Supplementary Tables.

Table S1. Off-Target LTR1 / GagD (LG) CRISPR Editing

gRNA	Guide Target (Protospacer mismatches) + PAM	*Gene* (Locus)	Treatment	% Indel (per pHIV-1 Strain)				Gene Function
				pCH040.c /2625	pCH106.c /2633	pTHRO.c /2626	pCH058.c /2960	
LTR-1	GCAGAACCACTCCCCAGGGCCTGG	*GRIK3* (1p35.1)	LTR-1	0%	0%	0%	0%	Glutamate ionotropic receptor kainate type subunit 3; glutamate neurotransmitter receptor subunit. (NG_011447.1)
			LG	0%	0%	0%	0%	
	AAAGAACAACACACCAGGGAGGGG	*CADPS2* (7q31.31)	LTR-1	0%	0%	0%	0%	Calcium dependent secretion activator 2; regulates exocytosis of synaptic vesicles in neurons and neuroendocrine cells. (NG_016215)
			LG	0%	0%	0%	0%	
	TAAAAACTACACACAAGGGCCAGG	Intronic (3q13.32)	LTR-1	0%	0%	0%	0%	(Accession AC021889)
			LG	0%	0%	0%	0%	
	AGGGAAACACACACCAGGGCCAGG	*GP6C* (13q31.1)	LTR-1	0%	0%	0%	0%	Glypican 6; glycosphlphophatidylinositol-anchored proteoglycan implicated in cell growth and cell division. (NG_011880.1)
			LG	0%	0%	0%	0%	
	TAGAACTACACCACCAGGGCCCGG	*CRX* (19q13.33)	LTR-1	0%	0%	0%	0%	Cone-rod homeobox; plays role in differentiation of photoreceptor cells and maintenance of cone & rod function. (AC008745.7)
			LG	0%	0%	0%	0%	

GagD	CACAGAGGCAAAAGACACCATGG	otopetrin 1 pseudogene (2p11.2)	GagD	0%	0%	0%	0%	Nonfunctional DNA segment derived from *OTOP1* (4p16.3), a gene that is required for the formation of inner ear otoliths. (NG_021568.4)
			LG	0%	0%	0%	0%	
	GATAGATGTAAAAGACACTGTGG	*ZNF420* (19q13.41)	GagD	0%	0%	0%	**n.d.**	Zinc finger protein 420; negatively regulates p53-mediated apoptosis especially in testes and ovaries. (AC008733.9)
			LG	0%	0%	0%	**n.d.**	
	GATAGATGTAAAAGACACCAGGG	*MLLT3* (9p22.1)	GagD	0%	0%	0%	0%	MLLT3 super elongation complex unit. (AK512635.8)
			LG	0%	0%	0%	0%	
	GAGAAATATAAAAGACACCATGG	Intronic (5q22.2)	GagD	0%	**n.d.**	0%	0%	(Accession AC137549.1)
			LG	0%	**n.d.**	0%	0%	
	TATAGCTGTAAAAACACCA-AGG	*PTPRG* (3p13.2)	GagD	0%	0%	0%	0%	Protein tyrosine phosphatase receptor type G; signaling molecule in mitosis, differentiation, and oncogenic transformation. (AC092502.2)
			LG	**n.d.**	0%	0%	0%	

n.d. not done

Table S2. Cloning Primers

Vector	gRNA	Forward Oligo (5'→3')[a]	Reverse Oligo (5'→3')[b]
px333	LTR-1	caccCAGAACTACACACCAGGGCC	aaacGGCCCTGGTGTGTAGTTCTG
	GagD	caccGATAGATGTAAAAGACACCA	aaacTGGTGTCTTTTACATCTATC
	DLTR-1	caccGACTGCTTAAGCCTCAATAA	aaacTTATTGAGGCTTAAGCAGTC
	DLTR-2	caccGCTTTATTGAGGCTTAAGCAG	aaacCTGCTTAAGCCTCAATAAAGC
	TatA2	caccTAGATCCTAACCTAGAGCCC	aaacGGGCTCTAGGTTAGGATCTA
	TatD	caccTCTCCTATGGCAGGAAGAAG	aaacCTTCTTCCTGCCATAGGAGA
	TatE	caccGAAGGAATCGAAGAAGAAGG	aaacCCTTCTTCTTCGATTCCTTC
	TatE2	caccGAAAGAATCGAAGAAGGAGG	aaacCCTCCTTCTTCGATTCTTTC
	TatF	caccCCGATTCCTTCGGGCCTGTC	aaacGACAGGCCCGAAGGAATCGG
	TatG	caccTCTCCGCTTCTTCCTGCCAT	aaacATGGCAGGAAGAAGCGGAGA
	TatH	caccGCTTAGGCATCTCCTATGGC	aaacGCCATAGGAGATGCCTAAGC
	TatI	caccCCGATTCCTTCGGGCCTGTC	aaacGACAGGCCCGAAGGAATCGG
pLentiCRISPR-RFP657	Control	caccGGAGACGTTTGTACGTCTCT	aaacAGAGACGTACAAACGTCTCC
	TatD	caccGTCTCCTATGGCAGGAAGAAG	aaacCTTCTTCCTGCCATAGGAGAC
	TatE	caccGGAAGGAATCGAAGAAGAAGG	aaacCCTTCTTCTTCGATTCCTTCC
pHIV-1NL4-3-Δnef-eGFP	ΔTatD	GAAGCGGGGGAGACAGCGACGAAGAGCTC	TGTCTCCCCCGCTTCTTCCTGCCATAG
	ΔTatE	TAGAAGAAAGAGACAGAGACAGATCCATTCG	TGTCTCTTTCTTCTATTCCTTCGGGCC

[a] Overhang sequences complementary to digested vector represented as lower case “cacc”
[b] Overhang sequences complementary to digested vector represented as lower case “aaac”

Table S3. PCR Primers

	Forward Oligo			Reverse Oligo		
PCR	**Template DNA (locus)**	**Primer Name**	**Sequence (5'→3')**	**Template DNA (locus)**	**Primer Name**	**Sequence (5'→3')**
TatDE Excision	HIV-1$_{HXB2}$ (5518-5537) HIV-1$_{CH040.c/2625}$ (5514-5533) HIV-1$_{CH106.c/2633}$ (5515-5534) HIV-1$_{TRJO.c/2851}$ (5542-5561) HIV-1$_{CH058.c/2960}$ (5535-5554)	Primer 300	AAGCCACCTTTGCCTAGTGT	HIV-1$_{HXB2}$ (8560-8580) HIV-1$_{CH040.c/2625}$ (8525-8545) HIV-1$_{THRO.c/2626}$ (8643-8663) HIV-1$_{REJO.c/2864}$ (8576-8596) HIV-1$_{TRJO.c/2851}$ (8586-8606)	Primer 301	CCAGAAGTTCCACAATCCTCG
	HIV-1$_{THRO.c/2626}$ (5549-5568)	Primer 302	AAGCCGCCTTTGCCTAGTGT	HIV-1$_{CH058.c/2960}$ (8546-8566)	Primer 303	CCAGAAGTTCCACAGTCCTCG
	HIV-1$_{REJO.c/2864}$ (5538-5557)	Primer 304	AAGCCACCTTTGCCTAGTAT	HIV-1$_{CH106.c/2633}$ (8580-8600)	Primer 305	CCAGAAGTTCCACTATCCTCG
TatDE Excision (nested)	HIV-1$_{HXB2}$ (5557-5578) HIV-1$_{CH040.c/2625}$ (5553-5574) HIV-1$_{CH106.c/2633}$ (5554-5575) HIV-1$_{THRO.c/2626}$ (5588-5609) HIV-1$_{REJO.c/2864}$ (5577-5598) HIV-1$_{TRJO.c/2851}$ (5581-5602) HIV-1$_{CH058.c/2960}$ (5574-5595)	Primer 300b	AGATGGAACAAGCCCCAGAAGA	HIV-1$_{HXB2}$ (8526-8552) HIV-1$_{CH040.c/2625}$ (8491-8517) HIV-1$_{CH106.c/2633}$ (8546-8572) HIV-1$_{THRO.c/2626}$ (8609-8635) HIV-1$_{REJO.c/2864}$ (8542-8568) HIV-1$_{TRJO.c/2851}$ (8552-8578) HIV-1$_{CH058.c/2960}$ (8512-8538)	Primer 301b	CAAGAGTAAGTCTCTCAAGCGGTGGTA
TatD Off-Target	chr11: 68022307-68022329	Primer 182-D2 Off F	TGAACTCCAAGGGTTCTAAGAT	chr11: 68022733-68022751	Primer 183-D2 Off R	TTGGAGAAGGCGTACAGG
	chr13: 93771074-93771102	Primer 194-D3 Off F	GTGCCAAACATATTGATAACACATACAC	chr13: 93771551-93771574	Primer 195-D3 Off R	CTCCTCAAAGCTCACCAAATTCC

	chr2: 216741039-216741061	Primer 274-D7 Off F	GTTTATAATTGCAGAAGCACTG	chr2: 216741522-216741539	Primer 275-D7 Off R	AACCCAATTCTGTCCGA
	chr12: 130325120-130325144	Primer 184-D9 Off F	AAAGTGGATGACAAGTTCTATCAG	chr12: 130325598-130325620	Primer 185-D9 Off R	AGACTTGAGATGAGAGAAACCC
	chr9: 83240840-83240862	Primer 247-D10 Off F	TCAAGGTTCTAAGTTTCTGGCA	chr9: 83241314-83241340	Primer 248-D10 Off R	CCAGAACATAAGCTGTAATGTAAACC
TatE Off-Target	chr18: 35964872-35964889	Primer 282-E3 Off F	TTCAGATGCTGGAGTAG	chr18: 35965355-35965372	Primer 283-E3 Off R	TTAGCTGGACTTGATGG
	chr19: 47991242-47991260	Primer 278-E4 Off F	GACCCAAACCAGCAGGAG	chr19: 47991721-47991742	Primer 279-E4 Off R	GGAGGAGGTGAAGAAGGAAGA
	chr4: 184272401-184272421	Primer 226-E7 Off F	CACACAGAGGCTTTGGAACT	chr4: 184272881-184272901	Primer 227-E7 Off R	ACTATGTACCCAGGCAGCTA
	chr8: 36453259-36453280	Primer 244-E12 Off F	GTACCTTAGAGGTTTCCAATC	chr8: 36453739-36453759	Primer 245-E12 Off R	TGAAGAATCAAATGCAACAC
	chr7: 124503052-124503072	Primer 373-E14 Off F	CCACAGTTTATATATTCCAC	chr7: 124503057-124503075	Primer 374-E14 Off R	CTTGAAGCCACCTTATAC
LTR-1 Off-Target	chr1: 36971727-36971747	Primer 254-LTR1 Off F	AAGACTTCTAGGTGGATAGC	chr1: 36972189-36972207	Primer 255-LTR1 Off R	CCTGTGGATTCTCCTTCA
	chr7: 122425478-122425495	Primer 256-LTR2 Off F	GTGGTCCCAAGCTACTC	chr7: 122425924-122425943	Primer 257-LTR2 Off R	GAGTTAAAGCAAGGGTCAG
	chr3: 117193688-117193710	Primer 258-LTR3 Off F	GTATTGCTTTAGACTCCTAAGT	chr3: 117194162-117194179	Primer 259-LTR3 Off R	CAAGATCTCGGCTCACT
	chr13: 94251055-94251072	Primer 260-LTR4 Off F	CCCAAATGCCCATCAAT	chr13: 94251526-94251545	Primer 261-LTR4 Off R	CACAAGTTCTGGGATTTGG
	chr19: 47786499-47786517	Primer 262-LTR11 Off F	TTACAGGCATATGTCACC	chr19: 47786908-47786928	Primer 263-LTR11 Off R	CTTGCTTAAACAAAGAACTG
GagD Off-Target	chr2: 91614689-91614709	Primer 264-Gag1 Off F	GGCATCACATCTGGGTTCAA	chr2: 91615200-91615221	Primer 265-Gag1 Off R	CTTTCTGCTTCTGGCCCTATC
	chr19: 36832404-36832427	Primer 367-Gag12 Off F	AGAATAGTAGTGATGCTGAACA	chr19: 36832409-36832427	Primer 368-Gag12 Off R	CCAGGGCTGCTATCATTT
	chr9: 20223152-20223174	Primer 369-Gag13 Off F	AGCAGCATTAAACTTGGAAAGG	chr9: 20223152-20223175	Primer 370-Gag13 Off R	AGTGTTGAGATTAGAGGTGTGAG
	chr5: 112567658-112567678	Primer 270-Gag4 Off F	TGCTAGGGCAGAGAGGATAA	chr5: 112568112-112568134	Primer 271-Gag4 Off R	GACGTTCTCATGGAATCTCACA
	chr3: 62330371-62330388	Primer 272-Gag11 Off F	ATATGGCCTGTGACCAA	chr3: 62330762-62330783	Primer 272-Gag11 Off R	GCTCAGTAAGATACCTCAGTA

Table S4. Experimental gRNA Targets and Specificities

gRNA Name	Target DNA Sequence (5' → 3')	% Sequence Conservation (n = 4004)	CRISPR*spec* Score†
LTR-1 (control)	GCAGAACTACACACCAGGGCC	27.12 %	6.937
GagD (control)	GATAGATGTAAAAGACACCA	11.14 %	4.071
DLTR-1 (control)	GACTGCTTAAGCCTCAATAA	29.57 %	3.364
DLTR-2 (control)	GCTTTATTGAGGCTTAAGCAG	28.94 %	2.266
TatG	TCTCCGCTTCTTCCTGCCAT	67.15 %	3.803
TatD	TCTCCTATGGCAGGAAGAAG	59.04 %	6.284
TatH	GCTTAGGCATCTCCTATGGC	51.12 %	4.662
$TatA_2$	TAGATCCTAACCTAGAGCCC	29.79%	7.327
TatI	GGCTCTAGGTTAGGATCTAC	25.90 %	6.844
TatE	GAAGGAATCGAAGAAGAAGG	15.26 %	3.090
TatF	CCGATTCCTTCGGGCCTGTC	6.19 %	6.467
$TatE_2$	GAAAGAATCGAAGAAGGAGG	5.82 %	6.021

†CRISPR*spec* score represents the specificity of the selected gRNA for HIV-1 based on a predefined off-target landscape within the human genome. Calculated using CRISPRoff webserver v1.1.

Table S5. Mosaic gRNA Coverage

gRNA Name	% Sequence Conservation (n = 4004 HIV-1 Strains)	Excised Base Pairs	Exons Disrupted
TatD	59.04 %	-	2
TatH[§]	51.12 %	-	1
TatE	15.26 %	-	3
TatDH[‡]	59.44 %	29	3
TatDE	62.19 %	2442	5
TatEH	56.24 %	2451	4

[§] 4 base pairs of TatH gRNA overlap with *rev*. Given that CRISPR cleavage, as determined by ICE, occurs 5+ bp distal to PAM, TatH was considered to disrupt 1 exon (tat_1).

[‡]Although TatDH is a composite of 2 highly conserved gRNAs, it overlaps in target recognition and multiplexing failed to increase the breadth of coverage.

Table S6. Off-Target TatDE CRISPR Editing

gRNA	Guide Target (Protospacer mismatches) + PAM	*Gene* (Locus)	Treatment	% Indel (per pHIV-1 Strain) pCH040.c /2625	pCH106.c /2633	pTHRO.c /2626	pCH058.c /2960	Gene Function
TatD	CCTCCCATGGCAGAAAGAAGTGG	*ALDH3B1* (11q13.2)	TatD	0%	0%	0%	0%	Aldehyde dehydrogenase aids in alcohol metabolism and lipid peroxidation (Accession NG_012282)
			TatDE	0%	0%	0%	0%	
	CTTCCCACGGCAGGAAGAACCGG	*GPC6* (13q31.3-q32.1)	TatD	0%	0%	0%	0%	Glypicans encode cell surface coreceptors for control of cell growth and division (Accession NG_011880)
			TatDE	0%	0%	0%	0%	
	CTTCCTAGGGCAGGAAGAAGGGG	*IGFBP5* (2q35)	TatD	0%	0%	0%	0%	Insulin-like growth factor-binding protein 5; function in humans under characterized (Accession AC007563)
			TatDE	0%	0%	0%	0%	
	TTCTCCTAGGAAGGAGGAAGAGG	Intronic (12q24.33)	TatD	0%	0%	0%	0%	(Accession AC127071)
			TatDE	0%	0%	0%	0%	
	AATCTTATGGCAGGAAGAAGAGG	Intronic (9q22.2-q31.1)	TatD	0%	0%	0%	0%	(Accession AL137847)
			TatDE	0%	0%	0%	0%	
TatE	GAAGAAAACAAAGAAGAAGGAGG	Intronic (18q12.2)	TatE	0%	0%	0%	0%	(Accession AC118757)
			TatDE	0%	0%	0%	0%	
	GAAGGAAGAGAAGAAGAAGGAGG	Intronic (19q13.33)	TatD	0%	0%	0%	0%	(Accession AC010330)
			TatDE	0%	0%	0%	0%	
	GAAGGAATCA-AGAAGAAGGAGG	Intronic (4q35.1)	TatD	0%	0%	0%	0%	(Accession AC079080)
			TatDE	0%	0%	0%	0%	
	GAAAGGAAGGAAGAAGAAGGGGG	Senescence gene region (8p11.2)	TatD	0%	0%	0%	0%	(Accession AP000078.1)
			TatDE	0%	0%	0%	0%	
	AAAGGAATCGAAGAAGAAGGGGG	Intronic (7q31.33)	TatD	0%	0%	0%	0%	(Accession AC005521)
			TatDE	0%	0%	0%	0%	(Accession AC005521)