Minimal Libraries and Synthetic Augmentation of CRISPR-Cas9 Screens for Drug Target Discovery

CRISPR Genome Engineering for Cellular Modelling and Screening

10th October 2024

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'Undruggable' cancer targets…

~3,000 gene-products are part of the “druggable genome”

~20,000 human protein-coding genes

Drugging the 'undruggable' cancer targets

Chi V. Dang ✉, E. Premkumar Reddy ✉, Kevan M. Shokat ✉ & Laura Soucek ✉

Nature Reviews Cancer **17**, 502–508 (2017) | Cite this article

Abstract

The term 'undruggable' was coined to describe proteins that could not be targeted pharmacologically. However, progress is being made to 'drug' many of these targets, and therefore more appropriate terms might be 'difficult to drug' or 'yet to be drugged'. Many desirable targets in cancer fall into this category, including the RAS and MYC oncogenes, and pharmacologically targeting these intractable proteins is now a key challenge in cancer research that requires innovation and the development of new technologies. In this Viewpoint article, we asked four scientists working in this field for their opinions on the most crucial advances, as well as the challenges and what the future holds for this important area of research.

'Undruggable' cancer targets… CRISPR-Cas9

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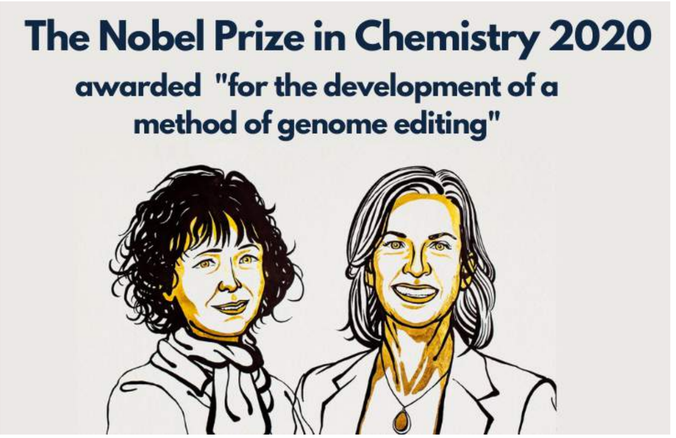
32k Accesses | **472** Citations | **81** Altmetric | Metrics

Abstract

The term 'undruggable' was coined to describe proteins that could not be targeted pharmacologically. However, progress is being made to 'drug' many of these targets, and therefore more appropriate terms might be 'difficult to drug' or 'yet to be drugged'. Many desirable targets in cancer fall into this category, including the RAS and MYC oncogenes, and pharmacologically targeting these intractable proteins is now a key challenge in cancer research that requires innovation and the development of new technologies. In this Viewpoint article, we asked four scientists working in this field for their opinions on the most crucial advances, as well as the challenges and what the future holds for this important area of research.

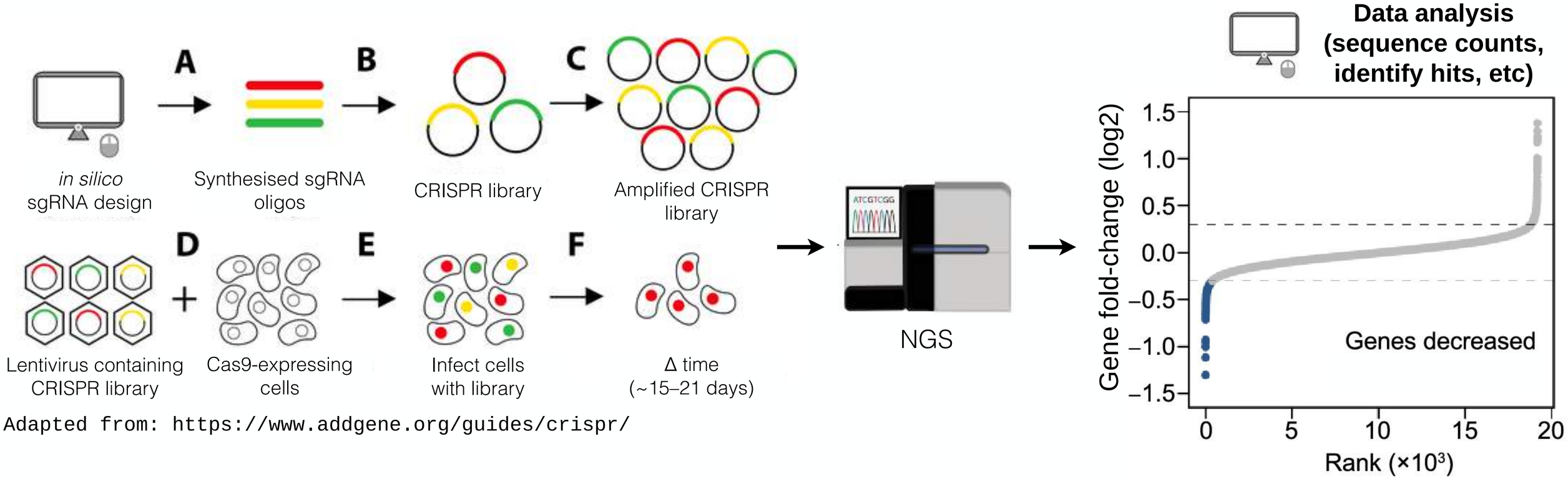
Functional genomic screens

- Zinc finger nucleases (ZFNs)
- Transcription activator-like effector nucleases (TALEN)
- RNA interference using small interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs)
- Clustered Regularly Interspaced Short Palindromic Repeats and associated endonuclease (CRISPR-Cas9)



Jinek et al. (2012). Science

CRISPR-Cas9 screen general pipeline



Adapted from: https://www.addgene.org/guides/crispr/

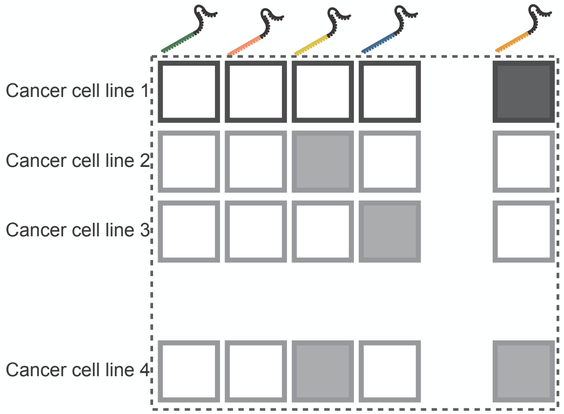
Key steps in CRISPR-Cas9 screening (gRNA design and data analysis) involves computational analyses

Using sequencing, you count the number of sgRNAs at the start and at the end. sgRNA whose cut lead to cell death are lost

Pre-clinical Discovery of Cancer Synthetic Lethal Interactions using CRISPR-Cas9 screens

Finding genetic biomarkers of essential genes in cancer

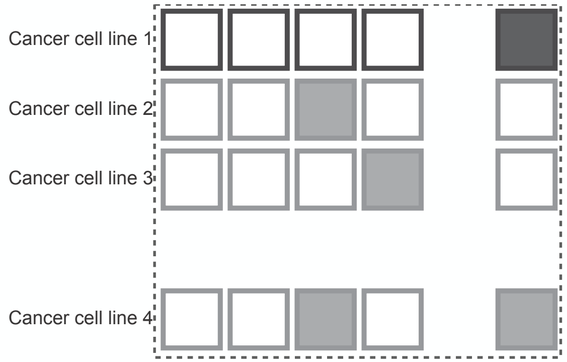
Gene Essentiality (CRISPR-Cas9)



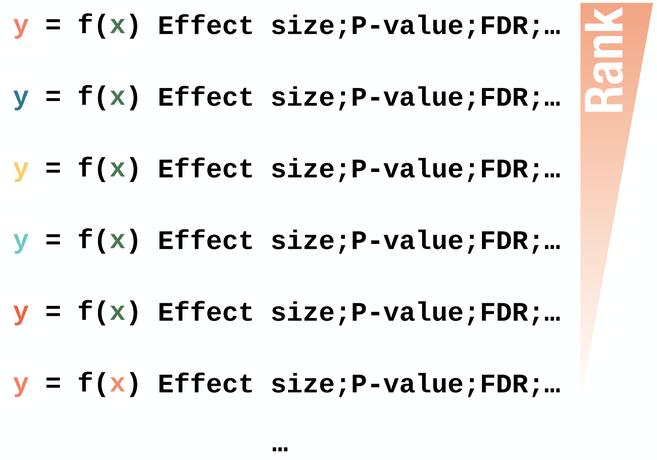
Continuous
(log2 fold-change)

$Y = f(X)$

Genetic Alterations (e.g. mutations)



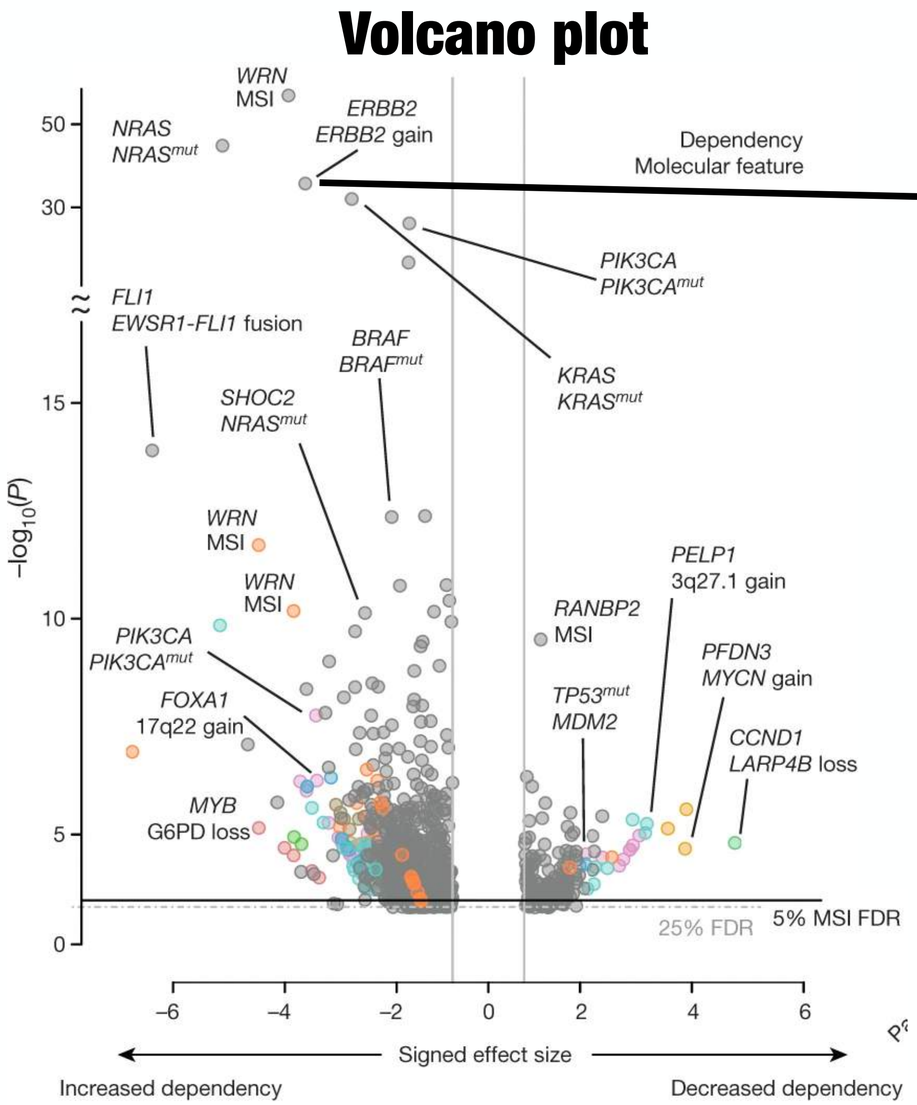
Binary
(1=present; 0=absent)

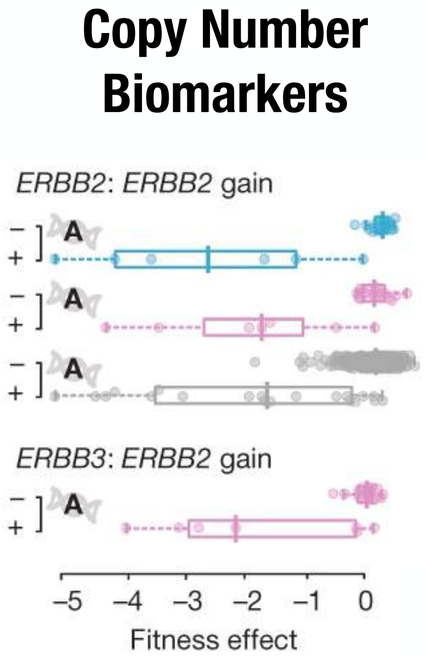


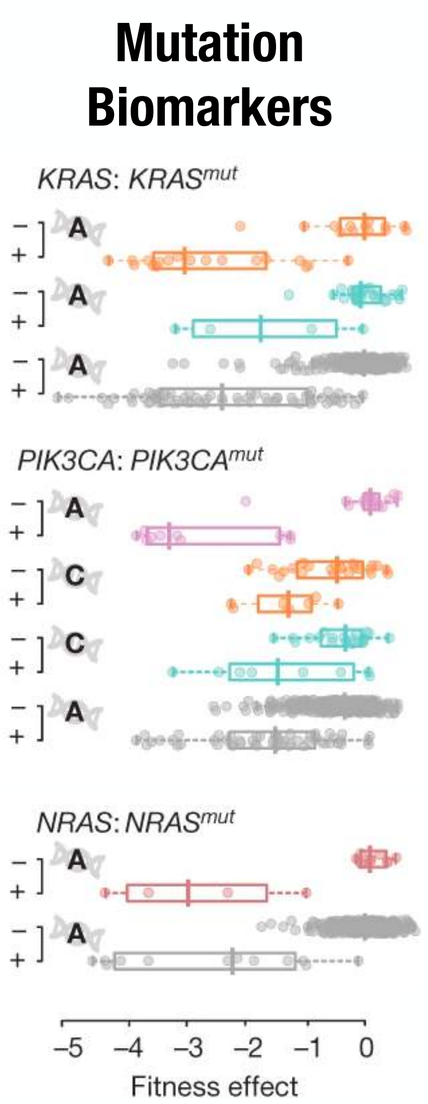
Systematic association between CRISPR-Cas9 gene essentiality and genetic alterations, e.g. such as mutations and copy-number alterations

Behan et al. (2019) Nature

One-way ANOVAs for systematic identification of cancer biomarkers

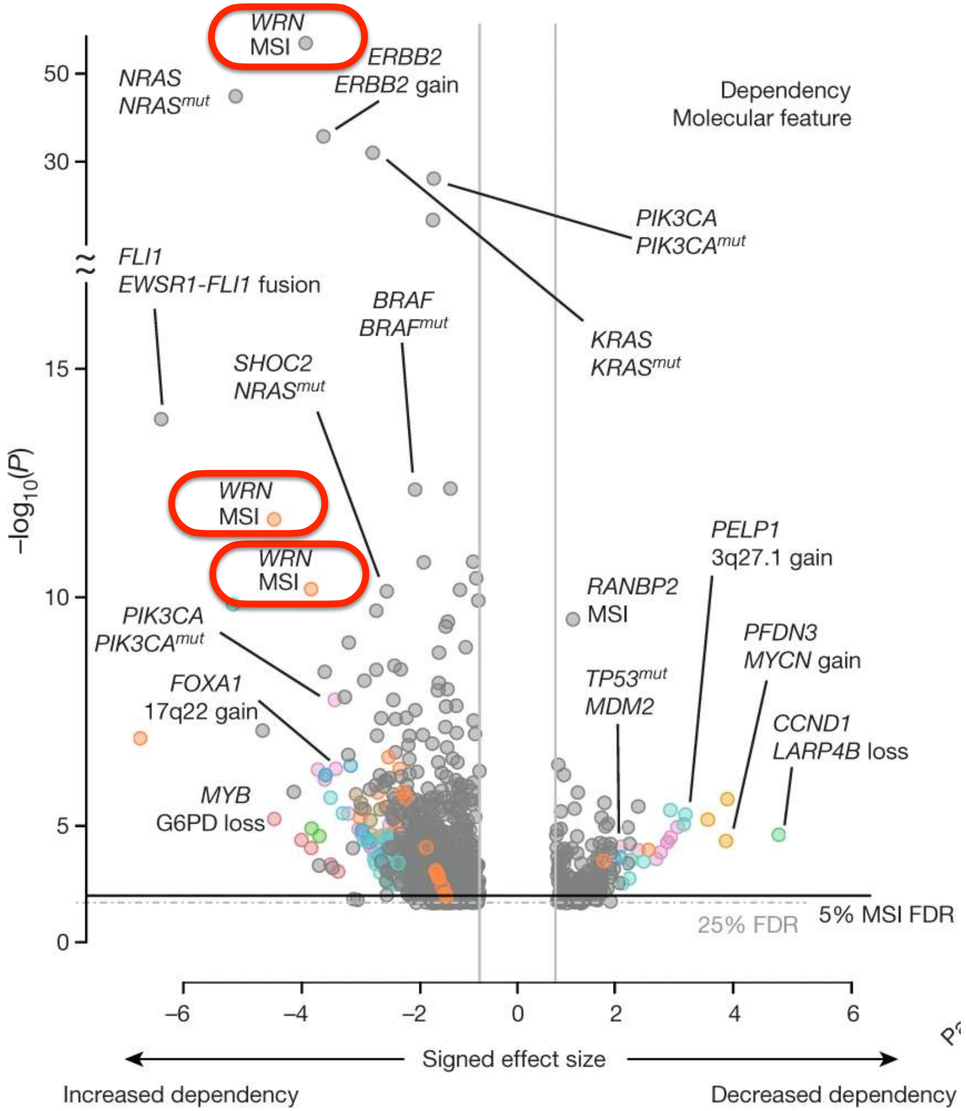






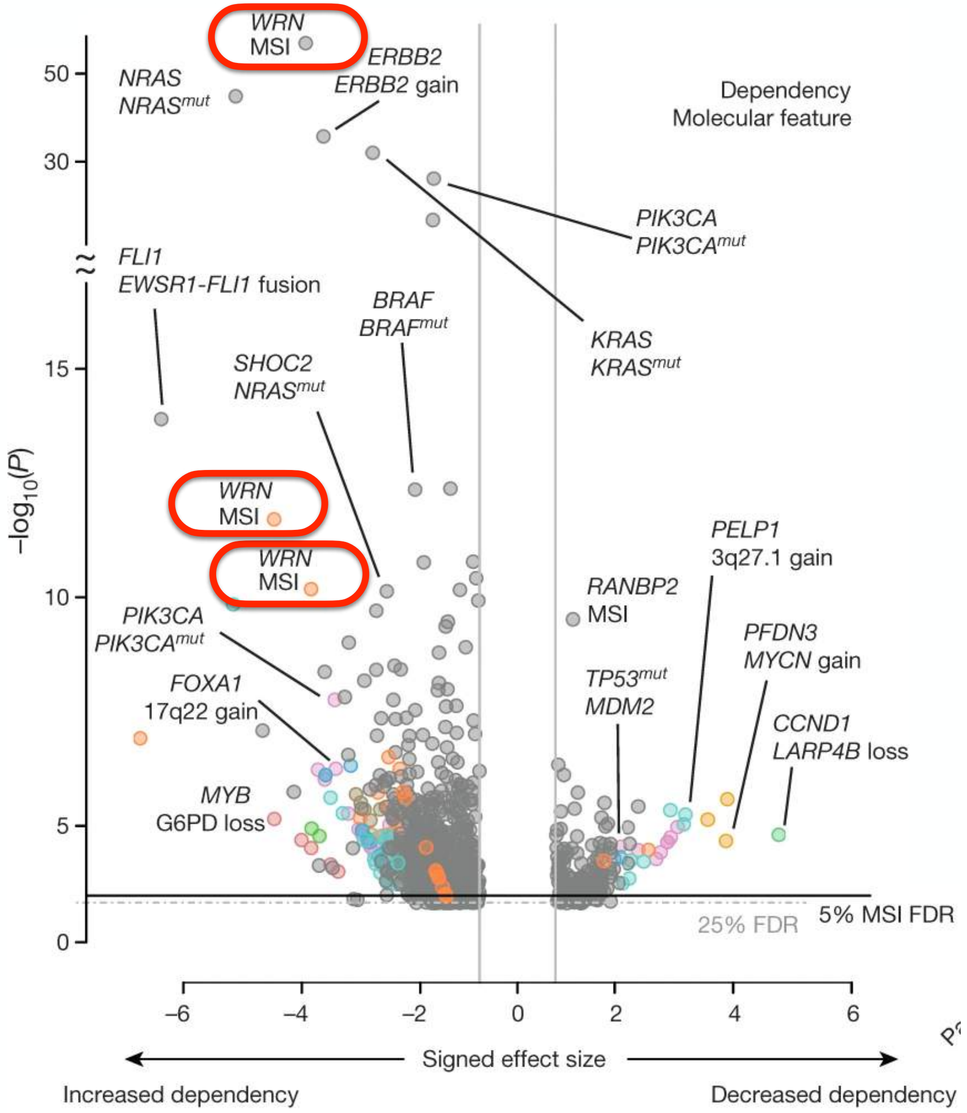
Behan et al. (2019) Nature

Synthetic lethal interaction between WRN and microsatellite instability



Behan et al. (2019) Nature

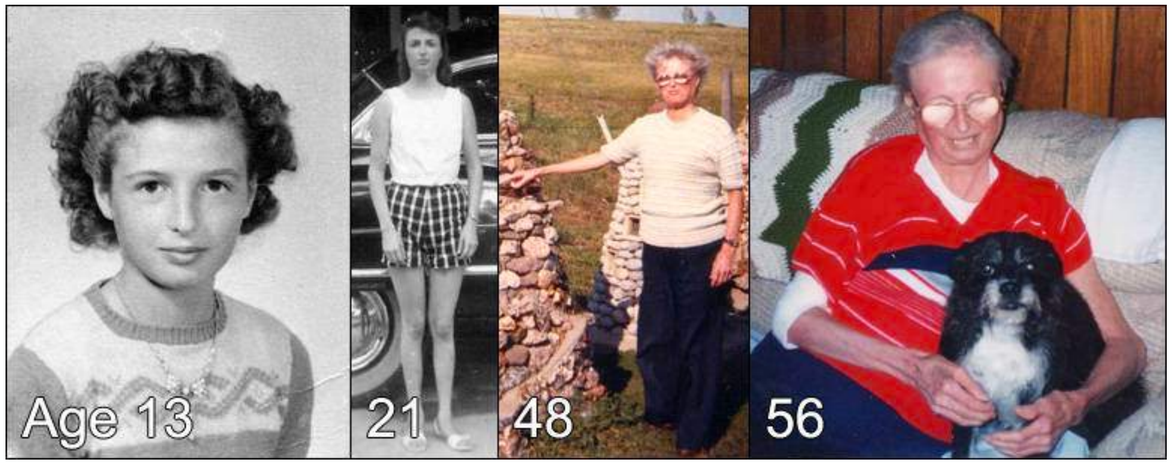
Werner Helicase and Werner syndrome



Several cancers with microsatellite instability (MSI) are sensitive Werner Syndrome RecQ Like Helicase (WRN) knockout

WRN is involved in DNA repair and maintenance

Werner Syndrome - premature ageing and increased risk of developing cancer



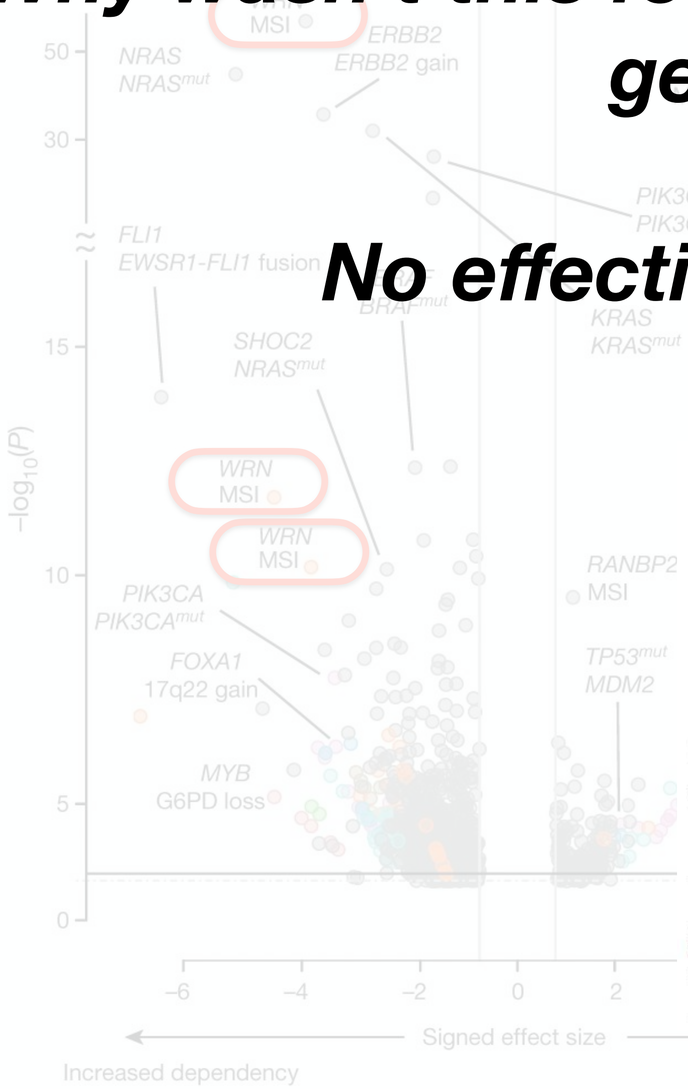
Behan et al. (2019) Nature

https://dlmp.uw.edu/research-center/werner/

Werner Helicase and Werner syndrome

Why wasn't this found with drug screens and only with functional genetic screens (CRISPR-Cas9)?

No effective drug is available for WRN inhibition



IDEAYA Announces Presentations at AACR Annual Meeting 2023 for Potential First-in-Class Synthetic Lethality Programs IDEE397 (MAT2A), IDE161 (PARG) and Werner Helicase

NEWS PROVIDED BY
IDEAYA Biosciences, Inc. →
14 Mar, 2023, 16:31 ET

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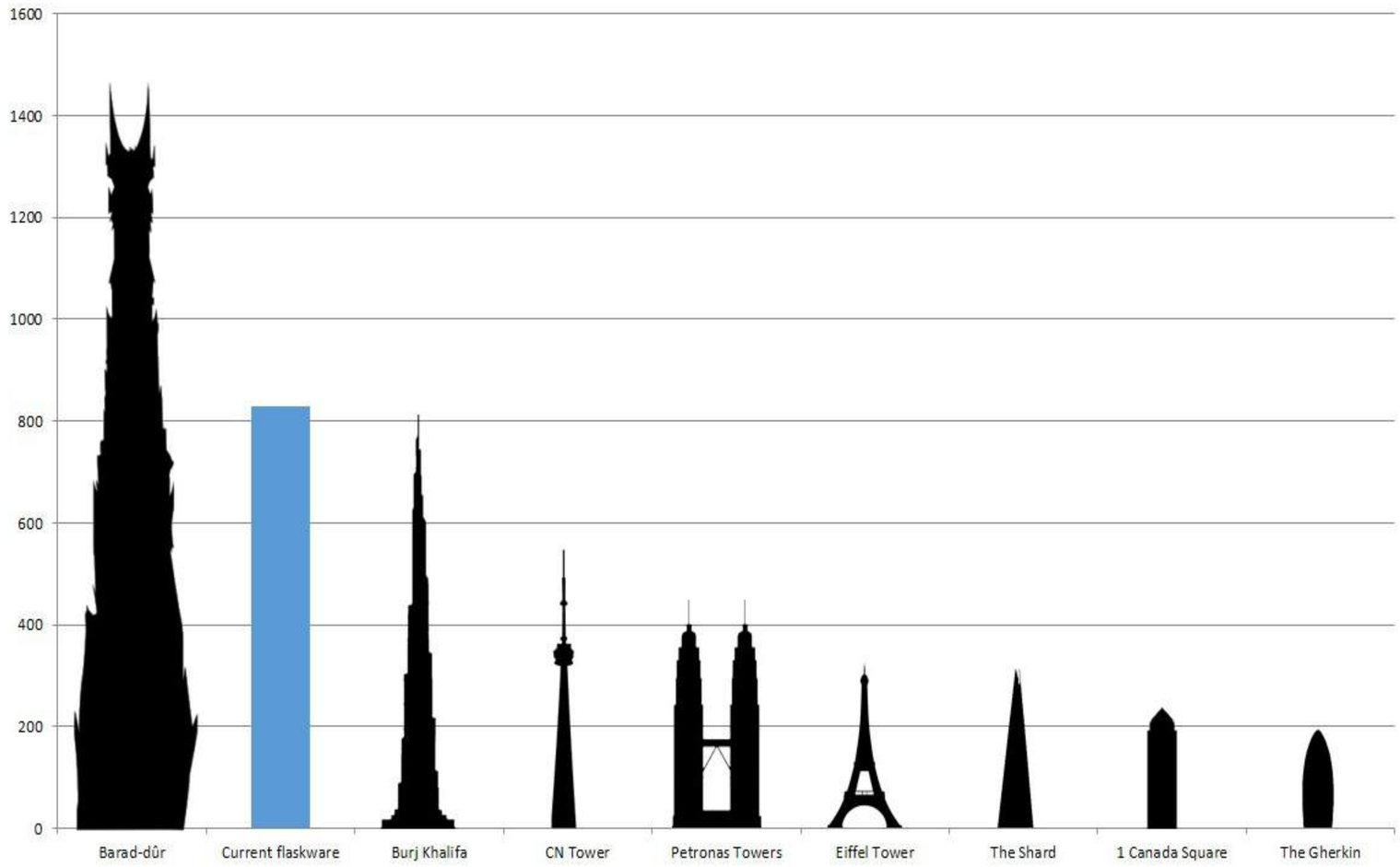
Behan et al. (2019) Nature

https://dlmp.uw.edu/research-center/werner/

Minimal genome-wide CRISPR-Cas9 screens

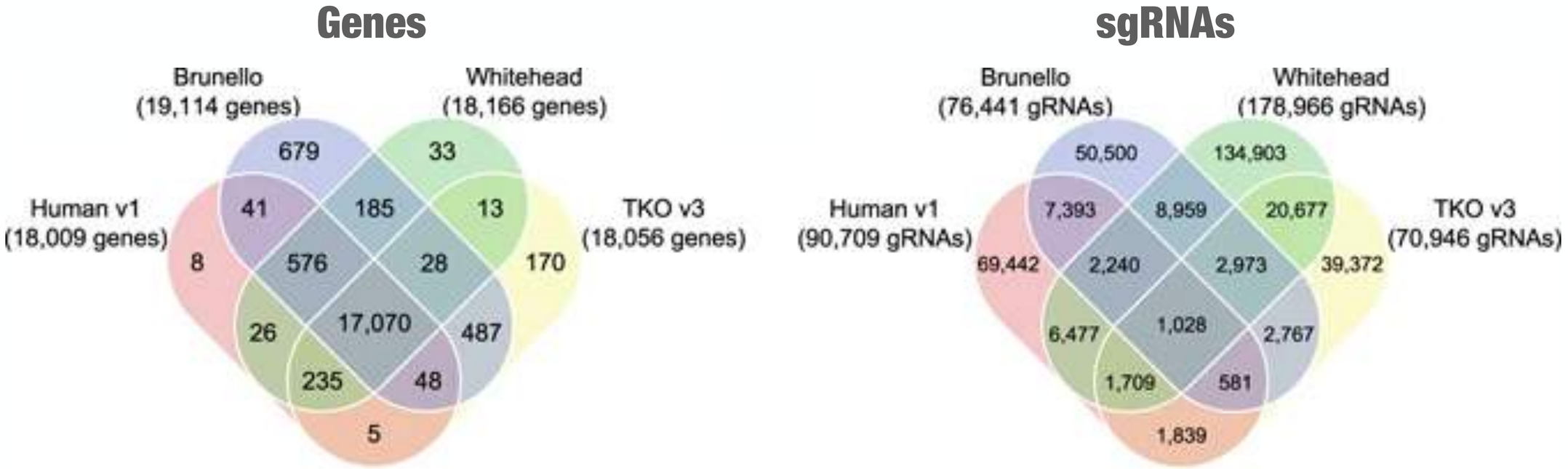
Gargantuan effort of CRISPR-Cas9 screening at scale

~10-15 five-layer flasks per cell line



Courtesy of CGaP and Fiona Behan

Multiple *in silico* CRISPR-Cas9 sgRNA library design



Ong SH, Li Y, Koike-Yusa H, Yusa K (2017) Optimised metrics for CRISPR-KO screens with second-generation gRNA libraries. Sci Rep 7: 7384

Although the number of genes targeted are largely the same the sgRNA used to target them is largely different across commonly used libraries

The field has converged, although this is still an active area of research, particularly to reduce the size of the sgRNA libraries.

Off-targets and on-target efficacy of sgRNAs

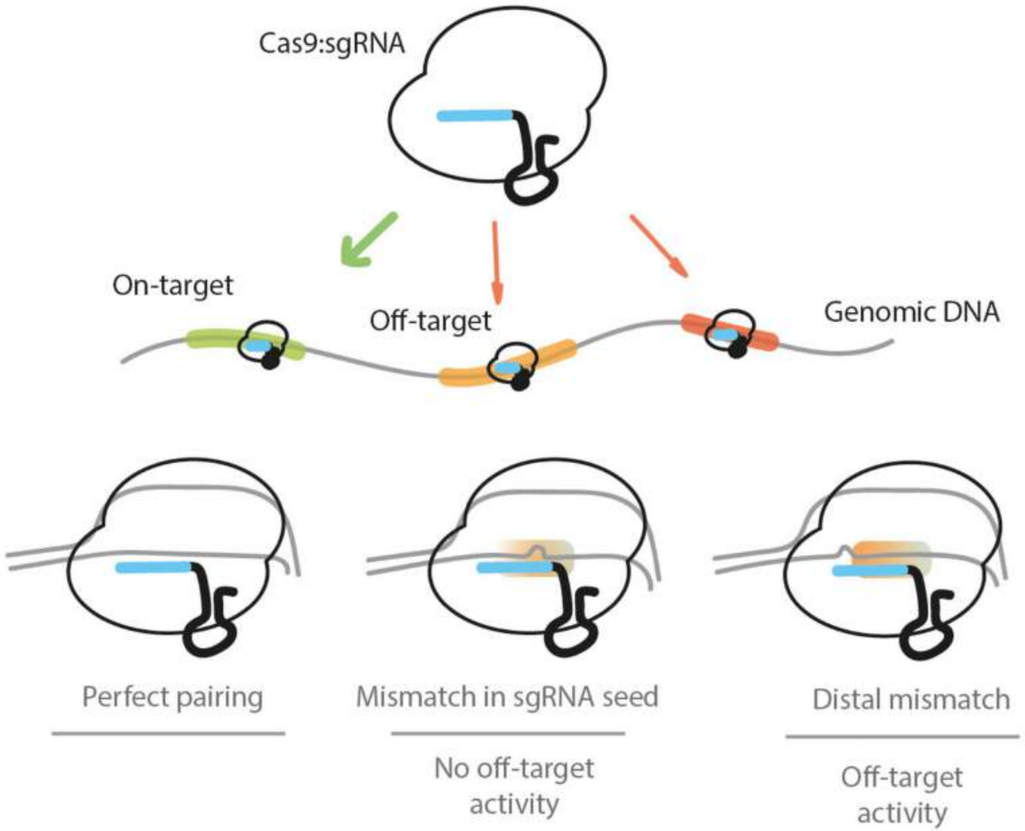
On-Target Efficiency

Precision and effectiveness of a sgRNA to direct the gene-editing enzyme to modify only the intended genomic location. High on-target efficiency ensures desired and accurate genetic alterations.

Off-Targets

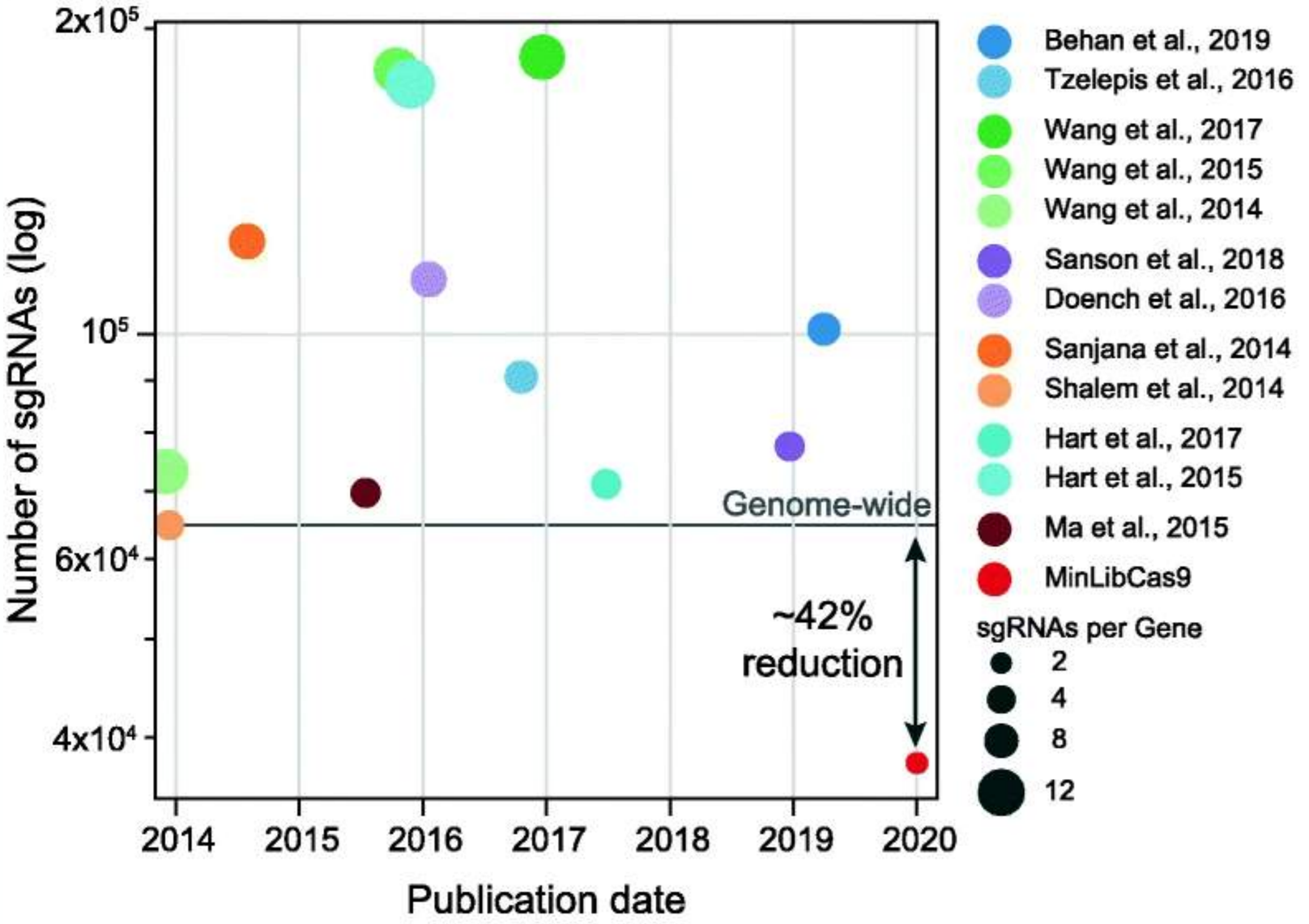
sgRNAs can bind to and cleave unintended genomic sites, potentially causing unwanted mutations and genomic instability.

Solution, **multiple sgRNAs per target gene** increases the robustness of CRISPR libraries



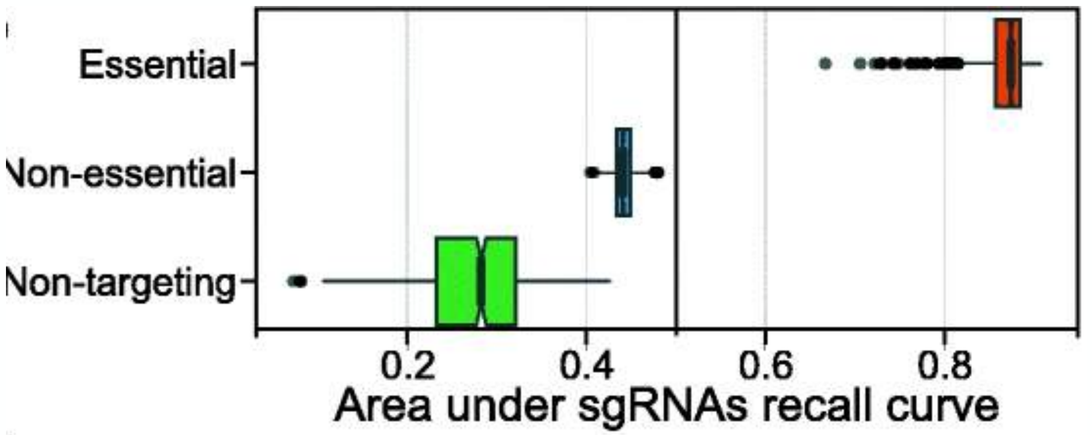
Vicente MM, et al. Front Cell Dev Biol. 2021;9: 718466. doi:10.3389/fcell.2021.718466

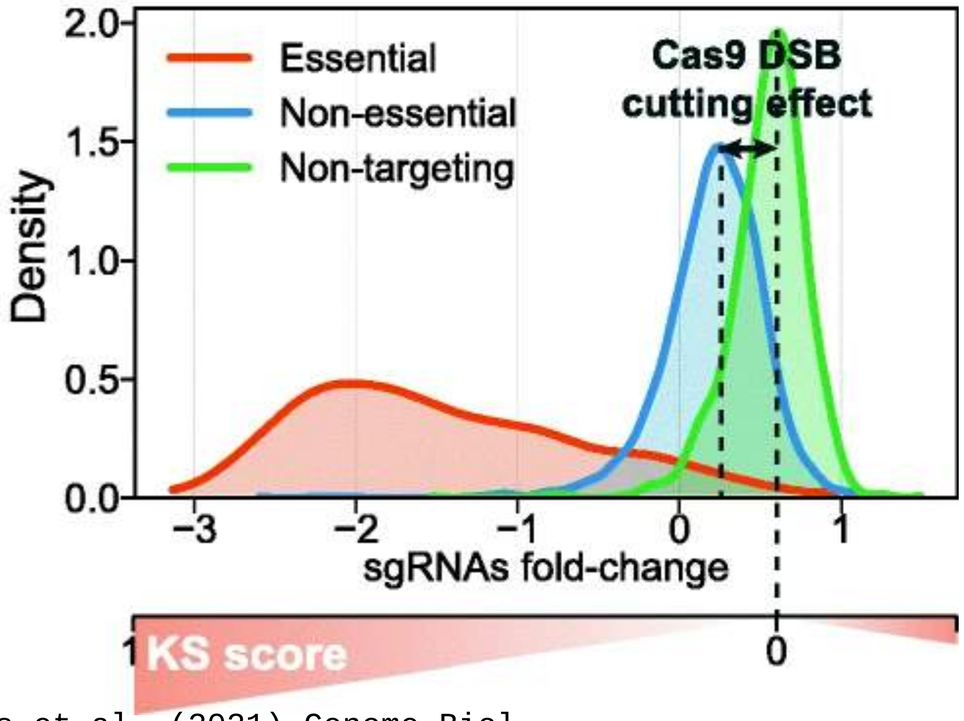
Minimal Genome-wide CRISPR-Cas9 library



Gonçalves et al. (2021) Genome Biol.

Prioritisation of guides with stronger “on-target” effects





Compiling multiple genome-wide CRISPR-Cas9 sgRNA libraries

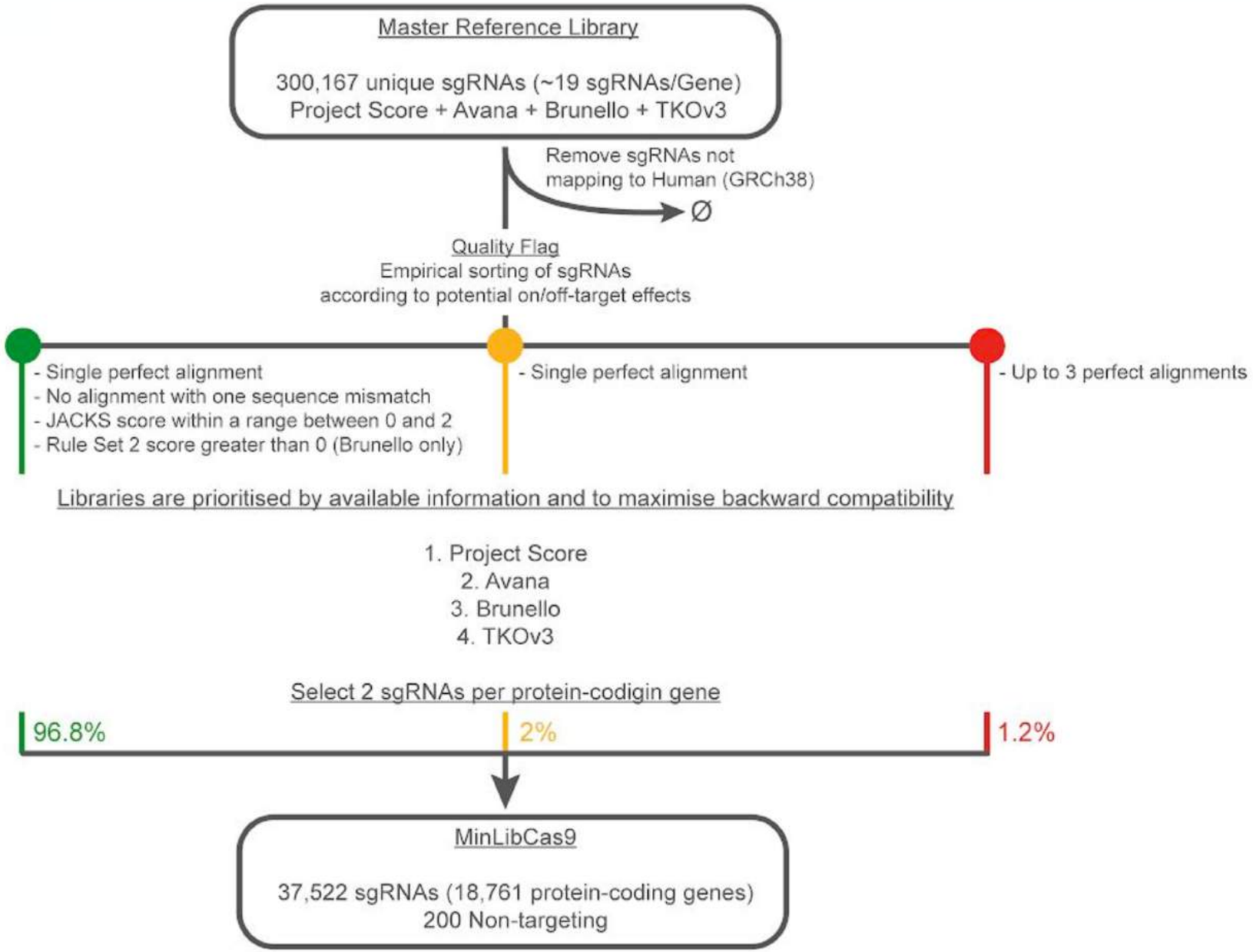
- Project Score - Kosuke Yusa V1.1; Avana; Brunello;TKOv3

Standardised annotation for 300,167 unique sgRNAs with a median of 19 sgRNA per gene

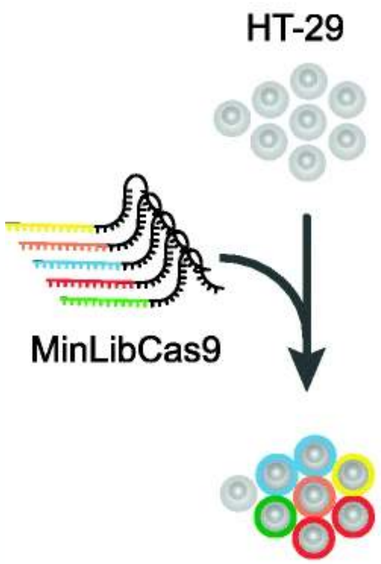
- KS-score
- Updated mapping to GRCh38
- Off-target summaries using WGE
- Multiple guide efficacy metrics (JACKS, Rule Set 2, FORECasT)
- CRISPOR scores, e.g. MIT specificity and CrisprScan

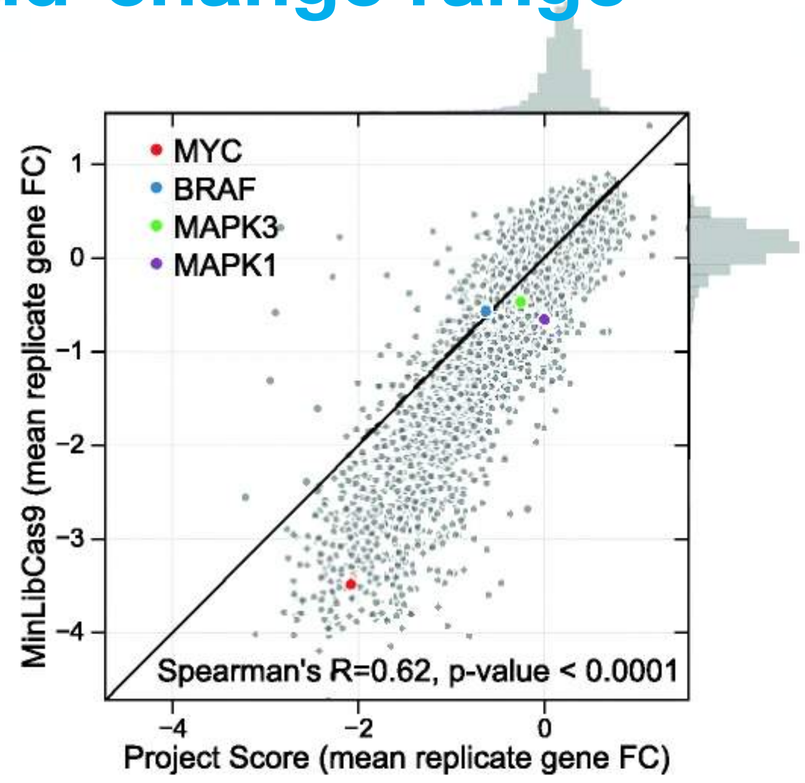
Gonçalves et al. (2021) Genome Biol.

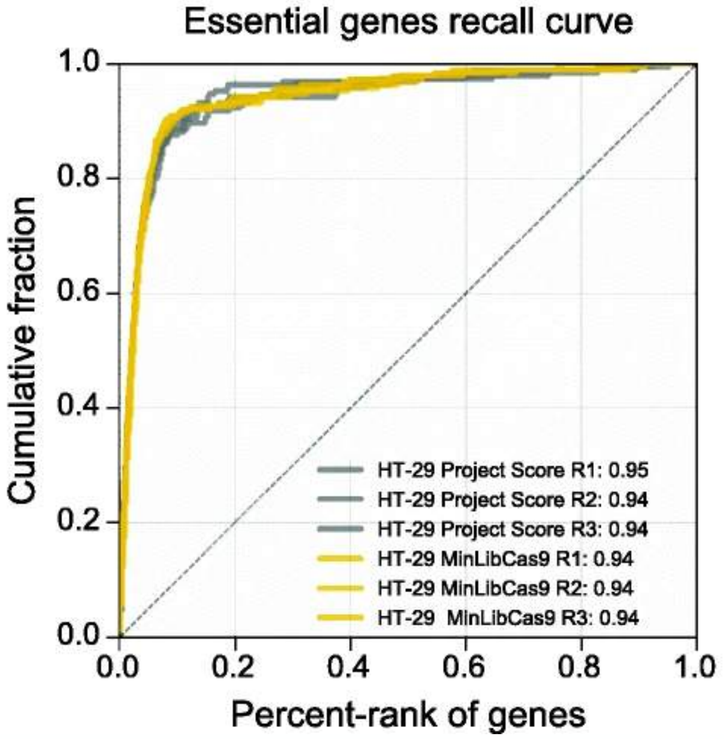
Data-driven design of a minimal genome-wide CRISPR-Cas9 lib.



MinLibCas9 recapitulates large genome-wide libraries and increases gene fold-change range





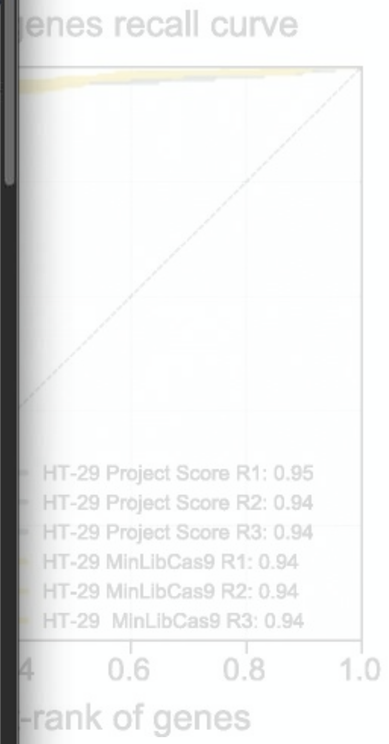


Synthesised and cloned the final MinLibCas9 library and re-screened the HT-29 colorectal cancer cell line

MinLibCas9 showed an higher fold-change range, improving the identification of cancer dependencies

Gonçalves et al. (2021) Genome Biol.

MinLibCas9 rec… …aries and increases gene …



Synthesised and clo… …d the HT-29 colorectal cancer cell line

MinLibCas9 showed … …ntification of cancer dependencies

Addgene: MinLibCas9

addgene.org/pooled-library/garnett-minlibcas9-li…

Relaunch to update

addgene

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Pooled Libraries / MinLibCas9

Garnett Lab MinLibCas9 Library

(Pooled Library #164896)

Print

Purpose

This human genome-wide CRISPR knockout library is designed with two single guide RNAs (sgRNA) per gene.

The minimal library has been optimized through empirical assessment using comprehensive sgRNA efficiency metrics.

Vector Backbone

pKLV2-U6gRNA(BbsI)-ccdB-PGKpuro2ABFP-W - does not express Cas9

Depositing Labs

Mathew Garnett

Publication

Goncalves et al Genome Biol. 2021 Jan 21;22(1):40. doi: 10.1186/s13059-021-02268-4. (How to cite)

Related items

From this article

Mathew Garnett

pKLV2-U6gRNA(BbsI)-ccdB-PGKpuro2ABFP-W

Additional resources

Guide to Using Pooled Libraries

All CRISPR Pooled Libraries

CRISPR Guide

Lentiviral Plasmids

Ordering

Item	Catalog #	Description	Quantity	Price (USD)	
Pooled Library	164896	MinLibCas9 Library†	1	$540	Add to Cart

Available to Academic and Nonprofits Only

† A Cas9 plasmid is NOT included with this item and will have to be ordered separately.

Library Details

Species: Human

Genes targeted: 18,761

gRNAs: 37,722

Controls: 200 non-targeting sgRNAs

Lentiviral Generation: 3rd

Resource Information

Protocols:

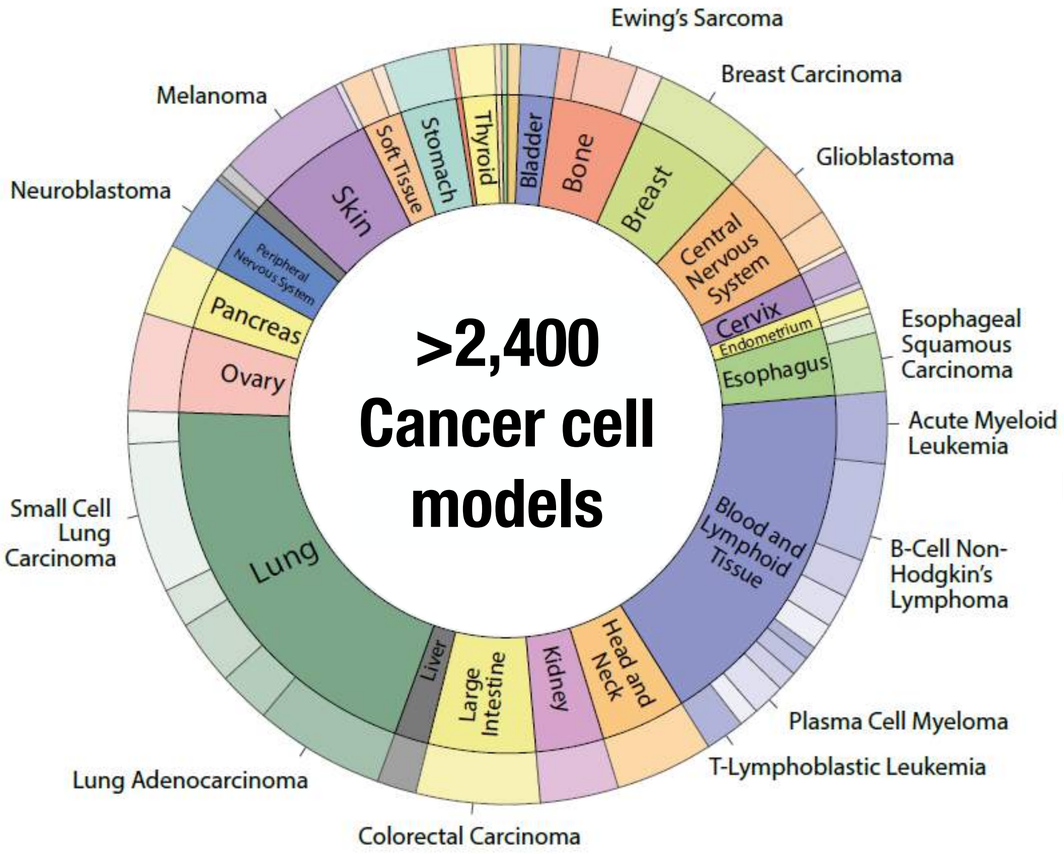
- Propagation of MinLibCas9 Library (DOC, 88 KB)
- Primers and PCR protocols to prepare samples of amplified library for deep sequencing verification (DOC, 133 KB)

Help Center

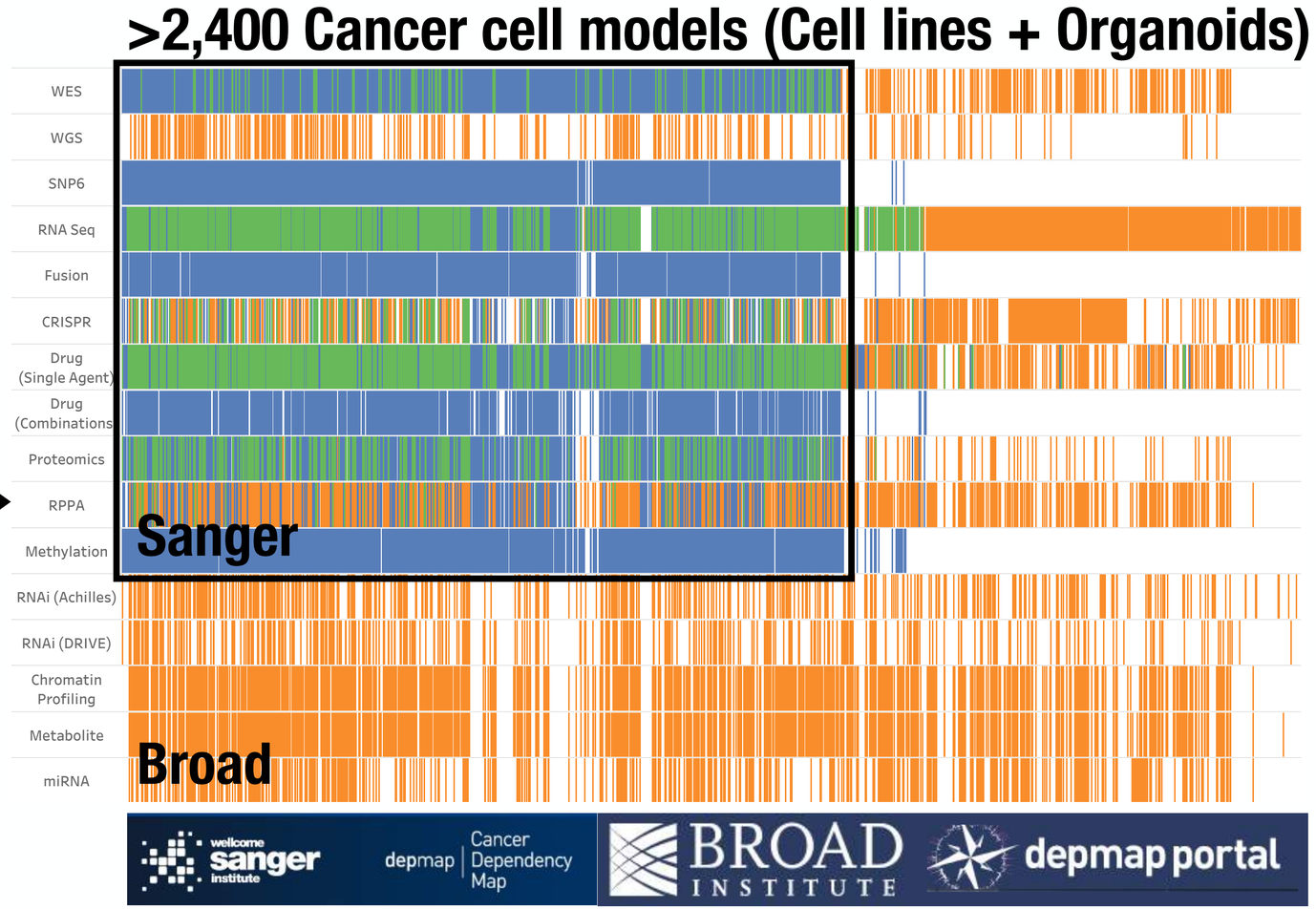
https://www.addgene.org/pooled-library/garnett-minlibcas9-library/

Multi-omics synthetic augmentation of CRISPR-Cas9 screens

Cancer Translation: Multi-omics



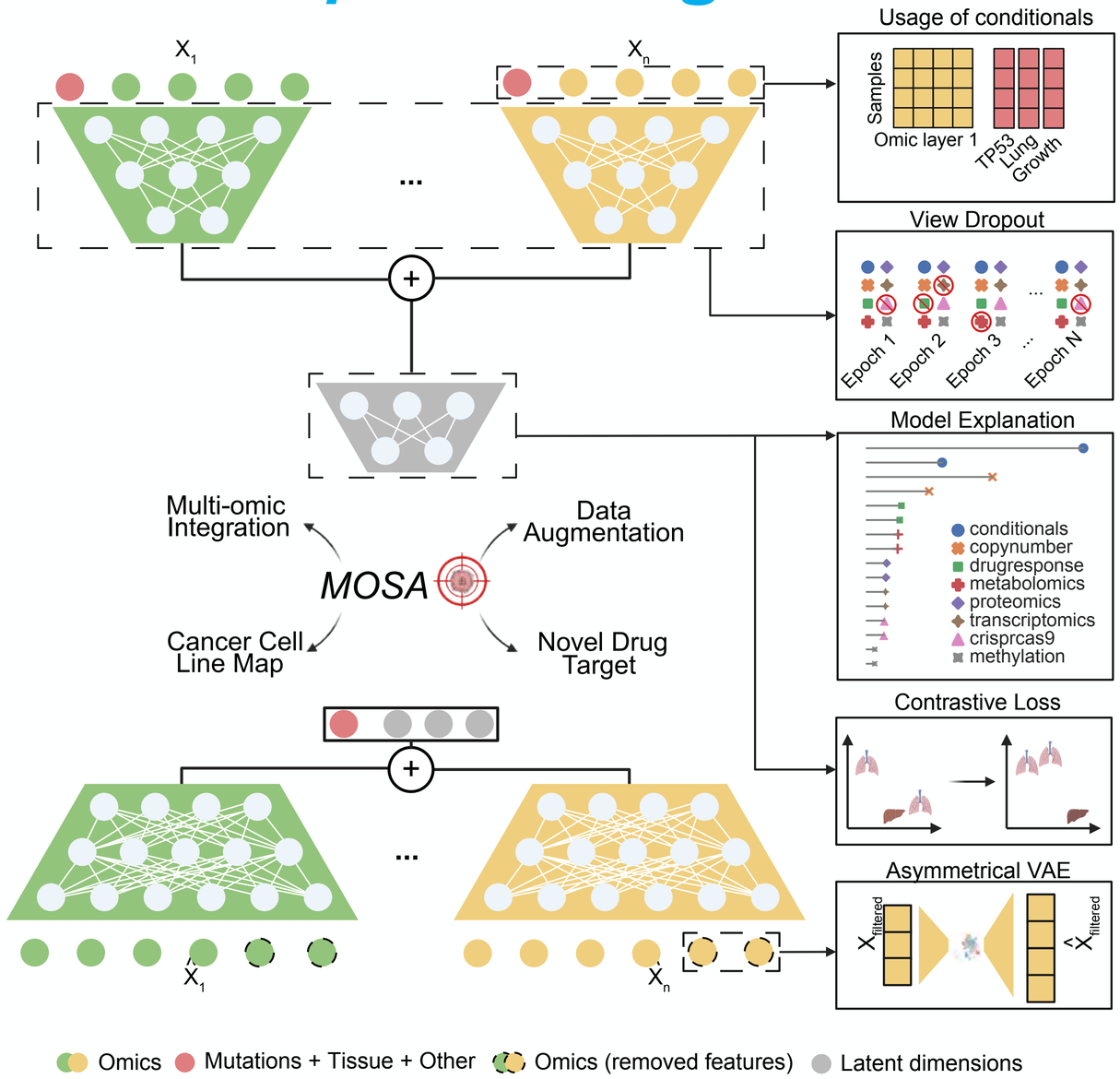
cellmodelpassports.sanger.ac.uk



Cancer gene, therapeutic biomarker and target discovery

Garnett et al. 2012 Nature
Iorio et al. 2016 Cell
Behan et al. 2019 Nature
van der Meer et al., 2019, Nucleic Acids Res
Gonçalves*, Poulos*, Cai* et al., 2022, Cancer Cell

Multi-Omic Synthetic Augmentation (MOSA)

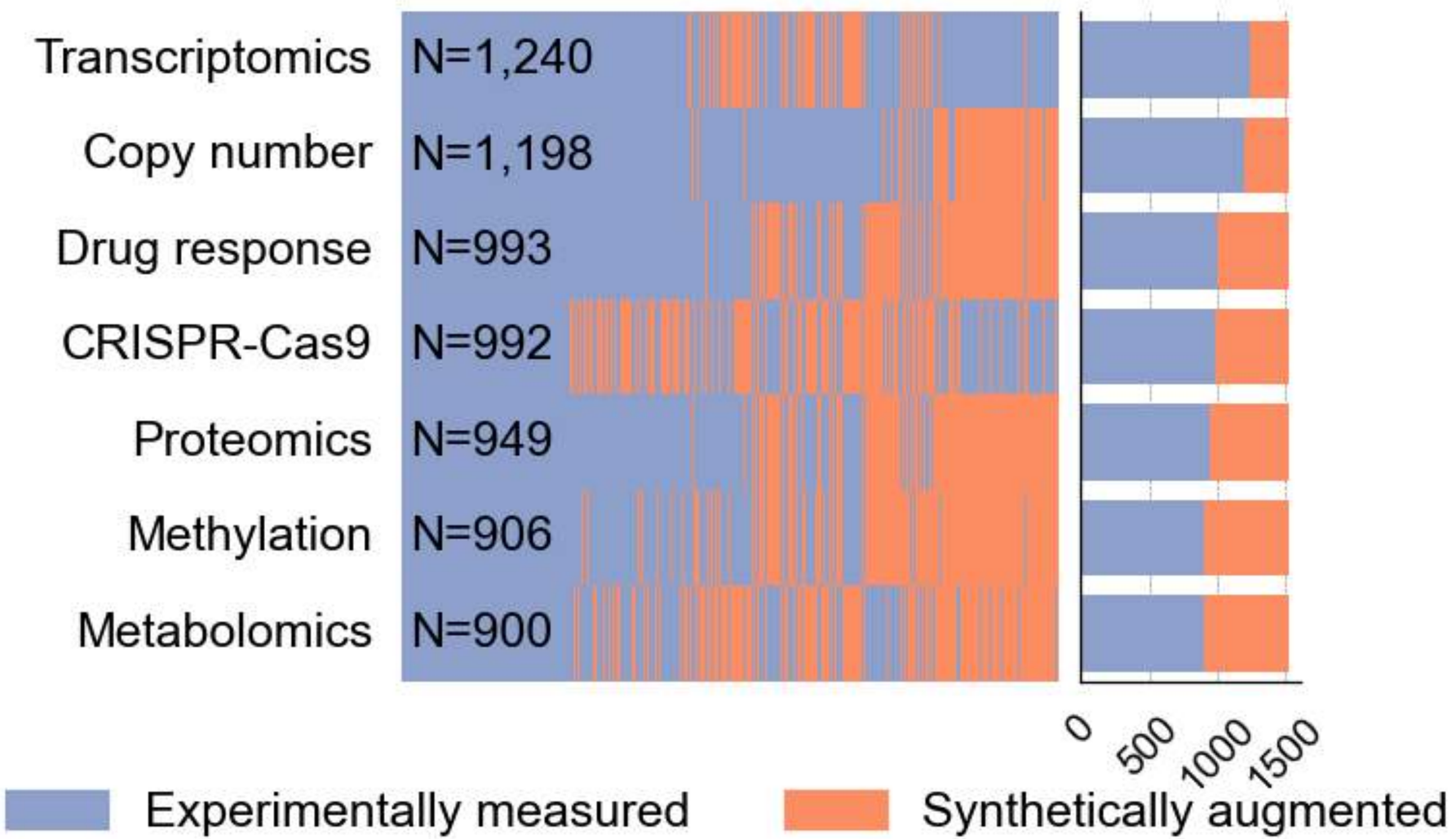


Unsupervised deep learning approach, i.e. variational autoencoder

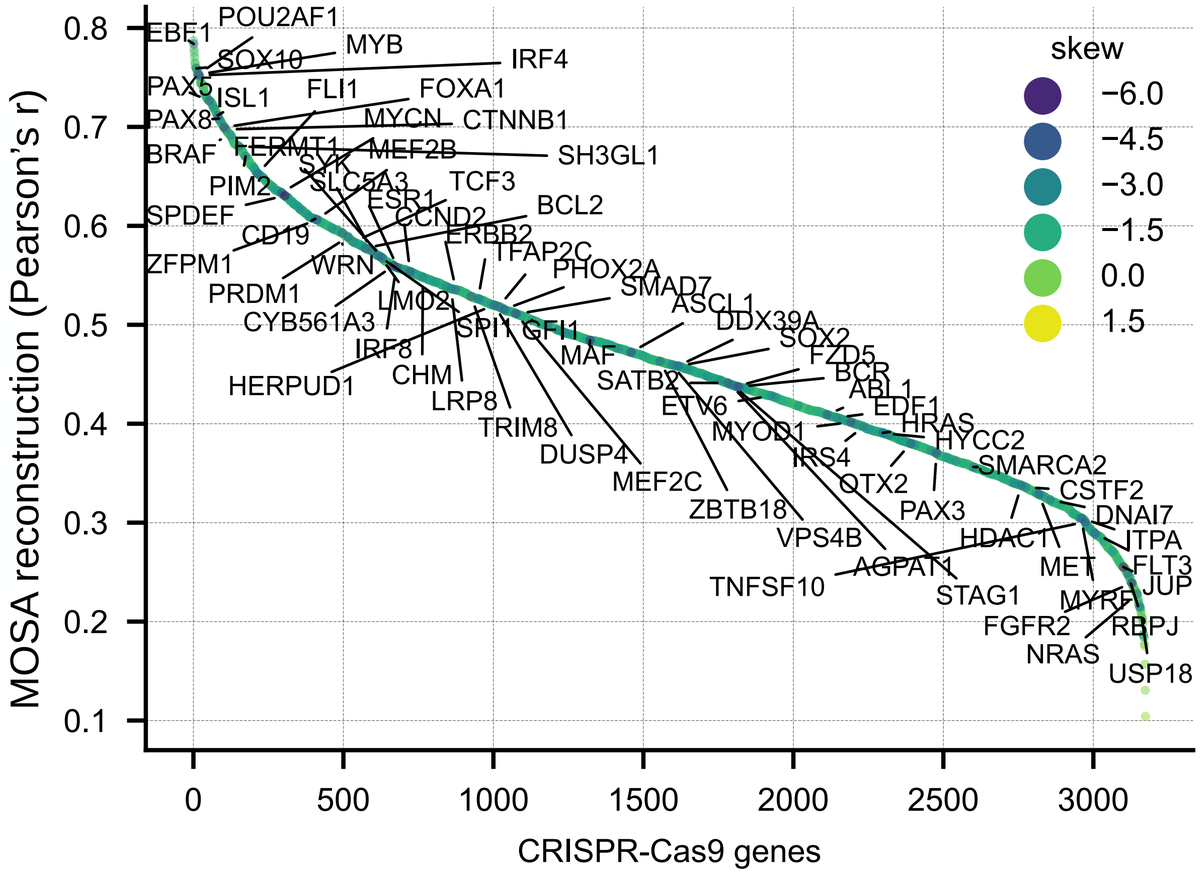
Cancer cell line generative model with the capacity of augmenting current datasets

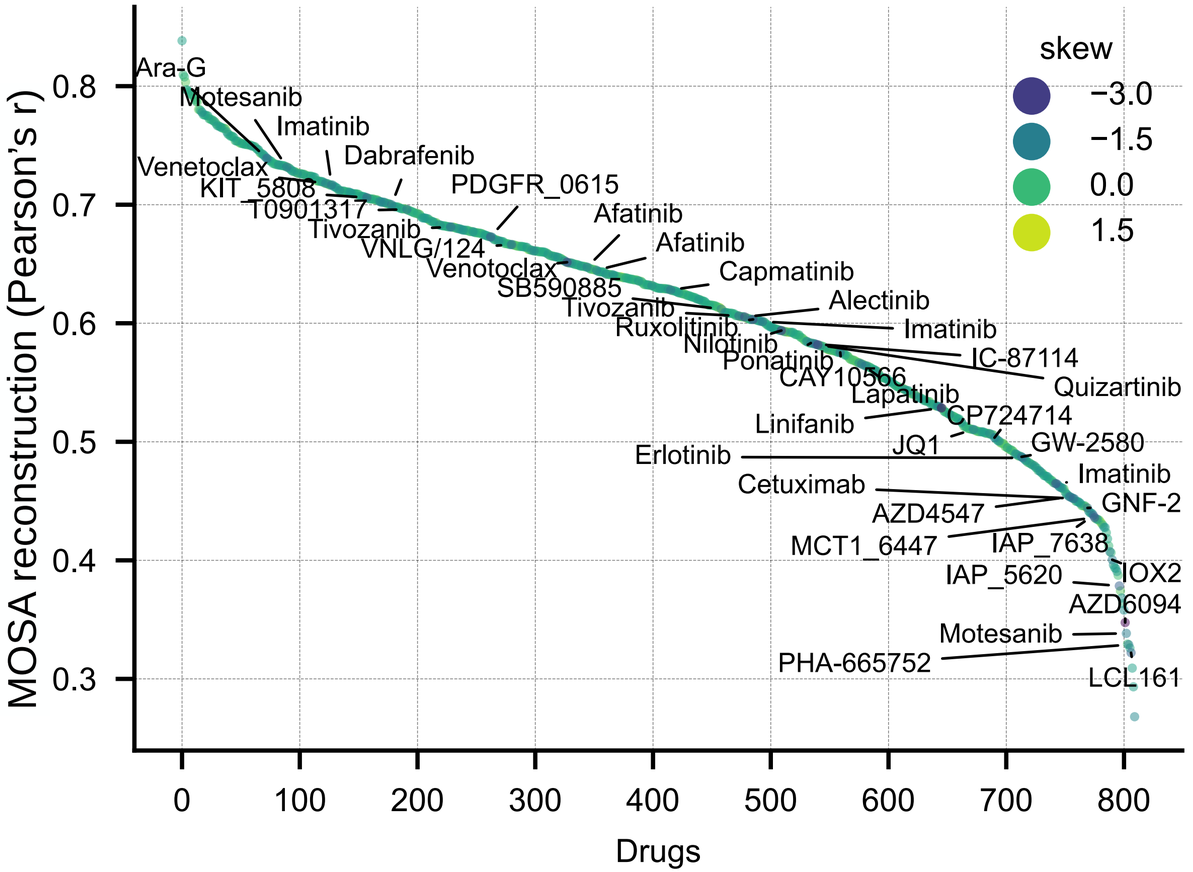
Cai Z*, Apolinário S*, et al. Synthetic augmentation of cancer cell line multi-omic datasets using unsupervised deep learning. bioRxiv. 2024. doi:10.1101/2024.06.26.600742

Synthetically augmented cancer cell lines multi-omics map



Overall reconstruction of drug response and CRISPR-Cas9



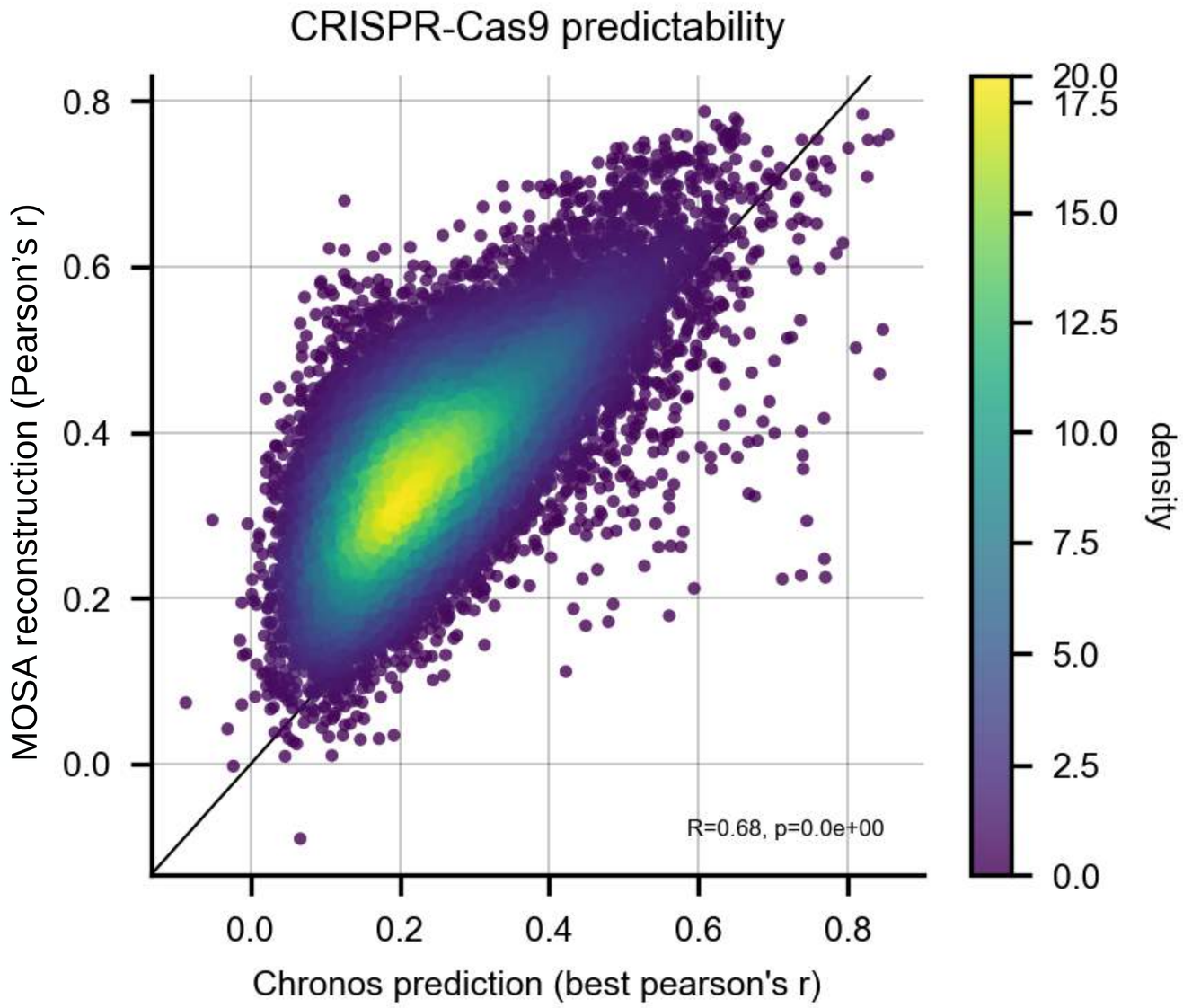


10-fold cross-validated dataset reconstruction

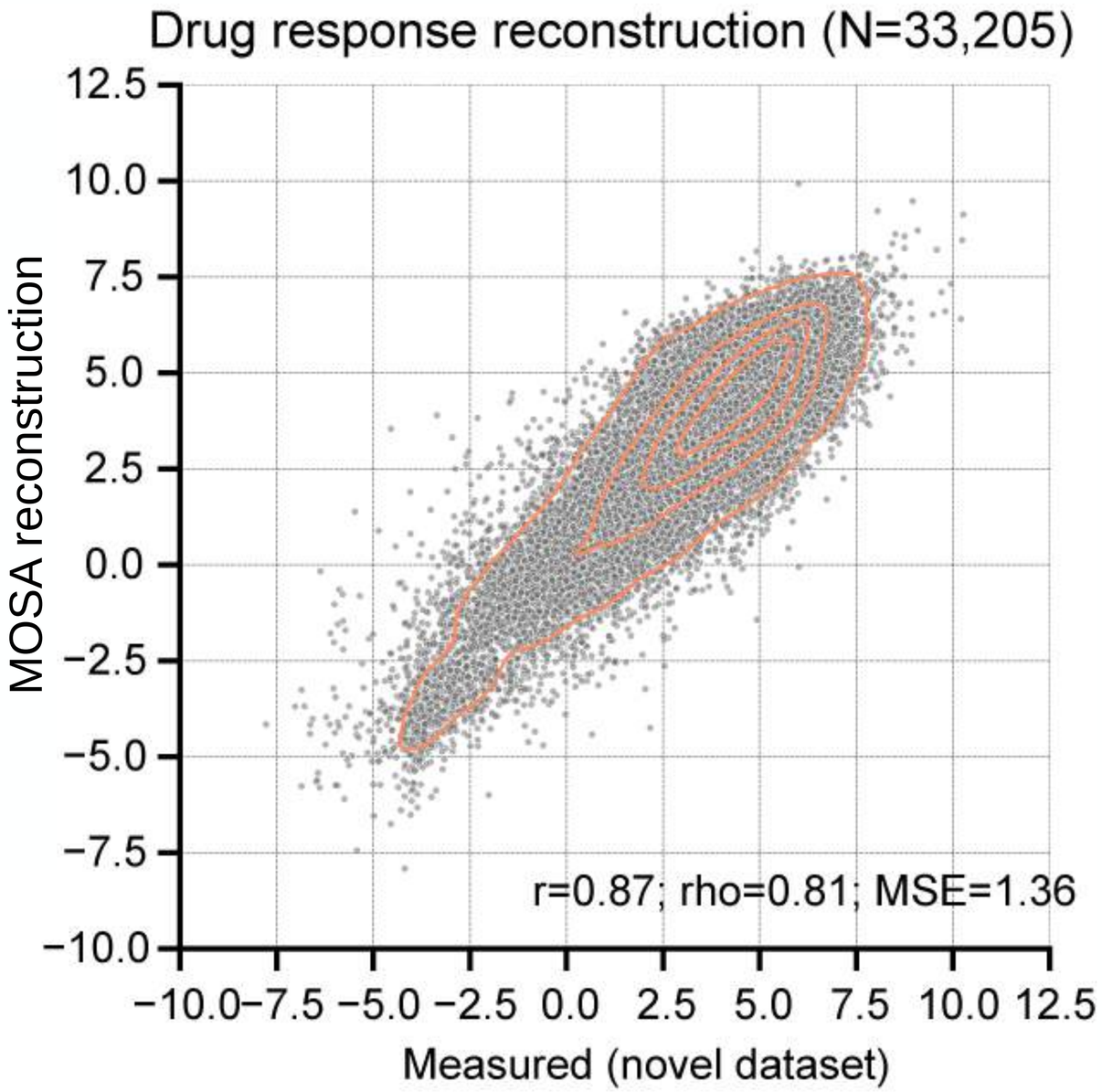
Comparison with predictive performance from Chronos - DepMap Portal

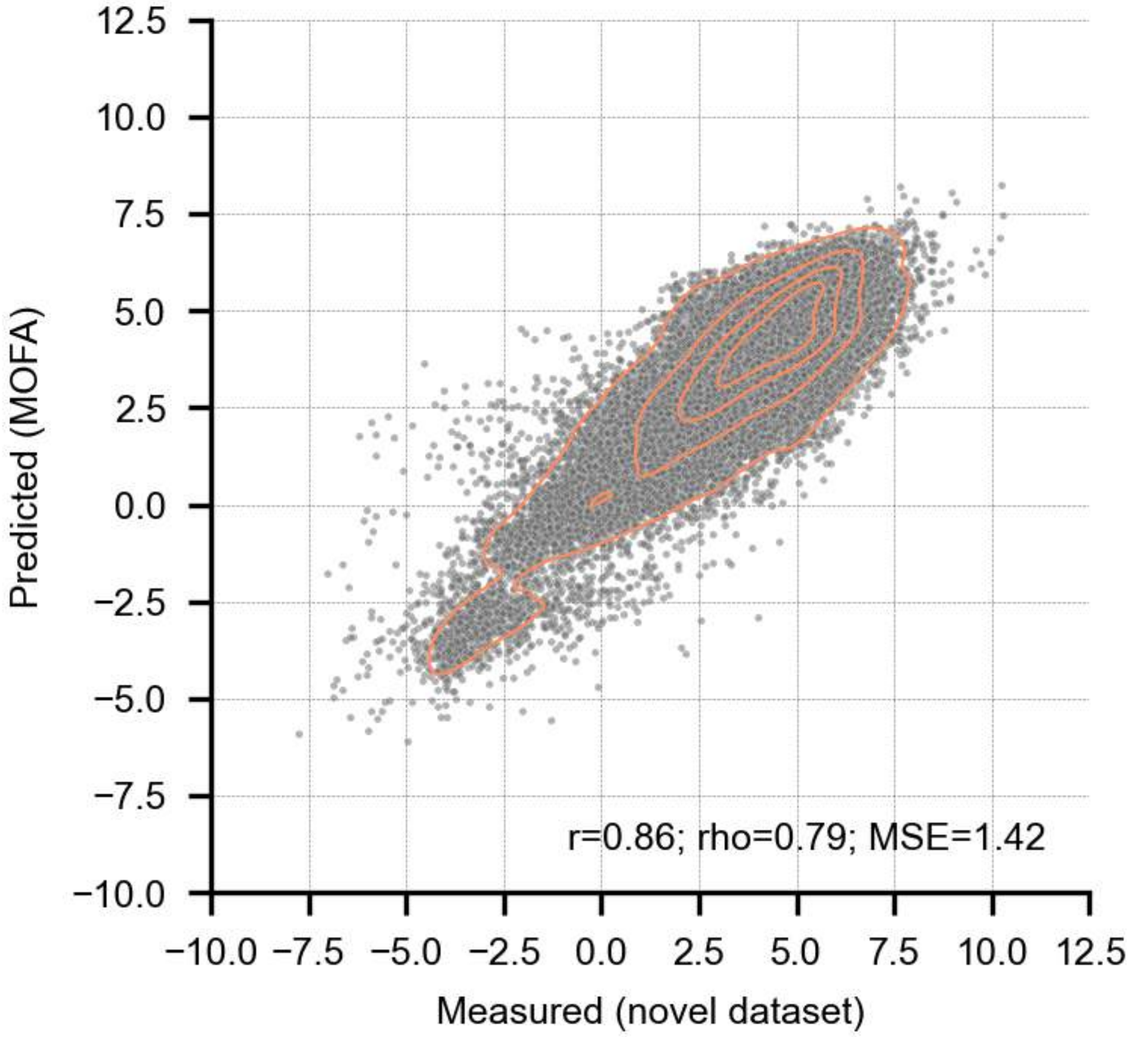
Deep-learning integration of all the multi-omics provides enhanced reconstruction of gene essentiality

Not a completely fair comparison as we use more biological data, including CRISPR-Cas9 screens

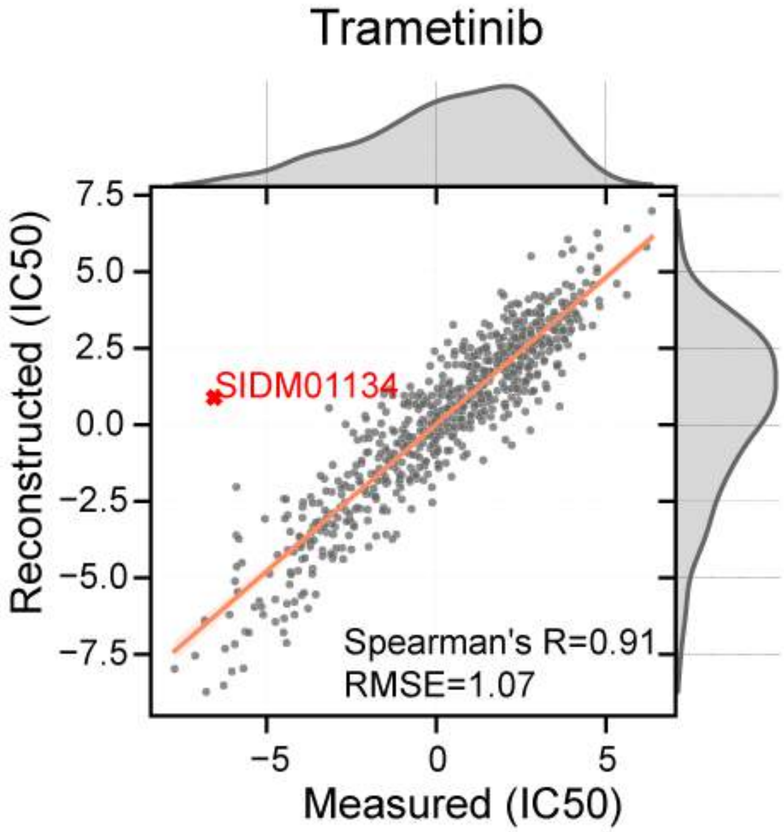


Successful reconstruction of new drug response screens



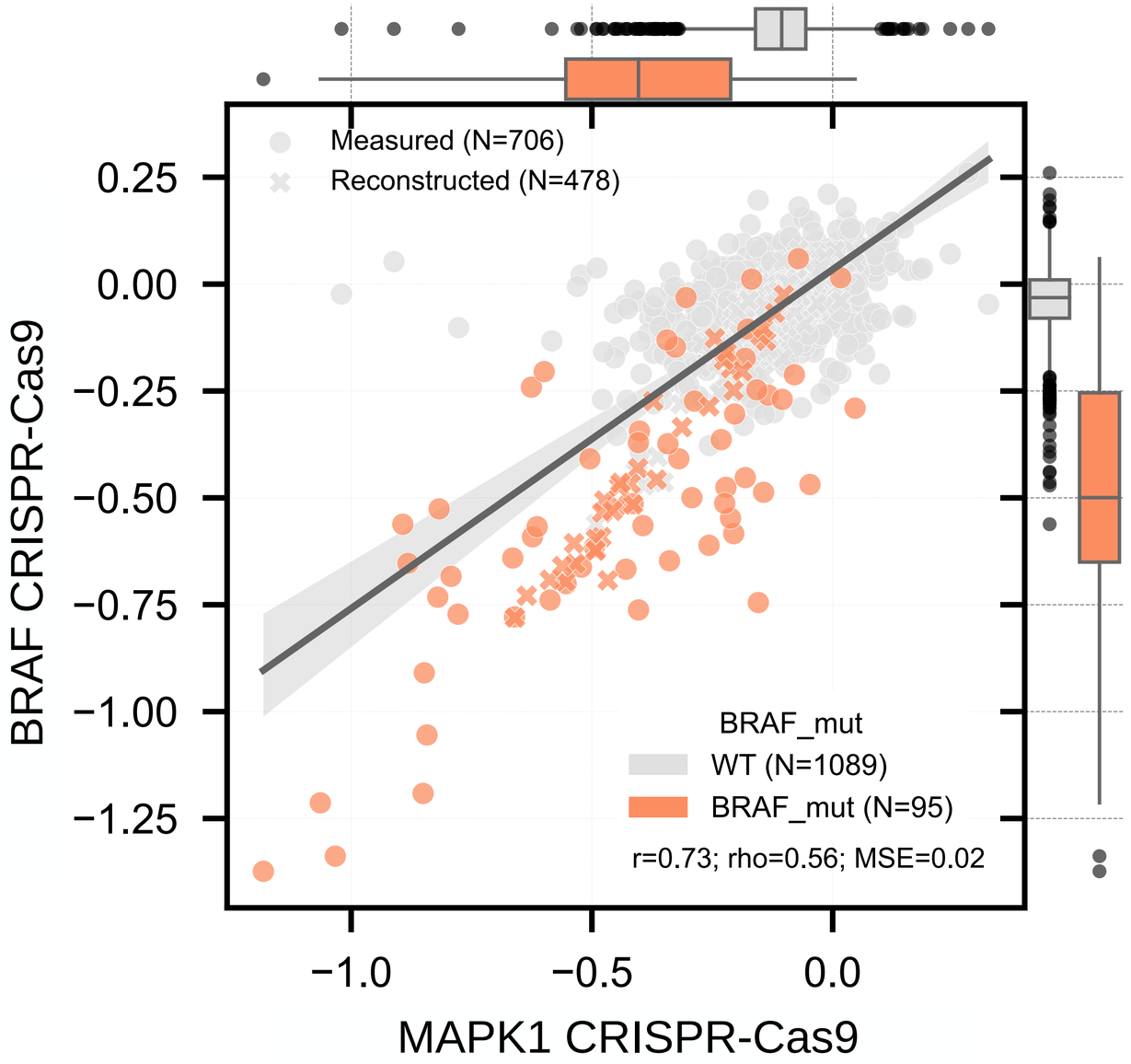


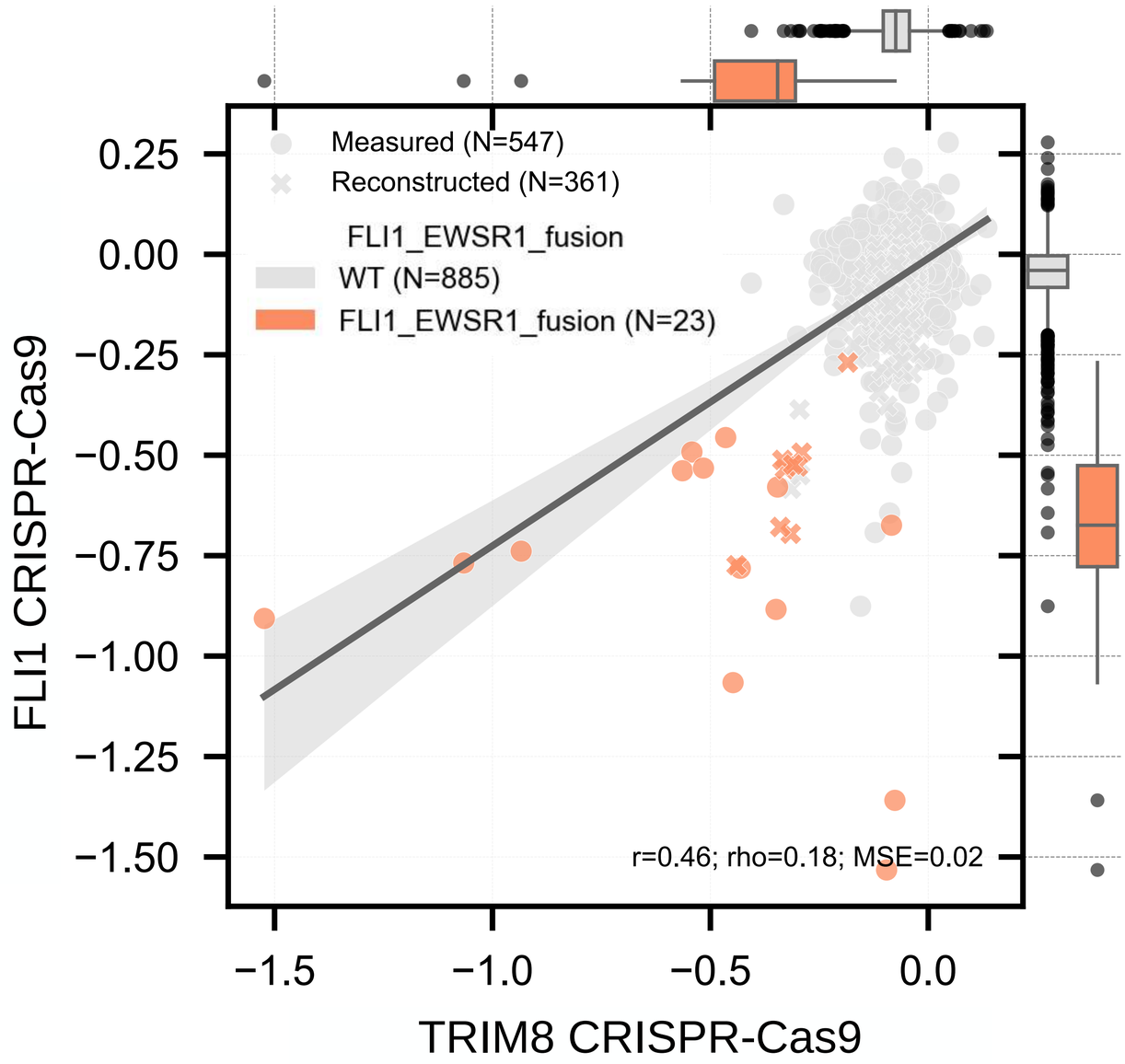
Inconsistencies between synthetic and original measurements



Inconsistencies revealed i) likely incorrect experimental measurements and ii) drugs (e.g. venetoclax) or classes of drugs (e.g. antiapoptotic inhibitors) without effective molecular biomarkers

MOSA synthetic generation of CRISPR-Cas9 screens



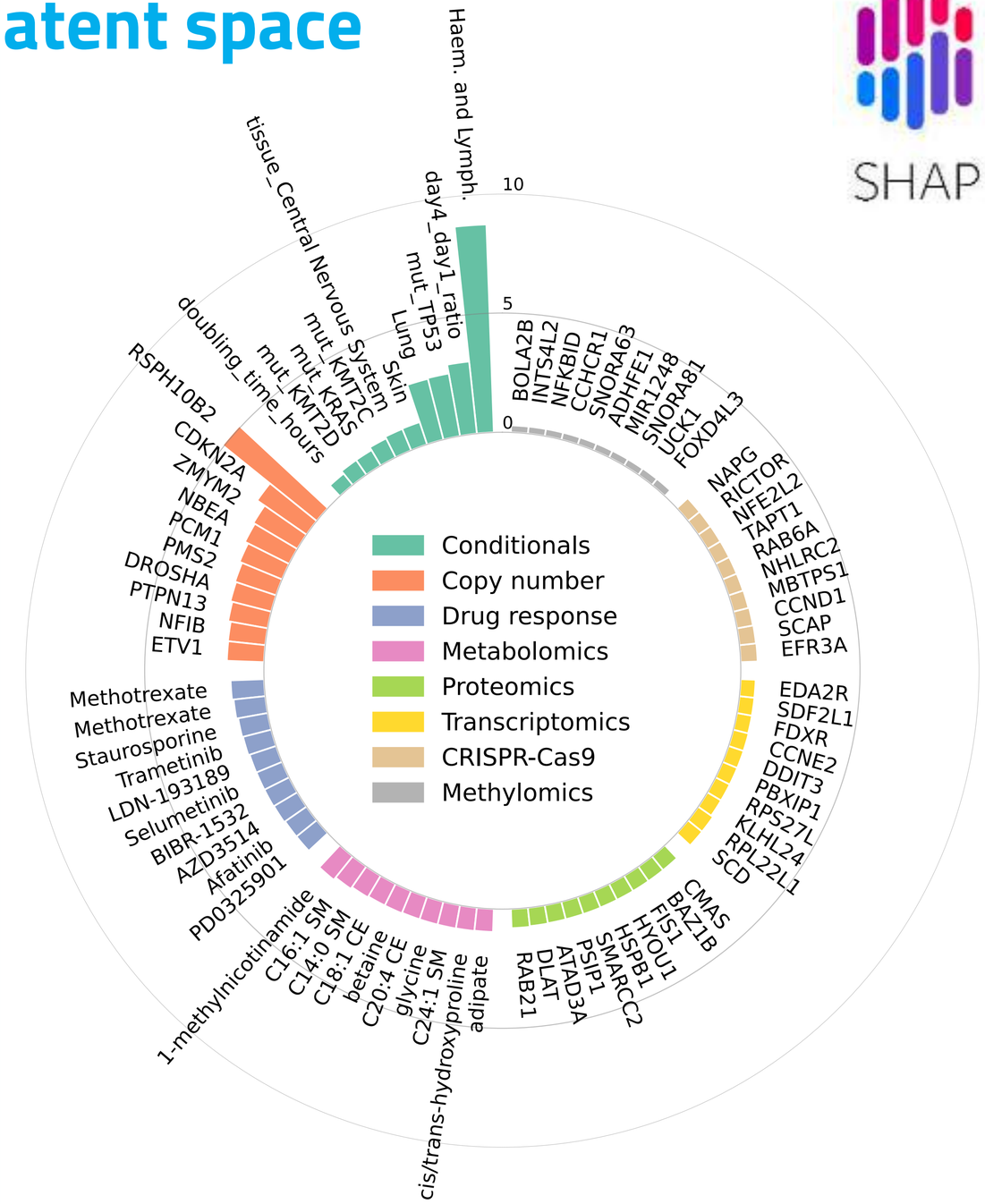


Features important for the multi-omic latent space

From the inputed features of each omic, rank those that are contributing the most to the variability of the cancer cell lines (latent space)

Clear dominance of previously expected features (e.g. Haem. and Lympho., growth rate, TP53 mutations)

Less expected and potentially novel feature associations (e.g. 1-methylnicotinamide)



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