Exploring gene functions on a sub-gene resolution

Technical Journal Club

14/09/2021

Stefano Sellitto

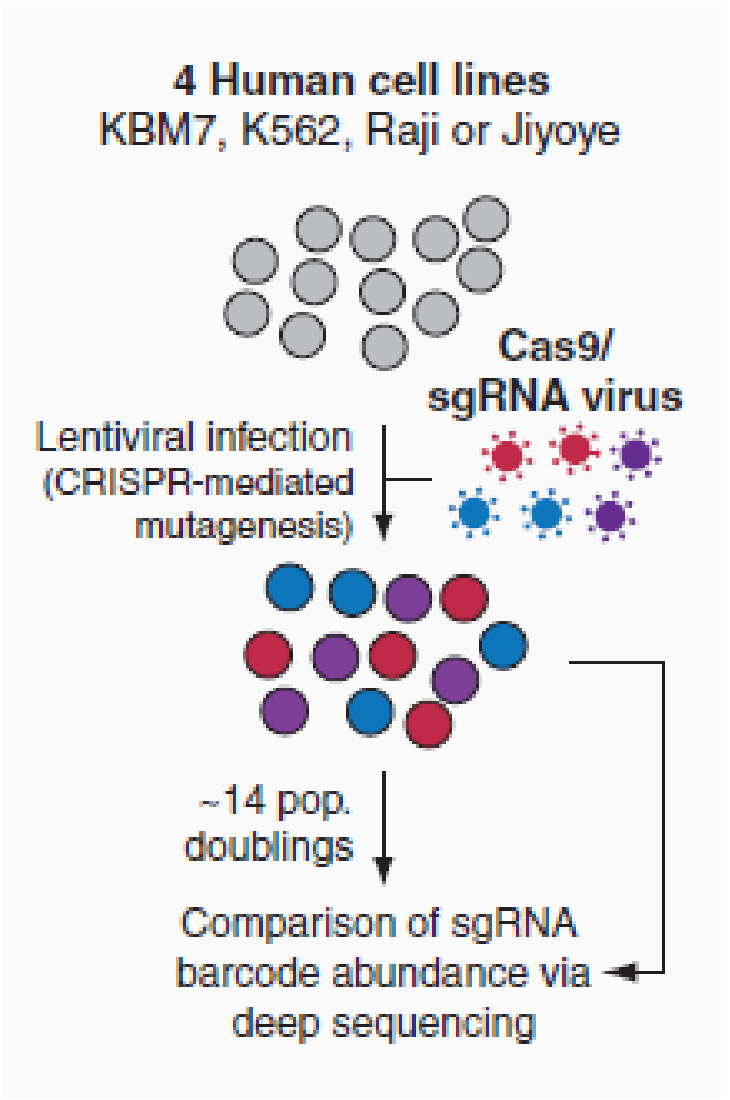
Functional screens using CRISPR-Cas9 techniques facilitate the identification of essential genes on a genome-wide scale.

GENOMICS

Identification and characterization of essential genes in the human genome

Tim Wang,[1,2,3,4] Kıvanç Birsoy,[1,2,3,4]* Nicholas W. Hughes,[3] Kevin M. Krupczak,[2,3,4] Yorick Post,[2,3,4] Jenny J. Wei,[1,2] Eric S. Lander,[1,3,5]†‡ David M. Sabatini[1,2,3,4,6]†‡

SCIENCE



To fully define the function of protein-coding essential genes, it is necessary to distinguish essential protein domains that directly contribute to cellular phenotypes.

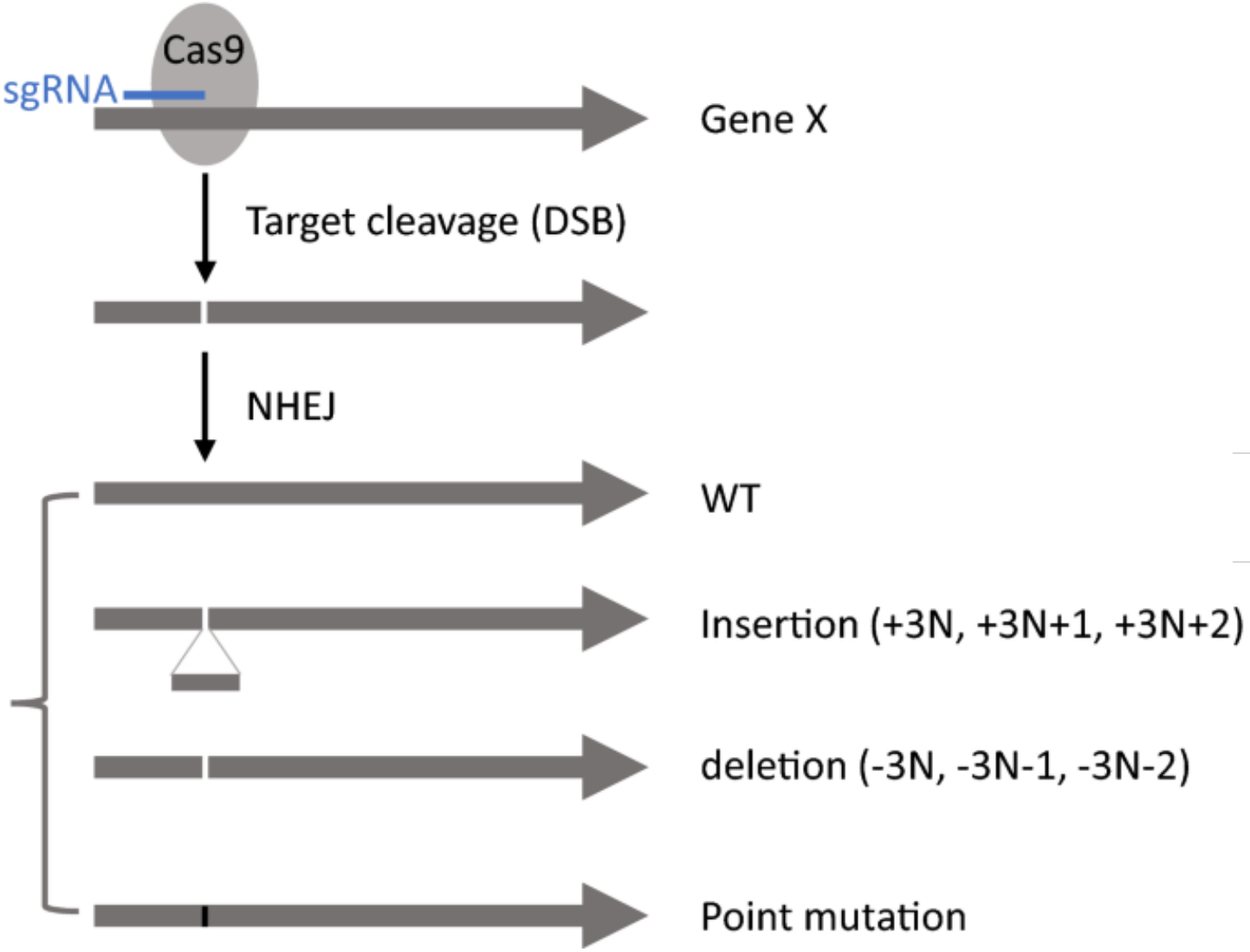
Typical CRISPR screen

sgRNAs library targeting all the genes within the genome

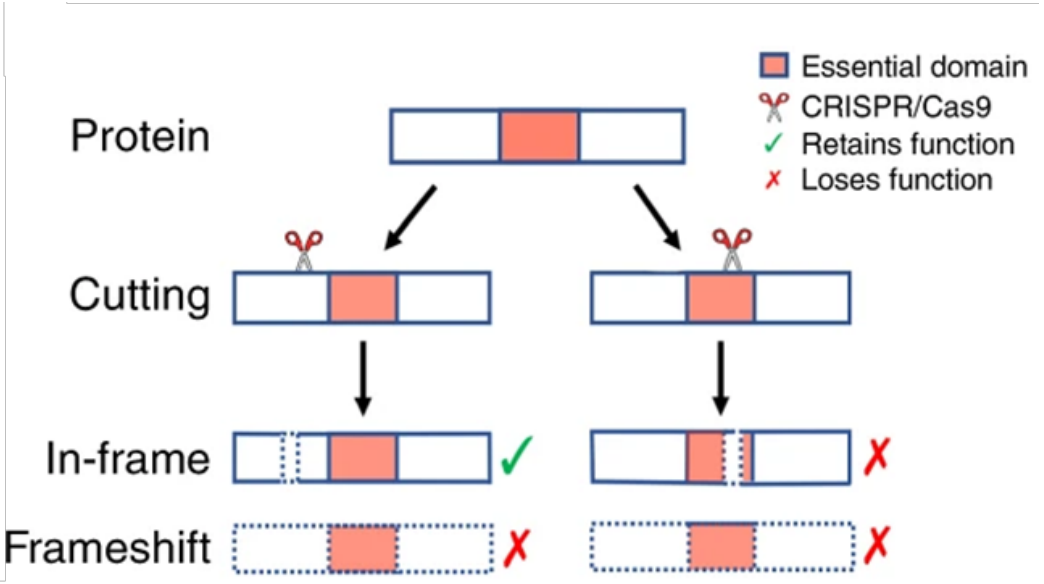
CRISPR tiling screen

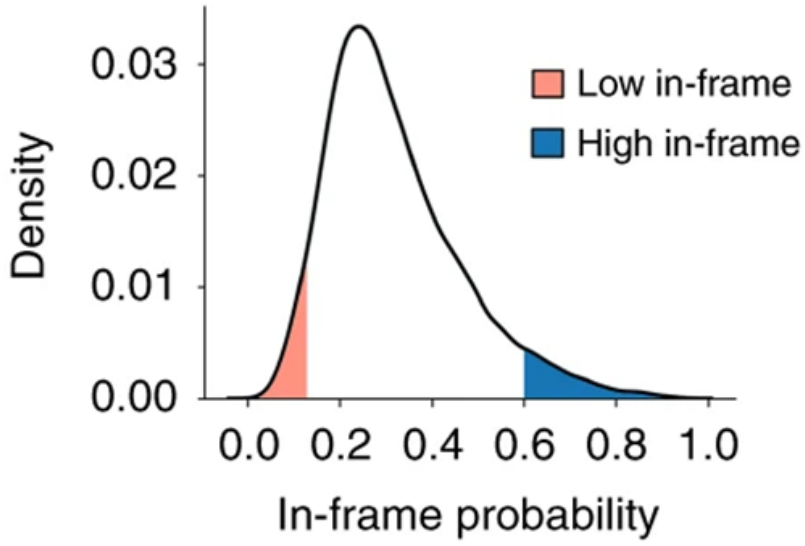
sgRNAs sub-library targeting regions within a gene or several genes

IN-FRAME VS OUT-OF-FRAME MUTATIONS



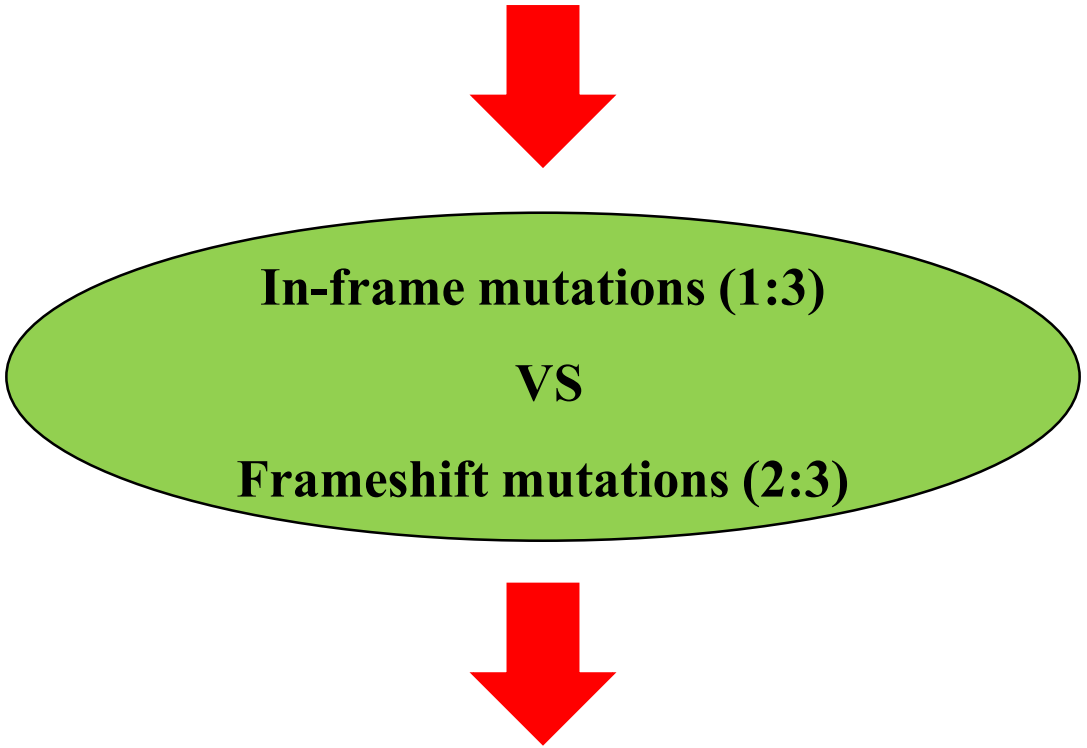
You, Ramachandra, and Jin 2020





He et al. 2019

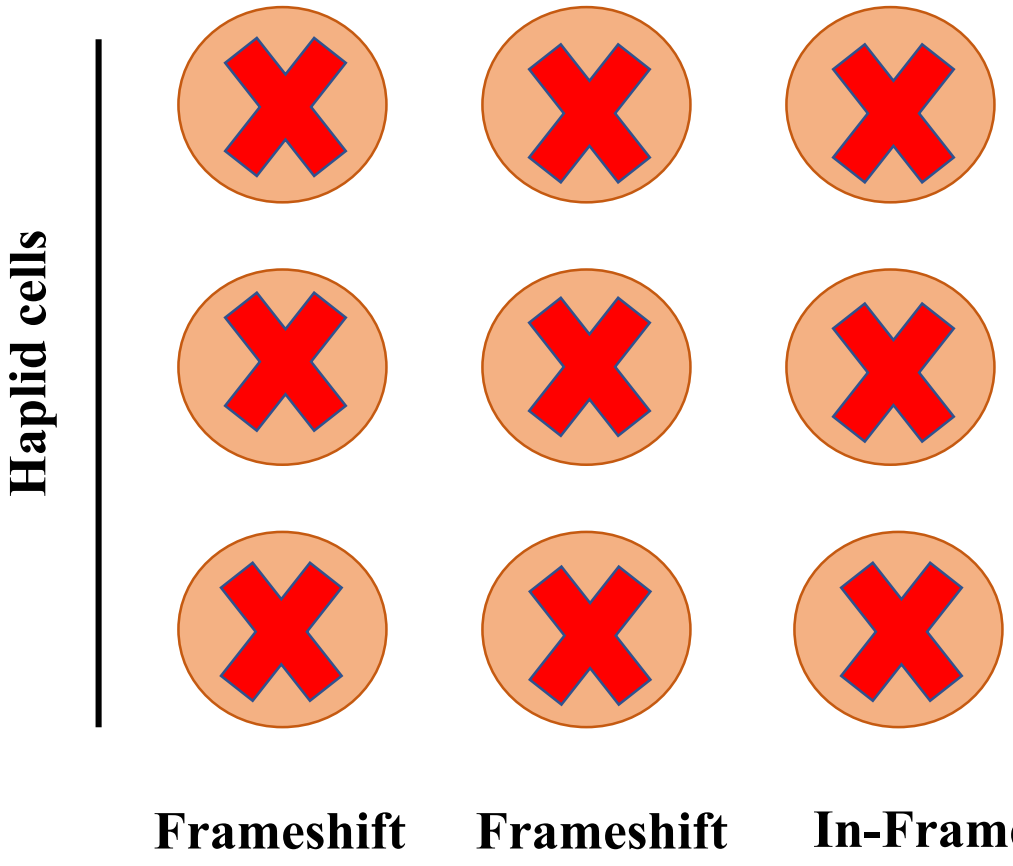
Proteins with small, in-frame indels in nonessential regions are likely to retain function. In contrast, proteins with indels in essential domains may display compromised protein function



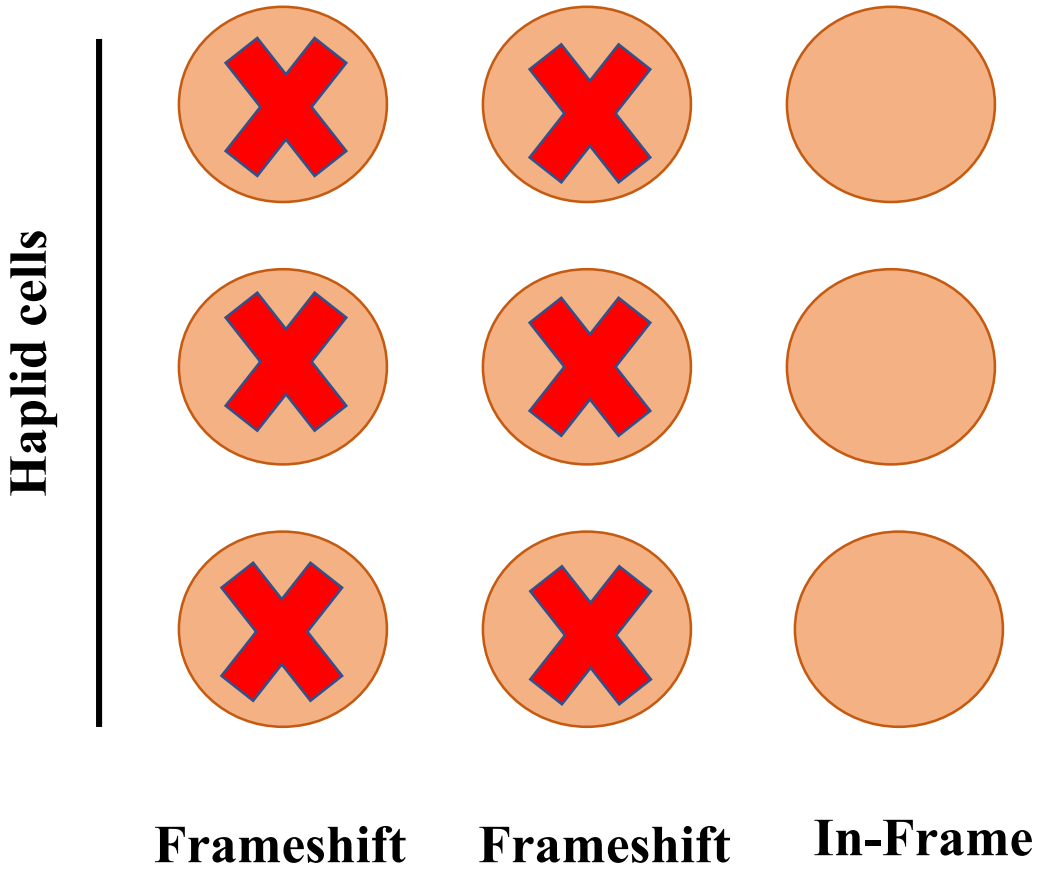
High population numbers and polyploid cells for optimal coverage and good statistical representation

9x COVERAGE

sgRNA against an essential region of an essential gene

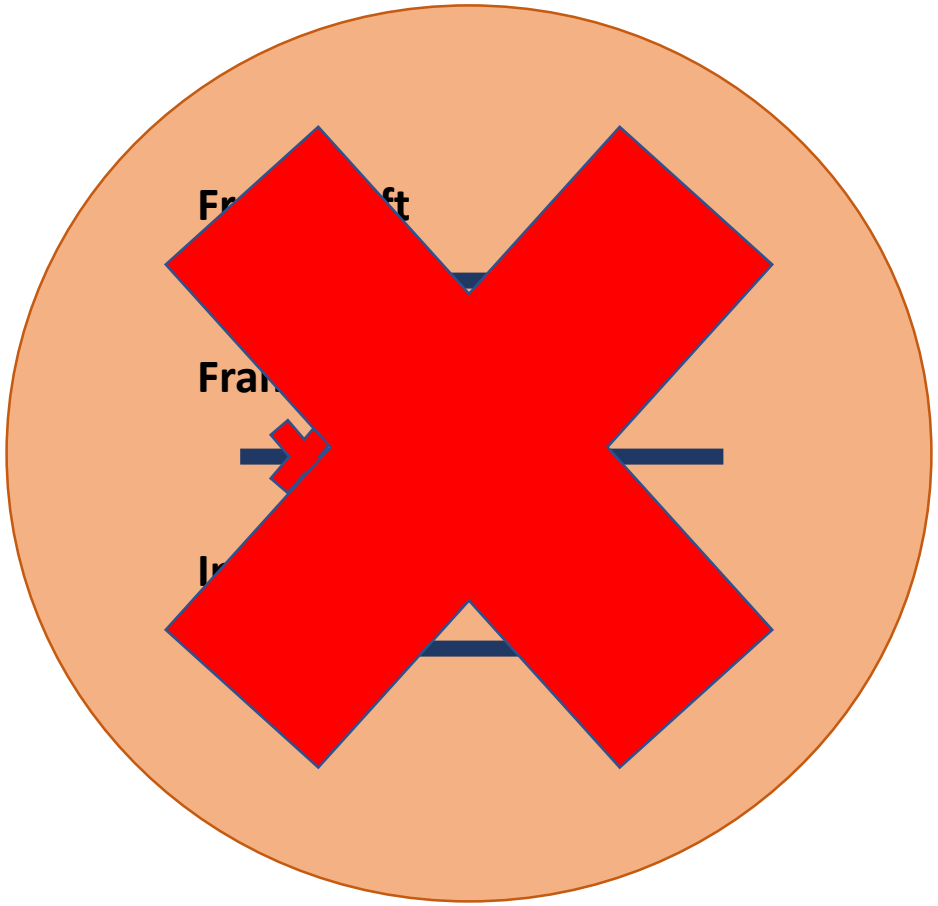


sgRNA against non essential region of an essential gene



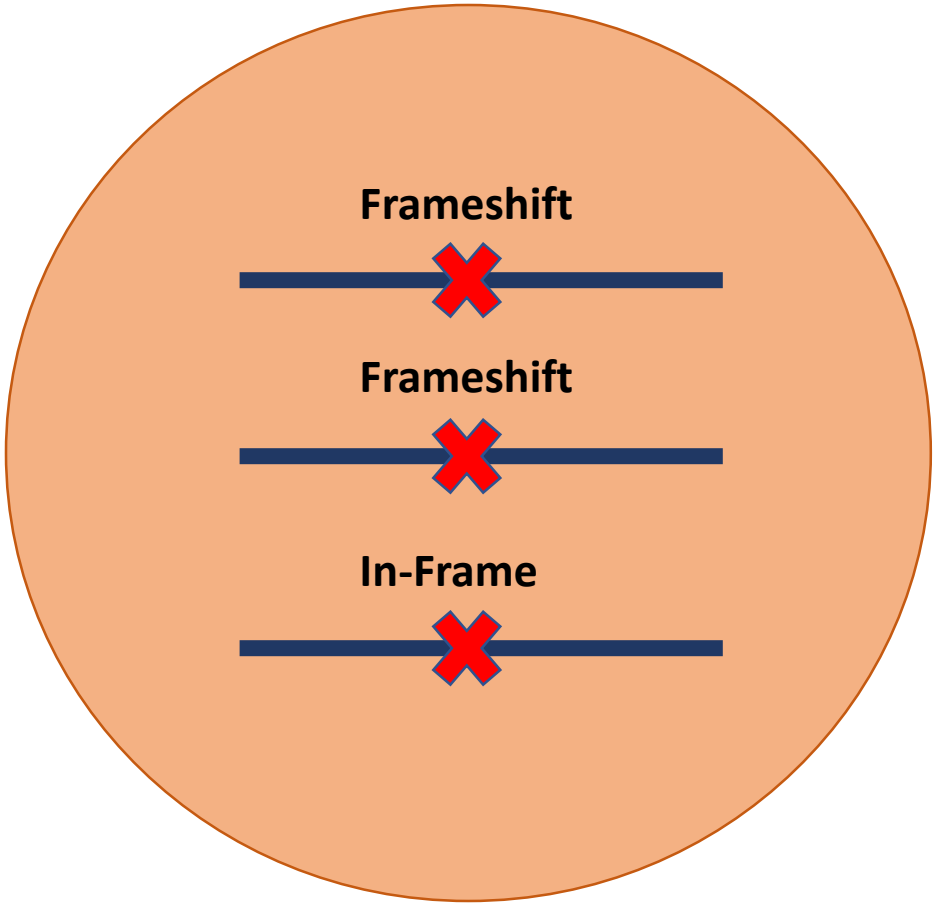
POLYPLOIDY

sgRNA against an essential region of an essential gene



Triploid cell

sgRNA against non essential region of an essential gene



Triploid cell

A CRISPR-Cas9 "tiling" screening strategy against encoding protein domains:

Nominate individual essential domains

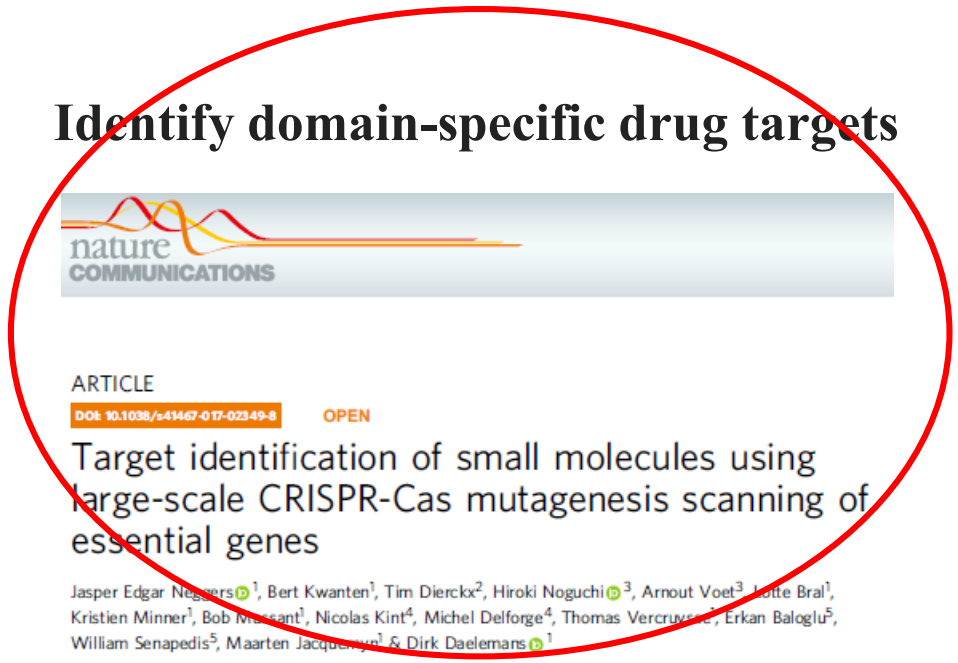

LETTERS

nature biotechnology

Discovery of cancer drug targets by CRISPR-Cas9 screening of protein domains

Junwei Shi[1,2], Eric Wang[1], Joseph P Milazzo[1], Zihua Wang[1], Justin B Kinney[1] & Christopher R Vakoc[1]

Identify domain-specific drug targets


nature COMMUNICATIONS

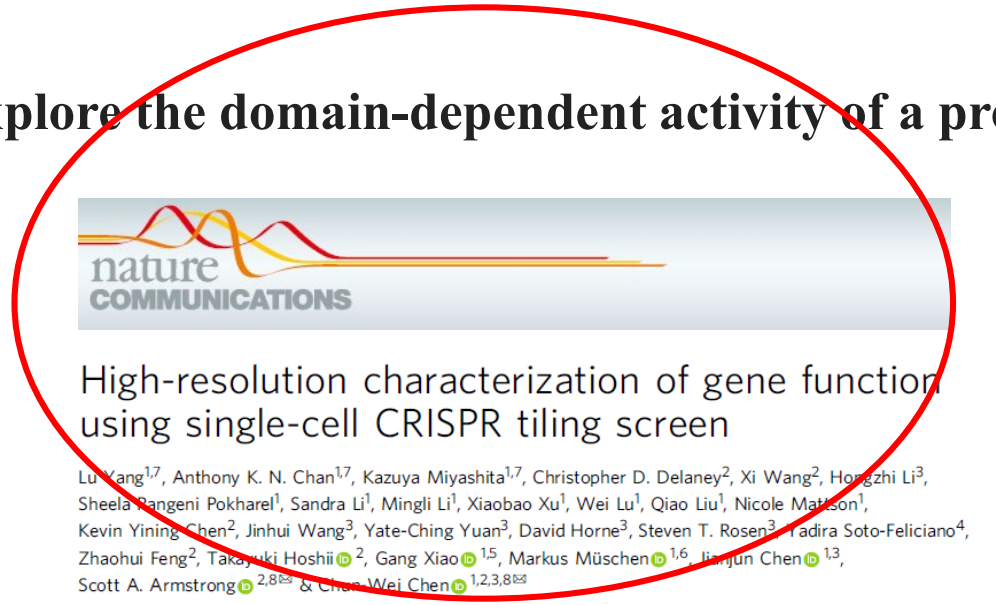
ARTICLE

DOI: 10.1038/s41467-017-02349-8 OPEN

Target identification of small molecules using large-scale CRISPR-Cas mutagenesis scanning of essential genes

Jasper Edgar Neggers[1], Bert Kwanten[1], Tim Dierckx[2], Hiroki Noguchi[3], Arnout Voet[3], Lotte Bral[1], Kristien Minner[1], Bob Maes[1], Nicolas Kint[4], Michel Delforge[4], Thomas Vercruysse[1], Erkan Baloglu[5], William Senapedis[5], Maarten Jacquemyn[1] & Dirk Daelemans[1]

Explore the domain-dependent activity of a protein


nature COMMUNICATIONS

High-resolution characterization of gene function using single-cell CRISPR tiling screen

Lu Yang[1,7], Anthony K. N. Chan[1,7], Kazuya Miyashita[1,7], Christopher D. Delaney[2], Xi Wang[2], Hongzhi Li[3], Sheela Pangeni Pokharel[1], Sandra Li[1], Mingli Li[1], Xiaobao Xu[1], Wei Lu[1], Qiao Liu[1], Nicole Mattson[1], Kevin Yining Chen[2], Jinhui Wang[3], Yate-Ching Yuan[3], David Horne[3], Steven T. Rosen[3], Yadira Soto-Feliciano[4], Zhaohui Feng[2], Takayuki Hoshii[2], Gang Xiao[1,5], Markus Müschen[1,6], Jianjun Chen[1,3], Scott A. Armstrong[2,8✉] & Chun-Wei Chen[1,2,3,8✉]

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Identify domain-specific drug targets by CRISPR tiling screens

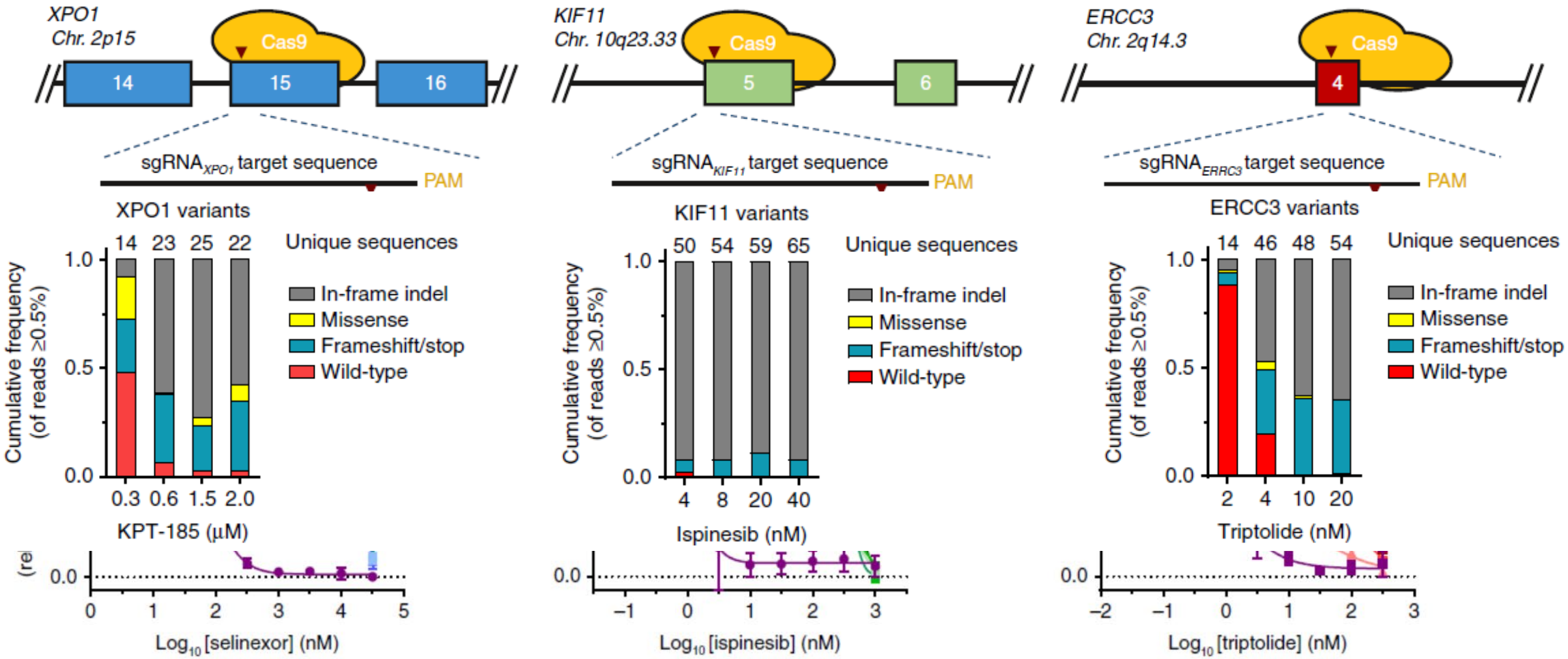
Identifying the cellular target of a chemical hit is a crucial step in drug discovery and development

Unraveling the molecular target of a small molecule drug still remains a challenging, laborious, and complex process

Deconvolution methods such as chemical proteomics → **Often ambiguous**

Typical genetic screens → **Miss gain-of-function mutations and drug-targeted essential proteins**

Rapid generation of drug-resistant variants with CRISPR-Cas9



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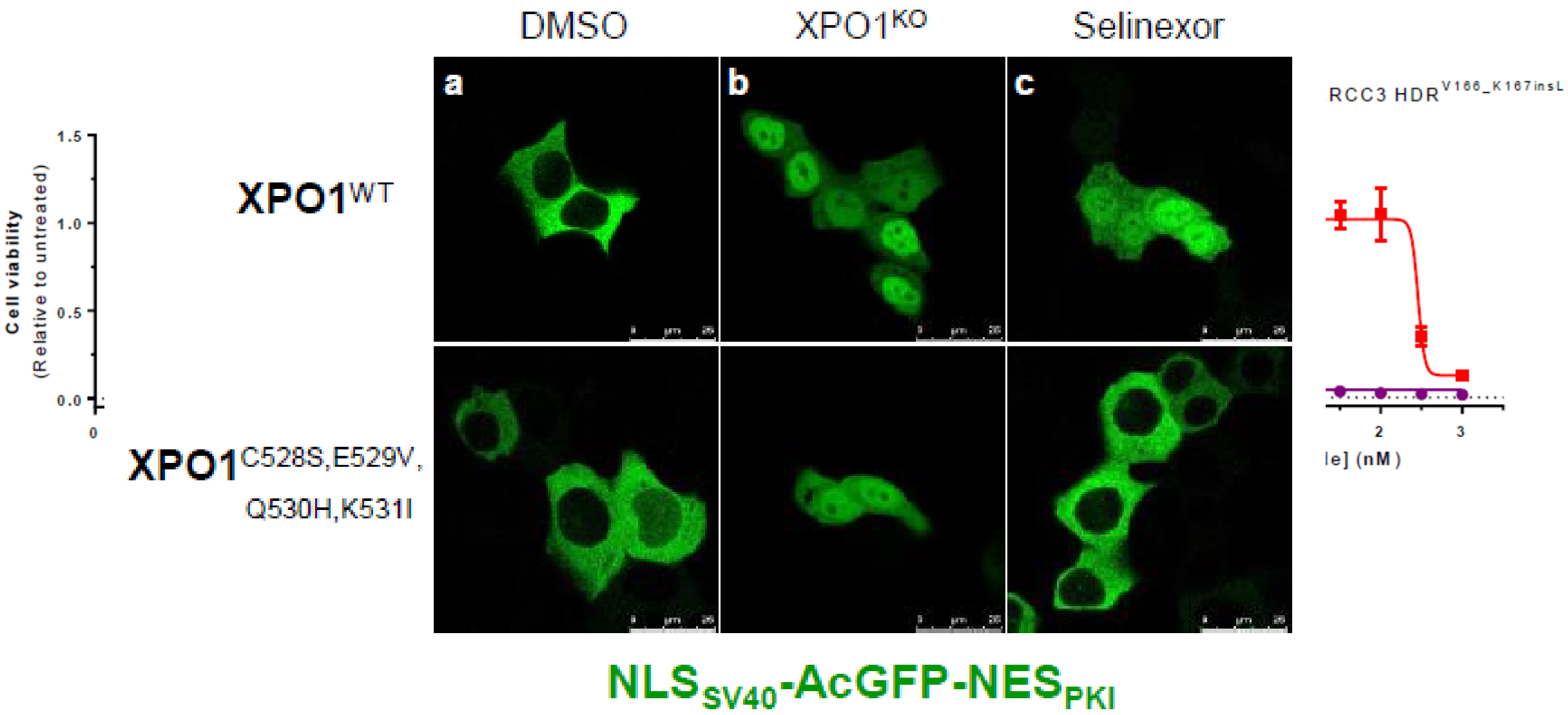
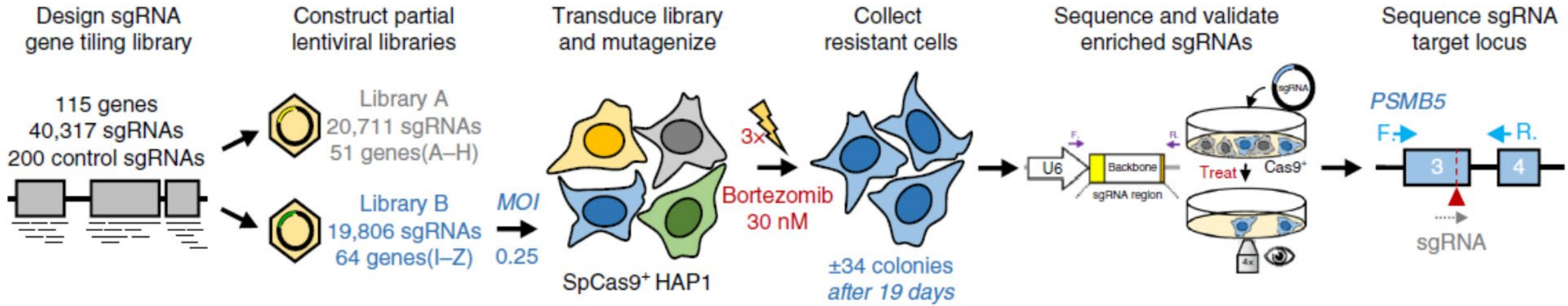
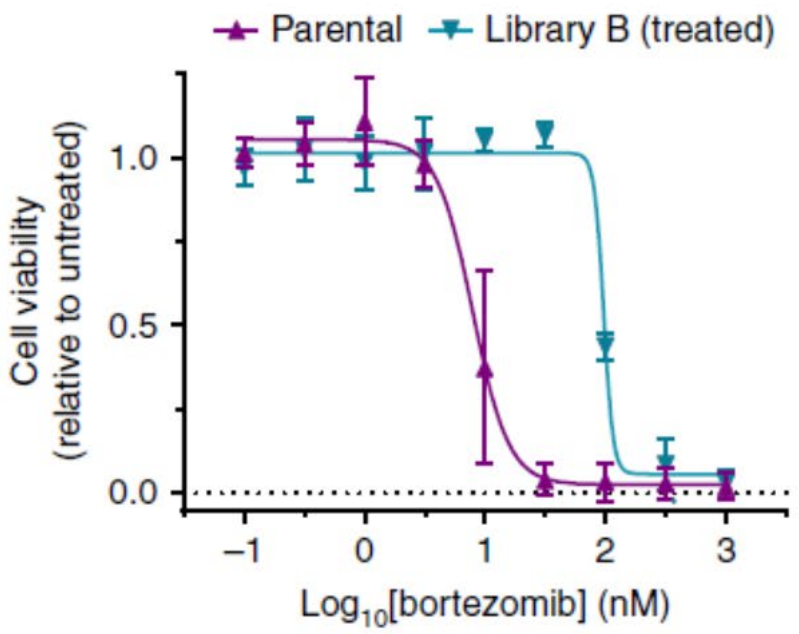
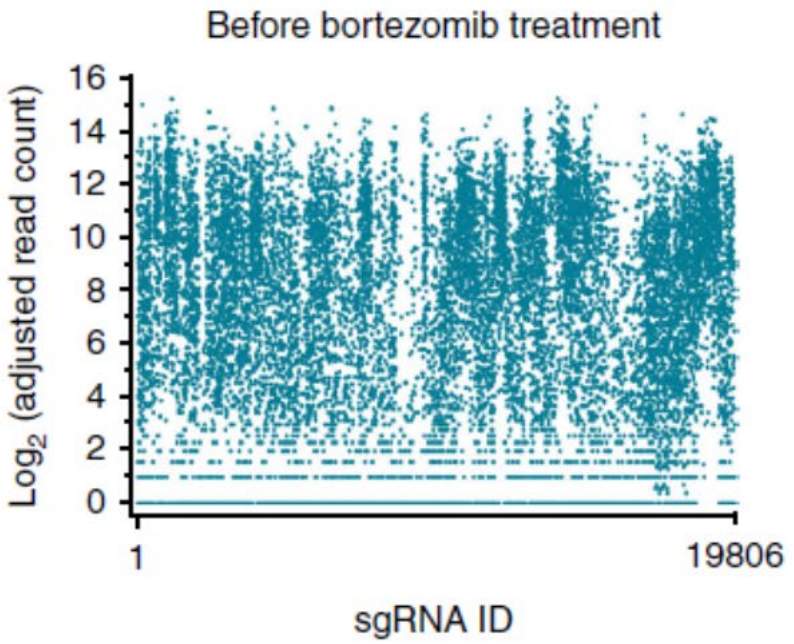
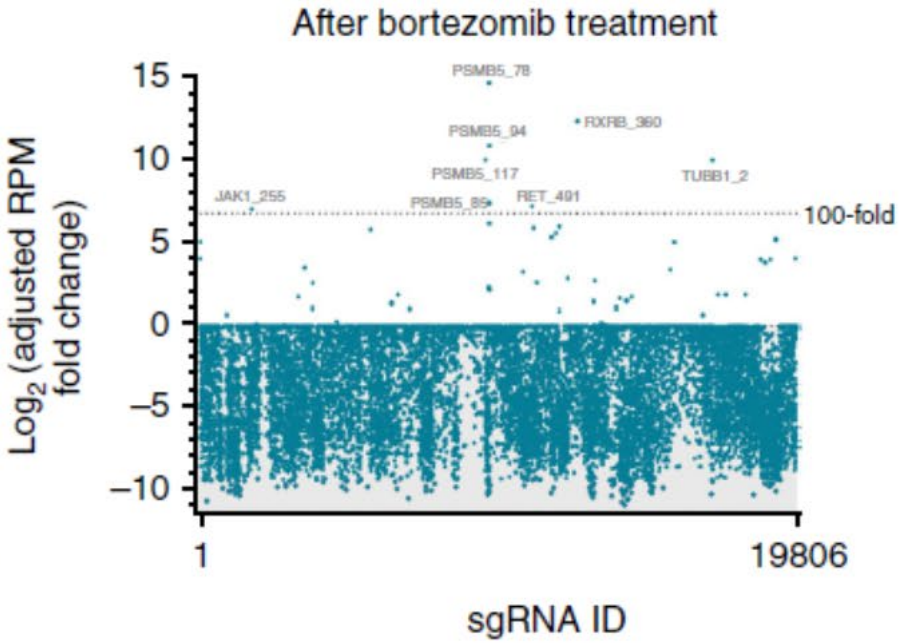


Targeting unknown drug resistances by sgRNA tiling of multiple genes





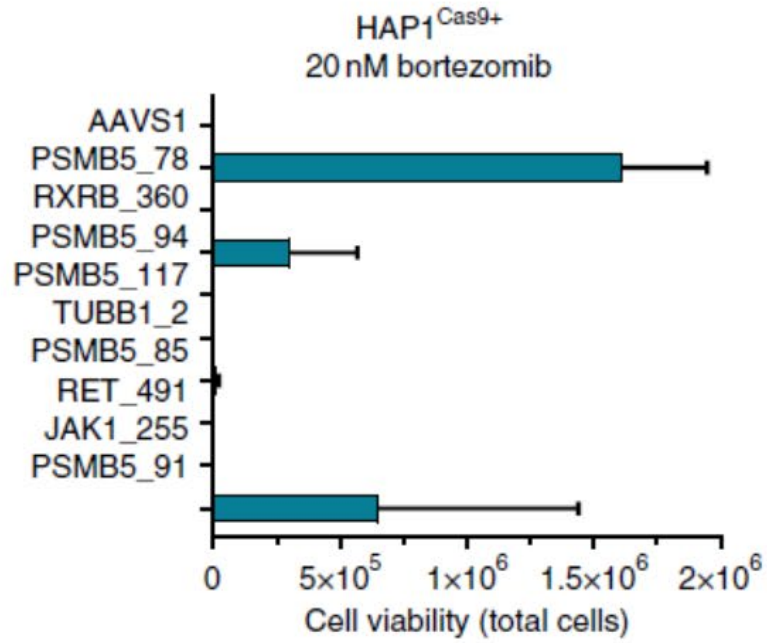




Targeting unknown drug resistances by sgRNA tiling of multiple genes

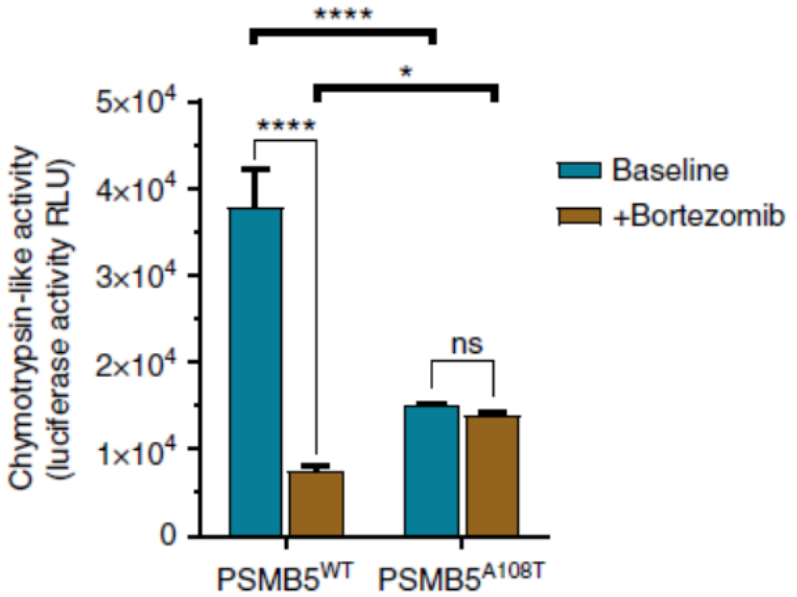
Enriched sgRNAs after bortezomib treatment

sgRNA ID	Cut site	Fold change	Frequency
PSMB5_78	*PSMB5*^{A79}	24,881	12.1%
RXRB_360	*RXRB*^{Q18}	4955	23.7%
PSMB5_94	*PSMB5*^{A108}	1761	23.6%
PSMB5_117	*PSMB5*^{Intron1}	959	22.4%
TUBB1_2	*TUBB1*^{Q11}	947	0.3%
PSMB5_85	*PSMB5*^{T80}	156	0.3%
RET_491	*RET*^{S795}	138	2.2%
JAK1_255	*JAK1*^{L511}	121	1.1%
PSMB5_91	*PSMB5*^{M104}	66	1.1%

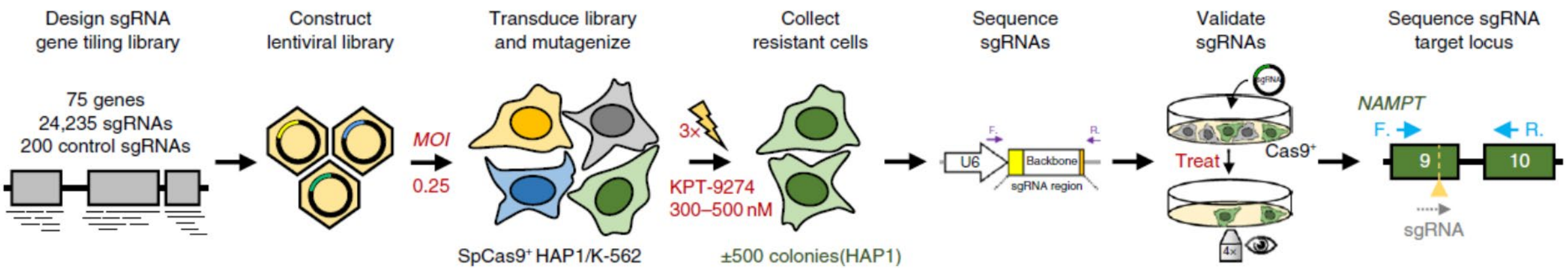


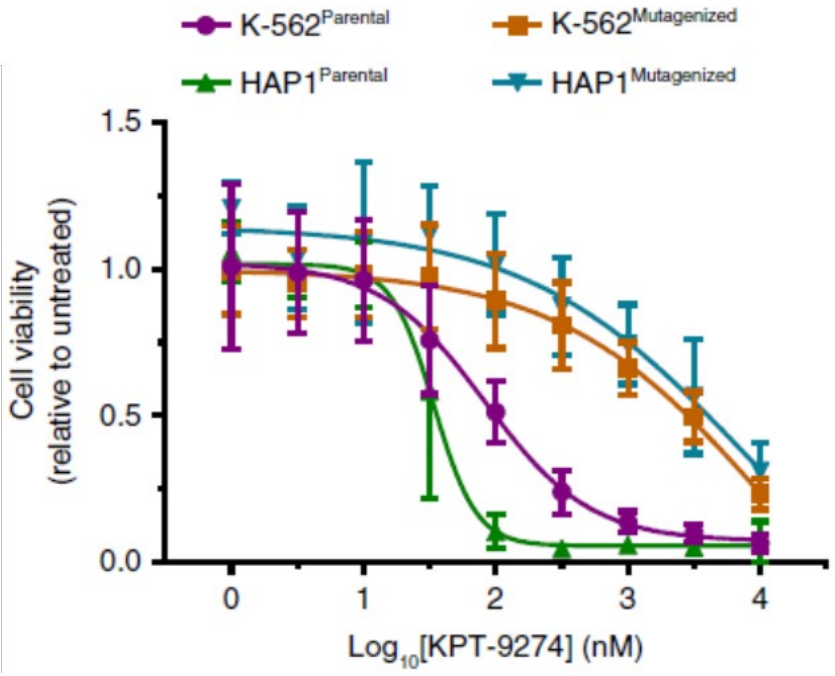
Mutations present in the bortezomib-resistant cell pool

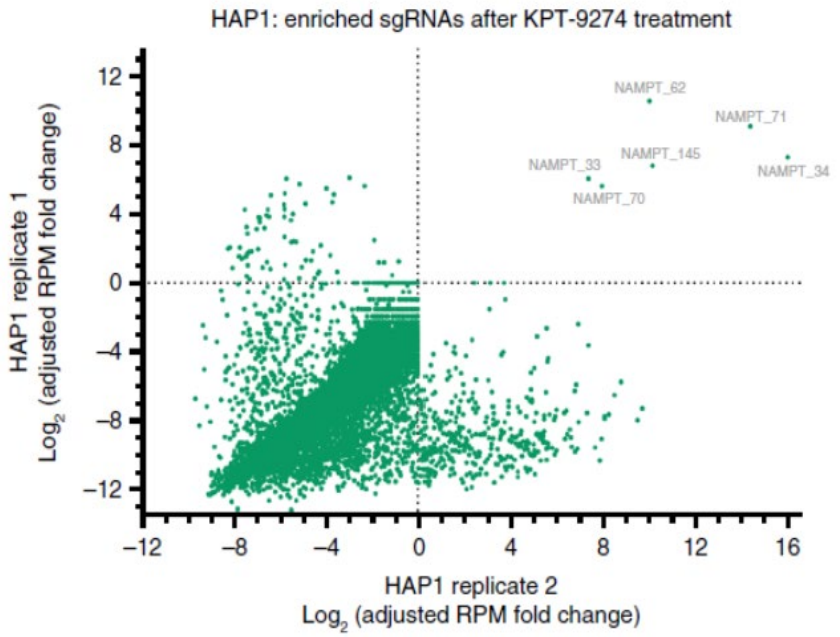
PSMB5 mutation	Type	Associated sgRNA	Frequency
A108T	SNV	PSMB5_94	36.2%
Intron1-del8	Deletion	PSMB5_117	34.0%
A79G, T80A	MNV	PSMB5_78; PSMB5_85	9.9%
R78_A79insS*LQLT	Insertion	PSMB5_78; PSMB5_85	9.0%
A108fs	Frameshift	PSMB5_94	2.3%
C111F	SNV	PSMB5_94	2.0%
M104I	SNV	PSMB5_91	0.9%
T80del	Deletion	PSMB5_78; PSMB5_85	0.6%
S77A	SNV	PSMB5_78; PSMB5_85	0.6%

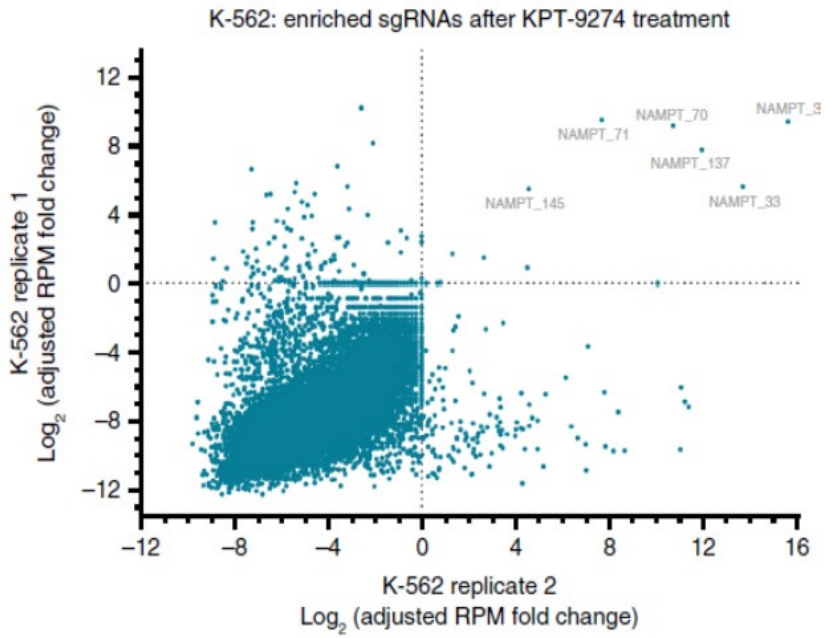


Identifying unknown drug cellular targets by sgRNA tiling of multiple genes



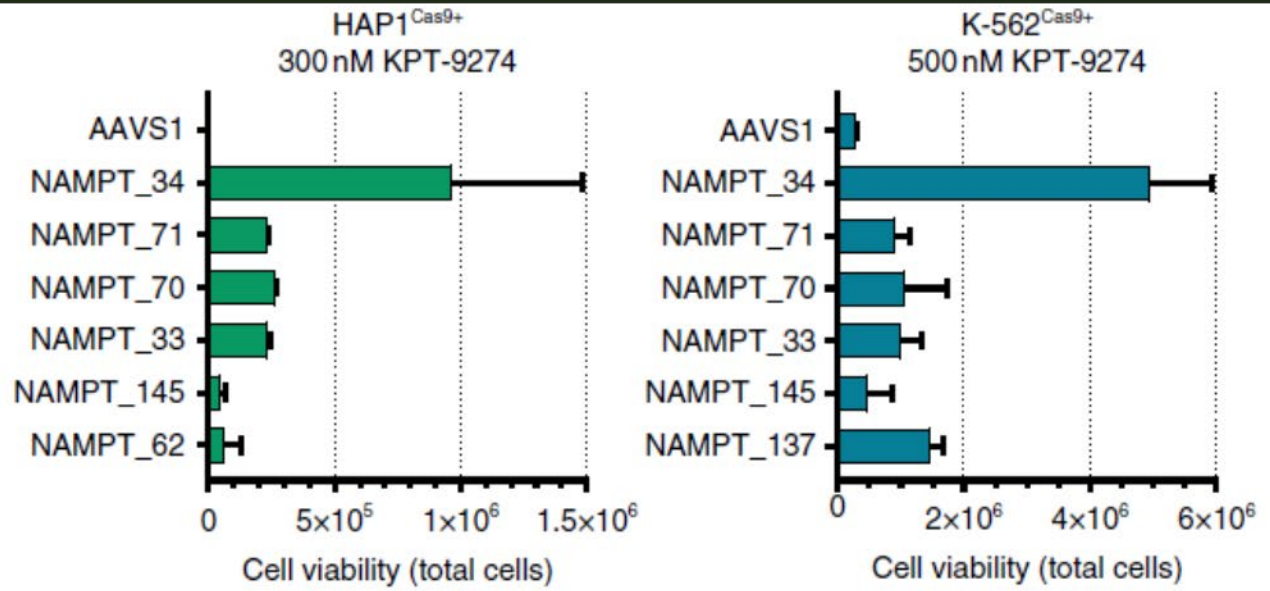






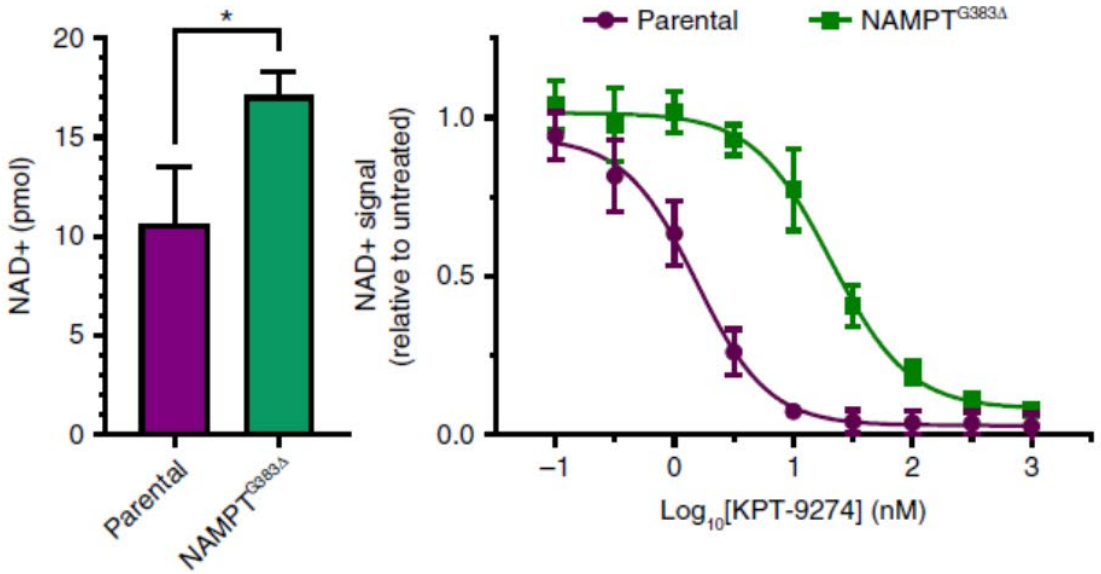
Identifying unknown drug cellular targets by sgRNA tiling of multiple genes

		HAP1 (averages)		K-562 (averages)	
sgRNA ID	Cut site	Log_2(FC)	Frequency	Log_2(FC)	Frequency
NAMPT_34	*NAMPT*^{G383}	11.66	35.65%	12.53	24.60%
NAMPT_71	*NAMPT*^{P238}	11.75	2.86%	8.63	0.06%
NAMPT_70	*NAMPT*^{Y240}	6.81	0.48%	9.98	1.08%
NAMPT_33	*NAMPT*^{S382}	6.70	0.02%	9.71	0.70%
NAMPT_145	*NAMPT*^{Y18}	8.46	6.72%	5.06	1.18%
NAMPT_62	*NAMPT*^{S275}	10.29	1.20%	–2.36	0.00%
NAMPT_137	*NAMPT*^{A14}	–4.04	0.00%	9.89	16.25%



Most prevalent NAMPT mutations detected in HAP1 replicate #1

NAMPT mutation	Type	Associated sgRNAs	Frequency
G383del	Deletion	NAMPT_33; NAMPT_34	51.90%
P238_Y240ins AAEHS	Insertion	NAMPT_70;NAMPT_71	2.53%
G217V	SNV	-	1.91%
P236_V237del	Deletion	NAMPT_70; NAMPT_71	1.64%
P236fs	Frameshift	NAMPT_70; NAMPT_71	1.02%
G239V	SNV	NAMPT_70; NAMPT_71	0.26%
G239_Y240insY	Insertion	NAMPT_70; NAMPT_71	0.24%
S275fs	Frameshift	NAMPT_62	0.17%
G239D	SNV	NAMPT_70;NAMPT_71	0.15%
S301S	Silent SNV	-	100.00%



Conclusions

- **Genetic variation introduced by targeted CRISPR-Cas-mediated NHEJ facilitates rapid selection of in-frame drug resistance mutations in cancer cells;**

- **This method can be applied as a genetic screening strategy using large tiling libraries to identify the direct cellular target protein of a small molecule.**

- **The sgRNA sequences directly annotate the genomic sequence containing the drug resistance conferring mutations, avoiding the need for large whole-exome sequencing to uncover the relevant resistance mutations.**

- **dCas9-cytidine deaminase (CRISPR X) induces site-specific mutagenesis not driving deletions or insertions with lower power in the detection of drug resistance mutations in non-haploid models.**

nature COMMUNICATIONS

High-resolution characterization of gene function using single-cell CRISPR tiling screen

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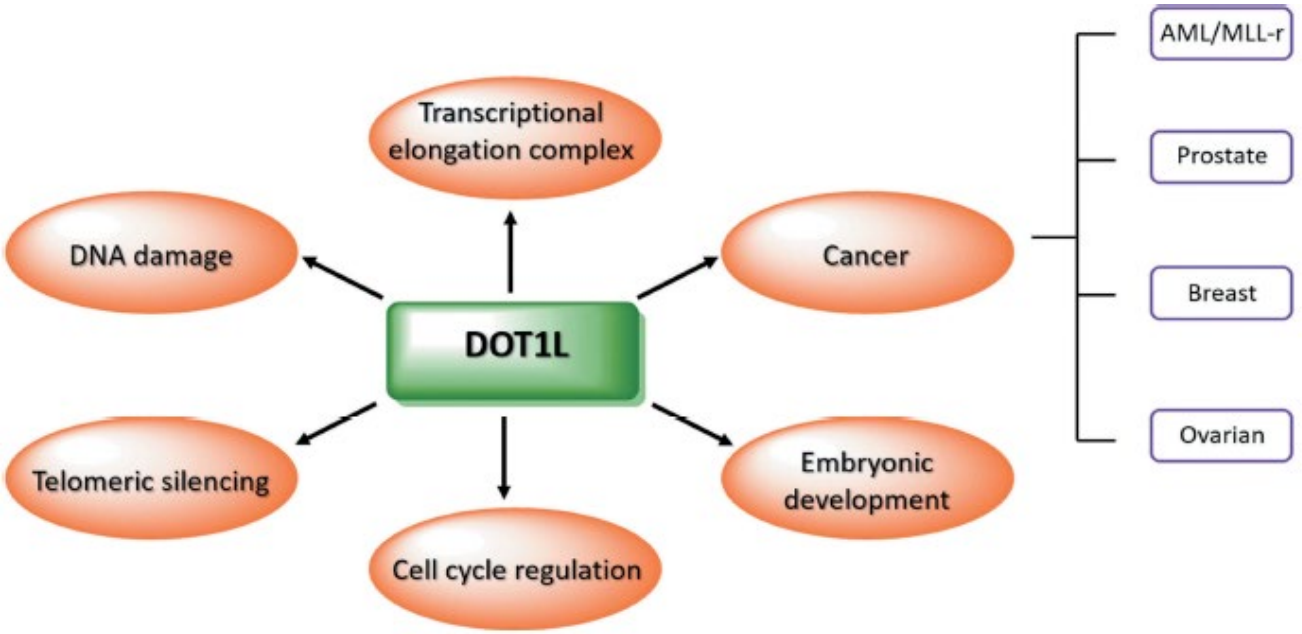
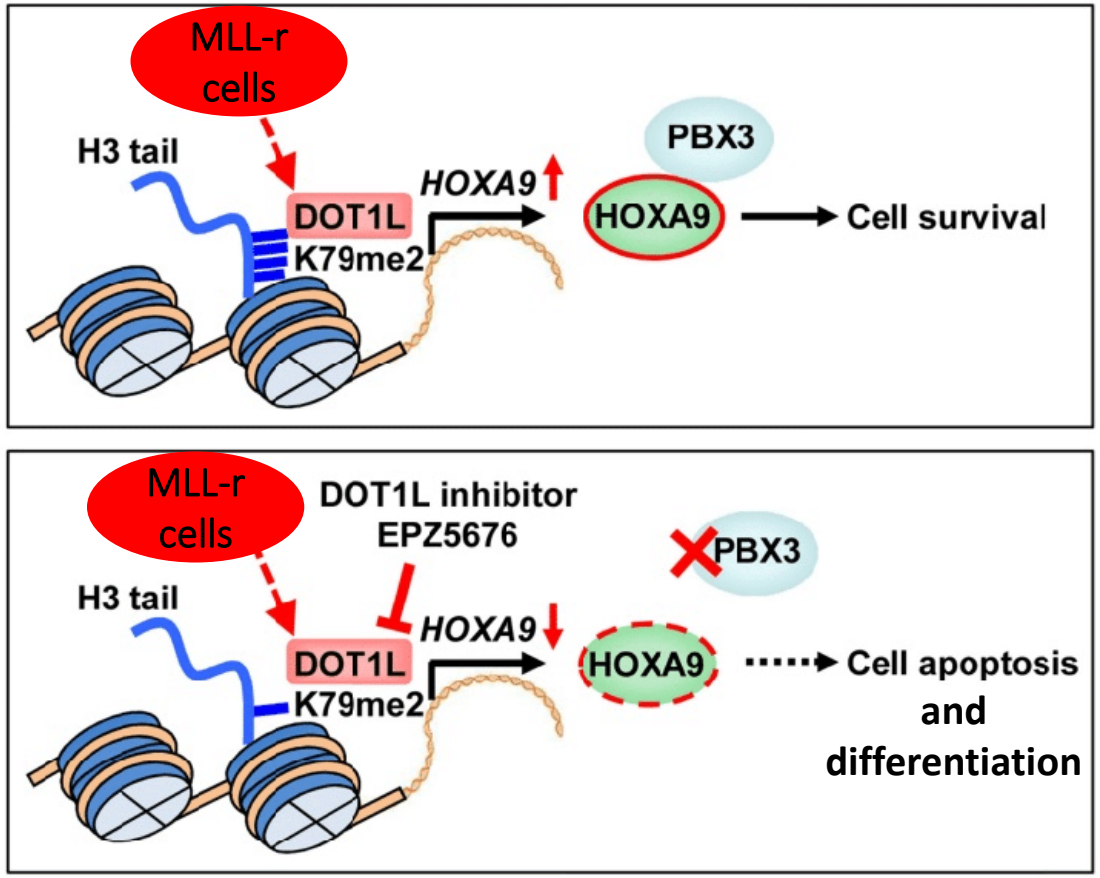
Explore the domain-dependent activity of a protein

DOT1L: a H3K79 methyltransferase in leukemia

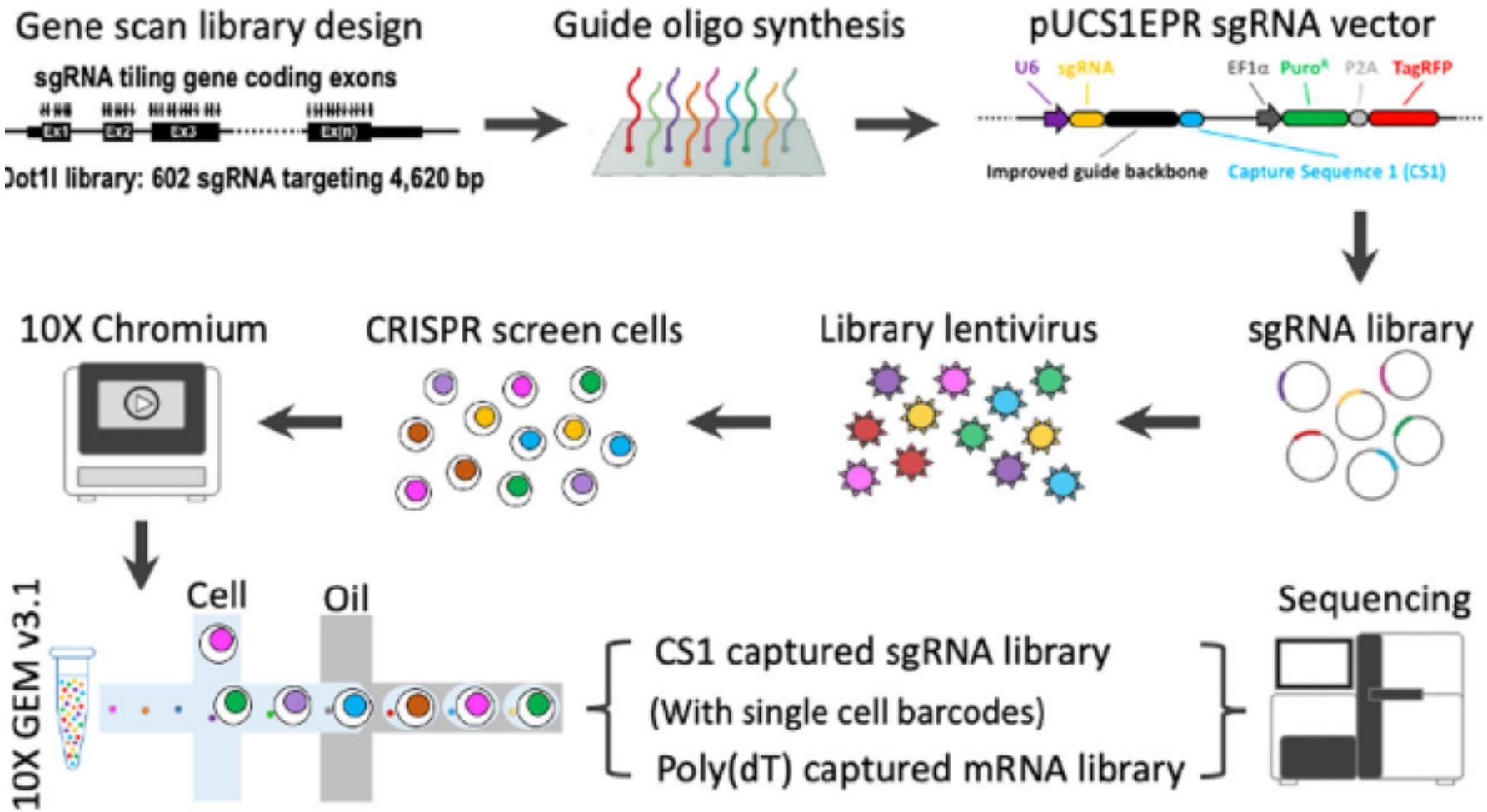


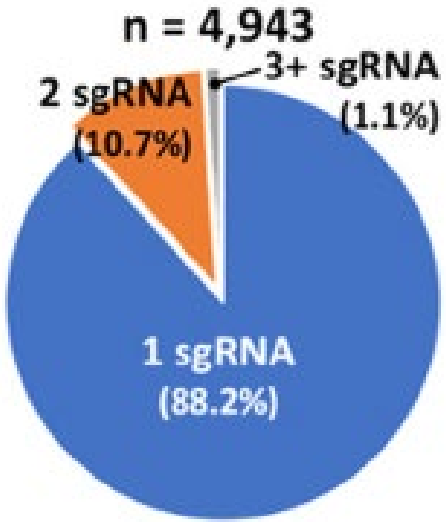


Sarno, Nebbioso, and Altucci 2020

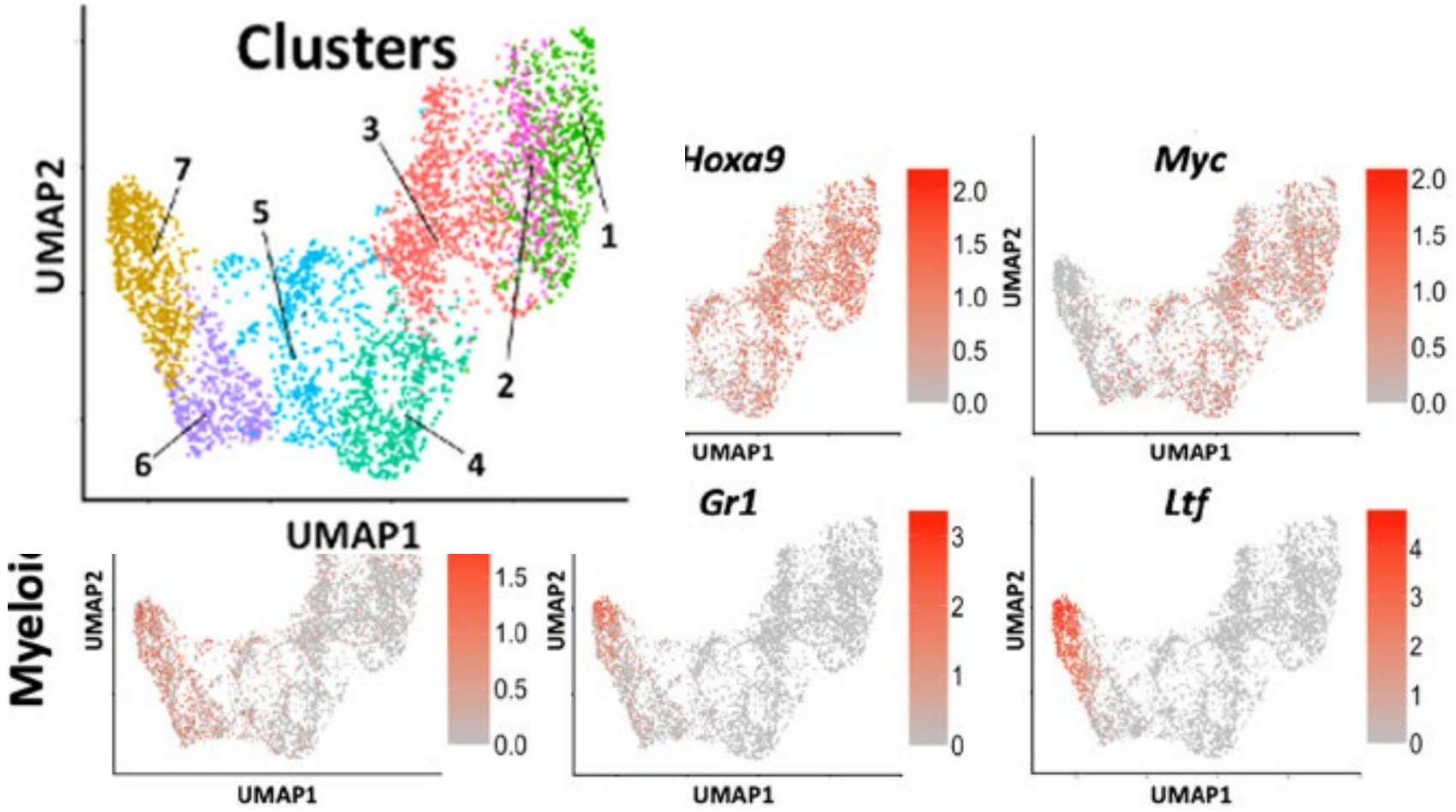
Zhang et al. 2018

Single-cell CRISPR gene tiling strategy: "sc-Tiling"





UMAP of DOT1L dependent-genes



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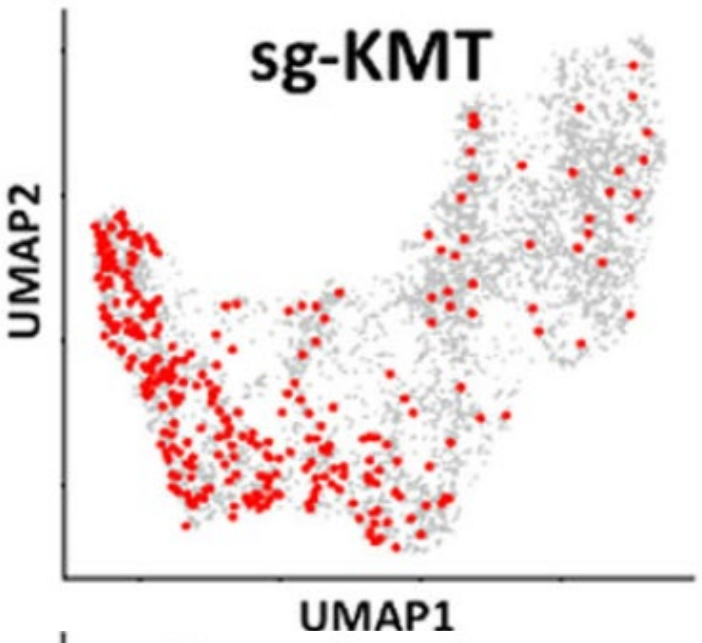
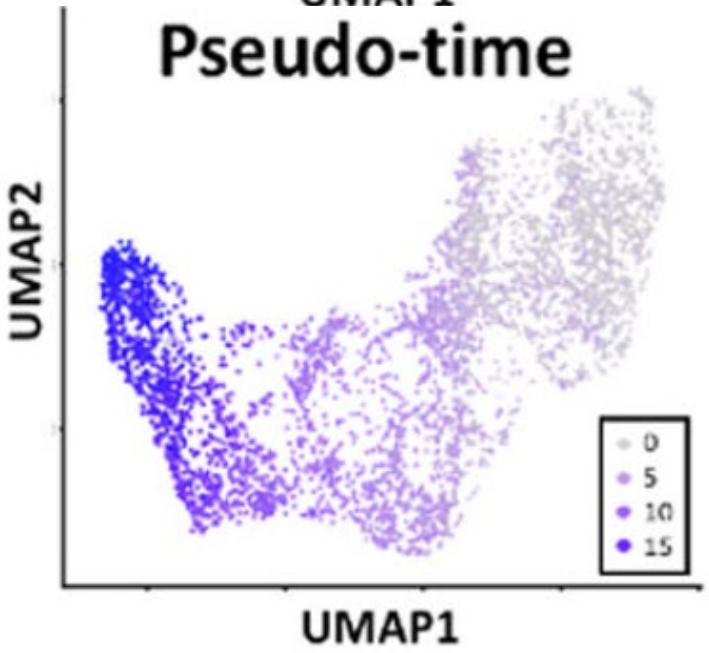
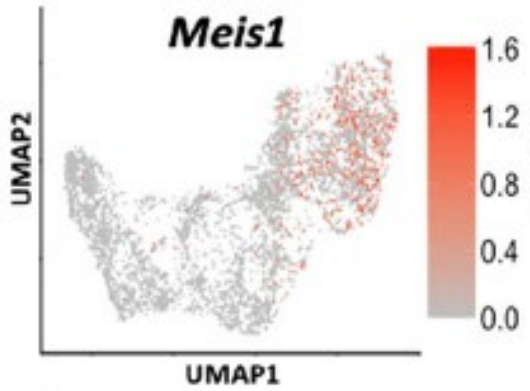
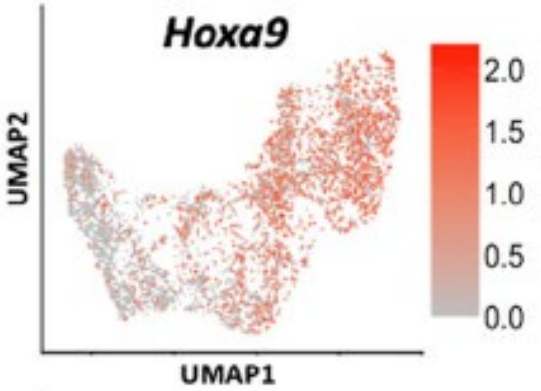
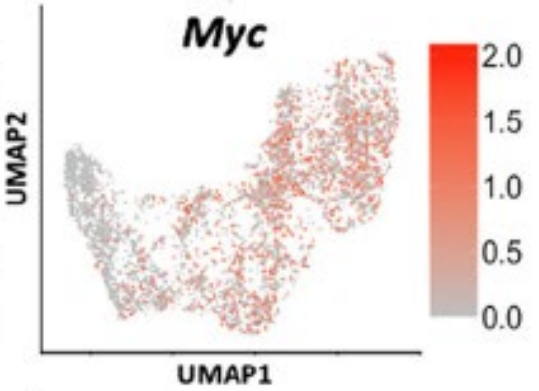




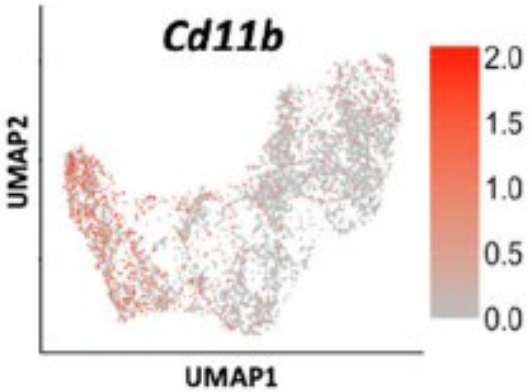
Leukemic

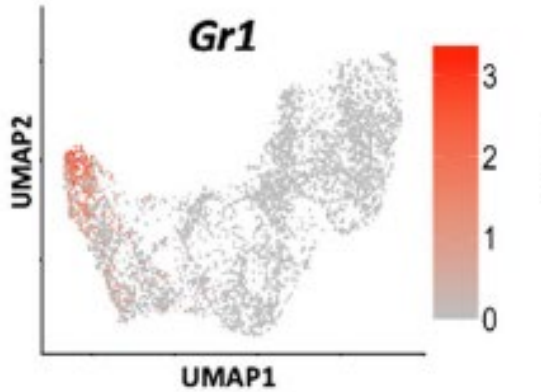


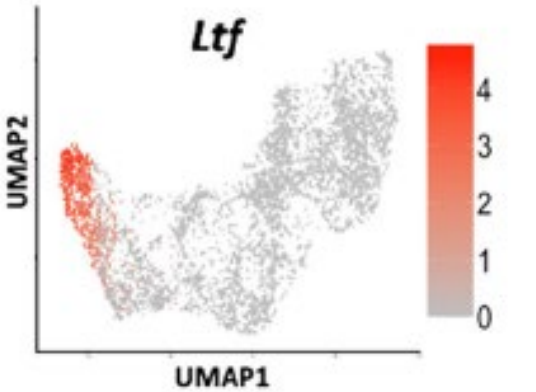




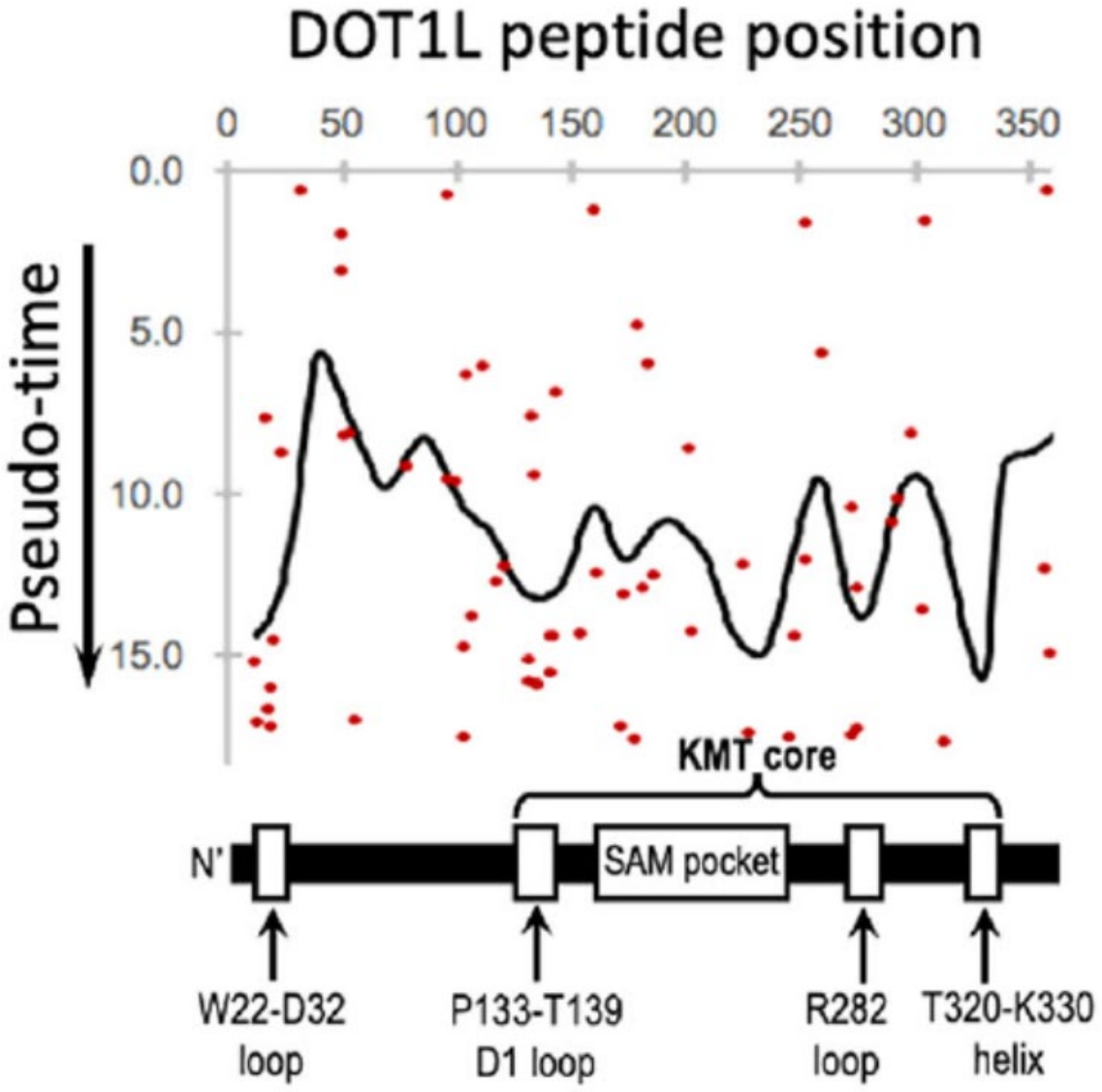
Myeloid

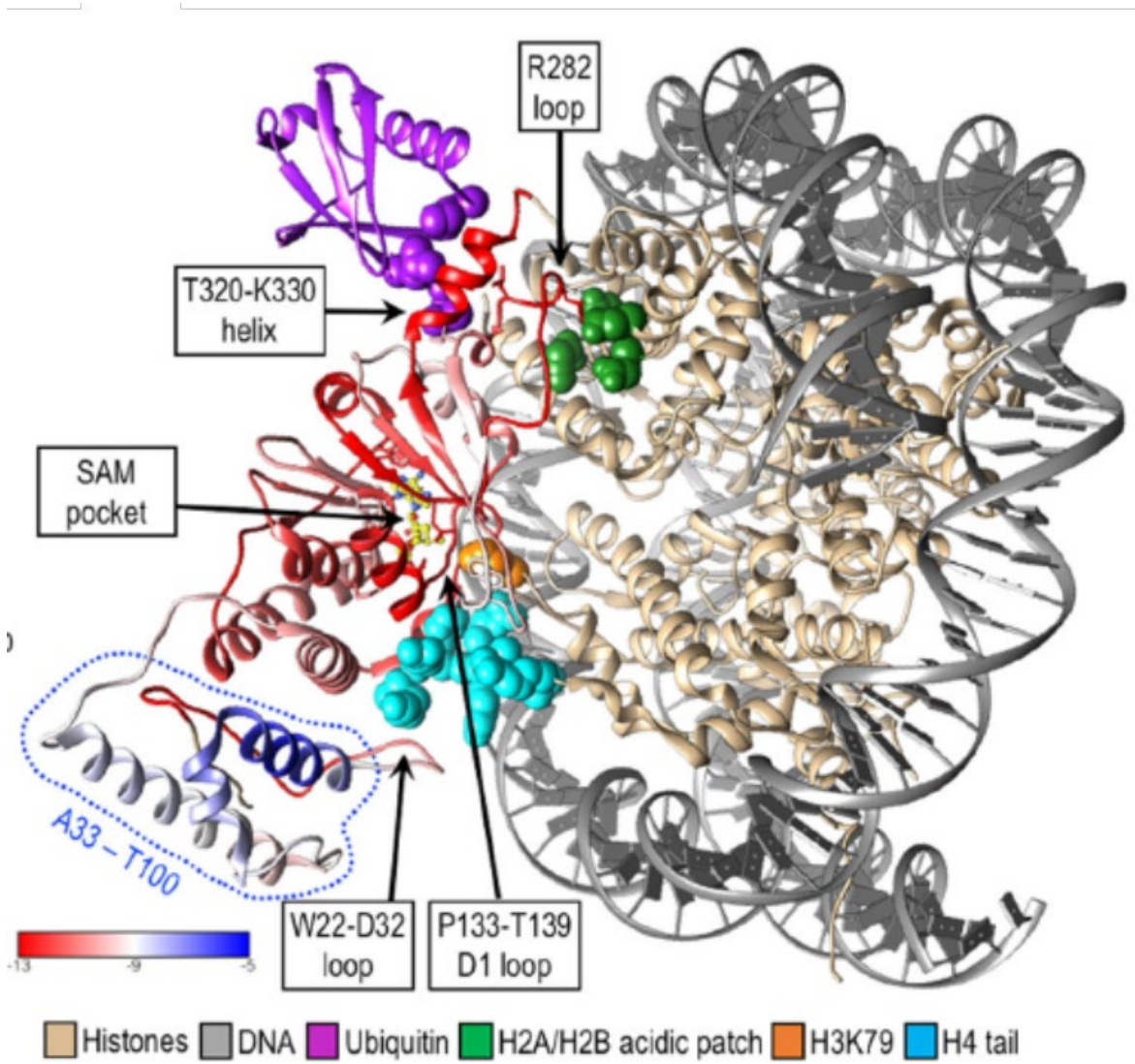




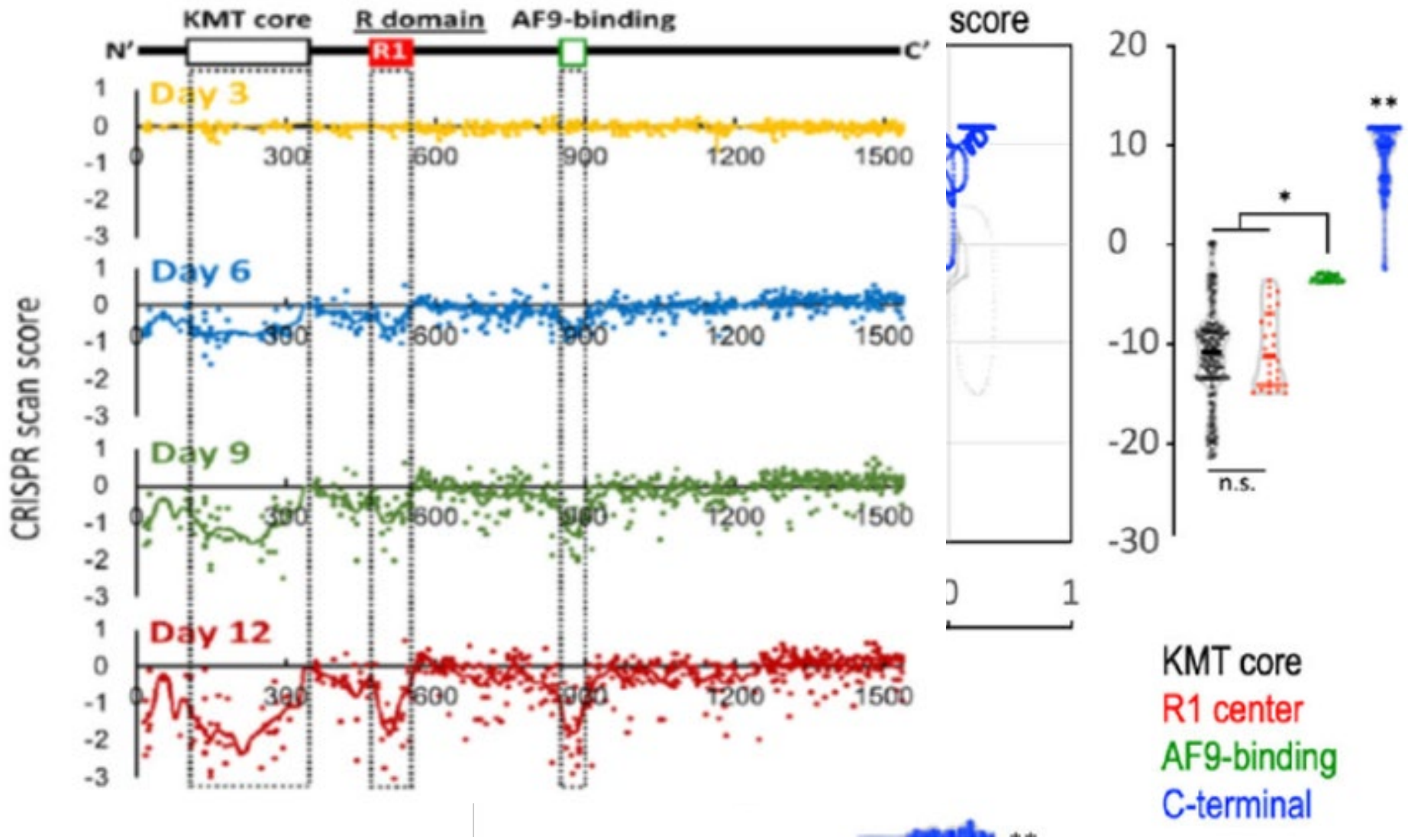


Structural and transcriptomic profiling of sc-Tiling

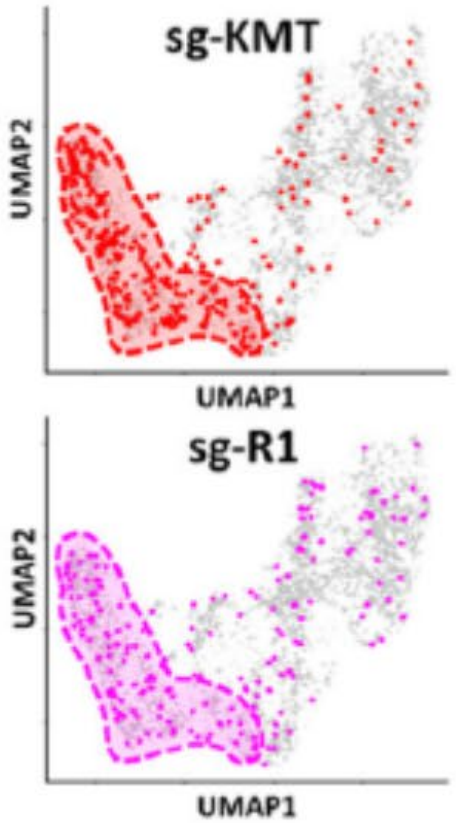


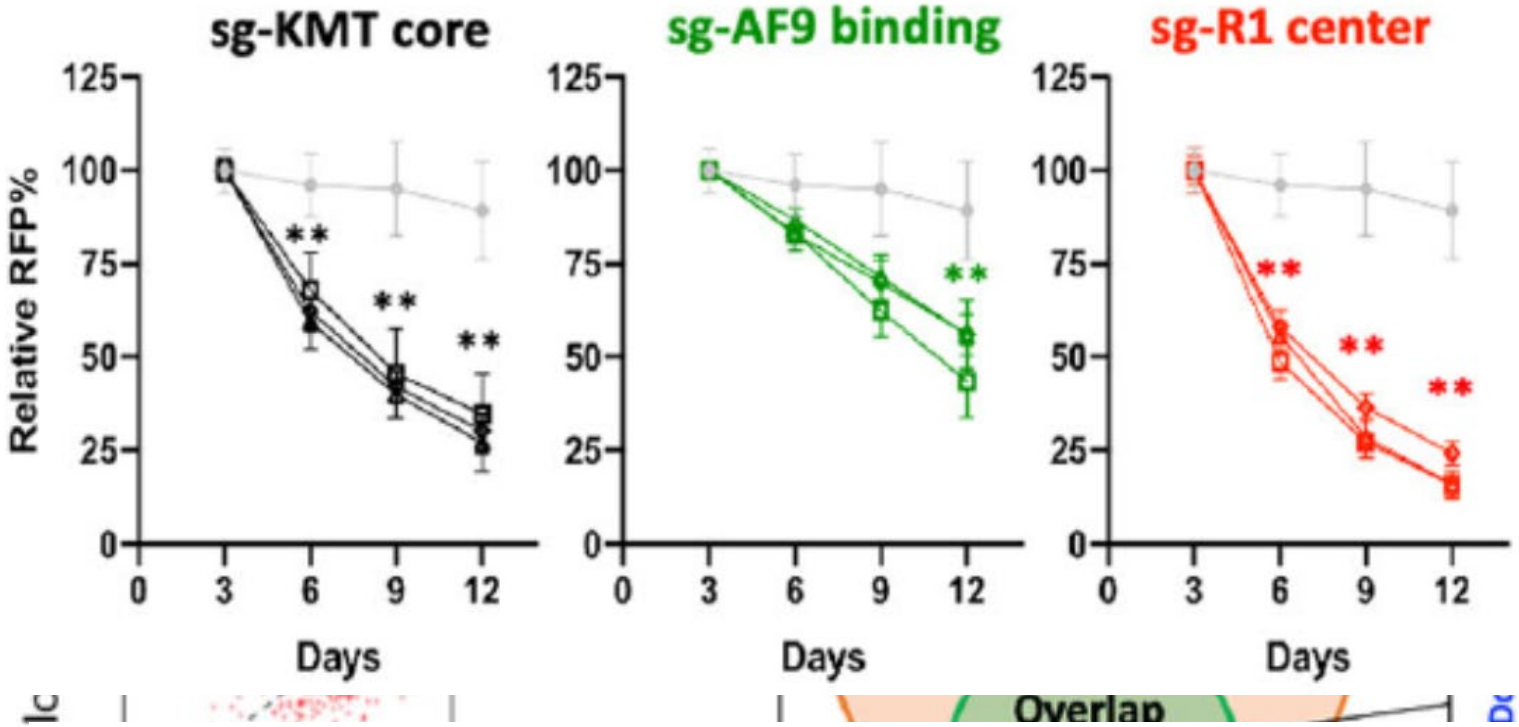


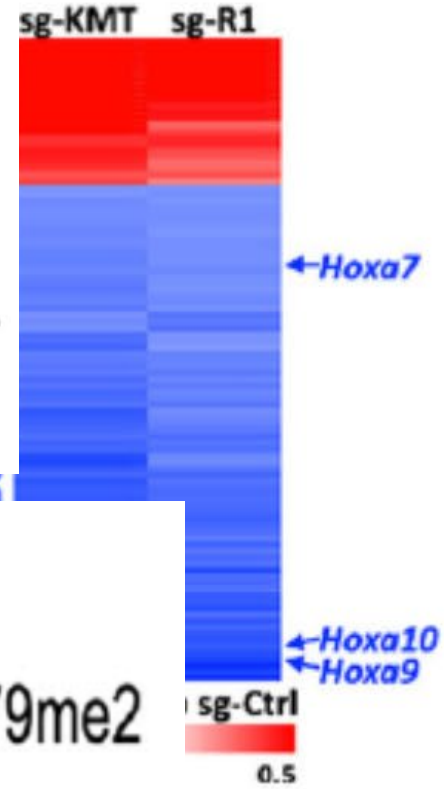
Structural and transcriptomic profiling of sc-Tiling

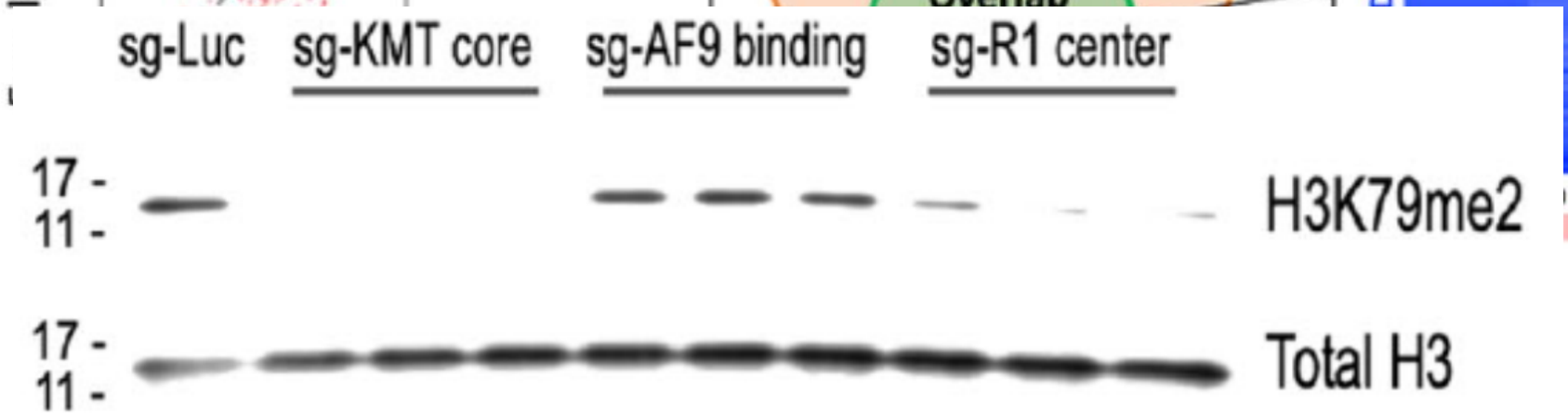


sc-Tiling pinpoints functional elements in DOT1L

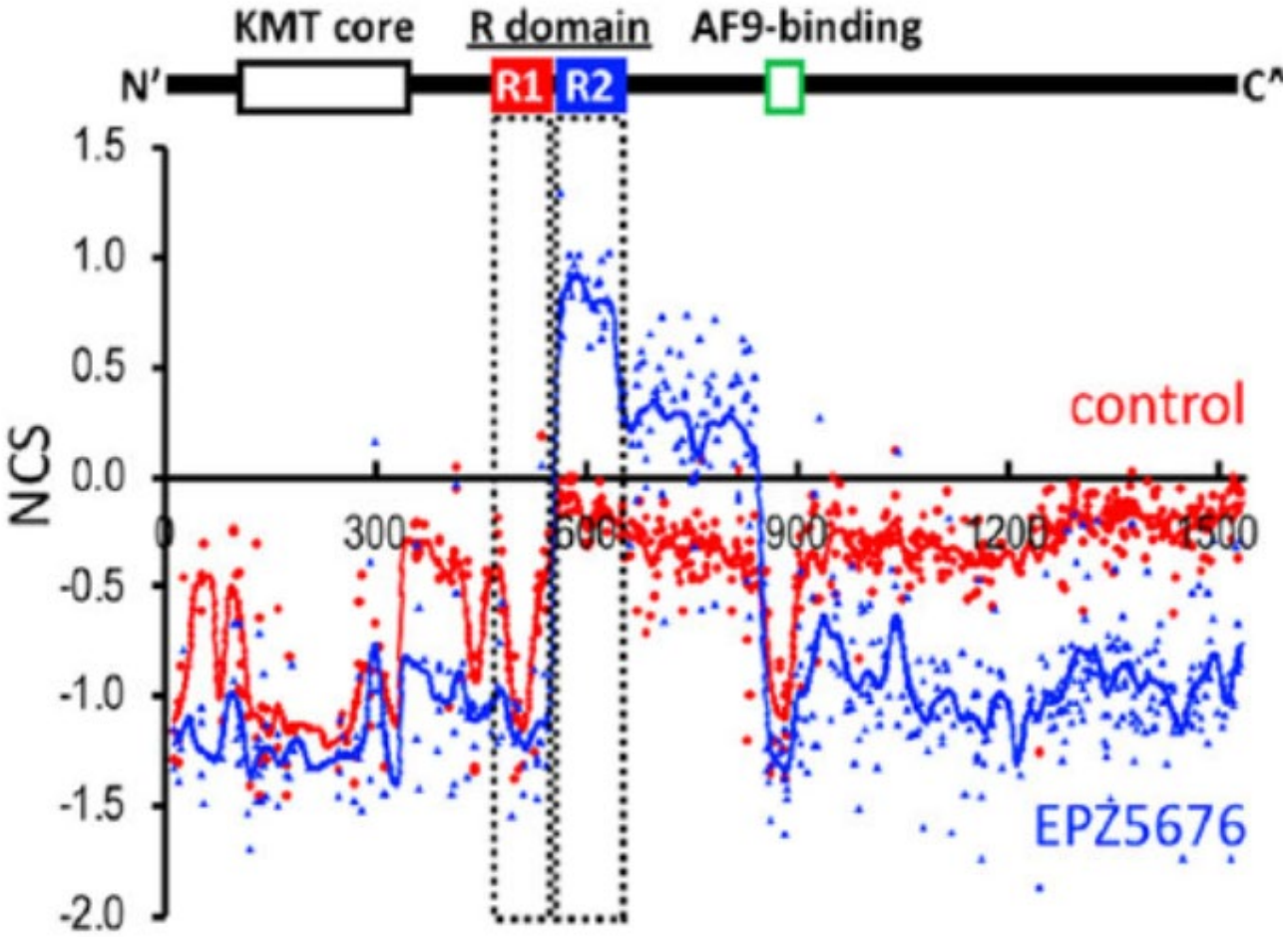


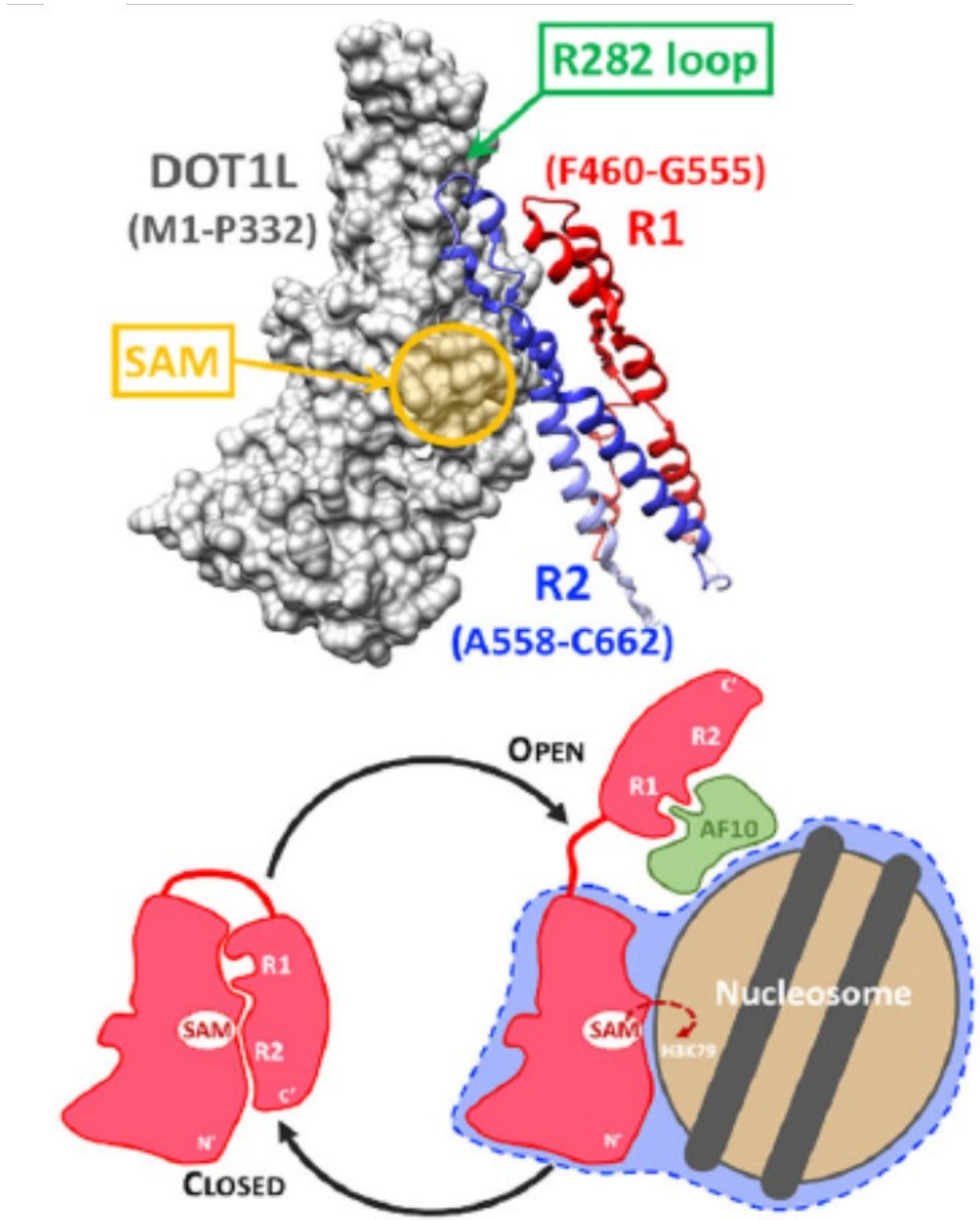




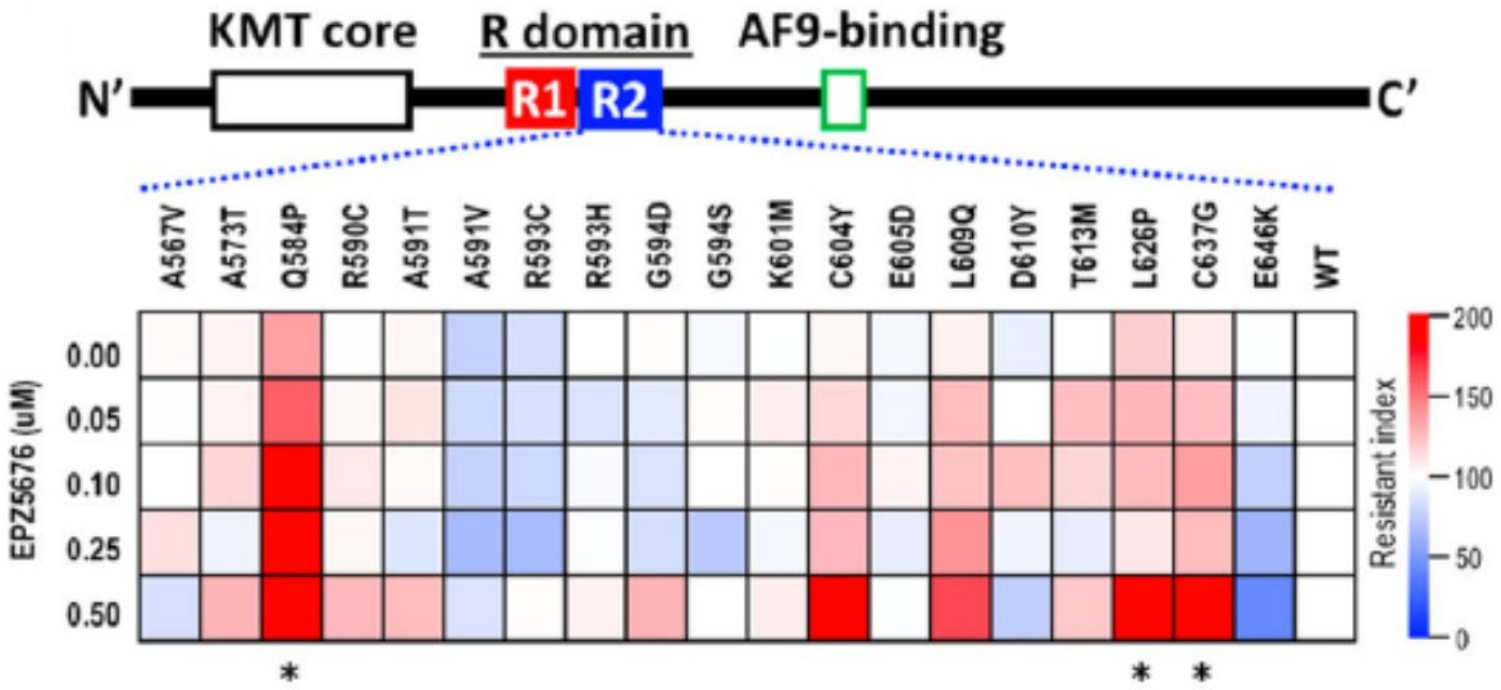


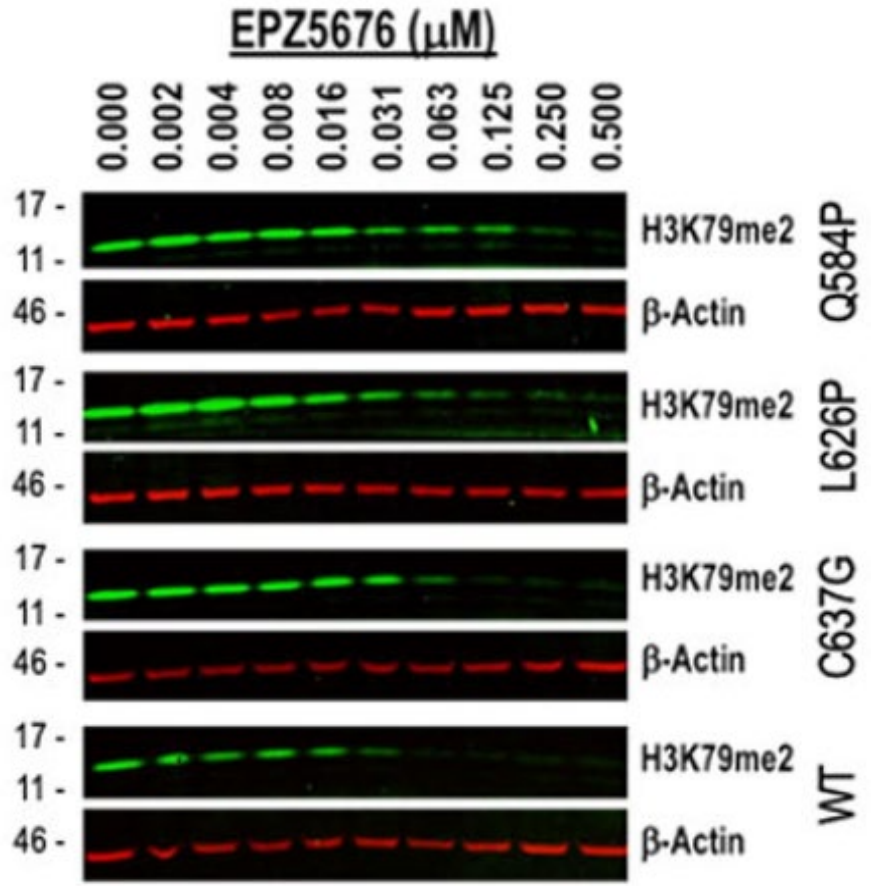
sc-Tiling predicts sub-elements functional interactions





sc-Tiling identifies new therapeutically relevant alleles





Conclusions

- **sc-Tiling integrates a CRISPR gene-tiling screen with single-cell transcriptomic and protein structural analyses.**

- **Sc-Tiling enables the dissection and the individual functional characterization of domains and sub-domains of a protein.**

- **sc-Tiling may complement the rapidly growing multi-omics databases to provide additional insights that bridge functional genomics, structural biology, and clinical investigation.**

THANK YOU FOR THE ATTENTION

References

He, Wei et al. 2019. “De Novo Identification of Essential Protein Domains from CRISPR-Cas9 Tiling-SgRNA Knockout Screens.” *Nature Communications* 10(1). http://dx.doi.org/10.1038/s41467-019-12489-8.

Neggers, Jasper Edgar et al. 2018. “Target Identification of Small Molecules Using Large-Scale CRISPR-Cas Mutagenesis Scanning of Essential Genes.” *Nature Communications* 9(1): 1–14. http://dx.doi.org/10.1038/s41467-017-02349-8.

Sarno, Federica, Angela Nebbioso, and Lucia Altucci. 2020. “DOT1L: A Key Target in Normal Chromatin Remodelling and in Mixed-Lineage Leukaemia Treatment.” *Epigenetics* 15(5): 439–53. https://doi.org/10.1080/15592294.2019.1699991.

Shi, Junwei et al. 2015. “Discovery of Cancer Drug Targets by CRISPR-Cas9 Screening of Protein Domains.” *Nature Biotechnology* 33(6): 661–67.

Yang, Lu et al. 2021. “High-Resolution Characterization of Gene Function Using Single-Cell CRISPR Tiling Screen.” *Nature Communications* 12(1): 4–12. http://dx.doi.org/10.1038/s41467-021-24324-0.

You, Yan, Sharmila G. Ramachandra, and Tian Jin. 2020. “A CRISPR-Based Method for Testing the Essentiality of a Gene.” *Scientific Reports* 10(1): 1–8. https://doi.org/10.1038/s41598-020-71690-8.

Zhang, Wu et al. 2018. “Inactivation of PBX3 and HOXA9 by Down-Regulating H3K79 Methylation Represses NPM1-Mutated Leukemic Cell Survival.” *Theranostics* 8(16): 4359–71.