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Article

Potential Role of APOBEC3 Family Proteins in SARS-CoV-2 Replication

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Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has acquired multiple mutations since its emergence. Analyses of the SARS-CoV-2 genomes from infected patients exhibit a bias toward C-to-U mutations, which are suggested to be caused by the apolipoprotein B mRNA editing enzyme polypeptide-like 3 (APOBEC3, A3) cytosine deaminase proteins. However, the role of A3 enzymes in SARS-CoV-2 replication remains unclear. To address this question, we investigated the effect of A3 family proteins on SARS-CoV-2 replication in THP-1 cells lacking *A3A* to *A3G* genes. The Wuhan, BA.1, and BA.5 variants had comparable viral replication in parent and *A3A*-to-*A3G*-null THP-1-ACE2 cells. On the other hand, the replication and infectivity of these variants were abolished in *A3A*-to-*A3G*-null THP-1-ACE2 cells in a series of passage experiments over 20 days. In contrast to previous reports, we observed no evidence for A3-induced SARS-CoV-2 mutagenesis in the passage experiments. Furthermore, our analysis of a large number of publicly available SARS-CoV-2 genomes did not reveal conclusive evidence for A3-induced mutagenesis. Taken together, our studies suggest that A3 family proteins can positively contribute to SARS-CoV-2 replication, however this effect is deaminase-independent.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for coronavirus disease 2019 (COVID-19). Since the first cases of novel coronavirus infection were detected in Wuhan, Hubei Province, China, in December 2019 [1,2], it spread rapidly worldwide. The World Health Organization (WHO) declared a Public Health Emergency of International Concern (PHEIC) as a COVID-19 pandemic on January 30, 2020 [3] and announced the termination of PHEIC on May 5, 2023 [4]. However, the COVID-19 pandemic has continued.

The apolipoprotein B mRNA editing enzyme polypeptide-like 3 (APOBEC3, A3) family of proteins are composed of seven DNA cytosine deaminases (A3A, A3B, A3C, A3D, A3F, A3G, and A3H proteins) in humans (reviewed in [5–8]). These A3 proteins are involved in an innate host defense mechanism against parasitic DNA-based elements (reviewed in [8–11]). Retroviruses are susceptible to cytosine to uracil (C-to-U) deamination caused by A3 family proteins because they produce single-stranded cDNA intermediates that act as the substrate for these enzymes (reviewed in [5–8,12]). Notably, human immunodeficiency virus type 1 (HIV-1) is the best characterized substrate for A3 family proteins. In primary $CD4^+$ T cells, at least four A3 enzymes (A3D, A3F, A3G, and only stable A3H) restrict HIV-1 replication by deaminating viral cDNA intermediates and physically blocking reverse transcription [13–22]. A3 enzymes recognize specific dinucleotide motifs for deamination, such as 5′-C<u>C</u> for A3G or 5′-T<u>C</u> for other A3 enzymes at target cytosine bases, which appear as 5′-<u>A</u>G or 5′-<u>A</u>A mutations in the genomic strand [15,17,23,24]. A3 protein-mediated mutations were observed in the genome of single-stranded DNA virus [Transfusion-transmitted

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virus (TTV)] (reviewed in [8,12]) and various double-stranded DNA viruses [Epstein-Barr virus (EBV), herpes simplex virus type 1 (HSV-1), alpha human papillomaviruses (α-HPV), and BK polyomavirus (BK PyV)] (reviewed in [8,11,12]). However, due to deaminase-independent mechanisms, the antiviral activity of A3 family proteins is not simply associated with its enzymatic activity [25–28].

Although A3 family proteins generally prefer single-stranded DNA for the deamination, several reports demonstrated that certain A3 proteins induce C-to-U mutations in single-stranded RNA substrates [29–34]. In addition, A3 protein-mediated mutations have been reported in the human coronavirus NL63 (HCoV-NL63) genome [35]. However, whether A3-induced mutations are associated with antiviral activity against RNA viruses remains unclear [35–37].

SARS-CoV-2 is continually evolving since its emergence in late 2019 [38–44]. C-to-U mutations are among the most frequent mutations accumulated in the SARS-CoV-2 genome [39–41,43,44]. This has been speculated to be due to the deaminase activity of APOBEC proteins. These mutations are thought to produce viruses that are more infectious and evade adaptive immunity. Recently, it has been reported that A3A protein is the potential source of C-to-U mutations in the SARS-CoV-2 genome [40]. Another *in vitro* study focusing on A1, A3A and A3G proteins suggested that A1 and A3A proteins (and to a much lesser extent A3G protein) have the capacity to mutate the SARS-CoV-2 genome, however these mutations do not impact viral replication. Unexpectedly, the expression of these editing enzymes promoted SARS-CoV-2 replication and propagation [45]. The authors suggested that APOBEC-induced mutations may provide a fitness advantage. Despite these illuminating studies, the role of A3 family proteins in SARS-CoV-2 replication is yet to be fully determined.

We have previously reported that A3H encoded in THP-1 is an unstable haplotype and it is not involved in the restriction to HIV-1 [21]. Further, we created THP-1 cells lacking *A3A*, *A3B*, *A3C*, *A3D*, *A3F*, and *A3G* genes (*A3A*-to-*A3G*-null) and characterized the susceptibility of *A3A*-to-*A3G*-null THP-1 cells to HIV-1 infection [21]. Importantly, these cell lines completely abolished restriction activity against Vif-deficient HIV-1 [21]. Therefore, we exploited the *A3A*-to-*A3G*-null THP-1 cells to create a version stably expressing ACE2 protein and examined the effect of A3 family proteins on SARS-CoV-2 replication.

2. Results

2.1. Creation of THP-1 and A3A-to-A3G-null THP-1 Cells Stably Expressing ACE2 Protein

It has been reported previously that A3C, A3F, and A3H proteins can inhibit HCoV-NL63 infection [35]. More recently, it has been shown that A3A protein and, to a significantly lesser extent, A3G protein are capable of inflicting C-to-U mutations in the SARS-CoV-2 genome. These studies have led to the speculation that A3 enzymes may be the potential source of the observed C-to-T mutations in the SARS-CoV-2 clinical isolates [40,45]. To address whether A3 family proteins are associated with SARS-CoV-2 mutation and restriction, we introduced the *ACE2* gene into THP-1 parent cells and their derivatives lacking the expression of A3A to A3G proteins (THP-1#11-4).

As shown in Figure 1A, ACE2 protein expression was not detected in THP-1 parent and THP-1#11-4 cells. However, 36% of THP-1 parental cells and 43% of THP-1#11-4 cells showed ACE2 protein expression after transduction. Next, *A3* mRNA expression levels in these ACE2-transduce THP-1 cells were quantified by RT-qPCR (Figure 1B). Except for a minor difference in the *A3H* mRNA expression, *A3A* to *A3G* mRNA expression levels remain consistent with the previous observation [21]. These data indicate that THP-1#11-4 cells stably express ACE2 protein and still fail to express the functional versions of any of these proteins.

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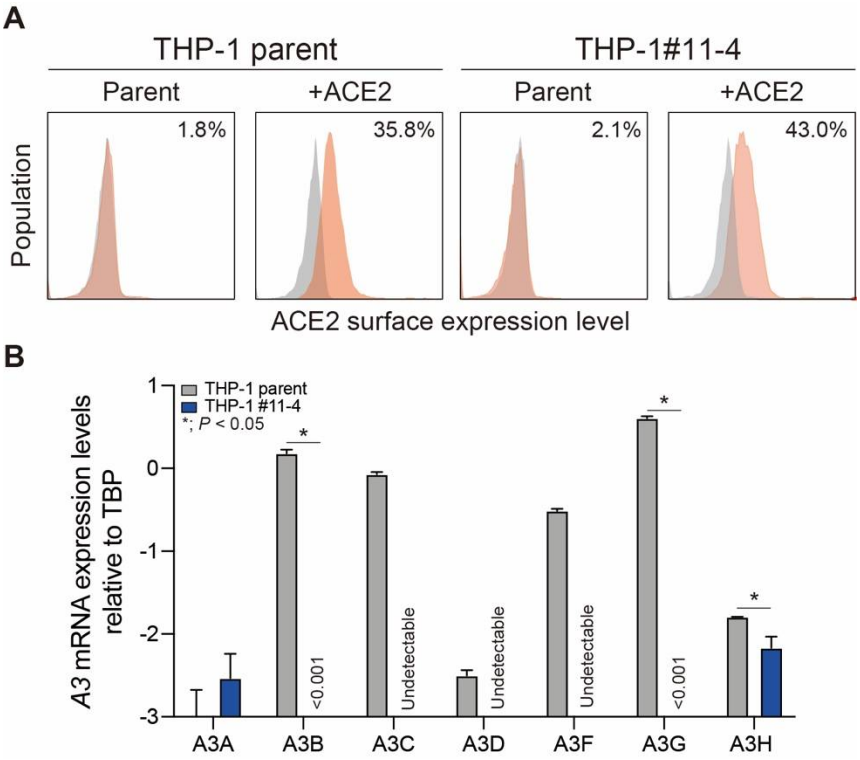

Figure 1. Validation of expression levels for ACE2 protein and *A3* mRNAs in THP-1 parent and *A3A*-to-*A3G*-null THP-1 cells. (**A**) ACE2 protein expression levels on the surface of THP-1 parent and #11-4 (*A3A*-to-*A3G*-null THP-1) cells. The *ACE2* gene was introduced by lentiviral vector and the expression levels of the surface ACE2 protein were detected by an anti-ACE2 polyclonal antibody (red). The number in each graph shows the percentage of $ACE2^+$ cells compared to those stained by isotype control (gray). (**B**) RT-qPCR data. *A3* mRNA expression levels were quantified by RT-qPCR and are normalized to *TBP* mRNA levels. Each bar represents the average of three independent experiments with Standard deviation (SD). Statistical significance was determined using the two-sided unpaired *t* test. *, $P < 0.05$ compared to THP-1 parent cells.

2.2. Effect of A3 Family Proteins on SARS-CoV-2 Replication

We next investigated viral replication in THP-1 parent and THP-1#11-4 cells stably expressing ACE2 protein. We did not observe viral (BA.1 and BA.5) replication in cells lacking ACE2 protein expression, indicating that ACE2 is required for viral replication in THP-1 cells (Figure 2A). However, the viral replication of these variant between THP-1 parent and THP-1#11-4 cells was comparable during 96 hours of time course (Figure 2A). We repeated the time course of viral replication assay until passage 5 (~20 days). In THP-1 parent, the Wuhan, BA.1, and BA.5 variants showed continuous viral replication until passage 5 (Figure 2B). Surprisingly, the lack of *A3A* to *A3G* genes in THP-1 diminished the viral replication of all variants and their viral RNAs were undetectable until passage 5 (Figure 2B). These data suggest that A3A to A3G proteins may be associated with long-term (~20 days) SARS-CoV-2 replication in THP-1 cells.

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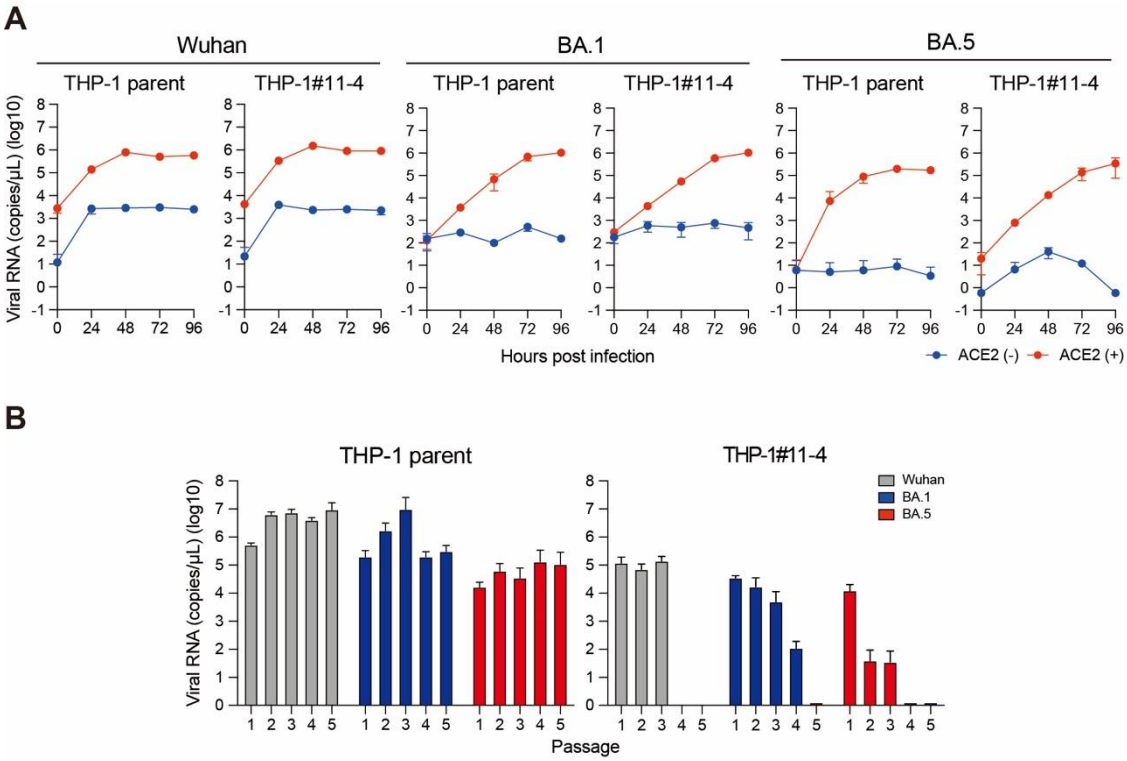

Figure 2. SARS-CoV-2 replication in THP-1 parent and *A3A*-to-*A3G*-null THP-1 cells. (**A**) Replication kinetics of the Wuhan, BA.1, and BA.5 variants produced from THP-1 parent and *A3A*-to-*A3G*-null THP-1 (*A3A*-to-*A3G*-null THP-1) cells without (blue line) or with (red line) ACE2 protein expression. The SARS-CoV-2 *N* gene was quantified by RT-qPCR to monitor the viral RNA copy number across the indicated timepoints. Each timepoint represents the average of four independent experiments with SD. (**B**) Passage experiments. The SARS-CoV-2 *N* gene in the cell culture supernatants produced from THP-1 parent or *A3A*-to-*A3G*-null THP-1 (*A3A*-to-*A3G*-null THP-1) cells at 96 hours postinfection of each passage was quantified by RT-qPCR to monitor the viral RNA copy number of the Wuhan (gray), BA.1 (blue), and BA.5 (red) variants. Each bar represents the average of three independent experiments with SD.

2.3. Effect of A3 Family Proteins on SARS-CoV-2 Infectivity

As mentioned above, the viral RNA of all variants tested became undetectable by 20 days postinfection, in contrast to that observed in THP-1 parent cells (Figure 2B). To know whether the Wuhan variant obtained from each passage was infectious, we performed plaque assay in VeroE6/TMPRSS2 (Figure 3). Consistent with viral replication results (Figure 2B), the number of PFU obtained from the Wuhan variant infection in THP-1 parent cells was increased during the passage experiments (Figure 3). However, *A3A*-to-*A3G* gene disruption caused a decrease in viral infectivity and it finally became undetectable (Figure 3). These data suggest that A3A to A3G proteins may contribute positively to the production of SARS-CoV-2 infectious particles from THP-1 cells.

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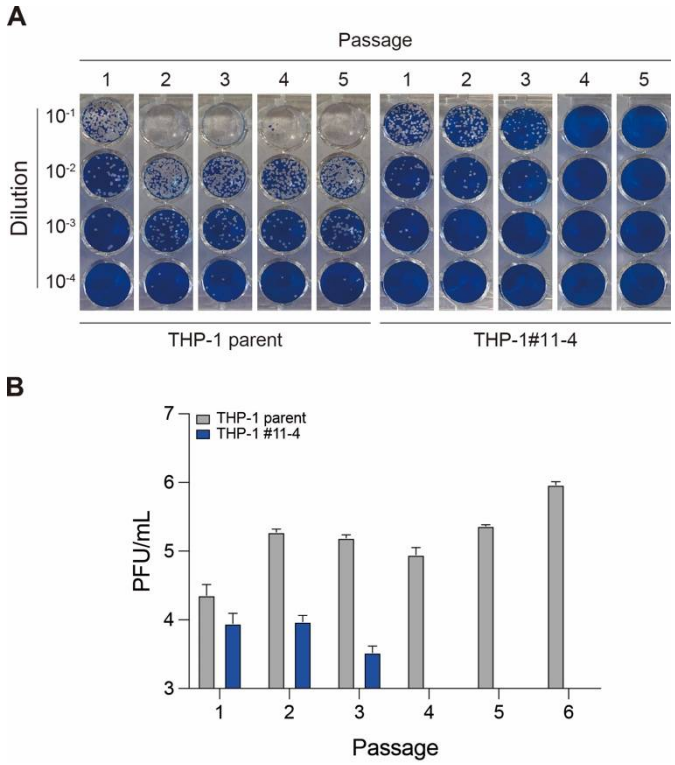

Figure 3. SARS-CoV-2 infectivity produced from THP-1 parent and *A3A*-to-*A3G*-null THP-1 cells during passage experiments.(**A**) Representative pictures of plaque assay. Cell culture supernatants obtained from the passage experiments for the Wuhan variant were also used for plaque assay with serial 10-times dilution. (**B**) PFU/ml of the Wuhan variant produced from THP-1 parent (gray) or *A3A*-to-*A3G*-null THP-1 (*A3A*-to-*A3G*-null THP-1) (blue) cells at 96 hours postinfection during passage experiments.

2.4. Effect of A3 Proteins on SARS-CoV-2 Mutagenesis

Finally, we asked whether A3 proteins contribute to C-to-U mutations in the SARS-CoV-2 genome. To address this question, we performed WGS analysis for viral RNA isolated from each passage. Our analysis revealed mutations in seven positions only two of which were C-to-T mutations, but none were in the TCA or TCT contexts that are known as APOBEC targets (Figure 4). Additionally, there was no mutational burden difference between the cells with and without *A3A* to *A3G* genes (Figure 4). These results indicate that A3 proteins do not play a role in SARS-CoV-2 mutagenesis. To investigate whether lack of A3-induced mutagenesis in SARS-CoV-2 is specific to THP-1 cells or it is a general feature of SARS-CoV-2 infection, we conducted a bioinformatics analysis of 40,000 whole genome SARS-CoV-2 sequences from NCBI. Our analysis involved the quantification of all possible 192 types of mutations followed by mutational signature deconvolution by NMF. We investigated models with up to 20 mutational signatures and could not find any mutational signatures closely resembling SBS2 [represented by TC(T/A)-to-TT(T/A)] or SBS13 [represented by TC(T/A)-to-TG(T/A)] as shown in Figure 5. However, we noted two signatures (S8 and S9), each represented by only one of the main SBS2 peaks (TCA-to-TTA in S8 and TCT-to-TTT in S9). Since such signatures containing only a single dominant peak have not been previously reported for any A3 enzymes, further studies are needed to provide evidence that these signatures are footprint of A3 enzymes.

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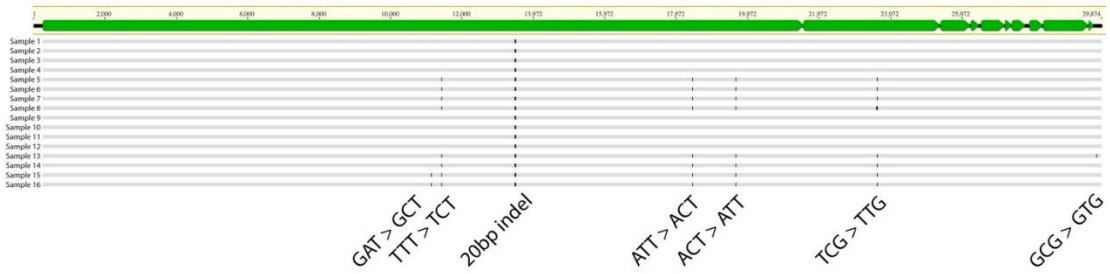

Figure 4. Analysis of mutations in SARS-CoV-2 genomes produced from THP-1 parent and *A3A*-to-*A3G*-null THP-1 cells during passage experiments. SARS-CoV-2 genomic RNA was isolated and subjected to WGS. Sample 1 to 4: Wuhan variant from passage 1 of THP-1 parent cells. Sample 5 to 8: Wuhan variant from passage 5 of THP-1 parent cells. Sample 9 to 12: Wuhan variant from passage 1 of *A3A*-to-*A3G*-null THP-1 cells. Sample 13 and 14: Wuhan variant from passage 3 of *A3A*-to-*A3G*-null THP-1 cells. Sample 15 and 16: Wuhan variant from passage 4 and passage 5 of *A3A*-to-*A3G*-null THP-1 cells. Green boxes on the top show each SARS-CoV-2 *ORF* gene with nucleotide position.

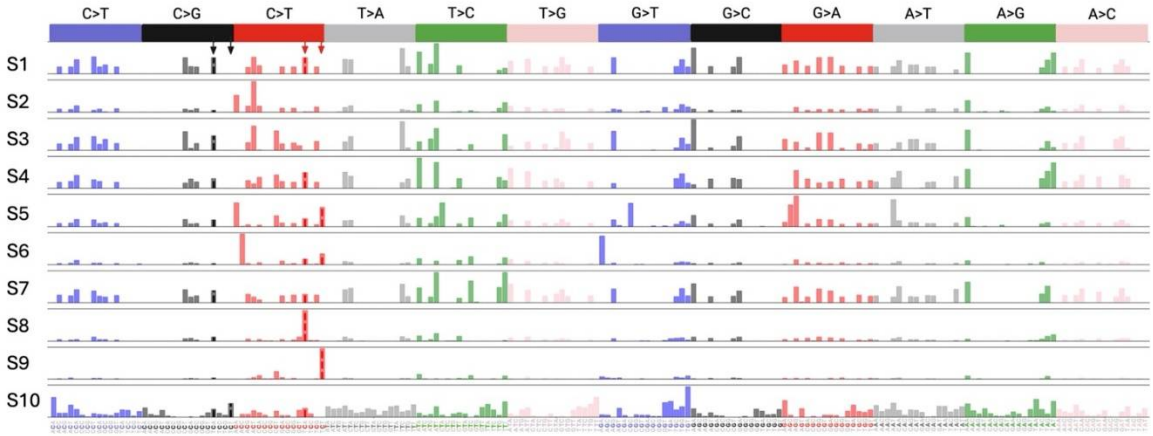

Figure 5. Analysis of mutational signatures in publicly available SARS-CoV-2 genomes. All possible 192 mutation types (NnN-to-NmN where n mutates to m and N:A/C/G/T) were quantified in a total of 38,830 whole genome SARS-CoV-2 sequences sampled between the years 2019 and 2022 and reprted in NCBI. These mutation counts were organized in a data matric of (38,830 by 192) and used as input for analysis using the Non-negative Matrix Factorization (NMF) method. Models with up to 20 components were built. Only a model with 10 component is shown for simplicity. None of the models showed signatures closely related to SBS2 and SBS13. Positions of the four major C>T and C>G peaks in SBS2 and SBS13 are shown by arrows.

3. Discussion

The role of A3 enzymes in SARS-CoV-2 mutations have been implicated in several studies [39–41,43,44], however little is known about the functional relevance of A3 proteins in SARS-CoV-2 infection. In this study, we examined the effect of A3 proteins on SARS-CoV-2 replication by conducting infection in THP-1 cells lacking A3 enzymes. We showed that the Wuhan, BA.1, and BA.5 variants had comparable viral RNA production in THP-1-ACE2 parent and THP-1 cells lacking the expression of A3A to A3G proteins during 96 hours of the time course (Figure 2A). However, A3 family proteins affected SAR-CoV-2 RNA production in the passage experiments for up to 20 days (Figure 2B). Further, the results of plaque assay showed that A3 family proteins might contribute to the production of infectious virus particles in THP-1 cells (Figure 3). Notably, the effect of A3 family proteins on SARS-CoV-2 replication is independent of C-to-U mutations (Figure 4). Taken together,

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our findings suggest that A3 family proteins may influence SARS-CoV-2 replication in a deaminase-independent manner.

C-to-U deamination by A3 family proteins is required to restrict HIV-1 [13,21–23,46–50]. However, deaminase-independent mechanisms also contribute to the anti-HIV-1 activity of A3 family proteins [14,21,48,49,51–55]. Indeed, deaminase-independent mechanisms are reported to be the predominant method of viral restriction against many viruses [35,37,56–59]. Several mechanisms have been proposed for the deaminase-independent action. First, the binding of A3F and A3G proteins to HIV-1 genomic RNA blocks the elongation of reverse transcription directly [14,49–51,55]. Second, a direct interaction between the A3G protein and HIV-1 Reverse transcriptase causes the disruption of cDNA synthesis [20,60]. Third, an interesting notion is that the A3B protein promotes stress granule formation through a protein kinase R signaling pathway that mediates translational shutdown in cells infected with diverse RNA viruses, such as Sendai virus, Polio virus, and Sindbis virus [61]. These findings indicate that deaminase-independent mechanisms mediate the interaction of the A3 proteins with viral and non-viral proteins. Therefore, the range of innate defense mechanisms by A3 family proteins may be broadly applied to numerous viruses more than those that have been reported so far.

THP-1-ACE2 cells mainly express *A3B*, *A3C*, *A3F*, and *A3G* mRNAs under normal cell culture conditions (Figure 1B). These proteins have the capacity to form high molecular mass ribonucleoprotein (HMM RNP) complexes [62–68]. Since HMM RNP complexes are composed of A3G-binding RNAs, A3G-binding proteins, and numerous cellular RNA-binding proteins [62–64,66–70], an essential negative factor(s) that can support SARS-CoV-2 replication may be sequestered in the HMM RNP complexes mediated by A3 proteins. Meanwhile, the myeloid cell line, THP-1 used in this study is a non-natural target cell of SARS-CoV-2 because this cell line does not express ACE2 and SARS-CoV-2 cannot replicate in this cell line (Figure 1A and 2A). As an alternative idea, the phenomenon observed in this study may be limited in THP-1 cells where A3 protein-mediated HMM RNP complexes may include an essential negative protein(s) for SARS-CoV-2 replication that is not expressed in non-natural target cells and the sequestration of this protein could support viral replication. Further investigation revealing the underlying mechanisms will be needed for understanding the role of A3 proteins in SARS-CoV-2 replication.

In summary, we found that A3 family proteins support SARS-CoV-2 replication in THP-1 independently of their enzymatic activity. Understanding the underlying mechanism may provide new insight into the interaction between A3 family proteins and coronaviruses. Further, A3 family proteins may be a potential therapeutic target for drug development to alleviate disease severity in respiratory diseases caused by coronaviruses.

4. Materials and Methods

4.1. Cell lines and Culture Conditions

THP-1 cells were provided by Dr. Andrea Cimarelli (INSERM, France) [71]. The generation and characterization of THP-1 Δ*A3A*-to-*A3G*#11-4 has been reported previously [21]. THP-1 cells and their derivatives were maintained in RPMI (Thermo Fisher Scientific, Cat# C11875500BT) with 10% fetal bovine serum (FBS) (NICHIREI, Cat#175012) and 1% penicillin/streptomycin (P/S) (Wako, Cat# 168-23191). GP2-293 cells (HEK293 expressing Moloney murine leukemia virus gag/pol protein; TAKARA, Cat# 631530) were maintained in high glucose Dulbecco's Modified Eagle Medium (DMEM, Wako, Cat# 044-29765) containing 10% FBS and 1% P/S. VeroE6/TMPRSS2 cells (VeroE6 cells stably expressing human TMPRSS2 protein; JCRB Cell Bank, JCRB1819) [72] were maintained in low glucose Dulbecco's Modified Eagle Medium (DMEM, Wako, Cat# 041-29775) containing 10% FBS, G418 (1 mg/ml; Wako, Cat#070-06803) and 1% P/S (Wako, Cat# 168-23191). All cells were maintained at 37°C with 5% CO_2.

4.2. Virus Preparation

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SARS-CoV-2 Wuhan variant (strain SARS-CoV-2/Hu/DP/Kng/19-020 strain, Genbank accession no. LC528232) [73,74] was provided by Drs. Tomohiko Takasaki and Jun-Ichi Sakuragi (Kanagawa Prefectural Institute of Public Health). SARS-CoV-2 Omicron BA.1 (strain TY38-873, GISAID ID: EPI_ISL_7418017) [74,75] variant was obtained from National Institute of Infectious Diseases. BA.5 (strain TKYS14631; GISAID ID: EPI_ISL_12812500) [76–78] variants were provided by Tokyo Metropolitan Institute of Public Health.

Virus propagation was performed as previously described [73,74,79,80]. Briefly, VeroE6/TMPRSS2 cells (5×10^6 cells) were seeded in a T-75 flask the day before infection. Virus was diluted in virus dilution buffer [1M HEPES, DMEM (low glucose), Non- essential Amino acid (gibco, Cat# 11140-050), 1% P/S] and the dilution buffer containing virus was added to the flask after removing the initial medium. After 1 hour of incubation at 37°C, the supernatant was replaced with 15 ml of 2% FBS/DMEM (low glucose) and cell culture was continued to incubate at 37°C until visible cytopathic effect (CPE) was clearly observed. Then, cell culture supernatant was collected, centrifuged at 300 × *g* for 10 minutes and frozen at –80°C as working virus stock. The titer of the prepared working virus was determined as the 50% tissue culture infectious dose ($TCID_{50}$) [76,80,81]. The day before infection, VeroE6/TMPRSS2 cells (10,000 cells) were seeded in a 96-well plate and infected with serially diluted working virus stocks. The infected cells were incubated at 37°C for 4 days and the appearance of CPEs in the infected cells was observed by a microscope. The value of $TCID_{50}$/ml was calculated by the Reed-Muench method [82].

4.3. A3 mRNA Quantification

Cells were harvested and washed with PBS twice. Then, total RNA was isolated by RNA Premium Kit (NIPPON Genetics, Cat# FG-81250) and cDNA was synthesized by Transcriptor Reverse Transcriptase (Roche, Cat# 03531287001) with random hexamer. RT-qPCR was performed using Power SYBR Green PCR Master Mix (Thermo Fisher Scientific, Cat# 4367659). Primers for each *A3* mRNA have been reported previously [83,84]. *A3A* forward: (5′-GAG AAG GGA CAA GCA CAT GG) and *A3A* reverse: (5′-TGG ATC CAT CAA GTG TCT GG). *A3B* forward: (5′-GAC CCT TTG GTC CTT CGA C) and *A3B* reverse: (5′-GCA CAG CCC CAG GAG AAG). *A3C* forward: (5′-AGC GCT TCA GAA AAG AGT GG) and *A3C* reverse: (5′-AAG TTT CGT TCC GAT CGT TG). *A3D* forward: (5′-ACC CAA ACG TCA GTC GAA TC) and *A3D* reverse: (5′-CAC ATT TCT GCG TGG TTC TC). *A3F* forward: (5′-CCG TTT GGA CGC AAA GAT) and *A3F* reverse: (5′-CCA GGT GAT CTG GAA ACA CTT). *A3G* forward: (5′-CCG AGG ACC CGA AGG TTA C) and *A3G* reverse: (5′-TCC AAC AGT GCT GAA ATT CG). *A3H* forward: (5′-AGC TGT GGC CAG AAG CAC) and *A3H* reverse: (5′-CGG AAT GTT TCG GCT GTT). *TATA-binding protein* (*TBP*) forward: (5′-CCC ATG ACT CCC ATG ACC) and *TBP* reverse: (5′-TTT ACA ACC AAG ATT CAC TGT GG). Fluorescent signals from resulting PCR products were acquired using a Thermal Cycler Dice Real Time System III (Takara). Finally, each *A3* mRNA expression level was represented as values normalized by *TBP* mRNA expression levels (Figure 1B).

4.4. ACE2 Transduction

pLV-EF1a-human ACE2-IRES-Puro [85] was used as a template to amplify human *ACE2* gene using primers, forward: (5′-NNN NNG TTA ACA CCA TGT CAA GCT CTT CCT GGC TCC TTC) and reverse: (5′-NNN NNC TCG AGC TAA AAG GAG GTC TGA ACA TCA TCA GTG). Then, the amplified human *ACE2* gene was inserted into pMSCVneo retroviral vector (Takara, Cat# 634401) at HpaI and XhoI site. The inserted human *ACE2* gene was Sanger sequenced (AZENTA) and the data were analyzed by Sequencher DNA sequence analysis software v5.5.6 (Gene Codes Corporation).

Retroviral transduction was performed as previously described in lentivirus [46,48,85]. VSV-G-pseudotyped virus expressing human ACE2 protein was generated by transfecting 4 μg of pMSCV-ACE2-neo plasmid and pVSV-G expression vector (Addgene, cat# 138479) using TransIT-LT1 reagent (Takara, Cat# MIR2306) into 293gp cells (3×10^6 cells). Forty-eight hours later, supernatants were harvested, filtered (0.45 μm filters, Merck, Cat# SLHVR33RB), and subjected to ultracentrifugation at 22,000 × *g* at 4°C for 2 hours. After resolving the viral pellets with 10% FBS/RPMI, the concentrated

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retrovirus was inoclulate into THP-1 cells and its derivatives (1.5×10^5 cells) and incubated at 37°C. At 72 hours posttransduction, the cells were selected by 1 mg/ml G418 (Wako, Cat#070-06803). G418-selected cells with relatively higher ACE2 expression were sorted by a FACS Aria II (BD Biosciences) and expanded. After expansion, the expression level of surface ACE2 was verified by a FACS Canto II (BD Biosciences). A goat anti-ACE2 polyclonal antibody (R&D Systems, Cat# AF933, 1:50) and an APC-conjugated donkey anti-goat IgG (R&D Systems, Cat# F0108, 1:50) were used for surface ACE2 staining (Figure 1A). Normal goat IgG (R&D Systems, Cat# AB-108-C, 1:100) was used as the negative control for this assay.

4.5. SARS-CoV-2 Infection

5×10^5 parent and *A3A*-to-*A3G*-null THP-1 cells were seeded into a 24-well plate, inoculated with SARS-CoV-2 (5,000 $TCID_{50}$) and incubated at 37°C for 1 hour. After washing with phosphate buffered saline (PBS), 1 ml of fresh cell culture medium was added. 15 µl of cell culture supernatant was harvested at the indicated timepoints and used for RT-qPCR to quantify the viral RNA copy number (see "RT-qPCR" section) (Figure 2A and B). For passage experiments, 10 µl of the cell culture supernatant at 96 hours postinfection was transferred to the next target cells. The passage of the cell culture supernatant was repeated until passage 5.

4.6. RT-qPCR for SARS-CoV-2 RNA

RT-qPCR was performed as previously described [81,85–89]. Briefly, 5 µl of culture supernatant was mixed with 5 µl of 2 × RNA lysis buffer [2% Triton X-100 (Nacalai Tesque, Cat#12969-25), 50 mM KCl, 100mM Tris-HCl (pH 7.4), 40% glycerol, 0.8 U/µl recombinant RNase inhibitor (Takara, Cat# 2313A)] and incubated at room temperature for 10 minutes. 90 µl of RNase free water was added and then 2.5 µl of diluted sample was used for real-time RT-PCR according to the manufacturer's protocol with One step TB green PrimeScript PLUS RT-PCR Kit (Takara, Cat# RR096A) and primers for *Nucleocapsid* (*N*) gene; Forward *N*, 5'-AGC CTC TTC TCG TTC CTC ATC-3' and Reverse *N*, 5'-CCG CCA TTG CCA GCC ATT C-3'. The viral RNA copy number was standardized using a SARS-CoV-2 direct detection RT-qPCR kit (Takara, Cat# RC300A). Fluorescent signals from resulting PCR products were acquired using a Thermal Cycler Dice Real Time System III (Takara).

4.7. Plaque Assay

Plaque assay was performed as previously described [79,87,90,91]. One day before infection, 1×10^5 VeroE6/TMPRSS2 cells were seeded into 24 well plate and infected with serial dilution of cell culture supernatants including SARS-CoV-2 (10, 100, 1000, and 10000-fold dilution, respectively) at 37°C for 1 hour. 3% FBS and 1.5% carboxymethyl cellulose (Wako, Cat# 039-1335) containing mounting solution was overlaid, followed by incubation at 37°C. At 3 days postinfection, the cell culture medium was removed, and the cells were washed with PBS three times and fixed with 4% paraformaldehyde phosphate (Nacalai Tesque, Cat# 09154-85). The fixed cells were washed with tap water, dried, and stained with 0.1% methylene blue (Nacalai Tesque, Cat# 22412-14) in water for 30 minutes. The stained cells were washed with tap water and dried. Finally, the number of plaques were counted and indicated as plaque forming unit (PFU)/ml (Figure 3).

4.8. SARS-CoV-2 Whole Genome Sequencing (WGS)

The WGS of SARS-CoV-2 RNA genome was performed as previously described [92]. Briefly, cDNA synthesis, viral sequence enrichment, library amplification, and indexing were performed using the QIAseq DIRECT SARC-CoV-2 kit (QIAgen, Cat# 333891) according to the manufacturer's protocol. After multiplexing with QIASeq DIRECT UDI Set-A (QIAgen), 25 µl library was prepared from each sample. The quality of the enriched libraries was evaluated by electrophoresis using TapeStation 4150 system (Agilent Technologies). The prepared libraries were subjected to sequencing using MiSeq reagent Micro and Nano Kits (Version 2, 300 cycles) in the MiSeq desktop sequencing system (Illumina). The data analysis was done as previously performed [92].

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4.9. SARS-CoV-2 Mutational Signature Analysis

We downloaded 40,000 whole genome SARS-CoV-2 sequences from NCBI. These sequences were sampled between the years 2019 and 2022 and were highly diverse in terms of geographic distribution. 38,830 of these sequences that had less than 5% insertion/deletion and/or non-A/C/G/T, were selected and aligned to the most common ancestor of SARS-CoV-2 as described by Kumar et al. [93]. For each sequence, the frequencies of all of the possible 192 mutation types (NnN-to-NmN where n mutates to m and N:A/C/G/T) were quantified. These mutation counts were organized in a data matric of (38,830 by 192) and used as input for analysis using the Non-negative Matrix Factorization (NMF) method. NMF is a matrix decomposition method used routinely to deconstruct mutational signatures in cancer and viral genomes [94–97]. Here, NMF was used to investigate if deconstruction of all SARS-CoV-2 sequences provides evidence for the existence of an APOBEC mutational signature similar to the known SBS2 and SBS13 signatures [94]. We built NMF models with up to 20 components to investigate the presence of SBS2 and SB13.

4.10. Statistical Analyses

GraphPad Prism software v8.4.3 was used for statistical analysis including two-tailed *t* tests (Figure 1B).

5. Conclusion

Our results suggest that A3 family proteins may contribute to SARS-CoV-2 replication in THP-1 cells. This effect of A3 family proteins on SARS-CoV-2 replication is independent of deaminase activity.

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References

1. Wu, F.; Zhao, S.; Yu, B.; Chen, Y.M.; Wang, W.; Song, Z.G.; Hu, Y.; Tao, Z.W.; Tian, J.H.; Pei, Y.Y.; et al. A new coronavirus associated with human respiratory disease in China. *Nature* **2020**, *579*, 265-269, doi:10.1038/s41586-020-2008-3.
2. Zhou, P.; Yang, X.L.; Wang, X.G.; Hu, B.; Zhang, L.; Zhang, W.; Si, H.R.; Zhu, Y.; Li, B.; Huang, C.L.; et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **2020**, *579*, 270-273, doi:10.1038/s41586-020-2012-7.
3. WHO. COVID-19 Public Health Emergency of International Concern (PHEIC) Global research and innovation forum (Feb 12, 2020), https://www.who.int/publications/m/item/covid-19-public-health-emergency-of-international-concern-(pheic)-global-research-and-innovation-forum.
4. WHO. Statement on the fifteenth meeting of the IHR (2005) Emergency Committee on the COVID-19 pandemic (May 5, 2023), https://www.who.int/news/item/05-05-2023-statement-on-the-fifteenth-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-coronavirus-disease-(covid-19)-pandemic.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 June 2024 doi:10.20944/preprints202406.1182.v1

5. Desimmie, B.A.; Delviks-Frankenberrry, K.A.; Burdick, R.C.; Qi, D.; Izumi, T.; Pathak, V.K. Multiple APOBEC3 restriction factors for HIV-1 and one Vif to rule them all. *J Mol Biol* **2014**, *426*, 1220-1245, doi:10.1016/j.jmb.2013.10.033.
6. Harris, R.S.; Dudley, J.P. APOBECs and virus restriction. *Virology* **2015**, *479-480C*, 131-145, doi:10.1016/j.virol.2015.03.012.
7. Ikeda, T.; Yue, Y.; Shimizu, R.; Nasser, H. Potential utilization of APOBEC3-mediated mutagenesis for an HIV-1 functional cure. *Front Microbiol* **2021**, *12*, 686357, doi:10.3389/fmicb.2021.686357.
8. Jonathan, M.; Ikeda, T. APOBEC3 family proteins as drivers of virus evolution. *Frontiers in Virology* **2023**, *3*, doi:10.3389/fviro.2023.1332010.
9. Holmes, R.K.; Malim, M.H.; Bishop, K.N. APOBEC-mediated viral restriction: not simply editing? *Trends Biochem Sci* **2007**, *32*, 118-128, doi:10.1016/j.tibs.2007.01.004.
10. Koito, A.; Ikeda, T. Apolipoprotein B mRNA-editing, catalytic polypeptide cytidine deaminases and retroviral restriction. *Wiley interdisciplinary reviews. RNA* **2012**, *3*, 529-541, doi:10.1002/wrna.1117.
11. Cheng, A.Z.; Moraes, S.N.; Shaban, N.M.; Fanunza, E.; Bierle, C.J.; Southern, P.J.; Bresnahan, W.A.; Rice, S.A.; Harris, R.S. APOBECs and herpesviruses. *Viruses* **2021**, *13*, doi:10.3390/v13030390.
12. Willems, L.; Gillet, N.A. APOBEC3 Interference during Replication of Viral Genomes. *Viruses* **2015**, *7*, 2999-3018, doi:10.3390/v7062757.
13. Newman, E.N.; Holmes, R.K.; Craig, H.M.; Klein, K.C.; Lingappa, J.R.; Malim, M.H.; Sheehy, A.M. Antiviral function of APOBEC3G can be dissociated from cytidine deaminase activity. *Current biology : CB* **2005**, *15*, 166-170, doi:10.1016/j.cub.2004.12.068.
14. Holmes, R.K.; Koning, F.A.; Bishop, K.N.; Malim, M.H. APOBEC3F can inhibit the accumulation of HIV-1 reverse transcription products in the absence of hypermutation. Comparisons with APOBEC3G. *J Biol Chem* **2007**, *282*, 2587-2595, doi:10.1074/jbc.M607298200.
15. Hultquist, J.F.; Lengyel, J.A.; Refsland, E.W.; LaRue, R.S.; Lackey, L.; Brown, W.L.; Harris, R.S. Human and rhesus APOBEC3D, APOBEC3F, APOBEC3G, and APOBEC3H demonstrate a conserved capacity to restrict Vif-deficient HIV-1. *J Virol* **2011**, *85*, 11220-11234, doi:10.1128/JVI.05238-11.
16. Wang, X.; Abudu, A.; Son, S.; Dang, Y.; Venta, P.J.; Zheng, Y.H. Analysis of human APOBEC3H haplotypes and anti-human immunodeficiency virus type 1 activity. *J Virol* **2011**, *85*, 3142-3152, doi:10.1128/JVI.02049-10.
17. Refsland, E.W.; Hultquist, J.F.; Harris, R.S. Endogenous origins of HIV-1 G to A hypermutation and restriction in the nonpermissive T cell line CEM2n. *PLoS Pathog* **2012**, *8*, e1002800, doi:10.1371/journal.ppat.1002800.
18. Ooms, M.; Brayton, B.; Letko, M.; Maio, S.M.; Pilcher, C.D.; Hecht, F.M.; Barbour, J.D.; Simon, V. HIV-1 Vif adaptation to human APOBEC3H haplotypes. *Cell Host Microbe* **2013**, *14*, 411-421, doi:10.1016/j.chom.2013.09.006.
19. Refsland, E.W.; Hultquist, J.F.; Luengas, E.M.; Ikeda, T.; Shaban, N.M.; Law, E.K.; Brown, W.L.; Reilly, C.; Emerman, M.; Harris, R.S. Natural polymorphisms in human APOBEC3H and HIV-1 Vif combine in primary T lymphocytes to affect viral G-to-A mutation levels and infectivity. *PLoS genetics* **2014**, *10*, e1004761, doi:10.1371/journal.pgen.1004761.
20. Pollpeter, D.; Parsons, M.; Sobala, A.E.; Coxhead, S.; Lang, R.D.; Bruns, A.M.; Papaioannou, S.; McDonnell, J.M.; Apolonia, L.; Chowdhury, J.A.; et al. Deep sequencing of HIV-1 reverse transcripts reveals the multifaceted antiviral functions of APOBEC3G. *Nat Microbiol* **2018**, *3*, 220-233, doi:10.1038/s41564-017-0063-9.
21. Ikeda, T.; Shimizu, R.; Nasser, H.; Carpenter, M.A.; Cheng, A.Z.; Brown, W.L.; Sauter, D.; Harris, R.S. APOBEC3 degradation is the primary function of HIV-1 Vif determining virion infectivity in the myeloid cell line THP-1. *mBio* **2023**, e0078223, doi:10.1128/mbio.00782-23.
22. Miyagi, E.; Opi, S.; Takeuchi, H.; Khan, M.; Goila-Gaur, R.; Kao, S.; Strebel, K. Enzymatically active APOBEC3G is required for efficient inhibition of human immunodeficiency virus type 1. *J Virol* **2007**, *81*, 13346-13353, doi:10.1128/JVI.01361-07.
23. Harris, R.S.; Bishop, K.N.; Sheehy, A.M.; Craig, H.M.; Petersen-Mahrt, S.K.; Watt, I.N.; Neuberger, M.S.; Malim, M.H. DNA deamination mediates innate immunity to retroviral infection. *Cell* **2003**, *113*, 803-809, doi:S0092867403004239 [pii].
24. Rathore, A.; Carpenter, M.A.; Demir, O.; Ikeda, T.; Li, M.; Shaban, N.M.; Law, E.K.; Anokhin, D.; Brown, W.L.; Amaro, R.E.; et al. The local dinucleotide preference of APOBEC3G can be altered from 5'-CC to 5'-TC by a single amino acid substitution. *J Mol Biol* **2013**, *425*, 4442-4454, doi:10.1016/j.jmb.2013.07.040.
25. Poulain, F.; Lejeune, N.; Willemart, K.; Gillet, N.A. Footprint of the host restriction factors APOBEC3 on the genome of human viruses. *PLoS Pathog* **2020**, *16*, e1008718, doi:10.1371/journal.ppat.1008718.
26. Vartanian, J.P.; Guetard, D.; Henry, M.; Wain-Hobson, S. Evidence for editing of human papillomavirus DNA by APOBEC3 in benign and precancerous lesions. *Science* **2008**, *320*, 230-233, doi:10.1126/science.1153201.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 June 2024 doi:10.20944/preprints202406.1182.v1

27. Verhalen, B.; Starrett, G.J.; Harris, R.S.; Jiang, M. Functional Upregulation of the DNA Cytosine Deaminase APOBEC3B by Polyomaviruses. *J Virol* **2016**, *90*, 6379-6386, doi:10.1128/JVI.00771-16.
28. Warren, C.J.; Van Doorslaer, K.; Pandey, A.; Espinosa, J.M.; Pyeon, D. Role of the host restriction factor APOBEC3 on papillomavirus evolution. *Virus Evol* **2015**, *1*, vev015, doi:10.1093/ve/vev015.
29. Sharma, S.; Patnaik, S.K.; Taggart, R.T.; Kannisto, E.D.; Enriquez, S.M.; Gollnick, P.; Baysal, B.E. APOBEC3A cytidine deaminase induces RNA editing in monocytes and macrophages. *Nat Commun* **2015**, *6*, 6881, doi:10.1038/ncomms7881.
30. Sharma, S.; Patnaik, S.K.; Taggart, R.T.; Baysal, B.E. The double-domain cytidine deaminase APOBEC3G is a cellular site-specific RNA editing enzyme. *Sci Rep* **2016**, *6*, 39100, doi:10.1038/srep39100.
31. Sharma, S.; Baysal, B.E. Stem-loop structure preference for site-specific RNA editing by APOBEC3A and APOBEC3G. *PeerJ* **2017**, *5*, e4136, doi:10.7717/peerj.4136.
32. Sharma, S.; Patnaik, S.K.; Kemer, Z.; Baysal, B.E. Transient overexpression of exogenous APOBEC3A causes C-to-U RNA editing of thousands of genes. *RNA Biol* **2017**, *14*, 603-610, doi:10.1080/15476286.2016.1184387.
33. Sharma, S.; Wang, J.; Alqassim, E.; Portwood, S.; Cortes Gomez, E.; Maguire, O.; Basse, P.H.; Wang, E.S.; Segal, B.H.; Baysal, B.E. Mitochondrial hypoxic stress induces widespread RNA editing by APOBEC3G in natural killer cells. *Genome Biol* **2019**, *20*, 37, doi:10.1186/s13059-019-1651-1.
34. Alqassim, E.Y.; Sharma, S.; Khan, A.; Emmons, T.R.; Cortes Gomez, E.; Alahmari, A.; Singel, K.L.; Mark, J.; Davidson, B.A.; Robert McGray, A.J.; et al. RNA editing enzyme APOBEC3A promotes pro-inflammatory M1 macrophage polarization. *Commun Biol* **2021**, *4*, 102, doi:10.1038/s42003-020-01620-x.
35. Milewska, A.; Kindler, E.; Vkovski, P.; Zeglen, S.; Ochman, M.; Thiel, V.; Rajfur, Z.; Pyrc, K. APOBEC3-mediated restriction of RNA virus replication. *Sci Rep* **2018**, *8*, 5960, doi:10.1038/s41598-018-24448-2.
36. Pauli, E.K.; Schmolke, M.; Hofmann, H.; Ehrhardt, C.; Flory, E.; Munk, C.; Ludwig, S. High level expression of the anti-retroviral protein APOBEC3G is induced by influenza A virus but does not confer antiviral activity. *Retrovirology* **2009**, *6*, 38, doi:10.1186/1742-4690-6-38.
37. Fehrholz, M.; Kendl, S.; Prifert, C.; Weissbrich, B.; Lemon, K.; Rennick, L.; Duprex, P.W.; Rima, B.K.; Koning, F.A.; Holmes, R.K.; et al. The innate antiviral factor APOBEC3G targets replication of measles, mumps and respiratory syncytial viruses. *J Gen Virol* **2012**, *93*, 565-576, doi:10.1099/vir.0.038919-0.
38. Wang, R.; Hozumi, Y.; Yin, C.; Wei, G.W. Mutations on COVID-19 diagnostic targets. *Genomics* **2020**, doi:10.1016/j.ygeno.2020.09.028.
39. Simmonds, P. Rampant C-->U Hypermutation in the Genomes of SARS-CoV-2 and Other Coronaviruses: Causes and Consequences for Their Short- and Long-Term Evolutionary Trajectories. *mSphere* **2020**, *5*, doi:10.1128/mSphere.00408-20.
40. Nakata, Y.; Ode, H.; Kubota, M.; Kasahara, T.; Matsuoka, K.; Sugimoto, A.; Imahashi, M.; Yokomaku, Y.; Iwatani, Y. Cellular APOBEC3A deaminase drives mutations in the SARS-CoV-2 genome. *Nucleic Acids Res* **2023**, *51*, 783-795, doi:10.1093/nar/gkac1238.
41. Di Giorgio, S.; Martignano, F.; Torcia, M.G.; Mattiuz, G.; Conticello, S.G. Evidence for host-dependent RNA editing in the transcriptome of SARS-CoV-2. *Sci Adv* **2020**, *6*, eabb5813, doi:10.1126/sciadv.abb5813.
42. Lythgoe, K.A.; Hall, M.; Ferretti, L.; de Cesare, M.; MacIntyre-Cockett, G.; Trebes, A.; Andersson, M.; Otecko, N.; Wise, E.L.; Moore, N.; et al. SARS-CoV-2 within-host diversity and transmission. *Science* **2021**, *372*, doi:10.1126/science.abg0821.
43. Kosuge, M.; Furusawa-Nishii, E.; Ito, K.; Saito, Y.; Ogasawara, K. Point mutation bias in SARS-CoV-2 variants results in increased ability to stimulate inflammatory responses. *Sci Rep* **2020**, *10*, 17766, doi:10.1038/s41598-020-74843-x.
44. Siqueira, J.D.; Goes, L.R.; Alves, B.M.; de Carvalho, P.S.; Cicala, C.; Arthos, J.; Viola, J.P.B.; de Melo, A.C.; Soares, M.A. SARS-CoV-2 genomic analyses in cancer patients reveal elevated intrahost genetic diversity. *Virus Evol* **2021**, *7*, veab013, doi:10.1093/ve/veab013.
45. Kim, K.; Calabrese, P.; Wang, S.; Qin, C.; Rao, Y.; Feng, P.; Chen, X.S. The roles of APOBEC-mediated RNA editing in SARS-CoV-2 mutations, replication and fitness. *Sci Rep* **2022**, *12*, 14972, doi:10.1038/s41598-022-19067-x.
46. Ikeda, T.; Molan, A.M.; Jarvis, M.C.; Carpenter, M.A.; Salamango, D.J.; Brown, W.L.; Harris, R.S. HIV-1 restriction by endogenous APOBEC3G in the myeloid cell line THP-1. *J Gen Virol* **2019**, *100*, 1140-1152, doi:10.1099/jgv.0.001276.
47. Browne, E.P.; Allers, C.; Landau, N.R. Restriction of HIV-1 by APOBEC3G is cytidine deaminase-dependent. *Virology* **2009**, *387*, 313-321, doi:10.1016/j.virol.2009.02.026.
48. Anderson, B.D.; Ikeda, T.; Moghadasi, S.A.; Martin, A.S.; Brown, W.L.; Harris, R.S. Natural APOBEC3C variants can elicit differential HIV-1 restriction activity. *Retrovirology* **2018**, *15*, 78, doi:10.1186/s12977-018-0459-5.
49. Gillick, K.; Pollpeter, D.; Phalora, P.; Kim, E.Y.; Wolinsky, S.M.; Malim, M.H. Suppression of HIV-1 infection by APOBEC3 proteins in primary human CD4(+) T cells is associated with inhibition of processive reverse transcription as well as excessive cytidine deamination. *J Virol* **2013**, *87*, 1508-1517, doi:10.1128/JVI.02587-12.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 June 2024 doi:10.20944/preprints202406.1182.v1

50. Ikeda, T.; Symeonides, M.; Albin, J.S.; Li, M.; Thali, M.; Harris, R.S. HIV-1 adaptation studies reveal a novel Env-mediated homeostasis mechanism for evading lethal hypermutation by APOBEC3G. *PLoS Pathog* **2018**, *14*, e1007010, doi:10.1371/journal.ppat.1007010.
51. Bishop, K.N.; Verma, M.; Kim, E.Y.; Wolinsky, S.M.; Malim, M.H. APOBEC3G inhibits elongation of HIV-1 reverse transcripts. *PLoS Pathog* **2008**, *4*, e1000231, doi:10.1371/journal.ppat.1000231.
52. Shaban, N.M.; Shi, K.; Lauer, K.V.; Carpenter, M.A.; Richards, C.M.; Salamango, D.; Wang, J.; Lopresti, M.W.; Banerjee, S.; Levin-Klein, R.; et al. The Antiviral and Cancer Genomic DNA Deaminase APOBEC3H Is Regulated by an RNA-Mediated Dimerization Mechanism. *Mol Cell* **2018**, *69*, 75-86 e79, doi:10.1016/j.molcel.2017.12.010.
53. Iwatani, Y.; Chan, D.S.; Wang, F.; Stewart-Maynard, K.; Sugiura, W.; Gronenborn, A.M.; Rouzina, I.; Williams, M.C.; Musier-Forsyth, K.; Levin, J.G. Deaminase-independent inhibition of HIV-1 reverse transcription by APOBEC3G. *Nucleic Acids Res* **2007**, *35*, 7096-7108, doi:10.1093/nar/gkm750.
54. Li, X.Y.; Guo, F.; Zhang, L.; Kleiman, L.; Cen, S. APOBEC3G inhibits DNA strand transfer during HIV-1 reverse transcription. *J Biol Chem* **2007**, *282*, 32065-32074, doi:10.1074/jbc.M703423200.
55. Belanger, K.; Savoie, M.; Rosales Gerpe, M.C.; Couture, J.F.; Langlois, M.A. Binding of RNA by APOBEC3G controls deamination-independent restriction of retroviruses. *Nucleic Acids Res* **2013**, *41*, 7438-7452, doi:10.1093/nar/gkt527.
56. Nguyen, D.H.; Gummuluru, S.; Hu, J. Deamination-independent inhibition of hepatitis B virus reverse transcription by APOBEC3G. *J Virol* **2007**, *81*, 4465-4472, doi:10.1128/JVI.02510-06.
57. Narvaiza, I.; Linfesty, D.C.; Greener, B.N.; Hakata, Y.; Pintel, D.J.; Logue, E.; Landau, N.R.; Weitzman, M.D. Deaminase-independent inhibition of parvoviruses by the APOBEC3A cytidine deaminase. *PLoS Pathog* **2009**, *5*, e1000439, doi:10.1371/journal.ppat.1000439.
58. Stavrou, S.; Zhao, W.; Blouch, K.; Ross, S.R. Deaminase-Dead Mouse APOBEC3 Is an In Vivo Retroviral Restriction Factor. *J Virol* **2018**, *92*, doi:10.1128/JVI.00168-18.
59. Boi, S.; Kolokithas, A.; Shepard, J.; Linwood, R.; Rosenke, K.; Van Dis, E.; Malik, F.; Evans, L.H. Incorporation of mouse APOBEC3 into murine leukemia virus virions decreases the activity and fidelity of reverse transcriptase. *J Virol* **2014**, *88*, 7659-7662, doi:10.1128/JVI.00967-14.
60. Wang, X.; Ao, Z.; Chen, L.; Kobinger, G.; Peng, J.; Yao, X. The cellular antiviral protein APOBEC3G interacts with HIV-1 reverse transcriptase and inhibits its function during viral replication. *J Virol* **2012**, *86*, 3777-3786, doi:10.1128/JVI.06594-11.
61. Manjunath, L.; Oh, S.; Ortega, P.; Bouin, A.; Bournique, E.; Sanchez, A.; Martensen, P.M.; Auerbach, A.A.; Becker, J.T.; Seldin, M.; et al. APOBEC3B drives PKR-mediated translation shutdown and protects stress granules in response to viral infection. *Nat Commun* **2023**, *14*, 820, doi:10.1038/s41467-023-36445-9.
62. Chiu, Y.L.; Witkowska, H.E.; Hall, S.C.; Santiago, M.; Soros, V.B.; Esnault, C.; Heidmann, T.; Greene, W.C. High-molecular-mass APOBEC3G complexes restrict Alu retrotransposition. *Proc Natl Acad Sci U S A* **2006**, *103*, 15588-15593, doi:10.1073/pnas.0604524103.
63. Kozak, S.L.; Marin, M.; Rose, K.M.; Bystrom, C.; Kabat, D. The anti-HIV-1 editing enzyme APOBEC3G binds HIV-1 RNA and messenger RNAs that shuttle between polysomes and stress granules. *J Biol Chem* **2006**, *281*, 29105-29119, doi:10.1074/jbc.M601901200.
64. Gallois-Montbrun, S.; Kramer, B.; Swanson, C.M.; Byers, H.; Lynham, S.; Ward, M.; Malim, M.H. Antiviral protein APOBEC3G localizes to ribonucleoprotein complexes found in P bodies and stress granules. *J Virol* **2007**, *81*, 2165-2178, doi:10.1128/JVI.02287-06.
65. Niewiadomska, A.M.; Tian, C.; Tan, L.; Wang, T.; Sarkis, P.T.; Yu, X.F. Differential inhibition of long interspersed element 1 by APOBEC3 does not correlate with high-molecular-mass-complex formation or P-body association. *J Virol* **2007**, *81*, 9577-9583, doi:10.1128/JVI.02800-06.
66. Soros, V.B.; Yonemoto, W.; Greene, W.C. Newly synthesized APOBEC3G is incorporated into HIV virions, inhibited by HIV RNA, and subsequently activated by RNase H. *PLoS Pathog* **2007**, *3*, e15, doi:10.1371/journal.ppat.0030015.
67. Gallois-Montbrun, S.; Holmes, R.K.; Swanson, C.M.; Fernandez-Ocana, M.; Byers, H.L.; Ward, M.A.; Malim, M.H. Comparison of cellular ribonucleoprotein complexes associated with the APOBEC3F and APOBEC3G antiviral proteins. *J Virol* **2008**, *82*, 5636-5642, doi:10.1128/JVI.00287-08.
68. Xiao, X.; Yang, H.; Arutiunian, V.; Fang, Y.; Besse, G.; Morimoto, C.; Zirkle, B.; Chen, X.S. Structural determinants of APOBEC3B non-catalytic domain for molecular assembly and catalytic regulation. *Nucleic Acids Res* **2017**, *45*, 7494-7506, doi:10.1093/nar/gkx362.
69. Shirakawa, K.; Takaori-Kondo, A.; Yokoyama, M.; Izumi, T.; Matsui, M.; Io, K.; Sato, T.; Sato, H.; Uchiyama, T. Phosphorylation of APOBEC3G by protein kinase A regulates its interaction with HIV-1 Vif. *Nat Struct Mol Biol* **2008**, *15*, 1184-1191, doi:10.1038/nsmb.1497.
70. Maeda, K.; Almofty, S.A.; Singh, S.K.; Eid, M.M.; Shimoda, M.; Ikeda, T.; Koito, A.; Pham, P.; Goodman, M.F.; Sakaguchi, N. GANP Interacts with APOBEC3G and Facilitates Its Encapsidation into the Virions To Reduce HIV-1 Infectivity. *J Immunol* **2013**, *191*, 6030-6039, doi:10.4049/jimmunol.1302057.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 June 2024 doi:10.20944/preprints202406.1182.v1

71. Berger, G.; Durand, S.; Fargier, G.; Nguyen, X.N.; Cordeil, S.; Bouaziz, S.; Muriaux, D.; Darlix, J.L.; Cimarelli, A. APOBEC3A is a specific inhibitor of the early phases of HIV-1 infection in myeloid cells. *PLoS Pathog* **2011**, *7*, e1002221, doi:10.1371/journal.ppat.1002221.
72. Matsuyama, S.; Nao, N.; Shirato, K.; Kawase, M.; Saito, S.; Takayama, I.; Nagata, N.; Sekizuka, T.; Katoh, H.; Kato, F.; et al. Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. *Proc Natl Acad Sci U S A* **2020**, *117*, 7001-7003, doi:10.1073/pnas.2002589117.
73. Fukuda, M.; Islam, M.S.; Shimizu, R.; Nasser, H.; Rabin, N.N.; Takahashi, Y.; Sekine, Y.; Lindoy, L.F.; Fukuda, T.; Ikeda, T.; et al. Lethal Interactions of SARS-CoV-2 with Graphene Oxide: Implications for COVID-19 Treatment. *Acs Appl Nano Mater* **2021**, *4*, 11881-11887, doi:10.1021/acsanm.1c02446.
74. Begum, M.M.; Ichihara, K.; Takahashi, O.; Nasser, H.; Jonathan, M.; Tokunaga, K.; Yoshida, I.; Nagashima, M.; Sadamasu, K.; Yoshimura, K.; et al. Virological characteristics correlating with SARS-CoV-2 spike protein fusogenicity. *Frontiers in Virology* **2024**, *4*, doi:10.3389/fviro.2024.1353661.
75. Suzuki, R.; Yamasoba, D.; Kimura, I.; Wang, L.; Kishimoto, M.; Ito, J.; Morioka, Y.; Nao, N.; Nasser, H.; Uriu, K.; et al. Attenuated fusogenicity and pathogenicity of SARS-CoV-2 Omicron variant. *Nature* **2022**, *603*, 700-705, doi:10.1038/s41586-022-04462-1.
76. Kimura, I.; Yamasoba, D.; Tamura, T.; Nao, N.; Suzuki, T.; Oda, Y.; Mitoma, S.; Ito, J.; Nasser, H.; Zahradnik, J.; et al. Virological characteristics of the SARS-CoV-2 Omicron BA.2 subvariants, including BA.4 and BA.5. *Cell* **2022**, *185*, 3992-4007 e3916, doi:10.1016/j.cell.2022.09.018.
77. Saito, A.; Tamura, T.; Zahradnik, J.; Deguchi, S.; Tabata, K.; Anraku, Y.; Kimura, I.; Ito, J.; Yamasoba, D.; Nasser, H.; et al. Virological characteristics of the SARS-CoV-2 Omicron BA.2.75 variant. *Cell Host Microbe* **2022**, doi:10.1016/j.chom.2022.10.003.
78. Islam, M.S.; Rabin, N.N.; Begum, M.M.; Goto, N.; Tagawa, R.; Nagashima, M.; Sadamasu, K.; Yoshimura, K.; Sekine, Y.; Ikeda, T.; et al. SARS-CoV-2 inactivation: Assessing the efficacy of GO-anchored filters versus various commercial masks. *RSC Appl Interfaces* **2024**, doi:10.1039/d3lf00250k.
79. Islam, M.S.; Fukuda, M.; Hossain, M.J.; Rabin, N.N.; Tagawa, R.; Nagashima, M.; Sadamasu, K.; Yoshimura, K.; Sekine, Y.; Ikeda, T.; et al. SARS-CoV-2 suppression depending on the pH of graphene oxide nanosheets. *Nanoscale Adv* **2023**, *5*, 2413-2417, doi:10.1039/d3na00084b.
80. Tamura, T.; Ito, J.; Uriu, K.; Zahradnik, J.; Kida, I.; Anraku, Y.; Nasser, H.; Shofa, M.; Oda, Y.; Lytras, S.; et al. Virological characteristics of the SARS-CoV-2 XBB variant derived from recombination of two Omicron subvariants. *Nat Commun* **2023**, *14*, 2800, doi:10.1038/s41467-023-38435-3.
81. Ito, J.; Suzuki, R.; Uriu, K.; Itakura, Y.; Zahradnik, J.; Kimura, K.T.; Deguchi, S.; Wang, L.; Lytras, S.; Tamura, T.; et al. Convergent evolution of SARS-CoV-2 Omicron subvariants leading to the emergence of BQ.1.1 variant. *Nat Commun* **2023**, *14*, 2671, doi:10.1038/s41467-023-38188-z.
82. Reed, L.J.; Muench, H. A simple method of estimating fifty per cent endopoints. *Am J Epidemiol* **1938**, *27*, 493-497, doi:10.1093/oxfordjournals.aje.a118408.
83. Refsland, E.W.; Stenglein, M.D.; Shindo, K.; Albin, J.S.; Brown, W.L.; Harris, R.S. Quantitative profiling of the full APOBEC3 mRNA repertoire in lymphocytes and tissues: implications for HIV-1 restriction. *Nucleic Acids Res* **2010**, *38*, 4274-4284, doi:gkq174 [pii]
84. 10.1093/nar/gkq174.
85. Burns, M.B.; Lackey, L.; Carpenter, M.A.; Rathore, A.; Land, A.M.; Leonard, B.; Refsland, E.W.; Kotandeniya, D.; Tretyakova, N.; Nikas, J.B.; et al. APOBEC3B is an enzymatic source of mutation in breast cancer. *Nature* **2013**, *494*, 366-370, doi:10.1038/nature11881.
86. Motozono, C.; Toyoda, M.; Zahradnik, J.; Saito, A.; Nasser, H.; Tan, T.S.; Ngare, I.; Kimura, I.; Uriu, K.; Kosugi, Y.; et al. SARS-CoV-2 spike L452R variant evades cellular immunity and increases infectivity. *Cell Host Microbe* **2021**, *29*, 1124-1136 e1111, doi:10.1016/j.chom.2021.06.006.
87. Meng, B.; Abdullahi, A.; Ferreira, I.; Goonawardane, N.; Saito, A.; Kimura, I.; Yamasoba, D.; Gerber, P.P.; Fatihi, S.; Rathore, S.; et al. Altered TMPRSS2 usage by SARS-CoV-2 Omicron impacts infectivity and fusogenicity. *Nature* **2022**, *603*, 706-714, doi:10.1038/s41586-022-04474-x.
88. Kimura, I.; Yamasoba, D.; Nasser, H.; Ito, H.; Zahradnik, J.; Wu, J.; Fujita, S.; Uriu, K.; Sasaki, J.; Tamura, T.; et al. Multiple mutations of SARS-CoV-2 Omicron BA.2 variant orchestrate its virological characteristics. *J Virol* **2023**.
89. Tamura, T.; Irie, T.; Deguchi, S.; Yajima, H.; Tsuda, M.; Nasser, H.; Mizuma, K.; Plianchaisuk, A.; Suzuki, S.; Uriu, K.; et al. Virological characteristics of the SARS-CoV-2 Omicron XBB.1.5 variant. *Nat Commun* **2024**, *15*, 1176, doi:10.1038/s41467-024-45274-3.
90. Tamura, T.; Mizuma, K.; Nasser, H.; Deguchi, S.; Padilla-Blanco, M.; Oda, Y.; Uriu, K.; Tolentino, J.E.M.; Tsujino, S.; Suzuki, R.; et al. Virological characteristics of the SARS-CoV-2 BA.2.86 variant. *Cell Host Microbe* **2024**, *32*, 170-180 e112, doi:10.1016/j.chom.2024.01.001.
91. Kimura, I.; Yamasoba, D.; Nasser, H.; Zahradnik, J.; Kosugi, Y.; Wu, J.; Nagata, K.; Uriu, K.; Tanaka, Y.L.; Ito, J.; et al. The SARS-CoV-2 spike S375F mutation characterizes the Omicron BA.1 variant. *iScience* **2022**, *25*, 105720, doi:10.1016/j.isci.2022.105720.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 June 2024 doi:10.20944/preprints202406.1182.v1

92. Saito, A.; Irie, T.; Suzuki, R.; Maemura, T.; Nasser, H.; Uriu, K.; Kosugi, Y.; Shirakawa, K.; Sadamasu, K.; Kimura, I.; et al. Enhanced fusogenicity and pathogenicity of SARS-CoV-2 Delta P681R mutation. *Nature* **2022**, *602*, 300-306, doi:10.1038/s41586-021-04266-9.
93. Rajib, S.A.; Ogi, Y.; Hossain, M.B.; Ikeda, T.; Tanaka, E.; Kawaguchi, T.; Satou, Y. A SARS-CoV-2 Delta variant containing mutation in the probe binding region used for RT-qPCR test in Japan exhibited atypical PCR amplification and might induce false negative result. *J Infect Chemother* **2022**, *28*, 669-677, doi:10.1016/j.jiac.2022.01.019.
94. Kumar, S.; Tao, Q.; Weaver, S.; Sanderford, M.; Caraballo-Ortiz, M.A.; Sharma, S.; Pond, S.L.K.; Miura, S. An Evolutionary Portrait of the Progenitor SARS-CoV-2 and Its Dominant Offshoots in COVID-19 Pandemic. *Mol Biol Evol* **2021**, *38*, 3046-3059, doi:10.1093/molbev/msab118.
95. Alexandrov, L.B.; Nik-Zainal, S.; Wedge, D.C.; Campbell, P.J.; Stratton, M.R. Deciphering signatures of mutational processes operative in human cancer. *Cell Rep* **2013**, *3*, 246-259, doi:10.1016/j.celrep.2012.12.008.
96. Lamb, K.D.; Luka, M.M.; Saathoff, M.; Orton, R.J.; Phan, M.V.T.; Cotten, M.; Yuan, K.; Robertson, D.L. Mutational signature dynamics indicate SARS-CoV-2's evolutionary capacity is driven by host antiviral molecules. *PLoS Comput Biol* **2024**, *20*, e1011795, doi:10.1371/journal.pcbi.1011795.
97. Zhu, B.; Xiao, Y.; Yeager, M.; Clifford, G.; Wentzensen, N.; Cullen, M.; Boland, J.F.; Bass, S.; Steinberg, M.K.; Raine-Bennett, T.; et al. Mutations in the HPV16 genome induced by APOBEC3 are associated with viral clearance. *Nat Commun* **2020**, *11*, 886, doi:10.1038/s41467-020-14730-1.
98. Alexandrov, L.B.; Kim, J.; Haradhvala, N.J.; Huang, M.N.; Tian Ng, A.W.; Wu, Y.; Boot, A.; Covington, K.R.; Gordenin, D.A.; Bergstrom, E.N.; et al. The repertoire of mutational signatures in human cancer. *Nature* **2020**, *578*, 94-101, doi:10.1038/s41586-020-1943-3.

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