

# A Phenotypic Variant of Small Cell Lung Cancer with Drug-Resistant and Immuno-modulatory Properties Identified by Computational Modeling

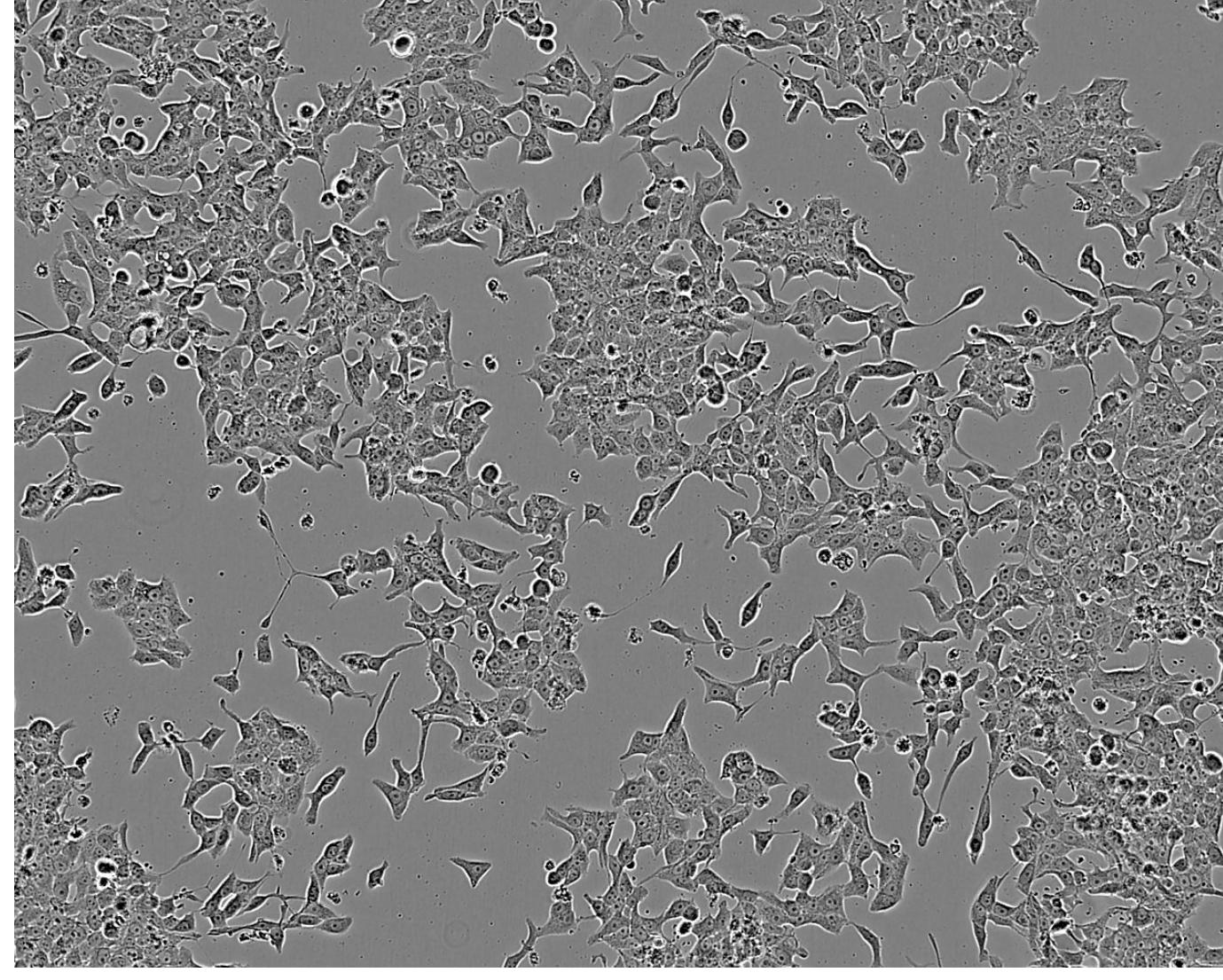
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## Introduction

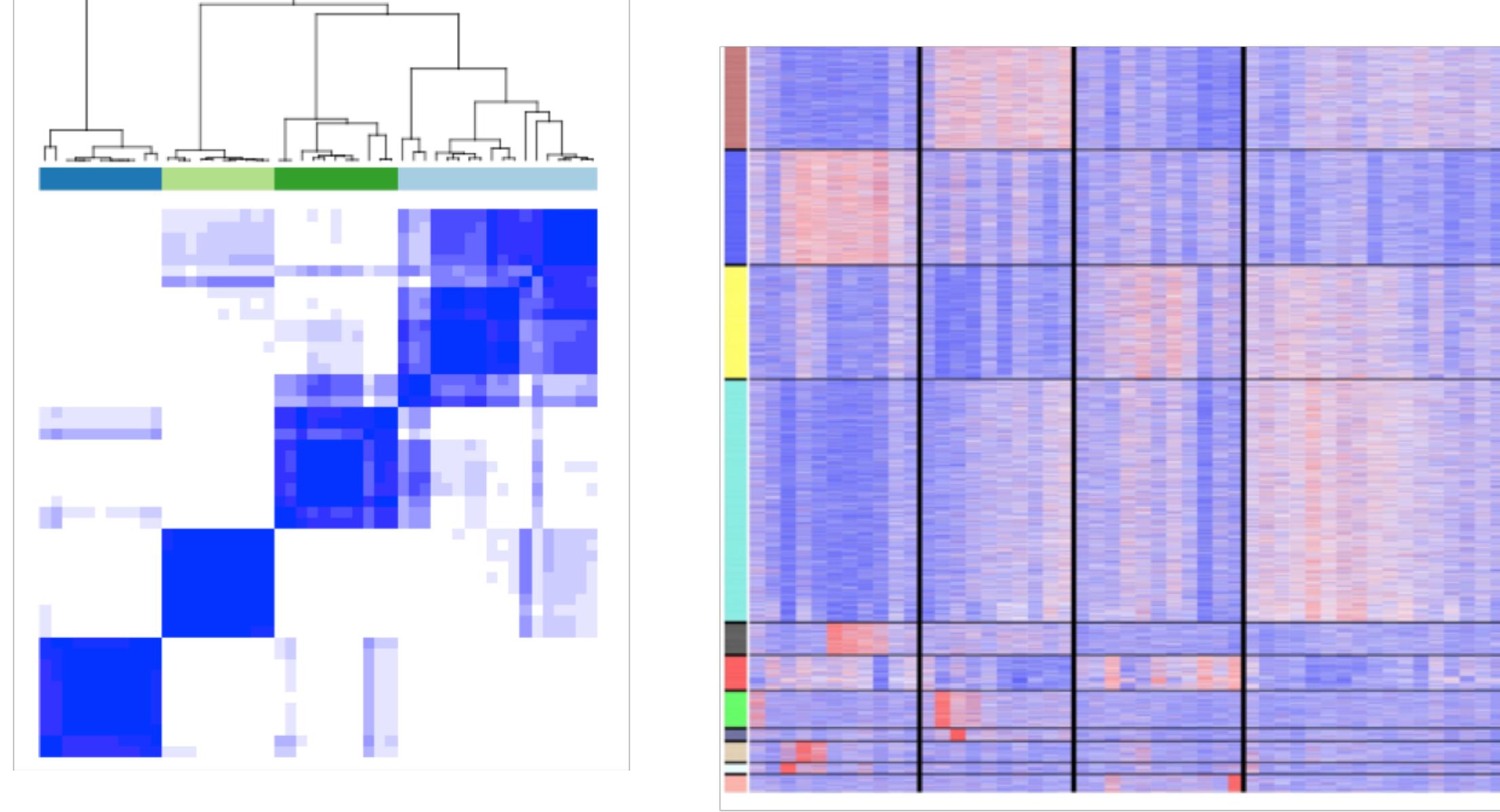
Small cell lung cancer (SCLC) is an aggressive tumor type with a strong ability to become resistant to all known treatments and to survive in diverse microenvironments. Proposals to stratify patients based on tumor phenotype have been met with resistance due to unclear clinical relevance, as the “small blue round” SCLC cells are extremely uniform by histopathology. More recently, however, it has become increasingly understood that SCLC tumors exhibit phenotypic heterogeneity implicated in the aggressiveness of the disease.

*By uncovering the mechanisms behind SCLC phenotypic diversity, we can develop a descriptive and functional model to reprogram SCLC towards sensitivity.*

One previously undefined subtype, here as NE Variant 2, shows gene enrichment and drug response that suggest it is an important subtype for understanding the recalcitrance of SCLC clinically. This study aims to distinguish NE Variant 2 from other computationally and empirically defined subtypes.

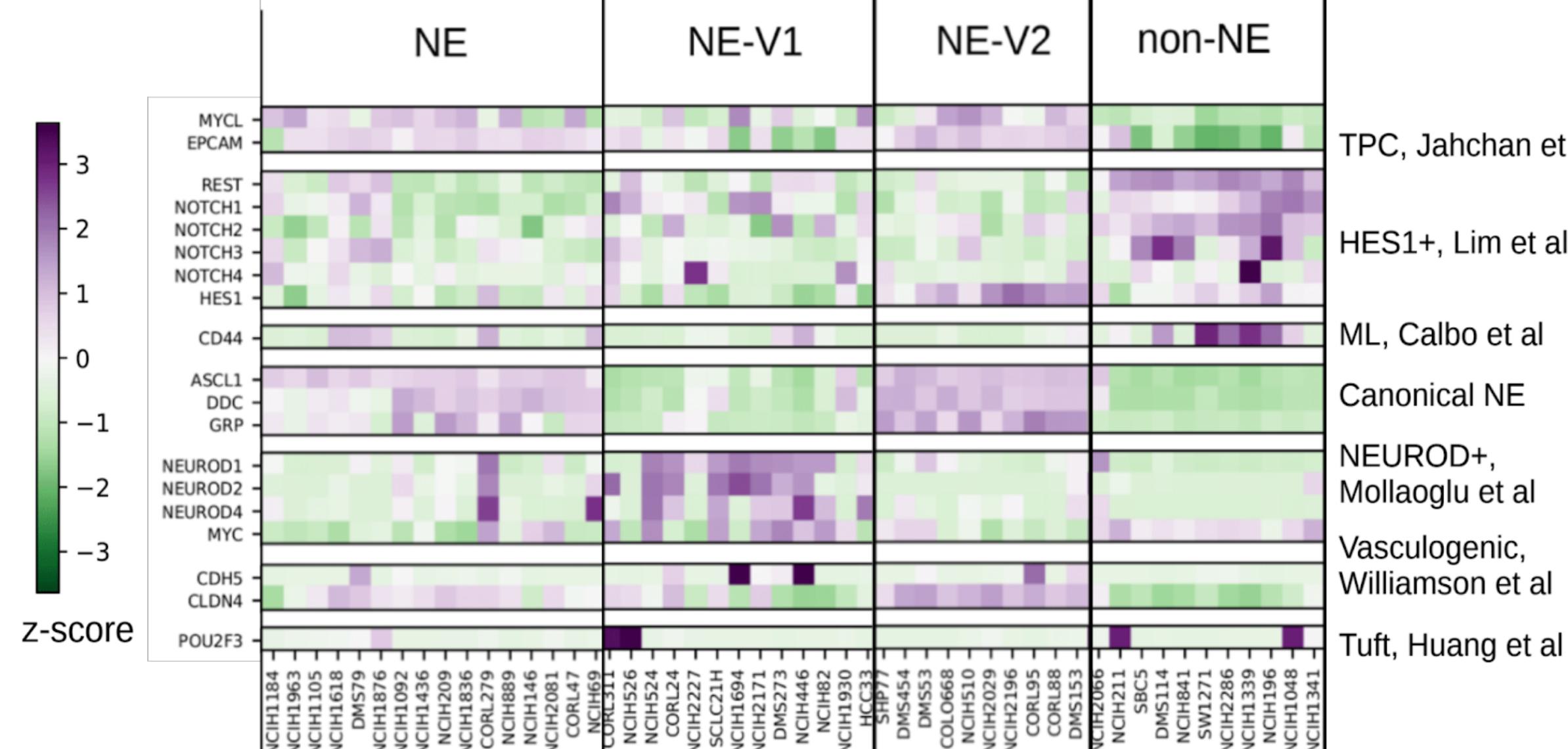


## Clustering by Samples and Genes Reveals Four Phenotypes defined by Gene Modules



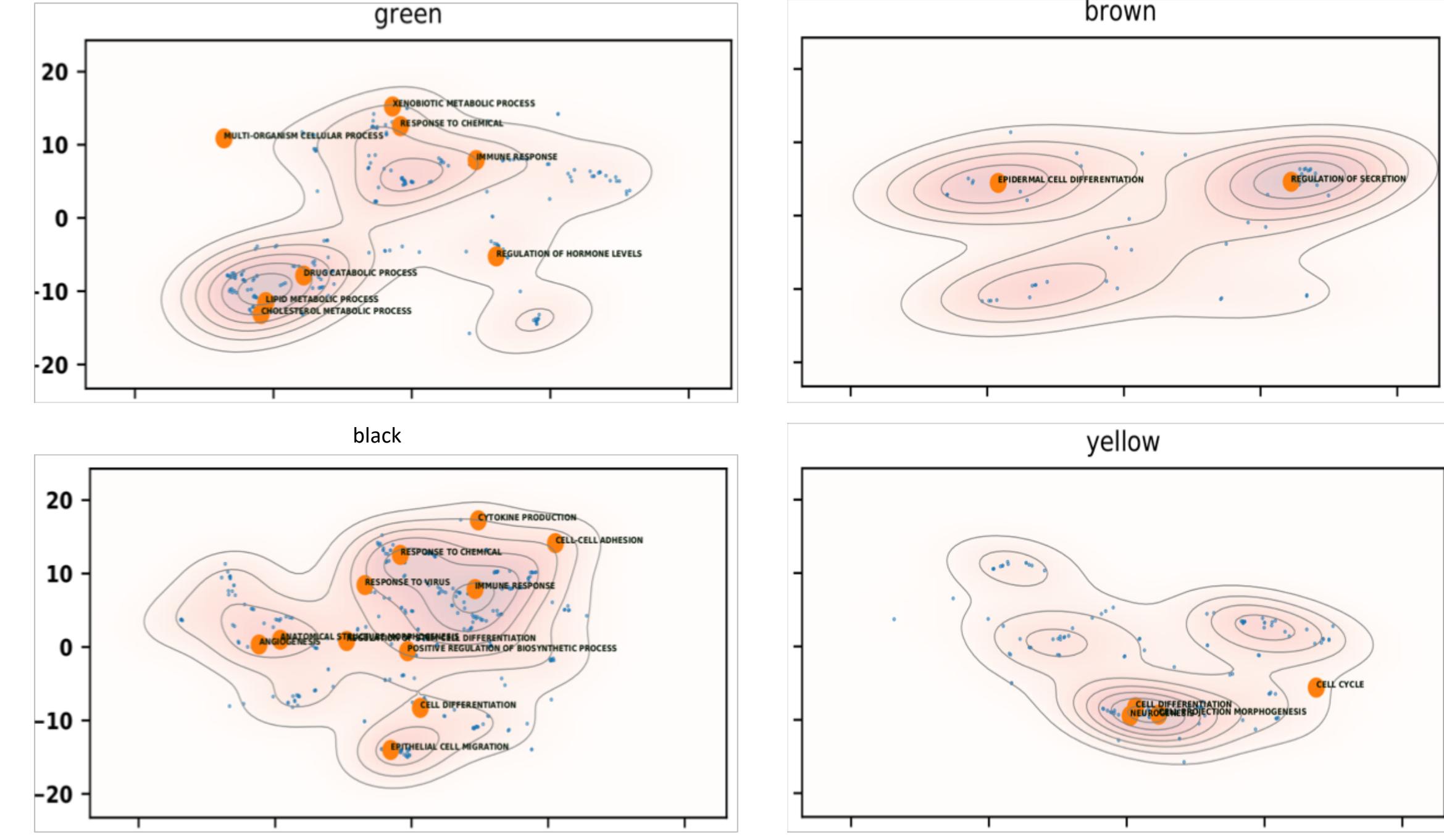
Consensus clustering with k-means algorithm and 1 — Pearson correlation distance metric on cell lines reveals four biologically relevant clusters.

## Current Biomarkers are Unable to Distinguish between Canonical NE and NE Variant 2 Subtypes



Biomarkers arranged by publication for previously defined SCLC phenotypes. NE, NE-V1, and NON-NE correspond with subtypes from literature. NE-V2 has not been previously defined.

## Gene Ontology Analysis Reveals NE Variant 2 Subtype is Enriched in Drug Metabolism and Immuno-modulatory Properties



Enrichment of gene ontology terms in co-expressed gene modules shown in phenospace (t-SNE). The green module, upregulated in NE-V2, suggests the subtype is enriched in drug metabolism and immuno-modulatory genes. Thus, we predicted NE-V2 would be better able to respond to stressors and drug perturbations.

## NE Variant 2 is More Drug Resistant than Other Subtypes to a Variety of Drugs

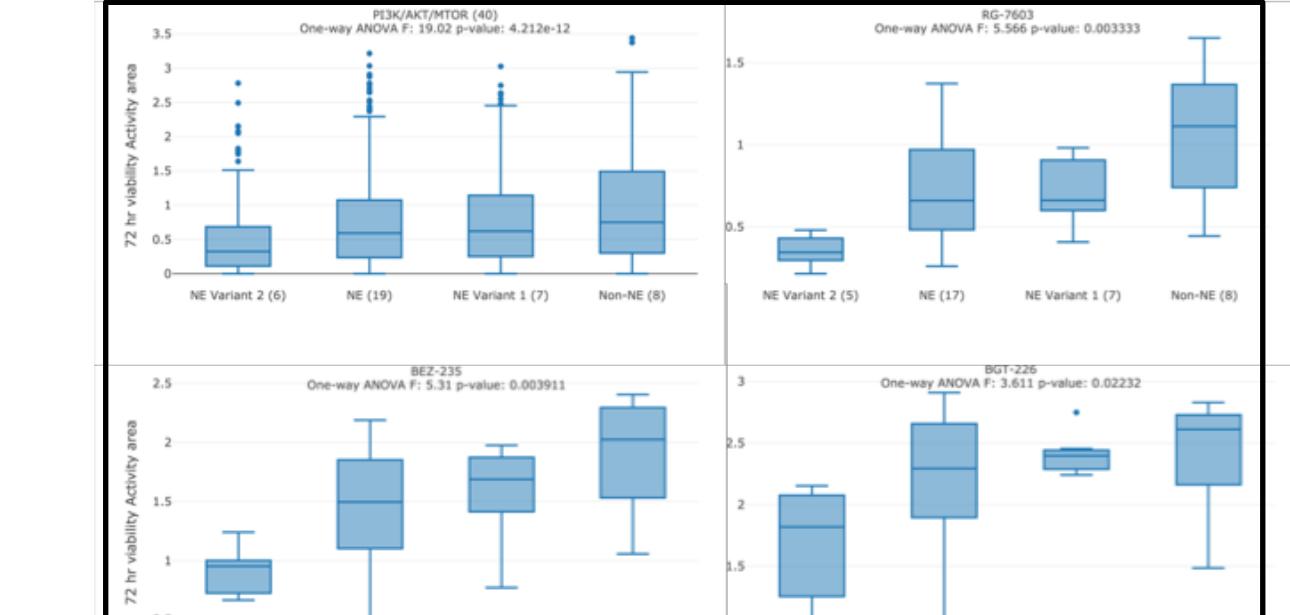
### Ranked Sensitivity of Subtypes by Average Activity Area

	NE	NEV1	NEV2	NON-NE
Most resistant ↑	103/ 19.8%	101/ 19.5%	<b>282/ 54.3%</b>	33/ 6.4%
↓ Most sensitive	213/ 21.6%	112/ 18.3%	95/ 19.1%	99/ 19.1%
	137/ 26.4%	<b>159/ 30.6%</b>	108/ 20.8%	115/ 22.2%
	66/ 12.7%	147/ 28.3%	34/ 6.6%	<b>272/ 52.7%</b>

One-way ANOVA F: 34.88 p-value: 7.319e-20

MEK Inhibitors

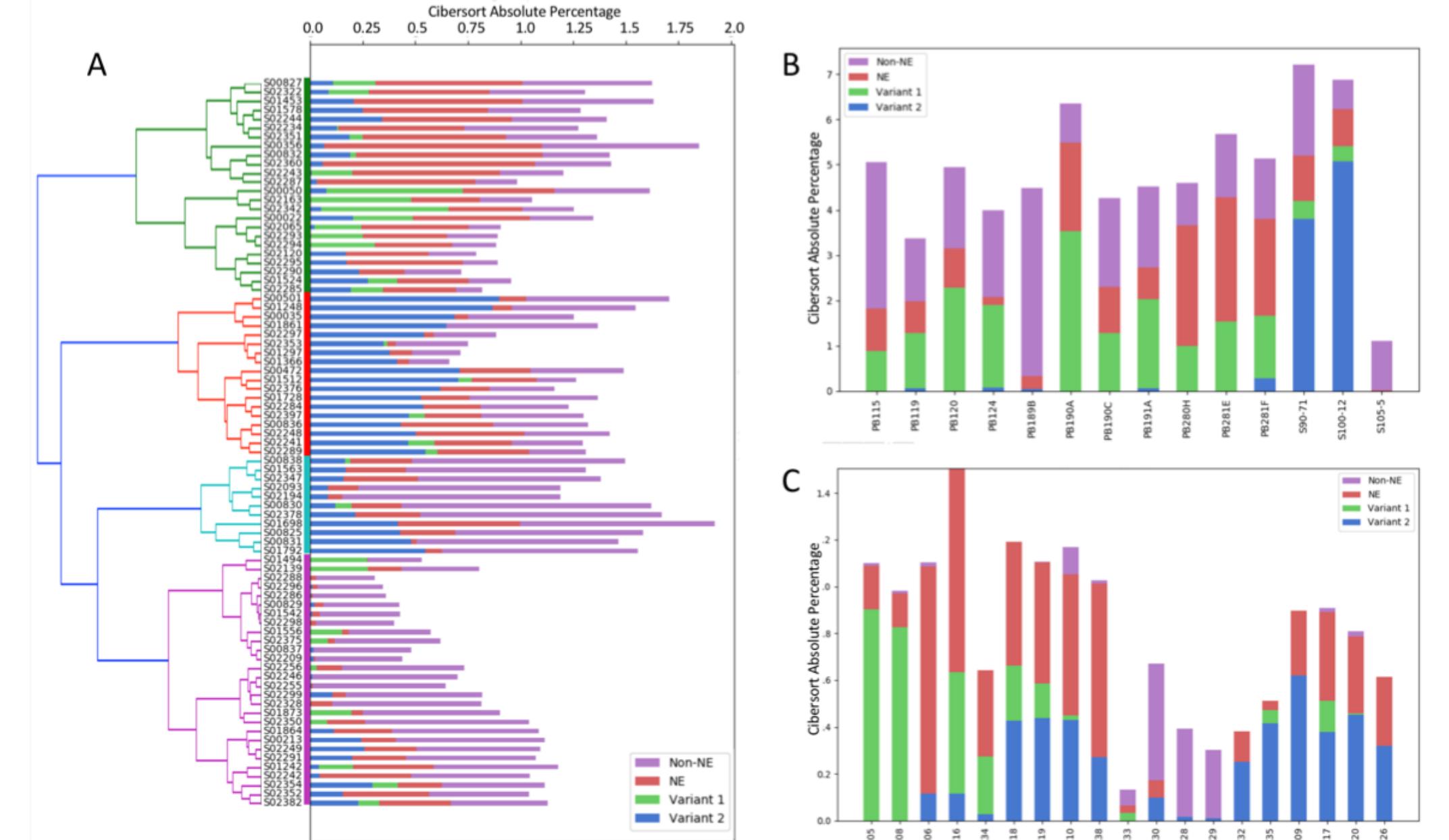
One-way ANOVA F: 34.88 p-value: 7.319e-20



Aurora Kinase Inhibitors

One-way ANOVA F: 12.97 p-value: 3.841e-08

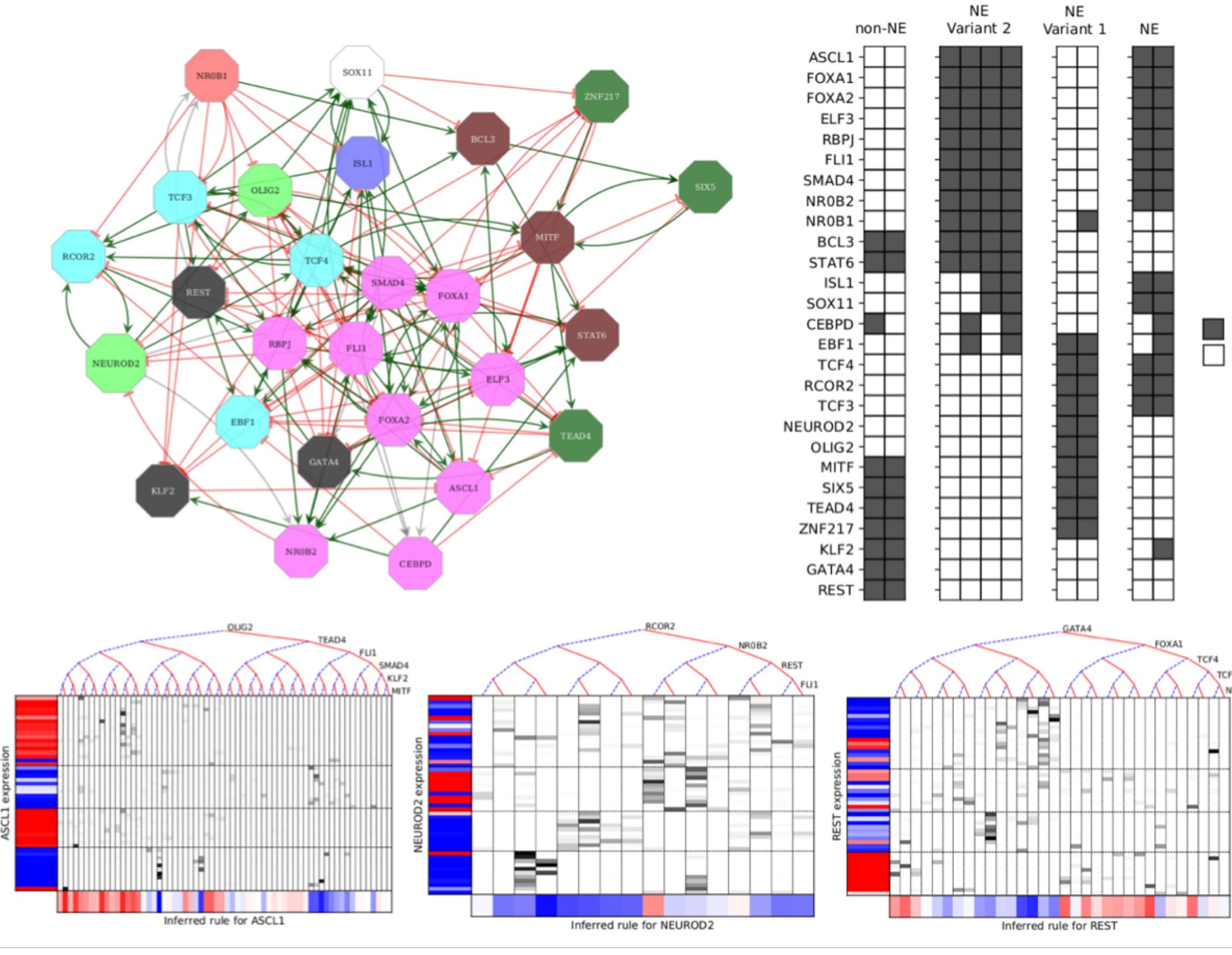
## CIBERSORT Analysis Suggests Subtypes are Represented in Varying Proportions in Human and Mouse SCLC Tumors



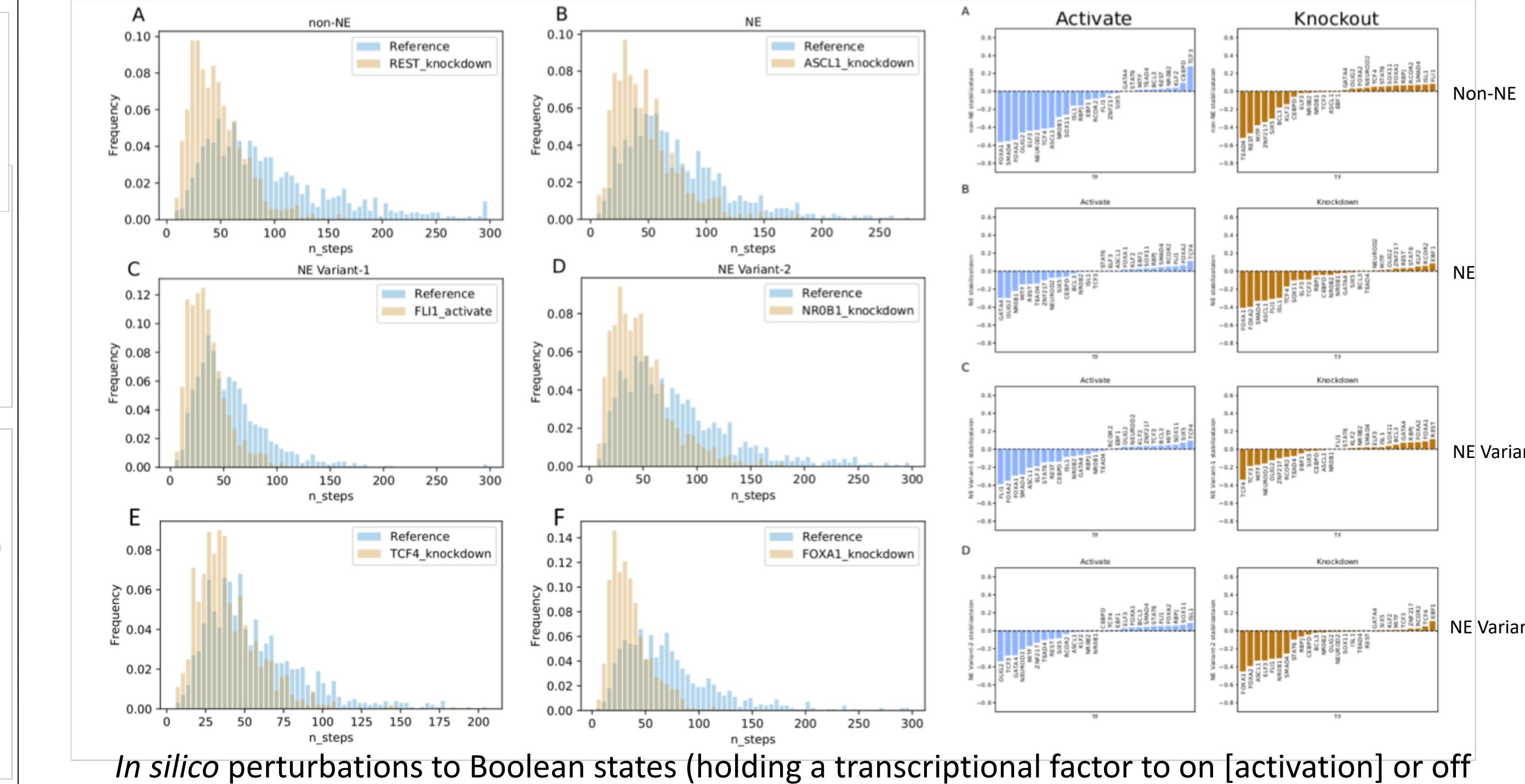
CIBERSORT, a computational algorithm for determining proportions of cell types in bulk populations, suggests the cell-line-defined subtypes are present in human and mouse tumors.

## In Silico Simulation of Gene Regulatory Network Recapitulates Four Subtypes

Transcriptional factor network simulated by probabilistic Boolean rules gives four attractor states that correlate with empirical subtypes. Probabilistic rules account for uncertainty in data, and avoid overfitting. *In silico* simulation suggests that subtypes arise from and are regulated by regulatory dynamics of transcription factors, rather than only mutational status of the cells.



## In Silico Perturbations Suggest Strategies for Reprogramming SCLC Cells toward Sensitivity



*In silico* perturbations to Boolean states (holding a transcriptional factor to on [activation] or off [knockdown] during simulation) shows stability of subtype states. Most destabilizing perturbations for each state suggest strategies for forcing transitions between states. This “reprogramming” method suggests an alternative to traditional, ineffective oncogene-targeted therapy for SCLC.

## Summary and References

- Consensus clustering identified four phenotypic clusters defined by WGCNA gene modules.
- There may be a relationship between phenotype and drug sensitivity, implicating NE Variant 2 as the most resistant SCLC subtype and thus a target for reprogramming strategies.
- NE Variant 2 may be able to modulate the immune system and better resist perturbations by drug through enriched drug metabolism.
- Stable attractors in the Boolean GRN uncover the mechanisms of phenotypic heterogeneity. We expect that manipulating these states can reprogram resistant SCLC phenotypes toward sensitivity.
- Computational analysis gives evidence for existence of cell-line-defined subtypes in human SCLC tumors, genetically engineered mouse models, and PDX/CDX mouse models.

(1) Barrentine C., et al. *Nature* (2012).

(2) George J., et al. *Nature* (2015).

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(4) Mollaoglu G., et al. *Cancer Cell* (2017).

(5) Polley E., et al. *J Natl Cancer Inst.* (2016).

(6) Udyavar A.R., et al. *Cancer Res.* (2017).

(7) Newman, et al. *Nature Methods* (2015).

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