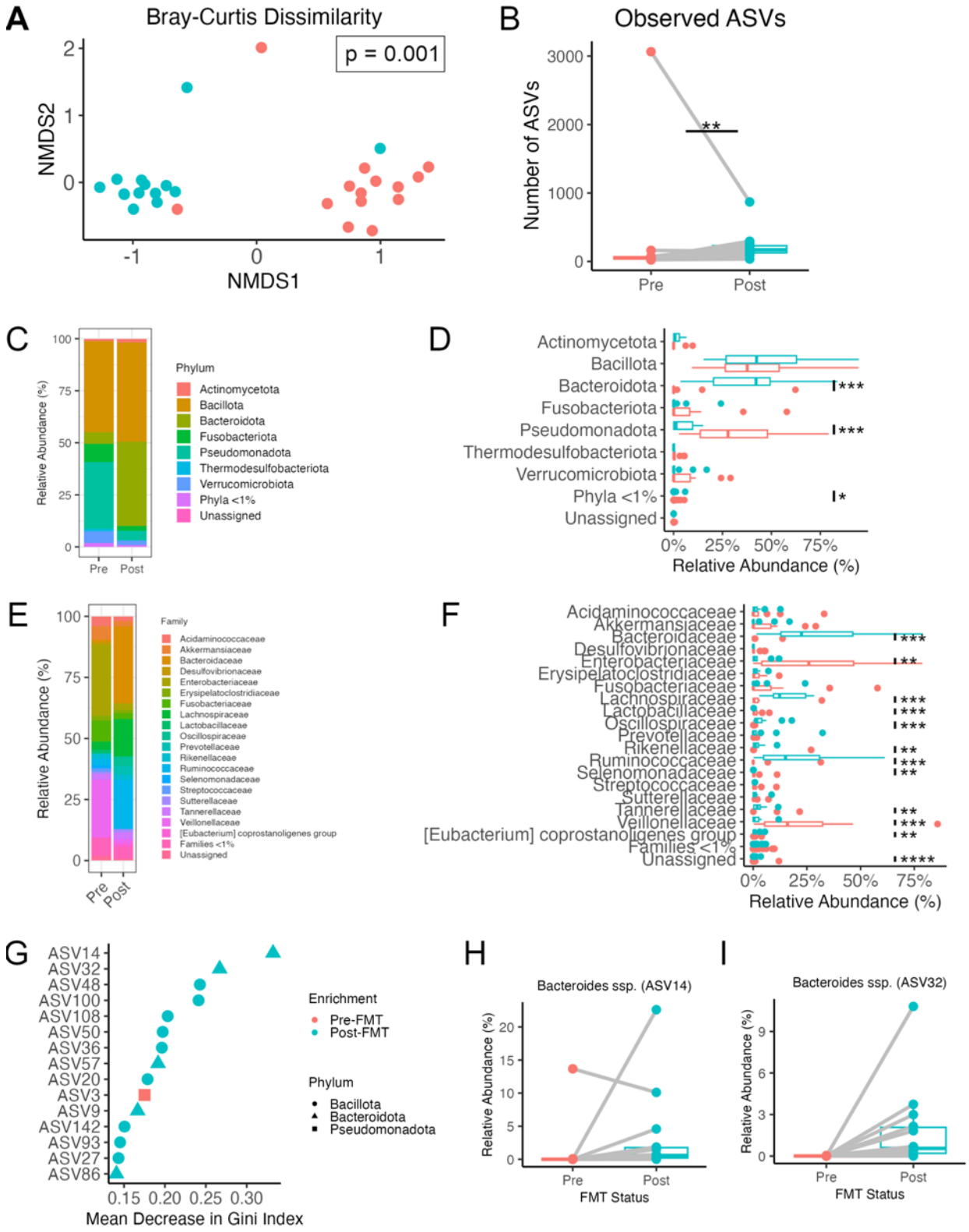
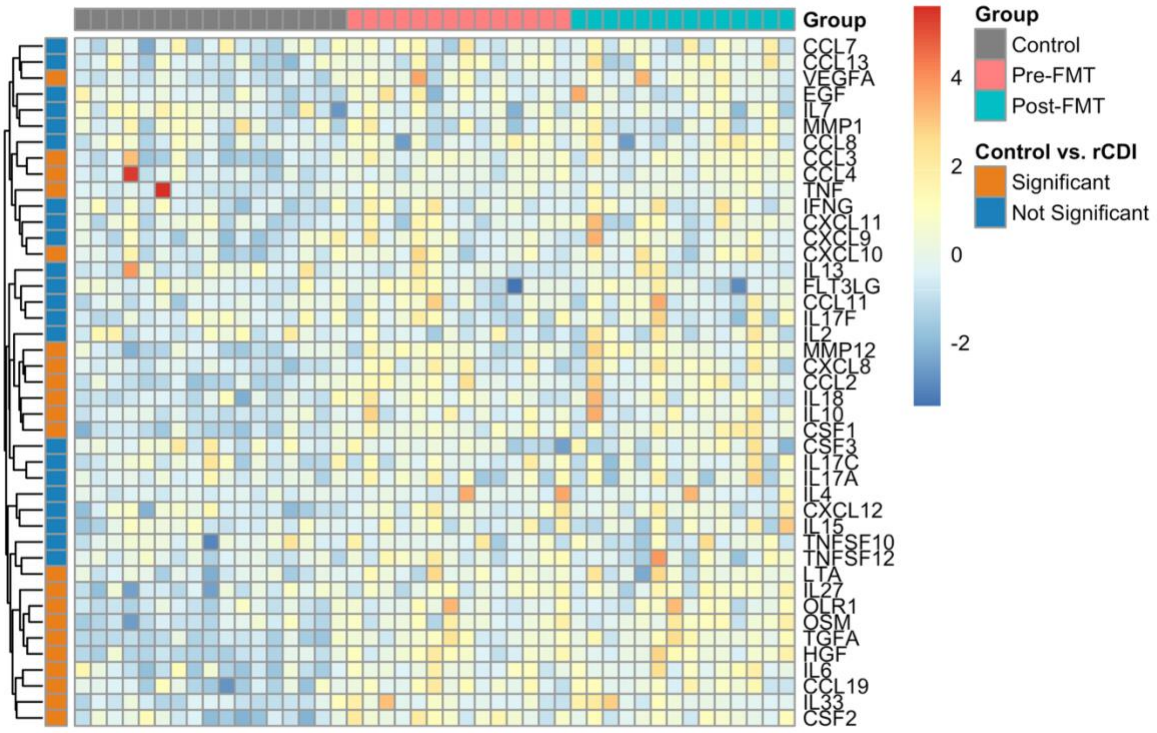
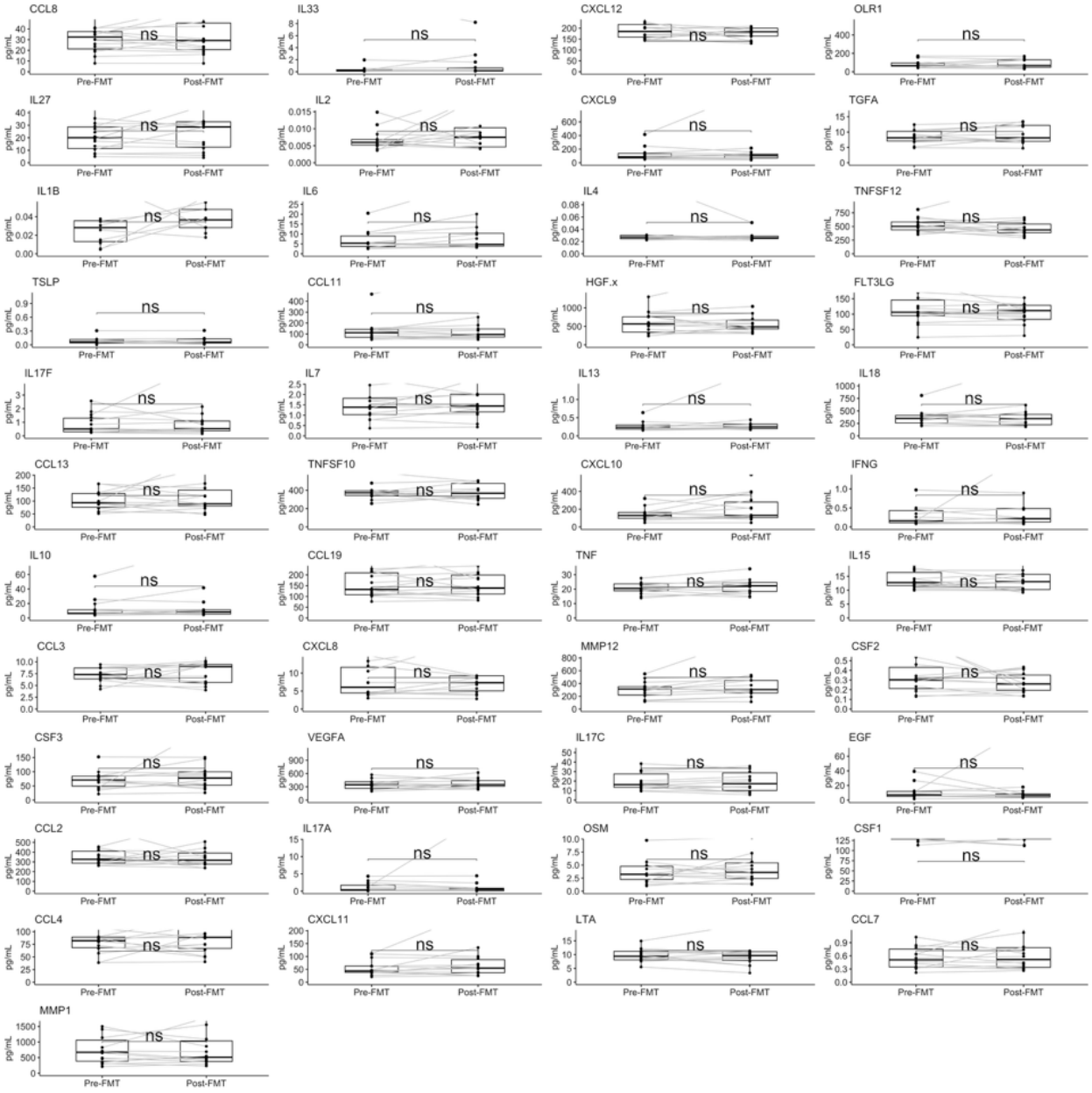

A
Bray-Curtis Dissimilarity
p = 0.001
NMDS2
NMDS1
B
Observed ASVs
Number of ASVs
**
Pre
Post
C
Relative Abundance (%)
Phylum
Actinomycetota
Bacillota
Bacteroidota
Fusobacteriota
Pseudomonadota
Thermodesulfobacteriota
Verrucomicrobiota
Phyla <1%
Unassigned
D
Relative Abundance (%)
E
Relative Abundance (%)
Family
F
Relative Abundance (%)
G
Mean Decrease in Gini Index
Enrichment
Pre-FMT
Post-FMT
Phylum
Bacillota
Bacteroidota
Pseudomonadota
H
Bacteroides ssp. (ASV14)
Relative Abundance (%)
FMT Status
I
Bacteroides ssp. (ASV32)
Relative Abundance (%)
FMT Status

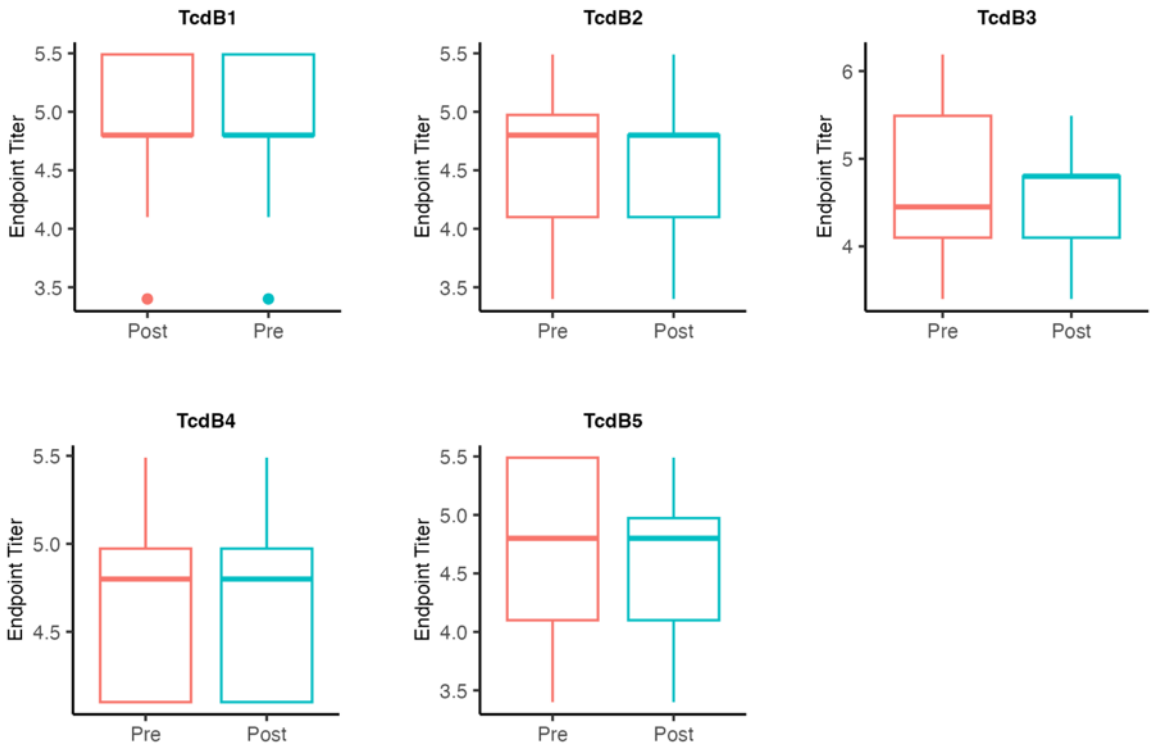
Supplemental Figure 1: FMT Significantly Alters the Stool Microbiome of Recurrent CDI Patients. A) Non-Metric Multidimensional Scaling (NMDS) plot of Bray-Curtis Dissimilarity among stool samples from patients pre- and post-FMT therapy (n=14 each). B) Microbiome richness (number of observed ASVs) for patient samples pre- and post-FMT. C-D) Phylum-level community composition, represented as either C) averaged relative abundance across samples within each group or D) boxplots representing the relative abundance median and quartiles for each group. E-F) Family-level community composition, represented as either E) averaged relative abundance across samples within each group or F) boxplots representing the relative abundance median and quartiles for each group. G) Ranked variable importance from a random forest model using ASV-level data to predict FMT status. H-I) Relative abundance plots for highly ranked ASVs from the random forest analysis. Lines represent paired patient samples pre- or post-FMT. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$



Supplemental Figure 2: Chemokine expression is significantly altered in recurrent CDI patients compared to controls. Heatmap of cytokines measured from biopsies of healthy control (n=17) or recurrent CDI patients (n=14 each pre- and post-FMT). Heatmap values represent Z scores of $\log_{10}$-transformed cytokine values. Chemokines were hierarchically clustered according to similarity. Significant chemokines between recurrent CDI (combined pre- and post) and healthy controls were calculated using a Wilcoxon ranked sum test and are indicated by the color key beside each row.

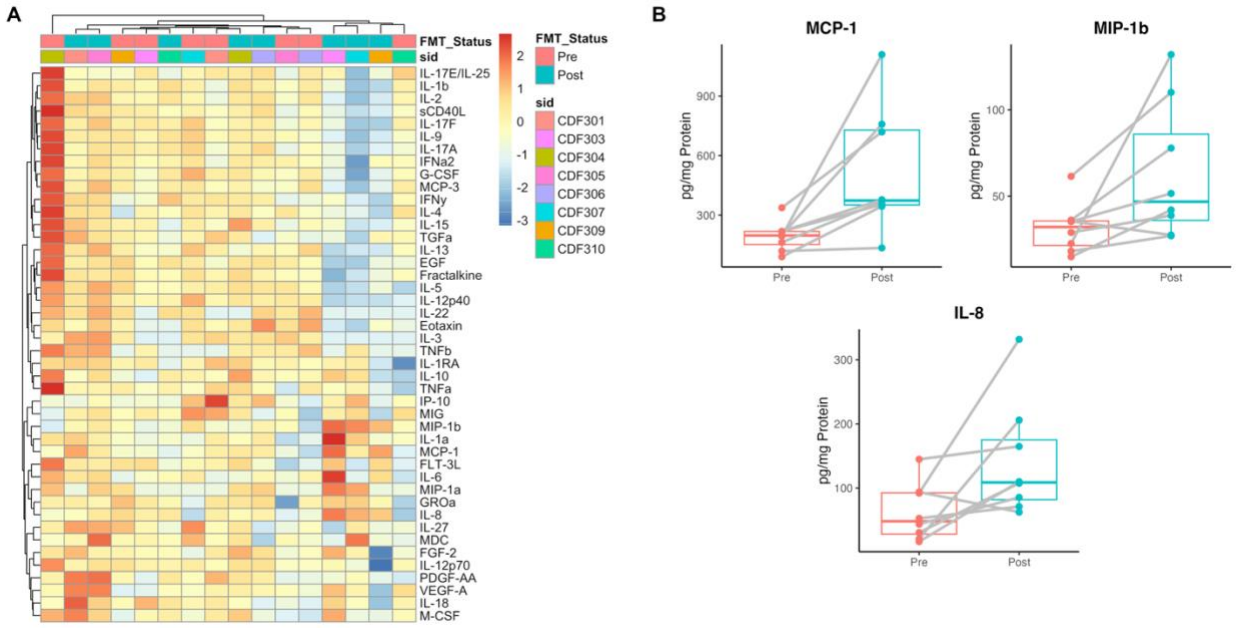


Supplemental Figure 3: Serum cytokines are unchanged between pre- and post-FMT samples. Cytokine concentrations from serum samples pre- and post-FMT (n=14 each) measured using a commercial multiplex proximity extension assay. Statistics derived from a two-tailed paired t test without adjustment for multiple comparisons. Nonsignificant p values are denoted as “ns”.



Supplemental Figure 4: Anti-TcdB antibodies are unchanged following FMT.

Endpoint titers of TcdB-specific IgG antibodies from pre- and post-FMT plasma samples (n=16 each). Titers were measured for each of the major TcdB subtypes via ELISA. Boxplots represent median and quartiles for each group. No comparisons were significant using a Wilcoxon rank sum test.



Supplemental Figure 5: Biopsy Cytokines are Broadly Unchanged Post-FMT. A) Heatmap of cytokines measured from biopsies collected a subset of subjects pre- and post-FMT (n=8 each). Cytokine concentrations were normalized to total protein within each sample before analysis. Heatmap values represent Z scores of $\log_{10}$-transformed cytokine values. Both samples and analytes were hierarchically clustered according to similarity. B) Boxplots of select chemoattractant cytokines that trended higher in post-FMT biopsies.

Supplemental Table 2: Details of Antibodies Used for Spatial Transcriptomics

Primary Antibody Specificity	Fluorochrome	Company	Catalog no.	Clone	Secondary Antibody	Fluorochrome	Company	Catalog No.
DNA	SYTO13	Invitrogen	S7575	-	-	-	-	-
PanCK	AF594	Novus Biologicals	NBP2-33200AF594	AE-1/AE-3	-	-	-	-
CD45	-	Dako	M0701	PD7/26 + 2B11	Goat Anti-mouse	AF532	Invitrogen	A1002
CD3	-	Cell Marque	103-R94	MRQ-39	Goat anti-rabbit	AF647	Invitrogen	A21245

Supplemental Table 3: Details of Antibodies Used for Flow Cytometry Analysis of Human PBMCs.

	SPECIFICITY	FLUOROCHROME	Company	Catalog no.	Clone
1	CD39	BUV615	BD Biosciences	751269	TU66
2	CD45	PerCP	Bio Legend	368506	2D1
3	CD3	BV510	Bio Legend	344828	SK7
4	CD4	RB744	BD Biosciences	570466	SK3
5	CD8	BUV496	BD Biosciences	741199	SK1
6	CD25	PE-Alexa Fluor700	Thermo-Fisher	MHCD2524	CD25-3G10
7	TCR γδ	PerCP-eFluor 710	Thermo-Fisher	46-9959-42	B1.1
8	CD14	Spark Blue 550	Bio Legend	367148	63D3
9	CD16	Spark blue 515	Bio Legend	302080	3G8
10	CD11c	eFluor 450	Thermo Fisher	48-0116-42	3.9
11	CD19	Spark NIR 685	Bio Legend	302270	HIB19
12	CD278 (ICOS)	BUV805	BD Biosciences	568038	C398.4A
13	CD28	BV605	Bio Legend	302968	CD28.2
14	CXCR5	BV711	BD Biosciences	740737	RF8B2
15	CCR7	BV421	Bio Legend	353208	G043H7
16	CD27	APC-H7	BD Biosciences	560222	M-T271
17	CD69	BV650	Bio Legend	310934	FN50
18	CD45RA	BUV395	BD Biosciences	740315	5H9
19	CD95 (FAS)	PE-Cy5	Bio Legend	305610	DX2
20	CD127	APC-R700	BD Biosciences	565185	hIL-7R-M21
21	CD137 (4-1BB)	PE-Dazzle594	Bio Legend	309826	4B4-1
22	CCR6	BUV737	BD Biosciences	612780	11A9
23	CCR5	BUV563	BD Biosciences	741401	2D7/CCR5
24	CD123	Super Bright 436	Thermo-Fisher	62-1239-42	6H6
25	CXCR3	PE-Cy7	Bio Legend	353720	G025H7
26	HLA-DR	PE-Fire810	Bio Legend	307683	L243
27	CD38	APC-Fire810	Bio Legend	303550	HIT2
28	b7 integrin	FITC	Bio Legend	321212	FIB504
29	PD-1 (CD 279	BV785	Bio Legend	329930	EH12.2H7
30	CD134 (OX40)	APC	BD Biosciences	563473	ACT3
31	CD154 (CD40L)	PE	BD Biosciences	555700	TRAP1
32	Viability	Live Dead UV Blue	Thermo Fisher	L34962	