

# HDAC11 regulates type I interferon signaling through defatty-acylation of SHMT2

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The smallest histone deacetylase (HDAC) and the only class IV HDAC member, HDAC11, is reported to regulate immune activation and tumorigenesis, yet its biochemical function is largely unknown. Here we identify HDAC11 as an efficient lysine defatty-acylase that is >10,000-fold more efficient than its deacetylase activity. Through proteomics studies, we hypothesized and later biochemically validated SHMT2 as a defatty-acylation substrate of HDAC11. HDAC11-catalyzed defatty-acylation did not affect the enzymatic activity of SHMT2. Instead, it affects the ability of SHMT2 to regulate type I IFN receptor ubiquitination and cell surface level. Correspondingly, HDAC11 depletion increased type I IFN signaling in both cell culture and mice. This study not only demonstrates that HDAC11 has an activity that is much more efficient than the corresponding deacetylase activity, but also expands the physiological functions of HDAC11 and protein lysine fatty acylation, which opens up opportunities to develop HDAC11-specific inhibitors as therapeutics to modulate immune responses.

HDAC11 | lysine fatty acylation | SHMT2 | IFNAR1 | interferon

**H**istone deacetylases (HDACs) regulate many biological functions by removing acetyl groups from lysine residues on proteins (1, 2). There are 18 HDACs in mammals, classified into four classes. Class III HDACs (known as sirtuins) are NAD<sup>+</sup>-dependent, while class I, II, and IV HDACs are all zinc-dependent. Class I HDACs, consisting of HDAC1, 2, 3, and 8, are homologous to yeast Rpd3. Class II HDACs are homologous to yeast Hda1 and are further divided into two subclasses: IIa (HDAC4, 5, 7, 9) and IIb (HDAC6, 10) (1, 3). Class IV HDAC is comprised solely of HDAC11 (4, 5).

HDAC11 is normally found in brain, testis, and immune cells such as antigen-presenting cells (APCs) (5, 6). Up-regulation of HDAC11 has been found in many cancer cells (7, 8) and interleukin-13 (IL-13) treated B cells (9). It has been reported that HDAC11 negatively regulates IL-10 production in APCs by binding to the IL-10 promoter and promoting histone deacetylation, thus influencing immune activation (10). However, the deacetylase activity of HDAC11 reported is rather weak, and its ability to directly deacetylate histones has not been demonstrated. The lack of efficient deacetylase activity has prevented the identification of more substrate proteins that could help to understand the biological function of HDAC11.

Recently, several HDACs have been shown to hydrolyze acyl lysine modifications that are distinct from acetyl lysine (11–15). In particular, SIRT5 can efficiently remove succinyl and malonyl groups (16, 17), while SIRT6 can efficiently remove long-chain fatty acyl groups in vitro and in vivo (18). Herein we identify a defatty-acylase activity of HDAC11 that is >10,000 times more efficient than its deacetylase activity. Two other laboratories at a similar time found the in vitro defatty-acylation activity (19, 20), and we coordinated the deposition of the results on BioRxiv. This potent defatty-acylase activity of HDAC11 led us to identify its substrate SHMT2, a lysine-acylated protein, as well as an immunoregulatory pathway mediated by HDAC11/SHMT2

interaction. These findings provide insights into the immunomodulatory function of HDAC11.

## Results

**HDAC11 Is an Efficient Lysine Defatty-Acylase.** To study the enzymatic activity of HDAC11, we first tested recombinant HDAC11 purified from HEK293T cells (*SI Appendix, Fig. S1A*) on H3K9 peptides bearing different acyl groups (Fig. 1A). Using an HPLC-based assay, we found that HDAC11 could efficiently remove long-chain fatty-acyl groups (myristoyl, palmitoyl, and 3-hydroxydodecanoyl) from H3K9 peptides (Fig. 1A and B). Surprisingly, HDAC11 failed to remove smaller acyl groups, including octanoyl and decanoyl, from H3K9 peptides (Fig. 1A and B). Many other acyl groups tested, including lipoyl, succinyl, and glutaryl, on H3K9 peptides were not HDAC11 substrates (Fig. 1A). We also used a few different peptide sequences. We detected demyristoylation activity of HDAC11 on H2BK12 and TNF $\alpha$  peptides, but did not detect any deacetylation activity on H2BK12 and  $\alpha$ -tubulin peptides (*SI Appendix, Fig. S1B*). Mouse HDAC11 also exhibited efficient demyristoylation activity (*SI Appendix, Fig. S1C*). Since N-terminal glycine myristoylation is a well-known posttranslational modification, we also tested whether HDAC11 could catalyze glycine demyristoylation. No glycine

## Significance

**HDAC11 is the only class IV member of the histone deacetylase (HDAC) family, and very little is known about its biological function. The work here reveals its efficient and physiologically relevant activity. The regulation of SHMT2 and interferon signaling expands the known biological function of protein lysine fatty acylation, which has only recently started to be appreciated. Furthermore, a compelling molecular mechanism is proposed to connect HDAC11 to immune response. The finding opens exciting opportunities to develop HDAC11-specific inhibitors to treat human diseases that would benefit from increased type I interferon signaling, such as viral infection, multiple sclerosis, and cancer.**

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The authors declare no conflict of interest.

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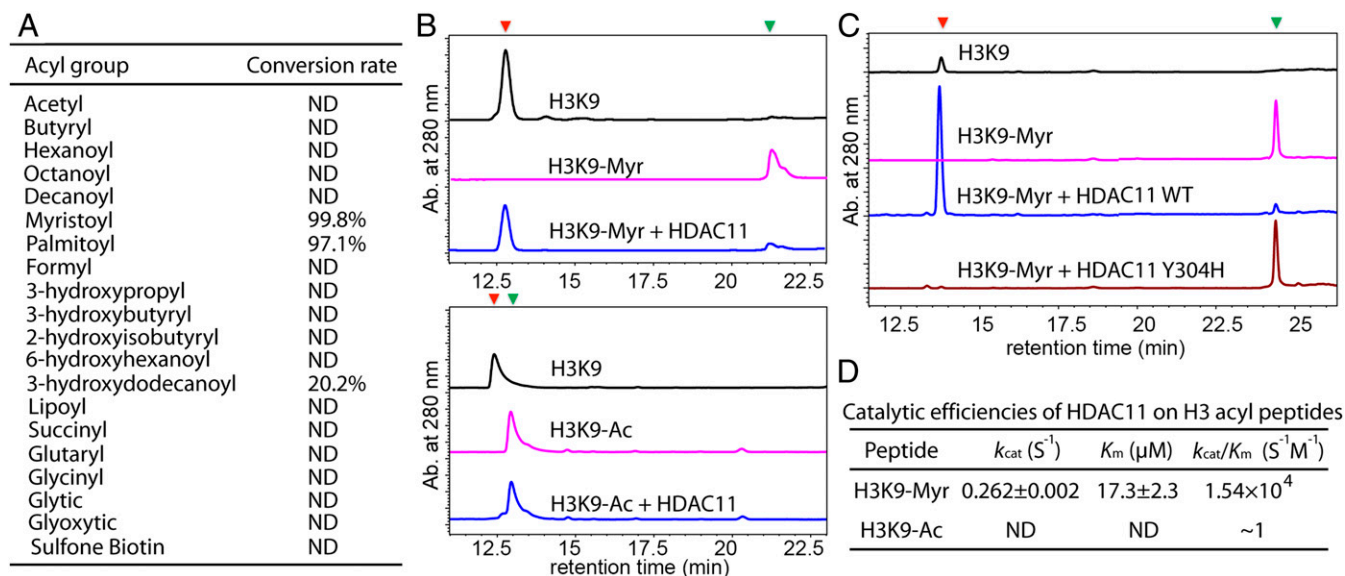
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**Fig. 1.** HDAC11 is an efficient lysine defatty-acylase. (A) HDAC11 activity on H3K9 peptides bearing different acyl groups. (B) Representative HPLC assay data of HDAC11 activity on myristoyl and acetyl H3K9 peptides. (C) Representative HPLC assay data of HDAC11 WT and Y304H mutant activity on myristoyl H3K9 peptide. (D) Kinetic parameters of HDAC11 on myristoyl and acetyl H3K9 peptides.

demyristoylation activity was detected on p21-activated kinase 2 (PAK2) and  $\alpha$  peptides (*SI Appendix, Fig. S1D*).

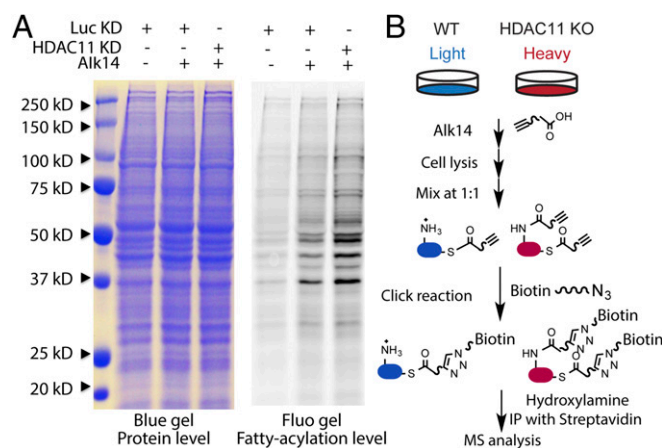
To rule out that the defatty-acylation activity was from a contaminating protein in the HDAC11 preparation, we further constructed and purified four catalytic mutants of HDAC11 that lack the general acid catalytic residue (Y304H) or zinc-binding residues (D181A, H183A, and D261A). Wild-type (WT) HDAC11, but not the catalytic mutants, could remove myristoyl group from the H3K9 peptide (Fig. 1C and *SI Appendix, Fig. S1E*). Furthermore, we also detected the demyristoylation activity of recombinant HDAC11 purified from *Saccharomyces cerevisiae* (*SI Appendix, Fig. S1F*).

We have previously identified SIRT2 and SIRT6 as functional lysine defatty-acylases (18, 21). Compared with SIRT2 and SIRT6, HDAC11 exhibited the most efficient and specific demyristoylation activity (*SI Appendix, Fig. S1G*). Kinetics studies (Fig. 1D) showed that the  $K_m$  value for the myristoyl H3K9 peptide was  $17.3 \mu M$ , and catalytic efficiency ( $k_{cat}/K_m$  value) is  $1.54 \times 10^4 M^{-1}s^{-1}$ . For deacetylation, we could not obtain the  $k_{cat}/K_m$  value because no activity was observed. But based on the detection limit of the HPLC assay, we estimated the upper limit of the  $k_{cat}/K_m$  value to be  $1 M^{-1}s^{-1}$ . Thus, the lysine defatty-acylase activity of HDAC11 is >10,000-fold more efficient than its deacetylase activity in vitro.

**Proteomic Approach Identifies Putative Defatty-Acylation Substrates of HDAC11.** To address whether the efficient defatty-acylation activity of HDAC11 is physiologically relevant, we first tested the effect of HDAC11 knockdown (KD) on global lysine fatty-acylation level in MCF-7 cells. Using a metabolic labeling method (*SI Appendix, Fig. S2A*) with the Alk14 probe (an alkyne-tagged fatty acid analog) for protein myristoylation and palmitoylation (18, 22), intracellular fatty-acylated proteins were labeled. The labeled proteins were then conjugated to a fluorescent tag (BODIPY-azide). After precipitating the proteins, hydroxylamine was used to remove cysteine acylation, and then the lysine-acylated proteins were resolved by SDS/PAGE and visualized by in-gel fluorescence. As shown in Fig. 2A and *SI Appendix, Fig. S2B*, HDAC11 KD cells had increased global hydroxylamine-resistant fatty-acylation compared with control cells, suggesting that HDAC11 may affect the lysine fatty acylation of many proteins.

We then set out to identify potential defatty-acylation substrates of HDAC11 using proteomics. We combined Alk14 labeling and stable isotope labeling with amino acid in cell culture

(SILAC) to identify proteins with increased Alk14 labeling in HDAC11 knockout (KO) HAP1 cells (Fig. 2B and *SI Appendix, Fig. S2C*). The SILAC experiments were carried out in two independent repeats. The HDAC11 KO cells were cultured with heavy amino acids, while the corresponding control cells were cultured with light amino acids. The cells were labeled with Alk14, and the alkyne-labeled proteins were conjugated to a biotin tag (biotin-azide) via click chemistry. The modified proteins were pulled down with streptavidin, treated with hydroxylamine, and analyzed by mass spectrometry. A protein with heavy/light (H/L) ratio higher than 1 in SILAC could be a potential substrate of HDAC11. To remove false positives and simplify downstream validation studies, we further filtered our data using the following criteria: H/L ratio  $\geq 1.2$ , H/L variability  $\leq 30\%$ , and with at least two unique peptides identified. Based on these criteria, we identified 7 and 71 proteins in the two repeats, respectively (see top 7 and 71 hits, respectively, listed in *SI*



**Fig. 2.** Proteomic approach identifies potential defatty-acylation substrates of HDAC11. (A) The effect of HDAC11 knockdown (KD) on global lysine fatty-acylation level in MCF-7 cells using a metabolic labeling method with Alk14. (B) Alk14 labeling and SILAC to identify proteins with increased acylation in cells with HDAC11 KO compared with WT cells.



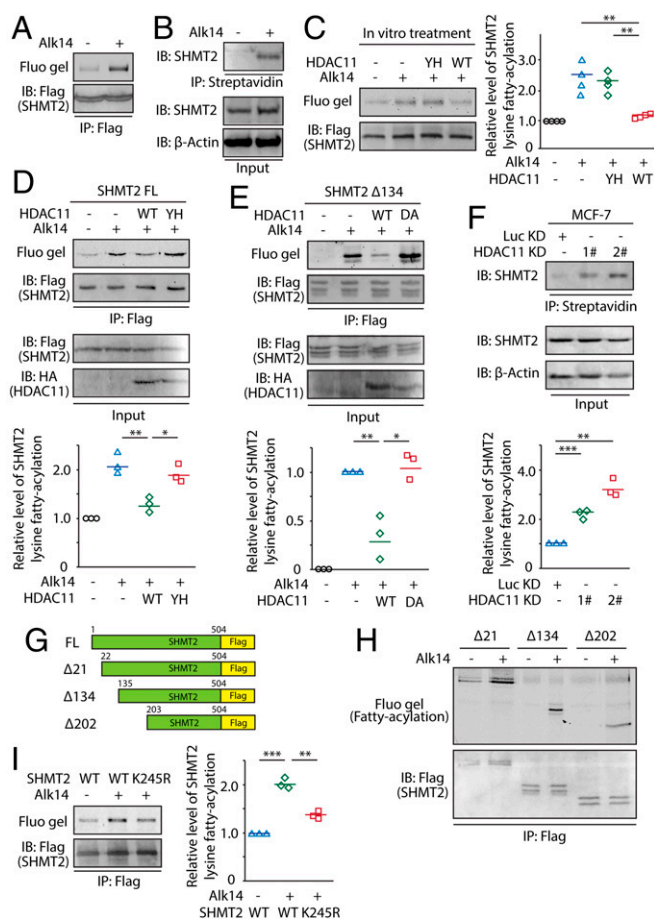
Appendix, Tables S1 and S2). Among them, only two proteins, serine hydroxymethyltransferase (SHMT) and protein LYRIC (MTDH), were identified from both SILACs. It should be noted that based on our previous experience, identifying the defatty-acylation substrates of HDACs by such proteomic experiments is very difficult because of the high false positive rates—many factors could cause the high false positive rates, such as hydroxylamine-resistant cysteine acylation and protein abundance change, as well as the low stoichiometry of the lysine fatty acylation. Thus, without further biochemical validation, none of the hits identified should be considered as true HDAC11 substrates. We focused on validation studies of SHMT here.

**SHMT2 Is a Defatty-Acylation Substrate of HDAC11 in Cells.** Two SHMTs, SHMT1 and SHMT2, are known in mammals. Examining the sequences of the SHMT proteins identified from the proteomics studies indicated that SHMT2 was the isoform identified in the proteomics. We first tested whether SHMT2 was fatty-acylated using Alk14 labeling (*SI Appendix, Fig. S2D*). Although some background fluorescent signal without Alk14 was detected, Alk14 treatment increased the fluorescent signal of Flag-tagged SHMT2 (Flag-SHMT2), but not SHMT1 (Fig. 3*A* and *SI Appendix, Fig. S2E*), suggesting that SHMT2 contained acylation. Moreover, we detected the acylation of endogenous SHMT2 in cells (Fig. 3*B*) using Alk14 labeling, biotin-azide conjugation, and streptavidin pulldown, followed by immunoblotting against SHMT2. Therefore, our data suggested that SHMT2 is a fatty-acylated protein. Coimmunoprecipitation studies showed that Flag-SHMT2 interacted with HA-HDAC11 when expressed in HEK 293T cells (*SI Appendix, Fig. S2F*).

To determine whether SHMT2 is a substrate of HDAC11, we first examined whether recombinant HDAC11 could remove the fatty-acylation on SHMT2 in vitro. Flag-SHMT2 was expressed in HEK 293T cells and labeled with Alk14. Immunoprecipitated SHMT2 was incubated with HDAC11 WT or the Y304H mutant of HDAC11. Then a fluorescent tag (BODIPY-azide) was conjugated using click chemistry, and the fatty acylation level of SHMT2 was detected using in-gel fluorescence. WT, but not mutant, HDAC11 significantly decreased the fluorescent signal on SHMT2 (Fig. 3*C*), suggesting that HDAC11 can directly remove the fatty acyl groups on SHMT2.

In HEK 293T cells, coexpression of SHMT2 and WT HDAC11 decreased the fatty-acylation level on SHMT2, compared with cells without HDAC11 overexpression or cells overexpressing the Y304H mutant (Fig. 3*D*). Due to the high background of full-length SHMT2 (Fig. 3*A* and *D*), we also conducted the experiment using a truncated SHMT2 (SHMT2  $\Delta$ 134), which lacked the mitochondrial localization signal and was used for identifying lysine fatty-acylation site (see below). SHMT2  $\Delta$ 134 had no detectable background fluorescence signal (Fig. 3*E*). WT HDAC11, but not the zinc-binding D181A mutant, decreased the fatty-acylation level on SHMT2  $\Delta$ 134 (Fig. 3*E*). Thus, the enzymatic activity of HDAC11 is required for controlling SHMT2 fatty acylation in cells. In MCF-7 cells, HDAC11 KD significantly increased the fatty-acylation level of the endogenous SHMT2, compared with control KD (Fig. 3*F*). Similar results were also observed in A549 cells (*SI Appendix, Figs. S2B* and *S3A*). Collectively, our data support the hypothesis that HDAC11 regulates the fatty-acylation level of SHMT2.

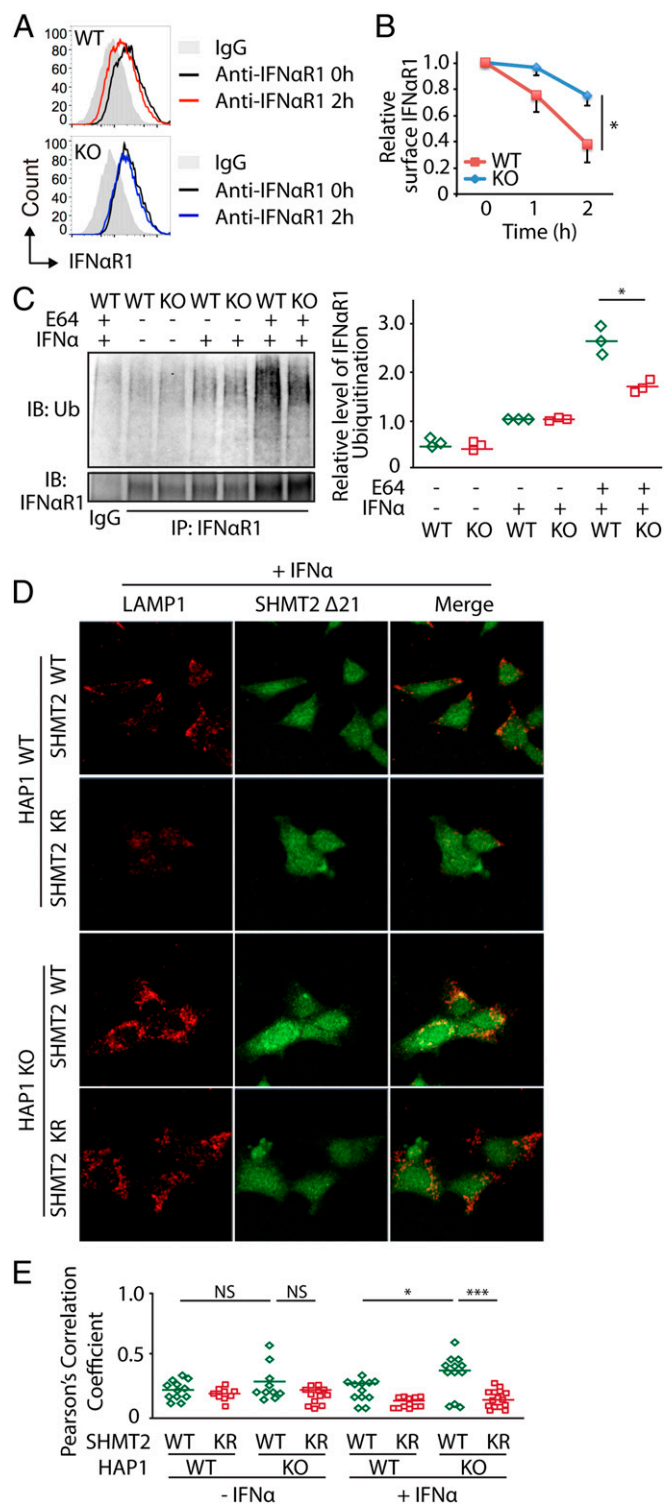
We next sought to determine which lysine residue of SHMT2 was the fatty-acylation site. We first constructed a series of N terminus truncated forms of SHMT2 ( $\Delta$ 21,  $\Delta$ 134, and  $\Delta$ 202) (Fig. 3*G*) to narrow down the modification region. All three truncated forms still had lysine fatty-acylation (Fig. 3*H*), suggesting the lysine modification site is localized between residues 203 and 504 of SHMT2, which contained 16 lysine residues. We mutated each of the 16 lysines (K) to arginine (R). Among them, 14 mutants were successfully expressed in HEK 293T cells (*SI Appendix, Fig. S3B*). Of the 14 mutants, only the K245R mutant significantly decreased the fatty acylation level, suggesting K245 is the major acylation site (Fig. 3*I* and *SI Appendix, Fig. S3B*).



**Fig. 3.** SHMT2 is a defatty-acylation substrate of HDAC11 in cells. (A) Alk14 labeling and In-gel fluorescence showing SHMT2 is a lysine fatty-acylated protein. (B) Fatty-acylation of endogenous SHMT2 in HEK293T cells was detected using Alk14 labeling, biotin-azide conjugation, and streptavidin pulldown, followed by immunoblotting against SHMT2. (C) SHMT2 Alk14 labeling after in vitro treatment with WT or the Y304H mutant (YH) of HDAC11. Quantification of lysine fatty-acylation levels was done with four biological replicates. (D and E) In-gel fluorescence showing that lysine fatty-acylation of SHMT2 full-length and  $\Delta$ 134 was regulated by WT but not the Y304H mutant (or D181A zinc-binding mutant) of HDAC11 in HEK 293T cells coexpressing SHMT2 and HDAC11. Quantification of lysine fatty-acylation levels was done with three biological replicates. (F) Fatty-acylation of endogenous SHMT2 increased after HDAC11 KD in MCF-7 cells. Quantification of the relative levels of lysine fatty-acylation was done with three biological replicates. (G) Schematic representation of Flag-tagged WT and truncated SHMT2 constructs. (H) In-gel fluorescence showing fatty-acylation was localized to residue 203–504 of SHMT2. (I) In-gel fluorescence showing K245 is the major fatty-acylation site of SHMT2. Quantification of lysine fatty-acylation levels was done for three biological replicates. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

Interestingly, this lysine residue is not conserved in SHMT1, which shares >60% identity with SHMT2 (*SI Appendix, Fig. S3C*). SHMT1 did not contain fatty acylation (*SI Appendix, Fig. S2D*), which further supported that K245 is the major lysine fatty acylation site in SHMT2.

**HDAC11 Defatty-Acylation of SHMT2 Regulates IFN $\alpha$ 1 Internalization and Stability.** We next investigated the biological function of SHMT2 lysine fatty acylation. SHMT2, a metabolic enzyme involved in one-carbon metabolism, catalyzes the reversible interconversion of serine and tetrahydrofolate to glycine and methylenetetrahydrofolate (23, 24). We first tested whether fatty-acylation might affect the enzymatic activity of SHMT2. The enzymatic activity



**Fig. 4.** HDAC11 defatty-acylation of SHMT2 regulates IFN $\alpha$ R1 cell surface level. (A) Cell surface IFN $\alpha$ R1 levels before and after 2 h IFN $\alpha$  treatment in HDAC11 WT and KO HAP1 cells monitored using flow cytometry. Images from one of three biological replicates are shown. (B) The relative surface IFN $\alpha$ R1 levels upon IFN $\alpha$  treatment in WT and HDAC11 KO HAP1 cells at indicated time points. Error bars represent SD in three biological replicates. (C) The ubiquitination level of IFN $\alpha$ R1 after treating HDAC11 WT and KO HAP1 cells with IFN $\alpha$ . Quantification of the relative level of IFN $\alpha$ R1 ubiquitination is shown on the Right. Statistical analysis: values with error bars indicate mean  $\pm$  SD of three replicates, and "NS" indicates no statistical difference. (D) Immunofluorescence showing the colocalization of SHMT2 $\alpha$  ( $\Delta$ 21) with LAMP1 in HAP1 HDAC11 WT and KO cells upon IFN $\alpha$  treatment. (E) Statistical analysis of the colocalization of SHMT2 $\alpha$  with LAMP1 with and without IFN $\alpha$  treatment using Pearson's coefficient. Each dot represents one cell; center lines represent the mean values. \* $P$  < 0.05, \*\*\* $P$  < 0.001.

of SHMT2 K245R mutant was not significantly different from that of WT SHMT2 (*SI Appendix, Fig. S4A*). Similarly, the enzymatic activity of SHMT2 was not significantly changed when coexpressed with either WT or the Y304H mutant of HDAC11 in 293T cells (*SI Appendix, Fig. S4B*). These observations suggested fatty-acylation did not affect SHMT2 enzymatic activity. In line with this, we also did not detect any difference in the oligomeric states of SHMT2 in HDAC11 knockdown cells (*SI Appendix, Fig. S4C*) (25).

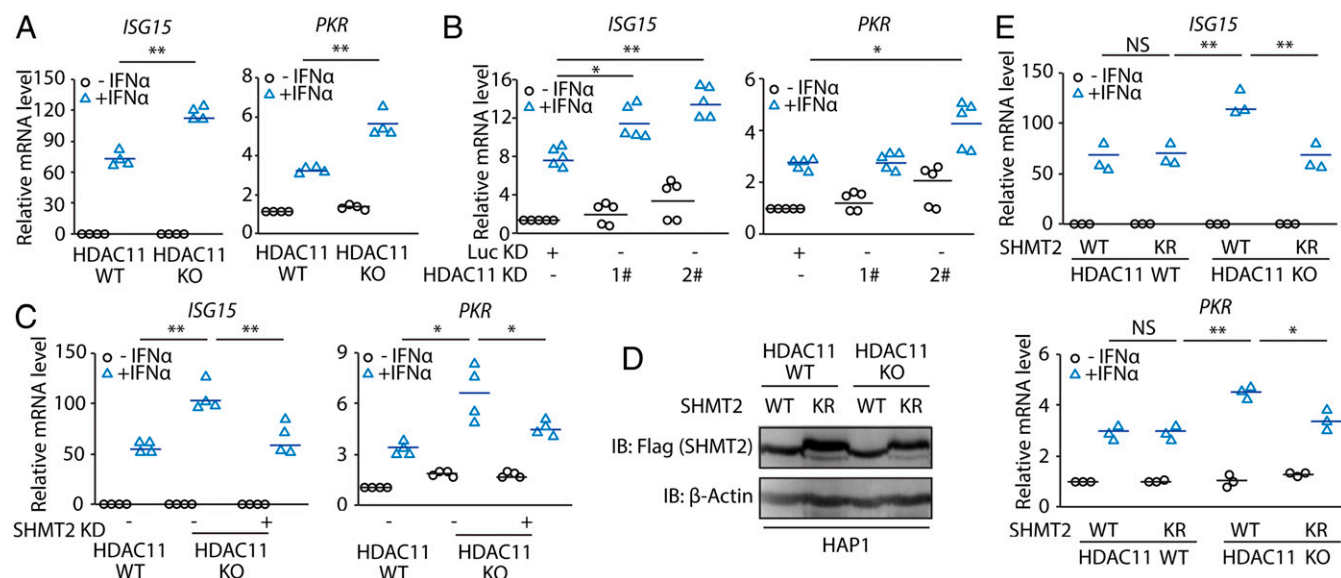
SHMT2 is mainly localized in the mitochondria, and the subcellular localization of WT SHMT2 and the K245R mutant in HDAC11 WT or KO HAP1 cells was not significantly different (*SI Appendix, Fig. S4D and E*). However, we did detect a small amount of SHMT2 that was localized in the cytosol (*SI Appendix, Fig. S4E*), which was consistent with previous reports (23, 26). Given that the natural cytosolic isoform of SHMT2 (also known as SHMT2 $\alpha$ , referred to as  $\Delta$ 21 mutant here) also had lysine fatty-acylation (Fig. 3E and *SI Appendix, Fig. S4F*), we believe that lysine fatty acylation mainly occurred in the cytosolic SHMT2 $\alpha$ . Cytosolic SHMT2 was known to direct the BRCC36-containing complex (BRISC, a complex with deubiquitination activity) to deubiquitinate type I IFN receptor chain 1 (IFN $\alpha$ R1) and thus decrease the internalization and increase the stability of IFN $\alpha$ R1 (26). We thus tested whether HDAC11 affects the internalization of IFN $\alpha$ R1. As shown in Fig. 4A and B, cell surface IFN $\alpha$ R1 significantly decreased upon IFN $\alpha$  treatment in WT, but not in HDAC11 KO cells. Given that the total IFN $\alpha$ R1 level was not significantly affected by HDAC11 KO (Fig. 4C), the data support the hypothesis that HDAC11 KO decreases the internalization or promoted the recycling of IFN $\alpha$ R1. Consistent with this, HDAC11 KO significantly decreased the ubiquitination level of IFN $\alpha$ R1 compared with WT cells (Fig. 4C).

Lysine fatty-acylation has been shown to regulate the subcellular localization of certain proteins (21, 27). We hypothesized that the lysine fatty-acylation of SHMT2 may also function to recruit SHMT2 to the plasma membrane, where IFN $\alpha$ R1 is normally localized. To our surprise, we did not observe any obvious plasma membrane localization of cytosolic SHMT2 $\alpha$ . However, interestingly, we found the colocalization of cytosolic SHMT2 $\alpha$  with late endosome/lysosome marker LAMP1 was significantly enhanced by HDAC11 KO under IFN $\alpha$  treatment (Fig. 4D and E). Importantly, for the SHMT2 $\alpha$  K245R mutant that cannot be acylated, HDAC11 KO did not increase its colocalization with LAMP1 under IFN $\alpha$  treatment (Fig. 4D and E). These results demonstrate that under IFN $\alpha$  treatment, lysine fatty-acylation of SHMT2 promotes its translocation to late endosome/lysosomes, where internalized IFN $\alpha$  is trafficked to. It is likely that by promoting SHMT2 late endosome/lysosome targeting and thus the deubiquitination of internalized IFN $\alpha$ R1, HDAC11 KO would promote the recycling of IFN $\alpha$ R1 back to the plasma membrane.

**HDAC11 Regulates Type I IFN Signaling.** IFN $\alpha$ R1 is important for type I IFN signaling, which controls the activation of many genes that are important for immune response (28). The cell surface level of IFN $\alpha$ R1 could affect the activation of downstream genes in type I IFN-treated cells (26, 29). Thus, HDAC11 may regulate type I IFN signaling via defatty-acylation of SHMT2. To test this, we measured expression of two classic IFN $\alpha$ -driven genes, *ISG15* and *PKR* (28). *ISG15* and *PKR* mRNA levels were increased in HDAC11 knockout cells more than those in WT HAP1 cells (Fig. 5A). To validate that the increase of IFN $\alpha$ R1 downstream genes was caused by the depletion of HDAC11, we reintroduced mouse HDAC11 in HDAC11 KO HAP1 cells. As expected, reexpressing mouse HDAC11 in HDAC11 KO HAP1 cells diminished the increase of *ISG15* and *PKR* expression (*SI Appendix, Fig. S5A*). Similarly, *ISG15* and *PKR* mRNA levels were higher in HDAC11 KD than in control KD MCF-7 cells (Fig. 5B).

(E) Statistical analysis of the colocalization of SHMT2 $\alpha$  with LAMP1 with and without IFN $\alpha$  treatment using Pearson's coefficient. Each dot represents one cell; center lines represent the mean values. \* $P$  < 0.05, \*\*\* $P$  < 0.001.





**Fig. 5.** HDAC11 regulates type I IFN signaling. (A) The relative mRNA levels of IFN $\alpha$ R1 downstream genes, *ISG15* and *PKR*, in IFN $\alpha$ -treated HDAC11 WT and KO HAP1 cells were quantified by RT-PCR and normalized to *GAPDH* expression. (B) The relative mRNA levels of *ISG15* and *PKR* in IFN $\alpha$ -treated control and HDAC11 KD MCF-7 cells were quantified by RT-PCR and normalized to *GAPDH* expression. (C) The effect of SHMT2 KD on the relative mRNA level of *ISG15* and *PKR* in HDAC11 KO HAP1 cells. (D) Western blot showing the overexpression of SHMT2 WT and K245R mutant in HDAC11 WT and KO HAP1 cells. (E) The effect of WT and the K245R mutant of SHMT2 on the relative mRNA level of *ISG15* and *PKR* in HDAC11 WT and KO HAP1 cells. \* $P < 0.05$ , \*\* $P < 0.01$ .

Furthermore, knockdown of SHMT2 or KIAA0157, key components of BRISC (26), diminished the increase of *ISG15* and *PKR* mRNA level in HDAC11 KO HAP1 cells (Fig. 5C and *SI Appendix, Fig. S5 B and C*), further supporting the idea that HDAC11 antagonizes type I IFN signaling via defatty-acylation of SHMT2 $\alpha$ , decreasing the recruitment of BRISC and increasing the ubiquitination of IFN $\alpha$ R1.

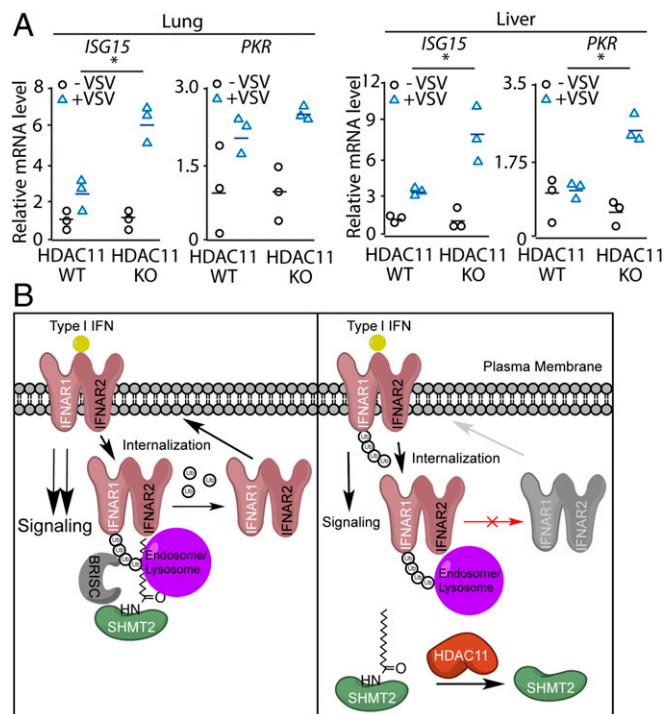
Type I IFN is released by mammalian cells in response to viral infections and helps cells to heighten their antiviral defense (28). Therefore, we investigated whether HDAC11 could affect the viral defense of cells. We treated WT and HDAC11 KO HAP1 cells with IFN $\alpha$  before infecting cells with lentiviruses carrying the GFP gene. The protein expression level of GFP would be reduced if there was increased antiviral defense due to increased IFN $\alpha$ R1 signaling in HDAC11 KO cells. Consistent with our hypothesis, HDAC11 KO cells had reduced GFP protein levels compared with WT HAP1 cells (*SI Appendix, Fig. S5D*).

We next investigated whether the effect of HDAC11 on type I IFN signaling is largely through lysine fatty-acylation of SHMT2. Using *ISG15* and *PKR* mRNA levels as the readout, we assessed the ability of WT SHMT2 and K245R mutant to affect the increase of IFN $\alpha$ R1 downstream genes in WT and HDAC11 KO HAP1 cells (Fig. 5D). In WT HAP1 cells, expressing WT SHMT2 and K245R mutant resulted in no significant changes of *ISG15* and *PKR* mRNA level after IFN $\alpha$  treatment (Fig. 5E). In contrast, expressing K245R mutant SHMT2, but not WT, compromised the increase of *ISG15* and *PKR* mRNA level in HDAC11 KO cells (Fig. 5E). These results support the hypothesis that the HDAC11 regulates type I IFN signaling via controlling lysine fatty-acylation of SHMT2.

To validate this phenotype in vivo, we further examined the IFN $\alpha$ R1 signaling in HDAC11 KO mice infected with vesicular stomatitis virus (VSV). The *ISG15* and *PKR* mRNA levels in the lung and liver were analyzed 2 d postinfection by VSV. As shown in Fig. 6A, VSV viral infection up-regulated levels of the *ISG15* and *PKR* mRNA in lung and liver tissues from VSV-infected HDAC11 KO mice compared with those from WT animals (Fig. 6A), consistent with the model that HDAC11 could antagonize type I IFN signaling. Unfortunately, we could not do the survival analysis because the virus was effectively cleared in both WT and HDAC11 KO mice.

## Discussion

In this study, we have identified HDAC11 as an efficient lysine defatty-acylase with catalytic efficiency >10,000-fold better than its



**Fig. 6.** HDAC11 regulates type I IFN signaling in vivo. (A) WT and HDAC11 KO mice were injected with VSV for 2 d, and the lung and liver were harvested for mRNA analysis. The relative mRNA levels of IFN $\alpha$ R1 downstream genes, *ISG15* and *PKR*, were quantified by RT-PCR and normalized to *GAPDH* expression. Bars indicate mean values. \* $P < 0.05$ . (B) Scheme showing the potential mechanism via which HDAC11 negatively regulates type I IFN signaling by controlling SHMT2 lysine fatty-acylation.

deacetylase activity. Consistent with two recent reports (19, 20) (we coordinated with these two groups and deposited manuscripts on BioRxiv on the same day), our data demonstrated that HDAC11 has an activity that is much more efficient than its deacetylase activity. HDAC8 was recently reported to have defatty-acylase activity, but the catalytic efficiency is only 2–3 folds better than that of deacetylation (12). The studies with HDAC11 suggest that the zinc-dependent HDACs are similar to the sirtuin family of HDACs, where several members preferentially recognize acyl lysine modifications other than acetyl lysine, while some can remove multiple modifications with similar efficiencies (11, 13). Among the zinc-dependent HDACs, class IIa HDACs lack detectable deacetylase activity (1). The finding that HDAC11 has an efficient activity suggests the possibility that class IIa HDACs may also remove currently unknown acyl lysine modifications.

Our study identified a posttranslational mechanism that regulates a multifunctional protein, SHMT2. SHMT2 is a mitochondrial enzyme involved in one carbon metabolism (23, 24). A shorter isoform, SHMT2 $\alpha$ , which lacks the mitochondrial localization sequence, is present in the cytosol and nucleus (21). The metabolic function of SHMT2 is thought to be critical for cancer cells (30). Recently, SHMT2 $\alpha$  was also reported to recruit the BRISC complex to IFN $\alpha$ R1 to promote the deubiquitination of IFN $\alpha$ R1 (26). Our study presented here indicates that SHMT2 $\alpha$  is regulated by lysine fatty acylation, which has only been known to occur on a few proteins in mammalian cells (18, 21, 27, 31, 32). Our data suggest that the deubiquitination of IFN $\alpha$ R1 by the BRISC complex likely happens at the late endosome/lysosome instead of plasma membrane and that lysine fatty acylation promotes SHMT2 $\alpha$  translocation to late endosome/lysosome upon type I IFN treatment and subsequent recruitment of the BRISC complex to IFN $\alpha$ R1. HDAC11 can remove the lysine fatty acylation on SHMT2 $\alpha$  and thus increase the ubiquitination of IFN $\alpha$ R1 and down-regulate IFN signaling (Fig. 6B).

Our results revealed an immune-regulatory function of HDAC11 by connecting it to the IFN-signaling pathway. It has been reported that HDAC11 overexpression suppresses LPS-stimulated IL-10 transcription, while HDAC11 knockdown increases LPS-stimulated IL-

10 transcription (10). This was explained by a model where HDAC11 binds to IL-10 promoter and deacetylates histones to suppress IL-10 transcription (10). In contrast, the HDAC11-IFN $\alpha$ R1 regulation that we discovered here is different. Our model is based on the more efficient defatty-acylase activity of HDAC11, while the previous model is based on the deacetylase activity of HDAC11. The two models may not be mutually exclusive, since it is possible that HDAC11's deacetylase activity could be increased upon chromatin binding, which allows for deacetylation of histones, while it functions efficiently as defatty-acylase in the cytosol. A similar mechanistic function has been reported for SIRT6, a member of the NAD<sup>+</sup>-dependent HDAC (27, 33).

We demonstrated here that the regulation of the IFN-signaling pathway is physiologically relevant for the antiviral response in human cell lines and in mice. Mice lacking HDAC11 exhibited increased IFN signaling. This finding significantly expands the physiological function of HDAC11 and opens up opportunities to develop HDAC11-specific inhibitors as therapeutics to treat disease where increased type I IFN signaling is beneficial, such as viral infection (34), multiple sclerosis (35), or cancer (36). The ability to target the HDAC11-SHMT2 node to up- or down-regulate type I IFN signaling may have significant power in modulating immune responses in a case-dependent manner for clinical benefits.

## Materials and Methods

Full details of materials and methods, including expression and purification of HDAC11, enzymatic activity assay, detection of lysine fatty acylation, SILAC proteomic experiments, and analysis of IFN $\alpha$ R1 internalization and ubiquitination, are provided in [SI Appendix](#).

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