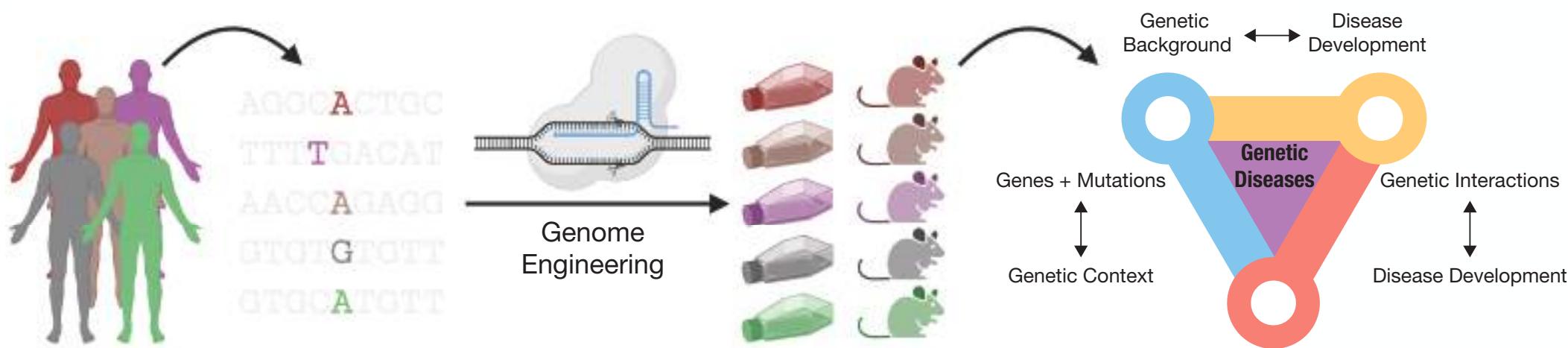


Functional studies of genetic variation using precision genome editing



Francisco J. Sánchez-Rivera

Assistant Professor of Biology at MIT

Koch Institute for Integrative Cancer Research at MIT

**WCS CRISPR Genome Informatics and
Computational Design and Analysis Course**

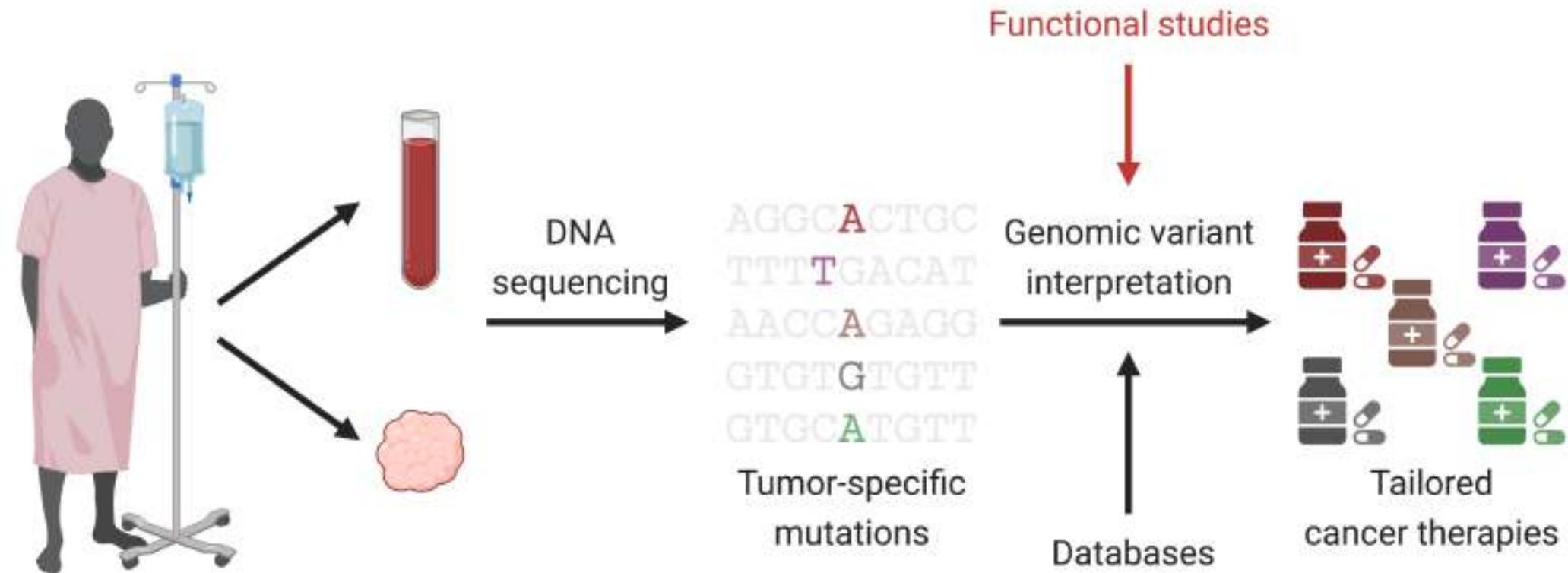


October 7th, 2024

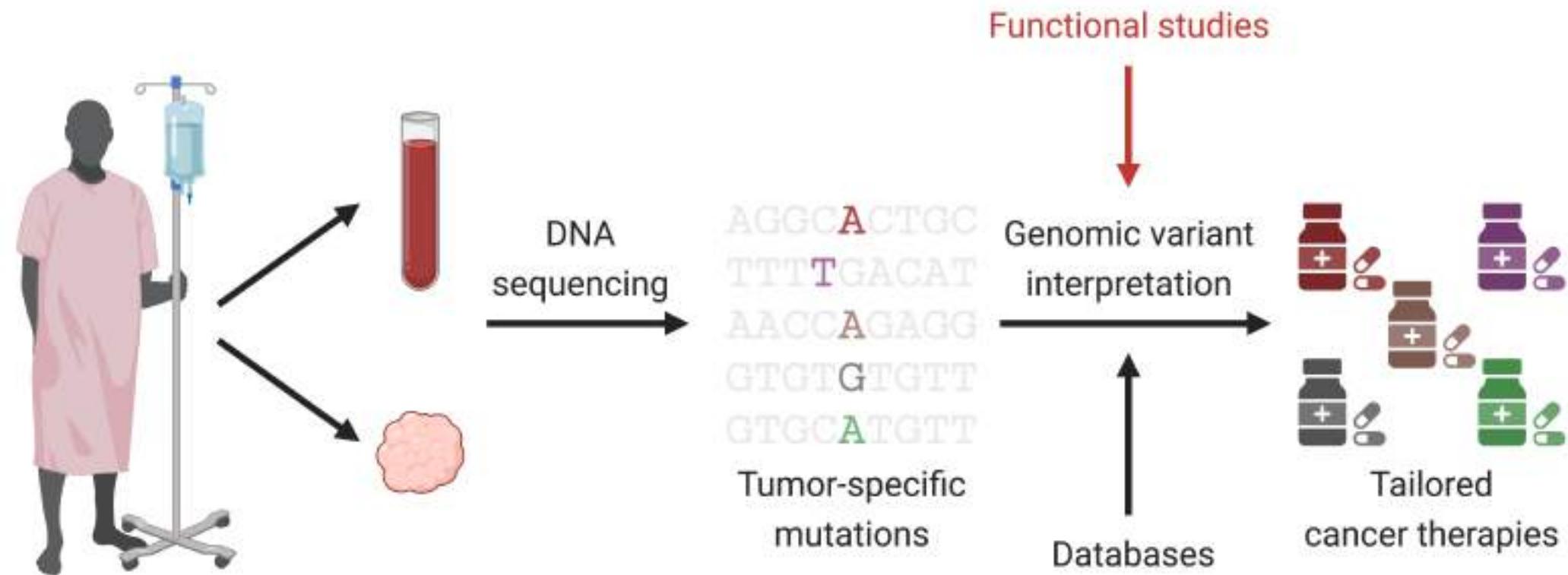
MIT Biology



Clinical DNA sequencing is informing cancer treatment in real time

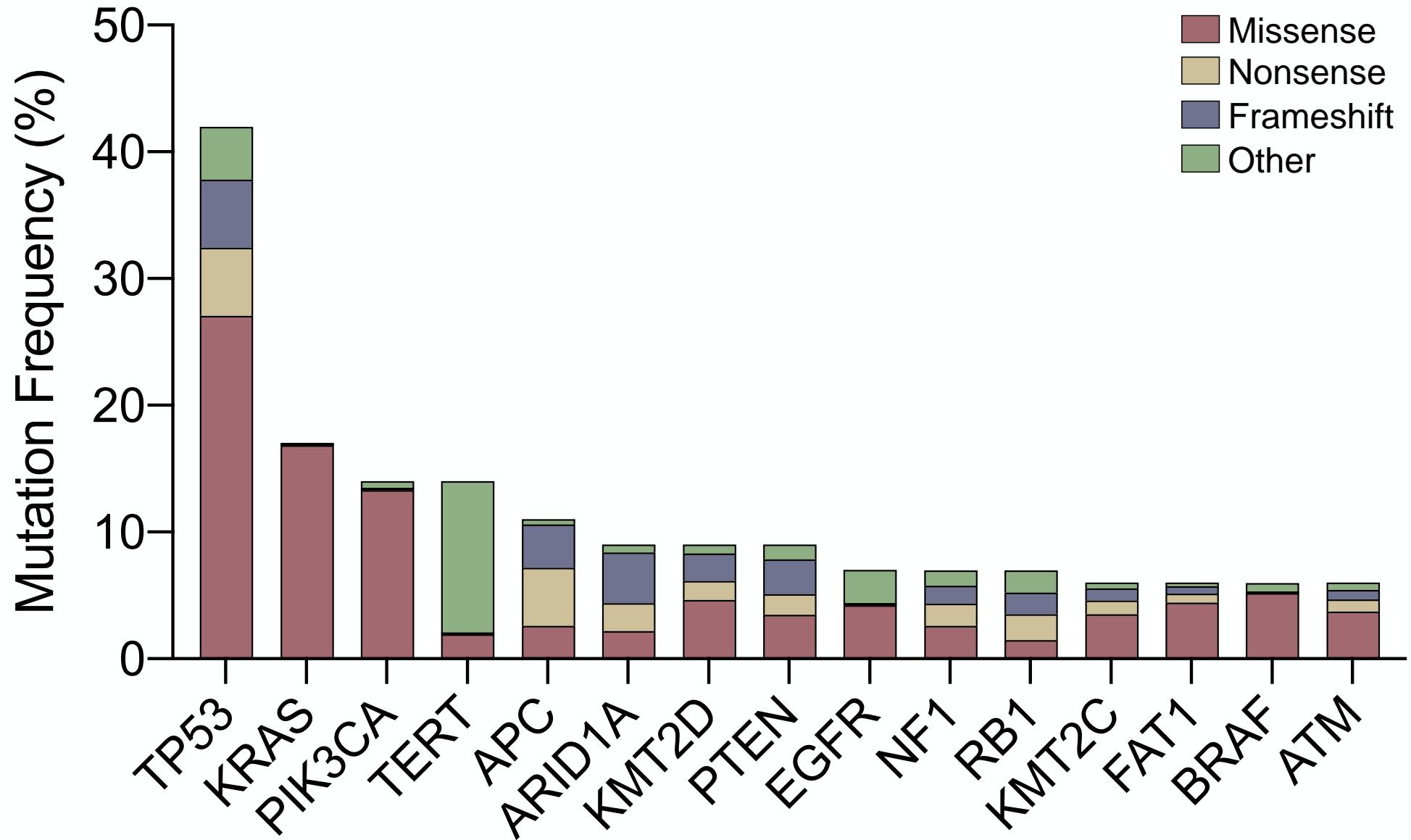


Clinical DNA sequencing is informing cancer treatment in real time



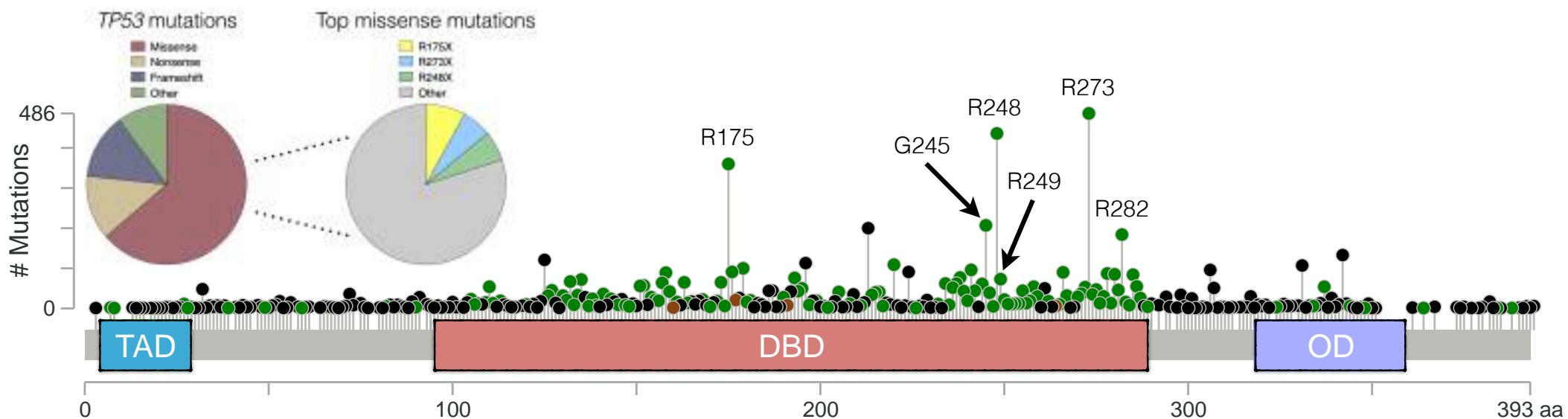
- Drugs targeting **specific mutant proteins** have shown promise in the clinic.
- Precision oncology requires knowledge of **variant function**.
- Most mutations represent **variants of unknown significance**.

Mutations can produce thousands of unique allelic variants



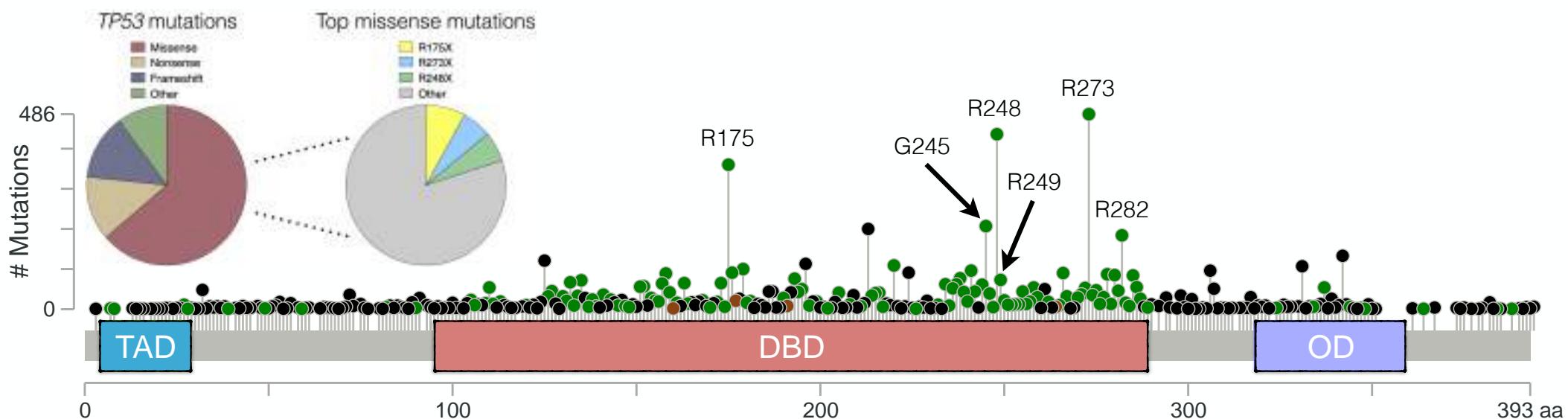
Source of mutation data: MSK-IMPACT (September 2019)

Remarkable diversity in the cancer mutational spectrum



Source of mutation data: TCGA and MSK-IMPACT

Remarkable diversity in the cancer mutational spectrum



Open questions in the p53 field and beyond:

1. Are all mutations functionally **redundant**?
2. What are the **mechanisms** through which these function?
3. Are any mutant proteins **required to sustain disease states**?

Answering these questions could have profound therapeutic implications.

TP53 mutations

Missense
Nonsense
Premature
Other



Top missense mutations

R175K
R273K
R248X
Other



R175

R248

G245

R273

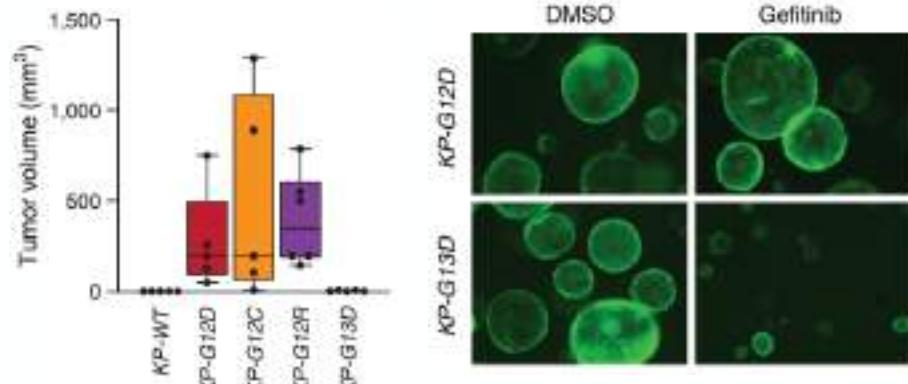
R249

R282

Are all genetic variants the same?

Variants can exhibit profound allele-specific phenotypes

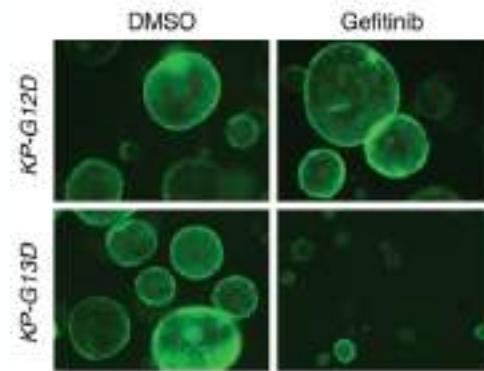
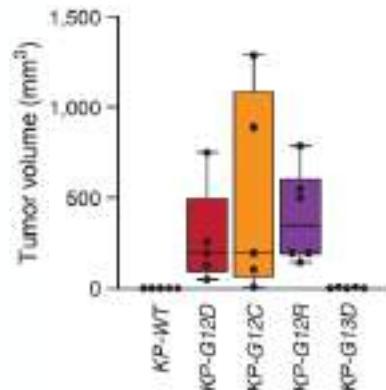
Kras allele-specific cancer phenotypes



Zafra et al. Cancer Discovery (2020)

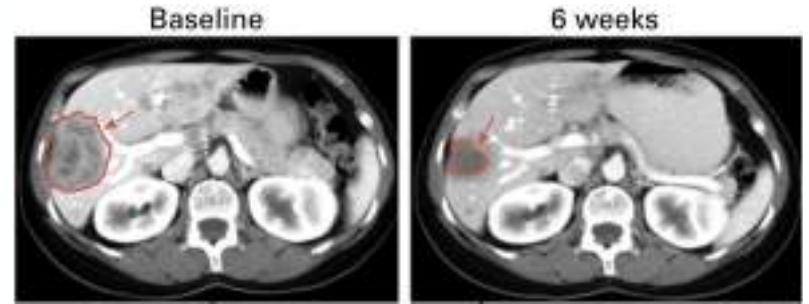
Variants can exhibit profound allele-specific phenotypes

Kras allele-specific cancer phenotypes



Zafra et al. Cancer Discovery (2020)

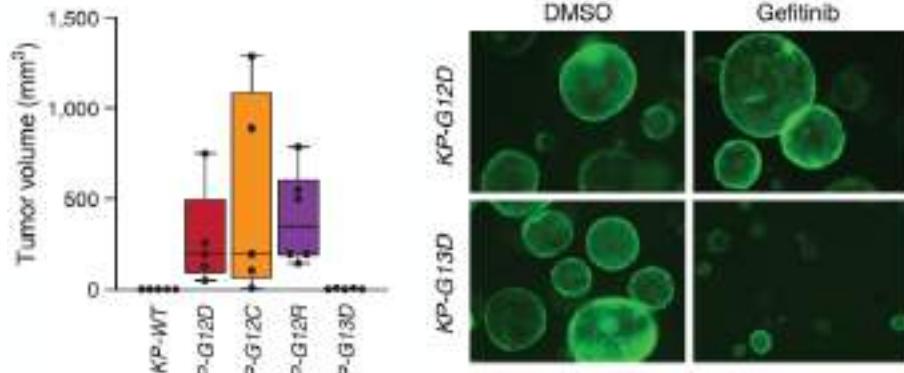
AKT allele-specific clinical outcomes



Hyman et al. Journal of Clinical Oncology (2017)

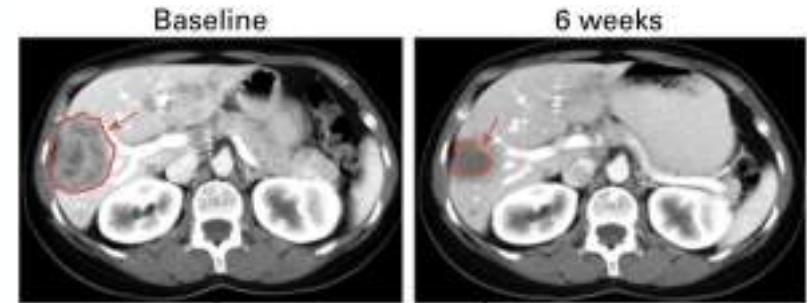
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Kras allele-specific cancer phenotypes



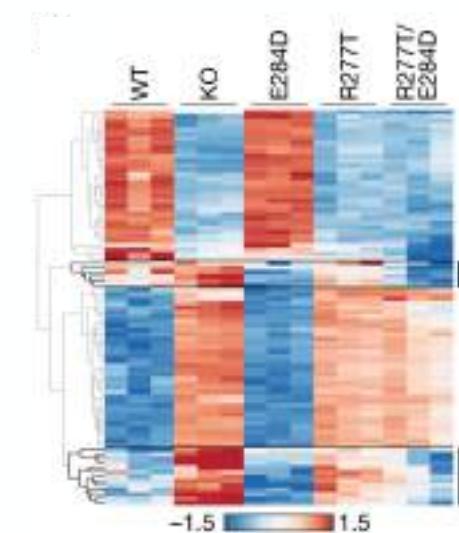
Zafra et al. Cancer Discovery (2020)

AKT allele-specific clinical outcomes



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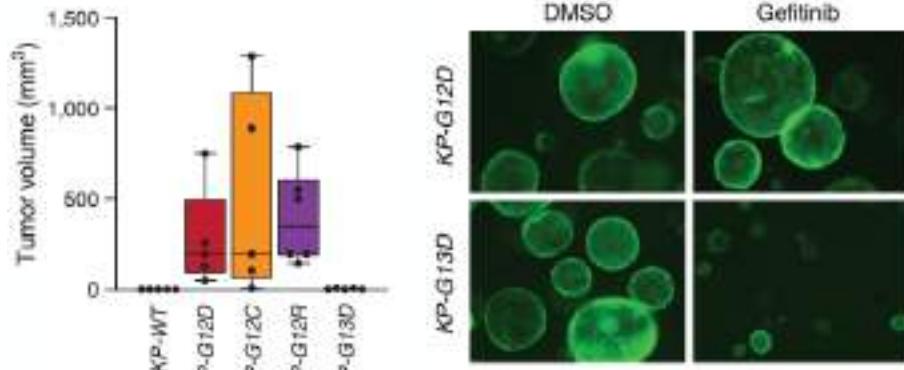
TP53 compound allele-specific phenotypes



Gorelick, Sánchez-Rivera ... Lowe, Reznik#, Taylor#. Nature (2020)

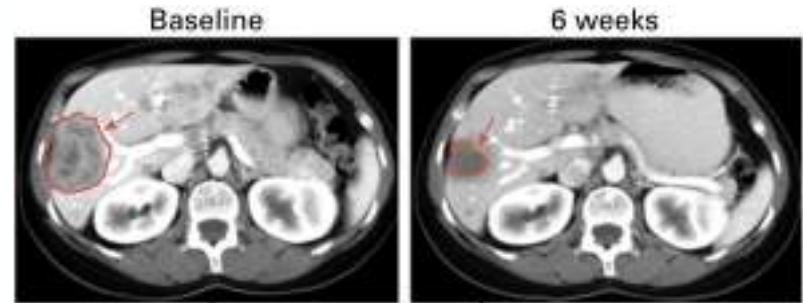
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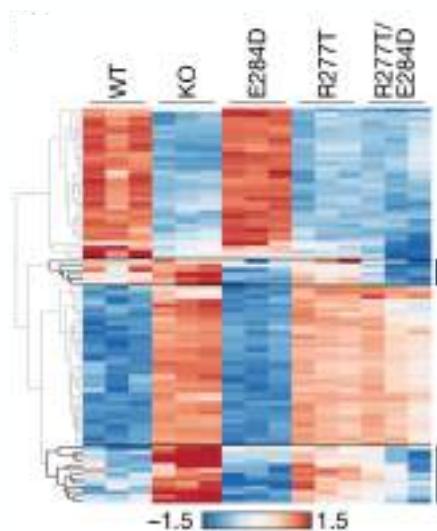
Zafra et al. Cancer Discovery (2020)

AKT allele-specific clinical outcomes



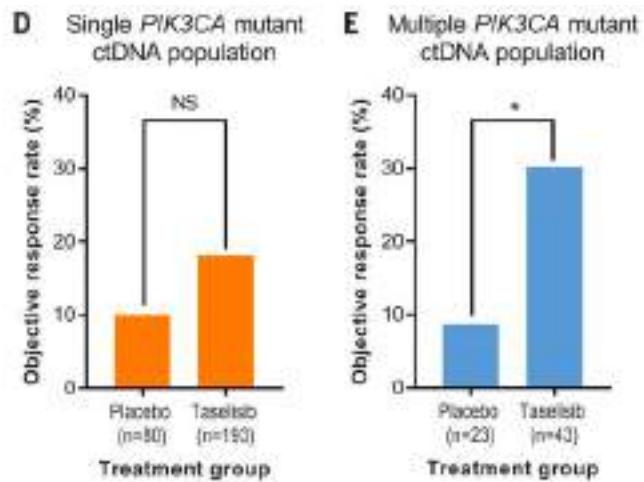
Hyman et al. Journal of Clinical Oncology (2017)

TP53 compound allele-specific phenotypes



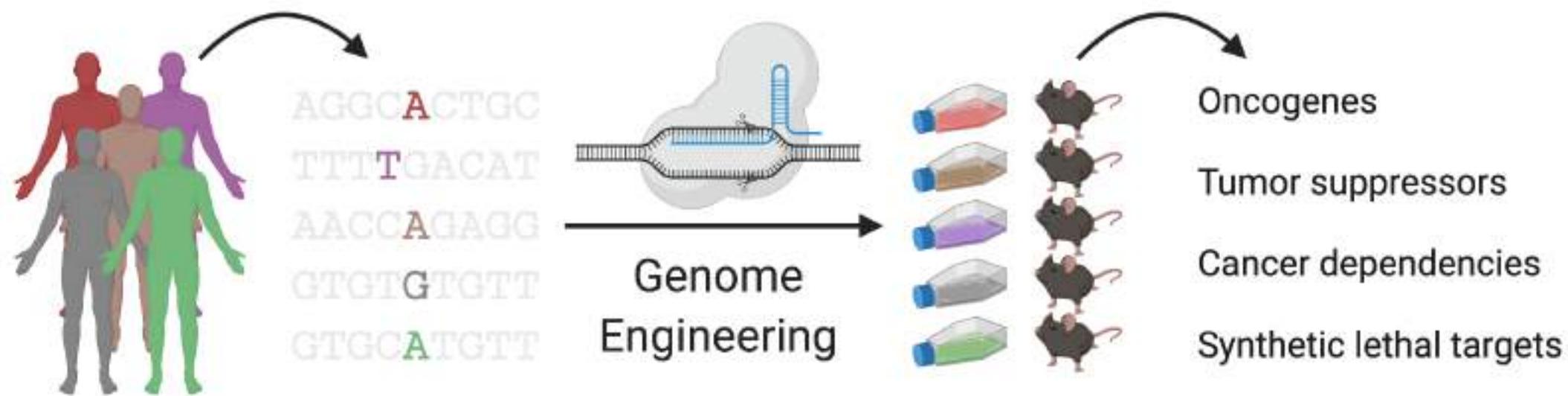
Gorelick, Sánchez-Rivera ... Lowe, Reznik#, Taylor#. Nature (2020)

PIK3CA compound allele-specific outcomes



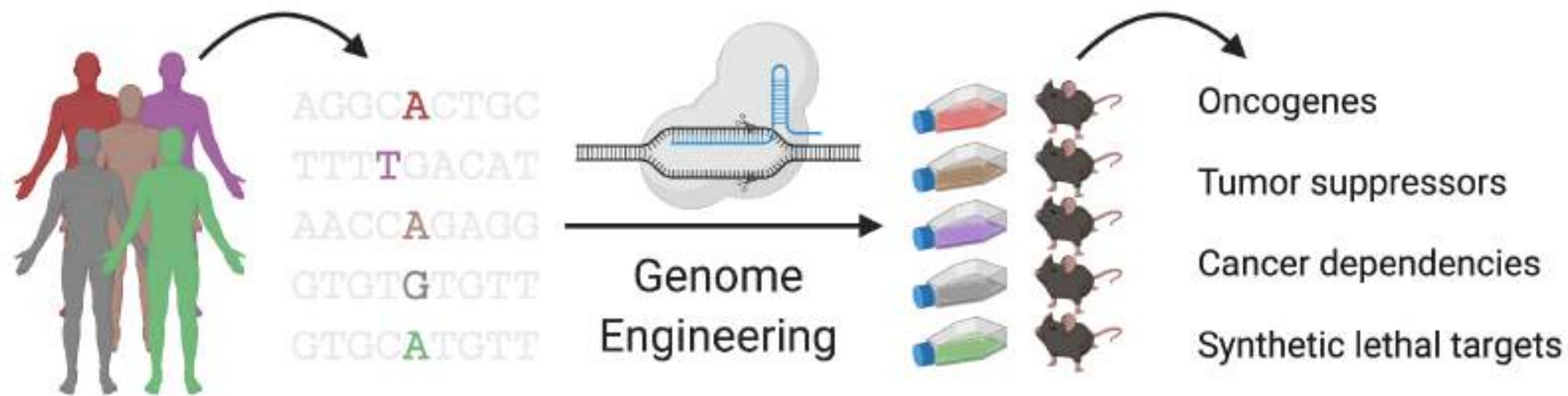
Vasan et al. Science (2019)

Probing gene and variant function using genome engineering



- CRISPR can be used to develop **genetically-defined models**.
- Developing **personalized models** requires **precision genome engineering**.
- The **Cas9 nuclease** remains a **relatively blunt** genome engineering tool.

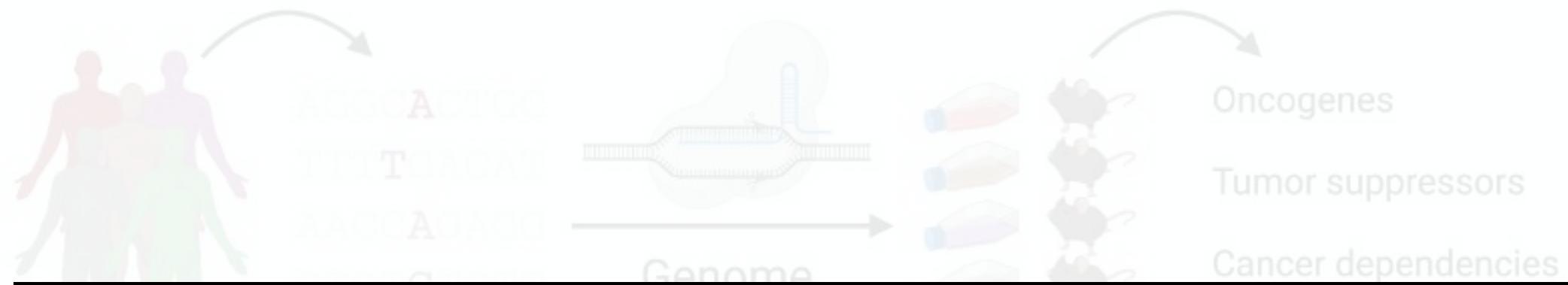
Probing gene and variant function using genome engineering



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Genetic Knockout \neq Cancer Variant

Probing gene and variant function using genome engineering



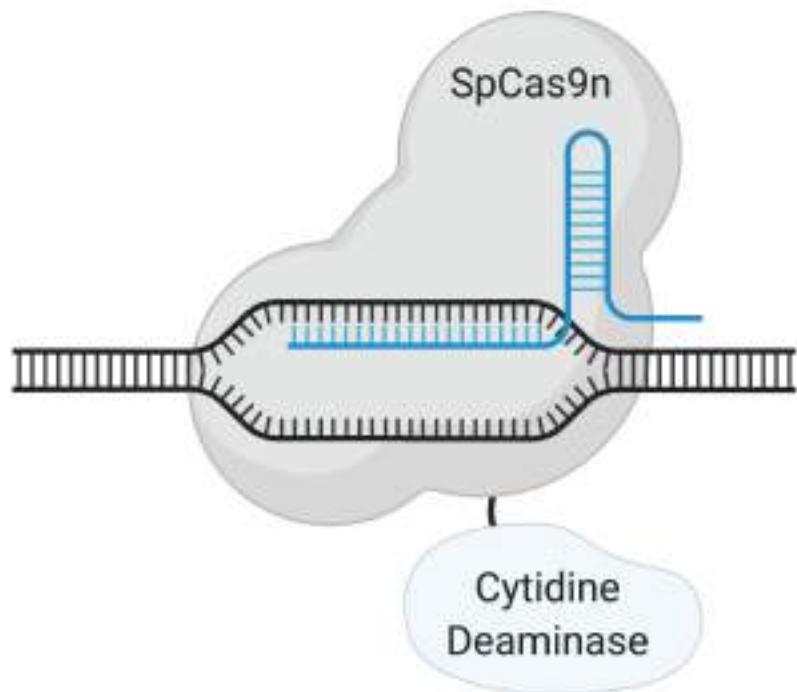
How can we interrogate specific variants?

CRISPR base editing allows efficient **cutting- and template-independent** genome engineering

GTTCGTGTTGTGCCTGCCCTGG



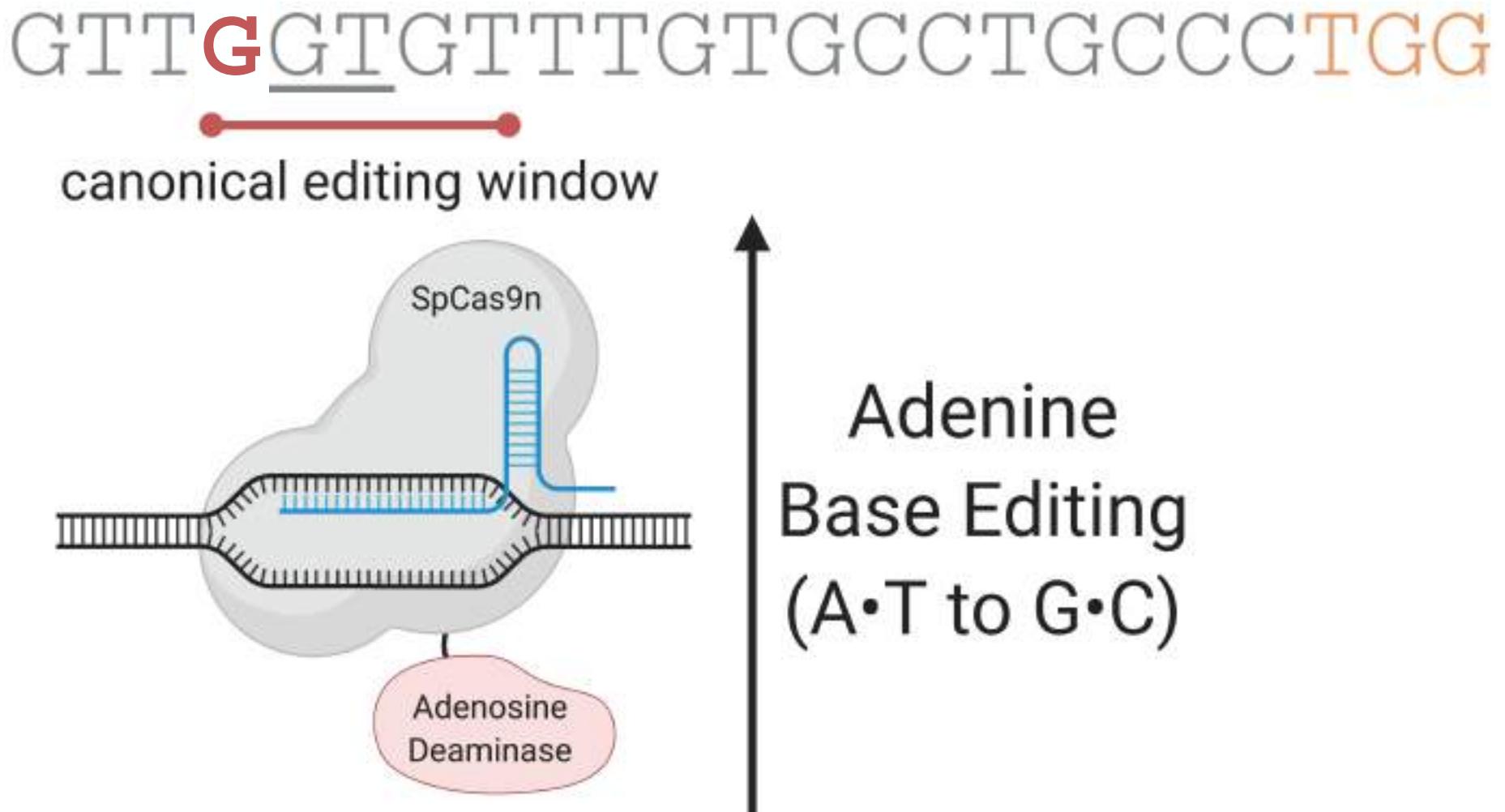
canonical editing window



Cytosine
Base Editing
(C•G to T•A)

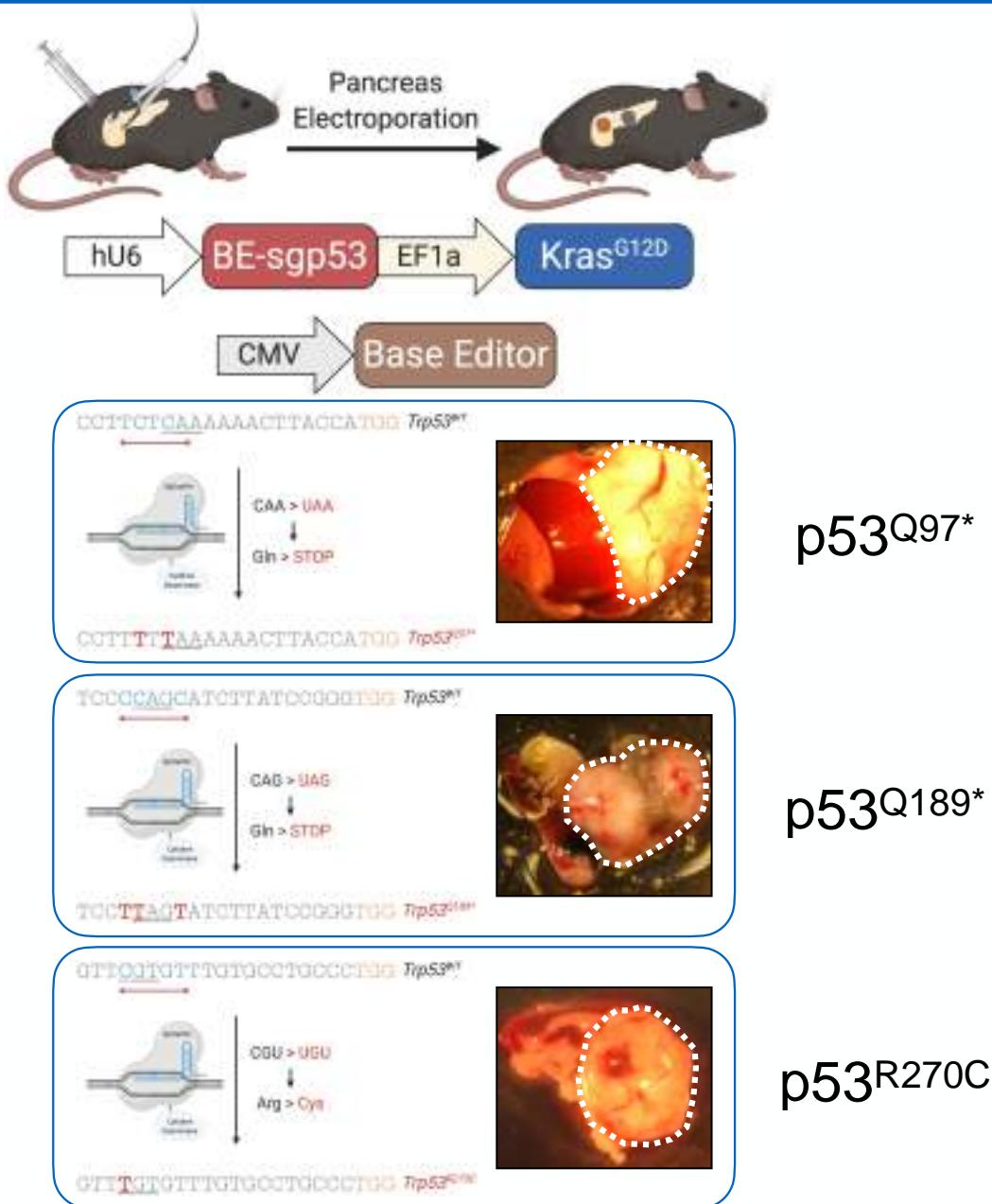
GTTTGTTGTGCCTGCCCTGG

CRISPR base editing allows efficient **cutting- and template-independent** genome engineering



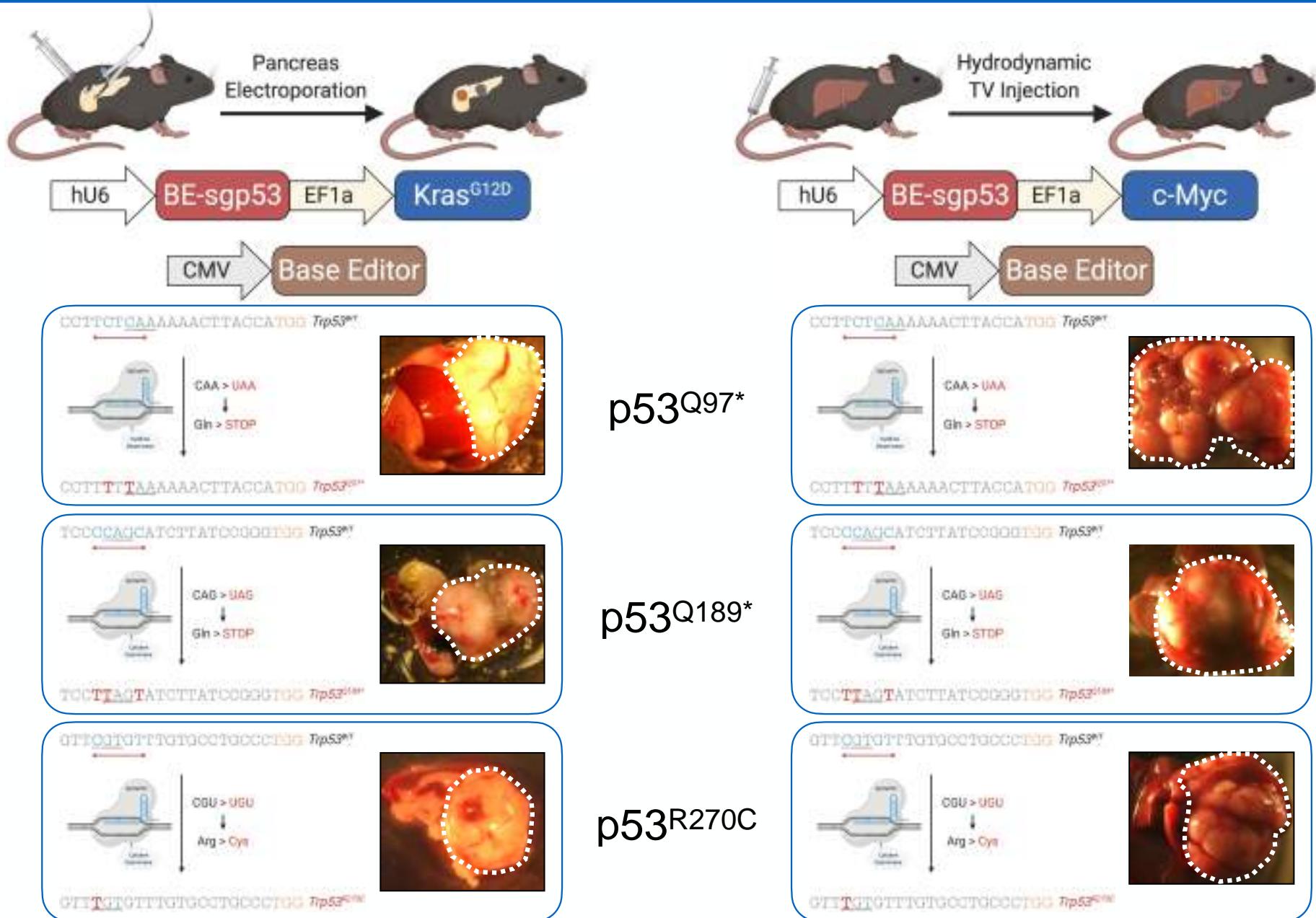
GT~~TG~~ATGTTGTGCCTGCCCTGG

Base editing can produce variant-specific mouse models

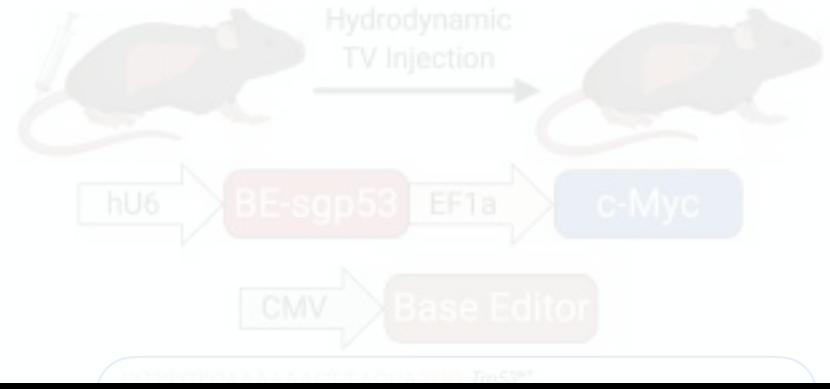
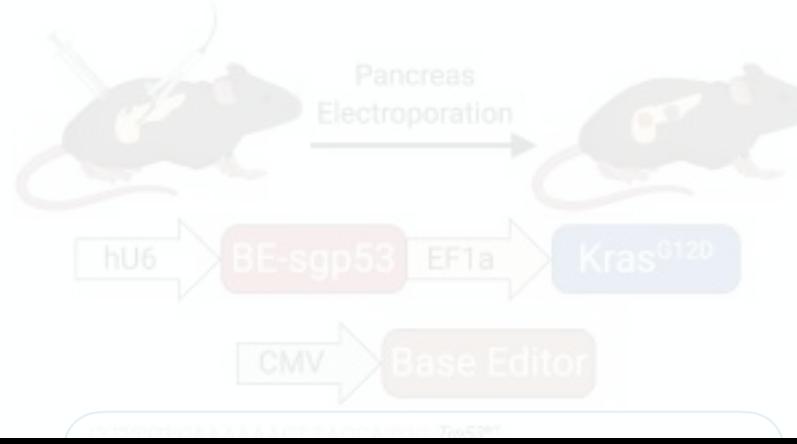


with Josef Leibold

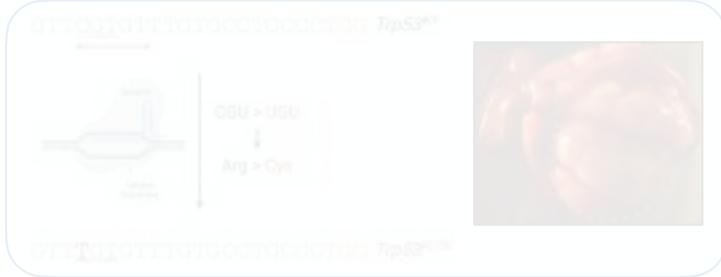
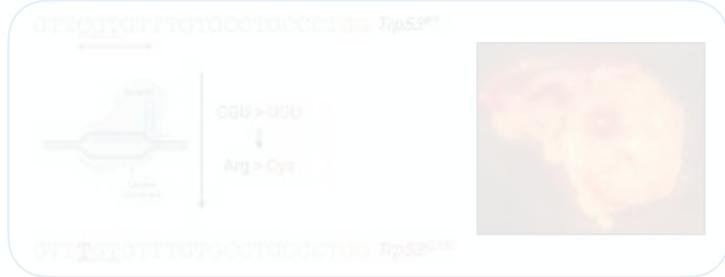
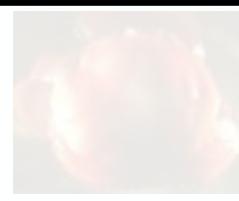
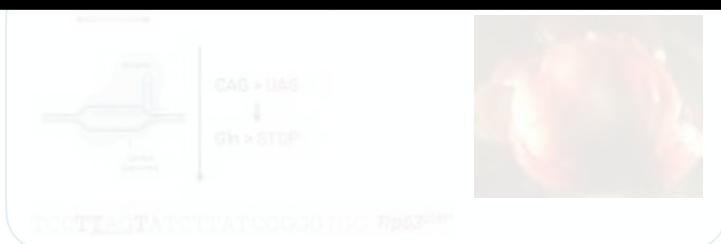
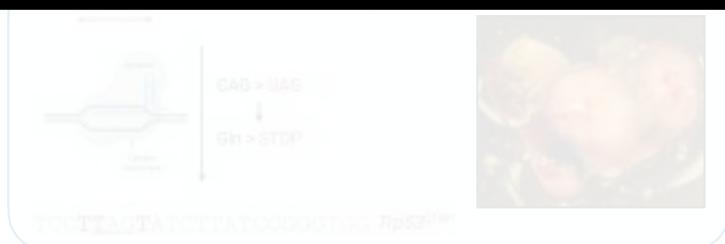
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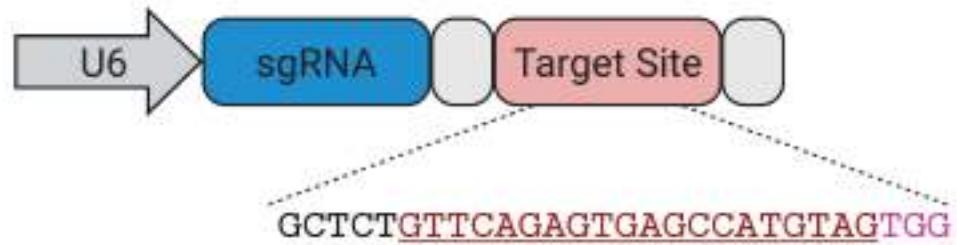
with Josef Leibold



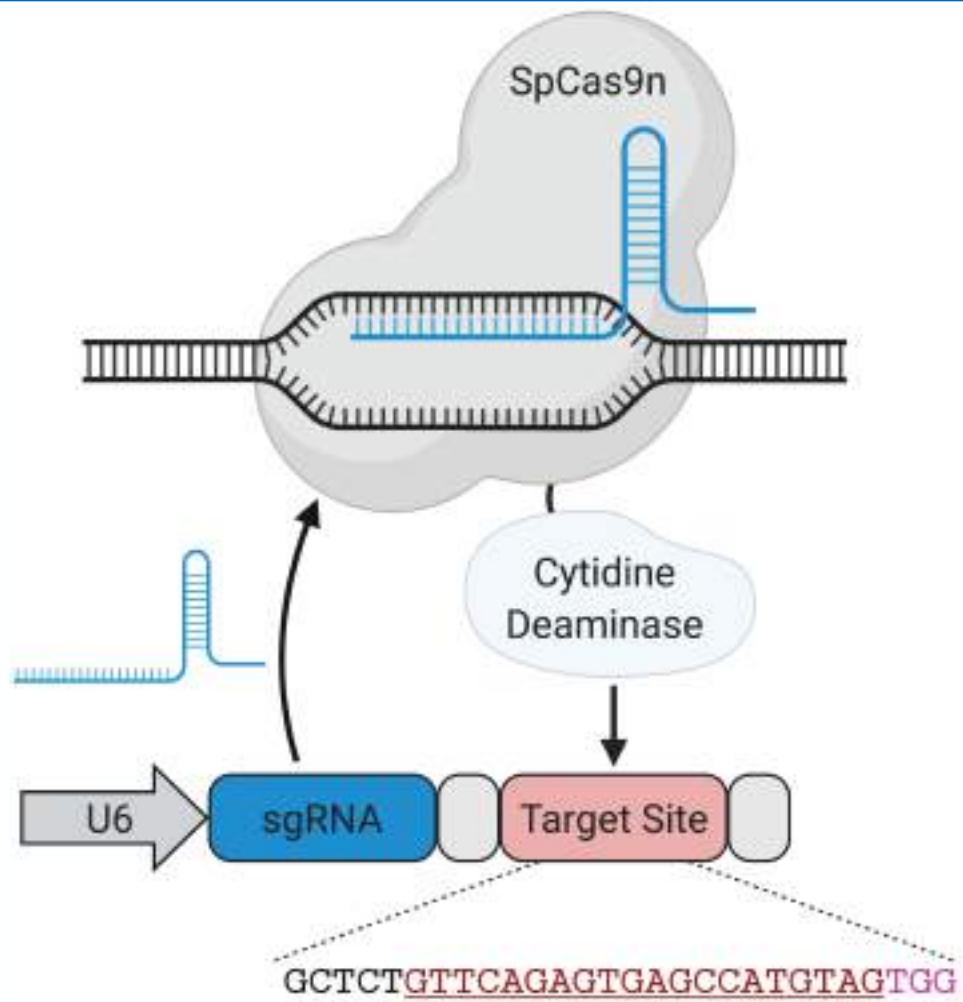
Promising technology, but is it scalable?



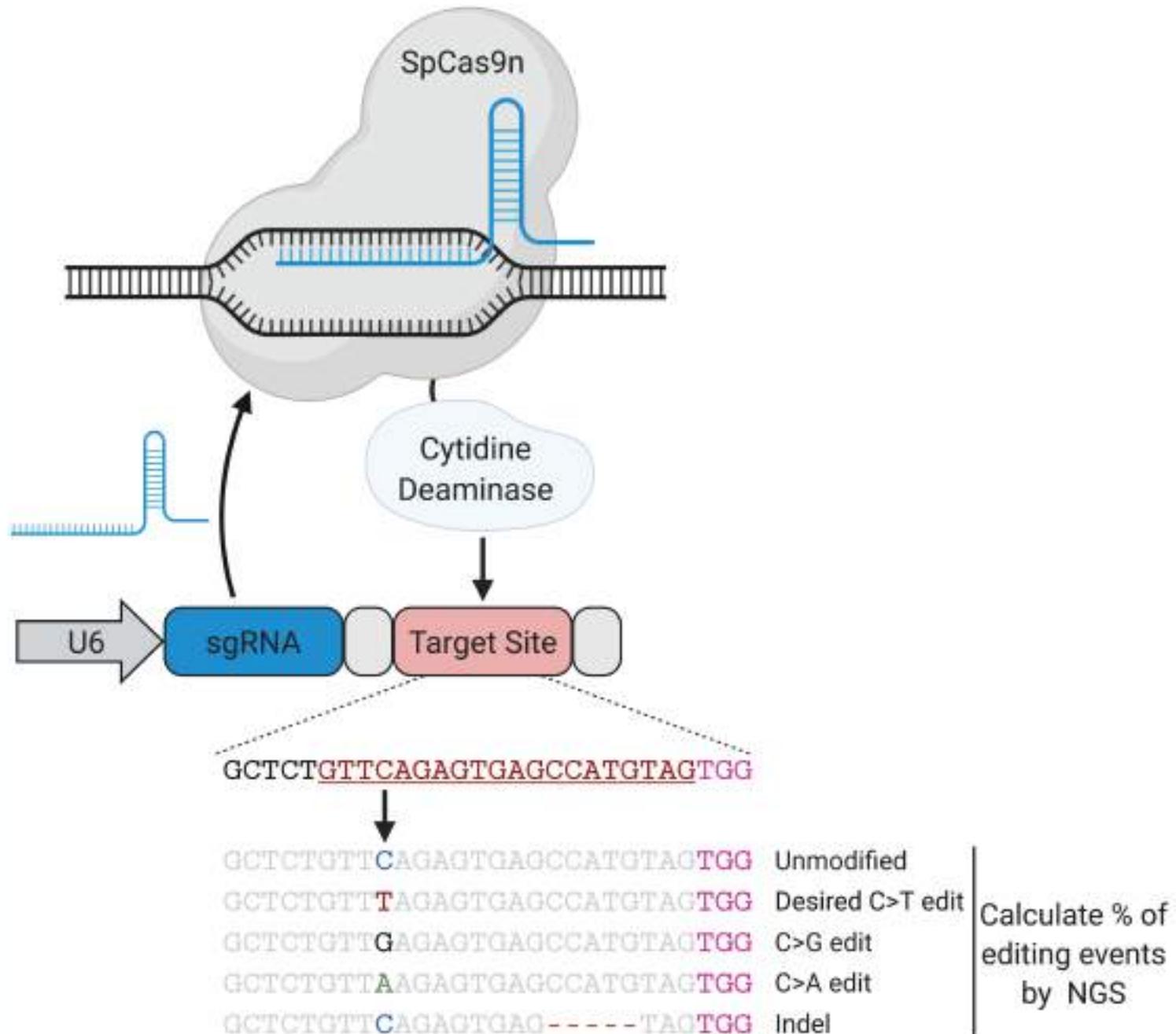
High-throughput evaluation of cancer-associated single nucleotide variants with base editing sensor libraries



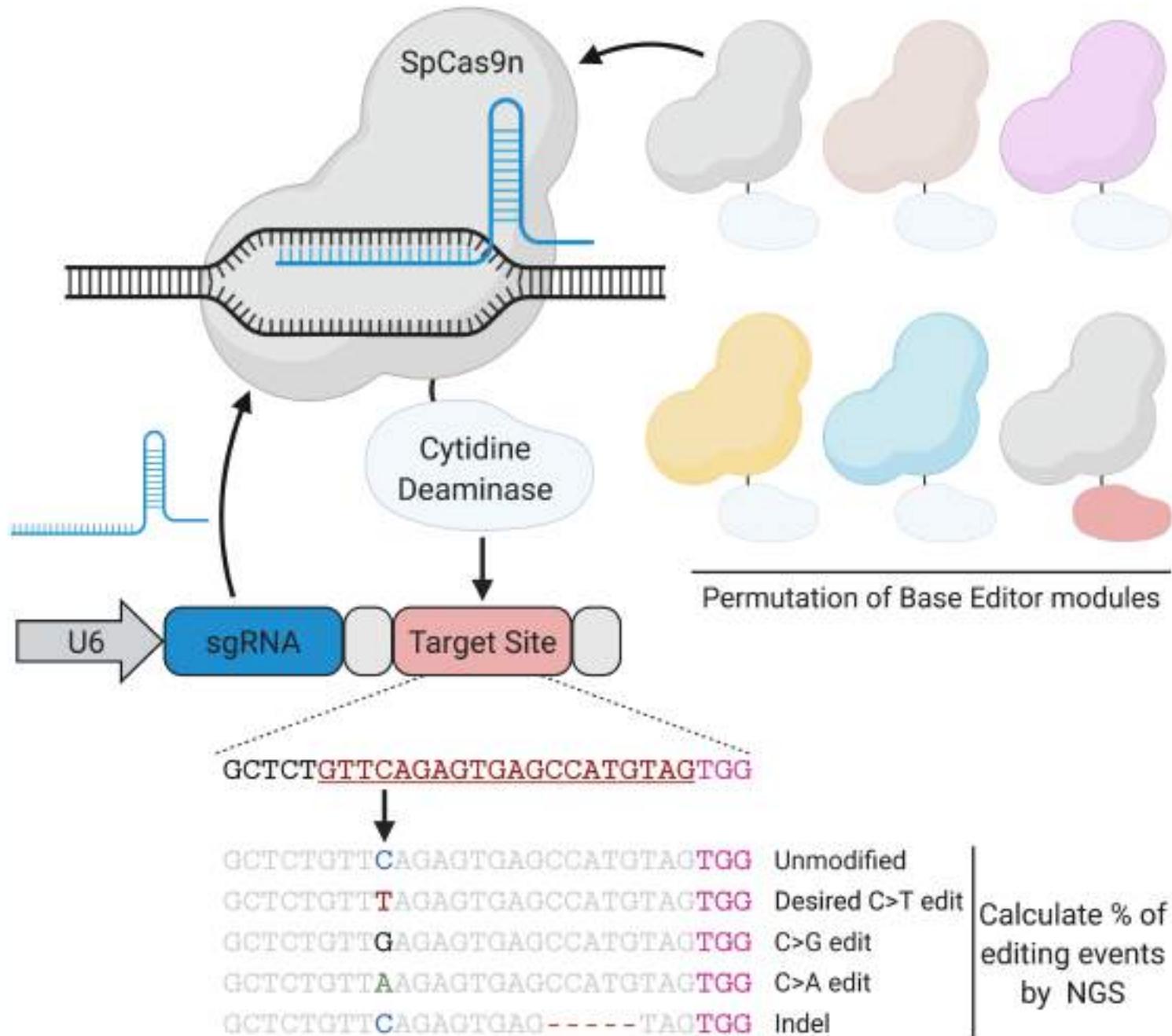
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High-throughput evaluation of cancer-associated single nucleotide variants with base editing sensor libraries

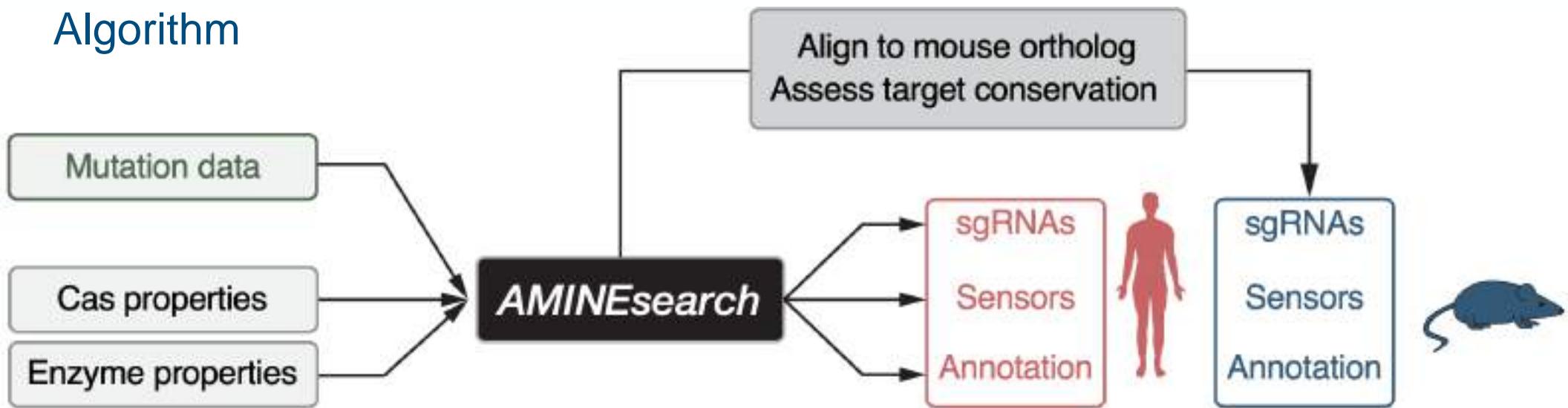


High-throughput evaluation of cancer-associated single nucleotide variants with base editing sensor libraries



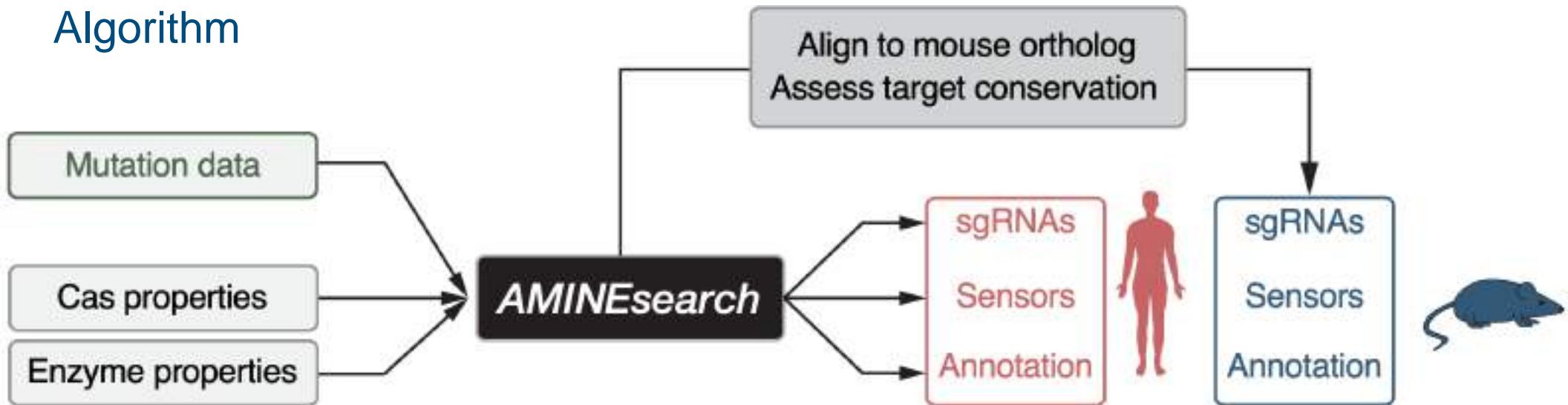
Modular pipeline for design of base editing libraries

Algorithm



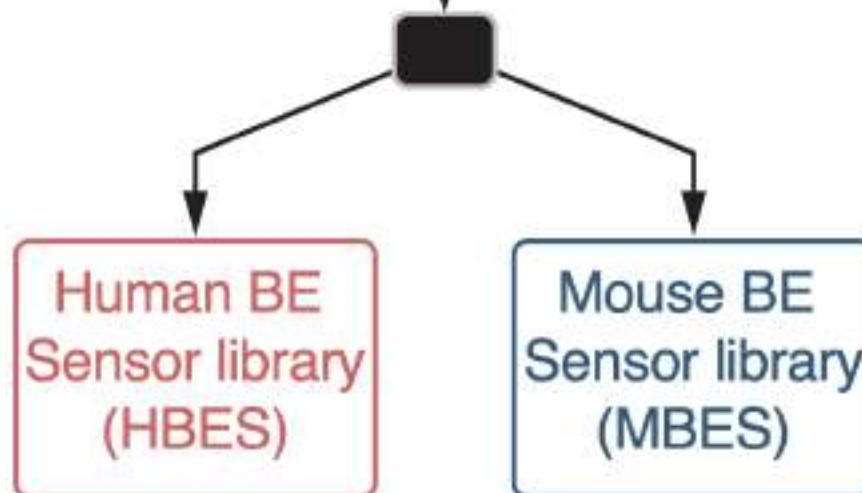
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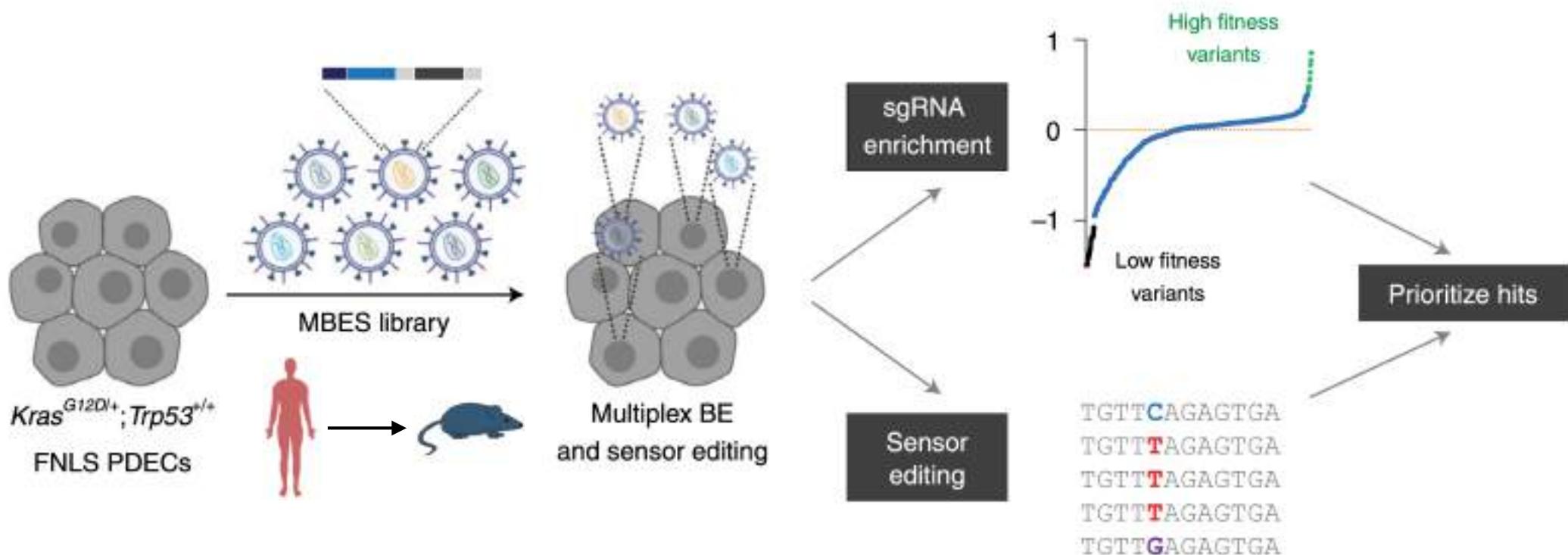


Implementation

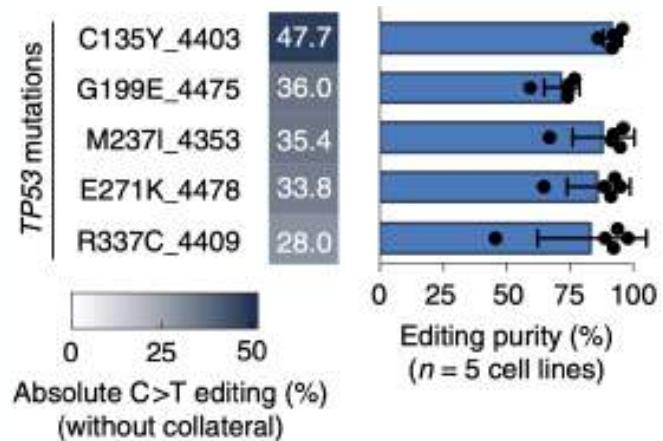
MSK-IMPACT
Clinical tumor sequencing



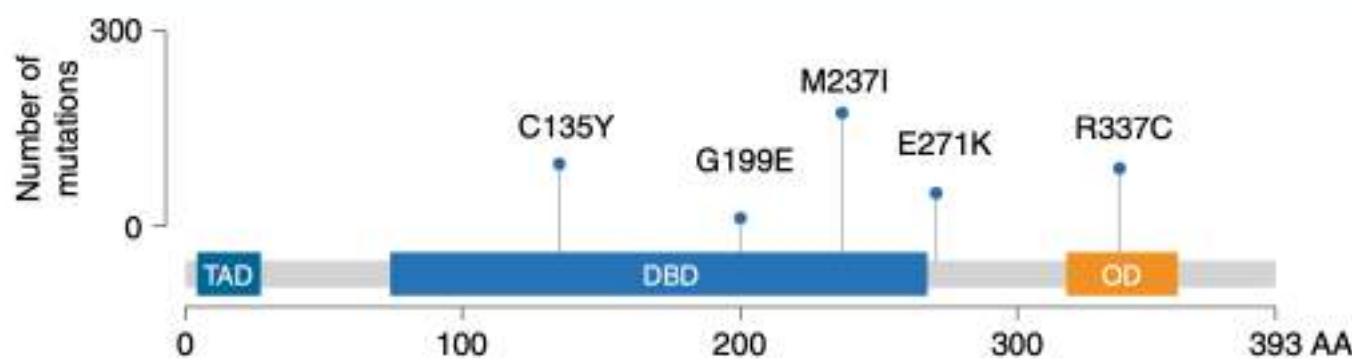
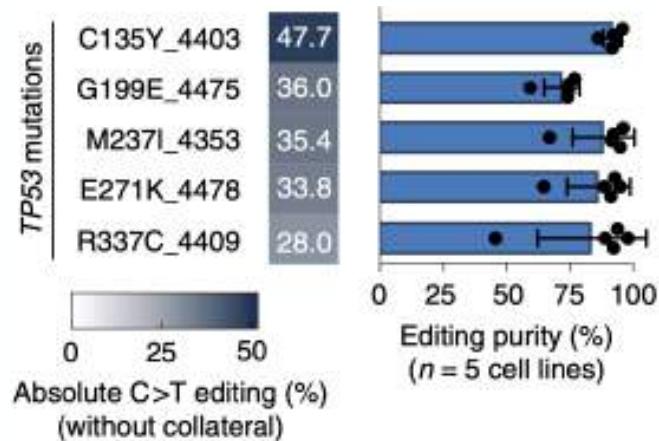
Massively parallel interrogation of genetic variants via pooled base editing screening



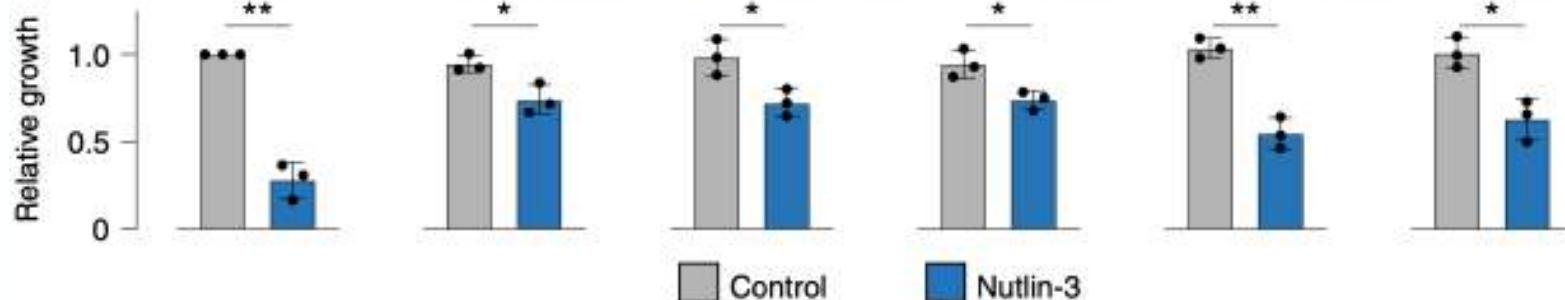
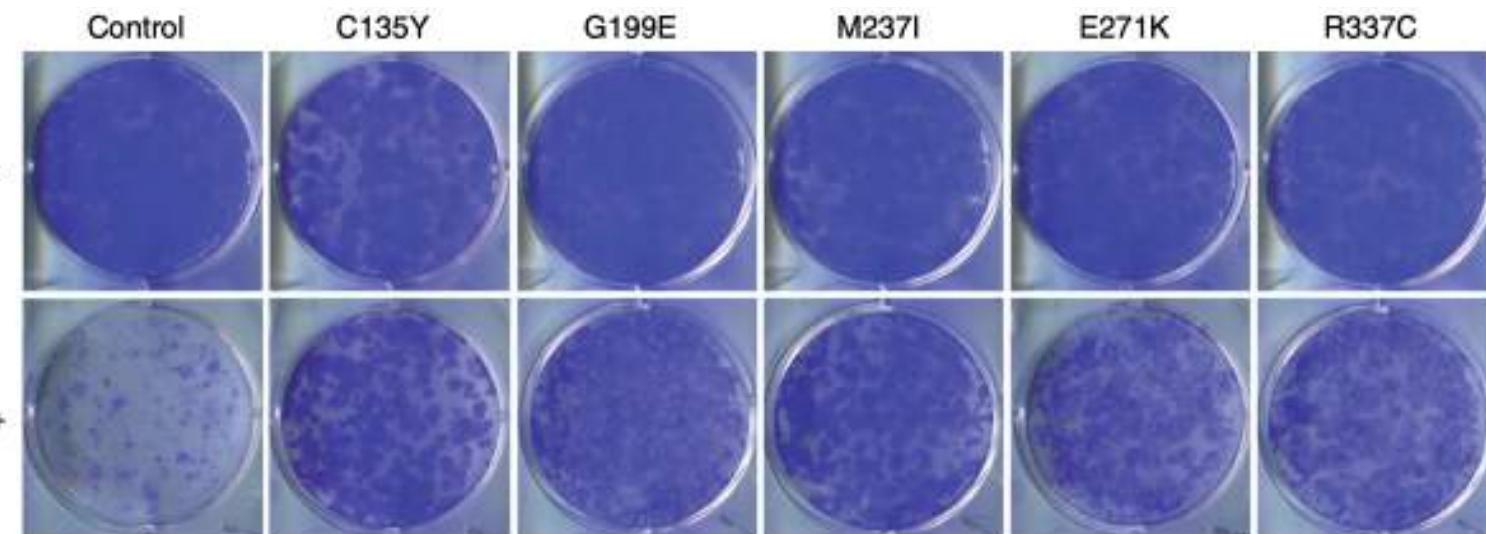
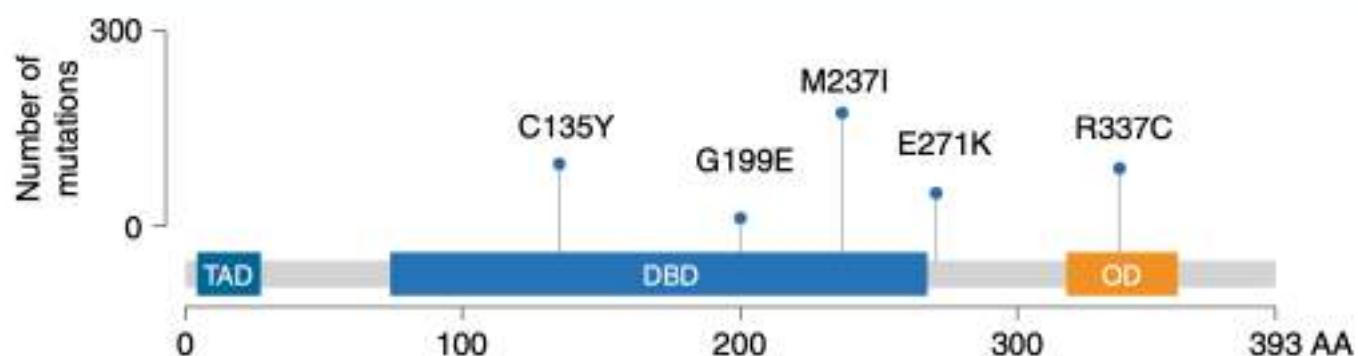
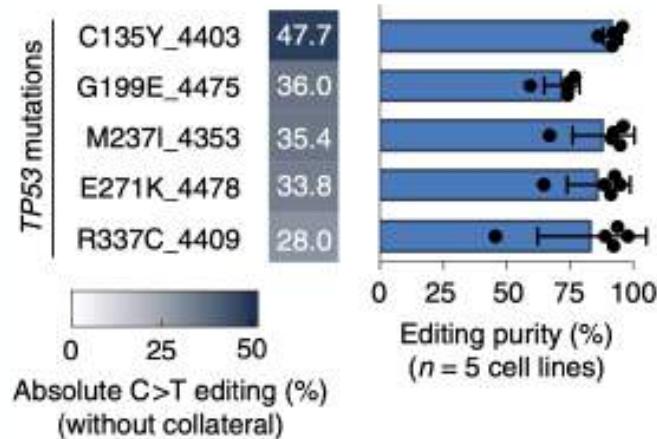
Functional validation of novel mutant p53 alleles



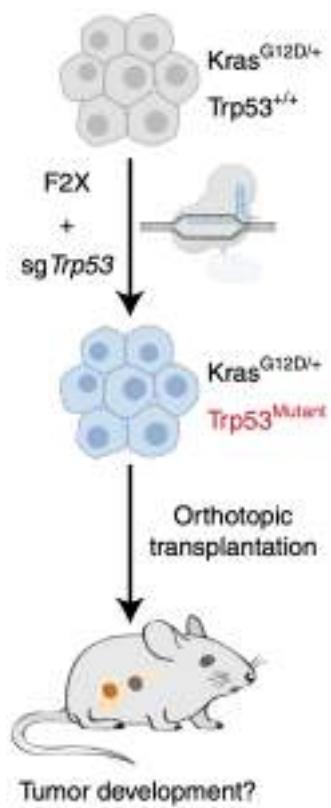
Functional validation of novel mutant p53 alleles



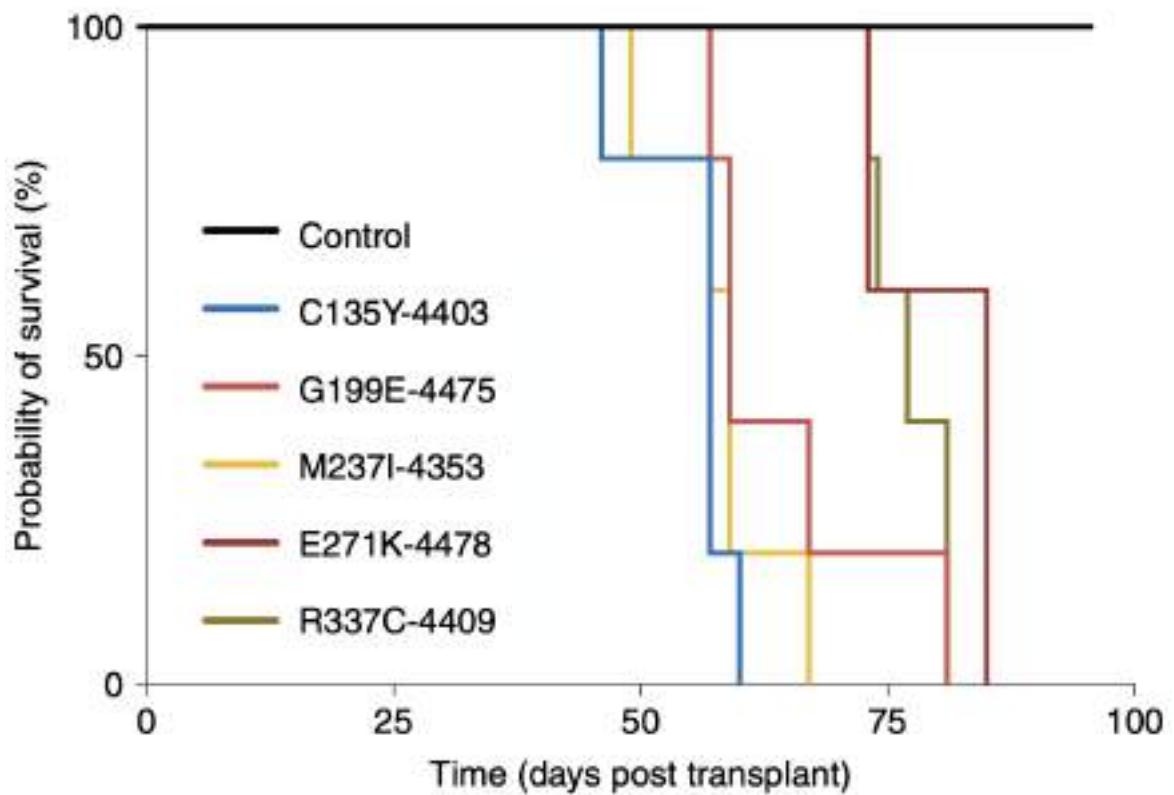
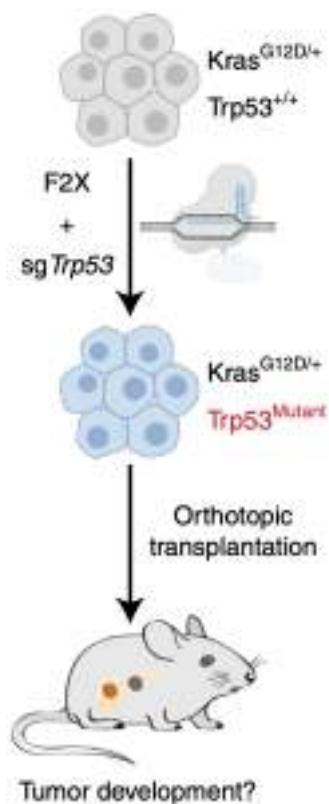
Functional validation of novel mutant p53 alleles



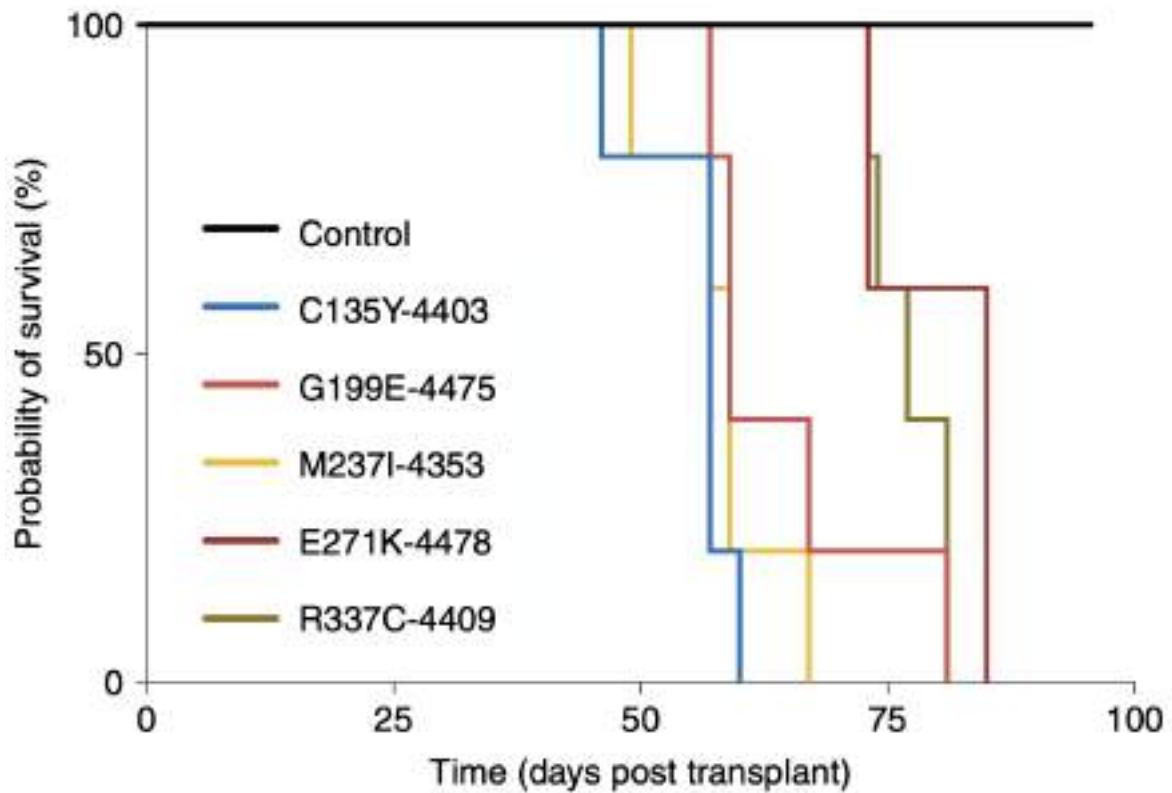
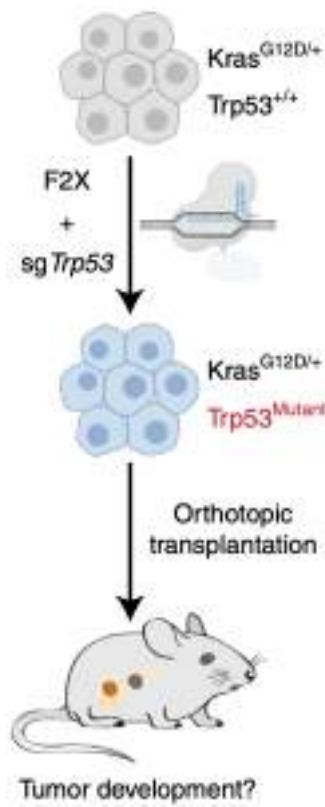
In vivo validation of novel mutant p53 alleles



In vivo validation of novel mutant p53 alleles



In vivo validation of novel mutant p53 alleles



Main takeaways

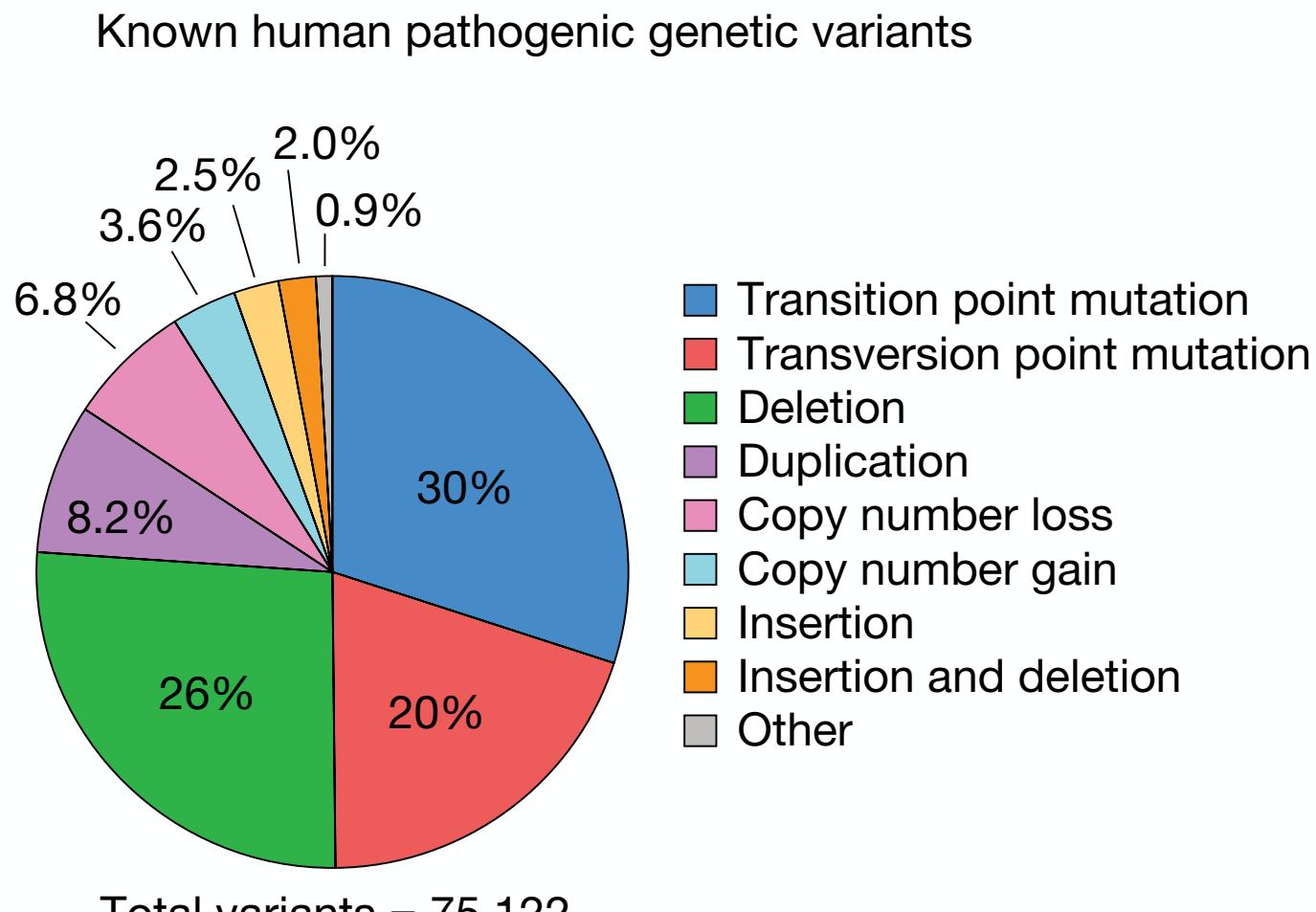
- **Rapid** *in vivo* interrogation of cancer-associated variants without any breeding.
- Base editing engineering of p53 mutations leads to **highly penetrant** disease.
- Not all p53 mutations appear to be functionally redundant.



Exciting findings and methods, but we were unable to model >50% of mutations.



Transition mutations account for just a fraction of human pathogenic variants

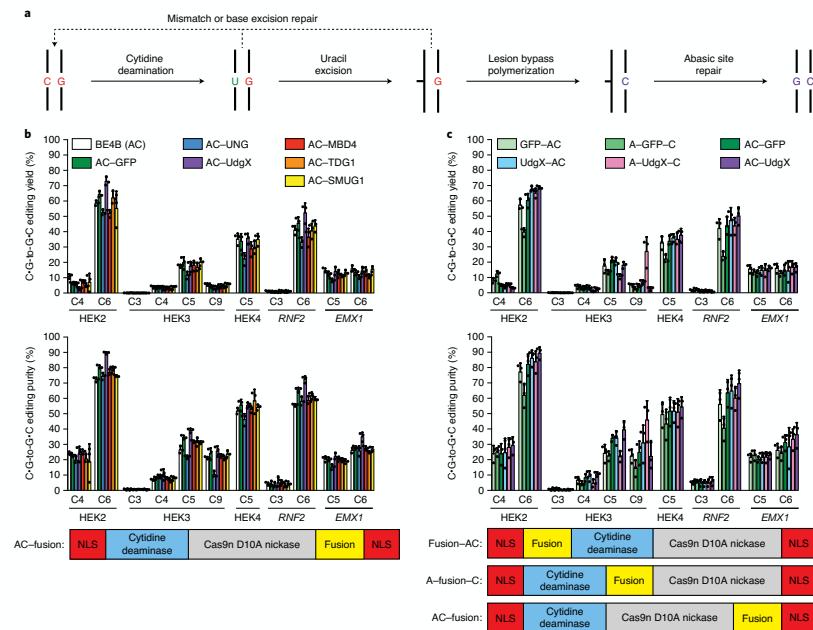


Anzalone et al.
Nature 2019

Transversion Base Editors expand the spectrum of mutations that can be targeted

Efficient C•G-to-G•C base editors developed using CRISPRi screens, target-library analysis, and machine learning

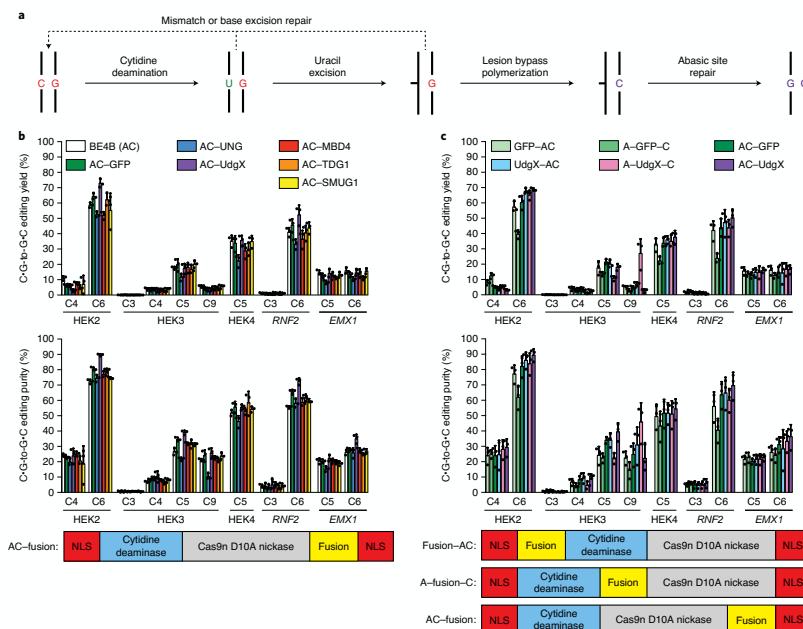
Luke W. Koblan ^{1,2,3,15}, Mandana Arbab ^{1,2,3,15}, Max W. Shen ^{1,2,3,4,15}, Jeffrey A. Hussmann ^{1,5,6,7,13,14}, Andrew V. Anzalone ^{1,2,3}, Jordan L. Doman ^{1,2,3}, Gregory A. Newby ^{1,2,3}, Dian Yang ^{5,7,13,14}, Beverly Mok ^{1,2,3}, Joseph M. Replogle ^{1,5,7,8,9,13,14}, Albert Xu ^{1,5,6,8,10}, Tyler A. Sisley ^{1,2}, Jonathan S. Weissman ^{1,5,7,8,13,14} , Britt Adamson ^{1,5,7,11,12}  and David R. Liu ^{1,2,3} 



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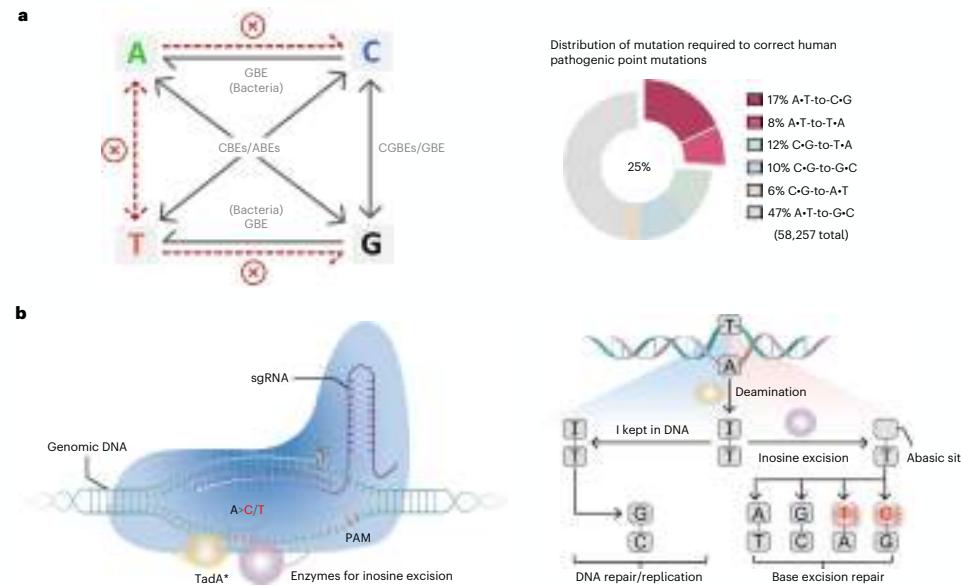
Adenine transversion editors enable precise, efficient A•T-to-C•G base editing in mammalian cells and embryos

Received: 12 January 2023

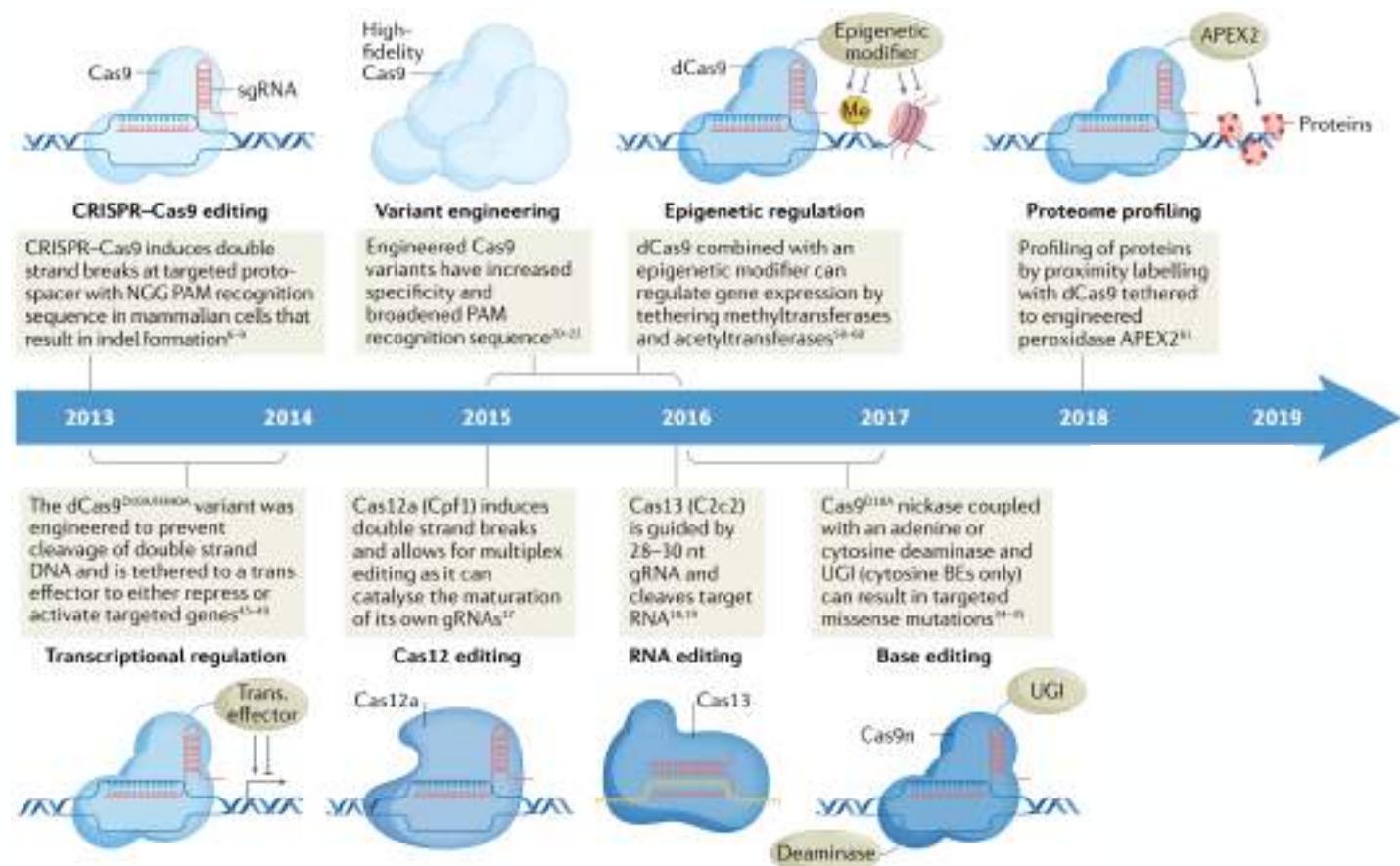
Accepted: 8 May 2023

Published online: 15 June 2023

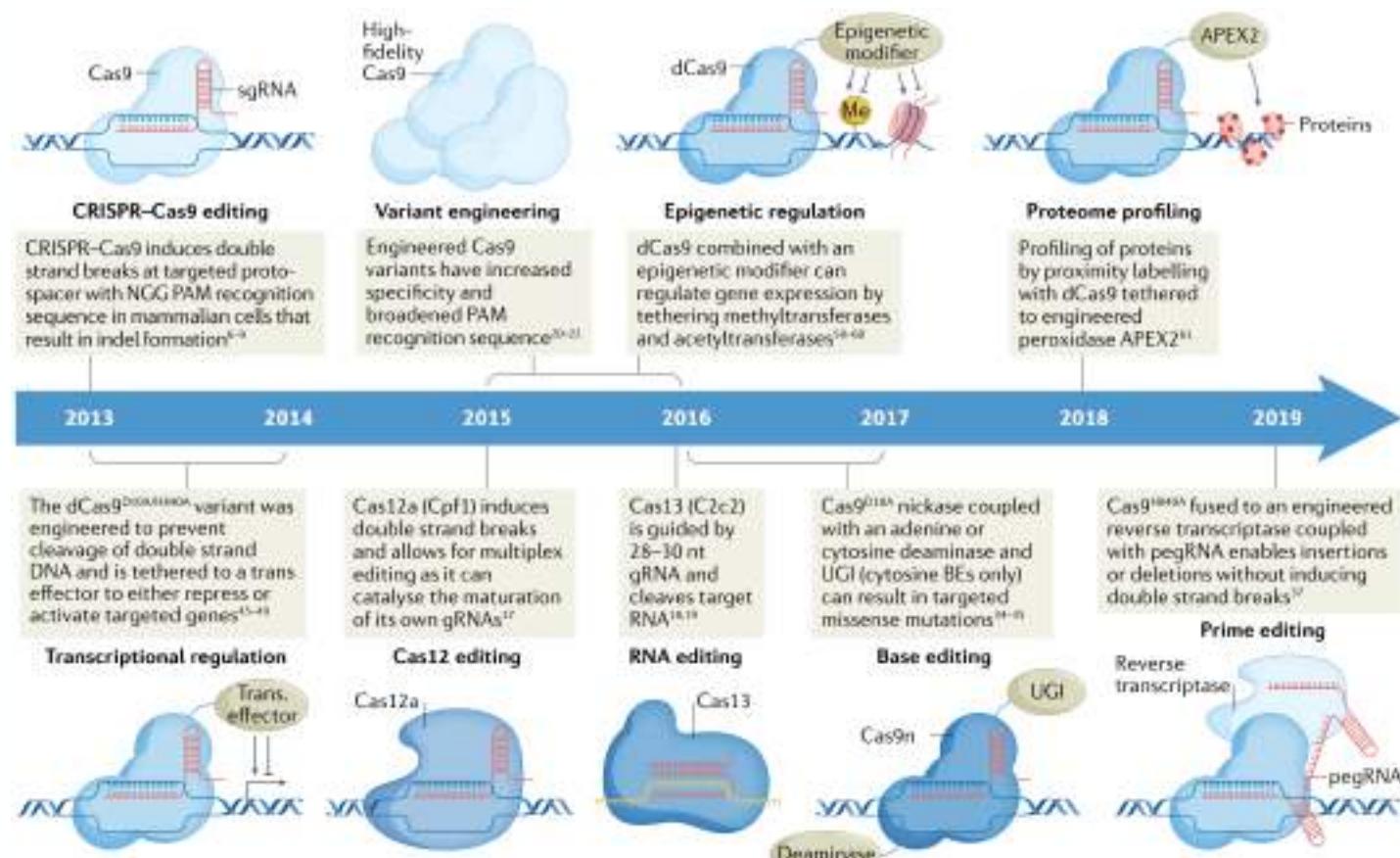
Liang Chen ^{1,8}, Mengjia Hong ^{1,8}, Changming Luan ^{1,8}, Hongyi Gao ¹, Gaomeng Ru ¹, Xinyuan Guo ¹, Dujuan Zhang ¹, Shun Zhang ¹, Changwei Li ², Jun Wu ¹, Peyton B. Randolph ^{3,4,5}, Alexander A. Sousa ^{3,4,5}, Chao Qu ¹, Yifan Zhu ¹, Yuting Guan ¹, Liren Wang ¹, Mingyao Liu ^{1,6}, Bo Feng ^{1,7}, Gaojie Song ^{1,8}, David R. Liu ^{1,3,4,5} and Dali Li ¹ 



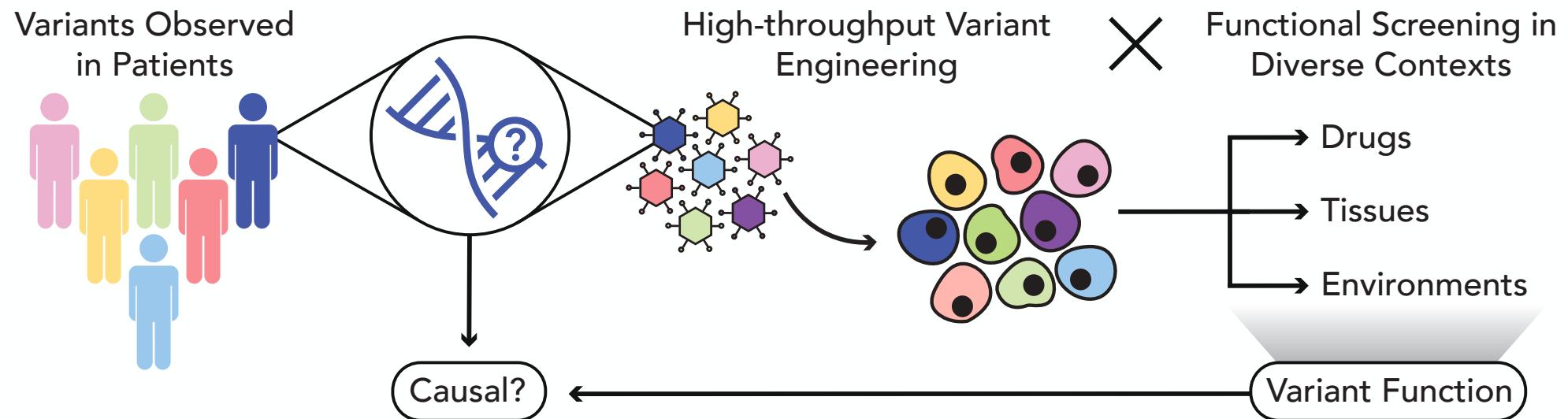
The rapidly evolving space of CRISPR-Cas biological engineering tools



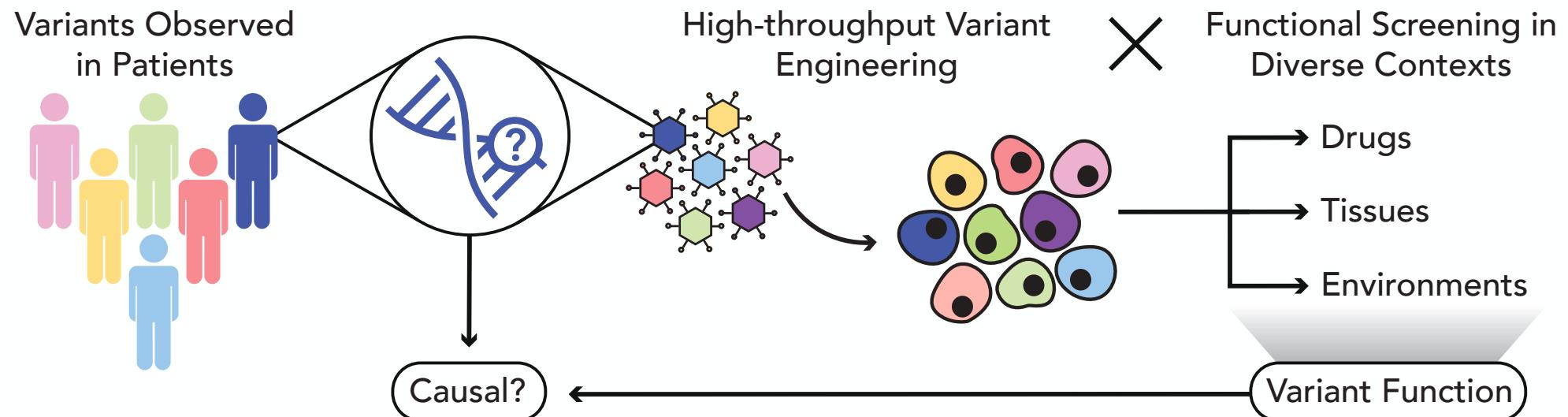
The rapidly evolving space of CRISPR-Cas biological engineering tools



We wanted to broadly and systematically apply this type of approach to interrogate many genes and variants



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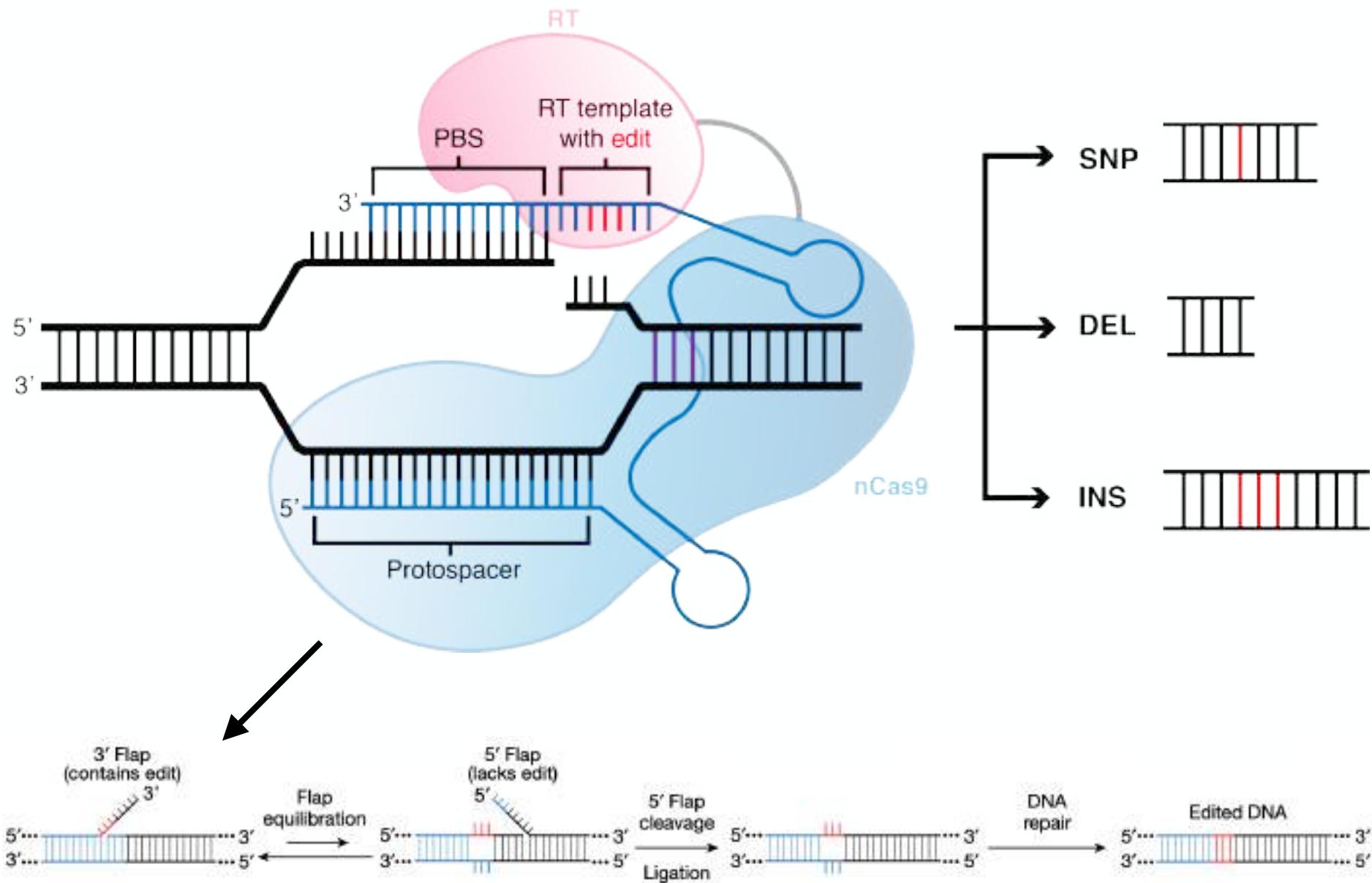


That's a tragedy...

Let me see what I can do!

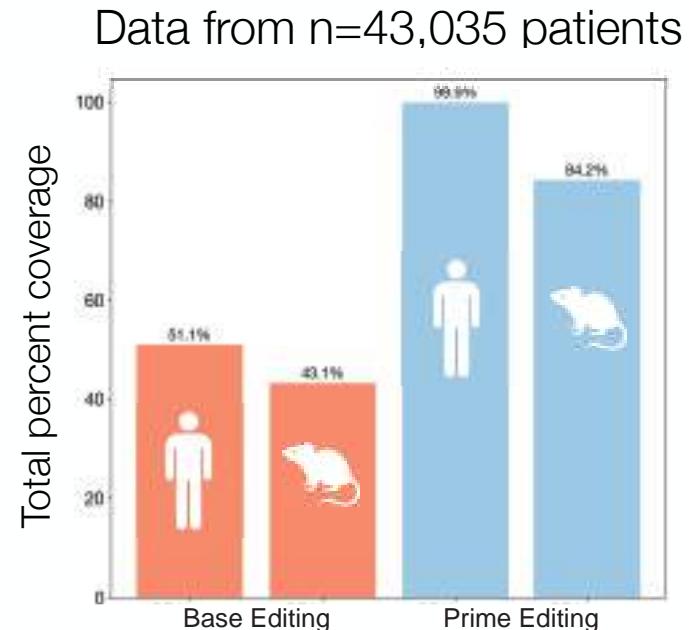
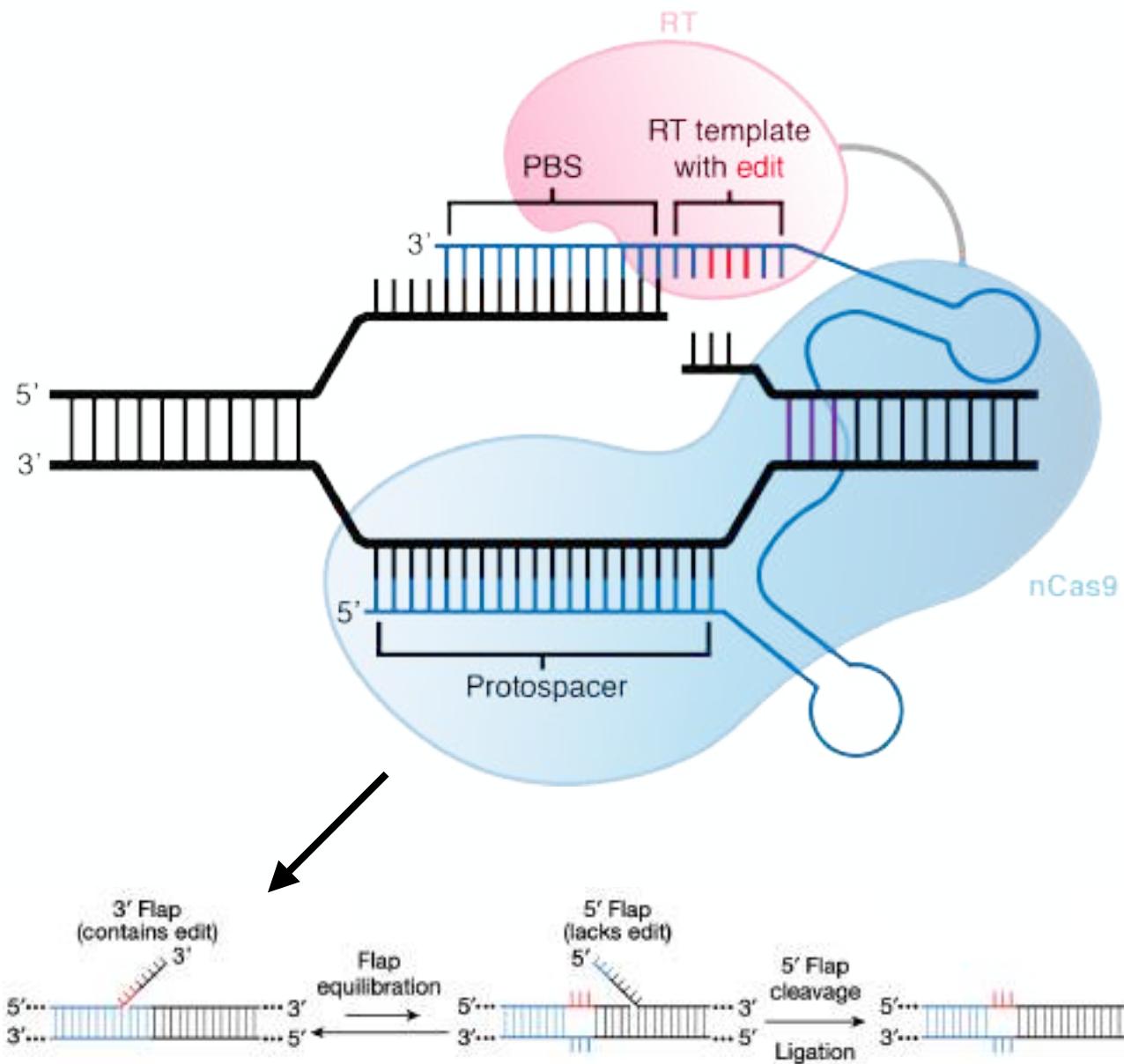
Sam Gould

Expanding the targetable cancer genome using prime editing



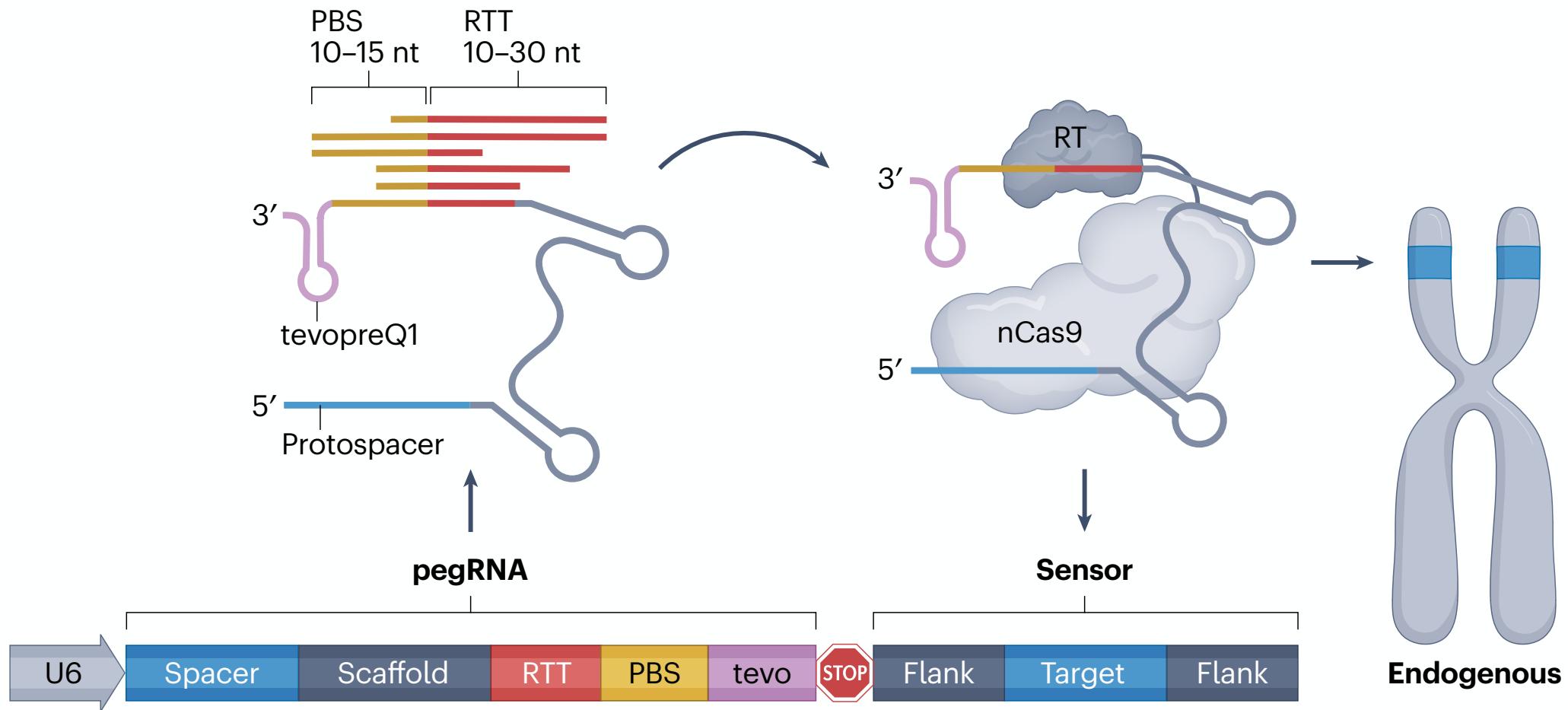
Based on Anzalone et al. Nature (2019)

Expanding the targetable cancer genome using **prime editing**

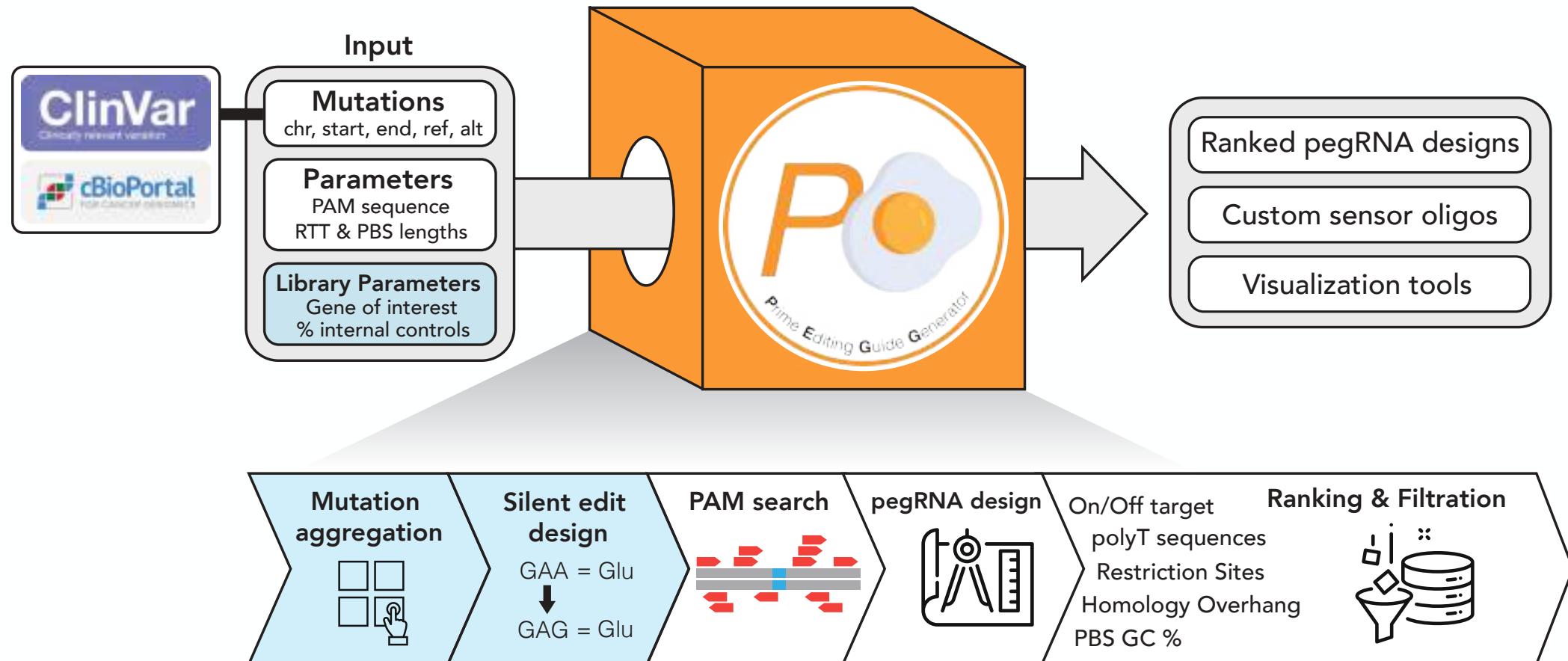


Based on Anzalone et al. Nature (2019)

A prime editing sensor construct to assess pegRNA editing

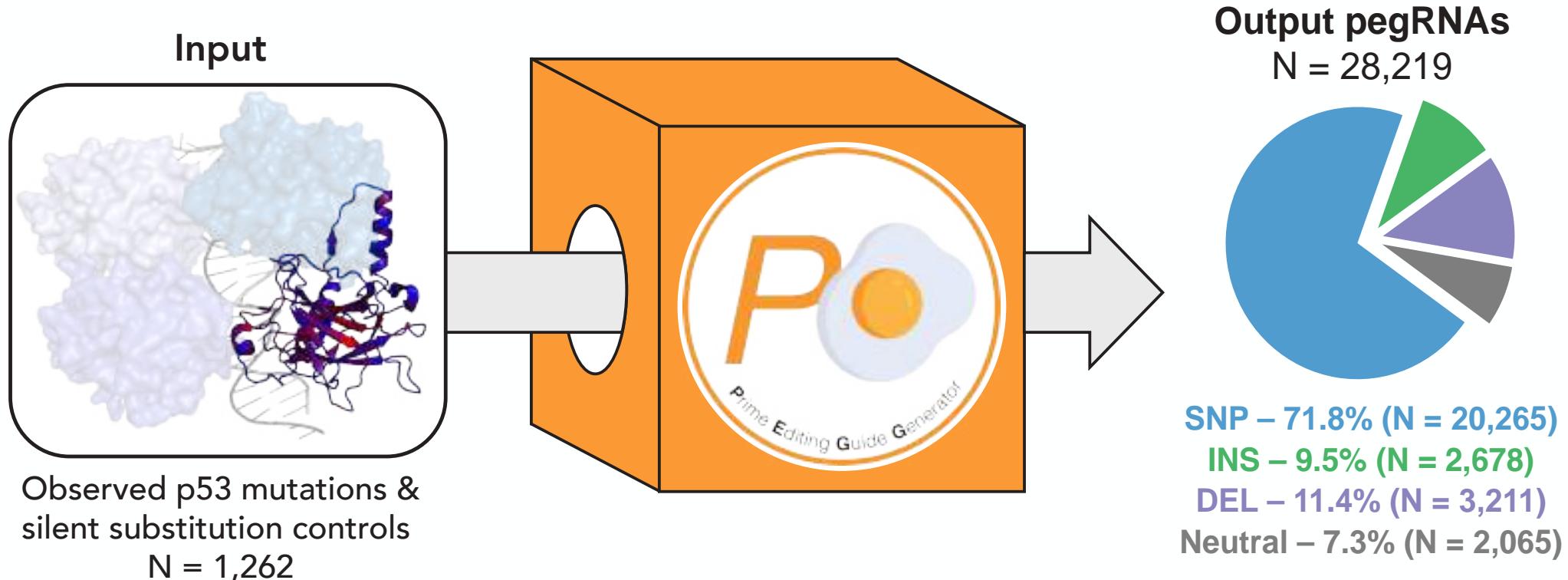


PEGG — a versatile pipeline for high-throughput pegRNA design

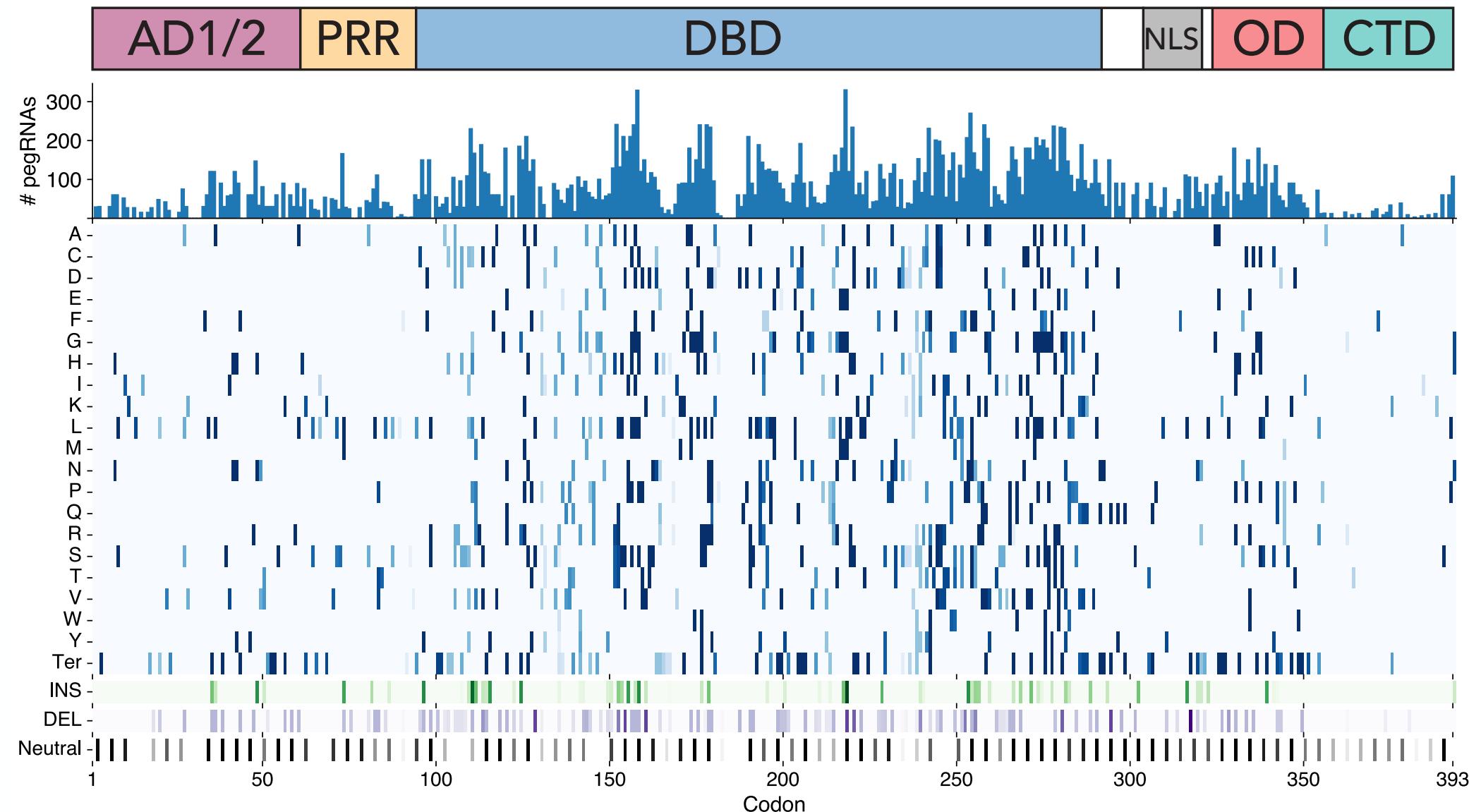


Want to use it? → pegg.readthedocs.io

We used PEGG to design ~30,000 pegRNAs against ~1,000 *TP53* variants



We used PEGG to design ~30,000 pegRNAs against ~1,000 *TP53* variants



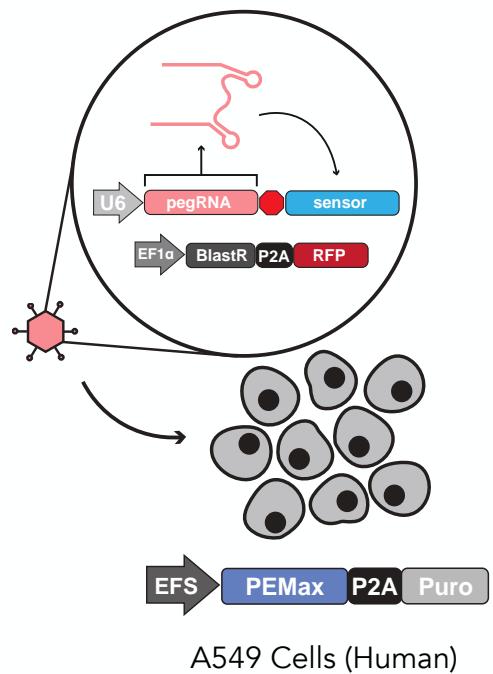
Key point → we can cover diverse types of single and multi-nucleotide mutations

Sensor-based measurements are representative of endogenous editing

PE sensors

E285Q
D259Y
M246R
M169I
H178R
R110G
R175H
E258E

Separate transductions

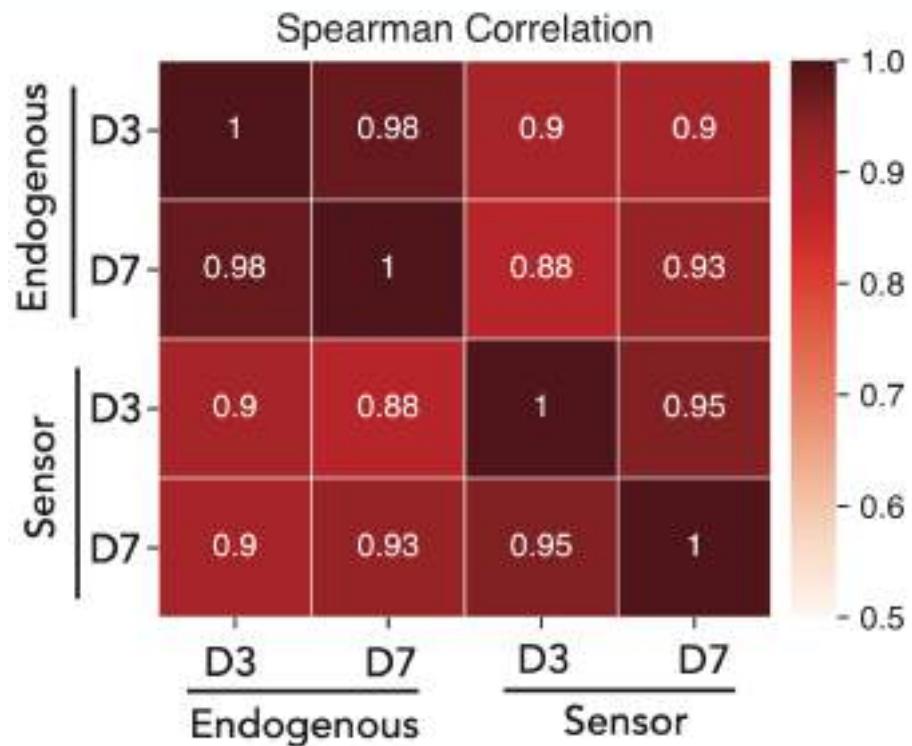
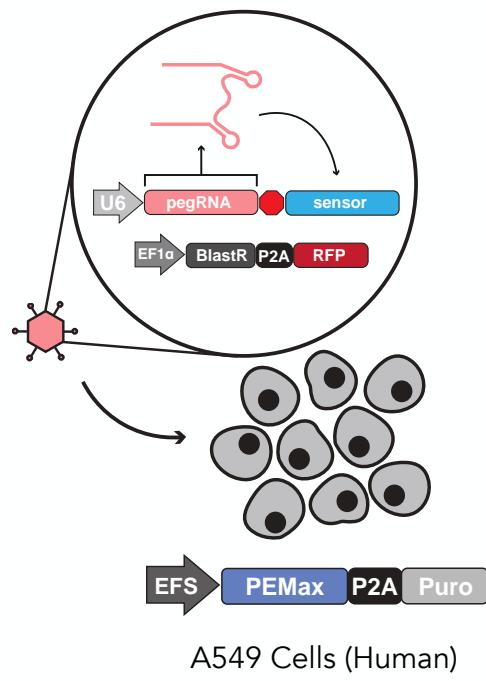


Sensor-based measurements are representative of endogenous editing

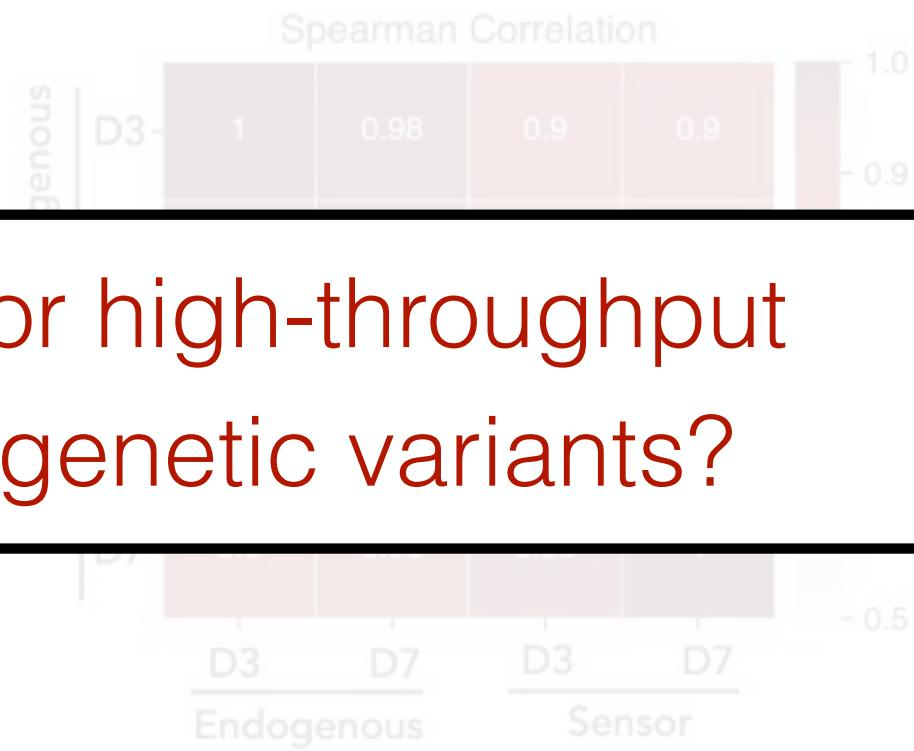
PE sensors

E285Q
D259Y
M246R
M169I
H178R
R110G
R175H
E258E

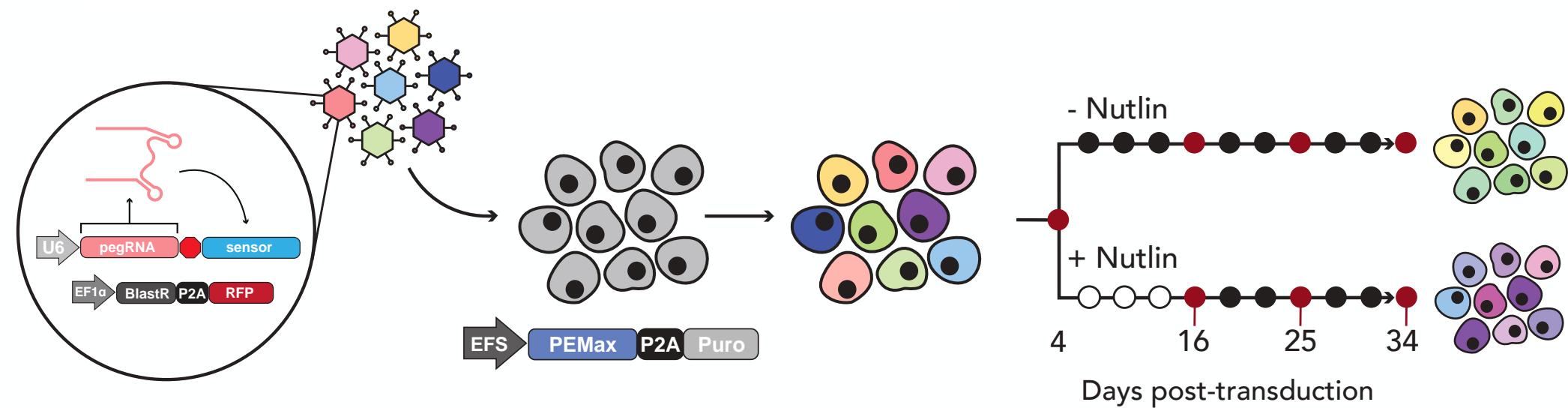
Separate transductions



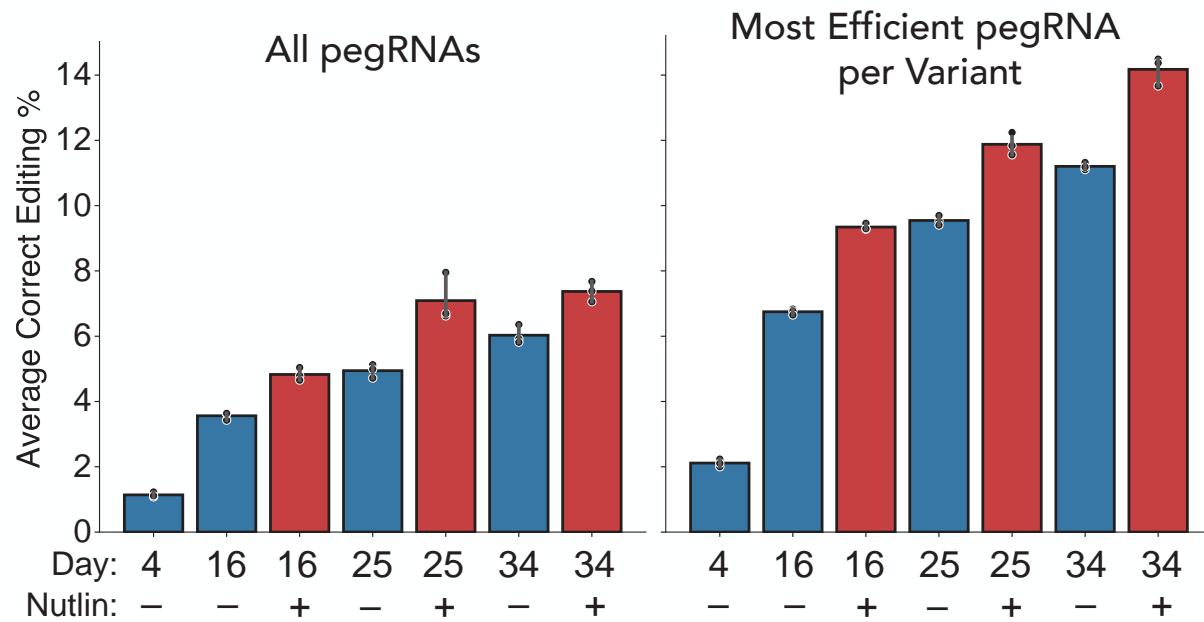
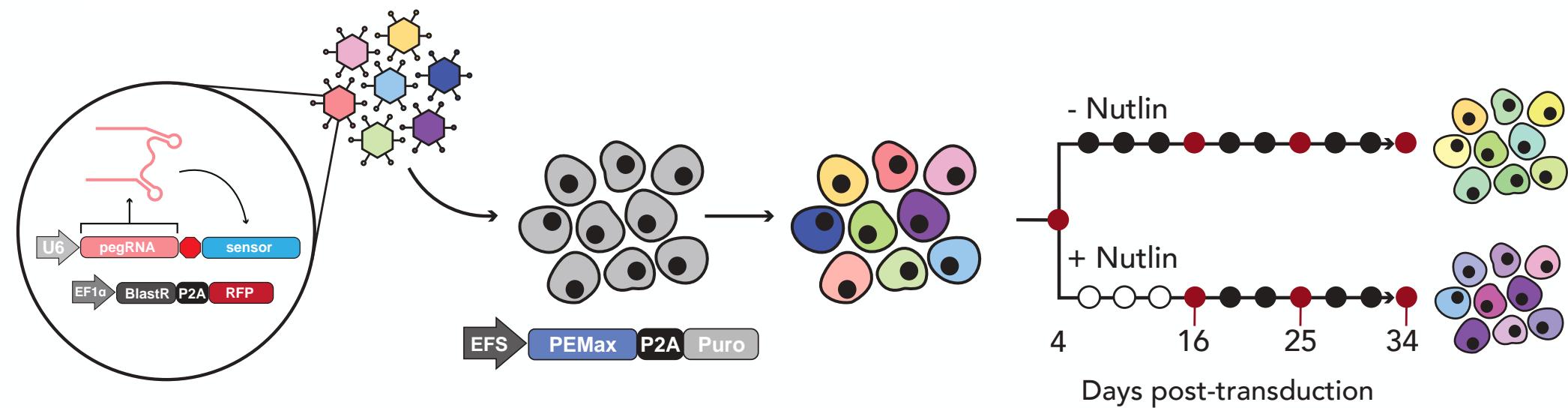
Can we scale this up for high-throughput evaluation of diverse genetic variants?



High throughput evaluation of *TP53* variants with prime editing



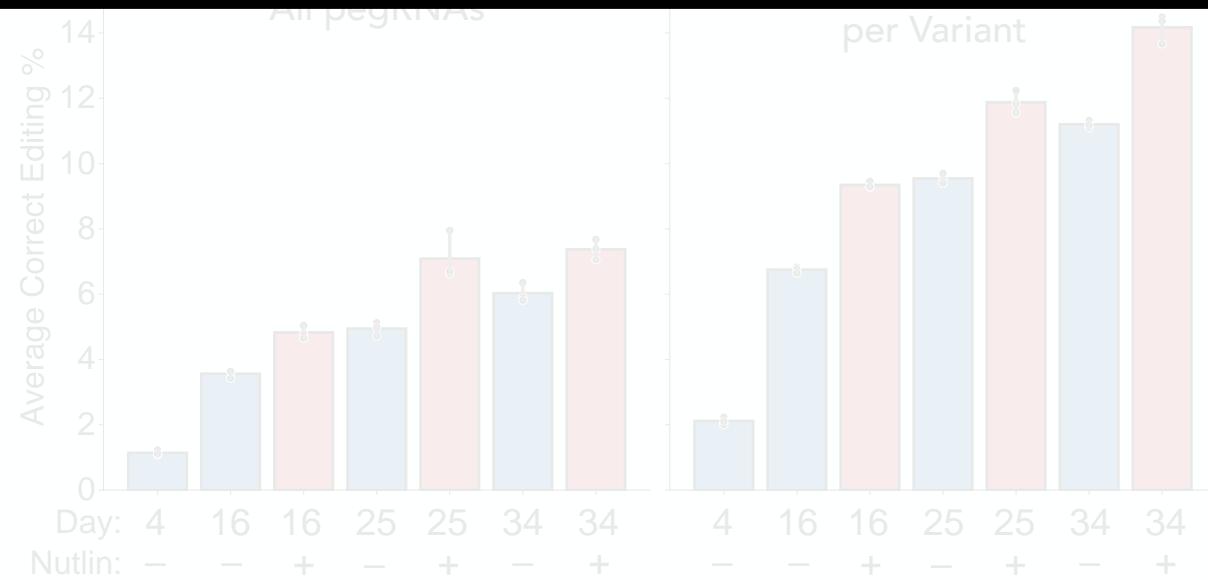
High throughput evaluation of *TP53* variants with prime editing



e

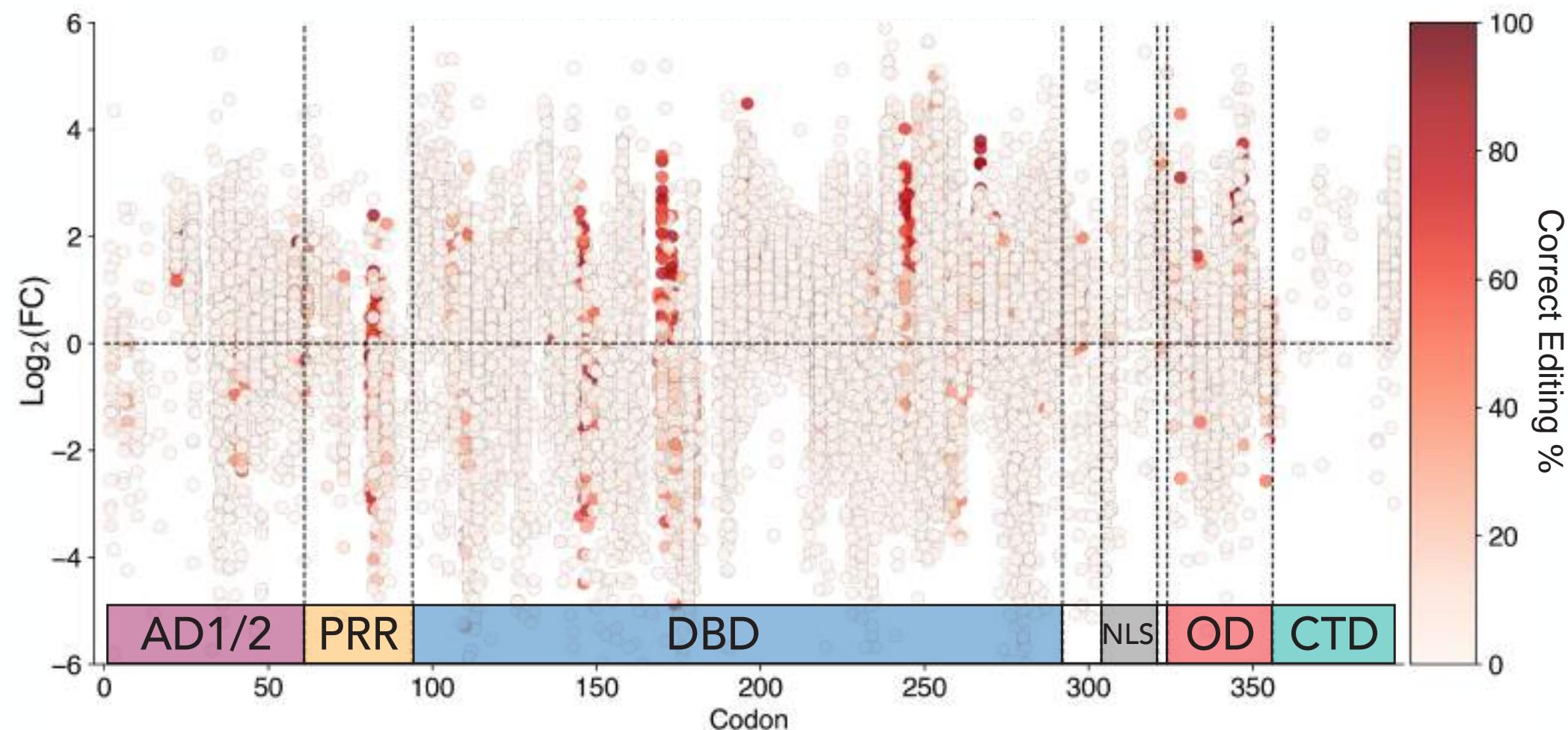


Can we use sensor editing measurements to extract true biological signals?



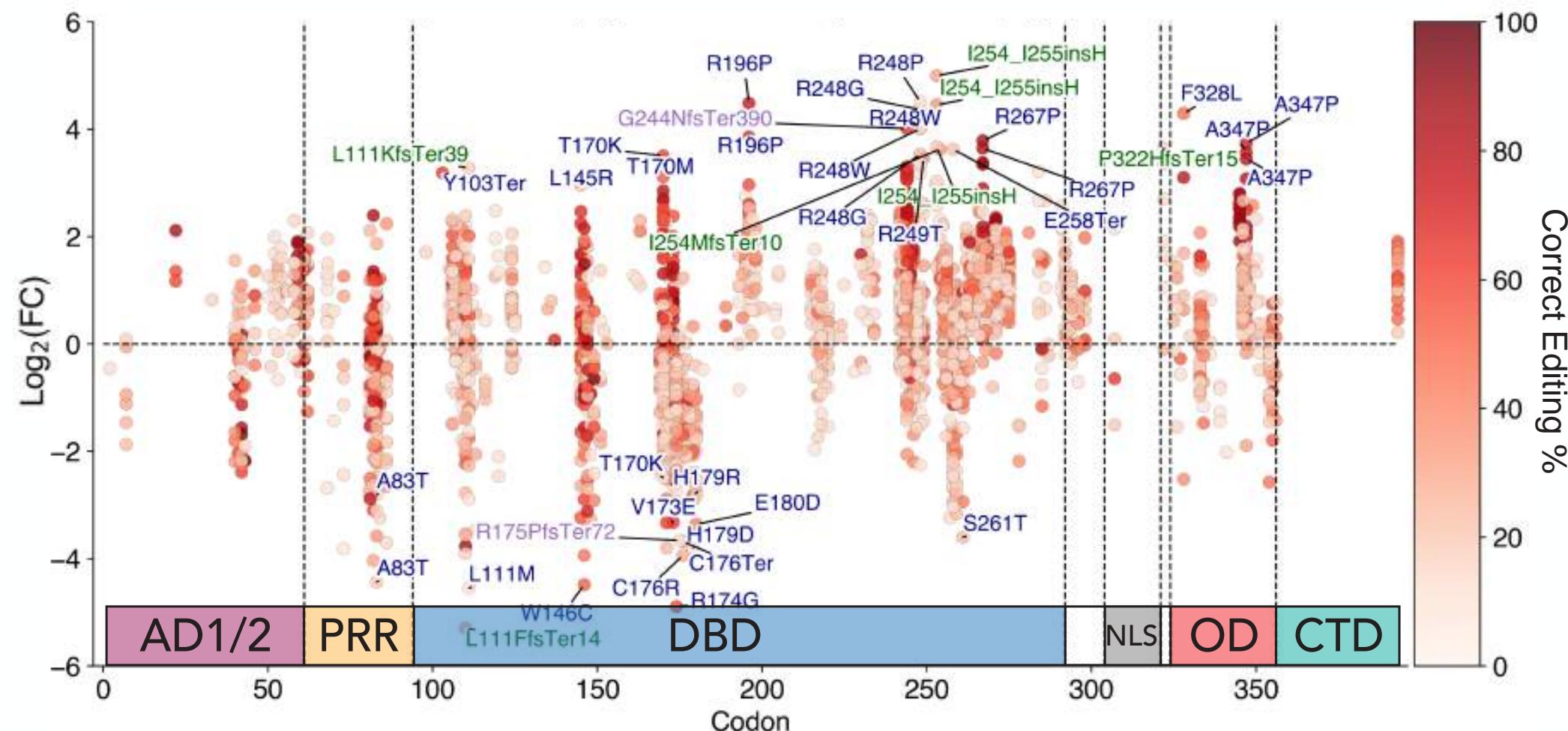
Sensor-based measurements can be used to calibrate screening data

Day 34 + Nutlin | **pegRNAs \geq 0% editing** | 18384/18851 pegRNAs



Sensor-based measurements can be used to calibrate screening data

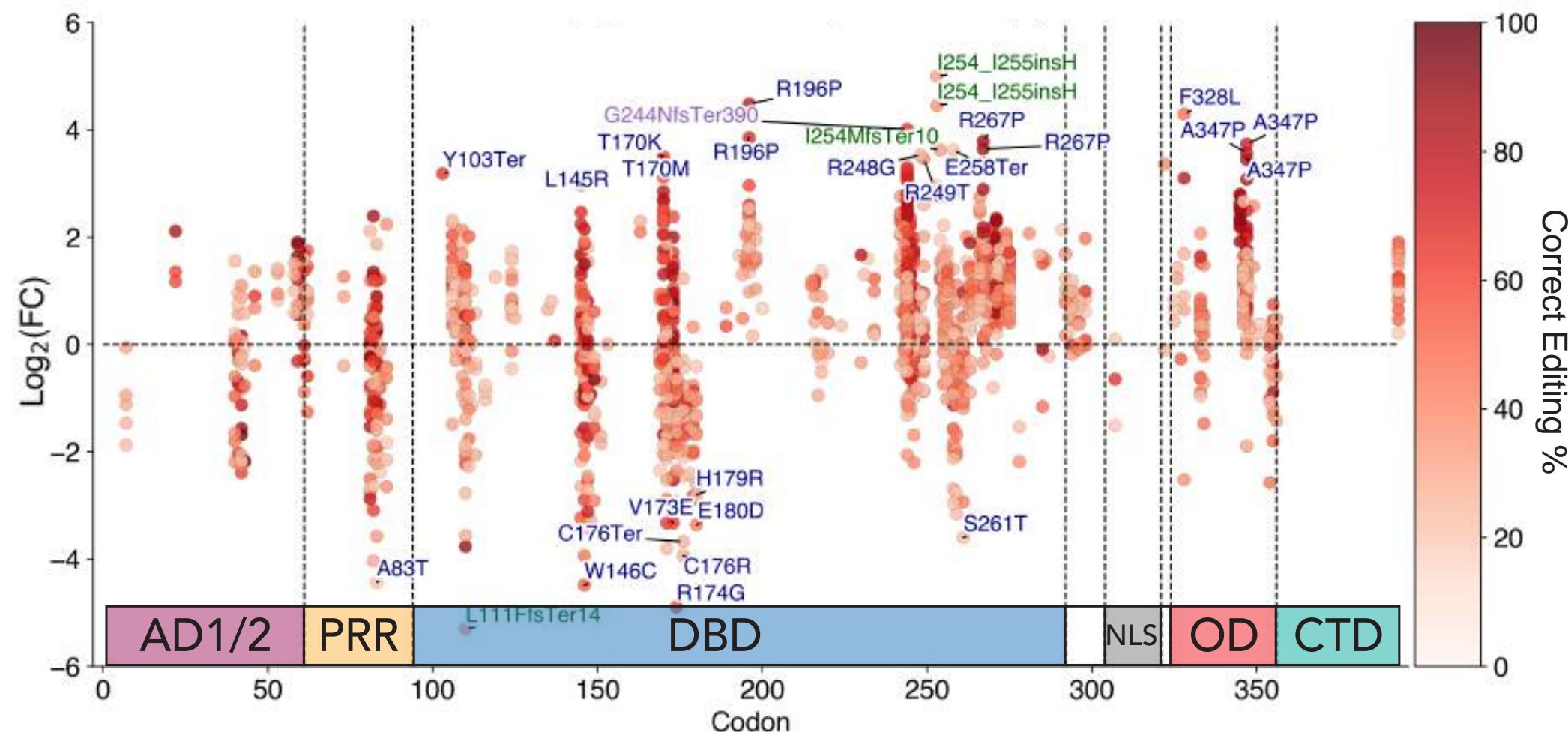
Day 34 + Nutlin | **pegRNAs $\geq 10\%$ editing** | 2465/18851 pegRNAs



Log₂(FC) ~ Fitness

Sensor-based measurements can be used to calibrate screening data

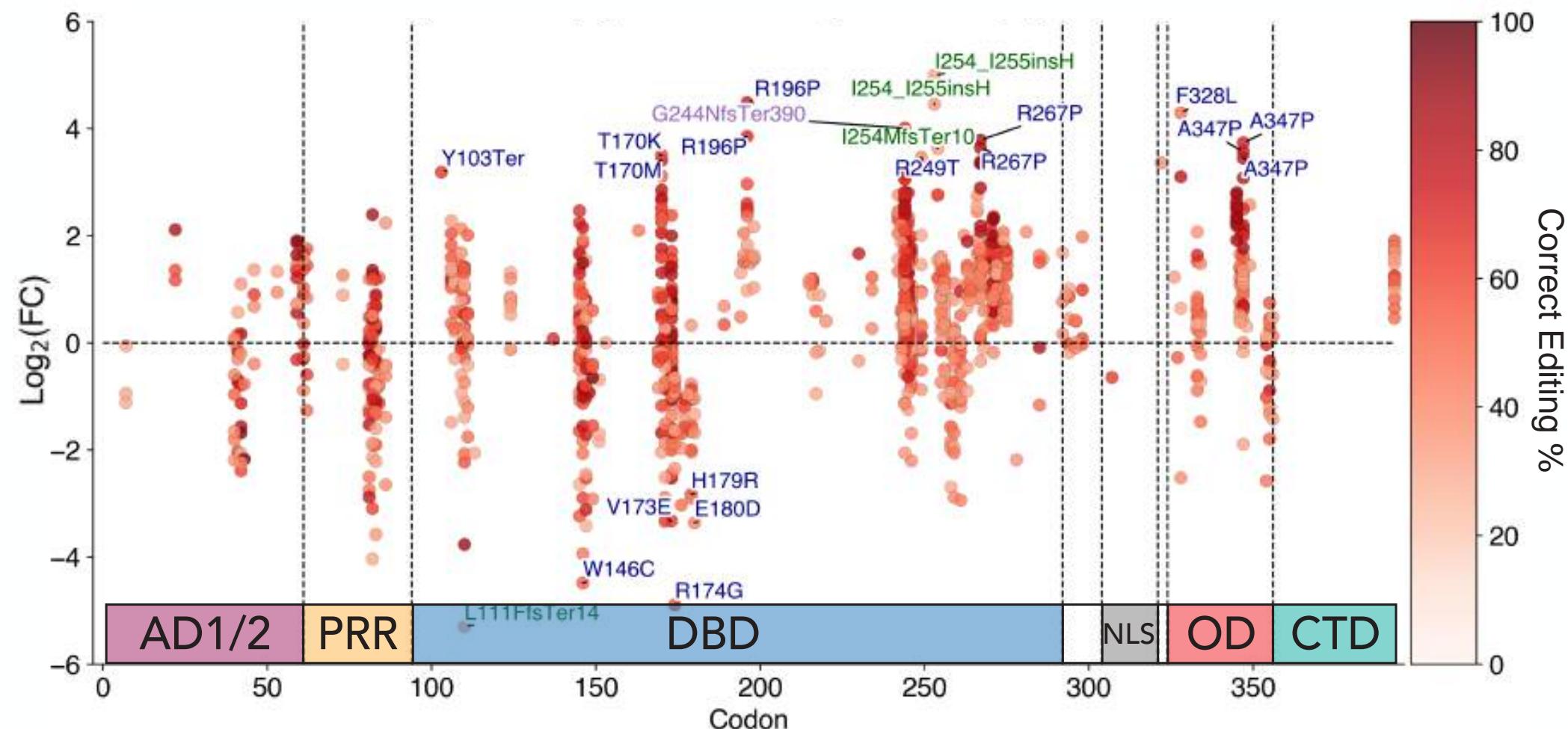
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Log₂(FC) ~ Fitness

Sensor-based measurements can be used to calibrate screening data

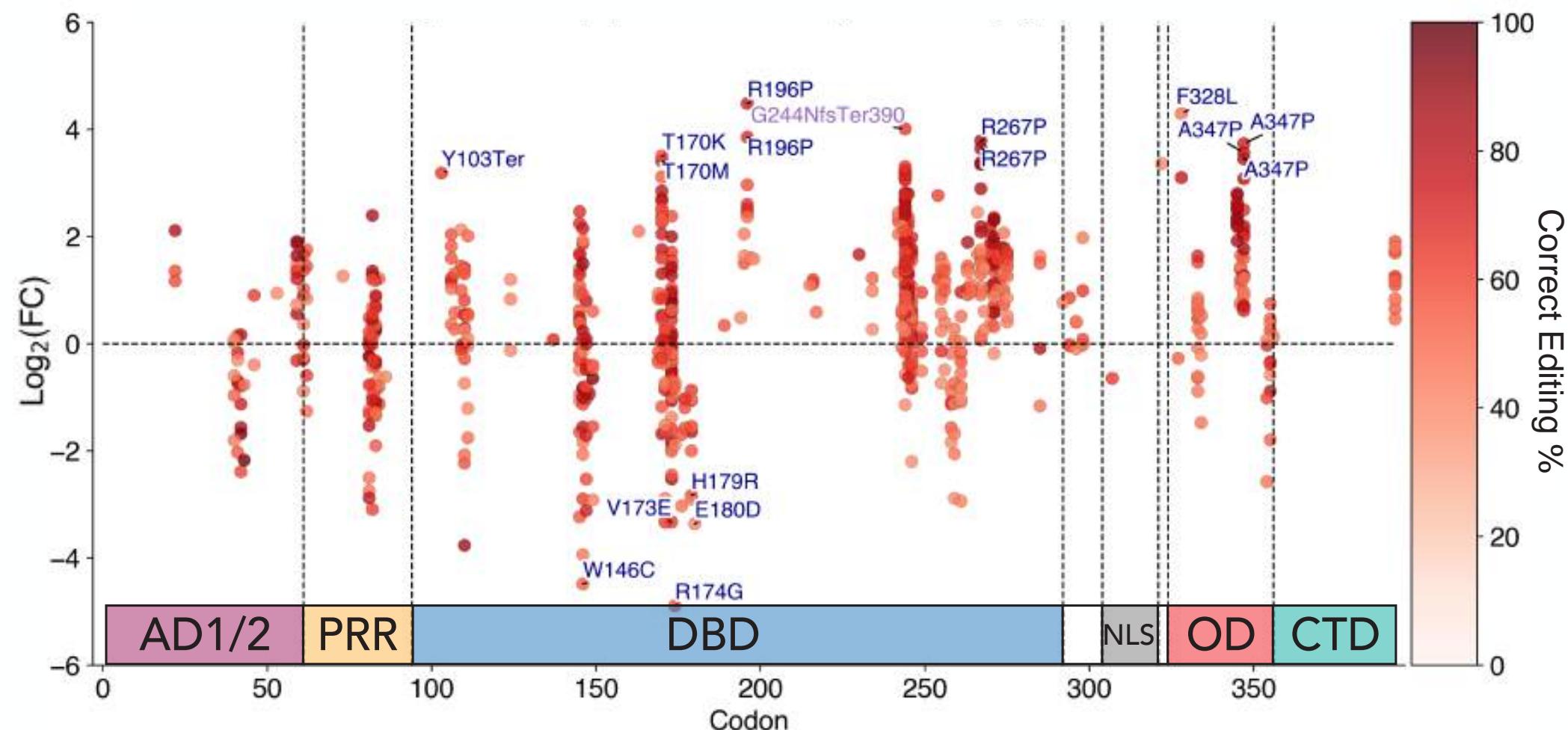
Day 34 + Nutlin | **pegRNAs \geq 30% editing** | 1085/18851 pegRNAs



$\text{Log}_2(\text{FC}) \sim \text{Fitness}$

Sensor-based measurements can be used to calibrate screening data

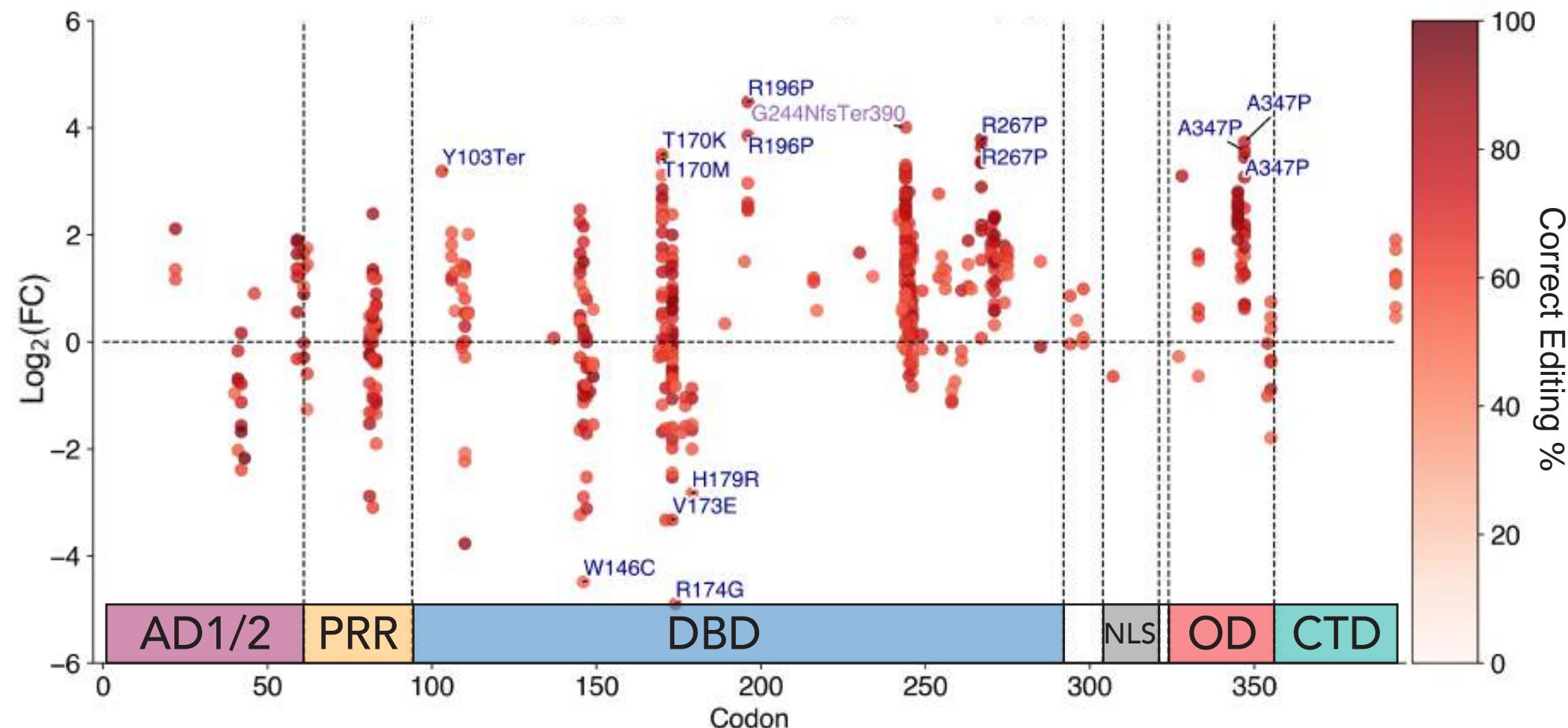
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Log₂(FC) ~ Fitness

Sensor-based measurements can be used to calibrate screening data

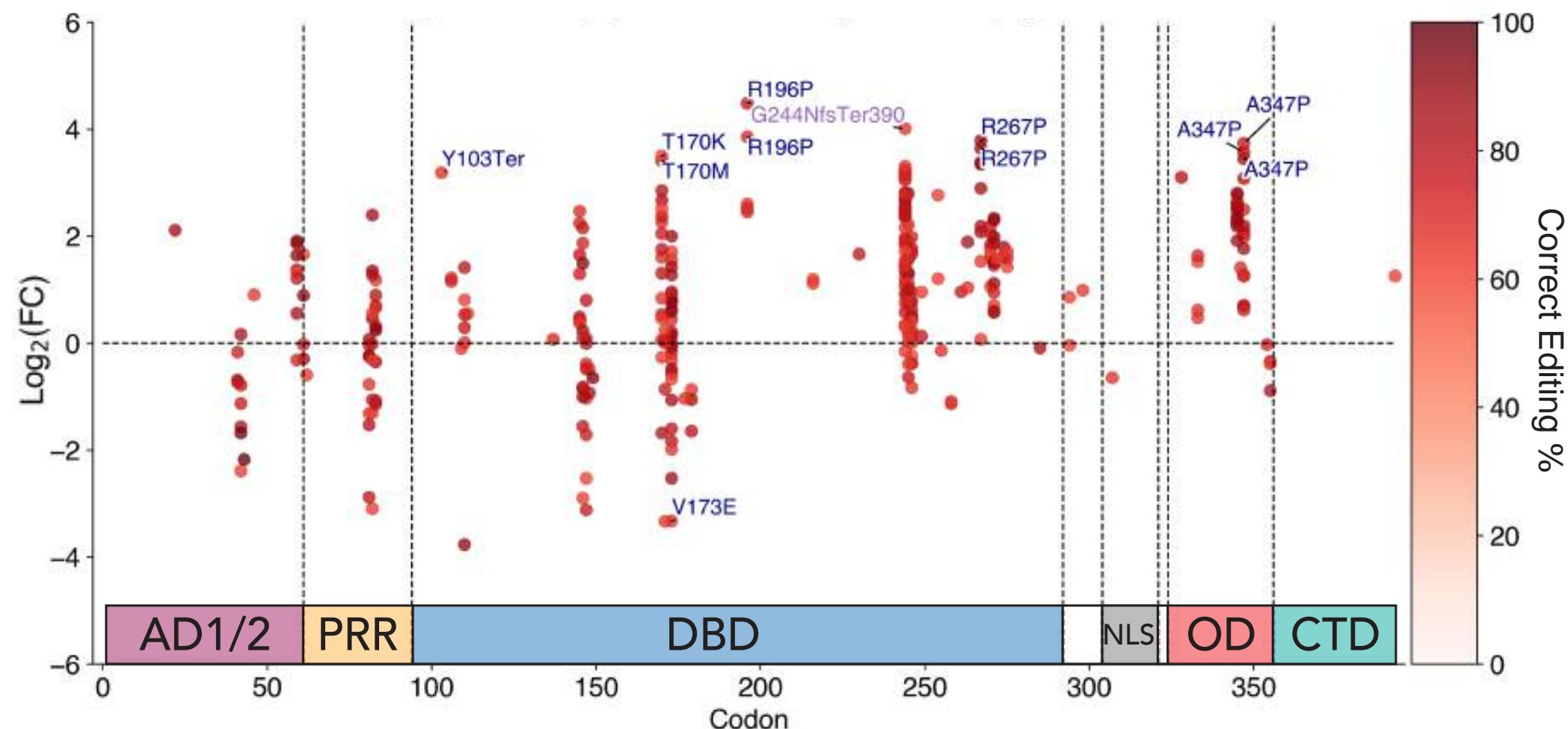
Day 34 + Nutlin | **pegRNAs \geq 50% editing** | 499/18851 pegRNAs



Log₂(FC) ~ Fitness

Sensor-based measurements can be used to calibrate screening data

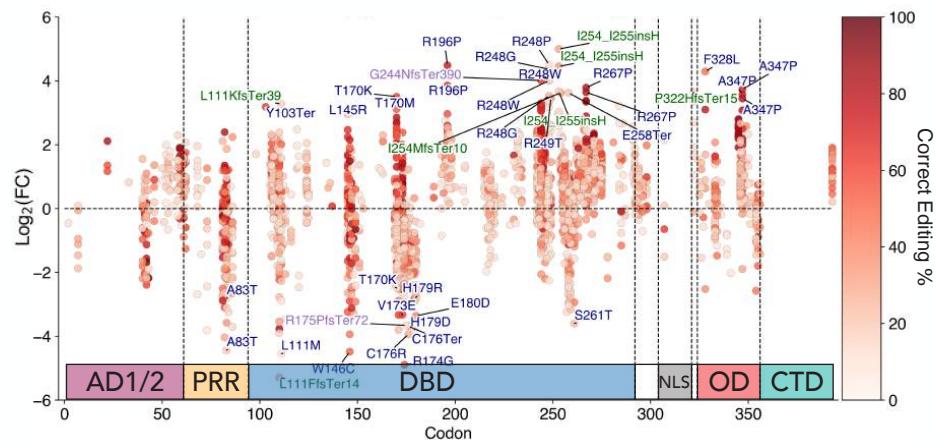
Day 34 + Nutlin | **pegRNAs \geq 60% editing** | 333/18851 pegRNAs



Log₂(FC) ~ Fitness

Sensor-based measurements can be used to calibrate screening data

Day 34 + Nutlin | **pegRNAs $\geq 10\%$ editing** | 2465/18851 pegRNAs



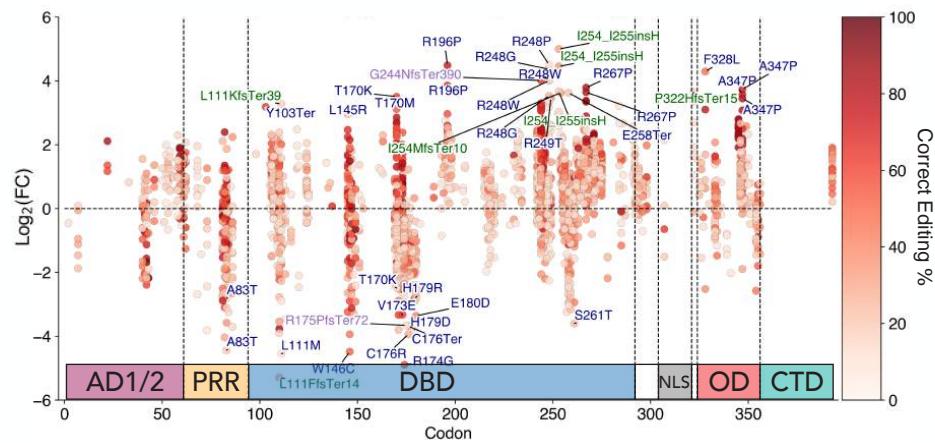
Sensor-based measurements can be used to calibrate screening data



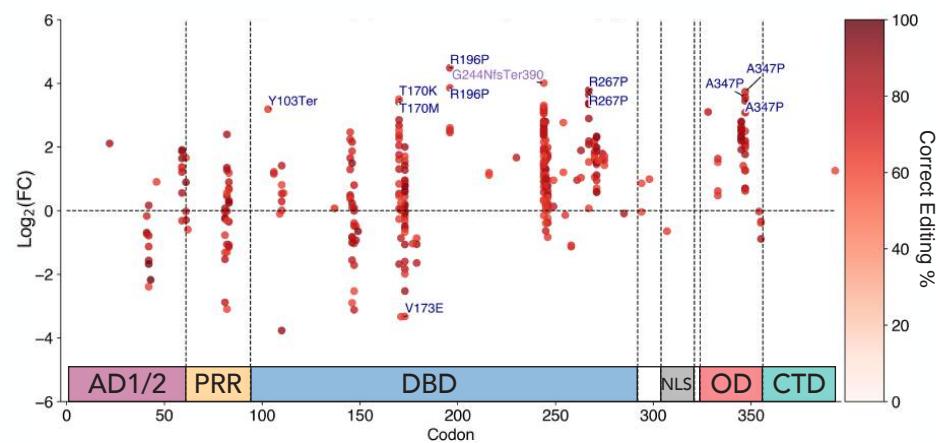
Data looks

less tragic now.

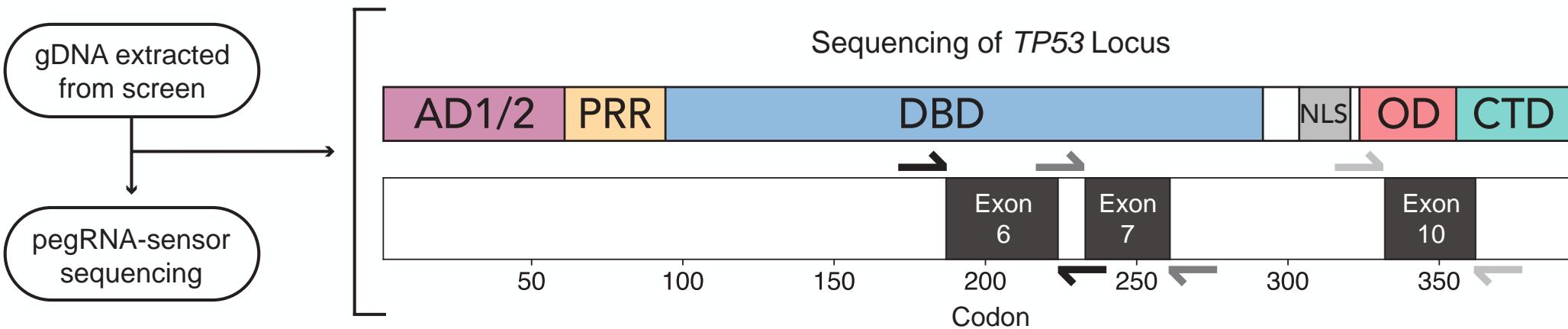
Day 34 + Nutlin | **pegRNAs \geq 10% editing** | 2465/18851 pegRNAs



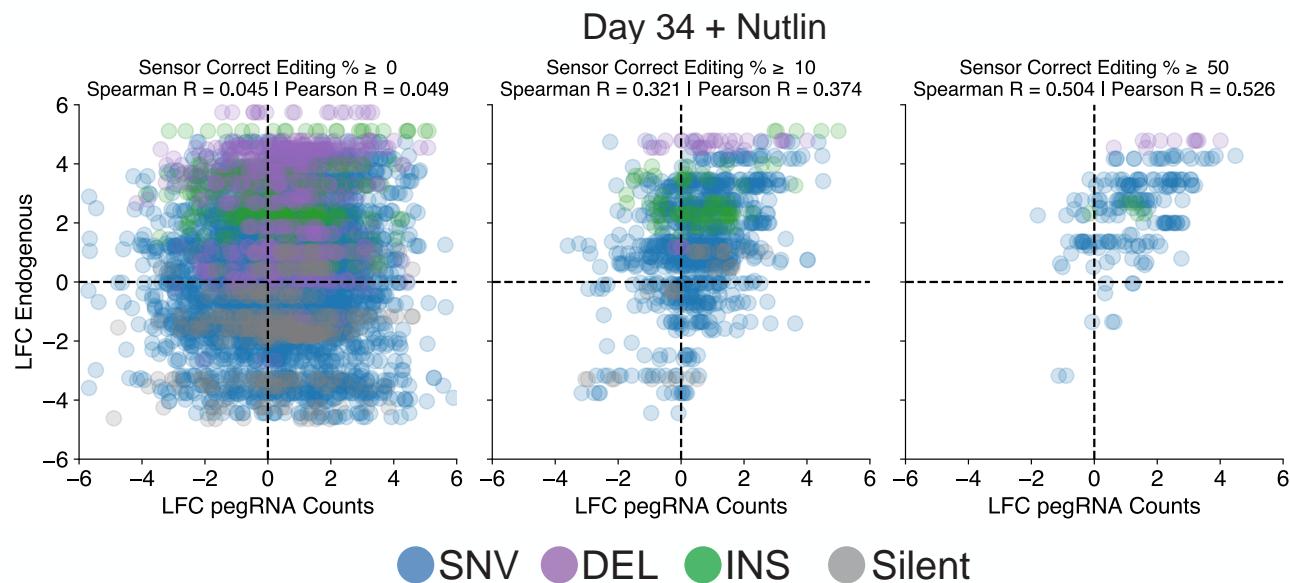
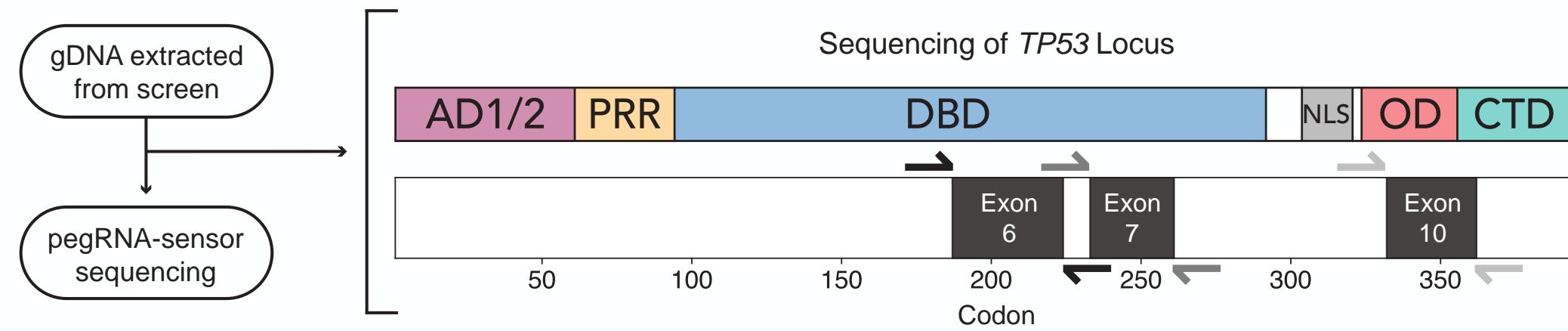
Day 34 + Nutlin | **pegRNAs \geq 60% editing** | 333/18851 pegRNAs



Sensor-based measurements recapitulate endogenous editing patterns

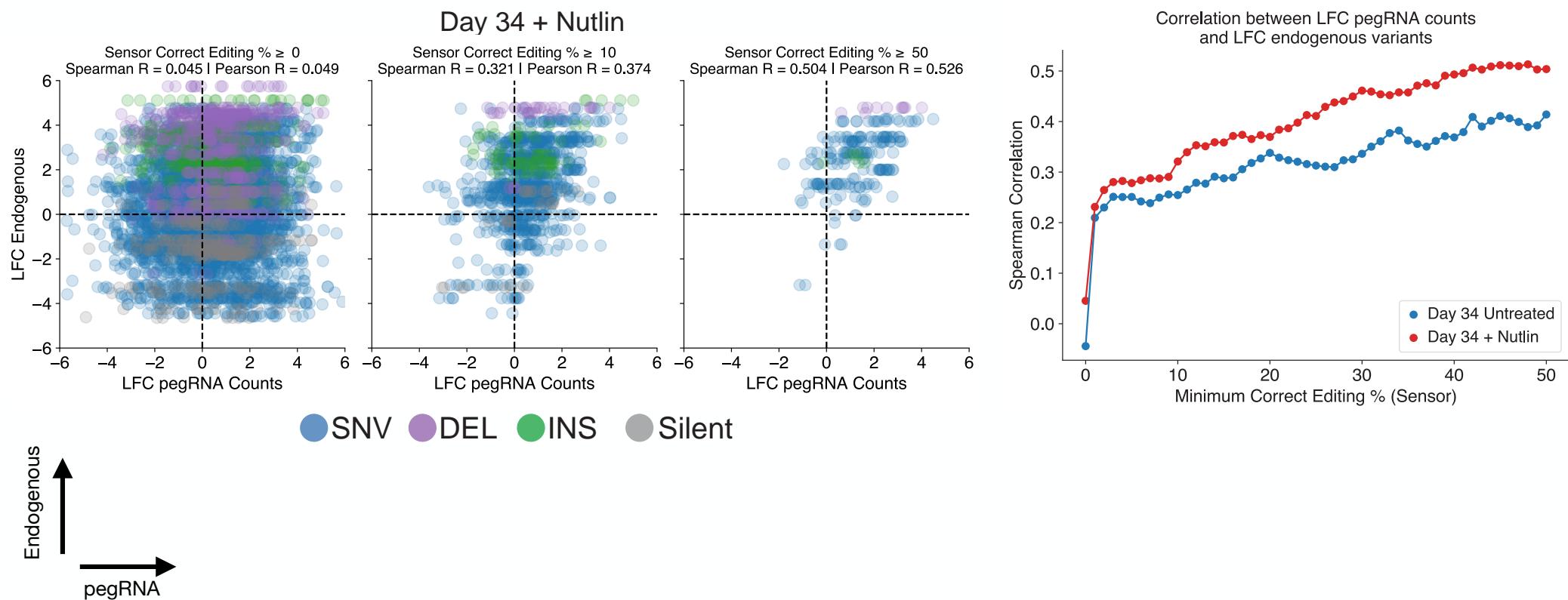
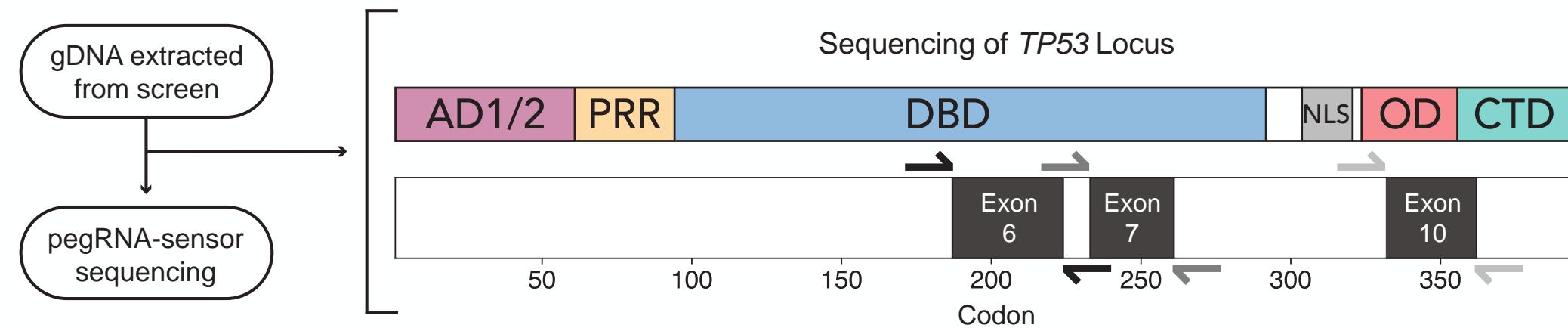


Sensor-based measurements recapitulate endogenous editing patterns

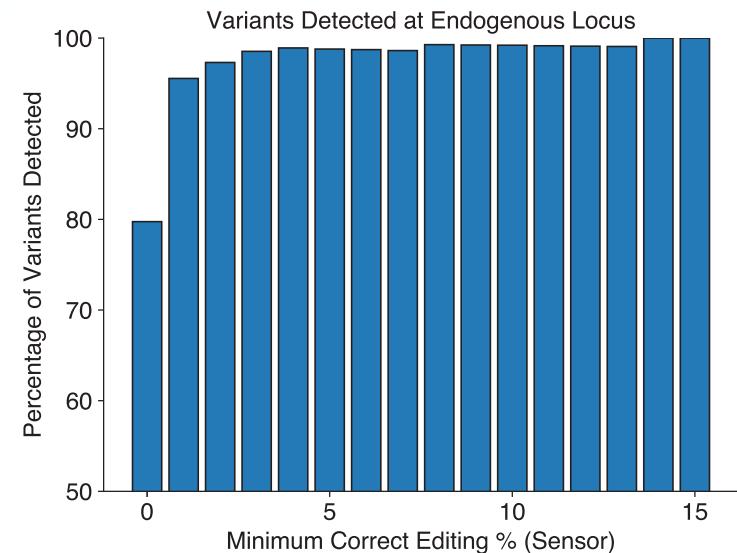
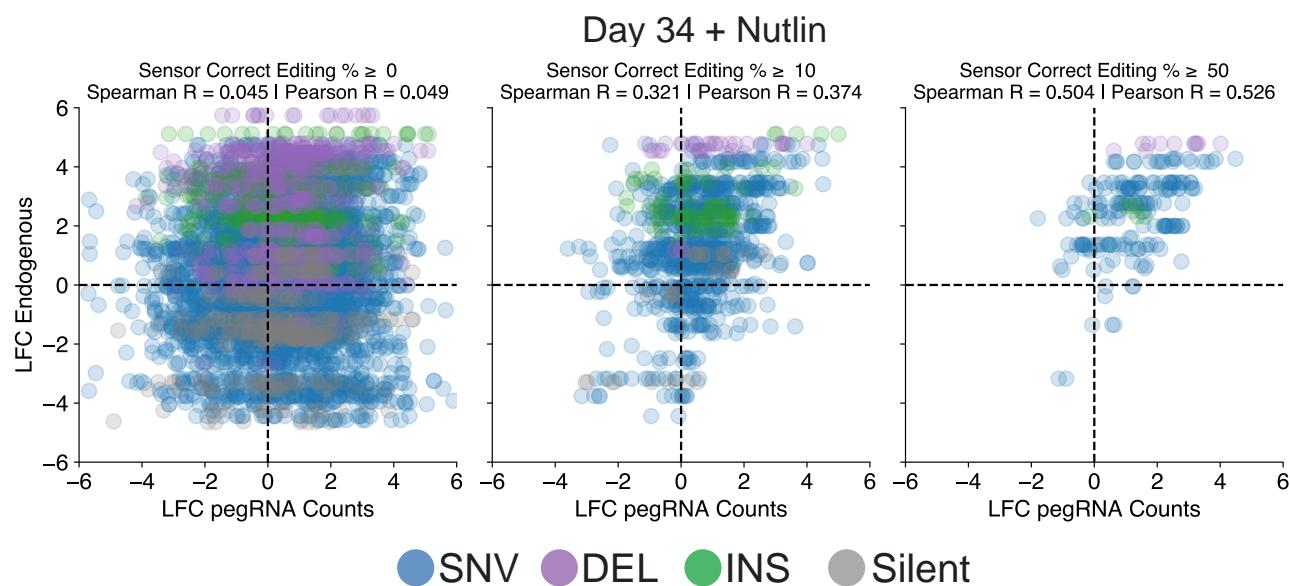
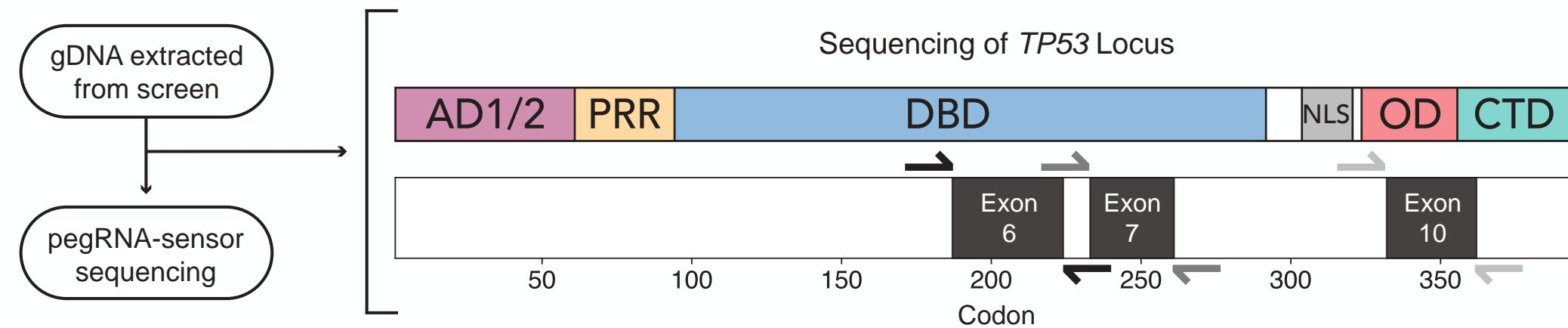


↑ Endogenous
→ pegRNA

Sensor-based measurements recapitulate endogenous editing patterns



Sensor-based measurements recapitulate endogenous editing patterns



↑ Endogenous
→ pegRNA

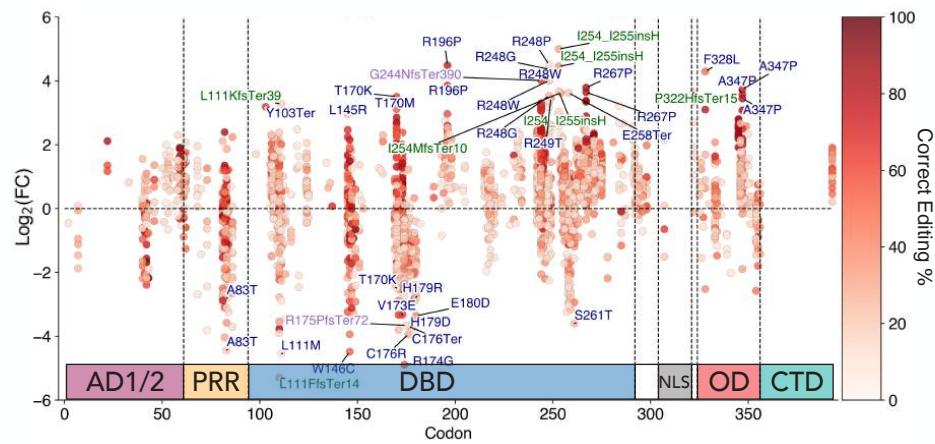
Sensor-based measurements can extract true biological signals



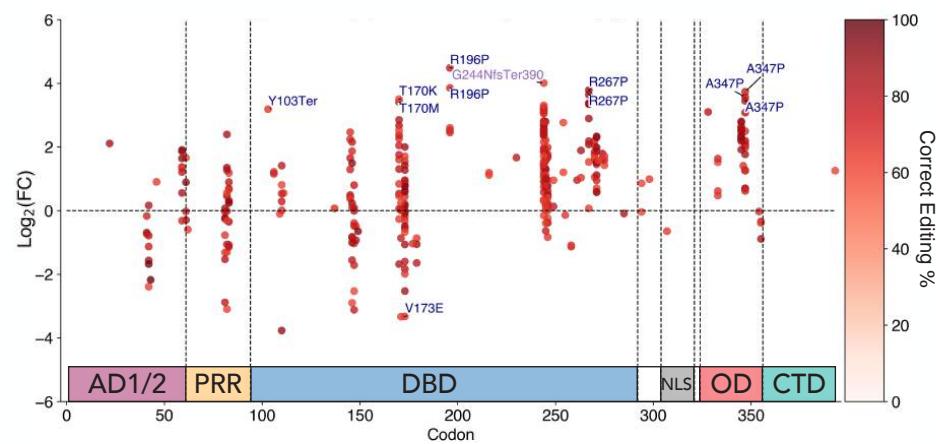
Data looks

less tragic now.

Day 34 + Nutlin | **pegRNAs \geq 10% editing** | 2465/18851 pegRNAs

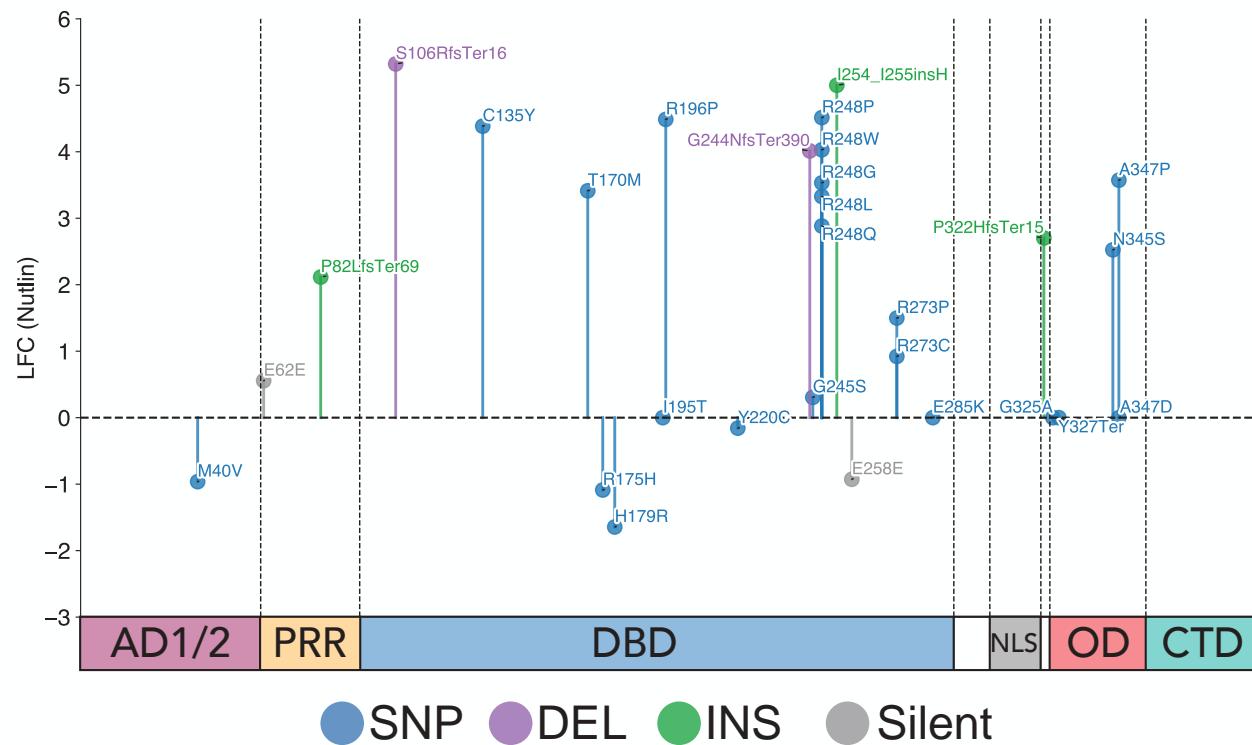


Day 34 + Nutlin | **pegRNAs \geq 60% editing** | 333/18851 pegRNAs

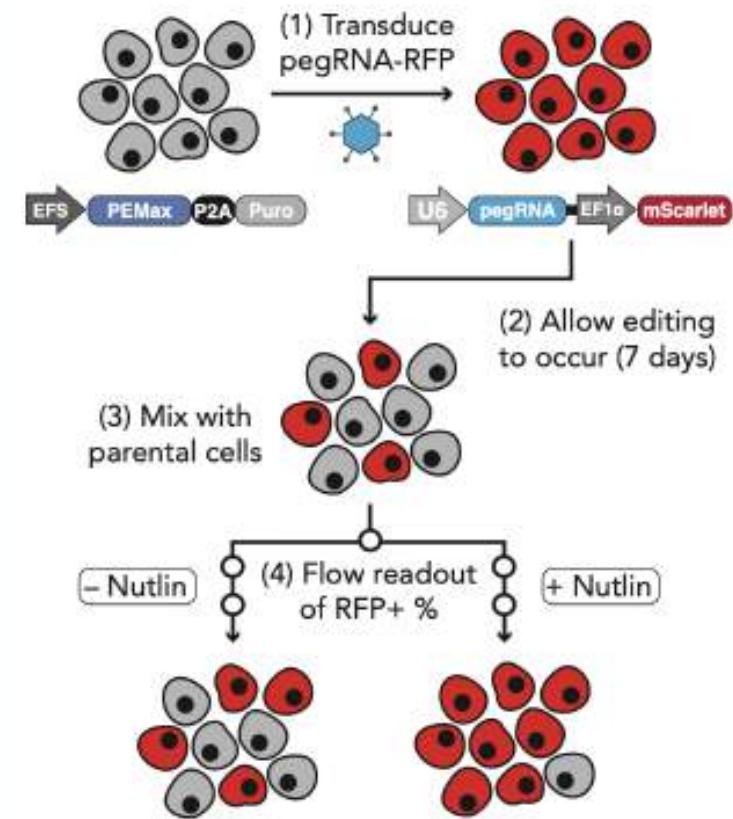
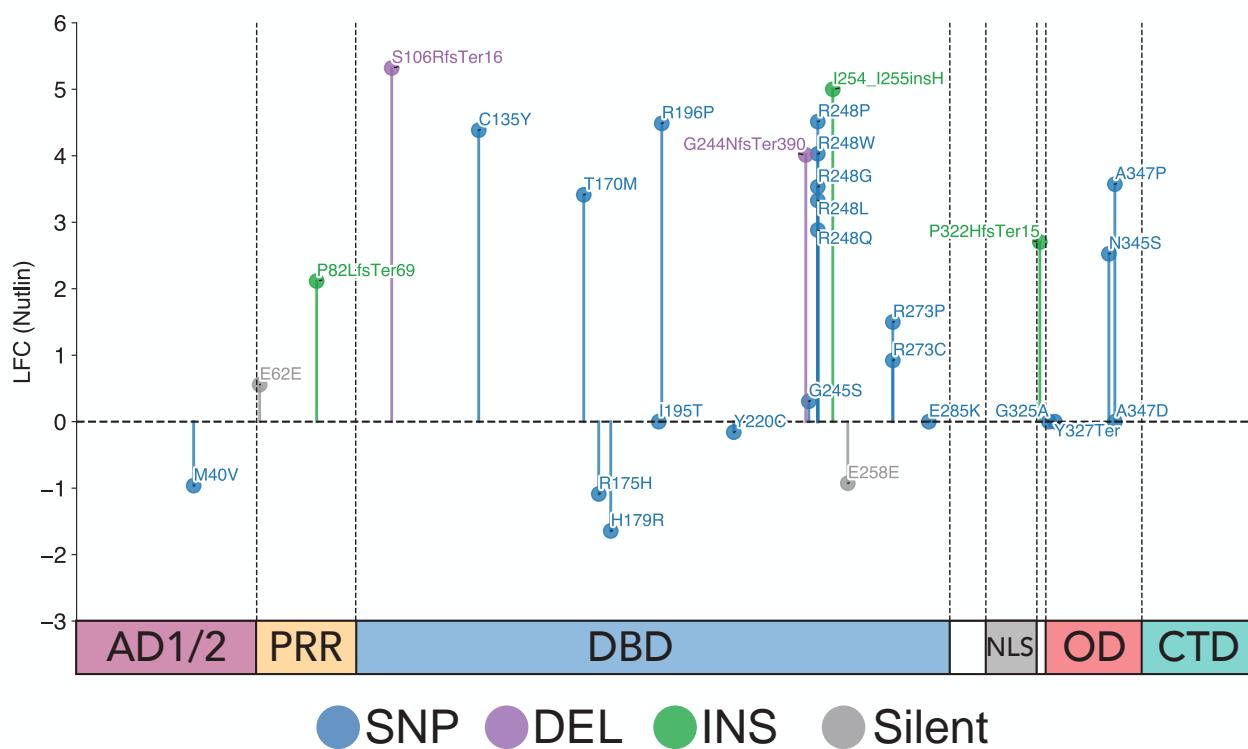


Are these *bona fide* pathogenic variants?

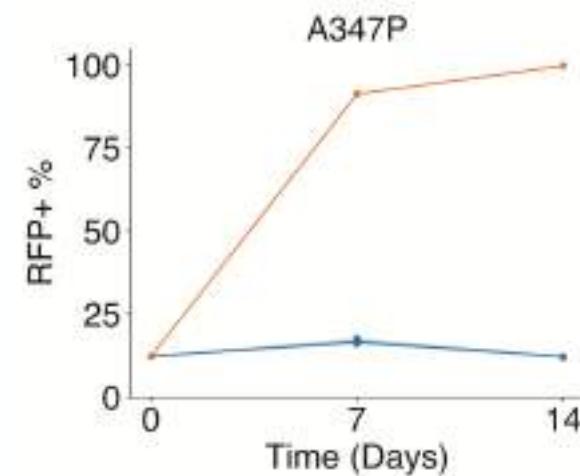
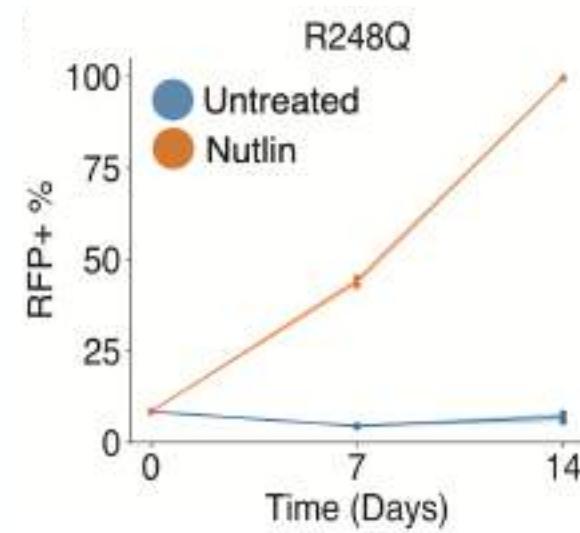
Variants affecting different p53 domains can differentially impact p53 function and cell fitness



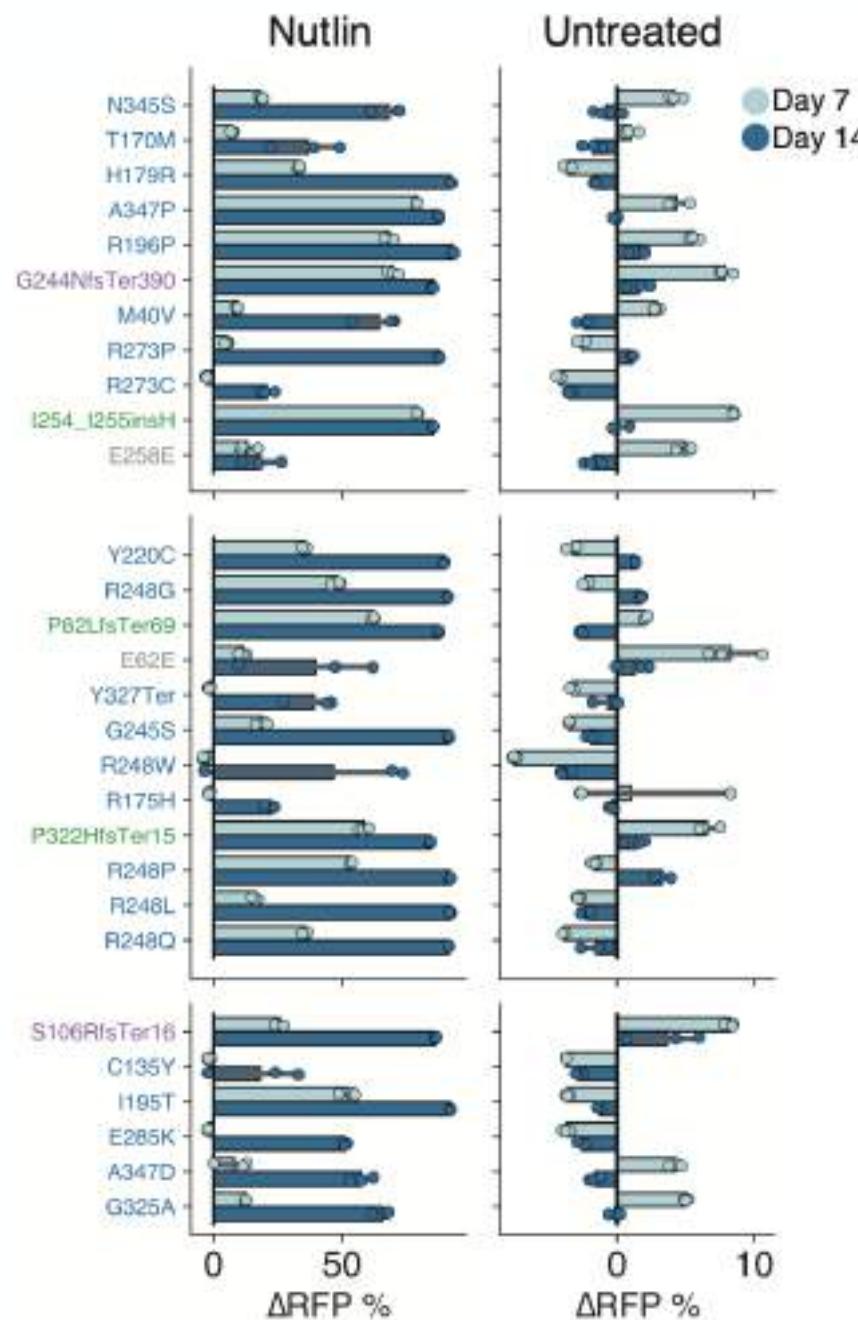
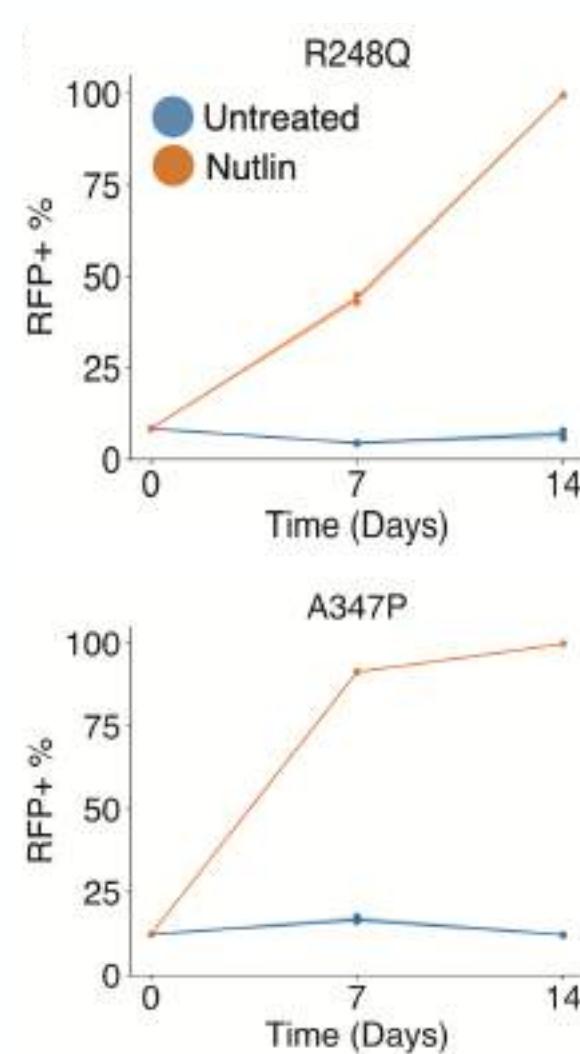
Variants affecting different p53 domains can differentially impact p53 function and cell fitness



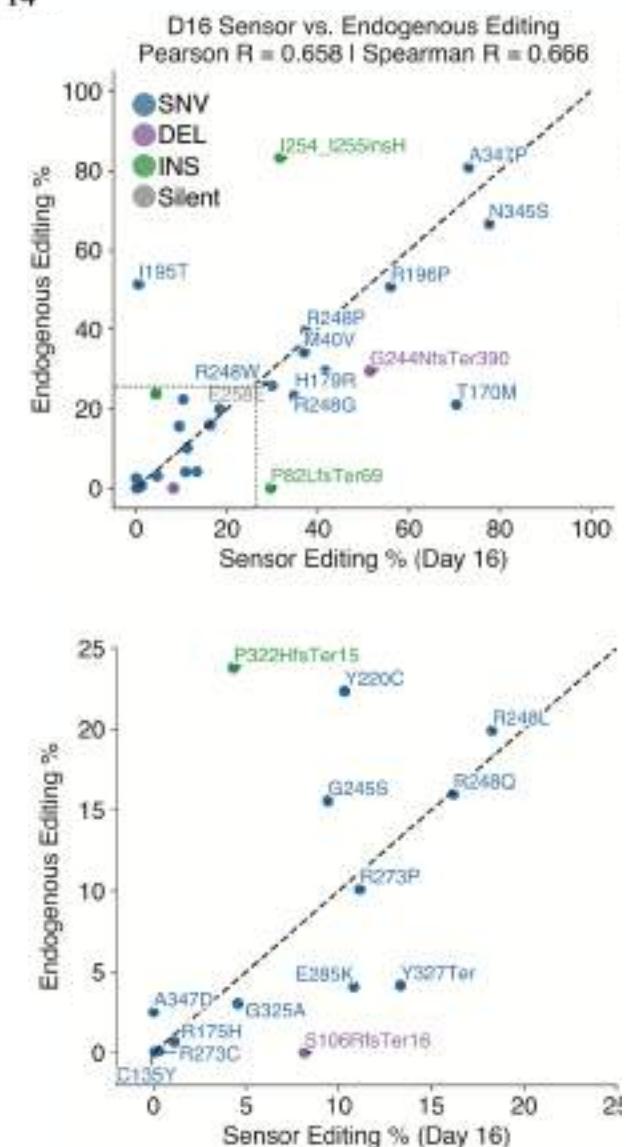
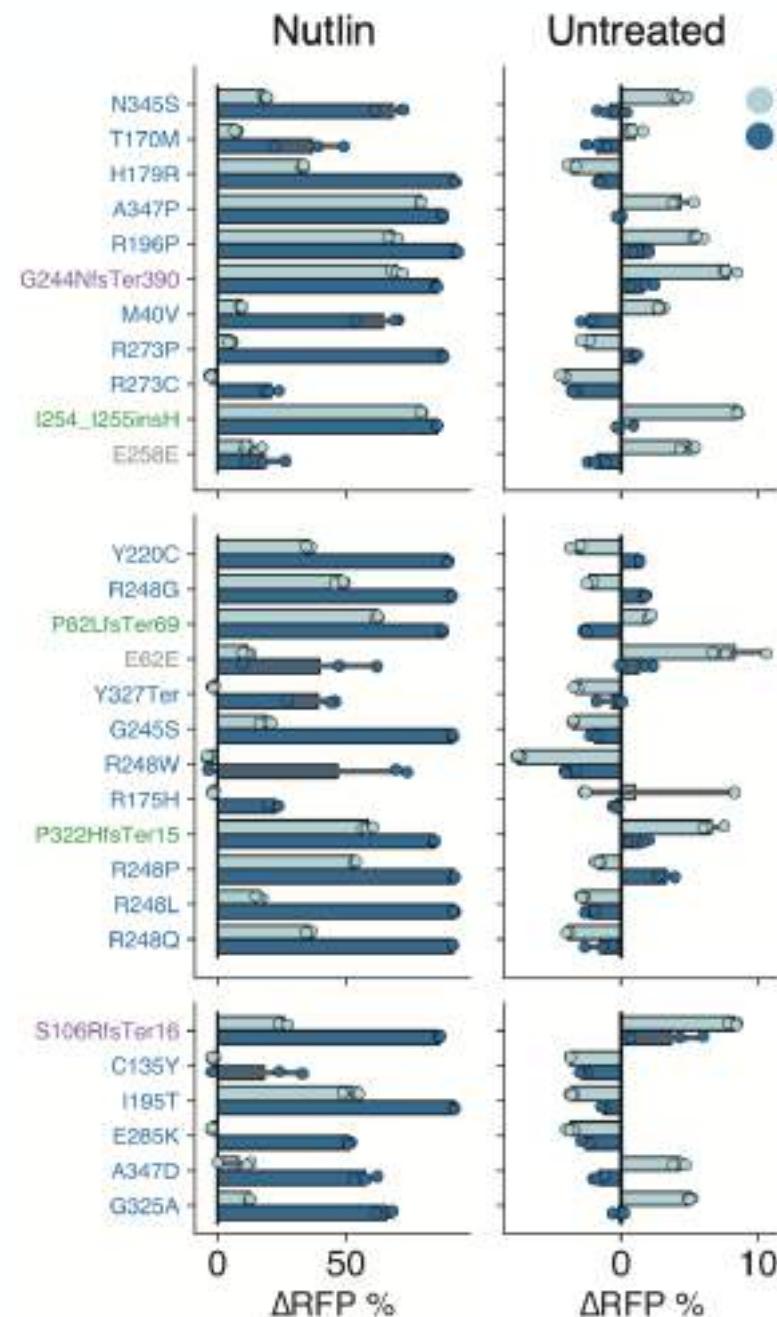
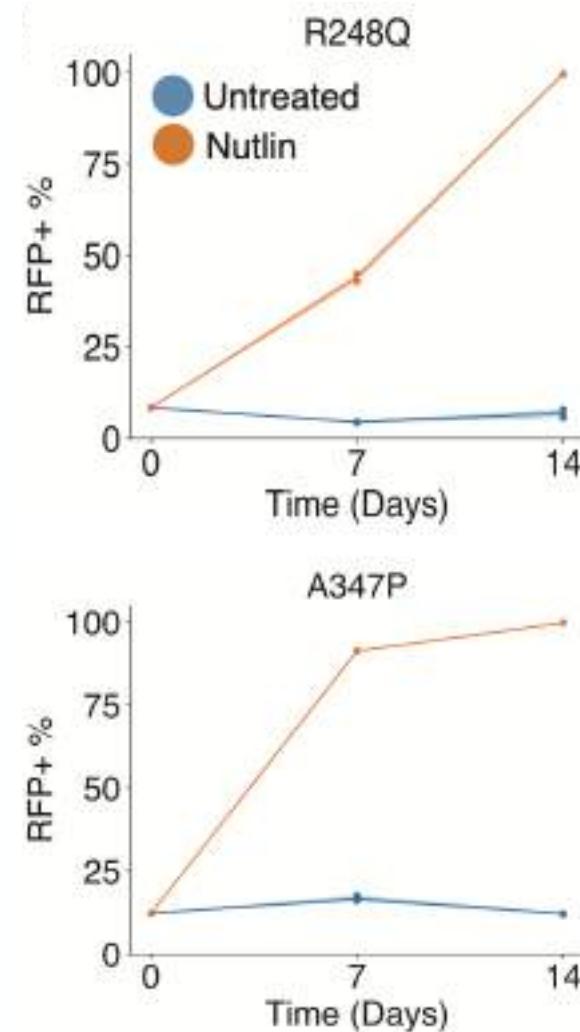
Variants affecting different p53 domains can differentially impact p53 function and cell fitness



Variants affecting different p53 domains can differentially impact p53 function and cell fitness



Variants affecting different p53 domains can differentially impact p53 function and cell fitness





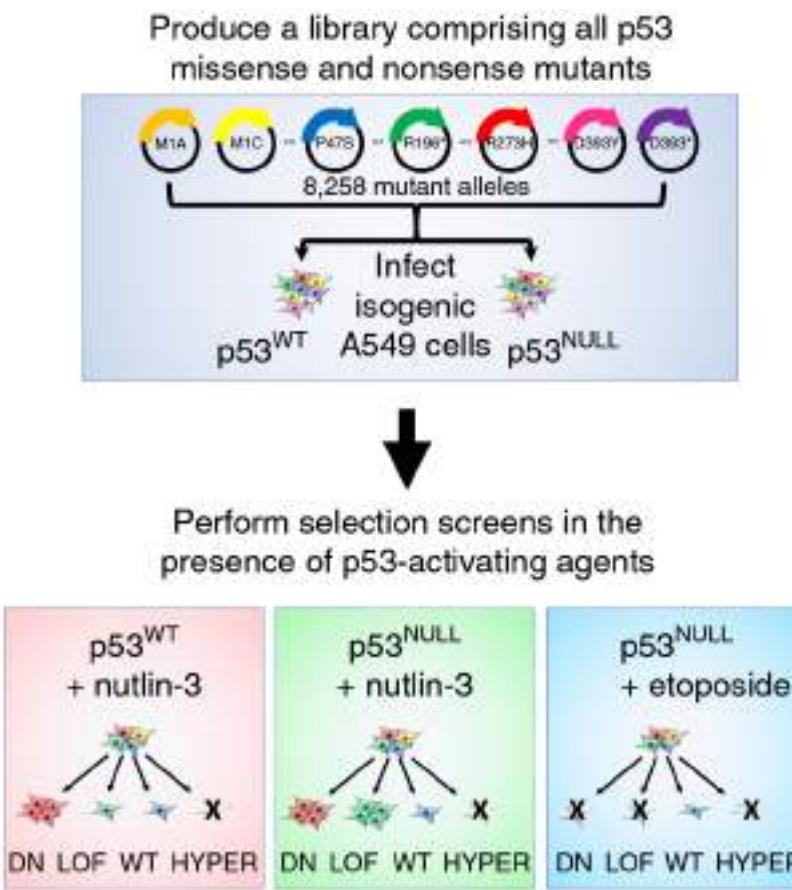
Is there real biological discovery potential here?

Is PE orthogonal to pooled cDNA screening?

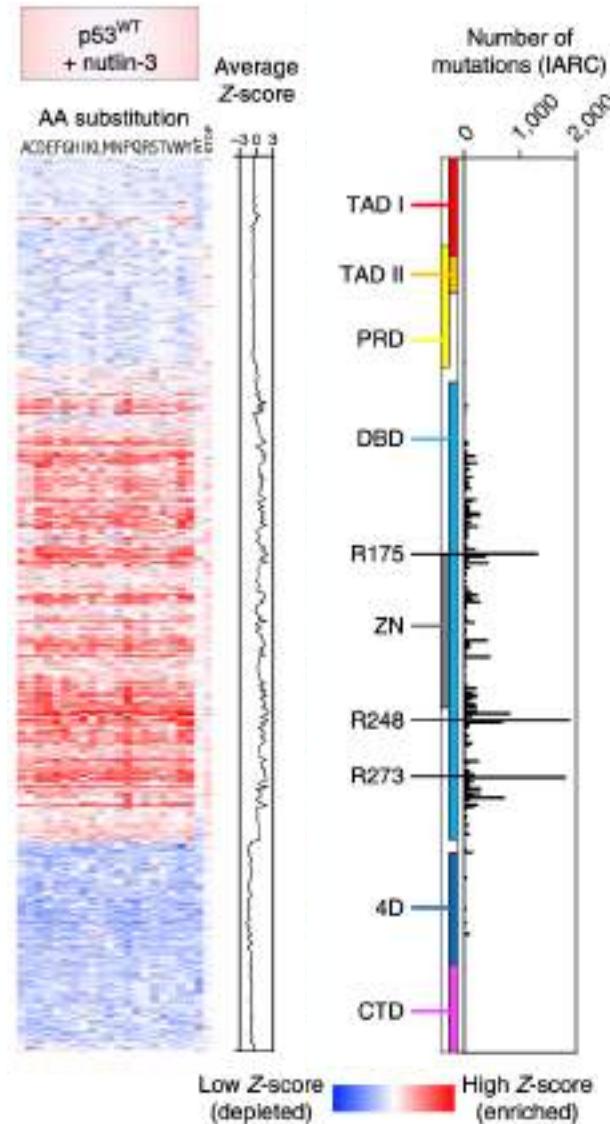
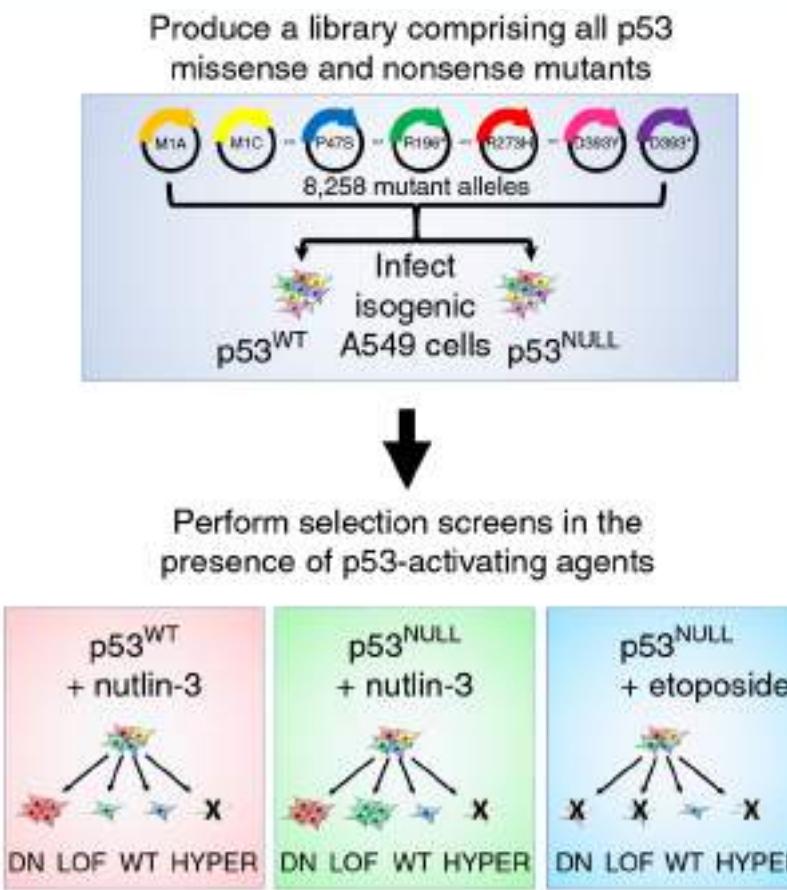


cDNA-based deep mutational scanning studies of p53 have suggested that most variants exhibit dominant negative activity

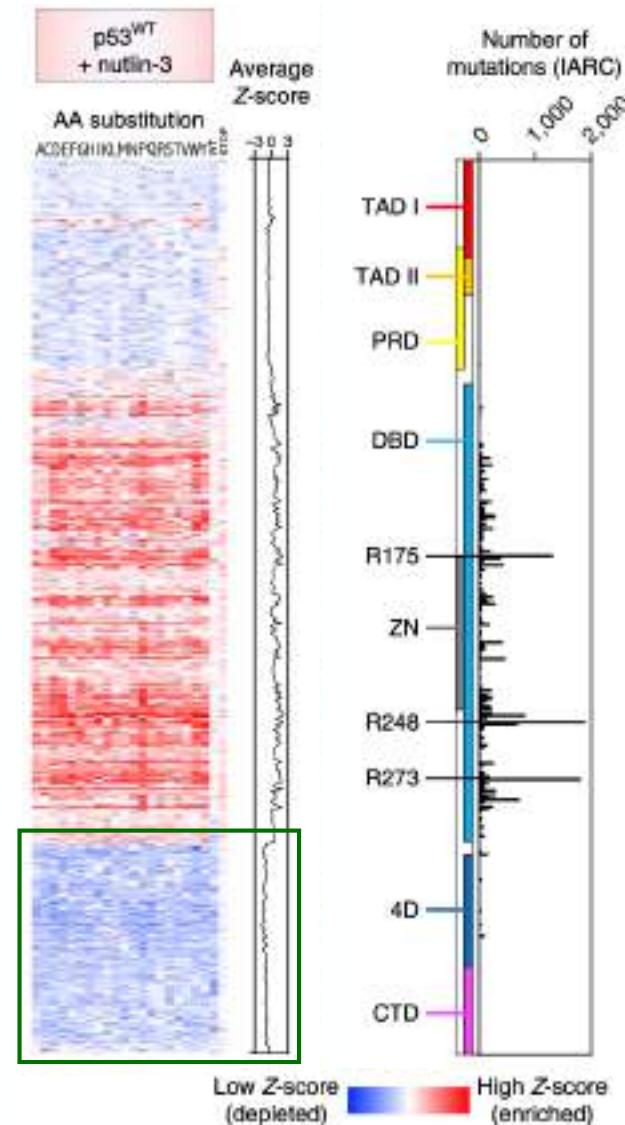
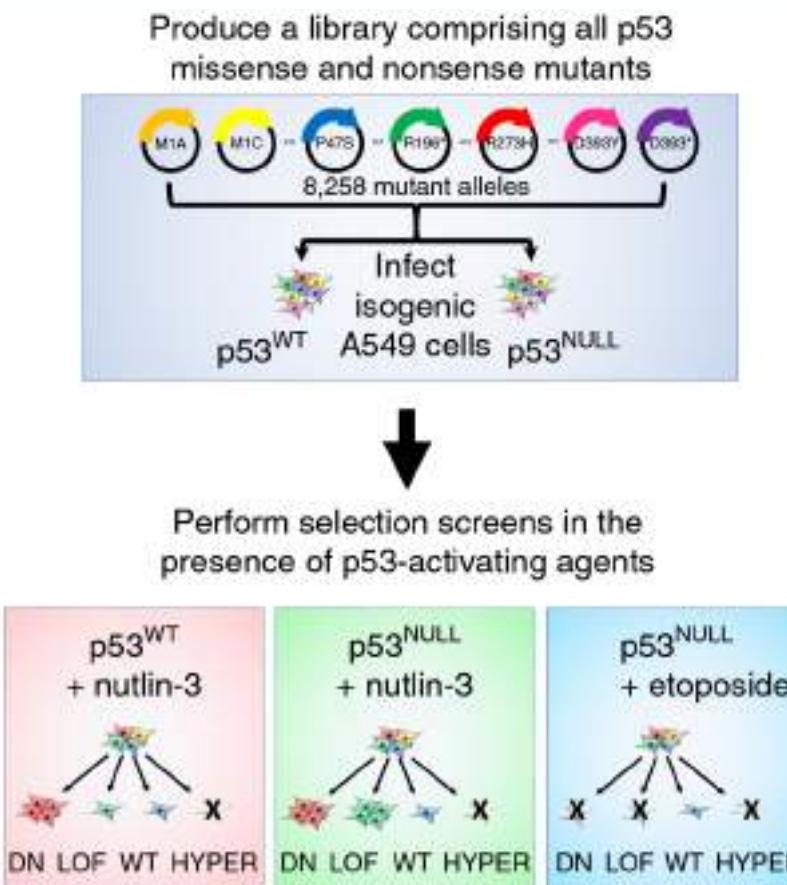
cDNA-based deep mutational scanning studies of p53 have suggested that most variants exhibit dominant negative activity



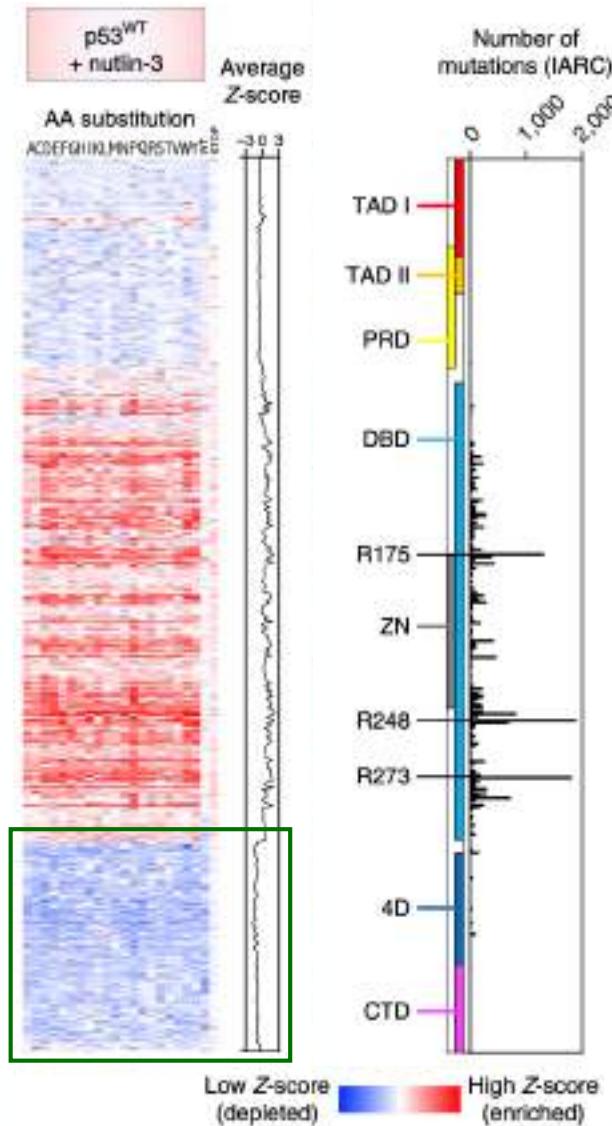
cDNA-based deep mutational scanning studies of p53 have suggested that most variants exhibit dominant negative activity



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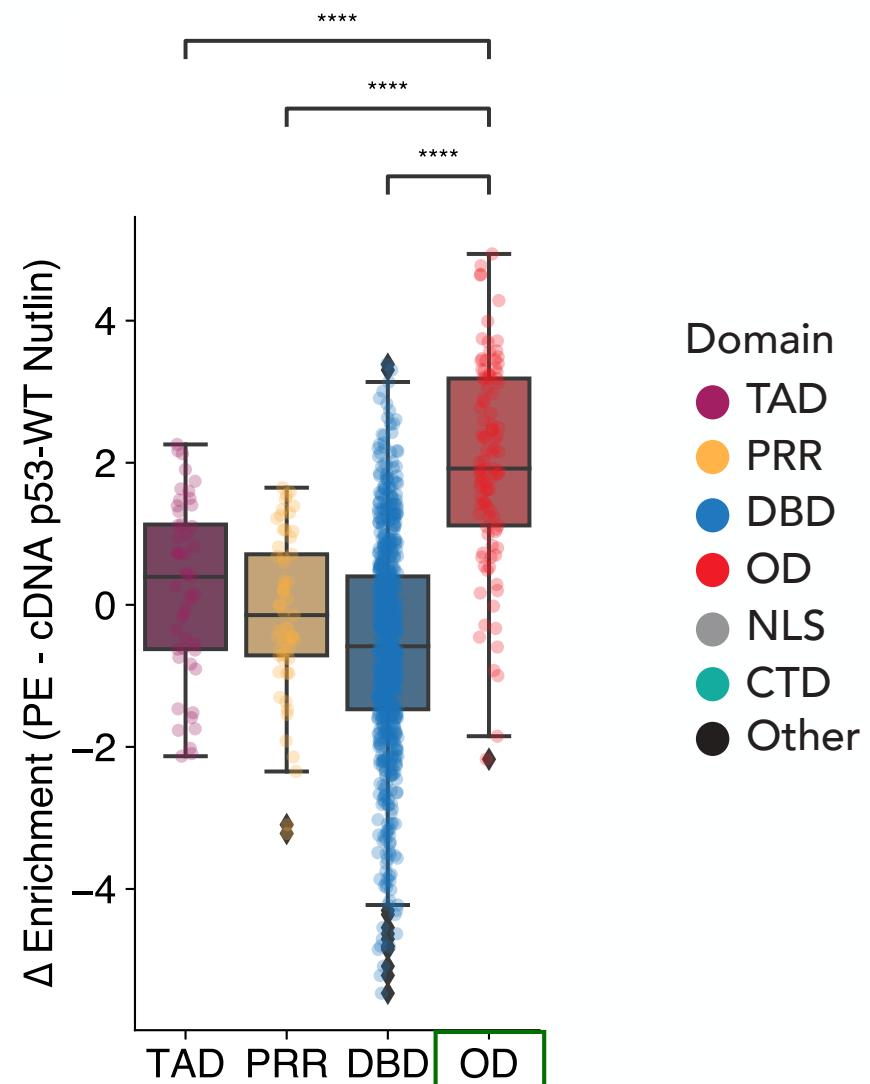


Comparative analysis of prime editing and cDNA screening datasets reveals pathogenic variants in the p53 oligomerization domain



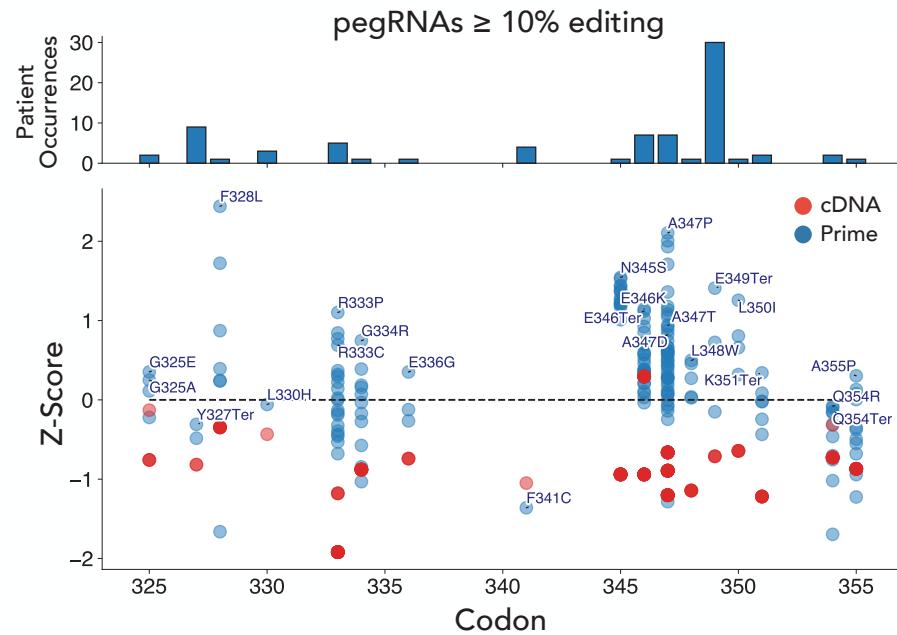
Giacomelli et al. Nat Genetics (2018)

≥ 30% editing

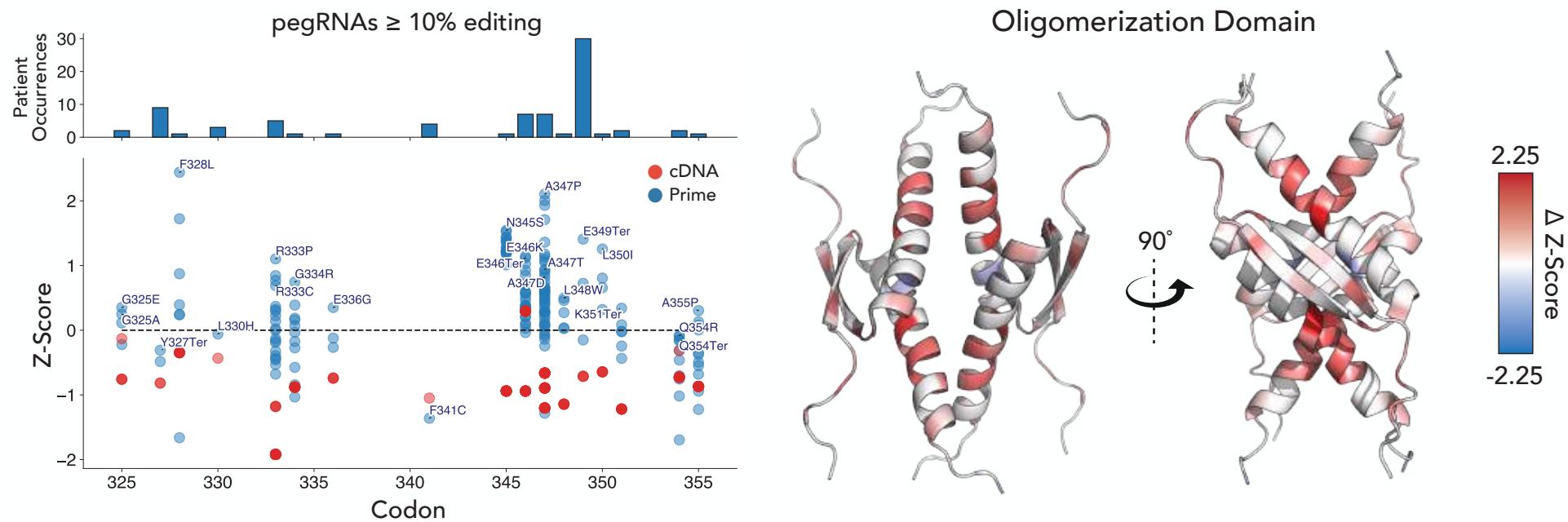


Gould et al. comparative analysis

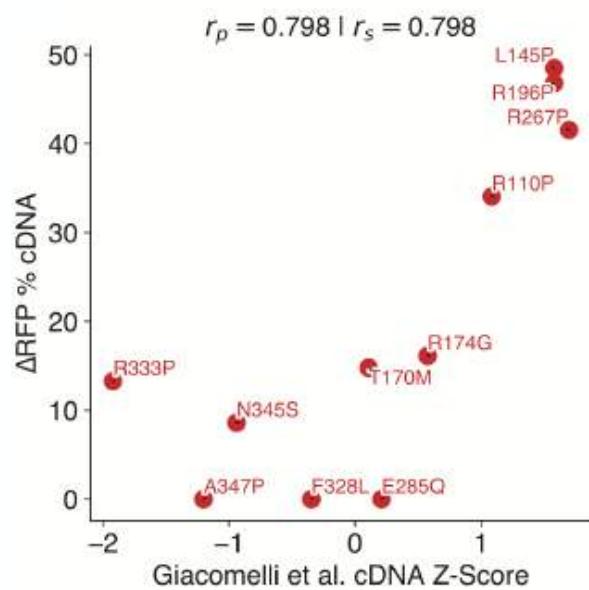
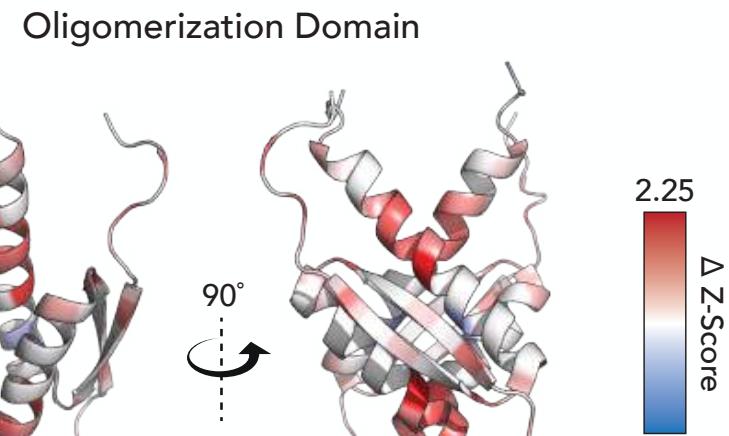
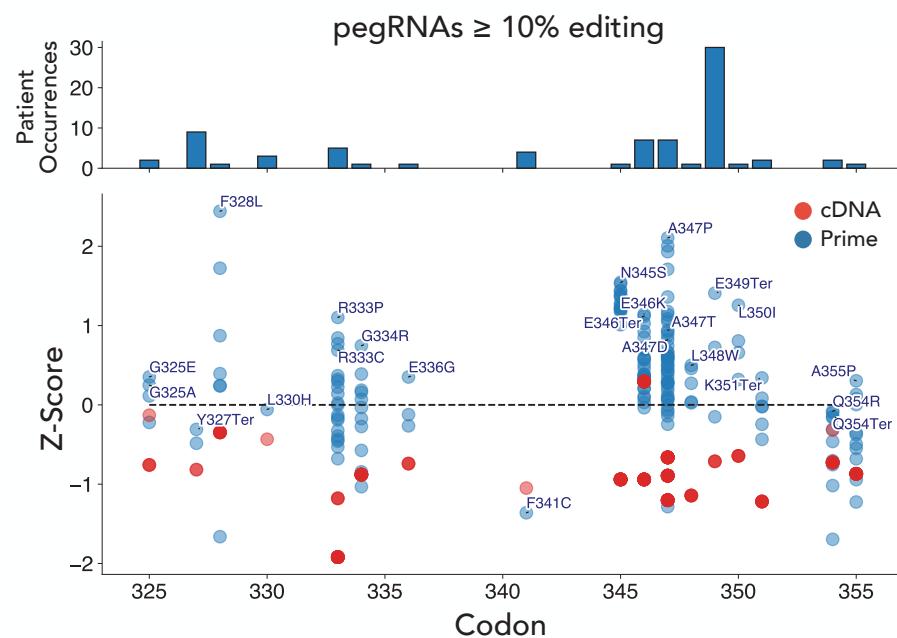
Comparative analysis of prime editing and cDNA screening datasets reveals pathogenic variants in the p53 oligomerization domain



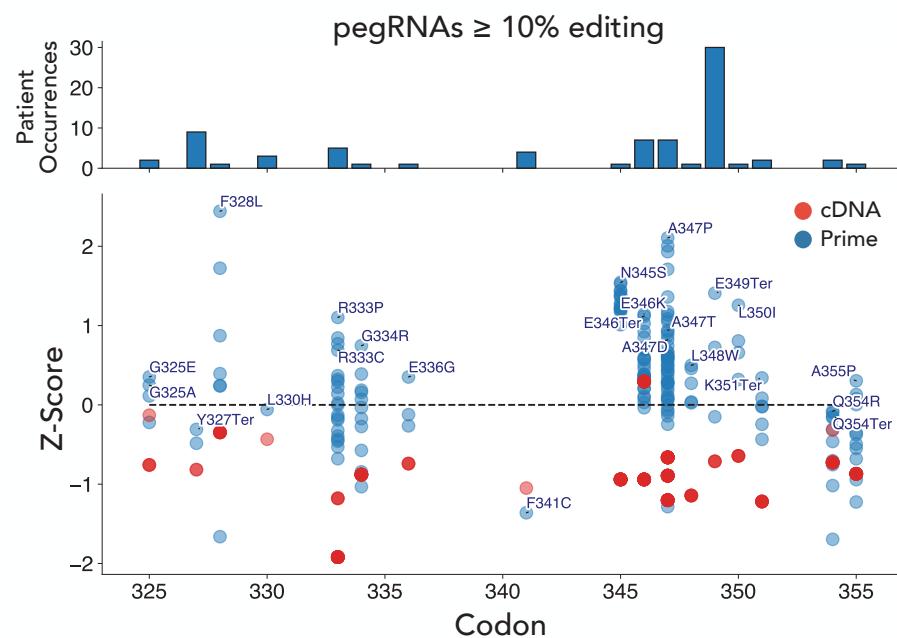
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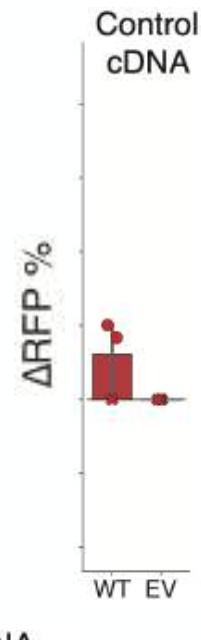
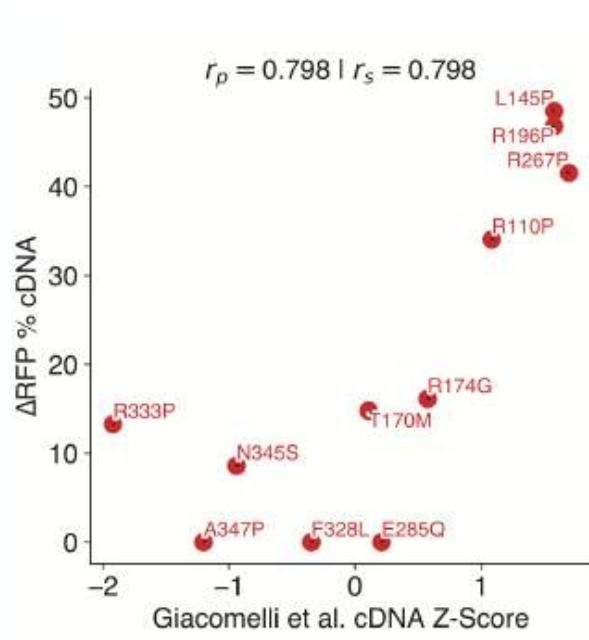
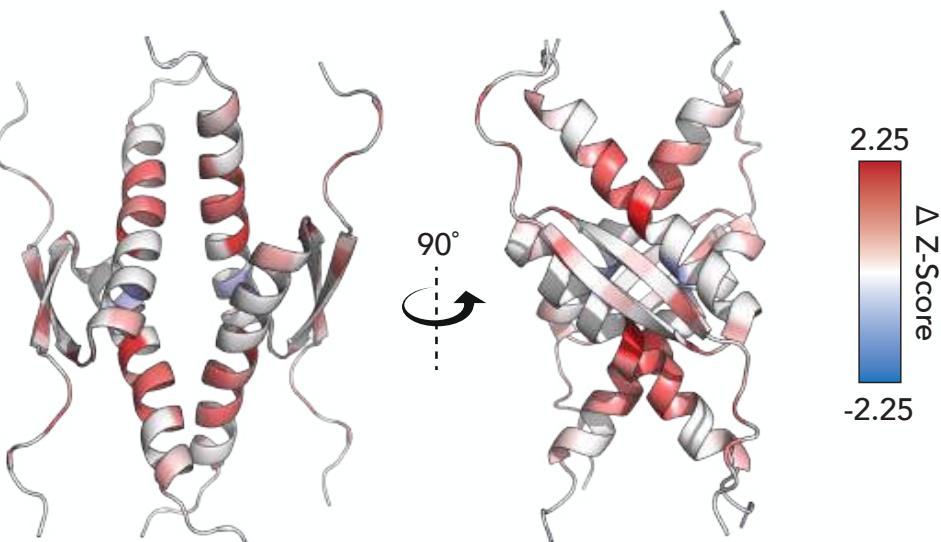
Comparative analysis of prime editing and cDNA screening datasets reveals pathogenic variants in the p53 oligomerization domain



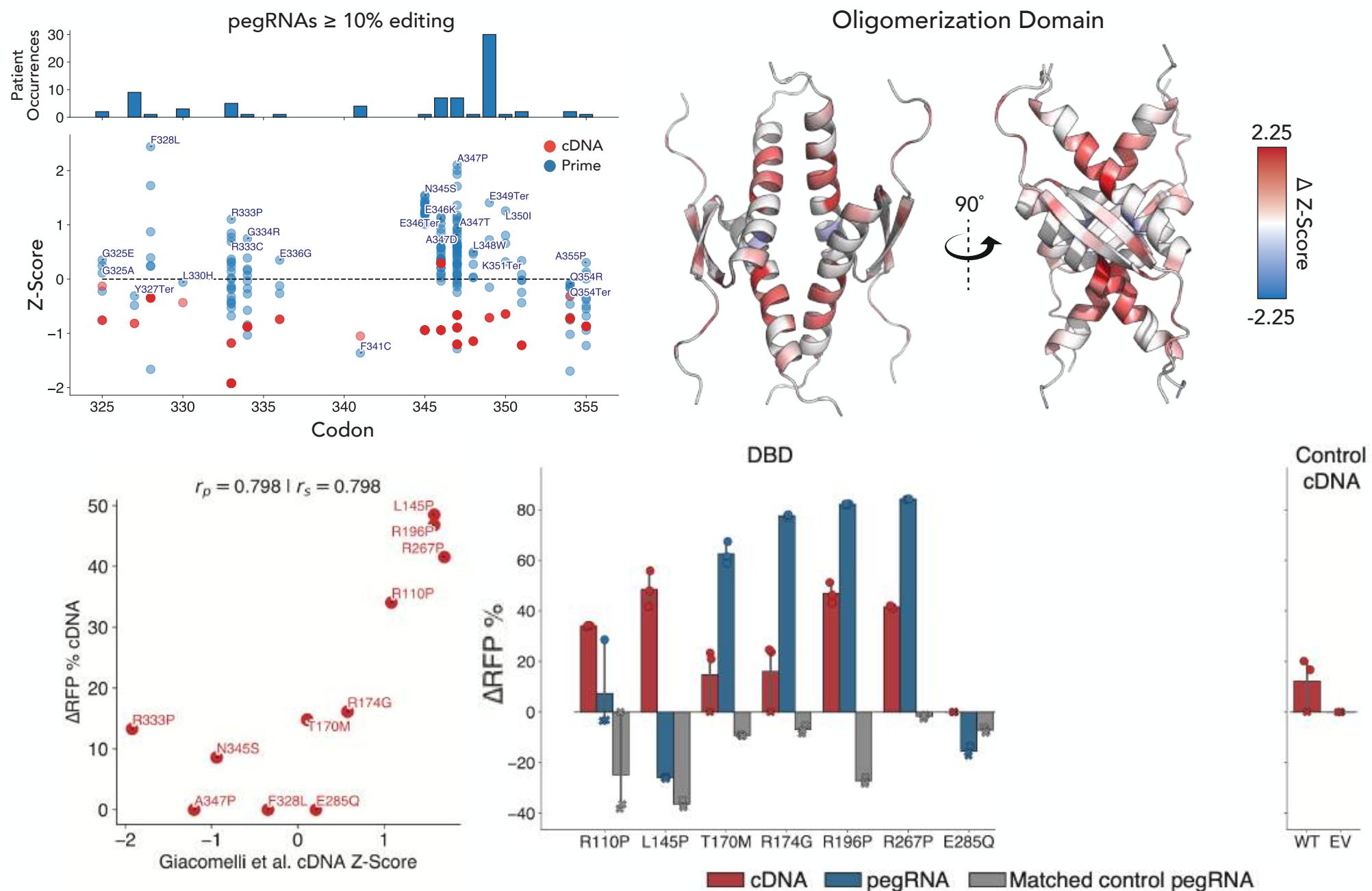
Comparative analysis of prime editing and cDNA screening datasets reveals pathogenic variants in the p53 oligomerization domain



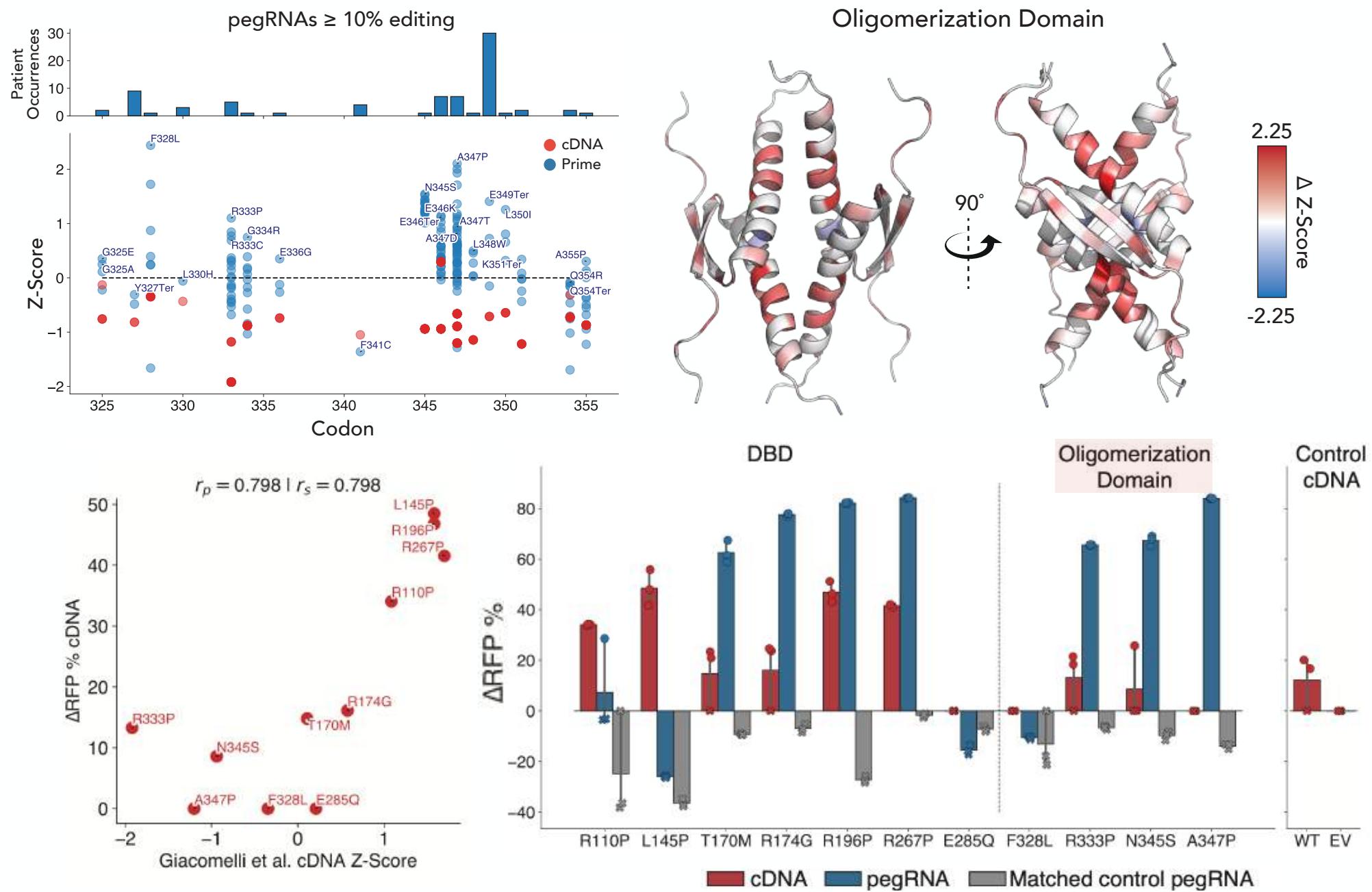
Oligomerization Domain



Comparative analysis of prime editing and cDNA screening datasets reveals pathogenic variants in the p53 oligomerization domain



Comparative analysis of prime editing and cDNA screening datasets reveals pathogenic variants in the p53 oligomerization domain



Comparative analysis of prime editing and cDNA screening datasets reveals pathogenic variants in the p53 oligomerization domain



Are these differences relevant to
human biology?



Mutations affecting the p53 OD are frequently observed in cancer patients and individuals with Li-Fraumeni Syndrome

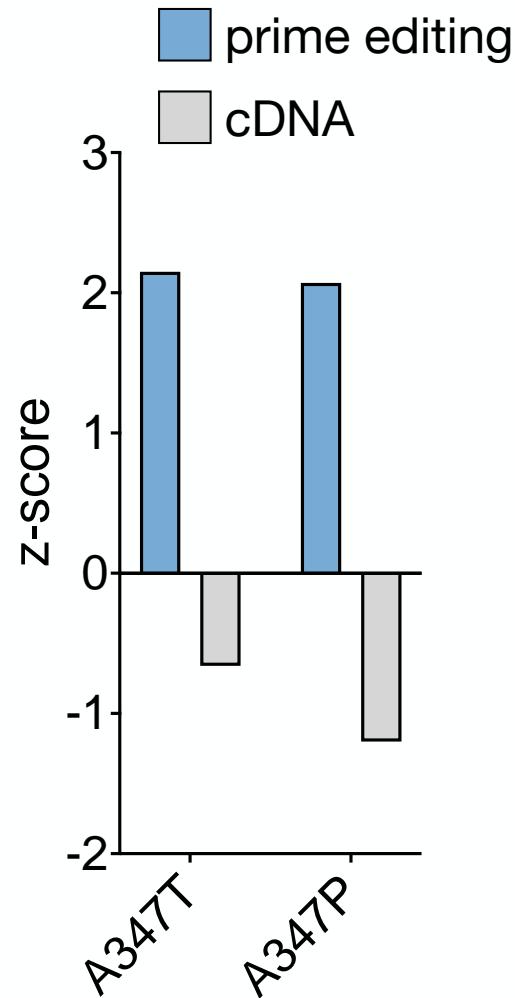
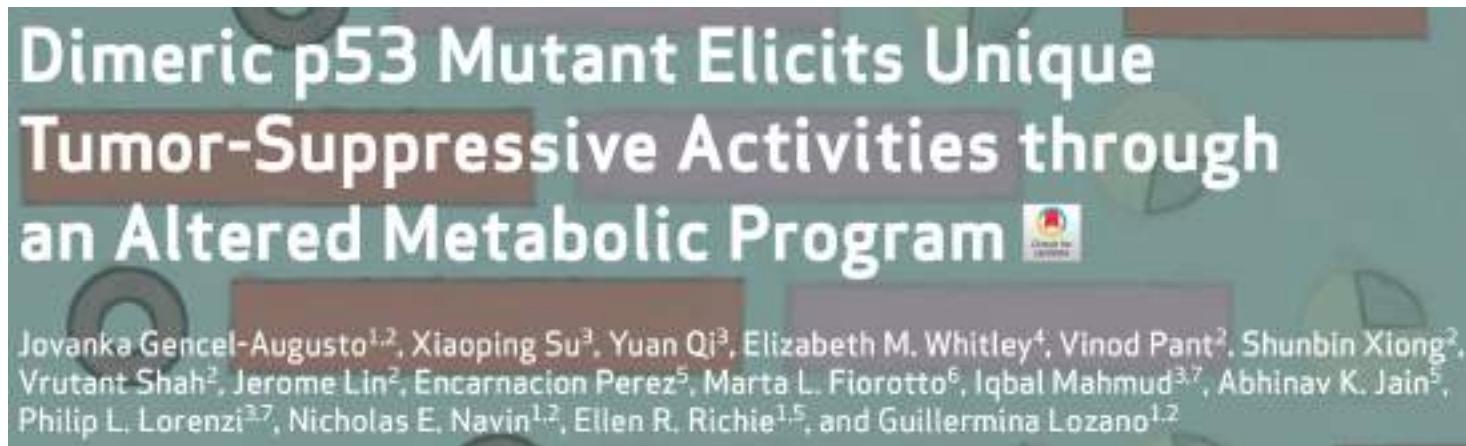
Li-Fraumeni Syndrome-Associated Dimer-Forming Mutant p53 Promotes Transactivation-Independent Mitochondrial Cell Death

Joshua H. Choe¹, Tatsuya Kawase^{1,2}, An Xu³, Asja Guzman¹, Aleksandar Z. Obradovic^{4,5}, Ana Maria Low-Calle¹, Bita Alaghebandan¹, Ananya Raghavan¹, Kaitlin Long¹, Paul M. Hwang⁶, Joshua D. Schiffman^{7,8}, Yan Zhu⁹, Ruiying Zhao³, Dung-Fang Lee^{3,10,11,12}, Chen Katz¹, and Carol Prives¹

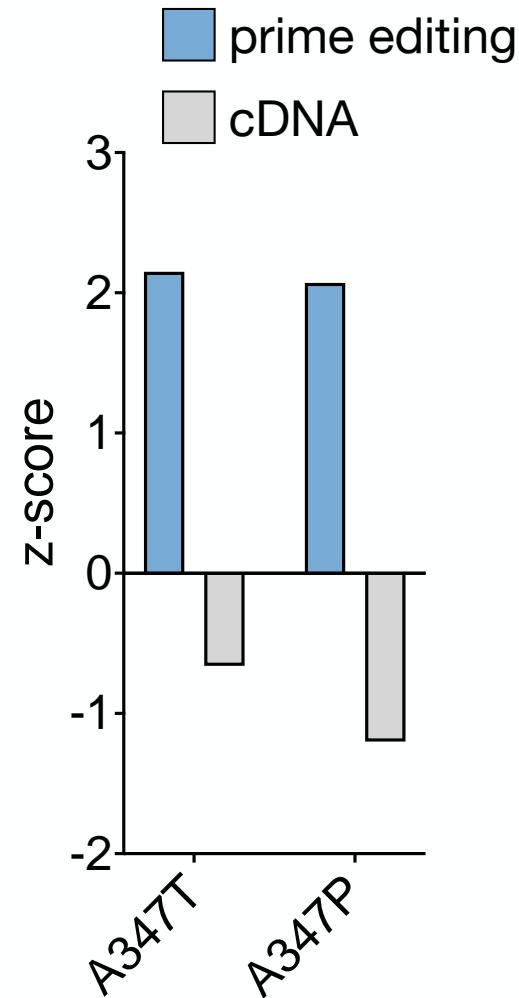
Dimeric p53 Mutant Elicits Unique Tumor-Suppressive Activities through an Altered Metabolic Program

Jovanka Gencel-Augusto^{1,2}, Xiaoping Su³, Yuan Qi³, Elizabeth M. Whitley⁴, Vinod Pant², Shunbin Xiong², Vrutant Shah², Jerome Lin², Encarnacion Perez⁵, Marta L. Fiorotto⁶, Iqbal Mahmud^{3,7}, Abhinav K. Jain⁵, Philip L. Lorenzi^{3,7}, Nicholas E. Navin^{1,2}, Ellen R. Richie^{1,5}, and Guillermo Lozano^{1,2}

Mutations affecting the p53 OD are frequently observed in cancer patients and individuals with Li-Fraumeni Syndrome



Mutations affecting the p53 OD are frequently observed in cancer patients and individuals with Li-Fraumeni Syndrome

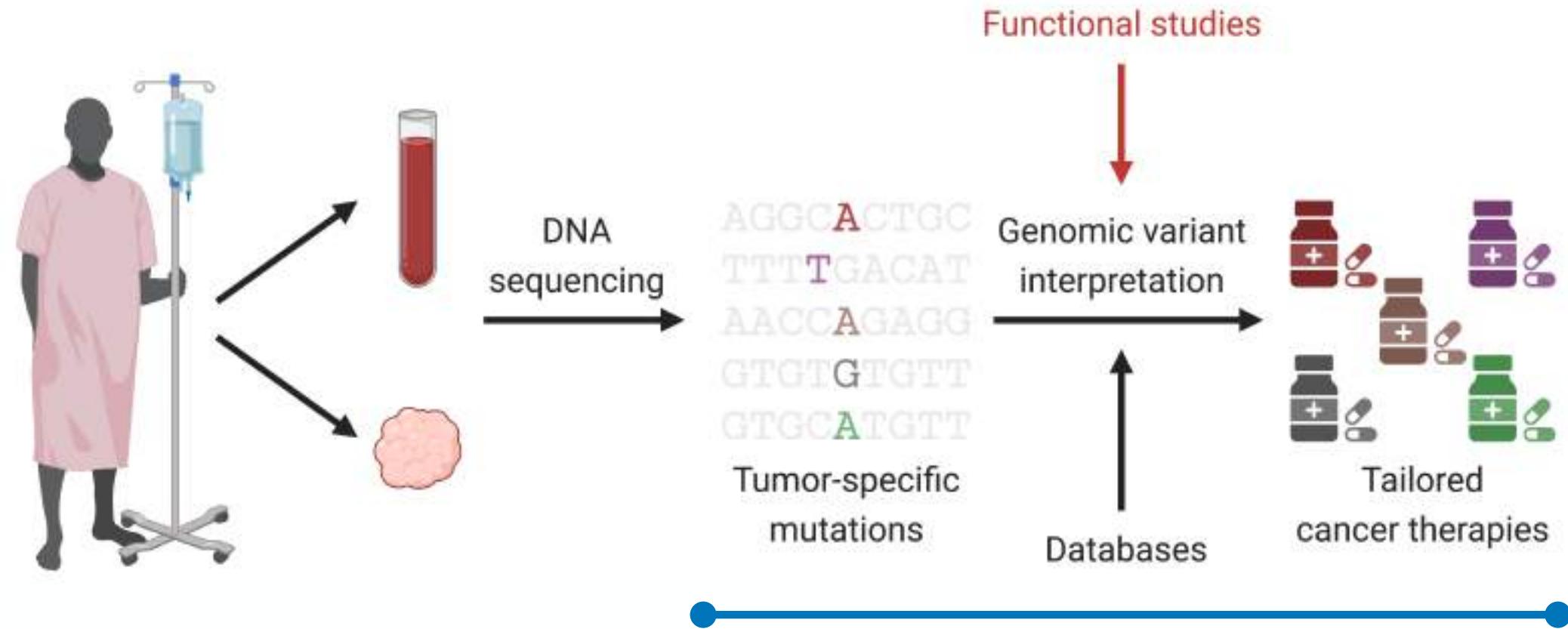


Mechanistic and structural basis → TP53 OD mutants act as obligate homodimers

Main takeaways

1. Physiological gene dosage, native protein stoichiometry, and protein-protein interactions are quite important.
2. There is significant functional heterogeneity in the mutant *TP53* allelic spectrum in Li-Fraumeni Syndrome and cancer.
3. This concept likely applies much more broadly beyond *TP53*.
4. We are now systematically expanding this approach in cancer.

Precision genome editing can link genetic variants with function



Precision genome editing

- Every cancer patient is unique
- Variant function can be shaped by context
- Next-gen drugs should exploit this knowledge

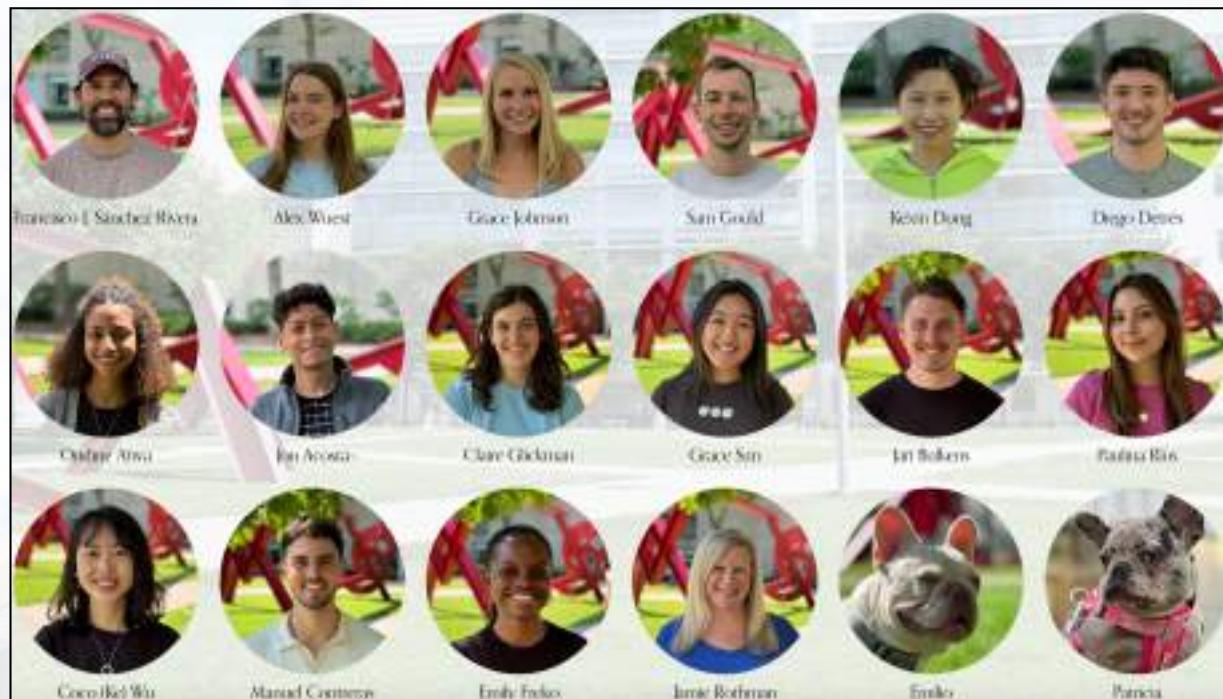
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Jamie Rothman; Administrative Assistant
Paulina Ríos; PhD Student
Grace Sun; Undergraduate Student
Coco (Ke) Wu; PhD Student; Co-Advised With Anders Hansen
Alexandra Wuest; Research Technician

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Calo Lab (MIT/Koch)
Dow Lab (Bianca Diaz, Alyna Katti)
Ed Reznik & Chai Bandlamudi (MSK)
Faltas Lab (Weill Cornell Medicine)
Hansen Lab (MIT/Koch)
Hemann Lab (MIT/Koch)
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KOCH INSTITUTE
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Research at MIT

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CENTER | MIT

Swanson Biotechnology Center
KOCH INSTITUTE CORE FACILITIES

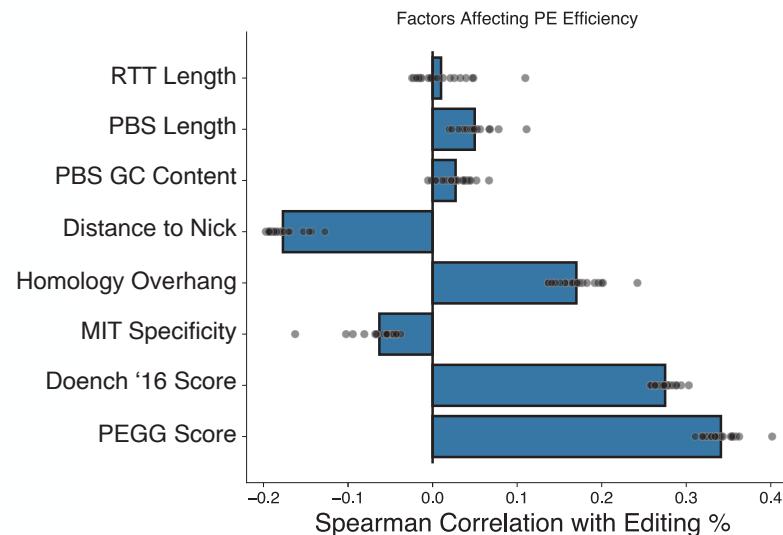
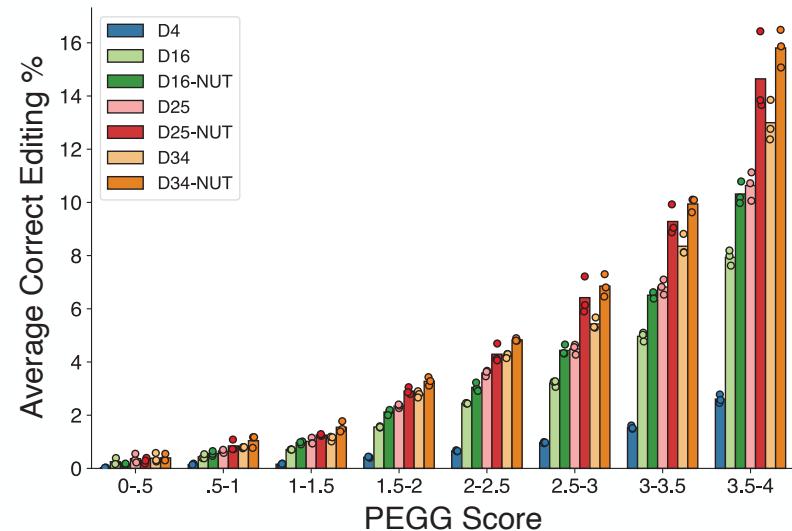
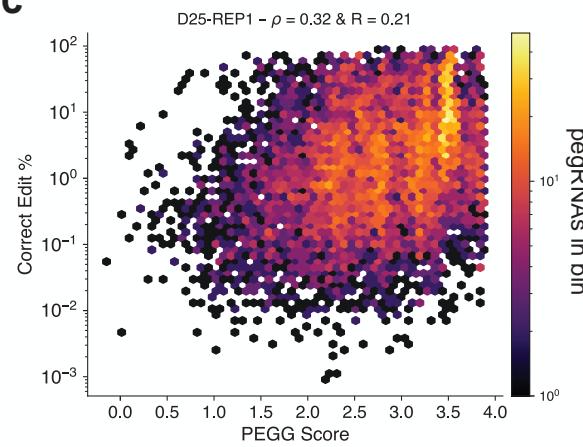
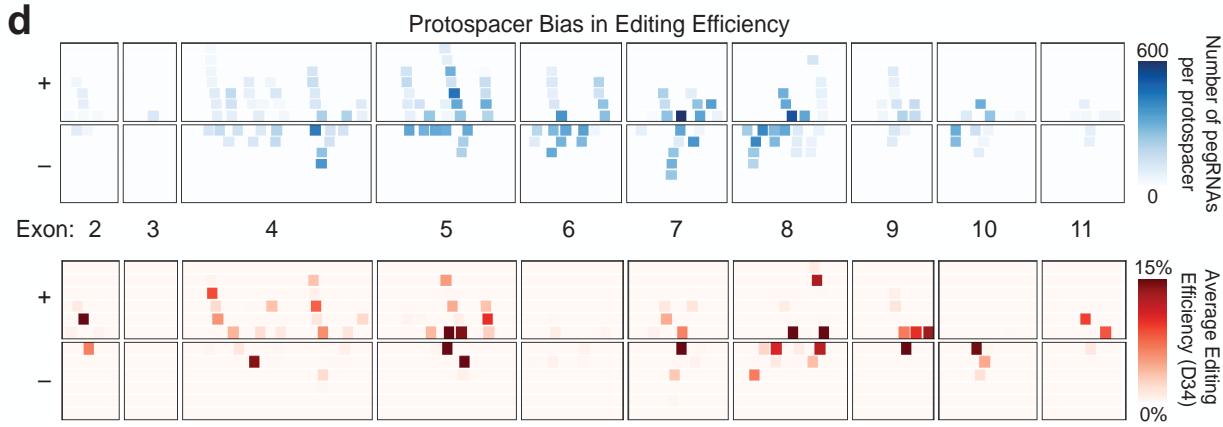
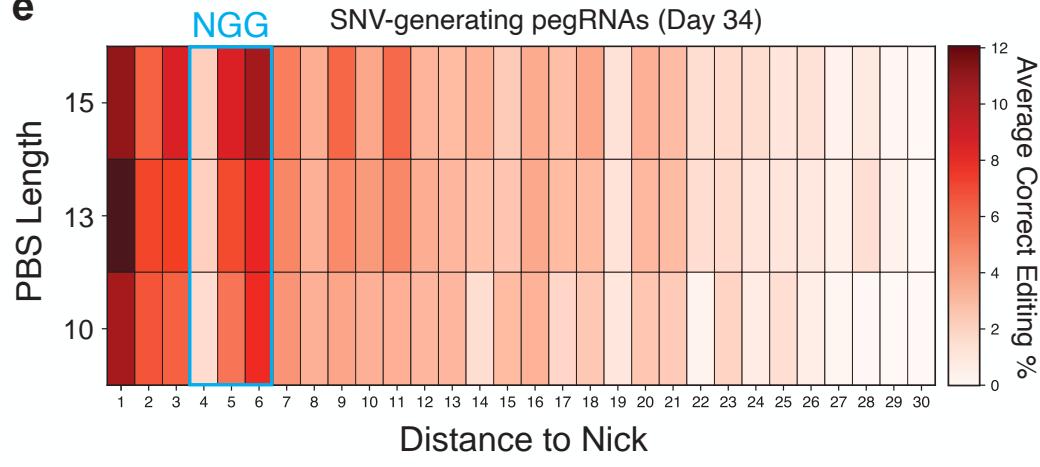
FRONTIER RESEARCH PROGRAM



Gould et al. Nat Biotech 2024

pegg.readthedocs.io

PEGG: Prime Editing Guide Generator

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