Expression-based variant impact phenotyping of somatic mutations in cancer

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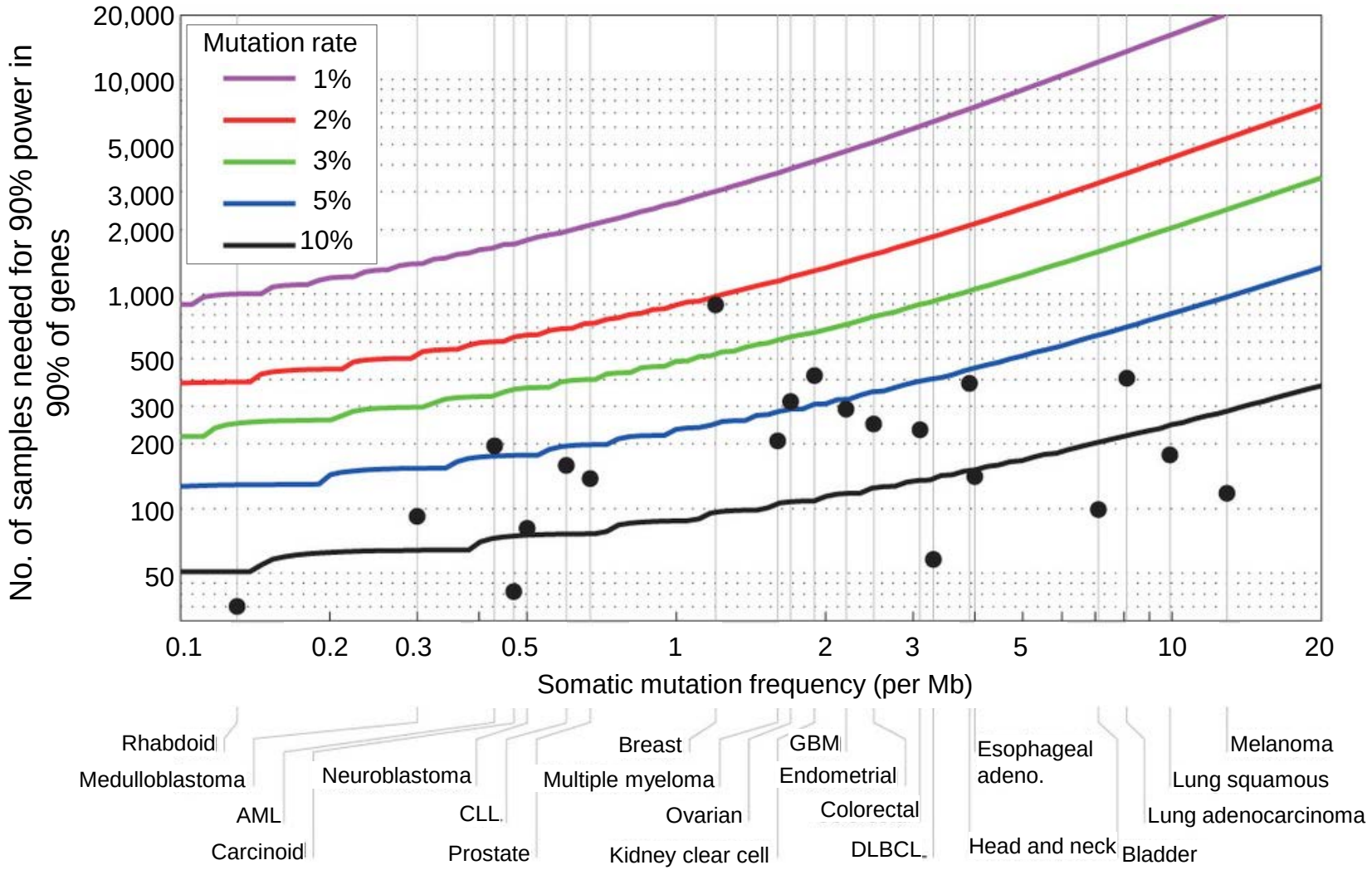
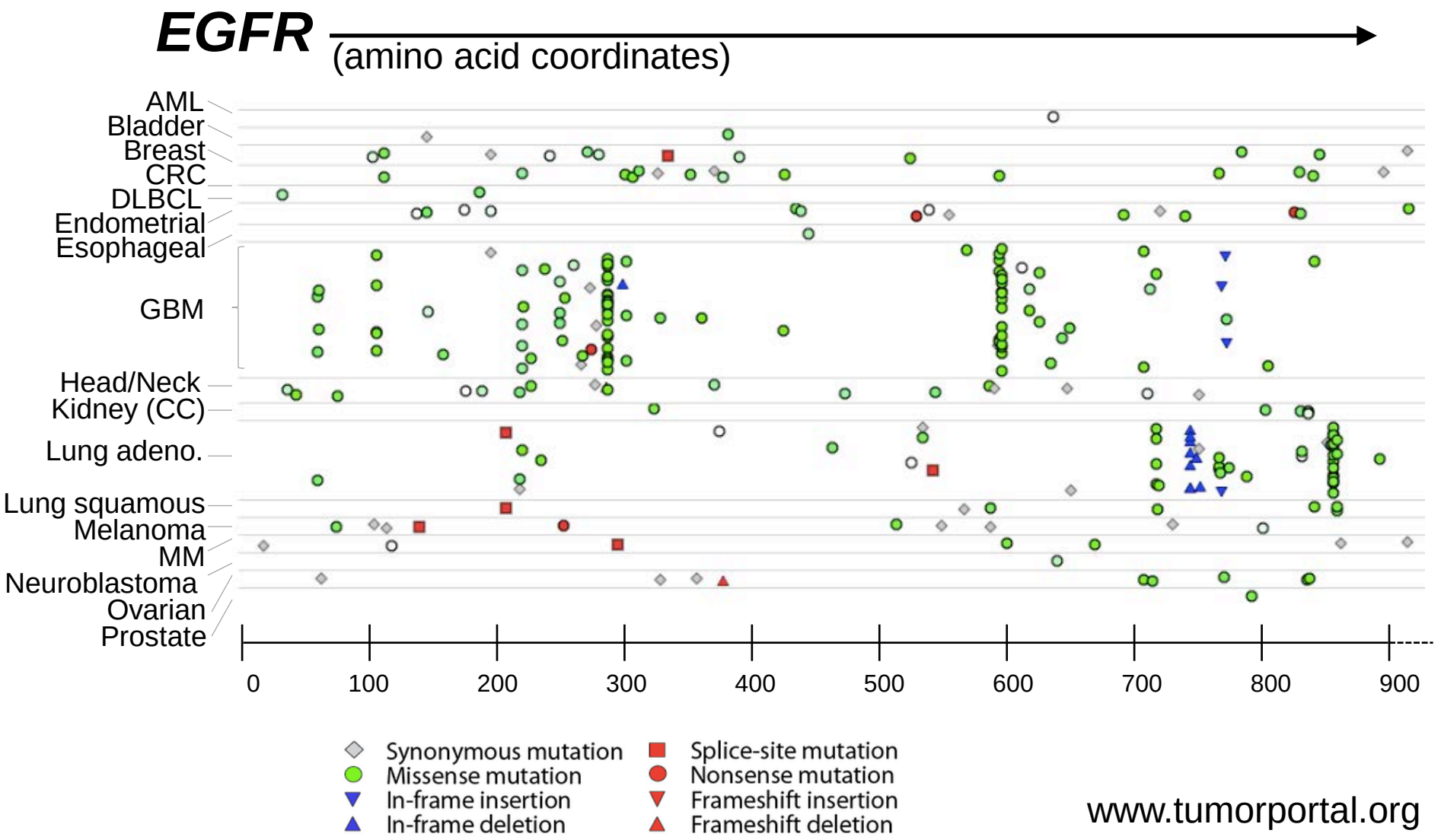
Cancer sequencing studies are underpowered to find all functionally relevant mutated genes



Lawrence et al. *Nature* 2014

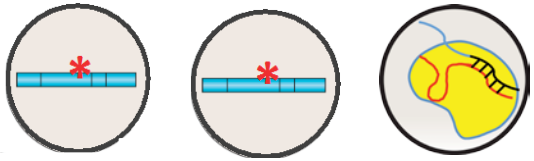
Sequencing studies are even more underpowered to find impactful variants from passengers



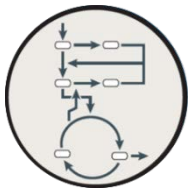
www.tumorportal.org

Approaches to high-throughput experimental variant impact phenotyping

Somatic variants from cancer genome studies



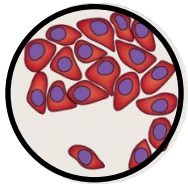
Variant-specific reagents: open-reading frame (ORF) or CRISPR



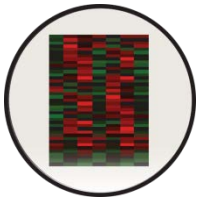
Genetic interactions

Multiplexed tumorigenesis

Pathway-specific assays



Morphologic profiling



Expression profiling

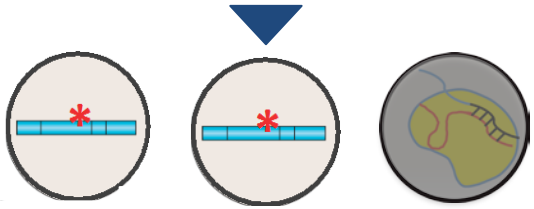
Gene-agnostic assays

TargetAccelerator
Disease variants to targets

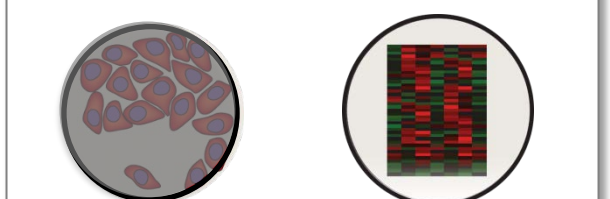
Lab of Jesse Boehm

Expression-based variant impact phenotyping

Somatic variants from cancer genome studies



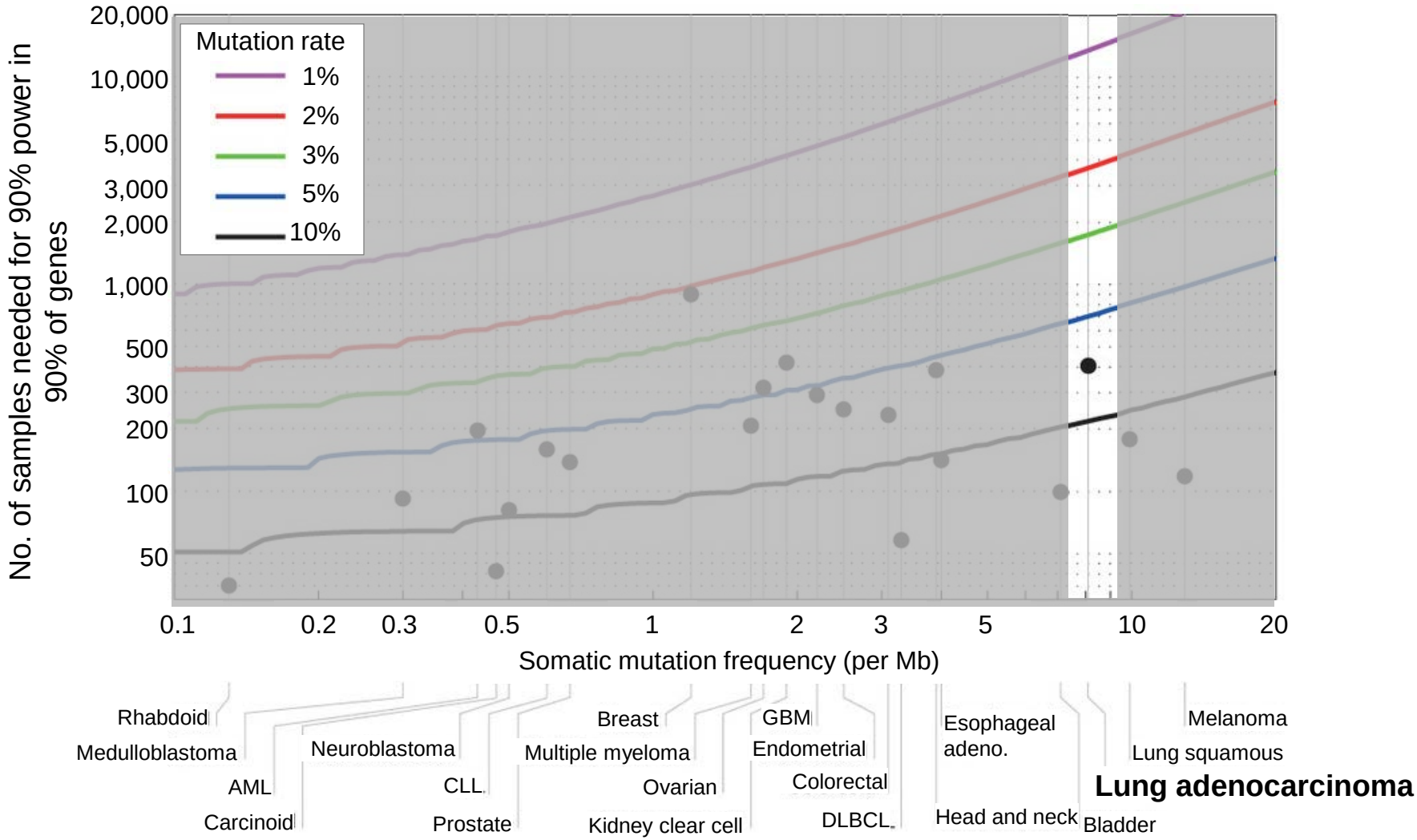
Variant-specific reagents: **open-reading frame (ORF)** or CRISPR



TargetAccelerator
Disease variants to targets

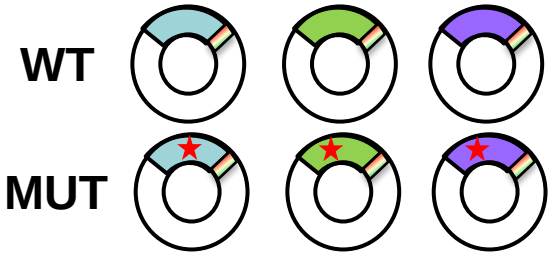
Lab of Jesse Boehm

Testing functional impact of somatic mutations in lung adenocarcinomas



Lawrence et al. *Nature* 2014

Approach: Compare gene expression changes upon introduction of WT and mutant variants



ORF library

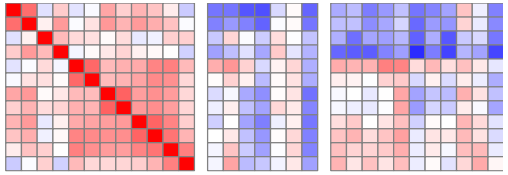
- 47 genes, 303 alleles
- Missense and indel variants found in >400 lung adenocarcinomas*
  - *KEAP1*: 38 alleles + WT
  - *KRAS*: 12 alleles + WT
  - *EGFR*: 11 alleles + WT

A549 lung adenocarcinoma cell line

384 well plates & replicates

96h

L1000 gene expression profiling



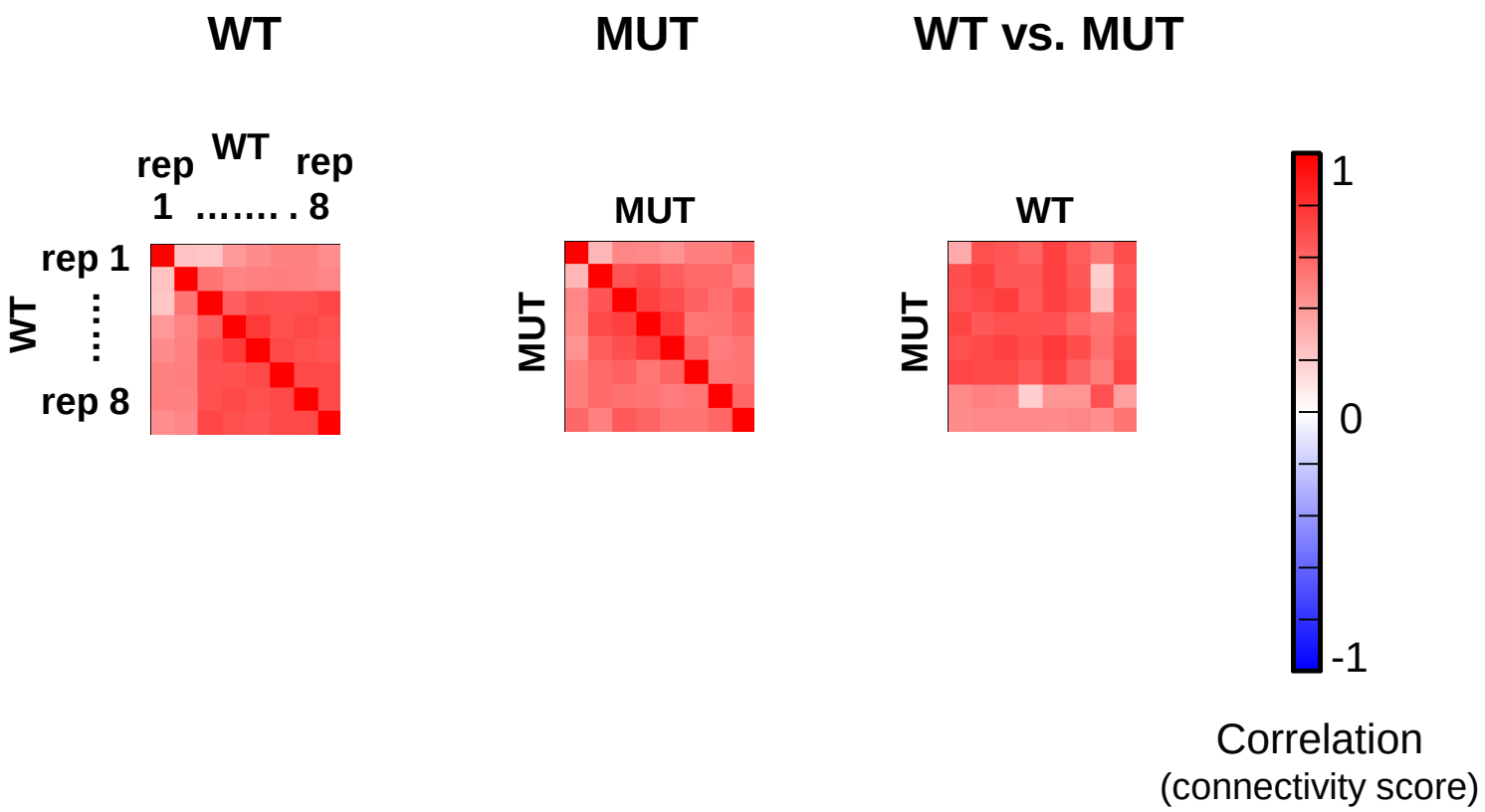
(luminex-based profiling of 1000 transcripts)

*Imielinski et al. 2012, TCGA Nature 2014

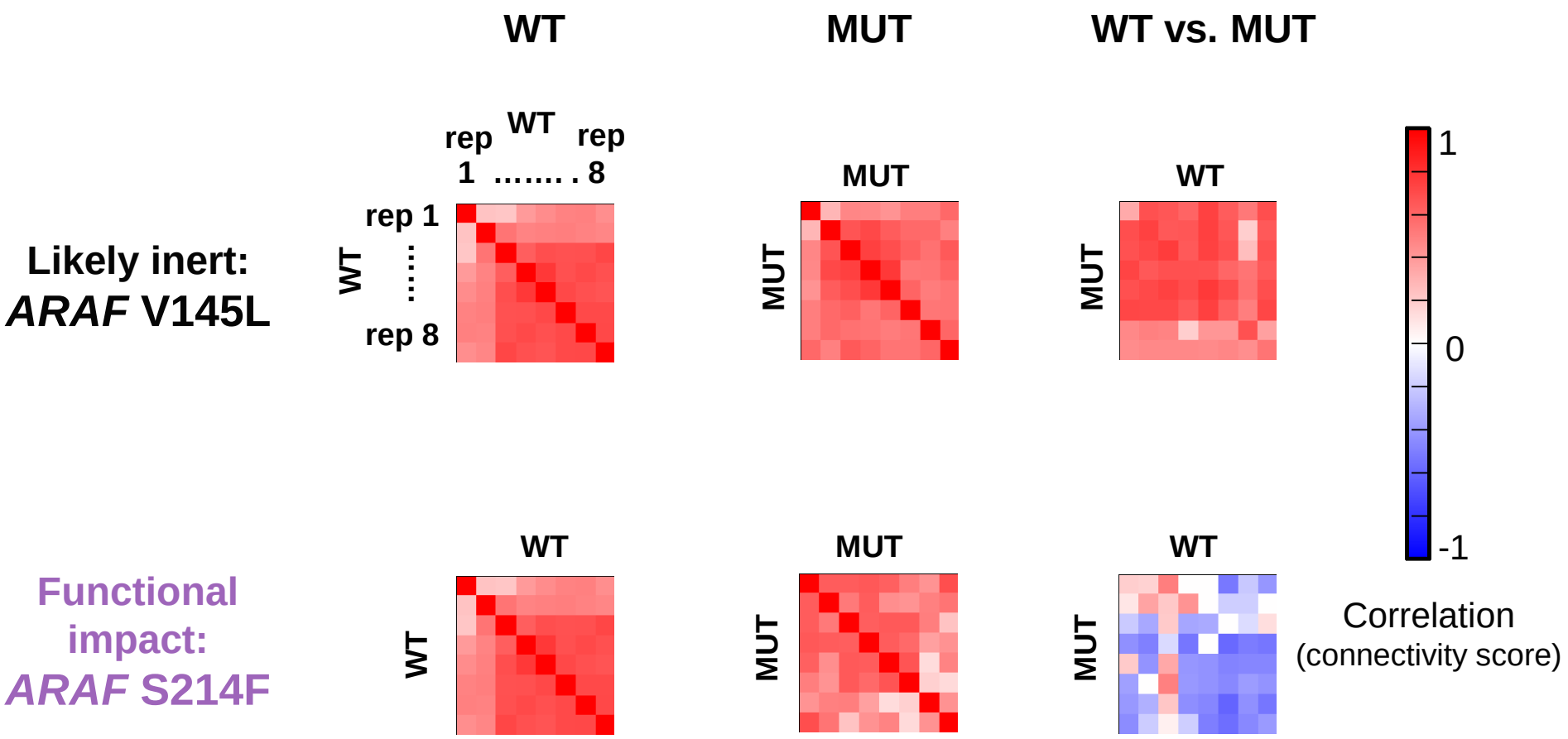
Broad Genetics Perturbation Platform
Broad Connectivity Map

Comparing changes in gene expression can predict variant impact

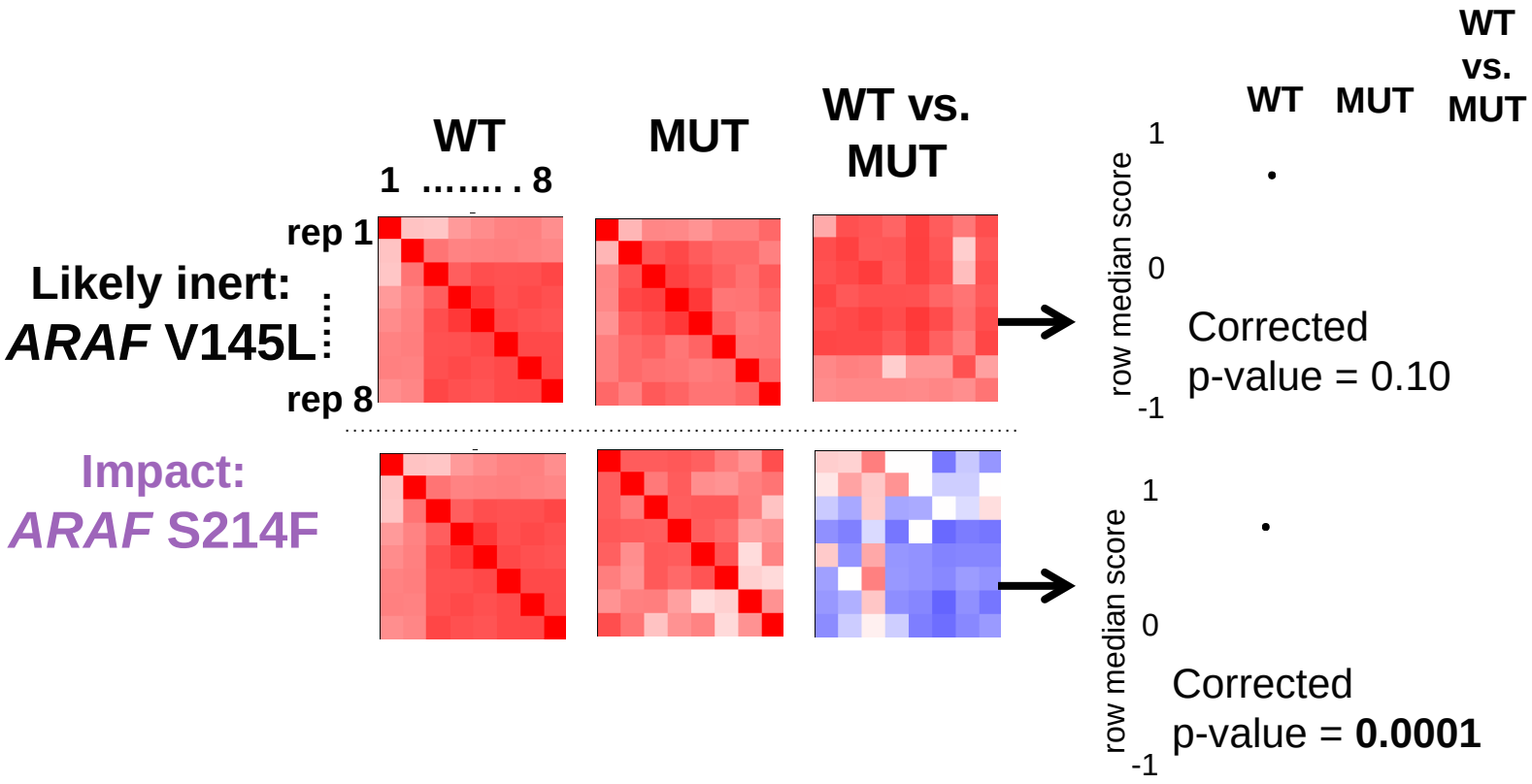
Likely inert:
***ARAF* V145L**



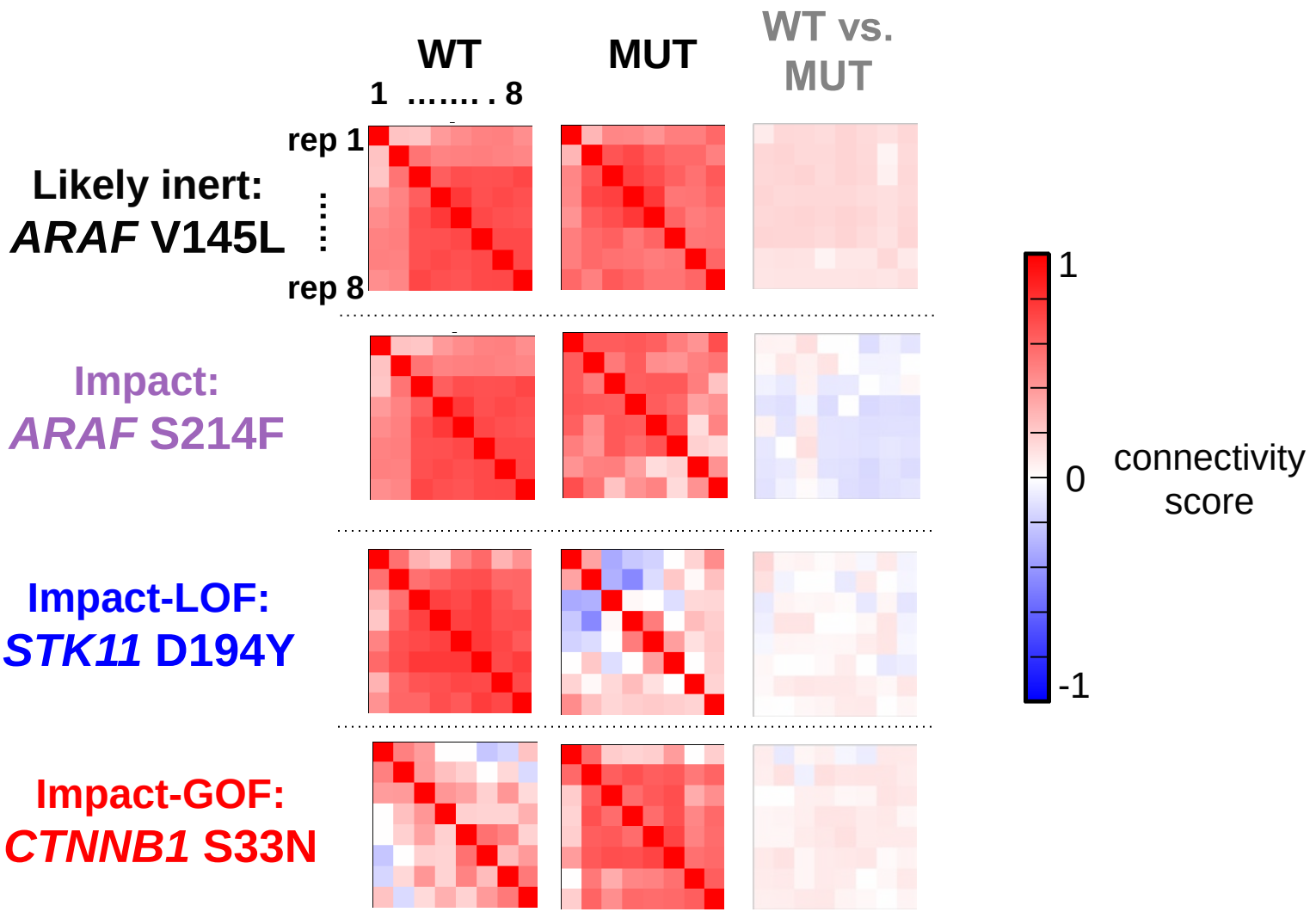
Comparing changes in gene expression can predict variant impact



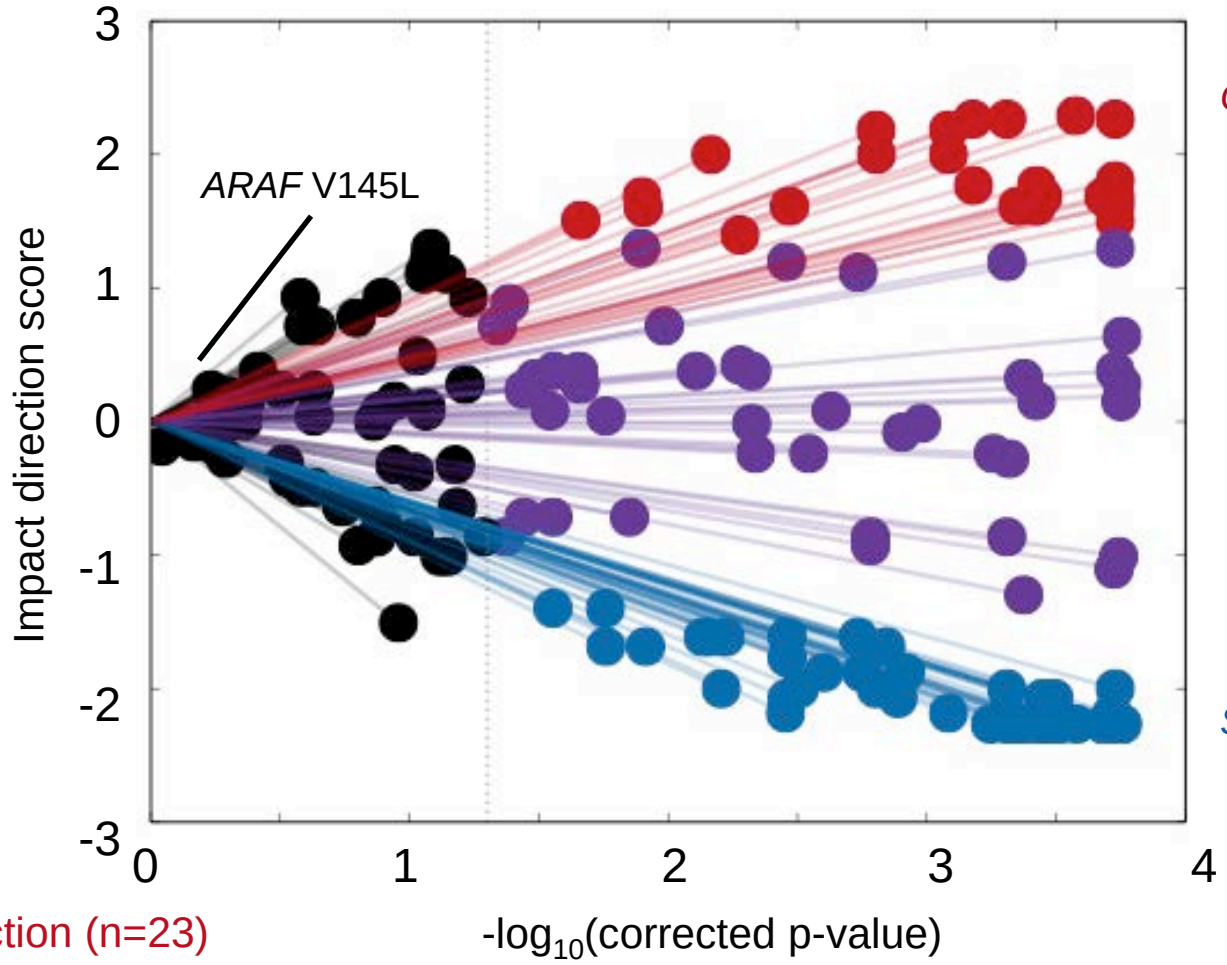
Variant impact prediction is determined by a Kruskal-Wallis test (FDR 5%)



Impact direction score can predict loss-of-function or gain-of-function change



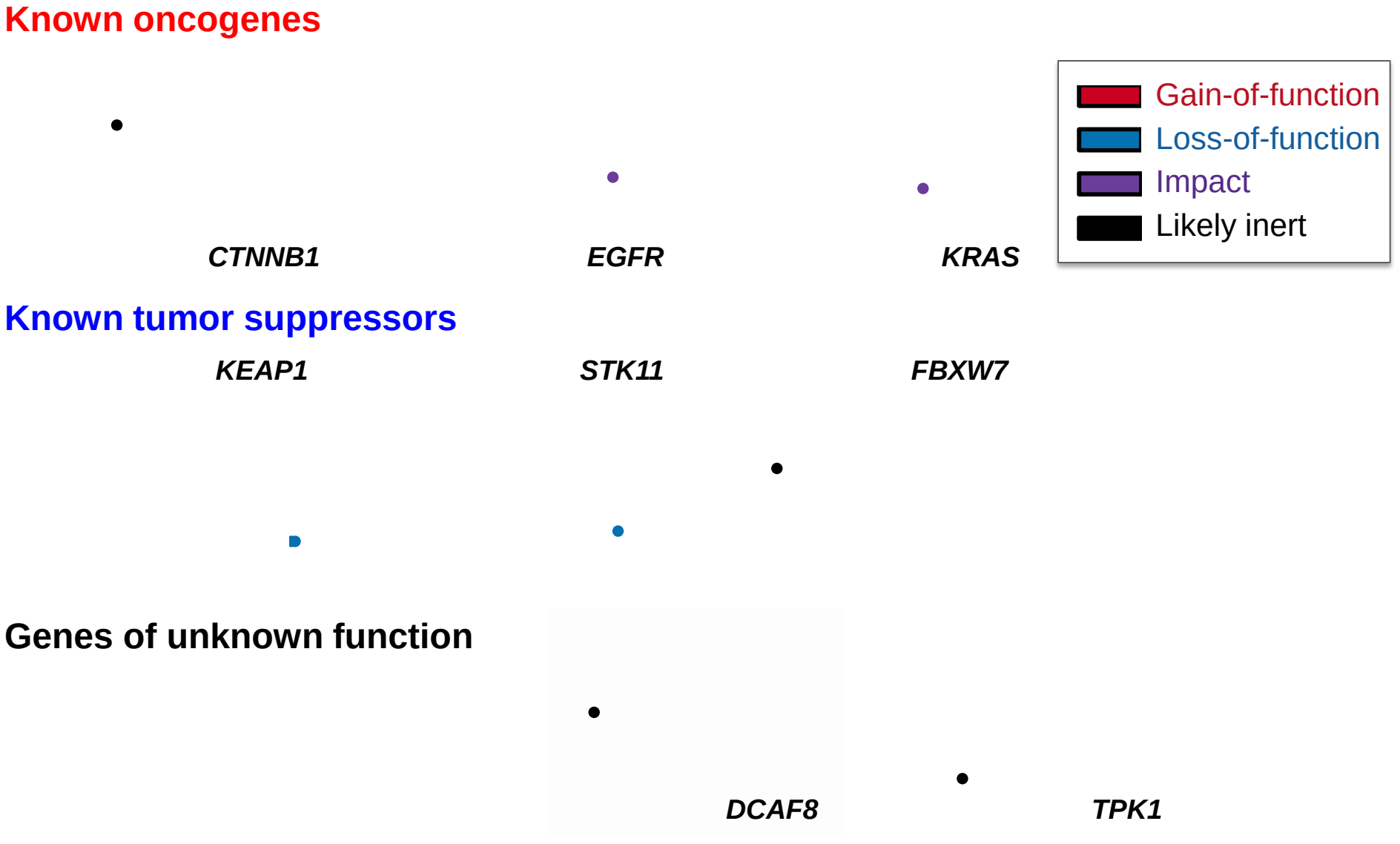
Sparkler plot of variant impact phenotyping for 151 lung cancer somatic mutations



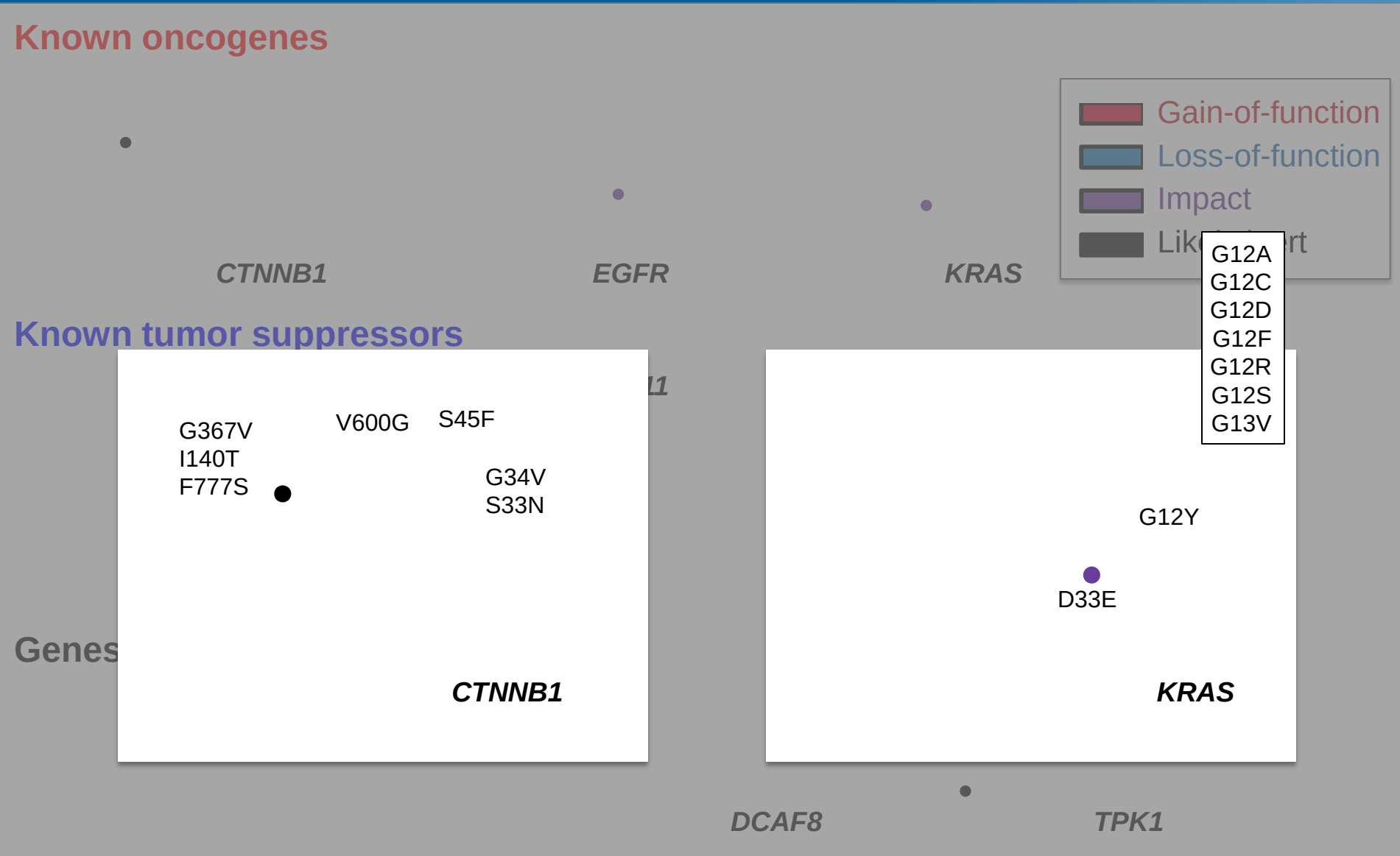
Gain-of-function (n=23)
Loss-of-function (n=49)
Impact (n=34)
Likely inert (n=45)

Ryo Sakai
Bang Wong

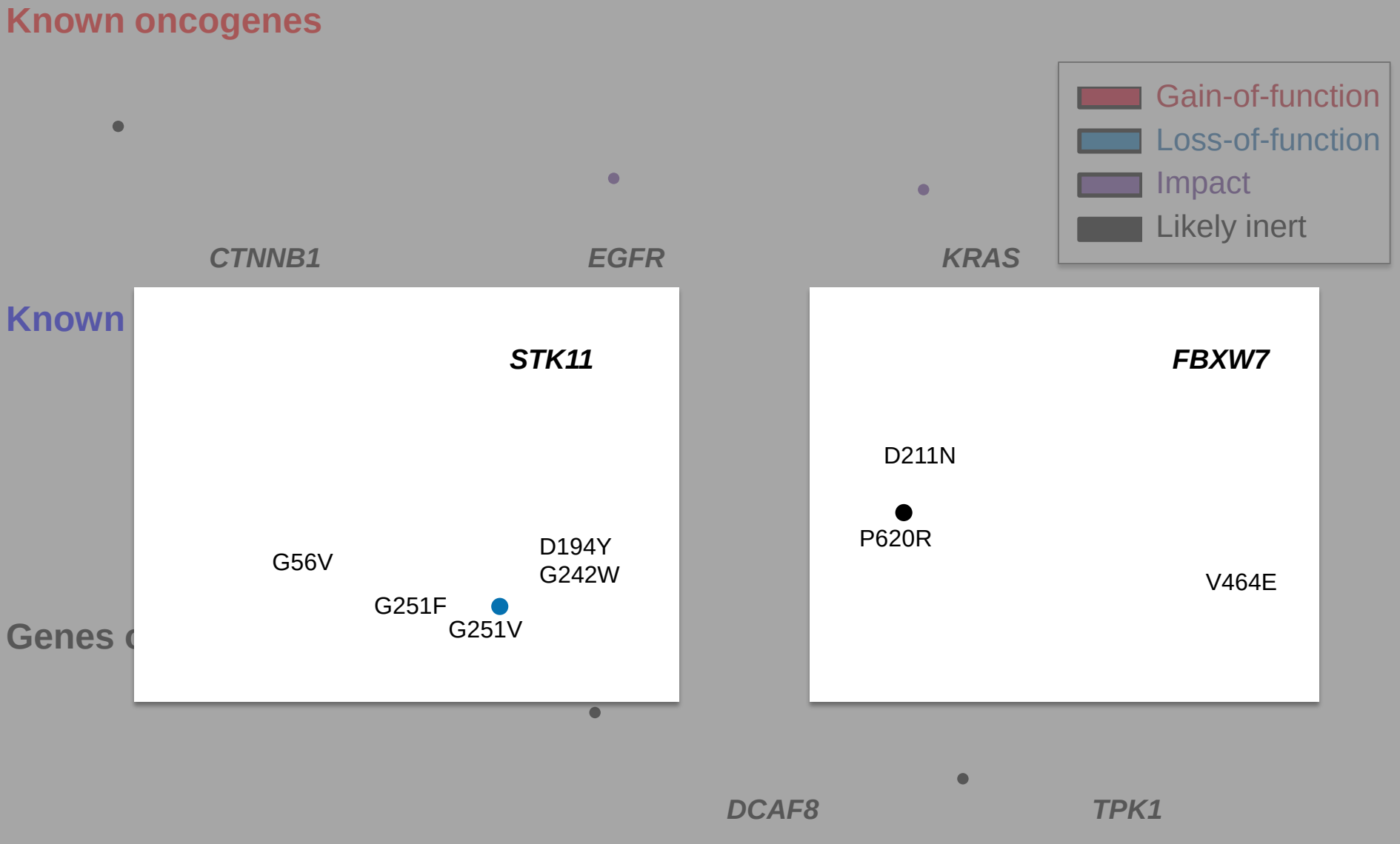
Gene-by-gene variant impact phenotyping



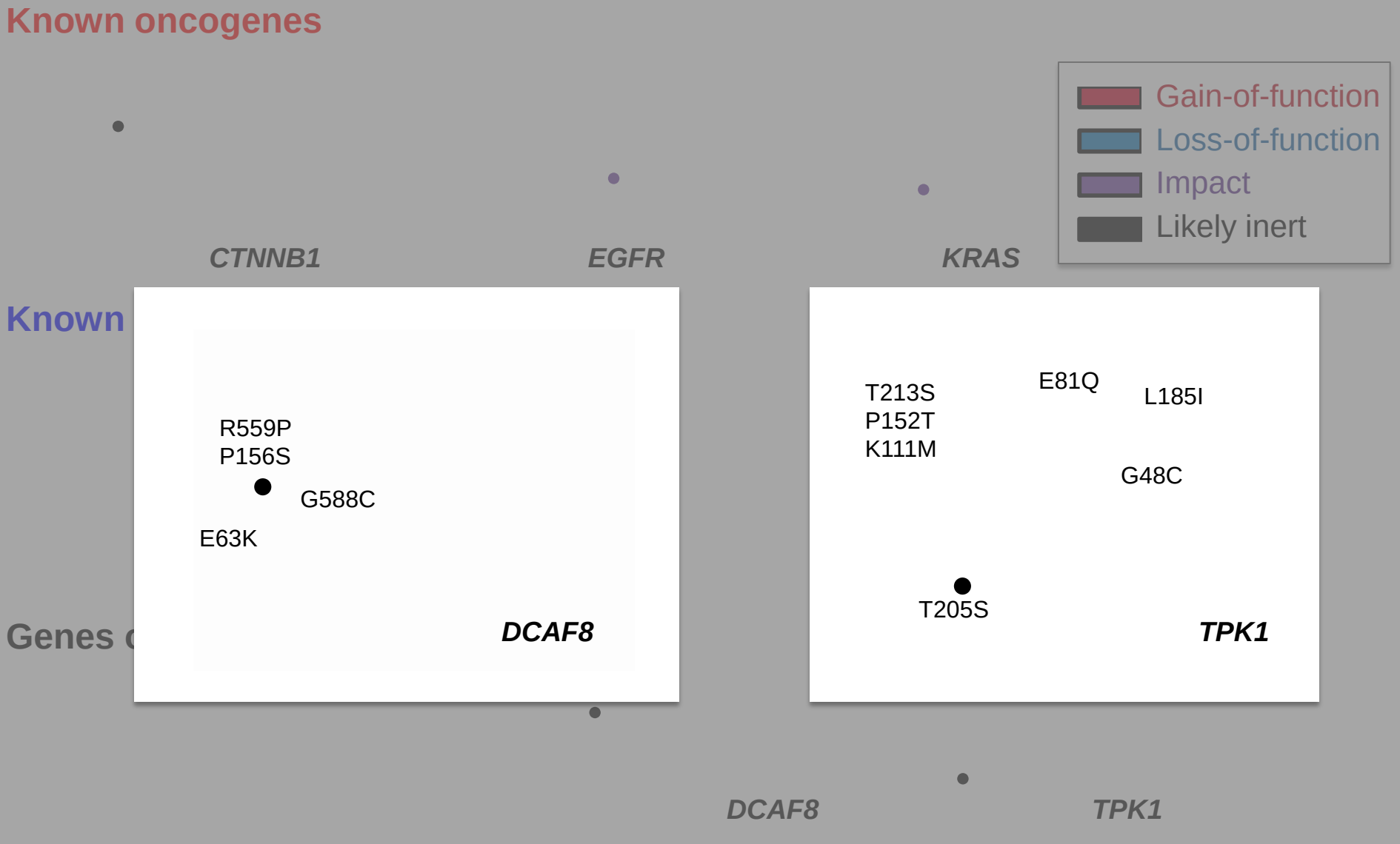
Gain- and change-of-function predictions of rare mutations in known oncogenes



Loss-of-function mutations in known tumor suppressor genes



Predicted impact of mutations in genes with unknown function



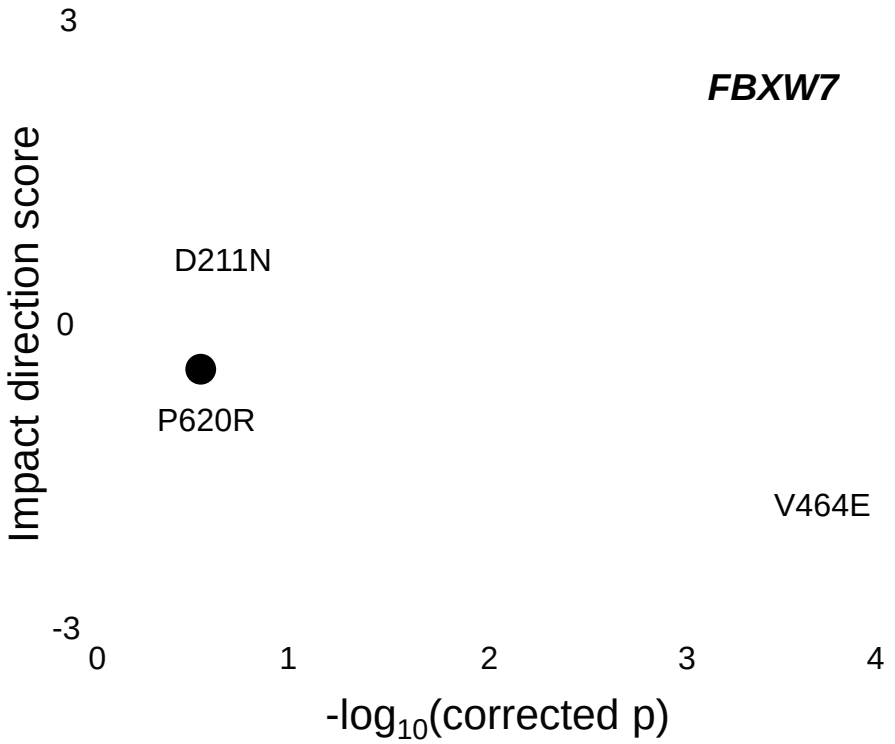
How do we know these predictions are correct?

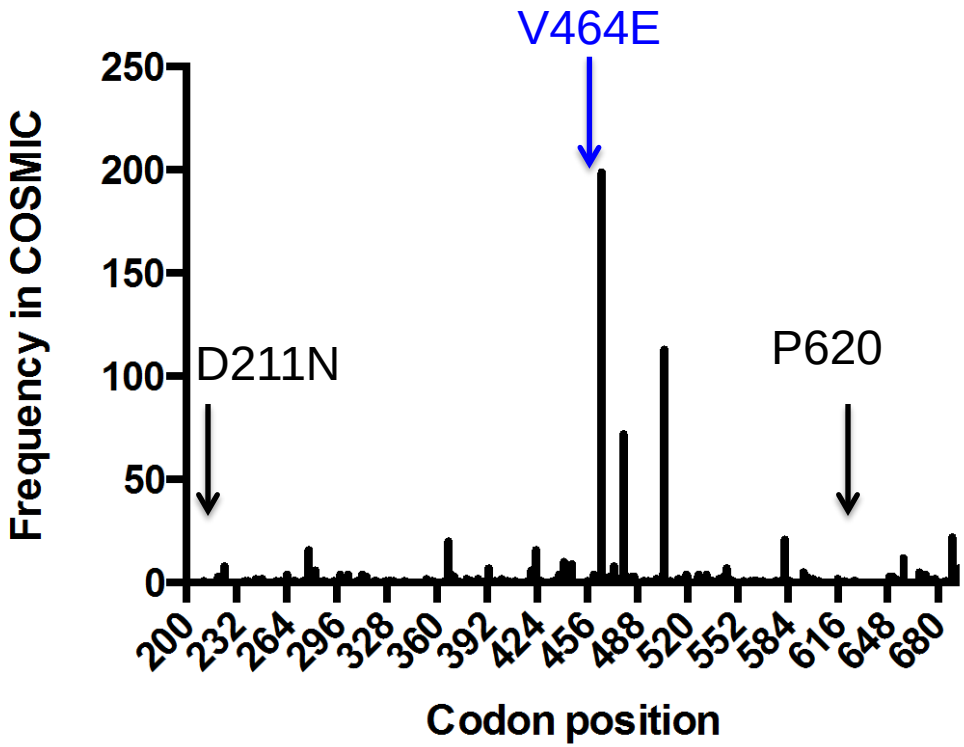
1) Literature Benchmarks
   - 100% accuracy with 21 previously studied mutations

2) Subsampling from high replicate experiment to assess false positive rate
   - FDR 5% cutoff gives 4.84% false positive calls

3) Correspondence with genetic patterns (e.g. hotspot)

Functional mutation in *FBXW7* is adjacent to a hotspot mutation





How do we know these predictions are correct?

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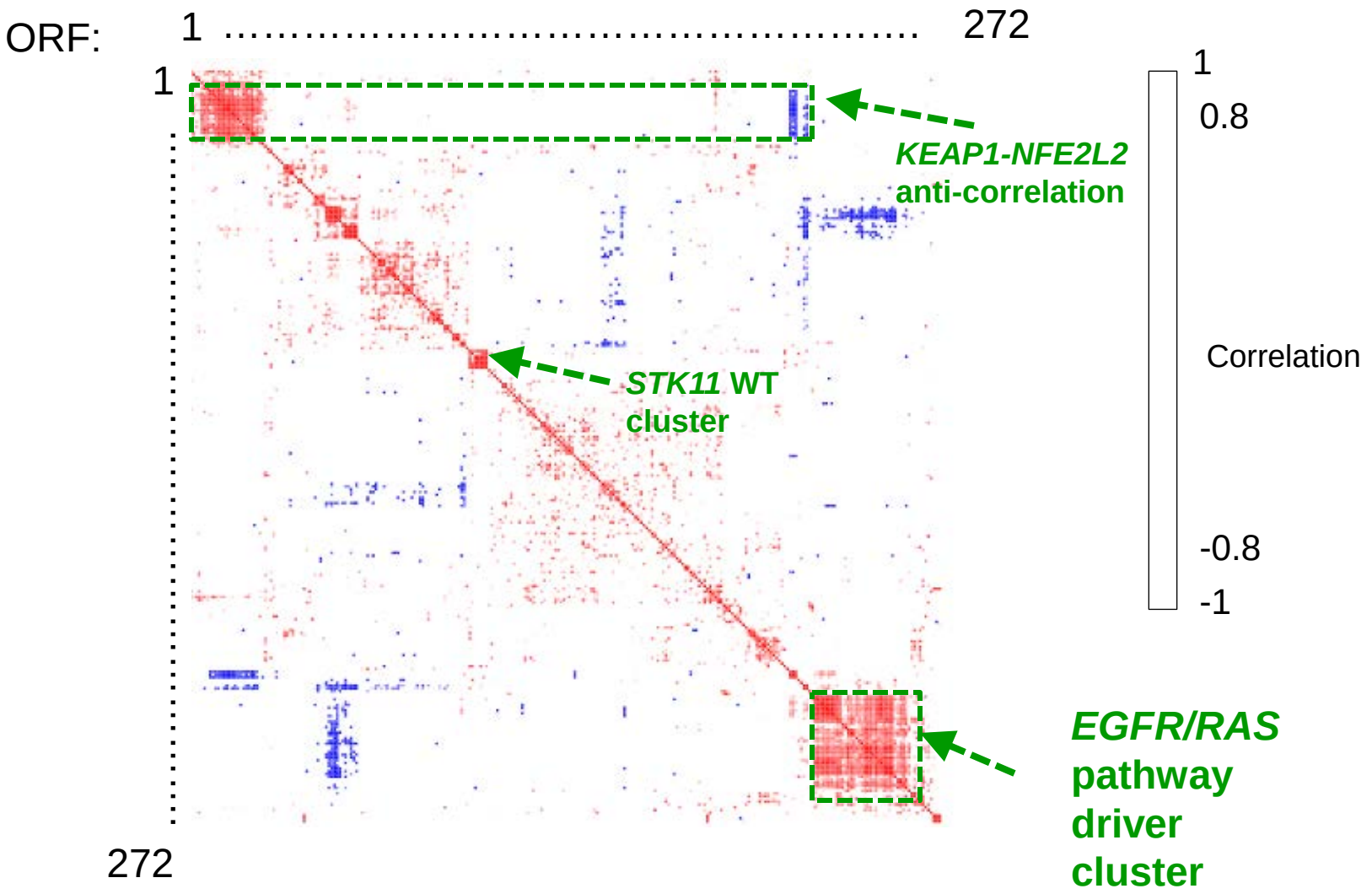
2) Simulation to assess false positive rate

3) Correspondence with genetic patterns (e.g. hotspot)

4) Signatures are similar to alleles of known gene function

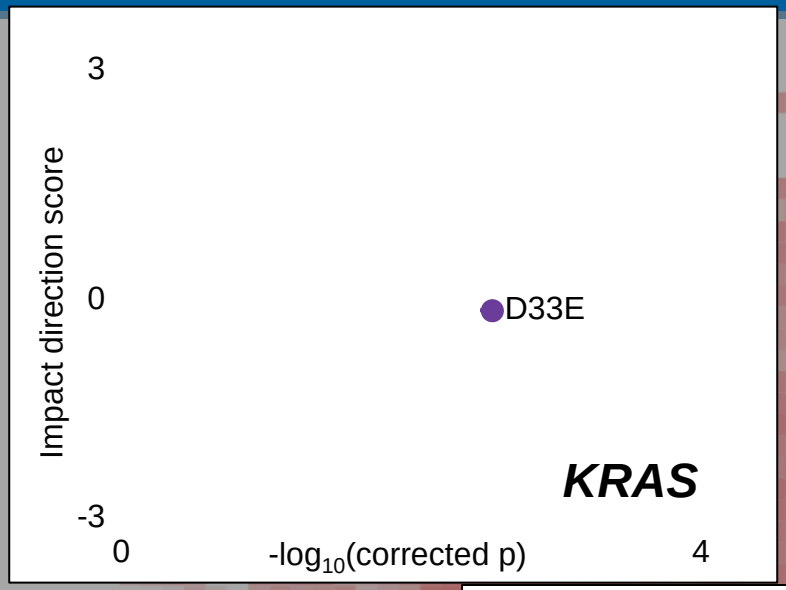
Identification of major transcriptional classes of lung adenocarcinoma genes

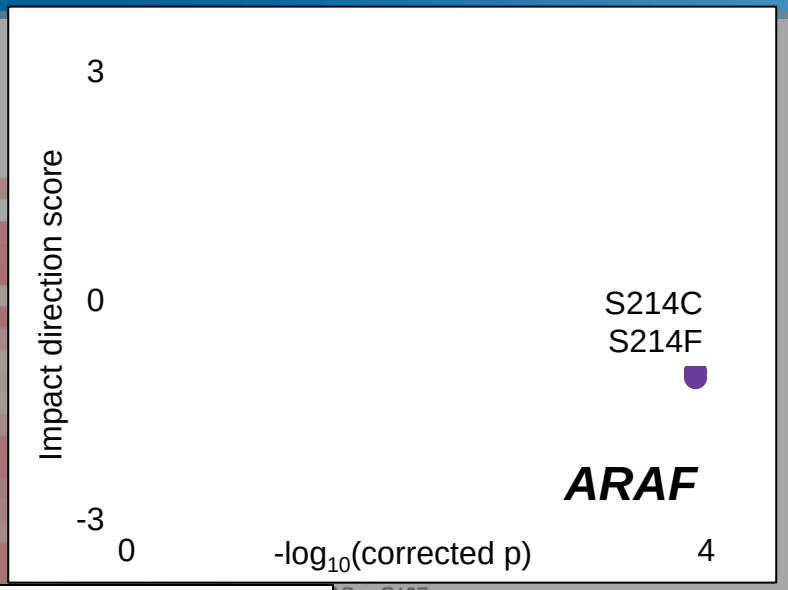


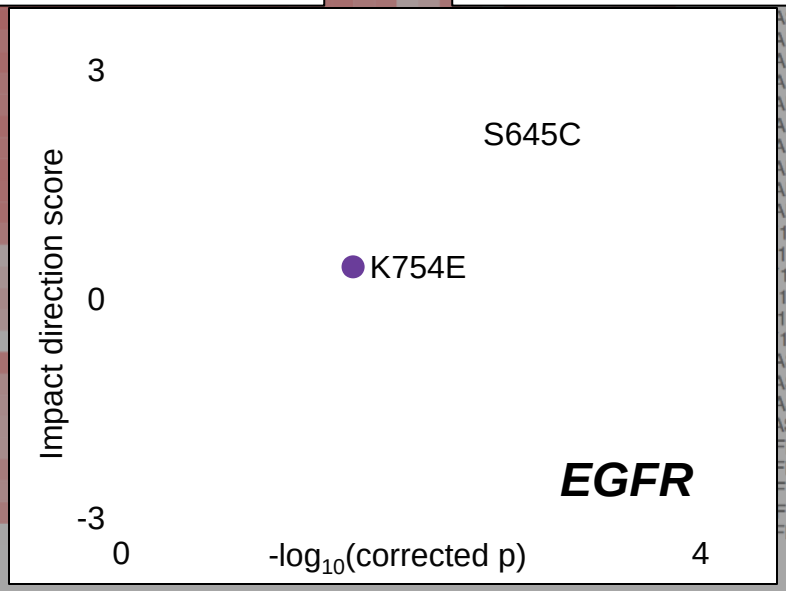
Rare variants cluster with known EGFR/RAS pathway drivers

BRAF_p.G466E
BRAF_p.A762E

Rare variants cluster with known EGFR/RAS pathway drivers



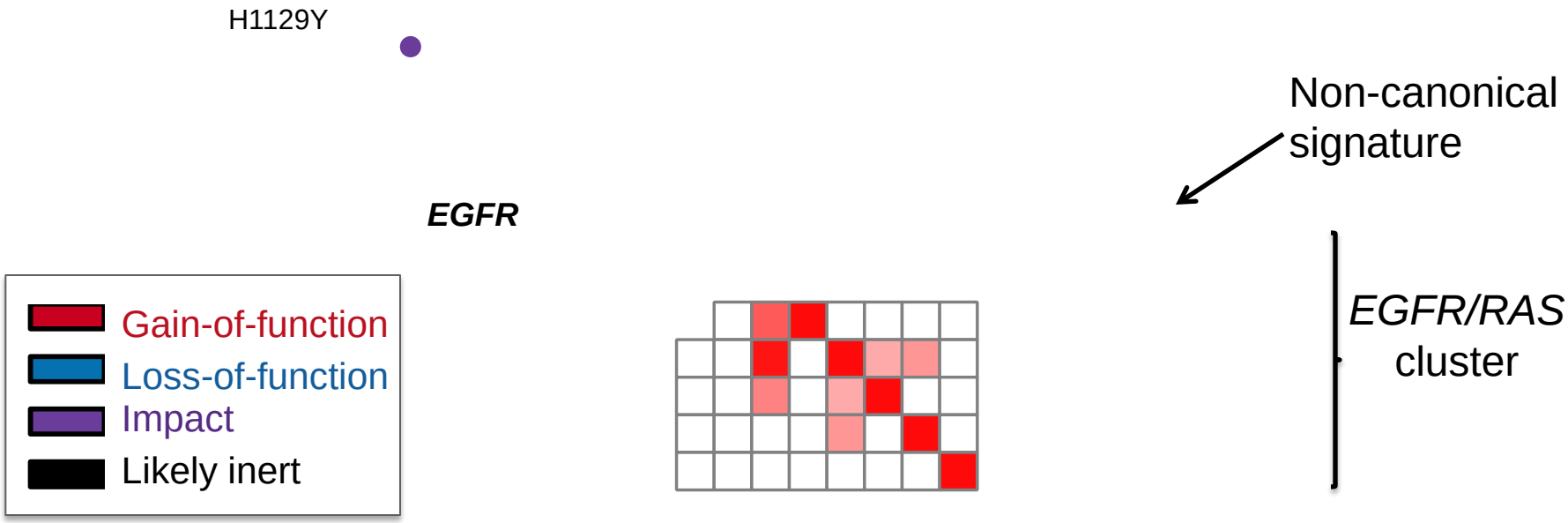




Gain-of-function
Loss-of-function
Impact
Likely inert

Unique signature of a rare non-canonical *EGFR* mutation is currently being investigated

EGFR
variants



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1) Literature Benchmarks

2) Simulation to assess false positive rate

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4) Signatures are similar to alleles of known gene function

5) Orthogonal assays
   - Genetic rescue screens, multiplex tumor xenografts, cell morphologic profiling

Variant impact phenotyping using a gene-agnostic assay

- Variant impact phenotyping is critical for understanding and treating cancer and other diseases.
- Gene-agnostic bioassays can more rapidly characterize variant impact
- This approach can be used for any gene, regardless of disease type and regardless of knowledge of gene function.

Jesse Boehm
Matthew Meyerson
Todd Golub

Yashaswi Shrestha
Candace Chouinard
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Brooks Lab positions available in cancer transcriptome analysis and functional genomics at UC Santa Cruz!
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