

Supplemental Material

Eliminating Antibody Polyreactivity through Addition of *N*-linked Glycosylation

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Table of Contents

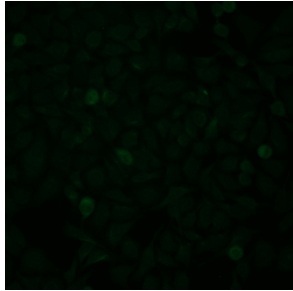
Supplementary Figures

- Figure S1. HEp-2 cell staining results for VRC07-I30Q-S58N, VRC07, and Synagis (Palivizumab, an anti-RSV antibody) at antibody concentration of 25µg/ml.
- Figure S2. UPLC profile of Fab glycans for five designed VRC07 glycan constructs.
- Figure S3. UPLC profile of Fab glycans for the five designed VRC07 glycan constructs treated with different combinations of exoglycosidases.
- Figure S4. HEp-2 cell staining results at antibody concentration of 25µg/ml.
- Figure S5. Sera antibody concentration-time profile for each VRC07 variant in the mice PK study.
- Figure S6. ELISA binding curves for VRC07 variants towards RSC3 gp120 core.

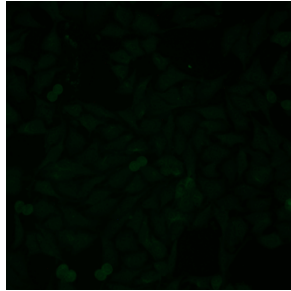
- Figure S7. Cardiophilin reactivity of VRC07 variants.
- Figure S8. Luminex AtheNA Multi-Lyte ANA test results for each VRC07 variant.
- Figure S9. HEp-2 cell staining results at antibody concentration of 50 μ g/ml.
- Figure S10. Differential scanning calorimetry results.
- Figure S11. Computational protocol for designing *N*-linked glycan on the protein surface.

Supplementary Table

- Table S1. Reactivity of VRC07 variants towards cardiophilins evaluated by an anti-cardiophilin ELISA assay.
- Table S2. Reactivity of VRC07 variants against nine different autoantigens.
- Table S3. Neutralization IC₅₀ (μ g/mL) of VRC07 variants against a panel of twenty different HIV-1 viruses.
- Table S4. AUC (Day*(μ g/mL)) of VRC07 variants in the mice pharmacokinetics study.
- Table S5. Construct names, mutations, and C β distances between polyreactive mutations and engineered glycans.
- Table S6. Reactivity of control VRC07 glycan variants toward cardiophilin evaluated by an anti-cardiophilin ELISA assay.
- Table S7. Reactivity of control VRC07 glycan variants toward nine different autoantigens.
- Table S8. Transitional temperatures of VRC07 variants determined by DSC.



VRC07

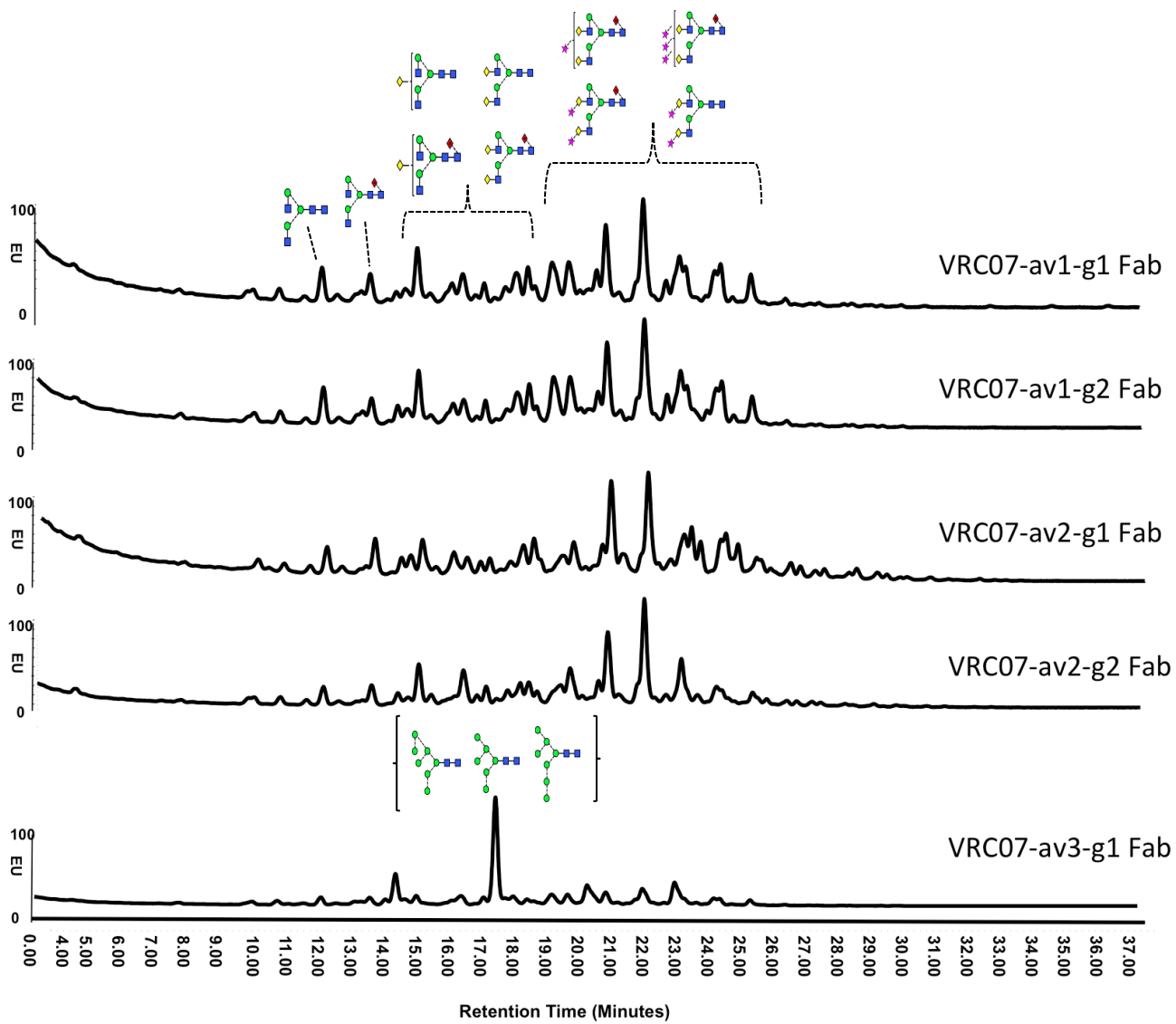


VRC07-I30Q_{HC}-S58N_{HC}



Synagis

Figure S1. HEp-2 cell staining results for VRC07-I30Q-S58N, VRC07, and Synagis (Palivizumab, an anti-RSV antibody) at antibody concentration of 25µg/ml.



■ N Acetyl Glucosamine
 ◆ Fucose
 ● Mannose
 ◆ Galactose
 ★ N-Acetyl Neuraminic acid

Figure S2. UPLC profile of Fab glycans for five designed VRC07 glycan constructs.

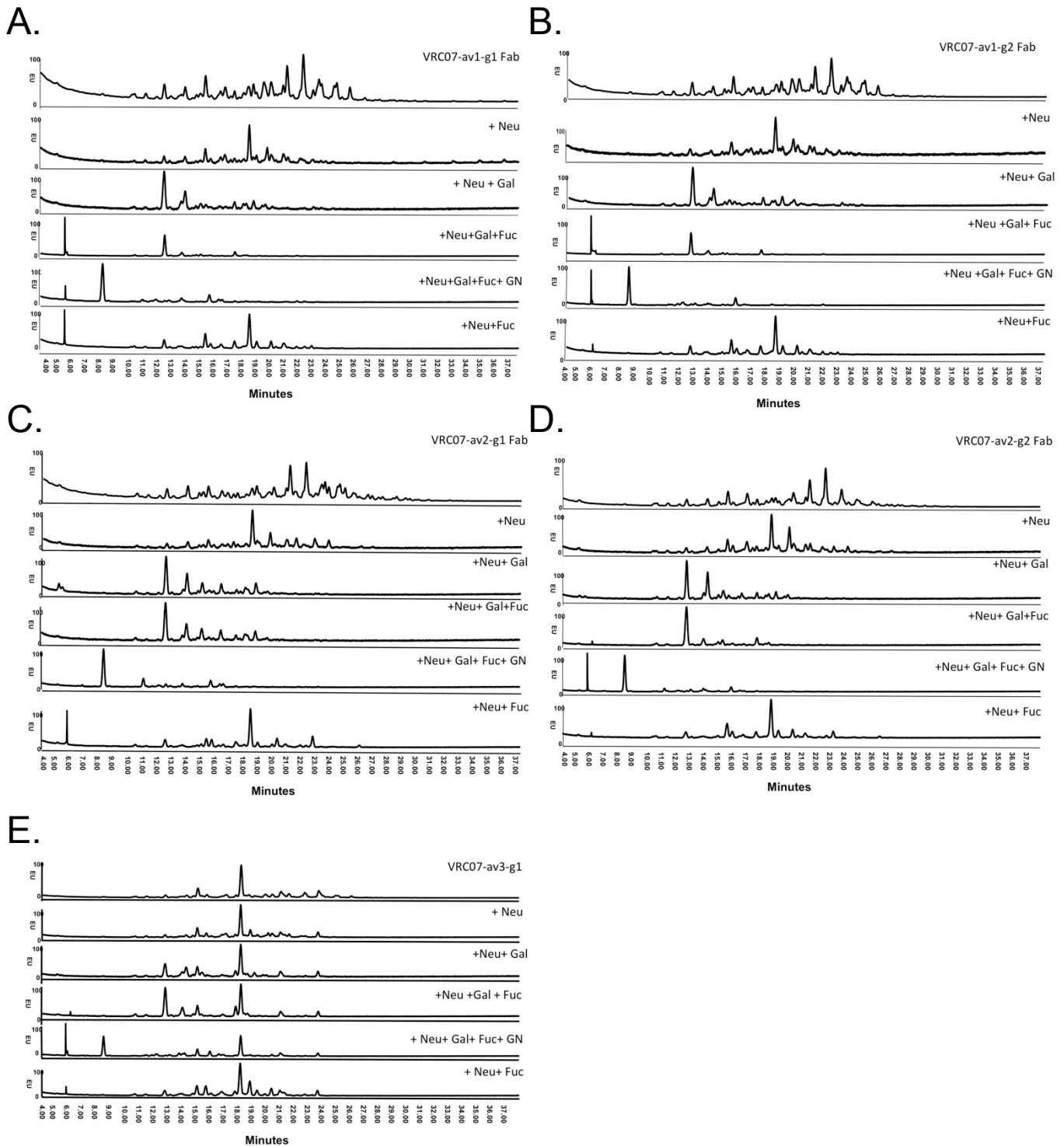
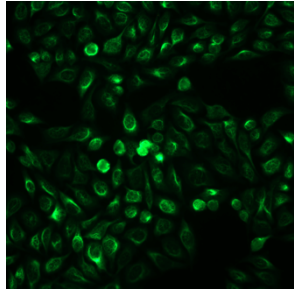


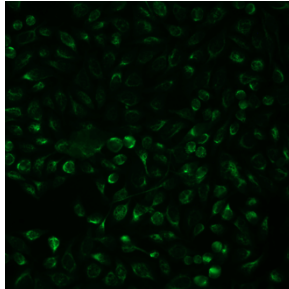
Figure S3. UPLC profile of Fab glycans for the five designed VRC07 glycan constructs treated with different combinations of exoglycosidases. (A) VRC07-av1-g1 (B) VRC07-av1-g2 (C) VRC07-av2-g1 (D) VRC07-av2-g2 (E) VRC07-av3-g3. Abbreviations: Neu: N-acetyl neuraminidase; Gal: Beta galactosidase; Fuc: Alpha fucosidase; GN: N-acetyl glucanosaminidase.

Polyreactive VRC07 variants

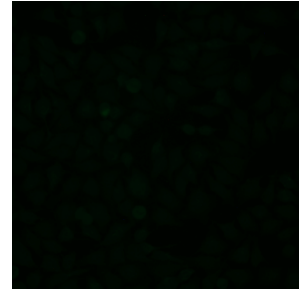
Single glycan added to polyreactive VRC07 variants



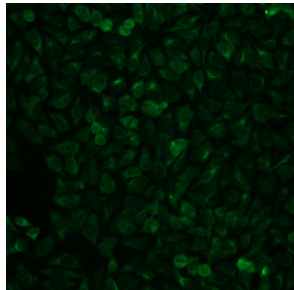
VRC07-av1



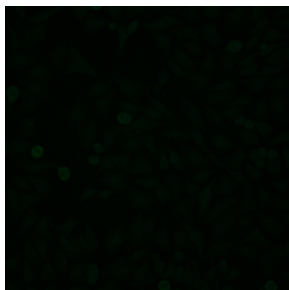
VRC07-av1-g1



VRC07-av1-g2



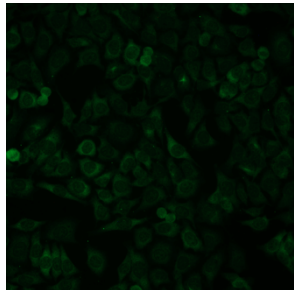
VRC07-av2



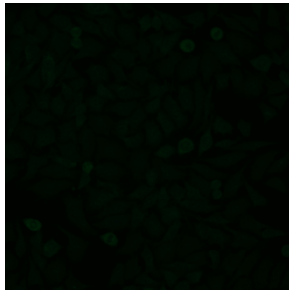
VRC07-av2-g1



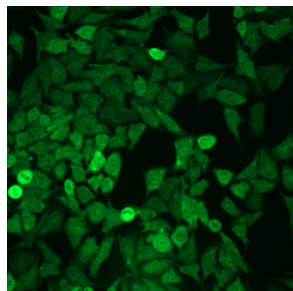
VRC07-av2-g2



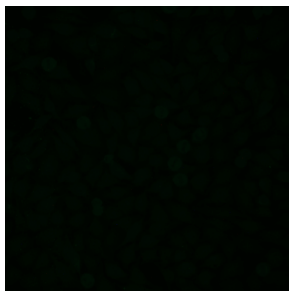
VRC07-av3



VRC07-av3-g1



2F5



Synagis

Figure S4. HEp-2 cell staining results at antibody concentration of 25µg/ml. Synagis, an anti-RSV monoclonal antibody, was used as the negative control. 2F5, a polyreactive HIV-1 antibody, was used as the positive control.

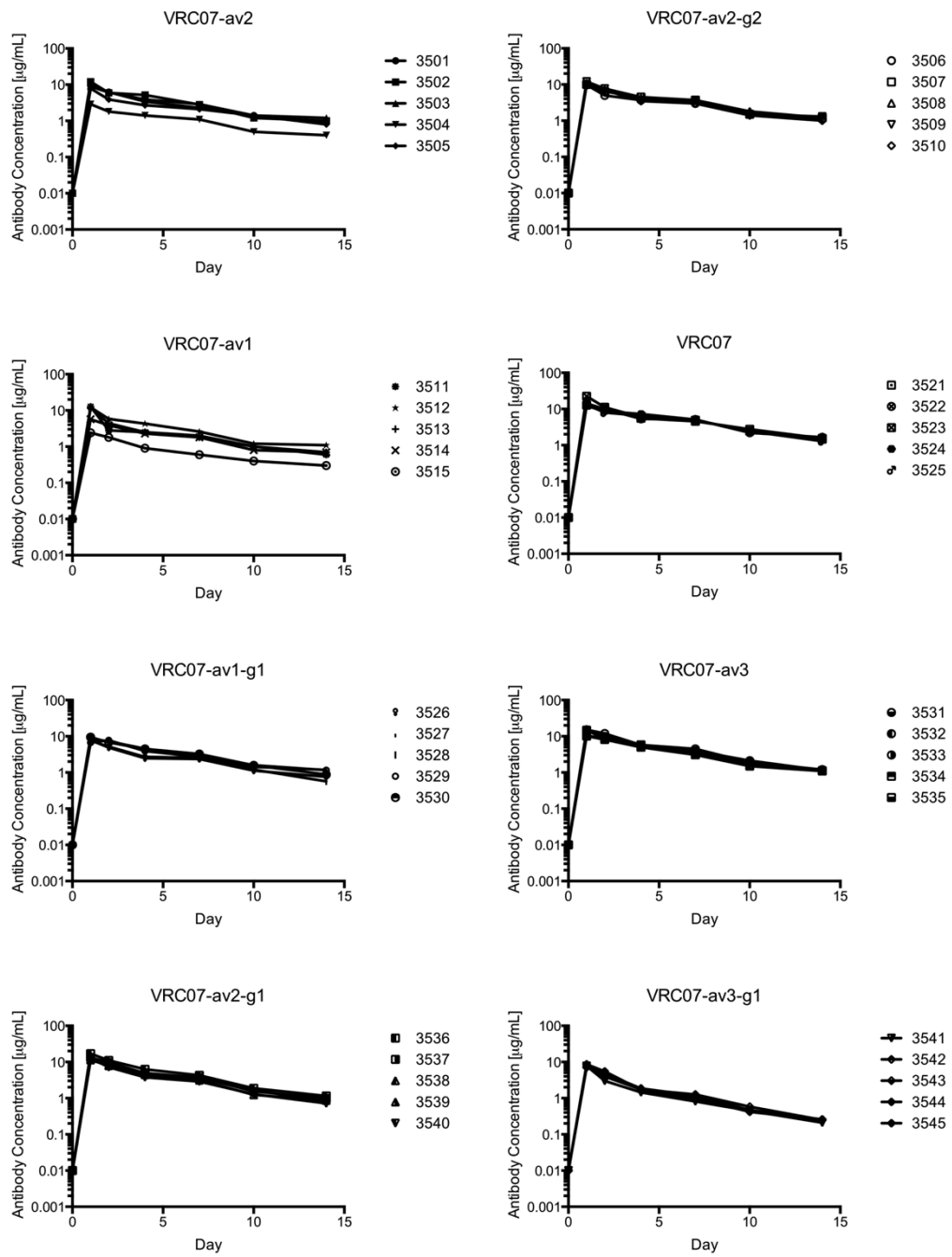


Figure S5. Sera antibody concentration-time profile for each VRC07 variant in the mice PK study.

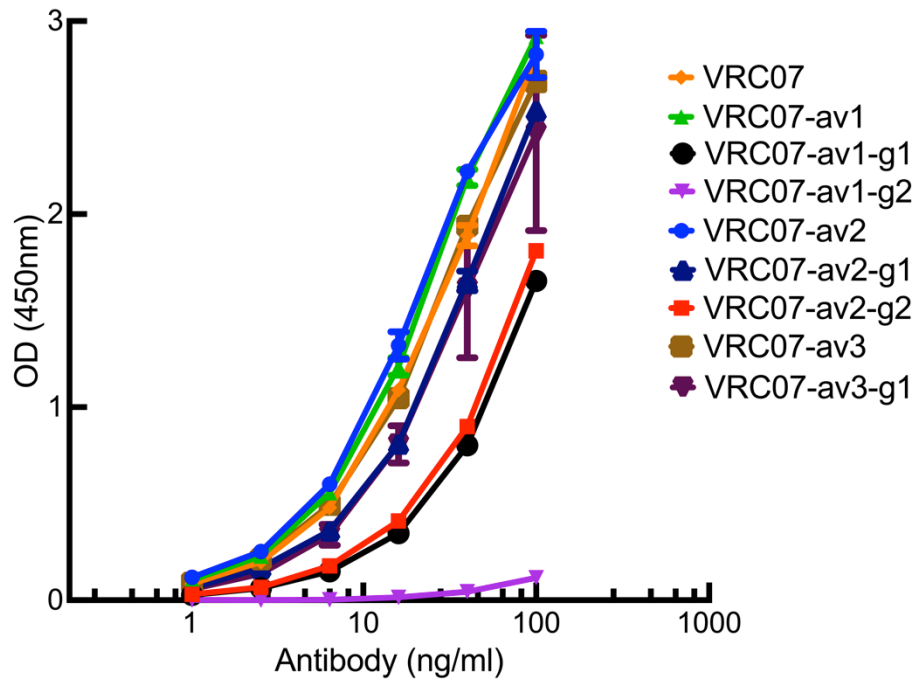


Figure S6. ELISA binding curves for VRC07 variants towards RSC3 gp120 core.

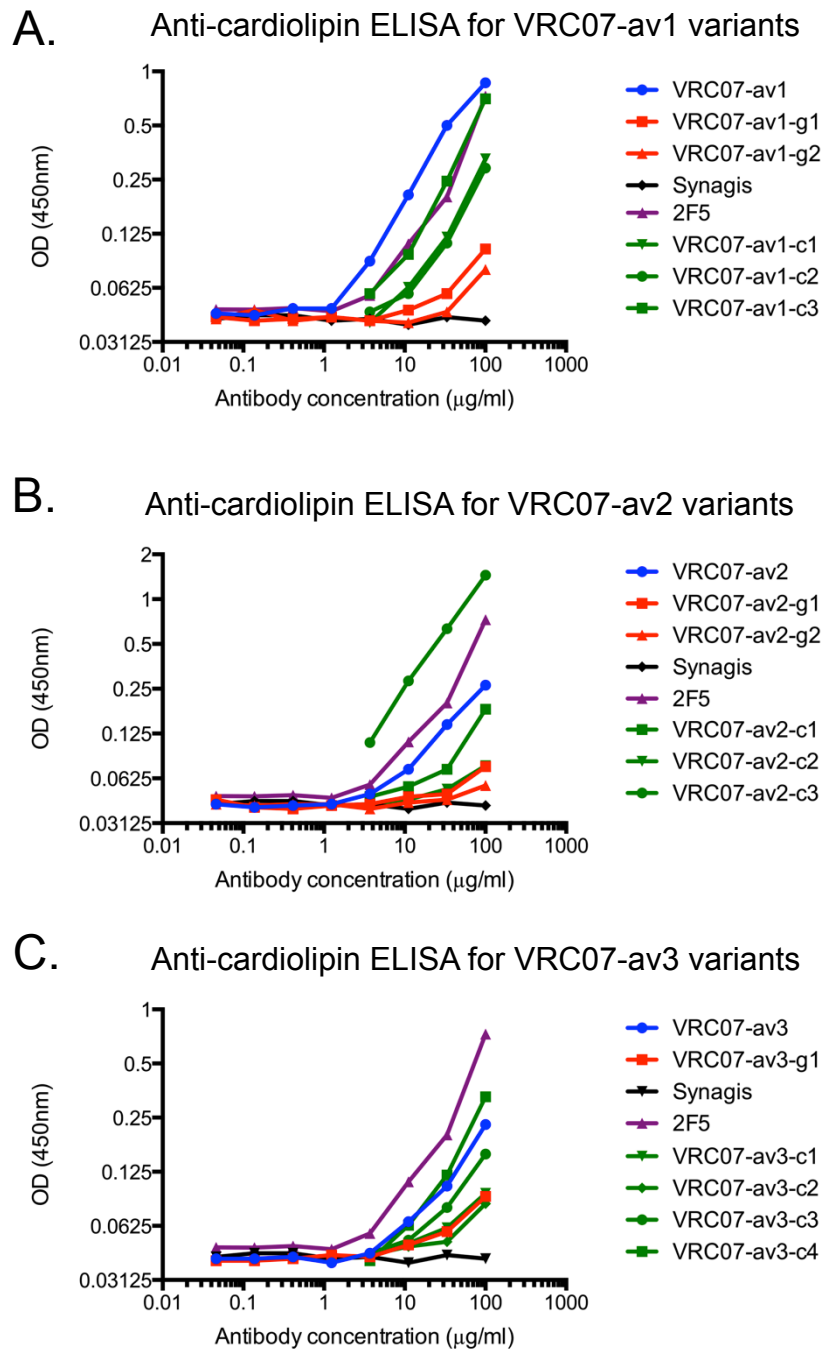


Figure S7. Cardiolipin reactivity of VRC07 variants. Shown is the cardiolipin ELISA results at different antibody concentrations for (A) VRC07-av1 and its glycan mutants (B) VRC07-av2 and its glycan mutants, and (C) VRC07-av3 and its glycan mutant. Synagis (Palivizumab), an anti-RSV monoclonal antibody, was used as a negative control. 2F5, a polyreactive HIV-1 antibody, was used as the positive control.

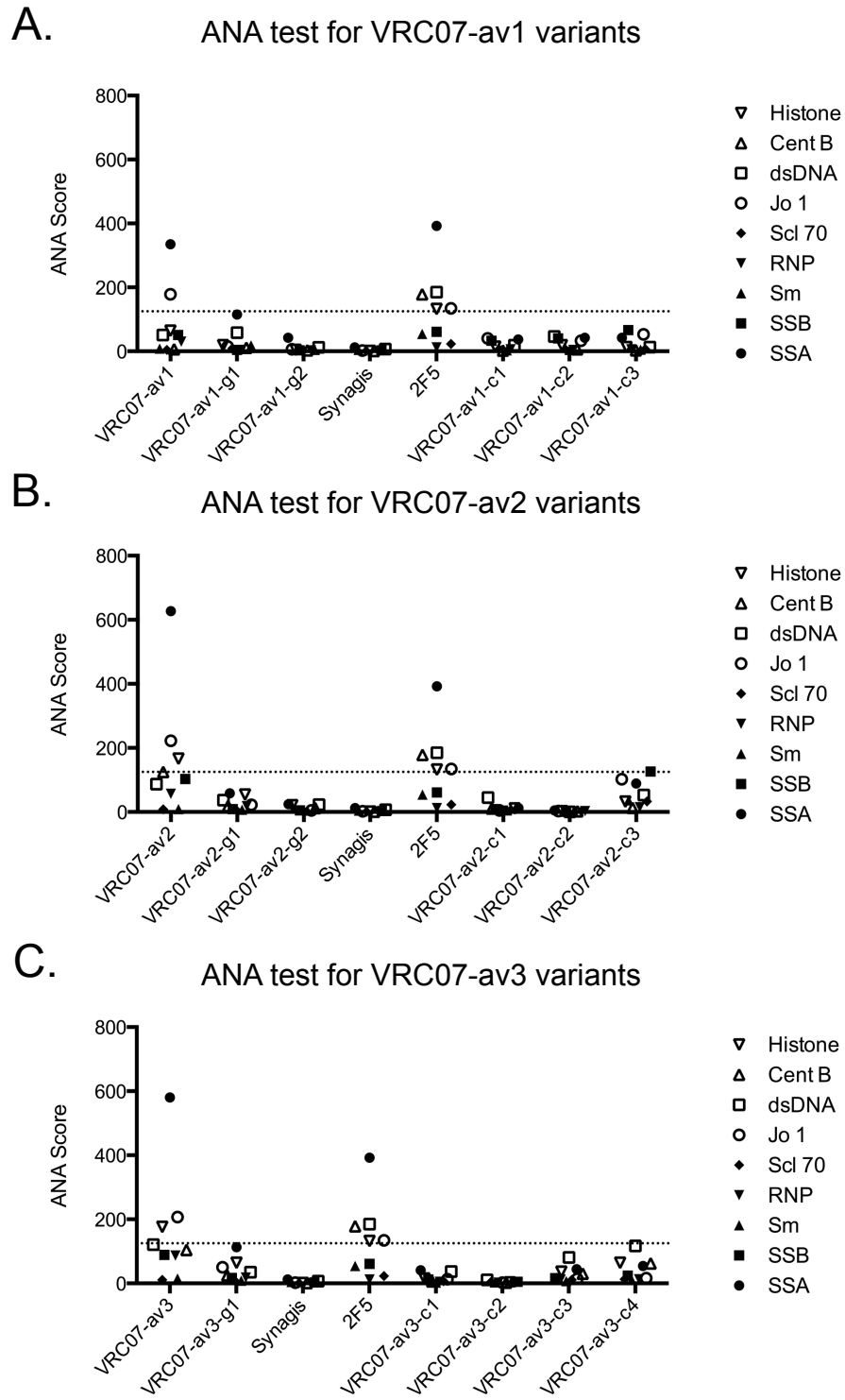


Figure S8. Luminex Athena Multi-Lyte ANA test results for each VRC07 variant. Shown is the Athena ANA results for (A) VRC07-av1 and its glycan mutants (B) VRC07-av2 and its glycan mutants, and (C) VRC07-av3 and its glycan mutant. The horizontal dash line indicates an ANA score cutoff of 125. Synagis (Palivizumab), an anti-RSV monoclonal antibody, was used as a negative control. 2F5, a polyreactive HIV-1 antibody, was used as the positive control.

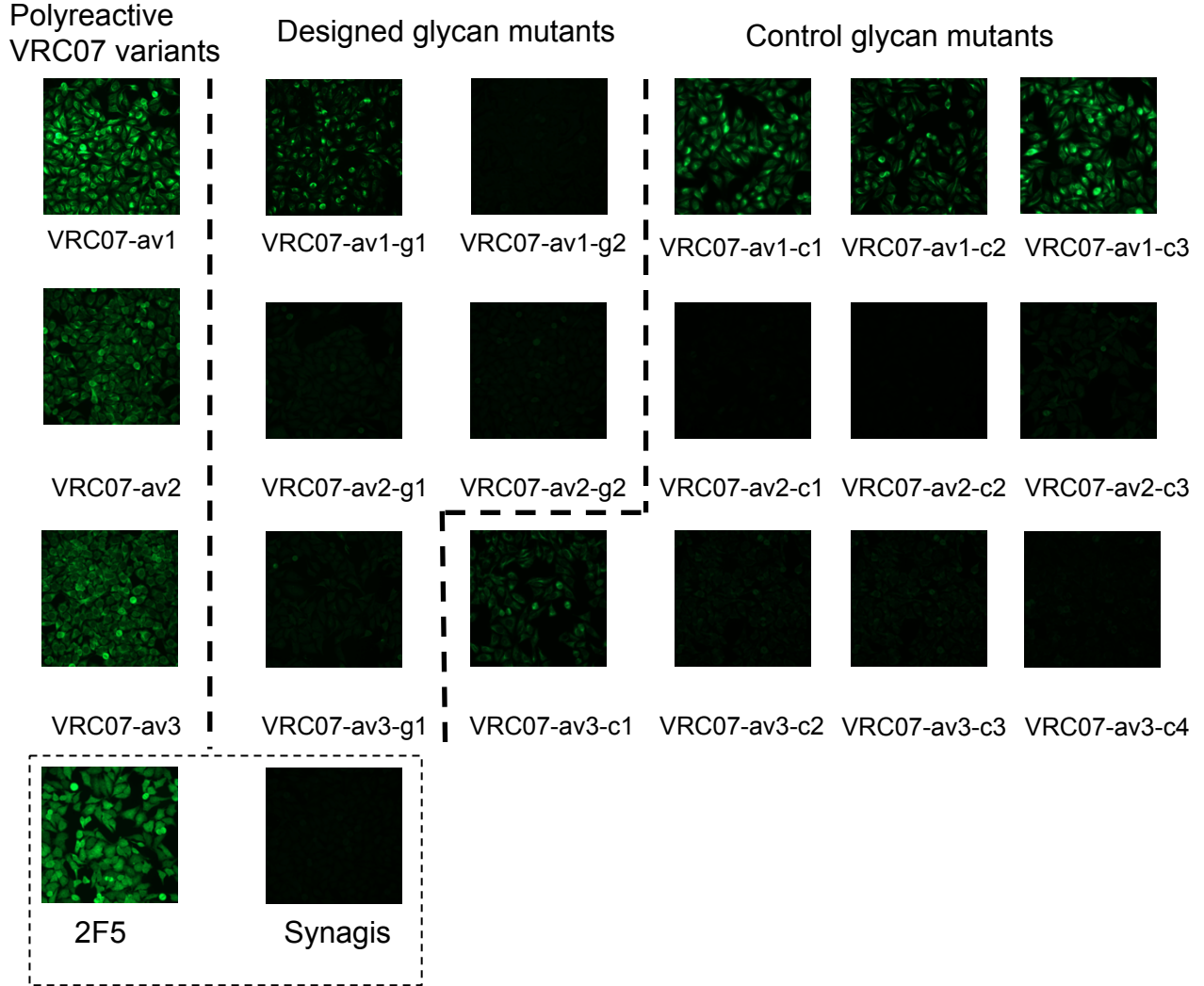


Figure S9. HEp-2 cell staining results at antibody concentration of 50µg/ml. Synagis, an anti-RSV monoclonal antibody, was used as the negative control. 2F5, a polyreactive HIV-1 antibody, was used as the positive control.

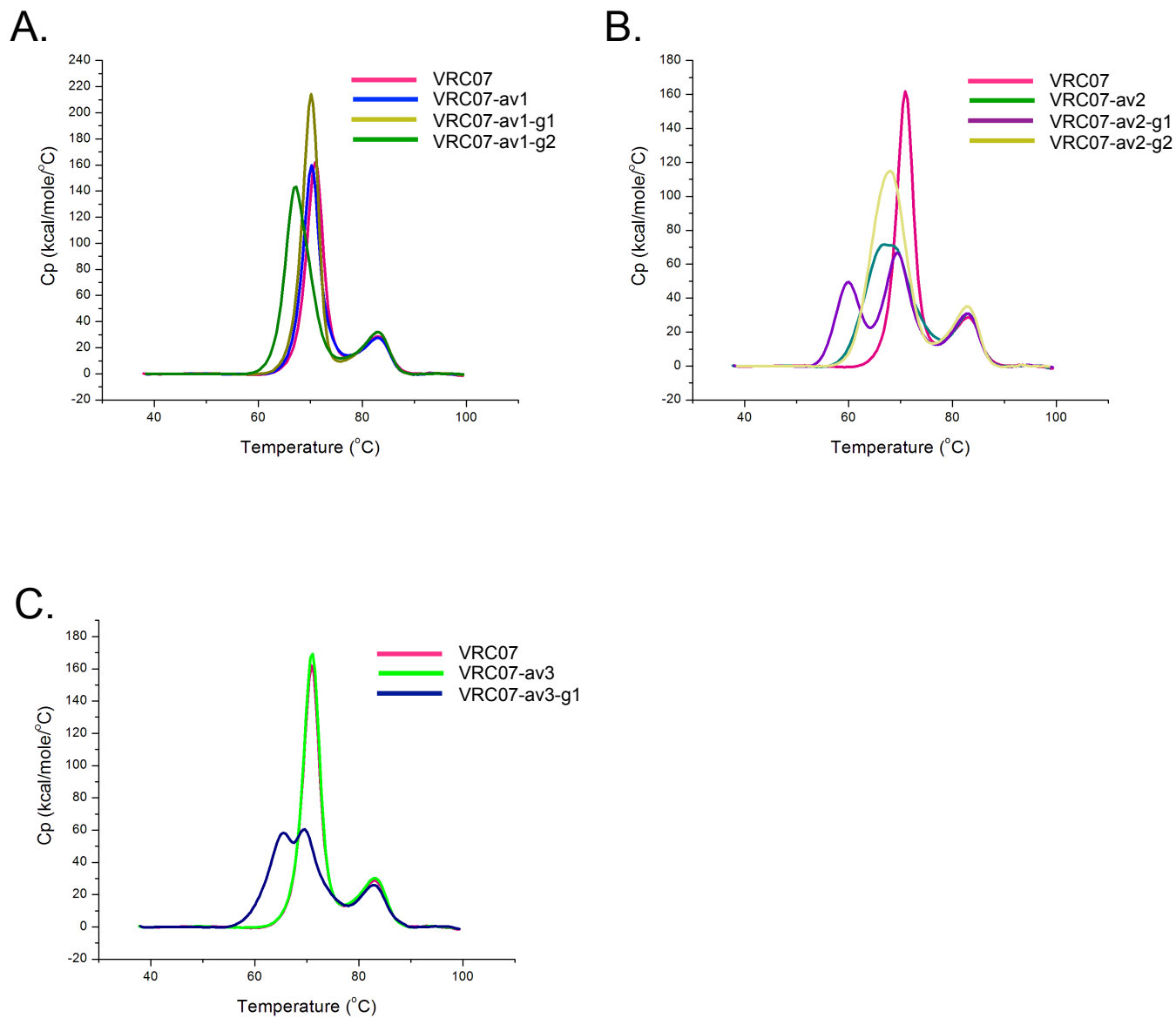


Figure S10. Differential scanning calorimetry results. (A) VRC07, VRC07-av1 and its glycan mutants, (B) VRC07, VRC07-av2 and its glycan mutants, and (C) VRC07, VRC07-av3 and its glycan mutants.

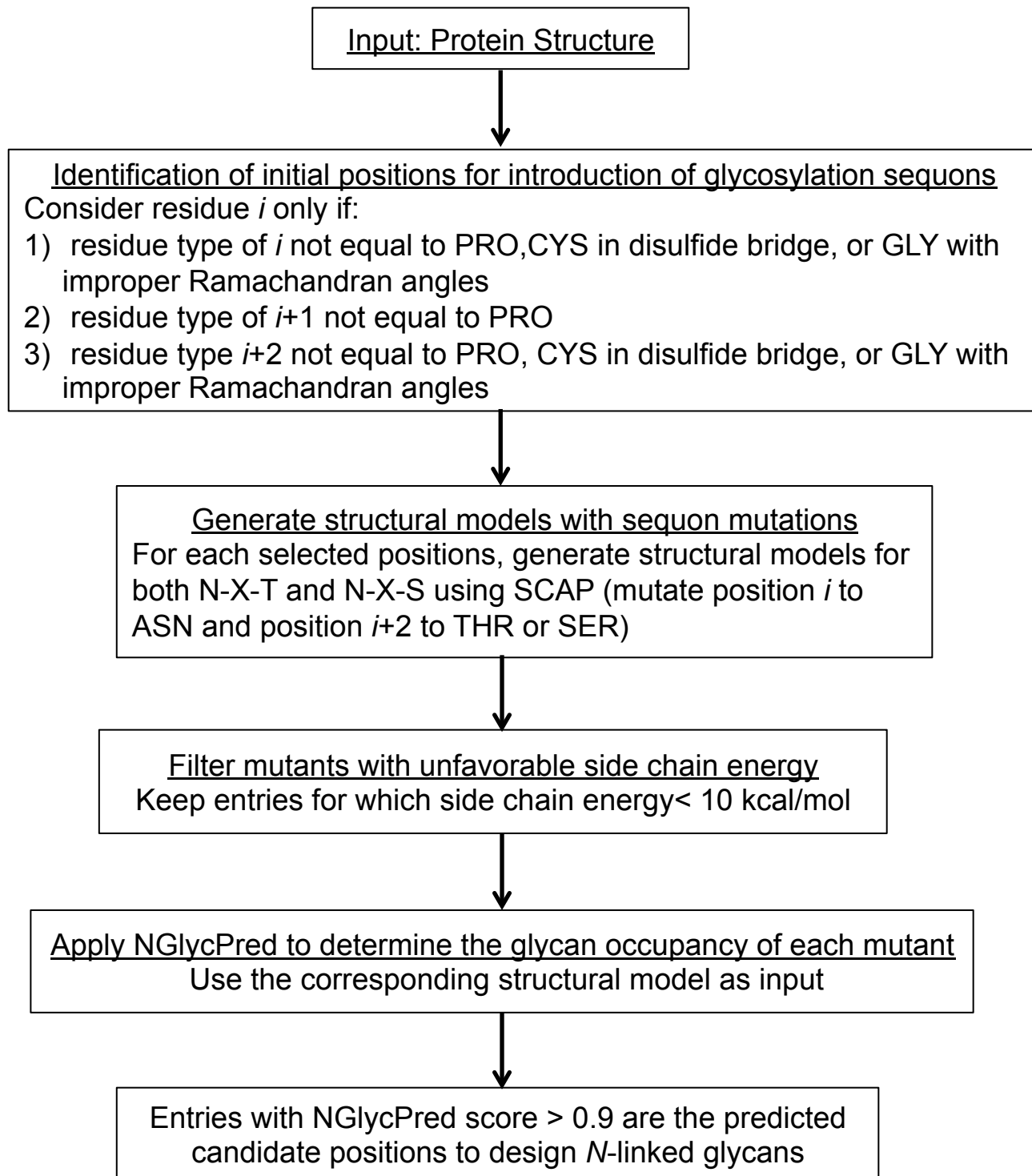


Figure S11. Computational protocol for designing *N*-linked glycan on the protein surface.

Table S1. Reactivity of VRC07 variants towards cardiolipins evaluated by an anti-cardiolipin ELISA assay.

	Antibody Concentration ($\mu\text{g/ml}$)							
	100	33.333	11.111	3.704	1.235	0.412	0.137	0.046
VRC07-av1	0.865	0.500	0.206	0.088	0.048	0.048	0.044	0.045
VRC07-av1-g1	0.103	0.058	0.047	0.041	0.043	0.042	0.041	0.043
VRC07-av1-g2	0.079	0.046	0.040	0.041	0.043	0.041	0.047	0.042
VRC07-av2	0.264	0.144	0.072	0.049	0.042	0.041	0.040	0.042
VRC07-av2-g1	0.075	0.049	0.047	0.042	0.041	0.039	0.040	0.045
VRC07-av2-g2	0.056	0.045	0.043	0.039	0.042	0.042	0.041	0.042
VRC07-av3	0.229	0.104	0.066	0.044	0.039	0.042	0.041	0.041
VRC07-av3-g1	0.091	0.058	0.049	0.042	0.043	0.041	0.040	0.040
VRC07	0.117	0.063	0.050	0.046	0.042	0.044	0.039	0.040
VRC07-I30Q-S58N	0.069	0.048	0.044	0.041	0.041	0.041	0.041	0.041
2F5	0.729	0.201	0.110	0.057	0.046	0.048	0.047	0.048
Synagis	0.041	0.043	0.039	0.042	0.041	0.044	0.044	0.042

An anti-RSV monoclonal antibody, Synagis (Palivizumab), was used as a negative control. 2F5, a polyreactive HIV-1 antibody, was used as a positive control. VRC07 and VRC07-I30Q-S58N were included in the assay for comparison. OD (450 nm) Values of equal to or greater than three fold of the background ELISA signal (0.0421) are highlighted red.

Table S2. Reactivity of VRC07 variants against nine different autoantigens.

Abs	µg/ml	SSA	SSB	Sm	RNP	Scl 70	Jo 1	dsDNA	Cent B	Histone
Neg Control		-	-	-	-	-	-	-	-	-
Pos Control 1		860	520						244	
Pos Control 2							259	1514		766
Pos Control 3				826	285	363				
VRC07-av1	50	335	51	8	30	4	178	51	6	64
	25	211	33	5	20	2	124	19	3	42
	12.5	138	22	4	13	2	88	15	3	32
	6.25	107	15	5	11	2	69	15	2	29
VRC07-av1-g1	50	115	4	18	4	9	15	58	11	19
	25	75	5	6	3	5	9	27	5	11
	12.5	54	5	5	2	4	5	15	3	7
	6.25	28	2	3	2	3	7	8	1	5
VRC07-av1-g2	50	42	3	4	3	2	6	12	3	4
	25	16	2	5	1	1	5	3	2	2
	12.5	16	3	3	2	2	3	5	0	2
	6.25	10	3	4	0	1	4	0	1	3
VRC07-av2	50	627	103	9	57	8	222	87	125	166
	25	491	82	6	50	5	155	74	111	161
	12.5	390	56	7	46	5	106	62	76	133
	6.25	297	37	4	41	4	70	53	54	114
VRC07-av2-g1	50	58	9	7	18	2	22	37	19	54
	25	33	6	5	10	2	11	21	8	29
	12.5	17	5	4	6	2	5	16	5	20
	6.25	18	4	2	5	2	6	14	4	11
VRC07-av2-g2	50	24	5	4	5	2	5	23	12	21
	25	18	3	5	2	1	6	10	7	12
	12.5	12	2	2	3	2	4	7	3	7
	6.25	11	5	3	2	2	2	3	3	6
VRC07-av3	50	580	89	15	87	11	207	121	104	176
	25	427	68	7	71	7	153	111	95	170
	12.5	336	52	4	61	6	110	92	73	146
	6.25	238	40	5	60	6	84	83	55	131
VRC07-av3-g1	50	113	17	8	18	3	50	35	25	64
	25	54	7	5	12	2	22	20	11	40
	12.5	25	6	4	9	2	12	17	7	26
	6.25	22	5	3	5	2	10	11	3	16
VRC07	247	27	7	54	5	85	88	48	98	247
	127	13	5	43	4	43	59	22	74	127
	62	9	6	33	4	20	41	12	51	62
	34	4	3	21	2	11	37	7	36	34
VRC07-I30Q-S58N	50	117	12	5	25	3	43	47	10	61
	25	61	8	4	20	3	19	37	6	47
	12.5	31	5	2	13	3	11	32	3	30
	6.25	25	4	3	8	2	5	14	2	18
Synagis	50	12	3	4	1	1	2	7	1	1
	25	10	2	2	1	1	2	0	0	1
	12.5	8	3	4	1	1	4	0	1	2
	6.25	15	1	6	0	0	3	0	1	1
2F5	50	392	61	54	12	23	134	185	178	132
	25	194	25	20	4	6	54	119	95	66
	12.5	111	15	12	2	3	33	75	54	40
	6.25	70	12	9	2	2	21	32	36	25

Reactivity of autoreactive VRC07 variants and glycan mutants with autoantigens was detected by the Luminex assay. An anti-RSV monoclonal antibody, Synagis (Palivizumab), was used as a negative control. 2F5, a polyreactive HIV-1 antibody, was used as a positive control. VRC07 and VRC07-I30Q-S58N were included in the assay for comparison. SSA, Sjogren's syndrome antigen A; SSB, Sjogren syndrome antigen B; Sm, Smith antigen; RNP, ribonucleoprotein; Scl 70, scleroderma 70; Jo1, antigen; CentrB, centromere B. Values of greater than 125 are highlighted red.

Table S3. Neutralization IC₅₀ (µg/mL) of VRC07 variants against a panel of twenty different HIV-1 viruses.

clade	virus	VRC07- av1	VRC07- av1-g1	VRC07- av1-g2	VRC07- av2	VRC07- av2-g1	VRC07- av2-g2	VRC07- av3	VRC07- av3-g1	VRC07
A	KER2008.12.SG3	0.256	>50	>50	0.188	0.656	7.170	0.446	2.270	0.585
A	Q23.17.SG3	0.064	3.48	37.4	0.07	0.072	0.2	0.073	0.208	0.074
A	UG037.8.SG3	0.051	2.83	36	0.054	0.068	0.112	0.075	0.153	0.045
AG	242-14.SG3	0.091	>50	>50	0.712	21.000	>50	1.590	20.100	2.840
B	7165.18.SG3	1.590	>50	>50	1.030	7.470	>50	3.720	16.000	4.340
B	AC10.29.SG3	0.529	16.8	>50	0.515	0.355	2.89	0.536	0.382	0.684
B	BG1168.01.SG3	0.044	>50	>50	0.087	1.580	>50	0.093	0.880	0.141
B	BL01.DG.SG3	>50	>50	>50	>50	>50	>50	>50	>50	>50
B	JRFLJB.SG3	0.013	0.454	>50	0.006	0.018	0.086	0.006	0.041	0.005
B	YU2.DG.SG3	0.010	3.210	>50	0.006	0.018	0.038	0.012	0.030	0.014
C	6631.V3.C10.SG3	0.282	>50	>50	2.920	>50	>50	9.480	>50	14.400
C	CAP210.E8.SG3	7.800	>50	>50	27.100	>50	>50	>50	>50	>50
C	DU151.02.SG3	0.149	14.2	>50	1.64	3.6	>50	1.65	3	4.37
C	DU172.17.SG3	0.055	27.800	>50	1.570	17.400	>50	0.264	1.250	0.338
C	DU422.01.SG3	>50	>50	>50	>50	>50	>50	>50	>50	>50
C	TV1.29.SG3	0.542	>50	>50	>50	>50	>50	>50	>50	>50
C	TZA125.17.SG3	0.493	>50	>50	>50	>50	>50	9.690	>50	22.000
C	ZM106.9.SG3	0.12	5.590	>50	0.146	0.111	1.590	0.167	0.126	0.119
C	ZM53.12.SG3	0.518	21	>50	0.385	0.266	1.8	0.258	0.324	0.405
D	57128.vrc15.SG3	0.267	>50	>50	0.441	1.970	>50	1.020	2.270	1.460
# of Viruses Neutralized		18	9	2	16	14	8	16	14	16
Median IC50		0.262	50.000	50.000	0.614	2.785	50.000	0.778	2.270	1.072
Geometric Mean		0.311	19.357	48.477	0.834	2.167	7.966	0.972	2.431	1.192

* A value of 50 was used to calculate the median and geometric for entries with IC₅₀ greater than 50.

Table S4. AUC (Day*($\mu\text{g}/\text{mL}$)) of VRC07 variants in the mice pharmacokinetics study.

Antibody	VRC07	VRC07- av1	VRC07- av1-g1	VRC07- av2	VRC07- av2-g1	VRC07- av2-g2	VRC07- av3	VRC07- av3-g1
	75.95	61.46	40.22	48.13	83.96	53.26	79.54	30.36
	79.01	58.84	51.33	60.79	67.98	57.57	62.72	32.02
	103.07	50.76	40.49	50.20	62.35	59.86	72.98	32.50
	78.24	31.12	47.68	17.24	58.10	62.29	57.88	28.74
	78.03	12.76	54.48	41.11	58.54	54.02	74.12	32.79
Average	82.86	42.99	46.84	43.49	66.19	57.40	69.45	31.28
SE	5.08	9.24	2.86	7.28	4.78	1.71	3.97	0.76

* SE: standard error

Table S5. Construct names, mutations, and C_β distances between polyreactive mutations and engineered glycans.

Construct Name	Heavy Chain Mutations	Light Chain Mutations	C _β Distances (Å)
VRC07-av1-c1	G54W-I30Q-S58N	R24N	37.7 ^a
VRC07-av1-c2	G54W-I30Q-S58N	S26N-Y28T	33.5 ^a
VRC07-av1-c3	G54W-I30Q-S58N	R45N-V47T	30.8 ^a
VRC07-av2-c1	M73N-S75T	AAAA N terminus	35.6 ^b
VRC07-av2-c2	R71N-M73T	AAAA N terminus	29.8 ^b
VRC07-av2-c3	-	AAAA N terminus-R45N-V47T	23.3 ^b
VRC07-av3-c1	M73N-S75T	A43R	25.3 ^c
VRC07-av3-c2	R71N-M73T	A43R	20.7 ^c
VRC07-av3-c3	-	A43R- R24N	26.2 ^c
VRC07-av3-c4	-	A43R-S26N-Y28T	25.3 ^c

^a C_β distance between HC W54 and engineered glycan. ^b C_β distance between LC A3 and engineered glycan. ^c C_β distance between LC A43 and engineered glycan.

Table S6. Reactivity of control VRC07 glycan variants toward cardiolipin evaluated by an anti-cardiolipin ELISA assay.

	Antibody Concentration ($\mu\text{g/ml}$)			
	100	33.333	11.111	3.704
VRC07-av1-c1	0.327	0.120	0.063	0.040
VRC07-av1-c2	0.290	0.111	0.058	0.046
VRC07-av1-c3	0.704	0.245	0.096	0.058
VRC07-av2-c1	0.182	0.072	0.055	0.047
VRC07-av2-c2	0.076	0.053	0.045	0.041
VRC07-av2-c3	1.451	0.634	0.282	0.109
VRC07-av3-c1	0.095	0.061	0.050	0.042
VRC07-av3-c2	0.083	0.051	0.048	0.043
VRC07-av3-c3	0.157	0.079	0.052	0.044
VRC07-av3-c4	0.327	0.120	0.063	0.040
Synagis	0.042	0.045	NA	NA

An anti-RSV monoclonal antibody, Synagis (Palivizumab), was used as a negative control. OD (450 nm) Values of equal to or greater than three fold of the background ELISA signal (0.05) are highlighted red. NA: not available.

Table S7. Reactivity of control VRC07 glycan variants toward nine different autoantigens.

Abs	µg/ml	SSA	SSB	Sm	RNP	Scl 70	Jo 1	dsDNA	Cent B	Histone
Neg Control		-	-	-	-	-	-	-	-	-
Pos Control 1									288	
Pos Control 2		901	970				347	565		292
Pos Control 3				467	479	355				
VRC07-av1-c1	50	37	33	3	6	6	40	19	2	14
	25	23	21	2	4	3	24	15	1	11
	12.5	15	13	2	3	4	12	10	2	7
	6.25	13	8	2	3	2	9	12	1	7
VRC07-av1-c2	50	42	40	4	4	5	33	46	6	19
	25	19	18	3	3	4	14	28	3	10
	12.5	12	11	3	2	2	9	19	3	7
	6.25	8	7	2	2	1	6	13	1	5
VRC07-av1-c3	50	42	66	5	6	7	53	13	3	13
	25	26	39	3	3	4	32	8	2	9
	12.5	22	29	2	3	2	23	8	2	7
	6.25	15	23	2	3	3	19	6	1	6
VRC07-av2-c1	50	12	9	5	4	7	5	45	11	11
	25	5	5	3	3	5	4	20	5	5
	12.5	4	5	2	2	2	3	13	3	4
	6.25	2	3	1	3	1	2	7	2	2
VRC07-av2-c2	50	5	2	2	3	3	3	0	2	2
	25	2	5	3	1	2	3	2	1	2
	12.5	3	6	2	1	2	2	2	1	1
	6.25	2	1	3	2	1	2	2	1	2
VRC07-av2-c3	50	89	126	36	15	33	102	53	13	32
	25	79	100	14	8	15	86	40	7	25
	12.5	69	86	10	8	11	72	34	5	22
	6.25	60	72	8	7	8	65	36	4	20
VRC07-av3-c1	50	41	12	2	5	7	13	37	6	15
	25	23	11	2	3	4	8	25	4	9
	12.5	12	3	3	3	3	2	18	2	6
	6.25	9	4	2	3	4	5	19	2	4
VRC07-av3-c2	50	3	5	3	2	4	2	11	2	3
	25	2	4	3	2	1	1	5	2	2
	12.5	1	3	3	3	1	2	7	1	2
	6.25	4	3	2	2	1	1	3	2	2
VRC07-av3-c3	247	44	16	7	12	11	14	81	30	36
	127	24	9	4	8	8	6	49	15	23
	62	18	7	3	7	6	5	47	12	19
	34	13	7	4	6	5	5	41	8	14
VRC07-av3-c4	50	54	24	10	13	13	16	117	62	64
	25	37	16	4	10	7	9	100	40	48
	12.5	19	8	3	6	5	6	59	25	28
	6.25	10	5	1	6	4	3	50	14	19

Reactivity of the control VRC07 variants with autoantigens was detected by the Luminex assay. SSA, Sjogren's syndrome antigen A; SSB, Sjogren syndrome antigen B; Sm, Smith antigen; RNP, ribonucleoprotein; Scl 70, scleroderma 70; Jo1, antigen; CentrB, centromere B. Values of greater than 125 are highlighted red.

Table S8. Transitional temperatures of VRC07 variants determined by DSC.

Antibody	T_{m1}	T_{m2}
VRC07	70.92	Not Observed
VRC07-av1	70.22	Not Observed
VRC07-av1-g1	70.16	Not Observed
VRC07-av1-g2	67.28	Not Observed
VRC07-av1-c1	68.65	Not Observed
VRC07-av1-c2	70.36	Not Observed
VRC07-av1-c3	65.36	69.52
VRC07-av2	66.91	Not Observed
VRC07-av2-g1	60.12	69.3
VRC07-av2-g2	68.14	Not Observed
VRC07-av2-c1	68.41	Not Observed
VRC07-av2-c2	62.24	68.92
VRC07-av2-c3	69.46	Not Observed
VRC07-av3	71.15	Not Observed
VRC07-av3-g1	65.51	69.68
VRC07-av3-c1	70.07	Not Observed
VRC07-av3-c2	66.84	Not Observed
VRC07-av3-c3	69.81	Not Observed
VRC07-av3-c4	71.15	Not Observed