

Deciphering Phenotypic Heterogeneity in Small Cell Lung Cancer

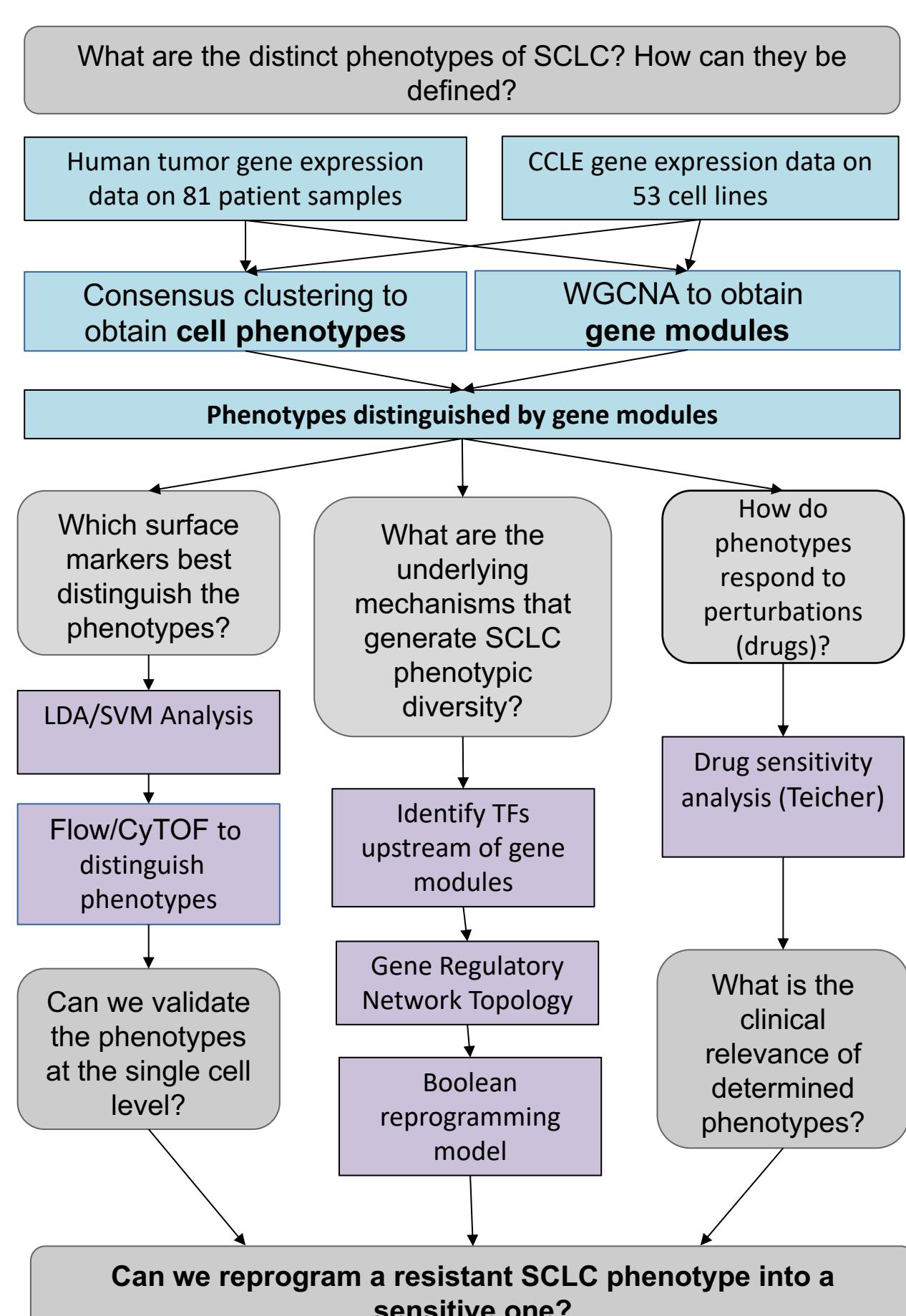
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Introduction

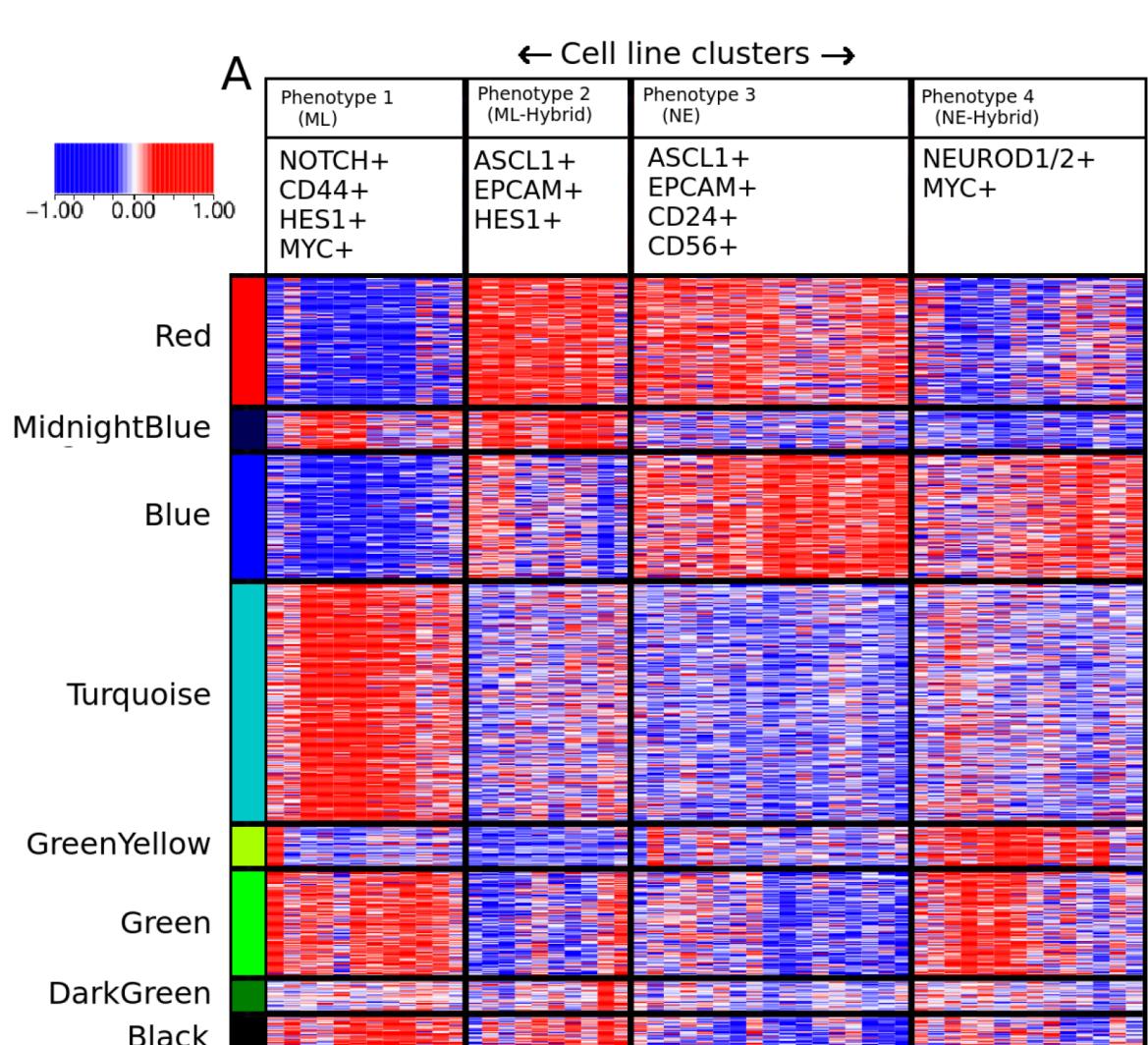
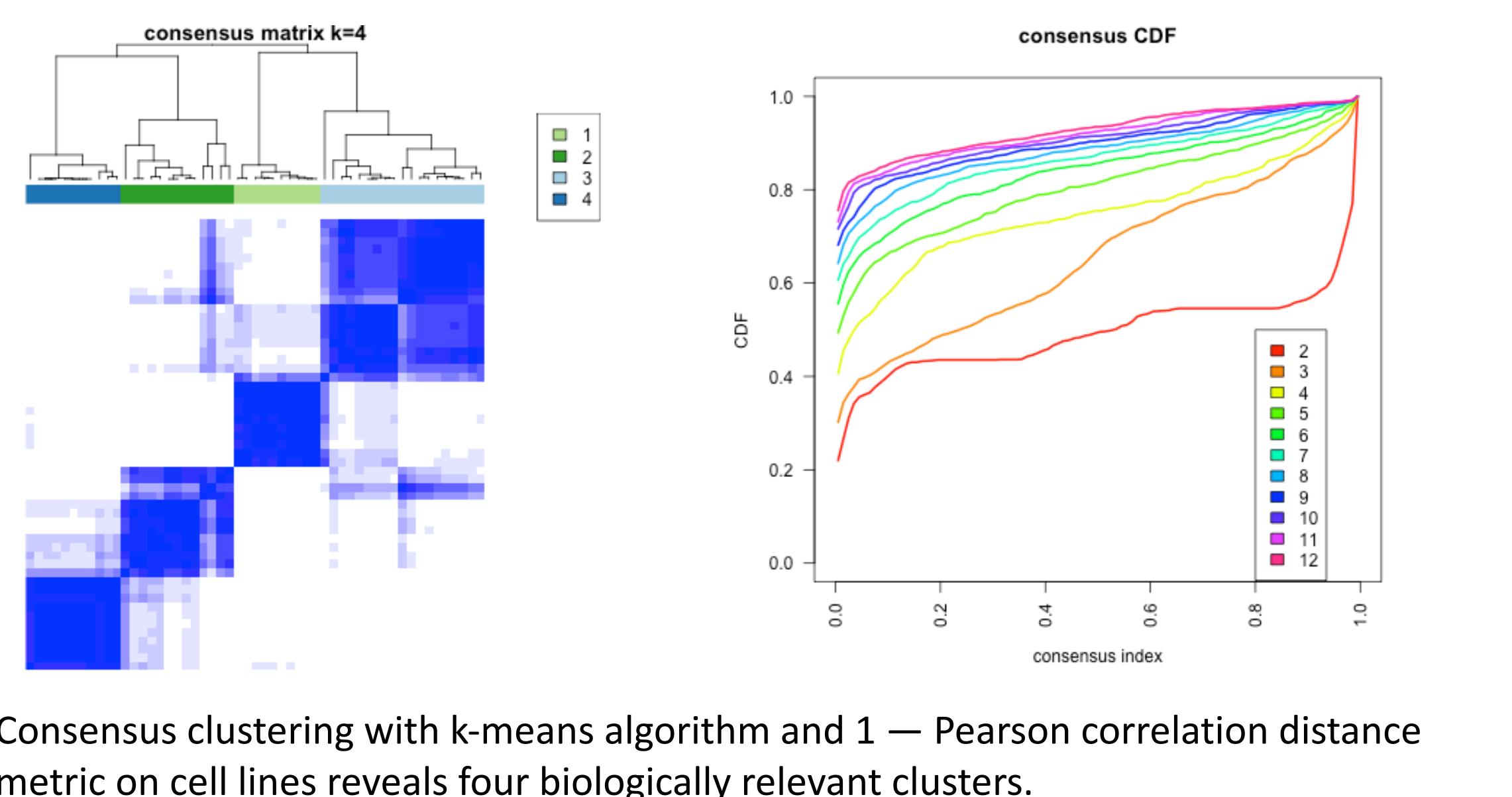
Small cell lung cancer (SCLC) is an aggressive tumor type with a strong ability to become resistant to all known treatments and to survive in diverse microenvironments. Proposals to stratify patients based on tumor phenotype have been met with resistance due to unclear clinical relevance, as the “small 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.

Pipeline for our SCLC phenotypic reprogramming strategy. The main questions this work addresses are highlighted in gray boxes.

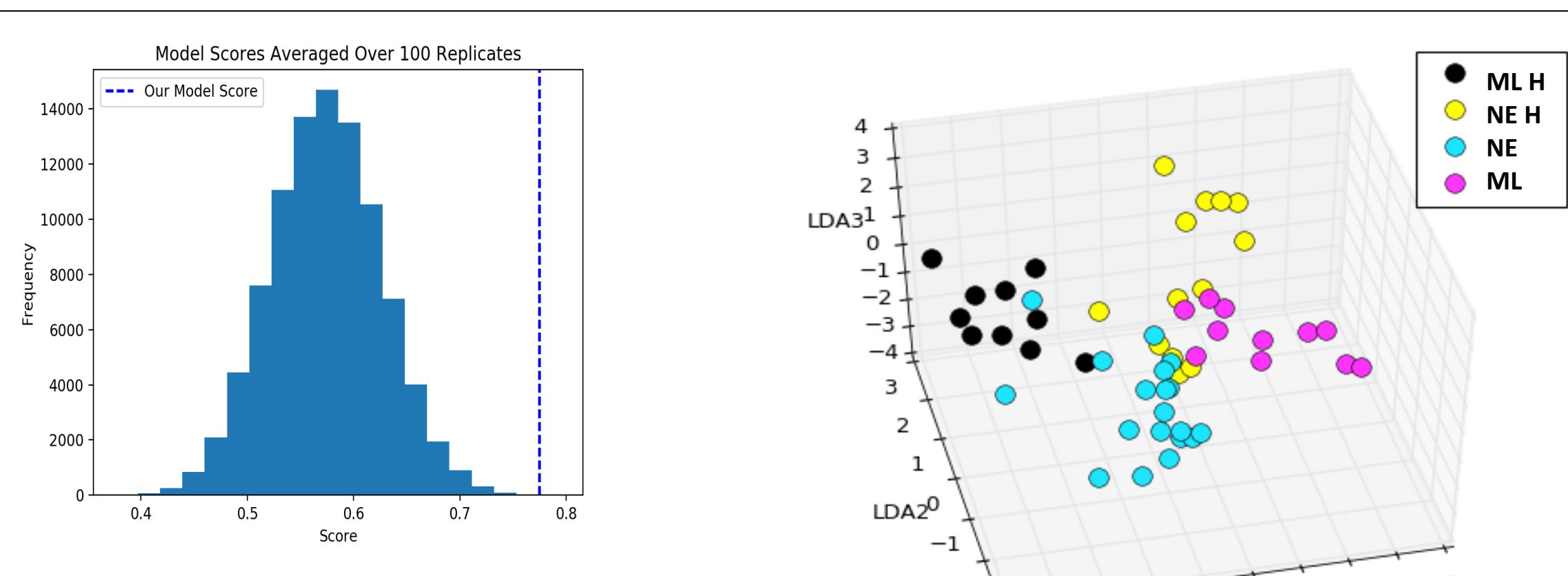


Clustering by Samples and Genes Reveals Four Phenotypes defined by Gene Modules



Phenotypic variation across A) 53 SCLC cell lines and B) 81 human tumor samples. Four distinct phenotypes generated by consensus clustering methods above (columns) are described by modules of co-expressed genes generated by Weighted Gene Co-Expression Network Analysis (WGCNA, rows). Similar patterns of co-expression can be seen in the human tumors.

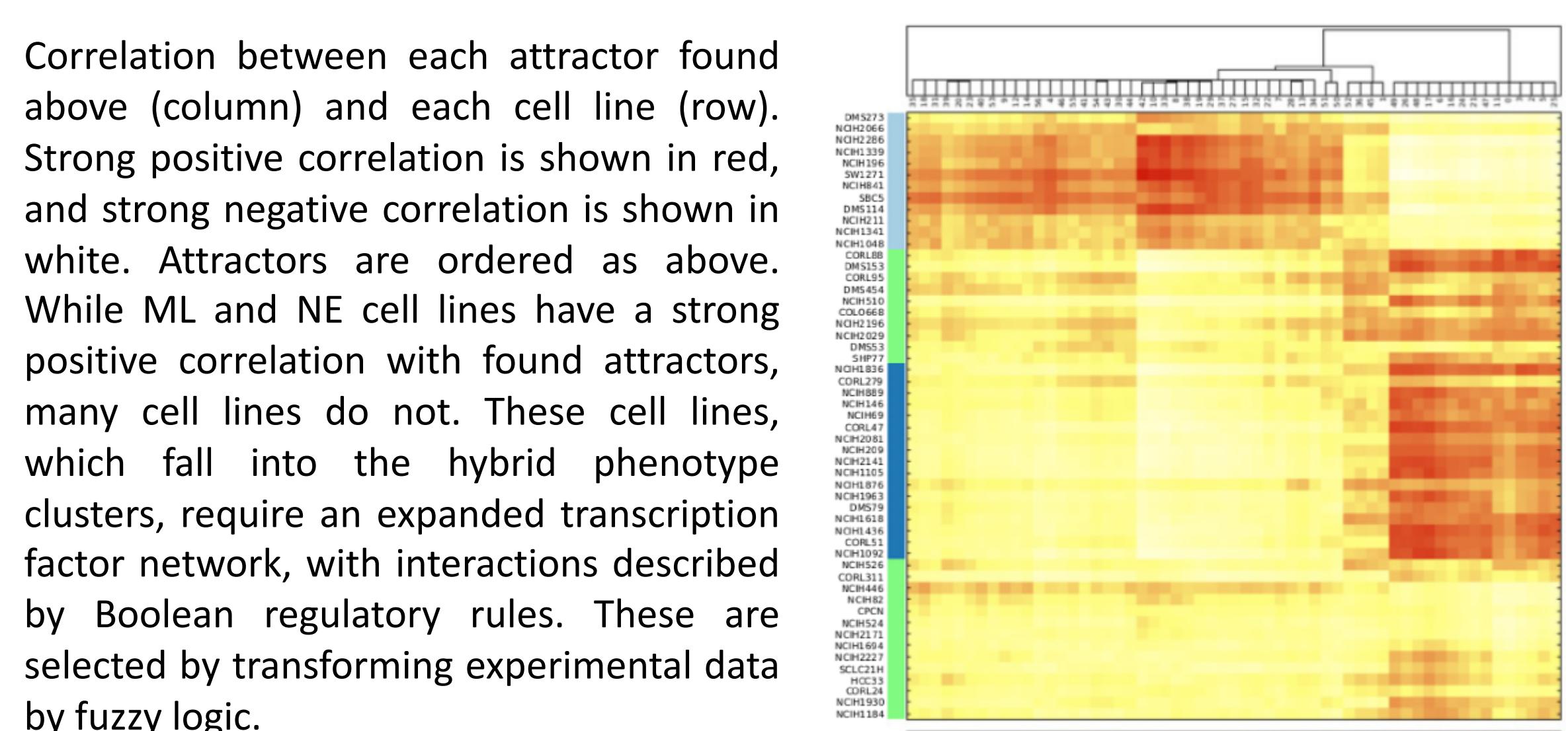
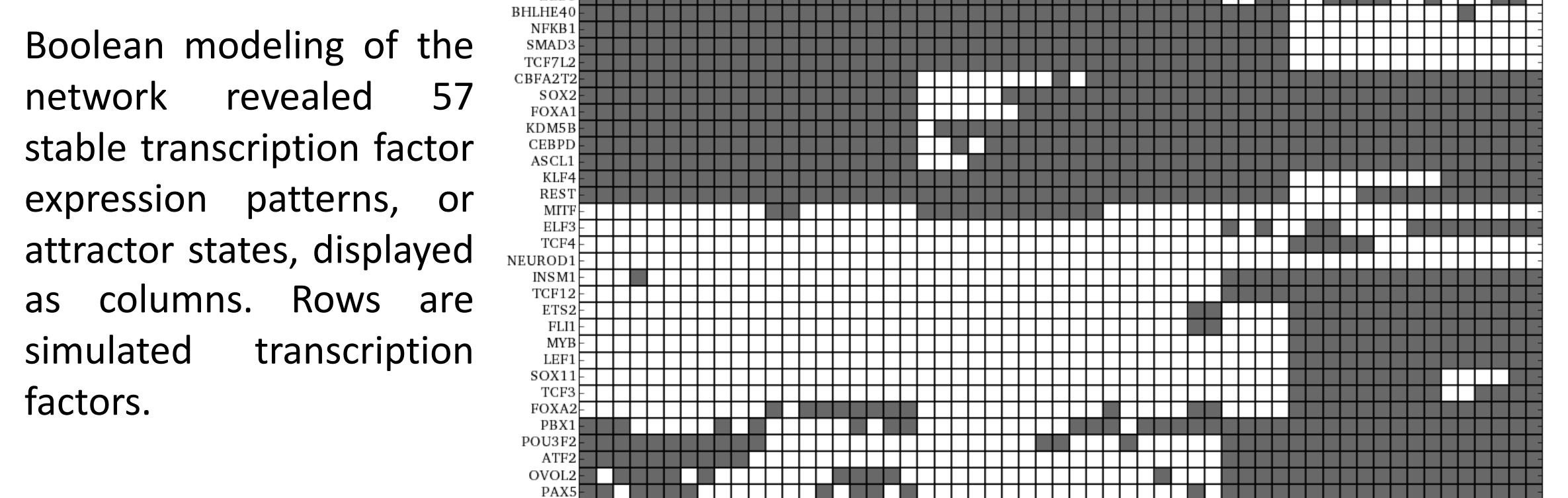
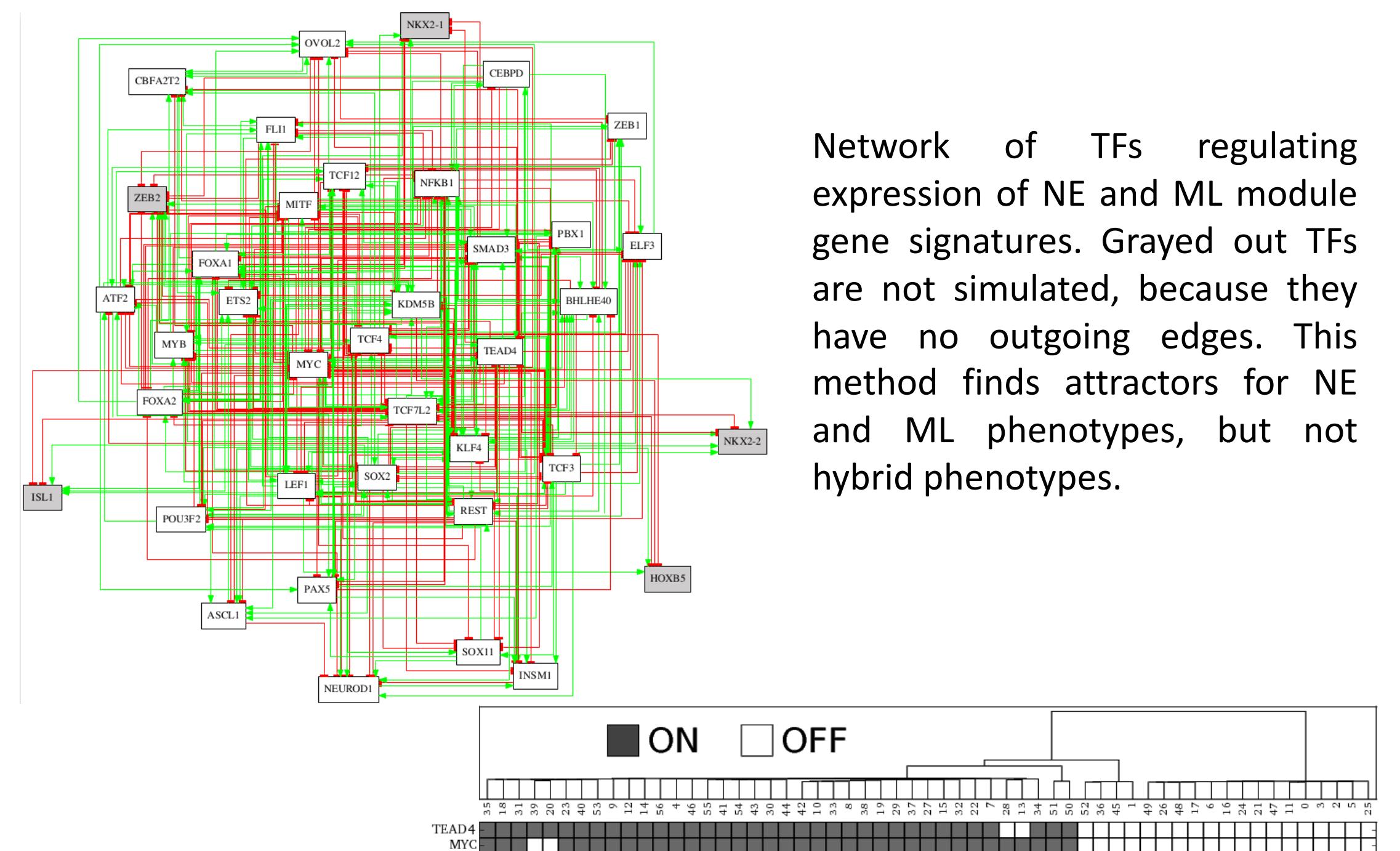
Support Vector Machine and Linear Discriminant Analysis



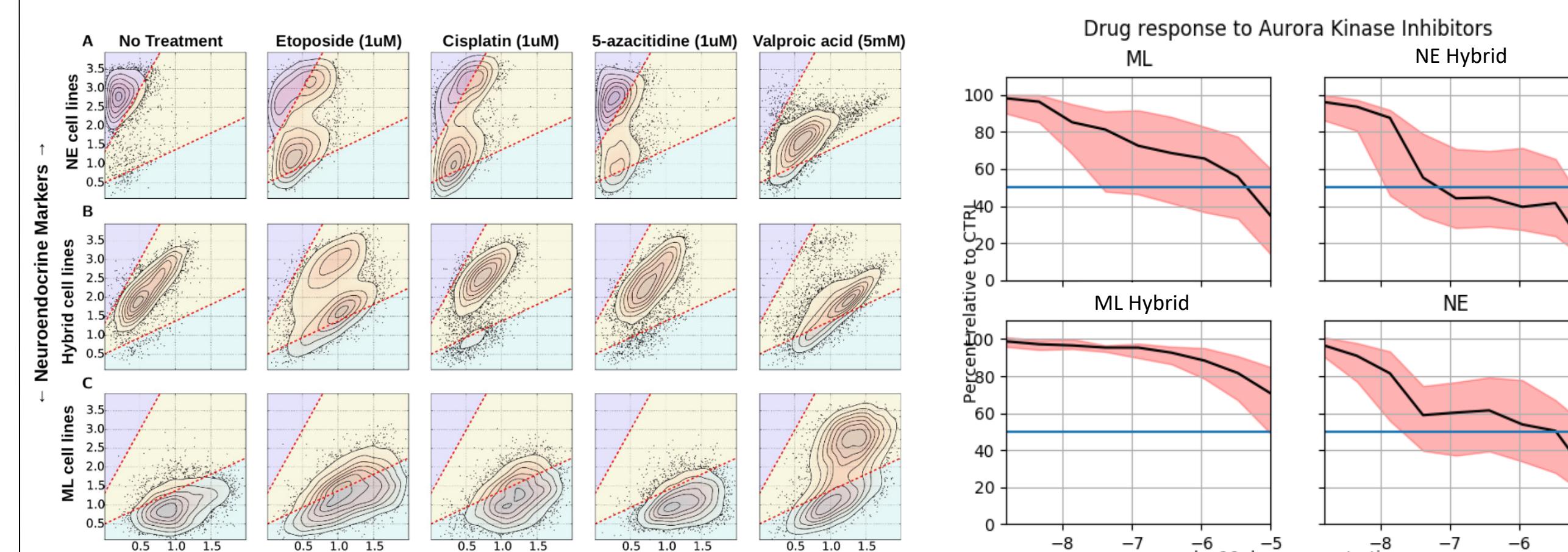
SVM models scored by accuracy using train and test subsets of gene expression data. Average score was calculated from 100 models generated by combinations of three surface markers from a curated list, and the score describes the model's ability to distinguish between phenotypes 1, 2, and 3. EphA2 was added to the marker list to distinguish phenotype 4, which has been used in previous work. Our marker set has a score much higher than average (blue dotted line).

Four phenotypes projected into a reduced-dimensional space by LDA. The model is able to separate the phenotypes. Flow cytometry will be used to validate these phenotypes at the single cell level.

Transcription Factor Network Topology

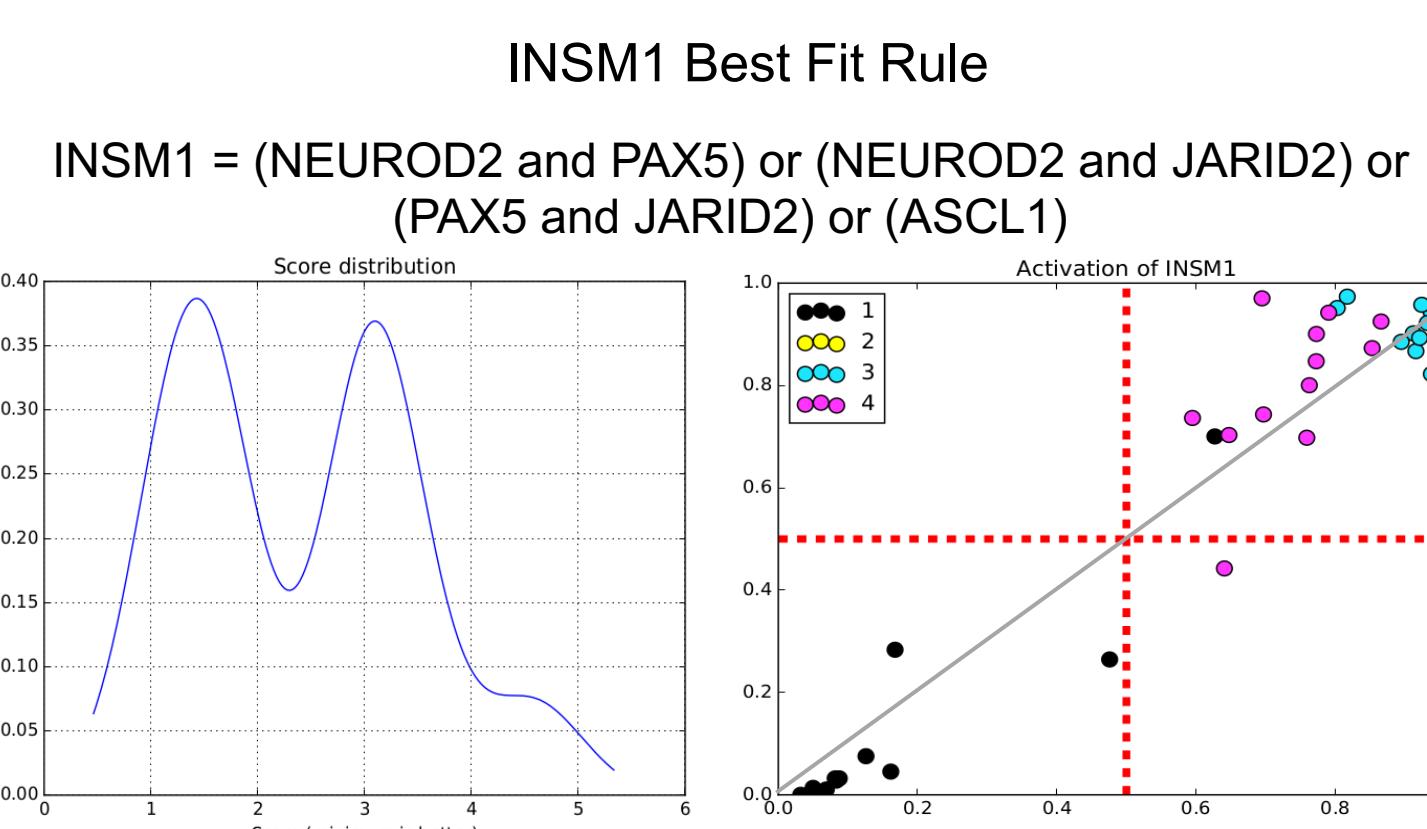


Drug Sensitivity Analysis and Phenotypic Plasticity



Using previously acquired data from Polley et al. on sensitivity of SCLC to various drugs, we analyzed dose response curves by phenotype. In a significant portion of drugs tested, the NE hybrid phenotype was most sensitive to drug, and the ML hybrid phenotype was most resistant, with NE and ML phenotypes having intermediate sensitivity. Notably, the NE hybrid cell lines showed increased sensitivity to aurora kinase inhibitors.

Reprogramming SCLC towards Sensitive Phenotypes



A genetic algorithm is used to generate optimal rules describing each node of the Boolean regulatory network. Using fuzzy logic and the generated rules, inputs to the node can be used to predict the normalized expression of that node across cell lines (x axis, right). This is then compared and scored against the true expression from data. The best rules have the lowest scores, and are better at explaining the experimental data.

Ensembles of reprogramming trajectories between phenotype attractors are scored for *in silico* reprogramming experiments based on likelihood of success, with given data.

Summary and References

- Consensus clustering identified four phenotypic clusters defined by WGCNA gene modules.
 - By building SVMs and LDA models based on combinations of a subset of cell surface markers, we are able to distinguish between the four phenotypes using only four markers, which can be used in flow cytometry to validate the phenotypes at the single cell level.
 - There may be a relationship between phenotype and drug sensitivity, implicating a hybrid phenotype as most resistant and thus a target for reprogramming strategies.
 - Stable attractors in the Boolean GRN uncover the mechanisms of phenotypic heterogeneity. We expect that manipulating these states will reprogram resistant SCLC phenotypes toward sensitivity.
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 (4) Iorio, F., et al. *Cell* (2016).
 (5) Mollaoglu G., et al. *Cancer Cell* (2017).
 (6) Polley E., et al. *J Natl Cancer Inst.* (2016).
 (7) Udyavar A.R., et al. *Cancer Res.* (2017).

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