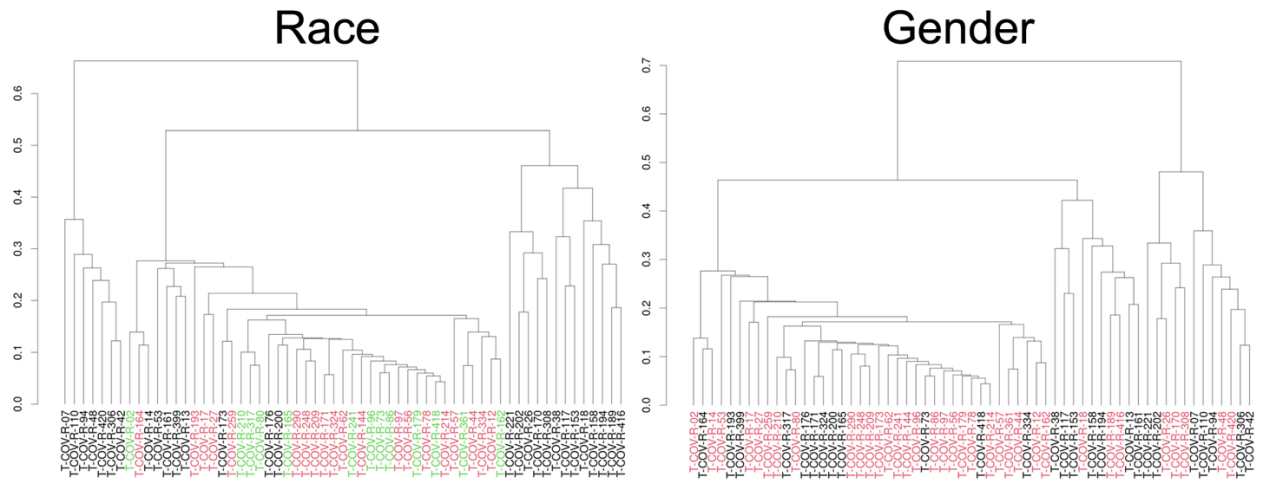
SUPPLEMENTARY INFORMATION

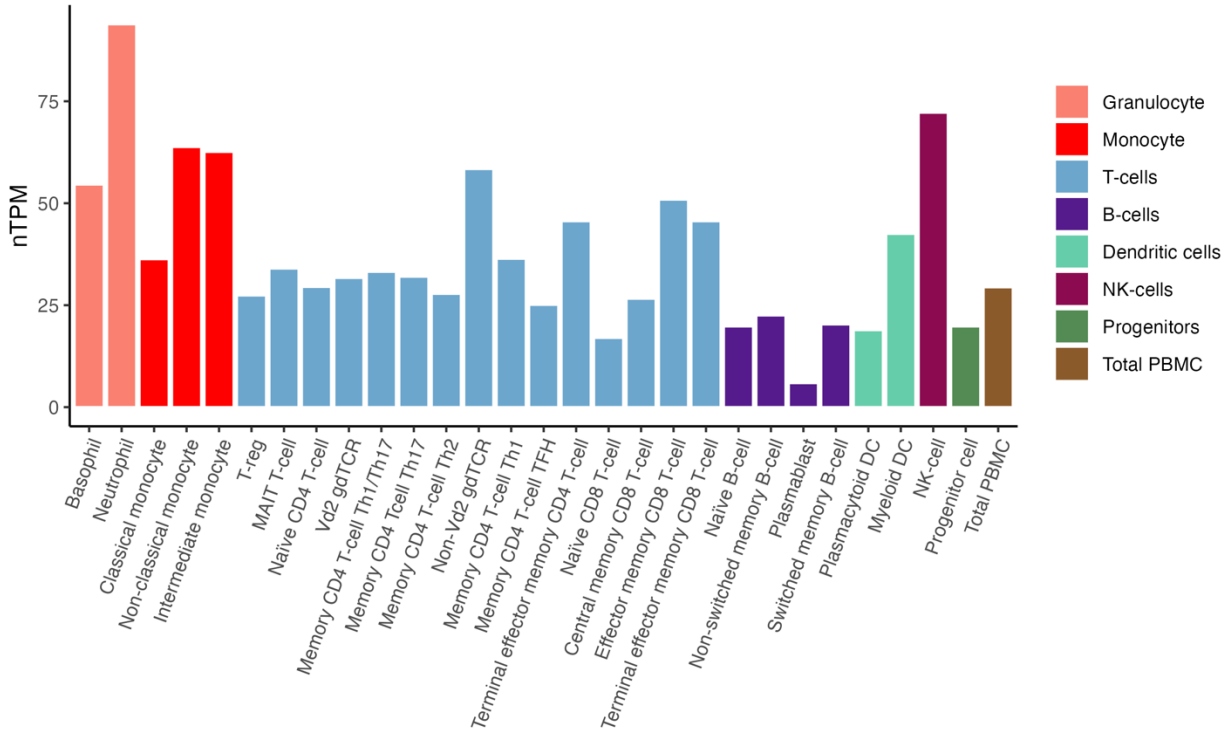
Methylation patterns of the nasal epigenome of hospitalized SARS-CoV-2 positive patients reveals insight into molecular mechanisms of COVID-19

Benjamin L. Spector, Boryana Koseva, Rebecca McLennan, Dithi Banerjee, Kamani Lankachandra, Todd Bradley, Rangaraj Selvarangan, Elin Grundberg

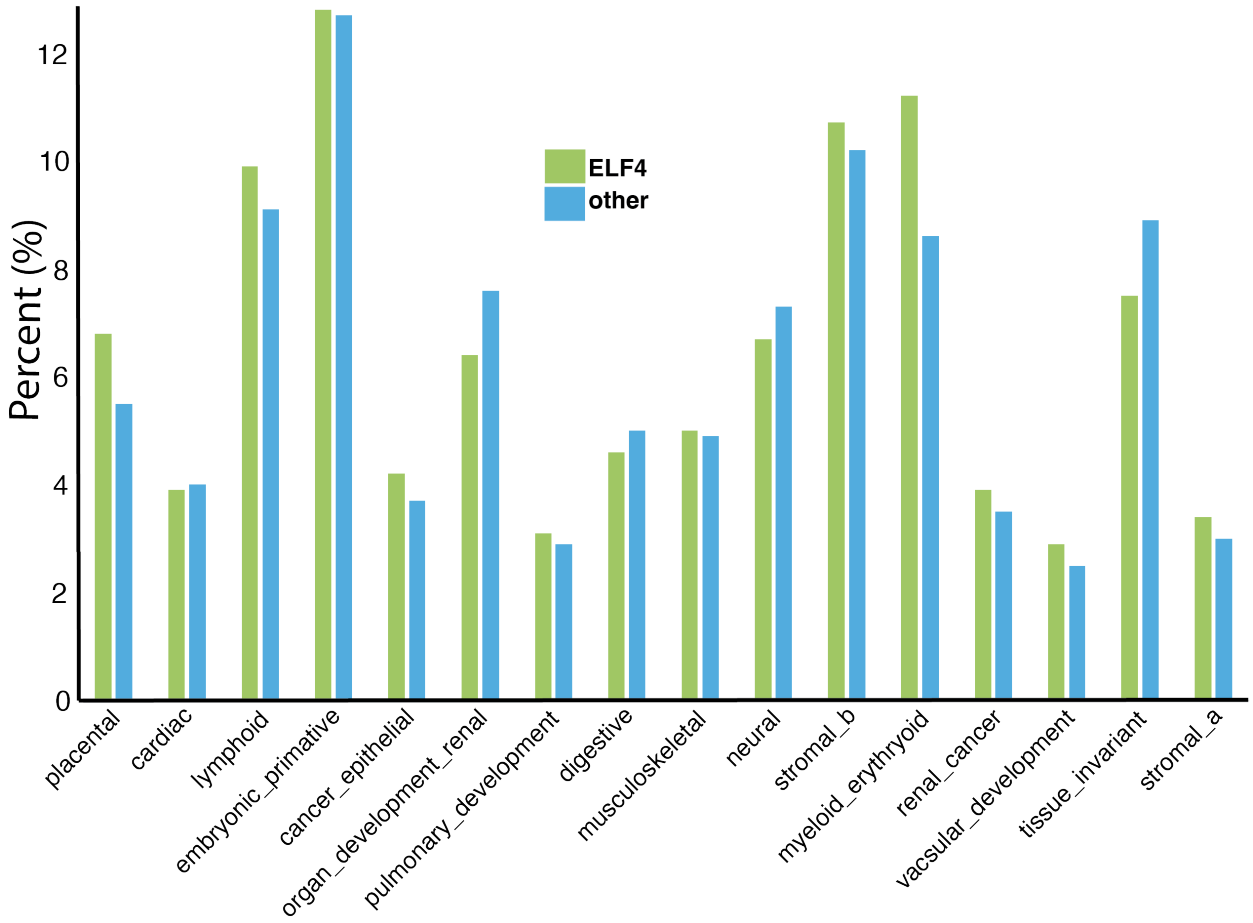
Table of contents



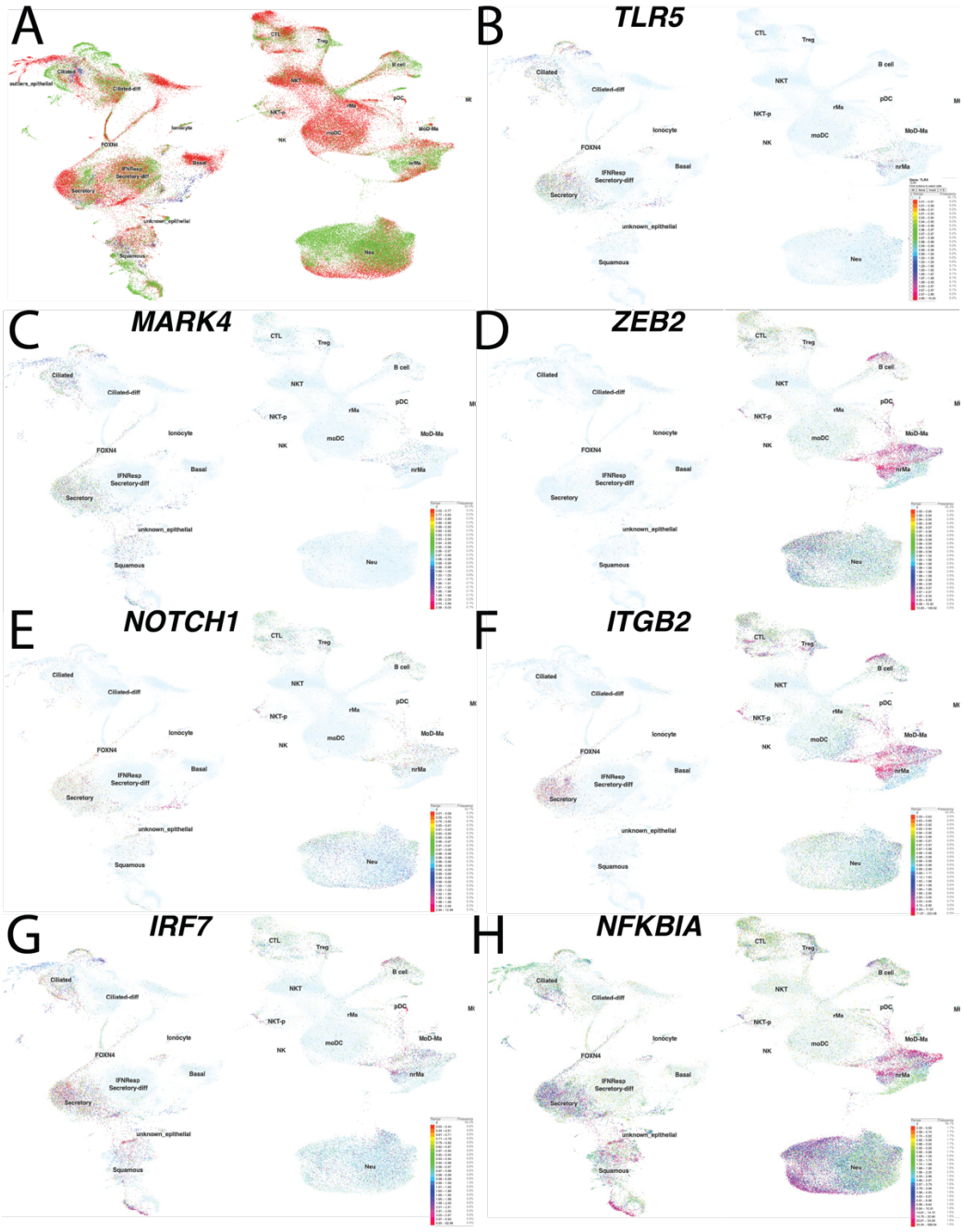
Supplemental Figure 1: Hierarchical clustering of COVID-19 positive individuals shows no clustering by race or gender. Hierarchical clustering was performed at the top 25th percentile most variable CpG methylation sites and showed no clustering structure by race (Black individuals = Black, Caucasian individuals = Green, Other races = Red), or gender (Female = Black, Male = Red).



Supplemental Figure 2: *ELF4* expressed broadly across immune cell types. Presented as normalized transcripts per million (y-axis). Granulocytes = pink, Monocytes = red, T-cells = blue, B-cells = purple, Dendritic cells = teal, NK cells = magenta, Progenitors = olive, Total PBMC = brown. Figure generated using dataset of Monaco *et al.* Image credit: Human Protein Atlas. Image available from: v23.proteinatlas.org/ENSG00000102034-ELF4/immune+cell



Supplemental Figure 3: **Distribution of *ELF4* transcription factor binding motifs across cell type-specific DHSs.** Among hypermethylated regions, a higher percentage of *ELF4* transcription factor binding motifs (blue) localize to myeloid specific DHSs as compared to hypermethylated DMRs that are not targets of the *ELF4* transcription factor (orange).



Supplemental Figure 4: CTL = Cytotoxic T lymphocytes, MC = Mast cell, moDC = Monocyte-derived dendritic cell, MoD-Ma = Monocyte-derived macrophage, Neu = Neutrophil, NK = Natural killer cell, NKT = NK T cell, NKT-p = Proliferating NKT cell, nrMA = non-resident macrophage, pDC = Plasmacytoid dendritic cell, rMA = Resident macrophage, Treg = Regulatory T cell. UMAP demonstrating differential gene expression by disease severity and cell-type in the nasopharynx. **(A)** Clustering by COVID-19 severity, Blue = Control, Red= Mild/moderate disease, Green = Critical disease. Gene expression as determined by scRNA-seq of **(B)** *TLR5,* **(C)** *MARK4*, **(D)** *ZEB2*, **(E)** *NOTCH1*, **(F)** *ITGB2*, **(G)** *IRF7*, **(H)** *NFKBIA.* Figure generated using the dataset of Chua *et al.* and the UCSC Cell Browser (https://covid-airways.cells.ucsc.edu) [44, 65].

Supplemental Table 1: Percent UMR and LMR overlapping regulatory elements by cell type in COVID-19 positive (severe + mild) and negative individuals.

	Overlapping single or multiple cell types				Overlapping single cell type only			
Cell Type	**COVID-19 Status**		[a]**Fold Difference**	[b]***p***	**COVID-19 Status**		[a]**Fold Difference**	[b]***p***
	Positive (%)	Negative (%)			Positive (%)	Negative (%)		
Immune								
UMR	64	62	1.0	0.0054	8	4	1.9	0.069
LMR	46	25	1.9	$< 2.2 \times 10^{-16}$	31	8	4.1	0.0037
Lymphoid								
UMR	49	48	1.0	0.051	5	3	1.8	0.21
LMR	24	15	1.6	$< 2.2 \times 10^{-16}$	11	4	2.6	5.6×10^{-16}
Myeloid								
UMR	33	32	1.0	0.0015	3	1	2.5	0.26
LMR	28	12	2.4	$< 2.2 \times 10^{-16}$	20	3	6.3	$< 2.2 \times 10^{-16}$
Pulmonary								
UMR	15	17	0.91	7.0×10^{-5}	0.6	0.7	0.83	1.0
LMR	7	10	0.66	$< 2.2 \times 10^{-16}$	2	3	0.27	$< 2.2 \times 10^{-16}$
Epithelial								
UMR	13	17	0.76	$< 2.2 \times 10^{-16}$	0.6	3	0.18	0.032
LMR	8	19	0.43	$< 2.2 \times 10^{-16}$	3	11	0.22	$< 2.2 \times 10^{-16}$

[a]Fold difference between COVID-19 positive *vs* negative patients
[b]*p*-value reflects chi-square examining COVID-19 positive *vs* negative patients

Supplemental Table 2: Percent UMR and LMR overlapping regulatory elements by cell type in severe and mild COVID-19.

	Overlapping single or multiple cell types				Overlapping single cell type only			
Cell Type	**COVID-19 Status**		[a]**Fold Difference**	[b]***p***	**COVID-19 Status**		[a]**Fold Difference**	[b]***p***
	Severe (%)	Mild (%)			Severe (%)	Mild (%)		
Immune								
UMR	64	64	1.0	0.84	9	9	1.1	0.87
LMR	44	46	0.97	2.5×10^{-6}	28	31	0.90	9.3×10^{-8}
Lymphoid								
UMR	49	49	1.0	0.86	6	6	1.1	0.89
LMR	24	24	1.0	0.74	10	11	0.96	0.25
Myeloid								
UMR	34	33	1.0	0.55	3	3	1.0	1.0
LMR	26	28	0.94	1.8×10^{-9}	18	20	0.87	1.1×10^{-7}
Pulmonary								
UMR	15	15	1.0	0.89	0.3	0.3	0.95	1.0
LMR	7	7	0.99	0.59	1	2	0.93	0.50
Epithelial								
UMR	13	13	1.0	0.45	2	0.6	2.8	0.32
LMR	9	8	1.1	4.8×10^{-8}	4	3	1.4	5.1×10^{-6}

[a]Fold difference between severe *vs* mild COVID-19 patients
[b]*p*-value reflects chi-square examining severe *vs* mild COVID-19 patients

Supplemental Table 7: PI3k/Akt pathway associated DMRs. Methylation difference reflects percent difference in mean regional methylation between hospitalized versus non-hospitalized COVID-19 subjects.

Gene	Chromosome	Start	Stop	Methylation difference (%)	*q*-value
AKT1	chr14	104796001	104796500	-18.38	2.22×10^{-22}
ISG15	chr1	1013751	1014250	-32.27	2.49×10^{-35}
ZEB2	chr2	144524251	144524750	-24.39	2.52×10^{-32}
SNAI1	chr20	49983251	49983750	-28.98	6.11×10^{-16}
TLR5	chr1	223133001	223133750	-15.09	1.22×10^{-16}
MARK4	chr19	45248251	45248750	-10.08	2.33×10^{-19}
NOTCH1	chr9	136549501	136550000	-20.24	2.23×10^{-16}
ITGB2	chr21	44923751	44924500	-22.09	1.08×10^{-33}
IRF7	chr11	615001	615500	-17.76	1.43×10^{-33}
NFKBIA	chr14	35402001	35402500	-15.80	1.02×10^{-16}

Supplemental Table 8: Shared differentially methylated genes in severe vs mild COVID-19 between our original dataset (Spector *et al.*) and genes noted by Pietzner *et al.* [58] to be regulated/co-expressed with *ELF5*.

Gene	Chromosome	Start	Stop	Methylation difference (%)	*q*-value
C1orf116	chr1	207031251	207031750	34.87	7.85×10^{-07}
VILL	chr3	37990251	37990750	17.54	3.61×10^{-18}
RHOH	chr4	40191751	40192250	29.49	2.50×10^{-10}
CCNI	chr4	77080751	77081250	13.20	2.87×10^{-08}
ANXA3	chr4	78553251	78553750	21.34	7.05×10^{-09}
PLAC8	chr4	83118751	83119250	30.91	4.96×10^{-07}
MEPE	chr4	87836501	87837000	13.73	3.61×10^{-07}
ALPK1	chr4	112300251	112300750	32.30	4.67×10^{-10}
ELOVL5	chr6	53346751	53347250	21.13	6.78×10^{-14}
PERP	chr6	138105751	138106250	18.51	4.76×10^{-07}
IFRD1	chr7	112448751	112449250	28.23	1.09×10^{-07}
PCM1	chr8	17921501	17922000	11.98	1.47×10^{-07}
NDRG1	chr8	133298251	133298750	11.05	3.19×10^{-06}
LY6D	chr8	142782251	142782750	22.30	6.48×10^{-14}
ANK3	chr10	60388751	60389250	12.02	4.12×10^{-07}
TRIM29	chr11	120134501	120135000	23.90	2.46×10^{-07}
NABP2	chr12	56223501	56224000	11.51	3.27×10^{-07}
SEMA4B	chr15	90203751	90204250	16.94	5.98×10^{-06}
ZKSCAN2	chr16	25255751	25256250	12.94	6.69×10^{-07}
GGT6	chr17	4560251	4560750	31.07	7.71×10^{-11}
ZNF750	chr17	82836001	82836500	24.21	8.27×10^{-17}
ZNF440	chr19	11815251	11815750	15.38	1.36×10^{-09}
PTK6	chr20	63534751	63535250	14.78	3.17×10^{-17}