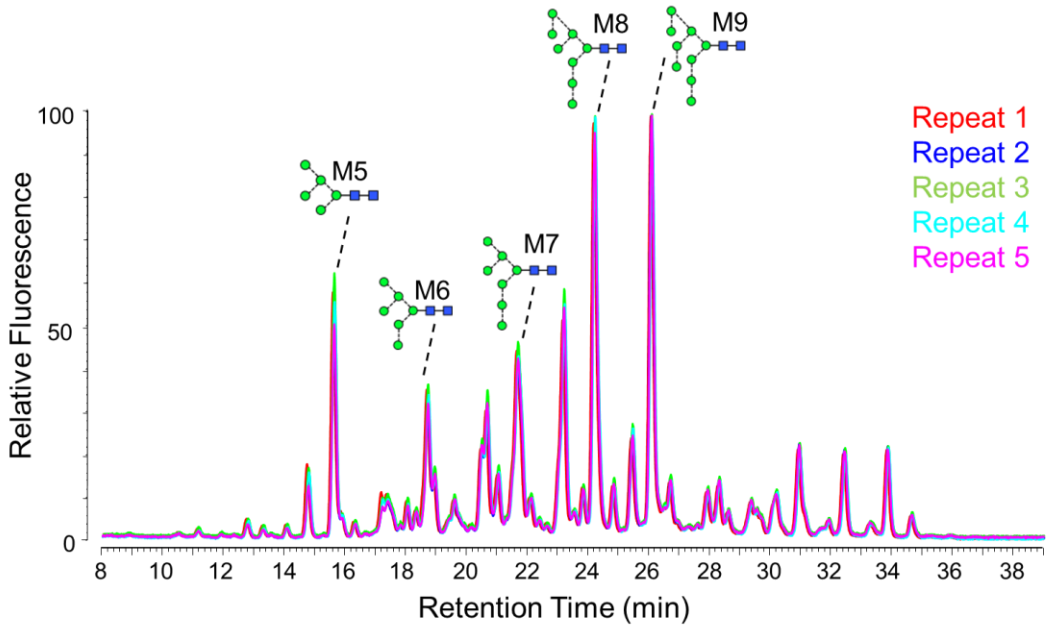
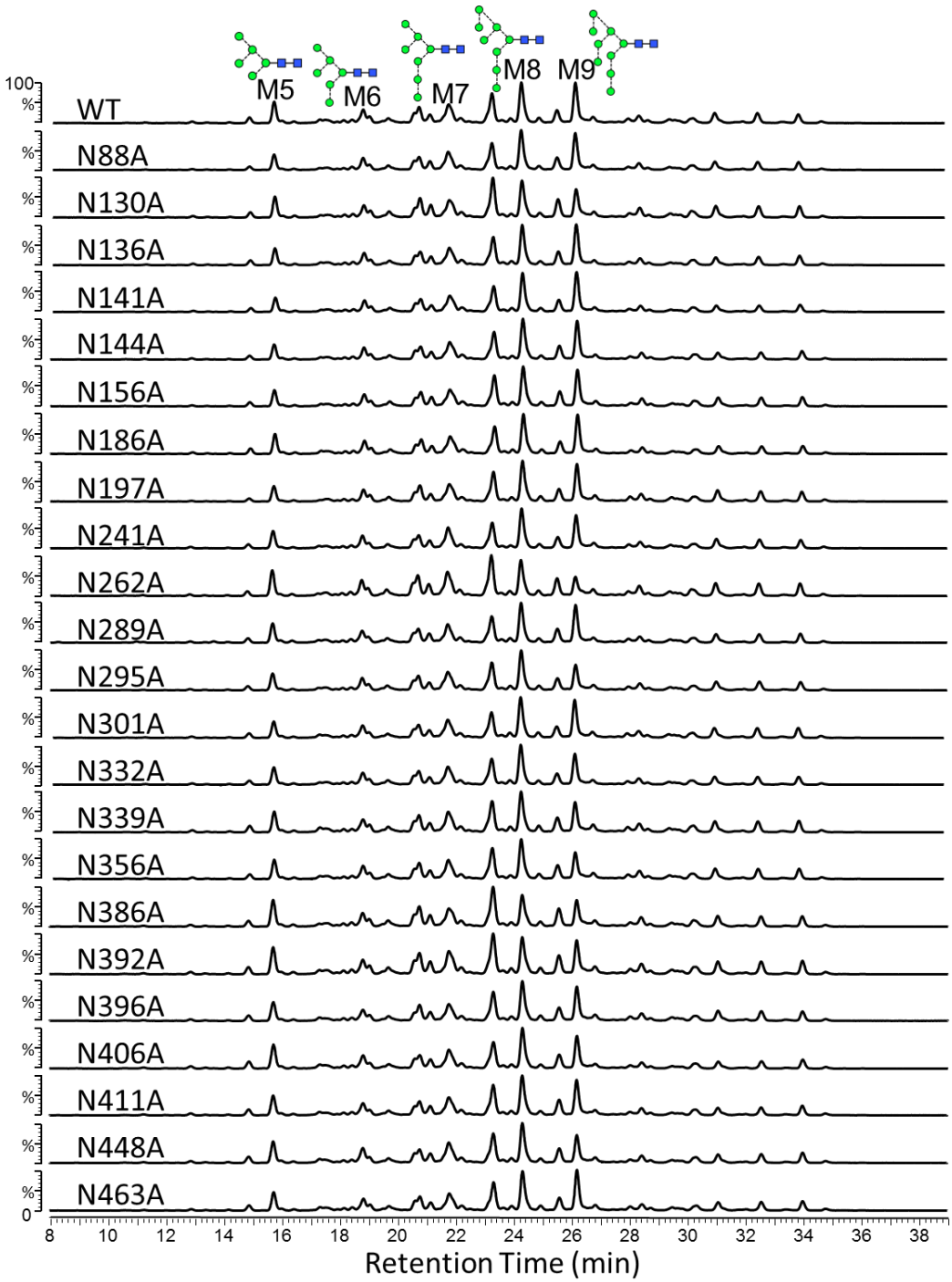
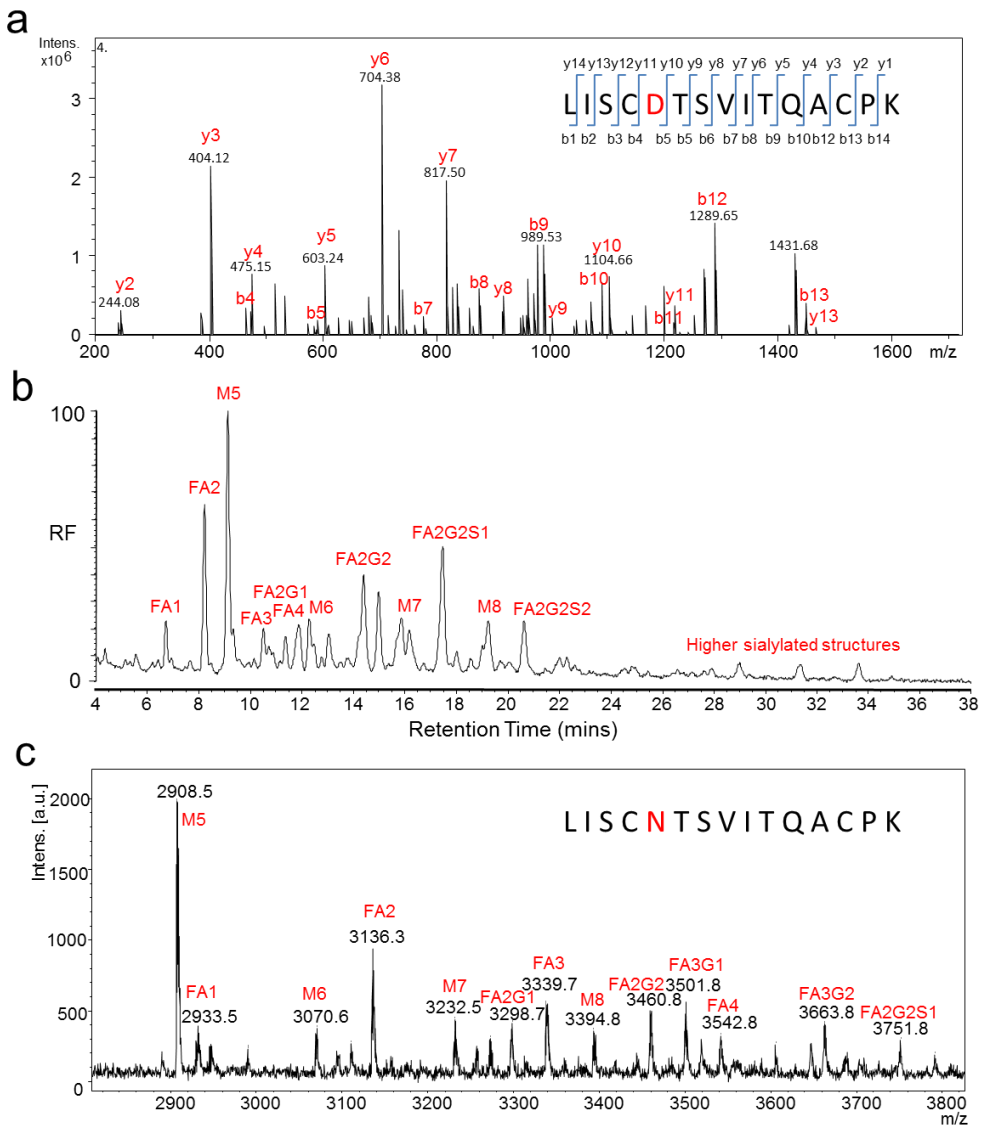


Supplementary Figure 1. Reproducibility of glycosylation profiles upon parallel expression.

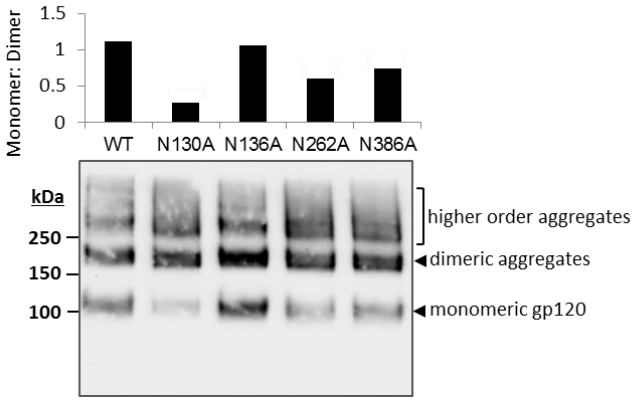
Wild-type $gp120_{BaL}$ was expressed, purified and analyzed five times in parallel, starting with the same passage of 293T cells. N-linked glycans were released from the five batches of wild-type $gp120_{BaL,}$ 2-AA labelled and analyzed by HILIC-UPLC. The five chromatograms are overlaid, and peaks corresponding to oligomannose-type glycans are indicated. Quantitation of the individual oligomannose-type glycan species by integration of the corresponding peaks was performed using Empower 3 software and values are reported in Supplementary Table 1. MX – $Man_xGlcNAc_2$.



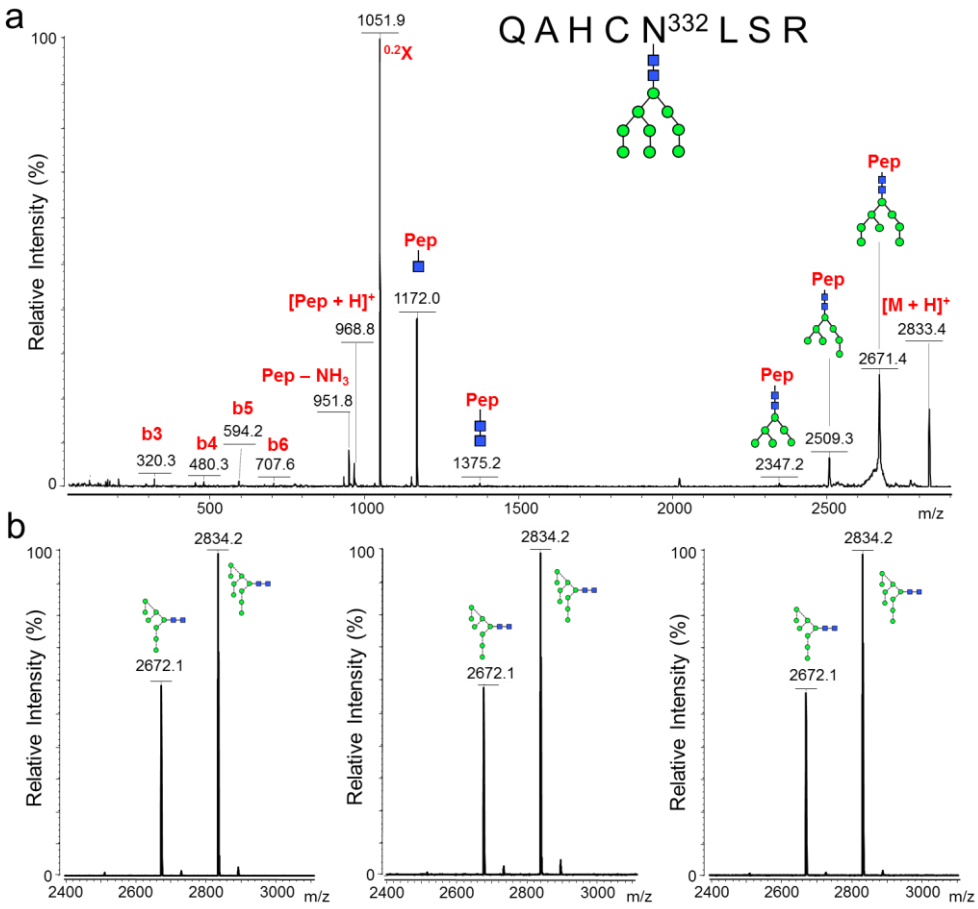
Supplementary Figure 2. HILIC-UPLC profiles of PNGS-deletion mutants. Monomeric wild-type gp120$_{BaL}$ and PNGS-deletion mutants were expressed in parallel in HEK 293T cells, followed by nickel-affinity purification. *N*-glycans were released from gel bands following SDS-PAGE using PNGase F, fluorescently labelled and analyzed by HILIC-UPLC. Integration of the chromatograms was performed using Empower 3 software. Peaks corresponding to oligomannose-type glycans (MX – $Man_xGlcNAc_2$) are indicated.



Supplementary Figure 3. Identification and glycoform characterization of the N197 glycosylation site. (**a**) ESI-LC-MS/MS spectrum of the deglycosylated tryptic peptide LISCDTSVITQACPK (deglycosylation by PNGase F causes conversion of N→D. Precursor mass was 847.4, $[M_{pep}+2]^{2+}$. Cysteine residues were modified with carbamidomethyl (+57). (**b**) HILIC-UPLC chromatogram of glycans released from the LISCNTSVITQACPK glycopeptides (following 2-AB labelling). (**c**) MALDI-MS spectrum of fraction containing LISCNTSVITQACPK glycopeptides. Observed masses correspond to $[M+H]^+$. RF; relative fluorescence.



Supplementary Figure 4. Western blot analysis of PNGS-deletion mutants. Culture media from N130A, N262A and N386A mutant expressions were assessed for the presence of aggregates. N136A is included as a control. Following non-reducing SDS-PAGE of cell culture supernatant, proteins were transferred to a PVDF membrane. The membrane was blocked in 5% non-fat milk in PBS + 0.05% Tween for 1 h at room temperature and then incubated with anti-His-HRP antibody (1:10,000 dilution; Life Technologies) in 5% non-fat milk in PBS + 0.05% Tween for 1 h. ECL substrate (GE Healthcare) was used for detection by chemiluminescence and measured using a Fujifilm Las-1000 Intelligent DarkBoxII to visualize the membranes. Densitometric evaluation of the bands corresponding to the monomeric and aggregated dimeric forms was performed using Image J software, and the ratios of monomer:dimer are shown.



Supplementary Figure 5. MALDI MS and MS/MS analysis of a N332-containing tryptic glycopeptide. Recombinant monomeric gp120, resolved by SDS-PAGE, was reduced and alkylated, digested with trypsin, and then fractionated by RP-HPLC. Fractions containing the QAHCNLSR glycopeptide were pooled and analyzed by MALDI-MS and MS/MS in positive ion mode. (**a**) MALDI MS/MS of the QAHCN^{332}LSR glycopeptide. The peptide contained a pyro-glutamine modification (mass difference –17 Da) and a carbamidomethyl modification of the cysteine (+57 Da). B fragment ions of the peptide are indicated. Characteristic fragmentation of the glycopeptide was also observed; a ^{0,2}X-ring cleavage of the inner *N*-acetylglucosamine produces a [Pep + H + 83]$^+$ peak, a Y-type cleavage of the chitobiose core produces a [Pep + H + 203]$^+$ peak, and loss of the amide side chain from the glycosylated asparagine produces a [Pep + H – 17]$^+$ peak. Y-type fragments ions resulting from cleavage of the glycans were also observed. (**b**) MALDI-MS analysis of the QAHCN^{332}LSR glycopeptides isolated from three separately expressed wild-type gp120$_{BaL}$ samples to determine any intrinsic variation of the glycoforms present.

Supplementary Table 1. Reproducibility of glycosylation profiles upon parallel expression[†]

	% of total glycans						
	M5	**M6**	**M7**	**M8**	**M9**	**M5-M9**	**Complex**
Mean	5.2	4.1	6.7	10.5	10.2	36.7	63.3
Standard Deviation	0.4	0.1	0.1	0.2	0.3	0.6	0.6
Coefficient of Variance[‡]	6.7	2.6	2.2	2.2	3.3	1.5	0.9

[†] Wild-type $gp120_{BaL}$ was expressed, purified and analyzed five times in parallel, starting with the same passage of 293T cells.

[‡] The coefficient of variance represents the standard deviation divided by the mean, and is expressed as a percentage. It is comparable with the % percentage change calculated for the PNGS mutants relative to wild-type.

Supplementary Table 2. Effect of PNGS-deletion on the abundance of oligomannose-type glycans within monomeric gp120$_{BaL}$

Mutant	% change[†]					
	M5	M6	M7	M8	M9	**Total**
N88A	-13	4	12	19	9	**9**
N130A	-4	-11	-6	-11	-32	**-15**
N136A	-13	1	6	11	12	**6**
N141A	-18	6	12	21	23	**13**
N144A	-17	8	18	21	15	**12**
N156A	-14	5	14	16	6	**7**
N186A	1	7	8	11	8	**8**
N197A	-25	-11	1	7	-1	**-3**
N241A	-5	8	23	15	-4	**8**
N262A	15	13	19	-16	-57	**-13**
N289A	-1	-6	0	7	1	**1**
N295A	-8	7	20	14	-28	**0**
N301A	-17	-5	0	9	0	**0**
N332A	3	7	15	21	-7	**8**
N339A	-6	-4	3	-3	-29	**-10**
N356A	0	7	8	3	-30	**-5**
N386A	23	3	-2	-24	-38	**-14**
N392A	8	-7	-1	-22	-32	**-15**
N396A	-2	-7	-4	4	-11	**-4**
N406A	26	6	7	8	-14	**4**
N411A	3	-3	6	7	-6	**2**
N448A	8	12	16	5	-28	**0**
N463A	-1	9	8	11	13	**9**
N295A + N332A	-6	1	18	-11	-54	**-17**
N295A + N339A	-15	0	16	-11	-54	**-18**
N295A + N386A	20	2	3	-34	-71	**-27**
N295A + N448A	5	6	23	3	-59	**-11**
K160N	20	33	29	11	0	**13**
A278S/T	19	7	2	-2	-5	**1**
N160N +N276	43	34	28	5	-3	**12**

[†]Corresponds to the % change, relative to wild-type, of the relative abundance (%) of each glycan species.

Supplementary Table 3. IC_{50} values for binding of N332-dependent and other conformation-dependent antibodies to $gp120_{BaL}$ PNGS-mutants

	IC_{50} (μg ml^{-1})					
	PGT121	**b12**	**PGT135**	**2G12**	**PGT128**	**17b**
WT	0.52	0.08	0.48	0.20	0.19	1.96
N88A	0.87	0.07	0.73	0.36	0.19	1.34
N130A	2.07	0.07	0.89	0.41	0.66	>40
N136A	0.58	0.07	0.61	0.30	0.20	1.38
N141A	0.80	0.08	0.53	0.35	0.24	1.43
N144A	0.77	0.09	0.39	0.24	0.20	1.66
N156A	1.23	0.08	0.26	0.15	0.38	6.70
N186A	0.85	0.09	0.74	0.13	0.24	1.49
N197A	0.95	0.09	0.55	0.16	0.33	2.62
N241A	0.53	0.09	0.55	0.22	0.18	1.64
N262A	0.91	0.08	1.53	0.29	0.52	>20
N289A	0.79	0.06	0.29	0.20	0.16	1.12
N295A	0.57	0.08	0.21	>20	0.19	2.83
N301A	0.42	0.08	0.29	0.21	2.39	2.37
N332A	>20	0.07	>20	0.23	9.60	1.31
N339A	0.30	0.07	0.16	0.21	0.20	1.01
N356A	0.31	0.06	0.44	0.97	0.21	1.25
N386A	0.40	0.04	0.24	0.58	0.20	>20
N392A	0.58	0.09	0.92	>20	0.19	2.85
N396A	0.29	0.08	0.30	0.44	0.19	4.91
N406A	0.32	0.09	0.37	0.23	0.23	1.01
N411A	0.28	0.08	0.34	0.24	0.22	1.52
N448A	0.45	0.09	0.36	0.20	0.21	3.63
N463A	0.45	0.09	0.32	0.29	0.17	1.48

No binding
Reduced binding plateau

Supplementary Table 4. Effect of deletion of the N262 glycosylation site on the abundance of oligomannose-type glycans within gp120s from different strains

Strain	% change					
	M5	**M6**	**M7**	**M8**	**M9**	**Total**
BaL	15	13	19	-16	-57	**-13**
BG505	9	10	3	-16	-54	**-9**
C22	18	-2	-13	-34	-47	**-14**
94UG103	-6	2	1	-12	-42	**-14**

Supplementary Table 5. Primers used for site-directed mutagenesis of $gp120_{BaL}$.

	Primer sequence (5′-to-3′)
Forwards flanking (Age1)	CGACACCGGTATGGACGCCATGAAG
Reverse flanking (Kpn1)	CATGGTACCCACCACGCTGCTGATG
BaL N88A	GAAGTAGAATTGGAAGCTGTGACAGAAAATTTTAAC
	GTTAAAATTTTCTGTCACAGCTTCCAATTCTACTTC
BaL N130A	CTGTGCGTGACCCTGGCTTGCACTGATTTGAGG
	CCTCAAATCAGTGCAAGCCAGGGTCACGCACAG
BaL N136A	GCACTGATTTGAGGGCTGCTACTAATGGGAAC
	GTTCCCATTAGTAGCAGCCCTCAAATCAGTGC
BaL N141A	GCTACTAATGGGGCCGACACCAACACCAC
	GTGGTGTTGGTGTCGGCCCCATTAGTAGC
BaL N144A	GGAACGACACCGCCACCACCAGCAGCAG
	CTGCTGCTGGTGGTGGCGGTGTCGTTCC
BaL N156A	GGCGAGATGAAGGCCTGCAGCTTCAAGATC
	GATCTTGAAGCTGCAGGCCTTCATCTCGCC
BaL N186A	GTGCCCATCGACGCCAACAGCAACAACC
	GGTTGTTGCTGTTGGCGTCGATGGGCAC
BaL N197A	CGCCTGATCAGCTGTGCCACCTCAGTCATTAC
	GTAATGACTGAGGTGGCACAGCTGATCAGGCG
BaL N241A	GAAAAGGACCATGTTCAGCTGTCAGCACAGTACAATG
	CATTGTACTGTGCTGACAGCTGAACATGGTCCTTTTC
BaL N262A	CAGCTGCTGCTGGCCGGCAGCCTGGC
	GCCAGGCTGCCGGCCAGCAGCAGCTG
BaL N289A	CATAATAGTACAGCTGGCTGAATCTGTAGAAATTAATTG
	CAATTAATTTCTACAGATTCAGCCAGCTGTACTATTATG
BaL N295A	GAATCTGTAGAAATTGCTTGTACAAGACCCAACAACAATACACGC
	GCGTGTATTGTTGTTGGGTCTTGTACAAGCAATTTCTACAGATTC
BaL N301A	GTACAAGACCCAACGCCAATACACGCAAGAGCATC
	GATGCTCTTGCGTGTATTGGCGTTGGGTCTTGTAC
BaL N332A	GAGAAATAATAGGAGATATAAGACAAGCACATTGTGCCCTTAGTAGAGCAAAATG
	CATTTTGCTCTACTAAGGGCACAATGTGCTTGTCTTATATCTCCTATTATTTCTC
BaL N339A	TAACCTTAGTAGAGCAAAATGGGCTGACACTCTGAACAAGATCGTG
	CACGATCTTGTTCAGAGTGTCAGCCCATTTTGCTCTACTAAGGTTA
BaL N356A	GCGAGCAGTTCGGCGCCAAGACCATCGTC
	GACGATGGTCTTGGCGCCGAACTGCTCGC
BaL N386A	GGAGGGGAATTTTTCTACTGTGCTTCAACACAACTGTTTAATAG
	CTATTAAACAGTTGTGTTGAAGCACAGTAGAAAAATTCCCCTCC
BaL N392A	CAACACAACTGTTTGCTAGTACTTGGAATG

	CATTCCAAGTACTAGCAAACAGTTGTGTTG
BaL N396A	GTTTAATAGTACTTGGGCTGTTACTGAAGAGTC
	GACTCTTCAGTAACAGCCCAAGTACTATTAAAC
BaL N406A	GAATGTTACTGAAGAGTCAGCTAACACTGTAG
	CTACAGTGTTAGCTGACTCTTCAGTAACATTC
BaL N411A	CAAATAACACTGTAGAAGCTAACACAATCACAC
	GTGTGATTGTGTTAGCTTCTACAGTGTTATTTG
BaL N448A	CAAATTCGCTGCAGCAGCGCCATCACCGGCC
	GGCCGGTGATGGCGCTGCTGCAGCGAATTTG
BaL N463A	CGGCCCAGAGGACGCCAAGACCGAGGTCTTC
	GAAGACCTCGGTCTTGGCGTCCTCTGGGCCG

Supplementary Table 6. Primers used for site-directed mutagenesis of BaL pseudovirus.

Mutation	Primer sequence (5′-to-3′)
Bal N130A	TCTGTGTTACTTTAGCTTGCACTGATTTGAG
	CTCAAATCAGTGCAAGCTAAAGTAACACAGA
Bal N262A	CAACTCAACTGCTGTTAGCTGGCAGTCTAGC
	GCTAGACTGCCAGCTAACAGCAGTTGAGTTG
Bal N295A	GAATCTGTAGAAATTGCTTGTACAAGACCCAAC
	GTTGGGTCTTGTACAAGCAATTTCTACAGATTC
Bal N301A	TGTACAAGACCCAACGCCAATACAAGAAAAAGT
	ACTTTTTCTTGTATTGGCGTTGGGTCTTGTACA
Bal N332A	GACAAGCACATTGTGCCCTTAGTAGAGCAA
	TTGCTCTACTAAGGGCACAATGTGCTTGTC
Bal N386A	GGAATTTTTCTACTGTGCTTCAACACAACTGTT
	AACAGTTGTGTTGAAGCACAGTAGAAAAATTCC
Bal N392A	TCAACACAACTGTTTGCTAGTACTTGGAATGTT
	AACATTCCAAGTACTAGCAAACAGTTGTGTTGA