Supporting Information

Figure S1. Amino acid alignment of Vif sequences derived from five major subtypes of HIV-1. Sequences were aligned using the Clustal W program. Dashes indicate identity with the NL4-3 *vif* sequence.

Figure S2. Optimization of plasmid DNA doses for transfection. (A and B) The mRNA levels of APOBEC3G (A) or APOBEC3F (B) endogenously expressed in PBMCs derived from two different donors, and of that expressed in cells transfected with serially diluted APOBEC3G or A108S-V231I polymorphic wild-type APOBEC3F expression plasmid (Kinomoto et al. 2007, Nucleic Acids Res. 35:2955-64) were compared. Using a real-time RT-PCR method as described below, introduction of 25 ng of APOBEC3G or of ~ 0.8 ng of APOBEC3F plasmid into 293T cells (closed red circle) reproduced the endogenous expression levels in PBMCs (upper panel). (C and D) In a parallel experiment, the infectivity of VSV-G-pseudotyped Δvif viruses produced from the transfected cells was examined by infecting 293T cells with the viruses and performing a luciferase assay. (E) Enhancement of virion infectivity by Vif, either from 1 μg of the luciferase reporter proviral construct pNL-Luc-E(-) (physiologically expressed Vif, [Tokunaga, et al. 2001, J. Virol., 75:6776-85]) or from the serially diluted Vif expression plasmid coupled with the VSV-G expression plasmid pHIT/G and the Vif-deficient luciferase reporter proviral construct pNL-Luc-F(-)E(-) in the presence of a fixed amount of the APOBEC3G plasmid (25 ng). The optimal dose of the Vif expression plasmid, reflecting its physiological expression, was 8 ng (closed red circle).

[Real-Time RT-PCR] Using the RNAqueous Kit (Ambion), total RNA was extracted from PBMCs from two donors or from 293T cells cotransfected with serially diluted APOBEC3G (200-1.5 ng) or APOBEC3F expression plasmid (200-0.2 ng), 0.1 μg of pHIT/G, 8 ng of NL-Vif expression plasmid, and 1 μg of pNL-Luc-F(-)E(-). Total RNA was treated with TURBO DNA-*free* (Ambion) according to the manufacturer's protocols. Real-time RT-PCR was performed with Mx3005P (Strategene) using the QuantiTect Probe RT-PCR (Qiagen) as described previously.

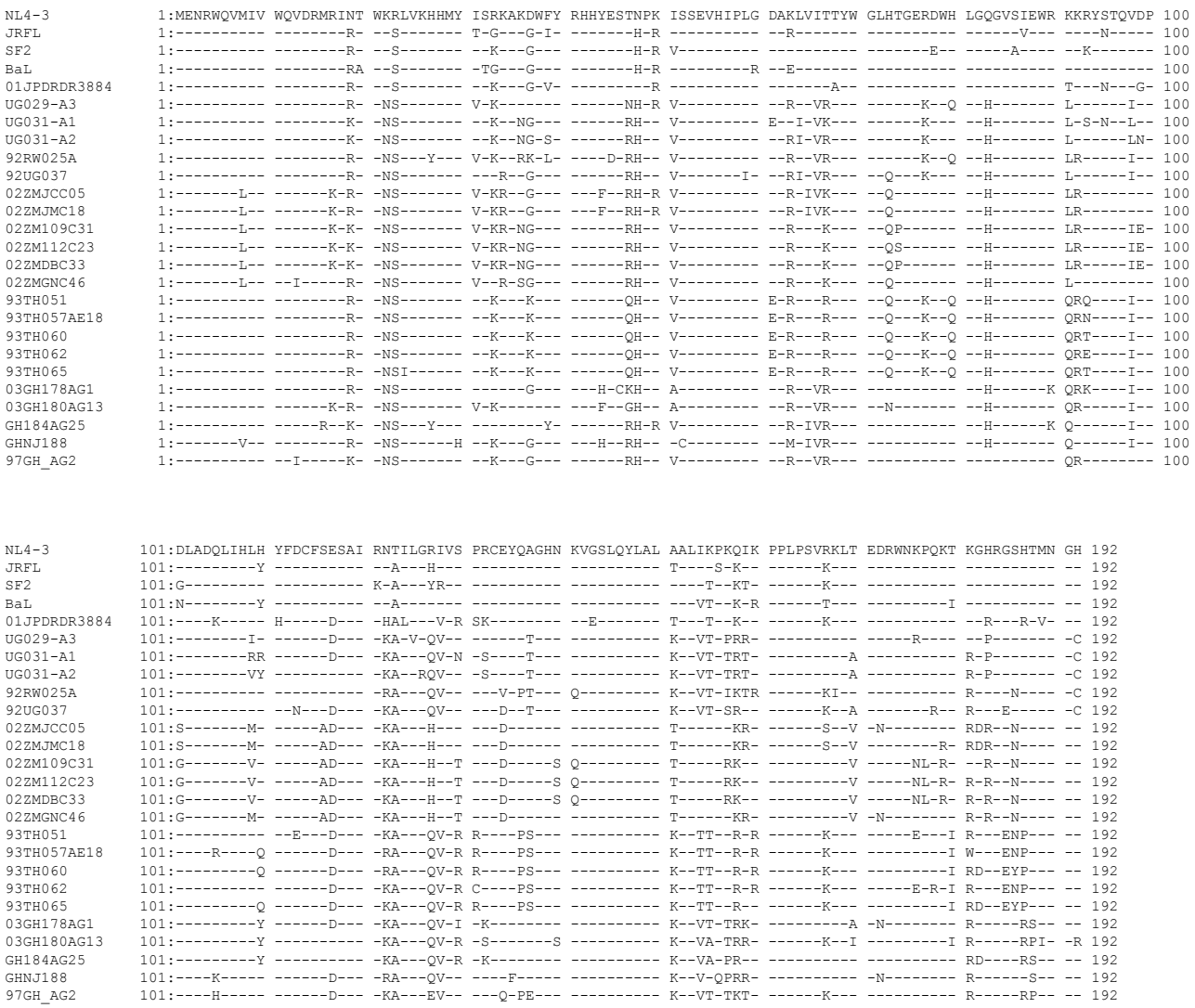
Figure S3. Further mutational analysis within the N-terminal (1-31) domain of subtype C–derived Vif protein. (A) N-terminal (1-31) amino acid alignment of Vif proteins expressed from NL4-3 (NL-Vif) and 02ZMDBC33 (DB-Vif) derived from subtypes B and C, respectively. Blue amino acid letters indicate consensus residues of each subtype. K/R in blue at position 17 of the DB-Vif protein shows that either lysine or arginine is conserved in 50% of all subtype C–derived Vif sequences. Asterisk at position 31 of DB-Vif shows that valine is highly conserved specifically in regionally circulating strains of subtype C viruses. (B and C) Functional testing for anti-APOBEC3G activity of the point mutant Vif proteins in the single-round replication assay described in Figure 2B. Results are representative of at least three independent triplicate experiments. RLU: relative light units. Data shown are mean ± SD; * $P <$ 0.01, *t*-test.

Figure S4. Binding activity of chimeric Vif proteins to Cul5 and EloC. 293T cells (7×10^5) were cotransfected using FuGENE6 with 400 ng each of pC-EloC-HA, pC-Cul5,

pC-Vif-FLAG-RRE, pCA-Rev, and empty vector. After 36 h, transfected cells were treated with 20 μM of MG-132 (Peptide Institute Inc.) for 9 h, and suspended in 500 μl of lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.5% NP-40, 20 μM MG-132 and complete protease inhibitor cocktail). The resultant lysates were clarified by brief centrifugation, pre-cleared with 30 μl of Protein G-Agarose (Sigma) for 1 h at 4°C, and then incubated with an anti-FLAG M2 Affinity gel. After 1 h at 4°C, the immune complexes were extensively washed with lysis buffer. Equal aliquots of the total and bound fractions were subjected to gel electrophoresis and transferred to a nitrocellulose membrane. The membranes were probed with an anti-HA rabbit polyclonal antibody (to detect EloC) (Sigma), a Cul5 antiserum (Santa Cruz), the anti-FLAG rabbit polyclonal antibody (to detect Vif), or the anti-β-actin mouse monoclonal antibody.

Figure S5. Replication kinetics of Vif recombinant viruses in PBMCs. PBMC (1×10^6) were infected overnight with 50 ng of p24 antigen of NL-WT, NL-DBvif, NL-DB(1-31)vif, or NL-Δvif viruses, washed extensively with PBS, and then cultured in fresh medium. Supernatants were sampled every 3 d, and p24 Gag antigen production was quantified by ELISA.

Figure S1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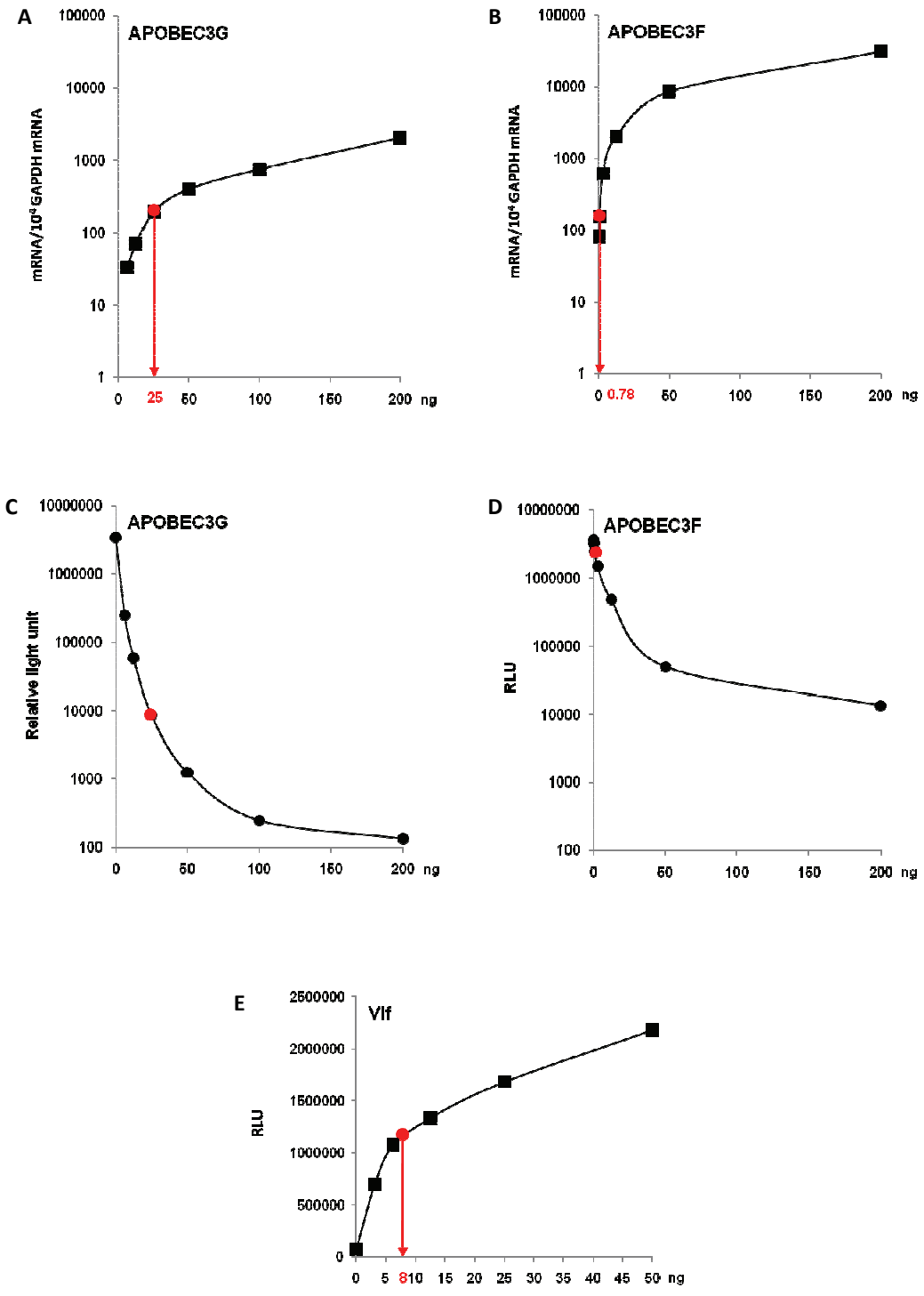
JRFL 1:---------- --------R- --S------- T-G---G-I- -------H-R ---------- --R------- ---------- ------V--- ----N----- 100
SF2 1:---------- --------R- --S------- --K---G--- -------H-R V--------- ---------- -------E-- -----A----- --K------- 100
BaL 1:---------- --------RA --S------- -TG---G--- -------H-R ---------R --E------- ---------- ---------- ---------- 100
01JPDRDR3884 1:---------- --------R- --S------- --K---G-V- ---------R ---------- -------A-- ---------- ---------- T---N---G- 100
UG029-A3 1:---------- --------R- -NS------- V-K------- ------NH-R V--------- --R--VR--- ------K--Q --H------- L------I-- 100
UG031-A1 1:---------- --------K- -NS------- --K--NG--- ------RH-- V--------- E--I-VK--- ------K--- --H------- L-S-N--L-- 100
UG031-A2 1:---------- --------K- -NS------- --K--NG-S- ------RH-- V--------- --RI-VR--- ------K--- --H------- L------LN- 100
92RW025A 1:---------- --------R- -NS---Y--- V-K--RK-L- ----D-RH-- V--------- --R--VR--- ------K--Q --H------- LR-----I-- 100
92UG037 1:---------- --------R- -NS------- ---R--G--- ------RH-- V-------I- --RI-VR--- --Q---K--- --H------- L------I-- 100
02ZMJCC05 1:-------L-- ------K-R- -NS------- V-KR--G--- ---F--RH-R V--------- --R-IVK--- --Q------- --H------- LR-------- 100
02ZMJMC18 1:-------L-- ------K-R- -NS------- V-KR--G--- ---F--RH-R V--------- --R-IVK--- --Q------- --H------- LR-------- 100
02ZM109C31 1:-------L-- ------K-K- -NS------- V-KR-NG--- ------RH-- V--------- --R---K--- --QP------ --H------- LR-----IE- 100
02ZM112C23 1:-------L-- ------K-K- -NS------- V-KR-NG--- ------RH-- V--------- --R---K--- --QS------ --H------- LR-----IE- 100
02ZMDBC33 1:-------L-- ------K-K- -NS------- V-KR-NG--- ------RH-- V--------- --R---K--- --QP------ --H------- LR-----IE- 100
02ZMGNC46 1:-------L-- --I-----R- -NS------- V--R-SG--- ------RH-- V--------- --R---K--- --Q------- --H------- L--------- 100
93TH051 1:---------- --------R- -NS------- --K---K--- ------QH-- V--------- E-R---R--- --Q---K--Q --H------- QRQ----I-- 100
93TH057AE18 1:---------- --------R- -NS------- --K---K--- ------QH-- V--------- E-R---R--- --Q---K--Q --H------- QRN----I-- 100
93TH060 1:---------- --------R- -NS------- --K---K--- ------QH-- V--------- E-R---R--- --Q---K--Q --H------- QRT----I-- 100
93TH062 1:---------- --------R- -NS------- --K---K--- ------QH-- V--------- E-R---R--- --Q---K--Q --H------- QRE----I-- 100
93TH065 1:---------- --------R- -NSI------ --K---K--- ------QH-- V--------- E-R---R--- --Q---K--Q --H------- QRT----I-- 100
03GH178AG1 1:---------- --------R- -NS------- ------G--- ---H-CKH-- A--------- --R--VR--- ---------- --H------K QRK----I-- 100
03GH180AG13 1:---------- ------K-R- -NS------- V-K------- ---F--GH-- A--------- --R--VR--- --N------- --H------- QR-----I-- 100
GH184AG25 1:---------- -----R--K- -NS---Y--- --------Y- ------RH-R V--------- --R-IVR--- ---------- --H------K Q------I-- 100
GHNJ188 1:-------V-- --------R- -NS------H --K---G--- ---H--RH-- -C-------- --M-IVR--- ---------- --H------- Q------I-- 100
97GH_AG2 1:---------- --I-----K- -NS------- --K---G--- ------RH-- V--------- --R--VR--- ---------- ---------- QR-------- 100

NL4-3 101:DLADQLIHLH YFDCFSESAI RNTILGRIVS PRCEYQAGHN KVGSLQYLAL AALIKPKQIK PPLPSVRKLT EDRWNKPQKT KGHRGSHTMN GH 192
JRFL 101:---------Y ---------- --A---H--- ---------- ---------- T----S-K-- ------K--- ---------- ---------- -- 192
SF2 101:G--------- ---------- K-A---YR-- ---------- ---------- ----T--KT- ------K--- ---------- ---------- -- 192
BaL 101:N--------Y ---------- --A------- ---------- ---------- ---VT--K-R ------T--- ---------I ---------- -- 192
01JPDRDR3884 101:----K----- H-----D--- -HAL---V-R SK-------- --E------- T---T--K-- ------K--- ---------- --R---R-V- -- 192
UG029-A3 101:--------I- ------D--- -KA-V-QV-- ------T--- ---------- K--VT-PRR- ---------- -----R---- --P------- -C 192
UG031-A1 101:--------RR ------D--- -KA---QV-N -S----T--- ---------- K--VT-TRT- ---------A ---------- R-P------- -C 192
UG031-A2 101:--------VY ---------- -KA--RQV-- -S----T--- ---------- K--VT-TRT- ---------A ---------- R-P------- -C 192
92RW025A 101:---------- ---------- -RA---QV-- ---V-PT--- Q--------- K--VT-IKTR ------KI-- ---------- R----N---- -C 192
92UG037 101:---------- --N---D--- -KA---QV-- ---D--T--- ---------- K--VT-SR-- ------K--A -------R-- R---E----- -C 192
02ZMJCC05 101:S-------M- -----AD--- -KA---H--- ---D------ ---------- T------KR- ------S--V -N-------- RDR--N---- -- 192
02ZMJMC18 101:S-------M- -----AD--- -KA---H--- ---D------ ---------- T------KR- ------S--V --------R- RDR--N---- -- 192
02ZM109C31 101:G-------V- -----AD--- -KA---H--T ---D-----S Q--------- T-----RK-- ---------V -----NL-R- --R--N---- -- 192
02ZM112C23 101:G-------V- -----AD--- -KA---H--T ---D-----S Q--------- T-----RK-- ---------V -----NL-R- R-R--N---- -- 192
02ZMDBC33 101:G-------V- -----AD--- -KA---H--T ---D-----S Q--------- T-----RK-- ---------V -----NL-R- R-R--N---- -- 192
02ZMGNC46 101:G-------M- -----AD--- -KA---H--T ---D------ ---------- T------KR- ---------V -N-------- R-R--N---- -- 192
93TH051 101:---------- --E---D--- -KA---QV-R R----PS--- ---------- K--TT--R-R ------K--- -----E---I R---ENP--- -- 192
93TH057AE18 101:----R----Q ------D--- -RA---QV-R R----PS--- ---------- K--TT--R-R ------K--- ---------I W---ENP--- -- 192
93TH060 101:---------Q ------D--- -RA---QV-R R----PS--- ---------- K--TT--R-R ------K--- ---------I RD--EYP--- -- 192
93TH062 101:---------- ------D--- -KA---QV-R C----PS--- ---------- K--TT--R-R ------K--- -----E-R-I R---ENP--- -- 192
93TH065 101:---------Q ------D--- -KA---QV-R R----PS--- ---------- K--TT--R-- ------K--- ---------I RD--EYP--- -- 192
03GH178AG1 101:---------Y ------D--- -KA---QV-I -K-------- ---------- K--VT-TRK- ---------A -N-------- R-----RS-- -- 192
03GH180AG13 101:---------Y ---------- -KA---QV-R -S-------S ---------- K--VA-TRR- ------K--I ---------I R-----RPI- -R 192
GH184AG25 101:---------Y ---------- -KA---QV-R -K-------- ---------- K--VA-PR-- ---------- ---------- RD----RS-- -- 192
GHNJ188 101:----K----- ------D--- -RA---QV-- ----F----- ---------- K--V-QPRR- ---------- -N-------- R------S-- -- 192
97GH_AG2 101:----H----- ------D--- -KA---EV-- ---Q-PE--- ---------- K--VT-TKT- ------K--- ---------- R-----RP-- -- 192

Figure S2


A
APOBEC3G
mRNA/10⁴ GAPDH mRNA
100000
10000
1000
100
10
1
0
25
50
100
150
200 ng
B
APOBEC3F
mRNA/10⁴ GAPDH mRNA
100000
10000
1000
100
10
1
0
0.78
50
100
150
200 ng
C
APOBEC3G
Relative light unit
10000000
1000000
100000
10000
1000
100
0
50
100
150
200 ng
D
APOBEC3F
RLU
10000000
1000000
100000
10000
1000
100
0
50
100
150
200 ng
E
Vif
RLU
2500000
2000000
1500000
1000000
500000
0
0
5
8
10
15
20
25
30
35
40
45
50 ng

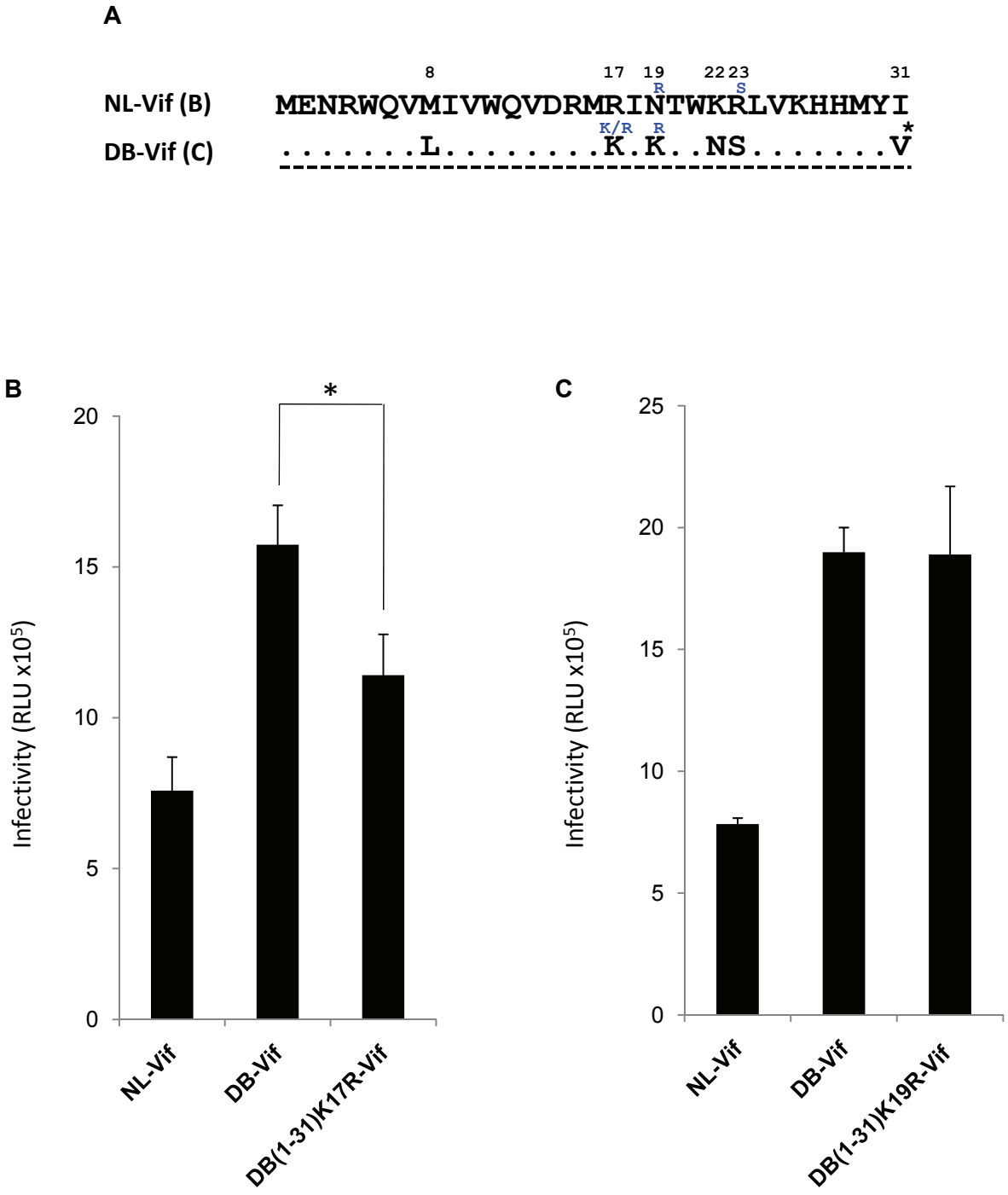

A
8
17
19
22
23
31
NL-Vif (B)
MENRWQVMIVWQVDRMRINTWKRLVKHHMYI
R
S
K/R
R
DB-Vif (C)
.....L.....K.K..NS.....V*
B
*
Infectivity (RLU x10^5)
20
15
10
5
0
NL-Vif
DB-Vif
DB(1-31)K17R-Vif
C
Infectivity (RLU x10^5)
25
20
15
10
5
0
NL-Vif
DB-Vif
DB(1-31)K19R-Vif

Figure S4

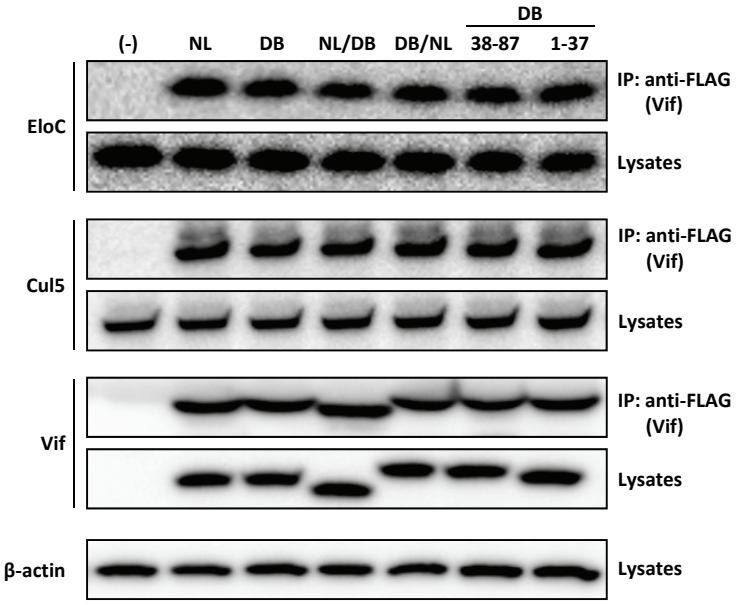
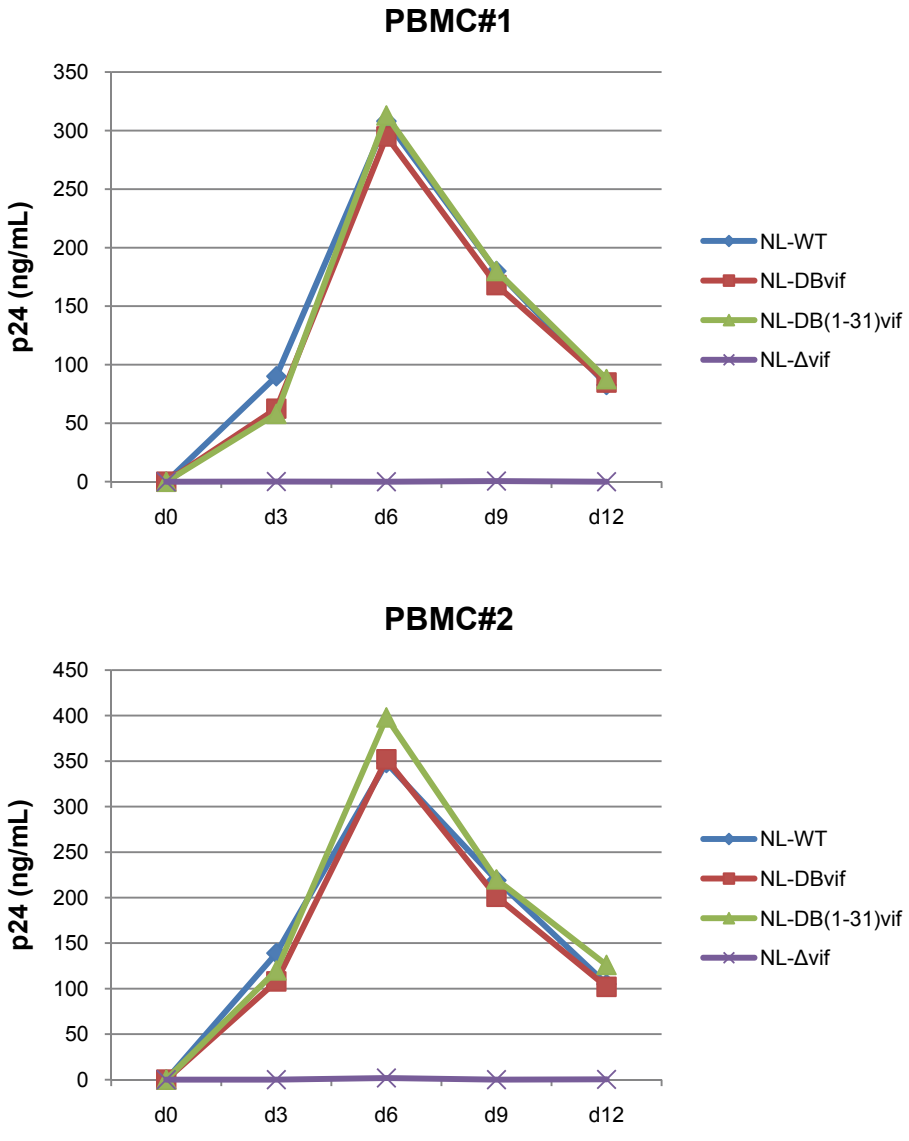

DB
(-)
NL
DB
NL/DB
DB/NL
38-87
1-37
EloC
IP: anti-FLAG (Vif)
Lysates
Cul5
IP: anti-FLAG (Vif)
Lysates
Vif
IP: anti-FLAG (Vif)
Lysates
β-actin
Lysates

Figure S5


PBMC#1
p24 (ng/mL)
350
300
250
200
150
100
50
0
d0
d3
d6
d9
d12
NL-WT
NL-DBvif
NL-DB(1-31)vif
NL-Δvif
PBMC#2
p24 (ng/mL)
450
400
350
300
250
200
150
100
50
0
d0
d3
d6
d9
d12
NL-WT
NL-DBvif
NL-DB(1-31)vif
NL-Δvif