XIX CONGRESSO NAZIONALE SIES 2026

# CRISPR e varianti di significato incerto nel cancro: scoperta di nuovi bersagli terapeutici e riposizionamento farmacologico

Francesco Iorio

Firenze | 4-6 marzo 2026
Palazzo degli Affari

# Disclosures of Francesco Iorio

| Company name | Research support | Employee | Consultant | Stockholder | Speakers bureau | Advisory board | Other |
|---|---|---|---|---|---|---|---|
| Open Targets | X | | | | | | |
| Nerviano Medical Sciences | x | | | | | | |
| CoSyne Therapeutics | | | x | | | | |
| Drug ReKindle | | | | | | x | |
| Mosaic Therapeutics | | | | | | | Former SAB member |
| AstraZeneca – Cancer Research Horizon Functional Genomics centre | | | | | | | Former SAB member |

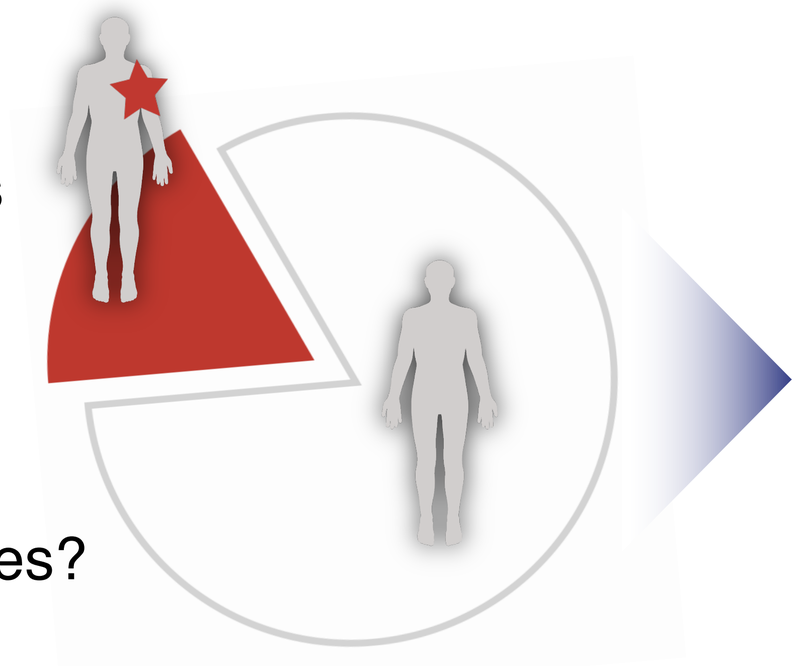
# Globally, most cancer genomes are “clinically silent”

## We need:

enhanced predictive markers

**to use existing drugs better**

to target non-mutated oncogenes?

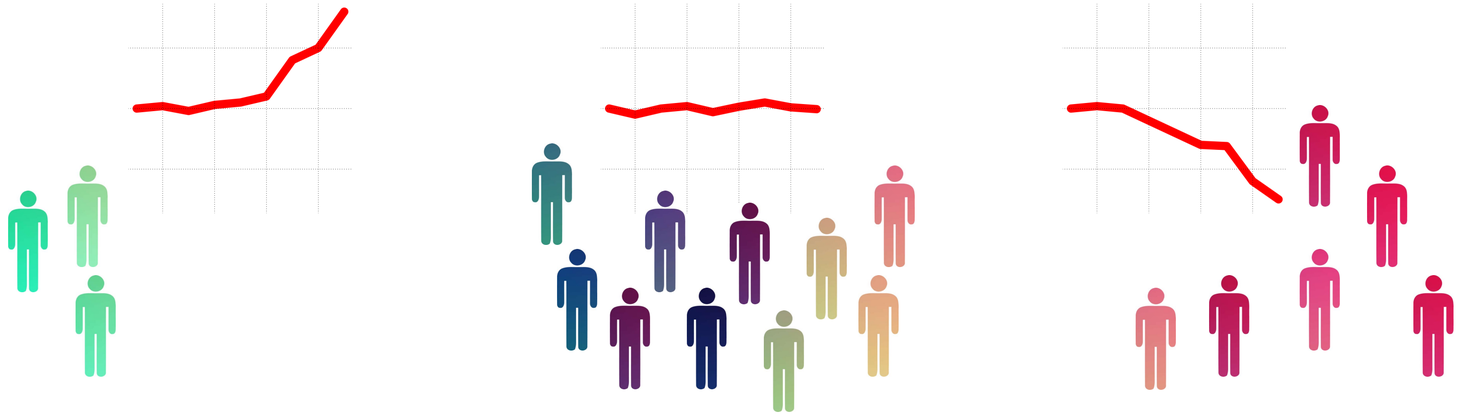


to find and prioritise
**new targets**
with robust
**predictive markers**

sources: MSK-IMPACT / NCI-MATCH

Actionable alterations

# ~90% of trialled new anti-cancer compounds fail to reach the market

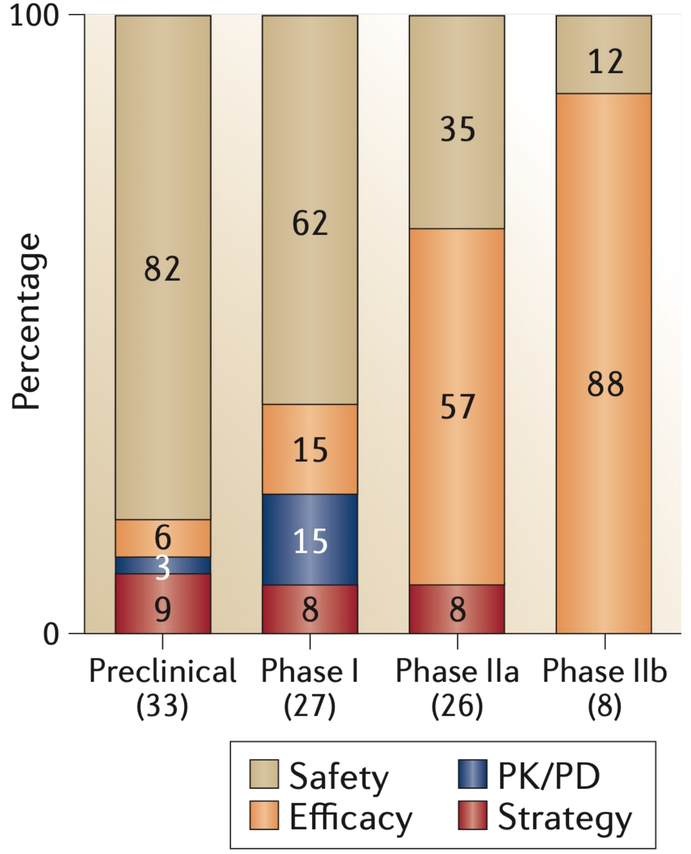


Therapeutic effect

No effect

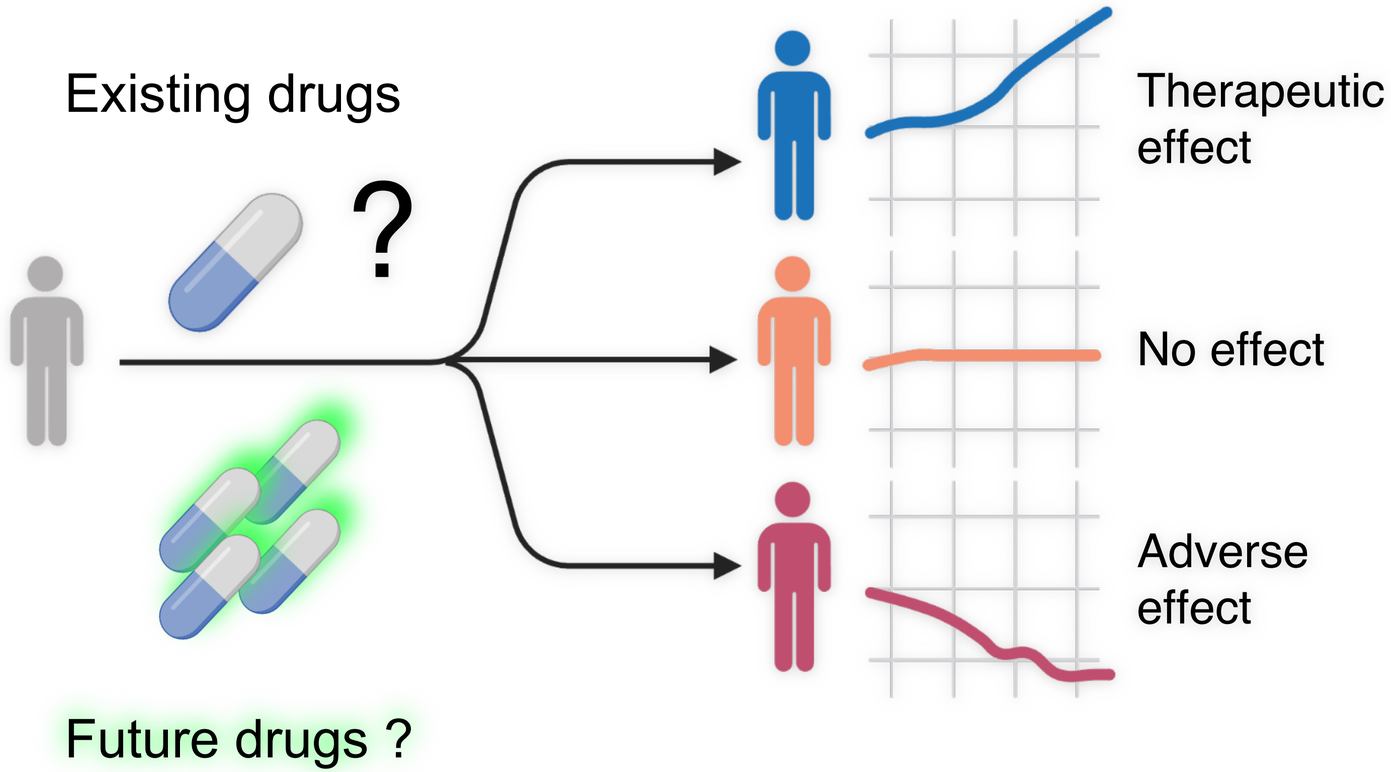
Adverse effect

## Most frequent reason for clinical trial failures = lack of efficacy




*adapted from Cook et al., Nat Rev Drug Discovery 2014*

# Precision oncology must anticipate future therapies




# ***The Cancer Dependency Map:***

*Identifying all dependencies in every cancer cell*

**Cancer dependencies =** essential genes and proteins that cancer cells (only) rely on for their survival, growth, or proliferation

cancer dependency map

depmap analytics

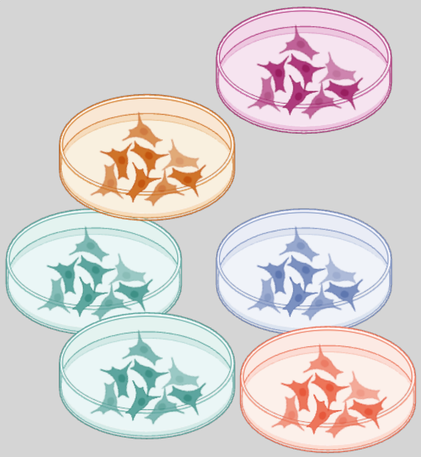
HUMAN TECHNOPOLE

wellcome sanger institute

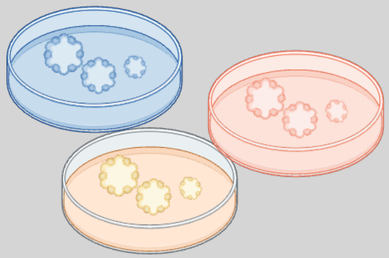
BROAD INSTITUTE

# *The Cancer Dependency Map Ecosystem*

**1,000s of Cancer Models**



cell lines

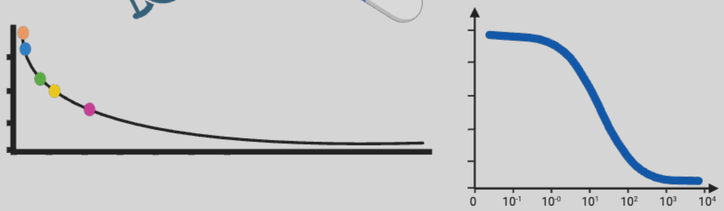


organoids

**Viability screens**

CRISPR-cas9

Drugs



**Multi-omic basal characterisation**

wellcome sanger institute

cancer dependency map

project score

cell model passports

CCLE Cancer Cell Line Encyclopedia

depmap

Project Achilles

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# Cancer DepMap Reveals Synthetic Lethal Interactions and Biomarker-Linked Targets

**1,000s of Cancer Models**

cell lines

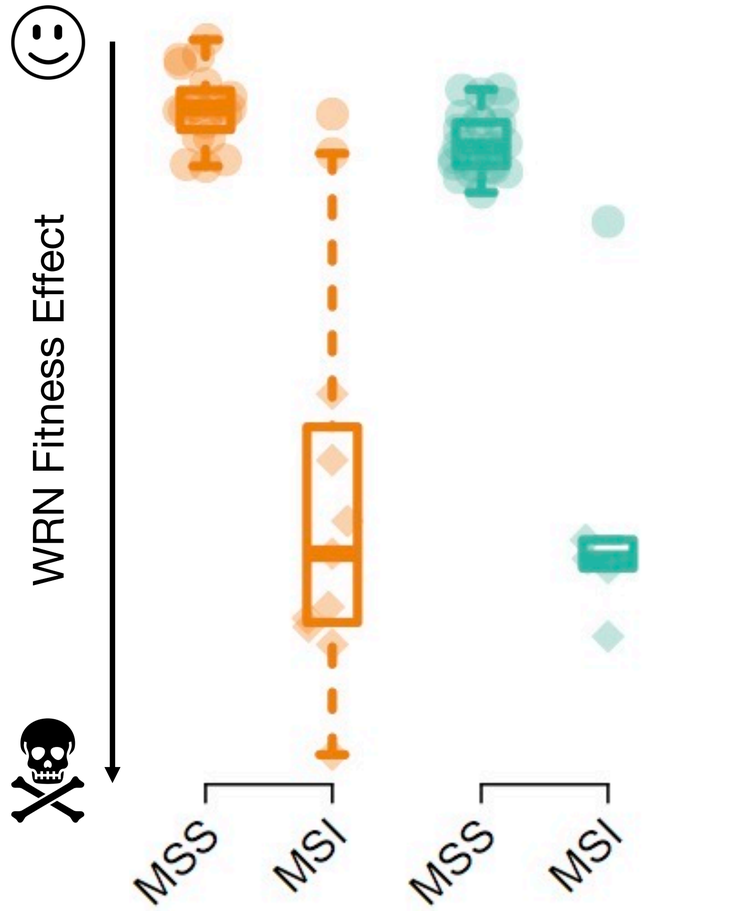
organoids

**Viability screens**

CRISPR-cas9

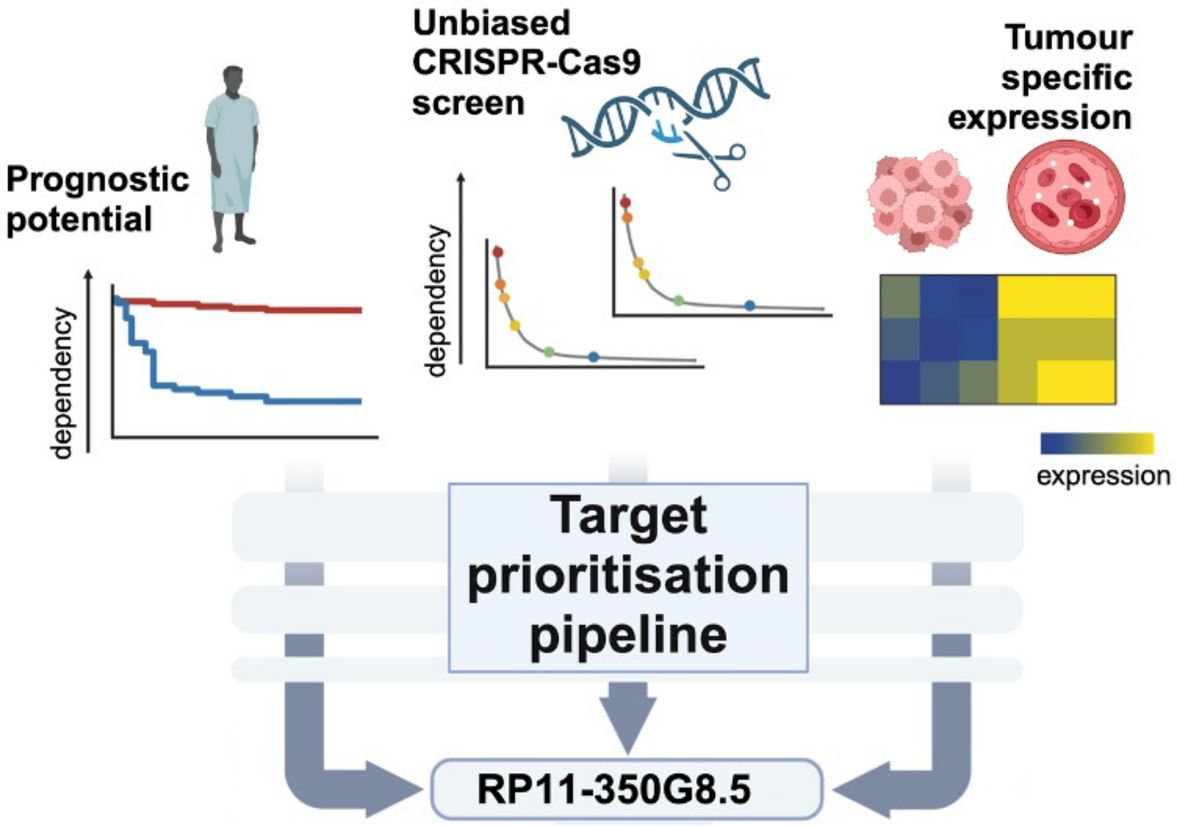
Drugs

**Multi-omic basal characterisation**



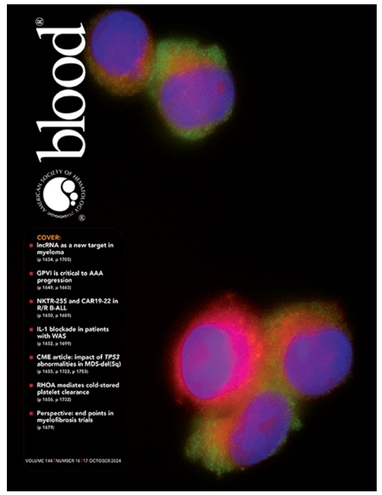

Pacini et al, ***Cancer Cell*** 2024;
Behan, Iorio et al, ***Nature*** 2019;

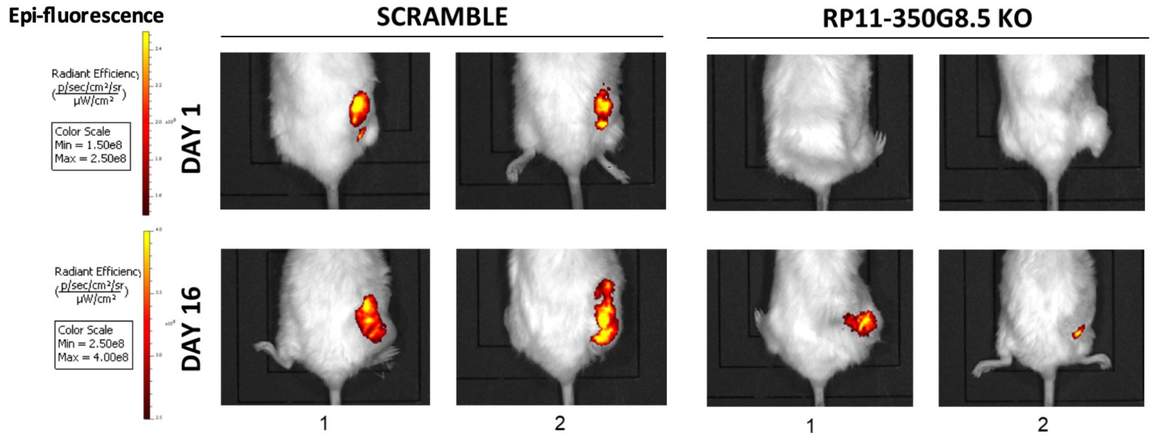
# lncRNA CRISPR screen and computational target prioritisation pipeline identifies RP11-350G8.5 as therapeutic target in Multiple Myeloma




O. Croci

P. Cremaschi




UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II

UMG Dubium sapientae initium

Grillone et al, ***Blood*** 2024

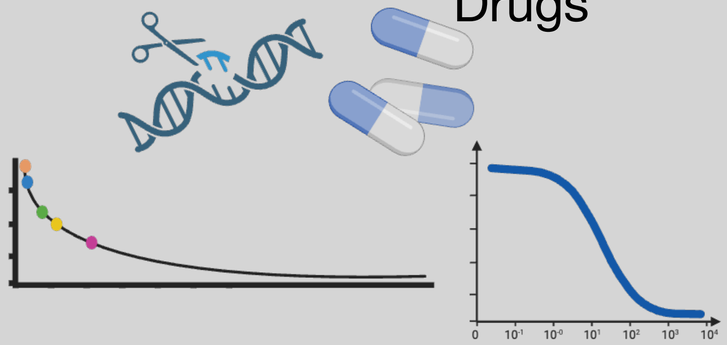
# HUGELY underdetermined problem

~2K of labels

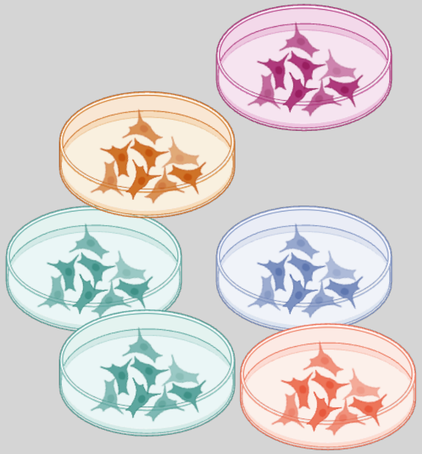
## Viability screens

CRISPR-cas9

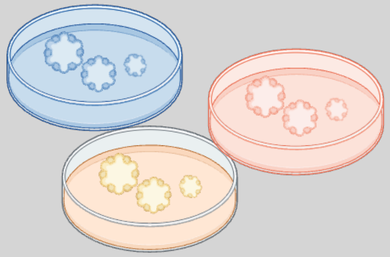
Drugs



## Cancer Models



cell lines

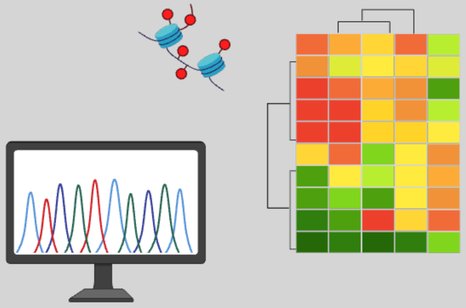


organoids

cancer dependency map

depmap

100K of features



**Multi-omic basal characterisation**

Pacini et al, ***Cancer Cell*** 2024;
Behan, Iorio et al, ***Nature*** 2019;

# Underdetermination can been mitigated by applying patient-guided, frequentist-informed feature curation/selection

## Cancer Models

cell lines

organoids

## Viability screens

CRISPR-cas9

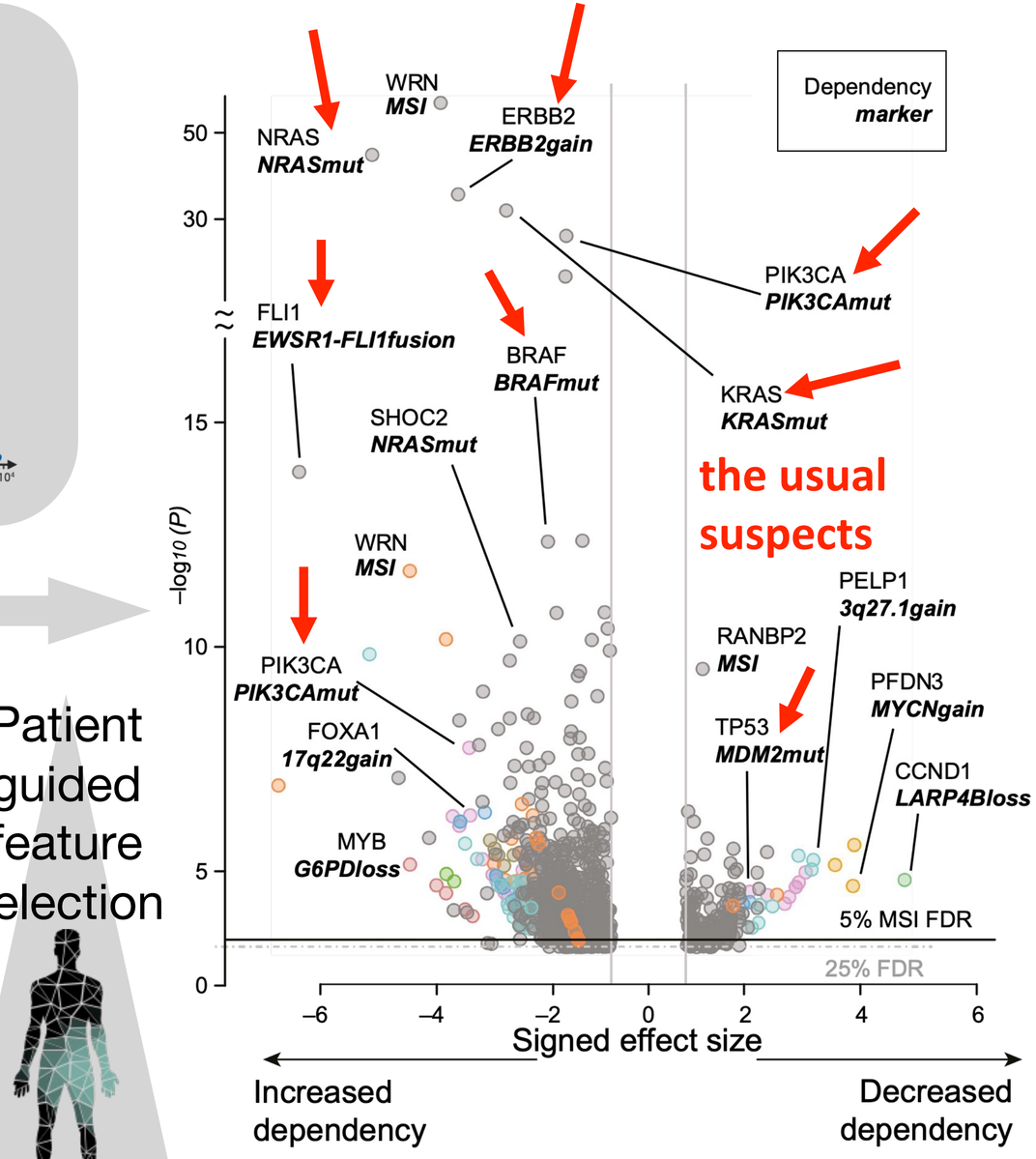
Drugs

cancer dependency map

depmap

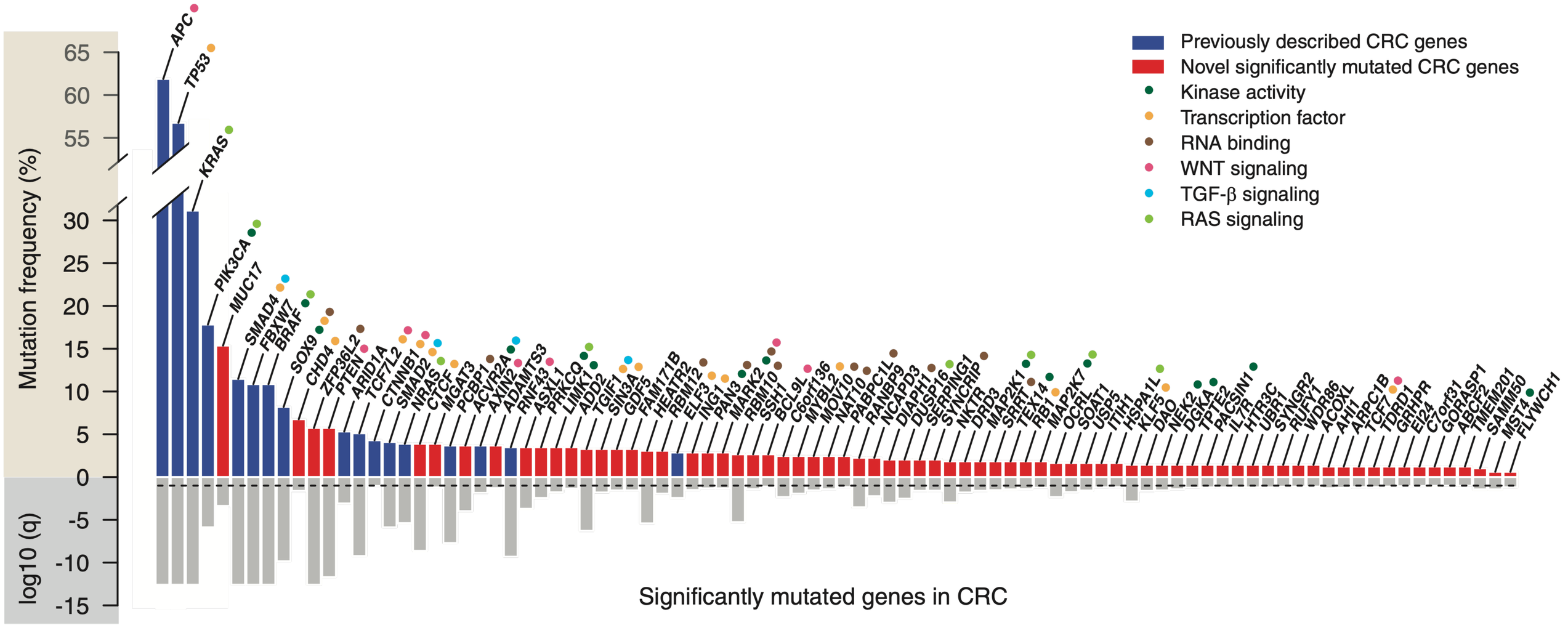
## Multi-omic basal characterisation

Patient guided feature selection




Pacini et al, ***Cancer Cell*** 2024;
Behan, Iorio et al, ***Nature*** 2019;
Iorio et al, ***Cell*** 2016;

# …but this overlooks long tails of less frequent genomic events

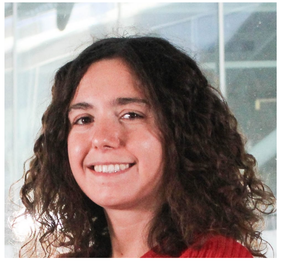



adapted from Giannakis et al, 2016

# *The CRISPR-enhanced VUS evaluation Idea*

- We use **DepMap data** to identify any VUS that might associate with strong dependency on the hosting gene (Dependency-Associated Mutations, DAMs)

- These dependencies represent **“self-addictions”, which are particularly relevant from a Target discovery prospective (as they come with an embedded predictive feature)**

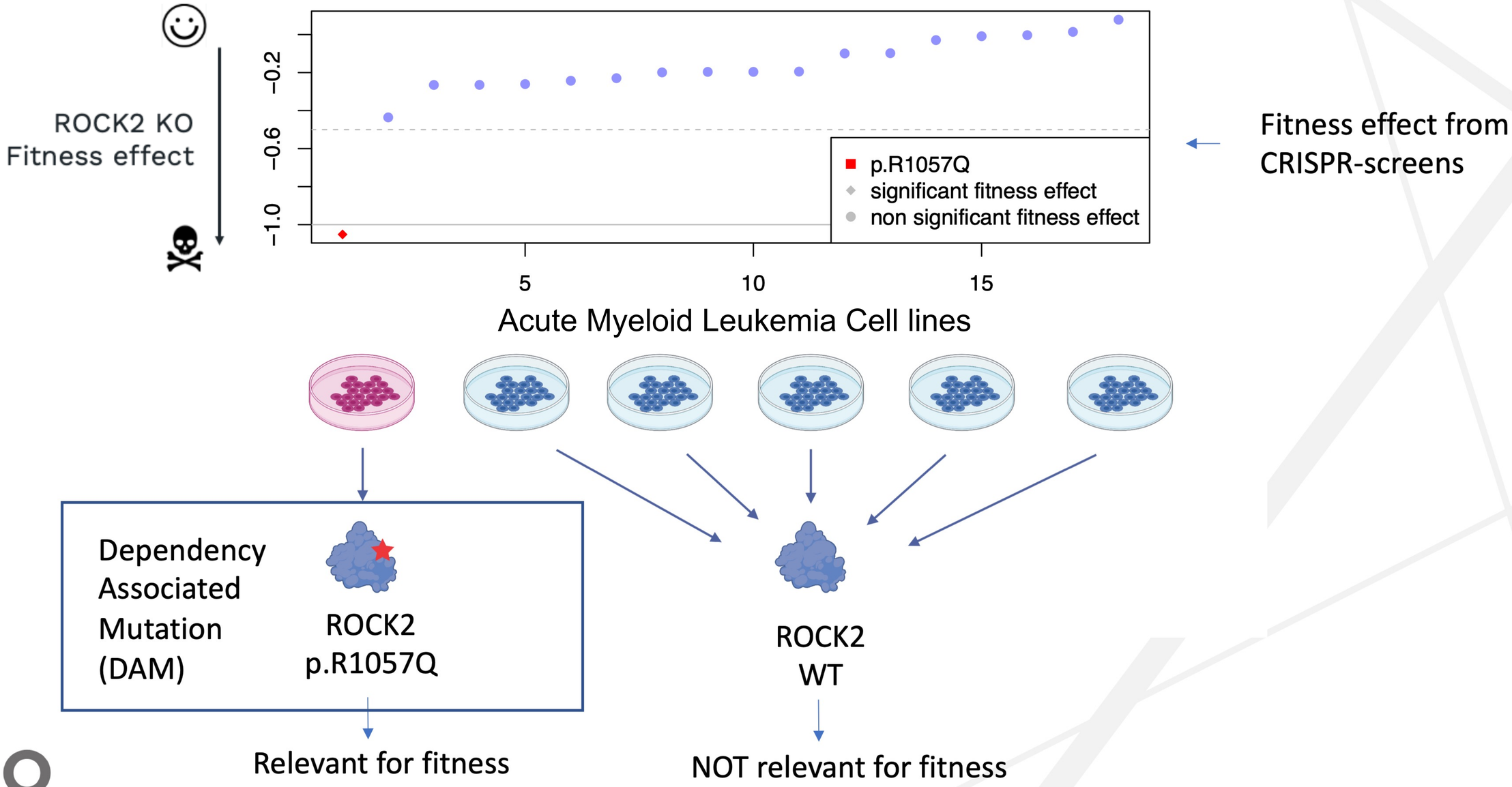


A. Savino

A. Oikonomou

# *Issue: lack of statistical power*

*Most VUSs appear only in one or very few cell lines*



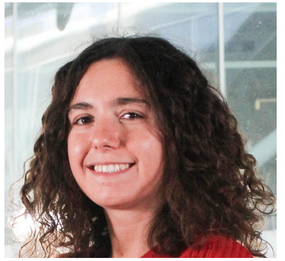
# *The CRISPR-enhanced VUS evaluation Idea*

- We use **DepMap data** to identify any VUS that might associate with strong dependency on the hosting gene (Dependency-Associated Mutations, DAMs)

- These dependencies represent **“self-addictions”, which are particularly relevant from a Target discovery prospective (as they come with an embedded predictive feature)**

**SOLUTION**

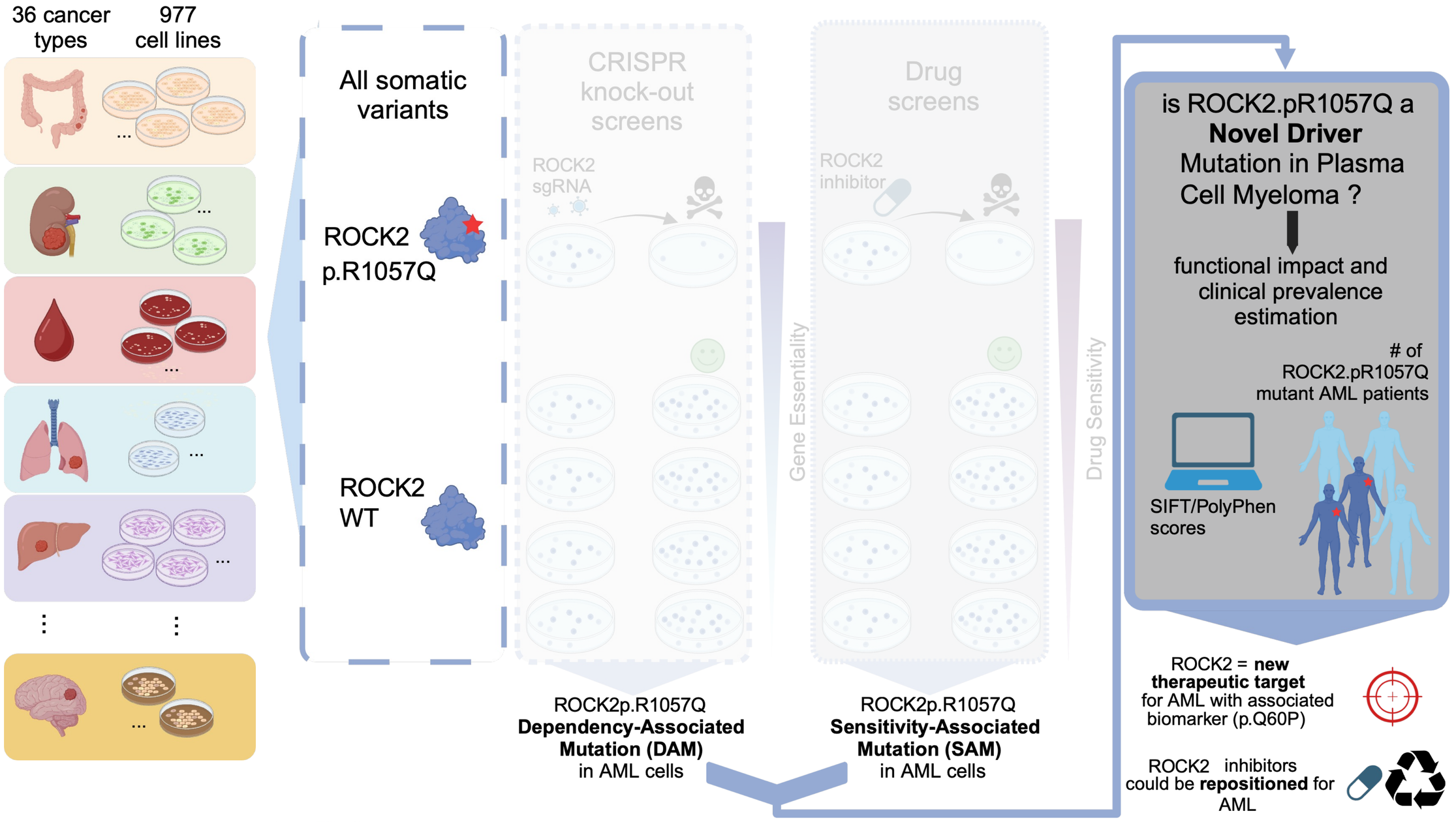
- We add further evidences like associations with drug sensitivity, sequence-based functional impact prediction and we estimate the clinical frequency a posteriori



A. Savino

A. Oikonomou

# *The CRISPR-VUS computational pipeline*




Savino et al. [Under Revision] - bioRxiv THE PREPRINT SERVER FOR BIOLOGY

## *We use a metric that is suitable also for singleton cases*

For a given set of variants ***X*** in a given gene ***g*** (whit $0 < |\boldsymbol{X}| \leq 10$) compute a *RankRatio* score as it follows

$$RankRatio(X) = \frac{\sum_{x \in X} r(x)}{(|X|^2 + |X|)/2}$$

where *r(x)* is the rank position of the cell line that harbours *x* when sorting all cell lines from a given tissue based on the dependency on ***g***

**Optimisation step**: subset of variants in a given gene ***g*** that minimises RankRatio(***X***)

Consider as DAMs all variants in ***X*** combinations such that RankRatio(***X***) < 1.6 (derived in a data driven way)

**Significance**:
rankRatio threshold is adaptively determined (max coverage of known oncogenic addictions)
the global FDR is determined by permutation test (restricting to < 20%)

# Example

## Gene: FLT3LG

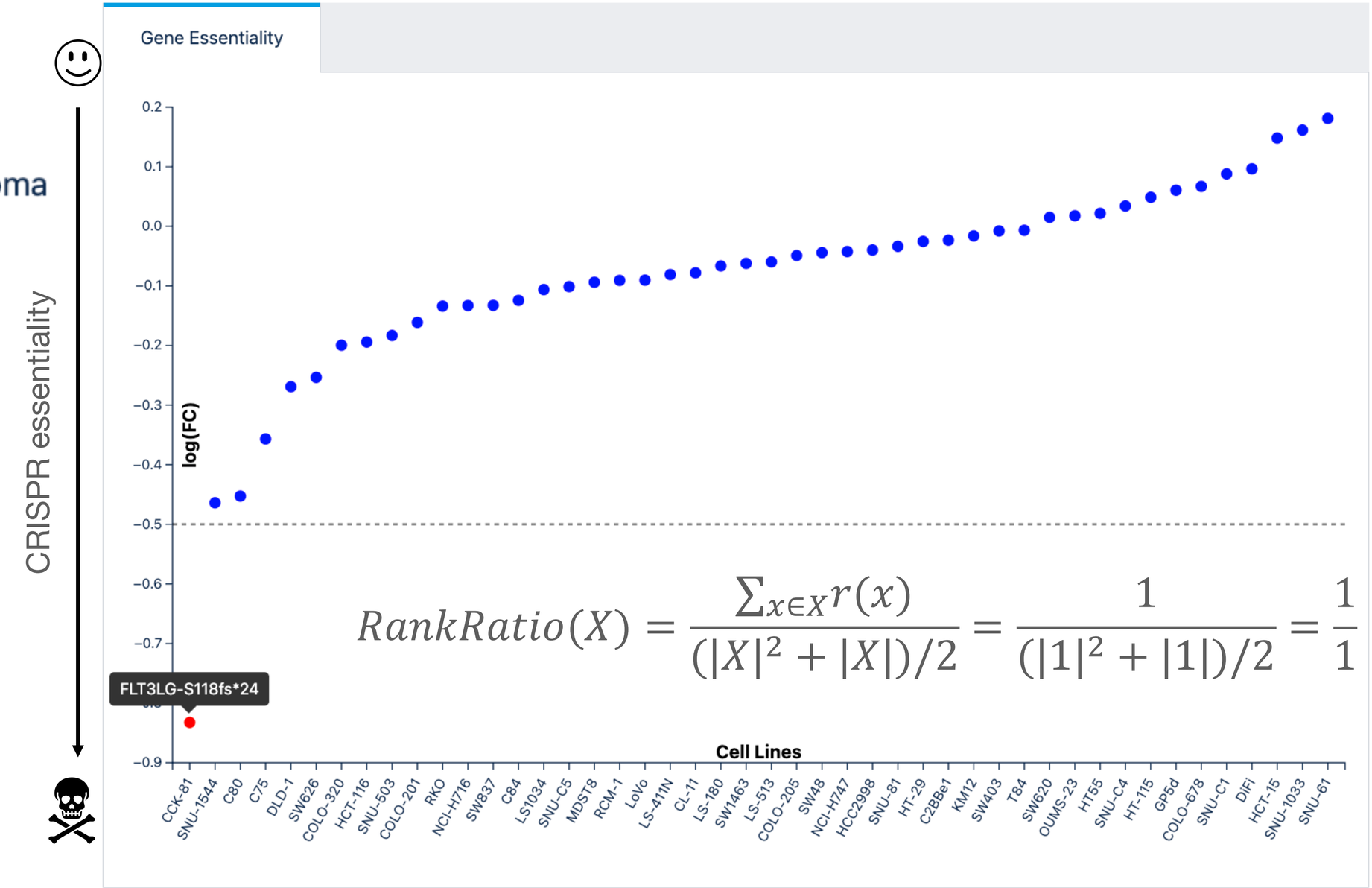
**Cancer Type:** Colorectal Carcinoma

**Rank Ratio:** 1

**All DAMs**

### Variants Status

- Present
- Absent




$$RankRatio(X) = \frac{\sum_{x \in X} r(x)}{(|X|^2 + |X|)/2} = \frac{1}{(|1|^2 + |1|)/2} = \frac{1}{1}$$

# Example

## Gene: BRAF

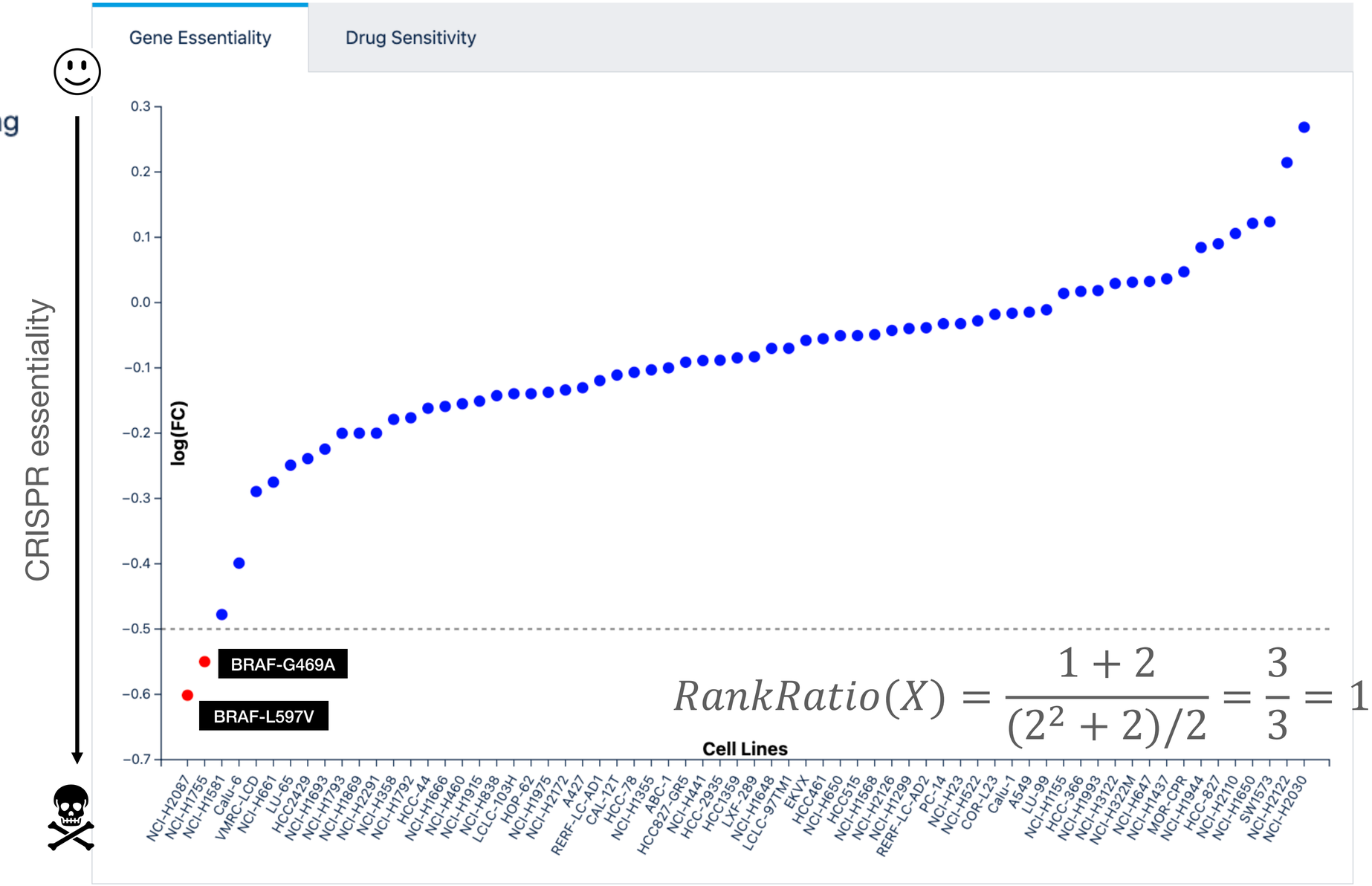
**Cancer Type:** Non-Small Cell Lung Carcinoma

**Rank Ratio:** 1

**All DAMs**

### Variants Status

- Present
- Absent




$$RankRatio(X) = \frac{1+2}{(2^2+2)/2} = \frac{3}{3} = 1$$

# Example

## Gene: PIK3CA

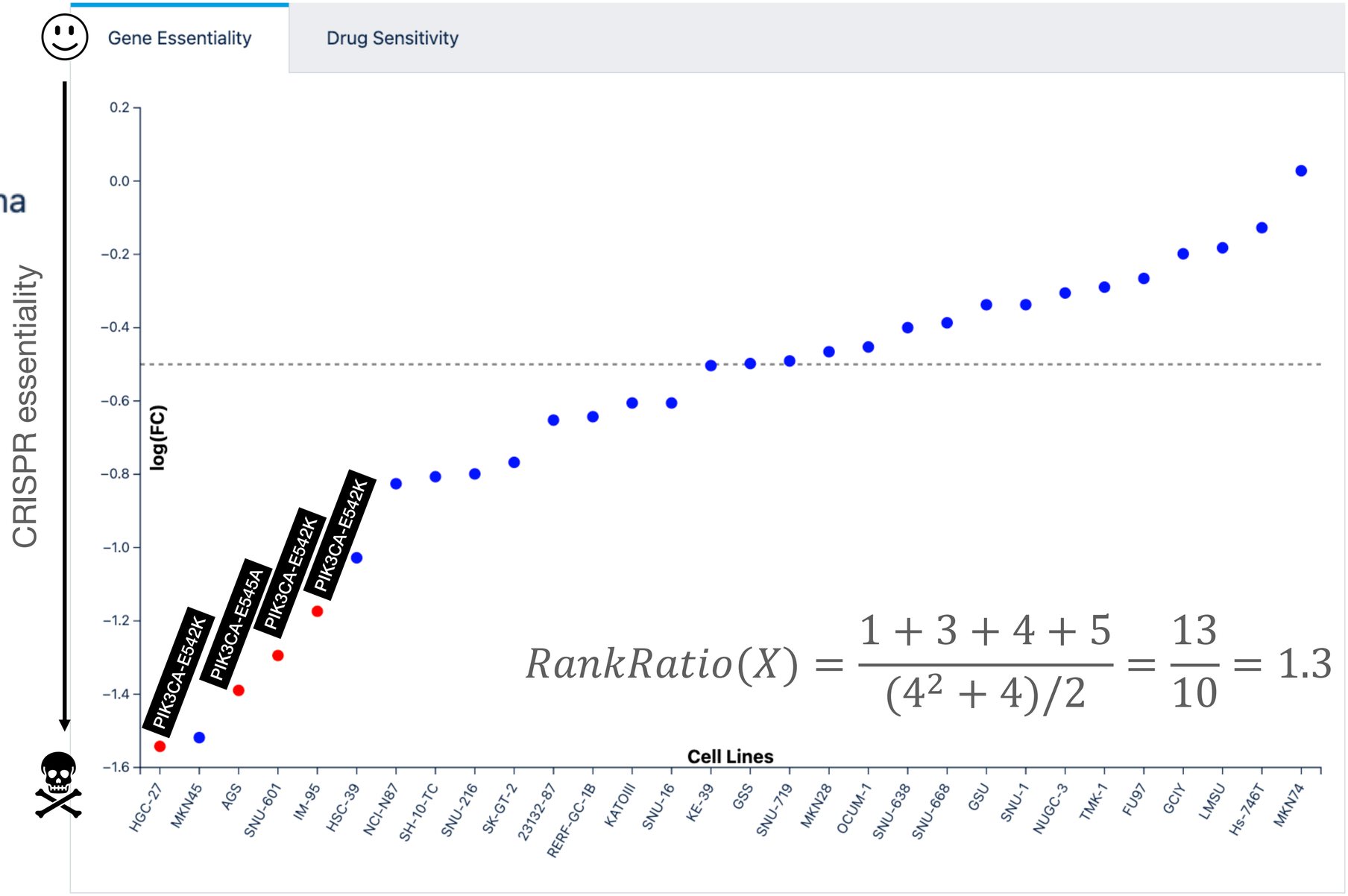
**Cancer Type:** Gastric Carcinoma

**Rank Ratio:** 1.3

**All DAMs**

### Variants Status

- Present
- Absent

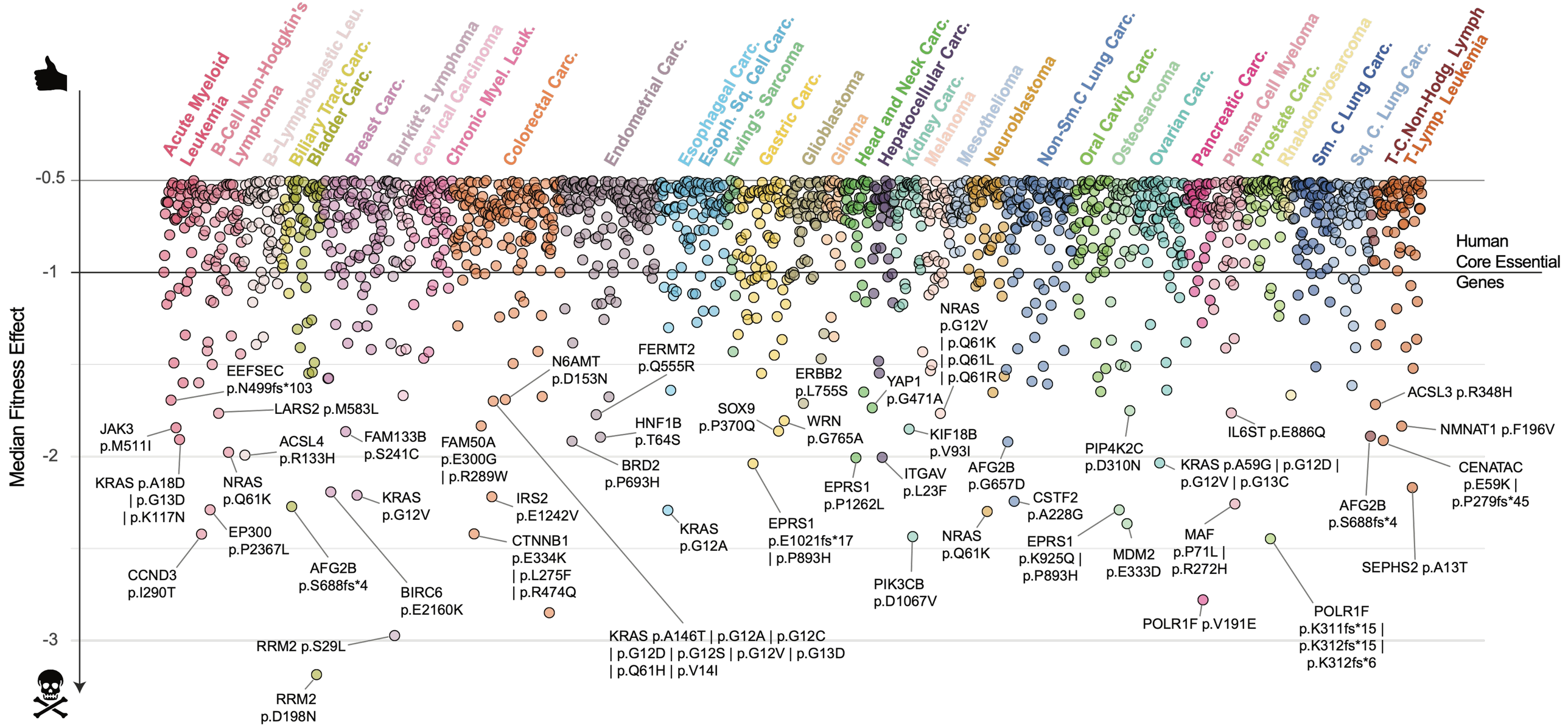



$$RankRatio(X) = \frac{1+3+4+5}{(4^2+4)/2} = \frac{13}{10} = 1.3$$

# *Further analytical details*

- Restricting to only missense/inframe/frameshift somatic variants
- Excluding genes with more than 10 syntactically different variants observed (likely to be TSGs or very large genes, ex. TTN)
- Excluding core-fitness/common-essential genes
- Considering only genes with fitness effect >= 1/2 of prior known essential genes in at least one cell line

# (Unbiased) PanCancer Catalogue of Dependency-Associated Mutations




Savino et al. bioRχiv THE PREPRINT SERVER FOR BIOLOGY
[Under Revision]

**1,773 cancer-type-specific hits, i.e Individual DAMs or optimal combinations of DAMs**

# *DAMs and DAM-bearing genes are highly tissue specific*

1,773 cancer-type specific hits

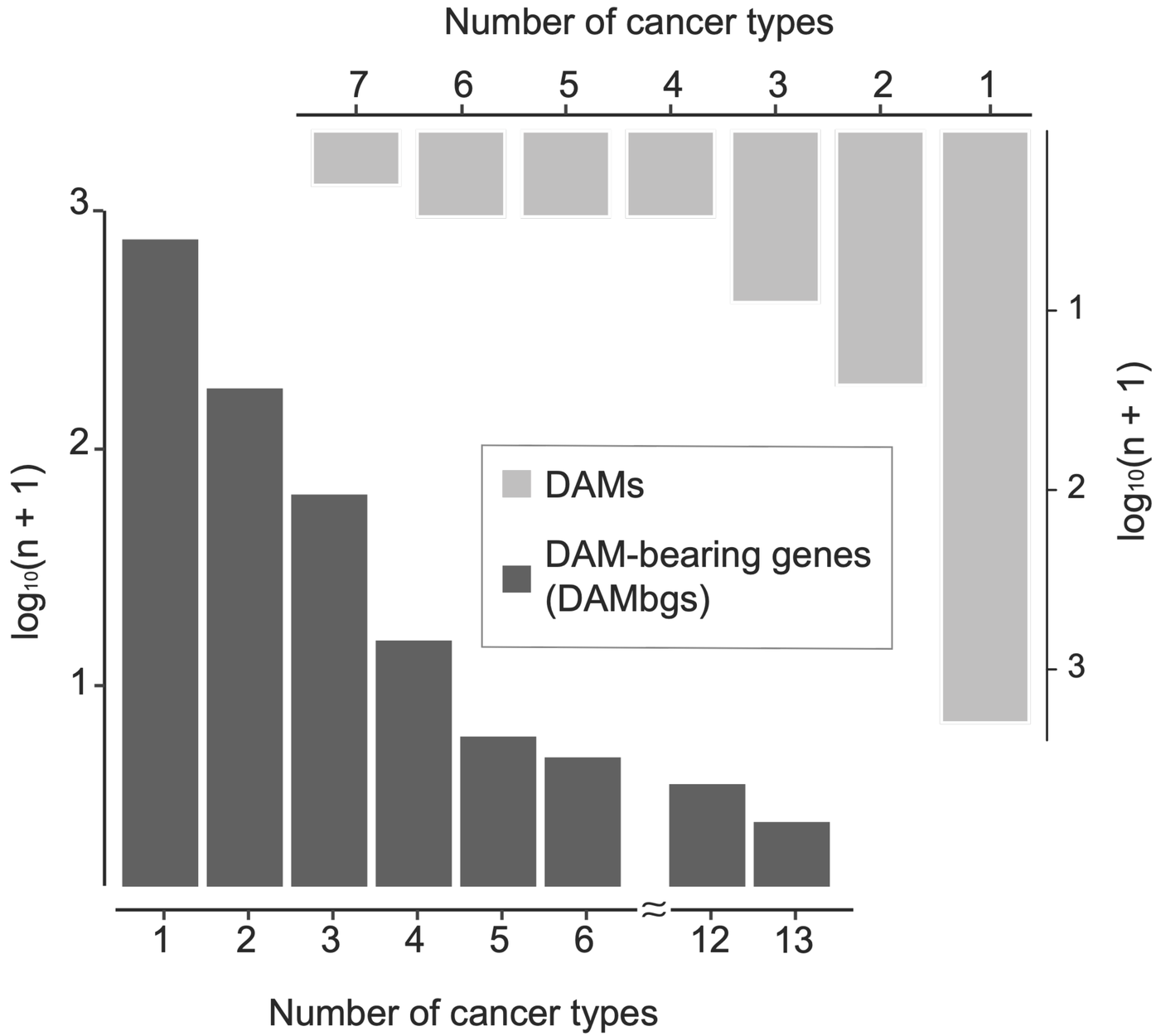
2,201 individual DAMs

**1,329 DAM-bearing genes**

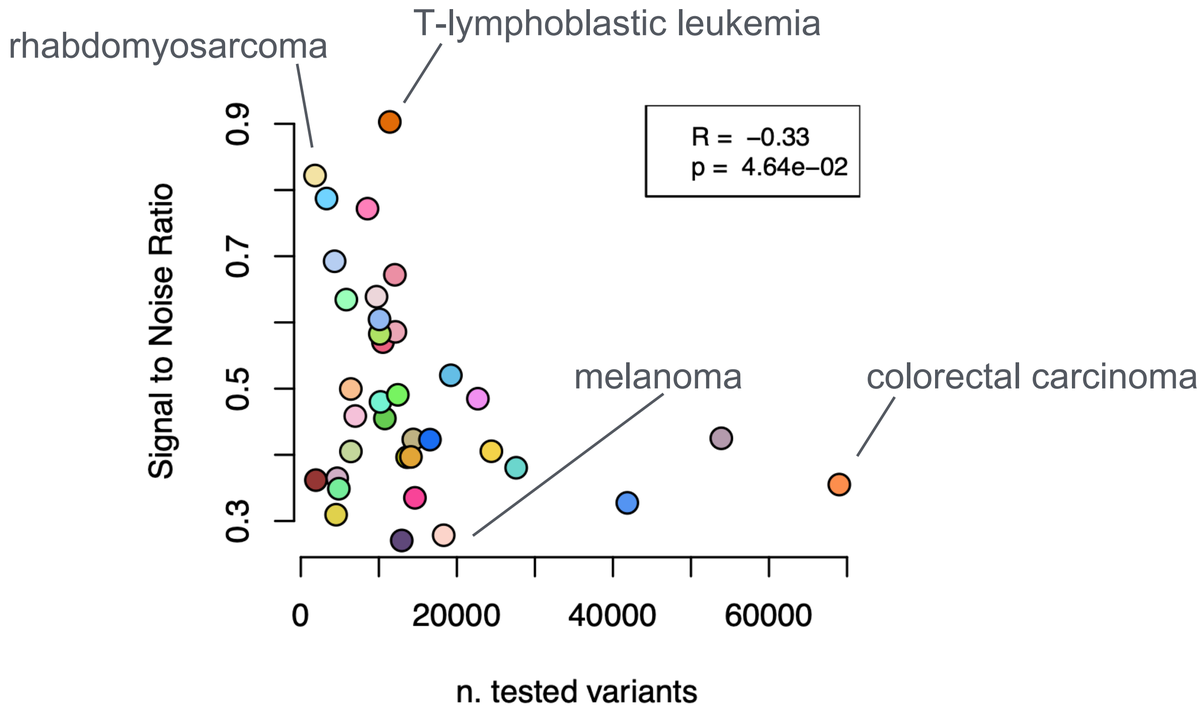
involved cell lines = **545** (55%)

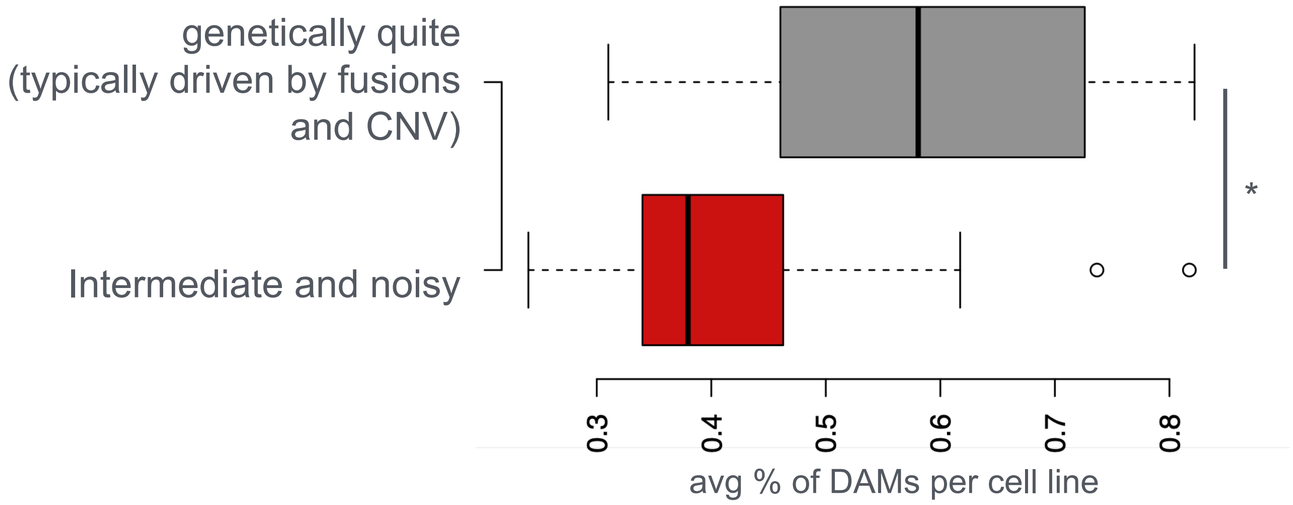
median n. hits harboured by a cell line = **2**

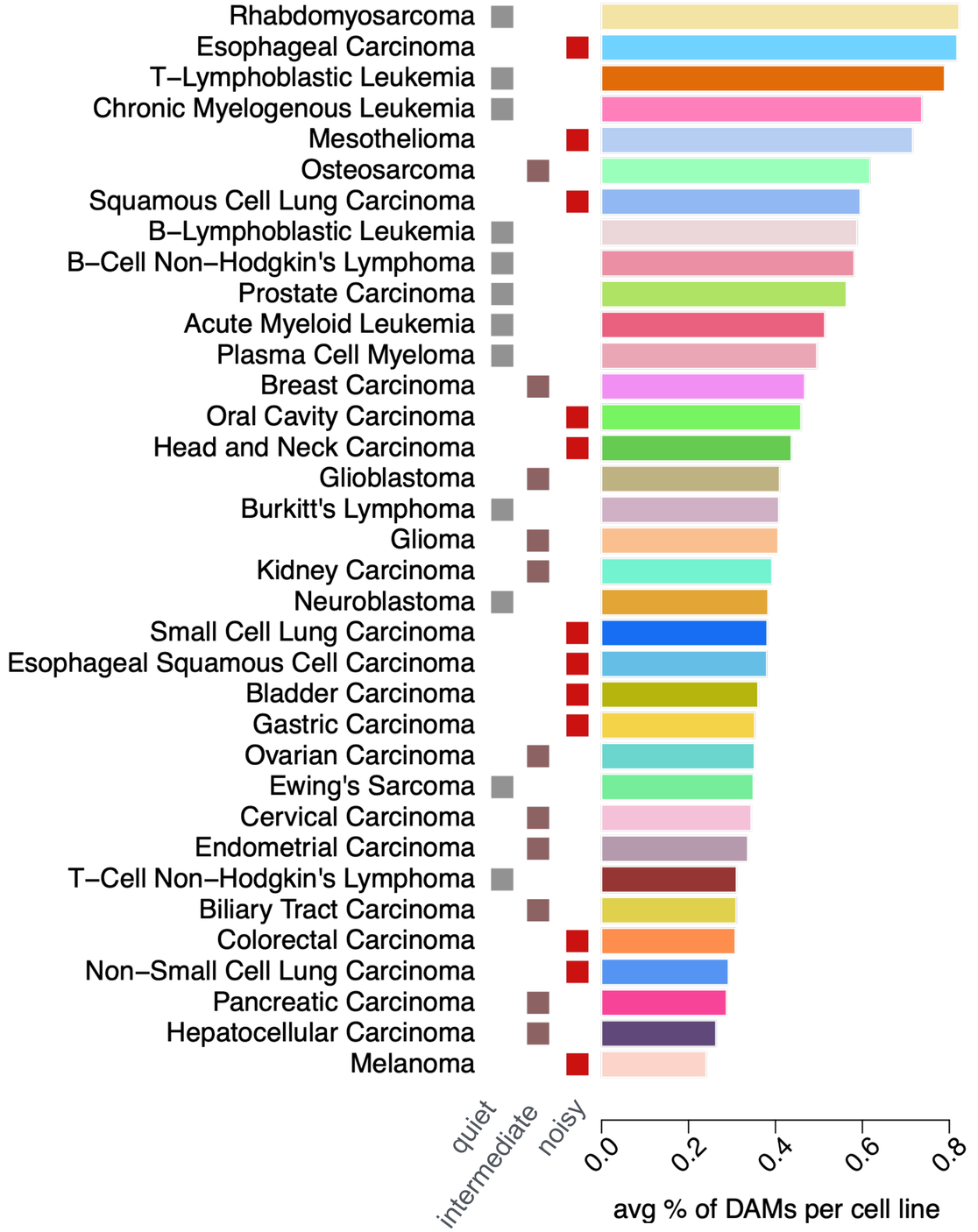
median n. of cell lines hosting a given hit = **1**



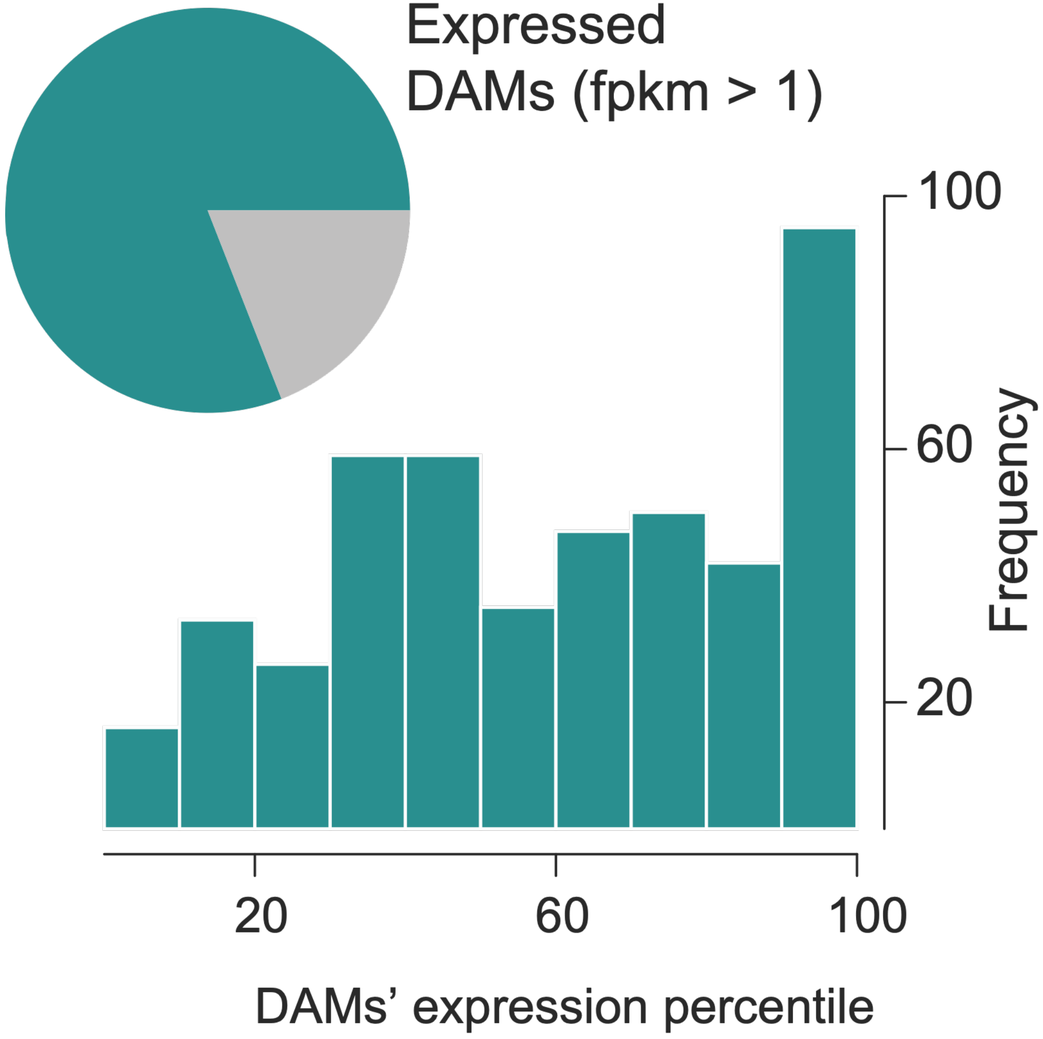
# *Genomically quieter Cancer-types have higher DAMs to VUSs ratio*



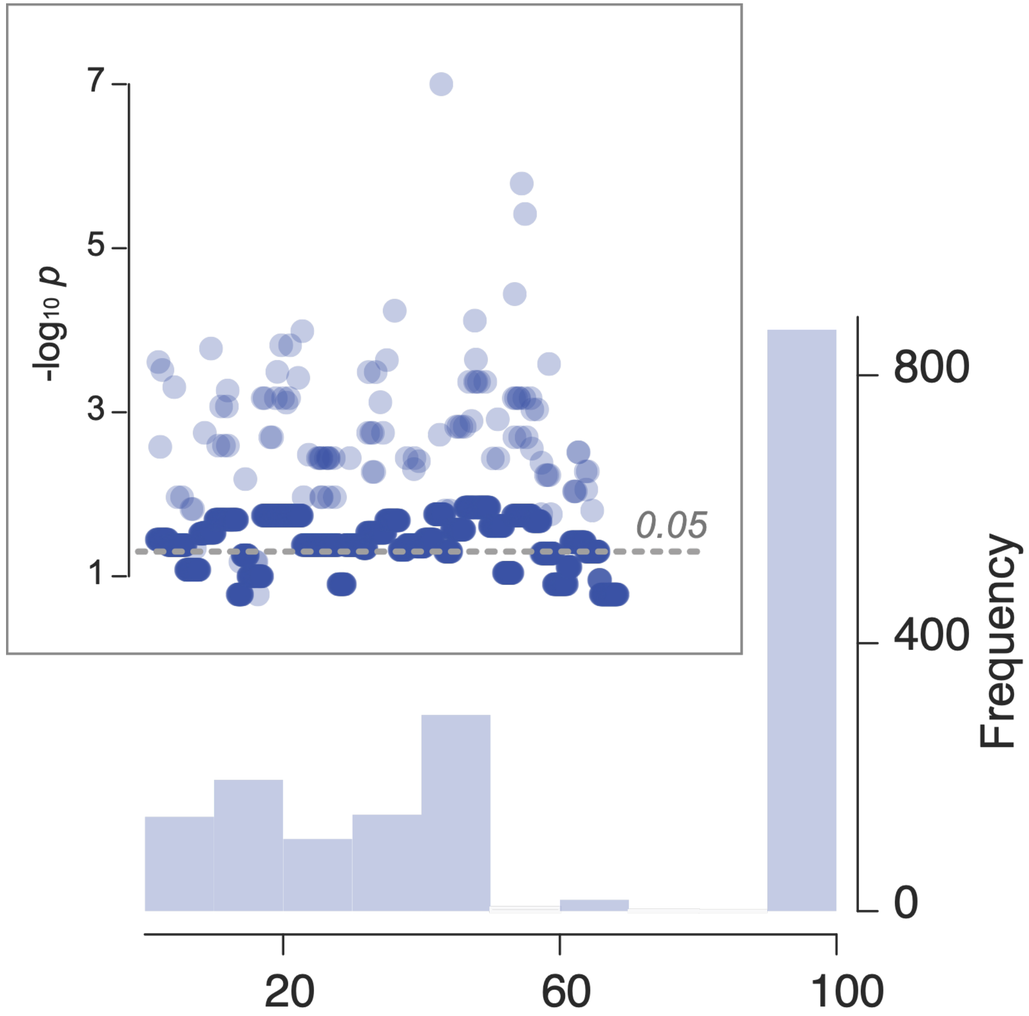



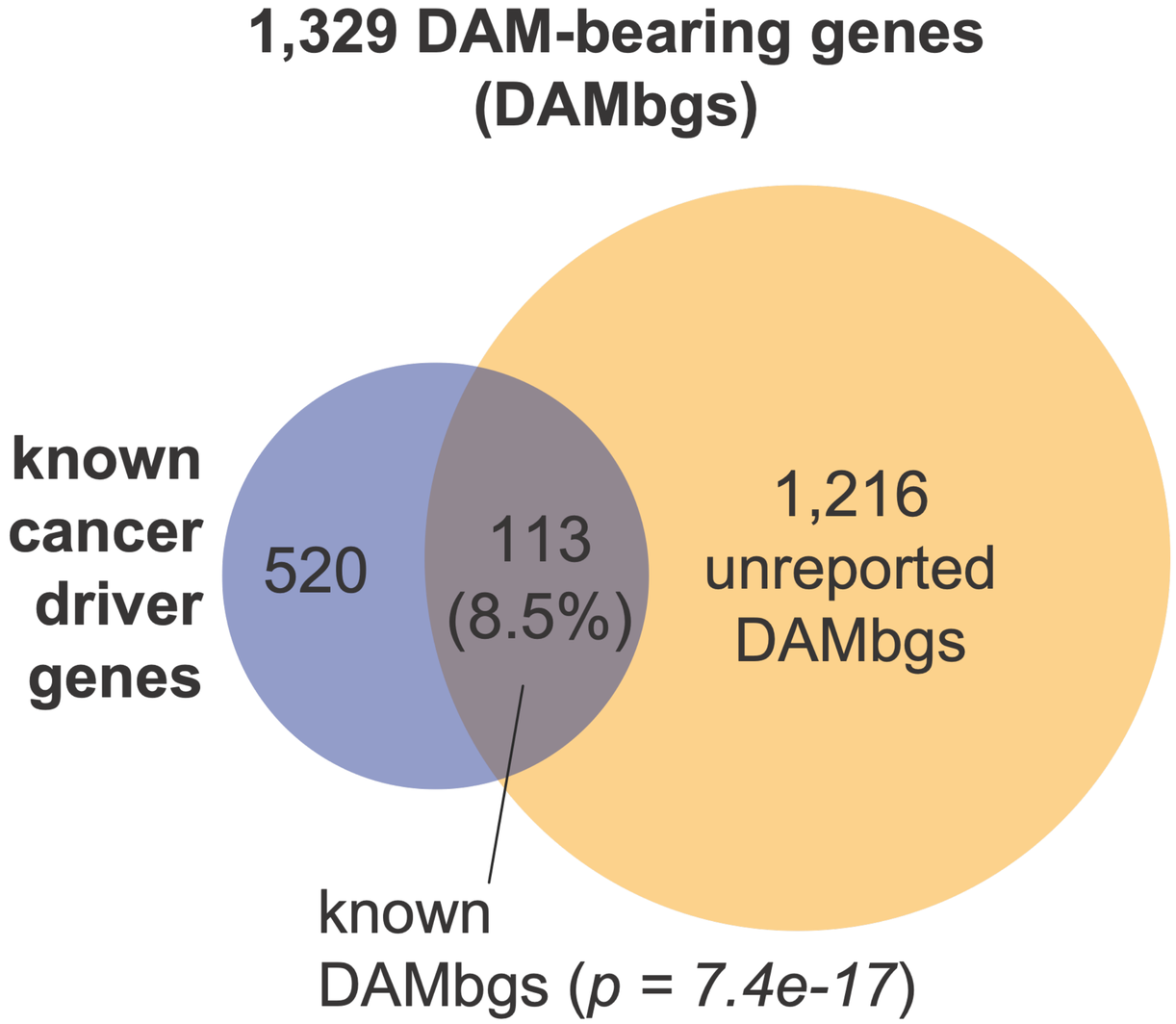
***DAMs are generally expressed, many are highly expressed***



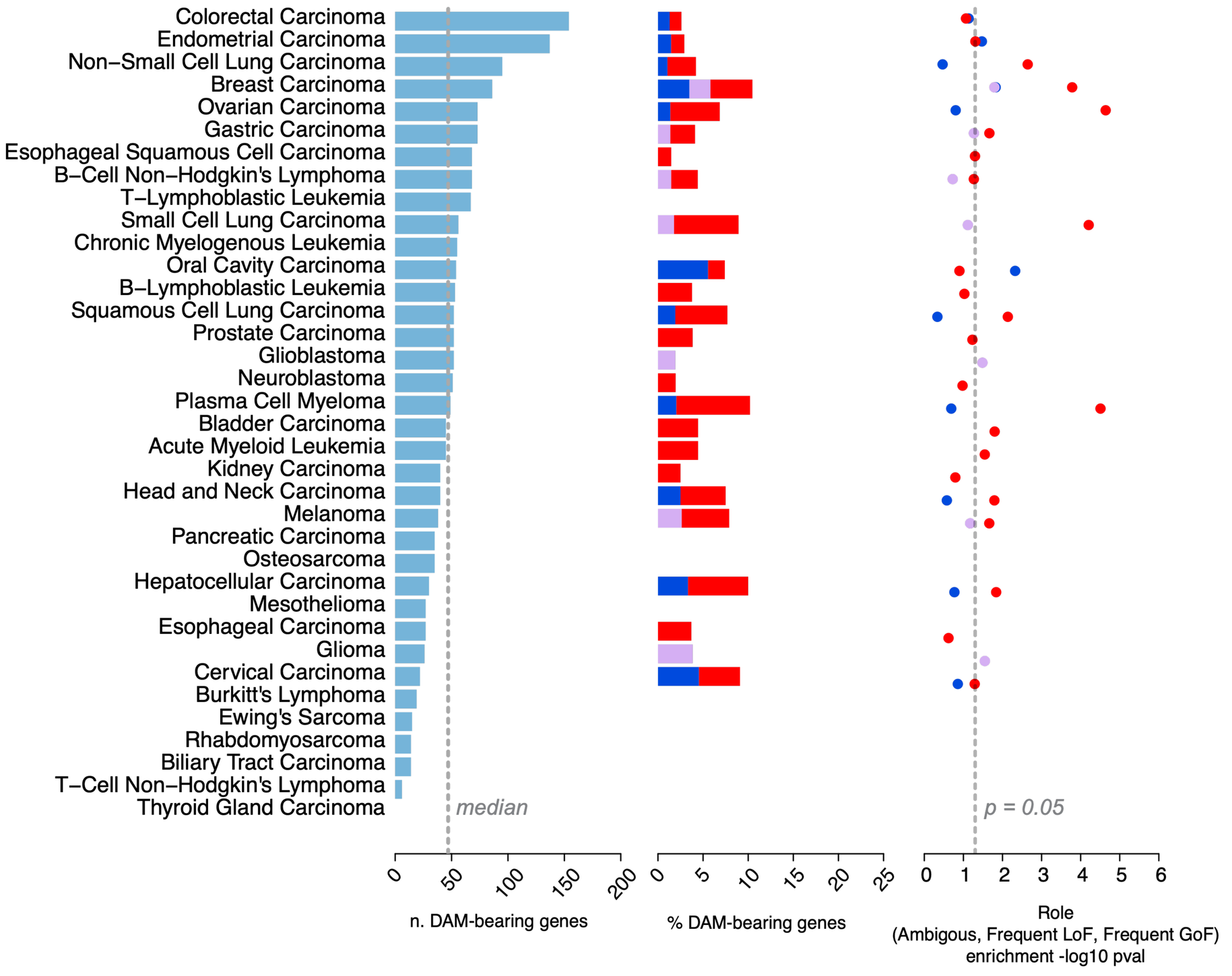

***…and there is a good match between DAM-hosting cell-lines and cell-lines that are dependent on the DAM-hosting gene***



# *DAM-bearing genes are enriched for known cancer drivers*



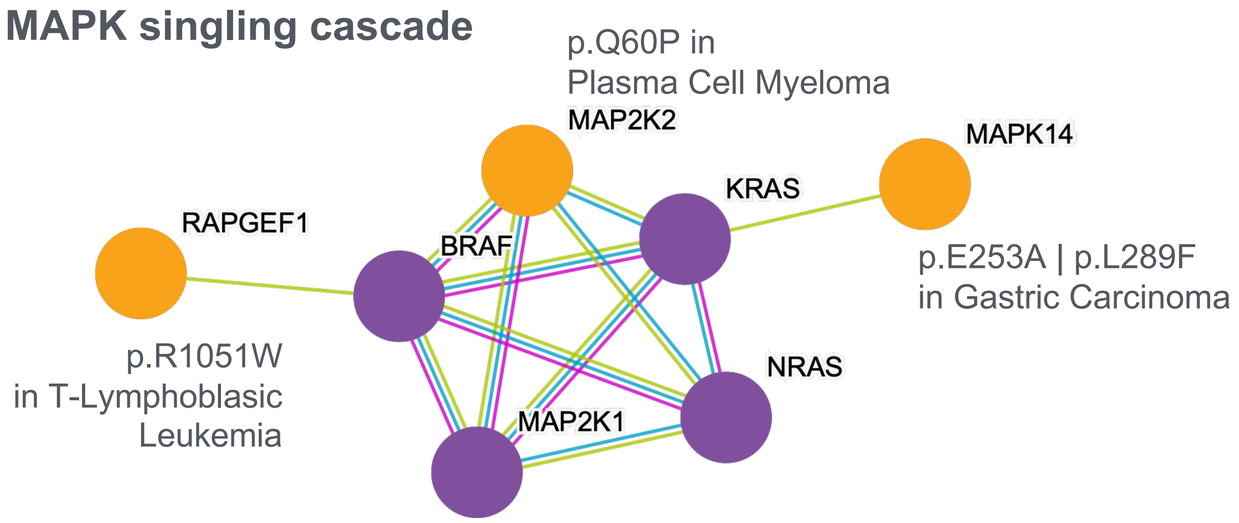
***DAMbgs are prominently enriched for GoF driver genes***

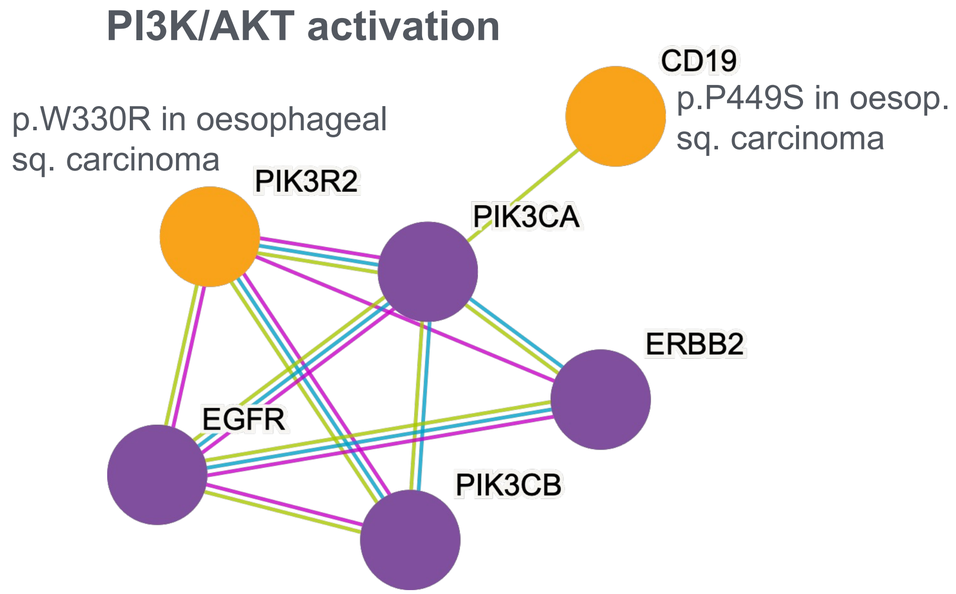


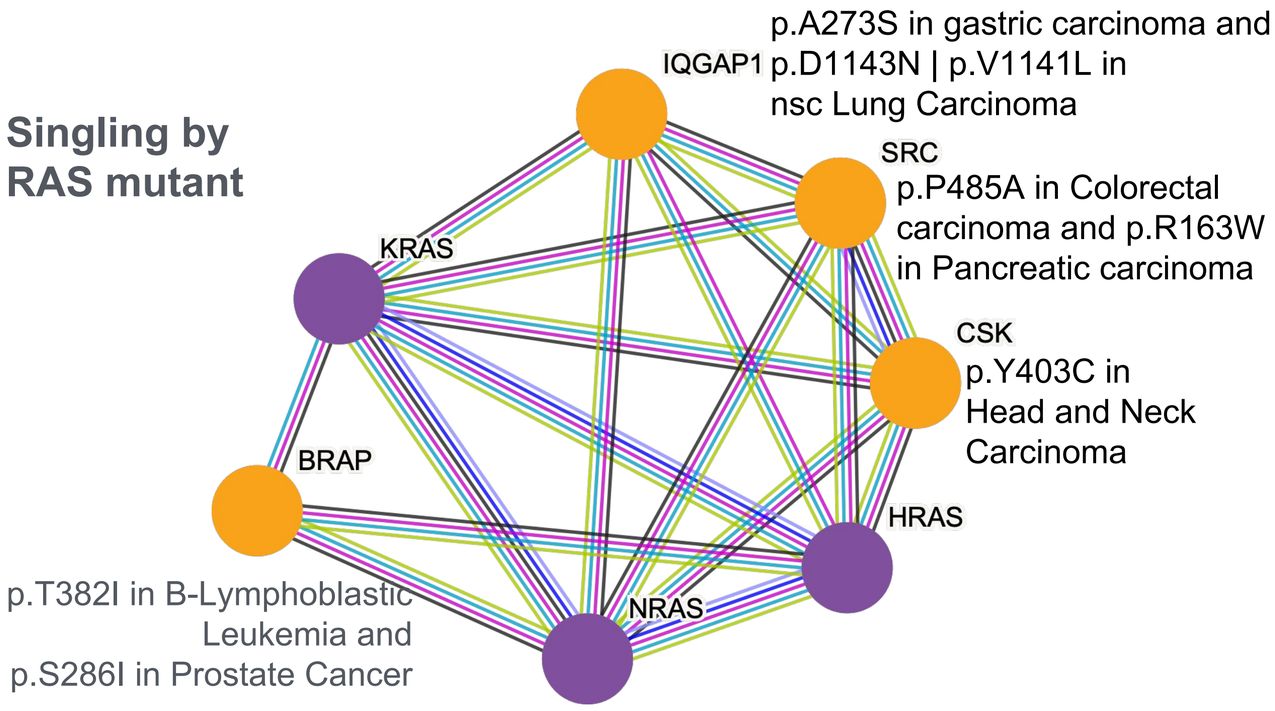

**Cancer driver role**

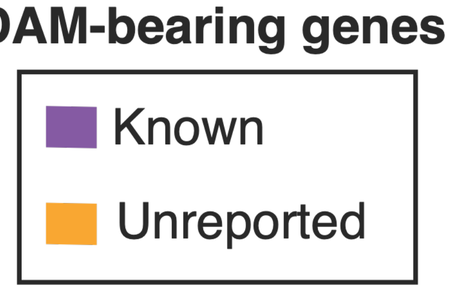
- Frequent GoF
- Frequent LoF
- Ambiguous

# *Dependency-Associated Mutations Converge on Canonical Cancer Pathways Highlighting Overlooked Nodes*



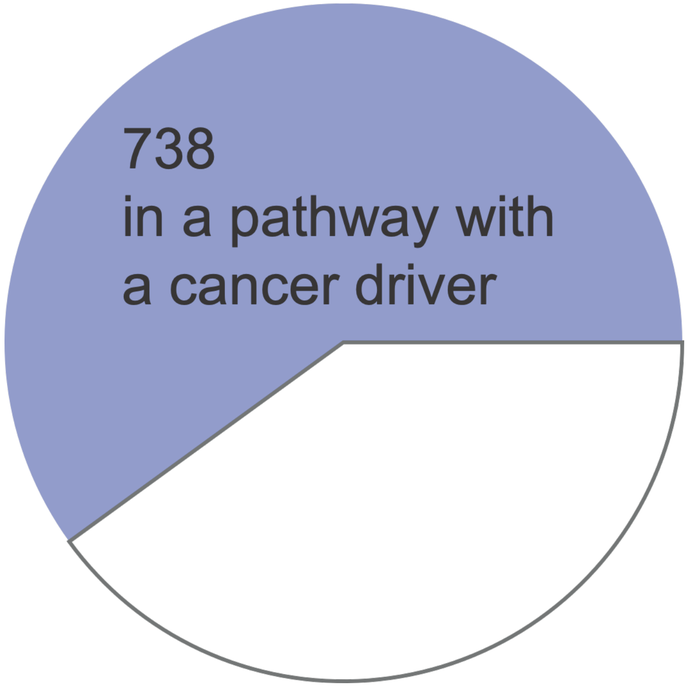



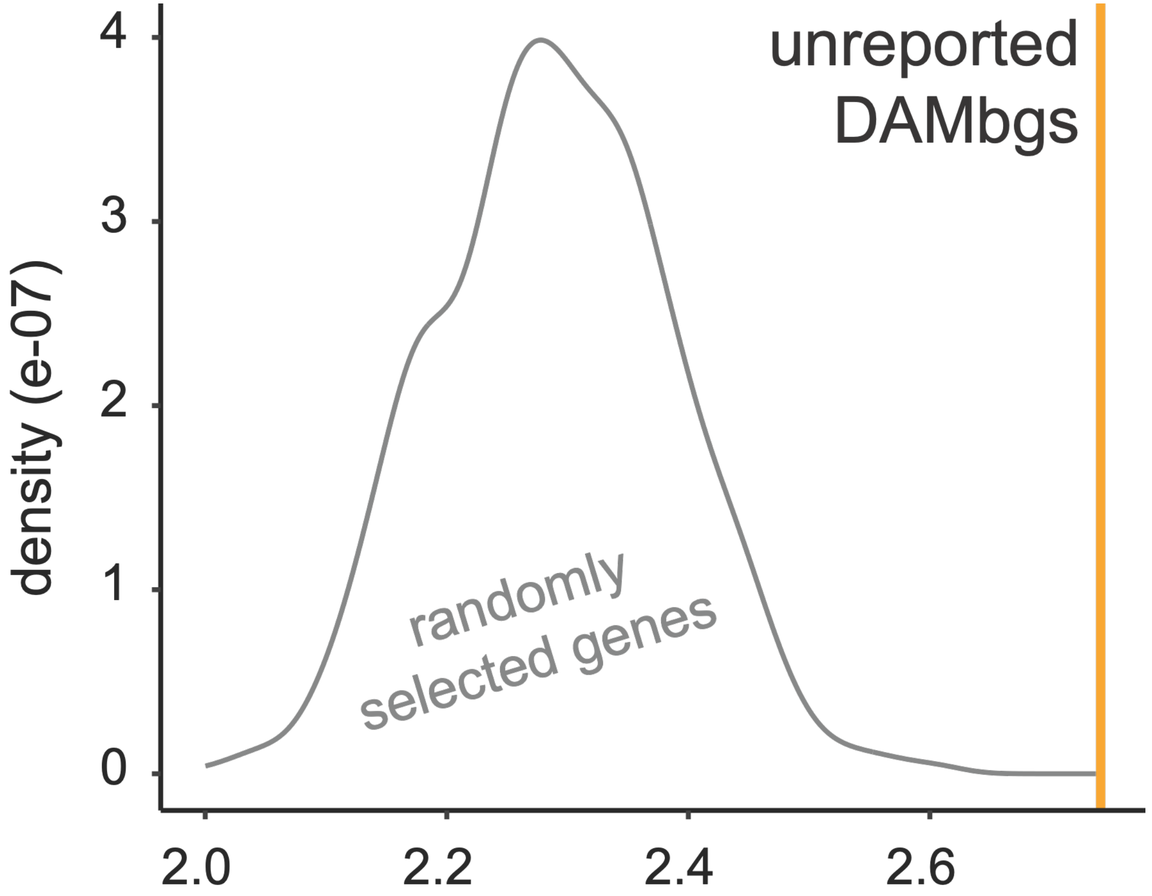
# *Unreported DAM-bearing genes are functionally connected to established cancer drivers*

**1,216 unreported DAM-bearing genes**

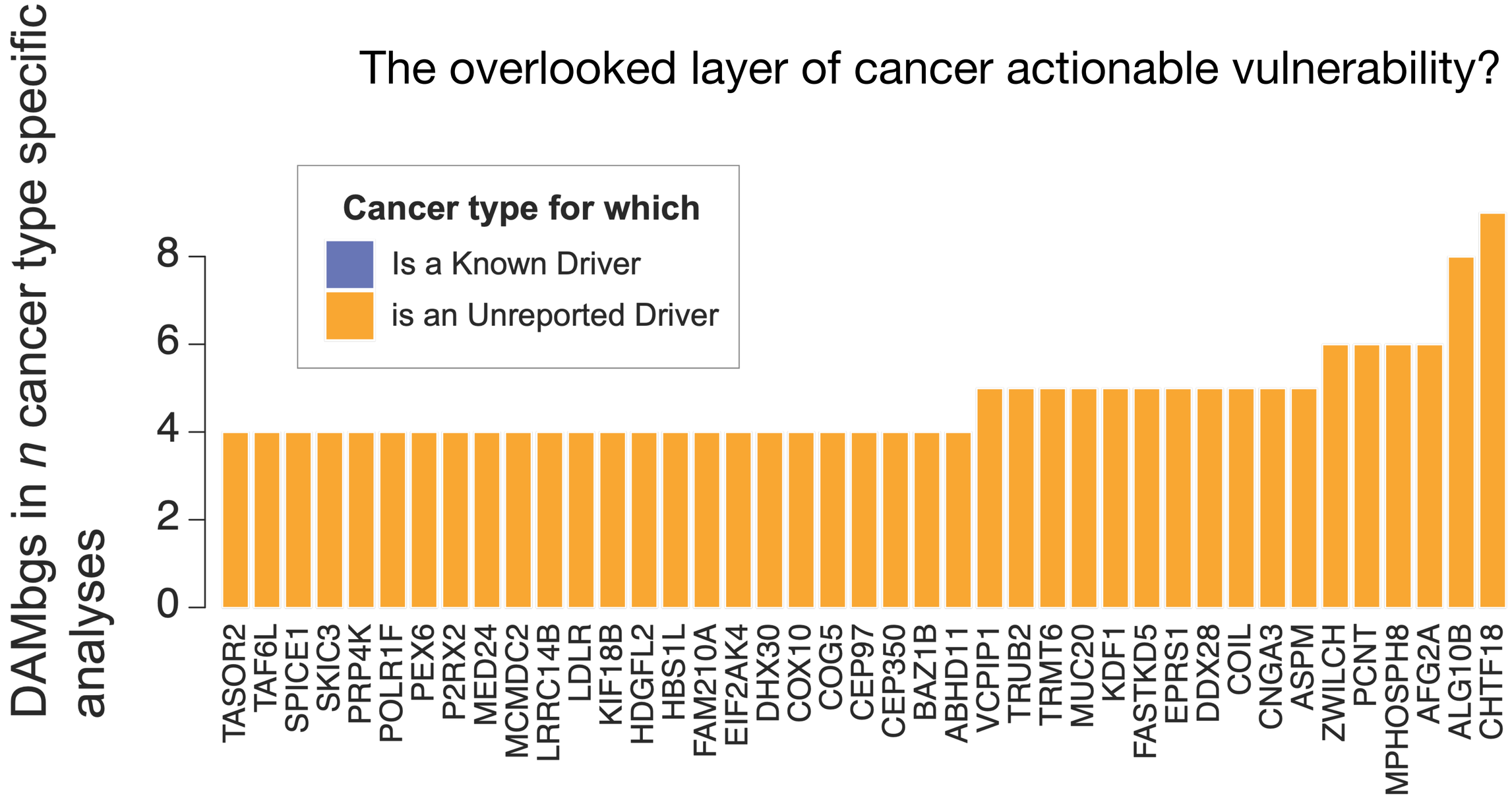



$p = 2.38 \times 10^{-70}$

expectation = 440

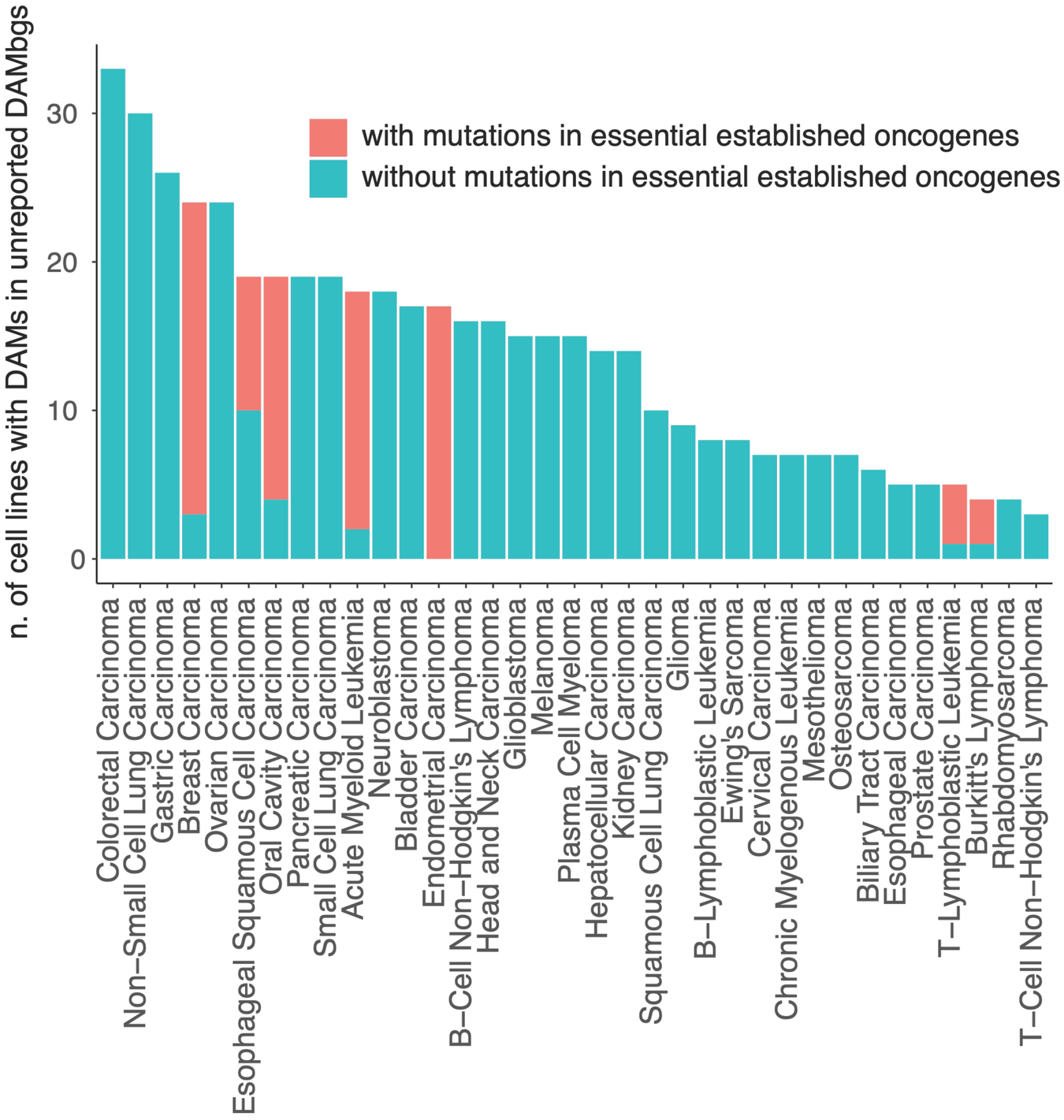


# *Most frequently detected DAM-bearing genes (unrported drivers)*

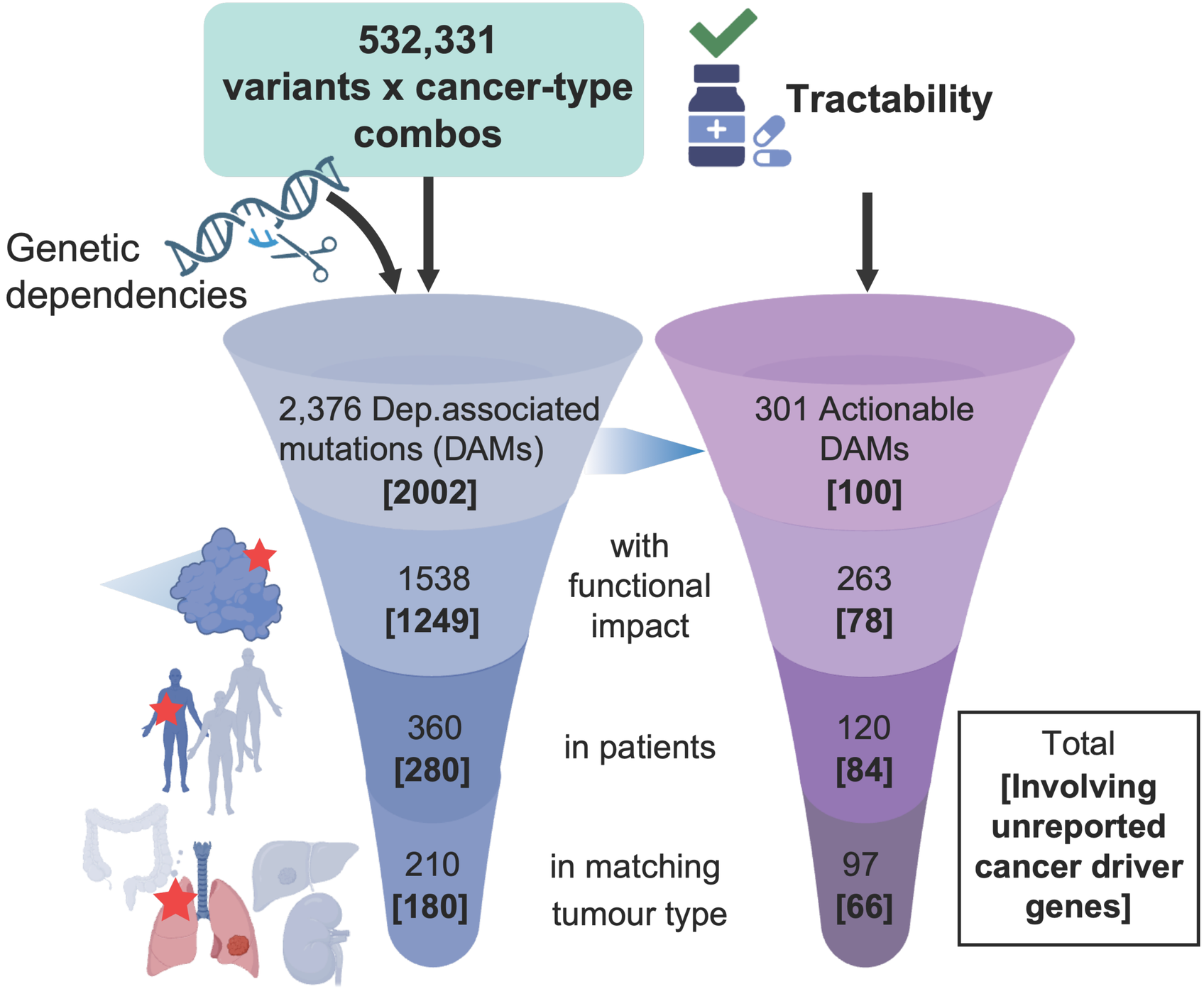


# *Most unreported DAMs are exclusive dependencies*

Vast majority of DAMs are found in cell lines where known activating driver mutations, typically conferring strong oncogenic dependency, are absent or not associated with hosting gene essentiality



**Many DAMs have functional impact,
are found in cancer patients and are
druggable**

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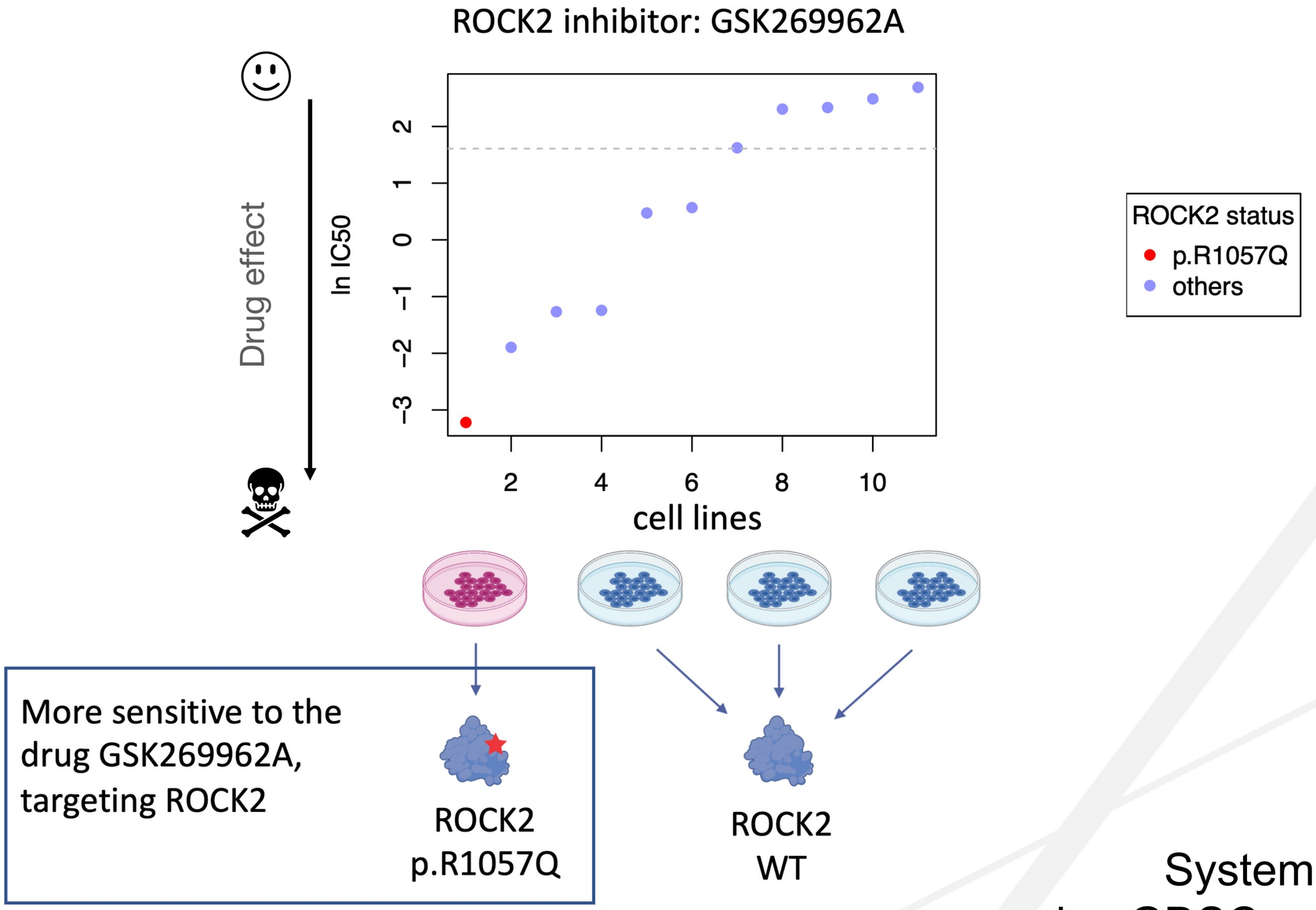
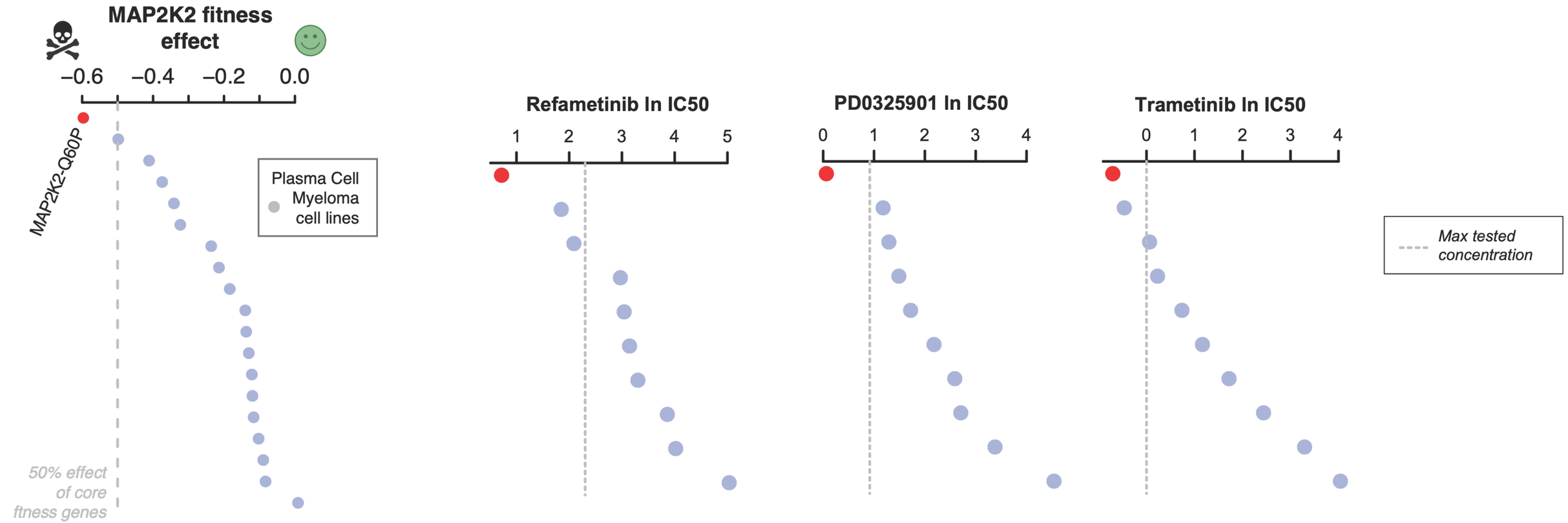
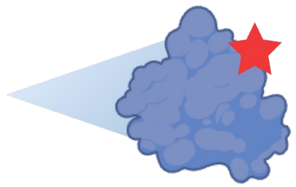
# *many DAMs are also associated with strong sensitivity to inhibitors of the mutated protein*




Systematic evaluation using GDSC and PRISM data

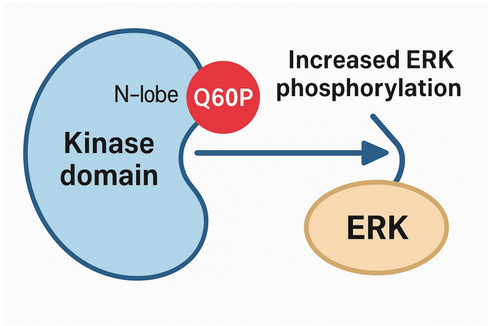
# *Rare variants define druggable dependencies in real patient populations*
# *ex. MAP2K2 Q60P in Plasma Cell Myeloma*



with SIFT/Polyphen predicted functional impact

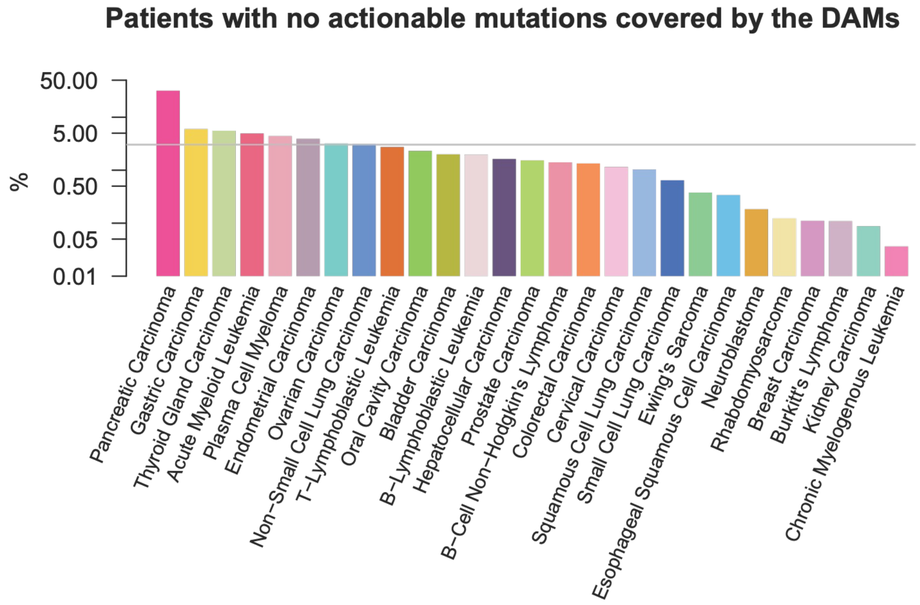
Detected in 6 PCM patients; MAP2K2 is not currently a PCM drive



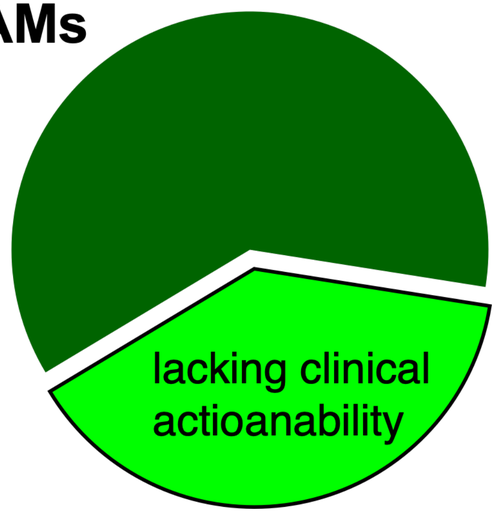

Mechanistically plausible

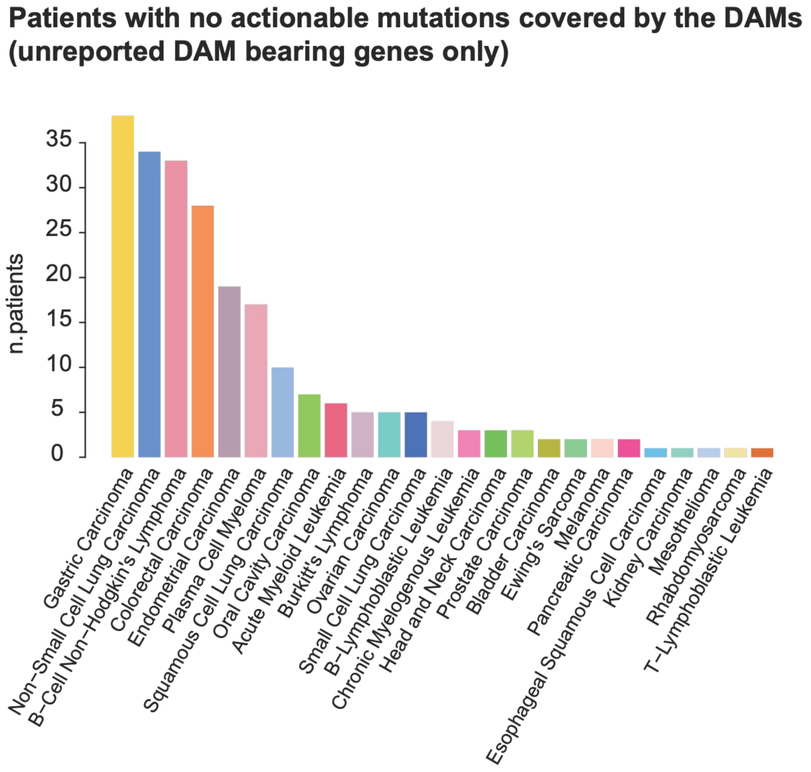
**drug repurposing opportunity with an immediate biomarker (Q60P)**

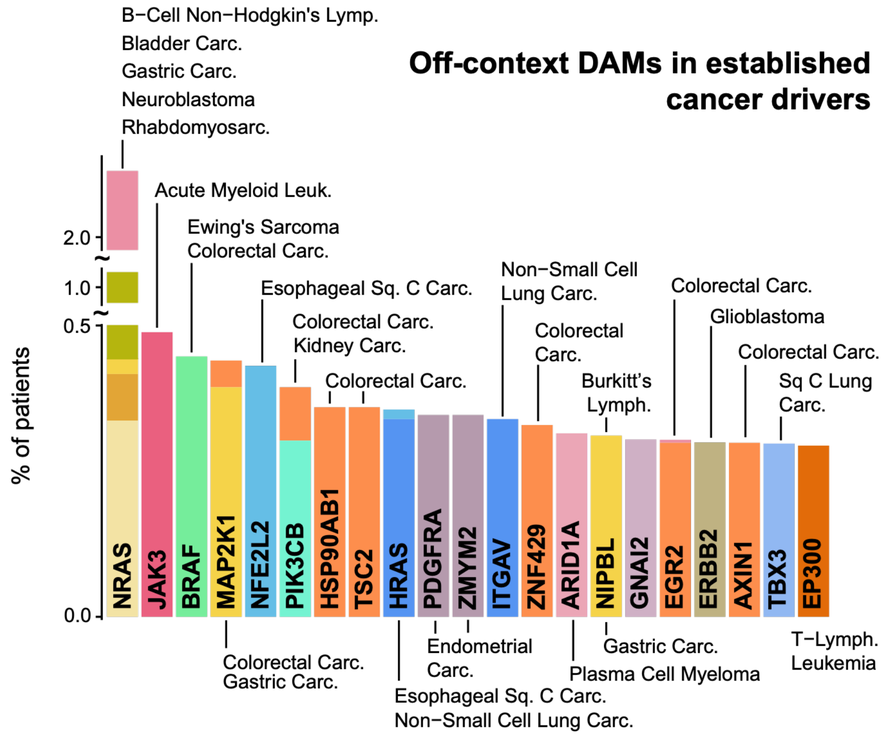
# *We uncover clinically relevant subcohorts that current oncology can't stratify*




**7,190 Patients with DAMs**

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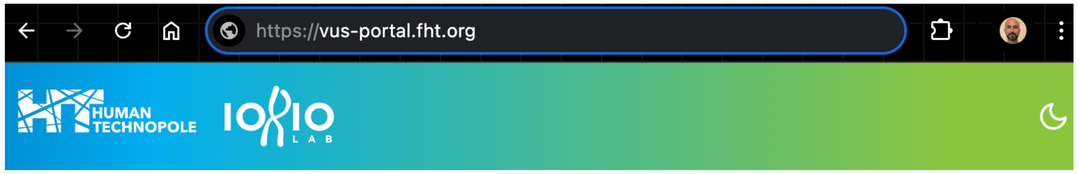


# *The CRISPR-VUS portal*

https://vus-portal.fht.org/

CRISPR VUS Portal

Search by gene | Show Top Variants | ← Back

Gene: NRAS

DAM Status
- is DAM

Polyphen
- Probably Damaging
- Possibly Damaging
- Benign
- Not Annotated

SIFT
- deleterious
- deleterious low confidence
- tolerated low confidence
- tolerated
- Not Annotated

#patients

sift
polyphen

Acute Myeloid Leukemia
B-Cell Non-Hodgkin's Lymphoma
B-Lymphoblastic Leukemia
Biliary Tract Carcinoma
Bladder Carcinoma
Breast Carcinoma
Burkitt's Lymphoma
Cervical Carcinoma
Chronic Lymphocytic Leukemia
Chronic Myelogenous Leukemia
Colorectal Carcinoma
Endometrial Carcinoma
Esophageal Carcinoma
Esophageal Squamous Cell Carcinoma
Ewing's Sarcoma
Gastric Carcinoma
Glioma
Glioblastoma
Head and Neck Carcinoma
Hepatocellular Carcinoma
Kidney Carcinoma
Melanoma
Mesothelioma
Neuroblastoma
Non-Small Cell Lung Carcinoma
Oral Cavity Carcinoma
Osteosarcoma
Ovarian Carcinoma
Pancreatic Carcinoma
Plasma Cell Myeloma
Prostate Carcinoma
Rhabdomyosarcoma
Small Cell Lung Carcinoma
Squamous Cell Lung Carcinoma
T-Cell Non-Hodgkin's Lymphoma
T-Lymphoblastic Leukemia
Thyroid Gland Carcinoma

NRAS-A146T
NRAS-A146V
NRAS-C186F
NRAS-G12D
NRAS-G12V
NRAS-K5R
NRAS-Q61H
NRAS-Q61K
NRAS-Q61L
NRAS-Q61R
All DAMs

CRISPR VUS Portal

Search by gene | Show Top Variants | ← Back

Gene: NRAS

**Cancer Type:** B-Cell Non-Hodgkin's Lymphoma
**Rank Ratio:** 1
**Variant:** NRAS-Q61K (is DAM: true)

Variant Status
- Present
- Absent

log(rc)

NRAS-Q61K

Cell Lines

OCI-Ly-19
SUVL
JVM-2
SCC-3
KARPAS-1718
RL
MC116
OCI-Ly18
H1
SU-DHL-10
Farage
KARPAS-422
U-2904
SU-DHL-4
WSU-NHL
BC-1
SU-DHL-5
RIDS-50
AS-KAW
DB
Ri-1
BC-3
KML-1
SU-DHL-8

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# CRISPR-enhanced assessment of variants of unknown significance nominates oncology therapeutic targets and drug repositioning opportunities

Aurora Savino, Athanasios Oikonomou, Riccardo R De Lucia, Miguel L Grau, Kathrina McCarten, Hanna Najgebauer, Umberto Perron, Luca Azzolin, Alexandra Livanova, Paolo Cremaschi, Nuria Lopez-Bigas, Andrea Sottoriva, Francesco Iorio

**doi:** https://doi.org/10.64898/2026.01.20.700565

**In response to reviewers' comments, we are performing a pooled prime-editing screen introducing ~2,000 DAMs into non-transformed immortalised cell lines to test their causal role in generating oncogenic fitness and targetable dependencies.**

# *Summary*

- CRISPR-based approach to identify **Dependency-Associated Mutations (DAMs):** VUS associated with self-addictions

- DAMs frequently affect **non-canonical driver genes**

- DAMs complement known oncogenic addictions, completing cancer pathways and revealing **novel vulnerabilities**, especially in **genomically quiet** tumours

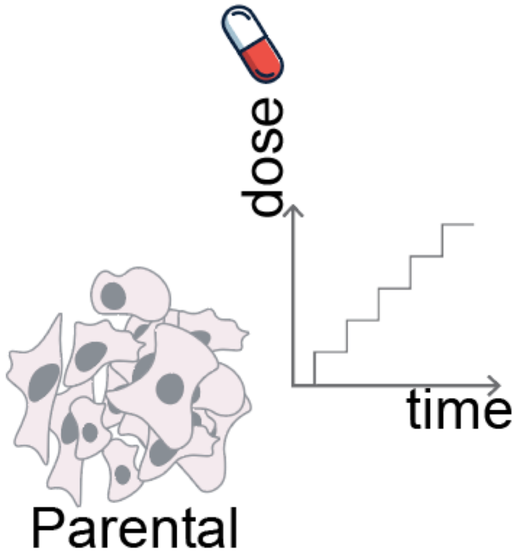
- A subset of DAMs also associate with increased drug sensitivity (**SAMs**), offering **variant-guided opportunities for repositioning**

- Many DAMs and SAMs are **observed in patient data**, albeit in minority subsets

- This strategy expands the reach of precision oncology to patients **beyond targetable genotype carriers**

# Cancer Drug Sensitivity-Recovery Map

## Identifying vulnerabilities of drug adapted cancer cells





New genetic dependencies
not present in the parental ?

1) targets for combinatorial therapies

2) mechanistic insights

# Progress to date

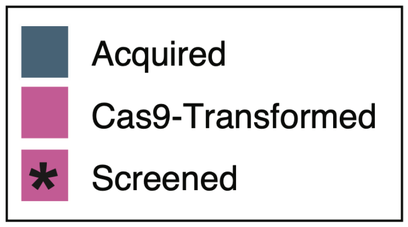
19 parental (drug sensitive) cell lines

89 drug-resistant clones

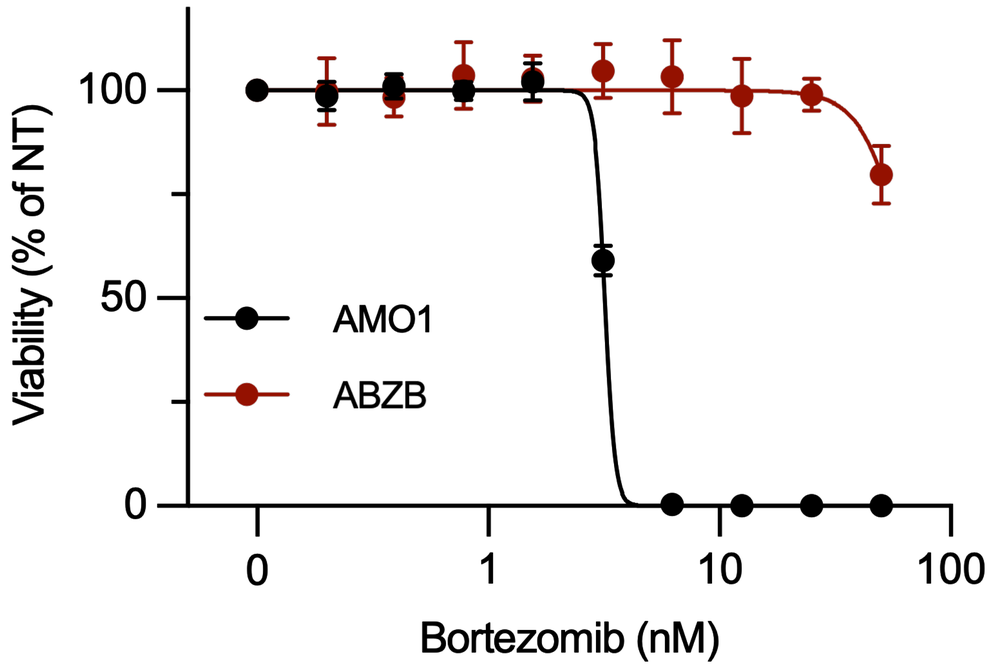
6 Cancer types

9 Drugs

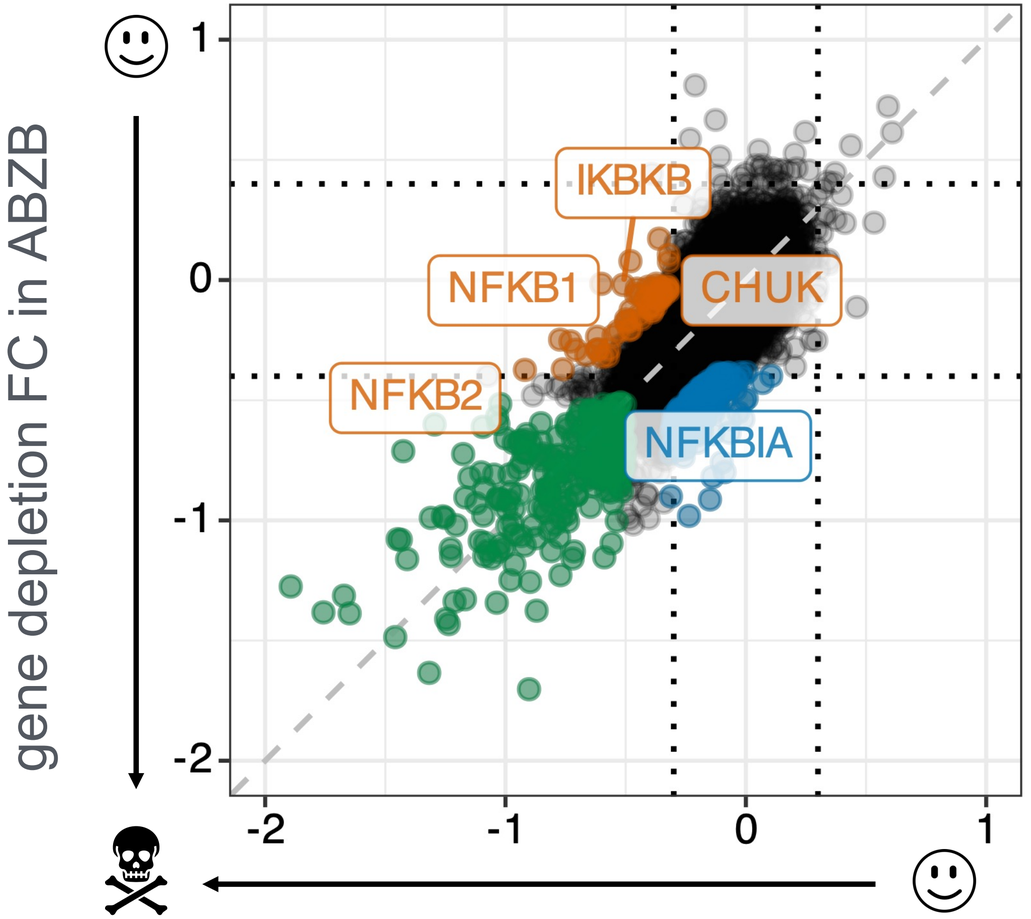
*N. Radic*



# Identifying secondary vulnerabilities of cancers with acquired drug resistance

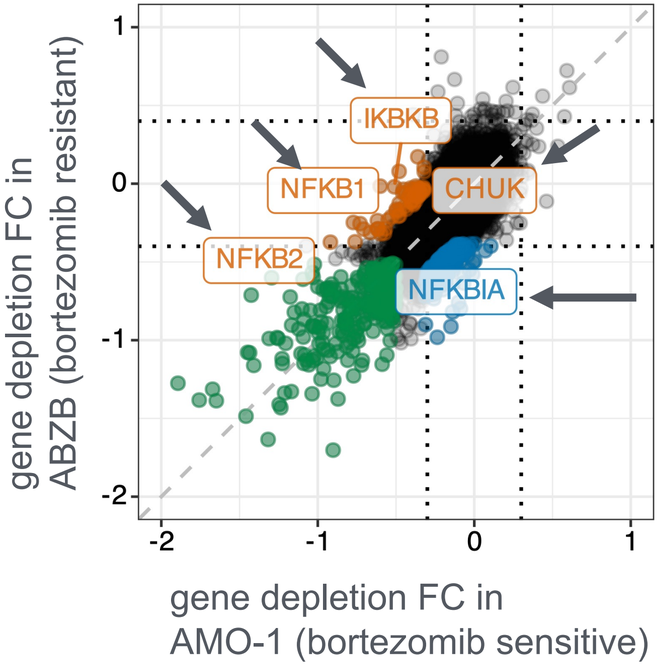


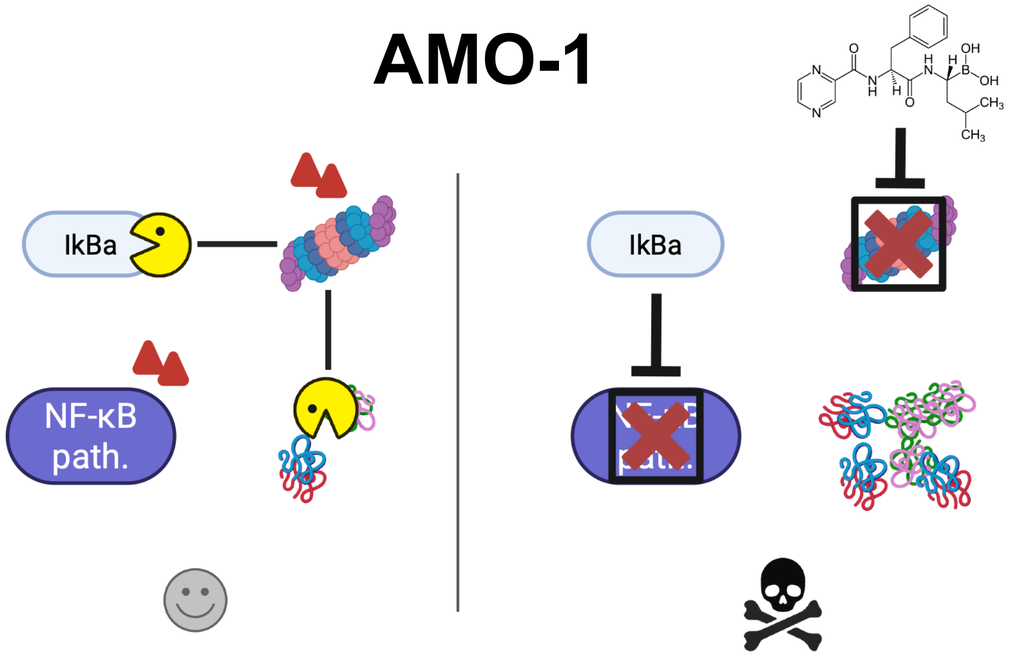

*N. Radic*



# NFKBIA ko is synthetic lethal with bortezomib resistance

## Expected hits for AMO-1



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