



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

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Introduction

Glioblastoma is one of the most deadliest forms of brain cancer. Its aggressiveness is attributed to its highly heterogeneous nature.

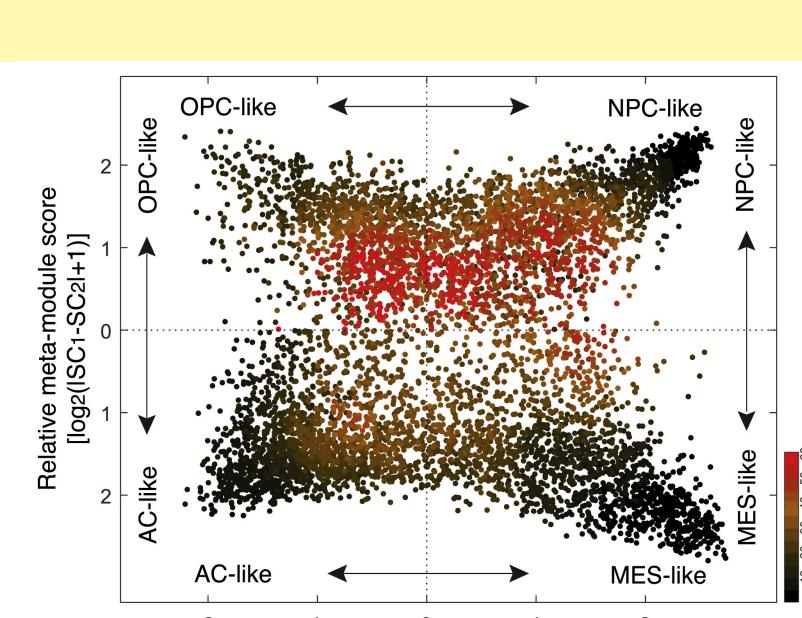
This heterogeneity can lead to very different patient outcomes and might need different clinical intervention and strategies. Hence it is important to study heterogeneity.

Initial characterization was done by Verhaak et al. using TCGA data.

4 subtypes found:

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

Are these cell states truly distinct and mutually exclusive?



Later, Neftel et al. leveraged single cell data to characterize 4 subtypes that resemble neurodevelopmental lineages:

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

Methods/Formulae

ssGSEA:

$$ES(G, S) = \sum_{i=1}^N [P_G^W(G, S, i) - P_{NG}(G, S, i)]$$

$$P_G^W(G, S, i) = \sum_{r \in G, j \leq i} \frac{|r_j|^\alpha}{\sum_{r_j \in G} |r_j|^\alpha} \quad P_{NG} = \sum_{r \notin G} \frac{1}{N - N_G}$$

J-Metric:

$$J = \frac{\sum_{x,y \in S_1} \rho_R(x, y)}{4N_1^2} + \frac{\sum_{x,y \in S_2} \rho_R(x, y)}{4N_2^2} - \frac{\sum_{x,y \in S_1, S_2} \rho_R(x, y)}{2N_1 N_2}$$

$\rho_R(x, y)$ = Spearman correlation of Gene x with Gene y
 S_i = Number of genes in set i
 N_i = Gene Set i

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

Antagonism is present in the context of Cell Cycle, Metabolism and Immune
NPC/PN - MES classification should be given more importance for therapeutic targeting efforts

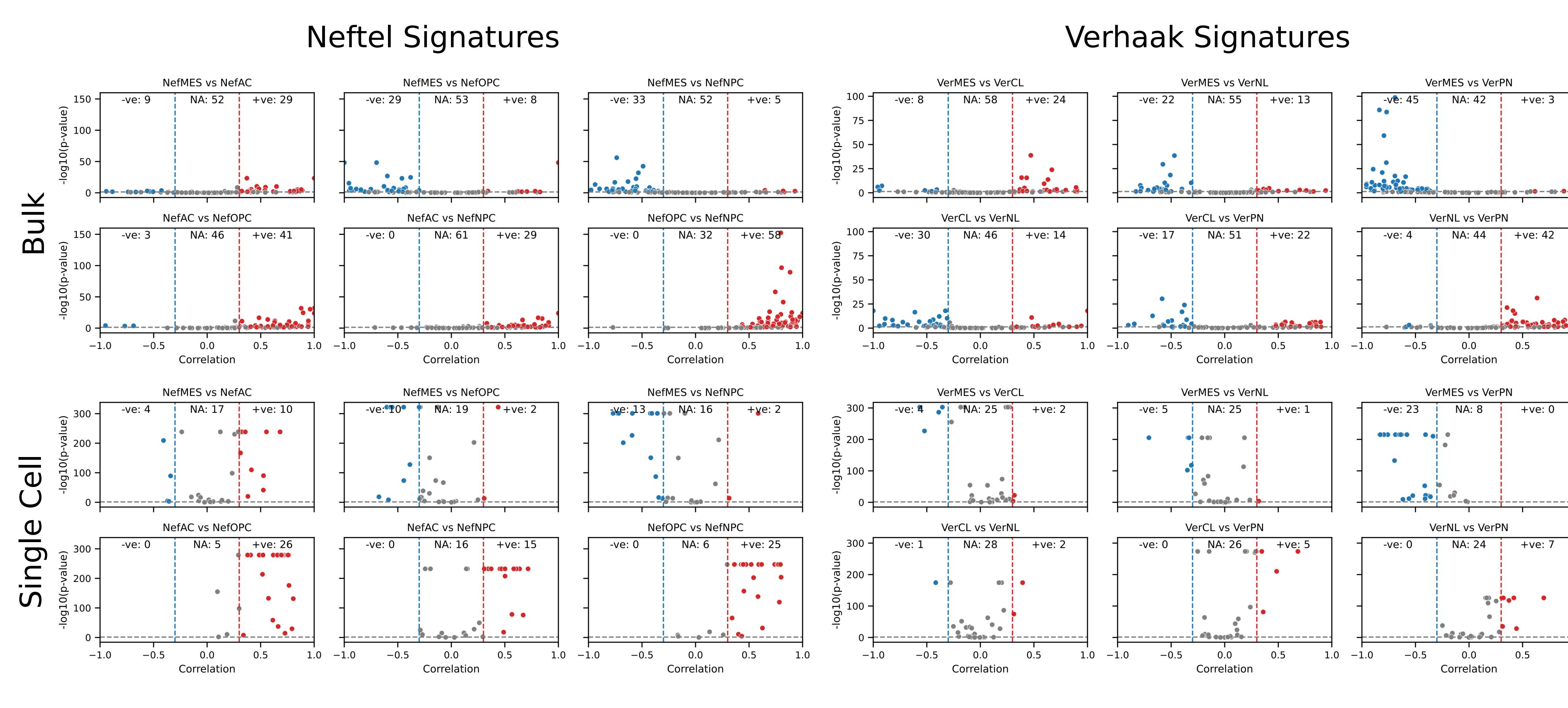
Future Directions

Constructing a Gene Regulatory Network and understanding the dynamics

Looking if the trends hold in other regulatory levels:

Methylation, Chromatin Configuration, Protein

Observed trend holds across multiple datasets

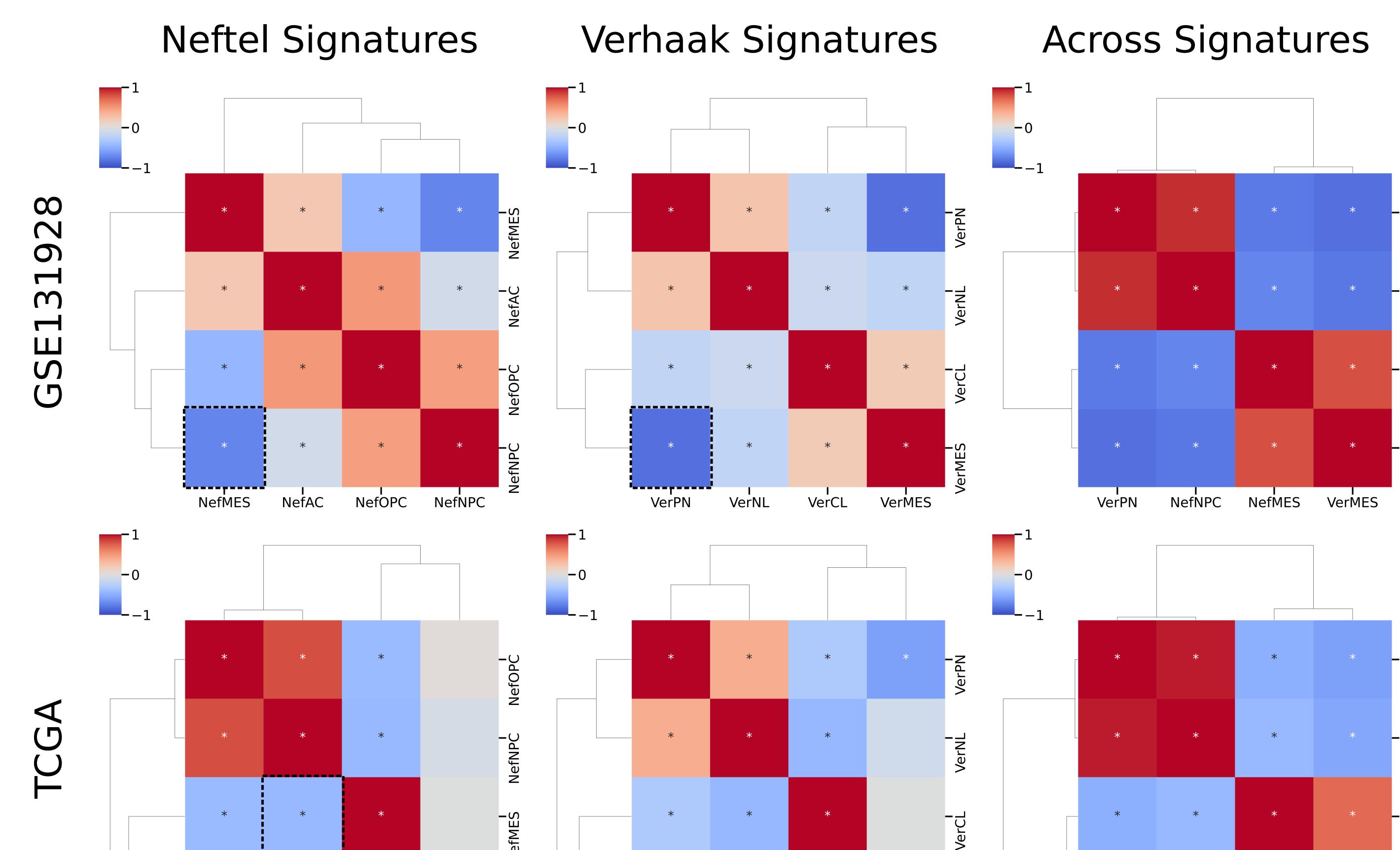


References

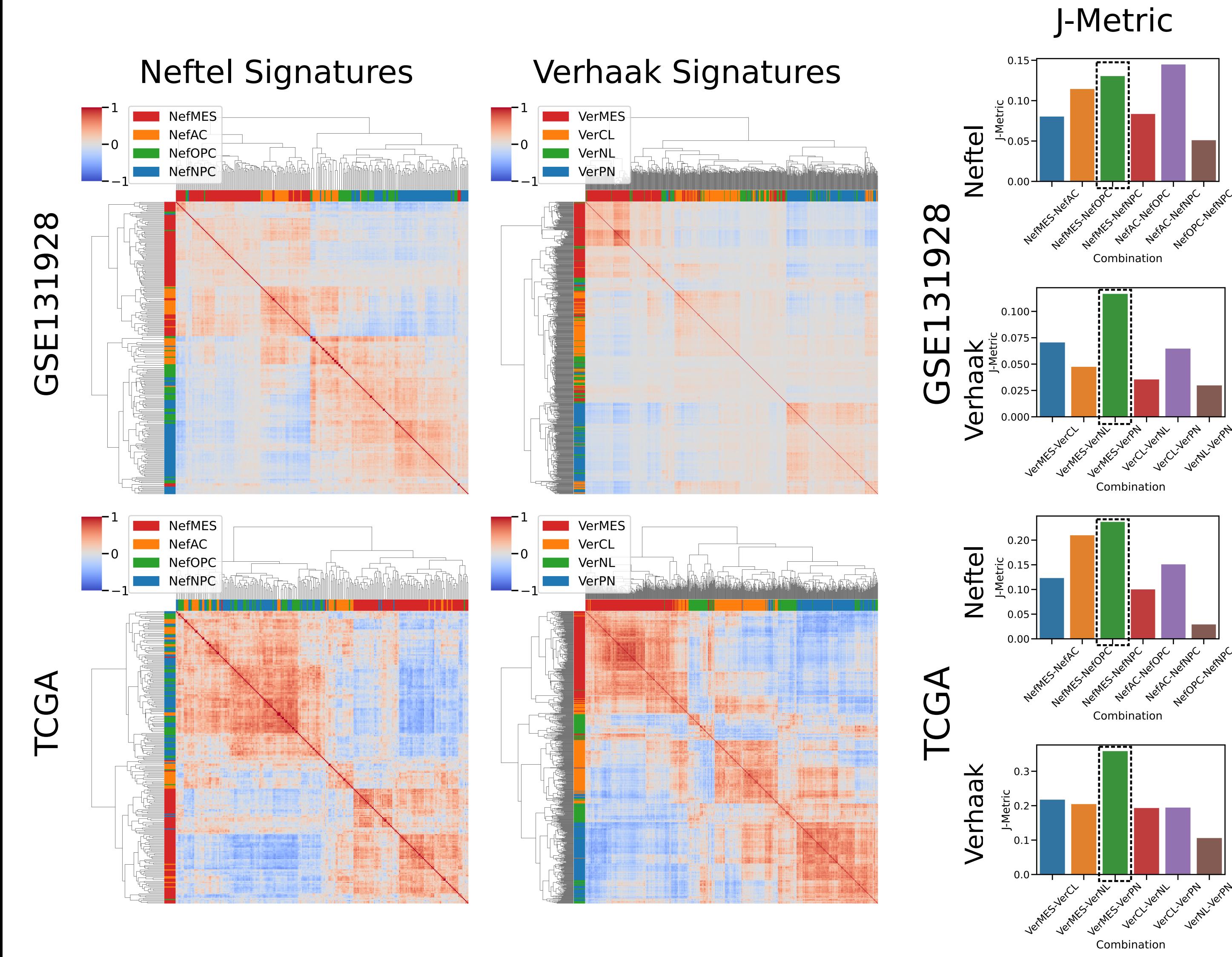
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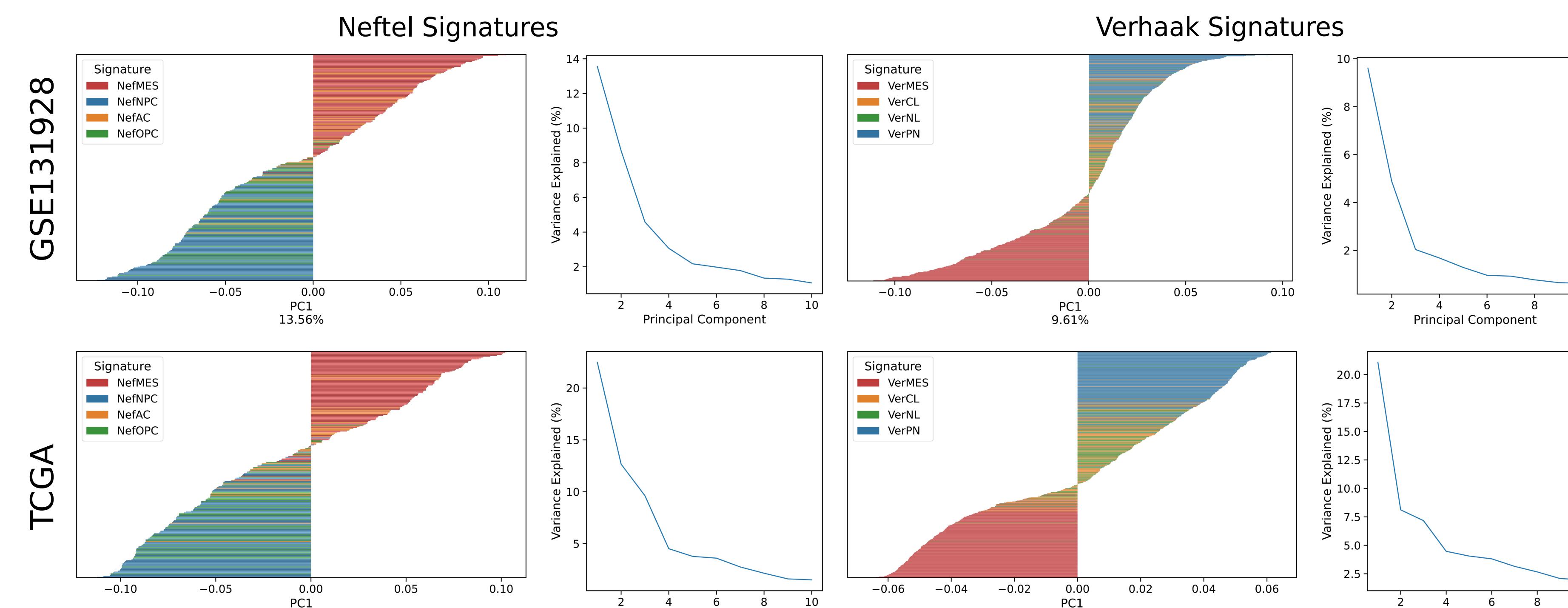
Gene Set Enrichment Analysis reveals not all states are distinctly mutually antagonistic



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

NefMES	95	1	0	1	14	1	0
NefAC	1	39	8	1	0	3	1
NefOPC	0	8	50	7	0	0	1
NefNPC	1	1	7	89	0	0	22
VerMES	14	0	0	0	216	0	0
VerCL	1	3	0	0	161	0	0
VerNL	0	1	1	1	0	0	128
VerPN	0	2	9	22	0	0	177

Trends are consistent in Proliferative, Metabolic and Immune axis NPC/PN - More Proliferative, MES- More glycolytic, MES- More susceptible to IBT

