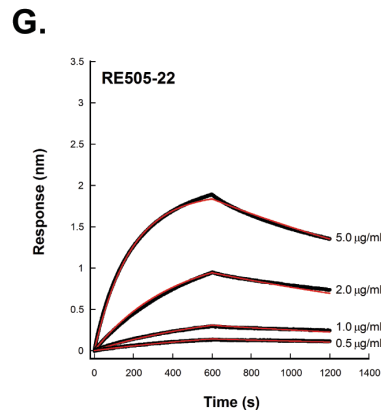
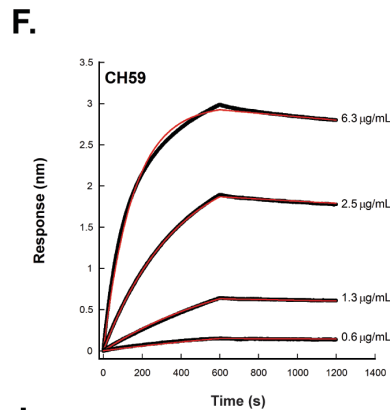
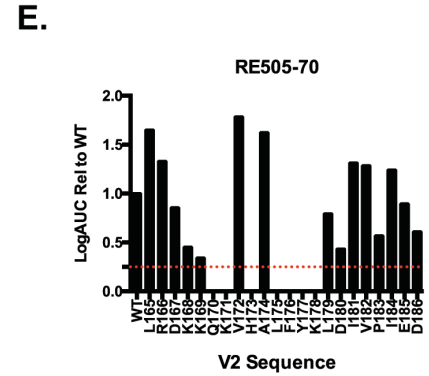
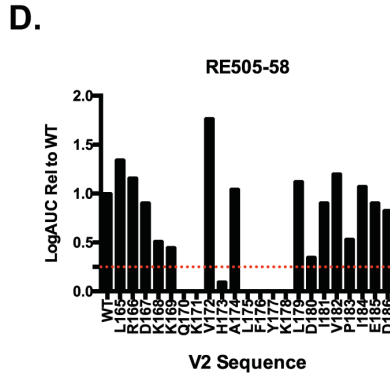
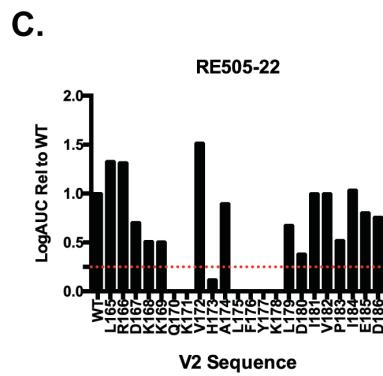
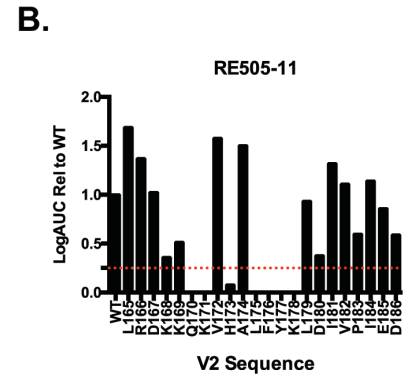
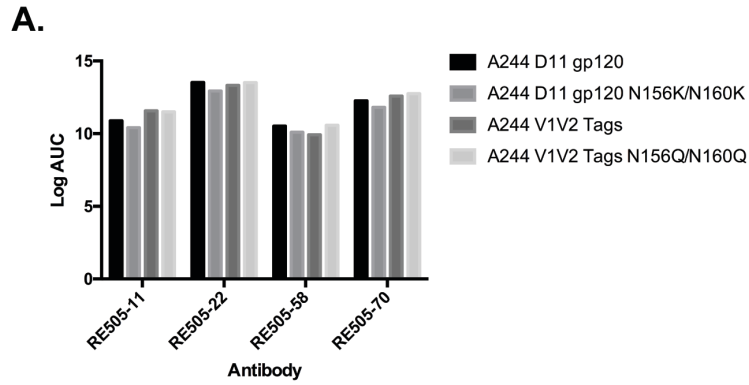
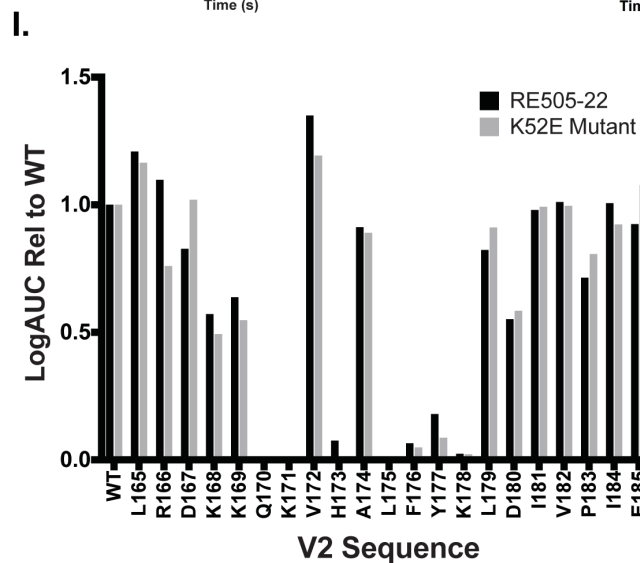


## **SUPPLEMENTARY MATERIALS**



**H.**

Antibody	Assoc. Rate, $k_a$ ( $\times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ )	Dissoc. Rate, $k_d$ ( $\times 10^{-4} \text{ s}^{-1}$ )	Dissoc. Const., $K_D$ (nM)
RE505-22	4.92	5.11	10.39
CH59	5.14	0.73	1.43



### **Figure S1. Binding and epitope mapping of antibodies**

(A) Binding to the V2 epitope by the chimeric humanized mouse antibodies was not dependent on V2 glycans. Binding was measured by ELISA and values reported as optical density (OD) at 450nm or the natural log of the area under the ELISA binding curve (LogAUC). (B-E) Epitopes of the chimeric humanized mouse antibodies defined by alanine scanning mutagenesis at each amino acid position in a V2 peptide (LRDKKQKVHALFYKLDIVPIED) for B) RE505-11 C) RE505-22 D) RE505-58 and E) RE505-70. Binding plotted as log of the area under the binding curve (LogAUC) of the alanine mutated V2 peptide relative to the wildtype V2 peptide LogAUC. (F-H) Bio-Layer Interferometry (BLI) sensorgrams of V2 peptide binding by F) CH59 Fab and G) RE505-22 Fab at multiple antibody concentrations. Sensorgrams are shown as black lines with the corresponding fitted curve shown as red lines. (H) The measured binding kinetics including association ( $k_a$ ) and dissociation ( $k_d$ ) rates and dissociation constants ( $K_d$ ) demonstrated a 7-fold reduction in binding affinity for RE505-22 relative to CH59 primarily driven by an increased dissociation rate. BLI measurements are representative of two independent experiments. (I) Epitope mapping of RE505-22 and the K52E mutant which introduces the CDR L2 ED motif. Epitopes were mapped by alanine scanning mutagenesis at each amino acid position in a V2 peptide (LRDKKQKVHALFYKLDIVPIED). Binding plotted as log of the area under the binding curve (LogAUC) of the alanine mutated V2 peptide relative to the wildtype V2 peptide LogAUC.

**Table S1. Epitope mapping by peptide microarray**

Antibody	Epitope	Peptide Sequence <sup>a</sup>	Binding Intensity (MFI)
RE504-46	C1/V1	THACVPTDPNPQEID	65,500
RE504-60	C1	DIISLWDQSLKPCVK	614
RE504-97	V3	RAFYTTKNIKGTIRQ	65,507
RE504-117	C1	CVPTDPNPQEIDLEN	57,107
RE504-125	gp120 conformational	N/A	negative
RE505-23	gp120 conformational	N/A	negative
RE505-27	gp120 conformational	N/A	negative
RE505-33	gp120 conformational	N/A	negative
RE505-11	V2 <sup>b</sup>	TEL RDKKQKVHALFY	101
RE505-58	V2 <sup>b</sup>	RDKKQKVHALFYKLD	42
RE505-70	V2 <sup>b</sup>	TEL RDKKQKVHALFY	35
RE505-22	V2 <sup>b</sup>	TEL RDKKQKVHALFY	1,856

a. Sequence of peptide with highest mean fluorescence intensity (MFI) in gp120 peptide microarray

b. V2 epitope was confirmed with ELISA (see Fig 2).

**Table S2. Antibody Sequences**

Heavy Chain Sequences					
Name	FW1	CDR1	FW2	CDR2	FW3
IGHV3-9	EVQLVESGGGLVQPGRSLRLSCAAS	GFTFDDYAM	HWVRQAPGKGLEWVSG	ISWNSGSI	GYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYC
CH59	EVQLVESGGGLVQPGRSLRLSCAAS	GFTFDD <b>G</b> AM	HWVRQAPGKGLEWVSG	ISWNS <b>N</b> II	<b>A</b> YADSVKGRFTISRDNAKNSLYL <b>E</b> MNSLR <b>V</b> EDTALYYC
RE505-11	EVQLVESGG <b>G</b> AMVQ <b>P</b> GR <b>A</b> LRLS <b>C</b> AAS	GFTFDDYAM	HWVRQ <b>P</b> PGKGLEWVSG	ISWNS <b>S</b> NI	GYADSVKGRFTISRDN <b>A</b> RNS <b>V</b> HMQMN <b>S</b> LR <b>I</b> EDT <b>A</b> FYYC
RE505-22	EVQLVESGGGLVQPGR <b>A</b> LRLS <b>C</b> AAS	GFTFDDYAM	HWVRQAPGKGLEWVSG	ISWNSG <b>S</b> R	GYADSVKGRFTISRDN <b>A</b> K <b>S</b> QY <b>L</b> QMN <b>S</b> LR <b>V</b> EDT <b>A</b> FYYC
RE505-58	EVQLVESGGGLVQPGRSLRLSCAAS	GFTFDDYAM	HWVRQ <b>V</b> PGKGLEWVSG	ISWNSGSI	GYADSVKGRFTISRDNAKNS <b>V</b> YLQMN <b>S</b> LR <b>P</b> GDT <b>A</b> VYYC
RE505-70	EVQLVESGG <b>D</b> MVQ <b>P</b> GR <b>A</b> LRLS <b>C</b> AAS	GFTFDDY <b>V</b> I	HWVRQ <b>P</b> PGKGLEWVSG	ISWNS <b>S</b> NI	GYADSVKGRFTISRDN <b>A</b> RNS <b>V</b> YMQMN <b>S</b> LR <b>I</b> EDT <b>A</b> FYYC

Name	CDR3	FW4
IGHV3-9	AKD-----	-----
CH59	AKDSPRGELPLNY	WGQGTLVTVSS
RE505-11	<b>T</b> RMNGTFD----Y	WGQGTLVTVSS
RE505-22	<b>A</b> RMNGTFD----Y	WGQGTLVTVSL
RE505-58	<b>S</b> KVHG <b>V</b> FE----N	WGQGILVTVSS
RE505-70	<b>T</b> RMNGTFD----Y	WGQGTLVTVSS

Light Chain Sequences					
Name	FW1	CDR1	FW2	CDR2	FW3
IGLV3-10	SYELTQPPSVSVSPGQARITCSGD	ALPKKY-	AYWYQQKSGQAPVLVIY	-- <b>EDS</b> --	KRPSGIPERFSGSSSGTMATLTI <b>S</b> G <b>A</b> Q <b>V</b> E <b>D</b> E <b>A</b> D <b>Y</b> I <b>C</b>
IGLV3-1*	QLVLTQSSSASFSLGASAKLTCTLS	SQHSTYT	IEWYQQPLKPPKYVME	LKKDGSH	STGDGIPDRFSGSSSGADRYLSISNIQPEDEAIYIC
RE505-11*	QLVLTQSSSASFSLGASAKLTCTLS	SQHSTYT	<b>V</b> EWY <b>Q</b> R <b>P</b> L <b>K</b> PP <b>K</b> F <b>V</b> ME	<b>L</b> T <b>K</b> DG <b>S</b> <b>Q</b>	<b>N</b> TGDGIPDRFSGSSSGADRY <b>L</b> TISNIQPEDEAIYIC
RE505-22*	QLVLTQSSSASFSLGASAKLTCTLS	SQHSTYT	IEWYQQPLKPP <b>K</b> F <b>V</b> ME	<b>L</b> R <b>K</b> DG <b>S</b> H	<b>N</b> TGDGIPDRFSGSSSGADRYLSISNIQPEDEAIYIC
RE505-58*	QLVLTQSSSASF <b>F</b> LGASAKLTCTLS	SQHSTYT	IEWYQQPLKPP <b>K</b> F <b>V</b> ME	LKKDGSH	STGDGIPDRFSGSSSGADRYLSISNIQPEDE <b>V</b> YIC
RE505_70*	QLVLTQSSSASFSLGASAKLTCTLS	SQHSTYT	<b>V</b> EWY <b>Q</b> R <b>P</b> L <b>K</b> PP <b>K</b> F <b>V</b> ME	<b>L</b> T <b>K</b> DG <b>S</b> <b>Q</b>	STGDGIPDRFSGSSSGADRY <b>L</b> TISNIQPEDEAIYIC

Name	CDR3	FW4
IGLV3-10	YSTDSSGNH----	-----
IGLV3-1*	GVD <b>T</b> IK <b>E</b> Q <b>F</b> V--	-----
RE505-11*	GVD <b>T</b> IK <b>G</b> Q <b>F</b> VYV	FGGGTKVTVL
RE505-22*	GVD <b>T</b> IK <b>E</b> Q <b>F</b> VYV	FGGGTKVTVL
RE505-58*	GVD <b>T</b> <b>V</b> <b>E</b> <b>E</b> Q <b>F</b> VYV	FGGGTKVTVL
RE505-70*	GVD <b>T</b> IK <b>G</b> Q <b>F</b> VYV	FGGGTKVTVL

\*Denotes usage of endogenous mouse lambda gene segments

Framework (FW) and CDR regions delineated using IMGT definition.

Mutations from germline V gene segment are emphasized in bold. ED motif is highlighted in gray box.

**Table S3. Neutralization Data**

Antibody	IC50 ( $\mu\text{g/ml}$ ) in TZM-bl Cells					
	MLV-SVA	Ce1086_B2	CM244.ec1	TH023.6	MW965.26	TV1.21
RE504-46	>50	>50	>50	>50	>50	>50
RE504-60	>50	>50	>50	>50	>50	>50
RE504-97	>50	>50	>50	0.16	>50	>50
RE504-117	>50	>50	>50	>50	>50	>50
RE504-125	>50	>50	>50	>50	>50	>50
RE505-23	>50	>50	>50	29.75	>50	>50
RE505-27	>50	>50	>50	0.17	>50	>50
RE505-33	>50	>50	>50	3.40	>50	>50
RE505-11	>50	>50	>50	>50	>50	>50
RE505-58	>50	>50	>50	>50	>50	>50
RE505-70	>50	>50	>50	>50	>50	>50
RE505-22	>50	>50	>50	46.05*	>50	>50
RE505-22_K52E^	>50	>50	>50	>50	>50	>50

\* IC50 reported as median value of two independent experiments

^ Mutant form of RE505-22 in which ED motif is introduced