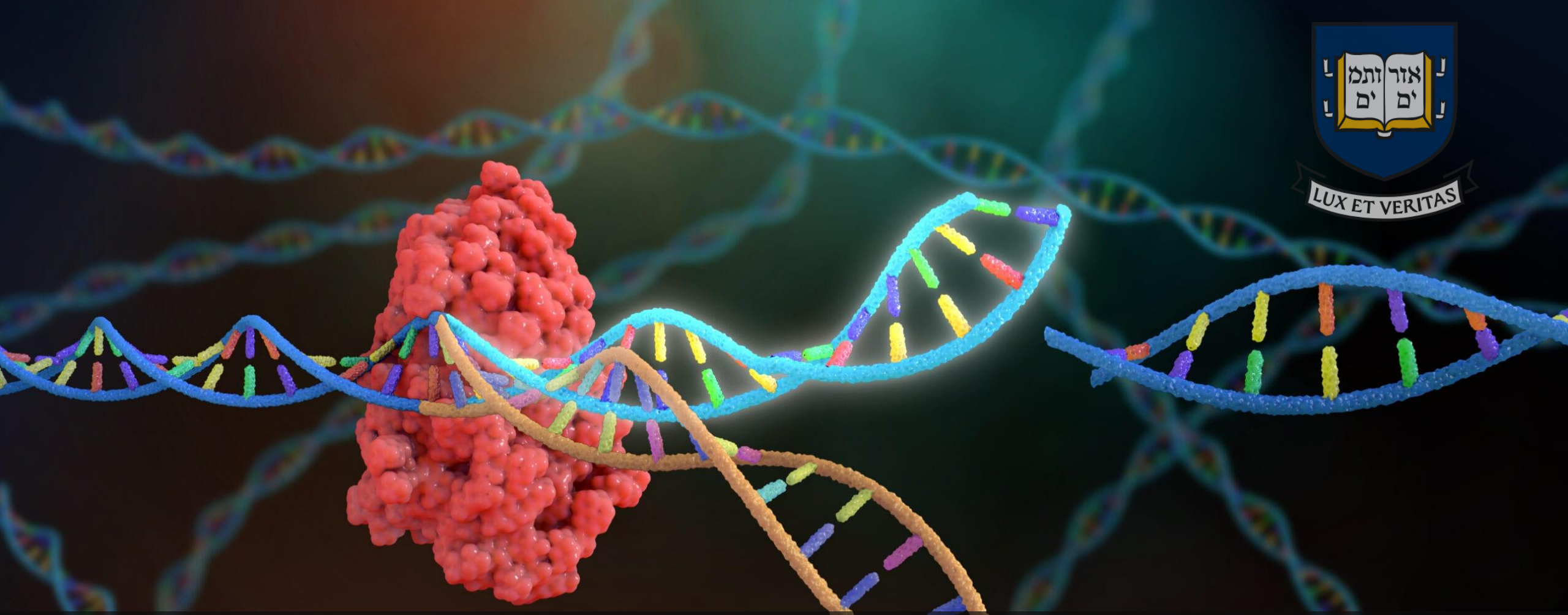


Intro to CRISPR Screen Analysis using "*MAGeCKFlute*" R package

B.S. Juan Martinez-Villalobos
Yale School of Medicine

February 20, 2025

Peer-to-Peer Teaching

Peer-to-Peer Teaching Spring 2025

Cushing/Harvey Medical Library, Yale University

Bioinformatics Support Hub

Sofia Fertuzinhos, PhD and Rolando Garcia-Milian, MLIS

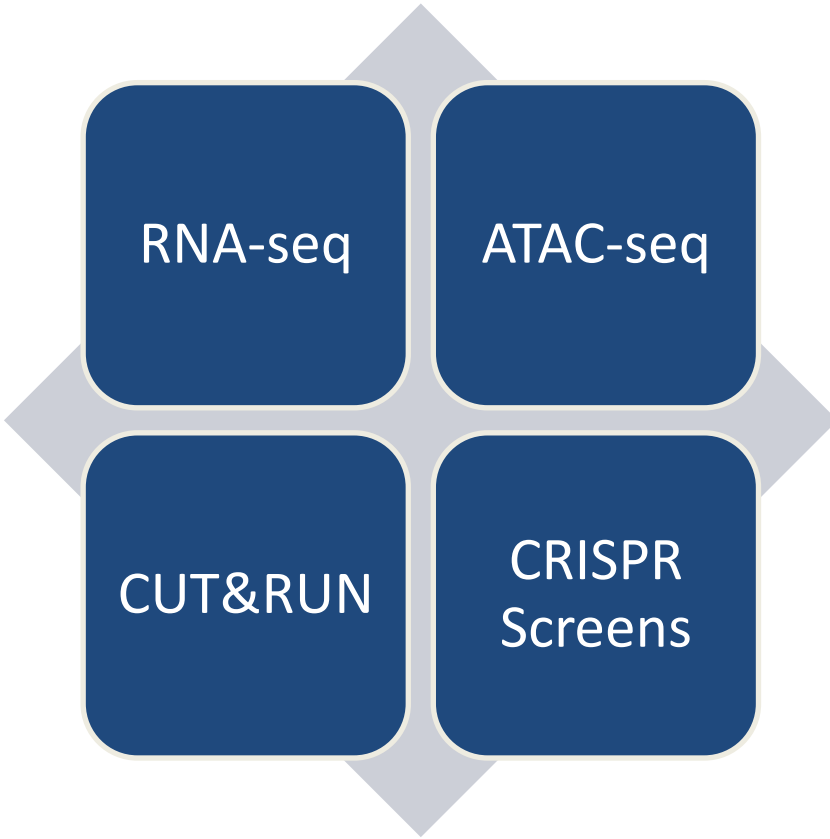
"Those having torches will pass them on to one another."

Submit your proposal!

About me

The Augert Lab
Yale Medicine – Department of Pathology

Small Cell Lung Cancer (SCLC)



Class structure

CRISPR Screens

Fundamentals Importance

Step-by-step

Common analysis workflows

Hands-on experience

MAGeCK Pipeline

Data analysis

CRISPR datasets

Best practices

Documenting analysis

CRISPR/Cas9 overview

Adaptive immunological mechanism

Cas9 endonuclease

Single-stranded guide RNA (sgRNA)

TP53

sgRNA

DNA strands

Cas9 endonuclease

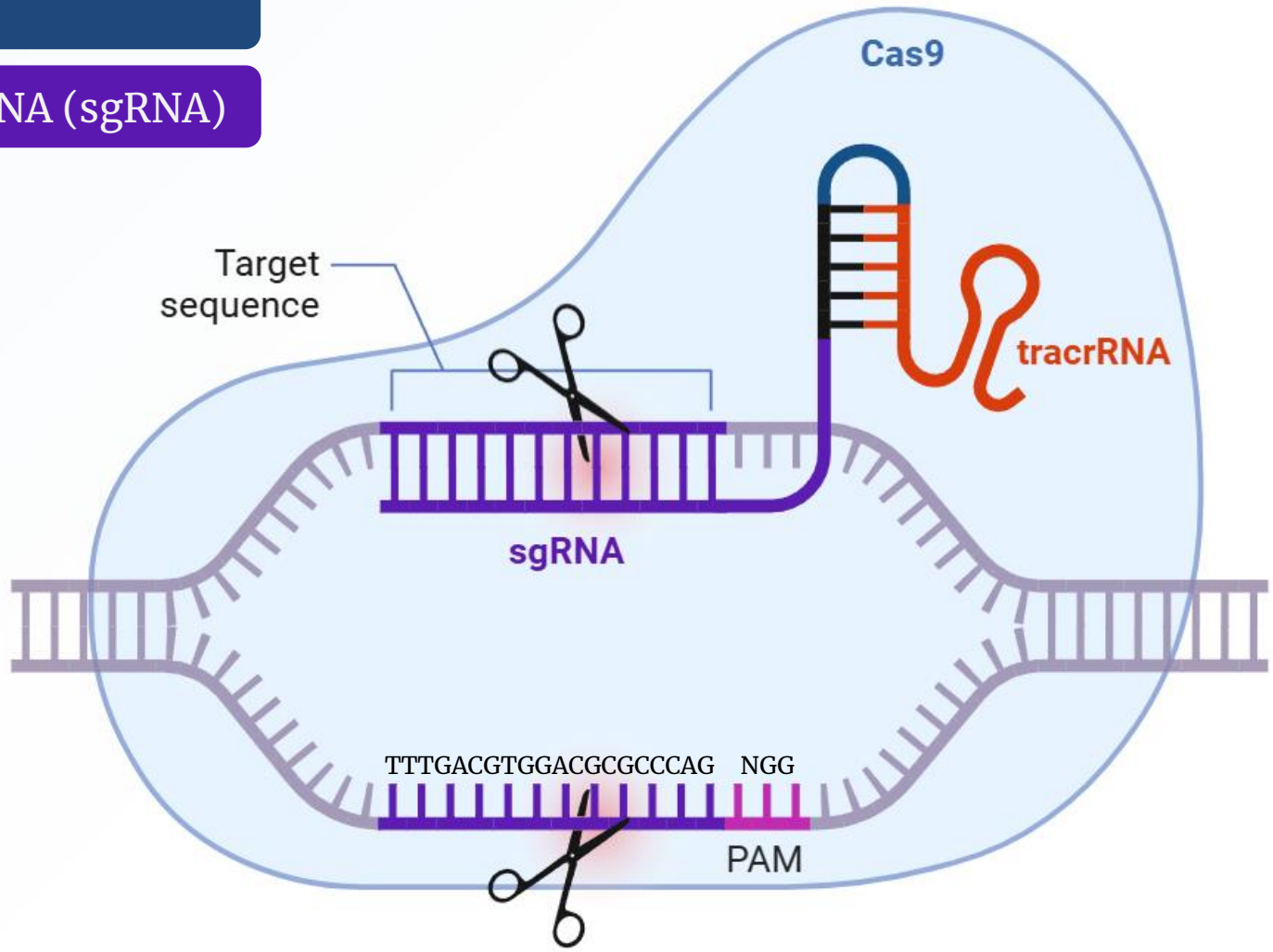
PAM

Cleaves

Non-homologous end joining (NHEJ)

Indel

KO



Kazemizadeh, H., et al. *Clin Transl Oncol* (2023)

CRISPR/Cas9 overview

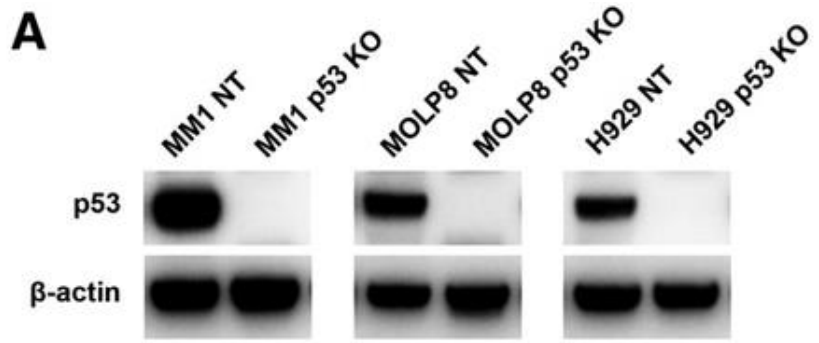
Adaptive immunological mechanism

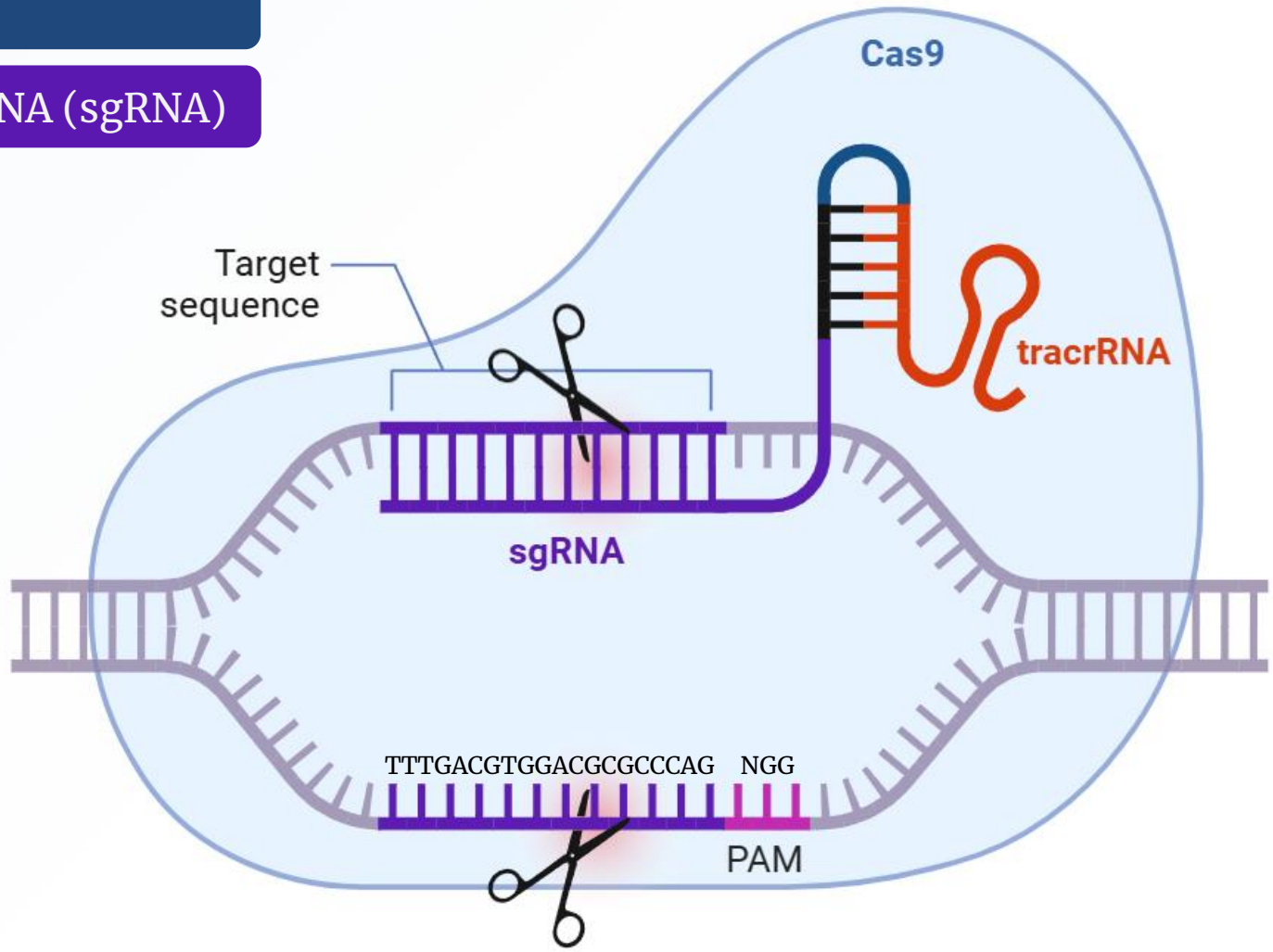
Cas9 endonuclease

Single-stranded guide RNA (sgRNA)

>**TP53**
CTCCTTGGTTCAAGTAATTCTCCTGCCTCAGACTCCAGAG
TAGCTGGGATTACAGGCGCCCGCCACCACGCCCAGCTAAT
TTTTTGTATTTTTAATAGAGATGGGGTTTCATCATGTTGG
CCAGGCTGGTCTCGAACTCCTGACCTCAGGTGATCCACCT
GCCTCAGCCTCCCAAAGTGCTGGGATTACAGGAGTCAGCC
ACCGCACCCAGCCCCAACTAATT[illegible]AGTAGAG
ACAGGGTTTTACCATGTT[illegible]AACTCT
TCACCTCAGGTGATCCAC[illegible]CATCTCAGCCTCCCAAAGTGT
TGGGATTAC**AGGCGTGAGCCACCGTGCCT**GGCCCTGGATT

Exon 1





Tu, Y. S., et al. *Neoplasia* (2017)

CRISPR Screen: Dual function of *MEN1*

nature genetics

Article

https://doi.org/10.1038/s41588-024-01874-9

In vivo CRISPR screens identify a dual function of MEN1 in regulating tumor–microenvironment interactions

Received: 10 November 2023

Accepted: 18 July 2024

Published online: 3 September 2024

Check for updates

Peiran Su[1,2,13], Yin Liu[3,13], Tianyi Chen[2,13], Yibo Xue[2], Yong Zeng[2], Guanghui Zhu[2,12], Sujun Chen[2,12], Mona Teng[1,2], Xinpei Ci[2], Mengdi Guo[2,4], Michael Y. He[2], Jun Hao[2], Vivian Chu[1,2], Wenxi Xu[2], Shivan Wang[2,5], Parinaz Mehdipour[6], Xin Xu[2], Saji… Nhu-An Pham[2], Bell Xi Wu[1,2], Pete… Najd Alshamlan[2], Maryam Khalil[2,7], … Chang Chen[8], Francis Burrows[9], … Shane Harding[1,2], Benjamin H. Lok[1,… Daniel D. De Carvalho[1,2], David G. B… Ming-Sound Tsao[1,2,7] & Houshe…

Functional genomic screens in tw… are limited in identifying therape… microenvironment. By comparing … two-dimensional culture with xen… line, we identified *MEN1* as the top… effects in vitro and in vivo. *MEN1* kno… does not impact cell proliferation in vitro but significantly promotes or inhibits tumor growth in immunodeficient or immunocompetent mice, respectively. Mechanistically, MEN1 knockout redistributes MLL1 chromatin occupancy, increasing H3K4me3 at repetitive genomic regions, activating double-stranded RNA expression and increasing neutrophil and CD8⁺ T cell infiltration in immunodeficient and immunocompetent mice, respectively. Pharmacological inhibition of the menin–MLL interaction reduces tumor growth in a CD8⁺ T cell-dependent manner. These findings reveal tumor microenvironment-dependent oncogenic and tumor-suppressive functions of *MEN1* and provide a rationale for targeting *MEN1* in solid cancers.

Tumor masses are mixtures of cancerous and normal cells that collectively form the tumor microenvironment (TME)[1]. Within the TME, various cell populations communicate through cytokines, chemokines and growth factors, further recruiting additional infiltrating cells, leading to increased tumor heterogeneity[2–4]. Advances in single-cell RNA sequencing (scRNA-seq) technology allow for the characterization of individual components within the TME, offering significant opportunities to enhance our understanding of tumor biology and cancer management[5,6]. Indeed, in recent years, an increasing number of studies has focused on targeting components of the TME, particularly the immune microenvironment[7,8].

The CRISPR screen is a powerful tool to identify vulnerabilities in cancer cells[9,10]. Although CRISPR screens have been extensively conducted in in vitro cell culture systems[11–13], including the Cancer Dependency Map (DepMap) project[14–16], the absence of the TME in these models has limited the ability to identify gene targets that modulate

A full list of affiliations appears at the end of the paper. e-mail: Ming.Tsao@uhn.ca; hansenhe@uhnresearch.ca

How did they authors identify *MEN1*?

MEN1 Protein-coding gene known for its role as tumor-suppressor. The gene consists of 10 exons and express 2.8 kb transcript

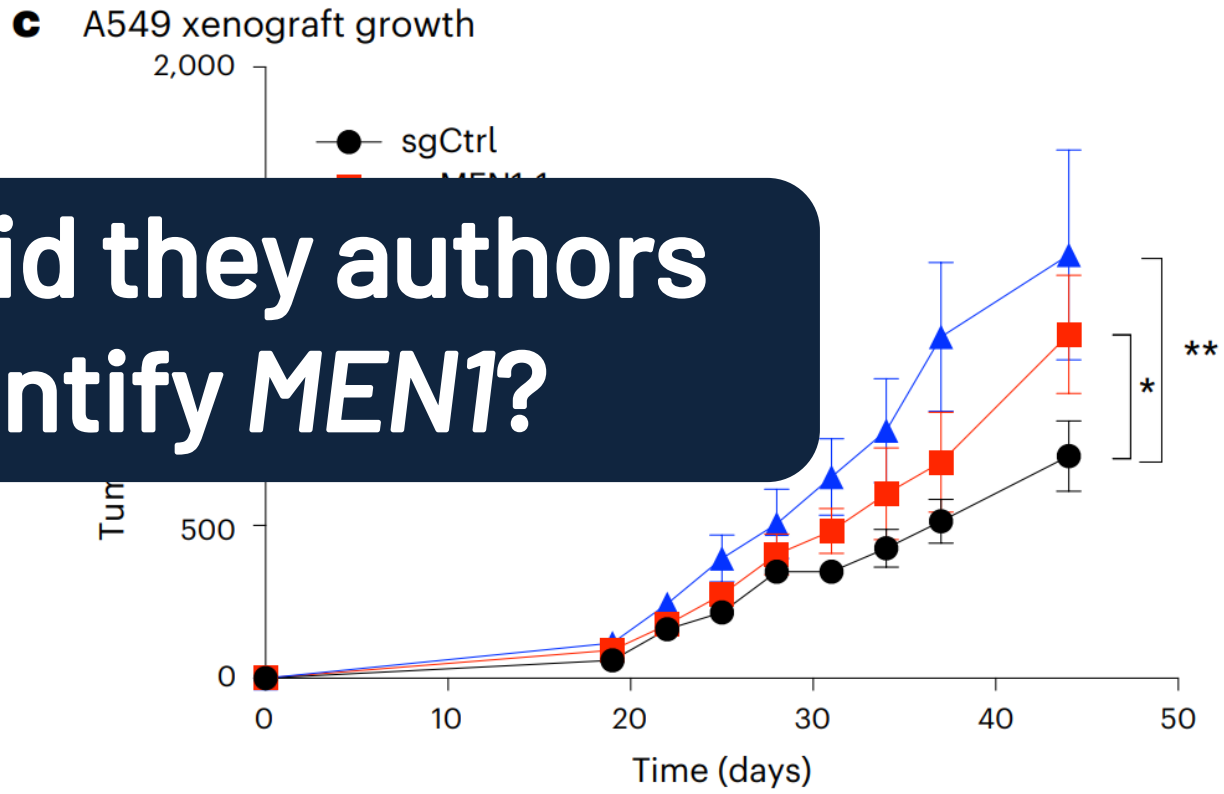
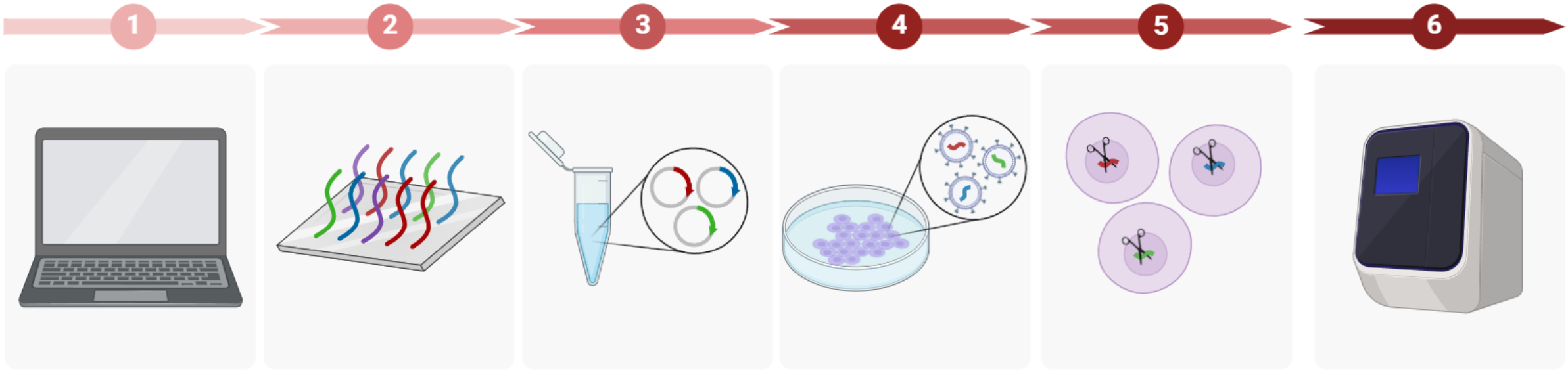


Figure 1C. Xenograft tumor growth curve in immunodeficient mice inoculated with control (sgCtrl) or MEN1 knockout (sgMEN1-1, sgMEN1-2) A549 cells.
Note: A549 (Lung cancer cell line) – Xenograft (Human tumor) – Immunodeficient (Defective T/B/NK)

Introduction to CRISPR Screens

A CRISPR screen is a high-throughput technique that uses CRISPR-Cas genome editing to systematically perturb genes across many cells, followed by selection and measurement to identify genes involved in specific biological processes or phenotypes.



Dual function of *MEN1*: Experimental design

nature genetics

Article

https://doi.org/10.1038/s41588-024-01874-9

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Functional genomic screens in two-dimensional cell culture models are limited in identifying therapeutic targets that influence the tumor microenvironment. By comparing targeted CRISPR–Cas9 screens in a two-dimensional culture with xenografts derived from the same cell line, we identified *MEN1* as the top hit that confers differential dropout effects in vitro and in vivo. *MEN1* knockout in multiple solid cancer types does not impact cell proliferation in vitro but significantly promotes or inhibits tumor growth in immunodeficient or immunocompetent mice, respectively. Mechanistically, MEN1 knockout redistributes MLL1 chromatin occupancy, increasing H3K4me3 at repetitive genomic regions, activating double-stranded RNA expression and increasing neutrophil and $CD8^+$ T cell infiltration in immunodeficient and immunocompetent mice, respectively. Pharmacological inhibition of the menin–MLL interaction reduces tumor growth in a $CD8^+$ T cell-dependent manner. These findings reveal tumor microenvironment-dependent oncogenic and tumor-suppressive functions of *MEN1* and provide a rationale for targeting *MEN1* in solid cancers.

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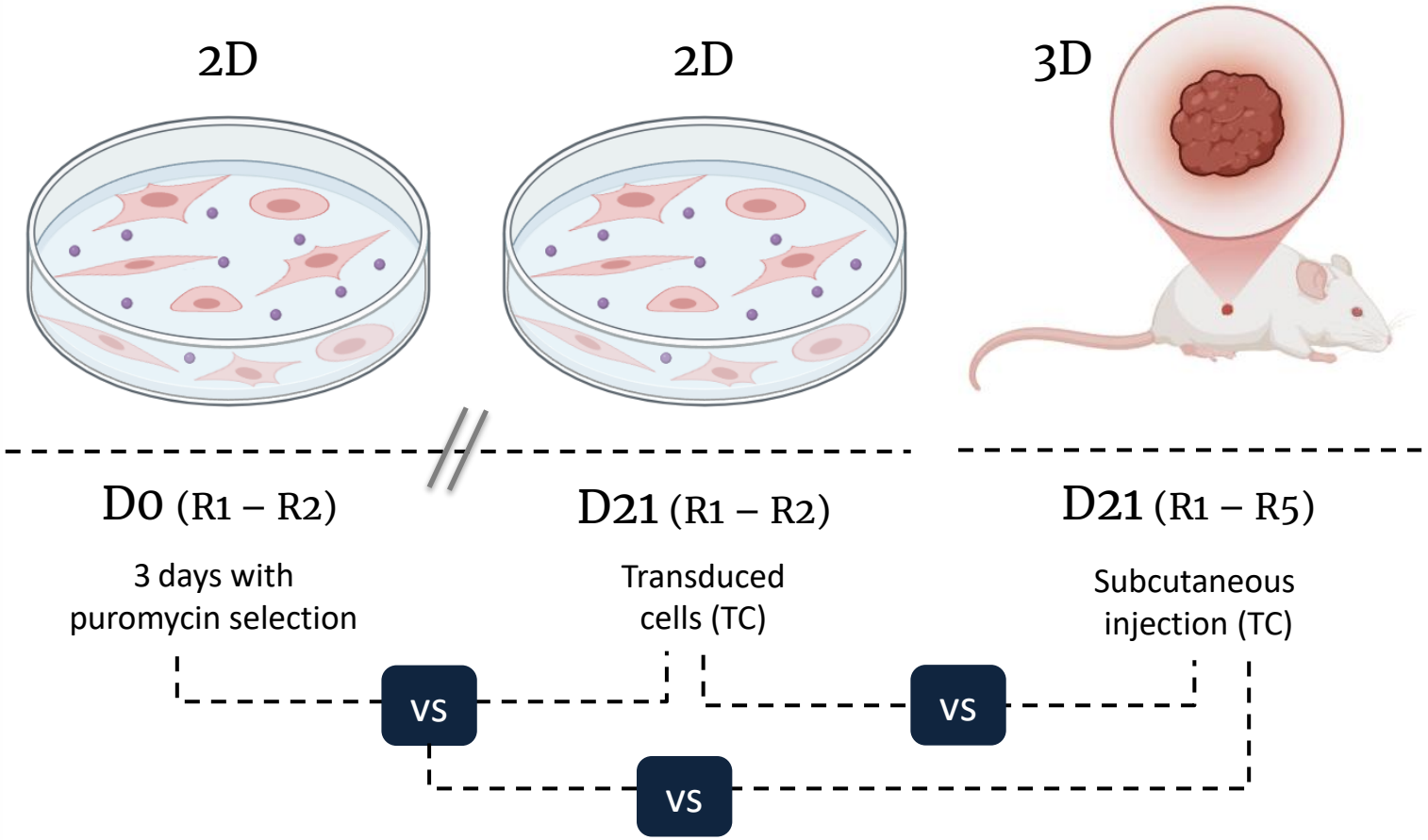


Library

Epi-Drug — 12,472 sgRNAs — 317 epigenetic regulators — 657 Drug Bank targets — 10 sgRNA per gene

CRISPR Screen – Key concepts

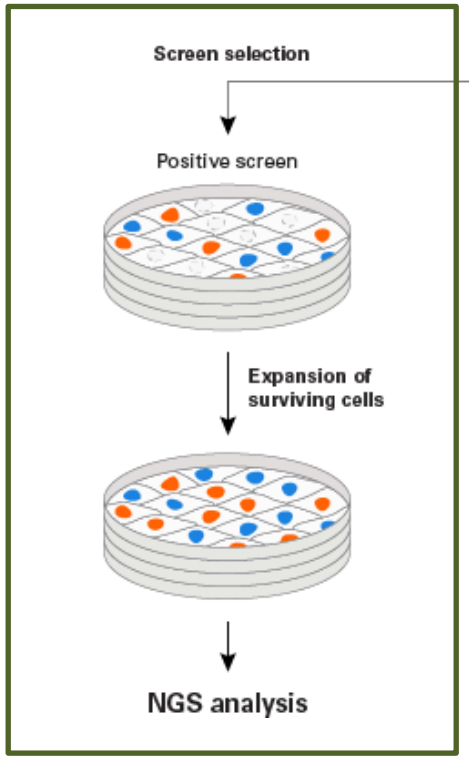
Useful for

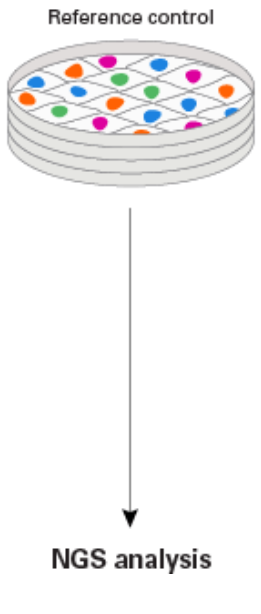
Identifying genes required for cancer cell survival

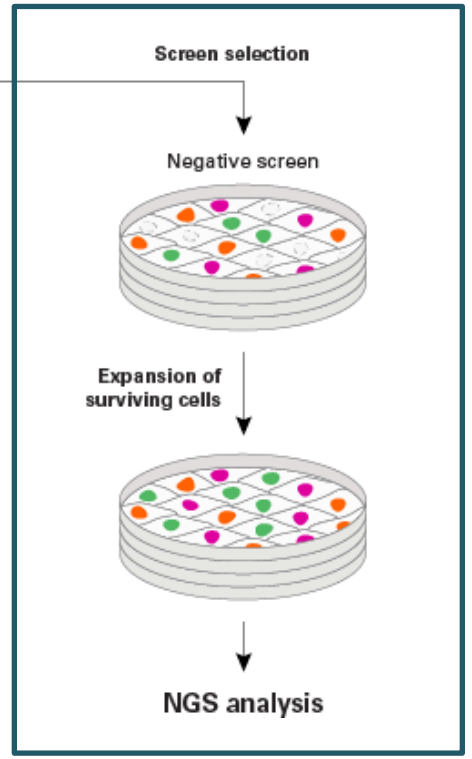
Finding genes that confer drug resistance

Chemotherapy

Interested in cells that survive







Interested in cells that die

Useful for

Identifying tumor suppressor genes

Finding genes that enhance metastasis

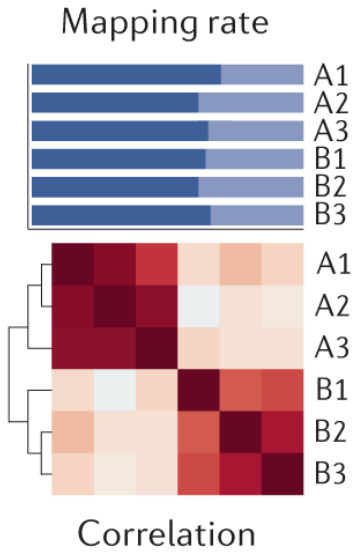
Growth in 3D

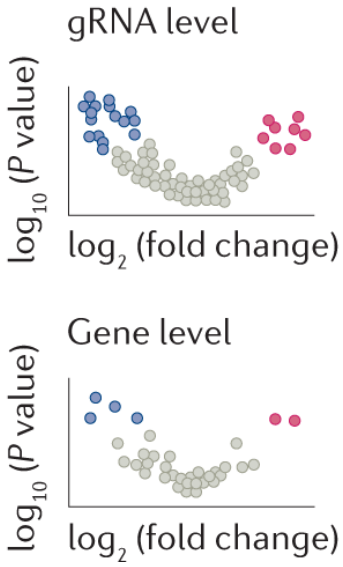
CRISPR Screen – NGS analysis

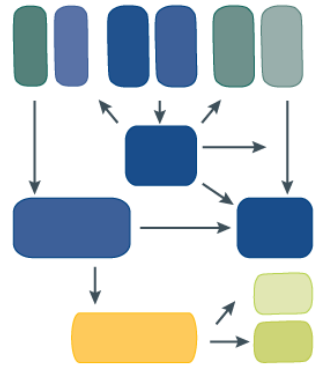
Workflow	Data processing →	Quality control →	Gene ranking →	Hit analysis →	Visual interpretation
Main tasks	• Read mapping • Derivation of count matrices	• Calculation of quality metrics • Quantification of consistency across replicates	• Statistical testing for differences in gRNA frequency • Aggregation of results and ranking of individual genes	• Gene set analysis for the hits of the screen • Overlay with regulatory networks and protein interactions	• Manual inspection of count data for hits across all experiments • Identification of outliers, biases and unexpected behaviour

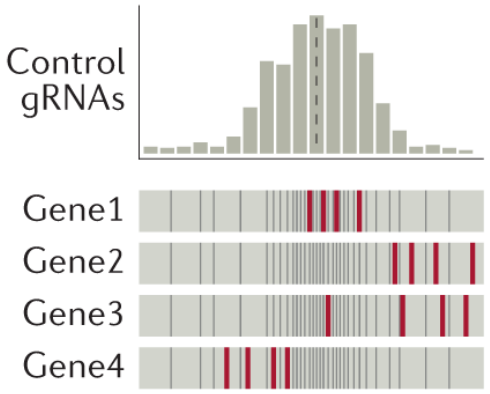
Example results

		Condition A			Condition B		
	Replicates	1	2	3	1	2	3
Gene A	gRNA1	845	749	985	24	49	17
	gRNA2	243	353	221	60	75	53
	gRNA3	748	695	680	98	76	110
	gRNA4	510	470	493	220	289	330
Gene B	gRNA1	330	280	291	660	623	725
	gRNA2	270	244	310	750	744	800
	gRNA3	440	410	398	921	963	898
	gRNA4	343	417	408	550	512	610









Bock, C.,0 *Nat Rev Methods Primers* (2022)