Interleukin-21 and anti-α4β7 dual therapy during ART promotes immunological and microbiome responses in SIV-infected macaques

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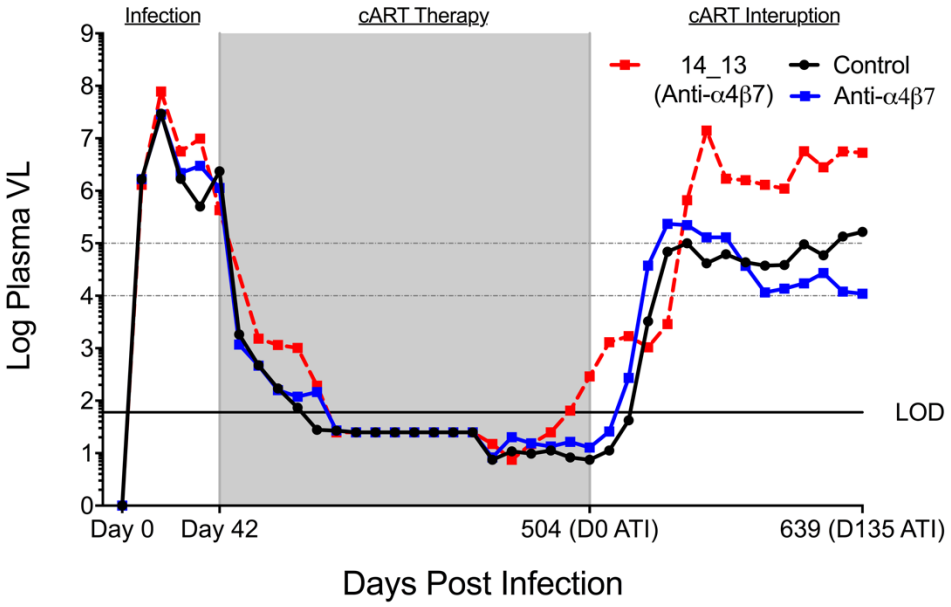
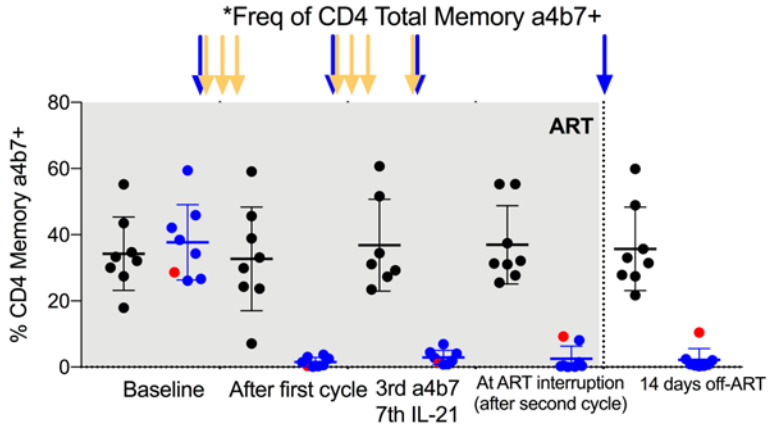
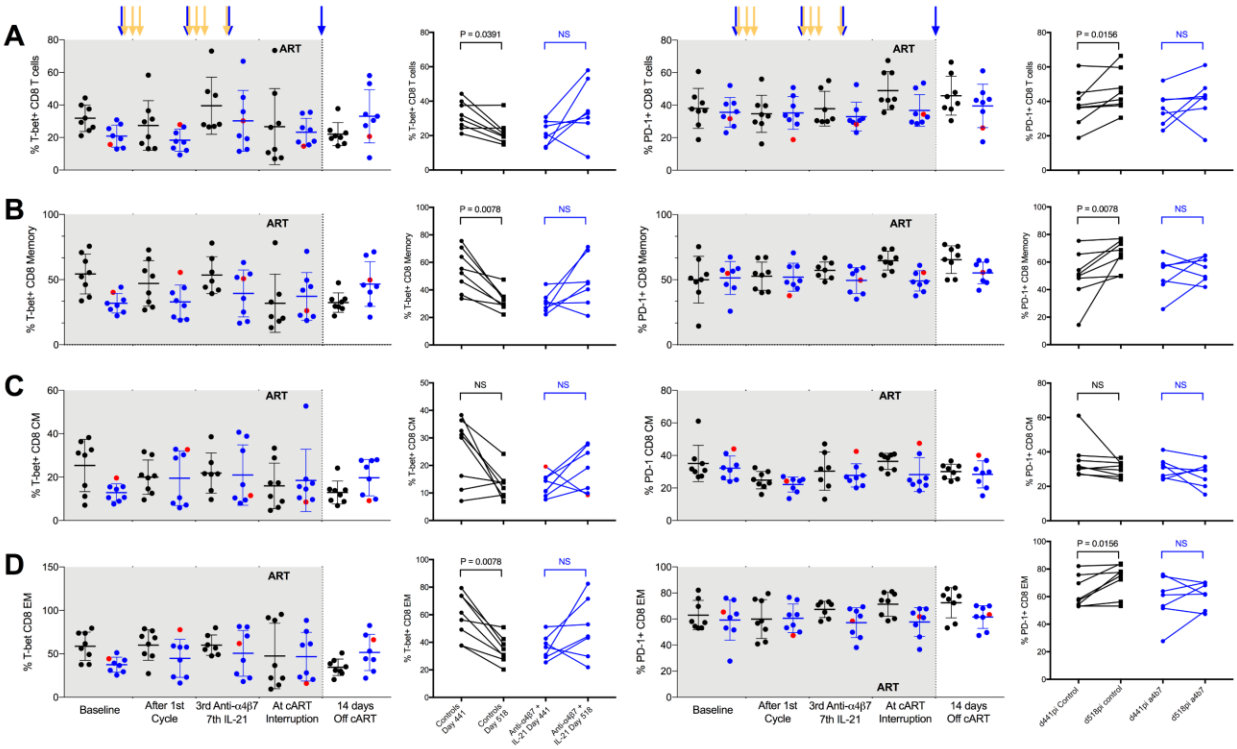
Supplemental Figures and Tables



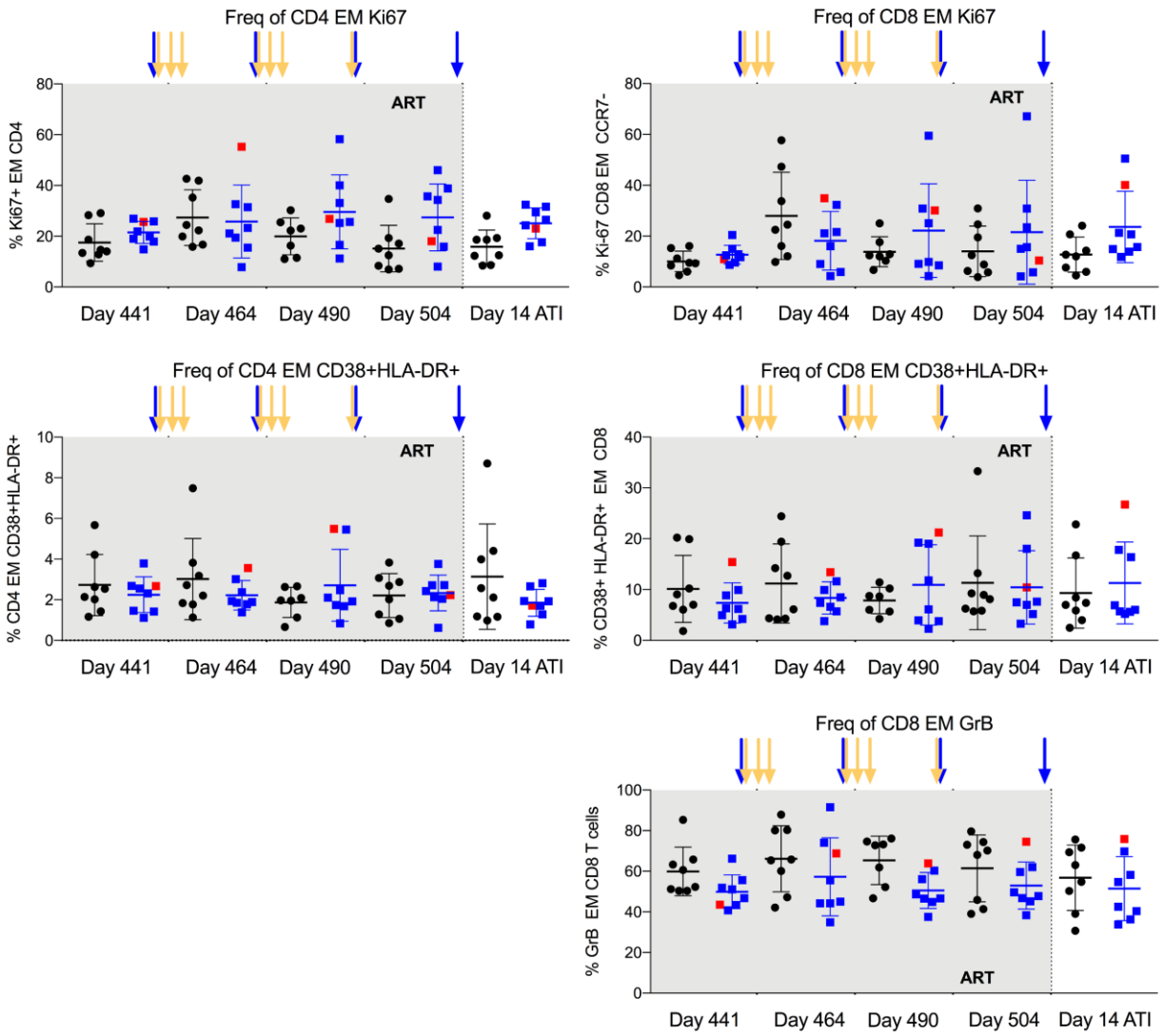
Supplemental Figure 1: Viral Loads with 14_13 shown separately from other treated individuals



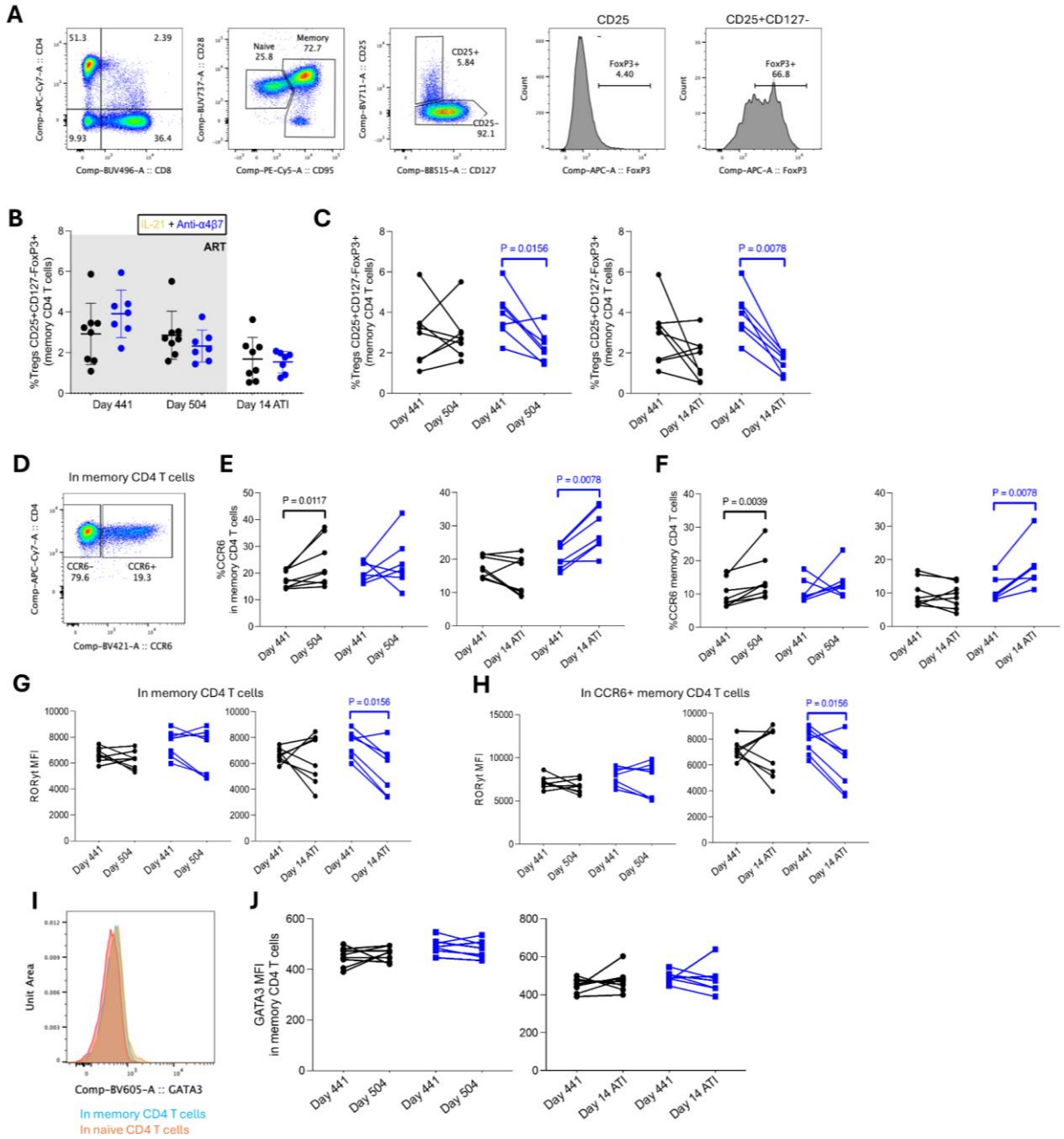
Supplemental Figure 2: Dual therapy effectively masks α4β7 integrin on CD4. Note: Excluded RM 14_13 is in red.



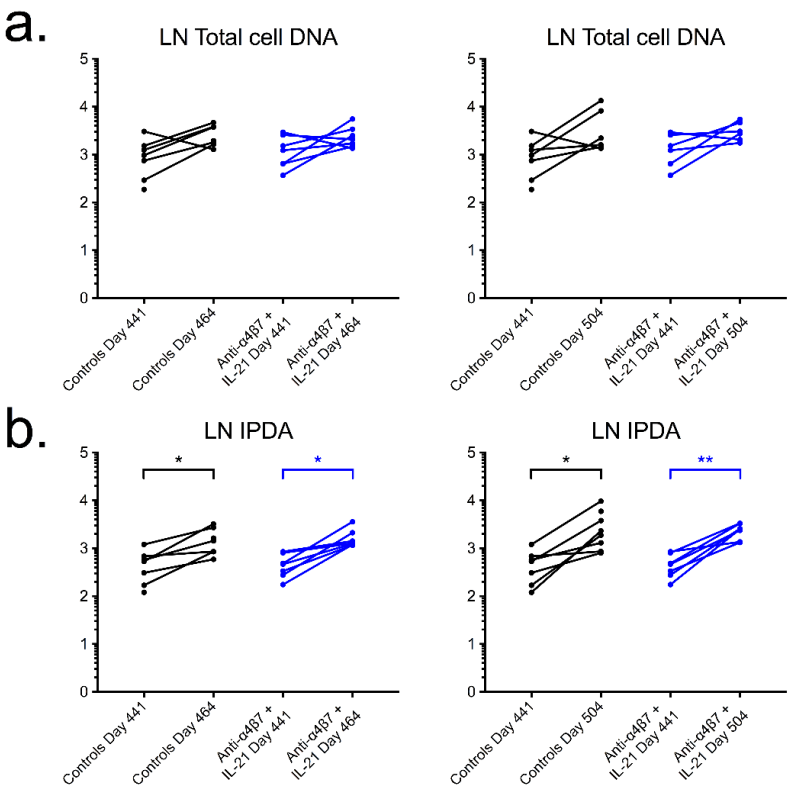
Supplemental Figure 3: T-bet and PD-1 expression on CD8+ T cell subsets in blood, including (A) total CD8+, (B) CD8+ memory, (C) CD8+ central memory, and (D) CD8+ effector memory T cells.



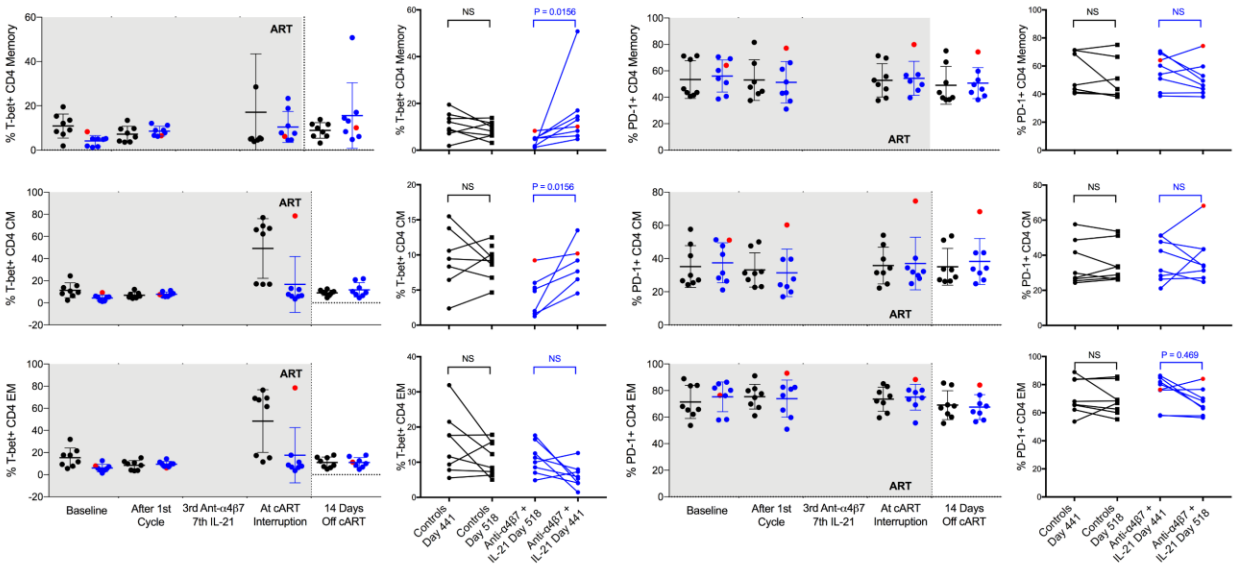
Supplemental Figure 4: Expression of Ki67, activation markers CD38 and HLA-DR, and Granzyme B on peripheral CD4+ and CD8+ effector memory T cell subsets.



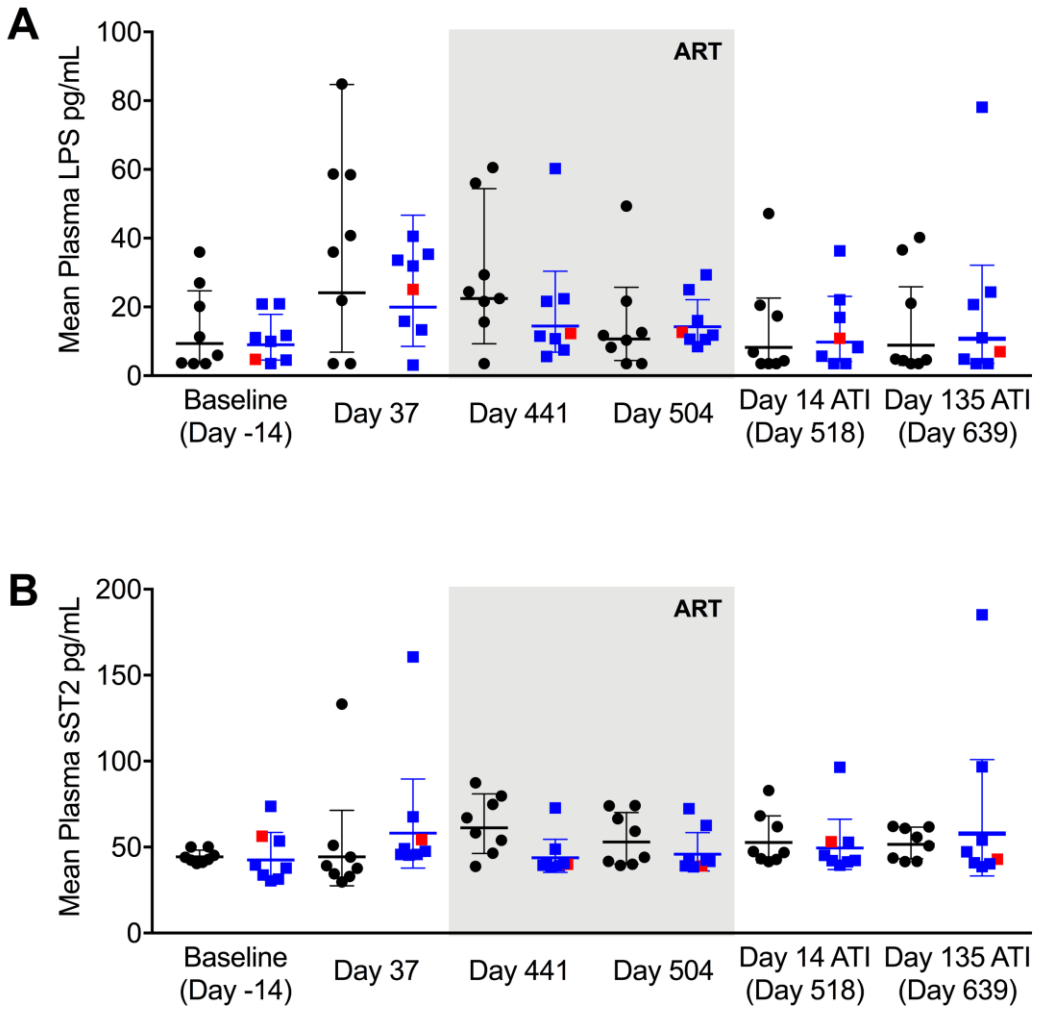
Supplemental Figure 5: Peripheral CD4+ T cell immunophenotyping. A) Gating strategy for T regulatory cells (CD25+CD127-FOXP3+); B, C) Longitudinal percent peripheral T regulatory T cells; D) Gating strategy for CCR6+ memory cells; E) percent CCR6+ of memory cells; F) percent CCR6+ memory cells of total peripheral CD4+ T cells; RORγt MFI on G) memory and H) CCR6+ memory CD4+ T cells; and I, J) GATA3 MFI on memory CD4+ T cells.



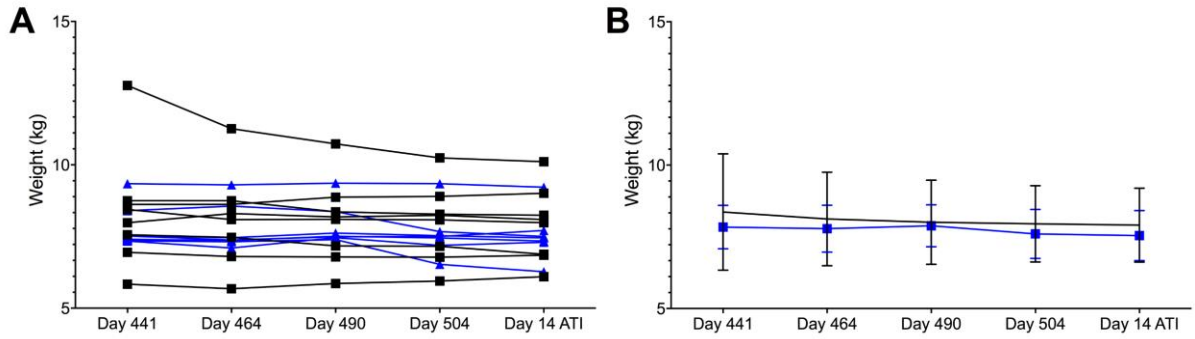
Supplemental Figure 6: Measures of lymph node reservoir size, including (A) total SIVgag DNA copies normalized and (B) intact proviral DNA Assay (IPDA), each normalized to 10^6 cells.



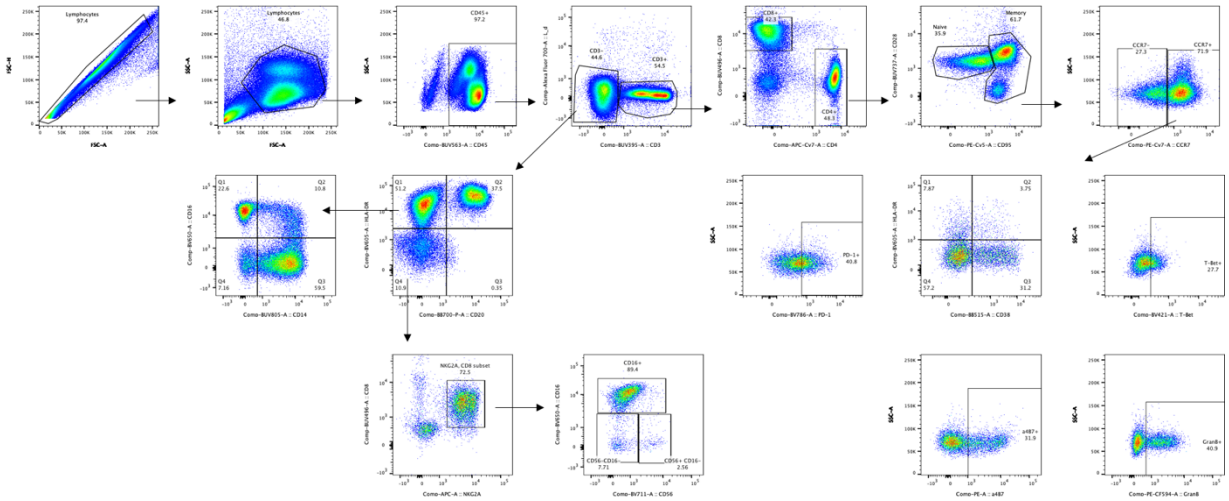
Supplemental Figure 7: LN Immune cell phenotypes, including T-bet and PD-1 expression. Statistics performed excluding 14_13 (red).



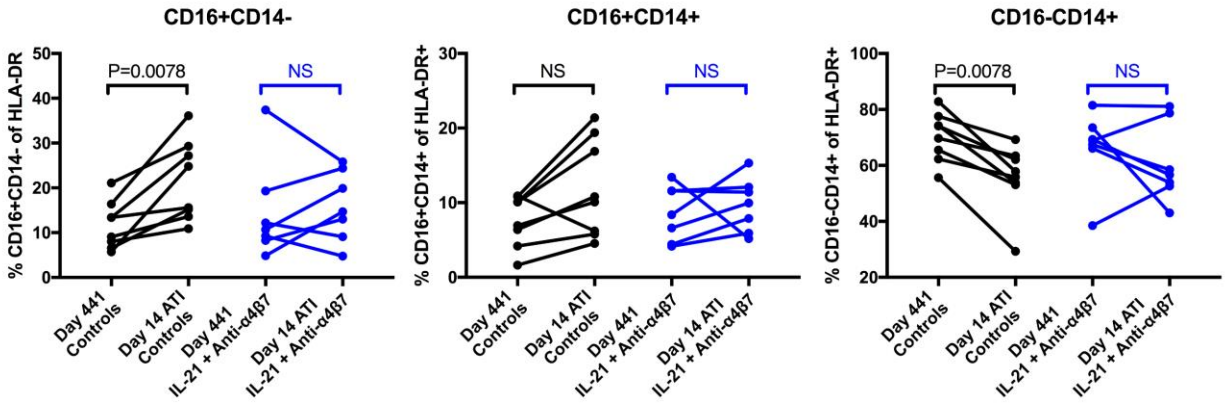
Supplemental Figure 8: Longitudinal plasma biomarkers of gut barrier function, including A) lipopolysaccharide and B) soluble ST2 levels



Supplemental Figure 9: Longitudinal macaque weights



Supplemental Figure 10: Gating Strategy



Supplemental Figure 11: Peripheral monocyte subsets

	Animal code	Age at infection (months)	Gender	Peak viremia VL (Log10 copies/mL)	Setpoint VL (d42pi; Log10 copies/mL)	freq CD4 CD95+ a4b7bright pre-infection	freq CD4 CD95+ a4b7bright last time	ADA mAb a4b7	A01
Control group	14C002	48	Female	8.23	6.45	21.1	19.8	+	-
	RFm12	117	Female	7.32	5.90	31.8	25.5	+	-
	13C081	57	Female	7.40	7.15	21.5	23.3	+	-
	RCr11	140	Female	6.98	6.32	29.8	29.6	-	+
	14C122	45	Female	7.18	5.83	35.6	25.6	-	-
	RIl13	104	Female	7.36	5.04	19.8	23.8	-	-
	14C289	44	Female	7.63	7.38	19.2	27.7	+	-
	14C141	46	Male	7.64	6.90	16.6	21.9	+	-
a4b7+IL-21 group	14_134	43	Female	6.70	5.28	23	20.6	-	-
	11_32	80	Female	7.56	6.18	37.7	41.5	-	-
	14C066	45	Male	7.46	7.04	22.1	27.2	-	-
	14_13	45	Female	7.89	5.63	24.1	24.6	-	-
	RFd12	128	Female	7.69	6.79	17.7	28.6	-	+
	RHy13	93	Female	7.88	6.11	25.2	24.5	-	-
	13M257	55	Female	7.71	6.83	22.3	20.2	-	-
	RWn12	118	Female	7.18	4.15	36.5	31.9	-	-

Supplemental Table 1: Rhesus macaque characteristics, including criteria utilized for appropriate stratification of animals into groups. “ADA mAb a4b7” indicates whether the RM tested +/- for pre-existent anti-anti-α4β7-specific antibodies, prior to infection. “A01” indicates MHC haplotype *Mamu*-A*01^{+}.

Phenotype panel 1					
Vendor	**Antibody**	**Fluorochrome**	**Cat #**	**Clone**	**Isotype**
Stemcell	CD38-FITC	FITC	60131FI	AT1	Mouse IgG1, κ
Biolegend	CD20-PerCpCy5.5	PerCpCy5.5	302326	2H7	Mouse IgG2b, κ
Beckman Coulter	NKG2a-APC	APC	A60797	Z199	IgG2b Mouse
Biolegend	CD4-APCCy7	APC Cy7	317418	OKT4	Mouse IgG2b, κ
NHP Reagent Resource	alpha4/beta 7 -PE	PE	PR-1421	A4B7R1	Rhesus IgG1 Fc
BD	CD45	BUV563	741414	D058-1283	Mouse IgG1, κ
BD	CD28-BUV737	BUV737	564438	CD28.2	Mouse C3H x BALB/c IgG1, κ
BD	CCR7-Cy7PE	Cy7PE	557648	3D12	Rat anti-human
BD	CD14-BUV805	BUV805	612902	M5E2	Mouse IgG2a, κ
Biolegend	T-bet	BV421	644816	4B10	Mouse IgG1, κ
BD	KI67-BV480	BV480	566109	B56	Mouse IgG1, κ
BD	HLADR-BV605	BV605	562845	G46-6	Mouse IgG2a, κ
BD	CD16-BV650	BV650	563692	3G8	Mouse BALB/c x DBA/2, also known as CD2F1 or CDF1 IgG1, κ
Biolegend	PD1-BV785	BV785	329930	EH12.2H7	Mouse IgG1, κ
BD	CD3-BUV395	BUV395	564117	SP34-2	Mouse BALB/c IgG1, λ
BD	CD8-BUV496	BUV496	564804	RPA-T8	Mouse IgG1, κ
BD	CD56-BV711	BV711	740781	B159	Mouse IgG1, κ
eBioscience	CXCR5-PE	PE	12-9185-42	MU5UBEE	Mouse / IgG2b, kappa
Invitrogen	GranB-PETR	PETR	GRB17	GB11	Mouse / IgG1
BD	CD95-Cy5PE	CY5PE	559773	DX2	Mouse C3H, also known as C3H/He, C3H/Bi IgG_1, κ

Supplemental Table 2: Antibody list used for fresh PBMCs and LN cells

Phenotype Panel 2

Vendor	Antibody	Fluorochrome	Cat #	Clone	Isotype
Thermofisher Scientific	CD127	Alexa Fluor 488	53-1278-42	eBioRDR5	Mouse IgG1, κ
BD	CCR6	BV421	565925	11A9	Mouse IgG1, κ
Thermofisher Scientific	GATA3	BV605	406-9966-42	TWAJ	Rat IgG2b, k
Biolegend	CD25	BV711	302636	BC96	Mouse IgG1, κ
Thermofisher Scientific	RORyt	PE	12-6988-82	AFKJS-9	Rat IgG2b, k
Biolegend	CD95	PE-Cy5	305610	DX2	Mouse IgG1, κ
BD	CD3	BUV395	564117	SP34-2	Mouse BALB/c IgG1, λ
BD	CD8	BUV496	612942	RPA-T8	Mouse IgG1, κ
BD	CD28	BUV737	612815	CD28.2	Mouse C3H x BALB/c IgG1, κ
Biolegend	FoxP3	Alexa Fluor® 647	320014	150D	Mouse IgG1, κ
Biolegend	CD4	APC-Cy7	317418	OKT4	Mouse IgG2b, κ
BD		Fixable Viability Stain 700	564997		

Supplemental Table 3: Second antibody list used for frozen PBMCs