Fig. S1. Amino acid sequence alignment of 16 VRC1-resistant viruses. The VRC01-sensitive HXBc2 and JRCSF gp120 virus envelope sequences are shown as references. In addition, the HXBc2 and 93TH057 core protein sequences are shown, as these proteins were used to solve the liganded crystal structures of b12 and VRC01, respectively. The gp120 contacts with VRC01, CD4, or b12 are indicated in blue, gold, or green, respectively. Open circles (\circ) denote gp120 main-chain-only contacts, open circles with rays (\diamondsuit) denote gp120-side-chain-only contacts, and filled circles (\bullet) denote both main-chain and side-chain contacts. Residues that vary from the reference sequence are highlighted in yellow. Residues that showed a significant change in VRC01 binding when mutated are shown in bold; the residue is underlined if the mutant virus displayed neutralization resistance to VRC01. Variations within the CD4 binding loop and it proximal region that contains the NXS/T motif resulting in additional N-glycosylation sites are highlighted in blue.

	 10	· · · · · · · · · · 20	 30	· · · · · · · · 40	···· ··· 50	•••• •••• • 60	···· ···· 70	•••• •••• • 80	•••• •••• 90	 100
HXB2R-K03455	MRVK-EKYQHLWRW		TCSAFFKLW	VTVYYCVDVW	KEDUUUT.ECD		муматнасу/	ΡΨΠΡΜΡΛΕΊΛΙ	VNVTENEN	WWKNDWVFOMH
JR CSF	MRVKGIRKNYOHLW									
HXBc2 Core								~		MWKNDMVEQMH
93TH057 Core										MWKNNMVEOMO
AY124970 BL01	MRVKEIRKNCLHLW	RWGTMLLGMLM								
EF210732 H086 8	MKVKGIRRNYQLLW							~		~
AP210 2 00 E8	MRVMGIQRNWQQWG									
u172 17	MRVMGILRSYQQWW									
u422_1	MRVRGIPRNWPQWW	IWGILGFWMII	ICRVVGNLDLW	VTVYYGVPVW	KEAKTTLFCA:	SDAKAYDKEVH	NVWATHACVI	PTDPNPQEIVL	ENVTENFNN	MWKNDMVDQMH
'V1 29	MRVMGTQKNCQQWW	IWGILGFWMLM	ICNTEDLW	VTVYYGVPVW	REAKTTLFCA:	SDAKAYETEVH	NVWATHACVI	PTDPNPQEIVL	GNVTENFNN	MWKNDMADQMH
ZA125 17	MRVKGILRNWQHRW	IWIWIILGFWMLM	ICNGNLW	VTVYYGVPVW	KEANAPLFCA:	SDAKAYEKEVH	NVWATHACVI	PTDPNPQELDL	VNVTENFNN	MWKNDMVDQMH
7128 02	MRVRGIERNYQHLW	WR-WGTMLLGILM	ICSAAEQLW	VTVYYGVPVW	KEATTTLFCA:	SDAKAYKAEAH	NIWATHACVI	PTDPNPQEIVL	GNVTENFN	MWKNDMVEQMH
U885764 X2088 9	MRARGTQRNWQHLW	KGGTLILGLVI	ICSASNNLW	VTVYYGVPAW	EDADTTLFCA	SDAKAYSTEKH	NVWATHACVI	PTDPDPQEIPL	ENVTENFN	MWKNNMVEQMH
242 14	MKVMGIQKNYPSFW	RWGMILFWIMM	ICNATNLW	VTVYYGVPVW	RDADTTLFCA	SDAKAYEKEVH	NVWATHACVI	PTDPNPQEIHL	KNVTEEFNN	MWKNNMVEQMH
250 4	MRVMGIQRNYPPLW	RWGTMIFWMMM	LCSAEKLW	VTVYYGVPVW	READTTLFCA	SDAKGYDTEAH	NVWATHACVI	PTDPRPQEMYL	ENVTENFNN	MWKNSMVEQMH
278_50	MRVMGIQKSYPLLW	SWGIIFWIM	MIGNANQMW	VTVYYGVPVW	RDAETTLFCA	SDAKAYDTEAH	NVWATHACVI	PTDPNPQEIHL	KNVTENFNN	MWKNAMVEQMN
20345 C1	MKVKETQMNWLNLW	RWGTLILGLVM	ICSASDKLW	VTVYYGVPVW	RDADTTLFCA:	SDAKVHSTEVH	NVWATHACVI	PTDPNPQEIHL	ENVTENFN	MW <mark>Q</mark> NKMAEQMQ
INE28	MRVRETQRNWPNLW	KWGTLILGLVI	ICSASDNLW	VTVYYGVPVW	RDADTTLFCA	SDAKAHEPEVH	NVWATHACVI	PTDPNPQEIHL	ENVTEYFNN	MWKNNMAEQMQ
817_V2_C59	MRVTGTQRNCQQWW	IWIWIILGFWWML	MMCKGEKLW	VTVYYGVPVW	KEATTTLFCA:	SDAKAYNPEMH	NVWATHACVI	PTDPNPQEMVL	ENVTENFNN	MW <mark>E</mark> NNMVEQMH
6540_V4_C1	MKVKGILRNCQQWW	IWGILGFWMVM	TYSVVGNLW	VTVYYGVPVW	KDAETTLFCA:	SDAKAYEREVH	NVWATHACVI	PTDPNPQEVIM	ENVTENFNN	MWKNNMVDQMH
5545_V4_C1	MRVKGILRNCQQWW	IWGILGFWMVM	<u>TYSV</u> VGNLW	VTVYYGVPVW	KDAETTLFCA:	SDAKAYEREVH	NVWATHACVI	PTDPNPQEVIM	ENVTENFNN	MWKNNMVDQMH
	Sign	al peptide								
						.		.		
	110 12	0 130	140	150		160	170	180		19

HXB2R-K03455 JR_CSF HXBc2 Core 93TH057 Core	VINLWDQSLKPC IISLWDQSLKPC	VKLTPLCVTLNCKDVNATNTTSSSEGMMER VKLTPLCVGAG	GEIKNC	SFNISTSIRGKVQKEYAFFYKLDIIPIDNDTTSYKL SFNITKSIRDKVQKEYALFYKLDVVPIDSKNNTKYRL
AY124970 BL01	IISLWDOSLKPC	VELTPLCVTLNCTDORKNTSPLGEAEKMEK	GEMKN	SFKVTTRLRNKVQKEYALFNKLDVVPIDNDNKTNTNYSSYKL
EF210732 H086 8		-		SFNITTSVKDKVOKEYALFYRLDIVPIDNTSYRL
CAP210 2 00 E8	IISLWDQSLKPC	VKLTPLCVTLNCSDATYNNGTNSTDT	MKI	SFNATTELRDKKKKEYALFYRLDIVPLKNESESQNFSEYIL
Du172 17	IISLWDQSLKPC	VKLTPLCVTLNCSDVKIKGTNATYNNATYNNN	NTISDMKN	SFNTTTEITDKKKKEYALFYKLDVVALDGKETNSTNSSEYRL
Du422_1	IISLWDQSLKPC	VKLTPLCVTLNCKNVNISANANATATLNSSMN	GEIKN	SFNTTTELRDKKQKVYALFYKPDVVPLNGGEHNETGEYIL
TV1 29				SFNVTTELRNKKHKEYALFYRLDIVSLNNKPNEYRL
TZA125 17				SFNITTEIRDKKKKEYSLFYRLDILPLDNSSESKNYSEYVL
57128 02	IISLWEQSLKPC	VKLTPLCVTLNCTNATATNATATSQNSTDGSN	KTVNTDTGMKN	SFNVTTDLKDKKRQDYALFYKLDVVQIDDKNTNGTNTNYRL
EU885764 X2088 9	IISLWDESLKPC	VMLTPLCVTLNCSAVNGTMN	EQMKN	TFNITTEIRDKKKEEYALFYKLDIEQISKTANSTTANSNYSSYRL
242 14	IISLWDQSLKPC	VKLTPLCVTLDCIEIRNNTDNITVDSN	MKGEITN	SFNMTTELRDKKQKVSAFFYRQDVVPVNSNQDNSSYRL
T250_4	IISLWDESLKPC	VKLTPLCVTLDCQAFNSSSHTNSS	IAMQEMKN	SFNVTTELRDKKKKEYSFFYKTDIEQINKNGRQYRL
T278_50	IISLWDQSLKPC	VKLTPLCVTLHCHNFTSSSNSSNSTVSPQMR	GEIQN	SFNTTTLLKDKKKKEYALFYRQDIVPLDTNGTNIDTNGTNSSQYML
620345_C1	VISLWDQSLKPC	IKLTPLCVTLSCTEAKFNETFNKIDNITKVSN	LTDEMRN	SFNMTTELRDKKQQVYALFYKLDIVPIDNSSEYRL
CNE28	VISLWDQSLKPC	VKLTPLCVTLNCTNAQFPNDTRTANASDKIGN	ITDEIKN	TFNITTEIRDKKQKAYALFYRLDIVPIEDKNNSSNEYRL
3817_V2_C59				SFNVTTEINDRKKKMHALFYRLDVVPLNKRNSSTNNNSNNESTMDYRL
6540_V4_C1	VISLWDQSLKPC	VKLTPLCVTLDCKNVNDTRNGNVTYNNTMN	EEIKN	SFNMTTELRDKKQKVSALFYRIDIVSLNSNSSDYRL
6545_V4_C1	IISLWDQSLKPC	VKLTPLCVTLDCRNVSDTRNGNVTYNNTMN	EEIKN	SFNMTTELRDKKQKVSALFYRIDIVSLNSNSSDYRL
		V1		V2

	. 200	. 210 220	230 240		· · · · · · · · · · · · · · · · · · · · · · · · · · · · · · · · ·	70 280		 300
	* *			0•				
HXB2R-K03455 JR_CSF HXBc2 Core	ISCNTSVITQACPKV	SFEPIPIHYCAPAGFAI	LKCNNKTFNGTGPCTNV LKCNNKTFNGKGQCKNV LKCNNKTFNGTGPCTNV	STVQCTHGIRPVVSTQ	LLLNGSLAEEKVV	VIRSVNFTDNAK VIRSDNFT D NAK	TIIVQLNESVKIN	JOTRPSNNT
93TH057 Core			LKCNDKNFNGTGPCKNV					
AY124970_BL01 EF210732 H086 8			LKCKDKKFNGTGRCENV					
CAP210 2 00 E8	~		LKCNNKTFNGTGPCNNV	~			~ ~	
Du172 17	~		LKCNNKTFNGTGPCNNV	~ ^				
Du422_1			LKCNNKTFNGTGPCNNV					
TV1_29			LKCNNKTFNGTGPCYNV					
TZA125_17			LKCKDKTFNGTGPCSNV					
57128_02 EU885764 X2088 9			LKCNNKTFNGTGPCKNV					
242 14	- ~		LKCNDKKFNGTGPCKNV	~ ~			~ ~	
T250 4			LKCNEKHFNGKGPCKNV					
т278_50			LKCRDKEFNGTGTCKNV					
620345_C1			LKCNDKNFNGTGPCKNV					
CNE28 3817 V2 C59	~		LKCNDVNFNGTGLCRNV LKCNSKTFNGTGPCTNV	~ ~ ~ ~				
6540 V4 C1			LKCNNETFNGTGPCHNV					
6545 V4 C1			LKCNNETFNGTGPCHNV					
	V2					Loop D		V3
	• -							
	. 310	. 320 330	340 350		370	380	 390	400
					* • 00• •• * *	• •		
HXB2R-K03455	RKRIRIORGPGRAFV	TIGKI.GNMROAHCNIS	SRAKWNNTLKQIASKLRE	OFGNNKTIIFK	CSSGGDPEIVTH	SFNCGGEFFYCN	STOLFNSTW-FNS	STWSTEGSNN
JR CSF			SRAQWNNTLKQIVEKLRE				STQLFNSTW	
HXBc2 Core			ARAKWNNTLKQIASKLRE	QFGNNKTIIFk	QSSGGDPEIVTH	SFNCGGEFFYCN	STQLFNSTW-FNS	JTWSTEGSNN
93TH057 Core			IGTKWNKVLKQVTEKLKE					
AY124970_BL01			RAHWNTTLGHIARKLRE					
EF210732_H086_8 CAP210 2 00 E8			IKTKWHEALKKIAKKLEE SEKAWNETLKKVVEKLVK					
Du172 17			SERAWNEILKRVVERLVR SRKKWNTTLQRVKEKLKE					
Du422 1			RETWNSTLIQVKEKLRE					
TV1_29			TDRWNKTLQQVMKKLGE					
TZA125_17			GRSDWNKTLQEVGKKLRE					
57128_02			RKEWNSTLQQVTKKLGS					
EU885764_X2088_9			RGKWVNITKNVKEQLWK					
242_14 T250_4			IKTEWNETLYQVVTRLRE SKREWNNTLQQVAAQLSK					
T278 50			GTEWNKTLHRVATKLKG					
620345 C1			IGTKWNETLRQVAKKLKE				TAQLFNSTWRGNE	
CNE28	RKSVRIGPGQVFY	RTGDIIGDIRQAYCEIN	IGTKWSEVLKQVTEKLKK	VTNK-TRIEF <mark>Q</mark> F	<mark>APA</mark> GGDLEITMH	HFNCRGEFFYCN	ISKLFNCTG	NSTTG
3817_V2_C59			RGEWNKTLQQVAEKLGN					
6540_V4_C1			IRTAWNNTLQKVVTRLRT				TSSLFNSTWPVSG TSSLFNSTWQVSG	
6545 V4 C1	RKSVHIAPGQAFY	Δ T C D L L C N L R O Δ IY (''''')/N						

		420 430	440	•••• ••• •• 450	 460		. 490		 510
				* * ☆○●	00 •🌣	FRPGGGDMRDNWRSEL			
HXB2R-K03455	TEGSDTITI	LPCRIKOIINMWOKVGK	AMYAPPISGOIRC	SSNITGLLLTRD	GGNSNNESEI	FREGGEDMRDNWRSELY	KYKVVKIEPLGV	JAPTKAKRRVV	OREKR
JR CSF						FRPGGG D MRDNWRSEL			
HXBc2 Core	TEGSDTITI	LPCRIKQIINMWQKVGK	AMYAPPISGQIRC	SSNITGLLLTRD	GGNSNNESEI	FRPGGGDMRDNWRSELY	KYKVVKIE		
93TH057 Core	CNGTITI	LPCKIKQIINMWQGTGQ	AMYAPPIDGKINC	VSNITGILLTRD	GGANNTSNET	FRPGGGNIKDNWRSELY	KYKVVQIE		
AY124970_BL01						FRP <mark>I</mark> GGNMRDNWRSELY			
EF210732_H086_8						FRP <mark>I</mark> GG <mark>E</mark> MRDNWRSELY			
CAP210_2_00_E8						FRPGGGDMKDNWRSELY			
Du172_17						FRPGGGDMRDNWRSELY			
Du422_1						FRPGGGNMKDNWRSELY			
TV1_29						FRPGGGDMRDNWRSELY			
TZA125_17						FRPGGGDMRDNWRSELY			
57128_02						FRPGGGDMRDNWRSEL			
EU885764_X2088_9						FRP <mark>T</mark> GGDMRDNWRSEL			
242_14						FRPGGGDMRDNWRSEL			
T250_4		~ ~ ~			~	FRPGGGDMRDNWRSEL	~		
T278_50						FRPIGGNMRDNWRSEL			
620345_C1 CNE28						FRP <mark>A</mark> GGDMRDNWRSELY FRPGGGNMKDNWRSELY			
3817 V2 C59						FRPGGGDMRDNWRSEL			
6540 V4 C1						FRP <mark>T</mark> GGDMRDNWRSEL			
6545 V4 C1						FRPTGGDMRDNWRSEL			
0010_V1_01				TOWITOTITI O				, 1 1 I I I I I I I I I I I I I I I I I	×1/11/1/
	V4	β20 / β21			V5 / β24	β24 / α5			
						Connection			