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Supplemental information

Cell-free DNA methylation-defined prognostic subgroups in small-cell lung cancer identified by leukocyte methylation subtraction

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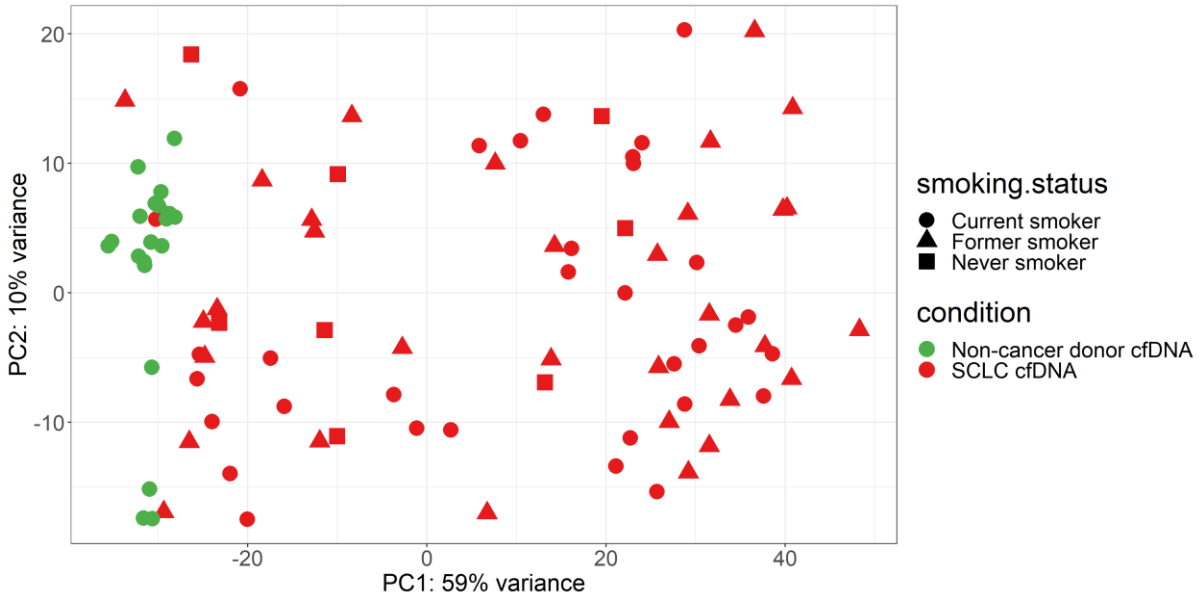
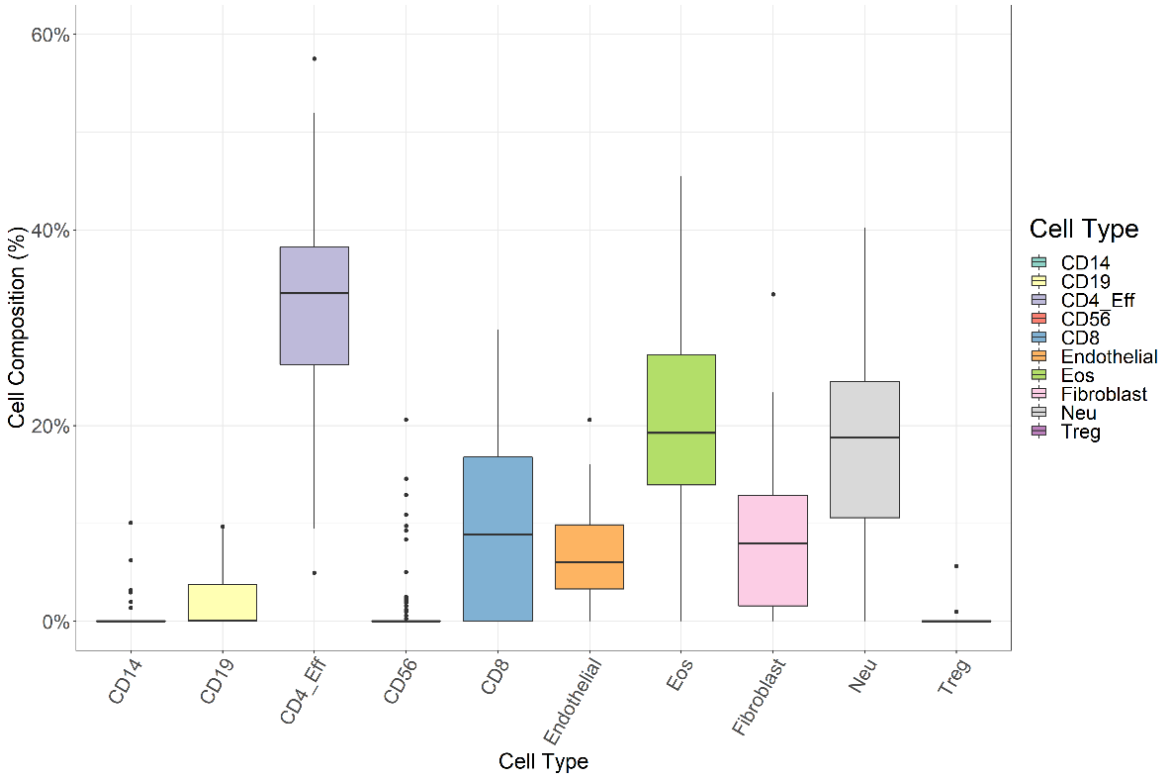
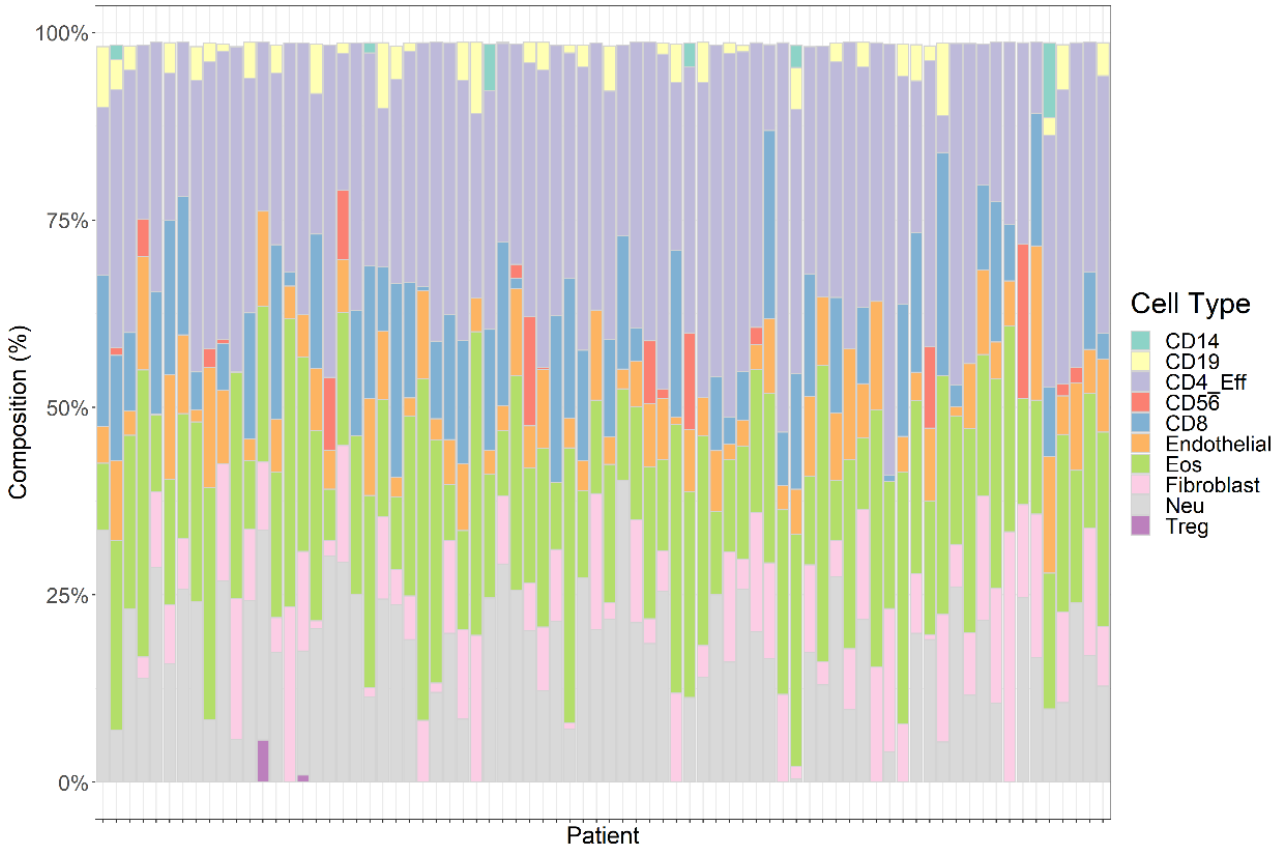
Supplemental Data – Figures & Tables



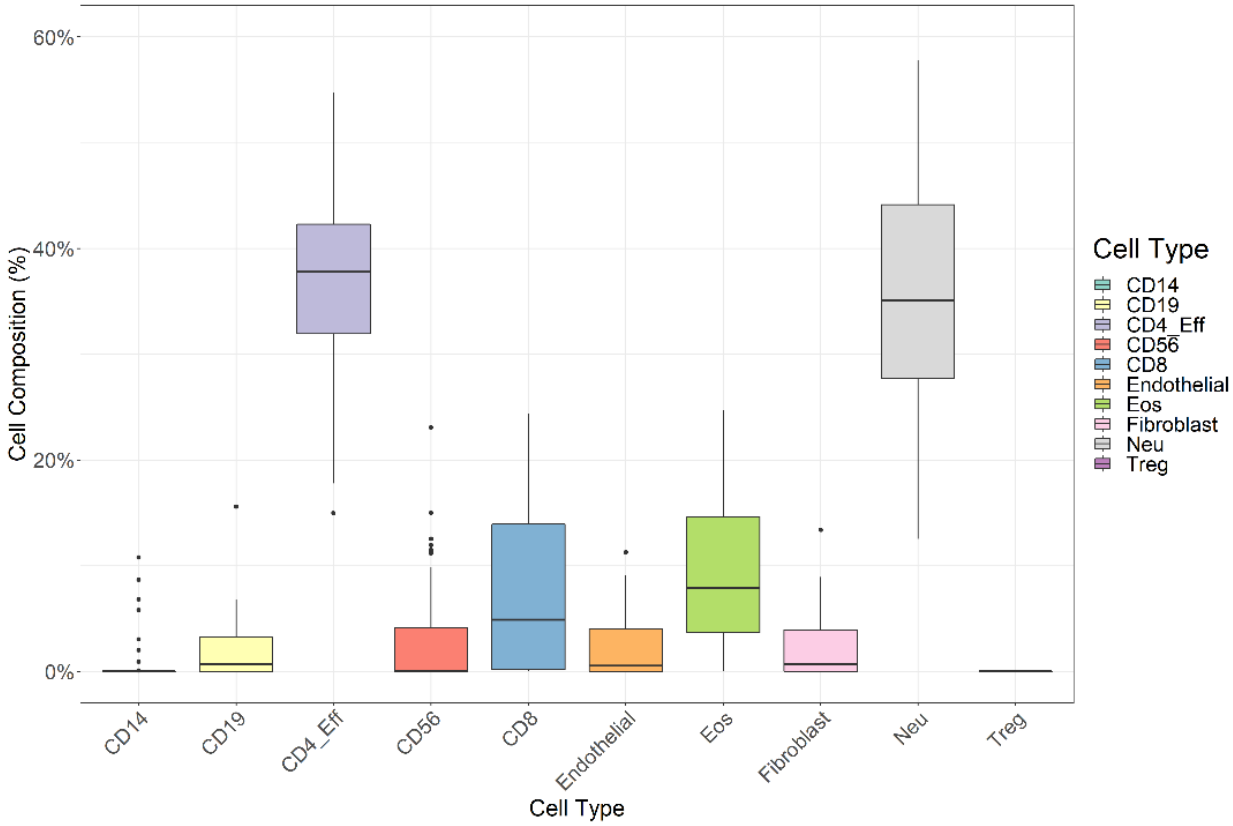
Supplemental Figure 1, related to Figure 1. Principal component analysis of genome-wide methylation profiles of total methylation cfDNA from SCLC patients (n=74) and non-cancer donors (n=20) by smoking status.



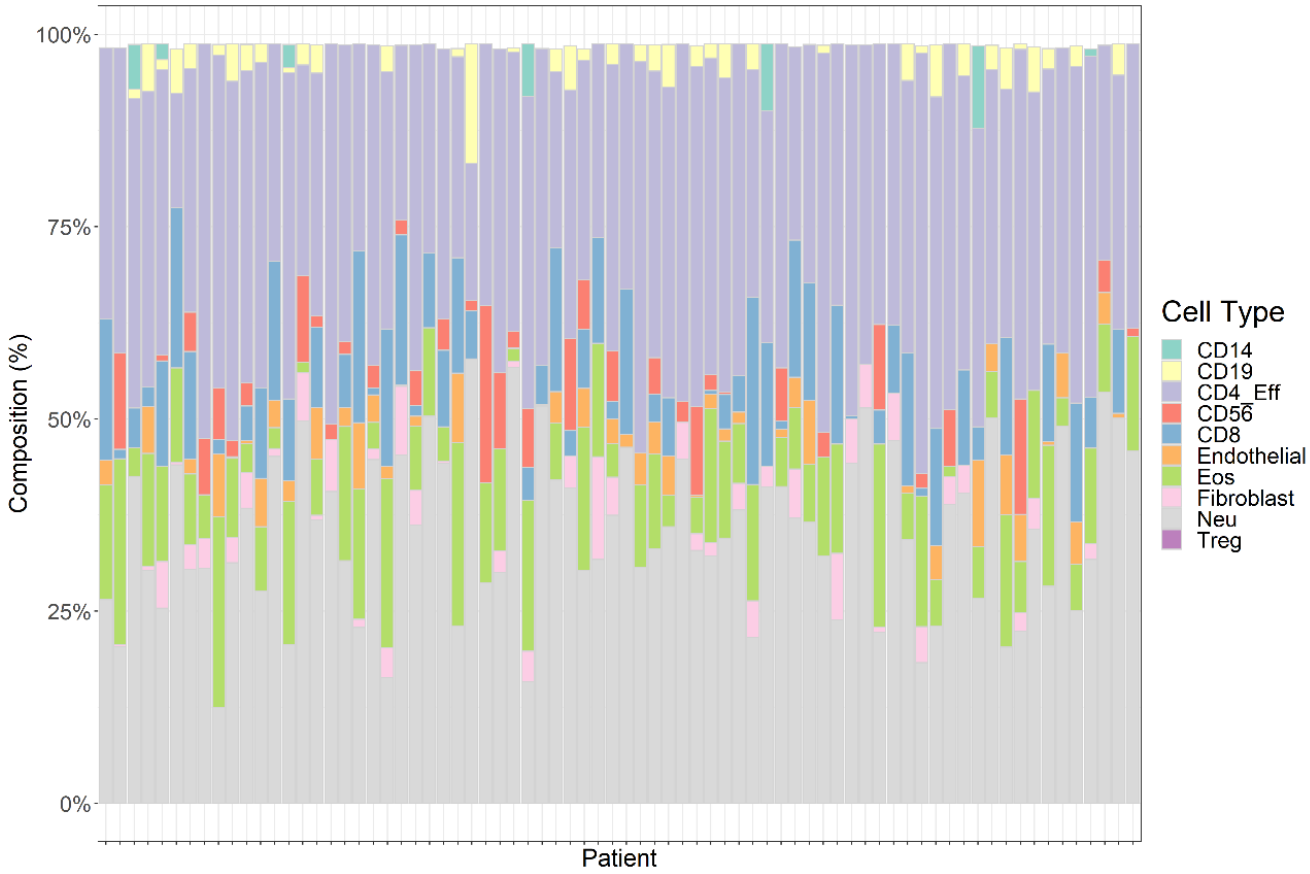
Supplemental Figure 2, related to Figure 2. MethylCIBERSORT composition analysis by total plasma cfDNA methylation, quantifying immune cell presence in the plasma by cell-type. Each box-plot represents the range of cell-composition (%) by cell type for all 74 SCLC patients.



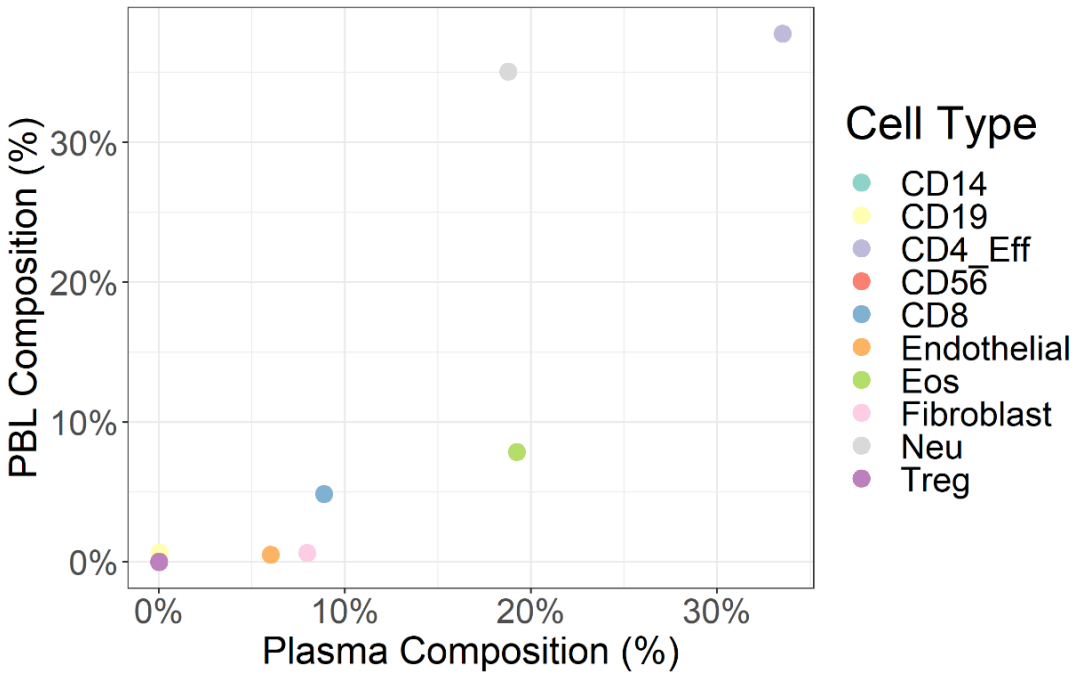
Supplemental Figure 3, related to Figure 2. MethylCIBERSORT composition analysis by total plasma cfDNA methylation, quantifying immune cell presence in the plasma by patient. Each stacked bar-plot represents each of the 74 SCLC patients.



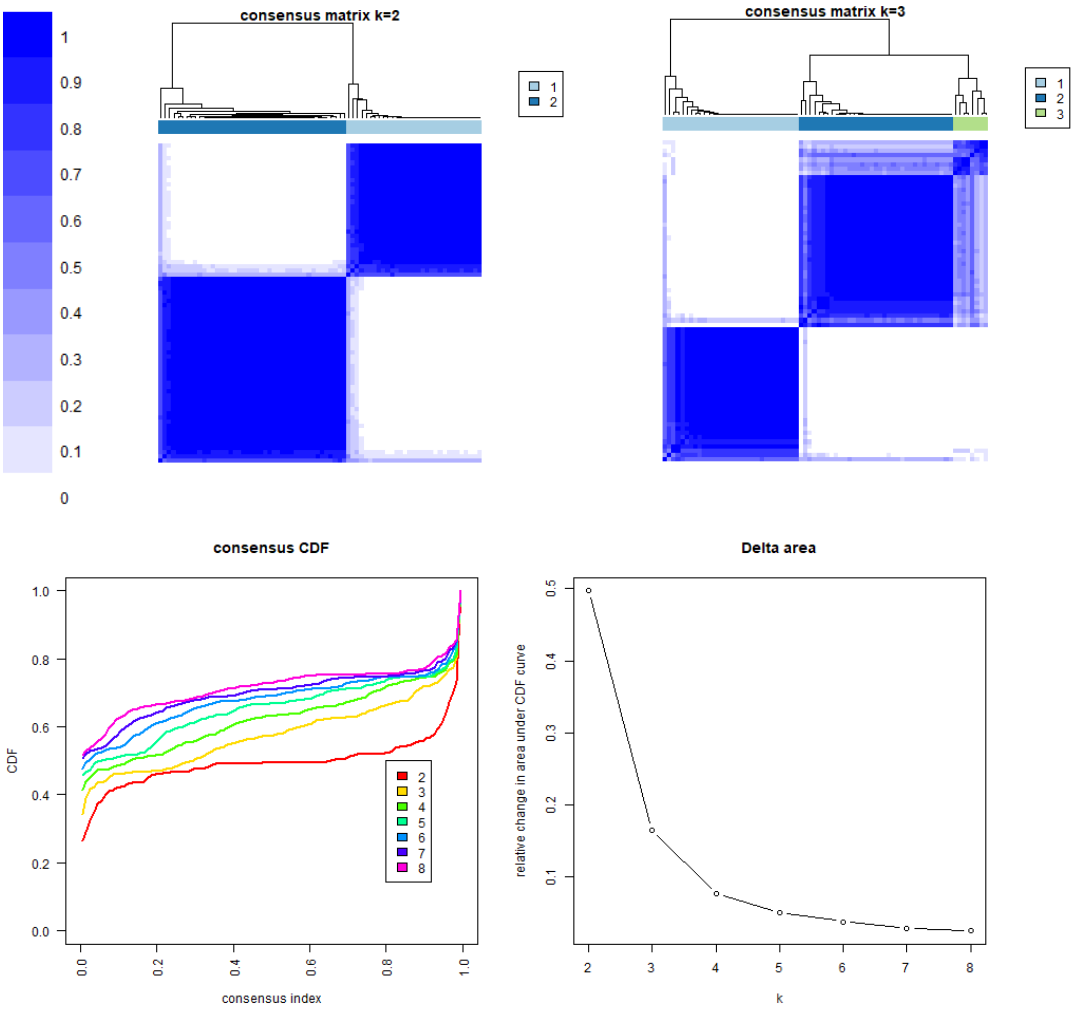
Supplemental Figure 4, related to Figure 2. MethylCIBERSORT composition analysis by PBL gDNA methylation, quantifying immune cell presence in the plasma by cell-type. Each box-plot represents the range of cell-composition (%) by cell type for all 74 SCLC patients.



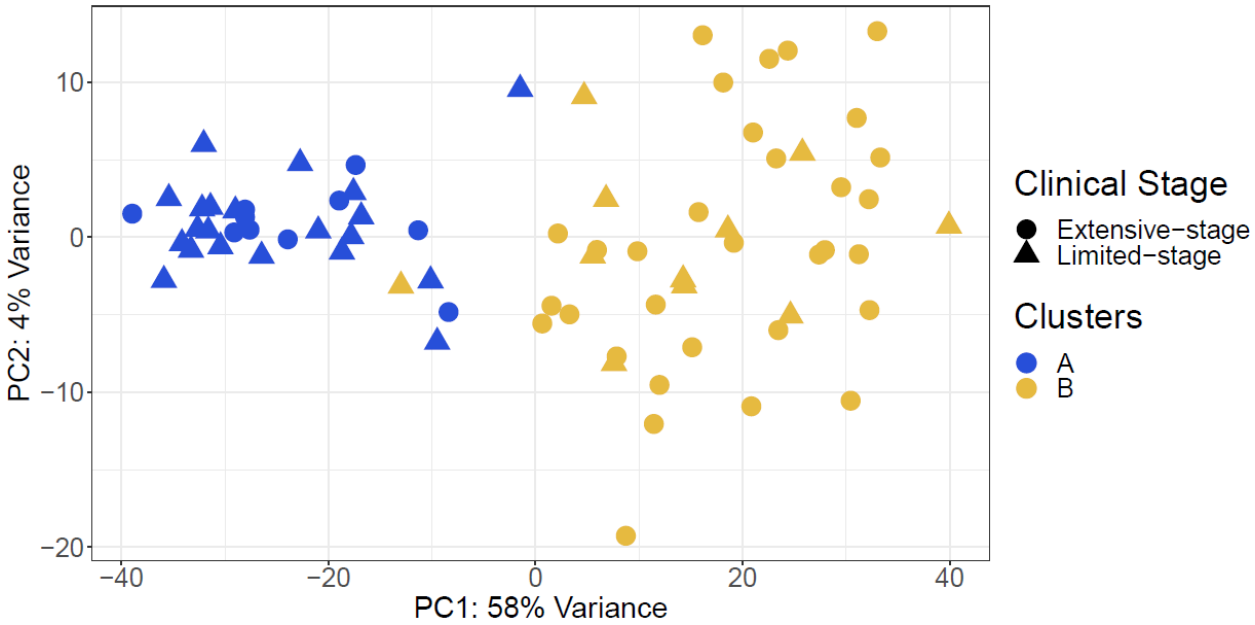
Supplemental Figure 5, related to Figure 2. MethylCIBERSORT composition analysis by PBL gDNA methylation, quantifying immune cell presence in the plasma by individual patient. Each stacked bar-plot represents each of the 74 SCLC patients.



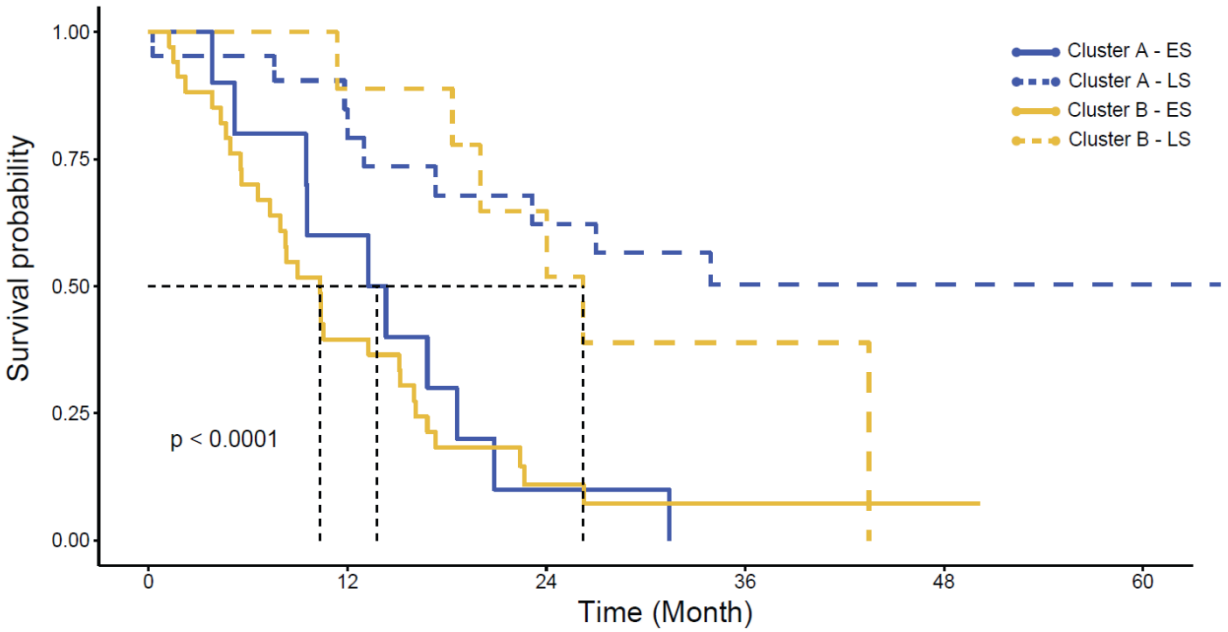
Supplemental Figure 6, related to Figure 2. Examining MethylCIBERSORT quantification of median immune cell composition (%) by methylation in SCLC total plasma and SCLC PBLs. Each dot represents an immune cell type and the x/y-axis represent the median immune cell composition (%) in either PBL genomic DNA or total plasma cfDNA for that cell type.



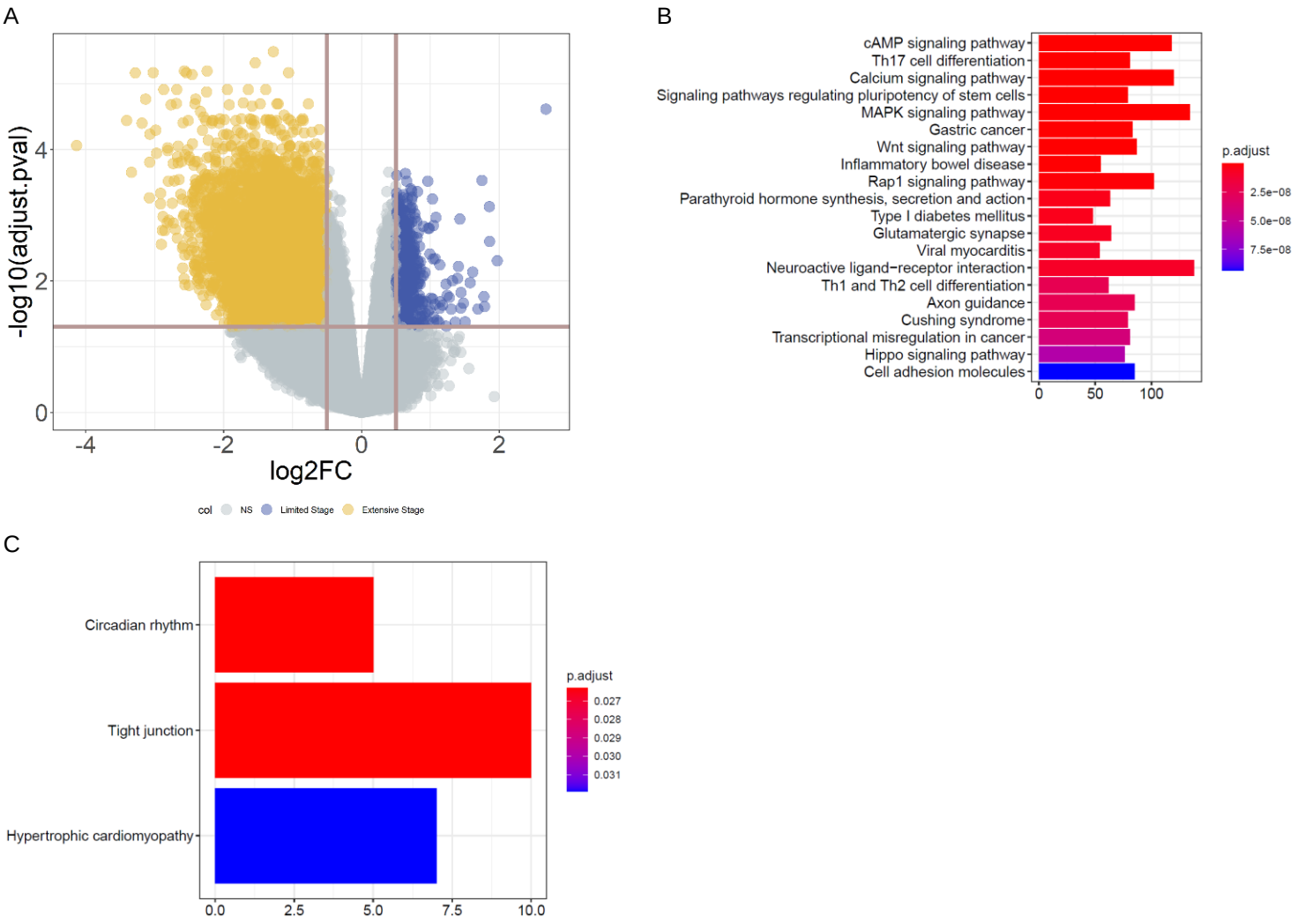
Supplemental Figure 7, related to Figure 3. Consensus clustering done on the PRIME-filtered cfDNA methylome data. Top images compare k=2 vs k=3 consensus clusters. For each k, consensus matrix plots depict consensus values on a white (less consensus) to blue (more consensus) colour scale, are ordered by the consensus clustering, which is shown as a dendrogram, and have samples' consensus clusters annotated. The plots on the bottom are empirical cumulative distribution function (CDF) plot highlights consensus distributions for each k.



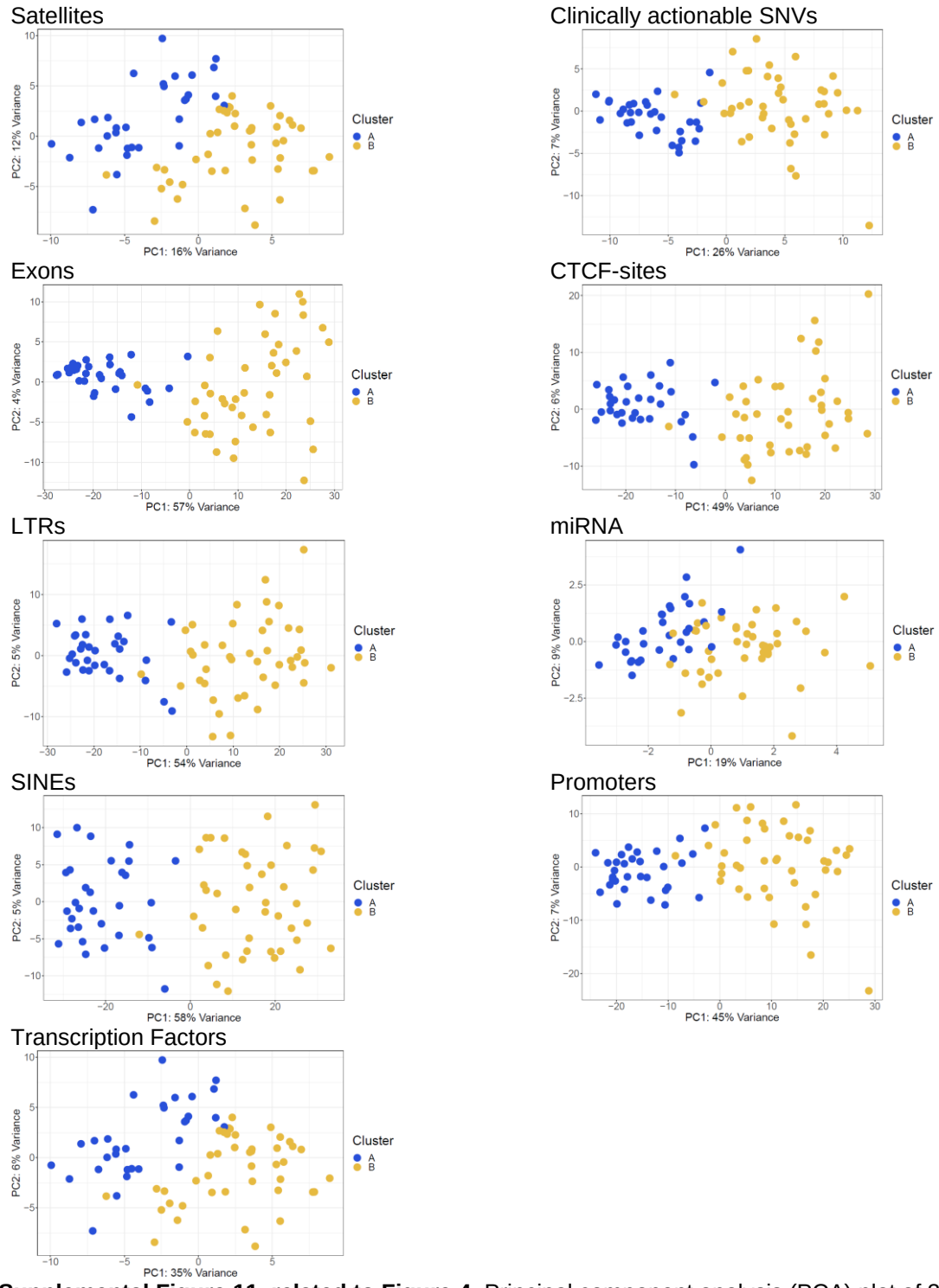
Supplemental Figure 8, related to Figure 3. Consensus clustering done on PRIME-filtered SCLC methylated cfDNA identified two clusters, A and B, with clinical stage annotated.



Supplemental Figure 9, related to Figure 3. Kaplan-Meier survival analysis on cluster A and B identified by consensus clustering stratified by either limited-stage or extensive-stage SCLC patients.



Supplementary Figure 10, related to Figure 3. The top-left figure (A) is a volcano plot of differentially methylated region (DMR) analysis between extensive-stage (ES) and limited-stage (LS). The horizontal line corresponds to p-adjusted = 0.05 and vertical lines correspond to log2 fold-change of +0.5 and -0.5. There are 907 hypermethylated DMRs in LS and 13,340 in ES. The top-right (B) and bottom-left (C) figures are KEGG pathway analysis corresponding to DMRs observed in ES and LS respectively.



Supplemental Figure 11, related to Figure 4. Principal component analysis (PCA) plot of 300bp PRIME-filtered windows subset to satellites (1,000/196,582 windows), clinically-actionable SNVs (919/196,582), exons (34,042/196,58), CTCF-sites (10,715/196,582), LTRs (25,917/196,582), miRNA (65/196,582), SINEs (64,027/196,582), promoters (11,702/196,582) and transcription factors (1,626/196,582).

Supplemental Table 1, related to Figure 1. Demographics by staging of SCLC patient cohort & non-cancer control participants. To test for association between continuous variables, the Mann-Whitney test was used and for categorical variables, Fishers exact test was used.

Small cell lung cancer patients						**Non-cancer**
label	**levels**	**Extensive-stage**	**Limited-stage**	**Total**	**p**	
Total N (%)		N = 44 (59.5)	N = 30 (40.5)	N = 74		N = 20
Age (years)	Median (IQR)	65.7 (60.8 to 74.5)	68.8 (61.9 to 75.0)	67.4 (61.3 to 75.1)	0.60	68 (70.0 to 78.0)
Sex	Female	12 (27.3)	16 (53.3)	28 (37.8)	0.03	12 (60%)
	Male	32 (72.7)	14 (46.7)	46 (62.2)		8 (40%)
Ethnicity	Asian	7 (20.0)	2 (6.9)	9 (14.1)	0.36	
	Caucasian	25 (71.4)	25 (86.2)	50 (78.1)		
	Other	3 (8.6)	2 (6.9)	5 (7.8)		
	(Missing)	9	1	10		
Smoking Status	Current smoker	20 (45.5)	13 (43.3)	33 (44.6)	0.39	20 (100%)
	Former smoker	21 (47.7)	12 (40.0)	33 (44.6)		-
	Never smoker	3 (6.8)	5 (16.7)	8 (10.8)		-
Smoking Pack Years	Median (IQR)	50.0 (30.0 to 60.0)	40.0 (30.0 to 50.0)	40.0 (30.0 to 54.2)	0.56	68 (59.7 to 91.7)
Thoracic Radiation	No	15 (34.1)	4 (13.3)	19 (25.7)	0.06	-
	Yes	29 (65.9)	26 (86.7)	55 (74.3)		
Any Systemic Therapy	No	0 (0.0)	2 (6.7)	2 (2.7)	0.16	-
	Yes	44 (100.0)	28 (93.3)	72 (97.3)		

Supplemental Table 2, related to Figure 3. Two-variable Cox-proportional hazard model of cluster A and B.

Dependent: Survival (time_os_mo, event)		all	HR (univariable)	HR (multivariable)
clusters	A	31 (100.0)	-	-
	B	43 (100.0)	2.02 (1.15-3.55, p=0.014)	1.25 (0.68-2.29, p=0.470)

Supplemental Table 3, related to Figure 3. Breakdown of Cluster A and B by Stage

	Limited-Stage	Extensive-Stage
Cluster A	21	10
Cluster B	11	32

Supplementary Table 4, related to Figure 3. Confusion matrix of a cross-validated elastic-net penalized regression model to classify LS/ES patients. The model was training using an 80/20 training/test split of the 74 samples. The balanced accuracy of the model is 85%.

	Reference	
Prediction	Extensive-Stage	Limited-Stage
Extensive-Stage	5	0
Limited-Stage	2	7