**SUPPLEMENTAL INFORMATION**

# Altered m$^6$A Modification of Specific Cellular Transcripts Affects *Flaviviridae* Infection

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**Figure S1: Related to Figure 1.**

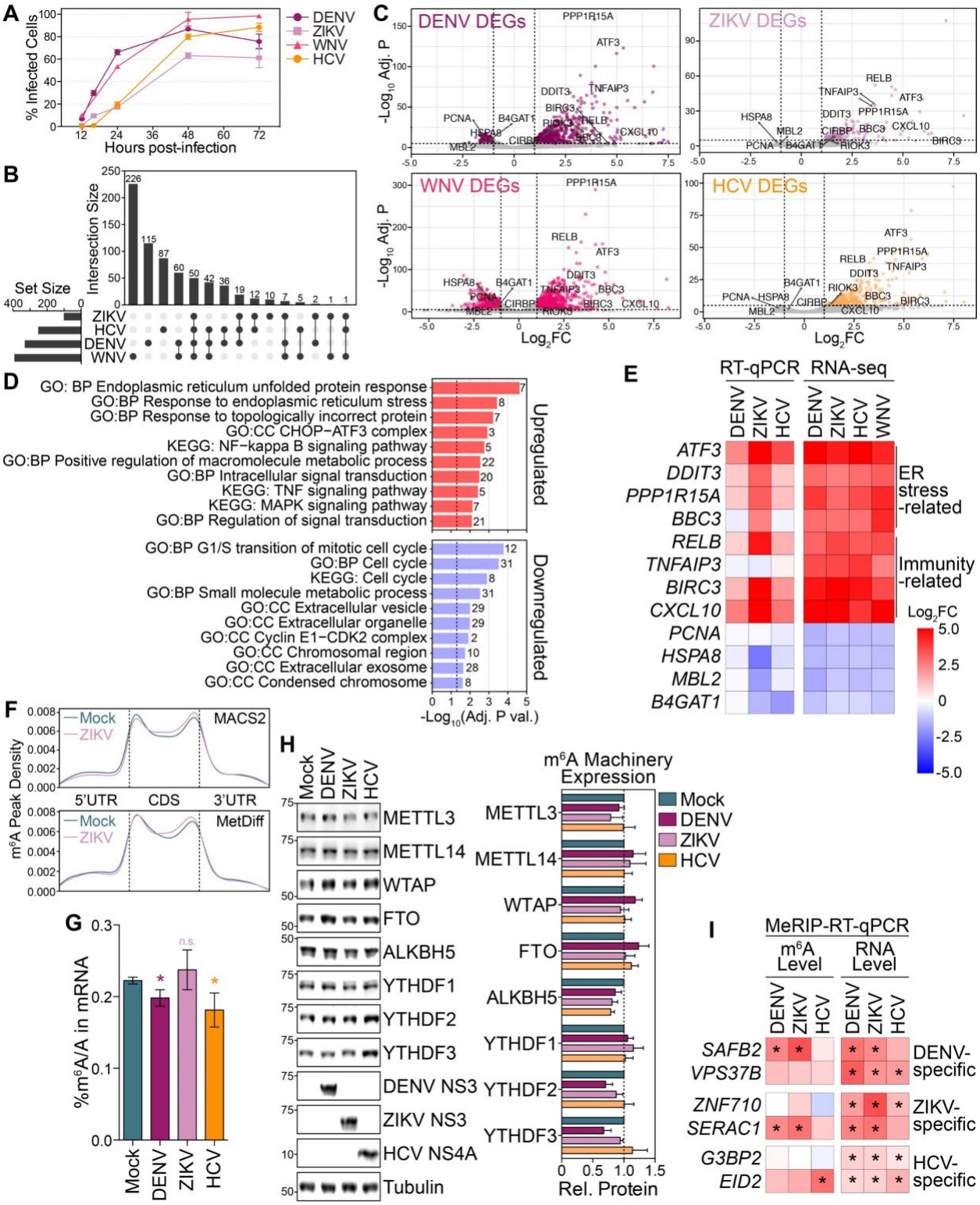

**Figure S1: Related to Figure 1.**

**(A)** Percent of Huh7 cells infected with DENV, ZIKV, WNV, and HCV at the indicated hours post-infection as determined by immunostaining of viral antigen and nuclei. >5000 cells counted for each condition.
**(B)** UpSet plot showing the number of differentially expressed genes (DEGs, $|Log_2FC| \geq 2$, adjusted $p < 0.05$) and those in common with DENV, ZIKV, WNV, and HCV infection as determined by RNA-seq analysis of input fractions from MeRIP-seq using DESeq2.
**(C)** Volcano plots of DEGs following infection by the indicated virus. Colored dots represent significant DEGs ($|Log_2FC| \geq 2$, adjusted $p < 0.05$). Example genes validated by RT-qPCR in (E) are named.
**(D)** Pathway enrichment (using gProfiler) for genes upregulated ($Log_2FC \geq 2$) by DENV, ZIKV, WNV, and HCV infection (top; red color) or downregulated ($Log_2FC \leq -0.5$) by DENV, WNV, and HCV infection (bottom; blue color). Few genes were significantly downregulated with ZIKV infection (see **C**), therefore we took the intersect of genes downregulated with the remaining viruses for this analysis. Numbers refer to the number of genes in each category.
**(E)** Heatmap of $Log_2FC$ in RNA levels for RT-qPCR validation at 48 hpi (left) of DEGs co-regulated by infection in Huh7 cells, as determined by RNA-seq (right). Values in RT-qPCR heatmaps were normalized to *GAPDH* and represent the mean of 3 biological replicates.
**(F)** Metagene plot of "methylated" DRAC motifs across transcripts in mock- and ZIKV-infected cells in 293T cells reanalyzed from Lichinchi et al. 2019b using the MACS2 and MeTDiff peak callers. DRAC motifs were considered methylated if they fell under a peak detected in at least two replicates.
**(G)** LC-MS/MS quantification of $m_6A$/A ratios in purified mRNA from mock- and virus-infected (48 hpi) Huh7 cells. Values are the mean ± SEM of three experiments. * $p < 0.05$, by unpaired Student's t test. n.s. = not significant.
**(H)** (Left) Representative immunoblot of protein expression of the cellular $m_6A$ machinery in mock- and virus-infected (48 hpi) Huh7 cells. (Right) Quantification of immunoblot analysis of the cellular $m_6A$ machinery relative to tubulin. Values are the mean ± SEM of 4 biological replicates.
**(I)** (Left) MeRIP-RT-qPCR analysis of relative $m_6A$ level of transcripts identified as having altered $m_6A$ modification with the indicated virus in DENV, ZIKV, and HCV-infected (48 hpi) Huh7 cells. (Right) RNA expression of these transcripts relative to *GAPDH* (right).
Values in heatmaps are the mean of 3 independent experiments. * $p < 0.05$, by unpaired Student's t test.

**Figure S2: Related to Figure 2.**

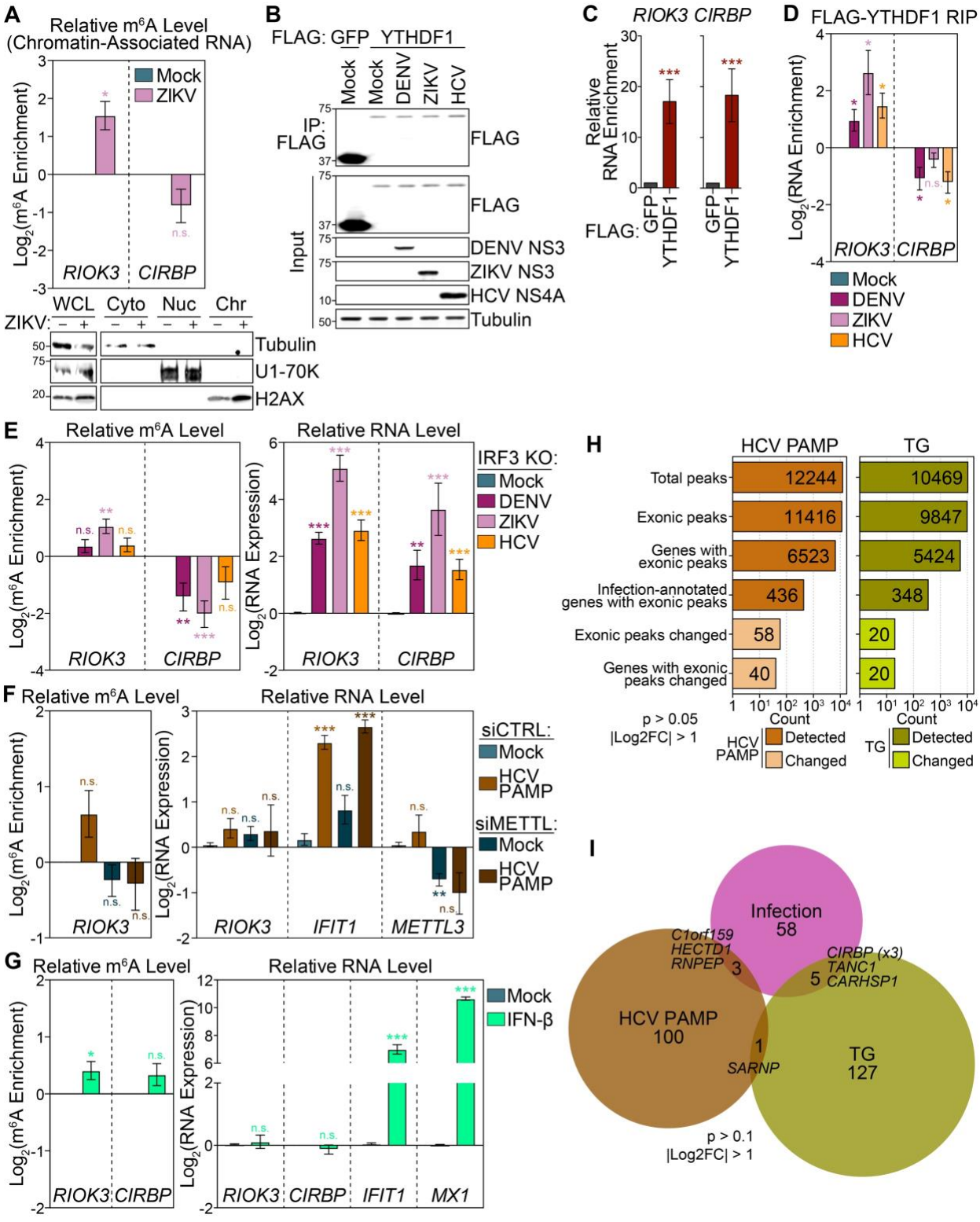

**Figure S2. Related to Figure 2.**

**(A)** (Left) MeRIP-RT-qPCR analysis of relative $m^6A$ level of chromatin-associated *RIOK3* and *CIRBP* RNA in mock- and ZIKV-infected (48 hpi) Huh7 cells. (Right) Immunoblot analysis of whole cell lysate (WCL), cytoplasmic (Cyto), nuclear (Nuc), and chromatin (Chr) fractions.
**(B)** Representative immunoblot of FLAG-immunoprecipitated (IP) and input fractions used for RT-qPCR analysis in (C) and (D).
**(C)** RT-qPCR analysis of enrichment of *RIOK3* and *CIRBP* RNA by immunoprecipitation of FLAG-YTHDF1 compared to FLAG-GFP in uninfected Huh7 cells stably expressing these constructs.
**(D)** RT-qPCR analysis of enrichment of *RIOK3* and *CIRBP* RNA by immunoprecipitation of FLAG-YTHDF1 in mock- or virus-infected (48 hpi) Huh7 cells stably expressing FLAG-YTHDF1. Relative enrichment was calculated as the percent of input for each sample relative to that in mock-infected cells.
**(E)** (Left) MeRIP-RT-qPCR analysis of relative $m^6A$ level of *RIOK3* and *CIRBP* in mock- and virus-infected (48 hpi) IRF3 KO Huh7 cells. (Right) RNA expression of *RIOK3* and *CIRBP* relative to *HPRT1* in mock- and virus-infected (48 hpi) IRF3 KO Huh7 cells.
**(F)** (Left) MeRIP-RT-qPCR analysis of relative $m^6A$ level of *RIOK3* in mock- and HCV PAMP-transfected (8 h) Huh7 cells treated with non-targeting control (CTRL) siRNA, or siRNAs targeting both *METTL3* and *METTL14*. (Right) RNA expression of *RIOK3*, a transcript known to be upregulated by HCV PAMP (*IFIT1),* and *METTL3*, relative to *HPRT1*.
**(G)** (Left) MeRIP-RT-qPCR analysis of relative $m^6A$ level of *RIOK3* and *CIRBP* in mock- and IFN-β-treated (24 h) Huh7 cells. (Right) RNA expression of *RIOK3*, *CIRBP*, and two transcripts known to be upregulated by IFN-β: *IFIT1* and *MX1,* relative to *HPRT1*.
**(H)** (Left) The number of $m^6A$ peaks and genes with $m^6A$ peaks detected in ≥ 2 samples of mock- or HCV PAMP-transfected (8 h; dark brown; MACS2 q-value < 0.05) Huh7 cells and peaks that change during HCV PAMP treatment (light brown, |peak – gene $Log_2FC$| ≥ 1, adjusted $p < 0.05$). (Right) The number of $m^6A$ peaks and genes with $m^6A$ peaks detected in ≥ 2 samples of mock- or thapsigargin (TG)-transfected (16 h; dark green; MACS2 q-value < 0.05) Huh7 cells and peaks that change during TG treatment (light green, |peak – gene $Log_2FC$| ≥ 1, adjusted $p < 0.05$). "Infection-annotated genes:" genes with known annotations for the Reactome Pathways 'Infectious Disease', 'Unfolded Protein Response', 'Interferon Signaling', or 'Innate Immune Signaling' in the database used by fgsea.
**(I)** Venn diagram of $m^6A$ peaks from the infection data (N = 31467, Figure 1B) that showed differences in IP enrichment with HCV PAMP or TG treatment. The 58 significantly changed exonic peaks with infection ($p < 0.05$, $|Log_2FC| > 1$, mean read count ≥ 10) are compared to the same regions in data from HCV PAMP- or TG-treated samples ($p < 0.1$, $|Log_2FC| > 1$, mean read count ≥ 10). All regions that met our thresholds for consideration in the HCV PAMP and TG data showed the same direction of change as with infection. *CSKND1* and *MST1,* which were in the 58 infection-altered peaks, both also showed $m^6A$ changes with TG treatment with a $p < 0.1$ but were slightly below the $Log_2FC$ threshold, and so are not part of the 5 overlap, while the same was true of *DDX39B* with HCV PAMP treatment. We note that the reproducibility of MeRIP-seq detection is low between different experiments (McIntyre et al., 2019); and for all the changes, the $m^6A$ regions do not necessarily correspond to the peaks summarized in (H).
Values are the mean ± SEM of 3 (A and F), 2 (C and D), 6 (E), and 5 (G) biological replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by unpaired Student's t test. n.s. = not significant.

**Figure S3: Related to Figure 3.**

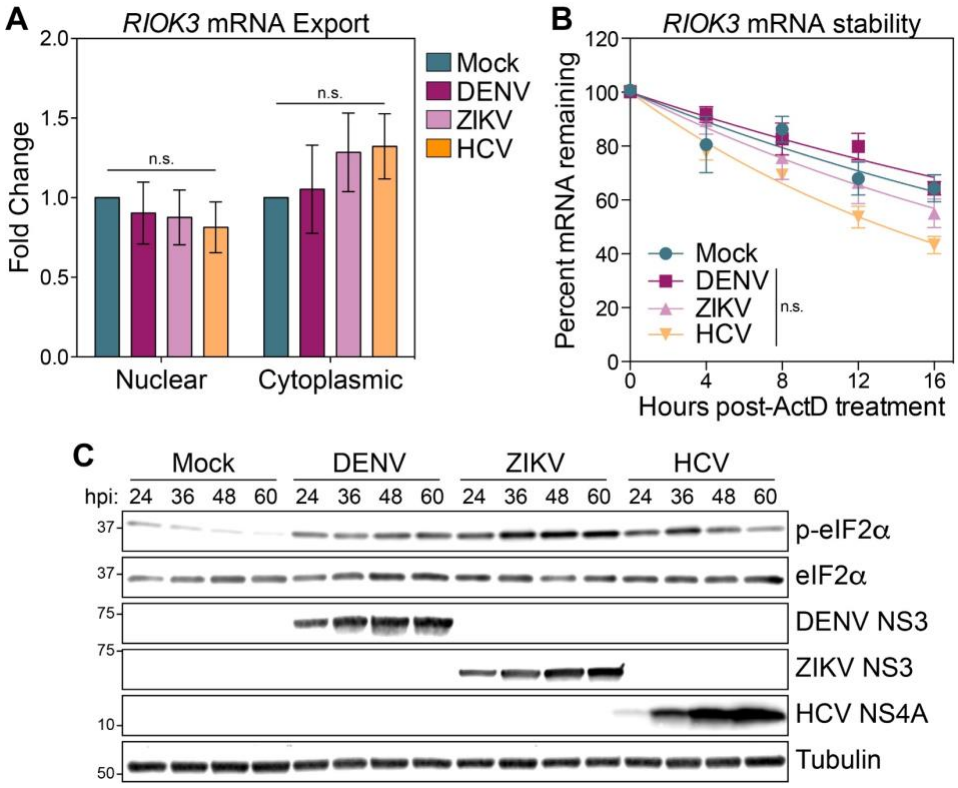


**Figure S3: Related to Figure 3.**

**(A)** RT-qPCR quantification of relative *RIOK3* RNA in nuclear and cytoplasmic fractions in mock- and virus-infected (48 hpi) Huh7 cells. n.s. = not significant by Student's t test.
**(B)** Measurement of *RIOK3* RNA in mock- and virus-infected Huh7 cells. At 36 hpi, cell culture media was replaced with media containing actinomycin D (ActD). RNA was harvested cells at the indicated times post-treatment and subjected to RT-qPCR to determine remaining relative RNA levels. n.s. = not significant by 2-way ANOVA.
**(C)** Immunoblot analysis of p-eIF2α and eIF2α levels in mock- and virus-infected Huh7 cells at the indicated time points. Data are representative of 3 biological replicates.
Values are the mean ± SEM from 4 (A) or 3 (B) biological replicates.

**Figure S4: Related to Figure 4.**

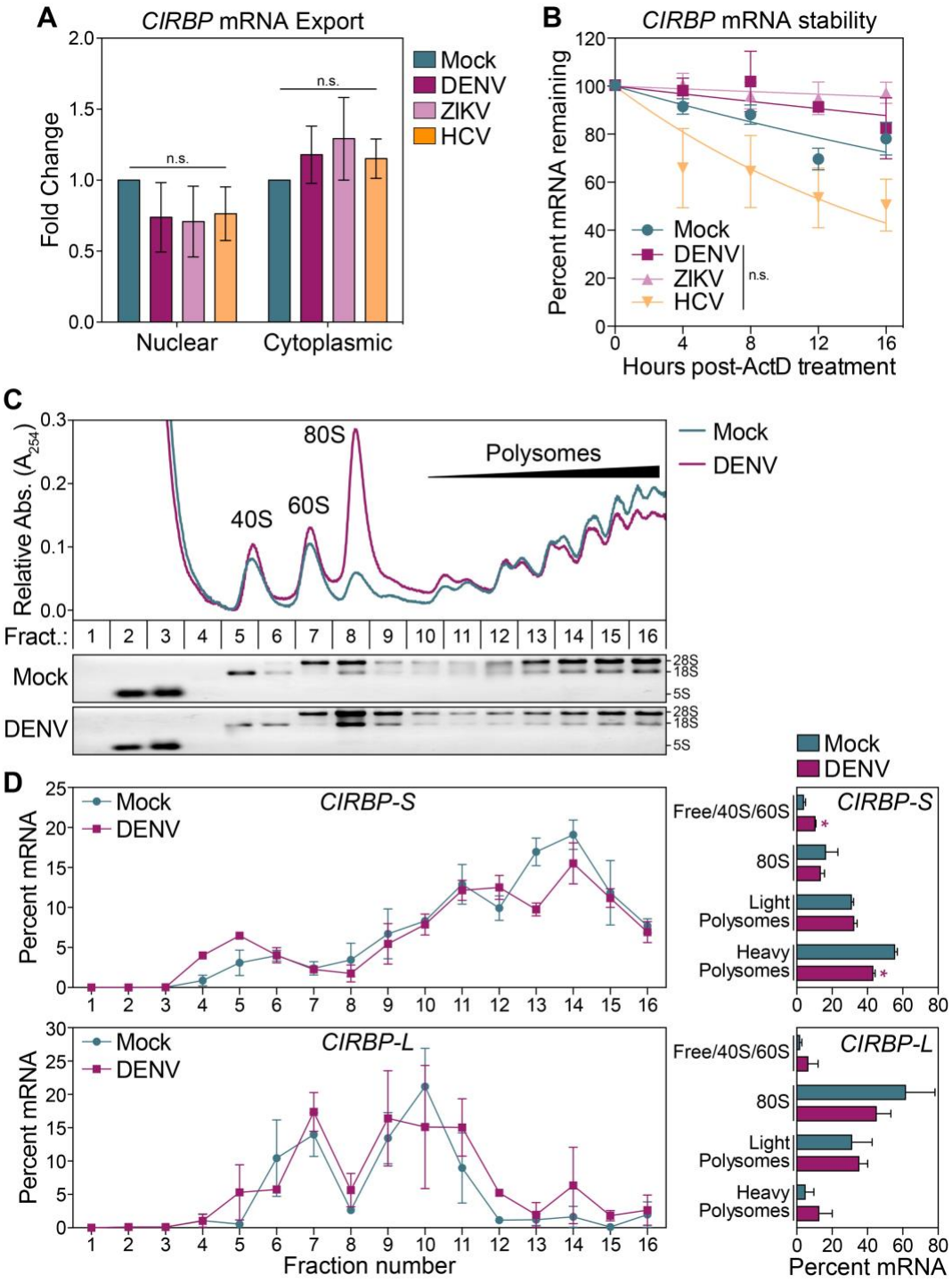

**Figure S4: Related to Figure 4.**

**(A)** RT-qPCR quantification of relative *CIRBP* RNA in nuclear and cytoplasmic fractions in mock- and virus-infected (48 hpi) Huh7 cells. n.s. = not significant by Student's t test.
**(B)** Measurement of *CIRBP* RNA in mock- and virus-infected Huh7 cells. At 36 hpi, cell culture media was replaced with media containing ActD. RNA was harvested cells at the indicated times post-treatment and subjected to RT-qPCR to determine remaining relative RNA levels. n.s. = not significant by 2-way ANOVA.
**(C)** (Top) Relative absorbance values of fractions 1-16 retrieved from ultracentrifugation of mock- and DENV-infected (48 hpi) Huh7 cell extracts over 15-50% sucrose gradients. (Bottom) RNA from each fraction separated on an agarose gel and visualized with ethidium bromide. rRNA bands are labeled. Data are representative of 2 biological replicates.
**(D)** (Left) RT-qPCR analysis of the indicated *CIRBP* isoforms in each fraction from (A). (Right) Percent of mRNA in each set of fractions. Free = fractions 1-4; 40/60/80S = fractions 5-9; Light polysomes = fractions 10-12; Heavy polysomes = fractions 13-16.
Values are the mean ± SEM from 4 (A), 3 (B), or 2 (D) biological replicates. * $p < 0.05$ by Student's t test. n.s. = not significant.

**Figure S5: Related to Figure 5.**

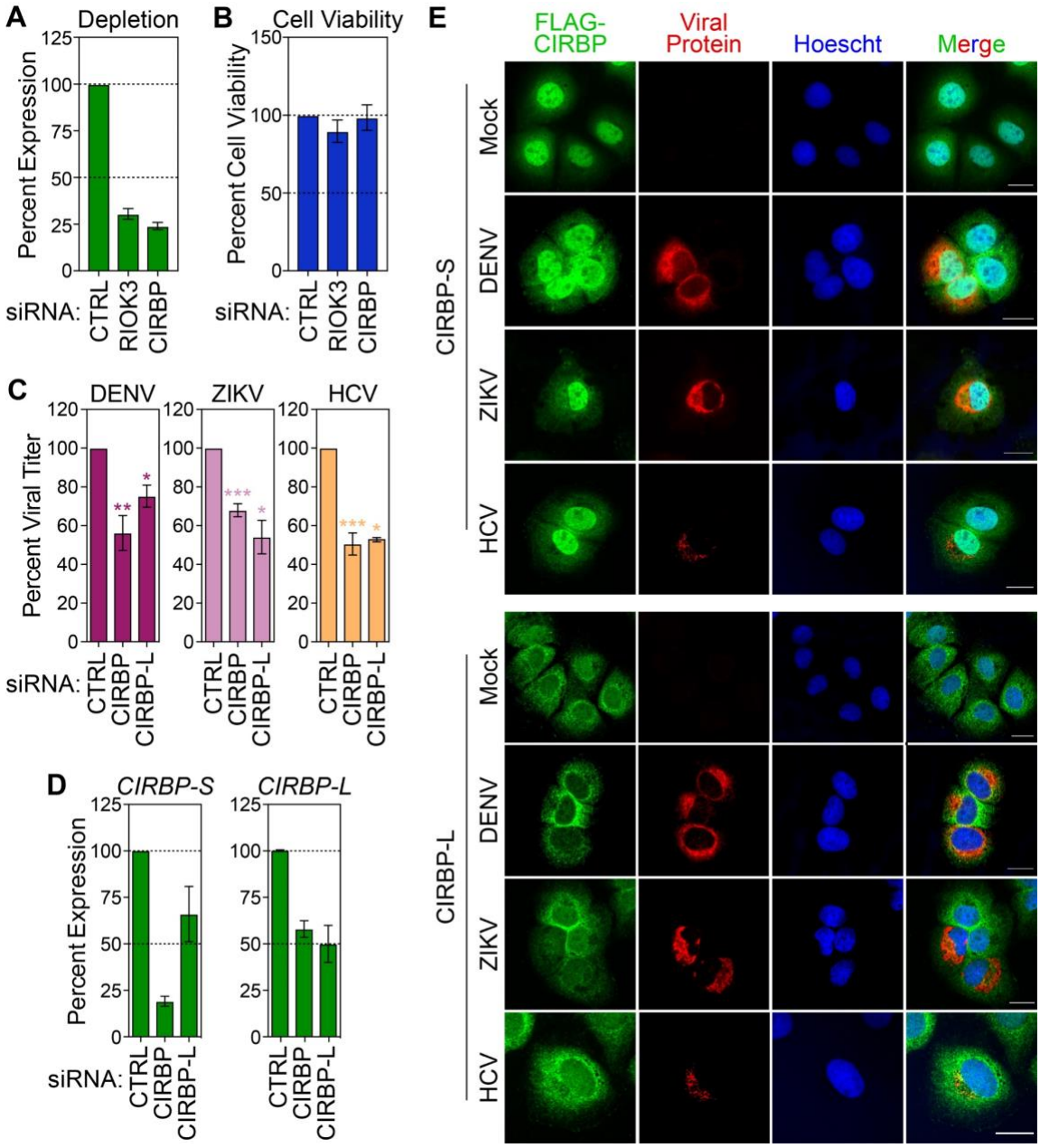

**Figure S5: Related to Figure 5.**

**(A)** (Top) RT-qPCR of the indicated transcripts relative to *GAPDH* at 72 hours post-transfection of the respective siRNAs compared to non-targeting control (siCTRL).
**(B)** Cell viability measured after siRNA depletion of indicated transcripts at 72 hours post-transfection of the respective siRNAs relative to that of siCTRL. Expression or cell viability in cells treated with siCTRL was set at 100%.
**(C)** Focus-forming assay of supernatants harvested from DENV, ZIKV, or HCV-infected (72 hpi) Huh7 cells treated with non-targeting control (CTRL) siRNA or siRNAs targeting both short and long (indicated by CIRPB), or only the large isoform of *CIRBP* (indicated by CIRBP-L).
**(D)** RT-qPCR of the short (*CIRBP-S*) or long (*CIRBP-L*) *CIRBP* transcripts relative to *GAPDH* at 72 hours post-transfection of siRNA compared to non-targeting control or isoform-specific siRNA used in (C).
**(E)** Immunofluorescence micrographs of mock- and virus-infected (48 hpi) Huh7 cells stably expressing FLAG-CIRBP-S or FLAG-CIRBP-L stained with antibodies against FLAG (green) or viral proteins (E protein for DENV and ZIKV, NS5A for HCV; red). Nuclei were stained with Hoechst (blue). Scale bar = 20 μM.
Values are the mean ± SEM of 4 (A), or 3 (B, C, and D) experiments. All viral infections for experiments in this figure were performed at a multiplicity of infection of 0.2. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by unpaired Student's t test. n.s. = not significant.

**Figure S6: Related to Figure 6.**

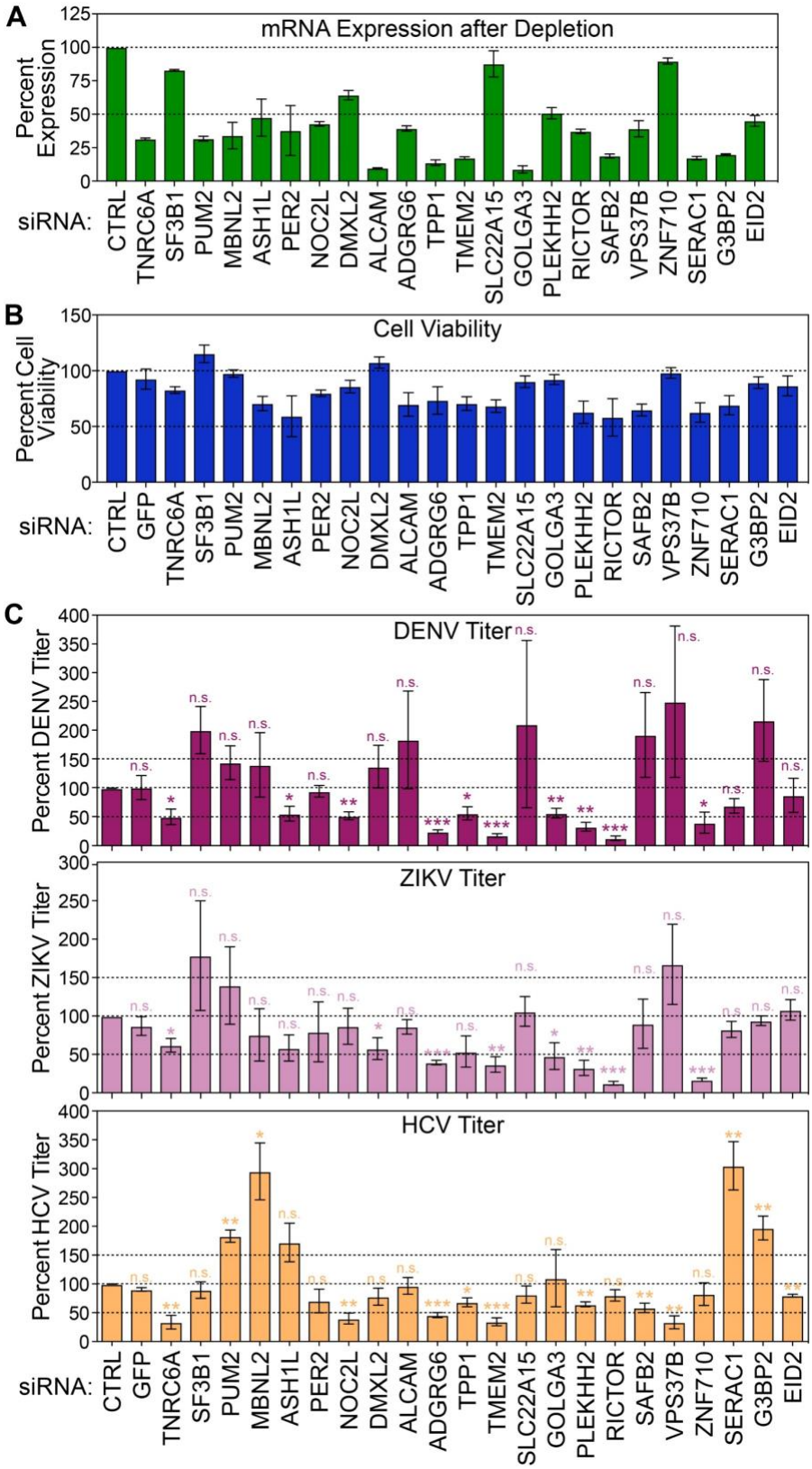

**Figure S6: Related to Figure 6.**

**(A)** RT-qPCR of the indicated transcripts relative to *GAPDH* at 72 hours post-transfection of the respective siRNA compared to non-targeting control (CTRL) siRNA.
**(B)** Cell viability measured after siRNA depletion of the indicated transcripts at 72 hours post-transfection of the respective siRNA relative to that of siCTRL.
**(C)** FFA of supernatants harvested at 48 hpi from Huh7 cells treated with the indicated siRNAs and infected with DENV (top), ZIKV (middle), or HCV (bottom). Viral titer in siCTRL treated cells was set at 100%.
All viral infections for experiments in this figure were performed at a multiplicity of infection of 0.2. All values are the mean ± SEM of 3 biological replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by unpaired Student's t test. n.s. = not significant.