Expanded View Figures

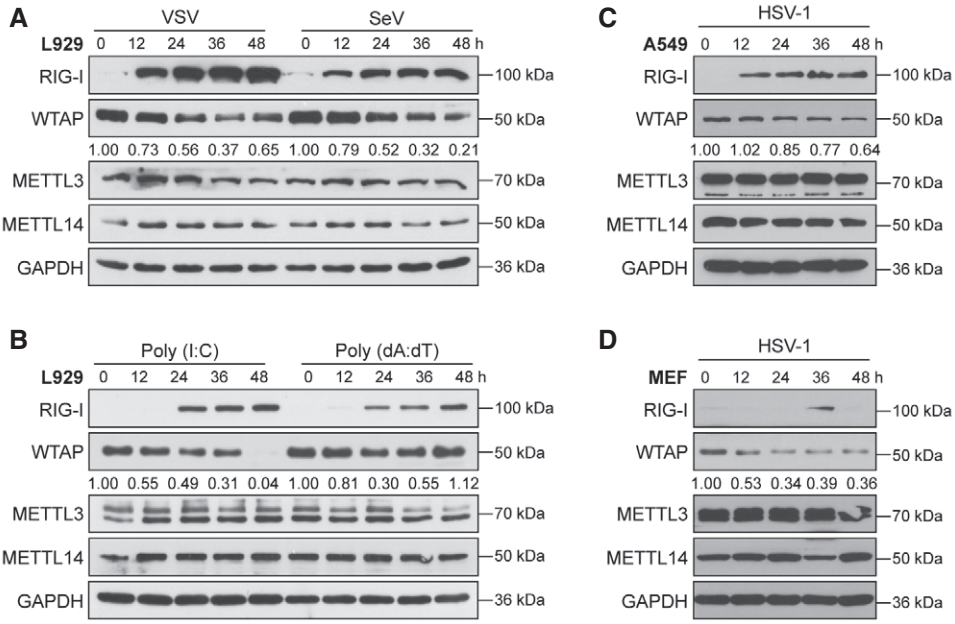

Figure EV1. WTAP protein is decreased in virus-infected or nucleic acid analog-treated cells.

A, B Immunoblot analyses of the protein abundance of the METTL3/METTL14/WTAP heterotrimer in L929 cells upon challenge with VSV-eGFP or SeV (A) and poly (I:C) or poly (dA:dT) (B) at the indicated time points. Data are representative of three independent biological experiments.

C, D Immunoblot analyses of the protein abundance of the METTL3/METTL14/WTAP heterotrimer in A549 cells (C) and MEFs (D) upon challenge with HSV-1 at the indicated time points. Data are representative of three independent biological experiments.

Source data are available online for this figure.

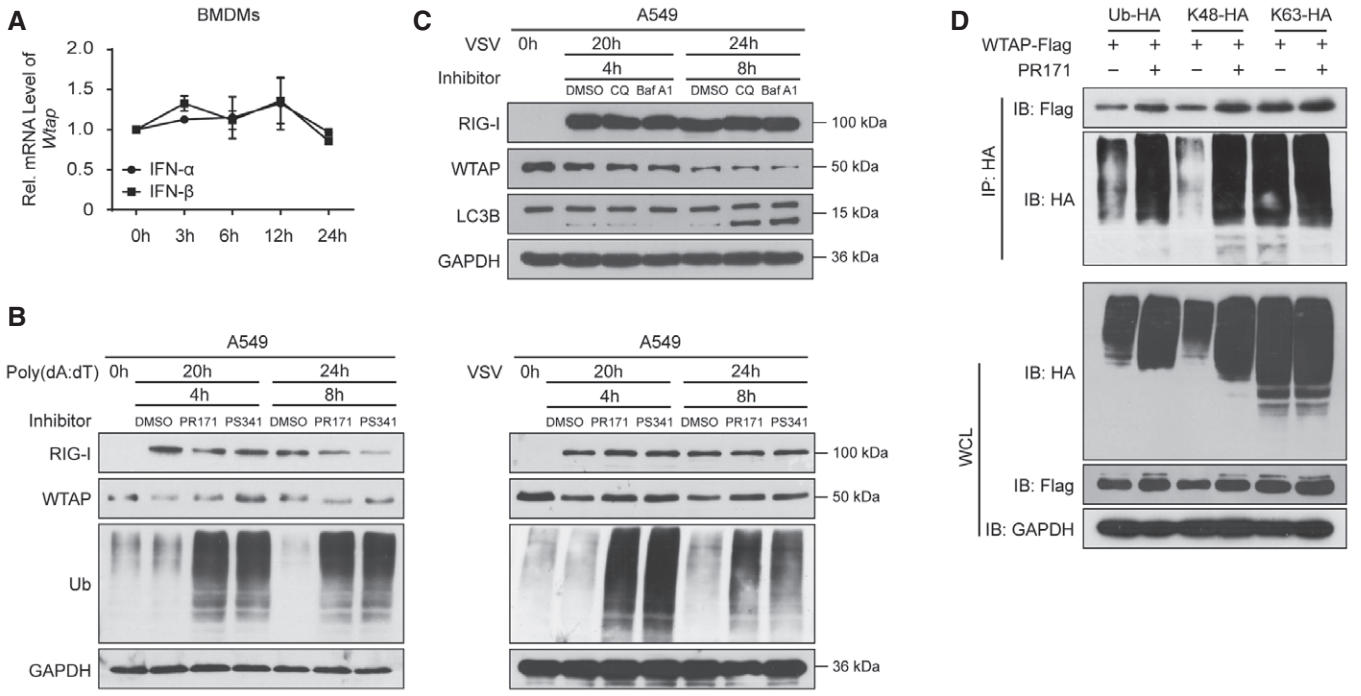

Figure EV2. WTAP is degraded through the K48-linked ubiquitination-proteasome pathway upon activation of IFN-I signaling.

A qRT–PCR analyses of *WTAP* mRNA expression in BMDMs stimulated with IFN-α or IFN-β at the indicated time points. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

B A549 cells were stimulated with poly (dA:dT) or infected with VSV for 16 h and then treated with PR-171 (10 μM) or PS-341 (10 μM) for additional 4 and 8 h. The cell lysates were analyzed by immunoblotting. Data are representative of three independent biological experiments.

C A549 cells were infected with VSV-eGFP for 16 h and then treated with CQ (50 mM) or Baf A1 (0.2 mM) for additional 4 and 8 h. The cell lysates were analyzed by immunoblotting. Data are representative of three independent biological experiments.

D Coimmunoprecipitation and immunoblot analyses of extracts of 293T cells transfected with various combinations of plasmids encoding FLAG-tagged WTAP, and HA-tagged K48-linked, K63-linked or wild-type ubiquitin, and treated with DMSO or PR-171 (10 μM), followed by immunoprecipitation with anti-HA antibody and immunoblot analysis with anti-Flag antibody. Data are representative of three independent biological experiments.

Source data are available online for this figure.

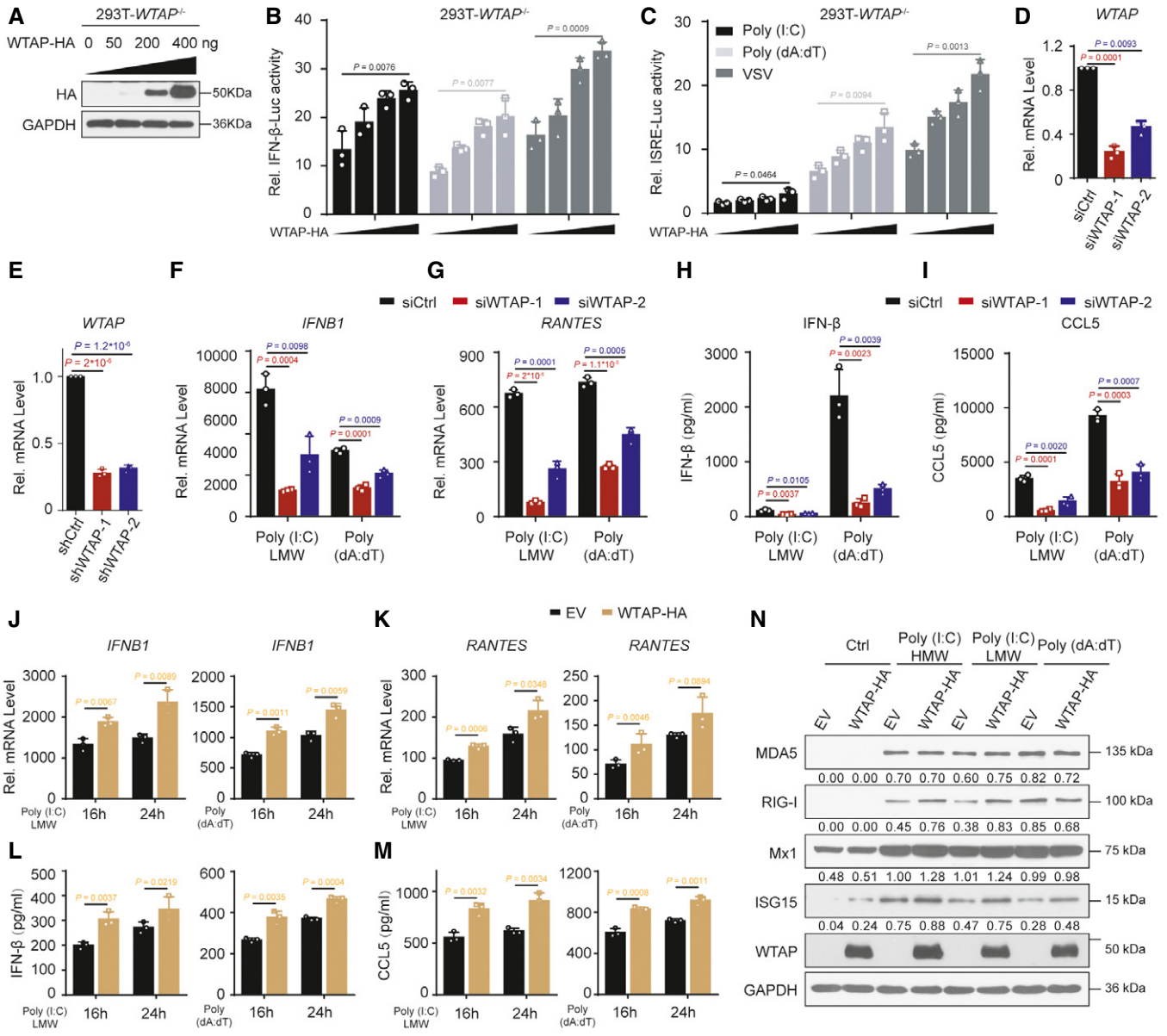

Figure EV3.

Figure EV3. WTAP positively regulates the antiviral immune responses.

A Immunoblot analyses of HA-tagged WTAP in *WTAP*$^{-/-}$ 293T cells transfected with increasing amounts of HA-tagged WTAP plasmid for 24 h. Data are representative of three independent biological experiments.

B, C IFN-β or ISRE luciferase activity in *WTAP*$^{-/-}$ 293T cells transfected with increasing amounts of WTAP plasmid for 24 h and then transfected with the indicated luciferase reporters, followed by treatments with intracellular poly(I:C), poly(dA:dT), or VSV-eGFP. The results are expressed relative to Renilla luciferase activity. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

D qRT–PCR analyses to detect the *WTAP* knockdown efficiency in A549 cells after transfection with Ctrl or WTAP-specific siRNAs for 48 h. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

E qRT–PCR analyses to detect the *WTAP* knockdown efficiency in A549 cells after transfection with Ctrl or WTAP-specific shRNAs. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

F, G qRT–PCR analyses of the mRNA abundances of *IFNB* (F) and *RANTES* (G) in A549 cells transfected with Ctrl or WTAP-specific siRNA, followed by treatments with poly(I:C)-LMW or poly(dA:dT) at the indicated time points. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

H, I ELISA analyses of IFN-β (H) and CCL5 (I) secretion in supernatants from A549 cells in (F). $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

J, K qRT–PCR analyses of the mRNA abundances of *IFNB* (J) and *RANTES* (K) in A549 cells transfected with empty vector (EV) or WTAP-HA plasmid, followed by treatment with poly(I:C)-LMW or poly(dA:dT) at the indicated time points. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

L, M ELISA analyses of IFN-β (L) and CCL5 (M) secretion in supernatants from A549 cells in (J). $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

N Immunoblot analyses to detect the protein expression of ISGs in A549 cells transfected with empty vector (EV) or WTAP-HA plasmid, followed by stimulation with poly(I:C)-HMW, poly(I:C)-LMW or poly(dA:dT) for 16 h with the indicated antibodies. Data are representative of three independent biological experiments.

Source data are available online for this figure.

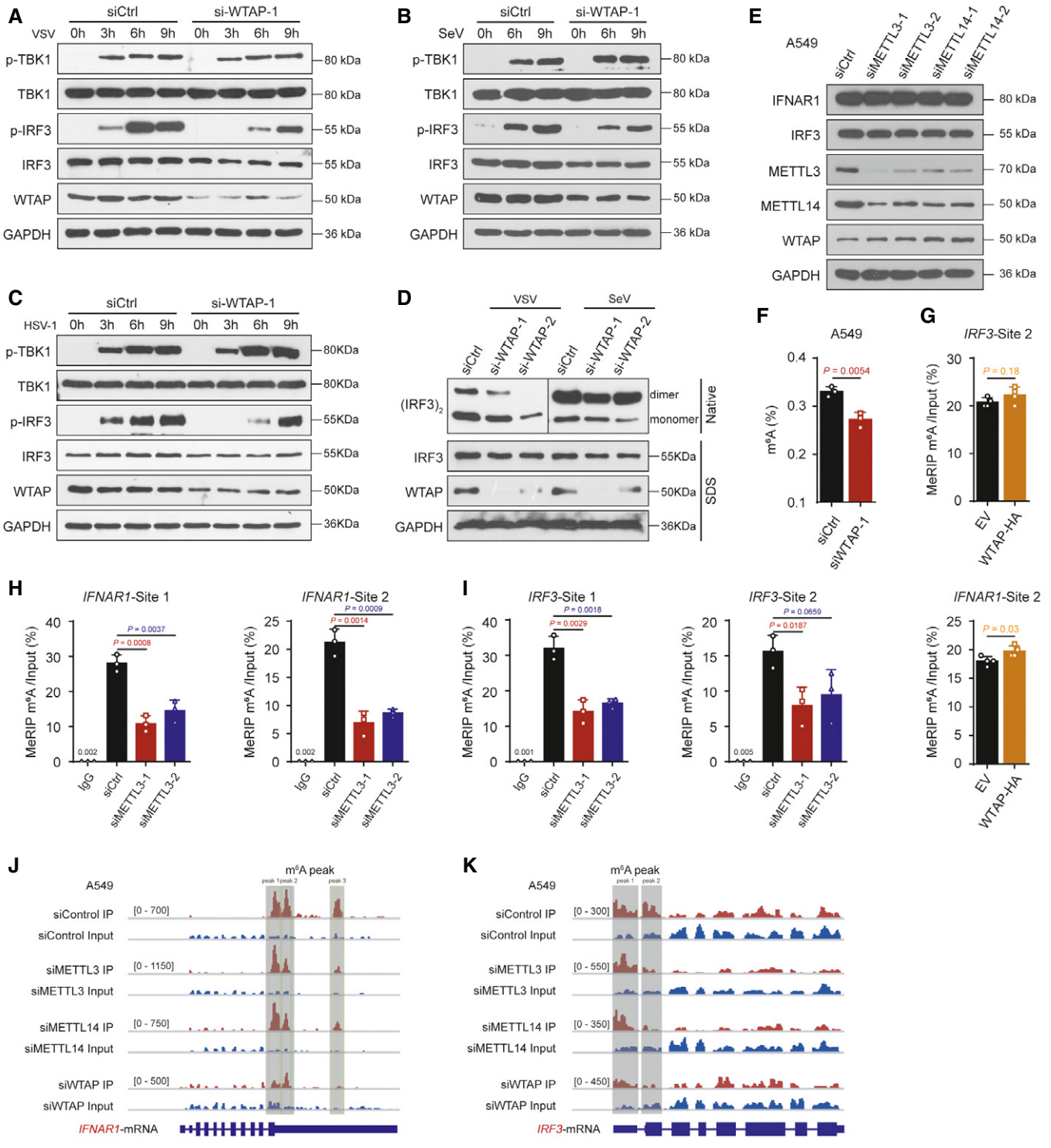

Figure EV4.

Figure EV4. WTAP maintains the protein abundance of IRF3 and IFNAR1 through m^{6}A modification.

A–C Immunoblot analyses of total and phosphorylated (p-) TBK1 and IRF3 in A549 cells transfected with Ctrl or WTAP-specific siRNA, followed by infection with VSV-eGFP (A), SeV (B), or HSV-1 (C) at different time points. Data are representative of three independent biological experiments.

D Immunoblot analyses of IRF3 dimerization in A549 cells transfected with Ctrl or WTAP-specific siRNA, followed by infection with VSV-eGFP or SeV for 6 h. Data are representative of three independent biological experiments.

E Immunoblot analyses to detect IRF3, IFNAR1, and WTAP protein abundance in A549 cells transfected with Ctrl and METTL3- or METTL14-specific siRNAs for 48 h. Data are representative of three independent biological experiments.

F ELISA quantification of m^{6}A levels in mRNAs extracted from A549 cells after transfection with Ctrl or WTAP-specific siRNA for 48 h. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

G Abundance of *IRF3* and *IFNAR1* transcripts (detection site 2) among mRNAs immunoprecipitated with anti-m^{6}A antibody from A549 cells after transfection with empty vector (EV) or HA-tagged WTAP plasmids for 24 h. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

H, I qRT–PCR analysis of the abundance of *IFNAR1* (H) and *IRF3* (I) transcripts (detection sites 1 and 2) among mRNAs immunoprecipitated with anti-m^{6}A antibody or IgG from A549 cells transfected with Ctrl and METTL3-specific siRNAs for 48 h. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

J, K IGV browser tracks showing that m^{6}A peaks were enriched in the 3' UTR of *IFNAR1* (J) and the 5' UTR of *IRF3* (K) transcripts from the m^{6}A-seq data of GSE55572. IP, m^{6}A-immunoprecipitation.

Source data are available online for this figure.

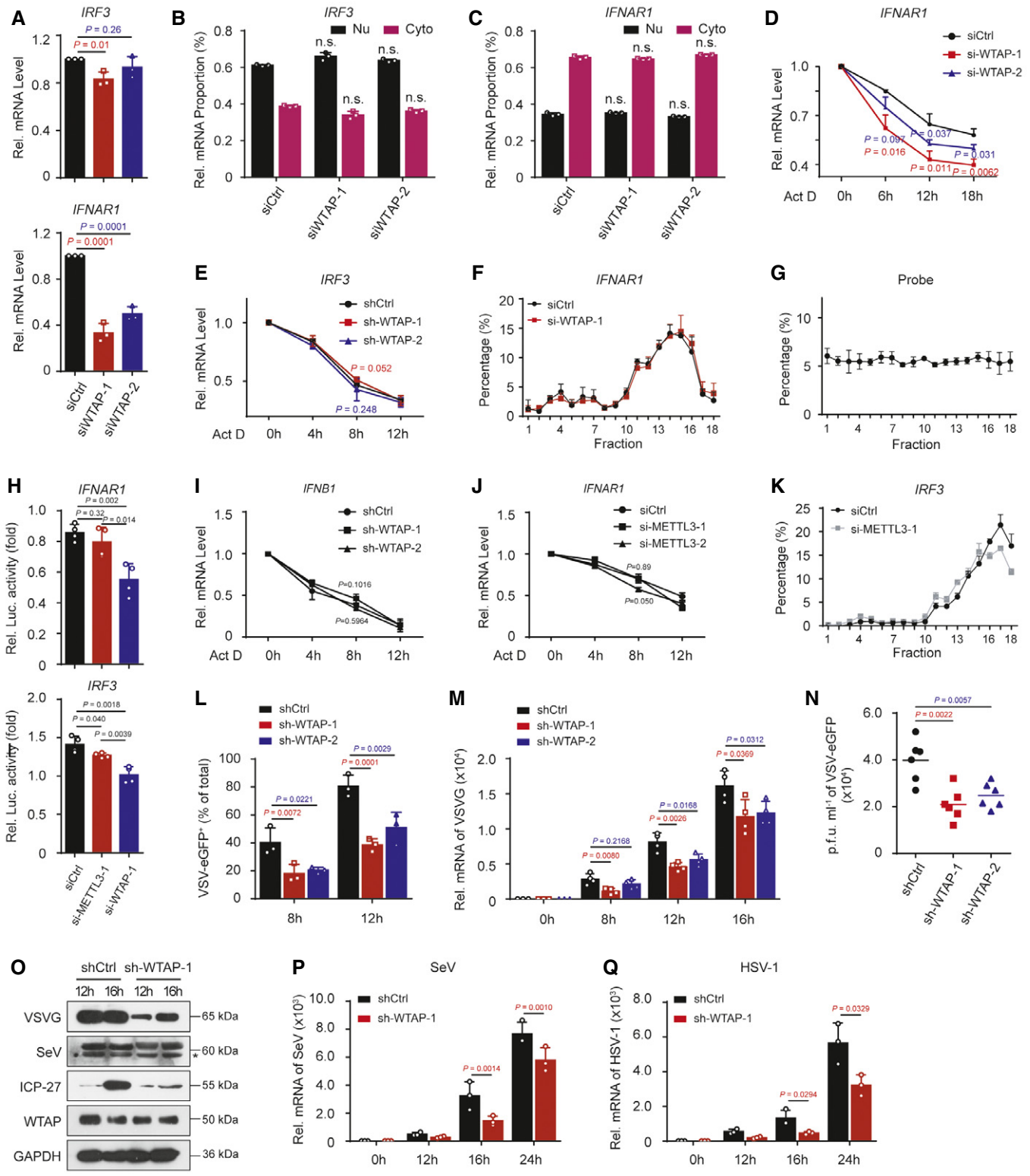

Figure EV5.

Figure EV5. WTAP and METTL3 have different effects on the fate of *IRF3* and *IFNAR1* mRNAs.

A qRT–PCR analyses of *IRF3* and *IFNAR1* mRNA expression in A549 cells after transfection with Ctrl or WTAP-specific siRNAs for 48 h. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

B, C qRT–PCR analyses of *IRF3* (B) and *IFNAR1* (C) mRNA in the nucleus (Nu) and cytoplasm (Cyto) of A549 cells transfected with Ctrl or WTAP-specific siRNAs for 48 h. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

D qRT–PCR analyses of *IFNAR1* mRNA expression in A549 cells after transfection with Ctrl or WTAP-specific siRNAs for 48 h, followed by treatment with Act D (5 μg/ml) for the indicated times. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

E qRT–PCR analyses of *IRF3* mRNA expression in shCtrl and shWTAP A549 cells, followed by treatment with Act D (5 μg/ml) for the indicated times. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

F qRT–PCR analyses of the proportion of *IFNAR1* mRNAs in polysome fractions from A549 cells transfected with Ctrl or WTAP-specific siRNAs. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

G qRT–PCR analyses of the proportion of RNA probes in polysome fractions from A549 cells. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

H Relative luciferase activities in METTL3- or WTAP-silenced A549 cells after transfection with reporter vectors bearing *IFNAR1*-3' UTR (above) or *IRF3*-5' UTR (below) with wild-type (WT) m^6A sites. Renilla luciferase activity was measured and normalized to firefly luciferase activity. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

I qRT–PCR analyses of *IFNB1* mRNA expression in shCtrl and shWTAP A549 cells, followed by treatment with Act D (5 μg/ml) for the indicated times. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

J qRT–PCR analyses of *IFNAR1* mRNA expression in A549 cells transfected with Ctrl or METTL3-specific siRNAs, followed by treatment with Act D (5 μg/ml) for the indicated times. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

K qRT–PCR analyses of the proportion of *IRF3* mRNAs in polysome fractions from A549 cells transfected with Ctrl or METTL3-specific siRNAs. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

L, M FACS (L) and qRT–PCR (M) analyses of cellular VSV loads in shCtrl and shWTAP A549 cells after VSV-eGFP infection for the indicated time points. $n = 3$ (D) or 4 (E) independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

N Plaque assay of VSV titers in cell supernatants after VSV-eGFP infection for 16 h. $n = 6$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

O Immunoblot analyses of VSV-G, SeV, and ICP-27 (HSV-1) proteins in shCtrl and shWTAP A549 cells after infection for the indicated time points. Data are representative of three independent biological experiments.

P, Q qRT–PCR analyses of cellular SeV (P) or HSV-1 (Q) loads in shCtrl and shWTAP A549 cells after SeV or HSV-1 infection for the indicated time points. $n = 3$ independent biological replicates, and error bars represent standard deviations. Data were compared using Student's *t*-test.

Source data are available online for this figure.