Figure S1
Related to Figure 1

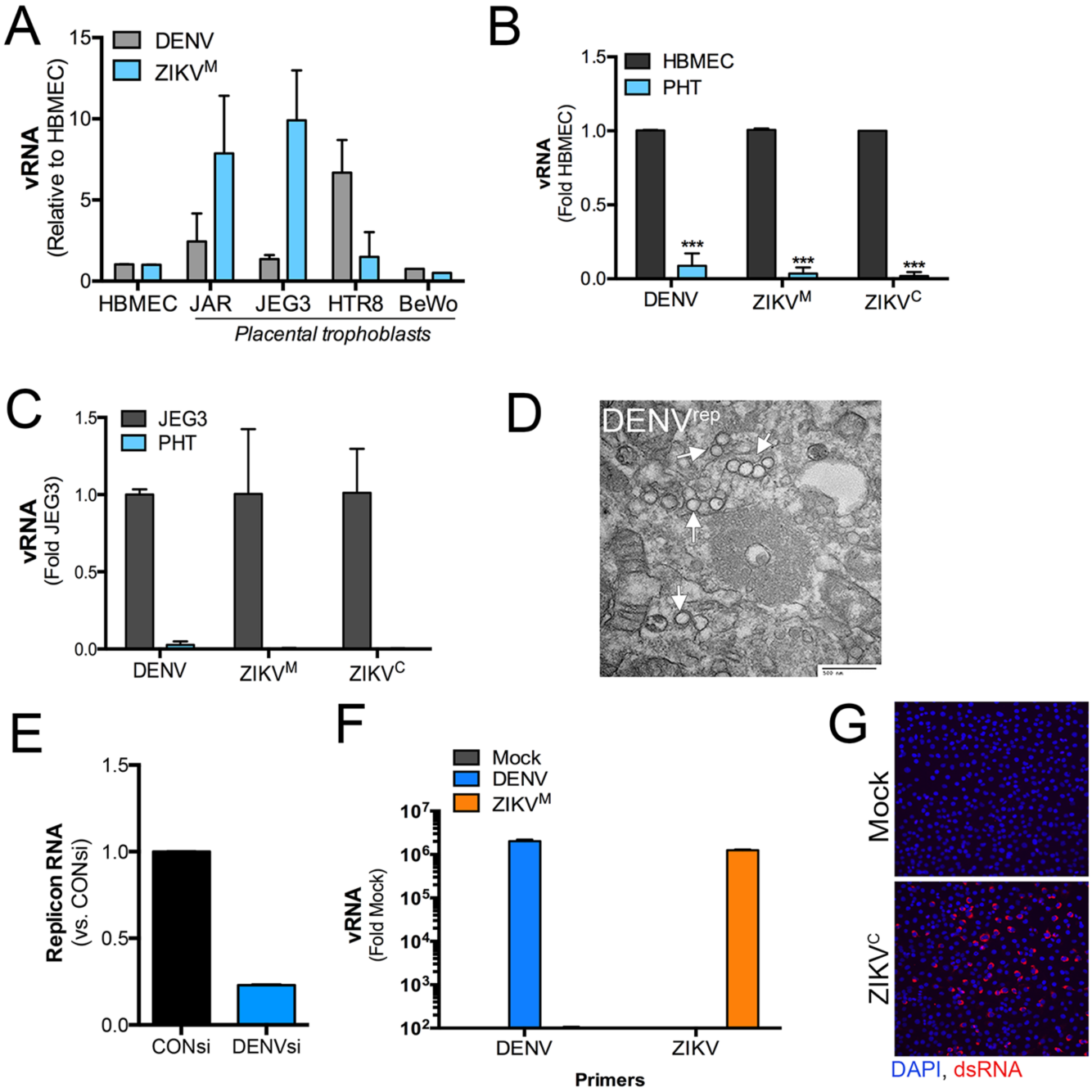


Figure S2
Related to Figure 2

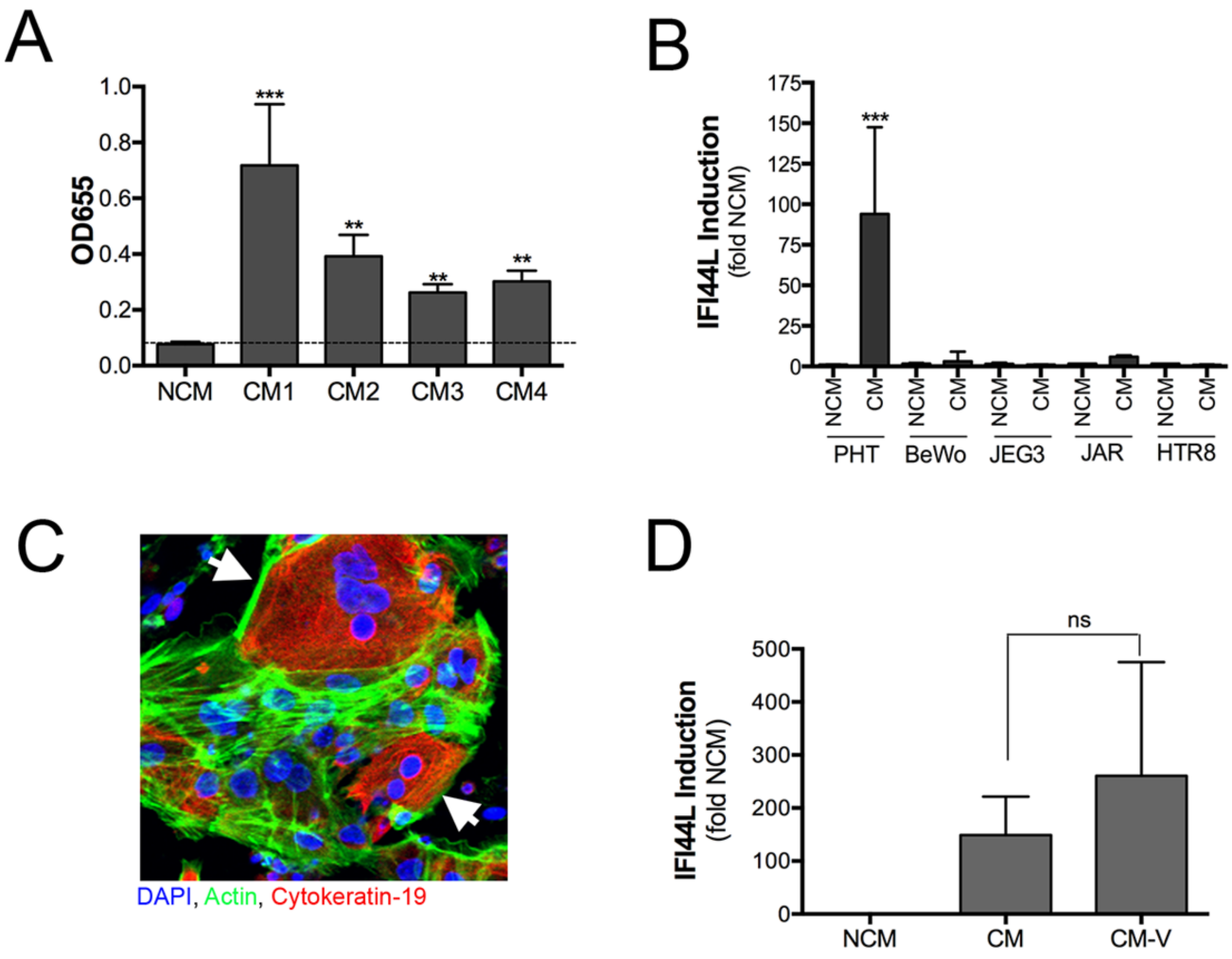
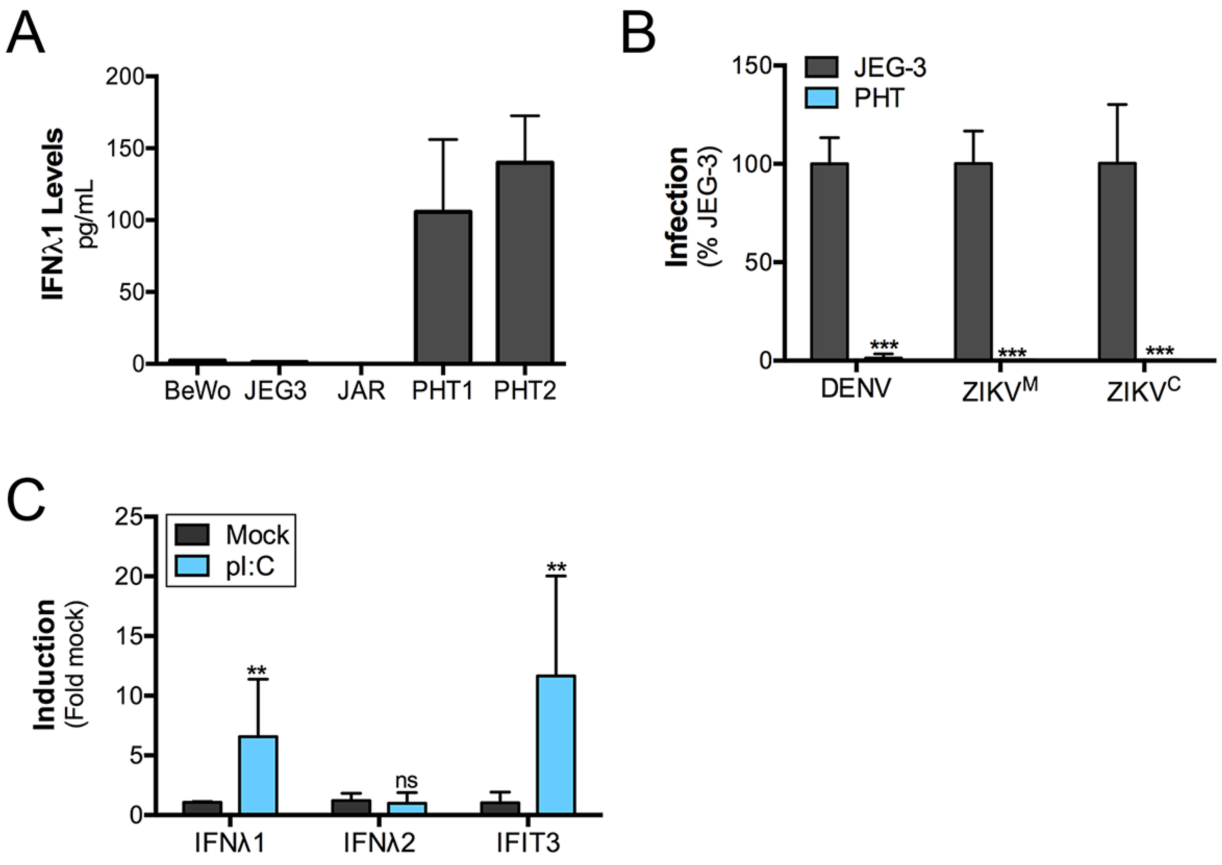


Figure S3
Related to Figure 3



Supplemental Data Legends

Figure S1. Related to Figure 1. **(A),** RT-qPCR for total vRNA in the indicated DENV or ZIKV^{M} infected trophoblast cells lines, or in HBMEC. Data are shown as a fold from HBMEC. **(B,C),** RT-qPCR for total vRNA in DENV, ZIKV^{M}, or ZIKV^{C} infected PHT cells, or in HBMEC (B) or JEG-3 (C) cells. Data are shown as a fold from HBMEC (B) or JEG-3 (C). **(D),** Transmission electron micrograph (TEM) from HeLa cells stably propagating a DENV subgenomic replicon. White arrows denote DENV-induced replication organelles. Scale, 500nm, at bottom right. **(E),** RT-qPCR for DENV vRNA in HeLa cells stably expressing a DENV replicon and transfected with a control siRNA (CONsi) or an anti-DENV siRNA (DENVsi). **(F),** RT-qPCR using DENV and ZIKV specific primers in HBMEC infected with DENV or ZIKV^{M}. Data are shown as a fold from mock-infected controls. **(G**), Fluorescence micrographs for dsRNA (using anti-dsRNA J2 antibody, in red) in HBMEC that were mock-infected (top) or infected with ZIKV^{C} (bottom) for 24 hrs. DAPI-stained nuclei are shown in blue. Data in (A-C) and (E-F) are shown as mean ± standard deviation.

Figure S2. Related to Figure 2. **(A),** SEAP levels in the cell culture medium of THP-1 ISG-Blue cells treated with NCM or four preparations of PHT CM using the QUANTI-Blue assay. Data are shown as OD655. **(B),** RT-qPCR for IFI44L in U2OS cells exposed to non-conditioned medium (NCM) or CM from the indicated trophoblast cell lines, or from PHT cells. Data are shown as a fold change from NCM matched control. Data are shown as mean ± standard deviation,*** $p<0.001$. **(C),** Confocal micrograph of PHT cells. DAPI-stained nuclei are shown in blue, actin is shown in green, and cytokeratin-19 is shown in red. White arrows denote multinucleated syncytiotrophoblasts. **(D),** The level of IFI44L induction from PHT CM depleted of exosomes as determined by RT-qPCR.

Figure S3. Related to Figure 3**. (A),** ELISA for IFNλ1 in the medium from the indicated trophoblast-derived cell lines and from two independent preparations of PHT cells. **(B),** RT-qPCR for total vRNA in DENV, $ZIKV^{M}$, or $ZIKV^{C}$ infected PHT cells, or in JEG-3 cells, which are matched to the data shown in Figure 3F. **(C),** RT-qPCR for IFNλ1, IFNλ2, and IFIT3 in mock-treated PHT cells or in cells incubated with 10μM pI:C ('floating'). Data are shown as mean ± standard deviation.

Table S1. Related to Figure 2. Expression of ISGs as determined by microarray analyses in 2fTGH (TGH) or U3A HT1080 cells treated with 100U of purified IFNβ (in grey) or PHT CM (in purple). Values shown are log2 fold changes from untreated control cells.

Table S2. Related to Figure 2. Expression of ISGs as determined by RNASeq analyses of two preparations of JEG-3 cells (in grey) and PHT cells (in blue). Shown are log2 fold changes and p values as determined by DeSeq2 differential expression analysis. Also shown are unique gene reads and RPKM.

Supplemental Experimental Procedures

Primer sequences

Primers for qPCR were as follows: GAPDH (5'- GAAGGTCGGAGTCAACGGATTT -3' and 5'-GAATTTGCCATGGGTGGAAT -3'); Actin (5'-ACTGGGACGACATGGAGAAAA-3' and 5'-GCCACACGCAGCTC-3'); IFI44L (5'-TGCAGAGAGGATGAGAATATC-3' and 5'-ACTAAAGTGGATGATTGCAG-3'); IFIT1 (5'-CAACCAAGCAAATGTGAGGA-3' and 5'-GGAGACTTGCCTGGTGAAAA-3'); IFNβ (5'-GAGCTACAACTTGCTTGGATTC-3' and 5'-CAAGCCTCCCATTCAATTGC-3'); IFNλ1 (5'-CGCCTTGGAAGAGTCACTCA-3' and 5'-GAAGCCTCAGGTCCCAATTC-3'); IFNλ2 (5'-ACATAGCCCAGTTCAAGTC-3' and 5'-GACTCTTCTAAGGCATCTTTG-3'); IFNAR1 (5'-CAGTTGAAAATGAACTACCTCC-3' and 5'-ACTTGAAAGGTCATGTTTGC-3'); IL28RA (5'-ATCCTCAGTTAACCTACACC-3' and 5'-CAGATACTCCACCACAAAAC-3'); OAS1 5'-ATAAAAGCAAACAGGTCTGG-3' and 5'-TCTGGCAAGAGATAGTCTTC-3'); ZIKV (5'-AGATGACTGCGTTGTGAAGC-3' and 5'-GAGCAGAACGGGACTTCTTC-3'); and DENV (5'-AGTTGTTAGTCTACGTGGACCGA-3' and 5'-CGCGTTTCAGCATATTGAAAG-3').

Differentiation assay

PHT differentiation was blocked by growing the cells in the presence of 1.5% dimethyl sulfoxide (DMSO) (Thirkill and Douglas, 1997). The levels of hCG in DMSO-exposed cells confirmed the attenuation of differentiation by DMSO. For BeWo cell differentiation, BeWo cells were exposed to 25 nM of forskolin for 24hr, and differentiation was confirmed by increased medium level of hCG. For EGF exposure, PHT medium was replaced 4hr post-plating and with medium containing 10ng/mL EGF (BD Biosciences), which was replenished with fresh medium containing EGF before medium was harvested at 48hr.

RNAi mediated silencing

HBMEC were reverse transfected with 25nM Mission siRNA Universal Negative Control (Sigma) or two pooled Silencer Select siRNAs targeting IL28RA (Ambion) using Dharmafect 1 (Dharmacon). At ~48hr post transfection, cells were exposed to PHT CM depleted of vesicles for 24hr followed by infection with $ZIKV^{C}$. At 24hr post-infection, RNA was isolated and infection analyzed by RT-qPCR.

Immunofluorescence and transmission electron microscopy

Cells cultured in chamber slides (LabTek, Nunc) were fixed in ice cold methanol, washed, incubated with primary antibody for 1h, followed by Alexa Fluor conjugated secondary antibody for 30 min (Figure S1G). Slides were mounted with VectaShield (Vector Laboratories) containing 4′,6-diamidino-2-phenylindole (DAPI). Images were captured on an Olympus IX83 inverted fluorescent microscope and analyzed using ImageJ.

For transmission electron microscopy, cells were fixed in 2.5% glutaraldehyde as previously described (Delorme-Axford et al., 2013). Micrographs were captured using a JEOL 1011 transmission electron microscope.

Neutralization assay

Conditioned medium diluted 4-fold, recombinant IFNβ (100 U/mL; PBL Source), or recombinant IFNλ1 and IFNλ2 (5ng/mL each; R&D Systems) was incubated with neutralizing antibodies against IFNβ (200ng/mL; R&D Systems), IFNλ1 and IFNλ2 (1μg/mL each; R&D Systems), or mouse IgG1 (1μg/mL; Sigma) for 1hr at room temperature then added to cells. After 24hr of exposure, the RNA was harvested and analyzed for induction of ISGs.

ELISA

Levels of IFNλ1, IFNλ2 (R&D Systems), and IFNβ (PBL Source) present in CM produced from individual PHT cell preparations were analyzed by ELISA, according to manufacturer protocol.

THP-1 cells expressing SEAP reporter

THP-1 cells stably expressing an interferon regulatory factor (IRF)-inducible SEAP reporter construct were obtained from Invivogen and were cultured in RPMI medium, 2mM L-glutamine, 10% FBS supplemented with 100 µg/ml Zeocin. SEAP levels in tissue culture medium were assessed according by QUANTI-Blue to the manufacturer's instructions.