

Applications of base editing and prime editing

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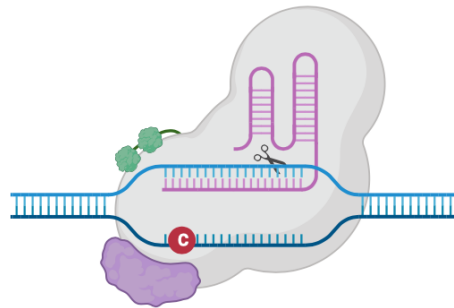
The variant to function problem

- Genome sequencing is uncovering DNA variants implicated in human disease at an increasing rate
- Linking DNA variants to their function is a major challenge, both clinically and for our foundational understanding of genetic disease
- This is particularly problematic for missense and non-coding variants

Fowler *et al*, *Genome Biology*, 2023 – Atlas of Variant Effects Alliance

Functional genomics approach

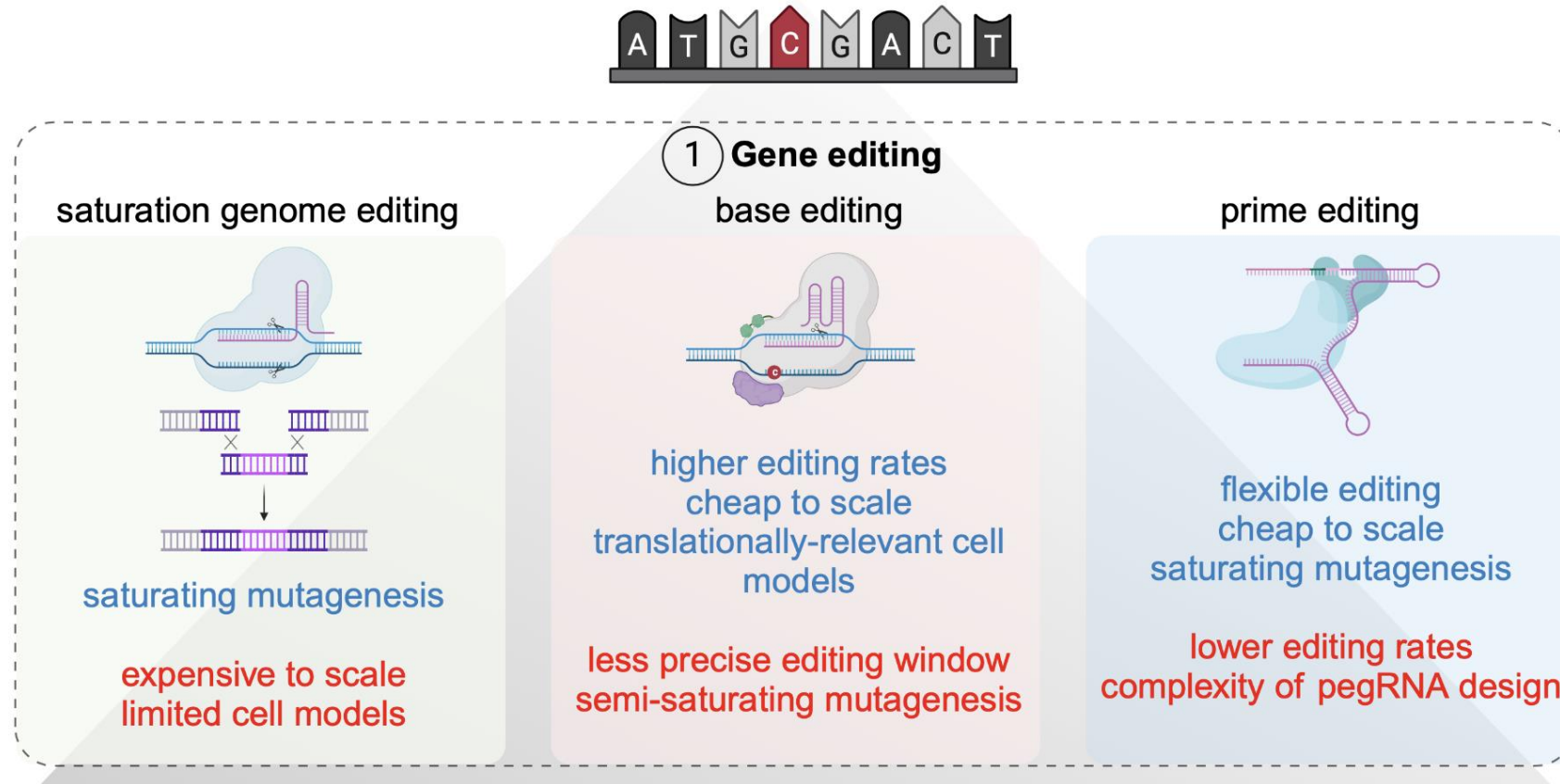
Build a map of variant effect in cancer to help translate genomics data into better treatments for patients



gene editing:

- *scalable*
- *rapid*
- *functional*
- *sensitive*
- *mechanistic insight*

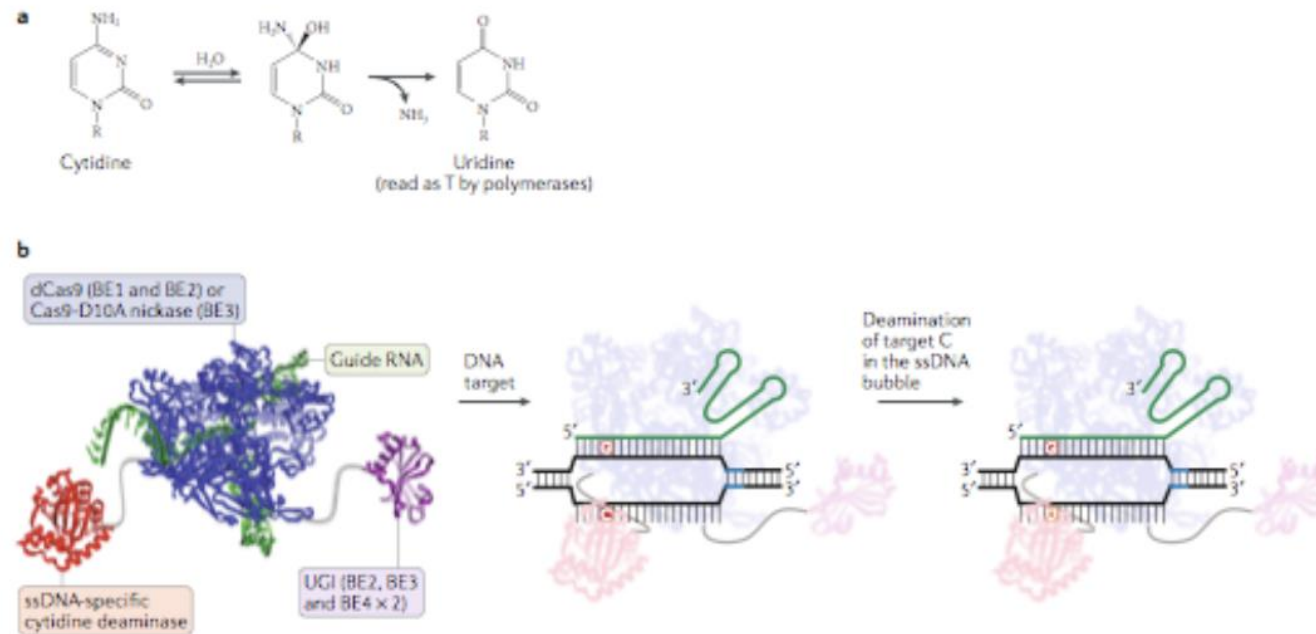
Functional interrogation of DNA variants at scale



Cooper, Obolenski, Waters, Bassett* & **Coelho***, *Cell Reports Methods*, 2024

Base editing

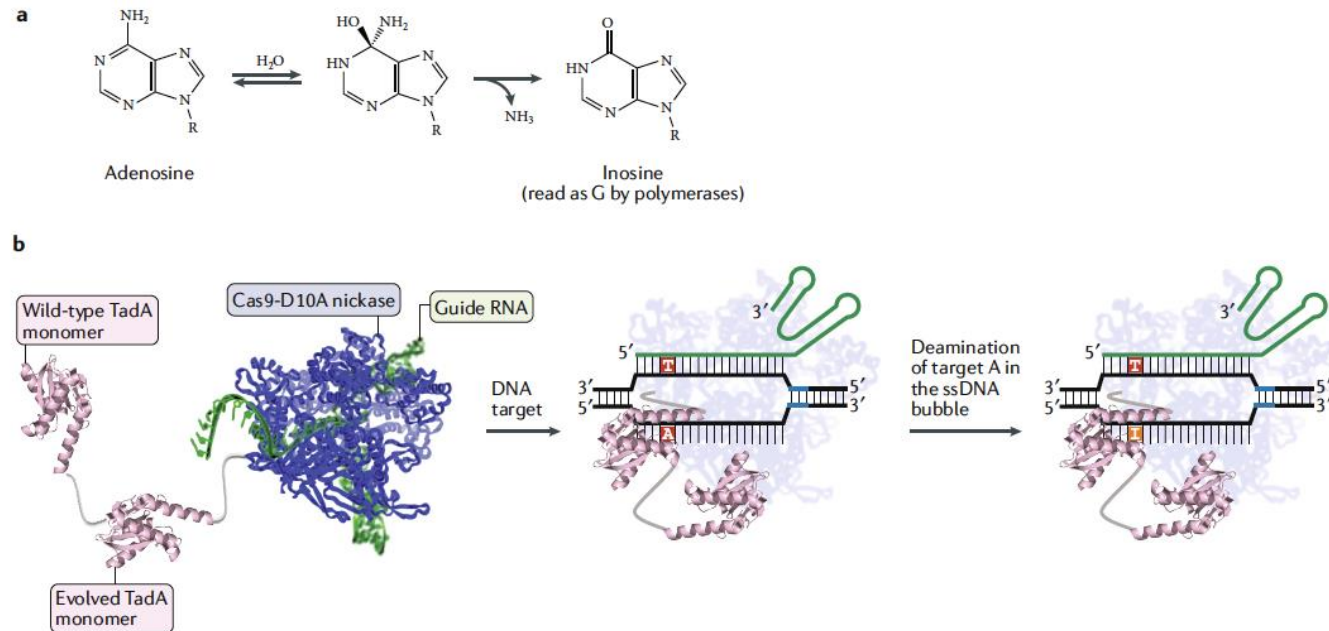
Base Editing (Cytosine; CBE)



Rees and Liu, Nature Reviews Genetic (2018)

Base editing

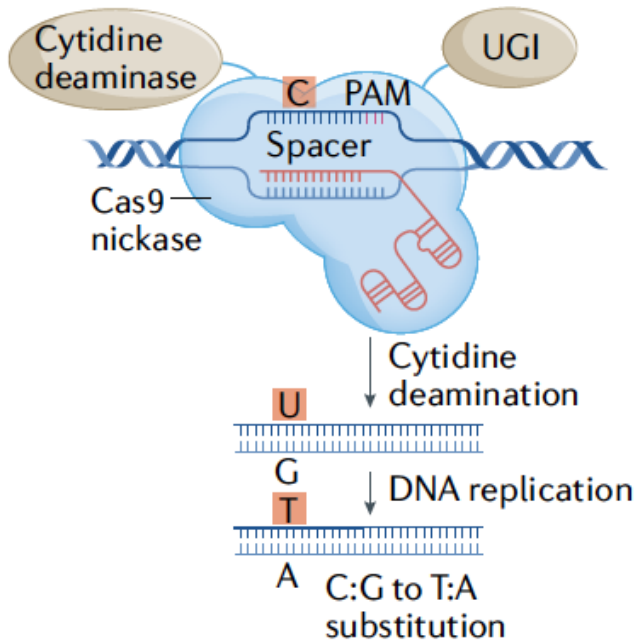
Base Editing (Adenine; ABE)



Rees and Liu, Nature Reviews Genetic (2018)

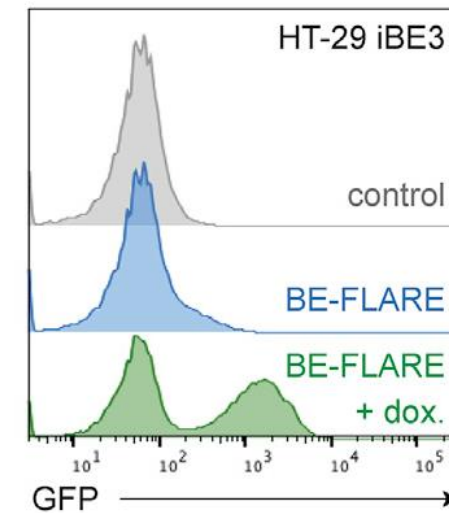
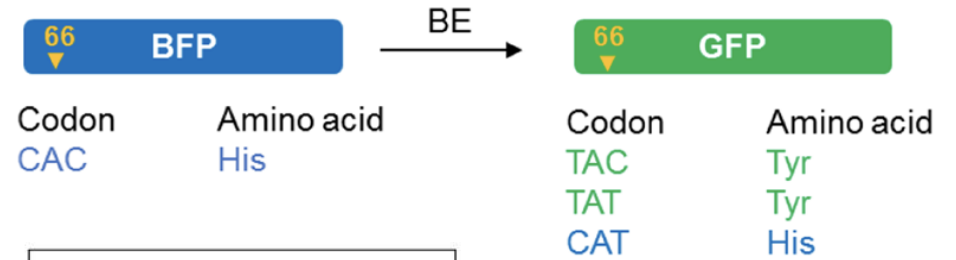
Base editing

CRISPR base editing



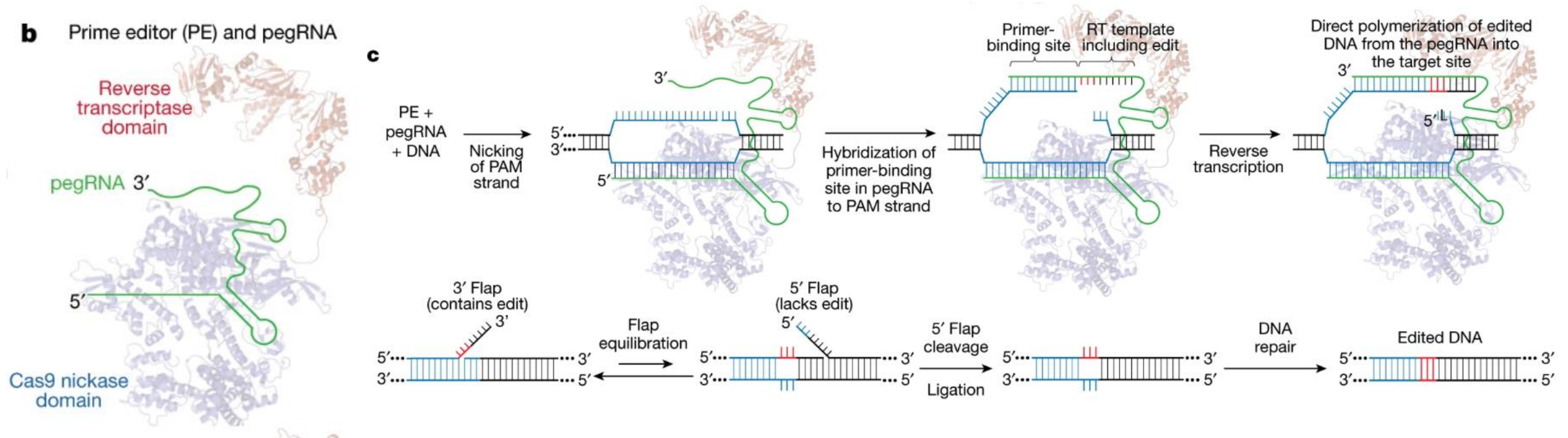
Bock, Datlinger, Chardon, **Coelho** *et al*,
Nature Reviews Methods Primer, 2022

BE-FLARE base editing activity reporter



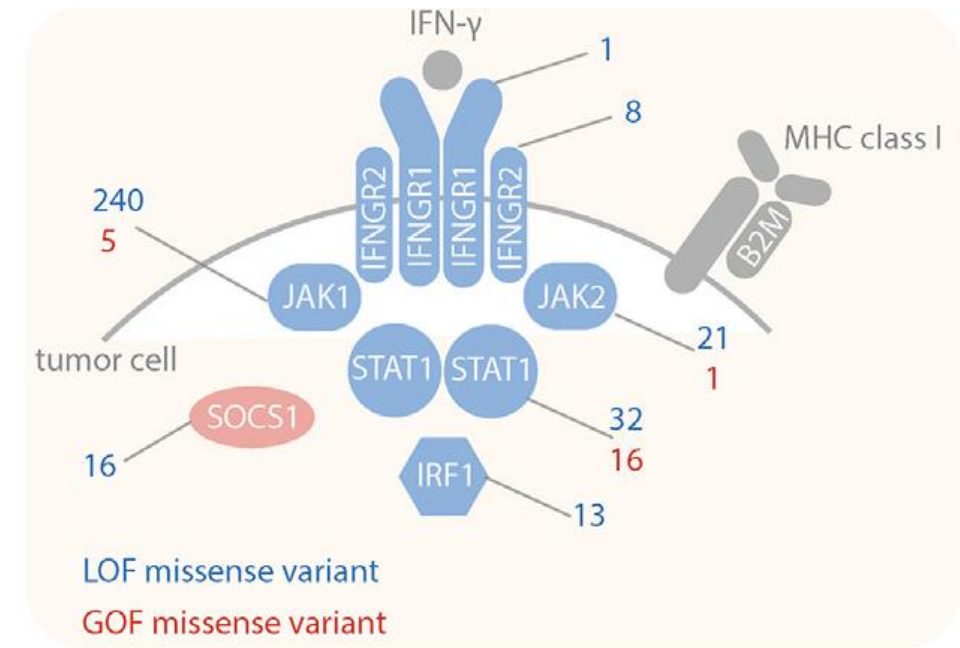
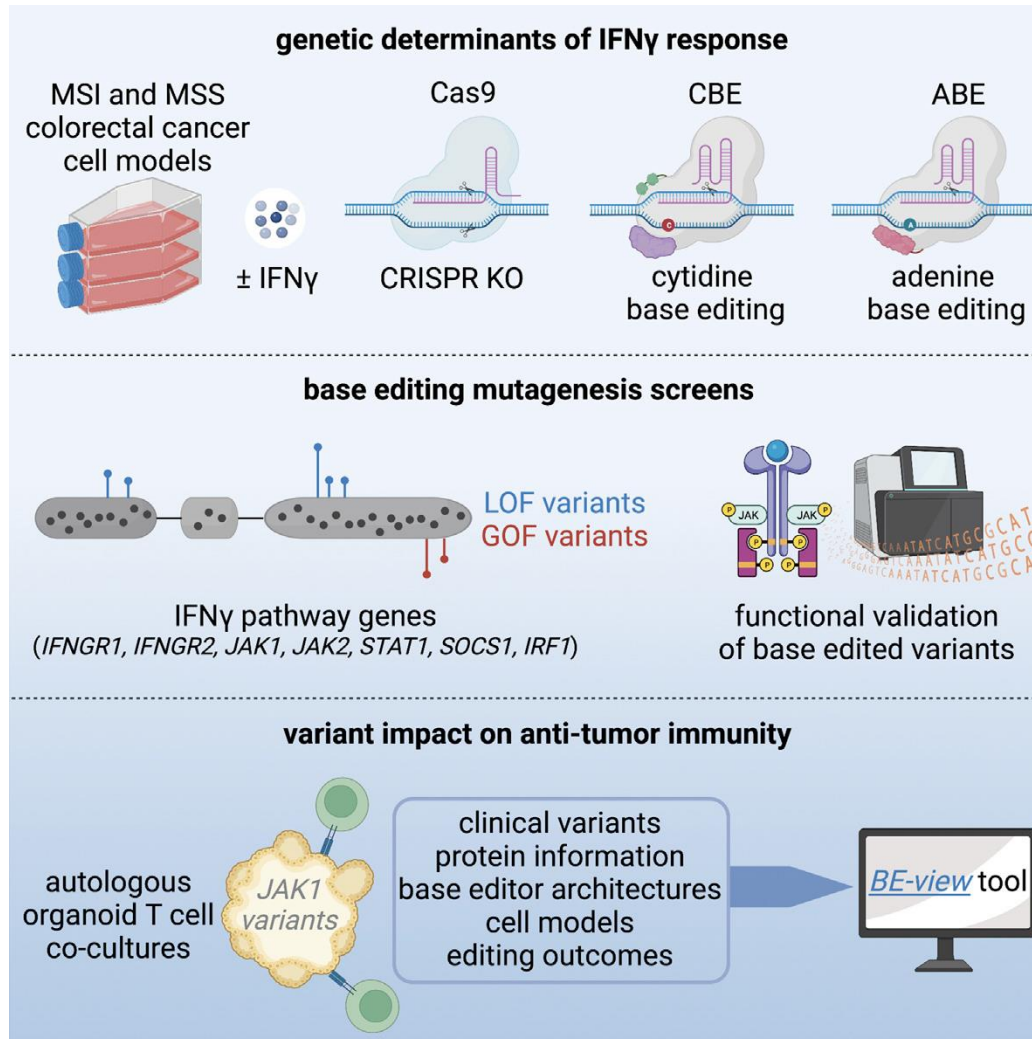
Coelho^{*} *et al*, BMC Biology, 2018

Prime editing



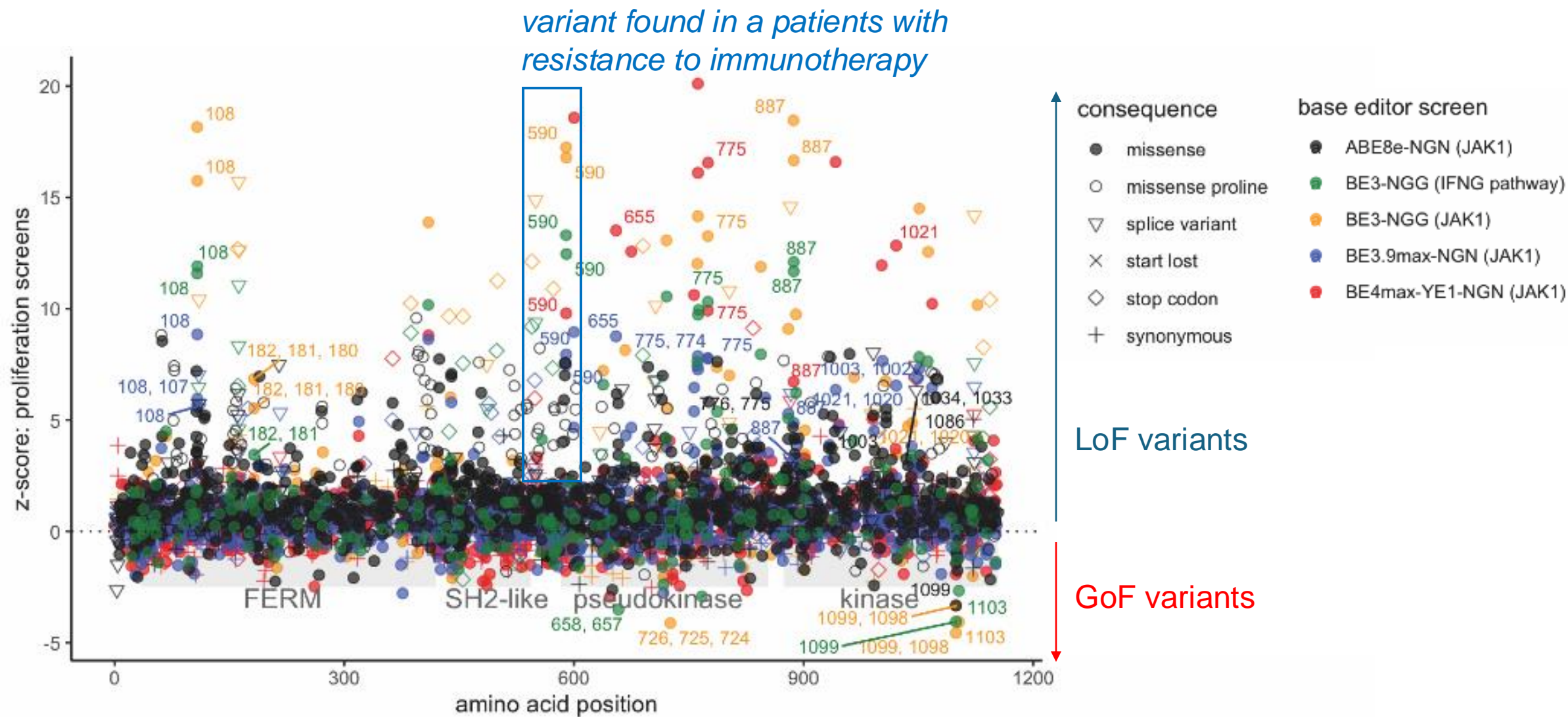
Anzalone, *Nature*, 2019

Pathway-level interrogation of variants affecting IFN- γ signaling

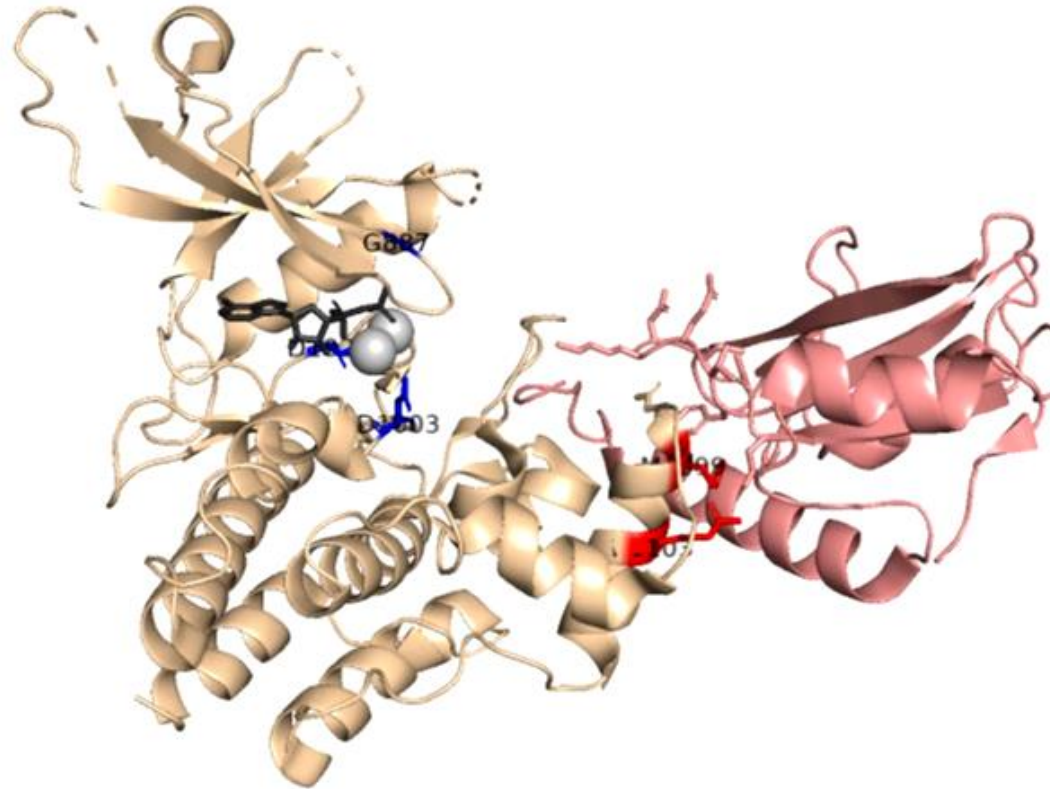


Coelho et al, Cancer Cell, 2023

Deep mutagenesis of *JAK1* using base editing



Mechanism of action of JAK1 LoF and GoF variants

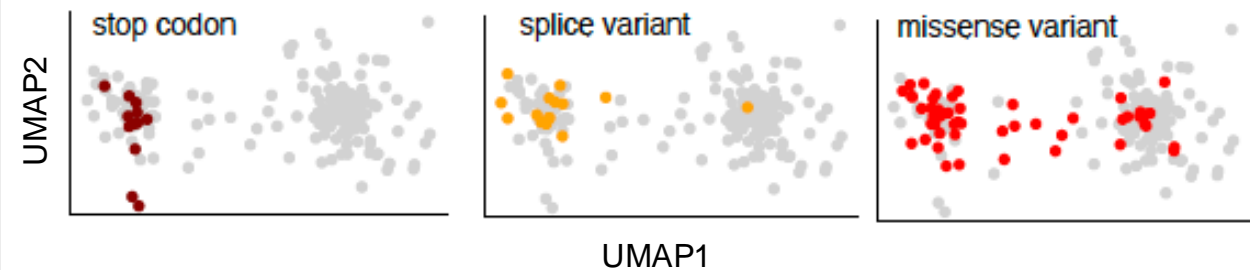
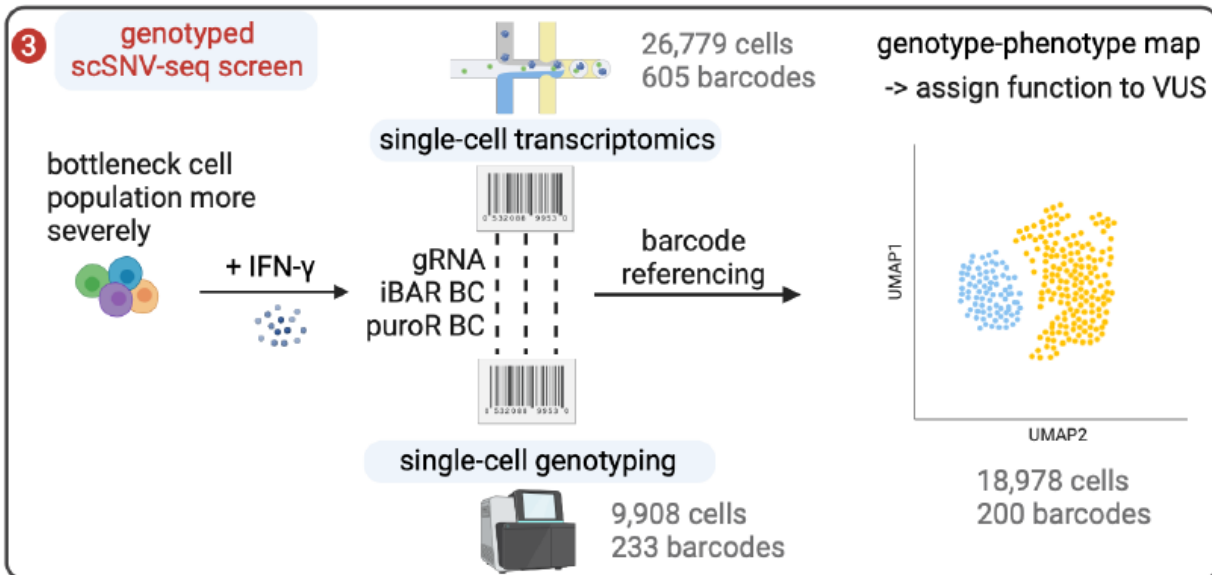
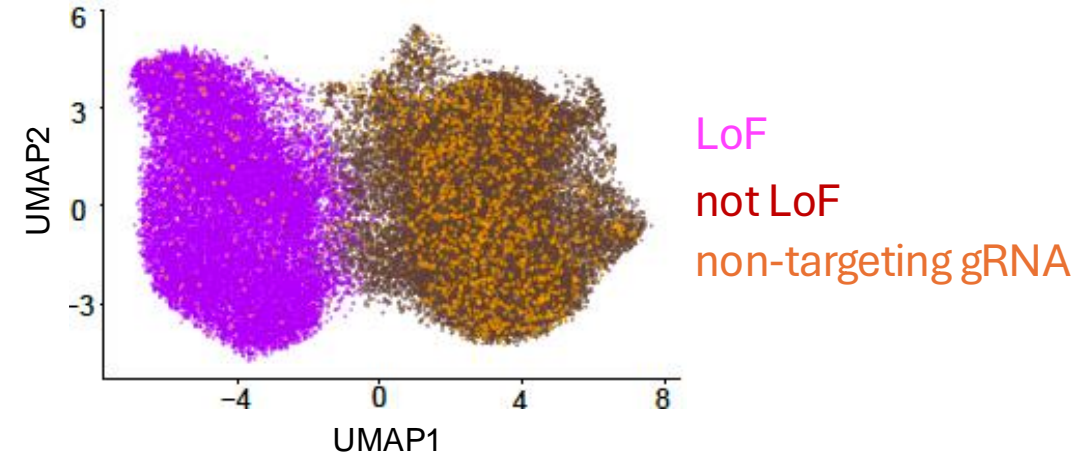
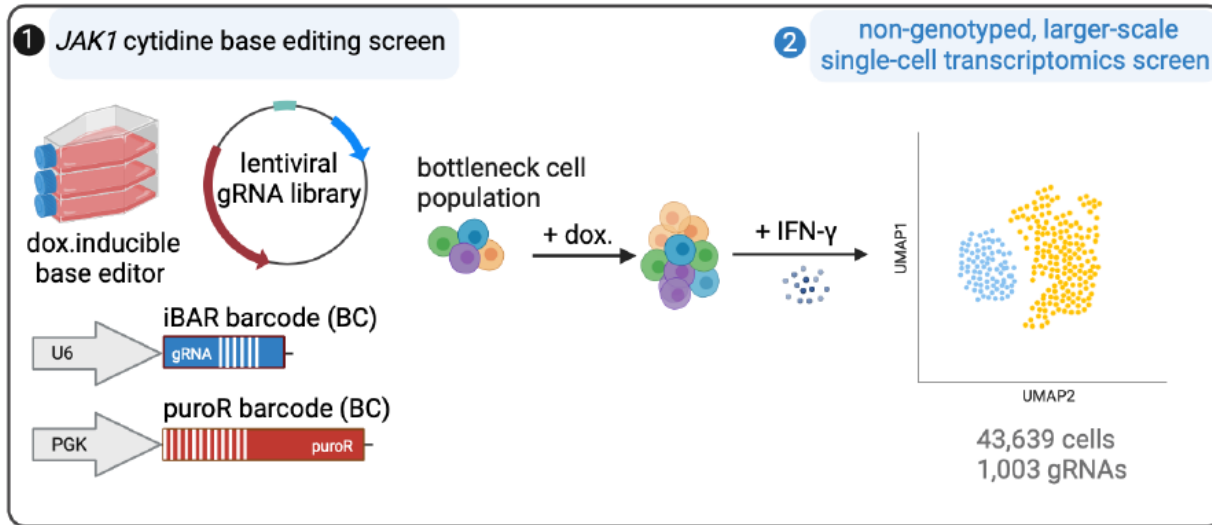


GOF
LOF
JAK1 (human)
SOCS1 (chicken)
ADP
Mg

PDB: 6C7Y

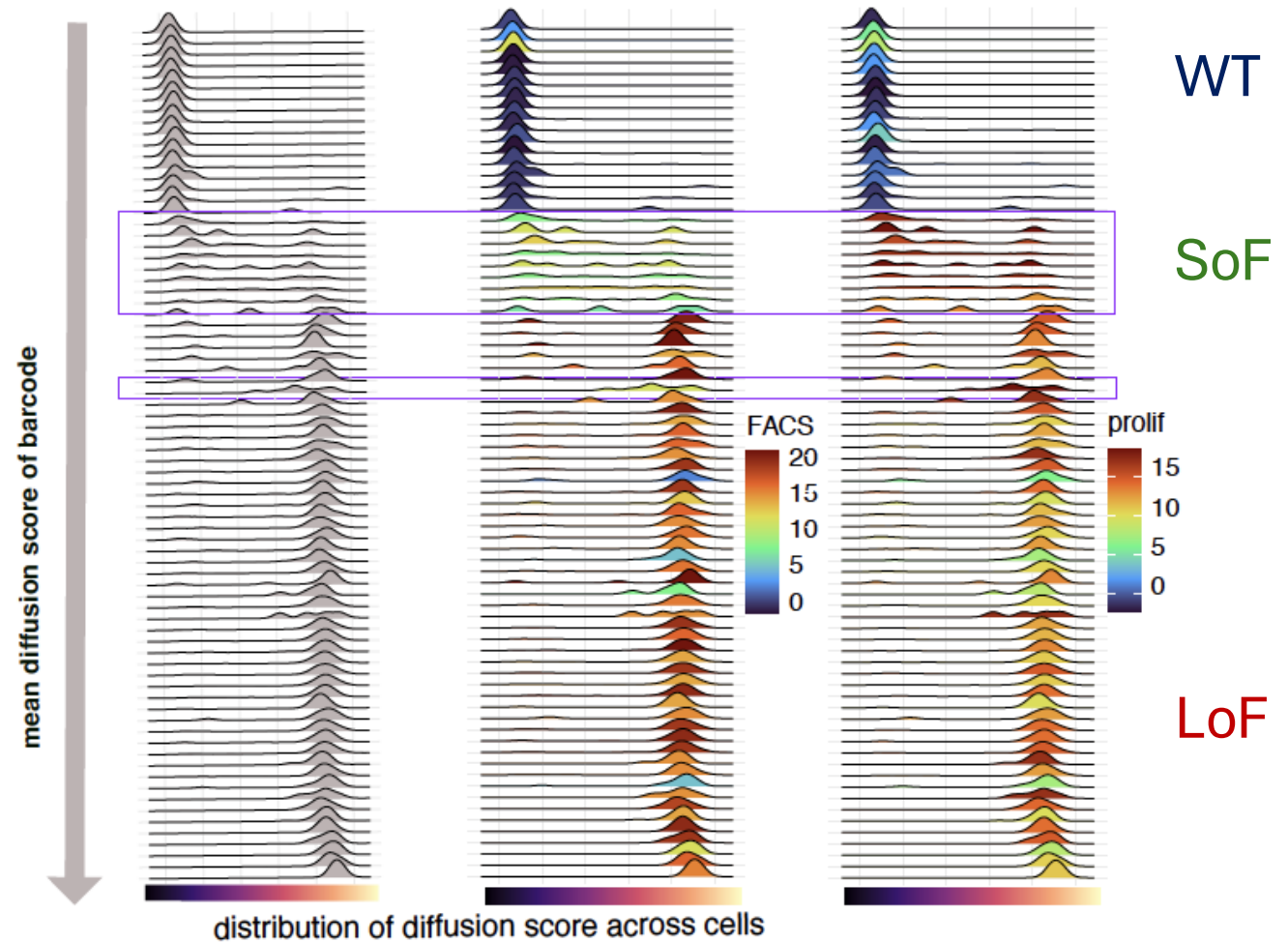
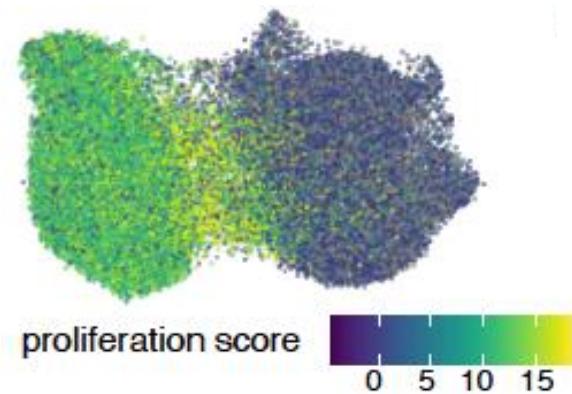
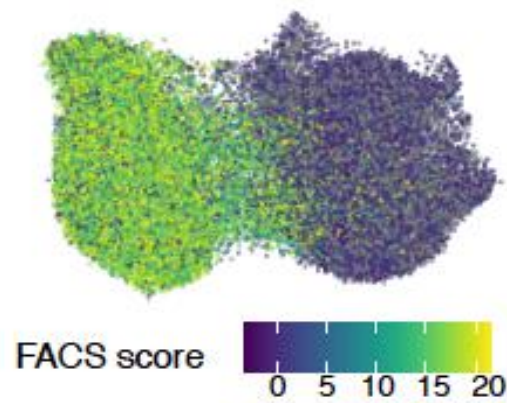
see also WRN base editing
Picco et al, *Cancer Discovery*, 2024

Transcriptomic readouts of variant effects in single cells



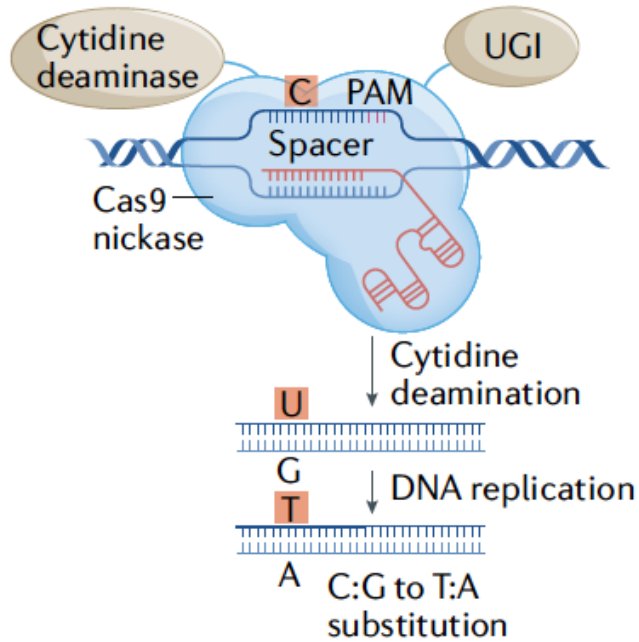
Cooper[‡], **Coelho[‡]**, Strauss[‡] *et al*, *Genome Biology*, 2024

scSNV-seq reveals cellular transcriptional heterogeneity



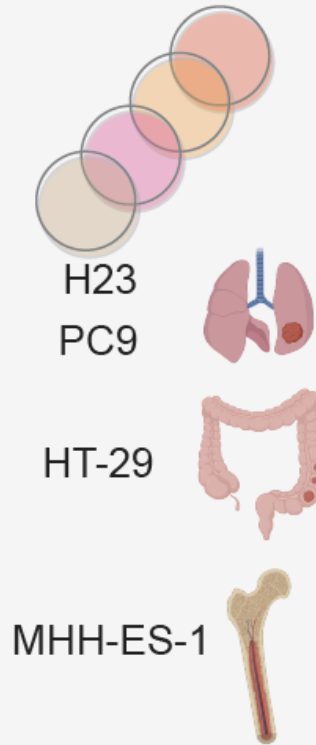
Base editing defines the genetic landscape of cancer drug resistance mechanisms

CRISPR base editing



Bock, Datlinger, Chardon, Coelho *et al*,
Nature Reviews Methods Primer, 2022

cancer cell models



base editing screens

gRNA libraries
11 cancer genes

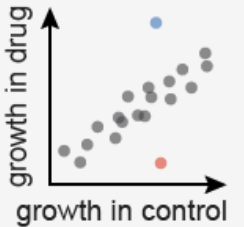
EGFR
KRAS
BRAF
MAP2K1
MAP2K2
PIK3CA
AKT1
MYC
BCL2
PARP1
PARP2

control

10 targeted drugs

Sotorasib
Adagrasib
Pictilisib
Trametinib
Cetuximab
Dabrafenib
Gefitinib
Osimertinib
Olaparib
Niraparib

proliferation

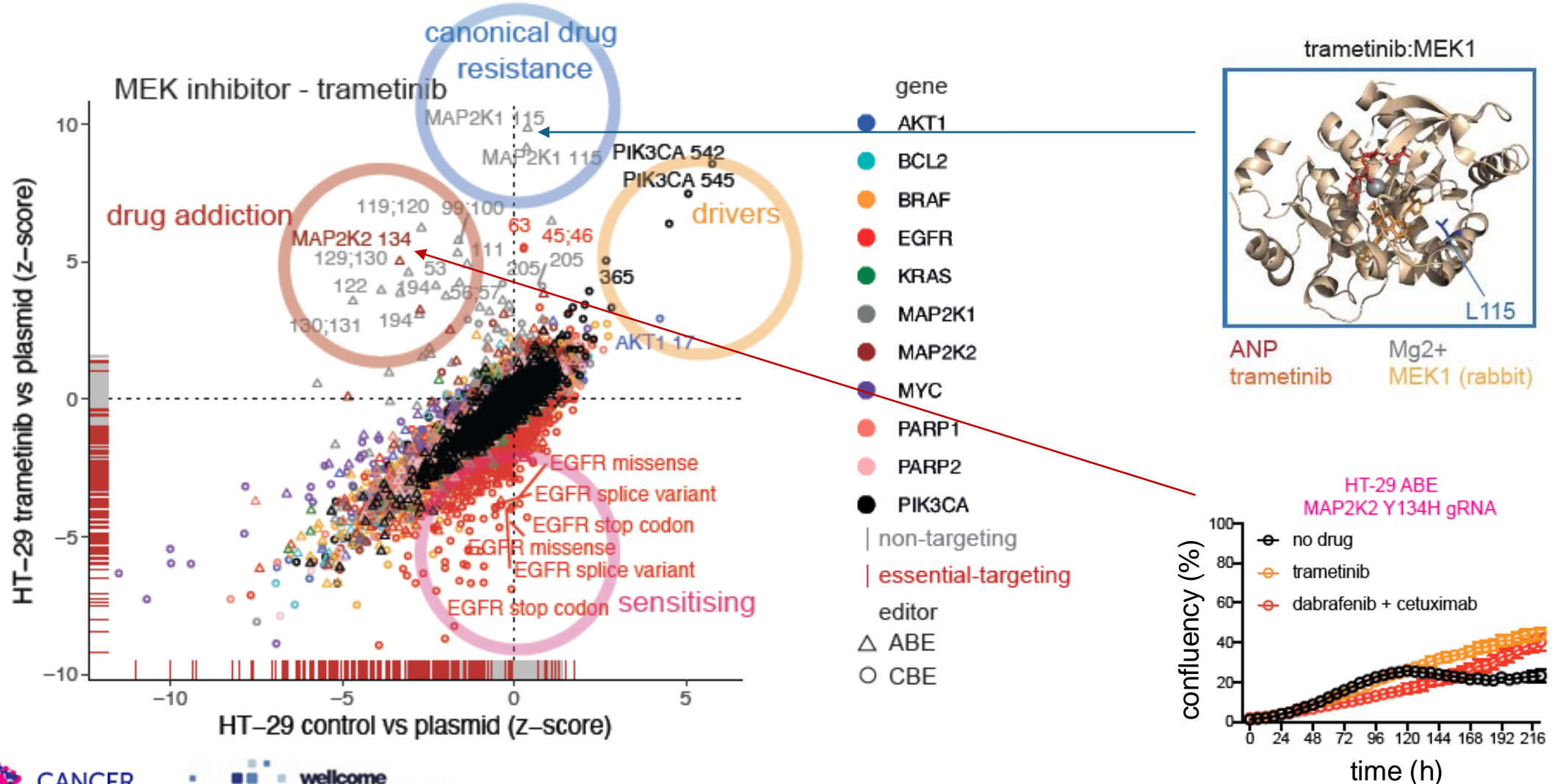


single-cell
transcriptomics

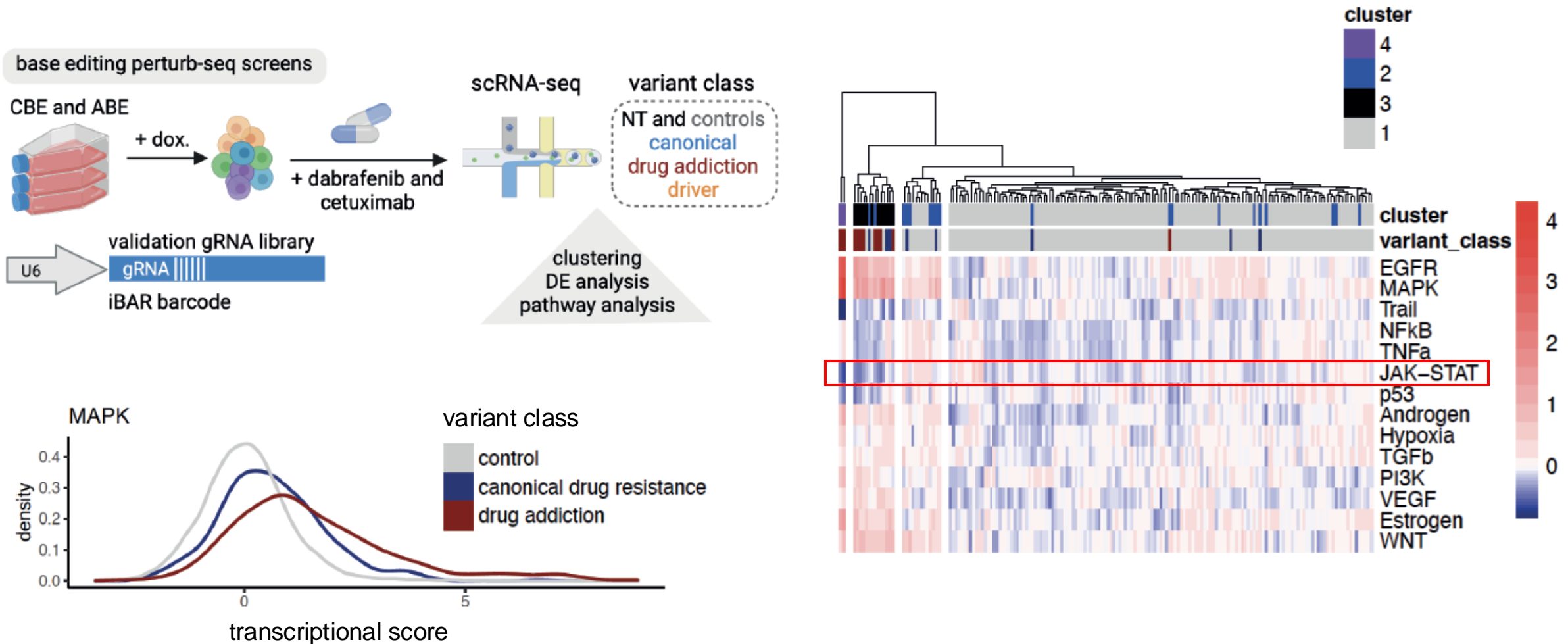
hits



Variants modulating drug sensitivity cluster into four functional classes

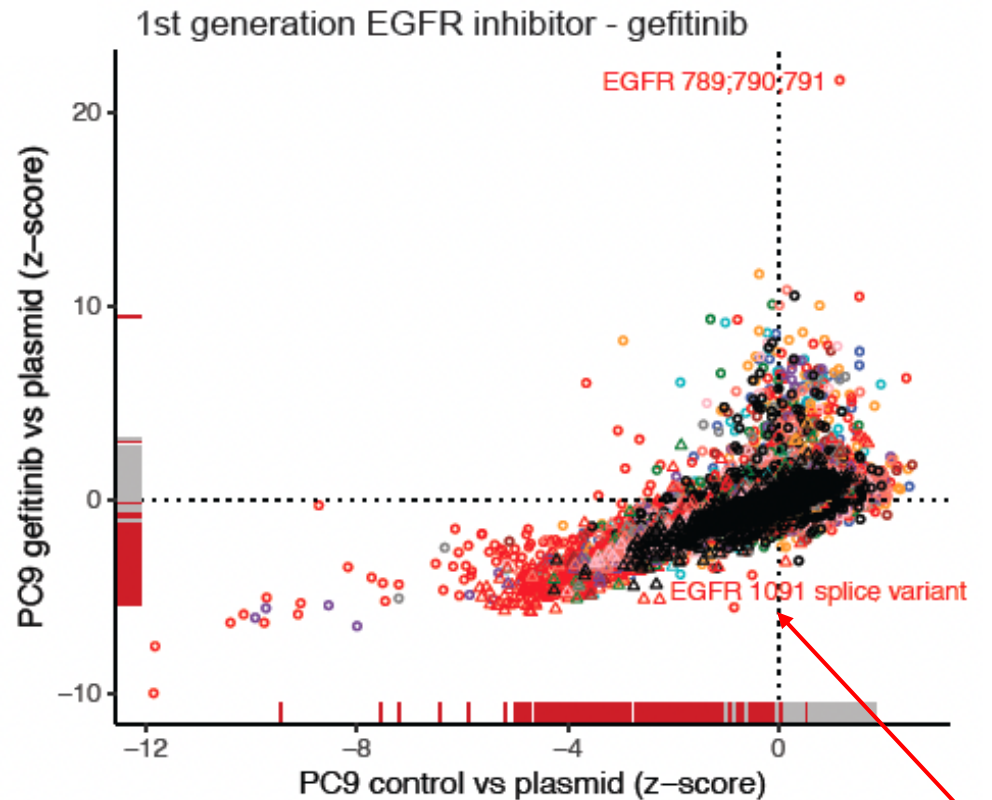


Single-cell RNA seq functionally defines drug resistant cell states

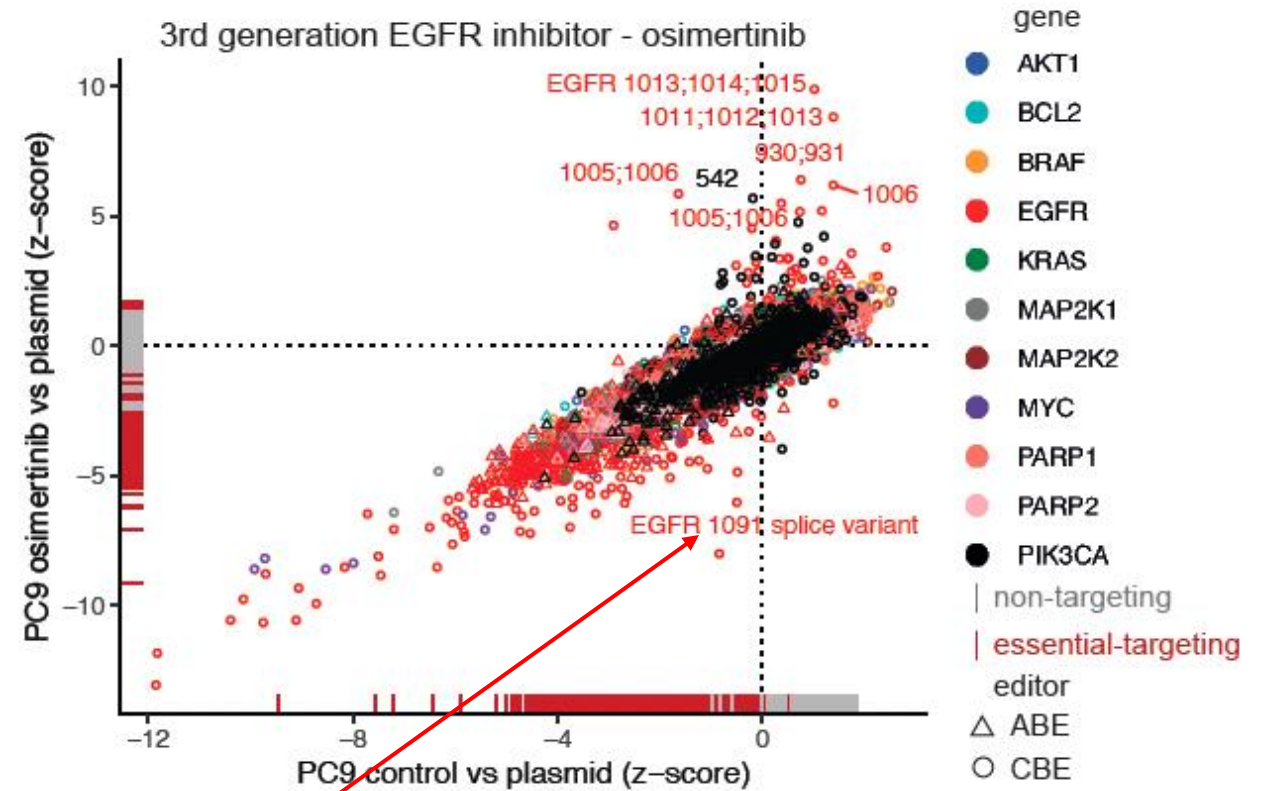


Variants affecting EGFR inhibitor sensitivity

EGFRi gefitinib



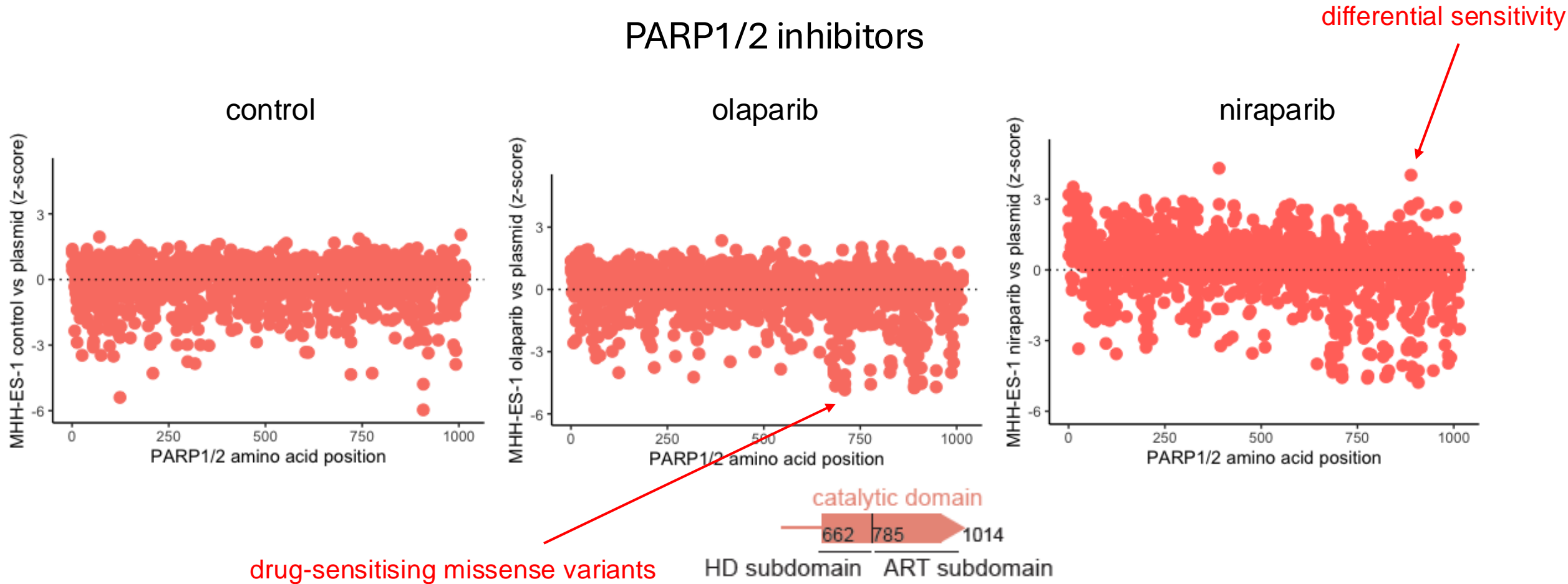
EGFRi osimertinib



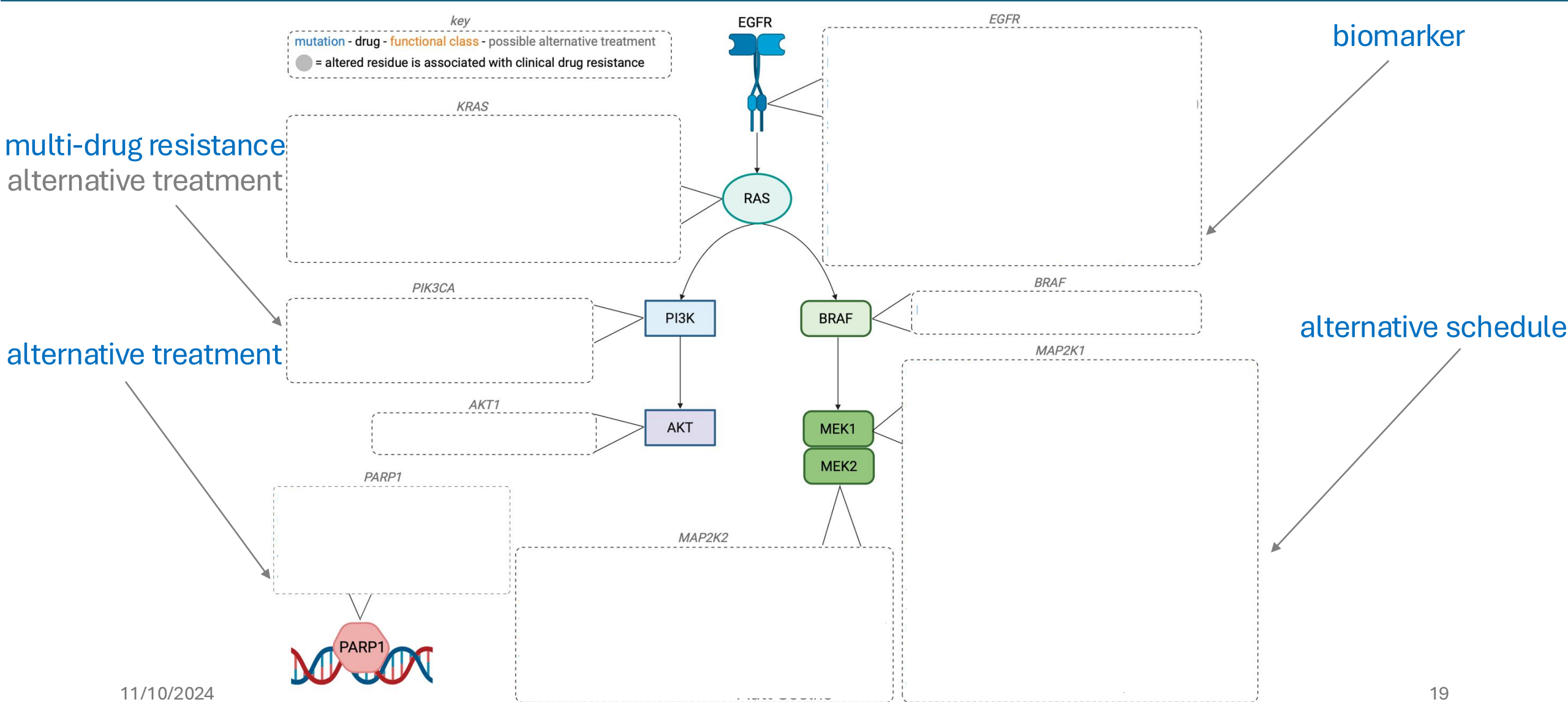
drug-sensitising

- Splice variant
- Not in the kinase domain

Drug-sensitising mutations in PARP1

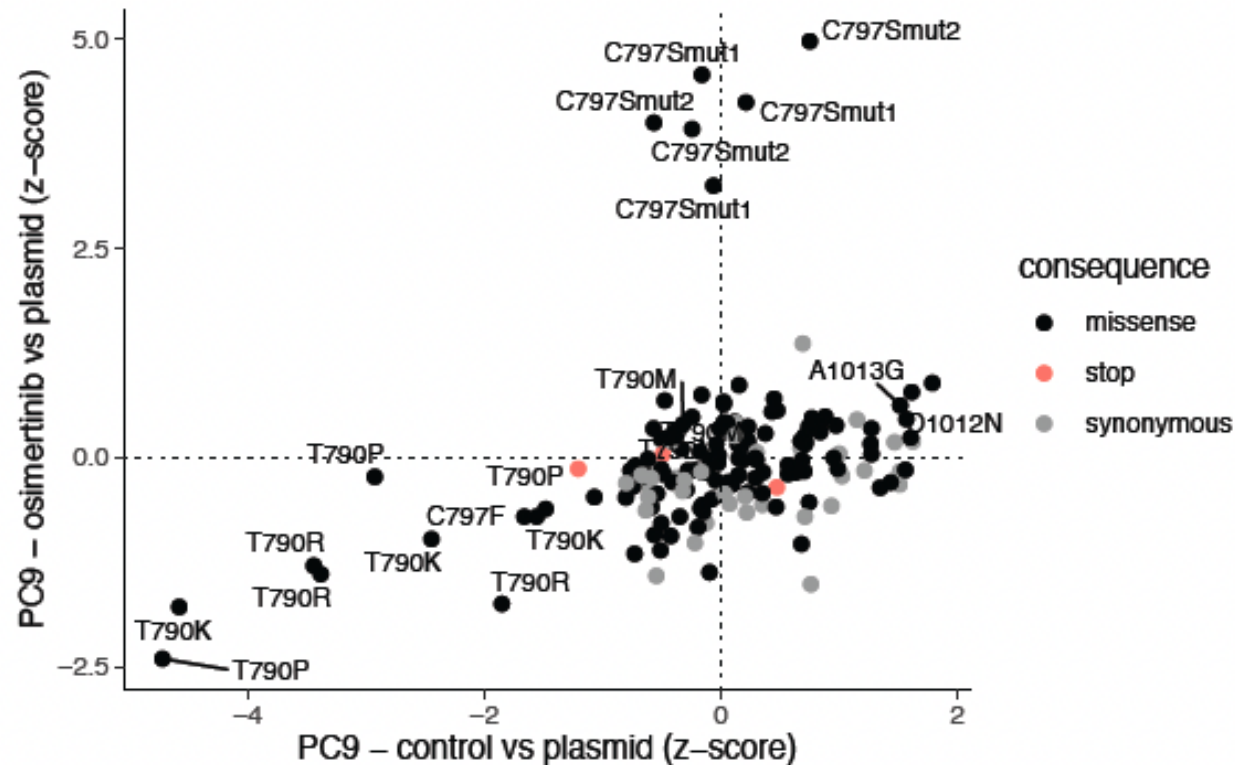


A variant function map indicates potential second-line therapies for drug resistant cancers

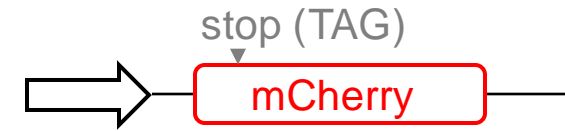


Prime editing screens of cancer variant function

EGFRi osimertinib resistance



reporter of prime editing

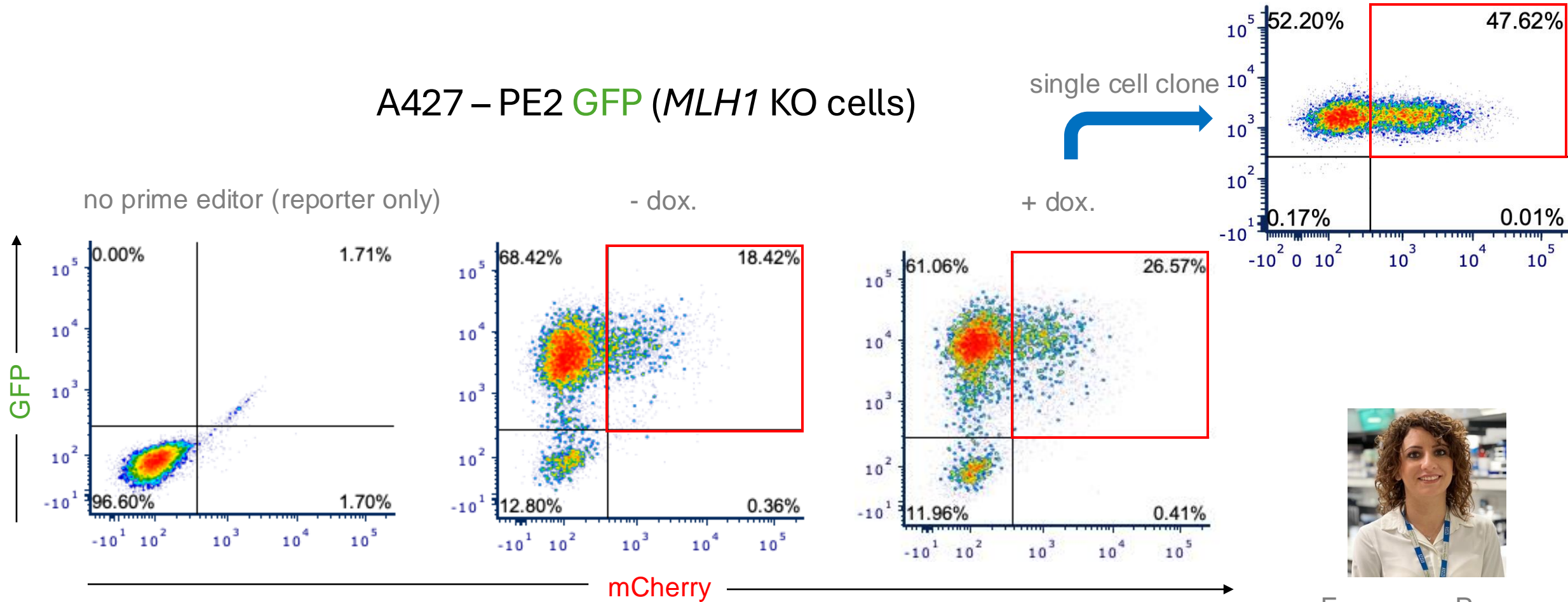


productive prime editing (mU6 -> epegRNA)



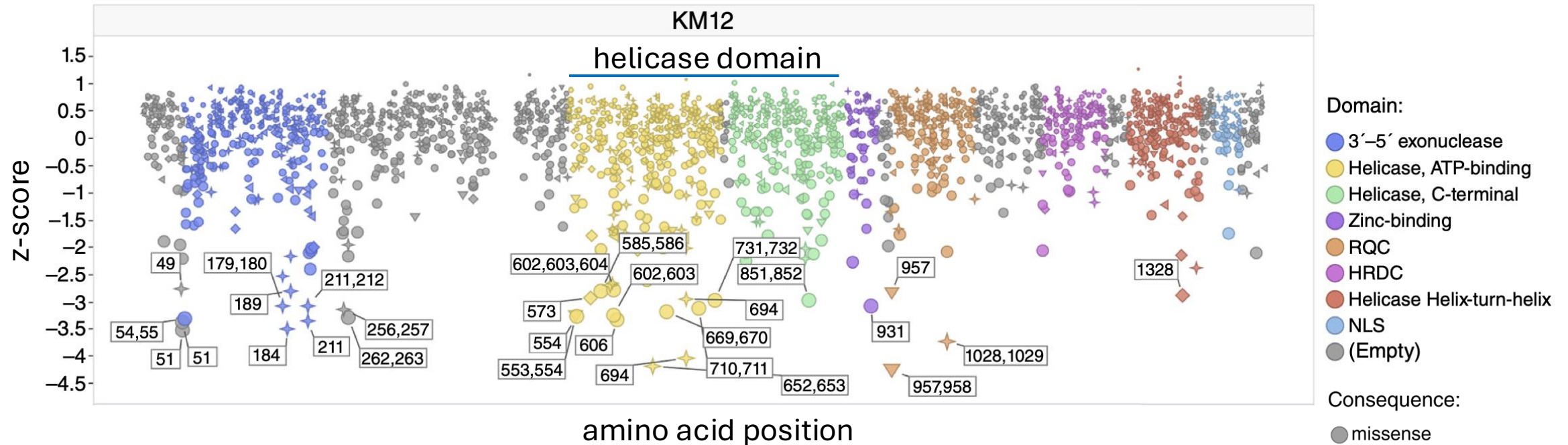
A fluorescent reporter of prime editing activity enables enrichment of edited cells

A427 – PE2 **GFP** (*MLH1* KO cells)



Francesca Perrone

Mutagenesis screens to guide drug design



Picco et al, *Cancer Discovery*, 2024

- Targeting synthetic lethal paralogues pairs with Dave Adams and Ishan Mehta (**CRUK Therapeutic Catalyst**)

Summary

1. Base editing can prospectively map variants that modulate cancer drug sensitivity across pathways in therapeutically-relevant cell models
2. Analysing multiple drugs in parallel can highlight possible of second-line treatments
3. Gene editing coupled to scRNA-seq can provide mechanistic insights into cancer variant function
4. Druggable domains can be identified and probed at high-resolution using base editing and prime editing
5. Functional data is needed to explain the occurrence drug resistance variants, and could help predict them for new drugs

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DNA Pipelines

Tim Halim
Youhani Samarakoon



CAMBRIDGE
CENTRE

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Open Targets



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RESEARCH
UK