

4 STATES \neq 4 DIMENSIONS: PRONEURAL-MESENCHYMAL ANTAGONISM DOMINATES THE PATTERNS OF PHENOTYPIC HETEROGENEITY IN GLIOBLASTOMA

CSB LAB SYMPOSIUM

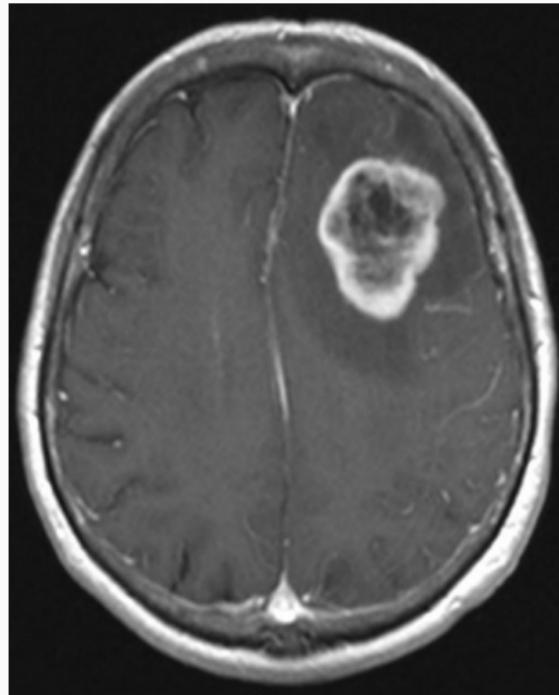
Harshavardhan BV

Advisors: Mohit Kumar Jolly (BE), Srimonta Gayen (DBG)

October 19, 2023

IMI, IISc Bangalore

GLIOBLASTOMA MULTIFORME(GBM)

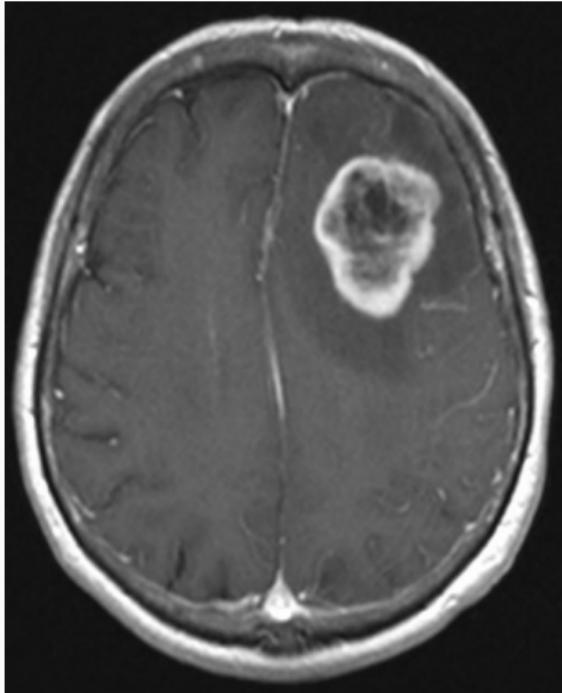


(a) MRI image of Glioblastoma¹

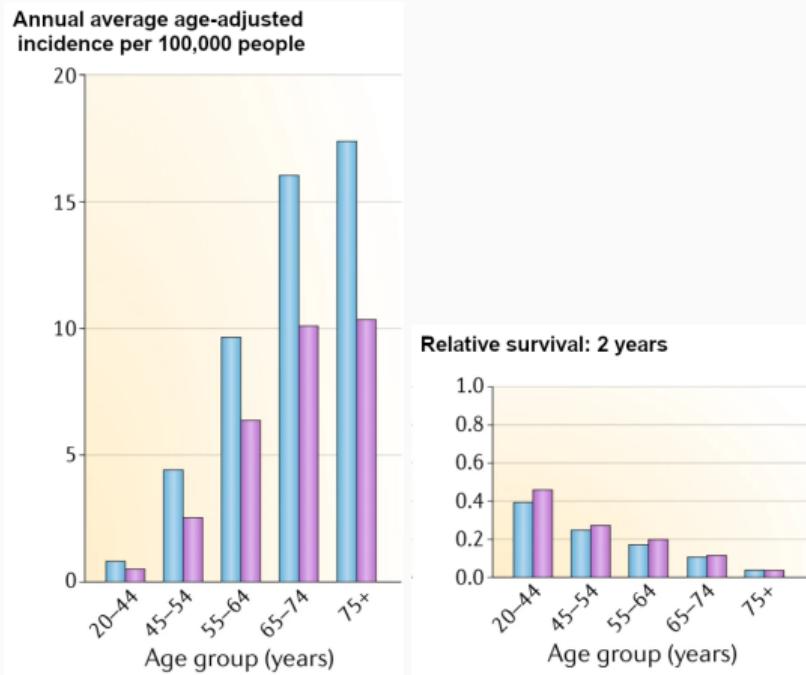
¹Tan et al., 2020

²Molinaro et al., 2019

GLIOBLASTOMA MULTIFORME(GBM)



(a) MRI image of Glioblastoma¹



(b) Incidence statistics for GBM²

(c) Survival statistics for GBM²

¹Tan et al., 2020

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MOLECULAR SUBTYPES OF GBM

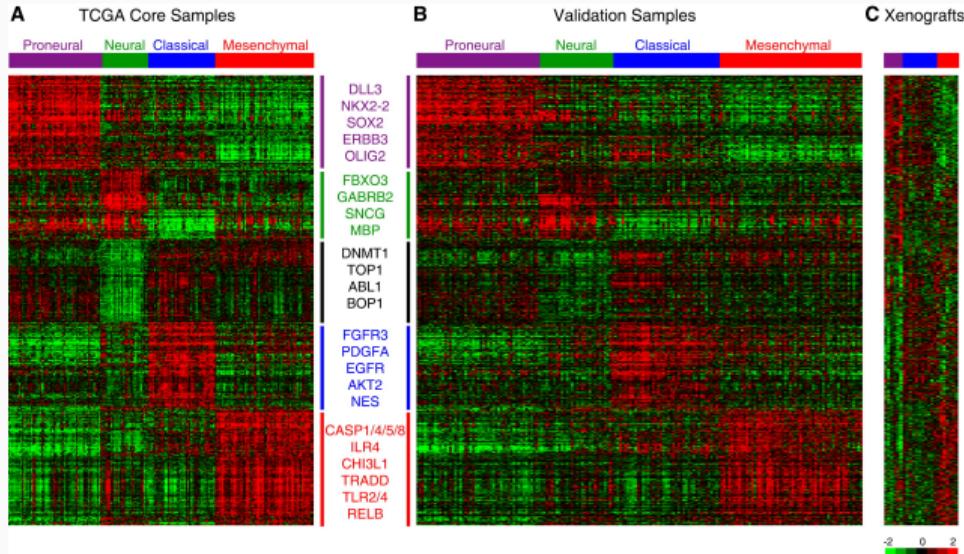
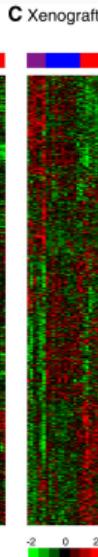
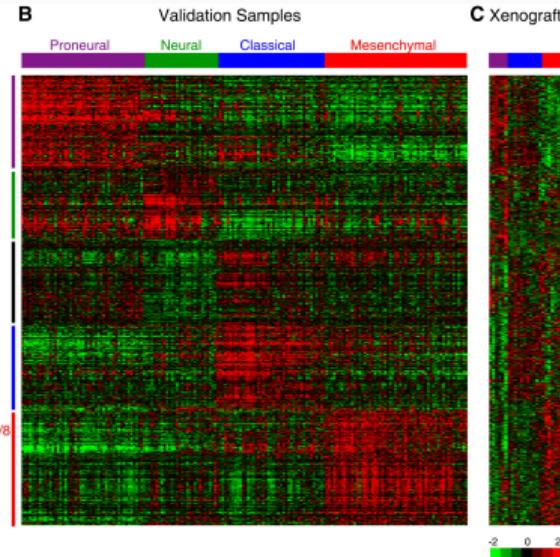
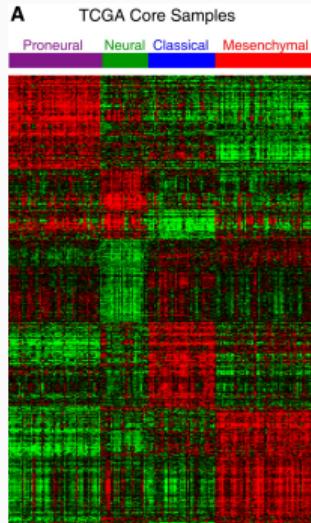


Figure 2: Cellular states defined for Glioblastoma (GBM)

MOLECULAR SUBTYPES OF GBM



(a) Cell states by Verhaak et al., 2010

(b) Cell states by Neftel et al., 2019

Figure 2: Cellular states defined for Glioblastoma (GBM)

ARE THESE CELL STATES TRULY DISTINCT AND MUTUALLY EXCLUSIVE?

METHOD FOR SCORING SIGNATURES

Scoring of Genesets: ssGSEA^a

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

Where,

- 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 x scores with y scores

^aBarbie et al., 2009.

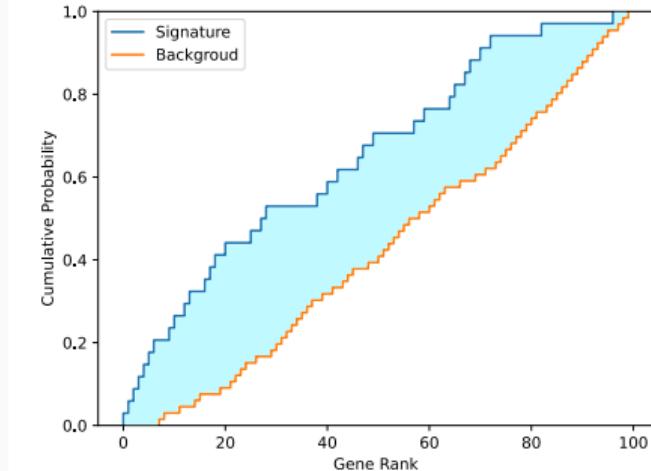


Figure 3: Schematic of ssGSEA

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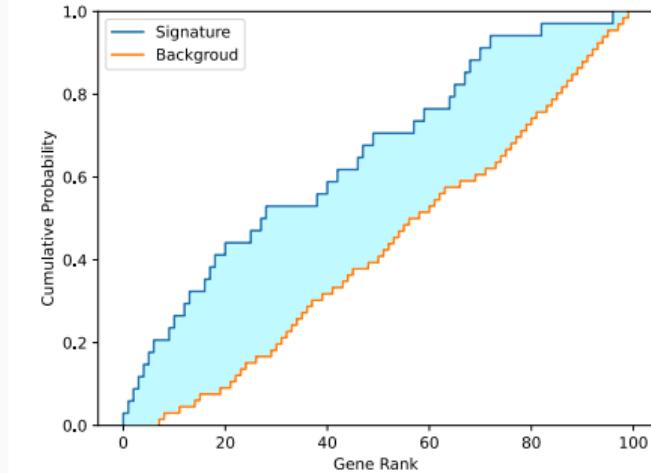


Figure 3: Schematic of ssGSEA

- Antagonistic $\implies \rho_r(x, y) = -ve$
- Independent $\implies \rho_r(x, y) \approx 0$

NOT ALL STATES ARE DISTINCTLY MUTUALLY ANTAGONISTIC (NEFTEL)

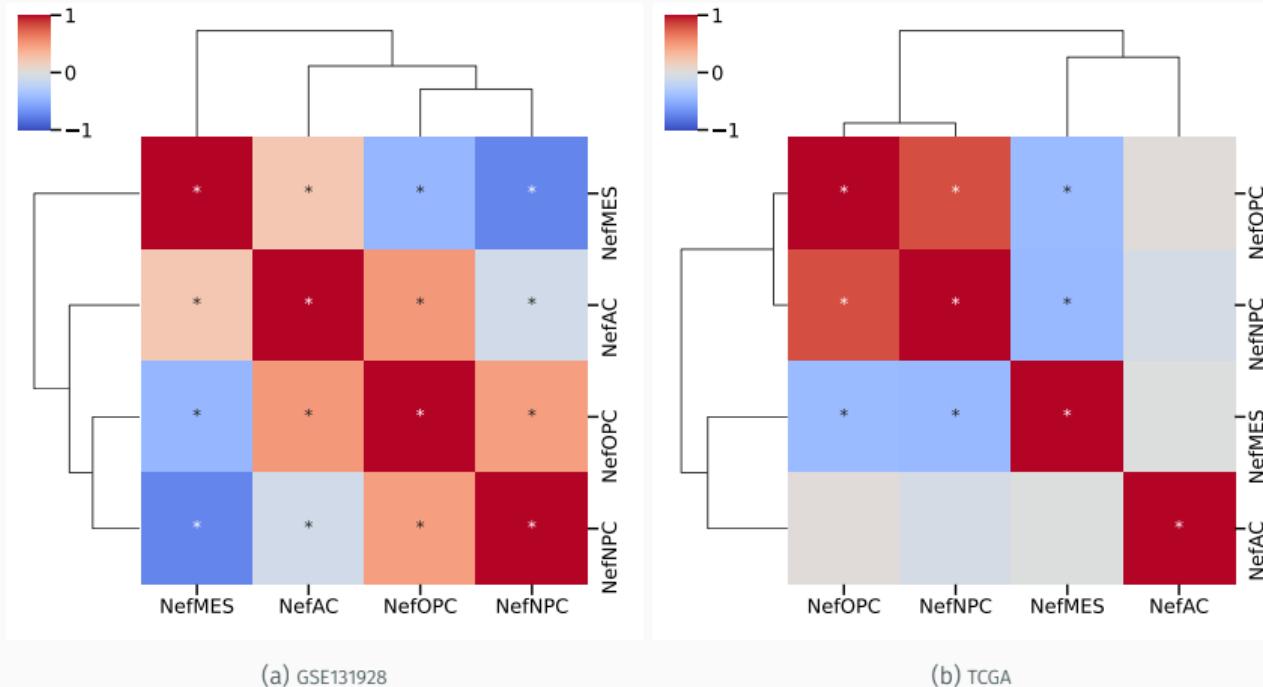


Figure 4: Correlation of ssGSEA/AUCell scores

NOT ALL STATES ARE DISTINCTLY MUTUALLY ANTAGONISTIC (VERHAAK)

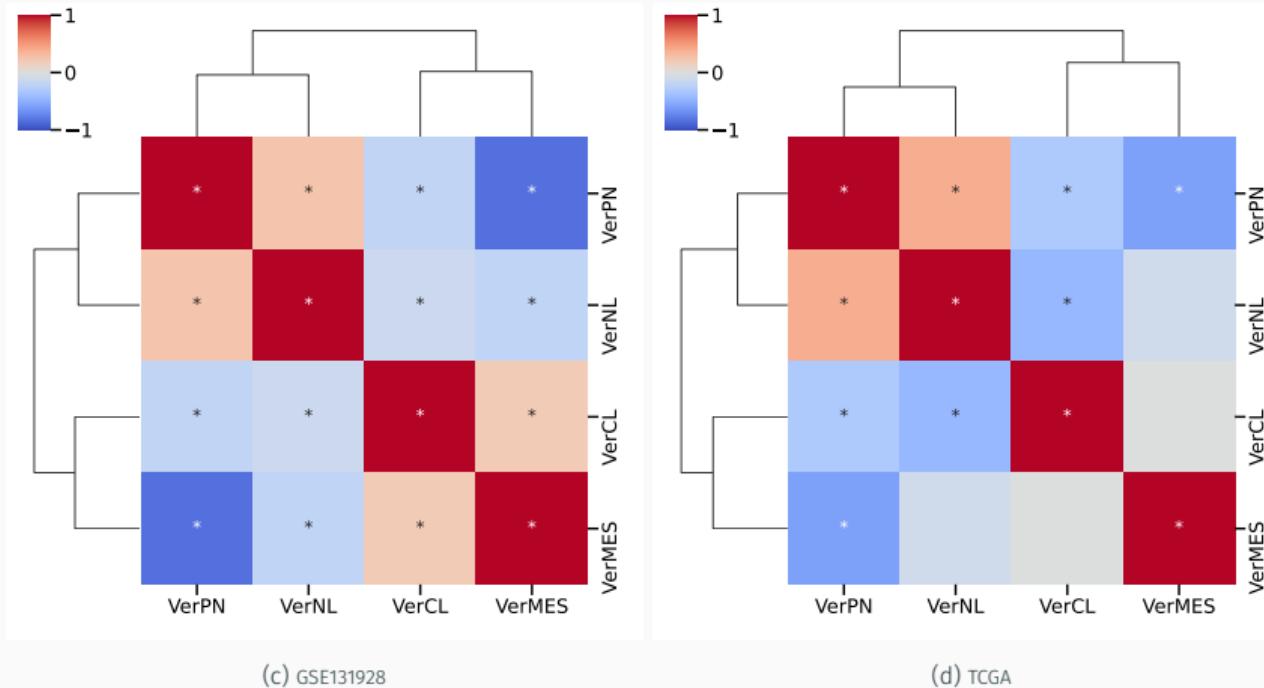


Figure 4: Correlation of ssGSEA/AUCell scores

SIMILAR STATES BETWEEN NEFTEL AND VERHAAK

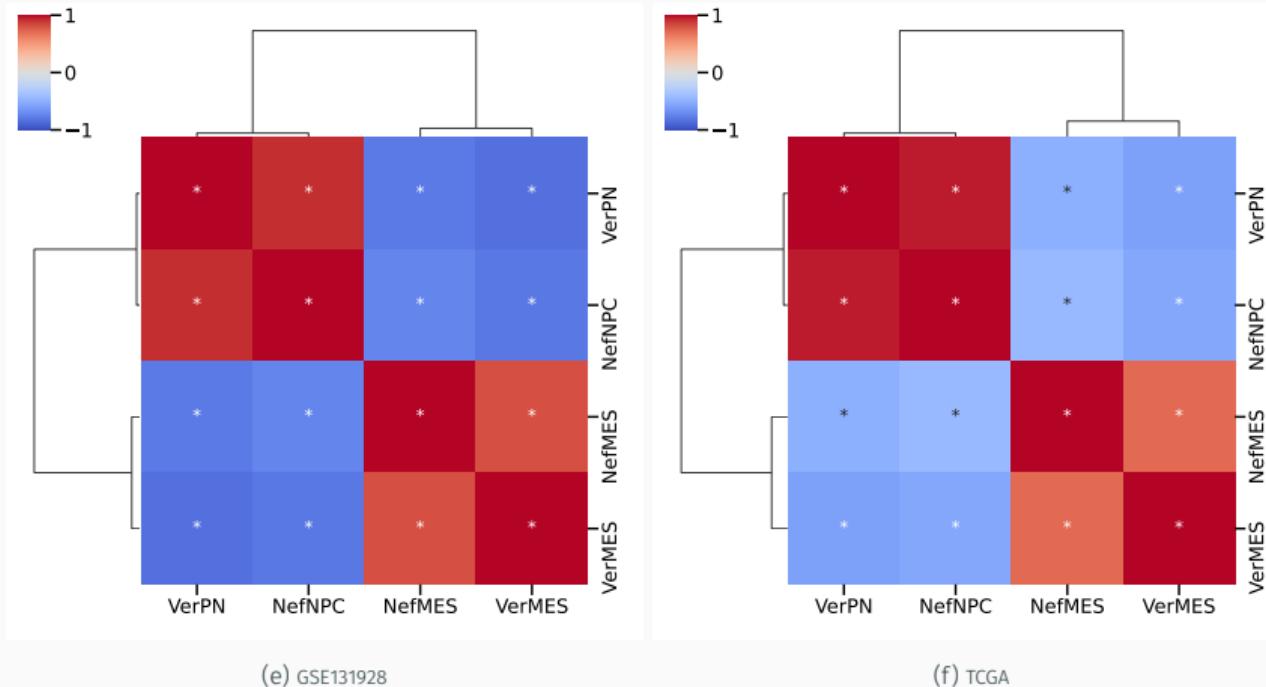
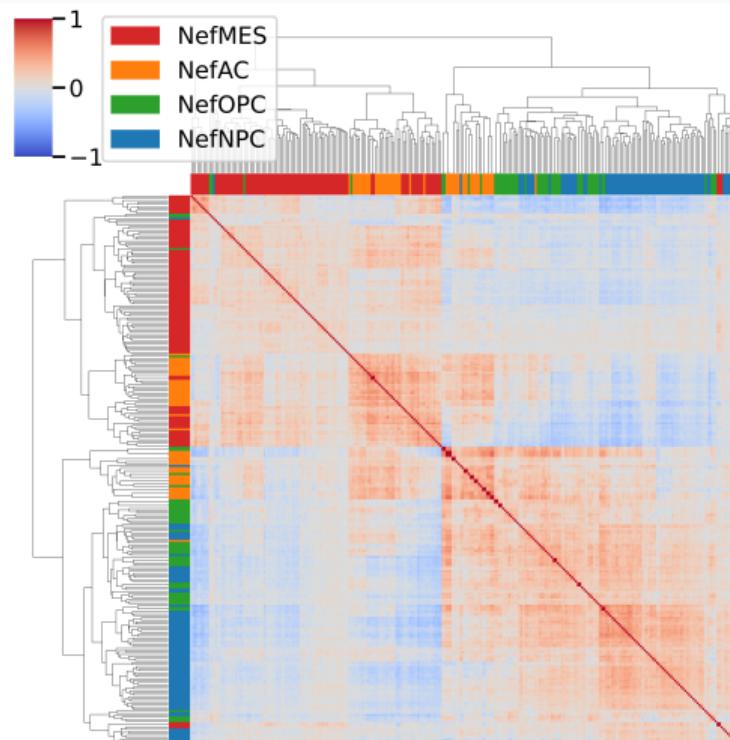
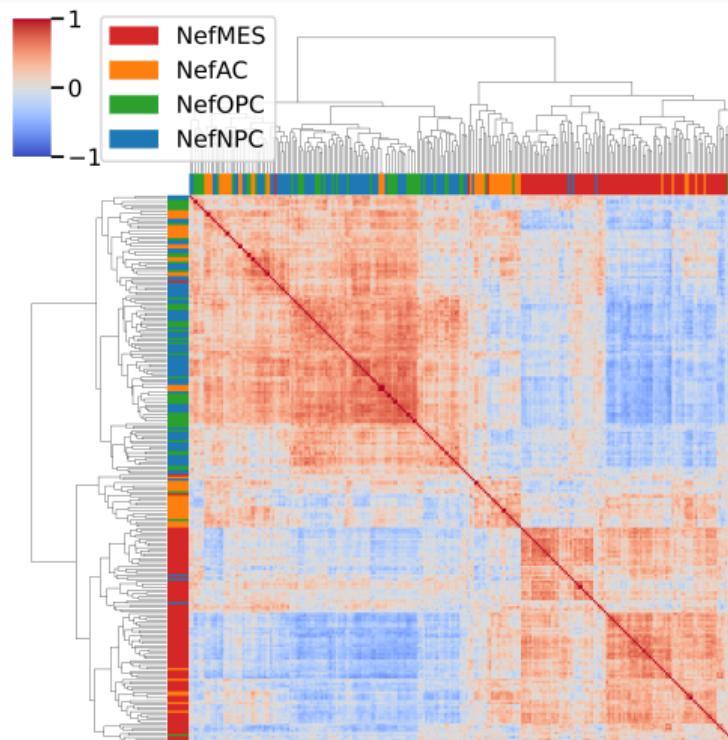


Figure 4: Correlation of ssGSEA/AUCell scores

CORRELATION OF EXPRESSION ALSO CAPTURES THE NPC-MES ANTAGONISM (NEFTEL)



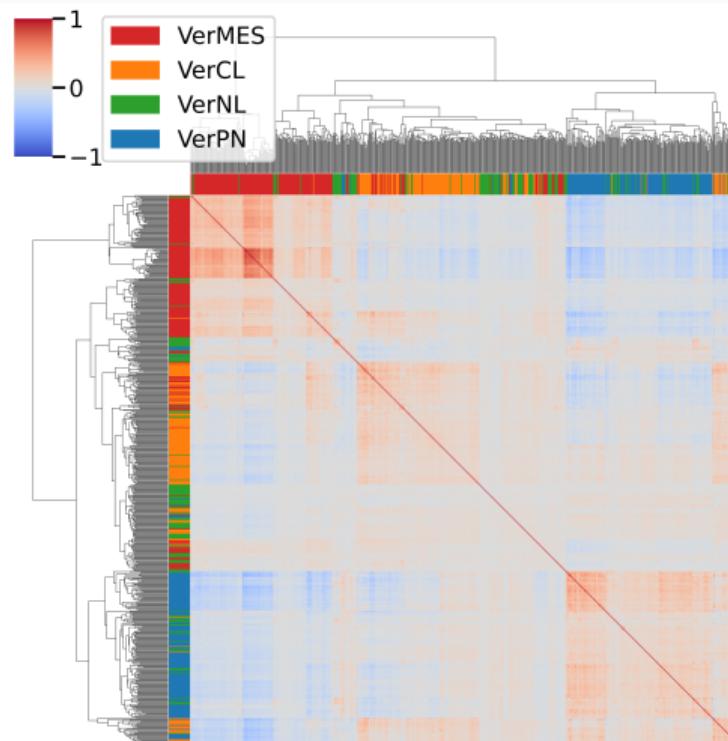
(a) GSE131928



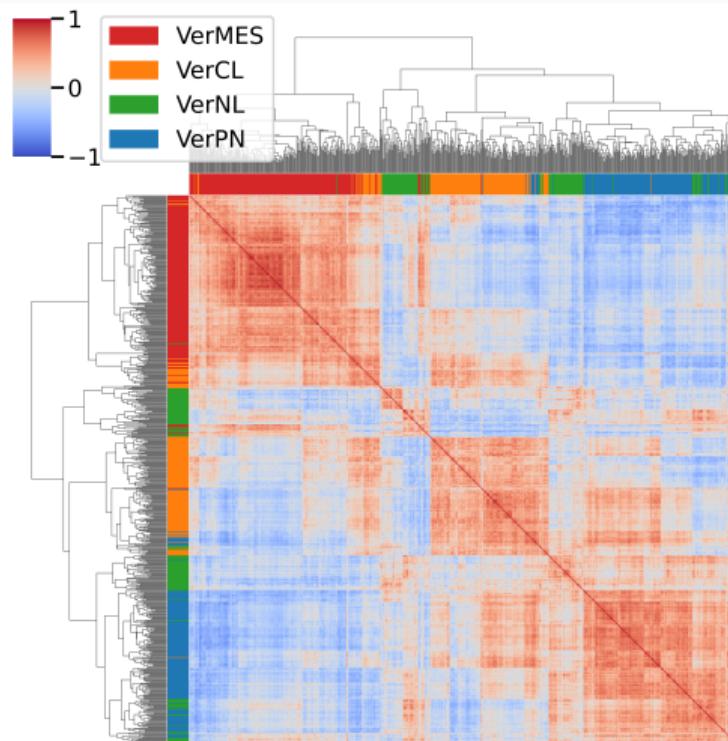
(b) TCGA

Figure 5: Correlation of signature expression

CORRELATION OF EXPRESSION ALSO CAPTURES THE PN-MES ANTAGONISM (VERHAAK)



(c) GSE131928



(d) TCGA

Figure 5: Correlation of signature expression

QUANTIFICATION OF ANTAGONISM IN CORRELATION

$$J = \underbrace{\sum_{x,y \in G_1} \frac{\rho_r(x,y)}{4N_1^2} + \sum_{x,y \in G_2} \frac{\rho_r(x,y)}{4N_2^2}}_{\text{Correlation within genesets}} - \underbrace{\sum_{x \in G_1, y \in G_2} \frac{\rho_r(x,y)}{2N_1 N_2}}_{\text{Correlation across genesets}}$$

Where,

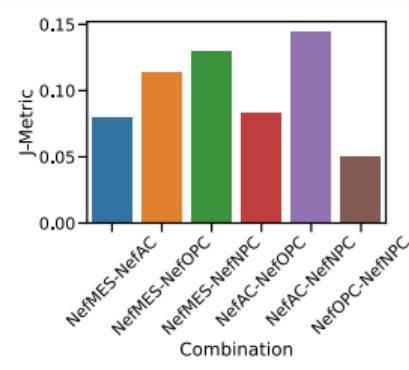
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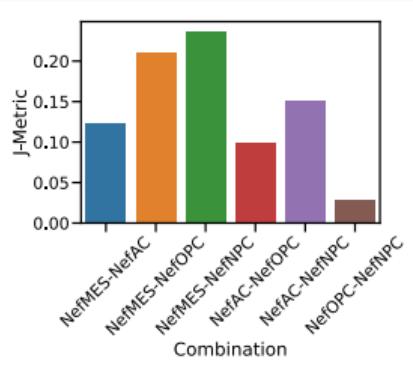
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(a) GSE131928 - Neftel



(b) TCGA - Neftel

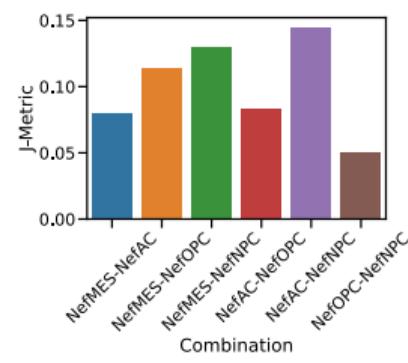
Figure 6: J metric of signature correlation

QUANTIFICATION OF ANTAGONISM IN CORRELATION

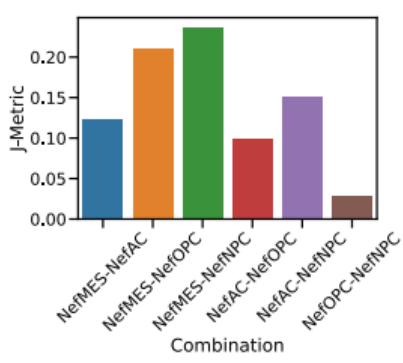
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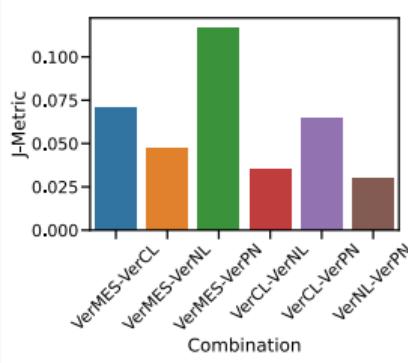
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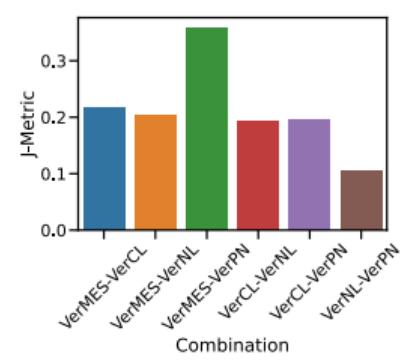
(a) GSE131928 - Neftel



(b) TCGA - Neftel



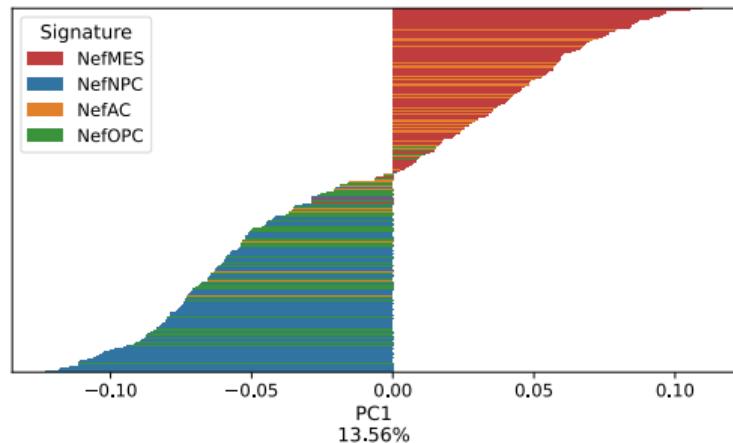
(c) GSE131928 - Verhaak



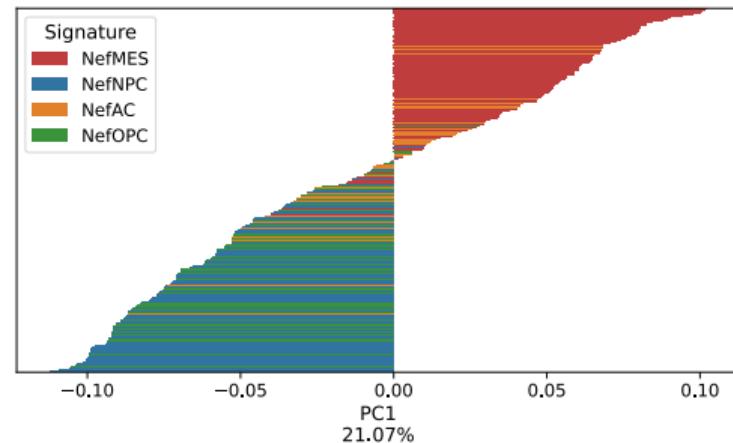
(d) TCGA - Verhaak

Figure 6: J metric of signature correlation

PC1 LOADINGS ARE DOMINATED BY NPC-MES ANTAGONISM (NEFTEL)



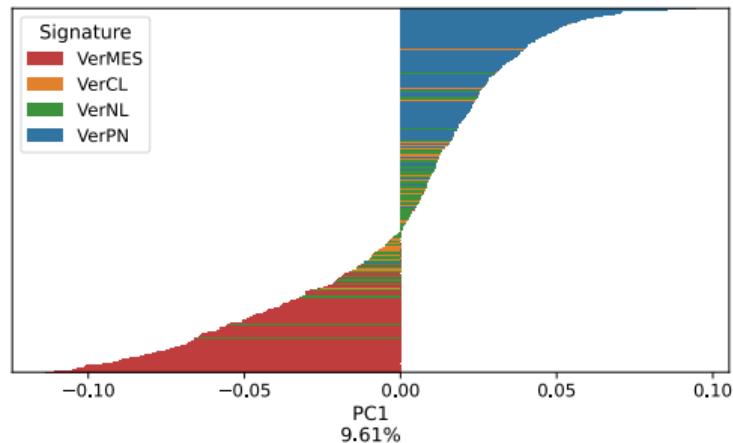
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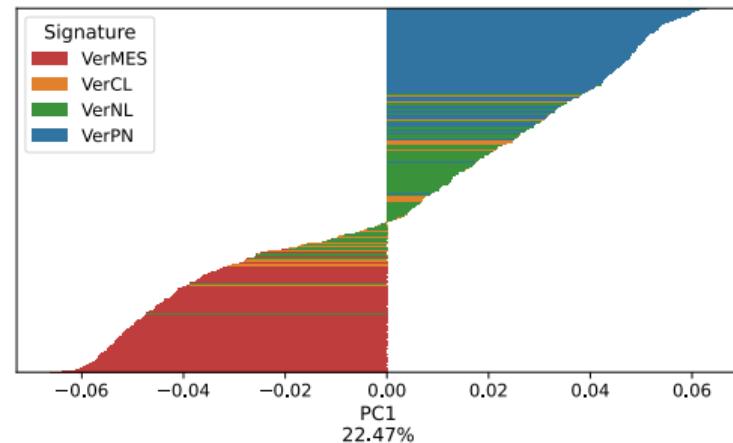
(b) TCGA

Figure 7: PC1 loadings - PCA on signature expression

PC1 LOADINGS ARE DOMINATED BY PN-MES ANTAGONISM (VERHAAK)



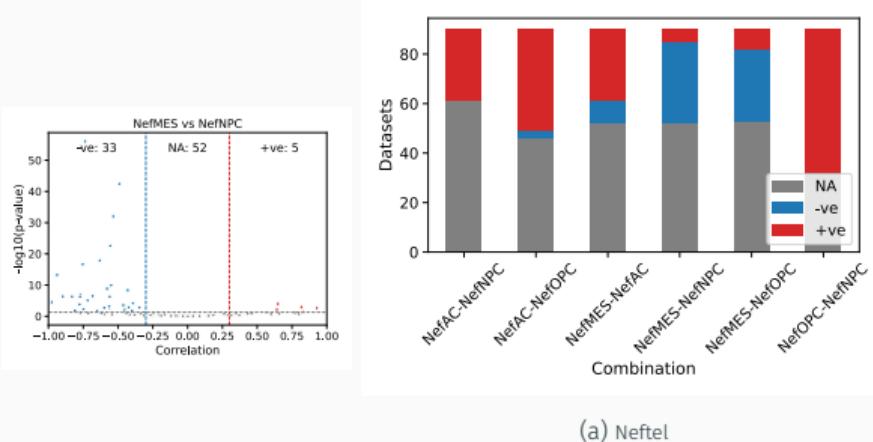
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(d) TCGA

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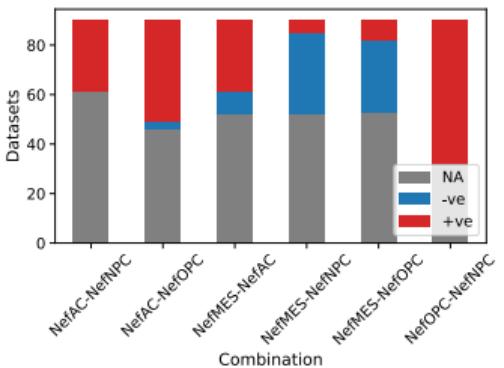
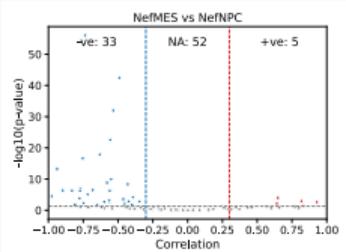
OBSERVED TREND HOLDS ACROSS MULTIPLE BULK RNASEQ DATASETS



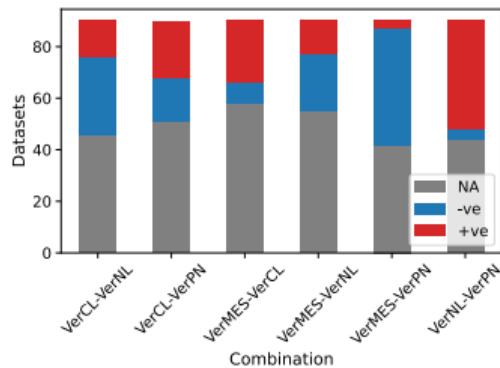
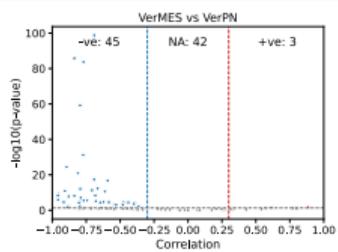
(a) Neftel

Figure 8: Metanalysis of Bulk datasets

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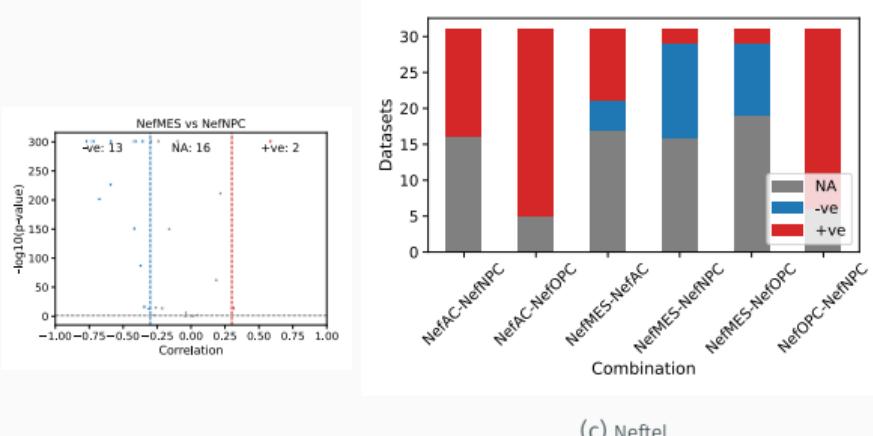
(a) Neftel



(b) Verhaak

Figure 8: Metanalysis of Bulk datasets

OBSERVED TREND HOLDS ACROSS MULTIPLE SINGLECELL RNASEQ DATASETS



(c) Neftel

Figure 8: Metanalysis of SingleCell datasets

OBSERVED TREND HOLDS ACROSS MULTIPLE SINGLECELL RNASEQ DATASETS

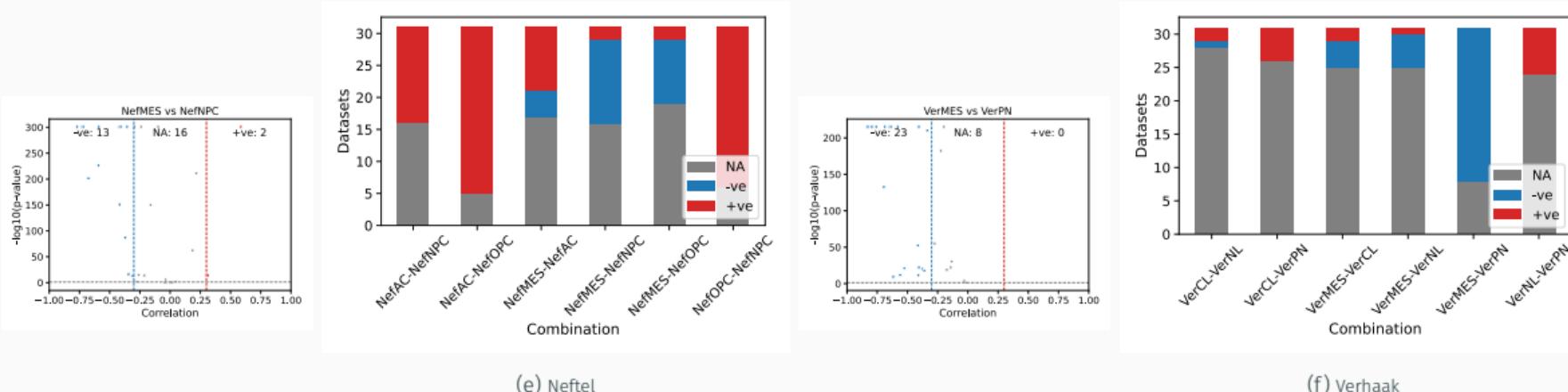
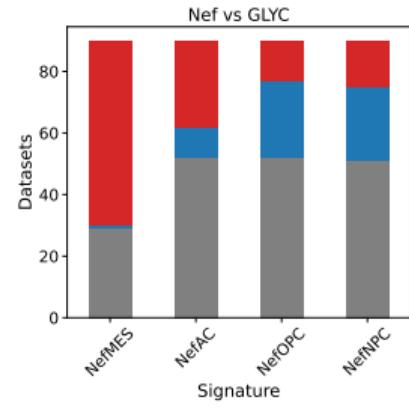
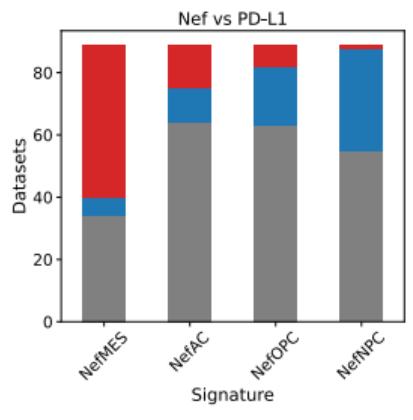


Figure 8: Metanalysis of SingleCell datasets

TRENDS ARE CONSISTENT IN METABOLIC AND IMMUNE AXIS



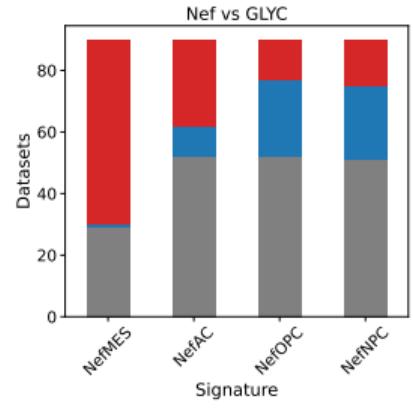
(a) Neftel - Glycolysis



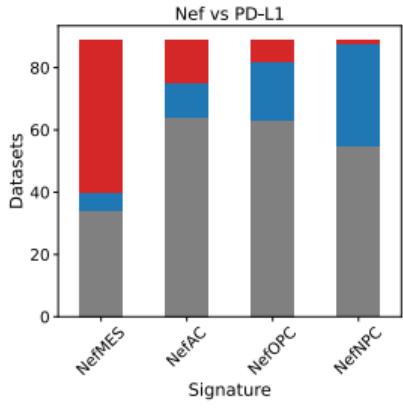
(b) Neftel - PD-L1

Figure 9: Correlation of subtypes with metabolism/immune axis

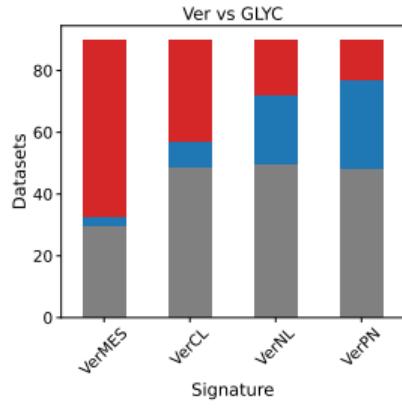
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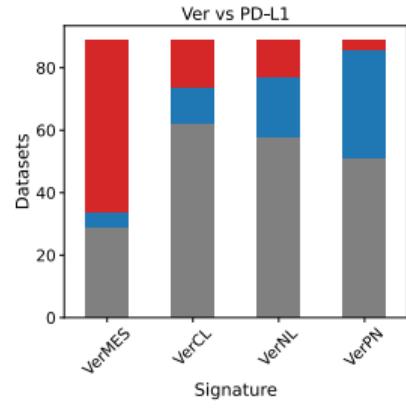
(a) Neftel - Glycolysis



(b) Neftel - PD-L1



(c) Verhaak - Glycolysis



(d) Verhaak - PD-L1

Figure 9: Correlation of subtypes with metabolism/immune axis

CONCLUSION

- We can't find the 4 states mentioned in Neftel or Verhaak to be distinct

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 - NPC vs MES - Neftel et al
 - PN vs MES - Verhaak et al

CONCLUSION

- We can't find the 4 states mentioned in Neftel or Verhaak to be distinct
- Most antagonistic pair
 - NPC vs MES - Neftel et al
 - PN vs MES - Verhaak et al
- NPC/PN - MES classification should be given more focus for therapeutic targetting efforts
 - Targetting similar states (NPC-OPC) can give similar response to differing degree
 - Targetting antagonistic states (NPC-MES) can give opposing response

- Why do we observe these trends? Can we explain them mechanistically?

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 - Construct a Gene regulatory network

- Why do we observe these trends? Can we explain them mechanistically?
 - Construct a Gene regulatory network
- Strengthen the argument across multiple regulatory levels

ACKNOWLEDGEMENTS



Thank You

P M R F
Prime Minister's Research Fellows

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