# Expanded View Figures

**Figure EV1. High salt is an instant promotion factor of viral infection.**

A, B Mice ($n$ = 5) were fed an NSD (0.45% NaCl) or HSD (4% NaCl) for 30 days and then intraperitoneally infected with VSV ($1 \times 10^8$ PFU per gram body, 48 h). VSV RNA levels in mouse blood (A) or spleen (B) were detected by RT-qPCR.

C, D Mice ($n$ = 5) were fed an NSD (0.45% NaCl) or HSD (4% NaCl) for 7 days and then intraperitoneally infected with VSV ($1 \times 10^8$ PFU per gram body, 48 h). VSV RNA levels in mouse blood (C) or different tissues (spleen, lung, liver, and kidney) (D) were detected by RT-qPCR.

E Western blot analysis of Caspase 3 and cleaved-Caspase 3 in RAW264.7 cells treated with additional NaCl (+51 mM) for 12, 24, and 36 h.

F RT–qPCR analysis of viral RNA levels in RAW264.7 cells infected with H1N1 or VSV (MOI = 1.0) for only 2 h immediately after addition of NaCl (+17, 34, and 51 mM).

G RAW264.7 cells were infected with VSV (MOI = 1.0 and 2.0, 24 h) immediately after addition of NaCl (+34 mM). VSV-G protein levels were analyzed by western blot.

H RAW264.7 cells were infected with H1N1 (MOI = 1.0, 24 h) immediately after addition of NaCl (+34 mM). H1N1-encoded HA protein levels were analyzed by western blot.

Data information: Data (A–D) show mean and SEM of five biological replicates; Data (E, G, H) are representative of at least two biological replicates; Data (F) represent mean and SD of four biological replicates. For all statistical testing: $P$-values were calculated using two-tailed unpaired Student's $t$-test. N.S, not significant ($P > 0.05$). *$P < 0.05$ and **$P < 0.01$.

Source data are available online for this figure.

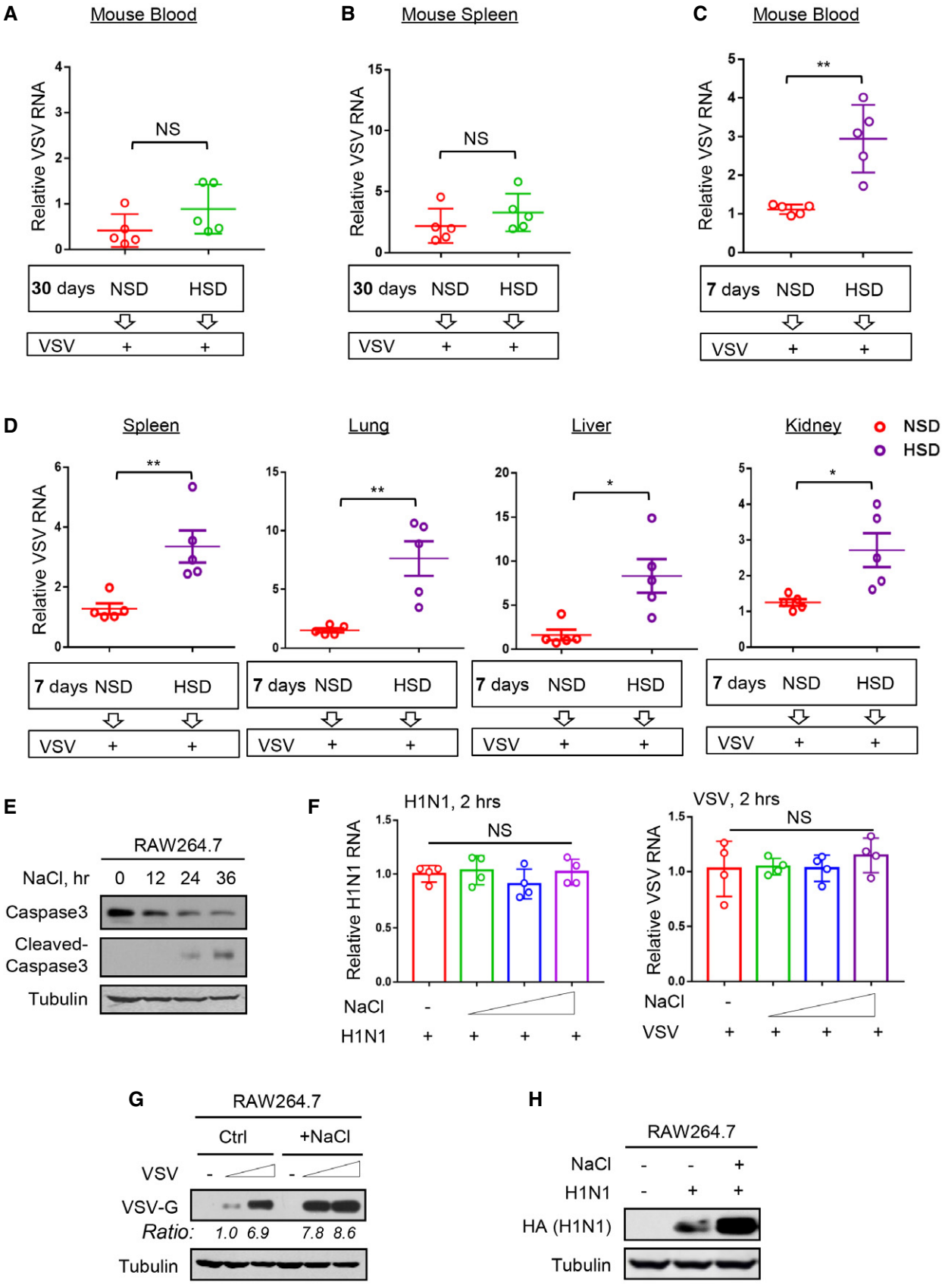
A
Mouse Blood
Relative VSV RNA
NS
30 days NSD HSD
VSV + +
B
Mouse Spleen
Relative VSV RNA
NS
30 days NSD HSD
VSV + +
C
Mouse Blood
Relative VSV RNA
**
7 days NSD HSD
VSV + +
D
Spleen
Lung
Liver
Kidney
NSD
HSD
Relative VSV RNA
**
**
*
*
7 days NSD HSD
VSV + +
E
RAW264.7
NaCl, hr 0 12 24 36
Caspase3
Cleaved-Caspase3
Tubulin
F
H1N1, 2 hrs
Relative H1N1 RNA
NS
NaCl -
H1N1 + + + +
VSV, 2 hrs
Relative VSV RNA
NS
NaCl -
VSV + + + +
G
RAW264.7
Ctrl +NaCl
VSV - - 
VSV-G
Ratio: 1.0 6.9 7.8 8.6
Tubulin
H
RAW264.7
NaCl - - +
H1N1 - + +
HA (H1N1)
Tubulin

**Figure EV1.**

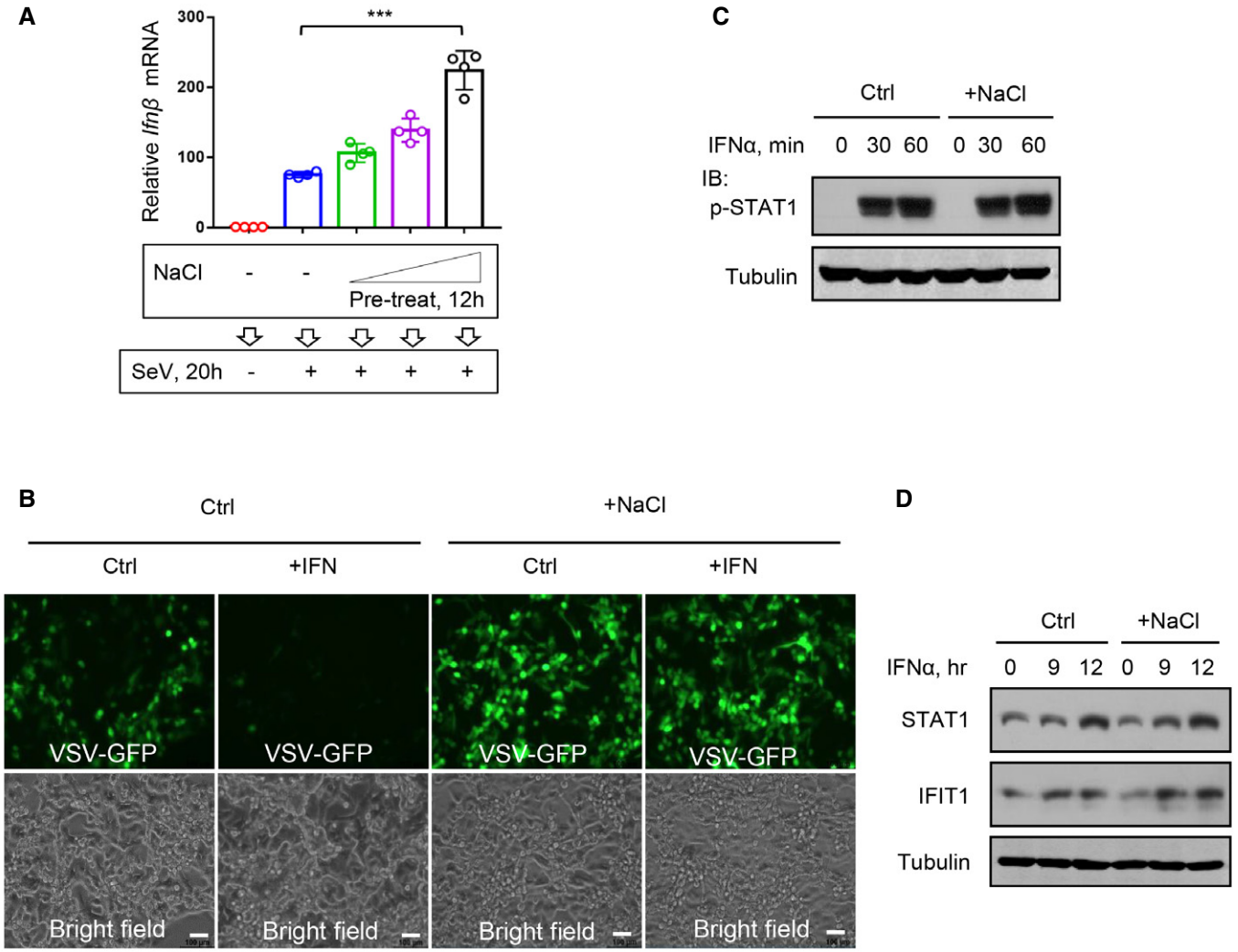


**Figure EV2. High salt does not affect IFN-I-induced signaling.**

A RT–qPCR analysis of IFNβ mRNA levels in HT1080 cells pretreated with additional NaCl (+17, 34, and 51 mM) for 12 h and then infected with SeV (MOI = 1.0) for 20 h.

B 2fTGH cells were treated with IFNα (50 IU/ml, 20 h) immediately after addition of NaCl (+34 mM). After washing, cells were infected with VSV-GFP (MOI = 0.1, 24 h) and then viruses were observed by fluorescence. Scale bars, 10 μm.

C Western blot analysis of STAT1-Y701 phosphorylation (p-STAT1) in HEK293T cells treated with additional NaCl (+34 mM, 12 h) and then stimulated with IFNα (1,000 IU/ml) for 30 and 60 min.

D Western blot analysis of STAT1 and IFIT1 levels in HEK293T cells stimulated with IFNα (1,000 IU/ml) immediately after addition of NaCl (+34 mM) as indicated.

Data information: Data (A) represent mean and SD of four biological replicates; Data (C, D) are representative of at least two biological replicates. For all statistical testing: *P*-values were calculated using two-tailed unpaired Student's *t*-test. ***$P < 0.001$.

Source data are available online for this figure.

**Figure EV3. High salt exacerbates viral infection by decreasing antiviral protein Viperin.**

A RAW264.7 cells were treated with mIFNβ (500 IU/ml) for 12 h immediately after addition of NaCl (+34 mM) or control $ddH_2O$ (Ctrl). Differentially expressed proteins were analyzed by a TMT2-plex Mass analysis (left). RT–qPCR was used to analyze mRNA levels of several top differential genes in RAW264.7 cells treated with mIFNβ (500 IU/ml) for 12 h (right). Data were shown as mean and SD of three biological replicates.

B, C Western blot analysis of Viperin in mouse splenocytes infected with VSV (B) or in THP1 cells infected with SeV (C) (MOI = 1.0, 12 and 24 h) immediately after addition of NaCl (+34 mM).

D, E Western blot analysis of Viperin in RAW264.7 treated with mIFNβ (300 IU/ml) (D) or in THP1 treated with IFNα (1,000 IU/ml) (E) for 12 h immediately after addition of NaCl (+17, 34, and 51 mM).

F Western blot analysis of Viperin in RAW264.7 treated with LPS (2.5 μg/ml, 12 and 24 h) immediately after addition of NaCl (+34 mM).

G RT–qPCR analysis of VSV RNA levels in 2fTGH cells transfected with control shRNAs (shCtrl) or shRNAs against Viperin (shViperin) and then infected with VSV (MOI = 1.0, 24 h) immediately after addition of NaCl (+34 mM).

H RT–qPCR analysis of VSV RNA levels in *Viperin*$^{+/+}$ or *Viperin*$^{-/-}$ 2fTGH cells infected with VSV (MOI = 1.0, 24 h) immediately after addition of NaCl (+34 mM).

I Western blot analysis of Flag-Viperin in *Rsad2*$^{-/-}$ MEF cells transfected with vectors or Flag-Viperin and then infected with VSV (MOI = 1.0, 24 h) immediately after addition of NaCl (+34 mM).

J Western blot analysis of VSV-G in A549 cells transfected with vectors or Flag-Viperin and then infected with VSV (MOI = 1.0, 24 h) immediately after addition of NaCl (+34 mM). VSV titers in culture supernatants were analyzed by the TCID 50 assay.

K Western blot analysis of Flag-HA-tagged (FH)-Viperin levels in stable FH-Viperin-expressing HeLa cells treated with additional NaCl (+34 mM) as indicated.

L Western blot analysis of HA-Viperin in HEK293T cells transfected with HA-Viperin and then treated with additional NaCl (+17, 34 mM) for 12 h.

M Flow cytometry analysis of eGFP-Viperin in HEK293T cells transfected eGFP-Viperin and then treated with additional NaCl (+17, 34 mM) for 12 h.

N Western blot analysis of FH-Viperin levels in stable FH-Viperin-expressing HeLa cells treated with additional NaCl (+17 and 34 mM), or $FeSO_4$ (20 and 40 μM), or $CaCl_2$ (20 and 40 μM) for 12 h.

O The densities of FH-Viperin protein bands in (N) were quantitated with the Image J. Data represent mean and SD of three biological replicates.

Data information: Data (A, G, H, J) represent mean and SD of three (A, J) or four (G, H) biological replicates; Data (B–F, I–L, N) are representative of at least two biological replicates. For all statistical testing, *P*-values were calculated using two-tailed unpaired Student's *t*-test. NS, not significant ($P > 0.05$). *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Source data are available online for this figure.

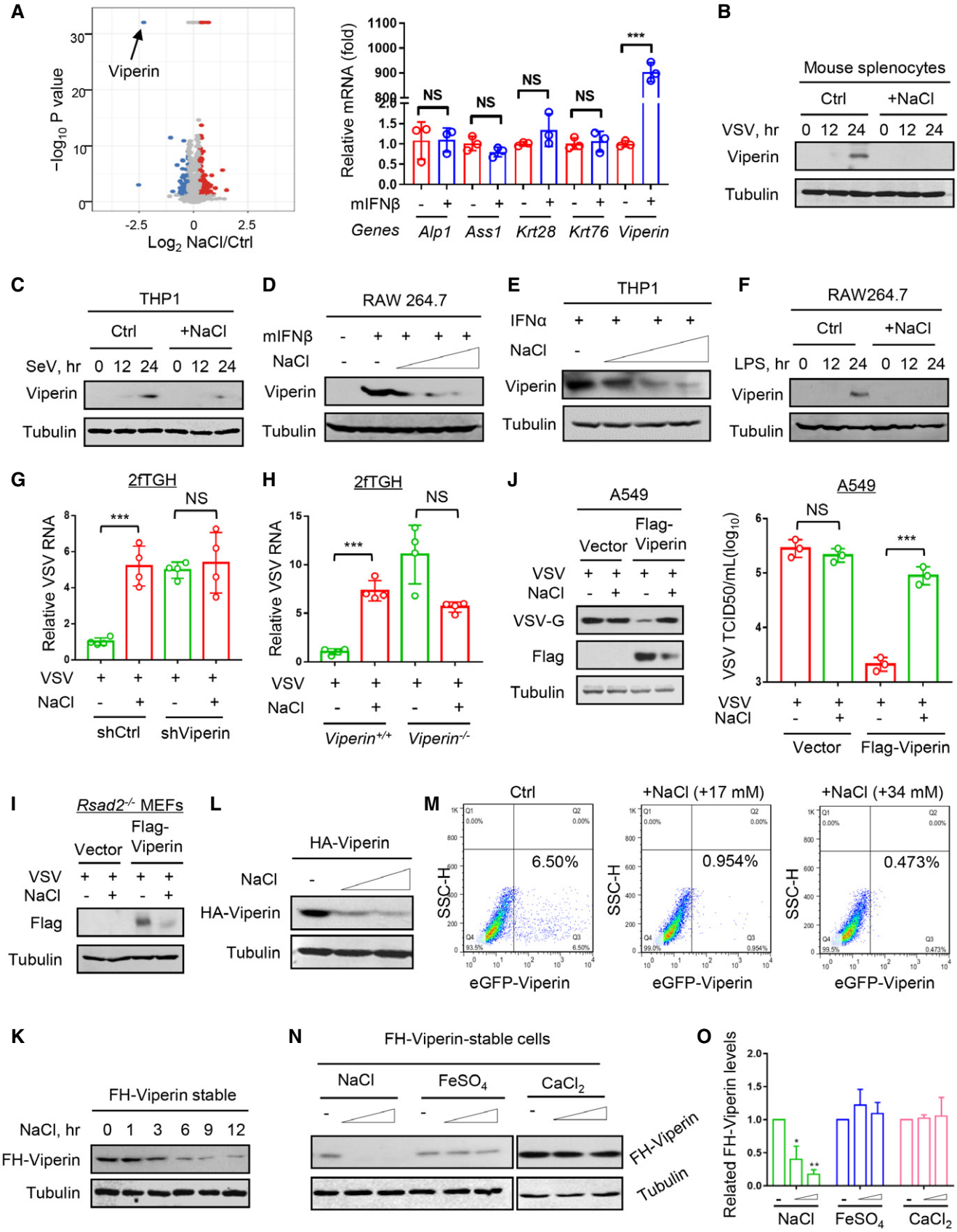


**Figure EV3.**

**Figure EV4. High salt downregulates USP33, which is a key deubiquitinase of Viperin.**

A Immunoprecipitation analysis of ubiquitination of Flag-Viperin in HEK293T cells co-transfected with Flag-Viperin and HA-Ub, and then treated with MG132 (10 μM) and additional NaCl (+34 mM) for 12 h.
B Western blot analysis of UBE4A in RAW264.7 cells treated with additional NaCl (+17, 34, and 51 mM) for 12 h.
C–E Western blot analysis of Flag-HA (FH)-USP33 (C), FH-USP1 (D), FH-UCHL1 (E) in HEK293T cells transfected with the corresponding plasmids and then treated with additional NaCl (+17 and 34 mM) for 12 h.
F Immunoprecipitation analysis of the interaction between Myc-Viperin and HA-USP33 in HEK293T cells co-transfected with Myc-Viperin and HA-USP33.
G Western blot analysis of Viperin in 2fTGH cells transfected with increasing amount of FH-USP33 and then treated with IFNα (5,000 IU/ml) for 15 h.
H Western blot analysis of Viperin in RAW264.7 transfected with empty vectors (Ctrl) or FH-USP33 and then treated with mIFNβ (500 and 1,000 IU/ml) for 12 h.
I Western blot analysis of Viperin in HeLa cells transfected with FH-USP33 and then treated with IFNα (1,000 IU/ml) for different times.
J Western blot analysis of USP33 protein levels in two clones (1# and 2#) of *Usp33*$^{+/+}$ and *Usp33*$^{-/-}$ MEF cells.
K Western blot analysis of FH-Viperin in HEK293T cells co-transfected FH-Viperin and FH-USP33 (wild type, WT; C194S and H673Q, DM).
L Immunoprecipitation analysis of Myc-Viperin ubiquitination in HEK293T cells co-transfected with Myc-Viperin, HA-Ub, and increasing amount of FH-USP33.
M Immunoprecipitation analysis of Flag-Viperin ubiquitination in HEK293T cells co-transfected with Flag-Viperin, HA-Ub, and shCtrl or shUSP33.
N Immunoprecipitation analysis of Flag-Viperin ubiquitination in HEK293T cells co-transfected with Flag-Viperin and shCtrl or shUSP33.
O Western blot analysis of Flag-Viperin levels in HEK293T cells co-transfected Flag-Viperin and FH-USP33, and then treated with CHX (50 μg/ml) as indicated.
P Western blot analysis of USP33 in RAW264.7 cells treated with additional NaCl (+17, 34, and 51 mM) for 12 h.
Q Western blot analysis of USP33 in 2fTGH cells treated with additional NaCl (+34 mM) for different times.
R Western blot analysis of USP33 in HepG2 cells treated with additional NaCl (+17, 34, and 51 mM) for 12 h.
S Western blot analysis of Myc-Viperin in HEK293T cells co-transfected with Myc-Viperin and shCtrl (–) or shUSP33, then treated with additional NaCl (+34 mM) for 12 h.
T Western blot analysis of VSV-G in *Usp33*$^{+/+}$ and *Usp33*$^{-/-}$ mouse liver cells infected with VSV (MOI = 1.0, 24 h) immediately after addition of NaCl (+34 mM).

Data information: Data (A–T) are representative of at least two biological replicates.

Source data are available online for this figure.

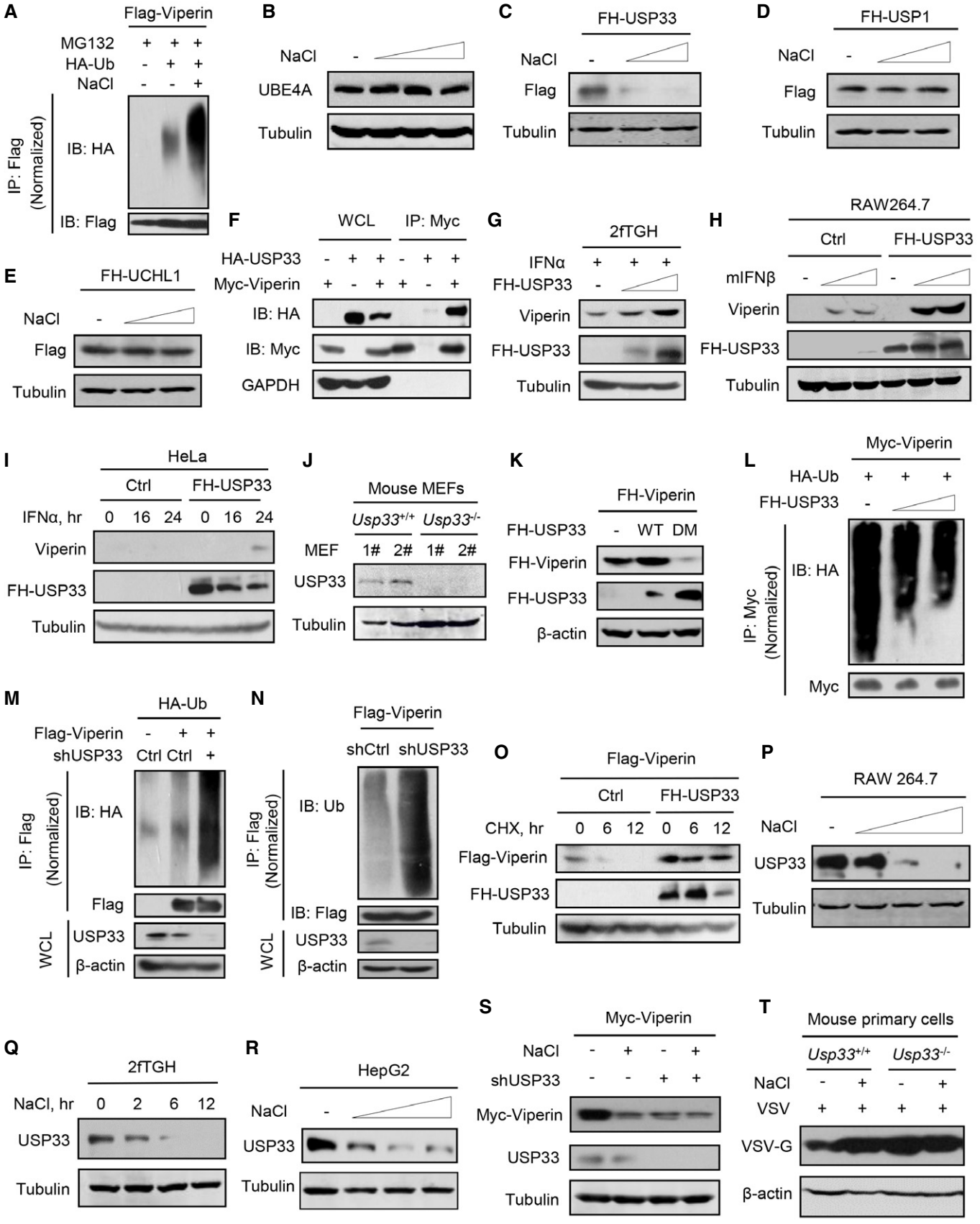


**Figure EV4.**

**Figure EV5. High salt stimulates degradation of ubiquitinated proteins via p97.**

A Immunoprecipitation analysis of FH-USP33 ubiquitination in HEK293T cells transfected with FH-USP33 and then treated with additional NaCl (+34 mM) for 12 h.
B Immunoprecipitation analysis of ubiquitination (pan-Ubi) of cellular proteins in HEK293T cells treated with additional NaCl (+17, 34, and 51 mM) for 12 h.
C, D Immunoprecipitation analysis of K48-linked (C) or K63-linked (D) ubiquitination of cellular proteins in RAW264.7 cells treated with additional NaCl (+17, 34, and 51 mM) for 12 h using a specific anti-K48-Ub chain or anti-K63-Ub chain antibody.
E Immunoprecipitation analysis of Myc-Viperin ubiquitination in HEK293T cells transfected with Myc-Viperin and then treated with NMS873 (3 and 5 μM) for 6 h.
F Western blot analysis of Viperin in RAW264.7 pretreated with NMS873 (1, 3 and 6 μM) for 2 h and then treated with mIFNβ (500 IU/ml) for 6 h.
G, H Western blot analysis of Viperin in RAW264.7 pretreated with NMS873 (10 μM) for 2 h and then treated with mIFNβ (500 and 1,000 IU/ml) for 6 h (G) or with mIFNβ (500 IU/ml) for 4 and 8 h (H).
I TCID50 assay of VSV titers in culture supernatants from HT1080 cells infected with VSV (MOI = 1.0, 24 h) immediately after addition of NaCl (+34 mM) and NMS873 (10 μM). Data represent mean and SD of three biological replicates. NS, not significant ($P > 0.05$). **$P < 0.01$ (two-tailed unpaired Student's *t*-test).
J Fluorescence microscopy of VSV with a green fluorescent protein gene (GFP) in HT1080 cells infected with VSV-GFP (MOI = 0.5, 24 h) immediately after addition of NaCl (+34 mM) and NMS873 (10 μM). Scale bars, 100 μm.
K Immunoprecipitation analysis of pan-serine phosphorylation (p-Ser) of V5-tagged p97 (V5-p97) in RAW264.7 cells transfected with V5-p97 and then treated with additional NaCl (+17, 34 and 51 mM) for 12 h using an anti-pan-serine phosphorylation antibody.
L, M Immunoprecipitation analysis of pan-acetylation (Ace) of p97 in RAW264.7 cells treated with mIFNβ (500 IU/ml) (L) or VSV (MOI = 1.0) (M) for indicated times.
N HEK293T cells with stable expression of FH-Viperin were used to make *Nr3c2* $^{+/+}$ and *Nr3c2* $^{-/-}$ cells using CRISPR-Cas9. Then cells were infected with VSV (MOI = 1.0) in media containing normal (CON) or reduced (Low, −34 mM) concentration of NaCl for 12 h. VSV-G and FH-Viperin levels were analyzed by western blot.

Data information: Data (A–H, K–N) are representative of at least two biological replicates; Data (I) represent mean and SD of three biological replicates. For all statistical testing, *P*-values were calculated using two-tailed unpaired Student's *t*-test. NS, not significant ($P > 0.05$). **$P < 0.01$.

Source data are available online for this figure.

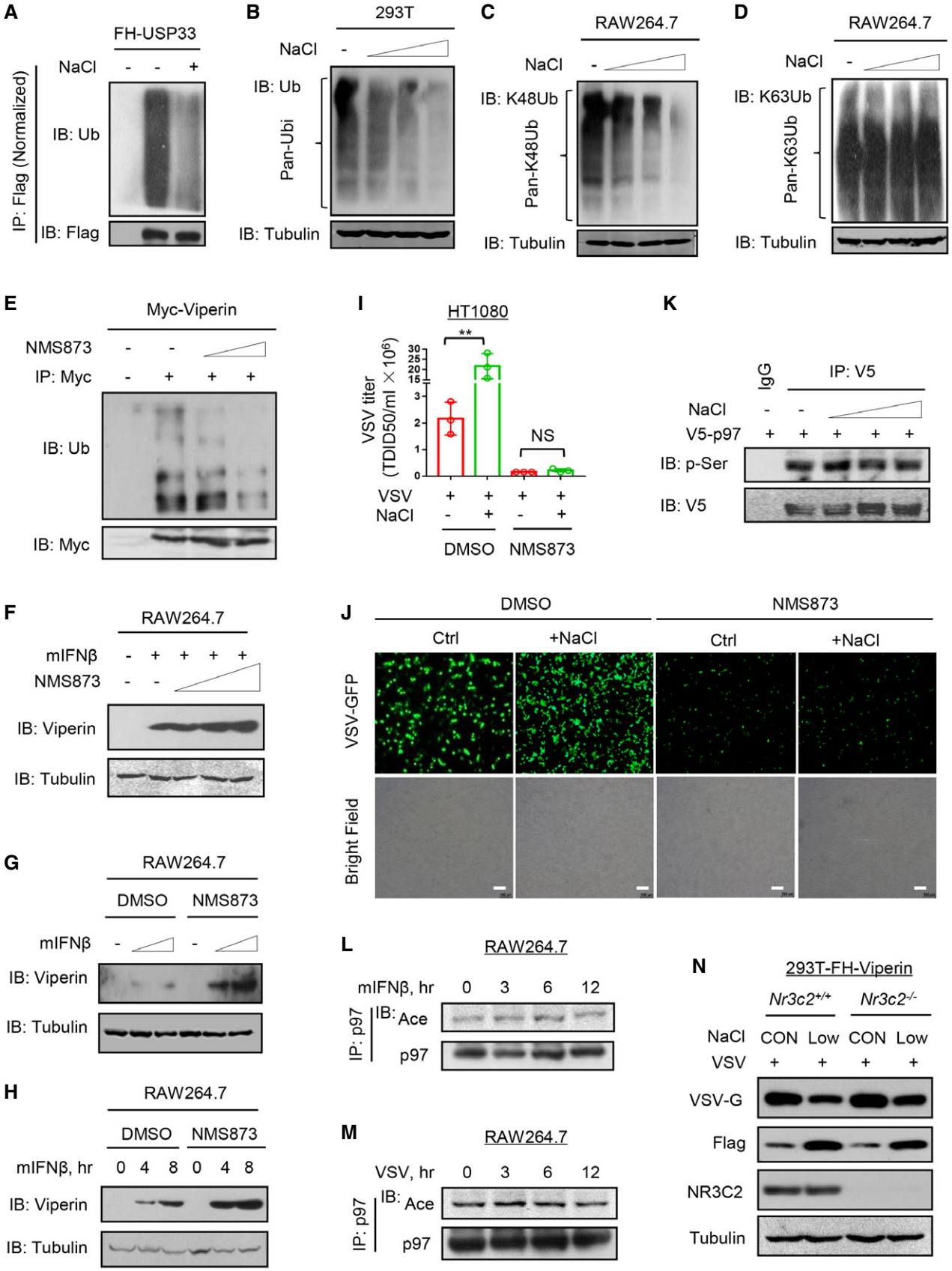


**Figure EV5.**