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Supplementary Materials for

XAF1 promotes anti-RNA virus immune responses by regulating chromatin accessibility

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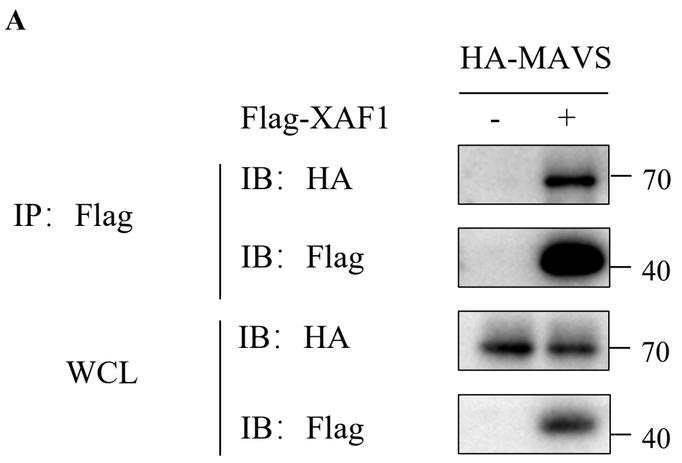
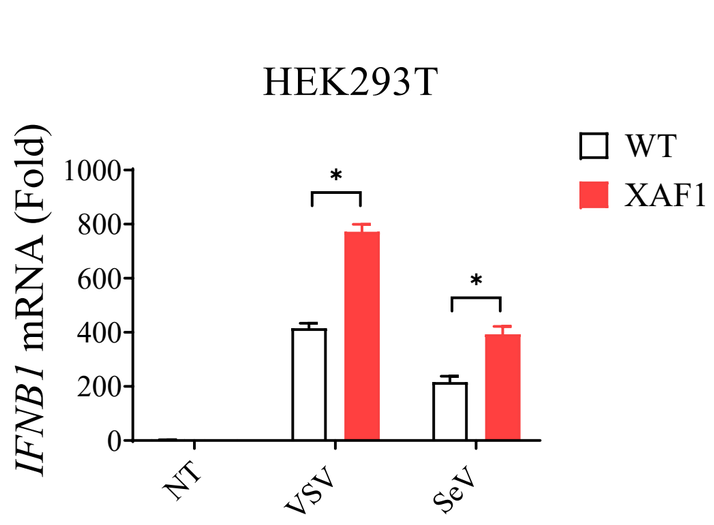
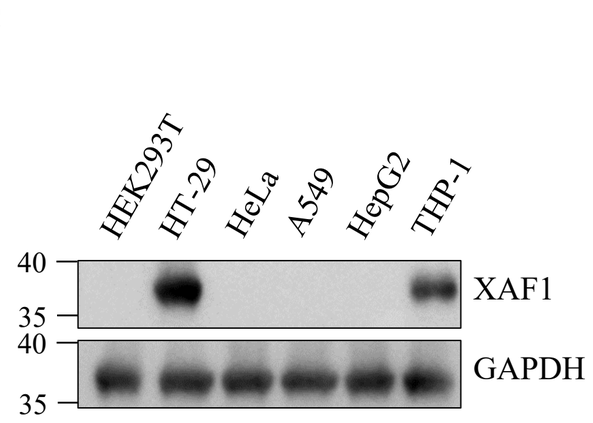
This PDF file includes:

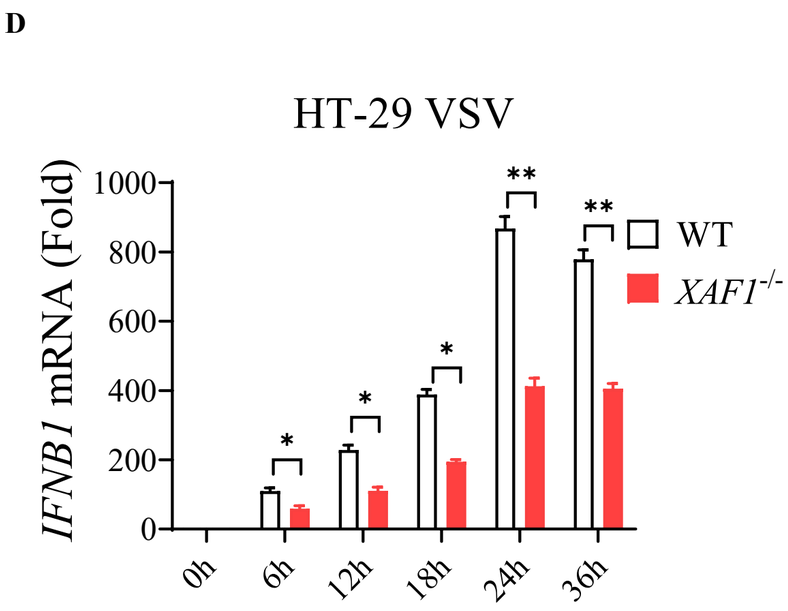
Other Supplementary Materials for this manuscript includes the following:

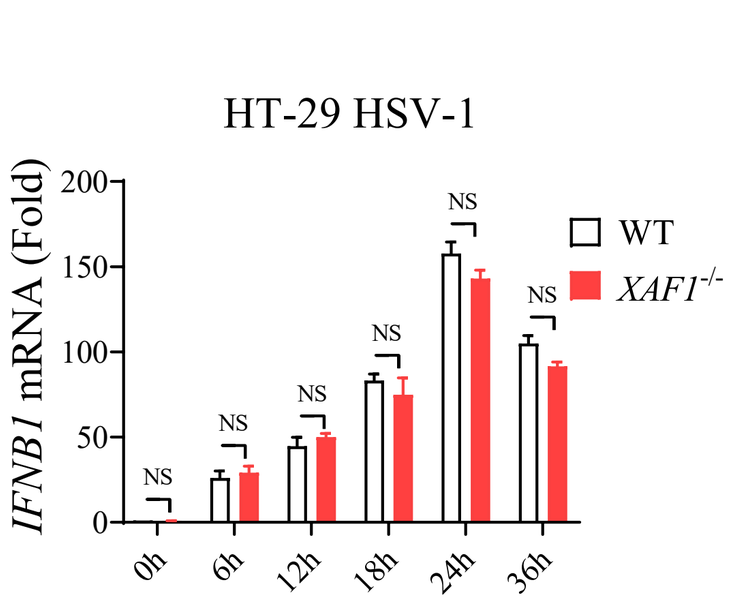
Supplementary Figure 1

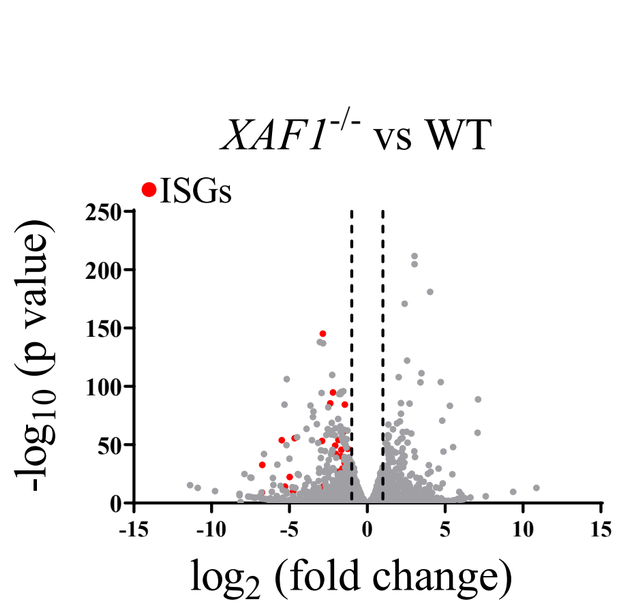


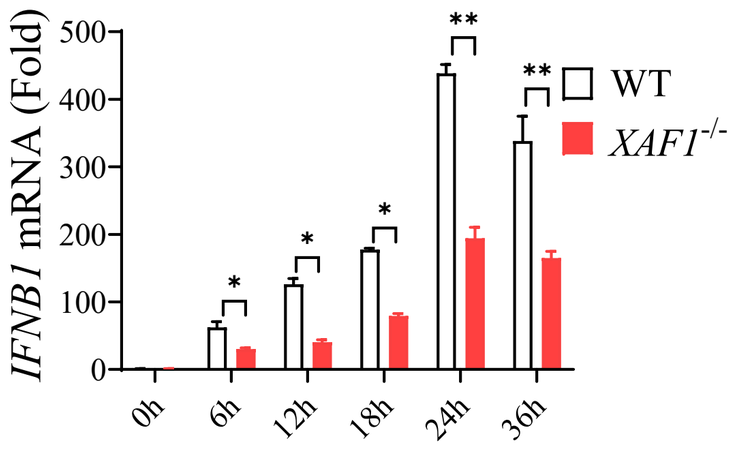


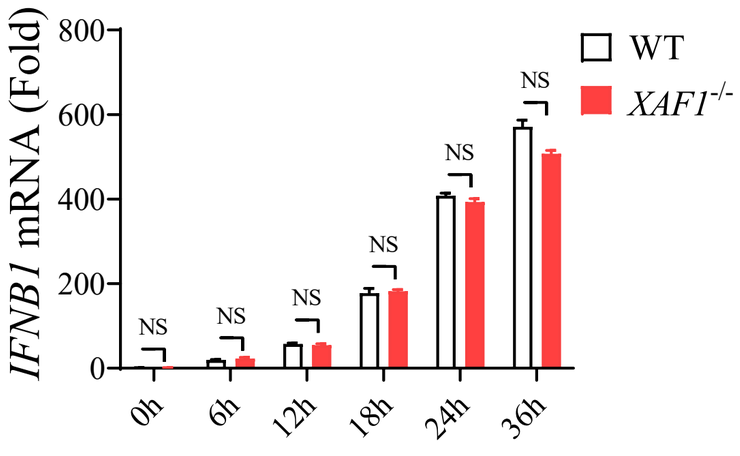


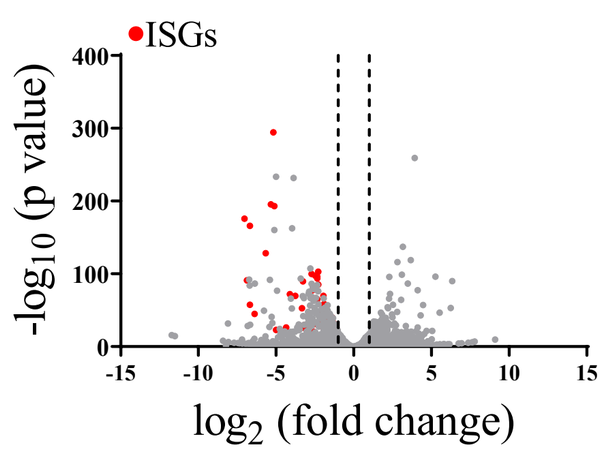


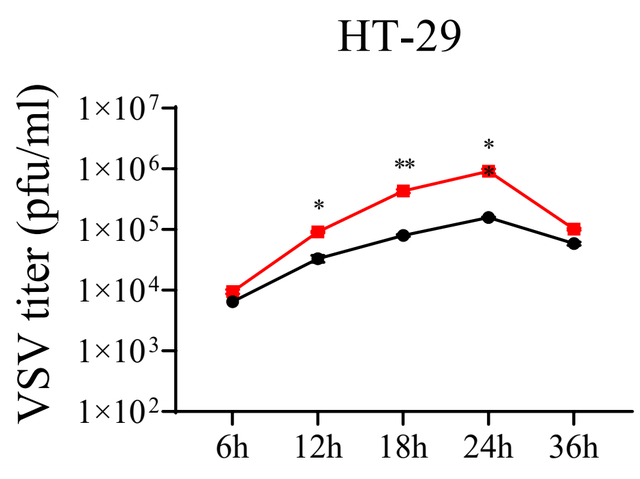


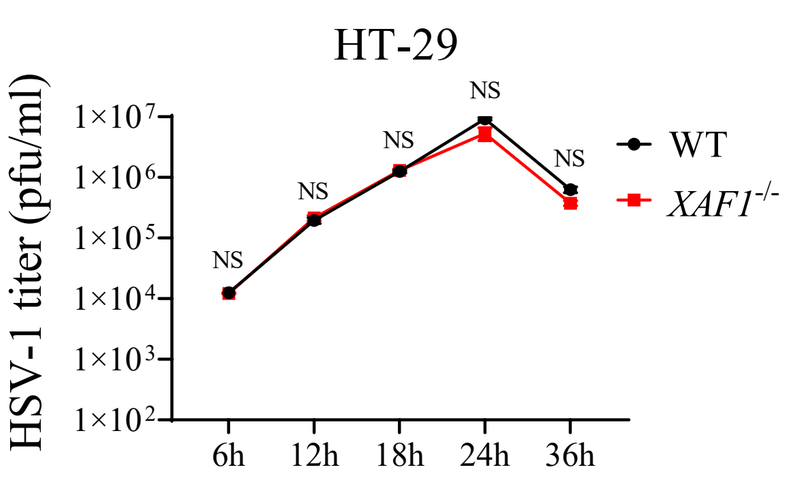


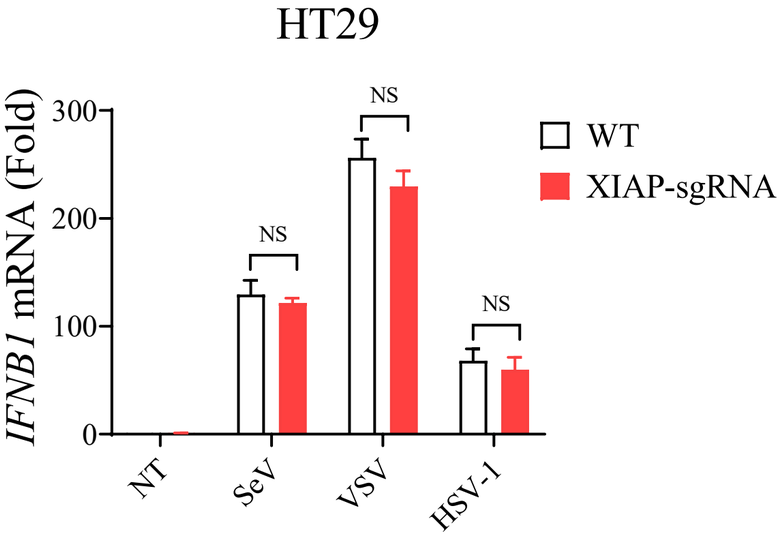


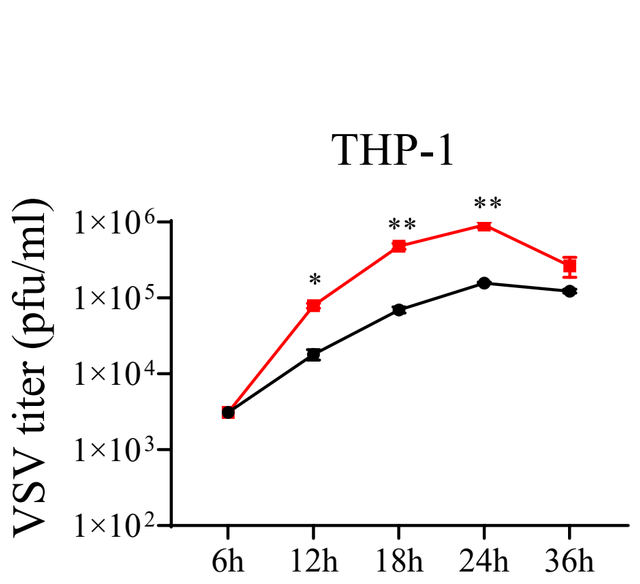


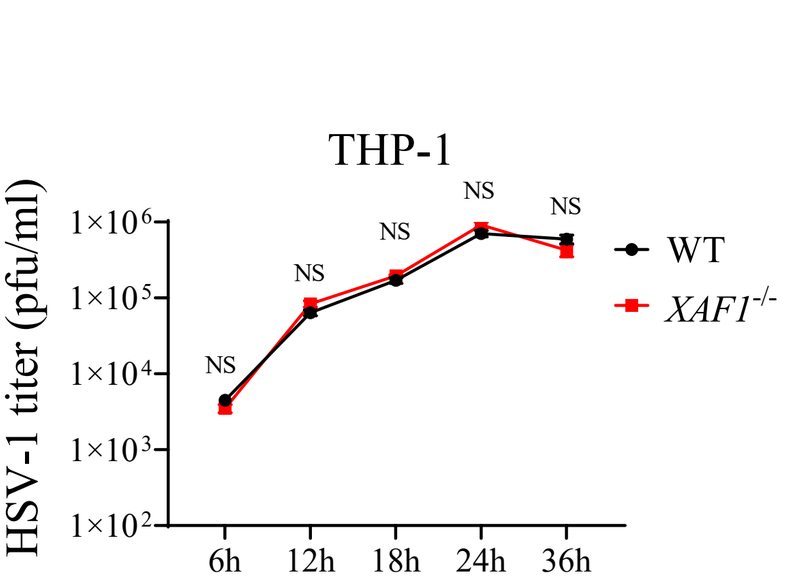












Supplementary figure 1

Fig. S1. XAF1 facilitates antiviral immune signaling . Related to Fig. 1.

(A) CoIP assay analysis of the interaction between XAF1 and MAVS.

(B) HEK293T cells were transfected Vector or XAF1. After 12h of VSV and SeV infection, qRT-PCR analyses of *IFNB1* mRNA was measured.

(C) Western blotting detected XAF1 protein level in HEK293T, HT-29, HeLa, A549, HepG2 and THP1 cells.

(D) WT and *XAF1* $^{-/-}$ HT-29 or THP-1 cells were NT (non-treated) or stimulated with VSV and HSV-1. *IFNB1* mRNA was measured by qRT-PCR.

(E) WT and *XAF1* $^{-/-}$ HT-29 or THP-1 cells were NT (non-treated) or stimulated with VSV and HSV-1. Viral loads was measured by plaque assay.

(F) A volcano plot showing changes in cellular transcripts levels in WT versus *XAF1*$^{-/-}$ HT-29 cells treated with VSV for 12 hours. The log2 fold change between WT versus *XAF1* $^{-/-}$ HT-29 cells and $-\log_{10}$ of the p value were represented in the x axis and y axis, respectively. Selected ISGs were marked in red. The p value was calculated using t test .

(G) HT-29 cells were transfected with control sgRNA or *XIAP* sgRNA. After 12h of VSV, SeV and HSV-1 infection, qRT-PCR analyses of *IFNB1* mRNA was measured.

Data are the mean ± SEM (n = 3) from three independent experiments, NS, not significant; $*p < 0.05$ and $**p < 0.01$, and $***p < 0.001$ (Student‘s t test: B, D to E and G). Data are representative of three (A to F) independent experiments with similar results.

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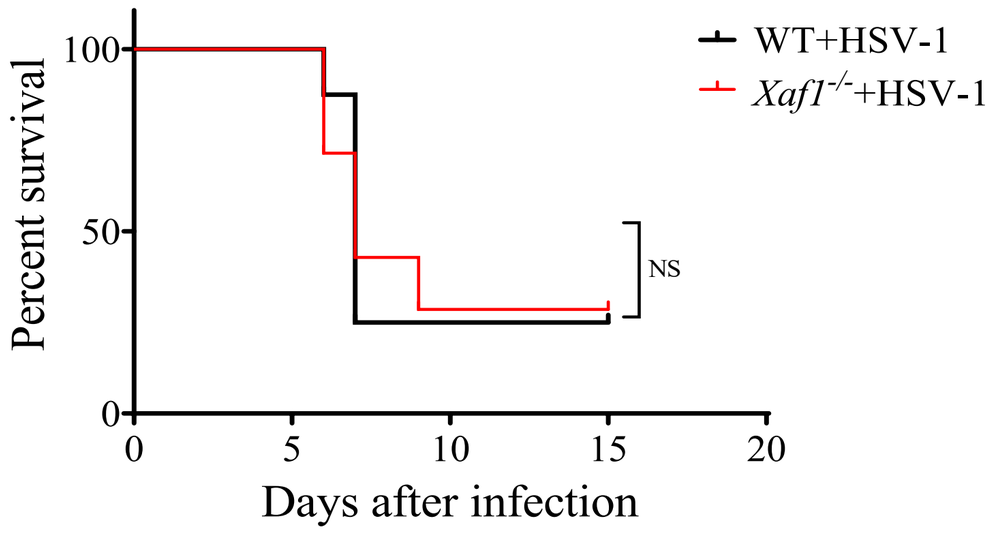

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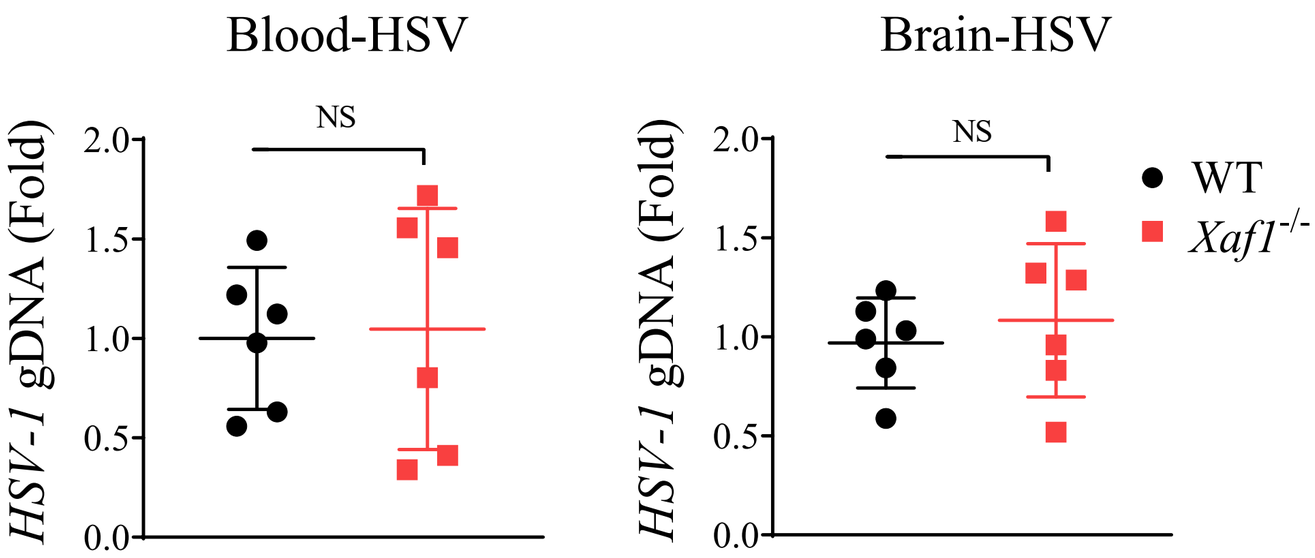

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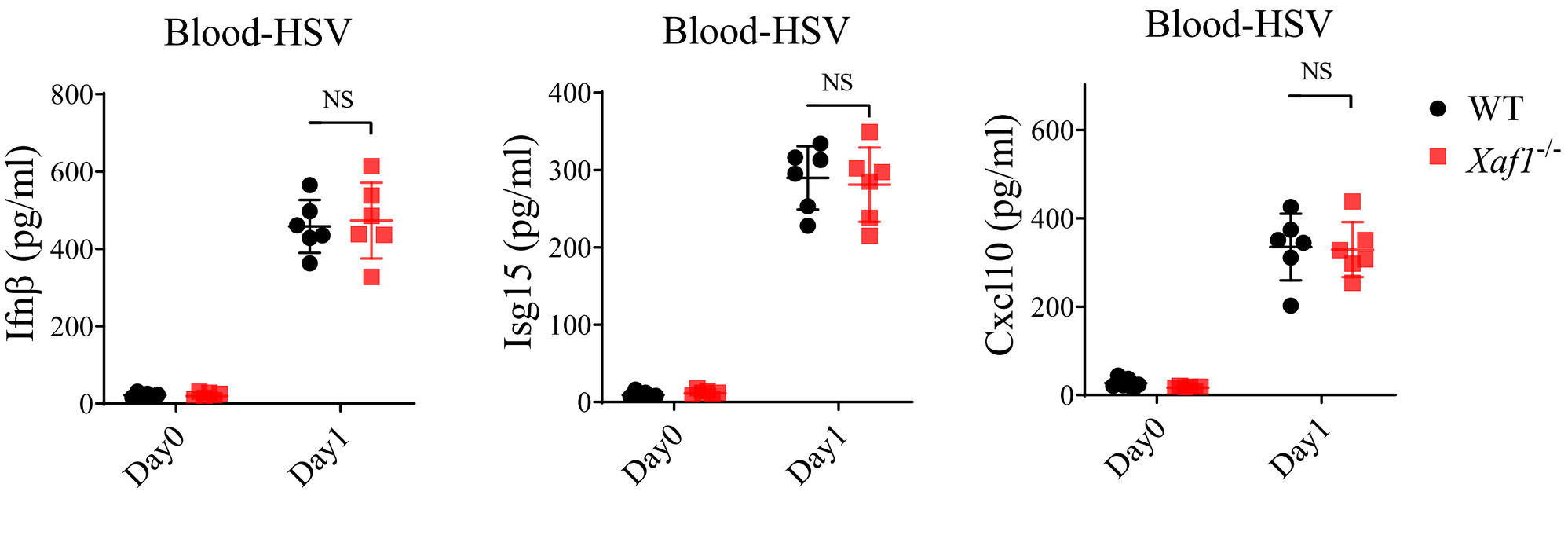

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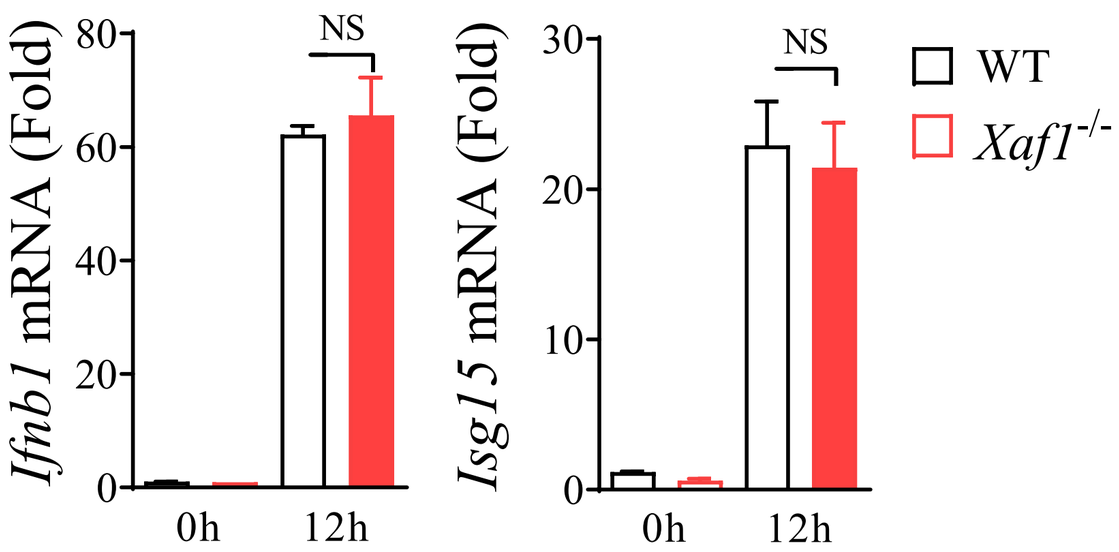

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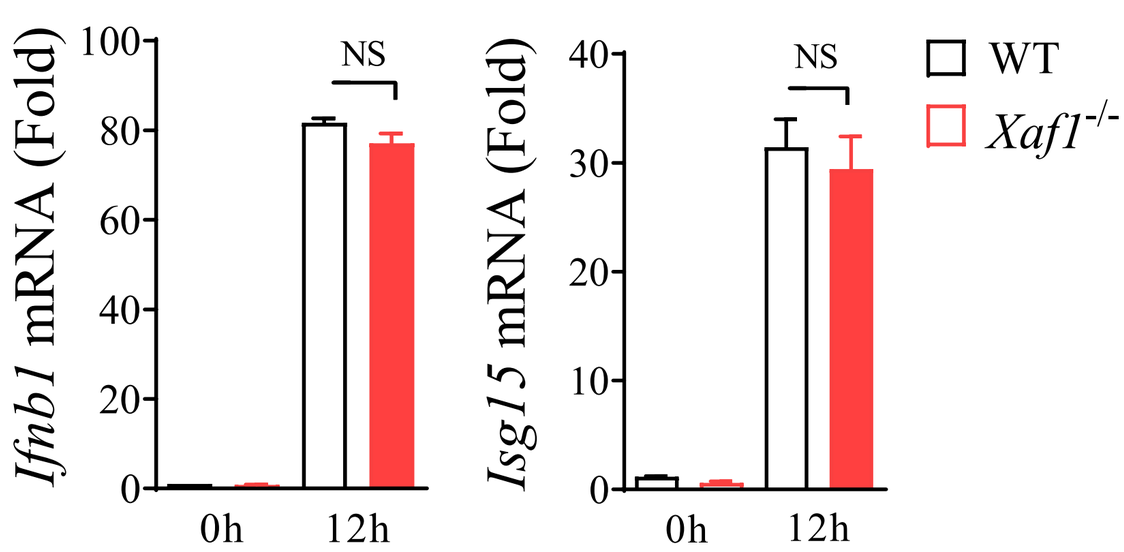


Supplementary figure 2

Fig. S2. XAF1 positively regulates IFN responses *in vivo*. Related to Fig. 2.

(A) WT and *Xaf1*$^{-/-}$ mice (n = 6 mice per group) were inoculated intra-venously with HSV-1. The survival rates of mice were recorded.

(B) Four days after HSV-1 infection, brains and blood of WT and *Xaf1*$^{-/-}$ mice were retrieved for qRT-PCR analysis of VSV genomic copies.

(C) Effects of XAF1 deficiency on HSV-1-induced serum levels of Ifnβ, Isg15 and Cxcl10. WT and *Xaf1*$^{-/-}$ mice were infected with HSV-1 (n = 6) for 6 hours before serum was measured by ELISA

(D and E) Peritoneal macrophages (PMs) and bone-marrow-derived macrophages (BMDMs) isolated from WT and *Xaf1*$^{-/-}$ mice were infected with HSV-1 for 12h. *Ifnb1* and *Isg15* mRNA was measured by qRT-PCR analysis.

Data are the mean ± SEM (n = 3) from three independent experiments, NS, not significant; $*p < 0.05$ and $**p < 0.01$, and $***p < 0.001$ (Student's t test: A to E). Data are representative of three independent experiments with similar results.

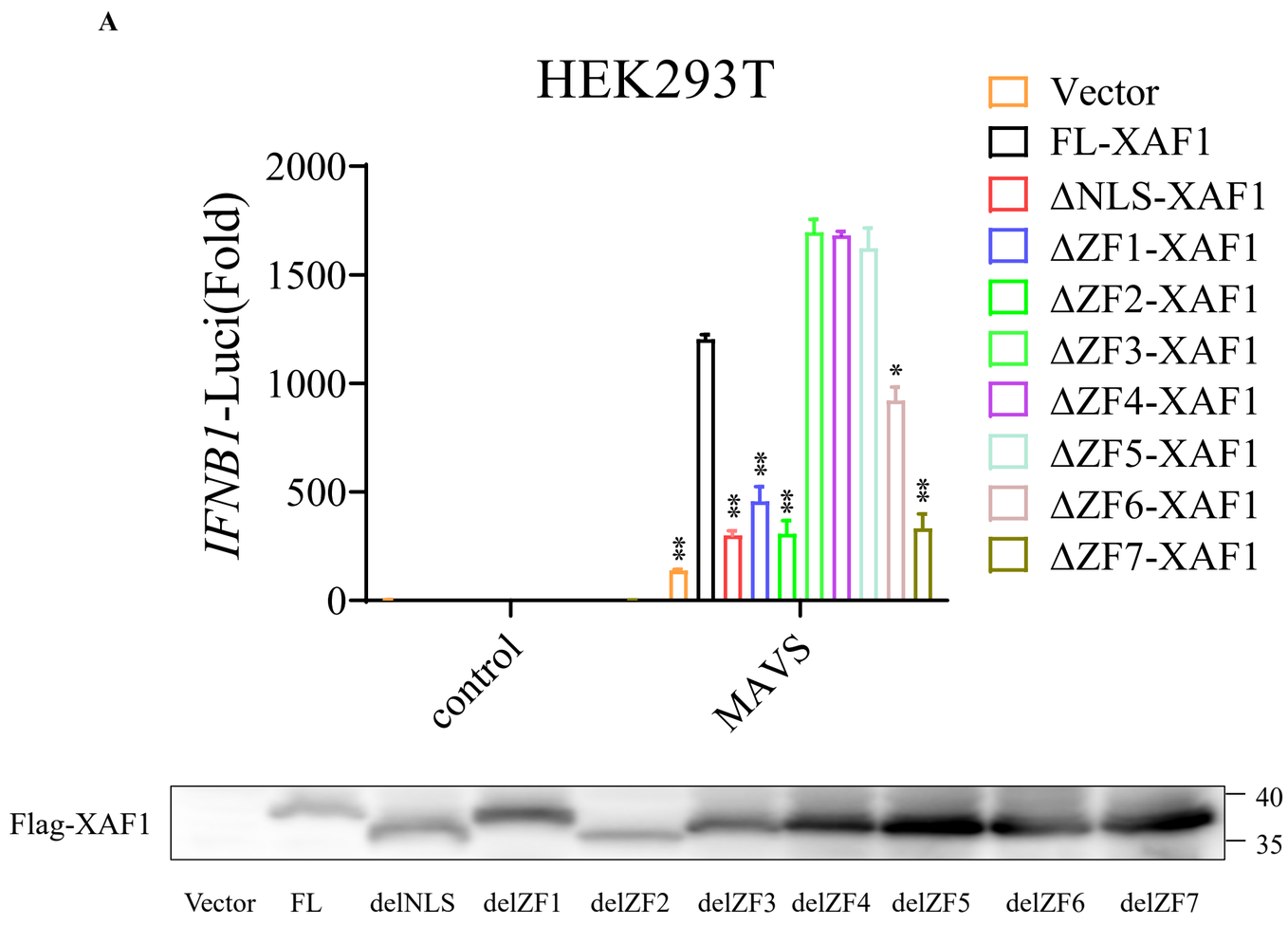
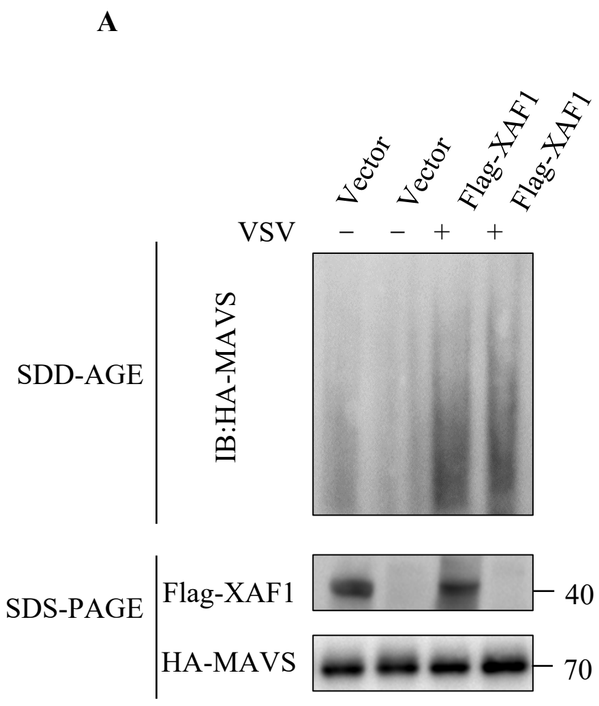
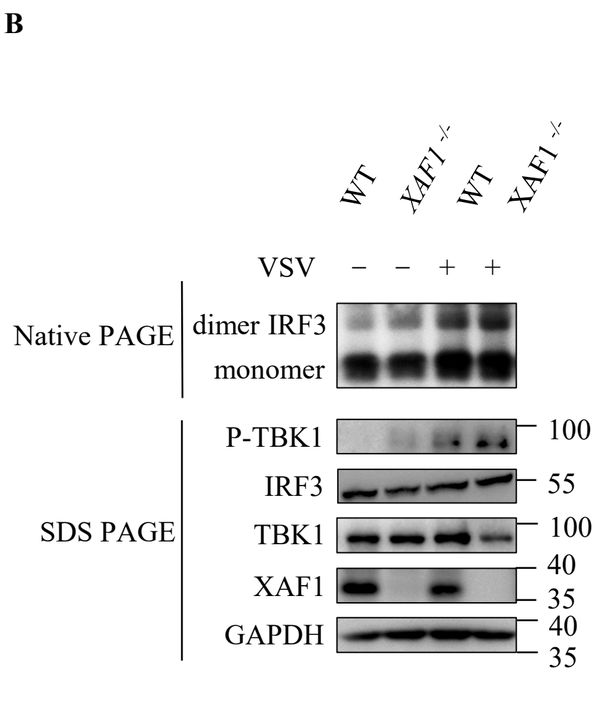


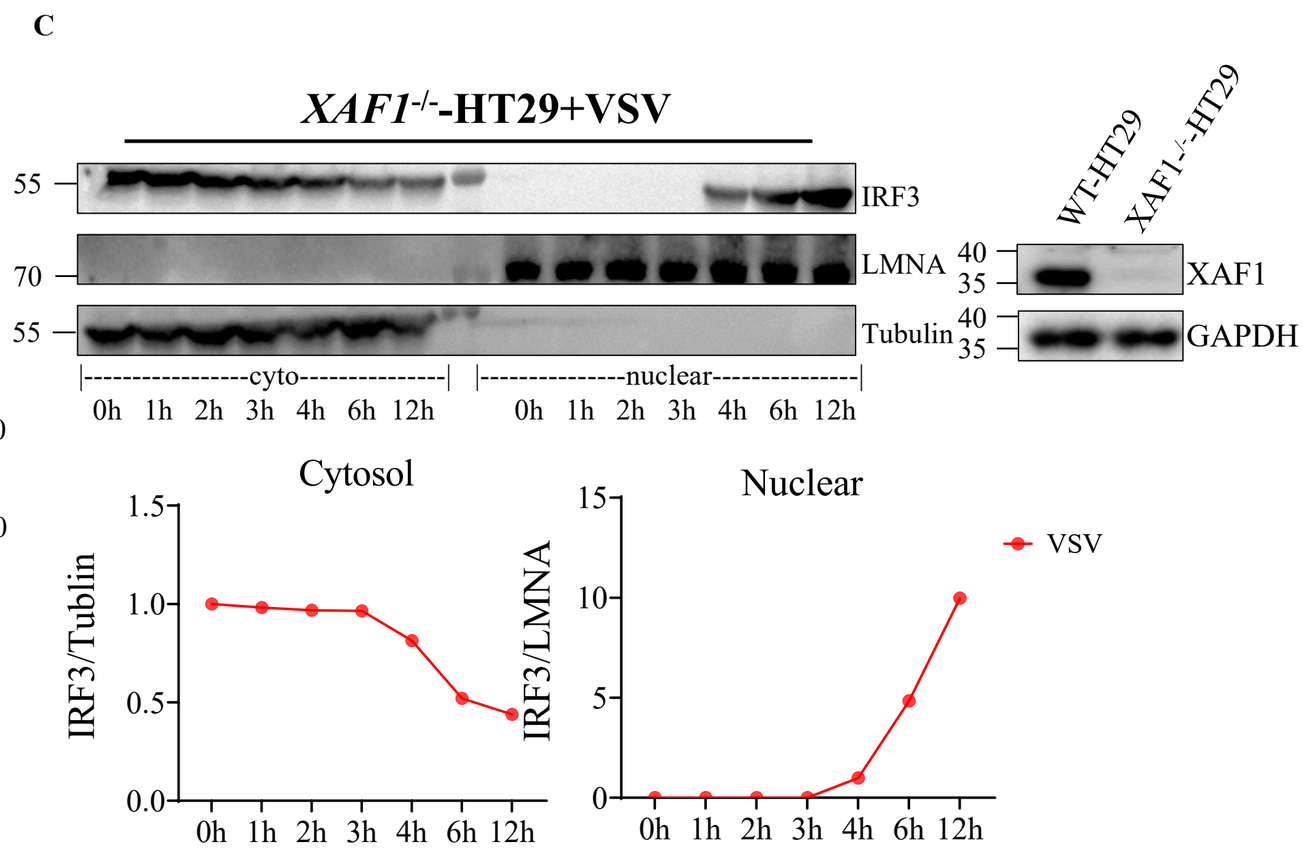
Fig. S3. XAF1 interacts with MAVS and TBK1. Related to Fig. 3.

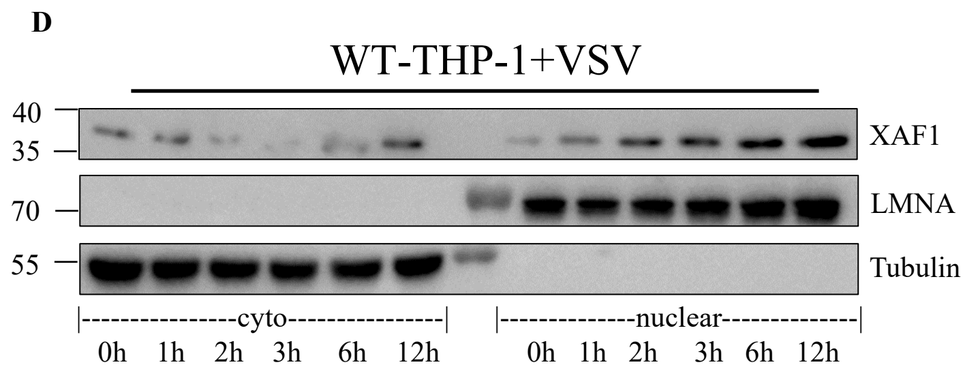
(A) Luciferase (Luc) activity in HEK293T cells transfected with 50 ng of the pRL-TK reporter, 50 ng luciferase reporter driven by promoters of genes encoding IFNβ together with Vector, NLS-, ZF1, ZF2, ZF3, ZF4, ZF5, ZF6 or ZF7-deletion XAF1 plasmids for 24 hours.

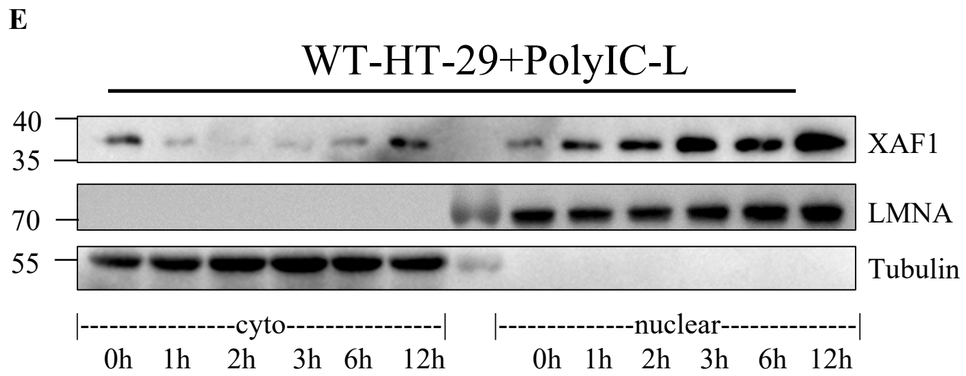
Data are the mean ± SEM (n = 3) from three independent experiments, NS, not significant; $*p < 0.05$ and $**p < 0.01$, and $***p < 0.001$ (Student's t test). Data are representative of three independent experiments with similar results.

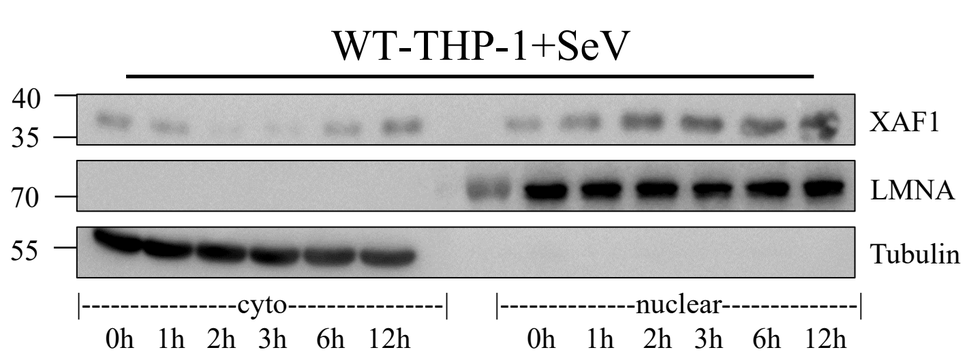


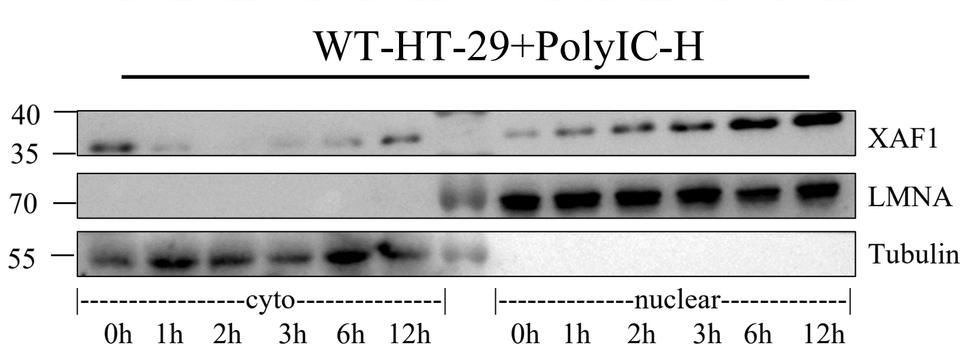


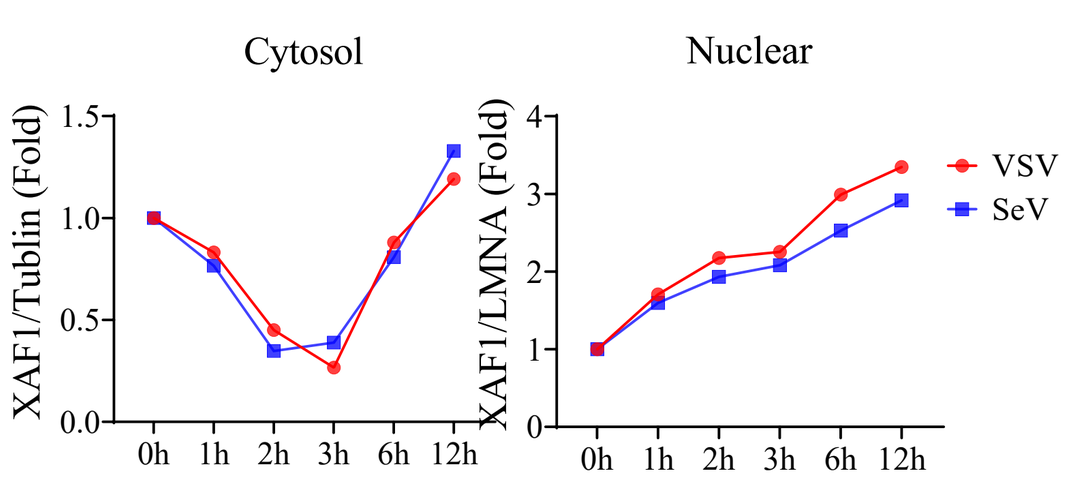


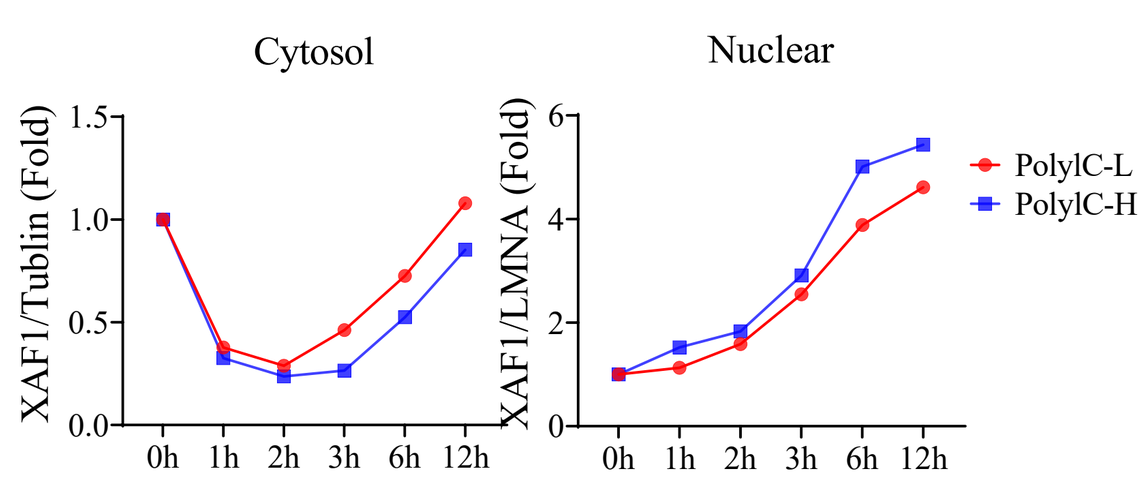


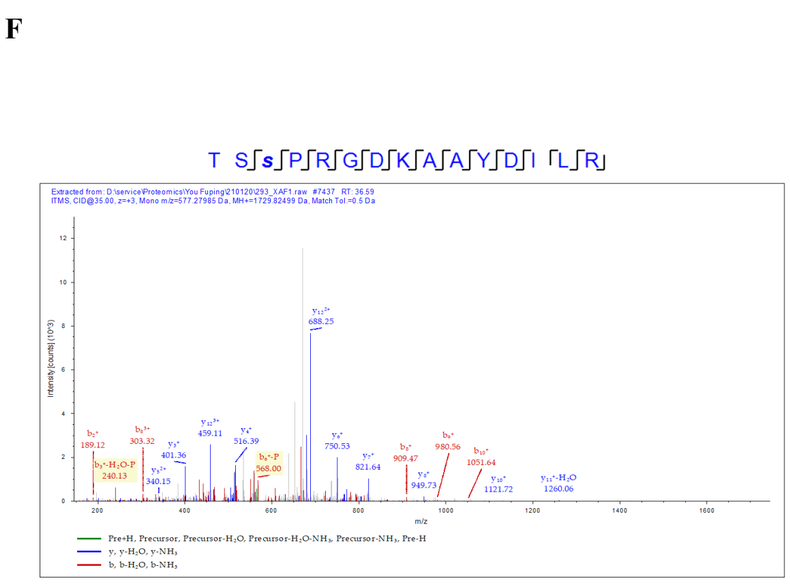


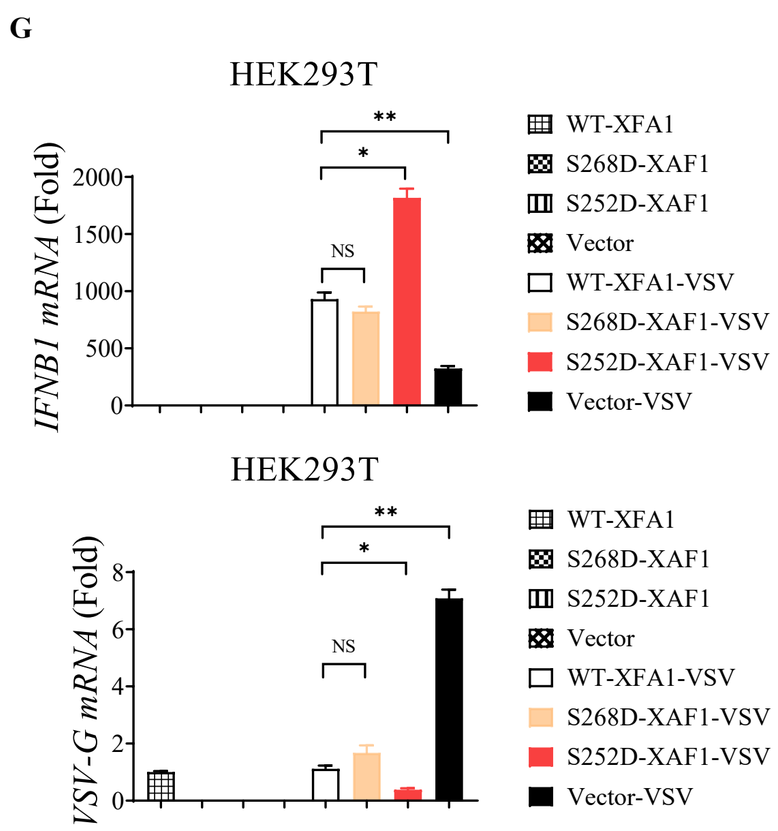


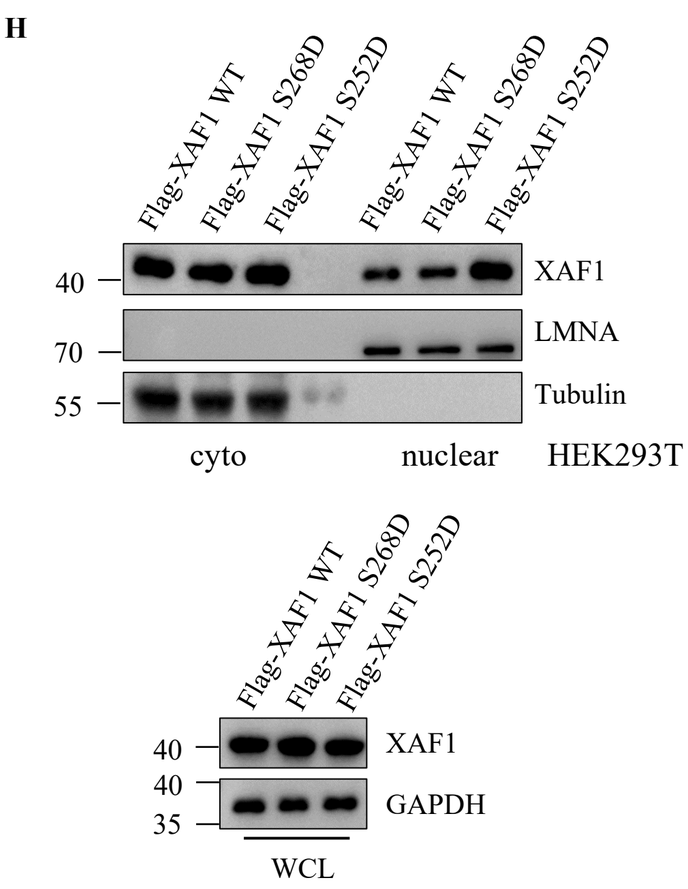












Supplementary figure 4

Fig. S4. TBK1 phosphorylates XAF1 at S252 and promotes early nuclear translocation of XAF1. Related to Fig. 4.

(A) The SDD-AGE and SDS-PAGE of HEK293T cells expressing Flag-XAF1 and HA-MAVS were treated with VSV for 6 hours

(B) Native PAGE and immunoblot of WT and *XAF1*$^{-/-}$ HT-29 cells after VSV infection for 6 hours. Monomer and dimer of IRF3 were assessed by IRF3-specific Ab. phosphorylation of TBK1 was assessed by phosphorylation -TBK1 Ab.

(C) Cytoplasmic and nuclear fractions were isolated, and then the time course of changes in IRF3 levels in each fraction after VSV infection in *XAF1*$^{-/-}$ HT-29 cells was tracked by Western blotting analysis. The relative expression of IRF3 (relative to GAPDH or LMNA) curve shows its dynamic changes in the cytoplasm and nucleus.

(D) Cytoplasmic and nuclear fractions were isolated, and then the time course of changes in XAF1 levels in each fraction after VSV and SeV infection in THP-1 cells was tracked by Western blotting analysis. The relative expression of XAF1 (relative to GAPDH or LMNA) curve shows its dynamic changes in the cytoplasm and nucleus.

(E) Cytoplasmic and nuclear fractions were isolated, and then the time course of changes in XAF1 levels in each fraction after transfecting ploy(I:C)-LMW and ploy(I:C) in HT-29 cells was tracked by Western blotting analysis. The relative expression of XAF1 (relative to GAPDH or LMNA) curve shows its dynamic changes in the cytoplasm and nucleus.

(F) Phosphorylation site of XAF1 by TBK1 was identified via mass spectrometry

(G and H) HEK293T cells were transfected with the indicated Vector, FL-XAF1, S252D-XAF1 and S268D-XAF1 plasmids for 24 hours. After 12 hours of VSV infection, qRT-PCR analyses of *IFNB1* mRNA (upper) and *VSV-G* mRNA (lower) was measured. Separation of nuclear and cytoplasmic fractions and Western blotting analyzed protein distribution in cytoplasm and nucleus.

Data are the mean ± SEM (n = 3) from three independent experiments, NS, not significant; $*p < 0.05$ and $**p < 0.01$, and $***p < 0.001$ (Student's t test: G). Data are representative of of two (F) or three independent (A to E, G and H) experiments with similar results.

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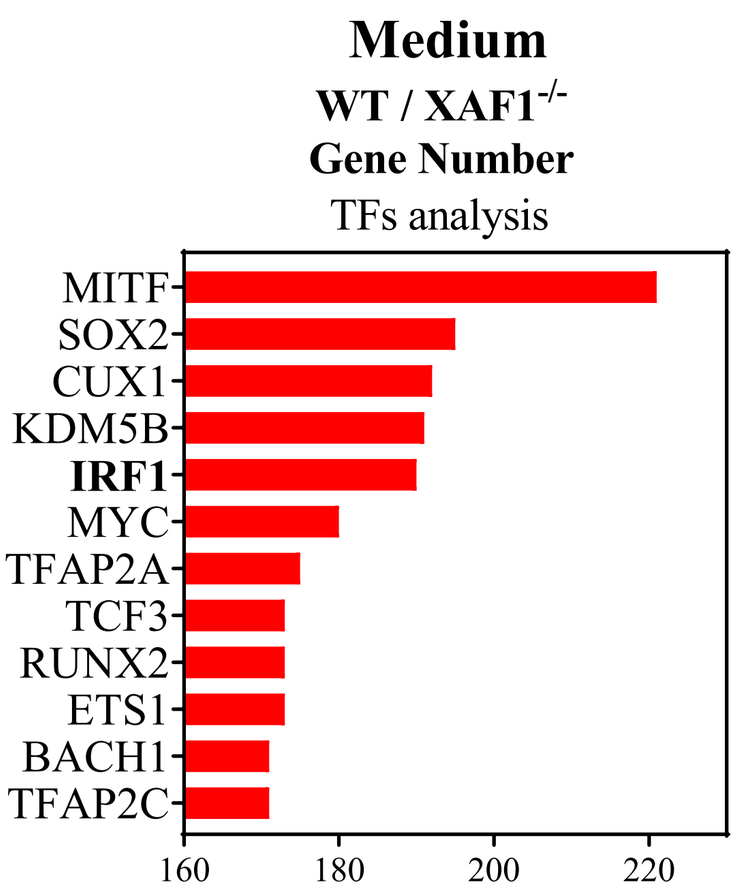

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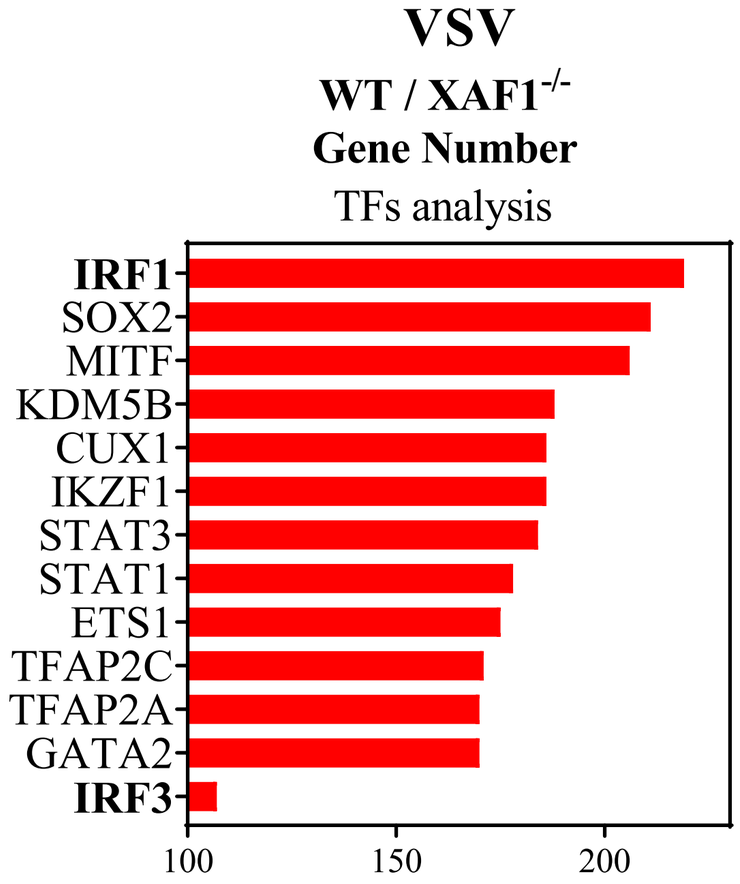

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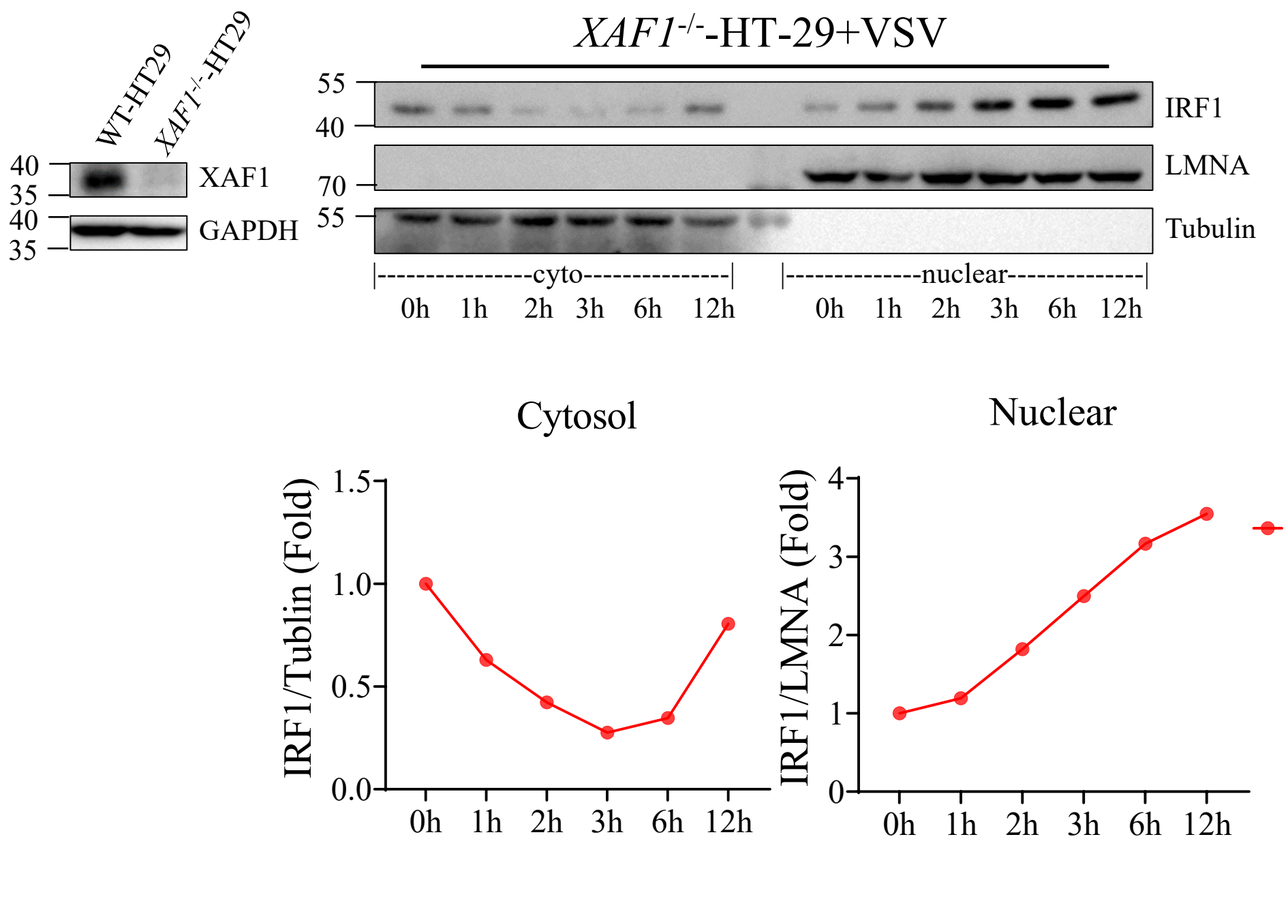

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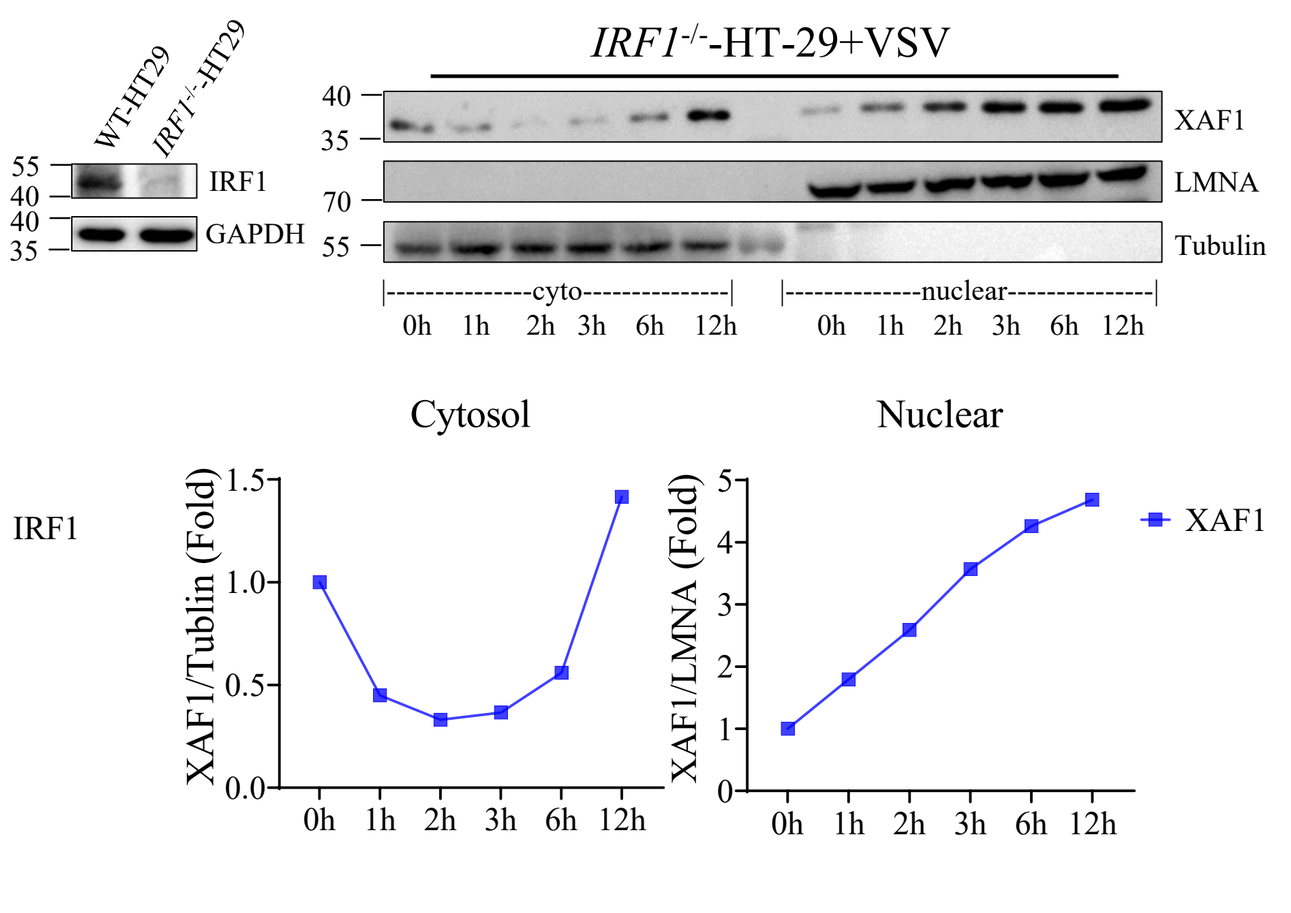

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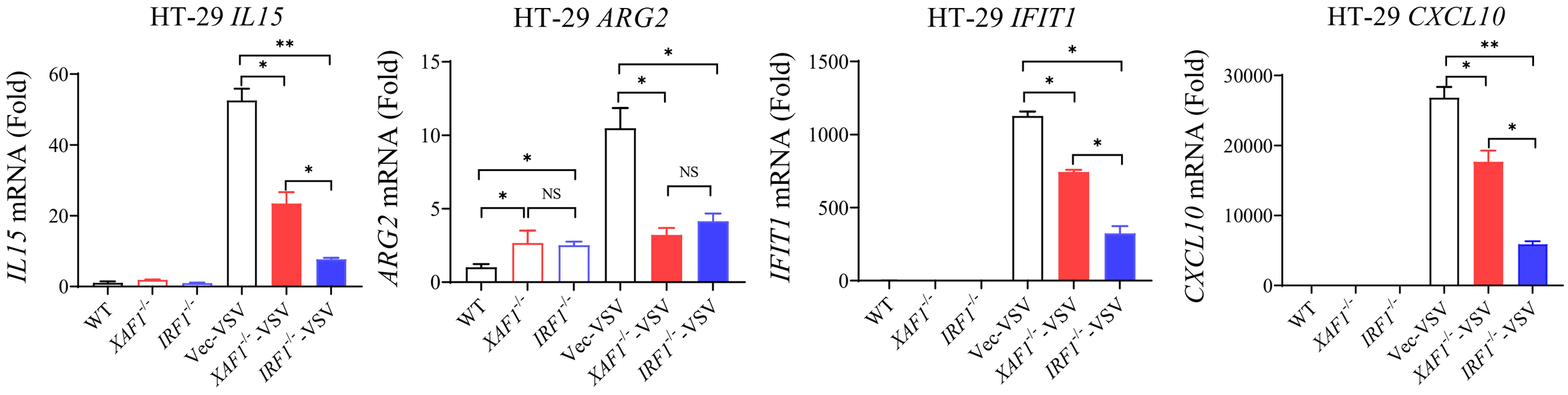


Supplementary figure 5

Fig. S5. XAF1 is required for induction of IRF1- and IRF3- target genes. Related to Fig. 5.

(A and B) Transcription factor enrichment analysis of differentially expressed down-regulated genes from RNA-seq of WT/*XAF1*$^{-/-}$ HT-29 cells infected without VSV by ChIP-X Enrichment Analysis Version 3 (https://maayanlab.cloud/chea3/#top)

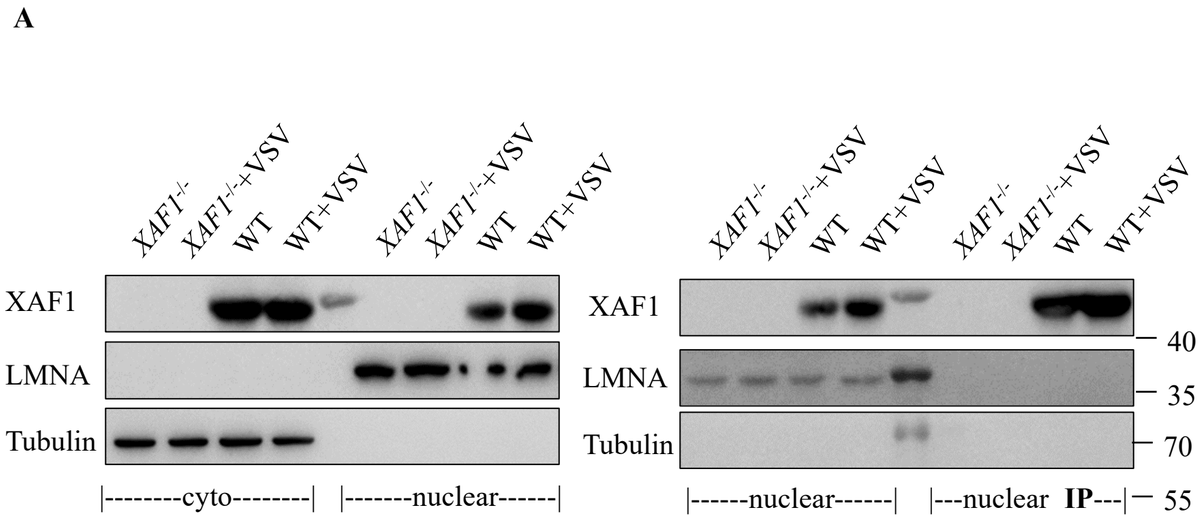
(C) Cytoplasmic and nuclear fractions were isolated, and then the time course of changes in IRF1 levels in each fraction after VSV infection in *XAF1*$^{-/-}$ HT-29 cells was tracked by Western blotting analysis. The relative expression of IRF1 (relative to GAPDH or LMNA) curve shows its dynamic changes in the cytoplasm and nucleus.

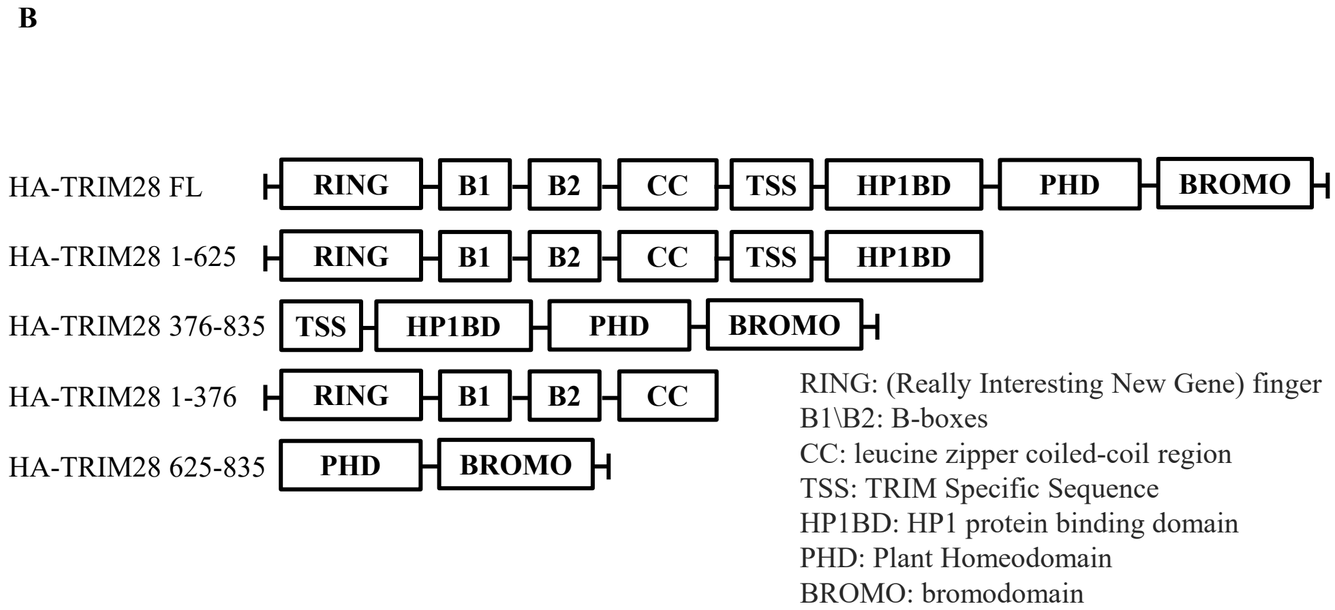
(D) Cytoplasmic and nuclear fractions were isolated, and then the time course of changes in XAF1 levels in each fraction after VSV infection in *IRF1*$^{-/-}$ HT-29 cells was tracked by Western blotting analysis. The relative expression of XAF1 (relative to GAPDH or LMNA) curve shows its dynamic changes in the cytoplasm and nucleus.

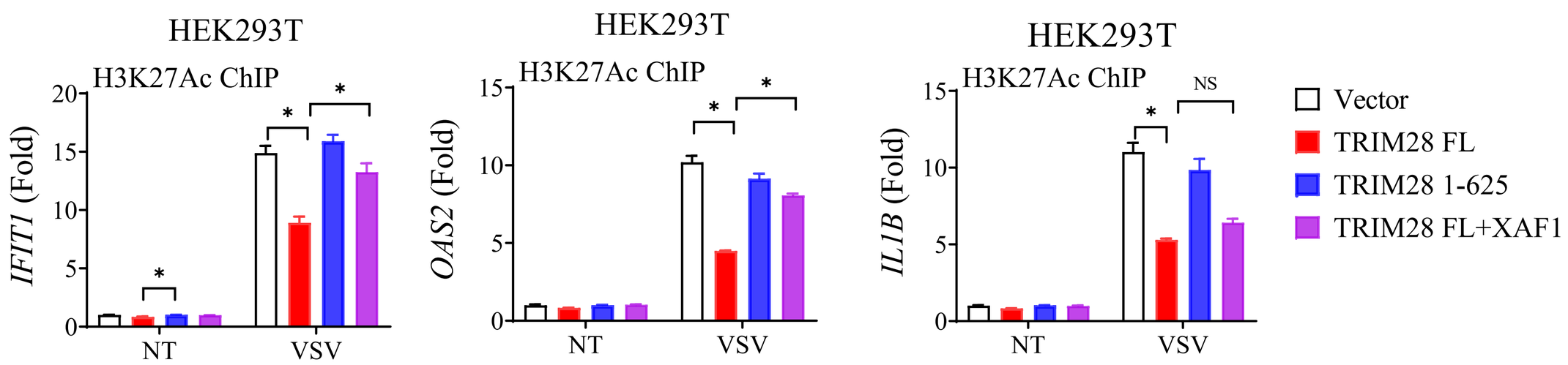
(E) qRT-PCR analysis of *IL15*, *ARG2*, *IFIT1* and *CXCL10* after NT (non-treated) or after stimulation with VSV for 12 hours in WT and *XAF1*$^{-/-}$ HT-29 cells.

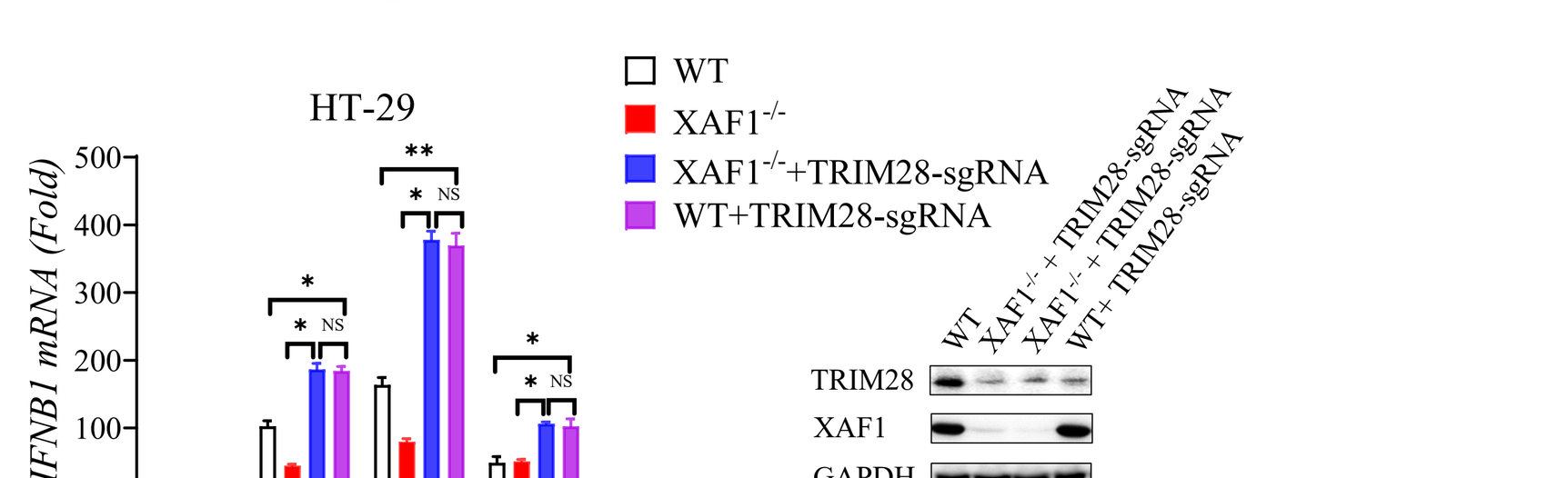
Data are the mean ± SEM (n = 3) from three independent experiments, NS, not significant; $*p < 0.05$ and $**p < 0.01$, and $***p < 0.001$ (Student's t test: E). Data are representative of of two (A and B) or three independent (C to E) experiments with similar results.

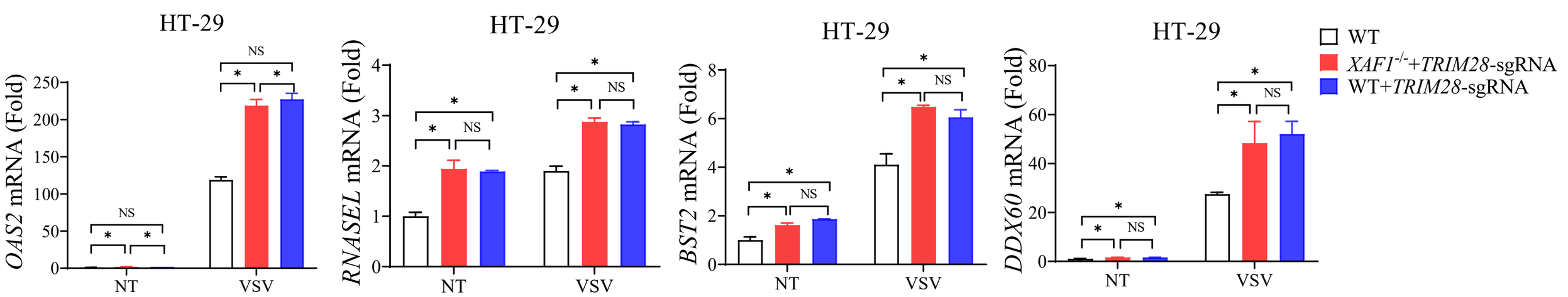
Supplementary Figure 6

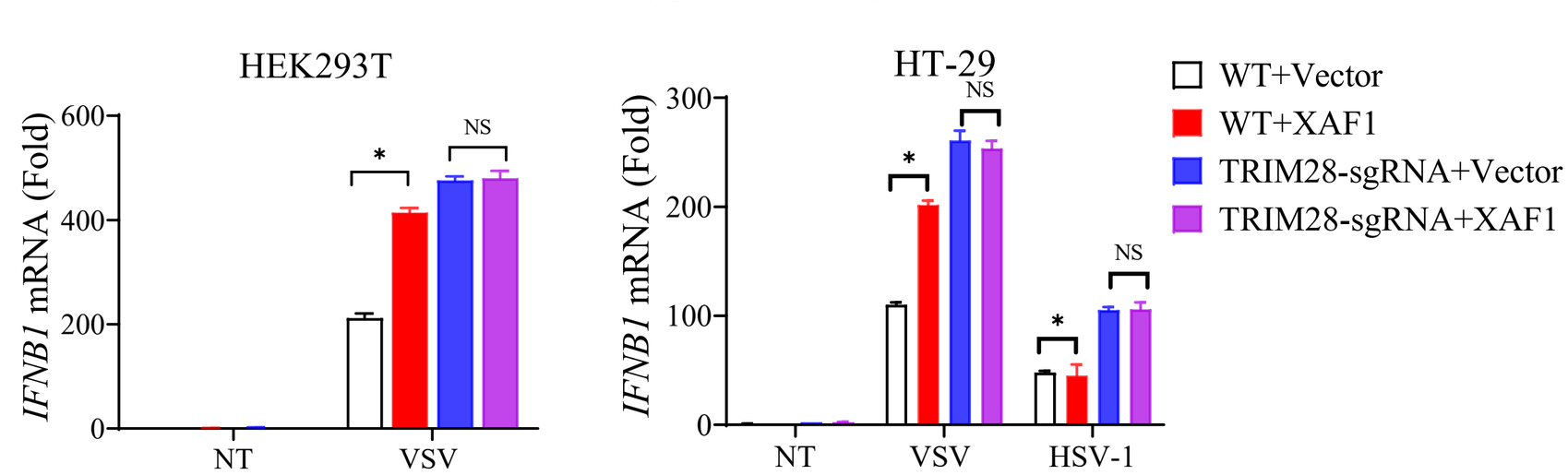


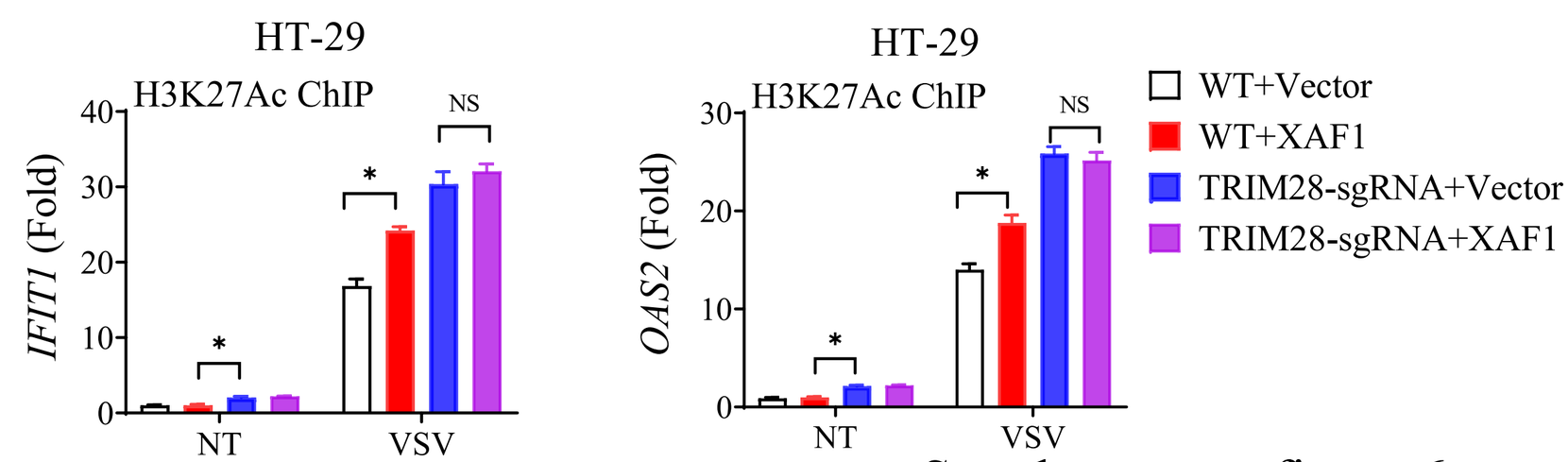












Supplementary figure 6

Fig. S6. XAF1 facilitates the immune responses by inhibiting TRIM28. Related to Fig. 6.

(A) Separation of nuclear and cytoplasmic fractions and Western blotting analyzed protein distribution in cytoplasm and nucleus (left) and detected protein enrichment in nucleus (right).

(B) Diagram detailing the series of truncations of TRIM28.

(C) Vector, TRIM28 FL, TRIM28 1-625 and TRIM28 FL+XAF1 HEK293T cells were NT (non-treated) or infected VSV for 3 hours, and ChIP-qPCR signal showing H3K27Ac occupancy of indicated genes

(D) qRT-PCR analysis of *IFNB1* after NT (non-treated) or after stimulation with VSV, SeV or HSV-1 for 12 hours in WT+Vector, WT+XAF1, TRIM28-sgRNA +Vector and TRIM28-sgRNA +XAF1 HT-29 cells.

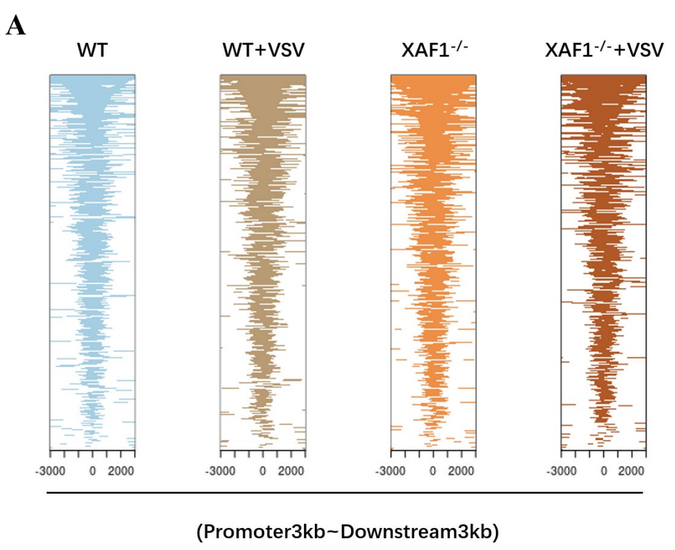
(E) qRT-PCR analysis of *OAS2* ,*RNASEL*, *BST2* and *DDX60* after NT (non-treated) or after being stimulated with VSV for 12h in WT, *XAF1*$^{-/-}$+*TRIM28*-sgRNA and WT+TRIM28-sgRNA HT-29 cells.

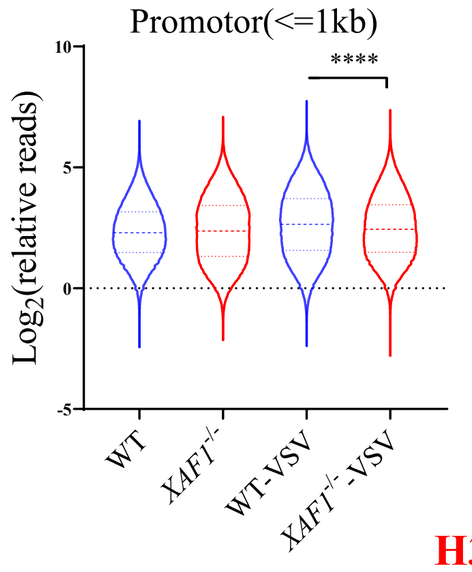
(F) qRT-PCR analysis of *IFNB1* after NT (non-treated) or after stimulation with VSV or HSV-1 for 12 hours in WT+Vector, WT+XAF1, TRIM28-sgRNA +Vector and TRIM28-sgRNA +XAF1 HT-29 cells.

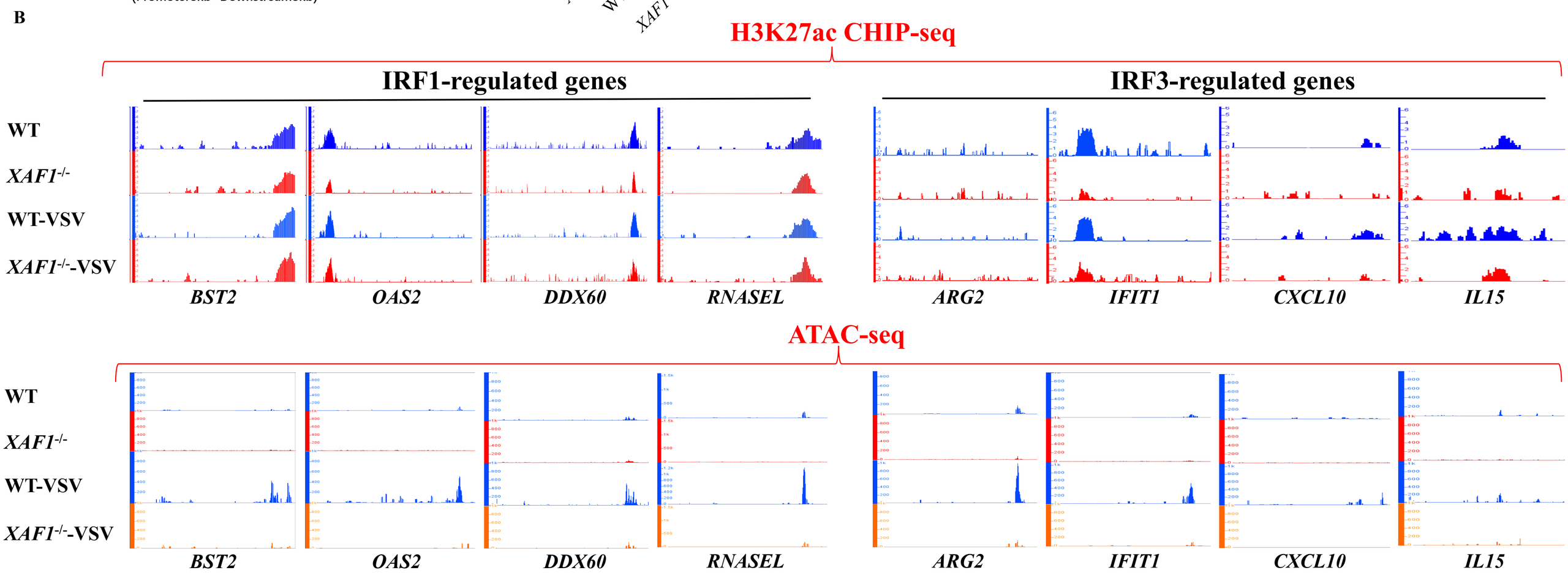
(G) WT+Vector, WT+XAF1, TRIM28-sgRNA +Vector and TRIM28-sgRNA +XAF1 HT-29 cells were NT (non-treated) or infected VSV for 3 hours, and ChIP-qPCR signal showing H3K27Ac occupancy of indicated genes.

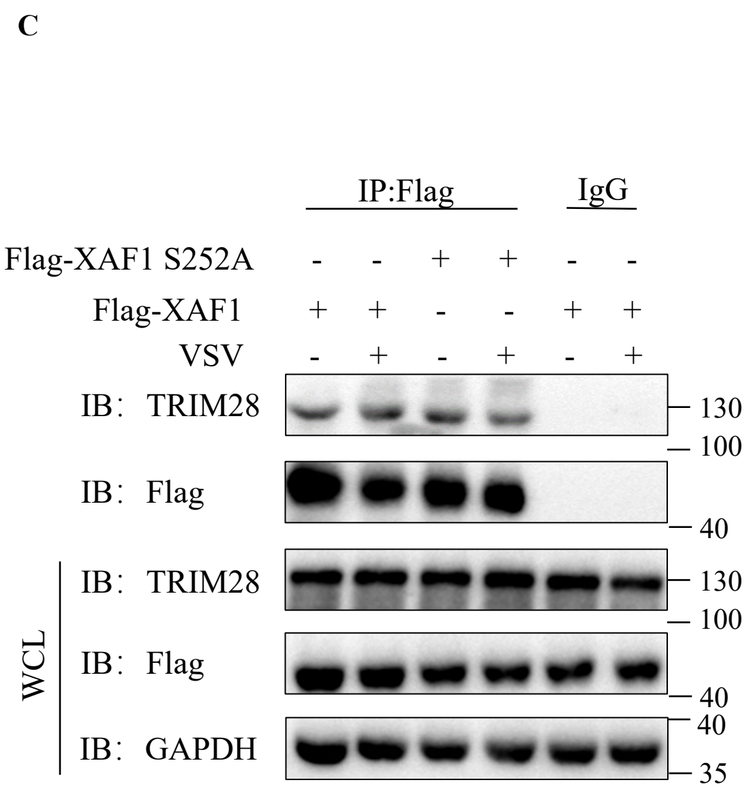
Data are the mean ± SEM (n = 3) from three independent experiments, NS, not significant; $*p < 0.05$ and $**p < 0.01$, and $***p < 0.001$ (Student's t test: C to G). Data are representative of three independent (C to G) experiments with similar results.

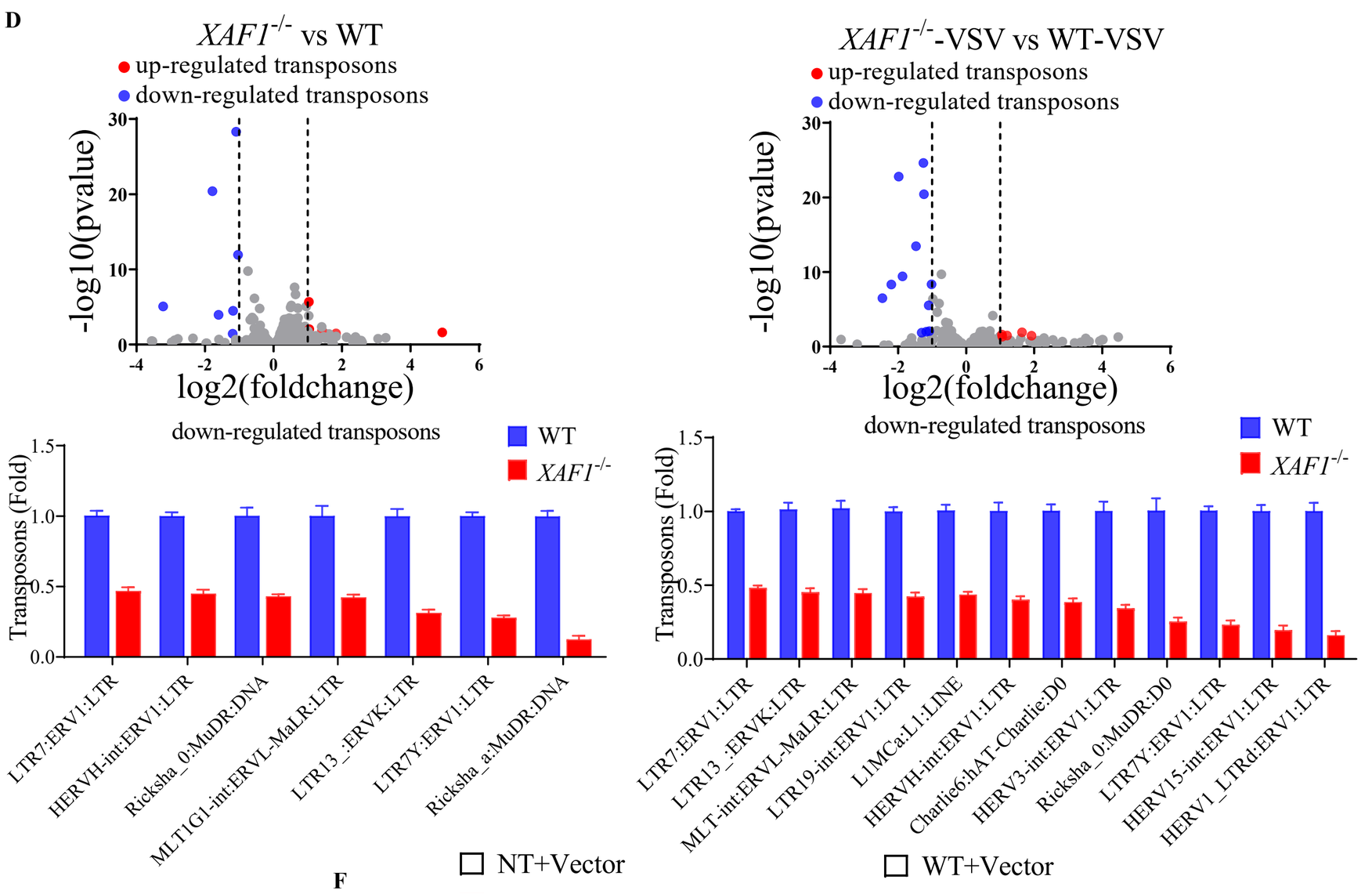
Supplementary Figure 7

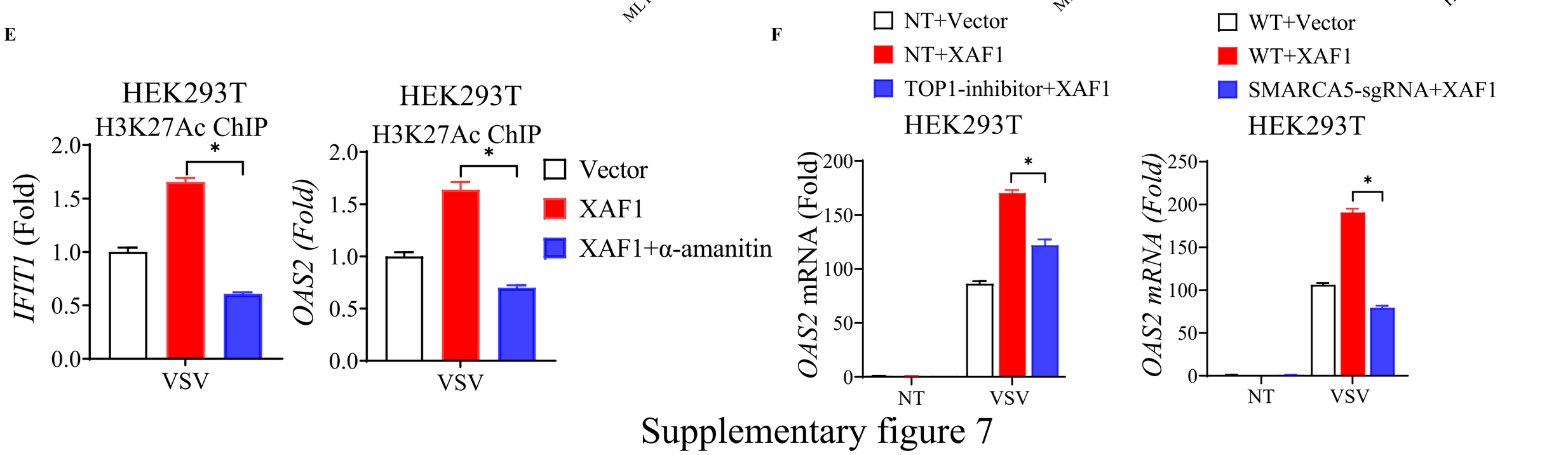












Supplementary figure 7

Fig. S7. XAF1 is recruited by IRF1 to inhibit TRIM28. Related to Fig. 7.

(A) Heatmap showing the ChIP-seq signal enrichment from promotor 3kd to downstream 3kb in WT and *XAF1*$^{-/-}$ HT-29 cells after NT (non-treated) or after being stimulated with VSV infection for 3h (left) and analysis of CHIP-seq RPKM level of Promoter<=1kb, Promoter (<= 1kb) (right).

(B) Genome browser views of ChIP-seq (upper) and ATAC-seq (lower) signal for the indicated genes in WT and *XAF1*$^{-/-}$ HT-29 cells after NT (non-treated) or after being stimulated with VSV infection for 3h.

(C) Western blotting showing the interaction between FL-XAF1 or S252A-XAF1 and TRIM28.

(D) A volcano plot showing changes in cellular transcripts levels in WT versus XAF1$^{-/-}$ HT-29 cells with or without VSV infection. The log2 fold change between WT versus XAF1$^{-/-}$ HT-29 cells and −log10 of the p value are represented in the x axis and y axis, respectively. Transposon was marked in blue (down-regulation) or red (up-regulation). The p value was calculated using t test. The down-regulated gene transposons were showed.

(E) Vector, XAF1 and XAF1 treated with α-amanitin(50 μg/ml) for 4h HEK293T cells were infected VSV for 3 hours, and ChIP-qPCR signal showing H3K27Ac occupancy of indicated genes.

(F) (left) Overexpression Vector or XAF1 in HEK293T cells for 18 hours, then treated with 60 μM TOP1 inhibitor (SW-044248) for 6 hours. qRT-PCR analysis of *OAS2* mRNA after NT(non-treated) or after stimulation with VSV for 12 hours. (right) qRT-PCR analysis of *OAS2* mRNA after NT(non-treated) or after stimulation with VSV for 12 hours in NT+Vector, NT+XAF1, and SMARCA5-sgRNA+XAF1 HEK293T cells.

Data are the mean ±, NS, not significant; ∗p < 0.05 and ∗∗p < 0.01, and ∗∗∗p < 0.001 (Student's t test: E and F). Data are representative of three independent (E and F) experiments with similar results.

Supplementary tables includes:

Table S1. MS data of MAVS-TurboID
Table S2. XAF1-phosphorylation site
Table S3. MS data of XAF1 interacted protein in nuclear
Table S4. Summary of primers used in this study