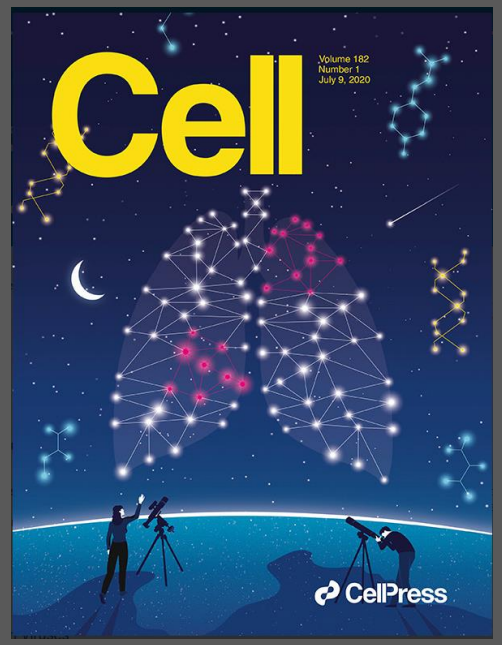
Proteogenomic Characterization Reveals Therapeutic Vulnerabilities in Lung Adenocarcinoma



Shankha Satpathy, PhD

On behalf of the CPTAC Lung Adenocarcinoma Working Group

Gillette*, Satpathy*.. Clinical Proteomic Tumor Analysis Consortium

CPTAC CLINICAL PROTEOMIC TUMOR ANALYSIS CONSORTIUM

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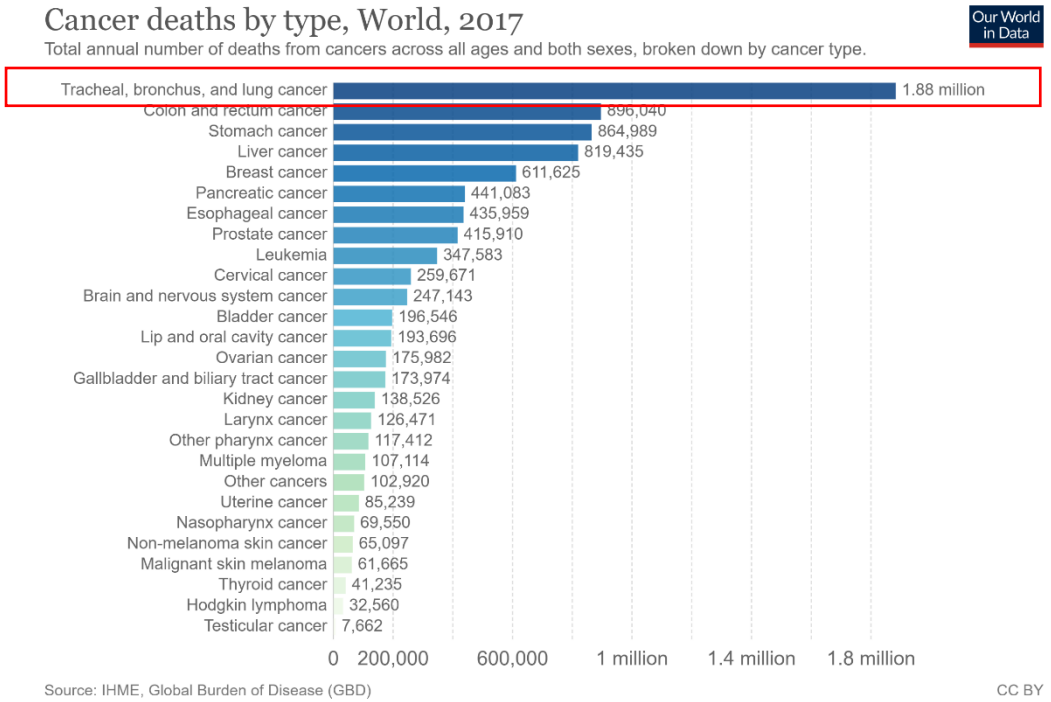
Lung cancer is of unparalleled clinical importance and is still organized by histopathologic subtype



Lung cancer is the leading cause of cancer death worldwide (1.8M cases; 1.6M deaths)

- Non-small cell lung cancer (85%)
  - Adenocarcinoma (40%)
  - Squamous cell carcinoma (30%)
  - Large cell carcinoma (10%)

- Small cell lung cancer (15%)

https://ourworldindata.org/cancer
https://lungevity.org/for-supporters-advocates/lung-cancer-statistics

Lung Adenocarcinoma

Clinical importance:

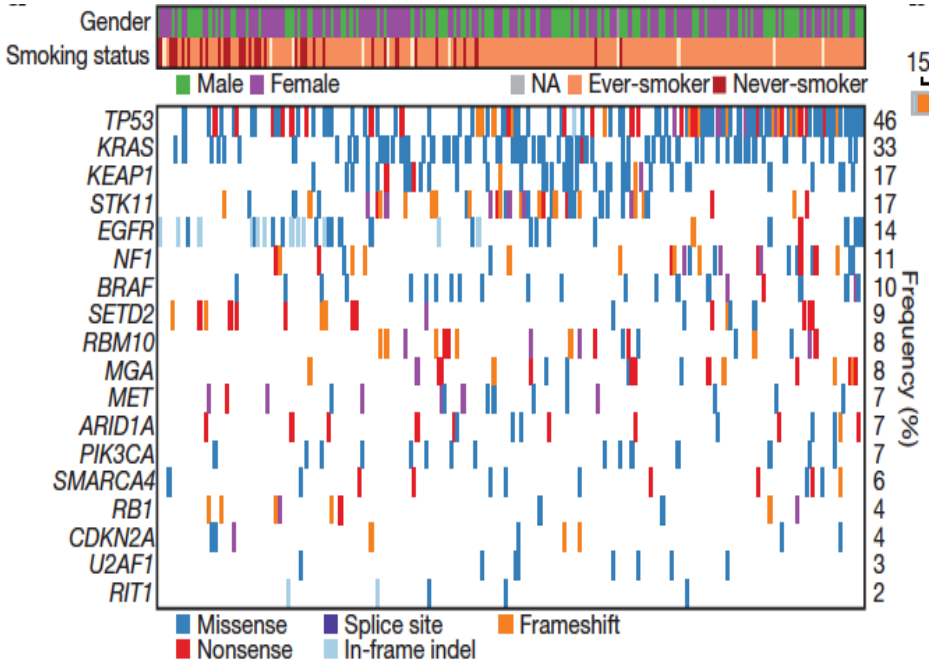
- Adenocarcinoma is the most common histologic subtype, accounting for ~40% of lung cancer and >500,000 deaths annually
- Most LUAD is smoking related, but it is the least smoking-related lung cancer
- Molecularly targeted therapies have improved treatment for patients with somatically activated oncogenes:
  mutant EGFR, translocated ALK; also translocated RET and ROS1 and mutant BRAF and ERBB2
- Most lung adenocarcinomas not targetable (lack an identifiable driver oncogene or harbor KRAS mutations)

Genomic Landscape

Smoking-related adenocarcinoma (~80%) has an exceptionally **high mutational burden**: 8 – 10 mutations / megabase
~ 1 mutation / megabase in non-smoking related LUAD
In total ~18 – 40 genes significantly mutated [TCGA; Tumor Portal]

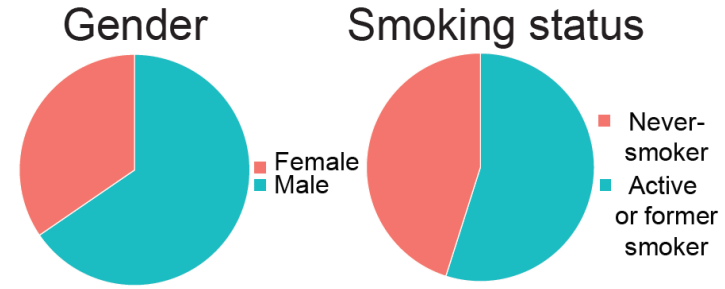
Oncogene mutations: KRAS (33%), EGFR (14%), BRAF (10%), PIK3CA (7%), MET (7%)
Tumor suppressor mutations: TP53 (46%), STK11 (17%), KEAP (17%), NF1 (11%), RB1 (4%), CDKN2A (4%)
RNA-splicing gene mutations: RBM10, U2AF1
Chromatin-modifying gene mutations: SETD2, ARID1A, SMARCA4 } 10%

Genomic alterations segregate differently in smoking- and nonsmoking-related adenocarcinomas

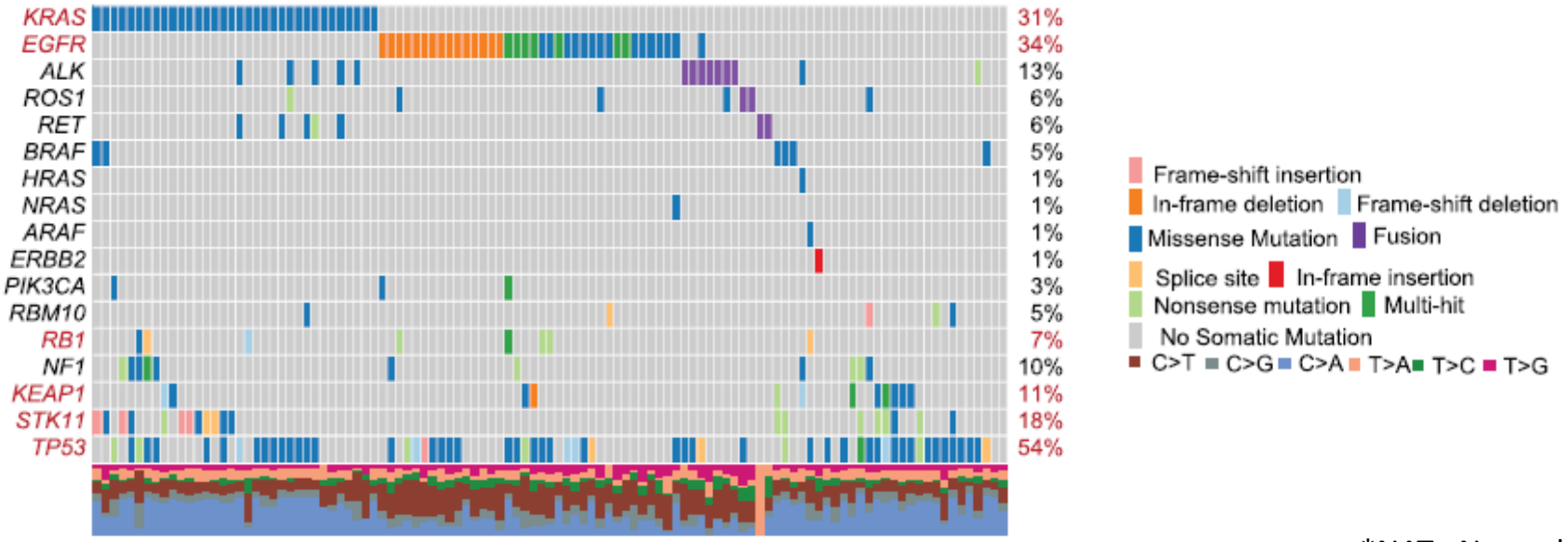


LUAD Discovery samples represent diverse Country of Origin, Smoking Status and Stage

Genomics and proteomics profiles were nearly complete for 110 LUADs & 101 NATs*

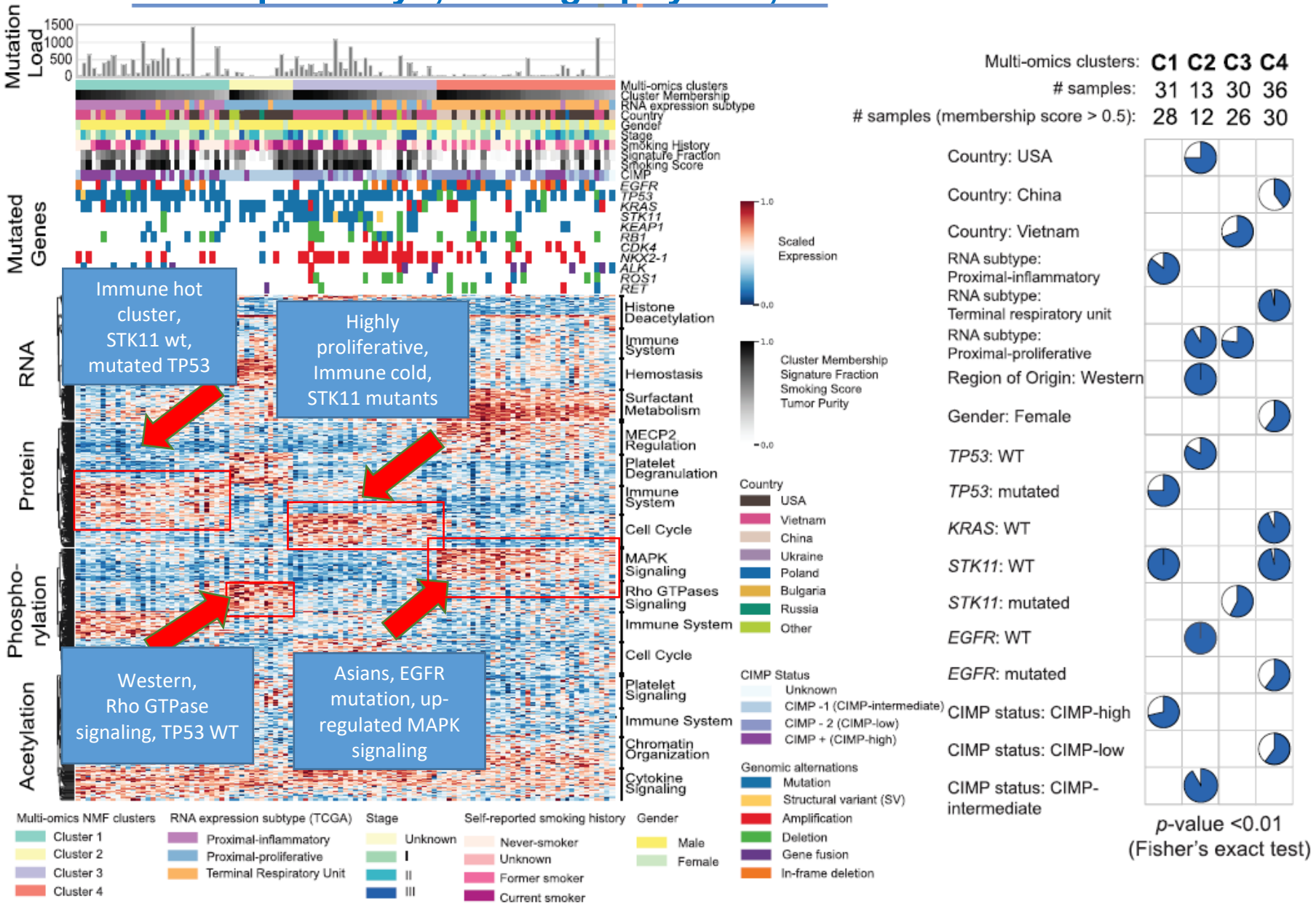


	Platform	Data type	Features
	Clinical metadata		15
	WXS WGS	Germline mutations	16,660
		Somatic mutations	32,250
		CNA	19,267
	Methylation array	DNA methylation	16,478
	RNA-seq	mRNA	18,099
	miRNA-seq	miRNA	2,585
	TMT Proteomics	Proteins	10,699
		Phosphorylation	41,188
		Acetylation	6,906

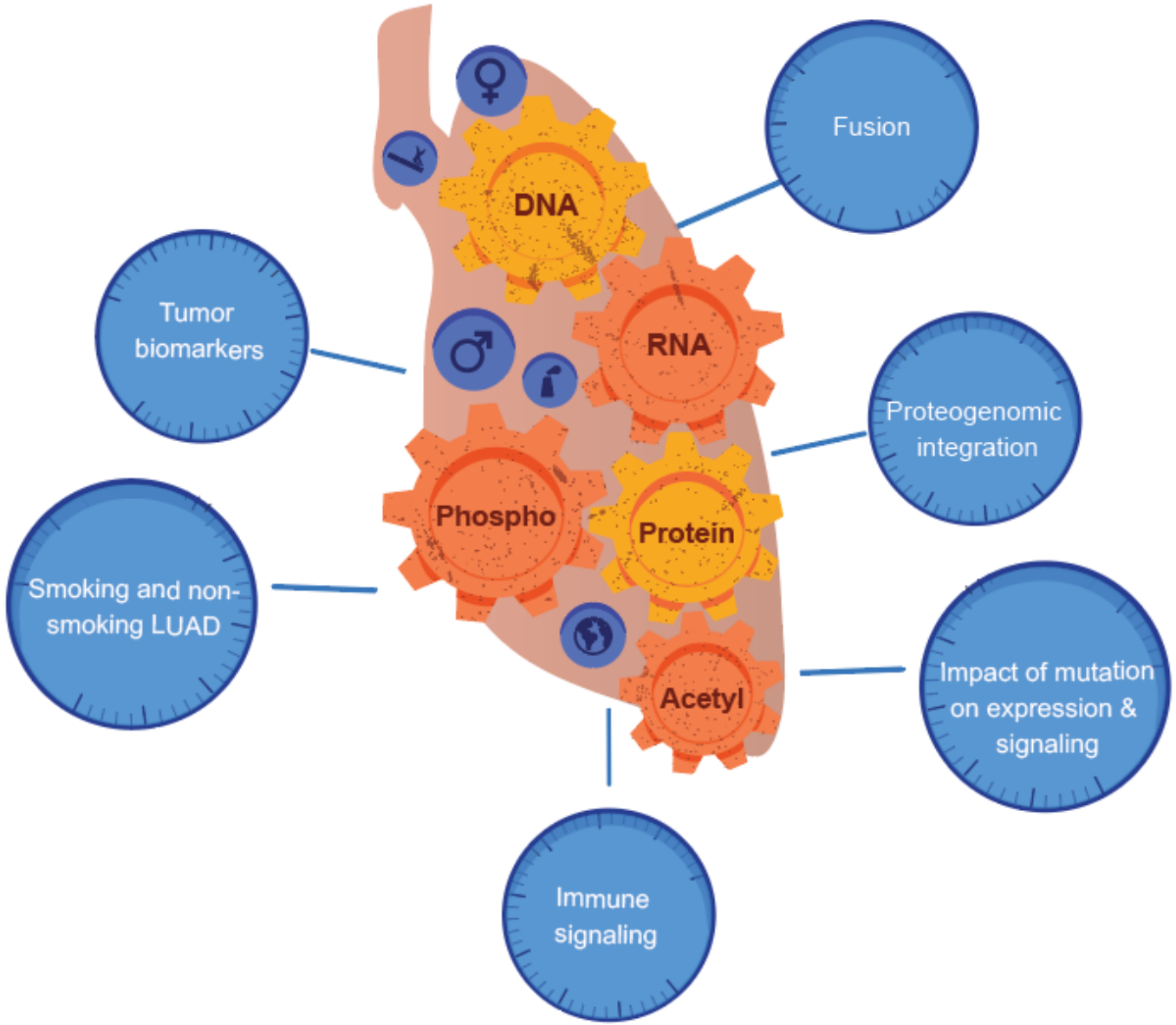


*NAT= Normal Adjacent Tissue

Multi-omics clustering (RNA, Protein, Phosphosite, Acetylsite) revealed 5 distinct clusters with unique characteristics for pathways, demography and, mutation status.



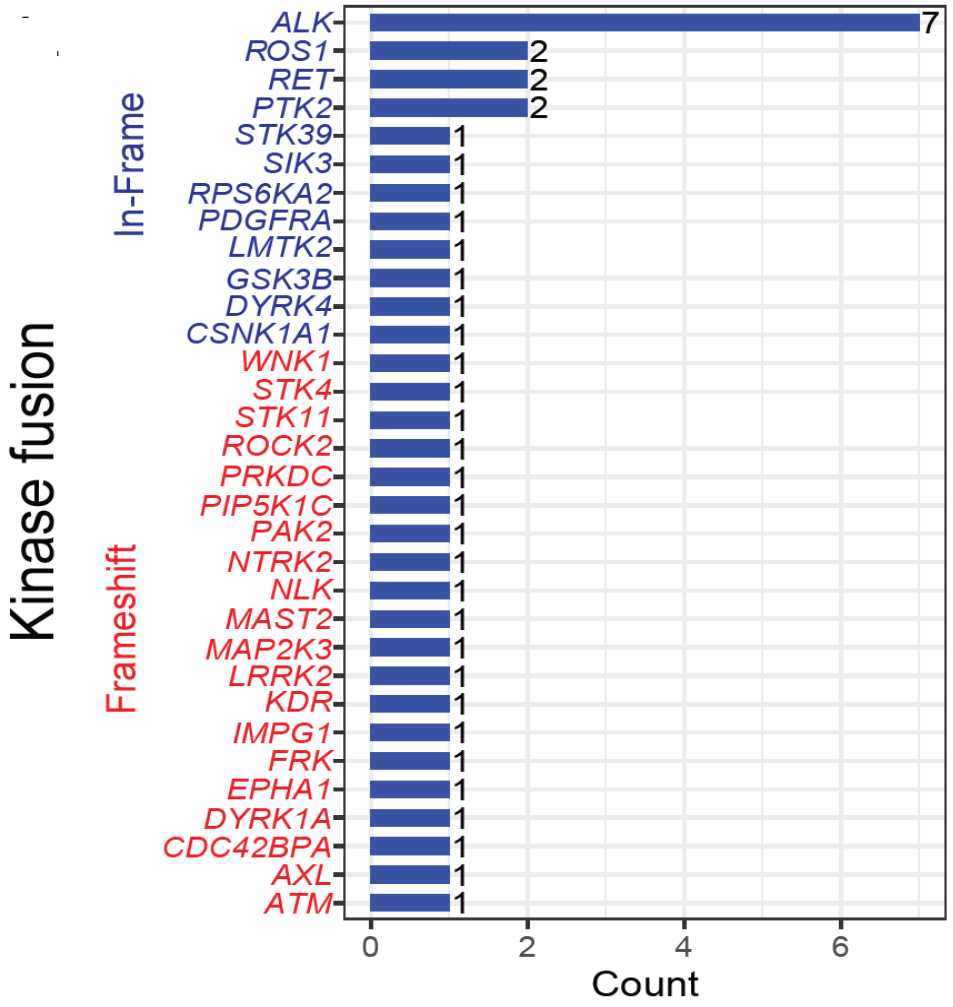
Key biological vignettes exemplified by this state-of-the art CPTAC LUAD effort



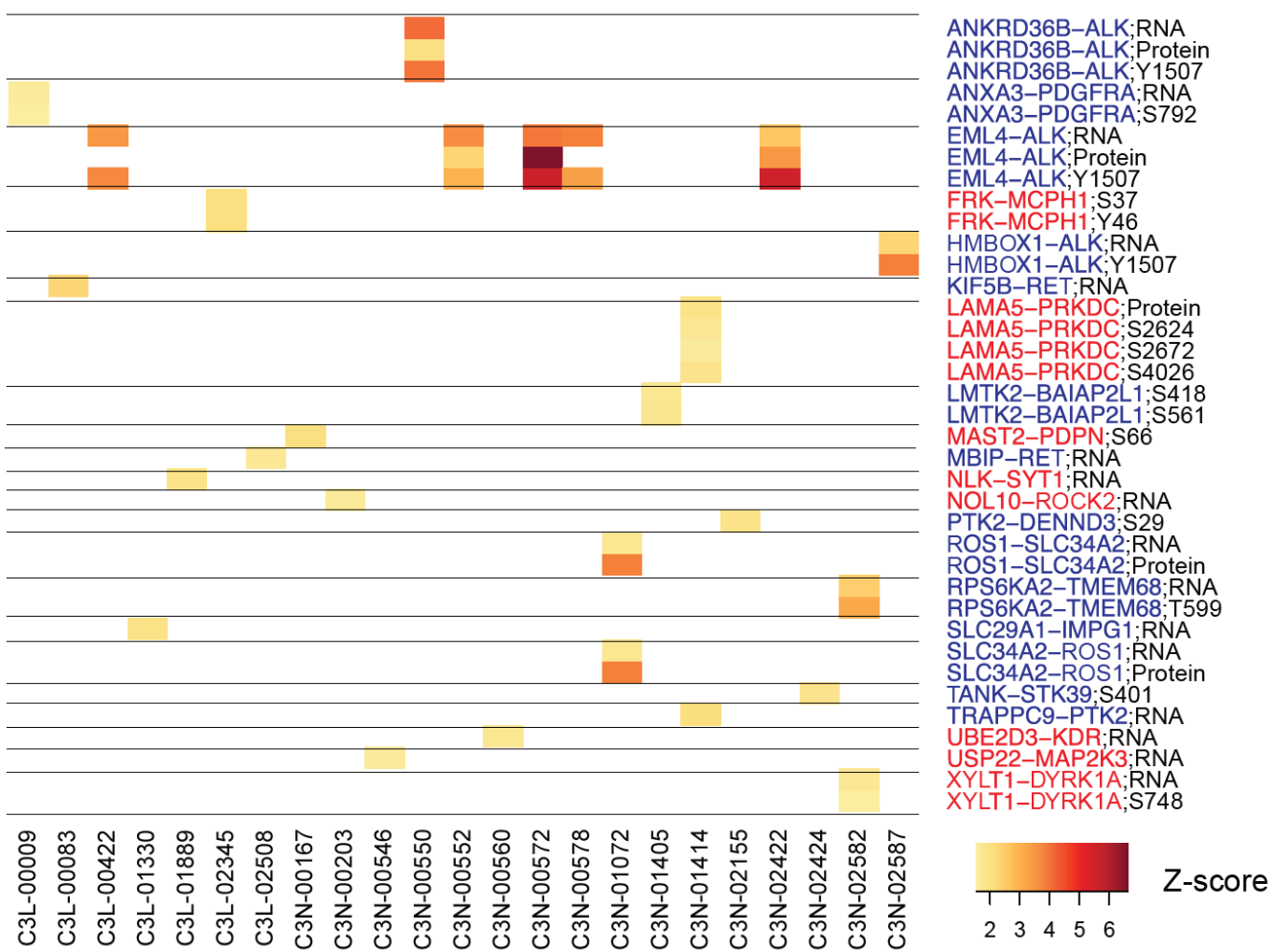
Global characterization of kinase fusions includes *ROS1*, *RET*, and many others

Outlier analysis suggested that more than half were likely functional

Fusions observed in this cohort

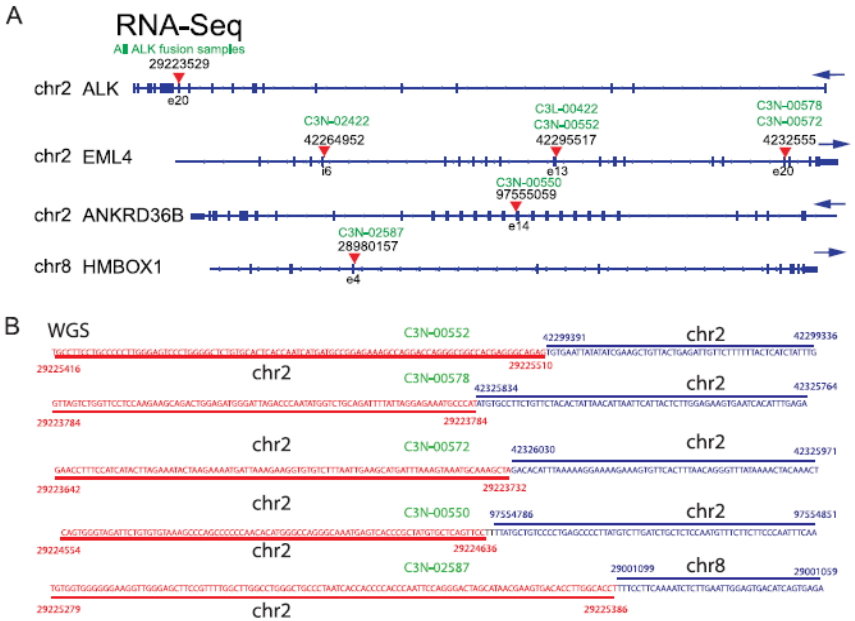


Outlier expression

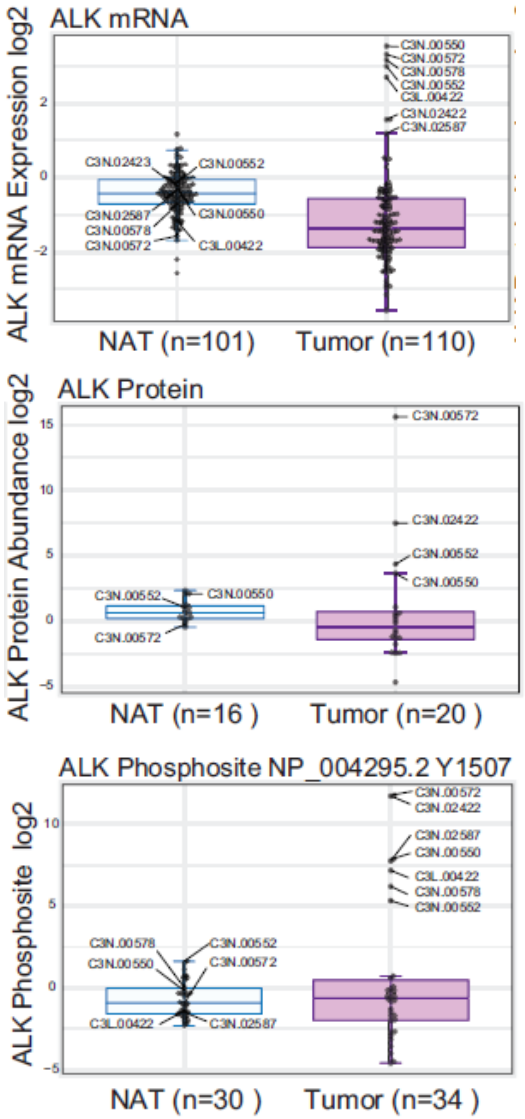


Novel *ALK* fusion partners are identified with spanning read support from WGS
Tyrosine phosphopeptides offer insights into biology and potential diagnostic & therapeutic targets

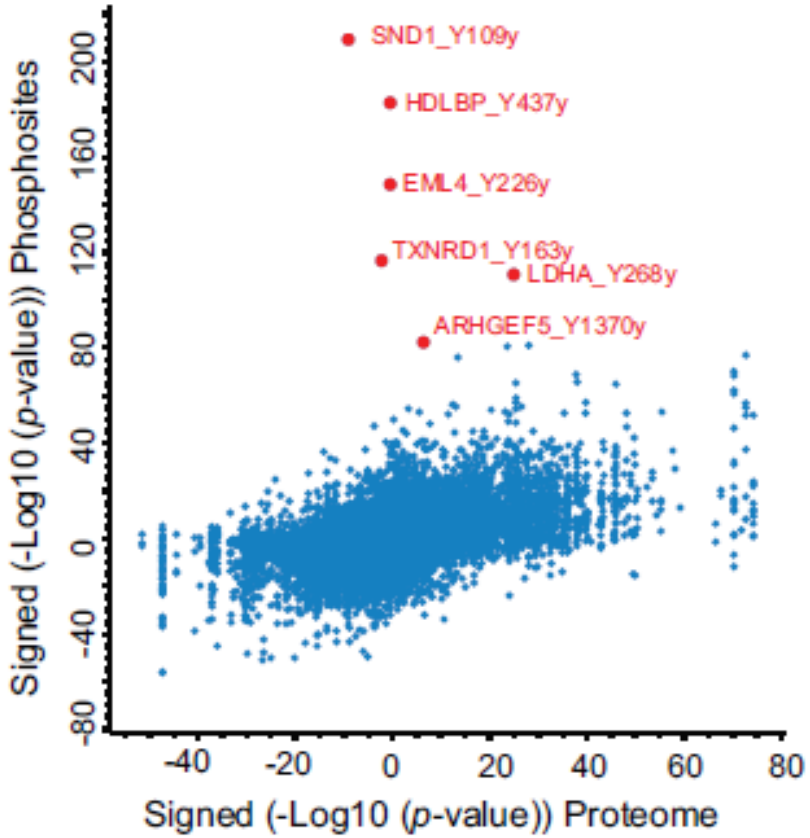
Fusion architecture



ALK outlier expression

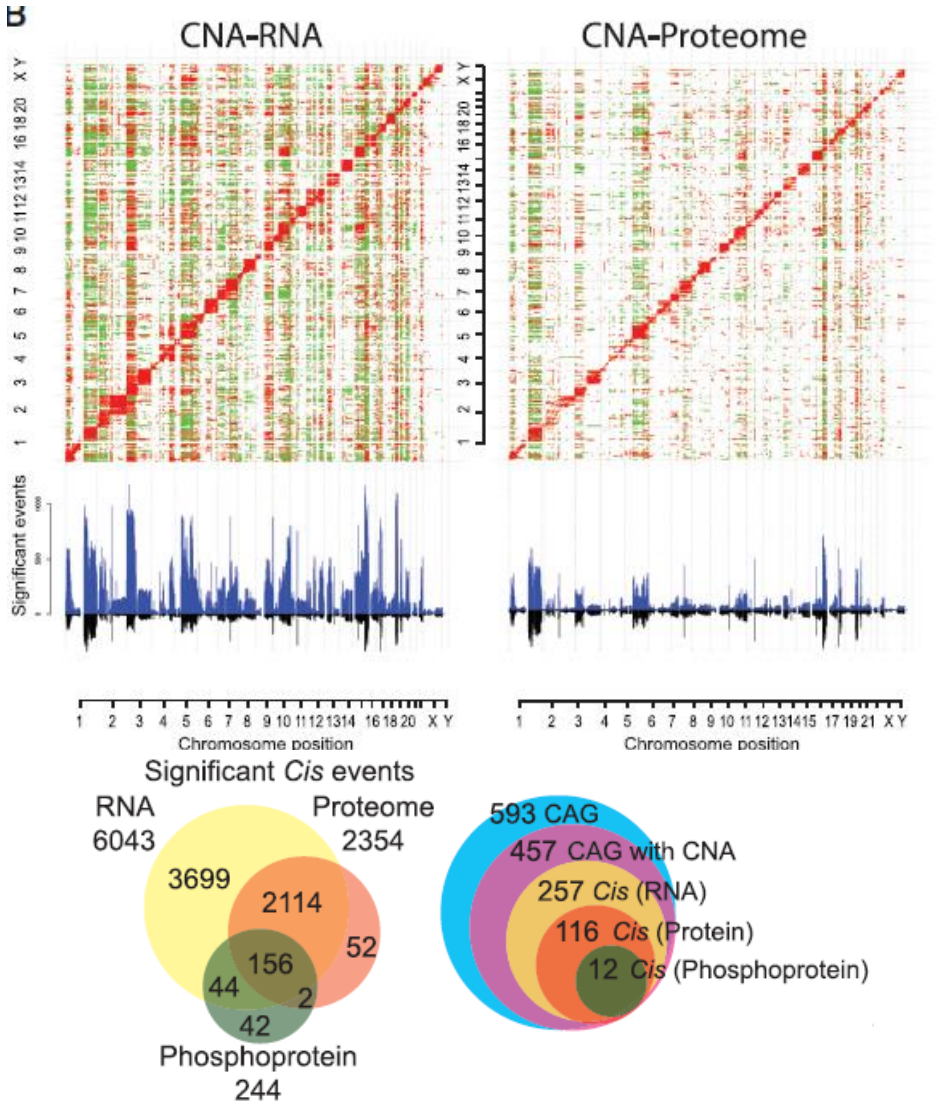


ALK fusion driven phosphosites

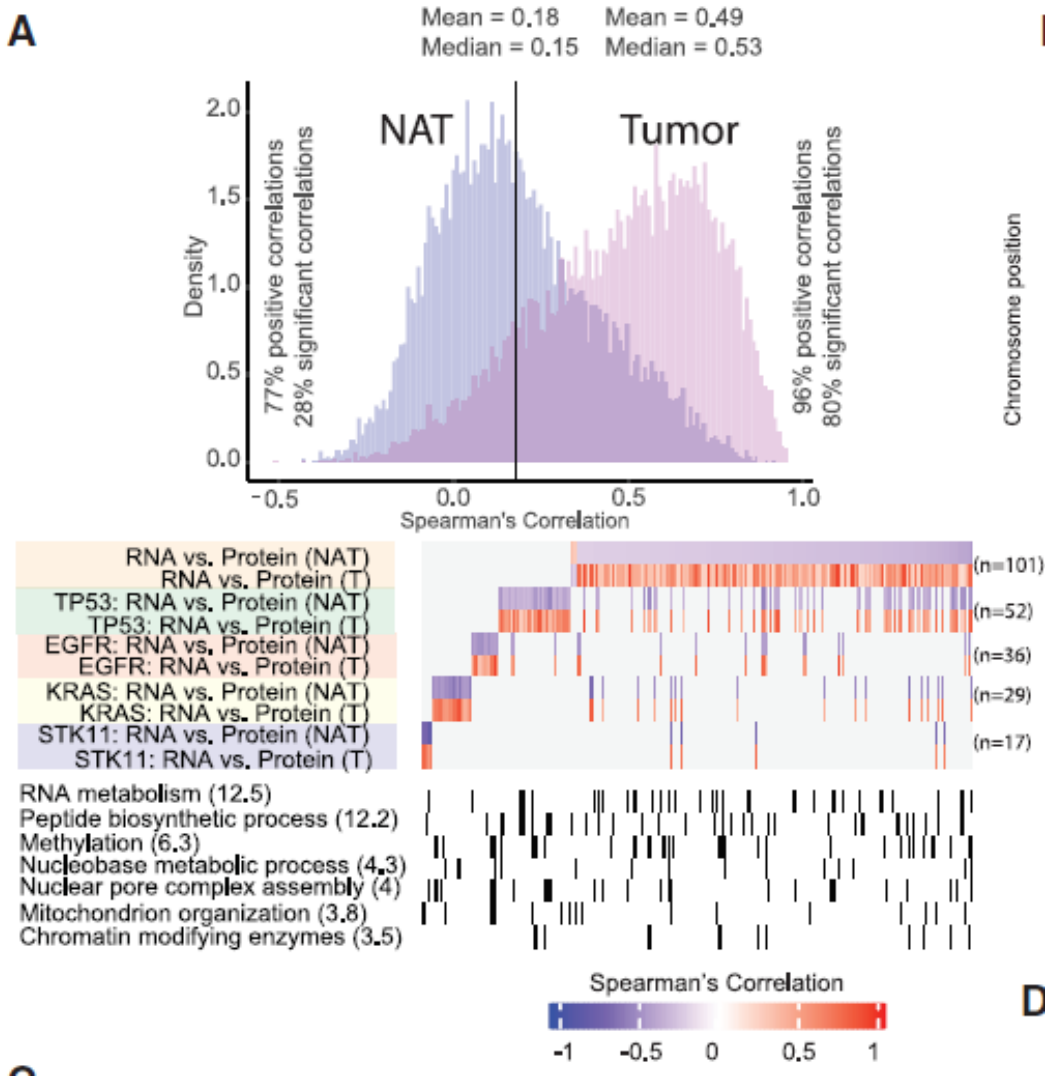


Proteogenomic integrative analysis prioritizes Copy number aberrations and highlights dichotomy in mRNA-protein correlation in tumors and NATs

Prioritizing copy number aberrations using proteomics

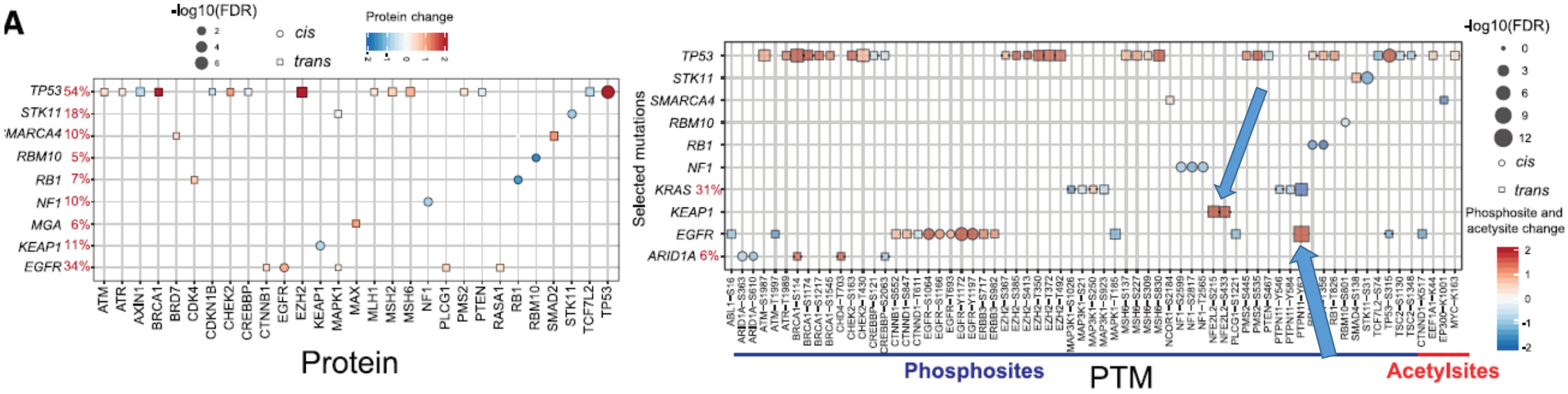


Differential mRNA-Protein correlation between tumors and NATs

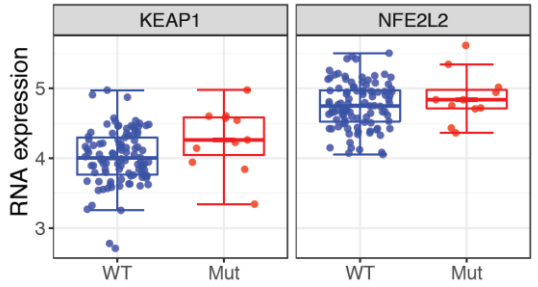


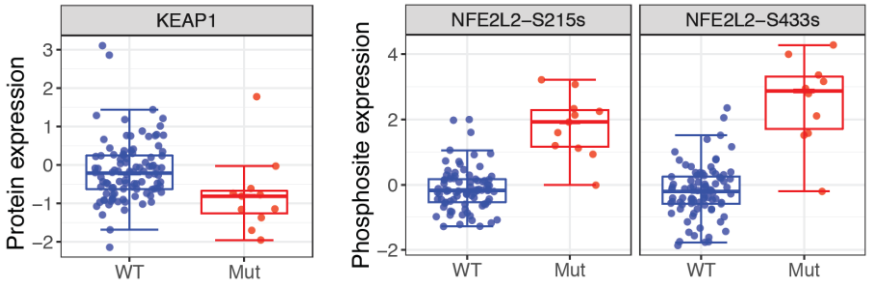
Impact of Somatic Mutation on the Proteogenomic Landscape

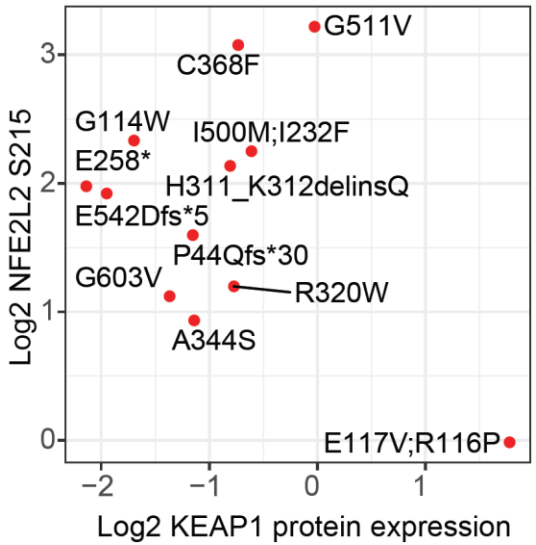
Impact of recurrent mutations on protein expression and PTM abundance

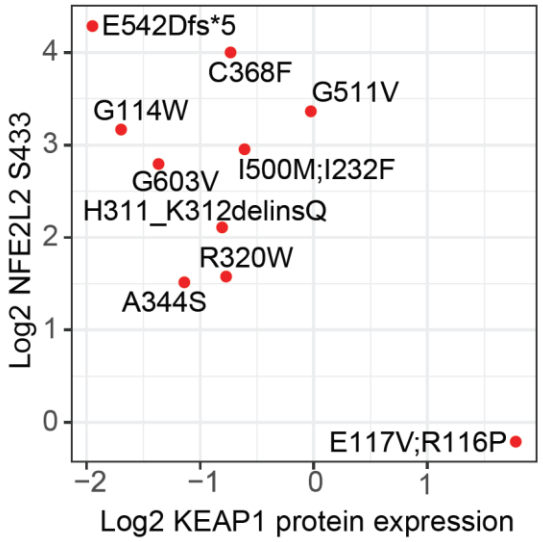


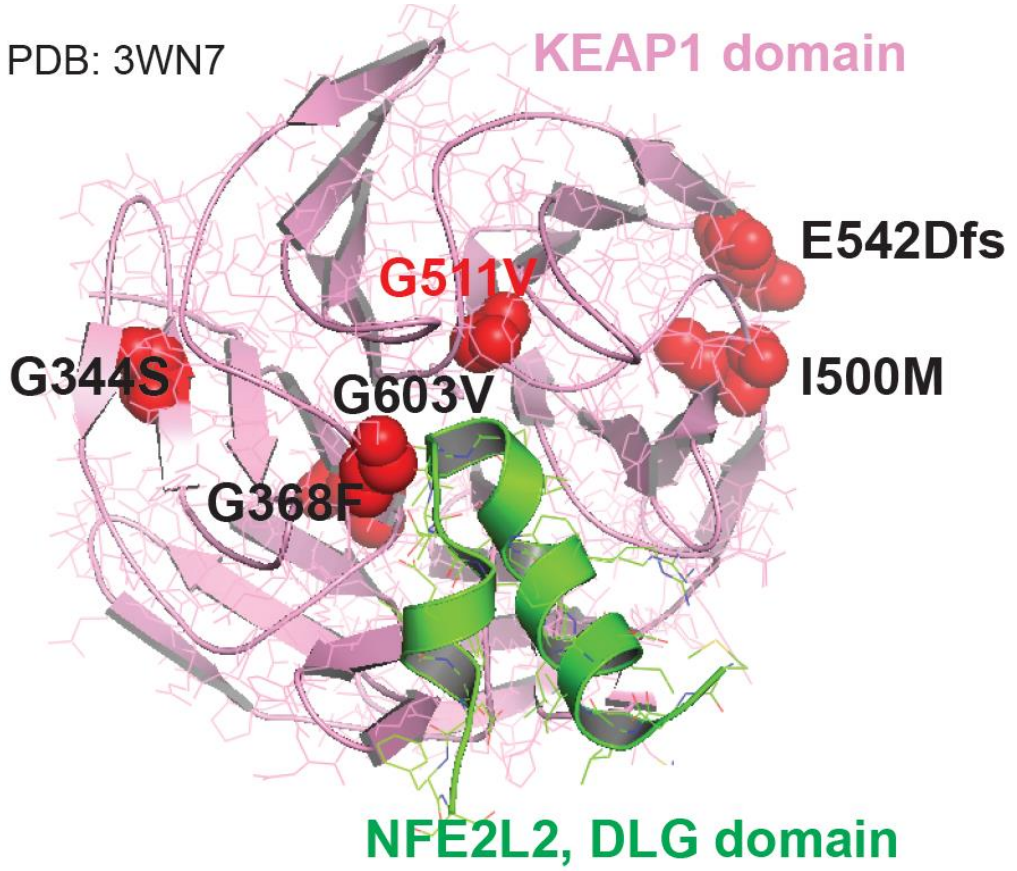
Deep proteogenomics exposes *KEAP1 / NFE2L2* (*NRF2*) biology and a putative novel *KEAP1 / NFE2L2* regulatory mechanism



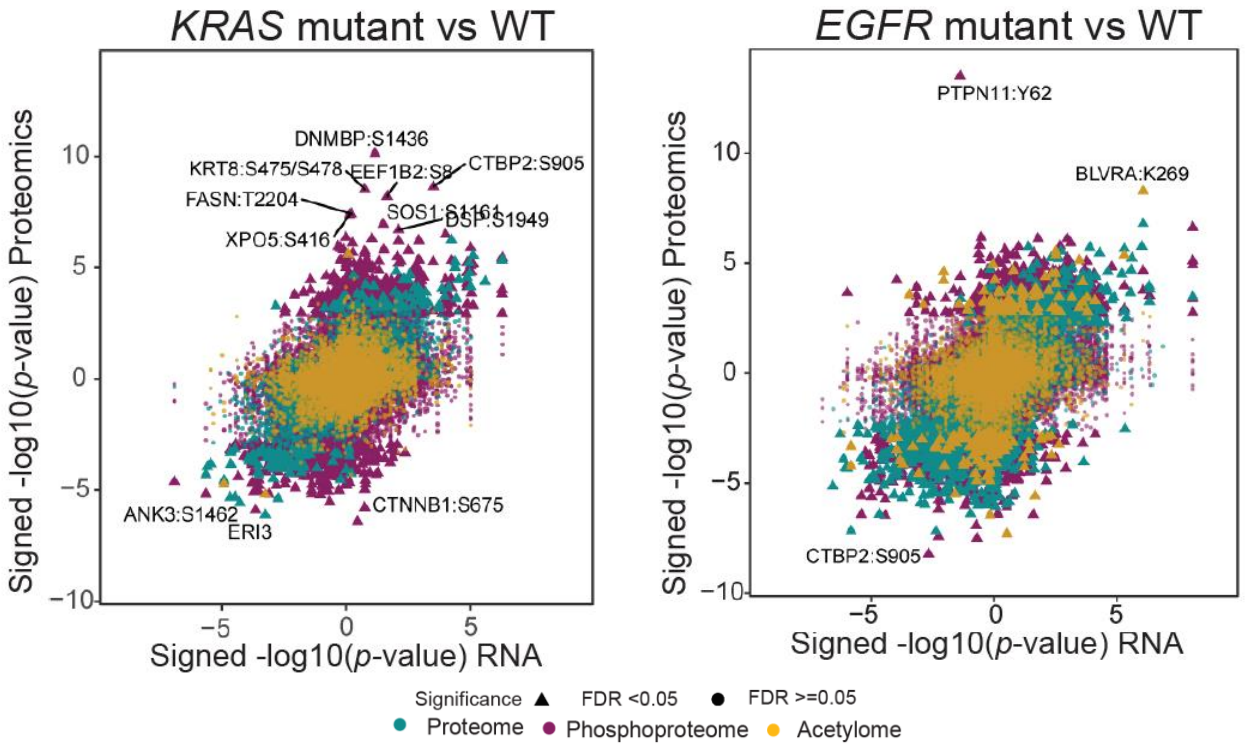


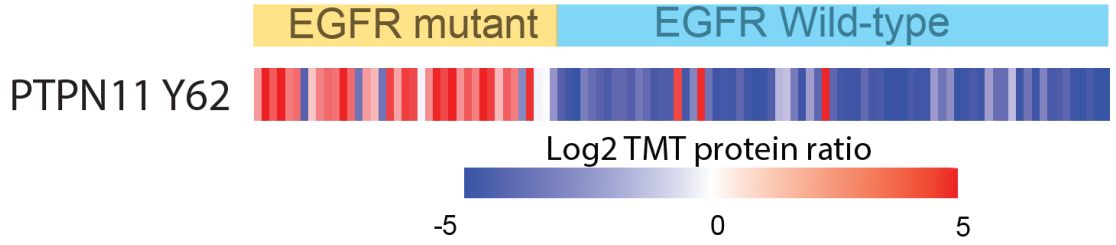


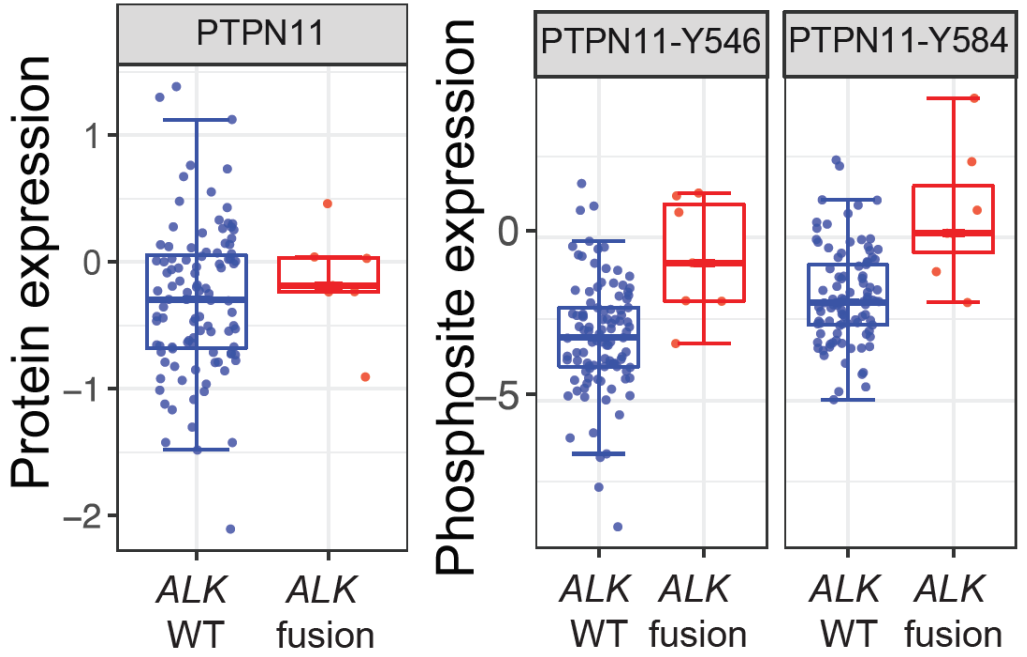




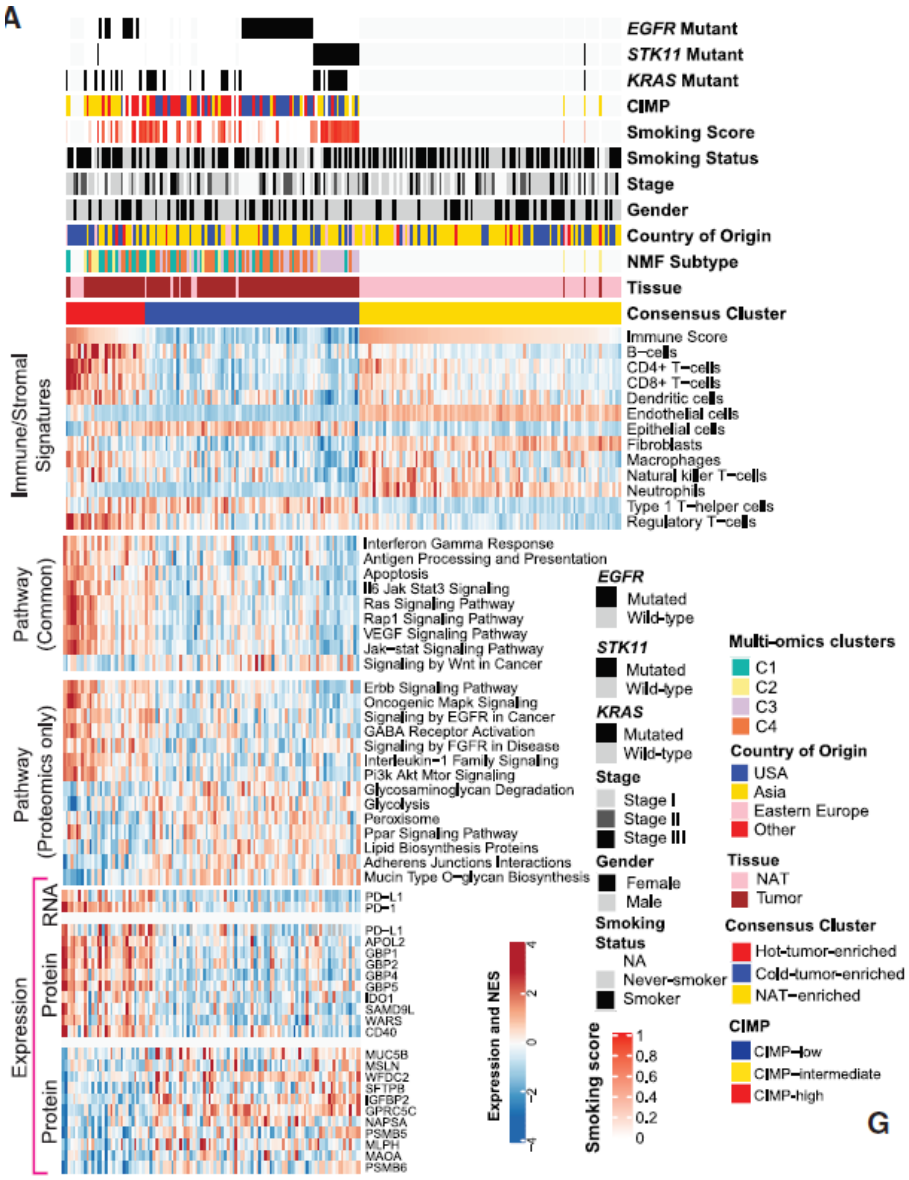
KRAS mutation association analysis highlights important outliers seen only in the phospho data
EGFR mutant tumors are associated with extreme outliers of PTPN11 (SHP2) phosphopeptide Y62

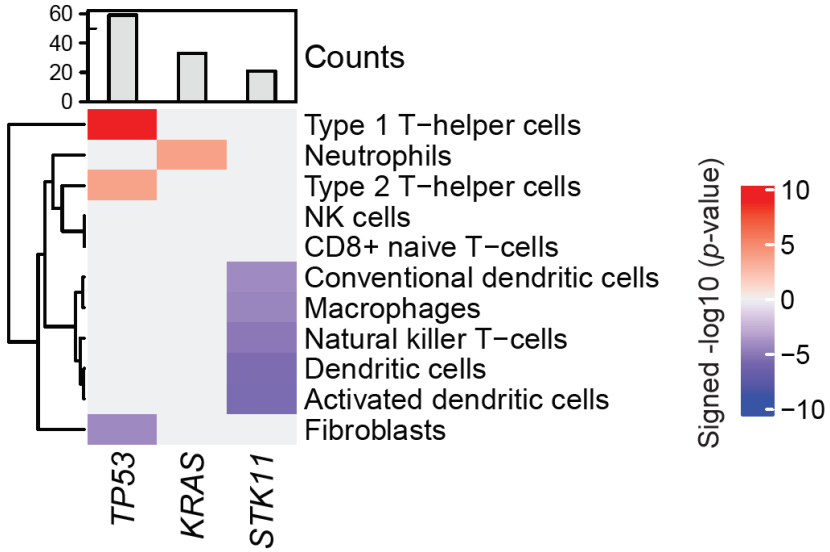


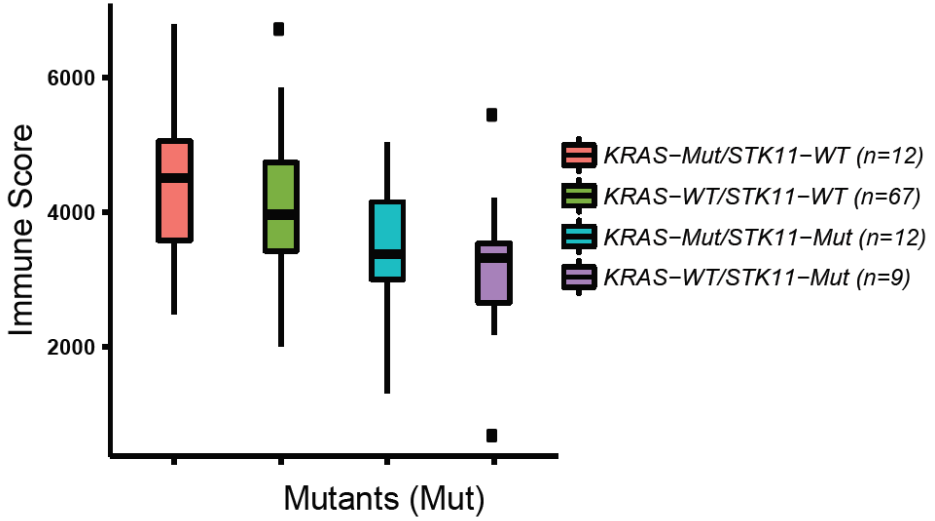




The immune landscape of LUAD shows “cold” and “hot” tumor and intermediate NAT clusters *STK11* mutant, *KRAS* WT tumors are especially “cold”



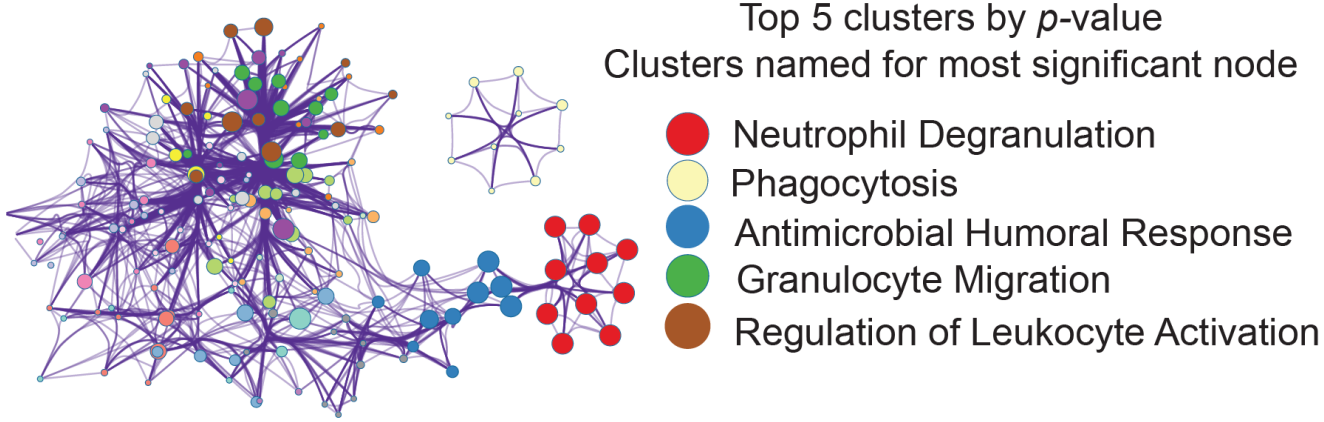




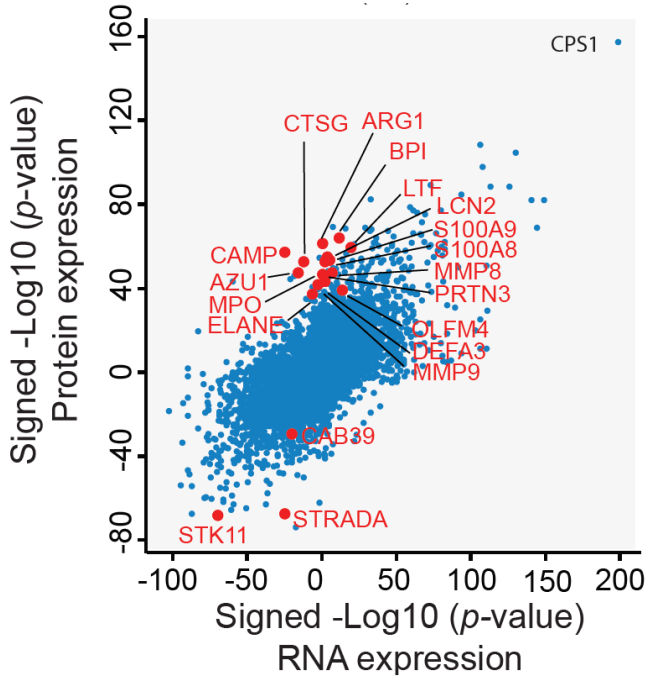
Neutrophil degranulation is a dominant *STK11* signature seen only in the proteome

Most of these proteins have well-established immunomodulatory functions

Proteins that drive unsupervised segregation of STK11 mutant samples are enriched for neutrophil degranulation



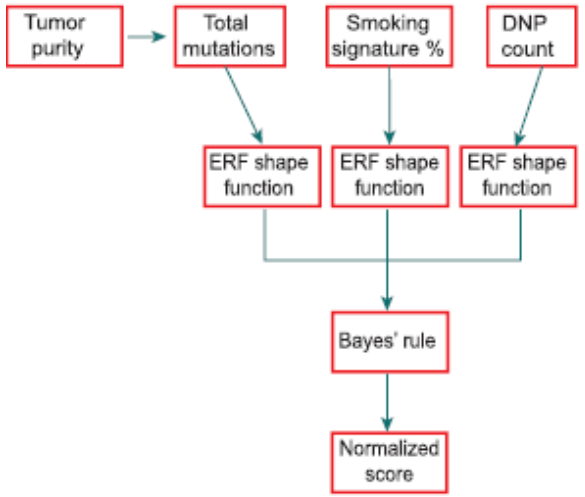
Differential regulation of neutrophil degranulation signature is exclusively captured in proteome



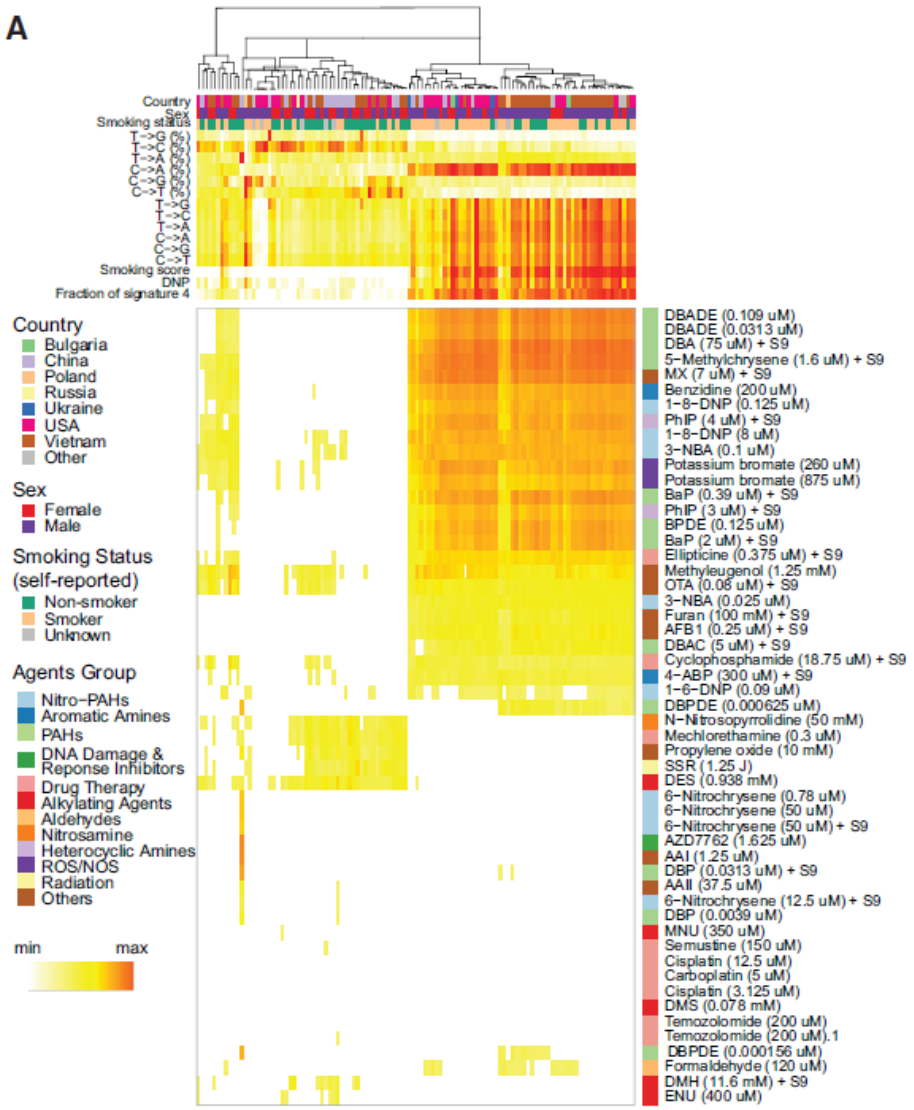
ICA analysis – Wenke Liu, David Fenyo

Evidence of environment exposure and oncogenic signaling in non-smokers

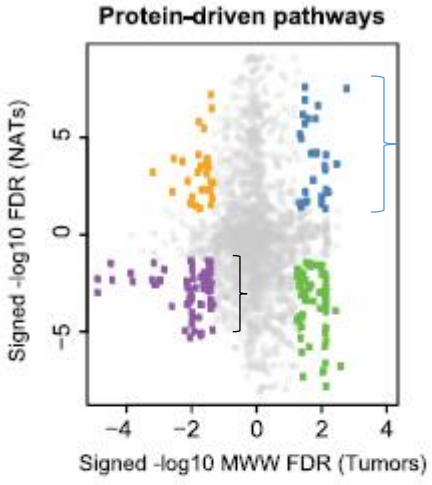
Computing smoking score



Differential chemical exposure signatures in smokers and non-smokers

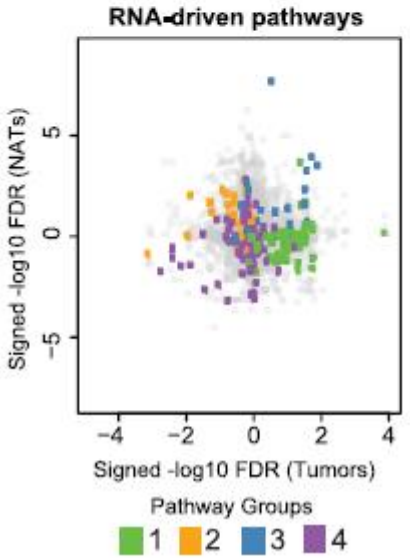


Differential pathways between samples with High smoking score vs. low smoking score



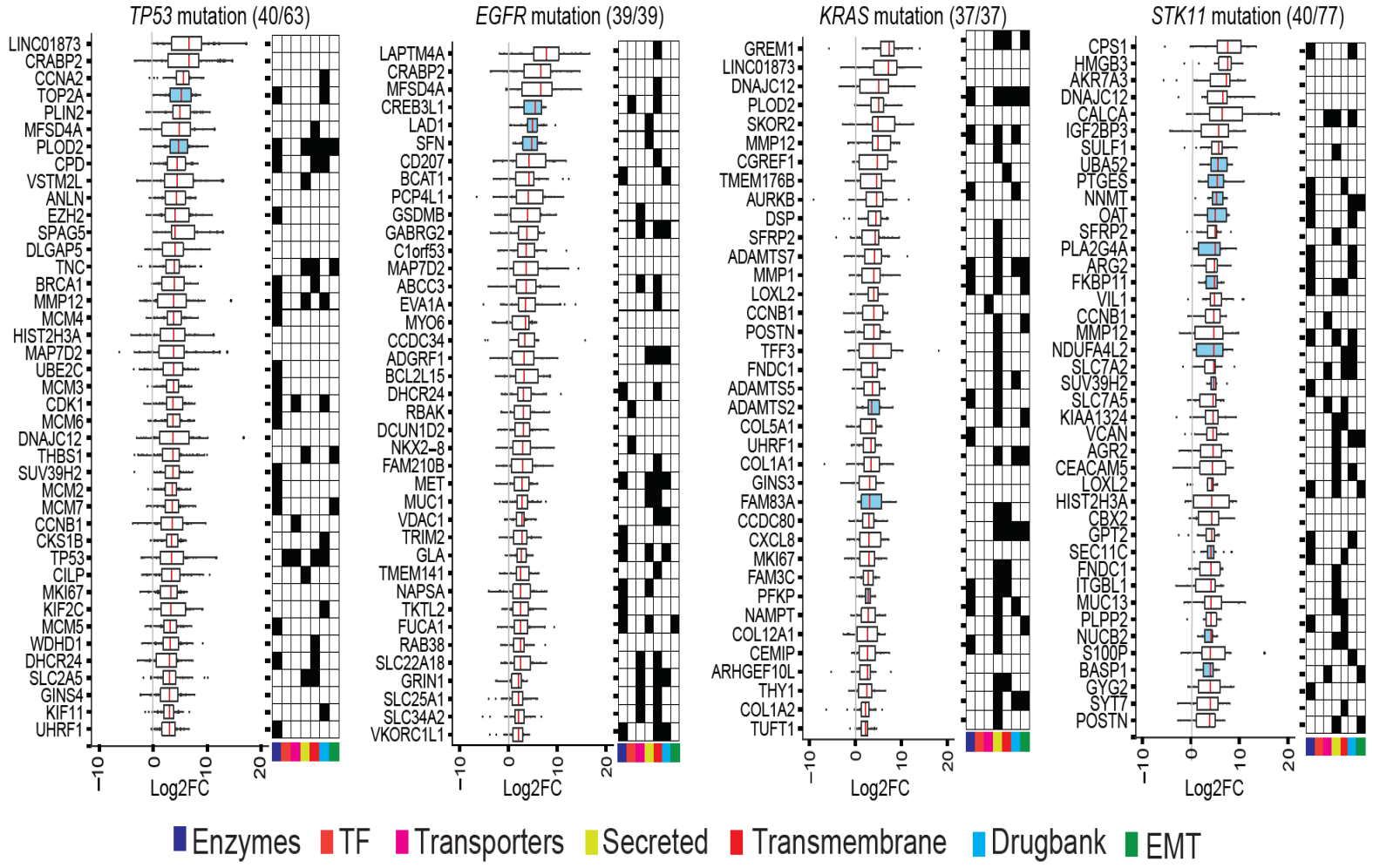
up/up
Ferroptosis
MYC response
UV response

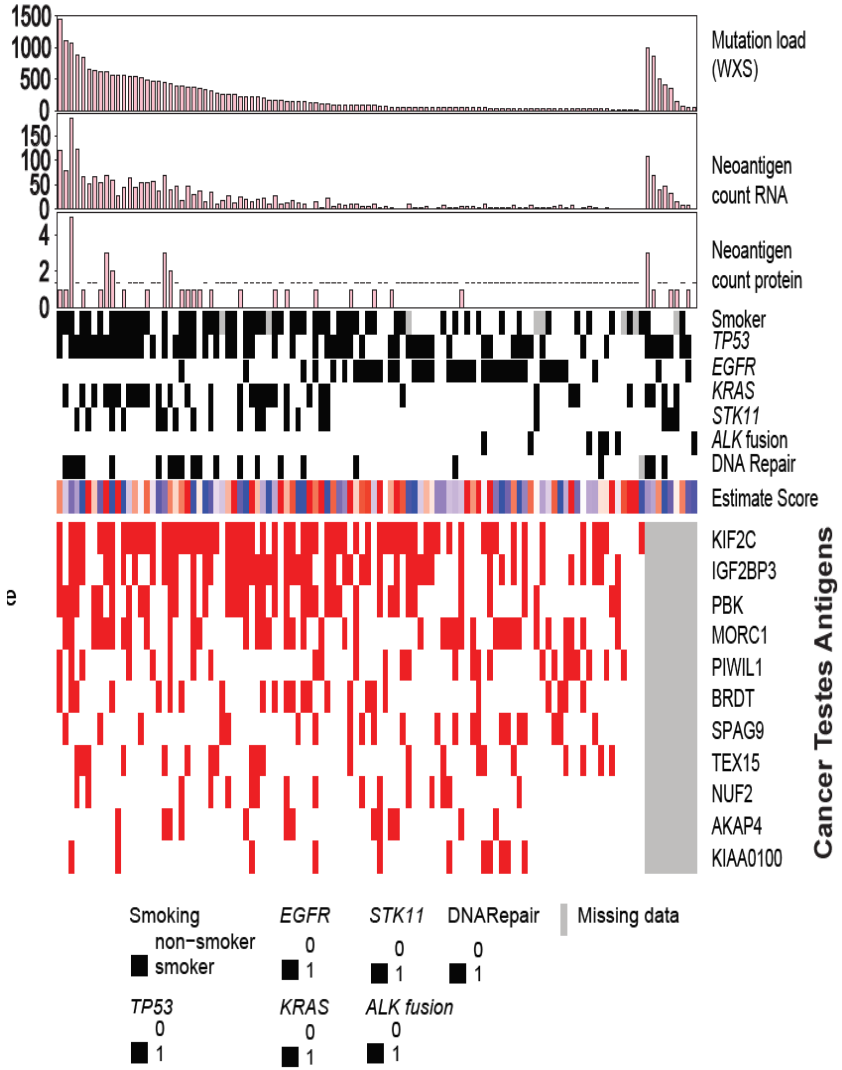
Down/down
HIPPO signaling
NF-KB signaling
IL signaling



Data provide a resource for global and subtype-specific LUAD biomarker development

Widely expressed Cancer-testis (CT) antigens are prime candidates as both biomarkers and immunogenic targets





Partial summary of findings

- The mutational landscape of this diverse sample set is largely familiar.
- Phosphoproteomics provides new, actionable insights into *KRAS*, *EGFR*, *KEAP1-NFF2* and other biology
- The immune landscape of LUAD is highly variable. *STK11* mutant tumors are especially cold. We have mechanistic hypotheses including the contribution of neutrophil degranulation to *STK11* mutant pauci-immune status.
- Cancerization of Normal adjacent tissue in smokers.
- Tumor-normal analyses provide candidate diagnostic markers, immunogenic and oncogenic targets, and potential insights into lung tumorigenesis

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  - Emily Boja
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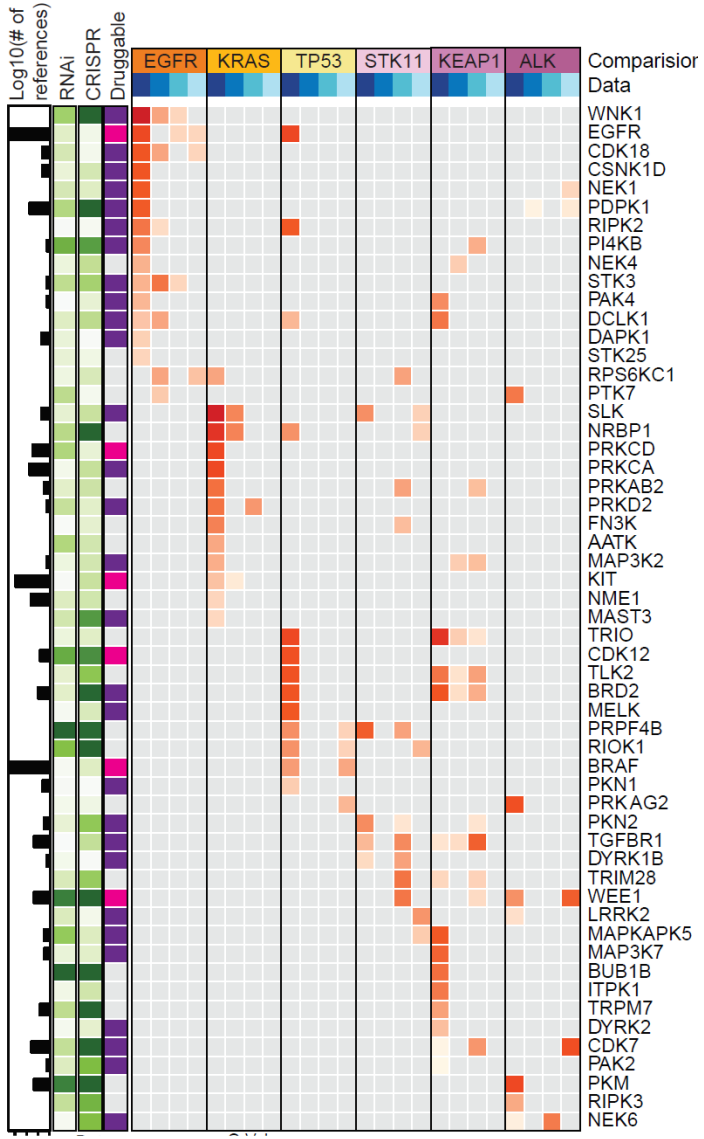
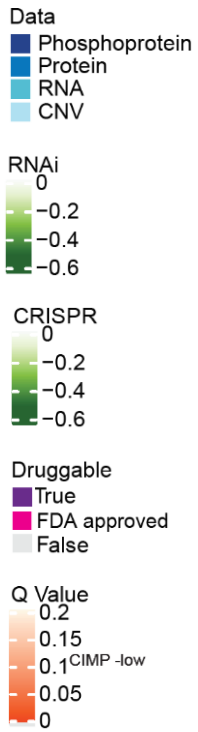
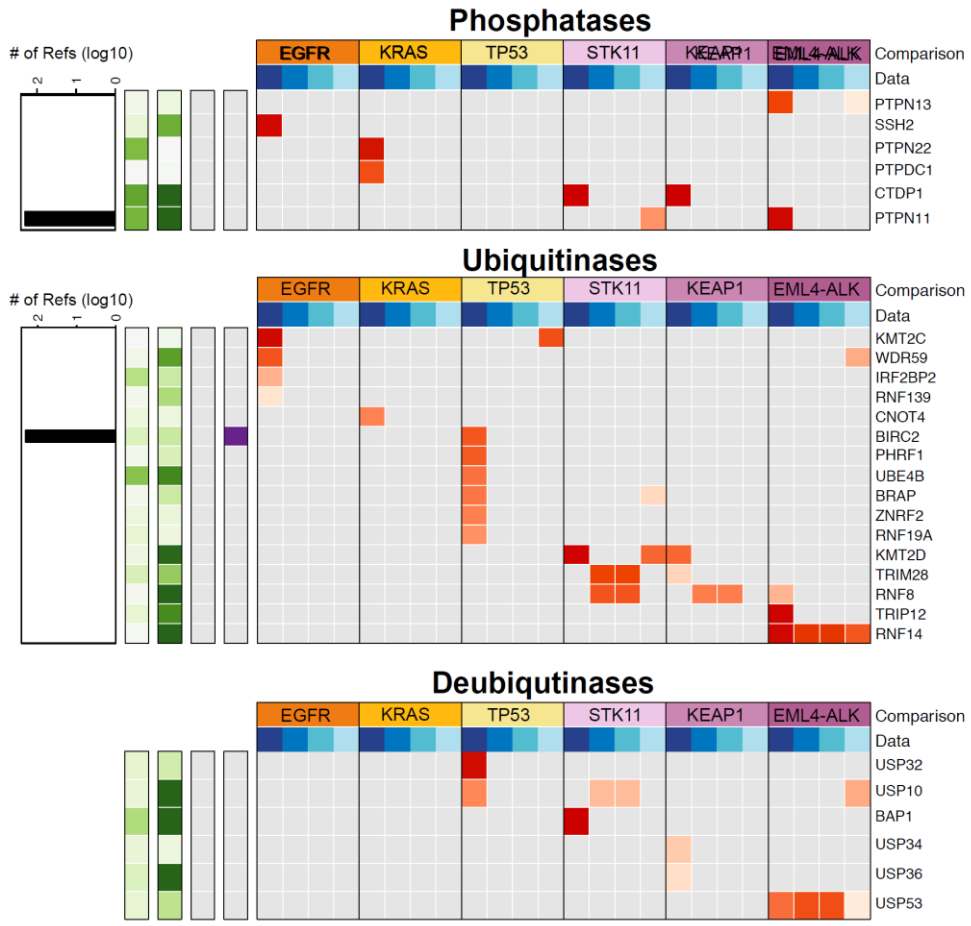
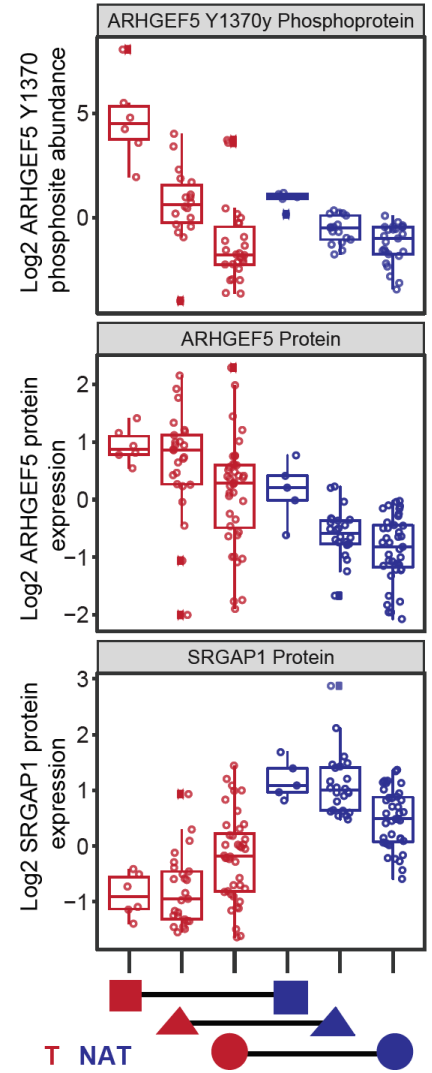
Kinase, phosphatase, ubiquitinase and deubiquitinase outlier analyses highlight the importance of post-translational modification data and nominate candidate therapeutic targets









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