

Table S1. List of the combined PNGS mutants.

Mutants	Mutated PNGS
M46	N625Q/ N463Q
197M.1	N197D /N301Q
197M.2	N197D /N625Q
197M.3	N197D / N463Q
197M.4	N197D / N463Q /N442Q
197M.5	N197D / N463Q /N625Q
197M.6	N197D /N625Q/N442Q
197M.7	N197D /N625Q/N442Q/N339Q
197M.8	N197D / N463Q /N625Q/N442Q/N339Q/N448
197M.9	N197D / N463Q /N625Q/N442Q/N339Q
197M.10	N197D / N463Q /N625Q/N442Q/N339Q/N466Q
197M.11	N197D / N463Q /N625Q/N442Q/N339Q /N466Q/N611Q

Figure S1. The alignment of gp120/41 (gp160) amino acid sequences of FE strain of HIV-1 against the HXB2 reference strain. Conserved residues in FE are shown as dots below the HXB2 sequence, and non-conserved residues are written out for FE. Asterisks indicate the locations of PNGS 197 and 463, both of which are conserved.

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HXB2 1 MRVKEKYQHLLRWGWRWGTMLLGMLMICSATEKLWVTVYGYVPVWKEATTTLCASDAKAYDTEVHNVWATHACVPTDPNPQEEVVLVNYTENFMWKNDM
FE      .TG.IRKNY.HL...I..F...V...VGN.....I..R.....E.*
HXB2 101VEQMIEDIISLWDQSLKPCVKLTPLCVSLKCTDLKNDNTINSSSGRMIMEKGEIKNCSFNISTSIRGKVQKEYAFFYKLDIIPIDND--TTSYKLTSCN
FE      .N...V.....T.E.NN---VTK.ET--YHERMK.M.....AT.EL.D.K.TV..L..R...L.LNSIDNSSKY.R.IN..
HXB2 198TSVITQACPKVSFEPIPIHYCAPAGFAILKCNNTFNGTGPCNTYSTVQCTHGIRPVVSTQLLNGSLAEEEVVIRSVNFTDNAKTIIVQLNTSVEINCT
FE      .A...T.D...T...Y...D.I...H...K.....I...II...ESI.N.V...H..Q...I..
HXB2 298RPNNNIRKRIRIQRGPGRAFVTIG.KIGNMRQAHCNISRAKWNNTLKQIASKLREQFGNNKTIIFKQSSGGDPEIVTSHSFCGGEFFVCNSTQLFNSTWF
FE      .G...SV.....QT.YAT.DI..DI.....NKI...E..QRVSK..A.H.Q...K.EP.....L..T.....R.....DTSG...E.YM
HXB2 397NSTWSTEGSNNTEGSDTITLPCRKQIINMWQKVGKAMYAPPISGQIRCSSNITGLLLTRDGG--NSNNESEIFRPGGGDMRDNRSELYKYKVVKIEPL
FE      S---N...S---I...R.....E.N.T.K.....LA.DT.GT.T...K.....*
HXB2 495GVAPTAKRRRVQREKRAVGIGALFLGFLGAAGSTMGAASMTLTVQARQLLSGIVQQQNNLLRAIEAQHLLQLTVWGIKQLQARILAVERYLKDQQLLG
FE      .E...E...V...V...I.....S.....T.V..I..
HXB2 595IWGCSQKLCITAVPWNASWSNKSLEQIWNHTTWMEWDREINNYTSLIHSLSIEESQNQQEKNEQELLELDKWSLWNWFNTNWLWYIKLFIMIVGGLYG
FE      .TQNE..DNM..Q..K..S...NT.YR.L.D...R..KD...D...I..I...I..
HXB2 695LRIVFAVLSIVNRVRQGYSPLSFQTHLPTPRGPDPRPEGIEEEGGERDRDRSIRLVNGLALIWDDLRLSLCLFSYHRLRDLILLIVTRIVELLGR-----
FE      .PT.N.G..ANLGR...Q.K...F..A..N...FI.VIA.V...R.SSLRGLQ
HXB2 788RWEEALKYVWVWLLQVWSQELKNSAVSLLNATAIAVAEGTDRVIEVYQGACRAIRHIPRRIRQGLERILL
FE      K..A...LGS.V...G...K.TI..VDTI.....I..LI..L...YN.....F.TA.Q

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Figure S2. The CD4bs nMAbs susceptibility for the Env mutants of B/AE-Env clones.

(A) B05, a clade B virus. B05 and all PNGS mutants were resistant to b12 at the highest concentration tested (25 $\mu\text{g/mL}$ MAbs) (B) GX74.20, a clade AE virus. The data represent the means of three independent experiments, and the error bars indicate the standard deviations from the means.

