SUPPLEMENTARY MATERIAL

Eribulin activates the cGAS-STING pathway *via* the cytoplasmic accumulation of mtDNA

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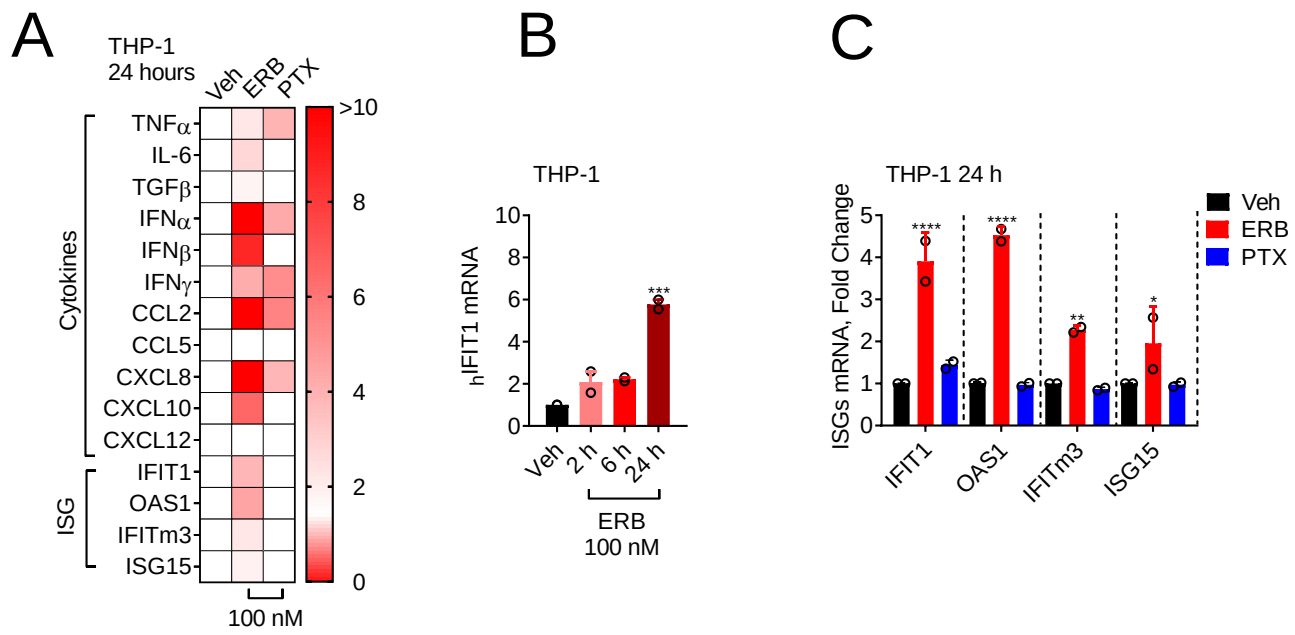

Figure S1. The effects of eribulin and paclitaxel on cytokine expression. (**A**) Heat map of relative human cytokine mRNA levels in THP-1 cells treated with 100 nM of eribulin (ERB) or paclitaxel (PTX) for 24 h as compared to DMSO. (**B**) IFIT1 mRNA in THP-1 cells treated with 100 nM eribulin for 2, 6, or 24 h compared to DMSO. Significance determined by vehicle compared 1-way ANOVA with Dunnett's posthoc test. (**C**) ISG (IFIT1, OAS1, IFITm3, and ISG15) mRNA in THP-1 cells treated with 100 nM eribulin or paclitaxel for 24 h compared to DMSO. Significance determined by 2-way ANOVA (gene * drug) with Tukey's posthoc test. Quantitative RT-PCR data are shown as individual points from two independent biological replicates with error bars denoting range. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

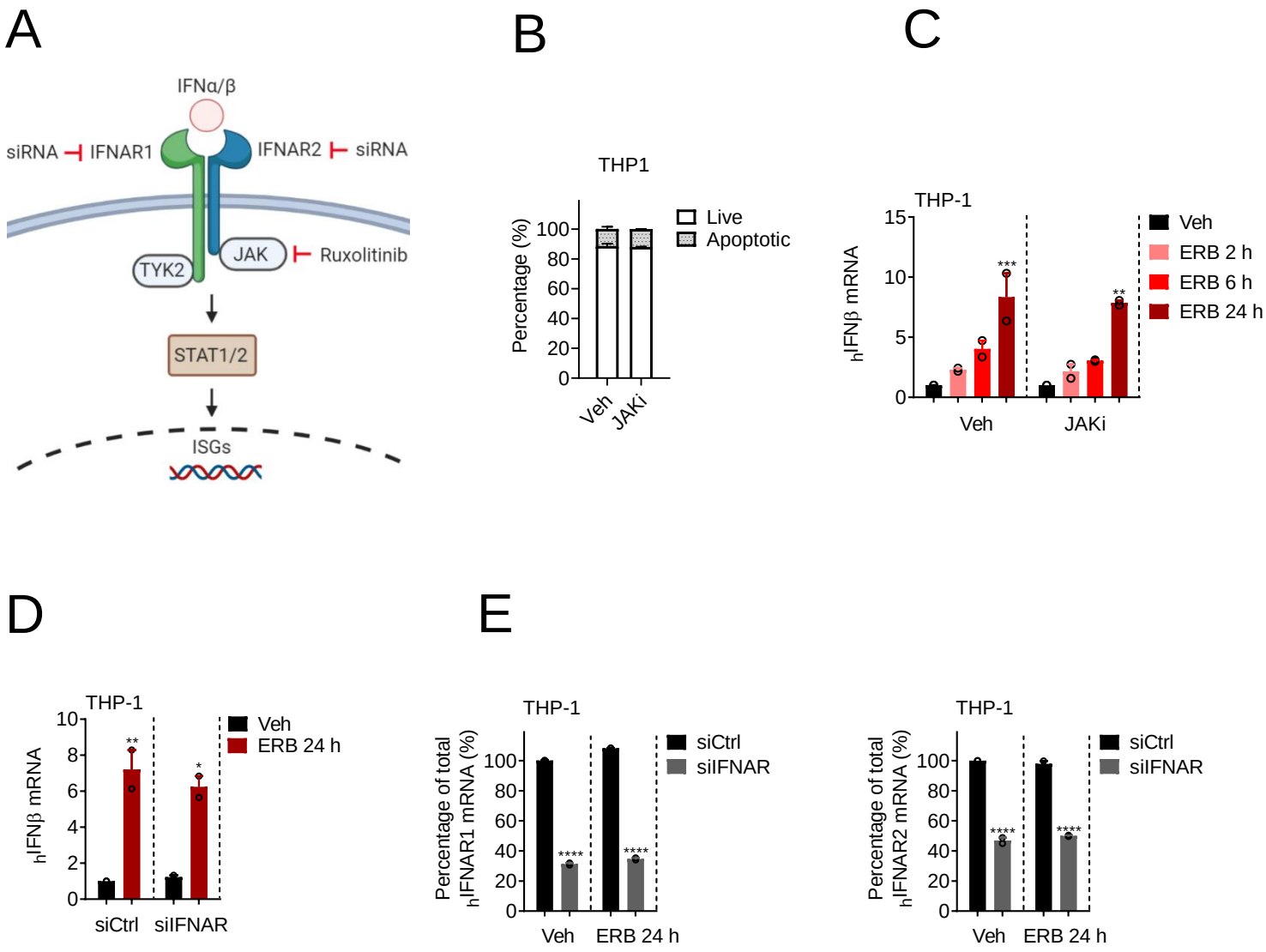

Figure S2. Eribulin-mediated expression of interferon stimulated genes is dependent on canonical IFN-mediated JAK signaling. (**A**) Canonical interferon receptor (IFNAR) signaling to promote ISG expression is mediated by JAK signaling and inhibited by the JAK inhibitor ruxolitinib or silencing of the IFNAR subunits. (**B**) Caspase 3/7 cleavage in THP-1 cells treated with 1 μM ruxolitinib (JAKi) for 24 h as compared to the DMSO vehicle from two independent experiments with errors denoting range. (**C**) IFNβ mRNA in THP-1 cells pretreated with 1 μM of ruxolitinib (JAKi) for 4 h and then treated with 100 nM eribulin for 2, 6, or 24 h as compared

to DMSO with the inhibitor still present. Significance determined by 2-way ANOVA (inhibitor * drug) with Tukey's posthoc test. (**D**) IFNβ and (**E**) IFNAR1/2 mRNA in THP-1 cells treated with control siRNA or siRNA for IFNAR1/2 for 48 h and then treated with 100 nM eribulin or vehicle for 24 h. Significance was determined by 2-way ANOVA (siRNA * drug) with Tukey's posthoc test. Quantitative RT-PCR data are shown as individual points from two independent biological replicates with error bars denoting range. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

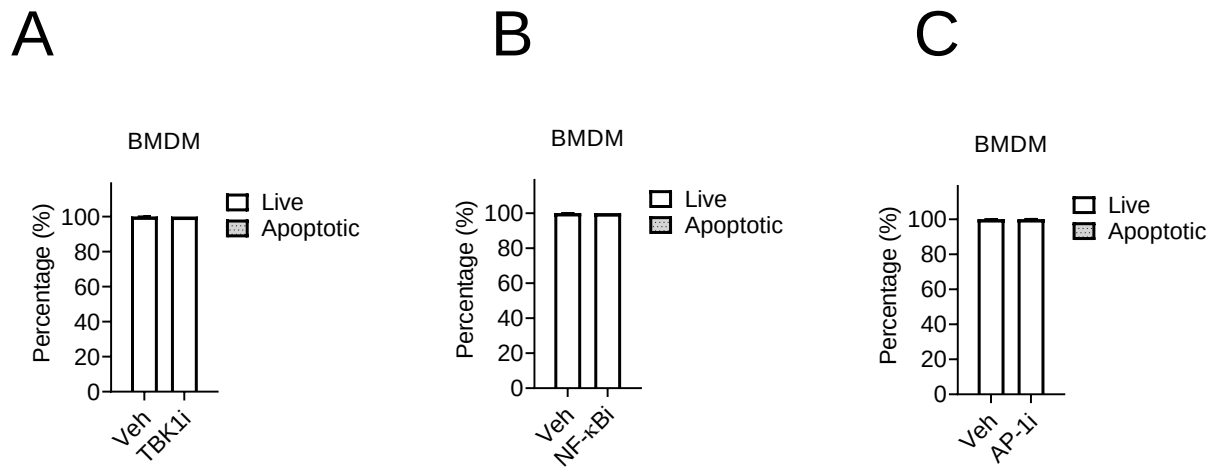

Figure S3. Pharmacological inhibitors do not increase apoptosis in BMDMs. Caspase 3/7 cleavage in BMDM cells treated with 1 µM (**A**) BX795 (TBK1i), (**B**) TPCA-1 (NF-κBi), or (**C**) SP600125 (AP-1i) for 24 h as compared to DMSO. The results are presented as percentage of live (white) or apoptotic (grey) cells from two independent experiments with error bars denoting range.

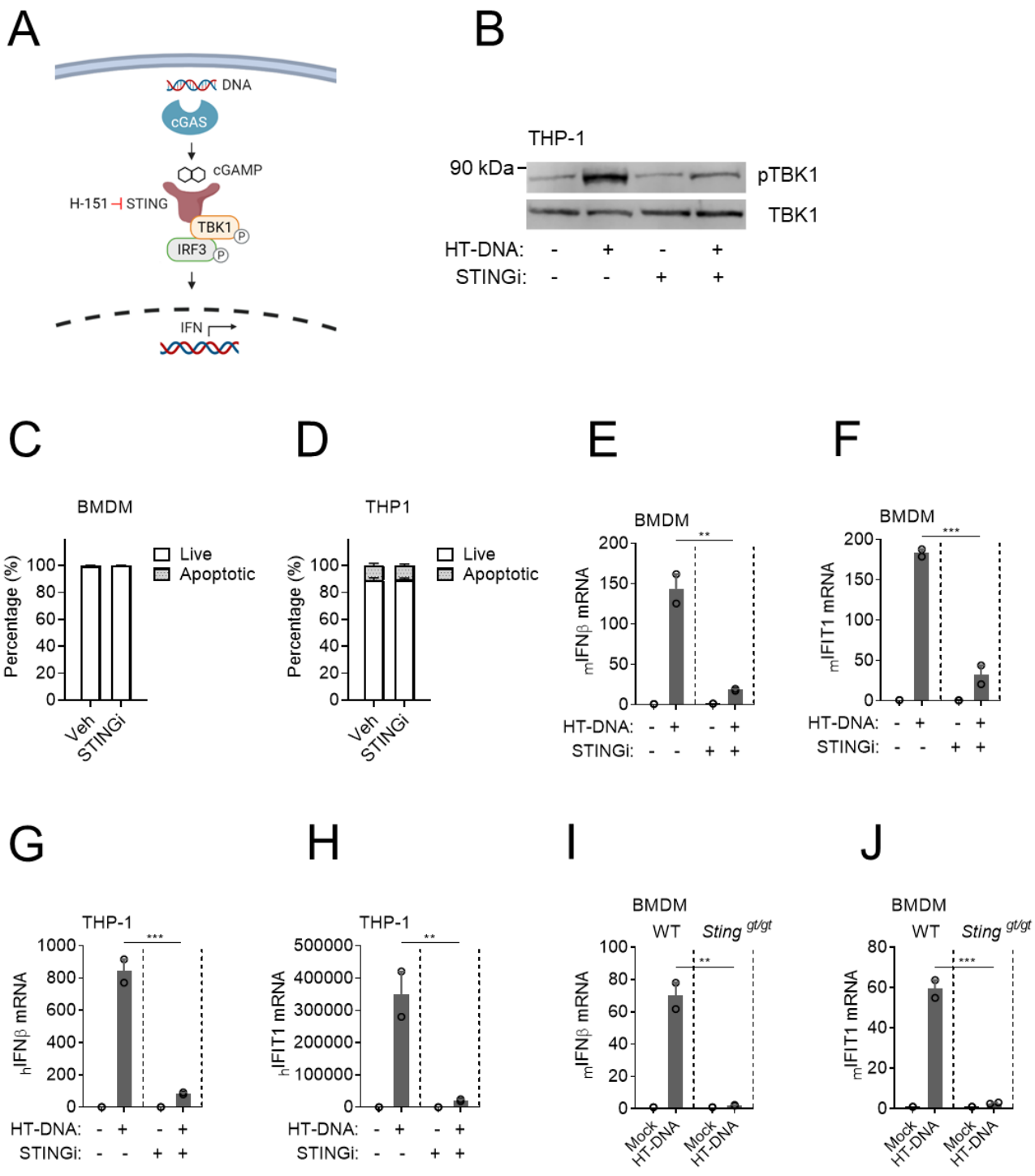

Figure S4. Inhibition of STING suppresses eribulin-mediated interferon β expression. (**A**) GAS-STING signaling pathway and pharmacological inhibition with H-151. (**B**) Immunoblot analysis

of phosphorylated TBK1 (pTBK1) and total TBK1 expression in THP-1 cells pretreated with 1 μM of the H-151 inhibitor for 4 h followed by transfection with HT-DNA for 24 h. (**C**, **D**) Caspase 3/7 cleavage in (**C**) BMDM and (**D**) THP-1 cells treated with DMSO (Veh) or 1 μM H-151 (STINGi) for 24 h. The results are presented as percentage of live (white) or apoptotic (gray) cells from two independent experiments with error bars denoting range. (**E**) Mouse IFNβ and (**F**) IFIT1 mRNA and human (**G**) IFNβ and (**H**) IFIT1 mRNA from BMDM and THP-1 cells respectively that have been pretreated with 1 μM of the H-151 STING inhibitor for 4 h and then transfected with 1 μg of herring testis DNA (HT-DNA) for 24 h with the inhibitor still present. Mouse IFNβ (**I**) and IFIT1 (**J**) mRNA in wild type or *Sting* $^{gt/gt}$ BMDMs transfected with 1 μg of herring testis DNA (HT-DNA) for 24 h. Quantitative RT-PCR data are shown as individual points from two independent biological replicates with error bars denoting range. Significance was determined by 2-way ANOVA (sting * HT-DNA) with Tukey's posthoc test. **$p < 0.01$, ***$p < 0.001$.

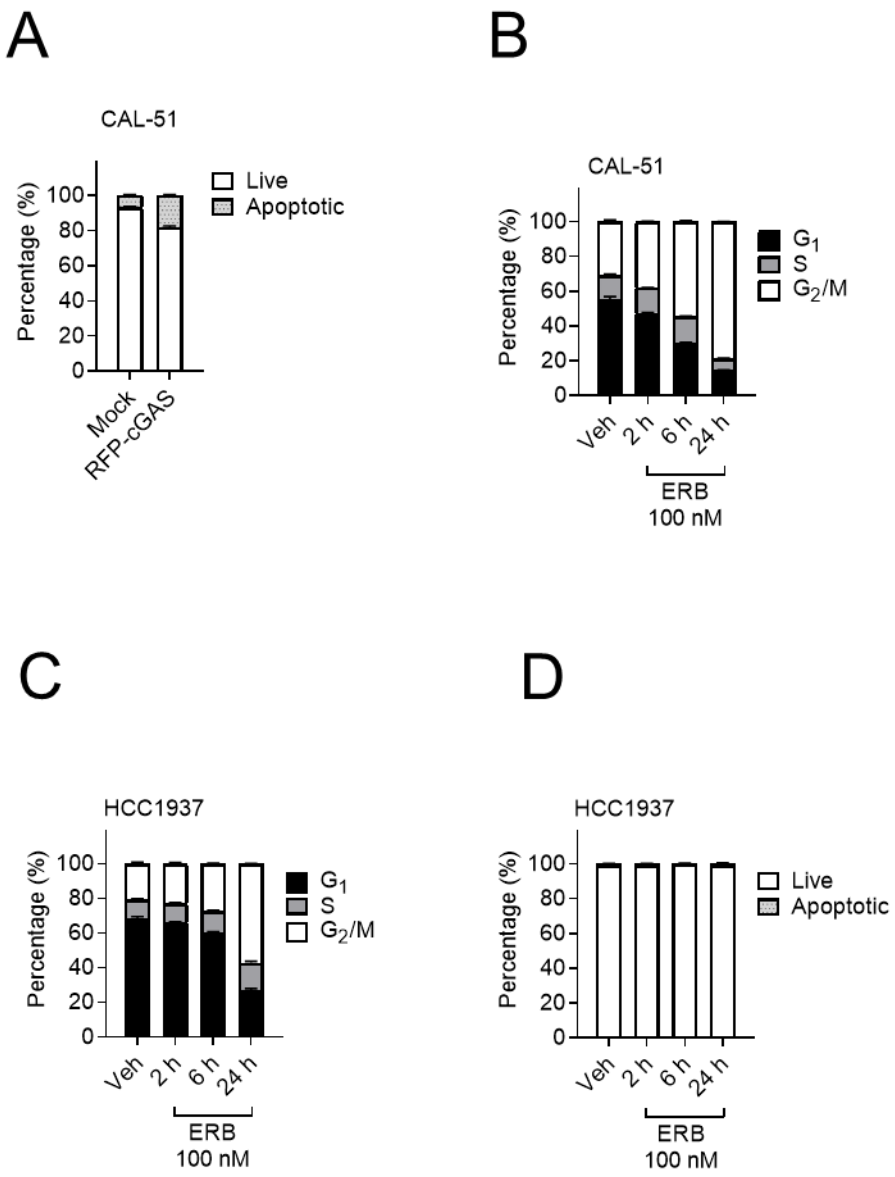

Figure S5. Eribulin-mediated expression of interferon stimulated genes in CAL-51 cells is dependent on the DNA sensor cGAS. (**A**) Caspase 3/7 cleavage in CAL-51 cells transfected with RFP-cGAS compared to mock transfected cells as percentage of live (white) or apoptotic (grey) cells from two independent experiments with error bars denoting range. (**B**, **C**) Cell cycle distribution of CAL-51 (**B**) or HCC1937 (**C**) cells treated with 100 nM eribulin for 2, 6, or 24 h as compared to DMSO as percentage of cells in G_1 (black), S (gray), or G_2/M (white) from two

independent experiments with error bars denoting range. (**D**) Caspase 3/7 cleavage of HCC1937 cells treated 100 nM eribulin or DMSO for 2, 6 or 24 h as percentage of live (white) or apoptotic (grey) cells from two independent experiments with errors denoting range.

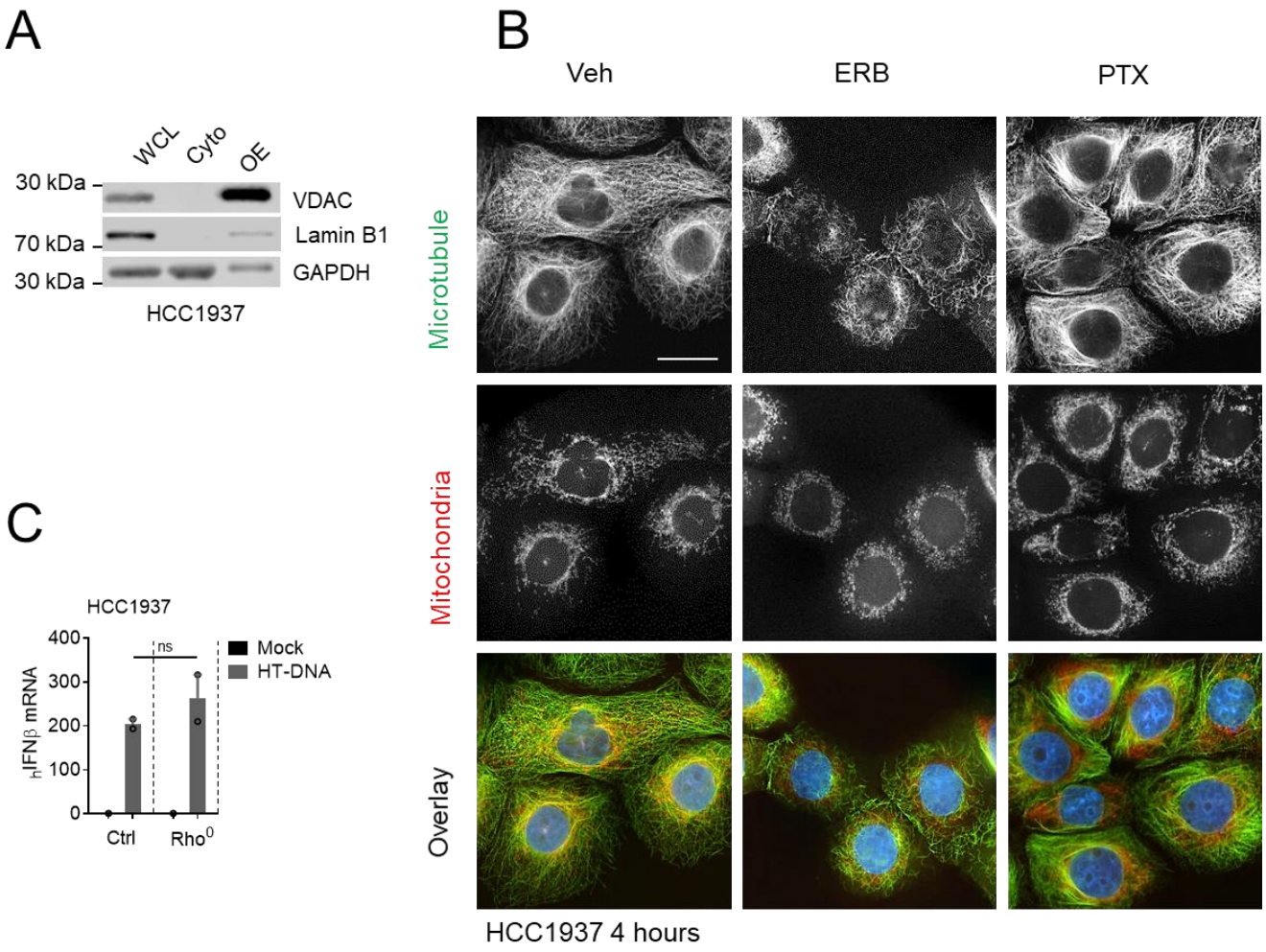

Figure S6. DNA sensing by the cGAS-STING pathway is retained in HCC1937 Rho^0 cells. (**A**) Immunoblot analysis of the human cytoplasmic marker GAPDH, the mitochondrial marker VDAC and the nuclear marker lamin B1 in HCC1937 whole cell lysate (WCL), cytoplasmic fraction (Cyto) and organelle enriched (OE) fraction generated by differential centrifugation. (**B**) Representative images of mitochondria (red), microtubules (green) and DNA (blue) in HCC1937 cells treated with 100 nM eribulin or paclitaxel for 4 h as compared to DMSO. Scale bar = 10 µm. (**C**) IFNβ mRNA in control and Rho^0 HCC1937 cells transfected with 1 µg of herring testis DNA for 24 h as compared to mock transfected cells. Individual points are shown and error bars

indicate the range from two independent experiments. Significance was determined by 2-way ANOVA (Rho * HT-DNA) with Tukey's posthoc test. ns – not significant

Table S1. **DNA oligonucleotides used in this study**.

All oligonucleotides were purchased from Sigma-Aldrich and validated by performing a Primer-Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi).

Gene name	**Forward and reverse oligonucleotide sequence (5' → 3') used in qRT-PCR analyses**
Human TNFα	CCAGGGCTCCAGGCGGTGCTTGTTC AACATGGGCTACAGGCTTGTCACTC
Human IL-6	AGGAGAAGATTCCAAAGATGTAGC CTCTTGTTACATGTCTCCTTTCTC
Human TGF-β	CCCAGCATCTGCAAAGCTC GTCAATGTACAGCTGCCGCA
Human IFNβ1	CAAGTGTCTCCTCCAAATTGCTCTC TCTCCTCAGGGATGTCAAAGTTCAT
Human IFNα4	CAGCCTGGGTAATAGGAGGGCCTTG AGGACAGAGATGGCTTGAGCCTTCT
Human IFNγ	CTAATTATTCGGTAACTGACTTGA ACAGTTCAGCCATCACTTGGA
Human CCL2	GAGAGGCTGAGACTAACCCAGA ATCACAGCTTCTTTGGGACACT
Human CCL5	GCTGTCATCCTCATTGCTACTG TGGTGTAGAAATACTCCTTGATGTG

Human CXCL8	TCTTGGCAGCCTTCCTGATTTCTGC ATAATTTCTGTGTTGGCGCAGTGTG
Human CXCL10	GCATTAGTAATCAACCTGTTAATCC TCCTTGCTAACTGCTTTCAGTAAAT
Human CXCL12	TGAGAGCTCGCTTTGAGTGA CACCAGGACCTTCTGTGGAT
Human IFIT1	CTGCCTATCGCCTGGATGGCTTTAA CTGTGAGGACATGTTGGCTAGAGCT
Human OAS1	CCAAGCTCAAGAGCCTCATC GAGCTCCAGGGCATACTGAG
Human IFITm3	CATTCGCCTACTCCGTGAAG ATGAGGATGCCCAGAATCAG
Human ISG15	GCGAACTCATCTTTGCCAGT AGCATCTTCACCGTCAGGTC
Human GAPDH	GCAAATTCCATGGCACCGT TCGCCCCACTTGATTTTGG
Human ACTb	CATGTACGTTGCTATCCAGGCT CTCCTTAATGTCACGCACGATT
Human HPRT	GAAAAGGACCCCACGAAGTGT AGTCAAGGGCATATCCTACAACAAA
Human PGK1	TGGACGTTAAAGGGAAGCGG GCTCATAAGGACTACCGACTTGG

Human RPS18	ATCACCATTATGCAGAATCCACG GACCTGGCTGTATTTTCCATCC
Human TBP	CCACTCACAGACTCTCACAAC CTGCGGTACAATCCCAGAACT
Human IFNAR1	ATTTACACCATTTCGCAAAGCTCAG TCCAAAGCCCACATAACACTATCTT
Human IFNAR2	ACCACTCCATTGTACCAACTCACT TGTGCTTCTCCACTCATCTGTGA
Human mtCOX-1	ATGACCCACCAATCACATGC ATCACATGGCTAGGCCGGAG
Human mtATP6	CCAAATATCTCATCAACAACCGACT AATGAGTGAGGCAGGAGTCCGAGGA
Human mtATP8	AATATTAAACACAAACTACCACCTACC TGGTTCTCAGGGTTTGTTATA
Human mtND1	CATCACCCTCTACATCACCGCCCCG TGAGTTTGATGCTCACCCTGATCAG
Human mtND4	TCCCTACAAATCTCCTTAATTATAA GAAGGGAGCCTACTAGGGTGTAGAA
Human mtND6	ACTCTTTCACCCACAGCACCAATCC TATTCTGAATTTTGGGGGAGGTTAT
Mouse Gapdh	TTCACCACCATGGAGAAGGC GGCATGGACTGTGGTCATGA

Mouse Ifnβ	CTGCGTTCCTGCTGTGCTTCTCCA TTCTCCGTCATCTCCATAGGGATC
Mouse Ifit1	TTCACATGGAAGCTGCTATTTGAAA TGCTCAGCTGCTCGCTCTGGATCAA
Mouse mt-Cox1	GCCCCAGATATAGCATTCCC GTTCATCCTGTTCCTGCTCC