Mutation	ROSETTA Justification
$Y100_AFP$	The *wt* TYR (Y) makes a hydrogen bond with *wt* GLY at 100_D backbone. The mutant PHE (F) residue takes on same rotamer as *wt*, but does not make an h-bond.
$N100_FF$	The mutation PHE (F) makes an inter-HCDR3 hydrophobic stacked with another designed PHE (F) at position 100_A. However, it loses h-bond with a neighbor *wt* ASN.
$N100_FL$	The LEU (L) packs well if there is a mutated PHE (F) at position 100_H, but that mutation is not preferred. However it packs well against *wt* PRO (P) at 99. Also packs well against LYS on antigen.
$N100_FY$	Same as $N100_FL$ and has an additional polar group facing towards solvent space. The Dunbrack score is also favored.
$D100_LL$	Favored Dunbrack. The h-bonds are all retained by the mutant ASN. Additional hydrogen bond made by the sp2 oxygen over to a GLU on antigen. Additional amine group facing solvent.
$Y100_QN$	The additional inter-HCDR3 loop h-bond that ASN (N) mutant requres that position 96 be a SER.
M101E	The solvation is really poor but makes an extra h-bond with *wt* TRP at position 104. It also makes another h-bond with a mutant SER at position 93.

Legend

Red	Mutation predicted was not beneficial to the interaction and was not made.
Grey	Mutation was predicted to have no effect.
Yellow	Mutation was predicted to be beneficial, but needs to be paired with other mutations.
Green	A single point mutation that was predicted to be beneficial.

Figure S1. Selection criteria for experimental characterization of suggested mutations by ROSETTA. Mutations were each inspected manually. Genes encoding antibodies containing the mutations indicated in green or yellow were synthesized and expressed.

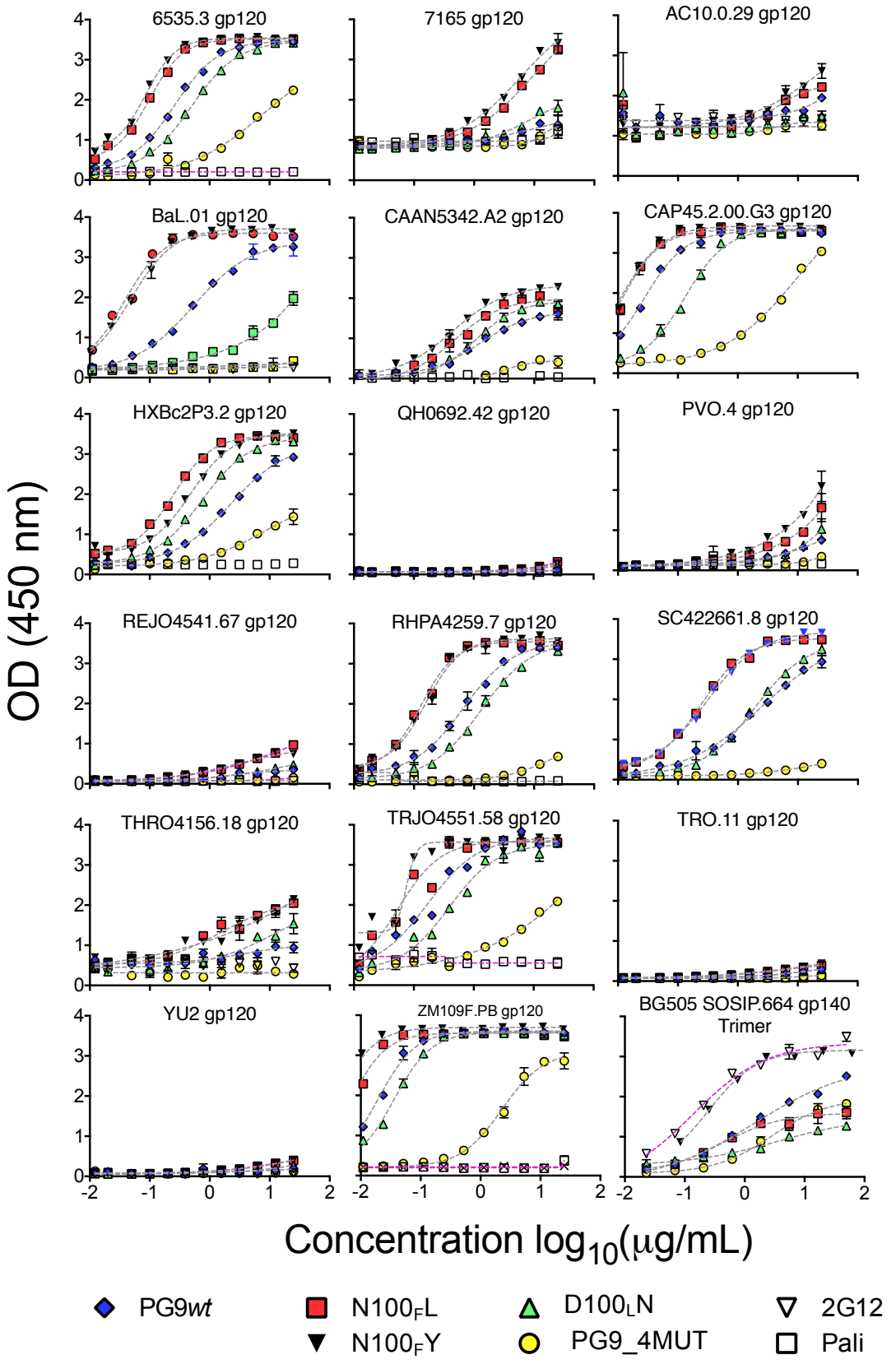

Figure S2. ELISA curves for binding to 18 HIV Env antigens including one recombinant trimer (BG505 SOSIP). Glycan-specific broadly neutralizing HIV mAb 2G12 is shown as a positive control; RSV neutralizing antibody palivizumab (Pali) is shown as a negative control.

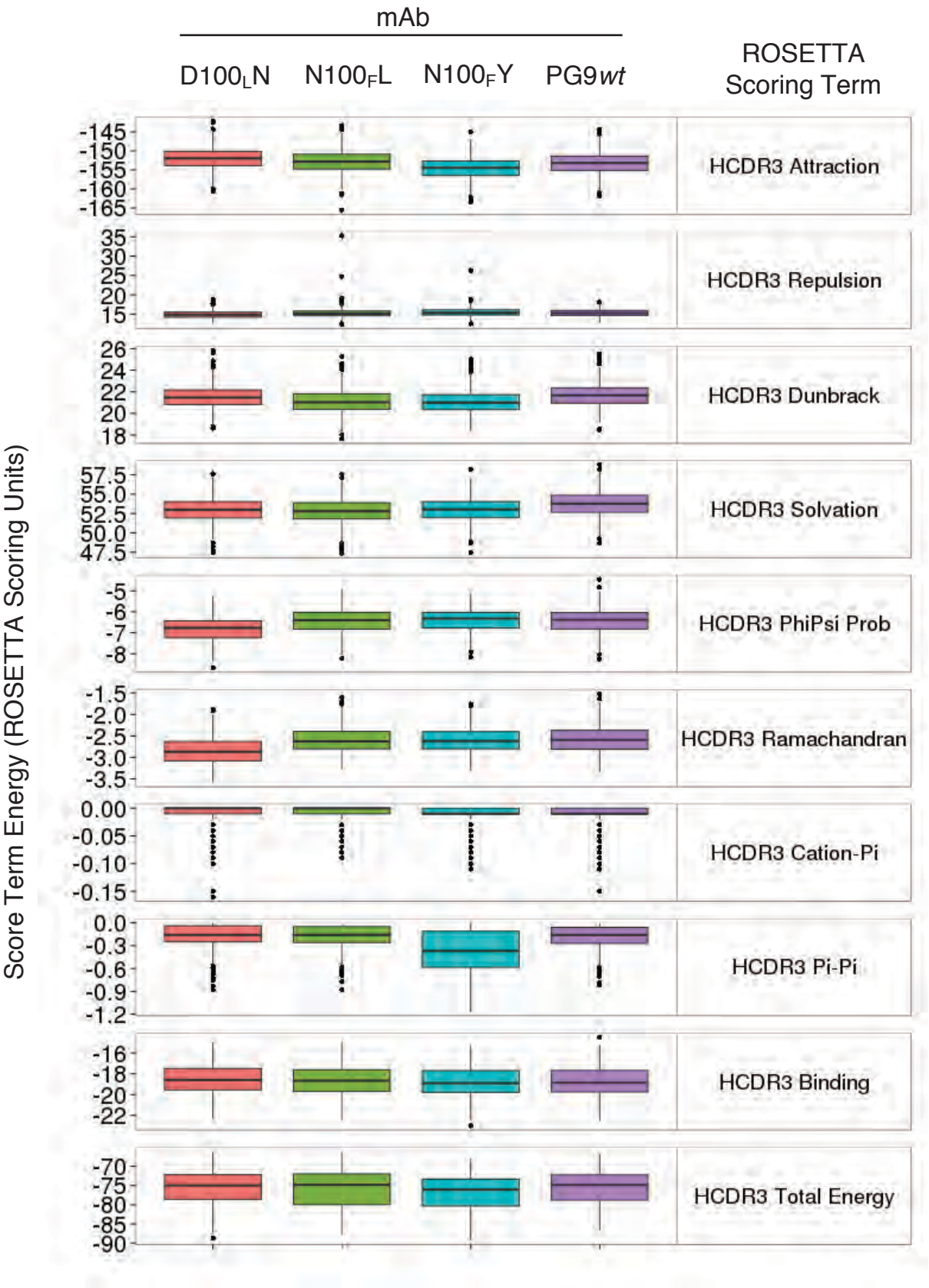

Figure S3. The contribution of individual scoring terms to the total energy score for the HCDR3 loop for each mutation. The predictive model used 1,000 simulations for each variant. Each scoring term for ROSETTA is shown in the y-axis panel. The y-axis value is the score for that energy term. Scoring terms are described in Table S2.

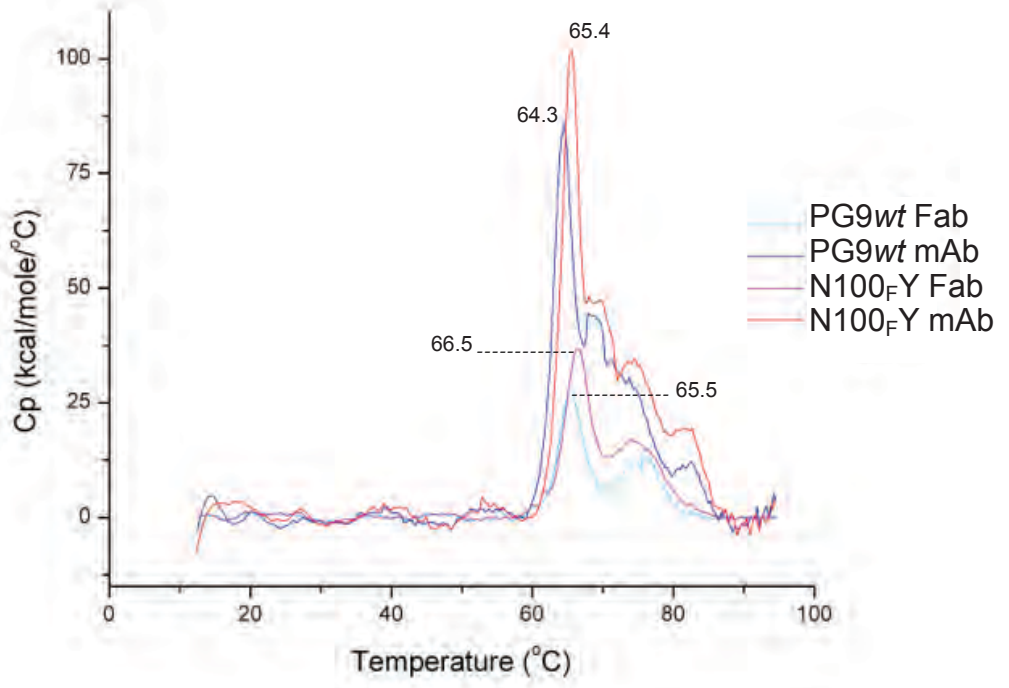

Figure S4. Normalized calorimetric recording of PG9*wt* Fab (cyan), PG9*wt* monoclonal antibody (blue), $N100_FY$ Fab (magenta), $N100_FY$ monoclonal antibody (red). The first peak melting temperature is labeled for each variant.

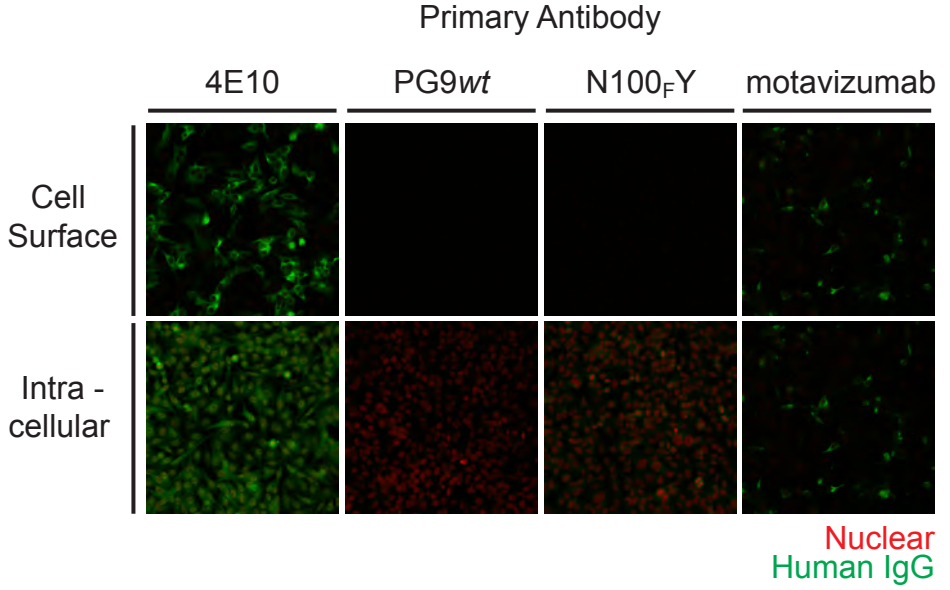

Figure S5. Cell-surface (non-permeabilized) or intracellular (permeabilized) staining for PG9*wt* or the $N100_FY$ variant. Using human HEp-2 cell line culture monolayers, the primary antibody was detected with a secondary Ab conjugated to Alexa Fluor 488 (green) and host cell DNA was detected with TO PRO3-iodine dye (red). The positive control HIV MPER-specific mAb 4E10, with known autoreactive phenotype, is shown in the first column. RSV antibody motavizumab is shown on the right column as a heterologous control for typical level of autoreactivity of an unrelated monoclonal antibody prepared in a similar manner.

Table S1. Data Collection and Refinement Statistics for PG9_N100$_F$Y

Data Collection	SSRL 11-1
Wavelength, Å	0.97945
Space group	$P2_1$
Unit cell a, b, c (Å)	57.7, 123.0, 69.9
α, β, γ (°)	90.0, 96.3, 90.0
Resolution (Å)*	40.0 – 2.3 (2.4 – 2.3)
Completeness*	98.3 (98.9)
Redundancy*	3.2 (3.2)
No. total reflections	166,000
No. unique reflections	42,379
I/σ_*	13.3 (1.9)
R_{merge}*†	0.06 (0.50)
R_{pim}*π	0.04 (0.32)
$CC_{1/2}$*ς	99.7 (48.6)
Refinement statistics	
Resolution (Å)	40.0 – 2.3
No. reflections total/R_{free}	42,379/2,000
R_{cryst}‡/R_{free}§	20.0/23.9
RMSD bond length (Å)	0.003
RMSD bond angles (°)	0.742
Protein atoms/solvent atoms	6,860/281
Wilson B-value ($Å^2$)	45.2
B-value overall/protein/solvent ($Å^2$)	53.0/52.5/55.7
Ramachandran Preferred %	97.7
Allowed %	100
Molprobity all-atom clashscore	1.99
PDB ID	4YAQ

* Values in parentheses are for the highest resolution shell.

† $R_{merge}= \Sigma|I-\langle I\rangle|/\Sigma\langle I\rangle$, where I is the observed intensity, and $\langle I\rangle$ is the average intensity of multiple observations of related reflections.

π $R_{pim} = \Sigma hkl\ (1/(n-1))1/2\ \Sigma i\ |\ Ihkl,i - \langle Ihkl\rangle\ |\ /\ \Sigma hkl\ \Sigma i\ Ihkl,I$, where Ihkl,i is the scaled intensity of the i^{th} measurement of reflection h, k, l, $\langle Ihkl\rangle$ is the average intensity for that reflection, and n is the redundancy

ς $CC_{1/2}$= correlation coefficient of half-datasets (*67*)

‡ $R_{cryst}= \Sigma hkl||Fobs|-|Fcalc||/\Sigma hkl|Fobs|$

§ R_{free} calculated as for R_{cryst} but for 5% of the reflections excluded from refinement

Table S2. ROSETTA scoring terms explained.

Scoring Term	Explanation of Scoring Term
Attraction	The van der Waals scoring term to indicate much attra ction residues have on each other.
Dunbrack	A statistical probability score indicating how often a side-chain configuration has been seen in the protein data bank
Repulsion	The van der Waals scoring term to indicate much repulsion residues have on each other.
Solvation	How well are hydrophobics packed away from solvent and hydrophilic groups are facing solvent
Ramachandran	A statistical probability of how well phi-psi angles fit into the Ramachandran plot
Total	A summation of all individual scoring terms to get a total score
ΔΔG	The change in total energy score when residues are moved out of complex
PhiPsi Prob.	A stastical probability score of how well a side-chain configuration has been seen given a phi-psi angle in the PDB
Cation-Pi	A score encompassing how the configuration of positive cation at the end of charged residues interact with pi orbitals
Pi-Pi	A score encompassing how two pi orbitals interact
HCDR3 Stabilization	The total score of residues only found in the HCDR3
Full Complex Stabilization	The total score of all residues
HCDR3 Binding	The contribution to ΔΔG by residues found in the HCDR3
Full Complex Binding	The ΔΔG for the entire complex