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RESEARCH ARTICLE

ZDHHC11 modulates innate immune response to DNA virus by mediating MITA–IRF3 association

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MITA is a central adaptor in innate immune responses to DNA viruses. The mechanisms responsible for recruitment of downstream kinase TBK1 and the transcription factor IRF3 to MITA remains enigmatic. Here we identified ZDHHC11, a member of DHHC palmitoyl transferase family, as a positive regulator of DNA virus-triggered signaling. Overexpression of ZDHHC11 activated the IFN-β promoter, while ZDHHC11-deficiency specifically impaired DNA virus HSV-1-induced transcription of downstream antiviral genes. *Zdhhc11*$^{-/-}$ mice exhibited lower serum cytokine levels and higher lethality after HSV-1 infection. Mechanistically, ZDHHC11 facilitated the optimal recruitment of IRF3 to MITA. Our findings support an important role for ZDHHC11 in mediating MITA-dependent innate immune responses against DNA viruses.
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Keywords: ZDHHC11; MITA; IRF3; innate immune response

INTRODUCTION

The innate immune response is crucial for defending the host against viral infection. On viral infection, structurally conserved viral molecules called pathogen-associated molecular patterns (PAMPs) are recognized by pattern-recognition receptors (PRRs) in host cells, which trigger signaling cascades that lead to the production of type I interferons (IFNs), proinflammatory cytokines and other downstream antiviral proteins. These effector proteins function to impede viral replication, get rid of virus-infected cells, and facilitate the initiation of adaptive immune response.[1,2]

Viral DNAs or RNAs serve as typical PAMPs to initiate innate immune responses. Cyclic GMP-AMP (cGAMP) synthase (cGAS), a nucleotidyl transferase family member, was found to be a cytoplasmic DNA sensor in mammalian cells and in mice.[3–6] Upon sensing viral DNA, cGAS catalyzes production of the second messenger molecule cGAMP, which associates with the adaptor MITA (also known as STING, ERIS or MPYS) in the endoplasmic reticulum (ER).[5,7–12] The cGAMP-bound MITA is translocated from the endoplasmic reticulum (ER) through the Golgi apparatus to perinuclear microsomal compartments, where MITA acts as an adaptor to bring IRF3 in close proximity to TBK1.[13] This protein aggregation causes TBK1-dependent phosphorylation and nuclear translocation of the transcriptional factor IRF3, eventually leading to induction of type I IFNs and downstream antiviral genes. However, the precise mechanisms underlying how IRF3 is recruited into the MITA-TBK1 signalosomes remains poorly understood.

In the present study, we found that ZDHHC11 is a novel regulator of MITA-dependent innate immune responses against DNA viruses. Overexpression of ZDHHC11 activates the IFN-β promoter in reporter assays, while Zdhhc11-deficiency impaired DNA- but not RNA virus-triggered production of type I IFNs and other cytokines. *Zdhhc11*$^{-/-}$ mice exhibited lower cytokine levels after HSV-1 infection and were more sensitive to HSV-1-induced death. Furthermore, we found that ZDHHC11 facilitated the association of MITA with IRF3. These data suggest that ZDHHC11 enhances MITA-mediated innate immune responses against DNA viruses by linking IRF3 to MITA.

MATERIALS AND METHODS

Reagents, antibodies, viruses and cells

SYBR (Bio-Rad, Hercules, CA, USA); RNase inhibitor (Thermo Fisher, Waltham, MA, USA); Poly(I:C) and lipofectamine 2000 (Thermo Fisher); GM-CSF (PeproTech, Rocky Hill, NJ, USA); polybrene (Merck, Darmstadt, Germany); mouse monoclonal

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antibodies against HA (Covance, Princeton, NJ, USA), Flag and β-actin (Sigma, St Louis, MO, USA); rabbit polyclonal antibodies against TBK1, and phospho-TBK1(S172) (Abcam, Cambridge, UK); rabbit polyclonal antibodies against IRF3 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and phospho-IRF3(S396) (Cell Signaling Technology, Danvers, MA, USA); MITA and phospho-STING/MITA (S366) (Cell Signaling Technology); ELISA kits for murine Ifn-α and Ifn-β (PBL, Piscataway, NJ, USA), and Tnfα and Il-6 (Biolegend, San Diego, CA, USA) were purchased from the indicated manufacturers. Herpes simplex virus 1(HSV-1), Sendi virus (SeV), Encephalomyocarditis virus (EMCV) were previously described.[12,14,15] HEK293 cells, HeLa cells and THP1 cells were obtained from ATCC (Manassas, VA, USA).

Expression screening

A collection of cDNA expression clones encoding ~10 000 independent human proteins were purchased from Origene. Clones were individually transfected into HEK293 cells along with an IFN-β promoter reporter plasmid for 20 h prior to luciferase assays.

Constructs

IFN-β, ISRE and NF-κB luciferase reporter plasmids and mammalian expression plasmids for HA-tagged cGAS, HA-tagged TBK1, HA- and Flag-tagged MITA, IRF3-5D, HA- and Flag-tagged IRF3 were previously described.[12,16,17] The complete coding region for human *ZDHHC11* was amplified from HEK293 cDNA and cloned in frame into the pRK-Flag to make the pRK-ZDHHC11-Flag mammalian expression vector. Similarly, the HA-tagged mammalian expression vector for ZDHHC11 was constructed. Expression constructs for truncations of human ZDHHC11 (aa1–197 and aa198–412), HA-MITA (aa1–190 and aa191–379) and Flag-IRF3 (aa1–140 and aa141–427) were PCR amplified and cloned into the pRK-Flag vector. Point mutations for ZDHHC11 mutants (D155A&H156A, C158S) were constructed according to the manual for QuickChange (Stratagene, La Jolla, CA, USA).

DNA oligonucleotide

B-DNA: 18 base-pair dsDNA with a sequence of poly(dA-dT)·poly(dT-dA).

Transfection and reporter assays

HEK293 cells were transfected using a standard calcium phosphate precipitation protocol as previously described.[18,19] MEFs, BMDMs were transfected using Lipofectamine 2000 according to procedures recommended by the manufacturer. To normalize transfection discrepancies, 0.01 μg pRL-TK (Renilla luciferase) reporter plasmid was included in each transfection. Luciferase assays were performed using a dual-specific luciferase assay kit (Promega, Madison, WI, USA).

RNAi

Double-stranded oligonucleotides corresponding to the target sequences were cloned into the pSuper.Retro-RNAi plasmid (Oligoengine, Seattle, WA, USA). The following sequences were targeted for ZDHHC11 mRNA: #1 5′-GAA GAT GTC AAG AAT ATG A-3′; #2 5′- GAT GAC CAC CTT TGA GTA T-3′.

RNAi-transduced stable THP1 cells

HEK293 cells were transfected with pGAG-Pol and pVSV-G packaging plasmids along with a control or ZDHHC11-RNAi retroviral plasmid. Cells were maintained in regular medium for 24 h and replaced with fresh medium for another 24 h. The recombinant virus-containing medium was then filtered and added to THP1 cells in the presence of polybrene (8 μg/ml). Infected cells were selected with puromycin (0.5–1.0 μg/ml) for 7 days before experiments.

Co-immunoprecipitation and immunoblot analysis

HEK293 cells (1×10^7) were lysed in 1 ml NP-40 buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride). Co-immunoprecipitation and immunoblot analyses were performed as previously described.[16,20–22]

***Zdhhc11* knockout mice**

Strategy for constructing the targeting vector is shown in Figure 2a. *Zdhhc11* knockout mice were generated in The Model Animal Research Center of Nanjing University. Linearized targeting vector was electroporated into the W4 ES cells, which was then selected in G418 containing medium. One positive clone was injected into the 129S6/SvEvTac blastocysts. Genotyping by PCR was performed using a combination of following primers: #1 5′- GCC TTT CGG AGT CCC CAC CTG TGG GA-3′, #2 5′-TCA TTG TAT GTC CCC CAT GGA GAG-3′, #3 5′-GGC TGG ACG TAA ACT C-3′. Amplification of the wild-type allele with primers #1 and #2 gives rise to a 437 bp fragment, while amplification of the mutant allele primer #1 and #3 leads to a 267-bp fragment. *Zdhhc11* knockout mice were maintained in the 129S6/SvEvTac background.

Ethics statement

All animal experiments were performed in accordance with the Wuhan University animal care and use committee guidelines (WDSKY0200902-2).

Preparations of MEFs, MLFs, BMDMs and BMDCs

Mouse embryonic fibroblasts (MEFs) were prepared from E13.5 embryos and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. Mouse lung fibroblasts (MLFs) were isolated from 6-week-old mice. Lungs were digested in HBSS (calcium and magnesium free) containing 20 μg/ml DNAse I (Sigma-Aldrich) and 10 μg/ml type II collagenase (Worthington, Lakewood, NJ, USA) at 37 °C for 3 h. Cell suspensions were filtered and then spun down. Cells were cultured in Ham's F-12/DMEM(1:1) supplemented with 10% fetal bovine serum. Bone marrow cells were

isolated from tibia and femur. For preparation of bone marrow-derived macrophages (BMDMs), bone marrow cells were cultured in 10% M-CSF-containing conditional medium from L929 cells for 3–5 days. For preparation of bone marrow-derived dendritic cells (BMDCs), the bone marrow cells were cultured in medium containing murine GM-CSF (50 ng/ml) and IL-4 (10 ng/ml) for 6–9 days.

Quantitative real-time PCR

Quantitative real-time PCR (qPCR) analyses were performed to measure mRNA levels of indicated genes using following primers. Data were presented as the relative abundance of indicated mRNA normalized to that of *GAPDH*. Primer sequences for qPCR assays are: Murine *Gapdh*: Forward-ACGGCCGCATCTTCTTGTGCA; Reverse-ACGGCCAAATCCGTTCACACC.

Murine *Ifnb1*: Forward-TCCTGCTGTGCTTCTCCACCACA; reverse-AAGTCCGCCCTGTAGGTGAGGTT.

Murine *Isg56*: Forward-ACAGCAACCATGGGAGAGAATGCTG; reverse-ACGTAGGCCAGGAGGTTGTGCAT.

Murine *Tnfa*: Forward-GGTGATCGGTCCCCAAAGGGATGA; reverse-TGGTTTGCTACGACGTGGGCT.

Murine *Il6*: Forward-TCTGCAAGAGACTTCCATCCAGTTGC; reverse-AGCCTCCGACTTGTGAAGTGGT.

Murine *Zdhhc11*: Forward-CCCAGAAGCCCAGGAAAACA; reverse-CTGAAAGGAGTGGAGGGGTG.

Human GAPDH: Forward-GAGTCAACGGATTTGGTCGT; reverse-GACAAGCTTCCCGTTCTCAG.

Human IFNB1: Forward-TCCTGCTGTGCTTCTCCACCACA; reverse-AAGTCCGCCCTGTAGGTGAGGTT.

Human IL6: forward-AGACAGCCACTCACCTCTTCAG; reverse-TTCTGCCAGTGCCTCTTTGCTG.

Human ZDHHC11: Forward-TCATCCCCTTCCCGTGCCGT; reverse-CGCCCTGGGCTCATCTGCAC.

HSV ICP22: Forward-TGTTTGGAGACCAGACGGTA; reverse-CATCGGAGATTTCATCATCG.

HSV ICP27: forward-GGCCTGATCGAAATCCTAGA; reverse-GTCAACTCGCAGACACGACT.

Viral infection in mice

Mice were infected intraperitoneal (i.p.) with HSV-1, SeV or EMCV. For ELISA assays, the mouse sera were collected at 6 h after viral infection; for survival records, the viability of the infected mice was monitored for consecutive 15 days; for viral titer of mouse brains measurement, the mouse brains were dropoff at the fifth day after viral infection, followed by homogenate in PBS. Then, 1/10 volume of the suspensions were collected for qPCR analysis of HSV-specific *ICP22* and *ICP27* mRNA levels. The remaining suspensions were centrifuged at $1620 \times g$ for 30 min and the supernatant were collected for plaque assays. Viral infection in mice was carried out in an ABSL-2 facility, and the protocols and procedures for mice study were approved by the Institutional Review Board of Wuhan Institute of Virology (WIV31201301).

Flow cytometry

Spleen, thymus, and peripheral lymph nodes (PLNs) were sampled from Zdhhc11+/+ and Zdhhc11−/− mice and single-cell suspensions were prepared. Cells were immunostained with indicated antibodies for 30 min on ice followed by flow cytometry analyses (Beckman CyAn ADP). Antibodies used were CD3-FITC, B220-APC, CD4-PerCP, CD8-PB, and CD25-FITC (BD Biosciences, San Jose, CA, USA).

Statistical analysis

As to mouse experiments, mice in each group were randomly selected. The sample size (n) of each experimental group is shown in each corresponding figure legend. GraphPad Prism (La Jolla, CA, USA) software was used for all statistical analyses. Quantitative data displayed as histograms are expressed as means ± s.d. (represented as error bars). Data were analyzed using a Student's unpaired t-test or multiple t-test. The number of asterisks represents the degree of significance with respect to P values. Statistical significance was set at a $P < 0.05$.

RESULTS

ZDHHC11 is a positive regulator of DNA virus-triggered signaling

To identify molecules that mediate the induction of IFN-β, we performed expression screens for cDNA clones that can activate the IFN-β promoter in reporter assays. These experiments led to the identification of two members of the aspartate-histidine-histidine-cysteine (DHHC) palmitoyl acyltransferase family, ZDHHC1 and ZDHHC11, which could markedly activate the IFN-β promoter in reporter assays (Supplementary Figure S1). We had reported that ZDHHC1 mediates dimerization of MITA to facilitate innate immune responses to DNA viruses.[14] Here we further investigated the functions and mechanisms of ZDHHC11 in DNA virus-triggered innate immune signaling. Overexpression of ZDHHC11 markedly activated the IFN-β promoter and NF-κB and mildly activated ISRE in a dose-dependent manner (Figure 1a). We next examined whether endogenous ZDHHC11 is required for DNA-virus-triggered signaling. We constructed two ZDHHC11-RNAi plasmids and established ZDHHC11-RNAi stable THP1 cell lines. The knockdown efficiencies of ZDHHC11 in these two cell lines were examined (Figure 1b). Knockdown of ZDHHC11 markedly impaired HSV-1-induced transcriptions of *Ifnb1* and *Il6* (Figure 1b), as well as phosphorylation of IRF3 but not TBK1 (Figure 1c).

To further unveil the functions of ZDHHC11 in DNA virus-triggered innate immune signaling, we generated *Zdhhc11* knockout mice (Figures 2a–c). Homozygous *Zdhhc11*$^{-/-}$ mice were born at the Mendelian ratio and did not show any developmental abnormality, suggesting that *Zdhhc11* is dispensable for mouse survival and development. Numbers and compositions of total T and B cells, or CD4$^+$ and CD8$^+$ T cells in peripheral lymph nodes, spleen, and thymus were comparable between *Zdhhc11*$^{-/-}$ mice and their wild-type littermates (Figure 2d), suggesting that *Zdhhc11* is not required for development of major types of immune cells. Next, we

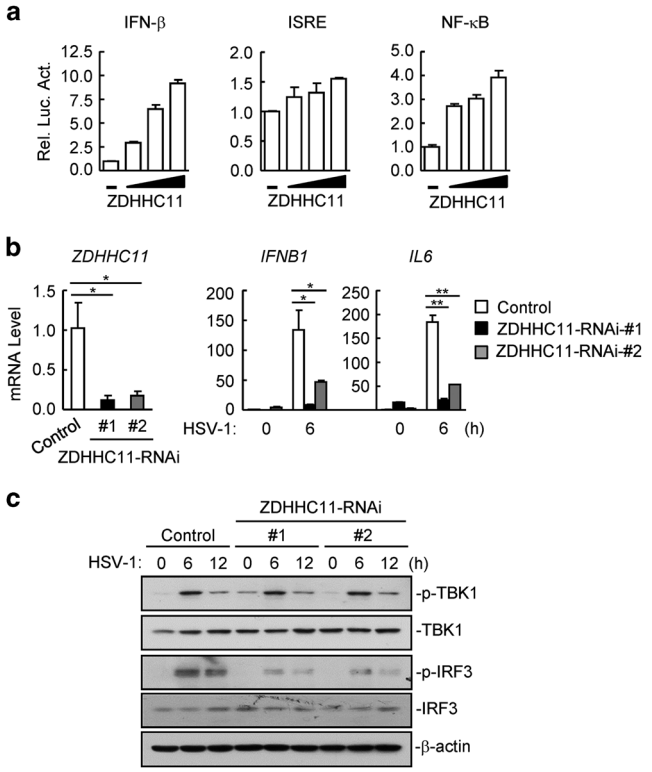

Figure 1 ZDHHC11 is a positive regulator of virus-triggered signaling. (**a**) Effects of overexpression of ZDHHC11 on activation of the IFN-β promoter. HEK293 cells were transfected with the indicated plasmids. Luciferase assays were performed 20 h after transfection. (**b**) Effects of knockdown of ZDHHC11 on HSV-1-induced transcriptions of *IFNB1* and *IL6* in THP1 cells. The control and ZDHHC11-RNAi stable THP1 cells were uninfected or infected with HSV-1 for 6 h, followed by qPCR analysis of *ZDHHC11*, *IFNB1* and *IL6* mRNAs. (**c**) Effects of knockdown of ZDHHC11 on HSV-1-induced phosphorylation of TBK1 and IRF3 in THP1 cells. The control and ZDHHC11-RNAi stable THP1 cells were infected with HSV-1 for the indicated times, followed by immunoblotting analysis with the indicated antibodies. qPCR, quantitative real-time PCR.

prepared mouse embryonic fibroblasts (MEFs), bone marrow-derived macrophages (BMDMs) and BMDCs from *Zdhhc11*$^{-/-}$ mice and their wild-type littermates, and examined the transcriptions of *Ifnb1* and downstream antiviral genes including *Isg56* and *Il6* induced by DNA virus HSV-1 and RNA virus Sendi virus (SeV) infection in these cells. The results showed that HSV-1- but not SeV-induced transcriptions of these genes were severely impaired in *Zdhhc11*$^{-/-}$ cells (Figure 3a). Furthermore, *Zdhhc11*-deficiency markedly impaired transcription of *Ifnb1* and *Il6* genes induced by transfected B-DNA (Figure 3b). Consistently, HSV-1-induced phosphorylation of IRF3 (Ser396) was markedly inhibited in *Zdhhc11*$^{-/-}$ BMDM and BMDC cells. However, levels of phosphorylated TBK1 (Ser172) and STING/MITA (Ser366), which are the respective markers for TBK1 and MITA activation, were not altered in *Zdhhc11*$^{-/-}$ BMDM and BMDC cells (Figure 3c). Interestingly, the expression of *Zdhhc11* was specifically induced by transfected B-DNA and DNA virus HSV-1 but not RNA virus SeV in all examined cells (Figure 3d, Supplementary Figure S2), which implied a role of ZDHHC11 in innate immune responses to DNA viruses. Taken together, these data suggest that ZDHHC11 is a positive regulator of innate immune responses to DNA viruses.

ZDHHC11 is crucial for host defense against HSV-1 infection in mice

To elucidate whether Zdhhc11 is essential for host defense against viral infection *in vivo*, 2-month-old wild-type and *Zdhhc11* knockout mice were infected with HSV-1 or SeV intraperitoneally (i.p.). The concentrations of serum cytokines including IFN-α, IFN-β, TNFα and IL-6 were much lower in *Zdhhc11*$^{-/-}$ mice compared to their wild-type littermates after HSV-1 but not SeV infection (Figure 4a). Furthermore, *Zdhhc11*$^{-/-}$ mice were more sensitive to HSV-1-induced death compared to wild-type littermates, while EMCV-induced death was comparable between the wild-type and knockout mice (Figure 4b). Consistently, *Zdhhc11*$^{-/-}$ mice exhibited higher HSV-1 gene transcription levels and viral titers in the brains than their wild-type littermates (Figures 4c and d). These results suggest that ZDHHC11 is essential for host defense against HSV-1 infection in mice.

ZDHHC11 acts on the level of MITA

To further explore the mechanisms on how ZDHHC11 modulates DNA virus-triggered innate immune response, we determined which component(s) in DNA virus-triggered signaling pathways that ZDHHC11 might act on. Reporter assays showed that knockdown of *ZDHHC11* significantly inhibited MITA- and cGAS plus MITA-, but not TBK1- or IRF3-5D-mediated activation of the IFN-β promoter (Figure 5a), while overexpression of ZDHHC11 markedly potentiated MITA-mediated activation of the IFN-β promoter (Figure 5b). Interestingly, all three ZDHHC11 mutants of impaired palmitoyl transferase activity, including ZDHHC11 (DH-AA$^{155/156}$), ZDHHC11(C158S) and ZDHHC11(ΔDHHC), potentiated MITA-mediated activation of ISRE as efficiently as wild-type ZDHHC11 (Figure 5b), suggesting that ZDHHC11 functions independently of its palmitoyl transferase activity in DNA virus-triggered signaling pathways, which is consistent with a previous study showing that ZDHHC11 cannot catalyze palmitoylation of MITA.[23] Co-immunoprecipitation experiments showed that ZDHHC11 interacted with MITA but not cGAS or TBK1 (Figure 5c). Domain mapping experiments indicated that the C-terminus of ZDHHC11 (aa198–412) and the N-terminal transmembrane domain of MITA (aa1–190) were required for their interaction (Figures 5d and e). Consistently, the C- but not N-terminus of ZDHHC11 potentiated MITA-mediated activation of ISRE in reporter assays (Figure 5f). These data suggest that ZDHHC11 modulates the activity of MITA to facilitate DNA virus-triggered innate immune response.

ZDHHC11 mediates the recruitment of IRF3 to MITA

The dimerization of MITA is important for its activation and subsequent translocation to the perinuclear microsomes,[5,10,24]

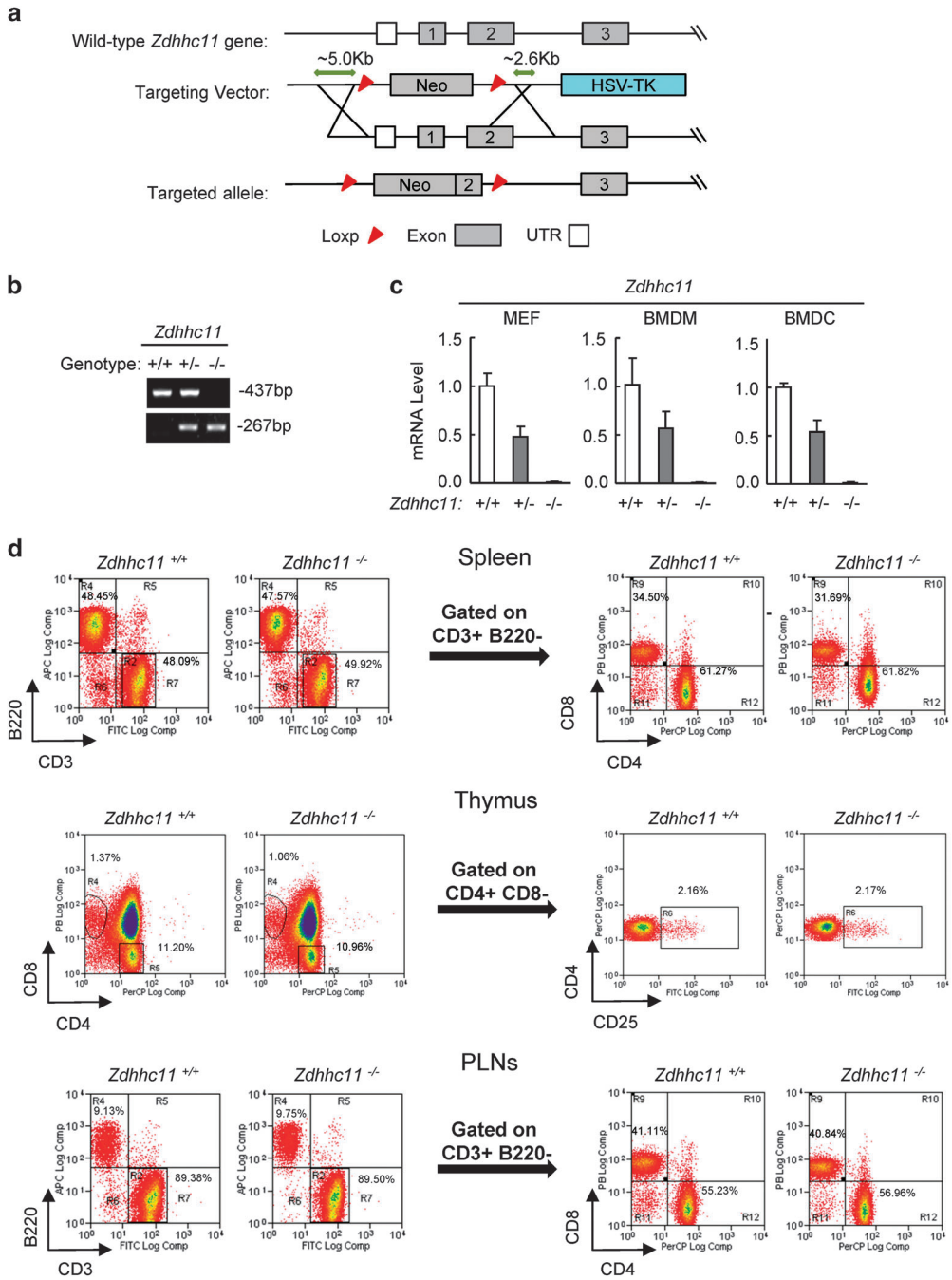

Figure 2 ZDHHC11-deficiency has no marked effects on development of the adaptive immune system. (**a**) A schematic representation of *Zdhhc11* targeting strategy. The first and 5′ half of the second exon of the *Zdhhc11* gene were replaced with a neomycin cassette, which results in a frame-shift mutation and loss of *Zdhhc11* expression. (**b**) Genotyping of *Zdhhc11* knockout mice. (**c**) qPCR analyses of *Zdhhc11* mRNA from the indicated mice. (**d**) Analyses of numbers and compositions of major immune cells in the indicated organs from *Zdhhc11*$^{+/+}$ and *Zdhhc11*$^{-/-}$ mice. Spleen, thymus and peripheral lymph nodes were homogenized and filtrated with nylon mesh. Red blood cells were lysed by ammonium chloride. Cells were stained with the indicated antibodies before FACS. qPCR, quantitative real-time PCR.

where the kinase TBK1 and the transcriptional factor IRF3 are simultaneously recruited in a MITA-dependent manner, followed by the phosphorylation of IRF3 by TBK1 and its subsequent nuclear translocation, leading to the ultimate expression of type I IFNs.[13,25] We have previously reported that ZDHHC1, another member of DHHC palmitoyl transferase family, modulates DNA virus-triggered innate immune signaling by promoting MITA dimerization.[14] We wondered whether ZDHHC11 functions in a similar mechanism. Co-immunoprecipitation experiments indicated that ZDHHC11 and its C-terminus markedly potentiated

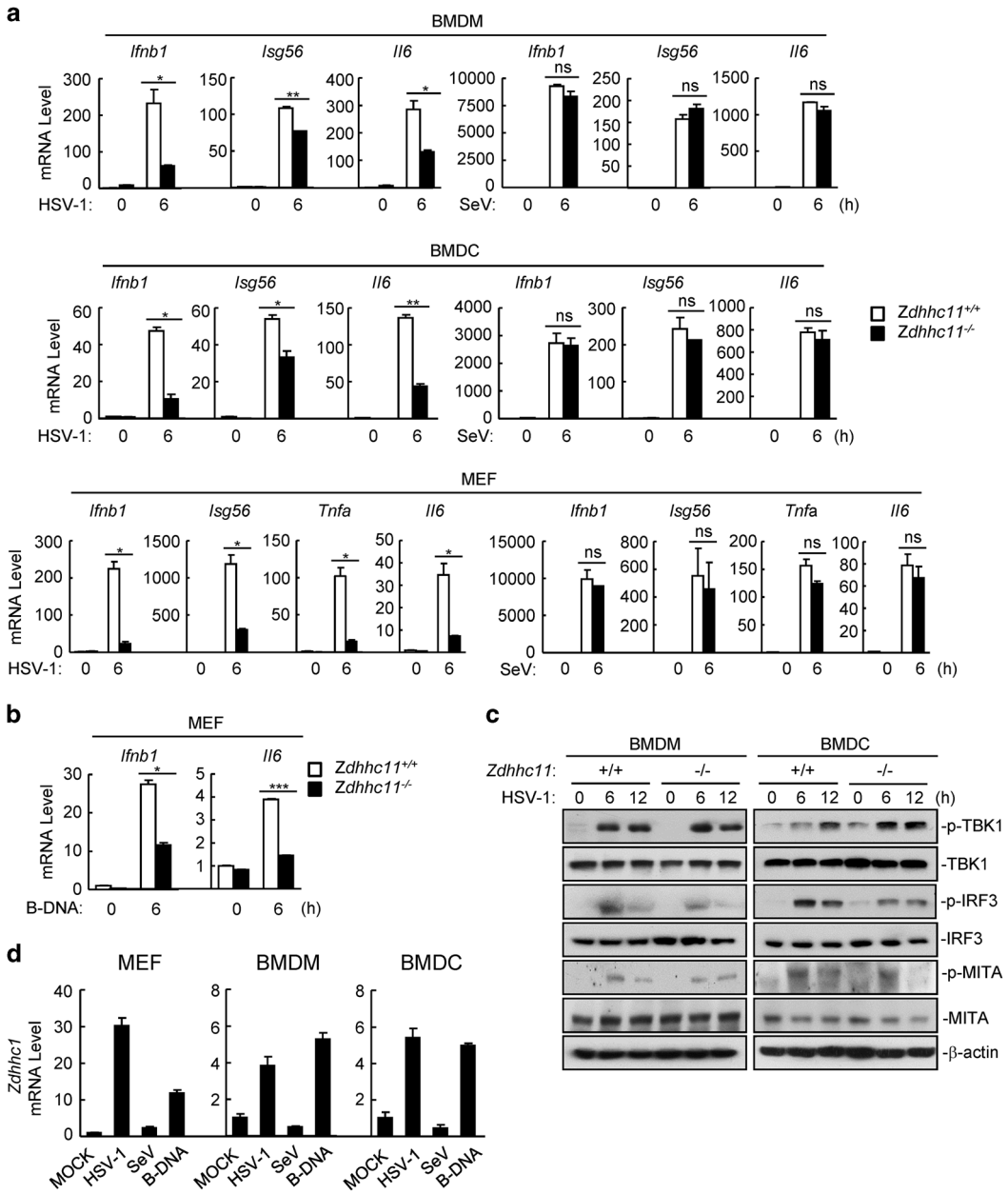

Figure 3 ZDHHC11 is essential for DNA virus-triggered innate immune signaling. (**a**) Effects of Zdhhc11-deficiency on HSV-1- or SeV-induced transcriptions of *Ifnb1* and downstream antiviral genes. *Zdhhc11*$^{+/+}$ and *Zdhhc11*$^{-/-}$ BMDMs, BMDCs and MEFs were infected with HSV-1 or SeV for 6 h, followed by qPCR analysis. (**b**) Effects of Zdhhc11-deficiency on transfected B-DNA-induced transcriptions of *Ifnb1* and *Il6* in MEFs. *Zdhhc11*$^{+/+}$ and *Zdhhc11*$^{-/-}$ MEFs were transfected with B-DNA for 6 h, followed by qPCR analysis of *Ifnb1* and *Il6* mRNAs. (**c**) Effects of Zdhhc11-deficiency on HSV-1-induced phosphorylation of TBK1(S172), IRF3(S396) and STING/MITA (S366). *Zdhhc11*$^{+/+}$ and *Zdhhc11*$^{-/-}$ BMDMs or BMDCs were infected with HSV-1 for the indicated times, followed by immunoblotting analysis with the indicated antibodies. (**d**) Effects of viral infection on transcriptional induction of *Zdhhc11*. The indicated cells were infected with HSV-1 or SeV, or transfected with B-DNA, and followed by qPCR analysis of *Zdhhc11* mRNAs. BMDCs, bone marrow-derived dendritic cells; BMDMs, bone marrow-derived macrophages; qPCR, quantitative real-time PCR.

the interaction of MITA with IRF3, but had no effects on the dimerization of MITA or MITA-TBK1 interaction (Figure 6a). Furthermore, endogenous co-immunoprecipitation experiments showed that ZDHHC11-deficiency impaired HSV-1-induced recruitment of IRF3 to MITA (Figure 6b). Domain mapping experiments showed that the C-terminus of ZDHHC11 (aa198–412), the region responsible for MITA association, was required for its interaction with IRF3 (Figure 6c). The C-terminus of IRF3 (aa141–427) was required for its interaction with ZDHHC11 (Figure 6d). These results suggest that ZDHHC11 is required for the optimal recruitment of IRF3 to MITA on DNA viral infection.

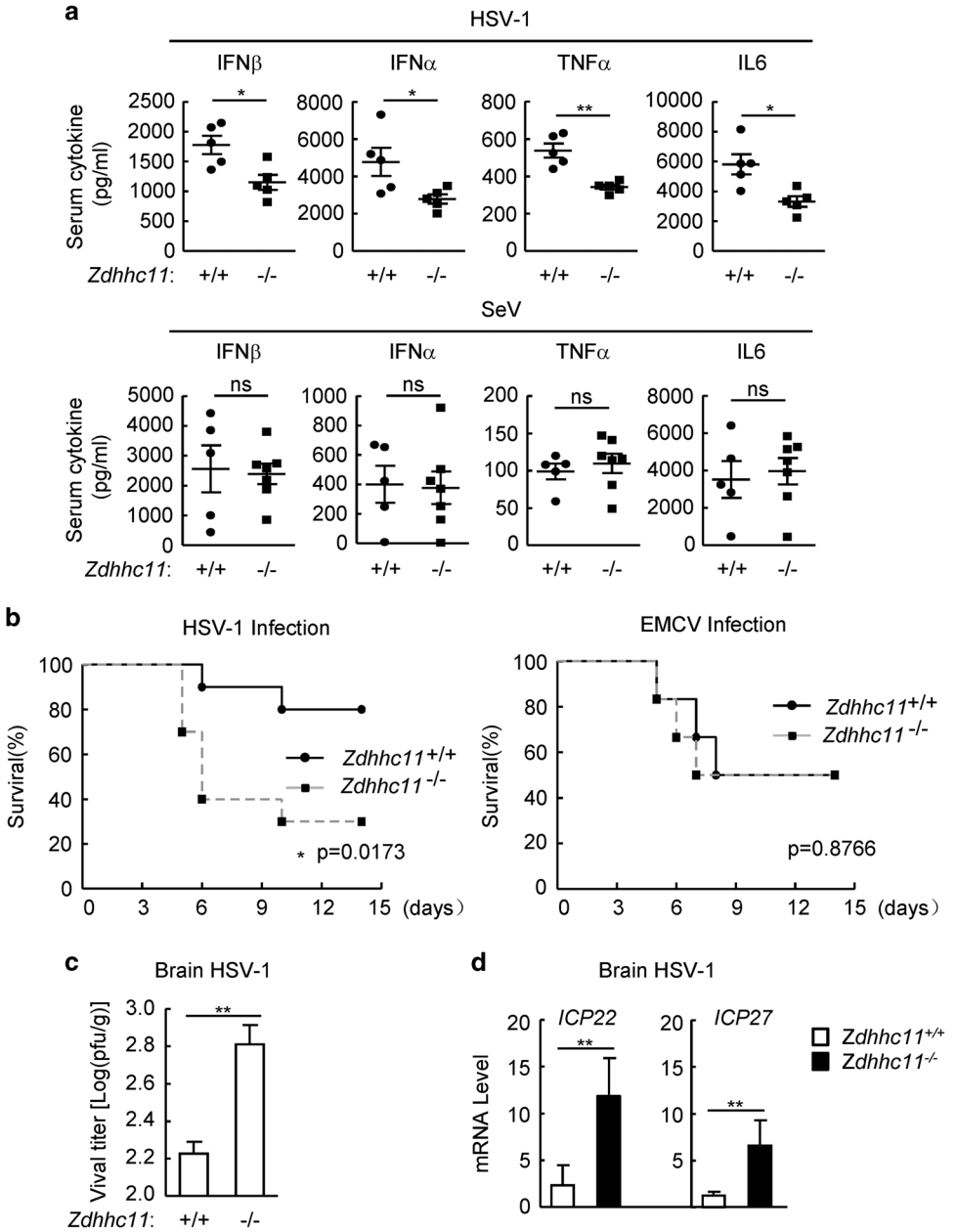

Figure 4 ZDHHC11 is essential for host defense against HSV-1 infection. (**a**) Effects of Zdhhc11-deficiency on expression of serum cytokines after viral infection. *Zdhhc11*$^{+/+}$ and *Zdhhc11*$^{-/-}$ mice were intraperitoneal (i.p.) infected with HSV-1 or SeV for 6 h, and then the sera were collected for measurement of cytokines by ELISA. (**b**) Effects of Zdhhc11-deficiency on HSV-1 or EMCV infection-induced death. *Zdhhc11*$^{+/+}$ and *Zdhhc11*$^{-/-}$ mice were intraperitoneal (i.p.) infected with HSV-1 at 1×10^7 p.f.u. per mouse ($n=10$ each) or with EMCV at 3×10^6 p.f.u. per mouse ($n=8$ each) and their survivals were recorded for 15 days. The survival curve was generated by Kaplan–Meier methods followed by long-rank test analysis. (**c**, **d**) Effects of ZDHHC11-deficiency on HSV-1 clearance *in vivo*. *Zdhhc11*$^{+/+}$ and *Zdhhc11*$^{-/-}$ mice ($n=4$ each) were intraperitoneal (i.p.) infected with HSV-1 at 1×10^7 p.f.u. per mouse. Brains were collected 5 days after infection for HSV-1 plaque assays and for quantifying transcripts of HSV-1 specific protein ICP-22 and ICP27. $^*P<0.05$; $^{**}P<0.01$ (Student's *t* test). p.f.u., plaque forming units.

DISCUSSION

The cGAS-MITA pathway is essential for innate immune response to DNA virus.[9,12,24,26] Upon detecting microbial DNAs by cGAS, MITA is activated and translocate from the ER via Golgi to perinuclear microsomes.[22] TBK1 is concurrently recruited to the same compartments in a MITA-dependent manner, which then activates the transcription factor IRF3.[13,18,27] A number of studies have characterized mechanisms in regulating immune responses to DNA viruses by modulating cGAS and MITA activity.[28–30] For example, CCP6 removes the polyglutamylation of cGAS and CCP5 hydrolyzes the monoglutamylation of cGAS, which coordinately regulate immune responses to DNA virus infection.[31] ZDHHC1 modulates the dimerization of MITA and recruits

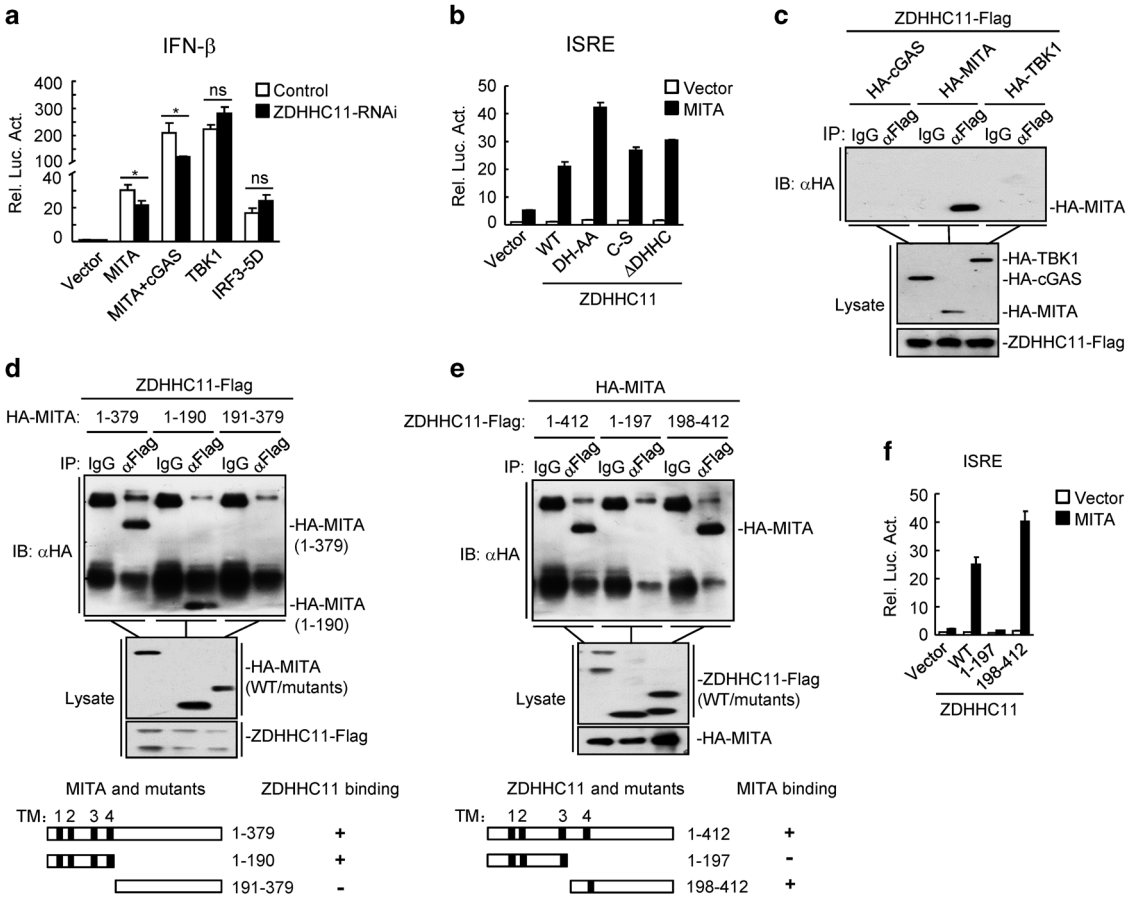

Figure 5 ZDHHC11 modulates MITA activity. (**a**) Effects of knockdown of ZDHHC11 on MITA- and cGAS plus MITA-, TBK1- and IRF3-5D-mediated activation of the IFN-β promoter. HEK293 cells were transfected with ZDHHC11-RNAi plasmids for 36 h, followed by re-transfection with the indicated plasmids for 24 h before luciferase assays were performed. (**b**) Effects of ZDHHC11 and its mutants on MITA-mediated activation of ISRE. HEK293 cells were transfected with the indicated plasmids for 24 h before luciferase assays were performed. (**c**) Interaction of ZDHHC11 with MITA. HEK293 cells were transfected with the indicated plasmids for 24 h, followed by co-immunoprecipitation and immunoblotting analysis with the indicated antibodies. (**d**, **e**) Domain mapping of the ZDHHC11 and MITA interaction. HEK293 cells were transfected with the indicated truncations of MITA and ZDHHC11 for 24 h before co-immunoprecipitation and immunoblotting analysis with the indicated antibodies. (**f**) Effects of ZDHHC11 and its truncation mutants on MITA-mediated activation of ISRE. HEK293 cells were transfected with the indicated plasmid for 24 h before luciferase assays were performed.

downstream components to MITA in response to DNA virus infection.[14] TRIM38 maintains cGAS sumoylated in uninfected or early-infected cells to prevents its polyubiquitination and degradation. Moreover, TRIM38 sumoylates MITA at K337 during early phase of DNA viral infection to promote its oligomerization-mediated activation and prevents its degradation.[28] SENP2 desumoylates both cGAS and MITA to facilitate their degradation at the late phase of DNA viral infection. We recently found iRhom2 recruits the translocon-associated protein TRAPβ to the MITA complex to facilitate trafficking of MITA from the endoplasmic reticulum to perinuclear microsomes upon DNA virus infection. iRhom2 also recruits the deubiquitinating enzyme EIF3S5 to maintain MITA's stability by removing its K48-linked poly-ubiquitin chains.[22] But little is known how IRF3 is recruited into the MITA signalosomes. In this study, we characterized ZDHHC11, a member of DHHC palmitoyl transferase family, enhances MITA-mediated innate immune responses to DNA viruses by linking IRF3 to MITA (for a working model, see Figure 6e). Several lines of evidence were provided to support this conclusion. Firstly, *Zdhhc11* was induced by DNA but not RNA viruses and its overexpression activated ISRE, NF-κB and the IFN-β promoter in reporter assays. Secondly, loss-of-function assays showed HSV-1- but not SeV-induced *Ifnb1* and downstream antiviral genes transcriptions were severely impaired in *Zdhhc11*$^{-/-}$ cells. HSV-1 induced phosphorylation of IRF3 but not phosphorylation of TBK1 or MITA was inhibited in *Zdhhc11*$^{-/-}$ BMDM and BMDC cells. This was distinct from the effects of ZDHHC1-deficiency that led to the impairment of HSV-1-induced phosphorylation of both TBK1 and IRF3,[14] implying that ZDHHC11 functions in a different way. Thirdly, host defense experiments showed that ZDHHC11 was essential for production of antiviral cytokines upon DNA virus infections, as *Zdhhc11*$^{-/-}$ mice were more susceptible to death. Subsequent experiments showed that ZDHHC11 interacted with MITA and IRF3 via its C-terminus, and the presence

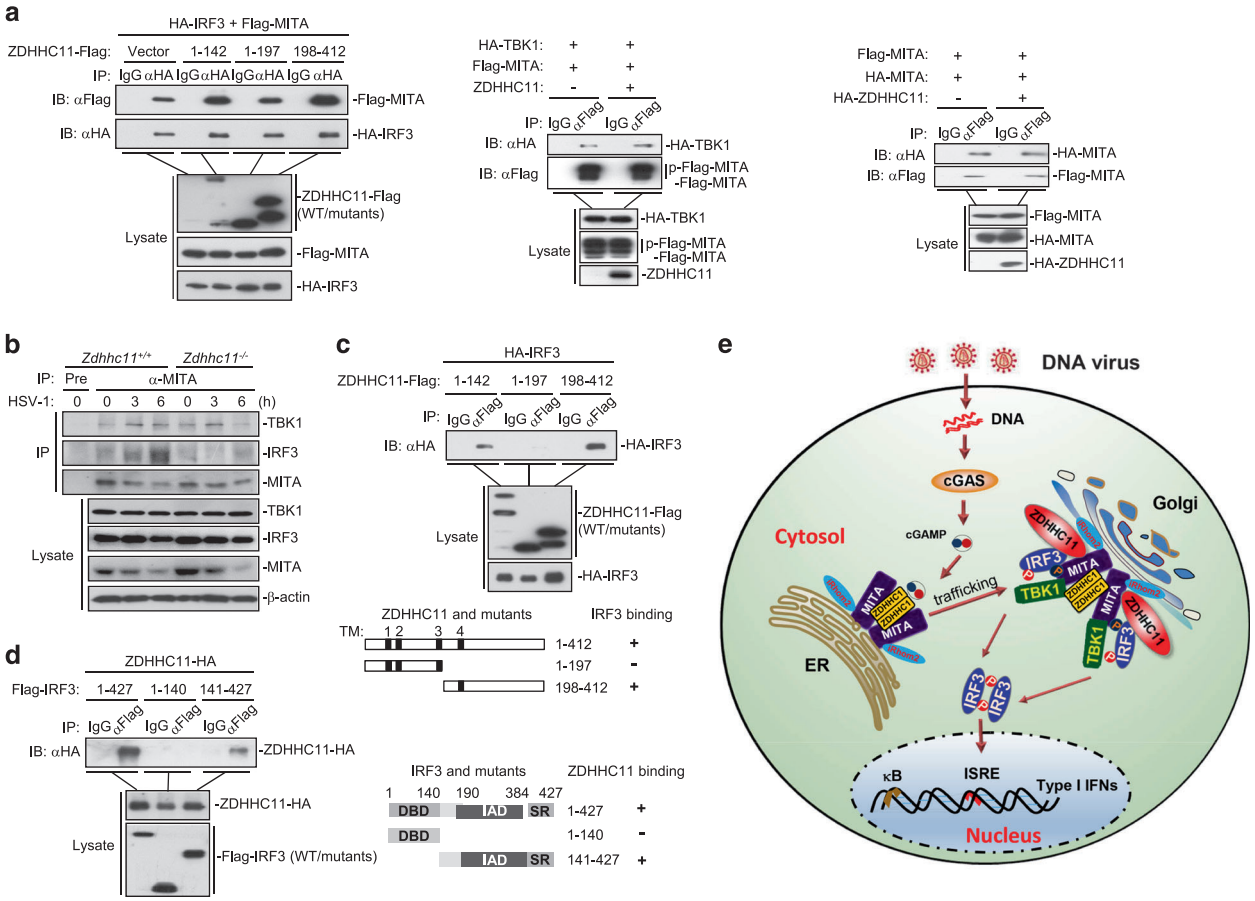

Figure 6 ZDHHC11 facilitates the recruitment of IRF3 to MITA. (**a**) Effects of ZDHHC11 or its truncation mutants on interactions of MITA with IRF3, TBK1 or itself. HEK293 cells were transfected with the indicated plasmids for 20 h before co-immunoprecipitation and immunoblotting analysis with the indicated antibodies. (**b**) Effects of ZDHHC11-deficiency on HSV-1-induced recruitment of TBK1 and IRF3. MLFs from *Zdhhc11*$^{+/+}$ and *Zdhhc11*$^{-/-}$ mice were treated with HSV-1 for the indicated times, followed by co-immunoprecipitation and immunoblotting analysis with the indicated antibodies. (**c**, **d**) Domain mapping of the ZDHHC11 and IRF3 interaction. HEK293 cells were transfected with the indicated expression plasmids before co-immunoprecipitation and immunoblotting analysis with the indicated antibodies. (**e**) A model on the role of ZDHHC11 in regulating MITA-mediated signaling. ZDHHC11, an adapter protein that facilitates the association of MITA with IRF3 at the perinuclear microsomes, enhances MITA-mediated innate immune responses against DNA viruses.

of ZDHHC11 promoted the association between IRF3 and MITA. Interestingly, IRF3 and MITA bind to the same domain of ZDHHC11. It's possible that IRF3 and MITA may interact with different regions in the C-terminal fragment of ZDHHC11 (aa198–412), which is fairly large. Alternatively, components in the complex may form hetero-oligomers and particular spatial structure to allow both IRF3 and MITA bind to the same domain of ZDHHC11. These data suggest that ZDHHC11 functions as an adapter protein to facilitate the assembly of the MITA signalosomes at the perinuclear microsomes.

The human genome encodes 23 DHHC proteins.[32,33] Although ZDHHC11 and ZDHHC1 share the highest homology among the DHHC-containing proteins and both of them are important for innate immune responses to DNA viruses, they act in a distinct manner in modulating MITA activity. The C-terminus of ZDHHC11 interacts with both MITA and IRF3 and promotes the recruitment of IRF3 to MITA, but ZDHHC1 does not bind to IRF3.[14] Instead, ZDHHC1 but not ZDHHC11 modulates the dimerization of MITA and promotes subsequent TBK1 recruitment and phosphorylation. Taken together, our studies suggest that ZDHHC11 and ZDHHC1 modulate MITA activity through distinct mechanisms.

It has been reported that palmitoylation of MITA is essential for its functions in host defense.[23] However, the putative palmitoyl transferase activities of ZDHHC11 as well as ZDHHC1 are not required for their abilities to modulate MITA-mediated signaling. It remains to be investigated whether other DHHC-containing enzymes are recruited to the MITA signalosomes to catalyze its palmitoylation. Recently, the ER adaptor SCAP was found to translocate from ER to perinuclear microsomes in a MITA-dependent manner.[34] Furthermore, the N-terminal transmembrane domain of SCAP interacts with MITA, and the C-terminal cytosolic domain of SCAP binds to IRF3, thus recruiting IRF3 into the MITA signalosomes. It remains to be investigated whether SCAP and

ZDHHC11 collaborate in assembling the MITA-TBK1-IRF3 complex at the perinuclear microsomes. Nevertheless, our study provides new clues on the mechanisms of MITA-mediated innate immune responses to DNA viruses.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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