Contents:

Appendix Figure S1----------2

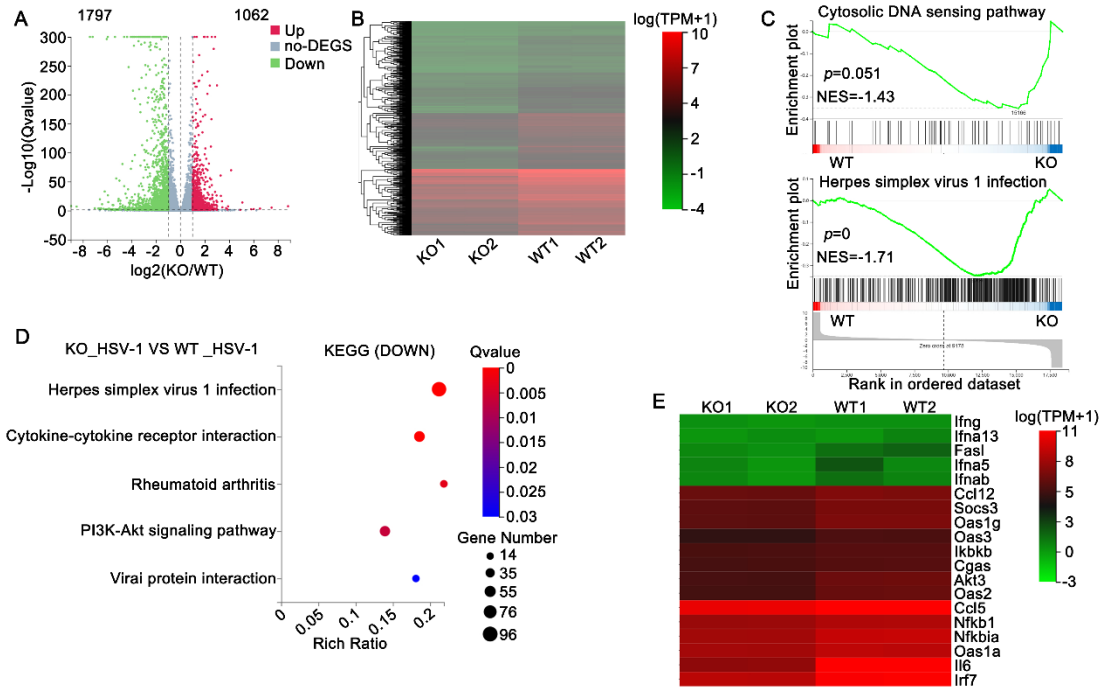
Appendix Figure S2----------3

Appendix Figure S3----------4-5

Appendix Figure S4----------6

Appendix Table S1----------7-8

Appendix Figure S1



Appendix Figure S1 RNF144A deficiency impairs the expression of type I IFNs, proinflammatory cytokines, and ISGs

(A) Volcano plots comparing gene expression in wild-type (WT) versus RNF144A-deficient (KO) MEFs after HSV-1 (MOI = 1) infection for 8 h.

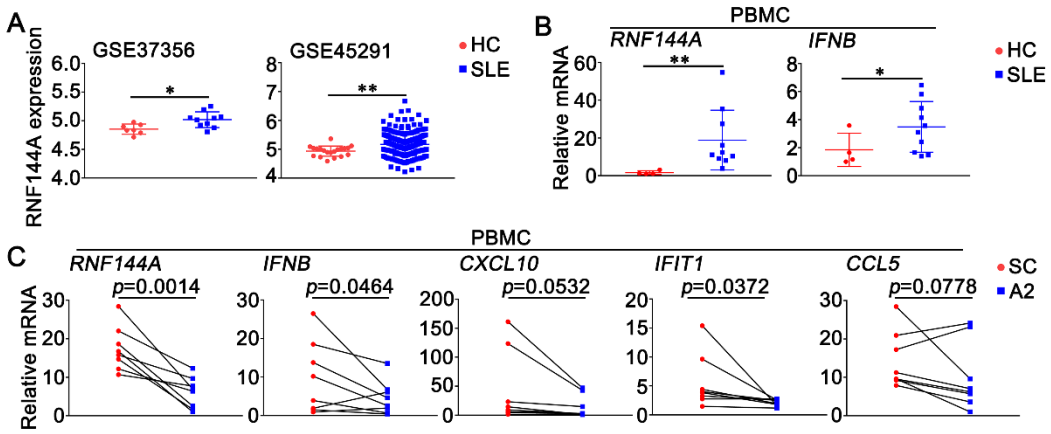
(B) Heatmap depiction of differentially expressed genes in wild-type (WT) versus RNF144A-deficient (KO) MEFs.

(C) GSEA analysis of cytosolic DNA sensing pathway and HSV-1 infection relative to wild-type (WT) versus RNF144A-deficient (KO) MEFs. Enrichment plots were shown along with the normalized enrichment score and p-value.

(D) KEGG analysis through Dr. Tom (https://biosys.bgi.com/) for genes significantly down-regulated (log2 $\geqslant$ 1, $p < 0.05$) after HSV-1 (MOI = 1) infection for 8 h.

(E) Heatmap view of mRNA variations in type I IFNs, ISGs, and proinflammatory cytokines (log2 $\geqslant$ 1).

Appendix Figure S2



Appendix Figure S2 RNF144A may act as a promoter of systemic lupus erythematous

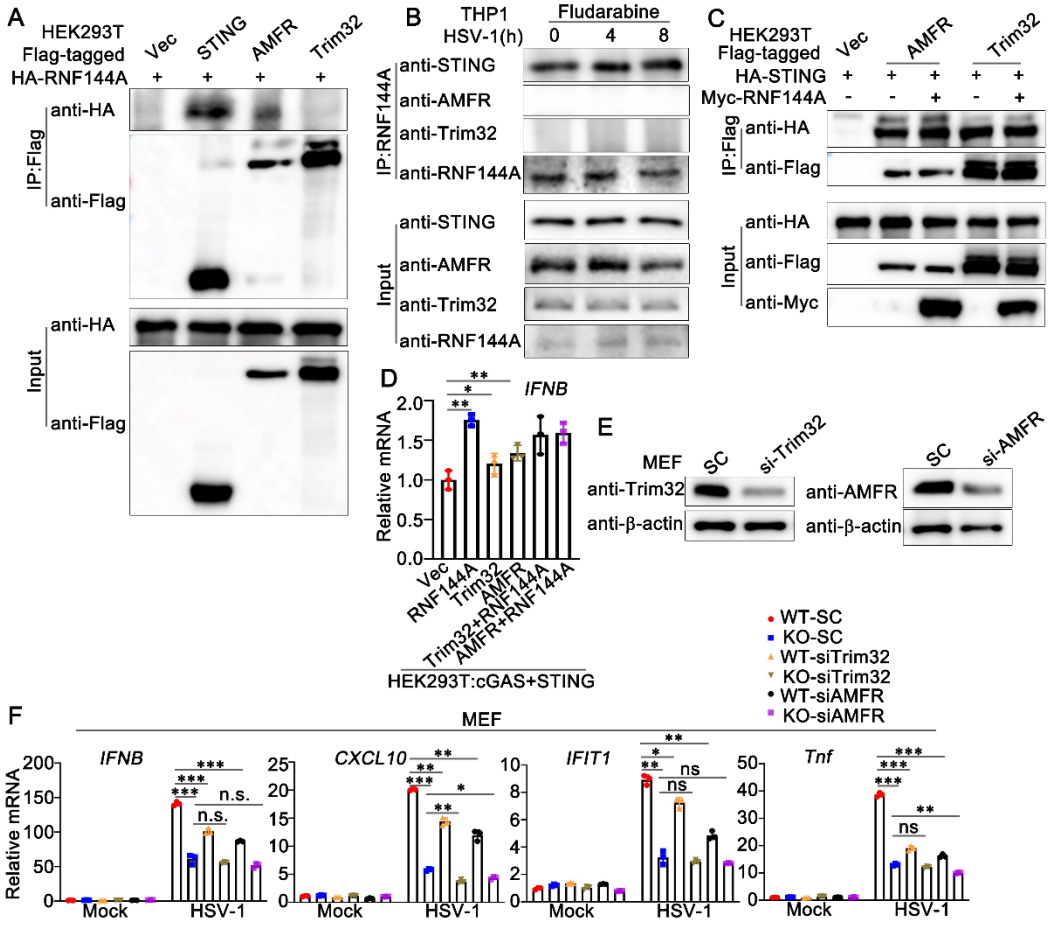
(A) The expression comparison of RNF144A in the monocytes and whole blood cells between SLE patients and healthy donors across four SLE cohorts.

(B) PBMCs were isolated from the blood sample of healthy donors (n=4) or SLE patients (n=10). The cells were lysed for real-time PCR analyses.

(C) PBMCs were isolated from the blood sample of SLE patients (n=8). PBMCs were transfected with control siRNA (SC) or RNF144A-specific siRNA (A2) for 24 h, Then, RNF144A, IFNB, CXCL10, IFIT1, and CCL5 mRNA were measured by real-time PCR.

Data information：*$p < 0.05$ and **$p < 0.01$; p values are calculated using two-tailed unpaired Student's t test. Data are representative of at least three independent biological replicates. Each data point represents an independent biological replicate. Error bars are presented as mean ± SD. Source data for this figure are available online.

Appendix Figure S3



Appendix Figure S3. The relationship between RNF144A, AMFR, and Trim32.

A) HEK293T cells were transfected with the indicated plasmids. At 24 h after transfection, immunoprecipitation (IP) and immunoblot (IB) assays were performed as indicated.

B) PMA-THP1 cells were treated with Fludarabine, and infected with HSV-1 for 0, 4, 8 h. The immunoprecipitation (IP) and immunoblot (IB) assays were performed as indicated.

C) HEK293T cells were transfected with indicated plasmids. At 24 h after transfection, immunoprecipitation (IP) and immunoblot (IB) assays were performed as indicated.

D) HEK293T cells were transfected with the indicated plasmids. At 24 h after transfection, IFN-β expression was detected by real-time PCR assays.

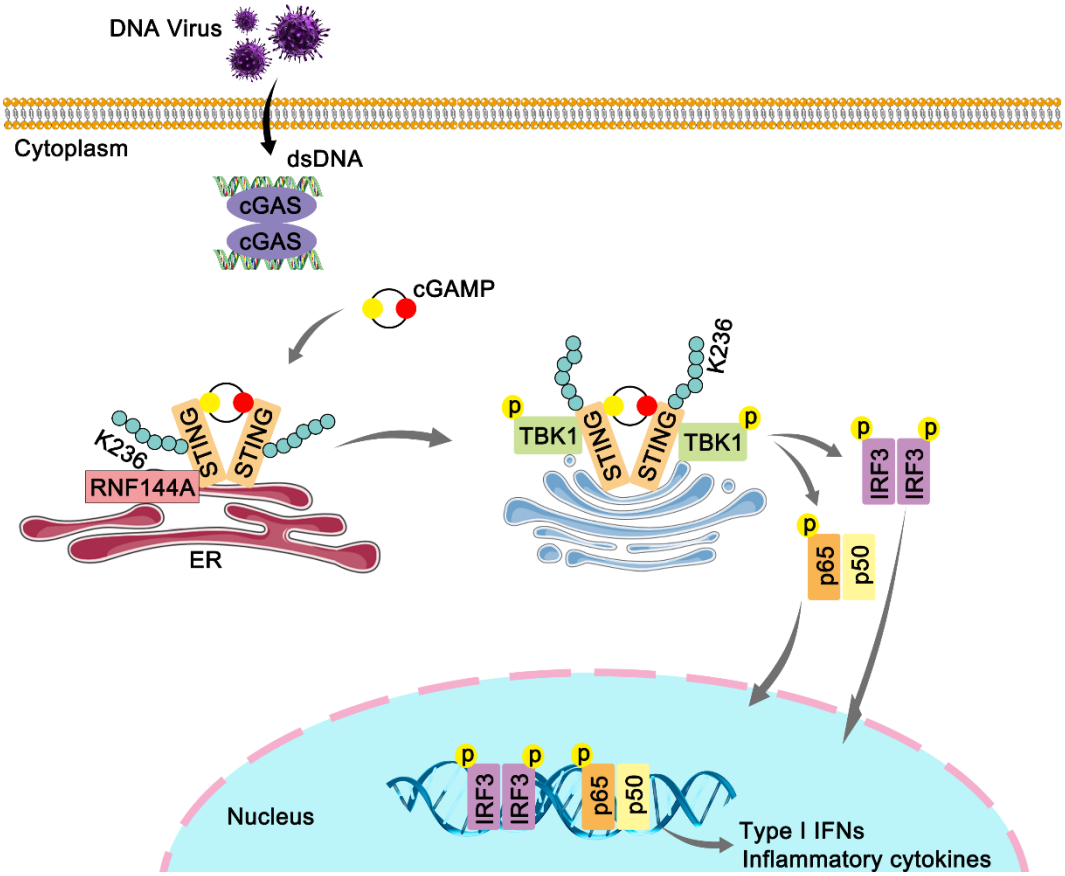
E) MEFs were transfected with control siRNA (SC), Trim32-specific siRNA (si-Trim32), or AMFR-specific siRNA (si-AMFR). At 24 h after transfection, the

immunoblot assays were performed as indicated.

F) MEFs were transfected with control siRNA (SC), Trim32-specific siRNA (si-Trim32), or AMFR-specific siRNA (si-AMFR). At 24 h after transfection, the cells were infected with HSV-1 (MOI=1) for 8 h and then the real-time PCR assays were performed as indicated.

Data information：*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and n.s., not significant ($p > 0.05$); p values are calculated using two-tailed unpaired Student's t test. Data are representative of at least three independent biological replicates. In (D, F), each data point represents a technical replicate. Error bars are presented as mean ± SD. Source data for this figure are available online.

Appendix Figure S4



Appendix Figure S4. The working model of the positive regulation of cGAS-STING signaling pathway by RNF144A.

Appendix Table S1 Primers for real-time PCR assays

Gene name	Primer sequence (5'→3') Forward（SP）	Primer sequence (5'→3') Reverse（AS）
Human *CCL5*	TACACCAGTGGCAAGTGCTC	ACACACTTGGCGGTTCTTTC
Human *CXCL10*	GGTGAGAAGAGATGTCTGAATCC	GTCCATCCTTGGAAGCACTGCA
Human *GAPDH*	TCAACGACCACTTTGTCAAGCTCA	GCTGGTGGTCCAGGTCTTACT
Human *IFIT1*	GCCATTTTCTTTGCTTCCCCTA	TGCCCTTTTGTAGCCTCCTTG
Human *IFNB*	CACGACAGCTCTTTCCATGA	AGCCAGTGCTCGATGAATCT
Human *RNF144A*	GAGCAGATGACAACCATAGCC	TGCACTCAATCTCGTTCTCCT
Human *TNF*	GGCGTGGAGCTGAGAGATAAC	GGTGTGGGTGAGGAGCACAT
Mouse *Ccl5*	TCACCATATGGCTCGGACACCAC	TTGGCACACACTTGGCGGTTC
Mouse *Cxcl10*	ATCATCCCTGCGAGCCTATCCT	GACCTTTTTTGGCTAAACGCTTTC
Mouse *Gapdh*	ACGGCCGCATCTTCTTGTGCA	ACGGCCAAATCCGTTCACACC
Mouse *Ifit1*	ACAGCAACCATGGGAGAGAATGCTG	ACGTAGGCCAGGAGGTTGTGCAT
Mouse *Ifnb*	TCCTGCTGTGCTTCTCCACCACA	AAGTCCGCCCTGTAGGTGAGGTT

Mouse *Il6*	GCTACCAAACTGGATATAATCAGGA	CCAGGTAGCTATGGTACTCCAGAA
HSV-1 gDNA	TGGGACACATGCCTTCTTGG	ACCCTTAGTCAGACTCTGTTACTTACCC
HSV-1 UL30	AGAGGGACATCCAGGACTTTGT	CAGGCGCTTGTTGGTGTAC