

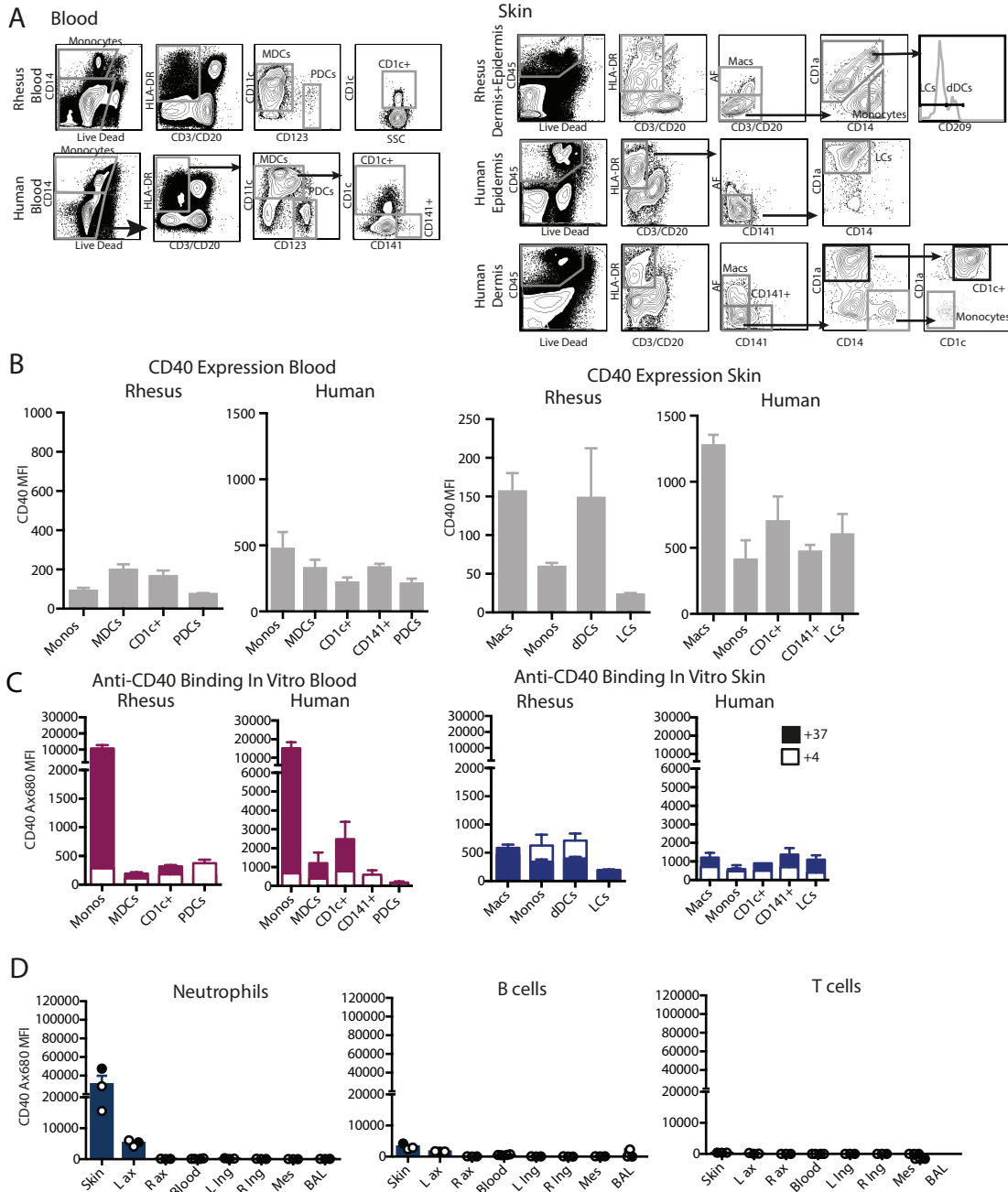
Cell Reports, Volume 28

Supplemental Information

**Monocytes Acquire the Ability
to Prime Tissue-Resident T Cells
via IL-10-Mediated TGF- β Release**

Elizabeth A. Thompson, Patricia A. Darrah, Kathryn E. Foulds, Elena Hoffer, Alayna Caffrey-Carr, Sophie Norenstedt, Leif Perbeck, Robert A. Seder, Ross M. Kedl, and Karin Loré

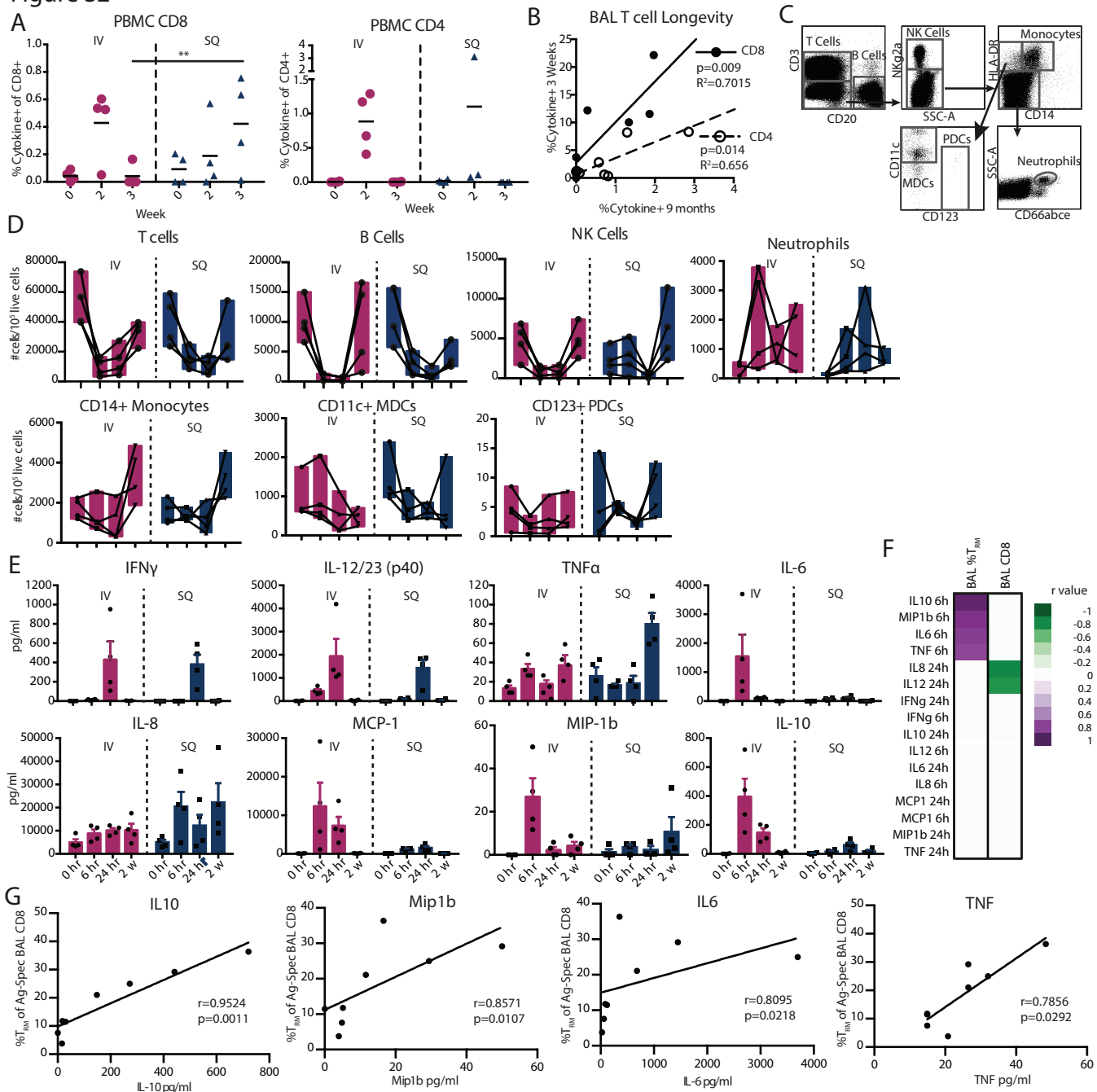
Figure S1



Supplemental Figure 1: CD40 expression and binding, Related to Figure 1

(A) Representative gating for rhesus and human APCs in blood and skin used throughout study. (B) Subsets identified above were evaluated for CD40 expression at baseline. (C) APCs were cultured in vitro for 24 hours with 1ug/ml anti-CD40 Alexa-680 at 37C (for Ab binding and internalization) or 4C (for Ab binding only) from human or rhesus blood and skin. (D) In vivo binding of anti-CD40 Alexa-680 following subcutaneous administration to Neutrophils, B cells, and T cells in different tissues. (Mean±SEM)

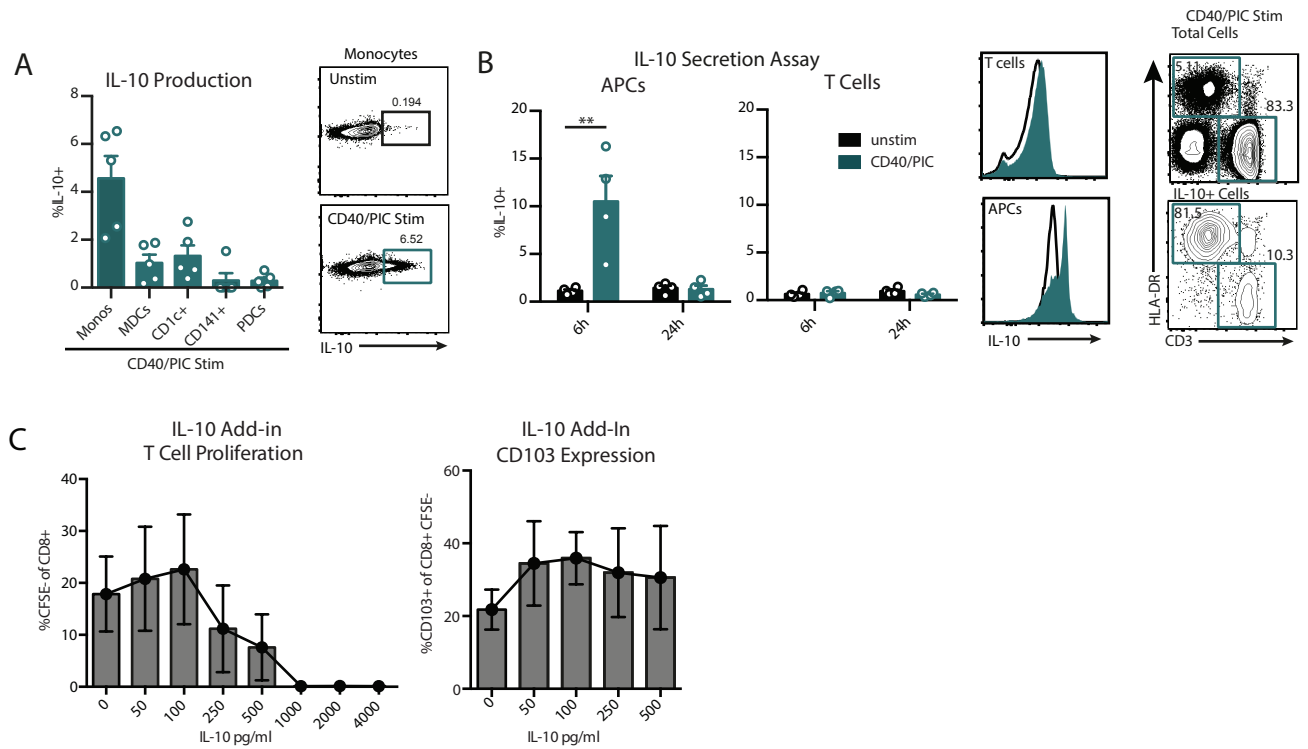
Figure S2



Supplemental Figure 2: Immunization of rhesus macaques with anti-CD40Ab and Poly IC:LC, Related to Figure 2

Rhesus macaques were immunized as described in Fig 2. Circulating PBMCs were monitored for antigen-specific T cells (2-3 weeks post immunization) and innate activity (6-24 hours after immunization). (A) Antigen-specific CD8 and CD4 T cell responses in PBMCs. (B) Correlation of antigen-specific CD8 and CD4 T cell responses in the BAL at 3 weeks and 9 months post immunization. (C) Gating scheme for evaluation of innate cell mobilization. (D) Frequencies of cell subsets in the blood following immunization as determined by flow cytometry. (E) Cytokine levels in plasma (pg/ml). (Mean \pm SEM) (F) All cytokines measured together with frequency antigen-specific BAL CD8 T cells and frequency of BAL T_{RM} were analyzed using a multivariate non-parametric Spearman's test. Correlations with a non-significant p value (>0.05) had Spearman's coefficient changed to 0. (G) Correlation of frequency of antigen-specific TRM in BAL at 3 weeks (Y-axis) with cytokines showing a significant correlation (X-axis).

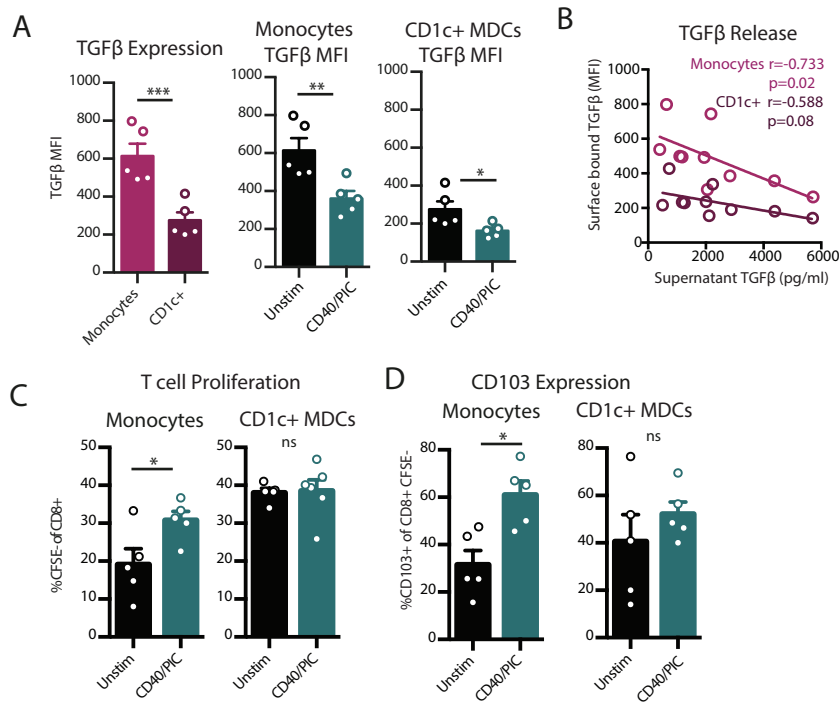
Figure S3



Supplemental Figure 3: Monocytes are main producer of IL-10, Related to Figure 3

(A) Blood APCs were stimulated overnight with anti-CD40Ab (1ug/ml) and Poly I:C (5ug/ml) (CD40/PIC) in the presence of brefeldin-A and stained for intracellular IL-10 (Mean±SEM and representative staining). (B) Blood APCs were cultured in a 1:5 ratio with naïve allogeneic T cells for 6 hours or 24 hours with or without anti-CD40Ab and Poly I:C. IL-10 secretion was captured on the cell surface for the final 45 minutes of stimulation using the cytokine secretion assay to account for potential bystander activation of T cells that would be blocked by brefeldin-A. Summary of %IL-10+ APCs and T cells (Mean±SEM). Representative plot showing histograms of IL-10 production within the T cell and APC compartment in unstimulated or stimulated conditions at 6 hours. Dot plots show total cell proportion of HLA-DR+ cells (APCs) and CD3+ cells (T cells) and proportion of subsets producing IL-10. (C) Human naïve CFSE labeled T cells were co-cultured with unstimulated allogeneic blood APCs and recombinant IL-10 was added in increasing concentrations. Frequency of proliferating CD8 T cells expressing CD103 after 6 days of culture (Mean±SEM)

Figure S4



Supplemental Figure 4: Monocytes are main producer of TGFβ, Related to Figure 4

(A) Surface expression of TGFβ1,2,3 after 6 hours in culture. (B) Correlation of secreted TGFβ1 (X-axis) and monocyte or CD1c+ MDC TGFβ MFI (Y-axis). (C-D) Purified CD14+ monocytes or CD1c+ MDCs were cultured with CFSE labeled allogenic naïve T cells. Frequency of proliferating CD8 T cells (C) and CD103 expression (D). (Mean±SEM)

Supplemental Table 1: Antibodies used for flow cytometry, Related to STAR Methods

Human Panels

Antibody	Fluorochrome	Clone	Company	Panel
CD20	Cy7APC	L27	BD	CD40 Binding/Expression
CD3	Cy7 APC	SP34-2	BD	CD40 Binding/Expression
CD40	FITC	5C3	Biolegend	CD40 Binding/Expression
CD11c	Cy7PE	3.9	Biolegend	CD40 Binding/Expression
CD123	PerCP Cy55	7G3	Biolegend	CD40 Binding/Expression
CD14	BV510	M5E2	Biolegend	CD40 Binding/Expression
HLA-DR	BV570	L243	Biolegend	CD40 Binding/Expression
CD1c	APC	AD5-8E7	Miltenyi	CD40 Binding/Expression
CD141	VioBlue	AD5-14H12	Miltenyi	CD40 Binding/Expression
CD1a	PE	SK9	BD	Human Skin
CD20	Cy7APC	L27	BD	Human Skin
CD3	Cy7APC	SP34-2	BD	Human Skin
CD45	BV605	HI30	BD	Human Skin
CD40	FITC	5C3	Biolegend	Human Skin
CD14	BV510	M5E2	Biolegend	Human Skin
HLA-DR	Cy55PE	Tü36	Life Tech	Human Skin
CD1c	APC	AD5-8E7	Miltenyi	Human Skin
CD141	VioBlue	AD5-14H12	Miltenyi	Human Skin
CD20	Cy7APC	L27	BD	IL-10 Secretion/Capture
CD3	Cy7APC	SP34-2	BD	IL-10 Secretion/Capture
CD11c	Cy7PE	3.9	Biolegend	IL-10 Secretion/Capture
CD14	BV510	M5E2	Biolegend	IL-10 Secretion/Capture
HLA-DR	TRPE	TU36	Life Tech	IL-10 Secretion/Capture
CD141	VioBlue	AD5-14H12	Miltenyi	IL-10 Secretion/Capture
CD1c	PE Vio770	AD5-8E7	Miltenyi	IL-10 Secretion/Capture
IL-10	PE	catch reagent	Miltenyi	IL-10 Secretion/Capture
CD20	Cy7 APC	L27	BD	IL-10 ICS
CD3	Cy7 APC	SP34-2	BD	IL-10 ICS
IL-10	APC	JES3-19F1	BD	IL-10 ICS
CD11c	Cy7 PE	3.9	Biolegend	IL-10 ICS
CD14	BV510	M5E2	Biolegend	IL-10 ICS
HLA-DR	BV570	L243	Biolegend	IL-10 ICS
CD123	PerCp Cy55	7G3	Biolgened	IL-10 ICS
CD1c	FITC	AD5-8E7	Miltenyi	IL-10 ICS
CD141	PE	AD5-14H12	Miltenyi	IL-10 ICS
CD103	PE Cy7	Ber-ACT8	BD	In vitro T cell
CD3	Cy7APC	SP34-2	BD	In vitro T cell
CD4	BV605	L200	BD	In vitro T cell
CD8	PE	RPA-T8	BD	In vitro T cell
CD20	Cy7APC	L27	BD	TGFb expression
CD3	Cy7APC	SP34-2	BD	TGFb expression
CD14	BV510	M5E2	Biolegend	TGFb expression
HLA-DR	TRPE	TU36	Life Tech	TGFb expression
CD141	VioBlue	AD5-14H12	miltenyi	TGFb expression
CD1c	PE Vio770	AD5-8E7	miltenyi	TGFb expression
TGFb1,2,3	AF700	1D11	R&D	TGFb expression

Rhesus Macaque Panels

Antibody	Fluorochrome	Clone	Company	Panel
CD3	Cy7APC	SP34-2	BD	Rhesus Innate Phenotype
CD70	PE-CF594	Ki-24	BD Horizon	Rhesus Innate Phenotype
CD80	BV605	L307.4	BD Horizon	Rhesus Innate Phenotype
CD123	PerCP Cy55	7G3	BD Pharmingen	Rhesus Innate Phenotype
NKG2a	PE	Z199	Beckman Coulter	Rhesus Innate Phenotype
CCR7	BV421	G043H7	Biolegend	Rhesus Innate Phenotype
CD11c	Cy7PE	3.9	BioLegend	Rhesus Innate Phenotype
CD16	BV570	38G	BioLegend	Rhesus Innate Phenotype
CD20	BV650	2H7	BioLegend	Rhesus Innate Phenotype
CD14	QD800	TüK4	Invitrogen	Rhesus Innate Phenotype
HLA-DR	Cy55PE	TU36	Invitrogen	Rhesus Innate Phenotype
BDCA1	FITC	AD5-8E7	Miltenyi	Rhesus Innate Phenotype
CD66	APC	TET2	Miltenyi	Rhesus Innate Phenotype
CD1a	PE	SK9	BD	Rhesus Tracking
CD209	PerCP Cy55	DCN46	BD	Rhesus Tracking
CD3	Cy7APC	SP34-2	BD	Rhesus Tracking
CD45	BV605	D058-1283	BD	Rhesus Tracking
CD11c	Cy7PE	3.9	Biolegend	Rhesus Tracking
CD123	BV510	6H6	Biolegend	Rhesus Tracking
CD14	BV785	M5E2	Biolegend	Rhesus Tracking
CD20	BV570	2H7	Biolegend	Rhesus Tracking
HLA-DR	TRPE	TU36	Life Tech	Rhesus Tracking
BDCA1	FITC	AD5-8E7	Miltenyi	Rhesus Tracking
CD66	APC	TET2	Miltenyi	Rhesus Tracking
CD3	Cy7APC	SP34-2	BD	Rhesus T cell
CD45RA	Cy7PE	L48	BD	Rhesus T cell
IFNg	FITC	B27	BD	Rhesus T cell
CD103	APC	2G5	Beckman Coulter	Rhesus T cell
CCR7	BV421	G043H7	Biolegend	Rhesus T cell
CD4	BV785	OKT4	BioLegend	Rhesus T cell
CD69	Cy5PE	FN50	BioLegend	Rhesus T cell
CD8	BV711	RPA-T8	BioLegend	Rhesus T cell
IL2	BV605	MQ1-17H12	BioLegend	Rhesus T cell
TNFa	BV650	MAB11	Biolegend	Rhesus T cell
CCR8	PerCp	19704	R&D	Rhesus T cell
CCR9	PE	112509	R&D	Rhesus T cell