SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table 1. Study cohort and demographics

Participant	Sex	Age (years)	CF or control	scRNA-seq	Totalseq ADT
A1_Ctrl	M	3.00	Control	Y	
B1_CF	M	2.99	CF	Y	
C1_CF	M	2.99	CF	Y	
D1_CF	M	3.03	CF	Y	
E2_CF	F	5.99	CF	Y	Y
F2_CF	F	6.02	CF	Y	Y
G2_CF	F	4.91	CF	Y	Y
H2_CF	F	5.89	CF	Y	Y
I2_CF	F	5.93	CF	Y	Y
J2_CF	M	5.05	CF	Y	Y
K2_CF	M	4.92	CF	Y	Y
L2_CF	M	5.95	CF	Y	Y

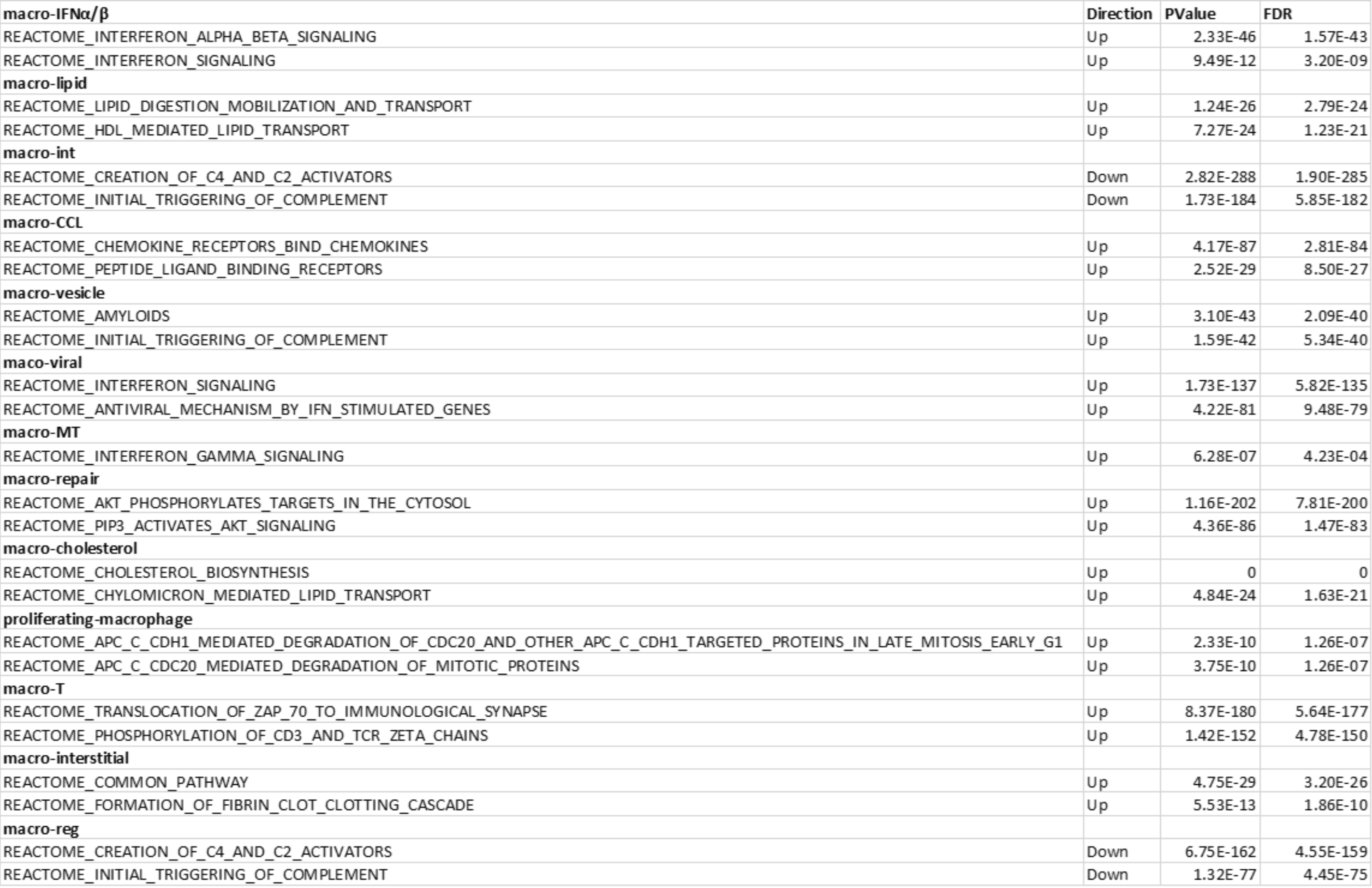
Supplementary Table 2. Concordance between HTO and genetic assignments.

HTO / genetic donor	donor_A	donor_B	donor_C	donor_D	donor_E	donor_F	donor_G	donor_H	Doublet	Unknown	Total
HTO_1	0	1620	3	1	1	0	9	0	16	15	1665
HTO_2	0	2	1	1	1	0	2763	2	49	30	2849
HTO_3	1	1	1	0	3267	0	0	2	17	8	3297
HTO_4	1	0	1663	0	0	0	0	0	7	2	1673
HTO_5	0	0	1	2	2	2913	0	1	30	8	2957
HTO_6	0	0	1	1861	0	1	0	1	8	6	1878
HTO_7	0	1	0	1	0	0	1	2310	14	6	2333
HTO_8	2274	1	3	4	2	1	3	2	30	81	2401
Doublet	181	163	248	216	380	330	273	281	1581	29	3682
Unknown	133	86	482	227	178	47	63	155	384	338	2093
Total	2590	1874	2403	2313	3831	3292	3112	2754	2136	523	24828

Supplementary Table 3. Flow cytometry antibody cocktail

Surface Marker	**Fluorophore**	**Clone**	**Final Dilution**
CD16	BUV395	3G8	1:400
SIRPα	BUV563	SE5A5	1:100
CD47	BUV737	B6H12	1:00
CD206	BV421	G10F5	1:50
HLADR	V500	G46-6	1:100
CD19	BV605	SJ25C1	1:100
CD8	BV650	RPA-T8	1:100
CD45	BV711	HI30	1:50
CD14	BV786	M5E2	1:25
EPCAM	BB515	EBA-1	1:100
CD3	FITC	SK7	1:50
PD1	BB700	EH12.1	1:100
CD66b	PE	19.2	1:100
CD15	PECF594	W6D3	1:100
CD11c	PECy7	B-ly6	1:50
CD63	A647	H5C6	1:50
CD4	A700	RPA-T4	1:25

Supplementary Table 4. REACTOME analysis of macrophage subclusters.



macro-IFNα/β	**Direction**	**PValue**	**FDR**
REACTOME_INTERFERON_ALPHA_BETA_SIGNALING	Up	2.33E-46	1.57E-43
REACTOME_INTERFERON_SIGNALING	Up	9.49E-12	3.20E-09
macro-lipid			
REACTOME_LIPID_DIGESTION_MOBILIZATION_AND_TRANSPORT	Up	1.24E-26	2.79E-24
REACTOME_HDL_MEDIATED_LIPID_TRANSPORT	Up	7.27E-24	1.23E-21
macro-int			
REACTOME_CREATION_OF_C4_AND_C2_ACTIVATORS	Down	2.82E-288	1.90E-285
REACTOME_INITIAL_TRIGGERING_OF_COMPLEMENT	Down	1.73E-184	5.85E-182
macro-CCL			
REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES	Up	4.17E-87	2.81E-84
REACTOME_PEPTIDE_LIGAND_BINDING_RECEPTORS	Up	2.52E-29	8.50E-27
macro-vesicle			
REACTOME_AMYLOIDS	Up	3.10E-43	2.09E-40
REACTOME_INITIAL_TRIGGERING_OF_COMPLEMENT	Up	1.59E-42	5.34E-40
maco-viral			
REACTOME_INTERFERON_SIGNALING	Up	1.73E-137	5.82E-135
REACTOME_ANTIVIRAL_MECHANISM_BY_IFN_STIMULATED_GENES	Up	4.22E-81	9.48E-79
macro-MT			
REACTOME_INTERFERON_GAMMA_SIGNALING	Up	6.28E-07	4.23E-04
macro-repair			
REACTOME_AKT_PHOSPHORYLATES_TARGETS_IN_THE_CYTOSOL	Up	1.16E-202	7.81E-200
REACTOME_PIP3_ACTIVATES_AKT_SIGNALING	Up	4.36E-86	1.47E-83
macro-cholesterol			
REACTOME_CHOLESTEROL_BIOSYNTHESIS	Up	0	0
REACTOME_CHYLOMICRON_MEDIATED_LIPID_TRANSPORT	Up	4.84E-24	1.63E-21
proliferating-macrophage			
REACTOME_APC_C_CDH1_MEDIATED_DEGRADATION_OF_CDC20_AND_OTHER_APC_C_CDH1_TARGETED_PROTEINS_IN_LATE_MITOSIS_EARLY_G1	Up	2.33E-10	1.26E-07
REACTOME_APC_C_CDC20_MEDIATED_DEGRADATION_OF_MITOTIC_PROTEINS	Up	3.75E-10	1.26E-07
macro-T			
REACTOME_TRANSLOCATION_OF_ZAP_70_TO_IMMUNOLOGICAL_SYNAPSE	Up	8.37E-180	5.64E-177
REACTOME_PHOSPHORYLATION_OF_CD3_AND_TCR_ZETA_CHAINS	Up	1.42E-152	4.78E-150
macro-interstitial			
REACTOME_COMMON_PATHWAY	Up	4.75E-29	3.20E-26
REACTOME_FORMATION_OF_FIBRIN_CLOT_CLOTTING_CASCADE	Up	5.53E-13	1.86E-10
macro-reg			
REACTOME_CREATION_OF_C4_AND_C2_ACTIVATORS	Down	6.75E-162	4.55E-159
REACTOME_INITIAL_TRIGGERING_OF_COMPLEMENT	Down	1.32E-77	4.45E-75

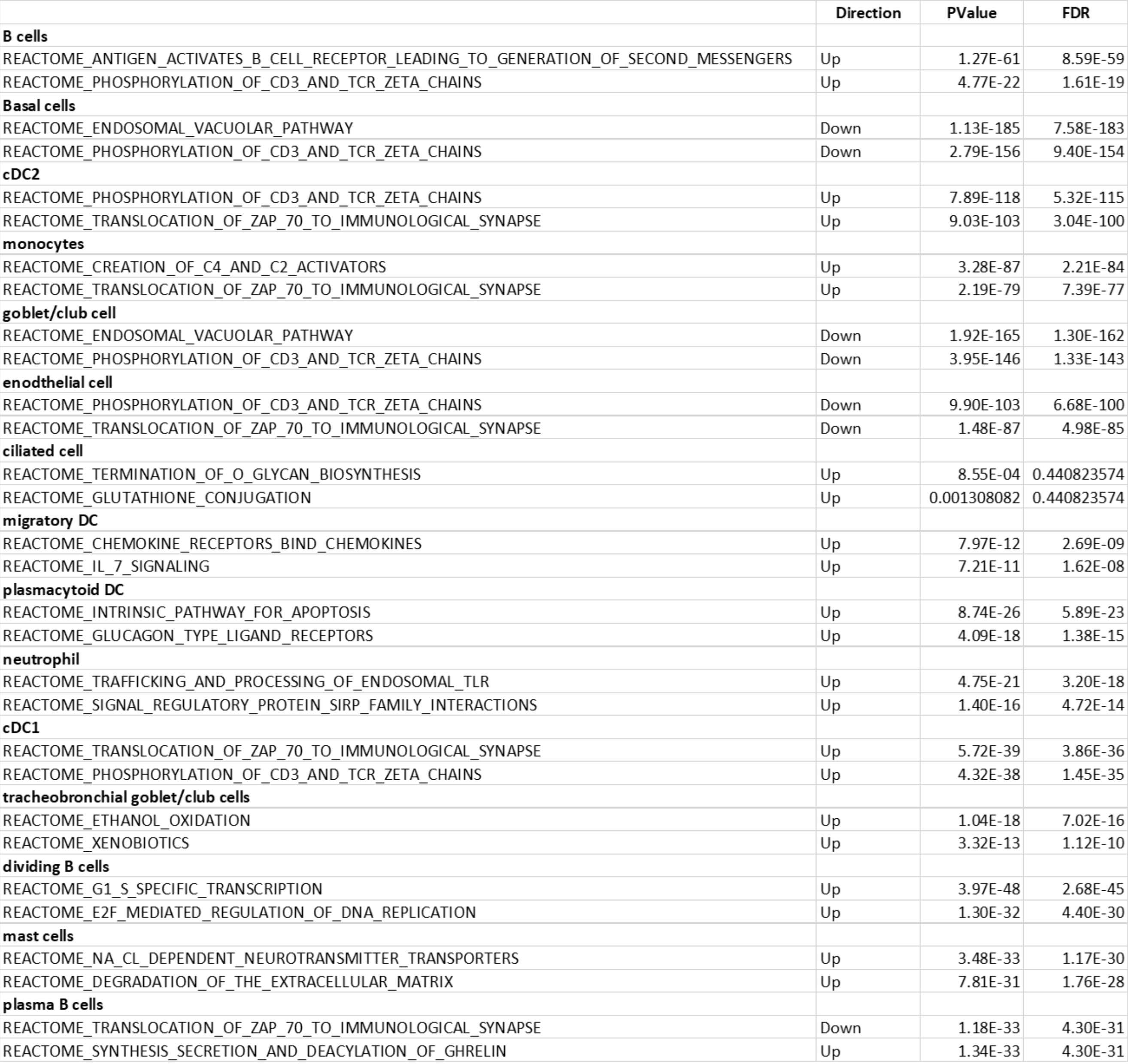
*the two most significant (FDR) pathways per functional subcluster are presented

Supplementary Table 5. REACTOME analysis of T/NK subclusters

CD4 T	**Direction**	**PValue**	**FDR**
REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES	Down	1.12E-06	7.56E-04
REACTOME_IMMUNOREGULATORY_INTERACTIONS_BETWEEN_A_LYMPHOID_AND_A_NON_LYMPHOID_CELL	Down	0.01666626	0.999955616
CD8 Trm			
REACTOME_IMMUNOREGULATORY_INTERACTIONS_BETWEEN_A_LYMPHOID_AND_A_NON_LYMPHOID_CELL	Up	1.46E-41	9.82E-39
REACTOME_TRANSLOCATION_OF_ZAP_70_TO_IMMUNOLOGICAL_SYNAPSE	Up	2.20E-29	7.42E-27
innate lymphocyte			
REACTOME_DEADENYLATION_OF_MRNA	Up	0.00189422	0.690751972
REACTOME_ACTIVATION_OF_THE_MRNA_UPON_BINDING_OF_THE_CAP_BINDING_COMPLEX_AND_EIFS_AND_SUBSEQUENT_BINDING_TO_43S	Up	0.00345795	0.690751972
CD8 GZMK			
REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES	Up	4.60E-15	3.10E-12
REACTOME_APOBEC3G_MEDIATED_RESISTANCE_TO_HIV1_INFECTION	Up	1.82E-14	6.14E-12
NK T cells			
REACTOME_IMMUNOREGULATORY_INTERACTIONS_BETWEEN_A_LYMPHOID_AND_A_NON_LYMPHOID_CELL	Up	1.48E-41	9.98E-39
REACTOME_OTHER_SEMAPHORIN_INTERACTIONS	Up	3.88E-12	1.31E-09
NK cells			
REACTOME_TRANSLOCATION_OF_ZAP_70_TO_IMMUNOLOGICAL_SYNAPSE	Down	8.52E-71	5.74E-68
REACTOME_PHOSPHORYLATION_OF_CD3_AND_TCR_ZETA_CHAINS	Down	1.56E-60	5.26E-58
γδ T cells			
REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES	Up	2.95E-09	1.99E-06
REACTOME_APOBEC3G_MEDIATED_RESISTANCE_TO_HIV1_INFECTION	Up	1.34E-07	4.50E-05
CD4 Treg			
REACTOME_CTLA4_INHIBITORY_SIGNALING	Up	1.55E-18	1.05E-15
REACTOME_IL_RECEPTOR_SHC_SIGNALING	Up	6.32E-15	2.13E-12
CD4 NFκB			
REACTOME_NFKB_IS_ACTIVATED_AND_SIGNALS_SURVIVAL	Up	1.62E-19	1.09E-16
REACTOME_RIP_MEDIATED_NFKB_ACTIVATION_VIA_DAI	Up	8.19E-14	2.76E-11
CD4 IFN			
REACTOME_INTERFERON_ALPHA_BETA_SIGNALING	Up	0	0
REACTOME_INTERFERON_SIGNALING	Up	1.33E-106	2.99E-104
proliferating NK/T			
REACTOME_G1_S_SPECIFIC_TRANSCRIPTION	Up	1.50E-261	1.01E-258
REACTOME_E2F_MEDIATED_REGULATION_OF_DNA_REPLICATION	Up	3.01E-123	1.01E-120
CD4 TFH			
REACTOME_PD1_SIGNALING	Up	3.17E-36	2.14E-33
REACTOME_IL_6_SIGNALING	Up	2.62E-35	8.84E-33

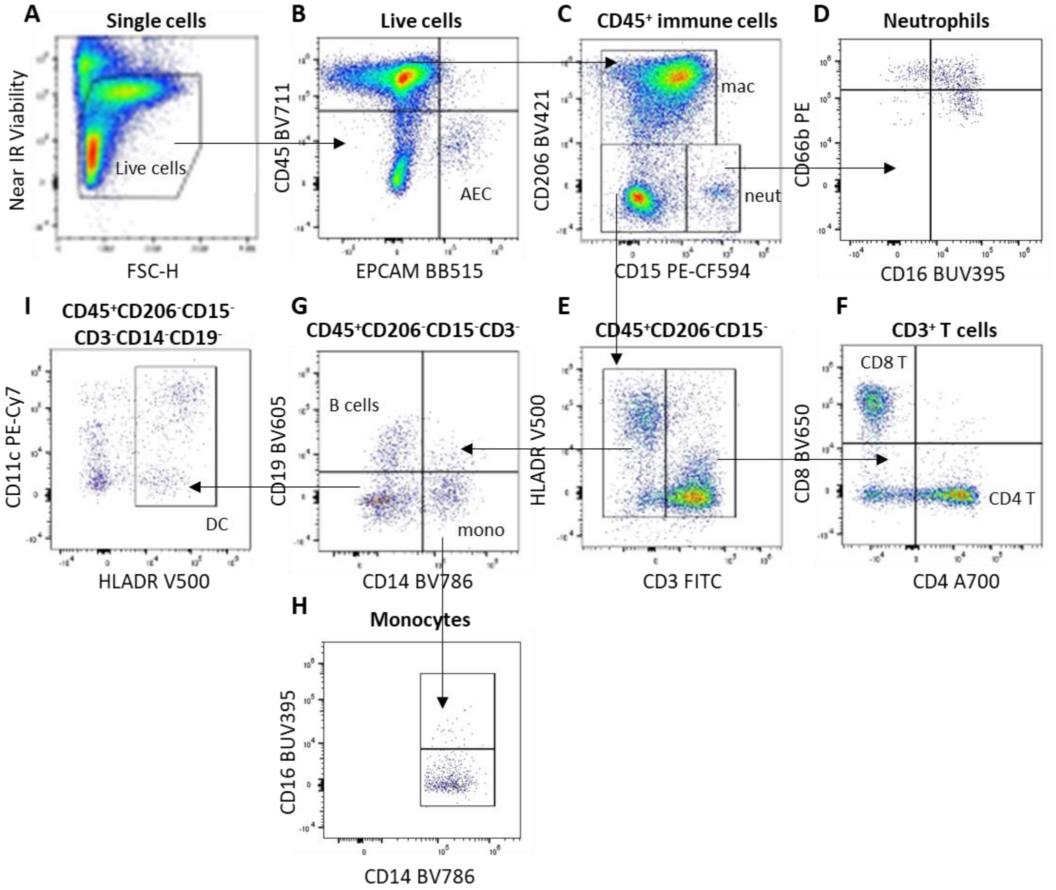
*the two most significant (FDR) pathways per functional subcluster are presented

Supplementary Table 6. REACTOME analysis of myeloid, B, and epithelial cell clusters

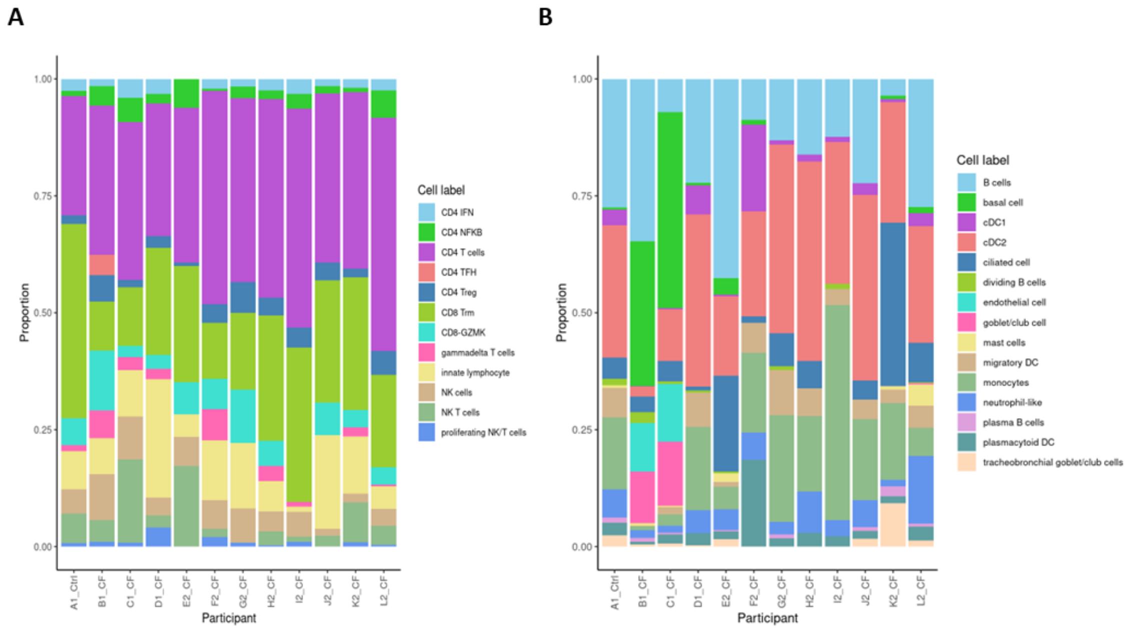


	Direction	PValue	FDR
B cells			
REACTOME_ANTIGEN_ACTIVATES_B_CELL_RECEPTOR_LEADING_TO_GENERATION_OF_SECOND_MESSENGERS	Up	1.27E-61	8.59E-59
REACTOME_PHOSPHORYLATION_OF_CD3_AND_TCR_ZETA_CHAINS	Up	4.77E-22	1.61E-19
Basal cells			
REACTOME_ENDOSOMAL_VACUOLAR_PATHWAY	Down	1.13E-185	7.58E-183
REACTOME_PHOSPHORYLATION_OF_CD3_AND_TCR_ZETA_CHAINS	Down	2.79E-156	9.40E-154
cDC2			
REACTOME_PHOSPHORYLATION_OF_CD3_AND_TCR_ZETA_CHAINS	Up	7.89E-118	5.32E-115
REACTOME_TRANSLOCATION_OF_ZAP_70_TO_IMMUNOLOGICAL_SYNAPSE	Up	9.03E-103	3.04E-100
monocytes			
REACTOME_CREATION_OF_C4_AND_C2_ACTIVATORS	Up	3.28E-87	2.21E-84
REACTOME_TRANSLOCATION_OF_ZAP_70_TO_IMMUNOLOGICAL_SYNAPSE	Up	2.19E-79	7.39E-77
goblet/club cell			
REACTOME_ENDOSOMAL_VACUOLAR_PATHWAY	Down	1.92E-165	1.30E-162
REACTOME_PHOSPHORYLATION_OF_CD3_AND_TCR_ZETA_CHAINS	Down	3.95E-146	1.33E-143
enodthelial cell			
REACTOME_PHOSPHORYLATION_OF_CD3_AND_TCR_ZETA_CHAINS	Down	9.90E-103	6.68E-100
REACTOME_TRANSLOCATION_OF_ZAP_70_TO_IMMUNOLOGICAL_SYNAPSE	Down	1.48E-87	4.98E-85
ciliated cell			
REACTOME_TERMINATION_OF_O_GLYCAN_BIOSYNTHESIS	Up	8.55E-04	0.440823574
REACTOME_GLUTATHIONE_CONJUGATION	Up	0.001308082	0.440823574
migratory DC			
REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES	Up	7.97E-12	2.69E-09
REACTOME_IL_7_SIGNALING	Up	7.21E-11	1.62E-08
plasmacytoid DC			
REACTOME_INTRINSIC_PATHWAY_FOR_APOPTOSIS	Up	8.74E-26	5.89E-23
REACTOME_GLUCAGON_TYPE_LIGAND_RECEPTORS	Up	4.09E-18	1.38E-15
neutrophil			
REACTOME_TRAFFICKING_AND_PROCESSING_OF_ENDOSOMAL_TLR	Up	4.75E-21	3.20E-18
REACTOME_SIGNAL_REGULATORY_PROTEIN_SIRP_FAMILY_INTERACTIONS	Up	1.40E-16	4.72E-14
cDC1			
REACTOME_TRANSLOCATION_OF_ZAP_70_TO_IMMUNOLOGICAL_SYNAPSE	Up	5.72E-39	3.86E-36
REACTOME_PHOSPHORYLATION_OF_CD3_AND_TCR_ZETA_CHAINS	Up	4.32E-38	1.45E-35
tracheobronchial goblet/club cells			
REACTOME_ETHANOL_OXIDATION	Up	1.04E-18	7.02E-16
REACTOME_XENOBIOTICS	Up	3.32E-13	1.12E-10
dividing B cells			
REACTOME_G1_S_SPECIFIC_TRANSCRIPTION	Up	3.97E-48	2.68E-45
REACTOME_E2F_MEDIATED_REGULATION_OF_DNA_REPLICATION	Up	1.30E-32	4.40E-30
mast cells			
REACTOME_NA_CL_DEPENDENT_NEUROTRANSMITTER_TRANSPORTERS	Up	3.48E-33	1.17E-30
REACTOME_DEGRADATION_OF_THE_EXTRACELLULAR_MATRIX	Up	7.81E-31	1.76E-28
plasma B cells			
REACTOME_TRANSLOCATION_OF_ZAP_70_TO_IMMUNOLOGICAL_SYNAPSE	Down	1.18E-33	4.30E-31
REACTOME_SYNTHESIS_SECRETION_AND_DEACYLATION_OF_GHRELIN	Up	1.34E-33	4.30E-31

*the two most significant (FDR) pathways per functional subcluster are presented



Supplementary Figure 1. Representative flow cytometry manual gating of BAL cells. **(A)** Live cells were negative for near IR viability dye. **(B)** Within the live single cell gate, immune cells were CD45^{+} and airway epithelial cells (AEC) were CD45^{-}EPCAM^{+}. **(C)** Macrophages were identified within the CD45^{+} immune cell gate by expression of CD206, and neutrophils were CD206^{-}CD15^{+}. **(D)** Within the neutrophil populations, three subtypes were identified: CD16^{-}CD66b^{high}, CD16^{+}CD66b^{high} and CD16^{+}CD66b$^{low.}$ **(E)** Within the CD45^{+}CD206^{-}CD15^{-} population, T cells were CD3^{+} and these were then identified as **(F)** CD4^{+} or CD8^{+} T cells. **(G)** Within the CD45^{+}CD206^{-}CD15^{-}CD3^{-} gate, monocytes were identified based on CD14 expression and B cells were CD14^{-}CD19^{+}. **(H)** A small proportion of monocytes were positive for CD16. **(I)** Finally, cells negative for all preceding markers (excluding CD45) were identified as DCs based on positive staining for HLADR.



Supplementary Figure 2. Proportions of subpopulations in each individual identified by a sub-clustering analysis of **(A)** T/NK cells and **(B)** all other cells (not macrophages, not T/NK cells). Data is expressed as a proportion of all cells in the parent clusters.