

# DECIPHERING THE LANDSCAPE OF CELL STATES IN GLIOBLASTOMA

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Harshavardhan BV

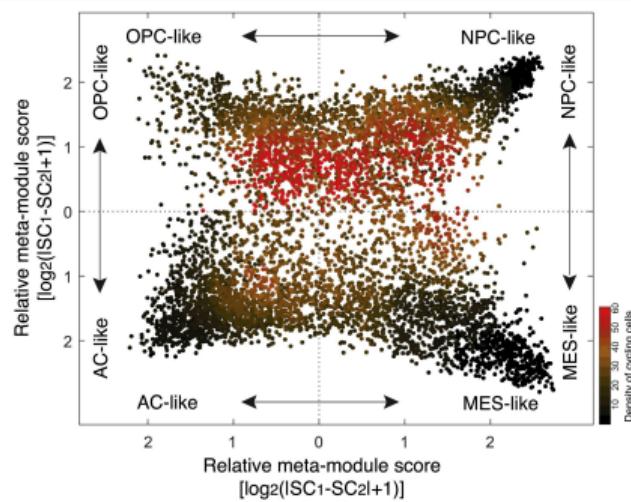
July 19, 2023

IISc Bangalore

## INTRODUCTION

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# MOLECULAR SUBTYPES OF GBM



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

Figure 1: Cellular states defined for Glioblastoma

# MOLECULAR SUBTYPES OF GBM

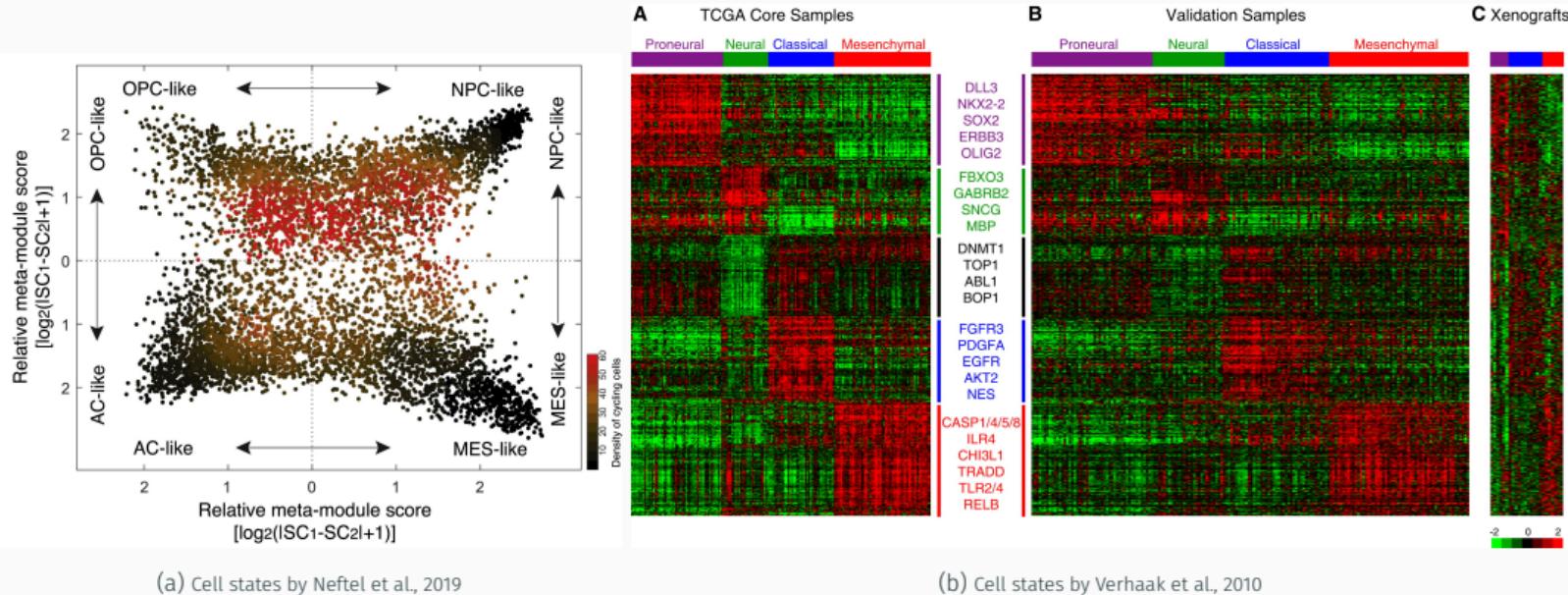


Figure 1: Cellular states defined for Glioblastoma

ARE THESE CELL STATES DISTINCT?

ARE THESE CELL STATES DISTINCT?

HOW DOES THIS HETEROGENEITY AND PLASTICITY AFFECT THERAPY?

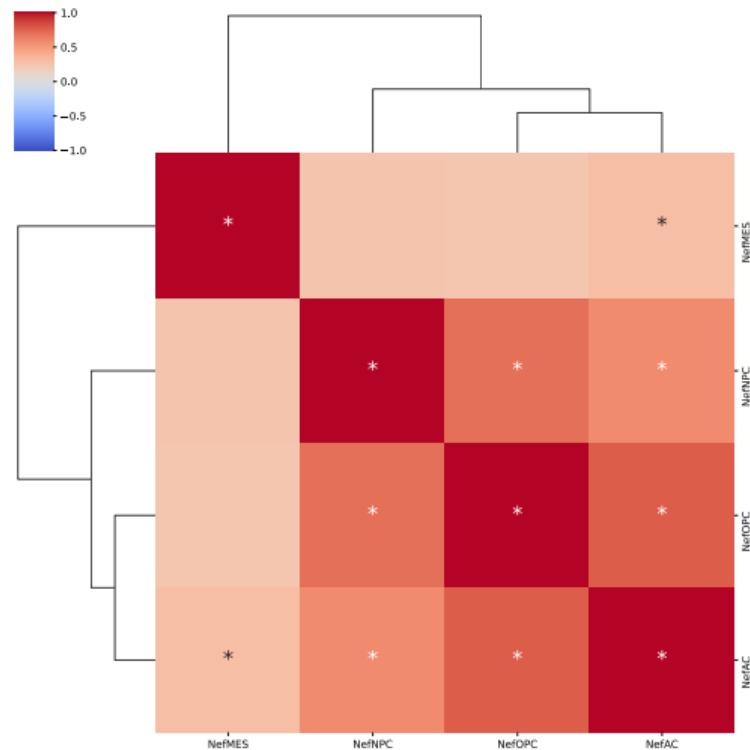
## DATASETS

Dataset	CellLine/Tumour	Bulk/SingleCell	Source
CCLE	CellLine	Bulk	Ghandi et al., 2019
TCGA	Tumour	Bulk	Brennan et al., 2013
GSE131928	Tumour	SingleCell	Neftel et al., 2019
GSE168004	CellLine	SingleCell	Hara et al., 2021

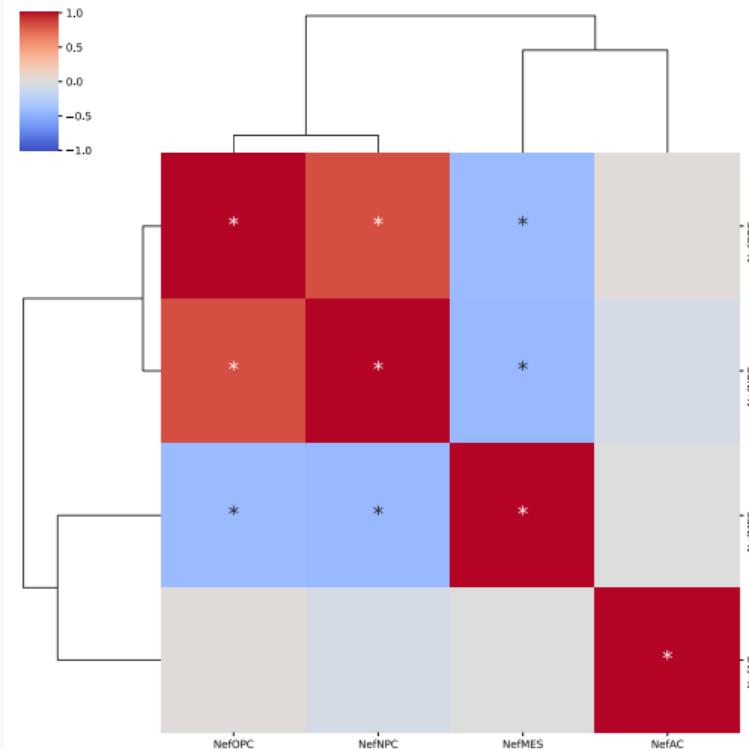
## PRELIMINARY RESULTS

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# GENE-SET ENRICHMENT - NEFTEL ET AL



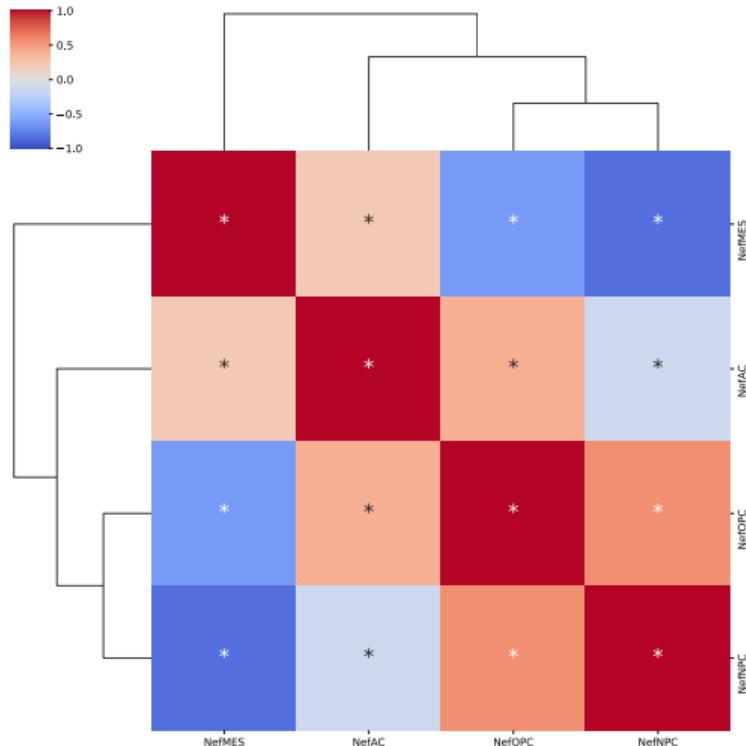
(a) CCLE (Bulk-CellLine)



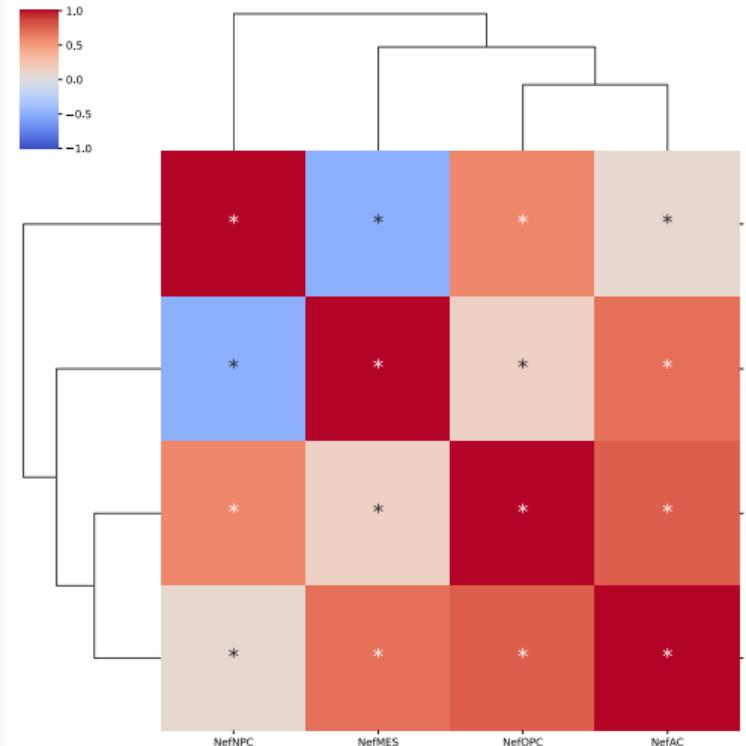
(b) TCGA (Bulk-Tumour)

Figure 2: Correlation of ssGSEA scores - Neftel

# GENE-SET ENRICHMENT - NEFTEL ET AL

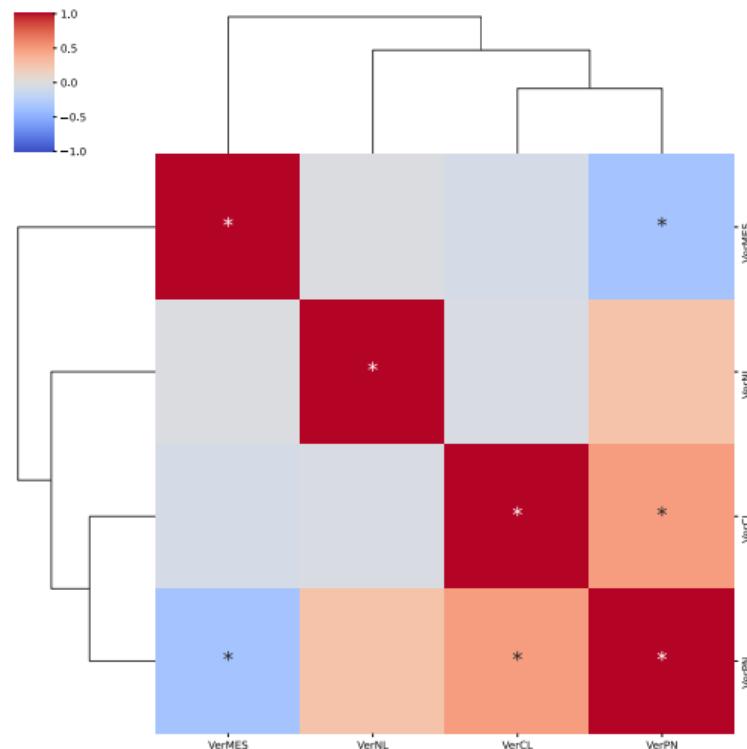


(c) GSE131928 (SingleCell-Tumour)

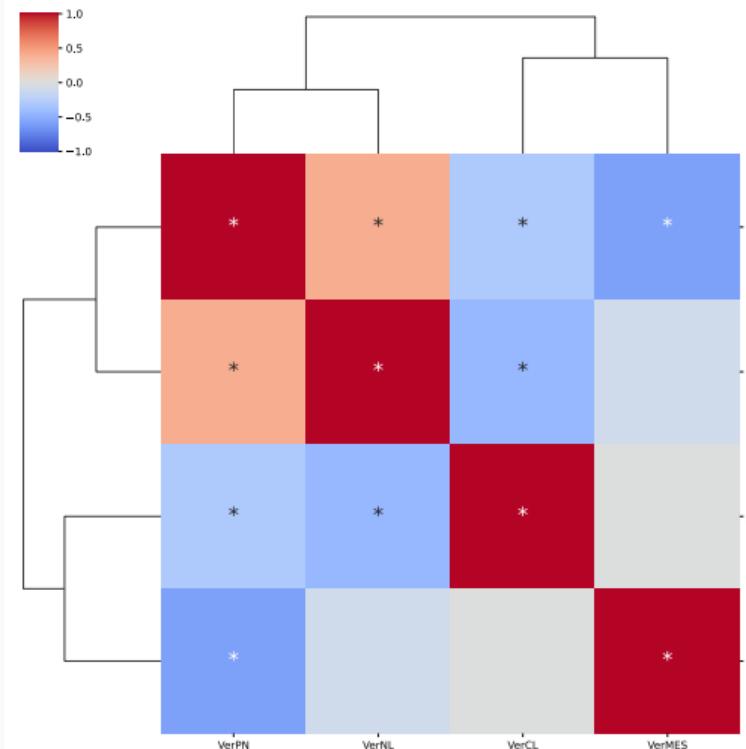


(d) GSE168004 (SingleCell-CellLine)

# GENE-SET ENRICHMENT - VERHAAK ET AL



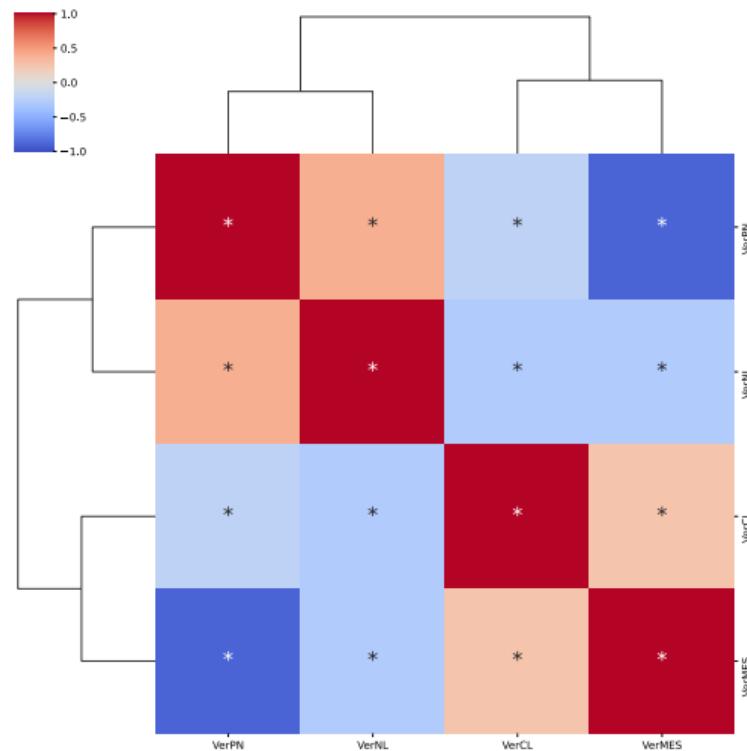
(e) CCLE (Bulk-CellLine)



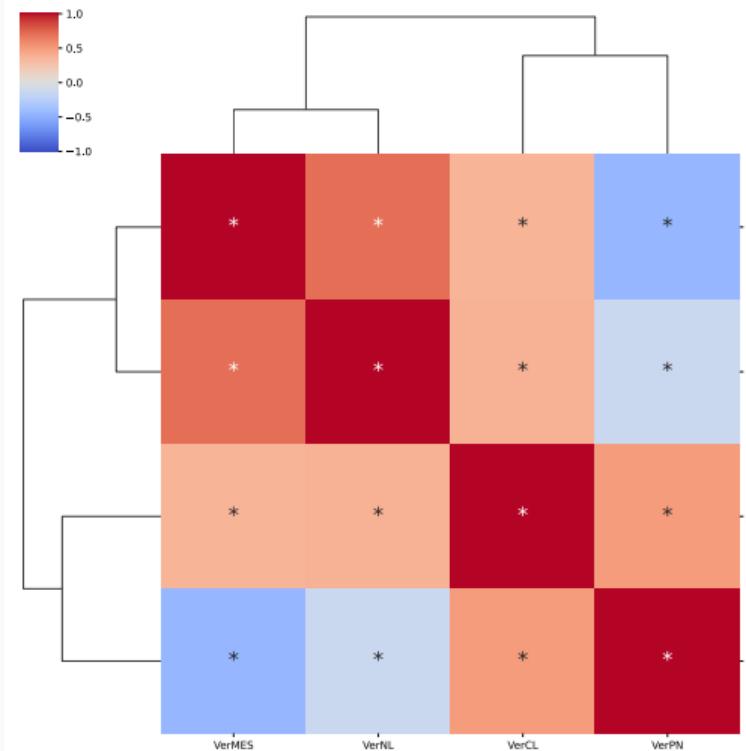
(f) TCGA (Bulk-Tumour)

Figure 2: Correlation of ssGSEA scores - Verhaak

# GENE-SET ENRICHMENT - VERHAAK ET AL



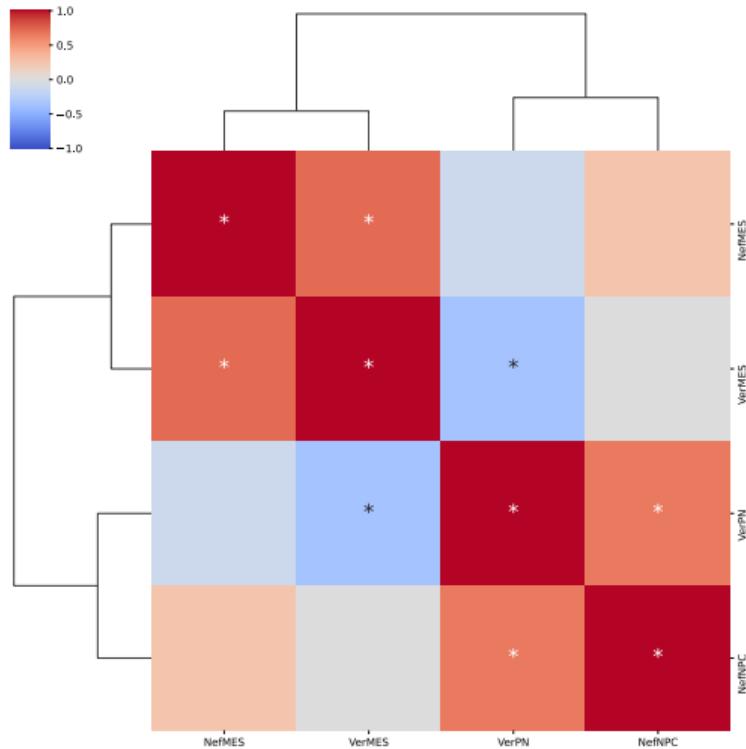
(g) GSE131928 (SingleCell-Tumour)



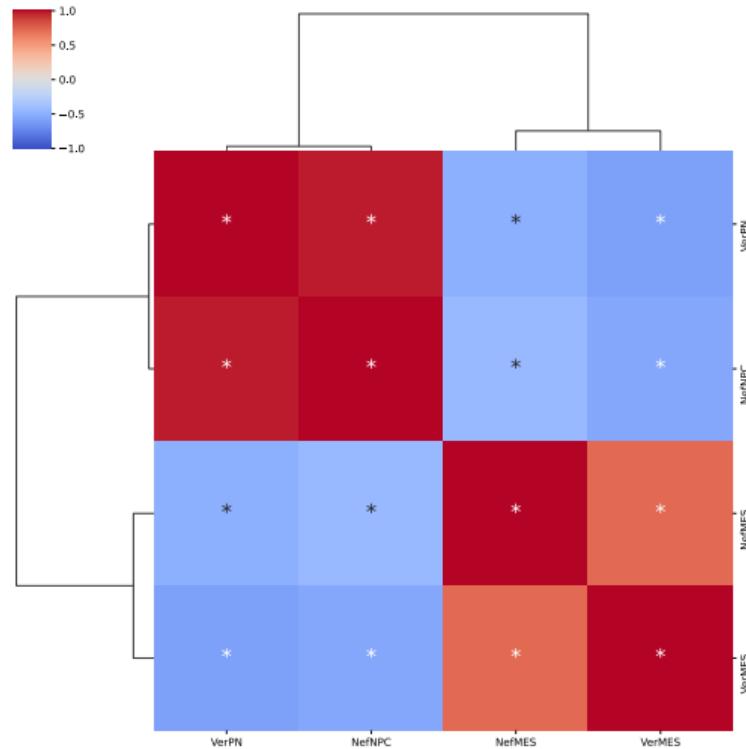
(h) GSE168004 (SingleCell-CellLine)

Figure 2: Correlation of AUCell scores - Verhaak

## GENE-SET ENRICHMENT - COMPARISON



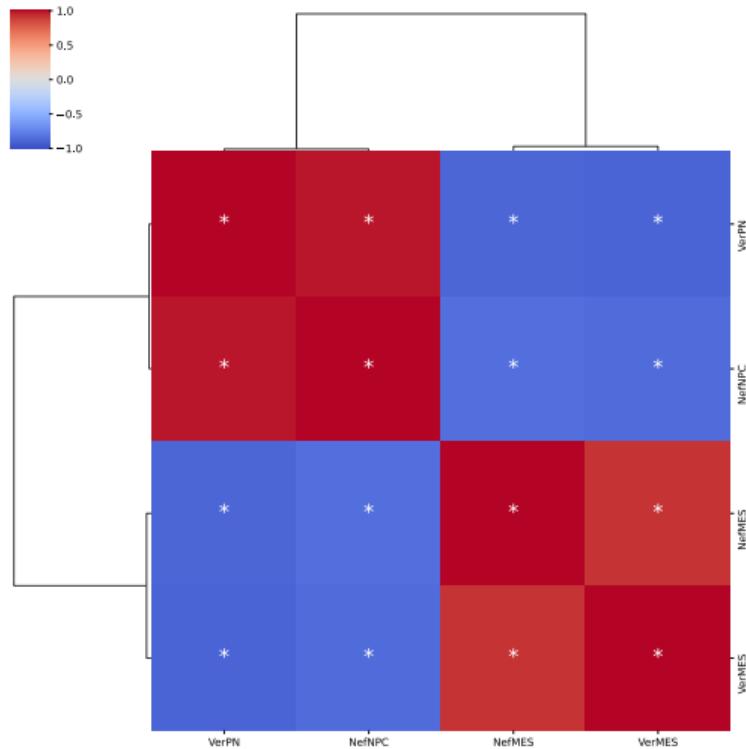
(i) CCLE (Bulk-CellLine)



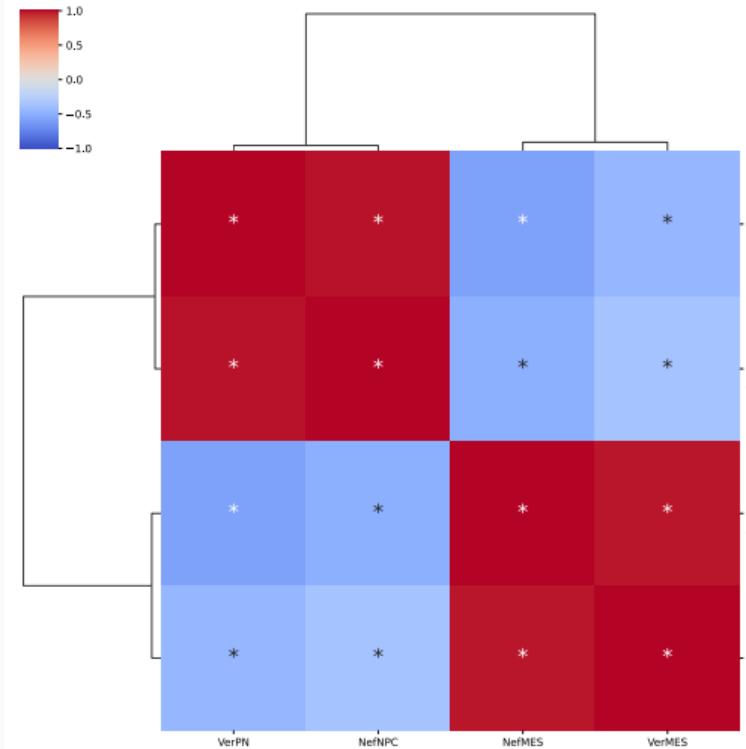
(j) TCGA (Bulk-Tumour)

Figure 2: Correlation of ssGSEA scores - Comparison

## GENE-SET ENRICHMENT - COMPARISON



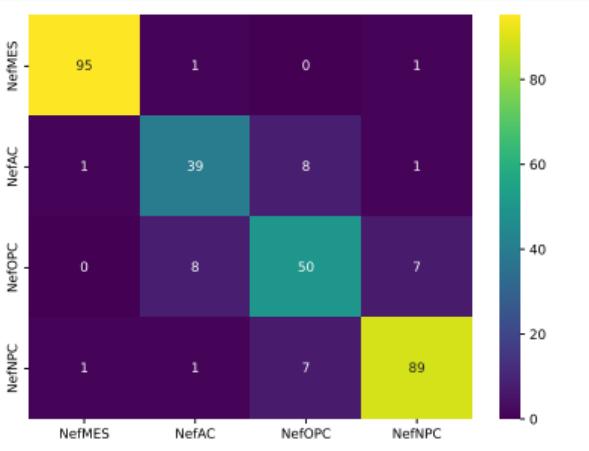
(k) GSE131928 (SingleCell-Tumour)



(l) GSE168004 (SingleCell-CellLine)

Figure 2: Correlation of AUCell scores- Comparison

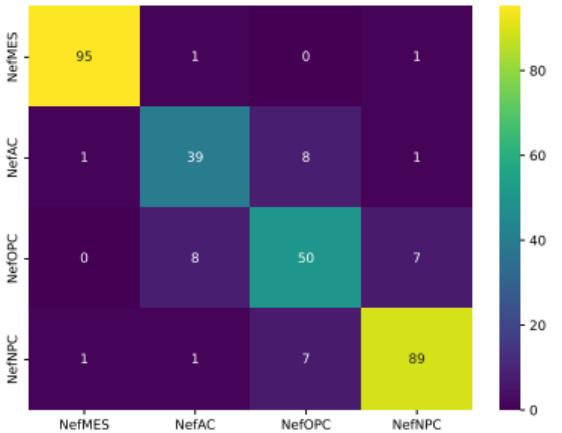
# SIGNATURES



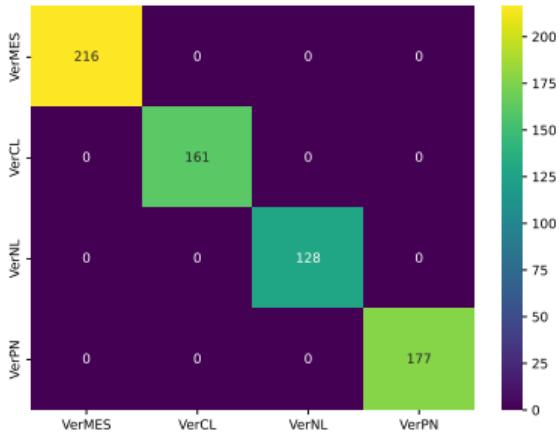
(a) Neftel et al signature

Figure 3: Overlap of genes in the signatures

# SIGNATURES



(a) Neftel et al signature



(b) Verhaak et al signature

Figure 3: Overlap of genes in the signatures

# SIGNATURES

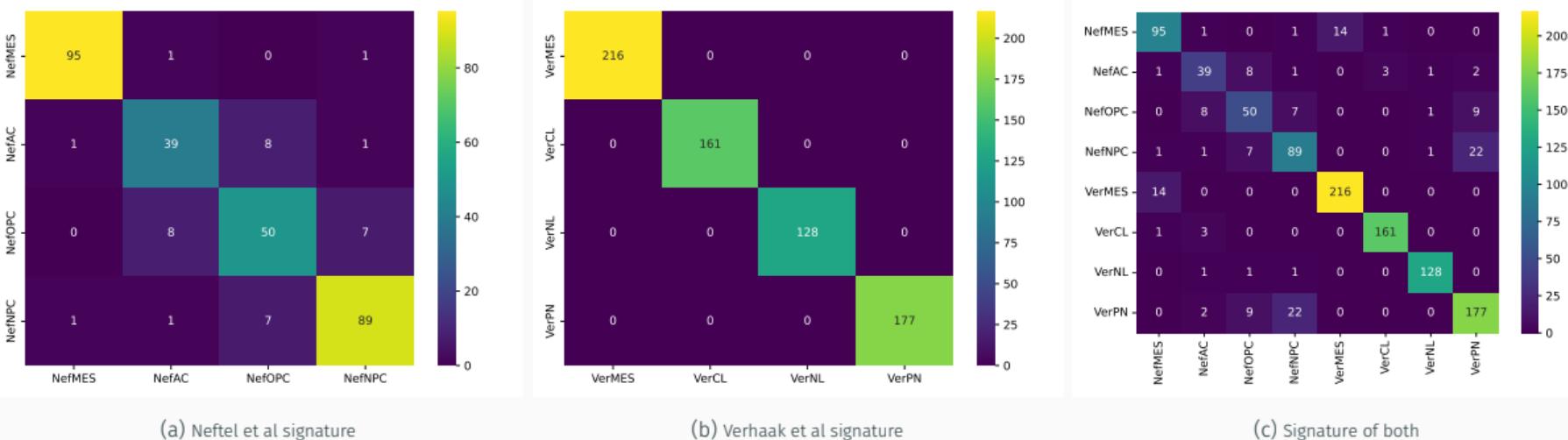


Figure 3: Overlap of genes in the signatures

## SIGNATURES

<b>NefNPC-VerPN</b>	ABAT	AMOTL2	ASCL1	BCAN	BEX1	CHD7	DBN1	DCX
	DLL3	FXYD6	MAP2	MARCKSL1	MLLT11	MYT1	PAK3	PFN2
	SCG3	SEZ6L	SOX11	SOX4	STMN1	STMN4		
<b>NefMES-VerMES</b>	ANXA1	ANXA2	CHI3L1	CLIC1	CTSB	EMP3	LGALS1	LGALS3
	NPC2	S100A11	SERPINE1	TIMP1	TNFRSF1A	WWTR1		

Table 1: Genes common between signatures

# SIGNATURE EXPRESSION - NEFTEL ET AL

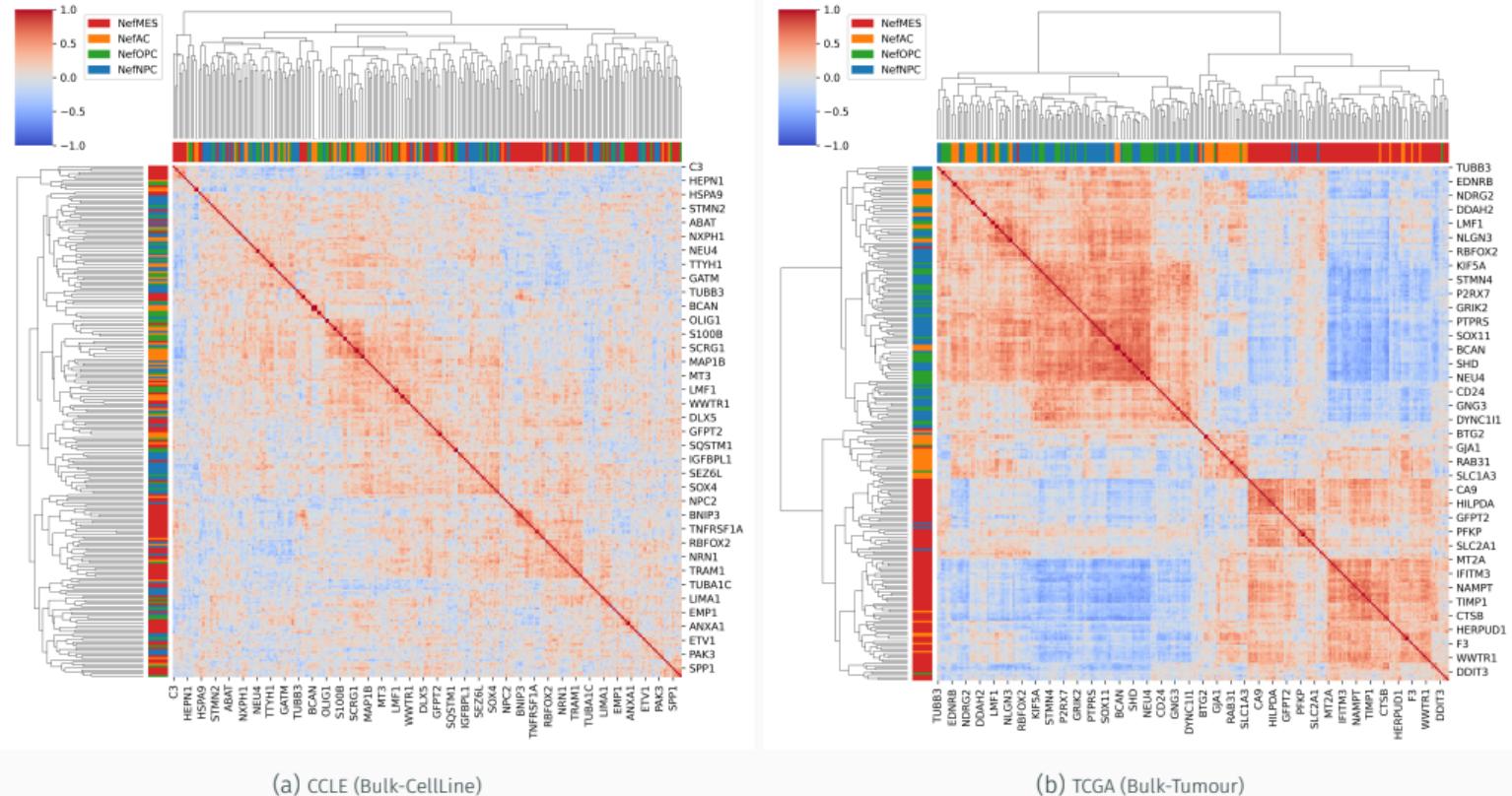
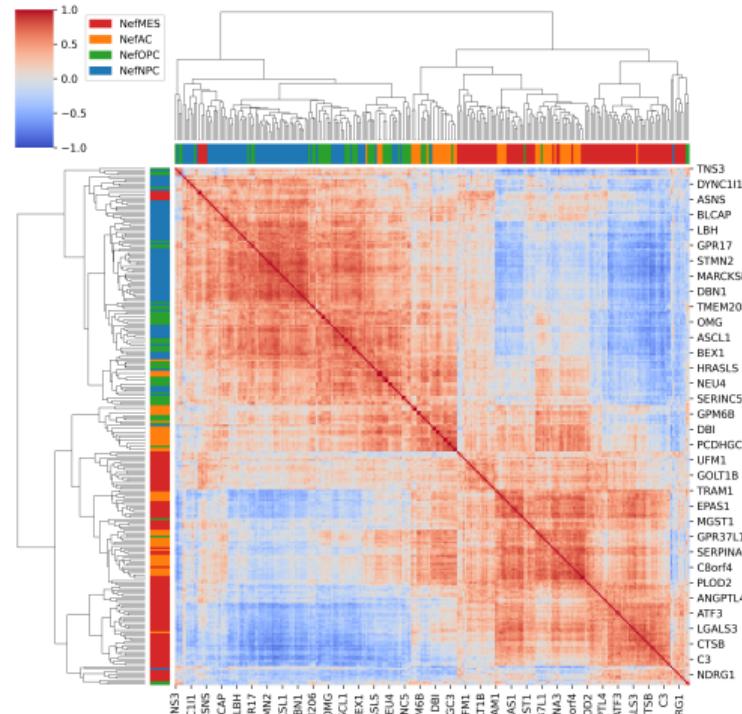
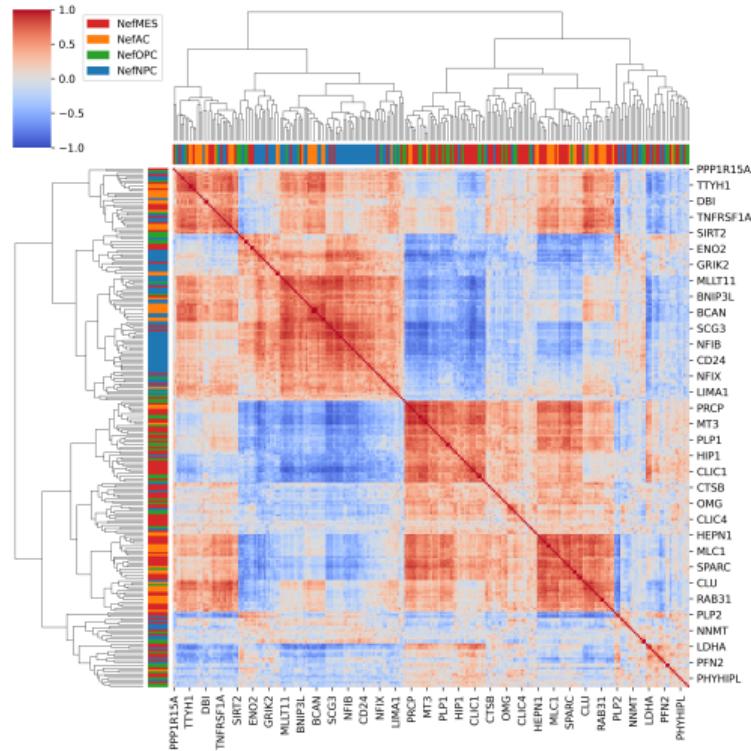


Figure 4: Correlation of signature expression - Neftel

# SIGNATURE EXPRESSION - NEFTEL ET AL



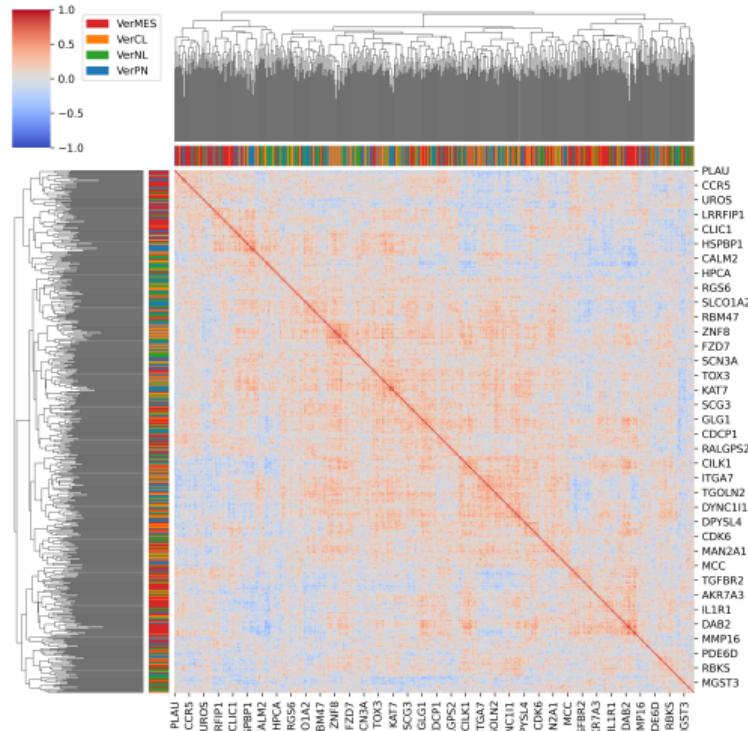
(c) GSE131928 (SingleCell-Tumour)



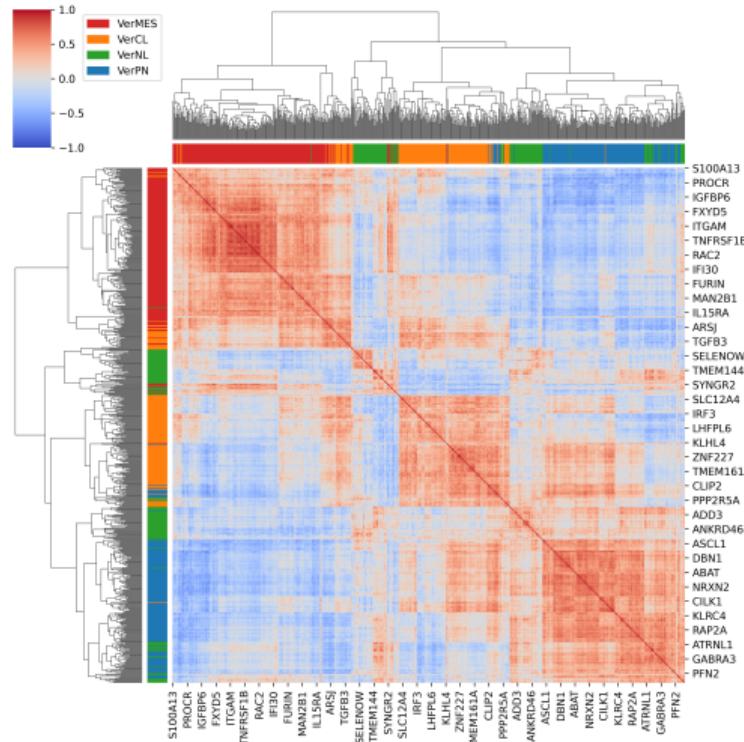
(d) GSE168004 (SingleCell-CellLine)

Figure 4: Correlation of signature expression - Neftel

# SIGNATURE EXPRESSION - VERHAAK ET AL



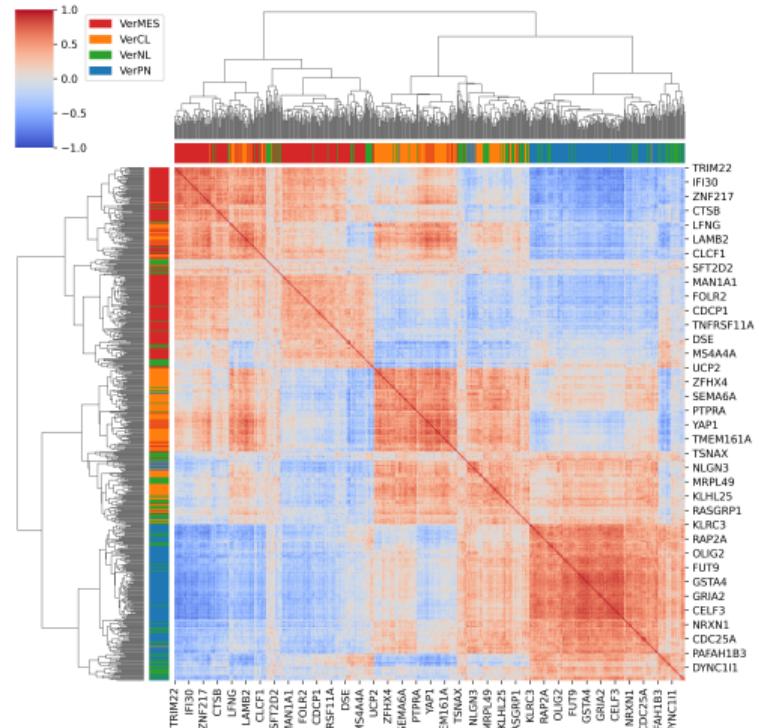
(e) CCLE (Bulk-CellLine)



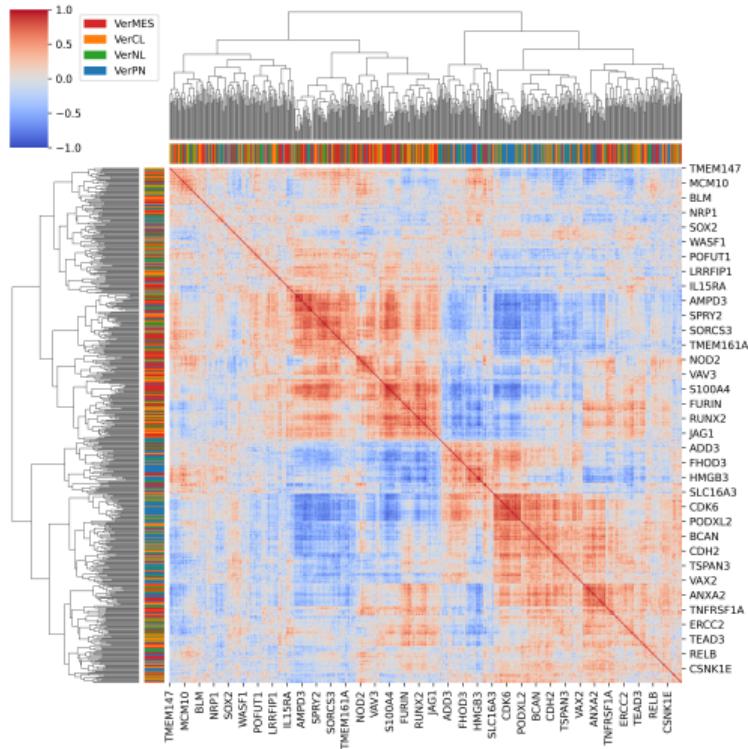
(f) TCGA (Bulk-Tumour)

Figure 4: Correlation of signature expression - Verhaak

# SIGNATURE EXPRESSION - VERHAAK ET AL



(g) GSE131928 (SingleCell-Tumour)



(h) GSE168004 (SingleCell-CellLine)

Figure 4: Correlation of signature expression - Verhaak

## PAIRWISE SIGNATURE EXPRESSION - METRIC

$$\text{Consistency} = \frac{1}{2} \left( \frac{\sum_{x,y \in S_1, S_1} \rho_R(x, y)}{2(N_1)^2} + \frac{\sum_{x,y \in S_2, S_2} \rho_R(x, y)}{2(N_2)^2} - \frac{\sum_{x,y \in S_1, S_2} \rho_R(x, y)}{N_1 N_2} \right)$$

Where,

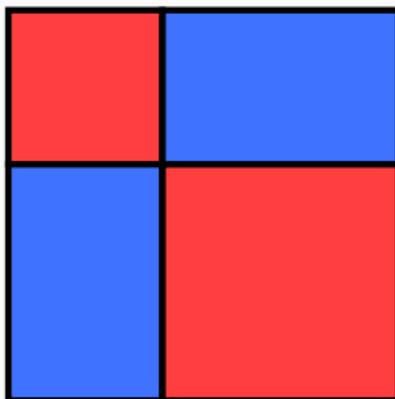
- $\rho_R(x, y)$  = Spearman correlation between x and y
- $S_i$  = Signature i
- $N_i$  = No. of genes in Signature i

## PAIRWISE SIGNATURE EXPRESSION - METRIC

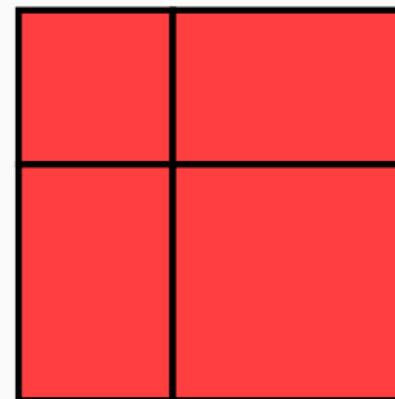
$$\text{Consistency} = \frac{1}{2} \left( \frac{\sum_{x,y \in S_1, S_1} \rho_R(x, y)}{2(N_1)^2} + \frac{\sum_{x,y \in S_2, S_2} \rho_R(x, y)}{2(N_2)^2} - \frac{\sum_{x,y \in S_1, S_2} \rho_R(x, y)}{N_1 N_2} \right)$$

Where,

- $\rho_R(x, y)$  = Spearman correlation between x and y
- $S_i$  = Signature i
- $N_i$  = No. of genes in Signature i



(a) Consistency = 1



(b) Consistency = 0

Figure 5: Consistency on example cases

# PAIRWISE SIGNATURE EXPRESSION - NEFTEL ET AL

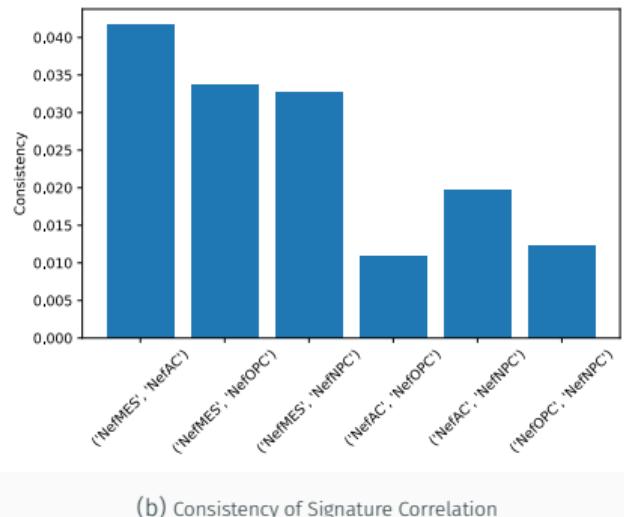
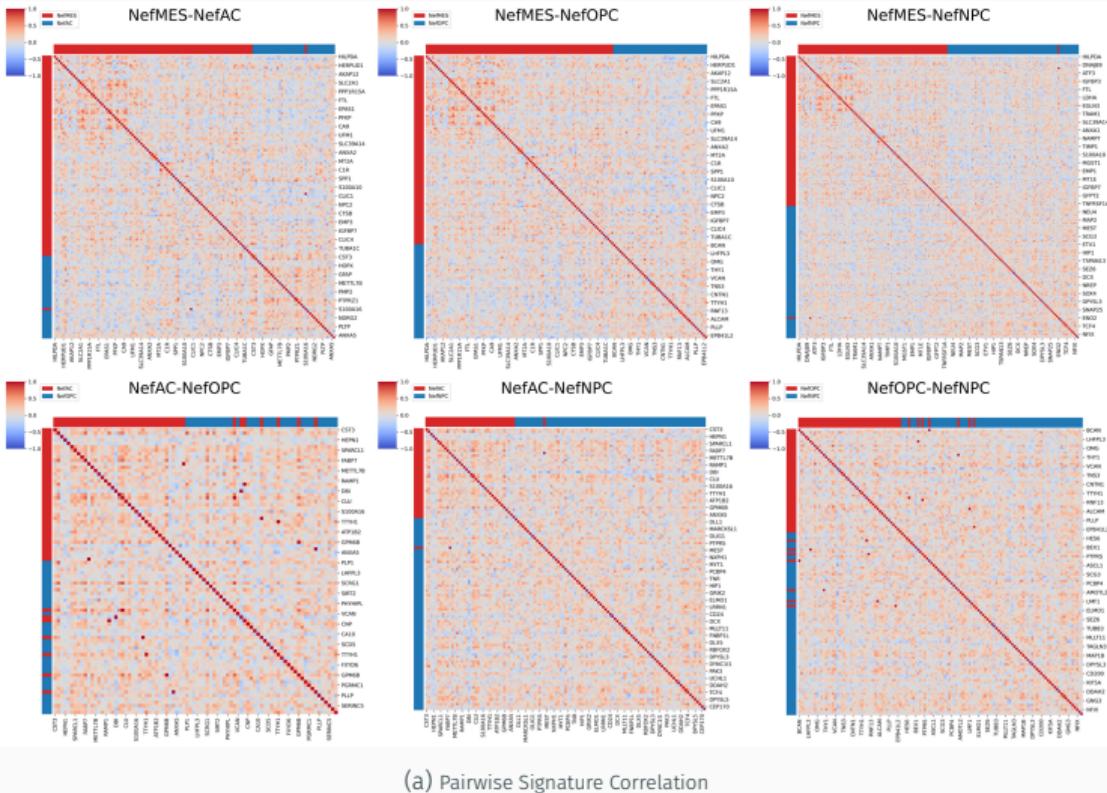
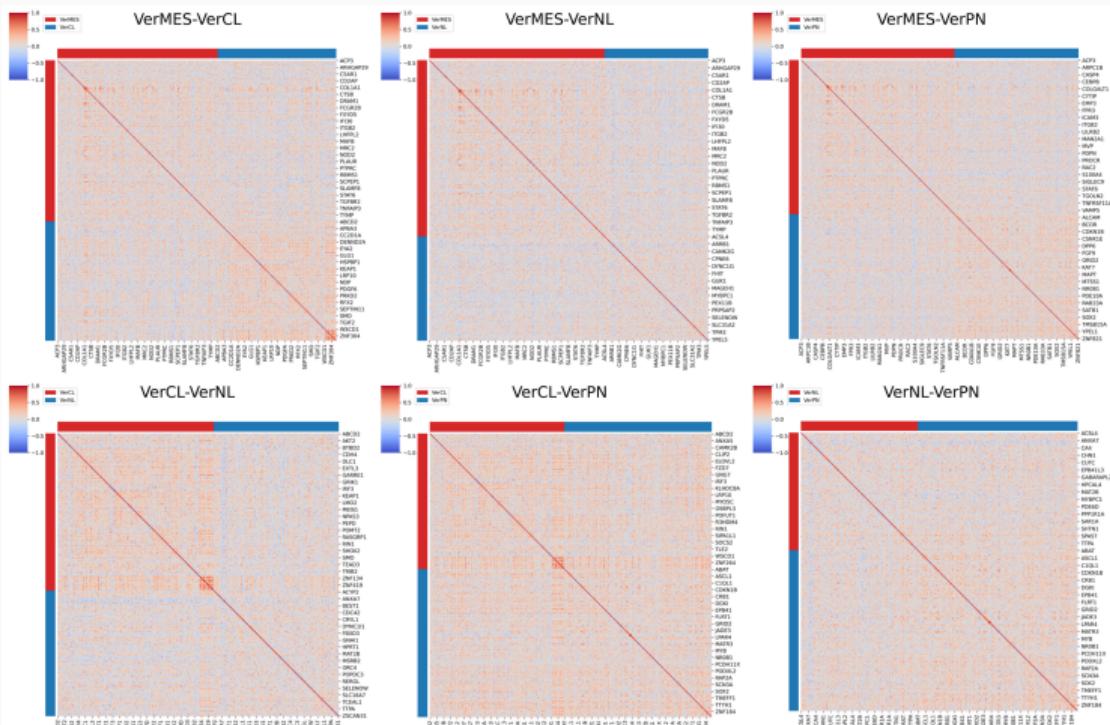
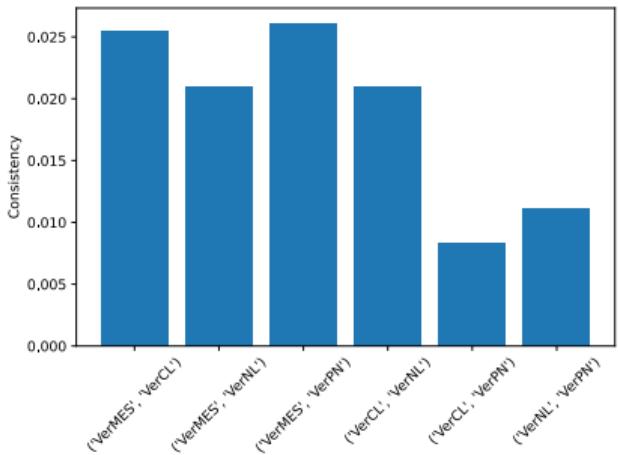


Figure 6: Pairwise correlation of signature expression - CCLE (Bulk-CellLine) - Neftel

PAIRWISE SIGNATURE EXPRESSION - VERHAAK ET AL



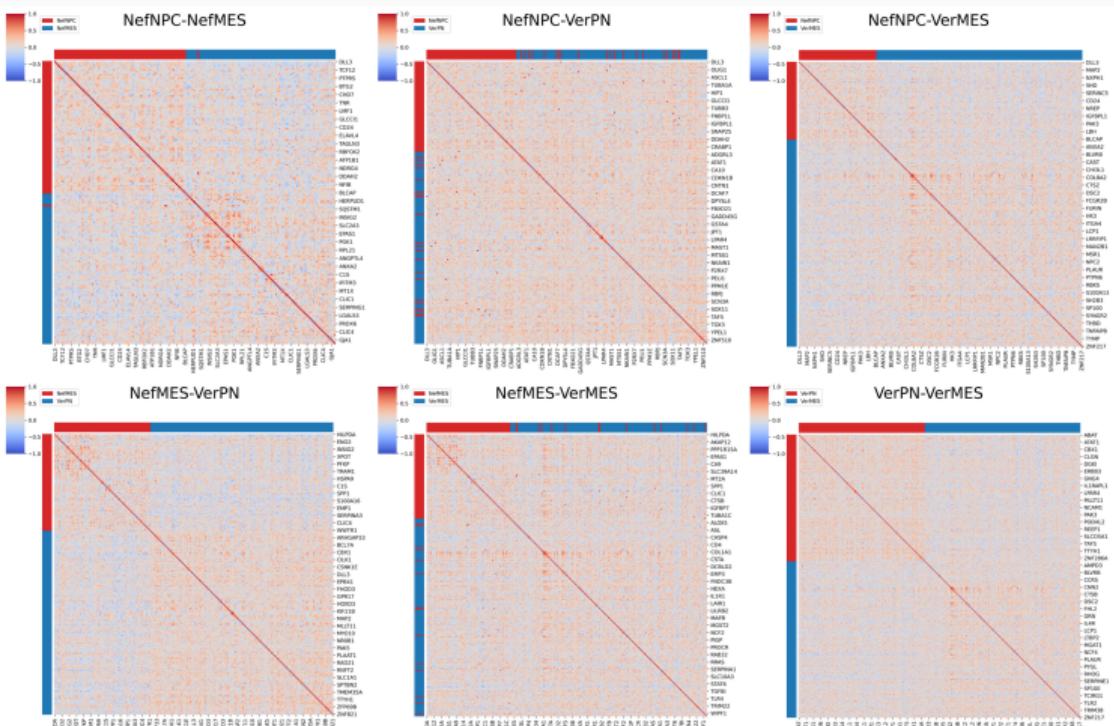
(c) Pairwise Signature Correlation



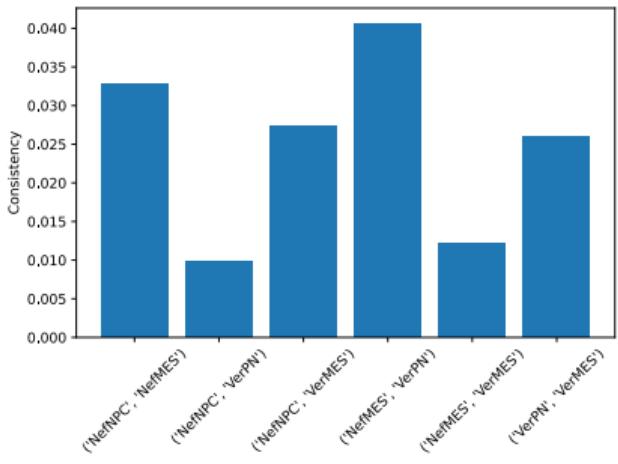
(d) Consistency of Signature Correlation

Figure 6: Pairwise correlation of signature expression - CCLE (Bulk-CellLine) - Verhaak

## PAIRWISE SIGNATURE EXPRESSION - COMPARISON



(e) Pairwise Signature Correlation



(f) Consistency of Signature Correlation

Figure 6: Pairwise correlation of signature expression - CCLE (Bulk-CellLine) - Comparison

# PAIRWISE SIGNATURE EXPRESSION - NEFTEL ET AL

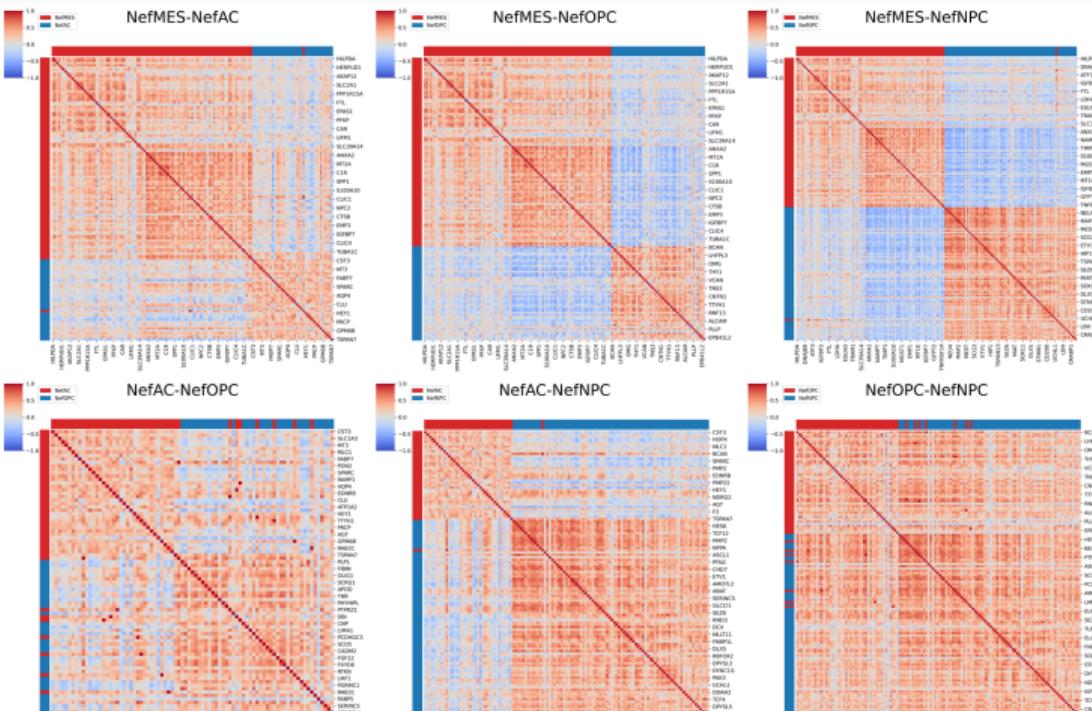
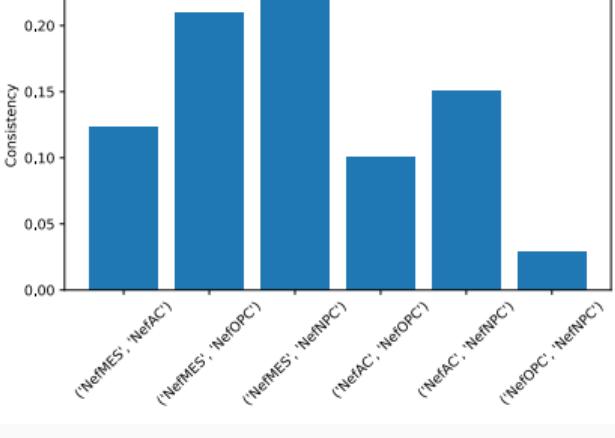


Figure 7: Pairwise correlation of signature expression - TCGA (Bulk-Tumour) - Neftel



(b) Consistency of Signature Correlation

# PAIRWISE SIGNATURE EXPRESSION - VERHAAK ET AL

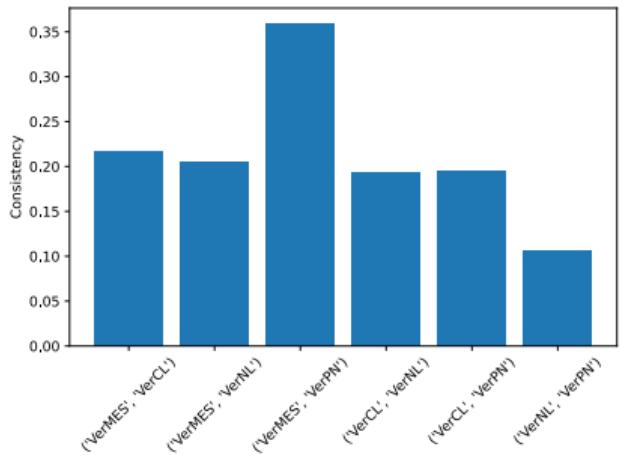
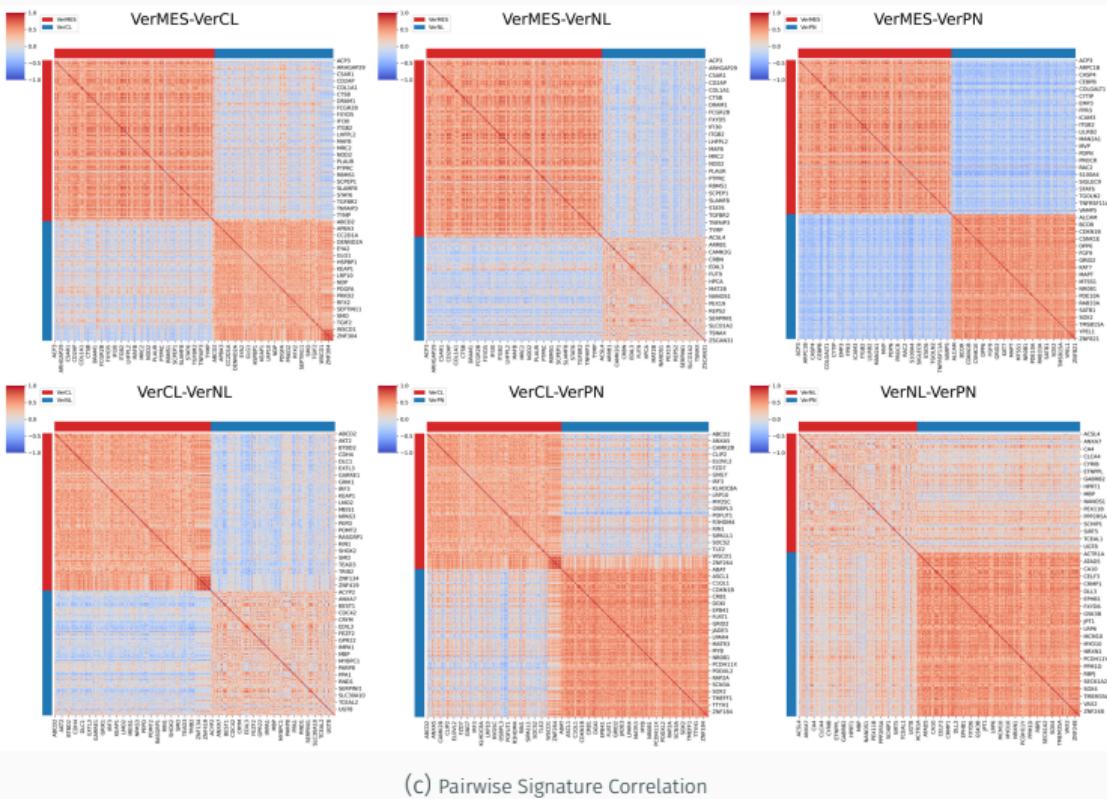


Figure 7: Pairwise correlation of signature expression - TCGA (Bulk-Tumour) - Verhaak

## PAIRWISE SIGNATURE EXPRESSION - COMPARISON

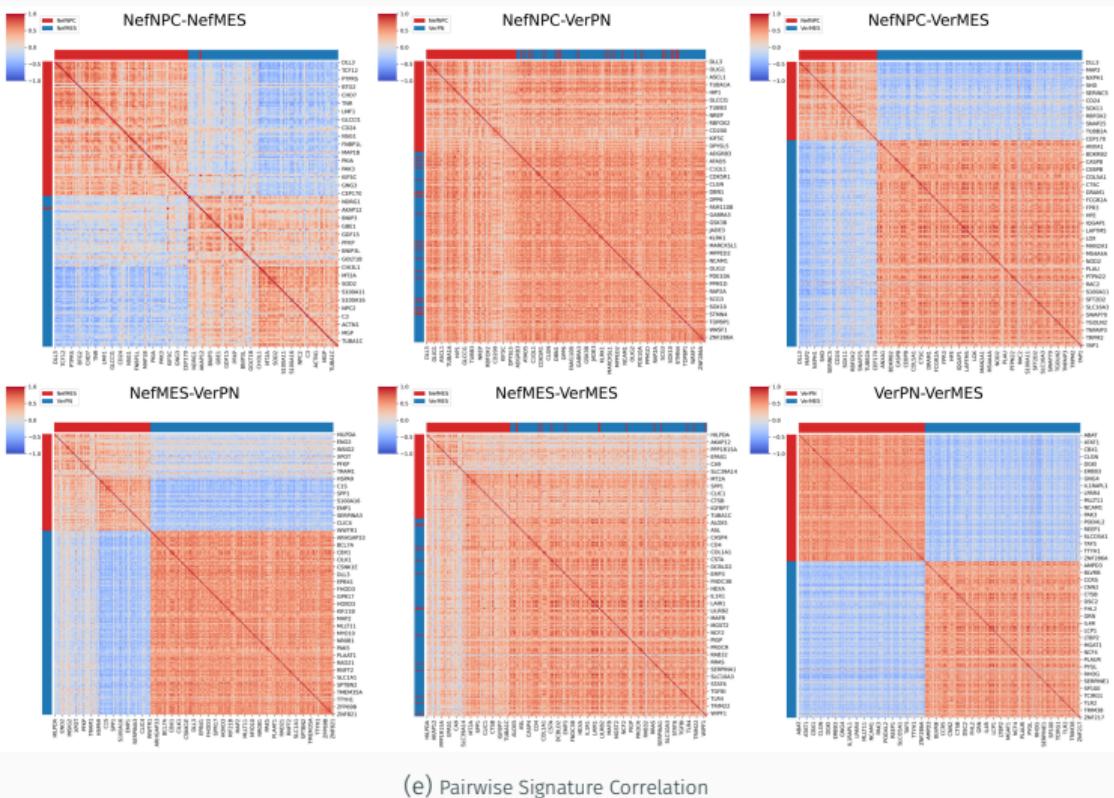
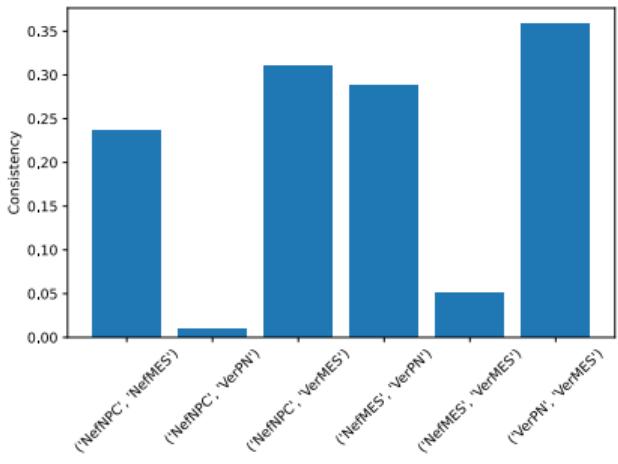


Figure 7: Pairwise correlation of signature expression - TCGA (Bulk-Tumour) - Comparison



(f) Consistency of Signature Correlation

# PAIRWISE SIGNATURE EXPRESSION - NEFTEL ET AL

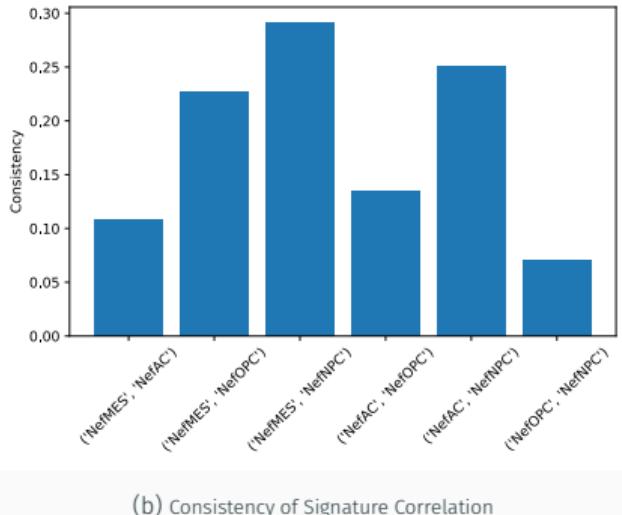
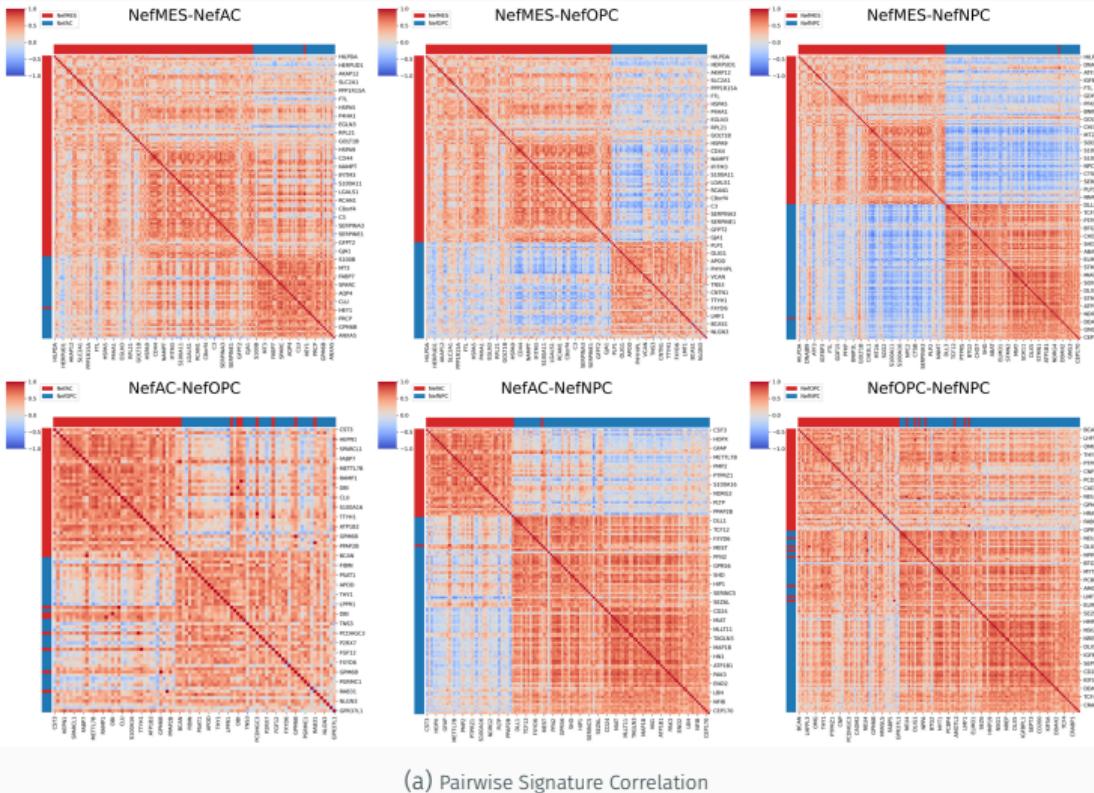
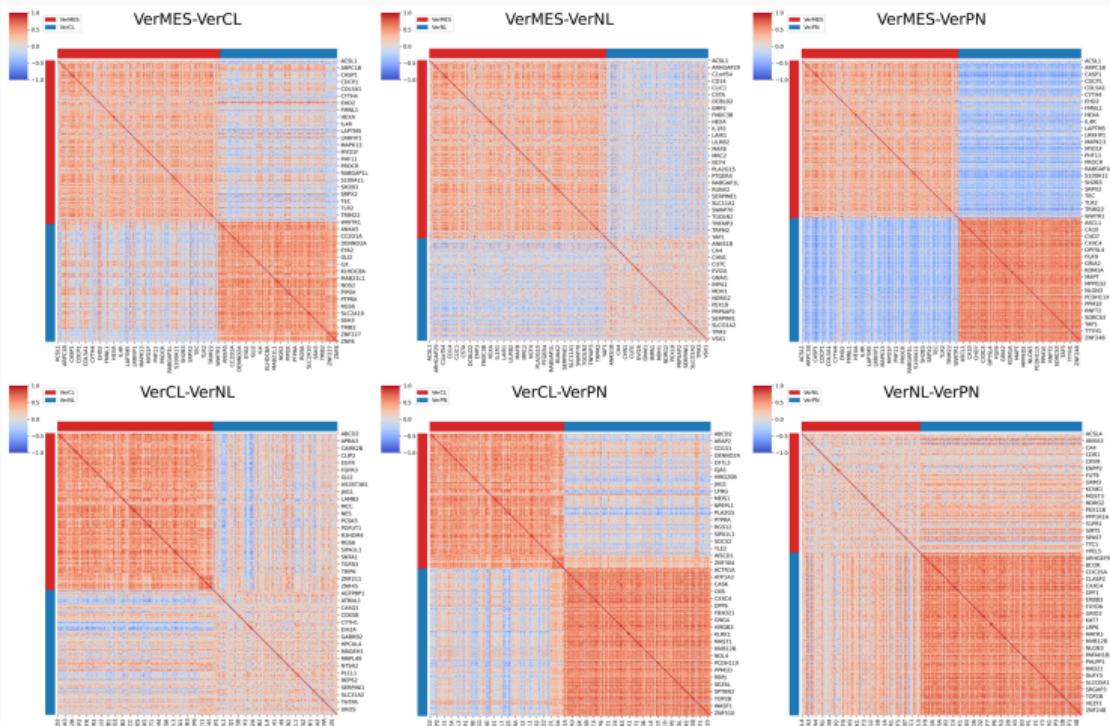
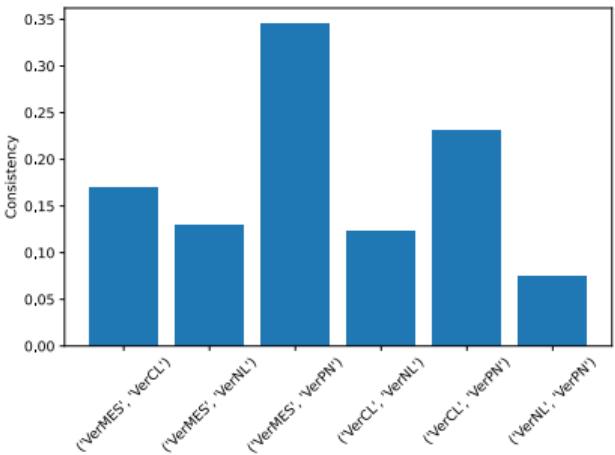


Figure 8: Pairwise correlation of signature expression - GSE131928 (SingleCell-Tumour) - Neftel

PAIRWISE SIGNATURE EXPRESSION - VERHAAK ET AL



(c) Pairwise Signature Correlation



(d) Consistency of Signature Correlation

Figure 8: Pairwise correlation of signature expression - GSE131928 (SingleCell-Tumour) - Verhaak

# PAIRWISE SIGNATURE EXPRESSION - COMPARISON

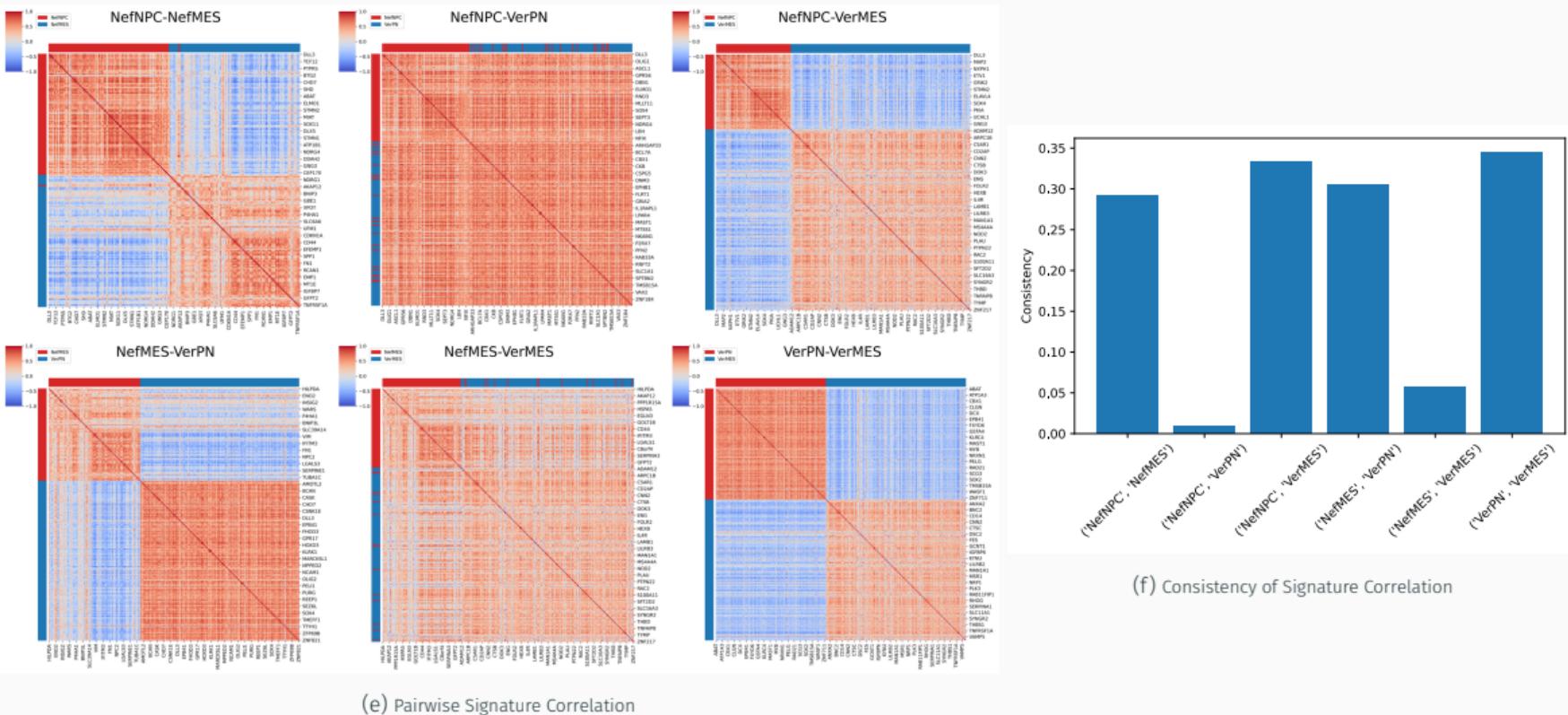
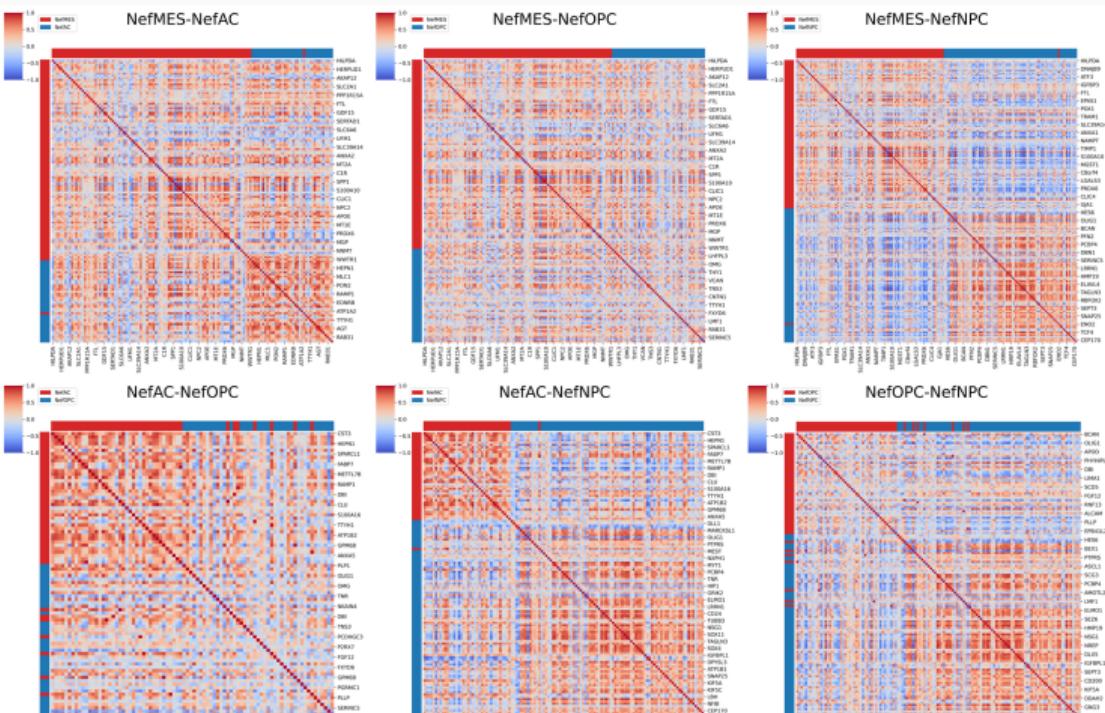
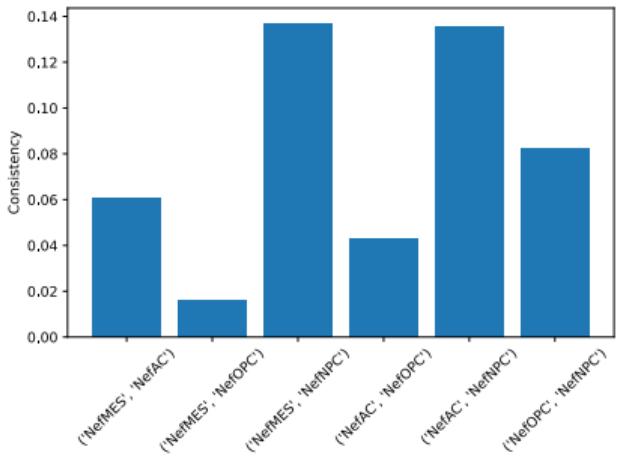


Figure 8: Pairwise correlation of signature expression - GSE131928 (SingleCell-Tumour) - Comparison

# PAIRWISE SIGNATURE EXPRESSION - NEFTEL ET AL



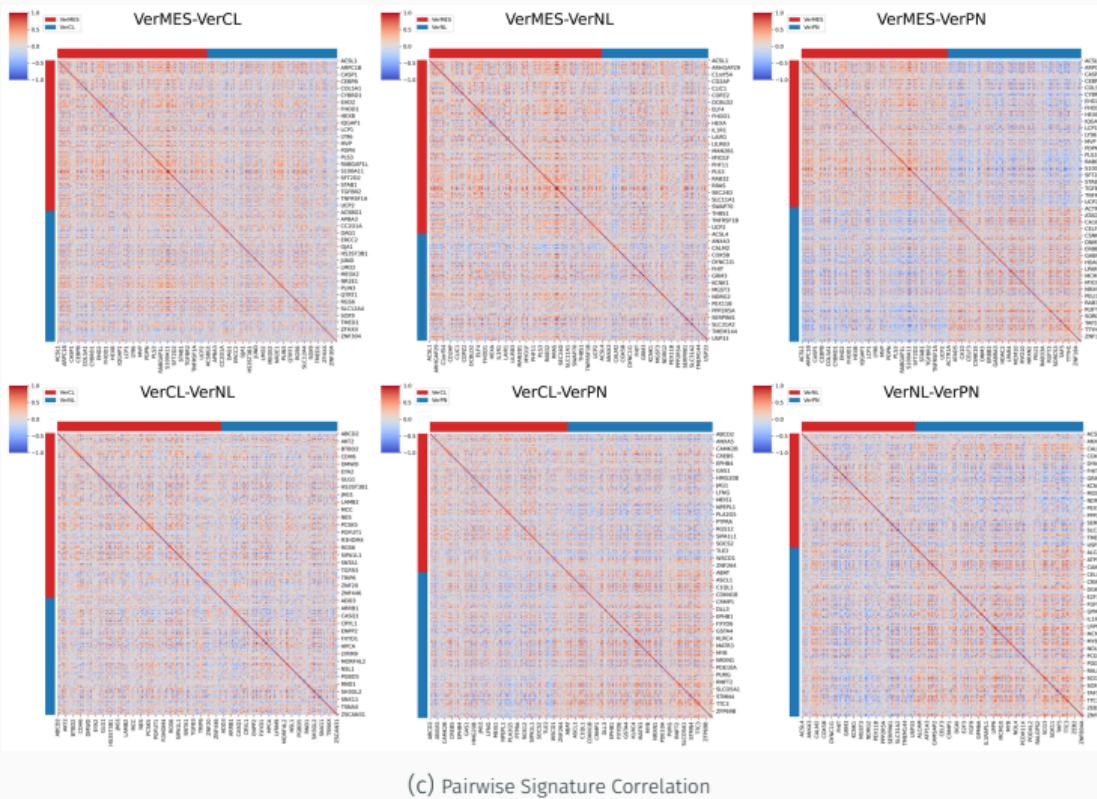
(a) Pairwise Signature Correlation



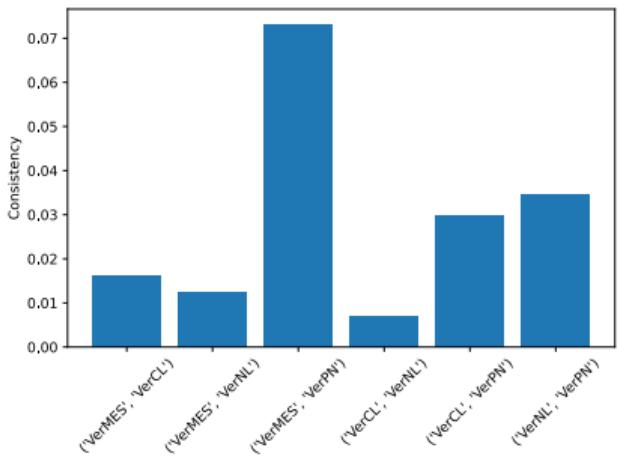
(b) Consistency of Signature Correlation

Figure 9: Pairwise correlation of signature expression - GSE168004 (SingleCell-CellLine) - Neftel

PAIRWISE SIGNATURE EXPRESSION - VERHAAK ET AL



### (c) Pairwise Signature Correlation



**(d) Consistency of Signature Correlation**

# PAIRWISE SIGNATURE EXPRESSION - COMPARISON

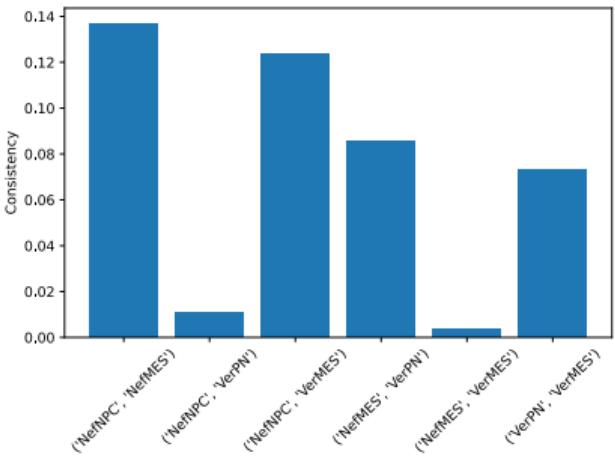
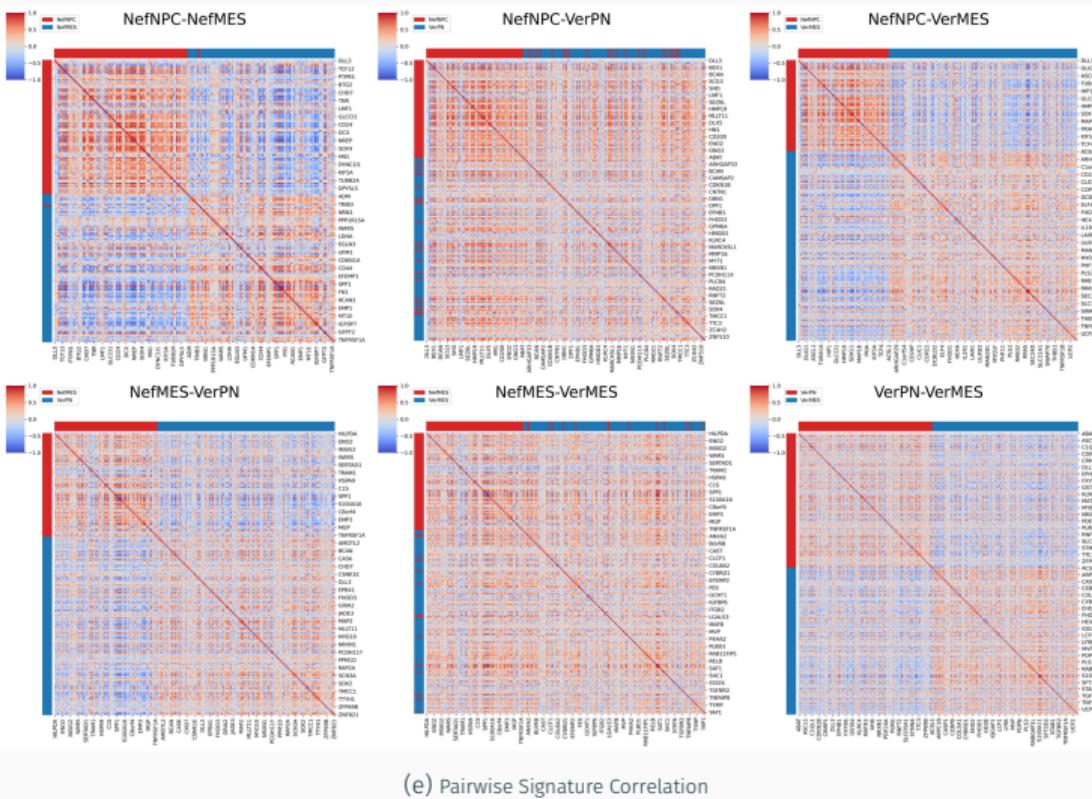
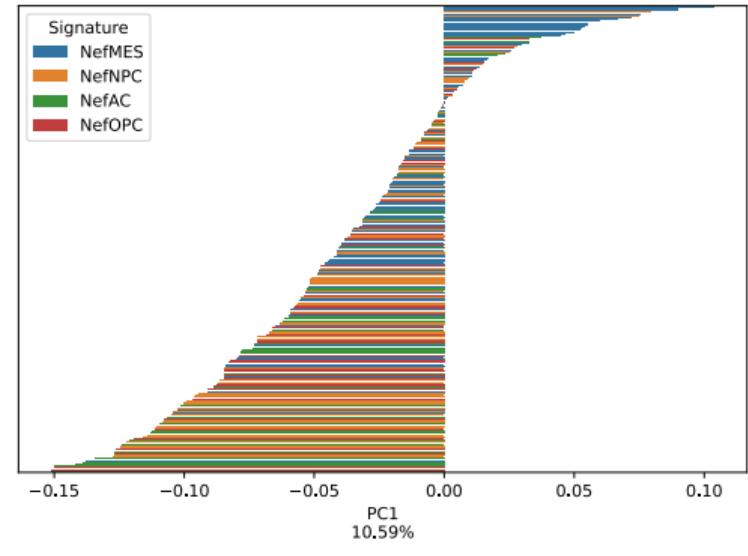
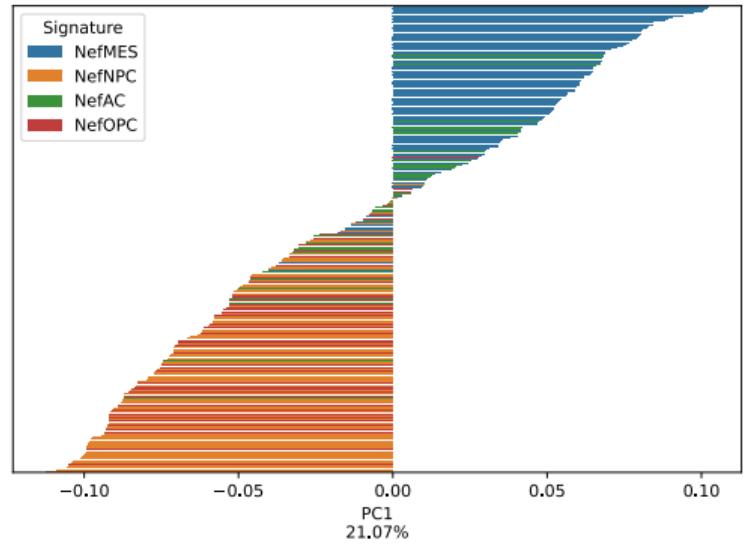


Figure 9: Pairwise correlation of signature expression - GSE168004 (SingleCell-CellLine) - Comparison

# PCA OF SIGNATURE - NEFTEL ET AL



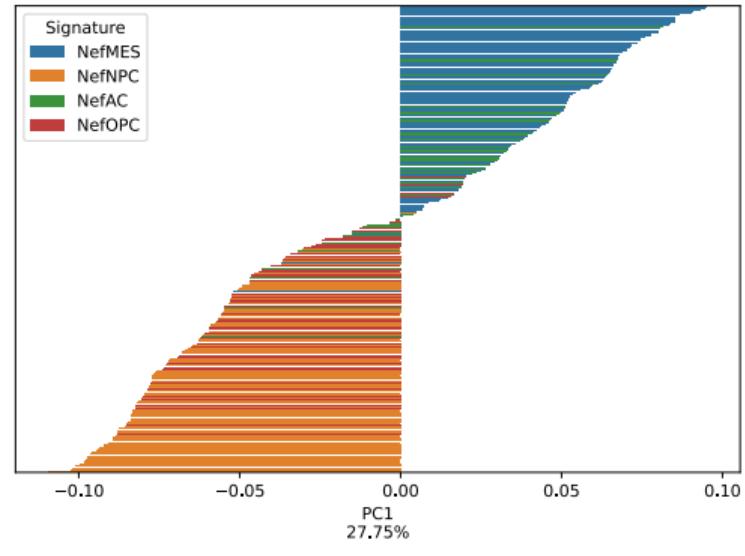
(a) CCLE (Bulk-CellLine)



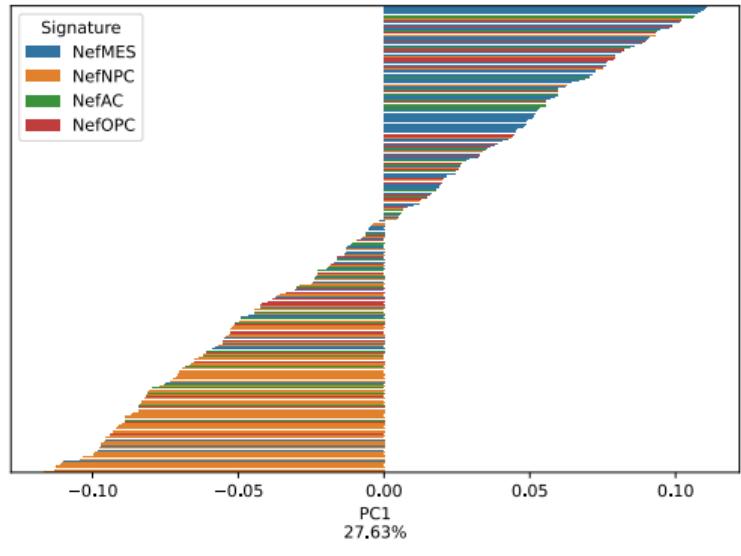
(b) TCGA (Bulk-Tumour)

Figure 10: Loadings of PC1 - PCA on signature expression - Neftel

# PCA OF SIGNATURE - NEFTEL ET AL

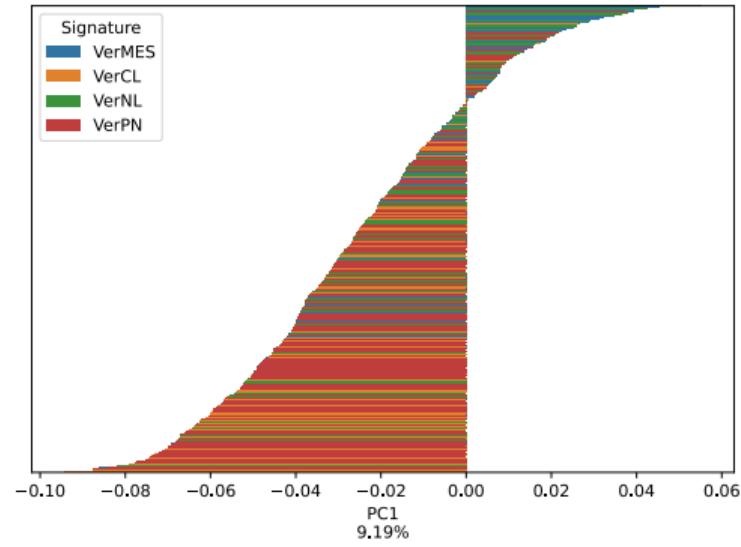


(c) GSE131928 (SingleCell-Tumour)

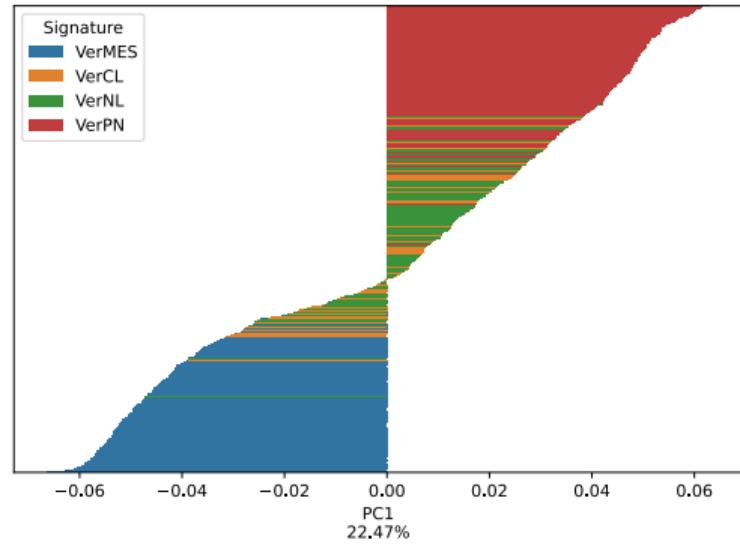


(d) GSE168004 (SingleCell-CellLine)

Figure 10: Loadings of PC1 - PCA on signature expression - Neftel



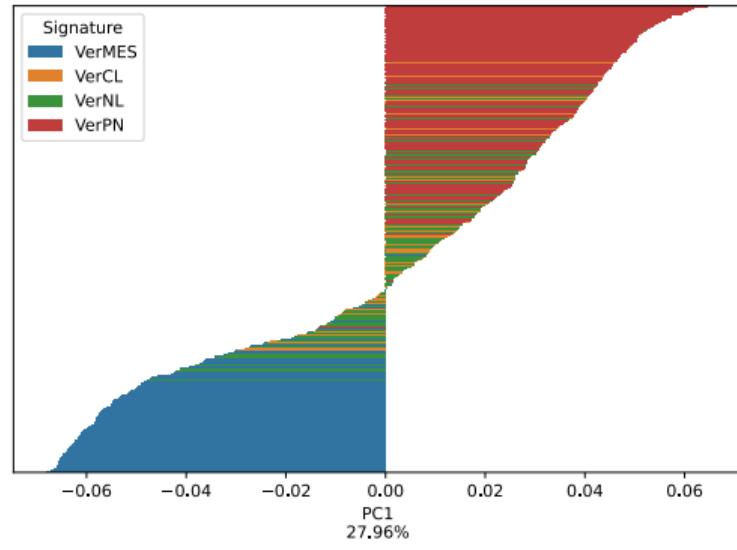
(e) CCLE (Bulk-CellLine)



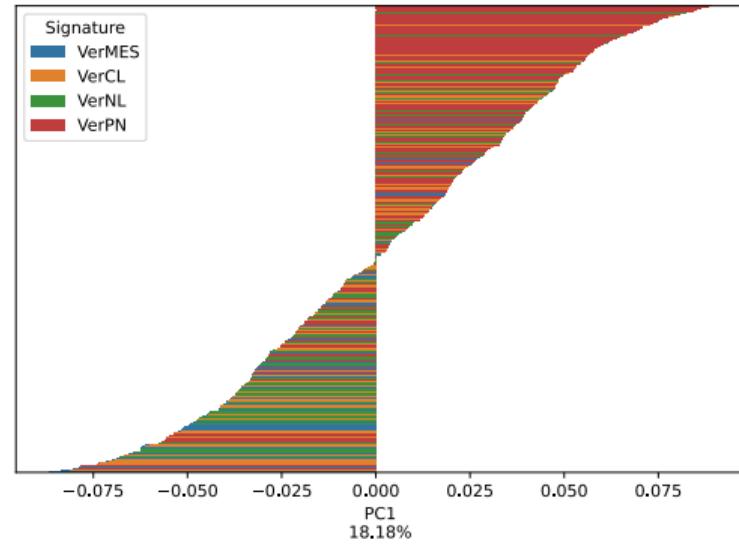
(f) TCGA (Bulk-Tumour)

Figure 10: Loadings of PC1 - PCA on signature expression - Verhaak

# PCA OF SIGNATURE - VERHAAK ET AL



(g) GSE131928 (SingleCell-Tumour)



(h) GSE168004 (SingleCell-CellLine)

Figure 10: Loadings of PCA on signature expression - Verhaak

# PCA VS GENE-SETS - NEFTEL ET AL

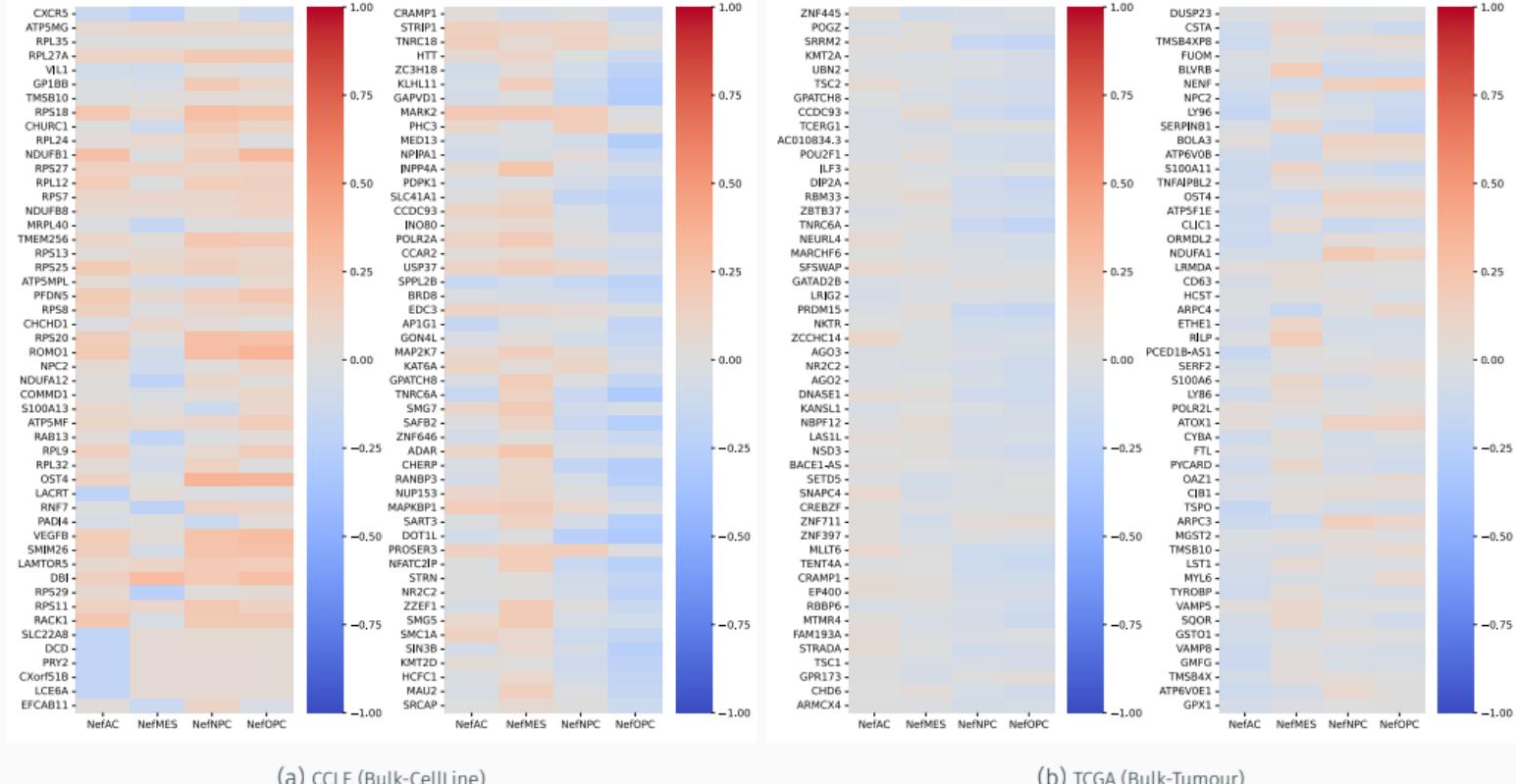
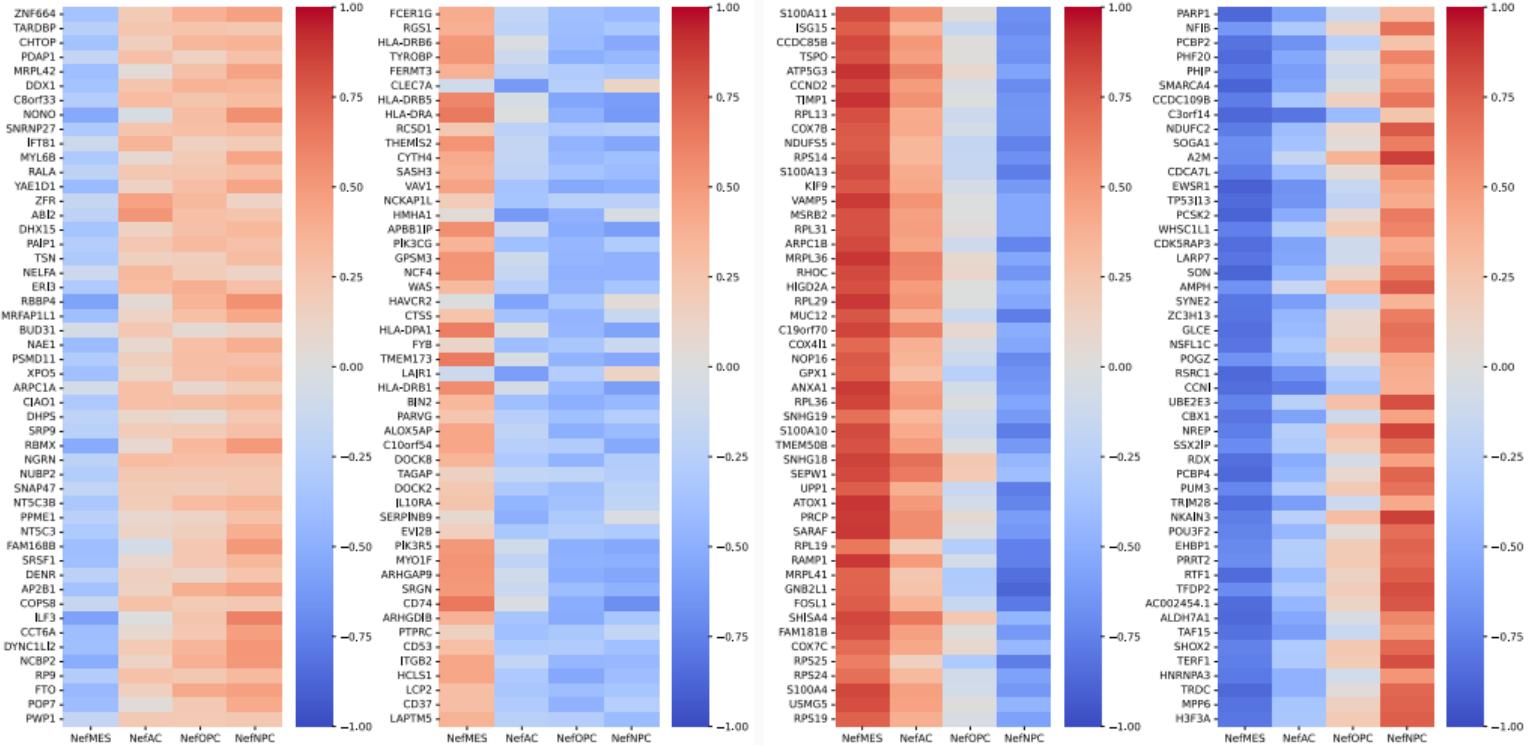


Figure 11: Correlation of top loadings of PCA with ssGSEA scores - Neftel

# PCA VS GENE-SETS - NEFTEL ET AL

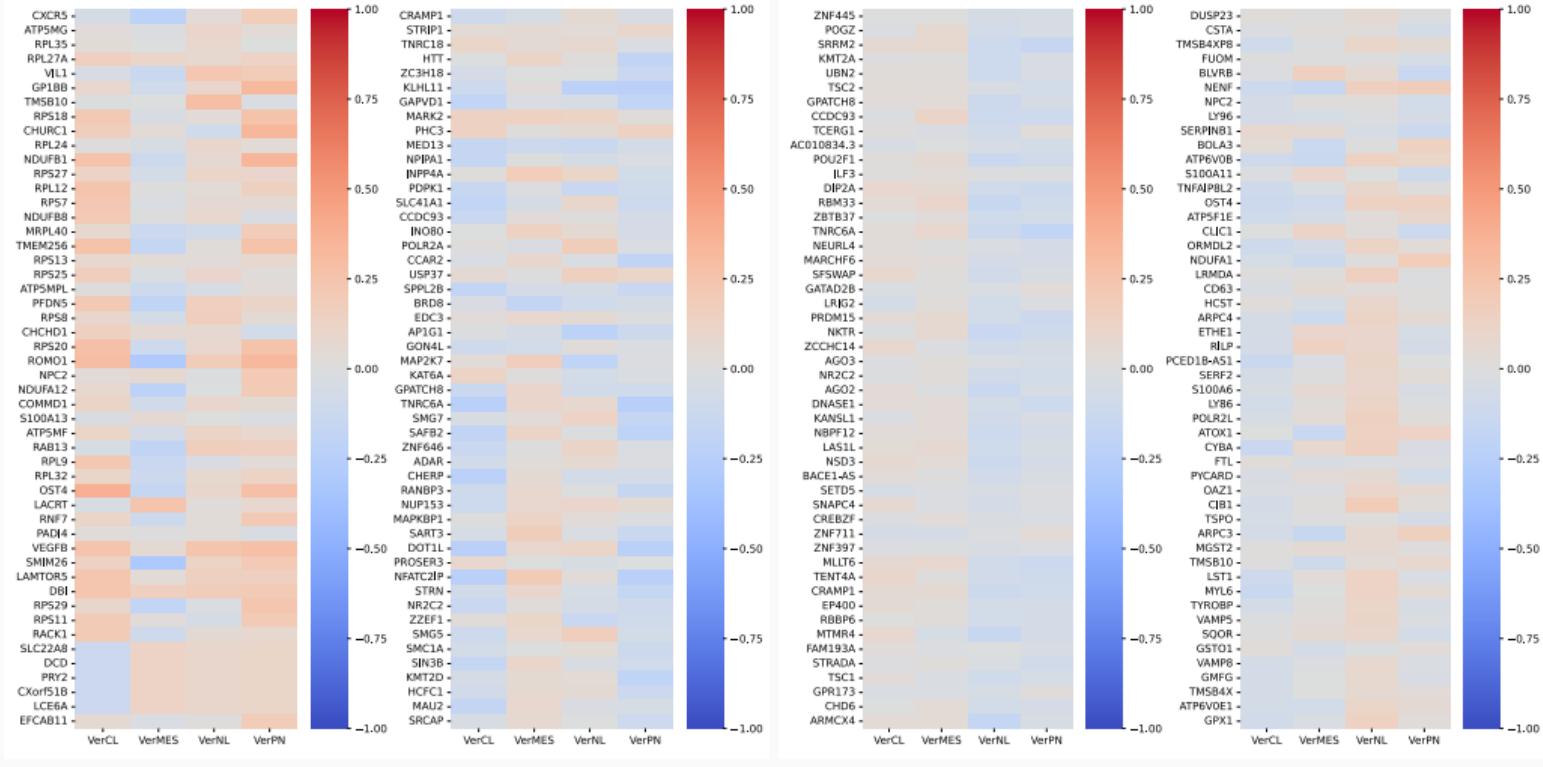


(c) GSE131928 (SingleCell-Tumour)

(d) GSE168004 (SingleCell-CellLine)

Figure 11: Correlation of top loadings of PCA with AUCCell scores - Neftel

# PCA VS GENE-SETS - VERHAAK ET AL



(e) CCLE (Bulk-CellLine)

(f) TCGA (Bulk-Tumour)

Figure 11: Correlation of top loadings of PCA with ssGSEA scores - Verhaak

# PCA VS GENE-SETS - VERHAAK ET AL

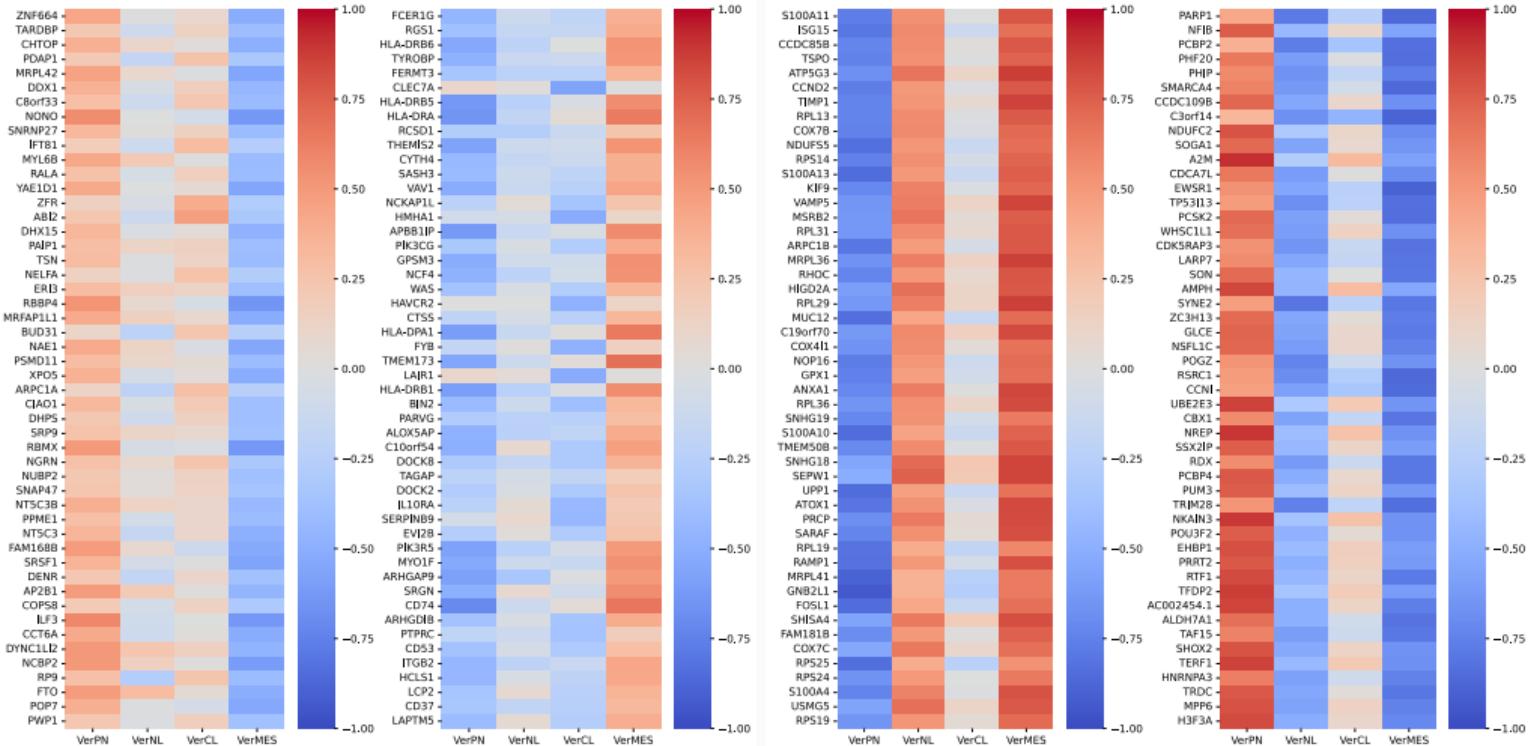


Figure 11: Correlation of top loadings of PCA with AUCell scores - Verhaak

Here be dragons!

# PAIRWISE PCA - NEFTEL

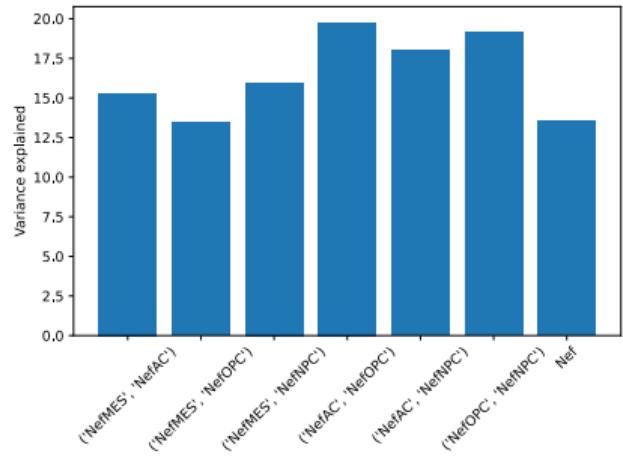
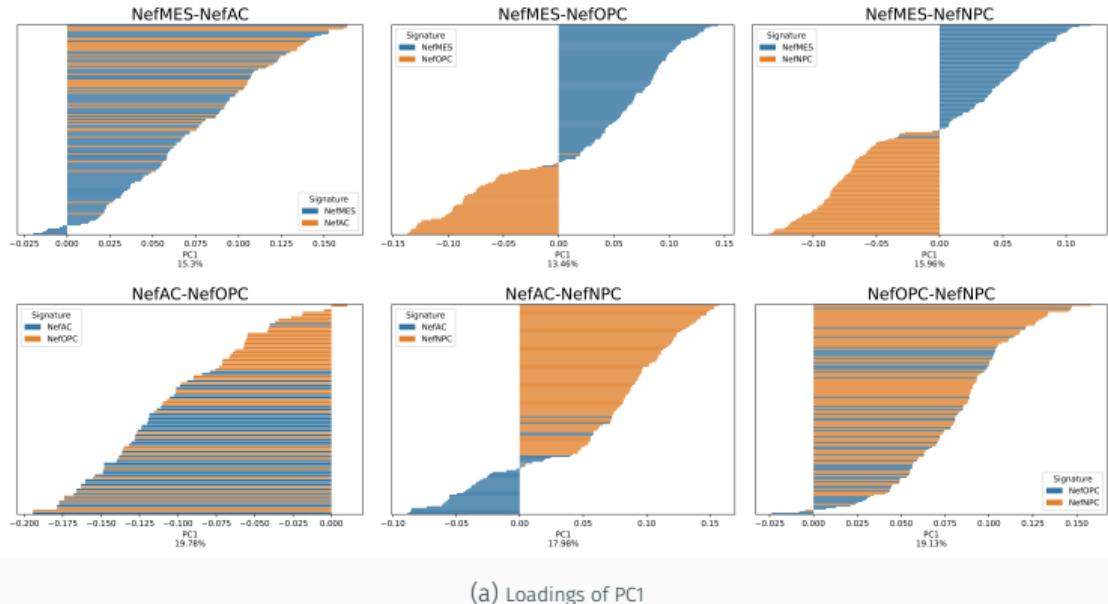


Figure 12: Pairwise PCA - GSE131928 (SingleCell-Tumor)

# PAIRWISE PCA - VERHAAK

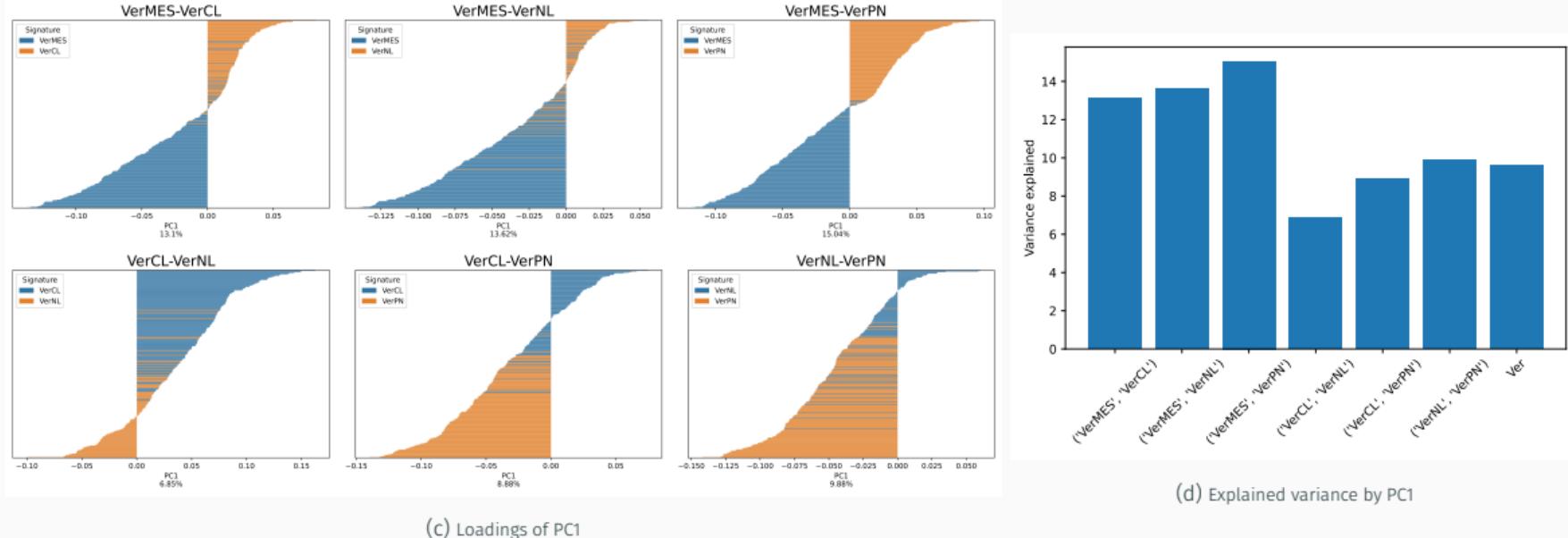
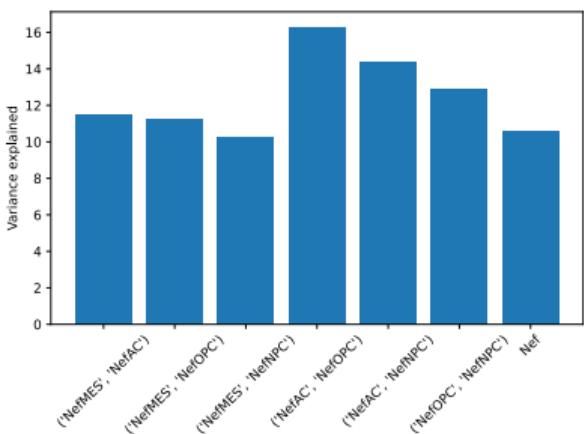
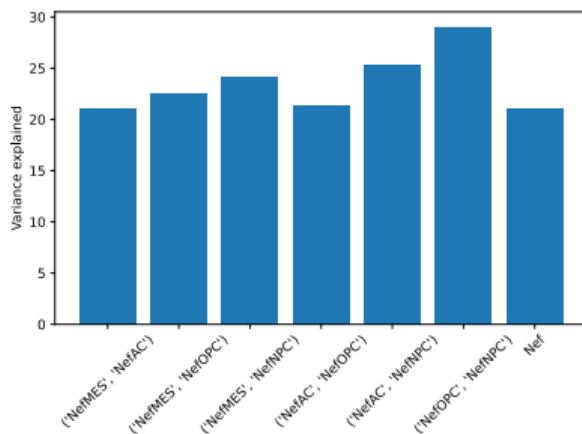


Figure 12: Pairwise PCA - GSE131928 (SingleCell-Tumor)

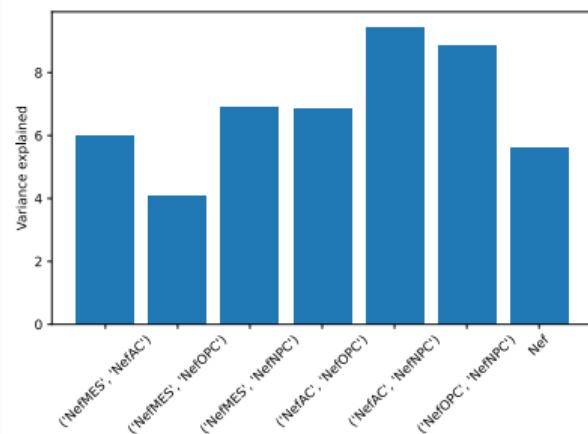
# PAIRWISE PCA - NEFTEL



(e) CCLE (Bulk-CellLine)



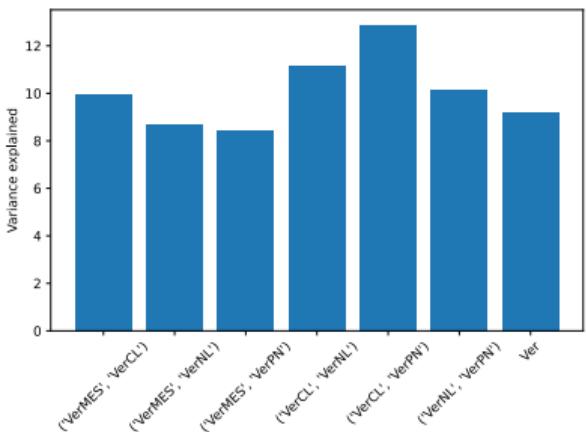
(f) TCGA (Bulk-Tumour)



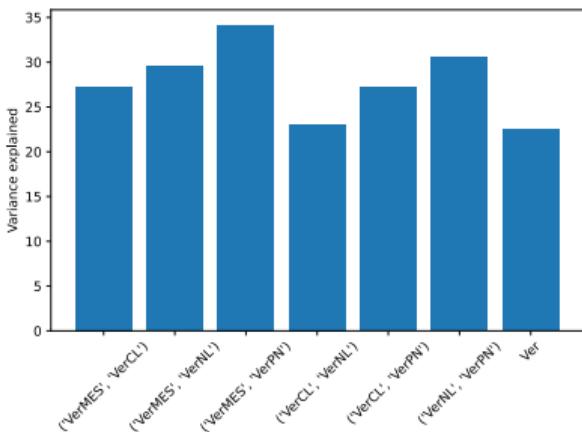
(g) GSE168004 (SingleCell-CellLine)

Figure 12: Explained variance by PC1 - Pairwise PCA - Neftel

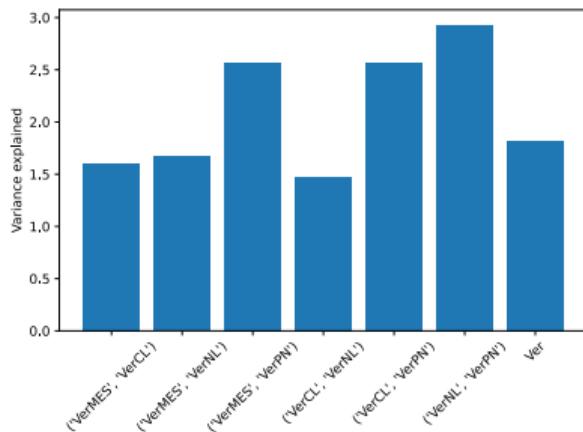
# PAIRWISE PCA - VERHAAK



(h) CCLE (Bulk-CellLine)



(i) TCGA (Bulk-Tumour)



(j) GSE168004 (SingleCell-CellLine)

Figure 12: Explained variance by PC1 - Pairwise PCA - Verhaak

## CONCLUSION

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

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- Most distinct axis
  - NPC vs MES - Neftel et al
  - PN vs MES - Verhaak et al

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- Most distinct axis
  - NPC vs MES - Neftel et al
  - PN vs MES - Verhaak et al
- Similar states between Neftel vs Verhaak
  - $\text{NPC} \approx \text{PN}$
  - $\text{MES} \approx \text{MES}$

- Establish that the other axes do not contribute much
- Construct a network based on literature which explains the observations

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