

Supplementary Material

1 Supplementary Figures and Tables

1.1 Supplementary Tables

Table S1. Equilibrium binding constants for IgG1 variants to RM and human FcyRs

	Rhesus macaque K _D equil (µM)									
FcγRIIa-1 Fc		FcyRIIa-2	FcyRIIa-4	FcyRIIb-1	$Fc\gamma RIIIa-1(I^{158})$	$Fc\gamma RIIIa-3(V^{158})$				
RhDH677.3	4.7	4.9	6.2	3.7	1.1	1.5				
Rh7B2	6.0	5.5	7.7	3.7	1.0	1.6				
RhDH827	5.7	5.5	6.9	3.7	1.3	1.9				
RM JR4	4.9	4.5	6.9	3.1	1.1	1.6				
RM DH614.1	5.0	4.7	6.8	3.0	0.8	1.3				
RM DH614.2	4.9	4.7	7.0	3.1	0.9	1.3				
RM DH614.3	4.4	4.2	6.5	2.8	0.9	1.4				
	Human K _D equil (µM)									
	FcγRIIa(¹	³¹) Fe	γ RIIa(H ¹³¹)	FcγRIIb FcγRIIIa(F ¹⁵⁸)		$Fc\gamma RIIIa(V^{158})$				
RhDH677.3	4.6		1.5	7.9	3.8	0.8				
Rh7B2	2.9		4.4	8.5	3.8	1.8				
RhDH827	4.6		3.7	7.2	4.1	2.4				
RM JR4	2.0		3.6	8.3	3.9	1.7				
RM DH614.1	2.7		3.5	6.6	3.9	1.6				
RM DH614.2	1.9		4.5	10.1	3.9	1.6				
RM DH614.3	1.7		3.9	6.9	3.9	1.4				

Table S2. Details of the RhDH677.3, the DH677.3, the RhDH827 and the DH827 interfaces based on the RhDH677.3 Fab-gp120_{93TH057}core_e-M48U1, the DH677.3 Fab-gp120_{93TH057}core_e-M48U1, the RhDH827 Fab-V2 peptide and the DH827 Fab-V2 peptide complex structures as calculated by the EBI PISA server (<u>http://www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver</u>). The values for the two copies in the asymmetric unit of the DH677.3 complex are averaged in the table while those of the first copy only (with M48U1 bound) are shown for the RhDH677.3 complex.

		RhDH677.3Fab	DH677.3 Fab -	RhDH827 Fab -	DH827 Fab -		
		-gp120 _{93TH057}	gp120 _{93TH057}	V2peptide	V2peptide		
		core _e -M48U1	core _e -M48U1				
	gp120 total	876	934	852	817		
	7/8-stranded β sheet	226	261	0	0		
	Layer 1	530	540	0	0		
Å 2	Layer 2	120	133	0	0		
	Layer 3	0	0	0	0		
a , 7	V2 loop	0	0	852	817		
Are	Heavy chain total	463	477	492	516		
burface A	FWR	0	0	0	0		
	CDR H1	10	18	51	53		
	CDR H2	96	81	184	195		
g	CDR H3	357	378	257	268		
rie	Light chain total	461	483	293	307		
Bu	FWR	59	90	2	1		
	CDR L1	246	229	124	138		
	CDR L2	28	28	37	35		
	CDR L3	128	136	130	133		
	Heavy and light chain total	924	960	785	823		

1.2 Supplementary Figures

			10	20	30		40	5052a	60	70	808	2abc	90	100ab	cdefghi	110
	Rh7B2	EVQLVES	GGGLVQPO	GSLRLSC	ASGFTFT	EYYMS WVR	QAPGKGLEWV	S YISKNO	EYSYYADSVE	G RFTISRD	NAKNSLSLQ	MSSLRAEDT	AVYYCTR A	DGLTYFS	ELLQYIFDL	WGQGVLVTVSS
	7B2	QVQLVQS	GGGVFKPG	GSLRLSC	ASGFTFT	EYYMT WVR	QAPGKGLEWL	A YISKNO	EYSKYSPSSN	G RFTISRD	NAKNSVFLQ	LDRLSADDT.	AVYYCAR A	DGLTYFS	ELLQYIFDL	WGQGARVTVSS
	RhUCA	EVQLVES	GGGLVQP	GSLRLSC	ASGFTFS	WVR	FWR2 QAPGKGLEWV	S	CDRH2	RFTISRD	NAKNSLSLQ	MSSLRAEDT.	AVYYCTR	CD	RH3	WGQGVLVTVSS
			10	20	27abc	def 30	40	5	0	60	70	80	90	10	0 107	
	Rh7B2	DIVMTQS	PDSLAVS	LGERVTIN	KSSQTLL	YSSNNRHSI	A WYQQKPGQ	APKLLIY	WASMRLS GV	PNRFSGSGS	GTDFTLTIS	GLQAEDVAV	YYC HOYSS	HPPT FG	GTKVEIK	
	7B2	ETTLTQS	PDSLAVS	PGERATIHO	KSSQTLL	YSSNNRHSI	A WYQQRPGQ	PPKLLLY	WASMRLS GV	PDRFSGSGS	GTDFTLTIN	NLQAEDVAI	YYC HQYSS	HPPT FG	HGTRVELR	
\frown	RhUCA	DIVMTQS	PDSLAVSI	LGERVTIN	C	DRL1	WYQQ K PGQ	APKLLIY	-CDRL2-	PNRFSG?GS	GTDFTLTIS	GLQAEDVAV	YYC	FG	GTKVEIK	
>			1.0		201	300		5/II	10 61		70	NUN/1550	1471	1	lilabadat	110
Γ.	Phoue	7 3 000	TUDECAR	WERCA SU	CRACCY	TTT CYDTX	WUPONBCOC	T FUNC W	NEWTONTON	OFFOC DUT	MTOTOTOTO	A VMET COT D	CEDTAUVVC	TAT VETT	AUCYPYEO	WCOCALUTURE
'	DUG	7.3 000	TUORCART	UOV DCA SU	NUCCENCCY	TEA CYDIN	WURON TCOC	TENMO M	NEWTONTON	OFFOC DUT	TTOMPCTCT	N VMPT TOT D	CEDTAUVV	TAT VOTT	ANUCYPYEO	WCOCTUTUES
~	DIG		LUSCAR	FWR1	KT CCVACCV	CDRH1	FWR2	T FUNC	CDRH2	INTER RAL	MATOREMON	FWR3	CEDWAUYY	AL INIL	-CDRH3	FWR4
\leq	K	IUCA QVQ	LIVE DGAL	KREGASVI	20	30	40	50	61	7	MIRDISISI	80	90	100	107	WGQGALVIVSS
S	Phoue	7 3 10	MTOSPEST	C CA CUCD	UTTTC DAS	OCECNYLA	WYOOKPCKUR	WILTY N	TTOS FUD	Drececeom	DETITICET	OPEDUATEV	CONVICAL	ET FCOC	TOTETH	
Ê	DH6	7 3 010	TTOCDEC	LEASUCD	UTTTC DAG	OCECNVLA	MAOOB BCKAR	FUTTY A	TTLYS EVER	EDFCCCCCC	DETETTOS	ODEDVATIV	C OKVNSAL	FT FCOC	TOLETY	
aj	DHO		MTOPPOP	FWR1	VIIIC RAS	CDRL1	FWR2	EVLII A	CDRL2-	DRI SGSGSGI	FWR3	OPEDVALLI	CDRL3	FI FGQG	WR4	
3	~	IUCA ALS	MIQSESS	LONDVGDK	VIIIC		WIQQREGRAE	Kulli	LVES	5KE 2626261	DE 1111331	MEPDAWI II	C	r GZG	IRLEIN	
ō			10	20	3	0	40	5052	a 60	70) 8	3082abc	90	100	abcd	110
р	RhDH8:	27 EVQLV	ESGGGLV	QPGGSLRL	SCAASGFTF	S NCAMH	VRQAPGKGLE	WVA VIS	HGSNKYYAD	YVKG RFTIS	RDNSKNML	LOMNNIKLE	DTAVYYCAL	EGGAPS	GWALDY WG	QGVLVTVSS
>	DH8:	27 EVQLV	ESGGGVV	QPGRSLRL	SCAASGFTF	S NCAMH	VRQAPGKGLE	WVA VIS	HGSNKYYAD	YVKG RFTIS	RDNSKSTV	LQMNSPRAE	DTAVYYCA	EGGAPS	GWALDY WG	QGTLVTVSS
	RhU	A EVQLV	ESGGGLV	FWR1 QPG <mark>G</mark> SLRL	SCAASGFTF	S CDRH1	FWR2-	AVWA	CDRH2	RFTIS	RDNSKNML	FWR3 LQMNNLKLE	DTAVYYCA		RH3	FWR4 GQGVLVTVSS
			10	20		30	40	50	60	70	80	2	90 95a	100	106a	
	RhDH82	27 SYELT	QSPS-VS	VSPGQPAS	ITC SGDKL	GDKYAC WY	QQKPGQ V PVI	VIY EDT	KRPS GIPERI	FSGSNSGNTA	TLTISGVE	GDEADYYC	QAWDSITH	V FGGGT	RLTVLG	
	DH82	7 SYELT	OPPS-VS	VSPGQTAS	ITC SGDKL	GDKYAC WY	COOKPGOSPVI	VIY EDT	KRPS GIPERI	FSGSNSGNTA	TLTISGTO	MDEADYYC	QAWDSIT-	V FGGGT	KLTVLG	
	RhUG	A SYELI	QSPS-VS	VSPGQPAS	ITC	W	QQKPGQ V PVI	VIY	GIPER	FSGSNSGNTA	TLTISGVE	GDEADYYC		FGGGT	RLTVLG	
S	Γ	110	1	20	130	140	150	160	170	180	190	20	0 3	210 214		
	rhkaj	pa RAVA	APSVFIF	PPSEDQVK	SGTVSVVCL	LNNFYPRE	ASVKWKVDGVI	KTGNSQE	SVTEQDSKDN	TYSLSSTLTL	SNTDYQSH	VYACEVTHQ	GLSSPVTKS	FNRGEC		
Ogi.	hkaj	pa RTVA	APSVFIF	PPSDEQLK	SGTASVVCL	LNNFYPRE	AKVQWKVDNAI	QSGNSQE	SVTEQDSKDS	TYSLSSTLTL	SKADYEKH	KVYACEVTHQ	GLSSPVTKS	FNRGEC		
E+ -	Į	110		20	120	1.40	1 5 0	с <mark>к</mark>	170	100	1.07			110		
ō×		de OBK	COLUMN E	DRCCPETO	130	TODEVDOIR	TOO	180	ITTDEVOENDIV	TOU LOU	Tal		OTTENT			
υσ	hlam	da OPKA	A DOUTT F	PROFELO	ANKATLVCL	TEDEVECAL	TUAWKADGNS	VNIGVET	TTPSKQSNNK	VARCEVICIT	DEOWKSHK	SISCOVTHEG	COUFFERINA	TECS		
0 <u></u>	manu		AF SVILLE.	FFSSEELY	ANALIVCI	ISDEIFGA	C	VIAGVEL	I I FORYONNE.	1449911911	FEYNCOL	SISCAALUEG	DIVERIVA	TECS		
		(1) A 10		120	1.40	150	1.60	17	1.0			200	21.0	222	220	240
	Contra and	14 12		130	140	150	100	17	180	19		200	210	223	230	240
	rnigg.	GSTRGE	SVEPLAP	SSRSTSES	TAALGCLVK	DIFPEPVIV	SWNSGSLISG	SVHTEPAV.	LQSSGLISLS:	SVVTVPSSSL	GTQTIVCN	VNHKPSNTKV	DKRVEIKT	GGGSKPP	TCPPCPAPE	LLGGPSV
υ ⁽¹)-	nige.	ASTRGE	SVEPLAP	SSKSTSGG	TAALGCLVK	DIFFEPVIV	C _H 1	VHIPPAV.	LØSSGLISTS:	SVVTVPSSSL	GTQTTICN	VNHKPSNTKV	DRRVEPRS	bkTh	inge —	LLGGPSV
шŌ	3	241	250	260	270	28	80 29	0	300	310	320	330	340	350	36	366
+	rhIgG	FLFPPR	PKDTLMI	SRTPEVTC	VVVDVSQED	PDVKFNWY	NGAEVHHAQI	KPRETQY	NSTYRVVSVL	TVTHQDWLNG	GKEYTCKVSN	KALPAPIQK	TISKDKGQI	PREPQVYT	LPPSREELI	KNQVSLT
	hIgG	FLFPPK	PKDTLMI	SRTPEVTC	VVVDVSHED	PEVKENWY	DGVEVHNAKT	KPREEQY	NSTYRVVSVL	TVLHQDWLNG	GKEYKCKVSN	NKALPAPIEK	TISKAKGO	PREPQVYT	LPPSREEMI	KNQVSLT
TUT		370	3.8	0	390	400	410	420	430	440	447					
UG	rhIcG	CLVKG	YPSDIV	EWESSCOP	ENTYKTTPP	VLDSDGSV	FLYSKLTVDKS	RWOOGNV	FSCSVMHEAT	INHYTOKSIS	VSPGK					
9	hIge	CLVKCE	VPSDIAU	EWESNCOP	ENNYKTTDD	VLDSDGST	T.VSKLTVDKS	RWOOGNU	ESCSVMHEAT.	INHYTOKELS	ST.SPCK					
				THE REAL PROPERTY	AALA AFE	- LUGGOGOE I	and a state a shirt	W. C. K. W.	CONTRACTOR	Total a Marging	a was					

Figure S1. Sequences for rhesusized variants of Rh7B2, RhDH677.3 and RhDH827. Variable domains of the three rhesusized mAbs with rhesusized sequence, human sequence and rhesus unmutated common ancestor sequence (RhUCA) for each antibody (top). Constant domains for the lambda and kappa light chains as compared to the human sequences (middle). Constant domains for the heavy chain (C_H1, C_H2, and C_H3) as compared to the human sequence. Residues that are different between macaque and human are highlighted in red. CDRs are colored and labelled as shown.

Supplementary Material



Figure S2. BLI sensorgrams (black) and curve fits (red) of binding of IgG1 variants to RM and human FcγRs. Antibodies were printed at two concentrations (300 and 150 nM), shown in side by side columns.



Figure S3. SPR binding curves (black) and curve fits (color) for the binding of IgG1 variants to HIV-1 Env antigens.



Figure S4. Comparison of the two copies of the RhDH677.3 Fab-gp120_{93TH057} core_e-M48U1 complex. Of the two copies present in asymmetric unit of the RhDH677.3 Fab-gp120_{93TH057} core_e-M48U1 complex only one had the CD4 mimetic M48U1 bound (complex 1). Despite this difference the root mean square deviation (RMSD) between the complex copies (RhDH677.3 Fab-gp120_{93TH057} core_e) is 1.4 Å for main chain residues of the full complex and 1.1 Å for the main chain residues of the variable part of the Fab and gp120 in the complex. In both copies the β -sandwich shows good rigidity and was found largely independent of CD4 bound status. In contrast mobile layers 1 and 2 have more flexible conformations that can vary with the presence or absence of CD4 or

gp41. The two complexes in the asymmetric unit of the RhDH677.3, complex 1 with the CD4 mimetic M48U1 bound and complex 2 with no CD4 mimetic bound, had almost identical heavy chain buried surface areas (BSAs) (complex 1, BSA of 404 Å² and complex 2, BSA of 401 Å². **Table S1**). But there were differences in how the two complexes arrived at these totals with complex 1 having slightly larger contributions from gp120 inner domain layers 1 and 2, 216 and 14 Å² versus 202 and 11 Å² respectively, and complex 2 having a higher contribution from the 7-stranded β-sandwich, 187 versus 174 Å². This difference in binding was more apparent in the light chain with complex 1 having a layer 1 BSA of 314 Å² and a layer 2 BSA of 105 Å² while complex 2 had a layer 1 BSA of 277 Å² and a layer 2 BSA of 121 Å²; the light chain 7-stranded β-sandwich BSAs were largely identical, 52 and 49 Å² respectively. The total RhDH677.3 gp120 BSA was lower for complex 2, 848 Å² versus 876 Å², even though contact residues were identical in both structures (layer 1 residues 53, 71-80 and 82, layer 2 residues 219-222, and 7-stranded β-sandwich residues 84, 223-224, 244-246, and 491-492). RhDH677.3's greater use of the 7-stranded β-sandwich in binding to gp120 in complex 2 may therefore be a consequence of conformational changes within layers 1 and 2 with and without M48U1 bound.



Figure S5. ADCC activities against gp120-coated cells obtained using a GTL-ADCC assay. ADCC activities are shown as area under the curve (AUC) for each mAb calculated from dilution curves (starting concentration 50 μ g/mL with 1:5 serial dilutions) against gp120-coated cells with PBMCs from an HIV-1-seronegative individual as effectors at an E:T ratio of 30:1. Each bar represents a different antibody. The experiment was performed once with two biological replicates.