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VIRUS-CELL INTERACTIONS

Long Noncoding RNA Metastasis-Associated Lung Adenocarcinoma Transcript 1 Promotes HIV-1 Replication through Modulating microRNAs in Macrophages

Zhihong Yuan,[a,b] Yunlong Huang,[c] Ruxana T. Sadikot[a,b]

[a]VA Nebraska Western Iowa Health Care System, Omaha, Nebraska, USA
[b]Division of Pulmonary, Critical Care & Sleep, Department of Internal Medicine, University of 0Nebraska Medical Center, Omaha, Nebraska, USA
[c]Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, Nebraska, USA

ABSTRACT Macrophages can serve as a reservoir for human immunodeficiency-1 (HIV-1) virus in host cells, constituting a barrier to eradication, even in patients who are receiving antiretroviral therapy. Although many noncoding RNAs have been characterized as regulators in HIV-1/AIDS-induced immune response and pathogenesis, only a few long noncoding RNAs (lncRNAs) have demonstrated a close association with HIV-1 replication, and the molecular mechanisms remain unknown. In this study, we investigated how lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), related microRNAs, and key inflammatory genes alter HIV-1 replication in macrophages. Our data show that HIV-1 infection modulates the expression of miR-155 and miR-150-5p in a time-dependent manner, which is regulated by MALAT1. MALAT1 induced suppressor of cytokine signaling 1 (SOCS1) expression by sponging miR-150-5p in HIV-1-infected macrophages and stimulated inflammatory mediators triggering receptor expressed on myeloid cells/cold inducible RNA binding protein (TREM 1/CIRP) ligand/receptor. The RNA immunoprecipitation (RIP) assay validated the direct interaction within the MALAT1/miR-150-5p/SOCS1 axis. HIV-1 infection-mediated upregulation of MALAT1, SOCS1, and HIV-1 Gag was attenuated by SN50 (an NF-κB p50 inhibitor). MALAT1 antisense oligonucleotides (ASOs) suppressed HIV-1 p24 production and HIV-1 Gag gene expression and decreased expression of miR-155 and SOCS1, as well as the production of proinflammatory cytokines by HIV-1-infected macrophages. In conclusion, HIV-1 infection induces MALAT1, which attenuates miR-150-5p expression and increases SOCS1 expression, promoting HIV-1 replication and reactivation. These data provide new insights into how MALAT1 alters the macrophage microenvironment and subsequently promotes viral replication and suggest a potential role for targeting MALAT1 as a therapeutic approach to eliminate HIV-1 reservoirs.

IMPORTANCE Viral reservoirs constitute an obstacle to curing HIV-1 diseases, despite antiretroviral therapy. Macrophages serve as viral reservoirs in HIV infection by promoting long-term replication and latency. Recent studies have shown that lncRNAs can modulate virus-host interactions, but the underlying mechanisms are not fully understood. In this study, we demonstrate how lncRNA MALAT1 contributes to HIV-1 replication through modulation of the miR-150/SOCS1 axis in human macrophages. Our findings have the potential to identify new therapies for eliminating HIV-1 reservoirs in immune cells.

KEYWORDS HIV-1, macrophages, MALAT1, miR-155, miR-150-5p, SOCS1, TREM-1

Human immunodeficiency virus (HIV)-1 infection is a significant global health issue affecting millions of people worldwide. HIV-1 infection and latency can contribute to end organ damage, leading to increased morbidity and mortality (1). While combination antiretroviral therapy (ART) can decrease the viral load and replication and slow

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Address correspondence to Ruxana T. Sadikot, rsadikot@unmc.edu.

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disease progression, it cannot reverse HIV-1 integration into target host cells and tissues. In addition to $CD4^+$ T lymphocytes, cells of the myeloid lineage, including macrophages, dendritic cells, and osteoclasts, are emerging as important target cells for HIV-1 infection in both lymphoid and nonlymphoid tissues and constitute persistent viral reservoirs (2). HIV reservoirs have been identified to lead to changes in macrophage phenotype and behavior and are known to promote a proinflammatory environment constituting a barrier to HIV eradication (3, 4). The development and function of tissue-resident macrophages, including those in the alveoli, perivascular space, and interstitial niche, are the crucial pathophysiological events leading to inflammation and cancer (5, 6). Understanding the molecular mechanisms that alter the effects of myeloid cells on HIV-1 reservoirs may guide future therapies aimed at eradicating HIV-1 infection and latency (7).

Noncoding RNAs (ncRNAs), such as microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), have been identified as key regulators of immune responses in viral infection (8, 9). In the context of HIV-1 infection, noncoding RNAs have been shown to modulate host-pathogen interactions. LncRNA is a class of RNA molecules that are longer than 200 bp and do not encode proteins but play crucial roles in various biological functions and disease processes, including inflammation and microbial infection (10–12). LncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) was initially discovered in metastatic carcinoma cells and was subsequently found to be highly upregulated in many types of cancer and inflammatory conditions (13, 14). As a highly conserved lncRNA in mammals, MALAT1 is specifically retained in nuclear speckles to modulate pre-mRNA processing and has been shown to modulate several key miRNAs. Increased expression of MALAT1 in HIV lymphocytes has been found to contribute to initial viral replication and disease progression. Mechanistically, MALAT1 sequesters histone trimethylase, releasing the epigenetic inhibition of the HIV-1 long terminal repeat (LTR) promoter responsible for latency (15). Although recent studies have indicated that MALAT1 modulates differentiation and activation of macrophages (16, 17), the role of MALAT1 in regulating HIV-1 infection in macrophages remains unclear.

As 20- to 24-nucleotide noncoding RNAs, miRNAs have been shown to regulate gene expression and cellular processes by targeting mRNA transcripts. They are involved in the clinical progression and pathogenesis of viral infections by modulating the interaction between virus and host cells, as well as homeostasis of the tissue microenvironment (18). Many miRNAs have been identified as dysregulated by HIV-1, including miR-155, miR-21, miR-30a, miR-150, miR-34a, and miR-146 (19–24). MiR-155 is a typical multifunctional miRNA that is closely linked to inflammatory response-related diseases, affecting various organs, including lung and liver (25, 26). MiR-155 activity has been shown to control multiple aspects of HIV-1 infectivity and innate immune response to the virus (21, 24). While miR-150 expression was reported to be downregulated in primary resting memory $CD4^+$ T cells and HIV-specific $CD8^+$ T cells (27–29), its role in HIV-1-infected macrophages and its impact on the host immune response have not been studied yet. Recent studies have shown that MALAT1 attenuates the expression of miR-150 in disease models (30–32). However, to date, there are no data linking MALAT1's sponging effect on miR-150 to HIV-1 infection.

In this study we investigated the role of MALAT1 in HIV-1-infected macrophages and its impact on the macrophage inflammatory phenotype by using culture systems that induce HIV-1 replication or reactivation. We found that HIV-1 infection upregulates MALAT1 in a time-dependent manner, which subsequently sponges miR-150, leading to increased expression of suppressor of cytokine signaling (SOCS). Mechanistically, we found that MALAT1 silencing using antisense oligonucleotides (ASOs) in HIV-1-infected macrophages alters miRNA expression and has beneficial effects. Therefore, we have identified that MALAT1 is a key modulator of miR-150, and the MALAT1/miR-150 signaling axis plays an important role in the macrophage inflammatory phenotype during HIV-1 replication. These results suggest that HIV-1-infected macrophages induce proinflammatory effects through modulation of lncRNAs and miRNAs, and understanding

related pathways might lead to more approaches for modulating the antiviral immune response and improving lung health in HIV-1-infected individuals.

RESULTS

HIV-1 infection induces MALAT1 expression in macrophages thorough NF-κB p50. To investigate the role of MALAT1 in HIV-1 infection, human monocyte-derived macrophages were infected with HIV-1, and then MALAT1 expression was quantitated. At 7 days postinfection, the expression of MALAT1 was significantly higher in HIV-1- infected macrophages than in control cells (Fig. 1A). A time course analysis showed that MALAT1 expression started increasing at 72 h postinfection compared to that in uninfected macrophages (Fig. 1B). To confirm the presence of HIV-1 infection in the cells, we used enzyme-linked immunosorbent assays (ELISAs) to demonstrate an increase in intracellular HIV-1 p24 production in the HIV-1-infected monocyte-derived macrophage (MDM) culture compared to that in the control uninfected cell culture, as shown in Fig. 1C. Together, these data suggest that HIV-1 infection upregulates MALAT1 expression in a time-dependent manner starting at 72 h post-HIV-1 infection of human macrophages.

In a subclone of U937 cells, U1 cells were used for latency induction experiments, which originally contained two copies of integrated HIV-1 DNA and were readily induced by stimulation with tumor necrosis factor alpha (TNF-α) or phorbol myristate acetate (PMA) (33–35). Since chronic infection induces reactivation of HIV-1 replication in U1 cells (36), to study the functional role of MALAT1 in this event, its expression level was quantified in U1 cells stimulated with TNF-α. At 24 h poststimulation, the expression level was significantly elevated in U1 cells (TNF-α concentration, 100 ng/mL) compared to that in untreated cells (Fig. 1D). The presence of HIV-1 infection was confirmed by upregulation of HIV-1 Gag mRNA expression noted after TNF-α treatment (Fig. 1E).

Next, we investigated the mechanism by which MALAT1 expression is induced in HIV-1-infected macrophages. Nuclear factor kappa B (NF-κB) and p53-binding sites have been identified in the proximal MALAT1 coding region (37). HIV-1 infection is also known to activate signaling pathways in viral latency and reactivation in myeloid cells (38–40). Therefore, we hypothesized that a subunit of NF-κB, p50, may bind to the MALAT1 promoter and regulate its expression in HIV-1-infected cells. To identify predicted p50 binding sites on MALAT1 promoter, we used the JASPAR database (http://jaspar.genereg.net/), which contains manually curated, nonredundant transcription factor (TF) binding profiles for TFs (41), as shown in Fig. 1F. To determine the functional role of p50 on MALAT1, we transfected U1 cells with siRNA against p50 before stimulation with TNF-α or treated HIV-1-infected monocyte-derived macrophages with SN50, a cell-permeable inhibitory peptide (41, 42). We confirmed that p50 expression was knocked down by siRNA transfection (Fig. 1G). Then, cells transfected with siRNA against p50 showed a significantly decreased expression of MALAT1 compared to cells transfected with mock siRNA (Fig. 1H). Consistent with the siRNA data, treatment with SN50 inhibitor peptide for 24 h significantly decreased NF-κB p50 translocation into the nucleus of macrophages compared to control peptide-treated cells (Fig. 1I). Importantly, when HIV-1-infected macrophages were exposed to SN50 peptide, HIV-1 Gag and MALAT1 expression, as determined by reverse transcription-quantitative PCR (qRT-PCR), or HIV-1 p24, as determined by ELISA, at 24 h postinfection, was significantly downregulated compared to the control peptide-treated group (Fig. 1J to L). Together, these findings show that MALAT1 is upregulated during HIV-1 infection and that p50 contributes to the regulation of MALAT1 in HIV-1-infected cells.

Differential expression of regulatory miRNAs in HIV-1-infected macrophages. It is increasingly recognized that miRNAs are involved in viral infection, latency, disease progression, and response to therapy (42). We have previously reported that altered expression levels of several regulatory miRNAs, including miR-155, miR-23a, miR-27a, and miR-21, in HIV-infected macrophages can alter the host immune response (43). However, the molecular mechanisms regulating these miRNAs and the impact of their induction on alterations in target genes remain unclear. Since MALAT1 regulates the expression of multiple miRNAs, we investigated the role of miRNAs (miR-150 and miR-

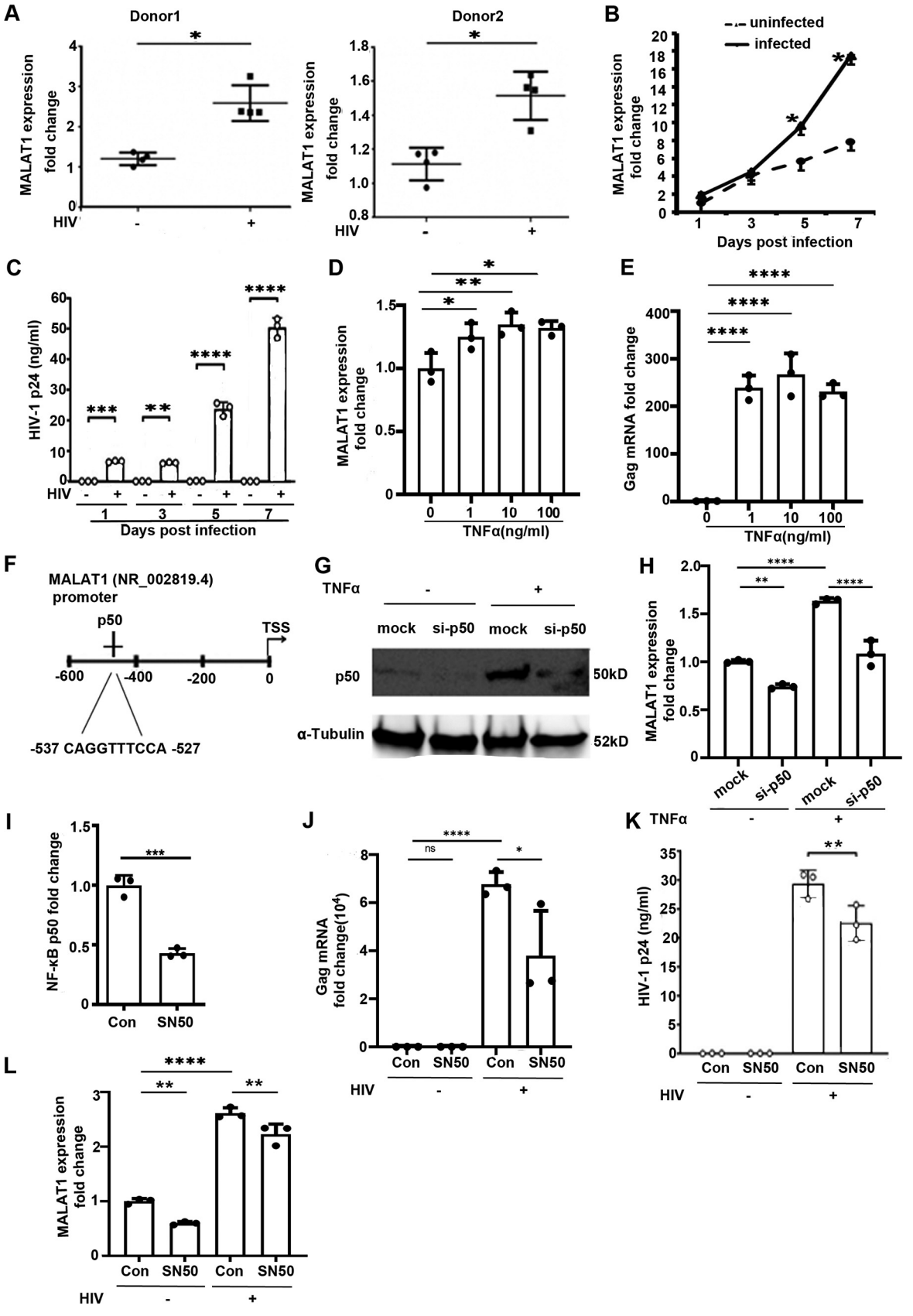

FIG 1 MALAT1 is induced in HIV-1-infected macrophages, and this induction is dependent on NF-κB p50. (A) MDMs (5×10^5/well) from two healthy donors were infected with an HIV-1 strain and cultured up to 7 days. Expression of MALAT1 was measured relative

(Continued on next page)

155) that have the potential to alter immune mechanisms and the contribution of MALAT1 to their expression. We infected human MDMs with HIV-1_{ADA}, an HIV-1 laboratory strain, and measured the expression of miR-155-5p and miR-155-3p using qRT-PCR. A time course experiment showed that after infection the expression level of miR-155-5p increased in a time-dependent manner. Then, 5 days postinfection, the miR-155-3p expression level reached the peak, following which it was downregulated (Fig. 2A). In the U1 cells exposed to different doses of TNF-α (0 to 100 ng/mL), the expression of miR-155-5p and miR-155-3p was also upregulated in a dose-dependent manner (Fig. 2B).

It has been previously reported that miR-150 is an immuno-miRNA that regulates immune functions, such as proliferation, apoptosis, and differentiation of NK, T, and B cells. Recent studies have shown that MALAT1 acts as a sponge for miR-150-5p, exerting its proinflammatory and proproliferative effects (44, 45). However, the role of miR-150 in HIV-1 infection is not fully understood. To determine its role, we employed a quantitative PCR (qPCR) assay to measure miR-150-5p expression in HIV-1-infected macrophages. We found that the miR-150-5p expression was reduced in HIV-1-infected MDMs compared to uninfected cells (Fig. 2C). TNF-α-induced U1 cells also showed downregulation of miR-150-5p in a dose-dependent manner (Fig. 2D). Similar results were obtained in human MDMs treated with recombinant HIV-1 viral protein Tat and gp120, which showed significantly reduced miR-150-5p expression compared to untreated cells (Fig. 2E).

These data show that the expression of miR-155 is upregulated, while miR-150-5p expression is downregulated in HIV-1-infected macrophages compared to uninfected cells, suggesting that these miRNAs may contribute to immunomodulatory effects in HIV-1-infected macrophages. Based on expression patterns of MALAT1 and miR-150-5p, we hypothesize that MALAT1 promotes HIV-1 replication by sponging miR-150-5p. First, we wanted to validate if there was direct binding between MALAT1 and miR-150-5p during HIV-1 infection. Bioinformatics analysis was performed to determine that there are potential complementary binding sites for miR-150-5p and MALAT1 (Fig. 2F). Next, we transfected U1 cells with miR-150-5p mimic, which increased miRNA expression as verified by reverse transcriptase quantitative PCR (RT-qPCR) (Fig. 2G). MiR-150-5p overexpression increased enrichment of MALAT1 in Argonaute (AGO) 2 complex, indicating interaction between MALAT1 and miR-150-5p 5p during reactivation of HIV-1 replication (Fig. 2H). These data showed direct interaction of miR-150-5p with MALAT1, indicating that MALAT1 may act as a sponge for miR-150-5p, thereby attenuating its effects.

HIV-1 infection leads to dysregulation of SOCS1 expression in macrophages. After finding altered expression of miR-155 and miR-150 in HIV-1 infection and reactivation models, we investigated their target genes. The suppressor of cytokine signaling (SOCS) family members serve as important immune regulators of cytokine signaling in immune cells and are modulated by miR-150 and miR-155. SOCS family members are induced upon viral infection to suppress the antiviral host defense. As negative regulators of cytokine signal transduction, SOCS proteins, such as SOCS1 and SOCS3, exert their provirulent effect by inhibiting interferons and other cytokines (46, 47). Studies by Ryo et al. have shown that SOCS1 directly binds to HIV-1 Gag and facilitates intracellular traffick-

FIG 1 Legend (Continued)
to ACTINB by qRT-PCR (n = 4/group). Data are the mean ± SD and were analyzed by two-tailed unpaired Student's t test. (B) Time course of MALAT1 expression in HIV-1-infected MDMs. Statistical analysis was performed using one-way ANOVA comparing within-time points. (C) Culture supernatant was harvested at the indicated time points after HIV-1 infection, and viral replication was measured by p24 ELISA in MDMs (n = 3). Statistical analysis: two-tailed unpaired Student's t test. (D and E) U1 cells were treated with the indicated dose of TNF-α or vehicle control. Expression of MALAT1 and Gag was measured by qRT-PCR. Statistical analysis: two-tailed unpaired Student's t test. (F) Schematic representation of NF-κB p50 binding sites in human MALAT1. (G) U1 cells transfected with siRNA against p50 and control siRNA and then treated with 10 ng/mL TNF-α for 24 h. Immunoblot assay was used to detect p50 expression in U1 cells expressing control or p50 siRNA. (H) qRT-PCR analysis of cells from panel G for MALAT1 expression. (I) MDMs (5×10^5/well) were treated with SN50 peptide inhibitor or control peptide (10 nM, 24 h). (J) MDMs (5×10^5/well) were infected with an HIV-1 strain and cultured up to 3 days and then treated with SN50 peptide inhibitor or control peptide (10 nM, 24 h). (K) The expression level of the Gag gene was measured by qRT-PCR. Viral replication was quantified by detecting p24 levels in the supernatant from panel J cells by ELISA. (L) The MALAT1 gene was measured by qRT-PCR. Data are presented as the mean ± SD of 3 to 4 biological replicates per group. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; and ****, $P < 0.0001$ denote significant differences. Student's t test: panels A, C, D, E, I, and K; one-way ANOVA with Tukey's multiple-comparison test: panels B, H, J, and L.

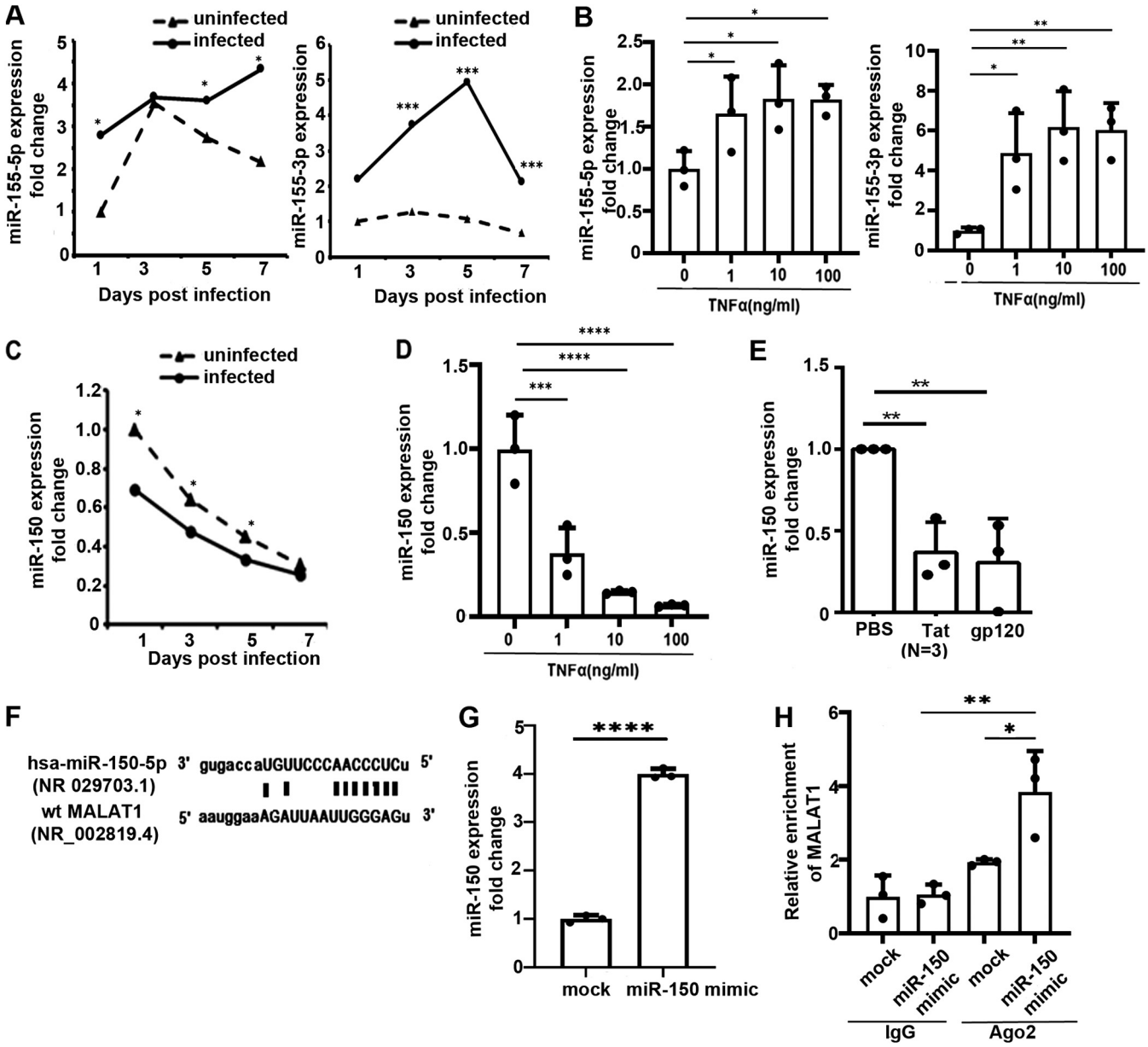

FIG 2 Macrophages infected with HIV-1 exhibited an increase in miR-155 expression and a decrease in miR-150-5p expression. (A) Time course of expression of miR-155-5p and miR-155-3p in HIV-1-infected MDMs cultured up to 7 days. (B) Expression of miR-155-5p and miR-155-3p in U1 cells treated with the indicated dose of TNF-α or vehicle control. (C) Expression of miR-150-5p in HIV-1-infected MDMs cultured up to 7 days. (D and E) U1 cells treated with the indicated dose of TNF-α or vehicle control (D) and MDMs treated with recombinant HIV-1 Tat and gp120 (100 ng/mL, 24 h) (n = 3) (E). (F) Identification of miR-150-5p target sites in MALAT1. (G) U1 cells transfected with miR-150-5p mimic or control. After transfection (48 h), cells were treated with 10 ng/mL TNF-α for 24 h. qRT-PCR was used to measure expression levels of miR-150-5p. (H) Reciprocal interaction between MALAT1 and miR-150-5p was detected by RNA immunoprecipitation (RIP) using anti-Ago2 and RT-qPCR. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; and ****, $P < 0.0001$ denote significant differences. One-way ANOVA with Tukey's multiple-comparison test: panels A, C, and H. Student's t test: panels B, D, E, and G.

ing and stability of SOCS1, resulting in the efficient production of HIV-1 particles (48). We measured the expression of SOCS1 by qRT-PCR and immunoblot assay in human macrophages following HIV-1 infection and found that both the transcript and protein levels were significantly elevated compared to those of control cells (Fig. 3A and B). Time course experiments further revealed that SOCS1 mRNA was significantly upregulated at 72 h postinfection (Fig. 3C). MiR-150 is also the regulator of cyclin T1 (CycT1), which is a host factor essential for HIV-1 replication in CD4$^+$ T cells and macrophages (22, 49). The importance of CycT1 and cellular positive transcription elongation factor b (P-TEFb) complex for HIV replication is well established, but the regulation of CycT1 expression during

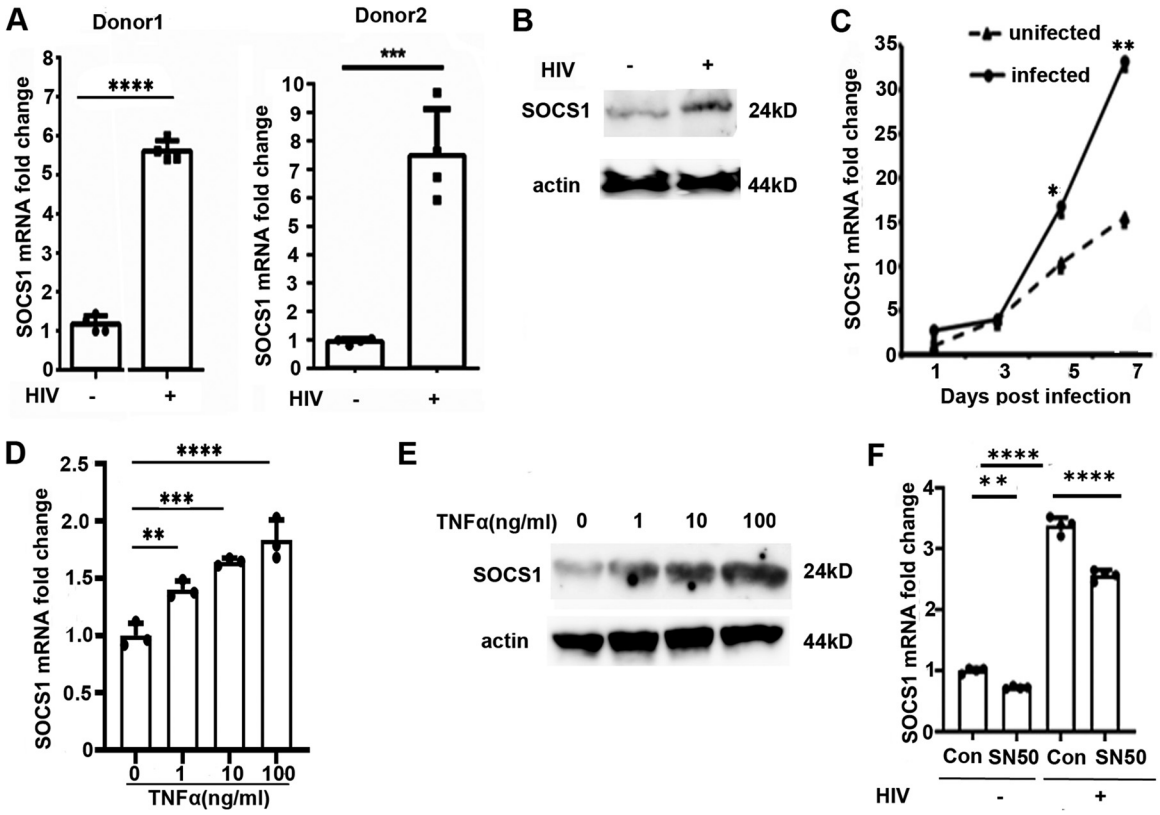

FIG 3 HIV-1-infection increased SOCS1 expression in macrophages. (A) MDMs from two healthy donors were infected with an HIV-1 strain and cultured up to 7 days. The expression level of SOCS1 was measured relative to ACTINB by qRT-PCR (n = 4/group). Data are the mean ± SD and were analyzed by two-tailed unpaired Student's t test. (B) An immunoblot assay was used to detect SOCS1 protein expression in MDMs infected with HIV-1. (C) Time course of SOCS1 expression in HIV-1-infected MDMs. Statistical analysis was performed using one-way ANOVA comparing within-time points. (D) U1 cells were treated with the indicated dose of TNF-α or vehicle control. The expression level of SOCS1 was measured relative to ACTINB by qRT-PCR (n = 3). (E) An immunoblot assay was used to detect SOCS1 expression in U1 cells treated with the indicated dose of TNF-α or vehicle control. (F) MDMs (5×10^5/well) were infected with an HIV-1 strain and cultured up to 3 days and then treated with SN50 peptide inhibitor or control peptide (10 nM, 24 h). SOCS1 expression was measured by qRT-PCR. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.00$; and ****, $P < 0.0001$ denote significant differences. Data are presented as the mean ± SD of 3 to 4 biological replicates per group. Student's t test: panels A and D. One-way ANOVA with Tukey's multiple-comparison test: panels C and F.

HIV infection in macrophages is less well characterized. In this study, the expression level of CycT1 increased in HIV-infected MDMs (see Fig. S1 in the supplemental material).

Next, we sought to confirm these findings using latently infected cells. After treatment (24 h), the qRT-PCR results revealed a dose-dependent increase in SOCS1 expression levels in response to TNF-α stimulation in U1 cells (Fig. 3D). Immunoblotting results further confirmed the upregulation of SOCS1 protein levels in U1 cells exposed to TNF-α (Fig. 3E). To investigate the upstream regulatory control of SOCS proteins, we examined whether it was regulated by NF-κB. Our results showed that NF-κB peptide inhibitor SN50 significantly suppressed the expression of SOCS1 in HIV-1-infected MDMs (Fig. 3F). Taken together, these results show that SOCS1 RNA and protein levels are increased in HIV-1 infection and reactivation, and this increase is dependent on NF-κB.

The expression of TREM-1/CIRP is upregulated in HIV-1-infected macrophages. Our previous studies have demonstrated that miR-155 modulates the expression of TREM-1, which leads to unrestrained inflammatory signaling in macrophages in response to LPS (50). Recent studies have shown that TREM-1 serves as a novel receptor for extracellular cold-inducible RNA-binding protein (eCIRP), a damage-associated molecular pattern molecule that triggers inflammatory responses during infections (51, 52). We and others have reported that elevated expression of TREM-1 propagates inflammation (53) and induces resistance to apoptosis in HIV-1-infected macrophages (54–56). However, the mechanisms by which expression of eCIRP and TREM-1 are regulated in response to

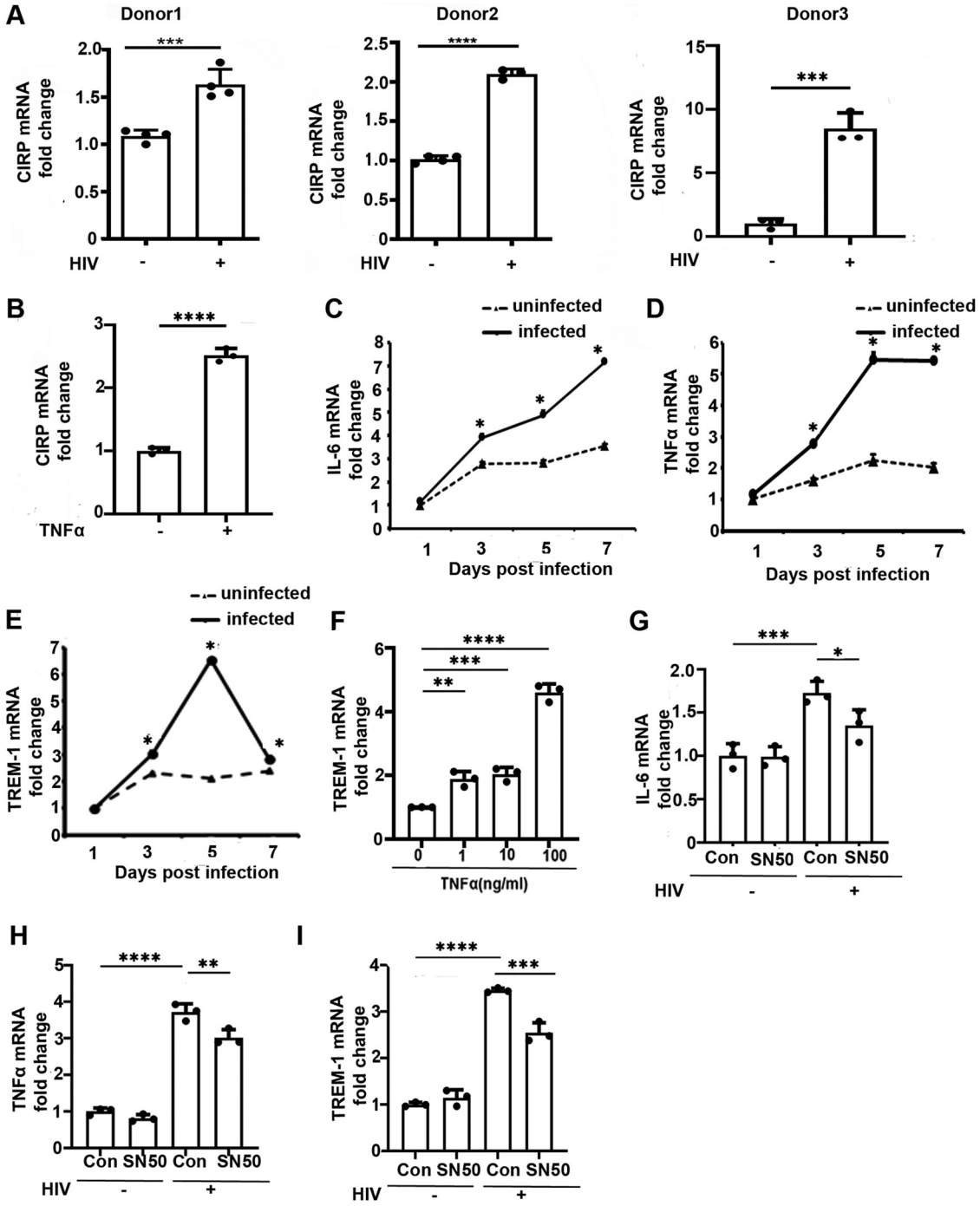

FIG 4 HIV-1-infection increased levels of inflammatory cytokines and TREM-1/CIRP in macrophages. (A) MDMs from three healthy donors were infected with an HIV-1 strain and cultured up to 7 days. Expression levels of CIRP was measured relative to ACTINB by qRT-PCR (n = 4/group). (B) U1 cells were treated with the indicated dose of TNF-α or vehicle control. Levels of CIRP were measured relative to ACTINB by qRT-PCR (n = 3). (C to E) Time course of TREM-1, IL-6, and TNF-α expression in HIV-1-infected MDMs. (F to I) MDMs (5×10^5/well) were infected with an HIV-1 strain and cultured up to 3 days and then treated with SN50 peptide inhibitor or control peptide (10 nM, 24 h). The expression level of TREM-1, IL-6, and TNF-α was measured by qRT-PCR. Data are presented as the mean $\pm$ SD of 3 to 4 biological replicates per group. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; and ****, $P < 0.0001$ denote significant differences. Student's t test: panels A, B, and F. One-way ANOVA with Tukey's multiple-comparison test: panels C, D, E, G, H, and I.

HIV-1 remain unclear. We investigated the expression pattern of CIRP in HIV-1-infected human MDMs and U1 cells. As illustrated in Fig. 4A, the expression of CIRP increased in HIV-1-infected macrophages from healthy donors compared to that of uninfected cells. Similarly, U1 cells showed a dose-dependent increase in CIRP expression following TNF-

α stimulation (Fig. 4B). TREM-1 expression increased in a time-dependent manner in MDMs infected with HIV-1_{ADA}. Since TREM-1 expression induces several proinflammatory cytokines, we investigated the effects of HIV-1 infection on the expression of inflammatory cytokines TNF-α and interleukin-6 (IL-6) in human macrophages using quantitative RT-PCR. Following infection, expression of TNF-α and IL-6 significantly increased in MDMs in a time-dependent manner compared to uninfected cells (Fig. 4C and D). The expression of the inflammation amplifier TREM-1 also increased (Fig. 4E). We have previously shown that TREM-1 expression is transcriptionally regulated by NF-κB p65 (57). Therefore, as expected, treatment with SN50 inhibited expression levels of IL-6, TNF-α, and TREM-1 in HIV-1-infected MDMs (Fig. 4F to I), suggesting that these factors are dependent on NF-κB. Taken together, these findings demonstrate that HIV-1 infection induces MALAT1, miR-155, SOCS1, TREM-1, and inflammatory cytokines in a NF-κB-dependent manner.

MALAT1 is essential for expression of proinflammatory miRNAs and mediators in macrophages. MALAT1 plays a crucial role in regulating multiple pathophysiological processes in inflammatory diseases and cancer (58). Our data show that cells with HIV-1 infection and reactivation have an inflammatory phenotype with increased expression of miR-155, TREM-1, SOCS1, and proinflammatory cytokines and reduced expression of miR-150-5p. We hypothesized that these effects are centrally regulated by MALAT1 through NF-κB. Synthetic antisense oligonucleotides (ASOs) are an important approach for the treatment of many genetic disorders and have been used in animal models and clinical trials (59). To determine whether MALAT1 is the central regulator of the proinflammatory pathways, HIV-1-infected MDMs were transfected with ASOs against MALAT1 and control ASOs. MALAT1 knockdown significantly suppressed HIV-1 Gag mRNA expression in macrophages and inhibited HIV p24 production in the supernatant (Fig. 5A to C), indicating the association between MALAT1 expression and viral replication. In addition, MALAT1 silencing downregulated expression levels of miR-155-5p, miR-155-3p, SOCS1, and CycT1, whereas it upregulated miR-150-5p expression (Fig. 5D to G; Fig. S2).

To investigate the effect of MALAT1 on miR-155- and miR-150-5p-mediated SOCS1 expression on HIV-1 reactivation, U1 cells were transfected with ASO against MALAT1 and control ASO, followed by treatment with TNF-α (10 ng/mL). Consistent with our previous findings in HIV-1-infected macrophages, attenuation of MALAT1 and Gag gene through a specific ASO was observed in TNF-α-treated U1 cells, as shown in Fig. 6A and B. MALAT1 silencing significantly inhibited the expression levels of miR-155-5p and miR-155-3p in macrophages following TNF-α stimulation (Fig. 6C and D); in contrast, the expression level of miR-150-5p significantly increased (Fig. 6E). Moreover, while TNF-α induced SOCS1 expression, SOCS1 mRNA levels decreased in MALAT1 ASO-transfected U1 cells compared to those in control-treated cells (Fig. 6F). The protein levels of SOCS1 in reactivated U1 cells were also downregulated compared to those of untreated cells (Fig. 6G). Additionally, MALAT1 silencing inhibited mRNA expression of SOCS3 and TREM-1 (Fig. 6H and I). Overexpression of miR-150-5p via mimic transfection resulted in the downregulation of SOCS1 expression in both mRNA and protein level in TNF-α-induced U1 cells (Fig. 6J and K). Collectively, these data show that MALAT1 specifically binds to miR-150-5p and modulates SOCS1 expression in HIV-1-infected macrophages and HIV-1 latently infected monocytes (U1 cells). Moreover, miR-150-5p overexpression enhanced the enrichment of SOCS1 in the AGO2 complex, indicating the interaction between SOCS1 and miR-150-5p during HIV-1 reactivation (Fig. 6L). Thus, there is a positive association between MALAT1 expression and the HIV-1 chronically infected state, which might influence the progression and pathogenesis of HIV-1-associated diseases. MALAT1 inhibition is a potential therapeutic approach to eliminate HIV reservoirs.

DISCUSSION

Noncoding RNAs have been implicated in modulation of viral immunity and antivirus host defense, and recent evidence suggests that ncRNAs, including lncRNA and miRNAs, can regulate HIV-1 infection and latency in host cells (60, 61). In this study, we aimed to investigate the role of MALAT1 and associated noncoding RNAs in modulating HIV-1 infection. We analyzed the expression pattern of MALAT1, miR-155, miR-150-5p, and the

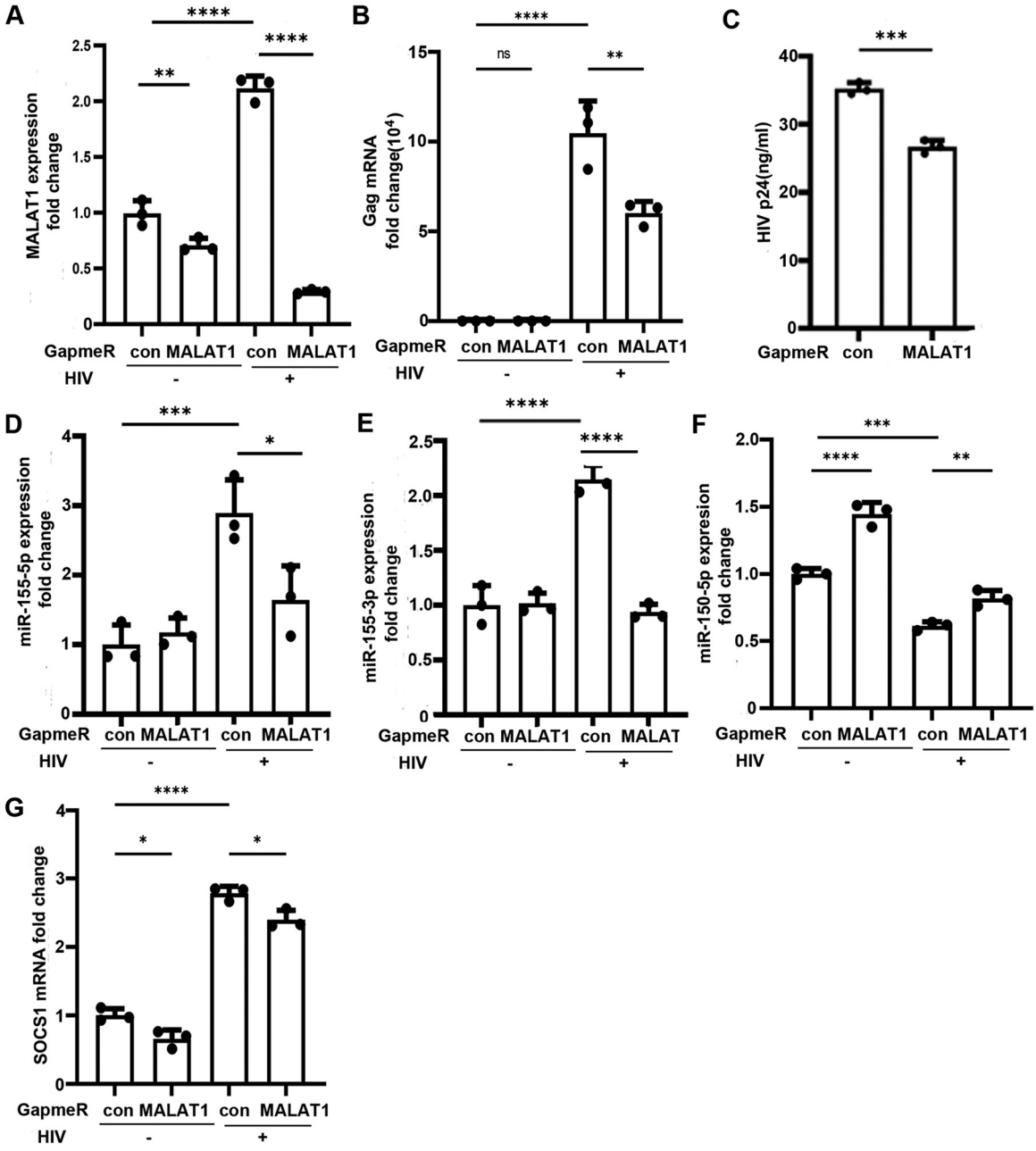

FIG 5 MALAT1 knockdown modulates the miR-150/SOCS1 axis in HIV-1-infected macrophages. MDMs from a healthy donor were infected with an HIV-1 strain and cultured up to 7 days and then transfected with ASO against MALAT1 or control ASO (10 nM, 48 h). (A) MALAT1 expression was measured relative to ACTINB by qRT-PCR (n = 3/group). (B) HIV-1 Gag expression was measured relative to ACTINB by qRT-PCR (n = 3/group). (C) Cell culture supernatant was sampled, and viral replication was measured with HIV p24 ELISA. (D to G) Expression of miR-155-5p, miR-155-3p, and miR-150-5p relative to U6 snRNA, as well as SOCS1 in HIV-1-infected MDMs transfected with LNA ASO against MALAT1 or control ASO. Data were presented as the mean ± SD of 3 to 4 biological replicates per group. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.00$; and ****, $P < 0.0001$ denote significant differences. One-way ANOVA with Tukey's multiple-comparison test: panels A, B, D, E, F and G. Student's t test: panel C.

inflammation suppressor SOCS1. Our results showed that lncRNA MALAT1 expression is required for HIV-1 replication and reactivation in macrophage reservoirs, which can influence the progression and pathology of HIV-1. Mechanistically, through sponging miR-150-5p, MALAT1 modulates SOCS1, priming the macrophage microenvironment for HIV-1 to form reservoirs (Fig. 7).

LncRNAs play a crucial role in modulating pathogen-specific immune responses, such as bacterial and viral infection (10). However, the molecular mechanisms of how

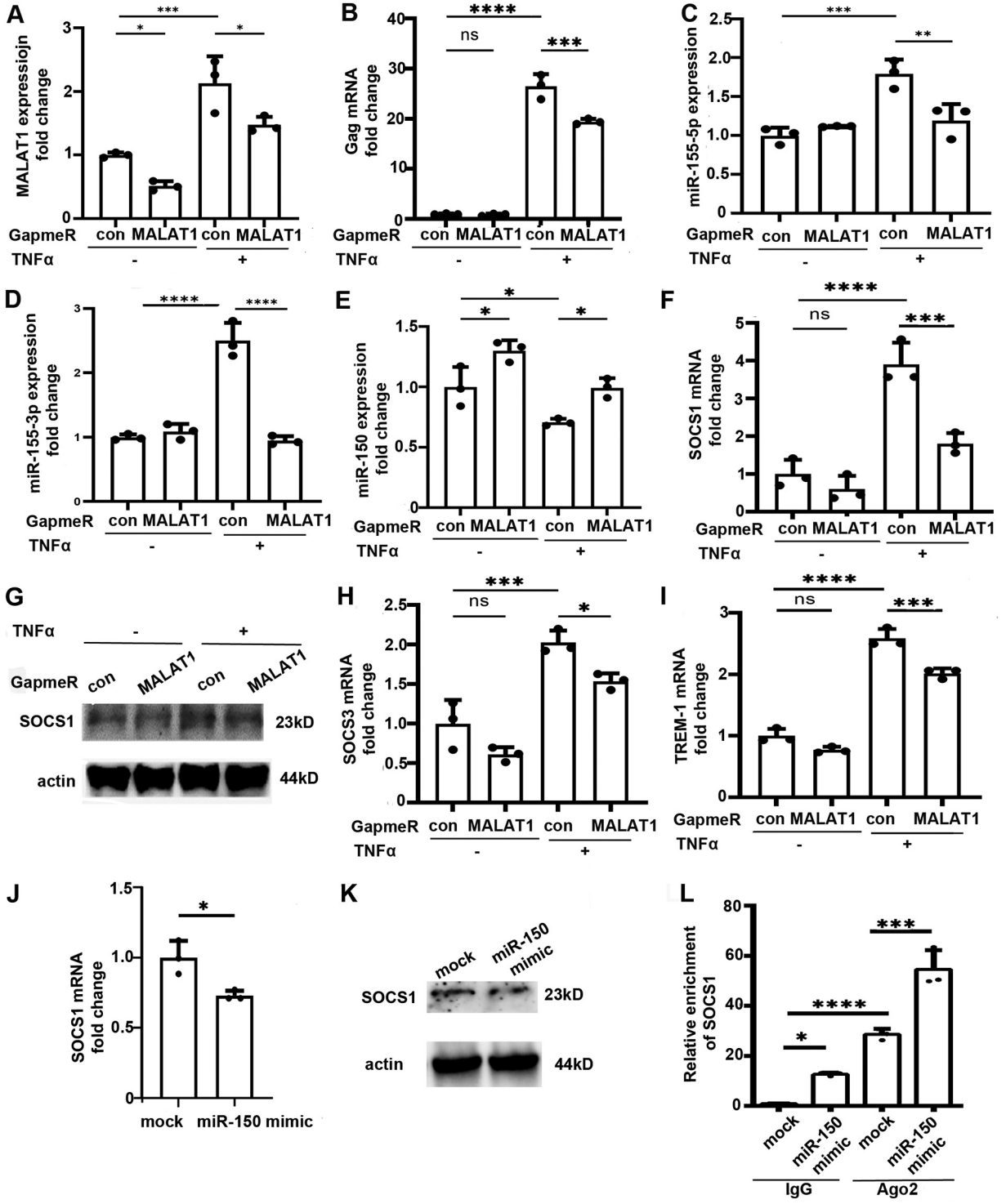

FIG 6 MALAT1 knockdown modulates the miR-150/SOCS1 axis in latently HIV-1-infected monocytes. U1 cells transfected with ASO against MALAT1 or control ASO. Posttransfection (48 h), cells were treated with 10 ng/mL TNF-α for 24 h. (A to F) qRT-PCR was used to measure expression levels of MALAT1 (A), Gag (B), miR-155-5p (C), miR-155-3p (D), miR-150-5p (E), and SOCS1 (F); $N = 3$. (G) The immunoblot assay was used to detect SOCS1 protein expression in U1 cells. (H and I) qRT-PCR assay of SOCS3 and TREM-1 in the indicated groups. U1 cells transfected with miR-150-5p mimic or control. Posttransfection (48 h), cells were treated with 10 ng/mL TNF-α for 24 h. (J) qRT-PCR was used to measure the expression level of SOCS1. (K) The immunoblot assay was used to detect SOCS1 protein expression. (L) Reciprocal interaction between SOCS1 and miR-150-5p was detected by RNA immunoprecipitation (RIP) using anti-Ago2 and qRT-PCR. Data were presented as the mean ± SD of 3 to 4 biological replicates per group. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; and ****, $P < 0.0001$ denote significant differences. One-way ANOVA with Tukey's multiple-comparison test: panels A to F, H, and I. Student's *t* test: panel J.

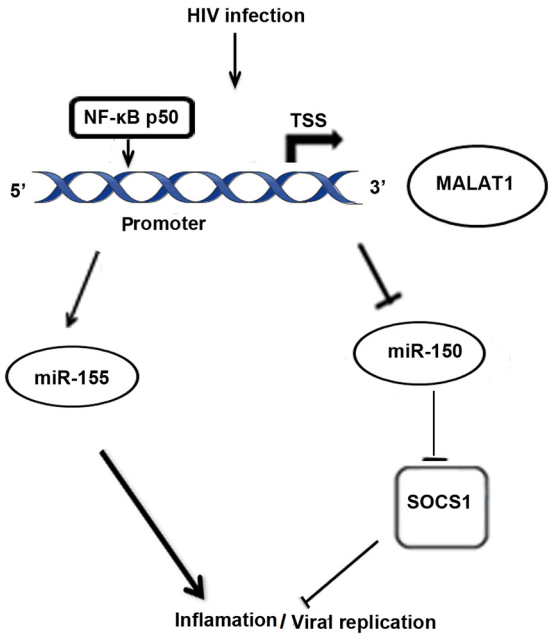

FIG 7 Schematic diagram describing the role of the MALAT1-miR-150-5p-SOCS1 axis in HIV-1-infected macrophages.

lncRNAs modulate HIV-1 infection in myeloid cells are not fully defined. Based on cellular machineries or viral components, lncRNAs either activate or repress HIV-1 replication. For example, LncRNA NEAT1, and NRON have been shown to be repressors HIV-1 infection (60–63), whereas lncRNA uc002yug.2 serves as an activator in HIV-1 latency (64). In this study, we identified MALAT1 as a key factor involved in maintaining HIV-1 activation in macrophages. In HIV-1-infected human macrophages, MALAT1 is expressed in a time-dependent manner, along with release of HIV antigen p24, suggesting that lncRNA MALAT1 activates HIV-1 infection. These findings were replicated in an HIV-1 chronic infection model. The expression of MALAT1 induced by TNF-α in U1 cells shows an association with increased HIV-1 Gag protein, indicating the reactivation of HIV-1 replication stimulated by MALAT1. Previous research has implicated miR-150 as an anti-HIV microRNA by targeting the 3′-untranscribed region (UTR) of HIV-1 transcripts in host defense (65), but the mechanisms are not well understood. In this study, we investigated whether MALAT1 interacts with miR-150 to modulate downstream target genes in HIV infection. Through knockdown studies and an RIP assay, we validated a direct binding between MALAT1 and miR-150. Previous studies have suggested that miR-150 inhibits the expression of SOCS1, although this association has not been established in HIV-1 infection. We found that miR-150 overexpression significantly reduced SOCS1 expression in HIV-1 chronically infected U1 monocytes. Our study shows that MALAT1 modulates miR-150/SOCS1 expression in HIV-1-infected macrophages. Therefore, our data suggest that the increased expression of MALAT1 via downregulation of miR-150 can modulate HIV-1 infection and reactivation in macrophages and thus has the potential to serve as a therapeutic approach for targeting HIV-1 reservoirs.

NF-κB activity has been shown to be associated with activation of various genes that regulate the transition between the activation and latency of HIV-1, including type I interferon signaling in HIV-1 5′ LTR in MDMs (66). NF-κB inhibitors have been widely explored to suppress inflammatory responses in conditions such as cancer, autoimmune disorders, and sepsis (67, 68). In this study, through bioinformatic prediction software, we identified the binding sites between MALAT1 promoter and NF-κB p50. NF-κB p50 silencing resulted in decreased expression of MALAT in TNF-α activated HIV-1-infected monocytes. To further investigate the role of p50, we employed a cell-permeable peptide inhibitor, SN50, which specifically blocks NF-κB p50 translocation to the nucleus.

Previous studies have validated the suppressive effects of SN50 on inflammation by targeting NF-κB activity in a sepsis model (69, 70). In our study, MALAT1 and downstream inflammatory signaling are found to be regulated by NF-κB in an HIV-1 infection model. Furthermore, SN50 significantly inhibited HIV p24 production, reduced MALAT1 expression, and suppressed miR-155 expression or inflammatory cytokine release in HIV-infected human MDMs that are dependent on MALAT1. These results suggest that SN50 has the potential to be employed as a therapeutic target against HIV-1 infection. Further *in vivo* investigation is needed to validate its effectiveness.

The suppressor of cytokine signaling (SOCS) family proteins consist of at least eight members, SOCS1 to 7. These proteins play an important role in balancing the activation of innate immunity (71, 72). Specifically, SOCS1 plays a vital role in the negative-feedback regulation of innate immunity via suppressing cytokine release in autoimmune diseases and sepsis (73). Viruses use the negative regulation of the SOCS1 protein to suppress the host's antiviral immune response (74, 75). Conversely, studies have suggested that SOCS1 in T lymphocytes contributes to HIV-1 replication and latency through posttranscriptional modifications (48). Other studies have reported that recombinant HIV-1 proteins upregulated SOCS expression in myeloid dendritic cells and mouse bone marrow-derived macrophages (76, 77). However, the role of SOCS1 in HIV-1 replication in human macrophages remains poorly understood. Considering the extensive heterogeneity of tissue macrophages, we chose to investigate human monocyte-derived macrophages, which are more physiologically representative and have been used in infection with laboratory HIV-1 strains. Our findings indicate that SOCS1 expression is time dependent post-HIV-1 infection in MDMs. Due to attenuated expression of miR-150, SOCS1 can promote HIV replication. Moreover, our data show that this inflammatory state is a result of MALAT1 expression and is NF-κB p50 dependent. Importantly, our work showed that MALAT1 silencing with ASOs results in a significant decrease in HIV-1 p24 antigen production from HIV-1-infected human macrophages. Treatment with peptide inhibitor SN50 significantly suppressed the expression of MALAT1 and downstream proinflammatory molecules.

In summary, our study defines the role of lncRNA MALAT1 in enhancing HIV-1 replication and reactivation in macrophages. The findings presented here demonstrate that MALAT1-induced alterations in miR-155 and miR-150/SOCS1 promote HIV-1 replication and survival in macrophages. Importantly inhibition of NF-κB p50 and MALAT1 in macrophages attenuated HIV-1 replication and reactivation. These findings reveal the critical role of lncRNA MALAT1 in promoting HIV-1 infection in macrophages, providing critical insights into the interaction between lncRNA and immune responses in HIV-1 infection. These findings also support development of appropriate biomarkers and therapeutic approaches to eradicate HIV reservoirs.

MATERIALS AND METHODS

Ethics statement. Human primary monocyte-derived macrophages (MDMs) were used in full compliance with the University of Nebraska Medical Center and National Institutes of Health ethical guidelines, with Institutional Review Board (IRB) approval no. 162-93-FB.

Chemicals and reagents. NF-κB p50 peptide inhibitor SN50, control peptide, and MALAT1 inhibitor compound MALAT1-IN-5 were purchased from Sigma-Aldrich (USA). Recombinant HIV IIIB oligomeric glycoprotein gp120 (baculovirus) (product no. 1061) and recombinant Tat HIV-1IIIB (product no. 1002) were obtained from ImmunoDX (USA). Antibodies against p50/p105, SOCS1, actin, and alpha-tubulin were obtained from Santa Cruz Biotechnology (USA). SiRNA against p50/p105, miR-150-5p mimic, and Cy3 dye-labeled pre-miR negative control no. 1 were purchased from Thermo Fisher Scientific (USA).

Primary cells, cell lines, and infection. Isolation of monocytes and culture of human MDMs were performed as previously described (43). Briefly, human peripheral blood mononuclear cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 50 ng/mL human macrophage colony-stimulating factor (M-CSF; Miltenyi Biotec, USA). MDMs were infected with HIV-1_{ADA}, a macrophage-tropic strain of HIV-1, at a multiplicity of infection (MOI) of 0.1 virus/target cell for 1, 3, 5, and 7 days, as described previously (78, 79) . This protocol was approved by the University of Nebraska Medical Center Institutional Review Board. The chronically infected HIV-1 promonocytic (U1) cell line was provided by the AIDS Reagent Program, National Institute of Health (33). U1 was cultured in RPMI 1640 containing 10% FBS and penicillin-streptomycin (100 U/mL).

ELISA. Supernatants were collected and protein levels of HIV-1 p24 were analyzed by in-house ELISAs (paired antibodies, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions, as previously described (80).

NF-κB p50 binding activity assay. HIV-1-infected macrophages were then treated with or without SN50 and control peptide for 24 h before the end of the incubation. After the indicated treatment, nuclear extracts were obtained from cells using a nuclear extraction kit (Active Motif, USA). The concentrations of proteins in the nuclear extracts were measured using a Pierce Bradford protein assay kit (Thermo Fisher, USA). The binding activity of NF-κB p50 was measured using a TransAM NF-κB p50 transcription factor assay kit (Active Motif) according to the manufacturer's instructions.

SiRNA, mimic, and ASO transfection. MALAT1 was knocked down in HIV-infected macrophages and U1 cells by transfecting MALAT1 (human) antisense locked nucleic acid (LNA) GapmeR (Qiagen, Germany) using HiPerFect reagents (Qiagen) and negative control A as the internal control. SiRNAs against NF-κB p50 were used to perform knockdown of target the gene through using HiPerFect reagent. MiR-150-5p mimic and Cy3 dye-labeled pre-miR negative control no. 1 were used for miRNA overexpression.

Quantitative real-time RT-PCR (q-RT PCR) for mRNA, LncRNA, and miRNA. Total cellular RNA was isolated using the mirVana miRNA isolation kit (Thermo Fisher Scientific, USA), and the concentration and quality of nucleic acids were measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Extracted RNA was reverse transcribed with Superscript IV master mix (Thermo Fisher Scientific). qPCR was performed with TaqMan universal PCR master mix (Thermo Fisher Scientific) as previously described (50). Primers for amplification of MALAT1, miR-155, miR-150-5p, TREM-1, TNF-α, IL-6, CIRP, SOCS1, SOCS3, and CycT1 were purchased from Thermo Fisher Scientific. Each sample was analyzed in triplicate using an ABI 7500 FAST PCR system (Thermo Fisher Scientific), and the expression values were normalized to the small housekeeping RNA U6 snRNA or ACTIN B (Thermo Fisher Scientific).

Western blot analysis. Western blot analysis was performed as previously described (43). Harvested cells were lysed with RIPA buffer (Millipore Sigma, USA) containing 1% Triton X-100 and protease inhibitors. Protein complex was detected using enhanced chemiluminescence reagents (Thermo Fisher Scientific, USA).

RNA immunoprecipitation (RIP) assay. The Magna RIP RNA-binding protein immunoprecipitation kit was used for performing the RIP assay according to the manufacturer's instructions (Millipore Sigma, USA). Briefly, cells were harvested and suspended with RIP lysis buffer. The whole-cell extract containing protein A/G magnetic beads was incubated with 5 μg of the antibody against Argonaute 2 (Ago2) (no. ab186733; Abcam, Cambridge, UK) and normal rabbit IgG (Millipore Sigma) at 4°C overnight. Agarose beads were washed, and immunoprecipitated RNA was extracted through protein K buffer and the RNeasy minikit (Qiagen, USA) and amplified with qRT-PCR. The results are presented as fold enrichment (normalized to normal IgG).

Statistical analysis. Statistical analyses were performed using Prism software version 7.0 (GraphPad Software). All experiments were repeated at least three to five times. Data are expressed as the mean ± standard deviation (SD), unless specified. Student's *t* tests were used for two-group comparisons, one-way analysis of variance (ANOVA) with Tukey's test was used for multiple-group comparisons, and a *P* value of <0.05 was considered significant.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, DOCX file, 0.1 MB.

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Z.Y. and R.T.S. developed the concept and design of the study. Z.Y. and Y.H. assisted with conducting experiments. Z.Y., Y.H., and R.T.S. provided analysis and interpretation of experiments and results. Z.Y. and R.T.S. wrote the manuscript.

We declare no conflict of interest.

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