Figure S1

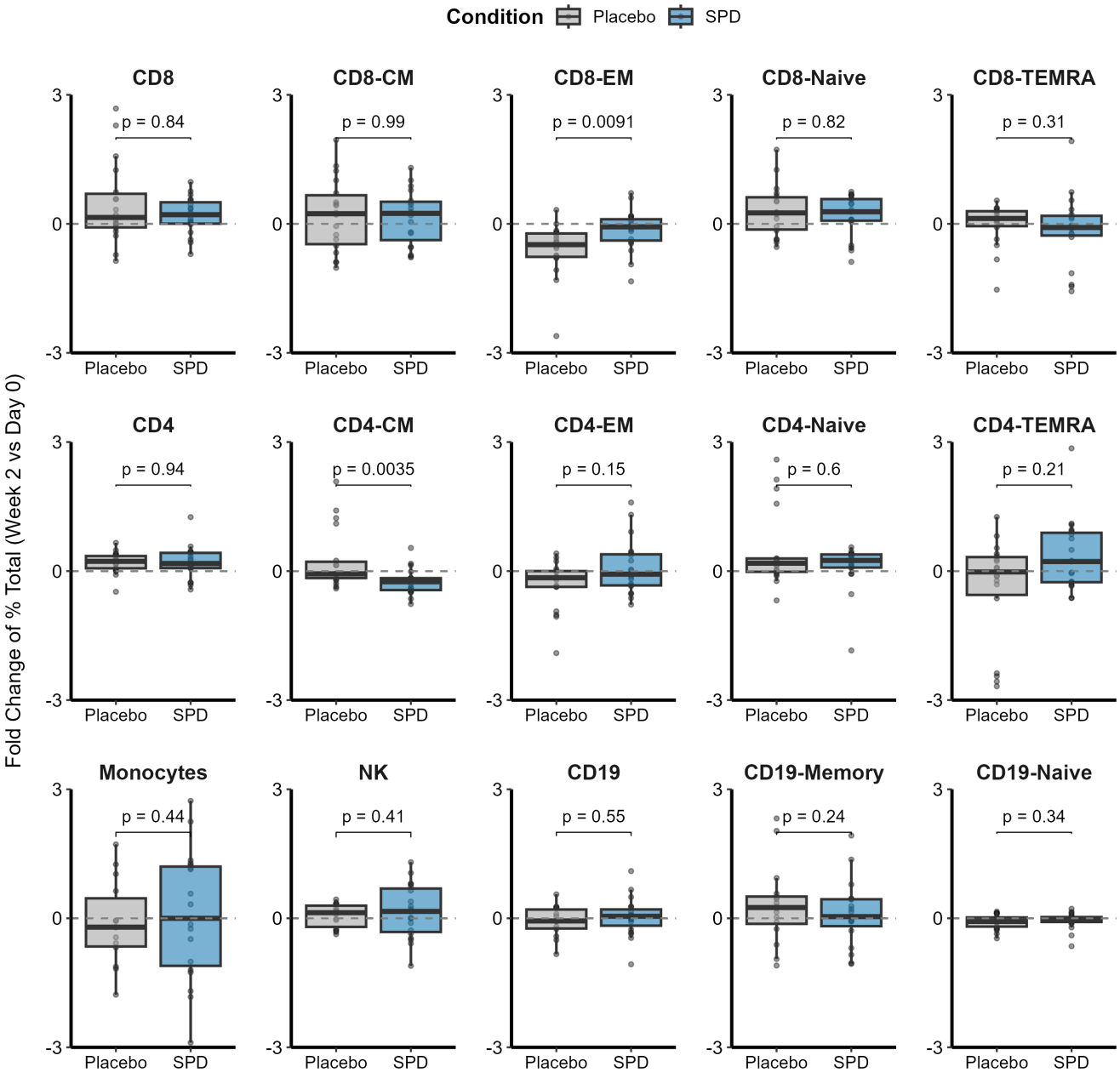


Figure S1: Immune cell subset profiling between Placebo and Spermidine Groups at 2 weeks (log2FC).

Figure S2

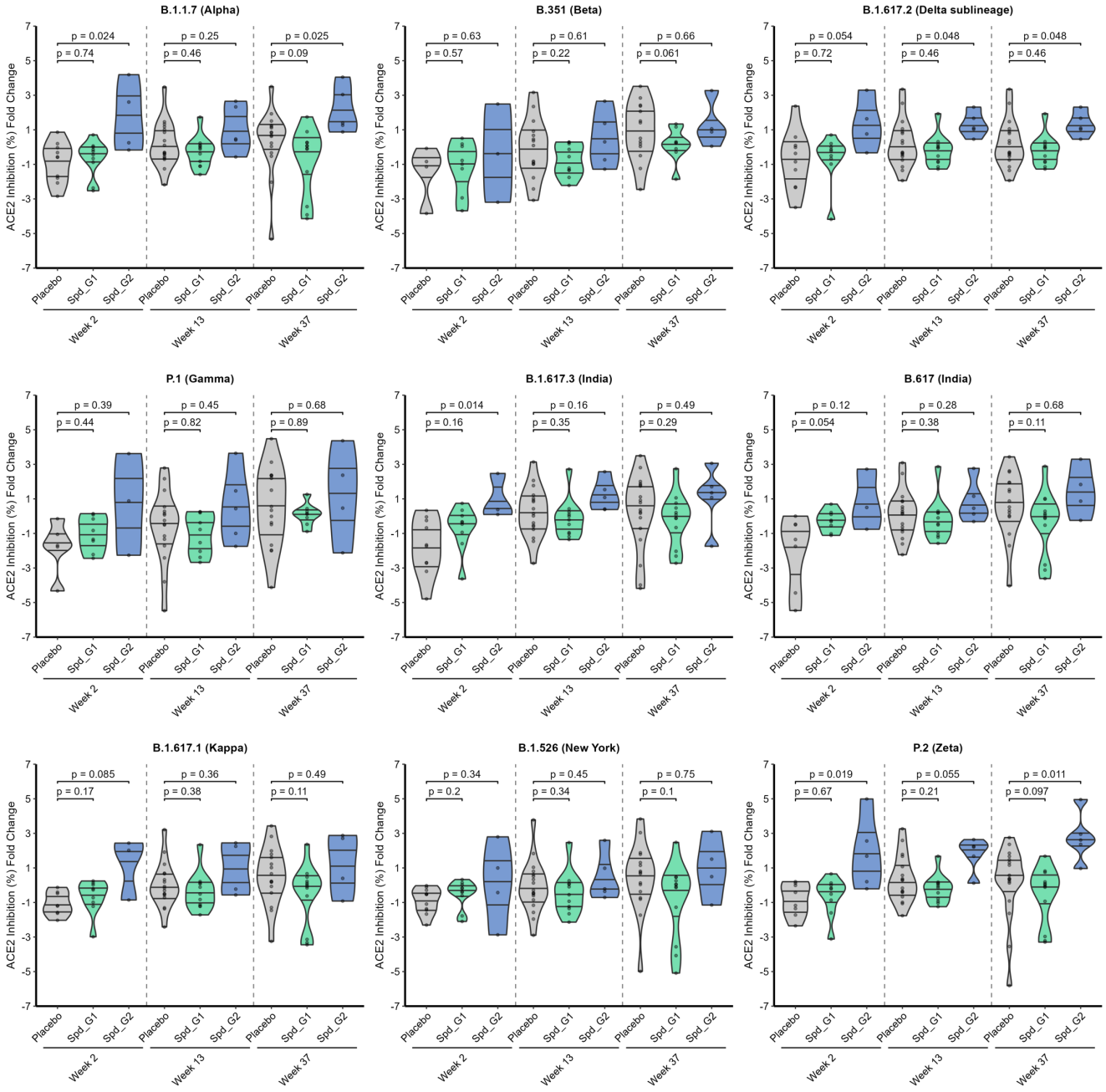


Figure S2: Enhanced ACE2 inhibition responses against SARS-CoV-2 variants following spermidine supplementation.

A-I: ACE2 inhibition (%) log2FC across all groups and timepoints, from neutralisation antibody assay against SARS-CoV-2 variants: **(A)** B.1.617 (India), **(B)** P.2 (Zeta), **(C)** B.1.1.7 (Alpha), **(D)** B.1.351 (Beta), **(E)** B.1.526 (New York), **(F)** B.1.617.2 (Delta), **(G)** B 1.617.1 (Kappa), **(H)** B.1.617.3 (India), **(I)** P.1 (Gamma). Annotations: Week 2 (W2); placebo and spermidine (Spd); group 1 (G1); group 2 (G2). Exact P values are reported. Outliers were retained in all statistical analyses; in some plots, extreme values were omitted from display for clarity. Data are presented as violin plots show median and IQR, with statistical comparisons using the two-sided Wilcoxon rank-sum test. Analyses in R 4.5.0; $\alpha = 0.05$.

Figure S3.

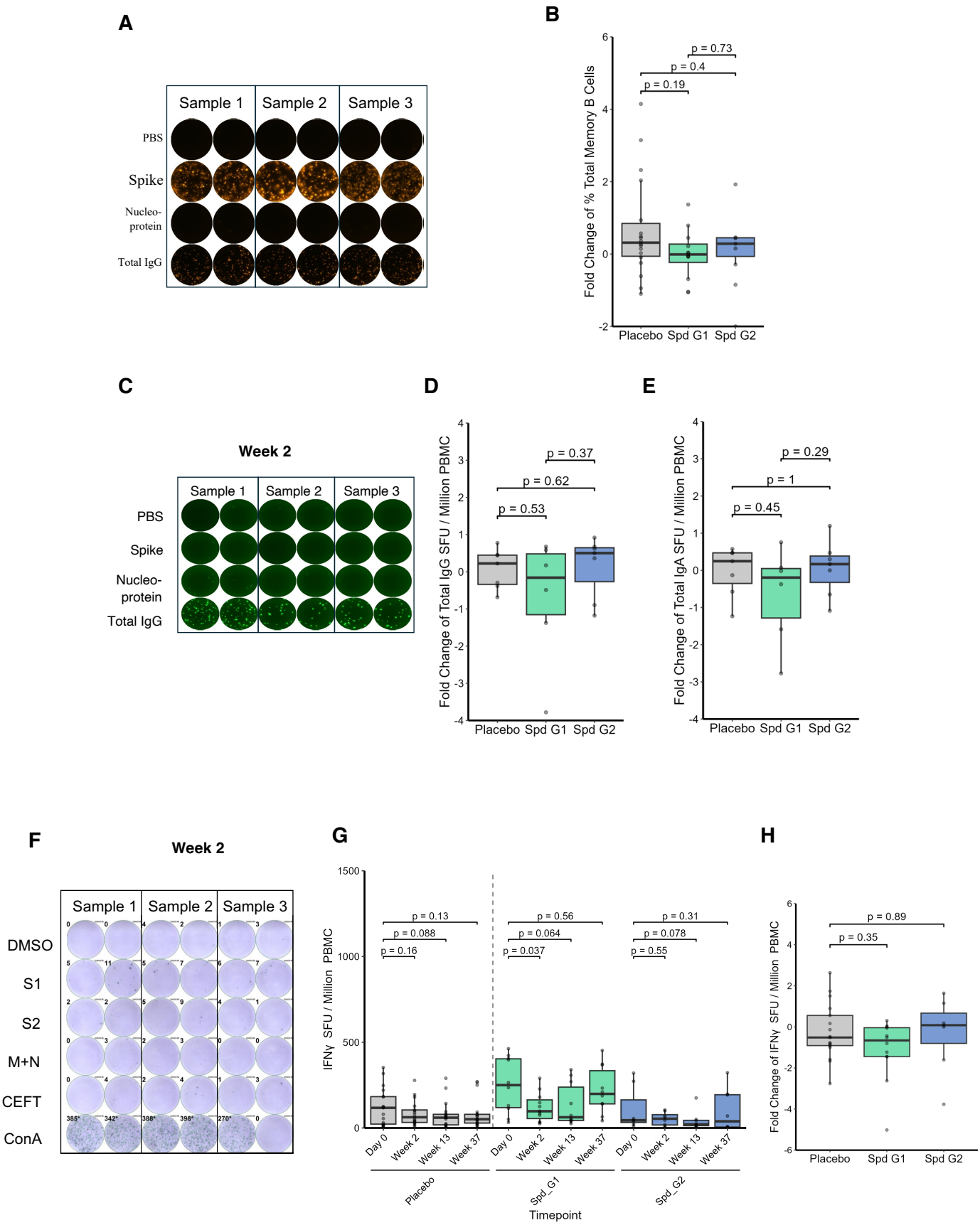

A
Sample 1
Sample 2
Sample 3
PBS
Spike
Nucleo-protein
Total IgG
B
Fold Change of % Total Memory B Cells
p = 0.73
p = 0.4
p = 0.19
Placebo
Spd G1
Spd G2
C
Week 2
Sample 1
Sample 2
Sample 3
PBS
Spike
Nucleo-protein
Total IgG
D
Fold Change of Total IgG SFU / Million PBMC
p = 0.37
p = 0.62
p = 0.53
Placebo
Spd G1
Spd G2
E
Fold Change of Total IgA SFU / Million PBMC
p = 0.29
p = 1
p = 0.45
Placebo
Spd G1
Spd G2
F
Week 2
Sample 1
Sample 2
Sample 3
DMSO
S1
S2
M+N
CEFT
ConA
G
IFNγ SFU / Million PBMC
p = 0.13
p = 0.088
p = 0.16
p = 0.56
p = 0.064
p = 0.037
p = 0.31
p = 0.078
p = 0.55
Day 0
Week 2
Week 13
Week 37
Placebo
Spd_G1
Spd_G2
Timepoint
H
Fold Change of IFNγ SFU / Million PBMC
p = 0.89
p = 0.35
Placebo
Spd G1
Spd G2

Figure S3: Spermidine supplementation does not affect memory B cells, antibody levels, or T cell responses following COVID-19 vaccination.

A: FluoroSpot example image for B cells in spike IgG plates. **B:** Memory B cell ($CD19^+$ $CD27^+IgD^{low}$) proportion $\log_2$ fold change at 2 weeks. **C:** FluoroSpot image example results for B cells in IgA and IgG plates. **D:** Total IgG (FluoroSpot) $\log_2$ fold change across groups from baseline at 2 weeks. **E:** Total IgA $\log_2$ fold change across groups from baseline at 2 weeks. **F:** ELISpot image example results for T cell plates stimulated with SARS-CoV-2 peptide pools representing the viral S1, S2, membrane (M) and nucleocapsid (N) regions. CEFT peptide pools and concanavalin A (ConA) were used as positive controls. **G:** *Ex vivo* IFN-γ ELISpot response raw data all groups and timepoints to total spike. **H:** *Ex vivo* IFN-γ ELISpot response $\log_2$ fold change at 2 weeks to total spike. Exact P values are reported. Outliers were retained in all statistical analyses; in some plots, extreme values were omitted from display for clarity. Data are presented as Box plots show median, IQR, and 1.5×IQR whiskers, with statistical comparisons using the two-sided Wilcoxon rank-sum test, except for panel F where the Wilcoxon signed-rank test was used. Analyses were conducted in R 4.5.0; $\alpha = 0.05$.

Figure S4

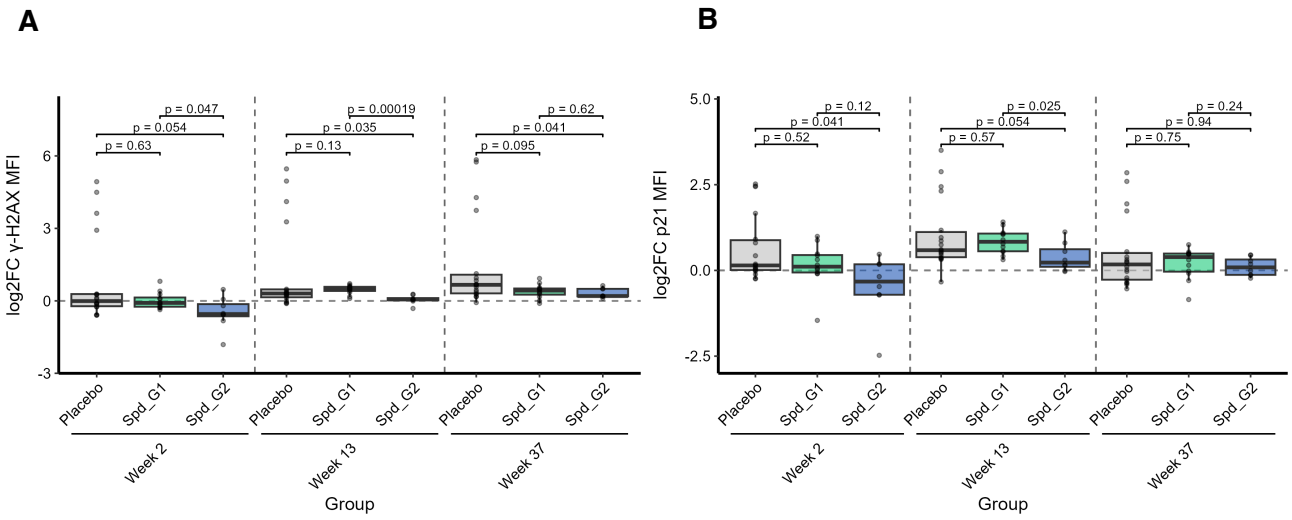


Figure S4: Spermidine treatment promotes senescent cell rejuvenation in vaccine non-responders, (Group 2, 'G2').

A-D: Senescence marker $\log_2$ fold change between day 0/basline and 2, 13 and 37 weeks for **(A)** γH2AX (MFI) **(B)** p-21 (MFI), in placebo (grey), Spd groups responders (G1, green) and non-responders (G2, blue) in PBMCs. For A-B, statistical comparisons were performed using the two-sided Wilcoxon rank-sum test. Statistical significance is indicated by asterisks (*, **, ***, **** for $P < 0.05$, 0.01, 0.001, 0.0001, respectively). Analyses were performed in R 4.5.0 with $\alpha = 0.05$.

Figure S5

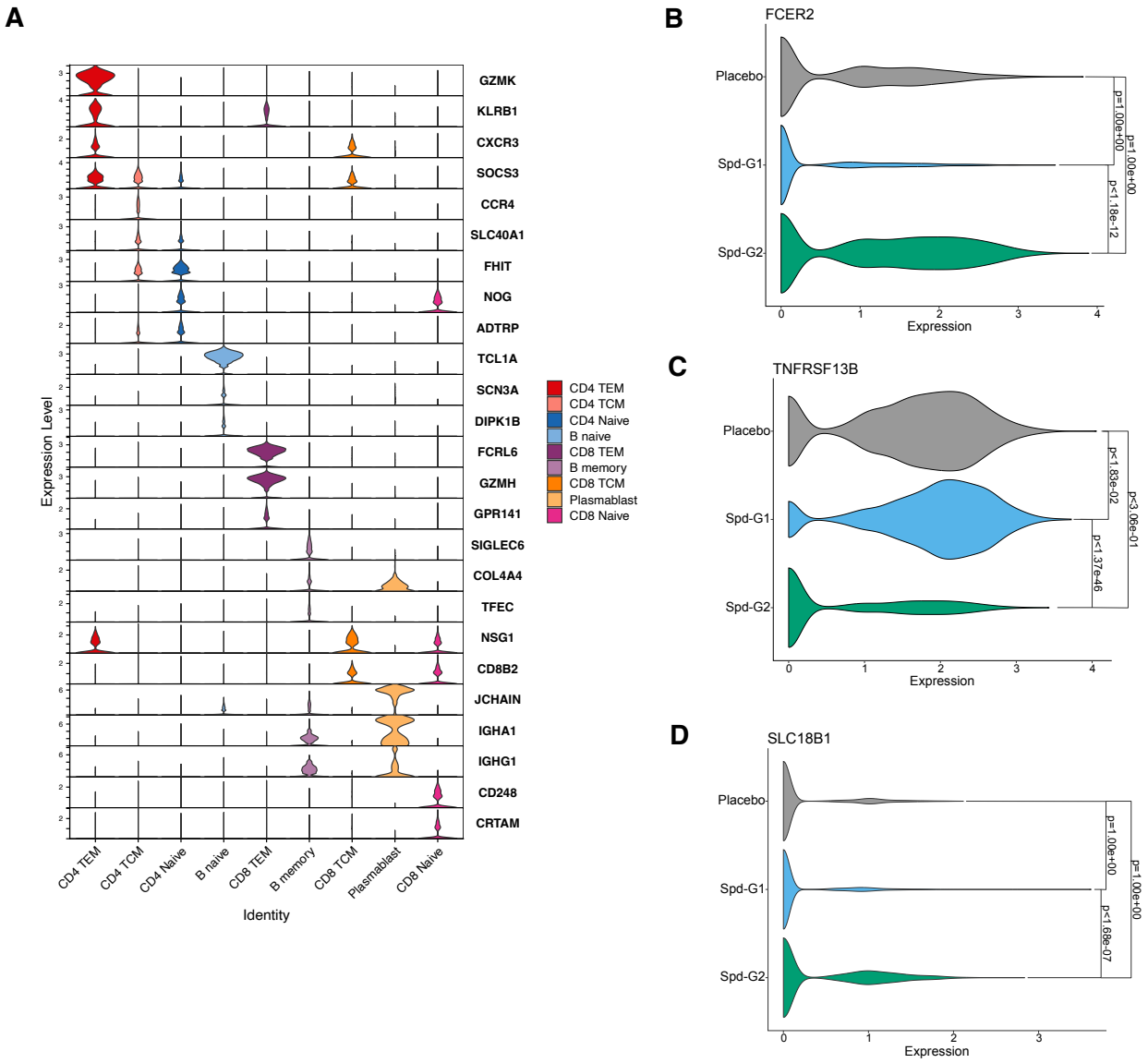


Figure S5: scRNAseq analysis revealed that spermidine treatment induces significant modifications in B cell pathways.

A: Violin plots showing normalized expression of the top three differentially expressed marker genes per identified cell type. **B-D:** Violin plots representing normalised gene expression for select genes differentially expressed between Placebo, G1 and G2 at baseline in the B memory cluster **(B)** FCER2 **(C)** TNFRSF13B **(D)** SLC18B1. Data are presented as violin and dotplots boxplots with statistical comparisons using the Wilcoxon rank-sum tests.

Figure S6

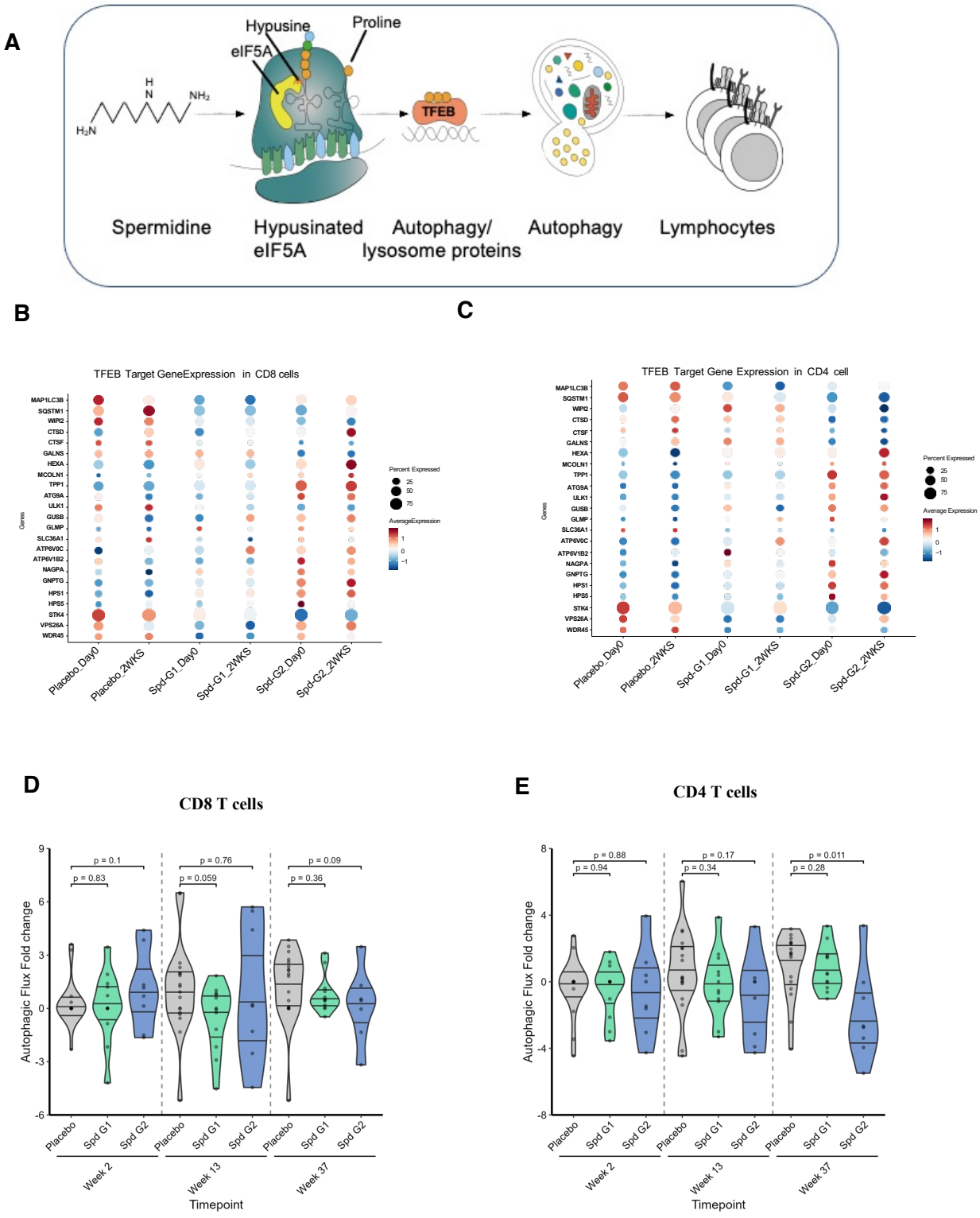
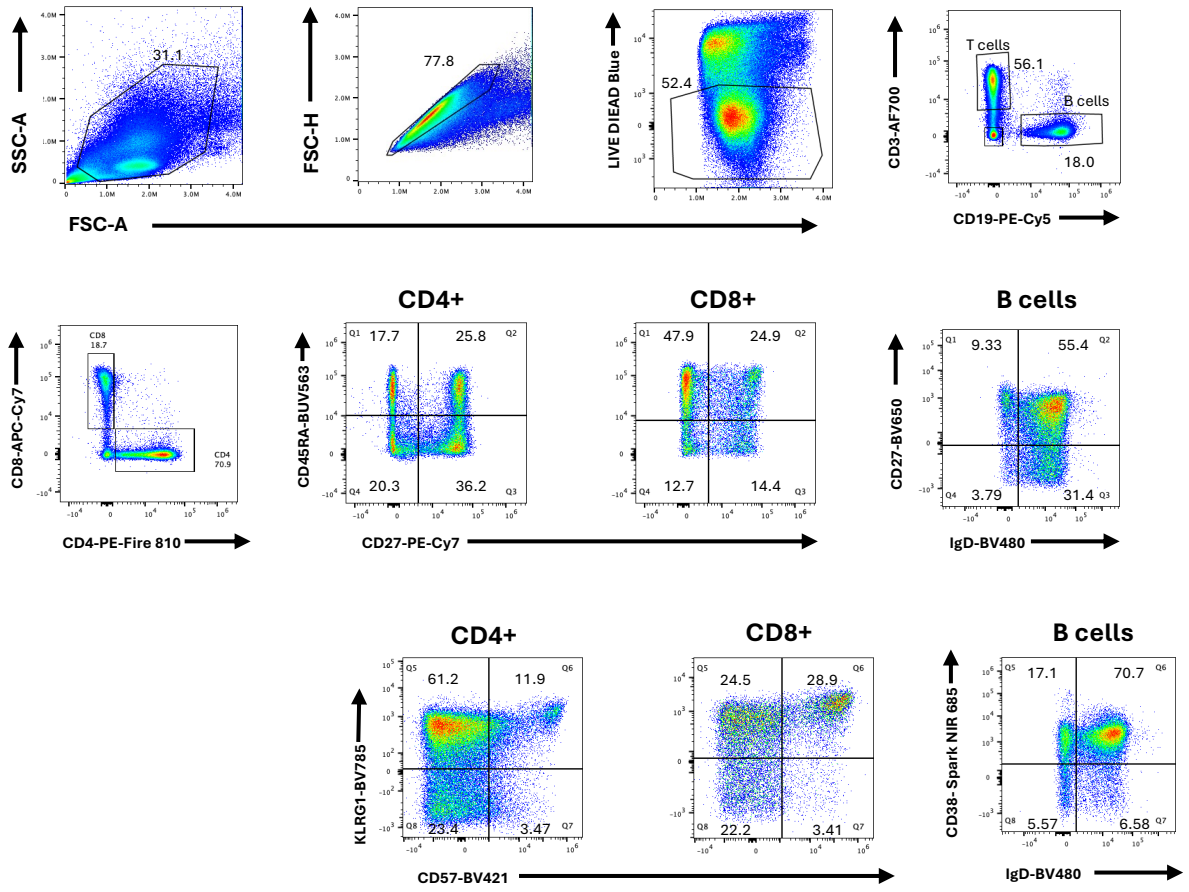


Figure S6: Spermidine supplementation does not induce TFEB or Autophagy in T Cell following COVID-19 vaccination.

A: Schematic representation of the proposed mechanism of action of spermidine supplementation in autophagy activation of immune cells. **B-C:** TFEB target gene expression (scaled) dot heatmaps and pathways of DEGs in **(B)** $CD8^+$ T cells **(C)** $CD4^+$ T cells. Annotations: Week 2 (W2); placebo and spermidine (Spd); group 1 (G1); group 2 (G2). **D-E:** Autophagic flux $\log_2$ fold change at all timepoints post spermidine supplementation in **(D)** CD8+ T cells and **(E)** $CD4^+$ T cells in spermidine Placebo (grey) (Spd) groups 1 (G1, green) and Spd group 2 (G2, blue). Annotations: spermidine (Spd); group 1 (G1); group 2 (G2). Exact P values are reported. Outliers were retained in all statistical analyses; in some plots, extreme values were omitted from display for clarity. Data are presented as violin plots showing median and IQR, with statistical comparisons using the two-sided Wilcoxon rank-sum test. Analyses in R 4.5.0; $\alpha = 0.05$.

Figure S7

A



B

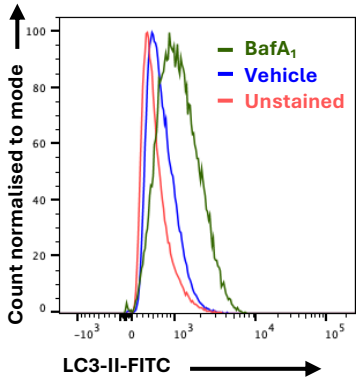


Figure S7: Gating strategies to determine cellular composition of B cells and CD4⁺ and CD8⁺ T cells. **A:** Gating strategy for $CD3^+$, $CD19^+$, $CD4^+$, $CD8^+$ subsets within live cells. **B:** Representative flow cytometry-based assay for LC3-II from PBMCs treated with or without bafilomycin A_1 ($BafA_1$) for 2 h prior to staining.

Figure S8

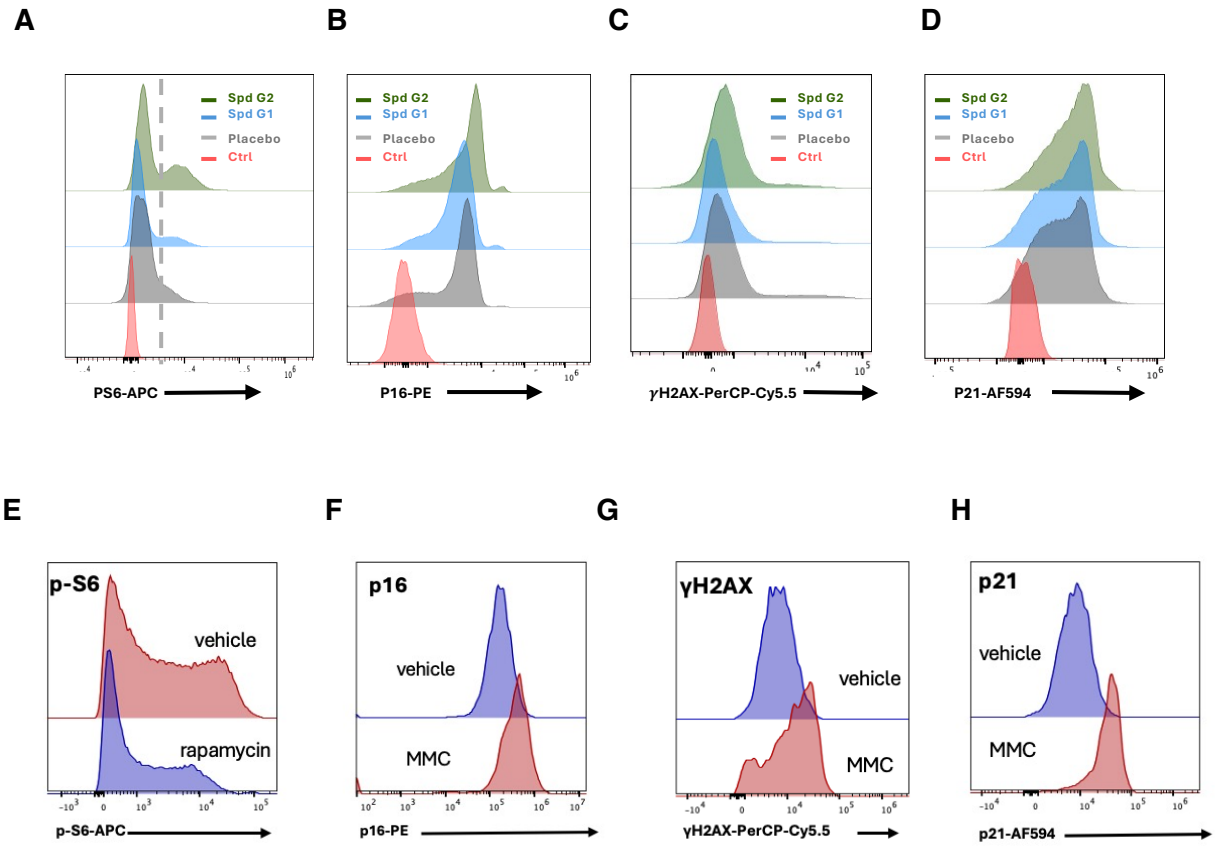


Figure S8: Flow Cytometry analysis of senescence markers at baseline /day0.

A-D: Representative flow cytometry for PBMC stained with antibodies targeting senescence markers, **(A)** pS6 **(B),** p16, (**C)** γ-H2AX and **(D)** p21 in placebo (grey), and the spermidine-treated (Spd) groups split into vaccine responders (G1, green) and non-responders (G2, blue). **E:** mTORC1 activity measured by phospho-S6 (p-S6) levels was assessed in human $CD4^+$ T cells activated for 3 days (1 µg/ml anti-CD3/28) in the presence of vehicle or 10 nM rapamycin (mTORC1 inhibitor). **F-H** Human dermal fibroblasts were treated with 100 nM mitomycin C (MMC) for 6 days and allowed to recover for a further 6 days to induce cellular senescence. Cells were then stained with antibodies targeting senescence markers, **(F)** p16**, (G)** p21, and **(H)** γ-H2AX, and measured by flow cytometry.

Supplementary Table 1: *Adverse reactions and COVID-19 cases.*

	Placebo (n=18)	**Spermidine (n=20)**
Serious adverse reactions (number)	0	0
Protocol-related adverse events (number)	0	0
PCR confirmed COVID-19 cases adverse events	4	7
Timepoint of PCR confirmed COVID-19 cases (number)		
Day 0/baseline to week 8	0	0
week 9	1	1
week 11	0	1
week 15	1	2
week 19	1	0
week 24	0	2
Non-COVID19 adverse events	2	2

Supplementary Table 2A : **Adjusted model (outcome ~ Group + Sex)**
Tests whether sex imbalance confounds the treatment effect.

Outcome	N	P_group	P_sex
Anti-spike IgG — Day 0	38	0.1381	0.635
Anti-spike IgG — Week 2	38	0.0488	0.9641
Anti-spike IgG — Week 13	38	0.0553	0.1878
Anti-spike IgG — Week 37	37	0.6115	0.0076
Memory B IgG ELISpot — Day 0	20	0.1654	0.8907
Memory B IgG ELISpot — Week 2	20	0.635	0.8529
nAb % Inhibition (Wuhan) — Day 0	38	0.9157	0.6046
nAb % Inhibition (Wuhan) — Week 2	37	0.0613	0.6732
nAb % Inhibition (Wuhan) — Week 13	35	0.3129	0.2976
nAb % Inhibition (Wuhan) — Week 37	38	0.7671	0.0033*

Supplementary Table 2B : Interaction model (outcome ~ Group × Sex)
Tests whether the treatment effect is modified by sex .

Outcome	N	P_group	P_sex	P_interaction
Anti-spike IgG — Day 0	38	0.2947	0.804	0.9079
Anti-spike IgG — Week 2	38	0.2936	0.794	0.7455
Anti-spike IgG — Week 13	38	0.2352	0.327	0.931
Anti-spike IgG — Week 37	37	0.38	0.163	0.4656
Memory B IgG ELISpot — Day 0	20	0.3005	0.91	0.8035
Memory B IgG ELISpot — Week 2	20	0.7401	0.949	0.9525
nAb % Inhibition (Wuhan) — Day 0	38	0.9875	0.661	0.9121
nAb % Inhibition (Wuhan) — Week 2	37	0.448	0.887	0.5494
nAb % Inhibition (Wuhan) — Week 13	35	0.3487	0.633	0.7045
nAb % Inhibition (Wuhan) — Week 37	38	0.7793	0.042*	0.9057

Supplementary Table 3: *List of antibodies and reagents.*

Antibody name	Description	Clone	Fluorophore	Source	Code/Catalogue number
CD3	Surface marker for T cells	UCHT1	BUV395	BD Biosciences	563546
CD11c	Surface marker for myeloid cells	B-ly6	BUV496	BD Biosciences	741139
CD45RA	Surface marker for T cells	HI100	BUV563	BD Biosciences	612927
CD19	Surface marker for B cells	SJ25C1	BUV615	BD Biosciences	612990
CD28	Surface marker for T cells	CD28.2	BUV661	BD Biosciences	741635
HLA-DR	Surface marker for monocytes	L203.rMAb	BUV737	BD Biosciences	752496
CD57	Surface marker for T cells and NK cells	NK-1	BV421	BD Biosciences	563896
NKG2A	Surface marker for NK cells	S19004C	Pacific Blue	BioLegend	375110
IgD	Surface marker for B cells	IA6-2	BV480	BD Biosciences	566187
CD45RO	Surface marker for T cells	UCHL1	BV510	BioLegend	304245
CD56	Surface marker for NK cells	HCD56	BV570	BioLegend	318329
CD27	Surface marker for T cells and B cells	O323	BV650	BioLegend	302827
KLRG1	Surface marker for T cells	2F1/KLRG1	BV785	BioLegend	138429
CCR7	Surface marker for T cells	G043H7	PerCP-Cy5.5	BioLegend	353219
CD16	Surface marker for monocytes	3G8	PE-Dazzle594	BioLegend	302053
CD127	Surface marker for T cells	A019D5	PE-Fire 700	BioLegend	351365
CD4	Surface marker for T cells	SK3	PE-Fire 810	BioLegend	344677

CD8	Surface marker for T cells	HIT8a	APC-Cy7	BioLegend	300925
CD14	Surface marker for B cells and monocytes	63D3	APC-Fire 810	BioLegend	367155
Perforin	Intracellular marker	B-D48	PE-Cy7	BioLegend	353316
Granzyme B	Intracellular marker	GB11	PE	eBioscience	12-8899-41
IFNγ	Intracellular marker	4S.B3	Ef506	ThermoFisher	69-7319-42
TNFα	Intracellular marker	MAb11	FITC	BioLegend	502906
IL-2	Intracellular marker	MQ1-17H12	BV650	BioLegend	500333
Phospho-S-6	Intracellular marker	cupk43k	APC	ThermoFisher Scientific	417-9007-42
P21	Intracellular marker	12D1	Alexa Fluor® 594	Cell Signaling	11850
P16	Intracellular marker	EPR1473	PE	abcam	ab209579
γH2Ax	Intracellular marker	N1-431	PerCP-Cy™5.5	BD Biosciences	564718