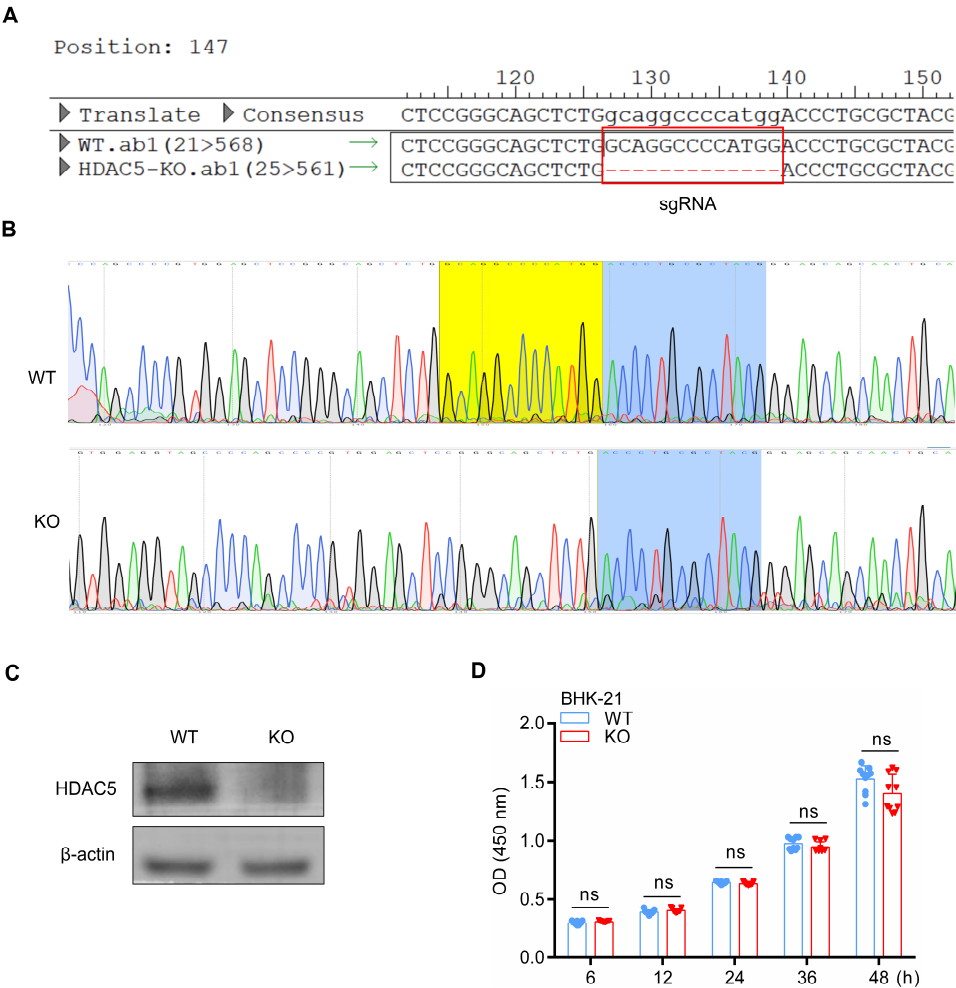


Supplementary Information



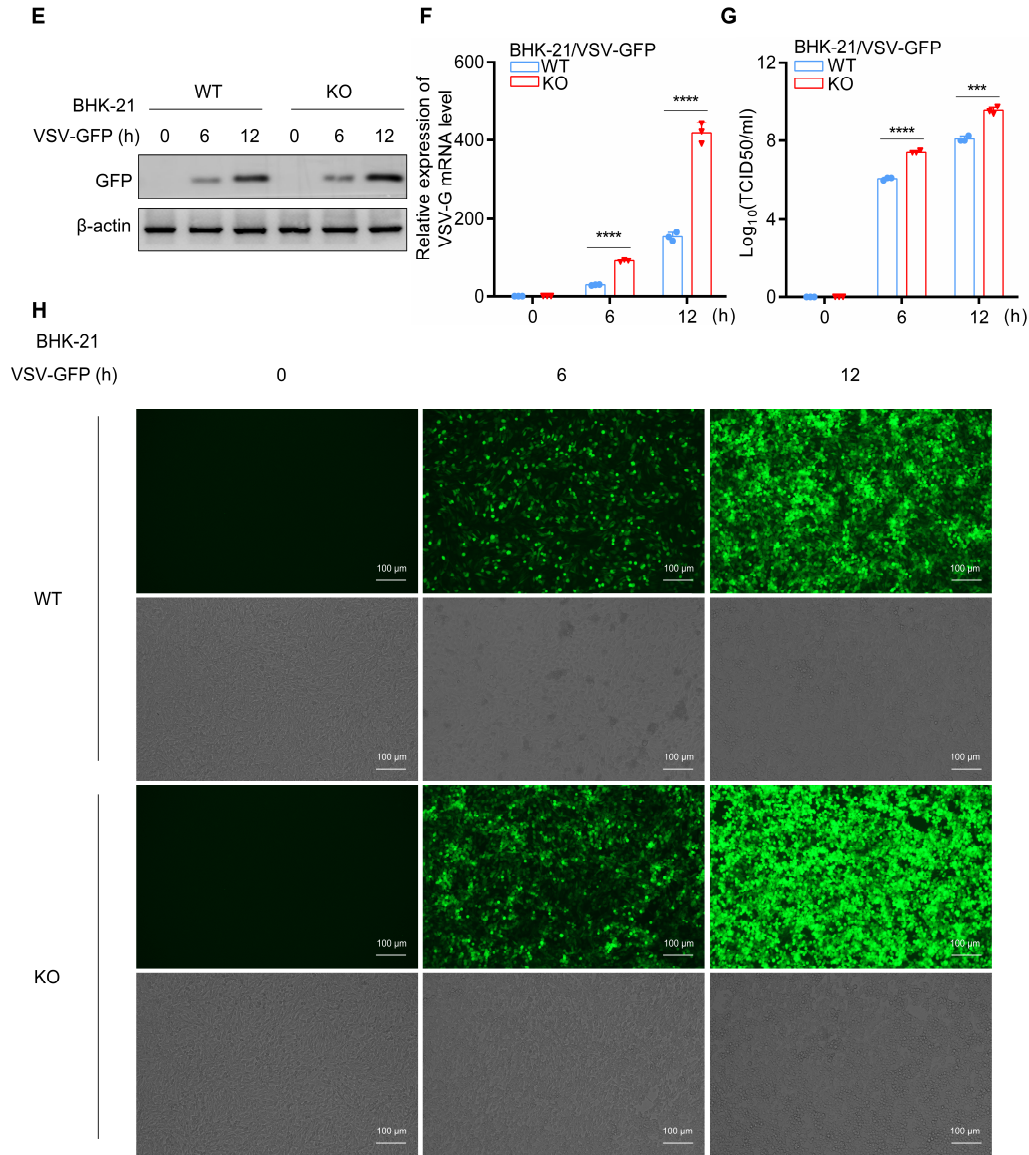


Figure S1. HDAC5 knockout promotes VSV replication. (A) The DNAMAN software was used to align the CDS sequences of WT and HDAC5-KO. The sgRNA sequence and mutations in the PAM motif are indicated by the red box. (B) Snapgene software was utilized to analysis the sequencing peaks of PCR products of WT and HDAC5-KO. (C) WT and HDAC5-KO BHK-21 cells were separately harvested. Western blotting was used to evaluate protein expression of HDAC5 and β -actin. (D) In 96-well plates, WT and HDAC5-KO BHK-21 cells were seeded. 10 μL of CCK8 reagent was used and cells were incubated for 60 minutes. Then, the cells were measured the absorbance at 450 nm. (E-H) WT and HDAC5-KO BHK-21 cells (1×10^6) were infected with VSV-GFP. Subsequently, cells and culture supernatants were separately harvested at the indicated time points. (E) Western blotting was used to evaluate β -actin and VSV-GFP protein expression. (F) RT-qPCR was applied to quantify the mRNA level of VSV-GFP. (G) TCID_{50} was employed to test viral titers of VSV-GFP. (H) Fluorescence microscopy was employed to observe and photograph viral replication of VSV-GFP. Groups were compared by unpaired Student's *t*-test. ns, not significant, $p > 0.05$, $p < 0.01^{**}$, $p < 0.001^{***}$, $p < 0.0001^{****}$.

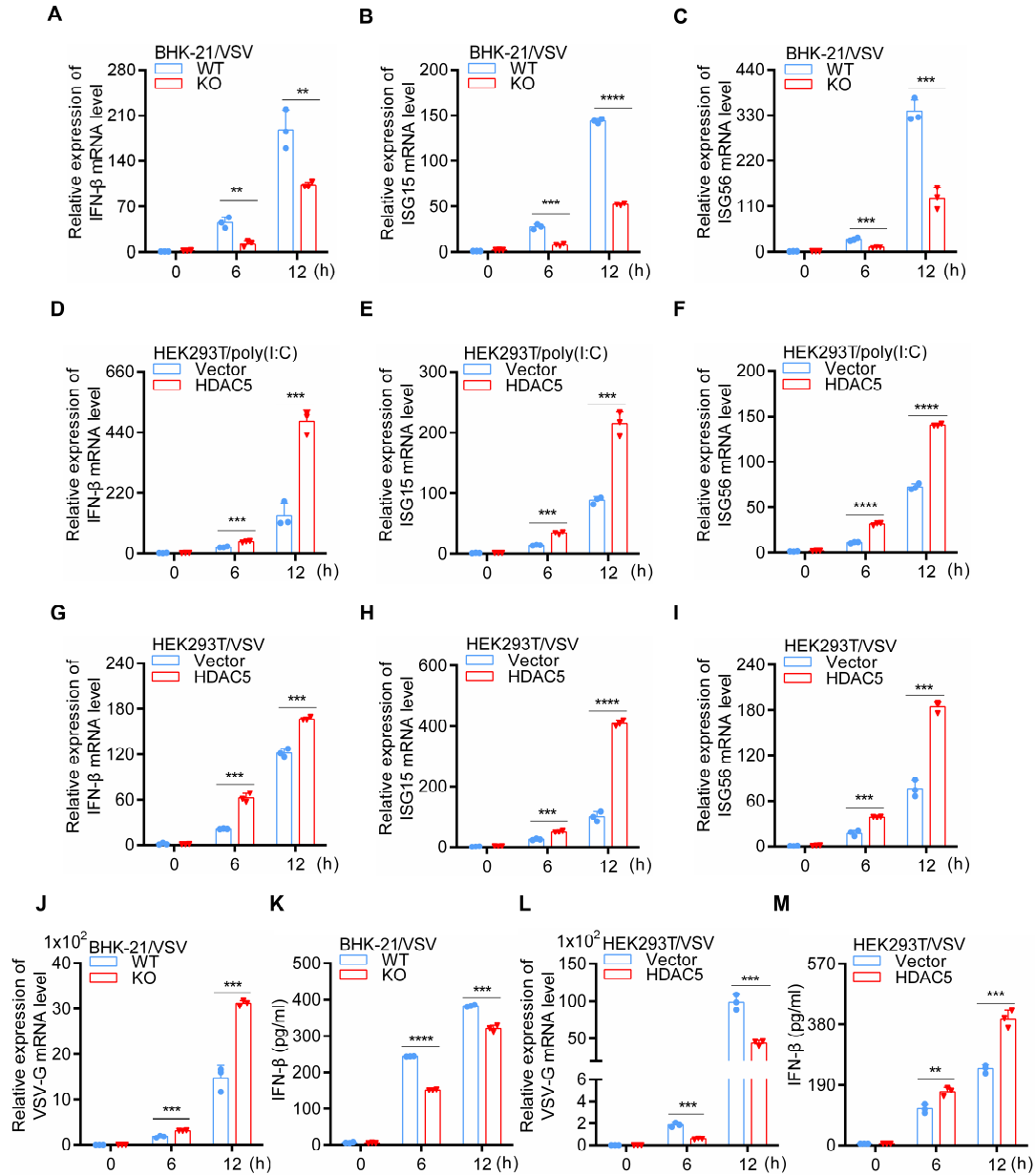


Figure S2. HDAC5 promotes poly(I:C)- and VSV-stimulated IFN-β signaling. (A-C) WT and HDAC5-KO BHK-21 cells (1×10^6) were infected with VSV. Subsequently, the cells were harvested separately at the indicated time points. RT-qPCR was applied to quantify the mRNA levels of IFN-β, ISG15, and ISG56. (D-F) HDAC5 or empty-vector plasmid (2 μg) was introduced into HEK293T cells (1×10^6) and incubated for 36 h, followed by poly(I:C) transfection. Subsequently, the cells were harvested separately at the indicated time points. RT-qPCR was applied to quantify the mRNA levels of IFN-β, ISG15, and ISG56. (G-I) HDAC5 or empty-vector plasmid (2 μg) was introduced into HEK293T cells (1×10^6) and incubated for 36 h, followed by VSV infection. Subsequently, the cells were harvested separately at the indicated time points. RT-qPCR was applied to quantify the mRNA levels of IFN-β, ISG15, and ISG56. (J, L) RT-qPCR was applied to quantify the mRNA levels of VSV-G (J as in A-C, L as in G-I). (K, M) Culture supernatants were separately harvested at the indicated time points, and ELISA was employed to detect IFN-β secretion (K as in A-C, M as in G-I). Groups were compared by unpaired Student's

t-test. ns, not significant, $p < 0.01$ **, $p < 0.001$ ***, $p < 0.0001$ ****.

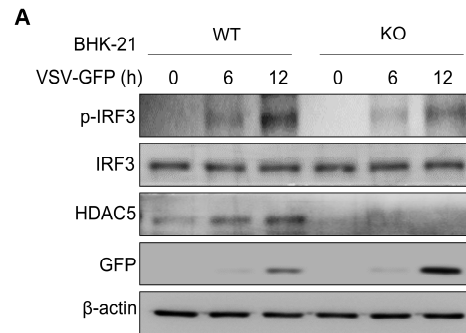


Figure S3. HDAC5 activates VSV-induced phosphorylation of IRF3. (A) WT and HDAC5-KO BHK-21 cells (1×10^6) were infected with VSV-GFP. Western blotting was used to evaluate protein expression of phosphorylated IRF3, total IRF3, HDAC5, VSV-GFP, and β -actin.