SAMHD1 suppresses innate immune responses to viral infections and inflammatory stimuli by inhibiting the NF-κB and interferon pathways

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Sterile alpha motif and HD-domain–containing protein 1 (SAMHD1) blocks replication of retroviruses and certain DNA viruses by reducing the intracellular dNTP pool. SAMHD1 has been suggested to down-regulate IFN and inflammatory responses to viral infections, although the functions and mechanisms of SAMHD1 in modulating innate immunity remain unclear. Here, we show that SAMHD1 suppresses the innate immune responses to viral infections and inflammatory stimuli by inhibiting nuclear factor-κB (NF-κB) activation and type I interferon (IFN-I) induction. Compared with control cells, infection of SAMHD1-silenced human monocytic cells or primary macrophages with Sendai virus (SeV) or HIV-1, or treatment with inflammatory stimuli, induces significantly higher levels of NF-κB activation and IFN-I induction. Exogenous SAMHD1 expression in cells or SAMHD1 reconstitution in knockout cells suppresses NF-κB activation and IFN-I induction by SeV infection or inflammatory stimuli. Mechanistically, SAMHD1 inhibits NF-κB activation by interacting with NF-κB1/2 and reducing phosphorylation of the NF-κB inhibitory protein IκBα. SAMHD1 also interacts with the inhibitor-κB kinase ε (IKKε) and IFN regulatory factor 7 (IRF7), leading to the suppression of the IFN-I induction pathway by reducing IKKε-mediated IRF7 phosphorylation. Interactions of endogenous SAMHD1 with NF-κB and IFN-I pathway proteins were validated in human monocytic cells and primary macrophages. Comparing splenocytes from *SAMHD1* knockout and heterozygous mice, we further confirmed SAMHD1-mediated suppression of NF-κB activation, suggesting an evolutionarily conserved property of SAMHD1. Our findings reveal functions of SAMHD1 in down-regulating innate immune responses to viral infections and inflammatory stimuli, highlighting the importance of SAMHD1 in modulating antiviral immunity.

SAMHD1 | viral infection | inflammatory stimuli | NF-kB | type I interferon

Sterile alpha motif and HD-domain–containing protein 1 (SAMHD1) was identified as a cellular restriction factor that inhibits HIV-1 infection in nondividing myeloid cells (1, 2). Human *SAMHD1* cDNA was initially cloned from dendritic cells (DCs) as a homolog of a mouse IFN–γ-inducible gene (3). As a deoxynucleoside triphosphate (dNTP) triphosphohydrolase (dNTPase) (4, 5), SAMHD1 inhibits diverse retroviruses and some DNA viruses by reducing the intracellular dNTP pool (6–12). SAMHD1 is a major regulator of DNA precursor pools in mammalian cells through its control of dNTP homeostasis and genome stability during the cell cycle (13, 14). Homozygous *SAMHD1* mutations are associated with Aicardi-Goutières syndrome (AGS), a hereditary autoimmune disease characterized by spontaneous IFN-I production and an up-regulation of IFN-stimulated genes (ISG) (15, 16). AGS is a severe inflammatory immune disease lacking effective treatments (16). Several studies have reported spontaneous induction of ISG transcripts in SAMHD1-deficient mouse cells (17–19) and hyperactivity of the IFN-I pathway in mouse macrophages with SAMHD1 knockdown (20). Although SAMHD1 has been implicated in negatively regulating IFN-I and inflammation responses (15, 17, 19), the mechanisms of SAMHD1 in modulating innate immunity are unclear.

Regulation of innate immune responses to microbial pathogens and inflammatory stimuli is critical for controlling infections and inflammation. The nuclear factor-κB (NF-κB) family of transcription factors is a master regulator of innate immune responses to microbial infections and inflammatory stimuli (21–23). The mammalian NF-κB family is composed of five members, including RelA/p65, RelB, c-Rel, NF-κB1/p50, and NF-κB2/p52, which form dimeric complexes to activate target gene expression. NF-κB1/p50 and NF-κB2/p52 are derived from proteolytic cleavage

Significance

Although the host protein SAMHD1 has been studied as a restriction factor to inhibit retroviral infection, it is unknown whether and how SAMHD1 regulates antiviral innate immune responses. Using *SAMHD1*-silenced human monocytic cell lines and primary macrophages, and primary splenocytes from *SAMHD1*-knockout mice, we revealed new functions and mechanisms of SAMHD1-mediated negative regulation of innate immune responses to viral infections and inflammatory stimuli. We also identified protein interactions between SAMHD1 and several key proteins in cellular signaling pathways that regulate inflammation and antiviral innate immune responses. Overall, we discovered an important role of SAMHD1 in regulating innate immune responses and defined the underlying mechanisms, which suggest that SAMHD1 might be a therapeutic target in controlling viral infections and inflammatory diseases.

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of their precursors, p105 and p100, respectively. Activation of NF-κB is mediated by an inhibitor-κB kinase (IKK) complex, which either directs canonical (classical) NF-κB signaling by degrading the IκB inhibitor and releasing p65/p50 dimers to the nucleus or causes partial p100 processing and nuclear translocation of RelB/p52 via a noncanonical (alternative) pathway (24). Canonical NF-κB signaling is activated by many extracellular or intracellular factors, including proinflammatory cytokines, bacterial products, and viruses. In contrast, the noncanonical pathway is induced by certain tumor necrosis factor (TNF) family cytokines. NF-κB signaling is modulated by many host and pathogen proteins (25), although whether and how SAMHD1 regulates NF-κB signaling is unknown.

The induction of IFN-I and inflammatory cytokines is a hallmark of the innate immune response to viral infections (22, 26). Activation of IFN-I by sensing viral RNA or DNA in infected cells causes phosphorylation of the transcription factors IFN regulatory factor (IRF) 3 and IRF7 by the TANK-binding kinase 1 (TBK1) and IKKε. Phosphorylated IRF3 and IRF7 in the cytosol forms homo- or hetero-dimers and then translocates into the nucleus to activate gene expression of IFN-I, including IFN-α and IFN-β. Secreted IFN-I by virus-infected cells can bind to IFN receptors on uninfected cells to further propagate the antiviral response. Viral infections can also activate the inflammasome through the NF-κB pathway, and NF-κB is also required for IFN-β induction. Overall, the interplay between the NF-κB and IFN-I–signaling pathways coordinately modulates antiviral innate immune responses (24). Despite extensive studies of SAMHD1-mediated viral restriction (27, 28), it remains unclear whether and how SAMHD1 directly regulates IFN-I signaling during viral infections.

Here we show that SAMHD1 suppresses the innate immune responses to Sendai virus (SeV) and HIV-1 infections and inflammatory stimuli by inhibiting activation of the NF-κB and IFN-I pathways. Our results reveal functions and mechanisms of SAMHD1 in down-regulating innate immune responses, suggesting a potential therapeutic target in controlling viral infections and autoimmune diseases.

Results

SAMHD1 Knockout Significantly Up-Regulates the NF-κB Pathway and Inflammatory Responses. We generated a monocytic THP-1 cell line with *SAMHD1* knockout (THP-1/KO) using CRISPR/Cas9 technology (29). Compared with control cells, three THP-1/KO cell clones showed significantly increased intracellular dNTP levels and cell proliferation, as well as altered cell-cycle status and reduced apoptosis (29). To understand the mechanisms and investigate the role of SAMHD1 in regulating immune-response–related genes, we performed Nanostring nCounter analyses of the gene expression profile in control and THP-1/KO cells. The analyzed gene array includes a comprehensive set of 594 human genes differentially expressed among 24 immunology-related gene networks (30). Bioinformatic analyses of the Nanostring data revealed that NF-κB signaling was the most significantly up-regulated pathway and that the inflammatory response was the most significantly activated biological process in THP-1/KO cells compared with control cells (*SI Appendix*, Fig. S1), suggesting an important function of SAMHD1 in negatively regulating NF-κB activation and the inflammatory response.

SAMHD1 Inhibits NF-κB Activation Induced by SeV Infection and Inflammatory Stimuli. To investigate the role of SAMHD1 in modulating the NF-κB and IFN-I pathways in the innate immune responses to viral infections, we utilized SeV, a single-stranded RNA virus that activates NF-κB and IRF3/7 (31, 32). We infected THP-1/KO and control cells with SeV and detected the expression of SeV proteins, NF-κB, and its regulators. Similar levels of SeV protein expression were detected in THP-1/KO and control cells (Fig. 1*A*), confirming that SAMHD1 expression does not affect SeV replication (33). SeV infection increased phosphorylation of the NF-κB inhibitor, IκBα, in THP-1/KO cells relative to control cells, suggesting that endogenous SAMHD1 suppresses NF-κB activation induced by SeV infection. The levels of total IκBα were higher in THP-1/KO cells relative to control cells, while SeV infection reduced total IκBα expression due to the degradation of phosphorylated IκBα. SeV infection increased the levels of p100/p52 in THP-1/KO cells, but not in control cells (Fig. 1*A*), suggesting that endogenous SAMHD1 may inhibit SeV-induced up-regulation of p100/p52. The levels of p105/p50 and p65 were comparable in THP-1/KO and control cells regardless of SeV infection (Fig. 1*A*).

To examine the effects of SAMHD1 on NF-κB activation and inflammatory responses, we treated THP-1/KO and control cells with the toll-like receptor 4 (TLR4) agonist lipopolysaccharide (LPS) and then measured *IκBα* and *TNF-α* mRNA levels as indicators of NF-κB activation. After 6 or 24 h of LPS treatment, the levels of p105/p50 and phosphorylated IκBα were increased in THP-1/KO cells compared to control cells (Fig. 1*B*), suggesting that SAMHD1 efficiently suppresses LPS-induced NF-κB activation via the canonical pathway. LPS treatment of control THP-1 cells also increased SAMHD1 expression levels (Fig. 1*B*), indicating an innate response of SAMHD1 to inflammation through NF-κB activation. Moreover, LPS-induced mRNA levels of *IκBα* and *TNF-α* were significantly higher in THP-1/KO cells than in control cells at 6 h posttreatment (Fig. 1 *C* and *D*), suggesting that SAMHD1 inhibits NF-κB activation in LPS-treated cells.

To examine the effect of exogenous SAMHD1 on NF-κB activation, we expressed exogenous SAMHD1 in HEK293T cells transfected with an NF-κB reporter and then measured the NF-κB activity after SeV infection. SAMHD1 overexpression significantly inhibited SeV-induced NF-κB activation in a dose-dependent manner (Fig. 2*A*), confirming SAMHD1-mediated inhibition of NF-κB activation. Proinflammatory cytokines, such as TNF-α and interleukin-1 beta (IL-1β), activate the canonical NF-κB pathway (23). To examine the effect of SAMHD1 on proinflammatory cytokine-induced NF-κB activation, we overexpressed SAMHD1 in HEK293T cells transfected with a NF-κB reporter, treated the cells with TNF-α or IL-1β, and then detected the NF-κB activity or IκBα phosphorylation. Compared with control cells, SAMHD1 overexpression significantly suppressed TNF-α– or IL-1β–induced NF-κB activation in a dose-dependent manner (Fig. 2 *B* and *C*). SAMHD1 overexpression in HEK293T cells significantly decreased IL-1β–induced *IκBα* and *TNF-α* mRNA levels (Fig. 2 *D* and *E*), while IL-1β treatment induced significantly higher levels of *IκBα* and *TNF-α* mRNA in THP-1/KO cells relative to control cells (*SI Appendix*, Fig. S2 *A* and *B*). We next compared the effect of TNF-α or IL-1β treatment on NF-κB expression in THP-1/KO and control cells over a time course. Both cytokine treatments induced rapid degradation of total IκBα and increased IκBα phosphorylation, although IκBα expression levels were altered during the time course, likely due to protein recycling in the cells. TNF-α– or IL-1β–induced IκBα phosphorylation was higher in THP-1/KO cells than in control cells at each time point posttreatment (*SI Appendix*, Fig. S2 *C* and *D*), suggesting that SAMHD1 inhibits NF-κB activation by reducing IκBα phosphorylation. TNF-α treatment also induced increased p100 expression in both control and THP-1/KO cells over the time course (*SI Appendix*, Fig. S2*C*).

To examine the effect of SAMHD1 on NF-κB DNA-binding activity, we performed an electrophoretic mobility shift assay (34) using THP-1/KO and control cells. Compared with mock-treated cells, TNF-α or IL-1β treatment induced the shift of NF-κB–DNA complexes with a high molecular weight (*SI Appendix*, Fig. S3). TNF-α treatment induced significantly higher amounts of the shifted NF-κB–DNA complexes in THP-1/KO cells relative to control cells, while the effect of IL-1β treatment was less pronounced (*SI Appendix*, Fig. S3). Thus, endogenous SAMHD1 inhibits NF-κB–DNA binding in THP-1 cells activated by proinflammatory cytokines, suggesting that SAMHD1 negatively regulates the activation of canonical NF-κB signaling.

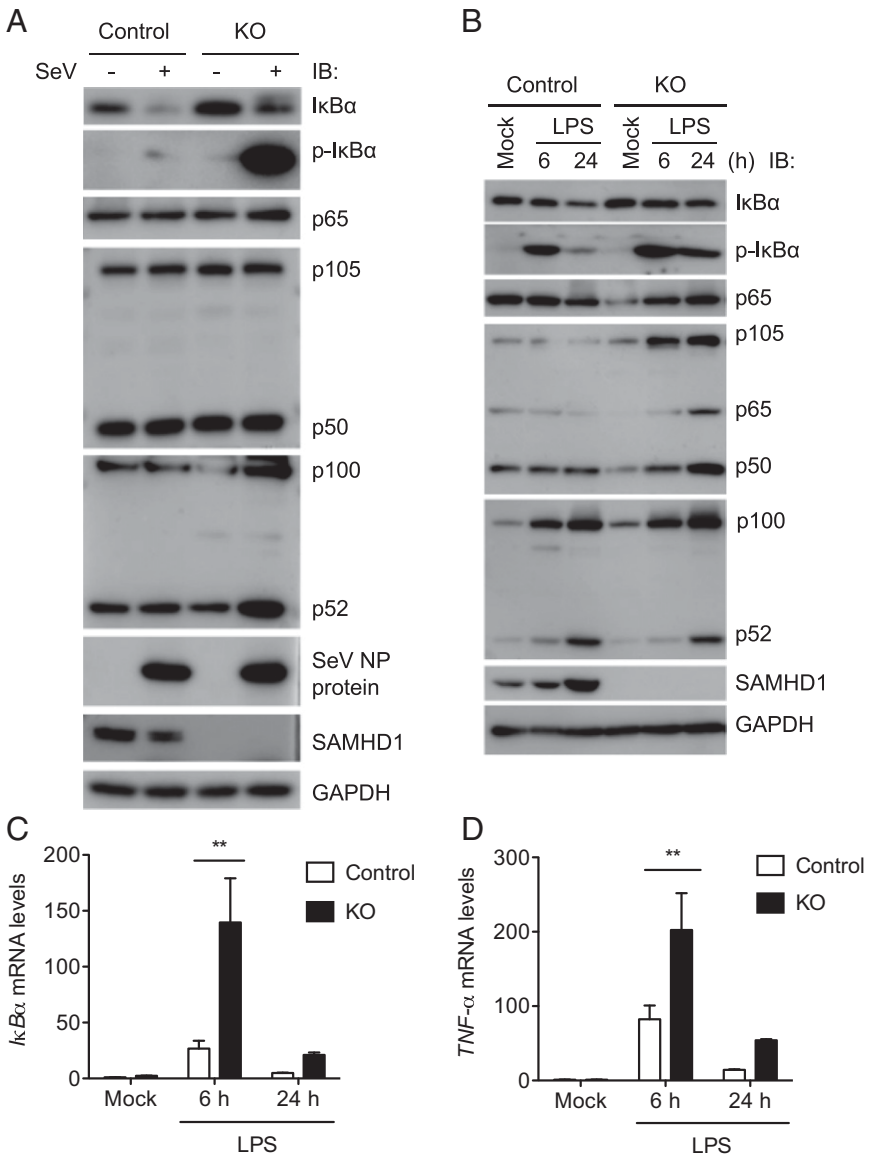

Fig. 1. Endogenous SAMHD1 inhibits NF-κB activation induced by SeV infection or LPS treatment. (*A*) Enhanced NF-κB activation induced by SeV infection in THP-1/KO cells (KO) compared with control cells. THP-1/KO and control cells were infected with SeV [multiplicity of infection (MOI) = 10] for 6 h. Cell lysates were analyzed by immunoblotting (IB) for the expression of NF-κB family proteins, SAMHD1, and SeV proteins. GAPDH was used as a loading control. (*B–D*) THP-1/KO and control cells treated with LPS (100 ng/mL) for 6 or 24 h or mock-treated. (*B*) IB of SAMHD1 and NF-κB family proteins in THP-1/KO and control cells treated with LPS or media (mock). The blot of p105/p50 had the residual p65 signal after reprobing. (*C* and *D*) Relative mRNA levels of *IκBα* and *TNF-α* in THP-1/KO and control cells were quantified by RT-qPCR. The data shown are from one representative experiment with biological replicate of $n = 3$, and each experiment was repeated at least three times. Statistical significance was determined using two-way ANOVA with Bonferroni posttest; $^{**}P \leq 0.01$. (*A–D*) Consistent results were obtained using an additional SAMHD1 KO THP-1 clone (*SI Appendix*, Figs. S7 and S8).

SAMHD1 Knockdown in Primary Human Macrophages Enhances NF-κB Activation and IFN-I Induction. To assess whether endogenous SAMHD1 in primary macrophages suppresses NF-κB activation and IFN-I induction, we measured the proinflammatory cytokines *TNF-α* and *IL-6* as well as *IFN-I* mRNA and protein levels in SeV-infected human monocyte-derived macrophages (MDM) with siRNA-mediated *SAMHD1* knockdown. Nonspecific siRNA transfected or mock-infected MDM were used as negative controls. The level of SAMHD1 protein was efficiently decreased by 70% in MDM transfected with *SAMHD1*-specific siRNA relative to nonspecific siRNA (Fig. 3*A*). Upon SeV infection, the SeV *nucleoprotein* mRNA level was slightly reduced in *SAMHD1*–down-regulated MDM compared with control cells (Fig. 3*B*), which could be attributed to IFN-I–mediated inhibition of SeV replication. In contrast, mRNA levels of *TNF-α*, *IL-6*, *IFN-α*, and *IFN-β* were significantly increased in *SAMHD1*–down-regulated MDM compared with control cells in the presence or absence of SeV infection (Fig. 3 *C–F*). Furthermore, the level of IFN-α protein was significantly increased in the supernatant from *SAMHD1*–down-regulated MDM relative to control cells (Fig. 3*G*). Consistent results were obtained using MDM from three different donors. Together, these data suggest that endogenous SAMHD1 in MDM efficiently suppresses SeV-induced NF-κB activation and IFN-I induction.

SAMHD1 Inhibits IRF7-Mediated Activation of the IFN-I Pathway. To understand the mechanisms of SAMHD1 in regulating IFN-I responses to viral infection or inflammation, we investigated the effect of SAMHD1 on the IFN-I pathway. SAMHD1 overexpression in HEK293T cells significantly inhibited SeV-induced IFN-β activation in a dose-dependent manner (Fig. 4*A*). Given the important roles of IRF3 and IRF7 in IFN-I induction (24), we examined their contributions to IFN-I suppression by SAMHD1. SAMHD1 was able to suppress the activity of an IFN-sensitive response element (ISRE) reporter activated by IRF7 overexpression, but not by IRF3 overexpression (Fig. 4*B*). SAMHD1 overexpression significantly inhibited IRF7-induced ISRE activation in a dose-dependent manner (*SI Appendix*, Fig. S4*A*), confirming the role of IRF7 in SAMHD1-mediated inhibition of IFN-I induction. As a specificity control, we observed that SAMHD1 overexpression did not suppress the ISRE activity (Fig. 4*B* and *SI Appendix*, Fig. S4*B*) or *IFN-I* mRNA expression (Fig. 4*C* and *SI Appendix*, Fig. S4*C*) induced by IRF3-5D, the constitutively active IRF3 phosphorylation mimetic (35).

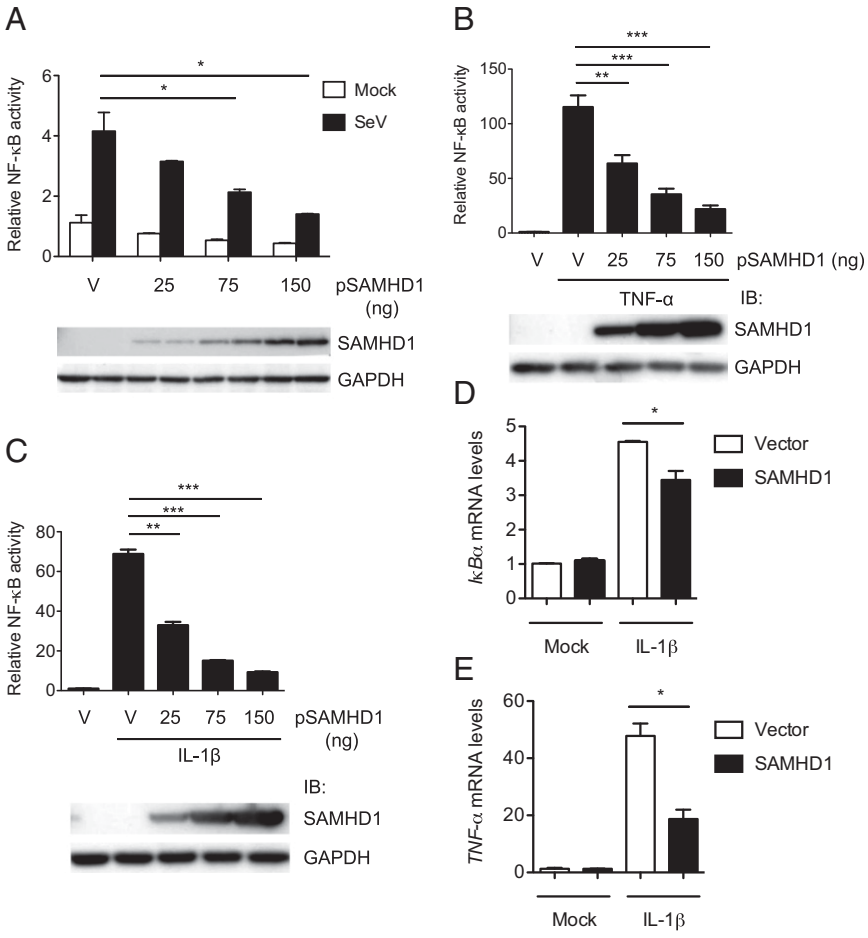

Fig. 2. Exogenous SAMHD1 inhibits NF-κB activation induced by SeV infection or inflammatory cytokines. (*A*) SAMHD1 overexpression in HEK293T cells suppresses NF-κB activation induced by SeV infection in a dose-dependent manner. HEK293T cells were transfected with increasing amounts of HA-tagged SAMHD1-expressing plasmid (pSAMHD1) or an empty vector (V) as indicated, together with a NF-κB-luciferase reporter and a renilla-TK reporter. At 24 h posttransfection, cells were infected with SeV (MOI = 1) for 6 h before dual luciferase assays. The IB results shown below the bar graph indicate the specific protein detection of the matched samples. (*B* and *C*) SAMHD1 overexpression in HEK293T cells suppresses NF-κB activation induced by TNF-α (*B*) or IL-1β (*C*). HEK293T cells were transfected as described in *A*. At 24 h posttransfection, cells were treated with TNF-α or IL-1β for 6 h before dual luciferase assays. (*D* and *E*) HEK293T cells overexpressing SAMHD1 or vector control cells were treated with IL-1β for 6 h, and mRNA levels of *IκBα* (*D*) or *TNF-α* (*E*) were quantified by RT-qPCR. All data are mean fold change ±SD from one representative experiment, biological replicates were $n = 3$, and each experiment was repeated at least three times. Statistical significance was determined by the two-tailed Student *t* test; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

Because IKKε- and TBK1-mediated phosphorylation of IRF7 is required for its dimerization, nuclear entry, and activity to direct *IFN-α* and *IFN-β* expression (26), we examined whether SAMHD1 inhibits IKKε- and/or TBK1-mediated IRF7 phosphorylation in HEK293T cells. SAMHD1 overexpression inhibited IRF7 phosphorylation mediated by IKKε (Fig. 4*D*), but not by TBK1 (*SI Appendix*, Fig. S4*D*), indicating that SAMHD1 suppresses the IRF7-activated IFN-I induction pathway by inhibiting IKKε-mediated IRF7 phosphorylation. Notably, coexpression of IKKε or TBK1 enhanced IRF7 expression (Fig. 4*D* and *SI Appendix*, Fig. S4*D*).

We next investigated the inhibitory effect of endogenous SAMHD1 on SeV-induced IFN-I activation in THP-1 cells. Upon SeV infection, we detected significantly higher levels of *IFN-I* mRNAs (*SI Appendix*, Fig. S5 *A* and *B*), phosphorylation of IRF3/7, and IRF7 expression in THP-1/KO cells relative to control cells (*SI Appendix*, Fig. S5*C*). Importantly, shRNA-mediated IRF7 knockdown in THP-1 control and THP-1/KO cells drastically decreased SeV-induced *IFN-α* and *IFN-β* mRNA expression (Fig. 4 *E*–*G*). Partial IRF7 knockdown in THP-1/KO cells (Fig. 4*E*) almost fully diminished *IFN-I* induction to levels similar to control cells without IRF7 knockdown (Fig. 4 *F* and *G*). These data demonstrate that IRF7 is critical for IFN-I induction in THP-1 cells, suggesting that SAMHD1 inhibits IRF7-mediated activation of the IFN-I pathway.

We also observed significantly higher levels of *IFN-α* and *IFN-β* mRNA in THP-1/KO cells relative to control cells at 6 h post LPS treatment (*SI Appendix*, Fig. S6 *A* and *B*). Upon LPS treatment, the levels of IRF7 expression, phosphorylation and dimerization, and IKKε expression were significantly higher in THP-1/KO cells relative to control cells (*SI Appendix*, Fig. S6 *C* and *D*). We further confirmed the results from SeV infection, LPS, or IL-1β treatment using different clones of THP-1 control and THP-1/KO cells (*SI Appendix*, Figs. S7 and S8). Thus, SAMHD1 suppresses IFN-I activation induced by viral infection or inflammatory stimuli.

SAMHD1 Reconstitution in THP-1/KO Cells Inhibits NF-κB Activation and IFN-I Induction. To further validate the results obtained using THP-1/KO cells, we reconstituted full-length SAMHD1 expression in these cells by retroviral transduction and henceforth refer to them as THP-1 SAMHD1 knock-in (THP-1/KI) cells (29). We induced NF-κB activation and IFN-I induction in these cells by LPS treatment and then measured the responses by quantifying mRNA levels of the proinflammatory cytokines *TNF-α* and *IL-6*, as well as *IFN-β*. The level of reconstituted SAMHD1 in THP-1/KI cells was similar to that in THP-1 control cells (*SI Appendix*, Fig. S9*A*). As expected, LPS treatment significantly increased mRNA levels of *TNF-α*, *IL-6*, and *IFN-β* in THP-1/KO cells transduced with the Lvx vector, while reconstitution of SAMHD1 expression significantly suppressed the induction of *TNF-α*, *IL-6*, and *IFN-β* mRNA by two-, six-, and fivefold, respectively (*SI Appendix*, Fig. S9 *B*–*D*). These results confirm the important role of SAMHD1 in suppressing NF-κB activation and IFN-I induction by inflammatory stimuli.

SAMHD1 Inhibits NF-κB Activation and IFN-I Responses in HIV-1–Infected THP-1 Cells. HIV-1 infection can activate NF-κB signaling to enhance viral gene expression driven by the HIV-1 promoter that contains NF-κB–binding sites (25). However, HIV-1 infection in myeloid cells does not trigger significant IFN-I responses unless SAMHD1 expression is down-regulated to enhance viral infection (36). To examine the effect of SAMHD1 on NF-κB and IFN-I signaling during HIV-1 infection, we performed HIV-1 infection of THP-1/KO and control cells. Single-cycle HIV-1 infection of

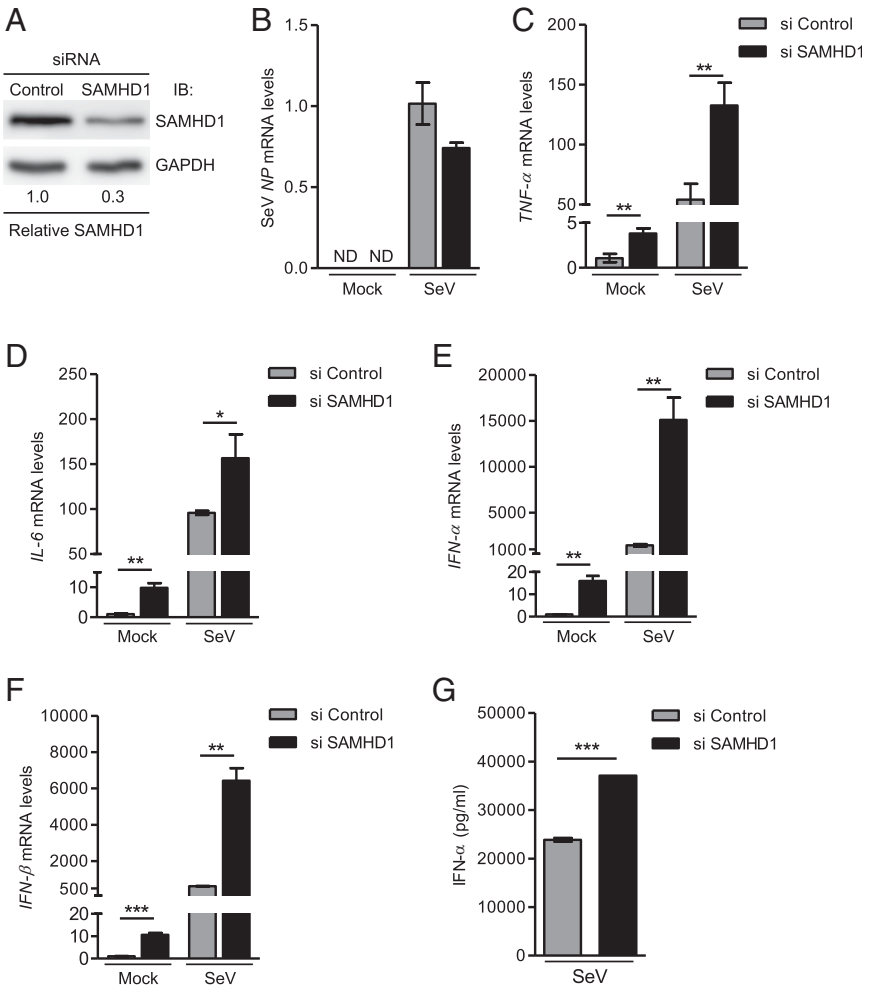

Fig. 3. SAMHD1 knockdown in primary human macrophages enhances NF-κB activation and IFN-I induction by SeV infection. Human primary monocyte-derived macrophages transfected with control siRNA (si Control) or SAMHD1-specific siRNA (si SAMHD1). Cells were harvested for (*A*) IB analysis of endogenous SAMHD1 levels. GAPDH was used as a loading control. (*B–F*) Cells were mock-infected or infected with SeV for 6 h (MOI = 20). Cells were harvested for RT-qPCR analysis of relative mRNA levels of SeV *nucleoprotein (NP), TNF-α, IL-6, IFN-α,* and *IFN-β*, respectively. For SeV *NP* mRNA analysis, si Control samples infected with SeV were set as 1. ND, not detectable. For all other mRNA analyses, the levels of mock-infected si Control samples were set as 1. (*G*) ELISA quantification of IFN-α protein levels in the supernatants of SeV-infected MDM at 24 h postinfection. The data shown are from one representative experiment with biological replicates ($n = 3$). Statistical significance was determined using an unpaired *t* test; $*P \leq 0.05$, $**P \leq 0.01$, and $***P \leq 0.001$.

nondifferentiated THP-1/KO cells was fourfold higher relative to control cells (Fig. 5*A*). Efficient HIV-1 infection in THP-1/KO cells significantly increased phosphorylation of IκBα in THP-1/KO cells relative to control cells (Fig. 5*B*), suggesting that endogenous SAMHD1 can suppress NF-κB activation during HIV-1 infection. The levels of total IκBα, IKKε, and TBK1 increased in THP-1/KO cells compared with control cells, while the level of p100/p52 slightly decreased (Fig. 5*B*).

To test whether SAMHD1 affects IFN-I signaling and activation of ISG during HIV-1 infection, we used THP1-Blue ISG cells to monitor IFN activation. THP1-Blue ISG cells were derived from THP-1 cells by stable integration of an IRF-inducible secreted embryonic alkaline phosphatase (SEAP) reporter construct, which allows the monitoring of ISG activation by determining the activity of SEAP. We generated a stable SAMHD1 knockdown cell line based on THP1-Blue ISG cells and confirmed SAMHD1 knockdown by immunoblotting (Fig. 5*C*). Single-cycle HIV-1 infection was significantly increased 16- to 23-fold in differentiated, nondividing SAMHD1 knockdown cells compared with control cells (Fig. 5*D*), which correlated with significantly increased ISG activation in SAMHD1 knockdown cells (Fig. 5*E*). As negative controls, inhibition of HIV-1 infection by the reverse transcriptase inhibitor nevirapine or by using heat-inactivated HIV-1 abolished viral infection and blocked ISG activation (Fig. 5 *D* and *E*). Thus, endogenous SAMHD1 inhibits ISG activation during HIV-1 infection.

SAMHD1 Inhibition of NF-κB and IFN-I Activation Is Independent of Its dNTPase Activity or Phosphorylation. Given that the dNTPase activity of SAMHD1 and its HIV-1 restriction function are negatively regulated by its phosphorylation at T592 (37–41), we examined whether the dNTPase activity or phosphorylation of SAMHD1 is required for its inhibition of NF-κB and IFN-I activation. To this end, we used the dNTPase-defective SAMHD1 mutant (H206R/D207N, or HD/RN) (42) and the phosphorylation-ablative mutant (T592A) (39). Overexpression of wild-type (WT) SAMHD1 or either of these mutants in HEK293T cells comparably inhibited IL-1β–induced NF-κB activation in a dose-dependent manner (*SI Appendix*, Fig. S10 *A* and *B*). SAMHD1 WT and mutants also similarly inhibited IRF7-mediated ISRE activation (*SI Appendix*, Fig. S10*C*). These results suggest that SAMHD1-mediated inhibition of NF-κB and IFN-I activation is independent of its dNTPase activity or T592 phosphorylation.

SAMHD1 Interacts with Several Key Proteins in the NF-κB and IFN-I Pathways. To examine the mechanisms of SAMHD1-mediated suppression of NF-κB and IFN-I activation, we investigated SAMHD1

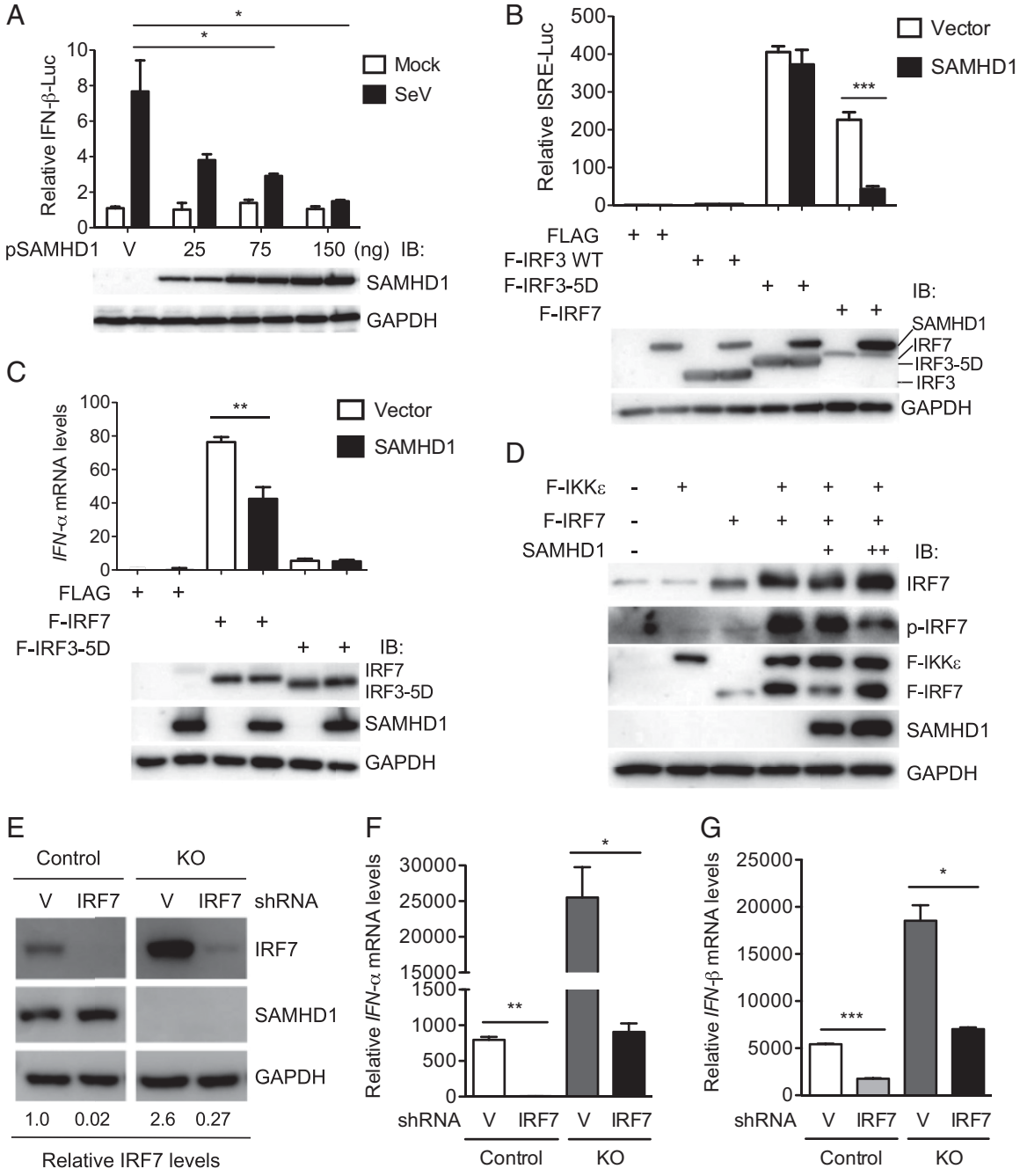

Fig. 4. SAMHD1 inhibits IRF7-activated IFN-I pathway. (*A*) SAMHD1 inhibits *IFN-β* promoter activity induced by SeV infection in a dose-dependent manner. HEK293T cells were transfected with increasing amounts of HA-SAMHD1–expressing plasmid or an empty vector (V) together with an *IFN-β* promoter luciferase reporter and a renilla-TK reporter. Cells were infected with SeV (MOI = 1) for 6 h before dual luciferase assays. (*B*) SAMHD1 inhibits ISRE reporter activity induced by IRF7, but not by WT IRF3 or IRF3-5D mutant. HEK293T cells were transfected with HA-SAMHD1, and the indicated plasmids together with an ISRE-luciferase reporter and a renilla-TK reporter. (*C*) SAMHD1 inhibits IRF7-mediated induction of *IFN-α* mRNA. HEK293T cells were cotransfected with plasmids encoding FLAG-vector, FLAG-IRF7, or FLAG-IRF3-5D and HA-SAMHD1. Lysates were harvested for IB, and RNA was extracted for RT-qPCR. (*A*–*C*) The IB results shown below the bar graphs indicate the specific protein detection of the matched samples. The data are mean fold change ±SD from one representative experiment and biological replicate was $n = 3$, and each experiment was repeated at least three times. Statistical significance was determined by the two-tailed Student *t* test; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. (*D*) SAMHD1 prevents IKKε-mediated phosphorylation of IRF7. HEK293T cells were cotransfected with plasmids encoding FLAG-IKKε, FLAG-IRF7, and increasing amounts of HA-SAMHD1 as indicated. At 24 h posttransfection, cells were lysed for IB for the indicated proteins. (*E*–*G*) IRF7 knockdown significantly reduces SeV-induced *IFN-I* mRNA expression in THP-1 cells. (*E*) THP-1 control and THP-1/KO cells were transduced with an IRF7-specific lentiviral shRNA vector or empty vector (V) control. IRF7 expression was assessed by IB 4 d after transduction. GAPDH was a loading control. The split blots of the same protein detection are from the same membrane with the same exposure. Similar IRF7 knockdown efficiency in these cells was obtained using a different IRF7 shRNA vector. (*F* and *G*) Cells transduced with IRF7-specific shRNA vector and the empty vector were infected with SeV (MOI = 10) for 6 h. RT-qPCR was used to quantify mRNA levels of *IFN-I*. *GAPDH* mRNA levels were used for normalization. (*F*) Relative *IFN-α* mRNA levels. (*G*) Relative *IFN-β* mRNA levels. $^{*}P < 0.05$, $^{**}P < 0.002$, and $^{***}P < 0.001$. The values of mock infection of THP-1 control cells transduced with shRNA empty vector were set as 1 to calculate relative mRNA levels. All mock-infected sample values were between 0.7 and 1.97 (not included in figure for clarity). The data shown are presented as mean fold change ±SD from one representative experiment and a biological replicate ($n = 2$), and each experiment was repeated at least three times. Statistical significance was determined using the two-tailed Student *t* test.

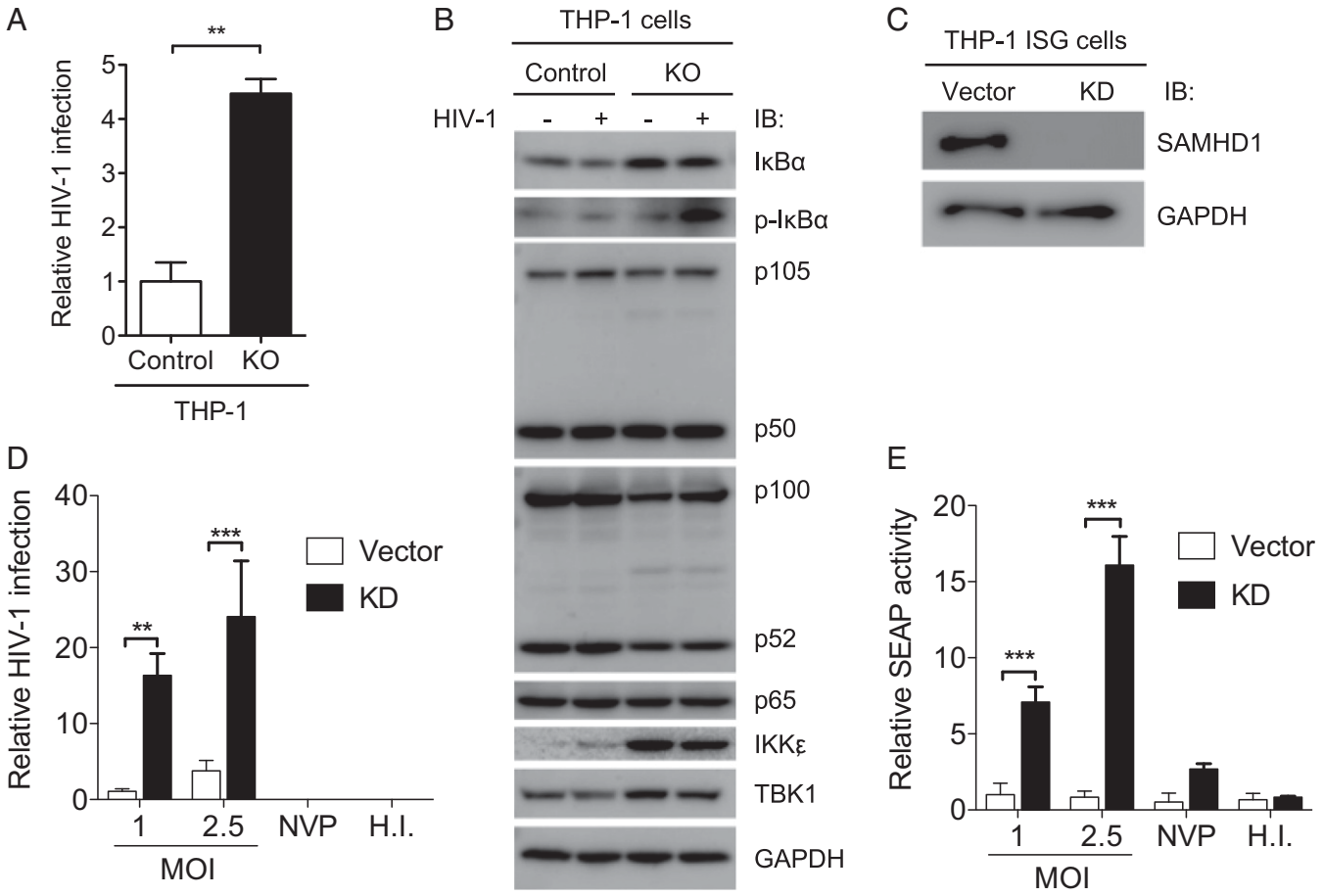

Fig. 5. SAMHD1 knockout or knockdown induces NF-κB and ISG activation in HIV-1–infected THP-1 cells. (*A*) Enhanced HIV-1 infection in THP-1/KO cells compared with control cells. Cells were infected with single-cycle HIV-1 pseudotyped with VSV-G (HIV-1-Luc/VSV-G, MOI = 2) for 24 h, and viral infection was determined by luciferase assays. $^{**}P = 0.0043$. (*B*) Cell lysates were analyzed at 6 h postinfection by IB for the expression of NF-κB family proteins and SAMHD1. GAPDH was used as a loading control. (*C*) THP1-Blue ISG reporter cells were transduced with SAMHD1-specific lentiviral shRNA vector or empty vector control. SAMHD1 knockdown (KD) was confirmed by IB. GAPDH was a loading control. (*D*) THP1-Blue ISG cells expressing vector or SAMHD1 shRNA (KD) were differentiated with phorbol 12-myristate 13-acetate (PMA) and infected with HIV-1-Luc/VSV-G at the indicated MOIs, and virus infection efficiency was analyzed after 48 h by luciferase assay. Treatment with the HIV-1 reverse transcriptase inhibitor nevirapine (NVP) or infection with a heat-inactivated virus (H.I.) was used as a negative control. (*E*) Detection of SEAP activity at 48 h postinfection as an indicator of ISG activation in cells infected and treated as in *B*. All samples were mock-subtracted. Error bars represent SD from duplicate samples. Two-way ANOVA with Bonferroni posttest was used to determine statistical significance; $^{**}P < 0.01$ and $^{***}P < 0.001$. Data are representative of two independent experiments.

interactions with key proteins in these pathways using immunoprecipitation (IP). IP of endogenous SAMHD1 showed its interactions with endogenous p50 and p100/p52 in THP-1 cells treated with or without IL-1β (Fig. 6*A*). At 30 min post IL-1β treatment, the amounts of SAMHD1-bound p50 and p100/p52 were reduced compared with those in mock controls, while the expression levels of p105 and p100/p52 were consistent over time (Fig. 6*A*), suggesting that SAMHD1 interactions with NF-κB proteins might be reduced upon NF-κB activation. Interestingly, endogenous SAMHD1 interacted with IκBα (Fig. 6 *A*–*C*), suggesting a potential mechanism of SAMHD1-mediated inhibition of IκBα phosphorylation in THP-1 control cells (*SI Appendix*, Fig. S2 *C* and *D*). IL-1β treatment or SeV infection of THP-1 control cells decreased the levels of endogenous IκBα, thereby reducing IκBα interaction with SAMHD1 (Fig. 6 *A* and *B*).

IP of overexpressed SAMHD1 in HEK293T cells confirmed its interactions with endogenous p105/p50 and p100/p52 (*SI Appendix*, Fig. S11 *A*–*C*). Although SAMHD1 overexpressed in HEK293T cells significantly inhibited p65-induced NF-κB activation in a dose-dependent manner (*SI Appendix*, Fig. S12 *A* and *B*), it did not interact with p65 regardless of SeV infection (*SI Appendix*, Fig. S12*C*), suggesting that the SAMHD1–p65 interaction is not required for SAMHD1-mediated NF-κB inhibition. During NF-κB activation through the canonical pathway, IKKβ phosphorylates IκBα to promote its proteasomal degradation through the E3 ubiquitin ligase complex containing β-TrCP (25). No interaction was observed between overexpressed SAMHD1 and β-TrCP in HEK293T cells (*SI Appendix*, Fig. S12*D*), suggesting that this interaction is not required for SAMHD1-mediated inhibition of IκBα phosphorylation.

We next examined whether SAMHD1 interacted with key IFN-I–signaling proteins in THP-1 cells with or without SeV infection. IP of endogenous SAMHD1 in THP-1 control cells revealed its interactions with endogenous IRF7, IKKε, and IκBα (Fig. 6*B*). Moreover, IP of exogenously expressed SAMHD1 confirmed its interaction with IRF7 overexpressed in HEK293T cells (*SI Appendix*, Fig. S11*D*). IP of IRF3, IRF7, TBK1, or IKKε individually overexpressed in HEK293T cells revealed that IRF7 and IKKε, but not IRF3 or TBK1, interacted with overexpressed SAMHD1 (*SI Appendix*, Fig. S11*E*), confirming the importance of IRF7 and IKKε in SAMHD1-mediated inhibition of the IFN-I pathway. We further validated endogenous SAMHD1 interactions with p52, IκBα, and IRF7 in primary human MDM from two healthy donors (Fig. 6*C*). An in vitro binding assay revealed direct interaction between purified recombinant SAMHD1

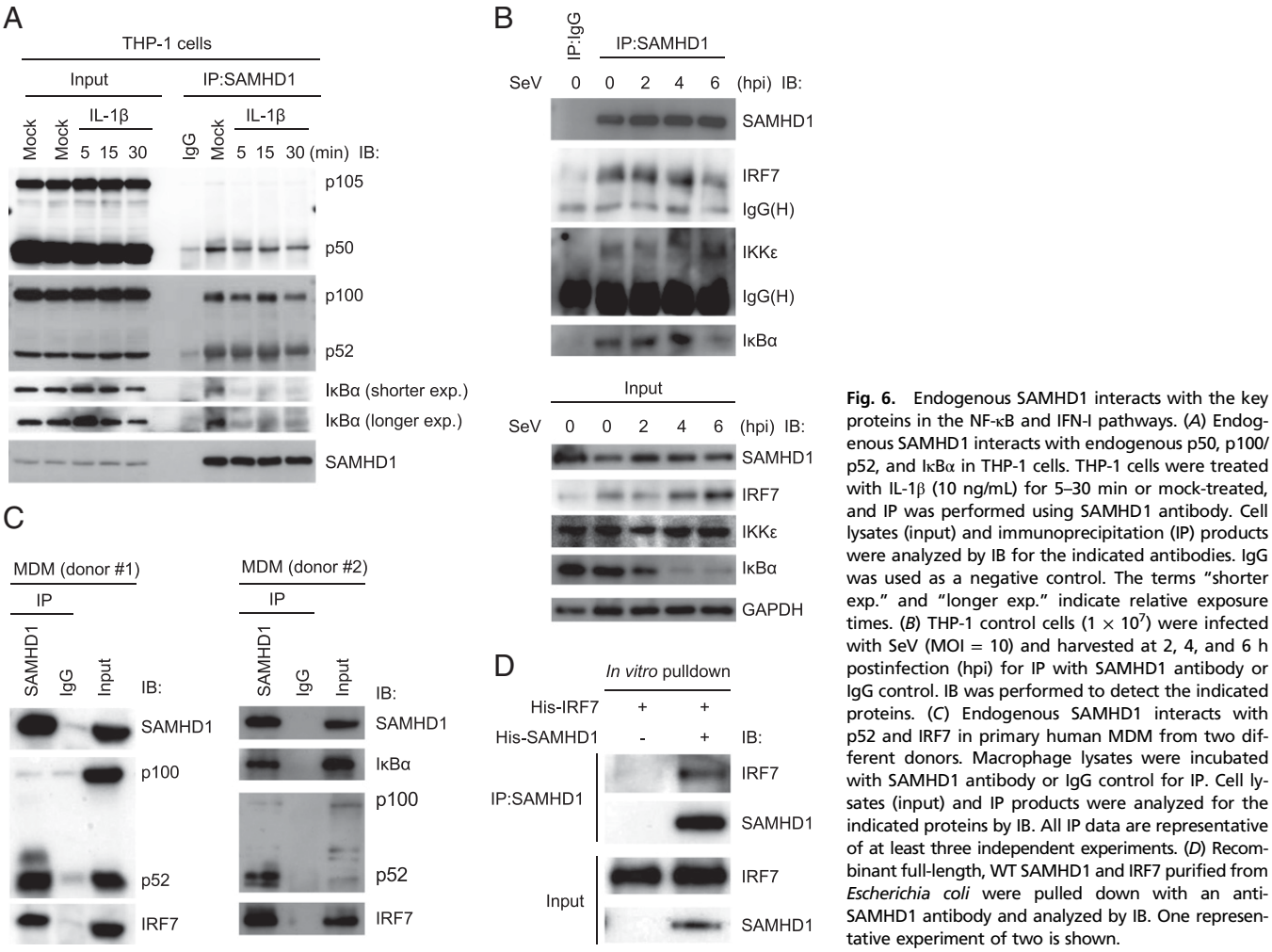

Fig. 6. Endogenous SAMHD1 interacts with the key proteins in the NF-κB and IFN-I pathways. (*A*) Endogenous SAMHD1 interacts with endogenous p50, p100/p52, and IκBα in THP-1 cells. THP-1 cells were treated with IL-1β (10 ng/mL) for 5–30 min or mock-treated, and IP was performed using SAMHD1 antibody. Cell lysates (input) and immunoprecipitation (IP) products were analyzed by IB for the indicated antibodies. IgG was used as a negative control. The terms "shorter exp." and "longer exp." indicate relative exposure times. (*B*) THP-1 control cells (1×10^7) were infected with SeV (MOI = 10) and harvested at 2, 4, and 6 h postinfection (hpi) for IP with SAMHD1 antibody or IgG control. IB was performed to detect the indicated proteins. (*C*) Endogenous SAMHD1 interacts with p52 and IRF7 in primary human MDM from two different donors. Macrophage lysates were incubated with SAMHD1 antibody or IgG control for IP. Cell lysates (input) and IP products were analyzed for the indicated proteins by IB. All IP data are representative of at least three independent experiments. (*D*) Recombinant full-length, WT SAMHD1 and IRF7 purified from *Escherichia coli* were pulled down with an anti-SAMHD1 antibody and analyzed by IB. One representative experiment of two is shown.

and IRF7 (Fig. 6*D*). Thus, SAMHD1 selectively interacts with several key proteins in the NF-κB and IFN-I pathways.

The HD Domain of SAMHD1 Is Required for IRF7 Interaction and IRF7-Induced ISRE Activation. To map the specific domains of SAMHD1 required for its interaction with IRF7, we performed co-immunoprecipitation assays using a series of truncated SAMHD1 mutants (M1–7) and full-length IRF7 (*SI Appendix*, Fig. S13*A*). IP of WT or mutant SAMHD1 overexpressed in HEK293T cells revealed that the mutants (M1 and M7) lacking the HD domain did not interact with IRF7 (*SI Appendix*, Fig. S13*B*), indicating that the interaction is dependent on the HD domain of SAMHD1. To assess whether the interaction is coupled with SAMHD1-mediated inhibition of IFN-I activation, we examined whether SAMHD1 mutants can suppress IRF7-induced ISRE activation. Interestingly, the SAMHD1 mutants (M1 and M7) lacking the HD domain also failed to suppress IRF7-induced ISRE activation (*SI Appendix*, Fig. S13*C*), suggesting that IRF7 interaction is required for SAMHD1-mediated inhibition of IFN-I signaling. Of note, the magnitude of the inhibition of ISRE activation mediated by WT SAMHD1 was less than that shown in Fig. 4*B*, which could be due to variable levels of SAMHD1 using different expression vectors.

Mouse SAMHD1 Inhibits TNF-α Responses in Primary Macrophages. Mouse SAMHD1 shares 72–74% protein sequence identity with human SAMHD1 (43). To determine if SAMHD1-mediated inhibition of TNF-α responses is evolutionarily conserved and applies to primary cells, we utilized mice deficient in SAMHD1 expression (17). Splenocytes were obtained from sibling SAMHD1 heterozygous (+/−) and knockout (−/−) mice and treated ex vivo with monophosphoryl lipid A (MPL-A) or dimethyl sulfoxide (DMSO) control and then intracellular TNF-α expression was analyzed by flow cytometry. MPL-A is a detoxified derivative of the *Salmonella minnesota* LPS and primarily signals via the TLR4-TRIF pathway (44). After 4 h of incubation with MPL-A or DMSO, the splenocytes were stained with the surface marker for macrophages (F4/80) and intracellular TNF-α (*SI Appendix*, Fig. S14). Baseline expression of TNF-α was less than 3% in $SAMHD1^{+/-}$ and $SAMHD1^{-/-}$ macrophages, while MPL-A stimulation induced a significant increase in the percentage of TNF-α–positive macrophages. There was significantly higher (approximately twofold) TNF-α–positive cells in $SAMHD1^{-/-}$ versus $SAMHD1^{+/-}$ macrophages (Fig. 7 and *SI Appendix*, Fig. S14). These data demonstrate that mouse SAMHD1 suppresses TNF-α responses in primary macrophages, suggesting an evolutionarily conserved property of SAMHD1.

Discussion

Balanced regulation of innate immune responses to viral infections and inflammatory stimuli is critical for the immune system's defense against viruses (22, 25, 28). In this study, we revealed that SAMHD1 suppresses the innate immune responses to viral infections and inflammatory stimuli by inhibiting the activation of the NF-κB and IFN-I–signaling pathways. To avoid inordinate innate immune responses to viral infections that might be detrimental to the host, SAMHD1-mediated inhibition of NF-κB and IFN-I signaling can negatively modulate these pathways activated by viral

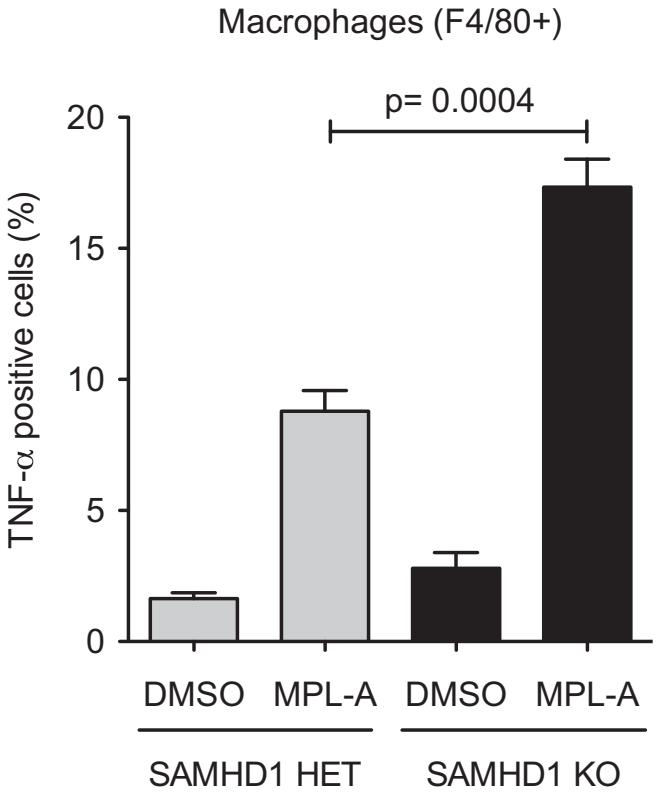

Fig. 7. Mouse SAMHD1 inhibits TNF-α responses in primary macrophages. Splenocytes isolated from sibling heterozygous (HET, *SAMHD1*$^{+/-}$) and knockout (KO, *SAMHD1*$^{-/-}$) mice (n = 3) were treated with DMSO (control) or MPL-A for 4 h, stained with surface markers for macrophages (F4/80) and intracellular TNF-α, and analyzed by flow cytometry. Summarized results of the percentages of TNF-α–positive macrophages treated with DMSO control or MPL-A are shown. The results are representative of two independent experiments performed with biological samples of n = 3. Statistical significance was determined by two-tailed t test.

infections, which is an important and previously unappreciated function of SAMHD1 (Fig. 8). Our results studying human SAMHD1 functions in cell lines and primary macrophages are consistent with previous ex vivo studies of mouse SAMHD1. Comparing control and SAMHD1-deficient mouse cells, Maelfait et al. (19) showed that SAMHD1-mediated HIV-1 restriction limits subsequent innate and adaptive immune responses, suggesting competition between cell-autonomous virus control and subsequent innate and adaptive immune responses. Although HIV-1 restriction by SAMHD1 is dependent on its dNTPase activity (11), our results indicate that the dNTPase activity is not required for SAMHD1-mediated inhibition of NF-κB and IFN-I signaling. Moreover, we confirm that SAMHD1 does not restrict SeV infection (33), suggesting that viral restriction is not required for SAMHD1-mediated inhibition of innate immune responses.

We demonstrate that endogenous SAMHD1 coprecipitates with several key proteins in the NF-κB and IFN-I pathways in THP-1 cells and human primary macrophages, suggesting that the interactions might be in the context of a multicomponent complex in cells. Baldauf et al. (12) showed substantial amounts of SAMHD1 in the nucleus and cytoplasm of primary human CD4$^+$ T cells and macrophages. It is therefore possible that SAMHD1 interacts with the NF-κB and IFN-I complex in both the nucleus and the cytoplasm. Furthermore, we demonstrate that SAMHD1, through its HD domain, directly interacts with IRF7 and that the interaction is required for SAMHD1-mediated inhibition of IFN-I activation. IRF-7 is the master regulator of IFN-dependent innate and adaptive immune responses (45), and NF-κB plays a critical role in modulating immunity (21). Our data highlight the importance of SAMHD1 interactions with the NF-κB and IFN-I–signaling proteins in negatively regulating innate immunity. It is plausible that SAMHD1 also affects other cell-signaling pathways involved in immune regulation. For example, using different approaches, a recent study suggests that the PI3K/AKT/IRF3-signaling pathway is important for the IFN-I response in SAMHD1-deficient THP-1 cells (46).

Given the significance of the NF-κB and IFN-I–signaling pathways in human pathobiology (21, 22, 25), our findings reveal the important role of SAMHD1 in regulating innate immunity and suggest a potential therapeutic target in controlling viral infections and autoimmune diseases. Significantly higher levels of serum TNF-α were detected in homozygous *SAMHD1* mutant patients compared with normal individuals (47), validating that SAMHD1 likely acts as an immunomodulator in down-regulating proinflammatory responses in humans. Our data suggest that silencing of SAMHD1 protein expression in monocytic cells leads to activation of NF-κB and IFN-I signaling upon viral infections or inflammatory stimuli. Targeting the NF-κB and IFN-I pathways could be an effective way to treat SAMHD1-defective patients with AGS. A recent case report shows that i.v. infusion of IL-6 antibodies (Tocilizumab) reversed cerebral vasculopathy in a patient with a homozygous *SAMHD1* mutation (48), suggesting that antiproinflammatory treatment targeting NF-κB activation may prevent disease progression of AGS patients. Furthermore, our findings on SAMHD1-mediated inhibition of NF-κB activation and IFN-I induction have implications in enhancing SAMHD1

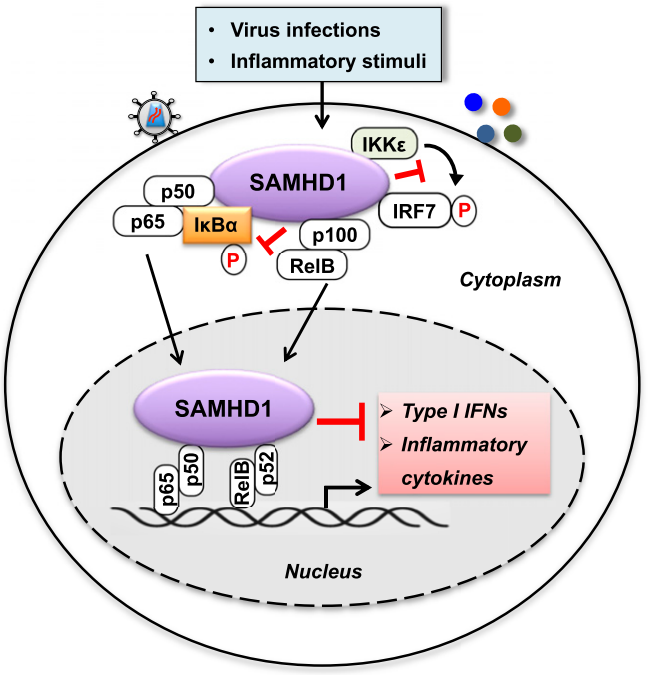

Fig. 8. Proposed molecular mechanisms and implications of SAMHD1-mediated inhibition of NF-κB activation and IFN-I induction. Endogenous SAMHD1 protein suppresses the innate immunity to viral infections and inflammatory stimuli (indicated with color dots outside of the cell) by inhibiting the activation of the NF-κB and IFN-I–signaling pathways. Mechanistically, SAMHD1 suppresses NF-κB activation by interacting with NF-κB1/2 and reducing phosphorylation of the NF-κB inhibitory protein IκBα. Through its interactions with IRF7 and IKKε (likely in the cytoplasm), SAMHD1 also suppresses the IFN-I induction pathway by reducing IKKε-mediated IRF7 phosphorylation, which is required for efficient *IFN-I* transcription. Balanced regulation of the innate immune responses to microbial pathogens and inflammatory stimuli is critical for controlling infections and autoimmune disorders. Our findings highlight the important role of SAMHD1 in regulating innate immunity, suggesting that SAMHD1 could be a therapeutic target in controlling viral infections and inflammatory diseases.

expression and activity in immune cells to control viral infections and inflammation.

Studies using *SAMHD1* KO mice suggest that SAMHD1 is critical for regulating the intracellular dNTP pool and intrinsic immunity against retroviral infection (17–19). Macrophages and DCs are professional antigen-presenting cells that bridge innate and adaptive immunity to microbial infections. We found that the effect of mouse SAMHD1-mediated suppression of TNF-α responses to MPL-A appeared to be specific to $F4/80^+$ macrophages and $CD11c^+$ DCs, as we did not observe significant MPL-A induction of TNF-α in $CD11c^-F4/80^-$ cells. MPL-A has been used in vaccine adjuvant formulations, such as the human papillomavirus vaccine Cervarix (49). Our data on mouse SAMHD1-mediated suppression of TNF-α responses to MPL-A suggest that SAMHD1 can regulate the downstream effects of TLR4-based vaccine adjuvants. It remains to be established whether activation of NF-κB and IFN-I pathways in *SAMHD1* KO mice by viral infections or inflammatory stimuli may lead to significantly higher responses relative to WT mice.

In summary, our results revealed functions and mechanisms of SAMHD1 in the regulation of innate immune responses to viral infections and inflammatory stimuli (Fig. 8). Our findings highlight the important role of SAMHD1 in negatively regulating innate immunity and suggest a potential therapeutic target in controlling viral infections and inflammatory diseases.

Materials and Methods

The Institutional Review Board (IRB) at the Ohio State University has approved the in vitro experiments involving human blood cells from de-identified healthy donors. The consent requirements for the de-identified blood samples were waived by IRB. Primary monocytes were isolated from buffy coats purchased from American Red Cross Blood Service. MDMs were differentiated from monocytes as described (50). THP-1 control and THP-1/KO cell lines have been described previously (29). SeV and HIV-1 infection assays were performed as described previously (32, 39). Detailed materials and methods can be found in *SI Appendix*.

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