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# HIV-1 restriction by endogenous APOBEC3G in the myeloid cell line THP-1

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**Abstract**

HIV-1 replication in CD4-positive T lymphocytes requires counteraction of multiple different innate antiviral mechanisms. Macrophage cells are also thought to provide a reservoir for HIV-1 replication but less is known in this cell type about virus restriction and counteraction mechanisms. Many studies have combined to demonstrate roles for APOBEC3D, APOBEC3F, APOBEC3G and APOBEC3H in HIV-1 restriction and mutation in CD4-positive T lymphocytes, whereas the APOBEC enzymes involved in HIV-1 restriction in macrophages have yet to be delineated fully. We show that multiple *APOBEC3* genes including *APOBEC3G* are expressed in myeloid cell lines such as THP-1. Vif-deficient HIV-1 produced from THP-1 is less infectious than Vif-proficient virus, and proviral DNA resulting from such Vif-deficient infections shows strong G to A mutation biases in the dinucleotide motif preferred by APOBEC3G. Moreover, Vif mutant viruses with selective sensitivity to APOBEC3G show Vif null-like infectivity levels and similarly strong APOBEC3G-biased mutation spectra. Importantly, *APOBEC3G*-null THP-1 cells yield Vif-deficient particles with significantly improved infectivities and proviral DNA with background levels of G to A hypermutation. These studies combine to indicate that APOBEC3G is the main HIV-1 restricting APOBEC3 family member in THP-1 cells.

## INTRODUCTION

Human cells have the potential to encode up to seven different single-stranded DNA cytosine deaminase enzymes of the APOBEC3 (A3) subfamily, APOBEC3A-D and APOBEC3F-H (A3A-D, A3F-H). These enzymes have overlapping functions in providing innate immune protection against a broad number of parasitic DNA-based elements (reviewed by [1–5]). Due to reverse transcription having obligate single-stranded cDNA replication intermediates, retroviruses are particularly sensitive to attack by A3 enzymes as cDNA strand C to U deamination events result in genomic strand G to A mutations. The best-studied example to date is the mechanism of HIV-1 restriction in which A3D, A3F, A3G and A3H have the capacity to mutate viral cDNA replication intermediates, as well as interfere with reverse transcription by deaminase-independent mechanisms (reviewed by [1–5]). Due to the intrinsic preferences of A3 enzymes for cytosine bases in specific dinucleotide motifs, 5′-CC (A3G) or 5′-TC (A3D, A3F and A3H), viral cDNA deamination events typically manifest as genomic strand 5′-GG to AG mutations or 5′-GA to AA mutations.

HIV-1, HIV-2 and non-human lentiviruses counteract this restriction mechanism through the virus-encoded virion infectivity factor (Vif), which nucleates the formation of an E3 ubiquitin ligase complex that binds and degrades restrictive A3 enzymes (reviewed by [1–5]). At the heart of this complex is Vif heterodimerization with the transcription cofactor CBF-$\beta$ [6, 7], which also interferes with expression of restrictive *A3* genes by preventing formation of CBF-$\beta$/RUNX transcriptional activation complexes [8]. Although this Vif-mediated counterdefense mechanism is certainly effective, a large body of evidence also indicates that A3 enzymes frequently escape degradation in CD4-positive T cells, package into assembling viral particles, deaminate viral cDNA replication intermediates, and ultimately contribute to HIV-1 genetic diversification including mutations

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**Abbreviations:** A3, APOBEC3, apolipoprotein mRNA editing catalytic subunit-like 3; gRNA, guide RNA; HIV-1, human immunodeficiency virus-type 1; m.o.i., multiplicity of infection; Vif, virion infectivity factor; VSV-G, vesicular stomatitis virus G protein.
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responsible for immune evasion and drug resistance (e.g. [9–13]).

HIV-1 also infects myeloid lineage cell types including macrophages, which may constitute an additional reservoir for virus replication and latency *in vivo* (reviewed by [14–17]). However, considerably less is known about A3 function in these cell types in comparison to the plethora of studies already done using T cells. Here we ask whether the A3 restriction mechanism works similarly or differently against Vif-deficient HIV-1 in the myeloid cell line THP-1. This cell line was selected for studies here because it has already proven to be a robust model system for prior HIV-1 studies including several on restriction factors (e.g. [18–21]). Interestingly, although multiple restrictive A3s are expressed in THP-1, infectivity data and G to A hypermutation patterns of a variety of different HIV-1 constructs in both endogenous *A3G*-expressing and *A3G*-null THP-1 lines combine to indicate that A3G is the predominant A3 family member capable of virus restriction in this system.

## RESULTS

### Multiple *APOBEC3* family members are expressed in THP-1 and other myeloid cell lines

Previous studies have reported mRNA expression of multiple *A3* family members including *A3F*, *A3G* and *A3H* in primary myeloid lineage cell types including macrophages and dendritic cells [18, 22–25]. To determine whether a similarly complex *A3* repertoire is expressed in a more experimentally tractable model, we first used established reverse transcription-quantitative PCR (RT-qPCR) assays [23, 24, 26] to quantify the mRNA levels of each of the seven human *A3* genes in the monocyte cell line THP-1. Relative to the housekeeping gene *TATA-binding protein* (*TBP*), significant levels of five different *A3* family member mRNAs were evident – *A3B*, *A3C*, *A3F*, *A3G* and *A3H* (Fig. 1a). Moreover, infection by HIV-$1_{IIIB}$ caused a modest but statistically significant increase in mRNA levels for *A3A*, *A3C*, *A3F*, *A3G* and *A3H*, but to lesser extents than reported previously for HIV-1 infection of primary CD4-positive T lymphocytes [27, 28].

To ask whether this mRNA expression profile is similar to those in other myeloid cell lines, we analysed *A3* expression levels in RNAseq data sets representing 72 different myeloid cell lines available through the Cancer Cell Line Encyclopedia (CCLE) [29]. These analyses revealed a similar overall expression pattern for most of the cell lines with high levels of *A3B*, *A3C* and *A3G* and varying amounts of other *A3* mRNAs (Fig. 1b). These gene expression studies combined to indicate that THP-1 may be a good model system for studies on A3 restriction in a myeloid lineage cell line.

### Vif-deficient HIV-1 is restricted in THP-1 cells

Next, we wanted to determine if the A3 enzymes expressed in THP-1 could functionally restrict virus infectivity. VSV-G pseudotyped Vif-proficient and Vif-deficient HIV-$1_{IIIB}$ stocks were produced using 293T cells, and m.o.i. were determined by titring on CEM-GXR reporter cells [30]. Equivalent amounts of each virus were used to infect THP-1 cells (m.o.i.=0.25). As controls, parallel infections were done using the T cell line SupT11 expressing an empty vector or A3G. SupT11 does not express any *A3* mRNA to significant levels [27] and, therefore, the empty vector line is expected to be fully permissive for replication of both viruses and the A3G expressing line will be non-permissive for Vif-deficient virus replication and permissive for Vif-proficient virus replication. In both cases, newly produced viruses were harvested after 48 h of incubation, and then infectivity was quantified using TZM-bl reporter cells. Short incubation durations of 48 h were chosen to enable estimates of the magnitude of virus restriction in a single round (or near single round) of virus replication.

As expected for cell lines expressing multiple restrictive A3 enzymes, the infectivity of Vif-proficient virus was higher than that of the Vif-deficient virus following production in THP-1 cells (Fig. 2a). A similar fold-difference was observed for the same viruses produced in the SupT11-A3G cell line and, also as expected, no infectivity difference was seen for viruses produced in the SupT11-vector cell line (Fig. 2a). These results were corroborated by anti-A3G immunoblots of protein extracts from these infected cell lines and from the resulting viral particles. For both the THP-1 and SupT11-A3G cell lines, A3G was clearly expressed in cells and incorporated into viral particles. Importantly, Vif caused A3G to be degraded, which resulted in lower steady-state levels of A3G in cells, lower amounts of A3G incorporated into particles, and higher overall infectivity levels in comparison to Vif-deficient reaction conditions. Similar results were obtained for a different HIV-1 strain (LAI), which produces a Vif protein that more effectively degrades human A3H [31] (Fig. 2b). These results therefore combined to implicate at least one A3 family member in HIV-1 restriction in THP-1 cells.

### Base substitution mutation spectra for Vif-deficient and Vif-proficient HIV-1 produced from THP-1 cells

To examine the base substitution mutation spectra, and particularly the dinucleotide context of G to A mutations, the HIV-$1_{IIIB}$ proviral DNA from the infectivity experiments described above in Fig. 2 was cloned and sequenced. Specifically, total genomic DNA was prepared from infected TZM-bl reporter cells, and the *pol* region of the virus was amplified by high-fidelity PCR, cloned and Sanger sequenced. As expected, the mutation levels were low in all Vif-proficient experimental conditions, likely due to degradation of restrictive A3 enzymes (WT in Fig. 3a–b). Vif-deficient viruses produced in SupT11-A3G cells had high frequencies of G to A mutations in A3G-preferred motifs (88 % GG to AG on the genomic strand due to CC to CU deamination events on the cDNA strand; Fig. 3a–b). Similarly high frequencies of G to A mutations in A3G-preferred motifs were evident in proviral DNA derived from THP-1 infections (85 %; Fig. 3a–b). In both the SupT11-A3G and THP-1 systems, most of the remaining G to A mutations occurred in GA to AA dinucleotide motifs (11 and 3 %, respectively). Based on prior

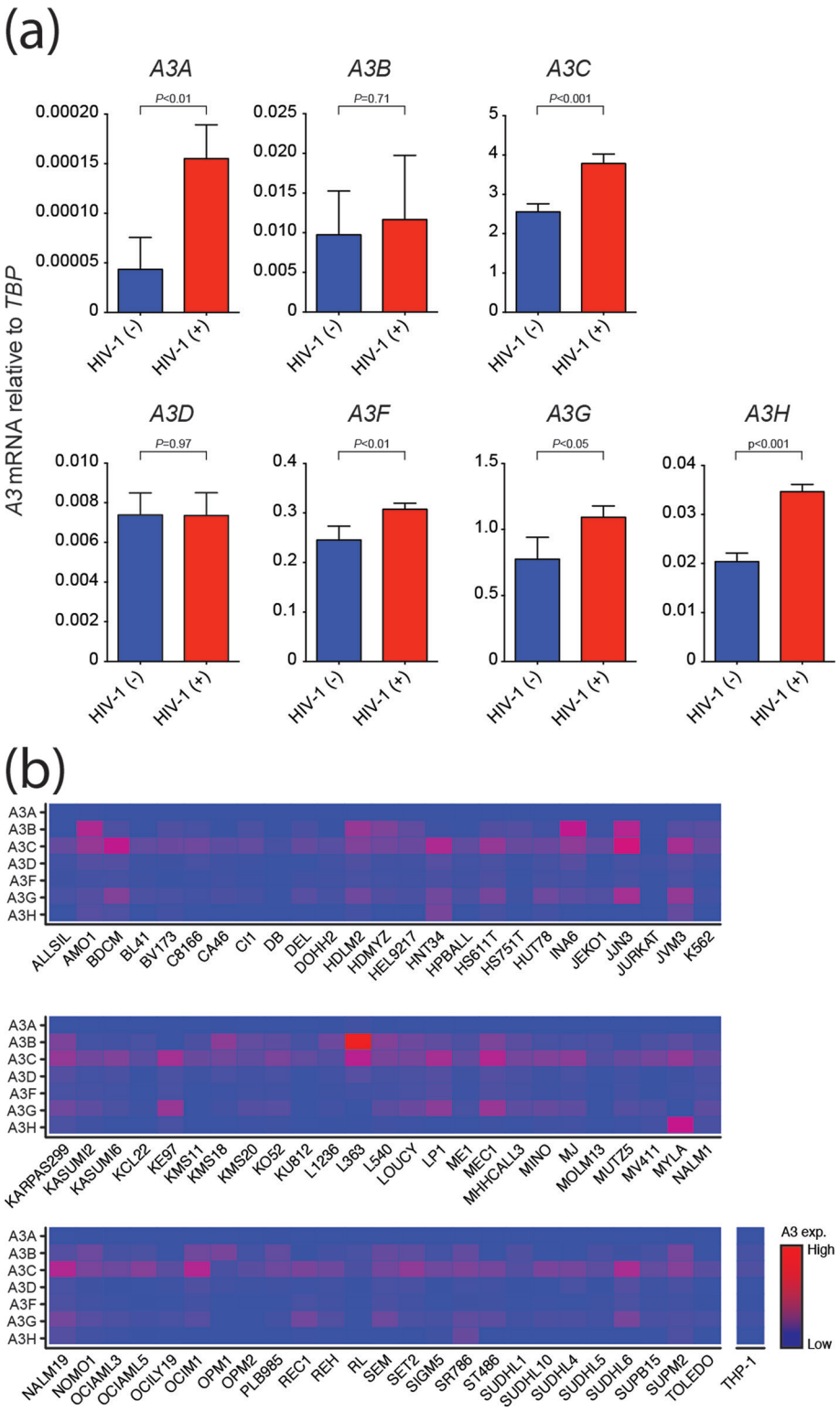


**Fig. 1.** Multiple *A3* genes are expressed in myeloid lineage cell lines. (a) *A3* mRNA levels relative to the housekeeping gene *TBP* in THP-1 cells+/-HIV-$1_{IIIB}$ infection (m.o.i.=0.25). Each histogram bar shows the mean+/-SD of three independent experiments (*P*-values obtained by Student's T-TEST). (b) *A3* mRNA levels relative to the housekeeping gene *TBP* in 72 different myeloid cell lines (RNAseq data from CCLE). Red indicates high expression levels and blue lower levels.

observations showing that A3G preferentially deaminates 5′-CC (~85 %) over 5′-TC (~15 %) [32–35], the distribution of G to A mutations in the THP-1 system may be attributable solely to enzymatic deamination by A3G; however, these sequencing results do not exclude the possibility of contributions from one or more A3 family members that prefer 5′-TC substrates.

## APOBEC3G causes the majority of Vif-deficient HIV-1 restriction in THP-1

To more precisely gauge the importance of A3G in the Vif-deficient HIV-1 restriction phenotype in THP-1 cells, we used Vif separation-of-function mutants that are completely sensitive to A3G and simultaneously fully capable of counteracting the other restrictive A3 enzymes. Specifically, penta-alanine (5A) substitution of Vif residues 40–44 (YRHHY in IIIB and NL4-3) renders HIV-1 susceptible to restriction by A3G but not by A3D, A3F or A3H [36, 37]. Similarly, dual-glutamine (2Q) substitution of Vif residues 26 and 27 (i.e. KH to QQ) renders HIV-1 sensitive to restriction by A3G but not by other A3 enzymes [38]. These separation-of-function Vif mutant viruses were used as molecular probes, along with Vif-deficient and Vif-proficient viruses in pseudo-single cycle experiments with THP-1, SupT11-vector and SupT11-A3G cell lines as described above.

We were surprised to find that the declines in infectivity of the 5A and 2Q Vif mutant viruses produced in THP-1 cells approach that of the fully Vif-null virus produced in parallel (left data sets in Fig. 4). Together with the strong hypermutation bias discussed above, these results further supported the notion that A3G may be the dominant restrictive A3 family member in THP-1 cells. In support of this possibility and confirming that the 5A and 2Q viruses are truly deficient in counteracting A3G, immunoblots showed that levels of A3G incorporated into 5A, 2Q and Vif-null particles are similar. As expected, these three Vif mutants all showed similarly high levels of A3G encapsidation and similarly low levels of infectivity following production in SupT11-A3G cells (right data sets in Fig. 4). The otherwise fully functional nature of these Vif mutant HIV-1 constructs was confirmed through observation of uniformly high levels of infectivity following production in SupT11-vector cells (middle data sets in Fig. 4).

## APOBEC3G signature mutations dominate the mutation spectra of Vif-mutant viruses

The infectivity data for the Vif separation-of-function viruses described above indicated that A3G might be the only source of A3 mutagenesis in THP-1 cells. To further investigate this possibility, the *pol* region of the proviruses from these infectivity studies was cloned and sequenced as described above.

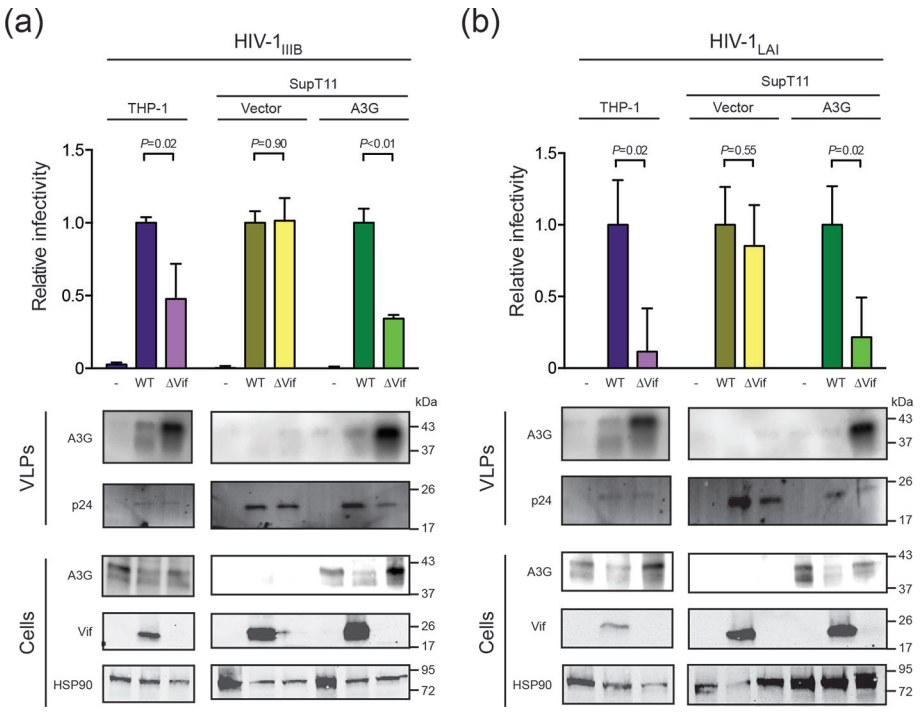


**Fig. 2.** Infectivity of Vif-proficient and Vif-deficient HIV-1 in THP-1 cells. (a) Infectivity of Vif-proficient and Vif-deficient HIV-$1_{IIIB}$ produced from THP-1 cells in comparison to the same viruses produced from SupT11 cells expressing either a control vector or A3G. Each histogram bar is the average of triplicate experiments +/-SD in which raw data are normalized to Vif-proficient virus infectivity (*P*-values obtained by Student's T-TEST). Immunoblots of the indicated cellular and viral proteins in whole cell extracts and virus-like particles (VLPs) with HSP90 and p24 as loading controls, respectively. (b) Experiments similar to those in (a) except a different HIV-1 strain was used (LAI).

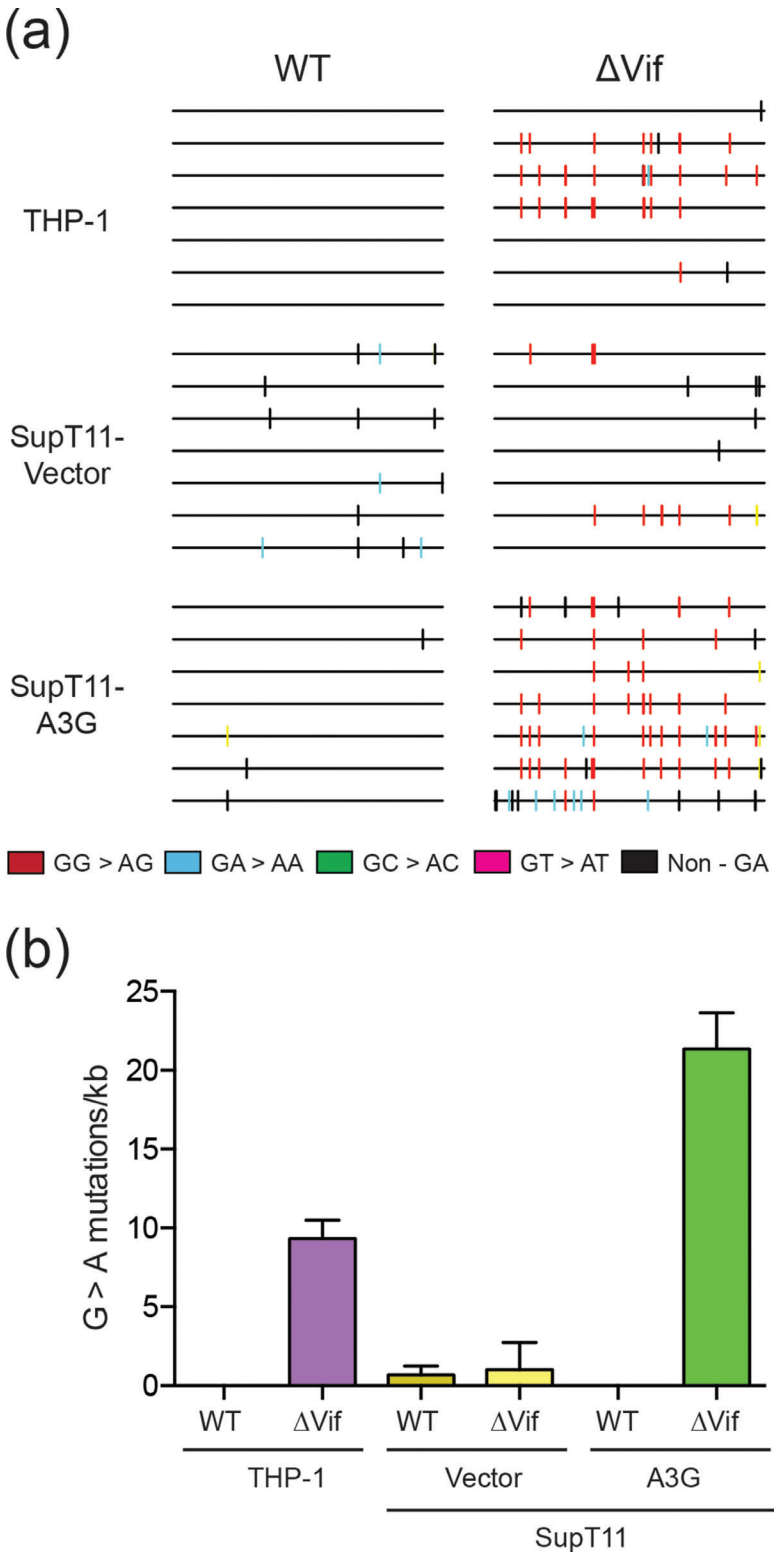


**Fig. 3.** Base substitution mutation spectra of Vif-proficient and Vif-deficient HIV-1 produced from THP-1 cells. (a) Dinucleotide contexts of G to A mutations in the *pol* region of the indicated HIV-$1_{IIIB}$ viruses produced from the indicated cell lines. Each G to A mutation is shown as a vertical line (tick) on top of individual sequences illustrated to scale on a 564 bp amplicon with colours corresponding to dinucleotide context. (b) Average G to A mutation loads in proviral DNA following Vif-proficient and Vif-deficient HIV-$1_{IIIB}$ production from THP-1 cells or SupT11 cells expressing either a control vector or A3G. Each histogram bar is the average +/-SD of triplicate infections.

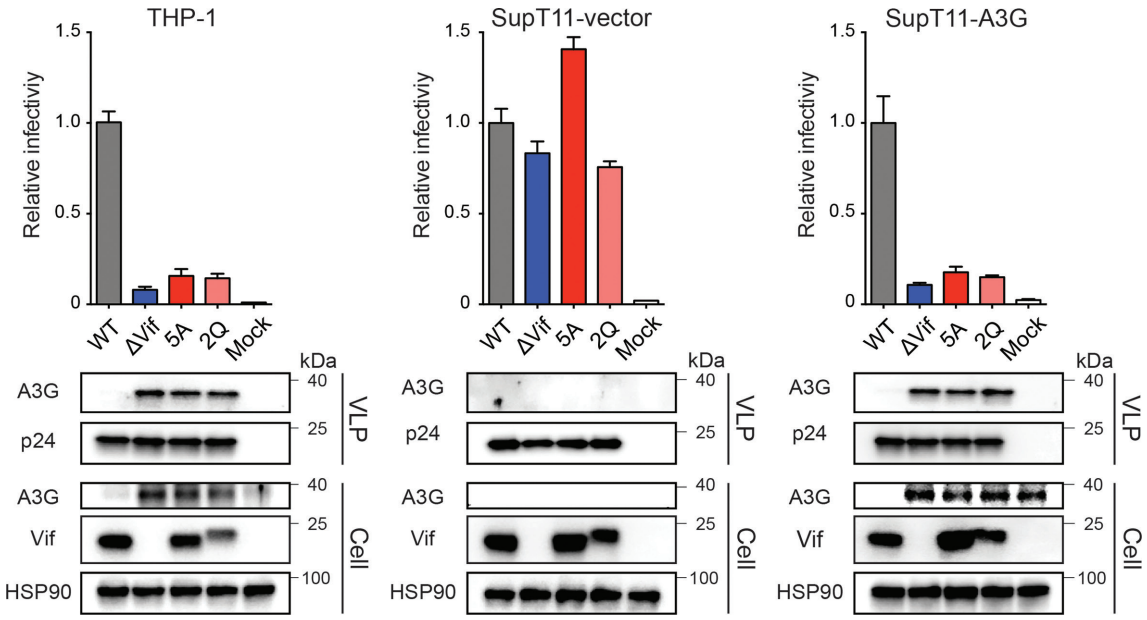


**Fig. 4.** A3G is the main Vif-counteracted APOBEC family member in THP-1 cells. Histograms showing relative infectivity levels for the indicated HIV-$1_{IIIB}$ viruses produced from THP-1, SupT11-vector or SupT11-A3G cells. The 5A and 2Q viruses are Vif mutants that are selectively sensitive to restriction by A3G (see text for additional details). Each histogram bar is the mean+/-SD of three independent experiments. Immunoblots are shown below for the indicated viral and cellular proteins in viral particle and whole cell extracts with HSP90 and p24 as loading controls, respectively.

Although the mutation levels were relatively low in Vif mutant viruses produced in THP-1 cells, the observed G to A mutations were predominantly within the GG to AG signature motif of A3G (Fig. 5a–b). Importantly, the relative proportion of mutations within GG to AG motifs was similar for Vif-null virus and the two Vif separation-of-function mutants 5A and 2Q (94±2 %, 90±3 % and 89±5 %, respectively; Fig. 5b). Moreover, high levels of G to A mutations within GG motifs were observed for all three Vif mutant viruses produced in SupT11-A3G cells and the relative proportions were similar to those observed in viruses from THP-1 cells (93±2, 92±3 and 92±1 %, respectively; Fig. 5a–b). The similarity of the results from THP-1 cells with a complex A3 repertoire and SupT11-A3G cells with only a single A3G protein provided further support to the idea that A3G may be the only functionally restrictive A3 enzyme in THP-1 cells.

### *A3G*-null THP-1 cells are semi-permissive for Vif-deficient HIV-1 and fully permissive for 5A and 2Q Vif separation-of-function mutants

To unambiguously address whether A3G might be the only HIV-1 restrictive deaminase family member in THP-1 cells, we used CRISPR to knock-out both copies of the endogenous *A3G* gene and performed infectivity and proviral DNA sequencing studies as described above. Two independent *A3G*-null clones were obtained as evidenced by genomic DNA sequences spanning the Cas9/gRNA cleavage site, with one clone harbouring two different 2 bp deletion mutations adjacent to the PAM site and the other two different insertion/deletion alleles (Fig. 6a). In both instances, RT-qPCR analyses showed that neither *A3G* nor flanking *A3* mRNA levels were affected by these mutations (Fig. 6b). However, also in both instances as predicted by the nature of the mutational events, A3G protein levels were undetectable by immunoblots indicating fully null alleles had been obtained (Fig. 6c). Next, pseudo-single cycle HIV-1 infectivity studies were conducted as described above with Vif-proficient, Vif-deficient, Vif-5A and Vif-2Q constructs. The THP-1 parental cell line yielded results similar to those described above in Fig. 4. In contrast, the *A3G*-null clones yielded two important results. First, the infectivity of the Vif-null virus increased from 10 % to approximately 50 %, but not completely to 100 % (Fig. 6d). Second, the infectivity of the 5A and the 2Q viruses recovered to 100 % in the *A3G*-null conditions (Fig. 6d). Moreover, for both the Vif-null and separation-of-function viruses, the A3G-biased hypermutation signature was no longer evident in proviral DNA sequences (Fig. 7). These results combined with the aforementioned data to indicate the existence of either a partial virus restriction activity from another A3 family member in this system (possibly a deamination-independent activity based on the near-exclusivity of the A3G-biased hypermutation data) or, alternatively, an A3 independent function for Vif that is genetically separable from amino acids required for A3G neutralization.

## DISCUSSION

We initiated these studies to ask whether the 'rules for HIV-1 restriction' by A3 enzymes are similar in myeloid and T cell lines. Prior work from our group and others had combined to

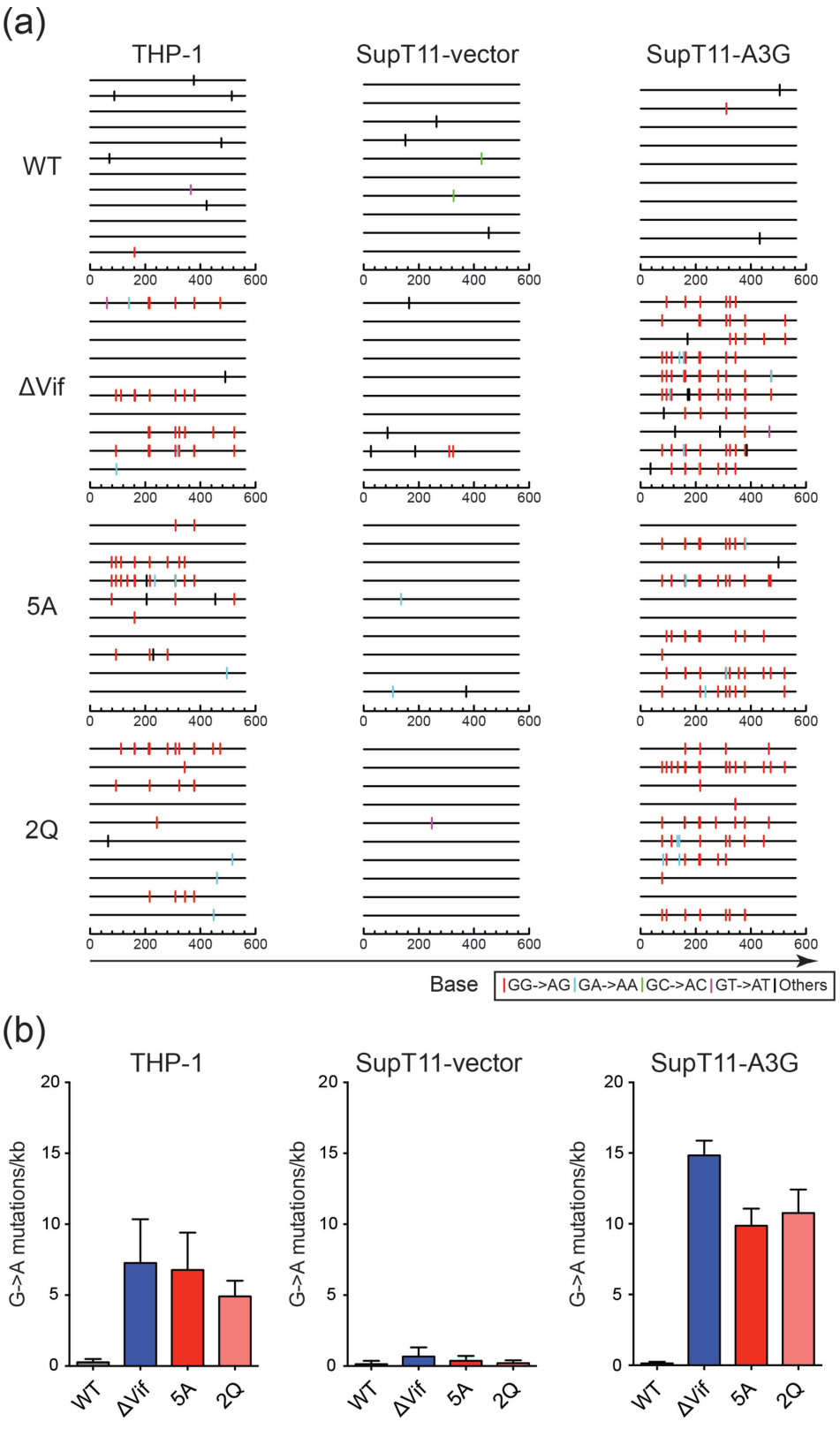


**Fig. 5.** A3G is the primary source of G to A mutations in THP-1 cells. (a) Dinucleotide contexts of G to A mutations in the *pol* region of the indicated HIV-$1_{IIIB}$ viruses produced from the indicated cell lines. Each G to A mutation is shown as a vertical line (tick) on top of individual sequences illustrated to scale on a 564 bp amplicon with colours corresponding to dinucleotide context. (b) Average G to A mutation loads +/-SD for three independent experiments for the viruses and conditions described in (a).

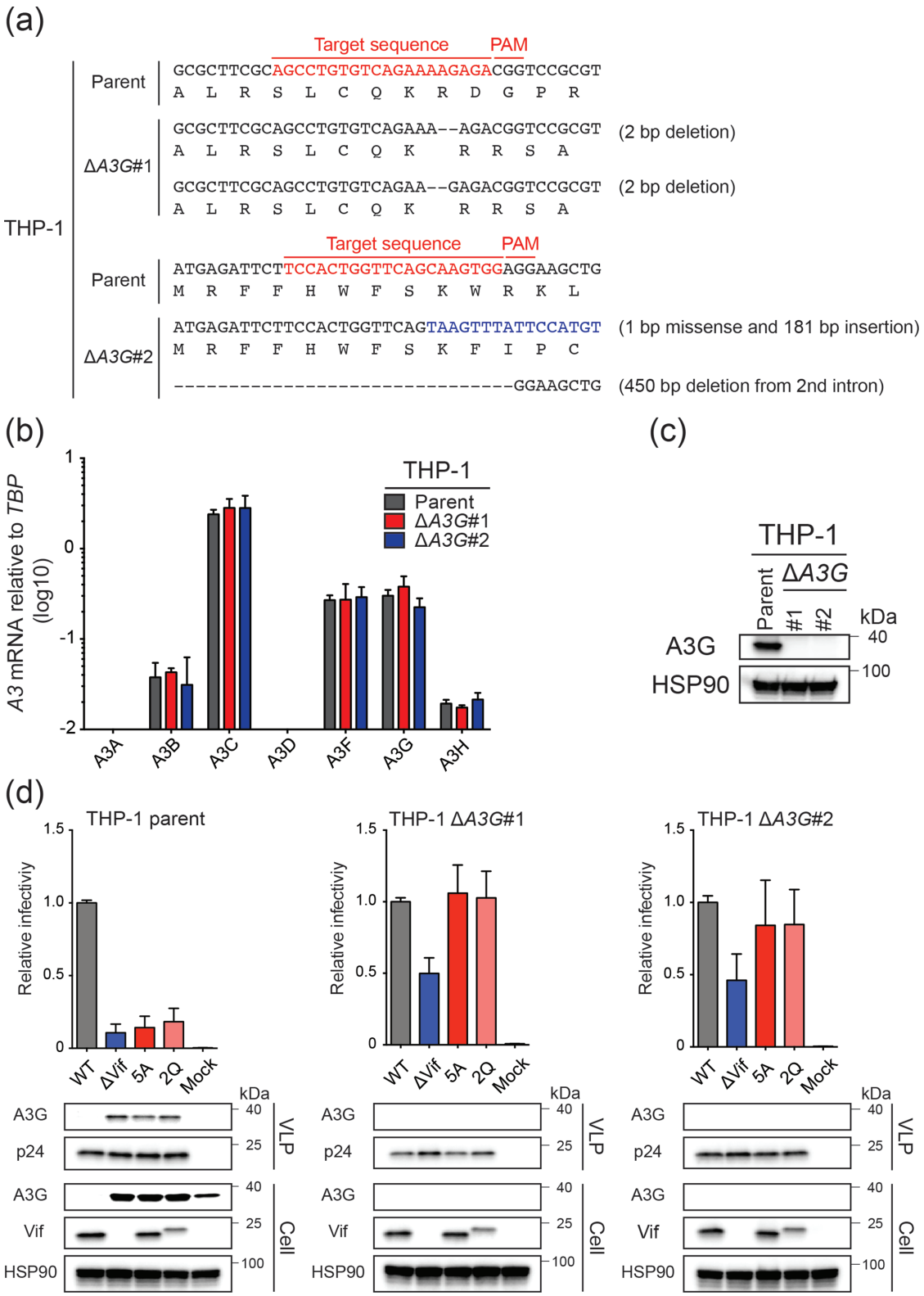


**Fig. 6.** HIV-1 infectivity phenotypes of parental and *A3G*-null THP-1 cells. (a) *A3G* exon 3 DNA sequences encompassing the gRNA binding site for the two alleles in each *A3G*-null clone. (b) RT-qPCR analyses of *A3* gene expression in parental and *A3G*-null THP-1 cells. Each histogram bar is the mean+/-SD of three independent experiments. (c) Immunoblot analysis of A3G in parental and *A3G*-null THP-1 cells. Anti-HSP90 was used to show equal loading of whole cell extracts from the indicated lines. (d) Histograms showing relative infectivities of the indicated HIV-$1_{IIIB}$ viruses produced from THP-1 or two independent *A3G*-null clonal derivatives. Each histogram bar is the mean+/-SD of three independent experiments. Immunoblots are shown below for the indicated viral and cellular proteins in viral particle and whole cell extracts with HSP90 and p24 as loading controls, respectively.

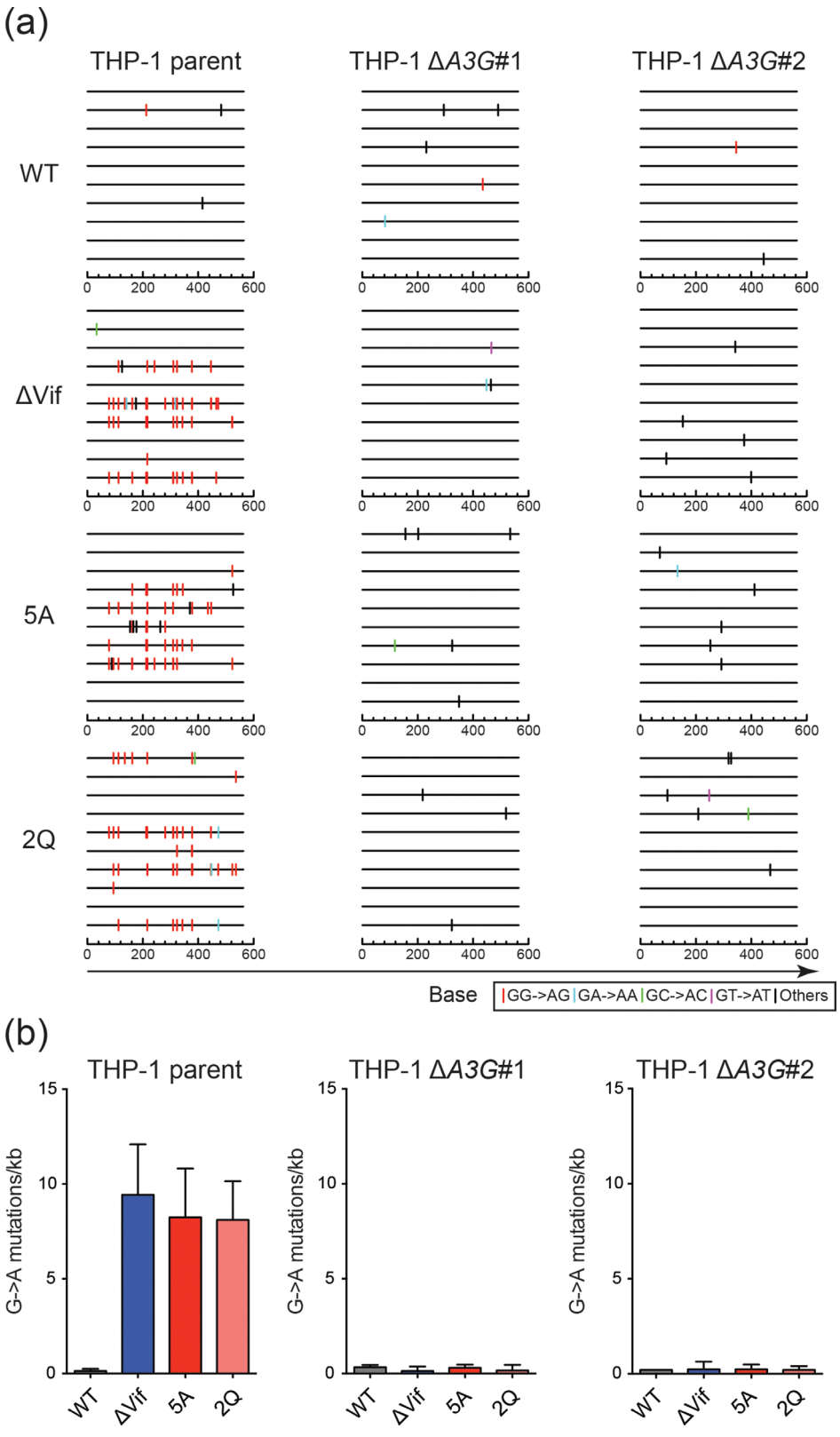


**Fig. 7.** *A3G* disruption in THP-1 erases G to A mutation bias in proviral DNA. (a) Dinucleotide contexts of G to A mutations in the *pol* region of the indicated HIV-$1_{IIIB}$ viruses produced from the indicated cell lines. Each G to A mutation is shown as a vertical line (tick) on top of individual sequences illustrated to scale on a 564 bp amplicon with colours corresponding to dinucleotide context. (b) Average G to A mutation loads +/-SD for three independent experiments for the viruses and conditions described in (a).

demonstrate that four different A3 enzymes, A3D, A3F, A3G and A3H (stable haplotypes only), contribute to Vif-deficient HIV-1 restriction and hypermutation in CD4-positive T cell lines and primary T-lymphocytes (e.g. [27, 31, 39]). Here, using the myeloid lineage cell line THP-1, we show that A3G is the main and possibly the only A3 family member capable of causing Vif-deficient HIV-1 restriction and G to A hypermutation. One approach revealed similar restriction and hypermutation phenotypes for Vif-null virus and two different Vif separation-of-function mutants (Figs 4 and 5). In particular, in all three of these conditions, emergent viruses showed similarly high levels of A3G signature GG to AG mutations and, importantly, similarly low levels of GA to AA mutations (i.e. low numbers that could be due to A3G or another family member). A complementary approach using *A3G*-null clones of the parental THP-1 cell line showed incomplete recovery of the infectivity of the Vif-null virus, yet full recovery of the infectivity of two Vif separation-of-function mutants (5A and 2Q) (Fig. 6). Moreover, proviral DNA sequences from the same experiments showed a near-complete erasure of the A3G-biased GG to AG hypermutation signature (Fig. 7). Taken together, we conclude that A3G is the dominant A3 family member capable of HIV-1 restriction and hypermutation in THP-1 cells. However, the reduced infectivity phenotype of the Vif-null virus produced in *A3G*-null clones indicated that THP-1 has at least one additional A3 enzyme exerting a largely deamination-independent restriction mechanism or that Vif may have an A3 independent function (that minimally does not require the A3G interaction surfaces defined by the 5A and 2Q amino acid substitutions).

Prior to the discovery of A3 enzymes in 2002 [40–42], multiple independent studies had demonstrated the importance of Vif for HIV-1 replication in primary macrophages [43–47]. Several studies also indicated a role for Vif in monocytic lineage cell lines [43, 45, 48–50]. However, since the discovery of A3 enzymes, the prime cellular targets of HIV-1 Vif, a disproportionate number of studies have addressed the Vif-A3 interaction using primary T lymphocytes and immortal T cell lines (reviewed by [1–5]). Comparatively fewer studies have focused on myeloid lineage cell types. First, multiple groups have shown that primary macrophages express a complex *A3* repertoire invariably including *A3G* [24, 25, 51–53]. Second, the Cimarelli group has implicated A3A in HIV-1 restriction in THP-1 and primary macrophages [18]. However, A3A was not addressed by our studies here because interferon was not used to induce *A3A* transcription [22, 24, 25, 51, 52, 54, 55]. Lastly and perhaps most relevant to our studies, Pathak and coworkers showed that a Vif mutant virus defective for interaction with A3G (5A also used here) has attenuated replication kinetics in cultures of primary monocyte-derived macrophages, whereas a Vif mutant defective for interaction with A3D and A3F was largely unaffected [56]. Moreover, it is important to note that this study also observed A3G signature-biased G to A hypermutations but likely due to difficulties engineering primary macrophages did not demonstrate a genetic requirement for endogenous A3G. Therefore, our studies and particularly the CRISPR knock-outs are important because they provide direct support to the idea that A3G is the predominant HIV-1 restrictive deaminase family member in macrophages and indicate that THP-1 cells may be a powerful model system for additional mechanistic studies.

Future studies should also consider the *A3* genotype of the host cells because, for instance, stable haplotypes of A3H are restrictive whereas unstable haplotypes are not [31, 39, 57, 58]. Moreover, the restriction capacity of the cellular A3 repertoire may be influenced by developmental stage and, especially, by the immune microenvironment including levels of various interferons, cytokines and chemokines [22–25, 51–55, 59–64]. For example, the overall antiviral state, including expression levels of several *A3* family members, is dramatically strengthened through activation of the type I interferon response [18, 22–25, 51–55, 64, 65]. Such studies may ultimately contribute to a greater understanding of potential reservoirs for HIV-1 infection and latency *in vivo*, and possibly also to prospects of activating antiviral restriction factors to suppress and/or clear virus infection.

## METHODS

### Cell lines and culture conditions

293T (CRL-3216) was obtained from American Type Culture Collection. TZM-bl (no. 8129) [66] was obtained from the NIH AIDS Reagent Program. CEM-GXR (CEM-GFP expressing CCR5) was provided by Dr Todd Allen (Harvard University, USA) [30]. THP-1 was provided by Dr Andrea Cimarelli (INSERM, France) [18]. Adherent cells were maintained in Dulbecco's modified Eagle medium supplemented with 10 % FBS and 0.5 % penicillin/streptomycin (P/S). Suspension cells were cultured in RPMI with 10 % FBS and 0.5 % P/S.

The creation and characterization of the permissive T cell line SupT11 has been reported [19, 35]. A transfer vector pTRPE-CCR5 was created by placing the coding region of CCR5 (AH005786.2) in pTRPE [67]. To create SupT11 stably expressing CCR5 (SupT11-CCR5), high titre lentiviral supernatant was generated as previously described [68] [kindly provided by Dr James L. Riley (University of Pennsylvania, USA)] and used for transduction. A single cell subclone of SupT11-CCR5 (clone no. 8) was isolated by limiting dilution and expanded. pcDNA3.1 and pcDNA3.1-A3G have been described [69]. To create SupT11-CCR5 cells stably expressing A3G, SupT11-CCR5 cells were electroporated with 20 µg of linearized pcDNA3.1-A3G or vector control, plated at several dilutions in 96-well plates and selected with 1 mg $ml^{-1}$ G418. Then, single cell clones of SupT11-CCR5-A3G and -vector were expanded and analysed by immunoblots for A3G expression (data now shown).

*A3G* specific guides for exon 3 (Fig. 6a) were designed using Synthego's CRISPR design tool (https://design.synthego.com/#/) and evaluated manually for specificity to *A3G* via an alignment with the most related members of the *A3* family. Oligos with ends compatible with the *Esp*3I sites in pLentiCRISPR1000 were ordered from IDT. The targeting constructs

were generated by annealing oligos [Δ*A3G* gRNA#1: (5′-CAC CGA GCC TGT GTC AGA AAA GAG A) and (5′-GAA CTC TCT TTT CTG ACA CAG GCT C). Δ*A3G* gRNA#2: (5′-CAC CGT CCA CTG GTT CAG CAA GTG G) and (5′-GAA CCC ACT TGC TGA ACC AGT GGA C)], and cloned by GoldenGate ligation into pLentiCRISPR1000 [70]. All constructs were confirmed by sequencing. 293T cells were transfected with the lentiCRISPR1000 targeting construct along with pΔ-NRF (HIV-1 *gag*, *pol*, *rev*, *tat* genes) and pMDG (VSV-G) expression vectors. After 48 h, virus-containing supernatants were filtered (0.45 µm, Millipore) and concentrated by centrifugation (26 200 ***g***, 4 ˚C, 2 h). Then, viral pellets were resuspended in 10 % FBS/RPMI and incubated with cells for 48 h before being placed under drug selection (1 µg $ml^{-1}$ puromycin). Single cell clones were isolated by limiting dilution of the drug-resistant cell pool, expanded and analysed for A3G expression by gnomic DNA sequencing, RT-qPCR and immunoblotting (Fig. 6a–c).

## RT-qPCR

Total RNA was extracted from THP-1 cells using the High Pure RNA Isolation Kit (Roche), and cDNAs were made using qScript cDNA SuperMix XL (Quantabio). After RT-qPCR was performed as described using specific primer–probe combinations for each *APOBEC* [23, 26].

## CCLE gene expression analysis

Gene expression information for relevant cell lines was obtained from the CCLE [29] (accessible online at https://portals.broadinstitute.org/ccle). Reads per kilobase per million (RPKM) values were obtained for all available haematopoietic and lymphoid cells lines from the latest CCLE preprocessing pipeline to quality control reads, align/assemble reads to a human reference genome, and quantify individual human gene expression RPKM values (CCLE_DepMap_18Q1_RNAseq_RPKM_20180214.gct). Data were formatted using the statistical analysis software R and RStudio, and visualized using the R package ggplot2 [71, 72].

## HIV-1 infectivity assays

Vif-proficient and Vif-deficient (X26 and X27) HIV-1 IIIB C200 proviral expression constructs have been reported [73]. An HIV-1 IIIB C200 variant encoding Vif Q26 and Q27 has been characterized [38] (here called 2Q mutant). An HIV-1 IIIB C200 Vif 5A variant ($^{40}$AAAAA$^{44}$) was created by digesting pNLCSFV3-5A proviral DNA construct ([74]; kindly provided by Dr Kei Sato, University of Tokyo, Japan) at *Swa*I and *Sal*I sites and similarly cloned into pIIIB C200 proviral construct. HIV-1 single-cycle assays using VSV-G pseudotyped viruses were performed as described previously with minor modifications [27, 75]. VSV-G pseudotyped viruses were generated by transfecting 2.4 µg of proviral DNA construct and 0.6 µg of VSV-G expression vector using TransIT-LT1 reagent (Mirus Bio) into 293T cells ($3.0\times10^6$). Then, 48 h later, supernatants were harvested, filtered by 0.45 µm filters and used to infect into $2.5\times10^4$ CEM-GXR reporter cells for m.o.i. determinations. $5\times10^5$ target cells were infected with a m.o.i. of 0.05 (for SupT11-CCR5-vector and SupT11-CCR5-A3G) or 0.25 (for THP-1), washed with PBS after 24 h of the infection, and then incubated for an additional 24 h. Then, 24 h later, supernatants were collected and filtered. The resulting viral particles were normalized by p24 ELISA (ZeptoMetrix) and viral infectivity was measured using TZM-bl cells.

## Hypermutation analysis

Hypermutation analyses were performed as described [27, 28]. Proviral DNAs were recovered by infecting virus produced in THP-1 or SupT11-CCR5 cells into TZM-bl or SupT11-CCR5 using Gentra Puregene Cell Kit (Qiagen) [75]. Following *Dpn*I digestion, the viral *pol* region was amplified by nested PCR with outer primers (876 bp) [(5′-TCC ART ATT TRC CAT AAA RAA AAA) and (5′-TTY AGA TTT TTA AAT GGY TYT TGA)] and inner primers (564 bp) [(5′-AAT ATT CCA RTR TAR CAT RAC AAA AAT) and (5′-AAT GGY TYT TGA TAA ATT TGA TAT GT)]. Tdata-word-spacing="data-word-spacing="0.2w"0.2w"he resulting 564 bp amplicon was cloned into CloneJet (ThermoScientific). At least seven independent clones were Sanger sequenced for each condition. Clones with identical mutations were eliminated.

## Immunoblots

Cells were lysed in 2.5×Laemmli sample buffer. Virions were dissolved in 2.5×Laemmli sample buffer after pelleting down using 20 % sucrose (26 200 ***g***, 4 ˚C, 2 h). Proteins in cell and viral lysates were separated by SDS-PAGE and transferred to PVDF membranes (LI-COR Biosciences). Membranes were blocked with 5 % milk in PBS containing 0.1 % Tween 20 and incubated with primary antibodies: mouse anti-HSP90 (Thermo Fisher Scientific, PA3-012); rabbit anti-APOBEC3B (RSH#10.87.13; first described in [76]); rabbit anti-A3G (NARP, #10201); mouse anti-Vif (319, NARP, #6459); mouse anti-p24 (183-H12-5C, NARP, #1513). Subsequently, the membranes were incubated with horseradish peroxidase (HRP) or fluorescent dye-conjugated secondary antibodies: IRDye 680LT anti-mouse IgG (LI-COR Biosciences, 926–68020); anti-rabbit IgG-HRP (Jackson ImmunoResearch, 111-035-144); anti-mouse IgG-HRP (Jackson ImmunoResearch, 715-035-150). HyGlo chemiluminescent HRP detection reagent (Denville Scientific) was used for HRP detection. Bands were visualized by using film exposure or the Odyssey Fc Imager (LI-COR Biosciences).

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Conflicts of interest

R. S. H. is a co-founder, shareholder and consultant of ApoGen Biotechnologies. The other authors have no conflicts of interest to declare.

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