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## Supplemental Information

# Tailored Immunogens Direct Affinity Maturation toward HIV Neutralizing Antibodies

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**Table S1. Related to Figure 1. Purpose of GT3 mutations.**

| Mutation in GT3 | Purpose | Mutation in GT6? | Mutation in GT8? |
|---|---|---|---|
| N276D | Eliminate 276 glycan | Y | Y |
| I277F | Stabilize loop D for VRC01-class binding | Y | Y |
| T278R | Stabilize loop D; possible VRC01-class contact | Y | Y |
| F353Y | Improve packing behind loop D | N | Y |
| N356D | Eliminate glycosylation site introduced by I358T | N | N |
| I358T | Improve packing on β-sheet; not VRC01 contact | Y | Y |
| N363P | Eliminate 363 glycan in BG505 that is uncommon in other HIV strains | N[#] | Y |
| T372M | Stabilize CD4-binding loop? | N | N |
| T373N | Add glycosylation site at position 373 | N | Y |
| T455V | Eliminate partially buried and unsatisfied hydroxyl? Possible contact | N | N |
| S460Y | Stabilize V5? Possible VRC01-class contact | N | Y |
| T464N | Eliminate the one V5 glycan in BG505 | N* | N* |

[#]GT6 lacks a glycan at 363 although it does not use P at that position.
*All V5 glycans have been removed from GT6 and GT8, by other mutations.

**Table S2. Related to Figure 1. Antigenic profile of GT3 SOSIP and native-like trimers**
Table of monovalent binding affinities as measured by SPR using Fabs as analytes and BG505 SOSIP, BG505 GT3 SOSIP, CD4bs-B SOSIP and CD4bs-C SOSIP as ligands.

Dissociation constant Kd (nM)

| | Mat-VRC01 | GL-VRC01 | Mat-12A12 | GL-12A12 | Mat-CH31 | GL-CH31 | Mat-3BNC60 | Mat-PGV04 | PGT145[a] | PGT151 | PGT121 | PGT128 | 35O22 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BG505 SOSIP** | 122 | NB | 59 | NB | 137 | NB | 62 | 197 | 6 | 25* | 29 | 12 | 276 |
| **BG505 N276D-SOSIP**[b] | 41 | - | 9 | - | 69 | - | 11 | 67 | - | 234 | 26 | 12 | 30 |
| **GT3 SOSIP** | 27* | 290 | 19* | 1500 | 38* | 2400* | 23* | 70* | 3.7 | 39* | 40 | 20 | 411 |
| **CD4bs-B N276D-SOSIP**[b] | 43 | - | 12 | - | 811 | - | 33 | 165 | 27 | 263 | 19 | 11 | 37 |
| **CD4bs-C N276D-SOSIP**[b] | 170 | - | 212 | - | 3200 | - | 10000 | 17000 | 18 | 214 | 20 | 10 | 25 |

* complex kinetics are poor fit to simple 1-to-1 binding model
[a] binding determined using IgG
[b] made in HEK 293S (-GnTI) cells
NB: no binding detected at ≥4μM

**Table S3. Related to Figure 3. LCDR1 characteristics for selected VRC01-class bnAbs.**

| Antibody | VK/VL gene | L-CDR1 sequence* | | L-CDR1 length | | LCDR1 deletion? |
|---|---|---|---|---|---|---|
| | | germline | mature | germline | mature | |
| 12A12 | VK1D-33 | QDISNY | QGIGSS | 6 | 6 | No |
| 12A21 | VK1D-33 | QDISNY | QGIGSS | 6 | 6 | No |
| 3BNC117 | VK1D-33 | QDISNY | GY | 6 | 2 | Yes: 4aa |
| 3BNC60 | VK1D-33 | QDISNY | GY | 6 | 2 | Yes: 4aa |
| VRC-CH31 | VK1D-33 | QDISNY | RGIGKD | 6 | 6 | No |
| NIH45-46 | VK3-20 | QSVSSSY | QSGS | 7 | 4 | Yes: 3aa |
| PGV19 | VL2-14 | SDVGGYNY | SDFRGFSS | 8 | 8 | No |
| PGV20 | VL2-14 | SDVGGYNY | STSV | 8 | 4 | Yes: 4aa |
| PGV04 | VK3-20 | QSVSSSY | SYGH | 7 | 4 | Yes: 3aa |
| VRC01 | VK3-20 | QSVSSSY | QYGS | 7 | 4 | Yes: 3aa |
| VRC18 | VK3-20[a] | QSVSSSY | QGILSNQ | 7 | 7 | No |
| VRC03 | VK3-20 | QSVSSSY | QGGNA | 7 | 5 | Yes: 2aa |
| VRC06 | VK3-20 | QSVSSSY | QGGNS | 7 | 5 | Yes: 2aa |
| VRC23 | VK3-15[b] | QSVSSN | QGVGSD | 6 | 6 | No |

*IMGT convention for kappa or lambda chains: L-CDR1 is the stretch between CXXX and XXW.
[a]Georgiev et al., 2013
[b]Zhou et al., 2015

**Table S4. Related to Figure 5. Primers used for antibody gene amplification.**

| Round | Chain | Direction | Sequence |
|---|---|---|---|
| 1 | Heavy | Forward | GAGGTGCAGCTGCAGGAGTCTGG |
| 1 | Heavy | Forward | CAGGTGCAGCTGGTGCAG |
| 1 | Heavy | Reverse | AGGGGGCTCTCGCAGGAGACGAGG |
| 1 | Heavy | Reverse | GGAAGGTGTGCACACCGCTGGAC |
| 1 | Heavy | Reverse | GGAAGGTGTGCACACCACTGGAC |
| 1 | Heavy | Reverse | GGAAGGTGTGCACACTGCTGGAC |
| 1 | Heavy | Reverse | AGACTGTGCGCACACCGCTGGAC |
| 1 | Heavy | Reverse | GAAAGTTCACGGTGGTTATATCC |
| 1 | Kappa | Forward | TGCTGCTGCTCTGGGTTCCAG |
| 1 | Kappa | Forward | ATTWTCAGCTTCCTGCTAATC |
| 1 | Kappa | Forward | TTTTGCTTTTCTGGATTYCAG |
| 1 | Kappa | Forward | TCGTGTTKCTSTGGTTGTCTG |
| 1 | Kappa | Forward | ATGGAATCACAGRCYCWGGT |
| 1 | Kappa | Forward | TCTTGTTGCTCTGGTTYCCAG |
| 1 | Kappa | Forward | CAGTTCCTGGGGCTCTTGTTGTTC |
| 1 | Kappa | Forward | CTCACTAGCTCTTCTCCTC |
| 1 | Kappa | Reverse | GATGGTGGGAAGATGGATACAGTT |
| 1 | Lambda | Forward | CAGGCTGTTGTGACTCAG |
| 1 | Lambda | Forward | CAACTTGTGCTCACTCAG |
| 1 | Lambda | Reverse | GTACCATYTGCCTTCCAGKCCACT |
| 2 | Heavy | Forward | CATCCTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCCCAGGTGCAGCTGGTGCAGTCTGG |
| 2 | Heavy | Forward | CATCCTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCCGAGGTGCAGCTGCAGGAGTCTGG |
| 2 | Heavy | Reverse | GGAAGACCGATGGGCCCTTGGTCGACGCTGAGGAGACGGTGACCGTGG |
| 2 | Heavy | Reverse | GGAAGACCGATGGGCCCTTGGTCGACGCTGAGGAGACTGTGAGAGTGG |
| 2 | Heavy | Reverse | GGAAGACCGATGGGCCCTTGGTCGACGCTGCAGAGACAGTGACCAGAG |
| 2 | Heavy | Reverse | GGAAGACCGATGGGCCCTTGGTCGACGCTGAGGAGACGGTGACTGAGG |
| 2 | Heavy | Reverse | GGAAGACCGATGGGCCCTTGGTCGACGCTGAGGAGACGGTGACCAGGG |
| 2 | Kappa | Forward | CATCCTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCCGAYATTGTGMTSACMCARWCTMCA |
| 2 | Kappa | Reverse | GAAGACAGATGGTGCAGCCACCGTACGTTTGATTTCCAGCTTGGTG |
| 2 | Kappa | Reverse | GAAGACAGATGGTGCAGCCACCGTACGTTTTATTTCCAGCTTGGTC |
| 2 | Kappa | Reverse | GAAGACAGATGGTGCAGCCACCGTACGTTTTATTTCCAACTTTGTC |
| 2 | Kappa | Reverse | GAAGACAGATGGTGCAGCCACCGTACGTTTCAGCTCCAGCTTGGTC |
| 2 | Lambda | Forward | CATCCTTTTTCTAGTAGCAACTGCACCGGTTCCTGGGCCCAGGCTGTTGTGACTCAG |
| 2 | Lambda | Forward | CATCCTTTTTCTAGTAGCAACTGCACCGGTTCCTGGGCCCAACTTGTGCTCACTCAG |
| 2 | Lambda | Reverse | GTTGGCTTGAAGCTCCTCACTCGAGAGGACAGTCAGTTTGGTTCC |
| 2 | Lambda | Reverse | GTTGGCTTGAAGCTCCTCACTCGAGAGGACAGTGACCTTGGTTCC |
| 2 | Lambda | Reverse | GTTGGCTTGAAGCTCCTCACTCGAGAGGACAGTCAATCTGGTTCC |
| 3 | Heavy | Forward | ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCCAGGGGGAAGACCGATGGGCCCTTGGTCGA |
| 3 | Kappa | Forward | ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCGGGAAGATGAAGACAGATGGTGCAGCCACCGT |
| 3 | Lambda | Forward | ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTGGCCTTGTTGGCTTGAAGCTCCTCACTCGA |
| 3 | All | Reverse | AGACGTGTGCTCTTCCGATCTXXXXXXXXXXGGTCATGTATCATCCTTTTTCTAGTAGCAACTG |
| 4 | All | Forward | CAAGCAGAAGACGGCATACGAGAGATCGGTCTCGGCATTCCTGCTGAAGATXXXXXXXXGTGACTGGAGTT CAGACGTGTGCTCTTCCGATC |
| 4 | All | Reverse | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTC |

*Note:* Red Xs in the Round 3 reverse primer indicate the position of well-specific barcodes. Blue Xs in the Round 4 reverse primer indicate the position of plate-specific barcodes.