

Supplementary Information for

Fc-mediated effector function contributes to the *in vivo* antiviral effect of an HIV neutralizing antibody

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FcR (M. mulatta)	mAb	KD (nM) KD Error k		kon(1/Ms)	kon Error	kdis(1/s)	kdis Error	Full X ²	Full R ²		
	VRC07-523	195.5	7.86E-09	3.99E+05	1.52E+04	7.79E-02	9.89E-04	0.0159	0.9969		
FcvRIII	VRC07-523LS	166.2	4.75E-09	4.01E+05	1.07E+04	6.67E-02	6.75E-04	0.0152	0.9979		
	VRC07-523LS/LALA	Poor binding; fits not obtained									
	VRC07-523LS/DEL	21.54	7.84E-10	2.50E+05	2.75E+03	5.37E-03	1.87E-04	0.0552	0.9993		
	VRC07-523	4200	1.47E-06	1.17E+05	4.06E+04	4.90E-01	2.34E-02	0.0023	0.9941		
FcvRIIA	VRC07-523LS	4190	1.70E-06	1.32E+05	5.32E+04	5.54E-01	2.90E-02	0.0026	0.9935		
	VRC07-523LS/LALA	Poor binding; fits not obtained									
	VRC07-523LS/DEL	1460	3.02E-07	2.24E+05	4.51E+04	3.26E-01	1.56E-02	0.0163	0.9888		
	VRC07-523	8270	6.67E-06	6.49E+04	5.22E+04	5.36E-01	3.47E-02	0.0025	0.9906		
FcvRIIB	VRC07-523LS	11700	1.32E-05	5.20E+04	5.85E+04	6.10E-01	4.12E-02	0.0016	0.9915		
i oyitaib	VRC07-523LS/LALA	Poor binding; fits not obtained									
	VRC07-523LS/DEL	1510	2.96E-07	2.09E+05	4.00E+04	3.16E-01	1.40E-02	0.0167	0.9896		
	VRC07-523	3.640	2.66E-10	3.64E+05	2.65E+04	1.33E-03	1.09E-05	0.0426	0.9956		
FcyRl	VRC07-523LS	3.305	1.90E-10	3.72E+05	2.12E+04	1.23E-03	8.47E-06	0.0316	0.9975		
,	VRC07-523LS/LALA	234.2	8.74E-09	1.73E+05	6.42E+03	4.06E-02	1.73E-04	0.0086	0.9984		
	VRC07-523LS/DEL	1.679	1.82E-10	2.38E+05	2.52E+04	4.00E-04	9.14E-06	0.1088	0.9968		
	VRC07-523	8.266	1.28E-10	5.70E+05	8.58E+03	4.71E-03	1.76E-05	0.1371	0.9944		
FcRn at pH6.0	VRC07-523LS	0.123	1.39E-11	3.65E+05	3.94E+03	4.50E-05	5.04E-06	0.1445	0.9991		
	VRC07-523LS/LALA	0.401	1.82E-11	3.71E+05	4.89E+03	1.49E-04	6.44E-06	0.205	0.9986		
	VRC07-523LS/DEL	0.337	1.88E-11	2.82E+05	3.70E+03	9.52E-05	5.15E-06	0.1168	0.9992		
	VRC07-523	760.1	3.24E-08	3.49E+05	1.41E+04	2.66E-01	3.70E-03	0.0707	0.9974		
FcRn at pH7.4	VRC07-523LS	55.05	2.49E-09	3.13E+05	5.97E+03	1.72E-02	7.06E-04	0.2386	0.9956		
	VRC07-523LS/LALA	59.49	2.66E-09	3.33E+05	6.86E+03	1.98E-02	7.87E-04	0.24	0.9948		
	VRC07-523LS/DEL	61.60	2.11E-09	3.34E+05	5.43E+03	2.06E-02	6.20E-04	0.1449	0.9967		



Figure S1: A) Binding kinetics of the antibodies to rhesus FcRn at pH6.0 or 7.4. Curves were fitted to a 1:1 binding model. Black lines show measured data and red lines are the fits. K_D values are shown within each graph. B) Summary of K_D, association and dissociation rates along with errors, and the fit parameters for all Fc receptors. C) Area under the curve for target-cell killing mediated by NK cells isolated from 3 rhesus macaques. D) AUC for phagocytosis performed on THP-1 cells. E, F) AUC for binding to C1q or C3c for serum isolated from three rhesus macaques. P-values were calculated by one-way ANOVA.



Figure S2: Comparison of pre-treatment viral loads across treatment groups. Baseline was calculated as mean of plasma viral load at 48h, 24h and 30 min before NAb infusion. P-values were calculated using one-way ANOVA.





Figure S3: Representative flow cytometry gating strategy of whole blood sample stained with lineage markers, Fcγ receptors and anti-Id against VRC07-523LS (5C9).



Figure S4: A) Levels of cell-surface bound NAb and B) Changes in cell subset frequency on B cells, CD4 T cells and CD8 T cells



Figure S5: a) Plasma viral loads pre-infusion and until day 1 post infusion b) Fold change in plasma viral load normalized to baseline shown for individual animals as wells as for group means





Figure S6: Cell-associated viral DNA. A) Sorted CD4+ T cells from frozen PBMC were assayed for levels of viral DNA at 30 mins before NAb infusion and 6h, days 1, 3 and 5 post NAb infusion. B) Slopes of change in cell-associated viral DNA load were calculated by linear regression analysis and p-values were calculated by one-way ANOVA.



Figure S7: Heatmap showing the SLEA z-score of genes in the NK cell- mediated ADCC pathway

NK cells		
NAME	FDR q-val	Genes
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY	1.14E-04	NFKB1,NFKBIA,BIRC2,NFKBIB,RELA,TAB2,MAPK12,TNFAIP3
	0.00142	NFKB2,FAS,NFKB1,RELB,GADD45B,TP53,MAP2K3,MAP3K8,RELA,MAP2K7,TAB2,MYC,MAPK12,TRAF2,MKNK2,PPP3CC,AKT3,FGFR1,MAP K8IP3,NR4A1,CACNA1E,CACNA1A,MAPK11,GADD45A,FGF2,MAPKAPK2,PPP3CA,MAP4K4,NRAS,MAPK7
KEGG_TYPE_I_DIABETES_MELLITUS	0.00544	FAS,HSPD1,CPE,LTA,HLA-DOB
KEGG_APOPTOSIS	0.00637	FAS,NFKB1,TP53,NFKBIA,BIRC2,TNFRSF10D,RELA,TNFSF10,TRAF2,CFLAR,PPP3CC,AKT3,IRAK2,TNFRSF10B,IRAK3,PIK3CD
KEGG_RIG_I_LIKE_RECEPTOR_SIGNALING_PATHWAY	0.00687	NFKB1,TANK,NFKBIA,NFKBIB,CYLD,RELA,MAPK12,TRAF2,TRAF3
KEGG_PROSTATE_CANCER	0.0101	NFKB1,TP53,NFKBIA,CREB1,TCF7,RELA,NKX3-1,MTOR,AKT3,FGFR1,PIK3CD,CCND1,IGF1R
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY 0.		NFKB1,NFKBIA,MAP2K3,MAP3K8,RELA,MAP2K7,TAB2,MAPK12,TRAF3,AKT3,PIK3CD,MAPK11,IRF5,TLR2,TBK1
KEGG T_CELL_RECEPTOR_SIGNALING_PATHWAY	0.0180	NFKB1,NFKBIA,NFKBIE,MAP3K8,NFKBIB,RELA,MAP2K7,MAPK12,BCL10,TEC,PPP3CC,PTPN6,AKT3,CTLA4,LCP2,DLG1,PIK3CD,ICOS,MAP K11,CARD11,PPP3CA,NRAS
KEGG_CYTOSOLIC_DNA_SENSING_PATHWAY	0.0197	NFKB1,NFKBIA,NFKBIB,RELA,POLR3A,POLR1C
KEGG_GRAFT_VERSUS_HOST_DISEASE	0.0211	FAS,HLA-DOB
KEGG SMALL_CELL_LUNG_CANCER	0.0257	NFKB1,TP53,NFKBIA,BIRC2,RELA,MYC,TRAF2,TRAF3,AKT3,PIK3CD,TRAF1,LAMA4,CCND1,TRAF5
KEGG B CELL RECEPTOR SIGNALING PATHWAY	0.0307	NFKB1,NFKBIA,NFKBIE,NFKBIB,RELA,BCL10,PPP3CC,PTPN6,AKT3,PIK3CD,CARD11,PPP3CA,CD72,NRAS
KEGG ALLOGRAFT REJECTION	0.0440	FAS,HLA-DOB
EGG EPITHELIAL CELL_SIGNALING_IN_HELICOBACTER PYLORI INFECTION	0.0496	NFKB1.NFKBIA.RELA.MAPK12.GIT1.MAPK11.ATP6V1C2
		NEKB1 TCE7, RELA MYC, STAT5A MTOR AKT3, PIK3CD, RPS6KB1, CCND1, ZBTB16, RARA, PPARD, NRAS, TCE7L2, LEE1, ARAE, PIK3CG, EIF4EB
KEGG_ACUTE_MYELOID_LEUKEMIA	0.0301	P1,PIK3CA
KEGG P53 SIGNALING PATHWAY	6.84E-04	FAS,GADD45B,TP53,CCNG2,CCND2,CD82,TP53I3,TNFRSF10B,CCND1,SESN1,SESN2,BID,SIAH1,GADD45A
KEGG LEISHMANIA INFECTION	0.0259	NFKB1,NFKBIA,NFKBIB,RELA,TAB2,MAPK12,PTPN6,IFNGR1,HLA-DOB,MAPK11,TLR2,IFNGR2,TGFB2,HLA-DOA,TRAF6,IRAK1,ITGAM,NCF4
KEGG_NEUROTROPHIN_SIGNALING_PATHWAY	0.0295	NFKB1,TP53,NFKBIA,NFKBIE,SH2B3,NFKBIB,RELA,MAP2K7,MAPK12,AKT3,IRAK2,IRAK3,PIK3CD,MAPK11,MAPKAPK2,CAMK4,NRAS,MAP K7,IRS2,YWHAE,PIK3CG,PLCG1,PIK3CA,FRS2
Monocytes		
NAME	FDR q-val	Genes
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	4.94E-04	TNFRSF18,TNFSF8,TNFRSF10B,CSF2RA,TNFRSF10C,FLT1,CXCL3,IL1R2,CCL4L2,IL4R,IL1B,CCL4,CSF1,IL2RG,CD40,IL21R,FAS,TNFRSF10 D,IL1A,IL7,IFNGR2,CCL22,CCL2,CCCR5,TNFSF15,TNFRSF19,IL10,CXCL16,TGFBR2,IL7R,IL6,TNFRSF6B,TNFRSF11A,CSF2RB,ACVR2A,CL CF1,VEGFB,PF4,IL1RAP,CCR1,TNFRSF9,IL3RA,IL6ST,IL12RB2,TNFRSF25,LIF,TNFRSF14,CCR7,CCL5,TGFB3,CCL20,TNFSF4,PDGFRB,OS M,IL23A,IL2A,IL2A,TNFRSF8,IFNAR2,PPBP,CCR5,TNF
KEGG_APOPTOSIS	0.0221	NFKB1,TNFRSF10B,BCL2L1,TNFRSF10C,NFKBIA,IL1B,BIRC2,BID,IRAK2,MYD88,FAS,CFLAR,TNFRSF10D,IL1A,PIK3R5,PIK3CD,NTRK1,BCL 2,AKT2,CSF2RB,RELA,TRAF2,IL1RAP,IL3RA
KEGG_RIBOSOME	0.0228	RPS12,RPS29,RPS26,RPL21,RPL31,RPL24,RPL22L1,RPL18A,RPL5,RPS6,RPSA,RPS8,RPL10A,FAU,RPL4,RPS13,RPS21,RPS24,RPL17,RPL 19,RPS10,RPS3A,RPL35,RPS27A,RPS5,RPL23,RPS15,RPL30,RPL27A,RPS20,RPS3,RPL8,RPL3,RPS23,RPL14,RPL26,RPS4Y1,RPL9,RSL24 D1,RPL28,RPL7,RPL117,RPL13,RPL7A,RPL13A
KEGG_SMALL_CELL_LUNG_CANCER	0.0153	NFKB1,BCL2L1,TRAF1,TRAF3,NFKBIA,FN1,BIRC2,E2F3,CCND1,LAMB3,ITGAV,PIAS2,LAMC1,MYC,PIK3R5,PIK3CD,ITGA6,LAMA5,BCL2,AKT 2,RELA,TRAF2,CDK6,CDKN2B,PIK3CG
KEGG_PATHWAYS_IN_CANCER	0.0188	NFKB1.NFKB2,BCL2L1,CSF2RA,TRAF1,MITF,TRAF3,NFKBIA,WNT5A,FN1,PPARG,BIRC2,PPARD,FZD7,NKX3- 1.BID,E2F3,JAK1,FH,CCND1,MMP9,FAS,LAMB3,ITGAV,PIAS2,LAMC1,MYC,PIK3R5,PIK3CD,NTRK1,STAT3,SP11,ITGA6,TCEB1,SMAD3,TGFB R2,ARAF,SLC2A1,IL6,LAMA5,BCL2,AKT2,CTNNB1,RALGDS,BRAF,HDAC2,EPAS1,VEGFB,RELA,TRAF2,RALA,CDK6,BCR,CDKN2B,HIF1A,PI K3CG,LEF1,XIAP,TGFB3
KEGG_AUTOIMMUNE_THYROID_DISEASE	0.0280	CD80,CD40,FAS,IL10,HLA-DQA1,HLA-DQB1,CD86,HLA-DPA1,CTLA4,CD28,HLA-DRB1
KEGG_GRAFT_VERSUS_HOST_DISEASE	0.0316	IL1B,CD80,FAS,IL1A,IL6,HLA-DQA1,HLA-DQB1,TNF,CD86,HLA-DPA1,KLRD1,CD28,HLA-DRB1
KEGG_ALLOGRAFT_REJECTION	0.0351	CD80,CD40,FAS,IL10,HLA-DQA1,HLA-DQB1,TNF,CD86,HLA-DPA1,CD28,HLA-DRB1
KEGG_HEMATOPOIETIC_CELL_LINEAGE	0.00198	CSF2RA,TFRC,IL1R2,IL4R,IL1B,CD14,CD44,CSF1,CD22,IL1A,IL7,GP1BA,ITGA6,IL7R,IL6,ANPEP,CD9,CD36,GP9,IL3RA,CD19,FCGR1A,MS4A 1,CD8A,ITGA2B,IL2RA,CD3E,TNF
KEGG_JAK_STAT_SIGNALING_PATHWAY	0.00239	BCL2L1,CSF2RA,JAK3,IL4R,CCND2,JAK1,IL2RG,IL21R,CCND1,IL7,PIAS2,CISH,IFNGR2,MYC,PIK3R5,SOCS3,PIK3CD,STAT3,IL10,IL7R,IL6,A KT2,CSF2RB,SOCS2,CLCF1,SPRED2,IL3RA,IL6ST,PIK3CG,IL12RB2,LIF,SPRY2,SPRY4,OSM,IL23A,IL2RA,IL24,IFNAR2,SOCS4,STAT4
KEGG_FOCAL_ADHESION	0.0423	SRC,ACTG1,FLT1,VAV1,FN1,CCND2,BIRC2,ACTN1,FLNB,CCND1,LAMB3,ITGAV,LAMC1,VAV3,PIK3R5,PIK3CD,ITGA6,VWF,RAPGEF1,LAMA 5,BCL2,AKT2,CTNNB1,BRAF,PXN,VEGFB,ACTN4,SHC2,ITGA9,PIK3CG,XIAP,PDGFRB,ITGA2B,LAMA2,ZYX,ITGB5,MYLK
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICIT	Y 0.0446	ICAM1,TNFRSF10B,TNFRSF10C,HCST,VAV1,BID,FAS,NFATC1,LCP2,TNFRSF10D,NFAT5,NCR1,IFNGR2,VAV3,PIK3R5,PIK3CD,ARAF,BRAF, CD247,SHC2,PIK3CG

Figure S8: Table showing the list of pathways and genes upregulated at 1h post infusion of VRC07-523LS/DEL NAb in NK cells and monocytes

VRC07-523LS

VRC07-523LS/DEL



Figure S9: Prozone effect *in vitro.* A) ADCC assays were performed using CEM-SHIV_{SF162P3} as targets (grey circles) and KHYG-Rh cell lines expressing $Fc\gamma$ RIII as effectors (orange circles). Y represents VRC07-523LS/DEL. Brackets are shown to indicate test conditions where cells were pre-incubated with the antibody and washed before adding to the ADCC assay. In some assays, additional NAb was added during the culture shown as +Y or +Y. Data are representative of at least 3 experiments. B) AUC for data in extended data figure 7a were calculated and analyzed by one way ANOVA with Tukey's correction. P values are presented as ns = not significant, * <0.05, **<0.0005, ***<0.0005 and ****<0.0001.

VRC07-523LS/LALA

10 15 20

E103

101

+10⁻¹ 20

A14V078

0 5

0 5 10 15

-6

10⁷

10

10

10¹

-6

Figure S10: Long-term follow-up of plasma viral load. Rhesus macaques were challenged iv with SHIV_{SF162P3} at week -6 and treated with 20 mg/kg of NAb at week 0 (shown as vertical dotted lines). Viral load is shown as black lines (left Y-axis) and circulating NAb levels in colored lines (right Y axis).

Α

SHIV	SF162P3	MRVKGIRKNY	QHLWRGGTLL	LGMLMICSAV	EKLWVTVYYG	VPVWKEATTT	LFCASDAKAY	DTEVHNVWAT	HACVPTDPNP	QEIVLENVTE	NFNMWKNNMV	EQMHEDIISL	WDQSLKPCVK	LTPLCVTLHC	TNLENATNTT	SSNWKEMNRG	150
	DGVK																
	HF7																
	HJR																
	A14V108																
	DFN3																
	A14V151																
	A11V149															N	
	A12V108																
SHIV	SF162P3	EIKNCSFNVT	TSIGNKMOKE	YALFYKLDVV	PIDSDNTSYN	LINCNTSVIT	OACPKVSFEP	IPIHYCAPAG	FAILKCNDKK	FNGSGPCINV	STVOCTHGIR	PVVSTOLLLN	GSLAEEGVVI	RSENFTDNVK	TIIVOLKESV	EINCTRPNNN	300
	DGVK				SN												
	HF7				N												
	HJR				N												
	A14V064				N												
	A14V108				N												
	DFN3				N												
	A11V149				N												
	A12V108				N												
SHIV	SF162P3	TRKSIPIGPG	KAFYATGDII	GDIRQAHCNI	SGEKWNNTLK	QIVTKLQAQF	ENKTIVFKQS	SGGDPEIVMH	SFNCGGEFFY	CNSTQLFNST	WNNTIGPNNT	NGTITLPCRI	KQIINRWQEV	GKAMYAPPIR	GQIRCSSNIT	GLLLTRDGGR	450
	DGVK																
	HF'/																
	A14V064																
	A14V108																
	DFN3																
	A14V151																
	A11V149																
	A12V108															K	
SHIV	SF162P3	EVSNTTEIFR	PGGGDMRDNW	RSELYKYKVV	KIEPLGVAPT	KAKRRVVQRE	KRAVTLGAVF	LGFLGAAGST	MGAASLTLTV	QARQLLSGIV	QQQNNLLRAI	EAQQHLLQLT	VWGIKQLQAR	VLAVERYLKD	QQLLGIWGCS	GKLICTTAVP	600
	DGVK																
	HF7																
	HJR																
	A14V064																
	A14V108																
	DEN3																
	A11V149																
	A12V108																
SHIV	SF162P3	WNASWSNKSL	DQIWNNMTWM	EWEREIGNYT	NLIYTLIEES	QNQQEKNEQE	LLELDKWASL	WNWFDISKWL	WYIKIFIMIV	GGLVGLRIVF	TVLSIVNRVR	QGYSPLSFQT	RFPAPRGLDR	PEGIEEEGGE	RDRDRSRPLV	HGLLALIWDD	750
	DGVK																
	HJR																
	A14V064																
	A14V108																
	DFN3																
	A14V151																
	A11V149																
	A12V108																
SHIV	SF162P3	LRSLCLFSYH	RLRDLILIAA	RIVELLGRRG	WEALKYWGNL	LQYWIQELKN	SAVSLFGAIA	IAVAEGTDRI	IEVAQRIGRA	FLHIPRRIRO	GLERILL 8	47					
	DGVK										T						
	HF7										T						
	HJR										T						
	A14V064				T						T						
	A14V108										T						
	DFN3										T						
	A14V151										T						
	A12V108										T						

Figure S11: Env sequencing. Plasma virus was sequenced from 3 animals per group at 48h prior to NAb infusion and following rebound. A) Animal Ids are highlighted based on NAb group (black for VRC07-523LS, red for LALA and blue for DEL). Sequences are shown here as an alignment of pre-infusion consensus sequences with the Env of SHIV_{SF162P3}. B) Sites showing evidence of selection after NAb infusion at a Bonferroni-adjusted Fisher's exact test *p* value < 10^{-12} are shown in blue, with contact sites for a related CD4bs NAb VRC01 shown in orange. C) Web logo plots are shown around the N197 site (blue highlight) at which significant selection was observed in most animals.