Supplementary Information for:

Targeting α4β7 integrin reduces mucosal transmission of SIV and protects GALT from infection

Siddappa N. Byrareddy[1,8], Brianne Kallam[1,8], James Arthos[2], Claudia Cicala[2], Fatima Nawaz[2], Joseph Hiatt[2], Ellen N. Kersh[3], Janet M. McNicholl[3], Debra Hanson[3], Keith A. Reimann[4], Markus Brameier[5], Lutz Walter[5], Kenneth Rogers[6], Ann E. Mayne[1], Paul Dunbar[1], Tara Villinger[1], Dawn Little[1], Tristram G. Parslow[1], Philip J. Santangelo[7], Francois Villinger[1,6], Anthony S. Fauci[2], and Aftab A. Ansari[1]

[1]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA 30322, USA

[2]Laboratory of Immunoregulation, National Institute of Allergy & Infectious Diseases, NIH, Bethesda, Maryland 20892

[3]Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia, 30333

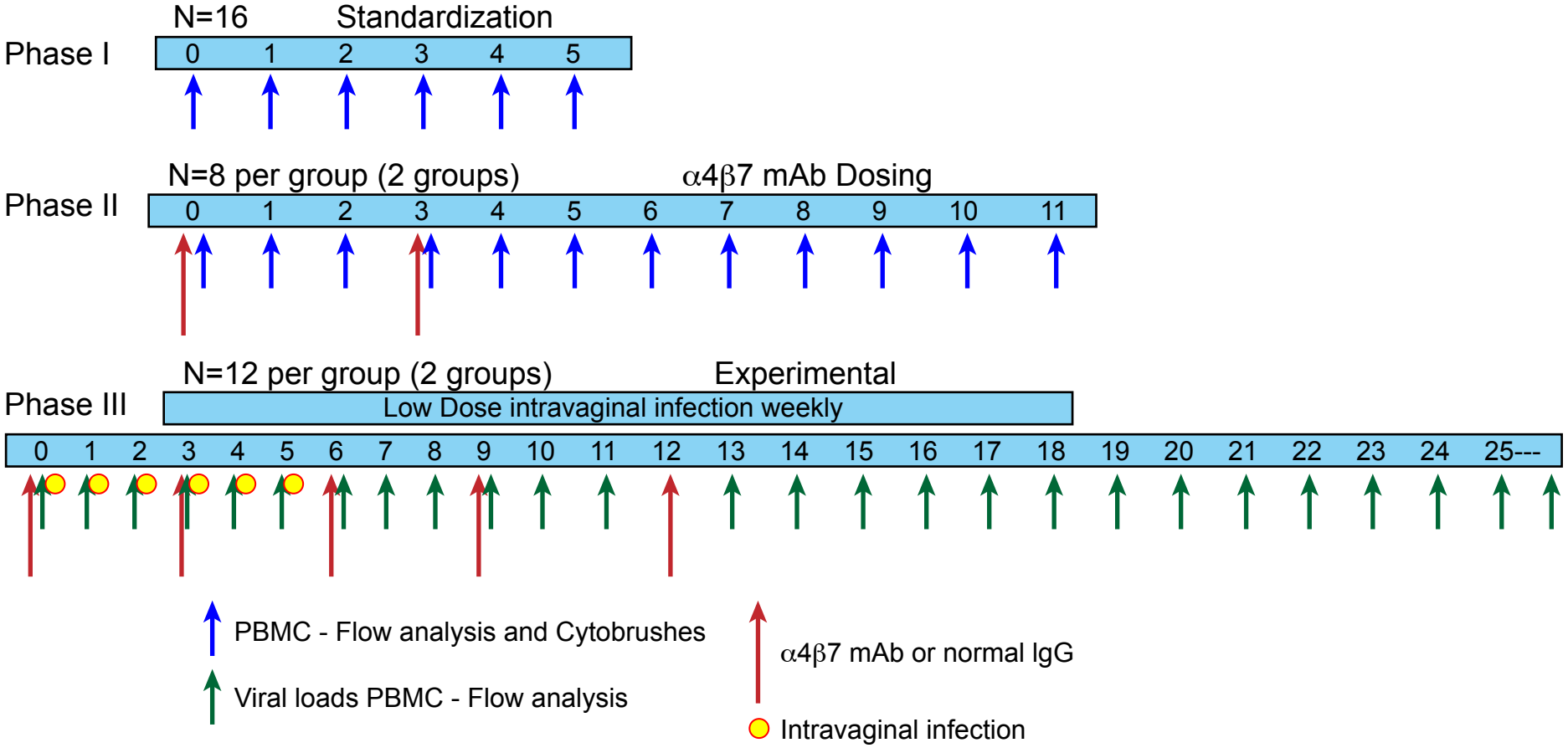
[4]Mass Biologics, University of Massachusetts Medical School, 460 Walk Hill St. Boston, MA 02126

[5]Primate Genetics Laboratory, German Primate Center, Leibniz-Institute for Primate Research, Göttingen, Germany

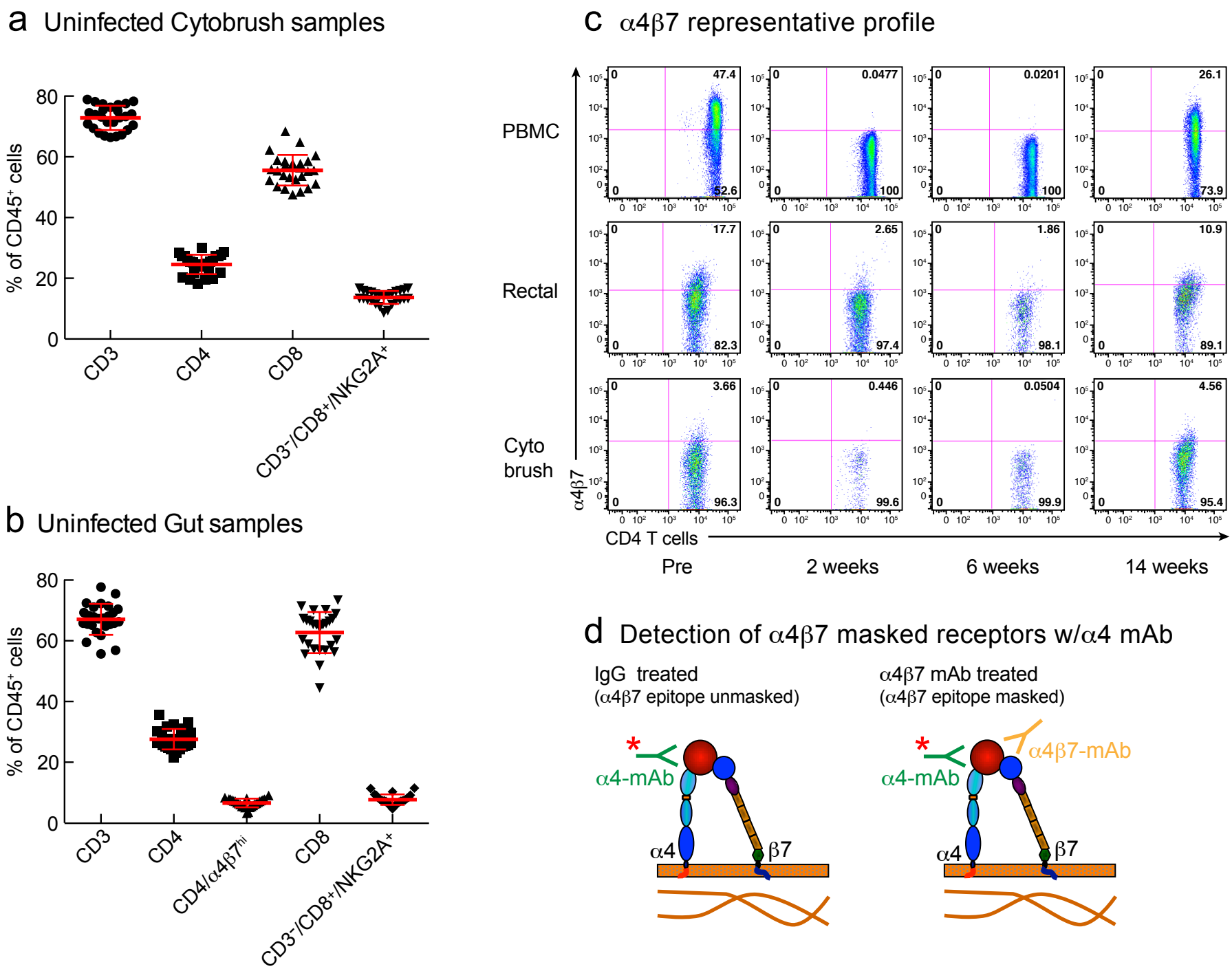
[6]Division of Pathology, Yerkes National Primate Research Center, Emory University, Atlanta, GA 30329, USA

[7]Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, 313 Ferst Drive, Atlanta, GA, 30680

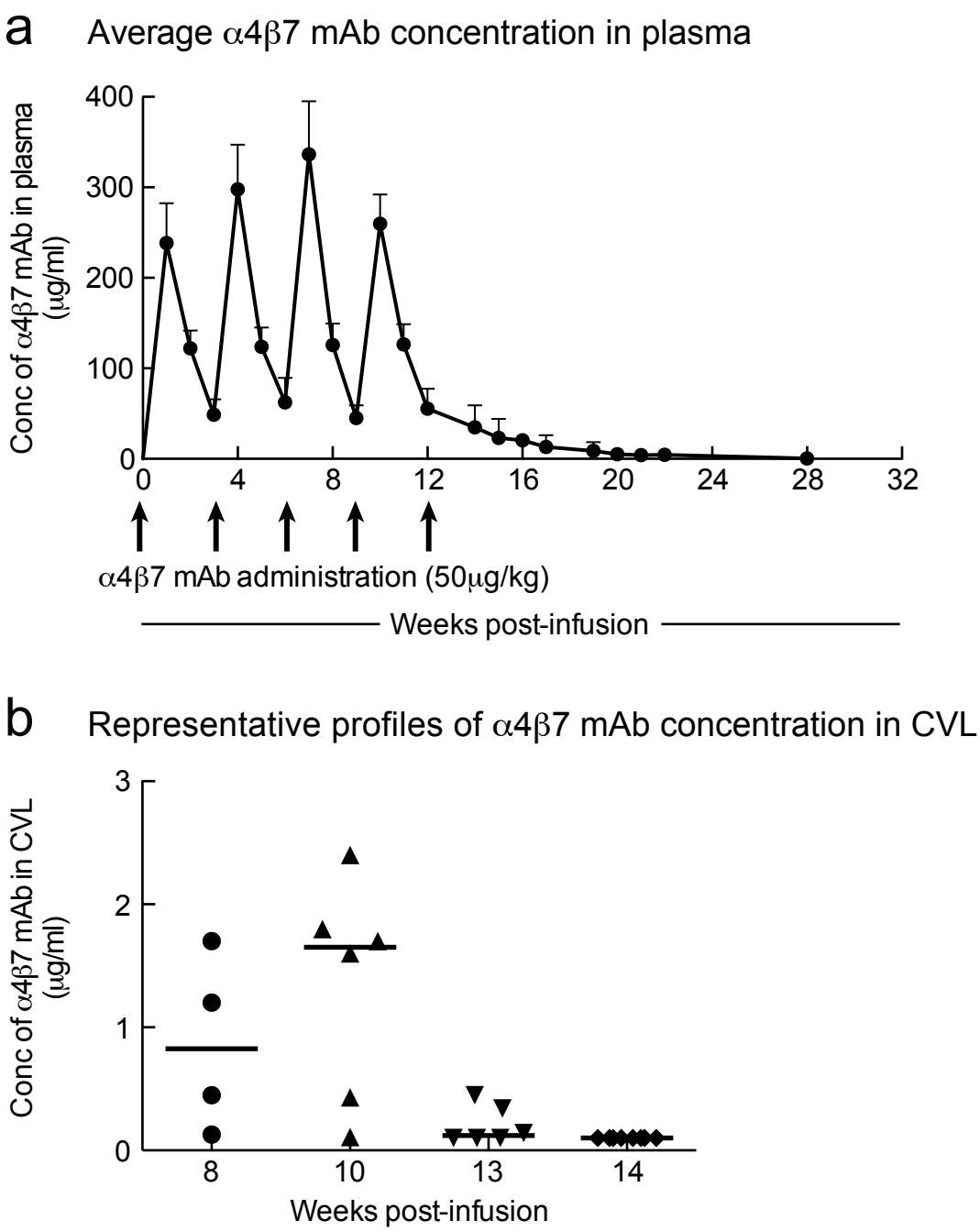
[8]These authors contributed equally.



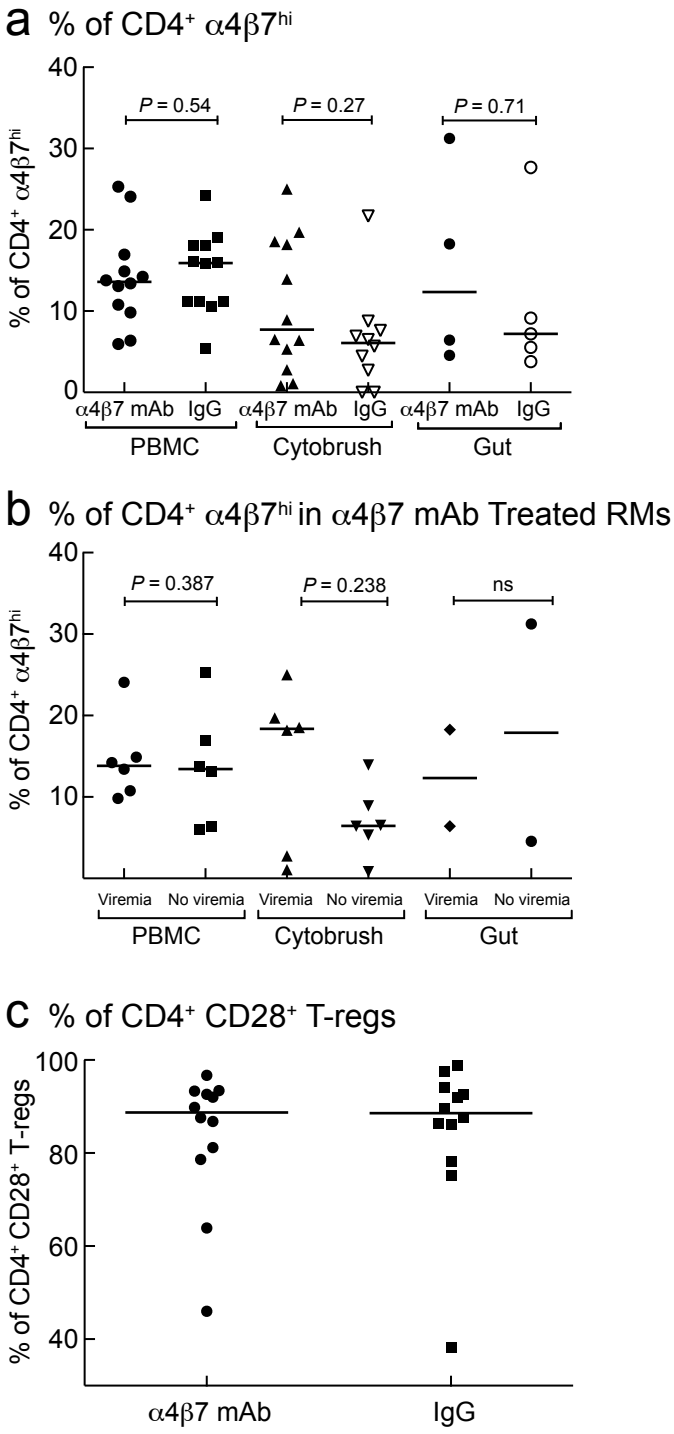
Supplementary Figure 1. Study design: The study protocol was performed in 3 phases. Phase I was performed to optimize the collection and analysis of cytobrush specimens (n=16 macaques). Data from Phase II was used to determine whether the α4β7-mAb administered intravenously at 50 mg/kg reached the cells within the female reproductive tract as defined by analysis of cytobrush specimens (n=8). Phase III (n=12 macaques per study group) involved the administration of either the α4β7-mAb or the rhesus IgG at 50 mg/kg every 3 weeks intravenously starting at day -3. Macaques were challenged IVAG with 1 ml of the stock virus (1:20 dilution, 36 ng p27) IVAG once a week until the macaques were confirmed to be infected. The challenges were terminated after 10/12 rhesus IgG treated macaques became infected.



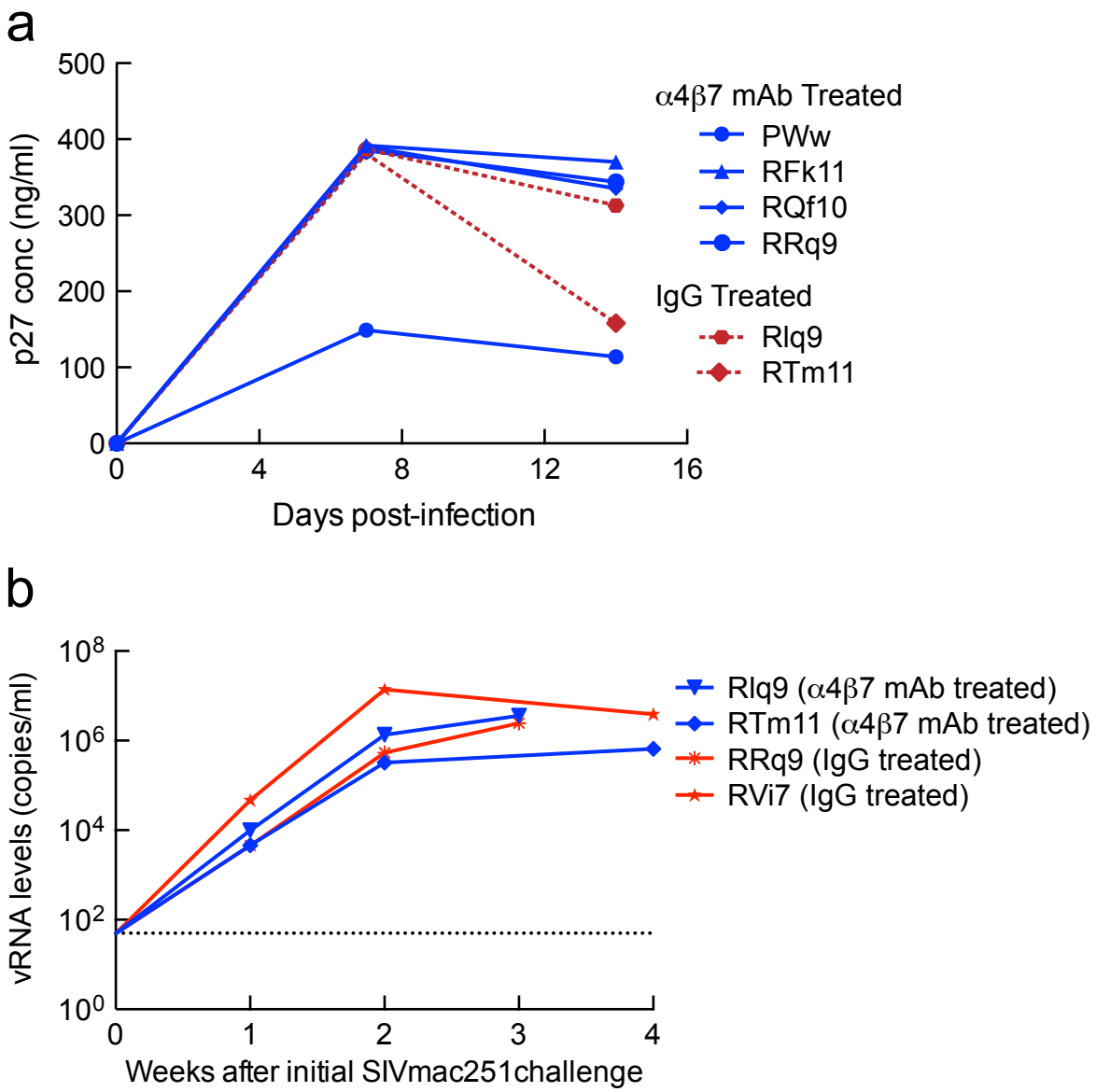
Supplementary Figure 2. Flow-cytometric analysis of PBMC, GIT biopsies and cytobrush specimens prior to and post treatment with the α4β7-mAb: Baseline studies comparing the frequencies of CD3+, CD4+, CD8+ and CD3-/CD8+/NKG2a+ subsets in the CD45+ gated population of cells obtained from cytobrush samples **(a)** and the corresponding gastro-intestinal biopsy tissues **(b)** from uninfected macaques prior to Phase I. Representative flow-cytometric profiles of α4β7 expression of CD4+-T cells from the PBMC, rectal biopsy and cytobrush cells obtained pre-infusion, and weeks 2, 6, and 14 post-infusion **(c)**. Such lack of detection of the α4β7-mAb staining at weeks 2 and 6 was not observed post-infusion of rhesus IgG (data not shown). A schematic that graphically illustrates the strategy used to detect masking of α4β7 expression using a labeled mAb against the α4 integrin, that targets an epitope distinct from the epitope targeted by the infused/unlabeled α4β7-mAb (d). Reactivity of the anti-α4 integrin mAb remained unchanged pre- and post- α4β7-mAb infusion (data not shown), and as previously described[10].



Supplementary Figure 3. Levels of the α4β7-mAb in plasma and CVL fluids: Levels of the α4β 7-mAb (μg/ml) in plasma samples from the 12 macaques that received the α4β7-mAb (50 mg/kg) intravenously starting day -3 and every 3 weeks (arrows). The data are mean +/- S. D. **(a)**. Levels of α4β7-mAb (ug/ml) in CVL fluids that were collected from animals RDg11 (+), RIz12 (+), RQm11 (+) and RRc9 (+/-) on week 8, animals ROc11 (+), RCw11 (+), RDg11 (+), RIz12 (+), RQm11* (+) and RRc9 (+/-) on week 10, animals RCw11 (+/-), RDg11 (+), RIz12 (+), ROc11 (+), RQm11 (+/-) and RRC9 (+/-) on week 13 and animals PWW (+/-), RFk11 (+/-), RLn12 (+/-), RQf10* (+/-), RRq9 (+/-) and RVi7(+/-) on week 14 (asterisk indicates samples collected during menses). The (+) indicated values > 0.1 ug/ml (significant) and the (+/-) indicated values just above the level of detection (non-significant) **(b)**.



Supplementary Figure 4. Baseline frequencies of CD4+α4β7^{hi} and Tregs: Baseline samples taken prior to treatment of PBMCs, cytobrush obtained mononuclear cells, and cells isolated from the GIT biopsy specimens, from the macaques that received α4β7-mAb (n=12) or rhesus IgG (n=12) were assayed for frequencies of CD4+ α4β7^{hi} cells (Mann-Whitney *U*-test; *P>0.05*) **(a)**. Comparison of the frequencies of CD4+ α4β7^{hi} cells in the macaques that received α4β7-mAb and became viremic vs. those that remained aviremic (Mann-Whitney *U*-test; *P>0.05*) **(b)**. Frequencies of Tregs prior to initiation of study **(c)**.



Supplementary Figure 5. *In vitro* and *in vivo* testing of susceptibility to infection: Anti-CD3/CD28 immunobead activated PBMCs from 2/12 macaques that received rhesus IgG (RIq9 and RTM11) and 4/6 that received α4β7-mAb (PWW, RFk11, RQf10 and RRq9) that were resistant to IVAG infection, were tested for their in vitro susceptibility to infection using an aliquot of the same SIVmac251 as described in the methods section. Levels of p27 (ng/ml) in the culture supernatant fluids **(a)**. Two animals from the control IgG group (RIq9 and RTm11) and 2 from the α 4β7-mAb group (RRq9 and RVi7) that were resistant to IVAG infection were challenged intra-rectally with 1 ml of the undiluted SIVmac251 stock virus and administered the same doses and schedule of α4β7-mAb or rhesus IgG, respectively as described in the methods section. Plasma viral loads (vRNA copies/ml) on these 4 macaques are illustrated as a function of time **(b)**.

Table 1a. MHC /TRIM5α Genotyping of Rhesus Macaques

RM name	MHC class I					TRIM5α			
	A1*	A2*	A3*	A4*	B*	Cyp A	Q	TFP	Restriction
IgG treated									
RBs9	003, 007				007, 022, 030, 031	x	x		Moderate
REo8	004, 008		13	14	012, 022, 030, 031, 038, 046, 047, 082				
RHy12	002, 004			14	021, 045, 060, 069	x	x		Moderate
RIq9	001, 023			14	001, 007, 019, 024, 030, 057, 082		x	x	Moderate
RKs11	004			14	012, 021, 022, 028, 030, 031, 045, 057, 060			xx	High
RLc12	004, 007			14	012, 021, 022, 028, 030, 031, 045, 057, 060, 068			xx	High
RRn11	006, 019			14	001, 047, 069		x	x	Moderate
RTm11	001, 004			14	012, 021, 028, 030, 038, 045, 046, 060, 082			xx	High
RVw10	001, 003				047, 082, 093	x		x	High
RWt9	001, 026			14	012, 022, 030, 031, 038, 047, 057, 082			xx	High
RZz10	002, 004			14	012, 022, 030, 031, 035, 049, 098		x	x	Moderate
RCd12	004,007			14	012, 022, 023, 030, 031, 057, 068		x	x	Moderate
α4β7 treated									
PWw	002, 008		13	02	012, 021, 022, 028, 030, 031, 045		x	x	Moderate
RCw11	004, 025			14	005, 012, 015, 022, 030, 031, 178			xx	High
RDg11	004			14	005, 012, 015, 022, 030, 049, 178			xx	High
RFk11	004, 025			14	012, 015, 022, 027, 030, 031, 044	x	x		Moderate
RIz12	006, 011			14	001, 007, 030, 046, 050, 065, 069, 078		xx		Susceptible
RLn12	001, 004			14	012, 022, 030, 031, 082	xx			High
ROc11	001, 004			14	012, 021, 028, 030, 045, 082			xx	High
RQf10	001, 041				004, 007, 030				
RQm11	004, 019			14	012, 022, 030, 031, 069		xx		Susceptible
RRc9	004			14	012, 022, 030, 031, 048	x	x		Moderate
RRq9	004, 026				006, 047, 071				
RVi7	004, 008		13	14	015, 030, 031, 043, 178			xx	High

Table 1b: FcRγ typing

RM name	CD16 (FcγRIII)			CD64 (FcγRI)							CD32b (FcγRIIb)	
	3A-1	3A-2	3A-3	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 1	Allele 2
				QVVRL	QVARL	EVVSL	QVVSF	QVVRF	QAVRL	QAVSL	(I59, L133)	(T59, P133)
IgG treated												
RBs9	x	x		x					x			
REo8	xx			xx								
RHy12	x	x		x					x			
RIq9		xx		x						x		
RKs11	x	x		xx								
RLc12	x	x		xx								
RRn11	x	x		xx								
RTm11		xx		xx								
RVw10	x	x										
RWt9		xx		xx								
RZz10	x	x		xx								
RCd12	xx			xx								
α4β7 treated												
PWw		x	x	xx							xx	
RCw11	x	x		xx							xx	
RDg11	xx			xx							xx	
RFk11	x	x		xx							xx	
RIz12	x	x		xx							xx	
RLn12		xx		xx							xx	
ROc11	x	x		x					x		xx	
RQf10	x	x		xx							xx	
RQm11		xx		x					x		xx	
RRc9	x	x		xx							xx	
RRq9	x	x					x		x		xx	
RVi7	x	x		xx							xx	

x, could also be allele combination 5 and 7 Q(V/A)V(R/S)(L/F)

Table 1c: KIR Genotypes of rhesus macaques

Animal Name	3D L01	3D L02	3D L04	3D L05	3D L06	3D L07	3D L08	3D L10	3D L11	3D S01	3D S02	3D S03	3D S04	3D S05	3D S06	3D S07	3DL W03	3DS W08	3DS W09
IgG treated RMs																			
RBs9	X						X				X		X						
REo8	X	X		X		X					X		X						
RHy12	X	X				X		X			X		X	X					
RIq9	X			X				X	X										
RKs11		ND				ND	X	X			X			X				X	
RLc12	X	X				X		X			X					X		X	
RRn11	X			X		X	X				X				X		X		
RTm11	X	X						X		X	X								
RVw10	X	X		X		X	X				X		X		X			X	
RWt9	X			X				X			X		X						
RZz10						X	X				X				X			X	
RCd12	X			X		X	X	X			X			X	X				
anti-α4β7 treated RMs																			
PWw		X		X			X		X		X			X					X
RCw11	X					X	X		X		X				X				
RDg11	X	X		X													X	X	
RFk11	X						X				X								
RIz12		X		X		X	X		X		X			X					X
RLn12	X						X							X				X	X
ROc11	X	X				X				X								X	
RQf10	X					X	X				X		X		X				X
RQm11	X	ND							ND	X									
RRc9	X			X			X				X				X				X
RRq9	X	X				X			X						X				
RVi7	X	ND				ND	X												

X, present, ND, not distinguishable

Supplementary Table 1. The results of MHC and TRIM5α genotyping (a) FcRγ genotyping (b) and KIR genotyping (c) of the macaques that received control rhesus IgG (n = 12) or the α4β7 mAb (n=12).

Table 2. Reproductive history of monkeys

Animal Name	Date of Birth	Apx. Age in yrs at time of challenge	# of Pregnancies (babies)	# mensing during viral challenge/ total challenges
IgG treated RMs				
RBs9	4/25/03	10	3(3)	0/3
REo8	5/17/01	12	0	1/2
RHy12	7/11/08	5	0	1/1
RIq9	4/16/03	10	5(5)	1/6
RKs11	6/11/06	7	1(1)	0/2
RLc12	5/19/07	6	0	0/2
RRn11	4/24/06	7	0	0/1
RTm11	4/20/06	7	0	1/6
RVw10	8/28/04	9	3(3)	1/4
RWt9	5/02/03	10	2(2)	0/4
RZz10	4/03/05	8	1(1)	0/3
RCd12	5/28/07	6	0	0/2
anti-α4β7 treated RMs				
PWw	5/15/96	17	3(3)	0/6
RCw11	4/04/07	6	0	0/6
RDg11	5/25/05	8	2(1)	1/5
RFk11	3/25/06	7	0	0/6
RIz12	8/15/08	5	0	1/5
RLn12	4/10/08	5	0	0/6
ROc11	4/23/05	8	1(1)	0/5
RQf10	8/07/03	10	1(1)	1/6
RQm11	4/19/06	7	1(1)	0/4
RRc9	4/23/02	11	2(2)	0/5
RRq9	4/18/03	10	4(4)	1/6
RVi7	2/13/00	13	3(3)	0/6

Supplementary Table 2. The date of birth, age at the time of initiation of study, the number of pregnancies (reproductive history) and the number of times during viral challenges/total viral challenges each monkey was mensing based on plasma levels of progesterone and estradiol for each of the 24 monkeys studied.