Supplementary Materials for

Vaccine targeting SIVmac251 protease cleavage sites protects macaques against vaginal infection

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This PDF file includes:

Supplemental methods (moved from main manuscript with added details)
Figures. S1 to S12
Tables S1 to S14 (a Table S14 is added with catalog numbers of antibodies used for FACS analysis)

Supplemental Methods

Viral load analysis. Viral RNA was isolated from plasma samples using the Maxwell 16 Viral Total Nucleic Acid Purification kit on the Maxwell 16MDx instrument (Promega, Madison WI). Viral RNA was then quantified using a highly sensitive qRT-PCR assay based on a previously published protocol (116).

Bio-Plex multiplexed cytokine/chemokine assay. We used a customized cytokine/chemokine panel targeting 14 (pro- or anti-) inflammatory cytokines/chemokines including Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), Macrophage Chemo-attractant Protein 1 (MCP-1), Monocyte Inflammatory Protein 1 alpha (MIP-1α), Monocyte Inflammatory Protein 1 beta (MIP-1β), CCL5/RANTES, IFN-γ inducible protein 10 (IP-10/CXCL-10), Tumor Necrosis Factor alpha (TNF-α), Interferon gamma (IFN-γ), IL-1α, IL-8, IL-10, IL-17a, IL-1β, and IL-6. Mucosal inflammatory responses and cellular recall responses (see below) were measured using cervicovaginal lavage (CVL) and PBMC culture supernatant samples, respectively. Antibodies and protein standards used in these assays are listed in Table S9. The CVL sample collection method was described previously(13). For the CVL assay, PBS with 1% BSA (Assay buffer) was used both as general diluent and blank controls, while RPMI-1640 Culture Medium (Sigma, R8758-6X500ML) was used as diluent for standards and blank controls for the PBMC supernatant samples. In order to generate the standard curves, we prepared a cytokine/chemokine protein standard pool with final concentrations listed in Table S10, using PBS with 1% BSA as diluent. We then prepared eight four-fold serial dilutions of this cytokine/chemokine protein standard pool and transferred 50 μL of each dilution to a 96-well Bio-Plex Pro^{TM} Flat bottom plate (BioRad, Catalog# 171025001). Final standard curves were optimized and generated using Bio-Plex Manager 6.1 software, where coefficient of variation (%CV) was no more than 30%

and percentage of recovery was at least 70. 20 µg of capture antibody for each cytokine/chemokine type was coupled to 1.25×10^7 Bio-Plex Pro™ Magnetic COOH Beads (BioRad, Catalog# MC10053-01) using Bio-Plex Amine Coupling Kit (Bio-Rad, Catalog# 171-406001) according to the manufacturer's suggestions and diluted to a final concentration of 5×10^6 beads/mL. 50 µL of undiluted sample was added to each well containing ~420,000 coupled magnetic beads/capture antibody type (1 in 600 dilutions) and incubated while shaking at high speed (~1000rpm) for 30 seconds, then at medium speed (~300rpm) for one hour on a plate shaker at room temperature. After washing the plate 3× (MAG3× setting on BioRad wash station) with wash buffer (PBS with 0.05% Tween20), 30 µl of biotinylated detection antibody at 1µg/ml was added to each well and again incubated while shaking for 30 minutes at room temperature. After another routine wash, 50 µl of Streptavidin-PE in 1:100 dilution of BioRad 1× stock (Catalog# 171304501) was added per well and incubated while shaking for 10 min at room temperature. A final wash was performed and afterwards 125 µl of assay buffer was added to each well. After shaking the plate for 10 seconds, we ran the plate on a Bio-Rad Bio-Plex™ 200 System. Each bead fluorescence intensity and corresponding protein concentration (pg/ml) were generated by Bio-Plex Manager 6.1 software. To take into account dilution variations during CVL sample collections, cytokine/chemokine concentrations were all normalized to total protein concentrations in the same sample. CVL total protein concentration was quantified using NanoOrange® protein quantitation kit (Thermo Fisher Scientific) according to the supplier's protocol.

MHC typing and Bio-Plex multiplexed antibody assay. Plasma IgG antibodies to SIV antigens were quantified by largely following the previously published protocols (22, 23) with slight modifications. Briefly, 20 µg of antigen peptide (synthesized by Genscript, Piscataway,

NJ) was coupled to 1.25 x 10^6 Bio-Plex Pro™ Magnetic COOH Beads (Bio-Rad) using a Bio-Plex Amine Coupling Kit (Bio-Rad). 50 µl plasma (1:80 diluted) was incubated with 2,500 beads/antigen type. SIV-specific IgG was detected with phycoerythrin-labelled mouse anti-monkey IgG (Southern Biotech, Birmingham, AL) at 5 µg/ml. Bead fluorescence intensities were acquired on the Bio-Plex 200 system (Bio-Rad) and converted to concentrations based on estimation using a PCS2 monoclonal antibody (National Microbiology Laboratory, Canada) as standard.

High-resolution MHC typing was conducted by Wisconsin National Primate Research Center Genetics Services (https://www.primate.wisc.edu/?page_id=4512).

IFN-γ enzyme-linked immunospot (ELISPOT) assay. Freshly isolated PBMCs (2-2.5 × 10^5/well) were stimulated for 16-18 hours in a 96-well pre-coated plate (ELISpotPLUS kit, MABTECH Inc., Mariemont, OH) at 37°C in a 5% CO_2 incubator. Stimulation was performed with PCS peptide pool, targeting subsets of PCSs (PCS1-4, PCS5-8 and PCS9-12, respectively), at 2.5µg/ml per individual peptide. The sequences of these peptides (Sigma-Aldrich) were listed in Table S11. Cells stimulated with 5.0 µg/ml of concanavalin A (Sigma Chemical, St. Louis, MO) were used as positive control. All tests were performed in duplicate. Wells were imaged with an AID ELISPOT reader (AID, Strassberg, Germany), and counted by AID EliSpot Reader version 3.2.3, with set parameters for spot size, intensity and gradient. Background (mean of wells without peptide) levels were subtracted from each well on the plate. A response was considered positive if the mean number of SFCs of duplicate sample wells exceeded twice the background and was >50 SFC per 1 × 10^6 cells.

Cell culture of PBMCs and PCS peptide stimulation for analysis of multiple cytokine secretions or T cell subsets. 2.0×10^6 PBMCs/ml in R10 media (RPMI1640-HyClone + 10%

FBS + 2% antibiotic/antimycotic, Thermo Fisher Scientific) were added into each well of a 96-well plate, and cultured in media only (no-stimulation negative control), in the presence of 0.001μg/ml PMA with 0.01μg/ml Ionomycin (positive control), or with 1.5μM SIV PCS peptide pools (Sigma-Aldrich, Table S11), overnight at 37°C with 5% CO_2. Cytokines secreted into the culture supernatants were analyzed by the Bio-Plex multiplexed cytokine assay as described above. For flow cytometry analysis (with intracellular staining) of T cell subsets, PBMC culture and stimulation were performed in the presence of protein transport inhibitors, 50nL GolgiStop™ (monensin) and 200nL GolgiPlug (brefeldin A).

Animal anesthesia. Sedation (ketamine alone, or ketamine/dexmedetomidine, atipamezole for reversal) was provided during the experimental procedures. For anesthesia, animals were immobilized against the front of the cage by a squeeze back mechanism, and anesthetized using up to 7 mg/kg ketamine (i.m.) and up to 0.03 mg/kg dexmedetomidine (i.m.) to be reversed at the conclusion of a procedure by up to 0.3 mg/kg atipamezole (i.v. or i.m.). Any additional anesthesia was administered only in consultation with a WNPRC veterinarian. Alternative anesthesia would only be used as directed by a WNPRC veterinarian. The duration of anesthesia was usually less than 45 minutes. Monitoring of anesthesia recovery was documented every 15 min until the animal was sitting upright, then every 30 minutes until the animal was fully recovered from the anesthesia.

Menstrual phase distributions of SIV challenges (standard)

Group	Animal ID	Number of challenges		Time of infection
		LT	N-LT	
Control	cy0774	1	1	LT
Control	cy0775	1	1	LT
Control	cy0776	0	1	N-LT
Control	cy0777	2	2	N-LT
Control	cy0778	3	3	-
Control	cy0779	3	3	-
Control	cy0780	2	2	LT
Control	cy0781	2	0	LT
PCS	cy0758	2	1	LT
PCS	cy0759	3	3	-
PCS	cy0760	3	3	-
PCS	cy0761	3	3	-
PCS	cy0762	3	3	-
PCS	cy0763	3	3	-
PCS	cy0764	1	1	LT
PCS	cy0765	3	3	-

LT: luteal phase; N-LT: non-luteal phase; - : not infected

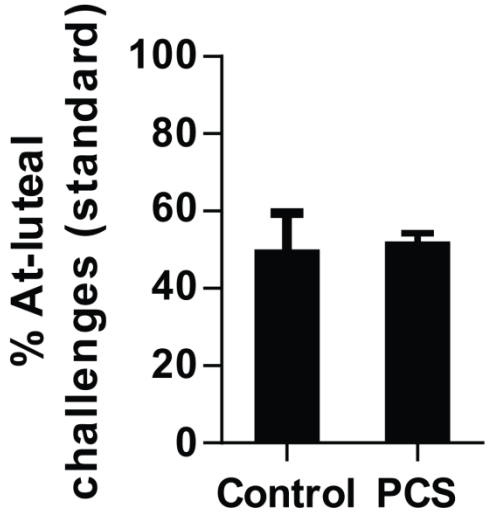

Fig. S1 Menstrual phase distributions of SIV challenges. Data were based on the standard challenge protocol (six challenges)

Menstrual phase distributions of SIV challenges (extended)

Group	Animal ID	Number of challenges		Time of infection
		LT	N-LT	
Control	cy0774	1	1	LT
Control	cy0775	1	1	LT
Control	cy0776	0	1	N-LT
Control	cy0777	2	2	N-LT
Control	cy0778	7	6	-
Control	cy0779	7	6	-
Control	cy0780	2	2	LT
Control	cy0781	2	0	LT
PCS	cy0758	2	1	LT
PCS	cy0759	7	6	LT
PCS	cy0760	6	7	-
PCS	cy0761	7	6	-
PCS	cy0762	4	5	LT
PCS	cy0763	6	7	-
PCS	cy0764	1	1	LT
PCS	cy0765	5	6	LT

LT: luteal phase; N-LT: non-luteal phase; - : not infected

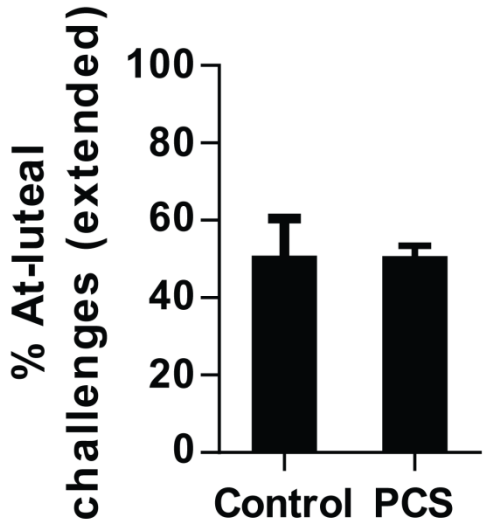

Fig. S2 Menstrual phase distributions of SIV challenges. Data were based on the extended (thirteen) challenges

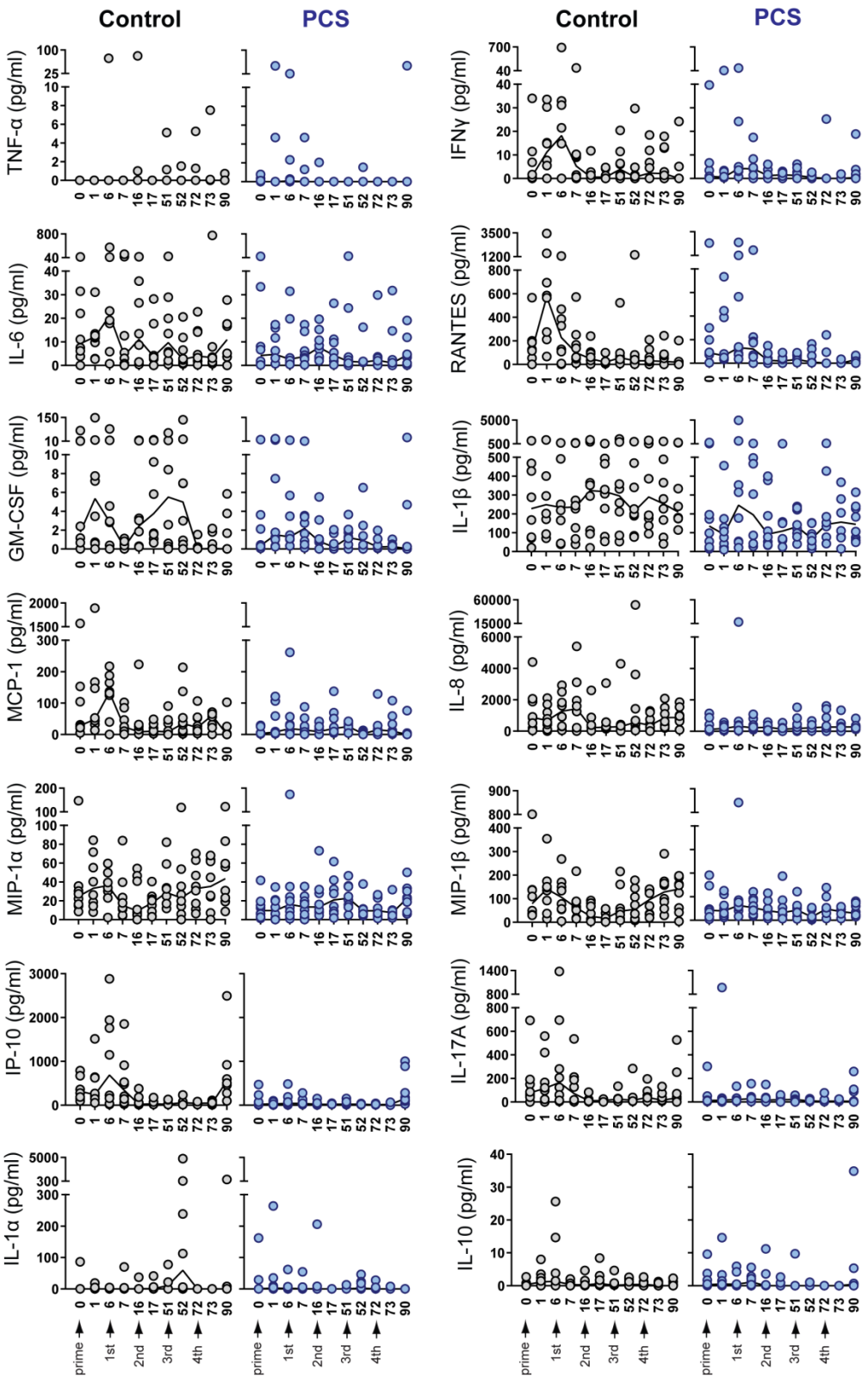

Fig. S3 Dynamics of inflammatory cytokines in cervicovaginal secretions. Cervicovaginal lavage (CVL) samples from the vaccination experiments illustrated in Fig. 1b were quantified by a Bio-Plex multiplexed cytokine assay, at indicated time points. Data are shown as values from individual animals with median line connection.

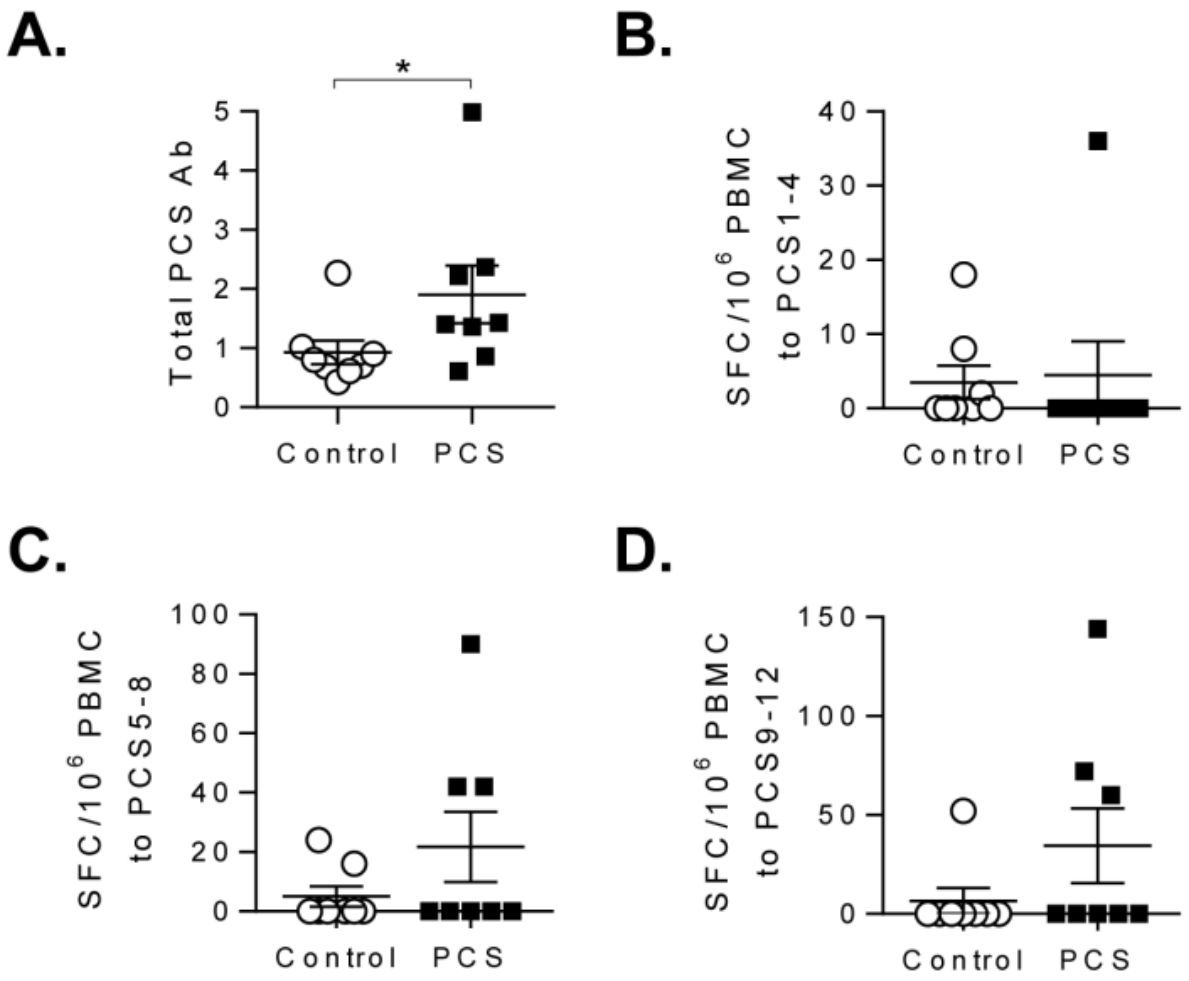

Figure S4. The PCS vaccine induced anti-PCS antibody and IFN-γ ELISPOT responses. (A). Plasma total anti-PCS antibodies at week 73 (peak time point). *$p < 0.05$ (Mann-Whitney's test). (B-D). Cellular antigen recall responses to PCS subset peptide pools in PBMCs isolated at week 73 were measured by IFN-γ ELISPOT assays. SFC, spot-forming cells. All data are presented as values from individual animals with mean ± SEM.

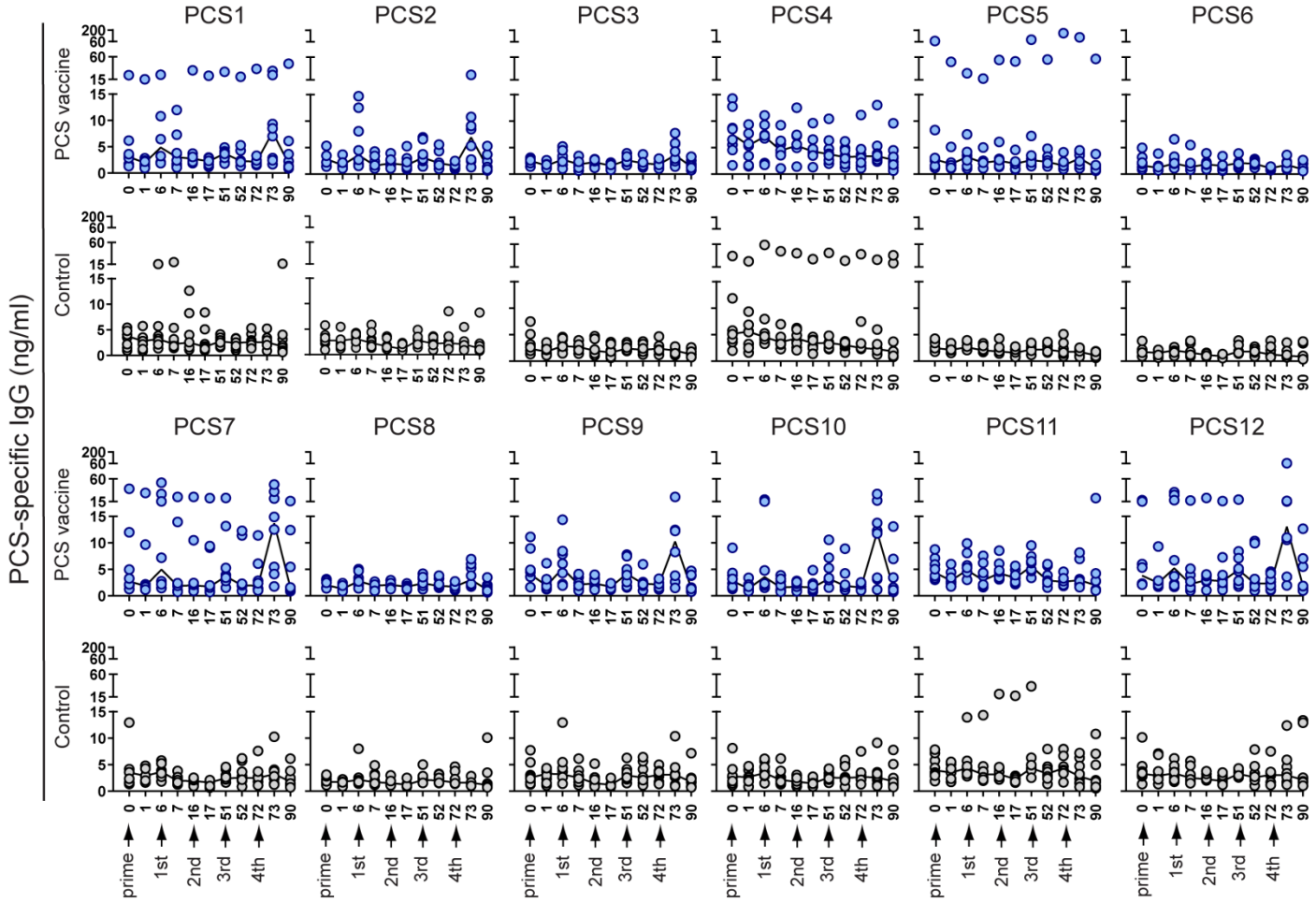

Fig. S5 Dynamics of plasma IgG antibodies to PCS peptide antigens. Plasma samples from the vaccination experiments illustrated in Fig. 1b were quantified for antibodies to each PCS peptide by a Bio-Plex multiplexed antibody assay, at indicated time points. Data are shown as values from individual animals with median line connection.

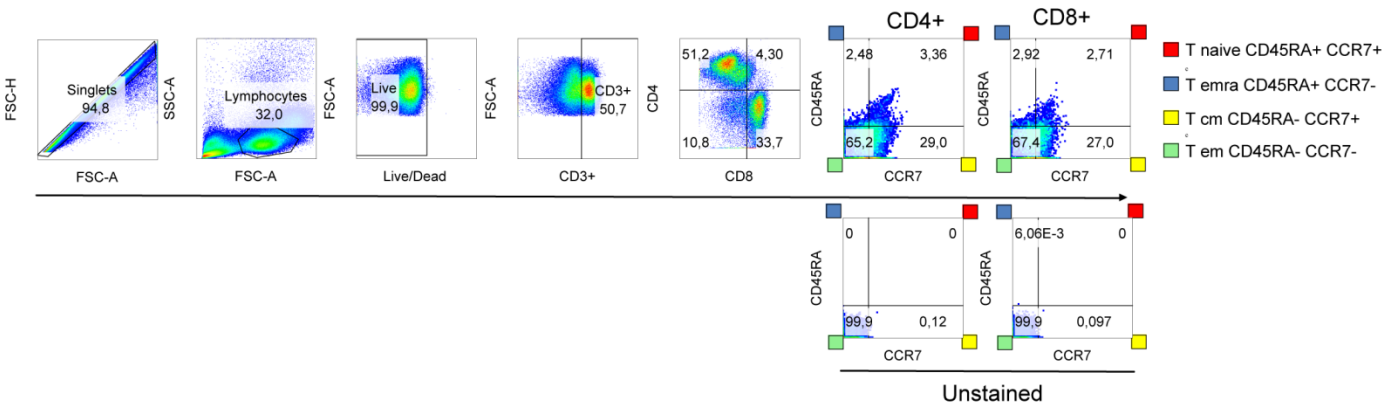

Fig. S6 Representative of gating strategy in defining T cell subsets.

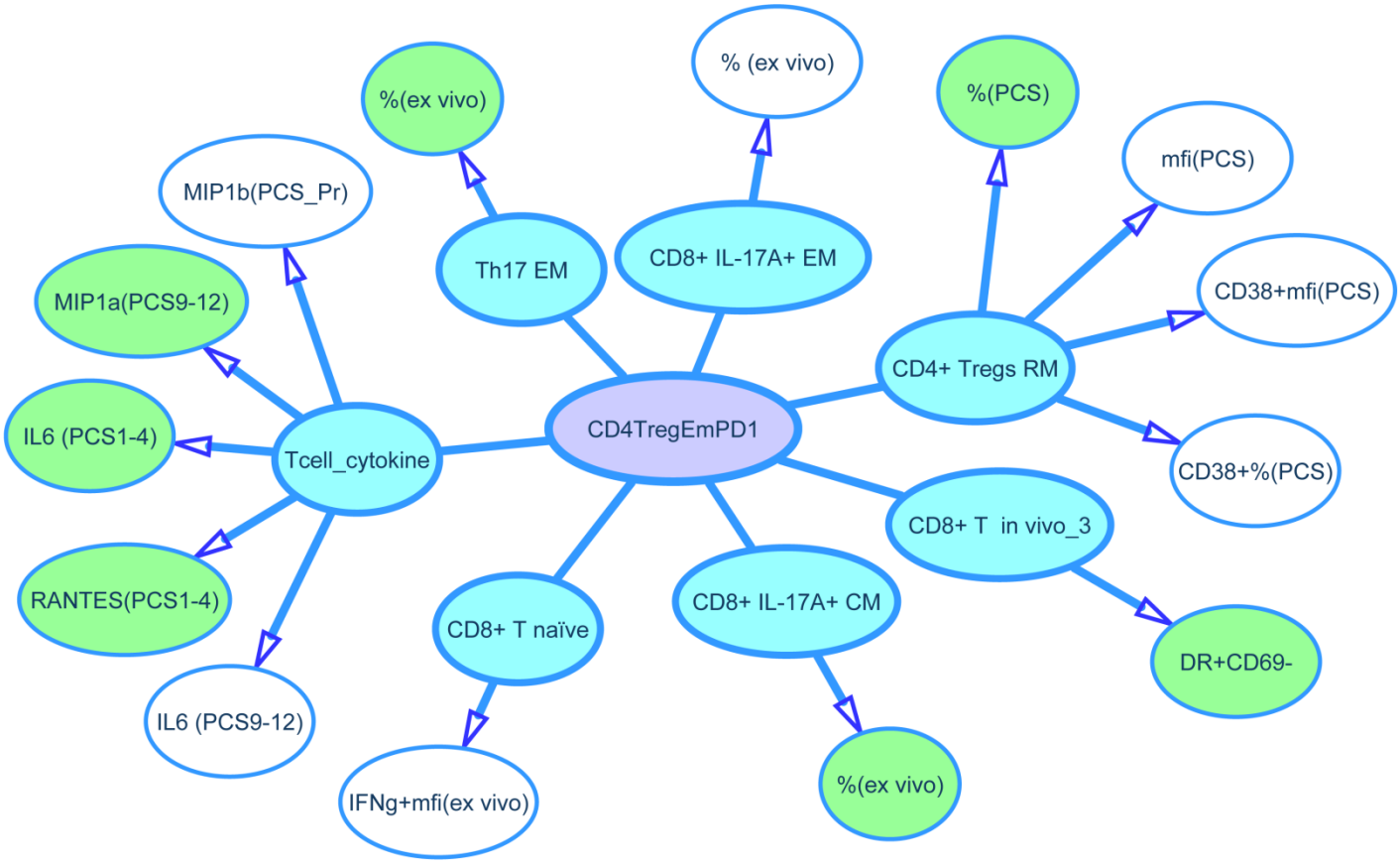

Figure S7 Correlations of immune factors in relation to CD4TregEmPD1. Different T cell subsets are in blue colored circles. Uncolored circles represent either frequency or expression intensity of immune activation or cytokine markers either under ex vivo conditions or after stimulated with PCS peptides. Green colored circles indicate variants that positively correlated with vaccine efficacy by Spearman rank analysis.

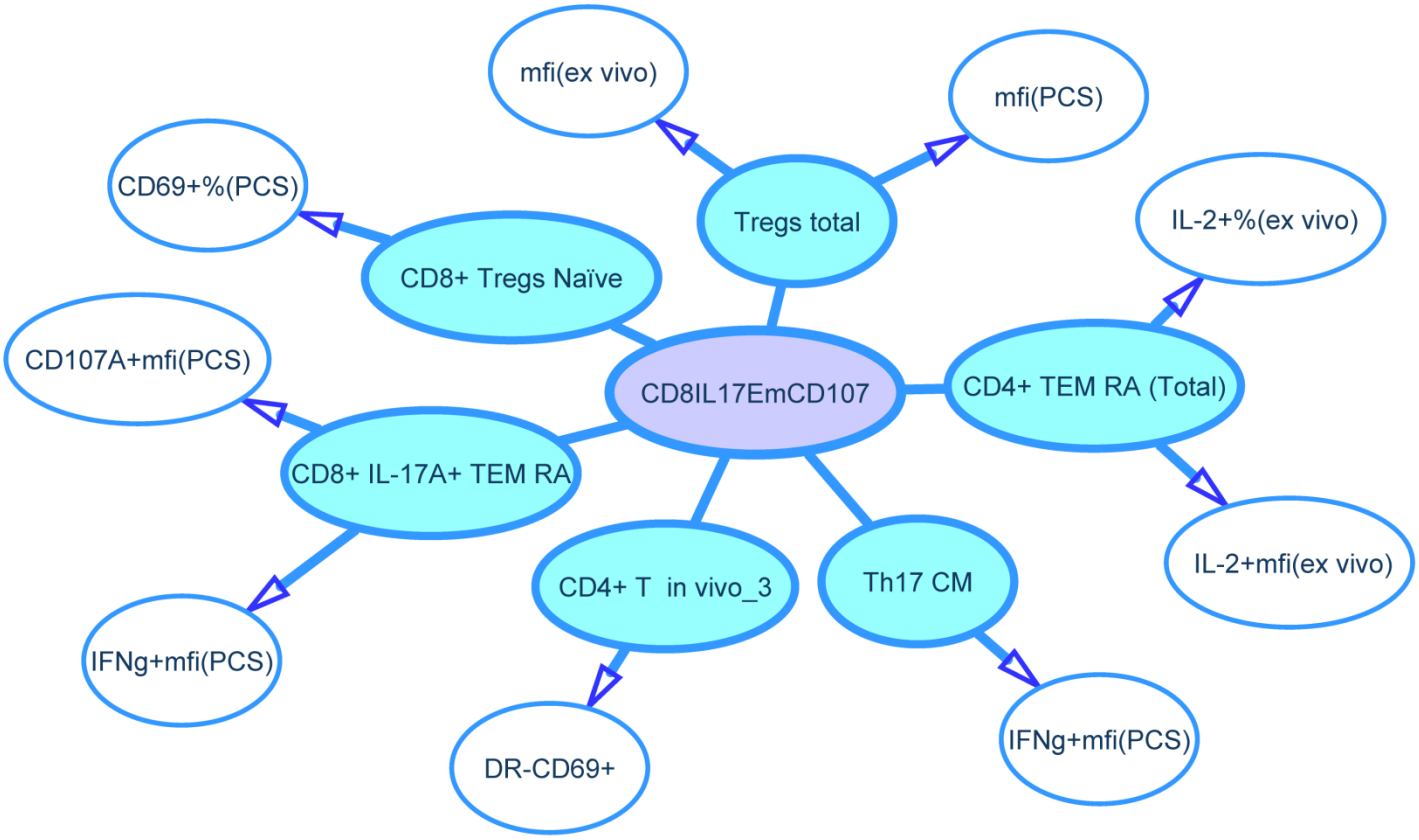

Figure S8. Correlations of immune factors in relation to CD8IL17EmCD107. Different T cell subsets are in blue colored circles. Uncolored circles represent either frequency or expression intensity of immune activation or cytokine markers either under ex vivo conditions or after stimulated with PCS peptides. Green colored circles indicate variants that positively correlated with vaccine efficacy by Spearman rank analysis.

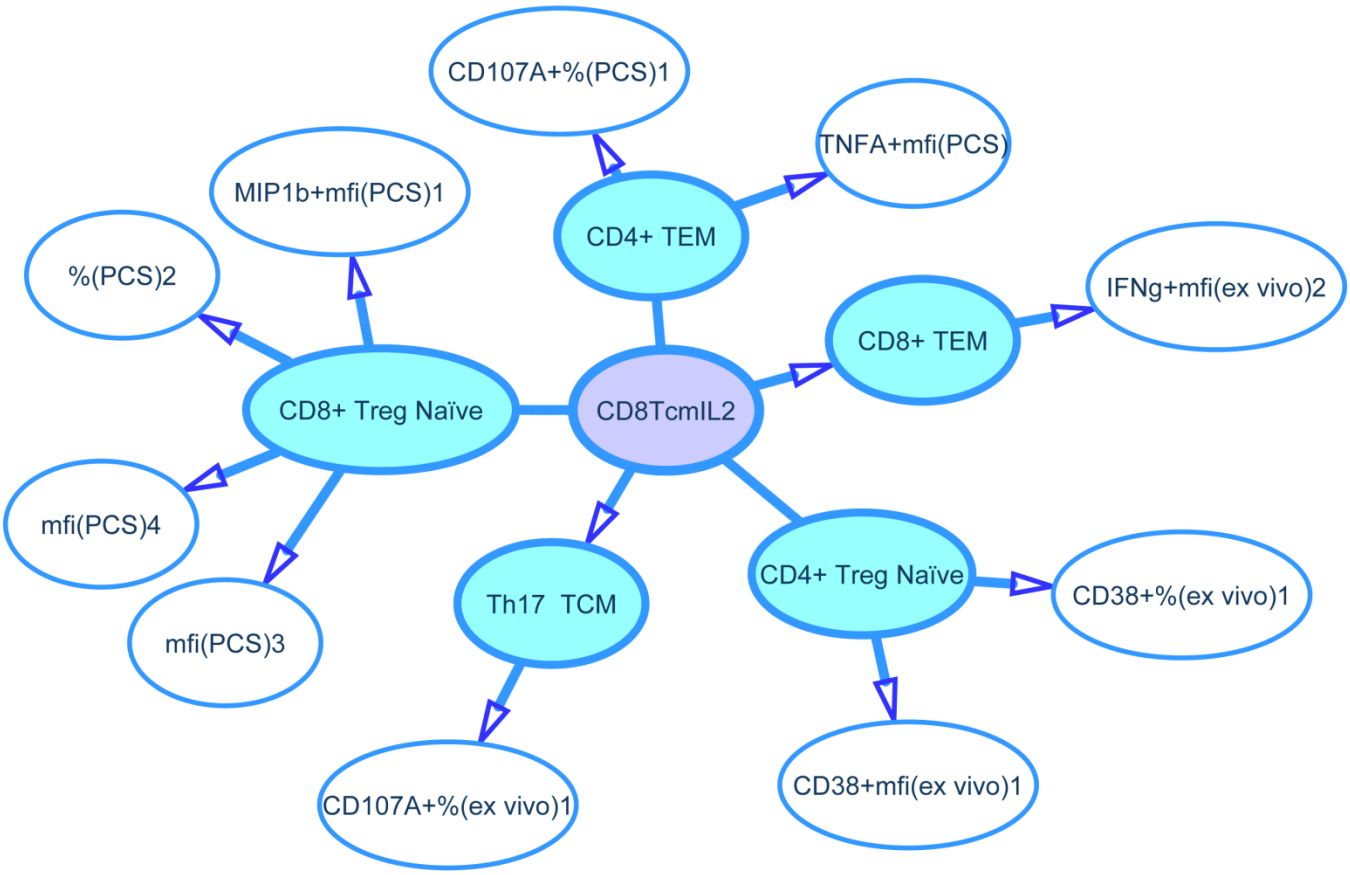

Figure S9. Correlations of immune factors in relation to CD8TcmIL2. Different T cell subsets are in blue colored circles. Uncolored circles represent either frequency or expression intensity of immune activation or cytokine markers either under ex vivo conditions or after stimulated with PCS peptides. Green colored circles indicate variants that positively correlated with vaccine efficacy by Spearman rank analysis.

Figure S10. Correlations of immune factors in relation to CD4TreNPD1m. Different T cell subsets are in blue colored circles. Uncolored circles represent either frequency or expression intensity of immune activation or cytokine markers either under ex vivo conditions or after stimulated with PCS peptides. Green colored circles indicate variants that positively correlated with vaccine efficacy by Spearman rank analysis.

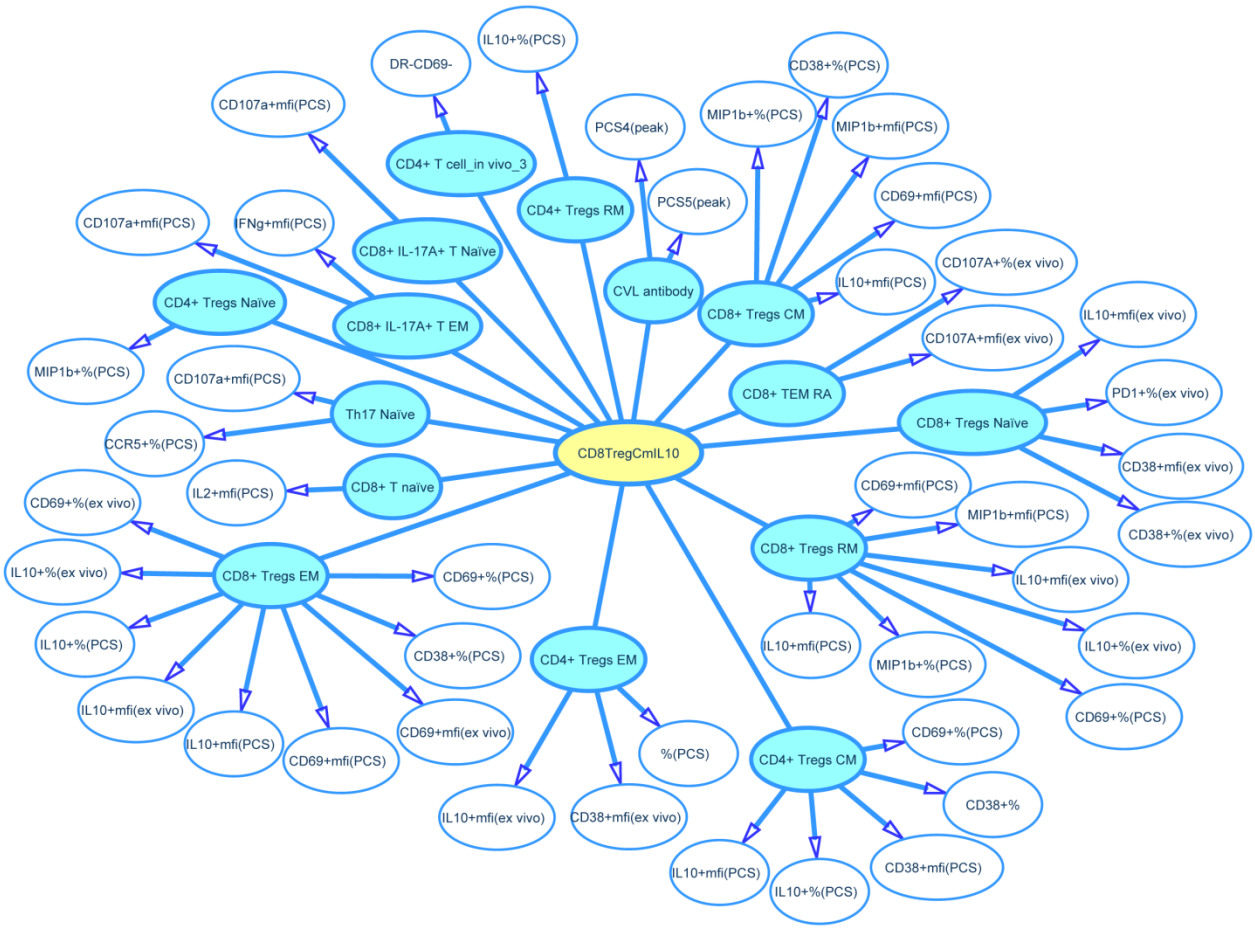

Figure S11 Correlations of immune factors in relation to CD8TregCmIL10. Different T cell subsets are in blue colored circles. Uncolored circles represent either frequency or expression intensity of immune activation or cytokine markers either under ex vivo conditions or after stimulated with PCS peptides. Green colored circles indicate variants that positively correlated with vaccine efficacy by Spearman rank analysis.

Figure S12. Correlations of immune factors in relation to CD4TreCmCD38. Different T cell subsets are in blue colored circles. Uncolored circles represent either frequency or expression intensity of immune activation or cytokine markers either under ex vivo conditions or after stimulated with PCS peptides. Green colored circles indicate variants that positively correlated with vaccine efficacy by Spearman rank analysis

Table S1 Animal characteristics

Group	Animal ID	Gender	Age (yrs)	Weight (kgs)	MHC class I	MHC class II
Control	cy0774	Female	6	4.9	M1/M4	M1/M4
Control	cy0775	Female	6	4.45	M1/M3	M1/M3
Control	cy0776	Female	6	3.5	M1/M2/1	M1/M1
Control	cy0777	Female	6	3.25	M1/M1	M1/M1
Control	cy0778	Female	6	4.35	M1/M2/1	M1/M3
Control	cy0779	Female	6	3.5	M3/M6	M2/M6/4/7
Control	cy0780	Female	6	3.9	M2/M2/1	M2/M1
Control	cy0781	Female	6	4.75	M4/6/M4	M4/6/M4
PCS	cy0758	Female	6	6.65	M6/M3/2	M6/M2
PCS	cy0759	Female	7	4.1	M1/M4	M1/M4
PCS	cy0760	Female	5	5.85	M1/M1	M1/M1
PCS	cy0761	Female	6	5.15	M4/M7	M4/M7
PCS	cy0762	Female	7	4.25	M3/M4/2	M3/M2
PCS	cy0763	Female	7	4.3	M1/M3	M1/M3
PCS	cy0764	Female	6	7	M1/M4	M1/M4
PCS	cy0765	Female	6	3.9	M3/M1	M3/M2

Table S2 MHC class I and II haplotype distributions between the control and the PCS vaccine groups

MHC I haplotype	No. of monkeys		P value[a]	MHC II haplotype	No. of monkeys		P value[a]
	Control	PCS			Control	PCS	
M1	5	5		M1	6	4	
Non-M1	3	3	1.0000	Non-M1	2	4	0.6084
M2	0	0		M2	2	3	
Non-M2	8	8	1.0000	Non-M2	6	5	1.0000
M3	2	3		M3	2	3	
Non-M3	6	5	1.0000	Non-M3	6	5	1.0000
M4	2	3		M4	2	3	
Non-M4	6	5	1.0000	Non-M4	6	5	1.0000
M5	0	0		M5	0	0	
Non-M5	8	8	1.0000	Non-M5	8	8	1.0000
M6	1	1		M6	0	1	
Non-M6	7	7	1.0000	Non-M6	8	7	1.0000
M7	0	1		M7	0	1	
Non-M7	8	7	1.0000	Non-M7	8	7	1.0000

a. Fisher's exact test.

Table S3 Kinetics of SIV acquistion

Group	Animal	Number of challenges (underlined) or days needed to acquire infection																																					
		1			2			3			4			5			6			7			8			9			10			11			12			13			
		0	6	10	14	20	24	28	34	38	42	48	52	56	62	66	70	76	80	84	90	94	98	104	108	112	118	122	126	132	136	140	146	150	154	160	164	168	174	178	182
Control	cy0774	○	○	○	○	○	○	●																																	
Control	cy0775	○	○	○	○	●																																			
Control	cy0776	○	●																																						
Control	cy0777	○	○	○	○	○	○	○	○	○ 39	○	○	● 53																												
Control	cy0778	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Control	cy0779	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Control	cy0780	○	○	○	○	○	○	○	○	○	○	○	●																												
Control	cy0781	○	○	○	○	●																																			
PCS	cy0758	○	○	○	○	○	○	○	○	● 39																															
PCS	cy0759	○	○	○	○	○	○	○	○	○ 39	○	○	○ 53	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●		
PCS	cy0760	○	○	○	○	○	○	○	○	○ 39	○	○	○ 53	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
PCS	cy0761	○	○	○	○	○	○	○	○	○ 39	○	○	○ 53	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
PCS	cy0762	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●												
PCS	cy0763	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
PCS	cy0764	○	○	○	○	○	○	●																																	
PCS	cy0765	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●							

Intravaginal SIVmac251 challenges were conducted every two weeks. Infection status was monitored on days 0, 6, 10 and 14 of each 14-day challenge round by determining plasma viral load. Numbers of challenges (underlined) or days relative to the time of the first challenge are indicated. For logistic reason, some animals were monitored on day 39 or 53 as labelled, one day later than originally designed. Empty circle: no detectable viral load. Solid circle: positive viral load.

Table S4. MHC class I and II haplotype distributions beteen infected and uninfected animals (Standard challenges)

MHC I haplotype	No. of monkeys		P value[a]	MHC II haplotype	No. of monkeys		P value[a]
	Infected	Uninfected			Infected	Uninfected	
M1	5	5		M1	6	4	
Non-M1	3	3	1.0000	Non-M1	2	4	0.6084
M2	1	0		M2	2	3	
Non-M2	7	8	1.0000	Non-M2	6	5	1.0000
M3	1	4		M3	1	4	
Non-M3	7	4	0.2821	Non-M3	7	4	0.2821
M4	3	2		M4	3	2	
Non-M4	5	6	1.0000	Non-M4	5	6	1.0000
M5	0	0		M5	0	0	
Non-M5	8	8	1.0000	Non-M5	8	8	1.0000
M6	1	1		M6	1	0	
Non-M6	7	7	1.0000	Non-M6	7	8	1.0000
M7	0	1		M7	1	1	
Non-M7	8	7	1.0000	Non-M7	7	7	1.0000

a. Fisher's exact test.

Table S5. MHC class I and II haplotype distributions between infected and uninfected animals (extended challenges)

MHC I haplotype	No. of monkeys		P value[a]	MHC II haplotype	No. of monkeys		P value[a]
	Infected	Uninfected			Infected	Uninfected	
M1	7	3		M1	7	3	
Non-M1	4	2	1.0000	Non-M1	4	2	1.0000
M2	1	0		M2	4	1	
Non-M2	10	5	1.0000	Non-M2	7	4	1.0000
M3	3	2		M3	3	2	
Non-M3	8	3	1.0000	Non-M3	8	3	1.0000
M4	4	1		M4	4	1	
Non-M4	7	4	1.0000	Non-M4	7	4	1.0000
M5	0	0		M5	0	0	
Non-M5	11	5	1.0000	Non-M5	11	5	1.0000
M6	1	1		M6	1	0	
Non-M6	10	4	1.0000	Non-M6	10	5	1.0000
M7	0	1		M7	0	1	
Non-M7	11	4	0.3125	Non-M7	11	4	0.3125

a. Fisher's exact test.

Table S6 Anti-SIVmac251 neutalizing antibody titer in TZM.bl cells (1/x)							
		SIVmac251.TCLA.15 (Tier 1A)		SIVmac251.30 (Tier 2)		MuLV	
Animal ID	Group	ID50	ID80	ID50	ID80	ID50	ID80
cy0758	PCS vaccine	<20	<20	<20	<20	<20	<20
cy0759	PCS vaccine	<20	<20	<20	<20	<20	<20
cy0760	PCS vaccine	<20	<20	<20	<20	<20	<20
cy0761	PCS vaccine	<20	<20	<20	<20	<20	<20
cy0762	PCS vaccine	<20	<20	<20	<20	<20	<20
cy0763	PCS vaccine	<20	<20	<20	<20	<20	<20
cy0764	PCS vaccine	<20	<20	<20	<20	<20	<20
cy0765	PCS vaccine	<20	<20	<20	<20	<20	<20
cy0774	control	<20	<20	<20	<20	<20	<20
cy0775	control	<20	<20	<20	<20	<20	<20
cy0776	control	<20	<20	<20	<20	<20	<20
cy0777	control	<20	<20	<20	<20	<20	<20
cy0778	control	<20	<20	<20	<20	<20	<20
cy0779	control	<20	<20	<20	<20	<20	<20
cy0780	control	<20	<20	<20	<20	<20	<20
cy0781	control	<20	<20	<20	<20	<20	<20

Table S7. Cytokine-producing antigen recall responses in PBMCs from the PCS vaccine group

Animal ID	Cytokines (pg/ml) secreted in response to PCS1-4														No. of challenges to cause infection
	TNF-α	IFN-γ	IL-6	RANTES	GM-CSF	IL-1β	MCP-1	IL-8	MIP-1α	MIP-1β	IL-10	IP-10	IL-17A	IL-1α	
cy0758	0.00	0.00	1.14	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	19.51	0.00	0.00	3
cy0759	43.24	0.00	0.00	29.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13
cy0760	0.00	4.84	3.53	219.59	0.00	0.00	0.00	33.47	0.00	106.80	1.34	237.04	0.00	0.00	Uninfected
cy0761	0.00	0.00	4.44	943.24	0.00	0.00	0.00	0.35	0.00	0.00	0.00	0.00	0.00	0.00	Uninfected
cy0762	0.00	0.00	0.00	47.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9
cy0763	0.00	1.13	22.40	2,498.02	0.00	0.00	0.00	8.53	0.00	563.57	0.00	0.00	0.00	0.00	Uninfected
cy0764	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.37	0.00	377.49	0.00	0.00	0.00	0.00	2
cy0765	0.00	0.00	0.00	0.00	19.28	0.00	0.00	285.19	0.00	0.00	0.00	179.60	0.00	0.00	11
Animal ID	Cytokines (pg/ml) secreted in response to PCS5-8														No. of challenges to cause infection
	TNF-α	IFN-γ	IL-6	RANTES	GM-CSF	IL-1β	MCP-1	IL-8	MIP-1α	MIP-1β	IL-10	IP-10	IL-17A	IL-1α	
cy0758	1.11	1.28	0.00	0.00	0.00	0.00	0.00	90.75	0.00	0.00	0.96	0.00	0.00	0.00	3
cy0759	55.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13
cy0760	122.64	6.62	6.30	573.69	14.18	0.00	0.00	366.19	0.00	201.33	0.91	323.34	0.00	0.00	Uninfected
cy0761	0.00	0.00	23.61	171.75	0.00	0.59	28.52	408.20	0.00	0.00	0.00	0.00	0.00	156.99	Uninfected
cy0762	12.36	14.15	16.79	326.20	9.24	3.85	0.00	49.05	20.63	0.00	0.00	0.00	168.14	15.61	9
cy0763	3.68	0.00	0.00	0.00	0.00	0.00	0.00	38.83	25.04	344.06	0.00	0.00	0.00	0.00	Uninfected
cy0764	25.73	0.00	0.00	0.00	0.00	0.00	0.00	165.60	0.00	465.20	0.00	0.00	0.00	0.00	2
cy0765	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	662.24	0.00	152.72	0.00	818.28	11
Animal ID	Cytokines (pg/ml) secreted in response to PCS9-12														No. of challenges to cause infection
	TNF-α	IFN-γ	IL-6	RANTES	GM-CSF	IL-1β	MCP-1	IL-8	MIP-1α	MIP-1β	IL-10	IP-10	IL-17A	IL-1α	
cy0758	0.00	0.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3
cy0759	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13
cy0760	0.00	1.02	47.45	365.24	0.00	6.90	62.93	238.46	56.57	261.44	0.00	249.40	0.00	101.05	Uninfected
cy0761	0.00	0.00	69.48	205.04	0.00	7.75	0.00	76.23	83.67	312.05	0.00	0.00	0.00	0.00	Uninfected
cy0762	0.00	22.46	14.56	340.55	15.59	1.51	0.00	203.23	1.20	0.00	8.14	0.00	142.50	0.00	9
cy0763	28.54	0.00	12.18	66.59	0.00	0.00	0.00	89.41	33.11	765.01	0.00	71.05	0.00	0.00	Uninfected
cy0764	19.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	126.15	0.00	0.00	0.00	0.00	2
cy0765	128.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	773.93	0.00	143.01	0.00	69.52	11

PBMCs collected at the peak time point (week 73) were stimulated with a pool of SIV peptides (PCS1-4, PCS5-8 or PCS9-12) and quantified for cytokine secretions by Bio-Plex multiplexed cytokine assay. Number of challenges to cause infection in each animal is also listed. The values of mediium only negative control were subtracted.

Table S8 Immune correlates of risk (CoRs) (p value not corrected for multiple comparison)

Immune response				Correlation with No. of challenges (Spearman rank)	
Type	Sub-type	Ag	Time point	Rho (r)	p
CVL mucosal cytokine	IL-1 α fold change	N/A	Peak/baseline	-0.6584	0.0056
CVL mucosal cytokine	IL-1 α fold change	N/A	Pre-challenge/baseline	-0.5435	0.0296
CVL mucosal cytokine	IL-1 α	N/A	Pre-challenge	-0.5168	0.0404
CVL mucosal IgG Ab	N/A	PCS1 peptide	Peak	-0.7075	0.0496
CVL mucosal IgG Ab	N/A	PCS3 peptide	Peak	-0.7075	0.0496
CVL mucosal IgG Ab	N/A	PCS4 peptide	Peak	-0.7075	0.0496
CVL mucosal IgG Ab	N/A	PCS5 peptide	Peak	-0.7807	0.0222
CVL mucosal IgG Ab	N/A	PCS9 peptide	Peak	-0.7075	0.0496
CD8+ T cells (ex vivo)	CCR7+CD45RA+% of CD8+	N/A	Pre-challenge	-0.8000	0.0171
CD4+CD25+FoxP3+ T cells (ex vivo)	CD38+% of CD4+CD25+FoxP3+CD45RA-CD127-	N/A	Pre-challenge	-0.8106	0.0147
CD4+CD25+FoxP3+ T cells (ex vivo)	CD38 MFI of CD4+CD25+FoxP3+CD45RA-CD127-	N/A	Pre-challenge	-0.8944	0.0027
CD8+CD25+FoxP3+ T cells (ex vivo)	CD127 MFI of CD8+CD25+FoxP3+CD45RA+	N/A	Pre-challenge	-0.7563	0.0299
CD8+CD25+FoxP3+ T cells (ex vivo)	CD38+% of CD8+CD25+FoxP3+CD45RA+CD127+	N/A	Pre-challenge	-0.8665	0.0054
CD8+CD25+FoxP3+ T cells (ex vivo)	CD38 MFI of CD8+CD25+FoxP3+CD45RA+CD127+	N/A	Pre-challenge	-0.8665	0.0054
CD8+CD25+FoxP3+ T cells (ex vivo)	IL-10+% of CD8+CD25+FoxP3+CD45RA+CD127+	N/A	Pre-challenge	-0.8106	0.0147
CD8+CD25+FoxP3+ T cells (ex vivo)	IL-10 MFI of CD8+CD25+FoxP3+CD45RA+CD127+	N/A	Pre-challenge	-0.8665	0.0054
CD8+CD25+FoxP3+ T cells (ex vivo)	MIP-1β+% of CD8+CD25+FoxP3+CD45RA+CD127+	N/A	Pre-challenge	-0.8113	0.0145
CD8+CD25+FoxP3+ T cells (ex vivo)	MIP-1β MFI of CD8+CD25+FoxP3+CD45RA+CD127+	N/A	Pre-challenge	-0.8318	0.0104
CD8+CD25+FoxP3+ T cells (ex vivo)	PD-1+% of CD8+CD25+FoxP3+CD45RA+CD127+	N/A	Pre-challenge	-0.8665	0.0054
CD8+CD25+FoxP3+ T cells (ex vivo)	PD-1 MFI of CD8+CD25+FoxP3+CD45RA+CD127+	N/A	Pre-challenge	-0.8106	0.0147
CD4+IL17A+ T cells (Ag recall)	CCR5+% of CD4+IL17A+CCR7+CD45RA+	PCS1-12 peptide pool	Pre-challenge	-0.7507	0.0319
CD8+IL17A+ T cells (Ag recall)	CCR5+% of CD8+IL17A+CCR7+CD45RA+	PCS1-12 peptide pool	Pre-challenge	-0.7278	0.0407
CD8+IL17A+ T cells (Ag recall)	CD69+% of CD8+IL17A+CCR7+CD45RA+	PCS1-12 peptide pool	Pre-challenge	-0.7278	0.0407
CD8+IL17A+ T cells (Ag recall)	CD69 MFI of CD8+IL17A+CCR7+CD45RA+	PCS1-12 peptide pool	Pre-challenge	-0.7278	0.0407
CD8+IL17A+ T cells (Ag recall)	CD107a MFI of CD8+IL17A+CCR7+CD45RA+	PCS1-12 peptide pool	Pre-challenge	-0.7507	0.0319
CD8+IL17A+ T cells (Ag recall)	IFN- γ MFI of CD8+IL17A+CCR7-CD45RA-	PCS1-12 peptide pool	Pre-challenge	-0.7507	0.0319
CD4+CD25+FoxP3+ T cells (Ag recall)	MIP-1 β+% of CD4+CD25+FoxP3+CD45RA+CD127+	PCS1-12 peptide pool	Pre-challenge	-0.7507	0.0319
CD4+CD25+FoxP3+ T cells (Ag recall)	CD38+% of CD4+CD25+FoxP3+CD45RA-CD127+	PCS1-12 peptide pool	Pre-challenge	-0.7507	0.0319
CD4+CD25+FoxP3+ T cells (Ag recall)	CD38 MFI of CD4+CD25+FoxP3+CD45RA-CD127+	PCS1-12 peptide pool	Pre-challenge	-0.7826	0.0217
CD8+CD25+FoxP3+ T cells (Ag recall)	CD38+% of CD8+CD25+FoxP3+CD45RA-CD127+	PCS1-12 peptide pool	Pre-challenge	-0.8665	0.0054
CD8+CD25+FoxP3+ T cells (Ag recall)	CD38 MFI of CD8+CD25+FoxP3+CD45RA-CD127+	PCS1-12 peptide pool	Pre-challenge	-0.8106	0.0147
CD8+CD25+FoxP3+ T cells (Ag recall)	IL-10 MFI of CD8+CD25+FoxP3+CD45RA-CD127+	PCS1-12 peptide pool	Pre-challenge	-0.7826	0.0217
CD8+CD25+FoxP3+ T cells (Ag recall)	MIP-1β MFI of CD8+CD25+FoxP3+CD45RA-CD127+	PCS1-12 peptide pool	Pre-challenge	-0.7826	0.0217
CD8+CD25+FoxP3+ T cells (Ag recall)	CD38+% of CD8+CD25+FoxP3+CD45RA-CD127-	PCS1-12 peptide pool	Pre-challenge	-0.7507	0.0319
CD8+CD25+FoxP3+ T cells (Ag recall)	IL-10+% of CD8+CD25+FoxP3+CD45RA-CD127-	PCS1-12 peptide pool	Pre-challenge	-0.7507	0.0319
CD8+CD25+FoxP3+ T cells (Ag recall)	IL-10 MFI of CD8+CD25+FoxP3+CD45RA-CD127-	PCS1-12 peptide pool	Pre-challenge	-0.7507	0.0319

Immune factors significantly correlating with number of challenges needed to cause infection were determined by Spearman rank correlation analysis. Ag: antigen (used to quantify antibody or to stimulate cells). CVL: cervicovaginal lavage wash. N/A: not applicable. Peak time point: Week 73 of vaccination schedule (1 week after final vaccination boost). Pre-challenge time point: Week 90 of vaccination schedule. Ab: antibody. Ex vivo: peripheral blood mononuclear cells (PBMCs) were not stimulated with Ag. Ag recall: PBMCs were stimulated with Ag in culture. MFI: mean fluorescent intensity. All analyses were conducted in the PCS vaccine group (n=8), except that CVL mucosal cytoine analyses were conducted in the PCS vaccine and control groups combined (n=16).

Table S9. The index of immune variants positively correlated with 6 features in the LASSO model

Featrue name	Feature variant	variant category	variant	Spearman Rho	P-value
CD4TregEmPD1	CD4+ Tregs EM PD1+%	Tcell_cytokine	IL6 (PCS1-4)	0.900	0.002
CD4TregEmPD1	CD4+ Tregs EM PD1+%	Tcell_cytokine	IL6 (PCS9-12)	0.780	0.022
CD4TregEmPD1	CD4+ Tregs EM PD1+%	Tcell_cytokine	RANTES(PCS1-4)	0.866	0.005
CD4TregEmPD1	CD4+ Tregs EM PD1+%	Tcell_cytokine	MIP1a(PCS9-12)	0.900	0.002
CD4TregEmPD1	CD4+ Tregs EM PD1+%	Tcell_cytokine	MIP1b(PCS_Pr)	0.738	0.037
CD4TregEmPD1	CD4+ Tregs EM PD1+%	Th17 EM	%(ex vivo)	0.866	0.005
CD4TregEmPD1	CD4+ Tregs EM PD1+%	CD8+ IL-17A+ CM	%(ex vivo)	0.845	0.008
CD4TregEmPD1	CD4+ Tregs EM PD1+%	CD8+ IL-17A+ EM	%(ex vivo)	0.845	0.008
CD4TregEmPD1	CD4+ Tregs EM PD1+%	CD4+ Tregs RM	%(PCS)	0.866	0.005
CD4TregEmPD1	CD4+ Tregs EM PD1+%	CD4+ Tregs RM	mfi(PCS)	0.751	0.032
CD4TregEmPD1	CD4+ Tregs EM PD1+%	CD4+ Tregs RM	CD38+%(PCS)	0.926	0.001
CD4TregEmPD1	CD4+ Tregs EM PD1+%	CD4+ Tregs RM	CD38+mfi(PCS)	0.780	0.022
CD4TregEmPD1	CD4+ Tregs EM PD1+%	CD8+ T naïve	IFNg+mfi(ex vivo)	0.794	0.019
CD4TregEmPD1	CD4+ Tregs EM PD1+%	CD8+ T in vivo_3	DR+CD69-	0.866	0.012
CD8IL17EmCD107	CD8+ IL17A+ TEM CD107a+%	CD4+ T in vivo_3	DR-CD69+	0.793	0.034
CD8IL17EmCD107	CD8+ IL17A+ TEM CD107a+%	CD8+ IL-17A+ TEM RA	CD107A+mfi(PCS)	0.956	0.000
CD8IL17EmCD107	CD8+ IL17A+ TEM CD107a+%	CD8+ IL-17A+ TEM RA	IFNg+mfi(PCS)	0.852	0.007
CD8IL17EmCD107	CD8+ IL17A+ TEM CD107a+%	Th17 CM	IFNg+mfi(PCS)	0.906	0.002
CD8IL17EmCD107	CD8+ IL17A+ TEM CD107a+%	Tregs total	mfi(ex vivo)	0.945	0.000
CD8IL17EmCD107	CD8+ IL17A+ TEM CD107a+%	Tregs total	mfi(PCS)	0.737	0.037
CD8IL17EmCD107	CD8+ IL17A+ TEM CD107a+%	CD8+ Tregs Naïve	CD69+%(PCS)	0.802	0.017
CD8IL17EmCD107	CD8+ IL17A+ TEM CD107a+%	CD4+ TEM RA (Total)	IL-2+%(ex vivo)	0.766	0.027
CD8IL17EmCD107	CD8+ IL17A+ TEM CD107a+%	CD4+ TEM RA (Total)	IL-2+mfi(ex vivo)	0.796	0.032
CD8TcmIL2	CD8+TCM IL2+%	Th17 TCM	CD107A+%(ex vivo)	0.730	0.040
CD8TcmIL2	CD8+TCM IL2+%	CD4+ Treg Naïve	CD38+%(ex vivo)	0.788	0.020
CD8TcmIL2	CD8+TCM IL2+%	CD4+ Treg Naïve	CD38+mfi(ex vivo)	0.788	0.020
CD8TcmIL2	CD8+TCM IL2+%	CD8+ Treg Naïve	%(PCS)	0.752	0.031
CD8TcmIL2	CD8+TCM IL2+%	CD8+ Treg Naïve	mfi(PCS)	0.792	0.019
CD8TcmIL2	CD8+TCM IL2+%	CD8+ Treg Naïve	MIP1b+mfi(PCS)	0.739	0.036
CD8TcmIL2	CD8+TCM IL2+%	CD8+ Treg Naïve	mfi(PCS)	0.788	0.020
CD8TcmIL2	CD8+TCM IL2+%	CD8+ TEM	IFNg+mfi(ex vivo)	0.803	0.016
CD8TcmIL2	CD8+TCM IL2+%	CD4+ TEM	CD107A+%(PCS)	0.873	0.005
CD8TcmIL2	CD8+TCM IL2+%	CD4+ TEM	TNFA+mfi(PCS)	0.755	0.030
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	cytokine(CVL)	RANTES(CF)	0.710	0.048
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	cytokine(CVL)	IL17A(CF)	0.710	0.048
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ IL-17A+ TEM RA	%(ex vivo)	0.759	0.029
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ IL-17A+ TEM RA	mfi(ex vivo)	0.741	0.036
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ IL-17A+ T naïve	IFNg+%(PCS)	0.757	0.030
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ Tregs	mfi(ex vivo)	0.888	0.003
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD4+ Tregs TEM	PD1+(mfi)	0.843	0.009
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD4+ Tregs TEM	CD38+%(PCS)	0.814	0.014
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD4+ Tregs TEM	CD38+mfi(PCS)	0.814	0.014
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD4+ Tregs TEM	PD1+% (PCS)	0.843	0.009
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD4+ Tregs TEM	PD1+mfi (PCS)	0.814	0.014
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD4 T naïve	Ki67%(ex vivo)	0.823	0.023
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD4+ TEM RA	Ki67mfi(ex vivo)	0.823	0.023
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD4+ TCM	Ki67%(ex vivo)	0.823	0.023
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD4+ TEM	Ki67%(ex vivo)	0.823	0.023
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD4+ TEM	Ki67mfi(ex vivo)	0.898	0.006
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ T naïve	Ki67%(ex vivo)	0.823	0.023
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ T naïve	Ki67mfi(ex vivo)	0.823	0.023
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ TEM RA	Ki67%(ex vivo)	0.898	0.006
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ TCM	Ki67%(ex vivo)	0.898	0.006
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ TCM	Ki67mfi(ex vivo)	0.898	0.006
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ TEM	Ki67%(ex vivo)	0.898	0.006
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ TEM	Ki67mfi(ex vivo)	0.898	0.006
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD4+ T naïve	%(PCS)	0.759	0.029
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD4+ T naïve	Ki67%(PCS)	0.759	0.029

Table S9. The index of immune variants positively correlated with the 6 features in the LASSO model (continued)

CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD4+ TEM RA	%(PCS)	0.715	0.046
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD4+ TCM	%(PCS)	0.811	0.015
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD4+ TCM	mfi(PCS)	0.952	0.000
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ T naïve	%(PCS)	0.719	0.044
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ T naïve	mfi(PCS)	0.952	0.000
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ T naïve	Ki67%(PCS)	0.820	0.013
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ TEM RA	%(PCS)	0.719	0.044
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ TEM RA	IFNg+%(PCS)	0.916	0.001
CD4TreNPD1m	CD4+Treg Naïve PD1+ mfi	CD8+ TEM	Ki67%(PCS)	0.804	0.016
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CVL antibody	PCS4(peak)	0.764	0.027
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CVL antibody	PCS5(peak)	0.733	0.039
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD4+ Tregs CM	CD38+%	0.959	0.000
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD4+ T cell_in vivo_3	DR-CD69-	0.794	0.033
CD8TregCmIL10	CD8+ Tregs CM IL10+%	Th17 Naïve	CCR5+%(PCS)	0.959	0.000
CD8TregCmIL10	CD8+ Tregs CM IL10+%	Th17 Naïve	CD107a+mfi(PCS)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ IL-17A+ T Naïve	CD107a+mfi(PCS)	0.959	0.000
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ IL-17A+ T EM	CD107a+mfi(PCS)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ IL-17A+ T EM	IFNg+mfi(PCS)	0.959	0.000
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD4+ Tregs EM	CD38+mfi(ex vivo)	0.875	0.004
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD4+ Tregs EM	IL10+mfi(ex vivo)	0.751	0.032
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs Naïve	CD38+%(ex vivo)	0.839	0.009
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs Naïve	CD38+mfi(ex vivo)	0.750	0.032
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs Naïve	IL10+mfi(ex vivo)	0.839	0.009
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs Naïve	PD1+%(ex vivo)	0.839	0.009
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs RM	IL10+%(ex vivo)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs RM	IL10+mfi(ex vivo)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs EM	CD69+%(ex vivo)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs EM	CD69+mfi(ex vivo)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs EM	IL10+%(ex vivo)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs EM	IL10+mfi(ex vivo)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD4+ Tregs Naïve	MIP1b+%(PCS)	0.959	0.000
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD4+ Tregs RM	IL10+%(PCS)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD4+ Tregs CM	CD38+mfi(PCS)	1.000	.
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD4+ Tregs CM	CD69+%(PCS)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD4+ Tregs CM	IL10+%(PCS)	0.839	0.009
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD4+ Tregs CM	IL10+mfi(PCS)	0.875	0.004
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD4+ Tregs EM	%(PCS)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs RM	CD69+%(PCS)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs RM	CD69+mfi(PCS)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs RM	IL10+mfi(PCS)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs RM	MIP1b+%(PCS)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs RM	MIP1b+mfi(PCS)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs CM	CD38+%(PCS)	0.839	0.009
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs CM	CD69+mfi(PCS)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs CM	IL10+mfi(PCS)	1.000	.
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs CM	MIP1b+%(PCS)	0.875	0.004
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs CM	MIP1b+mfi(PCS)	1.000	.
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs EM	CD38+%(PCS)	0.959	0.000
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs EM	CD69+%(PCS)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs EM	CD69+mfi(PCS)	0.756	0.030
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs EM	IL10+%(PCS)	0.959	0.000
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ Tregs EM	IL10+mfi(PCS)	0.959	0.000
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ TEM RA	CD107A+%(ex vivo)	0.765	0.027
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ TEM RA	CD107A+mfi(ex vivo)	0.765	0.027
CD8TregCmIL10	CD8+ Tregs CM IL10+%	CD8+ T naïve	IL2+mfi(PCS)	0.711	0.048
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ Tregs CM	IL10+%(PCS)	0.959	0.000
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CVL antibody	PCS4(peak)	0.733	0.039
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CVL antibody	PCS5(peak)	0.764	0.027
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD4+ T cell_in vivo_3	DR-CD69-	0.794	0.033
CD4TreCmCD38	CD4+ Tregs CM CD38+%	Th17 Tnaive	CCR5+%(PCS)	1.000	.
CD4TreCmCD38	CD4+ Tregs CM CD38+%	Th17 TEM RA	CD38+mfi(PCS)	0.750	0.032
CD4TreCmCD38	CD4+ Tregs CM CD38+%	Th17 TEM RA	CD107A+mfi(PCS)	0.750	0.032

Table S9. The index of immune variants positively correlated with 6 features in the LASSO model (continued)

CD4TreCmCD38	CD4+ Tregs CM CD38+%	Th17 TEM RA	IFNg+%(PCS)	0.750	0.032
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ IL-17A+ Tnaive	CD107A+mfi(PCS)	1.000	.
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD4+ Tregs EM	CD38+%(ex vivo)	0.750	0.032
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD4+ Tregs EM	CD38+mfi(ex vivo)	0.839	0.009
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD4+ Tregs EM	IL10+mfi(ex vivo)	0.783	0.022
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ Tregs Naïve	CD38+%(ex vivo)	0.875	0.004
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ Tregs Naïve	IL10+%(ex vivo)	0.750	0.032
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ Tregs Naïve	IL10+mfi(ex vivo)	0.875	0.004
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ Tregs Naïve	PD1+%(ex vivo)	0.875	0.004
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ Tregs CM	CD38+mfi(PCS)	0.750	0.032
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ Tregs CM	IL10+mfi(PCS)	0.959	0.000
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ Tregs CM	MIP1b+%(PCS)	0.839	0.009
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ Tregs CM	MIP1b+mfi(PCS)	0.959	0.000
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ Tregs EM	CD38+%(PCS)	1.000	.
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ Tregs EM	CD38+mfi(PCS)	0.756	0.030
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ Tregs EM	IL10+%(PCS)	1.000	.
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ Tregs EM	IL10+mfi(PCS)	1.000	.
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ T naïve	mfi(ex vivo)	0.713	0.047
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ TEM RA	CD107A+%(ex vivo)	0.819	0.013
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ TEM RA	CD107A+mfi(ex vivo)	0.819	0.013
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ TEM RA	IL2+mfi(ex vivo)	0.770	0.043
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD4+ T naïve	CD107A+%(PCS)	0.756	0.030
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD4+ T naïve	IL2+mfi(PCS)	0.761	0.028
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ T naïve	CD107A+%(PCS)	0.756	0.030
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ T naïve	Ki67mfi(PCS)	0.756	0.030
CD4TreCmCD38	CD4+ Tregs CM CD38+%	CD8+ TEM RA	IL2+mfi(PCS)	0.756	0.030

- P values are not corrected for multiple comparison.

Table S10. A List of capture and detection antibodies, and protein standards used in the Bio-Plex Multiplexed cytokine/chemokine assays

Protein Standards		
Name	**Catalog No.**	**Manufacturer**
Recombinant Human CXCL8/IL-8 Protein	208-IL-10	R&D system
Recombinant Human CCL5/RANTES Protein	278-RN-10	R&D system
Recombinant Human GM-CSF Protein	215-GM	R&D system
Recombinant Human Interferon Gamma Protein	RIFNG50	ThemoFisher scientific
Recombinant Human IL-1 beta/IL-1F2	201-LB	R&D system
Recombinant Human IL-6 Protein	206-IL-10	R&D system
Recombinant Human IL-10 (aa 19-178) Protein	1064-IL	R&D system
Recombinant Human IL-17A Protein	317-ILB	R&D system
Recombinant Human CXCL10/IP-10	266-IP	R&D system
Recombinant Human CCL2/MCP-1 Protein	279-MC	R&D system
Recombinant Human CCL3/MIP-1 alpha protein	270-LD-10	R&D system
Recombinant Human CCL4/MIP-1 beta Protein	271-BME-10	R&D system
Recombinant Human TNF-alpha Protein	210-TA-20	R&D system
Recombinant Human IL-1 alpha	200-LA	R&D system
Capture Antibodies		
Name	**Catalog No.**	**Manufacturer**
Human CXCL8/IL-8 MAb	M801	ThermoFisher Scientific
Human CCL5/RANTES PAb	P230E	ThermoFisher Scientific
Rat Anti-Human GM-CSF-UNLB	10111-01	SouthernBiotech
Human IFNγ MAb	M700A	ThermoFisher Scientific
Human IL-1beta/ IL-1F2 Antibody	MAB601-500	R&D System
Human IL-6 MAb	M620	ThermoFisher Scientific
Rat Anti-Human IL-10-UNLB	10100-01	SouthernBiotech
Human/Primate IL-17/IL-17A Antibody	MAB317-500	R&D System
Human IP-10/ CXCL10/CRG-2 Antibody	MAB266-500	R&D System
Human MCP-1/CCL2/JE Antibody	MAB679-500	R&D System
Human MIP-1α/CCL3Antibody	AF-270-NA	R&D System
Human MIP-1β/CCL4 Antibody	MAB271-100	R&D System
Human TNFα MAb	M303	ThermoFisher Scientific
Human IL-1 alpha/IL-1F1 Mab	MAB200	R&D system
Detection Antibodies		
Name	**Catalog No.**	**Manufacturer**
Human CXCL8/IL-8 MAb, Biotin-labeled	M802B	ThermoFisher Scientific
Human CCL5/RANTES MAb, Biotin-labeled	M230B	ThermoFisher Scientific
Rat Anti-Human GM-CSF-BIOT	10112-08	SouthernBiotech
Human IFNγ MAb, Biotin-labeled	M701B	ThermoFisher Scientific
Human IL-1beta/IL-1F2 Biotinylated Antibody	BAF201	R&D System
Human IL-6 MAb, Biotin-labeled	M621B	ThermoFisher Scientific
Rat Anti-Human IL-10-BIOT	10110-08	SouthernBiotech
Human/Primate IL-17/IL-17A Biotinylated Antibody	BAF317	R&D System
Human IP-10/CXCL10/CRG-2 Biotinylated Antibody	BAF266	R&D System
Human MCP-1/CCL2/JE Biotinylated Antibody	BAF279	R&D System
Human MIP-1α/CCL3 Biotinylated Antibody	BAF270	R&D System
Human MIP-1β/CCL4 Biotinylated Antibody	BAF271	R&D System
Human TNFα MAb, Biotin-labeled	M302B	ThermoFisher Scientific
Human IL-1 alpha/IL-1F1 Biotinylated	BAF200	R&D system

Table S11 Final concentration of the standard pool in the Bio-Plex Multiplexed cytokine/chemokine assays

Name	Concentration in pg/mL
Recombinant Human CXCL8/IL-8 Protein	27000
Recombinant Human CCL5/RANTES Protein	18500
Recombinant Human GM-CSF Protein	17000
Recombinant Human Interferon Gamma Protein	10500
Recombinant Human IL-1 beta/IL-1F2	19500
Recombinant Human IL-6 Protein	9500
Recombinant Human IL-10 (aa 19-178) Protein	28000
Recombinant Human IL-17A Protein	38000
Recombinant Human CXCL10/IP-10	24000
Recombinant Human CCL2/MCP-1 Protein	26500
Recombinant Human CCL3/MIP-1 alpha protein	27500
Recombinant Human CCL4/MIP-1 beta Protein	9000
Recombinant Human TNF-alpha Protein	18000
Recombinant Human IL-1 alpha	160000

Table S12. SIV PCS peptides used to stimulate PBMCs for testing antigen recall responses

Peptide	Peptide Sequence
PCS1-1	[H]APSSGRGGNYPVQQI[OH]
PCS1-2	[H]SGRGGNYPVQQIGGN[OH]
PCS1-3	[H]RGGNYPVQQIGGNYV[OH]
PCS2-1	[H]GGPGQKARLMAEALK[OH]
PCS2-2	[H]GQKARLMAEALKEAL[OH]
PCS2-3	[H]KARLMAEALKEALAP[OH]
PCS3-1	[H]LAPVPIPFAAAQQRG[OH]
PCS3-2	[H]VPIPFAAAQQRGPRK[OH]
PCS3-3	[H]IPFAAAQQRGPRKPI[OH]
PCS4-1	[H]MAKCPDRQAGFLGLG[OH]
PCS4-2	[H]CPDRQAGFLGLGPWG[OH]
PCS4-3	[H]DRQAGFLGLGPWGKK[OH]
PCS5-1	[H]GPWGKKPRNFPMAQVHQGLM[OH]
PCS5-2	[H]YGQMPRQTGGFFRPWSMGKE[OH]
PCS5-3	[H]KPRNFPMAQVHQGLM[OH]
PCS6-1	[H]YGQMPRQTGGFFRPW[OH]
PCS6-2	[H]MPRQTGGFFRPWSMG[OH]
PCS6-3	[H]RQTGGFFRPWSMGKE[OH]
PCS7-1	[H]WSMGKEAPQFPHGSS[OH]
PCS7-2	[H]GKEAPQFPHGSSASG[OH]
PCS7-3	[H]EAPQFPHGSSASGAD[OH]
PCS8-1	[H]LQGGDRGFAAPQFSL[OH]
PCS8-2	[H]GDRGFAAPQFSLWRR[OH]
PCS8-3	[H]RGFAAPQFSLWRRPV[OH]
PCS9-1	[H]LTALGMSLNFPIAKV[OH]
PCS9-2	[H]LGMSLNFPIAKVEPV[OH]
PCS9-3	[H]MSLNFPIAKVEPVKV[OH]
PCS10-1	[H]KDPIEGEETYYTDGS[OH]
PCS10-2	[H]IEGEETYYTDGSCNK[OH]
PCS10-3	[H]GEETYYTDGSCNKQS[OH]
PCS11-1	[H]LVSQGIRQVLFLEKI[OH]
PCS11-2	[H]QGIRQVLFLEKIEPA[OH]
PCS11-3	[H]IRQVLFLEKIEPAQE[OH]
PCS12-1	[H]NQGQYMNTPWRNPAE[OH]
PCS12-2	[H]QYMNTPWRNPAEERE[OH]
PCS12-3	[H]MNTPWRNPAEEREKL[OH]

Table S13. Flow cytometry panel design

T cell Panel 1		
Marker	**Fluorochrome**	**Clone**
Live/Dead	Blue (405)	reactive dye
CD3	V500	SP34-2
CD4	BV605	L200
CD8	V450	RP8-T4
CCR7	A700	150503
CD127 (IL-7)	PE-CF594	HIL-7R-M2
CD107a	PE-Cy5	H4:A3
IL-2	FITC	MQ1-17H12
IFNγ	BV786	4S.B3
TNFα	APC	MAb11
Ki67	PE-Cy7	B56
MIP1b	PE	D21-1351
CD45RO	APC-H7	UCHL1
IL-17A	BV650	N49-653
Marker	**Fluorochrome**	**Clone**
T cell Panel 2		
Marker	**Fluorochrome**	**Clone**
Live/Dead	Blue (405)	reactive dye
CD3	V500	SP34-2
CD4	BV605	L200
CD8	APC-Cy7	RP8-T4
CCR7	Alexa700	150503
CD38	FITC	HB7
PD-1	BV650	M1H4
CD69	APC	FN50
Foxp3	V450	259D/C7
CD127	Pe-CF594	HIL-7R-M2
IL-10	BV711	JES3-9D7
CD25	PE-CY5	M-A251
CD45RA	PE-Cy7	L48
MIP1b **Live/Dead**	PE **Blue (405)**	D21-1351 **reactive dye**
CD3	V500	SP34-2
CD4	BV605	L200
CD8	V450	RP8-T4
CCR7	A700	150503
CD127 (IL-7)	PE-CF594	HIL-7R-M2
MIP1b	PE	D21-1351
CD45RO	APC-H7	UCHL1
IL-17A	BV650	N49-653
CD195 (CCR5)	PE-Cy7	2D7/CCR5
CD38	FITC	HB7
CD69	APC	FN50
CD107a	PE-Cy5	H4:A3
IFNγ	BV786	4S.B3

Table S14. List of fluorescent flow cytometry antibodies

Name	Company	Catalogue Number
LIVE/DEAD™ Fixable Blue Dead Cell Stain Kit, for 405 nm	ThermoFisher	L23105
CD3 V500 (SP34-2)	BD Biosciences	560770
CD4 BV605 (L200)	BD Biosciences	562843
CD8 V450 (RP8-T4)	BD Biosciences	560347
CD127 PE-CF594 (HIL-7R-M2)	BD Biosciences	562397
CD107a PE-Cy5 (H4A3)	BD Biosciences	555802
IFNg BV786 (4S.B3)	BD Biosciences	563731
CD195 (CCR5)	BD Biosciences	557752
BCL-6 PE	BD Biosciences	561522
CD45RO APC-H7	BD Biosciences	561137
IL-17A BV650	BD Biosciences	563746
CD38 FITC HB7	BD Biosciences	340909
CD69 APC FN50	BD Biosciences	555533
Foxp3 V450 259D/C7	BD Biosciences	560459
Foxp3 Human buffer	BD Pharmaringen	560098
IL-10 JES3-9D7	BD Biosciences	564050
CD25 M-A251	BD Biosciences	555433
PD-1 BV650 M1H4	BD Biosciences	564324
Cytoperm/Cytofix Plus permeabiliziation solution kit with BD Golgistop/plug	BD Biosciences	554715
Brilliant Stain Buffer	BD Biosciences	563794