Supporting Information:

Global N-glycan Site Occupancy of HIV-1 gp120 by Metabolic Engineering and High-Resolution Intact Mass Spectrometry

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Materials and Methods

Expression and Purification of monomeric gp120: BG505 SOSIP.664 gp120 was transiently expressed in HEK 293F cells in presence of 20 µmol/l kifunensine and purified by nickel affinity chromatography (HisTrap HP column, GE Healthcare) according to the manufacturer's instructions. The protein was buffer exchanged into PBS and concentrated using 50 kDa cutoff centrifugal spin filters (Vivaspin, Sartorius AG) and then purified by size exclusion chromatography) using a HiLoadTM 16/600 SuperdexTM 200pg column (GE Healthcare). Protein purity was confirmed using sodium dodecyl sulfate polyacrylamide gel electrophoresis. For reference experiments, gp120 was deglycosylated using EndoH (Endoglycosidase H) according to manufacturer's instructions. Afterwards, glycosylated proteins were concentrated to 10 µmol/L using 50 kDa cutoff centrifugal spin filters (Vivaspin, Sartorius AG) for subsequent analysis by mass spectrometry.

Glycan Release and Purification: Release and labelling of the N-linked glycans from gp120 was performed as previously described[1]. Briefly, N-glycans were released from 20 µg via in-gel digestion with Peptide -N-Glycosidase F (PNGase F). PNGaseF and gp120 were incubated for 16 hours at 37ºC. N-glycans were purified with Spe-ed Amide 2 cartridges (Applied Separations) prior to analysis. O-glycans were released by reductive β-elimination as previously described[2]. Briefly, β-elimination solution (50 mM NaOH and 1 M NaBH4 in water) was added to 100 µg gp120 protein sample and incubated at 50°C for 16 hours. Reaction was stopped with several drops of glacial acetic acid and O-glycans were purified using porous graphitized carbon solid phase extraction cartridges prepared in-house. Samples were stored at -4°C until analysed by mass spectrometry. Sample preparation reporting is in accordance with MIRAGE guidelines[3].

Ion Mobility Mass Spectrometry: Immediately prior to mass spectrometry analysis, released glycans were desalted with a Nafion® 117 membrane as described[4]. Samples were removed, 5 µl of 1:1 (v:v) methanol:water LC-MS grade) containing 0.05 M ammonium phosphate was added to promote phosphate adduct formation. Samples were introduced into a Synapt G2Si ion mobility mass spectrometer (Waters, UK). Released N-glycans were analysed by direct static infusion and parent ions were mass selected in the quadrupole prior to collision induced dissociation in the transfer region (post ion mobility separation). Ion mobility was used to separate high-mannose isomers as described[5]. O-glycan IM-MS analysis was used for improved detection as previously reported[6]. Instrument setting were as follows: capillary voltage, 1-1.8 kV; sample cone, 100 V; extraction cone, 25 V; cone gas, 40 l/h; source temperature, 80°C; trap collision voltage, 4 V; transfer collision voltage, 4-100 V; trap DC bias, 40-60 V; wave velocity, 650 m/s; wave height, 40 V; trap gas flow, 2 ml/min; IMS gas flow, 80 ml/min. Data was acquired and processed with MassLynx v4.1 and Driftscope software (Waters, Manchester, UK).

Native Mass Spectrometry: Purified wild-type and N332A gp120 (10 µM) was initially buffer exchanged into 1M ammonium acetate, pH 6.9 with Bio-Spin™ 6 columns (Bio-Rad). Additional de-salting was achieved with three sequential washes of 1M ammonium acetate using 10 kDa MWCO centrifugal spin filters (Millipore). Samples were introduced into a Q Exactive™ hybrid quadrupole-Orbitrap™ mass spectrometer (Thermo Fisher

Scientific, Germany) modified for the transmission and detection of high molecular weight ions[7]. Positive Ions were generated by static nanoelectrospray using gold-coated capillaries prepared in-house. Data was obtained with a wide acquisition window (2,000-15,000 m/z) and desolvation was achieved with HCD voltage applied (150 V). Additional instrument settings were as follows: capillary voltage = 1.1 kV; source temperature = 60°C; max injection time = 50; S-lens RF = 100; C-trap entrance lens = 5.8. Spectra were obtained with 10 microscans, averaged over 50 scans. Data was processed using XCalibur 2.1 software (Thermo Fisher Scientific, Germany) and masses were assigned manually using in-house software. Deconvolution was done with UniDec software[8].

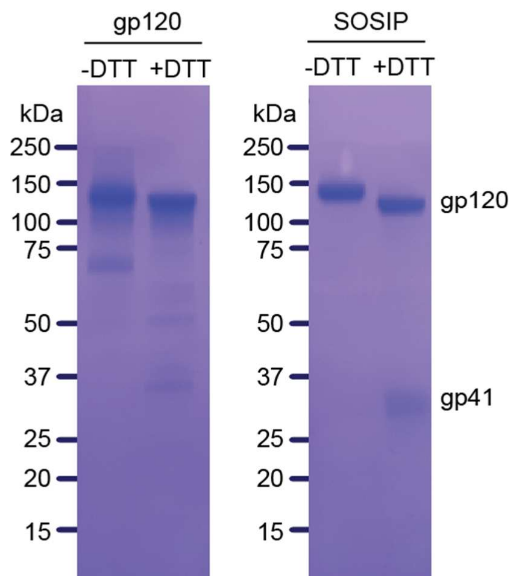

Figure S1. SDS-PAGE gel of purified +kifunensine expressed BG505 gp120 monomer and recombinant ectodomain of the HIV envelope trimer (BG505 SOSIP.664). Difference in migration between reduced and non-reduced gp120 monomer is attributed to number of bound SDS. Molecular mass of gp120 from SOSIP trimers (+DTT) is equivalent to monomeric gp120.

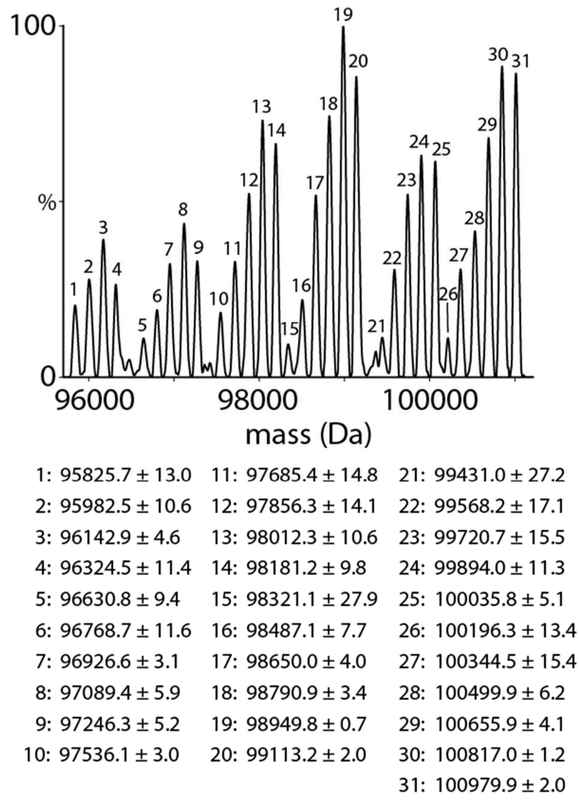

Figure S2. Deconvoluted zero-charge mass spectrum of gp120 (kif+) glycoforms with mass assignments and standard deviation.

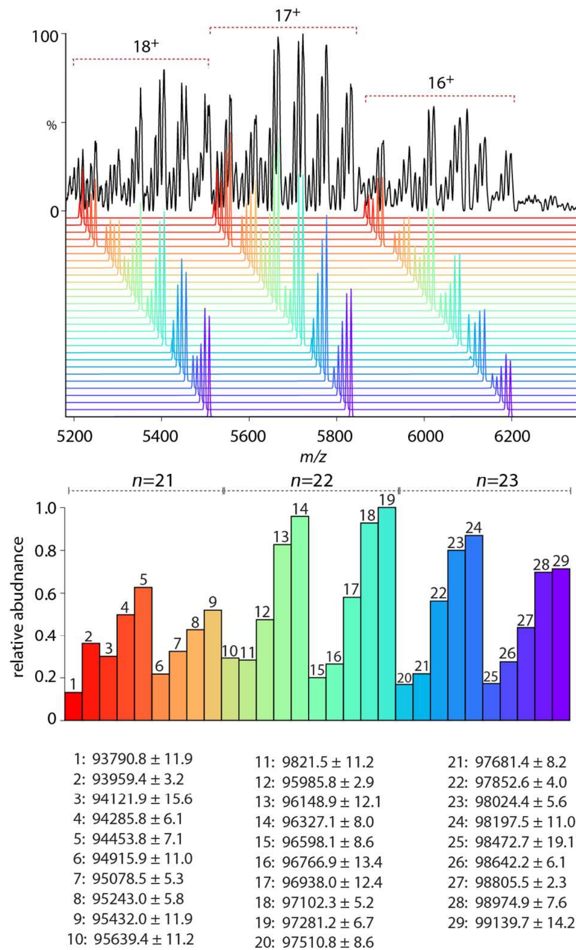

Figure S3. Intact and deconvoluted mass spectra of N332A gp120 (kif+) with mass assignments.

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