7/13/16

SUPPLEMENTARY FIGURES

Negative Feedback Regulation of HIV-1 by Gene Editing Strategy

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Table SI

primer	sequence
1. Cloning pX260-LTR-Cas9 constructs	
Kpn1-LTR(-454)-F	5'-**GGTACC**TGGAAGGGCTAATTTGG-3'
Kpn1-LTR(-120)-F	5'**-GGTACC**TCGAGCTTTCTACAAGG-3'
Xba1-LTR(-80)-F	5'**-TCTAGA**GGAGGTGTGGCCTGGGC-3'
LTR(+66)-**Nco1**-R	5'**-CCATGG**TAAGCAGTGGGTTCC-3'
2. Cloning lentiLTR(-80/+66)-Cas9-Blast construct	
Nhe1-LTR(-80)-F	5'**-GCTAGC**GGAGGTGTGGCCTGGGC-3'
LTR(+66)-**Xba1**-R	5'**-TCTAGA**TAAGCAGTGGGTTCC-3'
3. PCRs	
LTR -417/F	5'-GATCTGTGGATCTACCACACACA-3'
LTR -19/R	5'-GCTGCTTATATGTAGCATCTGAG-3'
LTR -374/F	5'-TTAGCAGAACTACACACCAGGGCC-3'
LTR +43/R	5'-CCGAGAGCTCCCAGGCTCAGATCT-3'
HIV-1 5'UTR +97/F	5'-AAGTAGTGTGTGCCCGTCTG-3'
HIV-1 5'UTR +235/R	5'-TCGAGAGATCTCCTCTGGCT-3'
HIV-1 Env +5828/F	5'- TCCTTGGGATGTTGATGATCT-3'
HIV-1 Env +5977/R	5'- TGGCCCAAACATTATGTACC-3'
b- actin/F	5'-CTACAATGAGCTGCGTGTGGC-3'
b-actin/R	5'-CAGGTCCAGACGCAGGATGGC-3'
4. Taqman qPCRs	
HIV-1 5'UTR F	5'-AAGTAGTGTGTGCCCGTCTG-3'
HIV-1 5'UTR R	5'-TCGAGAGATCTCCTCTGGCT-3'
HIV-1 5'UTR probe	5'-FAM-CTGTTCGGGCGCCACTGCTA-ZEN-IowaBlackFQ-3'
HIV-1 env F	5'- TCCTTGGGATGTTGATGATCT-3'
HIV-1 env F	5'- TGGCCCAAACATTATGTACC-3'
HIV-1 env probe	5'-FAM-TGGTGGTTGCTTCTTTCCACACA-ZEN-IowaBlackFQ-3'
Hs b-globin F	5'-CCCTTGGACCCAGAGGTTCT-3'
Hs b-globin R	5'-CGAGCACTTTCTTGCCATGA-3'
Hs b-globin probe:	5'-FAM-GCGAGCATCTGTCCACTCCTGATGCTGTTATGGGCGCTCGC-ZEN-IowaBlackFQ-3'
Hs b-actin F	5'-TGGACTTCGAGCAAGAGATG-3'
Hs b-actin R	5'-GAAGGAAGGCTGGAAGAGTG-3'
Hs b-actin probe:	5'-FAM-CGGCTGCTTCCAGCTCCTCC-ZEN-IowaBlackFQ-3'

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Position and nucleotide sequences of gRNA A/B targets within the LTR (highlighted in green, PAM in red) and LTR specific primers used in PCR on TZM-bl and in vitro infected Jurkat cells genomic DNA (highlighted in blue) in the reference HIV-1 NL4-3 genome. Sequences and sizes of LTR specific PCR products (full-length and truncated) and predicted edited fragment.

Figure S2. A representative agarose gel analyzing LTR specific PCR reactions used for quantification of Cas9/gRNA mediated LTR excision efficiency in experiments using the Jurkat 2D10 reporter cell line from Figures 3 and 4.

Figure S3. Position and nucleotide composition of LTR gRNA A/B targets (highlighted in green, PAM in red) and LTR specific primers used to analyze excision by PCR in Jurkat 2D10 cells (highlighted in blue) in the reference HIV-1 NL4-3 genome. Nucleotide sequences and sizes of amplicons (full-length and truncated LTR DNA) and predicted excised DNA fragment are shown.

Figure S4. **(a)** A representative fluorescence microscopy images of tranduced/infected Jurkat cells at day 5 of infection. Expression of BFP is indicative of the presence a vector expressing gRNAs. HIV-1 infection was monitored by the leve of GFP. **(b)** Quantitative comparison of cell numbers at various time points between the control and experimental samples treated with LTR-Cas9.

Figure S5. Primary human fetal astrocytes and microglia were transduced with lentiviral cocktails containing: lenti-LTR-80/+66-Cas9 (MOI 10), lenti-KLV-BFP-LTR A, B (MOI 3.3 of each). At day 3 post-transduction cells were infected with HIV-$1_{NL4\text{-}3\text{-}GFP\text{-}P2A\text{-}Nef/VSV\text{-}G}$ at MOI 1. One week after HIV-1 infection cells were harvested and viral expression levels were quantified by GFP

expression in flow cytometry **(a)** viral DNA levels **(b)** and viral RNA **(c)** by Taqman qPCR and qRT-PCRs using primer set and probe specific for Gag gene.

Figure S1

TZMbl

```
                                                    F [-413/-391](T361)
TGGAAGGGCTAATTTGGTCCCAAAAAAGACAAGAGATCCTTGATCTGTGGATCTACCACACACAAGGCTA
                                          LTR A [-347/-328]PAM
CTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGATCAGATATCCACTGACCTTTGGATGGTGCTTC
AAGTTAGTACCAGTTGAACCAGAGCAAGTAGAAGAGGCCAATGAAGGAGAGAACAACAGCTTGTTACACC
CTATGAGCCAGCATGGGATGGAGGACCCGGAGGGAGAAGTATTAGTGTGGAAGTTTGACAGCCTCCTAGC
                                  PAM LTR B [-143/-124]
ATTTCGTCACATGGCCCGAGAGCTGCATCCGGAGTACTACAAAGACTGCTGACATCGAGCTTTCTACAAG
                                                          R [-41/-19](T363)
GGACTTTCCGCTGGGGACTTTCCAGGGAGGTGTGGCCTGGGCGGGACTGGGGAGTGGCGAGCCCTCAGAT
                                          ┌Transcription start[+1]
GCTACATATAAGCAGCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGC
TCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTCAAAGTAGTGTG
```

PCR product full length LTR -413/-19: 395bp

```
GATCTGTGGATCTACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGATCA
GATATCCACTGACCTTTGGATGGTGCTTCAAGTTAGTACCAGTTGAACCAGAGCAAGTAGAAGAGGCCAA
TGAAGGAGAGAACAACAGCTTGTTACACCCTATGAGCCAGCATGGGATGGAGGACCCGGAGGGAGAAGTA
TTAGTGTGGAAGTTTGACAGCCTCCTAGCATTTCGTCACATGGCCCGAGAGCTGCATCCGGAGTACTACA
AAGACTGCTGACATCGAGCTTTCTACAAGGGACTTTCCGCTGGGGACTTTCCAGGGAGGTGTGGCCTGGG
CGGGACTGGGGAGTGGCGAGCCCTCAGATGCTACATATAAGCAGC
```

+SpCas9/gRNA LTR A+LTR B:

```
GATCTGTGGATCTACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGATCA
GATATCCACTGAC
```

Edited fragment: 190bp

```
↓CTTTGGATGGTGCTTCAAGTTAGTACCAGTTGAACCAGAGCAAGTAGAAGAGGCCAATGAAGGAGAGAA
CAACAGCTTGTTACACCCTATGAGCCAGCATGGGATGGAGGACCCGGAGGGAGAAGTATTAGTGTGGAAG
TTTGACAGCCTCCTAGCATTTCGTCACATGGCCCGAGAGCTGCATCCGGAG↓

TACTACAAAGACTGCTGACATCGAGCTTTCTACAAGGGACTTTCCGCTGGGGACTTTCCAGGGAGGTGTG
GCCTGGGCGGGACTGGGGAGTGGCGAGCCCTCAGATGCTACATATAAGCAGC
```

PCR product truncated LTR -413/-19: 205bp

```
GATCTGTGGATCTACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGATCA
GATATCCACTGACTACTACAAAGACTGCTGACATCGAGCTTTCTACAAGGGACTTTCCGCTGGGGACTTT
CCAGGGAGGTGTGGCCTGGGCGGGACTGGGGAGTGGCGAGCCCTCAGATGCTACATATAAGCAGC
```

Figure S2

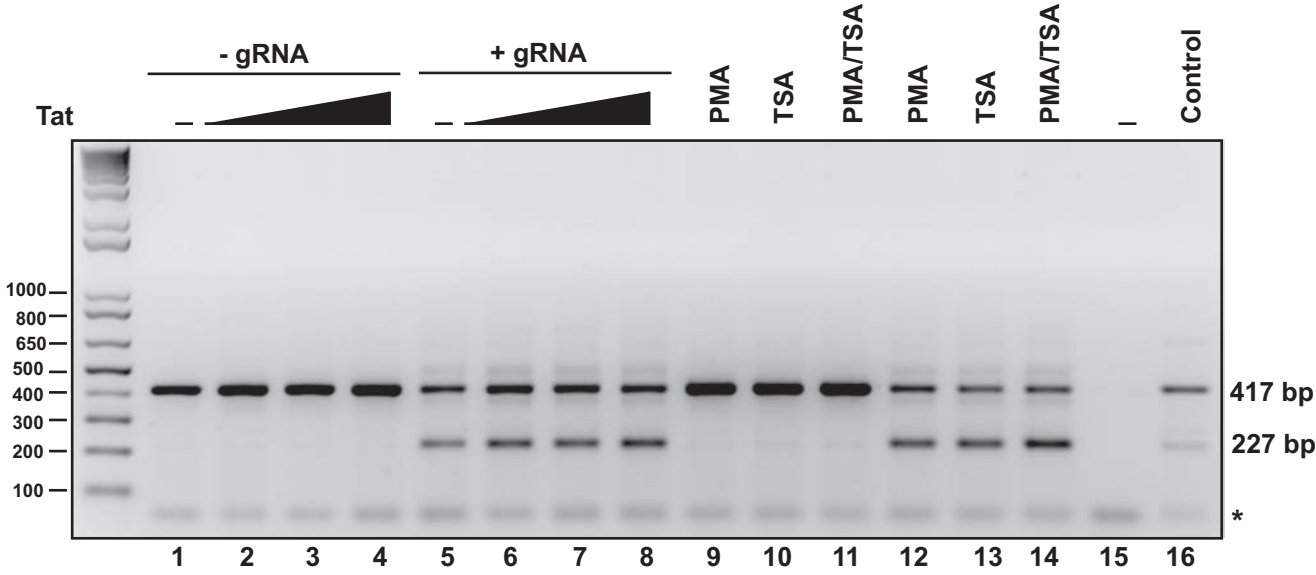

- gRNA
+ gRNA
Tat
–
PMA
TSA
PMA/TSA
PMA
TSA
PMA/TSA
–
Control
1000
800
650
500
400
300
200
100
417 bp
227 bp
*
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

Figure S3

Jurkat 2D10

Sequence:

```
TGGAAGGGCTAATTTGGTCCCAAAAAAGACAAGAGATCCTTGATCTGTGGATCTACCACACACAAGGCTA
              F [-375/-352]                LTR A [-347/-328]PAM
CTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGATCAGATATCCACTGACCTTTGGATGGTGCTTC
AAGTTAGTACCAGTTGAACCAGAGCAAGTAGAAGAGGCCAATGAAGGAGAGAACAACAGCTTGTTACACC
CTATGAGCCAGCATGGGATGGAGGACCCGGAGGGAGAAGTATTAGTGTGGAAGTTTGACAGCCTCCTAGC
                                PAM LTR B [-143/-124]
ATTTCGTCACATGGCCCGAGAGCTGCATCCGGAGTACTACAAAGACTGCTGACATCGAGCTTTCTACAAG
GGACTTTCCGCTGGGGACTTTCCAGGGAGGTGTGGCCTGGGCGGGACTGGGGAGTGGCGAGCCCTCAGAT
                                        ┌Transcription start[+1] R [+19/+43]
GCTACATATAAGCAGCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGC
TCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTCAAAGTAGTGTG
```

PCR product full length LTR -375/+43: 417bp

```
TTGGCAGAACTACACACCAGGGCCAGGGATCAGATATCCACTGACCTTTGGATGGTGCTTCAAGTTAGTA
CCAGTTGAACCAGAGCAAGTAGAAGAGGCCAATGAAGGAGAGAACAACAGCTTGTTACACCCTATGAGCC
AGCATGGGATGGAGGACCCGGAGGGAGAAGTATTAGTGTGGAAGTTTGACAGCCTCCTAGCATTTCGTCA
CATGGCCCGAGAGCTGCATCCGGAGTACTACAAAGACTGCTGACATCGAGCTTTCTACAAGGGACTTTCC
GCTGGGGACTTTCCAGGGAGGTGTGGCCTGGGCGGGACTGGGGAGTGGCGAGCCCTCAGATGCTACATAT
AAGCAGCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGG
```

+SpCas9/gRNA LTR A+LTR B:

```
TTGGCAGAACTACACACCAGGGCCAGGGATCAGATATCCACTGAC
```

Edited fragment: 190bp

```
↓CTTTGGATGGTGCTTCAAGTTAGTACCAGTTGAACCAGAGCAAGTAGAAGAGGCCAATGAAGGAGAGAA
CAACAGCTTGTTACACCCTATGAGCCAGCATGGGATGGAGGACCCGGAGGGAGAAGTATTAGTGTGGAAG
TTTGACAGCCTCCTAGCATTTCGTCACATGGCCCGAGAGCTGCATCCGGAG↓
```

PCR product truncated LTR -375/-43: 227bp

```
TTGGCAGAACTACACACCAGGGCCAGGGATCAGATATCCACTGACTACTACAAAGACTGCTGACATCGAG
CTTTCTACAAGGGACTTTCCGCTGGGGACTTTCCAGGGAGGTGTGGCCTGGGCGGGACTGGGGAGTGGCG
AGCCCTCAGATGCTACATATAAGCAGCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATCTG
AGCCTGGGAGCTCTCTGG
```

Figure S4

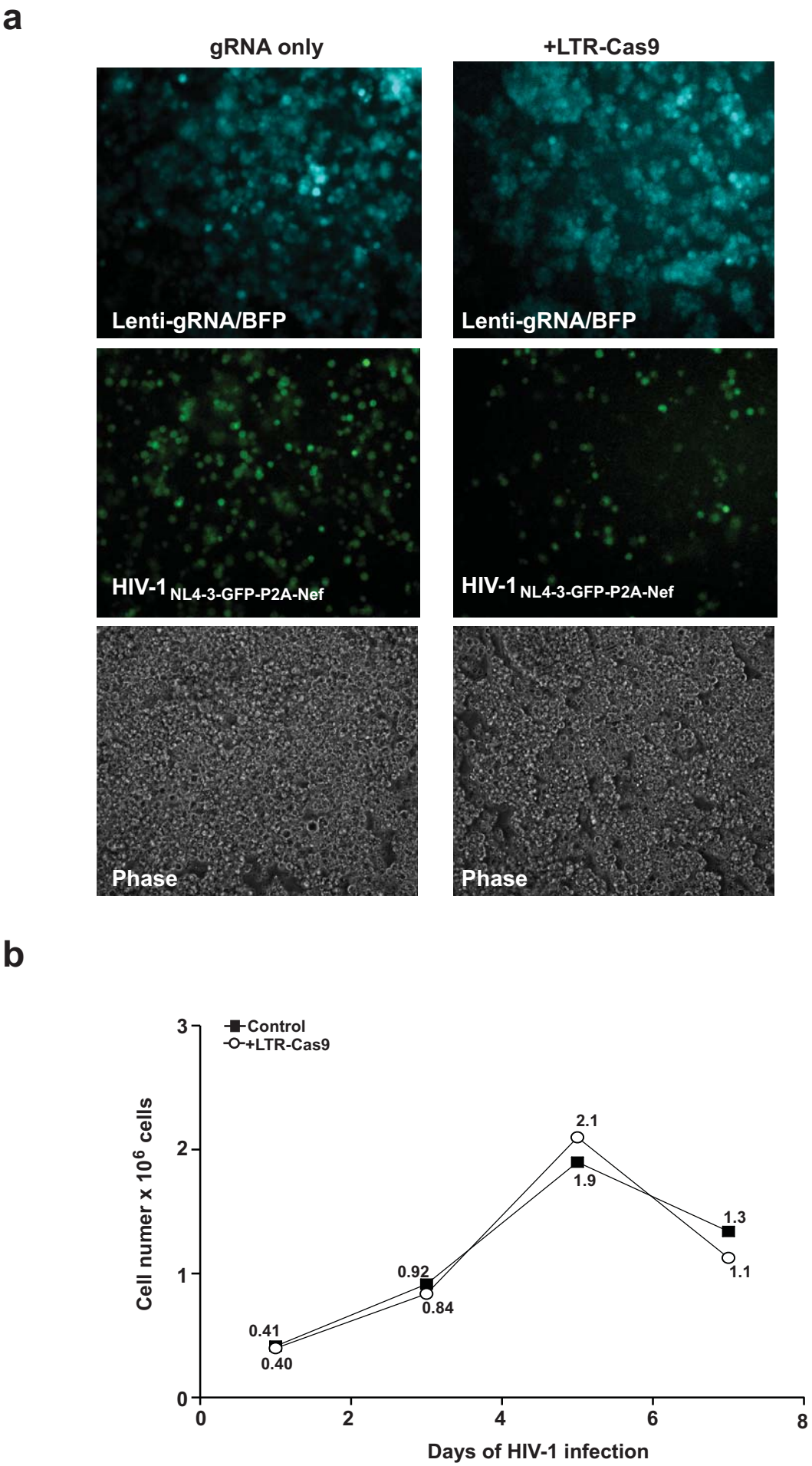
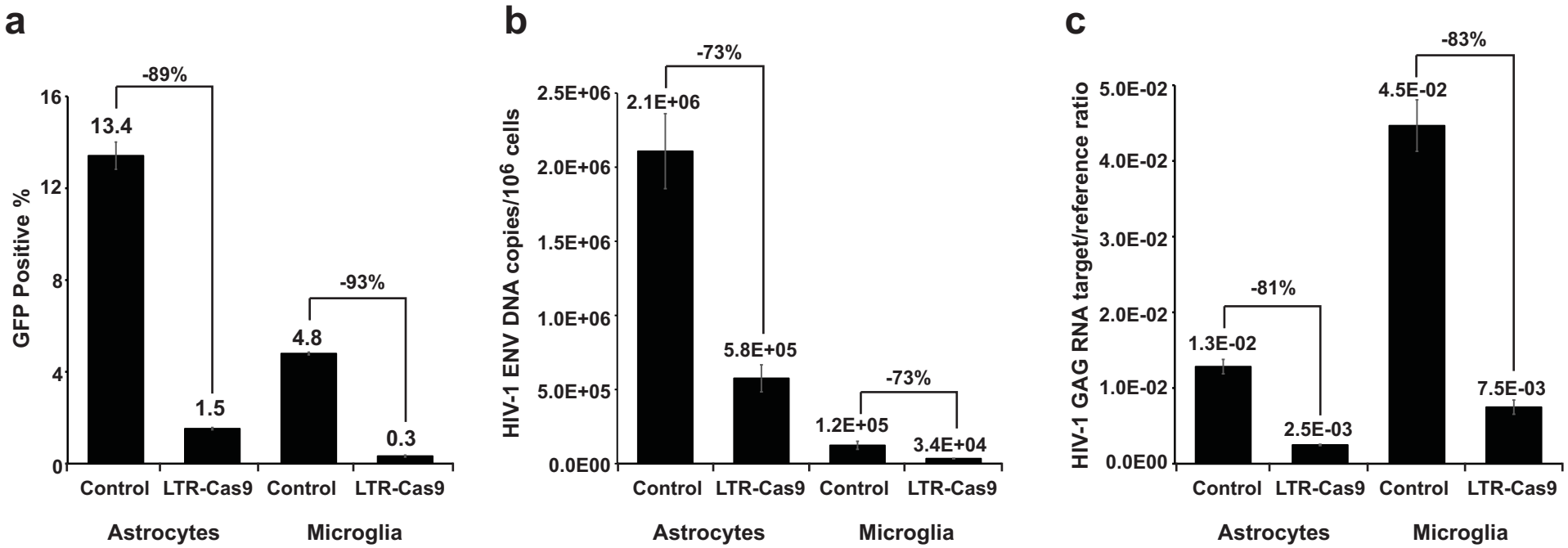

a
gRNA only
+LTR-Cas9
Lenti-gRNA/BFP
Lenti-gRNA/BFP
HIV-1 NL4-3-GFP-P2A-Nef
HIV-1 NL4-3-GFP-P2A-Nef
Phase
Phase
b
Control
+LTR-Cas9
Cell numer x 10^6 cells
Days of HIV-1 infection
0
1
2
3
0
2
4
6
8
0.41
0.40
0.92
0.84
2.1
1.9
1.3
1.1

Figure S5


a
-89%
-93%
GFP Positive %
16
12
8
4
0
13.4
1.5
4.8
0.3
Control
LTR-Cas9
Control
LTR-Cas9
Astrocytes
Microglia
b
-73%
-73%
HIV-1 ENV DNA copies/10^6 cells
2.5E+06
2.0E+06
1.5E+06
1.0E+06
5.0E+05
0.0E00
2.1E+06
5.8E+05
1.2E+05
3.4E+04
Control
LTR-Cas9
Control
LTR-Cas9
Astrocytes
Microglia
c
-83%
-81%
HIV-1 GAG RNA target/reference ratio
5.0E-02
4.0E-02
3.0E-02
2.0E-02
1.0E-02
0.0E00
4.5E-02
1.3E-02
2.5E-03
7.5E-03
Control
LTR-Cas9
Control
LTR-Cas9
Astrocytes
Microglia