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Supplemental Information

HIV-1 Envelope Glycan Composition as a Key Determinant of Efficient Virus Transmission via DC-SIGN and Resistance to Inhibitory Lectins

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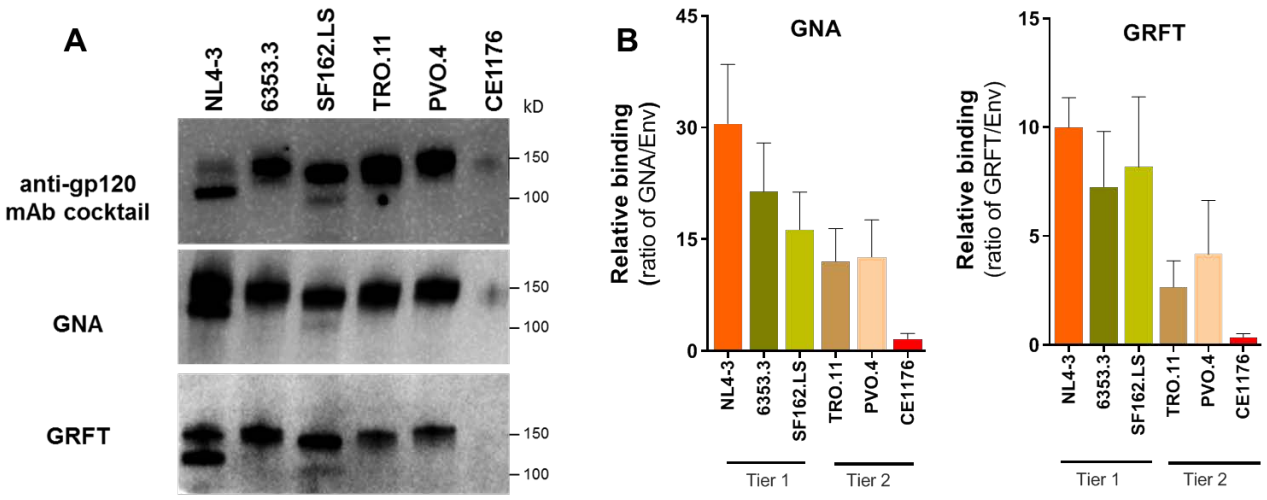

Figure S1: Varying oligosaccharide contents of Env from different HIV-1 isolates, Related to Figure 2.

(**A**) Oligosaccharide contents of Env from tier 1 to tier 3 subtype B viruses were probed by mannose-binding lectins GNA (recognizing Manα1-3 and Manα1-6) and GRFT (recognizing Manα1-2). Virus lysate were subjected to Western blotting and reacted with an anti-gp120 mAb cocktail, GNA, or GRTF. (**B**) The levels of lectin binding to Env were quantified relative to anti-gp120 Env bands for the respective viruses and expressed as ratios of GNA/Env or GRFT/Env. Averages and standard errors from two independent experiments are shown. Subtype C CE1176 lacking specific PNGSs that are required for recognition by these mannose-specific lectins was included as a negative control.

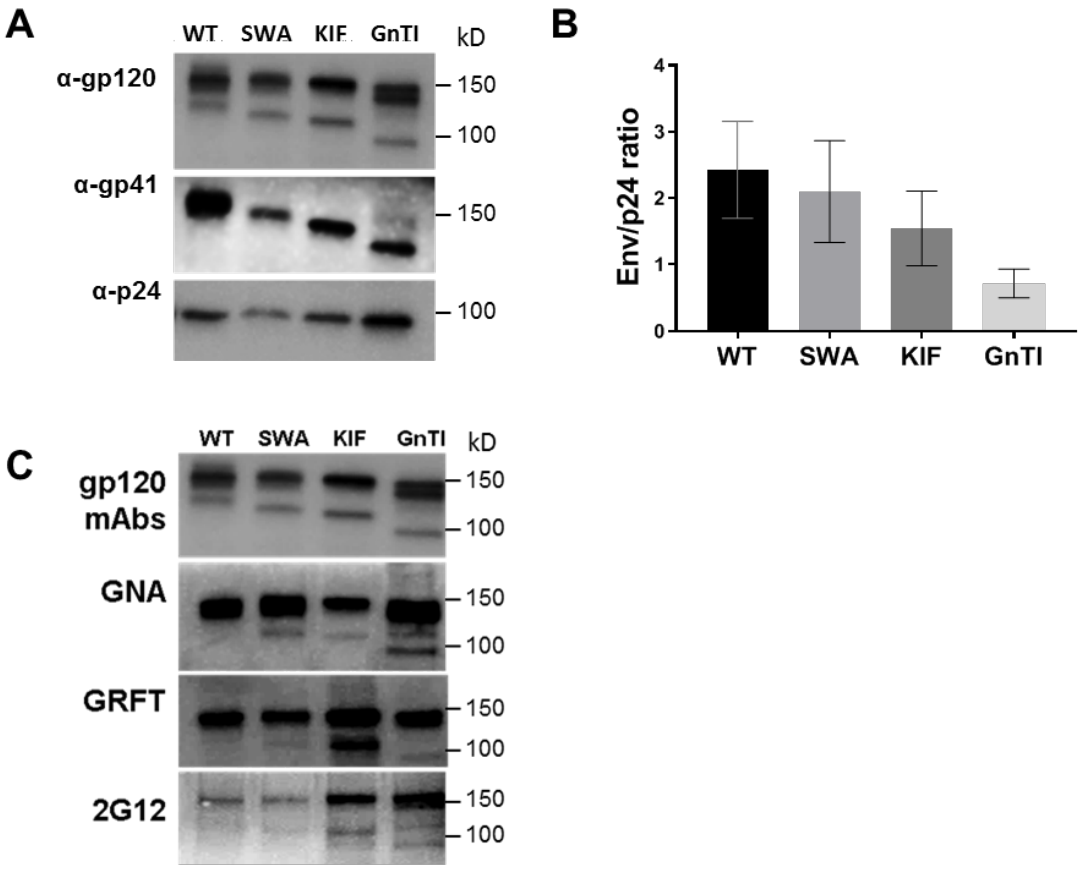

Figure S2: Alterations in molecular mass, incorporation, and glycan content of Env associated with virions produced in the presence of glycosidase inhibitors or in GnTI-/- cells, Related to Figure 3A.

(**A**) Changes in the molecular mass of Env from HIV-1 JRFL expressed in HEK293T cells that were left untreated (WT) vs treated with kifunensine (KIF) or swainsonine (SWA) or in HEK293S GnTI$^{-/-}$ cells. Western blots were done under reduced condition and probed with mAbs to gp120, gp41, or p24. Blots from one experiment are shown. (**B**) Relative levels of Env incorporation into KIF, SWA, and GnTI viruses vs WT as determined by the ratio of Env/p24 band intensities in Western blots probed with mAbs to gp120 and p24. Means and standard deviations from 2 repeated experiments are shown. **(C)** Changes in glycan composition of JRFL Env from sucrose-pelleted virions produced under different conditions as detected using oligomannose-specific lectins (GNA specific for terminal Manα1–3 + Manα1–6 and GRFT specific for terminal Manα1–2) or mAb 2G12. Virus lysates containing the same amount of Env was run on SDS-PAGE under reducing condition, blotted, and probed with an anti-gp120 mAb cocktail, GNA, GRFT, or 2G12. The reactivity of the upper and lower Env bands with lectins or 2G12 were quantified relative to the anti-gp120 mAb cocktail band for each virus and shown in Figure 3A. Western blot experiments were each repeated 2 to 3 times, and blots from one set of experiments are shown.

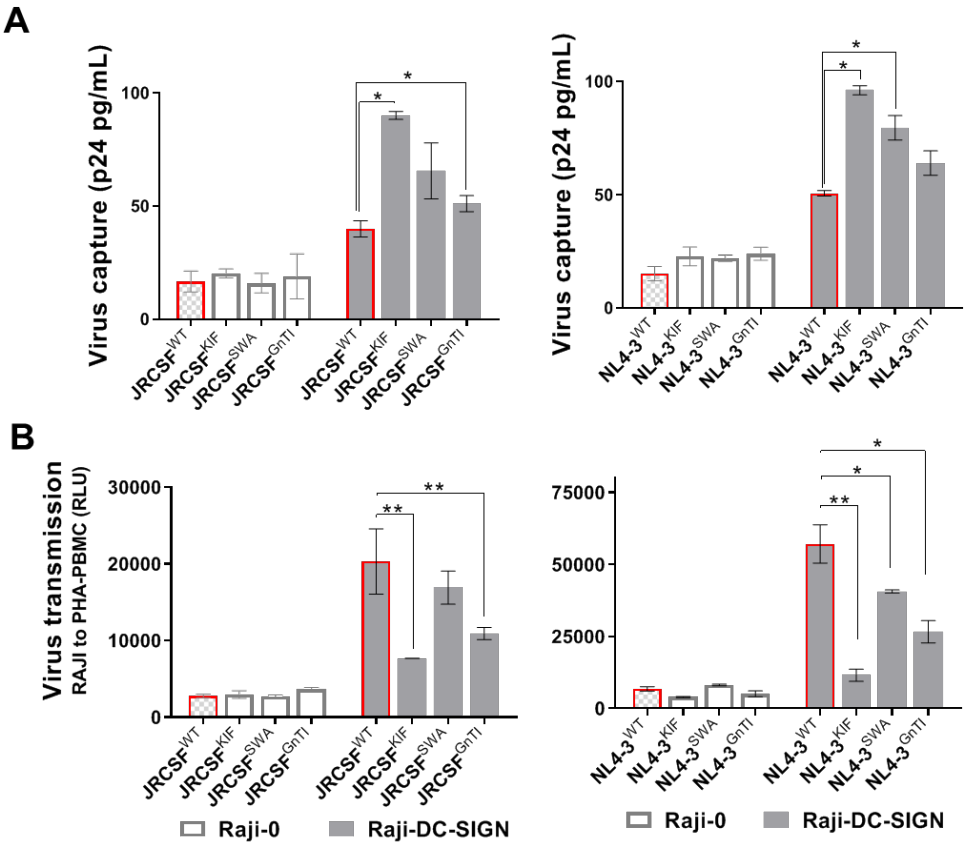

Figure S3: DC-SIGN-mediated capture and transmission of glycan modified viruses, Related to Figure 3B and Figure 3C.

(**A**) Virus capture by RAJI cells expressing DC-SIGN. Infectious HIV-1 molecular clones expressing JRCSF Env (R5) or NL4.3 Env (X4) were produced in transfected 293T cells in the presence of kifunensine or swansonine or in GnTI-/- 293S cells. RAJI cells with and without DC-SIGN were treated for 2 hours with virus (10 ng p24/ml), washed extensively to remove free virions, and then lysed. Virus capture was measured by p24 ELISA. (**B**) Virus transmission from DC-SIGN+ RAJI cells to PHA-stimulated PBMCs. RAJI cells with and without DC-SIGN were treated with infectious molecular clones JRCSF or NL4-3 bearing a Renilla luciferase reporter gene. After extensive washing to remove free virions, RAJI cells were co-cultured with PHA-stimulated PBMCs for 48 hours. Virus infection in PBMCs was detected by luciferase activity (RLU). Each experiment was repeated three times with different PBMC donors; average and standard deviation are shown. Statistical significance was determined by paired ratio t-test (*p<0.05, **p<0.01).

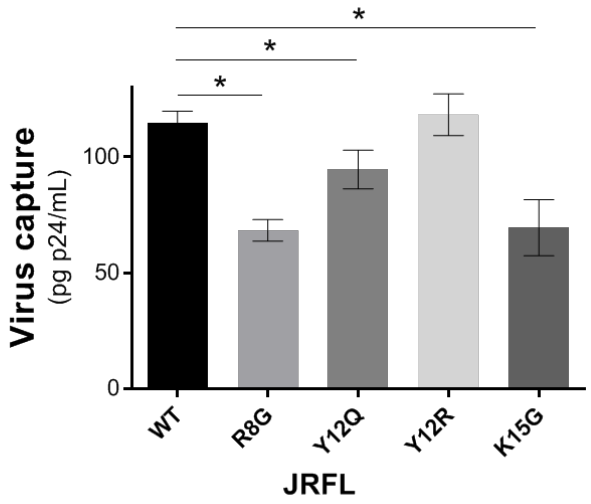

Figure S4: DC-SIGN-mediated capture of JRFL WT vs SP mutants, Related to Figure 5A. DC-SIGN+ Raji cells were treated with virus (10 ng p24/ml) for 2 hours. After extensive washing to remove free virus, cell-associated p24 protein was measured by ELISA. Mean and SD from four repeated experiments are shown. Data were analyzed using unpaired t test (* p<0.05).

TRANSPARENT METHODS

Cell lines and lectins

Cell lines 293T/17 and 293S GnTI$^{-/-}$ were obtained from the American Type Culture Collection (ATCC); TZM-bl cell line was obtained from Dr. John C. Kappes and Dr. Xiaoyun Wu, through the NIH AIDS Reagent Program (NARP), Division of AIDS, NIAID, NIH (Derdeyn et al., 2000). These cell lines were maintained in Dulbecco's modified eagle medium containing 10% heat-inactivated fetal bovine serum (FBS), penicillin/streptomycin (100 U/mL), MEM nonessential amino acids (0.1 mM), 1M HEPES, and L-glutamine. Cell monolayers were disrupted at confluence by treatment with 0.25% trypsin in 1 mM EDTA. Parental Raji (Raji-0) and Raji cells expressing DC-SIGN (DC-SIGN+ Raji), obtained through the NARP from Drs. Li Wu and Vineet N. Kewal Ramani, were cultured in RPMI1640 with 10% heat-inactivated FCS, MEM nonessential amino acids, and penicillin/streptomycin (both at 100 U/mL) (Wu et al., 2004).

GNA lectin was purchased from Vector Labs and checked for cell toxicity with Promega CellTiter Glo luminescent cell-viability assay prior to use in the study. GRFT was a gift from Dr. Barry O'Keefe (NCI, NIH) and also was obtained through the NARP (Mori et al., 2005).

Viruses

HIV-1 pseudoviruses expressing different Env strains were generated in 293T cells, as previously reported (Jan et al., 2018), by co-transfecting two plasmids: a plasmid encoding Env under a CMV promotor (pCAGGS-JRFL.JB) and the pNL4-

3.Luc.R-E- HIV-1 backbone plasmid. The pNL4-3.Luc.R-E- vector was obtained through the NARP from Dr. Nathaniel Landau (Connor et al., 1995). Glycan-modified pseudoviruses were produced in 293T cells in the presence of 25 μM kifunensine (Sigma Aldrich) or 20 μM swainsonine (Sigma Aldrich), or in 293S GnTI$^{-/-}$ cells. Viruses were checked for infectivity in TZM.bl cells.

Single- or double-point mutations were introduced to Env in the pCAGGS-JRFL expression vector by a multi-step, overlapping PCR mutagenesis strategy (Heckman and Pease, 2007). The SP mutants were cloned into pCAGGS using KpnI and MfeI restriction enzymes. The glycan-knockout and other gp120 mutants (N160K, N301Y, N301YN332K, I184G, A204E, M434G) were generated similarly using KpnI and Xho I restriction sites.

Primary cells

CD4 T cells and monocytes were isolated from peripheral blood mononuclear cells (PBMCs) in leukopacks (purchased from the New York Blood Center) using the Stem Cell Technologies EasySep human CD4+ T cell isolation kit or the Miltenyi Biotec monocyte isolation kit, respectively. CD4 T cells were stimulated with PHA (phytohemagglutinin) and maintained in RPMI-1640 medium supplemented with IL-2 (25U/mL). Monocytes were differentiated to monocyte-derived dendritic cells (MDDCs) by culturing in RPMI 1640 supplemented with 2mM L-glutamine, 5mM HEPES buffer, penicillin/streptomycin, 10% fetal bovine serum, recombinant human GM-CSF (20 ng/ml; Peprotech), and 20 ng/mL recombinant human IL-4 (20 ng/mL; Peprotech) for 7 days. MDDCs were analyzed for DC-SIGN expression by flow cytometry with FITC-

labeled mouse anti-human CD209 (DC-SIGN) or its isotype control. Similarly, levels of DC-SIGN expression on DC-SIGN+ Raji cells were also verified by flow cytometry before use.

Virus capture and transmission

For the virus capture assay, Raji-0 or DC-SIGN+ Raji cells (1×10^5/mL) were incubated with viruses (10 ng p24/mL) for 2 hours, washed to remove unbound viruses, and lysed in 1% Empigen for 1 hour at 56°C. After cell debris was removed by centrifugation, cell-associated p24 levels were determined by in-house p24 ELISA, as described in (Hioe et al., 1997).

For transmission experiments, Raji cells or MDDCs (1×10^5 cells/mL) were treated with viruses (150,000 RLU) for 2 hours, washed extensively, and then co-cultured with CD4+ target cells (TZM.bl or CD4 T cells) for 48 hours. To assess transmission inhibition by GRFT or GNA, virus was pretreated with titrated amounts of lectin for 1 hour and then incubated with DC-SIGN+ Raji cells for 2 hours. For inhibition by mannan, DC-SIGN+ Raji cells were pretreated with mannan (100 µg/mL) for 1 hour, washed, and incubated with virus for 2 hours. Virus transmission to CD4+ cells was detected by luciferase activity.

Virus internalization and degradation by MDDCs

MDDCs were incubated with virus (20 ng p24/mL) for 4 hours at 37°C, washed with warm PBS, and then treated with 0.05% trypsin for 5 minutes to remove surface-bound viral particles. The cells were lysed with ThermoFisher RIPA buffer containing

1% Triton X-100. Viral internalization was measured by quantifying p24 concentrations in the cell lysates by p24 ELISA.

To measure the kinetics of virus degradation, MDDCs were incubated with virus (10 ng p24/mL) for 2 hours at 37°C and washed 3 times with cold RPMI-1640 medium supplemented with 10% FBS to remove unbound virus. The cells were plated in triplicate in a 96-well plate at 3×10^5 cells/mL; the temperature was raised to 37°C, and virus degradation was determined at different time intervals by lysing the cells with 1% Empigen and measuring the amount of remaining p24 by ELISA.

Lectin-probed Western blotting

To evaluate the sugar composition of Env obtained from different viruses, Western blot analyses were performed with specific lectins and antibodies, as described (Upadhyay et al., 2018). Virus particles were pelleted through 20% sucrose by ultracentrifugation or concentrated using Lenti-X100 Concentrator (Clontech), treated with Laemmli buffer with 2-mercaptoethanol, and then boiled for 5 minutes. Virus lysates were resolved by Bio-Rad SDS-PAGE, blotted onto nitrocellulose membranes using the iBlot 2 Dry Blotting System from ThermoFisher, and then probed with antibodies or lectins. Env contents were estimated using a known amount of recombinant gp120 protein analyzed in the same blots. A cocktail of anti-human anti-gp120 mAbs (anti-V3 2219, anti-V3 2558, anti-C2 841, anti-C2 1006-30D, anti-C5 722; 1µg/mL each) and a cocktail of anti-gp41 MAbs (181-D, 240-D, 246-D, 167–7, 1367, 2295, 2556; 1µg/mL each) were both used to detect Env. p24-specific mAb (91–5) was used to detect Gag p24. Env glycan contents were probed by mannose-specific lectins

(biotinylated *Galanthus nivalis* GNA lectin or His-tagged GRFT lectin) or mAb 2G12, followed by HRP-tagged neutravidin, anti-His antibodies, or anti-IgG antibodies, and developed with a luminescence substrate (BioRad Clarity Max Western ECL Substrate). Band intensities were quantified using Image Lab Software Version 5.0 (Bio-Rad). Levels of GNA and GRFT binding to Env were calculated relative to the intensity of the corresponding Env band detected with anti-gp120 mAbs.

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