

4 states \neq 4 dimensions: Neural-progenitor-like – Mesenchymal antagonism dominates the patterns of phenotypic heterogeneity in Glioblastoma

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Introduction

Verhaak et al. using TCGA data.

4 subtypes found:

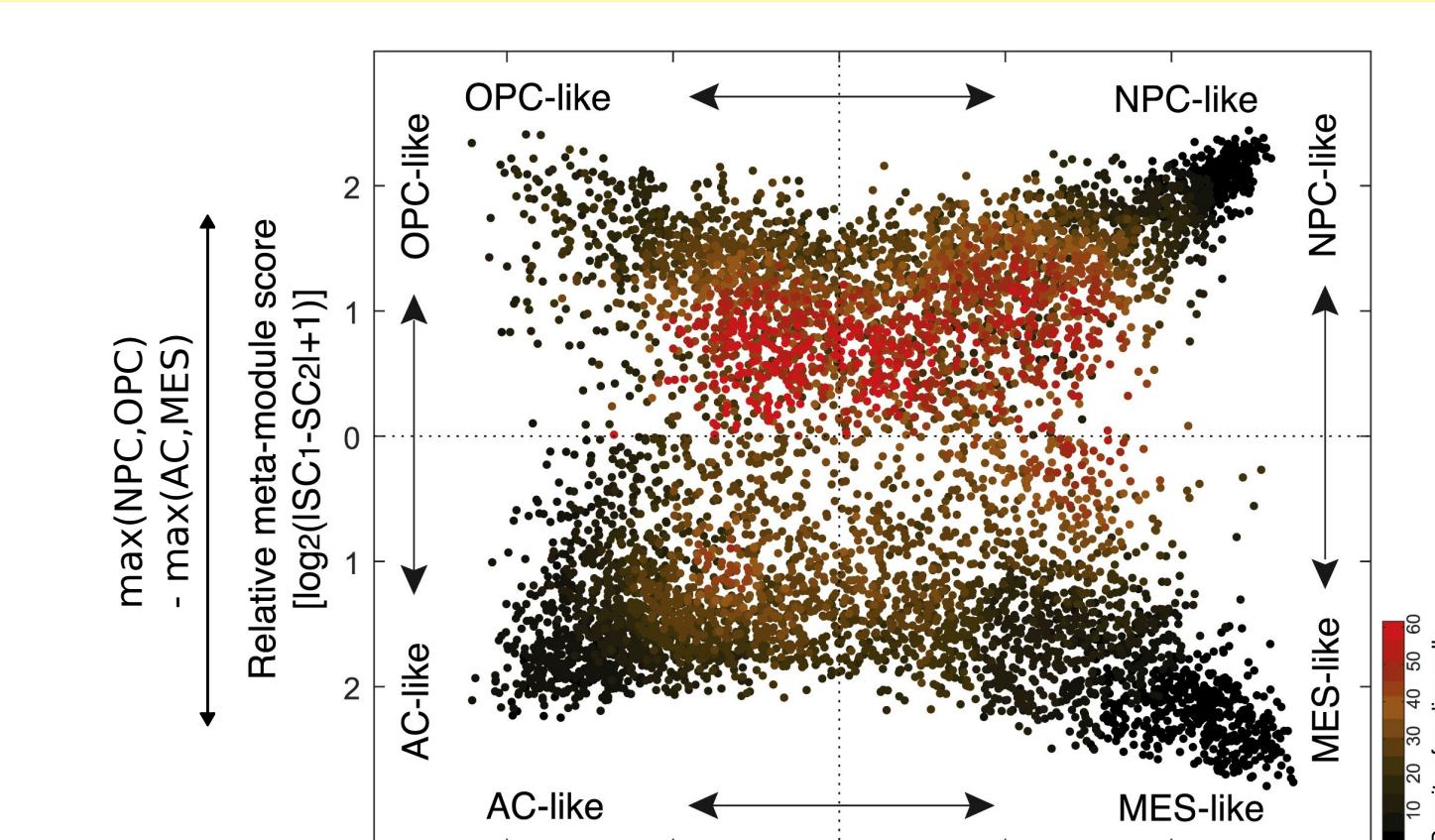
- Proneural (VerPN)
- Neural (VerNL)
- Classical (VerCL)
- Mesenchymal (VerMES)

Neftel et al. using single cell data.

resembling neurodevelopmental lineages:

- Neural-Progenitor-like (NefNPC)
- Oligodendrocyte-Progenitor-like (NefOPC)
- Astrocyte-like (NefAC)
- Mesenchymal (NefMES)

Are these cell states truly distinct and mutually exclusive?



Methods/Formulae

ssGSEA:

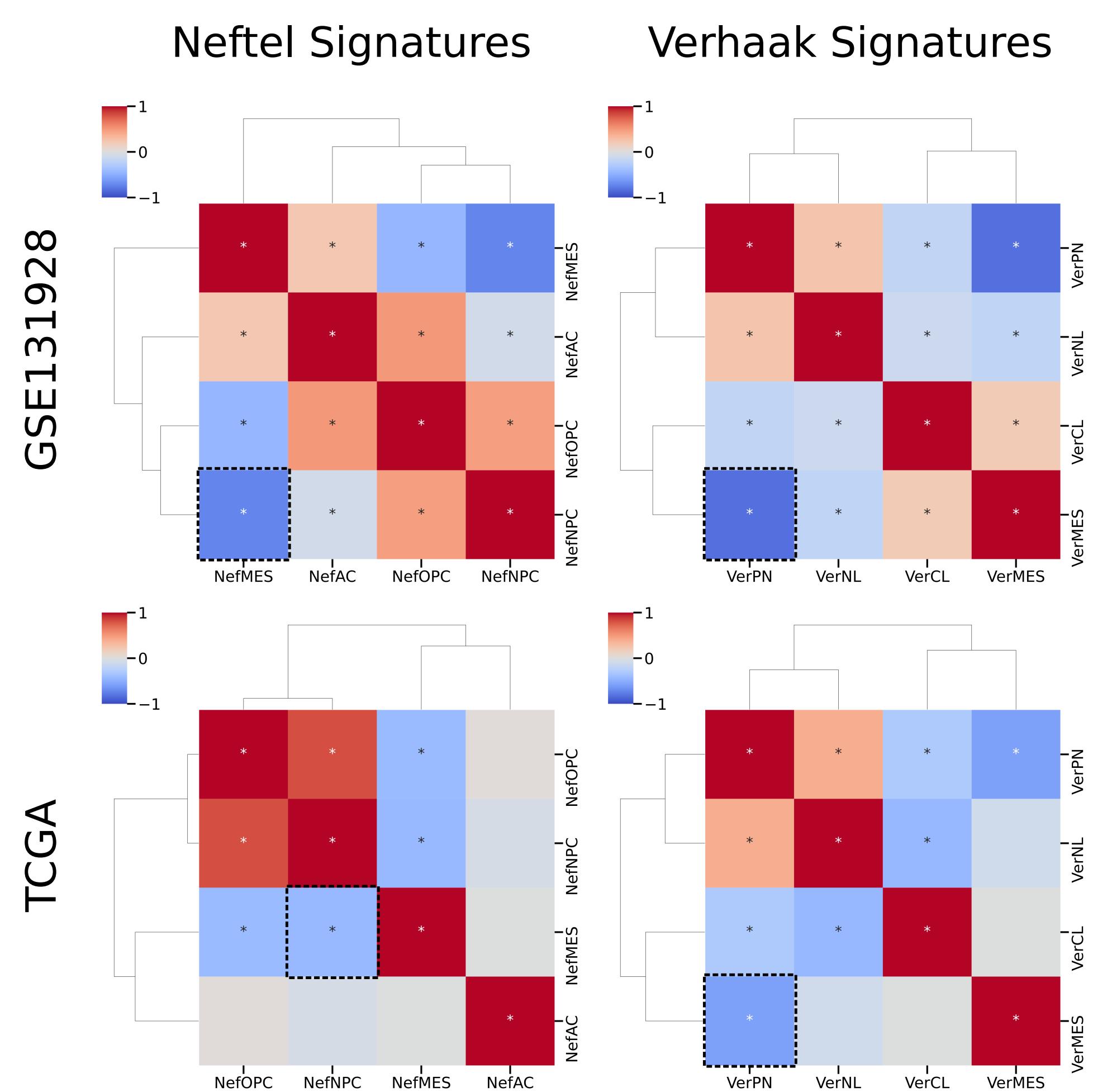
$$ES(G, S) = \sum_{i=1}^N \left[\sum_{r \in G, j \leq i} \frac{|r_j|^{1/4}}{\text{ECDF of genes in signature}} - \sum_{r \notin G, j \leq i} \frac{1}{N - N_G} \right]$$

J-Metric:

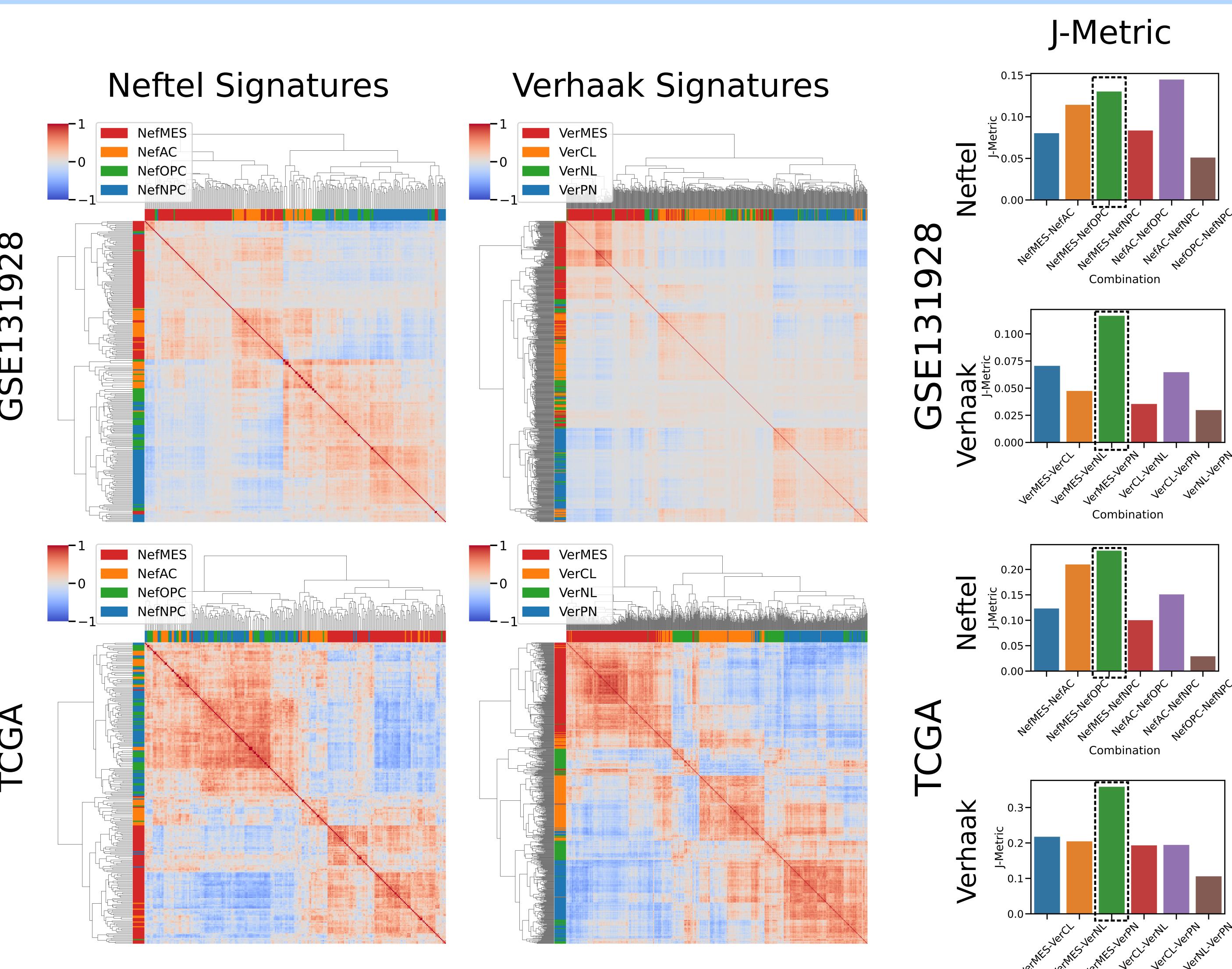
$$J = \sum_{x,y \in G_1} \frac{\rho_r(x,y)}{4N_1^2} + \sum_{x,y \in G_2} \frac{\rho_r(x,y)}{4N_2^2} - \sum_{x \in G_1, y \in G_2} \frac{\rho_r(x,y)}{2N_1 N_2}$$

G_i = Gene Set i
 N_i = Number of genes in set i
 r_j = Rank of Gene j
 $\rho_r(x, y)$ = Spearman correlation of Gene x with Gene y

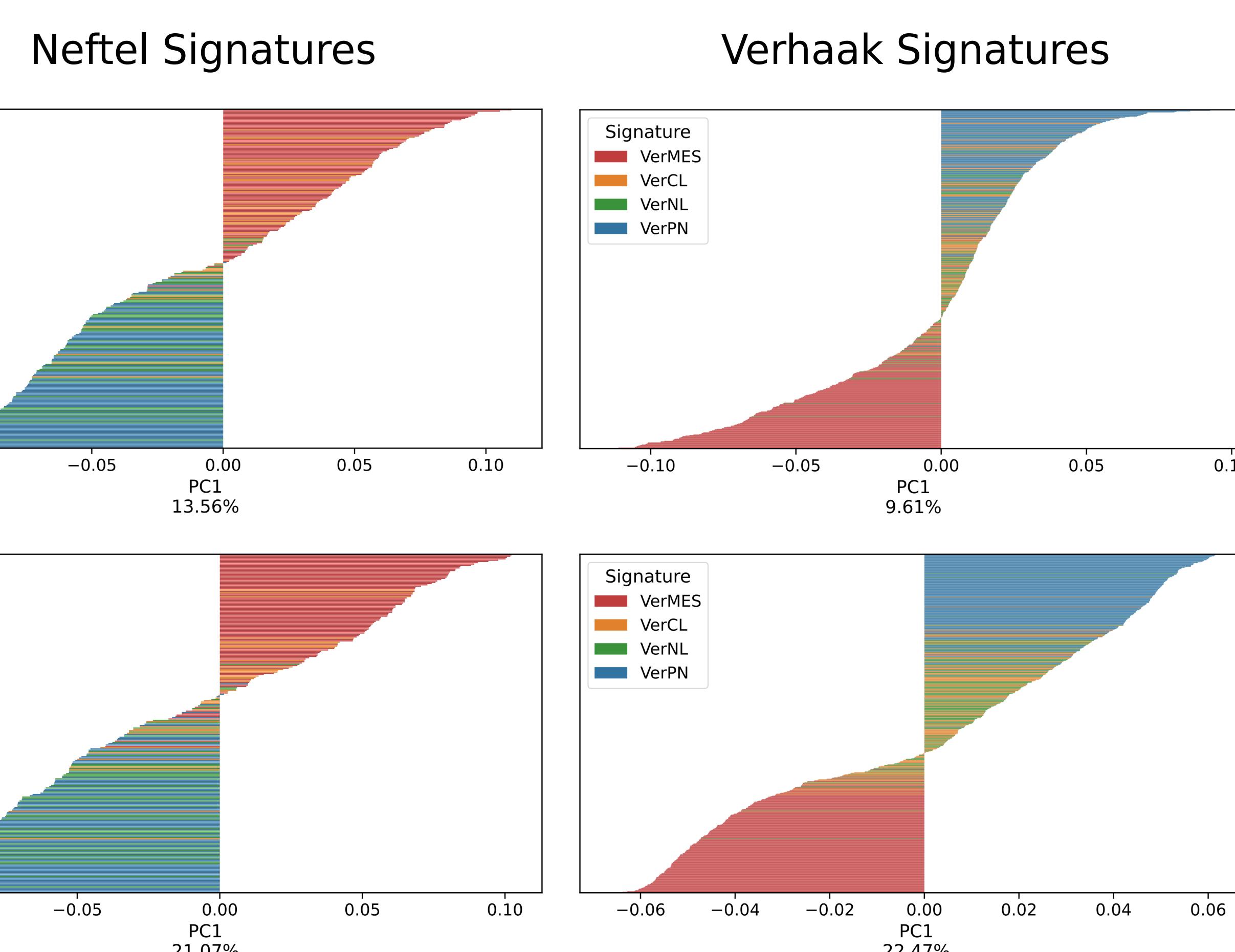
Scoring of signatures reveals not all states are distinctly mutually antagonistic



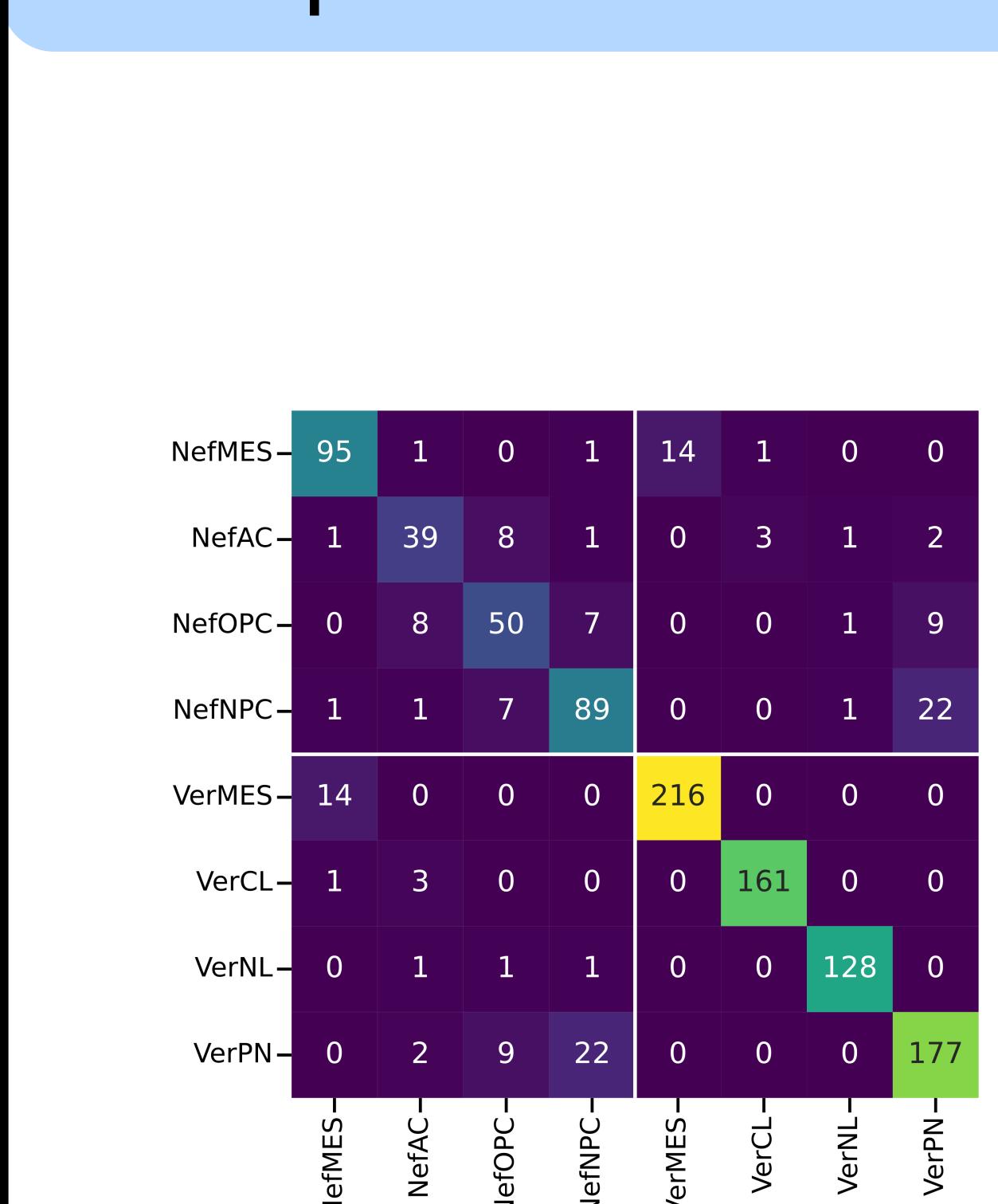
Correlation of Expression levels also captures the NPC/PN-MES antagonism



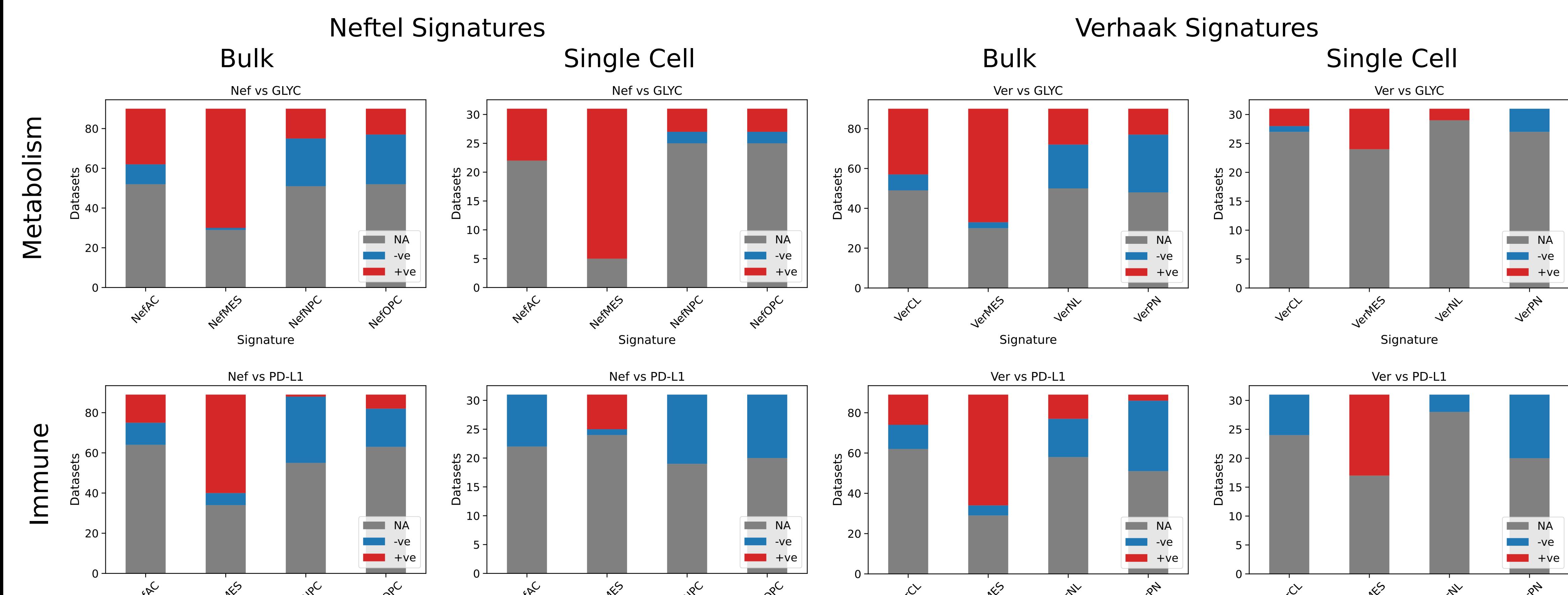
PC1 loadings are dominated by NPC/PN-MES antagonism



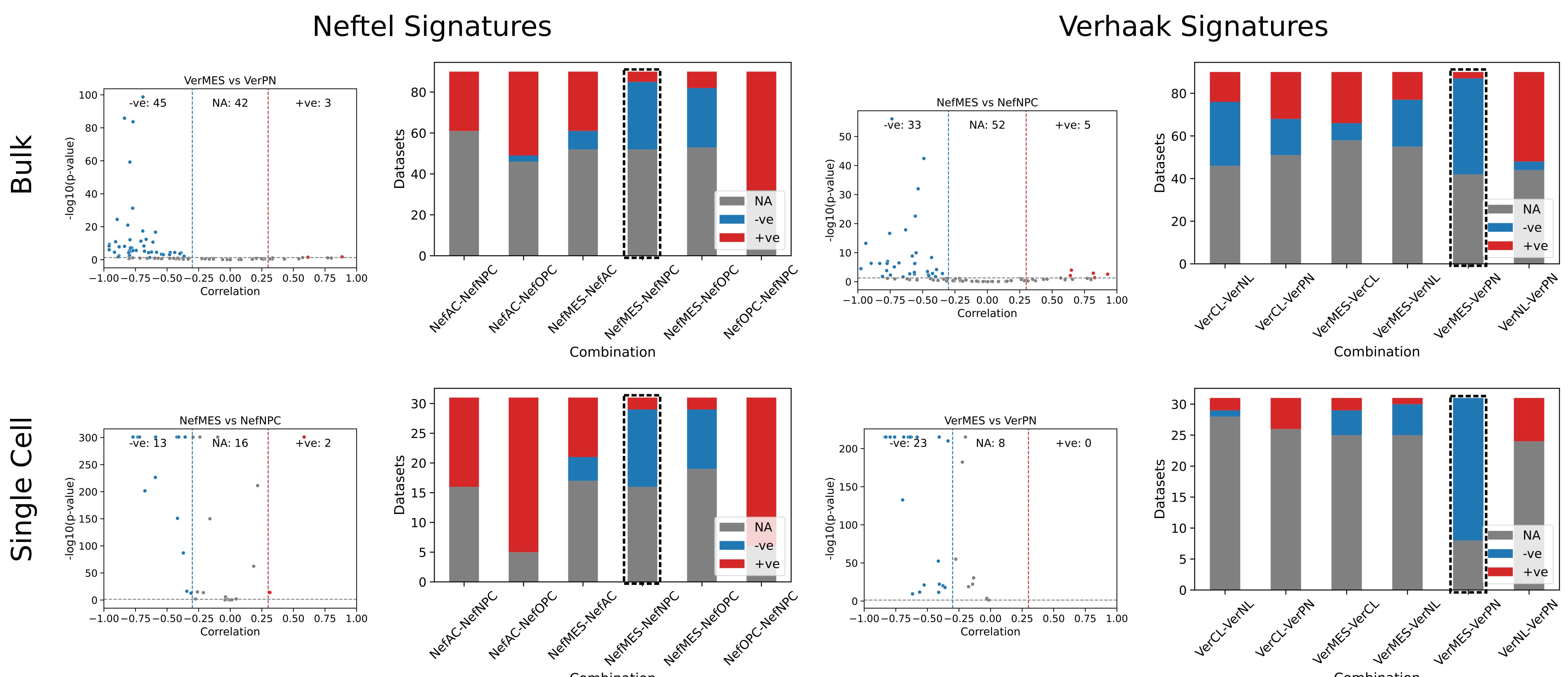
Overlap in signature sets is not sufficient to explain the trends



Trends are consistent in Metabolic and Immune axis MES- More glycolytic, MES- More immune evasive



Observed trend holds across multiple RNASeq datasets



Conclusion

- We can't find all the 4 states mentioned in Neftel or Verhaak to be truly distinct
- Most antagonistic pair:
 - In Neftel signatures: NefNPC vs NefMES
 - In Verhaak signatures: NefPN vs VerMES
- Similar states between the signatures:
 - NefNPC = VerPN
 - VerMES = VerMES
- NPC/PN - MES classification should be given more focus for therapeutic targeting efforts

Future Directions

- Constructing a Gene Regulatory Network and understanding the dynamics
- Looking if the trends hold in other regulatory levels: Methylation, Chromatin Configuration, Protein

References

- Verhaak, R. G., et al. (2010). Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in pdgfra, idh1, egfr, and nf1. *Cancer Cell*, 17(1), 98–110.
- Neftel, C., et al. (2019). An integrative model of cellular states, plasticity, and genetics for glioblastoma. *Cell*, 178(4), 835–849.e21.
- Barbie, D., et al. (2009). Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature* 462, 108–112.