a

I	gGag1		gGag3	
#	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA		AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	
1	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
2	GCTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT	AGATGAGAGTACCAAGG --AAGTGACATAGCAGG	-2
3	GTTAAAAGAGACCATC- ATGAGGAAGCTGCAGAA	-1	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
4	GTTAAAAGAGACCATCAC ATGAGGAAGCTGCAGAA	+1	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
5	GTTAAAAGAGACCA--- -TGAGGAAGCTGCAGAA	-4	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
6	GTTAAAAGAG------- ------AAGCTGCAGAA	-13	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
7	GTTAAAAGAGACCATCA ATGGGAAGCTGCAGAA	NS	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
8	GTTAAAAGAGAGGA--- -------AGCTGCAGAA	-10	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
9	GTTAAAAGAGACCA--- ---------GGCAGAA	-13/NS	AGATGAGAGAACCAAGG -GAAGTGACATAGCAGG	-1
10	GTTAAAAGAG------- -----GAAGCTGCAGAA	-12	AGATGAGAGAACCAAGG --AAGTGACATAGCAGG	-2
11	GTTAAAAGAGACCATC- -TGAGGAAGCTGCAGAA	-2	AGATGAGAGAACCAAGG --GTGTGACATAGCAGG	-2/NS
12	GTTAAAAGAGACCA--- ----------GAG	-17/NS	AGATGAGAGAACCAAGG --GAGTGACATAGCAGG	-2/NS
13	GTTAAAAGAGACCATC- ----------	-50	AGATGAGAGAACCAAGG C----------	NS/-23
14	GTTAAAAGAGACCTA-- --GAGGAAGCTGCAGAA	NS/-4	---------- ---AGTGACATAGCAGG	-24
15	GTTAAAAGAGACCAGGCC ATGAGGAAGCTGCAGAA	NS/+1	AGATGAGAG------- ----------	-44
16	GTTAAA---------- --GAGGAAGCTGCAGAA	-13	---------- GGAAGTGACATAGCAGG	-28
17	GTTAAAAGAGACCATCA -TGAGGAAGCTGCAGAA	-1	AGATGAGAGAACCAAGG GCGAAGTGACATAGCAGG	+1
18	GTTAAAAGAGACCACT- --GAGGAAGCTGCAGAA	-3	AGATGAGAGAACCAAGG -GAAGTGACATAGCAGG	-1
19	GTTAAAAGAGACCATCA		GGAAGTGACATAGCAGG	EX
20	GTTAAAAGAGACCATCA		GGAAGTGACATAGCAGG	EX
21	----------		----------	EX -156
22	GTTAAAAGAGACC----		----------AGCAGG	EX -15
23	GTTAAAAGAGGAAGCTTAACA		GGAAGTGACATAGCAGG	EX +4

b

II	gGag1		gGag3	
#	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA		AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	
1	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
2	GTTAAAAGGGACCATCA ATGAGGAAGCTGCAGAA	WT	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
3	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
4	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT	AGA---------- ----------	-31
5	GGTAA---------- ------AAGCTGCAGAA	-18	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
6	GTTAAAAGAGACCA--- ATGAGGAAGCTGCAGAA	-3	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
7	GTTAAAAGAGACCAT-- ----------CAGAA	-14	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
8	GTTAAAAGA-------- --GAGGAAGCTGCAGAA	-10	AGATGAGAGAACCAAGG --AAGTGACATAGCAGG	-2
9	GTTAAAAGAGACC---- ATGAGGAAGCTGCAGAA	-4	---------- ----GGGACATAGCAGG	-36
10	GTTAAAAGAGACCATCA ----------	-17	AGATGAGAGAACCAAGG --AAGTGACATAGCAGG	-2
11	GTT---------- --TAGGAAGCTGCAGAA	-16/NS	AGG---------- GGAAGTGACATAGCAGG	NS/-14
12	GTTAAAAGAGACCATATGTTACTA ATGAGGAAGCTGCAGAA	NS/+7	AGATGAGAGAACCAAGG --AAGTGACATAGCAGG	-2
13	GTTAAAAGAGACCATCC ATGAGGAAGCTGCAGAA	NS	AGATGAGAGAAC------ ----------	-45
14	---------- --------GCTGCAGAA	-31	AGATGAGAGAACCAAGG GATCGAAGGGACATAGCAGG	+4/NS
15	---------- ------AAGCTGCAGAA	-73	AGATGAGAGAACCAAGG ----------CAGG	-13
16	GTTAAAA---------- -----GAAGCTGCAGAA	-15	AGATGAGAGAACCAAG- -----TGACATAGCAGG	-6
17	GTTAAAAGAGACCATTAGG ATGAGGAAGCTGCAGAA	NS/+2	AGATGAGAGAACCAAGG GCGGAAGTGACATAGCAGG	+2
18	GTTAAAAGAGACCATCA ----------CAGAA	-12	AGATGAGAGAACCAAGG GTGGAAGTGACATAGCAGG	+2
19	GTTAAAAGAGACCATC-		GGAAGTGACATAGCAGG	EX -1
20	GTTAAAAGAGACCATCA		GGAAGTGACATAGCAG	EX
21	GTTAAAAGAGACTA---		----------AGCAGG	EX -14
22	GTTAAAAGAGACCATCA		GGAAGTGACATAGCAGG	EX
23	GTTAAAAGAGACCATCA		----------GG	EX -15

c

III	gGag1		gGag3	
#	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA		AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	
1	GTTAAAAGAGACCAT-- --GAGGAAGCTGCAGAA	-4	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
2	GTTAAAAGAGACCCA-- ATGAGGAAGCTGCAGAA	NS/-2	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
3	GTTA---------- ----------GC	-28/NS	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
4	GTTAAAAGAG------- -----GAAGCTGCAGAA	-11	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
5	CA---------- ATGAGGAAGCTGCAGAA	NS/-15	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
6	GTTAAAAGAGACCATC- ATGAGGAAGCTGCAGAA	-1	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
7	GTTAAAAGAGACCCA-- ATGAGGAAGCTGCAGAA	NS/-2	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
8	GTTAAAAGAGACC---- ----------GAA	-18	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
9	G---------- ----------AGGAA	-28/NS	AGATGTGAGAACCAAGG GGAAGTGACATAGCAGG	WT
10	---------- ----------	-36	AGATGAGAGAACCAAGG GGAAGTGACATAGCAGG	WT
11	GTTAAAAGAGACC---- ATGAGGAAGCTGCAGAA	-4	AGATGAGAGAACCAAGG -AAAGTGACATAGCAGG	-1/NS
12	GTTAAAAGAGACCCC-- -TGAGGAAGCTGCAGAA	NS/-3	AGATGAGAGAACCAAGG -----TGACATAGCAGG	-5
13	GTTAAAAGGCA------ ATGAGGAAGCTGCAGAA	-6	---------- ----------ATG	-32
14	GTTAAAAGAGACCATC- -------AGCTGCAGAA	-8	AGATGAGAGAACCAAGG -----GAGCATAGCAGG	-5/NS
15	GTTAAAAGAGACCAT-- ----------	-20	AGATGAGAGAACCAAGG AAGGAAGTGACATAGCAGG	+2
16	GTTAAAAGA-------- --GAGGAAGCTGCAGAA	-10	AGATGAGAGAAAAC--- --------CATAGCAGG	NS/-11
17	---------- ----------	-38	AGATGAGAGAACCAAGG -GAAGTGACATAGCAGG	-1
18	GTTAA---------- ------AAGGTGCAGAA	-18	AGATGAGAGAACCAAGG --AAGTGACATAGCAGG	-2
19	G----------		----------GAGCAG	EX -27/NS
20	GTTAAAAGAGACCATC-		----------GAGCAGG	EX -11/NS
21	GTTAAAAGAGACCATCA		GGAAGTGACATAGCAGG	EX
22	GTTAAAAGAGACCATCA		GGAAGTGACATAGCAGG	EX
23	GTTAAAAGAGACCATCA		GGAAGTGACATAGCAGG	EX
24	GTTAAAAGAGACCATC-		GGAAGTGACATAGCAGG	EX -1

Figure S1. Sequence analysis of gGag1+gGag3 targeted HIV-rtTA DNA. SupT1 cells stably expressing Cas9+gGag1+gGag3 were infected with HIV-rtTA and cellular DNA was isolated at 7 days post-infection as described in Figure 3 (n=3; **a-c**). The gRNA target regions were amplified by PCR, the PCR products were TA cloned and 23-24 cloned fragments were sequenced. Sequences were aligned to the wild-type reference HIV-LAI sequence (highlighted in yellow; PAM sequence in bold) and 17 nucleotides on either side of the Cas9 cleavage sites (indicated by black arrows) are shown. Wild-type (WT; green), mutation (white) and excision (EX; blue) products are indicated (mutations in red; NS, nucleotide substitution; -n, n nucleotides deleted; +n, n nucleotides inserted).

a HIV-LAI

I	gGag1	
#	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	
1	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
2	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
3	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
4	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
5	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
6	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
7	GTTAAAA---------- --GAGGAAGCTGCAGAA	-12
8	GTTAAAAGAGACCATC- ATGAGGAAGCTGCAGAA	-1
9	GTTA---------- ----------	-57
10	GTTAAAAGAGACCATCAC CTGAGGAAGCTGCAGAA	+1/NS
11	GTTAAAAGAGACAGAAT GGGAGGAAGCTGCAGAA	NS
12	GTTAAAAGA-------- -----GAAGCTGCAGAA	-13
13	GT---------- ---------CTGCAGAA	-24
14	GTTAAAAGAGA------ ---------CTGCAGAA	-15
15	GTTCCAAGAGACCGTT- ATAAGGAAGCTGCAGAA	NS/-1
16	GTTAAAAGAGACCTCAT ----CGAAGCTGCAGAA	NS/-4
17	GTTAA---------- ATGAGGAAGCTGCAGAA	-12
18	GTTAAAAGAGACCATC- ----------GCAGAA	-11
19	GTTA---------- ----------AGAG	-26/NS
20	GTTAAAAGAGACCTTCT ATGAGGAAGCTGCAGAA	NS
21	GTTAAAA---------- --GAGGAAGCTGCAGAA	-12
22	GTTAAAAGAGACC---- ATGAGGAAGCTGCAGAA	-14
23	GTTAAAAG-------- ----------	-44

b

II	gGag1	
#	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	
1	GTTAAAAGAGACCATCA ATGAGGAAGCTGCGGAA	WT
2	GTTAAAAGAGACCATC- ATGAGGAAGCTGCAGAA	-1
3	GTTAAAAGAGACC---- ATGAGGAAGCTGCAGAA	-4
4	GTTAAAAGAGAC----- ---------CTGCAGAA	-14
5	GTTAAA---------- ---AGGAAGCTGCAGAA	-14
6	GTTAAAAGAGACCATC- ATGAGGAAGCTGCAGTA	-1
7	GTTAAAAGAGACCATCAGA ATGAGGAAGCTGCAGAA	+2
8	GTTAAAAGAGACCATC- ATGAGGAAGCTGCAGAA	-1
9	GTTAAAAGAGACC---- ATGAGGAAGCTGCAGAA	-4
10	GTTAAAAGAGACCATCA -----CCAGCTGCAGAA	-5/NS
11	---------- ----------	-40
12	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
13	---------- ----GGAAGCTGCAGAA	-22
14	GTTAAAAGAGACA---- ATGAGGAAGCTGCAGAA	-4
15	GTTAAAAGAGACA---- ATGAGGAAGCTGCAGAA	-4
16	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1

c

III	gGag1	
#	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	
1	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
2	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
3	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
4	GTTAAAAGAGACGAA-- ATGAGGAAGCTGCAGAA	NS/-2
5	GTTAAAAGAGACCA--- ATGAGGAAGCTGCAGAA	-3
6	GCTAAAAGAGACCATCACCCT ATGAGGAAGCTGCAGAA	+4
7	GTTAAAAGAGACCATCTT ATGAGGAAGCTGCAGAA	NS/+1
8	GTTA---------- ----------CAGAA	-19
9	GTTAAAAGAGACA---- ATGAGGAAGCTGCAGAA	-4
10	---------- ----------	-79
11	GTTAAAAGAGAC----- ---------CTGCAGAA	-14
12	GTTAAAAGAGACCATC- ATGAGGAAGCTGCAGAA	-1
13	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1

d HIV-rtTA

I	gGag1	
#	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	
1	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
2	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
3	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
4	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
5	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
6	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
7	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
8	GTTAGAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
9	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
10	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
11	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
12	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
13	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
14	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
15	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
16	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
17	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
18	---------- ----------	-67
19	---------- --------GCTGCAGAA	-26
20	GTTAAAAGAGACCATC- ATGAGGAAGCTGCAGAA	-1
21	GTTAAAAGAGACCATCAACC ATGAGGAAGCTGCAGAA	+3
22	GTTAAAAGAG------- ----------	-34
23	GTTAAAAGAGACCATCC ATGAGGAAGCTGCAGGA	NS
24	GTTAAAAGAGACCATCA GTGAGGAAGCTGCAGAA	NS

e

II	gGag1	
#	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	
1	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
2	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
3	GTTAAAAG---------- -------AGCTGCAGAA	-16
4	GTTAAAAGAGACCATC- ------GAGCTGCAGAA	-7/NS
5	GTTAAAAGAGACCAT-- ATGAGGAAGCTGCAGAA	-2
6	GTTAAAAGAGACC---- ATGAGGAAGCTGCAGAA	-4
7	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
8	GTTAAAAGAGACC---- ATGAGGAAGCTGCAGAA	-4
9	GTTAAAAGAGACCATC- ATGAGGAAGCTGCAGAA	-1
10	GTTAAAAGAGACCA--- ----------GCAGAA	-14
11	G---------- ----------	-42
12	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
13	GTTAAAAG---------- -------AGCTGCAGAA	-16

f

III	gGag1	
#	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	
1	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
2	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
3	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
4	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
5	GTTAAAAGAGACCATC- ATGAGGAAGCTGCAGAA	-1
6	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
7	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
8	GTTAAAAGAGACCATCAC ATGAGGAAGCTGCAGAA	+1
9	GTTAAAAGAGACCATC- ATGGGGAAGCTGCAGAA	-1/NS
10	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
11	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
12	GTTAAAAGAGACC---- ATGAGGAAGCTGCAGAA	-4
13	GTTAAAAGAGACCAT-- CTGAGGAAGCTGCAGAA	-2/NS
14	---------- ----------	-149
15	---------- ----------	-76
16	---------- -----GAAGTTGCAGAA	-111/NS

Figure S2. Sequence analysis of gGag1+gTatRev targeted proviral DNA. SupT1 cells stably expressing Cas9+gGag1+gTatRev were infected with HIV-LAI (n=3; **a-c**) or HIV-rtTA (n=3; **d-f**) and cellular DNA was isolated at 7 days post-infection as described in Figure 5. The gGag1 target region was amplified through PCR, PCR products were TA cloned and 13-24 clones were sequenced. Sequences are shown as described in Supplementary Figure S1.

a HIV-LAI

I	gGag1	
#	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	
1	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
2	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
3	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
4	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
5	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
6	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
7	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
8	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
9	GTTAAAAGAGACCATCA -----GAAGCTGCAGAA	-5
10	---------- --GAGGAAGCTGCAGAA	-22
11	GTTAAAAGAGAC----- ----TTAAGCTGCAGAA	-9/NS
12	GTTAAA---------- ---AGGAAGCTGCAGAA	-14
13	GTTAAAAGAGACA---- ATGAGGAAGCTGCAGAA	NS/-4
14	---------- ----------	-80
15	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
16	GTTA---------- ----------GCAGAA	-24
17	GTTAAAAGAGACC---- ATGAGGAAGCTGCAGAA	-4
18	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
19	GTTAAAAGAGACCAT-- ---AGGAAGCTGCAGAA	-5
20	GTTAAAAGAGACCATCAGA ATGAGGAAGCTGCAGAA	+2
21	GTTAAAAGAGACC---- ----GGAAGCTGCAGAA	-8
22	GTTAAAAGAGACC---- ATGAGGAAGCTGCAGAA	-4
23	GTTAAAAGAGAC----- ATGAGGAAGCTGCAGAA	-5

b

II	gGag1	
#	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	
1	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
2	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
3	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
4	GTTAAAAGAGACCATCA --GAGGAAGCTGCAGAA	-2
5	GTTAAAAGAGACCA--- ATGAGGAAGCTGCAGAA	-3
6	GTTAAAAGAGACA---- ATGAGGAAGCTGCAGAA	NS/-4
7	GTTAAAAGAGACC---- ATGAGGAAGCTGCAGAA	-4
8	GTTAAAAGAGACCATC- ATGAGGAAGCTGCAGAA	-1
9	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
10	GTTAAAAGAGACCATCA ----------AGCAGAA	-10/NS
11	GTTAAAAGAGACCATCA ----------	-17
12	GTTAAAAGAGACCATCA -----TAAGCTGCAGAA	-5/NS
13	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
14	GTTAAA---------- ---AGGAAGCTGCAGAA	-14
15	GTCAAAAGAGACCATC- ATGAGGAAGCTGCAGAA	-1
16	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1

c

III	gGag1	
#	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	
1	GTTAAAAGAGACCATC- ATGAGGAAGCTGCAGAA	-1
2	GTTAAAAGAGAC----- ----TCAAGCTGCAGAA	-9/NS
3	GTTAAAAGAGACCATCCCATT CTGAGGAAGCTGCAGAA	NS/+4
4	GTTAAAAGAGACCATC- ---AGGAAGCTGCAGAA	-4
5	G---------- ----------	-35
6	GTTAAAAGA-------- ATGAGGAAGCTGCAGAA	-8
7	GTTAAAAGAGACCATCAGG ATGAGGAAGCTGCAGAA	+2
8	GTTAAAAGAGACC---- ATGAGGAAGCTGCAGAA	-4
9	---------- ATGAGGAAGCTGCAGAA	-19
10	GTTA---------- ----------CGACAGAA	-22
11	GTTAAAAGAGACCA--- -----GGAGCTGCAGAA	-8/NS
12	GTT---------- ---------CTGCAGAA	-23
13	GTTAAAAGAGA------ -------AGCTGCAGAA	-13
14	---------- ----------	-42
15	---------- ATGAGGAAGCTGCAGAA	-33

d HIV-rtTA

I	gGag1	
#	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	
1	GTTAGAAGAGACCATCA ATGAGGGAGCTGCAGAA	WT
2	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
3	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
4	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
5	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
6	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
7	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
8	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
9	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
10	GTTAAAAGAGAC----- ------AAGCTGCAGAA	-11
11	GTTAAAAGAGACCATCTTTTAAC ATGAGGAAGCTGCAGAA	NS/+6
12	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
13	GTTAAAAGAGACCATCAGA ATGAGGAAGCTGCAGAA	+2
14	GTTAAAAGAGAC----- ---------CTGCAGAA	-14
15	GTTAAAAGAG------- ------TGGCTGCAGAA	-13/NS
16	GTTAAA---------- ---AGCGGGCTGCAGAA	-14/NS
17	GTTAAAAGAGACCT--- CTGAGGAAGCTGCAGAA	NS/-3
18	GTTAAAAGAGACCATC- ATGAGGAAGCTGCAGAA	-1
19	GTTAAAA---------- --GAGGGAGCTGCAGAA	-12
20	GTTAAAAGAGAC----- ATGAGGAAGCTGCAGAA	-5
21	---------- --------GCCGCAGAA	-25/NS

e

II	gGag1	
#	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	
1	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
2	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
3	GTTAAAAGAGACCATCATTTGT ATGAGGAAGCTGCAGAA	+5
4	GTTAAGA---------- ATGAGGAAGCTGCAGAA	-10
5	---------- ----------	-129
6	GTTAAAAGAGACC---- ATGAGGAAGCTGCAGAA	-4
7	G---------- ----GGAAGCTGCAGAA	-20
8	GTTAAAAGA-------- --GAGGAAGCTGCAGAA	-10
9	GTTAAAAGAGACCATCATTTGT ATGAGGAAGCTGCAGAA	+5
10	GTTAAA---------- ---AGGAAGCTGCAGAA	-14
11	GTTAAAAGAGACCATC- ATGAGGAAGCTGCAGAA	-1
12	GTTAAAAGAGACCATCAAA ATGAGGAAGCTGCAGAA	+2
13	---------- ----------	-79
14	GTTAAAAGAGACCATC- ATGAGGAAGCTGCAGAA	-1
15	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
16	GTTAAA---------- ---AGGAAGCTGCAGAA	-14

f

III	gGag1	
#	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	
1	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
2	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
3	GTTAAAAGAGACCATCA ATGAGGAAGCTGCAGAA	WT
4	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
5	GTTAAAAGAGACCATCAGA ATGAGGAAGCTGCAGAA	+2
6	GTTAAA---------- ---AGGAAGCTGCAGAA	-14
7	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
8	GTTAAAAGAGAC----- --AGGGAAGCTGCAGAA	-7/NS
9	GTTAAA---------- ---AGGAAGCTGCAGAA	-14
10	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1
11	GTTAAAAGAGACCAT-- ----------	-20
12	GTTAA---------- ---AGGAAGCTGCAGAA	-15
13	GTTAAAAGAGACCA--- --AAGGAAGCTGCAGAA	-5/NS
14	GTTAAAAGAGAA----- AGGAGGAAGCTGCAGAA	NS/-5
15	GTTAAAAGAGACCATCAA ATGAGGAAGCTGCAGAA	+1

Figure S3. Sequence analysis of gGag1+gEnv2 targeted proviral DNA. SupT1 cells stably expressing Cas9+gGag1+gEnv2 were infected with HIV-LAI (n=3; **A-C**) or HIV-rtTA (n=3; **D-F**) and cellular DNA was isolated at 7 days post-infection (as described in Figure 4). The gGag1 target region was amplified through PCR, PCR products were TA cloned and 15-23 clones were sequenced. Sequences are shown as described in Supplementary Figure S1.

Table S1. Primers and probe used for PCR and qPCR analysis

name	sequence
gGag1-forward (a)	ACCTAGAACTTTAAATGCATGG
gGag3-reverse (b)	CGGTCTACATAGTCTCTAAAGG
gTatRev-forward (c)	GAGCCAGTAGATCCTAGAC
gTatRev-reverse (d)	CTACTACTAATGCTGCTATTGC
gEnv2-forward (c)	GCAAAGAGAAGAGTGGTG
gEnv2-reverse (d)	AGCCAGGATTCTTGCC
gGag1-probe	CCCATGTTTTCAGCATTATCAGAAGGAGCC

Table S2. Selected gRNAs targeting HIV-1

Name	Target Sequence + PAM (underlined)	Orientation
gGag1	GTTAAAAGAGACCATCAATGAGG	Sense
gGag3	CCAAGGGGAAGTGACATAGCAGG	Antisense
gTatRev	CCTATGGCAGGAAGAAGCGGAGA	Antisense
gEnv2	GGAGCAGCAGGAAGCACTATGGG	Sense