

Online Supplement

Methods

Sample preparation and sequencing

PBMC and granulocyte samples were counted using the Countess™ Automated Cell Counter (Invitrogen, Carlsbad, CA) with trypan blue for evaluating the cell viability. PBMC and granulocyte samples were mixed at a 1:1.2 ratio. Mixed samples were prepared for single-cell RNA sequencing using the Chromium Single-Cell 5' Reagent version 1.1 kit (PN 1000165) and Chromium Single-Cell Controller (10x Genomics, CA). Cells were subsequently loaded into the Single-Cell G Chip (PN 1000120) for gel bead-in-emulsion (GEM) generation and barcoding, targeting for a cell recovery of 10,000 cells per sample. Reverse transcription, RT clean-up, and cDNA amplification were performed to isolate and amplify cDNA for downstream 5' gene or enriched V(D)J library construction according to the manufacturer's protocol. Libraries were constructed using the Chromium Single-Cell 5' reagent kit v1.1 (PN 1000165) and 5' Library Construction Kit (PN 1000020) according to the manufacturer's protocol. For sequencing, the library concentration was assessed using a Qubit 2.0 Fluorometer and dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's protocol. Single-cell 5' gene expression libraries were sequenced using an Illumina NovaSeq 6000 system following standard protocols for a read length of 2 x 100 bp.

Supplementary Table 1. Effect of biologic therapy on inflammatory markers

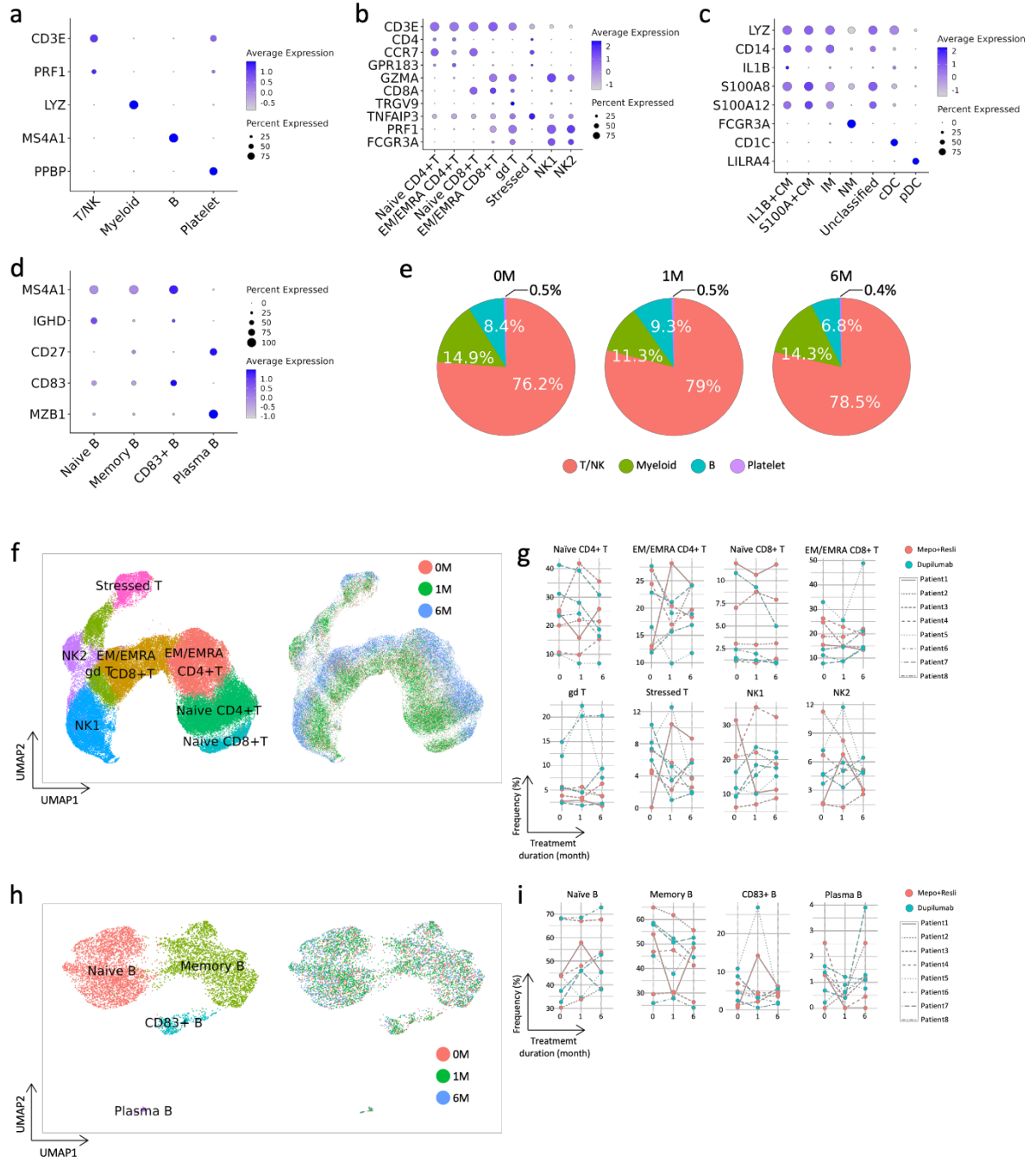
No.	Age/ sex	Drug	FeNO (ppb)			Sputum eosinophil (%)			Blood eosinophils, absolute count (proportion)			Blood neutrophils, absolute count (proportion)		
			0M	1M	6M	0M	1M	6M	0M	1M	6M	0M	1M	6M
1	43/F	Mepolizumab	.	.	155	76	47	.	2856 (23.8)	103 (1.9)	30 (0.4)	6372 (53.1)	3775 (69.9)	5472 (72.0)
2	74/F	Mepolizumab	49	41	29	90	5	.	682 (12.4)	70 (1.4)	50 (0.9)	2277 (41.4)	2510 (50.2)	2576 (46.0)
3	50/F	Reslizumab	86	75	94	1	0	.	509 (7.6)	50 (0.6)	46 (0.6)	3551 (53)	5354 (64.5)	4736 (61.5)
4	62/F	Reslizumab	57	70	79	47	31	.	929 (12.9)	166 (2.6)	20 (0.3)	4370 (60.7)	3605 (59.1)	4877 (73.9)
5	74/F	Dupilumab	62	20	39	53	53	.	482 (7.3)	400 (7.4)	284 (4.9)	3946 (59.8)	3218 (59.6)	3323 (57.3)
6	58/M	Dupilumab	96	50	24	30	.	.	312 (5.2)	1794 (21.1)	252 (4.0)	3072 (51.2)	3392 (39.9)	3415 (54.2)
7	62/M	Dupilumab	12	.	11	4	.	7	283 (2.5)	598 (4.9)	552 (4.8)	6735 (59.6)	7283 (59.7)	7625 (66.3)
8	46/M	Dupilumab	135	55	24	0	.	0	202 (2.3)	2232 (15.5)	1782 (17.3)	6802 (77.3)	8640 (60)	5583 (54.2)

Supplementary Table 2. Frequencies of S1 CD4⁺ T subtypes across patients with dupilumab treatment.

No.	Naïve, S1	TCM, S1	EM, S1	EM/EMRA, S1
5	14.3%	19.0%	38.9%	27.8%
6	2.71%	44.0%	23.9%	29.4%
7	4.41%	47.2%	31.1%	17.3%
8	2.32%	49.3%	19.8%	28.6%

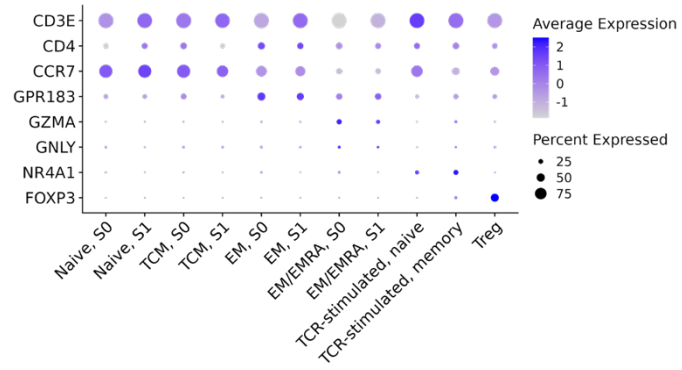
Supplementary Fig. 1. Changes in transcriptional profiles of blood immune cells at least one month after treatment with biologics

(a) Dot plots of markers for annotating T/NK cells, myeloid cells, B cells, and platelets. Dot plots of markers for subtypes of (b) T/NK cells, (c) myeloid cells, and (d) B cells. (e) Pie charts representing the frequencies of T/NK cells, myeloid cells, B cells, and platelets according to the treatment duration. (f) UMAP plots and (g) line plots of T/NK cells. (h) UMAP plots and (i) line plots of B cells. (f-i) The UMAP plots show the subtypes (left panel) and their distribution according to the treatment duration (right panel), and the line plots display the frequencies of the subtypes, with the x-axis and y-axis representing the treatment duration and frequency, respectively. Each line type represents a patient, and the colors represent the biologics. They were treated with, mepolizumab/reslizumab or dupilumab. EM/EMRA and gd T cells represent terminally differentiated effector memory and gamma-delta T cells, respectively.



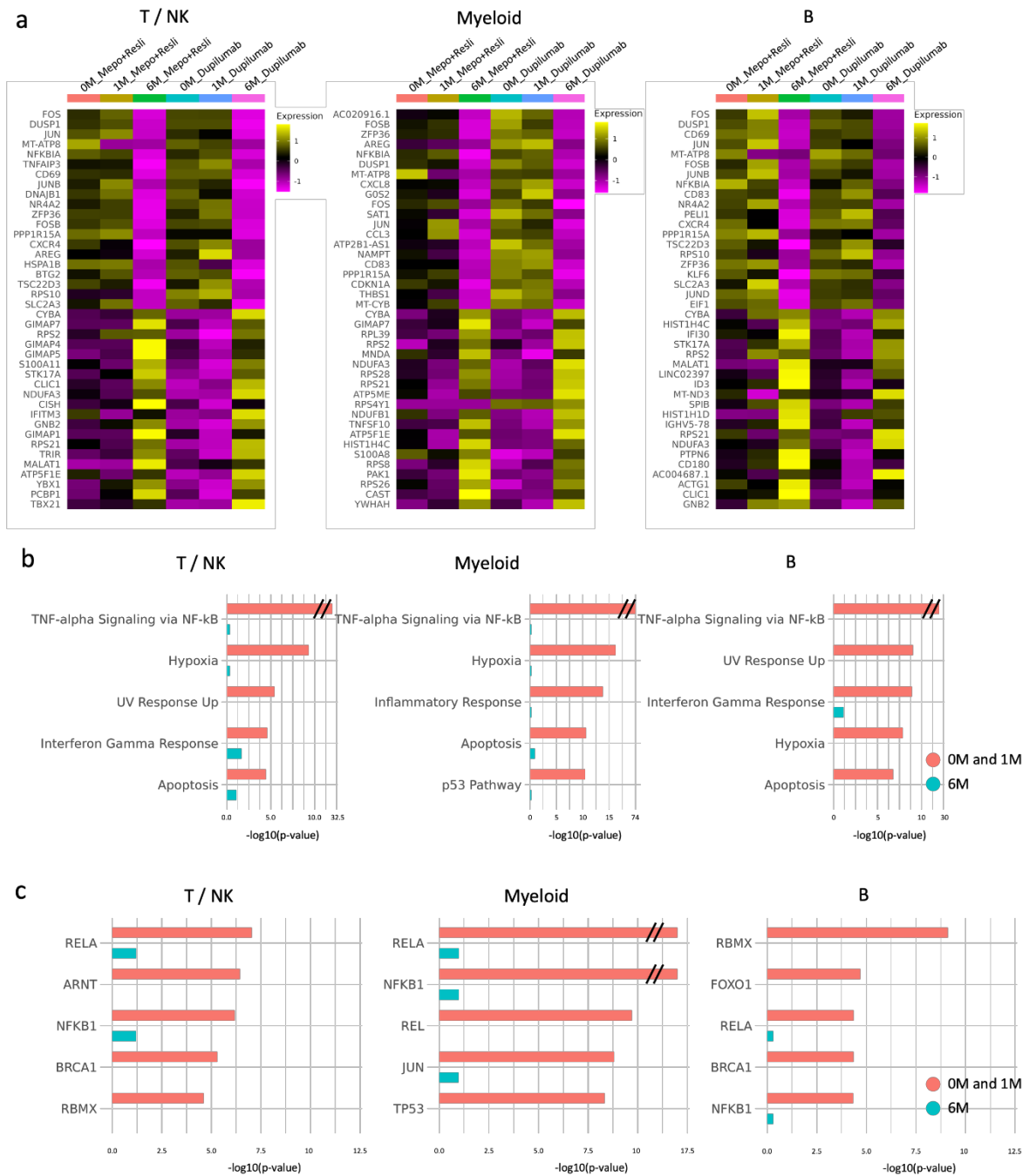
Supplementary Fig. 2. Dot plot of markers for annotating subtypes of CD4⁺ T cells

Dot plot displaying the average expression and percent expressed of corresponding markers across subtypes of CD4⁺ T cells. S0 and S1 represent State 0 and 1, respectively.



Supplementary Fig. 3. Downregulation of genes involved in the NF- κ B pathway in T/NK, myeloid, and B cells

(a) Heatmap displaying the expression levels of DEGs that are significantly different between 6M and 0/1M of T/NK (left panel), myeloid (middle panel), and B cells (right panel) categorized by treatment and duration. The top 20 downregulated and upregulated DEGs in 6M cells compared with 0/1M cells are shown on the y-axis. (b) The top 5 MSigDB Hallmark 2020 pathways and their p-values associated with downregulated DEGs at 6M of T/NK (left panel), myeloid (middle panel), and B cells (right panel). The y-axis represents the names of the pathways, and the x-axis represents $-\log_{10}$ of their p-values. Each pathway has two bars representing the p-values calculated by upregulated DEGs at 0/1M (red) and 6M (turquoise). (c) Top 5 TRRUST transcription factors and their p-values associated with downregulated DEGs at 6M (red colored) of T/NK (left panel), myeloid (middle panel), and B cells (right panel). The turquoise bars represent p-values of the corresponding pathways calculated by upregulated DEGs at 6M.



Supplementary Fig. 4. Effect of biologics on communication of CD4⁺ T cells with other cell types based on their mechanisms of action

(a) Raster plots representing the differences in the number of signaling pathway networks among cell types between 6M and 0/1M according to all biologics (left panel), the mepolizumab/reslizumab group (middle panel), and the dupilumab group (right panel). The x-axis and y-axis represent cell types as receptors and ligands, respectively, in a signaling pathway network. The differences are colored ranging from blue (+14 connections) to red (-14 connections). Blue color represents an increase in the number of networks at 6M, and red color represents a decrease in the number of networks at 6M compared with that at 0/1M. (b) Chord plots of MHC-II signaling pathway network at 0/1M and 6M of all patients. (c) Chord plots of the CD40 signaling pathway network at 0/1M and 6M of all patients. S0 and S1 represent State 0 and 1, respectively.

