Supplemental Information Titles and Legends

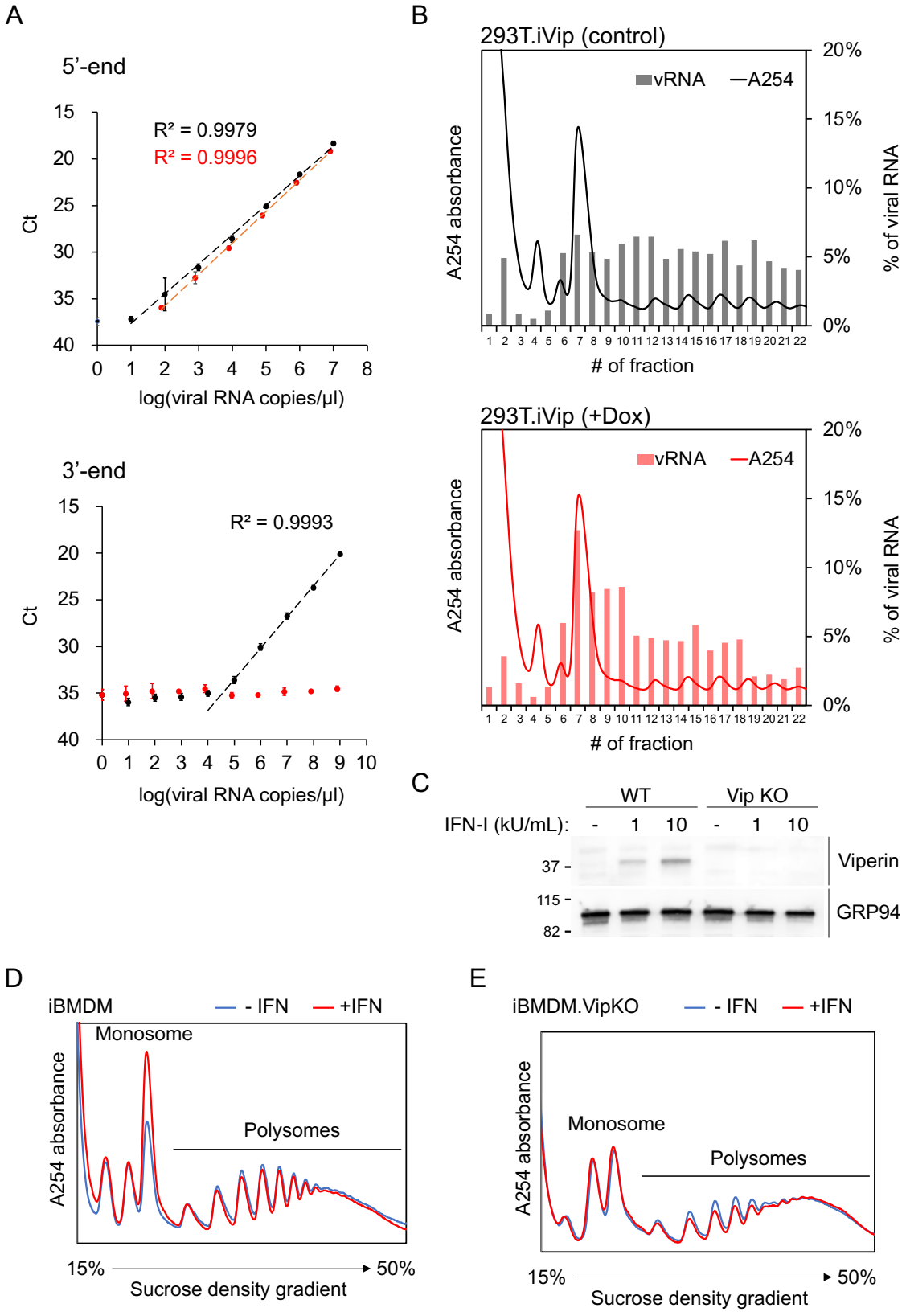

Figure S1, Related to Figure 1. Viperin is required for translation inhibition in viral infection and during type I interferon response.

(A) Mean Ct values for ZIKV primers targeting the 5' and 3' ends of the viral RNA tested for two biological replicates with 10-fold dilutions of *in vitro* transcribed full-length (black) and truncated (red) ZIKV RNAs. The truncated ZIKV RNA lacks the sequence targeted by the 3' end primer. The analytical sensitivity of detection for the 5' and 3' ends are 12 and ~30,000 copies/µl respectively.

(B) Translation of ZIKV RNA in 293T.iVip cells was determined by sucrose density gradient velocity sedimentation and qRT-PCR. Cells were treated with doxycycline (Dox) for 24 h and then infected with ZIKV at an MOI of 1 for 24 h. Cell lysates were fractionated by sucrose density gradient velocity sedimentation. Viral RNA in sucrose density gradient fractions were determined by qRT-PCR.

(C) Immunoblot analysis of viperin in macrophage cell lines derived from WT (iBMDM) and viperin knockout (iBMDM.VipKO) mice. Cells were treated with type I interferon (IFN-I) for 24 h. Cell lysates were analyzed by immunoblotting with anti-viperin and anti-GRP94 antibodies. kU, kilounit.

(D-E) Polysome profile analysis of iBMDM and iBMDM.VipKO cells. Cells were treated with IFN-I (10,000 U/mL) for 8 h. Cell lysates were cleared by centrifugation, loaded onto a 15-50% sucrose gradient, and subjected to ultracentrifugation. Absorbance was monitored at 254 nm to record the polysome profile. The monosome and polysome pools are indicated. a.u., arbitrary unit.

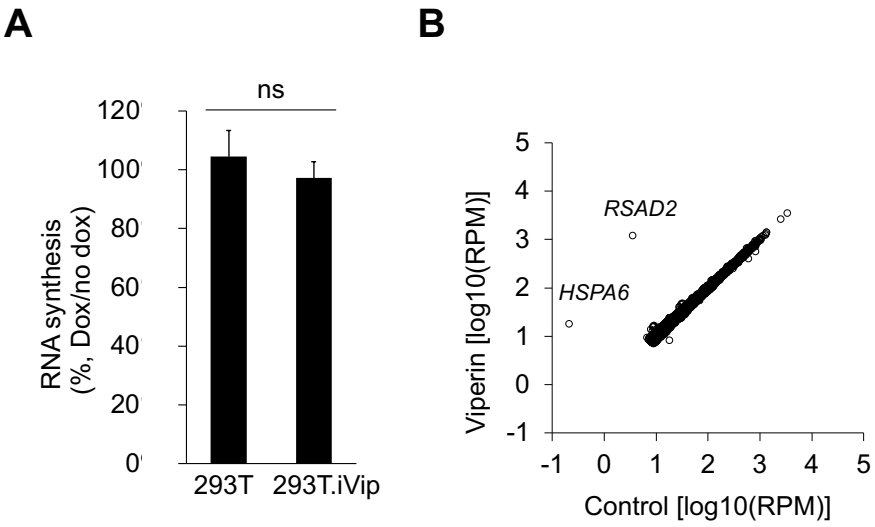

Figure S2, Related to Figure 1. Viperin does not regulate cellular RNA level.
(A) Transcription analysis of 293T.iVip cells. Cells were treated with doxycycline (Dox) for 24 h. Newly synthesized RNA was labeled by 5-ethynyluridine (5-EU) and stained by the Click chemistry reaction with azide-fluor 488 and analyzed by flow cytometry. Data represent the mean fluorescence intensity ratio between Dox-treated and mock treated cells. Data are shown as mean ± SD of three biological repeats (n = 3) and analyzed by unpaired Student's t test. ns, not significant.
(B) Scatter plots of RNA-seq expression data for differentially expressed genes with mock vs viperin transfection in 293T cells (Pearson correlation coefficient, r =0.999). Two significantly up-regulated mRNAs are labeled. *RSAD2* is the transgene encoding viperin protein, and *HSPA6* is a Lipofectamine-induced artifact (Plautz et al., 2011). RPM, reads per million.

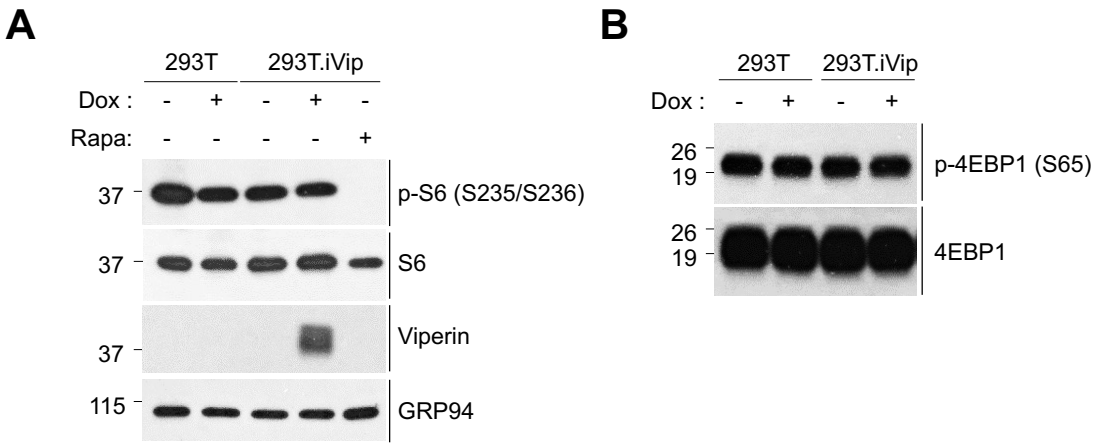

Figure S3, Related to Figure 5. Viperin does not regulate the mTORC1 signaling pathway

Immunoblot analysis of the phosphorylation of S6 (A) and 4EBP-1 (B) in 293T and 293T.iVip cells treated with doxycycline (Dox) for 24 h. Cell lysates were analyzed by immunoblotting with anti-phospho-4EBP1 (S65), anti-4EBP1, anti-phospho-S6 (S235/S236), anti-S6, anti-viperin and anti-GRP94 antibodies. The mTORC inhibitor rapamycin (Rapa) serves as a positive control.

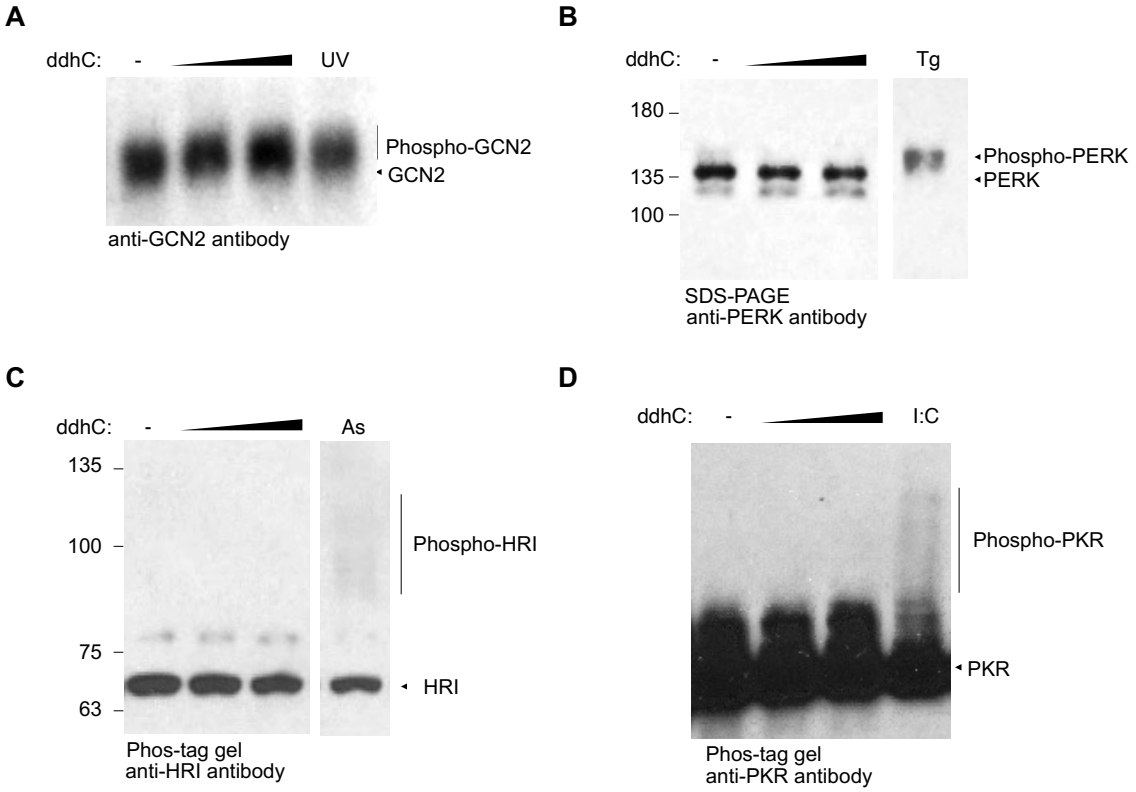

Figure S4, Related to Figure 5. ddhC activates the eIF2α kinase GCN2.
Immunoblot analysis of the phosphorylation of four eIF2α kinases (A) GCN2, (B) PERK, (C) HRI and (D) PKR. 293T cells were treated with 0.5 mM and 1 mM ddhC for 24 h and eIF2α kinase activators, UV irradiation (UV), thapsigargin (Tg), arsenite (As) and poly(I:C) (I:C). Cell lysates were analyzed by Phos-tag gel or SDS-PAGE and immunoblotting with anti-GCN2, anti-PERK, anti-HRI and anti-PKR antibodies. Irrelevant lanes were spliced out between lane 3 and 4 in (B) and (C).

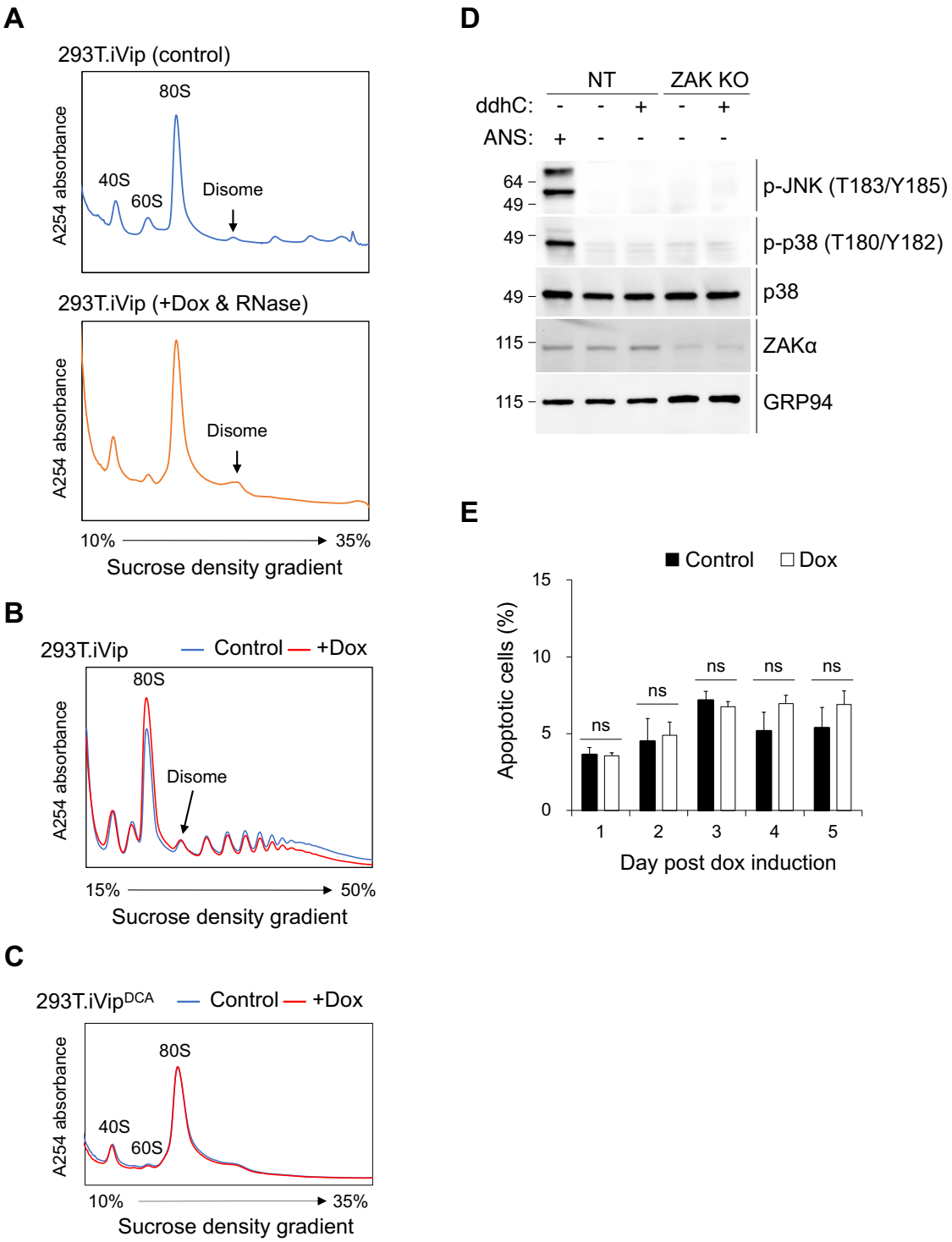
A
293T.iVip (control)
80S
40S
60S
Disome
A254 absorbance
293T.iVip (+Dox & RNase)
Disome
A254 absorbance
10%
35%
Sucrose density gradient
B
293T.iVip
Control
+Dox
80S
Disome
A254 absorbance
15%
50%
Sucrose density gradient
C
293T.iVipDCA
Control
+Dox
80S
40S
60S
A254 absorbance
10%
35%
Sucrose density gradient
D
NT
ZAK KO
ddhC: - - + - +
ANS: + - - - -
64
49
p-JNK (T183/Y185)
49
p-p38 (T180/Y182)
49
p38
115
ZAKα
115
GRP94
E
Control
Dox
Apoptotic cells (%)
15
10
5
0
ns
ns
ns
ns
ns
1
2
3
4
5
Day post dox induction

Figure S5, Related to Figure 6. Viperin expression and ddhC treatment induce RNase-resistant disome formation but not the ribosome stress response (RSR).
(A) Polysome profile analysis of the formation of RNase-resistant disomes in 293T.iVip cells. Cell were treated with doxycycline (Dox) for 24 h. Cell lysate was cleared by centrifugation, digested by RNase, loaded onto a 10-35% sucrose gradient, and subjected to ultracentrifugation. Mock treated cell lysate was directly loaded onto a gradient as a control. Absorbance was monitored at 254 nm to record the polysome profile. The ribosomal subunits (40S and 60S), monosome (80S) and disome peaks are indicated.
(B) Polysome profile analysis in 293T.iVip cells. Cell were treated with Dox for 24 h. Cell lysates were cleared by centrifugation, loaded onto a 15-50% sucrose gradient, and subjected to ultracentrifugation. Absorbance was monitored at 254 nm to record the polysome profile. The 80S monosome and disome pools are indicated.
(C) Polysome profile analysis in 293T.iVip^{DCA} cells. Cell were treated with Dox for 24 h. Cell lysates were cleared by centrifugation, digested by RNase, loaded onto a 10-35% sucrose gradient, and subjected to ultracentrifugation. Absorbance was monitored at 254 nm to record the polysome profile.
(D) Immunoblot analysis of the phosphorylation of JNK and p38. 293T cells were treated with ddhC for 24 h. Cell lysates were analyzed by immunoblotting with anti-phospho-JNK (T183/Y185), anti-phospho-p38 (T180/Y182), anti-p38, anti-ZAK and anti-GRP94. Anisomycin (ANS) serves as a positive control.
(E) 293T.iVip cells were treated with 0.5 μg/mL doxycycline (Dox) for indicated days, stained by Annexin V-FITC and propidium iodide according to the manufacturer's instruction (Invitrogen, BMS500FI) and analyzed by flow cytometry. Data are shown as mean ± SD of three biological repeats (n = 3) and analyzed by unpaired Student's t test. ns, not significant.

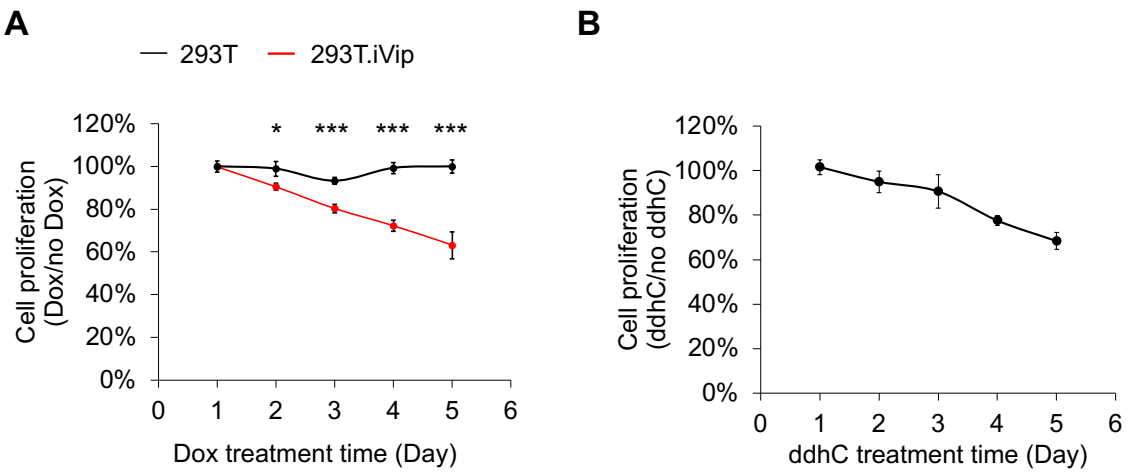

Figure S6, Related to Figure 6. Viperin expression and ddhC treatment inhibit cell proliferation.

(A) 293T and 293T.iVip cells were treated with doxycycline (Dox) for indicated days, stained by CellTrace CFSE Cell Proliferation Kit (Invitrogen) according to the manufacturer's instruction and analyzed by flow cytometry. Data are shown as mean ± SD of three biological repeats (n = 3) and analyzed by unpaired Student's t test. *$p < 0.05$, ***$p < 0.001$.

(B) 293T cells were treated with ddhC for indicated days, stained by CellTrace™ CFSE Cell Proliferation Kit (Invitrogen) according to the manufacturer's instruction and analyzed by flow cytometry. Data are shown as mean ± SD of three biological repeats (n = 3).

Table S1, Related to Figures 1 and 7. Primer list for qRT-PCR

Gene	Primer sequence
ZIKA 5' end	**F:** CATCGCTTGGCTTTTGGGAA
	R: TGCTGACTCCTATGCACCTG
ZIKA middle	**F:** CCTTAGAGGGCTTCCAGTGC
	R: ACGTGAAGTGAAGGTGGCAT
ZIKA 3' end	**F:** ACCTATCCACCCAAGTTCGC
	R: ACTAGCAGGCCTGACAACAC
WNV_{KUNV}	**F:** CTTGGATGATCGCTTTGCCA
	R: CCATCCGGTTGATGGTTTCC
GAPDH	**F:** ACAACTTTGGTATCGTGGAAGG
	R: GCCATCACGCCACAGTTTC