Supplementary Information

Early Treatment of SIV+ macaques with an $\alpha_4\beta_7$ mAb alters virus distribution and preserves CD4$^+$ T cells in later stages of infection

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The authors declare no conflict of interest.

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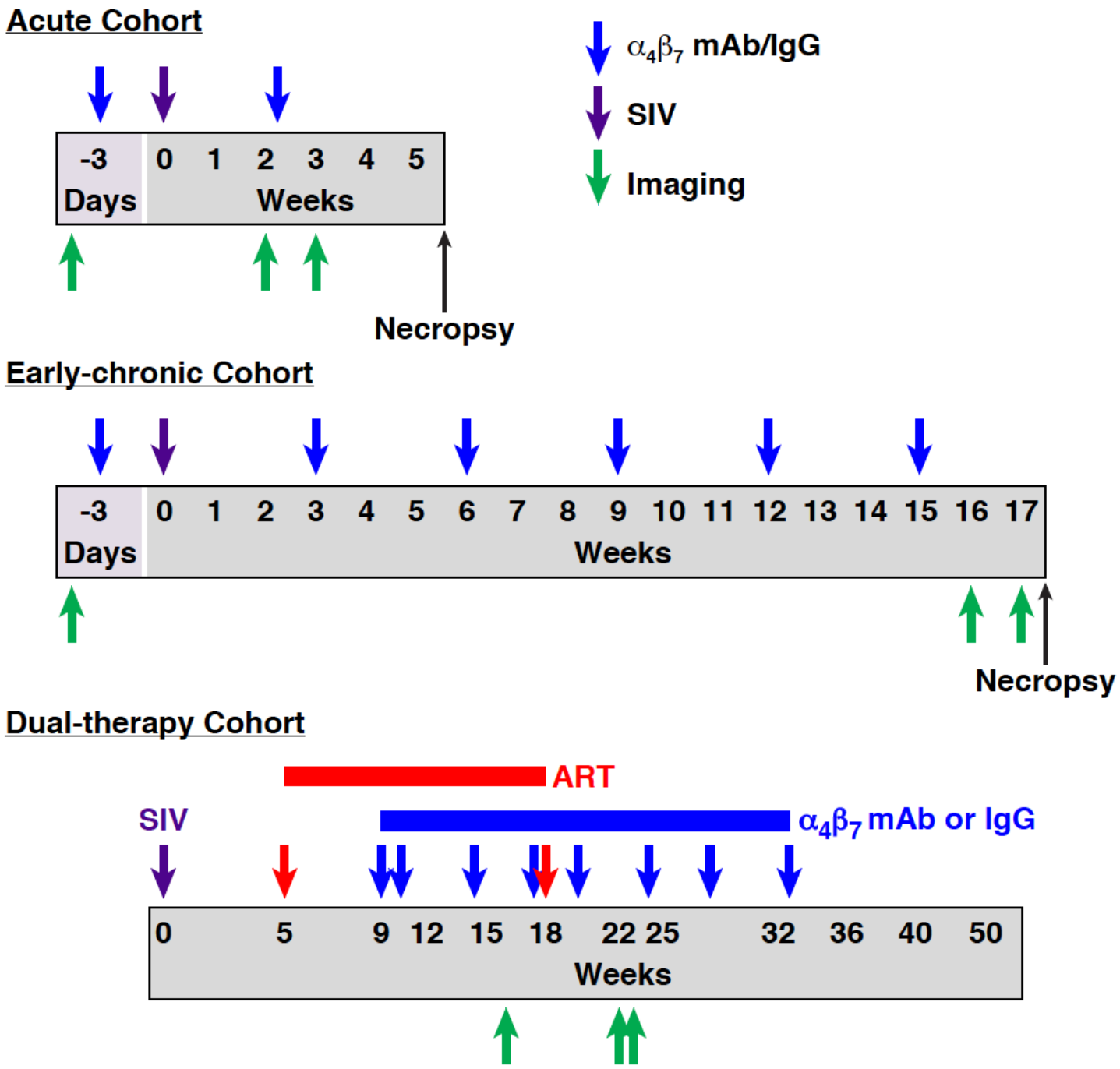
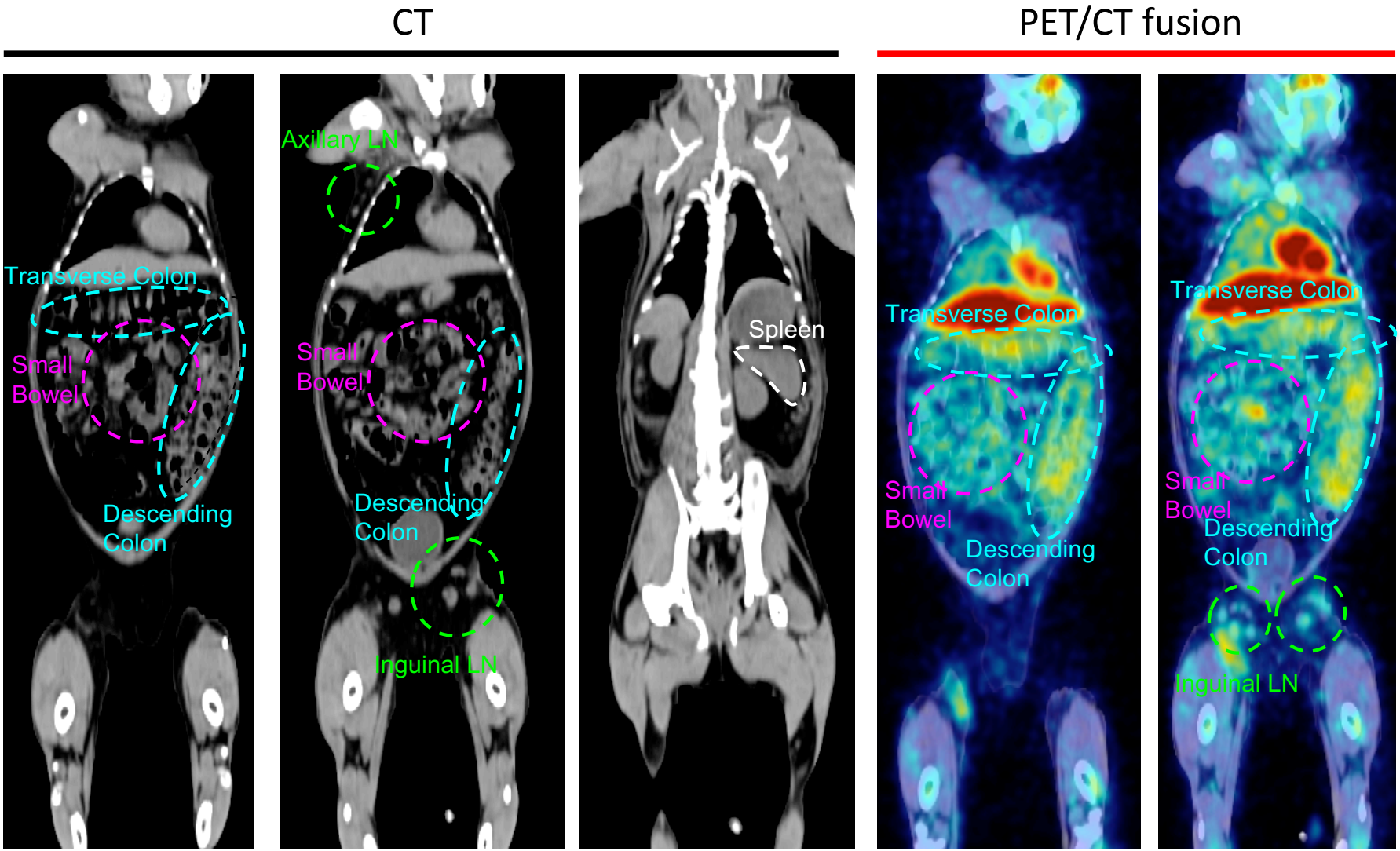
Keywords: HIV, PET/CT Imaging, Integrin $\alpha_4\beta_7$

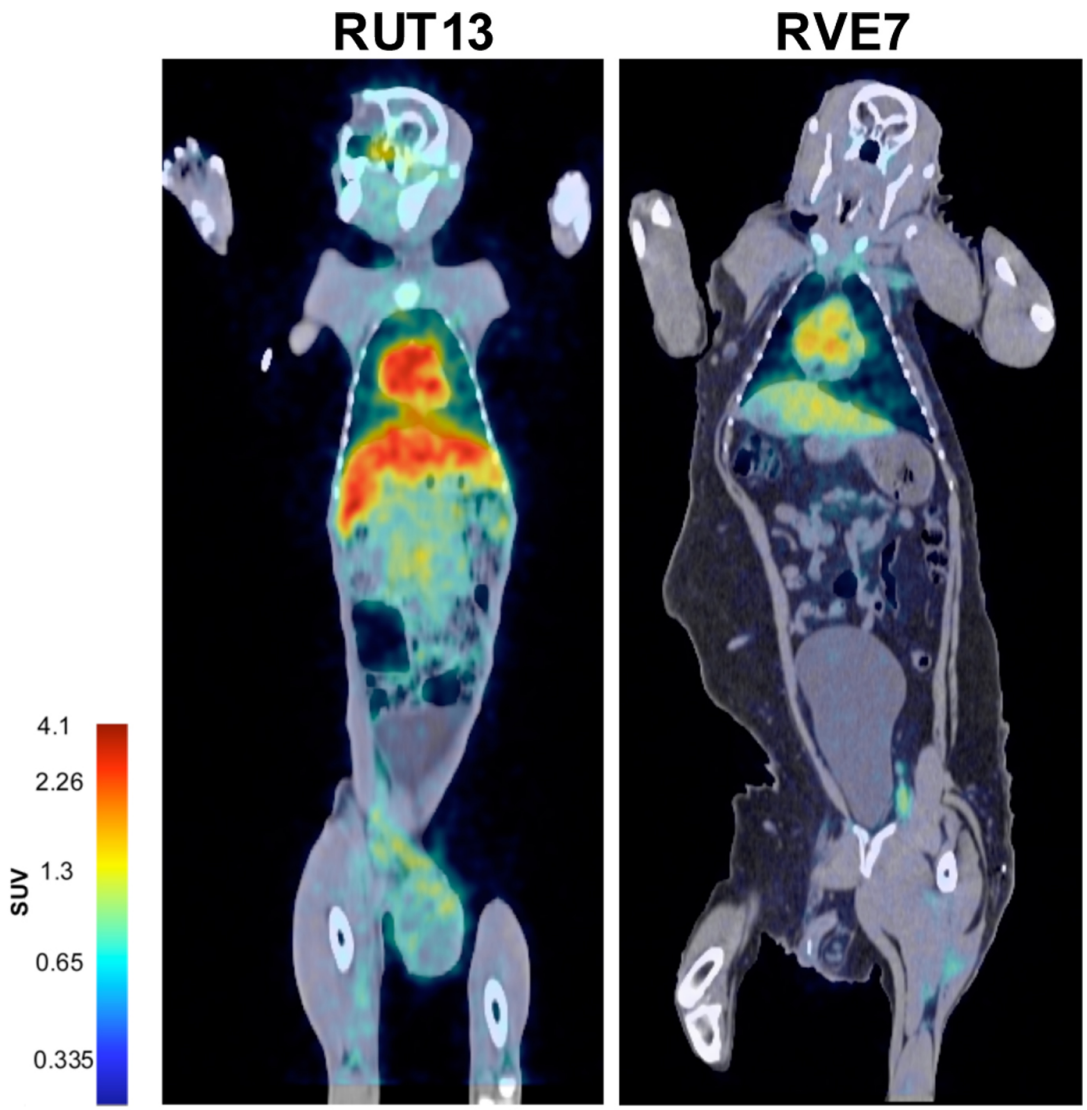
Supplementary Figure 1



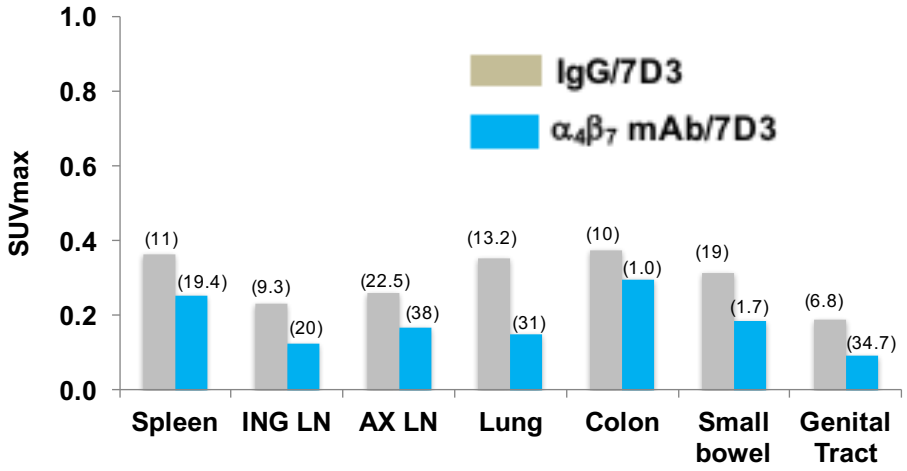
Supplementary Figure 2



Supplementary Figure 3

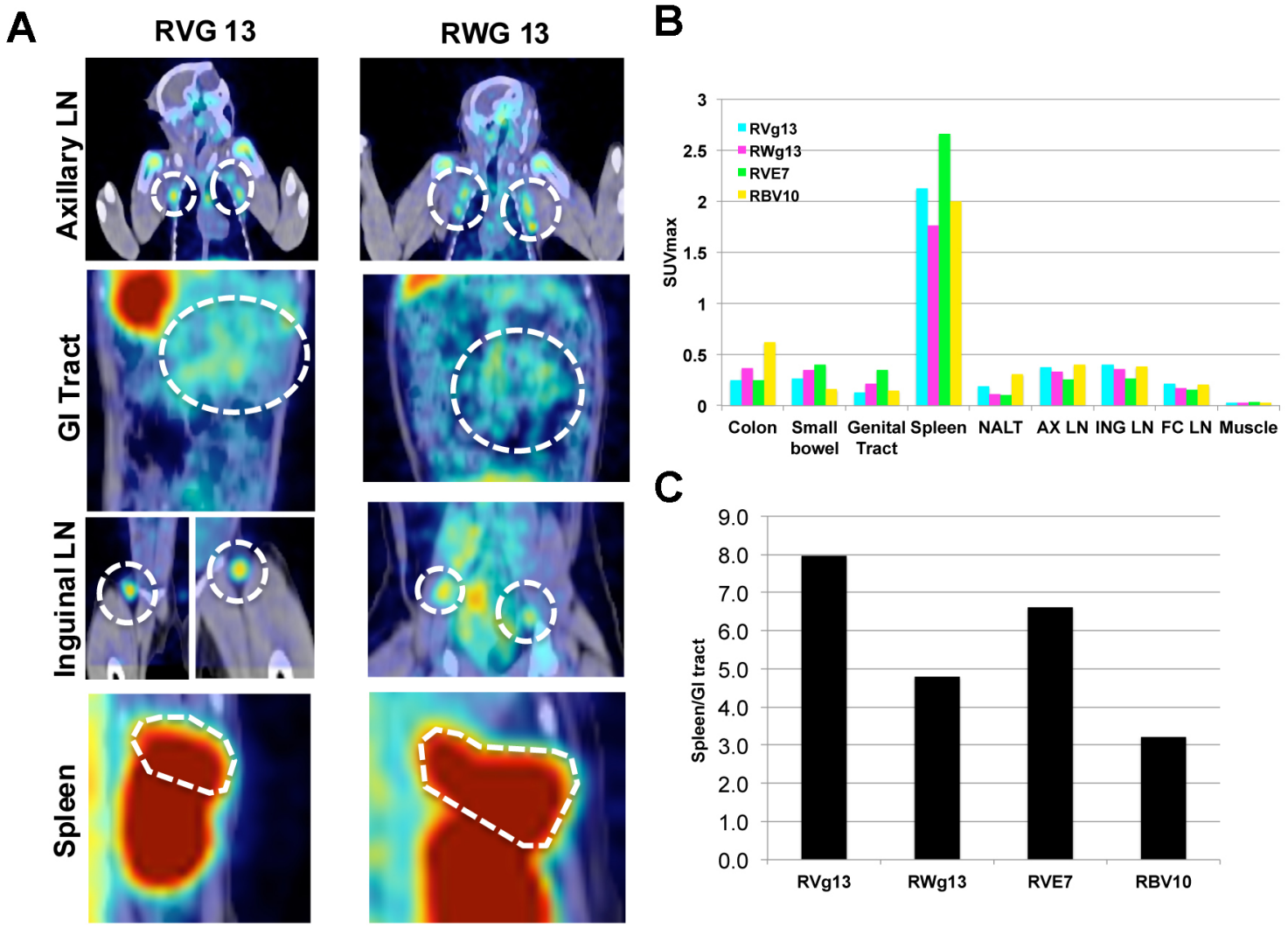


Supplementary Figure 4



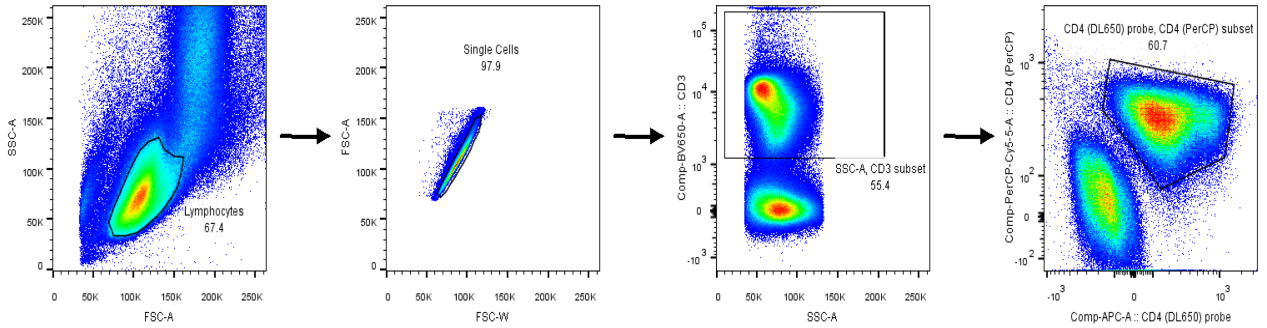
Week 2

Supplementary Figure 5



Supplementary Figure 6

a



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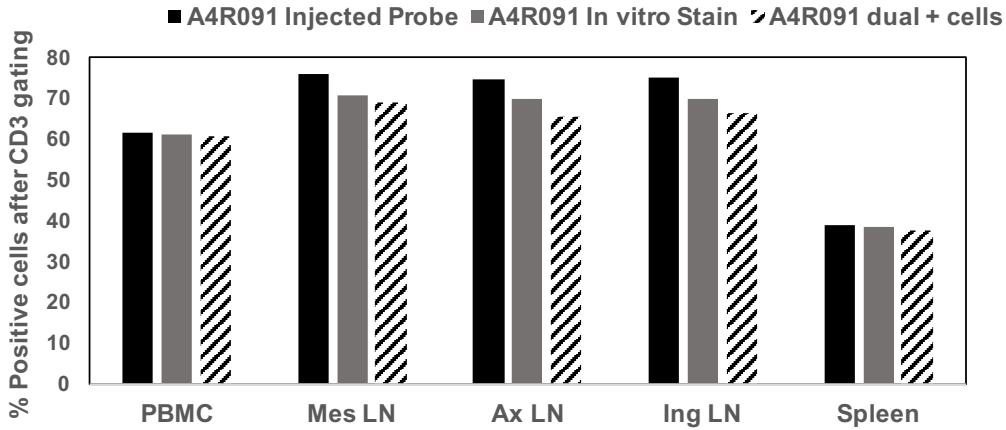
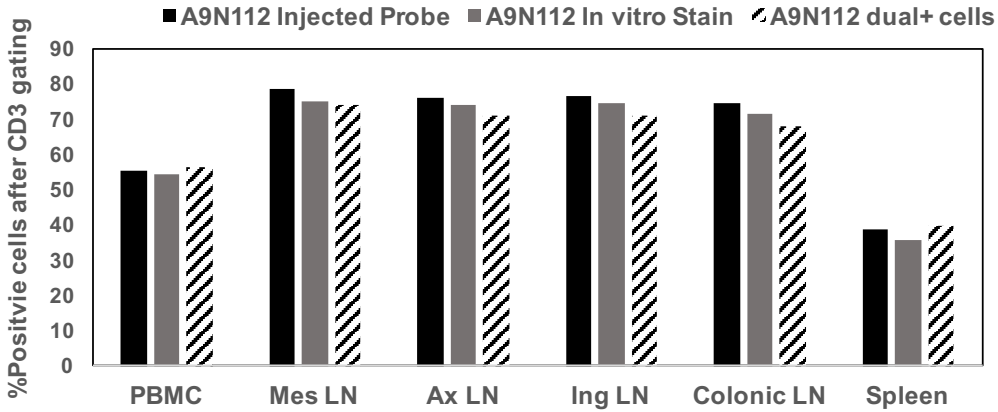
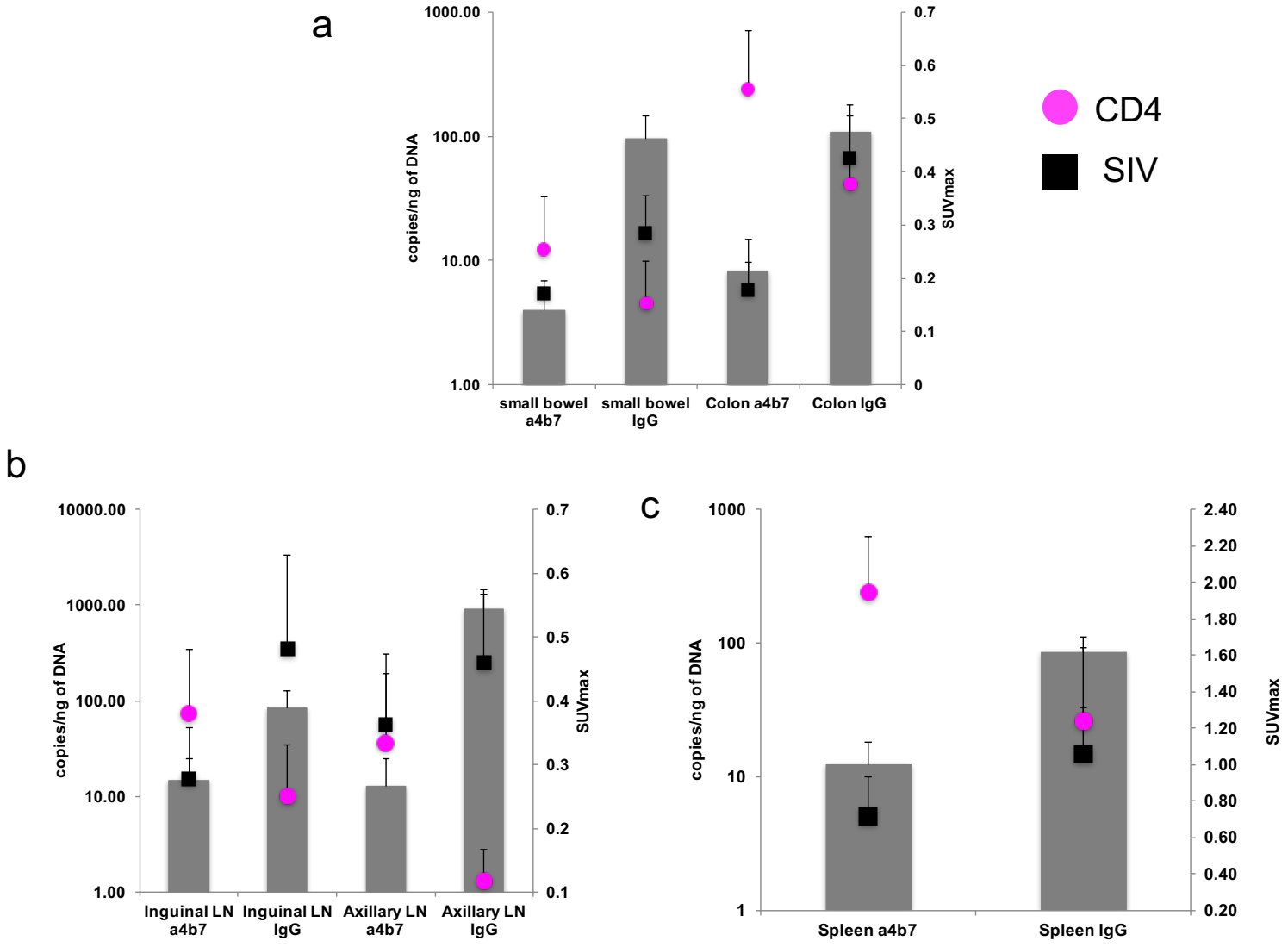




c

Animal ID	Tissue	Weight (g)	Probe+ cells/g of tissue	Probe+ cells	SUV total	cells/SUV	SNR=2
A4R091	Spleen	7	9.86E+07	6.90E+08	9857	7.00E+04	3500
A9N112	Spleen	7	8.82E+07	6.18E+08	9576	6.45E+04	3225
					Minimum number of cells detected with SNR=2		3363

Supplementary Figure 7



Supplementary Table 1. Animal, infection status and treatment descriptions at the time of imaging and blood CD4 counts

Animal name	Sex	Infection Route	Peak Viral Loads (copies/mL)	Imaging target	Probe	Imaging time after infection Weeks	IgG treated	α4β7 treated	Blood CD4 counts/μl
Acute									
RPj15	M	i.r.	8,112,781	SIV env	Modified 7D3 mAb	2&3	-	+	1386
RQl15	M	i.r.	1,817,007	SIV env	Modified 7D3 mAb	2&3	-	+	2064
RPg15	M	i.r.	9,515,745	SIV env	Modified 7D3 mAb	2&3	+	-	1659
RSq14	M	i.r	25,448,804	SIV env	Modified 7D3 mAb	2&3	+	-	2120
RUp14	F	i.r.	4,892,795	CD4	$F(ab')_2$ derived from CD4R1	2&5	+	-	845
RVo14	F	i.r.	20,930,170	CD4	$F(ab')_2$ derived from CD4R1	2&5	+	-	803
ROe15	F	i.r.	43,130,899	CD4	$F(ab')_2$ derived from CD4R1	2&5	-	+	1042
ROr14	F	i.r.	3,973,501	CD4	$F(ab')_2$ derived from CD4R1	2&5	-	+	641
Chronic									
RDg11	F	IVAG	7,926,573	SIV env	Modified 7D3 mAb	24	-	+	1224
RCw11	F	IVAG	257,966	SIV env	Modified 7D3 mAb	20	-	+	860
RLc12	F	IVAG	3,403,352	SIV env	Modified 7D3 mAb	20	+	-	480
RRn11	F	IVAG	401,692	SIV env	Modified 7D3 mAb	24	+	-	398
RUT13	M	IV	2,640,000	SIVenv	Modified 7D3 mAb	13	-	-	NA
RZB9	F	IV	579,000	CD4	$F(ab')_2$ derived from CD4R1	73	-	-	190
RTI12	F	IV	131,000	CD4	$F(ab')_2$ derived from CD4R1	73	-	-	179
ART+ α4β7									
ROq14	F	IV	936,191	SIV env/CD4	Modified 7D3 mAb/CD4R1	16/22/23	-	+	1628
RIv 14	F	IV	3,252,220	SIV env/CD4	Modified 7D3 mAb/CD4R1	16/22/23	-	+	3312
RLo14	F	IV	3,835,684	SIV env/CD4	Modified 7D3 mAb/CD4R1	16/22/23	+	-	944
RUs14	F	IV	10,950,452	SIV env/CD4	Modified 7D3 mAb/CD4R1	16/22/23	+	-	944
Uninfected									
RHg7	M	NA	NA	SIV	Modified 7D3 mAb	NA	-	-	1382
RVe7	M	NA	NA	SIV	Modified 7D3 mAb	NA	-	-	501
RVe7	M	NA	NA	CD4	$F(ab')_2$ derived from CD4R1	NA	-	-	501
RVg13	F	NA	NA	CD4	$F(ab')_2$ derived from CD4R1	NA	-	-	1702
RWg13	F	NA	NA	CD4	$F(ab')_2$ derived from CD4R1	NA	-	-	501
RBv10	M	NA	NA	CD4	$F(ab')_2$ derived from CD4R1	NA	-	-	1009
ROv15	F	NA	NA	CD4	$F(ab')_2$ derived from CD4R1	NA		+	2204
RPf16	M	NA	NA	CD4	$F(ab')_2$ derived from CD4R1	NA		+	525
ROa16	M	NA	NA	CD4	$F(ab')_2$ derived from CD4R1	NA		+	1318
RZq15	F	NA	NA	CD4	$F(ab')_2$ derived from CD4R1	NA		+	1262

Note: ROv15, RPf16, ROa16, and RZq15 were imaged for CD4 before and during α4β7 treatment.

Supplementary Table 2. Immuno-PET anti-gp120 SUVmax data from the animals imaged ($\alpha_4\beta_7$ mAb or IgG) during an acute infection.

2 weeks PI				SUV max values							
Treatment and Ligand	Animal	Colon	Small bowel	Genital Tract	Spleen	NALT	AX LN	ING LN	FC LN	Muscle	Lung
IgG anti-gp120	RPG15	0.347	0.271	0.178	0.334	0.413	0.302	0.247	0.298	0.055	0.321
IgG anti-gp120	RSQ14	0.400	0.355	0.196	0.390	0.337	0.219	0.217	0.240	0.055	0.387
α4β7mAb anti-gp120	RPJ15	0.298	0.188	0.116	0.287	0.230	0.214	0.143	0.200	0.054	0.180
α4β7mAb anti-gp121	RQL15	0.293	0.184	0.070	0.218	0.185	0.122	0.106	0.151	0.054	0.115
3 weeks PI											
Treatment and Ligand	Animal	Colon	Small bowel	Genital Tract	Spleen	NALT	AX LN	ING LN	FC LN	Muscle	Lung
IgG anti-gp120	RPG15	0.488	0.473	0.420	0.817	0.673	0.470	0.428	0.470	0.058	0.488
IgG anti-gp120	RSQ14	0.431	0.316	0.070	0.855	0.200	0.166	0.154	0.154	0.055	0.280
α4β7mAb anti-gp120	RPJ15	0.234	0.181	0.293	0.396	0.338	0.162	0.167	0.184	0.056	0.260
α4β7mAb anti-gp120	RQL15	0.248	0.266	0.187	0.441	0.247	0.188	0.136	0.159	0.055	0.250

Supplementary Table 3. Immuno-PET anti-gp120 SUVMean data at 2 weeks post-infection ($\alpha_4\beta_7$ mAb or IgG), from two different axial cross-sections within the animals imaged.

			SUVmean	
Treatment and Ligand	Animal	Upper	Lower	Average GI
IgG anti-gp120	RPG15	0.187	0.135	0.161
IgG anti-gp120	RSQ14	0.195	0.166	0.181
α4β7mAb anti-gp120	RPJ15	0.150	0.100	0.125
α4β7mAb anti-gp120	RQL15	0.120	0.060	0.090

Supplementary Table 4. Immuno-PET anti-gp120 SUVmax data from the 4 chronically infected animals, treated with $\alpha_4\beta_7$ mAb or IgG.

	SUVmax					
	NALT	Spleen	Ax LN	Ing LN	Lungs	GI
RLC12 (IgG)	1.327	1.593	0.523	0.881	0.731	1.368
RDG11 (α4β7mAb)	0.944	1.198	0.493	0.380	0.591	0.728
RRN11(IgG)	1.095	1.407	0.734	0.522	1.079	0.913
RCW11 (α4β7mAb)	0.945	0.976	0.609	0.451	0.536	0.523

Supplementary Table 5. Immuno-PET anti-gp120 SUVMean data from the GIT of 4 chronically infected animals, treated with $\alpha_4\beta_7$ mAb or IgG.

	SUVmean	
	colon	small bowel
RLC12(IgG)	0.57	0.26
RRN11(IgG)	0.35	0.20
RCW11 (α4β7mAb)	0.10	0.20
RDG11(α4β7mAb)	0.10	0.29

Supplementary Figure 1. Schematic of experimental design for PET/CT measurements during the acute, early-chronic and dual therapy cohorts.
Schematics of: $\alpha_4\beta_7$ mAb treatment, and imaging acquisition in the acute infection cohort. $\alpha_4\beta_7$ mAb treatment, and imaging acquisition in the early-chronic infection cohort. $\alpha_4\beta_7$ mAb and ART treatment, and imaging acquisition in the dual-therapy cohort. Detailed descriptions of the experimental protocols can be found in Ansari et. al. 2011, Byrareddy et.al. 2014, and Byrareddy et.al. 2016.

Supplementary Figure 2. CT and PET/CT fusion images of a chronically infected (SIV) macaque probed with an anti-gp120 mAb.
CT images (left) and PET/CT fusion images of an SIV infected macaque (RID9) probed with ^{64}Cu/DOTA labeled, PEG conjugated SIV gp120 mAb clone 7D3. Organs and tissues of interest are labelled for reference.

Supplementary Figure 3. Immuno PET image comparison of an infected vs. uninfected macaque of a chronically infected (SIV) macaque probed with an anti-gp120 mAb.
Immuno-PET assisted frontal image analysis of a chronically SIV infected animal (RUT13)(left) and an uninfected animal (RVe7)(right) 36 hrs. post administration of ^{64}Cu labeled anti-SIVgp120 monoclonal antibody (clone 7D3). Background signals in the uninfected animal appear primarily in heart and liver.

Supplementary Figure 4. Tissue specific localization of gp120 in $\alpha_4\beta_7$ mAb-treated, acutely infected macaques by immuno-PET/CT image analysis.
Anti-gp120 immuno-PET/CT images from two macaques pretreated with $\alpha_4\beta_7$ mAb (RQl15, RPj15) and two animals pretreated with control IgG (RSq14, RPg15) at week 2 The average SUVmax for the spleen, inguinal lymph-nodes (ING LN), axillary lymph-nodes (AX LN), lung, colon, small bowel, and genital tract were determined for control IgG treated (grey) and $\alpha_4\beta_7$ mAb treated macaques (blue). Median coefficient of variation (CV) indicated in parenthesis in panels.

Supplementary Figure 5. Immuno-PET/CT images of CD4$^+$ cells in uninfected macaques.
Four uninfected animals were subjected to immuno-PET/CT image analysis following administration of ^{64}Cu-labeled F(ab')$_2$ fragment from anti CD4 clone OKT-4A. **(a)** Representative images from two macaques (RVg13 and RWg13), with separate panels displaying axillary LNs, GI tract, Inguinal LNs, and spleen (each highlighted by a white broken line). **(b)** SUVmax values from RVg13, RWg13 along with 2 additional animals, RVE7 and RBv10, for the colon, small bowel, genital tract, spleen, nasal associated lymphoid tissues (NALT), axillary lymph-node (AX LN), inguinal lymph-node (AX LN) facio-cranial lymph-nodes (FC LN) and for background, muscle. **(c)** The ratio of the maximum signal in the spleen to the maximum signal in the GI tract is shown for each of the 4 animals.

Supplementary Figure 6. Specificity and sensitivity of the Immuno-PET CD4 probe.
(**a**) Gating strategy for dual labeled CD4 cells. CD4 staining in panel 4 (right), in vivo stain with 64Cu/Dylight 650 labeled anti-CD4 F(ab')$_2$ OKT4 (x-axis), in vitro stain with PerCP anti CD4 L200 (y-axis). (**b**) The specificity of the probe was determined by using a combination of 64Cu and Dylight 650 labeled anti-CD4 F(ab')$_2$ probes in two non-infected animals. Post PET/CT

imaging, the cells were isolated, and subsequently labeled with a second (different epitope) anti-CD4 antibody and a CD3 antibody. The number of CD4+ cells after CD3 gating for both the in vivo PET/fluorescent probe and the second anti-CD4 antibody were measured and plotted, as well as the cell population percentage that was double positive for both antibodies. (**c**) Sensitivity of in vivo detection for spleen was estimated. The number of probe positive cells per gram of tissue, the mass of the spleen measured and the number of probe positive cells is shown. From PET images, the total SUV within the spleen was measured and the number of cells per SUV is provided. Using a signal to noise ratio (SNR) of 0.05 (2X the background) the minimum number of cell detected is shown.

Supplementary Figure 7. Comparison of Immuno-PET derived SIV and CD4$^+$ cell imaging data with levels of pro-viral DNA. PCR measurements of pro-viral DNA levels (included in Fig. 1) compared with the SUV values from PET derived measurements of SIV (n=4) and CD4 cells (n=6). DNA measurements were pooled from both acute and early-chronic $\alpha_4\beta_7$ cohorts. SUV measurements were pooled for both SIV gp120 and CD4. Bar graphs represent pro-viral DNA levels (# copies/ng DNA), the black squares represent the mean +/- SD of the SUVmax data for SIV and the magenta circles represent the mean +/- SD of the SUVmax data for SIV levels. (**a**) small bowel and colon tissues (**b**) inguinal and axillary lymph-nodes and (**c**) spleen. Mean +/- SD are indicated.