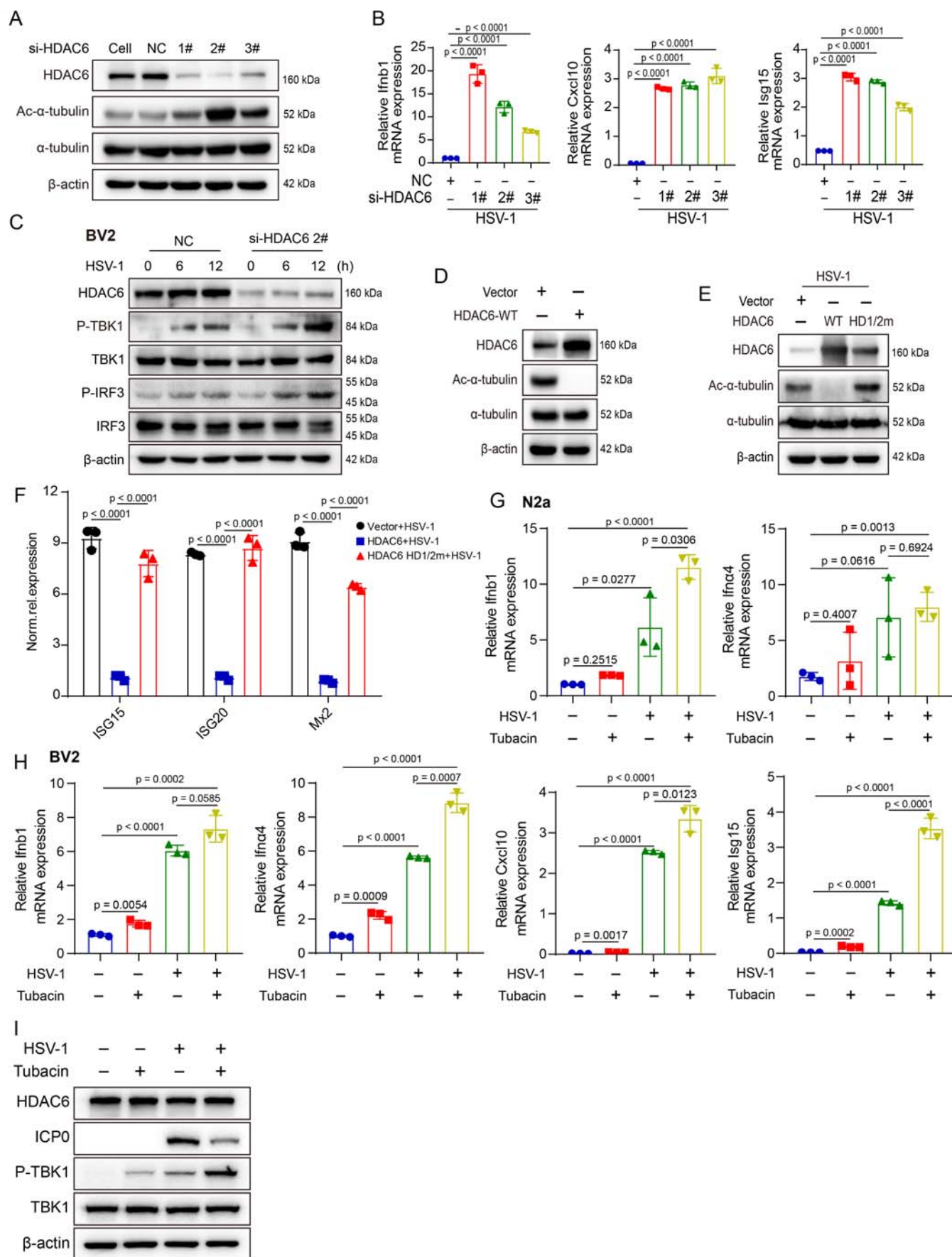


Expanded View Figures

Figure EV1. HDAC6 negatively regulates IFN production.

(A, B) N2a cells were transfected with negative control (NC) or HDAC6 siRNAs for 72 h. Knockdown efficacy of HDAC6 siRNAs in N2a cells (A). RT-qPCR analysis of the mRNA expression of *Ifnb1*, *Cxcl10* and *Isg15* in N2a cells transfected with HDAC6 siRNAs for 72 h followed by infection with HSV-1 (MOI = 3) for 12 h (B). Data were analyzed using the unpaired *t* test, which are shown as mean $\pm$ SD (*n* = 3 biological replicates). (C) Western blot analysis of the indicated proteins in BV2 cells infected with HSV-1 (MOI = 3) for different time points. (D–F) N2a cells were transfected with vector, HDAC6 WT or deacetylase activity mutation (HD1/2 m) for 72 h followed by infection without or with HSV-1 (MOI = 3) for 12 h. (D, E) Western blot analysis of the indicated proteins in cells. (F) The mRNA expression of *Isg15*, *Isg20* and *Mx2* was detected by RT-qPCR assay. Data were analyzed using the unpaired *t*-test, which are shown as mean $\pm$ SD (*n* = 3 biological replicates). (G, H) Effect of tubacin on IFN production. N2a cells (G) and BV2 cells (H) were infected with HSV-1 (MOI = 3) in the presence or absence of tubacin (1 μ M) for 12 h, and RT-qPCR analysis was performed to detect the expression of *Ifnb1*, *ifna4*, *cxcl10*, *Isg15* genes. Data were analyzed using the unpaired *t*-test, which are shown as mean $\pm$ SD (*n* = 3 biological replicates). (I) Western blot analysis of tubacin's effect on TBK1 activation. Source data are available online for this figure.



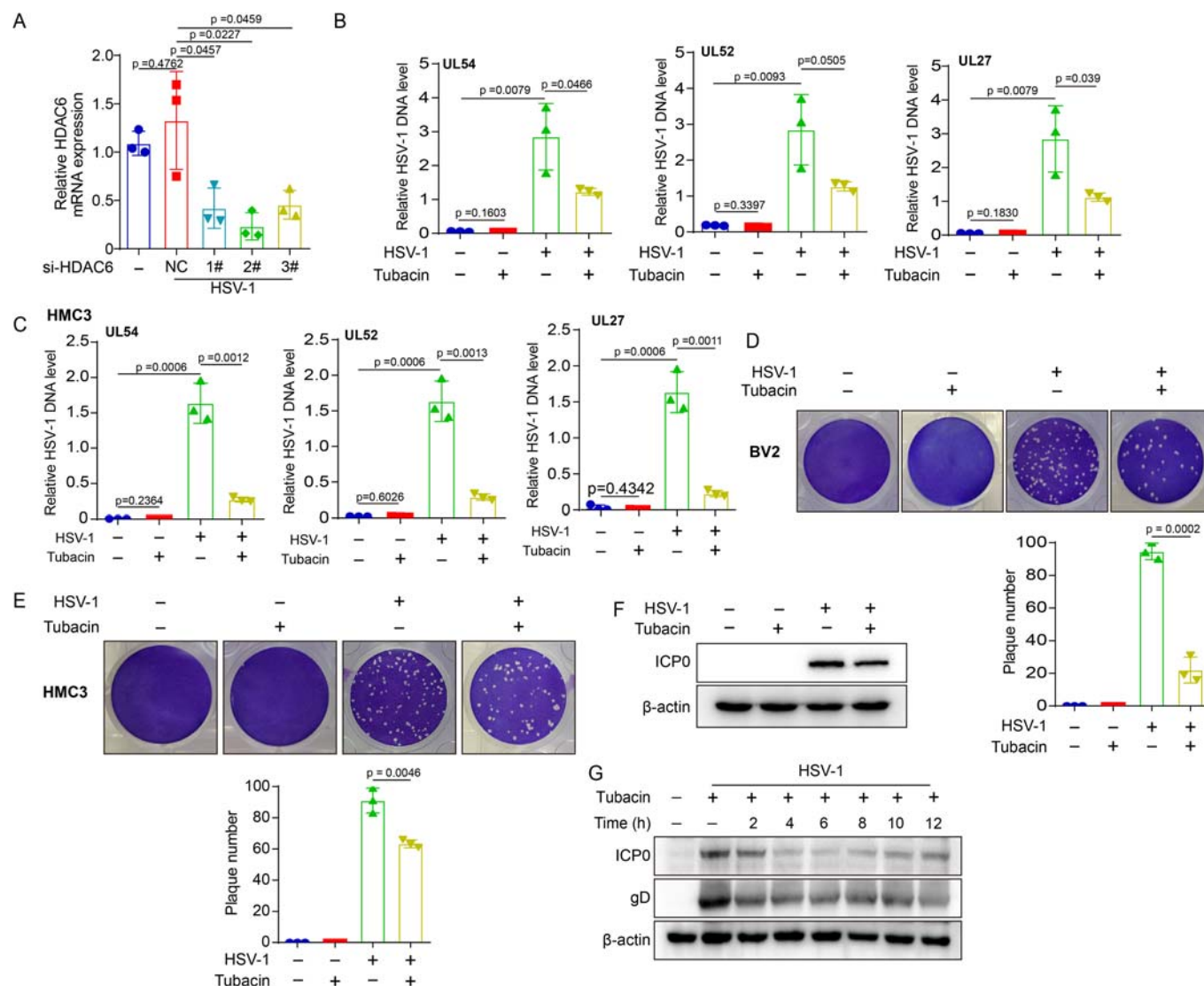


Figure EV2. HDAC6 promotes HSV-1 infection.

(A, B) RT-qPCR analysis of the mRNA expression of *HDAC6* in N2a cells transfected with *HDAC6* siRNA and infected with HSV-1 (MOI = 3) for 12 h. Data were analyzed using the unpaired t test, which are shown as mean $\pm$ SD ($n = 3$ biological replicates). (B, C) The DNA copy number of viral genes in BV2 (B) and HMC3 cells (C) infected with HSV-1 (MOI = 3) in the presence or absence of tubacin (1 μ M) for 12 h. Data were analyzed using the unpaired t test, which are shown as mean $\pm$ SD ($n = 3$ biological replicates). (D, E) Viral plaque assays of BV2 (D) and HMC3 cells (E) treated with or without HSV-1 (MOI = 3) in the presence or absence of tubacin (1 μ M) for 12 h. The number of plaques was calculated. Data were analyzed using the unpaired t test, which are shown as mean $\pm$ SD ($n = 3$ biological replicates). (F, G) Western blot assay was performed to assess the levels of ICP0 and gD in N2a cells infected with HSV-1 (MOI = 3) for 12 h followed by treatment with tubacin (1 μ M) in the indicated times of HSV-1 infection. Source data are available online for this figure.

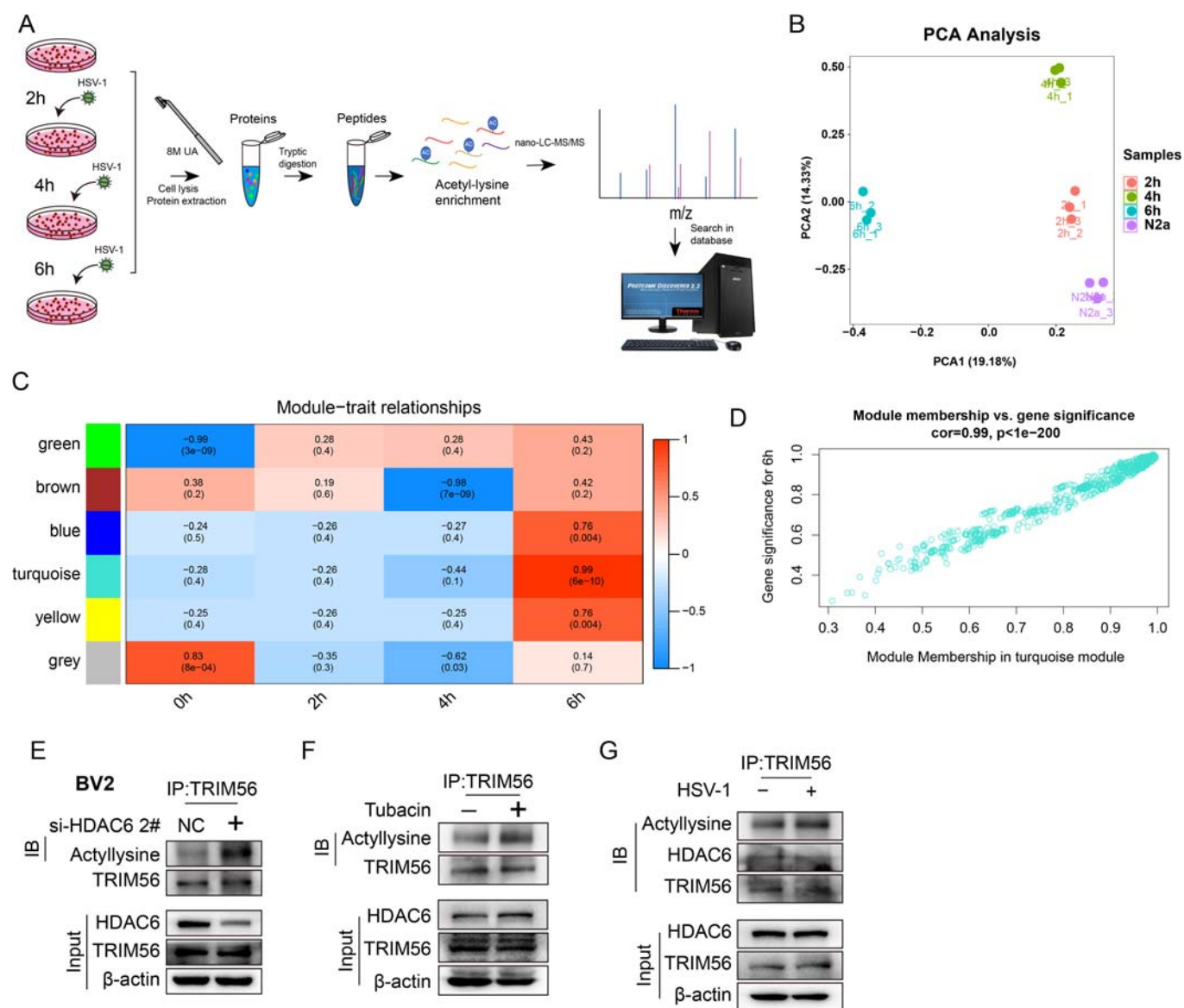


Figure EV3. HDAC6 regulates TRIM56 deacetylation in early stages of HSV-1 infection.

(A) Workflow diagram illustrating the process of profiling various time points in the acetylome of N2a cells infected with HSV-1 (MOI = 10). (B) PCA analysis of each sample. (C) Each row corresponds to a module feature point, and each column corresponds to the HSV-1 (MOI = 10) infected time point. The right panel represents the range of correlations: red indicates positive correlations and blue indicates negative correlations; numbers indicate correlations and significance. (D) Correlations between sites within the turquoise module and highly correlated sites following a 6 h infection. (E–G) Immunoprecipitation analysis of the acetylation of TRIM56 in BV2 cells transfected with or without HDAC6 siRNA for 72 h (E), and treated with or without tubacin (1 μM) (F), and infected with HSV-1 (MOI = 3) for 12 h (G). Source data are available online for this figure.

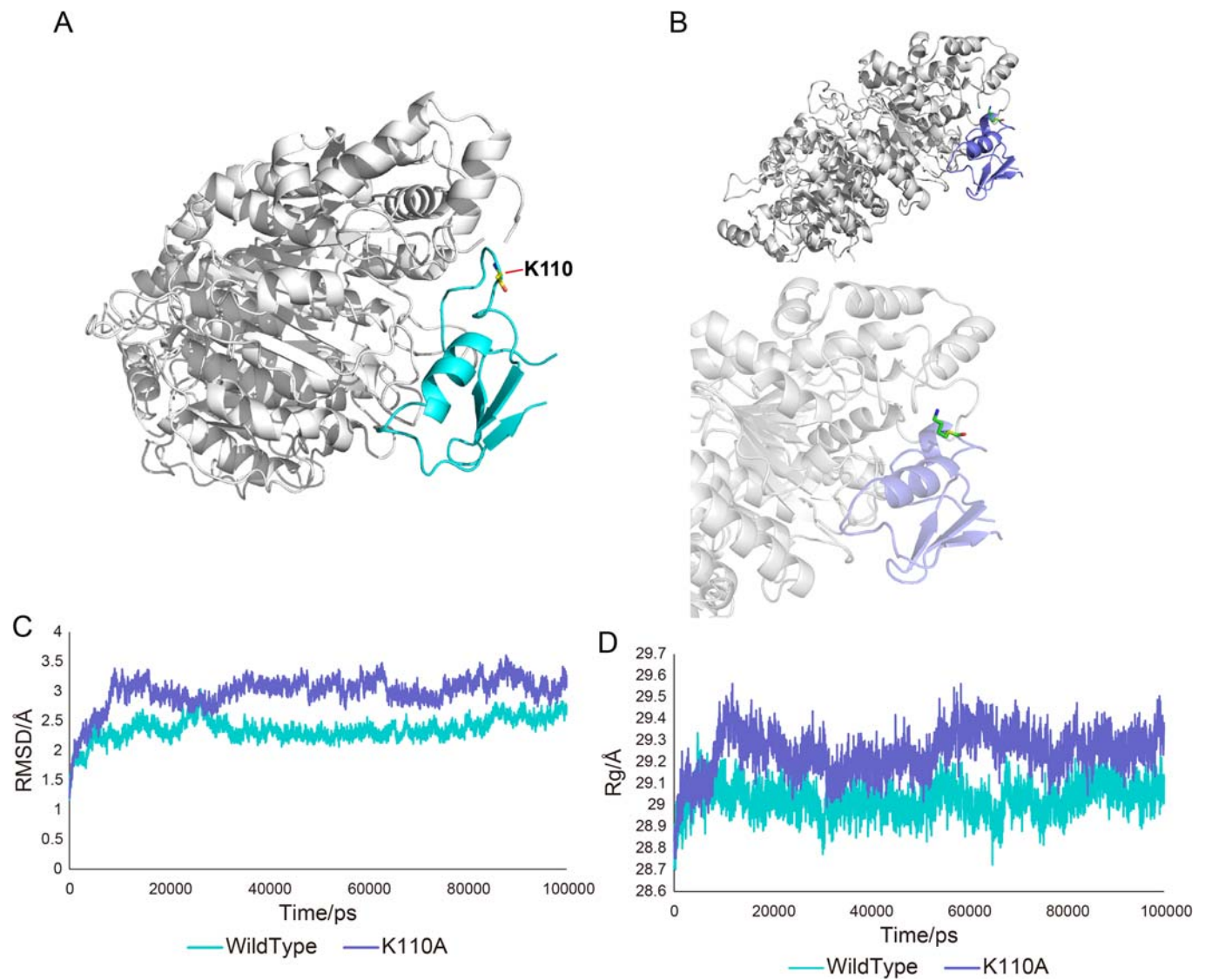
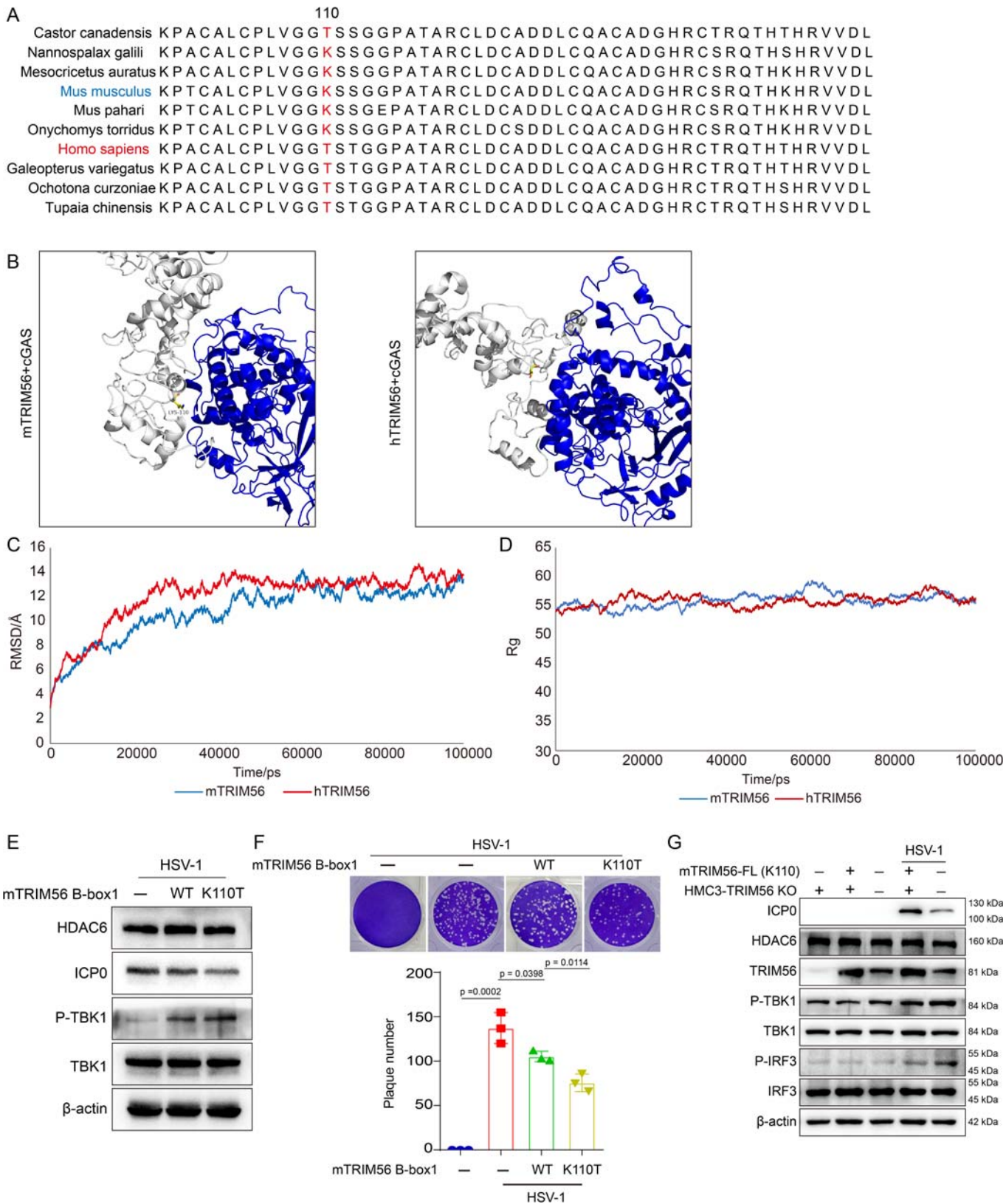


Figure EV4. TRIM56 positively regulates IFN production.

(A, B) Models depicting the predictable binding between HDAC6 CD1-CD2 (grey) and TRIM56 B box1 domain (cyan) (F) or TRIM56 K110A (G). (C, D) The plot comparing root mean square deviation (RMSD) (K) or Radius of gyration (Rg) (L) versus simulation time (ps) for WT and K110A mutant. Source data are available online for this figure.



◀ **Figure EV5. Species-specific amino acids at position 110 of TRIM56 K110 lead to different IFN responses.**

(A) Alignment of amino acids 98–149 of TRIM56 B-box1 from different species. (B) Molecular docking conformation of murine full-length TRIM56 K110 (mTRIM56) or K110T human full-length TRIM56 T110 (hTRIM56) (grey) and cGAS crystals (blue). (C) Plot of root mean square deviation (RMSD) versus simulation time (ps) for mTRIM56 and hTRIM56. (D) Rg comparison across the 100 ns molecular dynamic simulation of mTRIM56 and hTRIM56. (E) N2a cells transfected with mTRIM56 B-box1 or B-box1 K110T for 72 h followed by infection with HSV-1 (MOI = 3) for 12 h, and then western blot analysis of the cGAS-STING signaling pathway. (F) Viral plaque assays of BV2 cells transfected with mTRIM56 or mTRIM56 K110T followed by infection with HSV-1 (MOI = 3) for 12 h. The number of plaques was calculated (right). Data were analyzed using the unpaired *t* test, which are shown as mean ± SD (*n* = 3 biological replicates). (G) Western blot analysis of TBK1 and IRF3 activation in HMC3 TRIM56-KO12 cells transfected with mTRIM56 and then infected with HSV-1 (MOI = 3) for 12 h. Source data are available online for this figure.