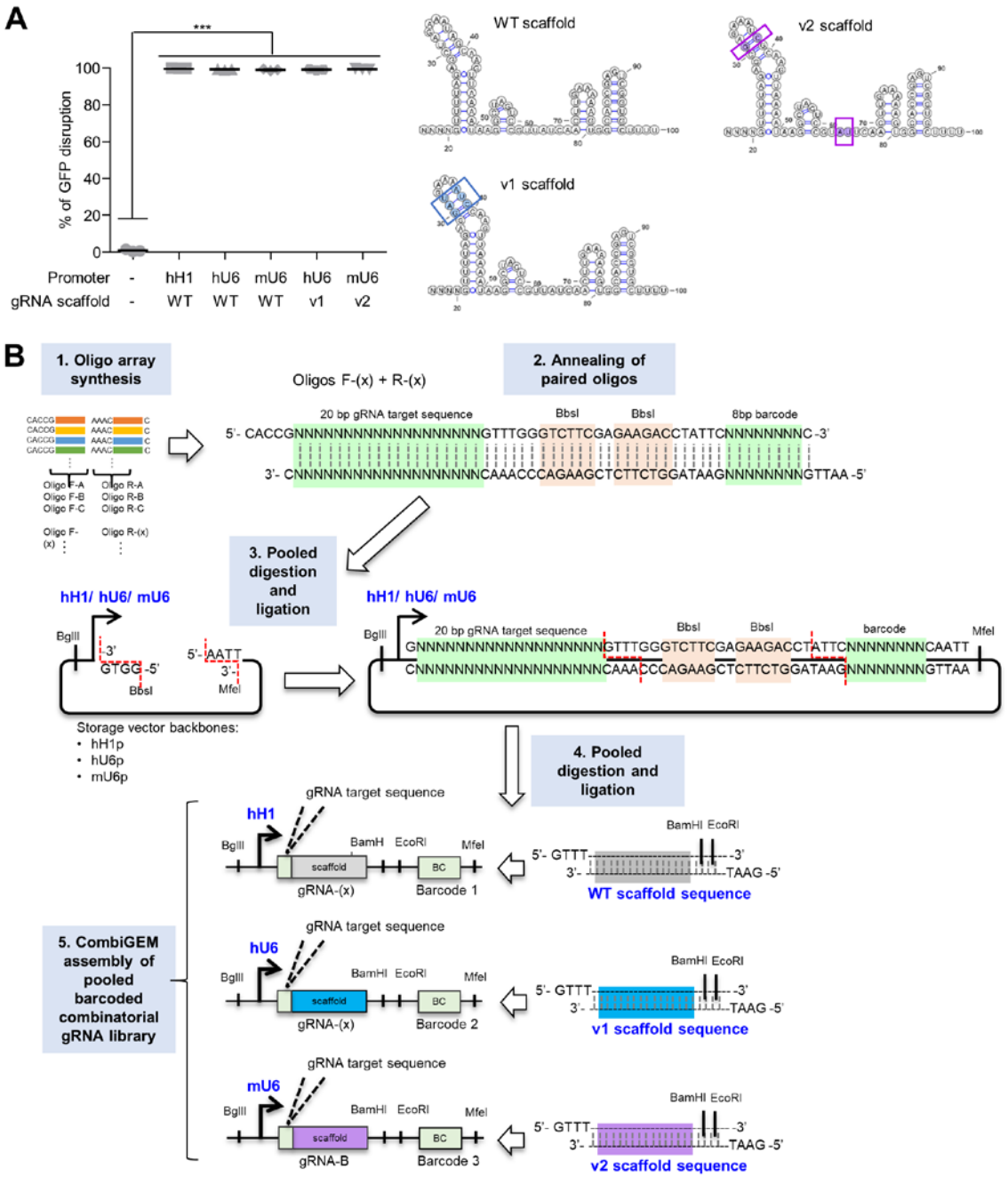

Oligonucleotide Library Synthesis
Infect cells with high-coverage sh/sgRNA library
Pooled screening
Count sh/sgRNAs by deep sequencing
Primary Hit Identification
Selected
Unselected
gene A
Neg control
• Select Active sh/sgRNAs
• Ligate to generate double library
Genetic Interaction Map
Buffering
Synergistic


Extensible assembly of gRNA combinations for CRISPR screens to discover drug synergy
Pairwise library
hH1
hU6
Barcodes
Drug 1
Drug 2
Two-drug synergy
CombiGEM-CRISPR v2.0
Three-way combinatorial library
hH1
hU6
mU6
Barcodes
Three-drug synergy
Drug 1
Drug 2
Drug 3
In vivo screen hit validation
Cancer model
Untreated
Three-drug combination-treated
Parkinson's disease model
Untreated
Two-drug combination-treated

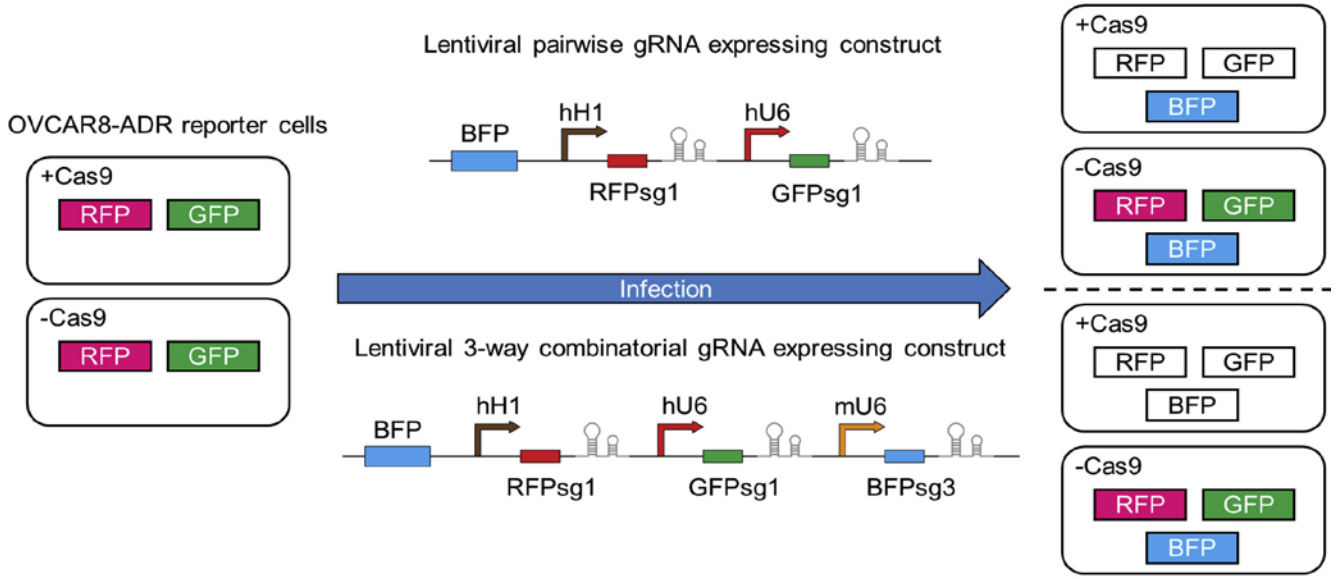
Screen Outlay

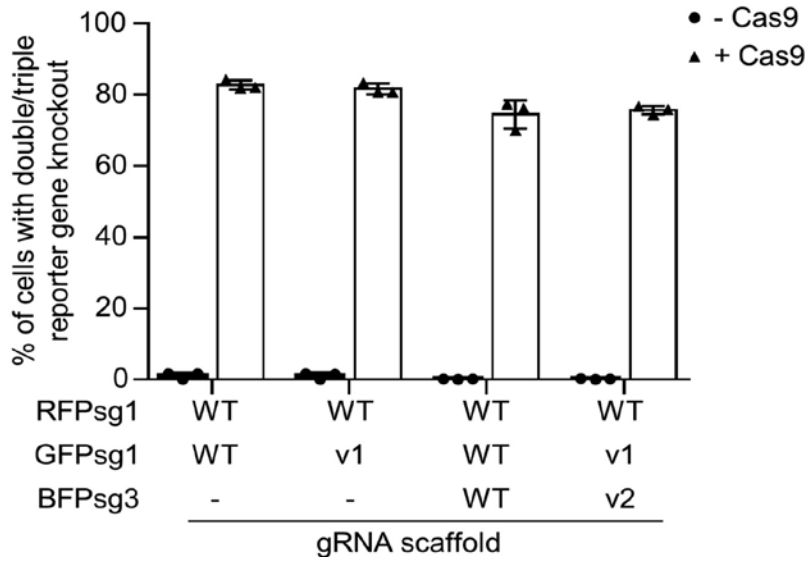


Cloning strategy

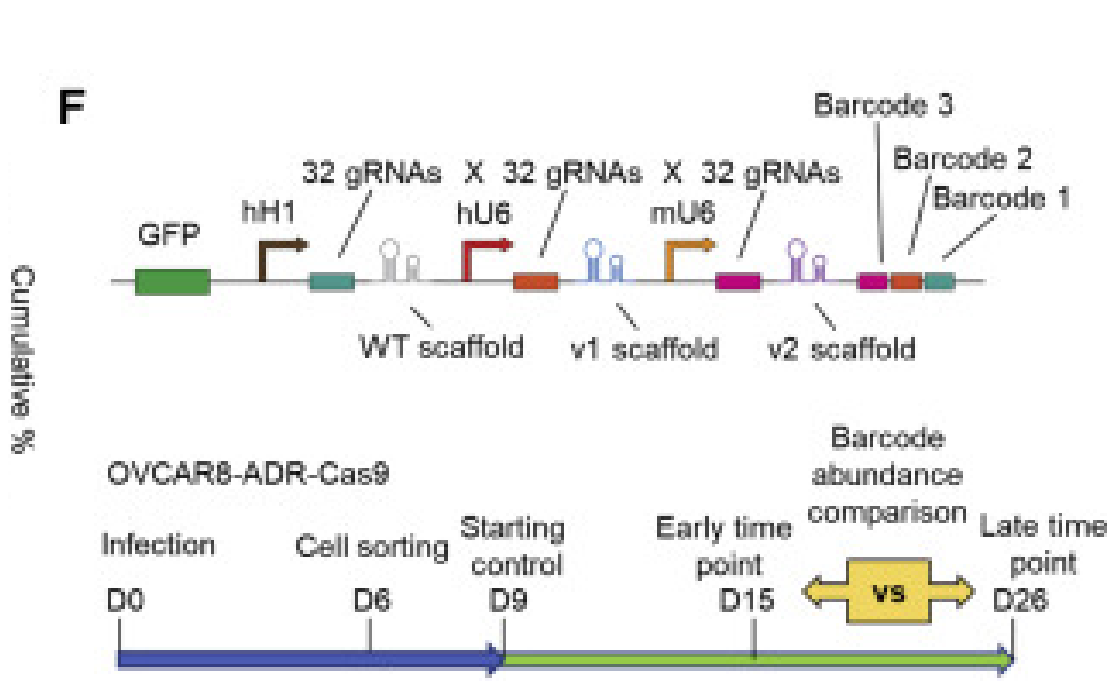


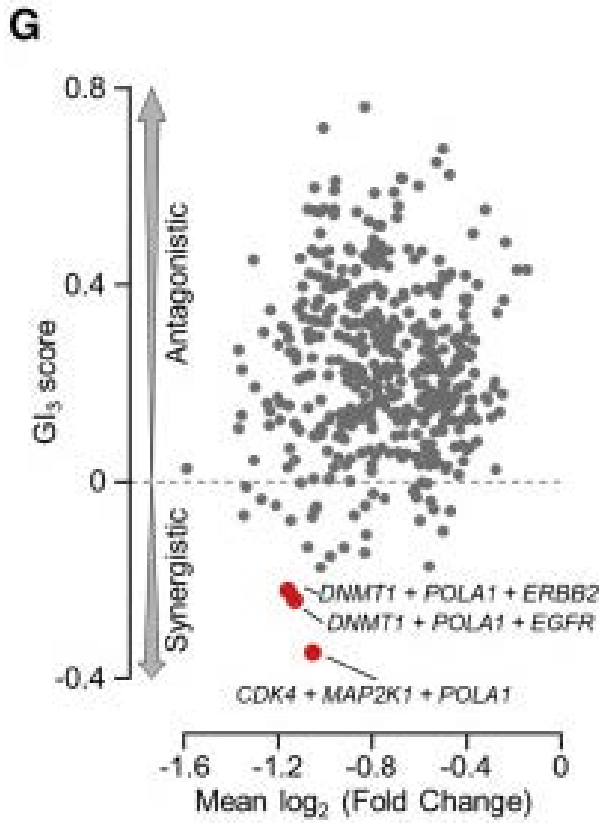
Proof of principle: Multiple gene knock out

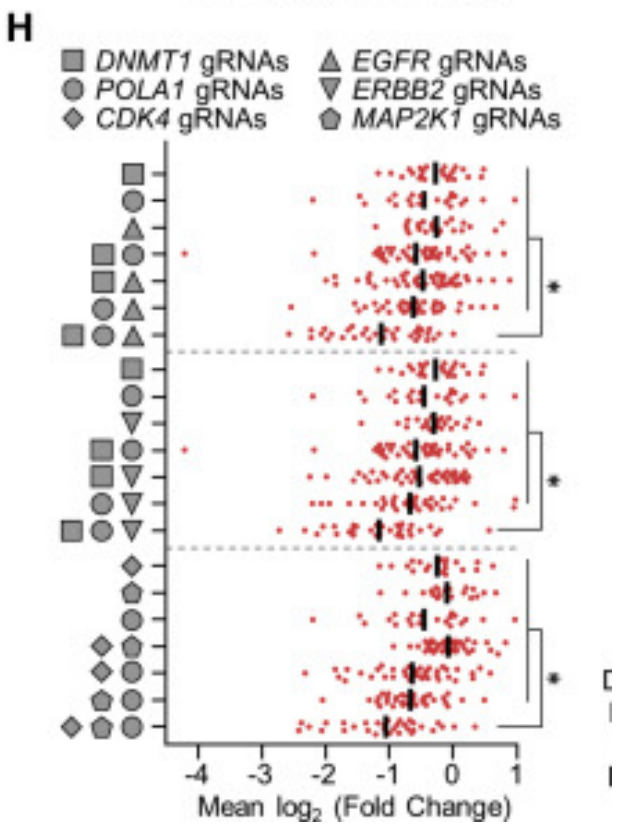




Triple gene knock out-Ovarian cancer



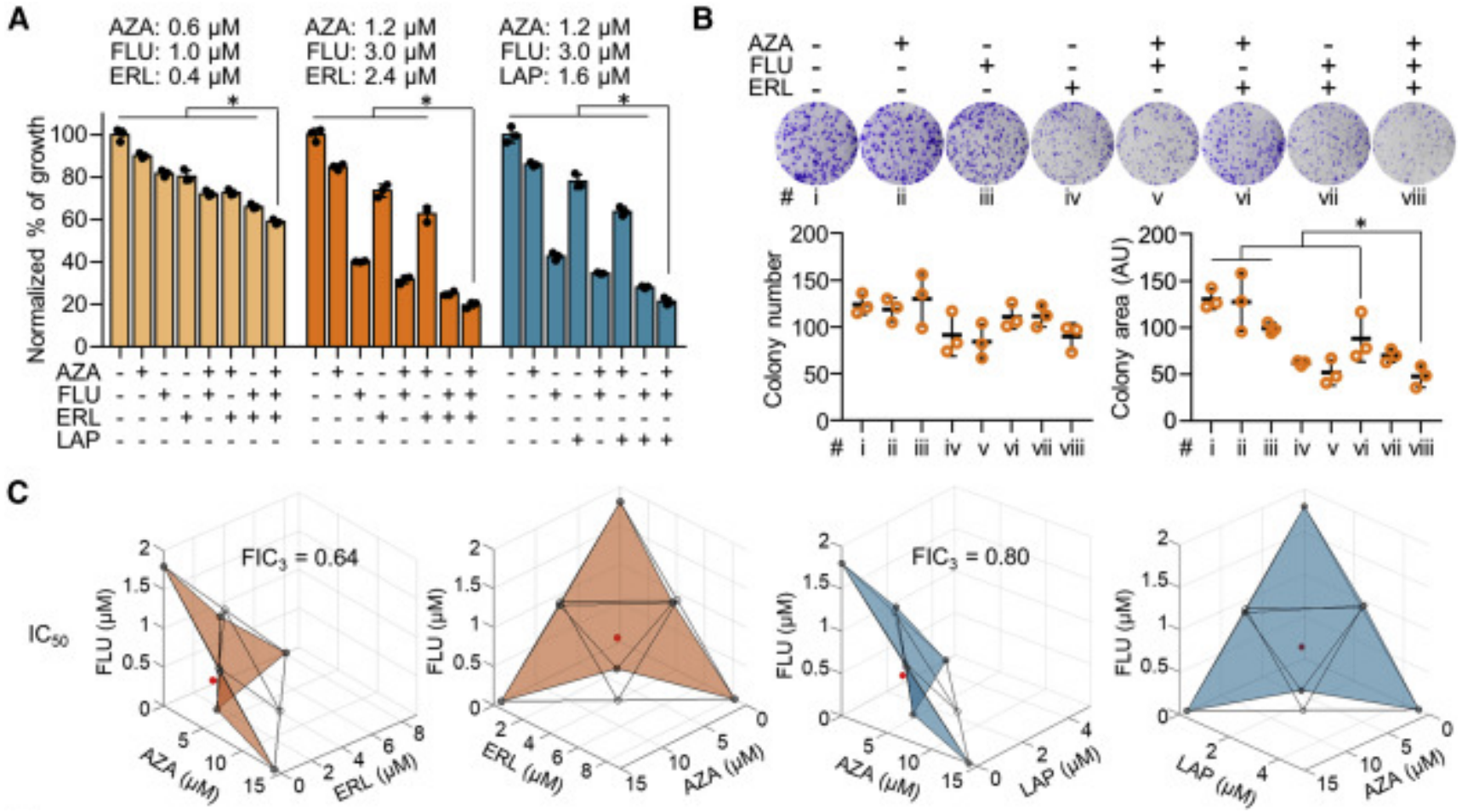




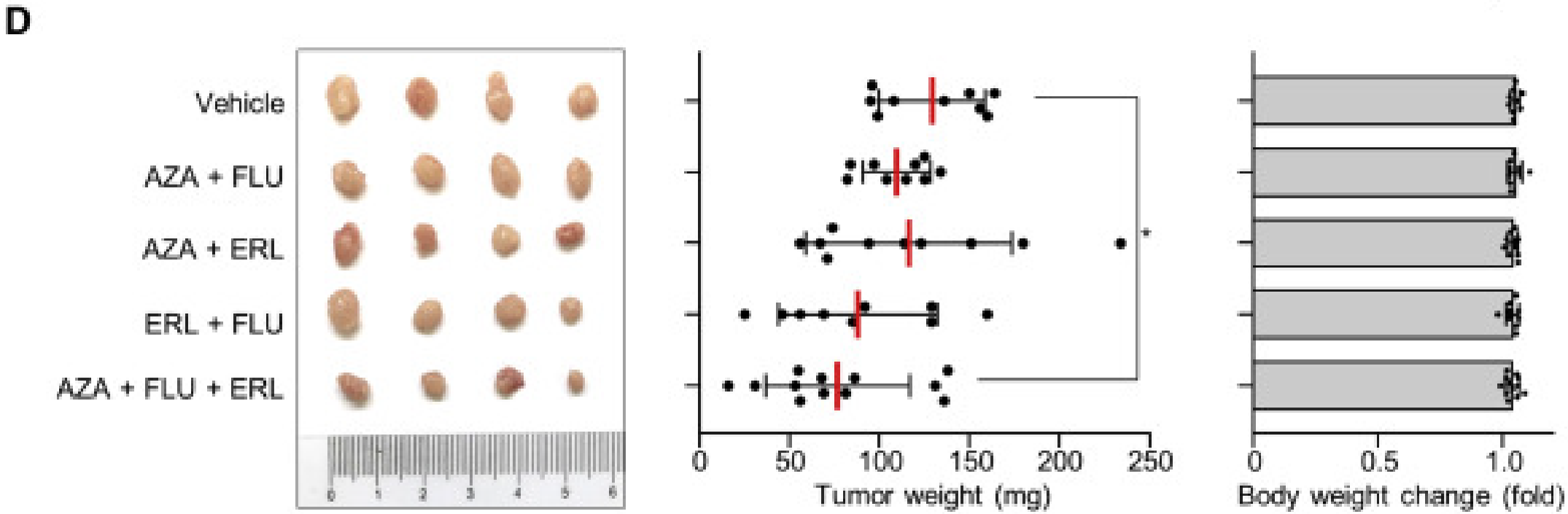
15 druggable genes were chosen

32x32x32gRNA were used

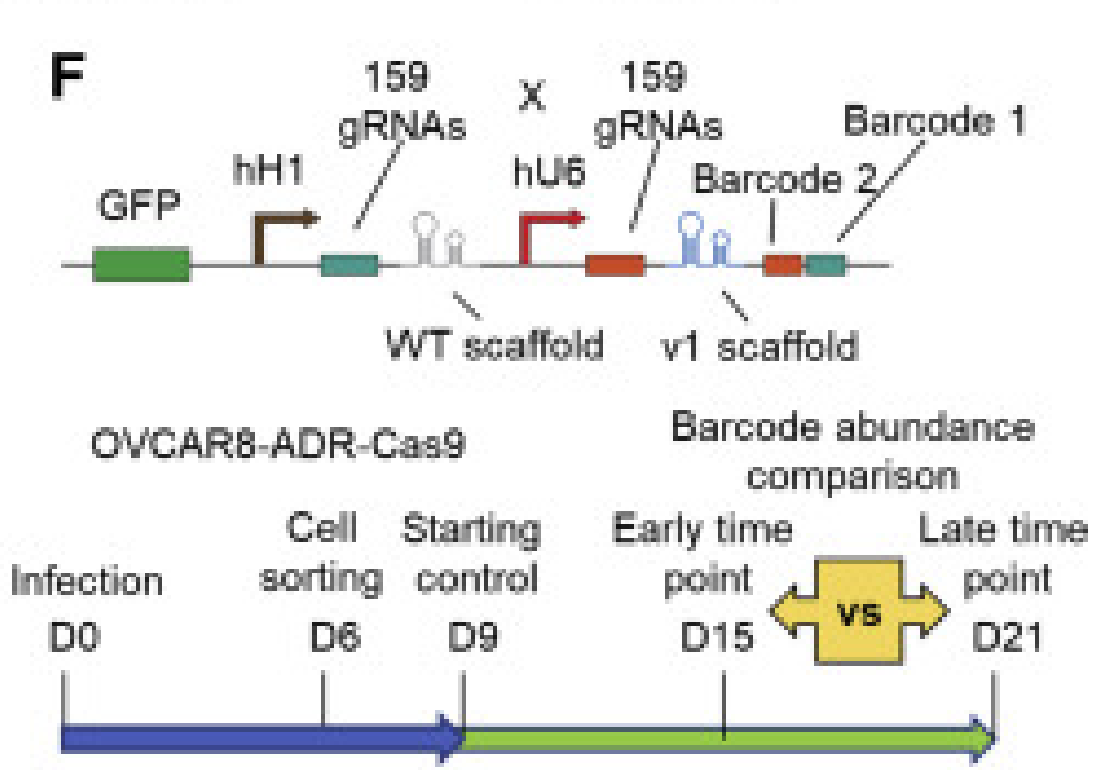
Validation of the hits in the screens using pharmacological inhibitors

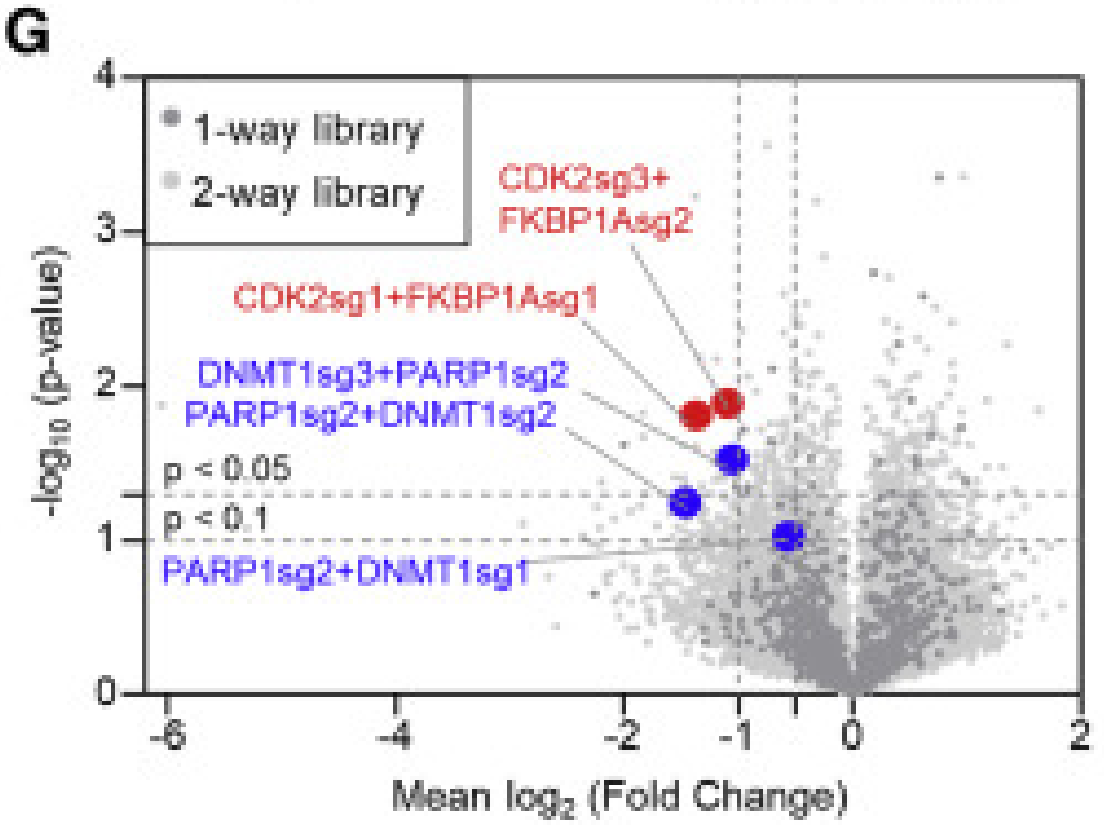


New combination therapy of drugs alters the tumor size in vivo

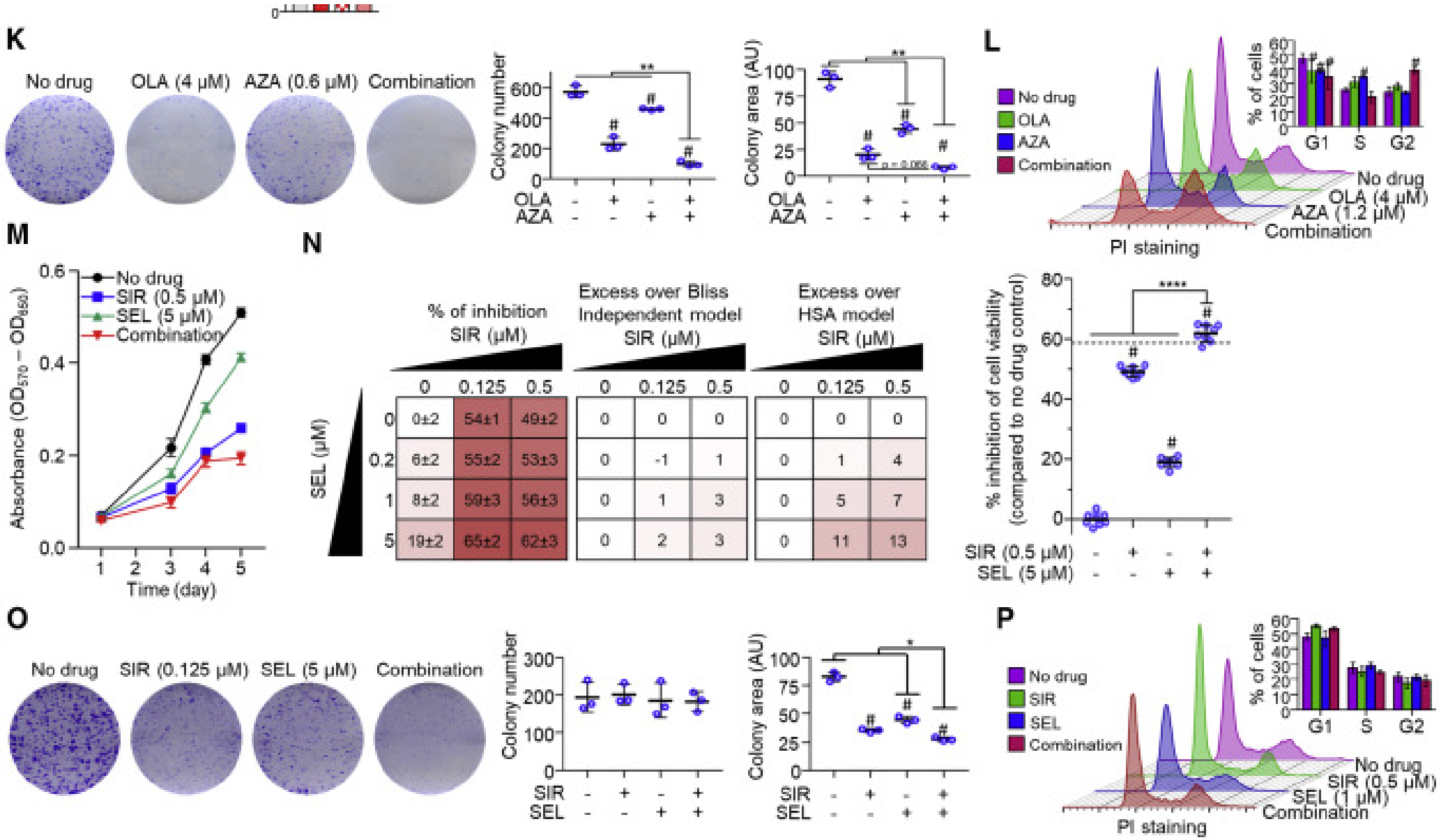


Double gene knock out-Ovarian cancer

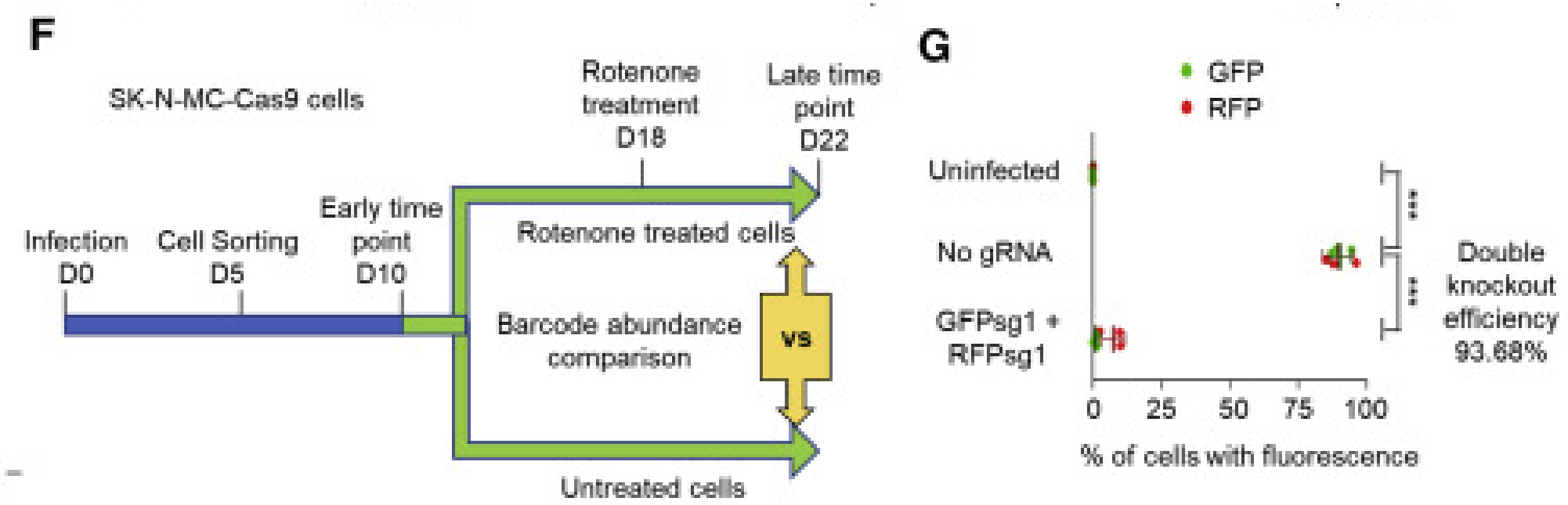




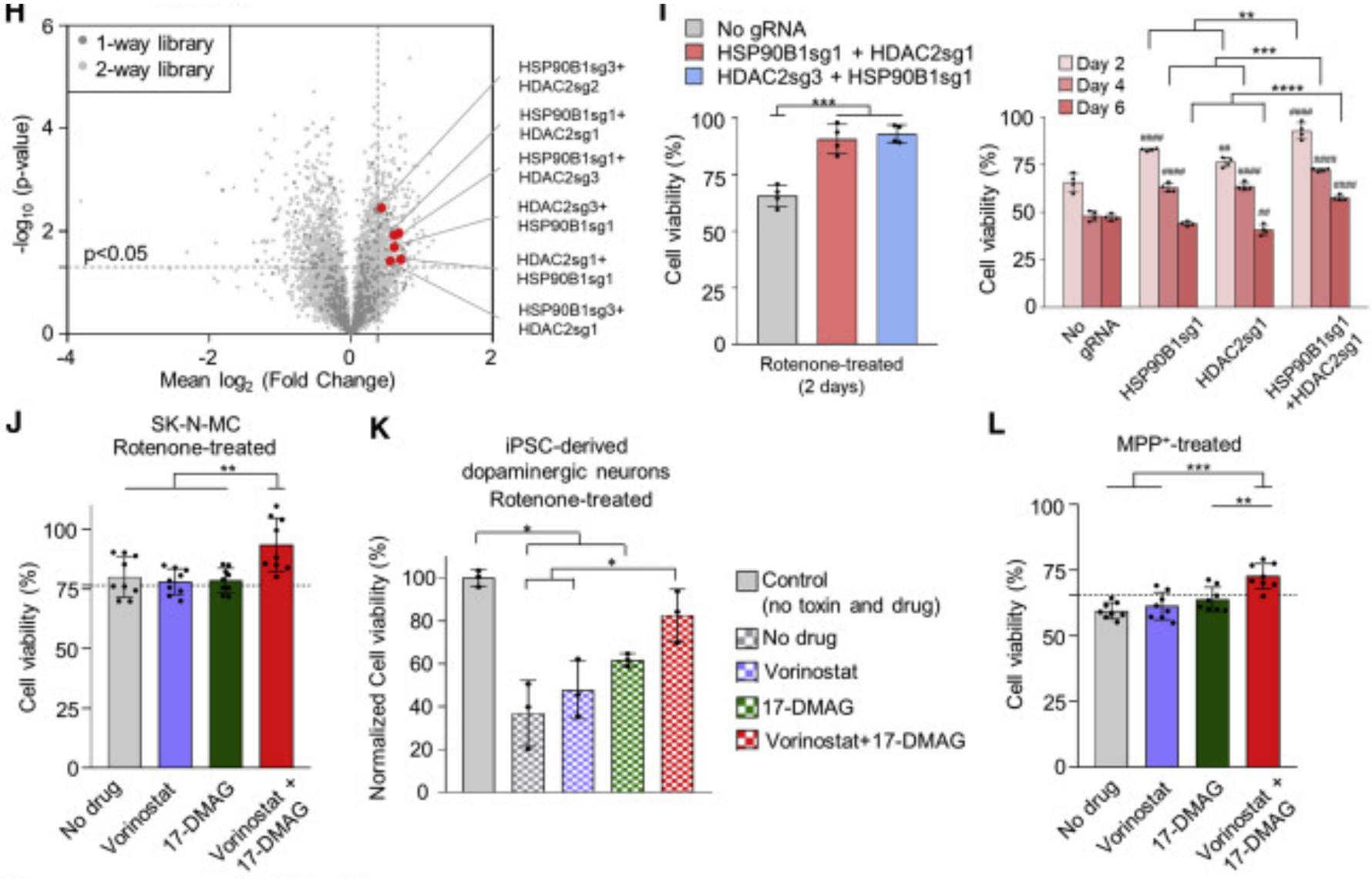
Validation of the hits in the screens using pharmacological inhibitors



Dual-Gene Knockout Screen Identifies a Drug Combination that Enhances Protection against PD Toxicity



Validation of the Hits

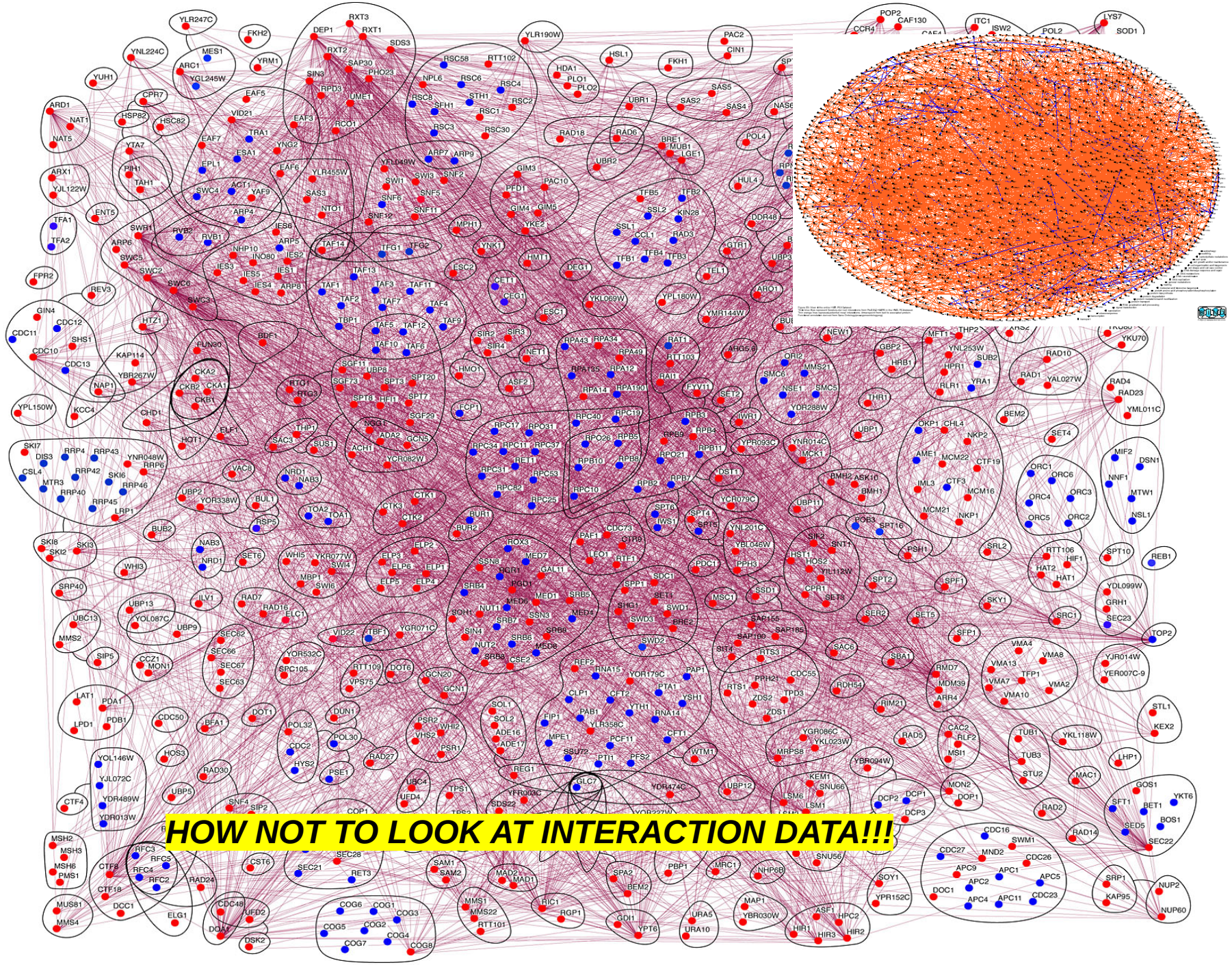


Conclusions

1. A nice strategy to identify novel combination of genes to identify new therapeutic targets.
2. Platfrom can be combined with CRISPRi to mimic drugs.
3. Platform can be combined with single cell RNA seq.

Questions:

1. Patient derived cell lines?
2. Efficiency of the guide RNAs


HOW NOT TO LOOK AT INTERACTION DATA!!!