Supplementary Material

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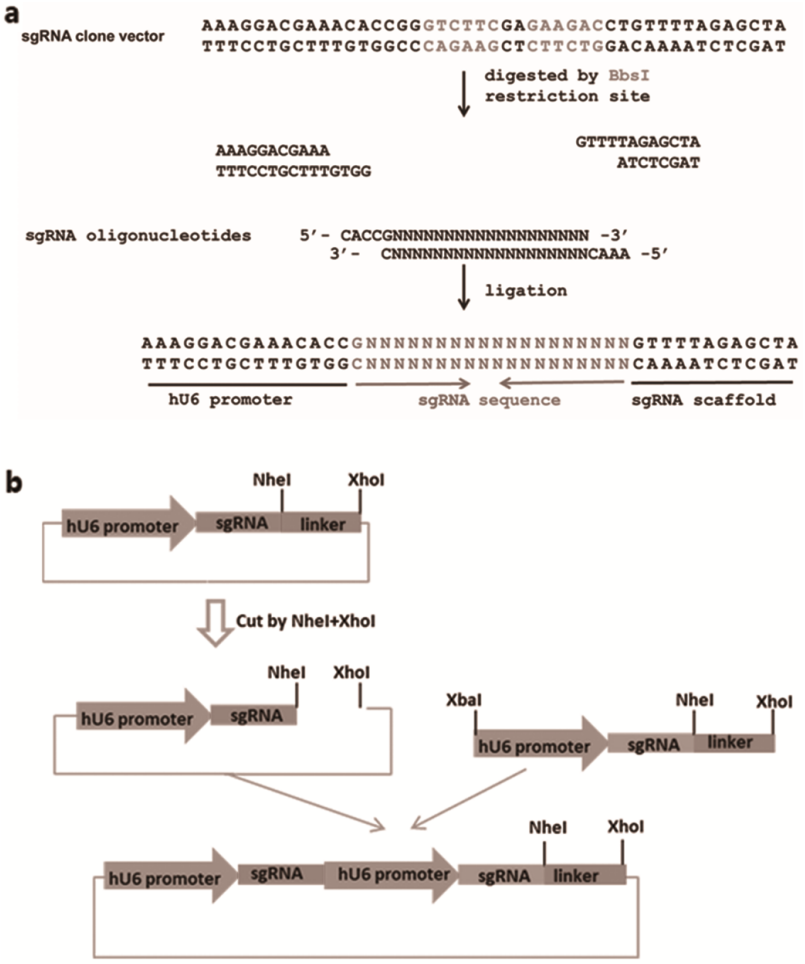
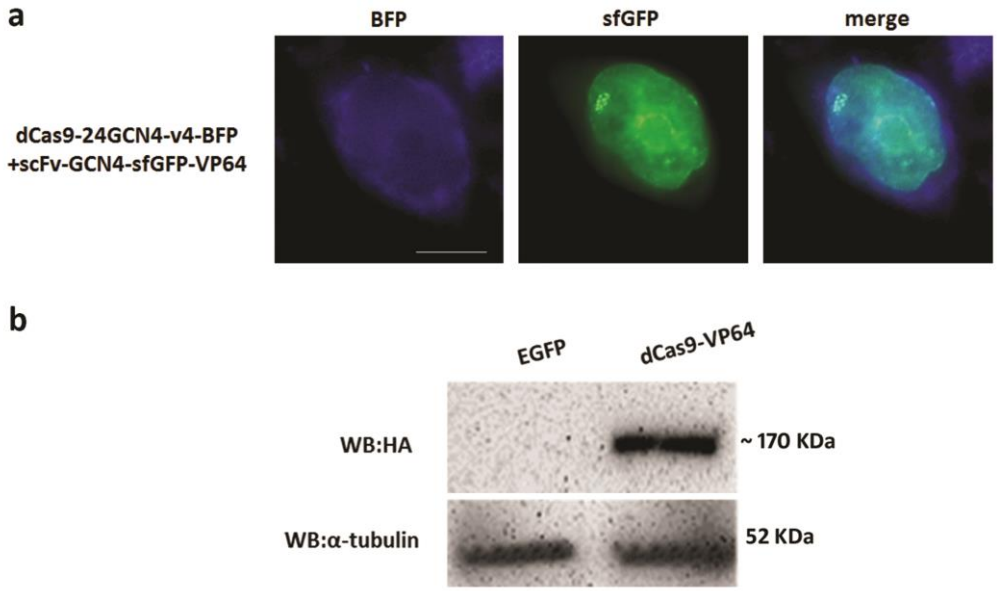
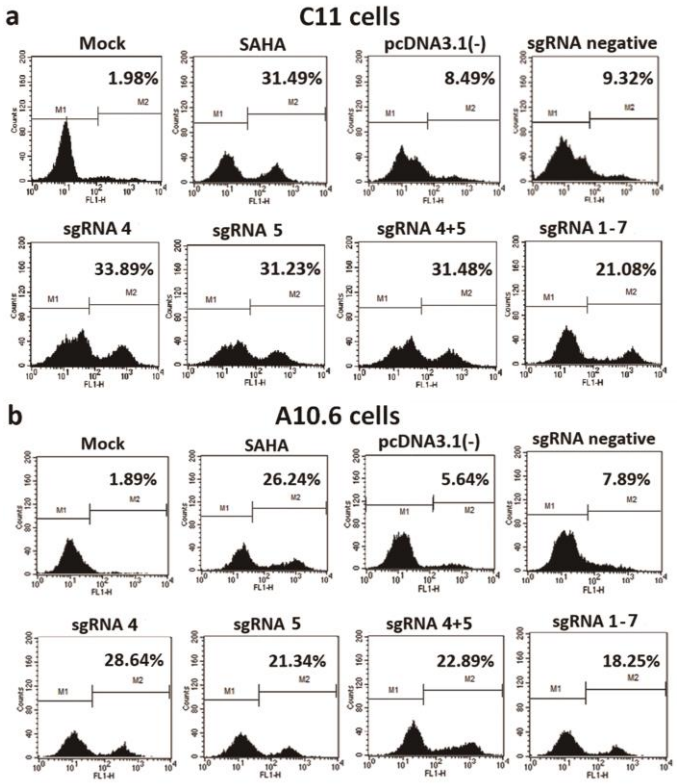


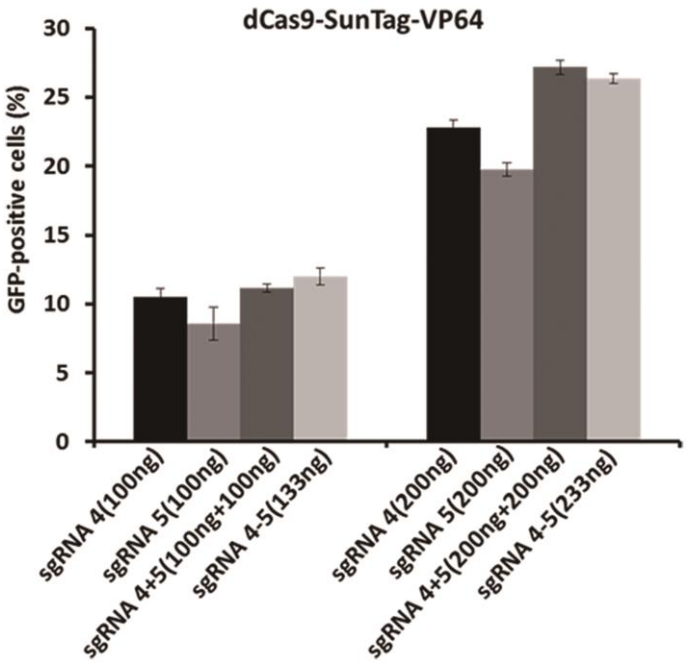
Supplementary Figure 1: sgRNA expression vector constructs. (a) The construct of individual sgRNA expression plasmid and (b) the construct of multiple sgRNAs coexpressed in one plasmid.



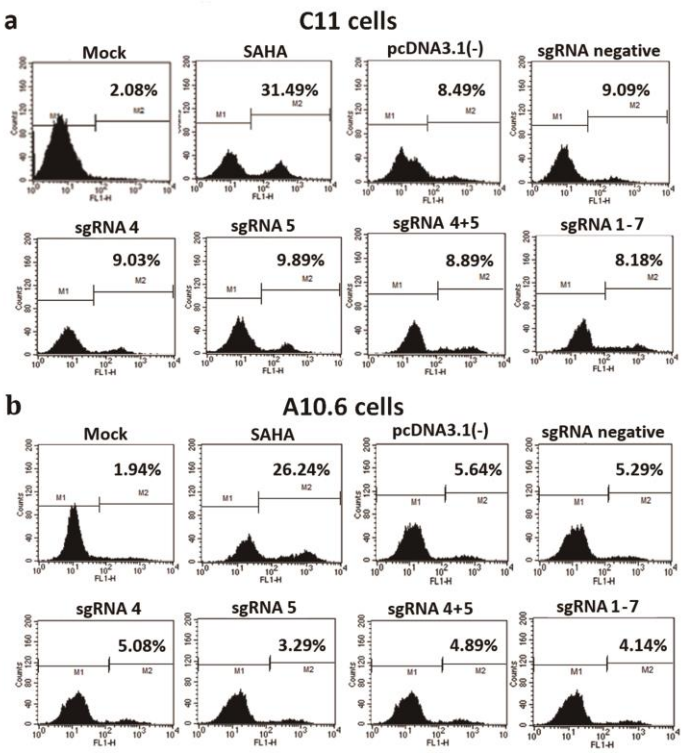
Supplementary Figure 2: The expression of dCas9-SunTag-VP64 system and dCas9-VP64 construct. (a) dCas9-24GCN4$_{-v4}$-BFP was co-expressed with scFv-GCN4-sfGFP-VP64 into HEK293T cells, and cells were imaged using confocal microscopy. Scale bars, 10 μm. (b) The expression of dCas9-VP64 in transfected HEK293T cells was confirmed by western blot for the C-terminal HA epitope tag. The EGFP expression plasmid does not contain the epitope tag. α-tubulin was used as internal control.



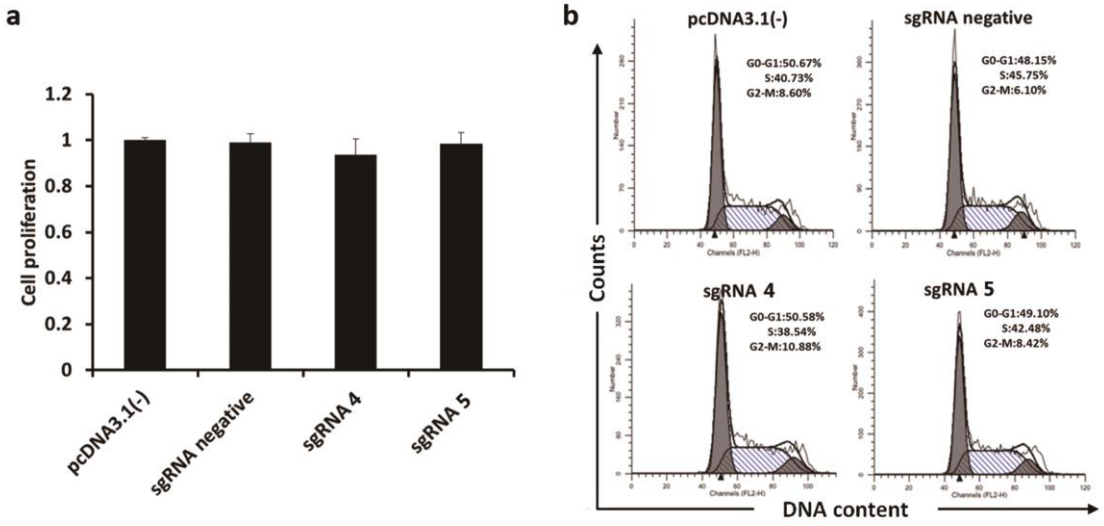
Supplementary Figure 3: Reactivation of HIV-1 in latently infected cells by dCas9-SunTag-VP64. (a) C11 cells were nucleofected with pcDNA3.1(-) construct or dCas9-SunTag-VP64 with indicated sgRNA (single sgRNA or combined sgRNAs) at indicated time. C11 cells untreated or treated with 0.5 μM SAHA were both used as control. At 72 h post transfection, the percentage of GFP-expressing cells was measured by flow cytometry. The results are presented as fluorescence histograms. (b) Similar experiment was performed in J-Lat clone A10.6 cells.



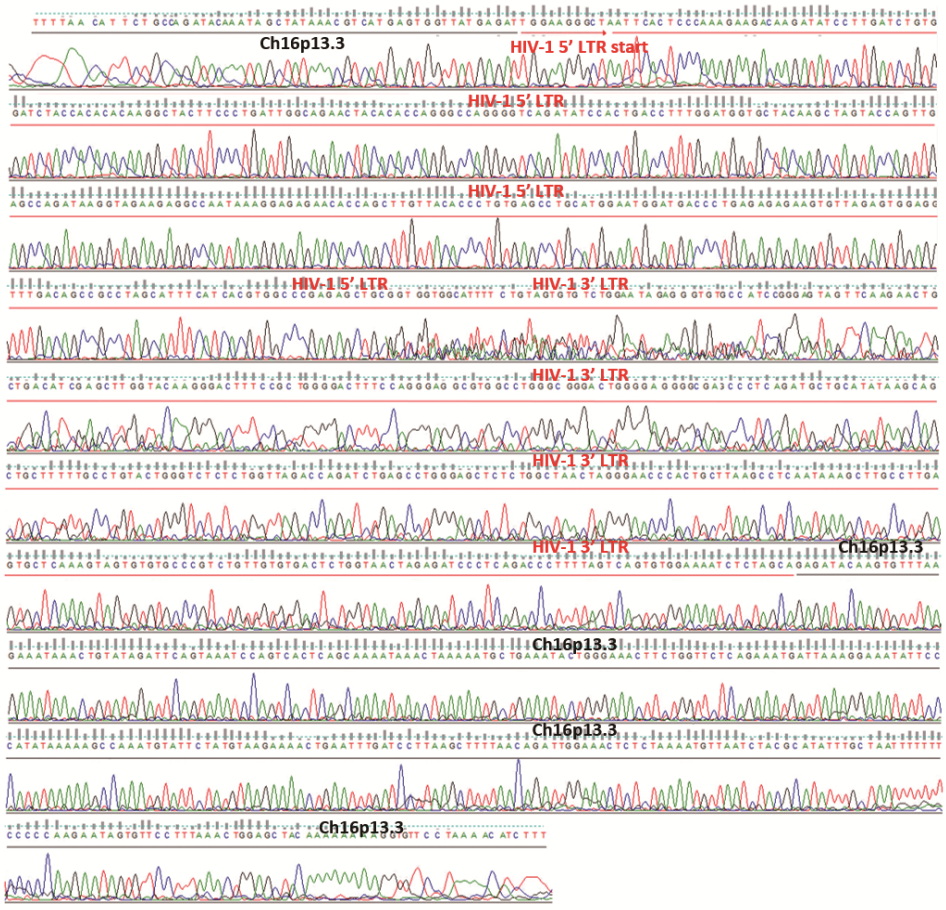
Supplementary Figure 4: Analysis of the synergistic effects of dCas9-SunTag-VP64 with combined sgRNAs in latently infected cells. C11 cells were nucleofected with dCas9-SunTag-VP64 and equal amount of each sgRNA. sgRNA empty plasmid was used to bring the total amount of plasmid to be equal (200 ng or 400ng) between the single sgRNA and the combined sgRNAs group. The amount DNA of dCas9-SunTag-VP64 kept constant in each group transfected with indicated sgRNA. pcDNA3.1(-) plasmid was used to fill up of a total of 3 μg according to manufacturer's instructions. At 72 h post transfection, the percentage of GFP-expressing cells was measured by flow cytometry. The results represent the mean ± SD of three independent experiments. sgRNA 4+5: expressed from two individual construct; sgRNA 4-5: co-expressed from single vector.



Supplementary Figure 5: dCas9-VP64 mediated the reversal of HIV-1 from latency in HIV-1 latently infected cells. (a) C11 cells were nucleofected with pcDNA3.1(-) construct or dCas9-VP64 with indicated sgRNA (single sgRNA or combined sgRNAs) at specified time. C11 cells untreated or treated with 0.5 μM SAHA were used as control. At 72 h post transfection, the percentage of GFP-expressing cells was measured by flow cytometry. The results are presented as fluorescence histograms. (b) We performed the same reactivation experiment in J-Lat clone A10.6 cells.



Supplementary Figure 6: Analysis of the cytotoxicity effects of dCas9-SunTag-VP64 in Jurkat T cells at 96 h post-transfection. Jurkat T cells were nucleofected with pcDNA3.1(-) construct or dCas9-SunTag-VP64 with indicated sgRNA at indicated time. At 96 h post-transfection, (a) Cell proliferation and (b) cell cycle distribution were detected, respectively. The data represent the mean ± SD of three independent experiments.



Supplementary Figure 7: DNA sequences of PCR products from latently infected C11 cells treated with CRISPR/Cas9. Latently infected C11 cells were transfected with CRISPR/Cas9 and indicated sgRNA. After three days transfection, genomic DNA from cells was extracted and subjected to PCR by primers located at outsides of the integrated sites in Ch16p13.3. The PCR products were cloned and sequenced.

Target	Name	Sequence	Position Relative to Transcriptional Start Site
5′LTR-U3	sgRNA 1	acaagatatccttgatctg	-427
5′LTR-U3	sgRNA 2	ttggcagaactacacacca	-375
5′LTR-U3	sgRNA 3	atatccactgacctttgga	-342
5′LTR-U3	sgRNA 4	gtggcccgagagctgcatc	-164
5′LTR-U3	sgRNA 5	gacatcgagcttgctacaa	-124
5′LTR-U3	sgRNA 6	tacaagggactttccgctg	-110
5′LTR-U3	sgRNA 7	ttccgctggggactttcca	-99
5′LTR-U3	sgRNA 8	cctgggcgggactggggag	-69
5′LTR-U3	sgRNA 9	agctgctttttgcctgtac	-21
5′LTR-R	sgRNA 10	ttagaccagatctgagcct	+12
5′LTR-U5	sgRNA 11	cccgtctgttgtgtgactc	+109
5′LTR-U5	sgRNA 12	cagacccttttagtcagtg	+146
—	sgRNA negative	atcgtttccgcttaacggcg	—

Supplementary Table 1: The sequence and position of the designed sgRNAs targeting the HIV-1 5′-LTR promoter are listed.

Chromosome	Closest gene	Homology score, %	Off-target sequence (PAM 5′-NGG included)	PAM proximal region match (mismatch)	Detection of indel
	HIV-1 5′ LTR	100%	GGTGGCCCGAGAGCTGCATCCGG	23	On-site target
Chr19	VSTM2B	91%	GGTGGCCTGgGAGCTGCATCTGG	12(1)	No
Chr18	ZBTB7C	83%	tGTttCCCcAGAGCTGCATCCaG	PAM-5′NAG	No
Chr16	ANKRD11	83%	GGcGGCggGgGAGCTGCATCTGG	12(1)	No
Chr2	C2orf48	83%	GGgGGCgtGgGAGCTGCATCAGG	12(1)	No
ChrX	GPM6B	87%	GcTGGCCaGAGAGCaGCATCCGG	12(1)	No
Chr1	C1orf174	87%	ccTGGCCCcAGAGCTGCATCCaG	PAM-5′NAG	No
Chr15	SEMA6D	91%	aGTGGCCCtAGAGCTGCATCAGG	12(1)	No
Chr2	HS6ST1	83%	GGaGaCCgGgGAGCTGCATCCGG	12(1)	No

Supplementary Table 2: Off-target analysis for CRISPR/Cas9-mediated targeting in C11 cells.

Chromosome	Closest gene	Forward primer	Reverse primer	Expected product size, bp
Chr19	VSTM2B	ATTTTTGTGTGTAGAGCCAGACC	CCCTTGTTTTCTGGCCGTGGC	225
Chr18	ZBTB7C	TGAGAGTTCTGGTTACTTGGGGT	TTGAAAAGTGCTGTGAAATGTGG	315
Chr16	ANKRD11	AACTGGAGTCAAAGAGAAGCTG	GAGTGTTCTTGGAGGAAGGAGTT	389
Chr2	C2orf48	GCTCTCAGACAAGGAATGACCCA	CTGGGCTCAGAGTAAAAAAGTGC	524
ChrX	GPM6B	GAAGAAGTTTGGTAGGGGAGATT	ATAGATTTTGTTGAGAACCCGCT	439
Chr1	C1orf174	CCTGGATTCTCCCGATTTTATTC	CTTAGCGAATTTCACAATCACTCCT	366
Chr15	SEMA6D	GGGGGAAGCTGACACAGAATAGACA	CAAAAGGGGTCCACTAAGCTTCACA	374
Chr2	HS6ST1	GGAGAGTTGGGGTGAGCAGA	GGAAGAGAATGAAATGAGCCAGACA	248

Supplementary Table 3: Primers information for CRISPR/Cas9 off-target analysis