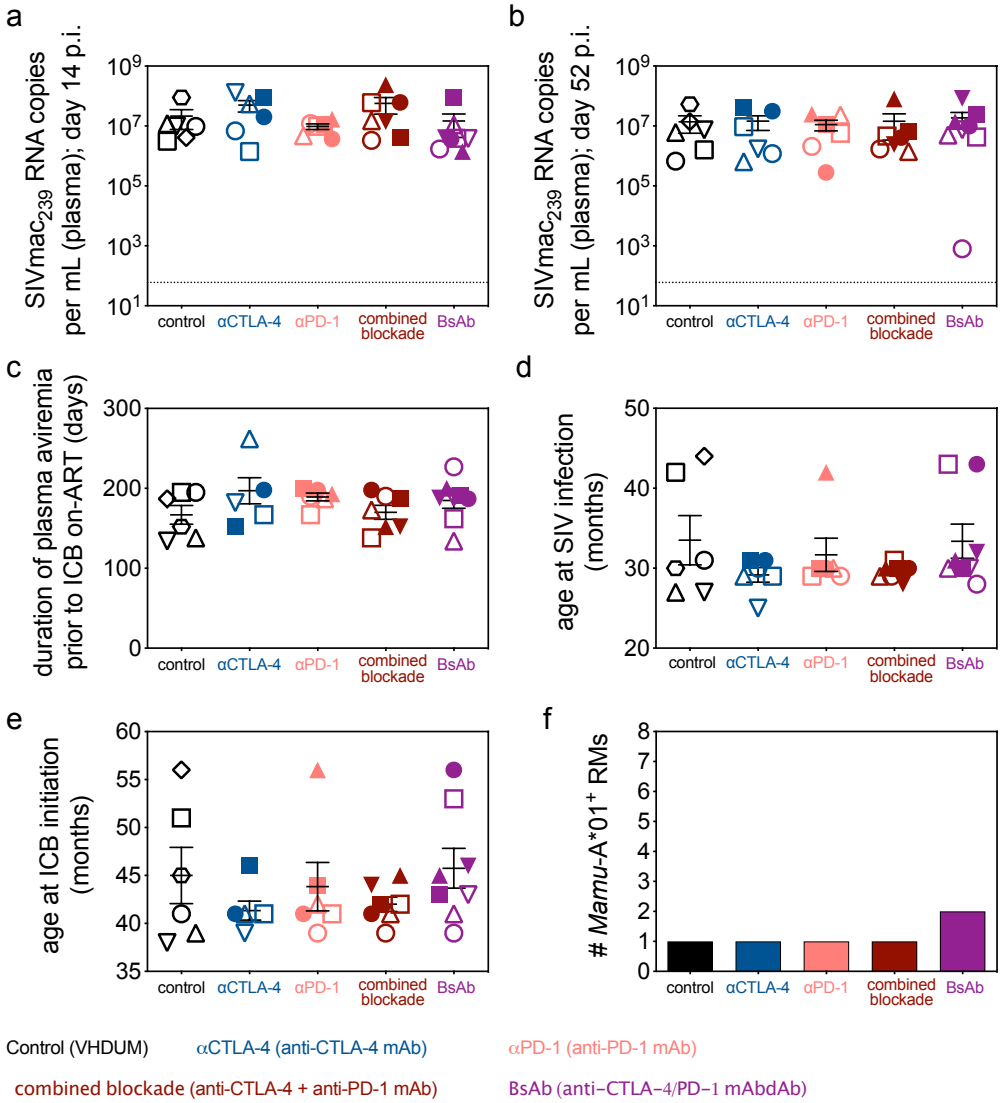
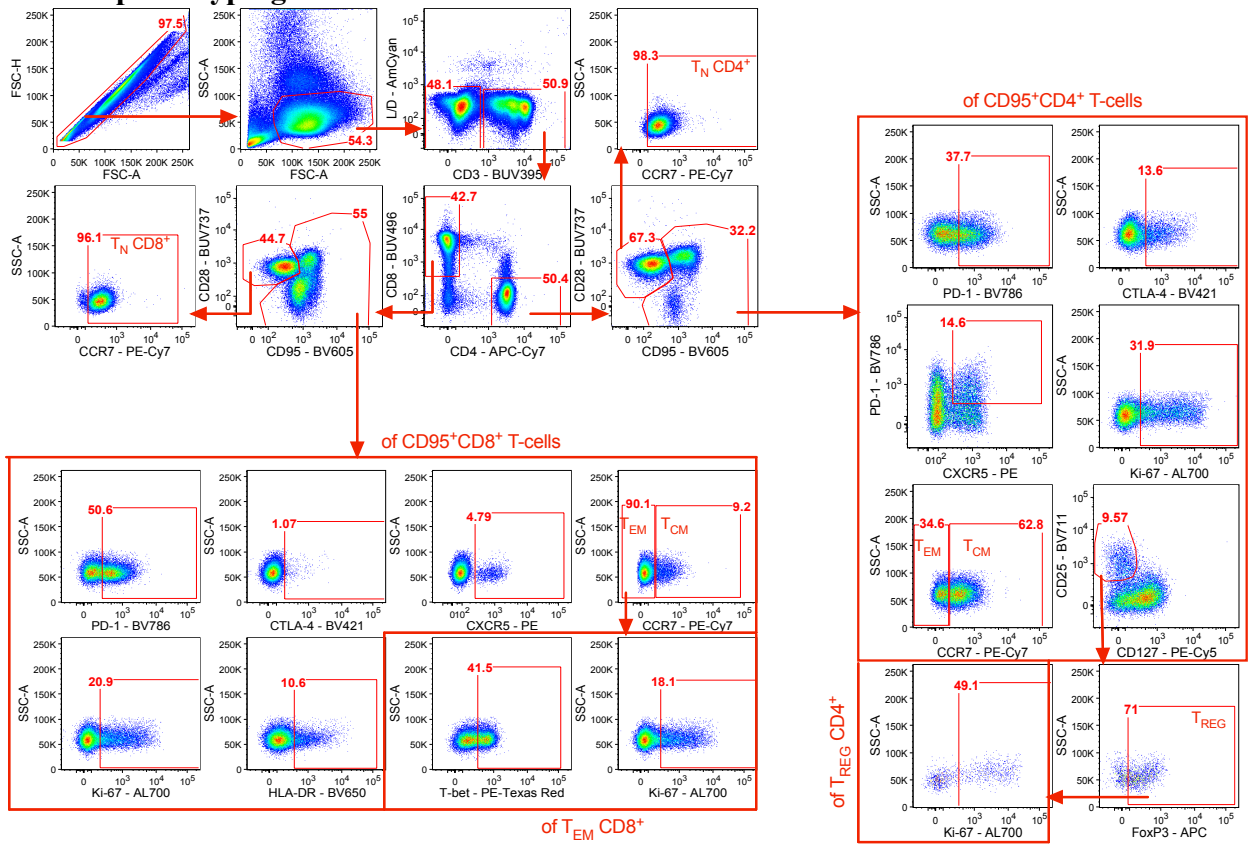
Supplementary Figure 1. Parameters used for ICB treatment group stratification.



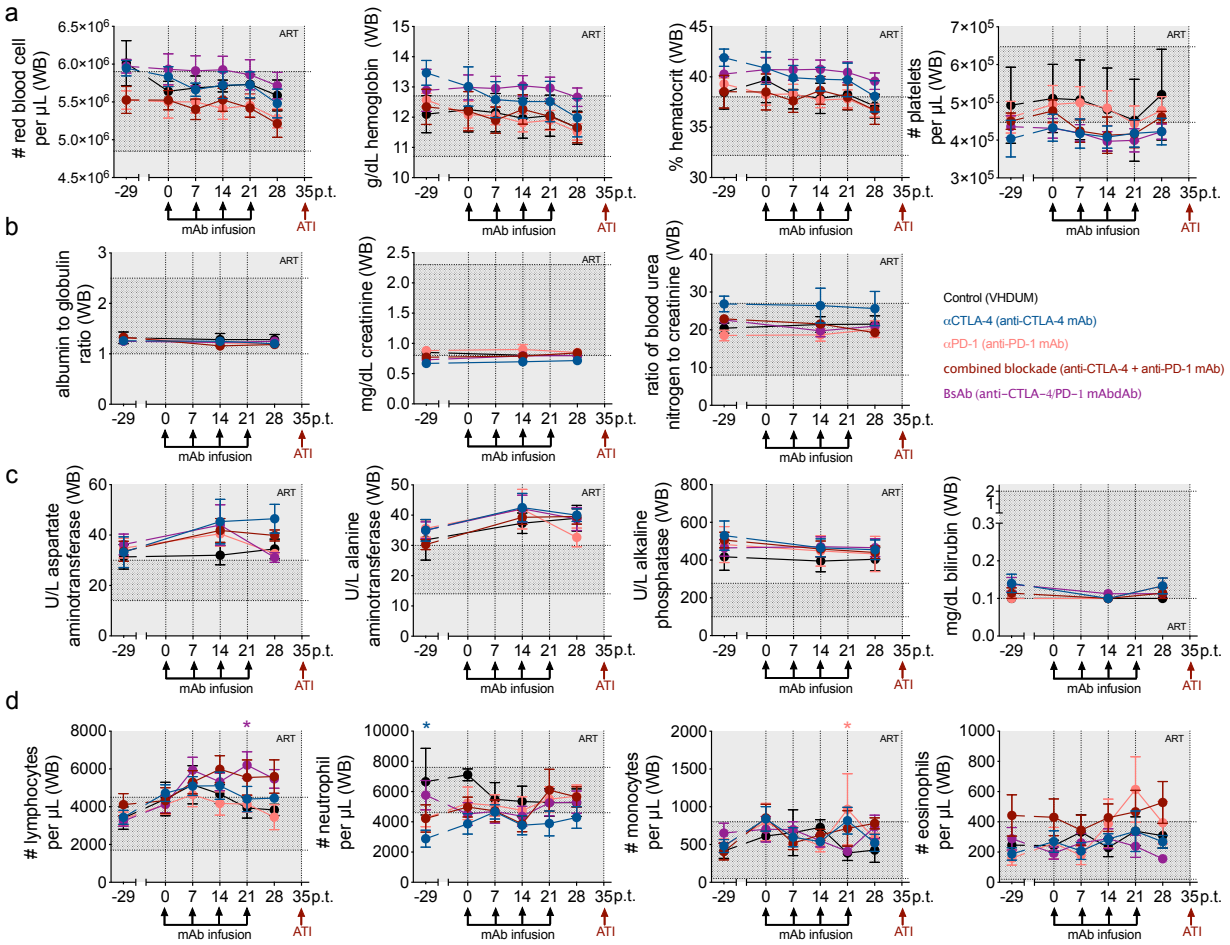
The plasma viral load (SIVmac$_{239}$ RNA copies per mL) was quantified by RT-qPCR during (**a**) acute infection (d14 p.i.) and (**b**) chronic infection prior to ART initiation (d52 p.i.). The hashed horizontal line indicates the assay's limit of detection (≤60 copies/mL). (**c**) The duration of on-ART virological suppression prior to ICB treatment was calculated from the first undetectable observation following ART initiation. The age of each RM was calculated relative to (**d**) SIV infection (d0 p.i.) and (**e**) to the initiation of ICB treatment (d0 p.t.). (**f**) All RMs were haplotyped for MHC repertoire (all were *Mamu*-B*08^{-} and -B*17^{-}) and the number of *Mamu*-A*01^{+} RMs is given per treatment group. Averaged data are presented as the mean ± SEM, and analyzed with a two-sided, two-way ANOVA with Dunn's correction for all treatment combinations. Individual RMs are indicated by shape with closed data points indicating animals with viral reactivation in plasma during ICB. All RMs are color-coded and grouped based on ICB therapy as follows with population sizes as indicated for all analyses: controls (n=6), black; αCTLA-4 (n=6), blue; αPD-1 (n=6), pink; combined blockade (n=7), red; and BsAb (n=8), purple.

Supplementary Figure 2. Representative gating strategy for longitudinal immunophenotyping.



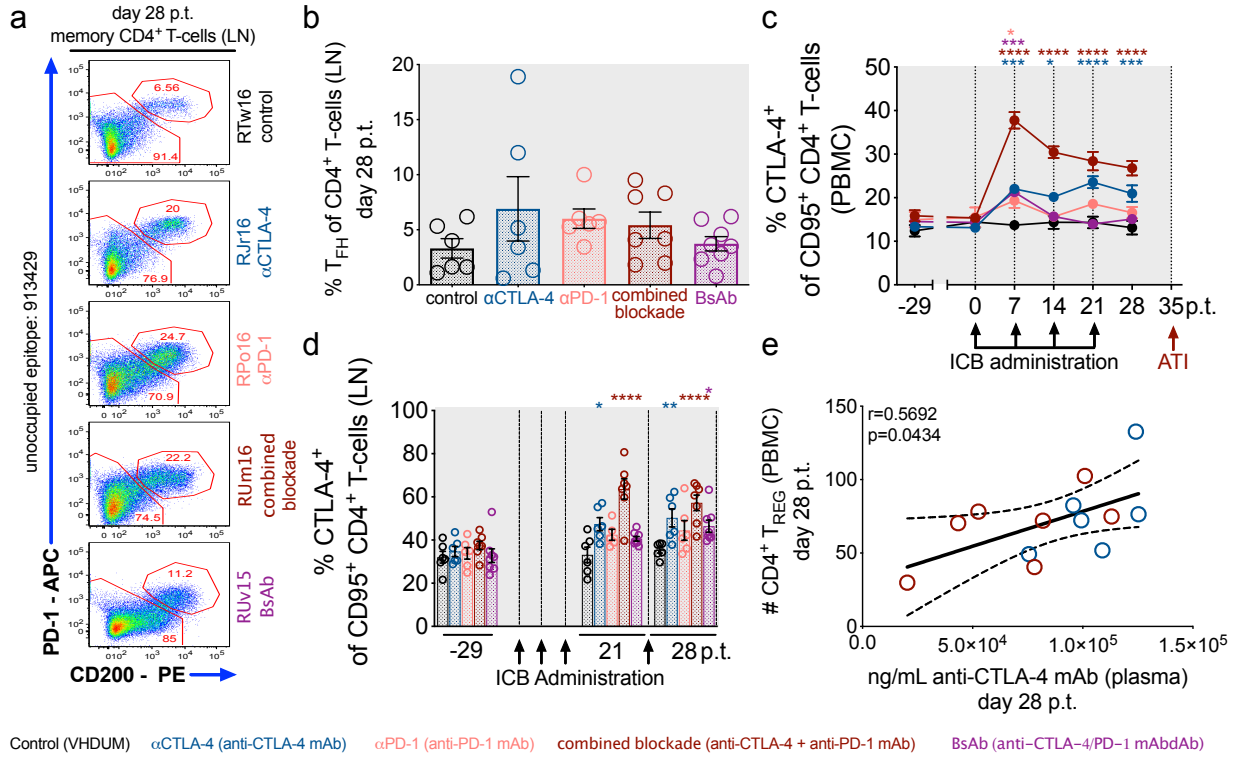
A representative gating strategy is given for the longitudinal immunophenotyping of blood and tissues. Red arrows indicated the successive hierarchy of gates and clusters of biomarkers within a common parental population are outlined with a red border and annotated appropriately. T-cell subsets of interest are annotated as follows: T_N, naïve; T_{EM}, effector-memory; T_{CM}, central-memory; and T_{REG}, regulatory T-cell. Shown are PBMCs at day -29 p.t. from RHj16.

Supplementary Figure 3. Combined blockade does not induce anemia, thrombocytopenia, nephrotoxicity, or hepatotoxicity.



(**a**) In WB, CBCs were employed to monitor for hematological indicators of anemia such as the red blood cell count (per µL), hemoglobin (g/dL), and hematocrit (%); whereas, the platelet count (per µL) was used to screen for thrombocytopenia. (**b**) Serum chemistries readouts for nephrotoxicity include the albumin to globulin ratio, creatinine (mg/dL), and the blood urea nitrogen (BUN) to creatinine ratio; furthermore, (**c**) aspartate aminotransferase (U/L), alanine aminotransferase (U/L), alkaline phosphatase (U/L), and bilirubin (mg/dL) were employed to assess hepatotoxicity. (**d**) Counts of leukocyte subsets (lymphocytes, neutrophils, monocytes, and eosinophils) per µL of whole blood (WB) are shown as quantified from a complete blood count (CBC). Horizontal, dashed lines with hashed shading indicate the normal range of values for indoors, juvenile RMs. All RMs are color-coded and grouped based on ICB therapy as follows with population sizes as indicated for all analyses: controls (n=6), black; αCTLA-4 (n=6), blue; αPD-1 (n=6), pink; combined blockade (n=7), red; and BsAb (n=8), purple. Averaged data are presented as the mean ± SEM, and were analyzed with a mixed-effects models with the Dunnett correction for multiple comparisons relative to controls. Color-coded statistical asterisks indicate significance between control animals and the treatment group of that color. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.

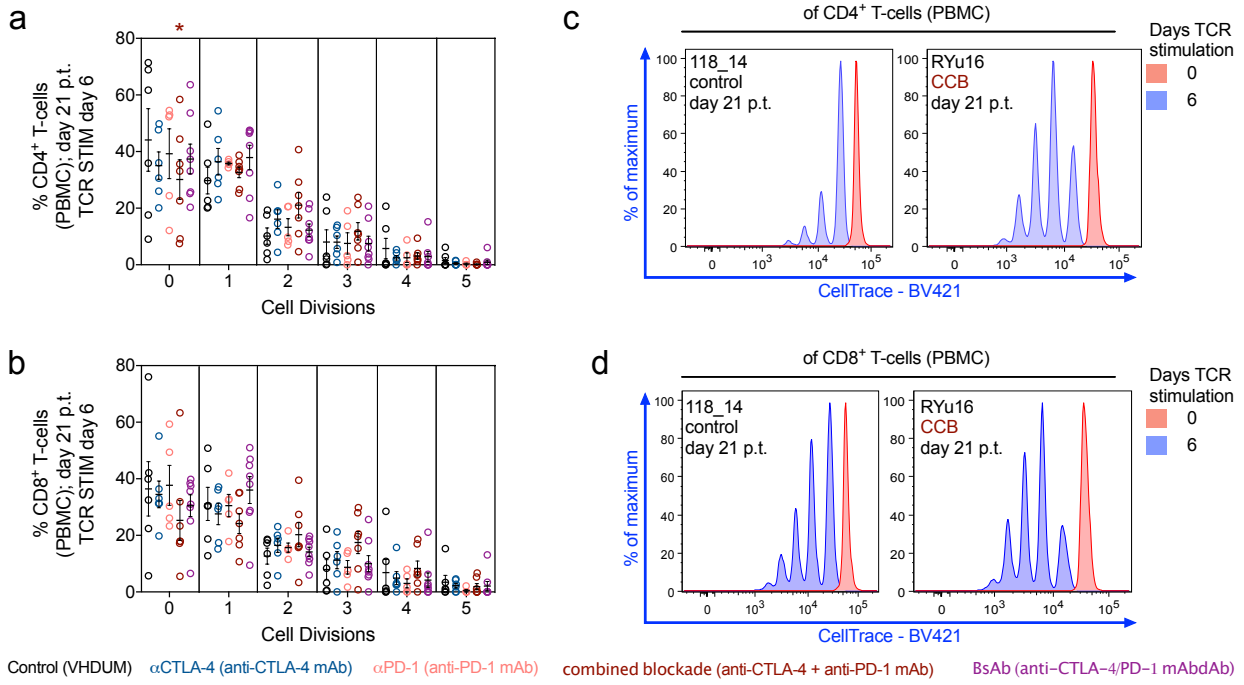
Supplementary Figure 4. Lymphoid T_{FH} are not depleted by PD-1 blockade and CTLA-4$^+$CD4$^+$ T-cells are expanded by CTLA-4 blockade.



Control (VHDUM) αCTLA-4 (anti-CTLA-4 mAb) αPD-1 (anti-PD-1 mAb) combined blockade (anti-CTLA-4 + anti-PD-1 mAb) BsAb (anti-CTLA-4/PD-1 mAbdAb)

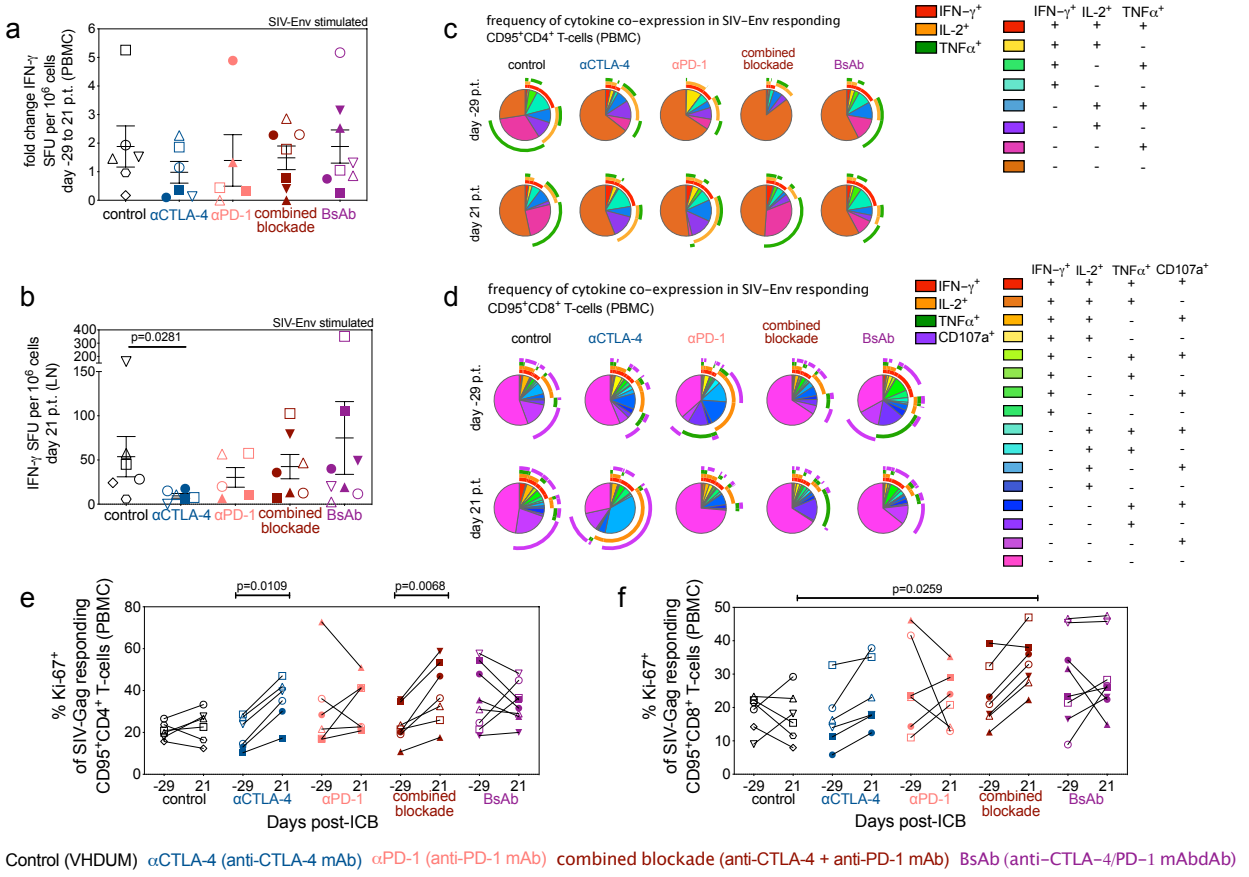
(**a**) A representative stain is given for the co-expression of CD200 and unmasked PD-1 (clone 913429) per T_{FH} discrimination within LN memory CD4$^+$ T-cells following the fourth ICB infusion (d28 p.t.) segmented by ICB treatment (indicated at right; 5 of 33 unique runs of single replicate; numbers indicate the frequency of parent), and (**b**) the frequency of T_{FH} was quantified in CD4$^+$ T-cells. By flow cytometry, the frequency of memory CD4$^+$ T-cells expressing CTLA-4 were measured longitudinally in (**c**) PBMCs and (**d**) LN. All RMs are color-coded and grouped based on ICB therapy as follows with population sizes as indicated for all phenotypic analyses: controls (n=6), black; αCTLA-4 (n=6), blue; αPD-1 (n=6), pink; combined blockade (n=7), red; and BsAb (n=8), purple. (**e**) The number of CD4$^+$ T_{REGs} per μL of PB was correlated against the contemporaneous concentration (ng/mL) of anti-CTLA-4 mAb in plasma at d28 p.t. (n=13). Averaged data are presented as the mean ± SEM, and were analyzed with a two-sided (**b**) Kruskal-Wallis test with Dunn's correction relative for multiple comparisons relative to controls, (**c,d**) a mixed-effects model with Dunnett's correction relative to controls, or (**e**) a Pearson correlation coefficient. Color-coded statistical asterisks indicate significance between control animals and the treatment group of that color. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001.

Supplementary Figure 5. Combined blockade reduces the frequency of non-dividing CD4$^+$ T-cells.



The frequency of cell divisions in (**a**) CD4$^+$ and (**b**) CD8$^+$ T-cells in PBMCs at day 21 p.t. was determined by CellTrace Violet labeling following 6 days of stimulation with IL-2, anti-CD3, anti-CD2, and anti-CD28 mAbs. All RMs are color-coded and grouped based on ICB therapy as follows with population sizes as indicated: controls (n=6), black; αCTLA-4 (n=6), blue; αPD-1 (n=5), pink; combined blockade (n=7), red; and BsAb (n=8), purple. Representative overlays are shown for CellTrace Violet labeling normalized as a percentage of maximum for (**c**) CD4$^+$ and (**d**) CD8$^+$ T-cells with the day of stimulation annotated by color (at right; 2 of 32 unique runs of single replicate). Undivided cells (0 cell divisions) are at right and are down-shifted relative to initial labeling. Each CellTrace peak extending left is quantified as a successive cell division. Averaged data are presented as the mean ± SEM, and were analyzed with a two-sided, two-way ANOVA with Dunnett's correction for multiple comparisons relative to controls. Color-coded asterisks indicate significance between controls and the ICB treatment group of that color. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.

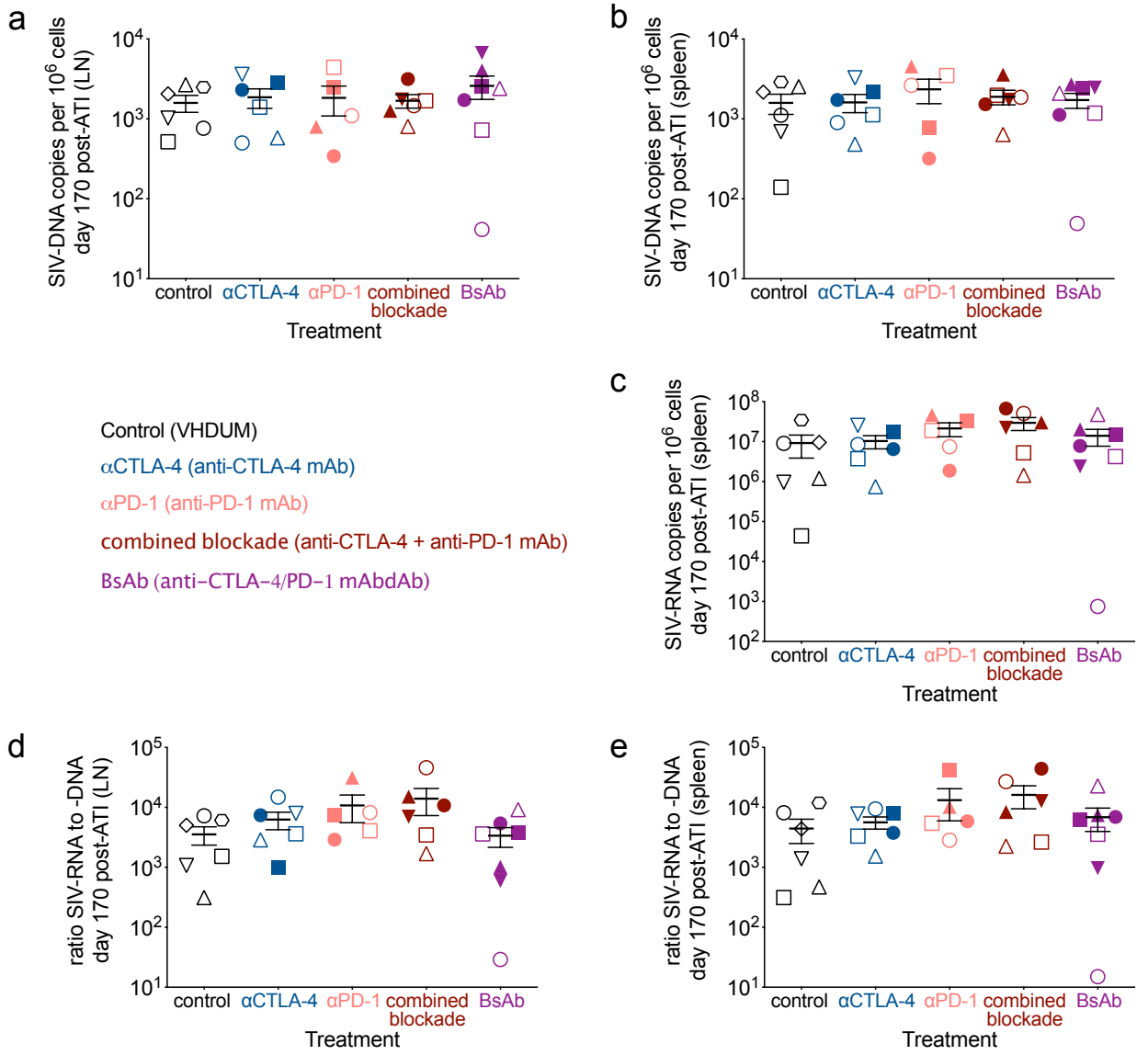
Supplementary Figure 6. SIV-specific cytokine responses in T-cells are not enhanced by ICB.



Control (VHDUM) αCTLA-4 (anti-CTLA-4 mAb) αPD-1 (anti-PD-1 mAb) combined blockade (anti-CTLA-4 + anti-PD-1 mAb) BsAb (anti-CTLA-4/PD-1 mAbdAb)

The number of interferon gamma (IFN-γ) spot forming units (SFU) per 10^6 mononuclear cells were quantified by ELISpot, performed in triplicate, upon SIV-Env stimulation in (**a**) PBMCs as a fold-change (d21 p.t. relative to d-29 p.t.) and (**b**) cross-sectionally in LN (d21 p.t.). Population sizes for all ELISpot analyses are as follows: controls (n=6), black; αCTLA-4 (n=6), blue; αPD-1 (n=6), pink; combined blockade (n=7), red; and BsAb (n=8), purple. From *ex vivo* SIV-Env-stimulated PBMCs with unstimulated background subtracted, the distribution of cytokine co-expression was determined via flow cytometry in memory (**c**) $CD4^+$ and (**d**) $CD8^+$ T-cells before (d-29 p.t.) and during (d21 p.t.; indicated at left) ICB administration (indicated above). Rainbow-colored inner pie wedges represent each Boolean combination of cytokine co-expression (annotated at far right); whereas, the stacked, outer concentric rings overlap with pie wedges that are positive for that cytokine (indicated at middle right): IFN-γ^+, red; IL-2^+, orange; TNFα^+, green; and CD107a$^+$, purple. The frequency of cycling cells (Ki-67^+) was quantified in responding (i.e. IFN-γ^+ and/or IL-2^+, and/or TNFα^+, and/or CD107a$^+$) PBMC memory (**e**) $CD4^+$ and (**f**) $CD8^+$ T-cells following SIV-Gag peptide stimulation. Population sizes for all phenotypic analyses are as follows: controls (n=6), black; αCTLA-4 (n=6), blue; αPD-1 (n=6), pink; combined blockade (n=7), red; and BsAb (n=8), purple. Each data point represents an individual animal, as indicated by shape, and those with ICB-related viral reactivation in plasma are represented as closed data points. Averaged data are presented as the mean ± SEM, and were analyzed with two-sided (**a**,**b**) a Mann-Whitney U test; (**c**,**d**) a Permutation test; or (**e**,**f**) a two-way ANOVA with Bonferroni's correction for multiple comparisons relative to baseline and controls. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001.

Supplementary Figure 7. Prior ICB treatment does not impact cell-associated SIV-DNA and SIV-RNA content in tissue following ART interruption.



By 12 replicate reaction RT-qPCR, the number of SIV-DNA copies per 10^6 cells was measured at necropsy (day 170 post-ATI) in bulk (**a**) axillary LN and (**b**) in spleen. (**c**) Likewise, the number of SIV-RNA copies per 10^6 cells was quantified in bulk spleen. From these data sets, the ratio of SIV-RNA to -DNA content was computed for both (**d**) axillary LN and (**e**) spleen. Each data point represents an individual animal, as indicated by shape, and those with prior ICB-related viral reactivation in plasma are represented as closed data points. All RMs are color-coded and grouped based on ICB therapy as follows with population sizes as indicated: controls (n=6), black; αCTLA-4 (n=6), blue; αPD-1 (n=5), pink; combined blockade (n=6), red; and BsAb (n=7), purple. Averaged data are presented as the mean ± SEM and were analyzed with a two-sided Mann-Whitney U test. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.

Supplementary Table 1. Screening of anti-drug antibodies in RMs prior to and during chronic infection for treatment stratification.

Animal ID	ICB Treatment	Annotated Symbol	ECL of anti-drug antibodies (plasma)[a] pre-infection (day -21 p.i.) αPD-1	αCTLA-4	control	chronic infection (day 52 p.i.) αPD-1	αCTLA-4	control	BsAb
RGi16	control	○	61	163	228	**176**	**253**	246	**268**
RJd16	control	□	54	103	120	71	**216**	185	192
RTw16	control	△	72	94	89	57	100	191	128
118_14	control	▽	69	94	138	74	141	172	168
RFu15	control	◇	74	67	92	**107**	67	170	92
RHj16	control	⬡	63	65	83	61	57	70	66
RHn16	αCTLA-4	○	**115**	**300**	**360**	56	**299**	**374**	**385**
RTj16	αCTLA-4	●	59	**376**	**410**	55	**363**	**327**	**379**
RJr16	αCTLA-4	□	**95**	**226**	149	53	157	179	185
RAq16	αCTLA-4	△	80	**283**	244	**112**	**208**	217	**334**
138_14	αCTLA-4	▽	67	154	160	**128**	**244**	**405**	**375**
RAi16	αCTLA-4	■	**96**	**236**	**520**	**105**	204	**340**	**227**
RWs16	αPD-1	○	61	82	132	54	100	241	187
RPo16	αPD-1	●	63	**517**	110	60	108	82	104
RPn16	αPD-1	□	68	98	118	61	**219**	177	**203**
RLt16	αPD-1	△	60	115	112	59	**248**	235	**463**
RFl16	αPD-1	■	78	**388**	**485**	58	**376**	**339**	**391**
82_13	αPD-1	▲	64	117	251	56	**953**	**980**	**950**
RVt16	combined	○	60	164	197	58	107	132	130
RUm16	combined	●	67	100	116	53	89	264	67
RYi16	combined	□	66	**247**	266	64	**257**	195	**275**
RVr16	combined	△	65	101	93	55	86	82	93
RYu16	combined	■	55	72	94	57	169	**328**	**308**
RZl16	combined	▲	74	**496**	**547**	69	**700**	**470**	**617**
RHs16	combined	▼	63	107	130	61	162	209	**268**
RKt16	BsAb	○	83	67	85	55	56	74	61
RZw15	BsAb	□	78	71	101	73	67	92	86
RZn16	BsAb	△	56	64	87	54	106	131	136
RUv15	BsAb	●	56	75	89	60	184	204	167
ROp16	BsAb	■	67	89	122	51	57	61	90
RFj16	BsAb	▲	82	146	134	61	73	110	91
RQt16	BsAb	▽	50	51	97	55	76	91	90
RKi16	BsAb	▼	62	84	189	67	84	223	138
	cutoff		**87**	**205**	**327**	**87**	**205**	**327**	**195**

[a]From plasma, the levels of cross-reactive anti-drug antibodies (ECL, electrochemiluminescence) were quantified by MSD assay against anti-PD-1 mAb, anti-CTLA-4 mAb, anti-CTLA-4/PD-1 mAbdAb, and the VHDUM (control) mAbdAb. ADAs were assayed in all RMs prior to infection (day -21 p.i.) and during chronic infection, prior to ART initiation (day 52 p.t.). Cutoff for positivity as listed at bottom of the table, and ECL values above the cutoff are indicated in bold font.

Supplementary Table 2. Clinical diagnoses and adverse events observed in ICB-treated RMs segmented by study phase.

Animal ID	Clinical observation	Study phase of observation[a]				ICB Treatment	Annotated Symbol
		pre-ART	on-ART	ICB[b]	post-ATI		
RJd16	Anemia		-57 p.t.			control	□
RTw16	Dermatitis		-76 p.t.			control	△
RTw16	Erythematous skin			0 p.t.		control	△
RTw16	Vomited during infusion			0 p.t.*		control	△
RTw16	Dermatitis			7 p.t.		control	△
RFu15	Lymphocytosis		-125 p.t.			control	◇
RHj16	Diarrhea				184 p.A.	control	⬡
RJr16	Inflammation at LN biopsy site		-32 p.t.			αCTLA-4	□
RAq16	Calcinosis cutis		-34 p.t.			αCTLA-4	△
RAq16	Debulking surgery for Calcinosis cutis		-31 p.t.			αCTLA-4	△
138_14	Hematoma at catheter site			8 p.t.*	14 p.A	αCTLA-4	▽
138_14	Anemia				14 p.A	αCTLA-4	▽
138_14	Epistaxis; *S. aureus* and *E. faecalis*				28 p.A.	αCTLA-4	▽
138_14	Enlarged heart, liver, and spleen with lung opacity[c]				44 p.A	αCTLA-4	▽
RAi16	Prolonged recovery post-infusion			14 p.t.*		αCTLA-4	■
RAi16	Idiopathic degenerative bone disease			0 p.t.		αCTLA-4	■
RWs16	Inflammation at LN biopsy site		-25 p.t.			αPD-1	○
RPo16	Inflammation at LN biopsy site		-26 p.t.			αPD-1	●
RLt16	Sneezing throughout infusion			14 p.t.*		αPD-1	△
RLt16	Thrombocytopenia				168 p.A.	αPD-1	△
82_13	Vomited prior to infusion			0 p.t.		αPD-1	▲
RUm16	Anemia			0 p.t.		combined	●
RYi16	Inflammation at LN biopsy site		-32 p.t.			combined	□
RVr16	Sterile hematoma		-110 p.t.			combined	△
RHs16	Diarrhea			22 p.t.*		combined	▼
ROp16	Inflammation at LN biopsy site		-21 p.t.			BsAb	■
ROp16	Vomited prior to infusion			8 p.t.		BsAb	■
RFj16	Anemia		-41 p.t.			BsAb	▲
RKi16	Vomited after infusion			14 p.t.*		BsAb	▼

[a]Timing of clinical observations by days post-infection (p.i.), post-treatment (p.t.), or post-ART interruption (p.A.) divided by study phase as follows: during acute/chronic infection prior to ART initiation ('pre-ART'); following ART initiation, but prior to intervention ('on-ART'); during ICB therapy while on-ART ('ICB'); and following ART interruption ('post-ATI').

[b]During the ICB phase, observations were made prior to infusion unless otherwise specified (*).

[c]Pathology reports suggest that symptoms could be related to chronic SIV infection or compromised cardiac function.

Supplementary Table 3. Summary of significant study readouts segmented by intervention.

Parameter[a]	Parental Population	Tissue	control	αCTLA-4	αPD-1	combined blockade	BsAb
Biological activity							
PD-1 occupancy	memory $CD4^+$	PBMC			↑↑↑↑	↑↑↑↑	↑↑↑↑
PD-1 occupancy	memory $CD8^+$	PBMC			↑↑↑↑	↑↑↑↑	↑↑↑↑
PD-1 occupancy	memory $CD4^+$	LN			↑↑↑↑	↑↑↑	↑↑
PD-1 occupancy	memory $CD8^+$	LN			↑↑↑↑	↑↑↑↑	↑↑↑↑
CTLA-4 expression	memory $CD4^+$	PBMC		↑↑↑↑	↑	↑↑↑↑	↑↑↑
CTLA-4 expression	memory $CD4^+$	LN		↑↑		↑↑↑↑	↑
T_{REG} expansion	memory $CD4^+$	PB		↑↑		↑↑↑↑	↑↑
Immunophenotype							
Cell cycling	memory $CD4^+$	PB		↑↑↑↑		↑↑↑↑	↑↑↑↑
Cell cycling	memory $CD8^+$	PB				↑↑↑	↑
T_{EM} expansion	$CD4^+$	PB				↑↑↑↑	↑↑↑
T_{EM} expansion	$CD8^+$	PB				↑	
Cell cycling	memory $CD4^+$	LN		↑↑↑↑		↑↑↑↑	↑
Cell cycling	memory $CD8^+$	LN		↑↑↑		↑↑↑↑	↑
Cell cycling	T_{EM} $CD4^+$	LN		↑↑↑		↑↑↑↑	↑↑
Activation	memory $CD4^+$	LN				↑↑	↑
Activation	memory $CD8^+$	LN					↑
SIV-specific responses							
IFN-γ ELISpot; SIV-Gag	bulk	PBMC		↓			
IFN-γ ELISpot; SIV-Env	bulk	PBMC					
IFN-γ ELISpot; SIV-Gag	bulk	LN					
IFN-γ ELISpot; SIV-Env	bulk	LN		↓			
Cytokine distribution; SIV-Gag	memory $CD4^+$	PBMC					
Cytokine distribution; SIV-Env	memory $CD8^+$	PBMC					
Latency reactivation							
Detectable viremia on-ART during ICB	by animal	plasma	0/6	2/6	3/6	4/7	4/8
Detectable viremia on-ART during ICB	by event	plasma	0/24	3/24	3/24	9/28	6/32
cell-associated SIV-RNA	bulk	PBMC				↑	
Viral reservoir content							
cell-associated SIV-DNA	T_{EM} $CD4^+$	LN		↓↓		↓↓	↓
IPDA[b]	$CD4^+$	LN				↓	↓
# $vRNA^+$	BCF	LN				↓	
# $vDNA^+$	BCF	LN					↓
# $vDNA^+$	TCZ	LN					↓
Virological rebound following ART interruption							
Delay in rebound of viremia		plasma			↑		
Peak viremia post-ATI							
Set point viremia post-ATI							
cell-associated SIV-RNA	bulk	LN					
cell-associated SIV-DNA	bulk	LN					

[a]A summary of significance for each tested parameter within the specified parental population of a given tissue (at left) stratified by treatment group (above). Directionality of the response is indicated as positive by the upward arrow (green) and as negative by the downward arrow (red). The strength of significance is indicated by the number of consecutive arrows, which follows the rule: ↑, $p<0.05$; ↑↑, $p<0.01$; ↑↑↑, $p<0.001$; ↑↑↑↑, $p<0.0001$. All significance is relative to controls unless otherwise noted and reflects the strongest observed significance for longitudinal measures.

[b]For IPDA in $CD4^+$ LN, the significance for combined blockade is $p=0.0860$.