bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

viruses

MDPI

Article

Editing of the TRIM5 gene decreases the permissiveness of human T lymphocytic cells to HIV-1

Kevin Désaulniers [1], Levine Ortiz [1], Caroline Dufour [1], Alix Claudel [1], Mélodie B.Plourde [1], Natacha Merindol [1], and Lionel Berthoux [1,*]

[1] Department of medical biology, Université du Québec à Trois-Rivières, Trois-Rivières, Québec, G9A 5H7, Canada.

* Correspondence: lionel.berthoux@uqtr.ca; Tel.: +1-819-376-5011 x4466 (L.B.)

Received: date; Accepted: date; Published: date

Abstract: TRIM5α is a cytoplasmic antiretroviral effector upregulated by type I interferons (IFN-I). We previously showed that two points mutations, R332G/R335G, in the retroviral capsid-binding region confer human TRIM5α the capacity to target and strongly restrict HIV-1 upon over-expression of the mutated protein. Here, we used CRISPR-Cas9-mediated homology-directed repair (HDR) to introduce these two mutations in the endogenous human TRIM5 gene. We found 6 out of 47 isolated cell clones containing at least one HDR-edited allele. One clone (Clone 6) had both alleles containing R332G but only one of the two alleles containing R335G. Upon challenge with an HIV-1 vector, clone 6 was significantly less permissive compared to unmodified cells, whereas the cell clones with monoallelic modifications were only slightly less permissive. Following IFN-β treatment, inhibition of HIV-1 infection in clone 6 was significantly enhanced (~40-fold inhibition). TRIM5α knockdown confirmed that HIV-1 was inhibited by the edited TRIM5 gene products. Quantification of HIV-1 reverse transcription products showed that inhibition occurred through the expected mechanism. In conclusion, we demonstrate the feasibility of potently inhibiting a viral infection through editing of innate effector genes. Our results also emphasize the importance of biallelic modification in order to reach significant levels of inhibition by TRIM5α.

Keywords: HIV-1; TRIM5α; genome editing; CRISPR; restriction factor; interferon

1. Introduction

Individuals infected with the human immunodeficiency virus type 1 (HIV-1) are treated with combination antiretroviral therapy (cART). Despite successfully reducing viral loads to undetectable levels in a large fraction of the treated patients [1], drugs administered under cART have significant side effects [2], complicating adherence. Moreover, they are not curative, as they do not target latently integrated HIV-1 that constitute the main reservoir [3], and are inefficient in some anatomical sanctuaries [4]. Genetic interventions offer the potential to durably suppress HIV-1 while avoiding the need for lifelong pharmacological treatments. The HIV-1 entry co-receptor CCR5 has been the most studied target for HIV-1 gene therapy. The goal is to phenotypically mimic the CCR5Δ32/Δ32 genotype of the donor used for the "Berlin patient" [5]. Zinc finger nucleases (ZFNs) have been designed to knock out the CCR5 gene, and phase I clinical trials demonstrated that patient-derived ZFN engineered T cells were effective in controlling HIV viral loads in some patients following treatment interruption [6]. Several clustered regularly interspaced short palindromic repeats (CRISPR)-based approaches have also been developed to edit the CCR5 gene [7-9]. However, results from the 'Essen patient' demonstrate that this approach alone will not be successful for patients that harbor even very small amounts of HIV that can use the CXCR4 co-receptor for entry into cells [10,11].

bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

44 Retrovirus infections may be inhibited by a family of innate immune effectors, also called
45 restriction factors [12,13]. These proteins may show some level of efficacy without external
46 stimulation ("intrinsic immunity"), but their expression is typically stimulated by type I interferons
47 (IFN-I). Restriction factors act at different stages of retroviral replication and through a variety of
48 mechanisms (reviewed in [14]). Tripartite motif containing protein 5 isoform α (TRIM5α) is a
49 cytoplasmic restriction factor encoded by the interferon stimulated gene (ISG) *TRIM5* [15,16]
50 (reviewed in [17]). TRIM5α targets retroviruses shortly after their entry into the cell's cytoplasm [18].
51 TRIM5α, like other members of the TRIM protein family, has RING, B-box and Coiled-coil domains
52 at its N-terminus [19]. The RING domain-associated ubiquitin ligase activity is instrumental in the
53 restriction mechanism, as it directs some viral components as well as TRIM5α itself to proteasomal
54 degradation [20,21]. The RING domain also promotes the formation of K63-linked ubiquitin chains
55 that play a role in the restriction process [22,23] and activate innate immune pathways mediated by
56 NF-κB, AP-1 and IFN-I [24-27]. At its C-terminus, TRIM5α has a SPRY domain whose sequence
57 determines retroviral target specificity [28,29]. Upon intercepting incoming retroviral cores, TRIM5α
58 binds to the capsid protein lattice that forms the outer side of the core. This in turn promotes the
59 multimerization of TRIM5α, greatly enhancing the avidity of TRIM5α-capsid interactions [30,31]. The
60 dimerization of TRIM5α and formation of higher-order multimers involve its central B-box and
61 Coiled-coil domains [32,33]. As a result, the viral core is destabilized and undergoes premature
62 disassembly [34-37], which disrupts reverse transcription of the viral genome [38-40]. The restriction
63 mechanism also includes sequestration of viral particles in cytoplasmic bodies [41,42].

64 Restriction by TRIM5α is species-specific, cell-type-specific and virus-specific [43-45]. Although
65 some HIV-1 variants are significantly restricted by human TRIM5α (huTRIM5α), in particular in
66 HLA:B27+ or B57+ elite controller patients [24,46,47], most HIV-1 strains are poorly sensitive to
67 huTRIM5α (< 2-fold). In contrast, many non-human primate orthologs of TRIM5α, such as rhesus
68 macaque TRIM5α (rhTRIM5α), restrict HIV-1 by 10- to 100-fold [48,49]. Attempts have been made to
69 generate mutants of huTRIM5α able to efficiently target HIV-1. For instance, investigators have
70 produced chimeric versions of huTRIM5α containing small motifs of the rhTRIM5α SPRY domain
71 [43,50]. Other studies introduced smaller changes in the SPRY domain based on the rhTRIM5α
72 sequence, leading to the discovery that mutations abrogating the positive charge at Arg332 increased
73 huTRIM5α targeting of HIV-1 [51,52]. Taking a different approach, our laboratory generated
74 huTRIM5α SPRY domain mutant libraries that were screened for their capacity to restrict HIV-1. This
75 led us to isolate the HIV-1 inhibitory mutation R335G, among other mutations [53,54]. Furthermore,
76 combining the R332G and R335G mutations yielded higher restriction levels compared with single
77 mutations [53,54]. When over-expressed through retroviral transduction, R332G-R335G huTRIM5α
78 inhibits the spread of HIV-1 by 20- to 40-fold and provides a survival advantage compared to
79 untransduced cells [53,55].

80 Over-expressing TRIM5α may have detrimental consequences *in vivo*, as this protein is involved
81 in processes such as inflammation [24,27] and autophagy [56]. Another caveat of lentiviral vector-
82 mediated TRIM5α transduction is the continued expression of the endogenous, wild-type WT
83 protein. TRIM5α proteins interact with each other, and presence of the non-restrictive WT protein
84 might interfere with the antiviral activity of the restrictive mutant [57,58]. Introducing the desired
85 mutations in *TRIM5* by gene editing represents an attractive alternative, as the therapeutic gene will
86 be expressed at physiological levels and in an IFN-I-dependent fashion. Assuming biallelic gene
87 editing, no WT protein would be co-expressed along with the therapeutical mutant. Previously, we
88 transfected plasmids encoding CRISPR components (Cas9 and guide RNA, gRNA) into HEK293T
89 cells, along with a donor DNA for HDR that bear the desired mutations [59], and obtained several
90 cell clones containing corrected alleles [60]. However, no antiviral effect was observed, which was
91 due to several possible reasons, including the fact that in this cell line *TRIM5* bears an additional
92 mutation that might inactivate its antiviral properties [60]. In this paper, we electroporated CRISPR
93 ribonucleoprotein (RNP) complexes and a mutation donor DNA into Jurkat T cells in order to
94 introduce the R332G and R335G mutations. Our results demonstrate that successfully *TRIM5*-edited
95 cells have decreased permissiveness to HIV-1, and that restriction is stimulated by IFN-I as expected.

bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

2. Materials and Methods

2.1. Cell culture

Cell lines were obtained from J. Luban (University of Massachusetts School of Medicine). Jurkat T lymphocytic cells were maintained in RPMI 1640 medium (HyClone, Thermo Fisher Scientific, Ottawa, ON, Canada). Cat CRFK cells and HEK293T cells were maintained in DMEM medium (HyClone). For HEK293T cells, medium was switched to RPMI at the time of virus production. All culture media were supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin (HyClone) and Plasmocin (InvivoGen, San Diego, CA). The Jurkat cells stably transduced with a lentivirus over-expressing R332G-R335G TRIM5α were described before [53].

2.2. TRIM5 editing

The CRISPR gRNA was designed using the Zhang Lab algorithm available online at crispr.mit.edu. The crRNA was synthesized by Integrated DNA Technologies (IDT, Kanata, ON, Canada) and targets the sequence 5′AGATAATATATGGGGCACGA. The previously described single-stranded donor DNA (ssDNA) that includes mutations encoding 332G and 335G as well as silent mutations [60] was also synthesized by IDT. We used the IDT ALT® CRISPR-Cas9 system to edit *TRIM5*. For each well, we prepared 1 μL of tracrRNA:crRNA:Cas9 RNP complex containing 22 pmol of crRNA:tracrRNA duplex and 18 pmol of Cas9 enzyme. The electroporation enhancer was diluted in IDTE buffer at a final concentration of 10.8 μM. We electroporated $3x10^5$ cells with 100 pmol of the donor ssDNA, 1 μL of the RNP complex and 2 μl of electroporation enhancer. We used the Neon transfection system (Thermo Fisher Scientific) with the following parameters: 1323 V, 10 ms and 3 pulses. The plates were incubated at 37°C, 5% CO_2 for 48 h.

2.3. Isolation and screening of cell clones

To isolate single cell clones, we seeded 6x 96-well plates with approximately 0.5 cell per well. For each well, we used 100 μL of RPMI 1640 medium supplemented with 12% FBS, 10% of conditioned medium, penicillin/streptomycin and Plasmocin. The plates were grown for 2-4 weeks, and we obtained 47 surviving clonal cell populations. To screen clones for HDR-mediated TRIM5 editing, genomic DNA was extracted using 30 μL of DirectPCR lysis reagent (Viagen Biotech, Los Angeles, CA) mixed with 30 μL of water containing 12 μg of proteinase K. Lysis was completed overnight at 55°C, then the lysate was heated to 85°C for 90 minutes to deactivate the proteinase K. HDR editing-specific PCR was performed on 2 μL of samples using the OneTaq polymerase (New England Biolabs, Whitby, ON, Canada) and primers T5a_mut_fwd (5′-AAATAATCTACGGGGCCGGCGGCACAG) and T5a_qPCR_rev (5′- CCAGCACATACCCCCAGGAT). PCR was performed using the following parameters: 30 sec at 94°C, 30 sec at 61.5°C, 30 sec at 68°C, 30 cycles. The reaction products were analyzed by electrophoresis on agarose gels.

2.4. HaeIII screening of edited clones and deep sequencing

Genomic DNA was extracted using the EZ-10 Spin Column Genomic DNA kit (BioBasic, Markham, ON, Canada) from the cell clones found to be positive in the first screen described above, resuspended in 50 μL and quantified with a NanoDrop spectrophotometer (Thermo Fisher Scientific). We first amplified the targeted *TRIM5* region by PCR using the OneTaq polymerase, with 5 μL of genomic DNA extraction and bar-labeled primers. Those primers, huTR5aGG_seq_FOR (5′-ACACTGACGACATGGTTCTACAATCCCTTAGCTGACCTGTTA) and huTR5aGG_seq_REV (5′-TACGGTAGCAGAGACTTGGTCTCCCCCAGGATCCAAGCAGTT), bind outside the 200 nt region aligning with the donor ssDNA. The PCR was run for 30 cycles using the following conditions: 94°C for 30 seconds, 63°C for 30 seconds and 68°C for 60 seconds. An aliquot of each PCR product was digested with HaeIII (New England Biolabs) at 37°C for 60 minutes. Reaction products were analyzed

bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

141 by performing electrophoresis on agarose gels. The PCR products (undigested) were sequenced on a
142 MiSeq apparatus at Genome Quebec (McGill University, Montreal, Canada) and results were analyzed
143 using Integrative Genomic Viewer, available online (http://software.broadinstitute.org/software/igv/).

144 *2.5. Virus production*

145 Plasmid DNA was prepared using the Plasmid Midi kit (Qiagen, Montréal, QC, Canada).
146 HEK293T cells were seeded into 10 cm culture dishes and transfected the next day using
147 polyethyleneimine (PolyScience, Niles, IL) with the following plasmids: pMD-G (5 µg), pCNCG (10 µg)
148 and pCIG3-N or pCIG3-B (10 µg) to produce N-MLV$_{GFP}$ and B-MLV$_{GFP}$, respectively; pMD-G (5 µg)
149 and pHIV-1$_{NL-GFP}$ (10 µg) to produce HIV-1$_{NL-GFP}$ [61,62]. The medium was changed 6 h after transfection
150 and virus-containing supernatants were harvested 24, 48 and 72 h later. Supernatants were clarified by
151 centrifuging for 10 min at 3,000 rpm then filtered through 0.45 µm filters (MilliporeSigma Millex
152 Durapore PVDF syringe filters, Thermo Fisher Scientific).

153 *2.6. Viral challenges*

154 Infections of Jurkat cells were performed in 96-well plates seeded at 10,000 cells per well the day
155 before. Where applicable, treatment with IFN-β (PeproTech, Rocky Hill, NJ) at a final concentration of
156 10 ng/ml was initiated 16 h prior to infection. For virus stock titrations, nonrestrictive CRFK cells seeded
157 the day before were exposed to serial dilutions of the virus preparations. 48 h post-infection, infected
158 Jurkat or CRFK cells were fixed in 2.5% formaldehyde. The percentage of GFP-positive cells was
159 determined on a FC500 MPL cytometer (Beckman Coulter, CA) with the FCS Express 6 analysis
160 software (De novo software, CA).

161 *2.7. Knockdowns*

162 Cells were knocked down for TRIM5α, or luciferase as a control, by lentiviral transduction of
163 pAPM vectors expressing miR30-based shRNAs [27], as extensively described in previous publications
164 [24,63]. Transduced cells were treated with puromycin at 1 µg/mL for one week; all mock-transduced
165 cells were killed in those conditions.

166 *2.8. HIV-1 cDNA quantification*

167 750,000 cells per well were seeded in 24-well plates using 0.6 mL of medium per well, 24 h prior to
168 infection. Where applicable, IFN-β (10 ng/mL), MG132 (Sigma-Aldrich, Oakville, ON, Canada; 1 µg/ml)
169 and nevirapine (Sigma-Aldrich; 40 or 80 µM) were added 16 h (IFN-β) or 4 h (MG132, nevirapine) prior
170 to infection. Infection was done with HIV-1$_{NL-GFP}$ passed through 0.2 µM filters (MilliporeSigma
171 Durapore) and treated with 20 U/mL DNaseI (New England Biolabs) for 60 minutes at 37°C. Cells were
172 infected for 6 h, then total cellular DNA was extracted using the EZ-10 Spin Column Genomic DNA kit
173 (BioBasic) and quantified using the NanoDrop spectrophotometer. HIV-1 cDNAs were amplified in
174 quantitative PCR (qPCR) reactions using primers specific for GFP or for the cellular gene GAPDH as a
175 control, as described before [64]. PCR reactions were done and in a final volume of 20 µL, containing
176 1X of SensiFast SYBR Lo-ROX kit (Bioline, Meridian Biosciences, Memphis, TN), 400 nM sense and
177 antisense primers and between 150 to 400 ng of DNA. After 3 min of incubation at 95°C, 40 cycles of
178 amplification were achieved as follows: 5 sec at 95°C, 10 sec at 60°C, 15 sec at 72°C in an Agilent
179 Mx3000P instrument. Reactions were performed in duplicate and the threshold cycle was determined
180 using the MxPro software (Agilent). HIV-1 cDNA levels were normalized to those of GAPDH, which
181 was amplified simultaneously with the same PCR parameters, using the ΔCt method. Relative HIV-1
182 cDNA copy numbers were then normalized to levels found in infected/untreated control (parental) cells
183 which were set at 100%.

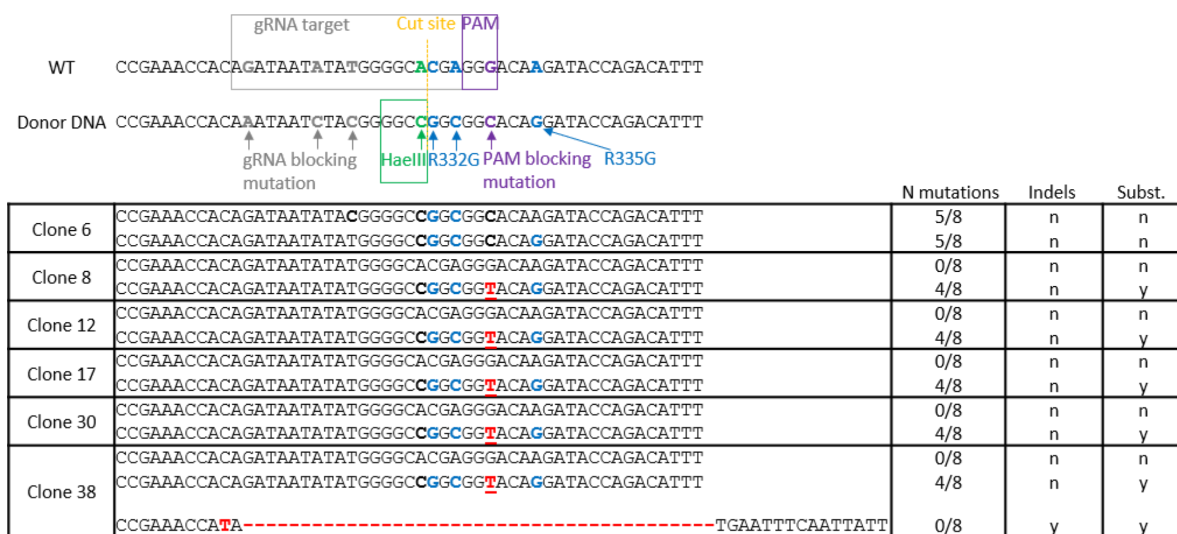
184

bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

3. Results

3.1. TRIM5 editing

Previously, we had designed three gRNAs leading to Cas9-mediated DNA cuts in the vicinity of Arg332 and Arg335 in *TRIM5* [60]. gRNA1 induces the cut closest to the desired mutations, specifically just upstream to the Arg332 triplet (Figure 1), and was the one used in this study. The HDR donor ssDNA, which is antisense to the gRNA and 200 nts long with homology arms of similar size, was the same as in the previous study [60]. Its central section contains the mutations to be introduced, as represented in Figure 1. In addition to mutations substituting arginine residues into glycine at positions 332 and 335, silent mutations are introduced to prevent resection of the HDR-corrected DNA by Cas9 through changes in both the gRNA binding site and the protospacer-adjacent motif (PAM). The silent mutations also create a HaeIII restriction site to facilitate subsequent screening (Figure 1).



	Sequence	N mutations	Indels	Subst.
Clone 6	CCGAAACCACAGATAATATACGGGGCCGGCGGCACAAGATACCAGACATTT	5/8	n	n
	CCGAAACCACAGATAATATATGGGGCCGGCGGCACAGGATACCAGACATTT	5/8	n	n
Clone 8	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCCGGCGGTACAGGATACCAGACATTT	4/8	n	y
Clone 12	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCCGGCGGTACAGGATACCAGACATTT	4/8	n	y
Clone 17	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCCGGCGGTACAGGATACCAGACATTT	4/8	n	y
Clone 30	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCCGGCGGTACAGGATACCAGACATTT	4/8	n	y
Clone 38	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCCGGCGGTACAGGATACCAGACATTT	4/8	n	y
	CCGAAACCATA--TGAATTTCAATTATT	0/8	y	y

Figure 1. *TRIM5* editing strategy and outcome. Top, the *TRIM5* region targeted for mutagenesis is aligned with the reverse-complement sequence of the donor ssDNA central region. The mutated nucleotides are shown in colors, and their purpose is indicated. The substitutions at Arg332 and Arg335 are the only nonsilent ones. Bottom, sequence of *TRIM5* alleles found in clonal Jurkat populations following electroporation of CRISPR-Cas9 RNPs and the ssDNA and PCR-based screening. For each cell population, alleles were found in equal amounts, except for clone 38, in which the relative amounts were 44% (WT allele), 24% (HDR-edited allele) and 32% (indel-containing allele). Substitutions leading to R332G and R335G mutations are in blue. Indels or undesirable substitutions are in red. The number of desired mutations for each allele is shown on the right, along with the presence (y) or absence (n) of insertions and deletions.

Jurkat cells were electroporated with CRISPR-RNP complexes. An aliquot of the transfected cells was subsequently analyzed for the presence of HDR-modified alleles. For this, we extracted DNA from the whole cell population and subjected it to PCR using primers designed to specifically amplify HDR-modified *TRIM5* [60]. We detected a PCR product of the expected size (157 nt-long) suggesting presence of the desired modifications. To isolate cellular clones of edited cells, we seeded the transfected cells in 96-well plates at 0.5 cell per well. We obtained 47 clonal Jurkat cell populations that were individually analyzed. An aliquot of each population was lysed and subjected to the HDR-edited-specific PCR assay. We found that 6 clones (13%) were positive in this screen: clones 6, 8, 12, 17, 30, 38 (Figure S1). We then performed a second PCR-based analysis on the 6 positive clones as well as 8 negative ones that were randomly selected. Specifically, we used PCR primers binding outside the genomic region complementary to the HDR, and the amplicons were then digested with HaeIII. We confirmed the presence HDR-mediated mutations in the 6 positive clones, as the HaeIII cut site was present in all of them (Figure S2). Clone 6 did not show a 304bp band corresponding to

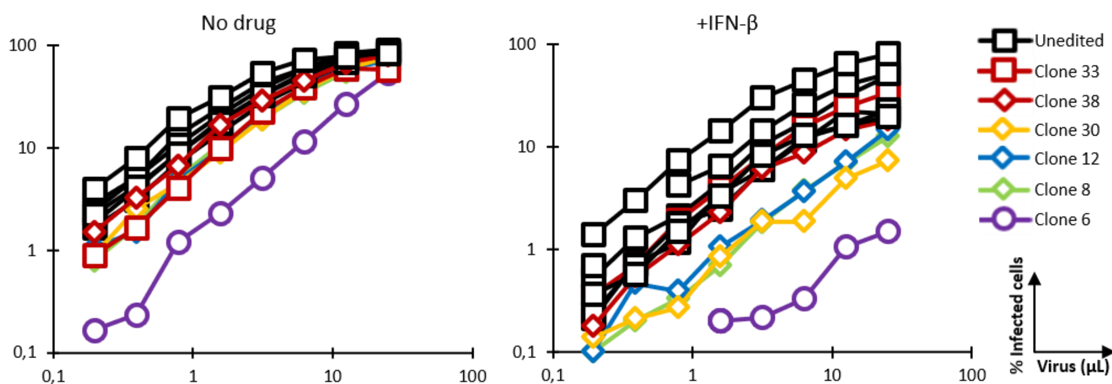
bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

220 an undigested amplicon, but only a strong 152bp band corresponding to the digestion products,
221 suggesting that both alleles were HDR-corrected for this clone but not for the other ones (Figure S2).

222 The 14 amplicons analyzed for the presence of the HaeIII cut site were also processed for MiSeq
223 sequencing. All cellular clones had two TRIM5 alleles of approximately equal abundance (≈50%),
224 with the exception of Clone 38 that seemingly had 3 alleles. Of the 6 positive clones after screening, 5
225 showed monoallelic editing including the insertion of both R332G and R335G mutations as well as
226 the formation of a HaeIII cut site, as expected (Figure 1). In each of these clones, a second *TRIM5*
227 allele had no mutation at all. In these monoallelically-modified clones, only 4 of the 8 intended
228 mutations were found. None of the HDR-modified alleles had the 3 desired mutations upstream of
229 the HaeIII-creating mutation; and all of them had an unexpected substitution in the PAM, G→T
230 instead of the intended G→C (both substitutions are silent). In addition to the modified and
231 unmodified alleles, clone 38 had an allele of unclear genesis, that contained a large deletion (Figure
232 1). Two the 8 negative clones randomly selected (31 and 33) had a single nucleotide deletion at
233 position -2 from the cut site (Figure S3). Clone 6 was the only clone to have both *TRIM5* alleles edited
234 by HDR. One of its alleles had 5 of the intended 8 mutations (R332G, R335G, HaeIII and PAM sites).
235 The other one also had 5 mutations, including R332G, but it lacked the R335G mutation (Figure 1).

236 *3.2. HIV-1 restriction activity in TRIM5-edited clones*

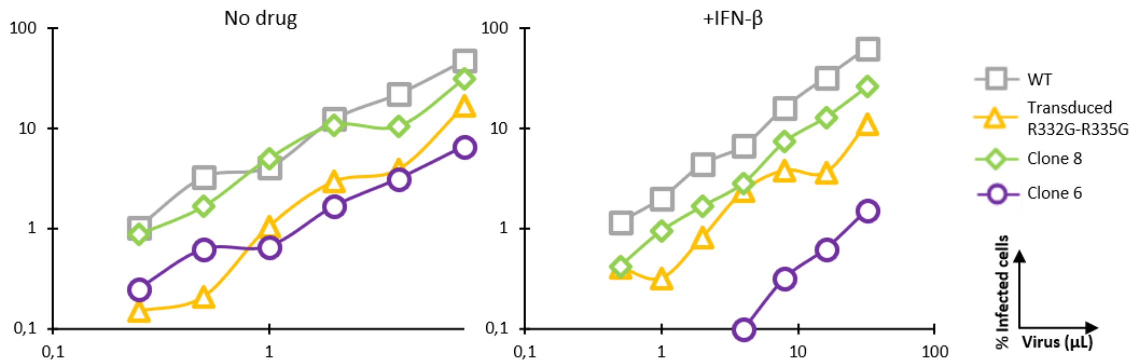
237 In order to determine permissiveness to HIV-1 of the gene-edited clones, as well as TRIM5α
238 stimulation by IFN-I, cells were infected in the absence or presence of IFN-β with increasing amounts
239 of VSV-G-pseudotyped HIV-1$_{NL-GFP}$, a GFP-expressing, propagation-incompetent ΔEnv/ΔNef version
240 of the HIV-1 clone NL4-3 [65]. The percentage of infected cells was determined by analyzing GFP
241 expression using flow cytometry. The titration curves were remarkably grouped for the 7 screen-
242 negative clones included in this experiment, suggesting an absence of strong clone-to-clone variation
243 in permissiveness to lentiviral transduction in Jurkat cells. In the absence of IFN-β, only clone 6
244 showed a significant decrease (≈10-fold) in permissiveness to HIV-1$_{NL-GFP}$, compared with the rest of
245 the clonal populations (Figure 2). In the presence of IFN-β, all cell populations showed decreased
246 permissiveness to HIV-1$_{NL-GFP}$, indicating that one or more ISGs inhibited HIV-1, as expected [12].
247 IFN-I treatment resulted in significant restriction of HIV-1$_{NL-GFP}$ in three clones (8, 12 and 30) that had
248 monoallelic HDR editing, compared to screen-negative clones (≈5-fold), and restriction was strikingly
249 strong in clone 6 cells (≈40-fold) (Figure 2). Clone 17 could not be included in this experiment, but we
250 found it to behave similarly to clone 30 in a separate infection experiment (Fig. S4).



251 **Figure 2.** Permissiveness of HDR-edited and unedited control Jurkat cell populations to infection with
252 HIV-1$_{NL-GFP}$. Cells were infected with increasing amounts of HIV-1$_{NL-GFP}$ in the absence (left) or
253 presence (right) of IFN-β (10 ng/ml), and the % of GFP-positive cells was determined 2 days later by
254 FACS. Results are presented for 5 clonal populations containing HDR-edited alleles (diamond
255 symbols and purple circles) and 7 randomly chosen negative clones (squares). Clones containing a
256 deletion in one of the alleles are shown in red.

bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

257 We then analyzed HIV-1 restriction in clones 6 and 8 on side-by-side with Jurkat cells stably
258 transduced with a retroviral vector expressing R332G-R335G huTRIM5α [53]. In the absence of IFN-
259 β, HIV-1$_{NL-GFP}$ was restricted in both clone 6 and in the retrovirally transduced cells expressing R332G-
260 R335G huTRIM5α, compared with the parental WT cells (Figure 3). In the presence of IFN-β, clone 6
261 showed a 50-fold reduction in permissiveness to infection compared to parental cells, and the
262 monoallelically HDR-modified clone 8 also showed a modest restriction effect. In contrast, HIV-1
263 restriction in the cells retrovirally transduced with R332G-R335G TRIM5α was not stimulated by IFN-
264 β (Figure 3), consistent with the transgene being expressed from a non-IFN-I-inducible promoter.



265 **Figure 3.** IFN-β treatment stimulates HIV-1 restriction by mutated endogenous TRIM5α but has no
266 effect on retrovirally transduced TRIM5α. Parental Jurkat cells, clone 6 and clone 8 cells, and cells
267 stably transduced with R332G-R335G were infected with increasing amounts of HIV-1$_{NL-GFP}$ in the
268 absence or presence of IFN-β, and the % of GFP-positive cells was determined 2 days later by FACS.

269 *3.3. Knockdown validation of TRIM5α antiviral function*

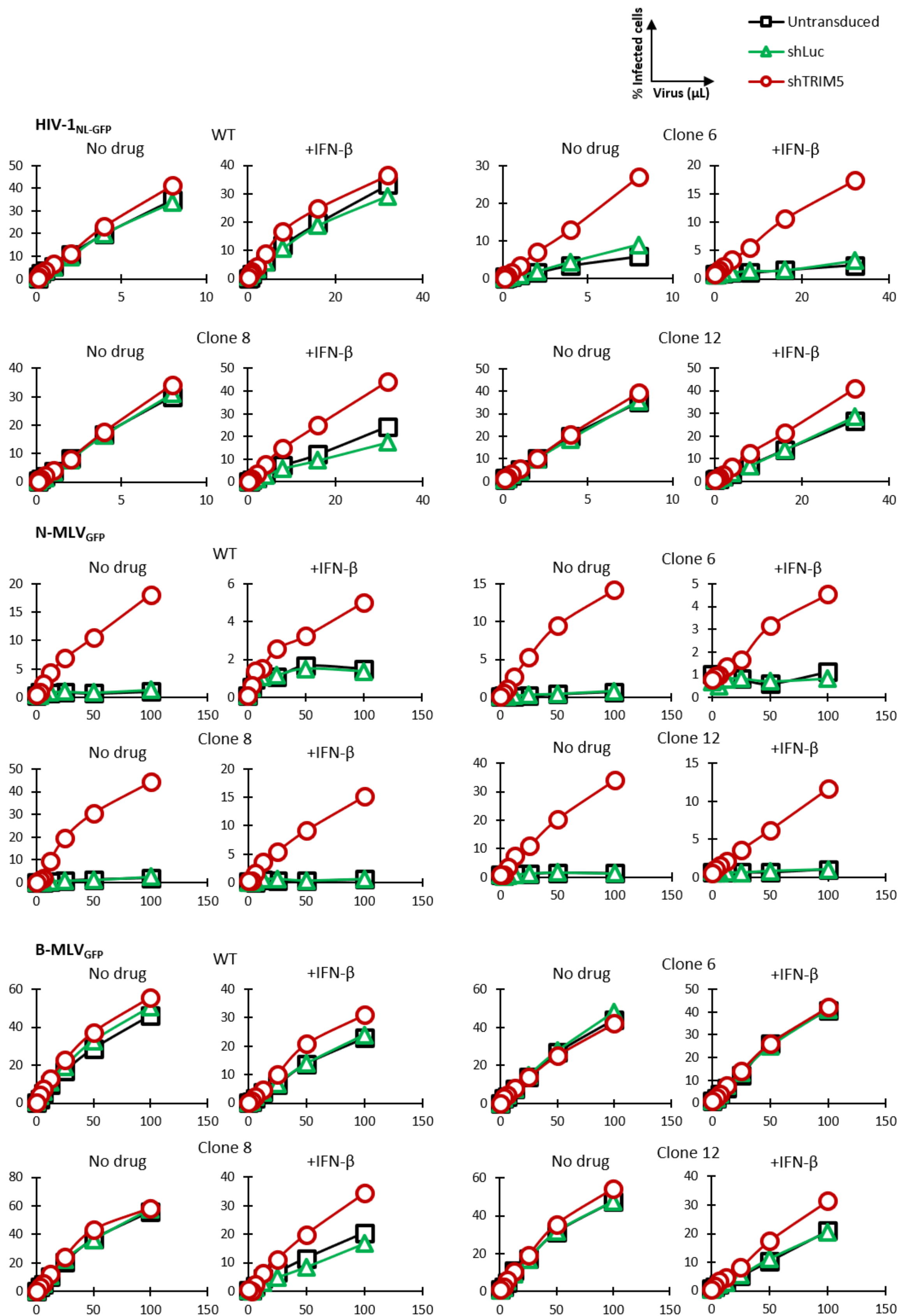
270 A cellular clone might show reduced permissiveness to HIV-1 due to an IFN-I-inducible factor
271 other than TRIM5α. In order to ensure that clone 6 low permissiveness to HIV-1 was due to
272 expression of the edited TRIM5α, we knocked down TRIM5α in these cells as well as in the unedited
273 parental (WT) cells. The shRNA used here binds to a different TRIM5α region than the one targeted
274 for mutagenesis. Untransduced cells were eliminated by antibiotic selection. Two monoallelically
275 *TRIM5*-edited clonal populations, clone 8 and clone 12, were also included in this experiment. Cells
276 were infected with increasing doses of HIV-1$_{NL-GFP}$ in the presence or absence of IFN-β. R332G-R335G
277 huTRIM5α retains the ability to restrict the N-tropic strains of murine leukemia virus (MLV), as
278 shown previously [53], whereas B-tropic MLV is not restricted by either WT or mutated huTRIM5α.
279 Thus, cells were also infected with N-MLV$_{GFP}$ and B-MLV$_{GFP}$ in order to assess TRIM5α restrictive
280 capabilities on a target other than HIV-1. We found that knocking down TRIM5α rescued HIV-1$_{NL-}$
281 $_{GFP}$ infection of clone 6 cells, both in the absence and the presence of IFN-β, though the effect was
282 stronger in the presence of IFN-β, as expected (Figure 4). In contrast, TRIM5α knockdown had no
283 effect on the infection of the unedited parental cells by HIV-1$_{NL-GFP}$. The control shRNA used, which
284 targets the nonhuman gene luciferase, did not affect permissiveness to HIV-1. Knocking down
285 TRIM5α also increased HIV-1$_{NL-GFP}$ infection of clones 8 and 12, which have only one R332G-R335G
286 *TRIM5* allele, but the effect was seen only in the presence of IFN-β and was smaller compared to
287 clone 6. In summary, TRIM5α knockdown-mediated rescue of HIV-1 infectivity in clones 6, 8 and 12
288 correlated with the extent of HIV-1 restriction as seen in Figure 2, and also correlated with the
289 enhancement of restriction by IFN-β. Thus, decreased permissiveness to HIV-1 in clones 6, 8 and 12
290 was due in large part to the antiviral effect of edited *TRIM5* alleles in these clones. N-MLV$_{GFP}$ infection
291 was strongly increased by knocking down TRIM5α in all cell populations and in the absence or
292 presence of IFN-β, indicating that all cell populations expressed restriction-competent TRIM5α alleles
293 (Figure 4). B-MLV$_{GFP}$ infection of parental cells as well as clone 8 and clone 12 cells was slightly
294 increased by knocking down TRIM5α, and only in the presence of IFN-β. This suggests with WT
295 huTRIM5α but not the R332G-R335G mutant has a weak inhibitory activity against B-MLV which is

bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

296 revealed by IFN-I treatment. Thus, *TRIM5* editing to allow HIV-1 targeting had little or no impact on
297 its capacity to restrict a different retrovirus.

bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

298



299 **Figure 4.** TRIM5α knockdown rescues HIV-1 from restriction in gene-edited cells. Parental Jurkat cells
300 and clone 6, 8 and 12 cells were lentivirally transduced with shRNAs targeting TRIM5α or Luc as a
301 control, or left untransduced. Cells were then infected with increasing amounts of HIV-1NL-GFP, N-

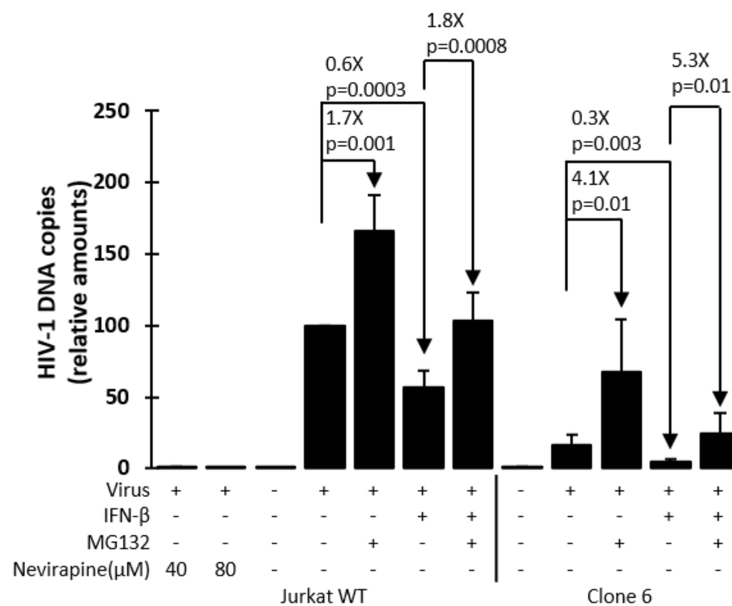
bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

302 MLV$_{GFP}$ or B-MLV$_{GFP}$ in the absence or presence of IFN-β. The % of GFP-positive cells was determined
303 2 days later by FACS.

304 *3.4. Mechanism of inhibition by edited TRIM5α*

305 TRIM5α-mediated restriction decreases retroviral cDNA synthesis by reverse transcription, and
306 this mechanism of restriction is counteracted by the proteasome-targeting drug MG132 [39,66]. qPCR
307 was used to analyze HIV-1 cDNA synthesis in clone 6 and in parental unmodified cells in the
308 presence or absence of IFN-β and MG132 (Figure 5). Following a short exposure to HIV-1$_{NL-GFP}$, total
309 cellular DNAs were prepared and qPCR was performed using primers complementary to the HIV-1
310 vector-specific gene GFP, which is representative of late reverse transcription cDNA. To adjust for
311 DNA amounts in the reactions, GAPDH DNA was quantified as well. Consistent with the known
312 mechanism of action for TRIM5α, we found HIV-1 cDNA amounts to be significantly smaller (≈6-
313 fold) in clone 6 cells, as compared to the parental cells, in the absence of drug (Figure 5). In the
314 presence of IFN-β, this effect was more pronounced, consistent with the stimulation of TRIM5α-
315 mediated restriction by IFN-I. Conversely, the reduction in HIV-1 cDNA amounts in clone 6 was only
316 2.5-fold compared to WT in the presence of MG132, showing that MG132 attenuated this restriction
317 phenotype, as expected. Accordingly, MG132 rescued HIV-1 cDNA synthesis by a larger magnitude
318 in clone 6 (4.1-fold) than in the WT cells (1.7-fold), in the absence of IFN-β. In conclusion, these results
319 show that HIV-1 infection is inhibited via the expected mechanism in the *TRIM5*-edited clone 6 cells.

320



321 **Figure 5.** HIV-1 cDNA synthesis is inhibited in *TRIM5*-edited clone 6 Jurkat cells but is rescued by
322 MG132 treatment. Parental (WT) Jurkat cells and clone 6 cells were treated or not as indicated with
323 IFN-β, MG132 or the reverse transcriptase inhibitor nevirapine (as a DNA contamination control).
324 Cells were then infected for 6 h with HIV-1$_{NL-GFP}$, followed by DNA extraction and qPCR with primers
325 specific for HIV-1 vector cDNA (GFP sequence) and for the cellular gene GAPDH for normalization
326 purposes. Results are presented as HIV-1 cDNA copy numbers adjusted according to GAPDH copy
327 numbers and normalized to the no-drug WT cells control which is set at 100%. P-values were
328 calculated using the Student's T-test.

329 **4. Discussion**

330 Despite an increased focus from the HIV-1 research community on cure research in recent years
331 [67], aiming in particular at eliminating latent reservoirs [3], we are still far from a sterilizing or
332 functional cure. Similarly, and despite some recent conceptual advances such as novel strategies to

bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

333 elicit the production of broadly-neutralizing antibodies [68], vaccine prospects remain seemingly out
334 of reach [69,70]. The field of HIV-1 drug research continues to show impressive progresses, such as
335 the ongoing emergence of long-acting antiviral drugs [71] and of the HIV-1 capsid protein as a novel
336 drug target [72]. However, curative treatments are still elusive, and multidrug resistance is a
337 persistent concern [73]. Thus, the search for alternative strategies is still a priority. HIV-1 is one of the
338 rare infectious diseases for which a genetic intervention is sensible. Indeed, it is a life-long infection
339 whose treatment is expensive, and which results in diminished life expectancy and lessened quality
340 of life, even in successfully treated patients [74-76]. If successful, a one-off genetic intervention would
341 represent an attractive option for HIV-1 patients. Most genetic strategies presently explored with the
342 aim of inhibiting HIV-1 involve the knockdown or knockout of the HIV-1 co-receptor CCR5, but this
343 approach is not without caveats (see introduction).

344 In this study, we demonstrate that editing of the intrinsic innate effector TRIM5α protects human
345 cells against infection by HIV-1. Although such an approach has been conceptualized before by us
346 and others [60,77], to the best of our knowledge this study constitutes the first proof-of-concept for
347 the protection against a pathogen provided by editing of an innate effector. Yet, we encountered a
348 major difficulty, in that biallelic modification seemed to be a rare event, with only one out of 6 HDR-
349 edited clones affecting both alleles. Clones with R332G-R335G introduced into only one of the two
350 alleles showed a mild restriction phenotype, which necessitated IFN-I treatment to be revealed. Co-
351 expression of restrictive and nonrestrictive TRIM5α alleles is expected to lead to a weak restriction
352 phenotype. Indeed, distinct TRIM5α proteins can form heterodimers [57,58], and thus a TRIM5α
353 lattice containing both capsid-targeting and -nontargeting monomers would bind the viral core
354 weakly. If TRIM5α is to be pursued in gene editing approaches to suppress HIV-1, it is crucial to
355 develop strategies that improve the rate of biallelic editing. When this project was initiated, co-
356 transfection of CRISPR components and donor DNA was the only option available for precise
357 genome editing. Methods for introducing discrete substitutions in eukaryotic genomes have vastly
358 improved and diversified since, and now include adeno-associated viral vector-mediated delivery of
359 the donor DNA for HDR [78]; various pharmacological, physical or genetic methods to improve HDR
360 rates [79-82]; and base editing, which does not require DNA cuts nor a donor DNA [83]. Taking
361 advantage of these recent innovations, we consider it likely that it will soon be possible to achieve
362 efficient biallelic editing of *TRIM5*, along with other restriction factor genes, in human cells, resulting
363 in a profound disruption of HIV-1 infectivity.

364 **Supplementary Materials:** The following are available online at www.mdpi.com/xxx/s1, Figure S1: HDR
365 editing-specific PCR screening of Jurkat cells transfected with CRISPR-Cas9 RNPs and donor ssDNA, Figure S2:
366 HaeIII screening of selected Jurkat clones, Figure S3: MiSeq sequencing results for all clones analyzed, Figure S4:
367 Permissiveness of HDR-edited clone 17 to HIV-1$_{NL-GFP}$ infection.

368 **Author Contributions:** Conceptualization, L.B.; methodology, K.D., L.O., L.B..; formal analysis, L.B., K.D., N.M.,
369 M.B.P., L.O..; investigation, K.D., L.B., L.O..; resources, C.D., A.C..; writing—original draft preparation, L.B. and
370 K.D.; writing—review and editing, L.B., N.M. and K.D.; supervision, L.B., M.B.P. and N.M.; project
371 administration, L.B.; funding acquisition, L.B. All authors have read and agreed to the published version of the
372 manuscript.

373 **Funding:** This research was funded by a grant from Fondation de l'Université du Québec à Trois-Rivières.

374 **Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the
375 study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to
376 publish the results.

377 **References**

378 1. Angel, J.B.; Parato, K.G.; Kumar, A.; Kravcik, S.; Badley, A.D.; Fex, C.; Ashby, D.; Sun, E.; Cameron,
379 D.W. Progressive human immunodeficiency virus-specific immune recovery with prolonged viral
380 suppression. *The Journal of infectious diseases* **2001**, *183*, 546-554, doi:10.1086/318547.

bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

381 2. Hagos, L.; Fessehaye, S.; Anand, I.S. Nature and prevalence of adverse drug reaction of antiretroviral
382 medications in Halibet National Referral Hospital: a retrospective study. *BMC pharmacology & toxicology*
383 **2019**, *20*, 24, doi:10.1186/s40360-019-0307-9.
384 3. Cohn, L.B.; Chomont, N.; Deeks, S.G. The Biology of the HIV-1 Latent Reservoir and Implications for
385 Cure Strategies. *Cell host & microbe* **2020**, *27*, 519-530, doi:10.1016/j.chom.2020.03.014.
386 4. Rose, R.; Nolan, D.J.; Maidji, E.; Stoddart, C.A.; Singer, E.J.; Lamers, S.L.; McGrath, M.S. Eradication of
387 HIV from Tissue Reservoirs: Challenges for the Cure. *AIDS research and human retroviruses* **2018**, *34*, 3-
388 8, doi:10.1089/AID.2017.0072.
389 5. Allers, K.; Hutter, G.; Hofmann, J.; Loddenkemper, C.; Rieger, K.; Thiel, E.; Schneider, T. Evidence for
390 the cure of HIV infection by CCR5Delta32/Delta32 stem cell transplantation. *Blood* **2011**, *117*, 2791-2799,
391 doi:blood-2010-09-309591 [pii]
392 10.1182/blood-2010-09-309591.
393 6. Tebas, P.; Stein, D.; Tang, W.W.; Frank, I.; Wang, S.Q.; Lee, G.; Spratt, S.K.; Surosky, R.T.; Giedlin, M.A.;
394 Nichol, G., et al. Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. *The New*
395 *England journal of medicine* **2014**, *370*, 901-910, doi:10.1056/NEJMoa1300662.
396 7. Xu, L.; Yang, H.; Gao, Y.; Chen, Z.; Xie, L.; Liu, Y.; Liu, Y.; Wang, X.; Li, H.; Lai, W., et al. CRISPR/Cas9-
397 Mediated CCR5 Ablation in Human Hematopoietic Stem/Progenitor Cells Confers HIV-1 Resistance In
398 Vivo. *Mol. Ther.* **2017**, *25*, 1782-1789, doi:10.1016/j.ymthe.2017.04.027.
399 8. Kang, H.; Minder, P.; Park, M.A.; Mesquitta, W.T.; Torbett, B.E.; Slukvin, II. CCR5 Disruption in
400 Induced Pluripotent Stem Cells Using CRISPR/Cas9 Provides Selective Resistance of Immune Cells to
401 CCR5-tropic HIV-1 Virus. *Mol. Ther. Nucleic Acids* **2015**, *4*, e268, doi:10.1038/mtna.2015.42.
402 9. Qi, C.; Li, D.; Jiang, X.; Jia, X.; Lu, L.; Wang, Y.; Sun, J.; Shao, Y.; Wei, M. Inducing CCR5Delta32/Delta32
403 Homozygotes in the Human Jurkat CD4+ Cell Line and Primary CD4+ Cells by CRISPR-Cas9 Genome-
404 Editing Technology. *Mol. Ther. Nucleic Acids* **2018**, *12*, 267-274, doi:10.1016/j.omtn.2018.05.012.
405 10. Verheyen, J.; Thielen, A.; Lubke, N.; Dirks, M.; Widera, M.; Dittmer, U.; Kordales, L.; Daumer, M.; Jong,
406 T.C.M.; Wensing, A.M.J., et al. Rapid rebound of a preexisting CXCR4-tropic HIV variant after
407 allogeneic transplantation with CCR5 delta32 homozygous stem cells. *Clinical infectious diseases : an*
408 *official publication of the Infectious Diseases Society of America* **2018**, 10.1093/cid/ciy565,
409 doi:10.1093/cid/ciy565.
410 11. Kordelas, L.; Verheyen, J.; Beelen, D.W.; Horn, P.A.; Heinold, A.; Kaiser, R.; Trenschel, R.; Schadendorf,
411 D.; Dittmer, U.; Esser, S. Shift of HIV tropism in stem-cell transplantation with CCR5 Delta32 mutation.
412 *N. Engl. J. Med.* **2014**, *371*, 880-882, doi:10.1056/NEJMc1405805.
413 12. Merindol, N.; Berthoux, L. Restriction Factors in HIV-1 Disease Progression. *Current HIV research* **2015**,
414 *13*, 448-461.
415 13. Schoggins, J.W.; Rice, C.M. Interferon-stimulated genes and their antiviral effector functions. *Curr Opin*
416 *Virol* **2011**, *1*, 519-525, doi:10.1016/j.coviro.2011.10.008.
417 14. Colomer-Lluch, M.; Ruiz, A.; Moris, A.; Prado, J.G. Restriction Factors: From Intrinsic Viral Restriction
418 to Shaping Cellular Immunity Against HIV-1. *Frontiers in immunology* **2018**, *9*, 2876,
419 doi:10.3389/fimmu.2018.02876.
420 15. Carthagena, L.; Parise, M.C.; Ringeard, M.; Chelbi-Alix, M.K.; Hazan, U.; Nisole, S. Implication of TRIM
421 alpha and TRIMCyp in interferon-induced anti-retroviral restriction activities. *Retrovirology* **2008**, *5*, 59.
422 16. Stremlau, M.; Owens, C.M.; Perron, M.J.; Kiessling, M.; Autissier, P.; Sodroski, J. The cytoplasmic body
423 component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature* **2004**, *427*, 848-853.

bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

424 17. Ganser-Pornillos, B.K.; Pornillos, O. Restriction of HIV-1 and other retroviruses by TRIM5. *Nature*
425 *reviews. Microbiology* **2019**, *17*, 546-556, doi:10.1038/s41579-019-0225-2.
426 18. Passerini, L.D.; Keckesova, Z.; Towers, G.J. Retroviral restriction factors Fv1 and TRIM5alpha act
427 independently and can compete for incoming virus before reverse transcription. *Journal of virology* **2006**,
428 *80*, 2100-2105.
429 19. Rajsbaum, R.; Garcia-Sastre, A.; Versteeg, G.A. TRIMmunity: the roles of the TRIM E3-ubiquitin ligase
430 family in innate antiviral immunity. *Journal of molecular biology* **2014**, *426*, 1265-1284,
431 doi:10.1016/j.jmb.2013.12.005.
432 20. Kutluay, S.B.; Perez-Caballero, D.; Bieniasz, P.D. Fates of Retroviral Core Components during
433 Unrestricted and TRIM5-Restricted Infection. *PLoS Pathog* **2013**, *9*, e1003214,
434 doi:10.1371/journal.ppat.1003214.
435 21. Rold, C.J.; Aiken, C. Proteasomal degradation of TRIM5alpha during retrovirus restriction. *PLoS Pathog*
436 **2008**, *4*, e1000074.
437 22. Imam, S.; Komurlu, S.; Mattick, J.; Selyutina, A.; Talley, S.; Eddins, A.; Diaz-Griffero, F.; Campbell, E.M.
438 K63-Linked Ubiquitin Is Required for Restriction of HIV-1 Reverse Transcription and Capsid
439 Destabilization by Rhesus TRIM5alpha. *Journal of virology* **2019**, *93*, doi:10.1128/JVI.00558-19.
440 23. Fletcher, A.J.; Christensen, D.E.; Nelson, C.; Tan, C.P.; Schaller, T.; Lehner, P.J.; Sundquist, W.I.; Towers,
441 G.J. TRIM5alpha requires Ube2W to anchor Lys63-linked ubiquitin chains and restrict reverse
442 transcription. *The EMBO journal* **2015**, *34*, 2078-2095, doi:10.15252/embj.201490361.
443 24. Merindol, N.; El-Far, M.; Sylla, M.; Masroori, N.; Dufour, C.; Li, J.X.; Cherry, P.; Plourde, M.B.;
444 Tremblay, C.; Berthoux, L. HIV-1 capsids from B27/B57+ elite controllers escape Mx2 but are targeted
445 by TRIM5alpha, leading to the induction of an antiviral state. *PLoS Pathog* **2018**, *14*, e1007398,
446 doi:10.1371/journal.ppat.1007398.
447 25. Nepveu-Traversy, M.E.; Berthoux, L. The conserved sumoylation consensus site in TRIM5alpha
448 modulates its immune activation functions. *Virus research* **2014**, *184C*, 30-38, doi:S0168-1702(14)00066-5
449 [pii]
450 10.1016/j.virusres.2014.02.013.
451 26. Na, L.; Tang, Y.D.; Wang, C.; Liu, C.; Wang, X. Rhesus monkey TRIM5alpha protein SPRY domain
452 contributes to AP-1 activation. *The Journal of biological chemistry* **2018**, *293*, 2661-2674,
453 doi:10.1074/jbc.RA117.000127.
454 27. Pertel, T.; Hausmann, S.; Morger, D.; Zuger, S.; Guerra, J.; Lascano, J.; Reinhard, C.; Santoni, F.A.; Uchil,
455 P.D.; Chatel, L., et al. TRIM5 is an innate immune sensor for the retrovirus capsid lattice. *Nature* **2011**,
456 *472*, 361-365, doi:10.1038/nature09976.
457 28. Stremlau, M.; Perron, M.; Welikala, S.; Sodroski, J. Species-specific variation in the B30.2(SPRY) domain
458 of TRIM5alpha determines the potency of human immunodeficiency virus restriction. *Journal of virology*
459 **2005**, *79*, 3139-3145.
460 29. Perez-Caballero, D.; Hatziioannou, T.; Yang, A.; Cowan, S.; Bieniasz, P.D. Human tripartite motif
461 5alpha domains responsible for retrovirus restriction activity and specificity. *Journal of virology* **2005**, *79*,
462 8969-8978.
463 30. Ganser-Pornillos, B.K.; Chandrasekaran, V.; Pornillos, O.; Sodroski, J.G.; Sundquist, W.I.; Yeager, M.
464 Hexagonal assembly of a restricting TRIM5alpha protein. *Proceedings of the National Academy of Sciences*
465 *of the United States of America* **2011**, *108*, 534-539, doi:10.1073/pnas.1013426108.

bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

466 31. Yu, A.; Skorupka, K.A.; Pak, A.J.; Ganser-Pornillos, B.K.; Pornillos, O.; Voth, G.A. TRIM5alpha self-
467 assembly and compartmentalization of the HIV-1 viral capsid. *Nature communications* **2020**, *11*, 1307,
468 doi:10.1038/s41467-020-15106-1.
469 32. Li, X.; Yeung, D.F.; Fiegen, A.M.; Sodroski, J. Determinants of the higher order association of the
470 restriction factor TRIM5alpha and other tripartite motif (TRIM) proteins. *The Journal of biological*
471 *chemistry* **2011**, *286*, 27959-27970, doi:M111.260406 [pii]
472 10.1074/jbc.M111.260406.
473 33. Keown, J.R.; Yang, J.X.; Douglas, J.; Goldstone, D.C. Characterisation of assembly and ubiquitylation
474 by the RBCC motif of Trim5alpha. *Scientific reports* **2016**, *6*, 26837, doi:10.1038/srep26837.
475 34. Black, L.R.; Aiken, C. TRIM5alpha disrupts the structure of assembled HIV-1 capsid complexes in vitro.
476 *Journal of virology* **2010**, *84*, 6564-6569, doi:JVI.00210-10 [pii]
477 10.1128/JVI.00210-10.
478 35. Zhao, G.; Zhang, P. CryoEM analysis of capsid assembly and structural changes upon interactions with
479 a host restriction factor, TRIM5alpha. *Methods in molecular biology* **2014**, *1087*, 13-28, doi:10.1007/978-1-
480 62703-670-2_2.
481 36. Stremlau, M.; Perron, M.; Lee, M.; Li, Y.; Song, B.; Javanbakht, H.; Diaz-Griffero, F.; Anderson, D.J.;
482 Sundquist, W.I.; Sodroski, J. Specific recognition and accelerated uncoating of retroviral capsids by the
483 TRIM5alpha restriction factor. *Proceedings of the National Academy of Sciences of the United States of*
484 *America* **2006**, *103*, 5514-5519.
485 37. Sastri, J.; Campbell, E.M. Recent insights into the mechanism and consequences of TRIM5alpha
486 retroviral restriction. *AIDS research and human retroviruses* **2011**, *27*, 231-238, doi:10.1089/AID.2010.0367.
487 38. Roa, A.; Hayashi, F.; Yang, Y.; Lienlaf, M.; Zhou, J.; Shi, J.; Watanabe, S.; Kigawa, T.; Yokoyama, S.;
488 Aiken, C., et al. RING domain mutations uncouple TRIM5alpha restriction of HIV-1 from inhibition of
489 reverse transcription and acceleration of uncoating. *Journal of virology* **2012**, *86*, 1717-1727,
490 doi:10.1128/JVI.05811-11.
491 39. Wu, X.; Anderson, J.L.; Campbell, E.M.; Joseph, A.M.; Hope, T.J. Proteasome inhibitors uncouple rhesus
492 TRIM5alpha restriction of HIV-1 reverse transcription and infection. *Proceedings of the National Academy*
493 *of Sciences of the United States of America* **2006**, *103*, 7465-7470.
494 40. Campbell, E.M.; Weingart, J.; Sette, P.; Opp, S.; Sastri, J.; O'Connor, S.K.; Talley, S.; Diaz-Griffero, F.;
495 Hirsch, V.; Bouamr, F. TRIM5alpha-Mediated Ubiquitin Chain Conjugation Is Required for Inhibition
496 of HIV-1 Reverse Transcription and Capsid Destabilization. *Journal of virology* **2016**, *90*, 1849-1857,
497 doi:10.1128/JVI.01948-15.
498 41. Nepveu-Traversy, M.E.; Demogines, A.; Fricke, T.; Plourde, M.B.; Riopel, K.; Veillette, M.; Diaz-
499 Griffero, F.; Sawyer, S.L.; Berthoux, L. A putative SUMO interacting motif in the B30.2/SPRY domain
500 of rhesus macaque TRIM5alpha important for NF-kappaB/AP-1 signaling and HIV-1 restriction.
501 *Heliyon* **2016**, *2*, e00056, doi:10.1016/j.heliyon.2015.e00056.
502 42. Campbell, E.M.; Perez, O.; Anderson, J.L.; Hope, T.J. Visualization of a proteasome-independent
503 intermediate during restriction of HIV-1 by rhesus TRIM5alpha. *The Journal of cell biology* **2008**, *180*, 549-
504 561.
505 43. Sawyer, S.L.; Wu, L.I.; Emerman, M.; Malik, H.S. Positive selection of primate TRIM5alpha identifies a
506 critical species-specific retroviral restriction domain. *Proceedings of the National Academy of Sciences of the*
507 *United States of America* **2005**, *102*, 2832-2837.

bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

508 44. Hatziioannou, T.; Perez-Caballero, D.; Yang, A.; Cowan, S.; Bieniasz, P.D. Retrovirus resistance factors
509 Ref1 and Lv1 are species-specific variants of TRIM5alpha. *Proceedings of the National Academy of Sciences*
510 *of the United States of America* **2004**, *101*, 10774-10779.
511 45. Bérubé, J.; Bouchard, A.; Berthoux, L. Both TRIM5alpha and TRIMCyp have only weak antiviral activity
512 in canine D17 cells. *Retrovirology* **2007**, *4*, 68.
513 46. Granier, C.; Battivelli, E.; Lecuroux, C.; Venet, A.; Lambotte, O.; Schmitt-Boulanger, M.; Delaugerre, C.;
514 Molina, J.M.; Chakrabarti, L.A.; Clavel, F., et al. Pressure from TRIM5alpha contributes to control of
515 HIV-1 replication by individuals expressing protective HLA-B alleles. *Journal of virology* **2013**, *87*, 10368-
516 10380, doi:10.1128/JVI.01313-13.
517 47. Battivelli, E.; Migraine, J.; Lecossier, D.; Yeni, P.; Clavel, F.; Hance, A.J. Gag cytotoxic T lymphocyte
518 escape mutations can increase sensitivity of HIV-1 to human TRIM5alpha, linking intrinsic and
519 acquired immunity. *Journal of virology* **2011**, *85*, 11846-11854, doi:JVI.05201-11 [pii]
520 10.1128/JVI.05201-11.
521 48. Song, B.; Javanbakht, H.; Perron, M.; Park, D.H.; Stremlau, M.; Sodroski, J. Retrovirus restriction by
522 TRIM5alpha variants from Old World and New World primates. *Journal of virology* **2005**, *79*, 3930-3937.
523 49. Wilson, S.J.; Webb, B.L.; Maplanka, C.; Newman, R.M.; Verschoor, E.J.; Heeney, J.L.; Towers, G.J.
524 Rhesus macaque TRIM5 alleles have divergent antiretroviral specificities. *Journal of virology* **2008**, *82*,
525 7243-7247, doi:10.1128/JVI.00307-08.
526 50. Walker, J.E.; Chen, R.X.; McGee, J.; Nacey, C.; Pollard, R.B.; Abedi, M.; Bauer, G.; Nolta, J.A.; Anderson,
527 J.S. Generation of an HIV-1-resistant immune system with CD34(+) hematopoietic stem cells transduced
528 with a triple-combination anti-HIV lentiviral vector. *Journal of virology* **2012**, *86*, 5719-5729,
529 doi:10.1128/JVI.06300-11.
530 51. Yap, M.W.; Nisole, S.; Stoye, J.P. A single amino acid change in the SPRY domain of human Trim5alpha
531 leads to HIV-1 restriction. *Current biology : CB* **2005**, *15*, 73-78.
532 52. Li, Y.; Li, X.; Stremlau, M.; Lee, M.; Sodroski, J. Removal of arginine 332 allows human TRIM5alpha to
533 bind human immunodeficiency virus capsids and to restrict infection. *Journal of virology* **2006**, *80*, 6738-
534 6744.
535 53. Pham, Q.T.; Bouchard, A.; Grutter, M.G.; Berthoux, L. Generation of human TRIM5alpha mutants with
536 high HIV-1 restriction activity. *Gene therapy* **2010**, *17*, 859-871, doi:gt201040 [pii]
537 10.1038/gt.2010.40.
538 54. Pham, Q.T.; Veillette, M.; Brandariz-Nunez, A.; Pawlica, P.; Thibert-Lefebvre, C.; Chandonnet, N.; Diaz-
539 Griffero, F.; Berthoux, L. A novel aminoacid determinant of HIV-1 restriction in the TRIM5alpha
540 variable 1 region isolated in a random mutagenic screen. *Virus research* **2013**,
541 10.1016/j.virusres.2013.01.013, doi:10.1016/j.virusres.2013.01.013.
542 55. Jung, U.; Urak, K.; Veillette, M.; Nepveu-Traversy, M.E.; Pham, Q.T.; Hamel, S.; Rossi, J.J.; Berthoux, L.
543 Preclinical Assessment of Mutant Human TRIM5alpha as an Anti-HIV-1 Transgene. *Human gene therapy*
544 **2015**, *26*, 664-679, doi:10.1089/hum.2015.059.
545 56. Ribeiro, C.M.; Sarrami-Forooshani, R.; Setiawan, L.C.; Zijlstra-Willems, E.M.; van Hamme, J.L.;
546 Tigchelaar, W.; van der Wel, N.N.; Kootstra, N.A.; Gringhuis, S.I.; Geijtenbeek, T.B. Receptor usage
547 dictates HIV-1 restriction by human TRIM5alpha in dendritic cell subsets. *Nature* **2016**, *540*, 448-452,
548 doi:10.1038/nature20567.

bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

549 57. Battivelli, E.; Migraine, J.; Lecossier, D.; Matsuoka, S.; Perez-Bercoff, D.; Saragosti, S.; Clavel, F.; Hance,
550 A.J. Modulation of TRIM5alpha activity in human cells by alternatively spliced TRIM5 isoforms. *Journal*
551 *of virology* **2011**, *85*, 7828-7835, doi:10.1128/JVI.00648-11.
552 58. Berthoux, L.; Sebastian, S.; Sayah, D.M.; Luban, J. Disruption of human TRIM5alpha antiviral activity
553 by nonhuman primate orthologues. *Journal of virology* **2005**, *79*, 7883-7888.
554 59. Kan, Y.; Ruis, B.; Takasugi, T.; Hendrickson, E.A. Mechanisms of precise genome editing using
555 oligonucleotide donors. *Genome research* **2017**, *27*, 1099-1111, doi:10.1101/gr.214775.116.
556 60. Dufour, C.; Claudel, A.; Joubarne, N.; Merindol, N.; Maisonnet, T.; Masroori, N.; Plourde, M.B.;
557 Berthoux, L. Editing of the human TRIM5 gene to introduce mutations with the potential to inhibit
558 HIV-1. *PLoS One* **2018**, *13*, e0191709, doi:10.1371/journal.pone.0191709.
559 61. Berthoux, L.; Sebastian, S.; Sokolskaja, E.; Luban, J. Cyclophilin A is required for TRIM5{alpha}-
560 mediated resistance to HIV-1 in Old World monkey cells. *Proceedings of the National Academy of Sciences*
561 *of the United States of America* **2005**, *102*, 14849-14853.
562 62. Masroori, N.; Merindol, N.; Berthoux, L. The interferon-induced antiviral protein PML (TRIM19)
563 promotes the restriction and transcriptional silencing of lentiviruses in a context-specific, isoform-
564 specific fashion. *Retrovirology* **2016**, *13*, 19, doi:10.1186/s12977-016-0253-1.
565 63. Malbec, M.; Pham, Q.T.; Plourde, M.B.; Letourneau-Hogan, A.; Nepveu-Traversy, M.E.; Berthoux, L.
566 Murine double minute 2 as a modulator of retroviral restrictions mediated by TRIM5alpha. *Virology*
567 **2010**, *405*, 414-423, doi:10.1016/j.virol.2010.06.021.
568 64. Veillette, M.; Bichel, K.; Pawlica, P.; Freund, S.M.; Plourde, M.B.; Pham, Q.T.; Reyes-Moreno, C.; James,
569 L.C.; Berthoux, L. The V86M mutation in HIV-1 capsid confers resistance to TRIM5alpha by abrogation
570 of cyclophilin A-dependent restriction and enhancement of viral nuclear import. *Retrovirology* **2013**, *10*,
571 25, doi:10.1186/1742-4690-10-25.
572 65. He, J.; Chen, Y.; Farzan, M.; Choe, H.; Ohagen, A.; Gartner, S.; Busciglio, J.; Yang, X.; Hofmann, W.;
573 Newman, W., et al. CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature* **1997**, *385*,
574 645-649, doi:10.1038/385645a0.
575 66. Anderson, J.L.; Campbell, E.M.; Wu, X.; Vandegraaff, N.; Engelman, A.; Hope, T.J. Proteasome
576 inhibition reveals that a functional preintegration complex intermediate can be generated during
577 restriction by diverse TRIM5 proteins. *Journal of virology* **2006**, *80*, 9754-9760.
578 67. Margolis, D.M.; Archin, N.M.; Cohen, M.S.; Eron, J.J.; Ferrari, G.; Garcia, J.V.; Gay, C.L.; Goonetilleke,
579 N.; Joseph, S.B.; Swanstrom, R., et al. Curing HIV: Seeking to Target and Clear Persistent Infection. *Cell*
580 **2020**, *181*, 189-206, doi:10.1016/j.cell.2020.03.005.
581 68. Thomas, J.; Ruggiero, A.; Paxton, W.A.; Pollakis, G. Measuring the Success of HIV-1 Cure Strategies.
582 *Frontiers in cellular and infection microbiology* **2020**, *10*, 134, doi:10.3389/fcimb.2020.00134.
583 69. Stephenson, K.E.; Wagh, K.; Korber, B.; Barouch, D.H. Vaccines and Broadly Neutralizing Antibodies
584 for HIV-1 Prevention. *Annual review of immunology* **2020**, *38*, 673-703, doi:10.1146/annurev-immunol-
585 080219-023629.
586 70. Del Moral-Sanchez, I.; Sliepen, K. Strategies for inducing effective neutralizing antibody responses
587 against HIV-1. *Expert review of vaccines* **2019**, *18*, 1127-1143, doi:10.1080/14760584.2019.1690458.
588 71. Swindells, S.; Andrade-Villanueva, J.F.; Richmond, G.J.; Rizzardini, G.; Baumgarten, A.; Masia, M.;
589 Latiff, G.; Pokrovsky, V.; Bredeek, F.; Smith, G., et al. Long-Acting Cabotegravir and Rilpivirine for
590 Maintenance of HIV-1 Suppression. *The New England journal of medicine* **2020**, *382*, 1112-1123,
591 doi:10.1056/NEJMoa1904398.

bioRxiv preprint doi: https://doi.org/10.1101/2020.12.07.413708; this version posted December 8, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

592 72. Bester, S.M.; Wei, G.; Zhao, H.; Adu-Ampratwum, D.; Iqbal, N.; Courouble, V.V.; Francis, A.C.;
593 Annamalai, A.S.; Singh, P.K.; Shkriabai, N., et al. Structural and mechanistic bases for a potent HIV-1
594 capsid inhibitor. *Science* **2020**, *370*, 360-364, doi:10.1126/science.abb4808.
595 73. Blair, H.A. Ibalizumab: A Review in Multidrug-Resistant HIV-1 Infection. *Drugs* **2020**, *80*, 189-196,
596 doi:10.1007/s40265-020-01258-3.
597 74. Katz, I.T.; Maughan-Brown, B. Improved life expectancy of people living with HIV: who is left behind?
598 *The lancet. HIV* **2017**, *4*, e324-e326, doi:10.1016/S2352-3018(17)30086-3.
599 75. Basavaraj, K.H.; Navya, M.A.; Rashmi, R. Quality of life in HIV/AIDS. *Indian journal of sexually*
600 *transmitted diseases and AIDS* **2010**, *31*, 75-80, doi:10.4103/0253-7184.74971.
601 76. Pozniak, A. Quality of life in chronic HIV infection. *The lancet. HIV* **2014**, *1*, e6-7, doi:10.1016/S2352-
602 3018(14)70003-7.
603 77. Koujah, L.; Shukla, D.; Naqvi, A.R. CRISPR-Cas based targeting of host and viral genes as an antiviral
604 strategy. *Seminars in cell & developmental biology* **2019**, *96*, 53-64, doi:10.1016/j.semcdb.2019.04.004.
605 78. Gaj, T.; Staahl, B.T.; Rodrigues, G.M.C.; Limsirichai, P.; Ekman, F.K.; Doudna, J.A.; Schaffer, D.V.
606 Targeted gene knock-in by homology-directed genome editing using Cas9 ribonucleoprotein and AAV
607 donor delivery. *Nucleic Acids Res* **2017**, *45*, e98, doi:10.1093/nar/gkx154.
608 79. Li, G.; Zhang, X.; Wang, H.; Liu, D.; Li, Z.; Wu, Z.; Yang, H. Increasing CRISPR/Cas9-mediated
609 homology-directed DNA repair by histone deacetylase inhibitors. *The international journal of biochemistry*
610 *& cell biology* **2020**, *125*, 105790, doi:10.1016/j.biocel.2020.105790.
611 80. Guo, Q.; Mintier, G.; Ma-Edmonds, M.; Storton, D.; Wang, X.; Xiao, X.; Kienzle, B.; Zhao, D.; Feder, J.N.
612 'Cold shock' increases the frequency of homology directed repair gene editing in induced pluripotent
613 stem cells. *Scientific reports* **2018**, *8*, 2080, doi:10.1038/s41598-018-20358-5.
614 81. Canny, M.D.; Moatti, N.; Wan, L.C.K.; Fradet-Turcotte, A.; Krasner, D.; Mateos-Gomez, P.A.;
615 Zimmermann, M.; Orthwein, A.; Juang, Y.C.; Zhang, W., et al. Inhibition of 53BP1 favors homology-
616 dependent DNA repair and increases CRISPR-Cas9 genome-editing efficiency. *Nat Biotechnol* **2018**, *36*,
617 95-102, doi:10.1038/nbt.4021.
618 82. Kurihara, T.; Kouyama-Suzuki, E.; Satoga, M.; Li, X.; Badawi, M.; Thiha; Baig, D.N.; Yanagawa, T.;
619 Uemura, T.; Mori, T., et al. DNA repair protein RAD51 enhances the CRISPR/Cas9-mediated knock-in
620 efficiency in brain neurons. *Biochemical and biophysical research communications* **2020**, *524*, 621-628,
621 doi:10.1016/j.bbrc.2020.01.132.
622 83. Molla, K.A.; Yang, Y. CRISPR/Cas-Mediated Base Editing: Technical Considerations and Practical
623 Applications. *Trends in biotechnology* **2019**, *37*, 1121-1142, doi:10.1016/j.tibtech.2019.03.008.

624

625 **Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional
626 affiliations.

© 2020 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

627

628

629

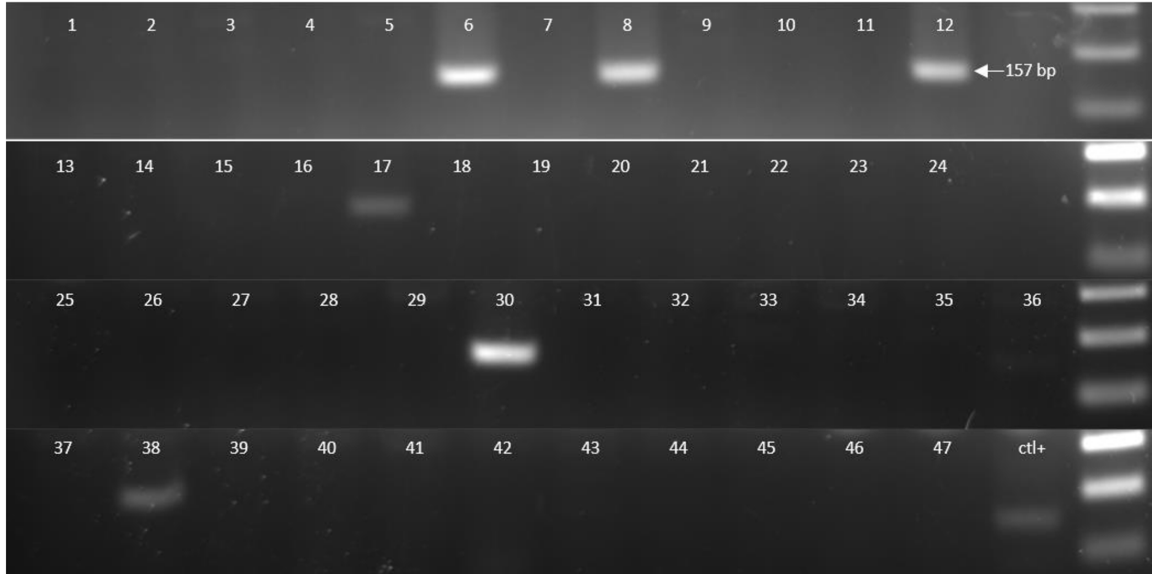


Figure S1. HDR editing-specific PCR screening of Jurkat cells transfected with CRISPR-Cas9 RNPs and donor ssDNA. 47 clonal cell populations were lysed and subjected to a PCR assay in which one of the primers is complementary to the correctly HDR-edited *TRIM5* region targeted for mutagenesis. A PCR product of the expected size was found in clones 6, 8, 12, 17, 30, 38. Ctl+ consisted of Jurkat cells following CRISPR components transfection but prior to the isolation of clones.

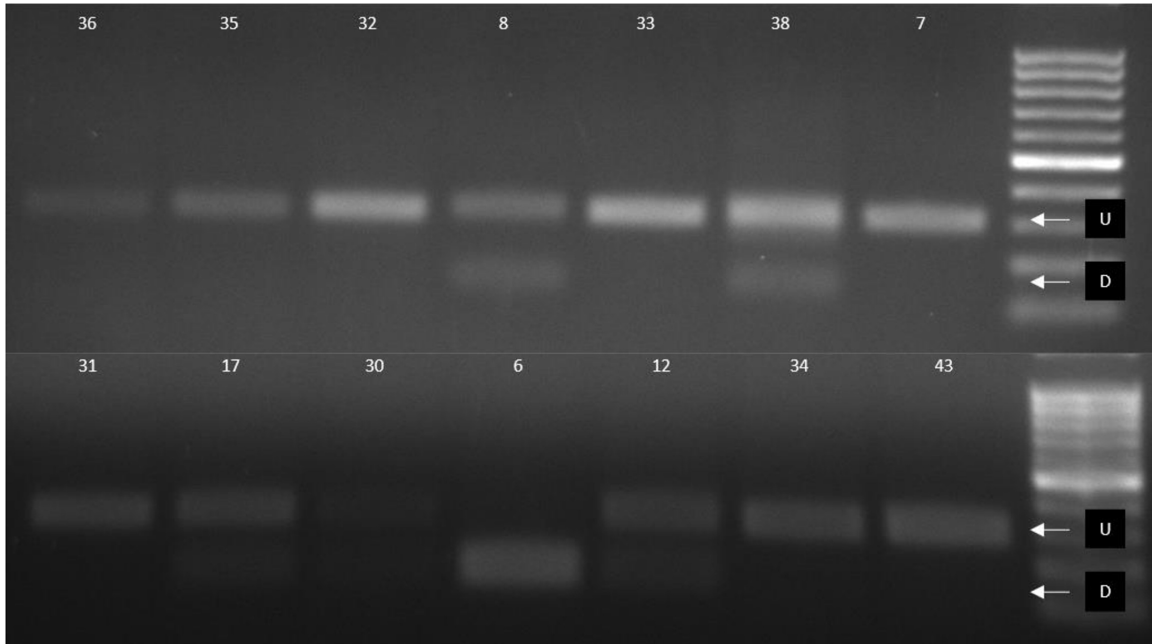
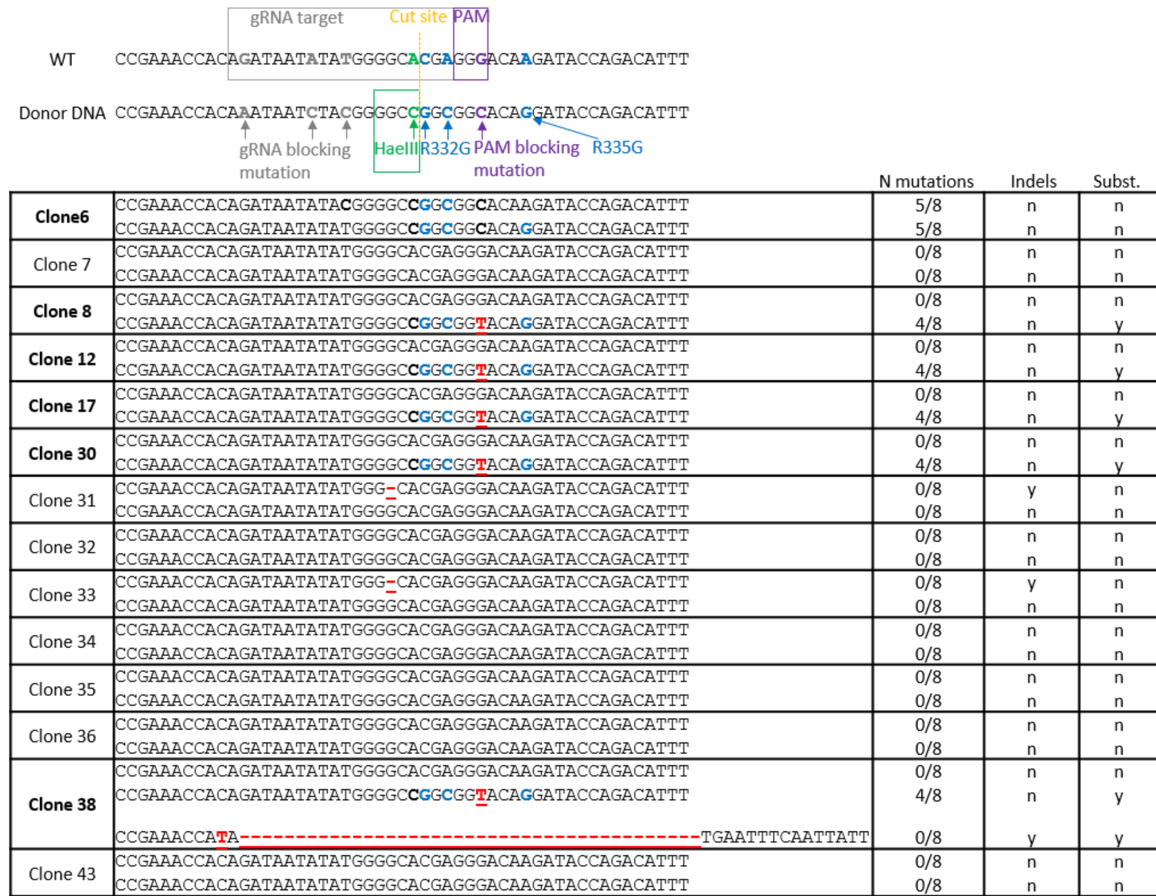


Figure S2. HaeIII screening of selected Jurkat clones. The 6 clones found to be positive in the HDR editing-specific PCR test, along with 8 randomly chosen negative clones, were subjected to a PCR assay using primers that bind outside of the genomic region complementary to the HDR donor DNA, followed by digestion with HaeIII. U and D indicates bands of the expected size for the undigested PCR product and the HaeIII digestion products, respectively.



	Sequence	N mutations	Indels	Subst.
Clone6	CCGAAACCACAGATAATATACGGGGCCGGCGGCACAAGATACCAGACATTT	5/8	n	n
	CCGAAACCACAGATAATATATGGGGCCGGCGGCACAGGATACCAGACATTT	5/8	n	n
Clone 7	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
Clone 8	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCCGGCGGTACAGGATACCAGACATTT	4/8	n	y
Clone 12	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCCGGCGGTACAGGATACCAGACATTT	4/8	n	y
Clone 17	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCCGGCGGTACAGGATACCAGACATTT	4/8	n	y
Clone 30	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCCGGCGGTACAGGATACCAGACATTT	4/8	n	y
Clone 31	CCGAAACCACAGATAATATATGGG-CACGAGGGACAAGATACCAGACATTT	0/8	y	n
	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
Clone 32	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
Clone 33	CCGAAACCACAGATAATATATGGG-CACGAGGGACAAGATACCAGACATTT	0/8	y	n
	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
Clone 34	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
Clone 35	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
Clone 36	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
Clone 38	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCCGGCGGTACAGGATACCAGACATTT	4/8	n	y
	CCGAAACCATA--TGAATTTCAATTATT	0/8	y	y
Clone 43	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n
	CCGAAACCACAGATAATATATGGGGCACGAGGGACAAGATACCAGACATTT	0/8	n	n

Figure S3. MiSeq sequencing results for all clones analyzed. This is an extended version of Figure 1, showing sequencing results for the 8 randomly chosen clones negative for HDR editing in the specific PCR test, in addition to the 6 clones found to be positive.

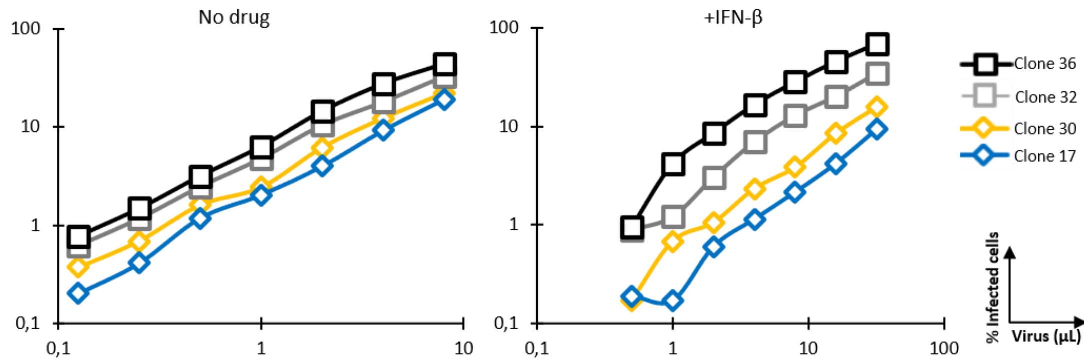


Figure S4. Permissiveness of HDR-edited clone 17 to HIV-1NL-GFP infection. Jurkat clonal cell populations were analyzed for permissiveness to HIV-1 infection. Clones 17 and 30 are monoallelically edited to express R332G-R335G TRIM5α whereas clones 32 and 36 are unedited. Cells were infected with increasing doses of HIV-1NL-GFP in the presence or not of IFN-β. The percentage of cells expressing GFP was determined by FACS two days later.