Supplementary Materials and methods

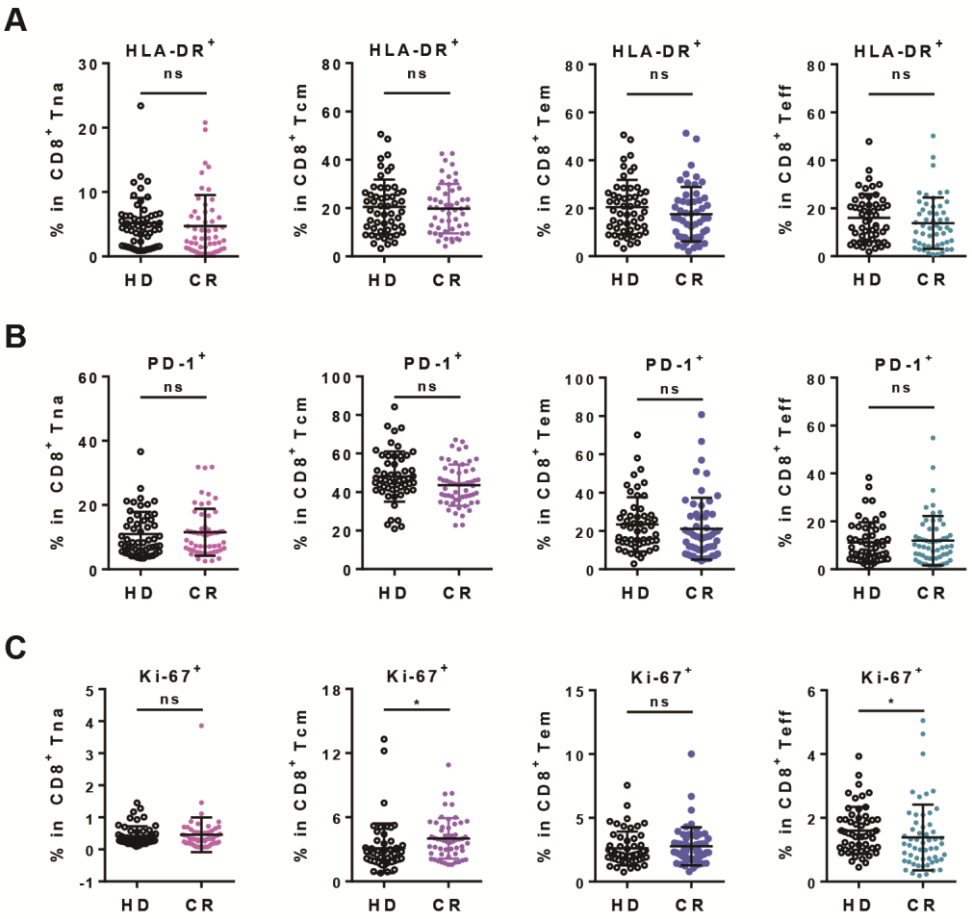
Lymphocyte response evaluation by flow cytometry

PBMCs were separated from the cell pellets after resuspension in PBS by density gradient centrifugation and then resuspended in complete RPMI 1640 medium containing 10% FBS (Gibco), 1% penicillin and 1% streptomycin. PBMCs were then divided for testing with 5 panels for analysis: panel 1 for T cell differentiation, proliferation and activation detection, panel 2 for Treg detection, panel 3 for B cell detection, panel 4 for circulating T follicular helper (cTfh) detection, and panel 5 for cell cytokine production detection. The PBMCs for panel 1 to panel 4 were directly used for staining, while the PBMCs for panel 5 were stimulated with 200 ng/ml PMA (Beyotime, China) and 2.5 μM ionomycin (Beyotime, China) in the presence of 1 μM monensin (BioLegend, USA) and 2.5 μg/ml brefeldin A at 37 ℃ in 5% CO_2 for 4.5 h before staining. PBMCs were stained with dead cell discrimination markers (eBioscience™ Fixable Viability Dye eFluor™ 506, FVD) and surface-staining antibodies in PBS at 4 ℃ for 30 min. After washing with PBS, the cells were fixed with fixation/permeabilization buffer (eBioscience) at 4 ℃ overnight and then stained with the respective panel of intracellular markers in permeabilization buffer at 4 ℃ for 30 min. The antibodies used are listed in supplementary Table S2. A BD LSR Fortessa flow cytometer (Becton Dickinson) was used to assess the stained cells, and data were analyzed using FlowJo V7.0.

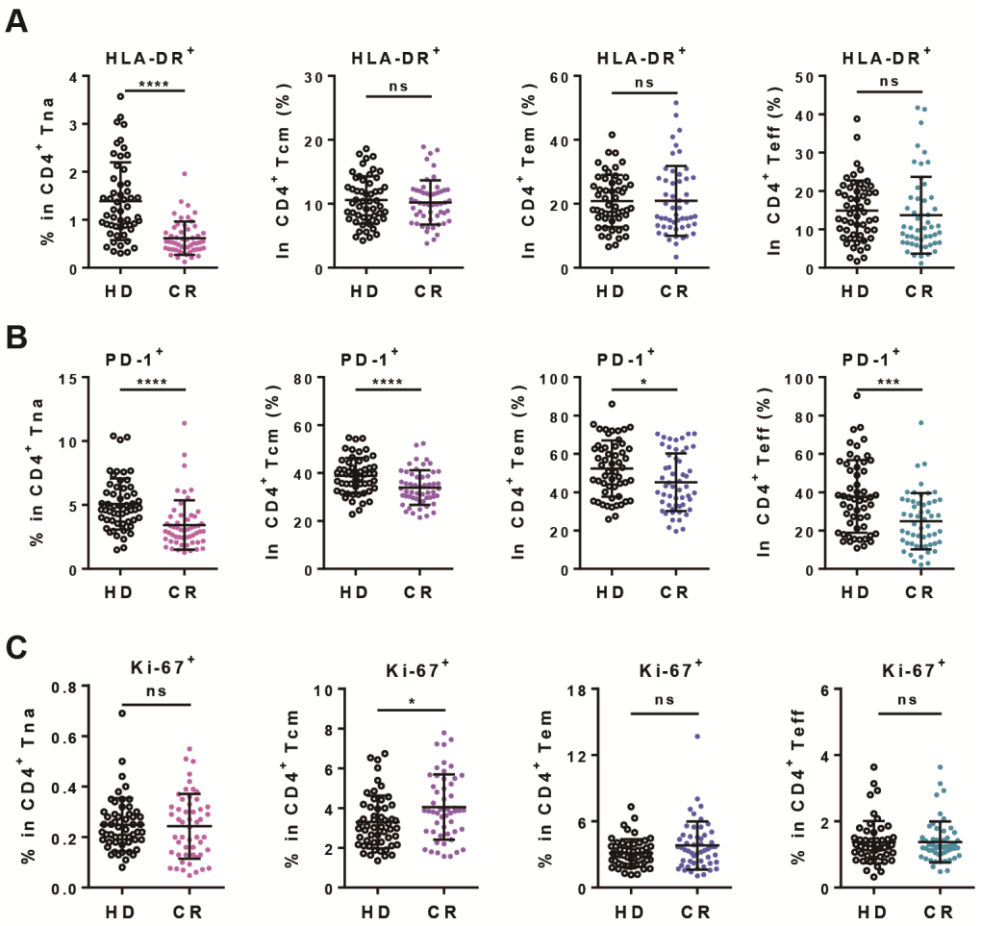
Measurement of total IgM and IgG in plasma

Total IgM and IgG in plasma were determined by ELISA as described in supplementary materials and methods. Briefly, each well of flat-bottom 96-well ELISA plates (Greiner Bio-one) were coated with 100 μl of 3 μg/ml unlabeled goat anti-human IgM or IgG polyclonal antibodies (Southern Biotechnology) and incubated at 4 ℃ overnight. Plates were blocked with 1% bovine serum albumin in PBS for 2 h, and 100 μl of serially diluted plasma was subsequently applied in blocking buffer. Alkaline phosphatase-labeled goat anti-human IgM or IgG polyclonal antibodies (Southern Biotechnology) and p-nitrophenyl phosphate substrate (Sigma) were used for detection. The absorbance was read by a microplate reader (Thermo Labsystems) at 405 nm.

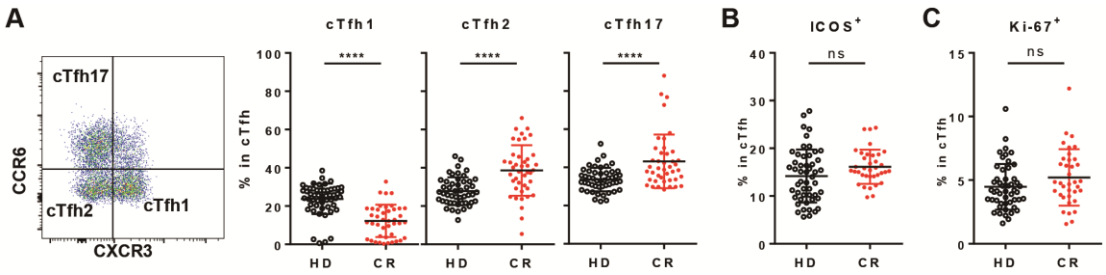
Supplementary figures



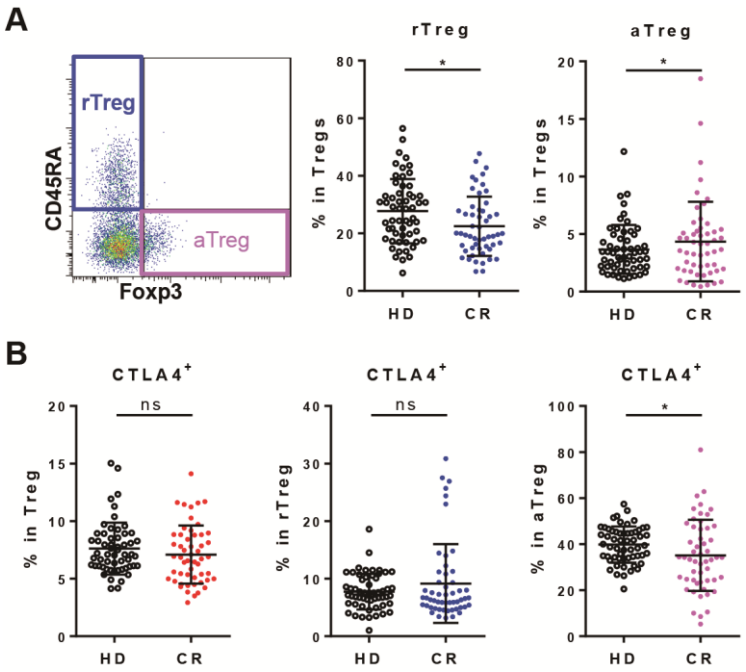
Supplementary Figure S1 Activation and proliferation of the Tna, Tcm, Tem and Teff subsets of CD8$^+$ T cells. Frequencies of HLA-DR$^+$ (A), PD-1$^+$ (B) and Ki-67$^+$ (C) in naïve T cells (Tna), central memory T cells (Tcm), effector memory T cell (Tem) and effector T cell (Teff) subsets of CD8$^+$ T cells in PBMCs of healthy donors (HD, n=55) and subjects clinically recovered from COVID-19 (CR, n=55). ns, non-significant; *, P<0.05.



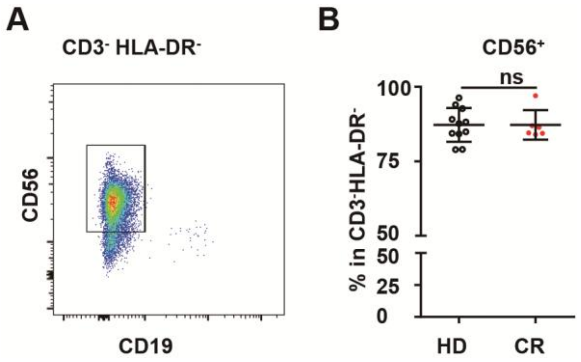
Supplementary Figure S2 Activation and proliferation of the Tna, Tcm, Tem and Teff subsets of CD4$^+$ T cells. Frequencies of HLA-DR$^+$ (A), PD-1$^+$ (B) and Ki-67$^+$ (C) in naïve T cells (Tna), central memory T cells (Tcm), effector memory T cell (Tem) and effector T cell (Teff) subsets of CD4$^+$ T cells in PBMCs of healthy donors (HD, n=55) and subjects clinically recovered from COVID-19 (CR, n=55). ns, non-significant; *, P<0.05; ***, p<0.001; ****, p<0.0001.



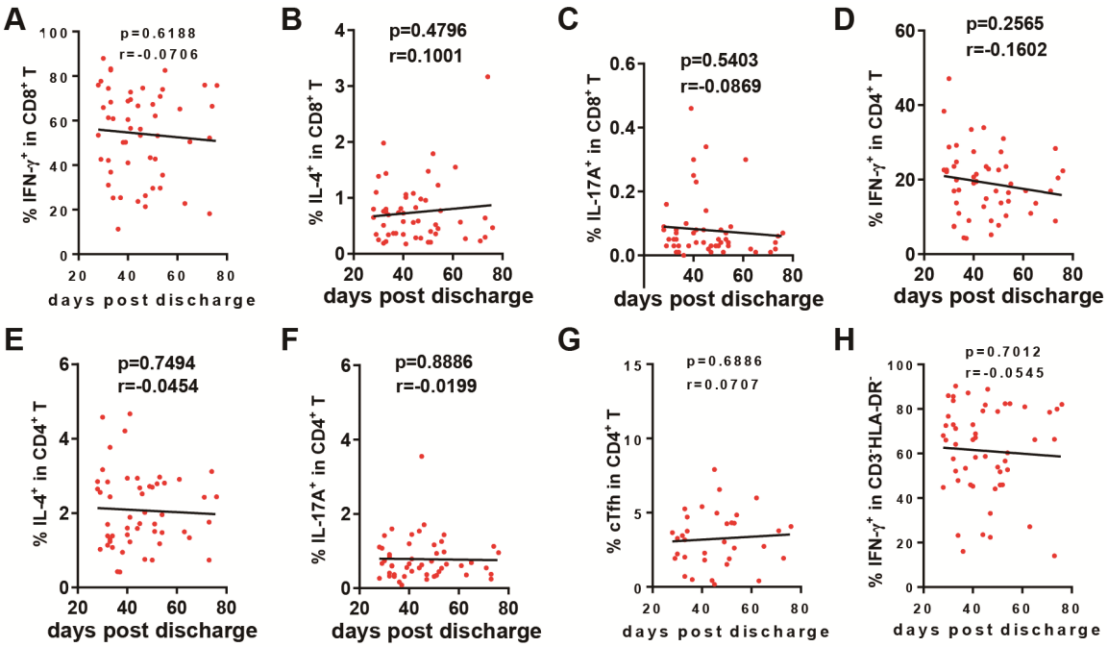
Supplementary Figure S3. Percentages of cTfh subsets and the activation, proliferation of cTfh in peripheral blood of HD and CR. A, Gating strategy and the percentages of cTfh subsets cTfh1 ($CXCR3^+$ $CCR6^-$), cTfh2 ($CXCR3^-$ $CCR6^-$) and cTfh17 ($CXCR3^-$ $CCR6^+$) of cTfh in PBMCs of healthy donors (HD, n=55) and subjects clinically recovered from COVID-19 (CR, n=36). B and C, The frequencies of $ICOS^+$ and $Ki\text{-}67^+$cells in cTfh. ns, non-significant; ***, p<0.001; ****, p<0.0001.



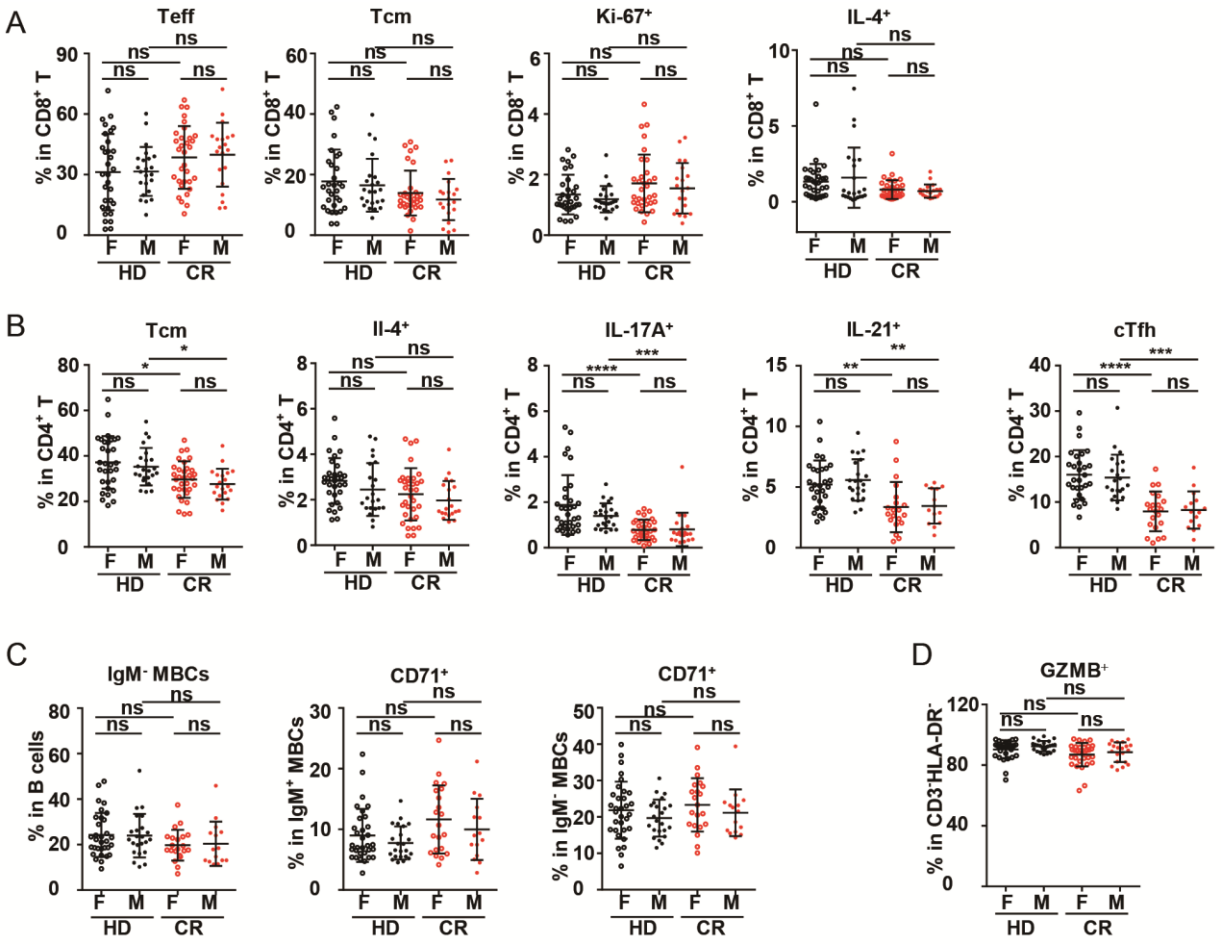
Supplementary Figure S4. Frequencies and phenotype of Tregs and Treg subsets in peripheral blood of HD and CR. A, Gating strategy and the frequencies of resting Treg (rTeg, $CD45RA^+Foxp3^{lo}$) and activated Treg (aTreg, $CD45RA^-Foxp3^{hi}$) in Treg. B, The frequencies of $CTLA4^+$ cells in Treg, rTreg and aTreg. ns, non-significant; *, P<0.05.



Supplementary Figure S5. Frequencies of CD56$^+$ NK cells in CD3$^-$HLADR$^-$ lymphocytes cells of peripheral blood. Gating strategy and the frequencies of CD56$^+$ NK cells (CD56$^+$CD19$^-$) in CD3$^-$HLADR$^-$ lymphocytes of PBMCs derived from HD (n=11) and CR (n=6). ns, non-significant.



Supplementary Figure S6. The correlation of lymphocytes response in CR cohort with days post discharge. The correlation of days post discharge in CR with frequency of IFN-γ$^+$ (A), IL-4$^+$ (B) and IL-17A$^+$ (C) in CD8$^+$ T cells were displayed in A-C. The correlation of days post discharge in CR with frequency of IFN-γ$^+$ (D), IL-4$^+$ (E), IL-17A$^+$ (F) and cTfh (G) in CD4$^+$ T cells were displayed in D-G. The correlation of days post discharge in CR with frequency of IFN-γ$^+$ in CD3$^-$HLA-DR$^-$ lymphocytes were displayed in H.



Supplementary Figure S7. Sex-based differences in phenotypic alterations and potential dysfunction of lymphocytes in the CR cohort. To elucidate the sex-based differences in lymphocyte changes in the CR cohort, the data from Figure 2 to Figure 5, which were significantly different between the HD and CR cohorts at the total cohort level, were reanalyzed. A, Frequencies of Teff, Tcm, Ki-67$^+$ and IL-4$^+$ CD8$^+$ T cells in PBMCs. B, Frequencies of Tcm, IL-4$^+$, IL-17A$^+$, IL-21$^+$ and cTfh cells in CD4$^+$ T cells in PBMCs. C, Frequency of IgM$^-$ MBCs in B cells, frequency of CD71$^+$ in IgM$^+$ MBCs and frequency of CD71$^+$ in IgM$^-$ MBCs. D, Frequencies of GZMB$^+$ in CD3$^-$HLA-DR$^-$ lymphocytes.

Supplementary tables

Supplementary Table S1 Clinical information of the CR cohort.

Final code	Sex	Age	Disease duration	Day of discharge	Day of testing	Days post discharge	SARS-CoV -2 antibody		Blood oxygen saturation	Symptoms in two weeks				Blood routine examination (10^9/L)			
							IgG	IgM		Fever	Shod of breath	Cough	Expectoration	Oxygen uptake	WBC	Neutrophils	Lymphocytes
1	M	43	35	2020-3-17	2020-4-14	28	+	+	99	-	-	-	-	-	6.22	3.81	1.95
2	F	46	28	2020-2-5	2020-4-14	44	+	+	99	-	-	+	-	-	6.52	1.59	1.8
3	F	50	38	2020-3-13	2020-4-14	32	+	-	99	-	-	-	-	-	5.54	3.66	1.33
4	M	68	46	2020-3-12	2020-4-14	39	+	-	99	-	-	-	-	-	5.68	3.47	1.91
5	F	40	32	2020-3-1	2020-4-14	54	+	+	99	-	-	-	-	-	5.21	3.88	1.03
6	F	48	41	2020-3-2	2020-4-14	73	+	-	99	-	-	-	-	-	6.06	2.71	2.94
7	F	39	17	2020-3-2	2020-4-14	45	+	+	98	-	-	-	-		6.26	4.12	1.64
8	M	45	17	2020-3-13	2020-4-14	61	+	+	99	-	-	-	-	-	5.06	2.64	1.81
9	M	60	37	2020-2-28	2020-4-14	41	+	-	98	-	-	-	-	-	3.75	2.12	1.26
10	F	34	30	2020-3-6	2020-4-14	41	+	-	98	-	-	-	-	-	5.55	3.06	1.88
11	M	32	28	2020-2-20	2020-4-14	29	+	-	95	-	-	-	-	-	6.56	4.12	2.07
12	M	55	22	2020-1-31	2020-4-14	32	+	-	96	-	-	-	-	-	5.33	1.9	3.01
13	F	54	23	2020-2-1	2020-4-14	38	+	-	99	-	-	-	-	-	4.78	2.85	1.48
14	M	35	40	2020-3-5	2020-4-14	40	+	+	98	-	-	-	-	-	6.9	3.44	2.95
15	F	57	16	2020-2-29	2020-4-14	41	+	-	99	-	+	-	-	-	5.69	3.15	2.01
16	F	27	45	2020-3-10	2020-4-14	44	+	-	99	-	-	-	-	-	5.29	2.7	2.2
17	F	31	22	2020-2-13	2020-4-14	37	+	+	99	-	-	-	-	-	6.36	3.18	2.32
18	F	51	35	2020-3-14	2020-4-14	40	+	+	99	-	-	-	-	-	3.95	2.22	1.41
19	F	34	44	2020-3-12	2020-4-14	51	+	+	98	-	-	-	-	-	7.22	4.46	2.21
20	F	68	21	2020-2-14	2020-4-14	74	+	+	99	-	-	-	-	-	4.08	3.22	0.64
21	M	60	14	2020-3-15	2020-4-14	47	+	+	99	-	-	-	-	-	6.31	3.76	2.01

22	F	31	54	2020-3-17	2020-4-14	55	+	+	98	-	-	-	-	-	7.24	3.45	3.12
23	F	39	16	2020-2-12	2020-4-14	73	+	+	96	-	-	-	-	-	6.09	3.56	2.06
24	M	37	22	2020-2-22	2020-4-15	33	+	-	99	-	-	-	-	-	6.96	4.78	1.76
25	M	70	18	2020-3-4	2020-4-15	34	+	-	98	-	+	-	-	-	7.13	4.38	2.08
26	F	51	45	2020-3-18	2020-4-15	44	+	-	99	-	-	-	+	-	7.98	5	2.44
27	F	40	27	2020-3-1	2020-4-15	46	+	-	99	-	-	-	-	-	5.17	3.13	1.66
28	F	51	25	2020-3-4	2020-4-15	41	+	-	99	-	-	-	-	-	3.82	1.92	1.42
29	F	61	28	2020-2-22	2020-4-15	36	+	+	98	-	-	-	-	-	6.85	3.74	2.39
30	F	67	44	2020-3-14	2020-4-15	32	+	-	98	-	+	-	-	-	7.32	3.68	2.9
31	M	56	34	2020-3-18	2020-4-15	34	+	+	99	-	-	-	-	-	7.96	4.81	2.5
32	M	51	42	2020-3-16	2020-4-15	63	+	-	98	-	-	-	-	-	8.58	6.74	1.38
33	F	61	14	2020-3-6	2020-4-15	28	+	+	98	-	-	-	-	-	6.13	3.43	2.15
34	M	31	43	2020-2-25	2020-4-15	53	+	-	98	-	-	+	-	-	6.38	4.06	2.01
35	M	53	28	2020-2-10	2020-4-15	40	+	+	99	-	-	-	-	-	5.19	2.86	2
36	F	38	19	2020-3-13	2020-4-15	50	+	-	99	-	-	-	-	-	5.02	2.78	1.87
37	F	40	39	2020-3-7	2020-4-15	65	+	-	99	-	-	-	-	-	5.49	2.8	2.33
38	M	54	34	2020-3-5	2020-4-15	51	+	-	96	-	-	-	-	-	5.15	2.79	1.9
39	F	56	34	2020-2-24	2020-4-15	33	+	-	98	-	-	-	-	+	6.11	3.43	1.97
40	F	38	40	2020-3-4	2020-4-15	49	+	+	99	-	-	-	-	-	4.88	2.38	2.07
41	F	43	29	2020-3-1	2020-4-15	52	+	-	99	-	-	-	-	-	8.86	5.14	3.09
42	F	57	24	2020-2-23	2020-4-16	71	+	+	99	-	-	+	-	-	6.02	3.67	1.94
43	M	34	43	2020-3-13	2020-4-16	45	+	-	99	-	-	+	-	-	3.97	2.06	1.6
44	F	61	27	2020-3-8	2020-4-16	76	+	-	99	-	-	+	+	-	4.98	3.24	1.4
45	F	57	60	2020-3-5	2020-4-16	62	+	-	99	-	-	-	-	-	6.63	4.71	1.45
46	M	55	31	2020-2-23	2020-4-16	32	+	-	96	-	+	+	+	-	7.17	5.34	1.37
47	M	51	31	2020-2-26	2020-4-16	30	+	-	99	-	-	-	-	-	4.56	2.46	1.54
48	M	38	48	2020-3-14	2020-4-16	54	+	-	99	-	-	-	-	-	6.1	3.8	1.82
49	F	51	18	2020-2-26	2020-4-16	46	+	+	99	-	-	-	-	-	4.88	2.82	1.54
50	M	56	59	2020-3-17	2020-4-16	33	+	-	96	-	-	-	-	-	5.24	2.17	2.63
51	M	61	16	2020-1-31	2020-4-16	29	+	+	99	-	-	-	-	-	3.93	1.83	1.75
52	F	39	33	2020-2-27	2020-4-16	53	+	-	99	-	-	-	-	-	5.32	4.94	3.61

53	F	52	54	2020-2-19	2020-4-16	50	+	-	99	-	-	-	-	-	6.66	3.67	2.56
54	F	70	13	2020-2-1	2020-4-16	33	+	-	99	-	-	-	-	-	5.57	3.18	1.9
55	F	65	15	2020-2-23	2020-4-16	30	+	-	99	-	-	-	-	-	5.46	2.94	2.1

+, positive; -, negative.

Cells filled in yellow color means the value was higher than normal range, while cells filled in blue color means the value was lower than normal range.

Supplementary Table S2 Reagents for flow cytometry.

Panel	Antibodies (clone)	Staining procedure	Source	Identifier
1	Fixable Viability Dye eFluor™ 506	surface	eBioscience	65-0866-14
	APC/Cyanine7 anti-human CD3 (HIT3a)	surface	BioLegend	300318
	Alexa Fluor® 700 anti-human CD4 (OKT4)	surface	BioLegend	317426
	Brilliant Violet 605™ anti-human CD8a (RPA-T8)	surface	BioLegend	301040
	FITC anti-human CD45RO (UCHL1)	surface	BioLegend	304242
	Pacific Blue™ anti-human CD27 (O323)	surface	BioLegend	302822
	PerCP/Cyanine5.5 anti-human HLA-DR (L243)	surface	BioLegend	307630
	Brilliant Violet 650™ anti-human CD38 (HB-7)	surface	BioLegend	356620
	PE/Dazzle™ 594 anti-human CD279 (PD-1) (EH12.2H7)	surface	BioLegend	329940
	PE-Cyanine7 anti human T-Bet (4B10)	intracellular	eBioscience	25-5825-82
	PE anti-human Ki-67 (Ki-67)	intracellular	BioLegend	350504
2	Fixable Viability Dye eFluor™ 506	surface		65-0866-14
	Alexa Fluor® 700 anti-human CD3 (SK7)	surface	BioLegend	344822
	Brilliant Violet 650™ anti-human CD4 (RPA-T4)	surface	BioLegend	300536
	Brilliant Violet 605™ anti-human CD8a (RPA-T8)	surface	BioLegend	301040
	FITC anti-human CD25 (BC96)	surface	BioLegend	302604
	PerCP/Cy5.5 anti-human CD127 (A019D5)	surface	BioLegend	351322
	PE/Cy7 anti-human CTLA4 (L3D10)	surface	BioLegend	349914
	APC/Cyanine7 anti-human CD45RA (HI100)	surface	BioLegend	304127
	Brilliant Violet 421™ anti-human FoxP3 (206D)	intracellular	BioLegend	320124
3	Fixable Viability Dye eFluor™ 506	surface	eBioscience	65-0866-14
	Alexa Fluor® 700 anti-human CD3 (SK7)	surface	BioLegend	344822
	Brilliant Violet 605™ anti-human CD19 (HIB19)	surface	BioLegend	302244
	FITC anti-human CD20 (2H7)	surface	BioLegend	302304
	PE/Cyanine7 anti-human CD27 (O323)	surface	BioLegend	302838
	Brilliant Violet 650™ anti-human CD38 (HB-7)	surface	BioLegend	356620
	PE/Dazzle™ 594 anti-human CD71 (CY1G4)	surface	BioLegend	334120
	PerCP/Cyanine5.5 anti-human HLA-DR (L243)	surface	BioLegend	307630
	APC anti-human CD275 (B7-H2, ICOSL) (2D3)	surface	BioLegend	309408
	PE anti-human IgM	surface	BioLegend	314508
4	Fixable Viability Dye eFluor™ 506	surface	eBioscience	65-0866-14
	APC/Cyanine7 anti-human CD3 (HIT3a)	surface	BioLegend	300318
	Brilliant Violet 650™ anti-human CD4 (RPA-T4)	surface	BioLegend	300536
	Brilliant Violet 605™ anti-human CD8a (RPA-T8)	surface	BioLegend	301040
	PerCP/Cyanine5.5 anti-human CD185 (CXCR5) (J252D4)	surface	BioLegend	356910
	PE/Dazzle™ 594 anti-human CD279 (PD-1) (EH12.2H7)	surface	BioLegend	329940
	Brilliant Violet 421™ anti-human/mouse/rat CD278 (ICOS)(C398.4A)	surface	BioLegend	313524

	APC anti-human CD196 (CCR6) (G034E3)	surface	BioLegend	353416
	Alexa Fluor® 700 anti-human CD183 (CXCR3) (G025H7)	surface	BioLegend	353742
	PE/Cyanine7 anti-human Ki-67 (Ki-67)	intracellular	BioLegend	350526
5	Fixable Viability Dye eFluor™ 506	surface	eBioscience	65-0866-14
	APC/Cyanine7 anti-human CD3 (HIT3a)	surface	BioLegend	300318
	Alexa Fluor® 700 anti-human CD4 (OKT4)	surface	BioLegend	317426
	Brilliant Violet 605™ anti-human CD8a (RPA-T8)	surface	BioLegend	301040
	PE/Cy7 anti-human IL-4 (MP4-25D2)	intracellular	BioLegend	500824
	PerCP/Cy5.5 anti-human IL-17A (BL168)	intracellular	BioLegend	512314
	PE/Dazzle™ 594 anti-human IFN-γ (B27)	intracellular	BioLegend	506530
	Brilliant Violet 650™ anti-human IL-2 (MQ1-17H12)	intracellular	BioLegend	500334
	PE anti-human IL-21 (3A3-N2)	intracellular	BioLegend	513004
	FITC anti-human/mouse Granzyme B (QA16A02)	intracellular	BioLegend	372206