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Kyoto University
ASK1 restores the antiviral activity of APOBEC3G by disrupting HIV-1 Vif-mediated counteraction

Kei Miyakawa1, Satoko Matsunaga1, Kazuhiko Kanou2, Atsushi Matsuawa3, Ryo Morishita1,4, Ayumi Kudoh1, Keisuke Shindo5, Masaru Yokoyama6, Hironori Sato6, Hirokazu Kimura2, Tomohiko Tamura7, Naoki Yamamoto8, Hidenori Ichijo3, Akifumi Takaori-Kondo5 & Akihide Ryo1

APOBEC3G (A3G) is an innate antiviral restriction factor that strongly inhibits the replication of human immunodeficiency virus type 1 (HIV-1). An HIV-1 accessory protein, Vif, hijacks the host ubiquitin–proteasome system to execute A3G degradation. Identification of the host pathways that obstruct the action of Vif could provide a new strategy for blocking viral replication. We demonstrate here that the host protein ASK1 (apoptosis signal-regulating kinase 1) interferes with the counteraction by Vif and revitalizes A3G-mediated viral restriction. ASK1 binds the BC-box of Vif, thereby disrupting the assembly of the Vif–ubiquitin ligase complex. Consequently, ASK1 stabilizes A3G and promotes its incorporation into viral particles, ultimately reducing viral infectivity. Furthermore, treatment with the antiretroviral drug AZT (zidovudine) induces ASK1 expression and restores the antiviral activity of A3G in HIV-1-infected cells. This study thus demonstrates a distinct function of ASK1 in restoring the host antiviral system that can be enhanced by AZT treatment.
The innate immune system is an evolutionarily conserved network that acts as a first-line defense against invading microbial pathogens and other potential threats to host cells. In addition to the nonspecific or broadly specific counteraction exerted by the physiological component of innate immunity, a more specific response is exerted by intracellular restriction factors, which belong to a group of interferon-stimulated genes. When interferons induce their transcription, restriction factors limit the replication of invading viruses. One such factor is an editing enzyme for nucleic acids, APOBEC3G (apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G, hereafter referred to as A3G). This protein severely restricts the replication of numerous viruses, including human immunodeficiency virus type 1 (HIV-1) and hepatitis B virus, by extensively deaminating cytosine residues in the viral genome during reverse transcription. This process introduces unnatural (cytosine-to-uracil) mutations in the minus-strand viral DNA, leading to either the destabilization of reverse transcripts or the production of defective viral proteins. A3G appears to inhibit the elongation of reverse transcripts by deaminase-independent mechanisms.

Although A3G is a potent antiviral molecule, HIV-1 has developed a specific accessory protein, Vif, which can counteract the antiviral activity of A3G. In infected cells, Vif forms an ubiquitin ligase complex with Cullin5 (CUL5), Elonging B/C (ELOB/C) and CBFβ that ubiquitinates and degrades A3G. In HIV-1 isolates lacking the Vif gene, A3G is efficiently incorporated into virions by interacting with viral nucleocapsid protein and viral RNA, severely limiting viral replication in the viral genome during reverse transcription. This process introduces unnatural (cytosine-to-uracil) mutations in the minus-strand viral DNA, leading to either the destabilization of reverse transcripts or the production of defective viral proteins. A3G appears to inhibit the elongation of reverse transcripts by deaminase-independent mechanisms.

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that ASK1 reduced the E3 ligase activity of Vif (including the autoubiquitination of Vif), which was accompanied by a reduction in the Vif–ELOC interaction (Fig. 2f). Moreover, ASK1 markedly reduced Vif ubiquitination of A3G (Fig. 2g). Although Mehle et al.\textsuperscript{33} have reported previously that phosphorylation of the Vif BC-box negatively regulates
Figure 2 | ASK1 inhibits the formation of Vif-E3 ubiquitin ligase complex. (a) The C-terminal domain (CT) of ASK1 is sufficient to impair Vif-mediated A3G degradation. HEK293 cells were cotransfected with plasmids encoding XP-ASK1 (500 ng), HA-A3G (10 ng) and Vif (50 or 100 ng). Protein expression was then detected using western blot analysis. The numerical values below the blot indicate the amounts of HA-A3G determined with densitometry. (b) The structural model of ASK1 CT (yellow) was generated and subjected to docking simulation with Vif (green, PDB: 4N9F). ELOC (purple) was overlaid onto the Vif–ASK1 model. The square shows the predicted inhibition by ASK1 of ELOC binding to the BC-Box of Vif. (c) The BC-box motif of Vif is important for the binding of ASK1. HEK293 cells were cotransfected with plasmids encoding ASK1-HA and Vif or the indicated mutants. Cell lysates were immunoprecipitated using an anti-Myc antibody. (d) Pull down with streptavidin beads

Vif–ELOB/C complex assembly, we could not detect any Vif phosphorylation by ASK1 (Supplementary Fig. 4). Our data suggest that ASK1 interacts with the BC-box motif of Vif and inhibits the formation of the E3 complex by interfering with the interaction between Vif and ELOB/C.

Nef does not affect ASK1-mediated Vif inactivation. Previous studies have demonstrated that ASK1 potently associates with Nef, another HIV accessory protein. Nef reduces the kinase activity of ASK1 to prevent tumour necrosis factor-α- and FAS-dependent apoptosis. We thus investigated whether Nef affects
ASK1-mediated Vif inactivation. Immunoblotting analysis revealed that Nef overexpression inhibited the autophosphorylation of ASK1 (phosphorylated Thr845 of ASK1), a hallmark of its kinase activity (Fig. 3a). Notably, irrespective of Nef expression, our data also showed that ASK1 effectively inhibited Vif-mediated A3G degradation (Fig. 3b), suggesting that the kinase activity of ASK1 is dispensable for its ability to inhibit Vif. Moreover, a kinase-negative (K709M) mutant of ASK1 also inhibited Vif-mediated A3G degradation, although a constitutively kinase-active ASK1 (ΔN) mutant exhibited a slightly higher ability to inhibit Vif via an unknown mechanism (Fig. 3c,d). These results suggest that ASK1 kinase activity is dispensable for, but has an additive effect in, inhibiting the function of Vif. This is indicative of the involvement of multiple mechanisms in the ASK1-mediated inhibition of Vif.

**ASK1 restricts HIV-1 replication via A3G reactivation.** To test whether ASK1 regulates viral infectivity by interfering with Vif function and stabilizing A3G, we performed a single-cycle viral infection assay using HIV-1NL4-3 and its Vif-deficient mutant virus collected from ASK1-expressing cell supernatants. Immunoblotting analysis of cell lysates and viral supernatants revealed that the expression of either ASK1 or its ΔN mutant suppressed the Vif-mediated degradation of A3G in cells, increasing the amount of A3G in virions to that seen with a Vif-deficient virus (Fig. 4a,b). Consistent with this result, the infectivity of viruses harvested from either ASK1- or ASK1 AN-overexpressing cells was much lower than that of control cells (Fig. 4c). Since the endogenous ASK1 levels in T cells were nearly undetectable in the normal state (Fig. 4d), we generated stable cell lines, CEM (A3G-positive) and CEMSS (A3G-negative), harbouring a tetracycline-inducible ASK1 gene, referred to hereafter as CEM-TetON-ASK1 and CEMSS-TetON-ASK1, respectively. Treatment with a tetracycline antibiotic, doxycycline (Dox), induced the expression of ASK1 in both cell lines at the physiological levels seen in PMA-treated 293 cells (Fig. 4d). Notably, Dox-induced ASK1 inhibited HIV-1 replication in CEM-TetON-ASK1 but not in CEMSS-TetON-ASK1 cells (Fig. 4e). Moreover, immunoblotting analysis demonstrated that the A3G level in virions was increased only in Dox-treated CEM-TetON-ASK1 cells (Fig. 4f). In addition, the number of G-to-A hypermutations in the viral genomes was markedly increased in these cells (Fig. 4g). Taken together, our data suggest that ASK1 restricts the replication of HIV-1 by promoting A3G incorporation into virions in human CD4+ T cells.

**AZT induces ASK1 and promotes the antiviral activity of A3G.** Generally, MAP3Ks act as stress-responsive kinases that quickly activate downstream cascades by sensing various stimuli such as cytokines, hormones and anticancer drugs. We wished to evaluate the pathophysiological significance of ASK1-mediated antiviral activity. Initially, we assessed whether approved antiretroviral drugs could induce ASK1 activity. Interestingly, the reverse transcriptase inhibitors azidothymidine/zidovudine (AZT) were found to induce ASK1 expression in peripheral blood mononuclear cells (PBMCs) and in the H9 CD4+ T-cell line.

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**Figure 3 | Nef does not affect ASK1-mediated Vif inactivation.** (a) Nef inhibits the autophosphorylation of ASK1. HEK293 cells were cotransfected with plasmids encoding XP-ASK1 (100 ng) and Nef-FLAG (100 or 200 ng). Cell lysates were subjected to western blot analysis using the indicated antibodies. (b) Nef does not alter the effect of ASK1 on Vif-mediated A3G degradation. HEK293 cells were cotransfected with plasmids encoding XP-ASK1 (500 ng), HA-A3G (10 ng), Vif (50 or 100 ng) and Nef-FLAG (1 μg). Protein expression was detected by western blot. The numerical values below the blot indicate the amounts of HA-A3G determined with densitometry. (c) HEK293 cells were transfected with the indicated ASK1 mutants (ΔN, constitutively active; K709M, kinase-dead). Cell lysates were subjected to western blotting against the indicated antibodies. (d) HEK293 cells were cotransfected with plasmids encoding HA-A3G (10 ng), Vif (50 or 100 ng) and with wild-type ASK1 or one of its kinase mutants (500 ng). Protein expression was detected by western blot. The numerical values below the blot indicate the amounts of HA-A3G as determined by densitometry. Full images for all western blots analysis are shown in Supplementary Fig. 5.
Figure 4 | ASK1 restricts HIV-1 replication via A3G reactivation. (a-c) ASK1 expression in virus-producing cells promotes A3G incorporation into virions and reduces infectivity. HEK293 cells were cotransfected with an HIV-1 molecular clone carrying a GFP reporter gene (pNL4-3ΔEnvΔVif-GFP) or its Vif-deficient mutant (pNL4-3ΔEnvΔVif-GFP) together with a plasmid encoding VSV-G, XP-ASK1 and HA-A3G. (a, b) Forty-eight hours after transfection, cell lysates and supernatants were harvested and analysed by western blotting against the indicated antibodies. The bar chart in (b) indicates the amounts of HA-A3G normalized by p24 levels in virions, as determined by densitometric analysis of western blots (n = 3, mean ± s.d.). (c) The CD4+ T-cell line (M8166) was infected with harvested and normalized virus for 2 days and infected (GFP-positive) cells were then measured by flow cytometry (n = 3, mean ± s.d.). *P < 0.05; **P < 0.01, two-tailed unpaired t-test. (d) Expression levels of ASK1 in DOX-treated or untreated CEM-TetON-ASK1 and CEMSS-TetON-ASK1 cells. 293 cells transfected with ASK1 or treated with PMA are shown as positive controls. (e) Forty-eight hours after transfection, cell lysates and supernatants were harvested and analysed by western blotting against the indicated antibodies. The bar chart in (b) indicates the amounts of HA-A3G normalized by p24 levels in virions, as determined by densitometric analysis of western blots (n = 3, mean ± s.d.). *P < 0.05; **P < 0.01, two-tailed unpaired t-test. (f) The incorporation of A3G into virions from indicated cells (at 8 d.p.i.) was detected by western blot. (g) The infected cells (CEM-TetON-ASK1) were harvested at 8 d.p.i. and subjected to G-to-A hypermutation analysis (n = 8, mean ± s.d.). **P < 0.01, two-tailed unpaired t-test. Full images for all western blots analysis are shown in Supplementary Fig. 6. d.p.i., days post infection; ELISA, enzyme-linked immunosorbent assay.

(5a,b). A previous report has indicated that the maximum serum AZT concentration after oral administration is ~10 μM (ref. 37). We next assessed the possibility that AZT treatment at physiological concentrations would activate the ASK1–A3G axis in HIV-infected cells. H9 cells were transfected with either ASK1-targeted short interfering RNA (siRNA) or control siRNA and then infected with HIV. At 2 days after infection, the cells were washed and additionally cultured for 24 h at the presence or absence of AZT at 10 μM (Fig. 5c). In this experiment, we used an AZT-resistant virus harbouring reverse transcriptase mutations (T69G, K70R, L74I, K103N, T215F and K219Q)38,39 to minimize the carry-over effect of AZT from the cell culture supernatants of producer cells. We found that A3G incorporation into virions was enhanced by transient AZT treatment of control cells, but this was not the case in ASK1-depleted cells (Fig. 5c). In accordance with the results for A3G amounts in virions, the infectivity of viruses derived from AZT-treated cells was significantly reduced; this reduction was blocked by ASK1 depletion in virus-producer
AZT can promote the antiviral activity of A3G by inducing ASK1. Taken together, our data show that AZT can promote the antiviral activity of A3G by inducing ASK1.

**Discussion**

We here demonstrate that ASK1 is an AZT-inducible host factor that negatively regulates the Vif-mediated degradation of A3G to restore intrinsic antiviral immunity to HIV-1 (a proposed model is depicted in Fig. 6). Since our preliminary studies have suggested that several external stimuli can inhibit Vif-mediated A3G degradation, we here targeted human protein kinases as responders to the external stimuli that regulate the functionality of Vif. A fluorescence-based screen ultimately identified ASK1 as acting as an ‘anti-Vif factor’ in terms of A3G protein stability. Indeed, cells overexpressing ASK1 showed a restored A3G antiviral function and rarely spread infectious viral particles in...
the secondary infection. Our study findings thus shed new light on the molecular link between ASK1 and Vif-mediated HIV-1 evasion of the host antiviral system and provide a better understanding of the role of a pre-existing antiretroviral drug in bolstering the host innate immune system.

Cellular regulatory mechanisms confer a sensitive, specific and robust response to external stimuli and initiate certain molecular events in cells. Such dynamic regulation is achieved through post-translational modifications (PTMs) including phosphorylation and ubiquitination. PTMs offer a dynamic way to regulate protein–protein interactions and protein activity, subcellular localization and stability. Thus, virus–host protein interactions can also be modulated by PTMs as a response to external stimuli such as chemicals, growth factors and cytokines. In this regard, Mehele et al. reported that the phosphorylation of Vif blocks assembly of the Vif–E3 complex. Moreover, the NEDD8 ubiquitin-like protein modification pathway also regulates the function of Vif and/or Vpx–E3 complex. Thus, many signalling pathways may antagonize the function of Vif or Vif–E3 complex to suppress Vif-mediated A3G degradation. Since the activity of protein kinases or ubiquitin ligases is basically regulated by the cellular context governed by external or internal signals, these results suggest that the functional interaction between viral accessory proteins and host restriction factors can be, at least in part, regulated by extracellular events.

ASK1 has been identified as MAP3K4 and is an effector of the external stimuli-triggered signalling that induces apoptosis. Concordant with the role of ASK1 in virus infection, ASK1 is involved in the apoptosis of cells infected by influenza A virus. Importantly, HIV-1 Nef is another ASK1-interacting protein that suppresses tumour necrosis factor-α-induced cell death in HIV-1-infected T-cell lines. Although Kumar et al. using a luciferase assay system, recently reported a dynamic interaction between Nef and ASK1, this interaction was only marginally detectable in our hands by conventional protein–protein interaction analysis. Our current analyses demonstrate an alternative role for ASK1 in HIV-1 infection, with ASK1 interacting with Vif and negatively modulating the function of Vif in terms of E3 ligase activity. Importantly, the kinase activity of ASK1 was found in our analysis to be dispensable for Vif counteraction because (i) the kinase-dead mutant of ASK1 could still inhibit Vif-mediated A3G degradation, and (ii) Nef, a suppressor of ASK1 kinase activity, did not affect the function of ASK1 directed against Vif. However, a constitutively active ASK1 mutant (AN) seems to have a larger inhibitory effect on Vif than its wild-type counterpart in cell-based assays. This may be the result of a higher Vif-binding affinity of AN ASK1 compared with wild type. In fact, our data show that ASK1 directly bound to the BC-box motif of Vif and blocked the interaction between Vif and ELOC/B. Consequently, assembly of the components of the Vif–E3 ubiquitin ligase complex failed to degrade A3G. Our computational docking simulation of the Vif–ASK1 interaction predicted that the binding interface for ASK1 within Vif belongs to a conserved region that is considered to be essential for formation of the E3 complex. ASK1 may exploit this weak point of Vif to effectively suppress its action.

We further found an unexpected ability of antiretroviral AZT to restore antiviral immunity by suppressing Vif function via ASK1. AZT is a chemical variant of the natural nucleoside thymidine formed by the addition of an azido group, and is a widely used nucleoside inhibitor that arrests reverse transcript synthesis of viral DNA. AZT is transported to mitochondria and affects mitochondrial metabolism, causing mitochondrial dysfunction that generates oxidative stress in cells. This internal stress may induce ASK1 to suppress the effects of Vif. Although the pathway(s) underlying ASK1 induction following AZT treatment are still unknown at present, ASK1 gene analysis in future studies may elucidate the molecular mechanisms by which ASK1 senses drugs.

In our current study, we mainly analysed the function of overexpressed ASK1 in 293 cells and T-cell lines. Since ASK1 is an apoptosis-regulating protein, it is not expressed or is expressed at very low levels in certain tissues and cells including PBMC and T cells at steady state. Our current data demonstrate that physiological concentrations of AZT stimulated the expression of ASK1 in T cells in which the ability of Vif to neutralize A3G was blocked (Fig. 5). Interestingly, this may not reflect a general effect of ASK1 to repress ELO/B/C-binding proteins, since the activity of VHL was not impaired by ASK1 (Supplementary Fig. 2). While our study shows a distinct polypharmacological effect of AZT, there are still many unanswered questions about the physiological role and clinical relevance of the ASK1–Vif interaction. To address these important questions, longitudinal research and fine-grained analysis should be conducted in the future.

In conclusion, we here demonstrate that ASK1 is a novel Vif-binding protein that negatively regulates Vif-mediated A3G degradation. The interaction between HIV-1 accessory proteins and host restriction factors is a potential target for the development of new antiviral drugs. Understanding the molecular mechanism of this interplay will provide new insights into the preservation of the intrinsic antiviral system and may be useful for the future therapeutic treatment of HIV infection.

**Methods**

**Plasmids.** Human MAP3Ks, ELO/B/C, CUL5 and CRBF genes were amplified from the Mammalian Gene Collection complementary DNA (cDNA) library and cloned into pcDNA-based vectors (Life Technologies, Gaithersburg, MD). The accession codes for the genes used are listed in Supplementary Table 1. HaeIII-digested pcDNA3.1+ plasmids were transformed into E. coli DH5α to obtain pBluescript II SK+ clones harbouring the desired inserts. Strain DH5α was used for colony PCR, followed by DNA extraction and sequencing.

**Cells and viruses.** HEK293, HEK293T (ATCC) and TZM-bl (NIH AIDS Reagent Program) cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS), MEM, CEMSS, H9, MEFs, and A59 cells. Cells were cultured in RPMI containing 10% fetal bovine serum. To generate CEM-Tet-ON ASK1 or CEMSS-Tet-ON ASK1 cells, parental cells were co-infected with VSV-G pseudotyped retroviral vectors expressing pRETRO-X-TREG-ASK1 and pRETRO-X-G418 (Clontech) and selected with G418 and puromycin. HIV-1 stocks were produced by transient transfection of HEK293T cells with the pNL4-3 (ref. 56) or AZT-resistant pNLGRINFQ (Clontech, Palo Alto, CA) to generate retroviral vector. Plasmids encoding HIV-1ΔNef, HIV-1ΔVif, human A3G and ubiquitin have been described. The Vif mutants were generated with PCR-based molecular cloning procedures. HIV-1ΔVif, Nef was amplified from a pNL4-3 molecular clone and subcloned into pX3FLAG-CMV-14 vector (Sigma-Aldrich, St Louis, MO). For in vitro protein synthesis, each cDNA was subcloned into pEUV vector (CellFree Sciences, Ehime, Japan).

**Ubiquitination and immunoprecipitation analysis.** HEK293 cells in six-well plates were transfected with vectors encoding XP-AS1 (500 ng), HA-A3G (50 ng), Vif (100 ng), and Myc-ubiquitin (2 µg) in (500 ng). Cells were washed with PBS and lysed with 2 µg of MGC12 (a proteasome inhibitor, Sigma-Aldrich) for 18 h before being harvested. At 48 h after transfection, cell lysates were subjected to 10% SDS-PAGE (plate 7A) containing protease inhibitor Complete mini (Roche Diagnostics, Basel, Switzerland). Cell lysates were immunoprecipitated with E28 (an anti-HA affinity gel) or 2 µg of anti-Vif antibody (Clone #319; NIH AIDS Reagent Program) 57,58 mixed with protein G sepharose (GE Healthcare, Healthcare, Little Chalfont, UK) and bound proteins were analysed by western blotting (Image 4A). The nitrocellulose membranes were probed with primary antibodies and horseradish peroxidase–conjugated secondary antibodies (GE Healthcare). The antibodies used including dilutions are listed in Supplementary Table 2. The proteins detected were visualized on a
FluorChem digital imaging system (Alpha Innotech, San Leandro, CA) and the band intensities were quantified with NIH ImageJ software.

**AlphaScreen and pull-down assays.** The wheat germ cell-free protein production with pEY vectors and AlphaScreen analysis has been described previously. In brief, DNA templates containing a biotin-ligating sequence or FLAG epitope were amplified by split-PCR with pEY-based vectors and corresponding primers and then used with the GenDecoder protein production system (CellFree Sciences). In this study, Vif proteins were co-expressed with untagged CBFI to stabilize the conformation of Vif protein. For the in vitro competitive pull-down assays, recombinant biotinylated Vif was pre-mixed with untagged ASK1 at a molar ratio of 1:1, 1:2, 1:4 or 1:8 for 5 min at room temperature and then mixed with equivalent amounts of FLAG-tagged CUL5, ELOB and ELOC proteins. After 1 h at 26°C, the mixture was processed for pull-down with streptavidin-coated magnetic beads (Merck Millipore). Bound proteins were detected with western blot analysis.

**Transfection-based single-round infection assays.** HEK293 cells in six-well plates were cotransfected with pNL4-3-3EAV-GFP or pNL4-3EAV-VIF-GFP (1 µg) and with vectors encoding VSV-G (400 ng), ASK1 (250 ng) and A3G (25 ng) and cultured for 2 days. The culture supernatants and cell lysates were subjected to western blotting analysis. The p24 antigens in supernatants were measured with an ELISA kit (Zepto Metrix, Buffalo, NY), and M8166 cells were infected with normalized virus (1 or 5 ng of p24 antigen) in 24-well plates for 24 h. Infectivity was calculated by counting the numbers of GFP-positive cells.

**Multicycle replication assays.** Cellular DNA from HIV-infected cells was extracted with a QIAamp DNA Mini Kit (Qiagen, Venlo, Netherlands) according to the manufacturer’s instructions. The extracted DNA was amplified using a primer pair HIV-1F (5'-AGGAGGCGCTGTAGATCTTAGCCACTT-3') and HIV-1R (5'-GGTGCTAGGGATCTCTAGTTAC-3') and with vector Vif proteins by competitive pull-down assays, recombinant biotinylated Vif was pre-mixed with untagged ASK1 at a molar ratio of 1:1, 1:2, 1:4 or 1:8 for 5 min at room temperature and then mixed with equivalent amounts of FLAG-tagged CUL5, ELOB and ELOC proteins. After 1 h at 26°C, the mixture was processed for pull-down with streptavidin-coated magnetic beads (Merck Millipore). Bound proteins were detected with western blot analysis.

**G-to-A hypermutation assays.** Cell-free translation was performed with 105 cells using Neon nucleofection system (Life Technologies) at 48 h before infection. Cells were infected with HIV-1NL4-3 (50 ng of p24 antigen) with or without 1 ng of p24 antigen and cultured for 18 h. DNA was isolated from infected cells and subjected to G-to-A hypermutation assays. The p24 antigens in supernatants were measured with an ELISA kit (Zepto Metrix, Buffalo, NY), and M8166 cells were infected with normalized virus (1 or 5 ng of p24 antigen) in 24-well plates for 24 h. Infectivity was calculated by counting the numbers of GFP-positive cells.

**Protein structure prediction and docking simulation.** The structural models for the CT domain of human ASK1 (ASK1 CT; 955–1374 amino acids) were obtained by I-TASSER software v2.1 (refs 64,65) or Molecular Operating Environment (MOE) (Chemical Computing Group, Montreal, Canada). To obtain the docking structure of Vif, this complex was subjected to the structural assembly simulations. To obtain the docking structure of Vif (PDB accession code 4N9F) and ASK1 CT, we used the docking simulation server ClusPro 2.0 (refs 66,67).

**Statistical analysis.** All graphs present the mean and s.d. The statistical significance of differences between two groups was tested by two-tailed unpaired t-test with Prism 6 software (GraphPad, La Jolla, CA). A P value of <0.05 was considered statistically significant.

**References**


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Author contributions

All authors contributed extensively to the work presented in this paper. K.M. designed the study, performed the experiments, analysed the data and wrote the manuscript. S.M. and R.K.M. performed the experiments and analysed the data. K.K., M.Y. and H.S. provided materials and discussed the data. A.K., H.K., T.T. and N.Y. analysed and performed the modelling and docking simulation analysis. A.M., K.S., H.I. and A.T.-K. performed the experiments and analysed the data. K.K., M.Y. and H.S. provided materials and discussed the data. A.R. designed and supervised the study, analysed the data and wrote the manuscript.

Additional information

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