

# Targeting DNA Methyltransferase 1 Limits Vesicular Stomatitis Virus Through Enhancing IRF3/IFN Signaling

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## Research

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# Abstract

## Background

Viruses develop strategies to escape from host anti-viral response in many aspects including hijacking host epigenetic factors. However, the relationship between host DNA methyltransferase 1 (DNMT1) and vesicular stomatitis virus (VSV) replication is largely unknown.

## Methods

In the present study, we performed Western Blotting, qRT-PCR, RNA interference, Methylation-specific PCR and Cell viability assay to uncover the role of DNMT1 in the process of VSV replication.

## Results

We have observed VSV infection enhances DNMT1 protein accumulation in macrophage cell. Furthermore, DNMT1 functional blocking and gene silencing limit VSV replication. Moreover, loss of DNMT1 increases interferon responses, including *Irf3* and IFN-stimulated genes (ISGs) upregulation. CpG islands (CGIs) of *Irf3* promoter region are demethylated after DNMT1 short-term inhibition with thioguanine, which is accompanied by *Irf3* transcript upregulation. Meanwhile, *Irf3* silencing largely reversed DNMT1 loss-suppressed VSV replication. What is more, the basal level of endogenous retrovirus (ERV) transcripts is required for thioguanine-induced ISGs.

## Conclusions

DNMT1 loss-induced IRF3 enhancement leads to interferon responses and subsequent VSV suppression, which provides a potential strategy to inhibit viral replication by targeting DNMT1 with its inhibitor.

## Background

Innate immune response plays a critical role in detecting viruses and subsequent mounting of adaptive immune responses [1]. It recognizes viruses by harnessing intrinsic pattern-recognition receptors (PRRs) [2–4]. As the key PRRs, retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and Toll-like receptors (TLRs) are critical for producing type I interferon (IFN-I), which activates Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway and induces IFN-stimulated genes (ISGs) [5]. After sensing of viruses, PRRs trigger downstream transcriptional cascades in chromatin level, which is mainly determined by epigenetic regulators [6]. Meanwhile, viruses also develop different strategies to target host epigenetic machinery to complete the life cycles. For instance, Respiratory syncytial virus infection elevates histone demethylase KDM5B to impair IFN-I production [7]. Epstein-Barr virus (EBV) infection not only downregulates the level of Tet methylcytosine dioxygenase 2 (TET2) but also upregulates DNMT1 level to maintain specific DNA methylation profile [8-11]. Additionally, HIV infection increases DNMT3b expression and *FOXP3* promoter methylation, thus influences the function of

regulatory T cells [12]. These evidences highlight that epigenetic machinery is deeply involved in the pathological processes after virus infection.

DNA methylation plays important roles in regulating gene expression through DNA methyltransferases (DNMTs). DNMTs silences gene expression through making CpG dinucleotides methylated [13-15]. There are three members of vertebrate DNMTs: DNMT1, DNMT3a and DNMT3b. The substrate of DNMT3a and DNMT3b are hemimethylated and unmethylated DNA, while DNMT1 prefers hemimethylated DNA and thus maintains the patterns of DNA methylation during the process of DNA replication [16,17]. It is reported that after hepatitis B virus (HBV) infection, host DNMTs is regulated by viral X protein to establish aberrant epigenetic modifications [18,19].

In the present study, we report VSV infection leads to DNMT1 protein accumulation in macrophage cell. Then, we examined VSV replication after DNMT1 functional blocking and gene silencing. Furthermore, in the context of DNMT1 inhibition, interferon responses, IRF3 expression, *Irf3* promoter DNA methylation and endogenous retrovirus (ERV) transcripts expression were studied to explore the underlying mechanisms.

## Materials And Methods

### Cell, Virus and reagents

Murine macrophage cell line RAW264.7 and HEK293 cell were cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO), supplemented with 10% fetal bovine serum (FBS, GIBCO), 100 IU penicillin/mL and 100 mg/mL streptomycin (GIBCO) at 37°C and 5% CO<sub>2</sub>. Vesicular Stomatitis virus serotype Indiana (VSV-IND) and VSV-eGFP were preserved in our laboratory. Anti-DNMT1 and anti-GAPDH antibody were purchased from Cell Signaling Technology (CST); VSV-G antibody was purchased from Abcam; Ultra-LEAFTM Purified anti-mouse IFN-β antibody was purchased from Biolegend; Thioguanine and Ruxolitinib was purchased from selleck; TLR3/dsRNA complex inhibitor was purchased from Calbiochem.

### Gene silencing and cell transfection

Plasmids psPAX2, pMD2G and pLV (shRNA-*Dnmt1*) or pLV (shRNA-*Irf3*) were co-transfected into HEK293T cells for lentivirus packaging. RAW264.7 cell was then transfected with the packaged lentivirus particles. The shRNA targeting murine *Dnmt1* is as follow: TATATGAAGACCTGATCAA-TACTCGAGTATTGATCAGGTCTTCATATA. shRNA targeting murine *Irf3* is as follow: GCGGTTAGCTGCTGACAATAGTTCAAGAGACTATTG- TCAGCAGCTAACCGC.

### qRT-PCR

Cells were collected after washing with PBS and RNA from the cells was extracted by using RNAiso Plus reagent. Then cDNA synthesis was done by using Reverse Transcriptase M-MLV. Real-time PCR assay was then performed to determine the transcriptional level of the genes. *Gapdh* gene was used as the

reference control for immune genes and *Hprt* gene was used as the reference control for ERV genes. The data was normalized to *Gapdh* and *Hprt* gene level separately. Method of  $2^{-\Delta\Delta Ct}$  has been used to present relative expression of each gene. (Table has listed all of the primers used in this paper).

### **Cell viability assay (CCK8 assay)**

Murine Raw264.7 cells were seeded into 96-well plates before treatment. Cells were treated with Thioguanine (1 $\mu$ M, 2.5 $\mu$ M, 5 $\mu$ M, 10 $\mu$ M) or DMSO for 6 and 12h. Metabolic activity of the cells was treated with CCK8 reagent 20min before testing, cell viability was measured and quantified by using TECAN plate spectrophotometer at 450 nm absorbance.

### **Western Blotting**

Culturing cells were washed with PBS twice and then lysed by lysis buffer supplemented with 0.01% EDTA, 0.1% Triton X-100 and protease inhibitor cocktail. After protein concentration determination, cell extracts were performed for Western blotting by using antibodies of DNMT1 (1:1000, CST), VSV-G (1:1000, Abcam), and GAPDH (1:1000, CST).

### **Methylation-specific PCR**

Genomic DNA extracted from cells were converted with 3.6M Bisulfite for 12h, and then purified using the DNA clean-up system. Bisulfite modified DNA was amplified using gene specific primers designed from the online software MethPrimer. Primers for methylated *Irf3* promoter region are: Forward 5'-TTGGTATTTAGTTTATTTGGTTCGC-3'(MF2) and Reserve 5'-CCCTTCGACGTTAAAACGA-T-3'(MR2). Primers for unmethylated *Irf3* promoter region are: Forward 5'-GGTATTTAGTTTATTTGGTTTGTGG-3'(UF2) and Reserve 5'-CACCCCTTCAACATTTAAAACAAT-3'(UR2).

### **Statistical analysis**

Unpaired Student's t-test as well as one-way ANOVA Tukey test were performed for the statistical analysis. \* P < 0.05 was considered as significant (\* P<0.05, \*\* P<0.01, \*\*\* P<0.001). Error bars indicate standard error.

## **Results**

### **DNMT1 inhibition impairs VSV replication**

In the present work, we have found that host DNMT1 protein level in murine macrophage cell RAW264.7 has accumulated shortly (6h and 12h) after VSV (MOI = 1) infection, which is shown by Western Blotting assay (Fig.1a). In order to explore if host DNMT1 is required in the process of virus replication, we pre-treated Raw264.7 cell with DNMT1 specific inhibitor Thioguanine (1 $\mu$ M, 2.5 $\mu$ M, 5 $\mu$ M, 10 $\mu$ M) for 3h, which was followed by VSV-eGFP infection (MOI=1) for 12h. After VSV-eGFP infection, the GFP fluorescence of the cells in each well have been detected by Tecan absorbance reader. The result shows that Thioguanine

treatment is able to significantly impair EGFP signal, which demonstrates VSV propagation has been impaired significantly by DNMT1 inhibition (Fig. 1b). Additionally, Thioguanine effect on VSV-eGFP propagation has also been displayed in fluorescence microscope observation (Fig. 1c). Furthermore, we treated macrophage cells with Thioguanine (1 $\mu$ M, 2.5 $\mu$ M, 5 $\mu$ M and 10 $\mu$ M) at different time points (6h and 12h), which was followed by CCK8 cell viability assay. We have found Thioguanine treatment could not affect cell viability at both time points, indicating the Thioguanine is not toxic to the cells (Fig. 1d). Moreover, in order to test if DNMT1 inhibition also affects VSV viral titer, we pre-treated the cells with Thioguanine 3h before VSV infection. 12h after VSV infection, cells were collected and VSV-G protein level was determined by performing Western Blotting assay. The results show that Thioguanine treatment significantly inhibits VSV replication (Fig. 1e). The above results demonstrate that DNMT1 pharmacological inactivation could impair VSV replication in host cells.

### ***Dnmt1* gene silencing impairs VSV replication**

To further confirm the role of DNMT1 during VSV replication, we also established a *Dnmt1* gene silencing macrophage cell line through expressing *Dnmt1* gene targeting shRNA, which is delivered by Lentivirus. Based on the examination of Western Blotting assay, and found DNMT1 protein has been largely reduced (Fig. 2a). The above assays demonstrate DNMT1 in macrophage cell has been successfully silenced. Then *Dnmt1* silencing cell line and a control cell line were infected with VSV (MOI=1). 12h after virus infection, cells were collected for the determination of VSV-G protein level. Western Blotting assay shows VSV-G protein level has been largely reduced in *Dnmt1* silencing group compared with control group (Fig. 2b). The above results demonstrate that *Dnmt1* silencing could also impair VSV replication in murine macrophage cells, suggesting a supportive role of DNMT1 during VSV replication.

### **Loss of DNMT1 causes interferon responses**

To explore the mechanism of DNMT1 loss-caused VSV suppression, we examined the level of IFN-stimulated genes (ISGs) after DNMT1 inhibition and *Dnmt1* gene silencing. We have found in qRT-PCR assay that Thioguanine treatment (10h) significantly upregulated the expression of a series of ISGs genes (Fig. 3a). This is also the case when *Dnmt1* gene was silenced, as *Dnmt1* knocking down induced the upregulation of a panel of ISGs (Fig. 3b). Furthermore, as ISGs expression is believed to be triggered by secreted IFN $\beta$ -activated JAK/STAT signaling, we tested *Ifnb1* expression by performing qRT-PCR assay and have found that *Ifnb1* mRNA level was highly enhanced after Thioguanine short term treatment (10h) or *Dnmt1* gene silencing (Fig. 3c and d). Moreover, we have also observed that IFN $\beta$  antibody blocking could fully reverse Thioguanine-induced ISG response, indicated by the transcriptional change of *Mx1* and *Ifit3* gene (Fig. 3e and f). What is more, the observation that JAK/STAT inhibitor ruxolitinib fully reversed Thioguanine-induced ISG response further confirmed Thioguanine short-term treatment actually activates type I interferon signaling (Fig. 3g and h). The above results demonstrate that Thioguanine-induced ISGs upregulation is mainly mediated through IFN $\beta$  secretion and subsequent JAK/STAT activation.

### **IRF3 plays a critical role to suppress VSV in the context of DNMT1 loss**

IRF3 is required in the production of IFN $\beta$ . In order to uncover DNMT1 loss-induced *Irf3* and ISGs upregulation, we detected *Irf3* level after DNMT1 inhibition, and found *Irf3* level was significantly upregulated by Thioguanine treatment (10h) (Fig. 4a), and this is also the case when *Dnmt1* was knocking down (Fig. 4b). The finding suggests Thioguanine-induced IRF3 may be involved in the above ISGs up-regulation and VSV repression. To confirm this, we silenced *Irf3* gene in the cells and have found *Irf3* gene silencing restored Thioguanine-impaired VSV-G level (Fig. 4c). At the same time, *Irf3* knocking down could also reverse *Dnmt1* silencing-impaired VSV-G level (Fig. 4d). Furthermore, based on CGIs searching through MethPrimer software, CGIs were rich in *Irf3* promoter region (-448 to -295 and -197 to -11). To explore if *Irf3* upregulation is due its promoter CGI demethylation, we performed Methylation-Specific PCR (MSP) assay (Fig. 4e). We have found the CGI methylation modification (-197 to -11 region) of *Irf3* promoter has been largely reduced after Thioguanine treatment (Fig. 4f). The above results demonstrate that IRF3 plays a critical role in VSV suppression in the context of DNMT1 loss, which could be explained by the CGI demethylation in *Irf3* promoter.

### **Thioguanine short-term treatment induces interferon response through dsRNA sensor TLR3 but does not increase endogenous retrovirus (ERV) transcripts**

It has been reported that long-term inhibition (Aza, 7-10 days) of DNMTs upregulates a series of endogenous retrovirus (ERV) transcripts, which interact with and activate TLR3/dsRNA complex, further upregulates ISGs in human cancer cells [20,21]. In the context of thioguanine short-term treatment (10h), we tested the level of murine ERV transcripts, but found ERV level was not significantly affected, indicating Thioguanine-induced ISGs upregulation is not due to the increase of ERV transcripts (Fig. 5a). However, Real-time PCR assay displays relative early amplification (CT value between 20-22) of murine ERV transcripts, suggesting the basal level of ERV may play a role (Fig. 5b). To confirm this, we blocked dsRNA sensor by treating the cells with TLR3/dsRNA complex inhibitor, which was followed by Thioguanine treatment. It shows that TLR3/dsRNA inhibitor is able to fully reverse Thioguanine-induced ISG (*Mx1* and *Ifit3*) upregulation (Fig. 5c and d). Moreover, we find TLR3/dsRNA inhibitor could also suppress the basal level of ISG (*Mx1* and *Ifit3*) expression without Thioguanine induction (Fig. 5c and d). These above results suggest the basal level of ERV transcripts is required for Thioguanine-induced ISGs upregulation.

## **Discussion**

Virus infection has been reported to regulate the expression of different epigenetic modifiers to establish persistent infection [7,8,22], which is consistent with our findings that DNMT1 protein is elevated shortly after VSV infection. So, it is reasonable to speculate DNMT1 is involved in the process of VSV replication, and this is verified by thioguanine-suppressed VSV replication. Interestingly, thioguanine not only blocks DNMT1 function, but also induces degradation of DNMT1 protein [23,24]. Additionally, DNMTs inhibitors has been approved to treat cancers, causing cell apoptosis [25]. Based on the above characteristic, cell viability assay was performed and we confirmed thioguanine has no toxicity to the cells within 12h treatment, excluding the possibility that the effect is due to the impaired cell viability. Furthermore, *Dnmt1*

silencing also suppressed VSV, which exclude the possibility that the anti-viral effect is a pharmacological off-target effects, as off-targets may produce undesirable non-specific cross-talks and effects [26].

Loss of DNMT1 induces ISGs upregulation. However, their promoters are rarely methylated, as classical CGIs were usually absent in their promoters [27]. Transcriptome analysis explains the absence of CGIs in ISG promoters is due to the requirement of nucleosome occupancy [28]. Actually, ISGs are directly regulated by IFN-stimulated gene factor 3 (ISGF3), which recognizes and binds to IFN-stimulated response elements (ISREs) in gene promoters [29-31]. ISGF3 is composed of signal transducer and activator of transcription 1 (STAT1), STAT2 and IFN-regulatory factor 9 (IRF9), triggered by IFN $\beta$  [32,33]. Indeed, we found IFN $\beta$  blocking and JAK/STAT inhibition fully reversed thioguanine-induced ISGs increase, which further suggests thioguanine does not affect ISGs expression directly. As expected, *Ifnb1* level is also induced by DNMT1 loss, but CGIs were absent in *Ifnb1* promoter [27]. Actually, *Ifnb1* transcription is mainly directed by IRF-3/7 homodimers and/or heterodimers [34, 35]. Then, we found *Irf3* level was upregulated after DNMT1 inhibition, which is due to the CGIs demethylation of *Irf3* promoter. It is reported that the increased (or overexpressed) level of IRF3 is able to enhance IRF3/IFN activity and induce downstream ISGs expression in different species, which may explain the above observation [36-38]. Importantly, we confirmed the critical role of IRF3 through the evidence that *Irf3* silencing largely reversed DNMT1 loss-caused VSV suppression.

Double-stranded RNA (dsRNA) triggers protective interferon response through cytosolic RNA sensors [39]. And long-term inhibition of DNMTs induces interferon in human cancer cells, which is mediated through endogenous retroviruses (ERV)-derived dsRNA. Additionally, cells with higher basal level of ERV is correlated with higher ISGs expression [20]. In our present work, we have tested the level of several commonly studied ERV transcripts in Raw264.7 cell and found they all display a relative early amplification, suggesting the possibility of high basal activation of RNA sensors, which may be further amplified by enhanced IRF3 (Fig. 5b). Furthermore, to explore the involved RNA sensors, we blocked the function of TLR3 and found TLR3 inhibition fully reverses thioguanine-induced ISGs level as well as the basal level of ISGs, demonstrating other RNA sensors like RIG-I and MDA5 may not be involved. Consistent with our above observation, MDA5 has been reported to recognize long dsRNA, which is absent in uninfected host cell [1]. Interestingly, we found that thioguanine short-term treatment does not change the levels of commonly studied murine ERV transcripts. This suggests the interferon response enhancement is not due to the increase of ERV transcripts, which is different from DNMTs long-term inhibition-caused effect [20].

## Conclusion

Collectively, we highlight the importance of host DNMT1 for VSV replication, and DNMT1 loss-caused VSV suppression is triggered by epigenetic elevated IRF3 and subsequent interferon responses. From this work we may speculate that the existence of host DNMT1 may gain time for the virus to replicate continuously in host cell through tightly controlling IFN activation.

# Abbreviations

VSV: Vesicular Stomatitis Virus; DNMT1: DNA methyltransferase1; CGIs: CpG islands; dsRNA: Double-stranded RNA; IRF3: IFN-regulatory factor 3; ISGs: IFN-stimulated genes; ISREs: IFN-stimulated response elements; ISGF3: IFN-stimulated gene factor 3; STAT: signal transducer and activator of transcription; ERV: Endogenous retroviruses; shRNA: short hairpin RNA; MSP: Methylation-Specific PCR; MOI: multiplicity of infection.

# Declarations

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Availability of data and materials

All data generated or analysed during this study are included in this published article.

## Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

Kui Zhao, Fei Gao, Ye Lin and Jing Lin carried out all experiments and drafted the manuscript. Bo Zhang, Deguang Song, Zi Li, Yungang Lan, Huijun Lu and Wenqi He analyzed data, discussed the results, and revised the manuscript. Jiyu Guan designed the study and supervised the project. Corresponding author: Jiyu Guan, jiygua@jlu.edu.cn. All authors have read and agreed to the published version of the manuscript.

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## Tables

**Table 1:** Primer sequence for real-time PCR in this manuscript

<i>lrf3</i>	F	TCCAGGGGCGCGGGACTTCGTAC
<i>lrf3</i>	R	ACATTGGGGCTTGGCAGTTGTTG
<i>lfnb1</i>	F	ATGAGTGGTGGTTGCAGGC
<i>lfnb1</i>	R	TGACCTTTCAAATGCAGTAGATTC
<i>Gapdh</i>	F	GGTGAAGGTCGGTGTGAACG
<i>Gapdh</i>	R	CTCGCTCCTGGAAGATGGTG
<i>VSV P</i>	F	GTGACGGACGAATGTCTCATAA
<i>VSV P</i>	R	TGATGAATGGATTGGGATAACA
<i>Dnmt1 (Primer Bank ID 31419356a1)</i>	F	AAGAATGGTGTGTCTACCGAC
<i>Dnmt1 (Primer Bank ID 31419356a1)</i>	R	CATCCAGGTTGCTCCCCTTG
<i>Mx1 (Primer Bank ID 6996930a1)</i>	F	GACCATAGGGGTCTTGACCAA
<i>Mx1 (Primer Bank ID 6996930a1)</i>	R	AGACTTGCTCTTTCTGAAAAGCC
<i>Mx2 (Primer Bank ID 7549779a1)</i>	F	GAGGCTCTTCAGAATGAGCAAA
<i>Mx2 (Primer Bank ID 7549779a1)</i>	R	CTCTGCGGTCAGTCTCTCT
<i>Ifi44 (Primer Bank ID 19527086a1)</i>	F	AACTGACTGCTCGCAATAATGT
<i>Ifi44 (Primer Bank ID 19527086a1)</i>	R	GTAACACAGCAATGCCTCTTGT
<i>Ifih1 (Primer Bank ID 23956208a1)</i>	F	AGATCAACACCTGTGGTAACACC
<i>Ifih1 (Primer Bank ID 23956208a1)</i>	R	CTCTAGGGCCTCCACGAACA
<i>Ifit2 (Primer Bank ID 6680363a1)</i>	F	AGTACAACGAGTAAGGAGTCACT
<i>Ifit2 (Primer Bank ID 6680363a1)</i>	R	AGGCCAGTATGTTGCACATGG
<i>Ifit3 (Primer Bank ID 6754288a2)</i>	F	CCTACATAAAGCACCTAGATGGC
<i>Ifit3 (Primer Bank ID 6754288a2)</i>	R	ATGTGATAGTAGATCCAGGCGT
<i>Syna (Primer Bank ID 333033800c1)</i>	F	ATGGTTCGTCCTTGGGTTTTTC
<i>Syna (Primer Bank ID 333033800c1)</i>	R	GTGTTGAGTGAGGTTTACCAGG
<i>Synb (Primer Bank ID 27734142a1)</i>	F	TGGGTCCTCTGTTTCGTCCTT
<i>Synb (Primer Bank ID 27734142a1)</i>	R	GGGAAGAGTTGGTATCACGTAGG
<i>eMLV env</i>	F	CCAGGGACCACCGACCCACCGT
<i>eMLV env</i>	R	TAGTCGGTCCCGGTAGGCTCG

<i>MMTV env</i>	<i>F</i>	<i>AGAGCGGAACGGACTCACCA</i>
<i>MMTV env</i>	<i>R</i>	<i>TCAGTGAAAGGTCGGATGAA</i>
<i>Emv2</i>	<i>F</i>	<i>CCTGGGTTTGCGGAAATGGCAC</i>
<i>Emv2</i>	<i>R</i>	<i>TTTGGCGTAGCCCTGCTTCTCG</i>
<i>Hprt</i>	<i>F</i>	<i>TTGTATACCTAATCATTATGCCGAG</i>
<i>Hprt</i>	<i>R</i>	<i>CATCTCGAGCAAGTCTTTCA</i>

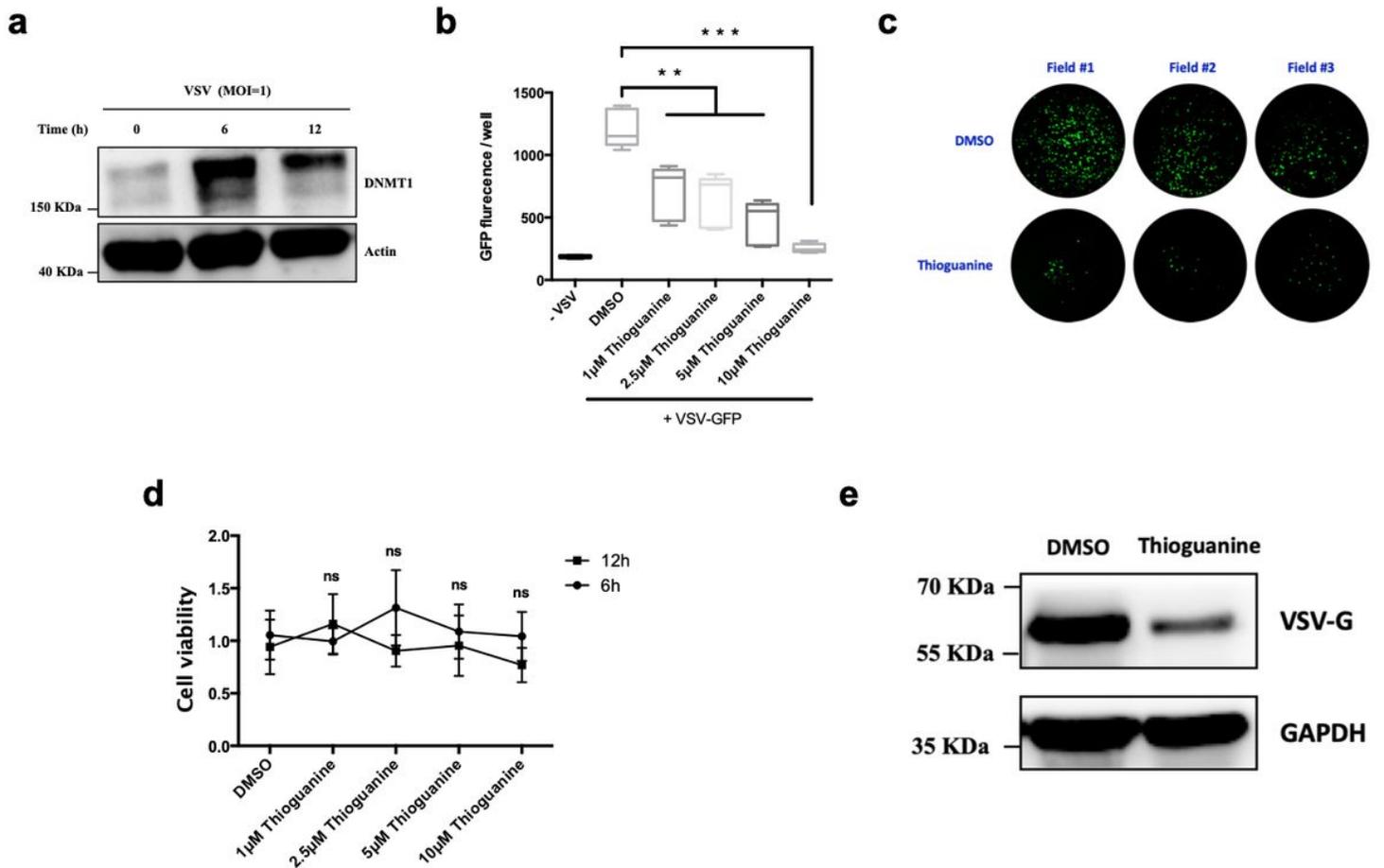
The sequences of primers marked with Primer Bank ID were obtained directly from Primer bank (<https://pga.mgh.harvard.edu/primerbank/index.html>)[1]

VSV P primer design is based on the published article[2]. eMLV env, MMTV env, Emv2 and Hprt primers design are based on the published article[3].

## Reference

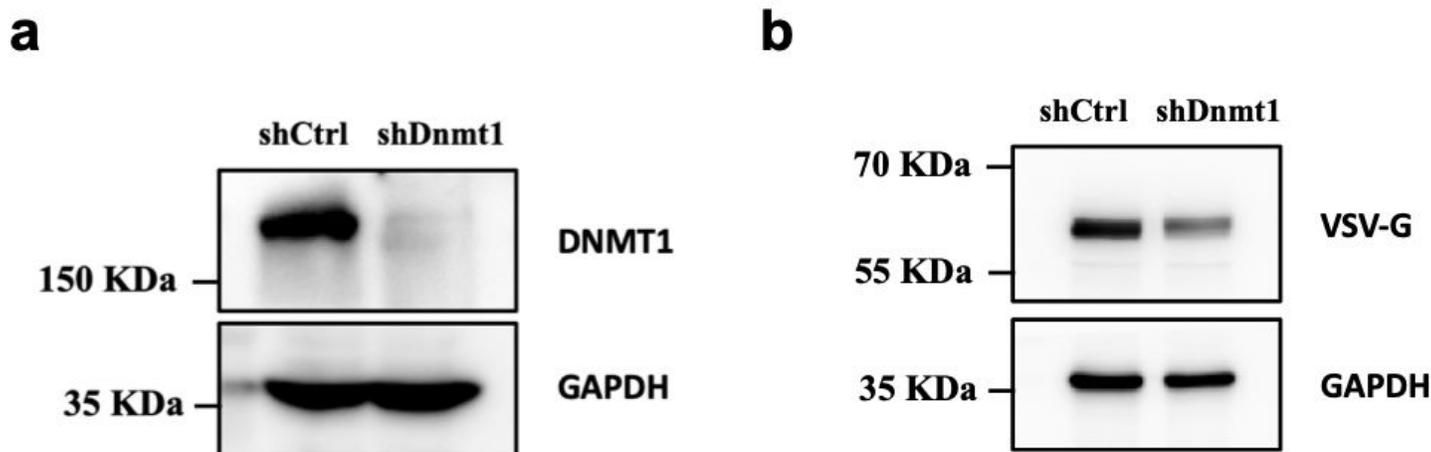
1. Spandidos A, Wang X, Wang H, Seed B, PrimerBank: a resource of human and mouse PCR primer pairs for gene expression detection and quantification. *Nucleic Acids Res.* 2010; 38: 792-799.
2. Das A, Dinh PX, Panda D, Pattnaik AK, Interferon-inducible protein IFI35 negatively regulates RIG-I antiviral signaling and supports vesicular stomatitis virus replication. *J. Virol.* 2014; 88: 3103–3113.
3. Young GR, Eksmond U, Salcedo R, Alexopoulou L, Stoye JP, Kassiotis G, Resurrection of endogenous retroviruses in antibody-deficient mice. *Nature.* 2012; 491: 774–778.

## Figures



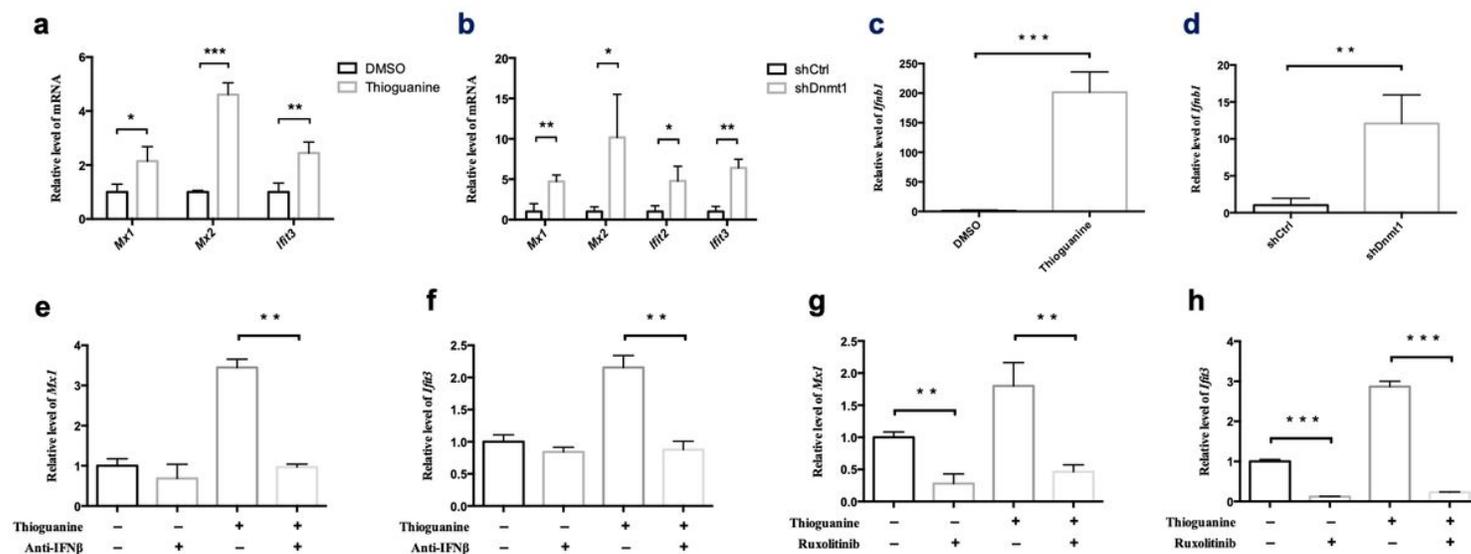
**Figure 1**

VSV replication was inhibited by Thioguanine treatment. a, Western Blotting assay was performed after RAW264.7 cells were infected with VSV (MOI=1) for indicated time points. b-c, Cells pre-treated with DNMT1 inhibitor thioguanine were infected by VSV-eGFP (MOI=1) for 12h. Different Thioguanine concentrations was set up (1µM, 2.5µM, 5µM, 10µM). The cells infected with VSV-eGFP displays EGFP green fluorescence, and cells were detected with Tecan absorbance reader and fluorescence microscope separately. Unpaired Student's t-test was performed for statistical analysis and the results were presented as means ± SEM (n=6). \*\* P<0.01, \*\*\* P<0.001. d, Cells pre-treated with 5µM thioguanine were tested by CCK8 assay to study cell viability. Unpaired Student's t-test were performed for the statistical analysis and results were presented as means ± SEM. ns, not significant. e, Cells pre-treated with 5µM thioguanine were infected with VSV (MOI=1) for 12h, then VSV-G level was determined.



**Figure 2**

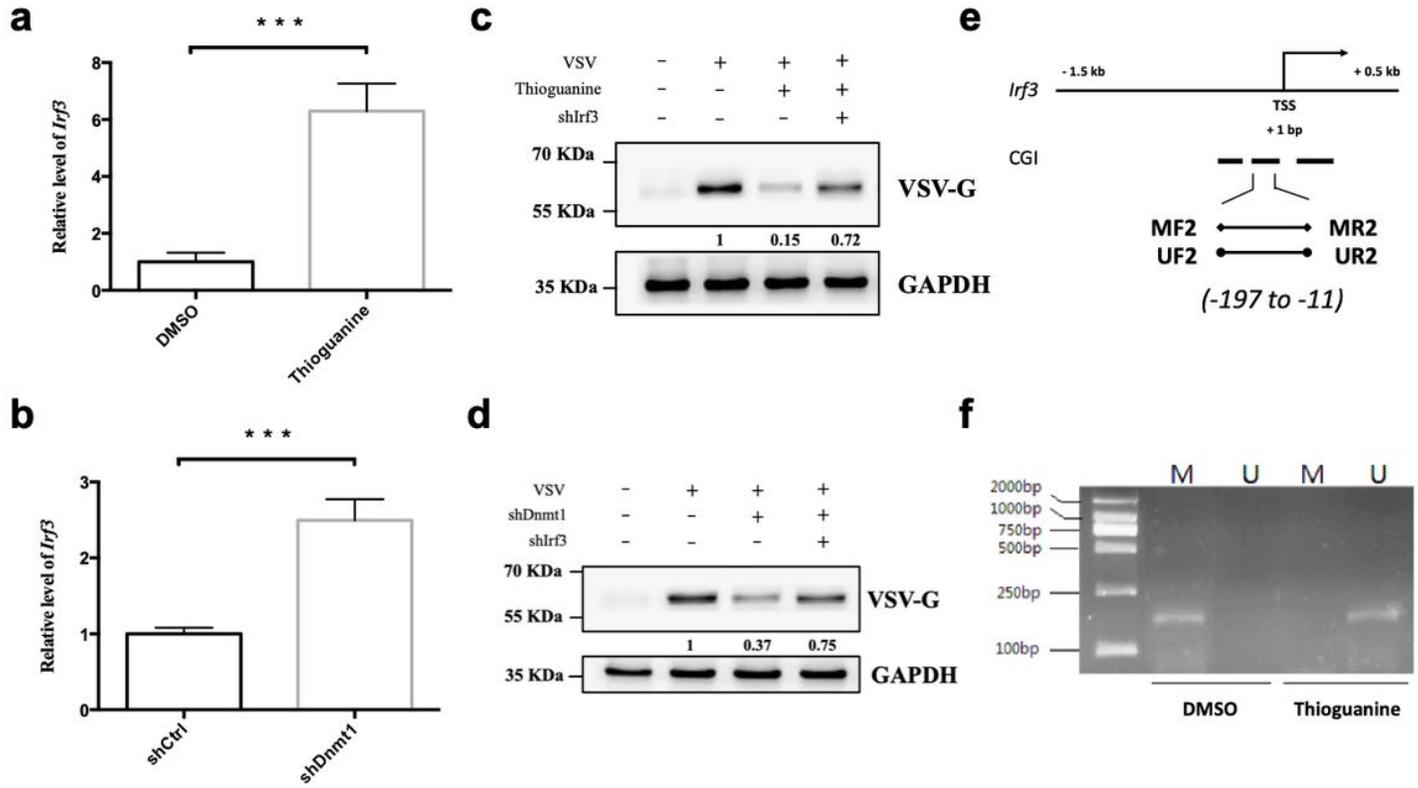
VSV replication was inhibited by Dnmt1 silencing. a, Cells were transfected with shRNA-Dnmt1-lentivirus which was followed by puromycin selecting to establish Dnmt1 silencing cell line, DNMT1 protein level was determined by Western blotting assay. b, Western blotting was performed to determine VSV-G protein level after VSV (MOI=1) infection for 12h.



**Figure 3**

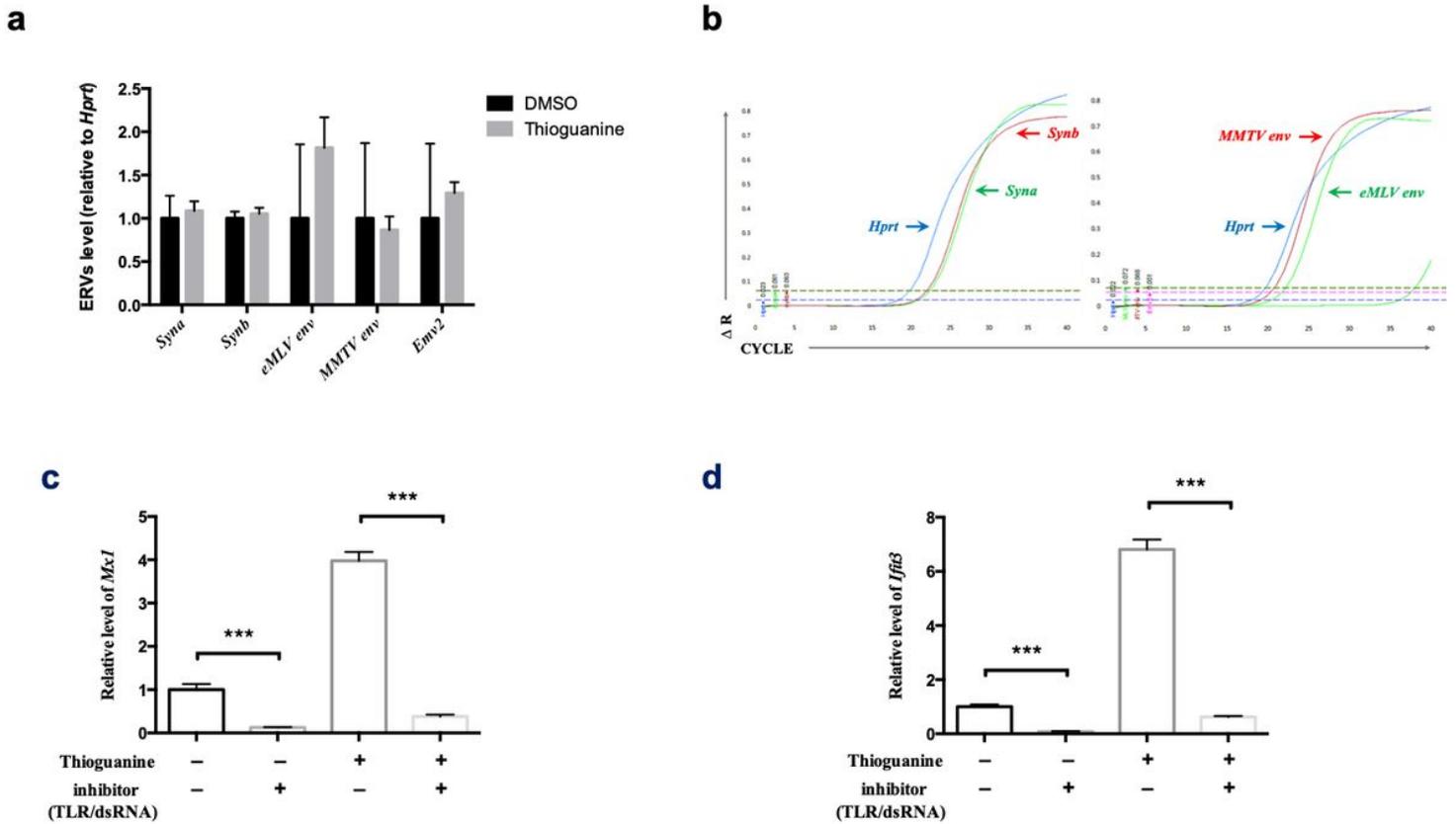
Loss of DNMT1 increase interferon responses. a-d, qRT-PCR assay was performed after RAW264.7 cells were treated with 5 $\mu$ M thioguanine for 10h, the mRNA level of ISGs (a) and *Ifn1* (c) were examined. qRT-PCR assay was performed with Dnmt1 silencing cells and control cells, the mRNA level of ISGs (b) and *Ifn1* (d) were examined. Unpaired Student's t-test was performed for statistical analysis and the results were presented as means  $\pm$  SEM (n=3). \* P<0.05, \*\* P<0.01, \*\*\* P<0.001. e-f, qRT-PCR assay was performed after cells were treated with thioguanine (5 $\mu$ M), anti-IFN $\beta$  blocking antibody (0.1 $\mu$ g/ml) and thioguanine/antibody combination, the mRNA level of *Mx1* (e) and *Ifi3* (f) were examined. g-h, qRT-PCR assay was performed after cells were treated with thioguanine (5 $\mu$ M), ruxolitinib (2 $\mu$ M) and thioguanine/

roxolitinib combination, mRNA level of Mx1 (g) and Ifit3(h) were examined. Data analysis was performed by one-way ANOVA Tukey test and the results were presented as means  $\pm$  SEM (n=3). \*\* P<0.01, \*\*\* P<0.001.



**Figure 4**

Loss of DNMT1-increased IRF3 is critical for VSV suppression. a-b, qRT-PCR assay was performed after cells were treated with 5 $\mu$ M thioguanine (a) or transfected with Dnmt1 silencing shRNA (b), mRNA level of *Irf3* was examined. Unpaired Student's t-test was performed for statistical analysis and the results were presented as means  $\pm$  SEM (n=3). \*\*\* P<0.001. c, *Irf3* silenced cells and control cells were pre-treated with 5 $\mu$ M thioguanine or DMSO before VSV (MOI=1) infection, Western Blotting was performed to detect VSV-G protein level. d, *Irf3* silenced cells and control cells were transfected with shRNA-Dnmt1-lentivirus or shRNA-control-lentivirus before VSV (MOI=1) infection, Western Blotting assay was performed to detect VSV-G protein level. e, Schematic presentation of *Irf3* promoter region and methylation-specific PCR (MSP) primers design. TSS, transcriptional start site. CGI, CpG islands. MF2 and MR2 represent primers for methylated fragment detection and UF2 and UR2 represent primers for unmethylated fragment detection. f, Genomic DNA from cells treated with 5 $\mu$ M thioguanine or DMSO were extracted, then treated with Bisulfite for MSP assay.



**Figure 5**

Thioguanine short-term treatment-induced interferon response is dependent on basal activity of TLR3, but not through ERV transcripts increase. a, qRT-PCR assay was performed after cells were treated with 5 $\mu$ M thioguanine for 10h. mRNA level of murine ERV transcripts were determined. b, Gene amplification curve. Real-time PCR assay displaying murine gene (Hprt, Syna, Synb, eMLV env, MMTV env and Emv2) amplification curve. c-d, qRT-PCR assay was performed after cells were treated with 5 $\mu$ M thioguanine, TLR/dsRNA complex inhibitor and thioguanine/inhibitor combination. mRNA level of *Mx1* (c) and *Ifit3* (d) were determined. Data analysis was performed by one-way ANOVA Tukey test and the results were presented as means  $\pm$  SEM (n=3). \*\*\* P<0.001.

## Supplementary Files

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